PGR2025-00003–USP 11,952,600

PGR2025-00004–USP 12,018,298

PGR2025-00006–USP 12,152,262

PGR2025-00009–USP 12,123,035

Petitioner Merck, Sharp & Dohme, LLC
Demonstratives

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Grounds

- § 112 Grounds
  - Lack of Benefit or Priority
  - Lack of written description
  - Lack of enablement
- § 103 Grounds
  - Obviousness of single substitution $PH20_{1\text{-}447}$ mutants (D320K, M313K, L317Q, S312T, S312N)

Overview

- **Claim Construction and Scope**
- **Unpredictability of Modifications to PH20**
- **The Claims Lack Written Description**
  - **No Disclosure of Representative Species**
  - **No Identification of Common Structural Features**
- **The Claims Are Not Enabled**
  - **Full Scope Can't be Practiced Using Mutagenesis or Homology Modeling**
  - **Dr. Petsko's Tolerance Analysis Does Not Enable Full Scope**
  - **The Claims Encompass Inoperative & Non-Useful Mutants Under Halozyme's Construction**
- **The '429 Patent Renders Obvious Single Substitution $PH20_{1-447}$ Mutants (D320K, M313K, L317Q, S312T, S312N)**

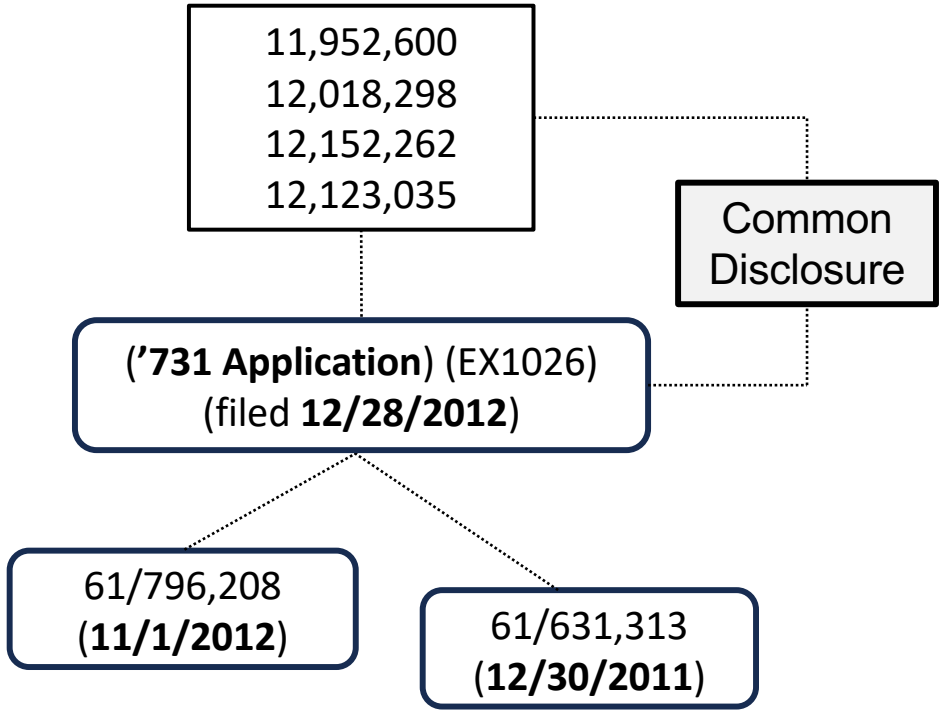
See, e.g., Pet., 48-60, 68-76, 79-83, 85-107; Reply, 4-7, 32-34, 39-42.

The Patents and the Common Disclosure

Related U.S. Application Data

(60) Division of application No. 17/327,568, filed on May 21, 2021, which is a continuation of application No. 15/226,489, filed on Aug. 2, 2016, now Pat. No. 10,865,400, which is a division of application No. 13/694,731, filed on Dec. 28, 2012, now Pat. No. 9,447,401, said application No. 17/327,568 is a continuation of application No. 13/694,731, filed on Dec. 28, 2012, now Pat. No. 9,447,401, said application No. 17/327,568 is a continuation of application No. 16/912,590, filed on Jun. 25, 2020, now Pat. No. 11,066,656, which is a continuation of application No. 15/226,489, filed on Aug. 2, 2016, now Pat. No. 10,865,400, and a division of application No. 13/694,731, filed on Dec. 28, 2012, now Pat. No. 9,447,401, said application No. 17/327,568 is a continuation of application No. 16/824,572, filed on Mar. 19, 2020, now Pat. No. 11,041,149, which is a continuation of application No. 15/226,489, filed on Aug. 2, 2016, now Pat. No. 10,865,400, which is a division of application No. 13/694,731, filed on Dec. 28, 2012, now Pat. No. 9,447,401.

(60) Provisional application No. 61/796,208, filed on Nov. 1, 2012, provisional application No. 61/631,313, filed on Dec. 30, 2011.



Merck's Experts



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Claim Construction and Scope

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Claims Share Common Form

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:
(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and
(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and
(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:
(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NO: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and
(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 313 with reference to amino acid positions set forth in SEQ ID NO: 3; and
(c) the modification at position 313 is a replacement selected from among A, H, K, L, P, R, and Y.

EX1001 (PGR2025-00004)('298 Patent)

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:
(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NO: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and
(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 317 with reference to amino acid positions set forth in SEQ ID NO: 3; and
(c) the modification at position 317 is a replacement selected from the group consisting of H, I, K, M, Q, R, and S.

EX1001 (PGR2025-00006)('262 Patent)

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 NOs: 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 312, with reference to amino acid positions set forth in SEQ ID NO:3;
the replacement at the position corresponding to residue 312 is selected from the group consisting of G, K, L, N, and T;
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 (PGR2025-00009)('035 Patent)

Institution Decisions: Claims Encompass Enzymatically Active PH20s

C. Analysis

We find that on the present record, the evidence supports a broad definition of "modified PH20 polypeptide" that includes active molecules.

I.D., 12 (PGR2025-00003)('600 Patent)

Here, the '600 patent defines "PH20" as a type of hyaluronidase enzyme and "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." Ex. 1001, 45:60–62. The '600

I.D., 13 (PGR2025-00003)('600 Patent)

posttranslational modifications." *Id.* at 46:15–18. The '600 patent provides a specific definition of the term "modified PH20 polypeptide" which

> refers to a PH20 polypeptide that contains at least one amino acid modification, such as at least one amino acid replacement as described herein, in its sequence of amino acids compared to a reference unmodified PH20 polypeptide. 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.

Id. at 48:38–53 (emphasis added).

