

Halozyme's Belated Attorney Argument in Sur-Reply Should Be Ignored

Petitioner now argues that, in addition to hyaluronidase activity, the claims require a different type of activity: a “spreading”/“diffusion” activity. Reply, 1-2. But the specification teaches that spreading/diffusion *is the result of* PH20’s hyaluronidase activity; it is not a separate enzymatic function.¹ For example, the specification teaches:

PO Sur-Reply (PGR2025-00039), 2-3

The modified PH20 polypeptides within claim 1’s genus also include those with multiple substitutions in the Hyal-EGF region of PH20. Before 2011, it was believed the Hyal-EGF domain mediated protein-protein interactions, and mutations to the Hyal-EGF domain substantially eliminated hyaluronidase activity in otherwise unaltered PH20 polypeptides.¹⁷⁴ Making multiple substitutions to the Hyal-EGF domain can alter its structure and thereby disrupt not only PH20’s hyaluronidase activity but any protein-protein interactions that might be involved in PH20’s spreading activity *in vivo*.¹⁷⁵

Pet. (PGR2025-00039), 66

Thus, *at a minimum*, to be used in the claimed methods, a modified PH20 polypeptide must be able to *hydrolyze* HA (*i.e.*, possess “hyaluronidase activity”). The common disclosure, however, indicates that enzymatic activity alone may be insufficient. It thus instructs skilled artisans to *further test* modified PH20 polypeptides to determine if they act as a “spreading or diffusing agent” by injecting each under the surface of the skin of a mouse and measuring “the ability of the PH20 polypeptide to act as a spreading agent.”¹⁵⁷

Pet. (PGR2025-00039), 24-25

As discussed in § IV.D.3(a), the common disclosure also explains that, while necessary, hyaluronidase activity may not be *sufficient* for a modified PH20 polypeptide to be able to increase delivery of another therapeutic agent.²⁰³ It thus instructs testing of modified PH20 polypeptides in a particular, *in vivo* assay to determine if they cause “spreading”/“diffusion,” which is necessary to enable increased delivery of another therapeutic agent.²⁰⁴ As the common disclosure explains: “[t]he ability of a PH20 polypeptide ... to act as a spreading or diffusing agent can be assessed” using a specified *in vivo* experiment in a mouse.²⁰⁵ Notably, this is a different test than the *in vitro* assay used to detect hyaluronidase activity.²⁰⁶ And, the common disclosure identifies only one mutant as having this

Pet. (PGR2025-00039), 76-77

PGR2025-00053
12,195,773

Halozyme Did Not Dispute Merck's "Soluble" Construction

1. A soluble human PH20 polypeptide comprising: a modification at a position aligned to position 320 with reference to the amino acid positions set forth in SEQ ID NO: 3, and 19 or less additional amino acid modifications, after alignment with SEQ ID NO: 3 to maximize the number of identical residues, wherein: modifications comprise insertions, deletions, and replacements, and the modification at position 320 is a replacement selected from the group consisting of H, K, and R.

EX1001 (PGR2025-00053)('773 Patent)

its Petition, Petitioner argued that the "common disclosure defines" this "soluble human PH20 polypeptide" via the following passage: "[a]s used herein, soluble human PH20 (sHuPH20) includes human PH20 polypeptides that lack a contiguous sequence of amino acids from the C terminus of human PH20 that includes all or a portion of the glycosylphosphatidylinositol (GPI) anchor sequence (C-terminally truncated PH20 polypeptides) such that upon expression, the polypeptides are soluble under physiological conditions." Pet., 18; EX1001, 45:24-30. For the purposes of this proceeding, Patentee does not contest Petitioner's position that this phrase is definitional. That is, soluble human PH20 polypeptides of the '773 patent

POR (PGR2025-00053), 32-33

Thus, per the common disclosure's definitions, a "soluble human PH20 polypeptide" is "a human PH20 polypeptide that (i) omits all or part of the C-terminal sequence 456-474 of SEQ ID NO:7, and (ii) is homogenous in an aqueous solution at physiological conditions."⁴⁸

Pet. (PGR2025-00053), 20

As used herein, "solubility" with reference to a protein refers to a protein that is homogenous in an aqueous solution, whereby protein molecules diffuse and do not sediment spontaneously. Hence a soluble protein solution is one in which there is an absence of a visible or discrete particle in a solution containing the protein, such that the particles cannot be easily filtered. Generally, a protein is soluble if there are no visible or discrete particles in the solution. For example, a protein is soluble if it contains no or few particles that can be removed by a filter with a pore size of 0.22 μm .

