U.S. PTO
61/631313
12/30/2011

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR §1.53(c).

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Ge H. Michael	Wei Shepard	San Diego, California San Diego, California

Additional inventors are being named on the 0 separately numbered sheets attached hereto

TITLE OF THE APPLICATION (500 characters max)

PH20 POLYPEPTIDE VARIANTS, FORMULATIONS AND USES THEREOF

CORRESPONDENCE ADDRESS

Direct all correspondence to:

[X] Customer Number: **13565**

[X] Firm *or* Individual Name	Stephanie Seidman McKenna Long & Aldridge LLP				
Address	4435 Eastgate Mall, Suite 400				
Address					
City	San Diego	State	CA	ZIP	92121
Country	United States	Telephone	(619) 595-8010	Fax	(619) 595-8135

ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification: *Number of Pages* 374

[X] Drawing(s): *Number of Sheets* 13 (1-2L)

X] Other: Compact Disc Transmittal Letter (1 page in duplicate) and a Return Postcard

[X] Sequence Listing: (2 CD's- Copy 1, Copy 2)

[ ] Application Data Sheet. See 37 CFR 1.76.

Fees Due:

[X] Filing Fee of $250 $250

[X] Application Size Fee: $1,860

Filing Fee of $250 ($125 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $310 ($155 for small entity for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[ ] Applicant Claims small entity status. See 37 CFR §1.27.

[ ] A check or money order is enclosed to cover the filing fees.

[X] The Commissioner is hereby authorized to charge the filing fee and any other fees or any unpaid amount that may be required in this application during its entire pendency, or credit any overpayment, to Deposit Account No. 50-0911. This sheet is filed in triplicate.

TOTAL FEE AMOUNT ($) $ 2,110.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature _____ Date December 30, 2011

Typed Name: Stephanie Seidman, Reg. No. 33,779

Telephone No.: (619) 595-8010

Docket No. 33320.03087.US70/ P3087

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
"Express Mail" Mailing Label Number: **EM 608820042 US**
Date of Deposit: **December 30, 2011**
I hereby certify that this paper is being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 CFR §1.10 on the date indicated above and is addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA, 22313-1450.

Karen Potter

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Karen Potter

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Wei *et al.*	Art Unit	: Not yet assigned
Serial No.	: Not yet assigned	Examiner	: Not yet assigned
Filed	: Herewith	Conf. No.	: Not yet assigned
Cust. No.	: 13565		

Title : PH20 POLYPEPTIDE VARIANTS, FORMULATIONS AND USES THEREOF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

COMPACT DISC TRANSMITTAL LETTER

Dear Sir:

Transmitted herewith are duplicate compact discs (CD-R) of a Sequence Listing (labeled Copy 1 and Copy 2). The computer-readable file on each of the aforementioned compact discs created on December 30, 2011, was made using an IBM-PC machine format with MS-Windows operating system compatibility. The computer-readable files on the two compact discs are identical, 3.15 megabytes in size, and are entitled P3087SEQ.001.txt.

☒ The Commissioner is hereby authorized to charge any fees that may be due in connection with this and the attached papers or with this application during its entire pendency to Deposit Account No. 50-0911. A duplicate of this sheet is enclosed.

Respectfully submitted,

Stephanie Seidman
Reg. No. 33,779

Attorney Docket No. 33320.03087.US70/ P3087
Address all correspondence to: 13565
Stephanie Seidman
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Karen Potter

PH20 POLYPEPTIDE VARIANTS, FORMULATIONS AND USES THEREOF

Incorporation by reference of Sequence Listing provided on compact discs

An electronic version on compact disc (CD-R) of the Sequence Listing is filed herewith in duplicate (labeled Copy # 1 and Copy # 2), the contents of which are incorporated by reference in their entirety. The computer-readable file on each of the aforementioned compact discs, created on December 30, 2011, is identical, 3.15 megabytes in size, and titled P3087SEQ.001.txt.

FIELD OF THE INVENTION

Modified PH20 hyaluronidase polypeptides, including modified polypeptides that exhibit increased stability and/or increased activity, are provided. Also provided are compositions and formulations and uses thereof.

BACKGROUND

Hyaluronan (hyaluronic acid; HA) is a polypeptide that is found in the extracellular matrix of many cells, especially in soft connective tissues. HA also is found predominantly in skin, cartilage, and in synovial fluid in mammals. Hyaluronan also is the main constituent of the vitreous of the eye. HA has a role in various physiological processes, such as in water and plasma protein homeostasis (Laurent TC et al (1992) *FASEB J* 6: 2397-2404). Certain diseases are associated with expression and/or production of hyaluronan. Hyaluronidases are enzymes that degrade hyaluronan. By catalyzing HA, hyaluronidases can be used to treat diseases or disorders associated with accumulation of HA or other glycosaminoglycans. Also, since HA is a major component of the interstitial barrier, hyaluronidase increases tissue permeability and therefore can be used to increase the dispersion and delivery of therapeutic agents. Various hyaluronidases have been used therapeutically (*e.g.* Hydase™, Vitrase™ and Wydase™), typically as dispersing and spreading agents in combination with other therapeutic agents. Many of these are ovine or bovine forms, which can be immunogenic for treatment of humans. Improved compositions of hyaluronidases that can be used for treatment are needed.

SUMMARY

Provided are modified PH20 polypeptides that have an altered property or properties compared to the PH20 polypeptide that do not have the modification(s). The modifications include amino acid replacement, deletion and/or insertions. Detailed structure/function of virtually each amino acid in a PH20 polypeptide is provided herein, as well as the identification of residues and loci that contribute to

Petitioner Merck, Ex. 1051, p. 6

alteration of a property, such as stability in particular conditions, is provided. Hence, provided are modified PH20 polypeptides that contain one or more amino acid replacements that result in a PH20 polypeptide that retains activity and/or exhibits increased or altered stability under a variety of conditions. Activity retained can be, for example, hyaluronidase activity that is as least about 40% or more of the PH20 polypeptide that does not include the replacement.

Provided are modified PH20 polypeptides that contain at least one amino acid replacement in an PH20 polypeptide, whereby the modified PH20 polypeptide exhibits increased stability compared to the PH20 polypeptide not containing the amino acid replacement. Increased stability can be manifested as increased resistance to one or more protein conditions that are denaturing to proteins. Stability of modified and unmodified PH20 is compared under the same conditions. Exemplary protein denaturation (or denaturing) conditions include, but are not limited to elevated temperature greater than 30°C or about 30 °C, agitation, low salt, including essentially or substantially or no salt, and presence of excipients that tend to denature proteins. Exemplary of such excipients are antiadherent(s), binder(s), coating(s), filler(s) and diluent(s), flavor(s), color(s), lubricant(s), glidant(s), preservative(s), detergent(s), sorbent(s) and combinations thereof.

The modified PH20 polypeptide can be one in which the unmodified form thereof has at least about 68% sequence identity to SEQ ID NO. 3 and further contains modifications that alter stability and/or can be a PH20 polypeptide that includes as many as about 100, 110, 120, 130, 150 amino acid differences from PH20 but retains enzymatic activity, particularly, at least about 40% of the activity of the unmodified PH20 polypeptide and exhibits increased stability, such as stability under denaturing conditions. Thus, included are modified PH20 polypeptides that have least 68% or about 68% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3. Included are modified PH20 polypeptides that have at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3. Exemplary of such modified PH20 polypeptides that contain amino acid replacement(s) is are any that contain the sequence of amino acid residues as set forth in any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, 72, 857, 859, 861, 870 or a sequence of amino acids that is at least 80%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, 72, 857, 859, 861, or 870.

Stability can be assessed based on a variety of parameters including hyaluronidase activity, solubility, aggregation and/or crystallization. Stability can be assessed in the presence of a denaturing condition. When stability of two or more polypeptides is compared, stability is assessed under the same conditions. In some instances, among the PH20 polypeptides provided herein, the modified PH20 polypeptide exhibits at least 120%, 130%, 135%, 140%, 145%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 350%, 400%, 500%, 1500%, 2000%, 3000%, 4000%, 5000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement(s).

Denaturing conditions include the presence of excipients that denature proteins. Exemplary of such conditions is the presence of a preservative, such as a phenolic preservative. Provided are modified PH20 polypeptides that exhibit increased stability in the presence of an anti-microbial effective amount of one or more phenolic preservatives. An anti-microbial effective amount is a total amount of one or more phenolic preservative agents can be expressed as a percentage (%) of mass concentration (w/v) that is or is between (or at least about or at about) 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive. Exemplary phenolic preservatives include, but are not limited to: phenol, metacresol (m-cresol), benzyl alcohol, and a paraben, such as methylparaben propylparaben, m-cresol, phenol or m-cresol and phenol. Exemplary of the stability achieved by provided modified PH20 polypeptides are those that exhibit at least 15% or about 15% of the hyaluronidase activity for at least 4 hours in the presence of preservative(s) compared to the modified PH20 polypeptide in absence of preservative. Activity is compared under the same conditions except for the presence of preservative(s). For example, provided are modified PH20 polypeptides that exhibit at least (or at least about) 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the hyaluronidase activity in the presence of a phenolic preservative(s) compared to absence of the same preservative(s). Thus, provided, among the modified PH20 polypeptides provided herein, are PH20 polypeptides that, by virtue of amino acid replacement(s), are phenophilic compared to PH20 polypeptides without such replacement. Included are modified PH20

polypeptides where the hyaluronidase activity is exhibited after at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks or more in the presence of the preservative(s) compared to the hyaluronidase activity of the modified PH20 polypeptide in the absence of preservative for the same time period and under the same conditions except for the presence of preservative(s).

Increased stability in a phenolic preservative can be exhibited under temperature conditions that include any temperature between, for example, 0°C and 40°C, such as between or about between 0°C to 40°C, 2°C to 6°C, 24°C to 32°C and 35°C to 40°C. Exemplary polypeptides exhibit increased stability at temperatures of between or about between 30°C to 45°C, 35° C to 45° C, 30° C to 37° C, 35° C to 37° C or 37° C to 42° C, each inclusive. The particular modified PH20 polypeptide and conditions depend upon the intended formulation, conditions to which the formulation will be exposed and/or intended application.

Particular and exemplary modified PH20 polypeptides include those that contain a single amino acid modification, such as replacement, and combinations of replacement, such as at least or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100 and more modifications. These include modified PH20 polypeptides that contain one or more amino acid replacements, where at least one replacement is at an amino acid position corresponding (*i.e.*, by alignment) to a position selected from among 10, 12, 20, 22, 26, 34, 36, 46, 50, 52, 58, 68, 70, 74, 82, 83, 84, 86, 97, 127, 131, 138, 142, 143, 144, 166, 169, 174, 193, 195, 196, 204, 205, 206, 213, 219, 234, 237, 238, 240, 249, 261, 267, 277, 279, 291, 309, 310, 314, 315, 317, 318, 347, 367, 375, 376, 399, 401, 407, 416, 419, 421, 431, 433, 439, 440, 443 or 445 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3. Exemplary of such modifications are at least one amino acid replacement selected from among replacement with:

G at a position corresponding to position 10; K at a position corresponding to position 12; S at a position corresponding to position 20; T at a position corresponding to position 22; M at a position corresponding to position 26; W at a position corresponding to position 34; N at a position corresponding to position 36; L at a

position corresponding to position 46; M at a position corresponding to position 50; T at a position corresponding to position 52; S at a position corresponding to position 52; C at a position corresponding to position 58; K at a position corresponding to position 58; R at a position corresponding to position 58; N at a position corresponding to position 58; Y at a position corresponding to position 58; P at a position corresponding to position 58; H at a position corresponding to position 58; P at a position corresponding to position 68; V at a position corresponding to position 70; E at a position corresponding to position 74; L at a position corresponding to position 82; N at a position corresponding to position 82; V at a position corresponding to position 83; Q at a position corresponding to position 83; S at a position corresponding to position 83; G at a position corresponding to position 83; N at a position corresponding to position 84; A at a position corresponding to position 86; K at a position corresponding to position 86; E at a position corresponding to position 97; L at a position corresponding to position 97; R at a position corresponding to position 127; R at a position corresponding to position 131; L at a position corresponding to position 138; K at a position corresponding to position 142; N at a position corresponding to position 142; P at a position corresponding to position 142; S at a position corresponding to position 142; T at a position corresponding to position 142; G at a position corresponding to position 143; K at a position corresponding to position 143; T at a position corresponding to position 144; Q at a position corresponding to position 166; T at a position corresponding to position 166; L at a position corresponding to position 169; G at a position corresponding to position 174; N at a position corresponding to position 174; Q at a position corresponding to position 193; T at a position corresponding to position 195; N at a position corresponding to position 195; E at a position corresponding to position 196; R at a position corresponding to position 196; P at a position corresponding to position 204; A at a position corresponding to position 205; E at a position corresponding to position 205; I at a position corresponding to position 206; A at a position corresponding to position 213; I at a position corresponding to position 219; M at a position corresponding to position 234; T at a position corresponding to position 237; H at a position corresponding to position 238; Q at a position corresponding to position 240; V at a position corresponding to position 249; A at a position corresponding to position 261; K at a position corresponding to position 261; T at a position corresponding to position 267; K at a position corresponding to

position 277; H at a position corresponding to position 279; V at a position corresponding to position 279; E at a position corresponding to position 309; Q at a position corresponding to position 310; Y at a position corresponding to position 314; Y at a position corresponding to position 315; N at a position corresponding to position 317; W at a position corresponding to position 317; D at a position corresponding to position 318; G at a position corresponding to position 347; A at a position corresponding to position 367; R at a position corresponding to position 375; R at a position corresponding to position 376; V at a position corresponding to position 399; E at a position corresponding to position 401; A at a position corresponding to position 407; L at a position corresponding to position 416; K at a position corresponding to position 419; H at a position corresponding to position 421; E at a position corresponding to position 431; T at a position corresponding to position 433; V at a position corresponding to position 433; C at a position corresponding to position 439; P at a position corresponding to position 440; G at a position corresponding to position 443; N at a position corresponding to position 445, with reference to amino acid residue positions set forth in SEQ ID NO:3. For example, the modified PH20 polypeptide can contain t least one amino acid replacement selected from among replacement with: T at a position corresponding to position 52, K at a position corresponding to position 58, R at a position corresponding to position 58, P at a position corresponding to position 68, V at a position corresponding to position 83, P at a position corresponding to position 204, A at a position corresponding to position 261, T at a position corresponding to position 267, K at a position corresponding to position 277 and H at a position corresponding to position 421, with reference to amino acid residue positions set forth in SEQ ID NO:3. An exemplary modified PH20 polypeptide is one that includes with P (or a conservative amino acid) at a position corresponding to position 204 in a PH20 polypeptide with reference to amino acid residue positions set forth in SEQ ID NO:3.

Among modified PH20 polypeptides provided herein that exhibit increased stability are those that exhibit increased hyaluronidase activity at the elevated temperature compared to the PH20 polypeptide not containing the amino acid replacement(s), such as at least 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500% or more hyaluronidase activity for at least 4 hours compared to the PH20 polypeptide not containing the amino acid replacement(s). Also among the polypeptides are those that exhibit activity, but

typically increased stability or other property, such as a modified PH20 polypeptide that exhibits at least 95%, 96 %, 97 %, 98 %, 99 %, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500% of the hyaluronidase activity for at least 4 hours at a temperature of between or about between 32°C to 37°C compared to the hyaluronidase activity of the modified PH20 polypeptide at a temperature of between or about between 2 °C to 8 °C, where activity is compared under the same conditions except for the differences in temperature. The hyaluronidase activity can be exhibited after at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks or more at elevated temperatures of between or about between 32°C to 37°C compared to the hyaluronidase activity of the modified PH20 polypeptide at a temperature between or about between 2 °C to 8 °C, where activity is compared for the same time period and under the same conditions except for the difference in temperature. Exemplary of such modified polypeptides are those that contain at least one amino acid replacement at an amino acid position corresponding to a position selected from among 1, 11, 12, 14, 20, 26, 29, 34, 50, 58, 70, 82, 83, 84, 86, 87, 140, 142, 143, 147, 152, 166, 167, 172, 174, 178, 193, 195, 206, 212, 213, 219, 233, 237, 240, 267, 277, 291, 292, 309, 313, 314, 317, 318, 347, 367, 368, 371, 374, 389, 392, 395, 396, 406, 419, 421, 439 and 443 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3. Exemplary mutations include, for example, replacement with R at a position corresponding to position 1; S at a position corresponding to position 11; I at a position corresponding to position 12; V at a position corresponding to position 14; S at a position corresponding to position 20; M at a position corresponding to position L with R at a position corresponding to position 29; W at a position corresponding to position 34; M at a position corresponding to position 50; K at a position corresponding to position 58; Q at a position corresponding to position 58; Q at a position corresponding to position 58; V at a position corresponding to position 70; L at a position corresponding to position 82; Q at a position corresponding to position 83; R at a position corresponding to position 84; A at a position corresponding to position 86; S at a position corresponding to position 87; K at a position corresponding to position 140; S at a position corresponding to position 142; T at a

position corresponding to position 142; K at a position corresponding to position 143; S at a position corresponding to position 147; T at a position corresponding to position 152; T at a position corresponding to position 166; D at a position corresponding to position 167; A at a position corresponding to position 172; G at a position corresponding to position 174; N at a position corresponding to position 174; R at a position corresponding to position 178; Q at a position corresponding to position 193; T at a position corresponding to position 195; I at a position corresponding to position 206; S at a position corresponding to position 212; A at a position corresponding to position 213; I at a position corresponding to position 219; G at a position corresponding to position 233; T at a position corresponding to position 237; A at a position corresponding to position 240; Q at a position corresponding to position 240; T at a position corresponding to position 267; E at a position corresponding to position 277; S at a position corresponding to position 291; H at a position corresponding to position 292; V at a position corresponding to position 292; S at a position corresponding to position 309; H at a position corresponding to position 313; S at a position corresponding to position 314; I at a position corresponding to position 317; T at a position corresponding to position 317; W at a position corresponding to position 317; R at a position corresponding to position 318; G at a position corresponding to position 347; A at a position corresponding to position 367; R at a position corresponding to position 368; S at a position corresponding to position 371; P at a position corresponding to position 374; A at a position corresponding to position 389; V at a position corresponding to position 392; A at a position corresponding to position 395; H at a position corresponding to position 396; N at a position corresponding to position 406; H at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 421; S at a position corresponding to position 421; A at a position corresponding to position 439; C at a position corresponding to position 439; and G at a position corresponding to position 443, with reference to amino acid positions set forth in SEQ ID NO:3.

Also provided are modified PH20 polypeptides that exhibit increased stability in low salt conditions, such as, for example, concentrations of NaCl of less than 100 mM, such as, but not limited to concentrations of NaCl less than 90 mM, 80mM, 70mM, 60 mM, 50 mM, 40 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM or less. Among the modified PH20 polypeptides are those that exhibit increased

hyaluronidase activity at lower concentrations of salt compared to the PH20 polypeptide not containing the amino acid replacement(s). Such activity includes, for example, at least more than 100%, or at least 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500% or more hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement(s). Exemplary of such modified PH20 polypeptides are those that exhibit at least 60% of the hyaluronidase activity in low concentrations of salt, such as between or about between 10 mM NaCl and 100 mM NaCl, inclusive (or comparable concentrations of other salts or mixtures of salts), compared to the hyaluronidase activity of the modified PH20 polypeptide in 150 mM NaCl, where activity is compared under the same conditions except for the difference in salt concentration.

Also provided are modified PH20 polypeptides that contain at least one amino acid replacement in a PH20 polypeptide, where the modified PH20 polypeptide exhibits increased hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement; and activity is compared under the same conditions. Among these are polypeptides, where the unmodified PH20 exhibits at least 68%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3, or the resulting modified PH20 exhibits such sequence identify to the sequence of amino acids set forth in SEQ ID NO:3. Exemplary of such modified PH20 polypeptides are any that have or contain a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, or 72, or a sequence of amino acids that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, or 72. In particular, provided are modified PH20 polypeptides that contain a sequence of amino acids set forth in SEQ ID NOS: 3, 7, 32-66, 69 or 72. Among the modified PH20 polypeptides are that that exhibit at least 120%, 130%, 135%, 140%, 145%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 350%, 400%, 500%, 1500%, 2000%, 3000%, 4000%, 5000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement. Activity can be assessed at any temperature, particular where activity is assessed at a temperature between or about between 2 °C to 8 °C. These modified PH20 polypeptides contain at least one amino acid replacement at an amino acid position corresponding to a position selected from among 1, 12, 15, 24, 26, 27, 29, 30,

31, 32, 33, 37, 39, 46, 48, 52, 58, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 84, 86, 87, 92, 93, 94, 97, 118, 120, 127, 131, 135, 141, 142, 147, 148, 150, 151, 152, 155, 156, 163, 164, 165, 166, 169, 170, 174, 198, 206, 209, 212, 213, 215, 219, 233, 234, 236, 238, 247, 257, 259, 260, 261, 263, 269, 271, 272, 276, 277, 278, 282, 291, 293, 305, 308, 309, 310, 313, 315, 317, 318, 320, 324, 325, 326, 328, 347, 353, 359, 371, 377, 380, 389, 392, 395, 399, 405, 407, 409, 410, 418, 419, 421, 425, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3. Exemplary modifications include at least one amino acid replacement selected from among replacement with: histidine (H) at a position corresponding to position 1; Q at a position corresponding to position 1; E at a position corresponding to position 12; T at a position corresponding to position 12; V at a position corresponding to position 15; E at a position corresponding to position 24; H at a position corresponding to position 24; E at a position corresponding to position 26; K at a position corresponding to position 26; K at a position corresponding to position 27; R at a position corresponding to position 27; E at a position corresponding to position 29; I at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; S at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; F at a position corresponding to position 32; G at a position corresponding to position 32; H at a position corresponding to position 32; W at a position corresponding to position 33; F at a position corresponding to position 37; N at a position corresponding to position 39; T at a position corresponding to position 39; R at a position corresponding to

position 46; F at a position corresponding to position 48; H at a position corresponding to position 48; N at a position corresponding to position 48; Q at a position corresponding to position 52; K at a position corresponding to position 58; Q at a position corresponding to position 58; W at a position corresponding to position 63; V at a position corresponding to position 67; H at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position 69; C at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; I at a position corresponding to position 69; L at a position corresponding to position 69; M at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; W at a position corresponding to position 69; Y at a position corresponding to position 69; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; N at a position corresponding to position 70; P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; R at a position corresponding to position 71; S at a position corresponding to position 71; M at a position corresponding to position 72; Q at a position corresponding to position 72; H at a position corresponding to position 73; L at a position corresponding to position 73; W at a position corresponding to position 73; A at a position corresponding to position 74; C at a position corresponding to position 74; G at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; F at a position corresponding to position 75; L at a position corresponding to position 75; R at a position corresponding to position 75; T at a position corresponding to position 75; G at a position corresponding to position 84; R at a position corresponding to position 84; A at a position corresponding to position 86; C at a position corresponding to position 87; T at a position corresponding to position 87; Y at a position corresponding to position 87; C at a position corresponding to position

92; I at a position corresponding to position 93; L at a position corresponding to position 93; R at a position corresponding to position 93; T at a position corresponding to position 93; R at a position corresponding to position 94; G at a position corresponding to position 97; Q at a position corresponding to position 118; F at a position corresponding to position 120; V at a position corresponding to position 120; Y at a position corresponding to position 120; H at a position corresponding to position 127; N at a position corresponding to position 127; G at a position corresponding to position 131; R at a position corresponding to position 131; V at a position corresponding to position 131; D at a position corresponding to position 135; G at a position corresponding to position 135; R at a position corresponding to position 135, with H at a position corresponding to position 141; Y at a position corresponding to position 141; R at a position corresponding to position 142; R at a position corresponding to position 147; V at a position corresponding to position 147; K at a position corresponding to position 148; G at a position corresponding to position 150; K at a position corresponding to position 151; L at a position corresponding to position 151; M at a position corresponding to position 151; Q at a position corresponding to position 151; R at a position corresponding to position 151; R at a position corresponding to position 152; G at a position corresponding to position 155; K at a position corresponding to position 155; D at a position corresponding to position 156; A at a position corresponding to position 163; E at a position corresponding to position 163; K at a position corresponding to position 163; R at a position corresponding to position 163; M at a position corresponding to position 164; D at a position corresponding to position 165; N at a position corresponding to position 165; A at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; Q at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; Y at a position corresponding to position 166; L at a position corresponding to position 169; R at a position corresponding to position 170; K at a position corresponding to position 174; D at a position corresponding to position 198; K at a position corresponding to position 206; L at a position corresponding to position 206; N at a position corresponding to position 212; M at a position corresponding to position 213; N at a position corresponding to position 213; M at a position corresponding to position 215; S at a position

corresponding to position 219; K at a position corresponding to position 233; R at a position corresponding to position 233; M at a position corresponding to position 234; R at a position corresponding to position 236; E at a position corresponding to position 237; S at a position corresponding to position 238; I at a position corresponding to position 247; T at a position corresponding to position 257; P at a position corresponding to position 259; Y at a position corresponding to position 260; K at a position corresponding to position 261; N at a position corresponding to position 261; K at a position corresponding to position 263; R at a position corresponding to position 263; A at a position corresponding to position 269; L at a position corresponding to position 271; M at a position corresponding to position 271; T at a position corresponding to position 272; D at a position corresponding to position 276; S at a position corresponding to position 276; Y at a position corresponding to position 276; K at a position corresponding to position 277; R at a position corresponding to position 277; T at a position corresponding to position 277; H at a position corresponding to position 278; K at a position corresponding to position 278; N at a position corresponding to position 278; R at a position corresponding to position 278; S at a position corresponding to position 278; T at a position corresponding to position 278; Y at a position corresponding to position 278; M at a position corresponding to position 282; V at a position corresponding to position 291; A at a position corresponding to position 293; C at a position corresponding to position 293; F at a position corresponding to position 293; M at a position corresponding to position 293; P at a position corresponding to position 293; Q at a position corresponding to position 293; V at a position corresponding to position 293; E at a position corresponding to position 305; G at a position corresponding to position 308; N at a position corresponding to position 308; E at a position corresponding to position 309; L at a position corresponding to position 309; N at a position corresponding to position 309; Q at a position corresponding to position 309; R at a position corresponding to position 309; T at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; K at a position corresponding to position 313; R at a position corresponding to position 313; H at a position corresponding to position 315; I at a position corresponding to position 317; K at a position corresponding to position 317; R at a position corresponding to position 317; M at a position corresponding to position 318; H at a position corresponding to position 320;

K at a position corresponding to position 320; R at a position corresponding to position 320; R at a position corresponding to position 324; A at a position corresponding to position 325; D at a position corresponding to position 325; E at a position corresponding to position 325; G at a position corresponding to position 325; H at a position corresponding to position 325; K at a position corresponding to position 325; M at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; S at a position corresponding to position 325; V at a position corresponding to position 326; I at a position corresponding to position 328; K at a position corresponding to position 328; L at a position corresponding to position 328; S at a position corresponding to position 328; Y at a position corresponding to position 328; G at a position corresponding to position 347; S at a position corresponding to position 347; V at a position corresponding to position 353; with T at a position corresponding to position 359; R at a position corresponding to position 371; P at a position corresponding to position 377; T at a position corresponding to position 377; W at a position corresponding to position 380; Y at a position corresponding to position 380; K at a position corresponding to position 389; M at a position corresponding to position 392; R at a position corresponding to position 395; M at a position corresponding to position 399; T at a position corresponding to position 399; W at a position corresponding to position 399; G at a position corresponding to position 405; D at a position corresponding to position 407; Q at a position corresponding to position 407; A at a position corresponding to position 409; Q at a position corresponding to position 409; T at a position corresponding to position 410; P at a position corresponding to position 418; F at a position corresponding to position 419; I at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; H at a position corresponding to position 421; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; K at a position corresponding to position 425; A at a position corresponding to position 431; H at a position corresponding to position 431; K at a position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to

position 431; L at a position corresponding to position 433; R at a position corresponding to position 433; T at a position corresponding to position 433; V at a position corresponding to position 433; K at a position corresponding to position 436; I at a position corresponding to position 437; M at a position corresponding to position 437; T at a position corresponding to position 438; V at a position corresponding to position 439; H at a position corresponding to position 440; R at a position corresponding to position 440; F at a position corresponding to position 441; R at a position corresponding to position 442; A at a position corresponding to position 443; M at a position corresponding to position 443; M at a position corresponding to position 445; P at a position corresponding to position 445; A at a position corresponding to position 446; D at a position corresponding to position 447; N at a position corresponding to position 447; and/or with Q at a position corresponding to position 447, with reference to amino acid positions set forth in SEQ ID NO:3. Among these polypeptides are those that exhibit at least 2.0-fold of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement.

Among these are modified PH20 polypeptides that contain comprising at least one amino acid replacement at an amino acid position corresponding to a position selected from among 24, 29, 31, 48, 58, 69, 70, 75, 84, 97, 165, 166, 271, 278, 317, 320, 325 and 326 with reference to positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3, such as modified PH20 polypeptides that contain at least one amino acid replacement selected from among replacement with: E at a position corresponding to position 24; E at a position corresponding to position 29; V at a position corresponding to position 31; N at a position corresponding to position 48; K at a position corresponding to position 58; Q at a position corresponding to position 58; A at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; A at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; H at a position corresponding to position 70; N at a position corresponding to position 70; R at a position corresponding to position 70; T at a position corresponding to position 70; V at a

position corresponding to position 70; L at a position corresponding to position 75; T at a position corresponding to position 75; G at a position corresponding to position 84; G at a position corresponding to position 97; D at a position corresponding to position 165; L at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; L at a position corresponding to position 271; H at a position corresponding to position 278; R at a position corresponding to position 278; K at a position corresponding to position 317; K at a position corresponding to position 320; E at a position corresponding to position 325, with G at a position corresponding to position 325; K at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; and V at a position corresponding to position 326; with reference to amino acid positions set forth in SEQ ID NO:3.

Also provided are modified PH20 polypeptides that contain at least one amino acid replacement in the PH20 polypeptide whose sequence is set forth in SEQ ID NO:7, a C-terminally truncated fragment thereof, a soluble fragment thereof, or in a PH20 polypeptide that has a sequence of amino acids that is at least 91% identical to the sequence of amino acids set forth in SEQ ID NO:7, where the at least one amino replacement(s) is at an amino acid position corresponding to a position selected from among 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 20, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 58, 59, 60, 61, 63, 65, 66, 67, 68, 69, 70, 71, 72, 73, 75, 75, 77, 79, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 96, 97, 98, 99, 102, 103, 104, 105, 106, 107, 108, 110, 114, 117, 118, 119, 120, 122, 124, 125, 127, 128, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 186, 192, 193, 195, 196, 197, 198, 200, 202, 204, 205, 206, 208, 209, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 224, 226, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 242, 245, 247, 248, 251, 253, 255, 256, 257, 258, 259, 260, 261, 263, 264, 265, 266, 267, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 297, 298, 300, 301, 302, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 320, 321, 323, 324, 325, 326, 327, 328, 331, 334, 335, 338, 339, 342, 343, 347, 348, 349, 351, 353, 356, 357, 358, 359, 360, 361, 367, 368, 369, 371, 373, 374, 375, 376, 377, 378,

379, 380, 381, 383, 385, 387, 388, 389, 391, 392, 393, 394, 395, 396, 397, 398, 399, 401, 403, 404, 405, 406, 407, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 425, 426, 427, 428, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3 or 7, where corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3; and provided that if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 13, 47, 131, or 219 the replacement is not replacement with an Alanine (A). Among these modified PH20 polypeptides are those that exhibit at least 40% of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement, where, as in all instances herein activity is compared under the same conditions. Included among these polypeptides are those that contain a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 and 72, including those in which the amino acid replacement is an amino acid replacement set forth in Table 3 below, and, including those that have at least one amino acid replacement at an amino acid position corresponding to a position selected from among 1, 6, 8, 9, 10, 11, 12, 14, 15, 20, 22, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 46, 47, 48, 49, 50, 52, 58, 59, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 79, 82, 83, 84, 86, 87, 89, 90, 92, 93, 94, 97, 102, 104, 107, 114, 118, 120, 127, 128, 130, 131, 132, 135, 138, 139, 140, 141, 142, 143, 146, 147, 148, 149, 150, 151, 152, 155, 156, 158, 160, 162, 163, 164, 165, 166, 167, 169, 170, 172, 173, 174, 175, 178, 179, 193, 195, 196, 198, 204, 205, 206, 209, 212, 213, 215, 219, 220, 221, 222, 232, 233, 234, 235, 236, 237, 238, 240, 247, 248, 249, 257, 258, 259, 260, 261, 263, 267, 269, 271, 272, 273, 274, 276, 277, 278, 279, 282, 283, 285, 287, 289, 291, 292, 293, 305, 307, 308, 309, 310, 313, 314, 315, 317, 318, 320, 321, 324, 325, 326, 328, 335, 347, 349, 351, 353, 356, 359, 367, 368, 369, 371, 373, 374, 375, 376, 377, 380, 381, 383, 385, 389, 392, 393, 395, 396, 399, 401, 404, 405, 406, 407, 409, 410, 412, 416, 418, 419, 421, 425, 427, 428, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446 or 447 with reference to amino acid positions set forth in SEQ ID NO:3. Exemplary of such replacements are those that contain at least one amino acid replacement selected from among replacement with: histidine (H) at a position corresponding to position 1; A at a position corresponding to position 1; E at a position corresponding to position 1; G at a position corresponding to position 1; K at a position corresponding to position 1; Q at

a position corresponding to position 1; R at a position corresponding to position 1; A at a position corresponding to position 6; M at a position corresponding to position 8; Q at a position corresponding to position 9; G at a position corresponding to position 10; H at a position corresponding to position 10; S at a position corresponding to position 11; E at a position corresponding to position 12; I at a position corresponding to position 12; K at a position corresponding to position 12; T at a position corresponding to position 12; V at a position corresponding to position 14; V at a position corresponding to position 15; M at a position corresponding to position 15; S at a position corresponding to position 20; T at a position corresponding to position 22; E at a position corresponding to position 24; H at a position corresponding to position 24; R at a position corresponding to position 24; A at a position corresponding to position 26; E at a position corresponding to position 26; K at a position corresponding to position 26; M at a position corresponding to position 26; Q at a position corresponding to position 26; R at a position corresponding to position 26; D at a position corresponding to position 27; K at a position corresponding to position 27; R at a position corresponding to position 27; R at a position corresponding to position 28; E at a position corresponding to position 29; I at a position corresponding to position 29; K at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; R at a position corresponding to position 29; S at a position corresponding to position 29; T at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; L at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; G at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; W at a position corresponding to position 31; C at a position corresponding to position 32; F at a position corresponding to position

32; G at a position corresponding to position 32; H at a position corresponding to position 32; W at a position corresponding to position 33; G at a position corresponding to position 33; W at a position corresponding to position 34; Q at a position corresponding to position 35; V at a position corresponding to position 35; H at a position corresponding to position 36; N at a position corresponding to position 36; F at a position corresponding to position 37; M at a position corresponding to position 37; Y at a position corresponding to position 38; A at a position corresponding to position 39; L at a position corresponding to position 39; N at a position corresponding to position 39; T at a position corresponding to position 39; L at a position corresponding to position 40; T at a position corresponding to position 41; L at a position corresponding to position 46; R at a position corresponding to position 46; D at a position corresponding to position 47; F at a position corresponding to position 47; T at a position corresponding to position 47; W at a position corresponding to position 47, with F at a position corresponding to position 48; H at a position corresponding to position 48; K at a position corresponding to position 48; N at a position corresponding to position 48; R at a position corresponding to position 49; D at a position corresponding to position 50; S at a position corresponding to position 50; M at a position corresponding to position 50; N at a position corresponding to position 52; Q at a position corresponding to position 52; R at a position corresponding to position 52; S at a position corresponding to position 52; T at a position corresponding to position 52; C at a position corresponding to position 58; K at a position corresponding to position 58; L at a position corresponding to position 58; P at a position corresponding to position 58; Q at a position corresponding to position 58; R at a position corresponding to position 58; H at a position corresponding to position 58; N at a position corresponding to position 58; Y at a position corresponding to position 58; N at a position corresponding to position 59; K at a position corresponding to position 63; L at a position corresponding to position 63; M at a position corresponding to position 63; R at a position corresponding to position 63; W at a position corresponding to position 63; V at a position corresponding to position 67; H at a position corresponding to position 68; P at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position 69; C at a position corresponding to position 69; E at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position

69; I at a position corresponding to position 69; L at a position corresponding to position 69; M at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; T at a position corresponding to position 69; W at a position corresponding to position 69; Y at a position corresponding to position 69; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; N at a position corresponding to position 70; P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; Y at a position corresponding to position 70; G at a position corresponding to position 71; N at a position corresponding to position 71; R at a position corresponding to position 71; S at a position corresponding to position 71; K at a position corresponding to position 72; M at a position corresponding to position 72; Q at a position corresponding to position 72; A at a position corresponding to position 73; H at a position corresponding to position 73; K at a position corresponding to position 73; L at a position corresponding to position 73; Q at a position corresponding to position 73; R at a position corresponding to position 73; T at a position corresponding to position 73; W at a position corresponding to position 73; A at a position corresponding to position 74; C at a position corresponding to position 74; E at a position corresponding to position 74; F at a position corresponding to position 74; G at a position corresponding to position 74; H at a position corresponding to position 74; K at a position corresponding to position 74; L at a position corresponding to position 74; M at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; F at a position corresponding to position 75; L at a position corresponding to position 75; M at position corresponding to position 75; R at a position corresponding to position 75; T at a position corresponding to position 75; L at a position corresponding to position 79; L at a position corresponding to position 82; N at a position corresponding to position 82; V at a position corresponding to position 83; Q

at a position corresponding to position 83; S at a position corresponding to position 83; G at a position corresponding to position 83; E at a position corresponding to position 84; F at a position corresponding to position 84; G at a position corresponding to position 84; N at a position corresponding to position 84; R at a position corresponding to position 84; A at a position corresponding to position 86; H at a position corresponding to position 86; K at a position corresponding to position 86; N at a position corresponding to position 86; S at a position corresponding to position 86; T at a position corresponding to position 86; W at a position corresponding to position 86; C at a position corresponding to position 87; G at a position corresponding to position 87; L at a position corresponding to position 87; M at a position corresponding to position 87; R at a position corresponding to position 87; S at a position corresponding to position 87; T at a position corresponding to position 87; V at a position corresponding to position 87; Y at a position corresponding to position 87; C at a position corresponding to position 89; A at a position corresponding to position 90; E at a position corresponding to position 90; H at a position corresponding to position 90; K at a position corresponding to position 90; N at a position corresponding to position 90; R at a position corresponding to position 90; C at a position corresponding to position 92; L at a position corresponding to position 92; I at a position corresponding to position 93; L at a position corresponding to position 93; Q at a position corresponding to position 93; R at a position corresponding to position 93; S at a position corresponding to position 93; T at a position corresponding to position 93; D at a position corresponding to position 94; Q at a position corresponding to position 94; R at a position corresponding to position 94; A at a position corresponding to position 97; C at an amino acid residue corresponding to position 97; D at a position corresponding to position 97; E at a position corresponding to position 97; G at a position corresponding to position 97; L at a position corresponding to position 97; S at a position corresponding to position 97; S at a position corresponding to position 102; T at a position corresponding to position 102; R at a position corresponding to position 104; L at a position corresponding to position 107; A at a position corresponding to position 114; Q at a position corresponding to position 118; H at a position corresponding to position 120; F at a position corresponding to position 120; I at a position corresponding to position 120; S at a position corresponding to position 120; V at a position corresponding to position 120; Y at a position corresponding to

position 120; E at a position corresponding to position 127; H at a position corresponding to position 127; N at a position corresponding to position 127; Q at a position corresponding to position 127; R at a position corresponding to position 127; I at a position corresponding to position 128; R at a position corresponding to position 130; G at a position corresponding to position 131; I at a position corresponding to position 131; M at a position corresponding to position 131; Q at a position corresponding to position 131; R at a position corresponding to position 131; V at a position corresponding to position 131; N at a position corresponding to position 132; L at a position corresponding to position 132; D at a position corresponding to position 135; G at a position corresponding to position 135; R at a position corresponding to position 135, with L at a position corresponding to position 138; T at a position corresponding to position 139; K at a position corresponding to position 140; H at a position corresponding to position 141; R at a position corresponding to position 141; S at a position corresponding to position 141; W at a position corresponding to position 141; Y at a position corresponding to position 141; D at a position corresponding to position 142; G at a position corresponding to position 142; K at a position corresponding to position 142; N at a position corresponding to position 142; P at a position corresponding to position 142; Q at a position corresponding to position 142; R at a position corresponding to position 142; S at a position corresponding to position 142; T at a position corresponding to position 142; G at a position corresponding to position 143; K at a position corresponding to position 143; R at a position corresponding to position 144; T at a position corresponding to position 144; P at a position corresponding to position 146; R at a position corresponding to position 146; A at a position corresponding to position 147; F at a position corresponding to position 147; L at a position corresponding to position 147; R at a position corresponding to position 147; S at a position corresponding to position 147; V at a position corresponding to position 147; H at a position corresponding to position 148; K at a position corresponding to position 148; Q at a position corresponding to position 148; T at a position corresponding to position 149; V at a position corresponding to position 149; A at a position corresponding to position 150; D at a position corresponding to position 150; G at a position corresponding to position 150; N at a position corresponding to position 150; S at a position corresponding to position 150; W at a position corresponding to position 150; Y at a position corresponding to position 150; A at a position

corresponding to position 151; H at a position corresponding to position 151; K at a position corresponding to position 151; L at a position corresponding to position 151; M at a position corresponding to position 151; Q at a position corresponding to position 151; R at a position corresponding to position 151; S at a position corresponding to position 151; T at a position corresponding to position 151; V at a position corresponding to position 151; W at a position corresponding to position 151; Y at a position corresponding to position 151; R at a position corresponding to position 152; T at a position corresponding to position 152; W at a position corresponding to position 152; D at a position corresponding to position 155; G at a position corresponding to position 155; K at a position corresponding to position 155; R at a position corresponding to position 155; D at a position corresponding to position 156; Q at a position corresponding to position 158; S at a position corresponding to position 158; S at a position corresponding to position 160; E at a position corresponding to position 162; A at a position corresponding to position 163; E at a position corresponding to position 163; K at a position corresponding to position 163; Q at a position corresponding to position 163; R at a position corresponding to position 163; S at a position corresponding to position 163; M at a position corresponding to position 164; V at a position corresponding to position 164; D at a position corresponding to position 165; F at a position corresponding to position 165; N at a position corresponding to position 165; S at a position corresponding to position 165; V at a position corresponding to position 165; A at a position corresponding to position 166; E at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; Q at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; W at a position corresponding to position 166; Y at a position corresponding to position 166; D at a position corresponding to position 167; L at a position corresponding to position 169; R at a position corresponding to position 170; A at a position corresponding to position 172; R at a position corresponding to position 173; G at a position corresponding to position 174; K at a position corresponding to position 174; N at a position corresponding to position 174; R at a position corresponding to position 174; T at a position corresponding to position 174; T at a position corresponding to position 175; K at a position corresponding to position 178; R at a position corresponding to position 178;

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K at a position corresponding to position 179; Q at a position corresponding to position 193; T at a position corresponding to position 195; N at a position corresponding to position 195; with E at a position corresponding to position 196; R at a position corresponding to position 196; with D at a position corresponding to position 198; P at a position corresponding to position 204; A at a position corresponding to position 205; E at a position corresponding to position 205; L at a position corresponding to position 205; T at a position corresponding to position 205; I at a position corresponding to position 206; K at a position corresponding to position 206; L at a position corresponding to position 206; R at a position corresponding to position 206; R at a position corresponding to position 209; N at a position corresponding to position 212; S at a position corresponding to position 212; A at a position corresponding to position 213; M at a position corresponding to position 213; N at a position corresponding to position 213; H at a position corresponding to position 215; M at a position corresponding to position 215; I at a position corresponding to position 219; K at a position corresponding to position 219; S at a position corresponding to position 219; H at a position corresponding to position 220; I at a position corresponding to position 220; L at a position corresponding to position 220; V at a position corresponding to position 220; Q at a position corresponding to position 221; G at a position corresponding to position 222; F at a position corresponding to position 232; g at a position corresponding to position 233; K at a position corresponding to position 233; R at a position corresponding to position 233; M at a position corresponding to position 234; A at a position corresponding to position 235; R at a position corresponding to position 236; C at a position corresponding to position 237; E at a position corresponding to position 237; H at a position corresponding to position 237; Q at a position corresponding to position 237; T at a position corresponding to position 237; E at a position corresponding to position 238; H at a position corresponding to amino acid position 238; S at a position corresponding to position 238; A at a position corresponding to position 240; Q at a position corresponding to position 240; I at a position corresponding to position 247; A at a position corresponding to position 248; V at a position corresponding to position 249; G at a position corresponding to position 257; T at a position corresponding to position 257; R at a position corresponding to position 257; N at a position corresponding to position 258; S at a position corresponding to position 258; P at a position corresponding to position 259; M at a position corresponding to

position 260; Y at a position corresponding to position 260; A at a position corresponding to position 261; K at a position corresponding to position 261; N at a position corresponding to position 261; K at a position corresponding to position 263; R at a position corresponding to position 263; T at a position corresponding to position 267; A at a position corresponding to position 269; L at a position corresponding to position 271; M at a position corresponding to position 271; D at a position corresponding to position 272; T at a position corresponding to position 272; H at a position corresponding to position 273; Y at a position corresponding to position 273; F at a position corresponding to position 274; D at a position corresponding to position 276; H at a position corresponding to position 276; M at a position corresponding to position 276; R at a position corresponding to position 276; S at a position corresponding to position 276; Y at a position corresponding to position 276; A at a position corresponding to position 277; E at a position corresponding to position 277; H at a position corresponding to position 277; K at a position corresponding to position 277; M at a position corresponding to position 277; N at a position corresponding to position 277; Q at a position corresponding to position 277; R at a position corresponding to position 277; S at a position corresponding to position 277; T at a position corresponding to position 277; E at a position corresponding to position 278; F at a position corresponding to position 278; G at a position corresponding to position 278; H at a position corresponding to position 278; K at a position corresponding to position 278; N at a position corresponding to position 278; R at a position corresponding to position 278; S at a position corresponding to position 278; T at a position corresponding to position 278; Y at a position corresponding to position 278; H at a position corresponding to position 279; M at a position corresponding to position 282; S at a position corresponding to position 283; H at a position corresponding to position 285; T at a position corresponding to position 287; S at a position corresponding to position 289; S at a position corresponding to position 291; V at a position corresponding to position 291; C at a position corresponding to position 292; F at a position corresponding to position 292; H at a position corresponding to position 292; K at a position corresponding to position 292; R at a position corresponding to position 292; V at a position corresponding to position 292; A at a position corresponding to position 293; C at a position corresponding to position 293; D at a position corresponding to position 293; F at a position corresponding to position 293; K at a

position corresponding to position 293; M at a position corresponding to position 293; P at a position corresponding to position 293; Q at a position corresponding to position 293; V at a position corresponding to position 293; Y at a position corresponding to position 293; G at a position corresponding to position 298; E at a position corresponding to position 305; G at a position corresponding to position 307; D at a position corresponding to position 308; G at a position corresponding to position 308; K at a position corresponding to position 308; N at a position corresponding to position 308; R at a position corresponding to position 308; E at a position corresponding to position 309; G at a position corresponding to position 309; H at a position corresponding to position 309; L at a position corresponding to position 309; M at a position corresponding to position 309; N at a position corresponding to position 309; Q, at a position corresponding to position 309; R at a position corresponding to position 309; S at a position corresponding to position 309; T at a position corresponding to position 309; V at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; Q at a position corresponding to position 310; S at a position corresponding to position 310; A at a position corresponding to position 313; G at a position corresponding to position 313; H at a position corresponding to position 313; K at a position corresponding to position 313; P at a position corresponding to position 313; R at a position corresponding to position 313; T at a position corresponding to position 313; Y at a position corresponding to position 313; with S at a position corresponding to position 314; Y at a position corresponding to position 314; A at a position corresponding to position 315; H at a position corresponding to position 315; Y at a position corresponding to position 315; A at a position corresponding to position 317; I at a position corresponding to position 317; K at a position corresponding to position 317; N at a position corresponding to position 317; Q at a position corresponding to position 317; R at a position corresponding to position 317; S at a position corresponding to position 317; T at a position corresponding to position 317; W at a position corresponding to position 317; D at a position corresponding to position 318; H at a position corresponding to position 318; K at a position corresponding to position 318; M at a position corresponding to position 318; R at a position corresponding to position 318; H at a position corresponding to position 320; K at a position corresponding to position 320; R at a position corresponding to position 320; R at a position corresponding to

position 321; S at a position corresponding to position 321; N at a position corresponding to position 324; R at a position corresponding to position 324; A at a position corresponding to position 325; D at a position corresponding to position 325; E at a position corresponding to position 325; G at a position corresponding to position 325; H at a position corresponding to position 325; K at a position corresponding to position 325; M at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; S at a position corresponding to position 325; V at a position corresponding to position 325; L at a position corresponding to position 326; V at a position corresponding to position 326; C at a position corresponding to position 328; G at a position corresponding to position 328; I at a position corresponding to position 328; K at a position corresponding to position 328; L at a position corresponding to position 328; S at a position corresponding to position 328; Y at a position corresponding to position 328; S at a position corresponding to position 335; A at a position corresponding to position 347; G at a position corresponding to position 347; S at a position corresponding to position 347; M at a position corresponding to position 349; R at a position corresponding to position 349; S at a position corresponding to position 351; V at a position corresponding to position 353; with H at a position corresponding to position 356; S at a position corresponding to position 356; E at a position corresponding to position 359; H at a position corresponding to position 359; T at a position corresponding to position 359; A at a position corresponding to position 367; G at a position corresponding to position 367; K at a position corresponding to position 367; S at a position corresponding to position 367; A at a position corresponding to position 368; E at a position corresponding to position 368; K at a position corresponding to position 368; L at a position corresponding to amino acid position 368; M at a position corresponding to amino acid position 368; R at a position corresponding to position 368; T at a position corresponding to amino acid position 368; H at a position corresponding to position 369; R at a position corresponding to position 369; F at a position corresponding to position 371; H at a position corresponding to position 371; K at a position corresponding to position 371; L at a position corresponding to position 371; R at a position corresponding to position 371; S at a position corresponding to position 371; M at a position corresponding to position 373; H at a position corresponding to position 374; P at a position corresponding to position 374; A at a position

corresponding to position 375; G at a position corresponding to position 375; K at a position corresponding to position 375; R at a position corresponding to position 375; D at a position corresponding to position 376; E at a position corresponding to position 376; Q at a position corresponding to position 376; R at a position corresponding to position 376; T at a position corresponding to position 376; V at a position corresponding to position 376; Y at a position corresponding to position 376; D at a position corresponding to position 377; E at a position corresponding to position 377; H at a position corresponding to position 377; K at a position corresponding to position 377; P at a position corresponding to position 377; R at a position corresponding to position 377; S at a position corresponding to position 377; T at a position corresponding to position 377; W at a position corresponding to position 380; Y at a position corresponding to position 380; S at a position corresponding to position 381; I at a position corresponding to position 383; K at a position corresponding to position 383; L at a position corresponding to position 383; S at a position corresponding to position 383; A at a position corresponding to position 385; Q at a position corresponding to position 385; V at a position corresponding to position 385; A at a position corresponding to position 389; G at a position corresponding to position 389; L at a position corresponding to position 389; K at a position corresponding to position 389; Q at a position corresponding to position 389; S at a position corresponding to position 389; A at a position corresponding to position 392; F at a position corresponding to position 392; M at a position corresponding to position 392; Q at a position corresponding to position 392; R at a position corresponding to position 392; V at a position corresponding to position 392; F at a position corresponding to position 393; M at a position corresponding to position 393; A at a position corresponding to position 395; H at a position corresponding to position 395; R at a position corresponding to position 395; A at a position corresponding to position 396; H at a position corresponding to position 396; Q at a position corresponding to position 396; S at a position corresponding to position 396; K at a position corresponding to position 399; M at a position corresponding to position 399; T at a position corresponding to position 399; V at a position corresponding to position 399; W at a position corresponding to position 399; A at a position corresponding to position 401; E at a position corresponding to position 401; A at a position corresponding to position 404; G at a position corresponding to position 405; F at a position corresponding to position 406;

N at a position corresponding to position 406; A at a position corresponding to position 407; D at a position corresponding to position 407; E at a position corresponding to position 407; F at a position corresponding to position 407; H at a position corresponding to position 407; Q at a position corresponding to position 407; P at a position corresponding to position 407; A at a position corresponding to position 409; Q at a position corresponding to position 409; T at a position corresponding to position 410; Q at a position corresponding to position 412; R at a position corresponding to position 412; V at a position corresponding to position 412; L at a position corresponding to position 416; E at a position corresponding to position 418; L at a position corresponding to position 418; P at a position corresponding to position 418; R at a position corresponding to position 418; V at a position corresponding to position 418; F at a position corresponding to position 419; H at a position corresponding to position 419; I at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; Y at a position corresponding to position 419; A at a position corresponding to position 421; H at a position corresponding to position 421; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; G at a position corresponding to position 425; K at a position corresponding to position 425; Q at a position corresponding to position 427; T at a position corresponding to position 427; L at a position corresponding to position 428; A at a position corresponding to position 431; G at a position corresponding to position 431; E at a position corresponding to position 431; H at a position corresponding to position 431; K at a position corresponding to position 431; L at a position corresponding to position 431; N at a position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to position 431; A at a position corresponding to position 433; H at a position corresponding to position 433; I at a position corresponding to position 433; K at a position corresponding to position 433; L at a position corresponding to position 433; R at a position corresponding to position 433; T at a position corresponding to position 433; V at a position corresponding to position 433; W at a position corresponding to position 433; K at a position corresponding to position 436;

I at a position corresponding to position 437; M at a position corresponding to position 437; A at a position corresponding to position 438; D at a position corresponding to position 438; E at a position corresponding to position 438; L at a position corresponding to position 438; N at a position corresponding to position 438; T at a position corresponding to position 438; A at a position corresponding to position 439; C at a position corresponding to position 439; K at a position corresponding to position 439; P at a position corresponding to position 439; Q at a position corresponding to position 439; T at a position corresponding to position 439; V at a position corresponding to position 439; D at a position corresponding to position 440; H at a position corresponding to position 440; M at a position corresponding to position 440; P at a position corresponding to position 440; R at a position corresponding to position 440; S at a position corresponding to position 440; A at a position corresponding to position 441; F at a position corresponding to position 441; C at a position corresponding to position 442; G at a position corresponding to position 442; R at a position corresponding to position 442; A at a position corresponding to position 443; E at a position corresponding to position 443; F at a position corresponding to position 443; G at a position corresponding to position 443; M at a position corresponding to position 443; N at a position corresponding to position 443; E at a position corresponding to position 444; H at a position corresponding to position 444; V at a position corresponding to position 444; H at a position corresponding to position 445; M at a position corresponding to position 445; N at a position corresponding to position 445; P at a position corresponding to position 445; Q at a position corresponding to position 445; S at a position corresponding to position 445; T at a position corresponding to position 445; V at a position corresponding to position 445; W at a position corresponding to position 445; A at a position corresponding to position 446; A at a position corresponding to position 446; M at a position corresponding to position 446; W at a position corresponding to position 446; D at a position corresponding to position 447; E at a position corresponding to position 447; G at a position corresponding to position 447; I at a position corresponding to position 447; N at a position corresponding to position 447; P at a position corresponding to position 447; Q at a position corresponding to position 447; T at a position corresponding to position 447, and/or replacement with V at a position corresponding to position 447, each with reference to amino acid positions set forth in SEQ ID NO:3. Among these modified

PH20 polypeptides are those that exhibit at least 40% of the activity of the PH20 does not contain the particular amino acid replacement. Activity can vary between, for example, 40% to 5000%, 40% to 2000%, 40% to 1000%, 40% to 500%, 40% to 100%, 80% to 2000%, 80% to 600%, 80% to 200%, 80% to 300%, and ranges in between of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement; and activity is compared under the same conditions. Such activity includes at least 50%, 60%, 70%, 80%, 90%, 100%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement, where, as in all instances herein, activity is compared under the same conditions.

In particular, provided are modified PH20 polypeptides that contain at least one amino acid replacement in a PH20 polypeptide set forth in SEQ ID NO:7, a C-terminally truncated fragment thereof or in a PH20 polypeptide that has a sequence of amino acids that is at least 91% identical to the sequence of amino acids set forth in SEQ ID NO:7 or a corresponding truncated fragment, where: the modified PH20 polypeptides exhibits less than 20% of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement, where activity is compared under the same conditions; the amino acid replacement(s) is at an amino acid position corresponding to a position selected from among 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 94, 95, 96, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 143, 144, 145, 149, 150, 152, 153, 154, 155, 156, 157, 158, 159, 161, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 197, 198, 199, 200, 201, 202, 203, 204, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 196, 297,

298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 331, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 408, 410, 411, 412, 413, 414, 415, 416, 417, 419, 420, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 434, 437, 438, 439, 440, 441, 442, 443, 444, or 447 with reference to amino acid positions set forth in SEQ ID NO:3 or 7;

corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3; and provided that:

(i) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 200, 333, 358 or 393 the replacement is not replacement with an Alanine (A).

(ii) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 111 or 249 the replacement is not replacement with an asparagine (N);

(iii) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 113 the replacement is not replacement with a glutamine (Q);

(iv) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 176 the replacement is not replacement with a glycine (G); and

(v) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 252 the replacement is not replacement with a threonine (T).

Exemplary are modified PH20 polypeptides that contain amino acid replacement(s) in a PH20 polypeptide that has a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 32-66, 69, 72, 856-861, 869 and 870 and any set forth in Table 5. The modified PH20 polypeptides can exhibit similar or the same activity as the PH20 without the modification, or can exhibit increased activity or activity that is less than 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% or less of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement. Included are

modified PH20 polypeptides of any of claims 1-61, wherein the amino acid replacements are in a PH20 polypeptide having a sequence of amino acids set forth any of SEQ ID NO: 3, 7, 69 or 72 provided that: (i) where the modified PH20 polypeptide includes only a single amino acid replacement the replacement does not corresponds to amino acid replacements V12A, N47A, D111N, E113Q, N131A, R176G, N200A, N219A, E249Q, R252T, N333A or N358A, with reference to amino acid positions set forth in SEQ ID NO:3;(ii) where the modified PH20 polypeptide includes only two amino acid replacements the replacements do not correspond to amino acid replacements P13A/L464W, N47A/N131A, N47A/N219A, N131A/N219A or N333A/N358A with reference to positions set forth in SEQ ID NO:3; and (iii) where the modified PH20 polypeptide includes only three amino acid replacements the replacements doe not correspond to amino acid replacements N47A/N131A/N219A, with reference to amino acid positions set forth in SEQ ID NO:3. These polypeptides and any provided herein and described above and below can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,49, 50, 51, 53, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 59,70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 82, 82, 83, 84, 85, 86, 87, 88, 89, 90, or more of the amino acid replacements. The modified PH20 polypeptides can include a signal sequence, including the native sequence or a heterologous sequence or a modified sequence, and also include a mature PH20 polypeptides that lack the signal sequence.

Provided are modified PH20 polypeptides that contain or have a sequence of amino acids set forth in any of SEQ ID NOS: 73-855 or a sequence of amino acids that exhibits at least 75%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a sequence of amino acids set forth in any of SEQ ID NOS: 73-855, where the modified PH20 polypeptide comprises at least one amino acid replacement compared to the sequence of amino acids set forth in SEQ ID NO:3.

The modified PH20 polypeptides provided herein can be substantially purified or isolated, can exhibit catalytic activity at neutral pH, can be secreted upon expression from cells and is soluble in the supernatant, and/or can include modified amino acids, such as a modification selected from among glycosylation, sialation, albumination, farnysylation, carboxylation, hydroxylation, conjugation to a polymer, such as pegylation or conjugation to dextran, conjugation to another moiety, such as a

multimerization domain, toxin, detectable label or drug, and phosphorylation. The modified PH20 polypeptide can be glycosylated, such as by containing at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues, where, for example, the three asparagine residues correspond to amino acid residues 200, 333 and 358 of SEQ ID NO:3. Multimerization domains include Fc domains.

Also provided are nucleic acid molecules that encode any of the modified PH20 polypeptides provided herein. Vectors, eukaryotic and prokaryotic, that contain the nucleic acid molecules are provided. The vectors include expression vectors and include mammalian vectors, including viral vectors. Viral vectors include adenovirus vectors, a retrovirus vectors, vaccinia virus vectors, herpes simplex virus and cytomegalovirus vectors and other such viral vectors. Of interest are oncolytic vectors that accumulate or are targeted to tumors. Also provided are cells that contain the nucleic acid molecules and cells that contain the vectors. The cells can be prokaryotic or eukaryotic, particularly mammalian cells, such as Chinese Hamster Ovary (CHO) cells.

Also provided are pharmaceutical compositions that contain any of the modified PH20 polypeptides provided herein or any of the nucleic acids or vectors provided herein. The compositions can be formulated with other agents and/or with other components, such as preservatives. The compositions can be formulated so that the components, particularly the PH20 and any other active agent, remain active or are stable under preselected conditions. In addition, as described herein, the PH20 polypeptides are modified so that they exhibit increased stability under various conditions. For example, provided are compositions in which the modified PH20 polypeptide is stable (*i.e.*, retain activity as described herein) at a temperature from or from about 2°C to 8°C, inclusive, for at least 1 month or is stable at a temperature from or from about 30°C to 42°C, inclusive, for at least 3 days. Provided are compositions in which the modified PH20 polypeptide in the composition is stable at a temperature from or from about 2°C to 8°C, inclusive, for at least 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months or 30 months. Also provided are compositions in which the modified PH20 polypeptide in the composition is stable at a temperature from or from about 30°C to

42°C, inclusive, for at least 3 days, t least 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 35 days, 40 days, 45 days, 50 days, 60 days or more. The pharmaceutical compositions can contain a pharmaceutically acceptable excipient.

The conditions, formulations, components, and modified PH20 polypeptide are chosen to achieve a desired stability. The pharmaceutical compositions can be formulated for direct administration or can require dilution, they can be formulated for multiple or single dosage administration. Exemplary compositions include concentrations of modified PH20 between or about between 0.1 μg/mL to 100 μg/mL, 1 μg/mL to 50 μg/mL or 1 μg/mL to 20 μg/mL, or 10 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 2000 U/mL, or 100 U/mL to 1000 U/mL. Exemplary salts include NaCl at a concentration, for example of less than or about or 200 mM, 180 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80mM, 70mM, 60 mM, 50 mM, 40 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM or less or between or about between 0.1 mM to 200 mM, 0.1mM to 100 mM, 120 mM to 200 mM, 10 mM to 50 mM, 10 mM to 90 mM, 80 mM to 200 mM, 80 mM to 140 mM, 50 mM to 100 mM, 80 mM to 100 mM, 50 mM to 80 mM, 100 mM to 140 mM or 120 mM to 140 mM.

The pharmaceutical compositions can contain an anti-microbially effective amount of a preservative or mixture of preservatives, such as one, two, three, four or more of a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s), such as, but not limited to, phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorohexidine, sodium dehydroacetate, o-cresol, p-cresol, chlorcresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, buytlparaben and any combinations thereof. Phenols include, for example, phenol, metacresol (m-cresol), benzyl alcohol, and parabens, such as methylparaben or propylparaben. Anti-microbial effective concentrations of one or more preservative agents (as a percentage (%) of mass concentration (w/v)) can be between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive. Exemplary thereof of are pharmaceutical compositions

where the preservatives are phenol, m-cresol or phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.1% to 0.25% phenol and between or about between 0.05% to 0.2% m-cresol, is between or about between 0.10% to 0.2% phenol and between or about between 0.6% to 01.8% m-cresol, between or about between 0.1% to 0.15% phenol and 0.8% t 0.15% m-cresol, is between or about between 0.10% to 0.15% phenol and between or about between 0.06 to 0.09% m-cresol or is between or about between 0.12% to 0.18% phenol and between or about between 0.14 to 0.22% m-cresol.

The pharmaceutical compositions can contain a further therapeutically active agent. The active agent can be formulated in the composition or provided as a combination with the PH20-containing composition, but in a separate composition for administration separately, sequentially, intermittently, simultaneously or together. Therapeutically active agents include, for example, an agent selected from among a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, and antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, a polypeptide, a protein, a nucleic acid, a drug, an organic molecule and a sleep inducer. Exemplary of such agents are antibodies, particularly monoclonal antibodies, an Immune Globulin preparation, a bisphosphonate, a cytokine, a chemotherapeutic agent, a coagulation factor and an insulin. Insulins include, for example, basal insulins and fast-acting insulin, such as regular insulin, particularly recombinant human insulin, and insulin analogs, such as insulin lispro, insulin aspart or insulin glulisine. Particular fast-acting insulin are

those with an A chain having a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in SEQ ID NO:863 or an insulin with an A chain with a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:864 and a B chain with a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:864 or an insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:865-867. The amount of fast acting insulin in the compositions can be empirically determined, but typically can be 10 U/mL to 1000 U/mL, 50 U/mL to 500 U/mL, 100 U/mL to 1000 U/mL or 500 U/mL to 1000 U/mL, inclusive.

Other therapeutic agents include, but are not limited to Adalimumabs, Agalsidase Betas, Alefacepts, Ampicillins, Anakinras, Antipoliomyelitic Vaccines, Anti-Thymocytes, Azithromycins, Becaplermins, Caspofungins, Cefazolins, Cefepimes, Cefotetans, Ceftazidimes, Ceftriaxones, Cetuximabs, Cilastatins, Clavulanic Acids, Clindamycins, Darbepoetin Alfas, Deaclizumabs, Diphtheria, Diphtheria antitoxins, Diphtheria Toxoids, Efalizumabs, Epinephrines, Erythropoietin Alphas, Etanercepts, Filgrastims, Fluconazoles, Follicle-Stimulating Hormones, Follitropin Alphas, Follitropin Betas, Fosphenyloins, Gadodiamides, Gadopentetates, Gatifloxacins, Glatiramers, GM-CSF's, Goserelins, Goserelin acetates, Granisetrons, Haemophilus Influenza B's, Haloperidols, Hepatitis vaccines, Hepatitis A Vaccines, Hepatitis B Vaccines, Ibritumomab Tiuxetans, Ibritumomabs, Tiuxetans, Immunoglobulins, Hemophilus influenza vaccines, Influenza Virus Vaccines, Infliximabs, Insulin lispro, 75% neutral protamine lispro (NPL)/25% insulin lispro, 50% neutral protamine Hagedorn (NPH)/ 50% regular insulin, 70% NPH/30% regular insulin; Regular insulin, NPH insulin, Ultra insulin, Ultralente insulin, and Insulin Glargines, Interferons, Interferon alpha, Interferon Betas, Interferon Gammas, Interferon alpha-2a, Interferon alpha 2-b, Interferon Alphacon, Interferon alpha-n, Interferon Betas, Interferon Beta-1a's, Interferon Gammas, Interferon alpha-con, Iodixanols, Iohexols, Iopamidols, Ioversols, Ketorolacs, Laronidases, Levofloxacins, Lidocaines, Linezolids, Lorazepams, Measles Vaccines, Measles virus, Mumps viruses, Measles-Mumps-Rubella Virus Vaccines, Rubella vaccines, Medroxyprogesterones, Meropenems, Methylprednisolones, Midazolams, Morphines, Octreotides, Omalizumabs, Ondansetrons, Palivizumabs, Pantoprazoles,

Pegaspargases, Pegfilgrastims, Peg-Interferon Alpha-2a's, Peg-Interferon Alpha-2b's, Pegvisomants, Pertussis vaccines, Piperacillins, Pneumococcal Vaccines and Pneumococcal Conjugate Vaccines, Promethazines, Reteplases, Somatropins, Sulbactams, Sumatriptans, Tazobactams, Tenecteplases, Tetanus Purified Toxoids, Ticarcillins, Tositumomabs, Triamcinolones, Triamcinolone Acetonides, Triamcinolone hexacetonides, Vancomycins, Varicella Zoster imrnunoglobulins, Varicella vaccines, other vaccines, Alemtuzumabs, Alitretinoins, Allopurinols, Altretamines, Amifostines, Anastrozoles, Arsenics, Arsenic Trioxides, Asparaginases, Bacillus Calmette-Guerin (BCG) vaccines, BCG Live, Bexarotenes, Bleomycins, Busulfans, Busulfan intravenous, Busulfan orals, Calusterones, Capecitabines, Carboplatins, Carmustines, Carmustines with Polifeprosans, Celecoxibs, Chlorambucils, Cisplatins, Cladribines, Cyclophosphamides, Cytarabines, Cytarabine liposomals, Dacarbazines, Dactinomycins, Daunorubicin liposomals, Daunorubicins, Daunomycins, Denileukin Diftitoxes, Dexrazoxanes, Docetaxels, Doxorubicins, Doxorubicin liposomals, Dromostanolone propionates, Elliott's B Solutions, Epirubicins, Epoetin alfas, Estramustines, Etoposides, Etoposide phosphates, Etoposide VP-16s, Exemestanes, Floxuridines, Fludarabines, Fluorouracils, 5-Fluorouracils, Fulvestrants, Gemcitabines, Gemtuzumabs, Ozogamicins, Gemtuzumab ozogamicins, Hydroxyureas, Idarubicins, Ifosfamides, Imatinib mesylates, Irinotecans, Letrozoles, Leucovorins, Levamisoles, Lomustines, CCNUs, Meclorethamines, Nitrogen mustards, Megestrols, Megestrol acetates, Melphalans, L-PAMs, Mercaptopurines, 6-Mercaptopurines, Mesnas, Methotrexates, Methoxsalens, Mitomycins, Mitomycin C's, Mitotanes, Mitoxantrones, Nandrolones, Nandrolone Phenpropionates, Nofetumomabs, Oprelvekins, Oxaliplatins, Paclitaxels, Pamidronates, Pegademases, Pentostatins, Pipobromans, Plicamycins, Mithramycins, Porfimers, Porfimer sodiums, Procarbazines, Quinacrines, Rasburicases, Rituximabs, Sargramostims, Streptozocins, Talcs, Tamoxifens, Temozolomides, Teniposides, Testolactones, Thioguanines, 6-Thioguanines, Triethylenethiophosphoramides (Thiotepas), Topotecans, Toremifenes, Trastuzumabs, Tretinoins, Uracil Mustards, Valrubicins, Vinblastines, Vincristines, Vinorelbines, Zoledronates, Acivicins, Aclarubicins, Acodazoles, Acronines, Adozelesins, Aldesleukins, Retinoic Acids, Alitretinoins, 9-Cis-Retinoic Acids, Alvocidibs, Ambazones, Ambomycins, Ametantrones, Aminoglutethimides, Amsacrines, Anaxirones, Ancitabines, Anthramycins, Apaziquones, Argimesnas, Asperlins, Atrimustines, Azacitidines,

Azetepas, Azotomycins, Banoxantrones, Batabulins, Batimastats, Benaxibines, Bendamustines, Benzodepas, Bicalutamides, Bietaserpines, Biricodars, Bisantrenes, Bisnafide Dimesylates, Bizelesins, Bortezomibs, Brequinars, Bropirimines, Budotitanes, Cactinomycins, Canertinibs, Caracemides, Carbetimers, Carboquones, Carmofurs, Carubicins, Carzelesins, Cedefingols, Cemadotins, Chiorambucils, Cioteronels, Cirolemycins, Clanfenurs, Clofarabines, Crisnatols, Decitabines, Dexniguldipines, Dexormaplatins, Dezaguanines, Diaziquones, Dibrospidiums, Dienogests, Dinalins, Disermolides, Dofequidars, Doxifluridines, Droloxifenes, Duazomycins, Ecomustines, Edatrexates, Edotecarins, Eflomithines, Elacridars, Elinafides, Elsamitrucins, Emitefurs, Enloplatins, Enpromates, Enzastaurins, Epipropidines, Eptaloprosts, Erbulozoles, Esorubicins, Etanidazoles, Etoglucids, Etoprines, Exisulinds, Fadrozoles, Fazarabines, Fenretinides, Fluoxymesterones, Flurocitabines, Fosquidones, Fostriecins, Fotretamines, Galarubicins, Galocitabines, Geroquinols, Gimatecans, Gimeracils, Gloxazones, Glufosfamides, Ilmofosines, Ilomastats, Imexons, Improsulfans, Indisulams, Inproquones, Interleukins, Interleukin-2s, recombinant Interleukins, Intoplicines, lobenguanes, Iproplatins, Irsogladines, Ixabepilones, Ketotrexates, L-Alanosines, Lanreotides, Lapatinibs, Ledoxantrones, Leuprolides, Leuprorelins, Lexacalcitols, Liarozoles, Lobaplatins, Lometrexols, Lonafarnibs, Losoxantrones, Lurtotecans, Mafosfamides, Mannosulfans, Marimastats, Masoprocols, Maytansines, Mechiorethamines, Melengestrols, Meiphalans, Menogarils, Mepitiostanes, Metesinds, Metomidates, Metoprines, Meturedepas, Miboplatins, Miproxifenes, Misonidazoles, Mitindomides, Mitocarcins, Mitocromins, Mitoflaxones, Mitogillins, Mitoguazones, Mitomalcins, Mitonafides, Mitoquidones, Mitospers, Mitozolomides, Mivobulins, Mizoribines, Mofarotenes, Mopidamols, Mubritinibs, Mycophenolic Acids, Nedaplatins, Neizarabines, Nemorubicins, Nitracrines, Nocodazoles, Nogalamycins, Nolatrexeds, Nortopixantrones, Ormaplatins, Ortataxels, Oteracils, Oxisurans, Oxophenarsines, Patubilones, Peldesines, Peliomycins, Pelitrexols, Pemetrexeds, Pentamustines, Peplomycins, Perfosfamides, Perifosines, Picoplatins, Pinafides, Piposulfans, Pirfenidones, Piroxantrones, Pixantrones, Plevitrexeds, Plomestanes, Porfiromycins, Prednimustines, Propamidines, Prospidiums, Pumitepas, Puromycins, Pyrazofurins, Ranimustines, Riboprines, Ritrosulfans, Rogletimides, Roquinimexs, Rufocromomycins, Sabarubicins, Safingols, Satraplatins, Sebriplatins, Semustines, Simtrazenes, Sizofirans, Sobuzoxanes, Sorafenibs, Sparfosates, Sparfosic Acids,

Sparsomycins, Spirogermaniums, Spiromustines, Spiroplatins, Squalamines, Streptonigrins, Streptovarycins, Sufosfamides, Sulofenurs, Tacedinalines, Talisomycins, Tallimustines, Tariquidars, Tauromustines, Tecogalans, Tegafurs, Teloxantrones, Temoporfins, Teroxirones, Thiamiprines, Tiamiprines, Tiazofurins, Tilomisoles, Tilorones, Timcodars, Timonacics, Tirapazamines, Topixantrones, Trabectedins, Ecteinascidin 743, Trestolones, Triciribines, Trilostanes, Trimetrexates, Triplatin Tetranitrates, Triptorelins, Trofosfarnides, Tubulozoles, Ubenimexs, Uredepas, Vaispodars, Vapreotides, Verteporfins, Vinbiastines, Vindesines, Vinepidines, Vinflunines, Vinformides, Vinglycinates, Vinleucinols, Vinleurosines, Vinrosidines, Vintriptols, Vinzolidines, Vorozoles, Xanthomycin A's, Guamecyclines, Zeniplatins, Zilascorbs [2-H], Zinostatins, Zorubicins, Zosuquidars, Acetazolamides, Acyclovirs, Adipiodones, Alatrofloxacins, Alfentanils, Allergenic extracts, Alpha 1-proteinase inhibitors, Aiprostadils, Amikacins, Amino acids, Aminocaproic acids, Aminophyllines, Amitriptylines, Amobarbitals, Amrinones, Analgesics, Anti-poliomyelitic vaccines, Anti-rabic serums, Anti-tetanus immunoglobulins, tetanus vaccines, Antithrombin III's, Antivenom serums, Argatrobans, Arginines, Ascorbic acids, Atenolols, Atracuriums, Atropines, Aurothioglucoses, Azathioprines, Aztreonams, Bacitracins, Baclofens, Basiliximabs, Benzoic acids, Benztropines, Betamethasones, Biotins, Bivalirudins, Botulism antitoxins, Bretyliums, Bumetanides, Bupivacaines, Buprenorphines, Butorphanols, Calcitonins, Calcitriols, Calciums, Capreomycins, Carboprosts, Carnitines, Cefaniandoles, Cefoperazones, Cefotaximes, Cefoxitins, Ceftizoximes, Cefuroximes, Chioramphenicols, Chioroprocaines, Chioroquines, Chlorothiazides, Chiorpromazines, Chondroitinsulfuric acids, Choriogonadotropin alfas, Chromiums, Cidofovirs, Cimetidines, Ciprofloxacins, Cisatracuriums, Clonidines, Codeines, Coichicines, Colistins, Collagens, Corticorelin ovine triflutates, Corticotrophins, Cosyntropins, Cyanocobalamins, Cyclosporines, Cysteines, Dacliximabs, Dalfopristins, Dalteparins, Danaparoids, Dantrolenes, Deferoxamines, Desmopressins, Dexamethasones, Dexmedetomidines, Dexpanthenols, Dextrans, Iron dextrans, Diatrizoic acids, Diazepams, Diazoxides, Dicyclomines, Digibinds, Digoxins, Dihydroergotamines, Diltiazems, Diphenhydramines, Dipyridamoles, Dobutamines, Dopamines, Doxacuriums, Doxaprams, Doxercalciferols, Doxycyclines, Droperidols, Dyphyllines, Edetic acids, Edrophoniums, Enalaprilats, Ephedrines, Epoprostenols, Ergocalciferols, Ergonovines, Ertapenems, Erythromycins, Esmolols, Estradiols, Estrogenics,

Ethacrynic acids, Ethanolamines, Ethanols, Ethiodized oils, Etidronic acids, Etomidates, Famotidines, Fenoldopams, Fentanyls, Flumazenils, Fluoresceins, Fluphenazines, Folic acids, Fomepizoles, Fomivirsens, Fondaparinuxs, Foscarnets, Fosphenytoins, Furosemides, Gadoteridols, Gadoversetamides, Ganciclovirs, Gentamicins, Glucagons, Glucoses, Glycines, Glycopyrrolates, Gonadorelins, Gonadotropin chorionics, Haemophilus B polysaccarides, Hemins, Herbals, Histamines, Hydralazines, Hydrocortisones, Hydromorphones, Hydroxocobalamins, Hydroxyzines, Hyoscyamines, Ibutilides, Imiglucerases, Indigo carmines, Indomethacins, Iodides, Iopromides, Iothalamic acids, loxaglic acids, loxilans, Isoniazids, Isoproterenols, Japanese encephalitis vaccines, Kanamycins, Ketamines, Labetalols, Lepirudins, Levobupivacaines, Levothyroxines, Lincomycins, Liothyronines, Luteinising hormones, Lyme disease vaccines, Mangafodipirs, Manthtols, Meningococcal polysaccharide vaccines, Meperidines, Mepivacaines, Mesoridazines, Metaraminols, Methadones, Methocarbamols, Methohexitals, Methyldopates, Methylergonovines, Metoclopramides, Metoprolols, Metronidazoles, Minocyclines, Mivacuriums, Morrhuic acids, Moxifloxacins, Muromonab-CD3s, Mycophenolate mofetils, Nafcillins, Nalbuphines, Nalmefenes, Naloxones, Neostigmines, Niacinamides, Nicardipines, Nitroglycerins, Nitroprussides, Norepinephrines, Orphenadrines, Oxacillins, Oxymorphones, Oxytetracyclines, Oxytocins, Pancuroniums, Panthenols, Pantothenic acids, Papaverines, Peginterferon alpha 2As, Penicillin Gs, Pentamidines, Pentazocines, Pentobarbitals, Perfiutrens, Perphenazines, Phenobarbitals, Phentolamines, Phenylephrines, Phenytoins, Physostigmines, Phytonadiones, Polymyxin, Pralidoximes, Prilocaines, Procainamides, Procaines, Prochiorperazines, Progesterones, Propranolols, Pyridostigmine hydroxides, Pyridoxines, Quinidines, Quinupristins, Rabies immunoglobulins, Rabies vaccines, Ranitidines, Remifentanils, Riboflavins, Rifampins, Ropivacaines, Samariums, Scopolamines, Seleniums, Sermorelins, Sincalides, Somatrems, Spectinomycins, Streptokinases, Streptomycins, Succinylcholines, Sufentanils, Sulfamethoxazoles, Tacrolimuss, Terbutalines, Teriparatides, Testosterones, Tetanus antitoxins, Tetracaines, Tetradecyl sulfates, Theophyllines, Thiamines, Thiethylperazines, Thiopentals, Thyroid stimulating hormones, Tinzaparins, Tirofibans, Tobramycins, Tolazolines, Tolbutamides, Torsemides, Tranexamic acids, Treprostinils, Trifluoperazines, Trimethobenzamides, Trimethoprims, Tromethamines, Tuberculins, Typhoid vaccines, Urofollitropins,

Urokinases, Vaiproic acids, Vasopressins, Vecuroniums, Verapamils, Voriconazoles, Warfarins, Yellow fever vaccines, Zidovudines, Zincs, Ziprasidone hydrochiorides, Aclacinomycins, Actinomycins, Adriamycins, Azaserines, 6-Azauridines, Carzinophilins, Chromomycins, Denopterins, 6 Diazo 5 Oxo-L-Norleucines, Enocitabines, Loxuridines, Olivomycines, Pirarubicins, Piritreximes, Pteropterins, Tagafurs, Tubercidins, Alteplases, Arcitumomabs, bevacizumabs, Botulinum Toxin Type A's, Botulinum Toxin Type B's, Capromab Pendetides, Daclizumabs, Dornase alphas, Drotrecogin alpahs, Imciromab Pentetates, Iodine-131's, an antibiotic agent; an angiogenesis inhibitor; anti-cataract and anti-diabetic retinopathy substances; carbonic anhydrase inhibitors; mydriatics; photodynamic therapy agents; prostaglandin analogs; growth factor; anti-neoplastics; anti-metabolites; anti-viral; amebicides and anti-protozoals; anti-tuberculosis and anti-leprotic; antitoxins and antivenins; antihemophilic factor, anti-inhibitor coagulent complex, antithrombin III, coagulations Factor V, coagulation Factor IX, plasma protein fraction, von Willebrand factor; antiplatelet agent a colony stimulating factor (CSF); an erythropoiesis stimulator; hemostatics and albumins; Immune Globulins; thrombin inhibitors; anticoagulants; a steroidal anti-inflammatory drug selected from among among alclomethasones, algestones, beclomethasones, betamethasones, budesonides, clobetasols, clobetasones, clocortolones, cloprednols, corticosterones, cortisones, cortivazols, deflazacorts, desonides, desoximetasones, dexamethasones, difluorosones, diflucortolones, difluprednates, enoxolones, fluazacorts, flucloronides, flumethasones, flunisolides, fluocinolones, fluocinonides, fluocortins, fluocortolones, fluorometholones, fluperolones, fluprednidenes, fluprednisolones, flurandrenolides, fluticasones, formocortals, halcinonides, halobetasols, halometasones, halopredones, hydrocortamates, hydrocortisones, loteprednol etabonate, mazipredones, medrysones, meprednisones, methylprednisolones, mometasone furoate, paramethasones, prednicarbates, prednisolones, prednisones, prednivals, prednylidenes, rimexolones, tixocortols and triamcinolones; Ducosanoids, prostaglandins, prostaglandin analogs, antiprostaglandins and prostaglandin precursors; miotics, cholinergics and anti-cholinesterase; and anti-allergenics.

The compositions and modified PH20 polypeptides can be used to treat any condition normally treated by the PH20 polypeptide or the therapeutically active agent. These include, for example, conditions in which hyaluronan plays are role. Hence provided are uses of the compositions and modified PH20 polypeptides for

treating a hyaluronan-associated disease or condition by administering any of the modified PH20 polypeptides or compositions provided herein. Hyaluronan-associated diseases and conditions include inflammatory disease and tumors or cancers, including a late-stage cancer, metastatic cancers and undifferentiated cancers, such as ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer and colon cancer. The PH20 polypeptide can be modified with a polymer such as a Pegylation moiety for such treatments.

Also provided are methods for increasing delivery of a therapeutic agent to a subject, by: administering to s subject any of the modified PH20 polypeptides or compositions provided herein, and administering the therapeutic agent. The therapeutic agent can be administered in the same compositions or separately, and can be administered before or after, simultaneously, or intermittently with administration of the PH20 polypeptide(s). Administration includes any route, including intravenous and subcutaneous administration, such as simultaneously, intermittently or subsequent to administration of the therapeutic agent. The therapeutic agents include any of those set forth above, elsewhere herein and/or known to those of skill in the art.

Also provided are methods for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents, by the modified PH20 polypeptides or compositions provided herein.

Also provided are pharmaceutical compositions provided herein for use in treating a hyaluronan-associated disease or disorder, for use in delivering a therapeutic agent to a subject, for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents, and for any other use of compositions containing PH20 polypeptides.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the amino acid sequence of full-length human PH20 (set forth in SEQ ID NO:7) and soluble C-terminal truncated variants thereof. The C-terminal amino acid residue of exemplary C-terminal truncated variants of full-length PH20 are indicated by bold. The complete amino acid sequences of exemplary C-termianl truncated variants of full-length PH20 also are provided in SEQ ID NOS: 3 and 32-66. The C-terminal amino acid residue of an exemplary soluble PH20, whose complete sequence is set forth in SEQ ID NO:3, also is indicated by underline. Exemplary, non-limiting, positions for amino acid replacements are indicated by highlighting. Corresponding positions can be identified by alignment of a sequence of interest with any of SEQ ID NOS: 3, 7 or 32-66, and in particular with SEQ ID NO:3.

Figure 2 depicts exemplary alignments of human soluble PH20 set forth in SEQ ID NO:3 with other PH20 polypeptides. A "*" means that the aligned residues are identical, a ":" means that aligned residues are not identical, but are similar and contain conservative amino acids residues at the aligned position, and a "." means that the aligned residues are similar and contain semi-conservative amino acid residues at the aligned position. Exemplary, non-limiting, corresponding positions for amino acid replacements are indicated by highlighting. For example, **Figure 2A** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with chimpanzee PH20 set forth in SEQ ID NO:10. **Figure 2B** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Rhesus monkey PH20 set forth in SEQ ID NO:12. **Figure 2C** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Cynomolgus monkey PH20 set forth in SEQ ID NO:14. **Figure 2D** depicts the alignment of human soluble PH20 set forth in SEQ ID NO:3 with bovine PH20 set forth in SEQ ID NO:16. **Figure 2E** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with mouse PH20 set forth in SEQ ID NO:20. **Figure 2F** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with rat PH20 set forth in SEQ ID NO:22. **Figure 2G** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with rabbit PH20 set forth in SEQ ID NO:24. **Figure 2H** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with guinea pig PH20 set forth in SEQ ID NO:29. **Figure 2I** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Fox PH20 set forth in SEQ ID NO:31. **Figure 2J** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Gibbon PH20 set forth in SEQ ID NO:857. **Figure**

2K depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Marmoset PH20 set forth in SEQ ID NO:859. **Figure 2L** depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Orangutan PH20 set forth in SEQ ID NO:861.

DETAILED DESCRIPTION

Outline

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A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, databases, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

As used herein, hyaluronidase refers to a class of enzymes that degrade hyaluronan. Hyaluronidases include, but are not limited to, bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1), hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36), and mammalian-type hyaluronidases (EC 3.2.1.35). Hyaluronidases include any of non-human origin including, but not limited to, murine, canine, feline, leporine, avian, bovine, ovine, porcine, equine, piscine, ranine, bacterial, and any from leeches, other parasites, and crustaceans. Exemplary human hyaluronidases include HYAL1, HYAL2, HYAL3, HYAL4, and PH20. Also included amongst hyaluronidases are soluble hyaluronidases, including, ovine and bovine PH20, and soluble PH20.

As used herein, PH20 refers to a type of hyaluronidase that occurs in sperm and is neutral-active. PH-20 occurs on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. PH20 includes those of any origin including, but not limited to, human, chimpanzee, Cynomolgus monkey, Rhesus monkey, murine, bovine, ovine, guinea pig, rabbit and rat origin. Exemplary PH20 polypeptides, including precursor and mature forms, include those from human (SEQ ID NO:6 and 7), chimpanzee (SEQ ID NO:8, 9, 10, 869 and 870), Rhesus monkey (SEQ ID NO:11 and 12), Cynomolgus monkey (SEQ ID NO:13 and 14), cow (*e.g.*, SEQ ID NOS:15-18); mouse (SEQ ID NO:19 and 20); rat (SEQ ID

NO:21 and 22); rabbit (SEQ ID NO:23 and 24); sheep (SEQ ID NOS:25-27), guinea pig (SEQ ID NO:29 and 30); fox (SEQ ID NO: 30 and 31); Gibbon (SEQ ID NO:856 and 857), Marmoset (SEQ ID NO:858 and 859) and orangutan (SEQ ID NO:860 and 861) . Reference to PH20 includes precursor PH20 polypeptides and mature PH20 polypeptides (such as those in which a signal sequence has been removed), truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NO:7, or the mature forms thereof. PH20 polypeptides also include those that contain chemical or posttranslational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, pegylation, albumination, glycosylation, farnysylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art. Examples of commercially available bovine or ovine soluble hyaluronidases are Vitrase® hyaluronidase (ovine hyaluronidase) and Amphadase® hyaluronidase (bovine hyaluronidase).

As used herein, a soluble PH20 refers to a polypeptide characterized by its solubility under physiologic conditions. Generally, a soluble PH20 lacks all or a portion of a glycophosphatidyl anchor (GPI), or does not otherwise sufficiently anchor to the cell membrane. Hence, upon expression from a cell, a soluble PH20 is secreted into the medium. Soluble PH20 proteins can be distinguished, for example, by its partitioning into the aqueous phase of a Triton X-114 solution warmed to 37 °C (Bordier et al., (1981) *J. Biol. Chem.*, 256:1604-7). Membrane-anchored, such as lipid anchored hyaluronidases, will partition into the detergent rich phase, but will partition into the detergent-poor or aqueous phase following treatment with Phospholipase-C. Included among soluble PH20 hyaluronidases are membrane anchored hyaluronidases in which one or more regions associated with anchoring of the hyaluronidase to the membrane has been removed or modified, where the soluble form retains hyaluronidase activity. Soluble hyaluronidases include recombinant soluble hyaluronidases and those contained in or purified from natural sources, such as, for example, testes extracts from sheep or cows. Exemplary of such soluble hyaluronidases are soluble human PH20 (SEQ ID NO: 3 or 32-66). Other soluble

hyaluronidases include ovine (SEQ ID NO:25-27) and bovine (SEQ ID NO:16 or 18) PH20.

As used herein, soluble human PH20 (sHuPH20) includes PH20 polypeptides that lack a contiguous sequence of amino acids from the C-terminus that includes all or a portion of the glycosylphospatidylinositol (GPI) anchor sequence (C-terminally truncated PH20 polypeptides) such that upon expression, the polypeptides are soluble under physiological conditions. Solubility can be assessed by any suitable method that demonstrates solubility under physiologic conditions. Exemplary of such methods is the Triton® X-114 assay, that assesses partitioning into the aqueous phase and that is described above. In addition, a soluble human PH20 polypeptide is, if produced in CHO cells, such as CHO-S cells, a polypeptide that is expressed and is secreted into the cell culture medium. Soluble human PH20 polypeptides, however, are not limited to those produced in CHO cells, but can be produced in any cell or by any method, including recombinant expression and polypeptide synthesis. Reference to secretion in CHO cells is definitional. Hence, if a polypeptide could be expressed and secreted in CHO cells and is soluble in the media, *i.e.* partitions into the aqueous phase when extracted with Triton® X-114, it is a soluble PH20 polypeptide whether or not it is so-produced. The precursor polypeptides for sHuPH20 polypeptides can include a signal sequence, such as a heterologous or non-heterologous (*i.e.* native) signal sequence. Exemplary of the precursors are those that include a signal sequence, such as the native 35 amino acid signal sequence at amino acid positions 1-35 (see, *e.g.*, amino acids 1-35 of SEQ ID NO:6).\

As used herein, modification is in reference to modification of a sequence of amino acids of a polypeptide or a sequence of nucleotides in a nucleic acid molecule and includes deletions, insertions, and replacements of amino acids and nucleotides, respectively. Modifications also can include post-translational modifications or other changes to the molecule that can occur due to conjugation or linkage, directly or indirectly, to another moiety. Methods of modifying a polypeptide are routine to those of skill in the art, such as by using recombinant DNA methodologies.

As used herein, "modified PH20 polypeptide" or "variant PH20 polypeptide" refers to a PH20 polypeptide that contains at least one amino acid replacement as described herein in its sequence of amino acids compared to a reference PH20 polypeptide. Exemplary reference PH20 polypeptides is a human PH20 polypeptide or allelic or species variants thereof or other variants, including mature and precursor

polypeptides. For example, exemplary reference PH20 polypeptides is a mature full length PH20 polypeptide set forth in SEQ ID NOS: 7, 69 or 72, or in C-terminally truncated forms thereof such as set forth in any of SEQ ID NOS: 3 and 32-66, or in a PH20 polypeptide that exhibits at least 68%, 69%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 3, 7, 32-66, 69 or 72. A modified PH20 polypeptide can have up to 150 amino acid replacements, so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity. Typically, a modified PH20 polypeptide contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acid replacements. It is understood that a modified PH20 polypeptide also can include any one or more other modifications, in addition to at least one amino acid replacement as described herein.

As used herein, an N-linked moiety refers to an asparagine (N) amino acid residue of a polypeptide that is capable of being glycosylated by post-translational modification of a polypeptide. Exemplary N-linked moieties of human PH20 include amino acids N47, N131, N200, N219, N333, N358 and N365 of the sequence of amino acids set forth in SEQ ID NO: 3 or 7 (corresponding to amino acid residues N82, N166, N235, N254, N368, N393 and N490 of human PH20 set forth in SEQ ID NO: 6).

As used herein, an N-glycosylated polypeptide refers to a PH20 polypeptide containing oligosaccharide linkage of at least three N-linked amino acid residues, for example, N-linked moieties corresponding to amino acid residues N200, N333 and N358 of SEQ ID NO:3 or 7. An N-glycosylated polypeptide can include a polypeptide where three, four, five and up to all of the N-linked moieties are linked to an oligosaccharide. The N-linked oligosaccharides can include oligomannose, complex, hybrid or sulfated oligosaccharides, or other oligosaccharides and monosaccharides.

As used herein, an N-partially glycosylated polypeptide refers to a polypeptide that minimally contains an N-acetylglucosamine glycan linked to at least three N-linked moieties. A partially glycosylated polypeptide can include various glycan forms, including monosaccharides, oligosaccharides, and branched sugar forms, including those formed by treatment of a polypeptide with EndoH, EndoF1, EndoF2 and/or EndoF3.

As used herein, "denaturing condition" or "denaturation condition" refers to any condition that, when exposed to a protein, results in the degradation of the protein, generally as a result of loss of the tertiary or secondary structure of the protein. Exemplary denaturing conditions include, but are not limited to, the presence of a stong acid or base, a concentrated inorganic salt, an organic solvent (*e.g.* alcohol or chloroform), elevated temperature (*e.g.* heat), the presence of excipients that can be denaturing (*e.g.* phenolic preservatives or detergent), and low or substantially no stabilizing agent that otherwise is required for stability of the protein (*e.g.* NaCl). Denaturaing conditions can result in effects such loss of activity, loss of solubility, aggregation and/or crystallization.

As used herein, "stable" or "stability" with reference to a formulation or a co-formulation provided herein refers to one in which a modified PH20 hyaluronidase therein is stable upon exposure to one or more denaturing conditions for at least 1 month at temperatures from or from about 2°C to 8°C, inclusive or for at least 3 days at a temperature from or from about 30°C to 42°C, inclusive.

As used herein, stability of a modified PH20 hyaluronidase means that it exhibits at least 20%, 30%, 40%, 50 %, 60 %, 70 %, 80 %, 90 % or more of the original hyaluronidase activity prior to exposure to the denaturing condition(s). Generally, a modified PH20 hyaluronidase is stable if it retains at least 50% or more of the hyaluronidase activity over the prolonged time and temperature. Assays to assess hyaluronidase activity are known to one of skill in the art and described herein.

As used herein, "increased stability" with reference to a modified PH20 hyaluronidase means that, when tested under the same denaturing condition(s), the modified PH20 hyaluronidase exhibits greater hyaluronidase activity compared to an unmodified PH20 hyaluronidase not containing the amino acid replacement(s). For example, a modified PH20 hyaluronidase exhibits at least or about at least 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 250%, 300%, 400%, 500% , 600%, 700%, 800%, 900%, 1000% or more of the activity of the unmodified or reference PH20 hyaluronidase.

As used herein, "elevated temperatures" refers to to temperatures that are greater than room temperature or ambient temperature. Generally, an elevated temperature is a temperature that is at least or greater or about 30 °C, such as 30 °C to 42 °C, and generally 32°C to 37°C or 35°C to 37°C, inclusive.

As used herein, room temperature refers to a range generally from about or at to 18 °C to about or at 32 °C. Those of skill in the art appreciate that room temperature varies by location and prevailing conditions. For example, room temperatures can be higher in warmer climates such as Italy or Texas.

As used herein, "conditions" refers to any parameter that can influence the activity or properties of a protein or agent. For purposes herein, conditions generally refer to the presence, including amount, of excipients, carriers or other components in a formulation other than the active agent (*e.g.* modified PH20 hyaluronidase); temperature; time of storage; storage vessel; properties of storage (*e.g.* agitation) and/or other conditions associated with exposure or use.

As used herein, recitation that proteins are "compared under the same conditions" means that different proteins are treated identically or substantially identically such that any one or more conditions that can influence the activity or properties of a protein or agent are not varied or not substantially varied between the test agents. For example, when the hyaluoronidase activity of a modified PH20 polypeptide is compared to an unmodified PH20 polypeptide any one or more conditions such as amount or concentration of the polypeptide; presence, including amount, of excipients, carriers or other components in a formulation other than the active agent (*e.g.* modified PH20 hyaluronidase); temperature; time of storage; storage vessel; properties of storage (*e.g.* agitation) and/or other conditions associated with exposure or use are identical or substantially identical between and among the compared polypeptides.

As used herein, "storage" means that a formulation is not immediately administered to a subject once prepared, but is kept for a period of time under particular conditions (*e.g.* particular temperature; time, liquid or lyophilized form) prior to use. For example, a liquid formulation can be kept for days, weeks, months or years, prior to administration to a subject under varied temperatures such as refrigerated (0° to 10° C, such as 2° to 8° C), room temperature (*e.g.* temperature up to 32° C, such as 18 °C to about or at 32 °C), or elevated temperature (*e.g.*, 30°C to 42°C, such as 32°C to 37°C or 35°C to 37°C).

As used herein, an "excipient" refers to a compound in a formulation of an active agent that does not provide the biological effect of the active agent when administered in the absence of the active agent. Exemplary excipients include, but are

not limited to, salts, buffers, stabilizers, tonicity modifiers, metals, polymers, surfactants, preservatives, amino acids and sugars.

As used herein, a stabilizing agent refers to compound added to the formulation to protect the modified PH20 polypeptide or other active agent from degradation, if necessary, such as due to denaturation conditions at which a formulation herein is exposed to when handled, stored or used. Thus, included are agents that prevent proteins from degradation from other components in the compositions. Exemplary of such agents are amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, inhibitors or substrates and other agents as described herein.

As used herein, an antimicrobial effectiveness test or preservative effectiveness test (PET) demonstrates the effectiveness of the preservative system in a product. A product is inoculated with a controlled quantity of specific organisms. The test then compares the level of microorganisms found on a control sample versus the test sample over a period of 28 days. Generally, target markets have differeing PET requirements. For example, the PET requirements of the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP) differ considerably. Parameters for performing an antimicrobial effectiveness test, including in different markets, are known to one of skill in the art as described herein.

As used herein, an anti-microbially or anti-microbial effective amount of a preservative refers to an amount of the preservative that kills or inhibits the propagation of microbial organisms in a sample that may be introduced from storage or use. For example, for multiple-dose containers, an anti-microbially effective amount of a preservative inhibits the growth of microorganisms that may be introduced from repeatedly withdrawing individual doses. USP and EP (EPA and EPB) have anti-microbial requirements that determine preservative effectiveness, and that vary in stringency. For example, an anti-microbial effective amount of a preservative is an amount such that at least a 1.0 $\log_{10}$ unit reduction in bacterial organisms occurs at 7 days following inoculation in an antimicrobial preservative effectiveness test (APET). In a particular example, an anti-microbial effective amount of a preservative is an amount such that at least a 1.0 $\log_{10}$ unit reduction in bacterial organisms occurs at 7 days following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 14 days following inoculation at least no further increase in bacterial organisms occurs after 28 days following inoculation;

and at least no increase in fungal organisms occurs after 7 days following inoculation. In a further example, an anti-microbial effective amount of a preservative is an amount such that at least a 1.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 24 hours following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 7 days following inoculation, no further increase in bacterial organisms occurs after 28 days following inoculation, at least a 1.0 $\log_{10}$ unit reduction of fungal organisms occurs at 14 days following inoculation, and at least no further increase in fungal organisms occurs after 28 days following inoculation. In an additional example, an anti-microbial effective amount of a preservative is an amount such that at least a 2.0 $\log_{10}$ unit reduction of bacterial organisms at 6 hours following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 24 hours following inoculation, no recovery of bacterial organisms occurs after 28 days following inoculation of the composition with the microbial inoculum, at least a 2.0 $\log_{10}$ unit reduction of fungal organisms occurs at 7 days following inoculation, and at least no further increase in fungal organisms occurs after 28 days following inoculation.

As used herein, "preservative" refers to a naturally occurring or synthetically or recombinantly produced substance that, when added to a molecule or protein composition, prevents microbial growth, including bacterial or fungal growth, in the composition.

As used herein, a "phenolic preservative" refers to a preservative that contains one hydroxyl group attached to an aromatic carbon ring, such as a benzene ring. Exemplary phenolic preservatives, include but are not limited to, phenol, m-cresol, p-hydroxybenzoic acid, methylparaben, ethylparaben, and propylparaben. For example, cresols, including meta-cresol (m-cresol), has a methyl group substituted onto the benzene ring of a phenol molecule.

As used herein, the term "detergent" is used interchangeably with the term "surfactant" or "surface acting agent." Surfactants are typically organic compounds that are amphiphilic, *i.e.*, containing both hydrophobic groups ("tails") and hydrophilic groups ("heads"), which render surfactants soluble in both organic solvents and water. A surfactant can be classified by the presence of formally charged groups in its head. A non-ionic surfactant has no charge groups in its head, whereas an ionic surfactant carries a net charge in its head. A zwitterionic surfactant contains a head with two oppositely charged groups. Some examples of common

surfactants include: Anionic (based on sulfate, sulfonate or carboxylate anions): perfluorooctanoate (PFOA or PFO), perfluorooctanesulfonate (PFOS), sodium dodecyl sulfate (SDS), ammonium lauryl sulfate, and other alkyl sulfate salts, sodium laureth sulfate (also known as sodium lauryl ether sulfate, or SLES), alkyl benzene sulfonate; cationic (based on quaternary ammonium cations): cetyl trimethylammonium bromide (CTAB) a.k.a. hexadecyl trimethyl ammonium bromide, and other alkyltrimethylammonium salts, cetylpyridinium chloride (CPC), polyethoxylated tallow amine (POEA), benzalkonium chloride (BAC), benzethonium chloride (BZT); Zwitterionic (amphoteric): dodecyl betaine; cocamidopropyl betaine; coco ampho glycinate; nonionic: alkyl poly(ethylene oxide), alkylphenol poly(ethylene oxide), copolymers of poly(ethylene oxide) and poly(propylene oxide) (commercially known as Poloxamers or Poloxamines), alkyl polyglucosides, including octyl glucoside, decyl maltoside, fatty alcohols (*e.g.*, cetyl alcohol and oleyl alcohol), cocamide MEA, cocamide DEA, polysorbates (Tween 20, Tween 80, *etc.*), Triton detergents, and dodecyl dimethylamine oxide.

As used herein, a "buffer" refers to a substance, generally a solution, that can keep its pH constant, despite the addition of strong acids or strong bases and external influences of temperature, pressure, volume or redox potential. Buffer prevents change in the concentration of another chemical substance, e.g proton donor and acceptor systems that prevent marked changes in hydrogen ion concentration (pH). The pH values of all buffers are temperature and concentration dependent. The choice of buffer to maintain a pH value or range can be empirically determined by one of skill in the art based on the known buffering capacity of known buffers. Exemplary buffers include but are not limited to, bicarbonate buffer, cacodylate buffer, phosphate buffer or Tris buffer. For example, Tris buffer (tromethamine) is an amine based buffer that has a pKa of 8.06 and has an effective pH range between 7.9 and 9.2. For Tris buffers, pH increases about 0.03 unit per °C temperature decrease, and decreases 0.03 to 0.05 unit per ten-fold dilution.

As used herein, activity refers to a functional activity or activities of a polypeptide or portion thereof associated with a full-length (complete) protein. Functional activities include, but are not limited to, biological activity, catalytic or enzymatic activity, antigenicity (ability to bind or compete with a polypeptide for binding to an anti-polypeptide antibody), immunogenicity, ability to form multimers, and the ability to specifically bind to a receptor or ligand for the polypeptide.

As used herein, hyaluronidase activity refers to the ability to enzymatically catalyze the cleavage of hyaluronic acid. The United States Pharmacopeia (USP) XXII assay for hyaluronidase determines hyaluronidase activity indirectly by measuring the amount of higher molecular weight hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37 °C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Reference Standard solution can be used in an assay to ascertain the relative activity, in units, of any hyaluronidase. *In vitro* assays to determine the hyaluronidase activity of hyaluronidases, such as PH20, including modified PH20 polypeptides, are known in the art and described herein. Exemplary assays include the microturbidity assay described herein that measures cleavage of hyaluronic acid by hyaluronidase indirectly by detecting the insoluble precipitate formed when the uncleaved hyaluronic acid binds with serum albumin. Reference Standards can be used, for example, to generate a standard curve to determine the activity in Units of the hyaluronidase being tested.

As used herein, neutral active refers to the ability of a PH20 polypeptide to enzymatically catalyze the cleavage of hyaluronic acid at neutral pH, such as at a pH between or about between pH 6.0 to pH 7.8.

As used herein, "increased activity" with reference to a modified PH20 hyaluronidase means that, when tested under the same conditions, the modified PH20 hyaluronidase exhibits greater hyaluronidase activity compared to an unmodified PH20 hyaluronidase not containing the amino acid replacement(s). For example, a modified PH20 hyaluronidase exhibits at least or about at least 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 250%, 300%, 400%, 500% , 600%, 700%, 800%, 900%, 1000% or more of the activity of the unmodified or reference PH20 hyaluronidase.

As used herein, the residues of naturally occurring α-amino acids are the residues of those 20 α-amino acids found in nature which are incorporated into protein by the specific recognition of the charged tRNA molecule with its cognate mRNA codon in humans.