I.D., 13-14 (PGR2025-00003)('600 Patent)

The definition of "modified PH20 polypeptide" in the '600 patent even permits up to 150 amino acid replacements but ***only*** "so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity." Ex. 1001, 48:44–46. That is, the definition of "modified PH20 polypeptide" in the '600 patent expressly requires some hyaluronidase activity. And Patent Owner's disclaimer of claims 5 and 6 does not impact the claim differentiation argument. The record reflects that the original issuance of these claims indicates that the Examiner allowed claim 1 with the understanding that it encompassed modified PH20 polypeptides with hyaluronidase activity, and there is no limitation in claim 1 limiting the PH20 polypeptides to inactive polypeptides with no hyaluronidase activity. *See* Ex. 1001, 309:2–17. On the current record, we therefore adopt the definition for "modified PH20 polypeptide" as recited in the '600 patent to encompass polypeptides with some hyaluronidase activity.[6]

I.D., 15-16 (PGR2025-00003)('600 Patent)

Halozyme: Claims Define D320K Variants and "Their Subvariants"

posttranslational modifications." *Id.* at 46:15–18. The '600 patent provides a specific definition of the term "modified PH20 polypeptide" which

> refers to a PH20 polypeptide that contains at least one amino acid modification, such as at least one amino acid replacement as described herein, in its sequence of amino acids compared to a reference unmodified PH20 polypeptide. 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.

Id. at 48:38–53 (emphasis added).

I.D., 13-14 (PGR2025-00003)('600 Patent)

The specification describes and characterizes the common structural features of the four D320 variants recited in the claims (D320K, D320R, D320H and D320S) and their 95% identical subvariants.

POR, 45

Sections V.A, C. Each claimed polypeptide must (i) include a defined substitution at position 320, and (ii) share at least 95% sequence identity with SEQ ID NOs: 3 or 32-66. EX2068, ¶¶254. POSAs would recognize, within this narrow genus, that the four variants and their subvariants are structurally homogeneous in that they share at least 95% identical primary structure, the D320 mutation, and for those that are active, the same PH20 tertiary structure and several common structural features, such as the same common residues critical for catalysis; moreover, POSAs can

POR, 63

"Modified PH20 Polypeptide" Does Not Have a "Purely Structural Meaning"

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:
(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and
(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and
(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

Based on this express definition, the current record does not support the interpretation of Dr. Triggs-Raine that the "term 'modified PH20 polypeptide,' therefore, has a purely structural meaning in the context of the specification." Ex. 2001 ¶ 68.[4] Indeed, when reproducing the definition from this column of the '600 patent, Dr. Triggs-Raine does not include any text after the first period, but the '600 patent text continues to detail specific elements required including a requirement that replacements in the PH20 polypeptide are permitted "so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity." Ex. 1001, 48:44–46. On this record, Dr. Triggs-Raine does not address this statement. And Dr. Triggs-Raines states "the modified PH20 polypeptides have multiple credible uses, including 'therapeutic uses of modified PH20 polypeptides that have the ability to degrade hyaluronan.'" Ex. 2001 ¶ 115.[5] That is, Dr. Triggs-Raines recognizes hyaluronidase as the primary utility for the modified PH20 polypeptides recited in claim 1.

I.D., 14-15 (PGR2025-00003)('600 Patent)

Claims Include Enzymatically Active PH20s

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:

(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and

(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and

(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

In its Institution Decision, the Board preliminarily found that the term "modified PH20 polypeptide" in the claims "encompass[ed] polypeptides with some hyaluronidase activity." ID, 15. Patentee does not dispute that the claims encompass PH20s with hyaluronidase activity. As set out below, however, Patentee does contend that the claims are defined by structure only, and encompass ***both*** enzymatically active and enzymatically inactive PH20s.

POR, 31

19 In the genus of polypeptides that are
20 defined by claim 1, would you agree that there
21 are enzymatically active, properly folded,
22 modified PH20 polypeptides?
23 A. Yes.

EX1131 (Petsko Dep.), 61:19-23; Reply, 5

Two Mutually Exclusive Types of Modified PH20s – Active or Inactive

98. The common disclosure describes two, mutually exclusive types of modified PH20 polypeptides. First, it says "active mutants" are those having at least 40% hyaluronidase activity of the unmodified form of PH20.[49] Then, it says that "inactive mutants" are mutants that do not have significant hyaluronidase activity, which it indicates is 20% or less of the activity of the unmodified PH20.[50] This is a fairly straightforward delineation of two categories of proteins that either have or do not have an activity (here hyaluronidase activity).

EX1003 (Hecht), ¶ 98; Pet., 23

131. The inventors appear to have stopped after making only single-replacement mutants. For example, are no mutants with a first mutation that led to its classification as an "active mutant" and that then acquired a second mutation. More importantly, there are no "double" or "triple" (or more) mutants that combined sets of single mutations classified as causing both "active" mutants" and "inactive" mutants or were within particular regions of the PH20 sequence. Consequently, there is no meaningful guidance in the common disclosure on what happens when different kinds mutations are combined, or different mutations in a region of the PH20 are combined.

EX1003 (Hecht), ¶ 131; Pet., 71

c. The common disclosure classifies modified PH20 polypeptides having the D320H, D320K, D320R or D320S substitutions as "active mutants" (*i.e.*, mutants that retained >40% enzymatic activity of unmodified $PH20_{1-447}$). Those four substitutions, for example, are listed only in Tables 3 and 9, which compile lists of "active mutants" (below).[110]

d. The common disclosure explains that a modified PH20 polypeptide can have modifications in addition to a first one (here the position 320 substitution). It then explains when an active mutant is further modified to have additional changes, those changes are selected to preserve hyaluronidase activity (*i.e.*, "[a] modified PH20 polypeptide can have up to 150 amino acid replacements, so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity.").[111] That is the case for the modified PH20 polypeptides being claimed—they all have at least a position 320 substitution that retained >40% hyaluronidase activity, which causes them to be classified as "active mutants," and each may have between zero and 14 or 22 additional modifications.

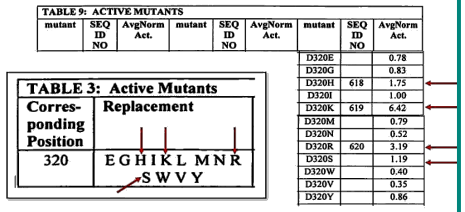


TABLE 9: ACTIVE MUTANTS

mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.	mutant	SEQ ID NO	AvgNorm Act.
						D320E		0.78
						D320G		0.83
						D320H	618	1.75
						D320I		1.00
						D320K	619	6.42
						D320M		0.79
						D320N		0.52
						D320R	620	3.19
						D320S		1.19
						D320W		0.40
						D320V		0.35
						D320Y		0.86

EX1133 (Naismith), ¶ 108; Reply, 4

Patent Owner – Common Structural Features of Active PH20s

The specification describes and characterizes the common structural features of the four D320 variants recited in the claims (D320K, D320R, D320H and D320S) and their 95% identical subvariants.