EX1026 ('731 App.), 56:31-38; Reply (PGR2025-00053), 2-3

Halozyme Ignores the Defined Meaning of “PH20”

1. A soluble human PH20 polypeptide comprising: a modification at a position aligned to position 320 with reference to the amino acid positions set forth in SEQ ID NO: 3, and 19 or less additional amino acid modifications, after alignment with SEQ ID NO: 3 to maximize the number of identical residues, wherein: modifications comprise insertions, deletions, and replacements, and the modification at position 320 is a replacement selected from the group consisting of H, K, and R.

EX1001 (PGR2025-00053)('773 Patent)

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), 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

EX1026 ('731 App.), 56:31-38, Pet. (PGR2025-00053), 28-29

Exemplary soluble PH20 polypeptides are C-terminal truncated human PH20 polypeptides that are mature (lacking a signal sequence), soluble and exhibit neutral activity, and that contain a contiguous sequence of amino acids set forth in SEQ ID NO:6 or SEQ ID NO:7 that minimally has a C-terminal truncated amino acid residue at or after amino acid residue 464 of the sequence of amino acids set forth in SEQ ID NO:6. For example, soluble PH20 polypeptides include C-terminal truncated polypeptides that minimally contain a contiguous sequence of amino acids 36-464 of SEQ ID NO:6, or includes 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 contiguous sequence of amino acids that has a C-terminal amino acid residue after amino acid 464 of SEQ ID NO:6 and retains hyaluronidase activity. Exemplary C-terminally truncated human PH20 polypeptides

are mature polypeptides (lacking a signal sequence) that include a contiguous sequence of amino acids set forth in SEQ ID NO:6 with a C-terminal residue after 464 such as 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. For example, exemplary C-terminal PH20 polypeptides have a sequence of amino

EX1026 ('731 App.), 85:4-22, Pet. (PGR2025-00053), 29-30; Reply 3

Halozyme Incorrectly Contends Folding Unnecessary for Solubility

II. CLAIM CONSTRUCTION

A. The Claims Are Structural, Not Functional

POR (PGR2025-00053), 37

As explained above, when properly construed, the claims do not require a specific function. Patentee emphasizes that, under established law, there is no requirement to describe or enable subject matter that is not claimed. *In re Entresto*,

POR (PGR2025-00053), 37

Even if Petitioner were correct to read the definition of “PH20” into “soluble human PH20 polypeptide,” (it is not), Petitioner’s argument still fails. The PH20 definition imposes no “properly folded” or any folding requirement. Petitioner thus has no basis to import that limitation into the claims. Like “soluble human PH20 polypeptide,” the specification uses “PH20” to refer to both active and inactive polypeptides, so Petitioner’s construction is inconsistent with the specification. EX1001, 118:30-48; 77:50-54.

PO Sur-Reply (PGR2025-00053), 3-4

Unfolded/Misfolded Proteins Often Become Insoluble

Proteins can fold or unfold inside chaperonins

Before protein molecules attain their native folded state they may expose hydrophobic patches to the solvent. Isolated purified proteins will aggregate during folding even at relatively low protein concentrations. Inside cells, where there are high concentrations of many different proteins, aggregation could therefore occur during the folding process. This is prevented by

EX1014, 99; Pet., 20, 74

39. Folded proteins generally are arranged to have a hydrophobic interior and a hydrophilic surface. The backbone or primary chain has a polar character, which is hydrophilic and not energetically favored within a hydrophobic environment.⁷ Proteins with native structures influenced by evolutionary pressure are typically soluble in an aqueous environment because of this arrangement of the protein's hydrophilic and hydrophobic structures. Changes induced by a protein's environment or to alteration of protein structure (*e.g.*, changes to its amino acid sequence that induce unfolding or misfolding of the protein) can cause hydrophobic portions of the protein that ordinarily reside within the hydrophobic interior of the protein structure to be exposed to solvent (water). When that happens the proteins will tend to aggregate (either through interactions with other proteins or through interactions between now-exposed hydrophobic residues or structures within a single protein molecule) and become insoluble.