As used herein, nucleic acids include DNA, RNA and analogs thereof, including peptide nucleic acids (PNA) and mixtures thereof. Nucleic acids can be single or double-stranded. When referring to probes or primers, which are optionally labeled, such as with a detectable label, such as a fluorescent or radiolabel, single-

stranded molecules are contemplated. Such molecules are typically of a length such that their target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous nucleotides of sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

As used herein, a peptide refers to a polypeptide that is from 2 to 40 amino acids in length.

As used herein, the amino acids which occur in the various sequences of amino acids provided herein are identified according to their known, three-letter or one-letter abbreviations (Table 1). The nucleotides which occur in the various nucleic acid fragments are designated with the standard single-letter designations used routinely in the art.

As used herein, an "amino acid" is an organic compound containing an amino group and a carboxylic acid group. A polypeptide contains two or more amino acids. For purposes herein, amino acids include the twenty naturally-occurring amino acids, non-natural amino acids and amino acid analogs (*i.e.*, amino acids wherein the α-carbon has a side chain).

As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are presumed to be in the "L" isomeric form. Residues in the "D" isomeric form, which are so designated, can be substituted for any L-amino acid residue as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem.*, 243: 3557-3559 (1968), and adopted 37 C.F.R. §§ 1.821-1.822, abbreviations for amino acid residues are shown in Table 1:

Table 1 – Table of Correspondence

SYMBOL		
1-Letter	**3-Letter**	**AMINO ACID**
Y	Tyr	Tyrosine
G	Gly	Glycine
F	Phe	Phenylalanine
M	Met	Methionine

SYMBOL		
1-Letter	**3-Letter**	**AMINO ACID**
A	Ala	Alanine
S	Ser	Serine
I	Ile	Isoleucine
L	Leu	Leucine
T	Thr	Threonine
V	Val	Valine
P	Pro	proline
K	Lys	Lysine
H	His	Histidine
Q	Gln	Glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	Tryptophan
R	Arg	Arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	Cysteine
X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence (Table 1) and modified and unusual amino acids, such as those referred to in 37 C.F.R. §§ 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues, to an amino-terminal group such as NH_2 or to a carboxyl-terminal group such as COOH.

As used herein, "naturally occurring amino acids" refer to the 20 L-amino acids that occur in polypeptides.

As used herein, "non-natural amino acid" refers to an organic compound that has a structure similar to a natural amino acid but has been modified structurally to mimic the structure and reactivity of a natural amino acid. Non-naturally occurring amino acids thus include, for example, amino acids or analogs of amino acids other than the 20 naturally-occurring amino acids and include, but are not limited to, the D-isostereomers of amino acids. Exemplary non-natural amino acids are described herein and are known to those of skill in the art.

As used herein, an isokinetic mixture is one in which the molar ratios of amino acids has been adjusted based on their reported reaction rates (see, *e.g.*, Ostresh et al., (1994) Biopolymers 34:1681).

As used herein, suitable conservative substitutions of amino acids are known to those of skill in this art and can be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al.* Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224). Such substitutions can be made in accordance with those set forth in TABLE 2 as follows:

TABLE 2

Original residue	Exemplary conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; Gln; Glu
Met (M)	Leu; Tyr; Ile
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Other substitutions also are permissible and can be determined empirically or in accord with known conservative substitutions.

As used herein, a DNA construct is a single or double stranded, linear or circular DNA molecule that contains segments of DNA combined and juxtaposed in a manner not found in nature. DNA constructs exist as a result of human manipulation, and include clones and other copies of manipulated molecules.

As used herein, a DNA segment is a portion of a larger DNA molecule having specified attributes. For example, a DNA segment encoding a specified polypeptide is a portion of a longer DNA molecule, such as a plasmid or plasmid fragment, which, when read from the 5' to 3' direction, encodes the sequence of amino acids of the specified polypeptide.

As used herein, the term polynucleotide means a single- or double-stranded polymer of deoxyribonucleotides or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and can be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. The length of a polynucleotide molecule is given herein in terms of nucleotides (abbreviated "nt") or base pairs (abbreviated "bp"). The term nucleotides is used for single- and double-stranded molecules where the context permits. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term base pairs. It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide can differ slightly in length and that the ends thereof can be staggered; thus all nucleotides within a double-stranded polynucleotide molecule can not be paired. Such unpaired ends will, in general, not exceed 20 nucleotides in length.

As used herein, "at a position corresponding to" or recitation that nucleotides or amino acid positions "correspond to" nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence listing, refers to nucleotides or amino acid positions identified upon alignment with the disclosed sequence to maximize identity using a standard alignment algorithm, such as the GAP algorithm. For purposes herein, alignment of a PH20 sequence is to the amino acid sequence set forth in any of SEQ ID NOS: 3, 7 or 32-66, and in particular SEQ ID NO:3. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides. In general, to identify corresponding positions, the sequences of amino acids are aligned so that the highest order match is obtained (see, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). Figure 2 exemplifies exemplary alignments and identification of exemplary corresponding residues for replacement.

As used herein, "sequence identity" refers to the number of identical or similar amino acids or nucleotide bases in a comparison between a test and a reference poly-

peptide or polynucleotide. Sequence identity can be determined by sequence alignment of nucleic acid or protein sequences to identify regions of similarity or identity. For purposes herein, sequence identity is generally determined by alignment to identify identical residues. The alignment can be local or global. Matches, mismatches and gaps can be identified between compared sequences. Gaps are null amino acids or nucleotides inserted between the residues of aligned sequences so that identical or similar characters are aligned. Generally, there can be internal and terminal gaps. Sequence identity can be determined by taking into account gaps as the number of identical residues/ length of the shortest sequence x 100. When using gap penalties, sequence identity can be determined with no penalty for end gaps (*e.g.* terminal gaps are not penalized). Alternatively, sequence identity can be determined without taking into account gaps as the number of identical positions/length of the total aligned sequence x 100.

As used herein, a "global alignment" is an alignment that aligns two sequences from beginning to end, aligning each letter in each sequence only once. An alignment is produced, regardless of whether or not there is similarity or identity between the sequences. For example, 50% sequence identity based on "global alignment" means that in an alignment of the full sequence of two compared sequences each of 100 nucleotides in length, 50% of the residues are the same. It is understood that global alignment also can be used in determining sequence identity even when the length of the aligned sequences is not the same. The differences in the terminal ends of the sequences will be taken into account in determining sequence identity, unless the "no penalty for end gaps" is selected. Generally, a global alignment is used on sequences that share significant similarity over most of their length. Exemplary algorithms for performing global alignment include the Needleman-Wunsch algorithm (Needleman *et al. J. Mol. Biol.* 48: 443 (1970). Exemplary programs for performing global alignment are publicly available and include the Global Sequence Alignment Tool available at the National Center for Biotechnology Information (NCBI) website (ncbi.nlm.nih.gov/), and the program available at deepc2.psi.iastate.edu/aat/align/align.html.

As used herein, a "local alignment" is an alignment that aligns two sequence, but only aligns those portions of the sequences that share similarity or identity. Hence, a local alignment determines if sub-segments of one sequence are present in another sequence. If there is no similarity, no alignment will be returned. Local

alignment algorithms include BLAST or Smith-Waterman algorithm (*Adv. Appl. Math.* 2: 482 (1981)). For example, 50% sequence identity based on "local alignment" means that in an alignment of the full sequence of two compared sequences of any length, a region of similarity or identity of 100 nucleotides in length has 50% of the residues that are the same in the region of similarity or identity.

For purposes herein, sequence identity can be determined by standard alignment algorithm programs used with default gap penalties established by each supplier. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non identities) and the weighted comparison matrix of Gribskov *et al. Nucl. Acids Res.* 14: 6745 (1986), as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Whether any two nucleic acid molecules have nucleotide sequences or any two polypeptides have amino acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical," or other similar variations reciting a percent identity, can be determined using known computer algorithms based on local or global alignment (*see e.g.*, wikipedia.org/wiki/Sequence_alignment_software, providing links to dozens of known and publicly available alignment databases and programs). Generally, for purposes herein sequence identity is determined using computer algorithms based on global alignment, such as the Needleman-Wunsch Global Sequence Alignment tool available from NCBI/BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&Page_TYPE=BlastHome); LAlign (William Pearson implementing the Huang and Miller algorithm (*Adv. Appl. Math.* (1991) 12:337-357)); and program from Xiaoqui Huang available at deepc2.psi.iastate.edu/aat/align/align.html. Generally, when comparing nucleotide sequences herein, an alignment with penalty for end gaps is used. Local alignment also can be used when the sequences being compared are substantially the same length.

Therefore, as used herein, the term "identity" represents a comparison or alignment between a test and a reference polypeptide or polynucleotide. In one non-limiting example, "at least 90% identical to" refers to percent identities from 90 to 100% relative to the reference polypeptide or polynucleotide. Identity at a level of

90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide or polynucleotide length of 100 amino acids or nucleotides are compared, no more than 10% (*i.e.*, 10 out of 100) of amino acids or nucleotides in the test polypeptide or polynucleotide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, *e.g.*, 10/100 amino acid difference (approximately 90% identity). Differences also can be due to deletions or turncations of amino acid residues. Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. Depending on the length of the compared sequences, at the level of homologies or identities above about 85-90%, the result can be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often without relying on software.

As used herein, an allelic variant or allelic variation references any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and can result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or can encode polypeptides having altered amino acid sequence. The term "allelic variant" also is used herein to denote a protein encoded by an allelic variant of a gene. Typically the reference form of the gene encodes a wildtype form and/or predominant form of a polypeptide from a population or single reference member of a species. Typically, allelic variants, which include variants between and among species typically have at least 80%, 90% or greater amino acid identity with a wildtype and/or predominant form from the same species; the degree of identity depends upon the gene and whether comparison is interspecies or intraspecies. Generally, intraspecies allelic variants have at least about 80%, 85%, 90% or 95% identity or greater with a wildtype and/or predominant form, including 96%, 97%, 98%, 99% or greater identity with a wildtype and/or predominant form of a polypeptide. Reference to an allelic variant herein generally refers to variations n proteins among members of the same species.

As used herein, "allele," which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two

identical alleles of a gene, the subject is said to be homozygous for that gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide or several nucleotides, and can include modifications such as substitutions, deletions and insertions of nucleotides. An allele of a gene also can be a form of a gene containing a mutation.

As used herein, species variants refer to variants in polypeptides among different species, including different mammalian species, such as mouse and human. Exemplary of species variants provided herein are primate PH20, such as, but not limited to, human, chimpanzee, macaque, cynomologus monkey, gibbon, orangutan, or marmoset. Generally, species variants have 70%, 75%. 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or sequence identity. Corresponding residues between and among species variants can be determined by comparing and aligning sequences to maximize the number of matching nucleotides or residues, for example, such that identity between the sequences is equal to or greater than 95%, equal to or greater than 96%, equal to or greater than 97%, equal to or greater than 98% or equal to greater than 99%. The position of interest is then given the number assigned in the reference nucleic acid molecule. Alignment can be effected manually or by eye, particularly, where sequence identity is greater than 80%.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized.

Preparations can be determined to be substantially free if they appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

Hence, reference to a substantially purified polypeptide, such as a substantially purified PH20 polypeptide refers to preparations of PH20 proteins that are substantially free of cellular material includes preparations of proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the term substantially free of cellular material includes preparations of enzyme proteins having less that about 30% (by dry weight) of non-enzyme proteins (also referred to herein as a contaminating protein), generally less than about 20% of non-enzyme proteins or 10% of non-enzyme proteins or less that about 5% of non-enzyme proteins. When the enzyme protein is recombinantly produced, it also is substantially free of culture medium, *i.e.*, culture medium represents less than about or at 20%, 10% or 5% of the volume of the enzyme protein preparation.

As used herein, the term substantially free of chemical precursors or other chemicals includes preparations of enzyme proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. The term includes preparations of enzyme proteins having less than about 30% (by dry weight), 20%, 10%, 5% or less of chemical precursors or non-enzyme chemicals or components.

As used herein, synthetic, with reference to, for example, a synthetic nucleic acid molecule or a synthetic gene or a synthetic peptide refers to a nucleic acid molecule or polypeptide molecule that is produced by recombinant methods and/or by chemical synthesis methods.

As used herein, production by recombinant means or using recombinant DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

As used herein, vector (or plasmid) refers to discrete elements that are used to introduce a heterologous nucleic acid into cells for either expression or replication thereof. The vectors typically remain episomal, but can be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection and use of such vehicles are well known to those of skill in the art.

As used herein, an expression vector includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Such additional segments can include promoter and terminator sequences, and optionally can include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

As used herein, vector also includes "virus vectors" or "viral vectors." Viral vectors are engineered viruses that are operatively linked to exogenous genes to transfer (as vehicles or shuttles) the exogenous genes into cells. Viral vectors include, but are not limited to, adenoviral vectors, retroviral vectors and vaccinia virus vectors.

As used herein, "operably" or "operatively linked" when referring to DNA segments means that the segments are arranged so that they function in concert for their intended purposes, *e.g.*, transcription initiates downstream of the promoter and upstream of any transcribed sequences. The promoter is usually the domain to which the transcriptional machinery binds to initiate transcription and proceeds through the coding segment to the terminator.

As used herein, a conjugate refers to a modified PH20 polypeptide linked directly or indirectly to one or more other polypeptides or chemical moieties. Such conjugates include fusion proteins, those produced by chemical conjugates and those produced by any other method whereby at least one modified PH20 polypeptide is linked, directly or indirectly to another polypeptide or chemical moiety so long as the conjugate retains hyaluronidase activity. Exemplary of conjugates provided herein include PH20 polypeptides linked directly or indirectly to a multimerization domain, such as an Fc moiety, a toxin, a label or a drug.

As used herein, a fusion protein refers to a polypeptide encoded by a nucleic acid sequence containing a coding sequence from one nucleic acid molecule and the coding sequence from another nucleic acid molecule in which the coding sequences are in the same reading frame such that when the fusion construct is transcribed and translated in a host cell, the protein is produced containing the two proteins. The two molecules can be adjacent in the construct or separated by a linker polypeptide that contains, 1, 2, 3, or more, but typically fewer than 10, 9, 8, 7, or 6 amino acids. The protein product encoded by a fusion construct is referred to as a fusion polypeptide. Exemplary of fusion polypeptides include Fc fusions.

As used herein, a polymer that is conjugated to a modified PH20 polypeptide refers to any polymer that is covalently or otherwise stably linked, directly or via a linker, to such polypeptide. Such polymers, typically increase serum half-life, and include, but are not limited to sialic moieties, pegylation moieties, dextran, and sugar and other moieties, such as for glycosylation.

As used herein, the term assessing or determining is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of a product, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of the activity. Assessment can be direct or indirect.

As used herein, a "composition" refers to any mixture of two or more products or compounds. It can be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous, or any combination thereof.

As used herein, a formulation refers to a composition containing at least one active pharmaceutical or therapeutic agent and one or more excipients.

As used herein, a co-formulation refers to a composition containing two or more active or pharmaceutical or therapeutic agents and one or more excipients. For

example, a co-formulation of a fast-acting insulin and a hyaluronan degrading enzyme contains a fast-acting insulin, a hyaluronan degrading enzyme, and one or more excipients.

As used herein, "a combination" refers to any association between two or among more items or elements. Exemplary combinations include, but are not limited to, two or more pharmaceutical compositions, a composition containing two or more active ingredients, such as two viruses, or a virus and an anticancer agent, such as a chemotherapeutic compound, two or more viruses, a virus and a therapeutic agent, a virus and an imaging agent, a virus and a plurality therapeutic and/or imaging agents, or any association thereof. Such combinations can be packaged as kits.

As used herein, a kit is a packaged combination, optionally, including instructions for use of the combination and/or other reactions and components for such use.

As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from cause or condition including, but not limited to, infections, acquired conditions, genetic conditions, and characterized by identifiable symptoms.

As used herein, a hyaluronan-associated disease, disorder or condition refers to any disease or condition in which hyaluronan levels are elevated as cause, consequence or otherwise observed in the disease or condition. Hyaluronan-associated diseases and conditions are associated with elevated hyaluronan expression in a tissue or cell, increased interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue. Hyaluronan-associated diseases, disorders or conditions can be treated by administration of a composition containing a hyaluronan degrading enzyme, such as a hyaluronidase, for example, a soluble hyaluronidase, either alone or in combination with or in addition to another treatment and/or agent. Exemplary diseases and conditions, include, but are not limited to, hyaluronan-rich cancers, for example, tumors, including solid tumors such as late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer and other cancers. Also exemplary of hyaluronan-associated diseases and conditions are diseases that are associated with elevated interstitial fluid pressure, such as diseases associated with disc pressure, and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury. Exemplary hyaluronan-associated diseases and

conditions include diseases and conditions associated with elevated interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, including cancers, disc pressure and edema. In one example, treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of increased interstitial fluid pressure (IFP), decreased vascular volume, and increased water content in a tissue.

As used herein, "treating" a subject with a disease or condition means that the subject's symptoms are partially or totally alleviated, or remain static following treatment. Hence treatment encompasses prophylaxis, therapy and/or cure. Prophylaxis refers to prevention of a potential disease and/or a prevention of worsening of symptoms or progression of a disease. Treatment also encompasses any pharmaceutical use of a modified interferon and compositions provided herein.

As used herein, a pharmaceutically effective agent or therapeutic agent includes any bioactive agent that can exhibits a therapeutic effect. Exemplary therapeutic agents are described herein. Therapeutic agents include, but are not limited to, for example, anesthetics, vasoconstrictors, dispersing agents, conventional therapeutic drugs, including small molecule drugs, including, but not limited to, bisphosphonates, and therapeutic proteins, including, but not limited to, insulin, IgG molecules, antibodies, cytokines and coagulation factors.

As used herein, "insulin" refers to a hormone, precursor or a synthetic or recombinant analog thereof that acts to increase glucose uptake and storage and/or decrease endogenous glucose production. Insulin and analogs thereof are well known to one of skill in the art, including in human and allelic and species variants thereof. Insulin is translated as a precursor polypeptide designated preproinsulin (110 amino acid for human insulin), containing a signal peptide that directs the protein to the endoplasmic reticulum (ER) wherein the signal sequence is cleaved, resulting in proinsulin. Proinsulin is processed further to release a C- or connecting chain peptide (a 31 amino acid C-chain in human insulin). The resulting insulin contains an A-chain (21 amino acid in length in human insulin; set forth in SEQ ID NO:862) and a B-chain (30 amino acid in length in human insulin; set forth in SEQ ID NO:863) which are cross-linked by disulfide bonds. A fully cross-linked human insulin contains three disulfide bridges: one between position 7 of the A-chain and position 7 of the B-chain, a second between position 20 of the A-chain and position 19 of the B-chain, and a third between positions 6 and 11 of the A-chain. Reference to an insulin

includes monomeric and multimeric insulins, including hexameric insulins, as well as humanized insulins. Exemplary insulin polypeptides are those of mammalian, including human, origin. Reference to insulin includes preproinsulin, proinsulin and insulin polypeptides in single-chain or two-chain forms, truncated forms thereof that have activity, and includes allelic variants and species variants of human insulin, variants encoded by splice variants, and other variants, such as insulin analogs. An exemplary insulin is human insulin having a sequence of amino acids of the A- and B-chains of human insulin are set forth in SEQ ID NOS: 862 and 863, respectively, and variants or analogs thereof that exhibit at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto to one or both of the A-chain or B-chain and that acts to increase glucose uptake and storage and/or decrease endogenous glucose production. A further exemplary insulin is porcine insulin having a sequence of amino acids for the preproinsulin as set forth in SEQ ID NO:864, whereby the A chain corresponds to amino acid residue positions 88-108 and the B-chain correspond to amino acid, and variants or analogs thereof that exhibit at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto to one or both of the A-chain or B-chain and that acts to increase glucose uptake and storage and/or decrease endogenous glucose production.

As used herein, "fast-acting insulin" refers to any insulin that exhibits peak insulin levels at or about not more than four hours following subcutaneous administration to a subject. Fast-acting insulin any insulin or any fast-acting insulin composition for acute administration to a diabetic subject in response to an actual, perceived, or anticipated hyperglycemic condition in the subject arising at the time of, or within about four hours following, administration of the fast-acting insulin (such as a prandial hyperglycemic condition resulting or anticipated to result from, consumption of a meal), whereby the fast-acting insulin is able to prevent, control or ameliorate the acute hyperglycemic condition. Fast-acting insulins include recombinant insulins and isolated insulins (also referred to as "regular" insulins) such as the insulin sold as human insulin, porcine insulins and bovine insulins, as well as rapid acting insulin analogs (also termed fast-acting insulin analogs herein) designed to be rapid acting by virtue of amino acid changes. Exemplary regular insulin preparations include, but are not limited to, human regular insulins, such as those sold under the trademarks Humulin® R, Novolin® R and Velosulin®, Insulin Human, USP and Insulin Human Injection, USP, as well as acid formulations of insulin, such as, for

example, Toronto Insulin, Old Insulin, and Clear Insulin, and regular pig insulins, such as Iletin II® (porcine insulin). Regular insulins typically have an onset of action of between 30 minutes to an hour, and a peak insulin level of 2-5 hours post administration.

As used herein, rapid acting insulin analogs (also called fast-acting insulin analogs) are insulins that have a rapid onset of action. Rapid insulins typically are insulin analogs that have been engineered, such as by the introduction of one or more amino acid substitutions, to be more rapid acting than regular insulins. Rapid acting insulin analogs typically have an onset of action of 10-30 minutes post injection, with peak insulin levels observed 30-90 minutes post injection. Exemplary rapid acting insulin analogs are analogs of human insulin containing one or more amino acid changes in the A-chain and/or B-chain of human insulin set forth in SEQ ID NO:862 or 863, respectively, and that exhibit an onset of action 10-30 minutes post injection with peak insulin levels observed 30-90 minutes post injection. Exemplary rapid acting insulin analogs include, but are not limited to, for example, insulin lispro (*e.g.* Humalog® insulin), insulin aspart (*e.g.* NovoLog® insulin), and insulin glulisine (*e.g.* Apidra® insulin) the fast-acting insulin composition sold as VIAject® and VIAtab® (see, *e.g.*, U.S. Pat. No. 7,279,457). The amino acid sequence of exemplary rapid acting insulin analogs have an A chain with a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in any of SEQ ID NOS:965-867. Also included are any other insulins that have an onset of action of 30 minutes or less and a peak level before 90 minutes, typically 30-90 minutes, post injection.

As used herein, a human insulin refers to an insulin that is synthetic or recombinantly produced based upon the human polypeptide, including allelic variants and analogs thereof.

As used herein, fast-acting human insulins or human fast-acting insulin compositions include any human insulin or composition of a human insulin that is fast-acting, but excludes non-human insulins, such as regular pig insulin.

As used herein, the terms "basal-acting insulins," or "basal insulins" refer to insulins administered to maintain a basal insulin level as part of an overall treatment regimen for treating a chronic condition such diabetes. Typically, a basal-acting insulin is formulated to maintain an approximately steady state insulin level by the controlled release of insulin when administered periodically (*e.g.* once or twice daily).

Basal-acting insulins include crystalline insulins (*e.g.* NPH and Lente®, protamine insulin, surfen insulin), basal insulin analogs (insulin glargine, HOE 901, NovoSol Basal) and other chemical formulations of insulin (*e.g.* gum arabic, lecithin or oil suspensions) that retard the absorption rate of regular insulin. As used herein, the basal-acting insulins can include insulins that are typically understood as long-acting (typically reaching a relatively low peak concentration, while having a maximum duration of action over about 20-30 hours) or intermediate-acting (typically causing peak insulin concentrations at about 4-12 hours after administration).

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease or other indication, are ameliorated or otherwise beneficially altered.

As used herein, therapeutic effect means an effect resulting from treatment of a subject that alters, typically improves or ameliorates the symptoms of a disease or condition or that cures a disease or condition. A therapeutically effective amount refers to the amount of a composition, molecule or compound which results in a therapeutic effect following administration to a subject.

As used herein, the term "subject" refers to an animal, including a mammal, such as a human being.

As used herein, a patient refers to a human subject exhibiting symptoms of a disease or disorder.

As used herein, amelioration of the symptoms of a particular disease or disorder by a treatment, such as by administration of a pharmaceutical composition or other therapeutic, refers to any lessening, whether permanent or temporary, lasting or transient, of the symptoms that can be attributed to or associated with administration of the composition or therapeutic.

As used herein, prevention or prophylaxis refers to methods in which the risk of developing disease or condition is reduced.

As used herein, a "therapeutically effective amount" or a "therapeutically effective dose" refers to the quantity of an agent, compound, material, or composition containing a compound that is at least sufficient to produce a therapeutic effect. Hence, it is the quantity necessary for preventing, curing, ameliorating, arresting or partially arresting a symptom of a disease or disorder.

As used herein, unit dose form refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art.

As used herein, a single dosage formulation refers to a formulation containing a single dose of therapeutic agent for direct administration. Single dosage formulations generally do not contain any preservatives.

As used herein, a multi-dose formulation refers to a formulation that contains multiple doses of a therapeutic agent and that can be directly administered to provide several single doses of the therapeutic agent. The doses can be administered over the course of minutes, hours, weeks, days or months. Multidose formulations can allow dose adjustment, dose-pooling and/or dose-splitting. Because multi-dose formulations are used over time, they generally contain one or more preservatives to prevent microbial growth.

As used herein, an "article of manufacture" is a product that is made and sold. As used throughout this application, the term is intended to encompass a therapeutic agent with a soluble PH20, such as esPH20, or an esPH20 alone, contained in the same or separate articles of packaging.

As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

As used herein, a "control" or "standard" refers to a sample that is substantially identical to the test sample, except that it is not treated with a test parameter, or, if it is a plasma sample, it can be from a normal volunteer not affected with the condition of interest. A control also can be an internal control. For example, a control can be a sample, such as a virus, that has a known property or activity.

As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an" agent includes one or more agents.

As used herein, the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive.

As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 5 bases" means "about 5 bases" and also "5 bases."

As used herein, "optional" or "optionally" means that the subsequently described event or circumstance does or does not occur, and that the description includes instances where said event or circumstance occurs and instances where it

does not. For example, an optionally substituted group means that the group is unsubstituted or is substituted.

As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) *Biochem.* 11:1726).

For clarity of disclosure, and not by way of limitation, the detailed description is divided into the subsections that follow.

B. PH20 Hyaluronidase

Provided herein are modified PH20 polypeptides. PH20 (also known as sperm surface protein, sperm adhesion molecule 1 or spam 1) is a hyaluronidase that hydrolyzes hyaluronan (also called hyaluronic acid, hyaluronate or HA) found in connective tissues such as the extracellular matrix. Hyaluronan polymers are composed of repeating disaccharides units, D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc), linked together via alternating β-1→4 and β-1→3 glycosidic bonds. Hyaluronan chains can reach about 25,000 disaccharide repeats or more in length and polymers of hyaluronan can range in size from about 5,000 to 20,000,000 Da in vivo. Hyaluronan, also called hyaluronic acid or hyaluronate, is a non-sulfated glycosaminoglycan that is widely distributed throughout connective, epithelial, and neural tissues. Hyaluronan is an essential component of the extracellular matrix and a major constituent of the interstitial barrier. PH20 is an endo-β-N-acetyl-hexosaminidase that hydrolyzes the β1→4 glycosidic bond of hyaluronic acid into various oligosaccharide lengths such as tetrasaccharides and hexasaccharides. PH20 has both hydrolytic and transglycosidase activities. In addition to degrading hyaluronic acid, PH20 also can degrade chondroitin sulfates, such as C4-S and C6-S. PH20 can exhibit hyaluronidase activity at acidic pH and neutral pH.

1. Structure

PH20 cDNA has been cloned from numerous mammalian species. Exemplary PH20 precursor polypeptides include, but are not limited to, human (SEQ ID NO:6 and encoded by SEQ ID NO:67), bovine (SEQ ID NOS:15 or 17), rabbit (SEQ ID NO:23), , Cynomolgus monkey (SEQ ID NO:13), guinea pig (SEQ ID NO:28), rat (SEQ ID NO:21), mouse (SEQ ID NO:19), chimpanzee (SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:869) Rhesus monkey (SEQ ID NO:11), Fox (SEQ ID NO:30), Gibbon (SEQ ID NO:856), Marmoset (SEQ ID NO:858) or orangutan (SEQ ID

NO:860) PH20 polypeptides. The mRNA transcript is typically translated to generate a precursor protein containing a 35 amino acid signal sequence at the N-terminus. Following transport to the ER, the signal peptide is removed to yield a mature PH20 polypeptide. Exemplary mature PH20 polypeptides include, but are not limited to, human (SEQ ID NO:7), bovine (SEQ ID NOS:16 or 18), rabbit (SEQ ID NO:24), Cynomolgus monkey (SEQ ID NO:14), guinea pig (SEQ ID NO:29), rat (SEQ ID NO:22), mouse (SEQ ID NO:20), chimpanzee (SEQ ID NO:10 or SEQ ID NO:870), Rhesus monkey (SEQ ID NO:12), Fox (SEQ ID NO:31), Gibbon (SEQ ID NO:857), Marmoset (SEQ ID NO:859) or orangutan (SEQ ID NO:861) PH20 polypeptides. For example, the human PH20 mRNA transcript is normally translated to generate a 509 amino acid precursor protein (SEQ ID NO:6) containing a 35 amino acid signal sequence at the N-terminus (amino acid residue positions 1-35 of SEQ ID NO:6). Thus, following transport to the ER and removal of the signal peptide, a 474 amino acid mature polypeptide with an amino acid sequence set forth in SEQ ID NO:7 is produced. Sequences of PH20 from ovine are also known (*see e.g.* SEQ ID NOS: 25-27).

In particular, human PH20 has a sequence of amino acids set forth in SEQ ID NO:6 and encoded by a sequence of nucleotides set forth in SEQ ID NO:67. The mature human PH20 lacking a signal sequence is set forth in SEQ ID NO:7. Allelic variants and other variants of PH20 are known. Other sequences of PH20 have been reported. For example, a PH20 variant is known as set forth in the precursor sequence set forth in SEQ ID NO:68 that contains an Ala at position 48 and a Trp at position 499, or the mature sequence thereof set forth in SEQ ID NO:69 containing the corresponding differences at positions 13 and 464, respectively, compared to the sequence set forth in SEQ ID NO:7 (*see e.g.*, Gmachl et al. (1993) FEBS Lett., 336:545-548; GenBank Accession No. AAC60607). Further, a natural variants of PH20 has been identified containing a Glutamine (Gln; Q) at position 5 as compared to the precursor sequence of amino acids set forth in SEQ ID NO:6 (*see e.g.* SEQ ID NO:70, see also Varela et al. (2011) Nature, 469:539-542). Another natural variant contains an Alanine (Ala; A) at position 47 compared to the sequence of amino acids set forth in SEQ ID NO:6 (as set forth in SEQ ID NO: 71) and corresponding to position 12 compared to the sequence of amino acids set forth in SEQ ID NO: 3 or 7 (as set forth in SEQ ID NO:72).

The sequence and structure of PH20 polypeptides is highly conserved. Sequence identity between and among PH20 proteins from various species is about 50% to 90%. The hydrophobic N-terminal signal sequence of 35 amino acids in length is generally conserved among PH20 hyaluronidase polypeptides. PH20 hyaluronidases contain a common core hyaluronidase domain region of about 340 amino acids in length that corresponds to amino acid residues 38-374 of the precursor human PH20 sequence set forth in SEQ ID NO:6. A mature PH20 polypeptide lacking the signal sequence and containing a contiguous sequence of amino acids having a C-terminal amino acid residue corresponding to amino acid residue 464 of SEQ ID NO:6 (*e.g.* amino acid residues corresponding to positions 36-464 of the amino acid sequence set forth in SEQ ID NO:6) is the minimal sequence required for hyaluronidase activity (*see e.g.* U.S. Patent No. 10/795,095; see also U.S. published application No. US20100143457).

Within the common hyaluronidase domain region at least 57 amino acids are conserved between and among species (*see e.g.* Arming *et al.* (1997) *Eur. J. Biochem.*, 247:810-814; en Have *et al.* (1998) *Reprod. Retil. Dev.*, 10:165-72; Chowpongpang *et al.* (2004) *Biotechnology Letters*, 26:1247-1252). For example, PH20 hyaluronidases contain 12 conserved cysteine residues corresponding to amino acid residue 25, 189, 203, 316, 341, 346, 352, 400, 402, 408, 423 and 429 of the sequence of amino acids set of a mature PH20 lacking the signal sequence such as set forth in SEQ ID NO: 3 or 7 (amino acid residues 60, 224, 238, 351, 376, 381, 387, 435, 437, 443, 458 or 464 of full-length human PH20 set forth in SEQ ID NO:6). Cysteine residues corresponding to 25 and 316 and cysteine residues corresponding to 189 and 203 form disulfide bridges. The other cysteine residues also form disulfide bridges, are involved in posttranslational protein maturation and/or in activity modulation. For example, further four disulfide bonds are formed between the cysteine residues C376 and C387; between C381 and C435; between C437 and C443; and between C458 and C464 of the polypeptide exemplified in SEQ ID NO:6 (corresponding to positions C341 and C352; between C346 and C400; between C402 and C408; and between C423 and C429 of the mature polypeptide set forth in SEQ ID NO:3 or 7, respectively).

Amino acid residues corresponding to amino acid residue D111, E113 and E249 of the sequence of amino acids set forth in SEQ ID NO: 3 or 7 are acidic residues part of the enzyme active site and are conserved between and among PH20

species. Amino acid residues R176, R246, R252 of the sequence of amino acids set forth in SEQ ID NO: 3 or 7 are also conserved between and among species and contribute to substrate binding and/or hyaluronidase activity. Amino acid mutations D111N, E113Q, R176G, E249N and R252T result in enzymes that have no detectable enzymatic activity or residual enzymatic activity (*see e.g.* Arming *et al.* (1997) *Eur. J. Biochem.*, 247:810-814).

The results herein confirm the requirement of PH20 amino acid residues for hyaluronidase activity corresponding to positions 25, 111, 113, 176, 189, 203, 246, 249, 252, 316, 341, 346, 352, 400, 402, 408, 423 and 429 of the sequence of amino acids set of a mature PH20 lacking the signal sequence such as set forth in SEQ ID NO: 3 or 7, since mutagenesis of these residues results in an enzyme that is not active (*e.g.* it is not expressed or is inactive when expressed, *see e.g.* Tables 5 and 10). The exception is that amino acid replacement corresponding to R176K and C316D resulted in mutants that generated some residual hyaluronidase activity.

Glycosylation also is required for PH20 hyaluronidase activity based on the recognition motif NxS or NxT. There are seven N-linked oligosaccharides at amino acid residues corresponding to positions N47, N131, N200, N219, N333, N358 and N365 of the sequence of amino acids set forth in SEQ ID NO: 3 or 7 (corresponding to amino acid residues N82, N166, N235, N254, N368, N393 and N490 of human PH20 set forth in SEQ ID NO: 6). In particular, at least N-linked glcosylation sites corresponding to amino acid residues N200, N333 and N358 are required for secretion and/or activity of the enzyme (*see e.g.* U.S. published application No. US20100143457). For example, PH20 polypeptide containing amino acid mutations N200A, N333A, N358A or N333A/N393A result in inactive proteins. Single mutations of glycosylation sites N47A, N131A, N219A, N47A/N131A, N47A/N219A, N131A/N291A retain activity. The N-linked glycosylation site corresponding to amino acid residue N368 of human PH20 set forth in SEQ ID NO:6 is conserved between and among species (*see e.g.* Chowpongpang *et al.* (2004) *Biotechnology Letters*, 26:1247-1252). Because amino acids 36 to 464 of SEQ ID NO:1 appears to contain the minimally active human PH20 hyaluronidase domain, the N-linked glycosylation site N-490 is not required for proper hyaluronidase activity. PH20 hyaluronidases also contains O-linked N-glycosylation sites. For example, human PH20 has one O-linked oligosaccharide at amino acid residue corresponding

to amino acid T440 of the sequence of amino acids set forth in SEQ ID NO:3 or 7 (corresponding to amino acid residue T475 in SEQ ID NO:6.

In addition to the catalytic sites, PH20 also contains a hyaluronan-binding site. Experimental evidence suggest that this site is located in the Peptide 2 region, which corresponds to amino acid positions 205-235 of the precursor polypeptide set forth in SEQ ID NO:6 and positions 170-200 of the mature polypeptide set forth in SEQ ID NO:3 or 7. This region is highly conserved among hyaluronidases and is similar to the heparin binding motif. Mutation of the arginine residue at position 176 (corresponding to the mature PH20 polypeptide set forth in SEQ ID NO:3 or 7) to a glycine results in a polypeptide with only about 1% of the hyaluronidase activity of the wild type polypeptide (Arming et al., (1997) Eur. J. Biochem. 247:810-814).

PH20 polypeptides contain a glycosyl phosphatidylinositol (GPI) anchor attached to the C-terminus of the protein that anchors the protein to the extracellular leaflet of the plasma membrane of cells. At least human, monkey, mouse and guinea pig PH20 are strongly attached to the plasma membrane via the GPI anchor, which can be released by treating with phosphatidylinositol-specific phospholipase C (PI-PLC; *see e.g.* Lin *et al.* (1994) *Journal of Cell Biology*, 125:1157-1163; Lin *et al.* (1993) *Proc. Natl. Acad. Sci.*, 90:10071-10075). Other PH20 enzymes, such as bovine PH20, are loosely attached to the plasma membrane and are not anchored via a phospholipase sensitive anchor. As discussed below, soluble active forms that, when expressed, are not attached to the membrane but are secreted can be generated by removal of all of a portion of the GPI anchor (*see also* U.S. Patent No. 10/795,095; U.S. published application No. US20100143457) .

GPI-anchored proteins, for example human PH20, are translated with a cleavable N-terminal signal peptide that directs the protein to the endoplasmic reticulum (ER). At the C-terminus of these proteins is another signal sequence that directs addition of a preformed GPI-anchor to the polypeptide within the lumen of the ER. Addition of the GPI anchor occurs following cleavage of the C-terminal portion at a specific amino acid position, called the ω-site (typically located approximately 20-30 amino acids from the C-terminus). Although there appears to be no consensus sequence to identify the location of the ω-site, GPI anchored proteins contain a C-terminal GPI-anchor attachment signal sequence or domain that typically contains a predominantly hydrophobic region of 8-20 amino acids, preceded by a hydrophilic spacer region of 8-12 amino acids immediately downstream of the ω-site. This

hydrophilic spacer region often is rich in charged amino acids and proline (White et al., (2000) J. Cell Sci. 113(Pt.4):721-727). More detailed analysis suggests that there is a region of approximately 11 amino acids before the ω-1 position that is characterized by a low amount of predicted secondary structure, a region around the cleavage site (ω-site), from ω-1 to ω+2 that is characterized by the presence of small side chain residues, the spacer region between positions ω+3 and ω+9, and a hydrophobic tail from ω+10 to the C-terminal end (Pierleoni et al., (2008) BMC Bioinformatics 9:392).

Although there is no GPI-anchor attachment signal consensus sequence, various *in silico* methods and algorithms have been developed that can be used to identify such sequences in polypeptides (see, *e.g.* Udenfriend et al. (1995) Methods Enzymol. 250:571-582; Eisenhaber et al., (1999) J. Biol. Chem. 292: 741-758; Kronegg and Buloz, (1999), "Detection/prediction of GPI cleavage site (GPI-anchor) in a protein (DGPI)," 129.194.185.165/dgpi/; Fankhauser et al., (2005) Bioinformatics 21:1846-1852; Omaetxebarria et al., (2007) Proteomics 7:1951-1960; Pierleoni et al., (2008) BMC Bioinformatics 9:392), including those that are readily available on bioinformatic websites, such as the ExPASy Proteomics tools site (expasy.ch/tools/). Thus, one of skill in the art can determine whether a PH20 polypeptide likely contains a GPI-anchor attachment signal sequence, and, therefore, whether the PH20 polypeptide is a GPI-anchored protein.

A GPI-anchor attachment signal sequence of human PH20 is located at amino acid positions 491-509 of the precursor polypeptide set forth in SEQ ID NO:6, and the ω-site is amino acid position 490. Thus, in this modeling of human PH20, amino acids 491-509 are cleaved following transport to the ER and a GPI anchor is covalently attached to the serine residue at position 490. A ω-site cleavage site of monkey PH20 is identified between Ser491 and Thr492 (Lin *et al.* (1993) *Proc. Natl. Acad. Sci*, (1993) 90:10071-10075). The covalent attachment of a GPI-anchor to the C-terminus of human PH20 and, therefore, the membrane-bound nature of PH20, has been confirmed using phosphatidylinositol-specific phospholipase C (PI-PLC) hydrolysis studies (*see, e.g.*, Lin *et al.*, (1994) *J. Biol. Chem.* 125:1157-1163). Phosphatidylinositol-specific phospholipase C (PI-PLC) and D (PI-PLD) hydrolyze the GPI anchor, releasing the PH20 polypeptide from the cell membrane. The resulting released PH20 polypeptide is, therefore, soluble.

2. Function

PH20 is normally expressed in sperm from a single testis-specific gene. PH20 is a sperm-associated protein involved in fertilization. PH20 is normally localized on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. PH20 is multifunctional and exhibits hyaluronidase activity, hyaluronan (HA) –mediated cell-signaling activity, and acts as a sperm receptor for the zona pellucida surrounding the oocyte when present on acrosome reacted (AR) sperm. For example, PH20 is naturally involved in sperm-egg adhesion and aids penetration by sperm of the layer of cumulus cells by digesting hyaluronic acid. In addition to being a hyaluronidase, PH20 also appears to be a receptor for HA-induced cell signaling, and a receptor for the zona pellucida surrounding the oocyte. Due to the role of PH20 in fertilization, PH20 can be used as an antigen for immunocontraception.

PH20 is a neutral active hyaluronidase, although it can exhibit acid-active activity in some cases. The hyaluronidase activity of PH20 is exhibited by the plasma membrane- and inner acrosomal membrane-associated PH20. The plasma membrane PH20 exhibits hyaluronidase activity only at neutral pH, while the inner acrosomal membrane-associated PH20 exhibits acid-active enzyme activity. The structural basis for these differences is due to the presence of two catalytic sites in PH20. A first catalytic site is designated the Peptide 1 region, corresponding to amino acid residues 142-172 of SEQ ID NO:6, which is involved in enzyme activity of PH20 at neutral pH. A second catalytic site is designated the peptide 3 region, corresponding to amino acid residues 277-297 of SEQ ID NO:6, which is involved in enzyme activity at lower pH. A change in the structure of the inner acrosomal membrane-associated PH20 occurs after the acrosome reaction, whereby PH20 is endoproteolytically cleaved but held together by disulfide bonds. The result of the endoproteolysis is that the peptide 3 region is activated and can thus effect neutral and acid-activity to PH20 (*see e.g.* Cherr et al. (2001) *Matrix Biology*, 20:515-525. Also, after the acrosome reaction, lower molecular weight forms are generated by release from the inner acrosomal membrane (*e.g.* a 53 k-Da soluble form of PH20 is generated in monkey). The lower molecular weight form(s) also is acid active.

The hyaluronidase activity of PH20 accounts for the spreading activity observed in by animal testes extracts that have been used clinically for decades to increase the dispersion and absorption of drugs (*see e.g.* Bookbinder et al. (2006) J Controlled Release, 114:230-241). For example, pharmaceutical preparations

containing hyaluronidase were developed as fractionated extracts from bovine testes for therapeutic use as spreading agents and in other applications (Schwartzman (1951) *J. Pediat.*, 39:491-502). Original bovine testicular extract preparations included, for example, extracts sold under the trademarks Wydase®, Hylase®, "Dessau," Neopermease®, Alidase® and Hyazyme®. It is now known that the spreading activity of testicular extract preparations are due to PH20 hyaluronidase activity. For example, in 2001 a sperm hyaluronidase in bull was identified as the hyaluronidase PH20 (Lalancette et al. (2001) Biol. Reprod., 65:628-36). By catalyzing the hydrolysis of hyaluronic acid, PH20 hyaluronidase lowers the viscosity of hyaluronic acid, thereby increasing tissue permeability. Hence, soluble forms of PH20 are used as a spreading or dispersing agent in conjunction with other agents, drug and proteins to enhance their dispersion an delivery, and to improve the pharmacokinetic and pharmacodynamic profile of the coadministered agent, drug or protein (*see e.g.* U.S. Patent No. 7,767,429; Bookbinder et al. (2006) J Controlled Release, 114:230-241).

3. Soluble PH20 Polypeptides

PH20 can exist in membrane-bound or membrane-associated form, or can be secreted into the media when expressed from cells, and thereby exist in soluble form. Soluble PH20 can be detected and discriminated from insoluble, membrane-bound PH20 using methods well known in the art, including, but not limited to, those using a Triton® X-114 assay. In this assay, soluble PH20 hyaluronidases partition into the aqueous phase of a Triton® X-114 solution warmed to 37 °C (Bordier *et al.*, (1981) *J. Biol. Chem.*, 256:1604-7) while membrane-anchored PH20 hyaluronidases partition into the detergent rich phase. Thus, in addition to using algorithms to assess whether a PH20 polypeptide is naturally GPI-anchored and hence membrane-bound, solubility experiments also can be performed.

Soluble PH20 enzymes include hyaluronidases that contain a GPI-anchor attachment signal sequence, but that are loosely attached to the membrane such that they do not contain a phospholipase sensitive anchor. For example, soluble PH20 polypeptides include ovine or bovine PH20. Various forms of such soluble PH20 hyaluronidases have been prepared and approved for therapeutic use in subjects, including humans. For example, animal-derived hyaluronidase preparations include Vitrase® (ISTA Pharmaceuticals), a purified ovine testicular hyaluronidase, and Amphadase® (Amphastar Pharmaceuticals), a bovine testicular hyaluronidase.

Soluble PH20 enzymes also include truncated forms of non-human or human

membrane-associated PH20 hyaluronidases that lack one or more amino acid residues of a glycosylphosphatidylinositol (GPI) anchor and that retain hyaluoronidase activity (*see e.g.* U.S. Patent No. 10/795,095; U.S. published application No. US20100143457). Thus, instead of having a GPI-anchor covalently attached to the C-terminus of the protein in the ER and being anchored to the extracellular leaflet of the plasma membrane, these polypeptides are secreted when expressed from cells and are soluble. In instances where the soluble hyaluronan degrading enzyme retains a portion of the GPI anchor attachment signal sequence, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid residues in the GPI-anchor attachment signal sequence can be retained, provided the polypeptide is soluble (*i.e.* secreted when expressed from cells) and active. Exemplary soluble hyaluronidases that are C-terminally truncated and lack all or a portion of the GPI anchor attachment signal sequence include, but are not limited to, PH20 polypeptides of primate origin, such as, for example, human and chimpanzee PH20 polypeptides. For example, soluble PH20 polypeptides can be made by C-terminal truncation of a polypeptide set forth in SEQ ID NOS:7, 10, 12, 14, 69, 72, 857, 859, 861 or 870 or variants thereof that exhibit at least 80%, 85%, 90%, 95% or more sequence identity to SEQ ID NO: 7, 10, 12, 14, 69, 72, 857, 859, 861 or 870, wherein the resulting polypeptide is active, soluble and lacks all or a portion of amino acid residues from the GPI-anchor attachment signal sequence.

Exemplary soluble PH20 polypeptides are C-terminal truncated human PH20 polypeptides that at least contain amino acids 36-464 of SEQ ID NO:6, or include a sequence of amino acids that has at least 85 %, for example at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % sequence identity to a sequence of amino acids that includes at least amino acids 36-464 of SEQ ID NO:6 and retain hyaluronidase activity. Exemplary C-terminally truncated human PH20 polypeptides include any that include at least amino acids 36-464 of SEQ ID NO:1 and are C-terminally truncated after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:6, or a variant thereof that exhibits at least 85 % sequence identity, such as at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % sequence identity thereto and retains hyaluronidase activity. Soluble PH20 polypeptides include any that has a sequence of amino acids set forth in SEQ ID NOS: 3 or 32-66 or a sequence of amino acids that

exhibits at least 85 % sequence identity, such as at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % sequence identity to the sequence of amino acids set forth in any of SEQ ID NOS: 3 or 32-66.

In particular, a soluble human PH20 polypeptide is a polypeptide that is truncated after amino acid 482 of the sequence set forth in SEQ ID NO:6. Such a polypeptide can be generated from a nucleic acid molecule containing a signal sequence and encoding amino acids 36-482, for example, as set forth in SEQ ID NO:1 (containing an IgG kappa signal sequence) or SEQ ID NO:67 (containing the native signal sequence). Post translational processing removes the signal sequence, leaving a 447 amino acid soluble recombinant human PH20 (SEQ ID NO:3). A product produced upon expression of a vector set forth in SEQ ID NO:4 or 5, and containing a nucleic acid molecule set forth in SEQ ID NO:67, results in a secreted product, designated rHuPH20, in the culture medium that exhibits heterogeneity at the C-terminus such that the product includes a mixture of species that can include any one or more of SEQ ID NOS: 3 and 44-48 in various abundance. Typically, rHuPH20 is produced in cells that facilitate correct N-glycosylation to retain activity, such as mammalian cells, for example CHO cells (*e.g.* DG44 CHO cells). Hylenex® (Halozyme) is a human recombinant hyaluronidase produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing nucleic acid encoding a truncated human PH20 polypeptide (designated rHuPH20).

C. MODIFIED PH20 POLYPEPTIDES

Provided herein are modified or variant PH20 polypeptides. The modified PH20 polypeptides provided herein exhibit altered activities or properties compared to a wildtype, native or reference PH20 polypeptide. For example, the PH20 polypeptides contain modifications compared to a wildtype, native or reference PH20 polypeptide set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870, or in a polypeptide that has a sequence of amino acids that is at least 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870. Included among the modified PH20 polypeptides provided herein are PH20 polypeptide that are active mutants, whereby the polypeptides exhibit at least 40% of the hyaluronidase activity of the corresponding PH20 polypeptide not containing the amino acid modification (*e.g.* amino acid replacement). In particular, provided herein are PH20 polypeptides that exhibit hyaluronidase activity and that exhibit increased

stability compared to the PH20 not containing the amino acid modification. Also provided are modified PH20 polypeptides that are inactive, and that can be used as antigens in contraception vaccines.

In particular, provided herein are PH20 polypeptides that contain modifications compared to a PH20 polypeptide set forth in SEQ ID NO: 3, 7, 32-66, 69 or 72, or a polypeptide that has a sequence of amino acids that is at least 68%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of SEQ ID NOS: 3, 7, 32-66, 69 or 72. For example, the modifications can be made in a PH20 polypeptide set forth as SEQ ID NOS: 10, 12, 14, 24, 857, 859, 861 or 870.

The modifications can be a single amino acid modification, such as single amino acid replacements (substitutions), insertions or deletions, or multiple amino acid modifications, such as multiple amino acid replacements, insertions or deletions. Exemplary of modification are amino acid replacements, including single or multiple amino acid replacements. The amino acid replacement can be a conservative substitution, such as set forth in Table 2, or a non-conservative substitution, such as any described herein. Modified PH20 polypeptides provided herein can contain at least or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more modified positions compared to the PH20 polypeptide not containing the modification.

The modifications described herein can be in any PH20 polypeptide. For example, the modifications are made in a human PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:7, SEQ ID NO:69 or SEQ ID NO:72; a bovine PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NOS:16 or 18; a rabbit PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:24; a Cynomolgus monkey PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:14; a guinea pig PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:29; a rat PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:22; a mouse PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:20; a chimpanzee PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:10 or 870; a Rhesus monkey PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:12; a Fox PH20

polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:31; a Gibbon PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:857; a Marmoset PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO: 859; an Orangutan PH20 polypeptide having a sequence of amino acids including or set forth in SEQ ID NO:861; or a sheep PH20 polypeptide having a sequence of amino acids including or set forth in any of SEQ ID NOS: 25-27; or in sequence variants or truncated variants that exhibit at least 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 7, 10, 12, 14, 16, 18, 20, 22, 24-27, 29, 31, 69, 72, 857, 859, 861 or 870.

In particular, provided herein are modified soluble PH20 polypeptides that are PH20 polypeptides containing a modification provided herein, and that when expressed from cells are secreted into the media as a soluble protein. For example, the modifications are made in a soluble PH20 polypeptide that is C-terminally truncated within or near the C-terminus portion containing the GPI-anchor signal sequence of a PH20 polypeptide that contains a GPI-anchor signal sequence. The C-terminal truncation can be a truncation or deletion of 8 contiguous amino acids at the C-terminus, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acids at the C-terminus, so long as the resulting C-terminally truncated polypeptide is secreted from cells *(e.g.* into the media) when expressed. In some examples, the modifications provided herein are made in a soluble PH20 polypeptide that is a C-terminally truncated polypeptide of SEQ ID NO:7, 10, 12, 14, 69, 72, 857, 859, 861 or 870 or a variant thereof that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 7, 10, 12, 14, 69, 72, 857, 859, 861 or 870. In particular, the modifications provided herein are made in a soluble or C-terminally truncated human PH20 polypeptide having the sequence of amino acids set forth in SEQ ID NOS: 3 or 32-66 or a sequence of amino acids that exhibits at least 70%, 75%, 80%, 85%, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % sequence identity to the sequence of amino acids set forth in any of SEQ ID NOS: 3 or 32-66. For example, modified PH20 polypeptides provided herein contain amino acid replacements or substitutions, additions or deletions, truncations

or combinations thereof with reference to the PH20 polypeptide set forth in SEQ ID NO:3.

Modifications also can be made in the corresponding precursor form containing a signal peptide of any of SEQ ID NOS: 3, 7, 10, 12, 14, 16, 18, 20, 22, 24-27, 29, 31, 32-66, 69, 72, 857, 859, 861 or 870. For example, modifications provided herein can be made in a precursor form set forth in any of SEQ ID NOS: 6, 8, 9, 11, 13, 15, 17, 19, 21, 23, 28, 30, 856, 858, 860 or 869 or in a variant thereof that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 6, 8, 9, 11, 13, 15, 17, 19, 21, 23, 28, 30, 856, 858, 860 or 869.

Generally, any modification, such as amino acid replacement, deletion or substitution, can be made in a PH20 polypeptide, with the proviso that the modification is not an amino acid replacement where the only modification is a single amino acid replacement that is V12A, N47A, D111N, E113Q, N131A, R176G, N200A, N219A, E249Q , R252T, N333A or N358A. Also, where the modified PH20 polypeptide contains only two amino acid replacements, the amino acid replacements are not P13A/L464W, N47A/N131A, N47A/N219A, N131A/N219A or N333A/N358A. In a further example, where the modified PH20 polypeptide contains only three amino acid replacements, the amino acid replacements are not N47A/N131A/N219A. Exemplary modifications provided herein are described in detail below.

For purposes herein, reference to positions and amino acids for modification, including amino acid replacement or replacements, herein are with reference to the PH20 polypeptide set forth in SEQ ID NO:3. It is within the level of one of skill in the art to make any of the modifications provided herein in another PH20 polypeptide by identifying the corresponding amino acid residue in another PH20 polypeptide, such as any set forth in SEQ ID NOS: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24-27, 38, 29, 30, 31, 32-66, 69, 72, 856, 857, 858, 859, 860, 861, 869 or 870 or a variant thereof that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24-27, 38, 29, 30, 31, 32-66, 69, 72, 856, 857, 858, 859, 860, 861, 869 or 870. Corresponding positions in another PH20 polypeptide can be identified by alignment of the PH20 polypeptide with the reference PH20 polypeptide set forth

in SEQ ID NO:3. For example, Figure 2 depicts alignment of exemplary PH20 polypeptides with SEQ ID NO:3, and identification of exemplary corresponding positions. Also, since SEQ ID NOS: 3, 7, 32-66, 69 and 72 are all forms of a mature human PH20 with a different C-terminal amino acid residue, the numbering of amino acid residues in any of SEQ ID NOS: 7, 32-66, 69 and 72 is the same as SEQ ID NO:3, and hence the corresponding residues of each are identical to that set forth in SEQ ID NO:3 (*see e.g.* Figure 1). For purposes of modification (*e.g.* amino acid replacement), the corresponding amino acid residue can be any amino acid residue, and need not be identical to the residue set forth in SEQ ID NO:3. Typically, the corresponding amino acid residue identified by alignment with residues in SEQ ID NO:3 is an amino acid residue that is identical to SEQ ID NO:3, or is a conservative or semi-conservative amino acid residue thereto (*see e.g.* Figure 2). It is also understood that the exemplary replacements provided herein can be made at the corresponding residue in a PH20 polypeptide, so long as the replacement is different than exists in the unmodified form of the PH20 polypeptide. Based on this description and the description elsewhere herein, it is within the level of one of skill in the art to generate a modified PH20 polypeptide containing any one or more of the described mutation, and test each for a property or activity as described herein.

Modifications in a PH20 polypeptide also can be made to a PH20 polypeptide that also contains other modifications, including modifications of the primary sequence and modifications not in the primary sequence of the polypeptide. For example, modification described herein can be in a PH20 polypeptides that is a fusion polypeptide or chimeric polypeptide. The modified PH20 polypeptides provided herein also include polypeptides that are conjugated to a polymer, such as a PEG reagent.

Also provided herein are nucleic acid molecules that encode any of the modified PH20 polypeptides provided herein. In particular examples, the nucleic acid sequence can be codon optimized, for example, to increase expression levels of the encoded sequence. The particular codon usage is dependent on the host organism in which the modified polypeptide is expressed. One of skill in the art is familiar with optimal codons for expression in mammalian or human cells, bacteria or yeast, including for example *E. coli* or Saccharomyces cerevisiae. For example, codon usage information is available from the Codon Usage Database available at kazusa.or.jp.codon (see Richmond (2000) Genome Biology, 1:241 for a description of

the database). See also, Forsburg (2004) Yeast, 10:1045-1047; Brown et al. (1991) Nucleic Acids Research, 19:4298; Sharp et al. (1988) Nucleic Acids Res., 12:8207-8211; Sharp et al. (1991) Yeast, 657-78. In some examples, the encoding nucleic acid molecules also can be modified to contain a heterologous signal sequence to alter (*e.g.* increased) expression and secretion of the polypeptide. Exemplary of a heterologous signal sequence is a nucleic acid encoding the IgG kappa signal sequence (set forth in SEQ ID NO:868).

The modified polypeptides and encoding nucleic acid molecules provided herein can be produced by standard recombinant DNA techniques known to one of skill in the art. Any method known in the art to effect mutation of any one or more amino acids in a target protein can be employed. Methods include standard site-directed or random mutagenesis of encoding nucleic acid molecules, or solid phase polypeptide synthesis methods. For example, nucleic acid molecules encoding a PH20 polypeptide can be subjected to mutagenesis, such as random mutagenesis of the encoding nucleic acid, error- prone PCR, site-directed mutagenesis, overlap PCR, gene shuffling, or other recombinant methods. The nucleic acid encoding the polypeptides can then be introduced into a host cell to be expressed heterologously. Hence, also provided herein are nucleic acid molecules encoding any of the modified polypeptides provided herein. In some examples, the modified PH20 polypeptides are produced synthetically, such as using solid phase or solutions phase peptide synthesis.

In the subsections below, exemplary modified PH20 polypeptide exhibiting altered properties and activities, and encoding nucleic acid molecules, provided herein are described.

1. Active Mutants

Provided herein are modified PH20 polypeptides that contain one or more amino acid replacements in a PH20 polypeptide and that exhibit hyaluronidase activity. The modified PH20 polypeptides can exhibit 40% to 5000% of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the polypeptide set forth in SEQ ID NOS: 3 or 7. For example, modified PH20 polypeptides provided herein exhibit at least 40% of the hyaluronidase activity, such as at least 50%, 60%, 70%, 80%, 90%, 100%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000% or more of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the corresponding polypeptide not containing the amino acid

modification (*e.g.* amino acid replacement), for example, a polypeptide set forth in SEQ ID NO:3 or 7. For example, exemplary positions that can be modified, for example by amino acid replacement or substitution, include, but are not limited to, any of positions corresponding to position 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 20, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 58, 59, 60, 61, 63, 65, 66, 67, 68, 69, 70, 71, 72, 73, 75, 75, 77, 79, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 96, 97, 98, 99, 102, 103, 104, 105, 106, 107, 108, 110, 114, 117, 118, 119, 120, 122, 124, 125, 127, 128, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 186, 192, 193, 195, 196, 197, 198, 200, 202, 204, 205, 206, 208, 209, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 224, 226, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 242, 245, 247, 248, 251, 253, 255, 256, 257, 258, 259, 260, 261, 263, 264, 265, 266, 267, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 297, 298, 300, 301, 302, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 320, 321, 323, 324, 325, 326, 327, 328, 331, 334, 335, 338, 339, 342, 343, 347, 348, 349, 351, 353, 356, 357, 358, 359, 360, 361, 367, 368, 369, 371, 373, 374, 375, 376, 377, 378, 379, 380, 381, 383, 385, 387, 388, 389, 391, 392, 393, 394, 395, 396, 397, 398, 399, 401, 403, 404, 405, 406, 407, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 425, 426, 427, 428, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446 or 447 with reference to amino acid positions set forth in SEQ ID NO:3. Typically, the amino acid residue that is modified (*e.g.* replaced) at the position corresponding to any of the above positions in a PH20 polypeptide is an identical residue, a conservative residue or a semi-conservative amino acid residue to the amino acid residue set forth in SEQ ID NO:3.

To retain hyaluronidase activity, modifications typically are not made at those positions that are less tolerant to change or required for hyaluronidase activity. For example, generally modifications are not made at a position corresponding to position 7, 16, 17, 18, 19, 21, 25, 53, 55, 56, 57, 62, 64, 76, 78, 80, 88, 95, 100, 101, 109, 111, 112, 113, 115, 116, 121, 123, 126, 129, 185, 187, 188, 189, 190, 191, 194, 199, 201, 203, 207, 210, 223, 225, 227, 228, 229, 241, 243, 244, 246, 249, 250, 252, 254, 262,

268, 282, 295, 296, 298, 299, 303, 319, 322, 329, 330, 332, 333, 336, 337, 340, 341, 344, 345, 346, 350, 352, 354, 355, 362, 363, 364, 365, 366, 370, 372, 382, 384, 386, 390, 400, 402, 408, 423, 424, 429, 430, 431, with reference to amino acid positions set forth in SEQ ID NO:3. Also, in examples where modifications are made at any of positions 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 20, 22, 23, 27, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 58, 59, 60, 61, 63, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 77, 79, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 94, 96, 98, 99, 102, 103, 104, 105, 106, 107, 108, 110, 114, 117, 118, 119, 122, 124, 125, 127, 128, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 143, 144, 145, 149, 150, 152, 153, 154, 155, 156, 157, 158, 159, 161, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 186, 192, 193, 195, 197, 198, 200, 202, 204, 206, 208, 209, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 224, 226, 230, 231, 232, 233, 234, 235, 236, 238, 239, 240, 242, 245, 247, 248, 251, 253, 255, 256, 257, 258, 260, 261, 263, 264, 265, 266, 267, 269, 270, 271, 272, 273, 274, 275, 276, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 297, 298, 300, 301, 302, 304, 305, 306, 307, 308, 310, 311, 312, 313, 314, 315, 316, 317, 318, 320, 321, 323, 324, 325, 326, 327, 331, 334, 335, 338, 339, 342, 343, 347, 348, 349, 351, 353, 356, 357, 358, 359, 360, 361, 367, 368, 369, 371, 373, 374, 375, 376, 377, 378, 379, 380, 381, 383, 385, 387, 388, 389, 391, 392, 393, 394, 395, 396, 397, 398, 399, 401, 403, 404, 405, 406, 410, 411, 412, 413, 414, 415, 416, 417, 419, 420, 422, 425, 426, 427, 428, 431, 432, 434, 437, 438, 439, 440, 441, 442, 443, 444, or 447 with reference to amino acid positions set forth in SEQ ID NO:3, the modification(s) is/are not the corresponding amino acid replacement(s) set forth in Table 5 or 10 herein. For example, if the modification is a modification at a position corresponding to position 2 with reference to SEQ ID NO:3, the modification is not replacement to a histidine (H), lysine (K), tryptophan (W) or tyrosine (Y).

Exemplary amino acid replacements at any of the above corresponding positions are set forth in Table 3. Reference to corresponding position in Table3 is with reference to positions set forth in SEQ ID NO:3. It is understood that the replacements can be made in the corresponding position in another PH20 polypeptide by alignment therewith with the sequence set forth in SEQ ID NO:3 (*see e.g.* Figure 1 and 2), whereby the corresponding position is the aligned position. In particular examples, the amino acid replacement(s) can be at the corresponding position in a

PH20 polypeptide as set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto, so long as the resulting modified PH20 polypeptide exhibits at least 40% of the hyaluronidase activity of the corresponding PH20 polypeptide not containing the amino acid replacement. In particular, the replacement(s) can be in a corresponding position in a human PH20 polypeptide, for example, any set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72, or a variant thereof that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 3, 7, 32-66, 69 or 72. In one example, any one or more of the replacements are in SEQ ID NO:3, so long as the resulting modified PH20 polypeptide exhibits at least 40% of the hyaluronidase activity of the PH20 polypeptide set forth in SEQ ID NO:3.

TABLE 3: Active Mutants

Corresponding Position	Replacement	Corresponding Position	Replacement	Corresponding Position	Replacement
1	A C E F G H K N P Q R S T V W	2	A C G I L P Q S T V	3	E H L Y
4	A I S T V	5	H	6	A H K L N Q R
7	M	8	I L M P	9	K L Q R S V
10	D E G H N Q R S W	11	D G H K S	12	A E I K L N R S T
13	H S T Y	14	D I M V	15	A M V
20	S	22	H M T Y	23	D
24	A E G H I K L M N R T V Y	26	A E G H I K M P Q R S T V W Y	27	A D E F H I K L P Q R S T W
28	A D E F I L M N P R S T V W	29	A E G H I K L M P R S T V W	30	A F G H K L M P Q R S T V W
31	A C G H I K L P R S T V W Y	32	A C F G H K L M N Q R S T V W Y	33	G M P Q R S T W
34	A E H K Q R W	35	F H L Q T V Y	36	A D G H K L N R T
37	F I K M P R W V	38	Y	39	A L N Q R T Y
40	L W	41	A C D E G H N T V W	42	A
43	N T	44	E	45	I K
46	A C E F H L M N R S T V Y	47	A D F G H K M Q R S T W Y	48	F G H I K M N Q R S V Y
49	I K R S V	50	A C D E H L M Q R S V Y	51	A N R S
52	N P Q R S T	54	A F N Q S V	58	C G H I K L N P Q R S W Y
59	Q N	60	K	61	F I M V
63	A H I K L M N R S T V W	65	R	66	H R
67	F L R V Y	68	E G H K L P Q R S T	69	A C E F G I L M P R T W Y
70	A C F G H K L N P R S T V Y	71	A D G H L M N Q R S	72	A D E H K L M Q R S Y

73	A C D G H K L M Q R S T W	74	A C E F G H K L M N P R S V W	75	A C F H L M N Q R S T Y
77	H K				
79	L T V	81	P	82	A E G H I L M N Q R S T V
83	F G H K L N Q R S T V	84	D E F G H I L M N P Q R T W Y	85	V
86	A D E F G H I K L M N P R S T V W	87	A C E G H I L M P Q R S T V Y	89	C K M P R W
90	A E G H I K L N Q R S T W	91	A Q R	92	C H L M T V
93	D E F G H I L M N P Q R S T V	94	A C D E F H L M N Q R S T	96	D L V
97	A C D E F G I L N P Q R S W Y	98	A C D E H I L M Q R S V W	99	A R S
102	A C E G H K L M N Q R S T W				
103	N	104	A C G I K M R S T	105	A C G H I P Q R S T W V
106	V				
107	F I L	108	G	110	V
114	A G H M S	117	D	118	H K L M N Q V
119	F P Q Y	120	D F G H I L N P R S T V W Y	122	M
124	H L R	125	A H R S	127	A E G H L M N Q R S T V W
128	A C G I K L Q R S W	130	I R	131	C E F G H I L M Q R S T V Y
132	A C E F H I K L N Q S T V Y	133	I	134	L T V
135	A C D F G H K L N Q R S W Y	136	A C D F H I M N Q R S T W	137	A C I T A C H I L M N R S W Y
139	A C D E F G H K L M R S T V	140	A C D F G H I K L M R V W Y	141	A D E F G H L M Q R S T V W Y
142	C D E G H I K L M N P Q R S T	143	C E G I K L N V	144	R T W
145	A C D E G H L M N P R	146	A C E G H I K N P Q R S T V Y	147	A C D F G I L M P Q R S V W Y
148	C F G H I K L Q R S T V W Y	149	C G K L M Q R S T V	150	A C D E F G I L N P R S W Y
151	A C G H K L M N Q R S T V W Y	152	A C F I M R T V W Y	153	I L S
154	I R T V	155	A C D F G H K L M R S T V W	156	A C D G I L M Q R S T V W
157	W	158	A F G H L Q S	159	A D E G H L M N Q R S V
160	C F G H I K L M N Q R S W V Y	161	A C D E R S V	162	A D E G H L M P Q R S V W Y
163	A E G K L Q R S T V W	164	L M V W	165	A C D F N R S V W Y
166	A C E F G H L N Q R T W Y	167	A D G H K M N P R S T Y	168	H
169	L R V	170	A Q N R V	171	I V
172	A C	173	Q N R	174	A G H K M N Q R S T V W Y
175	E H T V Y	176	K L	177	V
178	G K M R				
179	A C E G I K L M N P R S T V	180	F G I K M	181	K M Q

182	L	183	EL	184	W
186	Y				
192	ST	193	FGQRSY	195	AGHILNQRS TWV
196	EGLNRSTWY	197	ADEFGHKLM QRSTW	198	ADEHLNQRS TWY
200	DT	202	M	204	PW
205	LRSTVWY	206	HIKLMQRST	208	ACKLMQRS TV
209	AEFGLNRST	211	LW		
212	NST	213	AEGHKLMN QRVWY		
214	Q	215	ADEGHKLM QRTVWY	217	M
218	FMV	219	ACDEHIKLM RSTW	220	ADHILMSTV
221	ACIMQTV	222	DFGIKLNRS V	224	I
226	W				
230	I	231	T	232	S
233	AFGKLRY	234	LM	235	AEGHKT
236	AGHKRS	237	ACEFHLNQR STW	238	DEHKQRST
239	N				
240	KAMPQRSV	242	F	245	H
247	ILM	248	AHWY	251	LMY
253	I	255	AGNQRS	256	AHLV
257	ACGIKLMNQ RTV	258	GHNRS	259	EGIKLNPQR STVWY
260	ADEGHLMQR SY	261	AFKMNQRTV W	263	AHKMRTV
264	AH	265	I	266	Y
267	MT	269	ACDS	270	MNST
271	FGLMSV	272	DMRST	273	HTY
274	AFS				
275	LV	276	CDEGHILMR SY	277	ACDEGHKM NQRSTY
278	AEFGHIKNR STVY	279	AHQRT	280	GQ
282	DGMQ	283	EPRST	284	AEGHLMNQ STY
285	AFGHMNQY	286	RSW	287	INT
288	LW	289	KS	290	IM
291	CQRSV	292	ACFGHKNPR VW	293	ACDFGKLM PQSVY
294	M				
297	A	298	GI	300	R
301	AV	302	IW	303	DV
304	GI	305	DEN	306	DES
307	GKNQSTVWY	308	DGHKNPRT	309	DEGHKLMN QRSTVW
310	AFGQRSVY	311	GHKQST	312	GKLNT
313	AEGH KLPRS TVY	314	ADHINQRST Y	315	AEGHKLMR TY
316	D	317	ADHIKMNQR STW	318	DFGHIKMNQ RST
320	EGHIKL MNR SWVY	321	ADHKRSTY	323	FIL

324	A D H M N R S	325	A D E G H K M N Q S V W	326	C K L V Y
327	M	328	A C G H I K L Q R S T V W Y	331	C E V
334	P T	335	S	338	Q
339	M	342	A	343	T V
347	A E G L M R S	348	D G S	349	A E K M N R T
351	A C I Q S	353	T V	356	A D H S
357	A C K S T	358	C G L T	359	D E H K M T V
360	T				
361	H	367	A C G K R S	368	A E G H K L M R S T V H R S
371	E F G H I K L M R S V	373	A E F K L M R S V	374	A H I M N P R S T V W Y
375	A G I K L M N R S T	376	A D E L M Q R S T V Y	377	D E H K P R S T
378	K N R	379	G H R S T	380	I L P T V W Y
381	E H K N Q R S V	383	A E H I K L M N S T V	385	A G H N Q R S T V
387	S	388	F H I M R T V W Y	389	A G H K L M P Q R S T Y
391	C	392	A F G K L M Q R S T V W Y	393	A D F H K L M N R S T
394	L W	395	A G H K R T W	396	A D H L Q R S T
397	R	398	L		
399	A C E K M N Q R S T V W	401	A E G Q N	403	F
404	A P T	405	A F G K M P Q R S W Y	406	A C E F G I N Q S T V Y
407	A D E F G H L M N P Q R V W	409	A D E G H I P Q R S T V	410	D K M N P Q R S T V Y
411	A H N P R S T V	412	D G H I L N Q P R S V W Y	413	A E H K N Q R S T
414	I K L M	415	G S W V Y	416	F G H I K L N Q R T V Y
417	I	418	A E F G I L M N P Q R S V Y	419	E F G H I K L N R S W Y
420	I P	421	A E G H I K L M N Q R S T Y	422	I T
425	G I K M N R S Y	426	E G K N P Q S Y	427	H I K Q S T
428	L M P T	431	A E G H I K L N Q R S V W Y	432	E G H N S V
433	A C D E G H I K L P R S T V W	434	F G I M V	435	A C E G H R S T V Y
436	C D E G H I K L M Q R S T W Y	437	A D G H I K L M Q R S Y	438	A C D E G L N P Q R S T V W
439	A C F G H K L P Q S T V W	440	A D E F G H I L M P R S V Y	441	A D F G H K L N Q S T V Y
442	C G H K L P Q R T V W Y	443	A E F G H L M N Q R S T W	444	D E F G H I K M N R V W Y
445	A G H L M N P Q R S T V W Y	446	A C D E G H I K L M Q R T V W	447	D E F G I L M N P Q R T V W

In particular examples, provided herein is a modified PH20 polypeptide containing an amino acid replacement or replacements at a position or positions corresponding to 1, 6, 8, 9, 10, 11, 12, 14, 15, 20, 22, 24, 26, 27, 28, 29, 30, 31, 32,

33, 34, 35, 36, 37, 38, 39, 40, 41, 46, 47, 48, 49, 50, 52, 58, 59, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 79, 82, 83, 84, 86, 87, 89, 90, 92, 93, 94, 97, 102, 104, 107, 114, 118, 120, 127, 128, 130, 131, 132, 135, 138, 139, 140, 141, 142, 143, 146, 147, 148, 149, 150, 151, 152, 155, 156, 158, 160, 162, 163, 164, 165, 166, 167, 169, 170, 172, 173, 174, 175, 178, 179, 193, 195, 196, 198, 204, 205, 206, 209, 212, 213, 215, 219, 220, 221, 222, 232, 233, 234, 235, 236, 237, 238, 240, 247, 248, 249, 257, 258, 259, 260, 261, 263, 267, 269, 271, 272, 273, 274, 276, 277, 278, 279, 282, 283, 285, 287, 289, 291, 292, 293, 305, 307, 308, 309, 310, 313, 314, 315, 317, 318, 320, 321, 324, 325, 326, 328, 335, 347, 349, 351, 353, 356, 359, 367, 368, 369, 371, 373, 374, 375, 376, 377, 380, 381, 383, 385, 389, 392, 393, 395, 396, 399, 401, 404, 405, 406, 407, 409, 410, 412, 416, 418, 419, 421, 425, 427, 428, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446 or 447 with reference to amino acid positions set forth in SEQ ID NO:3. For example, the amino acid positions can be replacements at positions corresponding to replacement of Leucine (L) at position 1 (L1), P6, V8, I9, P10, N11, V12, F14, L15, A20, S22, F24, L26, G27, K28, F29, D30, E31, P32, L33, D34, M35, S36, L37, F38, S39, F40, I41, I46, N47, A48, T49, G50, G52, V58, D59, Y63, I67, D68, S69, I70, T71, G72, V73, T74, V75, I79, K82, I83, S84, G86, D87, L89, D90, A92, K93, K94, T97, V102, N104, M107, E114, T118, A120, D127, V128, K130, N131, R132, E135, Q138, Q139, Q140, N141, V142, Q143, L146, T147, E148, A149, T150, E151, K152, Q155, E156, E158, A160, K162, D163, F164, L165, V166, E167, I169, K170, G172, K173, L174, L175, N178, H179, H193, K195, K196, G198, F204, N205, V206, K209, D212, D213, S215, N219, E220, S221, T222, T232, Q233, Q234, S235, P236, V237, A238, T240, V247, R248, E249, P257, D258, A259, K260, S261, L263, A267, T269, I271, V272, F273, T274, Q276, V277, L278, K279, S282, Q283, E285, V287, T289, G291, E292, T293, G305, L307, S308, I309, M310, M313, K314, S315, L317, L318, D320, N321, E324, T325, I326, N328, T335, Q347, Q349, V351, I353, N356, S359, P367, D368, N369, A371, Q373, L374, E375, K376, G377, F380, T381, R383, K385, E389, E392, Q393, S395, E396, Y399, S401, S404, T405, L406, S407, K409, E410, A412, D416, D418, A419, D421, A425, G427, A428, D431, F433, P436, P437, M438, E439, T440, E441, E442, P443, Q444, I445, F446 or Y447 with reference to amino acid positions set forth in SEQ ID NO:3.

Exemplary of amino acid replacements in the modified PH20 polypeptides provided herein include, but are not limited, replacement with: histidine (H) at a position corresponding to position 1; A at a position corresponding to position 1; E at

a position corresponding to position 1; G at a position corresponding to position 1; K at a position corresponding to position 1; Q at a position corresponding to position 1; R at a position corresponding to position 1; A at a position corresponding to position 6; M at a position corresponding to position 8; Q at a position corresponding to position 9; G at a position corresponding to position 10; H at a position corresponding to position 10; S at a position corresponding to position 11; E at a position corresponding to position 12; I at a position corresponding to position 12; K at a position corresponding to position 12; T at a position corresponding to position 12; V at a position corresponding to position 14; V at a position corresponding to position 15; M at a position corresponding to position 15; S at a position corresponding to position 20; T at a position corresponding to position 22; E at a position corresponding to position 24; H at a position corresponding to position 24; R at a position corresponding to position 24; A at a position corresponding to position 26; E at a position corresponding to position 26; K at a position corresponding to position 26; M at a position corresponding to position 26; Q at a position corresponding to position 26; R at a position corresponding to position 26; D at a position corresponding to position 27; K at a position corresponding to position 27; R at a position corresponding to position 27; R at a position corresponding to position 28; E at a position corresponding to position 29; I at a position corresponding to position 29; K at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; R at a position corresponding to position 29; S at a position corresponding to position 29; T at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; L at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; G at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; W at a

position corresponding to position 31; C at a position corresponding to position 32; F at a position corresponding to position 32; G at a position corresponding to position 32; H at a position corresponding to position 32; W at a position corresponding to position 33; G at a position corresponding to position 33; W at a position corresponding to position 34; Q at a position corresponding to position 35; V at a position corresponding to position 35; H at a position corresponding to position 36; N at a position corresponding to position 36; F at a position corresponding to position 37; M at a position corresponding to position 37; Y at a position corresponding to position 38; A at a position corresponding to position 39; L at a position corresponding to position 39; N at a position corresponding to position 39; T at a position corresponding to position 39; L at a position corresponding to position 40; T at a position corresponding to position 41; L at a position corresponding to position 46; R at a position corresponding to position 46; D at a position corresponding to position 47; F at a position corresponding to position 47; T at a position corresponding to position 47; W at a position corresponding to position 47, with F at a position corresponding to position 48; H at a position corresponding to position 48; K at a position corresponding to position 48; N at a position corresponding to position 48; R at a position corresponding to position 49; D at a position corresponding to position 50; S at a position corresponding to position 50; M at a position corresponding to position 50; N at a position corresponding to position 52; Q at a position corresponding to position 52; R at a position corresponding to position 52; S at a position corresponding to position 52; T at a position corresponding to position 52; C at a position corresponding to position 58; K at a position corresponding to position 58; L at a position corresponding to position 58; P at a position corresponding to position 58; Q at a position corresponding to position 58; R at a position corresponding to position 58; H at a position corresponding to position 58; N at a position corresponding to position 58; Y at a position corresponding to position 58; N at a position corresponding to position 59; K at a position corresponding to position 63; L at a position corresponding to position 63; M at a position corresponding to position 63; R at a position corresponding to position 63; W at a position corresponding to position 63; V at a position corresponding to position 67; H at a position corresponding to position 68; P at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position 69; C at a position corresponding to position 69; E at a position

corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; I at a position corresponding to position 69; L at a position corresponding to position 69; M at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; T at a position corresponding to position 69; W at a position corresponding to position 69; Y at a position corresponding to position 69; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; N at a position corresponding to position 70; P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; Y at a position corresponding to position 70; G at a position corresponding to position 71; N at a position corresponding to position 71; R at a position corresponding to position 71; S at a position corresponding to position 71; K at a position corresponding to position 72; M at a position corresponding to position 72; Q at a position corresponding to position 72; A at a position corresponding to position 73; H at a position corresponding to position 73; K at a position corresponding to position 73; L at a position corresponding to position 73; Q at a position corresponding to position 73; R at a position corresponding to position 73; T at a position corresponding to position 73; W at a position corresponding to position 73; A at a position corresponding to position 74; C at a position corresponding to position 74; E at a position corresponding to position 74; F at a position corresponding to position 74; G at a position corresponding to position 74; H at a position corresponding to position 74; K at a position corresponding to position 74; L at a position corresponding to position 74; M at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; F at a position corresponding to position 75; L at a position corresponding to position 75; M at position corresponding to position 75; R at a position corresponding to position 75; T at a position corresponding to position 75; L at a position corresponding to position 79; L at a position corresponding to

position 82; N at a position corresponding to position 82; V at a position corresponding to position 83; Q at a position corresponding to position 83; S at a position corresponding to position 83; G at a position corresponding to position 83; E at a position corresponding to position 84; F at a position corresponding to position 84; G at a position corresponding to position 84; N at a position corresponding to position 84; R at a position corresponding to position 84; A at a position corresponding to position 86; H at a position corresponding to position 86; K at a position corresponding to position 86; N at a position corresponding to position 86; S at a position corresponding to position 86; T at a position corresponding to position 86; W at a position corresponding to position 86; C at a position corresponding to position 87; G at a position corresponding to position 87; L at a position corresponding to position 87; M at a position corresponding to position 87; R at a position corresponding to position 87; S at a position corresponding to position 87; T at a position corresponding to position 87; V at a position corresponding to position 87; Y at a position corresponding to position 87; C at a position corresponding to position 89; A at a position corresponding to position 90; E at a position corresponding to position 90; H at a position corresponding to position 90; K at a position corresponding to position 90; N at a position corresponding to position 90; R at a position corresponding to position 90; C at a position corresponding to position 92; L at a position corresponding to position 92; I at a position corresponding to position 93; L at a position corresponding to position 93; Q at a position corresponding to position 93; R at a position corresponding to position 93; S at a position corresponding to position 93; T at a position corresponding to position 93; D at a position corresponding to position 94; Q at a position corresponding to position 94; R at a position corresponding to position 94; A at a position corresponding to position 97; C at an amino acid residue corresponding to position 97; D at a position corresponding to position 97; E at a position corresponding to position 97; G at a position corresponding to position 97; L at a position corresponding to position 97; S at a position corresponding to position 97; S at a position corresponding to position 102; T at a position corresponding to position 102; R at a position corresponding to position 104; L at a position corresponding to position 107; A at a position corresponding to position 114; Q at a position corresponding to position 118; H at a position corresponding to position 120; F at a position corresponding to position 120; I at a position corresponding to position 120; S at a position corresponding to position

120; V at a position corresponding to position 120; Y at a position corresponding to position 120; E at a position corresponding to position 127; H at a position corresponding to position 127; N at a position corresponding to position 127; Q at a position corresponding to position 127; R at a position corresponding to position 127; I at a position corresponding to position 128; R at a position corresponding to position 130; G at a position corresponding to position 131; I at a position corresponding to position 131; M at a position corresponding to position 131; Q at a position corresponding to position 131; R at a position corresponding to position 131; V at a position corresponding to position 131; N at a position corresponding to position 132; L at a position corresponding to position 132; D at a position corresponding to position 135; G at a position corresponding to position 135; R at a position corresponding to position 135, with L at a position corresponding to position 138; T at a position corresponding to position 139; K at a position corresponding to position 140; H at a position corresponding to position 141; R at a position corresponding to position 141; S at a position corresponding to position 141; W at a position corresponding to position 141; Y at a position corresponding to position 141; D at a position corresponding to position 142; G at a position corresponding to position 142; K at a position corresponding to position 142; N at a position corresponding to position 142; P at a position corresponding to position 142; Q at a position corresponding to position 142; R at a position corresponding to position 142; S at a position corresponding to position 142; T at a position corresponding to position 142; G at a position corresponding to position 143; K at a position corresponding to position 143; R at a position corresponding to position 144; T at a position corresponding to position 144; P at a position corresponding to position 146; R at a position corresponding to position 146; A at a position corresponding to position 147; F at a position corresponding to position 147; L at a position corresponding to position 147; R at a position corresponding to position 147; S at a position corresponding to position 147; V at a position corresponding to position 147; H at a position corresponding to position 148; K at a position corresponding to position 148; Q at a position corresponding to position 148; T at a position corresponding to position 149; V at a position corresponding to position 149; A at a position corresponding to position 150; D at a position corresponding to position 150; G at a position corresponding to position 150; N at a position corresponding to position 150; S at a position corresponding to position 150; W at a position corresponding to

position 150; Y at a position corresponding to position 150; A at a position corresponding to position 151; H at a position corresponding to position 151; K at a position corresponding to position 151; L at a position corresponding to position 151; M at a position corresponding to position 151; Q at a position corresponding to position 151; R at a position corresponding to position 151; S at a position corresponding to position 151; T at a position corresponding to position 151; V at a position corresponding to position 151; W at a position corresponding to position 151; Y at a position corresponding to position 151; R at a position corresponding to position 152; T at a position corresponding to position 152; W at a position corresponding to position 152; D at a position corresponding to position 155; G at a position corresponding to position 155; K at a position corresponding to position 155; R at a position corresponding to position 155; D at a position corresponding to position 156; Q at a position corresponding to position 158; S at a position corresponding to position 158; S at a position corresponding to position 160; E at a position corresponding to position 162; A at a position corresponding to position 163; E at a position corresponding to position 163; K at a position corresponding to position 163; Q at a position corresponding to position 163; R at a position corresponding to position 163; S at a position corresponding to position 163; M at a position corresponding to position 164; V at a position corresponding to position 164; D at a position corresponding to position 165; F at a position corresponding to position 165; N at a position corresponding to position 165; S at a position corresponding to position 165; V at a position corresponding to position 165; A at a position corresponding to position 166; E at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; Q at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; W at a position corresponding to position 166; Y at a position corresponding to position 166; D at a position corresponding to position 167; L at a position corresponding to position 169; R at a position corresponding to position 170; A at a position corresponding to position 172; R at a position corresponding to position 173; G at a position corresponding to position 174; K at a position corresponding to position 174; N at a position corresponding to position 174; R at a position corresponding to position 174; T at a position corresponding to position 174; T at a position corresponding to position 175; K at a

position corresponding to position 178; R at a position corresponding to position 178; K at a position corresponding to position 179; Q at a position corresponding to position 193; T at a position corresponding to position 195; N at a position corresponding to position 195; with E at a position corresponding to position 196; R at a position corresponding to position 196; with D at a position corresponding to position 198; P at a position corresponding to position 204; A at a position corresponding to position 205; E at a position corresponding to position 205; L at a position corresponding to position 205; T at a position corresponding to position 205; I at a position corresponding to position 206; K at a position corresponding to position 206; L at a position corresponding to position 206; R at a position corresponding to position 206; R at a position corresponding to position 209; N at a position corresponding to position 212; S at a position corresponding to position 212; A at a position corresponding to position 213; M at a position corresponding to position 213; N at a position corresponding to position 213; H at a position corresponding to position 215; M at a position corresponding to position 215; A at a position corresponding to position 219; I at a position corresponding to position 219; K at a position corresponding to position 219; S at a position corresponding to position 219; H at a position corresponding to position 220; I at a position corresponding to position 220; L at a position corresponding to position 220; V at a position corresponding to position 220; Q at a position corresponding to position 221; G at a position corresponding to position 222; F at a position corresponding to position 232; g at a position corresponding to position 233; K at a position corresponding to position 233; R at a position corresponding to position 233; M at a position corresponding to position 234; A at a position corresponding to position 235; R at a position corresponding to position 236; C at a position corresponding to position 237; E at a position corresponding to position 237; H at a position corresponding to position 237; Q at a position corresponding to position 237; T at a position corresponding to position 237; E at a position corresponding to position 238; H at a position corresponding to amino acid position 238; S at a position corresponding to position 238; A at a position corresponding to position 240; Q at a position corresponding to position 240; I at a position corresponding to position 247; A at a position corresponding to position 248; V at a position corresponding to position 249; G at a position corresponding to position 257; T at a position corresponding to position 257; R at a position corresponding to position 257; N at a position corresponding to

position 258; S at a position corresponding to position 258; P at a position corresponding to position 259; M at a position corresponding to position 260; Y at a position corresponding to position 260; A at a position corresponding to position 261; K at a position corresponding to position 261; N at a position corresponding to position 261; K at a position corresponding to position 263; R at a position corresponding to position 263; T at a position corresponding to position 267; A at a position corresponding to position 269; L at a position corresponding to position 271; M at a position corresponding to position 271; D at a position corresponding to position 272; T at a position corresponding to position 272; H at a position corresponding to position 273; Y at a position corresponding to position 273; F at a position corresponding to position 274; D at a position corresponding to position 276; H at a position corresponding to position 276; M at a position corresponding to position 276; R at a position corresponding to position 276; S at a position corresponding to position 276; Y at a position corresponding to position 276; A at a position corresponding to position 277; E at a position corresponding to position 277; H at a position corresponding to position 277; K at a position corresponding to position 277; M at a position corresponding to position 277; N at a position corresponding to position 277; Q at a position corresponding to position 277; R at a position corresponding to position 277; S at a position corresponding to position 277; T at a position corresponding to position 277; E at a position corresponding to position 278; F at a position corresponding to position 278; G at a position corresponding to position 278; H at a position corresponding to position 278; K at a position corresponding to position 278; N at a position corresponding to position 278; R at a position corresponding to position 278; S at a position corresponding to position 278; T at a position corresponding to position 278; Y at a position corresponding to position 278; H at a position corresponding to position 279; M at a position corresponding to position 282; S at a position corresponding to position 283; H at a position corresponding to position 285; T at a position corresponding to position 287; S at a position corresponding to position 289; S at a position corresponding to position 291; V at a position corresponding to position 291; C at a position corresponding to position 292; F at a position corresponding to position 292; H at a position corresponding to position 292; K at a position corresponding to position 292; R at a position corresponding to position 292; V at a position corresponding to position 292; A at a position corresponding to position 293; C at a

position corresponding to position 293; D at a position corresponding to position 293; F at a position corresponding to position 293; K at a position corresponding to position 293; M at a position corresponding to position 293; P at a position corresponding to position 293; Q at a position corresponding to position 293; V at a position corresponding to position 293; Y at a position corresponding to position 293; G at a position corresponding to position 298; E at a position corresponding to position 305; G at a position corresponding to position 307; D at a position corresponding to position 308; G at a position corresponding to position 308; K at a position corresponding to position 308; N at a position corresponding to position 308; R at a position corresponding to position 308; E at a position corresponding to position 309; G at a position corresponding to position 309; H at a position corresponding to position 309; L at a position corresponding to position 309; M at a position corresponding to position 309; N at a position corresponding to position 309; Q, at a position corresponding to position 309; R at a position corresponding to position 309; S at a position corresponding to position 309; T at a position corresponding to position 309; V at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; Q at a position corresponding to position 310; S at a position corresponding to position 310; A at a position corresponding to position 313; G at a position corresponding to position 313; H at a position corresponding to position 313; K at a position corresponding to position 313; P at a position corresponding to position 313; R at a position corresponding to position 313; T at a position corresponding to position 313; Y at a position corresponding to position 313; with S at a position corresponding to position 314; Y at a position corresponding to position 314; A at a position corresponding to position 315; H at a position corresponding to position 315; Y at a position corresponding to position 315; A at a position corresponding to position 317; I at a position corresponding to position 317; K at a position corresponding to position 317; N at a position corresponding to position 317; Q at a position corresponding to position 317; R at a position corresponding to position 317; S at a position corresponding to position 317; T at a position corresponding to position 317; W at a position corresponding to position 317; D at a position corresponding to position 318; H at a position corresponding to position 318; K at a position corresponding to position 318; M at a position corresponding to position 318; R at a position corresponding to position 318; H at a position corresponding to

position 320; K at a position corresponding to position 320; R at a position corresponding to position 320; R at a position corresponding to position 321; S at a position corresponding to position 321; N at a position corresponding to position 324; R at a position corresponding to position 324; A at a position corresponding to position 325; D at a position corresponding to position 325; E at a position corresponding to position 325; G at a position corresponding to position 325; H at a position corresponding to position 325; K at a position corresponding to position 325; M at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; S at a position corresponding to position 325; V at a position corresponding to position 325; L at a position corresponding to position 326; V at a position corresponding to position 326; C at a position corresponding to position 328; G at a position corresponding to position 328; I at a position corresponding to position 328; K at a position corresponding to position 328; L at a position corresponding to position 328; S at a position corresponding to position 328; Y at a position corresponding to position 328; S at a position corresponding to position 335; A at a position corresponding to position 347; G at a position corresponding to position 347; S at a position corresponding to position 347; M at a position corresponding to position 349; R at a position corresponding to position 349; S at a position corresponding to position 351; V at a position corresponding to position 353; with H at a position corresponding to position 356; S at a position corresponding to position 356; E at a position corresponding to position 359; H at a position corresponding to position 359; T at a position corresponding to position 359; A at a position corresponding to position 367; G at a position corresponding to position 367; K at a position corresponding to position 367; S at a position corresponding to position 367; A at a position corresponding to position 368; E at a position corresponding to position 368; K at a position corresponding to position 368; L at a position corresponding to amino acid position 368; M at a position corresponding to amino acid position 368; R at a position corresponding to position 368; T at a position corresponding to amino acid position 368; H at a position corresponding to position 369; R at a position corresponding to position 369; F at a position corresponding to position 371; H at a position corresponding to position 371; K at a position corresponding to position 371; L at a position corresponding to position 371; R at a position corresponding to position 371; S at a position corresponding to position 371; M at a position

corresponding to position 373; H at a position corresponding to position 374; P at a position corresponding to position 374; A at a position corresponding to position 375; G at a position corresponding to position 375; K at a position corresponding to position 375; R at a position corresponding to position 375; D at a position corresponding to position 376; E at a position corresponding to position 376; Q at a position corresponding to position 376; R at a position corresponding to position 376; T at a position corresponding to position 376; V at a position corresponding to position 376; Y at a position corresponding to position 376; D at a position corresponding to position 377; E at a position corresponding to position 377; H at a position corresponding to position 377; K at a position corresponding to position 377; P at a position corresponding to position 377; R at a position corresponding to position 377; S at a position corresponding to position 377; T at a position corresponding to position 377; W at a position corresponding to position 380; Y at a position corresponding to position 380; S at a position corresponding to position 381; I at a position corresponding to position 383; K at a position corresponding to position 383; L at a position corresponding to position 383; S at a position corresponding to position 383; A at a position corresponding to position 385; Q at a position corresponding to position 385; V at a position corresponding to position 385; A at a position corresponding to position 389; G at a position corresponding to position 389; L at a position corresponding to position 389; K at a position corresponding to position 389; Q at a position corresponding to position 389; S at a position corresponding to position 389; A at a position corresponding to position 392; F at a position corresponding to position 392; M at a position corresponding to position 392; Q at a position corresponding to position 392; R at a position corresponding to position 392; V at a position corresponding to position 392; F at a position corresponding to position 393; M at a position corresponding to position 393; A at a position corresponding to position 395; H at a position corresponding to position 395; R at a position corresponding to position 395; A at a position corresponding to position 396; H at a position corresponding to position 396; Q at a position corresponding to position 396; S at a position corresponding to position 396; K at a position corresponding to position 399; M at a position corresponding to position 399; T at a position corresponding to position 399; V at a position corresponding to position 399; W at a position corresponding to position 399; A at a position corresponding to position 401; E at a position corresponding to position 401; A at a

position corresponding to position 404; G at a position corresponding to position 405; F at a position corresponding to position 406; N at a position corresponding to position 406; A at a position corresponding to position 407; D at a position corresponding to position 407; E at a position corresponding to position 407; F at a position corresponding to position 407; H at a position corresponding to position 407; Q at a position corresponding to position 407; P at a position corresponding to position 407; A at a position corresponding to position 409; Q at a position corresponding to position 409; T at a position corresponding to position 410; Q at a position corresponding to position 412; R at a position corresponding to position 412; V at a position corresponding to position 412; L at a position corresponding to position 416; E at a position corresponding to position 418; L at a position corresponding to position 418; P at a position corresponding to position 418; R at a position corresponding to position 418; V at a position corresponding to position 418; F at a position corresponding to position 419; H at a position corresponding to position 419; I at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; Y at a position corresponding to position 419; A at a position corresponding to position 421; H at a position corresponding to position 421; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; G at a position corresponding to position 425; K at a position corresponding to position 425; Q at a position corresponding to position 427; T at a position corresponding to position 427; L at a position corresponding to position 428; A at a position corresponding to position 431; G at a position corresponding to position 431; E at a position corresponding to position 431; H at a position corresponding to position 431; K at a position corresponding to position 431; L at a position corresponding to position 431; N at a position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to position 431; A at a position corresponding to position 433; H at a position corresponding to position 433; I at a position corresponding to position 433; K at a position corresponding to position 433; L at a position corresponding to position 433; R at a position corresponding to position 433; T at a position corresponding to

position 433; V at a position corresponding to position 433; W at a position corresponding to position 433; K at a position corresponding to position 436; I at a position corresponding to position 437; M at a position corresponding to position 437; A at a position corresponding to position 438; D at a position corresponding to position 438; E at a position corresponding to position 438; L at a position corresponding to position 438; N at a position corresponding to position 438; T at a position corresponding to position 438; A at a position corresponding to position 439; C at a position corresponding to position 439; K at a position corresponding to position 439; P at a position corresponding to position 439; Q at a position corresponding to position 439; T at a position corresponding to position 439; V at a position corresponding to position 439; D at a position corresponding to position 440; H at a position corresponding to position 440; M at a position corresponding to position 440; P at a position corresponding to position 440; R at a position corresponding to position 440; S at a position corresponding to position 440; A at a position corresponding to position 441; F at a position corresponding to position 441; C at a position corresponding to position 442; G at a position corresponding to position 442; R at a position corresponding to position 442; A at a position corresponding to position 443; E at a position corresponding to position 443; F at a position corresponding to position 443; G at a position corresponding to position 443; M at a position corresponding to position 443; N at a position corresponding to position 443; E at a position corresponding to position 444; H at a position corresponding to position 444; V at a position corresponding to position 444; H at a position corresponding to position 445; M at a position corresponding to position 445; N at a position corresponding to position 445; P at a position corresponding to position 445; Q at a position corresponding to position 445; S at a position corresponding to position 445; T at a position corresponding to position 445; V at a position corresponding to position 445; W at a position corresponding to position 445; A at a position corresponding to position 446; A at a position corresponding to position 446; M at a position corresponding to position 446; W at a position corresponding to position 446; D at a position corresponding to position 447; E at a position corresponding to position 447; G at a position corresponding to position 447; I at a position corresponding to position 447; N at a position corresponding to position 447; P at a position corresponding to position 447; Q at a position corresponding to position 447; T at a position corresponding to position 447, and/or replacement with

V at a position corresponding to position 447, each with reference to amino acid positions set forth in SEQ ID NO:3.

Any of the above modified PH20 polypeptides provided herein can exhibit altered, such as improved or increased, properties compared to the corresponding PH20 polypeptide not containing the amino acid modification (*e.g.* amino acid replacement). For example, the altered activities or properties can be an increased catalytic activity and/or an increased stability under denaturing conditions.

a. Increased Activity

Provided herein are modified or variant PH20 polypeptides that contain one or more amino acid replacement in a PH20 polypeptide and that exhibit increased hyaluronidase activity compared to the corresponding PH20 polypeptide not containing the amino acid replacement(s), for example, the PH20 polypeptide set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In particular, the modified or variant PH20 polypeptides provided herein exhibit increased hyaluronidase activity compared to the corresponding PH20 polypeptide not containing the amino acid replacement, for example, the PH20 polypeptide set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72 and in particular the PH20 polypeptide set forth in SEQ ID NO:3.

The modified PH20 polypeptide can exhibit hyaluronidase activity that is at least or about at least or 120%, 130%, 135%, 140%, 145%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 350%, 400%, 500%, 1500%, 2000%, 3000%, 4000%, 5000% of the hyaluronidase activity of the corresponding PH20 polypeptide not containing the amino acid replacement(s), for example the PH20 polypeptide set forth in any of any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof, under the same conditions. For example, the hyaluronidase activity is increased at least or about at least 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, 300-fold, 400-fold or more.

In particular examples, the modified PH20 polypeptides contain an amino acid replacement at one or more amino acid positions identified as being associated with increased hyaluronidase activity. As described herein, such positions have been

identified using mutagenesis and selection or screening methods to identify those positions that result in increased hyaluronidase activity. The PH20 polypeptide also can contain other modifications, such as other amino acid replacements, that alone are not associated with increased activity so long as the resulting modified PH20 polypeptide exhibits increased hyaluronidase polypeptide compared to the PH20 not containing the amino acid modification(s), such as amino acid replacement(s). The modified PH20 polypeptide provided herein can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,49, 50, 51, 53, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 59,70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 82, 82, 83, 84, 85, 86, 87, 88, 89, 90, or more amino acid replacements. Additional modifications, such as insertions or deletions, also can be included. The amino acid replacement can be in a PH20 polypeptide as set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. For example, the replacements can be in a human PH20 polypeptide, for example, any set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72 or a variant thereof.

For example, the modified PH20 polypeptides provided herein contain an amino acid replacement (substitution) at one or more amino acid positions corresponding to positions 1, 12, 15, 24, 26, 27, 29, 30, 31, 32, 33, 37, 39, 46, 48, 52, 58, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 84, 86, 87, 92, 93, 94, 97, 118, 120, 127, 131, 135, 141, 142, 147, 148, 150, 151, 152, 155, 156, 163, 164, 165, 166, 169, 170, 174, 198, 206, 209, 212, 213, 215, 219, 233, 234, 236, 238, 247, 257, 259, 260, 261, 263, 269, 271, 272, 276, 277, 278, 282, 291, 293, 305, 308, 309, 310, 313, 315, 317, 318, 320, 324, 325, 326, 328, 347, 353, 359, 371, 377, 380, 389, 392, 395, 399, 405, 407, 409, 410, 418, 419, 421, 425, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 445, 446 or 447 with reference to amino acid positions set forth in SEQ ID NO:3. For example, the amino acid positions can be replacements at positions corresponding to replacement of Leucine (L) at position 1 (L1), V12, L15, F24, L26, G27, F29, D30, E31, P32, L33, L37, S39, I46, A48, G52, V58, Y63, I67, D68, S69, I70, T71, G72, V73, T74, V75, S84, G86, D87, A92, K93, K94, T97, T118, A120, D127, N131, E135, N141, V142, T147, E148, T150, E151, K152, Q155, E156, D163, F164, L165, V166, I169, K170, L174, G198, V206, K209, D212, D213, S215, N219, Q233, Q234,

P236, A238, V247, P257, A259, K260, S261, L263, T269, I271, V272, Q276, V277, L278, S282, G291, T293, G305, S308, I309, M310, M313, S315, L317, L318, D320, E324, T325, I326, N328, Q347, I353, S359, A371, G377, F380, E389, E392, S395, Y399, T405, S407, K409, E410, D418, A419, D421, A425, D431, F433, P436, P437, M438, E439, T440, E441, E442, P443, I445, F446 or Y447 with reference to amino acid positions set forth in SEQ ID NO:3. Exemplary of such modified PH20 polypeptides are polypeptides that exhibit at least 1.5-fold or more the activity of the corresponding PH20 polypeptide not containing the amino acid replacement.