As Patentee's experts explain, based on the patent's description and extensive mutagenesis data, POSAs would have understood modified PH20 polypeptides within the scope of the claims to include a common mutation at position 320 to one of four specified amino acids and 95% identity in primary structure (*i.e.*, amino acid sequence). EX2068, ¶¶287-288; EX2070, ¶¶239-247. They also would have understood the claimed PH20s exhibit the same mechanism of action and share the same characteristic PH20 alpha-beta barrel tertiary structure, including a common active site and the same key catalytic residues, absent changes of the type the patent identifies as inactivating. *Id.*

PO Response, 45-46

The record developed since Institution establishes that specification discloses the common structural features based on that primary structure and the related mutagenesis data:

- 95% sequence identity to the primary structure of the recited SEQ IDs;
- The D320 mutation;
- And for actives:
  - A PH20 alpha-beta barrel fold, with known catalytic and binding sites.
  - Lack of the inactivating changes identified in the disclosure.

PO Sur-Reply, 21

Enablement and Written Description Must Be Met for the Full Claim Scope

Amgen v. Sanofi, 598 U.S. 594, 610 (2023)

If a patent claims an entire class of processes, machines, manufactures, or compositions of matter, the patent's specification **must enable a person skilled in the art to make and use the entire class**. In other words, **the specification must enable the full scope of the invention as defined by its claims.** The more one claims, the more one must enable.

Duke v. Sandoz, 160 F.4th 1305, 1310 (Fed. Cir. 2025)

Whether a patent provides **adequate written description** for a claim presents a question of fact. See Gen. Hosp. Corp. v. Sienna Biopharmaceuticals, Inc., 888 F.3d 1368, 1371 (Fed. Cir. 2018). **That question is whether the patent specification discloses that a person of ordinary skill in the art would conclude "the inventor possesse[d] the full scope of the invention" at the time of their patent application**. *LizardTech, Inc. v. Earth Res. Mapping, Inc.*, 424 F.3d 1336, 1345 (Fed. Cir. 2005)

Enablement and Written Description Must Be Met for the Full Claim Scope

> For the sake of simplicity, however, and given the robustness of the patent's teachings, the Patentee assumes—without conceding—that the specification must enable POSAs to reliably distinguish, create, and utilize the full range of active and, if Patentee's construction is accepted, inactive modified PH20 polypeptides encompassed by the claims. For the reasons stated below, it does.

POR, 44

> Patentee may contend the claims should be read as encompassing both alternative embodiments (*i.e.*, "active" and "inactive" mutants). Reading the claims in that manner is incorrect. It also exacerbates the § 112 problems, as every claim still necessarily includes (and thus must describe and enable) the full sub-genus of "active mutants" defined by claims 5 and 6.[64]

Pet. 26

Lack of Restrictions on Locations (other than one) or Types of Changes

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:

(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and

(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and

(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

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.

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 truncations 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 amino acids selected to replace the target positions on the particular protein being optimized can be either all of the remaining 19 amino acids, or a more restricted group containing only selected amino acids. In some methods provided herein, each amino acid that is replaced is independently replaced by 19 of the remaining amino acids or by less than 19 of the remaining amino acids, such as 10, 11, 12, 13, 14, 15, 16, 17 or 18 of the remaining amino acids.

EX1026 ('731 App.), 53:1-3; 68:25-31, 144:9-14, Pet., 19-20, Reply, 5, 11

Lack of Restrictions on Locations (other than one) or Types of Changes

119. The claim language does not require the additional changes (besides the position 320 change) to be restricted to any region of the polypeptide. In addition, the common disclosure explains that changes can be additions, deletions or replacements, and for replacements, can be at any position in the sequence and to any other amino acid (*i.e.*, 19 alternatives).[60]

EX1003 (Hecht), ¶ 119, Pet., 19-20, 32-34, Reply, 5

46. I agree with Dr. Hecht and the other experts that the sequence identity claim parameters do not restrict the location(s) or type(s) of amino acid changes permitted in each modified PH20 polypeptide, other than the substitution required at position 320.[18] Provided that the entire amino acid sequence of a polypeptide satisfies the numerical threshold of the sequence identity claim parameters and has one of the position 320 substitutions, it can contain an amino acid sequence with any (or all) of the following types of changes:

(i) changes at any position in the amino acid sequence (*e.g.*, at highly conserved positions or positions known to be necessary for activity);

(ii) a change that is a substitution can replace any wild-type amino acid with one of the 19 alternative amino acids; and

(iii) changes can delete or add one or several amino acids, and those deletions or additions can occur anywhere within the amino acid sequence.

EX1133 (Naismith), ¶ 46, Reply, 1

Lack of Restrictions on Locations (other than one) or Types of Changes

Q. Sure. The changes that constitute the difference that translates into the 5% difference from the amino acid sequences of the wild-type PH20, there could be substitutions, deletions, or additions of amino acids. Right?

A. Yes. Those are possibilities.

EX1130 (Simpson Dep.), 90:25-91:12, Reply, 5

Q. And do you understand those changes can be -- one kind of change could be a substitution of the amino acid?

A. Yes.

Q. Another kind could be a deletion of an amino acid?

A. Yes, I think so.

Q. And a third kind would be an addition of an amino acid. That would also count as a change that gets calculated as part of the sequence identity?

A. If it doesn't cross the threshold.

Q. Correct.

A. Yes.

EX1131 (Petsko Dep.), 54:20-55:8, Reply, 5

Claims Have Immense Breadth

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:

(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and

(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and

(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

The terms used in the claims are either expressly defined in the specification of the common disclosure or are used with their common and ordinary meaning. Consequently, no term requires an express construction to assess the grounds in this Petition. A clear understanding of the ***breadth*** of the claims, however, is important to assess the grounds. Specifically, each claim captures a massive genus of structurally distinct mutant PH20 polypeptides that is neither adequately described in nor enabled by the common disclosure of the '731 Application and the '600 Patent.

Pet., 17-18

Size of Sequence Space is Undisputed

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:

(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and

(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and

(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

Claim	SEQ ID/ % Identity	PH20 length	# Changes	Pos. 320 Choices	Add'l Changes	# Distinct Polypeptides
1	**3** /95%	447	22	4	21	1.50×10^{63}
	66 /95%	465	23	4	22	1.35×10^{66}
2	**3** /95%	447	22	1	21	3.76×10^{62}
3	**35** / 96%	433	17	4	16	6.14×10^{49}
4	**32** / 95%	430	21	4	20	1.76×10^{60}

Pet. 20-21; EX1003 (Hecht), ¶¶ 120, 122; EX1004 (Park), ¶¶ 168-171

Q. Okay. And so based on those parameters of the 447 amino acid sequence, 95 percent sequence identity, along with 22 changes and four options at position 320, that translates into 1.5 times 1063 different polypeptide sequences, right?