EX1003 (PGR2025-00053)(Hecht), ¶ 39; Pet., 39

152. Insolubility (other than in a test environment) is typically manifested by the protein forming aggregates in an aqueous solution. These aggregates may or may not be visible to the naked eye. Aggregation can occur when hydrophobic patches (*e.g.*, hydrophobic structures or side chains) on the protein that are ordinarily buried in the protein interior become exposed to the hydrophilic aqueous environment. Rather than remain exposed in the aqueous environment, the hydrophobic patches will strongly favor non-specific binding to other hydrophobic patches available in the environment (*e.g.*, different hydrophobic patches within the same protein, or interactions between two (or more) modified proteins). This phenomenon is explained in textbooks, like Branden & Tooze (below):

EX1003 (PGR2025-00053)(Hecht), ¶ 152; Pet., 39

No Data from Testing Mutants for Solubility – Only Theoretical Assays

As used herein, soluble human PH20 (sHuPH20) includes human PH20 polypeptides that lack a contiguous sequence of amino acids from the C-terminus of human PH20 that includes all or a portion of the glycosylphosphatidylinositol (GPI) anchor sequence (C-terminally truncated PH20 polypeptides) such that upon expression, the polypeptides are soluble under physiological conditions. For example, soluble human PH20 polypeptides are C-terminally truncated polypeptides of human PH20 set forth as SEQ ID NO:6 in its precursor form or in SEQ ID NO:7 in its mature form lacking the signal sequence, or allelic variants thereof (*e.g.* set forth in any of SEQ ID NOS: 68-72). 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

EX1026 ('731 App.), 56:31-38; Pet. (PGR2025-00053), 18-20

207. As I discussed previously (§ 151), a multiply-modified human PH20 polypeptide that exhibits significant hyaluronidase activity in the assay described in the common disclosure may be expected to be soluble, though this could not be confirmed until the mutant's solubility is tested. The same expectation would not follow for multiply-modified human PH20 polypeptides that show no activity in the hyaluronidase assay being used to screen the mutants. Instead, to determine which of the "inactive" mutants (if they were produced at all) are soluble proteins, they would each need to be tested in a solubility assay. This presents significant practical challenges, as the solubility assay illustrated in the common disclosure requires significantly larger quantities of the produced PH20 mutants than would be made by the transfected cells used to create the library that was screened for hyaluronidase activity. For example, Bordier reported using 0.2 mg to 1.0 mg/mL protein samples in its Triton® X-114 assay that was the solubility assay.¹⁷¹

EX1003 (Hecht)(PGR2025-00053), ¶ 207; Pet. (PGR2025-00053), 40, 65

No Examples of Soluble Multiply-Modified PH20s

163. As I discussed above (§ II.B.2), including additional substitutions after a first may adversely impact the structure modified by the first substitution, may affect the region of the protein having that first change, or may introduce impactful changes in an unrelated part of the protein. The ripple effect of changes throughout the protein can alter the structure of the protein in a way that impacts its solubility and other characteristics of the protein. Because the common disclosure has no examples of any PH20 with multiple substitutions and does not characterize the effects of the mutations on the PH20 protein structure or solubility, a skilled artisan could not realistically predict whether an effect observed in an active single-modified soluble human PH20 polypeptide would be observed for the trillions and trillions of modified human PH20 polypeptides that incorporate that first mutation plus the myriad other sets of 2 to 20 additional substitutions.

EX1003 (Hecht), ¶ 163; Pet. (PGR2025-00053), 74

193. The remainder of the common disclosure is largely hypothetical. This includes its descriptions about possible PH20 polypeptides that include one of the tested single substitutions, but then might have numerous additional, unidentified changes. Unlike its description of single-replacement PH20₁₋₄₄₇ mutants, the common disclosure identifies no examples of soluble human PH20 polypeptides with multiple amino acid substitutions at different positions (*i.e.*, specific amino acids being inserted into two or more different positions of the same PH20 polypeptide) that rendered active proteins. This appears to be the case because no such multiply-modified soluble human PH20 polypeptides appear to have actually been made or tested. That seems consistent with the common disclosure's