Exemplary of amino acid replacements in the modified PH20 polypeptides provided herein include, but are not limited, replacement: with histidine (H) at a position corresponding to position 1; Q at a position corresponding to position 1; E at a position corresponding to position 12; T at a position corresponding to position 12; V at a position corresponding to position 15; E at a position corresponding to position 24; H at a position corresponding to position 24; E at a position corresponding to position 26; K at a position corresponding to position 26; K at a position corresponding to position 27; R at a position corresponding to position 27; E at a position corresponding to position 29; I at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; S at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; F at a position corresponding to position 32; G at a position corresponding to position 32; H at a position corresponding to position 32; W at a position corresponding to position 33; F at a position corresponding to position 37; N at a position corresponding to position 39; T at a position corresponding to position 39; R at a position corresponding to position 46; F at a position corresponding to position

48; H at a position corresponding to position 48; N at a position corresponding to position 48; Q at a position corresponding to position 52; K at a position corresponding to position 58; Q at a position corresponding to position 58; W at a position corresponding to position 63; V at a position corresponding to position 67; H at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position 69; C at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; I at a position corresponding to position 69; L at a position corresponding to position 69; M at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; W at a position corresponding to position 69; Y at a position corresponding to position 69; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; N at a position corresponding to position 70; P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; R at a position corresponding to position 71; S at a position corresponding to position 71; M at a position corresponding to position 72; Q at a position corresponding to position 72; H at a position corresponding to position 73; L at a position corresponding to position 73; W at a position corresponding to position 73; A at a position corresponding to position 74; C at a position corresponding to position 74; G at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; F at a position corresponding to position 75; L at a position corresponding to position 75; R at a position corresponding to position 75; T at a position corresponding to position 75; G at a position corresponding to position 84; R at a position corresponding to position 84; A at a position corresponding to position 86; C at a position corresponding to position 87; T at a position corresponding to position 87; Y at a position corresponding to position 87; C at a position corresponding to position 92; I at a position corresponding to position 93; L

at a position corresponding to position 93; R at a position corresponding to position 93; T at a position corresponding to position 93; R at a position corresponding to position 94; G at a position corresponding to position 97; Q at a position corresponding to position 118; F at a position corresponding to position 120; V at a position corresponding to position 120; Y at a position corresponding to position 120; H at a position corresponding to position 127; N at a position corresponding to position 127; G at a position corresponding to position 131; R at a position corresponding to position 131; V at a position corresponding to position 131; D at a position corresponding to position 135; G at a position corresponding to position 135; R at a position corresponding to position 135, with H at a position corresponding to position 141; Y at a position corresponding to position 141; R at a position corresponding to position 142; R at a position corresponding to position 147; V at a position corresponding to position 147; K at a position corresponding to position 148; G at a position corresponding to position 150; K at a position corresponding to position 151; L at a position corresponding to position 151; M at a position corresponding to position 151; Q at a position corresponding to position 151; R at a position corresponding to position 151; R at a position corresponding to position 152; G at a position corresponding to position 155; K at a position corresponding to position 155; D at a position corresponding to position 156; A at a position corresponding to position 163; E at a position corresponding to position 163; K at a position corresponding to position 163; R at a position corresponding to position 163; M at a position corresponding to position 164; D at a position corresponding to position 165; N at a position corresponding to position 165; A at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; Q at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; Y at a position corresponding to position 166; L at a position corresponding to position 169; R at a position corresponding to position 170; K at a position corresponding to position 174; D at a position corresponding to position 198; K at a position corresponding to position 206; L at a position corresponding to position 206; N at a position corresponding to position 212; M at a position corresponding to position 213; N at a position corresponding to position 213; M at a position corresponding to position 215; S at a position corresponding to position 219; K at a position corresponding to

position 233; R at a position corresponding to position 233; M at a position corresponding to position 234; R at a position corresponding to position 236; E at a position corresponding to position 237; S at a position corresponding to position 238; I at a position corresponding to position 247; T at a position corresponding to position 257; P at a position corresponding to position 259; Y at a position corresponding to position 260; K at a position corresponding to position 261; N at a position corresponding to position 261; K at a position corresponding to position 263; R at a position corresponding to position 263; A at a position corresponding to position 269; L at a position corresponding to position 271; M at a position corresponding to position 271; T at a position corresponding to position 272; D at a position corresponding to position 276; S at a position corresponding to position 276; Y at a position corresponding to position 276; K at a position corresponding to position 277; R at a position corresponding to position 277; T at a position corresponding to position 277; H at a position corresponding to position 278; K at a position corresponding to position 278; N at a position corresponding to position 278; R at a position corresponding to position 278; S at a position corresponding to position 278; T at a position corresponding to position 278; Y at a position corresponding to position 278; M at a position corresponding to position 282; V at a position corresponding to position 291; A at a position corresponding to position 293; C at a position corresponding to position 293; F at a position corresponding to position 293; M at a position corresponding to position 293; P at a position corresponding to position 293; Q at a position corresponding to position 293; V at a position corresponding to position 293; E at a position corresponding to position 305; G at a position corresponding to position 308; N at a position corresponding to position 308; E at a position corresponding to position 309; L at a position corresponding to position 309; N at a position corresponding to position 309; Q at a position corresponding to position 309; R at a position corresponding to position 309; T at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; K at a position corresponding to position 313; R at a position corresponding to position 313; H at a position corresponding to position 315; I at a position corresponding to position 317; K at a position corresponding to position 317; R at a position corresponding to position 317; M at a position corresponding to position 318; H at a position corresponding to position 320; K at a position corresponding to position 320; R at a position

corresponding to position 320; R at a position corresponding to position 324; A at a position corresponding to position 325; D at a position corresponding to position 325; E at a position corresponding to position 325; G at a position corresponding to position 325; H at a position corresponding to position 325; K at a position corresponding to position 325; M at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; S at a position corresponding to position 325; V at a position corresponding to position 326; I at a position corresponding to position 328; K at a position corresponding to position 328; L at a position corresponding to position 328; S at a position corresponding to position 328; Y at a position corresponding to position 328; G at a position corresponding to position 347; S at a position corresponding to position 347; V at a position corresponding to position 353; with T at a position corresponding to position 359; R at a position corresponding to position 371; P at a position corresponding to position 377; T at a position corresponding to position 377; W at a position corresponding to position 380; Y at a position corresponding to position 380; K at a position corresponding to position 389; M at a position corresponding to position 392; R at a position corresponding to position 395; M at a position corresponding to position 399; T at a position corresponding to position 399; W at a position corresponding to position 399; G at a position corresponding to position 405; D at a position corresponding to position 407; Q at a position corresponding to position 407; A at a position corresponding to position 409; Q at a position corresponding to position 409; T at a position corresponding to position 410; P at a position corresponding to position 418; F at a position corresponding to position 419; I at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; H at a position corresponding to position 421; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; K at a position corresponding to position 425; A at a position corresponding to position 431; H at a position corresponding to position 431; K at a position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to position 431; L at a position corresponding to position 433; R at a

position corresponding to position 433; T at a position corresponding to position 433; V at a position corresponding to position 433; K at a position corresponding to position 436; I at a position corresponding to position 437; M at a position corresponding to position 437; T at a position corresponding to position 438; V at a position corresponding to position 439; H at a position corresponding to position 440; R at a position corresponding to position 440; F at a position corresponding to position 441; R at a position corresponding to position 442; A at a position corresponding to position 443; M at a position corresponding to position 443; M at a position corresponding to position 445; P at a position corresponding to position 445; A at a position corresponding to position 446; D at a position corresponding to position 447; N at a position corresponding to position 447; and/or with Q at a position corresponding to position 447;, each with reference to amino acid positions set forth in SEQ ID NO:3. The modified PH20 polypeptides can contain any one or more of the recited amino acid substitutions, in any combination, with or without additional modifications, so long at the PH20 polypeptide exhibits hyaluronidase activity compared PH20 polypeptide not containing the modification(s), such as increased hyaluronidase activity, for example, at least 1.5-fold increased hyaluronidase activity.

In some examples, provided herein are modified PH20 polypeptides containing one or more amino acid replacement(s) at a position corresponding to position 24, 29, 31, 48, 58, 69, 70, 75, 84, 97, 165, 166, 271, 278, 317, 320, 325, or 326 with reference to positions set forth in SEQ ID NO:3. For example, exemplary amino acid replacements include, but are not limited to, replacement with: E at a position corresponding to position 24; E at a position corresponding to position 29; V at a position corresponding to position 31; N at a position corresponding to position 48; K at a position corresponding to position 58; Q at a position corresponding to position 58; A at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; A at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; H at a position corresponding to position 70; N at a position corresponding to position 70; R at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; L

at a position corresponding to position 75; T at a position corresponding to position 75; G at a position corresponding to position 84; G at a position corresponding to position 97; D at a position corresponding to position 165; L at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; L at a position corresponding to position 271; H at a position corresponding to position 278; R at a position corresponding to position 278; K at a position corresponding to position 317; K at a position corresponding to position 320; E at a position corresponding to position 325, with G at a position corresponding to position 325; K at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; V at a position corresponding to position 326; each with reference to amino acid positions set forth in SEQ ID NO:3. The modified PH20 polypeptides can contain any one or more of the recited amino acid substitutions, in any combination, with or without additional modifications, so long at the PH20 polypeptide exhibits hyaluronidase activity compared PH20 polypeptide not containing the modification(s), such as increased hyaluronidase activity, for example, at least 2.0-fold increased hyaluronidase activity.

b. Increased Stability

Provided herein are PH20 polypeptides that exhibit increased stability. In particular, the PH20 polypeptides exhibit increased stability in vivo and/or in vitro. For example, the PH20 polypeptides can exhibit increased stability under various storage conditions. The modified PH20 polypeptides provided herein that exhibit increased stability display, among other parameters, increased resistance to denaturation conditions, including but not limited to, denaturation conditions caused by temperature (*e.g.* elevated temperature such as heat), agitation, no or low salt, and/or presence of excipients. Exemplary of excipients include, but are not limited to, antiadherents, binders, coatings, fillers and diluents, flavors, colors, lubricants, glidants, preservatives, sorbents or sweetners. For example, various excipients, such as preservatives, can act as protein denaturing agents. Modified PH20 polypeptides provided herein that exhibit increased protein stability exhibit reduced aggregation, reduced precipitation and/or increased activity when exposed to a denaturation condition compared to the corresponding PH20 not containing the amino acid replacement. For example, modified PH20 polypeptides provided herein exhibit at least or at least about or 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%,

110%, 120%, 130%, 140%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500% or more increased activity when exposed to a denaturation condition compared to the corresponding PH20 polypeptide not containing the amino acid replacement when exposed to the same denaturation condition.

The PH20 polypeptides provided herein that exhibit increased stability are modified or variant PH20 polypeptides that contain an amino acid replacement (substitution), deletion or insertion or other modification. Typically, the PH20 polypeptides provided herein that exhibit increased stability contain one or more amino acid replacement in a PH20 polypeptide compared to the corresponding PH20 polypeptide not containing the amino acid replacement(s), for example, the PH20 polypeptide set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. In particular, the modified or variant PH20 polypeptides provided herein exhibit increased stability compared to the corresponding PH20 polypeptide not containing the amino acid replacement, for example, the PH20 polypeptide set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72 and in particular the PH20 polypeptide set forth in SEQ ID NO:3.

In particular examples, the modified PH20 polypeptides contain an amino acid replacement at one or more amino acid positions identified as being associated with increased stability. As described herein, such positions can be identified using mutagenesis and selection or screening methods to identify those positions that result in stability (*e.g.* increased activity) of the polypeptide compared to the corresponding PH20 not containing the modification upon exposure to one or more denaturation conditions. The PH20 polypeptide also can contain other modifications, such as other amino acid replacements, that alone are not associated with conferring stability, so long as the resulting modified PH20 polypeptide exhibits increased stability under one or more denaturation conditions compared to the PH20 not containing the amino acid modification(s), such as amino acid replacement(s), and exhibits hyaluronidase activity. The modified PH20 polypeptide provided herein can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,49, 50, 51, 53, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 59,70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 82, 82, 83, 84, 85, 86, 87, 88, 89, 90, or more amino acid replacements.

Additional modifications, such as insertions or deletions, also can be included. The amino acid replacement can be in a PH20 polypeptide as set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%< 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. For example, the replacements can be in a human PH20 polypeptide, for example, any set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72 or a variant thereof.

Exemplary of modified PH20 polypeptides provided herein are PH20 polypeptides that exhibit increased stability upon exposure to phenol compounds, high temperature (heat), and/or lack of NaCl.

i. Phenophiles

Provided herein are modified PH20 polypeptides that exhibit increased stability in the presence of phenolic compounds. Multidose formulations must contain antimicrobial preservatives to protect them from microbial contamination. For parenteral drug products, including insulin and other therapeutic agents, the most common preservatives are phenolic compounds, such as phenol, metacresol (m-cresol), benzyl alcohol, and parabens including methylparaben and propylparaben. The preservatives typically must be present at sufficient concentrations to satisfy regulatory rules. For example, regulatory requirements assert that the antimicrobial efficacy of the formulation must satisfy the preservative efficacy test (PET) requirements of the target markets. Currently different regulatory agencies have different pharmacopeial criteria for antimicrobial effectiveness for pharmaceutical products designed for multiple dosing. The PET requirements of the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP) differ considerably, imposing additional constraints in developing multidose formulations. Table 4 shows the criteria for injectable drugs to meet USP and EP criteria. Typically, formulations that meet EP (EPA or EPB) anti-microbial requirements contain more preservative than those formulated only to meet USP anti-microbial requirements.

Table 4. USP and EP requirement for antimicrobial effectiveness testing

Requirement		United States	Europe	
	Timepoint	USP	EPB (Minimum)	EPA (Preferred)

Bacterial Log Reduction*	6 h			2
	24 h		1	3
	7 d	1.0	3	No recovery
	14 d	3.0	No increase	No recovery
	28 d	No increase	No increase	No recovery
Fungal Log Reduction*	7 d	No increase		2
	14 d	No increase	1	No increase
	28 d	No increase	No increase	No increase

* Log_{10} unit reduction from initial measured inoculum; No increase: not more than 0.5 log_{10} unit increase than previously measured value.

Anti-microbial preservatives are known to interact with proteins resulting in aggregations and negative effects on stability. Thus, although a necessary component, preservatives pose a significant problem in the development of stable, multidose formulations of proteins because they typically induce aggregation of the protein in aqueous solution. In particular, increasing or high amounts of preservatives can negatively impact the stability of a protein, including effects on physical stability (aggregation or precipitation) that can impact protein activity. For example, to meet the EP preservative efficacy requirements, relatively high amounts of phenolic compounds, such as phenol or m-cresol, can be required, which can influence stability of the protein formulation. For example, preservatives such as phenol, m-cresol, and benzyl alcohol have been shown to induce aggregation of human growth hormone (Maa and Hsu (1996) *Int. J. Pharm.* 140:155–168), recombinant interleukin-1 receptor (Remmele (1998) *Pharm. Res.*15:200–208), human insulin-like growth factor I (Fransson (1997) *Pharm. Res.* 14:606–612), rhIFN-γ (Lam (1997) *Pharm. Res.* 14:725–729) and cytochrome c (Singh *et al.* (2011) *J. Pharm Sci.*, 100:1679-89). The destabilizing effect that preservatives have on proteins in solution has been a limiting factor in the development of multidose formulations, and to date, most protein therapeutics have been formulated for single use only.

Like most other protein therapeutics, PH20 hyaluronidase, such as rHuPH20, rapidly loses activity in the presence of preservatives, likely due to unfolding of the protein and subsequent aggregate formation. For example, as shown in the Examples herein, preservatives reduce PH20 enzymatic activity, particularly at elevated temperatures (see also U.S. Provisional Appl. No.61/520,962). For example,

following incubation with 0.4% m-cresol for 4 hours, PH20 (*e.g.* rHuPH20) retains only about 10% of its activity (*see e.g.* Example 5). When incubated in the presence of 0.1% phenol and 0.15% or 0.315% m-cresol for 6 days at 37 °C, PH20 (*e.g.* rHuPH20) retains about 0% to 15% activity, depending on the presence of other excipients or amounts of excipients in the formulation (*see e.g.* Examples 9 and 10). For example, the presence of a higher concentration of salt generally increases the stability of PH20. In particular, the melting temperature of PH20, such as rHuPH20, is reduced significantly when phenolic preservatives, such as m-Cresol, are added to the formulation. For example, the unfolding temperature of rHuPH20 is reduced from 44°C to 24 °C. The lower PH20 unfolding temperatures leads to increased PH20 aggregation, especially at elevated temperatures, and reduced enzyme activity. The destabilizing effect is likely due to the hydrophobic nature of the phenolic preservatives. The hydrophobicity of the phenolic compounds can lead to interaction with rHuPH20 through nonspecific binding to the protein, ultimately perturbing the structural integrity of rHuPH20. This translates to a significant loss of rHuPH20 enzymatic activity in the presence of preservatives.

The modified PH20 polypeptides provided herein that exhibit increased stability to phenolic preservatives exhibit more than 15% enzymatic activity in the presence of at least one phenolic preservative for at least 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks or more compared to the enzymatic activity of the modified PH20 polypeptide in the absence of preservative for the same time period and under the same conditions (except for the presence of preservative). In some examples, the modified PH20 polypeptides provided herein exhibit at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more enzymatic activity in the presence of a phenolic preservative compared to absence of preservative. For example, the phenolic preservative compound can be phenol, metacresol (m-cresol), benzyl alcohol, and/or parabens including methylparaben or propylparaben.

In particular examples, the increased stability to preservative is exhibited under temperature conditions of between or about between 0°C to 40°C, such as between or about between 2°C to 6°C, 24°C to 32°C or 35°C to 40°C, and generally at or about at 4°C or 5°C, 30°C or 37°C. It is understood that since high temperature

also can have a destabilizing effect on PH20 activity (see below), the percentage enzymatic activity of a modified PH20 polypeptide provided herein in the presence of preservative is greater at lower temperatures than at higher temperatures.

Generally, the modified PH20 polypeptides provided herein exhibit the increased stability, and the noted enzymatic activities, in the presence of an anti-microbial effective amount of preservative that kills or inhibits the propagation of microbial organisms in a sample of the composition. For example, the modified PH20 polypeptides provided herein exhibit the increased stability in the presence of an anti-microbial effective amount of preservative that kills or inhibits the propagation of microbial organisms such that at least a 1.0 log10 unit reduction in bacterial organisms occurs at 7 days following inoculation. In some examples, the modified PH20 polypeptides provided herein exhibit the increased stability in the presence of an anti-microbial effective amount of preservative that kills or inhibits the propagation of microbial organisms such that, when tested in an antimicrobial preservative effectiveness test (APET), following inoculation of the composition with a microbial inoculum there is at least a 1.0 log10 unit reduction in bacterial organisms at 7 days following inoculation, at least a 3.0 log10 unit reduction of bacterial organisms at 14 days following inoculation, at least no further increase in bacterial organisms after 28 days following inoculation, and at least no increase in fungal organisms after 7 days following inoculation. In other examples, the modified PH20 polypeptides provided herein exhibit the increased stability in the presence of an anti-microbial effective amount of preservative that kills or inhibits the propagation of microbial organisms such that, when tested in an antimicrobial preservative effectiveness test (APET), following inoculation of the composition with a microbial inoculum there is at least a 1.0 log10 unit reduction of bacterial organisms at 24 hours following inoculation, at least a 3.0 log10 unit reduction of bacterial organisms at 7 days following inoculation, no further increase in bacterial organisms after 28 days following inoculation, at least a 1.0 log10 unit reduction of fungal organisms at 14 days following inoculation, and at least no further increase in fungal organisms after 28 days following inoculation. In yet another example, the modified PH20 polypeptides provided herein exhibit the increased stability in the presence of an anti-microbial effective amount of the preservative that kills or inhibits the propagation of microbial organisms such that, when tested in an antimicrobial preservative effectiveness test (APET), following inoculation of the composition with

a microbial inoculum there is at least a 2.0 log10 unit reduction of bacterial organisms at 6 hours following inoculation, at least a 3.0 log10 unit reduction of bacterial organisms at 24 hours following inoculation, no recovery of bacterial organisms after 28 days following inoculation of the composition with the microbial inoculum, at least a 2.0 log10 unit reduction of fungal organisms at 7 days following inoculation, and at least no further increase in fungal organisms after 28 days following inoculation.

For example, the modified PH20 polypeptides provided herein exhibit the increased stability, and above recited enzymatic activity, in the presence of a total amount of one or more phenolic preservative agents as a percentage (%) of mass concentration (w/v) that is or is between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive.

Generally, modified PH20 polypeptides provided herein exhibit increased stability in the presence of m-cresol and/or phenol. For example, modified PH20 polypeptides provided herein exhibit increased stability in the presence of m-cresol in an amount as a % of mass concentration (w/v) in a formulation containing the modified PH20 polypeptide of between or about between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 %. In other examples, modified PH20 polypeptides provided herein exhibit increased stability in the presence of phenol in an amount at a % of mass concentration (w/v) in a formulation containing the modified PH20 polypeptide of between or about between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % m-cresol. In further examples, modified PH20 polypeptides provided herein exhibit increased stability in the presence of phenol and m-cresol in an amount as a % of mass concentration (w/v) in a formulation containing the modified PH20 polypeptide of between or about between 0.05 % to 0.25 % phenol and between or about between 0.05 % to 0.3 % m-cresol, between or about between 0.10 % to 0.2 % phenol and between or about between 0.6 % to 0.18 % m-cresol, between or about between 0.1 % to 0.15 % phenol and 0.8 % t 0.15 % m-cresol, between or about between 0.10 % to 0.15 % phenol and between or about between 0.06 to 0.09 % m-cresol or between or about between 0.12 % to 0.18 % phenol and between or about between 0.14 to 0.22 % m-cresol.

In examples herein, modified PH20 polypeptides exhibit more than 15%, such as at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more enzymatic activity in the presence of at least about between or between 0.3 % to 0.4 %, inclusive, m-cresol and/or phenol for at least 4 hours at 37°C compared to the enzymatic activity of the modified PH20 polypeptide in the absence of the preservative for the same time period and under the same conditions (except for the presence of preservative). For example, modified PH20 polypeptides exhibit more than 15%, such as at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more enzymatic activity in the presence of about or 0.4 % m-cresol for at least 4 hours at 37°C compared to the enzymatic activity of the modified PH20 polypeptide in the absence of the preservative for the same time period and under the same conditions (except for the presence of preservative). Modified PH20 polypeptides provided herein also exhibit more than 15%, such as at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more enzymatic activity in the presence of at least about between or between 0.2 % to 0.4 %, inclusive, m-cresol and/or phenol for at least 1 day, 2 days, 3 days, 4 days, 5 days or 6 days at 37°C compared to the enzymatic activity of the modified PH20 polypeptide in the absence of preservative for the same time period and under the same conditions (except for the presence of preservative). For example, modified PH20 polypeptides provided herein exhibit more than 15%, such as at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more enzymatic activity in the presence of about or 0.10% phenol and about or 0.15% m-cresol for at least 1 day, 2 days, 3 days, 4 days, 5 days or 6 days at 37°C compared to the enzymatic activity of the modified PH20 polypeptide in the absence of preservative for the same time period and under the same conditions (except for the presence of preservative). In other examples, modified PH20 polypeptides provided herein exhibit more than 15%, such as at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more enzymatic activity in the presence of about or 0.315% m-cresol for at least 1 day, 2 days, 3 days, 4 days, 5 days or 6 days, generally for at least 6 days, at 37°C compared to the enzymatic activity of the modified PH20 polypeptide in the absence

of preservative for the same time period and under the same conditions (except for the presence of preservative).

For example, such modified PH20 polypeptides provided herein that exhibit increased stability to phenol compounds contain an amino acid replacement (substitution) at one or more amino acid positions corresponding to positions 10, 12, 20, 22, 26, 34, 36, 46, 50, 52, 58, 68, 70, 74, 82, 83, 84, 86, 97, 127, 131, 138, 142, 143, 144, 166, 169, 174, 193, 195, 196, 204, 205, 206, 213, 219, 234, 237, 238, 240, 249, 261, 267, 277, 279, 291, 309, 310, 314, 315, 317, 318, 347, 367, 375, 376, 399, 401, 407, 416, 419, 421, 431, 433, 439, 440, 443 or 445 with reference to amino acid positions set forth in SEQ ID NO:3. For example, the amino acid positions can be replacements at one or more positions corresponding to replacement of (P) at position 10 (P10), V12, A20, S22, L26, D34, S36, I46, G50, G52, V58, D68, I70, t74, K82, I83, S84, Q86, T97, D127, N131, Q138, V142, Q143, L144, V166, I169, L174, H193, K195, K196, F204, N205, V206, D213, N219, Q234, V237, A238, T240, E249, S261, A267, V277K279, G291, I309, M310, K314, S315, L317, Q347, P367, E375, K376, Y399, S401, S407, D416, A419, D421, D431, F433, E439, T440, P443 or I445 with reference to amino acid positions set forth in SEQ ID NO:3.

Exemplary of amino acid replacements in the modified PH20 polypeptides provided herein include, but are not limited, replacement with: G at a position corresponding to position 10; K at a position corresponding to position 12; S at a position corresponding to position 20; T at a position corresponding to position 22; M at a position corresponding to position 26; W at a position corresponding to position 34; N at a position corresponding to position 36; L at a position corresponding to position 46; M at a position corresponding to position 50; T at a position corresponding to position 52; S at a position corresponding to position 52; C at a position corresponding to position 58; K at a position corresponding to position 58; R at a position corresponding to position 58; N at a position corresponding to position 58; Y at a position corresponding to position 58; P at a position corresponding to position 58; H at a position corresponding to position 58; P at a position corresponding to position 68; V at a position corresponding to position 70; E at a position corresponding to position 74; L at a position corresponding to position 82; N at a position corresponding to position 82; V at a position corresponding to position 83; Q at a position corresponding to position 83; S at a position corresponding to position 83; G at a position corresponding to position 83; N at a position

corresponding to position 84; A at a position corresponding to position 86; K at a position corresponding to position 86; E at a position corresponding to position 97; L at a position corresponding to position 97; R at a position corresponding to position 127; R at a position corresponding to position 131; L at a position corresponding to position 138; K at a position corresponding to position 142; N at a position corresponding to position 142; P at a position corresponding to position 142; S at a position corresponding to position 142; T at a position corresponding to position 142; G at a position corresponding to position 143; K at a position corresponding to position 143; T at a position corresponding to position 144; Q at a position corresponding to position 166; T at a position corresponding to position 166; L at a position corresponding to position 169; G at a position corresponding to position 174; N at a position corresponding to position 174; Q at a position corresponding to position 193; T at a position corresponding to position 195; N at a position corresponding to position 195; E at a position corresponding to position 196; R at a position corresponding to position 196; P at a position corresponding to position 204; A at a position corresponding to position 205; E at a position corresponding to position 205; I at a position corresponding to position 206; A at a position corresponding to position 213; I at a position corresponding to position 219; M at a position corresponding to position 234; T at a position corresponding to position 237; H at a position corresponding to position 238; Q at a position corresponding to position 240; V at a position corresponding to position 249; A at a position corresponding to position 261; K at a position corresponding to position 261; T at a position corresponding to position 267; K at a position corresponding to position 277; H at a position corresponding to position 279; V at a position corresponding to position 279; E at a position corresponding to position 309; Q at a position corresponding to position 310; Y at a position corresponding to position 314; Y at a position corresponding to position 315; N at a position corresponding to position 317; W at a position corresponding to position 317; D at a position corresponding to position 318; G at a position corresponding to position 347; A at a position corresponding to position 367; R at a position corresponding to position 375; R at a position corresponding to position 376; V at a position corresponding to position 399; E at a position corresponding to position 401; A at a position corresponding to position 407; L at a position corresponding to position 416; K at a position corresponding to position 419; H at a position corresponding to position 421; E at a

position corresponding to position 431; T at a position corresponding to position 433; V at a position corresponding to position 433; C at a position corresponding to position 439; P at a position corresponding to position 440; G at a position corresponding to position 443; N at a position corresponding to position 445, each with reference to amino acid residue positions set forth in SEQ ID NO:3.

The amino acid replacement(s) can be in a PH20 polypeptide as set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%< 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. For example, the replacement(s) can be in a human PH20 polypeptide, for example, any set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72 or a variant thereof.

In particular, provided herein is a modified PH20 polypeptide that contains an amino acid replacement with P at a position corresponding to amino acid residue 204 with reference to SEQ ID NO:3. For example, provided herein is a modified PH20 polypeptide that contains an amino acid replacement F204P in a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 69, 72 or 32-66, or a sequence of amino acids that exhibits at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3, 7, 69, 72 or 32-66. In another example, provided herein is a modified PH20 polypeptide that contains an amino acid replacement F204P in a sequence of amino acids set forth in SEQ ID NO:10, 12, 14, 857, 859, 861 or 870 or a sequence that exhibits at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 10, 12, 14, 857, 859, 861 or 870. In a further example, provided herein is a modified PH20 polypeptide that contains an amino acid replacement F205P in a sequence of amino acids set forth in SEQ ID NO:24, or a sequence that exhibits at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24.

ii. Thermophiles

At elevated temperatures, PH20 hyaluronidases can lose activity. Provided herein are modified PH20 polypeptides that exhibit increased stability at elevated temperatures of between or about between 30°C to 45°C, inclusive, such as between or about between 35° C to 42° C, in particular at or about 37° C. For example, provided herein are modified PH20 polypeptides that are stable at elevated temperatures greater than 32° C such as 35° C to 45° C, 37° C to 42° C and in

particular at or about 37° C for at least 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, at least 5 days, at least 6 days or at least 7 days. Modified PH20 polypeptides that exhibit stability at elevated temperatures can be used in applications where temperatures are elevated, can fluctuate or increase. This can occur, for example, in methods of administration utilizing pumps or other continuous infusion devices.

In particular, modified PH20 polypeptides provided herein that exhibit stability at elevated temperatures exhibit increased hyaluronidase activity at elevated temperature compared to the corresponding PH20 polypeptide not containing the modification, *e.g.* amino acid replacement. The PH20 polypeptides can exhibit increased hyaluronidase activity upon incubation at elevated temperatures greater than 32° C such as 35° C to 45° C or 37° C to 42° C, in particular at or about 37° C for at least 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, at least 5 days, at least 6 days or at least 7 days compared to the corresponding PH20 polypeptide not containing the modification incubated under the same conditions. For example, the hyaluronidase activity can be increased at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500% or more compared to the corresponding PH20 polypeptide not containing the modification incubated under the same conditions. For example, the hyaluronidase activity can be increased at least 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 3-fold, 4-fold, 5-fold or more compared to the corresponding PH20 polypeptide not containing the modification incubated under the same conditions.

In other examples, modified PH20 polypeptides provided herein that exhibit stability at elevated temperatures retain hyaluronidase activity at elevated temperature compared to the activity of the modified PH20 polypeptide incubated at non-elevated temperatures under the same conditions (except for the differences in temperature). For example, modified PH20 polypeptides exhibit greater than or about 50%, such as greater than or at least 55%, 60 %, 65 %, 70 %, 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or 100% of the activity at elevated temperatures greater than 32° C such as 35° C to 45° C or 37° C to 42° C, in particular at or about 37° C compared to the activity of the PH20 at non-elevated temperatures of between or about between 2° C to 8° C. In some examples, modified PH20 polypeptides provided herein that exhibit stability at elevated temperatures exhibit increased

activity at elevated temperature compared to the activity of the modified PH20 polypeptide incubated at non-elevated temperatures under the same conditions (except for the differences in temperature). For example, modified PH20 polypeptides exhibit greater than or about 10% increased activity, such as greater than or at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500% or more of activity at elevated temperatures greater than 32° C such as 35° C to 45° C or 37° C to 42° C, in particular at or about 37° C compared to the activity of the PH20 at non-elevated temperatures of between or about between 2° C to 8° C. For example, modified PH20 polypeptides exhibit greater than or at least about 1.1-fold the hyaluronidase activity, such as greater than or at least 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 3-fold, 4-fold, 5-fold or more of activity at elevated temperatures greater than 32° C such as 35° C to 45° C or 37° C to 42° C, in particular at or about 37° C compared to the activity of the PH20 at non-elevated temperatures of between or about between 2° C to 8° C.

For example, such modified PH20 polypeptides provided herein that exhibit increased stability at elevated temperatures contain an amino acid replacement (substitution) at one or more amino acid positions corresponding to positions 1, 11, 12, 14, 20, 26, 29, 34, 50, 58, 70, 82, 83, 84, 86, 87, 140, 142, 143, 147, 152, 166, 167, 172, 174, 178, 193, 195, 206, 212, 213, 219, 233, 237, 240, 267, 277, 291, 292, 309, 313, 314, 317, 318, 347, 367, 368, 371, 374, 389, 392, 395, 396, 406, 419, 421, 439 or 443 with reference to amino acid positions set forth in SEQ ID NO:3. For example, the amino acid positions can be replacements at one or more positions corresponding to replacement of (L) at position 1 (L1), N11, V12, F14, A20, L26, F29, D34, G50, V58, I70, K82, I83, S84, Q86, D87, Q140, V142, Q143, T147, K152, V166, E167, G172, L174, N178, H193, K195, V206, D212, D213, N219, Q233, V237, T240, A267, V277, G291, E292, I309, M313, K314, L317, L318, Q347, P367,D368, A371, L374, E389, E392, S395, E396, L406, A419, D421, E439 or P443, with reference to amino acid positions set forth in SEQ ID NO:3. The resulting modified PH20 polypeptide exhibits increased stability at elevated temperatures greater than 32° C such as 35° C to 45° C, 37° C to 42° C and in particular at or about 37° C for at least 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, at least 5 days, at least 6 days, at least 7 days or more.

Exemplary of amino acid replacements in the modified PH20 polypeptides provided herein include, but are not limited, replacement with: R at a position corresponding to position 1; S at a position corresponding to position 11; I at a position corresponding to position 12; V at a position corresponding to position 14; S at a position corresponding to position 20; M at a position corresponding to position L with R at a position corresponding to position 29; W at a position corresponding to position 34; M at a position corresponding to position 50; K at a position corresponding to position 58; Q at a position corresponding to position 58; Q at a position corresponding to position 58; V at a position corresponding to position 70; L at a position corresponding to position 82; Q at a position corresponding to position 83; R at a position corresponding to position 84; A at a position corresponding to position 86; S at a position corresponding to position 87; K at a position corresponding to position 140; S at a position corresponding to position 142; T at a position corresponding to position 142; K at a position corresponding to position 143; S at a position corresponding to position 147; T at a position corresponding to position 152; T at a position corresponding to position 166; D at a position corresponding to position 167; A at a position corresponding to position 172; G at a position corresponding to position 174; N at a position corresponding to position 174; R at a position corresponding to position 178; Q at a position corresponding to position 193; T at a position corresponding to position 195; I at a position corresponding to position 206; S at a position corresponding to position 212; A at a position corresponding to position 213; I at a position corresponding to position 219; G at a position corresponding to position 233; T at a position corresponding to position 237; A at a position corresponding to position 240; Q at a position corresponding to position 240; T at a position corresponding to position 267; E at a position corresponding to position 277; S at a position corresponding to position 291; H at a position corresponding to position 292; V at a position corresponding to position 292; S at a position corresponding to position 309; H at a position corresponding to position 313; S at a position corresponding to position 314; I at a position corresponding to position 317; T at a position corresponding to position 317; W at a position corresponding to position 317; R at a position corresponding to position 318; G at a position corresponding to position 347; A at a position corresponding to position 367; R at a position corresponding to position 368; S at a position corresponding to position 371; P at a position corresponding to position 374;

A at a position corresponding to position 389; V at a position corresponding to position 392; A at a position corresponding to position 395; H at a position corresponding to position 396; N at a position corresponding to position 406; H at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 421; S at a position corresponding to position 421; A at a position corresponding to position 439; C at a position corresponding to position 439; or G at a position corresponding to position 443, each with reference to amino acid residue positions set forth in SEQ ID NO:3.

The amino acid replacement(s) can be in a PH20 polypeptide as set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto. For example, the replacement(s) can be in a human PH20 polypeptide, for example, any set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72 or a variant thereof.

iii. Absence of Salt

PH20 denatures in the presence of low salt or not salt. Thus, PH20 requires a high salt concentration of between or about between 140 mM to 200 mM to maintain stability. Other therapeutic agents, for example insulin, exhibit decreased solubility and increased crystallization/aggregation in the presence of high salt. Thus, the high salt requirements of PH20 can affect the solubility and/or activity of co-formulated therapeutic agents, while the presence of low salt can decrease the activity of PH20. This can create problems for generating PH20 co-formulations.

Provided herein are modified PH20 polypeptides that exhibit increased stability in the presence of low concentrations of NaCl less than 100 mM, for example, less than 90 mM, 80mM, 70mM, 60 mM, 50 mM, 40 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM or less. Generally, the modified PH20 polypeptides provided herein exhibit stability in the presence of low concentrations of NaCl of between or about between 10 mM NaCl and 100 mM NaCl, such as between or about between 15 mM to 80 mM NaCl. The modified PH20 polypeptides provided herein that exhibit stability at low concentrations of NaCl less than 100 mM or less exhibit increased hyaluronidase activity compared to the corresponding PH20 not containing the modification(s) (*e.g.* amino acid replacements). For example, For example, modified PH20 polypeptides exhibit greater than or about 10% increased activity, such as greater than or at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%,

200%, 300%, 400%, 500% or more of activity at low concentrations of NaCl less than 100 mM compared to the activity of the corresponding PH20 not containing the amino acid modification(s) (*e.g.* amino acid replacement(s) under the same conditions. For example, modified PH20 polypeptides exhibit greater than or at least about 1.1-fold the hyaluronidase activity, such as greater than or at least 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 3-fold, 4-fold, 5-fold or more of activity at low concentrations of NaCl less than 100 mM compared to the activity of the corresponding PH20 not containing the amino acid modification(s) (*e.g.* amino acid replacement(s) under the same conditions.

2. Inactive Mutants

Provided herein are modified PH20 polypeptides that contain one or more amino acid replacements in a PH20 polypeptide and that are inactive, whereby the polypeptides do not exhibit hyaluronidase activity. The modified PH20 polypeptides provided herein that are inactive generally exhibit less than 10% of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the polypeptide set forth in SEQ ID NOS: 3 or 7. For example, modified PH20 polypeptides provided herein that are inactive exhibit less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% or less of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the corresponding polypeptide not containing the amino acid modification (*e.g.* amino acid replacement), for example, a polypeptide set forth in SEQ ID NO:3 or 7.

For example, provided herein are PH20 polypeptides that are inactive and that are modified, for example by amino acid replacement or substitution, compared to a wildtype or reference PH20 polypeptide. For example, a modified PH20 polypeptide provided herein that is inactive contains one or more amino acid replacements at position(s) corresponding to position 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 94, 95, 96, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 143, 144, 145, 149, 150, 152, 153, 154, 155, 156, 157, 158, 159, 161, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191,

192, 193, 194, 195, 197, 198, 199, 200, 201, 202, 203, 204, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 196, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 331, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 408, 410, 411, 412, 413, 414, 415, 416, 417, 419, 420, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 434, 437, 438, 439, 440, 441, 442, 443, 444, or 447 with reference to amino acid positions set forth in SEQ ID NO:3, so long as the resulting modified PH20 polypeptide is inactive and exhibits less than 10% of the hyaluronidase activity of the corresponding PH20 polypeptide not containing the amino acid replacement. Typically, the amino acid residue that is modified (*e.g.* replaced) at the position corresponding to any of the above positions in a PH20 polypeptide is an identical residue, a conservative residue or a semi-conservative amino acid residue to the amino acid residue set forth in SEQ ID NO:3.

Exemplary amino acid replacements at any of the above corresponding positions are set forth in Table 5. Reference to corresponding position in Table 5 is with reference to positions set forth in SEQ ID NO:3. It is understood that the replacements can be made in the corresponding position in another PH20 polypeptide by alignment therewith with the sequence set forth in SEQ ID NO:3 (*see e.g.* Figures 1 and 2), whereby the corresponding position is the aligned position. The amino acid replacement(s) can be at the corresponding position in a PH20 polypeptide as set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870 or a variant thereof having at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity thereto, so long as the resulting modified PH20 polypeptide is inactive. For example, the replacement(s) can be in a corresponding position in a human PH20 polypeptide, for example, any set forth in any of SEQ ID NOS: 3, 7, 32-66, 69 or 72, or a variant

thereof. In particular, any one or more of the replacements are in SEQ ID NO:3, so long as the resulting modified PH20 polypeptide is inactive and exhibits less than 10% of the hyaluronidase activity of the PH20 polypeptide set forth in SEQ ID NO:3

TABLE 5: Inactive Mutants

Corresponding Position	Replacement	Corresponding Position	Replacement	Corresponding Position	Replacement
2	HKWY	3	AGKPTV	4	DEFGLPWY
5	DGILMNPQRTVWY	6	EFTVY	7	CDFGHIKLQRSTWY
8	DEGHNRSW	9	CDEGNP	10	FILMY
11	ACFILPTWY	12	GHW	13	EGILMV
14	AEGHKNPQW	15	EFGKNPQRSY	16	ACDEFGHKMPRSTY
17	DEGHILNPQRSTVWY	18	CDFGHILMPQSTVY	19	ACFGHILMPQRSVWY
20	DEFHKLNPRTVY	21	ACDEGHILMRSTVW	22	CEGKP
23	AFLMNPRSTV	25	DEFGHIKLNPRSTVY	27	C
33	CDHNVY	34	ILNSTV	35	ADGPRS
36	CFVWY	37	CEGNS	38	EGKLNQRTW
39	CDFW	40	ADEGKNRSTV	41	Q
42	DEHIKLMPQRSTV	43	AEFGIKLQRV	44	ACFGHILNQRSTWY
45	ADFGPW	46	PW	47	V
48	P	49	CDGHP	50	V
51	CFIMPTWY	52	CEFWY	53	ACDEGHLNPQRSTWY
54	DEGPRY	55	ADGHNPQRTVY	56	ACEGHIKLPRSTVW
57	ADFGIMPQRVW	58	A	59	AEILMPRTVWY
60	ADFGHILNPQSTVY	61	AEFGHNPQRTWY	62	ACDFIKLMPQRSTVY
63	CGP	64	ACDEFGHIKLPQRSTVW	65	ACDGHIKNRSTVWY
66	ACDEGIKLNPSTV	67	DEGPRTW	68	ACGILPVY
69	NT	70	Q	71	P
72	CFHIPVW	73	P	75	DGP
76	ACFGIKLPQRSTVW	77	DELPQRTV	78	ADIMPTY
79	ADFGHKNPSWY	80	ADEFGIKLMNRSTVY	81	ACEGHLNPSVWY
82	YEK	84	Y	85	ACDEFGHNQST
86	CP	87	P	88	ACEFGIKLMPRSTVY
89	ADEGQSTWY	90	CG	91	DEFGHILT
92	EFHKPQRWY	94	GP	95	ACEFGHKLMPQSVWY
96	SVHPRSTW	98	P	99	CEGINPVW

100	CEFGNPRST WY	101	ACFHIKLMN QRST	102	P
103	AEFGHILQRT VWY	104	FPW	105	CMN
106	ACDFHLMNP SWY	107	ACHKPQSVW	108	DEFKLMPQT VY
109	CDELMRTW	110	FKLMPW	111	HIQ
112	CEGHLNPS	113	RV	114	ILPTV
115	ACDFGHIKL MRSVY	116	ACDEGHILN PQSVW	117	DGIKNQRSV W
118	CDEGPRWY	119	AKILNPR	121	ACEFGHKL MPWY
122	A CEFIKQRS TV	123	ACDEHLMPQ RSTVY	124	CDEFN
125	CDGLNW	126	FHILNPY	127	K
128	EP	129	ACDEGHLPQ STVW	130	CDGHLNST WY
131	P	132	P	133	DEFGHLMNP RTVW
134	ACDFGHKPQ RSW	135	P	136	P
137	FGHNPRWY	138	V	139	P
143	CHPRST	144	AEFIKPQSV Y	145	TW
149	E	149	P	150	V
152	L	153	EFMPRTV	154	DEGPSWY
155	PY	156	P	157	ACDEGHIKL M PQRSTV
158	DKPRY	159	WY	161	W
163	CP	164	ACDEGHNPQ R	165	CHPT
166	D	167	V	168	ACDEFGKLP RSVWY
169	ADFGHKNPQ STY	170	CDEGMPWY	171	CDHMNRSW Y
172	DEILPQTVW Y	173	DEGHILMPS VWY	174	P
175	CDGKPRS	176	ACEFGHIPQ STVW	177	ACDFGHLM QRSTVW
178	EILVW	180	ACEPRS	181	ACDEFHIKL RS
182	ACDEHNPQR STVY	183	CDEGIKNPQ RSV	184	ACDEFGHKL MPRSV
185	ADEFGIKPRS TVWY	186	ADGHIKLNP QRSVW	187	AFGHILMNQ RSTVWY
188	ACFGHLMNP QRSTVW	189	AEGHKLMNP RSTVWY	190	CEFGHKLNQ RSTVW
191	AEFGKLMPQ RSTVWY	192	CFGKLMNPQ RVWY	193	ADKLMPV
194	ACILPSTV	195	S	197	C
198	VW	199	EGHIKLPRS W	200	AFGHKLMP QRSWY
201	AFLMNPRST VW	202	AEFGHKNPQ RVWY	203	ADEGHLMN QRSTV
204	ACEGHIKQR ST	206	CDFGPY	207	AFGMPQRST VW
208	DGPW	209	CP	210	ACDEGKMN PSTVWY
211	CFGHIKMPR	212	AGHIKLMPV	213	PS

	STVW		W		
214	ACDEGHKNP RSTY	215	CP	216	DEGHIKLMN PQRTV
217	ACGHPQSTV W	218	AIKLPSV	219	P
220	GKNPRW	221	DEHKPR	222	PY
223	CDEGHKLPQ RSTVWY	224	ADEFGMPQR STWY	225	ADEGHKPQ RTVW
226	ACDEFGLNQ RSTVWY	227	AFGHIKLMP QRTVWY	228	AEFGHLMNP RSTW
229	EFGKLPQTV W	230	AEGHKMNPR STVWY	231	ACDFGHIKL PQRSV
232	CGHKLNPQV Y	233	DIPST	234	ADEGHNPST VW
235	FLMRWY	236	CILNQTY	238	FGLPVWY
239	CFGHILPRST VWY	240	EFGNWY	241	ACDEGIPRS TVW
242	ACDGILMPR STVW	243	CDFGHLMPQ RSWY	244	ADGIVY
245	ACFLPQRST V	246	ACDEGHIKL MPSTVW	247	ACFHNPQRS TWY
248	CDEGIMPT	249	AGHIKMQSY	250	CFGHKLMN PQRSTVW
251	DFGHKPSTW	252	ADEFGHIKL NPSTY	253	ADEGHLMN QRSW
254	CDEGIKLPQ RTVWY	255	CDLPVW	256	CDEG[
257	D	258	LPVW	260	CP
261	P	262	ADE GHIKQR STVWY	263	EFPQW
264	DEFG LMRTV WY	265	ADFGHKLM NQRS	266	ACGHMPQR STVW
267	DGHIKNRSW	268	ACFGHKLNP QSTVW	269	EKLMNPQR
270	ACEFGHIPY	271	ADEHKTW	272	HLNPW
273	ACDGILPQS VW	274	CEGHNQWY	275	AFGIKLMQT VW
276	FPW	278	MP	279	ACFGLWY
280	DI MNRSTVW	281	ADGHIKNPQ RSVW	282	FLVWY
283	ACDFW	284	CDFW	284	CIP
285	KPRTV	286	ACDFHKMPT Y	287	ACDEGKLNP QRS
288	DEFGHIKPRT	289	ACEGHLPQR SY	290	DQY
291	ACDEFMNTW Y	292	ILT	293	EN
294	AEGHKLNPQ RSTW	295	CGHILNPTV Y	296	CFGIKMQRS TVWY
297	CEHLNPQRS TY	298	CELMNPQST WY	299	ACDFGHLM PQT
300	ACDEFLMNP QSTVW	301	EGHKMNPQR SWY	302	CDEFGHLMP RSTY
303	ACDEFGKLM RWY	304	ACDGIMNPQ STVY	305	LPQRSTVY
306	ACHILVWY	307	CIP	308	CFLMVWY
310	CEFKL	311	CEFILPVW	312	CEMVW
313	C	314	CLW	315	CIV
316	EGIKLMPRST	317	G P	318	CPW

	VWY				
319	CEFGHIKMP QRSVWY	320	CPV	321	EMP
322	CDEGILNPRS TVW	323	ACEGHKNRS TV	324	CFPVWY
325	CREGHNW	327	AEFGHNQRS TVWY	329	CFGHIKLNQ RSTVWY
330	ACDEGILMN PRSVW	331	ACDEFHKQR STWY	332	ACDEFGHKL NPRSTY
333	GHIKPRSTW Y	334	ACDEGMNRS	335	FGHIKLPVW Y
336	AEFGKNPRS TVWY	337	CFGIKLMRT W	338	CDEFGHIKL PRTV
339	DEFGHLNPS TVWY	340	ACDEFGHKP RSTVW	341	AEGHKLMN QRST VY
342	DEFHKLMPQ RTY	343	CDFIPW	344	FGHLMNPQ RSTWY
345	A CEHKNQRT VY	346	ADFGIKLMP RSTVW	347	CFIPTVW
348	CHILPQRTV WY	349	DFGPVWY	350	ADEFHKLM NPRSTVY
351	CDEFHNRWY	352	ADEFGKMPQ RSTVWY	353	CFGHKLMQ RSW
354	CDEGHIKLM PQSVWY	355	DFGHLMNPQ RSTVWY	356	CGKLPRTV W
357	DEFGLMQR	358	EHIKPQRW	359	AFGLPW
360	ACEFGIKLM PQRV	361	ACEGMNPQR SVW	362	ACEGHKLM NPRSTVW
363	ACDEFGHIP QRSTVW	364	ACDEFGKLM PRSTVY	365	ACDEGMNP QRSTWY
366	ACEFGKMPQ RTW	367	EFILMQV	368	CPW
369	CEFIKLPQV W	370	ADEGHKLNP QRSVY	371	P
372	ADEFGHKLN PRSTVW	373	CPW	374	DE
375	CFPVY	376	IPW	377	CILV
378	DEFILMQTW Y	379	ACEFILMW	380	CDEGQRS
381	GLPWY	382	EGHKLMNPQ RSTWY	383	GP
384	CFMQST	385	CLMPWY	386	ACFGHILMN QRSTVY
387	CEFGHILMN VWY	388	CGPQ	389	FV
390	ACEFGHLNP RSTVWY	391	ADGHKNPQR STVWY	392	CP
393	CP	394	ADEGIKNPQ RSTV	395	C;,[
396	CFGIPY	397	ACEFGILMP QTV	398	ACEGHILNP RSTVWY
399	DP	400	ADEFGILMP QRSTVY	401	CFHKRWY
402	ADEFLMPQR STVWY	403	ACEGHKLM NPQRT	404	CDFGHLMN RVWY
405	CIV	406	PR	408	AEFGIKLPR STVWY
410	W	411	DEFG	412	EH
413	HIKLP	414	ADEGHKRST	415	CDEP

416	C S	417	A D E F G H K M P Q R	419	D P
420	A D F G H K L N R S T W Y	422	C D G H L M N Q R S Y	423	A D E F G H L M P Q R S T V W
424	A C E G H N Q R S W Y	425	E L P W Y	426	C F M R
427	A C F L P V W Y	428	A C D E G H N R S Y	429	A D K L N P S T V W Y
430	A D E L M N S T V	431	P	432	C F I K L M P Y
434	H K P Q R W	437	T	438	Y
439	N R	440	Q	441	R
442	M N S	443	D		

3. Additional Modifications

The modified PH20 polypeptides include those that contain chemical or posttranslational modifications. In some examples, modified PH20 polypeptides provided herein do not contain chemical or posttranslational modifications. Chemical and post-translational modifications include, but are not limited to, pegylation, sialation, albumination, glycosylation, farnysylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art. Also, in addition to any one or more amino acid modifications, such as amino acid replacements, provided herein, modified PH20 polypeptides provided herein can be conjugated or fused to any moiety using any method known in the art, including chemical and recombinant methods, providing the resulting polypeptide retains hyaluronidase activity.

For example, in addition to any one or more amino acid modifications, such as amino acid replacements, provided herein, modified PH20 polypeptides provided herein also can contain other modifications that are or are not in the primary sequence of the polypeptide, including, but not limited to, modification with a carbohydrate moiety, a polyethylene glycol (PEG) moiety, a silation moiety, an Fc domain from immunoglobulin G, or any other domain or moiety. For example, such additional modifications can be made to increase the stability or serum half-life of the protein.

a. Decreased immunogenicity

The modified PH20 polypeptides provided herein can be made to have decreased immunogenicity. Decreased immunogenicity can be effected by sequence changes that elimiminate antigenic epitopes from the polypeptide or by altering post-translational modifications. One of skill in the art is familiar with methods of identifiying antigenic epitopes in a polypeptide (*see e.g.* Liang *et al.* (2009) *BMC Bioinformatics*, 10:302; Yang *et al.* (2009) *Rev. Med. Virol.*, 19:77-96). In some

examples, one or more amino acids can be modified in order to remove or alter an antigenic epitope.

In another example, altering the glycosylation of a protein also can effect immunogenecity. For example, altering the glycosylation of the peptide is contemplated, so long as the polypeptides minimally contain at least N-acetylglucosamine at amino acid residues corresponding to amino acid residues set forth as N200, N333 and N358 of SEQ ID NO:3 or 7.

For example, the PH20 polypeptides can be modified such that they lack fucose, particularly bifucosylation.. In particular, the PH20 polypeptides provided herein are not bifucosylated. This can be achieved by expressing and producing the PH20 polypeptide in a host cells, typically insect host cells, that do not effect bifucosylation. Fucose is a deoxyhexose that is present in a wide variety of organisms, including mammals, insects and plants. Fucosylated glycans are synthesized by fucosyl-tranferases. See, *e.g.*, Ma et al., *Glycobiology*, 15(2):158R-184R, (2006); Nakayama et al., *J. Biol. Chem.*, 276:16100-16106 (2001); and Sturla et al., *Glycobiology*, 15(10):924-935 (2005). In humans, fucose frequently exists as a terminal modification to glycan structures, and the presence of fucose α1,6-linked to N-acetylglucosamine has been shown to be important in glycoprotein processing and recognition. In insects, N-glycan core structures exhibit bifucosylation with α1,6- and α1,3-linkages. Insect cell core fucosylation with α1,3-linkages generates a carbohydrate epitope that is immunogenic in humans (see, *e.g.*, US Patent Application No. 20070067855). For example, PH20 polypeptides provided herein can be generated in host cells that are incapable of bifucosylating the polypeptide. Thus, while insect cells or other cells that bifucosylate can be used for expression of the polypeptides, typically mammalian cells, such as CHO cells, are used.

In some examples, defucosylated, or fucose-deficient PH20 polypeptides can be generated in insect cells with modified glycosylation pathways, through the use of baculovirus expression vectors containing eukaryotic oligosaccharide processing genes, thereby creating "mammalianized" insect cell expression systems (see, *e.g.*, US Patent No. 6,461,863). Alternatively, antigenicity can be eliminated by expression of PH20 polypeptides in insect cells lacking α1,3-fucosylatransferase (FT3) (see, *e.g.*, US Patent Application No. 20070067855). In other examples, defucosylated or fucose-deficient PH20 polypeptides can be generated, for example, in cell lines that produce defucosylated proteins, including Lec13 CHO cells deficient in protein

fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); U.S. Pat. Appl. No. 2003/0157108; and WO 2004/056312), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004)).

b. Conjugation to polymers

In some examples, the modified PH20 polypeptides provided herein are conjugated to polymers. Exemplary polymers that can be conjugated to the PH20 polypeptides, include natural and synthetic homopolymers, such as polyols (*i.e.* poly-OH), polyamines (*i.e.* poly-NH_2) and polycarboxylic acids (*i.e.* poly-COOH), and further heteropolymers *i.e.* polymers comprising one or more different coupling groups *e.g.* a hydroxyl group and amine groups. Examples of suitable polymeric molecules include polymeric molecules selected from among polyalkylene oxides (PAO), such as polyalkylene glycols (PAG), including polyethylene glycols (PEG), methoxypolyethylene glycols (mPEG) and polypropylene glycols, PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched polyethylene glycols (PEGs), polyvinyl alcohol (PVA), polycarboxylates, polyvinylpyrrolidone, poly-D,L-amino acids, polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextrans including carboxymethyl-dextrans, heparin, homologous albumin, celluloses, including methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, carboxyethylcellulose and hydroxypropylcellulose, hydrolysates of chitosan, starches such as hydroxyethyl-starches and hydroxypropyl-starches, glycogen, agaroses and derivatives thereof, guar gum, pullulan, inulin, xanthan gum, carrageenan, pectin, alginic acid hydrolysates and bio-polymers.

Typically, the polymers are polyalkylene oxides (PAO), such as polyethylene oxides, such as PEG, typically mPEG, which have few reactive groups capable of cross-linking. Typically, the polymers are non-toxic polymeric molecules such as (methoxy)polyethylene glycol (mPEG) which can be covalently conjugated to the PH20 polypeptides (*e.g.*, to attachment groups on the protein surface) using a relatively simple chemistry.

Suitable polymeric molecules for attachment to the PH20 polypeptides include, but are not limited to, polyethylene glycol (PEG) and PEG derivatives such as methoxy-polyethylene glycols (mPEG), PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched PEGs, and polyethylene oxide (PEO)

(*see e.g.* Roberts et al., *Advanced Drug Delivery Review* 2002, 54: 459-476; Harris and Zalipsky (eds.) "Poly(ethylene glycol), Chemistry and Biological Applications" ACS Symposium Series 680, 1997; Mehvar et al., *J. Pharm. Pharmaceut. Sci.,* 3(1):125-136, 2000; Harris and Chess (2003) *Nat Rev Drug Discov.* 2(3):214-21; and Tsubery, *J Biol. Chem* 279(37):38118-24, 2004). The polymeric molecule can be of a molecular weight typically ranging from about 3 kDa to about 60 kDa. In some embodiments the polymeric molecule that is conjugated to a PH20 polypeptide provided herein has a molecular weight of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 kDa.

Various methods of modifying polypeptides by covalently attaching (conjugating) a PEG or PEG derivative (*i.e.* "PEGylation") are known in the art (*see e.g.*, U.S. 2006/0104968; U.S. 5,672,662; U.S. 6,737,505; and U.S. 2004/0235734). Techniques for PEGylation include, but are not limited to, specialized linkers and coupling chemistries (*see e.g.*, Harris, *Adv. Drug Deliv. Rev.* 54:459-476, 2002), attachment of multiple PEG moieties to a single conjugation site (such as via use of branched PEGs; *see e.g.*, Veronese et al., *Bioorg. Med. Chem. Lett.* 12:177-180, 2002), site-specific PEGylation and/or mono-PEGylation (*see e.g.*, Chapman et al., Nature Biotech. 17:780-783, 1999), and site-directed enzymatic PEGylation (*see e.g.*, Sato, *Adv. Drug Deliv. Rev.,* 54:487-504, 2002) (see, also, for example, Lu and Felix (1994) *Int. J. Peptide Protein Res.* 43:127-138; Lu and Felix (1993) *Peptide Res.* 6:142-6, 1993; Felix *et al.* (1995) *Int. J. Peptide Res.* 46:253-64; Benhar *et al.* (1994) *J. Biol. Chem.* 269:13398-404; Brumeanu *et al.* (1995) *J Immunol.* 154:3088-95; see also, Caliceti *et al.* (2003) *Adv. Drug Deliv. Rev.* 55(10):1261-77 and Molineux (2003) *Pharmacotherapy* 23 (8 Pt 2):3S-8S). Methods and techniques described in the art can produce proteins having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 PEG or PEG derivatives attached to a single protein molecule (*see e.g.*, U.S. 2006/0104968).

Numerous reagents for PEGylation have been described in the art. Such reagents include, but are not limited to, N-hydroxysuccinimidyl (NHS) activated PEG, succinimidyl mPEG, mPEG2-N-hydroxysuccinimide, mPEG succinimidyl alpha-methylbutanoate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, mPEG carboxymethyl 3-hydroxybutanoic acid succinimidyl ester, homobifunctional PEG-succinimidyl propionate, homobifunctional PEG propionaldehyde, homobifunctional PEG butyraldehyde, PEG maleimide, PEG hydrazide, p-nitrophenyl-carbonate PEG, mPEG-benzotriazole carbonate,

propionaldehyde PEG, mPEG butryaldehyde, branched mPEG2 butyraldehyde, mPEG acetyl, mPEG piperidone, mPEG methylketone, mPEG "linkerless" maleimide, mPEG vinyl sulfone, mPEG thiol, mPEG orthopyridylthioester, mPEG orthopyridyl disulfide, Fmoc-PEG-NHS, Boc-PEG-NHS, vinylsulfone PEG-NHS, acrylate PEG-NHS, fluorescein PEG-NHS, and biotin PEG-NHS (*see e.g.*, Monfardini et al., Bioconjugate Chem. 6:62-69, 1995; Veronese et al., *J. Bioactive Compatible Polymers* 12:197-207, 1997; U.S. 5,672,662; U.S. 5,932,462; U.S. 6,495,659; U.S. 6,737,505; U.S. 4,002,531; U.S. 4,179,337; U.S. 5,122,614; U.S. 5,183,550; U.S. 5,324, 844; U.S. 5,446,090; U.S. 5,612,460; U.S. 5,643,575; U.S. 5,766,581; U.S. 5,795, 569; U.S. 5,808,096; U.S. 5,900,461; U.S. 5,919,455; U.S. 5,985,263; U.S. 5,990, 237; U.S. 6,113,906; U.S. 6,214,966; U.S. 6,258,351; U.S. 6,340,742; U.S. 6,413,507; U.S. 6,420,339; U.S. 6,437,025; U.S. 6,448,369; U.S. 6,461,802; U.S. 6,828,401; U.S. 6,858,736; U.S. 2001/0021763; U.S. 2001/0044526; U.S. 2001/0046481; U.S. 2002/0052430; U.S. 2002/0072573; U.S. 2002/0156047; U.S. 2003/0114647; U.S. 2003/0143596; U.S. 2003/0158333; U.S. 2003/0220447; U.S. 2004/0013637; US 2004/0235734; U.S. 2005/000360; U.S. 2005/0114037; U.S. 2005/0171328; U.S. 2005/0209416; EP 01064951; EP 0822199; WO 00176640; WO 0002017; WO 0249673; WO 9428024; and WO 0187925).

D. PRODUCTION OF MODIFIED PH20 POLYPEPTIDES AND ENCODING NUCLEIC ACID MOLECULES

Polypeptides of a modified PH20 polypeptide set forth herein can be obtained by methods well known in the art for protein purification and recombinant protein expression. Polypeptides also can be synthesized chemically. Modified or variant, including truncated forms, can be engineered from a wildtype polypeptide using standard recombinant DNA methods. For example, modified PH20 polypeptides can be engineered from a wildtype polypeptide, such as by site-directed mutagenesis.

1. Isolation or Preparation of Nucleic Acids Encoding PH20 Polypeptides

Polypeptides can be cloned or isolated using any available methods known in the art for cloning and isolating nucleic acid molecules. Such methods include PCR amplification of nucleic acids and screening of libraries, including nucleic acid hybridization screening, antibody-based screening and activity-based screening. For example, when the polypeptides are produced by recombinant means, any method known to those of skill in the art for identification of nucleic acids that encode desired genes can be used. Any method available in the art can be used to obtain a full length

or partial (*i.e.*, encompassing the entire coding region) cDNA or genomic DNA clone encoding a PH20, such as from a cell or tissue source.

Methods for amplification of nucleic acids can be used to isolate nucleic acid molecules encoding a desired polypeptide, including for example, polymerase chain reaction (PCR) methods. Exemplary of such methods include use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp). A nucleic acid containing material can be used as a starting material from which a desired polypeptide-encoding nucleic acid molecule can be isolated. For example, DNA and mRNA preparations, cell extracts, tissue extracts, fluid samples (*e.g.* blood, serum, saliva), samples from healthy and/or diseased subjects can be used in amplification methods. The source can be from any eukaryotic species including, but not limited to, vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, and other primate sources. Nucleic acid libraries also can be used as a source of starting material. Primers can be designed to amplify a desired polypeptide. For example, primers can be designed based on expressed sequences from which a desired polypeptide is generated. Primers can be designed based on back-translation of a polypeptide amino acid sequence. If desired, degenerate primers can be used for amplification. Oligonucleotide primers that hybridize to sequences at the 3' and 5' termini of the desired sequence can be uses as primers to amplify by PCR sequences from a nucleic acid sample. Primers can be used to amplify the entire full-length PH20, or a truncated sequence thereof, such as a nucleic acid encoding any of the soluble PH20 polypeptides provided herein. Nucleic acid molecules generated by amplification can be sequenced and confirmed to encode a desired polypeptide.

Additional nucleotide sequences can be joined to a polypeptide-encoding nucleic acid molecule, including linker sequences containing restriction endonuclease sites for the purpose of cloning the synthetic gene into a vector, for example, a protein expression vector or a vector designed for the amplification of the core protein coding DNA sequences. Furthermore, additional nucleotide sequences specifying functional DNA elements can be operatively linked to a polypeptide-encoding nucleic acid molecule. Examples of such sequences include, but are not limited to, promoter sequences designed to facilitate intracellular protein expression, and secretion sequences, for example heterologous signal sequences, designed to facilitate protein secretion. Such sequences are known to those of skill in the art. For example, exemplary heterologous signal sequences include, but are not limited to, human and

mouse kappa IgG heterologous signal sequences set forth in SEQ ID NO: 868. Additional nucleotide residues sequences such as sequences of bases specifying protein binding regions also can be linked to enzyme-encoding nucleic acid molecules. Such regions include, but are not limited to, sequences of residues that facilitate or encode proteins that facilitate uptake of an enzyme into specific target cells, or otherwise alter pharmacokinetics of a product of a synthetic gene.

In addition, tags or other moieties can be added, for example, to aid in detection or affinity purification of the polypeptide. For example, additional nucleotide residues sequences such as sequences of bases specifying an epitope tag or other detectable marker also can be linked to enzyme-encoding nucleic acid molecules. Exemplary of such sequences include nucleic acid sequences encoding a His tag or Flag Tag.

The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art can be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pCMV4, pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene, La Jolla, CA). Other expression vectors include the HZ24 expression vector exemplified herein (*see e.g.* SEQ ID NOS:4 or 5). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. Insertion can be effected using TOPO cloning vectors (Invitrogen, Carlsbad, CA).

If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules can be enzymatically modified. Alternatively, any site desired can be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers can contain specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and protein gene can be modified by homopolymeric tailing.

Recombinant molecules can be introduced into host cells via, for example, transformation, transfection, infection, electroporation and sonoporation, so that many copies of the gene sequence are generated. In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated protein gene,

cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene can be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

In addition to recombinant production, modified PH20 polypeptides provided herein, can be produced by direct peptide synthesis using solid-phase techniques (*see e.g.*, Stewart et al. (1969) Solid-Phase Peptide Synthesis, WH Freeman Co., San Francisco; Merrifield J (1963) J Am Chem Soc., 85:2149-2154). In vitro protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments of a polypeptide can be chemically synthesized separately and combined using chemical methods.

2. Generation of Mutant or Modified Nucleic Acid and Encoding Polypeptides

The modifications provided herein can be made by standard recombinant DNA techniques such as are routine to one of skill in the art. Any method known in the art to effect mutation of any one or more amino acids in a target protein can be employed. Methods include standard site-directed mutagenesis (using *e.g.* a kit, such as QuikChange available from Stratagene) of encoding nucleic acid molecules, or by solid phase polypeptide synthesis methods.

3. Vectors and Cells

For recombinant expression of one or more of the desired proteins, such as any modified PH20 polypeptide described herein, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence. The necessary transcriptional and translational signals also can be supplied by the native promoter for enzyme genes, and/or their flanking regions.

Also provided are vectors that contain a nucleic acid encoding the enzyme. Cells containing the vectors also are provided. The cells include eukaryotic and prokaryotic cells, and the vectors are any suitable for use therein. Generally, the cell is a cell that is capable of effecting glyosylation of the encoded protein.

Prokaryotic and eukaryotic cells containing the vectors are provided. Such cells include bacterial cells, yeast cells, fungal cells, Archea, plant cells, insect cells and animal cells. The cells are used to produce a protein thereof by growing the above-described cells under conditions whereby the encoded protein is expressed by the cell, and recovering the expressed protein. For purposes herein, for example, the enzyme can be secreted into the medium.

A host cells strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing can impact the folding and/or function of the polypeptide. Different host cells, such as, but not limited to, CHO (DG44, DXB11, CHO-K1), HeLa, MCDK, 293 and WI38 have specific cellular machinery and characteristic mechanisms for such post-translational activities and can be chosen to ensure the correct modification and processing of the introduced protein. Generally, the choice of cell is one that is capable of introducing N-linked glycosylation into the expressed polypeptide. Hence, eukaryotic cells containing the vectors are provided. Exemplary of eukaryotic cells are mammalian Chinese Hamster Ovary (CHO) cells. For example, CHO cells deficient in dihydrofolate reductase (*e.g.* DG44 cells) are used to produce polypeptides provided herein. Note that bacterial expression of an PH20 polypepyideprovided herein will not result in a catalytically active polypeptide, but when combined with proper glycosylation machinery, the PH20 can be artificially glycosylated.

Provided are vectors that contain a sequence of nucleotides that encodes the modified PH20 polypeptide, coupled to the native or heterologous signal sequence, as well as multiple copies thereof. The vectors can be selected for expression of the enzyme protein in the cell or such that the enzyme protein is expressed as a secreted protein.

A variety of host-vector systems can be used to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus and other viruses); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities.

Depending on the host-vector system used, any one of a number of suitable transcription and translation elements can be used.

Any methods known to those of skill in the art for the insertion of DNA fragments into a vector can be used to construct expression vectors containing a chimeric gene containing appropriate transcriptional/translational control signals and protein coding sequences. These methods can include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequences encoding protein, or domains, derivatives, fragments or homologs thereof, can be regulated by a second nucleic acid sequence so that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins can be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for a desired protein. Promoters which can be used include but are not limited to the SV40 early promoter (Bernoist and Chambon, *Nature 290*:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al. Cell 22*:787-797 (1980)), the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature 296*:39-42 (1982)); prokaryotic expression vectors such as the β-lactamase promoter (Jay *et al.*, (1981) *Proc. Natl. Acad. Sci. USA 78*:5543) or the *tac* promoter (DeBoer *et al.*, *Proc. Natl. Acad. Sci. USA 80*:21-25 (1983)); see also "Useful Proteins from Recombinant Bacteria": in Scientific American *242*:79-94 (1980)); plant expression vectors containing the nopaline synthetase promoter (Herrara-Estrella *et al.*, *Nature 303*:209-213 (1984)) or the cauliflower mosaic virus 35S RNA promoter (Garder *et al.*, *Nucleic Acids Res. 9*:2871 (1981)), and the promoter of the photosynthetic enzyme ribulose bisphosphate carboxylase (Herrera-Estrella *et al.*, *Nature 310*:115-120 (1984)); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter, and the following animal transcriptional control regions that exhibit tissue specificity and have been used in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell 38*:639-646 (1984); Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 50*:399-409 (1986); MacDonald, *Hepatology 7*:425-515 (1987)); insulin gene control region which is

active in pancreatic beta cells (Hanahan *et al.*, *Nature 315*:115-122 (1985)), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell 38*:647-658 (1984); Adams *et al.*, *Nature 318*:533-538 (1985); Alexander *et al.*, *Mol. Cell Biol. 7*:1436-1444 (1987)), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell 45*:485-495 (1986)), albumin gene control region which is active in liver (Pinckert *et al.*, *Genes and Devel. 1*:268-276 (1987)), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol. 5*:1639-1648 (1985); Hammer *et al.*, *Science 235*:53-58 1987)), alpha-1 antitrypsin gene control region which is active in liver (Kelsey *et al.*, *Genes and Devel. 1*:161-171 (1987)), beta globin gene control region which is active in myeloid cells (Magram *et al.*, *Nature 315*:338-340 (1985); Kollias *et al.*, *Cell 46*:89-94 (1986)), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead *et al.*, *Cell 48*:703-712 (1987)), myosin light chain-2 gene control region which is active in skeletal muscle (Shani, *Nature 314*:283-286 (1985)), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the hypothalamus (Mason *et al.*, *Science 234*:1372-1378 (1986)).

In a specific embodiment, a vector is used that contains a promoter operably linked to nucleic acids encoding a desired protein, or a domain, fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). Depending on the expression system, specific initiation signals also are required for efficient translation of a PH20 sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where the initiation codon and upstream sequences of PH20 or soluble forms thereof are inserted into the appropriate expression vector, no additional translational control signals are needed. In cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf et al. (1994) Results Probl Cell Differ 20:125-62; Bittner et al. (1987) Methods in Enzymol, 153:516-544).

Exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pQE expression vectors (available from Qiagen, Valencia, CA; see also literature published by Qiagen describing the system). pQE vectors have a phage T5 promoter (recognized by *E. coli* RNA polymerase) and a double lac operator repression module to provide tightly regulated, high-level expression of recombinant proteins in *E. coli,* a synthetic ribosomal binding site (RBS II) for efficient translation, a 6XHis tag coding sequence, t_0 and T1 transcriptional terminators, ColE1 origin of replication, and a beta-lactamase gene for conferring ampicillin resistance. The pQE vectors enable placement of a 6xHis tag at either the N- or C-terminus of the recombinant protein. Such plasmids include pQE 32, pQE 30, and pQE 31 which provide multiple cloning sites for all three reading frames and provide for the expression of N-terminally 6xHis-tagged proteins. Other exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pET expression vectors (see, U.S. patent 4,952,496; available from Novagen, Madison, WI; see, also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T7lac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b and pET19b (Novagen, Madison, WI), which contain a His-Tag™ leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column, the T7-lac promoter region and the T7 terminator.

Typically, vectors can be plasmid, viral, or others known in the art, used for expression of the modified PH20 polypeptide *in vivo* or *in vitro*. For example, the modified PH20 polypeptide is expressed in mammalian cells, including, for example, Chinese Hamster Ovary (CHO) cells.. Exemplary of a vector for mammalian cell expression is the HZ24 expression vector. The HZ24 expression vector was derived from the pCI vector backbone (Promega). It contains DNA encoding the Beta-lactamase resistance gene (AmpR), an F1 origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), and an SV40 late polyadenylation signal (SV40). The expression vector also has an internal ribosome entry site (IRES) from the ECMV virus (Clontech) and the mouse dihydrofolate reductase (DHFR) gene.

Viral vectors, such as adenovirus, retrovirus or vaccinia virus vectors, can be employed. In some examples, the vector is a defective or attenuated retroviral or

other viral vector (see U. S. Patent No. 4,980,286). For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217: 581-599 (1993)). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA.

In some examples, viruses armed with a nucleic acid encoding a modified PH20 polypeptide can facilitate their replication and spread within a target tissue for example. The target tissue can be a cancerous tissue whereby the virus is capable of selective replication within the tumor. The virus can also be a non-lytic virus wherein the virus selectively replicates under a tissue specific promoter. As the viruses replicate, the coexpression of the PH20 polypeptide with viral genes will facilitate the spread of the virus in vivo.

4. Expression

Modified PH20 polypeptides can be produced by any method known to those of skill in the art including *in vivo* and *in vitro* methods. Desired proteins can be expressed in any organism suitable to produce the required amounts and forms of the proteins, such as for example, needed for administration and treatment. Expression hosts include prokaryotic and eukaryotic organisms such as *E.coli*, yeast, plants, insect cells, mammalian cells, including human cell lines and transgenic animals. Expression hosts can differ in their protein production levels as well as the types of post-translational modifications that are present on the expressed proteins. The choice of expression host can be made based on these and other factors, such as regulatory and safety considerations, production costs and the need and methods for purification.

Many expression vectors are available and known to those of skill in the art and can be used for expression of proteins. The choice of expression vector will be influenced by the choice of host expression system. In general, expression vectors can include transcriptional promoters and optionally enhancers, translational signals, and transcriptional and translational termination signals. Expression vectors that are used for stable transformation typically have a selectable marker which allows selection and maintenance of the transformed cells. In some cases, an origin of replication can be used to amplify the copy number of the vector.

Modified PH20 polypeptides also can be utilized or expressed as protein fusions. For example, an enzyme fusion can be generated to add additional functionality to an enzyme. Examples of enzyme fusion proteins include, but are not limited to, fusions of a signal sequence, a tag such as for localization, *e.g.* a his_6 tag or

a myc tag, or a tag for purification, for example, a GST fusion, and a sequence for directing protein secretion and/or membrane association.

For long-term, high-yield production of recombinant proteins, stable expression is desired. For example, cell lines that stably express a modified PH20 polypeptide can be transformed using expression vectors that contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant cells of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell types.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M et al. (1977) Cell, 11:223-32) and adenine phosphoribosyltransferase (Lowy I et al. (1980) Cell, 22:817-23) genes, which can be employed in TK- or APRT- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection. For example, DHFR, which confers resistance to methotrexate (Wigler M et al. (1980) Proc. Natl. Acad. Sci, 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin F et al. (1981) J. Mol. Biol., 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively, can be used. Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of typtophan or hisD, which allows cells to utilize histinol in place of histidine (Hartman SC and RC Mulligan (1988) Proc. Natl. Acad. Sci, 85:8047-51). Visible markers, such as but not limited to, anthocyanins, beta glucuronidase and its substrate, GUS, and luciferase and its substrate luciferin, also can be used to identify transformants and also to quantify the amount of transient or stable protein expression attributable to a particular vector system (Rhodes CA et al. (1995) Methods Mol. Biol. 55:121-131).

The presence and expression of PH20 polypeptides can be monitored. For example, detection of a functional polypeptide can be determined by testing the conditioned media for hyaluronidase enzyme activity under appropriate conditions.

Exemplary assays to assess the solubility and activity of expressed proteins is provided herein.

a. Prokaryotic Cells

Prokaryotes, especially *E. coli*, provide a system for producing large amounts of proteins. Transformation of *E. coli* is simple and rapid technique well known to those of skill in the art. Expression vectors for *E.coli* can contain inducible promoters, such promoters are useful for inducing high levels of protein expression and for expressing proteins that exhibit some toxicity to the host cells. Examples of inducible promoters include the lac promoter, the trp promoter, the hybrid tac promoter, the T7 and SP6 RNA promoters and the temperature regulated λPL promoter.

Proteins, such as any provided herein, can be expressed in the cytoplasmic environment of *E. coli*. The cytoplasm is a reducing environment and for some molecules, this can result in the formation of insoluble inclusion bodies. Reducing agents such as dithiothreotol and β-mercaptoethanol and denaturants, such as guanidine-HCl and urea can be used to resolubilize the proteins. An alternative approach is the expression of proteins in the periplasmic space of bacteria which provides an oxidizing environment and chaperonin-like and disulfide isomerases and can lead to the production of soluble protein. Typically, a leader sequence is fused to the protein to be expressed which directs the protein to the periplasm. The leader is then removed by signal peptidases inside the periplasm. Examples of periplasmic-targeting leader sequences include the pelB leader from the pectate lyase gene and the leader derived from the alkaline phosphatase gene. In some cases, periplasmic expression allows leakage of the expressed protein into the culture medium. The secretion of proteins allows quick and simple purification from the culture supernatant. Proteins that are not secreted can be obtained from the periplasm by osmotic lysis. Similar to cytoplasmic expression, in some cases proteins can become insoluble and denaturants and reducing agents can be used to facilitate solubilization and refolding. Temperature of induction and growth also can influence expression levels and solubility, typically temperatures between 25 °C and 37 °C are used. Typically, bacteria produce aglycosylated proteins. Thus, if proteins require glycosylation for function, glycosylation can be added *in vitro* after purification from host cells.

b. Yeast Cells

Yeasts such as *Saccharomyces cerevisae, Schizosaccharomyces pombe, Yarrowia lipolytica, Kluyveromyces lactis* and *Pichia pastoris* are well known yeast expression hosts that can be used for production of proteins, such as any described herein. Yeast can be transformed with episomal replicating vectors or by stable chromosomal integration by homologous recombination. Typically, inducible promoters are used to regulate gene expression. Examples of such promoters include GAL1, GAL7 and GAL5 and metallothionein promoters, such as CUP1, AOX1 or other *Pichia* or other yeast promoter. Expression vectors often include a selectable marker such as LEU2, TRP1, HIS3 and URA3 for selection and maintenance of the transformed DNA. Proteins expressed in yeast are often soluble. Co-expression with chaperonins such as Bip and protein disulfide isomerase can improve expression levels and solubility. Additionally, proteins expressed in yeast can be directed for secretion using secretion signal peptide fusions such as the yeast mating type alpha-factor secretion signal from *Saccharomyces cerevisae* and fusions with yeast cell surface proteins such as the Aga2p mating adhesion receptor or the *Arxula adeninivoran*s glucoamylase. A protease cleavage site such as for the Kex-2 protease, can be engineered to remove the fused sequences from the expressed polypeptides as they exit the secretion pathway. Yeast also is capable of glycosylation at Asn-X-Ser/Thr motifs.

c. Insects and Insect Cells

Insect cells, particularly using baculovirus expression, are useful for expressing polypeptides such as PH20 polypeptides. Insect cells express high levels of protein and are capable of most of the post-translational modifications used by higher eukaryotes. Baculovirus have a restrictive host range which improves the safety and reduces regulatory concerns of eukaryotic expression. Typical expression vectors use a promoter for high level expression such as the polyhedrin promoter of baculovirus. Commonly used baculovirus systems include the baculoviruses such as Autographa californica nuclear polyhedrosis virus (AcNPV), and the bombyx mori nuclear polyhedrosis virus (BmNPV) and an insect cell line such as Sf9 derived from Spodoptera frugiperda, Pseudaletia unipuncta (A7S) and Danaus plexippus (DpN1). For high-level expression, the nucleotide sequence of the molecule to be expressed is fused immediately downstream of the polyhedrin initiation codon of the virus. Mammalian secretion signals are accurately processed in insect cells and can be used to secrete the expressed protein into the culture medium. In addition, the cell lines

Pseudaletia unipuncta (A7S) and Danaus plexippus (DpN1) produce proteins with glycosylation patterns similar to mammalian cell systems. Exemplary insect cells are those that have been altered to reduce immunogenicity, including those with "mammalianized" baculovirus expression vectors and those lacking the enzyme FT3.

An alternative expression system in insect cells is the use of stably transformed cells. Cell lines such as the Schnieder 2 (S2) and Kc cells (Drosophila melanogaster) and C7 cells (Aedes albopictus) can be used for expression. The Drosophila metallothionein promoter can be used to induce high levels of expression in the presence of heavy metal induction with cadmium or copper. Expression vectors are typically maintained by the use of selectable markers such as neomycin and hygromycin.

d. Mammalian expression

Mammalian expression systems can be used to express proteins including PH20 polypeptides. Expression constructs can be transferred to mammalian cells by viral infection such as adenovirus or by direct DNA transfer such as liposomes, calcium phosphate, DEAE-dextran and by physical means such as electroporation and microinjection. Expression vectors for mammalian cells typically include an mRNA cap site, a TATA box, a translational initiation sequence (Kozak consensus sequence) and polyadenylation elements. IRES elements also can be added to permit bicistronic expression with another gene, such as a selectable marker. Such vectors often include transcriptional promoter-enhancers for high-level expression, for example the SV40 promoter-enhancer, the human cytomegalovirus (CMV) promoter and the long terminal repeat of Rous sarcoma virus (RSV). These promoter-enhancers are active in many cell types. Tissue and cell-type promoters and enhancer regions also can be used for expression. Exemplary promoter/enhancer regions include, but are not limited to, those from genes such as elastase I, insulin, immunoglobulin, mouse mammary tumor virus, albumin, alpha fetoprotein, alpha 1 antitrypsin, beta globin, myelin basic protein, myosin light chain 2, and gonadotropic releasing hormone gene control. Selectable markers can be used to select for and maintain cells with the expression construct. Examples of selectable marker genes include, but are not limited to, hygromycin B phosphotransferase, adenosine deaminase, xanthine-guanine phosphoribosyl transferase, aminoglycoside phosphotransferase, dihydrofolate reductase (DHFR) and thymidine kinase. For example, expression can be performed in the presence of methotrexate to select for only those cells expressing the DHFR

gene. Fusion with cell surface signaling molecules such as TCR-ζ and $Fc_{\varepsilon}RI$-γ can direct expression of the proteins in an active state on the cell surface.

Many cell lines are available for mammalian expression including mouse, rat human, monkey, chicken and hamster cells. Exemplary cell lines include but are not limited to CHO, Balb/3T3, HeLa, MT2, mouse NS0 (nonsecreting) and other myeloma cell lines, hybridoma and heterohybridoma cell lines, lymphocytes, fibroblasts, Sp2/0, COS, NIH3T3, HEK293, 293S, 2B8, and HKB cells. Cell lines also are available adapted to serum-free media which facilitates purification of secreted proteins from the cell culture media. Examples include CHO-S cells (Invitrogen, Carlsbad, CA, cat # 11619-012) and the serum free EBNA-1 cell line (Pham *et al.*, (2003) *Biotechnol. Bioeng. 84*:332-42.). Cell lines also are available that are adapted to grow in special mediums optimized for maximal expression. For example, DG44 CHO cells are adapted to grow in suspension culture in a chemically defined, animal product-free medium.

e. Plants

Transgenic plant cells and plants can be used to express proteins such as any described herein. Expression constructs are typically transferred to plants using direct DNA transfer such as microprojectile bombardment and PEG-mediated transfer into protoplasts, and with agrobacterium-mediated transformation. Expression vectors can include promoter and enhancer sequences, transcriptional termination elements and translational control elements. Expression vectors and transformation techniques are usually divided between dicot hosts, such as *Arabidopsis* and tobacco, and monocot hosts, such as corn and rice. Examples of plant promoters used for expression include the cauliflower mosaic virus promoter, the nopaline syntase promoter, the ribose bisphosphate carboxylase promoter and the ubiquitin and UBQ3 promoters. Selectable markers such as hygromycin, phosphomannose isomerase and neomycin phosphotransferase are often used to facilitate selection and maintenance of transformed cells. Transformed plant cells can be maintained in culture as cells, aggregates (callus tissue) or regenerated into whole plants. Transgenic plant cells also can include algae engineered to produce hyaluronidase polypeptides. Because plants have different glycosylation patterns than mammalian cells, this can influence the choice of protein produced in these hosts.

5. Purification

Host cells transformed with a nucleic acid sequence encoding a modified PH20 polypeptide can be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell is generally secreted, but may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid encoding PH20 can be designed with signal sequences that facilitate direct secretion of PH20 through prokaryotic or eukaryotic cell membrane.

Thus, method for purification of polypeptides from host cells will depend on the chosen host cells and expression systems. For secreted molecules, proteins are generally purified from the culture media after removing the cells. For intracellular expression, cells can be lysed and the proteins purified from the extract. When transgenic organisms such as transgenic plants and animals are used for expression, tissues or organs can be used as starting material to make a lysed cell extract. Additionally, transgenic animal production can include the production of polypeptides in milk or eggs, which can be collected, and if necessary, the proteins can be extracted and further purified using standard methods in the art.

Proteins, such as modified PH20 polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange. Affinity purification techniques also can be utilized to improve the efficiency and purity of the preparations. For example, antibodies, receptors and other molecules that bind PH20 hyaluronidase enzymes can be used in affinity purification. For example, soluble PH20 can be purified from conditioned media.

Expression constructs also can be engineered to add an affinity tag to a protein such as a myc epitope, GST fusion or His_6 and affinity purified with myc antibody, glutathione resin and Ni-resin, respectively. Such tags can be joined to the nucleotide sequence encoding a soluble PH20 as described elsewhere herein, which can facilitate purification of soluble proteins. For example, a modified PH20 polypeptide can be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow

purification on immobilized immunoglobulin and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle Wash.). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the expressed PH20 polypeptide is useful to facilitate purification. One such expression vector provides for expression of a fusion protein containing a PH20 polypeptide in and an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography), while the enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein.

Purity can be assessed by any method known in the art including gel electrophoresis, orthogonal HPLC methods, staining and spectrophotometric techniques. The expressed and purified protein can be analyzed using any assay or method known to one of skill in the art, for example, any described in Section F. These include assays based on the physical and/or functional properties of the protein, including, but not limited to, analysis by gel electrophoresis, immunoassay and assays of hyaluronidase activity.

Depending on the expression system and host cells used, the resulting polypeptide can be heterogeneous due to peptidases present in the culture medium upon production and purification. For example, culture of soluble PH20 in CHO cells can result in a mixture of heterogeneous polypeptides.

6. Modification of Polypeptides by PEGylation

Polyethylene glycol (PEG) has been widely used in biomaterials, biotechnology and medicine primarily because PEG is a biocompatible, nontoxic, water-soluble polymer that is typically nonimmunogenic (Zhao and Harris, *ACS Symposium Series* 680: 458-72, 1997). In the area of drug delivery, PEG derivatives have been widely used in covalent attachment (i. e., "PEGylation") to proteins to reduce immunogenicity, proteolysis and kidney clearance and to enhance solubility (Zalipsky, Adv. Drug Del. Rev. 16:157-82, 1995). Similarly, PEG has been attached to low molecular weight, relatively hydrophobic drugs to enhance solubility, reduce toxicity and alter biodistribution. Typically, PEGylated drugs are injected as solutions.

A closely related application is synthesis of crosslinked degradable PEG networks or formulations for use in drug delivery since much of the same chemistry used in design of degradable, soluble drug carriers can also be used in design of

degradable gels (Sawhney *et al.*, *Macromolecules* 26: 581-87, 1993). It also is known that intermacromolecular complexes can be formed by mixing solutions of two complementary polymers. Such complexes are generally stabilized by electrostatic interactions (polyanion-polycation) and/or hydrogen bonds (polyacid-polybase) between the polymers involved, and/or by hydrophobic interactions between the polymers in an aqueous surrounding (Krupers *et al.*, *Eur. Polym J.* 32:785-790, 1996). For example, mixing solutions of polyacrylic acid (PAAc) and polyethylene oxide (PEO) under the proper conditions results in the formation of complexes based mostly on hydrogen bonding. Dissociation of these complexes at physiologic conditions has been used for delivery of free drugs (*i.e.*, non-PEGylated). In addition, complexes of complementary polymers have been formed from both homopolymers and copolymers.

Numerous reagents for PEGylation have been described in the art. Such reagents include, but are not limited to, reaction of the polypeptide with N-hydroxysuccinimidyl (NHS) activated PEG, succinimidyl mPEG, $mPEG_2$-N-hydroxysuccinimide, mPEG succinimidyl alpha-methylbutanoate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, mPEG carboxymethyl 3-hydroxybutanoic acid succinimidyl ester, homobifunctional PEG-succinimidyl propionate, homobifunctional PEG propionaldehyde, homobifunctional PEG butyraldehyde, PEG maleimide, PEG hydrazide, p-nitrophenyl-carbonate PEG, mPEG-benzotriazole carbonate, propionaldehyde PEG, mPEG butryaldehyde, branched $mPEG_2$ butyraldehyde, mPEG acetyl, mPEG piperidone, mPEG methylketone, mPEG "linkerless" maleimide, mPEG vinyl sulfone, mPEG thiol, mPEG orthopyridylthioester, mPEG orthopyridyl disulfide, Fmoc-PEG-NHS, Boc-PEG-NHS, vinylsulfone PEG-NHS, acrylate PEG-NHS, fluorescein PEG-NHS, and biotin PEG-NHS (*see e.g.*, Monfardini *et al.*, *Bioconjugate Chem.* 6:62-69, 1995; Veronese *et al.*, *J. Bioactive Compatible Polymers* 12:197-207, 1997; U.S. 5,672,662; U.S. 5,932,462; U.S. 6,495,659; U.S. 6,737,505; U.S. 4,002,531; U.S. 4,179,337; U.S. 5,122,614; U.S. 5,324, 844; U.S. 5,446,090; U.S. 5,612,460; U.S. 5,643,575; U.S. 5,766,581; U.S. 5,795, 569; U.S. 5,808,096; U.S. 5,900,461; U.S. 5,919,455; U.S. 5,985,263; U.S. 5,990, 237; U.S. 6,113,906; U.S. 6,214,966; U.S. 6,258,351; U.S. 6,340,742; U.S. 6,413,507; U.S. 6,420,339; U.S. 6,437,025; U.S. 6,448,369; U.S. 6,461,802; U.S. 6,828,401; U.S. 6,858,736; U.S. 2001/0021763; U.S. 2001/0044526; U.S. 2001/0046481; U.S. 2002/0052430; U.S. 2002/0072573; U.S. 2002/0156047;

U.S. 2003/0114647; U.S. 2003/0143596; U.S. 2003/0158333; U.S. 2003/0220447; U.S. 2004/0013637; US 2004/0235734; WO0500360; U.S. 2005/0114037; U.S. 2005/0171328; U.S. 2005/0209416; EP 01064951; EP 0822199; WO 00176640; WO 0002017; WO 0249673; WO 9428024; and WO 0187925).

In one example, the polyethylene glycol has a molecular weight ranging from about 3 kD to about 50 kD, and typically from about 5 kD to about 30 kD. Covalent attachment of the PEG to the drug (known as "PEGylation") can be accomplished by known chemical synthesis techniques. For example, the PEGylation of protein can be accomplished by reacting NHS-activated PEG with the protein under suitable reaction conditions.

While numerous reactions have been described for PEGylation, those that are most generally applicable confer directionality, utilize mild reaction conditions, and do not necessitate extensive downstream processing to remove toxic catalysts or bi-products. For instance, monomethoxy PEG (mPEG) has only one reactive terminal hydroxyl, and thus its use limits some of the heterogeneity of the resulting PEG-protein product mixture. Activation of the hydroxyl group at the end of the polymer opposite to the terminal methoxy group is generally necessary to accomplish efficient protein PEGylation, with the aim being to make the derivatised PEG more susceptible to nucleophilic attack. The attacking nucleophile is usually the epsilon-amino group of a lysyl residue, but other amines also can react (*e.g.* the N-terminal alpha-amine or the ring amines of histidine) if local conditions are favorable. A more directed attachment is possible in proteins containing a single lysine or cysteine. The latter residue can be targeted by PEG-maleimide for thiol-specific modification. Alternatively, PEG hydrazide can be reacted with a periodate oxidized hyaluronan-degrading enzyme and reduced in the presence of $NaCNBH_3$. More specifically, PEGylated CMP sugars can be reacted with a hyaluronan-degrading enzyme in the presence of appropriate glycosyl-transferases. One technique is the "PEGylation" technique where a number of polymeric molecules are coupled to the polypeptide in question. When using this technique the immune system has difficulties in recognizing the epitopes on the polypeptide's surface responsible for the formation of antibodies, thereby reducing the immune response. For polypeptides introduced directly into the circulatory system of the human body to give a particular physiological effect (*i.e.* pharmaceuticals) the typical potential immune response is an IgG and/or IgM response, while polypeptides which are inhaled through the

respiratory system (*i.e.* industrial polypeptide) potentially can cause an IgE response (*i.e.* allergic response). One of the theories explaining the reduced immune response is that the polymeric molecule(s) shield(s) epitope(s) on the surface of the polypeptide responsible for the immune response leading to antibody formation. Another theory or at least a partial factor is that the heavier the conjugate is, the more reduced immune response is obtained.

Typically, to make the PEGylated PH20 polypeptide provided herein, PEG moieties are conjugated, via covalent attachment, to the polypeptides. Techniques for PEGylation include, but are not limited to, specialized linkers and coupling chemistries (*see e.g.*, Harris, *Adv. Drug Deliv. Rev.* 54:459-476, 2002), attachment of multiple PEG moieties to a single conjugation site (such as via use of branched PEGs; *see e.g.*, Veronese *et al.*, *Bioorg. Med. Chem. Lett.* 12:177-180, 2002), site-specific PEGylation and/or mono-PEGylation (*see e.g.*, Chapman *et al.*, *Nature Biotech.* 17:780-783, 1999), and site-directed enzymatic PEGylation (*see e.g.*, Sato, *Adv. Drug Deliv. Rev.*, 54:487-504, 2002). Methods and techniques described in the art can produce proteins having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 PEG or PEG derivatives attached to a single protein molecule (*see e.g.*, U.S. 2006/0104968).

As an exemplary illustration of the PEGylation of an illustrative method for making PEGylated PH20 polypeptide, PEG aldehydes, succinimides and carbonates have each been applied to conjugate PEG moieties, typically succinimidyl PEGs, to rHuPH20. For example, rHuPH20 has been conjugated with exemplary succinimidyl monoPEG (mPEG) reagents including mPEG-Succinimidyl Propionates (mPEG-SPA), mPEG-Succinimidyl Butanoates (mPEG-SBA), and (for attaching "branched" PEGs) mPEG2-N-Hydroxylsuccinimide. These PEGylated succinimidyl esters contain different length carbon backbones between the PEG group and the activated cross-linker, and either a single or branched PEG group. These differences can be used, for example, to provide for different reaction kinetics and to potentially restrict sites available for PEG attachment to rHuPH20 during the conjugation process.

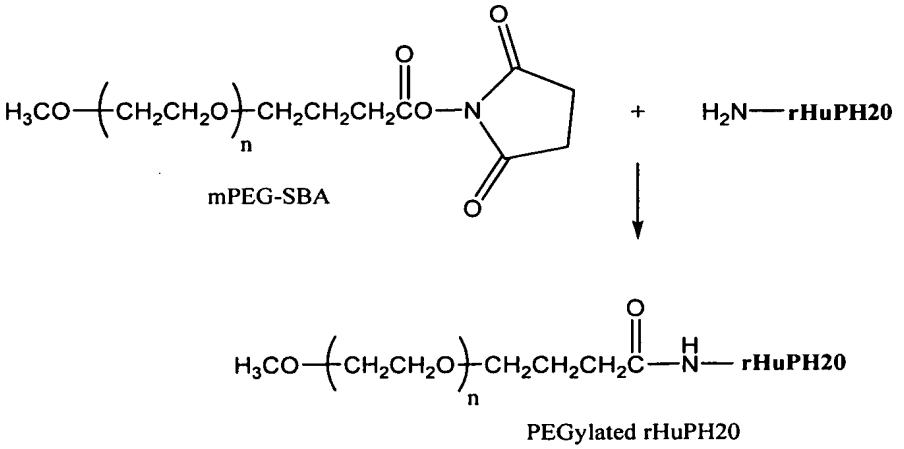
Succinimidyl PEGs (as above) containing either linear or branched PEGs can be conjugated to PH20. PEGs can used to generate PH20s reproducibly containing molecules having, on the average, between about three to six or three to six PEG molecules per hyaluronidase. Such PEGylated rHuPH20 compositions can be readily purified to yield compositions having specific activities of approximately 25,000 or

30,000 Unit/mg protein hyaluronidase activity, and being substantially free of non-PEGylated PH20 (less than 5 % non-PEGylated).

Using various PEG reagents, exemplary versions of a PEGylated PH20 polypeptide can be prepared, for example, using mPEG-SBA (30 kD), mPEG-SMB (30 kD), and branched versions based on mPEG2-NHS (40 kD) and mPEG2-NHS (60 kD). PEGylated versions of PH20 can be generated using NHS chemistries, as well as carbonates, and aldehydes, using each of the following reagents: mPEG2-NHS-40K branched, mPEG-NHS-10K branched, mPEG-NHS-20K branched, mPEG2-NHS-60K branched; mPEG-SBA-5K, mPEG-SBA-20K, mPEG-SBA-30K; mPEG-SMB-20K, mPEG-SMB-30K; mPEG-butyrldehyde; mPEG-SPA-20K, mPEG-SPA-30K; and PEG-NHS-5K-biotin. PEGylated PH20 also can be prepared using PEG reagents available from Dowpharma, a division of Dow Chemical Corporation; including PH20 polypeptides PEGylated with Dowpharma's p-nitrophenyl-carbonate PEG (30 kDa) and with propionaldehyde PEG (30 kDa).

In one example, the PEGylation includes conjugation of mPEG-SBA, for example, mPEG-SBA-30K (having a molecular weight of about 30 kDa) or another succinimidyl esters of PEG butanoic acid derivative, to a PH20 polypeptide. Succinimidyl esters of PEG butanoic acid derivatives, such as mPEG-SBA-30K readily couple to amino groups of proteins. For example, covalent conjugation of m-PEG-SBA-30K and rHuPH20 (which is approximately 60 KDa in size) provides stable amide bonds between rHuPH20 and mPEG, as shown in Scheme 1, below.

Scheme 1



Typically, the mPEG-SBA-30K or other PEG is added to the PH20 polypeptide at a PEG:polypeptide molar ratio of 10:1 in a suitable buffer, *e.g.* 130 mM NaCl /10 mM HEPES at pH 6.8 or 70 mM phosphate buffer, pH 7, followed by

sterilization, *e.g.* sterile filtration, and continued conjugation, for example, with stirring, overnight at 4 °C in a cold room. In one example, the conjugated PEG- PH20 is concentrated and buffer-exchanged.

Other methods of coupling succinimidyl esters of PEG butanoic acid derivatives, such as mPEG-SBA-30K are known in the art (*see e.g.*, U.S. 5,672,662; U.S. 6,737,505; and U.S. 2004/0235734). For example, a polypeptide, such as a PH20 polypeptide, can be coupled to an NHS activated PEG derivative by reaction in a borate buffer (0.1 M, pH 8.0) for one hour at 4 °C. The resulting PEGylated protein can be purified by ultrafiltration. Another method reacts polypeptide with mPEG-SBA in deionized water to which triethylamine is added to raise the pH to 7.2-9. The resulting mixture is stirred at room temperature for several hours to complete the PEGylation.

Methods for PEGylation of PH20 polypeptides, including, for example, animal-derived hyaluronidases and bacterial hyaluronan-degrading enzymes, are known to one of skill in the art. See, for example, European Patent No. EP 0400472, which describes the PEGylation of bovine testes hyaluorindase and chondroitin ABC lyase. Also, U.S. Publication No. 2006014968 describes PEGylation of a human hyaluronidase derived from human PH20. For example, the PEGylated hyaluronan-degrading enzyme generally contains at least 3 PEG moieties per molecule. In some examples, the PH20 polypeptide contains three to six PEG molecules. In other examples, the enzyme can have a PEG to protein molar ratio between 5:1 and 9:1, for example, 7:1.

E. Pharmaceutical Compositions and Formulations, Dosages and Administration

Pharmaceutical compositions of any of the modified PH20 polypeptides are provided herein for administration. Pharmaceutically acceptable compositions are prepared in view of approvals for a regulatory agency or other agency prepared in accordance with generally recognized pharmacopeia for use in animals and in humans. Typically, the compounds are formulated into pharmaceutical compositions using techniques and procedures well known in the art (*see e.g.*, Ansel *Introduction to Pharmaceutical Dosage Forms*, Fourth Edition, 1985, 126).

In particular, provided herein are pharmaceutical compositions that are stable as a liquid formulation for prolonged periods of time for at least 1 month at temperatures from or from about 2°C to 8°C, inclusive or for at least 3 days at a

temperature from or from about 30°C to 42°C, inclusive, for at least 3 days. Pharmaceutical compositions formulations, in particular liquid formulations, can be limited by the stability of the active agent, which can be susceptible to effects of storage conditions (time or length of storage, temperature and/or agitation) and/or formulation components contained in the composition. Hence, the stable pharmaceutical compositions generally contain a modified PH20 polypeptide as described in Section C.1.b that exhibits increased stability manifested as an increased resistance to one or more protein denaturation conditions. Such protein denaturation conditions can include, but are not limited to, elevated temperature greater than or equal to or about 30°C, agitation, low or no salt, and presence of excipients. The increased stability is characterized by improved storage time, decreased fragmentation, and/or decreased aggregate formation, while still retaining the activity of the active agent(s), *e.g.* the PH20 hyaluronidase. Such formulations can be provided as "ready-to use" liquid formulations without further reconstitution and/or without any requirement for further dilution. In some examples, the formulations also can be prepared in lyophilized or concentrated form.

Pharmaceutical compositions containing a modified PH20 polypeptide can be co-administered with another therapeutic agent. In such examples, the modified PH20 polypeptides can be formulated separately as a pharmaceutical composition and administered prior to, simultaneously with, intermittently or subsequently with a second composition containing an active therapeutic agent. In other examples, modified PH20 polypeptides can be co-formulated with pharmaceutical formulations of other therapeutic agents.

In particular, provided herein are co-formulations containing a modified PH20 polypeptide as described herein and a therapeutic agent that is a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, and antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a

electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, a polypeptide, a protein, a nucleic acid, a drug, an organic molecule or a sleep inducer. For example, modified PH20 polypeptides provided herein can be co-formulated with an antibody such as a monoclonal antibody, an Immune Globulin, an antibiotic, a bisphosphonate, a cytokine, a chemotherapeutic agent, a coagulation factor or an insulin. Exemplary therapeutic agents that can be co-formulated with a modified PH20 polypeptide are described in described in Section G. In particular, provided herein are co-formulations containing a modified PH20 polypeptide and an insulin, such as a fast-acting insulin, for example, a regular insulin or a fast-acting (rapid-acting) insulin analog. The co-formulations provided herein include stable co-formulations, whereby the active agents, *i.e.* the modified PH20 polypeptide and the therapeutic agent, exhibit increased stability and retain activity for prolonged periods as described herein.

Formulations containing PH20 provided herein, including separate formulations thereof and co-formulations, are stable for prolonged periods of time, including at varied temperatures and under varied storage or use conditions such as agitation. For example, the formulations provided herein are stable and retain activity of active agent(s) (*e.g.* PH20 hyaluronidase) at "refrigerator" conditions, for example, at 2° C to 8° C, such as at or about 4° C, for at least at least 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months or 30 months or more. In another example, the formulations provided herein are stable and retain activity of active agent(s) (*e.g.* PH20 hyaluornidase) at room temperature for example at 18° C to 32° C, generally 20° C to 32° C, such as 28° C to 32° C, for at least 2 weeks to 1 year, for example, at least 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, at least 7 months, at least 8 months, at least 9 months, or at least 1 year or more. In a further example, the formulations provided herein are stable and retain activity of active agent(s) (*e.g.* PH20 hyaluronidase) at elevated

temperatures of about or greater than 30 ° C, generally from or from about 30°C to 42°C, such as 32°C to 37°C or 35°C to 37°C or about or 37°C for at least 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 35 days, 40 days, 45 days, 50 days, 60 days or more.

Compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, and sustained release formulations. A composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and other such agents. Topical formulations also are contemplated. The formulation should suit the mode of administration.

1. Formulations – liquids, injectables, emulsions

The formulation generally is made to suit the route of administration. Parenteral administration, generally characterized by injection or infusion, either subcutaneously, intramuscularly, intravenous or intradermally is contemplated herein. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. For example, the compositions containing a modified PH20 polypeptide, formulated separately or co-formulated with another therapeutic agent, can be provided as a pharmaceutical preparation in liquid form as solutions, syrups or suspensions. In liquid form, the pharmaceutical preparations can be provided as a concentrated preparation to be diluted to a therapeutically effective concentration before use. Generally, the preparations are provided in a dosage form that does not require dilution for use. In another example, pharmaceutical preparations can be presented in lyophilized form for reconstitution with water or other suitable vehicle before use.

Injectables are designed for local and systemic administration. For purposes herein, local administration is desired for direct administration to the affected interstitium. The solutions can be either aqueous or nonaqueous. If administered

intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

The concentration of the pharmaceutically active compound is adjusted so that an injection or infusion provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art. The unit-dose parenteral preparations can be packaged in, for example, an ampoule, a cartridge, a vial or a syringe with a needle. The volume of liquid solution or reconstituted powder preparation, containing the pharmaceutically active compound, is a function of the disease to be treated and the particular article of manufacture chosen for package. All preparations for parenteral administration must be sterile, as is known and practiced in the art. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject.

Pharmaceutical compositions can include carriers or other excipients. For example, pharmaceutical compositions provided herein can contain any one or more of a diluents(s), adjuvant(s), antiadherent(s), binder(s), coating(s), filler(s), flavor(s), color(s), lubricant(s), glidant(s), preservative(s), detergent(s), sorbent(s) or sweetner(s) and a combination thereof or vehicle with which a modified PH20 polypeptide is administered. For example, pharmaceutically acceptable carriers or excipients used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Formulations, including liquid preparations, can be prepared by conventional means with pharmaceutically acceptable additives or excipients.

Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, generally in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and sesame oil. Water is a typical carrier when the

pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions also can be employed as liquid carriers, particularly for injectable solutions. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Suspending and dispersing agents include, but are not limited to, sorbitol syrup, cellulose derivatives or hydrogenated edible fats, sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include, but are not limited to, lecithin or acacia. Detergents include, but are not limited to, Polysorbate 80 (TWEEN 80). Non-aqueous vehicles include, but are not limited to, almond oil, oily esters, or fractionated vegetable oils. Anti-microbial agent or preservatives include, but are not limited to, methyl or propyl-p-hydroxybenzoates or sorbic acid, m-cresol, phenol. A diluent includes, but is not limited to, lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose. A lubricant includes, but is not limited to, magnesium stearate, calcium stearate or talc. A binder includes, but is not limited to, starch, natural gums, such as gum acacia, gelatin, glucose, molasses, polyvinylpyrrolidine, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Isotonic agents include, but are not limited to, sodium chloride and dextrose. Buffers include, but are not limited to, phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. A sequestering or chelating agent of metal ions include EDTA. Other suitable pharmaceutical excipients include, but are not limited to, starch, glucose, lactose, dextrose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, saline, water, and ethanol. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment. A composition, if desired, also can contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, or pH buffering agents, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, stabilizers, solubility enhancers, and other such agents such as for example, sodium acetate, sodium phosphate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

In particular, antimicrobial agents (*e.g.* preservatives) in bacteriostatic or fungistatic concentrations (*e.g.* an anti-microbial effective amount) can be added to parenteral preparations packaged in multiple-dose containers, which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride.