A. Assuming there are no errors in the calculation. I haven't verified it.

Q. Okay. And you don't have a basis for, today, disputing that number as accurate?

A. As I said, I don't disagree.

EX1131 (Petsko Dep.), 60:24-61:7; Reply, 4

Claims Do Not Define Narrow Set of Sequences

As discussed in Section V.A-C, the challenged claims recite a narrow and structurally homogeneous genus of four PH20 variants with one of four substitutions at position D320, as well as subvariants of each that must maintain at least 95% sequence identity. The scope of the claims is extremely narrow. While Petitioner

PO Response, 65

They would. As an initial matter, the challenged claims do not encompass an "immense genus" of unrelated polypeptides, as Petitioner contends (Pet., 42–45), but instead four variants with the D320 mutation and a narrowly defined and structurally homogeneous set of PH20 subvariants with 95% sequence identity. *See*

PO Response, 63

Indeed, unlike *Ariad* (and *Abbvie*) the patent claims here are exceedingly narrow, and in no way "claim[] all solutions" or even a tiny fraction of the possible solutions to degrading HA with modified PH20s. EX2068, ¶¶284-286. The patent claims only what was invented; that is what written description asks.

PO Response, 53

Claim	SEQ ID/ % Identity	PH20 length	# Changes	Pos. 320 Choices	Add'l Changes	# Distinct Polypeptides
1	**3** /95%	447	22	4	21	1.50×10^{63}
	66 /95%	465	23	4	22	1.35×10^{66}
2	**3** /95%	447	22	1	21	3.76×10^{62}
3	**35** / 96%	433	17	4	16	6.14×10^{49}
4	**32** / 95%	430	21	4	20	1.76×10^{60}

Pet. 20-21; EX1003 (Hecht), ¶¶ 120, 122; EX1004 (Park), ¶¶ 168-171

Total Changes	Total Sequences	#
1	4	4.0E+00
2	32,832	3.3E+04
3	134,430,624	1.3E+08
4	366,099,399,360	3.7E+11
5	746,019,051,045,840	7.5E+14
6	1,213,325,384,620,950,000	1.2E+18
7	1,640,618,140,904,970,000,000	1.6E+21
8	1,897,023,318,926,400,000,000,000	1.9E+24
9	1,914,807,912,541,340,000,000,000,000	1.9E+27
10	1,713,965,838,159,220,000,000,000,000,000	1.7E+30
11	1,377,514,344,128,570,000,000,000,000,000,000	1.4E+33
12	1,004,082,728,292,990,000,000,000,000,000,000,000	1.0E+36
13	669,304,811,967,966,000,000,000,000,000,000,000,000	6.7E+38
14	410,850,184,577,259,000,000,000,000,000,000,000,000,000	4.1E+41
15	233,627,022,815,683,000,000,000,000,000,000,000,000,000,000	2.3E+44
16	123,697,721,013,477,000,000,000,000,000,000,000,000,000,000,000	1.2E+47
17	61,253,565,224,361,100,000,000,000,000,000,000,000,000,000,000,000	6.1E+49

Reply, 8

Petition: Claims Require Active PH20s

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:
(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and
(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and
(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

The claim language reinforces that they are limited to the "active mutant" embodiment.

First, every claim requires each modified PH20 polypeptide in its scope to have one of four replacements at position 320 that yielded an "active mutant" as a single-replacement $PH20_{1-447}$ polypeptide (*i.e.*, D320H, D320K, D320R, or D320S). These mutants are listed in Table 3 and reported as having >40% activity in Table 9.[61]

Second, claims 5 and 6 restrict the genus of active mutants in claim 1 (*i.e.* those with at least 40% activity) to active mutant modified PH20 polypeptides that have at least 100% or 120% of the activity of unmodified PH20, respectively.

Third, the specification defines a "modified PH20 polypeptide" as "a PH20 polypeptide that contains at least one modification," but can also "have up to 150 changes, so long as the resulting modified PH20 polypeptide ***exhibits hyaluronidase activity.***"[62] This aligns with the specification's prophetic methodology for discovering PH20 polypeptides with multiple changes, which starts with one substitution that yields an "active mutant," randomly introduces another, and then screens to find "double mutants" that ***retained*** hyaluronidase activity.[63] This tracks the claims, which require one substitution and permit others.

Pet., 25-26

Enzymatic Activity Requires Properly Folded PH20s

[12] When I say "enzymatically active" in this context, I am not referring to a specific level of enzymatic activity. Rather, I am using enzymatic activity as a proxy for a properly folded protein. Secretion is another proxy that could be used for a properly folded protein; proteins that do not fold will generally not be secreted.

EX2070 (Petsko Dec.), ¶ 128, FN12; Reply, 1, 3, 5, 13

46. The specific amino acids in specific positions of an amino acid sequence also play a critical role in inducing the folding of the protein into its destined structure, as well as in maintaining that structure.[11]

55. Introducing multiple mutations into the amino acid sequence of a protein is a different story. Because so many secondary structures are dependent on particular patterns of amino acids with the correct spacing and character of amino acids at different positions, changing many amino acids simultaneously risks disrupting the pattern necessary to induce formation of the original secondary structure. Eliminating or altering a secondary structure can prevent the protein from folding and be highly destabilizing to the overall protein structure.[21]

EX1004 (Hecht), ¶¶ 46, 55; Pet., 15-16, 58, 60; Reply, 7, 23-24, 27

96. A modified PH20 polypeptide must preserve most if not all aspects of the tertiary structure of the wild-type PH20 protein to exhibit hyaluronidase activity.[84] In Dr. Petsko's terminology, it must be "properly folded."[85] This includes not just the structure of the active site, but the spatial positioning of particular residues and structural motifs within it, as well as the precise structural relationships necessary for the protein to bind to and hydrolyze the HA substrate.[86] Only particular amino acid sequences may yield the precise structure-function relationship that is needed. From the literature before 2011, these include: (a)

EX1133 (Naismith), ¶ 96; Reply, 3, 13, 16

POSAs Think of "PH20" As a Folded Protein

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:

(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and

(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and

(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

EX1001, 119:13-33; 79:27-29. Third, Petitioner contends that because wild type PH20, which is a membrane-bound sperm cell protein, is active and properly folded, "PH20" must be too. But the claims are directed to ***modified*** PH20 polypeptides which are C-terminally truncated and not membrane-bound or in sperm.

PO Sur-reply, 3

homologous to Hyal-1. POSAs think of PH20 as a folded protein, and POSAs think of 95% identical variants as proteins typically with the same tertiary structure but with 5% variability in the residues in that tertiary structure. POSAs do not think of modified PH20s as 10^60 strings of text that happen to be 95% identical to one another.

EX2070 (Petsko Dec.), ¶ 345; Reply, 3, 12

93. Dr. Petsko makes this point in his declaration. In ¶ 345 of his declaration, he states that "***POSAs think of PH20 as a folded protein***…" and not just "strings of text that happen to be 95% identical to one another." It is folding that gives rise to the spatial arrangement of amino acids linked by peptide bonds (the sequence). Dr. Petsko makes this point and echoes the definition of the PH20 ***protein*** that the patent uses:[80]

I agree with Dr. Petsko that a POSA would understand the presence of the word "PH20" in the phrase "modified PH20 polypeptide" to be indicating that the modified PH20 polypeptides being claimed are folded proteins.