EX1003 (Hecht), ¶ 193; Pet. (PGR2025-00053), 52

PGR2025-00052
12,264,345

No Response to Written Description Ground Based on Combination

(b) The Common Disclosure Does Not Describe a Genus of Modified PH20 Polypeptides with Multiple Modifications Restricted to the “Hyaluronidase Domain”

Likewise here, there are **no examples** (prophetic or actual) of modified PH20 polypeptides with multiple modifications **restricted within** positions 3-339 of SEQ ID NO:7, much less one with a position 313 replacement.¹¹² The common

The common disclosure also never portrays its examples of **singly-modified** PH20 mutants as targeting only the “hyaluronidase domain.” It states the opposite—“...**each of residues** (...1-447 of SEQ ID NO:3...) was changed to one of about 15 amino acid residues...”.¹¹⁵ Thus, it reports mutants with single substitutions at many positions **outside** positions 3-339 (*i.e.*, 83 for “active” and 98 for “inactive”), and many positions **within** the 3-339 region have no “active” (67) or “inactive” (27) mutants.¹¹⁶

Petition (PGR2025-00052), 43-46

214. I did not find any description in the common disclosure of a process that intentionally restricted multiple modifications to within the “common hyaluronidase domain” in a single PH20 polypeptide. For example, as I discussed above (§ 107), the protocol described in Example 2 did not restrict the sequential single replacements to only the “common hyaluronidase domain” in the human PH20₁₋₄₄₇ protein. Instead, it said it targeted **every** position in the 1-447 sequence of the PH20₁₋₄₄₇ polypeptide for replacements.¹⁹⁸

215. Neither of the theoretical mutagenesis protocols (§§ 211-212) is focused on production and discovery of modified PH20 polypeptide with two or more modifications restricted within the “common hyaluronidase domain.” The first protocol does not focus on any particular region of the PH20 sequence for mutations. Instead, it describes making a first library, selecting candidates for further mutagenesis based on retained hyaluronidase activity, then repeating these steps.¹⁹⁹ The protocol will select mutants with substitutions at random positions within the entire PH20 sequence in each round that collectively resulted in a mutant with enzymatic activity.

EX1003 (Hecht) (PGR2025-00052), 43-46; Pet. 44-45

US-457 Contradicts Dr. Simpson's Portrayal of PH20 Truncation Mutants

Terminus (precursor)	Terminus (mature)	pH 7.4	pH 5.5	Normalized Activity (pH 7.4)	Normalized Activity (pH 5.5)	Simpson Classification	US-457 Classification	Table
482	447	2.03175	1.647	100%	100%	Active	Active	6
465	430	0.441	0.468	22%	28%	Inactive	Active	6
466	431	0.000	0.212	0%	13%	Inactive	Active	6
467	432	0.545	0.900	27%	55%	Reduced Activity	Active	6
468	433	0.860	0.916	42%	56%	Reduced Activity	Active	6
472	437	0.545	0.900	27%	55%	Active	Active	6
490	455	0.819	1.238	40%	75%	Reduced Activity	Active	6
491	456	0.365	0.563	18%	34%	Reduced Activity	Active	6
491	456	0.851	0.729	42%	44%	Reduced Activity	Active	6A
492	457	n/t	n/t	n/a	n/a	Reduced Activity	-	none
493	458	0.689	0.851	34%	52%	Reduced Activity	Active	6
494	459	n/t	n/t	n/a	n/a	Reduced Activity	-	none
495	460	0.689	0.770	34%	47%	Reduced Activity	Active	6
496	461	n/t	n/t	n/a	n/a	Reduced Activity	-	none
497	462	0.176	0.216	9%	13%	Reduced Activity	Active	6
497	462	0.365	0.162	18%	10%	Reduced Activity	Active	6A
498	463	0.527	0.203	26%	12%	Inactive/Little Activity	Low expression	6A
499	464	0.020	0.045	1%	3%	Inactive/Little Activity	Low expression	6
499	464	0.486	0.122	24%	7%	Inactive/Little Activity	Low expression	6A
500	465	0.365	0.162	18%	10%	Inactive/Little Activity	Low expression	6A
501	466	0.446	0.122	22%	7%	None	Active	6
502	467	0.608	0.324	30%	20%	None	Active	6
503	468	1.094	0.648	54%	39%	None	Active	6

Note:

The information in this sheet is from Published U.S. Patent Application 2010/0143457 ("US-457"), Example 5, ¶¶ 360-362 (Table 6) and EX2068 (Simpson Decl.), ¶ 188.