The volume of the formulations, including the separately formulated or co-formulated PH20-containing formulations provided herein, can be any volume suitable for the container in which it is provided. In some examples, the formulations are provided in a vial, syringe, pen, reservoir for a pump or a closed loop system, or any other suitable container. For example, the formulations provided herein are between or about between 0.1 mL to 500 mL, such as 0.1 mL to 100 mL, 1 mL to 100 mL, 0.1 mL to 50 mL, such as at least or about at least or about or 0.1 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL, 15 mL, 20 mL, 30 mL, 40 mL, 50 mL or more.

a. Lyophilized Powders

Of interest herein are lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

The sterile, lyophilized powder is prepared by dissolving a compound of enzyme in a buffer solution. The buffer solution may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. A liquid formulation as described herein above can be prepared. The resulting mixture is sterile filtered or treated to remove particulates and to insure sterility, and apportioned into vials for lyophilization. For example, the lyophilized powder can be prepared by dissolving an excipient, such as dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent, in a suitable buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art. Then, a selected enzyme is added to the resulting mixture, and stirred until it dissolves.

Each vial is made to contain a single dosage or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature. Reconstitution of this lyophilized powder with

an appropriate buffer solution provides a formulation for use in parenteral administration.

b. Exemplary Formulations

Single dose formulations of PH20 are known in the art. For example, Hylenex® recombinant (hyaluronidase human injection) contains, per mL, 8.5 mg NaCl (145 mM), 1.4 mg dibasic sodium phosphate (9.9 mM), 1.0 mg human albumin, 0.9 mg edetate disodium (2.4 mM), 0.3 mg $CaCl_2$ (2.7 mM) and NaOH to adjust the pH to 7.4. Other formulations of human soluble hyaluronidase, such as the rHuPH20 formulations described in U.S. Pat. Pub. No. US2011/0053247, include 130 mM NaCl, 10 mM Hepes, pH 7.0; or 10 mM histidine, 130 mM NaCl, pH 6.0.

In addition to a therapeutically effective amount of a modified PH20 polypeptide and/or other therapeutic agent, exemplary pharmaceutical compositions provided herein, including separately formulated- and co-formulated-PH20 containing formulations, contain a concentration of NaCl and are prepared at a requisite pH to maintain the stability of the active agent(s) (*e.g.* PH20 hyaluronidase and/or other co-formulated therapeutic agent). For multi-dose formulations and other formulations stored for a prolonged time, the compositions generally also contain one or more preservatives. Further stabilizing agents and other excipients also can be included. Exemplary components are described below.

i. NaCl

In examples herein, the pharmaceutical compositions provided herein contain a concentration of sodium chloride (NaCl) to maintain the stability of the active agent(s) (*e.g.* PH20 hyaluornidase). NaCl is generally required to retain PH20 stability and activity. Low salt concentrations of generally less than 120 mM can have deleterious effects on PH20 activity over time and depending on temperature conditions. Hence, the absence of NaCl or a low concentration of NaCl can result in instability of the protein. In some examples herein, however, modified PH20 polypeptides that exhibit increased stability in the absence of low of no NaCl (*see e.g.* Section C.1.b.iii) are not susceptible to denaturation. Also, the presence of NaCl can have differeing effects on other therapeutic agents. For example, the solubility of insulin and insulin analogs tends to increase with lower salt concentration (*e.g.* <140 mM) and high salt concentrations can result in crystallization/aggregation of insulin, especially at lower temperatures (*see e.g.* U.S. Provisional Appl. No. 61/520,962). Thus, pharmaceutical compositions provided herein are prepared in accordance with

the requirements of the active agent(s). It is within the level of one of skill in the art to assess the stability of the active agent(s) in the formulation and under various storage conditions (*see e.g.* Section F). In particular examples herein, the pharmaceutical compositions, including the separately formulated or co-formulated PH20-containing formulations provided herein, contain NaCl at a concentration of between or about between 10 mM to 200 mM, such as 10 mM to 50 mM, 50 mM to 200 mM, 50 mM to 120 mM, 50 mM to 100 mM, 50 mM to 90 mM, 120 mM to 160 mM, 130 mM to 150 mM, 80-140 mM, 80 mM to 120 mM, 80 mM to 100 mMM, 80 mM to 160 mM, 100 mM to 140 mM, 120 mM to 120 mM or 140 mM to 180 mM.

ii. pH and Buffer

In examples herein, the pharmaceutical compositions provided herein are prepared at a pH to maintain the stability of the active agent(s) (*e.g.* PH20 hyaluornidase). For example, the pharmaceutical compositions provided herein are prepared at a pH of between or about between 6.5 to 7.8 such as between or about between 6.5 to 7.2, 7.0 to 7.8, 7.0 to 7.6 or 7.2 to 7.4. Reference to pH herein is based on measurement of pH at room temperature. It is understood that the pH can change during storage over time, but typically will remain between or between about pH 6.5 to or to about 7.8. For example, the pH can vary by ± 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.3, 1.4, 1.5 or more. Exemplary co-formulations provided herein have a pH of or of about 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2, 7.5 ± 0.2 or 7.6 ± 0.2 when prepared. If necessary, pH can be adjusted using acidifying agents to lower the pH or alkalizing agents to increase the pH. Exemplary acidifying agents include, but are not limited to, acetic acid, citric acid, sulfuric acid, hydrochloric acid, monobasic sodium phosphate solution, and phosphoric acid. Exemplary alkalizing agents include, but are not limited to, dibasic sodium phosphate solution, sodium carbonate, or sodium hydroxide.

The compositions are generally prepared using a buffering agent that maintains the pH range. Any buffer can be used in formulations provided herein so long as it does not adversely affect the stability of the active agent(s) (*e.g.* PH20 hyaluronidase), and supports the requisite pH range required. Examples of particularly suitable buffers include Tris, succinate, acetate, phosphate buffers, citrate, aconitate, malate and carbonate. Those of skill in the art, however, will recognize that formulations provided herein are not limited to a particular buffer, so long as the buffer provides an acceptable degree of pH stability, or "buffer capacity" in the range

indicated. Generally, a buffer has an adequate buffer capacity within about 1 pH unit of its pK (Lachman *et al.* 1986). Buffer suitability can be estimated based on published pK tabulations or can be determined empirically by methods well known in the art. The pH of the solution can be adjusted to the desired endpoint within the range as described above, for example, using any acceptable acid or base.

Buffers that can be included in the co-formulations provided herein include, but are not limited to, Tris (Tromethamine), histidine, phosphate buffers, such as dibasic sodium phosphate, and citrate buffers. Such buffering agents can be present in the co-formulations at concentrations between or about between 1 mM to 100 mM, such as 10 mM to 50 mM or 20 mM to 40 mM, such as at or about 30 mM. For example, such buffering agents can be present in the co-formulations in a concentration of or about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, or more.

iii. Preservative(s)

In examples herein, multi-dose formulations or formulations stored for prolonged periods contain an anti-microbially effective amount of preservative or mixture of preservatives in an amount to have a bacteriostatic or fungistatic effect. In particular examples, the preservatives are present in a sufficient concentration to provide the anti-microbial requirements of, for example, the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP), including the EP anti-microbial requirements (EPA) and the preferred EP anti-microbial requirements (EPB) (see Table 4). Since the presence of preservatives, and in particular phenolic preservatives, can have deleterious effects on the stability of PH20, such formulations typically contain a modified PH20 polypeptide that exhibits increased stability in the presence of preservatives, such as any described in Section C.1.b.i herein. Generally, the amount maintains the stability of the active agent(s) (*e.g.* PH20 hyaluoronidase).

An anti-microbial effective amount of preservative is an amount that exhibits anti-microbial activity by killing or inhibiting the propagation of microbial organisms in a sample of the composition as assessed in an antimicrobial preservative effectiveness test (APET). One of skill in the art is familiar with the antimicrobial preservative effectiveness test and standards to be meet under the USP and EPA or EPB in order to meet minimum requirements. In general, the antimicrobial

preservative effectiveness test involves challenging a composition with prescribed inoculums of suitable microorganisms, *i.e.*, bacteria, yeast and fungi, storing the inoculated preparation at a prescribed temperature, withdrawing samples at specified intervals of time and counting the organisms in the sample (see, Sutton and Porter, (2002) *PDA Journal of Pharmaceutical Science and Technology* 56(11);300-311; The United States Pharmacopeial Concention, Inc., (effective January 1, 2002), *The United States Pharmacopeia 25th Revision*, Rockville, MD, Chapter <51> Antimicrobial Effectiveness Testing; and European Pharmacopoeia, Chapter 5.1.3, Efficacy of Animicrobial Preservation). The microorganisms used in the challenge generally include three strains of bacteria, namely *E. coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027) and *Staphylococcus aureus* (ATCC No. 6538), yeast (*Candida albicans* ATCC No. 10231) and fungus (*Aspergillus niger* ATCC No. 16404), all of which are added such that the inoculated composition contains 10^5 or 10^6 colony forming units (cfu) of microorganism per mL of composition. The preservative properties of the composition are deemed adequate if, under the conditions of the test, there is a significant fall or no increase, as specified in Table 3 in the number of microorganisms in the inoculated composition after the times and at the temperatures prescribed. The criteria for evaluation are given in terms of the log reduction in the number of viable microorganism as compared to the initial sample or the previous timepoint.

Non-limiting examples of preservatives that can be included in the co-formulations provided herein include, but are not limited to, phenol, meta-cresol (m-cresol), methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol (2-bromo-2-nitropropane-1,3-diol), phenylmercuric acetate, glycerol (glycerin), imidurea, chlorohexidine, sodium dehydroacetate, ortho-cresol (o-cresol), para-cresol (p-cresol), chlorocresol, cetrimide, benzethonium chloride, ethylparaben, propylparaben or butylparaben and any combination thereof. For example, formulations provided herein can contain a single preservative. In other examples, the formulations contain at least two different preservatives or at least three different preservatives. For example, formulations provided herein can contain two preservatives such as L-phenylalanine and m-cresol, L-phenylalanine and methylparaben, L-phenylalanine and phenol, m-cresol and methylparaben, phenol and methylparaben, m-cresol and phenol or other similar combinations. In one example,

the preservative in the formulation contains at least one phenolic preservative. For example, the formulation contains phenol, m-cresol or phenol and m-cresol.

In the formulations provided herein, the total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the formulation can be, for example, between from or between about from 0.1% to 0.4%, such as 0.1% to 0.3%, 0.15% to 0.325%, 0.15% to 0.25%, 0.1% to 0.2%, 0.2% to 0.3%, or 0.3% to 0.4%. Generally, the formulations contain less than 0.4% (w/v) preservative. For example, the co-formulations provided herein contain at least or about at least 0.1% , 0.12%, 0.125%, 0.13%, 0.14%, 0.15%, 0.16% 0.17%, 0.175%, 0.18%, 0.19%, 0.2%, 0.25%, 0.3%, 0.325%, 0.35% but less than 0.4% total preservative.

In some examples, the formulations provided herein contain between or between about 0.1% to 0.25% phenol, and between or about between 0.05% to 0.2% m-cresol, such as between or about between 0.10% to 0.2% phenol and between or about between 0.6% to 0.18% m-cresol or between or about between 0.1% to 0.15% phenol and between or about between 0.8% to 0.15% m-cresol. For example, formulations provided herein contain or contain about 0.1% phenol and 0.075% m-cresol; 0.1% phenol and 0.15% m-cresol; 0.125% phenol and 0.075% m-cresol; 0.13% phenol and 0.075% m-cresol; 0.13% phenol and 0.08% m-cresol; 0.15% phenol and 0.175% m-cresol; or 0.17% phenol and 0.13% m-cresol.

iv. Stabilizers

In examples herein, the pharmaceutical compositions provided herein optionally can contain one or more other stabilizing agent to maintain the stability of the active agent(s) (*e.g.* PH20 hyaluronidase). Included among the types of stabilizers that can be contained in the formulations provided herein are amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, and other agents. The formulations provided herein contain at least one stabilizer. For example, the formulations provided herein contain at least one, two, three, four, five, six or more stabilizers. Hence, any one or more of an amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, and other agents can be included in the formulations herein. Generally, the formulations herein contain at least contain a surfactant and an appropriate buffer. Optionally, the formulations provided herein can contain other additional stabilizers. Other components include,

for example, one or more tonicity modifiers, one or more anti-oxidation agents, or other stabilizer.

Exemplary amino acid stabilizers, amino acid derivatives or amines include, but are not limited to, L-Arginine, Glutamine, glycine, Lysine, Methionine, Proline, Lys-Lys, Gly-gly, Trimethylamine oxide (TMAO) or betaine. Exemplary of sugars and polyols include, but are not limited to, glycerol, sorbitol, mannitol, inositol, sucrose or trehalose. Exemplary of salts and buffers include, but are not limited to, magnesium chloride, sodium sulfate, Tris such as Tris (100 mM), or sodium Benzoate. Exemplary surfactants include, but are not limited to, poloxamer 188 (*e.g.* Pluronic® F68), polysorbate 80 (PS80), polysorbate 20 (PS20). Other stabilizers include, but are not limited to, hyaluronic acid (HA), human serium albumin (HSA), phenyl butyric acid, taurocholic acid, polyvinylpyrolidone (PVP) or zinc.
In particular examples herein, the formulations contain one or more detergents, such as surfactants, to maintain the stability of the active agent(s) (*e.g.* PH20 hyaluronidase). For example, surfactants can inhibit aggregation of PH20 and minimize absorptive loss. The surfactants generally are non-ionic surfactants. Surfactants that can be included in the formulations herein include, but are not limited to, partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol, or sorbitol, poloxamers and polysorbates. For example, exemplary surfactants in the - formulations herein include any one or more of poloxamer 188 (PLURONICS® such as PLURONIC® F68), TETRONICS®, polysorbate 20, polysorbate 80, PEG 400, PEG 3000, Tween® (*e.g.* Tween® 20 or Tween® 80), Triton® X-100, SPAN®, MYRJ®, BRIJ®, CREMOPHOR®, polypropylene glycols or polyethylene glycols. In some examples, the formulations herein contain poloxamer 188, polysorbate 20, polysorbate 80, generally poloxamer 188 (pluronic F68). The formulations provided herein generally contain at least one surfactant, such as 1, 2 or 3 surfactants.

In the formulations provided herein, the total amount of the one or more surfactants as a percentage (%) of mass concentration (w/v) in the formulation can be, for example, between from or between about from 0.005% to 1.0%, such as between from or between about from 0.01% to 0.5%, such as 0.01% to 0.1% or 0.01% to 0.02%. Generally, the formulations contain at least 0.01% surfactant and contain less than 1.0%, such as less than 0.5% or less than 0.1% surfactant. For example, the formulations provided herein can contain at or about 0.001%, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%,

0.065%, 0.07%, 0.08%, or 0.09%. In particular examples, the formulations provided herein contain or contain about 0.01% to or to about 0.05% surfactant.

Tonicity modifiers can be included in the formulation provided herein to produce a solution with the desired osmolarity. The formulations provided herein have an osmolarity of between or about between 245 mOsm/kg to 305 mOsm/kg. For example, the osmolarity is or is about 245 mOsm/kg, 250 mOsm/kg, 255 mOsm/kg, 260 mOsm/kg, 265 mOsm/kg, 270 mOsm/kg, 275 mOsm/kg, 280 mOsm/kg, 285 mOsm/kg, 290 mOsm/kg, 295 mOsm/kg, 300 mOsm/kg or 305 mOsm/kg. In some examples, the formulations have an osmolarity of or of about 275 mOsm/kg. Tonicity modifiers include, but are not limited to, glycerin, NaCl, amino acids, polyalcohols, trehalose, and other salts and/or sugars. The particular amount can be empirically determined in order to retain enzyme activity, and/or tonicity.

In other instances, glycerin (glycerol) is included in the formulations. For example, formulations provided herein typically contain less than 60 mM glycerin, such as less than 55 mM, less than 50 mM, less than 45 mM, less than 40 mM, less than 35 mM, less than 30 mM, less than 25 mM, less than 20 mM, less than 15 mM, 10 mM or less. The amount of glycerin typically depends on the amount of NaCl present: the more NaCl present in the formulation, the less glycerin is required to achieve the desired osmolarity. Thus, for example, in formulations containing higher NaCl concentrations, little or no glycerin need be included in the formulation. In contrast, in formulations containing slightly lower NaCl concentrations, glycerin can be included. For example, formulations provided herein can contain glycerin at a concentration of 40 mM to 60 mM, such as less than 50 mM, such as 20 mM to 50 mM, for example at or about 50 mM.

The formulations provided herein also can contain antioxidants to reduce or prevent oxidation, in particular oxidation of the PH20 polypeptide. For example, oxidation can be effected by high concentrations of surfactant or hyaluronan oligomers. Exemplary antioxidants include, but are not limited to, cysteine, tryptophan and methionine. In particular examples, the anti-oxidant is methionine. The formulations provided herein can include an antioxidant at a concentration from between or from about between 5 mM to or to about 50 mM, such as 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM. For example, methionine can be provided in the formulations herein at a concentration from between or from about between 5 mM to or to about 50 mM, such as 5 mM to 40 mM, 5 mM to 20 mM or 10

mM to 20 mM. For example, an antioxidant, for example methionine, can be included at a concentration that is or is about 5 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM. In some examples, the formulations contain 10 mM to 20 mM methionine, such as or about 10 mM or 20 mM methionine.

The formulations provided herein also can contain an amino acid stabilizer, which contributes to the stability of the preparation. The stabilizer can be a non-polar or basic amino acids. Exemplary non-polar and basic amino acids include, but are not limited to, alanine, histidine, arginine, lysine, ornithine, isoleucine, valine, methionine, glycine and proline. For example, the amino acid stabilizer is glycine or proline, typically glycine. The stabilizer can be a single amino acid or it can be a combination of 2 or more such amino acids. The amino acid stabilizers can be natural amino acids, amino acid analogues, modified amino acids or amino acid equivalents. Generally, the amino acid is an L-amino acid. For example, when proline is used as the stabilizer, it is generally L-proline. It is also possible to use amino acid equivalents, for example, proline analogues. The concentration of amino acid stabilizer, for example glycine, included in the formulation ranges from 0.1 M to 1 M amino acid, typically 0.1 M to 0.75 M, generally 0.2 M to 0.5 M, for example, at least at or about 0.1 M, 0.15 M, 0.2 M, 0.25 M, 0.3 M, 0.35 M, 0.4 M, 0.45 M, 0.5 M, 0.6 M, 0.7 M, 0.75 M or more. The amino acid, for example glycine, can be used in a form of a pharmaceutically acceptable salt, such as hydrochloride, hydrobromide, sulfate, acetate, etc. The purity of the amino acid, for example glycine, should be at least 98 %, at least 99 %, or at least 99.5 % or more.

In examples herein, if necessary, hyaluronidase inhibitors are included in a formulation to stabilize PH20, in particular to the effects of in the presence of otherwise destabilizing agents and conditions, such as, for example, low salt, high pH, the presence of preservatives and elevated temperatures. Such a component generally is not required for pharmaceutical compositions containing a modified PH20 polypeptide as provided herein that exhibits increased stability under such conditions. When provided, the hyaluronidase inhibitor is provided at least at its equibilibrium concentraton. One of skill in the art is familiar with various classess of hyaluronidase inhibitors (*see e.g.* Girish *et al.* (2009) *Current Medicinal Chemistry*, 16:2261-2288, and references cited therein). One of skill in the art knows or can determine by

standard methods in the art the equilibrium concentration of a hyaluronidase inhibitor in a reaction or stable composition herein.

An exemplary hyaluronidase inhibitor for use in the compositions herein is hyaluronan (HA). Hyaluronic acid (HA, also known as hyaluronan and hyaluronate) is the natural substrate for PH20. HA is a non-sulfated glycosaminoglycan that is widely distributed throughout connective, epithelial, and neural tissues. It is a polymer of up to 25,000 disaccharide units, themselves composed of D-glucuronic acid and D-N-acetylglucosamine. The molecular weight of HA ranges from about 5 kDa to 200,000 kDa. Any size HA can be used in the compositions as a stabilizer. In some examples, the HA is a disaccharide, composed of D-glucuronic acid and D-N-acetylglucosamine. In other examples, the HA is an oligosaccharide, such as a tetrasaccharide, containing 2 repeating disaccharide units, or alternatively, the HA used in the co-formulations provided herein can contain multiple repeating disaccharide units, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more disaccharide units. In other example, the HA used in the formulations provided herein has a molecular weight that is from or from about 5 kDa to or to about 5,000 kDa; from or from about 5 kDa to or to about 1,000 kDa; from or from about 5 kDa to or to about 500 kDa; or from or from about 5 kDa to or to about 200 kDa. Exemplary HA oligosaccharides for use in the formulations herein have a molecular weight of or of about 6.4 kDa, 74.0 kDa. or 234.4 kDa. The formulations can contain 1 mg/mL to 20 mg/mL HA, 8 mg/nL to 12 mg/nL, such as at least or about 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, 10 mg/mL, 11 mg/mL, 12 mg/mL, 13 mg/mL, 14 mg/mL, 15 mg/mL, 16 mg/mL, 17 mg/mL, 18 mg/mL, 19 mg/mL or 20 mg/mL or more HA. In some examples, the molar ratio of HA to PH20 is or is about 100,000:1, 95,000:1, 90,000:1, 85,000:1, 80,000:1, 75,000:1, 70,000:1, 65,000:1, 60,000:1, 55,000:1, 50,000:1, 45,000:1, 40,000:1, 35,000:1, 30,000:1, 25,000:1, 20,000:1, 15,000:1, 10,000:1, 5,000:1, 1,000:1, 900:1, 800:1, 700:1, 600:1, 500:1, 400:1, 300:1, 200:1, or 100:1 or less.

In some examples, a nicotininc compound is used as a stabilizing agent. Nicotinic compounds include, but are not limited to, nicotinamide, nicotinic acid, niacin, niacinamide, vitamin B3 and/or salts thereof and/or any combination thereof. In particular applications, the stabilizing agent can include a nicotinic compound an an amino acid or amino acids (*see e.g.* International published PCT Appl. No.

WO2010149772). For example, the amino acid can be arginine, glutamic acid and/or salts thereof or combinations thereof.

2. Compositions for Other Routes of Administration

Depending upon the condition treated other routes of administration, such as topical application, transdermal patches, oral and rectal administration are also contemplated herein.

For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories include solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The typical weight of a rectal suppository is about 2 to 3 gm. Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration. Formulations suitable for rectal administration can be provided as unit dose suppositories. These can be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

For oral administration, pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets can be coated by methods well-known in the art.

Formulations suitable for buccal (sublingual) administration include, for example, lozenges containing the active compound in a flavored base, usually sucrose

and acacia or tragacanth; and pastilles containing the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Topical mixtures are prepared as described for the local and systemic administration. The resulting mixture can be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

The compounds or pharmaceutically acceptable derivatives thereof may be formulated as aerosols for topical application, such as by inhalation (see, *e.g.*, U. S. Patent Nos. 4,044,126,4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will typically have diameters of less than 50 microns, or less than 10 microns.

The compounds can be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients also can be administered.

Formulations suitable for transdermal administration are provided. They can be provided in any suitable format, such as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches contain the active compound in optionally buffered aqueous solution of, for example, 0.1 to 0.2 M concentration with respect to the active compound. Formulations suitable for transdermal administration also can be delivered by iontophoresis (see, *e.g.*, *Pharmaceutical Research* 3(6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound.

Pharmaceutical compositions also can be administered by controlled release formulations and/or delivery devices (see, *e.g.*, in U.S. Patent Nos. 3,536,809;

3,598,123; 3,630,200; 3,845,770; 3,847,770; 3,916,899; 4,008,719; 4,687,610; 4,769,027; 5,059,595; 5,073,543; 5,120,548; 5,354,566; 5,591,767; 5,639,476; 5,674,533 and 5,733,566).

3. Dosages and Administration

The modified PH20 polypeptides provided herein can be formulated as pharmaceutical compositions for single dosage or multiple dosage administration. The PH20 polypeptide is included in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration can be determined empirically by testing the polypeptides in known *in vitro* and *in vivo* systems such as by using the assays provided herein or known in the art (*see e.g.*, Taliani et al. (1996) *Anal. Biochem.*, 240: 60-67; Filocamo et al. (1997) *J Virology*, 71: 1417-1427; Sudo et al. (1996) *Antiviral Res.* 32: 9-18; Buffard et al. (1995) *Virology,* 209:52-59; Bianchi et al. (1996) *Anal. Biochem.*, 237: 239-244; Hamatake et al. (1996) *Intervirology* 39:249-258; Steinkuhler et al. (1998) *Biochem.*, 37:8899-8905; D'Souza et al. (1995) *J Gen. Virol.,* 76:1729-1736; Takeshita et al. (1997) *Anal. Biochem.*, 247:242-246; see also *e.g.*, Shimizu et al. (1994) *J. Virol.* 68:8406-8408; Mizutani et al. (1996) *J. Virol.* 70:7219-7223; Mizutani et al. (1996*) Biochem. Biophys. Res. Commun.*, 227:822-826; Lu et al. (1996*) Proc. Natl. Acad. Sci (USA)*, 93:1412-1417; Hahm et al., (1996) *Virology*, 226:318-326; Ito et al. (1996) *J. Gen. Virol.,* 77:1043-1054; Mizutani et al. (1995) *Biochem. Biophys. Res. Commun.*, 212:906-911; Cho et al. (1997) *J. Virol. Meth.* 65:201-207 and then extrapolated therefrom for dosages for humans.

The amount of a modified PH20 to be administered for the treatment of a disease or condition can be determined by standard clinical techniques. In addition, *in vitro* assays and animal models can be employed to help identify optimal dosage ranges. The precise dosage, which can be determined empirically, can depend on the particular enzyme, the route of administration, the type of disease to be treated and the seriousness of the disease.

Hence, it is understood that the precise dosage and duration of treatment is a function of the disease being treated and can be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values also can vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and

the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or use of compositions and combinations containing them. The compositions can be administered hourly, daily, weekly, monthly, yearly or once. Generally, dosage regimens are chosen to limit toxicity. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or bone marrow, liver or kidney or other tissue dysfunctions. Conversely, the attending physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

Typically, a therapeutically effective dose of a modified PH20 enzyme is at or about 10 Unit (U) to 500,000 Units, 100 Units to 100,000 Units, 500 Units to 50,000 Units, 1000 Units to 10,000 Units, 5000 Units to 7500 Units, 5000 Units to 50,000 Units, or 1,000 Units to 10,000 Units, generally 1,000 to 50,000 Units, in a stabilized solution or suspension or a lyophilized from. For example, a PH20 polypeptide, can be administered at a dose of at least or about at least or 10 U, 20 U, 30 U, 40 U, 50 U, 100 U, 150 U, 200 U, 250 U, 300 U, 350 U, 400 U, 450 U, 500 U, 600 U, 700 U, 800 U, 900 U, 1000 U, 2,000 U, 3,000 U, 4,000 Units, 5,000 U or more. The formulations can be provided in unit-dose forms such as, but not limited to, ampoules, syringes and individually packaged tablets or capsules.

The PH20 enzyme can be administered alone, or with other pharmacologically effective agent or therapeutic agent, in a total volume of 0.1 -100 ml, 1 -50 ml, 10- 50 ml, 10-30 ml, 1-20 ml, or 1-10 ml, typically 10-50 ml. Typically, volumes of injections or infusions of a PH20-containing composition are at least or at least about 0.01 mL, 0.05 mL, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 ml, 10 ml, 20 ml, 30 ml, 40 ml, 50 ml or more. The formulations provided herein contain a modified PH20 polypeptide in an amount between or about between 30 Units/mL to 3000 U/mL, 300 U/mL to 2000 U/mL or 600 U/mL to 2000 U/mL or 600 U/mL to 1000 U/mL, such as at least or about at least 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 200 U/mL, 300 U/mL, 400 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/ml, 2000 U/mL or 3000 U/mL. For example, the formulations provided herein contain a PH20 that is in an amount that is at least 100 U/mL to 1000 U/mL, for example at least or about at least or about or 600 U/mL.

The PH20 polypeptide can be provided as a stock solution in an amount that is at least or about or is 100 U/ml, 150 U/ml, 200 U/ml, 300 U/ml, 400 U/ml, 500 U/mL, 600 U/mL, 800 U/mL or 1000 U/mL, or can be provided in a more concentrated form, for example in an amount that is at least or about or is 2000 U/ml, 3000 Units/ml, 4000 U/ml, 5000 U/ml, 8000 U/ml, 10,000 U/mL or 20,000 U/mL for use directly or for dilution to the effective concentration prior to use. The PH20 polypeptide compositions can be provided as a liquid or lyophilized formulation.

When the PH20 is co-formualted with a therapeutic agent, dosages can be provided as a ratio of amount of a PH20 polypeptide to therapeutic agent administered. For example, a PH20 polypeptide can be administered at 1 hyaluronidase U/therapeutic agent U (1:1) to 50:1 or more, for example, at or about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1 or more.

The formulations provided herein, including co-formulations and/or stable formulations, can be prepared for single dose administration, multiple dose administration or continuous infusion administrations. Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e. g., U. S. Patent No. 3,710,795) is also contemplated herein.

For example, formulations of pharmaceutically therapeutically active compounds and derivatives thereof are provided for administration to humans and animals in unit dosage forms or multiple dosage forms. For example, compounds can be formulated as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. Each unit dose contains a predetermined quantity of therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit dose forms can be administered in fractions or multiples thereof. A multiple dose form is a plurality of identical unit dosage forms packaged in a single container to be administered in segregated unit dose form. Examples of multiple dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit doses that are not segregated in packaging.

Generally, dosage forms or compositions containing active ingredient in the range of 0.005% to 100% with the balance made up from non-toxic carrier can be prepared.

Compositions provided herein typically are formulated for administration by subcutaneous route; although other routes of administration are contemplated, such as any route known to those of skill in the art including intramuscular, intraperitineal, intravenous, intradermal, intralesional, intraperitoneal injection, epidural, vaginal, rectal, local, otic, transdermal administration or any route. Formulations suited for such routes are known to one of skill in the art. Administration can be local, topical or systemic depending upon the locus of treatment. Local administration to an area in need of treatment can be achieved by, for example, but not limited to, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant. Compositions also can be administered with other biologically active agents, either sequentially, intermittently or in the same composition.

The most suitable route in any given case depends on a variety of factors, such as the nature of the disease, the tolerance of the subject to a particular administration route, the severity of the disease, and the particular composition that is used. Typically, the compositions provided herein are administered parenterally. In some examples, modified PH20 polypeptide compositions are administered so that they reach the interstitium of skin or tissues, thereby degrading the interstitial space for subsequent delivery of a therapeutic agent. Thus, in some examples, direct administration under the skin, such as by subcutaneous administration methods, is contemplated. Thus, in one example, local administration can be achieved by injection, such as from a syringe or other article of manufacture containing a injection device such as a needle. In another example, local administration can be achieved by infusion, which can be facilitated by the use of a pump or other similar device. Other modes of administration also are contemplated. For example, modified PH20 polypeptides, included conjugated forms with increased half-life such as PEGylated forms thereof, can be administered intravenously. Pharmaceutical compositions can be formulated in dosage forms appropriate for each route of administration.

Administration methods can be employed to decrease the exposure of selected modified PH20 polypeptides to degradative processes, such as proteolytic degradation and immunological intervention via antigenic and immunogenic responses. Examples of such methods include local administration at the site of treatment. PEGylation of

therapeutics has been reported to increase resistance to proteolysis, increase plasma half-life, and decrease antigenicity and immunogenicity. Examples of PEGylation methodologies are known in the art (see for example, Lu and Felix, Int. J. Peptide Protein Res., 43: 127-138, 1994; Lu and Felix, Peptide Res., 6: 142-6, 1993; Felix et al., Int. J. Peptide Res., 46 : 253-64, 1995; Benhar et al., J. Biol. Chem., 269: 13398-404, 1994; Brumeanu et al., J Immunol., 154: 3088-95, 1995; see also, Caliceti et al. (2003) Adv. Drug Deliv. Rev. 55(10):1261-77 and Molineux (2003) Pharmacotherapy 23 (8 Pt 2):3S-8S). PEGylation also can be used in the delivery of nucleic acid molecules in vivo. For example, PEGylation of adenovirus can increase stability and gene transfer (see, *e.g.*, Cheng et al. (2003) Pharm. Res. 20(9): 1444-51).

Various other delivery systems are known and can be used to administer selected PH20 polypeptides, such as but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor mediated endocytosis, and delivery of nucleic acid molecules encoding selected PH20 polypeptides such as retrovirus delivery systems.

Hence, in certain embodiments, liposomes and/or nanoparticles also can be employed with administration of soluble PH20 polypeptides. Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 angstroms containing an aqueous solution in the core.

Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios, the liposomes form. Physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

Liposomes interact with cells via different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and

neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. Varying the liposome formulation can alter which mechanism is operative, although more than one can operate at the same time. Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use herein, and such particles can be easily made.

4. Exemplary PH20-Insulin Co-Formulation

Provided herein are stable co-formulations of a fast acting insulin, such as a rapid acting (fast-acting) insulin analog, and a modified PH20 polypepide.

In particular, the modified PH20 polypeptide is a modified PH20 polypeptide that exhibits increased stability under denaturation conditions, such as any set forth in Sections C.1.b. In particular, the PH20 polypeptide is a modified PH20 polypeptide that exhibits increased stability to one or more phenolic preservatives, such as any set forth in Section C.1.b.i. For example, the PH20 polypeptide is a modified PH20 polypeptide that contains an amino acid replacement with P at a position corresponding to position 204 with reference to amino acid positions set forth in SEQ ID NO:3.

The fast acting insulin can be a regular insulin or a rapid acting (fast-acting) insulin analog. Insulin is a polypeptide that when processed is composed of 51 amino acids containing an A- and B- chain. Generally, insulin contains an A-chain of about 21 amino acids and a B-chain of about 30 amino acids. The A- and B- chains are linked by disulfide bridges. Exemplary regular insulins include, for example, a human insulin (with an A chain having a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in SEQ ID NO:863) or a porcine insulin (with an A chain having a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:864 and a B chain having a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID

NO:864). Exemplary fast-acting insulin analogs are insulin variants that contain one or more amino acid modifications compared to a human insulin set forth in SEQ ID NO: 862 and 863 (A and B chains). For example, exemplary insulin analogs are known to one of skill in the art, and include, but are not limited to, glulisine set forth in SEQ ID NO:862 (A-chain) and having a B- chain that is a variant of SEQ ID NO:863 (B-chain; LysB3, GluB29), HMR-1 153 set forth in SEQ ID NO:862 (A-chain) and having a B-chain that is a variant of SEQ ID NO:863 (B-chain; LysB3, IleB28), insulin aspart set forth in SEQ ID NO:862 (A-chain) and having a B-chain that is a variant of SEQ ID NO:863 (B-chain; AspB28), and insulin lispro set forth in SEQ ID NO:862 (A-chain) and having a B-chain that is a variant of SEQ ID NO:863 (B-chain; LysB28, ProB29). In every instance above, the nomenclature of the analogs is based on a description of the amino acid substitution at specific positions on the A or B chain of insulin, numbered from the N-terminus of the chain, in which the remainder of the sequence is that of natural human insulin. (A and B chains), Exemplary of such analog forms, are set forth in SEQ ID NOS:862 (A-chain) and having a B-chain set forth in any of SEQ ID NOS: 865-867.

The co-formulations are stable as a liquid formulation for prolonged periods of time for at least 1 month at temperatures from or from about 2°C to 8°C, inclusive or for at least 3 days at a temperature from or from about 30°C to 42°C, inclusive, for at least 3 days. For example, the co-formulations are stable and and retain activity of the PH20 hyaluronidase and hyaluronidase at "refrigerator" conditions, for example, at 2° C to 8° C, such as at or about 4° C, for at least at least 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months or 30 months or more. In another example, the formulations provided herein are stable and retain activity of the PH20 hyaluornidase and insulin at room temperature for example at 18° C to 32° C, generally 20° C to 32° C, such as 28° C to 32° C, for at least 2 weeks to 1 year, for example, at least 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, at least 7 months, at least 8 months, at least 9 months, or at least 1 year or more. In a further example, the formulations provided herein are stable and retain activity of active of the PH20 hyaluronidase and insulin at elevated temperatures of about or greater than 30 ° C, generally from or from about 30°C to 42°C, such as

32°C to 37°C or 35°C to 37°C or about or 37°C for at least 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 35 days, 40 days, 45 days, 50 days, 60 days or more.

Assays to assess stability of active agents are well-known to one of skill in the art. Section F provides exemplary assays to asses stability of PH20 hyaluronidase. The stability of insulin can be assessed using similar methods well-known to one of skill in the art. For example, insulin stability and solubility can be assessed by visual assessment (*e.g.* including changes in color, clarity, presence of aggregates or clumping and material adhesion, or frosting), acid clarification, optical microscopy, reversed phase high performance liquid chromatography (RP-HPLC), *in vitro* or *in vivo* bioassays and denaturing and non-denaturing size exclusion chromatography (SEC). *In vitro* or *in vivo* bioassays for insulin activity include, but are not limited to, a competitive binding assay using cells expressing insulin receptors (*e.g.* human placental cell membranes) and a radiolabeled insulin (*see e.g.* Weiss *et al.,* (2001) *J. Biol. Chem.* 276:40018-40024; Duttaroy *et al.*, (2005) *Diabetes* 54:251-258); insulin-stimulated glucose uptake (Louveau *et al.,* (2004) *J Endocrin.* 181:271-280, Duttaroy *et al.*, (2005) *Diabetes* 54:251-258); assess to assess glucose production in the presence of insulin (Wang *et al.,* (2000) *J. Biochem.*, 275:14717-14721, Duttaroy *et al.*, (2005) *Diabetes* 54:251-258); and studies using diabetic and/or healthy animal models (Atkinson *et al.,* (1999) *Nature Med.* 5:601-604; Nagoya-Shibata-Yasuda (NSY) mice, Zucker diabetic fatty (ZDF) rats and Gato-Katazaki (GK) rats (Cefalu (2006) *ILAR Journal* 47:186-198).

Exemplary of such formulations contain 100 U/mL to 1000 U/mL of a modified PH20 polypeptide, and in particular at or about or at least 600 U/mL; 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at or at least or about 100 U/mL; NaCl at a concentration of between or about between 80-140 mM; a pH of between or about between 7.0 to 7.8; a buffering agent that maintains the pH range of between or about between 7.0 to 7.8; 0.1 % to 0.4 % preservative as a mass concentration (w/v). Optionally, a further stabilizing agent can be included. For example, the co-formulations provided herein contain 1 mM to 100 mM of a buffering agent. For example, the co-formulations provided herein contain 0.005 % to 0.5 % surfactant. Exemplary co-formulations provided herein also can contain less than 60 mM glycerin (glycerol) and 2 mM to or to about 50 mM of an antioxidant.

The following stable formulations are exemplary only and provide a platform from which minor adjustments can be made. It is understood that very small changes in the concentrations of the various excipients and other components (*e.g.* ± 15 % of the stated concentrations), or small changes in pH, can be made while retaining some if not all of the insulin solubility and stability and PH20 stability. Further changes also can be made by adding or removing excipients. For example, the type of stabilizing surfactant can be changed.

For example, the exemplary co-formulations herein contain contain 100 U/mL to 1000 U/mL of a modified PH20 polypeptide, and in particular at least or about at least or or about 600 U/mL; 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at least or about at least or or about 100 U/mL of a fast-acting insulin; from or from about 10 mM to or to about 50 mM Tris (*e.g.* from or from about 20 mM to 40 mM Tris, such as or as about 20 mM, 25 mM, 30 mM, 35 mM or 40 mM); from or from about 80 mM to or to about 140 mM NaCl (*e.g.* at or about 80 mM, 90 mM, 100 mM, 110 mM 120 mM, 130 mM, 140 mM, 150 mM or 160 mM NaCl); from or from about 2 mM to or to about 50 mM methionine (*e.g.* at or about 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM methionine); from or from about 0 mM to or to about 50 mM glycerin (*e.g.* at or about 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM glycerin); from or from about 0.005 % to or to about 0.5 % poloxamer 188, such as 0.01% to 0.05% (*e.g.* at or about 0.01%, 0.02%, 0.03%, 0.04% or 0.05% poloxamer 188); from or from about 0.05% to or to about 0.25% phenol (*e.g.* at or about 0.1%, 0.12%, 0.125%, 0.13%, 0.14%, 0.15%, 0.16% or 0.17% phenol); and from or from about 0.05% to or to about 0.4% m-cresol (*e.g.* at or about 0.075%, 0.08%, 0.09%, 0.1%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16% or 0.17% m-cresol). The formulations are prepared with a pH from or from about 7.0 to or to about 7.6 (*e.g.* at or about pH 7.0, 7.1, 7.2, 7.3, 7.4, 7.5 or 7.6). In further examples, zinc is included at a concentration of or about 0.017 mg/100 U, 0.018 mg/100 U, 0.02 mg/100 U, 0.022 mg/100 U or 0.024 mg/100 U insulin.

In particular examples, the fast acting insulin is insulin aspart, insulin lispro or insulin glulisine. Exemplary of the co-formulations provided herein that contain a modified PH20 polypeptide and insulin lispro are those that contain from or about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 70 mM to or to about 100 mM NaCl (*e.g.* at or about 80 mM or 100 mM NaCl); from or from about 10 mM to or to about 30 mM methionine (*e.g.* at or about 10 mM or 20 mM

methionine); from or from about 40 mM to or to about 60 mM glycerin (*e.g.* at or about 50 mM glycerin); from or from about 0.005 % to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01 % poloxamer 188); from or from about 0.017 mg zinc/100 U insulin to or to about 0.024 mg zinc/100 U insulin (*e.g.* 0.017 mg zinc/100 U insulin, 0.018 mg/100 U, 0.02 mg/100 U, 0.022 mg/100 U or 0.024 mg zinc/100 U insulin); from or from about 0.08 % to or to about 0.17 % phenol (*e.g.* 0.1 %, 0.125 % or 0.13 % phenol); and from or from about 0.07% to or to about 0.17 % m-cresol (*e.g.* 0.075 %, 0.08 %, 0.13 % or 0.15 % m-cresol). For example, the co-formulations can contain at or about 0.1% phenol and 0.015 % m-cresol; at or about 0.125 % phenol and 0.075% m-cresol; at or about 0.13 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.08 % m-cresol; or at or about 0.17% phenol and 0.13 % m-cresol. Such formulations of insulin lispro and a modified PH20 polypeptide are prepared with a pH of or about 7.0 to or to about 7.5 (typically a pH of or about pH 7.2).

Exemplary of the co-formulations provided herein that contain a modified PH20 polypeptide and insulin aspart are those that contain from or from about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 70 mM to or to about 120 mM NaCl (*e.g.* at or about 80 mM or 100 mM NaCl); from or from about 2 mM to or to about 30 mM, such as 2 mM to 10 mM, 5 mM to 30 mM methionine (*e.g.* at or about 5 mM, 10 mM or 20 mM methionine); from or from about 0.005% to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01% poloxamer 188); from or from about 0.08 % to or to about 0.17 % phenol (*e.g.* 0.1%, 0.125% or 0.13% phenol); and from or from about 0.07 % to or to about 0.17% m-cresol (*e.g.* 0.075 %, 0.08 %, 0.13 % or 0.15 % m-cresol). For example, the co-formulations can contain at or about 0.1 % phenol and 0.015 % m-cresol; at or about 0.125 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.08 % m-cresol; or at or about 0.17 % phenol and 0.13 % m-cresol. Such formulations of insulin aspart and a modified PH20 polypeptide, such as are prepared with a pH of or about 7.0 to or to about 7.6 (typically a pH of or about pH 7.4 or 7.3).

A further exemplary formulation provided herein that contains a modified PH20 polypeptide and insulin aspart are those that do not contain phenol. Such exemplary formulations contain from or from about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 70 mM to or to about 120 mM NaCl

(*e.g.* at or about 80 mM or 100 mM NaCl); from or from about 2 mM to or to about 30 mM, such as 2 mM to 10 mM, 5 mM to 30 mM methionine (*e.g.* at or about 5 mM, 10 mM or 20 mM methionine); from or from about 0.005% to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01% poloxamer 188); and from or from about 0.07 % to or to about 0.4% m-cresol, such as from or from about).2% to 0.4% m-creso (*e.g.* 0.3%, 0.315%, 0.35%, 0.4% m-cresol). Such formulations of insulin aspart and a modified PH20 polypeptide, such as are prepared with a pH of or about 7.0 to or to about 7.6 (typically a pH of or about pH 7.4 or 7.3).

Exemplary of the co-formulations provided herein that contain a modified PH20 polypeptide and insulin glulisine are those that contain from or from about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 100 mM to or to about 150 mM NaCl (*e.g.* at or about 100 mM or 140 mM NaCl); from or from about 10 mM to or to about 30 mM methionine (*e.g.* at or about 10 mM or 20 mM methionine); from or from about 40 mM to or to about 60 mM glycerin (*e.g.* at or about 50 mM glycerin); from or from about 0.005 % to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01 % poloxamer 188); from or from about 0.08% to or to about 0.17% phenol (*e.g.* 0.1 %, 0.125 % or 0.13 % phenol); and from or from about 0.07 % to or to about 0.17% m-cresol (*e.g.* 0.075 %, 0.08 %, 0.13 % or 0.15 % m-cresol). For example, the co-formulations can contain at or about 0.1 % phenol and 0.015 % m-cresol; at or about 0.125 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.08 % m-cresol; or at or about 0.17 % phenol and 0.13 % m-cresol. Such formulations of insulin glulisine and a modified PH20 polypeptide are prepared with a pH of or about 7.0 to or to about 7.6 (typically a pH of or about pH 7.4).

5. Packaging, Articles of Manufacture and Kits

Pharmaceutical compounds of modified PH20 polypeptides, or nucleic acids encoding such polypeptides, or a derivative or variant thereof can be packaged as articles of manufacture containing packaging material, a pharmaceutical composition which is effective for treating a disease or disorder, and a label that indicates that the molecule is to be used for treating the disease or disorder. Combinations of a selected modified PH20 polypeptide, or derivative or variant thereof and an therapeutic agent also can be packaged in an article of manufacture.

The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products are well known to

those of skill in the art. See, for example, U.S. Patent Nos. 5,323,907, 5,052,558 and 5,033,252, each of which is incorporated herein in its entirety. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment. The articles of manufacture can include a needle or other injection device so as to facilitate administration (*e.g.* sub-epidermal administration) for local injection purposes. A wide array of formulations of the compounds and compositions provided herein are contemplated including a modified PH20 polypeptide and a therapeutic agent, such as a fast-acting insulin, known to treat a particular disease or disorder. The choice of package depends on the PH20 and/or therapeutic agent, and whether such compositions will be packaged together or separately. In one example, the PH20 can be packaged as a mixture with the therapeutic agent. In another example, the components can be packaged as separate compositions

Modified PH20 polypeptides, therapeutic agents and/or articles of manufacture thereof also can be provided as kits. Kits can include a pharmaceutical composition described herein and an item for administration provided as an article of manufacture. For example a PH20 polypeptide can be supplied with a device for administration, such as a syringe, an inhaler, a dosage cup, a dropper, or an applicator. The compositions can be contained in the item for administration or can be provided separately to be added later. The kit can, optionally, include instructions for application including dosages, dosing regimens and instructions for modes of administration. Kits also can include a pharmaceutical composition described herein and an item for diagnosis. For example, such kits can include an item for measuring the concentration, amount or activity of the selected protease in a subject.

F. Methods of Assessing PH20 Activity and Stability

Assays can be used to assess the stability and activity of the PH20 polypeptides provided herein. The assays can be used to assess the hyaluronidase activity of the PH20 polypeptide under particular conditions, temperature, and/or over time. Such assays can be used, for example, to determine the stability of the PH20 polypeptide under specific denaturation conditions, including, but not limited to, elevated temperatures greater than or about or 30°C (*e.g.* 30°C to 42°C such as or about 37°C), agitation, presence of excipients or low or no NaCl (salt). For example,

stability under specific conditions can be monitored by assessing activity, solubility, and stability (*e.g.* formation of aggregates, etc.) in the absence of exposure to the denaturation condition and then at various time points thereafter in the presence of the condition. Hence, stability can be assessed over time. Stability also can be assessed by comparing any one or more of activity, solubility or aggregation in the presence of one or more denaturation conditions compared to a native, wildtype or reference PH20 polypeptide. The assays also can be used make minor adjustments to the formulations provided herein while retaining the stability of both active agents.

1. Hyaluronidase Activity

The activity of a modified PH20 polypeptide can be assessed using methods well known in the art. For example, the USP XXII assay for hyaluronidase determines activity indirectly by measuring the amount of undegraded hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37° C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Hyaluronidase Reference Standard (USP) or National Formulary (NF) Standard Hyaluronidase solution can be used in an assay to ascertain the activity, in units, of any hyaluronidase. In one example, activity is measured using a microturbidity assay. This is based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin. The activity is measured by incubating hyaluronidase with sodium hyaluronate (hyaluronic acid) for a set period of time (*e.g.* 10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after an additional development period. The decrease in turbidity resulting from hyaluronidase activity on the sodium hyaluronate substrate is a measure of hyaluronidase enzymatic activity.

In another example, hyaluronidase activity is measured using a microtiter assay in which residual biotinylated hyaluronic acid is measured following incubation with hyaluronidase (*see e.g.* Frost and Stern (1997) *Anal. Biochem.* 251:263-269, U.S. Pat. Publication No. 20050260186). The free carboxyl groups on the glucuronic acid residues of hyaluronic acid are biotinylated, and the biotinylated hyaluronic acid substrate is covalently coupled to a microtiter plate. Following incubation with hyaluronidase, the residual biotinylated hyaluronic acid substrate is detected using an avidin-peroxidase reaction, and compared to that obtained following reaction with hyaluronidase standards of known activity.

Other assays to measure hyaluronidase activity also are known in the art and can be used in the methods herein (*see e.g.* Delpech *et al.*, (1995) *Anal. Biochem.* 229:35-41; Takahashi *et al.*, (2003) *Anal. Biochem.* 322:257-263).

Many hyaluronidase assays have been based upon the measurement of the generation of new reducing N-acetylamino groups (Bonner and Cantey, Clin. Chim. Acta 13:746-752, 1966), or loss of viscosity (De Salegui et al., Arch. Biochem. Biophys.121:548-554, 1967) or turbidity (Dorfman and Ott, J. Biol. Chem. 172:367, 1948). With purified substrates all of these methods suffice for determination of the presence or absence of endoglucosamidic activity.

Substantially purified glycosaminoglycan substrates can also be used in a Gel Shift Assay. Glycosaminoglycans are mixed with recombinant PH20, such as a soluble PH20, to test for endoglucosidase activity that results in a shift in substrate mobility within the gel. Exemplary of such substrates include, but are not limited to, chondroitin-4 and 6 sulfate, dermatan sulfate, heparan-sulfate, which can be obtained from Sigma Chemical. Human umbilical cord Hyaluronan can be obtained from ICN. For example, each test substrate can be diluted to at or about 0.1 mg/ml in a buffer range from pH 3.5-7.5. In such an exemplary assay, at or about 10 µl samples of purified soluble PH20 or conditioned media from PH20 expressing cells can be mixed with at or about 90 µl of test substrate in desired buffer and incubated for 3 hours at 37 °C. Following incubation, samples are neutralized with sample buffer (Tris EDTA pH 8.0, Bromophenol Blue and glycerol) followed by electrophoresis. Glycosaminoglycans can be detected using any method known in the art, for example, glycosaminoglycans can be detected by staining the gels using 0.5% Alcian Blue in 3% Glacial Acetic Acid overnight followed by destaining in 7% Glacial Acetic Acid. Degradation is determined by comparison of substrate mobility in the presence and absence of enzyme.

Hyaluronidase activity can also be detected by substrate gel zymography (Guentenhoner et al., 1992, Matrix 388-396). In this assay a sample is applied to a SDS-PAGE gel containing hyaluronic acid and the proteins in the sample separated by electrophoresis. The gel is then incubated in an enzyme assay buffer and subsequently stained to detect the hyaluronic acid in the gel. Hyaluronidase activity is visualized as a cleared zone in the substrate gel.

The ability of a PH20 polypeptide, including a modified PH20 polypeptide provided herein, to act as a spreading or diffusing agent also can be assessed. For

example, trypan blue dye can be injected subcutaneously with or without a PH20 polypeptide into the lateral skin on each side of nude mice. The dye area is then measured, such as with a microcaliper, to determine the ability of the PH20 polypeptide to act as a spreading agent (U.S. Pat. Pub. No. 20060104968).

The functional activity of a PH20 polypeptide can be compared and/or normalized to a reference standard using any of these assays. This can be done to determine what a functionally equivalent amount of a PH20 polypeptide is. For example, the ability of a PH20 polypeptide to act as a spreading or diffusing agent can be assessed by injecting it into the lateral skin of mice with trypan blue, and the amount required to achieve the same amount of diffusion as, for example, 100 units of a Hyaluronidase Reference Standard, can be determined. The amount of PH20 polypeptide required is, therefore, functionally equivalent to 100 hyaluronidase units.

2. Solubility

Solubility of a PH20 polypeptide can be determined by any method known to one of the skill in the art. One method for determining solubility is by detergent partitioning. For example, a soluble PH20 polypeptide can be distinguished, for example, by its partitioning into the aqueous phase of a Triton® X-114 solution at 37 °C (Bordier et al., (1981) *J. Biol. Chem.*, 256:1604-1607). Membrane-anchored polypeptides, such as lipid-anchored hyaluronidases, including GPI-anchored hyaluronidases, will partition into the detergent-rich phase, but will partition into the detergent-poor or aqueous phase following treatment with Phospholipase C. Phospholipase C is an enzyme that cleaves the phospho-glycerol bond found in GPI-anchored proteins. Treatment with PLC will cause release of GPI-linked proteins from the outer cell membrane.

3. Purity, Crystallization or Aggregation

The stability of a PH20 polypeptide provided herein also can be assessed using other methods and assays known in the art. In addition to assessing stability based on hyaluronidase activity, stability can be assessed by visual inspection, percent recovery, protein purity and apparent melting temperature.

For example, protein purity can be measured by reversed phase high performance liquid chromatography (RP-HPLC). Protein purity, as determined by RP-HPLC, is the percent of the main PH20 protein peak present, as compared to all of the protein species present. Thus, RP-HPLC, and similar methods known to one of skill in the art, can assess degradation of the enzyme. Protein purity can be assessed

over time. Protein purity also can be assessed in the presence of one or more denaturation conditions and in varying amounts thereof. Percent recovery also can be determined as the relative percentage of the polypeptide under various conditions (denaturation conditions, time of storage, mode of storage such as vessel or container, or other similar parameters that can be altered) as compared to a reference sample. PH20 polypeptide stability also can be determined by measuring the oxidation of the hyaluronidase by RP-HPLC. Percent oxidation is a measure of sum of the peak areas of the major (ox-1) and minor (ox-2) peaks.

In one example, the melting temperature of a PH20 polypeptide, such as a modified PH20 polypeptide, can be determined by measuring the hydrodynamic radius of particles by dynamic light scattering under various conditions (*e.g.* denaturation conditions or other storage conditions). An increase in particle size and a decrease in the melting temperature indicates denaturation and subsequent aggregation of the hyaluronidase.

Other methods known to one of skill in the art that can be used to determine the stability of the hyaluronidase in the co-formulations provided herein, include polyacrylamide gel electrophoresis (PAGE), immunoblotting, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, circular dichroism (CD) and dye-based fluorescence assays.

4. Pharmacodynamics/Pharmacokinetics

The effect of administration of a PH20 polypeptide, such as a modified PH20 polypeptide, alone or in combination with another therapeutic agent, on the pharmacokinetic and pharmacodynamic properties of any administered agent also can be assessed *in vivo* using animal model and/or human subjects, such as in the setting of a clinical. Pharmacokinetic or pharmacodynamic studies can be performed using animal models or can be performed during studies with patients administered with a PH20 polypeptide or modified PH20 polypeptide.

Animal models include, but are not limited to, mice, rats, rabbits, dogs, guinea pigs and non-human primate models, such as cynomolgus monkeys or rhesus macaques. In some instances, pharmacokinetic or pharmacodynamic studies are performed using healthy animals. In other examples, the studies are performed using animal models of a disease for which therapy with hyaluronan is considered, such as animal models of any hyaluronan-associated disease or disorder, for example a tumor model.

The pharmacokinetic properties of a PH20 polypeptide, such as a modified PH20 polypeptide, can be assessed by measuring such parameters as the maximum (peak) concentration (Cmax), the peak time (*i.e.* when maximum concentration occurs; Tmax), the minimum concentration (*i.e.* the minimum concentration between doses; Cmin), the elimination half-life (T1/2) and area under the curve (*i.e.* the area under the curve generated by plotting time versus concentration; AUC), following administration. The absolute bioavailability of the hyaluronidase can be determined by comparing the area under the curve of hyaluronidase following subcutaneous delivery (AUCsc) with the AUC of hyaluronidase following intravenous delivery (AUCiv). Absolute bioavailability (F), can be calculated using the formula: F = ([AUC]sc × dosesc) / ([AUC]iv × doseiv). A range of doses and different dosing frequency of dosing can be administered in the pharmacokinetic studies to assess the effect of increasing or decreasing concentrations enzyme, such as modified PH20 polypeptide, in the dose.

G. Methods of Treatment and Combination Therapy

Provided herein are methods and uses of any of the modified PH20 polypeptides provided herein that exhibit hyaluronidase activity based on its ability to degrade glycosaminoglycan(s) such as hyaluronan. Due to such activity, the modified PH20 polypeptides can be used as a spreading factor to increase the delivery and/or bioavailability of subcutaneously administered therapeutic agents. For example, modified PH20 polypeptides can be used to increase the delivery of therapeutic agents such as antibodies (*e.g.* monoclonal antibodies), cytokines, Immune Globulin, an Insulin, or coagulation factors, for increasing penetration of chemotherapeutic agents into solid tumors. The modified PH20 polypeptides also can be used to treat a hyaluronan-disease or disorder that is characterized by an excess or accumulation of hyaluronan. For example, modified PH20 polypeptides provided herein can be used to for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for treating cellulite; and/or for treating a proliferative disorder.

Other methods and uses of a modified PH20 polypeptide include any that are known to one of skill in the art. For example, various forms of PH20 hyaluronidases have been prepared and approved for therapeutic use in humans. For example, animal-derived hyaluronidase preparations include Vitrase® (ISTA Pharmaceuticals), a purified ovine testicular hyaluronidase, and Amphadase® (Amphastar

Pharmaceuticals), a bovine testicular hyaluronidase. Hylenex® (Halozyme Therapeutics) is a human recombinant hyaluronidase produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing nucleic acid encoding for soluble rHuPH20 (*see e.g.* U.S. Patent No. 7,767,429). Approved therapeutic uses for hyaluronidase include use as an adjuvant to increase the absorption and dispersion of other therapeutic agents, for hypodermoclysis (subcutaneous fluid administration), and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents. In addition to these indications hyaluronidases can be used as a therapeutic or cosmetic agent for the treatment of additional diseases and conditions. For example, hyaluronidase is commonly used, for example, for peribulbar block in local anesthesia prior ophthalmic surgery. The presence of the enzyme prevents the need for additional blocks and speeds the time to the onset of akinesia (loss of eye movement). Peribulbar and sub-Tenon's block are the most common applications of hyaluronidase for ophthalmic procedures. Hyaluronidase also can promote akinesia in cosmetic surgery, such as blepharoplasties and face lifts. It is understood that soluble PH20 hyaluronidases provided herein, including esPH20 hyaluronidases, can be used in any method of treatment or combination therapy for which a PH20 hyaluronidase is used (*see e.g.*, U.S. Publication Nos. US20040268425; US20050260186; US20060104968; and U.S. Appl. Serial Nos. 12/381,844, 12/386,249, 12/387,225 and 12/386,222, incorporated by reference in their entirety).

Exemplary, non-limiting, methods and uses are described in the following subsections.

1. Methods of Delivering Therapeutic Agents

As noted above, hyaluronidase is a spreading or diffusing substance that modifies the permeability of connective tissue through the hydrolysis of hyaluronic acid, a polysaccharide found in the intercellular ground substance of connective tissue, and of certain specialized tissues, such as the umbilical cord and vitreous humor. When no spreading factor is present, materials injected subcutaneously, such as drugs, proteins, peptides and nucleic acid, spread very slowly. Co-injection with hyaluronidase, however, can cause rapid spreading. The rate of diffusion is proportional to the amount of enzyme, and the extent of diffusion is proportional to the volume of solution.

Modified PH20 polypeptides provided herein can be used to promote or enhance the delivery agents and molecules to any of a variety of mammalian tissues *in*

vivo. It can be used to facilitate the diffusion and, therefore, promote the delivery, of small molecule pharmacologic agents as well as larger molecule pharmacologic agents, such as proteins, nucleic acids and ribonucleic acids, and macromolecular compositions than can contain a combination of components including, but not limited to, nucleic acids, proteins, carbohydrates, lipids, lipid-based molecules and drugs (*see e.g.* U.S. Publication Nos. US20040268425; US20050260186; and US20060104968). Modified PH20 polypeptides can be co-administered and/or co-formulated with a therapeutic agent to improve the bioavailability as well as pharmacokinetic (PK) and/or pharmacodynamic (PD) characteristics of co-formulated or co-administered agents. PK/PD parameters that can be improved by using soluble PH20, such as esPH20, include such measures as C_{max} (the maximal concentration of agent achieved following absorption in, *e.g.*, the bloodstream), T_{max} (the time required to achieve maximal concentration), $T_{1/2}$ (the time required for the concentration to fall by half), C_{min} (the minimal concentration of agent following metabolism and excretion), AUC (area under the curve of concentration versus time, a measure of the overall amount of bioavailability), concentrations in various tissues of interest (including, *e.g.*, the rate of achieving desired concentrations, the overall levels, and the duration of maintaining desired levels), and E_{max} (the maximal effect achieved).

The methods of treatment provided herein include combination therapies with a therapeutic agent for the treatment of a disease or disorder for which the therapeutic agent threats. Any therapeutic agent that ameliorates and or otherwise lessens the severity of a disease or condition can be combined with a modified PH20 polypeptide provided herein in order to increase the bioavailability of such therapeutic agent. In particular, modified PH20 polypeptides provided herein can be used in each and all of the combinations described in applications *see e.g.*, U.S. Publication Nos. US20040268425; US20050260186; US20060104968 and U.S. Appl. Serial Nos. 12/381,844, 12/386,249, 12/387,225 and 12/386,222 in place of the disclosed hyaluronidase or hyaluronidase degrading enzyme.

Modified PH20 polypeptides can be administered prior, subsequently, intermittently or simultaneously to the therapeutic agent preparation. Generally, the modified PH20 polypeptide is administered prior to or simultaneously with administration of the therapeutic agent preparation to permit the PH20 to degrade the hyaluronic acid in the interstitial space. The PH20 can be administered at a site different from the site of administration of the therapeutic molecule or the soluble

PH20 can be administered at a site the same as the site of administration of the therapeutic molecule.

Examples of pharmaceutical, therapeutic and cosmetic agents and molecules that can be administered with hyaluronidase include, but are not limited to, a chemotherapeutic or anticancer agent, an analgesic agent, an antibiotic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, an anti-arthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasitic agent, an antihistamine agent, an alpha-adrenergic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostatic agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a cosmetic or esthetic agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, an electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sleep inducer, a sympathomimetic agent, a tranquilizer agent, a urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, or an angiotensin converting enzyme inhibitor agent, and any combination thereof. In particular, therapeutic agents include antibodies, including monoclonal antibodies, bisphosphonates, insulins, coagulation factors, cytokines and Immun Globulins.

For example, modified PH20 polypeptides provided herein can be used to increase the delivery of chemotherapeutic agents. Hyaluronidases have also been used to enhance the activity of chemotherapeutics and/or the accessibility of tumors to chemotherapeutics (Schuller et al., 1991, *Proc. Amer. Assoc. Cancer Res.* 32:173, abstract no. 1034; Czejka et al., 1990, *Pharmazie* 45:H.9; Baumgartner et al. (1988) *Reg. Cancer Treat.* 1:55-58; Zanker et al. (1986) *Proc. Amer. Assoc. Cancer Res.* 27:390). Combination chemotherapy with hyaluronidase is effective in the treatment of a variety of cancers including urinary bladder cancer (Horn et al., 1985, *J. Surg. Oncol.* 28:304-307), squamous cell carcinoma (Kohno et al., 94, *J. Cancer Res. Oncol.* 120:293-297), breast cancer (Beckenlehner et al., 1992, *J. Cancer Res. Oncol.* 118:591-596), and gastrointestinal cancer (Scheithauer et al., 1988, *Anticancer Res.* 8:391-396). In this example, the modified PH20 hyaluronidase enhances penetration

of chemotherapeutic or other anti-cancer agents into solid tumors, thereby treating the disease.

Compositions containing soluble PH20 can be injected intratumorally with anti-cancer agents or intravenously for disseminated cancers or hard to reach tumors. The anticancer agent can be a chemotherapeutic, an antibody, a peptide, or a gene therapy vector, virus or DNA. Additionally, hyaluronidase can be used to recruit tumor cells into the cycling pool for sensitization in previously chemorefractory tumors that have acquired multiple drug resistance (St Croix et al., (1998) *Cancer Lett* September 131(1): 35-44).

Exemplary anti-cancer agents that can be administered after, coincident with or before administration of a soluble PH20, such as an esPH20, include, but are not limited to Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoins (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrines; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrenes; Bisantrenes; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines; Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines; Diaziquones; Dibrospidiums; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins; Enpromates; Enzastaurins; Epipropidines; Epirubicins; Epoetin alfas; Eptaloprosts;

Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds; Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines; Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones; Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines; Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils; Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas; Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofosines; Ilomastats; Imatinib mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as; Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas; Interferons; Interleukin-2s and other Interleukins (including recombinant Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines; Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones; Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles; Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs; Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans; Marimastats; Masoprocols; Maytansines; Mechlorethamines; Mechlorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols; Melphalans; MelphalanslL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates; Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides; Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins; Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes; Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols; Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins; Nelzarabines; Nemorubicins; Nitracrines; Nocodazoles; Nofetumomabs; Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins; Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines; Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims; Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins; Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans; Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers; Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidiums;

Pumitepas; Puromycins; Pyrazofurins; Quinacrines; Ranimustines; Rasburicases; Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs; Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins; Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs; Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines; Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins; Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-TG; Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars; Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfins; Temozolomides; Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines; Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones; Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes; Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones; Tretinoins/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates; Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas; Valrubicins; Valspodars; Vapreotides; Verteporfins; Vinblastines; Vincristines; Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols; Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles; Xanthomycin A's (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins; Zoledronate; Zorubicins; and Zosuquidars, for example:

Aldesleukins (*e.g.* PROLEUKIN®); Alemtuzumabs (*e.g.* CAMPATH®); Alitretinoins (*e.g.* PANRETIN®); Allopurinols (*e.g.* ZYLOPRIM®); Altretamines (*e.g.* HEXALEN®); Amifostines (*e.g.* ETHYOL®); Anastrozoles (*e.g.* ARIMIDEX®); Arsenic Trioxides (*e.g.* TRISENOX®); Asparaginases (*e.g.* ELSPAR®); BCG Live (*e.g.* TICE® BCG); Bexarotenes (*e.g.* TARGRETIN®); Bevacizumab (AVASTIN®); Bleomycins (*e.g.* BLENOXANE®); Busulfan intravenous (*e.g.* BUSULFEX®); Busulfan orals (*e.g.* MYLERAN™); Calusterones (*e.g.* METHOSARB®); Capecitabines (*e.g.* XELODA®); Carboplatins (*e.g.* PARAPLATIN®); Carmustines (*e.g.* BCNU®, BiCNU®); Carmustines with Polifeprosans (*e.g.* GLIADEL® Wafer); Celecoxibs (*e.g.* CELEBREX®); Chlorambucils (*e.g.* LEUKERAN®); Cisplatins (*e.g.* PLATINOL®); Cladribines (*e.g.* LEUSTATIN®, 2-CdA®); Cyclophosphamides (*e.g.* CYTOXAN®, NEOSAR®); Cytarabines (*e.g.* CYTOSAR-U®); Cytarabine liposomals (*e.g.* DepoCyt®); Dacarbazines (*e.g.* DTIC-Domeυ): Dactinomycins (*e.g.* COSMEGEN®); Darbepoetin

Alfas (*e.g.* ARANESP®); Daunorubicin liposomals (e. g. DANUOXOME®); Daunorubicins/Daunomycins (*e.g.* CERUBIDINE®); Denileukin Diftitoxes (*e.g.* ONTAK®); Dexrazoxanes (*e.g.* ZINECARD®); Docetaxels (*e.g.* TAXOTERE®); Doxorubicins (*e.g.* ADRIAMYCIN®, RUBEX®); Doxorubicin liposomals, including Docorubicin HCL liposome injections (*e.g.* DOXIL®); Dromostanolone propionates (*e.g.* DROMOSTANOLONE® and MASTERONE® Injection); Elliott's B Solutions (*e.g.* Elliott's B Solution®); Epirubicins (*e.g.* ELLENCE®); Epoetin alfas (*e.g.* EPOGEN®); Estramustines (*e.g.* EMCYT®); Etoposide phosphates (*e.g.* ETOPOPHOS®); Etoposide VP-16s (*e.g.* VEPESID®); Exemestanes (*e.g.* AROMASIN®); Filgrastims (*e.g.* NEUPOGEN®); Floxuridines (*e.g.* FUDR®); Fludarabines (*e.g.* FLUDARA®); Fluorouracils incl. 5-FUs (*e.g.* ADRUCIL®); Fulvestrants (*e.g.* FASLODEX®); Gemcitabines (*e.g.* GEMZAR®); Gemtuzumabs/Ozogamicins (*e.g.* MYLOTARG®); Goserelin acetates (*e.g.* ZOLADEX®); Hydroxyureas (*e.g.* HYDREA®); Ibritumomabs/Tiuxetans (*e.g.* ZEVALIN®); Idarubicins (*e.g.* IDAMYCIN®); Ifosfamides (*e.g.* IFEX®); Imatinib mesylates (*e.g.* GLEEVEC®); Interferon alfa-2as (*e.g.* ROFERON-A®); Interferon alfa-2bs (*e.g.* INTRON A®); Irinotecans (*e.g.* CAMPTOSAR®); Letrozoles (*e.g.* FEMARA®); Leucovorins (*e.g.* WELLCOVORIN®, LEUCOVORIN®); Levamisoles (*e.g.* ERGAMISOL®); Lomustines/CCNUs (*e.g.* CeeBU®); Mechlorethamines/Nitrogen mustards (*e.g.* MUSTARGEN®); Megestrol acetates (*e.g.* MEGACE®); Melphalans/L-PAMs (*e.g.* ALKERAN®); Mercaptopurine incl. 6-MPs (*e.g.* PURINETHOL®); Mesnas (*e.g.* MESNEX®); Methotrexates; Methoxsalens (*e.g.* UVADEX®); Mitomycin Cs (*e.g.* MUTAMYCIN®, MITOZYTREX®); Mitotanes (*e.g.* LYSODREN®); Mitoxantrones (*e.g.* NOVANTRONE®); Nandrolone Phenpropionates (*e.g.* DURABOLIN-50®); Nofetumomabs (*e.g.* VERLUMA®); Oprelvekins (*e.g.* NEUMEGA®); Oxaliplatins (*e.g.* ELOXATIN®); Paclitaxels (*e.g.* PAXENE®, TAXOL®); Pamidronates (*e.g.* AREDIA®); Pegademases (*e.g.* ADAGEN®); Pegaspargases (*e.g.* ONCASPAR®); Pegfilgrastims (*e.g.* NEULASTA®); Pentostatins (*e.g.* NIPENT®); Pipobromans (*e.g.* VERCYTE®); Plicamycin/Mithramycins (*e.g.* MITHRACIN®); Porfimer sodiums (*e.g.* PHOTOFRIN®); Procarbazines (*e.g.* MATULANE®); Quinacrines (*e.g.* ATABRINE®); Rasburicases (*e.g.* ELITEK®); Rituximabs (*e.g.* RITUXAN®); Sargramostims (*e.g.* PROKINE®); Streptozocins (*e.g.* ZANOSAR®); Sunitinib Malates (*e.g.* SUTENT®); Talcs (*e.g.* SCLEROSOL®); Tamoxifens (*e.g.*

NOLVADEX®); Temozolomides (*e.g.* TEMODAR®); Teniposides/VM-26s (*e.g.* VUMON®); Testolactones (*e.g.* TESLAC®); Thioguanines incl. 6-TG; Thiotepas (*e.g.* THIOPLEX®); Topotecans (*e.g.* HYCAMTIN®); Toremifenes (*e.g.* FARESTON®); Tositumomabs (*e.g.* BEXXAR®); Trastuzumabs (*e.g.* HERCEPTIN®); Tretinoins/ATRA (*e.g.* VESANOID®); Uracil Mustards; Valrubicins (*e.g.* VALSTAR®); Vinblastines (*e.g.* VELBAN®); Vincristines (*e.g.* ONCOVIN®); Vinorelbines (*e.g.* NAVELBINE®); and Zoledronates (*e.g.* ZOMETA®).

For example, exemplary antibiotic agents include, but are not limited to, Aminoglycosides; Amphenicols; Ansamycins; Carbacephems; Carbapenems; Cephalosporins or Cephems; Cephamycins; Clavams; Cyclic lipopeptides; Diaminopyrimidines; Ketolides; Lincosamides; Macrolides; Monobactams; Nitrofurans; Oxacephems; Oxazolidinones; Penems, thienamycins and miscellaneous beta-lactams; Penicillins; Polypeptides antibiotics; Quinolones; Sulfonamides; Sulfones; Tetracyclines; and other antibiotics (such as Clofoctols, Fusidic acids, Hexedines, Methenamines, Nitrofurantoins Nitroxolines, Ritipenems, Taurolidines, Xibomols).

Also included among exemplary therapeutic agents are coagulation factors or other blood modifiers such as antihemophilic factors, anti-inhibitor coagulent complexes, antithrombin III, coagulations Factor Vh, coagulation Factor VIII, coagulation Factor IX, plasma protein fractions, von Willebrand factors; antiplatelet agents (including, for example, abciximabs, anagrelides, cilostazols, clopidogrel bisulfates, dipyridamoles, epoprostenols, eptifibatides, tirofibans; colony stimulating factors (CSFs) (including, for example, Granulocyte CSFs and Granulocyte Macrophage CSFs); erythropoiesis stimulators (including, for example, erythropoietins such as darbepoetin alfas) and epoetin alfas; hemostatics and albumins (including, for example, aprotinins, combinations of antihemophilic factors and plasma, Desmopressin Acetates, and albumins); immune globulins, as well as hepatitis B immune globulins; thrombin inhibitors (including for example direct thrombin inhibitors and lepirudin), and drotecogin alfas; anticoagulants (including, for example, dalteparins, enoxaperins and other heparins, and warfarins).

Exemplary antibodies or other therapeutic agents include, but are not limited to, Cetuximab (IMC-C225; Erbitux®); Trastuzumab (Herceptin®); Rituximab (Rituxan®; MabThera®); Bevacizumab (Avastin®); Alemtuzumab (Campath®;

Campath-1H®; Mabcampath®); Panitumumab (ABX-EGF; Vectibix®); Ranibizumab (Lucentis®); Ibritumomab; Ibritumomab tiuxetan (Zevalin ®); Tositumomab; Iodine I 131 Tositumomab (BEXXAR®); Catumaxomab (Removab®); Gemtuzumab; Gemtuzumab ozogamicine (Mylotarg®); Abatacept (CTLA4-Ig; Orencia®); Belatacept (L104EA29YIg; LEA29Y; LEA); Ipilimumab (MDX-010; MDX-101); Tremelimumab (ticilimumab; CP-675,206); PRS-010 (*see e.g.* US20090042785); PRS-050 (US7585940; US20090305982); Aflibercept (VEGF Trap, AVE005; Holash *et al.*, (2002) *PNAS* 99:11393-11398); Volociximab (M200); F200 (Chimeric (human/murine) IgG4 Fab fragment of Volociximab (M200)); MORAb-009 Mouse/human chimeric IgG1(US20050054048); Soluble fusion protein:Anti-mesothelin Fv linked to a truncated Pseudomonas exotoxin A (SS1P (CAT-5001); US20070189962); Cixutumumab (IMC-A12); Nimotuzumab (h-R3) (Spicer (2005) *Curr Opin Mol Ther* 7:182-191); Zalutumumab (HuMax-EGFR; Lammerts van Bueren *et al.* (2008) *PNAS* 105:6109-14); Necitumumab IMC-11F8 (Li *et al.* (2008) *Structure* 16:216-227); Sym004 (Pederson *et al.* 2010 *Cancer Res* 70:588-597); and mAb-425.

In particular, therapeutic agents include, but are not limited to, immunoglobulins, Interferon beta, Interferon alpha-2as, Interferon alpha-1s, Interferon alpha-n3s, Interferon beta-1, Interferon beta-1as, Interferon gamma-1bs, Peg-interferon alpha-2 and Peginterferon alpha-2bs, insulin, a bisphosphate (*e.g.* Pamidronates or Zoledronates), Docetaxels, Doxorubincins, Doxorubicin liposomals and bevacizumabs.

Other exemplary therapeutic agents that can be combined by co-administration and/or co-formulation with a soluble PH20, such as an esPH20, include, but are not limited to, Adalimumabs, Agalsidase Betas, Alefacepts, Ampicillins, Anakinras, Antipoliomyelitic Vaccines, Anti-Thymocytes, Azithromycins, Becaplermins, Caspofungins, Cefazolins, Cefepimes, Cefotetans, Ceftazidimes, Ceftriaxones, Cetuximabs, Cilastatins, Clavulanic Acids, Clindamycins, Darbepoetin Alfas, Deaclizumabs, Diphtheria, Diphtheria antitoxins, Diphtheria Toxoids, Efalizumabs, Epinephrines, Erythropoietin Alphas, Etanercepts, Filgrastims, Fluconazoles, Follicle-Stimulating Hormones, Follitropin Alphas, Follitropin Betas, Fosphenyloins, Gadodiamides, Gadopentetates, Gatifloxacins, Glatiramers, GM-CSF's, Goserelins, Goserelin acetates, Granisetrons, *Haemophilus Influenza* B's, Haloperidols, Hepatitis vaccines, Hepatitis A Vaccines, Hepatitis B Vaccines, Ibritumomab Tiuxetans,

Ibritumomabs, Tiuxetans, Immunoglobulins, Hemophilus influenza vaccines, Influenza Virus Vaccines, Infliximabs, Insulins, Insulin Glargines, Interferons, Interferon alphas, Interferon Betas, Interferon Gammas, Interferon alpha-2a's, Interferon alpha-2b's, Interferon alpha-1's, Interferon alpha-n3's, Interferon Betas, Interferon Beta-1a's, Interferon Gammas, Interferon alpha-consensus, Iodixanols, Iohexols, Iopamidols, Ioversols, Ketorolacs, Laronidases, Levofloxacins, Lidocaines, Linezolids, Lorazepams, Measles Vaccines, Measles virus, Mumps viruses, Measles-Mumps-Rubella Virus Vaccines, Rubella vaccines, Medroxyprogesterones, Meropenems, Methylprednisolones, Midazolams, Morphines, Octreotides, Omalizumabs, Ondansetrons, Palivizumabs, Pantoprazoles, Pegaspargases, Pegfilgrastims, Peg-Interferon Alfa-2a's, Peg-Interferon Alfa-2b's, Pegvisomants, Pertussis vaccines, Piperacillins, Pneumococcal Vaccines and Pneumococcal Conjugate Vaccines, Promethazines, Reteplases, Somatropins, Sulbactams, Sumatriptans, Tazobactams, Tenecteplases, Tetanus Purified Toxoids, Ticarcillins, Tositumomabs, Triamcinolones, Triamcinolone Acetonides, Triamcinolone hexacetonides, Vancomycins, Varicella Zoster immunoglobulins, Varicella vaccines, other vaccines, Alemtuzumabs, Alitretinoins, Allopurinols, Altretamines, Amifostines, Anastrozoles, Arsenics, Arsenic Trioxides, Asparaginases, Bacillus Calmette-Guerin (BCG) vaccines, BCG Live, Bexarotenes, Bleomycins, Busulfans, Busulfan intravenous, Busulfan orals, Calusterones, Capecitabines, Carboplatins, Carmustines, Carmustines with Polifeprosans, Celecoxibs, Chlorambucils, Cisplatins, Cladribines, Cyclophosphamides, Cytarabines, Cytarabine liposomals, Dacarbazines, Dactinomycins, Daunorubicin liposomals, Daunorubicins, Daunomycins, Denileukin Diftitoxes, Dexrazoxanes, Docetaxels, Doxorubicins, Doxorubicin liposomals, Dromostanolone propionates, Elliott's B Solutions, Epirubicins, Epoetin alfas, Estramustines, Etoposides, Etoposide phosphates, Etoposide VP-16s, Exemestanes, Floxuridines, Fludarabines, Fluorouracils, 5-Fluorouracils, Fulvestrants, Gemcitabines, Gemtuzumabs, Ozogamicins, Gemtuzumab ozogamicins, Hydroxyureas, Idarubicins, Ifosfamides, Imatinib mesylates, Irinotecans, Letrozoles, Leucovorins, Levamisoles, Lomustines, CCNUs, Mechlorethamines, Nitrogen mustards, Megestrols, Megestrol acetates, Melphalans, L-PAMs, Mercaptopurines, 6-Mercaptopurines, Mesnas, Methotrexates, Methoxsalens, Mitomycins, Mitomycin C's, Mitotanes, Mitoxantrones, Nandrolones, Nandrolone Phenpropionates, Nofetumomabs, Oprelvekins, Oxaliplatins, Paclitaxels, Pamidronates, Pegademases,

Pentostatins, Pipobromans, Plicamycins, Mithramycins, Porfimers, Porfimer sodiums, Procarbazines, Quinacrines, Rasburicases, Rituximabs, Sargramostims, Streptozocins, Talcs, Tamoxifens, Temozolomides, Teniposides, Testolactones, Thioguanines, 6-Thioguanines, Triethylenethiophosphoramides (Thiotepas), Topotecans, Toremifenes, Trastuzumabs, Tretinoins, Uracil Mustards, Valrubicins, Vinblastines, Vincristines, Vinorelbines, Zoledronates, Acivicins, Aclarubicins, Acodazoles, Acronines, Adozelesins, Aldesleukins, Retinoic Acids, Alitretinoins, 9-Cis-Retinoic Acids, Alvocidibs, Ambazones, Ambomycins, Ametantrones, Aminoglutethimides, Amsacrines, Anaxirones, Ancitabines, Anthramycins, Apaziquones, Argimesnas, Asperlins, Atrimustines, Azacitidines, Azetepas, Azotomycins, Banoxantrones, Batabulins, Batimastats, Benaxibines, Bendamustines, Benzodepas, Bicalutamides, Bietaserpines, Biricodars, Bisantrenes, Bisnafide Dimesylates, Bizelesins, Bortezomibs, Brequinars, Bropirimines, Budotitanes, Cactinomycins, Canertinibs, Caracemides, Carbetimers, Carboquones, Carmofurs, Carubicins, Carzelesins, Cedefingols, Cemadotins, Chiorambucils, Cioteronels, Cirolemycins, Clanfenurs, Clofarabines, Crisnatols, Decitabines, Dexniguldipines, Dexormaplatins, Dezaguanines, Diaziquones, Dibrospidiums, Dienogests, Dinalins, Disermolides, Dofequidars, Doxifluridines, Droloxifenes, Duazomycins, Ecomustines, Edatrexates, Edotecarins, Eflomithines, Elacridars, Elinafides, Elsamitrucins, Emitefurs, Enloplatins, Enpromates, Enzastaurins, Epipropidines, Eptaloprosts, Erbulozoles, Esorubicins, Etanidazoles, Etoglucids, Etoprines, Exisulinds, Fadrozoles, Fazarabines, Fenretinides, Fluoxymesterones, Flurocitabines, Fosquidones, Fostriecins, Fotretamines, Galarubicins, Galocitabines, Geroquinols, Gimatecans, Gimeracils, Gloxazones, Glufosfamides, Ilmofosines, Ilomastats, Imexons, Improsulfans, Indisulams, Inproquones, Interleukins, Interleukin-2s, recombinant Interleukins, Intoplicines, lobenguanes, Iproplatins, Irsogladines, Ixabepilones, Ketotrexates, L-Alanosines, Lanreotides, Lapatinibs, Ledoxantrones, Leuprolides, Leuprorelins, Lexacalcitols, Liarozoles, Lobaplatins, Lometrexols, Lonafarnibs, Losoxantrones, Lurtotecans, Mafosfamides, Mannosulfans, Marimastats, Masoprocols, Maytansines, Mechiorethamines, Melengestrols, Meiphalans, Menogarils, Mepitiostanes, Metesinds, Metomidates, Metoprines, Meturedepas, Miboplatins, Miproxifenes, Misonidazoles, Mitindomides, Mitocarcins, Mitocromins, Mitoflaxones, Mitogillins, Mitoguazones, Mitomalcins, Mitonafides, Mitoquidones, Mitospers, Mitozolomides, Mivobulins, Mizoribines, Mofarotenes, Mopidamols, Mubritinibs, Mycophenolic

Acids, Nedaplatins, Neizarabines, Nemorubicins, Nitracrines, Nocodazoles, Nogalamycins, Nolatrexeds, Nortopixantrones, Ormaplatins, Ortataxels, Oteracils, Oxisurans, Oxophenarsines, Patubilones, Peldesines, Peliomycins, Pelitrexols, Pemetrexeds, Pentamustines, Peplomycins, Perfosfamides, Perifosines, Picoplatins, Pinafides, Piposulfans, Pirfenidones, Piroxantrones, Pixantrones, Plevitrexeds, Plomestanes, Porfiromycins, Prednimustines, Propamidines, Prospidiums, Pumitepas, Puromycins, Pyrazofurins, Ranimustines, Riboprines, Ritrosulfans, Rogletimides, Roquinimexs, Rufocromomycins, Sabarubicins, Safingols, Satraplatins, Sebriplatins, Semustines, Simtrazenes, Sizofirans, Sobuzoxanes, Sorafenibs, Sparfosates, Sparfosic Acids, Sparsomycins, Spirogermaniums, Spiromustines, Spiroplatins, Squalamines, Streptonigrins, Streptovarycins, Sufosfamides, Sulofenurs, Tacedinalines, Talisomycins, Tallimustines, Tariquidars, Tauromustines, Tecogalans, Tegafurs, Teloxantrones, Temoporfins, Teroxirones, Thiamiprines, Tiamiprines, Tiazofurins, Tilomisoles, Tilorones, Timcodars, Timonacics, Tirapazamines, Topixantrones, Trabectedins, Ecteinascidin 743, Trestolones, Triciribines, Trilostanes, Trimetrexates, Triplatin Tetranitrates, Triptorelins, Trofosfamides, Tubulozoles, Ubenimexs, Uredepas, Vaispodars, Vapreotides, Verteporfins, Vinbiastines, Vindesines, Vinepidines, Vinflunines, Vinformides, Vinglycinates, Vinleucinols, Vinleurosines, Vinrosidines, Vintriptols, Vinzolidines, Vorozoles, Xanthomycin A's, Guamecyclines, Zeniplatins, Zilascorbs [2-H], Zinostatins, Zorubicins, Zosuquidars, Acetazolamides, Acyclovirs, Adipiodones, Alatrofloxacins, Alfentanils, Allergenic extracts, Alpha 1-proteinase inhibitors, Aiprostadils, Amikacins, Amino acids, Aminocaproic acids, Aminophyllines, Amitriptylines, Amobarbitals, Amrinones, Analgesics, Anti-poliomyelitic vaccines, Anti-rabic serums, Anti-tetanus immunoglobulins, tetanus vaccines, Antithrombin III's, Antivenom serums, Argatrobans, Arginines, Ascorbic acids, Atenolols, Atracuriums, Atropines, Aurothioglucoses, Azathioprines, Aztreonams, Bacitracins, Baclofens, Basiliximabs, Benzoic acids, Benztropines, Betamethasones, Biotins, Bivalirudins, Botulism antitoxins, Bretyliums, Bumetanides, Bupivacaines, Buprenorphines, Butorphanols, Calcitonins, Calcitriols, Calciums, Capreomycins, Carboprosts, Carnitines, Cefaniandoles, Cefoperazones, Cefotaximes, Cefoxitins, Ceftizoximes, Cefuroximes, Chioramphenicols, Chioroprocaines, Chioroquines, Chlorothiazides, Chiorpromazines, Chondroitinsulfuric acids, Choriogonadotropin alfas, Chromiums, Cidofovirs, Cimetidines, Ciprofloxacins, Cisatracuriums, Clonidines, Codeines, Coichicines, Colistins, Collagens, Corticorelin

ovine triflutates, Corticotrophins, Cosyntropins, Cyanocobalamins, Cyclosporines, Cysteines, Dacliximabs, Dalfopristins, Dalteparins, Danaparoids, Dantrolenes, Deferoxamines, Desmopressins, Dexamethasones, Dexmedetomidines, Dexpanthenols, Dextrans, Iron dextrans, Diatrizoic acids, Diazepams, Diazoxides, Dicyclomines, Digibinds, Digoxins, Dihydroergotamines, Diltiazems, Diphenhydramines, Dipyridamoles, Dobutamines, Dopamines, Doxacuriums, Doxaprams, Doxercalciferols, Doxycyclines, Droperidols, Dyphyllines, Edetic acids, Edrophoniums, Enalaprilats, Ephedrines, Epoprostenols, Ergocalciferols, Ergonovines, Ertapenems, Erythromycins, Esmolols, Estradiols, Estrogenics, Ethacrynic acids, Ethanolamines, Ethanols, Ethiodized oils, Etidronic acids, Etomidates, Factor VIII's, Famotidines, Fenoldopams, Fentanyls, Flumazenils, Fluoresceins, Fluphenazines, Folic acids, Fomepizoles, Fomivirsens, Fondaparinuxs, Foscarnets, Fosphenytoins, Furosemides, Gadoteridols, Gadoversetamides, Ganciclovirs, Gentamicins, Glucagons, Glucoses, Glycines, Glycopyrrolates, Gonadorelins, Gonadotropin chorionics, Haemophilus B polysaccarides, Hemins, Herbals, Histamines, Hydralazines, Hydrocortisones, Hydromorphones, Hydroxocobalamins, Hydroxyzines, Hyoscyamines, Ibutilides, Imiglucerases, Indigo carmines, Indomethacins, Iodides, lopromides, Iothalamic acids, loxaglic acids, loxilans, Isoniazids, Isoproterenols, Japanese encephalitis vaccines, Kanamycins, Ketamines, Labetalols, Lepirudins, Levobupivacaines, Levothyroxines, Lincomycins, Liothyronines, Luteinising hormones, Lyme disease vaccines, Mangafodipirs, Manthtols, Meningococcal polysaccharide vaccines, Meperidines, Mepivacaines, Mesoridazines, Metaraminols, Methadones, Methocarbamols, Methohexitals, Methyldopates, Methylergonovines, Metoclopramides, Metoprolols, Metronidazoles, Minocyclines, Mivacuriums, Morrhuic acids, Moxifloxacins, Muromonab-CD3s, Mycophenolate mofetils, Nafcillins, Nalbuphines, Nalmefenes, Naloxones, Neostigmines, Niacinamides, Nicardipines, Nitroglycerins, Nitroprussides, Norepinephrines, Orphenadrines, Oxacillins, Oxymorphones, Oxytetracyclines, Oxytocins, Pancuroniums, Panthenols, Pantothenic acids, Papaverines, Peginterferon-alpha (*e.g.* interferon alpha 2a or 2b), Penicillin Gs, Pentamidines, Pentazocines, Pentobarbitals, Perfiutrens, Perphenazines, Phenobarbitals, Phentolamines, Phenylephrines, Phenytoins, Physostigmines, Phytonadiones, Polymyxin bs, Pralidoximes, Prilocaines, Procainamides, Procaines, Prochiorperazines, Progesterones, Propranolols, Pyridostigmine hydroxides, Pyridoxines, Quinidines,

Quinupristins, Rabies immunoglobulins, Rabies vaccines, Ranitidines, Remifentanils, Riboflavins, Rifampins, Ropivacaines, Samariums, Scopolamines, Seleniums, Sermorelins, Sincalides, Somatrems, Spectinomycins, Streptokinases, Streptomycins, Succinylcholines, Sufentanils, Sulfamethoxazoles, Tacrolimuss, Terbutalines, Teriparatides, Testosterones, Tetanus antitoxins, Tetracaines, Tetradecyl sulfates, Theophyllines, Thiamines, Thiethylperazines, Thiopentals, Thyroid stimulating hormones, Tinzaparins, Tirofibans, Tobramycins, Tolazolines, Tolbutamides, Torsemides, Tranexamic acids, Treprostinils, Trifluoperazines, Trimethobenzamides, Trimethoprims, Tromethamines, Tuberculins, Typhoid vaccines, Urofollitropins, Urokinases, Vaiproic acids, Vasopressins, Vecuroniums, Verapamils, Voriconazoles, Warfarins, Yellow fever vaccines, Zidovudines, Zincs, Ziprasidone hydrochiorides, Aclacinomycins, Actinomycins, Adriamycins, Azaserines, 6-Azauridines, Carzinophilins, Chromomycins, Denopterins, 6-Diazo-5-Oxo-L-Norleucines, Enocitabines, Loxuridines, Olivomycines, Pirarubicins, Piritreximes, Pteropterins, Tagafurs, Tubercidins, Alteplases, Arcitumomabs, bevacizumabs, Botulinum Toxin Type A's, Botulinum Toxin Type B's, Capromab Pendetides, Daclizumabs, Dornase alfas, Drotrecogin alfas, Imciromab Pentetates, and Iodine-131's.

Delivery of Insulin

Methods provided herein include methods of co-administering a modified PH20 polypeptide and an insulin to increase subsutaneous delivery of the insulin, such as a fast-acting insulin. Such methods include methods of direct administration, and closed pump and continuous infusion methods. For example, exemplary of insulins that can be administered with a modified PH20 hyaluronidase provided herein are fast-acting insulins or insulin analogs. For example, a co-administered insulin includes a regular insulin, insulin aspart, insulin lispro, insulin glulisine or other similar analog variants. Exemplary of insulin are insulins that contain an A chain set forth in SEQ ID NO:862 and a B chain set forth in SEQ ID NO:863 or variants that contain one or more amino acid modifications compared to a human insulin set forth in SEQ ID NO: 862 and 863 (A and B chains). For example, exemplary insulin analogs are known to one of skill in the art, and include, but are not limited to, those set forth in SEQ ID NOS:862 (A-chain) and having a B-chain set forth in any of SEQ ID NOS: 865-867.

The co-formulations can be administered subcutaneously to treat any condition that is amenable to treatment with insulin. Therapeutic uses include, but are

not limited to, treatment for type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, and for glycemic control in critically ill patients. For example, the co-formulations of a fast acting insulin and hyaluronan degrading enzyme can be administered subcutaneously in discrete doses, such as via a syringe or insulin pen, prior to a meal as prandial insulin therapy in subjects with diabetes to achieve glycemic control. The co-formulations also can be administered subcutaneously or intraperitonally using an insulin pump or in the context of a closed loop system to continuously control blood glucose levels throughout the day and night and/or to control post-prandial glycemic excursions. It is within the skill of a treating physician to identify such diseases or conditions.

For any disease or condition, including all those exemplified above, for which a fast-acting insulin is indicated or has been used and for which other agents and treatments are available, the co-formulations can be used in combination therewith. Depending on the disease or condition to be treated, exemplary combinations include, but are not limited to, combination with anti-diabetic drugs, including, but not limited to, sulfonylureas, biguanides, meglitinides, thiazolidinediones, alpha-glucosidase inhibitors, peptide analogs, including glucagon-like peptide (GLP) analogs and, gastric inhibitory peptide (GIP) analogs and DPP-4 inhibitors. In another example, the co-formulations of a fast acting insulin and modified PH20 polypeptide described herein can be administered in combination with, prior to, intermittently with, or subsequent to, with one or more other insulins, including fast-acting insulin, and basal-acting insulins.

2. Methods of Hyaluronan-Associated Diseases and Conditions (*e.g.* Tumors)

In particular, PH20 hyaluronidase can be used to treat hyaluronan-associated diseases or conditions. Typically, hyaluronan-associated diseases and conditions are associated with elevated hyaluronan expression in a tissue, cell, or body fluid (*e.g.* tumor tissue or tumor-associated tissue, blood, or interstitial space) compared to a control, *e.g.* another tissue, cell or body fluid. The elevated hyaluronan expression can be elevated compared to a normal tissue, cell or body fluid, for example, a tissue, cell or body fluid that is analogous to the sample being tested, but isolated from a different subject, such as a subject that is normal (*i.e.* does not have a disease or condition, or does not have the type of disease or condition that the subject being tested has), for example, a subject that does not have a hyaluronan-associated disease

or condition. The elevated hyaluronan expression can be elevated compared to an analogous tissue from another subject that has a similar disease or condition, but whose disease is not as severe and/or is not hyaluronan-associated or expresses relatively less hyaluronan and thus is hyaluronan-associated to a lesser degree. For example, the subject being tested can be a subject with a hyaluronan-associated cancer, where the HA amounts in the tissue, cell or fluid are relatively elevated compared to a subject having a less severe cancer, such as an early stage, differentiated or other type of cancer. In another example, the cell, tissue or fluid contains elevated levels of hyaluronan compared to a control sample, such as a fluid, tissue, extract (*e.g.* cellular or nuclear extract), nucleic acid or peptide preparation, cell line, biopsy, standard or other sample, with a known amount or relative amount of HA, such as a sample, for example a tumor cell line, known to express relatively low levels of HA, such as exemplary tumor cell lines described herein that express low levels of HA, for example, the HCT 116 cell line, the HT29 cell line, the NCI H460 cell line, the DU145 cell line, the Capan-1 cell line, and tumors from tumor models generated using such cell lines.

Hyaluoronan- associated diseases and conditions include those associated with high interstitial fluid pressure, such as disc pressure, proliferative disorders, such as cancer and benign prostatic hyperplasia, and edema. Edema can result from or be manifested in, for example, from organ transplant, stroke or brain trauma. Proliferative disorders include, but are not limited to, cancer, smooth muscle cell proliferation, systemic sclerosis, cirrhosis of the liver, adult respiratory distress syndrome, idiopathic cardiomyopathy, lupus erythematosus, retinopathy, *e.g.*, diabetic retinopathy or other retinopathies, cardiac hyperplasia, reproductive system associated disorders, such as benign prostatic hyperplasia (BPH) and ovarian cysts, pulmonary fibrosis, endometriosis, fibromatosis, harmatomas, lymphangiomatosis, sarcoidosis, desmoid tumors. Cancers include solid and lymphatic/blood tumors and metastatic disease, and undifferentiated tumors. The tumors amenable to treatment typically exhibit cellular and/or stromal expression of a hyaluronan, compared to a non-cancerous tissue of the same tissue type or compared to a non-metastatic tumor of the same tumor-type. Cancers include any one or more of ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, other gastric cancers, non-small cell lung cancer, breast cancer, brain cancer and colon cancer.

Modified PH20 polypeptides provided herein, such as PEGylated forms thereof, can be used to treat tumors. Thus, in addition to its indirect anticancer effects, hyaluronidases also have direct anticarcinogenic effects. Hyaluronidase prevents growth of tumors transplanted into mice (De Maeyer et al., 1992, *Int. J. Cancer* 51:657-660) and inhibits tumor formation upon exposure to carcinogens (Pawlowski et al., 1979, *Int. J. Cancer* 23:105-109; Haberman et al., 1981, Proceedings of the 17th Annual Meeting of the American Society of Clinical Oncology, Washington, D.C., 22:105, abstract no. 415). PH20 hyalurondiase has been shown to treat various tumors (*see e.g.* U.S. published application nos. US2010/0003238 and U.S. Applic. Serial No. 13/135,817).

The hyaluronan-rich cancer can be a cancer in which the cancer cells produce HALOs, cancers that have elevated expression of hyaluronan (as determined by immunostaining, *e.g.* histological staining of sections from the tumor), cancers that have elevated HAS2 (Hyaluronan synthase 2), cancers that do not produce hyaluronidase (HYAL1) *in vitro*. Hyaluronan-rich cancers can be identified by any method for assessing hyaluronan expression, and other known methods for assaying protein/mRNA expression.

Several hyaluronan-rich cancers have been identified. In some cases, hyaluronan expression correlates with poor prognosis, for example, decreased survival rate and/or recurrence-free survival rate, metastases, angiogenesis, cancer cell invasion into other tissues/areas, and other indicators of poor prognosis. Such correlation has been observed, for example, in hyaluronan-rich tumors including ovarian cancer, SCC, ISC, prostate cancer, lung cancer, including non-small-cell lung cancer (NSCLC), breast cancer, colon cancer and pancreatic cancer (see, for example, Maarit et al., *Cancer Research*, 60:150-155 (2000); Karvinen et al., *British Journal of Dermatology*, 148:86-94 (2003); Lipponen et al., *Eur. Journal of Cancer*, 849-856 (2001); Pirinen et al., *Int. J. Cancer*: 95: 12-17 (2001); Auvinen et al., *American Journal of Pathology*, 156(2):529-536 (2000); Ropponen et al., *Cancer Research*, 58: 342-347 (1998)). Thus, hyaluronan-rich cancers can be treated by administration of a hyaluronidase, such as a soluble PH20, to treat one or more symptoms of the cancer. Hyaluronan-rich tumors include, but are not limited to, prostate, breast, colon, ovarian, stomach, head and neck and other tumors and cancers.

Other hyaluornan-associated diseases or conditions that are associated with excess glycosaminoglycans and that can be treated with a modified PH20 polypeptide provided herein include, but are not limited to, cardiovascular disease (*e.g.* following ischemia reperfusion; in arteriosclerosis); vitrectomy and ophthalmic disorders and conditions (*e.g.* in methods to liquefy the vitreous humor of the eye; reduce postoperative pressure; other ocular surgical procedures such as glaucoma, vitreous and retina surgery and in corneal transplantation); in hypodermoclysis (*i.e.* infusion of fluids and electrolytes into the hypodermis of the skin); cosmetic applications (*e.g.*, in the treatment of cellulite, "pigskin" edema or "orange peel" edema); organ transplantation (*e.g.* associated with interstitial edemas in connection with grafting of an organ); pulmonary disease.

3. Other uses

In further examples of its therapeutic use, modified PH20 polypeptides provided herein, can be used for such purposes as an antidote to local necrosis from paravenous injection of necrotic substances such as vinka alkaloids (Few et al. (1987) *Amer. J. Matern. Child Nurs.* 12, 23-26), treatment of ganglion cysts (Paul et al. (1997) *J Hand Surg.* 22 (2): 219-21) and treatment of tissue necrosis due to venous insufficiency (Elder et al. (1980) *Lancet* 648-649). Modified PH20 polypeptides also can be used to treat ganglion cysts (also known as a wrist cyst, Bible cyst, or dorsal tendon cyst), which are the most common soft tissue mass of the hand and are fluid filled sacs that can be felt below the skin.

Modified PH20 polypeptides can be used in the treatment of spinal cord injury by degrading chondroitin sulfate proteoglycans (CSPGs). Following spinal cord injury, glial scars containing CSPGs are produced by astrocytes. CSPGs play a crucial role in the inhibition of axon growth. In addition, the expression of CSPG has been shown to increase following injury of the central nervous system (CNS). Soluble PH20 also can be utilized for the treatment of herniated disks in a process known as chemonucleolysis. Chondroitinase ABC, an enzyme cleaving similar substrates as hyaluronidase, can induce the reduction of intradiscal pressure in the lumbar spine. There are three types of disk injuries. A protruded disk is one that is intact but bulging. In an extruded disk, the fibrous wrapper has torn and the NP has oozed out, but is still connected to the disk. In a sequestered disk, a fragment of the NP has broken loose from the disk and is free in the spinal canal. Chemonucleolysis is

typically effective on protruded and extruded disks, but not on sequestered disk injuries.

4. Contraception

Modified PH20 polypeptides provided herein can be used as vaccines in contraceptive applications. PH20 is present in the male reproductive tract, and is expressed in both the testis and epididymis and is present in sperm. PH20 plays a role in fertilization by facilitating entry of the sperm through the cumulus layer surrounding the unfertilized egg. PH20 also is able to bind to hyaluronic acid (HA) on the the zona pellucida during early phases of fertilization. This binding also initiates intracellular signaling that aids in the acrosome reaction. Immunization with PH20 has been show to be an effective contraceptive in male guinea pigs (Primakoff et al. (1988), Tung et al. 1997). It also has been shown to be an effective contraceptive in female guinea pigs due to the generation of anti-PH20 antibodies that prevent sperm and egg binding. In examples herein, the modified PH20 polypeptides can be inactive enzymes, such as any described in Sections C.2. The polypeptides can be administered directly or can be administered as a recombinant virus to deliver the antigen.

H. EXAMPLES

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1

GENERATION OF RECOMBINANT HUMAN PH20 HYALURONIDASE (rHuPH20)

A. Generation of a soluble rHuPH20-expressing cell line

A recombinant human PH20 hyaluronidase designated rHuPH20 was generated as described in published U.S. Patent Application No. US20110053247. Briefly, the pCI-PH20-IRES-DHFR-SV40pa (HZ24) plasmid (set forth in SEQ ID NO:5) was used to transfect Chinese Hamster Ovary (CHO cells) (*see e.g.* U.S. Patent Nos. 7,76,429 and 7,781,607 and U.S. Publication No. 2006-0104968). The HZ24 plasmid vector for expression of soluble rHuPH20 contains a pCI vector backbone (Promega), DNA encoding amino acids 1-482 of human PH20 hyaluronidase (SEQ ID NO:2), an internal ribosomal entry site (IRES) from the ECMV virus (Clontech), and the mouse dihydrofolate reductase (DHFR) gene. The pCI vector backbone also includes DNA encoding the Beta-lactamase resistance gene (AmpR), an f1 origin of

replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), a chimeric intron, and an SV40 late polyadenylation signal (SV40). The DNA encoding the soluble rHuPH20 construct contains an NheI site and a Kozak consensus sequence prior to the DNA encoding the methionine at amino acid position 1 of the native 35 amino acid signal sequence of human PH20, and a stop codon following the DNA encoding the tyrosine corresponding to amino acid position 482 of the human PH20 hyaluronidase set forth in SEQ ID NO:2, followed by a BamHI restriction site.

Non-transfected DG44 CHO cells growing in GIBCO Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), were seeded at 0.5 x 10^6 cells/ml in a shaker flask in preparation for transfection. Cells were grown at 37 °C in 5 % CO_2 in a humidified incubator, shaking at 120 rpm. Exponentially growing non-transfected DG44 CHO cells were tested for viability prior to transfection.