EX1133 (Naismith), ¶ 93; Reply 3

POSAs Think of "PH20" as a Folded Protein

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,

EX1026 ('731 Application), 50:31-32, Reply, 2-3

16 And at the bottom of the page, do you see
17 a definition of the word "PH20" around Line 31?
18 A. Yes.
19 Q. And what is the definition of PH20 that
20 the common disclosure provides?
21 A. (Reading:)
22 As used herein, PH20
23 refers to a type of
24 hyaluronidase that occurs in
25 sperm and is neutral-active.

EX1130 (Simpson Dep.), 60:16-25; Reply, 3

13 Q. So when the PH20 is on the sperm surface,
14 is it a folded protein?
15 A. Yes.
16 Q. And it's enzymatically active. Correct?
17 A. In normal biology, surface-associated PH20
18 would be enzymatically active.
19 Q. And it's -- because it is properly folded,
20 it is enzymatically active. Right?
21 MS. MARTIN: Object to form.
22 A. Properly folded PH20 should have enzymatic
23 activity, yes.
24 Q. Okay. And this is how the common
25 disclosure defines the term "PH20." Right?
2 A. PH20 is defined as a type of hyaluronidase
3 that occurs in sperm and is neutral-active.
4 It explains further that it occurs on the
5 sperm surface. In this particular paragraph, the
6 folded structure is not specified.
7 Q. But it would just be the folded structure
8 that occurs naturally with the PH20?
9 A. If PH20 is active on the sperm surface, it
10 is assumed to be folded.

EX1133 (Naismith), ¶ 94 (citing EX1130 (Simpson Dep), 61:13-62:10), Reply, 3

Proper Construction is Properly Folded, Enzymatically Active PH20s

Because "PH20" and "modified PH20 polypeptide" are ***both*** defined terms,[5] Halozyme's assertions[6] warrant addressing their ***combined*** meaning.

Halozyme cannot dispute the preamble is limiting—its arguments rest on the ***preambular*** phrase "modified PH20 polypeptide."[7] Halozyme's expert (Dr. Simpson) testified that "PH20" ***provides context*** to "modified PH20 polypeptide."[8] Another Halozyme expert, Dr. Petsko, provided that context: "***POSAs think of PH20 as a folded protein***."[9] Dr. Simpson also testified PH20's definition[10] led POSAs to view "PH20s" as properly folded and enzymatically active proteins.[11] Giving effect to both defined terms, the preamble requires a "***properly folded*** PH20 polypeptide with ***at least one modification***."

The Petition explained ***other*** claim language restricts them to active PH20s:

- the disclosure portrays "active" and "inactive" mutants as mutually exclusive embodiments;
- paragraphs (a) and (b) impose 95%+ sequence identity and position 320 modification requirements; and
- paragraph (c) requires a 320 substitution that yields only "active mutants."[12]

POSAs thus read the claims ***as a whole*** as being directed to properly folded, enzymatically active PH20s.[13]

Reply, 2-4

Claims as a Whole Require PH20s with Activity

posttranslational modifications." *Id.* at 46:15–18. The '600 patent provides a specific definition of the term "modified PH20 polypeptide" which

> refers to a PH20 polypeptide that contains at least one amino acid modification, such as at least one amino acid replacement as described herein, in its sequence of amino acids compared to a reference unmodified PH20 polypeptide. 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.

Id. at 48:38–53 (emphasis added).

I.D., 12-14

The Petition explained ***other*** claim language restricts them to active PH20s:

- the disclosure portrays "active" and "inactive" mutants as mutually exclusive embodiments;
- paragraphs (a) and (b) impose 95%+ sequence identity and position 320 modification requirements; and
- paragraph (c) requires a 320 substitution that yields only "active mutants." [12]

POSAs thus read the claims ***as a whole*** as being directed to properly folded, enzymatically active PH20s.[13]

Halozyme's exposition of defined terms ignores "PH20"[14] and gives it no weight—that is legal error.[15]

Reply, 3-4

Halozyme Sur-Reply Assertions Regarding "PH20"

in sperm and is neutral-active." EX1001, 45:56-57. Second, like "modified PH20 polypeptide," the specification uses "PH20" to refer to both active and inactive polypeptides, so Petitioner's construction is inconsistent with the specification. EX1001, 119:13-33; 79:27-29. Third, Petitioner contends that because wild type

PO Sur-Reply, 2-3

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 or exhibit low or diminished hyaluronidase activity. The modified PH20 polypeptides provided herein that are inactive generally exhibit less than 20%, such as less than 10%, of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the polypeptide set forth in SEQ ID NO: 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

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

EX1001 (PGR2025-00003)('600 Patent), 79:26-30; PO Sur-Reply, 2-3

EX1001 (PGR2025-00003)('600 Patent), 119:12-33; PO Sur-Reply, 2-3

Merck's Position: Claims Require Enzymatic Activity

Petitioner for itself still claims 40% activity is required (Reply, 4, fn. 13), Dr. Naismith does not: he testified that PH20s with far less than 40% activity are within the claim's scope. EX2301, 194:9-14, 197:10-21, 192:13-198:21.

PO Sur-Reply, 2

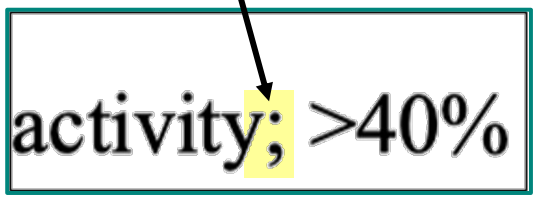
as inactive. The disclosure is nothing more than a research plan, lacking any blaze marks, while the claims improperly seek to capture any enzymatically active, multiply-mutated PH20 polypeptides that might be discovered now or in the future.

Second, regarding enablement, the common disclosure suffers equally fatal problems. It neither describes nor characterizes ***any*** modified PH20 with 2 or more substitutions. It provides no guidance about which ***combinations*** of substitutions should be made, much less affirmatively guides the selection of those that do yield enzymatically active proteins. And the only disclosed process for making PH20 mutants with multiple substitutions is a prophetic, "iterative" research plan. That plan explicitly requires the same type of "trial-and-error"

Pet., 2-3

TIP, 1375. Merck's position is that the claims require PH20s with hyaluronidase activity; >40% value is the disclosure's classification threshold.

Reply, 4 n.13



Protein and Polypeptide Are Used Interchangeably

88. Dr. Petsko also confirmed that POSAs frequently use the terms "polypeptide" and "protein" interchangeably.[73] I note that Drs. Simpson and Petsko both interchangeably used the terms "polypeptide" and "protein" when they described the "modified PH20 polypeptides" being claimed by the patent. Some examples include the following (all emphases added):

- EX2068 (Simpson Dec.), ¶ 283: "[I]t is helpful to compare and contrast ***the claimed proteins*** from those that fall outside the scope of the claims";
- EX2068 (Simpson Dec.), ¶ 353: "POSAs would evaluate the scope of the claims by considering the variation across ***the set of claimed proteins***";
- EX2068 (Simpson Dec.), ¶ 376: "[P]ermit the POSA to make and use the full scope of ***the claimed proteins***";
- EX2070 (Petsko Dec.), ¶ 50: "POSAs would have understood that ***the set of claimed proteins*** share at least 95% of their primary structure, with less than 5% variation, and the recited modification at position 320"; and
- EX2070 (Petsko Dec.), ¶ 423: "To illustrate the structural similarities and differences within ***the set of claimed proteins***, I generated the figures below."