EX1133 (Naismith), ¶ 217Appx. B-6;
Reply, 35

Modifications to PH20s Are Unpredictable

Modifications to PH20 Are Unpredictable

- **Most single-substitution PH20₁₋₄₄₇ mutants impair, reduce, or eliminate activity in unexplained ways**
 - Even single modifications can result in inactivity, aggregation, instability, failure to secrete
- **Large swings in activity for truncated PH20s confirms long-range effects of modifications in C-terminal region**
 - Long-range effects contribute to epistasis
- **High variability among active mutants at same position confirms unpredictable long-range effects of mutations**
 - Effects of individual substitutions are not explained in disclosure
- **Multiple Modifications Amplify Unpredictable Effects of Mutations and Introduce Epistatic Effect**
 - Observed even in α - β Barrel motifs; epistatic effects are unpredictable

See e.g., Pet., 10-11, 41-45, 52-54, 73-75; Reply, 4-6, 7-9, 20, 23-33.

Amino Acid Sequence vs. Folded Protein

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SEQ ID NO: 3      moltype = AA  length = 447
FEATURE          Location/Qualifiers
REGION           1..447
                 note = Mature PH20 36-482
source           1..447
                 mol_type = protein
                 organism = Homo sapiens

SEQUENCE: 3
LNFRAPPVIP NVPFLWAWNA PSEFCLGKFD EPLDMSLFSF IGSPRINATG QGVTFIFYVDR 60
LGYYPYIDSI TGVTVNGGIP QKISLQDHL D KAKKDITFYM PVDNLGMAVI DWEWRPTWA 120
RNWPKPDVYK NRSIELVQQQ NVQLSLTEAT EKAKQEF EKA GKDFLVETIK LGKLLRPNHL 180
WGYYLFPCY NHYKPGYN GSCFNVEIKR NDDL SWLWNE STALYPSIYL NTQQSPVAAT 240
LYVRNRVREA IRVSKIPDAK SPLPVFAYTR IVFTDQVLKF LSQDELVYTF GETVALGASG 300
IVIWGTL SIM RSMKSCLLD NYMETILNPY IINVT LAAKM CSQVLCQEQG VCIRKNWNSS 360
DYLHLNPDNF AIQLEKGGKF TVRGKPTLED LEQFSEK FYC SCYSTLSCKE KADV KDTDAV 420
DVCIADGVCI DAFLKPPMET EEPQIFY 447
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EX1026 ('731 App.), SEQ ID NO:3



EX2070 (Petsko), ¶ 131; POR, 12

The Sequence Space of the Claims

1. A modified PH20 polypeptide, comprising one or more amino acid modifications in an unmodified PH20 polypeptide, wherein:

the unmodified PH20 polypeptide consists of the amino acid sequence selected from the group consisting of SEQ ID NO: 3, 7 and 32-66;

amino acid modifications are selected from the group consisting of amino acid replacements(s), deletion(s), and/or insertion(s);

the modified PH20 polypeptide comprises an amino acid replacement at a position corresponding to residue 307, with reference to amino acid positions set forth in SEQ ID NO:3;

the replacement at the position corresponding to residue 307 is selected from the group consisting of G, K, N, Q, S, T, V, W, and Y;

corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide having the amino acid sequence of SEQ ID NO:3; and

the modified PH20 polypeptide has at least 91% sequence identity to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 7 and 32-66.

EX1001 ('590 Patent), Claim 1

Claims	SEQ ID/ % Identity	PH20 length	# Changes	Pos. 307 Choices	Add'l Changes	# of Distinct Polypeptides
1	3 /91	447	40	9	39	1.26×10^{107}
	7 /91	474	42	9	41	5.69×10^{112}
2	35 /95	433	21	1	20	5.08×10^{59}
3	66 /91	465	41	9	40	1.27×10^{110}
4	35 /91	433	38	9	37	9.02×10^{101}

Pet., 21 (citing EX1004 (Park), ¶ 179, App'x F)

**Claim Parameter Sequence Space
(between 10^{59} to 10^{112} different
sequences)**

EX1133 (Naismith), ¶¶ 78-79; Reply, 5, 21, 27-28