Sixty million viable cells of the non-transfected DG44 CHO cell culture were pelleted and resuspended to a density of 2 $\times 10^7$ cells in 0.7 mL of 2x transfection buffer (2x HeBS: 40 mM Hepes, pH 7.0, 274 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, 12 mM dextrose). To each aliquot of resuspended cells, 0.09 mL (250 µg) of the linear HZ24 plasmid (linearized by overnight digestion with Cla I (New England Biolabs) was added, and the cell/DNA solutions were transferred into 0.4 cm gap BTX (Gentronics) electroporation cuvettes at room temperature. A negative control electroporation was performed with no plasmid DNA mixed with the cells. The cell/plasmid mixes were electroporated with a capacitor discharge of 330 V and 960 µF or at 350 V and 960 µF.

The cells were removed from the cuvettes after electroporation and transferred into 5 mL of Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), and allowed to grow in a well of a 6-well tissue culture plate without selection for 2 days at 37 °C in 5 % CO_2 in a humidified incubator.

Two days post-electroporation, 0.5 mL of tissue culture media was removed from each well and tested for the presence of hyaluronidase activity, using the microturbidity assay described in Example 8.

Table 6: Initial Hyaluronidase Activity of HZ24 Transfected DG44 CHO

cells at 40 hours post-transfection		
	Dilution	**Activity (Units/ml)**
Transfection 1 330V	1 to 10	0.25
Transfection 2 350V	1 to 10	0.52
Negative Control	1 to 10	0.015

Cells from Transfection 2 (350V) were collected from the tissue culture well, counted and diluted to 1 $\times 10^4$ to 2 $\times 10^4$ viable cells per mL. A 0.1 mL aliquot of the cell suspension was transferred to each well of five, 96 well round bottom tissue culture plates. One hundred microliters of CD-CHO media (GIBCO) containing 4 mM GlutaMAX™-1 supplement (GIBCO™, Invitrogen Corporation) and without hypoxanthine and thymidine supplements were added to the wells containing cells (final volume 0.2 mL). Ten clones were identified from the 5 plates grown without methotrexate.

Table 7. Hyaluronidase activity of identified clones

Plate/Well ID	Relative Hyaluronidase
1C3	261
2C2	261
3D3	261
3E5	243
3C6	174
2G8	103
1B9	304
2D9	273
4D10	302

Six HZ24 clones were expanded in culture and transferred into shaker flasks as single cell suspensions. Clones 3D3, 3E5, 2G8, 2D9, 1E11, and 4D10 were plated into 96-well round bottom tissue culture plates using a two-dimensional infinite dilution strategy in which cells were diluted 1:2 down the plate, and 1:3 across the plate, starting at 5000 cells in the top left hand well. Diluted clones were grown in a background of 500 non-transfected DG44 CHO cells per well, to provide necessary growth factors for the initial days in culture. Ten plates were made per subclone, with 5 plates containing 50 nM methotrexate and 5 plates without methotrexate.

Clone 3D3 produced 24 visual subclones (13 from the no methotrexate treatment, and 11 from the 50 nM methotrexate treatment. Significant hyaluronidase activity was measured in the supernatants from 8 of the 24 subclones (>50 Units/mL), and these 8 subclones were expanded into T-25 tissue culture flasks. Clones isolated from the methotrexate treatment protocol were expanded in the presence of 50 nM

methotrexate. Clone 3D35M was further expanded in 500 nM methotrexate giving rise to clones producing in excess of 1,000 Units/ml in shaker flasks (clone 3D35M; or Gen1 3D35M). A master cell bank (MCB) of the 3D35M cells was then prepared.

B. Production Gen2 Cells Containing Soluble human PH20 (rHuPH20)

The Gen1 3D35M cell line described in Example 1.A was adapted to higher methotrexate levels to produce generation 2 (Gen2) clones. 3D35M cells were seeded from established methotrexate-containing cultures into CD CHO medium containing 4mM GlutaMAX-1™ and 1.0 μM methotrexate. The cells were adapted to a higher methotrexate level by growing and passaging them 9 times over a period of 46 days in a 37 °C, 7% CO_2 humidified incubator. The amplified population of cells was cloned out by limiting dilution in 96-well tissue culture plates containing medium with 2.0 μM methotrexate. After approximately 4 weeks, clones were identified and clone 3E10B was selected for expansion. 3E10B cells were grown in CD CHO medium containing 4 mM GlutaMAX-1™ and 2.0 μM methotrexate for 20 passages. A master cell bank (MCB) of the 3E10B cell line was created and frozen and used for subsequent studies.

Amplification of the cell line continued by culturing 3E10B cells in CD CHO medium containing 4 mM GlutaMAX-1™ and 4.0 μM methotrexate. After the 12th passage, cells were frozen in vials as a research cell bank (RCB). One vial of the RCB was thawed and cultured in medium containing 8.0 μM methotrexate. After 5 days, the methotrexate concentration in the medium was increased to 16.0 μM, then 20.0 μM 18 days later. Cells from the 8th passage in medium containing 20.0 μM methotrexate were cloned out by limiting dilution in 96-well tissue culture plates containing CD CHO medium containing 4 mM GlutaMAX-1™ and 20.0 μM methotrexate. Clones were identified 5-6 weeks later and clone 2B2 was selected for expansion in medium containing 20.0 μM methotrexate. After the 11th passage, 2B2 cells were frozen in vials as a research cell bank (RCB).

The resultant 2B2 cells are dihydrofolate reductase deficient (dhfr-) DG44 CHO cells that express soluble recombinant human PH20 (rHuPH20). The soluble PH20 is present in 2B2 cells at a copy number of approximately 206 copies/cell. Southern blot analysis of Spe I-, Xba I- and BamH I/Hind III-digested genomic 2B2 cell DNA using a rHuPH20-specific probe revealed the following restriction digest profile: one major hybridizing band of ~7.7 kb and four minor hybridizing bands (~13.9, ~6.6, ~5.7 and ~4.6 kb) with DNA digested with Spe I; one major hybridizing

band of ~5.0 kb and two minor hybridizing bands (~13.9 and ~6.5 kb) with DNA digested with Xba I; and one single hybridizing band of ~1.4 kb observed using 2B2 DNA digested with BamH I/Hind III.

C. Production of Gen2 soluble rHuPH20 in 300 L Bioreactor Cell Culture

A vial of HZ24-2B2 was thawed and expanded from shaker flasks through 36L spinner flasks in CD-CHO media (Invitrogen, Carlsbad, CA) supplemented with 20 μM methotrexate and GlutaMAX-1™ (Invitrogen). Briefly, the vial of cells was thawed in a 37 °C water bath, media was added and the cells were centrifuged. The cells were re-suspended in a 125 mL shake flask with 20 mL of fresh media and placed in a 37 °C, 7% CO_2 incubaor. The cells were expanded up to 40 mL in the 125 mL shake flask. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 125 mL spinner flask in a 100 mL culture volume. The flask was incubated at 37 °C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 250 mL spinner flask in 200 mL culture volume, and the flask was incubated at 37 °C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 1 L spinner flask in 800 mL culture volume and incubated at 37 °C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL the culture was expanded into a 6 L spinner flask in 5000 mL culture volume and incubated at 37 °C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL the culture was expanded into a 36 L spinner flask in 32 L culture volume and incubated at 37 °C, 7% CO_2.

A 400 L reactor was sterilized and 230 mL of CD-CHO media was added. Before use, the reactor was checked for contamination. Approximately 30 L cells were transferred from the 36L spinner flasks to the 400 L bioreactor (Braun) at an inoculation density of 4.0 × 10^5 viable cells per ml and a total volume of 260L. Parameters were temperature setpoint, 37 °C; Impeller Speed 40-55 RPM; Vessel Pressure: 3 psi; Air Sparge 0.5- 1.5 L/Min.; Air Overlay: 3 L/ min. The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Also, during the run nutrient feeds were added. At 120 hrs (day 5), 10.4L of Feed #1 Medium (4× CD-CHO + 33 g/L Glucose + 160 mL/L Glutamax-1™ + 83 mL/L Yeastolate + 33 mg/L rHuInsulin) was added. At 168 hours (day 7), 10.8 L of Feed #2 (2× CD-CHO + 33 g/L Glucose + 80 mL/L Glutamax-1™ + 167 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 36.5°C. At 216 hours (day 9), 10.8 L of Feed #3 (1× CD-CHO + 50 g/L

Glucose + 50 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 1.80 g/L Sodium Butyrate) was added, and culture temperature was changed to 36° C. At 264 hours (day 11), 10.8 L of Feed #4 (1× CD-CHO + 33 g/L Glucose + 33 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 35.5 °C. The addition of the feed media was observed to dramatically enhance the production of soluble rHuPH20 in the final stages of production. The reactor was harvested at 14 or 15 days or when the viability of the cells dropped below 40%. The process resulted in a final productivity of 17,000 Units per ml with a maximal cell density of 12 million cells/mL. At harvest, the culture was sampled for mycoplasma, bioburden, endotoxin and viral *in vitro* and *in vivo*, Transmission Electron Microscopy (TEM) and enzyme activity.

The culture was pumped by a peristaltic pump through four Millistak filtration system modules (Millipore) in parallel, each containing a layer of diatomaceous earth graded to 4-8 μm and a layer of diatomaceous earth graded to 1.4-1.1 μm, followed by a cellulose membrane, then through a second single Millistak filtration system (Millipore) containing a layer of diatomaceous earth graded to 0.4-0.11 μm and a layer of diatomaceous earth graded to <0.1 μm, followed by a cellulose membrane, and then through a 0.22 μm final filter into a sterile single use flexible bag with a 350 L capacity. The harvested cell culture fluid was supplemented with 10 mM EDTA and 10 mM Tris to a pH of 7.5. The culture was concentrated 10× with a tangential flow filtration (TFF) apparatus using four Sartoslice TFF 30 kDa molecular weight cut-off (MWCO) polyether sulfone (PES) filter (Sartorious) , followed by a 10× buffer exchange with 10 mM Tris, 20mM Na_2SO_4, pH 7.5 into a 0.22 μm final filter into a 50 L sterile storage bag.

The concentrated, diafiltered harvest was inactivated for virus. Prior to viral inactivation, a solution of 10% Triton® X-100, 3% tri (n-butyl) phosphate (TNBP) was prepared. The concentrated, diafiltered harvest was exposed to 1% Triton® X-100, 0.3% TNBP for 1 hour in a 36 L glass reaction vessel immediately prior to purification on the Q column.

D. Purification of Gen2 soluble rHuPH20

A Q Sepharose (Pharmacia) ion exchange column (9 L resin, H= 29 cm, D= 20 cm) was prepared. Wash samples were collected for a determination of pH, conductivity and endotoxin (LAL) assay. The column was equilibrated with 5 column volumes of 10 mM Tris, 20 mM Na2SO4, pH 7.5. Following viral

inactivation, the concentrated, diafiltered harvest was loaded onto the Q column at a flow rate of 100 cm/hr. The column was washed with 5 column volumes of 10 mM Tris, 20 mM Na2SO4, pH 7.5 and 10 mM Hepes, 50 mM NaCl, pH7.0. The protein was eluted with 10 mM Hepes, 400 mM NaCl, pH 7.0 into a 0.22 μm final filter into sterile bag. The eluate sample was tested for bioburden, protein concentration and hyaluronidase activity. A_{280} absorbance reading were taken at the beginning and end of the exchange.

Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed. A Phenyl-Speharose (PS) column (19-21 L resin, H=29 cm, D= 30 cm) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate, 0.1 mM CaCl2, pH 7.0. The protein eluate from the Q sepharose column was supplemented with 2M ammonium sulfate, 1 M potassium phosphate and 1 M $CaCl_2$ stock solutions to yield final concentrations of 5 mM, 0.5 M and 0.1 mM, respectively. The protein was loaded onto the PS column at a flow rate of 100 cm/hr and the column flow thru collected. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate and 0.1 mM CaCl2 pH 7.0 at 100 cm/hr and the wash was added to the collected flow thru. Combined with the column wash, the flow through was passed through a 0.22 μm final filter into a sterile bag. The flow through was sampled for bioburden, protein concentration and enzyme activity.

An aminophenyl boronate column (Prometics) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate. The PS flow through containing purified protein was loaded onto the aminophenyl boronate column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate, pH 7.0. The column was washed with 20 mM bicine, 0.5 M ammonium sulfate, pH 9.0. The column was washed with 20 mM bicine, 100 mM sodium chloride, pH 9.0. The protein was eluted with 50 mM Hepes, 100 mM NaCl, pH 6.9 and passed through a sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

The hydroxyapatite (HAP) column (Biorad) was prepared. The wash was collected and tested for pH, conductivity and endotoxin (LAL assay). The column

was equilibrated with 5 mM potassium phosphate, 100 mM NaCl, 0.1 mM $CaCl_2$, pH 7.0. The aminophenyl boronate purified protein was supplemented to final concentrations of 5 mM potassium phosphate and 0.1 mM $CaCl_2$ and loaded onto the HAP column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM $CaCl_2$. The column was next washed with 10 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM $CaCl_2$. The protein was eluted with 70 mM potassium phosphate, pH 7.0 and passed through a 0.22μm sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

The HAP purified protein was then passed through a viral removal filter. The sterilized Viosart filter (Sartorius) was first prepared by washing with 2 L of 70 mM potassium phosphate, pH 7.0. Before use, the filtered buffer was sampled for pH and conductivity. The HAP purified protein was pumped via a peristaltic pump through the 20 nM viral removal filter. The filtered protein in 70 mM potassium phosphate, pH 7.0 was passed through a 0.22 μm final filter into a sterile bag. The viral filtered sample was tested for protein concentration, enzyme activity, oligosaccharide, monosaccharide and sialic acid profiling. The sample also was tested for process related impurities.

The protein in the filtrate was then concentrated to 10 mg/mL using a 10 kD molecular weight cut off (MWCO) Sartocon Slice tangential flow filtration (TFF) system (Sartorius). The filter was first prepared by washing with 10 mM histidine, 130 mM NaCl, pH 6.0 and the permeate was sampled for pH and conductivity. Following concentration, the concentrated protein was sampled and tested for protein concentration and enzyme activity. A 6× buffer exchange was performed on the concentrated protein into the final buffer: 10 mM histidine, 130 mM NaCl, pH 6.0. Following buffer exchange, the concentrated protein was passed though a 0.22 μm filter into a 20 L sterile storage bag. The protein was sampled and tested for protein concentration, enzyme activity, free sulfydryl groups, oligosaccharide profiling and osmolarity. For lot number WRS2 used as a standard in the assays described below, the results showed that the test description for appearance was clear and colorless; the pH was 7.4; the endotoxin level was <0.01 EU/mL; the osmolarity was 308 mOsm/Kg; the density was 1.005 g/mL; the rHuPH20 content was 1.3 ppm; and the hyaluronidase activity was 145 USP U/mL.

The sterile filtered bulk protein was then asceptically dispensed at 20 mL into 30 mL sterile Teflon vials (Nalgene). The vials were then flash frozen and stored at -20 ± 5°C.

EXAMPLE 2

GENERATION OF PH20 MUTANT LIBRARY

A. Cloning and Mutagenesis

In this example, a human hyaluronidase PH20 library was created by cloning DNA encoding human PH20 into a plasmid followed by transfection and protein expression.

The library was created by mutagenesis of a PH20 template that is a codon optimized version of PH20 with an Ig Kappa leader sequence. Specifically, for generating the library of variants, the HZ24-PH20(OHO)-IRES-SEAP expression vector (set forth in SEQ ID NO:4) was used as a template, which contains the sequence of nucleotides encoding PH20 set forth in SEQ ID NO:1, which encodes a precursor PH20 set forth in SEQ ID NO:2 or a mature PH20 set forth in SEQ ID NO:3 lacking residues 1-22 corresponding to the IgK signal sequence. The backbone of the vector was derived from the original HZ24 vector containing the DHFR selection marker (see Example 1and SEQ ID NO:5) with the addition of an IgK leader sequence and codon optimization. The expression vector also was modified to contain the gene for secreted alkaline phosphatase (SEAP). Hence, in addition to sequence encoding PH20, the HZ24-PH20(OHO)-IRES-SEAP expression vector also contains an internal ribosome entry site (EMCV IRES) that is linked to the coding sequence for the gene for secreted alkaline phosphatase (SEAP), and a single CMV promoter that drives expression of PH20 and SEAP in the construct.

The first library was made to generate encoded variant proteins wherein each of residues 23-469 of SEQ ID NO:2 (corresponding to positions 1-447 of SEQ ID NO:3) was changed to one of about 15 amino acid residues, such that each member contained a single amino change. The resulting library contained 6753 variant members, each containing a single amino acid mutation compared to residues 23-469 of SEQ ID NO:2 or residues 1-447 of SEQ ID NO:3. Glycerol stocks of the resulting library was prepared and stored at -80°C. The amino acids substitutions and corresponding mutated codons in the libraries are listed in Table 8, below. Each member was expressed and screened for hyaluoridase activity as described below.

TABLE 8: PH20 Variant Library						
L001A	Y066V	R132Q	G198W	V265I	I331Q	F398A
L001C	I067C	R132S	G198Y	V265K	I331R	F398C
L001D	I067D	R132T	Y199A	V265L	I331S	F398E
L001E	I067E	R132V	Y199C	V265M	I331T	F398G
L001F	I067F	R132Y	Y199E	V265N	I331W	F398H
L001G	I067G	S133A	Y199G	V265P	I331Y	F398I
L001H	I067H	S133D	Y199H	V265Q	I332A	F398L
L001K	I067L	S133E	Y199I	V265R	I332C	F398N
L001N	I067N	S133F	Y199K	V265S	I332D	F398P
L001P	I067P	S133G	Y199L	V265W	I332E	F398R
L001Q	I067Q	S133H	Y199N	V265Y	I332F	F398S
L001R	I067R	S133I	Y199P	F266A	I332G	F398T
L001S	I067T	S133L	Y199Q	F266C	I332H	F398V
L001T	I067V	S133M	Y199R	F266D	I332K	F398W
L001V	I067W	S133N	Y199S	F266G	I332L	F398Y
L001W	I067Y	S133P	Y199T	F266H	I332N	Y399A
N002A	D068A	S133R	Y199W	F266L	I332P	Y399C
N002C	D068C	S133T	N200A	F266M	I332R	Y399D
N002F	D068E	S133V	N200D	F266P	I332S	Y399E
N002G	D068G	S133W	N200F	F266Q	I332T	Y399G
N002H	D068H	I134A	N200G	F266R	I332Y	Y399K
N002I	D068I	I134C	N200H	F266S	N333A	Y399M
N002K	D068K	I134D	N200K	F266T	N333E	Y399N
N002L	D068L	I134F	N200L	F266V	N333G	Y399P
N002P	D068P	I134G	N200M	F266W	N333H	Y399Q
N002Q	D068Q	I134H	N200P	F266Y	N333I	Y399R
N002S	D068R	I134K	N200Q	A267D	N333K	Y399S
N002T	D068S	I134L	N200R	A267E	N333L	Y399T
N002V	D068T	I134P	N200S	A267G	N333M	Y399V
N002W	D068V	I134Q	N200T	A267H	N333P	Y399W
N002Y	D068Y	I134R	N200V	A267I	N333R	C400A
F003A	S069A	I134S	N200W	A267K	N333S	C400D
F003E	S069C	I134T	N200Y	A267L	N333T	C400E
F003G	S069E	I134V	G201A	A267M	N333V	C400F
F003H	S069F	I134W	G201E	A267N	N333W	C400G
F003I	S069G	E135A	G201F	A267P	N333Y	C400I
F003K	S069I	E135C	G201H	A267R	V334A	C400L

TABLE 8: PH20 Variant Library						
F003L	S069L	E135D	G201K	A267S	V334C	C400M
F003M	S069M	E135F	G201L	A267T	V334D	C400P
F003N	S069N	E135G	G201M	A267V	V334E	C400Q
F003P	S069P	E135H	G201N	A267W	V334G	C400R
F003R	S069R	E135K	G201P	Y268A	V334H	C400S
F003S	S069T	E135L	G201Q	Y268C	V334L	C400T
F003T	S069V	E135N	G201R	Y268F	V334M	C400V
F003V	S069W	E135P	G201S	Y268G	V334N	C400Y
F003Y	S069Y	E135Q	G201T	Y268H	V334P	S401A
R004A	I070A	E135R	G201V	Y268K	V334Q	S401C
R004D	I070C	E135S	G201W	Y268L	V334R	S401D
R004E	I070F	E135W	S202A	Y268N	V334S	S401E
R004F	I070G	E135Y	S202E	Y268P	V334T	S401F
R004G	I070H	L136A	S202F	Y268Q	V334Y	S401G
R004I	I070K	L136C	S202G	Y268R	T335A	S401H
R004L	I070L	L136D	S202H	Y268S	T335C	S401K
R004M	I070N	L136F	S202K	Y268T	T335F	S401L
R004N	I070P	L136G	S202M	Y268V	T335G	S401N
R004P	I070Q	L136H	S202N	Y268W	T335H	S401Q
R004S	I070R	L136I	S202P	T269A	T335I	S401R
R004T	I070S	L136M	S202Q	T269C	T335K	S401T
R004V	I070T	L136N	S202R	T269D	T335L	S401W
R004W	I070V	L136P	S202T	T269E	T335N	S401Y
R004Y	I070Y	L136Q	S202V	T269G	T335P	C402A
A005D	T071A	L136R	S202W	T269K	T335Q	C402D
A005G	T071C	L136S	S202Y	T269L	T335S	C402E
A005H	T071D	L136T	C203A	T269M	T335V	C402F
A005I	T071E	L136W	C203D	T269N	T335W	C402G
A005L	T071G	V137A	C203E	T269P	T335Y	C402L
A005M	T071H	V137C	C203G	T269Q	L336A	C402M
A005N	T071L	V137E	C203H	T269R	L336E	C402P
A005P	T071M	V137F	C203L	T269S	L336F	C402Q
A005Q	T071N	V137G	C203M	T269V	L336G	C402R
A005R	T071P	V137H	C203N	T269Y	L336H	C402S
A005S	T071Q	V137I	C203P	R270A	L336K	C402T
A005T	T071R	V137L	C203Q	R270C	L336M	C402V
A005V	T071S	V137N	C203R	R270D	L336N	C402W
A005W	T071V	V137P	C203S	R270E	L336P	C402Y

TABLE 8: PH20 Variant Library						
A005Y	T071Y	V137Q	C203T	R270F	L336R	Y403A
P006A	G072A	V137R	C203V	R270G	L336S	Y403C
P006D	G072C	V137S	C203W	R270H	L336T	Y403E
P006E	G072D	V137T	F204A	R270I	L336V	Y403F
P006F	G072E	V137W	F204C	R270M	L336W	Y403G
P006G	G072F	V137Y	F204E	R270N	L336Y	Y403H
P006H	G072H	Q138A	F204G	R270P	A337C	Y403K
P006K	G072I	Q138C	F204H	R270Q	A337F	Y403L
P006L	G072K	Q138E	F204I	R270S	A337G	Y403M
P006N	G072L	Q138F	F204K	R270T	A337H	Y403N
P006Q	G072M	Q138G	F204L	R270V	A337I	Y403P
P006R	G072P	Q138H	F204M	R270Y	A337K	Y403Q
P006S	G072Q	Q138I	F204P	I271A	A337L	Y403R
P006T	G072R	Q138L	F204Q	I271D	A337M	Y403S
P006V	G072S	Q138M	F204R	I271E	A337N	Y403T
P006W	G072T	Q138N	F204S	I271F	A337P	S404A
P006Y	G072V	Q138R	F204T	I271G	A337R	S404C
P007A	G072W	Q138S	F204V	I271H	A337S	S404D
P007C	G072Y	Q138V	F204W	I271K	A337T	S404F
P007D	V073A	Q138W	N205A	I271L	A337V	S404G
P007F	V073C	Q138Y	N205D	I271M	A337W	S404H
P007G	V073D	Q139A	N205E	I271P	A338C	S404L
P007H	V073F	Q139C	N205F	I271R	A338D	S404M
P007I	V073G	Q139D	N205G	I271S	A338E	S404N
P007K	V073H	Q139E	N205K	I271T	A338F	S404P
P007L	V073K	Q139F	N205L	I271V	A338G	S404R
P007M	V073L	Q139G	N205M	I271W	A338H	S404T
P007Q	V073M	Q139H	N205P	V272A	A338I	S404V
P007R	V073P	Q139K	N205R	V272C	A338K	S404W
P007S	V073Q	Q139L	N205S	V272D	A338L	S404Y
P007T	V073R	Q139M	N205T	V272E	A338P	T405A
P007V	V073S	Q139P	N205V	V272G	A338Q	T405C
P007W	V073T	Q139R	N205W	V272H	A338R	T405F
P007Y	V073W	Q139S	N205Y	V272K	A338S	T405G
V008A	T074A	Q139T	V206C	V272L	A338T	T405I
V008D	T074C	Q139V	V206D	V272M	A338V	T405K
V008E	T074E	Q140A	V206F	V272N	K339D	T405L
V008G	T074F	Q140C	V206G	V272P	K339E	T405M

TABLE 8: PH20 Variant Library						
V008H	T074G	Q140D	V206H	V272R	K339F	T405P
V008I	T074H	Q140F	V206I	V272S	K339G	T405Q
V008L	T074K	Q140G	V206K	V272T	K339H	T405R
V008M	T074L	Q140H	V206L	V272W	K339L	T405S
V008N	T074M	Q140I	V206M	F273A	K339M	T405V
V008P	T074N	Q140K	V206P	F273C	K339N	T405W
V008Q	T074P	Q140L	V206Q	F273D	K339P	T405Y
V008R	T074R	Q140M	V206R	F273G	K339R	L406A
V008S	T074S	Q140R	V206S	F273H	K339S	L406C
V008T	T074V	Q140S	V206T	F273I	K339T	L406D
V008W	T074W	Q140V	V206Y	F273L	K339V	L406E
I009A	V075A	Q140W	E207A	F273P	K339W	L406F
I009C	V075C	Q140Y	E207F	F273Q	K339Y	L406G
I009D	V075D	N141A	E207G	F273R	M340A	L406I
I009E	V075F	N141D	E207H	F273S	M340C	L406N
I009G	V075G	N141E	E207I	F273T	M340D	L406P
I009H	V075H	N141F	E207K	F273V	M340E	L406Q
I009K	V075L	N141G	E207L	F273W	M340F	L406R
I009L	V075M	N141H	E207M	F273Y	M340G	L406S
I009N	V075N	N141L	E207P	T274A	M340H	L406T
I009P	V075P	N141M	E207Q	T274C	M340K	L406V
I009Q	V075Q	N141P	E207R	T274E	M340L	L406Y
I009R	V075R	N141Q	E207S	T274F	M340P	S407A
I009S	V075S	N141R	E207T	T274G	M340R	S407D
I009T	V075T	N141S	E207V	T274H	M340S	S407E
I009V	V075W	N141T	E207W	T274L	M340T	S407F
P010D	V075Y	N141V	I208A	T274N	M340V	S407G
P010E	N076A	N141W	I208C	T274P	M340W	S407H
P010F	N076C	N141Y	I208D	T274Q	C341A	S407L
P010G	N076D	V142C	I208E	T274R	C341E	S407M
P010H	N076F	V142D	I208G	T274S	C341G	S407N
P010I	N076G	V142E	I208K	T274V	C341H	S407P
P010L	N076I	V142G	I208L	T274W	C341K	S407Q
P010M	N076K	V142H	I208M	T274Y	C341L	S407R
P010N	N076L	V142I	I208P	D275A	C341M	S407T
P010Q	N076P	V142K	I208Q	D275C	C341N	S407V
P010R	N076Q	V142L	I208R	D275E	C341Q	S407W
P010S	N076R	V142M	I208S	D275F	C341R	C408A

TABLE 8: PH20 Variant Library						
P010T	N076S	V142N	I208T	D275G	C341S	C408E
P010W	N076T	V142P	I208V	D275I	C341T	C408F
P010Y	N076V	V142Q	I208W	D275K	C341V	C408G
N011A	N076W	V142R	K209A	D275L	C341W	C408I
N011C	G077D	V142S	K209C	D275M	C341Y	C408K
N011D	G077E	V142T	K209D	D275Q	S342A	C408L
N011E	G077F	Q143C	K209E	D275R	S342D	C408N
N011F	G077H	Q143E	K209F	D275S	S342E	C408P
N011G	G077K	Q143F	K209G	D275T	S342F	C408R
N011H	G077L	Q143G	K209L	D275V	S342G	C408S
N011I	G077M	Q143H	K209N	D275W	S342H	C408T
N011K	G077N	Q143I	K209P	Q276C	S342I	C408V
N011L	G077P	Q143K	K209R	Q276D	S342K	C408W
N011P	G077Q	Q143L	K209S	Q276E	S342L	C408Y
N011S	G077R	Q143M	K209T	Q276F	S342M	K409A
N011T	G077S	Q143N	K209V	Q276G	S342P	K409C
N011W	G077T	Q143P	K209W	Q276H	S342Q	K409D
N011Y	G077V	Q143R	K209Y	Q276I	S342R	K409E
V012A	G077Y	Q143S	R210A	Q276L	S342T	K409G
V012D	G078A	Q143T	R210C	Q276M	S342Y	K409H
V012E	G078C	Q143V	R210D	Q276P	Q343C	K409I
V012G	G078D	Q143Y	R210E	Q276R	Q343D	K409L
V012H	G078H	L144A	R210G	Q276S	Q343E	K409P
V012I	G078I	L144E	R210K	Q276V	Q343F	K409Q
V012K	G078K	L144F	R210L	Q276W	Q343G	K409R
V012L	G078L	L144G	R210M	Q276Y	Q343I	K409S
V012M	G078M	L144I	R210N	V277A	Q343L	K409T
V012N	G078P	L144K	R210P	V277C	Q343M	K409V
V012P	G078Q	L144N	R210S	V277D	Q343P	K409W
V012R	G078R	L144P	R210T	V277E	Q343R	A412Y
V012S	G078S	L144Q	R210V	V277G	Q343S	E410D
V012T	G078T	L144R	R210W	V277H	Q343T	E410G
V012W	G078V	L144S	R210Y	V277K	Q343V	E410I
P013A	G078Y	L144T	N211A	V277L	Q343W	E410K
P013E	I079A	L144V	N211C	V277M	Q343Y	E410L
P013F	I079D	L144W	N211F	V277N	V344E	E410M
P013G	I079F	L144Y	N211G	V277Q	V344F	E410N
P013H	I079G	S145A	N211H	V277R	V344G	E410P

TABLE 8: PH20 Variant Library						
P013I	I079H	S145C	N211I	V277S	V344H	E410Q
P013L	I079K	S145D	N211K	V277T	V344I	E410R
P013M	I079L	S145E	N211L	V277Y	V344L	E410S
P013Q	I079N	S145F	N211M	L278A	V344M	E410T
P013R	I079P	S145G	N211P	L278E	V344N	E410V
P013S	I079R	S145H	N211R	L278F	V344P	E410W
P013T	I079S	S145L	N211S	L278G	V344Q	E410Y
P013V	I079T	S145M	N211T	L278H	V344R	K411A
P013W	I079V	S145N	N211V	L278I	V344S	K411D
P013Y	I079W	S145P	N211W	L278K	V344T	K411E
F014A	I079Y	S145R	D212A	L278M	V344W	K411F
F014D	P080A	S145T	D212E	L278N	V344Y	K411G
F014E	P080D	S145V	D212G	L278P	L345A	K411H
F014G	P080E	S145W	D212H	L278R	L345C	K411I
F014H	P080F	L146A	D212I	L278S	L345D	K411L
F014I	P080G	L146C	D212K	L278T	L345E	K411N
F014K	P080I	L146E	D212L	L278V	L345G	K411P
F014M	P080K	L146G	D212M	L278Y	L345H	K411R
F014N	P080L	L146H	D212N	K279A	L345K	K411S
F014P	P080M	L146I	D212P	K279C	L345N	K411T
F014Q	P080N	L146K	D212Q	K279D	L345P	K411V
F014R	P080R	L146N	D212S	K279F	L345Q	K411W
F014T	P080S	L146P	D212T	K279G	L345R	A412D
F014V	P080T	L146Q	D212V	K279H	L345T	A412E
F014W	P080V	L146R	D212W	K279L	L345V	A412G
L015A	P080Y	L146S	D213A	K279P	L345W	A412H
L015E	Q081A	L146T	D213E	K279Q	L345Y	A412I
L015F	Q081C	L146V	D213G	K279R	C346A	A412L
L015G	Q081E	L146Y	D213H	K279S	C346D	A412N
L015K	Q081F	T147A	D213K	K279T	C346F	A412P
L015M	Q081G	T147C	D213L	K279V	C346G	A412Q
L015N	Q081H	T147D	D213M	K279W	C346I	A412R
L015P	Q081L	T147F	D213N	K279Y	C346K	A412S
L015Q	Q081M	T147G	D213P	F280D	C346L	A412V
L015R	Q081N	T147I	D213Q	F280E	C346M	A412W
L015S	Q081P	T147L	D213R	F280G	C346P	D413A
L015T	Q081R	T147M	D213S	F280H	C346Q	D413E
L015V	Q081S	T147P	D213V	F280I	C346R	D413F

TABLE 8: PH20 Variant Library						
L015W	Q081V	T147Q	D213W	F280L	C346S	D413G
L015Y	Q081W	T147R	D213Y	F280M	C346T	D413H
W016A	Q081Y	T147S	L214A	F280N	C346V	D413I
W016C	K082A	T147V	L214C	F280P	C346W	D413K
W016D	K082E	T147W	L214D	F280Q	Q347A	D413L
W016E	K082G	T147Y	L214E	F280R	Q347C	D413N
W016F	K082H	E148C	L214G	F280S	Q347E	D413P
W016G	K082I	E148F	L214H	F280T	Q347F	D413Q
W016H	K082L	E148G	L214K	F280V	Q347G	D413R
W016K	K082M	E148H	L214N	F280W	Q347I	D413S
W016L	K082N	E148I	L214P	L281A	Q347L	D413T
W016M	K082P	E148K	L214Q	L281D	Q347M	D413W
W016P	K082Q	E148L	L214R	L281F	Q347P	V414A
W016R	K082R	E148P	L214S	L281G	Q347R	V414D
W016S	K082S	E148Q	L214T	L281H	Q347S	V414E
W016T	K082T	E148R	L214V	L281I	Q347T	V414F
W016Y	K082V	E148S	L214Y	L281K	Q347V	V414G
A017D	K082W	E148T	S215A	L281N	Q347W	V414H
A017E	K082Y	E148V	S215C	L281P	Q347Y	V414I
A017G	I083E	E148W	S215D	L281Q	E348C	V414K
A017H	I083F	E148Y	S215E	L281R	E348D	V414L
A017I	I083G	A149C	S215G	L281S	E348G	V414M
A017L	I083H	A149E	S215H	L281V	E348H	V414Q
A017N	I083K	A149F	S215K	L281W	E348I	V414R
A017P	I083L	A149G	S215L	L281Y	E348L	V414S
A017Q	I083N	A149K	S215M	S282A	E348M	V414T
A017R	I083P	A149L	S215P	S282C	E348P	V414Y
A017S	I083Q	A149M	S215Q	S282D	E348Q	K415A
A017T	I083R	A149P	S215R	S282E	E348R	K415C
A017V	I083S	A149Q	S215T	S282F	E348S	K415D
A017W	I083T	A149R	S215V	S282G	E348T	K415E
A017Y	I083V	A149S	S215W	S282L	E348V	K415G
W018C	I083Y	A149T	W216D	S282M	E348W	K415L
W018D	S084D	A149V	W216E	S282P	E348Y	K415M
W018F	S084E	A149W	W216G	S282Q	Q349A	K415P
W018G	S084F	A149Y	W216H	S282R	Q349D	K415Q
W018H	S084G	T150A	W216I	S282T	Q349E	K415R
W018I	S084H	T150C	W216K	S282V	Q349F	K415S

TABLE 8: PH20 Variant Library						
W018L	S084I	T150D	W216L	S282W	Q349G	K415T
W018M	S084L	T150E	W216M	S282Y	Q349H	K415V
W018P	S084M	T150F	W216N	Q283A	Q349K	K415W
W018Q	S084N	T150G	W216P	Q283C	Q349L	K415Y
W018R	S084P	T150I	W216Q	Q283D	Q349M	D416C
W018S	S084Q	T150L	W216R	Q283E	Q349N	D416F
W018T	S084R	T150N	W216T	Q283F	Q349P	D416G
W018V	S084T	T150P	W216V	Q283G	Q349R	D416H
W018Y	S084W	T150R	W216Y	Q283H	Q349S	D416I
N019A	S084Y	T150S	L217A	Q283L	Q349T	D416K
N019C	L085A	T150V	L217C	Q283N	Q349V	D416L
N019F	L085C	T150W	L217E	Q283P	Q349W	D416N
N019G	L085D	T150Y	L217G	Q283R	Q349Y	D416Q
N019H	L085E	E151A	L217H	Q283S	G350A	D416R
N019I	L085F	E151C	L217I	Q283T	G350D	D416S
N019L	L085G	E151G	L217M	Q283W	G350E	D416T
N019M	L085H	E151H	L217P	Q283Y	G350F	D416V
N019P	L085K	E151K	L217Q	D284A	G350H	D416W
N019Q	L085N	E151L	L217R	D284C	G350K	D416Y
N019R	L085P	E151M	L217S	D284E	G350L	T417A
N019S	L085Q	E151N	L217T	D284G	G350M	T417D
N019V	L085R	E151Q	L217V	D284H	G350N	T417E
N019W	L085S	E151R	L217W	D284I	G350P	T417F
N019Y	L085T	E151S	L217Y	D284L	G350R	T417G
A020D	L085V	E151T	W218A	D284M	G350S	T417H
A020E	Q086A	E151V	W218D	D284N	G350T	T417I
A020F	Q086C	E151W	W218F	D284P	G350V	T417K
A020G	Q086D	E151Y	W218G	D284Q	G350Y	T417L
A020H	Q086E	K152A	W218H	D284S	V351A	T417M
A020K	Q086F	K152C	W218I	D284T	V351C	T417P
A020L	Q086G	K152F	W218K	D284V	V351D	T417Q
A020N	Q086H	K152G	W218L	D284Y	V351E	T417R
A020P	Q086I	K152I	W218M	E285A	V351F	T417S
A020Q	Q086K	K152L	W218P	E285F	V351G	T417W
A020R	Q086L	K152M	W218Q	E285G	V351H	D418A
A020S	Q086M	K152N	W218R	E285H	V351I	D418C
A020T	Q086N	K152P	W218S	E285K	V351L	D418E
A020V	Q086P	K152R	W218T	E285M	V351N	D418F

TABLE 8: PH20 Variant Library						
A020Y	Q086R	K152S	W218V	E285N	V351Q	D418G
P021A	Q086S	K152T	N219A	E285P	V351R	D418I
P021C	Q086T	K152V	N219C	E285Q	V351S	D418L
P021D	Q086V	K152W	N219D	E285R	V351W	D418M
P021E	Q086W	K152Y	N219E	E285S	V351Y	D418N
P021G	D087A	A153C	N219G	E285T	C352A	D418P
P021H	D087C	A153E	N219H	E285V	C352D	D418Q
P021I	D087E	A153F	N219I	E285W	C352E	D418R
P021K	D087G	A153G	N219K	E285Y	C352F	D418S
P021L	D087H	A153H	N219L	L286A	C352G	D418V
P021M	D087I	A153I	N219M	L286C	C352K	D418Y
P021R	D087L	A153K	N219P	L286D	C352M	A419D
P021S	D087M	A153L	N219R	L286E	C352P	A419E
P021T	D087P	A153M	N219S	L286F	C352Q	A419F
P021V	D087Q	A153P	N219T	L286G	C352R	A419G
P021W	D087R	A153Q	N219W	L286H	C352S	A419H
S022A	D087S	A153R	E220A	L286K	C352T	A419I
S022C	D087T	A153S	E220D	L286M	C352V	A419K
S022D	D087V	A153T	E220G	L286P	C352W	A419L
S022E	D087Y	A153V	E220H	L286R	C352Y	A419N
S022G	H088A	A153W	E220I	L286S	I353A	A419P
S022H	H088C	K154A	E220K	L286T	I353C	A419R
S022K	H088E	K154C	E220L	L286W	I353E	A419S
S022L	H088F	K154D	E220M	L286Y	I353F	A419T
S022M	H088G	K154E	E220N	V287A	I353G	A419W
S022N	H088I	K154G	E220P	V287C	I353H	A419Y
S022P	H088K	K154H	E220R	V287D	I353K	V420A
S022R	H088L	K154I	E220S	V287E	I353L	V420D
S022T	H088M	K154L	E220T	V287F	I353M	V420F
S022V	H088P	K154P	E220V	V287G	I353Q	V420G
S022Y	H088R	K154R	E220W	V287I	I353R	V420H
E023A	H088S	K154S	S221A	V287K	I353S	V420I
E023D	H088T	K154T	S221C	V287L	I353T	V420K
E023F	H088V	K154V	S221D	V287N	I353V	V420L
E023G	H088Y	K154W	S221E	V287P	I353W	V420N
E023H	L089A	K154Y	S221G	V287Q	R354C	V420P
E023L	L089C	Q155A	S221H	V287R	R354D	V420R
E023M	L089D	Q155C	S221I	V287S	R354E	V420S

TABLE 8: PH20 Variant Library						
E023N	L089E	Q155D	S221K	V287T	R354G	V420T
E023P	L089G	Q155F	S221L	Y288D	R354H	V420W
E023Q	L089K	Q155G	S221M	Y288E	R354I	V420Y
E023R	L089M	Q155H	S221P	Y288F	R354K	D421A
E023S	L089N	Q155K	S221Q	Y288G	R354L	D421E
E023T	L089P	Q155L	S221R	Y288H	R354M	D421G
E023V	L089Q	Q155M	S221T	Y288I	R354P	D421H
E023W	L089R	Q155P	S221V	Y288K	R354Q	D421I
F024A	L089S	Q155R	T222A	Y288L	R354S	D421K
F024C	L089T	Q155S	T222D	Y288P	R354V	D421L
F024E	L089W	Q155T	T222E	Y288Q	R354W	D421M
F024G	L089Y	Q155V	T222F	Y288R	R354Y	D421N
F024H	D090A	Q155W	T222G	Y288S	K355D	D421Q
F024I	D090C	Q155Y	T222I	Y288T	K355F	D421R
F024K	D090E	E156A	T222K	Y288V	K355G	D421S
F024L	D090G	E156C	T222L	Y288W	K355H	D421T
F024M	D090H	E156D	T222N	T289A	K355L	D421W
F024N	D090I	E156G	T222P	T289C	K355M	D421Y
F024P	D090K	E156I	T222R	T289E	K355N	V422A
F024R	D090L	E156K	T222S	T289G	K355P	V422C
F024T	D090N	E156L	T222V	T289H	K355Q	V422D
F024V	D090P	E156M	T222W	T289K	K355R	V422E
F024Y	D090Q	E156P	T222Y	T289L	K355S	V422G
C025D	D090R	E156Q	A223C	T289M	K355T	V422H
C025E	D090S	E156R	A223D	T289N	K355V	V422I
C025F	D090T	E156S	A223E	T289P	K355W	V422L
C025G	D090W	E156T	A223G	T289Q	K355Y	V422M
C025H	K091A	E156V	A223H	T289R	N356A	V422N
C025I	K091D	E156W	A223K	T289S	N356C	V422P
C025K	K091E	F157A	A223L	T289V	N356D	V422Q
C025L	K091F	F157C	A223P	T289Y	N356F	V422R
C025N	K091G	F157D	A223Q	F290A	N356G	V422S
C025P	K091H	F157E	A223R	F290C	N356H	V422T
C025R	K091I	F157G	A223S	F290D	N356K	V422W
C025S	K091L	F157H	A223T	F290G	N356L	V422Y
C025T	K091N	F157I	A223V	F290H	N356P	C423A
C025V	K091Q	F157K	A223W	F290I	N356Q	C423D
C025Y	K091R	F157L	A223Y	F290K	N356R	C423E

TABLE 8: PH20 Variant Library						
L026A	K091S	F157M	L224A	F290L	N356S	C423F
L026E	K091T	F157P	L224D	F290M	N356T	C423G
L026G	K091Y	F157Q	L224E	F290Q	N356V	C423H
L026H	A092C	F157R	L224F	F290R	N356W	C423L
L026I	A092E	F157S	L224G	F290S	W357A	C423M
L026K	A092F	F157T	L224I	F290T	W357C	C423P
L026M	A092G	F157V	L224M	F290V	W357D	C423Q
L026P	A092H	F157W	L224P	F290Y	W357E	C423R
L026Q	A092K	E158A	L224Q	G291A	W357F	C423S
L026R	A092L	E158C	L224R	G291C	W357G	C423T
L026S	A092M	E158D	L224S	G291D	W357K	C423V
L026T	A092P	E158F	L224T	G291E	W357L	C423W
L026V	A092Q	E158G	L224V	G291F	W357M	I424A
L026W	A092R	E158H	L224W	G291H	W357P	I424C
L026Y	A092T	E158K	L224Y	G291L	W357Q	I424E
G027A	A092V	E158L	Y225A	G291M	W357R	I424G
G027C	A092W	E158N	Y225D	G291N	W357S	I424H
G027D	A092Y	E158P	Y225E	G291P	W357T	I424K
G027E	K093D	E158Q	Y225G	G291Q	W357V	I424L
G027F	K093E	E158R	Y225H	G291R	N358C	I424N
G027H	K093F	E158S	Y225K	G291S	N358D	I424Q
G027I	K093G	E158V	Y225L	G291T	N358E	I424R
G027K	K093H	E158Y	Y225P	G291V	N358G	I424S
G027L	K093I	K159A	Y225Q	G291W	N358H	I424T
G027P	K093L	K159D	Y225R	G291Y	N358I	I424V
G027Q	K093M	K159E	Y225S	E292A	N358K	I424W
G027R	K093N	K159F	Y225T	E292C	N358L	I424Y
G027S	K093P	K159G	Y225V	E292F	N358P	A425C
G027T	K093Q	K159H	Y225W	E292G	N358Q	A425D
G027W	K093R	K159L	P226A	E292H	N358R	A425E
K028A	K093S	K159M	P226C	E292I	N358S	A425G
K028D	K093T	K159N	P226D	E292K	N358T	A425I
K028E	K093V	K159Q	P226E	E292L	N358V	A425K
K028F	K094A	K159R	P226F	E292N	N358W	A425L
K028G	K094C	K159S	P226G	E292P	S359A	A425M
K028I	K094D	K159V	P226L	E292Q	S359C	A425N
K028L	K094E	K159W	P226N	E292R	S359D	A425P
K028M	K094F	K159Y	P226Q	E292T	S359E	A425R

TABLE 8: PH20 Variant Library						
K028N	K094G	A160C	P226R	E292V	S359F	A425S
K028P	K094H	A160F	P226S	E292W	S359G	A425V
K028R	K094L	A160G	P226T	T293A	S359H	A425W
K028S	K094M	A160H	P226V	T293C	S359K	A425Y
K028T	K094N	A160I	P226W	T293D	S359L	D426A
K028V	K094P	A160K	P226Y	T293E	S359M	D426C
K028W	K094Q	A160L	S227A	T293F	S359P	D426E
F029A	K094R	A160M	S227F	T293G	S359R	D426F
F029C	K094S	A160N	S227G	T293K	S359T	D426G
F029E	K094T	A160Q	S227H	T293L	S359V	D426I
F029G	D095A	A160R	S227I	T293M	S359W	D426K
F029H	D095C	A160S	S227K	T293N	S360A	D426L
F029I	D095E	A160V	S227L	T293P	S360C	D426M
F029K	D095F	A160W	S227M	T293Q	S360E	D426N
F029L	D095G	A160Y	S227P	T293S	S360F	D426P
F029M	D095H	G161A	S227Q	T293V	S360G	D426Q
F029P	D095K	G161C	S227R	T293Y	S360I	D426R
F029R	D095L	G161D	S227T	V294A	S360K	D426S
F029S	D095M	G161E	S227V	V294C	S360L	D426Y
F029T	D095P	G161H	S227W	V294E	S360M	G427A
F029V	D095Q	G161I	S227Y	V294G	S360N	G427C
F029W	D095S	G161K	I228A	V294H	S360P	G427F
D030A	D095V	G161L	I228E	V294K	S360Q	G427H
D030E	D095W	G161M	I228F	V294L	S360R	G427I
D030F	D095Y	G161Q	I228G	V294M	S360T	G427K
D030G	I096A	G161R	I228H	V294N	S360V	G427L
D030H	I096C	G161S	I228K	V294P	D361A	G427P
D030K	I096D	G161T	I228L	V294Q	D361C	G427Q
D030L	I096E	G161V	I228M	V294R	D361E	G427R
D030M	I096F	G161W	I228N	V294S	D361G	G427S
D030P	I096G	K162A	I228P	V294T	D361H	G427T
D030Q	I096H	K162D	I228Q	V294W	D361L	G427V
D030R	I096L	K162E	I228R	A295C	D361M	G427W
D030S	I096N	K162F	I228S	A295D	D361N	G427Y
D030T	I096P	K162G	I228T	A295E	D361P	V428A
D030V	I096R	K162H	I228W	A295F	D361Q	V428C
D030W	I096S	K162L	Y229E	A295G	D361R	V428D
E031A	I096T	K162M	Y229F	A295H	D361S	V428E

TABLE 8: PH20 Variant Library						
E031C	I096V	K162P	Y229G	A295I	D361V	V428F
E031G	I096W	K162Q	Y229H	A295L	D361W	V428G
E031H	T097A	K162R	Y229I	A295N	D361Y	V428H
E031I	T097C	K162S	Y229K	A295P	Y362A	V428L
E031K	T097D	K162V	Y229L	A295Q	Y362C	V428M
E031L	T097E	K162W	Y229N	A295S	Y362E	V428N
E031N	T097F	K162Y	Y229P	A295T	Y362G	V428P
E031P	T097G	D163A	Y229Q	A295V	Y362H	V428R
E031R	T097I	D163C	Y229R	A295Y	Y362K	V428S
E031S	T097L	D163E	Y229S	L296A	Y362L	V428T
E031T	T097N	D163F	Y229T	L296C	Y362M	V428Y
E031V	T097P	D163G	Y229V	L296F	Y362N	C429A
E031W	T097Q	D163H	Y229W	L296G	Y362P	C429D
E031Y	T097R	D163K	L230A	L296I	Y362R	C429G
P032A	T097S	D163L	L230E	L296K	Y362S	C429I
P032C	T097W	D163P	L230G	L296M	Y362T	C429K
P032F	T097Y	D163Q	L230H	L296P	Y362V	C429L
P032G	F098A	D163R	L230I	L296Q	Y362W	C429M
P032H	F098C	D163S	L230K	L296R	L363A	C429N
P032K	F098D	D163T	L230M	L296S	L363C	C429P
P032L	F098E	D163V	L230N	L296T	L363D	C429R
P032M	F098G	D163W	L230P	L296V	L363E	C429S
P032N	F098H	F164A	L230R	L296W	L363F	C429T
P032Q	F098I	F164C	L230S	L296Y	L363G	C429V
P032R	F098L	F164D	L230T	G297A	L363H	C429W
P032S	F098M	F164E	L230V	G297C	L363I	C429Y
P032T	F098P	F164G	L230W	G297E	L363P	I430A
P032V	F098Q	F164H	L230Y	G297H	L363Q	I430D
P032W	F098R	F164L	N231A	G297I	L363R	I430E
P032Y	F098S	F164M	N231C	G297L	L363S	I430G
L033C	F098V	F164N	N231D	G297N	L363T	I430H
L033D	F098W	F164P	N231F	G297P	L363V	I430K
L033G	Y099A	F164Q	N231G	G297Q	L363W	I430L
L033H	Y099C	F164R	N231H	G297R	H364A	I430M
L033I	Y099E	F164S	N231I	G297S	H364C	I430N
L033M	Y099F	F164V	N231K	G297T	H364D	I430P
L033N	Y099G	F164W	N231L	G297V	H364E	I430R
L033P	Y099I	L165A	N231P	G297W	H364F	I430S

TABLE 8: PH20 Variant Library						
L033Q	Y099L	L165C	N231Q	G297Y	H364G	I430T
L033R	Y099N	L165D	N231R	A298C	H364K	I430V
L033S	Y099P	L165F	N231S	A298E	H364L	I430W
L033T	Y099Q	L165G	N231T	A298G	H364M	D431A
L033V	Y099R	L165H	N231V	A298I	H364P	D431E
L033W	Y099S	L165N	T232A	A298L	H364R	D431G
L033Y	Y099T	L165P	T232C	A298M	H364S	D431H
D034A	Y099V	L165Q	T232F	A298N	H364T	D431I
D034E	Y099W	L165R	T232G	A298P	H364V	D431K
D034G	M100C	L165S	T232H	A298Q	H364Y	D431L
D034H	M100E	L165T	T232K	A298R	L365A	D431N
D034I	M100F	L165V	T232L	A298S	L365C	D431P
D034K	M100G	L165W	T232M	A298T	L365D	D431Q
D034L	M100K	L165Y	T232N	A298V	L365E	D431R
D034N	M100L	V166A	T232P	A298W	L365G	D431S
D034P	M100N	V166C	T232Q	A298Y	L365I	D431V
D034Q	M100P	V166D	T232R	S299A	L365M	D431W
D034R	M100Q	V166E	T232S	S299C	L365N	D431Y
D034S	M100R	V166F	T232V	S299D	L365P	A432C
D034T	M100S	V166G	T232Y	S299E	L365Q	A432E
D034V	M100T	V166H	Q233A	S299F	L365R	A432F
D034W	M100V	V166L	Q233C	S299G	L365S	A432G
M035A	M100W	V166N	Q233D	S299H	L365T	A432H
M035D	M100Y	V166P	Q233F	S299I	L365V	A432I
M035F	P101A	V166Q	Q233G	S299L	L365W	A432K
M035G	P101C	V166R	Q233I	S299M	L365Y	A432L
M035H	P101F	V166T	Q233K	S299P	N366A	A432M
M035I	P101G	V166W	Q233L	S299Q	N366C	A432N
M035L	P101H	V166Y	Q233P	S299R	N366E	A432P
M035N	P101I	E167A	Q233R	S299T	N366F	A432R
M035P	P101K	E167D	Q233S	S299Y	N366G	A432S
M035Q	P101L	E167F	Q233T	G300A	N366K	A432V
M035R	P101M	E167G	Q233V	G300C	N366L	A432Y
M035S	P101N	E167H	Q233W	G300D	N366M	F433A
M035T	P101Q	E167K	Q233Y	G300E	N366P	F433C
M035V	P101R	E167L	Q234A	G300F	N366Q	F433D
M035Y	P101S	E167M	Q234C	G300L	N366R	F433E
S036A	P101T	E167N	Q234D	G300M	N366S	F433G

TABLE 8: PH20 Variant Library						
S036C	P101Y	E167P	Q234E	G300N	N366T	F433H
S036D	V102A	E167R	Q234G	G300P	N366V	F433I
S036F	V102C	E167S	Q234H	G300Q	N366W	F433K
S036G	V102E	E167T	Q234L	G300R	P367A	F433L
S036H	V102G	E167V	Q234M	G300S	P367C	F433P
S036K	V102H	E167Y	Q234N	G300T	P367E	F433R
S036L	V102K	T168A	Q234P	G300V	P367F	F433S
S036N	V102L	T168C	Q234R	G300W	P367G	F433T
S036P	V102M	T168D	Q234S	I301A	P367H	F433V
S036R	V102N	T168E	Q234T	I301E	P367I	F433W
S036T	V102P	T168F	Q234V	I301G	P367K	L434F
S036V	V102Q	T168G	Q234W	I301H	P367L	L434G
S036W	V102R	T168H	S235A	I301K	P367M	L434H
S036Y	V102S	T168K	S235E	I301L	P367Q	L434I
L037A	V102T	T168L	S235F	I301M	P367R	L434K
L037C	V102W	T168P	S235G	I301N	P367S	L434M
L037E	D103A	T168R	S235H	I301P	P367V	L434N
L037F	D103E	T168S	S235K	I301Q	P367W	L434P
L037G	D103F	T168V	S235L	I301R	D368A	L434Q
L037I	D103G	T168W	S235M	I301S	D368C	L434R
L037K	D103H	T168Y	S235P	I301V	D368E	L434S
L037M	D103I	I169A	S235Q	I301W	D368G	L434T
L037N	D103L	I169D	S235R	I301Y	D368H	L434V
L037P	D103N	I169F	S235T	V302C	D368K	L434W
L037R	D103Q	I169G	S235V	V302D	D368L	L434Y
L037S	D103R	I169H	S235W	V302E	D368M	K435A
L037T	D103S	I169K	S235Y	V302F	D368P	K435C
L037V	D103T	I169L	P236A	V302G	D368R	K435E
L037W	D103V	I169N	P236C	V302H	D368S	K435F
F038A	D103W	I169P	P236E	V302I	D368T	K435G
F038C	D103Y	I169Q	P236G	V302L	D368V	K435H
F038E	N104A	I169R	P236H	V302M	D368W	K435I
F038G	N104C	I169S	P236I	V302P	D368Y	K435L
F038K	N104F	I169T	P236K	V302R	N369A	K435P
F038L	N104G	I169V	P236L	V302S	N369C	K435R
F038M	N104H	I169Y	P236N	V302T	N369E	K435S
F038N	N104I	K170A	P236Q	V302W	N369F	K435T
F038P	N104K	K170C	P236R	V302Y	N369H	K435V

TABLE 8: PH20 Variant Library						
F038Q	N104L	K170D	P236S	I303A	N369I	K435W
F038R	N104M	K170E	P236T	I303C	N369K	K435Y
F038S	N104P	K170G	P236W	I303D	N369L	P436C
F038T	N104R	K170I	P236Y	I303E	N369P	P436D
F038W	N104S	K170L	V237A	I303F	N369Q	P436E
F038Y	N104T	K170M	V237C	I303G	N369R	P436G
S039A	N104V	K170N	V237E	I303K	N369S	P436H
S039C	N104W	K170P	V237F	I303L	N369T	P436I
S039D	L105A	K170Q	V237G	I303M	N369V	P436K
S039F	L105C	K170R	V237H	I303P	N369W	P436L
S039G	L105D	K170V	V237L	I303R	F370A	P436M
S039L	L105E	K170W	V237N	I303S	F370D	P436Q
S039M	L105G	K170Y	V237P	I303V	F370E	P436R
S039N	L105H	L171A	V237Q	I303W	F370G	P436S
S039P	L105I	L171C	V237R	I303Y	F370H	P436T
S039Q	L105M	L171D	V237S	W304A	F370I	P436W
S039R	L105N	L171G	V237T	W304C	F370K	P436Y
S039T	L105P	L171H	V237W	W304D	F370L	P437A
S039V	L105Q	L171I	V237Y	W304G	F370N	P437D
S039W	L105R	L171M	A238D	W304I	F370P	P437F
S039Y	L105S	L171N	A238E	W304L	F370Q	P437G
F040A	L105T	L171P	A238F	W304M	F370R	P437H
F040D	L105V	L171Q	A238G	W304N	F370S	P437I
F040E	L105W	L171R	A238H	W304P	F370V	P437K
F040G	G106A	L171S	A238K	W304Q	F370Y	P437L
F040I	G106C	L171V	A238L	W304R	A371C	P437M
F040K	G106D	L171W	A238P	W304S	A371E	P437Q
F040L	G106E	L171Y	A238Q	W304T	A371F	P437R
F040N	G106F	G172A	A238R	W304V	A371G	P437S
F040Q	G106H	G172C	A238S	W304Y	A371H	P437T
F040R	G106I	G172D	A238T	G305C	A371I	P437W
F040S	G106L	G172E	A238V	G305D	A371K	P437Y
F040T	G106M	G172I	A238W	G305E	A371L	M438A
F040V	G106N	G172L	A238Y	G305F	A371M	M438C
F040W	G106P	G172M	A239C	G305H	A371P	M438D
F040Y	G106S	G172P	A239F	G305K	A371R	M438E
I041A	G106V	G172Q	A239G	G305L	A371S	M438G
I041C	G106W	G172R	A239H	G305N	A371T	M438L

TABLE 8: PH20 Variant Library						
I041D	G106Y	G172S	A239I	G305P	A371V	M438N
I041E	M107A	G172T	A239K	G305Q	A371W	M438P
I041F	M107C	G172V	T240K	G305R	I372A	M438Q
I041G	M107D	G172W	A239L	G305S	I372D	M438R
I041H	M107F	G172Y	A239N	G305T	I372E	M438S
I041N	M107G	K173D	A239P	G305V	I372F	M438T
I041P	M107H	K173E	A239R	G305Y	I372G	M438V
I041Q	M107I	K173G	A239S	T306A	I372H	M438W
I041R	M107K	K173H	A239T	T306C	I372K	M438Y
I041S	M107L	K173I	A239V	T306D	I372L	E439A
I041T	M107P	K173L	A239W	T306E	I372N	E439C
I041V	M107Q	K173M	A239Y	T306F	I372P	E439F
I041W	M107R	K173N	T240A	T306G	I372R	E439G
G042A	M107S	K173P	T240E	T306H	I372S	E439H
G042C	M107V	K173Q	T240F	T306I	I372T	E439K
G042D	M107W	K173R	T240G	T306L	I372V	E439L
G042E	A108D	K173S	T240L	T306P	I372W	E439N
G042H	A108E	K173V	T240M	T306R	Q373A	E439P
G042I	A108F	K173W	T240N	T306S	Q373C	E439Q
G042K	A108G	K173Y	T240P	T306V	Q373E	E439R
G042L	A108H	L174A	T240Q	T306W	Q373F	E439S
G042M	A108K	L174C	T240R	T306Y	Q373G	E439T
G042P	A108L	L174G	T240S	L307C	Q373H	E439V
G042Q	A108M	L174H	T240V	L307E	Q373K	E439W
G042R	A108N	L174K	T240W	L307F	Q373L	T440A
G042S	A108P	L174M	T240Y	L307G	Q373M	T440D
G042T	A108Q	L174N	L241A	L307I	Q373N	T440E
G042V	A108R	L174P	L241C	L307K	Q373P	T440F
S043A	A108S	L174Q	L241D	L307N	Q373R	T440G
S043D	A108T	L174R	L241E	L307P	Q373S	T440H
S043E	A108V	L174S	L241F	L307Q	Q373T	T440I
S043F	A108Y	L174T	L241G	L307R	Q373V	T440L
S043G	V109A	L174V	L241I	L307S	Q373W	T440M
S043H	V109C	L174W	L241K	L307T	L374A	T440P
S043I	V109D	L174Y	L241P	L307V	L374D	T440Q
S043K	V109E	L175C	L241Q	L307W	L374E	T440R
S043L	V109F	L175D	L241R	L307Y	L374G	T440S
S043N	V109G	L175E	L241S	S308C	L374H	T440V

TABLE 8: PH20 Variant Library						
S043P	V109H	L175F	L241T	S308D	L374I	T440Y
S043Q	V109L	L175G	L241V	S308F	L374M	E441A
S043R	V109M	L175H	L241W	S308G	L374N	E441C
S043T	V109P	L175K	Y242A	S308H	L374P	E441D
S043V	V109Q	L175N	Y242C	S308K	L374R	E441F
P044A	V109R	L175P	Y242D	S308L	L374S	E441G
P044C	V109T	L175R	Y242F	S308M	L374T	E441H
P044E	V109W	L175S	Y242G	S308N	L374V	E441K
P044F	V109Y	L175T	Y242I	S308P	L374W	E441L
P044G	I110A	L175V	Y242K	S308R	L374Y	E441N
P044H	I110C	L175W	Y242L	S308T	E375A	E441Q
P044I	I110D	L175Y	Y242M	S308V	E375C	E441R
P044L	I110F	R176A	Y242P	S308W	E375F	E441S
P044N	I110G	R176C	Y242R	S308Y	E375G	E441T
P044Q	I110H	R176E	Y242S	I309D	E375I	E441V
P044R	I110K	R176F	Y242T	I309E	E375K	E441Y
P044S	I110L	R176G	Y242V	I309G	E375L	E442C
P044T	I110M	R176H	Y242W	I309H	E375M	E442G
P044W	I110N	R176I	V243A	I309K	E375N	E442H
P044Y	I110P	R176K	V243C	I309L	E375P	E442K
R045A	I110R	R176L	V243D	I309M	E375R	E442L
R045D	I110S	R176P	V243F	I309N	E375S	E442M
R045F	I110V	R176Q	V243G	I309Q	E375T	E442N
R045G	I110W	R176S	V243H	I309R	E375V	E442P
R045H	D111C	R176T	V243L	I309S	E375Y	E442Q
R045I	D111E	R176V	V243M	I309T	K376A	E442R
R045K	D111G	R176W	V243P	I309V	K376D	E442S
R045M	D111H	P177A	V243Q	I309W	K376E	E442T
R045P	D111I	P177C	V243R	I309Y	K376G	E442V
R045Q	D111K	P177D	V243S	M310A	K376I	E442W
R045S	D111L	P177F	V243T	M310C	K376L	E442Y
R045T	D111M	P177G	V243W	M310E	K376M	P443A
R045V	D111P	P177H	V243Y	M310F	K376P	P443D
R045W	D111Q	P177L	R244A	M310G	K376Q	P443E
R045Y	D111R	P177M	R244D	M310K	K376R	P443F
I046A	D111S	P177Q	R244G	M310L	K376S	P443G
I046C	D111T	P177R	R244H	M310N	K376T	P443H
I046E	D111V	P177S	R244I	M310P	K376V	P443I

TABLE 8: PH20 Variant Library						
I046F	D111W	P177T	R244K	M310Q	K376W	P443L
I046H	D111Y	P177V	R244M	M310R	K376Y	P443M
I046L	W112C	P177W	R244N	M310S	G377C	P443N
I046M	W112D	P177Y	R244P	M310V	G377D	P443Q
I046N	W112E	N178A	R244Q	M310W	G377E	P443R
I046P	W112F	N178D	R244S	M310Y	G377F	P443S
I046R	W112G	N178E	R244T	R311A	G377H	P443T
I046S	W112H	N178G	R244V	R311C	G377I	P443W
I046T	W112I	N178I	R244W	R311E	G377K	Q444C
I046V	W112L	N178K	R244Y	R311F	G377L	Q444D
I046W	W112N	N178L	N245A	R311G	G377M	Q444E
I046Y	W112P	N178M	N245C	R311H	G377P	Q444F
N047A	W112Q	N178P	N245F	R311I	G377R	Q444G
N047D	W112R	N178R	N245G	R311K	G377S	Q444H
N047F	W112S	N178S	N245H	R311L	G377T	Q444I
N047G	W112V	N178T	N245I	R311P	G377V	Q444K
N047H	W112Y	N178V	N245K	R311Q	G377Y	Q444L
N047I	E113A	N178W	N245L	R311S	G378D	Q444M
N047K	E113C	N178Y	N245P	R311T	G378E	Q444N
N047L	E113D	H179A	N245Q	R311V	G378F	Q444R
N047M	E113F	H179C	N245R	R311W	G378I	Q444V
N047P	E113G	H179E	N245S	S312A	G378K	Q444W
N047Q	E113H	H179G	N245T	S312C	G378L	Q444Y
N047R	E113L	H179I	N245V	S312E	G378M	I445A
N047S	E113P	H179K	N245W	S312F	G378N	I445C
N047T	E113Q	H179L	R246A	S312G	G378Q	I445D
N047V	E113R	H179M	R246C	S312H	G378R	I445G
N047W	E113S	H179N	R246D	S312K	G378S	I445H
N047Y	E113T	H179P	R246E	S312L	G378T	I445K
A048C	E113V	H179R	R246G	S312M	G378V	I445L
A048E	E113W	H179S	R246H	S312N	G378W	I445M
A048F	E113Y	H179T	R246I	S312P	G378Y	I445N
A048G	E114A	H179V	R246K	S312Q	K379A	I445P
A048H	E114C	H179W	R246L	S312R	K379C	I445Q
A048I	E114D	L180A	R246M	S312T	K379E	I445R
A048K	E114G	L180C	R246P	S312V	K379F	I445S
A048L	E114H	L180E	R246S	S312W	K379G	I445T
A048M	E114I	L180F	R246T	M313A	K379H	I445V

TABLE 8: PH20 Variant Library						
A048N	E114L	L180G	R246V	M313C	K379I	I445W
A048P	E114M	L180H	R246W	M313D	K379L	I445Y
A048Q	E114P	L180I	V247A	M313E	K379M	F446A
A048R	E114R	L180K	V247C	M313F	K379N	F446C
A048S	E114S	L180M	V247F	M313G	K379R	F446D
A048V	E114T	L180N	V247H	M313H	K379S	F446E
A048W	E114V	L180P	V247I	M313K	K379T	F446G
A048Y	E114W	L180R	V247L	M313L	K379V	F446H
T049A	E114Y	L180S	V247M	M313P	K379W	F446I
T049C	W115A	L180T	V247N	M313R	F380A	F446K
T049D	W115C	L180W	V247P	M313S	F380C	F446L
T049F	W115D	W181A	V247Q	M313T	F380D	F446M
T049G	W115F	W181C	V247R	M313V	F380E	F446Q
T049H	W115G	W181D	V247S	M313Y	F380G	F446R
T049I	W115H	W181E	V247T	K314A	F380I	F446T
T049K	W115I	W181F	V247W	K314C	F380L	F446V
T049L	W115K	W181H	V247Y	K314D	F380P	F446W
T049N	W115L	W181I	R248A	K314H	F380Q	Y447D
T049P	W115M	W181K	R248C	K314I	F380R	Y447E
T049R	W115P	W181L	R248D	K314L	F380S	Y447F
T049S	W115R	W181M	R248E	K314N	F380T	Y447G
T049V	W115S	W181N	R248G	K314P	F380V	Y447I
T049W	W115V	W181Q	R248H	K314Q	F380W	Y447K
G050A	W115Y	W181R	R248I	K314R	F380Y	Y447L
G050C	R116A	W181S	R248L	K314S	T381A	Y447M
G050D	R116C	W181V	R248M	K314T	T381E	Y447N
G050E	R116D	G182A	R248P	K314V	T381F	Y447P
G050F	R116E	G182C	R248S	K314W	T381G	Y447Q
G050H	R116G	G182D	R248T	K314Y	T381H	Y447R
G050L	R116H	G182E	R248V	S315A	T381K	Y447T
G050M	R116I	G182H	R248W	S315C	T381L	Y447V
G050P	R116L	G182L	R248Y	S315E	T381N	Y447W
G050Q	R116N	G182M	E249A	S315G	T381P	
G050R	R116P	G182N	E249G	S315H	T381Q	
G050S	R116Q	G182P	E249H	S315I	T381R	
G050V	R116S	G182Q	E249I	S315K	T381S	
G050W	R116T	G182R	E249K	S315L	T381V	
G050Y	R116V	G182S	E249L	S315M	T381W	

TABLE 8: PH20 Variant Library					
Q051A	R116W	G182T	E249M	S315P	T381Y
Q051C	P117D	G182V	E249P	S315R	V382E
Q051D	P117E	G182Y	E249Q	S315T	V382G
Q051F	P117F	Y183A	E249R	S315V	V382H
Q051H	P117G	Y183C	E249S	S315W	V382I
Q051I	P117H	Y183D	E249T	S315Y	V382K
Q051K	P117I	Y183E	E249V	C316A	V382L
Q051M	P117K	Y183G	E249W	C316D	V382M
Q051N	P117N	Y183I	E249Y	C316E	V382N
Q051P	P117Q	Y183K	A250C	C316G	V382P
Q051R	P117R	Y183L	A250F	C316I	V382Q
Q051S	P117S	Y183N	A250G	C316K	V382R
Q051T	P117T	Y183P	A250H	C316L	V382S
Q051W	P117V	Y183Q	A250K	C316M	V382T
Q051Y	P117W	Y183R	A250L	C316P	V382W
G052A	P117Y	Y183S	A250M	C316R	V382Y
G052C	T118C	Y183V	A250N	C316S	R383A
G052E	T118D	Y183W	A250P	C316T	R383E
G052F	T118E	Y184A	A250Q	C316V	R383F
G052H	T118G	Y184C	A250R	C316W	R383G
G052K	T118H	Y184D	A250S	C316Y	R383H
G052L	T118K	Y184E	A250T	L317A	R383I
G052N	T118L	Y184F	A250V	L317C	R383K
G052P	T118M	Y184G	A250W	L317D	R383L
G052Q	T118N	Y184H	I251C	L317G	R383M
G052R	T118P	Y184K	I251D	L317H	R383N
G052S	T118Q	Y184L	I251F	L317I	R383P
G052T	T118R	Y184M	I251G	L317K	R383S
G052W	T118V	Y184P	I251H	L317M	R383T
G052Y	T118W	Y184R	I251K	L317N	R383V
V053A	T118Y	Y184S	I251L	L317P	R383W
V053C	W119A	Y184V	I251M	L317Q	G384A
V053D	W119D	Y184W	I251P	L317R	G384C
V053E	W119E	L185A	I251Q	L317S	G384D
V053G	W119F	L185D	I251S	L317T	G384E
V053H	W119G	L185E	I251T	L317W	G384F
V053L	W119I	L185F	I251V	L318C	G384H
V053N	W119K	L185G	I251W	L318D	G384I

TABLE 8: PH20 Variant Library

V053P	W119L	L185I	I251Y	L318F	G384K
V053Q	W119N	L185K	R252A	L318G	G384L
V053R	W119P	L185N	R252D	L318H	G384M
V053S	W119Q	L185P	R252E	L318I	G384P
V053T	W119R	L185R	R252F	L318K	G384Q
V053W	W119S	L185S	R252G	L318M	G384R
V053Y	W119V	L185T	R252H	L318N	G384S
T054A	W119Y	L185V	R252I	L318P	G384T
T054D	A120C	L185W	R252K	L318Q	K385A
T054E	A120D	L185Y	R252L	L318R	K385C
T054F	A120F	F186A	R252N	L318S	K385G
T054G	A120G	F186D	R252P	L318T	K385H
T054H	A120H	F186G	R252S	L318W	K385L
T054I	A120I	F186H	R252T	L319C	K385M
T054M	A120L	F186I	R252V	L319E	K385N
T054N	A120N	F186K	R252Y	L319F	K385P
T054P	A120P	F186L	V253A	L319G	K385Q
T054Q	A120R	F186N	V253D	L319H	K385R
T054R	A120S	F186P	V253E	L319I	K385S
T054S	A120T	F186Q	V253G	L319K	K385T
T054V	A120V	F186R	V253H	L319M	K385V
T054Y	A120W	F186S	V253I	L319P	K385W
I055A	A120Y	F186V	V253L	L319Q	K385Y
I055C	R121A	F186W	V253M	L319R	P386A
I055D	R121C	F186Y	V253N	L319S	P386C
I055F	R121D	P187A	V253P	L319V	P386F
I055G	R121E	P187F	V253Q	L319W	P386G
I055H	R121F	P187G	V253R	L319Y	P386H
I055L	R121G	P187H	V253S	D320C	P386I
I055N	R121H	P187I	V253T	D320E	P386L
I055P	R121K	P187L	V253W	D320F	P386M
I055Q	R121L	P187M	S254C	D320G	P386N
I055R	R121M	P187N	S254D	D320H	P386Q
I055S	R121P	P187Q	S254E	D320I	P386R
I055T	R121S	P187R	S254G	D320K	P386S
I055V	R121T	P187S	S254I	D320L	P386T
I055Y	R121V	P187T	S254K	D320M	P386V
F056A	R121W	P187V	S254L	D320N	P386Y

TABLE 8: PH20 Variant Library					
F056C	R121Y	P187W	S254N	D320P	T387C
F056E	N122A	P187Y	S254P	D320R	T387E
F056G	N122C	D188A	S254Q	D320S	T387F
F056H	N122E	D188C	S254R	D320V	T387G
F056I	N122F	D188F	S254T	D320W	T387H
F056K	N122I	D188G	S254V	D320Y	T387I
F056L	N122K	D188H	S254W	N321A	T387K
F056N	N122L	D188L	S254Y	N321D	T387L
F056P	N122M	D188M	K255A	N321E	T387M
F056R	N122P	D188N	K255C	N321G	T387N
F056S	N122Q	D188P	K255D	N321H	T387Q
F056T	N122R	D188Q	K255G	N321I	T387S
F056V	N122S	D188R	K255H	N321K	T387V
F056W	N122T	D188S	K255L	N321L	T387W
Y057A	N122V	D188T	K255N	N321M	T387Y
Y057D	N122W	D188V	K255P	N321P	L388A
Y057E	W123A	D188W	K255Q	N321R	L388C
Y057F	W123C	C189A	K255R	N321S	L388F
Y057G	W123D	C189E	K255S	N321T	L388G
Y057I	W123E	C189G	K255T	N321V	L388H
Y057L	W123G	C189H	K255V	N321Y	L388I
Y057M	W123H	C189K	K255W	Y322C	L388M
Y057P	W123L	C189L	K255Y	Y322D	L388P
Y057Q	W123M	C189M	I256A	Y322E	L388Q
Y057R	W123P	C189N	I256C	Y322F	L388R
Y057S	W123Q	C189P	I256D	Y322G	L388S
Y057T	W123R	C189R	I256E	Y322H	L388T
Y057V	W123S	C189S	I256G	Y322I	L388V
Y057W	W123T	C189T	I256H	Y322L	L388W
V058A	W123V	C189V	I256L	Y322N	L388Y
V058C	W123Y	C189W	I256M	Y322P	E389A
V058D	K124A	C189Y	I256N	Y322R	E389F
V058G	K124C	Y190C	I256P	Y322S	E389G
V058H	K124D	Y190E	I256Q	Y322T	E389H
V058I	K124E	Y190F	I256R	Y322V	E389I
V058K	K124F	Y190G	I256T	Y322W	E389K
V058L	K124G	Y190H	I256V	M323A	E389L
V058N	K124H	Y190K	I256W	M323C	E389M

TABLE 8: PH20 Variant Library

V058P	K124I	Y190L	P257A	M323E	E389P
V058Q	K124L	Y190N	P257C	M323F	E389Q
V058R	K124N	Y190P	P257D	M323G	E389R
V058S	K124P	Y190Q	P257G	M323H	E389S
V058W	K124R	Y190R	P257I	M323I	E389T
V058Y	K124S	Y190S	P257K	M323K	E389V
D059A	K124T	Y190T	P257L	M323L	E389Y
D059E	K124V	Y190V	P257M	M323N	D390A
D059G	K124W	Y190W	P257N	M323P	D390C
D059H	P125A	N191A	P257Q	M323R	D390E
D059I	P125C	N191E	P257R	M323S	D390F
D059L	P125D	N191F	P257S	M323T	D390G
D059M	P125G	N191G	P257T	M323V	D390H
D059N	P125H	N191K	P257V	E324A	D390L
D059P	P125I	N191L	P257W	E324C	D390N
D059Q	P125L	N191M	D258A	E324D	D390P
D059R	P125N	N191P	D258E	E324F	D390R
D059T	P125Q	N191Q	D258G	E324G	D390S
D059V	P125R	N191R	D258H	E324H	D390T
D059W	P125S	N191S	D258I	E324L	D390V
D059Y	P125T	N191T	D258L	E324M	D390W
R060A	P125V	N191V	D258N	E324N	D390Y
R060D	P125W	N191W	D258P	E324P	L391A
R060F	P125Y	N191Y	D258Q	E324R	L391C
R060G	K126A	H192C	D258R	E324S	L391D
R060H	K126D	H192F	D258S	E324V	L391G
R060I	K126E	H192G	D258T	E324W	L391H
R060K	K126F	H192K	D258V	E324Y	L391K
R060L	K126G	H192L	D258W	T325A	L391N
R060N	K126H	H192M	D258Y	T325C	L391P
R060P	K126I	H192N	A259E	T325D	L391Q
R060Q	K126L	H192P	A259G	T325E	L391R
R060S	K126M	H192Q	A259I	T325G	L391S
R060T	K126N	H192R	A259K	T325H	L391T
R060V	K126P	H192S	A259L	T325I	L391V
R060Y	K126Q	H192T	A259M	T325K	L391W
L061A	K126R	H192V	A259N	T325M	L391Y
L061E	K126S	H192W	A259P	T325N	E392A

TABLE 8: PH20 Variant Library

L061F	K126T	H192Y	A259Q	T325Q	E392C
L061G	K126V	H193A	A259R	T325R	E392F
L061H	K126W	H193C	A259S	T325S	E392G
L061I	K126Y	H193D	A259T	T325V	E392K
L061M	D127A	H193F	A259V	T325W	E392L
L061N	D127E	H193G	A259W	I326A	E392M
L061P	D127F	H193K	A259Y	I326C	E392P
L061Q	D127G	H193L	K260A	I326D	E392Q
L061R	D127H	H193M	K260C	I326E	E392R
L061T	D127K	H193P	K260D	I326G	E392S
L061V	D127L	H193Q	K260E	I326H	E392T
L061W	D127M	H193R	K260G	I326K	E392V
L061Y	D127N	H193S	K260H	I326L	E392W
G062A	D127Q	H193T	K260L	I326N	E392Y
G062C	D127R	H193V	K260M	I326P	Q393A
G062D	D127S	H193Y	K260P	I326R	Q393C
G062F	D127T	Y194A	K260Q	I326S	Q393D
G062I	D127V	Y194C	K260R	I326V	Q393F
G062K	D127W	Y194E	K260S	I326W	Q393G
G062L	V128A	Y194F	K260V	I326Y	Q393H
G062M	V128C	Y194G	K260W	L327A	Q393I
G062P	V128E	Y194I	K260Y	L327D	Q393K
G062Q	V128F	Y194L	S261A	L327E	Q393L
G062R	V128G	Y194N	S261E	L327F	Q393M
G062S	V128H	Y194P	S261F	L327G	Q393N
G062T	V128I	Y194Q	S261G	L327H	Q393P
G062V	V128K	Y194R	S261I	L327M	Q393R
G062Y	V128L	Y194S	S261K	L327N	Q393S
Y063A	V128P	Y194T	S261L	L327Q	Q393T
Y063C	V128Q	Y194V	S261M	L327R	F394A
Y063G	V128R	Y194W	S261N	L327S	F394D
Y063H	V128S	K195A	S261P	L327T	F394E
Y063I	V128W	K195E	S261Q	L327V	F394G
Y063K	V128Y	K195F	S261R	L327W	F394I
Y063L	Y129A	K195G	S261T	L327Y	F394K
Y063M	Y129C	K195H	S261V	N328A	F394L
Y063N	Y129D	K195I	S261W	N328C	F394N
Y063P	Y129E	K195L	P262A	N328D	F394P

TABLE 8: PH20 Variant Library

Y063R	Y129G	K195N	P262D	N328G	F394Q
Y063S	Y129H	K195Q	P262E	N328H	F394R
Y063T	Y129L	K195R	P262F	N328I	F394S
Y063V	Y129M	K195S	P262G	N328K	F394T
Y063W	Y129P	K195T	P262H	N328L	F394V
Y064A	Y129Q	K195V	P262I	N328Q	F394W
Y064C	Y129R	K195W	P262K	N328R	S395A
Y064D	Y129S	K195Y	P262Q	N328S	S395C
Y064E	Y129T	K196A	P262R	N328T	S395D
Y064F	Y129V	K196C	P262S	N328V	S395E
Y064G	Y129W	K196D	P262T	N328W	S395G
Y064H	K130C	K196E	P262V	N328Y	S395H
Y064I	K130D	K196G	P262W	P329C	S395K
Y064K	K130E	K196I	P262Y	P329F	S395L
Y064L	K130G	K196L	L263A	P329G	S395M
Y064P	K130H	K196N	L263E	P329H	S395P
Y064Q	K130I	K196P	L263F	P329I	S395R
Y064R	K130L	K196R	L263G	P329K	S395T
Y064S	K130N	K196S	L263H	P329L	S395V
Y064T	K130Q	K196T	L263K	P329N	S395W
Y064V	K130R	K196V	L263M	P329Q	S395Y
Y064W	K130S	K196W	L263N	P329R	E396A
P065A	K130T	K196Y	L263P	P329S	E396C
P065C	K130V	P197A	L263Q	P329T	E396D
P065D	K130W	P197C	L263R	P329V	E396F
P065F	K130Y	P197D	L263S	P329W	E396G
P065G	N131C	P197E	L263T	P329Y	E396H
P065H	N131E	P197F	L263V	Y330A	E396I
P065I	N131F	P197G	L263W	Y330C	E396L
P065K	N131G	P197H	P264A	Y330D	E396P
P065N	N131H	P197K	P264D	Y330E	E396Q
P065R	N131I	P197L	P264E	Y330F	E396R
P065S	N131L	P197M	P264F	Y330G	E396S
P065T	N131M	P197Q	P264G	Y330I	E396T
P065V	N131P	P197R	P264H	Y330L	E396V
P065W	N131Q	P197S	P264L	Y330M	E396Y
P065Y	N131R	P197T	P264M	Y330N	K397A
Y066A	N131S	P197W	P264N	Y330P	K397C

TABLE 8: PH20 Variant Library					
Y066C	N131T	G198A	P264R	Y330R	K397E
Y066D	N131V	G198C	P264S	Y330S	K397F
Y066E	N131Y	G198D	P264T	Y330V	K397G
Y066G	R132A	G198E	P264V	I331V	K397I
Y066H	R132C	G198H	P264W	Y330W	K397L
Y066I	R132E	G198L	P264Y	I331A	K397M
Y066K	R132F	G198N	V265A	I331C	K397N
Y066L	R132H	G198P	V265C	I331D	K397P
Y066N	R132I	G198Q	V265D	I331E	K397Q
Y066P	R132K	G198R	V265E	I331F	K397R
Y066R	R132L	G198S	V265F	I331H	K397S
Y066S	R132N	G198T	V265G	I331K	K397T
Y066T	R132P	G198V	V265H	I331L	K397V

2. Expression

For expression of each mutant, HZ24-PH20-IRES-SEAP plasmid DNA containing cDNA encoding one of the variant PH20 or encoding wildtype PH20 was transfected into monolayer CHO-S cells (Invitrogen, Cat. No. 11619-012) using Lipofectamine 2000 (Invitrogen, Cat. No. 11668-027) according to the protocol suggested by the manufacture. Before transfection, CHO-S cells were seeded the night before transfection and grown in DMEM with 10% FBS to be 80% confluent the next day. Then, the medium of the CHO-S cells was replaced with Opti-MEM. A mixture of plasmid DNA and lipofectamine was made (0.2 μg DNA and 0.5 μL Lipofetamine). The Lipofectamine/DNA mixture was added to CHO-S cells and incubated overnight. The next day, the cells were supplemented with CD-CHO serum free media (Invitrogen, Cat. No. 10743-029). Supernatant from transfected cells was collected at various time points after transfection, and generally 96 hours after transfection. The supernatant, containing the variant PH20 protein or wildtype PH20 having a sequence of amino acids set forth in SEQ ID NO:3, was stored at -20 ^{0}C. Activity of supernatants were screened as described in the following examples.

EXAMPLE 3

SCREENING OF LIBRARY WITH A HYALURONIDASE ACTIVITY ASSAY TO IDENTIFY ACTIVITY MUTANTS

In this example, supernatant of expressed PH20 variants generated in Example 2 were screened using a hyaluronidase activity assay to assess activity of each mutant. In addition, activity of the secreted alkaline phosphatase (SEAP) was also measured to allow for normalizing PH20 activity of the expressed mutants to the PH20 wildtype. Active and inactive mutants were identified.

1. Generation of Biotinylated HA (bHA) Substrate

A 1.2-MDa HA (Lifecore) was biotinylated for use as a substrate in the hyaluronidase activity assay. First, 1.2 grams (g) of 1.2 MDa HA was dissoved at 4°C in 600 mL ddH_20 for a week at a concentration of 2 mg/mL with stirring. Next, 645.71 mg Biotin Hydrazide was dissolved in 100 mL DMSO to a concentration of 25 mM (6.458 mg/mL, 247.8 mg in 38.37 mL DMSO). The biotin solution was warmed briefly at 37 °C until the solution was clear. Also, 368.61 mg Sulfo-NHS in 20 mL ddH_2O was dissolved to make a 100X solution (18.4 mg/mL Sulfo-NHS). A 30 mM (1000X) water-soluble carbodiimide EDC solution was made by dissolving 17.63 mg EDC in 3 mL ddH20 at a concentration of 5.7513 mg/mL right before the reaction was started.

To four (4) 1000-mL sterile capped bottles, the following components were added at room temperature (RT) and in the following order with stirring: 1) 200 mL of 2 mg/mL HA solution; 2) 80 mL of 0.5M MES, pH 5.0 with gentle mixing; and 3) 91.6 mL of ddH_20 with gentle mixing. Next, 24 mL of 25 mM Biotin-Hydrazide and 4 mL of 100X Sulfo-NHS solution were added sequentially. Immediately, 500 µL EDC was added. After the addition of each component, the solution was mixed by inverting three times and stirring. After the addition of the last component, the solution was mixed by stirring overnight at 4°C. Then, Guanidine hydrochloride was added to a final concentration of 4 M by adding 38.2 g per 100 mL and was allowed to dissolve completely before adjusing the solution volume to 600 mL with ddH_2O.

For dialysis, 200 mL from each batch of the conjugated HA guanidine hydrochloride solution was transferred into dialysis membranes. Over the course of three days, dialyze against ddH_20 with a change in ddH_20 at least six times. The resulting volume of about 840 mL was adjusted to a final volume of 1000 mL with ddH_20. The final concentration of the biotinylated hyaluronan (bHA) was 0.4 mg/mL.

2. Hyaluronidase Activity Assay

The enzyme assay was a modification of the method described by Frost *et al.* (1997) (A Microtiter-Based Assay for Hyaluronidase Activity Not Requiring

Specialized Reagents. Analytical Biochemistry (1997) 251:263-269) that provides a measure of PH20 hyaluronidase activity.

First, biotinylated HA (bHA) substrate was bound to plastic microtiter plates to generate assay plates. Briefly, 100 µl of b-HA at 1 mg/mL in 0.5 M carbonate buffer (pH 9.6) was dispensed into each well of a high bind microplate (Immunolon 4 HBX extra high binding; Thermo Scientific). The plate was covered with a plate sealer and stored between 2-8°C for 24-48 hours.

Then, the assay plate was washed with 1 X phosphate buffered saline (PBS) wash buffer containing 0.05% (v/v) Tween 20 (PBST). PBST was generated from 1X PBS (generated from Catalog No. P5368, Sigma (10 mM Phosphate Buffer, 2.7 mM Potassium Chloride, 137 mM Sodium Chloride, pH 7.4) by placing the contents of one packet of PBS into a 1-L graduated cylinder with 800 mL deionized water, dissolved by stirring or shaking and adding sufficient quantity of water to 1 L) by adding 500 µl Tween 20 (Catalog No. 6505; EMD Bioscience) to 900 mL of 1 X PBS and adding sufficient quantity of water to 1 L. Washing was done using the BioTek ELx405 Select CW plate washer (BioTek) by washing five (5) times with 300 µl PBST wash buffer per well for each wash. At the end of each wash, the plate was tapped on a paper towel to remove excess liquid from each well. Prior to incubation with samples, 200 µl Blocking Buffer (1.0% w/v Bovine Serum Albumin (BSA) in PBS) was added to each well and the assay plate was incubated at 37°C for approximately 1 hour prior. The Blocking buffer was generated by adding 2.5g of BSA (Catalog No. 001-000-162; Jackson Immuno Research) to 200 mL 1 X PBS, stirring, adding a sufficient quantity of 1 X PBS to 250 mL and filtering through an 0.2 µM PES filter unit.

Transfected variant or wildtype PH20 supernatants generated as described in Example 1 were diluted in duplicate 1:25 in assay diluent buffer (pH 7.4 HEPES buffer; 10 mM HEPES, 50 mM NaCl, 1 mM CaCL2, 1 mg/mL BSA, pH 7.4, 0.05% Tween-20) in uncoated 4XHB high bound microplates. For the standard curve, 1:3 serial dilutions of rHuPH20 (generated as described in Example 1 with a specific activity of 145 U/mL) were made in assay diluent buffer in duplicate starting from 3 U/mL for standards as follows: 3 U/mL, 1 U/mL, 1/3 U/mL, 1/9 U/mL, 1/27 U/mL, 1/81 U/mL, and 1/243 U/mL. One hundred microliters (100 µl) of each standard and sample was transferred to the assay plates and incubated for approximately 1.5 hours at 37°C.

After the incubation, the plate was washed with PBST using the BioTek ELx405 Select CW plate washer by washing five (5) times with 300 µl PBST wash buffer per well for each wash. At the end of each wash, the plate was tapped on a paper towel to remove excess liquid from each well. Then, 100 µl of 1:5000 diluted Streptavidin-HRP (SA-HRP) was added to each well of the plate and incubated at ambient temperature for approximately 1 hour. For the dilution, a 1 mg/mL stock of Streptavidin-HRP conjugate (Catalog No. 21126; Thermo Scientific) was diluted 1:5000 into dilution buffer (1 mg/mL BSA, 0.025% Tween20, 137 mM NaCl, 20 mM Tris pH 7.5). After the incubation, the plate was washed with PBST using the BioTek ELx405 Select CW plate washer by washing five (5) times with 300 µl PBST wash buffer per well for each wash. At the end of each wash, the plate was tapped on a paper towel to remove excess liquid from each well. Then, 100 µl of TMB solution (Catalog No. 52-00-03, KPL; ambient temperature and protected from light) was added to each well for approximately five (5) minutes at room temperature or until an optimal color development was yielded. To stop the reaction, 100 µl 1.0 N Sulfuric Acid or TMB Stop solution (Catalog No. 50-85-06) was added to each well and the plates tapped to mix. Optical density was measured at 450 nm within 30 minutes of adding the stop solution. Since more PH20 in a standard or sample would lead to less bHA available to bind SA-HRP, the optical density (450 nm) value was inversely proportional to the concentration of hyaluronidase activity in each specimen.

3. SEAP Activity

Activity of secreted alkaline phosphatase (SEAP) in the cell culture supernatant also was measured using a colorimetric assay of placental alkaline phosphatase using *p*NPP as a phosphatase substrate (Anaspec SensoLyte pNPP SEAP kit; Catalog No. 72144, Anaspec) according to the manufacturer's instructions. The absorbance signal was measured at optical density (OD) of 405 nm.

The criteria for the high throughput (HTP) screening was that the transfected supernatant resulted in a SEAP signal of $\geq$ 0.1 and a signal for the rHuPH20 wildtype control of $\geq$ 1 U/mL. Also, the criteria for each screen was that the standard curves have a signal to noise ratio (S/N) for the 0 U/mL standard versus the 3 u/mL standard at OD450 was $\geq$ 5-fold, had less than three (3) standards with a coefficient of variation (CV) $\geq$ 10%, and that at least four (4) of the standards were in the linear range.

EXAMPLE 4

SELECTED PH20 VARIANTS WITH ALTERED HYALURONIDASE ACTIVITY

Each generated variant was screened for hyaluonidase activity as described in Example 3. The SEAP expression was used to normalize PH20 activity of each variant to the PH20 wildtype. Mutants were identified that exhibited altered hyaluronidase activity compared to wildtype.

1. Active Mutants

Active mutants were selected whereby at least one duplicate sample exhibited greater than 40% of wildtype activity when normalized to SEAP activity. The identified active mutants are set forth in Table 9. The Table sets forth the amino acid replacement compared to the sequence of amino acids of PH20 set forth in SEQ ID NO:3. The amino acid sequence of exemplary mutants also is set forth by reference to a SEQ ID NO. The Table also sets forth the average hyaluronidase activity of tested duplicates normalized by SEAP values compared to average of wildtype PH20 activities in each plate, which were also normalized by their own SEAP values. For example, a value of 0.40 indicates that the variant exhibits 40% of the hyaluronidase activity of wildtype PH20, a value of 1 indicates that the variant exhibits a similar hyaluronidase activity of wildtype and a value of 3.00 indicates that the variant exhibits 300% of the hyaluronidase activity of wildtype PH20 or 3-fold increased activity compared to wildtype.

The results in Table 9 show that over 600 tested mutants exhibit activity that is increased compared to wildtype. For example, about 536 mutants exhibit 120% or greater than 120% of the hyaluronidase activity of wildtype PH20 and about 75 of the mutants exhibit 300% or greater than 300% of the hyaluronidase activity of wildtype PH20. In particular, the results in Table 9 show that that hyaluronidase activity compared to wildtype of mutant S69A is about 22-fold; mutant S69R is about 14-fold; mutant I70A is about 27-fold; mutant I70K is about 14-fold; mutant I70R is about 14-fold; and mutant I271L is about 10-fold.