EX1133 (Naismith), ¶ 88; Reply 3

Modifications to PH20 Are Unpredictable

- **Most single-substitution $PH20_{1-447}$ 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, 42-45, 52-55, 73-75; Reply, 5-6, 8-9, 20, 23-32.

Amino Acid Sequence vs. Folded Protein

```
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 QGVTIFYVDR  60
LGYYPYIDSI TGVTVNGGIP QKISLQDHLD KAKKDITFYM PVDNLGMAVI DWEEWRPTWA  120
RNWKPKDVYK NRSIELVQQQ NVQLSLTEAT EKAKQEFEKA GKDFLVETIK LGKLLRPNHL  180
WGYYLFPDCY NHHYKKPGYN GSCFNVEIKR NDDLSWLWNE STALYPSIYL NTQQSPVAAT  240
LYVRNRVREA IRVSKIPDAK SPLPVFAYTR IVFTDQVLKF LSQDELVYTF GETVALGASG  300
IVIWGTLSIM RSMKSCLLLD NYMETILNPY IINVTLAAKM CSQVLCQEQG VCIRKNWNSS  360
DYLHLNPDNF AIQLEKGGKF TVRGKPTLED LEQFSEKFYC SCYSTLSCKE KADVKDTDAV  420
DVCIADGVCI DAFLKPPMET EEPQIFY                                      447
```

EX1026 ('731 App.), SEQ ID NO:3



EX2070 (Petsko), ¶ 131; POR, 12

The Sequence Space of the Claims

1. A modified PH20 polypeptide comprising an amino acid sequence, wherein:

(a) at least 95% of the residues of the amino acid sequence of the modified PH20 polypeptide are identical to the residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 when the sequence of the modified PH20 polypeptide is aligned at positions corresponding to the sequence selected from the group consisting of SEQ ID NOs: 3 and 32-66 to maximize identical residues, and wherein terminal gaps are treated as non-identical; and

(b) the amino acid sequence of the modified PH20 polypeptide comprises an amino acid modification at a position corresponding to position 320 with reference to amino acid positions set forth in SEQ ID NO: 3; and

(c) the modification at position 320 is a replacement selected from among H, K, Rand S.

EX1001 (PGR2025-00003)('600 Patent)

Claim	SEQ ID/ % Identity	PH20 length	# Changes	Pos. 320 Choices	Add'l Changes	# Distinct Polypeptides
1	**3** /95%	447	22	4	21	1.50×10^{63}
	66 /95%	465	23	4	22	1.35×10^{66}
2	**3** /95%	447	22	1	21	3.76×10^{62}
3	**35** / 96%	433	17	4	16	6.14×10^{49}
4	**32** / 95%	430	21	4	20	1.76×10^{60}

Pet., 21 (citing EX1004 Park), ¶ 171, App'x F)

Claim Parameter Sequence Space (between 10^{49} to 10^{66} different sequences)

EX1133 (Naismith), ¶¶ 78-79, Reply, 5-6, 21, 27

Challenges in Protein Folding – Alternative Outcomes

64. As Dr. Dobson's figures illustrate, in order to fold stably into the native three-dimensional structure (tertiary), the naturally occurring amino acid sequence must navigate a complex pathway with various branches at key steps. Some of the key steps where the protein (or portions of it) can come off the track that leads to a native three-dimensional structure are:

a. The cell may not complete the synthesis of the encoded amino acid sequence.

b. The unfolded nascent polypeptide chain may form disordered aggregates or be degraded into fragments.

c. Partially folded forms of the sequence may lead to disordered or ordered aggregates.

d. The natively folded proteins may form various types of aggregates, or may unfold and be degraded within the extracellular environment.[42]

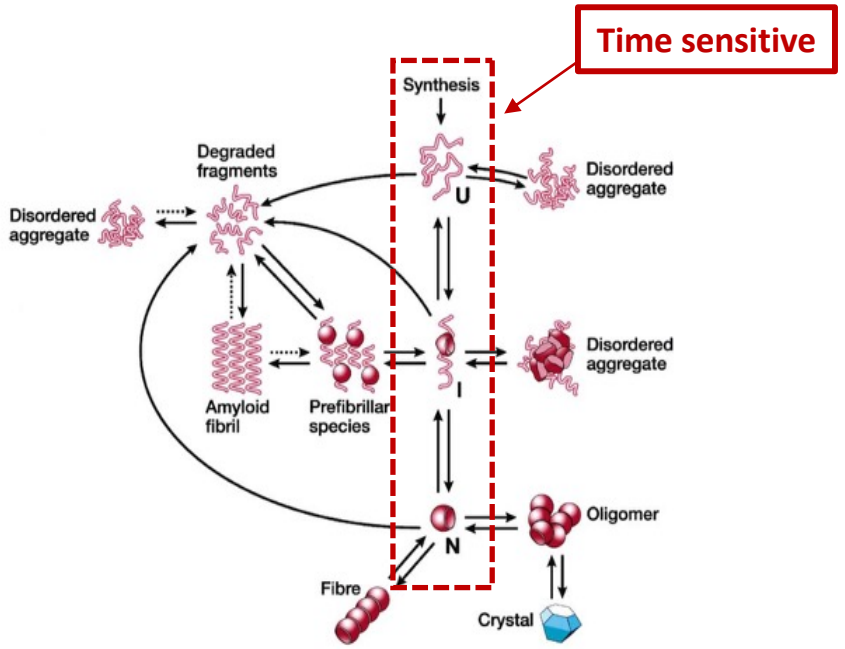


Fig. 5. Schematic representation of some of the states accessible to a polypeptide chain following its synthesis on a ribosome. The relative populations of the different states depend on the kinetics and thermodynamics of the various equilibria shown in the diagram. In living systems the fate of a given protein molecule is closely regulated by molecular chaperones and other quality control mechanisms, rather as metabolic pathways in cells are controlled by enzymes and associated molecules such as cofactors. From [36].