TABLE 9: ACTIVE MUTANTS

mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
L001A	74	0.95	Q140G		0.73	T293F	561	1.94
L001C		0.89	Q140H		0.84	T293G		1.00
L001E	75	0.55	Q140I		0.75	T293K	562	1.35

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
L001F		0.41	Q140K	343	0.93	T293L		1.00
L001G	76	0.62	Q140L		0.51	T293M	563	2.29
L001H	73	1.90	Q140M		0.80	T293P	564	1.64
L001K	77	1.39	Q140R		0.85	T293Q	565	1.83
L001N		0.87	Q140V		0.61	T293S		0.89
L001P		0.92	Q140W		0.59	T293V	566	2.15
L001Q	78	3.27	Q140Y		0.41	T293Y	567	1.49
L001R		0.72	N141A		1.12	V294M		0.41
L001S		0.74	N141D		1.09	A298G	568	0.43
L001T		0.99	N141E		0.67	A298I		0.41
L001V		1.00	N141F		0.81	G300R		0.42
L001W		0.88	N141G		1.15	I301A		0.88
N002A		0.61	N141H	344	2.03	I301V		0.88
N002C		0.4	N002I		0.37	V287N		0.35
G291C		0.27	G297A		0.57	V302W		0.46
N002G		0.44	N141L		0.61	V302I		0.45
N002L		0.46	N141M		0.48	I303V		0.47
N002P		0.54	N141Q		1.16	W304G		1.13
N002Q		0.84	N141R	345	1.40	W304I		1.17
N002S		0.78	N141S	346	0.72	G305D		1.00
N002T		1.05	N141T		0.45	G305E	569	1.62
N002V		0.65	N141V		0.50	T306D		0.76
F003E		0.42	N141W	347	0.83	T306E		0.52
F003H		0.68	N141Y	348	1.55	T306S		1.02
F003L		0.59	V142C		0.61	L307K		0.43
F003Y		0.50	V142D	349	0.71	L307N		0.76
R004A		0.73	V142E		0.87	L307Q		0.61
R004I		0.54	V142G	350	0.98	L307S		0.86
R004S		0.60	V142H		1.11	L307T		1.08
R004T		0.66	V142I		0.81	L307V		0.48
R004V		1.09	V142K	351	1.40	L307W		0.64
A005H		0.44	V142L		0.75	L307Y		0.60
P006A	80	0.78	V142M		0.76	S308D	571	0.92
P006H		0.58	V142N	352	0.98	S308G	572	1.73
P006K		0.80	V142P	353	0.88	S308H		1.15
P006L		0.76	V142Q	354	1.04	S308K	573	1.33
P006N		0.40	V142R	355	1.53	S308N	574	2.33

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
P006Q		0.89	V142S	356	0.93	S308P		0.65
P006R		0.56	V142T	357	1.19	S308R	575	1.34
P007M		0.57	Q143E		0.77	S308T		0.72
V008I		1.17	Q143G	358	0.62	I309D		0.72
V008L		0.53	Q143I		0.44	I309E	576	1.99
V008M	81	0.47	Q143K	359	1.30	I309G	577	1.44
V008P		0.33	I009Q		0.4	I303D		0.34
I009K		0.69	Q143L		0.56	I309H	578	1.30
I009L		1.08	Q143N		0.73	I309K		0.98
I009R		0.53	Q143V		0.57	I309L	579	1.72
I009S		0.98	L144T	361	1.02	I309M		1.47
I009V		0.84	L144W		0.79	I309N	581	3.11
P010D		0.62	S145A		0.58	I309Q	582	1.64
P010E		0.66	S145C		0.44	I309R	583	2.27
P010G	83	0.55	S145D		0.48	I309S	584	1.16
P010H	84	0.43	S145E		0.56	I309T	585	2.09
P010N		0.55	S145G		0.94	I309V	586	0.60
P010Q		0.89	S145H		0.56	I309W		0.88
P010R		0.73	S145L		0.44	M310A	587	1.50
P010S		0.55	S145M		0.56	M310G	588	2.73
P010W		0.59	S145N		0.58	M310Q	589	0.59
N011D		0.54	S145P		1.04	M310R		0.50
N011G		0.45	S145R		0.97	M310S	590	1.61
N011H		0.69	L146A		0.52	M310V		0.70
N011K		0.58	L146C		0.42	R311G		0.53
N11S		0.39	G305N		0.36	L307G	570	0.32
M310F		0.30	M310Y		0.38	R311G		0.54
V012A		0.56	L146E		0.50	R311H		0.48
V012E	86	1.86	L146G		0.62	R311K		0.72
V012I	87	0.68	L146H		0.78	R311Q		0.43
V012K	88	0.65	L146I		0.82	R311S		0.84
V012L		0.44	L146K		0.84	R311T		0.52
V012N		0.46	L146N		0.57	S312G		0.49
V012R		0.50	L146P	362	0.93	S312N		1.26
V012S		0.75	L146Q		0.84	S312T		0.75
V012T	89	1.50	L146R	363	1.47	M313A	591	1.34

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
P013H		0.46	L146S		0.71	M313E		0.63
P013S		0.68	L146T		0.74	M313G	592	0.56
P013T		0.90	L146V		0.84	M313H	593	1.23
P013Y		0.51	L146Y		0.80	M313K	594	2.85
F014D		0.64	S312K		0.38	S312L		0.38
F014I		0.42	T147A	364	1.20	M313L		1.05
F014M		0.47	T147C		0.47	M313P	595	1.11
F014V	90	0.46	T147D		0.71	M313R	596	2.30
L015A		0.65	T147F	365	1.24	M313S		0.88
L015M	92	0.45	T147G		1.05	M313T	597	0.67
L015V	91	2.20	T147I		0.85	M313V		0.99
A020S	93	0.50	T147L	366	1.30	M313Y		1.12
S022H		0.57	T147M		0.79	K314A		0.82
S022M		0.49	T147P		1.09	K314D		0.53
S022T	94	0.48	T147Q		1.29	K314H		1.10
S022Y		0.45	T147R	367	2.11	K314I		0.54
E023D		0.97	T147S	368	1.27	K314N		0.57
F024A		0.69	T147V	369	2.04	K314Q		0.62
F024E	95	3.99	T147W		0.97	K314R		0.95
F024G		0.75	T147Y		1.04	K314S	599	0.61
F024H	96	2.07	E148C		0.66	K314T		0.61
F024I		0.70	E148F		0.42	K314Y	600	0.45
F024K		0.96	E148G		1.05	S315A	601	0.85
F024L		0.62	E148H	370	1.24	S315E		0.41
F024M		0.85	E148I		0.73	S315G		0.72
F024N		0.60	E148K	371	1.63	S315H	602	2.04
F024R	97	1.22	E148L		0.85	S315K		0.62
F024T		1.18	E148Q	372	1.44	S315L		0.42
F024V		1.15	E148R		0.97	S315M		0.63
F024Y		0.90	E148S		1.15	S315R		1.04
L026A	98	1.30	E148T		0.82	S315T		0.97
L026E	99	3.22	E148V		0.99	S315Y	603	0.50
L026G		0.81	E148W		0.43	C316D		0.41
L026H		0.97	E148Y		0.95	L317A	604	1.27
L026I		0.51	A149C		1.15	L317D		0.61
L026K	100	1.88	A149G		0.52	L317H		1.05
L026M	101	1.43	A149K		0.51	L317I	605	1.76

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
L026P		0.55	A149L		0.88	L317K	606	5.11
L026Q	102	1.44	A149M		0.88	L317M		1.20
L026R	103	1.43	A149Q		1.15	L317N	607	0.73
L026S		0.78	A149R		1.02	L317Q	608	1.67
L026T		0.87	A149S		1.08	L317R	609	2.41
L026V		0.52	A149T	373	1.24	L317S	610	1.03
L026W		0.53	A149V	374	1.34	L317T	611	0.93
L026Y		0.52	T150A	375	1.21	L317W	612	0.84
G027A		0.79	T150C		0.70	L318D	614	0.46
G027D	104	1.22	T150D	376	1.24	L318F		0.51
G027E		1.18	T150E		1.05	L318G		0.49
G027F		0.61	T150F		0.71	L318H	615	0.45
G027H		1.11	T150G	377	2.19	L318I		0.70
G027I		0.41	T150I		0.52	L318K	616	1.36
G027K	105	2.71	T150L		0.70	L318M		1.68
G027L		0.76	T150N	378	0.91	L318N		0.52
G027P		0.46	T150P		0.88	L318Q		0.71
G027Q		1.12	T150R		0.90	L318R	617	1.34
G027R	106	1.88	T150S	379	0.92	L318S		0.71
G027S		0.94	T150W	380	1.25	L318T		0.63
G027T		0.61	T150Y	381	1.36	D320E		0.78
G027W		0.76	E151A	382	1.27	D320G		0.83
K028A		0.78	E151C		1.00	D320H	618	1.75
K028D		0.62	E151G		1.06	D320I		1.00
K028E		0.54	E151H	383	1.34	D320K	619	6.42
K028F		0.75	E151K	384	2.05	D320M		0.79
K028I		0.55	E151L	385	1.03	D320N		0.52
K028L		0.51	E151M	386	1.26	D320R	620	3.19
K028M		0.67	E151N		0.95	D320S		1.19
K028N		0.58	E151Q	387	2.01	D320W		0.40
K028P		0.40	D320L		0.37	D320V		0.35
K028R	107	0.71	E151R	388	1.61	D320Y		0.86
K028S		0.46	E151S	389	1.28	N321A		1.01
K028T		0.68	E151T	390	1.21	N321D		1.25
K028V		0.76	E151V	391	1.38	N321H		0.92
K028W		0.51	E151W	392	1.31	N321K		1.29
F029A		0.90	E151Y	393	1.31	N321R	621	1.23

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
F029E	108	4.03	K152A		0.51	N321S	622	1.26
F029G		1.05	K152C		0.52	N321T		0.64
F029H		0.82	K152F		0.61	N321Y		0.40
F029I	109	1.53	K152I		0.65	M323F		0.64
F029K	110	1.34	K152M		0.75	M323I		0.55
F029L	111	2.36	K152R	394	1.85	M323L		0.55
F029M	112	2.08	K152T		1.20	E324A		0.59
F029P	113	3.79	K152V		0.82	E324D		1.15
F029R	114	1.24	K152Y		0.67	E324H		0.79
F029S	115	2.21	A153I		0.93	E324M		0.50
F029T	116	0.85	A153L		0.51	E324N	623	1.01
F029V	117	1.65	K154R		0.86	E324R	624	2.28
F029W		0.48	K154T		0.83	E324S		0.62
D030A		1.12	K154V		0.46	T325A	625	1.87
D030F		0.84	Q155A		0.91	T325D	626	1.78
D030G	118	2.02	Q155C		0.60	T325E	627	4.03
D030H	119	1.69	Q155D	397	1.49	T325G	628	4.21
D030K	120	2.63	Q155F		0.70	T325H	629	3.45
D030L	121	1.32	Q155G	398	1.61	T325K	630	4.37
D030M	122	1.85	Q155H		1.03	T325M	631	2.11
D030P		1.19	Q155K	399	1.57	T325N	632	4.64
D030Q		0.84	Q155L		0.86	T325Q	633	5.08
D030R	123	1.82	Q155M		0.97	T325S	634	3.19
D030S	124	1.62	Q155R	400	1.27	T325V	635	1.24
D030T		0.57	Q155S		0.77	T325W		0.62
D030V		0.46	Q155T		0.76	I326K		0.95
D030W		0.62	Q155V		0.73	I326L	636	1.50
E031A	125	2.05	Q155W		0.91	I326V	637	6.29
E031C	126	2.95	E156A		0.79	I326Y		0.77
E031G	127	1.27	E156D	401	1.95	L327M		0.52
E031H	128	2.74	E156G		0.49	N328A		0.67
E031I	129	3.89	E156I		0.51	N328C	638	1.25
E031K	130	3.13	E156L		0.43	N328G	639	0.56
E031L	131	2.62	E156M		0.87	N328H		0.88
E031P	132	1.51	E156Q		0.84	N328I		1.85
E031R	133	2.27	E156R		0.43	N328K	640	2.12
E031S	134	1.70	E156S		0.62	N328L	641	2.01

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
E031T	135	3.96	E156T		0.69	N328Q		1.13
E031V	136	4.57	E156V		0.45	N328R		0.68
E031W	137	1.26	E156W		0.49	N328S	643	2.22
E031Y		1.13	F157W		0.61	N328T		0.59
P032A		0.92	E158A		0.56	N328V		1.16
P032C	138	0.40	E158F		0.51	N328Y	644	1.66
P032F	139	2.71	E158H		0.54	I331V		0.94
I326C		0.39	I326S		0.95	N328W		0.33
I331C		0.27	I331E		0.34	V334T		0.39
P032G	140	1.60	E158L		0.44	V334P		0.46
P032H	141	2.08	E158Q	402	1.25	T335S	645	0.47
P032K		1.04	E158S	403	0.95	A338Q		0.63
P032L		0.82	K159A		0.64	K339M		0.61
P032M		0.67	K159D		0.52	S342A		0.68
P032N		0.70	K159E		0.49	Q343T		0.49
P032Q		1.11	K159H		0.74	Q343V		0.51
P032R		1.17	K159L		0.62	Q347A	646	0.78
P032S		1.01	K159M		0.66	Q347E		0.78
P032T		0.77	K159N		0.73	Q347G	647	2.68
P032V		0.81	K159Q		0.92	Q347M		0.61
P032W		0.54	K159R		0.88	Q347R		0.55
P032Y		1.01	K159S		0.67	Q347S	648	2.38
L033G	143	0.57	K159V		0.41	E348D		0.67
L033M		0.69	A160C		0.61	E348G		0.55
L033P		0.87	A160F		0.79	E348S		0.44
L033Q		0.45	A160G		0.75	Q349A		0.47
L033R		0.61	A160H		0.47	Q349E		0.83
L033S		0.48	A160I		0.43	Q349K		0.93
L033T		0.45	A160K		0.91	Q349M	649	0.70
L033W	142	1.58	A160L		0.67	Q349N		0.44
D034A		0.38	M035Q		0.37	M035V		0.37
D034E		0.58	A160M		0.77	Q349R	650	0.73
D034H		0.41	A160N		0.56	Q349T		0.49
D034K		0.54	A160Q		0.65	V351A		1.14
D034Q		0.59	A160R		0.89	V351S	651	0.92
D034R		1.17	A160S	404	1.35	I353T		0.42
D034W	144	0.46	A160V		0.73	I353V	652	1.61

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
M035F		0.87	A160Y		1.07	N356A		0.41
M035H		0.60	G161A		0.99	N356D		0.79
M035L		0.52	G161C		0.44	N356H	653	0.82
M035T		0.83	G161D		0.86	N356S	654	0.46
M035Y		0.78	G161E		0.49	W357A		0.80
S036A		0.45	G161R		0.48	W357C		0.67
S036D		0.32	S036N	148	0.38	L037W		0.36
S036G		0.64	G161S		0.77	W357S		0.41
S036H	147	0.54	G161V		0.42	W357T		0.62
S036K		0.83	K162A		0.50	N358C		0.66
S036L		0.71	K162D		0.77	N358G		0.41
S036R		1.09	K162E	405	0.51	N358T		0.58
Q347L		0.39	V351C		0.35	V351I		0.36
V351Q		0.34	W357K		0.36	N358L		0.38
S036T		0.51	K162G		0.56	S359D		0.45
L037F	149	3.33	K162H		0.62	S359E	655	1.05
L037I		0.62	K162L		0.54	S359H	656	0.44
L037K		0.43	K162M		1.04	S359K		0.66
L037M	150	1.46	K162P		0.64	S359M		0.63
L037P		0.63	K162Q		0.58	S359T	657	2.11
L037R		0.51	K162R		0.52	S359V		0.65
L037V		0.57	K162S		0.47	S360T		0.50
F038Y	151	1.29	K162V		0.52	P367A	658	0.55
S039A	152	1.06	K162W		1.01	P367C		0.83
S039L	153	0.80	K162Y		0.72	P367G	659	0.47
S039N	154	2.32	D163A	406	1.52	P367K	660	0.57
S039Q		1.10	D163E	407	1.63	P367R		0.46
S039R		0.56	D163G		1.15	P367S	661	0.52
S039T	155	1.57	D163K	408	1.90	D368A	662	1.34
S039Y		0.56	D163L		1.18	D368E	663	1.28
F040L	156	0.92	D163Q	409	1.40	D368G		0.49
F040W		1.11	D163R	410	1.80	D368H		0.96
I041A		0.67	D163S	411	1.34	D368K	664	1.31
I041C		0.53	D163T		1.13	D368L	665	0.64
I041D		0.78	D163V		0.76	D368M	666	0.78
I041E		0.51	F164L		1.13	D368R	667	1.31
I041G		0.76	F164M	412	1.66	D368S		0.93

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
I041H		0.77	F164V	413	1.23	D368T	668	0.80
I041N		0.40	S043N		0.34	D361H		0.37
I041T	157	1.47	F164W		0.72	D368V		0.41
I041V		0.73	L165A		0.48	N369H	669	1.33
I041W		0.66	L165D	414	5.79	N369R	670	0.55
G042A		0.64	L165F	415	1.23	N369S		0.54
S043T		0.43	L165N	416	2.19	A371E		1.05
P044E		0.59	L165R		0.59	A371F	671	0.52
R045I		0.45	L165S	417	1.31	A371H	672	1.20
R045K		0.53	L165V	418	1.22	A371I		0.50
I046A		1.04	L165W		1.14	A371K	673	1.76
I046C		0.37	A371G		0.38	L374W		0.34
I046E		0.43	L165Y		0.66	A371L	674	0.57
I046F		0.73	V166A	419	2.85	A371M		0.57
I046H		0.82	V166C		1.16	A371R	675	1.51
I046L	158	1.08	V166E	420	1.28	A371S	676	1.45
I046M		1.00	V166F	421	1.67	A371V		0.94
I046N		0.66	V166G		1.11	Q373A		0.65
I046R	159	2.29	V166H	422	1.74	Q373E		0.81
I046S		0.64	V166L	423	4.38	Q373F		0.62
I046T		0.55	V166Q	424	3.61	Q373K		0.73
I046V		1.01	V166R	425	5.56	Q373L		0.84
I046Y		0.76	V166T	426	4.26	Q373M	677	1.43
N047A		0.48	V166W	427	1.26	Q373R		0.68
N047D	160	0.82	V166Y	428	2.08	Q373S		0.87
N047F	161	1.32	E167A		0.84	Q373V		1.05
N047G		0.82	E167D	429	0.69	L374A		0.60
N047H		1.16	E167G		0.60	L374H	678	1.42
N047K		0.67	E167H		0.89	L374I		0.80
N047M		0.77	E167K		0.91	L374M		1.11
N047Q		0.69	E167M		0.87	L374N		0.43
N047R		0.84	E167N		0.83	L374P	679	0.43
N047S		0.85	E167P		0.58	L374R		0.83
N047T	162	1.49	E167R		1.02	L374S		0.58
N047W	163	0.63	E167S		1.17	L374T		0.47
N047Y		0.45	E167T		0.59	L374V		0.56
A048F	164	2.51	E167Y		0.55	L374Y		0.66

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
A048G		0.83	T168H		0.46	E375A	680	0.42
A048H	165	1.99	I169L	430	2.08	E375G	681	0.90
A048I		0.64	I169R		0.54	E375K	682	1.49
A048K	166	1.28	I169V		0.74	E375L		0.46
A048M		0.76	K170N		0.72	E375M		0.54
A048N	167	4.25	K170R	431	2.58	E375N		0.81
A048Q		1.05	K170V		0.58	E375R	683	0.43
A048R		0.66	L171I		0.73	E375S		0.77
A048S		1.06	L171V		0.64	E375T		1.17
A048V		0.60	G172A	432	1.20	K376A		0.95
A048Y		0.81	G172C		1.03	K376D	684	0.78
T049I		0.42	K173N		0.44	K376E	685	0.88
T049K		0.85	K173R	433	0.82	K376M		0.46
T049R	168	1.41	L174A		1.20	K376Q	686	0.69
T049S		0.92	L174G	434	0.40	K376R	687	0.67
T049V		0.45	L174K	435	2.39	K376S		0.80
G050A		0.93	L174M		0.79	K376T	688	0.53
G050C		0.41	L174N	436	1.36	K376V	689	0.58
G050D	169	1.37	L174Q		0.99	K376Y	690	0.42
G050E		0.78	L174R	437	1.50	G377D	691	1.35
G050H		0.74	L174S		0.85	G377E	692	0.59
G050L		0.43	L174T	438	1.12	G377H	693	1.49
G050M	171	0.47	L174V		0.62	G377K	694	1.50
G050Q		0.86	L174W		0.78	G377P	695	2.30
G050R		0.86	L174Y		1.06	G377R	696	1.28
G050S	170	1.24	L175E		0.43	G377S	697	1.80
G050V		0.3	Q051A		0.34	Q051R		0.36
G050Y		0.58	L175H		0.57	G377T	698	3.83
Q051N		0.60	L175T	439	1.43	G378K		1.22
Q051S		0.46	L175V		0.94	G378N		0.64
G052N	172	0.89	L175Y		0.66	G378R		1.03
G052P		0.43	R176K		0.67	K379G		0.52
G052Q	173	3.71	N178G		0.85	K379H		0.57
G052R	174	0.53	N178K	440	0.85	K379R		0.74
G052S	175	1.32	N178M		0.88	K379S		0.46
E375I		0.36	K376L		0.37	K379T		0.4
F380V		0.39	F380T		0.39	M035Q	145	0.37

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
G052T	176	0.49	N178R	441	1.10	F380I		0.56
T054A		0.43	H179A		1.06	F380L		0.67
T054F		0.56	H179C		0.94	F380P		0.47
T054N		0.48	H179E		0.62	F380W	699	2.15
T054Q		0.91	H179G		0.86	F380Y	700	1.50
T054S		0.70	H179I		0.90	T381H		0.48
T054V		0.66	H179K	442	1.39	T381K		1.06
V058C	177	0.55	H179L		0.73	T381N		0.51
V058G		0.54	H179M		0.63	T381Q		0.84
V058H	183	1.09	H179N		0.96	T381R		0.87
V058I		0.57	H179P		0.44	T381S	701	0.87
V058K	178	4.08	H179R		0.96	T381V		0.89
V058L	179	1.54	H179S		0.51	R383A		0.51
V058N	184	0.49	H179T		0.43	R383E		0.51
V058P	180	0.90	H179V		0.42	R383H		0.71
V058Q	181	4.54	L180F		0.59	R383I	702	0.71
V058R	182	1.92	L180G		0.62	R383K	703	1.30
V058S		0.83	L180K		0.44	R383L	704	1.31
V058W		0.65	L180M		0.64	R383M		0.61
V058Y	185	1.07	W181M		0.88	R383N		0.77
D059Q		0.40	L061F		0.3	T381E		0.35
D059N	186	1.27	W181Q		0.88	R383S	705	0.87
R060K		0.69	G182L		0.90	R383T		0.98
L061I		0.42	Y183L		0.70	R383V		1.05
L061M		0.73	F186Y		0.59	K385A	706	1.12
L061V		0.59	H192S		0.49	K385G		0.62
Y063A		0.63	H192T		0.50	K385H		0.50
Y063H		1.07	H193G		0.68	K385N		0.41
Y063I		1.03	H193Q	443	0.82	K385Q	707	0.73
Y063K	187	1.36	H193S		0.42	K385R		0.94
Y063L		1.33	H193Y		0.58	K385S		1.05
Y063M	189	1.32	K195A		0.51	K385T		0.46
Y063N		0.96	K195G		0.45	K385V	708	0.43
Y063R	190	1.40	K195H		0.45	T387S		0.93
Y063S		1.00	K195I		0.50	L388F		0.92
Y063T		1.07	K195L		0.45	L388H		0.47
Y063V		0.43	K195N	445	0.74	L388I		0.98

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
Y063W	191	1.53	K195Q		0.71	L388M		0.79
P065R		0.57	K195R		0.85	L388R		0.60
Y066H		0.47	K195S		0.42	L388T		0.51
Y066R		0.51	K195T	444	0.58	L388V		0.78
I067F		1.00	K195W		0.49	L388W		0.77
I067L		0.45	K196E	446	0.43	L388Y		1.18
I067R		0.24	D068G		0.37	E392W		0.31
I067V	192	1.80	K196G		0.41	E389A	709	1.14
I067Y		0.55	K196L		0.65	E389G	710	0.91
D068E		0.72	K196R	447	0.58	E389H		1.17
D068H	193	2.06	K196S		0.68	E389K	712	1.91
D068K		1.08	K196T		1.18	E389L	711	0.65
D068L		0.43	K196W		0.55	E389M		0.60
D068P	194	0.50	P197A		0.81	E389P		0.75
D068Q	195	1.67	P197D		0.58	E389Q	713	0.69
D068R		0.70	P197E		0.52	E389R		0.94
D068S		0.81	P197F		0.48	E389S		1.08
D068T		0.75	P197G		0.75	E389T		0.70
S069A	196	22.06	P197H		0.62	E389Y		0.77
S069C	197	1.97	P197K		0.99	L391C		0.90
S069E	198	1.48	P197L		0.56	E392A	715	0.58
S069F	199	8.75	P197M		1.03	E392F	716	0.54
S069G	200	6.06	P197Q		0.69	E392G		1.00
S069I	201	3.12	P197R		0.58	E392K		0.66
S069L	202	3.44	P197S		0.70	E392L		0.80
S069M	203	2.67	P197T		0.41	E392M	717	1.54
S069P	204	8.14	G198A		0.80	E392Q	718	1.01
S069R	205	14.06	G198D	448	1.99	E392R	719	0.66
S069T	206	0.58	G198E		0.49	E392S		0.52
S069W	207	2.18	G198H		0.84	E392T		0.72
S069Y	208	2.71	G198L		0.48	E392V	720	1.27
I070A	209	27.00	G198N		0.80	E392Y		0.92
I070C	210	2.57	G198Q		0.55	Q393A		1.26
I070F	211	5.69	G198R		0.58	Q393D		0.45
I070G	212	6.22	G198S		0.76	Q393F	721	1.23
I070H	213	9.09	G198T		0.41	Q393H		1.05
I070K		14.64	G198Y		0.81	Q393K		0.80

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
I070L	215	3.05	N200D		0.46	Q393L		0.91
I070N	216	6.19	S202M		0.40	Q393M	722	0.80
I070P	217	3.03	F204P	449	0.63	Q393N		0.72
I070R	218	13.95	N205A	450	1.30	Q393R		0.74
I070S	219	3.63	N205D		0.85	Q393S		1.15
I070T	220	5.43	N205E	451	1.94	Q393T		0.41
I070V	221	6.34	N205F		0.52	F394L		0.56
I070Y	222	1.26	N205G		0.79	F394W		0.41
T071A		0.86	N205K		0.76	S395A	723	1.10
T071D		0.50	N205M		0.58	S395G		0.77
T071G	223	1.41	N205P		0.75	S395H	724	0.56
T071H		0.93	N205R		0.54	S395K		0.96
T071L		1.09	N205S		0.80	S395R	725	1.98
T071M		0.89	N205T	453	0.85	E396A	726	0.52
T071N	224	1.21	N205V		0.49	E396D		0.64
T071Q		0.68	N205W		0.41	E396H	727	0.47
T071R	225	2.17	V206H		0.50	E396Q	728	0.73
T071S	226	1.54	V206I	454	0.94	E396R		0.61
G072A		0.45	V206K	455	1.75	E396S	729	0.61
G072D		0.60	V206L	456	1.57	E396T		0.89
S395W		0.4	S395T		0.39	E396L		0.39
G072E		0.69	V206M		0.43	Y399A		1.01
G072H		0.46	V206R	457	1.30	Y399C		0.46
G072K	227	1.39	V206S		0.72	Y399E		1.49
G072L		0.43	G072Y		0.35	S407L		0.4
G072M	228	3.11	V206T		0.59	Y399K	730	1.94
G072Q	229	2.33	I208A		0.62	Y399M	731	2.70
G072R		0.65	I208C		0.48	Y399N		0.52
G072S		0.51	I208K		0.91	Y399Q		1.18
V073A	230	1.38	I208L		0.84	Y399R		1.20
V073C		0.84	I208M		0.88	Y399S		1.01
V073D		0.94	I208Q		0.77	Y399T	732	2.40
V073G		1.17	I208R		1.14	Y399V	733	1.44
V073H	231	1.54	I208S		0.62	Y399W	734	1.92
V073K	232	1.42	I208T		1.01	S401A	735	0.82
V073L	233	1.59	I208V		1.07	S401E	736	0.46
V073M		0.68	K209A		0.53	S401N		0.42

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
V073Q	234	0.96	K209E		0.46	Y403F		0.62
V073R	235	0.72	K209G		0.44	S404A	737	0.63
V073S		0.86	K209N		0.50	S404P		0.64
K297R		0.34	F398L		0.35	S401G		0.38
S401Q		0.39	S404T		0.37	T405F		0.36
V073T	236	1.34	K209R	458	0.68	T405A		0.56
V073W	237	1.91	K209S		0.50	T405G	738	2.32
T074A	238	2.28	K209T		0.50	T405K		0.74
T074C	239	2.18	D212N	459	1.52	T405M		0.48
T074E	240	1.38	D212S	460	0.93	T405P		0.64
T074F	241	1.43	D212T		0.76	T405Q		0.75
T074G	242	2.75	D213A	461	0.85	T405R		0.60
T074H	243	1.40	D213E		0.79	T405S		0.94
T074K	244	1.29	D213G		0.81	T405W		0.73
T074L	245	1.43	D213H		0.75	T405Y		0.44
T074M	246	0.52	D213K		0.82	L406A		0.70
T074N	247	2.12	D213L		0.56	L406C		0.98
T074P	248	2.45	D213M	462	1.56	L406E		0.73
T074R	249	2.22	D213N	463	1.53	L406F	739	1.42
T074S	250	1.80	D213Q		1.04	L406G		1.00
T074V	251	2.27	D213R		0.92	L406I		0.61
T074W	252	2.13	D213V		0.47	L406N	740	0.76
V075A		0.71	D213W		0.49	L406Q		0.93
V075C		0.46	D213Y		0.49	L406S		0.47
V075F	253	2.00	L214Q		0.57	L406T		0.83
V075H		0.62	S215A		0.74	L406V		0.87
V075L	254	5.22	S215D		0.62	L406Y		0.74
V075M	255	1.16	S215E		0.74	S407A	741	1.16
V075N		0.81	S215G		0.88	S407D	742	1.52
V075Q		1.51	S215H	464	0.91	S407E	743	1.38
V075R	256	3.02	S215K		0.99	S407F	744	1.42
V075S		0.76	S215L		0.60	S407G		0.75
V075T	257	4.34	S215M	465	1.77	S407H	745	1.34
V075Y		0.63	S215Q		0.79	S407M		0.74
G077H		0.32	G077K		0.32	K411H		0.33
I079L	258	1.44	S215R		0.71	S407N		0.72
I079T		0.79	S215T		0.80	S407P	747	0.94

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
I079V		1.01	S215V		0.69	S407Q	746	1.71
Q081P		0.60	S215W		0.52	S407R		1.04
K082A		0.94	W216Y		0.48	S407V		0.56
K082E		0.50	L217M		0.51	S407W		0.41
K082G		0.64	W218F		0.57	K409A	748	2.18
K082H		0.44	N219A	466	1.29	K409D		0.65
K082I		1.01	N219C		0.43	K409E		0.62
K082L	259	0.87	N219D		0.75	K409G		0.50
K082M		0.58	N219E		0.95	K409H		0.64
K082N	260	0.96	N219H		0.97	K409I		0.51
K082Q		0.76	N219I	467	0.60	K409P		0.48
K082R		0.85	N219K	468	1.45	K409Q	749	3.33
K082S		0.62	N219L		0.72	K409R		0.84
K082T		0.56	N219M		1.02	K409S		0.72
K082Y		0.32	I083H		0.4	I083K		0.30
K082V		0.57	N219R		1.10	K409T		0.63
I083F		0.57	N219S	469	2.48	K409V		0.48
I083G	264	1.05	N219T		0.82	A412Y		0.66
I083L		0.93	N219W		0.48	E410D		0.47
I083N		0.82	E220A		0.75	E410K		0.70
I083Q	262	1.07	E220H	470	1.40	E410M		0.42
I083R		0.45	E220I	471	1.34	E410N		0.67
I083S	263	0.79	E220L	472	1.45	E410P		0.73
I083T		0.95	E220S		0.62	E410Q		0.85
I083V	261	0.99	E220T		0.91	E410R		0.61
S084D		0.98	E220V	473	1.35	E410S		0.81
S084E	265	0.52	S221A		0.72	E410T	750	1.54
S084F	266	0.72	S221C		0.59	E410V		0.65
S084G	267	8.68	S221M		0.46	E410Y		0.62
S084H		0.96	S221Q	474	1.37	K411A		0.48
S084I		0.90	S221T		0.94	K411N		1.02
S084L		0.92	S221V		1.04	K411P		0.42
S084M		0.77	T222D		0.43	K411R		0.97
S084N	268	0.89	T222F		0.43	K411S		1.21
S084P		0.57	T222G	475	0.49	K411T		0.63
S084Q		0.86	T222K		0.75	K411V		0.99
S084R	269	1.89	T222L		0.64	A412D		0.74

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
S084T		0.82	T222N		0.80	A412G		0.80
S084W		0.86	T222R		0.75	A412I		0.81
S084Y		0.30	E220D		0.39	E220M		0.36
S221I		0.35	T222I		0.4	P226W		0.51
L085V		0.42	T222S		0.63	A412L		0.65
Q086A	270	2.70	T222V		0.79	A412N		0.86
Q086D		0.88	L224I		0.61	A412P		0.77
Q086E		1.18	L230I		0.87	A412R	752	0.66
Q086F		0.54	N231T		1.10	A412S		0.86
Q086G		1.02	T232F	476	0.73	A412V	753	0.53
Q086H	271	1.70	T232S		0.76	A412W		0.54
Q086I		0.65	Q233A		0.71	D413E		0.52
Q086K	272	0.97	Q233F		0.53	D413K		0.42
Q086L		0.92	Q233G	477	0.46	D413N		0.94
Q086M		1.06	Q233K	478	1.69	D413R		0.50
Q086N	273	1.28	Q233L		0.69	D413T		0.41
Q086P		0.42	Q233R	479	1.50	V414I		1.12
Q086R		0.93	Q233Y		0.50	V414M		0.53
Q086S	274	0.85	Q234M	480	1.65	K415G		0.40
Q086T	275	0.58	S235A	481	0.47	K415S		0.42
Q086V		0.97	S235E		1.00	K415W		0.42
Q086W	276	1.21	S235G		0.95	D416F		0.41
D087A		1.00	S235H		0.44	D416G		0.67
D087C	277	1.77	S235K		0.53	D416H		0.57
D087E		0.86	S235T		0.66	D416I		0.63
D087G	278	1.00	P236A		1.07	D416K		0.76
D087H		0.72	P236G		1.09	D416L	754	0.75
D087I		0.53	P236H		0.46	D416N		0.73
D087L	279	0.55	P236K		0.71	D416Q		0.83
D087M	280	0.58	P236R	482	3.09	D416R		0.46
D087P		0.31	Q234L		0.40	V237C	483	0.35
D087Q		1.05	P236S		0.91	D416T		0.85
D087R	281	1.28	V237A		0.90	D416V		0.59
D087S	282	0.99	V237E	484	1.93	D416Y		0.40
D087T	283	1.70	V237F		0.41	T417I		1.22
A412H		0.39	A412Q	751	0.35	D413A		0.38
D413H		0.31	A413Q		0.38	D413S		0.39

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
V414K		0.3	V414L		0.36	K415Y		0.39
K415V		0.39	D418G		0.45			
D087V	284	0.66	V237H	485	0.75	D418A		0.92
D087Y	285	2.72	V237L		1.12	D418E	755	1.31
L089C	286	1.46	V237N		0.67	D418F		0.81
L089R		0.34	L089W		0.26	L089P		0.38
L089K		0.45	V237Q	486	1.46	D418G		0.45
L089M		0.63	V237R		0.71	D418I		0.99
D090A	287	1.48	V237S		1.03	D418L	756	1.28
D090E	288	1.15	V237T	487	1.01	D418M		1.09
D090G		0.41	V237W		0.52	D418N		0.91
D090H	289	1.24	A238D		0.75	D418P	757	2.11
D090I		1.10	A238E	488	0.59	D418Q		1.05
D090K	290	1.36	A238H	489	0.60	D418R	758	1.18
D090L		1.15	A238K		0.60	D418S		0.78
D090N	291	1.18	A238Q		1.02	D418V	759	1.43
D090Q		1.11	A238R		0.49	D418Y		0.97
D090R	292	1.49	A238S	490	2.62	A419E		0.45
D090S		1.15	A238T		0.44	A419F	760	2.17
D090T		1.02	T240K		1.13	A419G		0.42
D090W		0.81	T240A	491	0.48	A419H	761	1.21
K091A		0.89	T240M		0.48	A419I	762	1.64
K091Q		0.43	T240P		0.56	A419K	763	1.88
K091R		0.67	T240Q	492	0.75	A419L		0.56
A092C	293	1.97	T240R		0.91	A419N		0.53
A092H		0.22	A239N		0.32	V421I		0.39
A092L	294	1.29	T240S		0.74	A419R	764	1.81
A092M		0.86	T240V		0.77	A419S	765	2.65
A092T		0.70	Y242F		1.08	A419W		0.69
A092V		1.09	N245H		0.50	A419Y	766	1.44
K093D		0.71	V247I	493	2.01	V420I		1.04
K093E		0.83	V247L		0.83	V420P		0.48
K093F		0.50	V247M		0.52	D421A	767	1.28
K093G		0.97	R248A	494	0.43	D421E		0.81
K093H		0.61	R248W		0.52	D421G		0.62
K093I	295	3.25	R248Y		0.67	D421H	768	1.98
R248H		0.4	I251Y		0.37	K255G		0.39

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
K093L	296	1.53	I251L		0.58	D421K	769	2.42
K093M		0.70	I251M		0.43	D421L		0.73
K093N		0.71	V253I		0.76	D421M		0.94
K093Q	297	0.84	K255A		0.40	D421N	770	1.89
K093R	298	1.52	K255N		0.52	D421Q	771	1.54
K093S	299	1.25	K255Q		0.91	D421R	772	2.21
K093T	300	3.93	K255R		0.71	D421S	773	2.12
K093V		0.24	K093P		0.38	K094C		0.33
K094A	304	0.64	K255S		0.43	D421T		0.80
K094D	301	0.93	I256A		0.42	D421Y		0.66
K094E		0.79	I256H		0.51	V422I		0.42
K094F		0.59	I256L		0.64	V422T		0.49
K094H		0.72	I256V		0.51	A425G	774	1.20
K094L		0.52	P257A		0.82	A425I		0.44
K094M		0.66	P257G	496	0.51	A425K	775	1.75
K094N		0.99	P257I		1.07	A425M		0.70
K094Q	302	1.22	P257K		0.92	A425N		0.46
K094R	303	3.94	P257L		0.69	A425R		0.49
K094S		0.94	P257M		0.90	A425S		0.47
K094T		1.14	P257N		0.69	D426E		0.62
I096D		0.69	P257Q		0.61	D426G		0.85
I096L		0.46	P257R	498	1.38	D426N		0.61
I096V		0.68	P257T	497	2.04	D426P		1.03
T097A	304	1.25	P257V		0.88	D426Q		0.42
T097C	305	0.53	D258H		0.84	D426Y		0.43
T097D	306	1.31	D258N	499	1.44	G427K		0.52
T097E	307	1.19	D258R		0.45	G427S		0.42
T097F		0.75	D258S	500	1.44	V428L	778	1.25
P257C		0.36	D258G		0.39	A425Y		0.39
D426K		0.26	D426S		0.36	G427T	777	0.35
G427H		0.35	G427I		0.54	G427Q	776	0.39
T097G	308	4.84	A259E		0.85	V428M		0.42
T097I		0.85	A259G		0.68	V428P		0.82
T097L	309	1.22	A259I		0.46	V428T		0.62
T097N		1.10	A259K		0.76	D431A	779	2.42
T097P		0.62	A259L		0.53	D431E	781	1.27
T097Q		1.17	A259N		0.49	D431G		0.55

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
T097R		0.95	A259P	501	1.54	D431H	782	3.13
T097S	310	1.21	A259Q		0.70	D431I		1.05
T097W		0.53	A259R		0.72	D431K	783	1.83
T097Y		0.74	A259S		0.63	D431L	784	0.62
F098A		0.60	A259T		0.51	D431N	785	1.30
F098C		0.58	A259V		0.41	D431Q	786	2.16
F098D		0.47	A259W		0.55	D431R	787	2.20
F098E		0.44	A259Y		0.51	D431S	788	1.91
F098H		1.06	K260A		0.66	D431V	789	1.52
F098I		0.52	K260D		0.41	D431W		0.56
F098L		0.58	K260E		0.58	D431Y		0.85
F098M		0.87	K260H		0.87	A432E		0.60
F098Q		0.65	K260L		0.60	A432G		0.52
P436C		0.39	E249V			A432H		0.34
F098R		0.72	K260M	502	0.85	A432N		0.51
F098S		0.56	K260Q		0.58	A432S		0.61
F098V		0.46	K260R		0.83	A432V		0.56
F098W		0.81	K260S		0.66	F433A	790	0.97
Y099A		0.33	K260G		0.37	R270T		0.40
Y099R		0.53	K260Y	503	1.73	F433C		0.69
Y099S		0.43	S261A	504	0.74	F433D		0.95
V102A		0.83	S261F		0.73	F433E		0.82
V102C		0.69	S261K	505	2.54	F433G		0.54
V102E		0.90	S261M		0.56	F433H	791	0.83
V102G		0.67	S261N	506	1.98	F433I	792	1.06
V102H		0.88	S261Q		0.76	F433K	793	1.36
V102K		1.03	S261R		1.19	F433L	794	1.87
V102L		0.71	S261T		0.66	F433P		0.95
V102M		0.77	S261V		0.48	F433R	795	1.63
V102N		1.02	S261W		0.44	F433S		0.86
V102Q		1.03	L263A		0.76	F433T	796	1.86
V102R		0.94	L263K	507	2.73	F433V	797	1.63
V102S	311	1.41	L263M		0.89	F433W	798	1.28
V102T	312	1.26	L263R	508	1.63	L434F		0.41
V102W		0.76	L263T		0.49	L434G		0.47
D103N		0.39	N104I		0.35	L263H		0.36
N104A		0.69	L263V		0.75	L434I		0.89

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
N104C		0.41	P264A		0.43	L434M		0.60
N104G		0.48	P264H		0.60	L434V		0.46
N104K		0.88	V265I		0.58	K435A		1.08
N104M		0.61	F266Y		0.58	K435C		0.53
N104R	313	1.25	A267M		0.45	K435E		0.78
N104S		1.03	A267T	509	1.34	K435G		0.64
N104T		0.71	T269A	510	1.63	K435H		1.05
L105A		0.54	T269C		0.75	K435R		1.01
L105G		0.51	T269D		0.76	K435S		1.03
L105I		0.94	T269S		1.01	K435T		0.73
L105P		0.84	R270M		0.46	K435V		0.44
L105Q		0.90	R270N		0.52	K435Y		0.50
L105R		0.65	R270S		0.69	P436D		1.19
L105S		0.61	I271F		0.72	P436E		0.74
L105T		0.51	I271G		1.29	P436G		1.19
L105W		0.34	L105C		0.33	L105H		0.36
L105V		0.99	I271L	511	10.62	P436H		0.72
G106V		0.43	V272E		0.39	V272M		0.31
M107F		0.91	I271M	512	3.24	P436I		0.84
M107I		0.67	I271S		0.42	P436K	799	2.05
M107L	314	1.32	I271V		1.05	P436L		0.63
A108G		0.47	V272D	513	1.36	P436M		0.61
I110V		0.51	V272R		0.74	P436Q		0.86
E114A	315	1.44	V272S		0.96	P436R		1.00
E114G		0.73	V272T	514	1.61	P436S		0.92
E114H		0.75	F273H	515	1.41	P436T		0.59
E114M		0.44	F273T		0.48	P436W		0.43
E114S		0.69	F273Y	516	0.90	P436Y		0.49
P117D		0.56	T274A		0.51	P437A		0.56
T118H		0.47	T274F	517	1.28	P437D		0.62
T118K		0.53	T274S		0.62	P437G		0.50
T118L		1.09	Q276C		0.88	P437H		1.11
T118M		0.53	Q276D	518	1.69	P437I	800	2.46
T118N		0.67	Q276E		1.05	P437K		0.83
T118Q	316	3.37	Q276H	519	1.20	P437L		0.51
T118V		0.79	Q276I		0.51	P437M	801	2.55
W119F		0.53	Q276L		0.48	P437Q		0.96

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
W119P		0.36	W119Q		0.72	D275L		0.24
W119Y		1.08	Q276M	520	1.14	P437R		0.85
A120D		0.76	Q276R	521	1.30	P437S		0.57
A120F	318	2.62	Q276S	522	1.63	P437Y		0.42
A120G		1.03	Q276Y	523	1.94	M438A	802	0.75
A120H	317	1.11	V277A	524	0.65	M438C		0.63
A120I	319	1.33	V277C		0.41	M438D	803	0.87
A120L		1.25	V277D		0.79	M438E	804	0.72
A120N		0.81	V277E	525	1.02	M438G		0.83
A120P		0.42	V277G		1.18	M438L	805	0.86
A120R		0.82	V277H	526	1.09	M438N	806	1.08
A120S	320	1.21	V277K	527	1.51	M438P		0.81
A120T		0.62	V277M	528	0.94	M438Q		0.85
A120V	321	1.53	V277N		1.15	M438R		0.99
A120W		0.59	V277Q	530	0.82	M438S		0.83
A120Y	322	1.95	V277R	531	1.63	M438T	807	3.99
N122M		0.56	V277S	532	0.83	M438V		0.85
K124L		0.34	K124H		0.35	P125A		0.36
K124R		0.62	V277T	533	1.94	M438W		0.57
P125H		0.43	V277Y		0.66	E439A	808	1.20
P125R		0.63	L278A		1.13	E439C	809	0.58
P125S		0.54	L278E	534	1.03	E439F		1.00
D127A		0.89	L278F	535	1.26	E439G		1.22
D127E	323	1.31	L278G	536	1.33	E439H		0.74
D127G		0.97	L278H	537	4.50	E439K	810	1.20
D127H	324	2.33	L278I		0.93	E439L		0.88
D127L		0.84	L278K	538	1.75	E439P	811	1.16
D127M		0.4	D275V		0.4	Q276G		0.36
D127N	325	1.69	L278N	539	1.74	E439Q	812	1.32
D127Q	326	1.21	L278R	540	5.87	E439S		1.02
D127R	327	0.51	L278S	541	1.67	E439T	813	1.15
D127S		0.77	L278T	542	1.66	E439V	814	1.57
D127T		1.11	L278V		0.44	E439W		0.62
D127V		0.56	L278Y	543	1.51	T440A		1.22
D127W		0.44	K279H		0.44	T440D	815	1.03
V128A		0.53	K279Q		0.84	T440E		1.00
V128C		0.68	K279R		1.10	T440F		0.85

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
V128G		0.49	K279T		0.86	T440G		0.86
V128I	328	1.25	F280G		0.47	T440H	816	3.00
V128K		1.16	F280Q		0.43	T440I		1.04
V128L		0.95	S282D		0.41	T440L		0.97
V128Q		0.55	S282G		0.54	T440M	817	1.08
V128R		0.74	S282M	545	2.64	T440P	818	0.88
V128S		0.53	S282Q		0.41	T440R	819	1.77
V128W		0.50	Q283E		0.63	T440S	820	1.17
K130I		0.50	Q283P		1.18	T440V		1.02
K130R	329	1.42	Q283R		0.59	T440Y		1.11
N131C		0.60	Q283S	546	1.73	E441A	821	1.47
N131E		0.44	Q283T		0.65	E441D		0.67
N131F		0.63	D284A		0.58	E441F	822	3.91
N131G	330	2.47	D284E		1.21	E441G		0.87
N131H		0.80	D284G		0.60	E441H		0.65
N131I	331	1.40	D284H		0.51	E441K		0.80
N131L		0.82	D284L		0.50	E441L		0.82
N131M	332	0.99	D284M		0.56	E441N		0.82
N131Q	333	1.24	D284N		0.40	E441Q		0.81
N131R	334	2.81	D284Q		0.95	E441S		0.79
N131S		0.76	D284S		0.99	E441T		0.66
N131T		1.02	E285F		0.47	E441V		0.54
N131V	335	2.08	E285G		0.52	E441Y		0.51
N131Y		0.85	E285H		1.30	E442C	823	1.38
R132A		0.68	E285M	547	0.43	E442G	824	0.51
R132C		0.58	E285N		0.40	E442H		0.76
R132E		0.70	E285Q		0.59	E442K		0.73
R132F		0.60	E285Y		0.99	E442P		0.91
R132H		0.66	L286S		0.46	E442Q		0.74
K279A		0.27	D284T		0.39	D284Y		0.37
E285A		0.34	L286R		0.53	L286W		0.38
R132I		0.56	V287I		0.51	E442R	825	3.94
R132K		1.05	V287T	548	0.50	E442T		0.61
R132L		0.76	Y288L		0.79	E442V		0.65
R132N	336	1.28	Y288W		0.49	E442Y		0.60
R132Q		0.69	T289K		0.75	P443A	826	1.63
R132S		0.79	T289S	549	0.48	P443E	827	1.07

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
R132T		0.61	F290I		0.41	P443F	828	0.70
R132V		0.73	F290M		1.03	P443G	829	1.12
R132Y		0.78	G291Q		0.80	P443H		1.08
S133I		0.54	G291R		0.45	P443L		1.19
I134L		1.04	G291S	550	0.41	P443M	830	1.99
I134T		0.60	G291V	551	1.63	P443N	831	1.25
I134V		1.08	E292A		0.66	P443Q		0.96
E135A		0.99	E292C	552	0.71	P443R		1.04
E135C		0.77	E292F	553	0.90	P443S		0.99
E135D	338	2.68	E292G		0.41	P443T		0.87
E135F		0.73	E292H	554	1.26	P443W		0.64
E442L		0.4	E442W		0.38	Q444M		0.37
E135G	339	2.79	E292K	555	1.27	Q444D		0.97
E135H		0.79	E292N		0.99	Q444E	832	1.19
E135K		1.15	E292P		1.05	Q444F		0.66
E135L		0.82	E292R	556	0.42	Q444G		0.93
E135N		0.56	E292V	557	1.28	Q444H	833	0.97
E135Q		1.59	E292W		0.83	Q444I		0.58
E135R	340	2.08	T293A	558	1.90	Q444K		1.03
E135S		1.13	T293C	559	1.67	Q444N		1.01
E135W		0.63	T293D	560	1.46	Q444R		0.85
E135Y		0.50	V137C		0.37	Q444V	834	1.12
L136A		0.73	V137S		0.36	Q444W		0.64
L136C		0.56	V137L		0.21	Q444Y		0.67
L136D		0.47	Q143C		0.28	I445A		0.97
L136F		0.96	L144R	360	0.26	I445G		0.98
L136H		1.00	K152W	396	0.37	I445H	835	1.35
L136I		0.65	A153S		0.34	I445L		1.06
L136M		1.05	K154I		0.38	I445M	836	1.57
L136N		0.48	E156C		0.35	I445N	837	1.24
L136Q		0.61	E158G		0.37	I445P	838	1.67
L136R		0.74	K159G		0.38	I445Q	839	1.26
L136S		0.80	A160W		0.39	I445R		1.08
L136T		0.72	G161V		0.42	I445S	840	1.21
L136W		1.11	D163W		0.38	I445T	841	1.38
V137A		0.48	D163F		0.39	I445V	842	1.25
V137I		1.01	L165C		0.27	I445W	843	0.69

TABLE 9: ACTIVE MUTANTS								
mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
V137T		0.51	V166N		0.47	I445Y		0.53
Q138A		0.69	E167F		0.31	F446A	844	1.58
Q138C		0.65	K170A		0.40	F446C		0.75
Q138H		0.71	K170Q		0.40	F446D		1.18
Q138I		0.54	K173Q		0.32	F446E		1.10
Q138L	341	0.59	L174H		0.38	F446G		1.12
Q138M		0.68	R176L		0.40	F446H		1.28
Q138N		0.61	P177V		0.36	F446I		1.06
Q138R		0.53	L180I		0.38	F446K		0.94
Q138S		0.48	W181K		0.29	F446L		0.93
Q138W		0.41	Y183E		0.32	F446M	845	1.31
Q138Y		0.60	Y184W		0.39	F446Q		0.72
Q139A		0.92	H193R		0.33	F446R		0.89
Q139C		0.44	H193F		0.38	F446T		0.89
Q139D		0.48	K195V		0.36	F446V		0.91
Q139E		0.94	K196N		0.39	F446W	846	1.40
Q139F		0.53	K196Y		0.39	Y447D	847	3.25
Q139G		0.65	P197W		0.39	Y447E	848	1.36
Q139H		0.56	G198W		0.29	Y447F		1.41
Q139K		0.73	N200T		0.37	Y447G	849	0.92
Q139L		0.70	F204W		0.39	Y447I		1.36
Q139M		0.95	N205L	452	0.39	Y447L		1.09
Q139R		0.79	N205Y		0.4	Y447M		0.90
Q139S		0.81	V206Q		0.33	Y447N	851	1.58
Q139T	342	1.31	K209F		0.4	Y447P	852	1.46
Q139V		0.77	K209L		0.38	Y447Q	853	2.37
Q140A		0.96	N211L		0.41	Y447R		1.12
Q140C		0.50	N211W		0.51	Y447T	854	1.90
Q140D		0.59	W218M		0.38	Y447V		1.38
Q140F		0.66	W218V		0.28	Y447W		1.07

2. Inactive Mutants

The other mutants that exhibited less than 20% hyaluronidase activity of wildtype PH20 in one of the duplicates were rescreened to confirm the dead mutants that are inactive. To confirm the inactive mutants, the hyaluronidase activity assay described in Example 2 was modified to incorporate an overnight 37°C substrate-

sample incubation step prior to measurement of enzymatic activity. The modified assay is intended to detect PH20 activities below 0.2 U/mL.

The preparation of the bHA coated plates and blocking of the plates prior to addition of the transfected variant supernatants or wildtype PH20 was the same as described in Example 3. The assay was modified as follows. First, transfected variant supernatants or wildypte PH20 not containing a mutation generated as described in Example 2 were diluted in duplicate 1:25 in assay diluent. For the standard curve, 1:3 serial dilutions of rHuPH20 (generated as described in Example 1) were made in assay diluent in duplicate starting from 0.1 U/mL down to 0.00014 U/mL. A blank well also was included. Then, 100 µl of the diluted samples or standard were added to pre-designated wells of the bHA-coated and blocked plate and allowed to incubate at 37°C overnight. After the incubation, the plates were washed and binding to bHA detected as described above in Example 3. Optical density was measured at 450 nm within 30 minutes of adding the stop solution.

The identified reconfirmed inactive mutants are set forth in Table 10. The Table sets forth the amino acid replacement compared to the sequence of amino acids of PH20 set forth in SEQ ID NO:3.

TABLE 10: Inactive Mutants

N002H	R060V	R121W	C189P	P236I	V287N	L336W	G377V
N002K	R060Y	R121Y	C189R	P236L	V287P	L336Y	G378D
N002W	L061A	N122A	C189S	P236N	V287Q	A337C	G378E
N002Y	L061E	N122C	C189T	P236Q	V287R	A337F	G378F
F003A	L061F	N122E	C189V	P236T	V287S	A337G	G378I
F003G	L061G	N122F	C189W	P236Y	Y288D	A337I	G378L
F003K	L061H	N122I	C189Y	A238F	Y288E	A337K	G378M
F003P	L061N	N122K	Y190C	A238G	Y288F	A337L	G378Q
F003T	L061P	N122Q	Y190E	A238L	Y288G	A337M	G378T
F003V	L061Q	N122R	Y190F	A238P	Y288H	A337R	G378W
R004D	L061R	N122S	Y190G	A238V	Y288I	A337T	G378Y
R004E	L061T	N122T	Y190H	A238W	Y288K	A337W	K379A
R004F	L061W	N122V	Y190K	A238Y	Y288P	A338C	K379C
R004G	L061Y	W123A	Y190L	A239C	Y288R	A338D	K379E
R004L	G062A	W123C	Y190N	A239F	Y288T	A338E	K379F
R004P	G062C	W123D	Y190Q	A239G	T289A	A338F	K379I
R004W	G062D	W123E	Y190R	A239H	T289C	A338G	K379L
R004Y	G062F	W123H	Y190S	A239I	T289E	A338H	K379M
A005D	G062I	W123L	Y190T	A239L	T289G	A338I	K379W
A005G	G062K	W123M	Y190V	A239P	T289H	A338K	F380C
A005I	G062L	W123P	Y190W	A239R	T289L	A338L	F380D

TABLE 10: Inactive Mutants							
A005L	G062M	W123Q	N191A	A239S	T289P	A338P	F380E
A005M	G062P	W123R	N191E	A239T	T289Q	A338R	F380G
A005N	G062Q	W123S	N191F	A239V	T289R	A338T	F380Q
A005P	G062R	W123T	N191G	A239W	T289S	A338V	F380R
A005Q	G062S	W123V	N191K	A239Y	T289Y	K339D	F380S
A005R	G062T	W123Y	N191L	T240E	F290D	K339E	T381G
A005T	G062V	K124C	N191M	T240F	F290Q	K339F	T381L
A005V	G062Y	K124D	N191P	T240G	F290Y	K339G	T381P
A005W	Y063C	K124E	N191Q	T240N	G291A	K339H	T381W
A005Y	Y063G	K124F	N191R	T240W	G291C	K339L	T381Y
P006E	Y063P	K124N	N191S	T240Y	G291D	K339N	V382E
P006F	Y064A	P125C	N191T	L241A	G291E	K339P	V382G
P006T	Y064C	P125D	N191V	L241C	G291F	K339S	V382H
P006V	Y064D	P125G	N191W	L241D	G291M	K339T	V382K
P006Y	Y064E	P125L	N191Y	L241E	G291N	K339V	V382L
P007C	Y064F	P125N	H192C	L241G	G291T	K339W	V382M
P007D	Y064G	P125W	H192F	L241I	G291W	K339Y	V382N
P007F	Y064H	K126F	H192G	L241P	G291Y	M340A	V382P
P007G	Y064I	K126H	H192K	L241R	E292I	M340C	V382Q
P007H	Y064K	K126I	H192L	L241S	E292L	M340D	V382R
P007I	Y064L	K126L	H192M	L241T	E292T	M340E	V382S
P007K	Y064P	K126N	H192N	L241V	T293E	M340F	V382T
P007L	Y064Q	K126P	H192P	L241W	T293N	M340G	V382W
P007Q	Y064R	K126Y	H192Q	Y242A	V294A	M340H	V382Y
P007R	Y064S	D127K	H192R	Y242C	V294E	M340K	R383G
P007S	Y064T	V128E	H192V	Y242D	V294G	M340P	R383P
P007T	Y064V	V128P	H192W	Y242G	V294H	M340R	G384C
P007W	Y064W	Y129A	H192Y	Y242I	V294K	M340S	G384F
P007Y	P065A	Y129C	H193A	Y242L	V294L	M340T	G384M
V008D	P065C	Y129D	H193D	Y242M	V294N	M340V	G384Q
V008E	P065D	Y129E	H193K	Y242P	V294P	M340W	G384S
V008G	P065G	Y129G	H193L	Y242R	V294Q	C341A	G384T
V008H	P065H	Y129H	H193M	Y242S	V294R	C341E	K385C
V008N	P065I	Y129L	H193P	Y242T	V294S	C341G	K385L
V008R	P065K	Y129P	H193V	Y242V	V294T	C341H	K385M
V008S	P065N	Y129Q	Y194A	Y242W	V294W	C341K	K385P
V008W	P065R	Y129S	Y194C	V243C	A295C	C341L	K385W
I009C	P065S	Y129T	Y194I	V243D	A295G	C341M	K385Y
I009D	P065T	Y129V	Y194L	V243F	A295H	C341N	P386A
I009E	P065V	Y129W	Y194P	V243G	A295I	C341Q	P386C
I009G	P065W	K130C	Y194S	V243H	A295L	C341R	P386F
I009N	P065Y	K130D	Y194T	V243L	A295N	C341S	P386G
I009P	Y066A	K130G	Y194V	V243M	A295P	C341T	P386H

TABLE 10: Inactive Mutants							
P010F	Y066C	K130H	K195S	V243P	A295T	C341V	P386I
P010I	Y066D	K130L	P197C	V243Q	A295V	C341Y	P386L
P010L	Y066E	K130N	G198V	V243R	A295Y	S342D	P386M
P010M	Y066G	K130S	G198W	V243S	L296C	S342E	P386N
P010Y	Y066I	K130T	Y199E	V243W	L296F	S342F	P386Q
N011A	Y066K	K130W	Y199G	V243Y	L296G	S342H	P386R
N011C	Y066L	K130Y	Y199H	R244A	L296I	S342K	P386S
N011F	Y066N	N131P	Y199I	R244D	L296K	S342L	P386T
N011I	Y066P	R132P	Y199K	R244G	L296M	S342M	P386V
N011L	Y066S	S133D	Y199L	R244I	L296Q	S342P	P386Y
N011P	Y066T	S133E	Y199P	R244V	L296R	S342Q	T387C
N011T	Y066V	S133F	Y199R	R244Y	L296S	S342R	T387E
N011W	I067D	S133G	Y199S	N245A	L296T	S342T	T387F
N011Y	I067E	S133H	Y199W	N245C	L296V	S342Y	T387G
V012G	I067G	S133L	N200A	N245F	L296W	Q343C	T387H
V012H	I067P	S133M	N200F	N245L	L296Y	Q343D	T387I
V012W	I067R	S133N	N200G	N245P	G297C	Q343F	T387L
P013E	I067T	S133P	N200H	N245Q	G297E	Q343I	T387M
P013G	I067W	S133R	N200K	N245R	G297H	Q343P	T387N
P013I	D068A	S133T	N200L	N245S	G297L	Q343W	T387V
P013L	D068C	S133V	N200M	N245T	G297N	V344F	T387W
P013M	D068G	S133W	N200P	N245V	G297P	V344G	T387Y
P013V	D068I	I134A	N200Q	R246A	G297Q	V344H	L388C
F014A	D068L	I134C	N200R	R246C	G297R	V344L	L388G
F014E	D068P	I134D	N200S	R246D	G297S	V344M	L388P
F014G	D068V	I134F	N200W	R246E	G297T	V344N	L388Q
F014H	D068Y	I134G	N200Y	R246G	G297Y	V344P	L388S
F014K	S069N	I134H	G201A	R246H	A298C	V344Q	E389F
F014N	S069T	I134K	G201F	R246I	A298E	V344R	E389V
F014P	I070Q	I134P	G201L	R246K	A298L	V344S	D390A
F014Q	T071P	I134Q	G201M	R246L	A298M	V344T	D390C
F014W	G072C	I134R	G201N	R246M	A298N	V344W	D390E
L015E	G072F	I134S	G201P	R246P	A298P	V344Y	D390F
L015F	G072H	I134W	G201R	R246S	A298Q	L345A	D390G
L015G	G072I	E135P	G201S	R246T	A298S	L345C	D390H
L015K	G072P	L136P	G201T	R246V	A298T	L345E	D390L
L015N	G072V	V137F	G201V	R246W	A298W	L345H	D390N
L015P	G072W	V137G	G201W	V247A	A298Y	L345K	D390P
L015Q	V073P	V137H	S202A	V247C	S299A	L345N	D390R
L015R	V075D	V137N	S202E	V247F	S299C	L345Q	D390S
L015S	V075G	V137P	S202F	V247H	S299D	L345R	D390T
L015Y	V075P	V137R	S202G	V247N	S299F	L345T	D390V
W016A	N076A	V137W	S202H	V247P	S299G	L345V	D390W

TABLE 10: Inactive Mutants							
W016C	N076C	V137Y	S202K	V247Q	S299H	L345Y	D390Y
W016D	N076F	Q138V	S202N	V247R	S299L	C346A	L391A
W016E	N076G	Q139P	S202P	V247S	S299M	C346D	L391D
W016F	N076I	Q143C	S202Q	V247T	S299P	C346F	L391G
W016G	N076K	Q143H	S202R	V247W	S299Q	C346G	L391H
W016H	N076L	Q143P	S202V	V247Y	S299T	C346I	L391K
W016K	N076P	Q143R	S202W	R248C	G300A	C346K	L391N
W016M	N076Q	Q143S	S202Y	R248D	G300C	C346L	L391P
W016P	N076R	Q143T	C203A	R248E	G300D	C346M	L391Q
W016R	N076S	L144A	C203D	R248G	G300E	C346P	L391R
W016S	N076T	L144E	C203E	R248I	G300F	C346R	L391S
W016T	N076V	L144F	C203G	R248M	G300L	C346S	L391T
W016Y	N076W	L144I	C203H	R248P	G300M	C346T	L391V
A017D	G077D	L144K	C203L	R248T	G300N	C346V	L391W
A017E	G077E	L144P	C203M	E249A	G300P	C346W	L391Y
A017G	G077L	L144Q	C203N	E249G	G300Q	Q347C	E392C
A017H	G077P	L144S	C203Q	E249H	G300S	Q347F	E392P
A017I	G077Q	L144V	C203R	E249I	G300T	Q347I	Q393C
A017L	G077R	L144Y	C203S	E249K	G300V	Q347P	Q393P
A017N	G077T	S145T	C203T	E249M	G300W	Q347T	F394A
A017P	G077V	S145W	C203V	E249Q	I301E	Q347V	F394D
A017Q	G078A	A149E	F204A	E249S	I301G	Q347W	F394E
A017R	G078D	A149P	F204C	E249Y	I301H	E348C	F394G
A017S	G078I	T150V	F204E	A250C	I301K	E348H	F394I
A017T	G078M	K152L	F204G	A250F	I301M	E348I	F394K
A017V	G078P	A153E	F204H	A250G	I301N	E348L	F394N
A017W	G078T	A153F	F204I	A250H	I301P	E348P	F394P
A017Y	G078Y	A153M	F204K	A250K	I301Q	E348Q	F394Q
W018C	I079A	A153P	F204Q	A250L	I301R	E348R	F394R
W018D	I079D	A153R	F204R	A250M	I301S	E348T	F394S
W018F	I079F	A153T	F204S	A250N	I301W	E348V	F394T
W018G	I079G	A153V	F204T	A250P	I301Y	E348W	F394V
W018H	I079H	K154D	V206C	A250Q	V302C	E348Y	S395C
W018I	I079K	K154E	V206D	A250R	V302D	Q349D	S395L
W018L	I079N	K154G	V206F	A250S	V302E	Q349F	S395M
W018M	I079P	K154P	V206G	A250T	V302F	Q349G	S395P
W018P	I079S	K154S	V206P	A250V	V302G	Q349P	E396C
W018Q	I079W	K154W	V206Y	A250W	V302H	Q349V	E396F
W018S	I079Y	K154Y	E207A	I251D	V302L	Q349W	E396G
W018T	P080A	Q155P	E207F	I251F	V302M	Q349Y	E396I
W018V	P080D	Q155Y	E207G	I251G	V302P	G350A	E396P
W018Y	P080E	E156P	E207M	I251H	V302R	G350D	E396Y
N019A	P080F	F157A	E207P	I251K	V302S	G350E	K397A

TABLE 10: Inactive Mutants							
N019C	P080G	F157C	E207Q	I251P	V302T	G350F	K397C
N019F	P080I	F157D	E207R	I251S	V302Y	G350H	K397E
N019G	P080K	F157E	E207S	I251T	I303A	G350K	K397F
N019H	P080L	F157G	E207T	I251W	I303C	G350L	K397G
N019I	P080M	F157H	E207V	R252A	I303D	G350M	K397I
N019L	P080N	F157I	E207W	R252D	I303E	G350N	K397L
N019M	P080R	F157K	I208D	R252E	I303F	G350P	K397M
N019P	P080S	F157L	I208G	R252F	I303G	G350R	K397P
N019Q	P080T	F157M	I208P	R252G	I303K	G350S	K397Q
N019R	P080V	F157P	I208W	R252H	I303L	G350T	K397T
N019S	P080Y	F157Q	K209C	R252I	I303M	G350V	K397V
N019V	Q081A	F157R	K209P	R252K	I303R	G350Y	F398A
N019W	Q081C	F157S	R210A	R252L	I303W	V351C	F398C
N019Y	Q081E	F157T	R210C	R252N	I303Y	V351D	F398E
A020D	Q081G	F157V	R210D	R252P	W304A	V351E	F398G
A020E	Q081H	E158D	R210E	R252S	W304C	V351F	F398H
A020F	Q081L	E158K	R210G	R252T	W304D	V351H	F398I
A020H	Q081N	E158P	R210K	R252Y	W304G	V351N	F398L
A020K	Q081P	E158R	R210M	V253A	W304I	V351R	F398N
A020L	Q081S	E158Y	R210N	V253D	W304M	V351W	F398P
A020N	Q081V	K159W	R210P	V253E	W304N	V351Y	F398R
A020P	Q081W	K159Y	R210S	V253G	W304P	C352A	F398S
A020R	Q081Y	G161W	R210T	V253H	W304Q	C352D	F398T
A020T	K082W	D163C	R210V	V253L	W304S	C352E	F398V
A020V	K082Y	D163P	R210W	V253M	W304T	C352F	F398W
A020Y	I083E	F164A	R210Y	V253N	W304V	C352G	F398Y
P021A	I083K	F164C	N211C	V253Q	W304Y	C352K	Y399D
P021C	S084Y	F164D	N211F	V253R	G305L	C352M	Y399P
P021D	L085A	F164E	N211G	V253S	G305P	C352P	C400A
P021E	L085C	F164G	N211H	V253W	G305Q	C352Q	C400D
P021G	L085D	F164H	N211I	S254C	G305R	C352R	C400E
P021H	L085E	F164N	N211K	S254D	G305S	C352S	C400F
P021I	L085F	F164P	N211M	S254E	G305T	C352T	C400G
P021L	L085G	F164Q	N211P	S254G	G305V	C352V	C400I
P021M	L085H	F164R	N211R	S254I	G305Y	C352W	C400L
P021R	L085N	L165C	N211S	S254K	T306A	C352Y	C400M
P021S	L085Q	L165H	N211T	S254L	T306C	I353C	C400P
P021T	L085S	L165P	N211V	S254P	T306H	I353F	C400Q
P021V	L085T	L165T	N211W	S254Q	T306I	I353G	C400R
P021W	Q086C	V166D	D212A	S254R	T306L	I353H	C400S
S022C	Q086P	E167V	D212G	S254T	T306V	I353K	C400T
S022E	D087P	T168A	D212H	S254V	T306W	I353L	C400V
S022G	H088A	T168C	D212I	S254W	T306Y	I353M	C400Y

TABLE 10: Inactive Mutants							
S022K	H088C	T168D	D212K	S254Y	L307C	I353Q	S401C
S022P	H088E	T168E	D212L	K255C		I353R	S401F
E023A	H088F	T168F	D212M	K255D	L307I	I353S	S401H
E023F	H088G	T168G	D212P	K255L	L307P	I353W	S401K
E023L	H088I	T168K	D212V	K255P	S308C	R354C	S401R
E023M	H088K	T168L	D212W	K255V	S308F	R354D	S401W
E023N	H088L	T168P	D213P	K255W	S308L	R354E	S401Y
E023P	H088M	T168R	D213S	I256C	S308M	R354G	C402A
E023R	H088P	T168S	L214A	I256D	S308V	R354H	C402D
E023S	H088R	T168V	L214C	I256E	S308W	R354I	C402E
E023T	H088S	T168W	L214D	I256G	S308Y	R354K	C402F
E023V	H088T	T168Y	L214E	I256P	M310C	R354L	C402L
C025D	H088V	I169A	L214G	P257D	M310E	R354M	C402M
C025E	H088Y	I169D	L214H	D258L	M310F	R354P	C402P
C025F	L089A	I169F	L214K	D258P	M310K	R354Q	C402Q
C025G	L089D	I169G	L214N	D258V	M310L	R354S	C402R
C025H	L089E	I169H	L214P	D258W	R311C	R354V	C402S
C025I	L089G	I169K	L214R	K260C	R311E	R354W	C402T
C025K	L089Q	I169N	L214S	K260P	R311F	R354Y	C402V
C025L	L089S	I169P	L214T	S261P	R311I	K355D	C402W
C025N	L089T	I169Q	L214Y	P262A	R311L	K355F	C402Y
C025P	L089W	I169S	S215C	P262D	R311P	K355G	Y403A
C025R	L089Y	I169T	S215P	P262E	R311V	K355H	Y403C
C025S	D090C	I169Y	W216D	P262F	R311W	K355L	Y403E
C025T	D090G	K170C	W216E	P262G	S312C	K355M	Y403G
C025V	K091D	K170D	W216G	P262H	S312E	K355N	Y403H
C025Y	K091E	K170E	W216H	P262I	S312M	K355P	Y403K
G027C	K091F	K170G	W216I	P262K	S312V	K355Q	Y403L
L033C	K091G	K170M	W216K	P262Q	S312W	K355R	Y403M
L033D	K091H	K170P	W216L	P262R	M313C	K355S	Y403N
L033H	K091I	K170W	W216M	P262S	K314C	K355T	Y403P
L033N	K091L	K170Y	W216N	P262T	K314L	K355V	Y403Q
L033V	K091N	L171C	W216P	P262V	K314W	K355W	Y403R
L033Y	K091T	L171D	W216Q	P262W	S315C	K355Y	Y403T
D034I	A092E	L171H	W216R	P262Y	S315I	N356C	S404C
D034L	A092F	L171M	W216T	L263E	S315V	N356G	S404D
D034N	A092H	L171N	W216V	L263F	C316E	N356K	S404F
D034S	A092K	L171R	L217A	L263P	C316G	N356L	S404G
D034T	A092P	L171S	L217C	L263Q	C316I	N356P	S404H
D034V	A092Q	L171W	L217G	L263W	C316K	N356R	S404L
M035A	A092R	L171Y	L217H	P264D	C316L	N356T	S404M
M035D	A092W	G172D	L217P	P264E	C316M	N356V	S404N
M035G	A092Y	G172E	L217Q	P264F	C316P	N356W	S404R

TABLE 10: Inactive Mutants							
M035P	K094G	G172I	L217S	P264G	C316R	W357D	S404V
M035R	K094P	G172L	L217T	P264L	C316S	W357E	S404W
M035S	D095A	G172P	L217V	P264M	C316T	W357F	S404Y
S036C	D095C	G172Q	L217W	P264R	C316V	W357G	T405C
S036F	D095E	G172T	W218A	P264T	C316W	W357L	T405I
S036V	D095F	G172V	W218I	P264V	C316Y	W357M	T405V
S036W	D095G	G172W	W218K	P264W	L317G	W357Q	L406P
S036Y	D095H	G172Y	W218L	P264Y	L317P	W357R	L406R
L037C	D095K	K173D	W218P	V265A	L318C	N358E	C408A
L037E	D095L	K173E	W218S	V265D	L318P	N358H	C408E
L037G	D095M	K173G	W218V	V265F	L318W	N358I	C408F
L037N	D095P	K173H	N219P	V265G	L319C	N358K	C408G
L037S	D095Q	K173I	E220G	V265H	L319E	N358P	C408I
F038E	D095S	K173L	E220K	V265K	L319F	N358Q	C408K
F038G	D095V	K173M	E220N	V265L	L319G	N358R	C408L
F038K	D095W	K173P	E220P	V265M	L319H	N358W	C408P
F038L	D095Y	K173S	E220R	V265N	L319I	S359A	C408R
F038N	I096A	K173V	E220W	V265Q	L319K	S359F	C408S
F038Q	I096C	K173W	S221D	V265R	L319M	S359G	C408T
F038R	I096G	K173Y	S221E	V265S	L319P	S359L	C408V
F038T	I096H	L174P	S221H	F266A	L319Q	S359P	C408W
F038W	I096P	L175C	S221K	F266C	L319R	S359W	C408Y
S039C	I096R	L175D	S221P	F266G	L319S	S360A	E410W
S039D	I096S	L175G	S221R	F266H	L319V	S360C	K411D
S039F	I096T	L175K	T222P	F266M	L319W	S360E	K411E
S039W	I096W	L175P	T222Y	F266P	L319Y	S360F	K411F
F040A	F098P	L175R	A223C	F266Q	D320C	S360G	K411G
F040D	Y099C	L175S	A223D	F266R	D320P	S360I	A412E
F040E	Y099E	R176A	A223E	F266S	D320V	S360K	A412H
F040G	Y099G	R176C	A223G	F266T	N321E	S360L	D413H
F040K	Y099I	R176E	A223H	F266V	N321M	S360M	D413I
F040N	Y099N	R176F	A223K	F266W	N321P	S360P	D413K
F040R	Y099P	R176G	A223L	A267D	Y322C	S360Q	D413L
F040S	Y099V	R176H	A223P	A267G	Y322D	S360R	D413P
F040T	Y099W	R176I	A223Q	A267H	Y322E	S360V	V414A
F040V	M100C	R176P	A223R	A267I	Y322G	D361A	V414D
I041Q	M100E	R176Q	A223S	A267K	Y322I	D361C	V414E
G042D	M100F	R176S	A223T	A267N	Y322L	D361E	V414G
G042E	M100G	R176T	A223V	A267R	Y322N	D361G	V414H
G042H	M100N	R176V	A223W	A267S	Y322P	D361M	V414K
G042I	M100P	R176W	A223Y	A267W	Y322R	D361N	V414R
G042K	M100R	P177A	L224A	Y268A	Y322S	D361P	V414S
G042L	M100S	P177C	L224D	Y268C	Y322T	D361Q	V414T

TABLE 10: Inactive Mutants							
G042M	M100T	P177D	L224E	Y268F	Y322V	D361R	K415C
G042P	M100W	P177F	L224F	Y268G	Y322W	D361S	K415D
G042Q	M100Y	P177G	L224G	Y268H	M323A	D361V	K415E
G042R	P101A	P177H	L224M	Y268K	M323C	D361W	K415P
G042S	P101C	P177L	L224P	Y268L	M323E	Y362A	D416C
G042T	P101F	P177M	L224Q	Y268N	M323G	Y362C	D416S
G042V	P101H	P177Q	L224R	Y268P	M323H	Y362E	T417A
S043A	P101I	P177R	L224S	Y268Q	M323K	Y362G	T417D
S043E	P101K	P177S	L224T	Y268S	M323N	Y362H	T417E
S043F	P101L	P177T	L224W	Y268T	M323R	Y362K	T417F
S043G	P101M	P177V	L224Y	Y268V	M323S	Y362L	T417G
S043I	P101N	P177W	Y225A	Y268W	M323T	Y362M	T417H
S043K	P101Q	N178E	Y225D	T269E	M323V	Y362N	T417K
S043L	P101R	N178I	Y225E	T269K	E324C	Y362P	T417M
S043Q	P101S	N178L	Y225G	T269L	E324F	Y362R	T417P
S043R	P101T	N178V	Y225H	T269M	E324P	Y362S	T417Q
S043V	V102P	N178W	Y225K	T269N	E324V	Y362T	T417R
P044A	D103A	N178Y	Y225P	T269P	E324W	Y362V	A419D
P044C	D103E	H179W	Y225Q	T269Q	E324Y	Y362W	A419P
P044F	D103F	L180A	Y225R	T269R	T325C	L363A	V420A
P044G	D103G	L180C	Y225T	R270A	T325R	L363C	V420D
P044H	D103H	L180E	Y225V	R270C	I326E	L363D	V420F
P044I	D103I	L180P	Y225W	R270E	I326G	L363E	V420G
P044L	D103L	L180R	P226A	R270F	I326H	L363F	V420H
P044N	D103Q	L180S	P226C	R270G	I326N	L363G	V420K
P044Q	D103R	W181A	P226D	R270H	I326W	L363H	V420L
P044R	D103T	W181C	P226E	R270I	L327A	L363I	V420N
P044S	D103V	W181D	P226F	R270P	L327E	L363P	V420R
P044T	D103W	W181E	P226G	R270Y	L327F	L363Q	V420S
P044W	D103Y	W181F	P226L	I271A	L327G	L363R	V420T
P044Y	N104F	W181H	P226N	I271D	L327H	L363S	V420W
R045A	N104P	W181I	P226Q	I271E	L327N	L363T	V420Y
R045D	N104W	W181K	P226R	I271H	L327Q	L363V	V422C
R045F	L105C	W181L	P226S	I271K	L327R	L363W	V422D
R045G	L105M	W181R	P226T	I271T	L327S	H364A	V422G
R045P	L105N	W181S	P226V	I271W	L327T	H364C	V422H
R045W	G106A	W181V	P226W	V272A	L327V	H364D	V422L
I046P	G106C	G182A	P226Y	V272H	L327W	H364E	V422M
I046W	G106D	G182C	S227A	V272L	L327Y	H364F	V422N
N047V	G106F	G182D	S227F	V272N	P329C	H364G	V422Q
A048P	G106H	G182E	S227G	V272P	P329F	H364K	V422R
T049C	G106L	G182H	S227H	V272W	P329G	H364L	V422S
T049D	G106M	G182N	S227I	F273A	P329H	H364M	V422Y

TABLE 10: Inactive Mutants							
T049G	G106N	G182P	S227K	F273C	P329I	H364P	C423A
T049H	G106P	G182Q	S227L	F273D	P329K	H364R	C423D
T049P	G106S	G182R	S227M	F273G	P329L	H364S	C423E
	G106W	G182S	S227P	F273I	P329N	H364T	C423F
Q051C	G106Y	G182T	S227Q	F273L	P329Q	H364V	C423G
Q051F	M107A	G182V	S227R	F273P	P329R	H364Y	C423H
Q051I	M107C	G182Y	S227T	F273Q	P329S	L365A	C423L
Q051M	M107H	Y183C	S227V	F273S	P329T	L365C	C423M
Q051P	M107K	Y183D	S227W	F273V	P329V	L365D	C423P
Q051T	M107P	Y183E	S227Y	F273W	P329W	L365E	C423Q
Q051W	M107Q	Y183G	I228A	T274C	P329Y	L365G	C423R
Q051Y	M107S	Y183I	I228E	T274E	Y330A	L365M	C423S
G052C	M107V	Y183K	I228F	T274G	Y330C	L365N	C423T
G052E	M107W	Y183N	I228G	T274H	Y330D	L365P	C423V
G052F	A108D	Y183P	I228H	T274N	Y330E	L365Q	C423W
G052W	A108E	Y183Q	I228L	T274Q	Y330G	L365R	I424A
G052Y	A108F	Y183R	I228M	T274W	Y330I	L365S	I424C
V053A	A108K	Y183S	I228N	T274Y	Y330L	L365T	I424E
V053C	A108L	Y183V	I228P	D275A	Y330M	L365W	I424G
V053D	A108M	Y184A	I228R	D275F	Y330N	L365Y	I424H
V053E	A108P	Y184C	I228S	D275G	Y330P	N366A	I424N
V053G	A108Q	Y184D	I228T	D275I	Y330R	N366C	I424Q
V053H	A108T	Y184E	I228W	D275K	Y330S	N366E	I424R
V053L	A108V	Y184F	Y229E	D275L	Y330V	N366F	I424S
V053N	A108Y	Y184G	Y229F	D275M	Y330W	N366G	I424W
V053P	V109C	Y184H	Y229G	D275Q	I331A	N366K	I424Y
V053Q	V109D	Y184K	Y229K	D275T	I331C	N366M	A425E
V053R	V109E	Y184L	Y229L	D275V	I331D	N366P	A425L
V053S	V109L	Y184M	Y229P	D275W	I331E	N366Q	A425P
V053T	V109M	Y184P	Y229Q	Q276F	I331F	N366R	A425W
V053W	V109R	Y184R	Y229T	Q276P	I331H	N366T	A425Y
V053Y	V109T	Y184S	Y229V	Q276W	I331K	N366W	D426C
T054D	V109W	Y184V	Y229W	L278M	I331Q	P367E	D426F
T054E	I110F	L185A	L230A	L278P	I331R	P367F	D426M
T054G	I110K	L185D	L230E	K279A	I331S	P367I	D426R
T054P	I110L	L185E	L230G	K279C	I331T	P367L	G427A
T054R	I110M	L185F	L230H	K279F	I331W	P367M	G427C
T054Y	I110P	L185G	L230K	K279G	I331Y	P367Q	G427F
I055A	I110W	L185I	L230M	K279L	I332A	P367V	G427L
I055D	D111H	L185K	L230N	K279W	I332C	D368C	G427P
I055G	D111I	L185P	L230P	K279Y	I332D	D368P	
I055H	D111Q	L185R	L230R	F280D	I332E	D368W	G427V
I055N	W112C	L185S	L230S	F280I	I332F	N369C	G427W

TABLE 10: Inactive Mutants							
I055P	W112E	L185T	L230T	F280L	I332G	N369E	G427Y
I055Q	W112G	L185V	L230V	F280M	I332H	N369F	V428A
I055R	W112H	L185W	L230W	F280N	I332K	N369I	V428C
I055T	W112L	L185Y	L230Y	F280R	I332L	N369K	V428D
I055V	W112N	F186A	N231A	F280S	I332N	N369L	V428E
I055Y	W112P	F186D	N231C	F280T	I332P	N369P	V428G
F056A	W112S	F186G	N231D	F280V	I332R	N369Q	V428H
F056C	E113R	F186H	N231F	F280W	I332S	N369V	V428N
F056E	E113V	F186I	N231G	L281A	I332T	N369W	V428R
F056G	E114I	F186K	N231H	L281D	I332Y	F370A	V428S
F056H	E114L	F186L	N231I	L281G	N333G	F370D	V428Y
F056I	E114P	F186N	N231K	L281H	N333H	F370E	C429A
F056K	E114T	F186P	N231L	L281I	N333I	F370G	C429D
F056L	E114V	F186Q	N231P	L281K	N333K	F370H	C429K
F056P	W115A	F186R	N231Q	L281N	N333P	F370K	C429L
F056R	W115C	F186S	N231R	L281P	N333R	F370L	C429N
F056S	W115D	F186V	N231S	L281Q	N333S	F370N	C429P
F056T	W115F	F186W	N231V	L281R	N333T	F370P	C429S
F056V	W115G	P187A	T232C	L281S	N333W	F370Q	C429T
F056W	W115H	P187F	T232G	L281V	N333Y	F370R	C429V
Y057A	W115I	P187G	T232H	L281W	V334A	F370S	C429W
Y057D	W115K	P187H	T232K	S282F	V334C	F370V	C429Y
Y057F	W115L	P187I	T232L	S282L	V334D	F370Y	I430A
Y057G	W115M	P187L	T232N	S282V	V334E	A371P	I430D
Y057I	W115R	P187M	T232P	S282W	V334G	A371W	I430E
Y057L	W115S	P187N	T232Q	S282Y	V334M	I372A	I430L
Y057M	W115V	P187Q	T232V	Q283A	V334N	I372D	I430M
Y057P	W115Y	P187R	T232Y	Q283C	V334R	I372E	I430N
Y057Q	R116A	P187S	Q233D	Q283D	V334S	I372F	I430S
Y057R	R116C	P187T	Q233I	Q283F	T335F	I372G	I430T
Y057V	R116D	P187V	Q233P	Q283W	T335G	I372H	I430V
Y057W	R116E	P187W	Q233S	D284C	T335H	I372K	D431P
V058A	R116G	P187Y	Q233T	D284I	T335I	I372L	A432C
D059A	R116H	D188A	Q234A	D284P	T335K	I372N	A432F
D059E	R116I	D188C	Q234D	E285K	T335L	I372P	A432I
D059I	R116L	D188F	Q234E	E285P	T335P	I372R	A432K
D059L	R116N	D188G	Q234G	E285R	T335V	I372S	A432L
D059M	R116P	D188H	Q234H	E285T	T335W	I372T	A432M
D059P	R116Q	D188L	Q234N	E285V	T335Y	I372V	A432P
D059R	R116S	D188M	Q234P	L286A	L336A	I372W	A432Y
D059T	R116V	D188N	Q234S	L286C	L336E	Q373C	L434H
D059V	R116W	D188P	Q234T	L286D	L336F	Q373P	L434K
D059W	P117D	D188Q	Q234V	L286F	L336G	Q373W	L434P

TABLE 10: Inactive Mutants							
D059Y	P117G	D188R	Q234W	L286H	L336K	L374D	L434Q
R060A	P117I	D188S	S235F	L286K	L336N	L374E	L434R
R060D	P117K	D188T	S235L	L286M	L336P	E375C	L434W
R060F	P117N	D188V	S235M	L286P	L336R	E375F	P437T
R060G	P117Q	D188W	S235R	L286T	L336S	E375P	M438Y
R060H	P117R	C189A	S235W	L286Y	L336T	E375V	E439N
R060I	P117S	C189E	S235Y	V287A	L336V	E375Y	E439R
R060L	P117V	C189G	P236C	V287C	R121G	K376I	T440Q
R060N	P117W	C189H	W119L	V287D	R121H	K376P	E441R
R060P	T118C	C189K	W119N	V287E	R121K	K376W	E442M
R060Q	T118D	C189L	W119P	V287G	R121L	G377C	E442N
R060S	T118E	C189M	W119R	V287K	R121M	G377I	E442S
R060T	T118G	C189N	R121A	V287L	R121P	G377L	P443D
T118R	T118P	T118W	R121C	R121F	G378D	G377V	G378E
T118Y	W119I	W119A	W119K	R121E	G378F	G378I	

EXAMPLE 5

ASSAY FOR HYALURONIDASE ACTIVITY UNDER TEMPERATURE AND PHENOPHILIC CONDITIONS

Supernatants from PH20 activity variants set forth in Table 9, as identified in Example 4, were tested for stability uner thermophilic and/or phenophilic conditions. The assay to measure hyaluronidase activity under temperature and phenophile conditions using biotinylated-HA (bHA) as substrate for measuring hyaluronidase activity was modified from the original assay described in Example 3 in that it incorporated a 4-hour 37°C incubation of samples with or without m-cresol prior to measurement of enzymatic activity. The assay was used to identify PH20 mutants with thermophilic properties (activity greater at 37° C condition than at 4° C) and/or with phenolphilic properties (greater activity in the presence of m-cresol than wildtype PH20).

1. Primary Screen

Prior to incubating samples with bHA, variant PH20 samples were diluted into designated wells of an uncoated 4XHB plate for pre-incubation at 37° C for 4 hours under the following conditions: 1) pre-incubation at 37° C with 0.4% m-cresol; and 2) pre-incubation at 37° C without 0.4% m-cresol. For the preincubation at 37° C with 0.4% m-cresol, a 1% m-cresol intermediate stock was prepared from 50% (v/v) m-cresol stock solution. Briefly, in a 2 mL Wheaton glass vial a 50% stock of m-cresol

(Fluka, Catalog No. 65996; Spectrum, Catalog No. C2773) was made in methanol based on the density (D=1.034 g/L). The vial was sealed and stored at -20°C with protection from light in small aliquotes. Then, the 1% intermediate stock was generated by dilution in HEPES assay buffer (10 mM HEPES, 50 mM NaCl, 1 mM CaCL2, 1 mg/mL BSA, pH 7.4, 0.05% Tween-20) daily immediately prior to use in a fume hood with vortexing.

Then, duplicates of transfected variant supernatant samples set forth in Table 9, generated as described above in Example 2, were each separately subjected to a 1:2.5 dilution of 1% m-cresol in HEPES assay buffer/ transfected supernatant to obtain 0.4% final concentraion of m-cresol. For the preincubation at 37° C without 0.4% m-cresol, transfected variant supernatant samples were subjected to a 1:2.5 dilution in HEPES assay buffer/transfected supernatant. In addition for each condition, an internal killing control was also tested by spiking in 3 U/mL of rHuPH20 in pH 7.4 HEPES buffer (generated as described in Example 1) that was diluted the same as described above for the transfected samples. The plates were sealed with plate sealers and incubated at 37° C for 4 hours.

The preparation of the bHA coated plates and blocking of the plates prior to addition of the transfected variant supernatants or wildtype PH20 was the same as described in Example 3. The assay was further modified as follows. First, samples were diluted in duplicate 1:10 in HEPES assay buffer in 4XHB plates. For each variant, the samples that were tested were 1) non-preincubated transfected variant supernatant (no incubation; 4 °C); 2) preincubated transfected variant supernatants preincubated at 37 °C for 4 hours with 0.4% m-cresol (Cresol); or 3) preincubated transfected variant supernatant preincubated at 37 °C for 4 hours without 0.4% m-cresol (no cresol; 37 °C). In addition, the spiked-in samples also were tested. A standard curve using rHuPH20 was made as described in Example 3 without m-cresol. One hundred microliters (100 µl) of each standard and sample was transferred to pre-designated wells of the bHA-coated and blocked plate and incubated for approximately 1.5 hours at 37°C. Thus, each variant sample in each sample was tested in quadruplicate due to the preincubation of duplicate samples of each transfected variant supernatants in the pre-incubation step and the further duplicate of each sample in the bHA assay.

After the incubation, the plates were washed and binding to bHA detected as described above in Example 3. Optical density was measured at 450 nm within 30 minutes of adding the stop solution.

The U/mL activity was calculated from the standard curve and compared. The results were depicted as the percent (%) activity remaining under each of the following parameter: ratio of activity at 1) 37 °C preincubation without m-cresol/4 °C; 2) 37 °C after preincubation with m-cresol/4 °C; and 3) 37 °C after preincubation with m-cresol/after preincubation at 37 °C without m-cresol. Initial phenophile hits for reconfirmation were identified as those that in a duplicate assay exhibited a percentage of remaining activity under condition 3) of $\geq$ 20% of the original activity at 37°C.

Initial Hits were rescreened using a 6-well plate rescreen assay. For the rescreen, plasmid DNA corresponding to the potential Hit was transformed into *E.coli* bacteria and plasmid DNA prepared and purified using MaxiPrep according to manufacturers instructions. The DNA sequence was confirmed.

The plasmid DNA was transfected into monolayer CHO-S cells (Invitrogen, Cat. No. 11619-012) grown on 6-well plates at a density of about 50-80% confluency using Lipofectamine 2000 (Invitrogen, Cat. No. 11668-027) according to the protocol suggested by the manufacture. Transfections were performed in duplicate. The cells were incubated at 37°C in a CO_2 incubator for 96 hours hours post-transfection before collecting the supernatant for the assay. As controls, cells also were transfected with the HZ24-PH20(OHO)-IRES-SEAP expression vector (SEQ ID NO:4) that contains a codon-optimized wildtype PH20 sequence (OHO). In addition, mock cells also were included as controls.

Ninety-Six (96) hours post-transfections, supernatant was collected from each sample, including the OHO and mock controls, and assayed for hyaluronidase activity under various conditions as described above: 1) non-preincubated transfected variant supernatant (no incubation; 4 °C); 2) preincubated transfected variant supernatants preincubated at 37 °C for 4 hours with 0.4% m-cresol (Cresol); or 3) preincubated transfected variant supernatant preincubated at 37 °C for 4 hours without 0.4% m-cresol (no cresol; 37 °C). Hyaluronidase activity was determined as described above using the bHA assay.

The results were assessed as described above. Absolute hyaluronidase activity (U/mL) was generated from the standard curve. In addition, percent activity was

determined as a ratio of activity at 37 °C/4 °C, 37 °C plus m-cresol/4 °C and 37 °C plus m-cresol/37 °C. The results are set forth in Tables 11 and 12 below.

TABLE 11: Absolute Hyaluronidase Activity						
Mutant	Noincubation (4 °C)		37 °C no cresol (37 °C)		37 °C with m-cresol (37 °C plus m-cresol)	
L001A	2.993	2.511	3.529	3.214	0.287	0.295
L001E	2.669	2.539	2.862	3.179	0.376	0.341
L001G	0.348	0.583	0.596	0.676	0.055	0.031
L001Q	5.135	6.443	6.133	5.719	0.621	0.636
L001R	5.603	4.390	6.576	7.042	0.458	0.396
P006A	2.965	3.208	4.088	3.495	0.404	0.435
V008M	1.376	1.401	1.856	1.678	0.000	0.008
I009Q	0.447	0.381	0.469	0.476	0.031	0.030
P010G	0.747	0.564	0.820	0.688	0.123	0.114
P010H	0.473	0.485	0.624	0.548	0.000	0.000
N011S	0.862	0.962	1.313	1.263	0.094	0.064
V012E	11.019	5.519	5.312	5.528	0.753	0.934
V012I	2.804	3.844	3.610	6.566	0.106	0.090
V012K	1.691	1.963	2.479	2.243	0.330	0.321
F014V	0.144	0.165	0.222	0.242	0.003	0.000
L015M	0.902	1.073	1.026	0.901	0.017	0.017
A020S	1.494	2.205	2.822	2.620	0.413	0.397
S022T	3.035	3.788	3.375	3.273	0.684	0.748
L026M	1.482	1.226	2.027	1.704	0.224	0.178
K028R	0.944	0.845	1.043	0.925	0.112	0.095
F029R	1.195	1.511	1.848	1.839	0.140	0.140
F029S	3.019	3.615	3.566	3.521	0.250	0.283
F029T	1.451	1.712	1.839	2.065	0.220	0.212
P032C	0.370	0.419	0.476	0.534	0.006	0.040
L033G	0.566	0.700	0.686	0.627	0.001	0.026
D034W	0.340	0.321	0.499	0.471	0.076	0.069
M035V	0.887	0.639	0.721	0.652	0.116	0.023
S036H	1.109	0.752	1.178	1.135	0.117	0.026
S036N	0.797	0.933	0.893	0.859	0.171	0.260
L037M	0.574	0.404	0.455	0.353	0.049	0.032
F040L	2.603	3.941	3.515	4.148	0.277	0.361
I046L	3.027	2.959	4.011	3.342	0.513	0.557
N047D	2.222	2.359	2.573	2.639	0.032	0.021
N047W	0.404	0.415	0.423	0.456	0.000	0.017
A048N	12.398	45.971	14.252	23.873	0.797	0.902
T049R	7.893	13.334	9.685	12.102	0.563	0.649
G050D	3.287	3.148	3.084	3.020	0.242	0.264
G050M	1.763	2.333	2.780	3.244	0.250	0.393
G052N	7.217	9.809	6.939	13.978	1.109	1.083
G052T	1.542	1.224	1.795	1.433	0.381	0.463
G052S	2.152	1.999	2.120	1.963	0.498	0.566
V058C	1.428	1.312	1.321	1.301	0.212	0.210
V058K	28.000	28.000	61.016	61.016	23.586	23.586
V058R	5.719	4.688	5.542	4.822	3.134	3.149
V058N	1.200	1.175	1.550	1.525	0.200	0.175
V058Y	1.040	0.770	1.071	1.088	0.388	0.454
V058Q	11.956	15.363	18.458	45.092	1.567	2.166
V058P	3.360	2.949	2.799	5.121	0.592	0.884
V058H	3.790	5.074	7.590	9.222	0.826	1.205

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D068P	0.215	0.215	0.213	0.180	0.001	0.184
S069T	1.927	2.179	2.671	2.671	0.289	0.240
I070P	1.284	1.593	1.306	1.589	0.010	0.032
I070V	1.818	2.437	3.099	3.335	0.433	0.363
V073Q	4.846	5.441	5.880	5.827	0.383	0.477
V073R	0.522	0.803	0.720	0.804	0.018	0.059
T074E	2.903	3.834	3.868	3.871	0.666	0.626
T074M	0.569	0.744	0.656	0.771	0.079	0.083
T074N	2.792	1.905	2.565	2.995	0.281	0.204
T074P	2.331	1.593	2.525	2.648	0.309	0.265
T074R	0.999	0.820	0.806	1.066	0.060	0.023
T074V	1.186	1.280	1.365	1.460	0.101	0.080
V075M	0.917	1.087	1.233	1.321	0.003	0.028
K082L	1.362	1.311	1.563	3.302	0.325	0.354
K082N	3.202	3.411	3.396	3.244	0.792	0.861
I083V	3.706	2.633	5.194	3.615	1.552	1.017
I083Q	2.376	1.946	2.665	3.674	0.720	0.510
I083S	0.841	1.054	0.880	1.005	0.235	0.268
I083G	2.276	2.443	2.418	1.866	0.545	0.601
S084E	1.470	1.484	1.834	1.683	0.115	0.115
S084F	1.179	1.212	0.982	1.103	0.025	0.000
S084N	2.255	1.888	3.268	2.476	0.597	0.547
S084R	8.534	14.779	10.230	30.016	1.117	1.494
Q086A	2.084	2.120	2.845	3.310	0.405	0.322
Q086H	1.187	1.000	1.218	1.296	0.087	0.065
Q086K	0.127	0.110	0.126	0.072	0.032	0.023
Q086S	2.528	2.082	2.539	2.149	0.173	0.241
Q086T	3.018	2.542	2.832	4.562	0.290	0.406
D087G	2.755	2.176	2.252	1.971	0.034	0.122
D087L	2.070	2.277	2.195	2.311	0.324	0.299
D087M	2.262	2.325	2.510	2.038	0.191	0.335
D087S	5.210	10.305	6.983	14.399	0.569	0.928
D087V	1.361	1.364	1.553	1.187	0.142	0.189
D090E	8.251	12.299	7.666	19.836	1.093	1.234
D090N	2.812	2.775	3.123	2.737	0.379	0.290
K093Q	2.491	2.065	2.267	1.971	0.132	0.131
K093R	2.986	2.862	3.094	2.842	0.362	0.465
K094D	2.393	2.088	2.071	2.132	0.135	0.211
K094R	1.407	1.542	1.764	1.676	0.158	0.166
T097C	0.330	0.618	0.545	0.505	0.044	0.087
T097D	0.520	0.565	0.643	0.664	0.055	0.073
T097E	1.096	1.410	1.394	1.623	0.217	0.262
T097L	0.899	1.198	1.065	1.241	0.246	0.300
N104R	2.508	2.356	2.876	2.790	0.279	0.238
A120H	2.155	2.551	2.028	2.883	0.168	0.199
D127R	0.264	0.339	0.149	0.199	0.105	0.068
V128I	3.120	3.313	3.546	3.401	0.389	0.504
N131M	15.335	20.678	27.143	15.899	0.505	0.447
N131R	8.195	8.748	7.724	8.392	1.645	1.626
N131V	1.656	1.870	2.280	1.962	0.233	0.214
R132L	3.306	3.235	3.259	2.966	0.337	0.430
Q138L	1.494	1.660	1.611	1.521	0.410	0.347

Q140K	2.829	4.065	4.996	4.464	0.546	0.559
N141R	1.290	1.320	1.334	1.527	0.058	0.035
N141S	2.201	2.708	2.900	2.966	0.135	0.164
N141W	1.475	1.568	1.927	1.643	0.100	0.105
V142D	2.552	2.186	2.914	3.193	0.128	0.067
V142G	1.357	1.796	1.597	1.621	0.211	0.219
V142K	3.532	2.381	3.867	3.681	0.571	0.575
V142N	0.432	0.567	0.672	0.589	0.103	0.087
V142P	4.624	7.213	7.722	7.021	1.074	1.081
V142Q	5.090	6.900	7.618	6.897	0.678	0.678
V142R	1.968	2.595	2.941	2.689	0.364	0.330
V142S	2.789	2.988	4.763	3.497	0.416	0.591
V142T	1.926	3.260	4.313	4.031	0.495	0.472
Q143G	3.922	4.903	5.632	4.846	0.782	0.780
Q143K	3.634	3.671	7.285	5.008	1.043	1.039
L144R	3.810	4.581	5.191	5.107	0.556	0.520
L144T	1.496	1.681	1.941	1.831	0.285	0.219
L146P	0.818	0.782	0.954	0.904	0.011	0.031
T147S	0.984	1.149	1.399	1.497	0.055	0.039
T150N	0.442	0.585	0.622	0.684	0.039	0.046
T150S	1.747	1.400	1.875	1.988	0.120	0.121
E151A	2.870	2.269	2.965	2.860	0.359	0.337
E151L	3.365	3.289	4.446	4.007	0.218	0.251
E151S	5.187	4.591	5.987	6.262	0.371	0.294
E151T	2.442	3.000	3.134	3.309	0.000	0.000
E151V	3.998	4.247	4.459	4.232	0.326	0.314
E151W	7.166	14.248	11.352	13.524	0.131	0.121
K152T	1.204	1.377	1.796	1.883	0.100	0.067
K152W	2.084	1.795	2.549	2.406	0.063	0.069
E158S	0.339	0.397	0.451	0.407	0.000	0.000
K162E	0.168	0.195	0.114	0.080	0.004	0.024
L165F	4.775	5.250	5.075	5.075	0.600	0.725
V166Q	1.883	2.507	2.937	2.958	0.392	0.324
V166T	0.993	1.315	1.821	1.800	0.231	0.235
E167D	0.811	0.910	1.109	1.480	0.111	0.056
I169L	1.812	1.796	2.540	2.196	0.335	0.341
K170R	1.578	2.054	2.536	1.995	0.209	0.201
G172A	0.413	0.581	0.692	0.777	0.052	0.056
K173R	1.654	1.551	1.766	2.083	0.173	0.156
L174G	0.184	0.087	0.210	0.230	0.026	0.031
L174N	1.616	2.276	2.494	2.872	0.331	0.543
L174T	0.552	0.566	0.689	0.820	0.090	0.050
N178K	2.931	4.375	4.891	4.513	0.258	0.362
N178R	8.160	13.820	16.287	20.033	0.665	0.790
H193Q	1.060	1.367	2.264	1.888	0.346	0.346
K195T	1.227	0.806	1.548	1.911	0.348	0.292
K195N	1.266	1.437	1.649	1.385	0.369	0.353
K196E	0.732	0.660	0.663	1.017	0.244	0.239
K196R	2.246	2.285	2.383	2.174	0.315	0.384
F204P	3.500	4.550	2.925	3.750	2.475	4.725
N205A	0.515	0.837	0.717	0.854	0.153	0.160
N205E	1.011	2.004	1.627	1.870	0.314	0.346

N205L	1.084	1.029	1.165	0.000	0.123	0.088
N205T	0.295	0.367	0.428	0.406	0.043	0.053
V206I	0.317	0.508	0.600	0.565	0.079	0.088
K209R	2.041	2.453	2.445	1.951	0.291	0.077
D212N	5.568	4.549	6.271	6.016	0.167	0.322
D212S	1.987	1.502	2.442	2.222	0.204	0.152
D213A	0.235	0.283	0.432	0.438	0.116	0.060
D213M	1.664	2.080	2.650	2.046	0.181	0.142
S215H	2.448	3.056	2.670	2.414	0.268	0.139
S215M	1.497	2.175	2.618	1.630	0.110	0.146
N219I	0.338	0.250	0.860	0.728	0.076	0.082
E220V	3.783	3.828	4.993	4.349	0.371	0.257
T222G	3.528	5.262	5.399	5.549	0.033	0.044
T232F	0.539	1.242	0.716	0.781	0.089	0.153
Q233G	0.041	0.095	0.115	0.121	0.000	0.000
Q234M	6.029	6.031	5.764	4.871	1.286	0.988
S235A	0.550	0.502	0.714	0.607	0.079	0.073
V237C	0.623	0.708	0.860	0.824	0.000	0.000
V237H	0.303	0.316	0.370	0.459	0.046	0.034
V237T	0.152	0.196	0.254	0.247	0.054	0.053
A238E	2.050	1.800	1.945	2.559	0.159	0.171
A238H	0.579	0.363	0.345	0.743	0.090	0.062
T240A	1.107	0.900	1.564	1.302	0.143	0.118
T240Q	0.333	0.510	0.542	0.617	0.080	0.085
R248A	2.274	2.499	2.575	3.115	0.027	0.075
E249V	3.001	3.894	4.284	4.325	0.655	0.712
P257G	3.981	4.452	4.985	5.022	0.039	0.034
K260M	0.719	0.960	0.839	0.935	0.072	0.068
S261A	3.253	3.117	1.872	2.686	1.264	1.451
S261K	6.089	5.421	9.860	6.297	1.583	1.437
S261N	14.149	40.257	20.219	14.303	2.115	1.917
A267T	0.052	0.095	0.102	0.106	0.036	0.041
F273H	0.340	0.436	0.417	0.519	0.025	0.031
F273Y	0.558	0.505	0.668	0.519	0.052	0.050
Q276H	2.706	1.877	2.027	1.997	0.181	0.201
Q276M	0.775	0.768	0.762	0.806	0.043	0.000
Q276R	6.080	9.717	7.383	14.593	0.807	1.281
Q276S	1.353	1.212	1.497	1.681	0.149	0.147
V277A	1.202	1.643	1.692	2.129	0.118	0.110
V277E	2.440	2.340	4.289	4.577	0.161	0.239
V277H	5.548	5.302	7.181	7.300	0.227	0.512
V277K	8.950	8.996	33.627	33.627	4.442	4.045
V277M	1.279	1.622	1.754	1.818	0.264	0.270
V277N	14.351	4.306	12.865	11.772	0.938	0.796
V277Q	5.459	5.461	6.547	6.343	0.373	0.493
V277R	18.300	12.038	17.581	20.641	2.737	2.023
V277S	14.351	10.444	9.509	15.135	0.727	0.716
V277T	8.412	7.804	8.497	11.184	0.679	0.871
L278E	4.416	2.795	3.330	2.800	0.170	0.202
L278G	7.502	7.456	9.173	7.760	0.596	0.612
K279H	0.888	1.087	1.234	1.339	0.185	0.269
V287T	0.580	0.667	0.843	0.832	0.139	0.100

T289S	0.783	1.019	0.819	1.001	0.008	0.007
G291S	0.227	0.322	0.419	0.385	0.051	0.016
G291V	3.662	3.707	4.131	5.599	0.821	0.706
E292C	1.344	1.599	1.711	1.617	0.138	0.144
E292F	6.106	4.697	8.422	6.216	0.520	0.363
E292H	2.620	3.316	4.458	3.830	0.389	0.451
E292R	2.810	2.178	3.155	2.829	0.398	0.339
E292V	0.891	1.121	1.453	1.494	0.193	0.177
T293A	1.986	3.110	2.546	1.789	0.086	0.076
A298G	0.161	0.274	0.342	0.236	0.030	0.022
L307G	0.616	0.661	0.726	0.605	0.000	0.000
S308D	0.264	0.325	0.337	0.344	0.014	0.010
S308K	0.651	0.722	0.826	0.716	0.011	0.000
S308N	3.995	4.406	6.808	6.128	0.386	0.362
I309E	3.166	2.819	3.921	3.663	0.637	0.528
I309G	6.651	5.429	6.824	6.194	0.503	0.400
I309L	0.326	0.403	0.501	0.431	0.048	0.047
I309M	2.809	2.473	3.467	3.383	0.278	0.239
I309N	4.865	5.191	5.444	5.054	0.380	0.327
I309S	10.719	28.759	18.217	158.604	0.748	1.367
I309T	3.052	2.509	2.989	3.735	0.228	0.207
I309V	1.705	1.292	1.929	1.787	0.029	0.062
M310G	4.514	6.397	7.568	7.084	0.866	0.915
M310Q	3.648	3.179	3.912	3.380	1.088	0.955
M313G	0.252	0.325	0.348	0.355	0.034	0.036
M313H	3.767	5.276	10.243	10.395	0.380	0.404
M313K	12.689	12.122	15.085	12.984	0.129	0.072
M313P	4.050	2.951	4.198	3.919	0.209	0.177
M313R	4.634	10.863	7.288	3.568	0.337	0.296
M313T	2.903	4.474	4.705	4.467	0.331	0.313
M313Y	1.063	1.262	1.276	1.300	0.096	0.089
K314S	2.848	4.450	4.042	5.879	0.391	0.533
K314Y	0.093	0.131	0.226	0.182	0.013	0.020
S315A	1.472	1.082	1.345	1.484	0.222	0.148
S315H	2.412	3.242	3.648	3.414	0.440	0.371
S315Y	0.279	0.626	0.477	0.362	0.146	0.143
L317A	3.254	2.845	4.019	3.776	0.280	0.317
L317I	1.078	1.524	2.021	1.687	0.257	0.180
L317K	12.129	9.382	11.668	12.591	0.402	0.445
L317N	2.907	3.066	3.703	3.717	0.445	0.540
L317R	8.631	15.187	20.585	15.106	0.796	0.857
L317S	11.586	29.267	10.535	25.114	1.637	1.613
L317T	1.338	1.073	1.953	1.656	0.136	0.018
L317W	0.810	1.128	1.326	1.665	0.158	0.171
L318D	1.750	1.970	1.847	1.930	0.322	0.322
L318H	1.073	0.806	1.072	1.005	0.046	0.074
L318R	2.856	3.464	4.583	4.187	0.258	0.260
N321R	3.069	4.409	5.059	4.946	0.482	0.426
N321S	0.683	0.710	0.700	0.772	0.058	0.035
E324N	4.309	2.530	4.508	3.321	0.348	0.303
T325E	1.071	1.270	1.337	1.352	0.193	0.143
N328G	0.379	0.504	0.747	0.553	0.031	0.040

N328Y	2.629	4.543	4.758	4.543	0.490	0.477
T335S	0.905	0.787	0.977	0.986	0.113	0.062
Q347A	8.316	11.961	8.432	11.508	0.918	1.266
Q347G	1.358	1.120	3.021	2.319	0.253	0.209
Q349M	1.493	1.629	1.486	1.760	0.178	0.217
Q349R	0.451	0.572	0.663	0.598	0.078	0.079
V351S	1.379	1.633	1.804	1.647	0.000	0.000
I353V	2.335	1.954	3.090	2.697	0.323	0.321
N356H	0.445	0.451	0.445	0.588	0.038	0.023
N356S	0.262	0.253	0.136	0.318	0.000	0.008
S359E	2.616	2.635	3.547	3.560	0.382	0.333
S359H	0.403	0.371	0.445	0.374	0.000	0.000
P367A	0.643	0.782	1.074	0.996	0.139	0.131
P367G	0.593	0.530	0.686	0.650	0.000	0.000
P367K	0.707	0.767	0.890	0.513	0.045	0.052
P367S	3.967	3.478	2.946	3.073	0.424	0.505
D368A	1.762	2.321	2.143	1.895	0.031	0.040
D368E	3.464	4.944	5.772	4.842	0.530	0.555
D368L	0.557	0.566	0.607	0.619	0.000	0.006
D368M	0.861	1.065	1.031	1.104	0.028	0.028
D368R	4.503	5.270	7.418	6.226	0.754	0.735
D368T	2.345	1.993	2.512	2.525	0.072	0.085
N369R	1.548	2.719	2.503	2.022	0.160	0.125
A371F	2.760	5.207	4.974	3.980	0.308	0.222
A371H	8.101	86.587	77.531	77.531	1.403	1.316
A371H	3.509	4.058	3.900	3.879	0.000	0.334
A371K	2.903	3.546	3.963	4.055	0.509	0.505
A371L	11.018	40.668	76.587	43.516	1.159	0.964
A371L	3.328	3.445	3.472	2.075	0.000	0.025
A371R	25.855	25.855	n/a	n/a	2.851	3.634
A371R	6.592	7.733	7.987	7.576	0.000	0.196
A371S	3.329	3.505	4.916	4.611	0.412	0.781
L374P	2.939	7.129	11.522	8.771	0.665	0.646
E375A	0.627	0.507	0.557	0.683	0.000	0.014
E375G	1.596	1.299	2.025	1.806	0.209	0.265
E375R	0.937	1.132	1.529	1.318	0.201	0.260
K376D	0.458	0.312	0.518	0.515	0.064	0.026
K376E	1.572	1.094	1.572	1.674	0.213	0.174
K376Q	0.727	0.940	0.910	0.846	0.116	0.102
K376R	2.086	1.351	1.704	2.690	0.539	0.279
K376T	0.847	1.001	1.026	1.135	0.153	0.064
K376V	0.834	0.861	1.036	1.021	0.033	0.026
K376Y	1.316	0.777	1.353	0.747	0.125	0.097
G377D	1.159	1.332	1.285	1.763	0.202	0.186
G377E	0.877	0.926	1.144	1.189	0.092	0.088
G377H	3.037	3.432	4.460	3.598	0.372	0.364
G377K	3.445	4.101	6.405	4.911	0.283	0.245
G377R	1.096	1.257	1.312	1.191	0.077	0.085
G377S	0.453	0.452	0.492	0.457	0.034	0.036
G377T	2.198	2.313	2.474	2.522	0.424	0.461
F380W	17.497	27.987	25.734	29.353	2.566	2.716
T381S	2.861	3.161	3.886	3.558	0.521	0.367