EX1133 (Naismith), ¶¶ 64-65 (citing EX1136, 11, Figure 5); Reply, 25

Challenges in Protein Folding – Limited Time to Fold

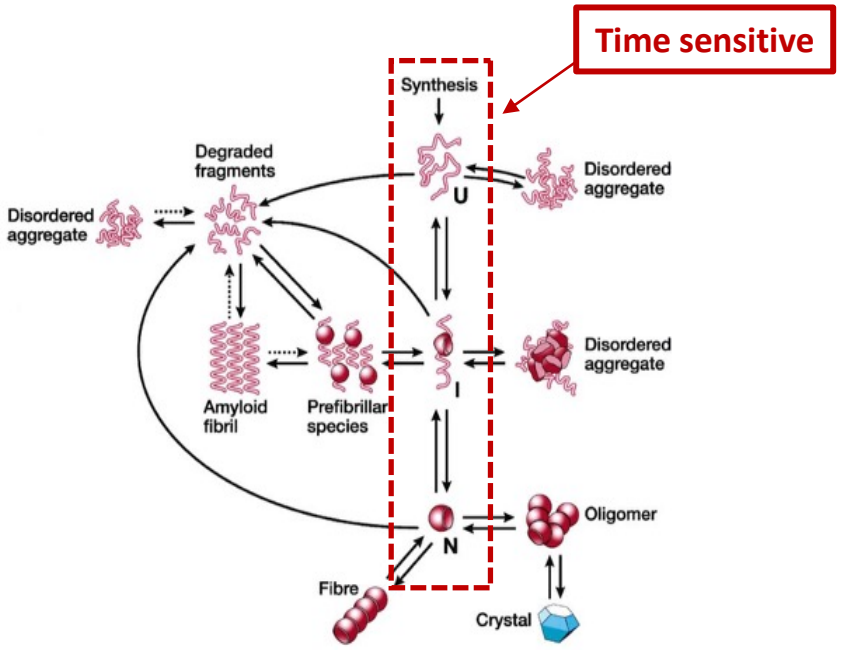


Fig. 5. Schematic representation of some of the states accessible to a polypeptide chain following its synthesis on a ribosome. The relative populations of the different states depend on the kinetics and thermodynamics of the various equilibria shown in the diagram. In living systems the fate of a given protein molecule is closely regulated by molecular chaperones and other quality control mechanisms, rather as metabolic pathways in cells are controlled by enzymes and associated molecules such as cofactors. From [36].

EX1133 (Naismith), ¶ 65 (citing EX1136, 11, Figure 5), Reply, 25

66. There is a time-dependence as well—the amino acid sequence must complete its folding pathway within a relatively short period of time. Otherwise, the amino acid sequence will be degraded. That degradation can occur within or outside the cell. Dr. Petsko illustrated the time sensitive nature of folding during his deposition:[43]

EX1133 (Naismith), ¶ 66; Reply, 25

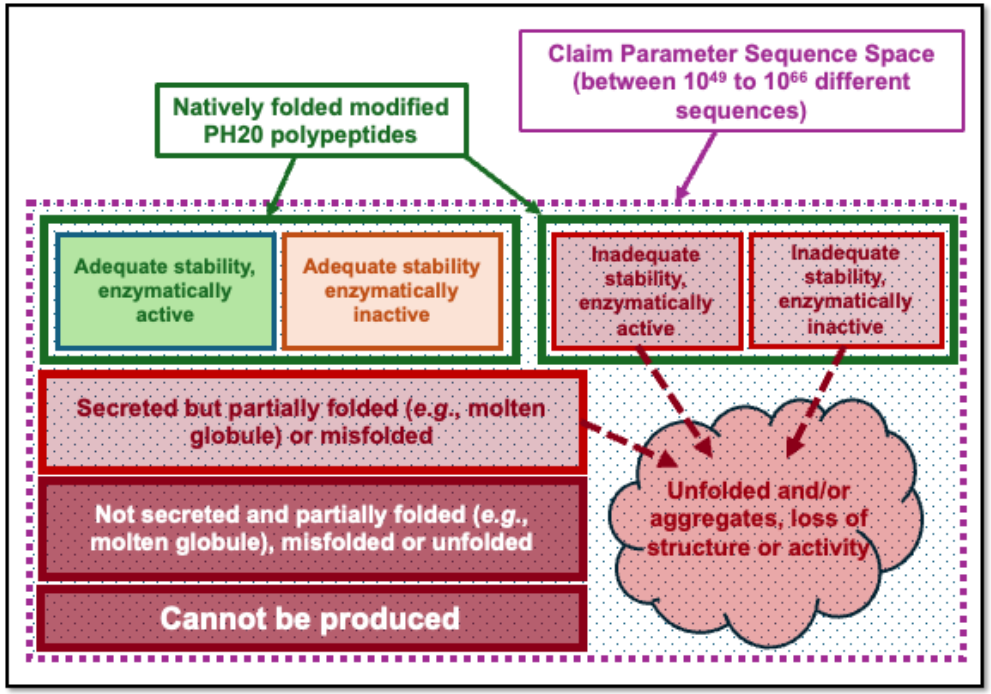
15 Proteins have to fold within a defined
16 time period, otherwise a partially folded protein
17 might accidentally get degraded while it hasn't
18 got enough time to complete the folding. There
19 are proteins, for example, that if they -- if
20 they weren't protected from that in some fashion,
21 would literally take minutes to hours to fold --
22 to be synthesized, much less fold. So that's an
23 issue.

EX1131 (Petsko Dep.), 43:15-23; Reply, 25

Outcomes for Sequences Other than Proper Folding

246. Dr. Hecht expressed concerns over use of the inactive mutant data to draw conclusions about PH20 folding,[275] and I share those concerns. The primary reason for this conclusion is that the common disclosure does not provide information that permits a POSA to determine ***why*** any particular single amino acid substitution in the $PH20_{1-447}$ wild type sequence exhibited reduced (< 20 %) hyaluronidase activity. A POSA cannot tell from what is reported in the common disclosure whether an inactive mutant was classified as such because (i) it was not successfully synthesized by the cell, (ii) it was not secreted, (iii) it did not fold within the cell, (iv) it properly folded and was secreted but unfolded after secretion due to instability, (v) it was secreted in a misfolded or partially folded state, or (vi) it was properly folded but exhibited impaired or reduced enzymatic activity.

EX1133 (Naismith), ¶ 246; Reply, 21, 32



EX1133 (Naismith), ¶¶ 77-79; Reply, 5-6, 21, 27

Unrestricted Mutations in PH20 Sequence Prevent Folding of Billions of PH20s

347. A POSA seeking to make a modified PH20 with the '600 patent in hand would not make a randomly generated combinatorial library of billions of sequences that happen to be at least 95% identical to SEQ ID NO: 3 and then screen it for activity, because it makes no sense to do so. It is difficult to imagine why anyone would do such a thing. If the sequences truly are random, then it is likely that a substantial number would include substantial modifications to residues known to be invariant because they are involved in catalysis, the active site, or in protein structure. A POSA would not make a library with billions of sequences that the POSA expects are not going to fold into PH20 proteins because it makes no sense to do that, and thanks to the '600 patent, the POSA has a much more sensible strategy.