R383I	1.959	6.936	10.340	6.820	0.655	0.513
R383S	2.429	2.548	3.228	3.044	0.339	0.321
K385A	0.479	0.669	0.604	0.754	0.028	0.000
K385Q	1.746	2.089	2.403	2.609	0.217	0.196
K385V	1.232	1.750	1.387	1.410	0.071	0.042
E389A	6.872	10.944	21.081	24.610	0.449	0.449
E389G	0.166	0.203	0.188	0.284	0.004	0.000
E389L	1.814	2.142	2.598	2.403	0.370	0.303
E389Q	2.547	3.432	3.459	3.423	0.411	0.437
E389S	1.847	2.640	3.059	2.456	0.000	0.007
E392A	1.797	1.370	2.021	2.133	0.147	0.136
E392F	1.575	1.407	1.821	2.023	0.071	0.079
E392Q	5.826	4.653	6.583	4.364	0.693	0.729
E392R	4.555	5.306	5.900	6.548	0.218	0.193
E392V	3.817	2.936	4.747	4.544	0.367	0.291
Q393F	1.754	2.186	2.455	2.222	0.260	0.226
Q393M	1.252	1.826	1.749	1.588	0.028	0.049
S395A	4.220	6.127	8.788	6.906	1.141	0.856
S395H	1.609	2.261	2.574	2.564	0.323	0.268
E396A	1.135	1.184	1.497	1.524	0.126	0.149
E396H	0.357	0.532	0.751	0.684	0.069	0.022
E396Q	1.310	1.625	1.611	1.559	0.162	0.160
E396S	3.375	5.709	5.274	6.380	0.146	0.129
Y399T	2.538	3.250	3.313	3.989	0.000	0.002
Y399V	2.738	2.697	3.028	3.129	0.484	0.557
Y399W	1.400	1.883	1.715	1.946	0.236	0.233
S401A	2.636	3.171	3.216	3.148	0.447	0.410
S401E	1.685	1.601	2.110	2.060	0.344	0.309
S404A	1.288	1.635	1.924	1.724	0.000	0.019
L406F	0.706	0.490	0.867	0.716	0.000	0.000
L406N	0.617	0.795	0.943	1.044	0.060	0.070
S407A	2.428	2.949	3.432	3.255	0.389	0.548
S407D	2.090	5.790	5.038	5.682	0.569	0.575
S407P	2.660	2.708	3.812	3.301	0.261	0.366
A412Q	2.001	2.918	2.925	2.902	0.279	0.247
A412R	4.562	5.132	6.390	6.347	0.570	0.596
A412V	2.581	3.451	3.789	3.511	0.189	0.189
D416L	0.610	0.817	0.737	1.043	0.130	0.160
D418R	4.541	4.847	5.347	5.438	0.406	0.583
A419H	10.409	20.311	25.109	38.221	2.214	2.293
A419K	12.835	10.298	24.536	208.289	2.556	3.173
D421A	5.968	5.617	6.094	16.940	0.761	0.764
D421H	48.012	48.012	160.106	32.481	16.300	28.113
D421K	5.527	5.225	6.864	5.346	0.523	0.725
D421N	9.060	8.635	10.039	8.645	1.502	1.422
D421Q	7.529	5.581	7.858	8.016	0.842	0.994
D421R	6.637	5.463	9.211	7.537	0.815	0.737
D421S	5.556	5.355	7.899	8.898	0.869	0.762
A425G	10.421	8.827	7.796	10.676	0.827	1.189
G427Q	1.008	1.252	1.342	1.230	0.031	0.106
G427T	1.330	1.380	1.664	1.643	0.080	0.065
V428L	2.138	2.769	2.930	3.029	0.053	0.030

D431E	2.810	2.220	1.972	2.112	0.519	0.438
D431H	2.154	3.185	4.017	3.028	0.294	0.301
D431K	8.123	16.953	19.563	11.575	2.272	2.339
D431L	1.211	1.215	1.564	1.448	0.164	0.170
D431N	11.819	12.063	16.358	15.131	1.601	1.399
D431Q	6.077	9.828	14.157	10.760	1.533	1.153
D431S	14.523	10.220	11.338	9.075	0.853	0.829
F433A	4.035	4.673	5.943	4.649	0.581	0.595
F433H	1.836	2.397	2.574	2.108	0.347	0.356
F433I	2.754	2.643	2.990	2.299	0.338	0.382
F433K	17.815	14.495	16.240	49.615	1.806	1.790
F433R	8.198	6.719	10.572	8.960	1.113	0.857
F433T	6.005	5.941	9.716	8.019	1.327	1.542
F433V	10.645	7.762	150.315	8.696	2.415	1.505
F433W	0.526	0.795	0.784	0.903	0.082	0.068
P437I	0.759	0.996	1.130	1.066	0.027	0.019
M438A	1.996	1.518	2.125	2.060	0.214	0.210
M438D	2.849	2.522	3.002	2.857	0.305	0.074
M438E	4.681	4.992	5.386	5.680	0.431	0.518
M438L	10.127	5.268	6.663	11.324	0.670	0.739
M438N	6.172	5.531	8.050	5.568	0.649	0.662
M438T	2.218	2.411	2.308	2.500	0.309	0.304
E439A	3.557	4.432	4.883	4.235	0.568	0.596
E439A	1.099	0.998	1.694	1.470	0.080	0.109
E439C	0.148	0.256	0.286	0.286	0.042	0.045
E439K	0.466	0.588	0.580	0.616	0.077	0.065
E439P	2.868	3.736	3.394	3.267	0.529	0.490
E439Q	1.070	0.848	1.087	1.080	0.116	0.115
E439T	1.965	1.889	2.179	2.323	0.313	0.263
T440D	4.148	4.443	4.931	3.533	0.568	0.651
T440H	2.317	1.982	3.297	2.595	0.147	0.196
T440M	3.397	3.305	2.878	2.873	0.254	0.367
T440P	3.562	3.593	3.987	3.277	0.540	0.566
T440S	2.522	2.207	2.533	2.895	0.283	0.284
E441F	1.402	1.407	1.813	1.560	0.204	0.178
E442G	2.871	3.340	3.193	3.347	0.327	0.367
P443E	0.907	0.710	0.856	0.928	0.044	0.063
P443F	1.830	2.370	2.683	2.321	0.301	0.286
P443G	4.077	2.921	9.751	4.614	0.835	0.756
Q444E	8.293	3.861	6.800	6.213	0.581	0.594
Q444H	3.823	3.936	5.746	4.710	0.486	0.513
Q444V	2.193	2.107	2.847	2.583	0.384	0.284
I445M	5.265	4.438	4.480	4.489	0.773	0.691
I445N	3.375	4.024	3.592	3.515	0.499	0.455
I445W	2.289	2.694	2.683	2.695	0.314	0.296
Y447E	2.373	2.464	2.363	2.685	0.391	0.345
Y447G	0.945	1.352	1.358	1.401	0.187	0.162
Y447P	0.991	1.383	1.379	1.490	0.190	0.183
positive control (OHO)	2.919	2.173	2.773	2.105	0.145	0.178
	3.984	4.463	4.215	4.823	0.189	0.253
	3	2.725	3	3.325	0.1	0.125
	2.501	2.883	2.370	3.158	0.452	0.522

	7.629	2.989	10.835	3.914	0.485	0.219
	5.783	5.356	2.609	3.643	0.542	0.402
	5.279	5.422	2.815	4.026	0.618	0.401
	4.775	4.385	2.845	3.327	0.718	0.540
	3.617	4.264	3.322	3.427	0.633	0.479
	5.881	4.511	5.518	4.359	0.743	0.848
	6.754	4.932	3.902	4.120	0.665	0.724
	3.911	3.494	3.911	5.179	0.726	0.841
	5.406	7.559	4.018	4.620	0.735	0.429
	4.015	3.887	3.9400	3.4080	0.3340	0.3410
	2.604	2.339	2.4430	2.3910	0.2350	0.2330
	3.736	3.473	3.6210	3.0560	0.3100	0.2770
	3.759	3.509	3.6330	3.0490	0.3600	0.3030

n/a (not available; *e.g.* beyond detection limit)

TABLE 12: Percent (%) Activity

	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
L001A	117.908	8.13	9.59	127.997	9.179	11.75
L001E	107.231	13.14	14.09	125.207	10.727	13.43
L001G	171.264	9.23	15.80	115.952	4.586	5.32
L001Q	119.435	10.13	12.09	88.763	11.121	9.87
L001R	117.366	6.96	8.17	160.410	5.623	9.02
P006A	137.875	9.88	13.63	108.946	12.446	13.56
V008M	134.884	0.00	0.00	119.772	0.477	0.57
I009Q	104.922	6.61	6.94	124.934	6.303	7.87
P010G	109.772	15.00	16.47	121.986	16.570	20.21
P010H	131.924	0.00	0.00	112.990	0.000	0.00
N011S	152.320	7.16	10.90	131.289	5.067	6.65
V012E	48.208	14.18	6.83	100.163	16.896	16.92
V012I	128.745	2.94	3.78	170.812	1.371	2.34
V012K	146.600	13.31	19.52	114.264	14.311	16.35
F014V	154.167	1.35	2.08	146.667	0.000	0.00
L015M	113.747	1.66	1.88	83.970	1.887	1.58
A020S	188.889	14.64	27.64	118.821	15.153	18.00
S022T	111.203	20.27	22.54	86.404	22.854	19.75
L026M	136.775	11.05	15.11	138.989	10.446	14.52
K028R	110.487	10.74	11.86	109.467	10.270	11.24
F029R	154.644	7.58	11.72	121.707	7.613	9.27
F029S	118.119	7.01	8.28	97.400	8.037	7.83
F029T	126.740	11.96	15.16	120.619	10.266	12.38
P032C	128.649	1.26	1.62	127.446	7.491	9.55
L033G	121.201	0.15	0.18	89.571	4.147	3.71
D034W	146.765	15.23	22.35	146.729	14.650	21.50
M035V	81.285	16.09	13.08	102.034	3.528	3.60
S036H	106.222	9.93	10.55	150.931	2.291	3.46
S036N	112.045	19.15	21.46	92.069	30.268	27.87
L037M	79.268	10.77	8.54	87.376	9.065	7.92
F040L	135.036	7.88	10.64	105.252	8.703	9.16
I046L	132.507	12.79	16.95	112.944	16.667	18.82

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
N047D	115.797	1.24	1.44	111.869	0.796	0.89
N047W	104.703	0.00	0.00	109.880	3.728	4.10
A048N	114.954	5.59	6.43	51.931	3.778	1.96
T049R	122.704	5.81	7.13	90.760	5.363	4.87
G050D	93.824	7.85	7.36	95.934	8.742	8.39
G050M	157.686	8.99	14.18	139.048	12.115	16.85
G052N	96.148	15.98	15.37	142.502	7.748	11.04
G052T	116.407	21.23	24.71	117.075	32.310	37.83
G052S	98.513	23.49	23.14	98.199	28.833	28.31
V058C	92.507	16.05	14.85	99.162	16.141	16.01
V058K	217.914	38.66	84.24	217.914	38.655	84.24
V058R	96.905	56.55	54.80	102.858	65.305	67.17
V058N	129.167	12.90	16.67	129.787	11.475	14.89
V058Y	102.981	36.23	37.31	141.299	41.728	58.96
V058Q	154.383	8.49	13.11	293.510	4.804	14.10
V058P	83.304	21.15	17.62	173.652	17.262	29.98
V058H	200.264	10.88	21.79	181.750	13.067	23.75
D068P	99.070	0.47	0.47	83.721	102.222	85.58
S069T	138.609	10.82	15.00	122.579	8.985	11.01
I070P	101.713	0.77	0.78	99.749	2.014	2.01
I070V	170.462	13.97	23.82	136.849	10.885	14.90
V073Q	121.337	6.51	7.90	107.094	8.186	8.77
V073R	137.931	2.50	3.45	100.125	7.338	7.35
T074E	133.241	17.22	22.94	100.965	16.172	16.33
T074M	115.290	12.04	13.88	103.629	10.765	11.16
T074N	91.870	10.96	10.06	157.218	6.811	10.71
T074P	108.323	12.24	13.26	166.227	10.008	16.64
T074R	80.681	7.44	6.01	130.000	2.158	2.80
T074V	115.093	7.40	8.52	114.063	5.479	6.25
V075M	134.460	0.24	0.33	121.527	2.120	2.58
K082L	114.758	20.79	23.86	251.869	10.721	27.00
K082N	106.059	23.32	24.73	95.104	26.541	25.24
I083V	140.151	29.88	41.88	137.296	28.133	38.63
I083Q	112.163	27.02	30.30	188.798	13.881	26.21
I083S	104.637	26.70	27.94	95.351	26.667	25.43
I083G	106.239	22.54	23.95	76.381	32.208	24.60
S084E	124.762	6.27	7.82	113.410	6.833	7.75
S084F	83.291	2.55	2.12	91.007	0.000	0.00
S084N	144.922	18.27	26.47	131.144	22.092	28.97
S084R	119.873	10.92	13.09	203.099	4.977	10.11
Q086A	136.516	14.24	19.43	156.132	9.728	15.19
Q086H	102.612	7.14	7.33	129.600	5.015	6.50
Q086K	99.213	25.40	25.20	65.455	31.944	20.91
Q086S	100.435	6.81	6.84	103.218	11.215	11.58
Q086T	93.837	10.24	9.61	179.465	8.900	15.97
D087G	81.742	1.51	1.23	90.579	6.190	5.61
D087L	106.039	14.76	15.65	101.493	12.938	13.13

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
D087M	110.964	7.61	8.44	87.656	16.438	14.41
D087S	134.031	8.15	10.92	139.728	6.445	9.01
D087V	114.107	9.14	10.43	87.023	15.922	13.86
D090E	92.910	14.26	13.25	161.281	6.221	10.03
D090N	111.060	12.14	13.48	98.631	10.596	10.45
K093Q	91.008	5.82	5.30	95.448	6.646	6.34
K093R	103.617	11.70	12.12	99.301	16.362	16.25
K094D	86.544	6.52	5.64	102.107	9.897	10.11
K094R	125.373	8.96	11.23	108.690	9.905	10.77
T097C	165.152	8.07	13.33	81.715	17.228	14.08
T097D	123.654	8.55	10.58	117.522	10.994	12.92
T097E	127.190	15.57	19.80	115.106	16.143	18.58
T097L	118.465	23.10	27.36	103.589	24.174	25.04
N104R	114.673	9.70	11.12	118.421	8.530	10.10
A120H	94.107	8.28	7.80	113.015	6.903	7.80
D127R	56.439	70.47	39.77	58.702	34.171	20.06
V128I	113.654	10.97	12.47	102.656	14.819	15.21
N131M	177.000	1.86	3.29	76.888	2.811	2.16
N131R	94.253	21.30	20.07	95.930	19.376	18.59
N131V	137.681	10.22	14.07	104.920	10.907	11.44
R132L	98.578	10.34	10.19	91.685	14.498	13.29
Q138L	107.831	25.45	27.44	91.627	22.814	20.90
Q140K	176.600	10.93	19.30	109.815	12.522	13.75
N141R	103.411	4.35	4.50	115.682	2.292	2.65
N141S	131.758	4.66	6.13	109.527	5.529	6.06
N141W	130.644	5.19	6.78	104.783	6.391	6.70
V142D	114.185	4.39	5.02	146.066	2.098	3.06
V142G	117.686	13.21	15.55	90.256	13.510	12.19
V142K	109.485	14.77	16.17	154.599	15.621	24.15
V142N	155.556	15.33	23.84	103.880	14.771	15.34
V142P	166.998	13.91	23.23	97.338	15.397	14.99
V142Q	149.666	8.90	13.32	99.957	9.830	9.83
V142R	149.441	12.38	18.50	103.622	12.272	12.72
V142S	170.778	8.73	14.92	117.035	16.900	19.78
V142T	223.936	11.48	25.70	123.650	11.709	14.48
Q143G	143.600	13.88	19.94	98.837	16.096	15.91
Q143K	200.468	14.32	28.70	136.421	20.747	28.30
L144R	136.247	10.71	14.59	111.482	10.182	11.35
L144T	129.746	14.68	19.05	108.923	11.961	13.03
L146P	116.626	1.15	1.34	115.601	3.429	3.96
T147S	142.175	3.93	5.59	130.287	2.605	3.39
T150N	140.724	6.27	8.82	116.923	6.725	7.86
T150S	107.327	6.40	6.87	142.000	6.087	8.64
E151A	103.310	12.11	12.51	126.047	11.783	14.85
E151L	132.125	4.90	6.48	121.830	6.264	7.63
E151S	115.423	6.20	7.15	136.397	4.695	6.40
E151T	128.337	0.00	0.00	110.300	0.000	0.00

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
E151V	111.531	7.31	8.15	99.647	7.420	7.39
E151W	158.415	1.15	1.83	94.919	0.895	0.85
K152T	149.169	5.57	8.31	136.747	3.558	4.87
K152W	122.313	2.47	3.02	134.039	2.868	3.84
E158S	133.038	0.00	0.00	102.519	0.000	0.00
K162E	67.857	3.51	2.38	41.026	30.000	12.31
L165F	106.283	11.82	12.57	96.667	14.286	13.81
V166Q	155.975	13.35	20.82	117.990	10.953	12.92
V166T	183.384	12.69	23.26	136.882	13.056	17.87
E167D	136.745	10.01	13.69	162.637	3.784	6.15
I169L	140.177	13.19	18.49	122.272	15.528	18.99
K170R	160.710	8.24	13.24	97.128	10.075	9.79
G172A	167.554	7.51	12.59	133.735	7.207	9.64
K173R	106.771	9.80	10.46	134.300	7.489	10.06
L174G	114.130	12.38	14.13	264.368	13.478	35.63
L174N	154.332	13.27	20.48	126.186	18.907	23.86
L174T	124.819	13.06	16.30	144.876	6.098	8.83
N178K	166.871	5.27	8.80	103.154	8.021	8.27
N178R	199.596	4.08	8.15	144.957	3.943	5.72
H193Q	213.585	15.28	32.64	138.113	18.326	25.31
K195T	126.161	22.48	28.36	237.097	15.280	36.23
K195N	130.253	22.38	29.15	96.381	25.487	24.57
K196E	90.574	36.80	33.33	154.091	23.500	36.21
K196R	106.100	13.22	14.02	95.142	17.663	16.81
F204P	83.571	84.62	70.71	82.418	126.000	103.85
N205A	139.223	21.34	29.71	102.031	18.735	19.12
N205E	160.930	19.30	31.06	93.313	18.503	17.27
N205L	107.472	10.56	11.35	0.000	#DIV/0!	8.55
N205T	145.085	10.05	14.58	110.627	13.054	14.44
V206I	189.274	13.17	24.92	111.220	15.575	17.32
K209R	119.794	11.90	14.26	79.535	3.947	3.14
D212N	112.626	2.66	3.00	132.249	5.352	7.08
D212S	122.899	8.35	10.27	147.936	6.841	10.12
D213A	183.830	26.85	49.36	154.770	13.699	21.20
D213M	159.255	6.83	10.88	98.365	6.940	6.83
S215H	109.069	10.04	10.95	78.992	5.758	4.55
S215M	174.883	4.20	7.35	74.943	8.957	6.71
N219I	254.438	8.84	22.49	291.200	11.264	32.80
E220V	131.985	7.43	9.81	113.610	5.909	6.71
T222G	153.033	0.61	0.94	105.454	0.793	0.84
T232F	132.839	12.43	16.51	62.882	19.590	12.32
Q233G	280.488	0.00	0.00	127.368	0.000	0.00
Q234M	95.605	22.31	21.33	80.766	20.283	16.38
S235A	129.818	11.06	14.36	120.916	12.026	14.54
V237C	138.042	0.00	0.00	116.384	0.000	0.00
V237H	122.112	12.43	15.18	145.253	7.407	10.76
V237T	167.105	21.26	35.53	126.020	21.457	27.04

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
A238E	94.878	8.17	7.76	142.167	6.682	9.50
A238H	59.585	26.09	15.54	204.683	8.345	17.08
T240A	141.283	9.14	12.92	144.667	9.063	13.11
T240Q	162.763	14.76	24.02	120.980	13.776	16.67
R248A	113.237	1.05	1.19	124.650	2.408	3.00
E249V	142.752	15.29	21.83	111.068	16.462	18.28
P257G	125.220	0.78	0.98	112.803	0.677	0.76
K260M	116.690	8.58	10.01	97.396	7.273	7.08
S261A	57.547	67.52	38.86	86.173	54.021	46.55
S261K	161.931	16.05	26.00	116.159	22.820	26.51
S261N	142.901	10.46	14.95	35.529	13.403	4.76
A267T	196.154	35.29	69.23	111.579	38.679	43.16
F273H	122.647	6.00	7.35	119.037	5.973	7.11
F273Y	119.713	7.78	9.32	102.772	9.634	9.90
Q276H	74.908	8.93	6.69	106.393	10.065	10.71
Q276M	98.323	5.64	5.55	104.948	0.000	0.00
Q276R	121.431	10.93	13.27	150.180	8.778	13.18
Q276S	110.643	9.95	11.01	138.696	8.745	12.13
V277A	140.765	6.97	9.82	129.580	5.167	6.70
V277E	175.779	3.75	6.60	195.598	5.222	10.21
V277H	129.434	3.16	4.09	137.684	7.014	9.66
V277K	375.721	13.21	49.63	373.799	12.029	44.96
V277M	137.138	15.05	20.64	112.084	14.851	16.65
V277N	89.645	7.29	6.54	273.386	6.762	18.49
V277Q	119.930	5.70	6.83	116.151	7.772	9.03
V277R	96.071	15.57	14.96	171.465	9.801	16.81
V277S	66.260	7.65	5.07	144.916	4.731	6.86
V277T	101.010	7.99	8.07	143.311	7.788	11.16
L278E	75.408	5.11	3.85	100.179	7.214	7.23
L278G	122.274	6.50	7.94	104.077	7.887	8.21
K279H	138.964	14.99	20.83	123.183	20.090	24.75
V287T	145.345	16.49	23.97	124.738	12.019	14.99
T289S	104.598	0.98	1.02	98.234	0.699	0.69
G291S	184.581	12.17	22.47	119.565	4.156	4.97
G291V	112.807	19.87	22.42	151.039	12.609	19.05
E292C	127.307	8.07	10.27	101.126	8.905	9.01
E292F	137.930	6.17	8.52	132.340	5.840	7.73
E292H	170.153	8.73	14.85	115.501	11.775	13.60
E292R	112.278	12.61	14.16	129.890	11.983	15.56
E292V	163.075	13.28	21.66	133.274	11.847	15.79
T293A	128.197	3.38	4.33	57.524	4.248	2.44
A298G	212.422	8.77	18.63	86.131	9.322	8.03
L307G	117.857	0.00	0.00	91.528	0.000	0.00
S308D	127.652	4.15	5.30	105.846	2.907	3.08
S308K	126.882	1.33	1.69	99.169	0.000	0.00
S308N	170.413	5.67	9.66	139.083	5.907	8.22
I309E	123.847	16.25	20.12	129.940	14.414	18.73

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
I309G	102.601	7.37	7.56	114.091	6.458	7.37
I309L	153.681	9.58	14.72	106.948	10.905	11.66
I309M	123.425	8.02	9.90	136.797	7.065	9.66
I309N	111.901	6.98	7.81	97.361	6.470	6.30
I309S	169.951	4.11	6.98	551.493	0.862	4.75
I309T	97.936	7.63	7.47	148.864	5.542	8.25
I309V	113.138	1.50	1.70	138.313	3.470	4.80
M310G	167.656	11.44	19.18	110.739	12.916	14.30
M310Q	107.237	27.81	29.82	106.323	28.254	30.04
M313G	138.095	9.77	13.49	109.231	10.141	11.08
M313H	271.914	3.71	10.09	197.024	3.886	7.66
M313K	118.882	0.86	1.02	107.111	0.555	0.59
M313P	103.654	4.98	5.16	132.802	4.516	6.00
M313R	157.272	4.62	7.27	32.845	8.296	2.72
M313T	162.074	7.04	11.40	99.844	7.007	7.00
M313Y	120.038	7.52	9.03	103.011	6.846	7.05
K314S	141.924	9.67	13.73	132.112	9.066	11.98
K314Y	243.011	5.75	13.98	138.931	10.989	15.27
S315A	91.372	16.51	15.08	137.153	9.973	13.68
S315H	151.244	12.06	18.24	105.305	10.867	11.44
S315Y	170.968	30.61	52.33	57.827	39.503	22.84
L317A	123.510	6.97	8.60	132.724	8.395	11.14
L317I	187.477	12.72	23.84	110.696	10.670	11.81
L317K	96.199	3.45	3.31	134.204	3.534	4.74
L317N	127.382	12.02	15.31	121.233	14.528	17.61
L317R	238.501	3.87	9.22	99.467	5.673	5.64
L317S	90.929	15.54	14.13	85.810	6.423	5.51
L317T	145.964	6.96	10.16	154.334	1.087	1.68
L317W	163.704	11.92	19.51	147.606	10.270	15.16
L318D	105.543	17.43	18.40	97.970	16.684	16.35
L318H	99.907	4.29	4.29	124.690	7.363	9.18
L318R	160.469	5.63	9.03	120.872	6.210	7.51
N321R	164.842	9.53	15.71	112.180	8.613	9.66
N321S	102.489	8.29	8.49	108.732	4.534	4.93
E324N	104.618	7.72	8.08	131.265	9.124	11.98
T325E	124.837	14.44	18.02	106.457	10.577	11.26
N328G	197.098	4.15	8.18	109.722	7.233	7.94
N328Y	180.981	10.30	18.64	100.000	10.500	10.50
T335S	107.956	11.57	12.49	125.286	6.288	7.88
Q347A	101.395	10.89	11.04	96.213	11.001	10.58
Q347G	222.459	8.37	18.63	207.054	9.013	18.66
Q349M	99.531	11.98	11.92	108.042	12.330	13.32
Q349R	147.007	11.76	17.29	104.545	13.211	13.81
V351S	130.819	0.00	0.00	100.857	0.000	0.00
I353V	132.334	10.45	13.83	138.025	11.902	16.43
N356H	100.000	8.54	8.54	130.377	3.912	5.10
N356S	51.908	0.00	0.00	125.692	2.516	3.16

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
E389A	306.767	2.13	6.53	224.872	1.824	4.10
E389G	113.253	2.13	2.41	139.901	0.000	0.00
E389L	143.219	14.24	20.40	112.185	12.609	14.15
E389Q	135.807	11.88	16.14	99.738	12.767	12.73
E389S	165.620	0.00	0.00	93.030	0.285	0.27
E392A	112.465	7.27	8.18	155.693	6.376	9.93
E392F	115.619	3.90	4.51	143.781	3.905	5.61
E392Q	112.993	10.53	11.89	93.789	16.705	15.67
E392R	129.528	3.69	4.79	123.407	2.947	3.64
E392V	124.365	7.73	9.61	154.768	6.404	9.91
Q393F	139.966	10.59	14.82	101.647	10.171	10.34
Q393M	139.696	1.60	2.24	86.966	3.086	2.68
S395A	208.246	12.98	27.04	112.714	12.395	13.97
S395H	159.975	12.55	20.07	113.401	10.452	11.85
E396A	131.894	8.42	11.10	128.716	9.777	12.58
E396H	210.364	9.19	19.33	128.571	3.216	4.14
E396Q	122.977	10.06	12.37	95.938	10.263	9.85
E396S	156.267	2.77	4.33	111.753	2.022	2.26
Y399T	130.536	0.00	0.00	122.738	0.050	0.06
Y399V	110.592	15.98	17.68	116.018	17.801	20.65
Y399W	122.500	13.76	16.86	103.346	11.973	12.37
S401A	122.003	13.90	16.96	99.275	13.024	12.93
S401E	125.223	16.30	20.42	128.670	15.000	19.30
S404A	149.379	0.00	0.00	105.443	1.102	1.16
L406F	122.805	0.00	0.00	146.122	0.000	0.00
L406N	152.836	6.36	9.72	131.321	6.705	8.81
S407A	141.351	11.33	16.02	110.376	16.836	18.58
S407D	241.053	11.29	27.22	98.135	10.120	9.93
S407P	143.308	6.85	9.81	121.898	11.088	13.52
A412Q	146.177	9.54	13.94	99.452	8.511	8.46
A412R	140.070	8.92	12.49	123.675	9.390	11.61
A412V	146.804	4.99	7.32	101.739	5.383	5.48
D416L	120.820	17.64	21.31	127.662	15.340	19.58
D418R	117.749	7.59	8.94	112.193	10.721	12.03
A419H	241.224	8.82	21.27	188.179	5.999	11.29
A419K	191.165	10.42	19.91	2022.616	1.523	30.81
D421A	102.111	12.49	12.75	301.584	4.510	13.60
D421H	333.471	10.18	33.95	67.652	86.552	58.55
D421K	124.190	7.62	9.46	102.316	13.562	13.88
D421N	110.806	14.96	16.58	100.116	16.449	16.47
D421Q	104.370	10.72	11.18	143.630	12.400	17.81
D421R	138.783	8.85	12.28	137.964	9.778	13.49
D421S	142.171	11.00	15.64	166.162	8.564	14.23
A425G	74.810	10.61	7.94	120.947	11.137	13.47
G427Q	133.135	2.31	3.08	98.243	8.618	8.47
G427T	125.113	4.81	6.02	119.058	3.956	4.71
V428L	137.044	1.81	2.48	109.390	0.990	1.08

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
S359E	135.589	10.77	14.60	135.104	9.354	12.64
S359H	110.422	0.00	0.00	100.809	0.000	0.00
P367A	167.030	12.94	21.62	127.366	13.153	16.75
P367G	115.683	0.00	0.00	122.642	0.000	0.00
P367K	125.884	5.06	6.36	66.884	10.136	6.78
P367S	74.263	14.39	10.69	88.355	16.433	14.52
D368A	121.623	1.45	1.76	81.646	2.111	1.72
D368E	166.628	9.18	15.30	97.937	11.462	11.23
D368L	108.977	0.00	0.00	109.364	0.969	1.06
D368M	119.744	2.72	3.25	103.662	2.536	2.63
D368R	164.735	10.16	16.74	118.140	11.805	13.95
D368T	107.122	2.87	3.07	126.693	3.366	4.26
N369R	161.693	6.39	10.34	74.366	6.182	4.60
A371F	180.217	6.19	11.16	76.436	5.578	4.26
A371H	957.055	1.81	17.32	89.541	1.697	1.52
A371H	111.143	0.00	0.00	95.589	8.610	8.23
A371K	136.514	12.84	17.53	114.354	12.454	14.24
A371L	695.108	1.51	10.52	107.003	2.215	2.37
A371L	104.327	0.00	0.00	60.232	1.205	0.73
A371R	#VALUE!	#VALUE!	11.03	#VALUE!	#VALUE!	14.06
A371R	121.162	0.00	0.00	97.970	2.587	2.53
A371S	147.672	8.38	12.38	131.555	16.938	22.28
L374P	392.038	5.77	22.63	123.033	7.365	9.06
E375A	88.836	0.00	0.00	134.714	2.050	2.76
E375G	126.880	10.32	13.10	139.030	14.673	20.40
E375R	163.180	13.15	21.45	116.431	19.727	22.97
K376D	113.100	12.36	13.97	165.064	5.049	8.33
K376E	100.000	13.55	13.55	153.016	10.394	15.90
K376Q	125.172	12.75	15.96	90.000	12.057	10.85
K376R	81.687	31.63	25.84	199.112	10.372	20.65
K376T	121.133	14.91	18.06	113.387	5.639	6.39
K376V	124.221	3.19	3.96	118.583	2.547	3.02
K376Y	102.812	9.24	9.50	96.139	12.985	12.48
G377D	110.871	15.72	17.43	132.357	10.550	13.96
G377E	130.445	8.04	10.49	128.402	7.401	9.50
G377H	146.855	8.34	12.25	104.837	10.117	10.61
G377K	185.922	4.42	8.21	119.751	4.989	5.97
G377R	119.708	5.87	7.03	94.749	7.137	6.76
G377S	108.609	6.91	7.51	101.106	7.877	7.96
G377T	112.557	17.14	19.29	109.036	18.279	19.93
F380W	147.077	9.97	14.67	104.881	9.253	9.70
T381S	135.827	13.41	18.21	112.559	10.315	11.61
R383I	527.820	6.33	33.44	98.328	7.522	7.40
R383S	132.894	10.50	13.96	119.466	10.545	12.60
K385A	126.096	4.64	5.85	112.706	0.000	0.00
K385Q	137.629	9.03	12.43	124.892	7.512	9.38
K385V	112.581	5.12	5.76	80.571	2.979	2.40

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
D431E	70.178	26.32	18.47	95.135	20.739	19.73
D431H	186.490	7.32	13.65	95.071	9.941	9.45
D431K	240.835	11.61	27.97	68.277	20.207	13.80
D431L	129.149	10.49	13.54	119.177	11.740	13.99
D431N	138.404	9.79	13.55	125.433	9.246	11.60
D431Q	232.960	10.83	25.23	109.483	10.716	11.73
D431S	78.069	7.52	5.87	88.796	9.135	8.11
F433A	147.286	9.78	14.40	99.486	12.798	12.73
F433H	140.196	13.48	18.90	87.943	16.888	14.85
F433I	108.569	11.30	12.27	86.984	16.616	14.45
F433K	91.159	11.12	10.14	342.290	3.608	12.35
F433R	128.958	10.53	13.58	133.353	9.565	12.75
F433T	161.799	13.66	22.10	134.977	19.229	25.96
F433V	1412.071	1.61	22.69	112.033	17.307	19.39
F433W	149.049	10.46	15.59	113.585	7.530	8.55
P437I	148.880	2.39	3.56	107.028	1.782	1.91
M438A	106.463	10.07	10.72	135.705	10.194	13.83
M438D	105.370	10.16	10.71	113.283	2.590	2.93
M438E	115.061	8.00	9.21	113.782	9.120	10.38
M438L	65.794	10.06	6.62	214.958	6.526	14.03
M438N	130.428	8.06	10.52	100.669	11.889	11.97
M438T	104.058	13.39	13.93	103.691	12.160	12.61
E439A	137.279	11.63	15.97	95.555	14.073	13.45
E439A	154.140	4.72	7.28	147.295	7.415	10.92
E439C	193.243	14.69	28.38	111.719	15.734	17.58
E439K	124.464	13.28	16.52	104.762	10.552	11.05
E439P	118.340	15.59	18.44	87.446	14.998	13.12
E439Q	101.589	10.67	10.84	127.358	10.648	13.56
E439T	110.891	14.36	15.93	122.975	11.322	13.92
T440D	118.877	11.52	13.69	79.518	18.426	14.65
T440H	142.296	4.46	6.34	130.928	7.553	9.89
T440M	84.722	8.83	7.48	86.929	12.774	11.10
T440P	111.931	13.54	15.16	91.205	17.272	15.75
T440S	100.436	11.17	11.22	131.174	9.810	12.87
E441F	129.315	11.25	14.55	110.874	11.410	12.65
E442G	111.216	10.24	11.39	100.210	10.965	10.99
P443E	94.377	5.14	4.85	130.704	6.789	8.87
P443F	146.612	11.22	16.45	97.932	12.322	12.07
P443G	239.171	8.56	20.48	157.960	16.385	25.88
Q444E	81.997	8.54	7.01	160.917	9.561	15.38
Q444H	150.301	8.46	12.71	119.665	10.892	13.03
Q444V	129.822	13.49	17.51	122.591	10.995	13.48
I445M	85.090	17.25	14.68	101.149	15.393	15.57
I445N	106.430	13.89	14.79	87.351	12.945	11.31
I445W	117.213	11.70	13.72	100.037	10.983	10.99
Y447E	99.579	16.55	16.48	108.969	12.849	14.00
Y447G	143.704	13.77	19.79	103.624	11.563	11.98

TABLE 12: Percent (%) Activity						
	duplicate 1			duplicate 2		
	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C	% activity at 37 °C/4 °C	% activity 37 °C + m-cresol/37 °C	% activity 37 °C + m-cresol/4 °C
Y447P	139.152	13.78	19.17	107.737	12.282	13.23
positive control (OHO)	94.998	5.23	4.97	96.871	8.456	8.19
	105.798	4.48	4.74	108.066	5.246	5.67
	100.000	3.33	3.33	82.7780	3.759	4.59
	94.762	19.07	18.07	109.539	16.529	18.11
	142.024	4.48	6.36	130.947	5.595	7.33
	45.115	20.77	9.37	68.017	11.035	7.51
	53.324	21.95	11.71	74.253	9.960	7.40
	59.581	25.24	15.04	75.872	16.231	12.31
	91.844	19.05	17.50	80.371	13.977	11.23
	93.828	13.47	12.63	96.630	19.454	18.80
	57.773	17.04	9.85	83.536	17.573	14.68
	100.000	18.56	18.56	148.226	16.239	24.07
	74.325	18.29	13.60	61.119	9.286	5.68
	98.132	8.48	8.32	87.677	10.006	8.77
	93.817	9.62	9.02	102.223	9.745	9.96
	96.922	8.56	8.30	87.993	9.064	7.98
	96.648	9.91	9.58	86.891	9.938	8.63

n/a (not available; *e.g.* beyond detection limit)

2. Summary of Results for F204P

For mutant F204P, the results above of tested supernatant from transient transfection of CHO-S cells incubated in the presence of m-cresol in a bHA enzymatic activity assay showed that the F204P mutant protein was highly resistant to 0.4% m-cresol treatment. The results showed that the activity that remained after 4 hours incubation with 0.4% m-cresol at 37°C was approximately equal to the activity observed when the enzyme was incubated at either 4°C or at 37°C alone. The positive control (WT PH20 – OHO) showed a reduction in activity of 75% and 83% on the day of the assay (as assayed from two different OHO transfections). This demonstrated that the F204P phenophile was able to retain 60% to 90% or greater of its activity above the residual activity of the wildtype PH20 control enzyme.

In order to confirm the stability of F204P to m-cresol treatment or temperature, a second transfection of F204P was performed in duplicate using CHO-S cells, and clarified supernatant was again tested for its stability at 4°C, at 37 °C for 4 hours with 0.4% m-cresol and at 37 °C for 4 hours without 0.4% m-cresol. The results confirmed that the F204P mutant enzyme retained a high amount of hyaluronidase activity after the 4 hour incubation in m-cresol. The results were

similar to the results seen in the first screening of the mutant, with F204P retaining anywhere from 57% to greater than 90% of its activity above the residual activity of the wildtype PH20 control enzyme after the 4 hour incubation.

A summary of the enzyme activity of F204P compared to the wildtype control is set forth in Table 13.

Table 13: Summary of Enzyme Activity

Transfection #	Remaining Activity after 4h incubation (37 °C + m-cre / 37 °C)		Net % Increase in Activity Over WT (37 °C)	Remaining Activity after 4h incubation (37 °C + m-cre / 4°C)		Net % Increase in Activity Over WT (4 °C)
	F204P	WT (OHO)		F204P	WT (OHO)	
1	73.6%	16.4%	57.2%	86.0%	25.3%	60.7%
2	122.3%	25.2%	97.1%	109.7%	16.6%	93.1%

EXAMPLE 6

LARGE SCALE EXPRESSION AND PURIFICATION OF PH20 HIT VARIANT

1. Expression and Purification

CHO-S cells were cultured in shaker flasks using CD-CHO media supplemented with GlutaMAX (8 mM). On the day of transfection, 15 flasks were prepared of approximately 300 mL volume containing the CHO-S cells at an approximate density of 1.0 x 10^6 cells/mL Each 300 mL flask was transfected using 375 µg of F204P mutant PH20 cDNA combined with 375 µL of Freestyle MAX transfection reagent. The transfected cells were then allowed to remain in culture for 96 hours, whereupon the cells and media were harvested and pooled. The cells were pelleted by centrifugation (4000g x 20'), and the supernatant retained for purification of the F204P protein (approximately 4.5 liters).

The crude supernatant was concentrated 10x using a 30 kDa Tangential flow filter (TFF) system (Millipore Pellicon XL, Bimax 30, 200 mL void volume; 50 cm^2 filter surface area) until the volume was approximately 450 mL. The permeate was saved for assay to detect flow through of the F204P protein. A free-flow buffer

exchange for the retentate was then performed using 4 liters of buffer (10 mM NaPO4; 25 mM NaCl, pH 7.2). The volume of the retentate was reduced again to approximately 200 mL, and then the remaining permeate in the system was purged (void volume ~200 mL) and the system was flushed using approximately 50 mL of buffer to yield a final concentrated product of approximately 450 mL.

An α-rHuPH20 affinity column was prepared by coupling antigen affinity purified Rabbit anti-rHuPH20 IgG to CNBr-activated Sepharose 4 Fast Flow (GE catalog No. 17-0981-01). Briefly, 0.7 g of pre-activated Sepharose 4 powder was suspended in 1 mM HCl in a 10 mL glass column for 30 minutes to allow the powder to swell. The solution was drained from the column and washed with 15 gel volumes (about 30 mL) of cold 1 mM HCl by gravity. The column was washed with 5 gel volumens of coupling buffer (0.1M $NaHCO_3$, 0.5M NaCl at pH 8.3). Next, 5 mg of Rabbit anti-rHuPH20 IgG at > 1.0 mg/mL in coupling buffer was added to the column at a protein/gel ratio of 2-3 mg/mL gel. The column was rotated head to head at 4°C overnight. The flow-through was collected for coupling efficiency determination. The gel was washed with 2 gel volumes of coupling buffer, and then was washed and resuspended in 1 M ethanolaminine pH 9.5 for 2 hours at room temperature to block unused activated sites. The gel was wash 6 times with 5 gel volumes per wash alternating coupling buffer and 0.1 NaAc, 0.5M NaCl, pH 4.5. The gel was then washed with 10 gel volumes of TBS (20 mM Tris-HCl, 0.15 M NaCl, pH 7.5). The coupling efficiency was determined (1-post-coupling protein concentration/pre-coupling protein concentration x 100%)/ The antibody coupled gel was stored in TBS with 0.02% NaN_3 at 4°C.

The concentrated supernatant product was subsequently loaded onto a α-rHuPH20 affinity column at an approximate rate of 5 mL/min. The elution was performed according to standard procedure using a GE™ AKTA FPLC purification system (GE Healthcare, Product No. 18-1900-26), whereby the protein was eluted via a low pH glycine wash (0.1M glycine-HCl, pH 2.5) in 1 mL fractions. Each fraction was immediately neutralized by the addition of 100 μL of 1M Tris, pH 7.5.

The eluted protein was assayed by resolving protein bands on a 4-20% SDS-PAGE gradient Tris-glycine gel. SeeBlue®Plus2Pre-stained MW standards (Life Teechnologies; Catalog No. LC5925) were used as molecular weight standards and 50 ng rHuPH20 (as described in Example 1) was used as a positive control. The polyacrylamide gel was stained with Instant Blue to show total protein from each

fraction. To confirm the bands on the gel are PH20, the gel was tranferred to a PVDF membrane (Invitrogen), which was subjected to Western Blot using a Rabbit anti-PH20 primary antibody generated by immunizing rabbits with rHuPH20 and an HRP-Goat anti-rabbit secondary antibody (Calbiochem, Cat. No. DC03L).

Then, the flow-through from the initial loading of the affinity column was re-loaded onto the column twice due to the low capacity of the affinity column. All fractions containing the protein were then combined resulting in a total volume that was approximately 13 mL. This product was then dialyzed overnight versus four liters of buffer (10 mM NaPO4, 140 mM NaCl, pH 7.2) using a Slide-A-Lyzer Dialysis Cassette G2 (20,000 MWCO) with a 15 mL capacity. The buffer was then changed and the product dialyzed against a second fresh four liters of the same buffer. The F204P protein was then concentrated using an Amicon Ultra Centrifugation column (Millipore; 10,000 MWCO) to a final volume of approximately 450 µL (10 minutes at 4000g).

2. Characterization of Protein

The purfied protein was characterized for its protein concentration, activity, and purity.

To determine the protein concentration of the purified protein, a quantification ELISA was performed as described in Example 7. Also, hyaluronidase activity was determined as described in Example 3. The protein concentration after centrifugation was estimated to be approximately 400 µg/mL. The purified protein also was resolved on a 4-20% SDS-PAGE gradient Tris-glycine gel, which was then stained with Instant Blue. The staining results demonstrated that the protein was essentially a single molecular weight protein of approximately 63 kDa, similar to the rHuPH20 control. No appreciable degradative products were detected by this method. Approximate yields of the protein at various timepoints and activity during the purification are described in Table 14.

TABLE 14: Characterization of Purification Steps

		Activity Assay		Quant ELISA Assay		
Purification Step	**Volume (mL)**	**Activity (U/mL)**	**Total Activity (U)**	**Protein Conc. (µg/mL)**	**Total Protein (µg)**	**Specific Activity (U/µg)**
Supernatant	4500	2.66	11,700	0.046	207	56.5

Conc. after TFF & Buffer Exchange	450	42	18,900	0.4	178	105.9
Pooled Fractions 5-7 after AC, Dialysis & Conc. – A280	0.45	11,741	5283	396	180	35.3

The purity of the purified protein was determined by Reverse Phase HPLC (RP-HPLC). The elution time from the reverse phase column was essentially identical as that observed with the recombinant human hyaluronidase (HUB), and provides a basis for crude estimation of the purity of the sample at approximately 80-90%.

EXAMPLE 7

Quantification Using ELISA

The quantification of PH20 or variants were performed using an ELISA that captures the protein using a monoclonal α-rHuPH20 capture antibody. Specifically, one day prior to performing the ELISA, 96-well 4HBX plates were coated with capture antibody (Protein G purified rabbit polyclonal anti-PH20 antibody generated by immunizing rabbits with rHuPH20; 1 mg/mL stock) at 1 μg/mL in 100 mM phosphate (pH 7.2) in a total volume of 100 μL per well. The plates were stored at 4°C overnight. On the next day, the plates were washed 5x with 1xPBS at 300 μL/well with a plate washer. After each wash, the plated were patted dry on paper towels. Then, the plates were blocked with 200 μL well PBS containing Tween 20 (1xPBST) at room temperature for 1 hour.

The standards and samples were added to the plate. For generation of the standard, a 1 mg/mL stock of rHuPH20 (Example 1) was freshly diluted to 50 μg/mL in HEPES pH 7.4 assay buffer as an intermediate stock. Then, for the standards, the 50 μg/mL stock was diluted in duplicates into 360 μL of 0.5xPBST at 300 ng/mL for the first standard (first row). For the other standard rows, 240 μL 0.5xPBST was added to each well, and 1:3 serial dilutions made. For the transfected supernatant samples, 360 μL per well was added in duplicate into the first row, and each were also serially diluted as described above into 0.5x PBST. For purified samples, 100 μL was added per well. The plates were incubated for 2 hours at room temperature. After incubation, the plates were washed 5x with 1xPBST at 300 μL/well using a plate washer. After each wash, the plated were patted dry on paper towels.

An HRP-conjugated anti-PH20 antibody was prepared for detection using an HRP conjugation kit (Pierce, Thermo-Fisher; Catlog No. 31489). 1 mg of a Protein G purified rabbit polyclonal antibody generated by immunizing rabbits with rHuPH20 was diluted in 1 mL PBS and 1 mL of 2 x carbonate kit buffer. Next, 100 µL of peroxidase was added to 1 mL of the above antibody solution, and was incubated at room temperature for 1 hour. Then, 10 µL NaBH4 stock was added in a fume hood, and the sample incubated at room temperature for 20 minutes. To quench the reaction, 20 µL of ethanolamine was added and incubated at room temperature for 15 minutes. to this, 1/25 volume 5% human serum albumin (0.1 mL syringe) was added to give a 2 mg/mL albumin stock reaction. The pH was adjusted to about 7.9 by addition of 250 µL of 1 M Tris pH 7.4. The concentration of the stock was 400 µg/mL. The stock solution was further diluted 1/10 in PBS Tween20 (0.05%) containing 0.5% human serum albumin and preservatives, and then was sterile filtered. The stock was stored at 4 °C or was frozen at -20 °C.

Antibodies were detecting using the HRP-conjugated anti-PH20 antibody that was diluted 1000x into 0.5x PBST. 100 µL of the diluted antibody was added to all wells of the plate and the plate incubated for a further 2 hours at room temperature. After incubation, the plates were washed 5x with 1xPBST at 300 µL/well using a plate washer. After each wash, the plated were patted dry on paper towels. Then, 100 µL/well of TMB substrate was added to each well and the reaction was stopped after 5-10 minutes by adding 100 µL of stop solution per well. The plate was read at OD_{450}.

EXAMPLE 8

Determination of enzymatic activity of PH20

Enzymatic activity of PH20 in samples such as cell cultures, purification fractions and purified solutions was determined using a turbidometric assay, which is based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin. The activity is measured by incubating PH20 with sodium hyaluronate (hyaluronic acid) for a set period of time (10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after a 30 minute development period. The decrease in turbidity resulting from enzyme activity on the sodium hyaluronate substrate is a measure of the PH20 enzymatic activity. The

method is run using a calibration curve generated with dilutions of a PH20 assay working reference standard, and sample activity measurements are made relative to this calibration curve.

Dilutions of the sample and standards were prepared in Enzyme Diluent Solution (70 mM NaCl, 0.1 % human serum albumin [HAS], 0.67 g/L gelatin hydrolysate in 25 mM PIPES buffer, pH 5.5). The samples were diluted to an appropriate concentration. Hyaluronic acid (HA, average MW of 20 – 50 kDa) from Lifecore Biomedical (Chaska, MN) also was prepared at 1 mg/ml in substrate solution that contains 25 mM PIPES, 70 mM NaCl at pH 5.5. Equal amounts of the above two solutions were mixed to prepare a 1 ml reaction mixture and incubated at 37°C for 30 min. The reaction was stopped by addition of 4 mL of Cetylpyridinium Chloride Solution (CPC, 5.0 mg/mL). After brief vortexing, the turbidity of the sample mixture was read at 640 nm and the activity was determined by fitting against a standard curve. Specific activity (Units/mg) was calculated by dividing the enzyme activity (U/ml) by the protein concentration (mg/mL).

EXAMPLE 9

STABILITY OF PH20 VARIANT IN PRESERVATIVE

To confirm the screening results, an amount estimated to be about 450 U/mL of the purified F204P protein as described in Example 6 was formulated in 10 mM sodium phosphate, pH 6.5, 120 mM NaCl, 10 mM methionine, 0.01% Pluronic F-68, 0.1% phenol and 0.15% m-cresol. A test article that also contained an amount estimated to be about 450 U/mL wild type rHuPH20 (generated as described in Example 1) in the same formulation was also prepared to serve as a control. Each formulation solution was aliquotted in 0.5 ml and filled into 2 mL USP Type I borosilicate glass with a chlorobutyl rubber stopper and an aluminum seal. The vials were incubated at 5°C, 30 °C or 37°C. Samples were withdrawn from the incubator at various times and enzymatic activity was measured as described in Example 8.

The results of the enzymatic activity measurements are shown in Table 15. As can be seen, the rHuPH20 wild type control showed a rapid decrease in activity when incubated at 37°C in the presence of phenolic preservatives. In contrast, the F204P mutant showed no significant loss in activity throughout the study. The results also show that activity of PH20 is retained after incubation for up to 4 weeks at 5°C and 30 °C compared to the activity of the rHuPH20 wildtype control not containing the

mutation. This result confirms that F204P tolerates EPB level of preservative (0.1% phenol and 0.15% m-cresol) and is stable at 37°C for at least up to 6 days at at 5°C and 30 °C for greater than one month.

TABLE 15: Stability of rHuPH20 wildtype and F204P mutant incubated at with preservative

ID	PH20 relative activity (%) at 5 °C			PH20 relative activity (%) at 30 °C			PH20 relative activity (%) at 37 °C		
	T0	2w	4w	6d	2w	4w	2d	4d	6d
F204P	100	-	91.8	84.1	100	96.6	105	91.1	95.9
wildtype control	100	-	81.9	66.7	61.7	60.5	48.6	29.6	15.2

EXAMPLE 10

STABILITY OF PH20 VARIANT AND INSULIN COFORMULATION

The PH20 variant F204P was tested for its stability in a coformulation containing an insulin analog (insulin aspart or insulin lispro).

In the tested coformulations, the insulin lispro was a commercial product (Insulin Lispro: Eli Lilly Humalog® (insulin Lispro) 100 U/mL, Lot A572364).

In the tested coformulations, the insulin aspart analog was a reprocessed aspart prepared by pooling 12 vials (10 mL each) of a commercial product (Insulin Aspart: Novo Nordisk, NovoRapid® (insulin Aspart), Lot XS60195), which was then concentrated using an Amicon Ultracel-10 K column concentrator until the final concentration was about 5 times the original concentration. The insulin analog was precipitated by addition of 1 M sodium acetate, pH 5.3 and 30 mM zinc chloride (ZnCl2, EMD, Cat No. ZX0065-1) at 1/10 of the protein solution volume. The solution was placed on ice for 30 minutes followed by centrifugation at 5600 rpm for 20 minutes in an Avanti J-E Centrifuge with JS-5.3 swinging bucket rotor (Beckman Coulter). The supernatant was decanted and the pellet was resuspended and washed with 20 mM sodium acetate, 2 mM zinc chloride, pH 5.5 solution. The resuspended solution was centrifuged as described above. The washing step was repeated a total of 5 times. A final wash was performed with 20 mM sodium acetate, pH 5.5 to remove all traces of zinc chloride. The resulting protein paste was dissolved with water containing 20 mM HCl. After complete dissolution, 250 mM Tris, pH 10.7 was added to a final Tris concentration of 20 mM. The pH of the resulting solution was adjusted such that the insulin analog was formulated as described below and the protein concentration was adjusted to about 15-20 mg/mL. An insulin analog

prepared in this way typically had a yield of about 90 %, with a residual preservative concentration at less than 100 times the starting material.

Briefly, three (3) formulations were generated each containing 600 Units (U) of PH20-F204P or wildtype rHuPH20 (generated as described in Example 1) for a total of 6 formulations as set forth in Table 16:

TABLE 16: Summary of Insulin Formulations

ID	pH	Buffer		Tonicity modifier	Anti-Ox		Metal	Surfactant	Preservatives		API	
		$NaPO_4$	Tris/HCl	NaCl	Methionine	Glycerin	Zn	F68	Phenol	m-Cresol	PH20 (U/mL)	Analog (mg/mL)
F1.Humalog + F204P	7.0-7.8	13.2 mM				173.7 mM	0.242 mM			0.315%	600	3.5
F2.Humalog + wt	7.0-7.8	13.2 mM				173.7 mM	0.242 mM			0.315%	600	3.5
F3.Aspart + F204P	7.3		30 mM	100 mM	5 mM			0.010%	0.100 %	0.150%	600	3.5
F4.Aspart + wt	7.3		30 mM	100 mM	5 mM			0.010%	0.100 %	0.150%	600	3.5
F5.Aspart + F204P	7.3		30 mM	100 mM	5 mM			0.010%		0.315%	600	3.5
F6.Aspart+ wt	7.3		30 mM	100 mM	5 mM			0.010%		0.315%	600	3.5

Each formulation solution was aliquoted in 0.5 mL and filled into 2 mL USP Type I borosilicate glass with a chlorobutyl rubber stopper and an aluminum seal. The vials were incubated at 5°C, 30°C and 37°C. Samples were withdrawn from the incubator at scheduled time points for enzymatic activity measures as described in Example 8.

The results of the enzymatic activity measurements for samples incubated at 37°C, 30°C and 5°C are shown in Tables 17-19, respectively. At 37°C, the enzymatic activity of samples containing wildtype rHuPH20 (F2, F4 and F6) were almost totally lost within two days of incubation. In contrast, after 6 days incubation at 37°C, formulation F3 and F5, which contains PH20-F204P, lost only about 10% and 30%, respectively. The PH20-F204P formulated in commercial Humalog (F1) lost most of its activity within 2 days at 37°C most likely due to lack of NaCl in the formulation.

A similar trend for enzymatic activities of amples incubated at 30°C was noted between the PH20-F204P and rHuPH20. For formulations that contain EPA preservative level, the differences between wild typ eand F204P were dramatic (Table 17; F1 and F5 vs. F2 and F6). When the preservative concentration was reduced to EPB level (F3 and F4), the F204P still outperformed wildtype rHuPH20, although

there was slighly higher rHuPH20 stability compared to EPA conditions. In both EPA and EPB preservative levels, PH20-F204P was able to maintain its activity up to 14 days at 30°C when 100 mM of NaCl was included in the formulation.

Table 17. Enzymatic activity of rHuPH20 wild type and F204P mutant incubated at 37 °C					
ID	PH20 activity U/mL, (% of remaining activity)				
	Initial Activity	2d	4d	6d	2w
F1.Humalog + F204P	583 (100%)	61 (10%)	15 (3%)	10 (2%)	-
F2.Humalog + wt	439 (100%)	4 (1%)	-	-	-
F3.Aspart + F204P	625 (100%)	613 (98%)	496 (79%)	570 (91%)	532 (85%)
F4.Aspart + wt	566 (100%)	58 (10%)	24 (4%)	4 (1%)	-
F5.Aspart + F204P	657 (100%)	484 (74%)	462 (70%)	478 (73%)	360 (55%)
F6.Aspart+ wt	596 (100%)	-1 (0%)	-	-	-

Table 18. Enzymatic activity of rHuPH20 wild type and F204P mutant incubated at 30°C				
ID	PH20 activity U/mL, (% of remaining activity)			
	Initial Activity	6d	2w	4w
F1.Humalog + F204P	583 (100%)	345 (59%)	250 (43%)	111 (19%)
F2.Humalog + wt	439 (100%)	1 (0%)	16 (4%)	-1
F3.Aspart + F204P	625 (100%)	601(96%)	650(104%)	579 (93%)
F4.Aspart + wt	566 (100%)	428 (76%)	390 (69%)	277 (49%)
F5.Aspart + F204P	657 (100%)	632 (96%)	655 (100%)	561 (85%)
F6.Aspart+ wt	596 (100%)	145 (24%)	65 (11%)	9 (1.5%)

TABLE 19: Enzymatic Activity at 5°C			
ID	PH20 activity (U/mL) at 5 °C		
	Initial Activity	2w	4w
F1.Humalog + F204P	583	544	565
F2.Humalog + wt	439	428	404
F3.Aspart + F204P	625	647	607
F4.Aspart + wt	566	580	496
F5.Aspart + F204P	657	695	574
F6.Aspart+ wt	596	583	519

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

CLAIMS:

1. A modified PH20 polypeptide, comprising at least one amino acid replacement in an PH20 polypeptide, wherein:

the modified PH20 polypeptide exhibits increased stability compared to the PH20 polypeptide not containing the amino acid replacement;

increased stability is manifested as increased resistance to one or more protein denaturation conditions; and

stability is compared under the same conditions.

2. The modified PH20 polypeptide of claim 1, wherein the protein denaturation condition is selected from among elevated temperature greater than 30°C, agitation, low or no salt, and presence of excipients.

3. The modified PH20 polypeptide of claim 2, wherein the excipient is selected from among one or more of an antiadherent(s), binder(s), coating(s), filler(s) and diluent(s), flavor(s), color(s), lubricant(s), glidant(s), preservative(s), detergent(s), sorbent(s) or sweetner(s) and a combination thereof.

4. The modified PH20 polypeptide of any of claims 1-3, wherein the modified PH20 polypeptide exhibits at least 68% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3.

5. The modified PH20 polypeptide of claim any of claims 1-4, wherein the modified PH20 polypeptide exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3.

6. The modified PH20 polypeptide of any of claims 1-5, wherein the amino acid replacement(s) is in a PH20 polypeptide that has a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, 72, 857, 859, 861, 870 or a sequence of amino acids that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, 72, 857, 859, 861, or 870.

7. The modified PH20 polypeptide of any of claims 1-6, wherein the amino acid replacement is in a PH20 polypeptide that has a sequence of amino acids set forth in SEQ ID NOS: 3, 7, 32-66, 69 or 72.

8. The modified PH20 polypeptide of any of claims 1-7, wherein:

stability is assessed based on hyaluronidase activity, solubility, aggregation or crystallization;

stability is assessed in the presence of the denaturation condition; and

stability is compared under the same conditions.

9. The modified PH20 polypeptide of any of claims 1-8, wherein stability is assessed based on hyaluronidase activity and the modified PH20 polypeptide exhibits at least 120%, 130%, 135%, 140%, 145%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 350%, 400%, 500%, 1500%, 2000%, 3000%, 4000%, 5000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement(s).

10. The modified PH20 polypeptide of any of claims 3-9, wherein the excipient is a preservative and the preservative is a phenolic preservative.

11. The modified PH20 polypeptide of any of claims 1-10, wherein the modified PH20 polypeptide exhibits increased stability in the presence of an anti-microbial effective amount of one or more phenolic preservatives.

12. The modified PH20 polypeptide of claim 1, wherein the anti-microbial effective amount is a total amount of one or more phenolic preservative agents as a percentage (%) of mass concentration (w/v) that is or is between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive.

13. The modified PH20 polypeptide of any of claims 10-12, wherein the phenolic preservative is selected from among phenol, metacresol (m-cresol), benzyl alcohol, and parabens.

14. The modified PH20 polypeptide of claim 13, wherein the paraben is methylparaben or propylparaben.

15. The modified PH20 polypeptide of any of claims 10-14, wherein the preservative is m-cresol, phenol or m-cresol and phenol.

16. The modified PH20 polypeptide of any of claims 1-15, wherein:

the modified PH20 polypeptide exhibits at least 15% of the hyaluronidase activity for at least 4 hours in the presence of preservative(s) compared to the modified PH20 polypeptide in absence of preservative; and

activity is compared under the same conditions except for the presence of preservative(s).

17. The modified PH20 polypeptide of any of claims 1-16, wherein the modified PH20 polypeptide exhibits at least 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or

more of the hyaluronidase activity in the presence of a phenolic preservative(s) compared to absence of preservative(s).

18. The modified PH20 polypeptide of claim 16 or claim 17, wherein the hyaluronidase activity is exhibited after at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks or more in the presence of the preservative(s) compared to the hyaluronidase activity of the modified PH20 polypeptide in the absence of preservative for the same time period and under the same conditions except for the presence of preservative(s).

19. The modified PH20 polypeptide of any of claims 15-18, wherein the modified PH20 polypeptide exhibits increased stability to a phenolic preservative under temperature conditions of between or about between 0°C to 40°C, 2°C to 6°C, 24°C to 32°C or 35°C to 40°C.

20. The modified PH20 polypeptide of any of claims 1-19, comprising at least one amino acid replacement at an amino acid position corresponding to a position selected from among 10, 12, 20, 22, 26, 34, 36, 46, 50, 52, 58, 68, 70, 74, 82, 83, 84, 86, 97, 127, 131, 138, 142, 143, 144, 166, 169, 174, 193, 195, 196, 204, 205, 206, 213, 219, 234, 237, 238, 240, 249, 261, 267, 277, 279, 291, 309, 310, 314, 315, 317, 318, 347, 367, 375, 376, 399, 401, 407, 416, 419, 421, 431, 433, 439, 440, 443 or 445 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3.

21. The modified PH20 polypeptide of any of claims 1-20, comprising at least one amino acid replacement selected from among replacement with:

G at a position corresponding to position 10; K at a position corresponding to position 12; S at a position corresponding to position 20; T at a position corresponding to position 22; M at a position corresponding to position 26; W at a position corresponding to position 34; N at a position corresponding to position 36; L at a position corresponding to position 46; M at a position corresponding to position 50; T at a position corresponding to position 52; S at a position corresponding to position 52; C at a position corresponding to position 58; K at a position corresponding to position 58; R at a position corresponding to position 58; N at a position corresponding to position 58; Y at a position corresponding to position 58; P at a position corresponding to position 58; H at a position corresponding to position

58; P at a position corresponding to position 68; V at a position corresponding to position 70; E at a position corresponding to position 74; L at a position corresponding to position 82; N at a position corresponding to position 82; V at a position corresponding to position 83; Q at a position corresponding to position 83; S at a position corresponding to position 83; G at a position corresponding to position 83; N at a position corresponding to position 84; A at a position corresponding to position 86; K at a position corresponding to position 86; E at a position corresponding to position 97; L at a position corresponding to position 97; R at a position corresponding to position 127; R at a position corresponding to position 131; L at a position corresponding to position 138; K at a position corresponding to position 142; N at a position corresponding to position 142; P at a position corresponding to position 142; S at a position corresponding to position 142; T at a position corresponding to position 142; G at a position corresponding to position 143; K at a position corresponding to position 143; T at a position corresponding to position 144; Q at a position corresponding to position 166; T at a position corresponding to position 166; L at a position corresponding to position 169; G at a position corresponding to position 174; N at a position corresponding to position 174; Q at a position corresponding to position 193; T at a position corresponding to position 195; N at a position corresponding to position 195; E at a position corresponding to position 196; R at a position corresponding to position 196; P at a position corresponding to position 204; A at a position corresponding to position 205; E at a position corresponding to position 205; I at a position corresponding to position 206; A at a position corresponding to position 213; I at a position corresponding to position 219; M at a position corresponding to position 234; T at a position corresponding to position 237; H at a position corresponding to position 238; Q at a position corresponding to position 240; V at a position corresponding to position 249; A at a position corresponding to position 261; K at a position corresponding to position 261; T at a position corresponding to position 267; K at a position corresponding to position 277; H at a position corresponding to position 279; V at a position corresponding to position 279; E at a position corresponding to position 309; Q at a position corresponding to position 310; Y at a position corresponding to position 314; Y at a position corresponding to position 315; N at a position corresponding to position 317; W at a position corresponding to position 317; D at a position corresponding to position 318; G at a position corresponding to position 347;

A at a position corresponding to position 367; R at a position corresponding to position 375; R at a position corresponding to position 376; V at a position corresponding to position 399; E at a position corresponding to position 401; A at a position corresponding to position 407; L at a position corresponding to position 416; K at a position corresponding to position 419; H at a position corresponding to position 421; E at a position corresponding to position 431; T at a position corresponding to position 433; V at a position corresponding to position 433; C at a position corresponding to position 439; P at a position corresponding to position 440; G at a position corresponding to position 443; N at a position corresponding to position 445, with reference to amino acid residue positions set forth in SEQ ID NO:3.

22. The modified PH20 polypeptide of any of claims 1-21, comprising at least one amino acid replacement selected from among replacement with:

T at a position corresponding to position 52, K at a position corresponding to position 58, R at a position corresponding to position 58, P at a position corresponding to position 68, V at a position corresponding to position 83, P at a position corresponding to position 204, A at a position corresponding to position 261, T at a position corresponding to position 267, K at a position corresponding to position 277 and H at a position corresponding to position 421, with reference to amino acid residue positions set forth in SEQ ID NO:3.

23. The modified PH20 polypeptide of any of claims 1-22, comprising replacement with P at a position corresponding to position 204 in a PH20 polypeptide with reference to amino acid residue positions set forth in SEQ ID NO:3.

24. The modified PH20 polypeptide of any of claims 1-23, wherein the modified PH20 polypeptide exhibits increased stability at elevated temperatures of between or about between 30°C to 45°C, 35° C to 45° C, 30° C to 37° C, 35° C to 37° C or 37° C to 42° C, each inclusive.

25. The modified PH20 polypeptide of claim 24, wherein:

the modified PH20 polypeptide exhibits increased hyaluronidase activity at the elevated temperature compared to the PH20 polypeptide not containing the amino acid replacement(s); and

activity is compared under the same conditions.

26. The modified PH20 polypeptide of any of claims 1-25, wherein the modified PH20 polypeptide exhibits at least 110%, 120%, 130%, 140%, 150%, 160%,

170%, 180%, 190%, 200%, 300%, 400%, 500% or more hyaluronidase activity for at least 4 hours compared to the PH20 polypeptide not containing the amino acid replacement(s).

27. The modified PH20 polypeptide of any of claims 1-26, wherein:

the modified PH20 polypeptide exhibits at least 95% of the hyaluronidase activity for at least 4 hours at a temperature of between or about between 32°C to 37°C compared to the hyaluronidase activity of the modified PH20 polypeptide at a temperature of between or about between 2 °C to 8 °C; and

activity is compared under the same conditions except for the differences in temperature.

28. The modified PH20 polypeptide of claim 27, wherein the modified PH20 polypeptide exhibits at least 96 %, 97 %, 98 %, 99 %, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500% or more of the hyaluronidase activity.

29. The modified PH20 polypeptide of claim 27 or claim 28, wherein:

the hyaluronidase activity is exhibited after at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks or more at elevated temperatures of between or about between 32°C to 37°C compared to the hyaluronidase activity of the modified PH20 polypeptide at a temperature between or about between 2 °C to 8 °C; and

activity is compared for the same time period and under the same conditions except for the difference in temperature.

30. The modified PH20 polypeptide of any of claims 1-29, comprising at least one amino acid replacement at an amino acid position corresponding to a position selected from among 1, 11, 12, 14, 20, 26, 29, 34, 50, 58, 70, 82, 83, 84, 86, 87, 140, 142, 143, 147, 152, 166, 167, 172, 174, 178, 193, 195, 206, 212, 213, 219, 233, 237, 240, 267, 277, 291, 292, 309, 313, 314, 317, 318, 347, 367, 368, 371, 374, 389, 392, 395, 396, 406, 419, 421, 439 and 443 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3.

31. The modified PH20 polypeptide of any of claims 1-30, comprising at least one amino acid replacement selected from among replacement with:

R at a position corresponding to position 1; S at a position corresponding to position 11; I at a position corresponding to position 12; V at a position corresponding to position 14; S at a position corresponding to position 20; M at a position corresponding to position L with R at a position corresponding to position 29; W at a position corresponding to position 34; M at a position corresponding to position 50; K at a position corresponding to position 58; Q at a position corresponding to position 58; Q at a position corresponding to position 58; V at a position corresponding to position 70; L at a position corresponding to position 82; Q at a position corresponding to position 83; R at a position corresponding to position 84; A at a position corresponding to position 86; S at a position corresponding to position 87; K at a position corresponding to position 140; S at a position corresponding to position 142; T at a position corresponding to position 142; K at a position corresponding to position 143; S at a position corresponding to position 147; T at a position corresponding to position 152; T at a position corresponding to position 166; D at a position corresponding to position 167; A at a position corresponding to position 172; G at a position corresponding to position 174; N at a position corresponding to position 174; R at a position corresponding to position 178; Q at a position corresponding to position 193; T at a position corresponding to position 195; I at a position corresponding to position 206; S at a position corresponding to position 212; A at a position corresponding to position 213; I at a position corresponding to position 219; G at a position corresponding to position 233; T at a position corresponding to position 237; A at a position corresponding to position 240; Q at a position corresponding to position 240; T at a position corresponding to position 267; E at a position corresponding to position 277; S at a position corresponding to position 291; H at a position corresponding to position 292; V at a position corresponding to position 292; S at a position corresponding to position 309; H at a position corresponding to position 313; S at a position corresponding to position 314; I at a position corresponding to position 317; T at a position corresponding to position 317; W at a position corresponding to position 317; R at a position corresponding to position 318; G at a position corresponding to position 347; A at a position corresponding to position 367; R at a position corresponding to position 368; S at a position corresponding to position 371; P at a position corresponding to position 374; A at a position corresponding to position 389; V at a position corresponding to position 392; A at a position corresponding to position 395; H at a position

corresponding to position 396; N at a position corresponding to position 406; H at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 421; S at a position corresponding to position 421; A at a position corresponding to position 439; C at a position corresponding to position 439; and G at a position corresponding to position 443, with reference to amino acid positions set forth in SEQ ID NO:3.

32. The modified PH20 polypeptide of any of claims 1-31, wherein the modified PH20 polypeptide exhibits increased stability in low concentrations of NaCl of less than 100 mM.

33. The modified PH20 polypeptide of claim 32, wherein the concentration of NaCl is less than 90 mM, 80mM, 70mM, 60 mM, 50 mM, 40 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM or less.

34. The modified PH20 polypeptide of claim 32 or claim 33, wherein:

the modified PH20 polypeptide exhibits increased hyaluronidase activity at low concentrations of salt compared to the PH20 polypeptide not containing the amino acid replacement(s); and

activity is compared under the same conditions.

35. The modified PH20 polypeptide of claim 34, wherein the modified PH20 polypeptide exhibits at least 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500% or more hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement(s).

36. The modified PH20 polypeptide of any of claims 1-35, wherein:

the modified PH20 polypeptide exhibits at least 60% of the hyaluronidase activity in low concentrations of salt of between or about between 10 mM NaCl and 100 mM NaCl, inclusive, compared to the hyaluronidase activity of the modified PH20 polypeptide in 150 mM NaCl; and activity is compared under the same conditions except for the difference in NaCl concentration.

37. A modified PH20 polypeptide, comprising at least one amino acid replacement in a PH20 polypeptide, wherein:

the modified PH20 polypeptide exhibits increased hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement; and

activity is compared under the same conditions.

38. The modified PH20 polypeptide of claim 37, wherein the modified PH20 polypeptide exhibits at least 68% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3.

39. The modified PH20 polypeptide of claim 37 or claim 38, wherein the modified PH20 polypeptide exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO:3.

40. The modified PH20 polypeptide of any of claims 37-39, wherein the amino acid replacement(s) is in a PH20 polypeptide that has a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, or 72, or a sequence of amino acids that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of SEQ ID NOS: 3, 7, 10, 12, 14, 24, 32-66, 69, or 72.

41. The modified PH20 polypeptide of any of claims 37-40, wherein the amino acid replacement is in a PH20 polypeptide that has a sequence of amino acids set forth in SEQ ID NOS: 3, 7, 32-66, 69 or 72.

42. The modified PH20 polypeptide of any of claims 37-41, wherein the modified PH20 polypeptide exhibits at least 120%, 130%, 135%, 140%, 145%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 350%, 400%, 500%, 1500%, 2000%, 3000%, 4000%, 5000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement.

43. The modified PH20 polypeptide of any of claims 37-42, wherein activity is assessed at a temperature between or about between 2 °C to 8 °C.

44. The modified PH20 polypeptide of any of claims 37-43, comprising at least one amino acid replacement at an amino acid position corresponding to a position selected from among 1, 12, 15, 24, 26, 27, 29, 30, 31, 32, 33, 37, 39, 46, 48, 52, 58, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 84, 86, 87, 92, 93, 94, 97, 118, 120, 127, 131, 135, 141, 142, 147, 148, 150, 151, 152, 155, 156, 163, 164, 165, 166, 169, 170, 174, 198, 206, 209, 212, 213, 215, 219, 233, 234, 236, 238, 247, 257, 259, 260, 261, 263, 269, 271, 272, 276, 277, 278, 282, 291, 293, 305, 308, 309, 310, 313, 315, 317, 318, 320, 324, 325, 326, 328, 347, 353, 359, 371, 377, 380, 389, 392, 395, 399, 405, 407, 409, 410, 418, 419, 421, 425, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3,

wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3. .

45. The modified PH20 polypeptide of any of claims 37-44, comprising at least one amino acid replacement selected from among replacement with:

histidine (H) at a position corresponding to position 1; Q at a position corresponding to position 1; E at a position corresponding to position 12; T at a position corresponding to position 12; V at a position corresponding to position 15; E at a position corresponding to position 24; H at a position corresponding to position 24; E at a position corresponding to position 26; K at a position corresponding to position 26; K at a position corresponding to position 27; R at a position corresponding to position 27; E at a position corresponding to position 29; I at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; S at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; F at a position corresponding to position 32; G at a position corresponding to position 32; H at a position corresponding to position 32; W at a position corresponding to position 33; F at a position corresponding to position 37; N at a position corresponding to position 39; T at a position corresponding to position 39; R at a position corresponding to position 46; F at a position corresponding to position 48; H at a position corresponding to position 48; N at a position corresponding to position 48; Q at a position corresponding to position 52; K at a position corresponding to position 58; Q at a position corresponding to position 58; W at a position corresponding to position 63; V at a position corresponding to position 67; H at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position

69; C at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; I at a position corresponding to position 69; L at a position corresponding to position 69; M at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; W at a position corresponding to position 69; Y at a position corresponding to position 69; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; N at a position corresponding to position 70; P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; R at a position corresponding to position 71; S at a position corresponding to position 71; M at a position corresponding to position 72; Q at a position corresponding to position 72; H at a position corresponding to position 73; L at a position corresponding to position 73; W at a position corresponding to position 73; A at a position corresponding to position 74; C at a position corresponding to position 74; G at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; F at a position corresponding to position 75; L at a position corresponding to position 75; R at a position corresponding to position 75; T at a position corresponding to position 75; G at a position corresponding to position 84; R at a position corresponding to position 84; A at a position corresponding to position 86; C at a position corresponding to position 87; T at a position corresponding to position 87; Y at a position corresponding to position 87; C at a position corresponding to position 92; I at a position corresponding to position 93; L at a position corresponding to position 93; R at a position corresponding to position 93; T at a position corresponding to position 93; R at a position corresponding to position 94; G at a position corresponding to position 97; Q at a position corresponding to position 118; F at a position corresponding to position 120; V at a position corresponding to position 120; Y at a position corresponding to position 120; H at a position corresponding to position 127; N at a position

corresponding to position 127; G at a position corresponding to position 131; R at a position corresponding to position 131; V at a position corresponding to position 131; D at a position corresponding to position 135; G at a position corresponding to position 135; R at a position corresponding to position 135, with H at a position corresponding to position 141; Y at a position corresponding to position 141; R at a position corresponding to position 142; R at a position corresponding to position 147; V at a position corresponding to position 147; K at a position corresponding to position 148; G at a position corresponding to position 150; K at a position corresponding to position 151; L at a position corresponding to position 151; M at a position corresponding to position 151; Q at a position corresponding to position 151; R at a position corresponding to position 151; R at a position corresponding to position 152; G at a position corresponding to position 155; K at a position corresponding to position 155; D at a position corresponding to position 156; A at a position corresponding to position 163; E at a position corresponding to position 163; K at a position corresponding to position 163; R at a position corresponding to position 163; M at a position corresponding to position 164; D at a position corresponding to position 165; N at a position corresponding to position 165; A at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; Q at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; Y at a position corresponding to position 166; L at a position corresponding to position 169; R at a position corresponding to position 170; K at a position corresponding to position 174; D at a position corresponding to position 198; K at a position corresponding to position 206; L at a position corresponding to position 206; N at a position corresponding to position 212; M at a position corresponding to position 213; N at a position corresponding to position 213; M at a position corresponding to position 215; S at a position corresponding to position 219; K at a position corresponding to position 233; R at a position corresponding to position 233; M at a position corresponding to position 234; R at a position corresponding to position 236; E at a position corresponding to position 237; S at a position corresponding to position 238; I at a position corresponding to position 247; T at a position corresponding to position 257; P at a position corresponding to position 259; Y at a position corresponding to position 260; K at a position corresponding to position 261;

N at a position corresponding to position 261; K at a position corresponding to position 263; R at a position corresponding to position 263; A at a position corresponding to position 269; L at a position corresponding to position 271; M at a position corresponding to position 271; T at a position corresponding to position 272; D at a position corresponding to position 276; S at a position corresponding to position 276; Y at a position corresponding to position 276; K at a position corresponding to position 277; R at a position corresponding to position 277; T at a position corresponding to position 277; H at a position corresponding to position 278; K at a position corresponding to position 278; N at a position corresponding to position 278; R at a position corresponding to position 278; S at a position corresponding to position 278; T at a position corresponding to position 278; Y at a position corresponding to position 278; M at a position corresponding to position 282; V at a position corresponding to position 291; A at a position corresponding to position 293; C at a position corresponding to position 293; F at a position corresponding to position 293; M at a position corresponding to position 293; P at a position corresponding to position 293; Q at a position corresponding to position 293; V at a position corresponding to position 293; E at a position corresponding to position 305; G at a position corresponding to position 308; N at a position corresponding to position 308; E at a position corresponding to position 309; L at a position corresponding to position 309; N at a position corresponding to position 309; Q at a position corresponding to position 309; R at a position corresponding to position 309; T at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; K at a position corresponding to position 313; R at a position corresponding to position 313; H at a position corresponding to position 315; I at a position corresponding to position 317; K at a position corresponding to position 317; R at a position corresponding to position 317; M at a position corresponding to position 318; H at a position corresponding to position 320; K at a position corresponding to position 320; R at a position corresponding to position 320; R at a position corresponding to position 324; A at a position corresponding to position 325; D at a position corresponding to position 325; E at a position corresponding to position 325; G at a position corresponding to position 325; H at a position corresponding to position 325; K at a position corresponding to position 325; M at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to

position 325; S at a position corresponding to position 325; V at a position corresponding to position 326; I at a position corresponding to position 328; K at a position corresponding to position 328; L at a position corresponding to position 328; S at a position corresponding to position 328; Y at a position corresponding to position 328; G at a position corresponding to position 347; S at a position corresponding to position 347; V at a position corresponding to position 353; with T at a position corresponding to position 359; R at a position corresponding to position 371; P at a position corresponding to position 377; T at a position corresponding to position 377; W at a position corresponding to position 380; Y at a position corresponding to position 380; K at a position corresponding to position 389; M at a position corresponding to position 392; R at a position corresponding to position 395; M at a position corresponding to position 399; T at a position corresponding to position 399; W at a position corresponding to position 399; G at a position corresponding to position 405; D at a position corresponding to position 407; Q at a position corresponding to position 407; A at a position corresponding to position 409; Q at a position corresponding to position 409; T at a position corresponding to position 410; P at a position corresponding to position 418; F at a position corresponding to position 419; I at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; H at a position corresponding to position 421; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; K at a position corresponding to position 425; A at a position corresponding to position 431; H at a position corresponding to position 431; K at a position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to position 431; L at a position corresponding to position 433; R at a position corresponding to position 433; T at a position corresponding to position 433; V at a position corresponding to position 433; K at a position corresponding to position 436; I at a position corresponding to position 437; M at a position corresponding to position 437; T at a position corresponding to position 438; V at a position corresponding to position 439; H at a position corresponding to position 440; R at a position corresponding to position 440; F at a

position corresponding to position 441; R at a position corresponding to position 442; A at a position corresponding to position 443; M at a position corresponding to position 443; M at a position corresponding to position 445; P at a position corresponding to position 445; A at a position corresponding to position 446; D at a position corresponding to position 447; N at a position corresponding to position 447; and/or with Q at a position corresponding to position 447, with reference to amino acid positions set forth in SEQ ID NO:3.

46. The modified PH20 polypeptide of any of claims 37-45, wherein the modified PH20 polypeptides exhibits at least 2.0-fold of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement.

47. The modified PH20 polypeptide of any of claims 37-46, comprising at least one amino acid replacement at an amino acid position corresponding to a position selected from among 24, 29, 31, 48, 58, 69, 70, 75, 84, 97, 165, 166, 271, 278, 317, 320, 325 and 326 with reference to positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3.

48. The modified PH20 polypeptide of any of claims 37-47, comprising at least one amino acid replacement selected from among replacement with:

E at a position corresponding to position 24; E at a position corresponding to position 29; V at a position corresponding to position 31; N at a position corresponding to position 48; K at a position corresponding to position 58; Q at a position corresponding to position 58; A at a position corresponding to position 69; F at a position corresponding to position 69; G at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; A at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; H at a position corresponding to position 70; N at a position corresponding to position 70; R at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; L at a position corresponding to position 75; T at a position corresponding to position 75; G at a position corresponding to position 84; G at a position corresponding to position 97; D at a position corresponding to position 165; L at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; L at a position

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corresponding to position 271; H at a position corresponding to position 278; R at a position corresponding to position 278; K at a position corresponding to position 317; K at a position corresponding to position 320; E at a position corresponding to position 325, with G at a position corresponding to position 325; K at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; and V at a position corresponding to position 326; with reference to amino acid positions set forth in SEQ ID NO:3.

49. A modified PH20 polypeptide, comprising at least one amino acid replacement in a PH20 polypeptide set forth in SEQ ID NO:7, a C-terminally truncated fragment thereof, or in a PH20 polypeptide that has a sequence of amino acids that is at least 91% identical to the sequence of amino acids set forth in SEQ ID NO:7, wherein:

the at least one amino replacement(s) is at an amino acid position corresponding to a position selected from among 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 20, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 58, 59, 60, 61, 63, 65, 66, 67, 68, 69, 70, 71, 72, 73, 75, 75, 77, 79, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 96, 97, 98, 99, 102, 103, 104, 105, 106, 107, 108, 110, 114, 117, 118, 119, 120, 122, 124, 125, 127, 128, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 186, 192, 193, 195, 196, 197, 198, 200, 202, 204, 205, 206, 208, 209, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 224, 226, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 242, 245, 247, 248, 251, 253, 255, 256, 257, 258, 259, 260, 261, 263, 264, 265, 266, 267, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 297, 298, 300, 301, 302, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 320, 321, 323, 324, 325, 326, 327, 328, 331, 334, 335, 338, 339, 342, 343, 347, 348, 349, 351, 353, 356, 357, 358, 359, 360, 361, 367, 368, 369, 371, 373, 374, 375, 376, 377, 378, 379, 380, 381, 383, 385, 387, 388, 389, 391, 392, 393, 394, 395, 396, 397, 398, 399, 401, 403, 404, 405, 406, 407, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 425, 426, 427, 428, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3 or 7;

corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3; and

provided that if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 13, 47, 131, or 219 the replacement is not replacement with an Alanine (A).

50. The modified PH20 polypeptide of claim 49, wherein:

the modified PH20 polypeptide exhibits at least 40% of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement; and

activity is compared under the same conditions.

51. The modified PH20 polypeptide of claim 49 or claim 50, wherein the amino acid replacement(s) is in a PH20 polypeptide that has a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 32-66, 69, or 72.

52. The modified PH20 polypeptide of any of claims 49-51, wherein the amino acid replacement is an amino acid replacement set forth in Table 3.

53. The modified PH20 polypeptide of any of claims 49-52, comprising at least one amino acid replacement at an amino acid position corresponding to a position selected from among 1, 6, 8, 9, 10, 11, 12, 14, 15, 20, 22, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 46, 47, 48, 49, 50, 52, 58, 59, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 79, 82, 83, 84, 86, 87, 89, 90, 92, 93, 94, 97, 102, 104, 107, 114, 118, 120, 127, 128, 130, 131, 132, 135, 138, 139, 140, 141, 142, 143, 146, 147, 148, 149, 150, 151, 152, 155, 156, 158, 160, 162, 163, 164, 165, 166, 167, 169, 170, 172, 173, 174, 175, 178, 179, 193, 195, 196, 198, 204, 205, 206, 209, 212, 213, 215, 219, 220, 221, 222, 232, 233, 234, 235, 236, 237, 238, 240, 247, 248, 249, 257, 258, 259, 260, 261, 263, 267, 269, 271, 272, 273, 274, 276, 277, 278, 279, 282, 283, 285, 287, 289, 291, 292, 293, 305, 307, 308, 309, 310, 313, 314, 315, 317, 318, 320, 321, 324, 325, 326, 328, 335, 347, 349, 351, 353, 356, 359, 367, 368, 369, 371, 373, 374, 375, 376, 377, 380, 381, 383, 385, 389, 392, 393, 395, 396, 399, 401, 404, 405, 406, 407, 409, 410, 412, 416, 418, 419, 421, 425, 427, 428, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446 or 447 with reference to amino acid positions set forth in SEQ ID NO:3.

54. The modified PH20 polypeptide of any of claims 49-53, comprising at least one amino acid replacement selected from among replacement with:

histidine (H) at a position corresponding to position 1; A at a position corresponding to position 1; E at a position corresponding to position 1; G at a

position corresponding to position 1; K at a position corresponding to position 1; Q at a position corresponding to position 1; R at a position corresponding to position 1; A at a position corresponding to position 6; M at a position corresponding to position 8; Q at a position corresponding to position 9; G at a position corresponding to position 10; H at a position corresponding to position 10; S at a position corresponding to position 11; E at a position corresponding to position 12; I at a position corresponding to position 12; K at a position corresponding to position 12; T at a position corresponding to position 12; V at a position corresponding to position 14; V at a position corresponding to position 15; M at a position corresponding to position 15; S at a position corresponding to position 20; T at a position corresponding to position 22; E at a position corresponding to position 24; H at a position corresponding to position 24; R at a position corresponding to position 24; A at a position corresponding to position 26; E at a position corresponding to position 26; K at a position corresponding to position 26; M at a position corresponding to position 26; Q at a position corresponding to position 26; R at a position corresponding to position 26; D at a position corresponding to position 27; K at a position corresponding to position 27; R at a position corresponding to position 27; R at a position corresponding to position 28; E at a position corresponding to position 29; I at a position corresponding to position 29; K at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; R at a position corresponding to position 29; S at a position corresponding to position 29; T at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; L at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; G at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; W at a position corresponding to position 31; C

at a position corresponding to position 32; F at a position corresponding to position 32; G at a position corresponding to position 32; H at a position corresponding to position 32; W at a position corresponding to position 33; G at a position corresponding to position 33; W at a position corresponding to position 34; Q at a position corresponding to position 35; V at a position corresponding to position 35; H at a position corresponding to position 36; N at a position corresponding to position 36; F at a position corresponding to position 37; M at a position corresponding to position 37; Y at a position corresponding to position 38; A at a position corresponding to position 39; L at a position corresponding to position 39; N at a position corresponding to position 39; T at a position corresponding to position 39; L at a position corresponding to position 40; T at a position corresponding to position 41; L at a position corresponding to position 46; R at a position corresponding to position 46; D at a position corresponding to position 47; F at a position corresponding to position 47; T at a position corresponding to position 47; W at a position corresponding to position 47, with F at a position corresponding to position 48; H at a position corresponding to position 48; K at a position corresponding to position 48; N at a position corresponding to position 48; R at a position corresponding to position 49; D at a position corresponding to position 50; S at a position corresponding to position 50; M at a position corresponding to position 50; N at a position corresponding to position 52; Q at a position corresponding to position 52; R at a position corresponding to position 52; S at a position corresponding to position 52; T at a position corresponding to position 52; C at a position corresponding to position 58; K at a position corresponding to position 58; L at a position corresponding to position 58; P at a position corresponding to position 58; Q at a position corresponding to position 58; R at a position corresponding to position 58; H at a position corresponding to position 58; N at a position corresponding to position 58; Y at a position corresponding to position 58; N at a position corresponding to position 59; K at a position corresponding to position 63; L at a position corresponding to position 63; M at a position corresponding to position 63; R at a position corresponding to position 63; W at a position corresponding to position 63; V at a position corresponding to position 67; H at a position corresponding to position 68; P at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position 69; C at a position corresponding to position 69; E at a position corresponding to position 69; F

at a position corresponding to position 69; G at a position corresponding to position 69; I at a position corresponding to position 69; L at a position corresponding to position 69; M at a position corresponding to position 69; P at a position corresponding to position 69; R at a position corresponding to position 69; T at a position corresponding to position 69; W at a position corresponding to position 69; Y at a position corresponding to position 69; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; N at a position corresponding to position 70; P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; V at a position corresponding to position 70; Y at a position corresponding to position 70; G at a position corresponding to position 71; N at a position corresponding to position 71; R at a position corresponding to position 71; S at a position corresponding to position 71; K at a position corresponding to position 72; M at a position corresponding to position 72; Q at a position corresponding to position 72; A at a position corresponding to position 73; H at a position corresponding to position 73; K at a position corresponding to position 73; L at a position corresponding to position 73; Q at a position corresponding to position 73; R at a position corresponding to position 73; T at a position corresponding to position 73; W at a position corresponding to position 73; A at a position corresponding to position 74; C at a position corresponding to position 74; E at a position corresponding to position 74; F at a position corresponding to position 74; G at a position corresponding to position 74; H at a position corresponding to position 74; K at a position corresponding to position 74; L at a position corresponding to position 74; M at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; F at a position corresponding to position 75; L at a position corresponding to position 75; M at position corresponding to position 75; R at a position corresponding to position 75; T at a position corresponding to position 75; L at a position corresponding to position 79; L at a position corresponding to position 82; N at a

position corresponding to position 82; V at a position corresponding to position 83; Q at a position corresponding to position 83; S at a position corresponding to position 83; G at a position corresponding to position 83; E at a position corresponding to position 84; F at a position corresponding to position 84; G at a position corresponding to position 84; N at a position corresponding to position 84; R at a position corresponding to position 84; A at a position corresponding to position 86; H at a position corresponding to position 86; K at a position corresponding to position 86; N at a position corresponding to position 86; S at a position corresponding to position 86; T at a position corresponding to position 86; W at a position corresponding to position 86; C at a position corresponding to position 87; G at a position corresponding to position 87; L at a position corresponding to position 87; M at a position corresponding to position 87; R at a position corresponding to position 87; S at a position corresponding to position 87; T at a position corresponding to position 87; V at a position corresponding to position 87; Y at a position corresponding to position 87; C at a position corresponding to position 89; A at a position corresponding to position 90; E at a position corresponding to position 90; H at a position corresponding to position 90; K at a position corresponding to position 90; N at a position corresponding to position 90; R at a position corresponding to position 90; C at a position corresponding to position 92; L at a position corresponding to position 92; I at a position corresponding to position 93; L at a position corresponding to position 93; Q at a position corresponding to position 93; R at a position corresponding to position 93; S at a position corresponding to position 93; T at a position corresponding to position 93; D at a position corresponding to position 94; Q at a position corresponding to position 94; R at a position corresponding to position 94; A at a position corresponding to position 97; C at an amino acid residue corresponding to position 97; D at a position corresponding to position 97; E at a position corresponding to position 97; G at a position corresponding to position 97; L at a position corresponding to position 97; S at a position corresponding to position 97; S at a position corresponding to position 102; T at a position corresponding to position 102; R at a position corresponding to position 104; L at a position corresponding to position 107; A at a position corresponding to position 114; Q at a position corresponding to position 118; H at a position corresponding to position 120; F at a position corresponding to position 120; I at a position corresponding to position 120; S at a position corresponding to position 120;

V at a position corresponding to position 120; Y at a position corresponding to position 120; E at a position corresponding to position 127; H at a position corresponding to position 127; N at a position corresponding to position 127; Q at a position corresponding to position 127; R at a position corresponding to position 127; I at a position corresponding to position 128; R at a position corresponding to position 130; G at a position corresponding to position 131; I at a position corresponding to position 131; M at a position corresponding to position 131; Q at a position corresponding to position 131; R at a position corresponding to position 131; V at a position corresponding to position 131; N at a position corresponding to position 132; L at a position corresponding to position 132; D at a position corresponding to position 135; G at a position corresponding to position 135; R at a position corresponding to position 135, with L at a position corresponding to position 138; T at a position corresponding to position 139; K at a position corresponding to position 140; H at a position corresponding to position 141; R at a position corresponding to position 141; S at a position corresponding to position 141; W at a position corresponding to position 141; Y at a position corresponding to position 141; D at a position corresponding to position 142; G at a position corresponding to position 142; K at a position corresponding to position 142; N at a position corresponding to position 142; P at a position corresponding to position 142; Q at a position corresponding to position 142; R at a position corresponding to position 142; S at a position corresponding to position 142; T at a position corresponding to position 142; G at a position corresponding to position 143; K at a position corresponding to position 143; R at a position corresponding to position 144; T at a position corresponding to position 144; P at a position corresponding to position 146; R at a position corresponding to position 146; A at a position corresponding to position 147; F at a position corresponding to position 147; L at a position corresponding to position 147; R at a position corresponding to position 147; S at a position corresponding to position 147; V at a position corresponding to position 147; H at a position corresponding to position 148; K at a position corresponding to position 148; Q at a position corresponding to position 148; T at a position corresponding to position 149; V at a position corresponding to position 149; A at a position corresponding to position 150; D at a position corresponding to position 150; G at a position corresponding to position 150; N at a position corresponding to position 150; S at a position corresponding to position 150; W at a position corresponding to

position 150; Y at a position corresponding to position 150; A at a position corresponding to position 151; H at a position corresponding to position 151; K at a position corresponding to position 151; L at a position corresponding to position 151; M at a position corresponding to position 151; Q at a position corresponding to position 151; R at a position corresponding to position 151; S at a position corresponding to position 151; T at a position corresponding to position 151; V at a position corresponding to position 151; W at a position corresponding to position 151; Y at a position corresponding to position 151; R at a position corresponding to position 152; T at a position corresponding to position 152; W at a position corresponding to position 152; D at a position corresponding to position 155; G at a position corresponding to position 155; K at a position corresponding to position 155; R at a position corresponding to position 155; D at a position corresponding to position 156; Q at a position corresponding to position 158; S at a position corresponding to position 158; S at a position corresponding to position 160; E at a position corresponding to position 162; A at a position corresponding to position 163; E at a position corresponding to position 163; K at a position corresponding to position 163; Q at a position corresponding to position 163; R at a position corresponding to position 163; S at a position corresponding to position 163; M at a position corresponding to position 164; V at a position corresponding to position 164; D at a position corresponding to position 165; F at a position corresponding to position 165; N at a position corresponding to position 165; S at a position corresponding to position 165; V at a position corresponding to position 165; A at a position corresponding to position 166; E at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; Q at a position corresponding to position 166; R at a position corresponding to position 166; T at a position corresponding to position 166; W at a position corresponding to position 166; Y at a position corresponding to position 166; D at a position corresponding to position 167; L at a position corresponding to position 169; R at a position corresponding to position 170; A at a position corresponding to position 172; R at a position corresponding to position 173; G at a position corresponding to position 174; K at a position corresponding to position 174; N at a position corresponding to position 174; R at a position corresponding to position 174; T at a position corresponding to position 174; T at a position corresponding to position 175; K at a

position corresponding to position 178; R at a position corresponding to position 178; K at a position corresponding to position 179; Q at a position corresponding to position 193; T at a position corresponding to position 195; N at a position corresponding to position 195; with E at a position corresponding to position 196; R at a position corresponding to position 196; with D at a position corresponding to position 198; P at a position corresponding to position 204; A at a position corresponding to position 205; E at a position corresponding to position 205; L at a position corresponding to position 205; T at a position corresponding to position 205; I at a position corresponding to position 206; K at a position corresponding to position 206; L at a position corresponding to position 206; R at a position corresponding to position 206; R at a position corresponding to position 209; N at a position corresponding to position 212; S at a position corresponding to position 212; A at a position corresponding to position 213; M at a position corresponding to position 213; N at a position corresponding to position 213; H at a position corresponding to position 215; M at a position corresponding to position 215; I at a position corresponding to position 219; K at a position corresponding to position 219; S at a position corresponding to position 219; H at a position corresponding to position 220; I at a position corresponding to position 220; L at a position corresponding to position 220; V at a position corresponding to position 220; Q at a position corresponding to position 221; G at a position corresponding to position 222; F at a position corresponding to position 232; g at a position corresponding to position 233; K at a position corresponding to position 233; R at a position corresponding to position 233; M at a position corresponding to position 234; A at a position corresponding to position 235; R at a position corresponding to position 236; C at a position corresponding to position 237; E at a position corresponding to position 237; H at a position corresponding to position 237; Q at a position corresponding to position 237; T at a position corresponding to position 237; E at a position corresponding to position 238; H at a position corresponding to amino acid position 238; S at a position corresponding to position 238; A at a position corresponding to position 240; Q at a position corresponding to position 240; I at a position corresponding to position 247; A at a position corresponding to position 248; V at a position corresponding to position 249; G at a position corresponding to position 257; T at a position corresponding to position 257; R at a position corresponding to position 257; N at a position corresponding to position 258; S at a position corresponding to position 258;

P at a position corresponding to position 259; M at a position corresponding to position 260; Y at a position corresponding to position 260; A at a position corresponding to position 261; K at a position corresponding to position 261; N at a position corresponding to position 261; K at a position corresponding to position 263; R at a position corresponding to position 263; T at a position corresponding to position 267; A at a position corresponding to position 269; L at a position corresponding to position 271; M at a position corresponding to position 271; D at a position corresponding to position 272; T at a position corresponding to position 272; H at a position corresponding to position 273; Y at a position corresponding to position 273; F at a position corresponding to position 274; D at a position corresponding to position 276; H at a position corresponding to position 276; M at a position corresponding to position 276; R at a position corresponding to position 276; S at a position corresponding to position 276; Y at a position corresponding to position 276; A at a position corresponding to position 277; E at a position corresponding to position 277; H at a position corresponding to position 277; K at a position corresponding to position 277; M at a position corresponding to position 277; N at a position corresponding to position 277; Q at a position corresponding to position 277; R at a position corresponding to position 277; S at a position corresponding to position 277; T at a position corresponding to position 277; E at a position corresponding to position 278; F at a position corresponding to position 278; G at a position corresponding to position 278; H at a position corresponding to position 278; K at a position corresponding to position 278; N at a position corresponding to position 278; R at a position corresponding to position 278; S at a position corresponding to position 278; T at a position corresponding to position 278; Y at a position corresponding to position 278; H at a position corresponding to position 279; M at a position corresponding to position 282; S at a position corresponding to position 283; H at a position corresponding to position 285; T at a position corresponding to position 287; S at a position corresponding to position 289; S at a position corresponding to position 291; V at a position corresponding to position 291; C at a position corresponding to position 292; F at a position corresponding to position 292; H at a position corresponding to position 292; K at a position corresponding to position 292; R at a position corresponding to position 292; V at a position corresponding to position 292; A at a position corresponding to position 293; C at a position corresponding to position 293; D at a position

corresponding to position 293; F at a position corresponding to position 293; K at a position corresponding to position 293; M at a position corresponding to position 293; P at a position corresponding to position 293; Q at a position corresponding to position 293; V at a position corresponding to position 293; Y at a position corresponding to position 293; G at a position corresponding to position 298; E at a position corresponding to position 305; G at a position corresponding to position 307; D at a position corresponding to position 308; G at a position corresponding to position 308; K at a position corresponding to position 308; N at a position corresponding to position 308; R at a position corresponding to position 308; E at a position corresponding to position 309; G at a position corresponding to position 309; H at a position corresponding to position 309; L at a position corresponding to position 309; M at a position corresponding to position 309; N at a position corresponding to position 309; Q, at a position corresponding to position 309; R at a position corresponding to position 309; S at a position corresponding to position 309; T at a position corresponding to position 309; V at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; Q at a position corresponding to position 310; S at a position corresponding to position 310; A at a position corresponding to position 313; G at a position corresponding to position 313; H at a position corresponding to position 313; K at a position corresponding to position 313; P at a position corresponding to position 313; R at a position corresponding to position 313; T at a position corresponding to position 313; Y at a position corresponding to position 313; with S at a position corresponding to position 314; Y at a position corresponding to position 314; A at a position corresponding to position 315; H at a position corresponding to position 315; Y at a position corresponding to position 315; A at a position corresponding to position 317; I at a position corresponding to position 317; K at a position corresponding to position 317; N at a position corresponding to position 317; Q at a position corresponding to position 317; R at a position corresponding to position 317; S at a position corresponding to position 317; T at a position corresponding to position 317; W at a position corresponding to position 317; D at a position corresponding to position 318; H at a position corresponding to position 318; K at a position corresponding to position 318; M at a position corresponding to position 318; R at a position corresponding to position 318; H at a position corresponding to position 320; K at a position corresponding to position 320;

R at a position corresponding to position 320; R at a position corresponding to position 321; S at a position corresponding to position 321; N at a position corresponding to position 324; R at a position corresponding to position 324; A at a position corresponding to position 325; D at a position corresponding to position 325; E at a position corresponding to position 325; G at a position corresponding to position 325; H at a position corresponding to position 325; K at a position corresponding to position 325; M at a position corresponding to position 325; N at a position corresponding to position 325; Q at a position corresponding to position 325; S at a position corresponding to position 325; V at a position corresponding to position 325; L at a position corresponding to position 326; V at a position corresponding to position 326; C at a position corresponding to position 328; G at a position corresponding to position 328; I at a position corresponding to position 328; K at a position corresponding to position 328; L at a position corresponding to position 328; S at a position corresponding to position 328; Y at a position corresponding to position 328; S at a position corresponding to position 335; A at a position corresponding to position 347; G at a position corresponding to position 347; S at a position corresponding to position 347; M at a position corresponding to position 349; R at a position corresponding to position 349; S at a position corresponding to position 351; V at a position corresponding to position 353; with H at a position corresponding to position 356; S at a position corresponding to position 356; E at a position corresponding to position 359; H at a position corresponding to position 359; T at a position corresponding to position 359; A at a position corresponding to position 367; G at a position corresponding to position 367; K at a position corresponding to position 367; S at a position corresponding to position 367; A at a position corresponding to position 368; E at a position corresponding to position 368; K at a position corresponding to position 368; L at a position corresponding to amino acid position 368; M at a position corresponding to amino acid position 368; R at a position corresponding to position 368; T at a position corresponding to amino acid position 368; H at a position corresponding to position 369; R at a position corresponding to position 369; F at a position corresponding to position 371; H at a position corresponding to position 371; K at a position corresponding to position 371; L at a position corresponding to position 371; R at a position corresponding to position 371; S at a position corresponding to position 371; M at a position corresponding to position 373; H at a position corresponding to

position 374; P at a position corresponding to position 374; A at a position corresponding to position 375; G at a position corresponding to position 375; K at a position corresponding to position 375; R at a position corresponding to position 375; D at a position corresponding to position 376; E at a position corresponding to position 376; Q at a position corresponding to position 376; R at a position corresponding to position 376; T at a position corresponding to position 376; V at a position corresponding to position 376; Y at a position corresponding to position 376; D at a position corresponding to position 377; E at a position corresponding to position 377; H at a position corresponding to position 377; K at a position corresponding to position 377; P at a position corresponding to position 377; R at a position corresponding to position 377; S at a position corresponding to position 377; T at a position corresponding to position 377; W at a position corresponding to position 380; Y at a position corresponding to position 380; S at a position corresponding to position 381; I at a position corresponding to position 383; K at a position corresponding to position 383; L at a position corresponding to position 383; S at a position corresponding to position 383; A at a position corresponding to position 385; Q at a position corresponding to position 385; V at a position corresponding to position 385; A at a position corresponding to position 389; G at a position corresponding to position 389; L at a position corresponding to position 389; K at a position corresponding to position 389; Q at a position corresponding to position 389; S at a position corresponding to position 389; A at a position corresponding to position 392; F at a position corresponding to position 392; M at a position corresponding to position 392; Q at a position corresponding to position 392; R at a position corresponding to position 392; V at a position corresponding to position 392; F at a position corresponding to position 393; M at a position corresponding to position 393; A at a position corresponding to position 395; H at a position corresponding to position 395; R at a position corresponding to position 395; A at a position corresponding to position 396; H at a position corresponding to position 396; Q at a position corresponding to position 396; S at a position corresponding to position 396; K at a position corresponding to position 399; M at a position corresponding to position 399; T at a position corresponding to position 399; V at a position corresponding to position 399; W at a position corresponding to position 399; A at a position corresponding to position 401; E at a position corresponding to position 401; A at a position corresponding to position 404; G at a

position corresponding to position 405; F at a position corresponding to position 406; N at a position corresponding to position 406; A at a position corresponding to position 407; D at a position corresponding to position 407; E at a position corresponding to position 407; F at a position corresponding to position 407; H at a position corresponding to position 407; Q at a position corresponding to position 407; P at a position corresponding to position 407; A at a position corresponding to position 409; Q at a position corresponding to position 409; T at a position corresponding to position 410; Q at a position corresponding to position 412; R at a position corresponding to position 412; V at a position corresponding to position 412; L at a position corresponding to position 416; E at a position corresponding to position 418; L at a position corresponding to position 418; P at a position corresponding to position 418; R at a position corresponding to position 418; V at a position corresponding to position 418; F at a position corresponding to position 419; H at a position corresponding to position 419; I at a position corresponding to position 419; K at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; Y at a position corresponding to position 419; A at a position corresponding to position 421; H at a position corresponding to position 421; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; G at a position corresponding to position 425; K at a position corresponding to position 425; Q at a position corresponding to position 427; T at a position corresponding to position 427; L at a position corresponding to position 428; A at a position corresponding to position 431; G at a position corresponding to position 431; E at a position corresponding to position 431; H at a position corresponding to position 431; K at a position corresponding to position 431; L at a position corresponding to position 431; N at a position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to position 431; A at a position corresponding to position 433; H at a position corresponding to position 433; I at a position corresponding to position 433; K at a position corresponding to position 433; L at a position corresponding to position 433; R at a position corresponding to position 433; T at a position corresponding to position 433; V at a position corresponding to position 433; W at a

position corresponding to position 433; K at a position corresponding to position 436; I at a position corresponding to position 437; M at a position corresponding to position 437; A at a position corresponding to position 438; D at a position corresponding to position 438; E at a position corresponding to position 438; L at a position corresponding to position 438; N at a position corresponding to position 438; T at a position corresponding to position 438; A at a position corresponding to position 439; C at a position corresponding to position 439; K at a position corresponding to position 439; P at a position corresponding to position 439; Q at a position corresponding to position 439; T at a position corresponding to position 439; V at a position corresponding to position 439; D at a position corresponding to position 440; H at a position corresponding to position 440; M at a position corresponding to position 440; P at a position corresponding to position 440; R at a position corresponding to position 440; S at a position corresponding to position 440; A at a position corresponding to position 441; F at a position corresponding to position 441; C at a position corresponding to position 442; G at a position corresponding to position 442; R at a position corresponding to position 442; A at a position corresponding to position 443; E at a position corresponding to position 443; F at a position corresponding to position 443; G at a position corresponding to position 443; M at a position corresponding to position 443; N at a position corresponding to position 443; E at a position corresponding to position 444; H at a position corresponding to position 444; V at a position corresponding to position 444; H at a position corresponding to position 445; M at a position corresponding to position 445; N at a position corresponding to position 445; P at a position corresponding to position 445; Q at a position corresponding to position 445; S at a position corresponding to position 445; T at a position corresponding to position 445; V at a position corresponding to position 445; W at a position corresponding to position 445; A at a position corresponding to position 446; A at a position corresponding to position 446; M at a position corresponding to position 446; W at a position corresponding to position 446; D at a position corresponding to position 447; E at a position corresponding to position 447; G at a position corresponding to position 447; I at a position corresponding to position 447; N at a position corresponding to position 447; P at a position corresponding to position 447; Q at a position corresponding to position 447; T at a position corresponding to position 447,

and/or replacement with V at a position corresponding to position 447, each with reference to amino acid positions set forth in SEQ ID NO:3.

55. The modified PH20 polypeptide of any of claims 49-54, wherein:

the modified PH20 polypeptide exhibits 40% to 5000%, 40% to 2000%, 40% to 1000%, 40% to 500%, 80% to 2000%, or 80% to 600% of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement; and

activity is compared under the same conditions.

56. The modified PH20 polypeptide of any of claims 49-55, wherein:

the modified PH20 polypeptide exhibits at least 50%, 60%, 70%, 80%, 90%, 100%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement; and

activity is compared under the same conditions.

57. A modified PH20 polypeptide, comprising at least one amino acid replacement in a PH20 polypeptide set forth in SEQ ID NO:7, a C-terminally truncated fragment thereof or in a PH20 polypeptide that has a sequence of amino acids that is at least 91% identical to the sequence of amino acids set forth in SEQ ID NO:7, wherein:

the modified PH20 polypeptides exhibits less than 20% of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement;

activity is compared under the same conditions;

the amino acid replacement(s) is at an amino acid position corresponding to a position selected from among 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 94, 95, 96, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 143, 144, 145, 149, 150, 152, 153, 154, 155, 156, 157, 158, 159, 161, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 197, 198, 199, 200, 201, 202, 203, 204, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229,

230, 231, 232, 233, 234, 235, 236, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 279, 280, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 196, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 331, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 408, 410, 411, 412, 413, 414, 415, 416, 417, 419, 420, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 434, 437, 438, 439, 440, 441, 442, 443, 444, or 447 with reference to amino acid positions set forth in SEQ ID NO:3 or 7;

corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide set forth in SEQ ID NO:3; and

provided that:

(i) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 200, 333, 358 or 393 the replacement is not replacement with an Alanine (A).

(ii) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 111 or 249 the replacement is not replacement with an asparagine (N);

(iii) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 113 the replacement is not replacement with a glutamine (Q);

(iv) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 176 the replacement is not replacement with a glycine (G); and

(v) if the modified PH20 polypeptide contains an amino acid replacement at a position corresponding to position 252 the replacement is not replacement with a threonine (T).

58. The modified PH20 polypeptide of 57, wherein the amino acid replacement(s) is in a PH20 polypeptide that has a sequence of amino acids set forth in any of SEQ ID NOS: 3, 7, 32-66, 69, or 72.

59. The modified PH20 polypeptide of claim 57 or claim 58, wherein the modified PH20 polypeptide exhibits less than 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% or less of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement.

60. The modified PH20 polypeptide of any of claims 57-59, wherein the amino acid replacement is an amino acid replacement set forth in Table 5.

61. The modified PH20 polypeptide of any of claims 1-60, wherein the modified PH20 does not consist of the sequence of amino acids set forth in any of SEQ ID NOS: 3, 6-66, 69, 72, 856-861, 869 or 870.

62. The modified PH20 polypeptide of any of claims 1-61, whereby the amino acid replacements are in a PH20 polypeptide having a sequence of amino acids set forth any of SEQ ID NO: 3, 7, 69 or 72 provided that:

(i) where the modified PH20 polypeptide includes only a single amino acid replacement the replacement does not corresponds to amino acid replacements V12A, N47A, D111N, E113Q, N131A, R176G, N200A, N219A, E249Q, R252T, N333A or N358A, with reference to amino acid positions set forth in SEQ ID NO:3;

(ii) where the modified PH20 polypeptide includes only two amino acid replacements the replacements do not correspond to amino acid replacements P13A/L464W, N47A/N131A, N47A/N219A, N131A/N219A or N333A/N358A with reference to positions set forth in SEQ ID NO:3; and

(iii) where the modified PH20 polypeptide includes only three amino acid replacements the replacements doe not correspond to amino acid replacements N47A/N131A/N219A, with reference to amino acid positions set forth in SEQ ID NO:3.

63. The modified PH20 polypeptide of any of claims 1-62, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,49, 50, 51, 53, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 59,70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 82, 82, 83, 84, 85, 86, 87, 88, 89, 90, or more of the amino acid replacements.

64. The modified PH20 polypeptide of any of claims 1-63 that is a mature PH20 polypeptide lacking the signal sequence.

65. A modified PH20 polypeptide, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 73-855 or a sequence of amino acids that exhibits at least 75%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a sequence of amino acids set forth in any of SEQ ID NOS: 73-855, wherein the modified PH20 polypeptide comprises at least one amino acid replacement compared to the sequence of amino acids set forth in SEQ ID NO:3.

66. The modified PH20 polypeptide of any of claims 1-65 that is substantially purified or isolated.

67. The modified PH20 polypeptide of any of claims 1-66 that exhibits catalytic activity at neutral pH.

68. The modified PH20 polypeptide of claim 67, wherein the modified PH20 polypeptide is secreted upon expression from cells and is soluble in the supernatant.

69. The modified PH20 polypeptide of any of claims 1-68 that is modified by modification selected from among glycosylation, sialation, albumination, farnysylation, carboxylation, hydroxylation and phosphorylation.

70. The modified PH20 polypeptide of claim 69, wherein the modified PH20 polypeptide is glycosylated, whereby the polypeptide comprises at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues.

71. The modified PH20 polypeptide of claim 70, wherein the three asparagine residues correspond to amino acid residues 200, 333 and 358 of SEQ ID NO:3.

72. The modified PH20 polypeptide of any of claims 1-71 that is modified by conjugation to a polymer.

73. The modified PH20 polypeptide of claim 72, wherein the polymer is dextran or PEG.

74. The modified PH20 polypeptide of any of claims 1-71, wherein the modified PH20 polypeptide is conjugated to a moiety selected from among a multimerization domain, toxin, detectable label or drug.

75. The modified PH20 polypeptide of claim 74, wherein the modified PH20 polypeptide is conjugated to an Fc domain.

76. A nucleic acid molecule, encoding a modified PH20 polypeptide of any of claims 1-68.

77. A vector, comprising the nucleic acid molecule of claim 76.

78. The vector of claim 77 that is a eukaryotic or a prokaryotic vector.

79. The vector of claim 77 or claim 78 that is a mammalian vector or a viral vector.

80. The vector of claim 79 that is a viral vector, wherein the viral vector is an adenovirus vector, a retrovirus vector or a vaccinia virus vector.

81. A cell, comprising the vector of any of claims 77-80.

82. The cell of claim 81 that is a mammalian cell.

83. The cell of claim 82, wherein the mammalian cell is a Chinese Hamster Ovary (CHO) cell.

84. A pharmaceutical composition, comprising a modified PH20 polypeptide of any of claims 1-36.

85. The pharmaceutical composition of claim 84, wherein the modified PH20 polypeptide in the composition is stable at a temperature from or from about 2°C to 8°C, inclusive, for at least 1 month or is stable at a temperature from or from about 30°C to 42°C, inclusive, for at least 3 days.

86. The pharmaceutical composition of claim 84 or claim 85, wherein the modified PH20 polypeptide in the composition is stable at a temperature from or from about 2°C to 8°C, inclusive, for at least 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months or 30 months.

87. The pharmaceutical composition of any of claim 84 or claim 85, wherein the modified PH20 polypeptide in the composition is stable at a temperature from or from about 30°C to 42°C, inclusive, for at least 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 35 days, 40 days, 45 days, 50 days, 60 days or more.

88. The pharmaceutical composition of claim 87, wherein the modified PH20 polypeptide is stable in the composition at or about 37°C for at least 3 days, 4 days, 5 days, 6 days, 7 days, 14 days, 21 days, or 30 days.

89. A pharmaceutical composition, comprising a modified PH20 polypeptide 37-75.

90. The pharmaceutical composition of any of claims 84-89, wherein the pharmaceutical composition comprises a pharmaceutically acceptable excipient.

91. The pharmaceutical composition of any of claims 84-90 that is for single dose administration

92. The pharmaceutical composition of any of claims 84-91 that is for multiple dose administration.

93. The pharmaceutical composition of any of claims 84-92, wherein the amount of modified PH20 is between or about between 0.1 μg/mL to 100 μg/mL, 1 μg/mL to 50 μg/mL or 1 μg/mL to 20 μg/mL.

94. The pharmaceutical composition of any of claims 84-93, wherein the amount of a modified PH20 is between or about between 10 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 2000 U/mL, or 100 U/mL to 1000 U/mL.

95. The pharmaceutical composition of any of claims 84-94, comprising NaCl at a concentration less than or about or 200 mM, 180 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80mM, 70mM, 60 mM, 50 mM, 40 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM or less.

96. The pharmaceutical composition of any of claims 84-95, comprising NaCl at a concentration between or about between 0.1 mM to 200 mM, 0.1mM to 100 mM, 120 mM to 200 mM, 10 mM to 50 mM, 10 mM to 90 mM, 80 mM to 200 mM, 80 mM to 140 mM, 50 mM to 100 mM, 80 mM to 100 mM, 50 mM to 80 mM, 100 mM to 140 mM or 120 mM to 140 mM.

97. The pharmaceutical composition of any of claims 84-96, comprising an anti-microbially effective amount of a preservative or mixture of preservatives.

98. The pharmaceutical composition of claim 97, wherein the preservative(s) is a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s).

99. The pharmaceutical composition of claim 97 or claim 98, wherein the preservative(s) is(are) selected from among phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorohexidine, sodium dehydroacetate, o-cresol, p-cresol, chlorcresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, buytlparaben and any combinations thereof.

100. The pharmaceutical composition of any of claims 97-99, wherein the composition contains a single preservative.

101. The pharmaceutical composition of any of claims 97-100, wherein the composition contains a mixture of preservatives that contains 2, 3 or 4 different preservatives.

102. The pharmaceutical composition of any of claims 84-101, comprising at least one phenolic preservative.

103. The pharmaceutical composition of any of claims 84-102, wherein the preservative(s) is(are) selected from among phenol, metacresol (m-cresol), benzyl alcohol, and parabens.

104. The pharmaceutical composition of claim 103, wherein the paraben is methylparaben or propylparaben.

105. The pharmaceutical composition of any of claims 97-104, wherein the anti-microbial effective amount is a total amount of one or more preservative agents as a percentage (%) of mass concentration (w/v) that is or is between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive.

106. The pharmaceutical composition of any of claims 97-105, wherein the preservatives are phenol, m-cresol or phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.1% to 0.25% phenol and between or about between 0.05% to 0.2% m-cresol, is between or about between 0.10% to 0.2% phenol and between or about between 0.6% to 01.8% m-cresol, between or about between 0.1% to 0.15% phenol and 0.8% t 0.15% m-cresol, is between or about between 0.10% to 0.15% phenol and between or about between 0.06 to 0.09% m-cresol or is between or about between 0.12% to 0.18% phenol and between or about between 0.14 to 0.22% m-cresol.

107. The pharmaceutical composition of any of claims 84-106, comprising a therapeutically active agent.

108. The pharmaceutical composition of claim 107, wherein the therapeutic agent is formulated with the composition or in a separate composition.

109. The pharmaceutical composition of claim 107 or claim 108, wherein the therapeutic agent is a polypeptide, a protein, a nucleic acid, a drug, a small molecule or an organic molecule.

110. The pharmaceutical composition of any of claims 107-109, wherein the therapeutically active agent is selected from among a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, and antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, or a sleep inducer.

111. The pharmaceutical composition of any of claims 107-110, wherein the therapeutic agent is selected from among an antibody, an Immune Globulin, a bisphosphonate, a cytokine, a chemotherapeutic agent, a coagulation factor and an insulin.

112. The pharmaceutical composition of claim 111, wherein the insulin is a fast-acting insulin.

113. The pharmaceutical composition of claim 112, wherein the fast-acting insulin is regular insulin or is an insulin analog.

114. The pharmaceutical composition of claim 112 or 113, wherein the fast-acting insulin is a regular insulin that is a human insulin or a pig insulin.

115. The pharmaceutical composition of any of claims 112-114, wherein the fast-acting insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in SEQ ID NO:863 or an insulin with an A chain with a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:864 and a B chain with a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:864.

116. The pharmaceutical composition of claim 112 or 113, wherein the fast-acting insulin is an insulin analog selected from among insulin lispro, insulin aspart or insulin glulisine.

117. The pharmaceutical composition of claim 116, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:865-867.

118. The pharmaceutical composition of any of claims 112-117, wherein the amount of fast-acting insulin is 10 U/mL to 1000 U/mL, 50 U/mL to 500 U/mL, 100 U/mL to 1000 U/mL or 500 U/mL to 1000 U/mL, inclusive.

119. The pharmaceutical composition of any of claims 107-111, wherein the therapeutic agent is selected from among a Adalimumabs, Agalsidase Betas, Alefacepts, Ampicillins, Anakinras, Antipoliomyelitic Vaccines, Anti-Thymocytes, Azithromycins, Becaplermins, Caspofungins, Cefazolins, Cefepimes, Cefotetans, Ceftazidimes, Ceftriaxones, Cetuximabs, Cilastatins, Clavulanic Acids, Clindamycins, Darbepoetin Alfas, Deaclizumabs, Diphtheria, Diphtheria antitoxins, Diphtheria Toxoids, Efalizumabs, Epinephrines, Erythropoietin Alphas, Etanercepts, Filgrastims, Fluconazoles, Follicle-Stimulating Hormones, Follitropin Alphas, Follitropin Betas, Fosphenyloins, Gadodiamides, Gadopentetates, Gatifloxacins, Glatiramers, GM-CSF's, Goserelins, Goserelin acetates, Granisetrons, Haemophilus Influenza B's, Haloperidols, Hepatitis vaccines, Hepatitis A Vaccines, Hepatitis B Vaccines, Ibritumomab Tiuxetans, Ibritumomabs, Tiuxetans, Immunoglobulins, Hemophilus influenza vaccines, Influenza Virus Vaccines, Infliximabs, Insulin lispro, 75% neutral protamine lispro (NPL)/25% insulin lispro, 50% neutral protamine Hagedorn (NPH)/ 50% regular insulin, 70% NPH/30% regular insulin; Regular insulin, NPH insulin, Ultra insulin, Ultralente insulin, and Insulin Glargines, Interferons, Interferon alpha, Interferon Betas, Interferon Gammas, Interferon alpha-2a, Interferon alpha 2-b, Interferon Alphacon, Interferon alpha-n, Interferon Betas, Interferon Beta-1a's, Interferon Gammas, Interferon alpha-con, Iodixanols, Iohexols, Iopamidols, Ioversols, Ketorolacs, Laronidases, Levofloxacins, Lidocaines, Linezolids, Lorazepams, Measles Vaccines, Measles virus, Mumps viruses, Measles-Mumps-Rubella Virus Vaccines, Rubella vaccines, Medroxyprogesterones, Meropenems, Methylprednisolones, Midazolams, Morphines, Octreotides, Omalizumabs, Ondansetrons, Palivizumabs, Pantoprazoles, Pegaspargases, Pegfilgrastims, Peg-

Interferon Alpha-2a's, Peg-Interferon Alpha-2b's, Pegvisomants, Pertussis vaccines, Piperacillins, Pneumococcal Vaccines and Pneumococcal Conjugate Vaccines, Promethazines, Reteplases, Somatropins, Sulbactams, Sumatriptans, Tazobactams, Tenecteplases, Tetanus Purified Toxoids, Ticarcillins, Tositumomabs, Triamcinolones, Triamcinolone Acetonides, Triamcinolone hexacetonides, Vancomycins, Varicella Zoster imrnunoglobulins, Varicella vaccines, other vaccines, Alemtuzumabs, Alitretinoins, Allopurinols, Altretamines, Amifostines, Anastrozoles, Arsenics, Arsenic Trioxides, Asparaginases, Bacillus Calmette-Guerin (BCG) vaccines, BCG Live, Bexarotenes, Bleomycins, Busulfans, Busulfan intravenous, Busulfan orals, Calusterones, Capecitabines, Carboplatins, Carmustines, Carmustines with Polifeprosans, Celecoxibs, Chlorambucils, Cisplatins, Cladribines, Cyclophosphamides, Cytarabines, Cytarabine liposomals, Dacarbazines, Dactinomycins, Daunorubicin liposomals, Daunorubicins, Daunomycins, Denileukin Diftitoxes, Dexrazoxanes, Docetaxels, Doxorubicins, Doxorubicin liposomals, Dromostanolone propionates, Elliott's B Solutions, Epirubicins, Epoetin alfas, Estramustines, Etoposides, Etoposide phosphates, Etoposide VP-16s, Exemestanes, Floxuridines, Fludarabines, Fluorouracils, 5-Fluorouracils, Fulvestrants, Gemcitabines, Gemtuzumabs, Ozogamicins, Gemtuzumab ozogamicins, Hydroxyureas, Idarubicins, Ifosfamides, Imatinib mesylates, Irinotecans, Letrozoles, Leucovorins, Levamisoles, Lomustines, CCNUs, Meclorethamines, Nitrogen mustards, Megestrols, Megestrol acetates, Melphalans, L-PAMs, Mercaptopurines, 6-Mercaptopurines, Mesnas, Methotrexates, Methoxsalens, Mitomycins, Mitomycin C's, Mitotanes, Mitoxantrones, Nandrolones, Nandrolone Phenpropionates, Nofetumomabs, Oprelvekins, Oxaliplatins, Paclitaxels, Pamidronates, Pegademases, Pentostatins, Pipobromans, Plicamycins, Mithramycins, Porfimers, Porfimer sodiums, Procarbazines, Quinacrines, Rasburicases, Rituximabs, Sargramostims, Streptozocins, Talcs, Tamoxifens, Temozolomides, Teniposides, Testolactones, Thioguanines, 6-Thioguanines, Triethylenethiophosphoramides (Thiotepas), Topotecans, Toremifenes, Trastuzumabs, Tretinoins, Uracil Mustards, Valrubicins, Vinblastines, Vincristines, Vinorelbines, Zoledronates, Acivicins, Aclarubicins, Acodazoles, Acronines, Adozelesins, Aldesleukins, Retinoic Acids, Alitretinoins, 9-Cis-Retinoic Acids, Alvocidibs, Ambazones, Ambomycins, Ametantrones, Aminoglutethimides, Amsacrines, Anaxirones, Ancitabines, Anthramycins, Apaziquones, Argimesnas, Asperlins, Atrimustines, Azacitidines, Azetepas, Azotomycins, Banoxantrones,

Batabulins, Batimastats, Benaxibines, Bendamustines, Benzodepas, Bicalutamides, Bietaserpines, Biricodars, Bisantrenes, Bisnafide Dimesylates, Bizelesins, Bortezomibs, Brequinars, Bropirimines, Budotitanes, Cactinomycins, Canertinibs, Caracemides, Carbetimers, Carboquones, Carmofurs, Carubicins, Carzelesins, Cedefingols, Cemadotins, Chiorambucils, Cioteronels, Cirolemycins, Clanfenurs, Clofarabines, Crisnatols, Decitabines, Dexniguldipines, Dexormaplatins, Dezaguanines, Diaziquones, Dibrospidiums, Dienogests, Dinalins, Disermolides, Dofequidars, Doxifluridines, Droloxifenes, Duazomycins, Ecomustines, Edatrexates, Edotecarins, Eflomithines, Elacridars, Elinafides, Elsamitrucins, Emitefurs, Enloplatins, Enpromates, Enzastaurins, Epipropidines, Eptaloprosts, Erbulozoles, Esorubicins, Etanidazoles, Etoglucids, Etoprines, Exisulinds, Fadrozoles, Fazarabines, Fenretinides, Fluoxymesterones, Flurocitabines, Fosquidones, Fostriecins, Fotretamines, Galarubicins, Galocitabines, Geroquinols, Gimatecans, Gimeracils, Gloxazones, Glufosfamides, Ilmofosines, Ilomastats, Imexons, Improsulfans, Indisulams, Inproquones, Interleukins, Interleukin-2s, recombinant Interleukins, Intoplicines, lobenguanes, Iproplatins, Irsogladines, Ixabepilones, Ketotrexates, L-Alanosines, Lanreotides, Lapatinibs, Ledoxantrones, Leuprolides, Leuprorelins, Lexacalcitols, Liarozoles, Lobaplatins, Lometrexols, Lonafarnibs, Losoxantrones, Lurtotecans, Mafosfamides, Mannosulfans, Marimastats, Masoprocols, Maytansines, Mechiorethamines, Melengestrols, Meiphalans, Menogarils, Mepitiostanes, Metesinds, Metomidates, Metoprines, Meturedepas, Miboplatins, Miproxifenes, Misonidazoles, Mitindomides, Mitocarcins, Mitocromins, Mitoflaxones, Mitogillins, Mitoguazones, Mitomalcins, Mitonafides, Mitoquidones, Mitospers, Mitozolomides, Mivobulins, Mizoribines, Mofarotenes, Mopidamols, Mubritinibs, Mycophenolic Acids, Nedaplatins, Neizarabines, Nemorubicins, Nitracrines, Nocodazoles, Nogalamycins, Nolatrexeds, Nortopixantrones, Ormaplatins, Ortataxels, Oteracils, Oxisurans, Oxophenarsines, Patubilones, Peldesines, Peliomycins, Pelitrexols, Pemetrexeds, Pentamustines, Peplomycins, Perfosfamides, Perifosines, Picoplatins, Pinafides, Piposulfans, Pirfenidones, Piroxantrones, Pixantrones, Plevitrexeds, Plomestanes, Porfiromycins, Prednimustines, Propamidines, Prospidiums, Pumitepas, Puromycins, Pyrazofurins, Ranimustines, Riboprines, Ritrosulfans, Rogletimides, Roquinimexs, Rufocromomycins, Sabarubicins, Safingols, Satraplatins, Sebriplatins, Semustines, Simtrazenes, Sizofirans, Sobuzoxanes, Sorafenibs, Sparfosates, Sparfosic Acids, Sparsomycins, Spirogermaniums, Spiromustines, Spiroplatins, Squalamines,

Streptonigrins, Streptovarycins, Sufosfamides, Sulofenurs, Tacedinalines, Talisomycins, Tallimustines, Tariquidars, Tauromustines, Tecogalans, Tegafurs, Teloxantrones, Temoporfins, Teroxirones, Thiamiprines, Tiamiprines, Tiazofurins, Tilomisoles, Tilorones, Timcodars, Timonacics, Tirapazamines, Topixantrones, Trabectedins, Ecteinascidin 743, Trestolones, Triciribines, Trilostanes, Trimetrexates, Triplatin Tetranitrates, Triptorelins, Trofosfamides, Tubulozoles, Ubenimexs, Uredepas, Vaispodars, Vapreotides, Verteporfins, Vinbiastines, Vindesines, Vinepidines, Vinflunines, Vinformides, Vinglycinates, Vinleucinols, Vinleurosines, Vinrosidines, Vintriptols, Vinzolidines, Vorozoles, Xanthomycin A's, Guamecyclines, Zeniplatins, Zilascorbs [2-H], Zinostatins, Zorubicins, Zosuquidars, Acetazolamides, Acyclovirs, Adipiodones, Alatrofloxacins, Alfentanils, Allergenic extracts, Alpha 1-proteinase inhibitors, Aiprostadils, Amikacins, Amino acids, Aminocaproic acids, Aminophyllines, Amitriptylines, Amobarbitals, Amrinones, Analgesics, Anti-poliomyelitic vaccines, Anti-rabic serums, Anti-tetanus immunoglobulins, tetanus vaccines, Antithrombin III's, Antivenom serums, Argatrobans, Arginines, Ascorbic acids, Atenolols, Atracuriums, Atropines, Aurothioglucoses, Azathioprines, Aztreonams, Bacitracins, Baclofens, Basiliximabs, Benzoic acids, Benztropines, Betamethasones, Biotins, Bivalirudins, Botulism antitoxins, Bretyliums, Bumetanides, Bupivacaines, Buprenorphines, Butorphanols, Calcitonins, Calcitriols, Calciums, Capreomycins, Carboprosts, Carnitines, Cefaniandoles, Cefoperazones, Cefotaximes, Cefoxitins, Ceftizoximes, Cefuroximes, Chioramphenicols, Chioroprocaines, Chioroquines, Chlorothiazides, Chiorpromazines, Chondroitinsulfuric acids, Choriogonadotropin alfas, Chromiums, Cidofovirs, Cimetidines, Ciprofloxacins, Cisatracuriums, Clonidines, Codeines, Coichicines, Colistins, Collagens, Corticorelin ovine triflutates, Corticotrophins, Cosyntropins, Cyanocobalamins, Cyclosporines, Cysteines, Dacliximabs, Dalfopristins, Dalteparins, Danaparoids, Dantrolenes, Deferoxamines, Desmopressins, Dexamethasones, Dexmedetomidines, Dexpanthenols, Dextrans, Iron dextrans, Diatrizoic acids, Diazepams, Diazoxides, Dicyclomines, Digibinds, Digoxins, Dihydroergotamines, Diltiazems, Diphenhydramines, Dipyridamoles, Dobutamines, Dopamines, Doxacuriums, Doxaprams, Doxercalciferols, Doxycyclines, Droperidols, Dyphyllines, Edetic acids, Edrophoniums, Enalaprilats, Ephedrines, Epoprostenols, Ergocalciferols, Ergonovines, Ertapenems, Erythromycins, Esmolols, Estradiols, Estrogenics, Ethacrynic acids, Ethanolamines, Ethanols, Ethiodized oils, Etidronic acids,

Etomidates, Famotidines, Fenoldopams, Fentanyls, Flumazenils, Fluoresceins, Fluphenazines, Folic acids, Fomepizoles, Fomivirsens, Fondaparinuxs, Foscarnets, Fosphenytoins, Furosemides, Gadoteridols, Gadoversetamides, Ganciclovirs, Gentamicins, Glucagons, Glucoses, Glycines, Glycopyrrolates, Gonadorelins, Gonadotropin chorionics, Haemophilus B polysaccarides, Hemins, Herbals, Histamines, Hydralazines, Hydrocortisones, Hydromorphones, Hydroxocobalamins, Hydroxyzines, Hyoscyamines, Ibutilides, Imiglucerases, Indigo carmines, Indomethacins, Iodides, lopromides, Iothalamic acids, loxaglic acids, loxilans, Isoniazids, Isoproterenols, Japanese encephalitis vaccines, Kanamycins, Ketamines, Labetalols, Lepirudins, Levobupivacaines, Levothyroxines, Lincomycins, Liothyronines, Luteinising hormones, Lyme disease vaccines, Mangafodipirs, Manthtols, Meningococcal polysaccharide vaccines, Meperidines, Mepivacaines, Mesoridazines, Metaraminols, Methadones, Methocarbamols, Methohexitals, Methyldopates, Methylergonovines, Metoclopramides, Metoprolols, Metronidazoles, Minocyclines, Mivacuriums, Morrhuic acids, Moxifloxacins, Muromonab-CD3s, Mycophenolate mofetils, Nafcillins, Nalbuphines, Nalmefenes, Naloxones, Neostigmines, Niacinamides, Nicardipines, Nitroglycerins, Nitroprussides, Norepinephrines, Orphenadrines, Oxacillins, Oxymorphones, Oxytetracyclines, Oxytocins, Pancuroniums, Panthenols, Pantothenic acids, Papaverines, Peginterferon alpha 2As, Penicillin Gs, Pentamidines, Pentazocines, Pentobarbitals, Perfiutrens, Perphenazines, Phenobarbitals, Phentolamines, Phenylephrines, Phenytoins, Physostigmines, Phytonadiones, Polymyxin, Pralidoximes, Prilocaines, Procainamides, Procaines, Prochiorperazines, Progesterones, Propranolols, Pyridostigmine hydroxides, Pyridoxines, Quinidines, Quinupristins, Rabies immunoglobulins, Rabies vaccines, Ranitidines, Remifentanils, Riboflavins, Rifampins, Ropivacaines, Samariums, Scopolamines, Seleniums, Sermorelins, Sincalides, Somatrems, Spectinomycins, Streptokinases, Streptomycins, Succinylcholines, Sufentanils, Sulfamethoxazoles, Tacrolimuss, Terbutalines, Teriparatides, Testosterones, Tetanus antitoxins, Tetracaines, Tetradecyl sulfates, Theophyllines, Thiamines, Thiethylperazines, Thiopentals, Thyroid stimulating hormones, Tinzaparins, Tirofibans, Tobramycins, Tolazolines, Tolbutamides, Torsemides, Tranexamic acids, Treprostinils, Trifluoperazines, Trimethobenzamides, Trimethoprims, Tromethamines, Tuberculins, Typhoid vaccines, Urofollitropins, Urokinases, Vaiproic acids, Vasopressins, Vecuroniums, Verapamils, Voriconazoles,

Warfarins, Yellow fever vaccines, Zidovudines, Zincs, Ziprasidone hydrochiorides, Aclacinomycins, Actinomycins, Adriamycins, Azaserines, 6-Azauridines, Carzinophilins, Chromomycins, Denopterins, 6 Diazo 5 Oxo-L-Norleucines, Enocitabines, Loxuridines, Olivomycines, Pirarubicins, Piritreximes, Pteropterins, Tagafurs, Tubercidins, Alteplases, Arcitumomabs, bevacizumabs, Botulinum Toxin Type A's, Botulinum Toxin Type B's, Capromab Pendetides, Daclizumabs, Dornase alphas, Drotrecogin alpahs, Imciromab Pentetates, Iodine-131's, an antibiotic agent; an angiogenesis inhibitor; anti-cataract and anti-diabetic retinopathy substances; carbonic anhydrase inhibitors; mydriatics; photodynamic therapy agents; prostaglandin analogs; growth factor; anti-neoplastics; anti-metabolites; anti-viral; amebicides and anti-protozoals; anti-tuberculosis and anti-leprotic; antitoxins and antivenins; antihemophilic factor, anti-inhibitor coagulent complex, antithrombin III, coagulations Factor V, coagulation Factor IX, plasma protein fraction, von Willebrand factor; antiplatelet agent a colony stimulating factor (CSF); an erythropoiesis stimulator; hemostatics and albumins; Immune Globulins; thrombin inhibitors; anticoagulants; a steroidal anti-inflammatory drug selected from among among alclomethasones, algestones, beclomethasones, betamethasones, budesonides, clobetasols, clobetasones, clocortolones, cloprednols, corticosterones, cortisones, cortivazols, deflazacorts, desonides, desoximetasones, dexamethasones, difluorosones, diflucortolones, difluprednates, enoxolones, fluazacorts, flucloronides, flumethasones, flunisolides, fluocinolones, fluocinonides, fluocortins, fluocortolones, fluorometholones, fluperolones, fluprednidenes, fluprednisolones, flurandrenolides, fluticasones, formocortals, halcinonides, halobetasols, halometasones, halopredones, hydrocortamates, hydrocortisones, loteprednol etabonate, mazipredones, medrysones, meprednisones, methylprednisolones, mometasone furoate, paramethasones, prednicarbates, prednisolones, prednisones, prednivals, prednylidenes, rimexolones, tixocortols and triamcinolones; Ducosanoids, prostaglandins, prostaglandin analogs, antiprostaglandins and prostaglandin precursors; miotics, cholinergics and anti-cholinesterase; and anti-allergenics.

120. The pharmaceutical composition of any of claims 84-119 that is a liquid composition.

121. A co-formulation, comprising:

a therapeutically effective amount of a modified PH20 polypeptide of any of claims 1-36; and

a therapeutically effective amount of a fast-acting insulin.

122. The co-formulation of claim 121, wherein the modified PH20 polypeptide in the formulation is stable at a temperature from or from about 2°C to 8°C, inclusive, for at least 1 month or is stable at a temperature from or from about 30°C to 42°C, inclusive, for at least 3 days.

123. The co-formulation of claim 121 or claim 122, wherein the modified PH20 polypeptide in the formulation is stable at a temperature from or from about 2°C to 8°C, inclusive, for at least 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months or 30 months.

124. The pharmaceutical composition of any of claim 121-123, wherein the modified PH20 polypeptide in the formulation is stable at a temperature from or from about 30°C to 42°C, inclusive, for at least 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 35 days, 40 days, 45 days, 50 days, 60 days or more.

125. The co-formulation of claim 124, wherein the modified PH20 polypeptide is stable in the composition at or about 37°C for at least 3 days, 4 days, 5 days, 6 days, 7 days, 14 days, 21 days, or 30 days.

126. The co-formulation of any of claims 121-125, wherein the amount of modified PH20 polypeptide is 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, or 400 U/mL to 800 U/mL.

127. The co-formulation of any of claims 121-126, wherein the fast-acting insulin is regular insulin or is an insulin analog.

128. The co-formulation of any of claims 121-127, wherein the fast-acting insulin is a regular insulin that is a human insulin or a pig insulin.

129. The co-formulation of any of claims 121-128, wherein the fast-acting insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in SEQ ID NO:863 or an insulin with an A chain with a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:864 and a B chain with a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:864.

130. The co-formulation of any of claims 121-127, wherein the fast-acting insulin is an insulin analog selected from among insulin lispro, insulin aspart or insulin glulisine.

131. The co-formulation of claim 130, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:865-867.

132. The co-formulation of any of claims 121-131, wherein the amount of fast-acting insulin is 10 U/mL to 1000 U/mL, 50 U/mL to 500 U/mL, 100 U/mL to 1000 U/mL or 500 U/mL to 1000 U/mL, inclusive.

133. The co-formulation of any of claims 121-132 that has a pH of between or about between 7.0 to 7.6.

134. The co-formulation of any of claims 121-133, comprising NaCl at a concentration between or about between 0.1 mM to 200 mM, 0.1mM to 100 mM, 120 mM to 200 mM, 10 mM to 50 mM, 10 mM to 90 mM, 80 mM to 200 mM, 80 mM to 140 mM, 50 mM to 100 mM, 80 mM to 100 mM, 50 mM to 80 mM, 100 mM to 140 mM or 120 mM to 140 mM.

135. The co-formulation of any of claims 121-134, comprising about or between about 80 mM to 140 mM NaCl.

136. The co-formulation of any of claims 121-135, comprising an anti-microbial effective amount of at least one preservative.

137. The co-formulation of any of claims 121-136, wherein the anti-microbial effective amount is a total amount of one or more preservative agents as a percentage (%) of mass concentration (w/v) that is or is between 0.05% to 0.6%, 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive.

138. The co-formulation of claim 136 or claim 137, wherein the preservative(s) is a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s).

139. The co-formulation of any of claims 136-138, wherein the preservative(s) is(are) selected from among phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorohexidine, sodium dehydroacetate,

o-cresol, p-cresol, chlorcresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, buytlparaben and any combinations thereof.

140. The co-formulation of any of claims 136-139, wherein the composition contains a single preservative.

141. The co-formulation of any of claims 136-139, wherein the composition contains a mixture of preservatives that contains 2, 3 or 4 different preservatives.

142. The co-formulation of any of claims 121-141, comprising at least one phenolic preservative.

143. The co-formulation of any of claims 136-142, wherein the preservative(s) is(are) selected from among phenol, metacresol (m-cresol), benzyl alcohol, and parabens.

144. The co-formulation of claim 143, wherein the paraben is methylparaben or propylparaben.

145. The co-formulation of any of claims 136-144, wherein the preservatives are phenol, m-cresol or phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.05% to 0.25% phenol and between or about between 0.05% to 0.2% m-cresol, is between or about between 0.10% to 0.2% phenol and between or about between 0.6% to 0.18% m-cresol, between or about between 0.1% to 0.15% phenol and 0.8% to 0.15% m-cresol, is between or about between 0.10% to 0.15% phenol and between or about between 0.06 to 0.09% m-cresol, is between or about between 0.12% to 0.18% phenol and between or about between 0.14 to 0.22% m-cresol, or is 0.3% to 0.4% m-cresol.

146. The sco-formulation of any of claims 121-145, comprising a surfactant in an amount as a % of mass concentration (w/v) in the formulation that is or is about 0.001%, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%. 0.08% or 0.9%.

147. The co-formulation of claim 146, wherein the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate.

148. The co-formulation of claim 146 or claim 147, wherein the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

149. The co-formulation of any of claims 146-148, wherein the surfactant is poloxamer 188 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.005 % to 0.5 % or 0.01% to 0.05%.

150. The co-formulation of any of claims 121-149, comprising a buffering agent that is a non-metal binding agent or is a metal binding agent.

151. The co-formulation of claim 150, wherein the buffering agent is selected from among Tris, histidine, phosphate and citrate.

152. The co-formulation of claim 150 or claim 151, wherein the buffering agent is Tris.

153. The co-formulation of any of claims 150-152, wherein the concentration of the buffering agent is between or is between about 1 mM to 100 mM, 10 mM to 50 mM or 20 mM to 40 mM.

154. The co-formulation of any of claims 121-153, comprising glycerin in a concentration less than 60 mM, less than 55 mM, less than 50 mM, less than 45 mM, less than 40 mM, less than 35 mM, less than 30 mM, less than 25 mM, less than 20 mM, less than 15 mM, less than 10 mM.

155. The co-formulation of any of claims 121-154, comprising an antioxidant.

156. The co-formulation of claim 155, wherein the antioxidant is selected from among cysteine, tryptophan and methionine.

157. The co-formulation of claim 155 or claim 156, wherein the antioxidant is at a concentration from between or from about between 2 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive.

158. The co-formulation of any of claims 121-157, comprising zing.

159. The co-formulation of claim 158, wherein the concentration of zinc is between or about between 0.001 to 0.1 mg per 100 units of insulin (mg/100U), 0.001 to 0.05 mg/100U or 0.01 to 0.05 mg/100U.

160. A closed loop system, comprising the co-formulation of any of claims 121-159.

161. An insulin pump, comprising the co-formulation of any of claims 121-159.

162. An insulin pen, comprising the co-formulation of any of claims 121-159.

163. A method for treating a hyaluronan-associated disease or condition, comprising administering to a subject a modified PH20 polypeptide of any of claims 1-75 or a pharmaceutical composition of any of claims 84-120.

164. The method of claim 163, wherein the hyaluronan-associated disease or condition is an inflammatory disease or a tumor or cancer.

165. The method of claim 164, wherein the tumor is a solid tumor.

166. The method of any of claims 163-165, wherein the hyaluronan-associated disease or condition is selected from among a late-stage cancer, metastatic cancers and undifferentiated cancers.

167. The method of any of claims 163-166, wherein the hyaluronan-associated disease or condition is selected from among ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer and colon cancer.

168. A method for treating diabetes, comprising administering to a subject a co-formulation of any of claims 121-159.

169. The method of claim 168, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus or gestational diabetes.

170. A method for increasing delivery of a therapeutic agent to a subject, comprising:

administering a subject a modified PH20 polypeptide of any of claims 1-75, a pharmaceutical composition of any of claims 84-120 or a co-formulation of any of claims 121-159; and

administering a therapeutic agent.

171. The method of claim 170, wherein the administration is subcutaneous.

172. The method of claim 170 or claim 171, wherein the modified PH20 polypeptide or composition comprising a modified PH20 polypeptide is administered prior to, simultaneously, intermittently or subsequent to administration of the therapeutic agent.

173. The method of any of claims 170-172, wherein the therapeutic agent is a polypeptide, a protein, a nucleic acid, a drug, a small molecule or an organic molecule.

174. The method of any of claims 170-173, wherein the therapeutic agent is selected from among a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, and antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a

beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, or a sleep inducer.

175. The method of any of claims 170-174, wherein the therapeutic agent is selected from among an antibody, an Immune Globulin, a bisphosphonate, a cytokine, a chemotherapeutic agent, a coagulation factor and an insulin.

176. The method of claim 175, wherein the insulin is a fast-acting insulin.

177. The method of claim 176, wherein the fast-acting insulin is regular insulin or is an insulin analog.

178. The method of claim 175 or 176, wherein the fast-acting insulin is a regular insulin that is a human insulin or a pig insulin.

179. The method of any of claims 175-178, wherein the fast-acting insulin is an insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:862 and a B chain having a sequence of amino acids set forth in SEQ ID NO:863 or an insulin with an A chain with a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:864 and a B chain with a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:864.

180. The method of claim 176 or 177, wherein the fast-acting insulin is an insulin analog selected from among insulin lispro, insulin aspart or insulin glulisine.

181. The method of claim 180, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:862 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:865-867.

182. The method of any of claims 170-174, wherein the therapeutic agent is selected from among a Adalimumabs, Agalsidase Betas, Alefacepts, Ampicillins, Anakinras, Antipoliomyelitic Vaccines, Anti-Thymocytes, Azithromycins, Becaplermins, Caspofungins, Cefazolins, Cefepimes, Cefotetans, Ceftazidimes, Ceftriaxones, Cetuximabs, Cilastatins, Clavulanic Acids, Clindamycins, Darbepoetin Alfas, Deaclizumabs, Diphtheria, Diphtheria antitoxins, Diphtheria Toxoids,

Efalizumabs, Epinephrines, Erythropoietin Alphas, Etanercepts, Filgrastims, Fluconazoles, Follicle-Stimulating Hormones, Follitropin Alphas, Follitropin Betas, Fosphenyloins, Gadodiamides, Gadopentetates, Gatifloxacins, Glatiramers, GM-CSF's, Goserelins, Goserelin acetates, Granisetrons, Haemophilus Influenza B's, Haloperidols, Hepatitis vaccines, Hepatitis A Vaccines, Hepatitis B Vaccines, Ibritumomab Tiuxetans, Ibritumomabs, Tiuxetans, Immunoglobulins, Hemophilus influenza vaccines, Influenza Virus Vaccines, Infliximabs, Insulin lispro, 75% neutral protamine lispro (NPL)/25% insulin lispro, 50% neutral protamine Hagedorn (NPH)/ 50% regular insulin, 70% NPH/30% regular insulin; Regular insulin, NPH insulin, Ultra insulin, Ultralente insulin, and Insulin Glargines, Interferons, Interferon alpha, Interferon Betas, Interferon Gammas, Interferon alpha-2a, Interferon alpha 2-b, Interferon Alphacon, Interferon alpha-n, Interferon Betas, Interferon Beta-1a's, Interferon Gammas, Interferon alpha-con, Iodixanols, Iohexols, Iopamidols, Ioversols, Ketorolacs, Laronidases, Levofloxacins, Lidocaines, Linezolids, Lorazepams, Measles Vaccines, Measles virus, Mumps viruses, Measles-Mumps-Rubella Virus Vaccines, Rubella vaccines, Medroxyprogesterones, Meropenems, Methylprednisolones, Midazolams, Morphines, Octreotides, Omalizumabs, Ondansetrons, Palivizumabs, Pantoprazoles, Pegaspargases, Pegfilgrastims, Peg-Interferon Alpha-2a's, Peg-Interferon Alpha-2b's, Pegvisomants, Pertussis vaccines, Piperacillins, Pneumococcal Vaccines and Pneumococcal Conjugate Vaccines, Promethazines, Reteplases, Somatropins, Sulbactams, Sumatriptans, Tazobactams, Tenecteplases, Tetanus Purified Toxoids, Ticarcillins, Tositumomabs, Triamcinolones, Triamcinolone Acetonides, Triamcinolone hexacetonides, Vancomycins, Varicella Zoster imrnunoglobulins, Varicella vaccines, other vaccines, Alemtuzumabs, Alitretinoins, Allopurinols, Altretamines, Amifostines, Anastrozoles, Arsenics, Arsenic Trioxides, Asparaginases, Bacillus Calmette-Guerin (BCG) vaccines, BCG Live, Bexarotenes, Bleomycins, Busulfans, Busulfan intravenous, Busulfan orals, Calusterones, Capecitabines, Carboplatins, Carmustines, Carmustines with Polifeprosans, Celecoxibs, Chlorambucils, Cisplatins, Cladribines, Cyclophosphamides, Cytarabines, Cytarabine liposomals, Dacarbazines, Dactinomycins, Daunorubicin liposomals, Daunorubicins, Daunomycins, Denileukin Diftitoxes, Dexrazoxanes, Docetaxels, Doxorubicins, Doxorubicin liposomals, Dromostanolone propionates, Elliott's B Solutions, Epirubicins, Epoetin alfas, Estramustines, Etoposides, Etoposide phosphates, Etoposide VP-16s, Exemestanes,

Floxuridines, Fludarabines, Fluorouracils, 5-Fluorouracils, Fulvestrants, Gemcitabines, Gemtuzumabs, Ozogamicins, Gemtuzumab ozogamicins, Hydroxyureas, Idarubicins, Ifosfamides, Imatinib mesylates, Irinotecans, Letrozoles, Leucovorins, Levamisoles, Lomustines, CCNUs, Meclorethamines, Nitrogen mustards, Megestrols, Megestrol acetates, Melphalans, L-PAMs, Mercaptopurines, 6-Mercaptopurines, Mesnas, Methotrexates, Methoxsalens, Mitomycins, Mitomycin C's, Mitotanes, Mitoxantrones, Nandrolones, Nandrolone Phenpropionates, Nofetumomabs, Oprelvekins, Oxaliplatins, Paclitaxels, Pamidronates, Pegademases, Pentostatins, Pipobromans, Plicamycins, Mithramycins, Porfimers, Porfimer sodiums, Procarbazines, Quinacrines, Rasburicases, Rituximabs, Sargramostims, Streptozocins, Talcs, Tamoxifens, Temozolomides, Teniposides, Testolactones, Thioguanines, 6-Thioguanines, Triethylenethiophosphoramides (Thiotepas), Topotecans, Toremifenes, Trastuzumabs, Tretinoins, Uracil Mustards, Valrubicins, Vinblastines, Vincristines, Vinorelbines, Zoledronates, Acivicins, Aclarubicins, Acodazoles, Acronines, Adozelesins, Aldesleukins, Retinoic Acids, Alitretinoins, 9-Cis-Retinoic Acids, Alvocidibs, Ambazones, Ambomycins, Ametantrones, Aminoglutethimides, Amsacrines, Anaxirones, Ancitabines, Anthramycins, Apaziquones, Argimesnas, Asperlins, Atrimustines, Azacitidines, Azetepas, Azotomycins, Banoxantrones, Batabulins, Batimastats, Benaxibines, Bendamustines, Benzodepas, Bicalutamides, Bietaserpines, Biricodars, Bisantrenes, Bisnafide Dimesylates, Bizelesins, Bortezomibs, Brequinars, Bropirimines, Budotitanes, Cactinomycins, Canertinibs, Caracemides, Carbetimers, Carboquones, Carmofurs, Carubicins, Carzelesins, Cedefingols, Cemadotins, Chiorambucils, Cioteronels, Cirolemycins, Clanfenurs, Clofarabines, Crisnatols, Decitabines, Dexniguldipines, Dexormaplatins, Dezaguanines, Diaziquones, Dibrospidiums, Dienogests, Dinalins, Disermolides, Dofequidars, Doxifluridines, Droloxifenes, Duazomycins, Ecomustines, Edatrexates, Edotecarins, Eflomithines, Elacridars, Elinafides, Elsamitrucins, Emitefurs, Enloplatins, Enpromates, Enzastaurins, Epipropidines, Eptaloprosts, Erbulozoles, Esorubicins, Etanidazoles, Etoglucids, Etoprines, Exisulinds, Fadrozoles, Fazarabines, Fenretinides, Fluoxymesterones, Flurocitabines, Fosquidones, Fostriecins, Fotretamines, Galarubicins, Galocitabines, Geroquinols, Gimatecans, Gimeracils, Gloxazones, Glufosfamides, Ilmofosines, Ilomastats, Imexons, Improsulfans, Indisulams, Inproquones, Interleukins, Interleukin-2s, recombinant Interleukins, Intoplicines, lobenguanes, Iproplatins, Irsogladines, Ixabepilones, Ketotrexates, L-

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Aztreonams, Bacitracins, Baclofens, Basiliximabs, Benzoic acids, Benztropines, Betamethasones, Biotins, Bivalirudins, Botulism antitoxins, Bretyliums, Bumetanides, Bupivacaines, Buprenorphines, Butorphanols, Calcitonins, Calcitriols, Calciums, Capreomycins, Carboprosts, Carnitines, Cefaniandoles, Cefoperazones, Cefotaximes, Cefoxitins, Ceftizoximes, Cefuroximes, Chioramphenicols, Chioroprocaines, Chioroquines, Chlorothiazides, Chiorpromazines, Chondroitinsulfuric acids, Choriogonadotropin alfas, Chromiums, Cidofovirs, Cimetidines, Ciprofloxacins, Cisatracuriums, Clonidines, Codeines, Coichicines, Colistins, Collagens, Corticorelin ovine triflutates, Corticotrophins, Cosyntropins, Cyanocobalamins, Cyclosporines, Cysteines, Dacliximabs, Dalfopristins, Dalteparins, Danaparoids, Dantrolenes, Deferoxamines, Desmopressins, Dexamethasones, Dexmedetomidines, Dexpanthenols, Dextrans, Iron dextrans, Diatrizoic acids, Diazepams, Diazoxides, Dicyclomines, Digibinds, Digoxins, Dihydroergotamines, Diltiazems, Diphenhydramines, Dipyridamoles, Dobutamines, Dopamines, Doxacuriums, Doxaprams, Doxercalciferols, Doxycyclines, Droperidols, Dyphyllines, Edetic acids, Edrophoniums, Enalaprilats, Ephedrines, Epoprostenols, Ergocalciferols, Ergonovines, Ertapenems, Erythromycins, Esmolols, Estradiols, Estrogenics, Ethacrynic acids, Ethanolamines, Ethanols, Ethiodized oils, Etidronic acids, Etomidates, Famotidines, Fenoldopams, Fentanyls, Flumazenils, Fluoresceins, Fluphenazines, Folic acids, Fomepizoles, Fomivirsens, Fondaparinuxs, Foscarnets, Fosphenytoins, Furosemides, Gadoteridols, Gadoversetamides, Ganciclovirs, Gentamicins, Glucagons, Glucoses, Glycines, Glycopyrrolates, Gonadorelins, Gonadotropin chorionics, Haemophilus B polysaccarides, Hemins, Herbals, Histamines, Hydralazines, Hydrocortisones, Hydromorphones, Hydroxocobalamins, Hydroxyzines, Hyoscyamines, Ibutilides, Imiglucerases, Indigo carmines, Indomethacins, Iodides, Iopromides, Iothalamic acids, Ioxaglic acids, Ioxilans, Isoniazids, Isoproterenols, Japanese encephalitis vaccines, Kanamycins, Ketamines, Labetalols, Lepirudins, Levobupivacaines, Levothyroxines, Lincomycins, Liothyronines, Luteinising hormones, Lyme disease vaccines, Mangafodipirs, Manthtols, Meningococcal polysaccharide vaccines, Meperidines, Mepivacaines, Mesoridazines, Metaraminols, Methadones, Methocarbamols, Methohexitals, Methyldopates, Methylergonovines, Metoclopramides, Metoprolols, Metronidazoles, Minocyclines, Mivacuriums, Morrhuic acids, Moxifloxacins, Muromonab-CD3s, Mycophenolate mofetils, Nafcillins, Nalbuphines, Nalmefenes, Naloxones,

Neostigmines, Niacinamides, Nicardipines, Nitroglycerins, Nitroprussides, Norepinephrines, Orphenadrines, Oxacillins, Oxymorphones, Oxytetracyclines, Oxytocins, Pancuroniums, Panthenols, Pantothenic acids, Papaverines, Peginterferon alpha 2As, Penicillin Gs, Pentamidines, Pentazocines, Pentobarbitals, Perfiutrens, Perphenazines, Phenobarbitals, Phentolamines, Phenylephrines, Phenytoins, Physostigmines, Phytonadiones, Polymyxin, Pralidoximes, Prilocaines, Procainamides, Procaines, Prochiorperazines, Progesterones, Propranolols, Pyridostigmine hydroxides, Pyridoxines, Quinidines, Quinupristins, Rabies immunoglobulins, Rabies vaccines, Ranitidines, Remifentanils, Riboflavins, Rifampins, Ropivacaines, Samariums, Scopolamines, Seleniums, Sermorelins, Sincalides, Somatrems, Spectinomycins, Streptokinases, Streptomycins, Succinylcholines, Sufentanils, Sulfamethoxazoles, Tacrolirnuss, Terbutalines, Teriparatides, Testosterones, Tetanus antitoxins, Tetracaines, Tetradecyl sulfates, Theophyllines, Thiamines, Thiethylperazines, Thiopentals, Thyroid stimulating hormones, Tinzaparins, Tirofibans, Tobramycins, Tolazolines, Tolbutamides, Torsemides, Tranexamic acids, Treprostinils, Trifluoperazines, Trimethobenzamides, Trimethoprims, Tromethamines, Tuberculins, Typhoid vaccines, Urofollitropins, Urokinases, Vaiproic acids, Vasopressins, Vecuroniums, Verapamils, Voriconazoles, Warfarins, Yellow fever vaccines, Zidovudines, Zincs, Ziprasidone hydrochiorides, Aclacinomycins, Actinomycins, Adriamycins, Azaserines, 6-Azauridines, Carzinophilins, Chromomycins, Denopterins, 6 Diazo 5 Oxo-L-Norleucines, Enocitabines, Loxuridines, Olivomycines, Pirarubicins, Piritreximes, Pteropterins, Tagafurs, Tubercidins, Alteplases, Arcitumomabs, bevacizumabs, Botulinum Toxin Type A's, Botulinum Toxin Type B's, Capromab Pendetides, Daclizumabs, Dornase alphas, Drotrecogin alpahs, Imciromab Pentetates, Iodine-131's, an antibiotic agent; an angiogenesis inhibitor; anti-cataract and anti-diabetic retinopathy substances; carbonic anhydrase inhibitors; mydriatics; photodynamic therapy agents; prostaglandin analogs; growth factor; anti-neoplastics; anti-metabolites; anti-viral; amebicides and anti-protozoals; anti-tuberculosis and anti-leprotic; antitoxins and antivenins; antihemophilic factor, anti-inhibitor coagulent complex, antithrombin III, coagulations Factor V, coagulation Factor IX, plasma protein fraction, von Willebrand factor; antiplatelet agent a colony stimulating factor (CSF); an erythropoiesis stimulator; hemostatics and albumins; Immune Globulins; thrombin inhibitors; anticoagulants; a steroidal anti-inflammatory drug selected from among

among alclomethasones, algestones, beclomethasones, betamethasones, budesonides, clobetasols, clobetasones, clocortolones, cloprednols, corticosterones, cortisones, cortivazols, deflazacorts, desonides, desoximetasones, dexamethasones, difluorosones, diflucortolones, difluprednates, enoxolones, fluazacorts, flucloronides, flumethasones, flunisolides, fluocinolones, fluocinonides, fluocortins, fluocortolones, fluorometholones, fluperolones, fluprednidenes, fluprednisolones, flurandrenolides, fluticasones, formocortals, halcinonides, halobetasols, halometasones, halopredones, hydrocortamates, hydrocortisones, loteprednol etabonate, mazipredones, medrysones, meprednisones, methylprednisolones, mometasone furoate, paramethasones, prednicarbates, prednisolones, prednisones, prednivals, prednylidenes, rimexolones, tixocortols and triamcinolones; Ducosanoids, prostaglandins, prostaglandin analogs, antiprostaglandins and prostaglandin precursors; miotics, cholinergics and anti-cholinesterase; and anti-allergenics.

183. A method for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents, comprising administering to a subject the modified PH20 polypeptide of any of claims 1-75 or a pharmaceutical composition of any of claims 84-120.

184. A pharmaceutical composition of any of claims 84-120 for use in treating a hyaluronan-associated disease or disorder.

185. A co-formulation of any of claims 121-159 for use in treating diabetes.

186. A pharmaceutical composition of any of claims 84-120 for use in delivering a therapeutic agent to a subject.

187. A pharmaceutical composition of any of claims 84-120 for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

ABSTRACT

Modified PH20 hyaluronidase polypeptides, including modified polypeptides that exhibit increased stability and/or increased activity, are provided. Also provided are compositions and formulations and uses thereof.

5

FIGURE 1

LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR 60

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RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180

WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240

LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG 300

IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS 360

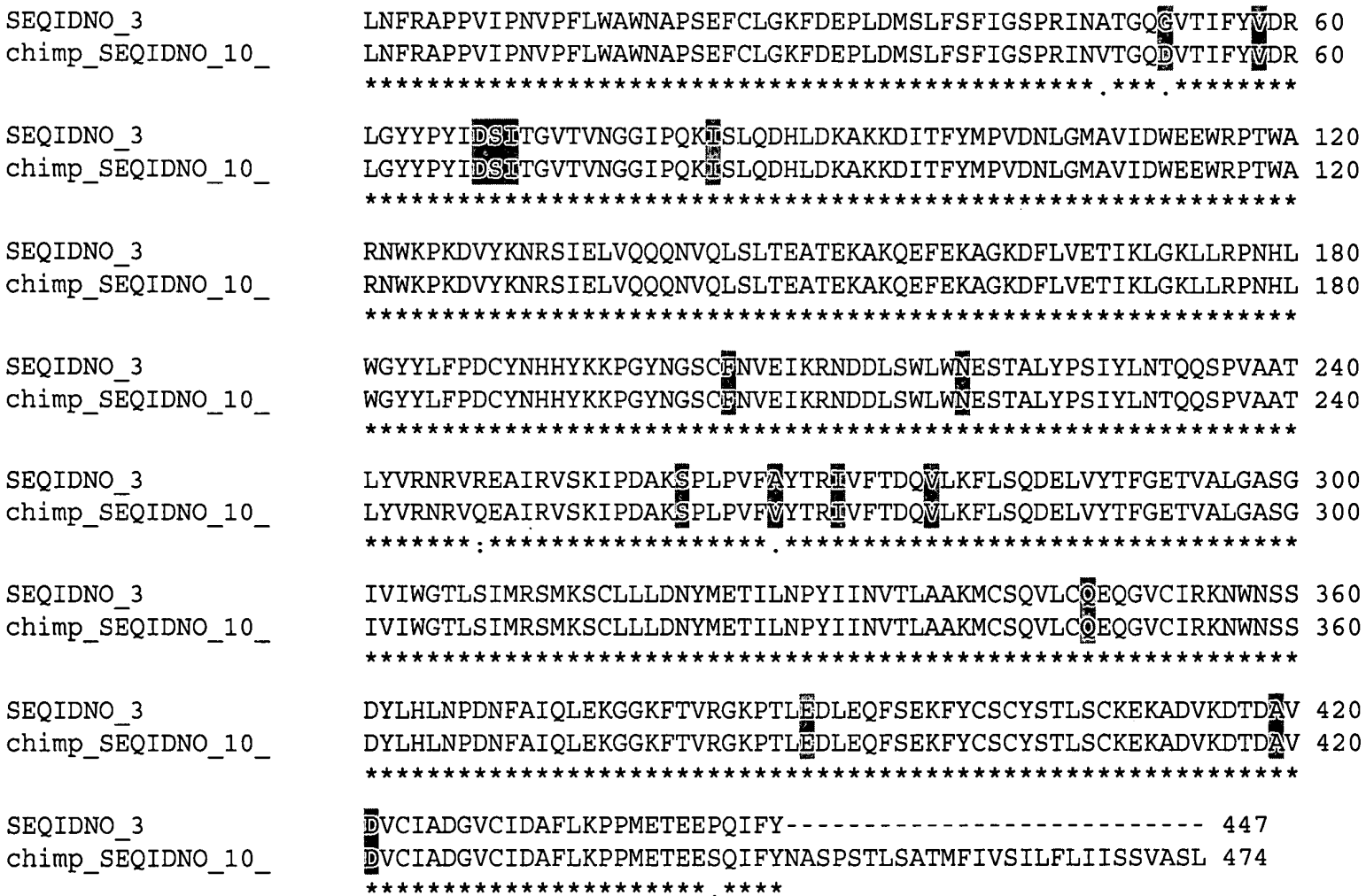
DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV 420

DVCIADGVC**IDAFLKPPMETEEPQIFYNASPSTLSATMFIVSILF**LIISSVASL 474

Sheet 1 of 13
Attorney Docket No. 33320.03087.US70/ P3087
Applicant: Wei *et al.*
Title: PH20 POLYPEPTIDE VARIANTS, FORMULATIONS AND USES THEREOF

Petitioner Merck, Ex. 1051, p. 380

FIGURE 2A



Sheet 2 of 13
Attorney Docket No. 33320.03087.US70/ P3087
Applicant: Wei *et al.*
Title: PH20 POLYPEPTIDE VARIANTS, FORMULATIONS AND USES THEREOF

FIGURE 2B

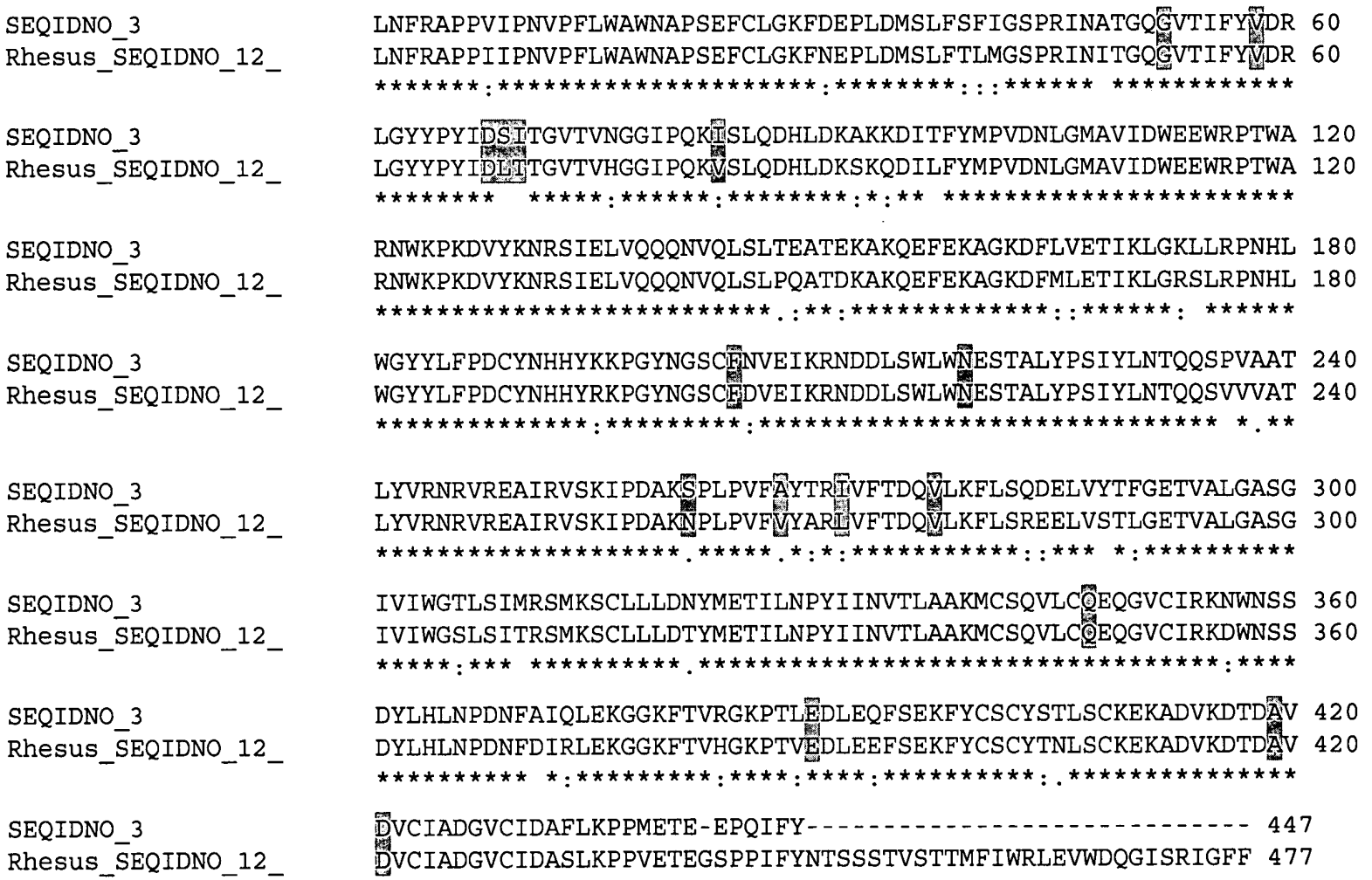

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SEQIDNO_3 LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
Rhesus_SEQIDNO_12_ LGYYPYIDLTTGVTVHGGIPQKVSLQDHLDKSKQDILFYMPVDNLGMAVIDWEEWRPTWA 120
SEQIDNO_3 RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
Rhesus_SEQIDNO_12_ RNWKPKDVYKNRSIELVQQQNVQLSLPQATDKAKQEFEKAGKDFMLETIKLGRSLRPNHL 180
SEQIDNO_3 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
Rhesus_SEQIDNO_12_ WGYYLFPDCYNHHYRKPGYNGSCFDVEIKRNDDLSWLWNESTALYPSIYLNTQQSVVVAT 240
SEQIDNO_3 LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG 300
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FIGURE 2C

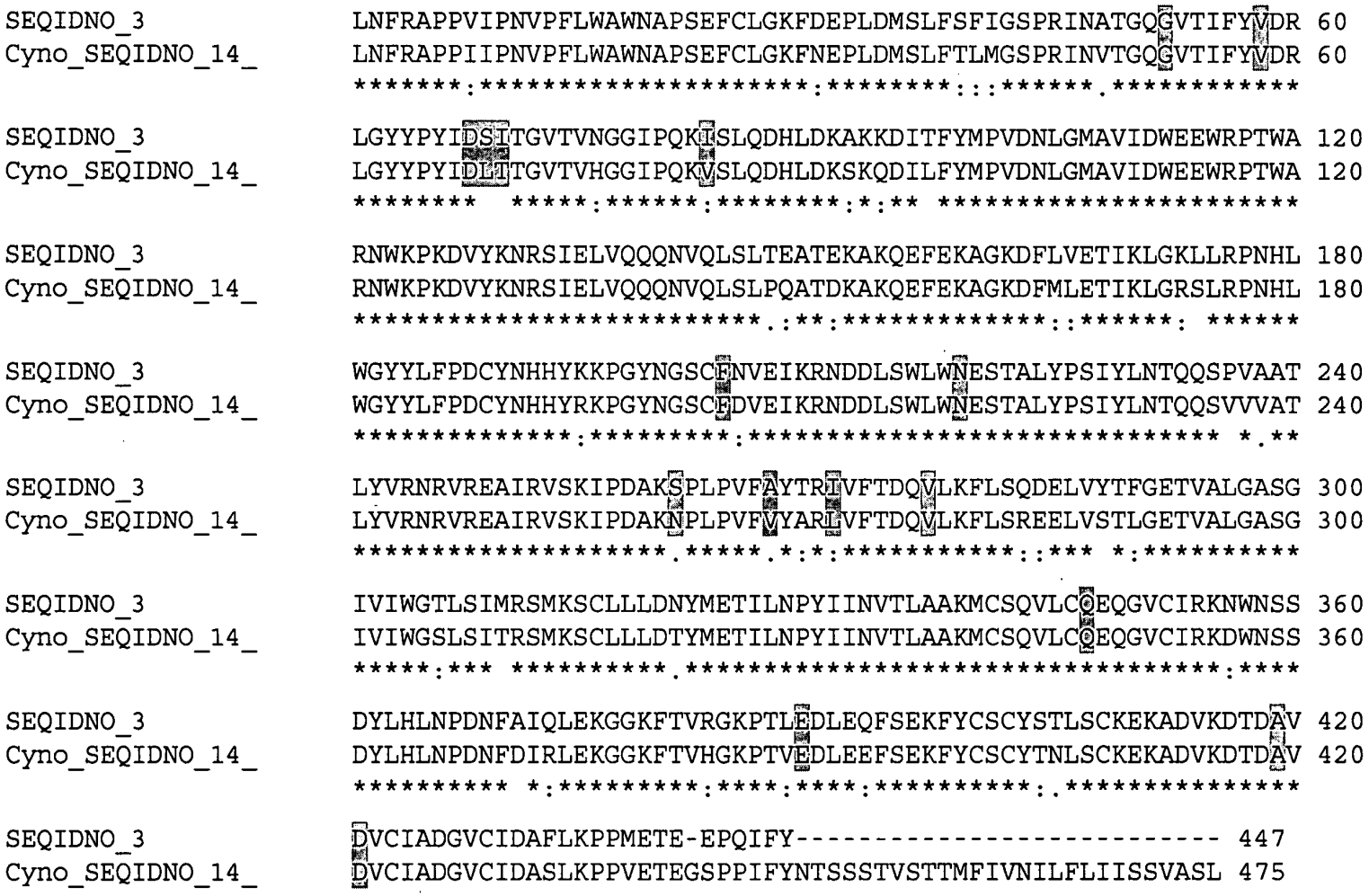
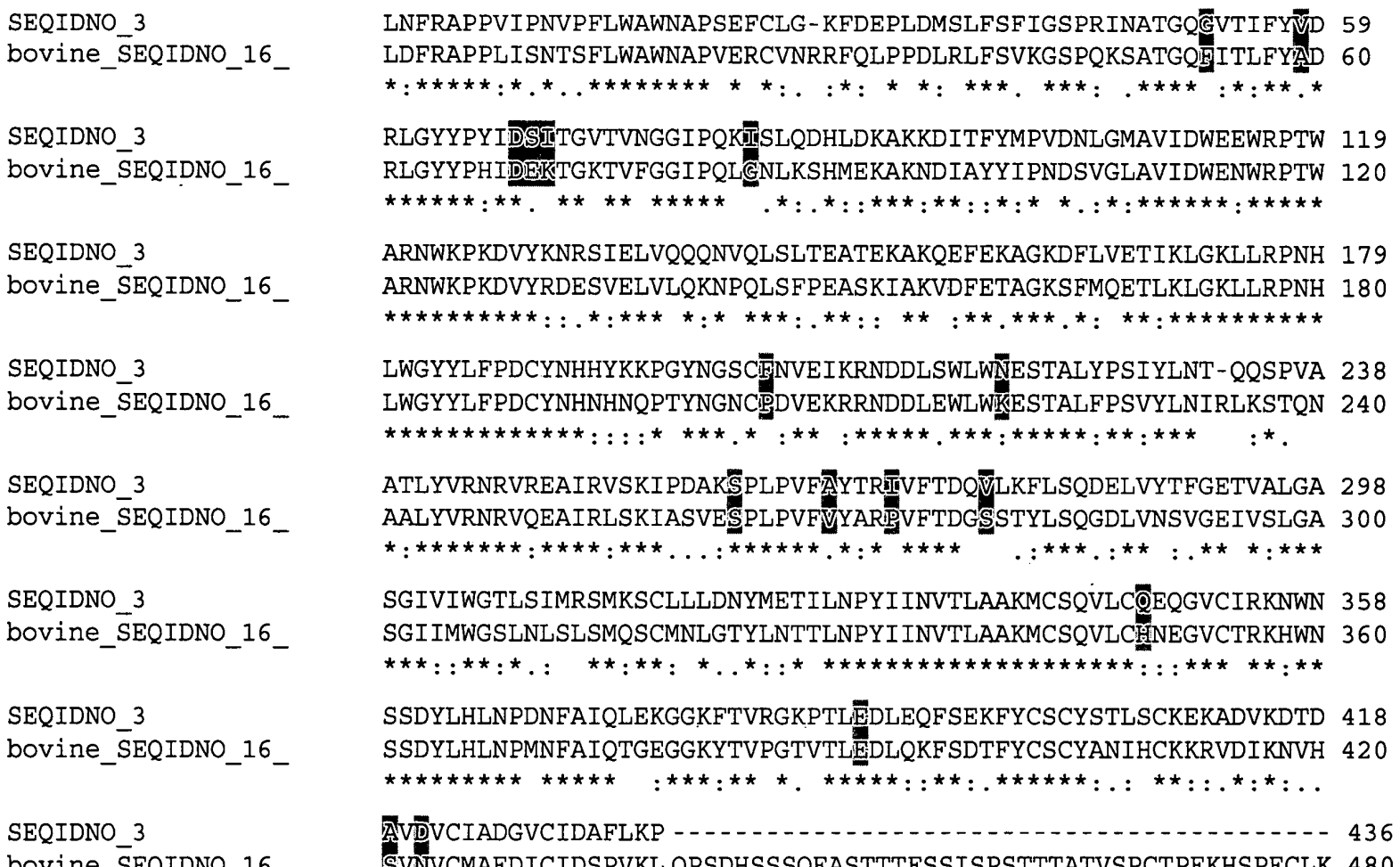
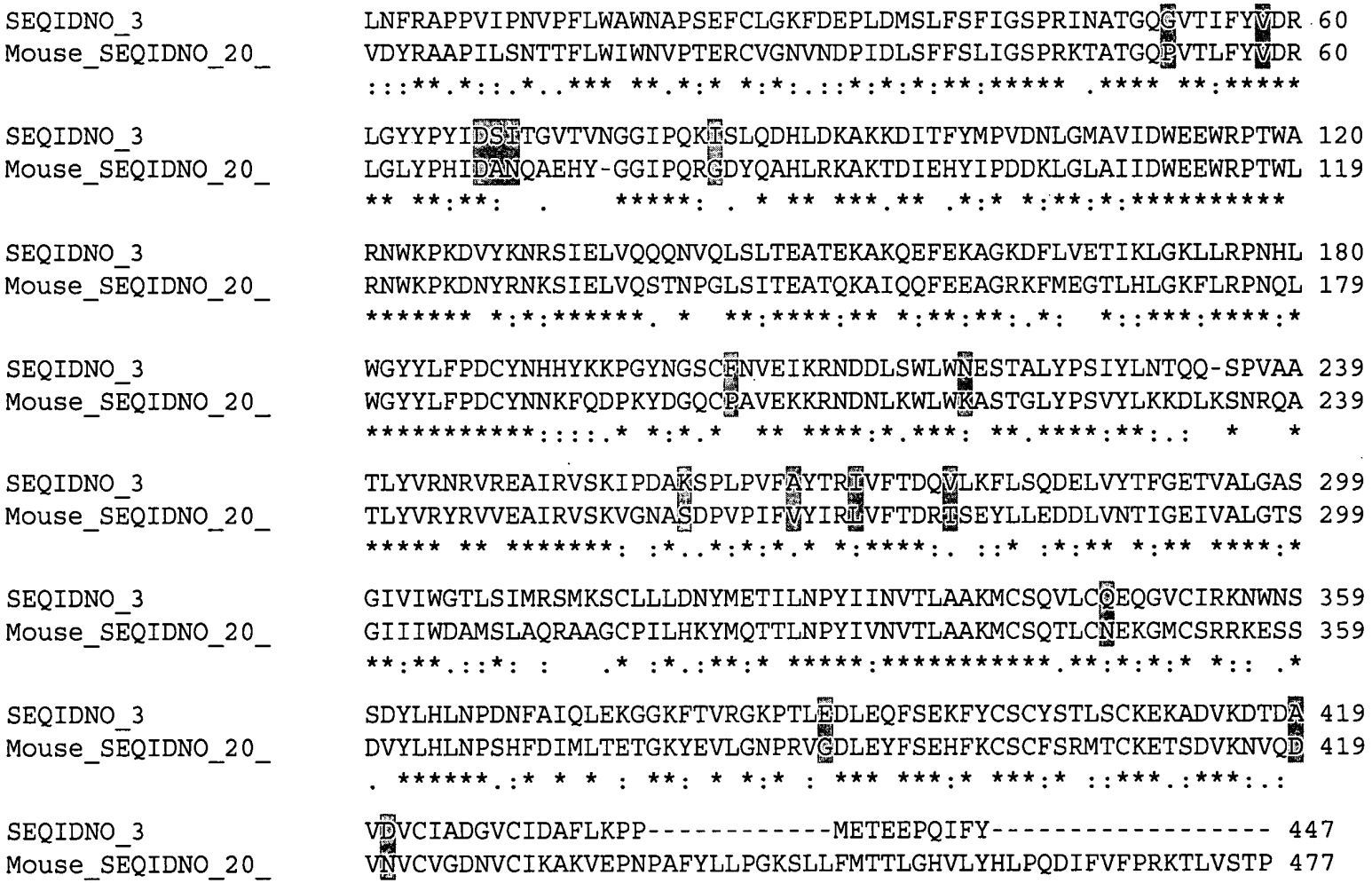

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SEQIDNO_3 LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
Cyno_SEQIDNO_14_ LGYYPYIDLTTGVTVHGGIPQKVSLQDHLDKSKQDILFYMPVDNLGMAVIDWEEWRPTWA 120
SEQIDNO_3 RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
Cyno_SEQIDNO_14_ RNWKPKDVYKNRSIELVQQQNVQLSLPQATDKAKQEFEKAGKDFMLETIKLGRSLRPNHL 180
SEQIDNO_3 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
Cyno_SEQIDNO_14_ WGYYLFPDCYNHHYRKPGYNGSCFDVEIKRNDDLSWLWNESTALYPSIYLNTQQSVVVAT 240
SEQIDNO_3 LYVRNRVREAIRVSKIPDAKSPLPVFAYTRLVFTDQVLKFLSQDELVYTFGETVALGASG 300
Cyno_SEQIDNO_14_ LYVRNRVREAIRVSKIPDAKNPLPVFVYARLVFTDQVLKFLSREELVSTLGETVALGASG 300
SEQIDNO_3 IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS 360
Cyno_SEQIDNO_14_ IVIWGSLSITRSMKSCLLLDTYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSS 360
SEQIDNO_3 DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV 420
Cyno_SEQIDNO_14_ DYLHLNPDNFDIRLEKGGKFTVHGKPTVEDLEEFSEKFYCSCYTNLSCKEKADVKDTDAV 420
SEQIDNO_3 DVCIADGVCIDAFLKPPMETE-EPQIFY---------- 447
Cyno_SEQIDNO_14_ DVCIADGVCIDASLKPPVETEGSPPIFYNTSSSTVSTTMFIVNILFLIISSVASL 475

FIGURE 2D



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FIGURE 2E



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Applicant: Wei *et al.*
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Petitioner Merck, Ex. 1051, p. 385

FIGURE 2F

```
SEQIDNO_3          LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR 60
Rat_SEQIDNO_22_    VDYRATPVLSDTTFVWVWNVPTEACVENVTEPIDLSFFSLIGSPRKTAIGQPVTLFYVDR 60
                   :::**.**:.:..*:*.**.*:* *: :. **:*:*:**:***** .* ** **:*****

SEQIDNO_3          LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
Rat_SEQIDNO_22_    LGNYPHIDAQQ-TEHHGGIPQKGDLTTHLVKAKEDVERYIPTDKLGLAIIDWEEWRPTWM 119
                   ** **:**:   .  :****** .*  ** ***:*:  *:*.*:**:*:**********

SEQIDNO_3          RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
Rat_SEQIDNO_22_    RNWTPKDIYRNKSIELVQAADPAINITEATVRAKAQFEGAAKEFMEGTLKLGKHIRPKHL 179
                   ***.***:*:*:******  :  :.:**** :** :** *.*:*:  *:**** :**:**

SEQIDNO_3          WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQ-SPVAA 239
Rat_SEQIDNO_22_    WGFYLFPDCYNNKFQVDNYDGQCPDVEKKRNDDLDWLWKESTGLYPSVYLKKDLKSSRKA 239
                   **:********::::  .*:*.* :** ******.***:***.****:**:.:  *.  *

SEQIDNO_3          TLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGAS 299
Rat_SEQIDNO_22_    TLYVRYRVLESIRVSKVSDESNPVPIFVYIRLVFTDHVSEYLLEDDLVNTIGEIVAQGTS 299
                   ***** ** *:*****:.* ..*:*:*.* *:****:* ::* :*:** *:** ** *:*

SEQIDNO_3          GIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNS 359
Rat_SEQIDNO_22_    GIIIWDAMSLAQRSAGCPILRQYMKTTLNPYIVNVTLAAKMCSQTLCKEKGMCSRKTESS 359
                   **:**.::*: :   .* :* :**:* *****:**********.**:*:*:* **. .*

SEQIDNO_3          SDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDA 419
Rat_SEQIDNO_22_    DAYLHLDPSSFSINVTEAGKYEVLGKPEVKDLEYFSEHFKCSCFSKMTCEETSDMRSIQD 419
                   . ****:*..*:*:: :.**: * *** ::*** ***:* ***:*.::*:*.:*::. :

SEQIDNO_3          VDVCIADGVCIDAFLKPP-----------METEEPQIFY----------------- 447
Rat_SEQIDNO_22_    VNVCMGDNVCIKATLGPNSAFHLLPGKGLLLMTTLAHILHHLPHDIFVFPWKMLVSTP 477
                   *:**:.*.***.* * *             : *  .:*::
```

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FIGURE 2G

```
SEQIDNO_3              LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR 60
Rabbit_SEQIDNO_24_     ANFRAPPVIPNVPFLWAWNAPTEFCLGKSGEPLDMSLFSLFGSPRKNKTGQGITIFYVDR 60
                        ********************:****** .*********::**** * ****:*******

SEQIDNO_3              LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
Rabbit_SEQIDNO_24_     LGYYPYIDPHTGAIVHGRIPQLGPLQQHLTKLRQEILYYMPKDNVGLAVIDWEEWLPTWL 120
                       ********. **. *:* ***  .**:** * :::* :*** **:*:******** ***

SEQIDNO_3              RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
Rabbit_SEQIDNO_24_     RNWKPKDIYRIKSIELVKSQHPQYNHSYATEKAKRDFEKAGKDFMEETLKLGRLLRPNHL 180
                       *******:*: :*****:.*: * . : ******::********: **:***:*******

SEQIDNO_3              WGYYLFPDCYNHHYKKP-GYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQ---SP 236
Rabbit_SEQIDNO_24_     WGYYLFPDCYNHHYDKPNLYKGSCFDIEKKRNDDLSWLWKESTALFPSVYLTSRARSATA 240
                       **************.**  *:****::* **********:*****:**:**.::    :.

SEQIDNO_3              VAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVAL 296
Rabbit_SEQIDNO_24_     LSKLYVVRNRVHEAIRVSKIPDDKSPLPNFVYTRLVFTDQIFQFLSHHDLVYTIGEIVAL 300
                       ::    *****:********** ***** *.***:*****:::***:.:****:** ***

SEQIDNO_3              GASGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKN 356
Rabbit_SEQIDNO_24_     GASGIVVWGSQSLARSMKSCLHLDNYMKTILNPYLINVTLAAKMCNQVLCQEQGVCTRKN 360
                       ******:**: *: ******* *****:******:**********.********** ***

SEQIDNO_3              WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKD 416
Rabbit_SEQIDNO_24_     WNPNDYLHLNPGNFAIQLGSNGTYKVDGKPTLTDLEQFSKNFQCSCYTNLNCKERTDMNN 420
                       **..*******.****** ..*.:.* ***** ******::* ****:.*.***::*:::

SEQIDNO_3              TDAVDVCIADGVCIDAFLKPPMETEEPQ------------------------------- 444
Rabbit_SEQIDNO_24_     VRTVNVCAVENVCIDTNVGPQAVTYAPKEKKDVAHILSNTTSINSSTTMSLPFPRKHVSG 480
                       . :*:** .:.****: : *   *  *:

SEQIDNO_3              -------------IFY-------------- 447
Rabbit_SEQIDNO_24_     CLLVLCMYSQYLNICYRLVAIGIQHGYYLK 510
                                  * *
```

FIGURE 2H

```
SEQIDNO_3                LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATG 50
GuineaPig_SEQIDNO_29_    -DKRAPPLIPNVPLLWVWNAPTEFCIGGTNQPLDMSFFSIVGTPRKNITG 49
                          : ****:*****:**.****:***:*  ::*****:**::*:** * **

SEQIDNO_3                QGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYM 100
GuineaPig_SEQIDNO_29_    QSITLYYVDRLGYYPYIDPHTGAIVHGGLPQLMNLQQHLRKSRQDILFYM 99
                         *.:*::************. **. *:**:** :.**:** *:::** ***

SEQIDNO_3                PVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTEAT 150
GuineaPig_SEQIDNO_29_    PTDSVGLAVIDWEEWRPTWTRNWRPKDIYRNKSIELVKSQHPQYNHSYAV 149
                         *.*.:*:************:***:***:*:*:*****:.*: * . : *.

SEQIDNO_3                EKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYN 200
GuineaPig_SEQIDNO_29_    AVAKRDFERTGKAFMLETLKLGKSLRPSSLWGYYLFPDCYNTHFTKPNYD 199
                           **::**::** *::**:**** ***. ********** *:.**;*:

SEQIDNO_3                GSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQ-SPVAATLYVRNRVRE 249
GuineaPig_SEQIDNO_29_    GHCPPIELQRNNDLQWLWNDSTALYPSVYLTSRVRSSQNGALYVRNRVHE 249
                         * *  :*::**:**.****:*******:**.::  *.  .:*******:*

SEQIDNO_3                AIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGAS 299
GuineaPig_SEQIDNO_29_    SIRVSKLMDDKNPLPIYVYIRLVFTDQTTFLELDDLVHSVGEIVPLGVS 299
                         :*****: * *.***::.* *:*****. .**. *:**::.** *.**.*

SEQIDNO_3                GIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQ 349
GuineaPig_SEQIDNO_29_    GIIIWGSLSLTRSLVSCIGLENYMKGTLLPYLINVTLAAKMCGQVLCKNQ 349
                         **:***:**: **: **: *:***:  * **:**********.****::*

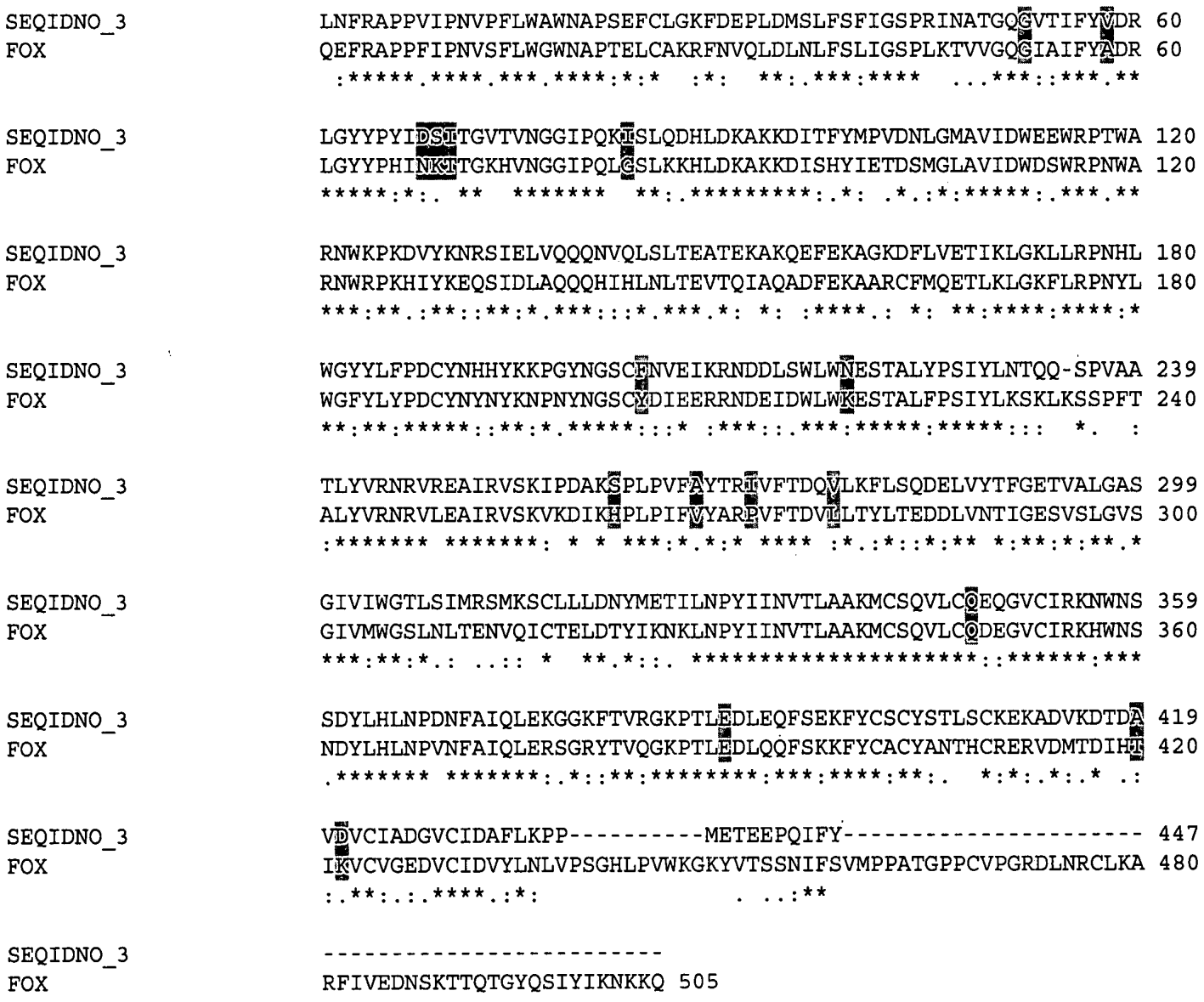
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GuineaPig_SEQIDNO_29_    GICTRKDWNTNTYLHLNATNFDIELQQNGKFVVHGKPSLEDLQEFSKNFH 399
                         *:* **:**:. *****. ** *:*::.***.*:***:****::**::*:

SEQIDNO_3                CSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFL---------KPPMET 440
GuineaPig_SEQIDNO_29_    CSCYTNVACKDRLDVHNVRSVNVCTANNICIDAVLNFPSLDDDDEPPITD 449
                         ****:.::**:: **::. :*:** *:.:****.*         :**:

SEQIDNO_3                EEPQ-------------------------------IFY------- 447
GuineaPig_SEQIDNO_29_    DTSQNQDSISDITSSAPPSSHILPKDLSWCLFLLSIFSQHWKYLL 494
                         : .*                                 **
```

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FIGURE 2I



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FIGURE 2J

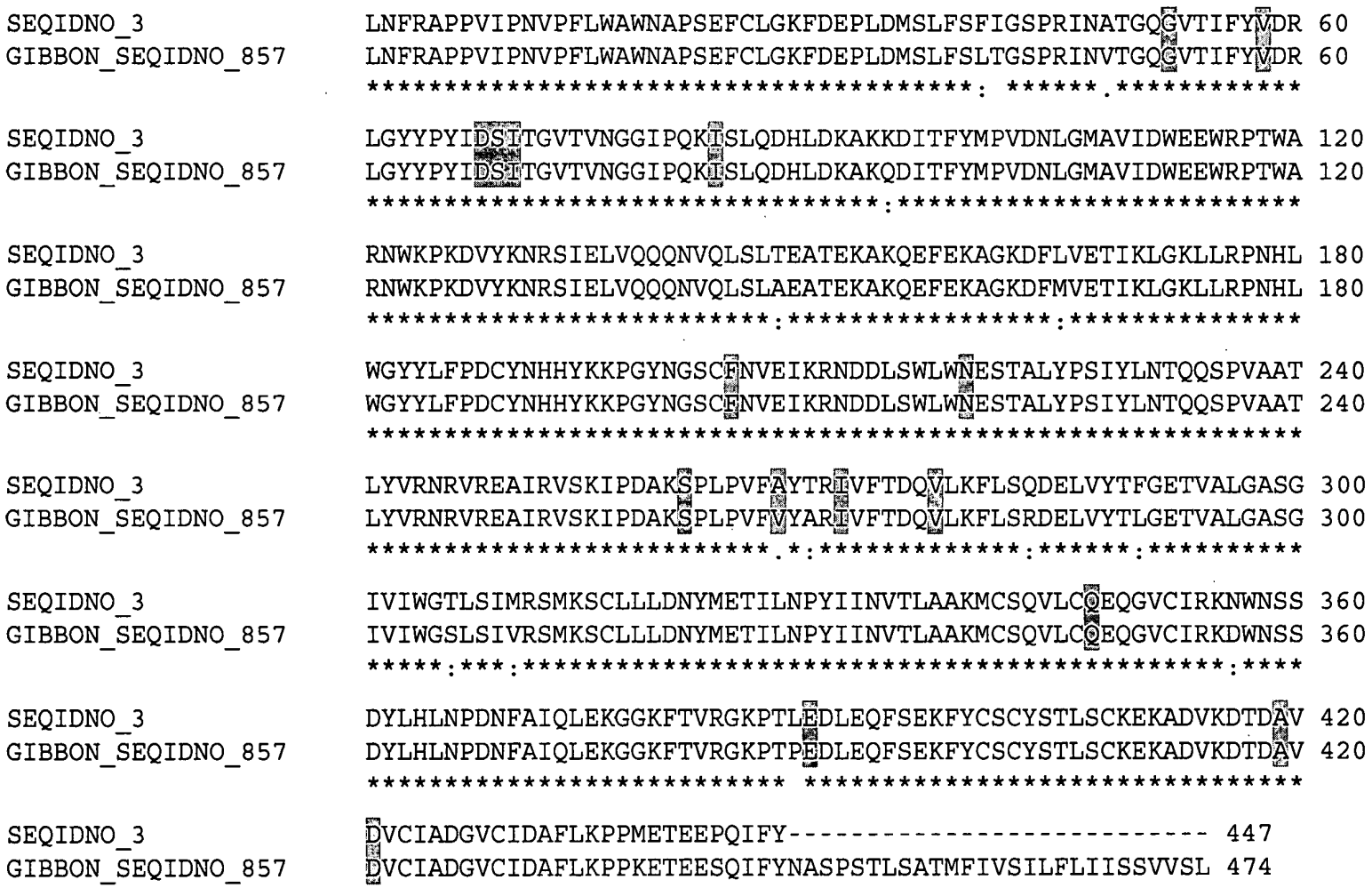

SEQIDNO_3 LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR 60
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SEQIDNO_3 LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
GIBBON_SEQIDNO_857 LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKQDITFYMPVDNLGMAVIDWEEWRPTWA 120
SEQIDNO_3 RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
GIBBON_SEQIDNO_857 RNWKPKDVYKNRSIELVQQQNVQLSLAEATEKAKQEFEKAGKDFMVETIKLGKLLRPNHL 180
SEQIDNO_3 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
GIBBON_SEQIDNO_857 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
SEQIDNO_3 LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG 300
GIBBON_SEQIDNO_857 LYVRNRVREAIRVSKIPDAKSPLPVFVYARIVFTDQVLKFLSRDELVYTLGETVALGASG 300
SEQIDNO_3 IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS 360
GIBBON_SEQIDNO_857 IVIWGSLSIVRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSS 360
SEQIDNO_3 DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV 420
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SEQIDNO_3 DVCIADGVCIDAFLKPPMETEEPQIFY---------- 447
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FIGURE 2K

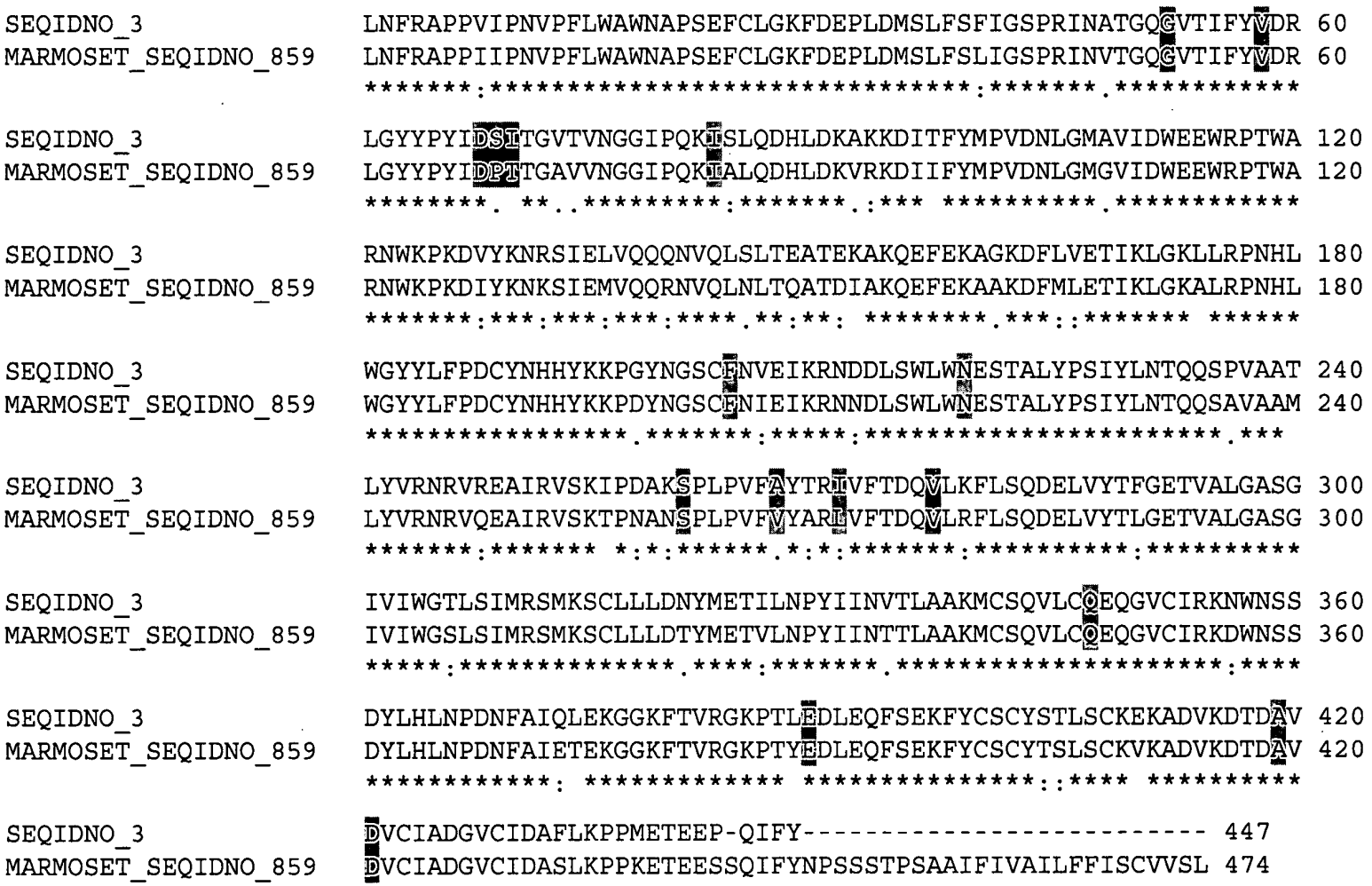
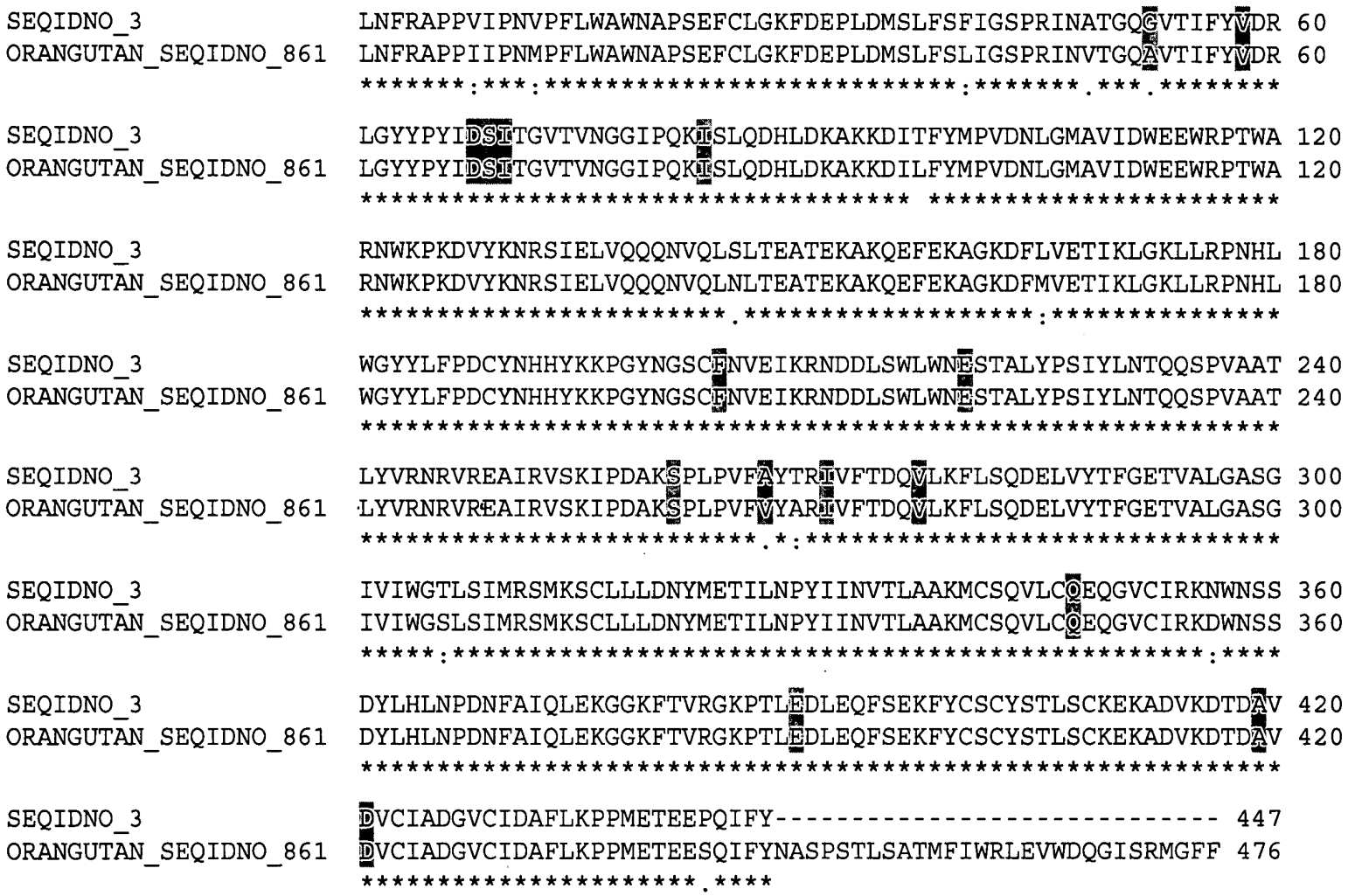

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SEQIDNO_3 LGYYPYIDSITGVTVNGGIPQKLSLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
MARMOSET_SEQIDNO_859 LGYYPYIDPTTGAVVNGGIPQKLALQDHLDKVRKDIIFYMPVDNLGMGVIDWEEWRPTWA 120
SEQIDNO_3 RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
MARMOSET_SEQIDNO_859 RNWKPKDIYKNKSIEMVQQRNVQLNLTQATDIAKQEFEKAAKDFMLETIKLGKALRPNHL 180
SEQIDNO_3 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
MARMOSET_SEQIDNO_859 WGYYLFPDCYNHHYKKPDYNGSCFNIEIKRNNDLSWLWNESTALYPSIYLNTQQSAVAAM 240
SEQIDNO_3 LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG 300
MARMOSET_SEQIDNO_859 LYVRNRVQEAIRVSKTPNANSPLPVFVYARLVFTDQVLRFLSQDELVYTLGETVALGASG 300
SEQIDNO_3 IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS 360
MARMOSET_SEQIDNO_859 IVIWGSLSIMRSMKSCLLLDTYMETVLNPYIINTTLAAKMCSQVLCQEQGVCIRKDWNSS 360
SEQIDNO_3 DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV 420
MARMOSET_SEQIDNO_859 DYLHLNPDNFAIETEKGGKFTVRGKPTYEDLEQFSEKFYCSCYTSLSCKVKADVKDTDAV 420
SEQIDNO_3 DVCIADGVCIDAFLKPPMETEEP-QIFY---------- 447
MARMOSET_SEQIDNO_859 DVCIADGVCIDASLKPPKETEESSQIFYNPSSSTPSAAIFIVAILFFISCVVSL 474

FIGURE 2L


SEQIDNO_3 LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR 60
ORANGUTAN_SEQIDNO_861 LNFRAPPIIPNMPFLWAWNAPSEFCLGKFDEPLDMSLFSLIGSPRINVTGQAVTIFYVDR 60
SEQIDNO_3 LGYYPYIDSITGVTVNGGIPQKLSLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA 120
ORANGUTAN_SEQIDNO_861 LGYYPYIDSITGVTVNGGIPQKLSLQDHLDKAKKDILFYMPVDNLGMAVIDWEEWRPTWA 120
SEQIDNO_3 RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL 180
ORANGUTAN_SEQIDNO_861 RNWKPKDVYKNRSIELVQQQNVQLNLTEATEKAKQEFEKAGKDFMVETIKLGKLLRPNHL 180
SEQIDNO_3 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
ORANGUTAN_SEQIDNO_861 WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT 240
SEQIDNO_3 LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG 300
ORANGUTAN_SEQIDNO_861 LYVRNRVREAIRVSKIPDAKSPLPVFVYARIVFTDQVLKFLSQDELVYTFGETVALGASG 300
SEQIDNO_3 IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS 360
ORANGUTAN_SEQIDNO_861 IVIWGSLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSS 360
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ORANGUTAN_SEQIDNO_861 DVCIADGVCIDAFLKPPMETEESQIFYNASPSTLSATMFIWRLEVWDQGISRMGFF 476

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61631313UA

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Doc Code: Computer Artifact Type Code: P

pages of specification and/or sequence listing and/or table ☐
Doc Code: Artifact Artifact Type Code: S

content unspecified or combined **2**
Doc Code: Artifact Artifact Type Code: U

☐ Stapled Set(s) Color Documents or B/W Photographs
Doc Code: Artifact Artifact Type Code: C

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Doc Code: Artifact Artifact Type Code: F

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Doc Code: Artifact Artifact Type Code: V

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Doc Code: Artifact Artifact Type Code: M

☐ Bound Document(s)
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Doc Code: Artifact Artifact Type Code X

☐ Other, description: _____
Doc Code: Artifact Artifact Type Code: Z

March 8, 2004

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