EX2070 (Petsko Dec.), ¶ 347, Reply, 7, 8, 35

142. The positions of any particular set of substitutions and the identity of the amino acids being inserted at those positions can dramatically influence the structure of the PH20 protein. For example, multiple substitutions could be made at locations within a sequence in PH20 responsible for forming an α-helix that will disrupt the pattern necessary to form that α-helix, or even convert it into a different secondary structure, like a β-sheet. See ¶¶ 55 to 60, above. The effects of such a dramatic change in one or more of the secondary structures of PH20 could not have been predicted in 2011 based on the guidance in the common disclosure. And because the claims encompass making up to 17 or 22 substitutions at any positions in PH20 (and changing wild type residues to any of the other amino acids at these positions), there are many, many billions (and more) of possible scenarios where the changes may materially affect the folding and maintenance of the secondary structure(s) within the PH20 polypeptide.

EX1003 (Hecht Dec.), ¶ 142; Pet. 43, 45, 53, 57

Mutations Can Disrupt Stability

62. Many proteins, particularly globular ones like PH20, are inherently unstable. Slight changes in pH or in temperature can convert a properly folded and active protein into an unfolded and inactive form (figure below).[24]

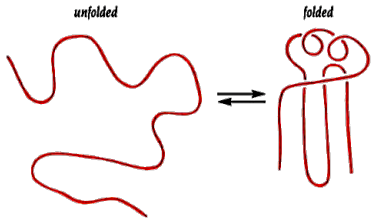


Figure 6.1 A polypeptide chain is extended and flexible in the unfolded, denatured state whereas it is globular and compact in the folded, native state.

63. The stability of a protein corresponds to the energy required to induce a transition from an unfolded state (an ensemble of disordered chains) to the folded state.[25] For many proteins, the total energy difference between the two states (the "free energy" difference) is small (e.g., 5-15 kcal/mol), which is not much greater than the energy contribution of a single hydrogen bond.[26]

EX1003 (Hecht), ¶ 62-63; Pet., 51; Reply, 26

Q. And is it possible for mutations made to the protein to shift the net-free-energy level to cause a protein to go from -- that had a tendency to be stable at one temperature to be unstable at that temperature with the mutations in it?
A. Yes. In case of some mutations, it is. That will depend to some extent on how intrinsically stable a fold is.
Q. Are you aware of instances where two or three mutations to a protein can shift that thermal stability of a protein that intrinsic?
A. Yes.

EX1131 (Petsko Dep.), 99:11-22; Reply, 26

Q. So your testimony is that a person of ordinary skill in the art in the 2011-2012 timeframe would be able to predict the change to a protein's net free energy of stabilization associated with a set of 10 or 15 or 20 mutations?
A. No. They would not be able to predict the number. They would be able to get a sense of whether those were likely to be destabilizing or not.

EX1131 (Petsko Dep.), 102:1-9; Reply, 26

Single Mutations Can Cause Unfolding and Aggregation

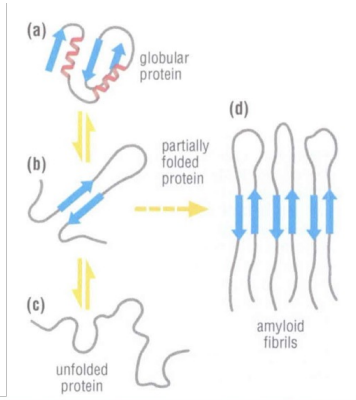


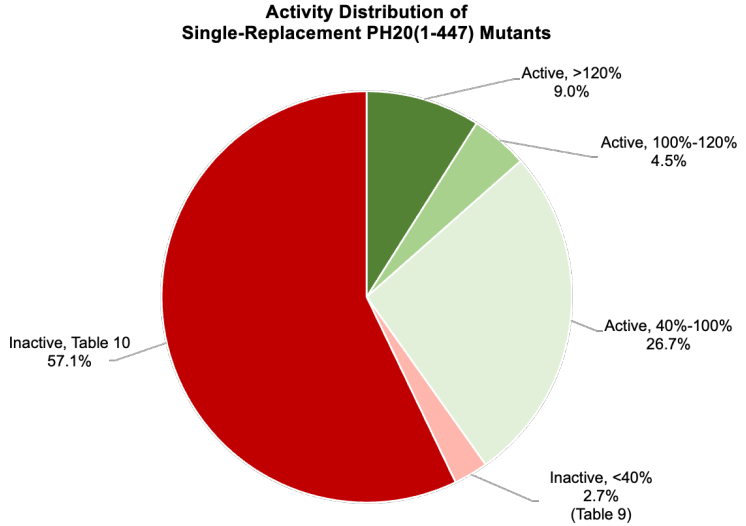
Figure 4-53 A possible mechanism for the formation of amyloid fibrils by a globular protein The correctly folded protein **(a)** is secreted from the cell. Under certain conditions, or because it contains a mutation, the protein unfolds partially **(b)** or completely **(c)**; the unfolded forms can also refold partially or completely. The partially unfolded form is prone to aggregation, which results in the formation of fibrils **(d)** and other aggregates that accumulate in the extracellular space.

EX2164 (Petsko Textbook), 160, Reply, 26

76. Dr. Petsko's textbook references Dr. Dobson's work and provides an illustration of the impact of even a single mutation to the structure and stability of a globular protein (*e.g.*, PH20).[57] In this example, the single mutation does not prevent secretion of the globular protein but disrupts its stability to the degree that the original folded protein partially or completely unfolds in the extracellular environment. After that occurs, the sequence can refold partially or completely. However, as Dr. Petsko's textbook explains, "the partially unfolded form is prone to aggregation, which results in the formation of fibrils [] and other aggregates that accumulate in the extracellular space."[58]

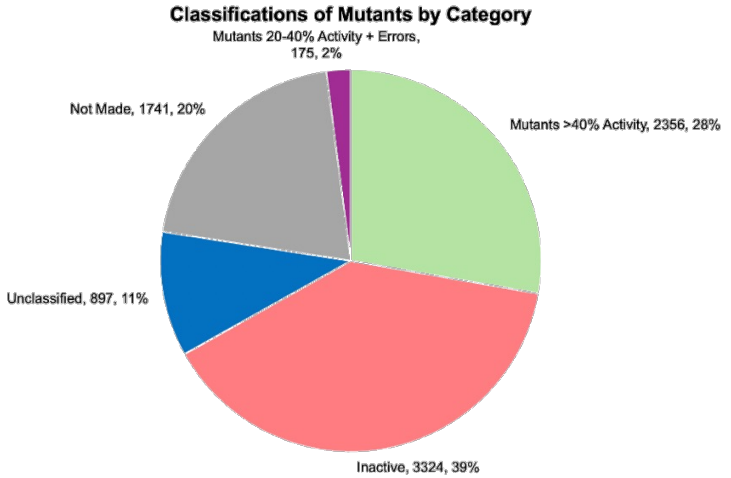
EX1133 (Naismith), ¶ 76; Reply, 26-27

Most Single Substitutions in PH20 Are Classified as "Inactive," "Not Made," or "Unclassified"



Activity vs. Unmodified PH20	Number	% of Tested (5916)
Active Mutants (Table 9)		
>120%	532	9.0%
100%-120%	267	4.5%
40%-100%	1577	26.7%
Inactive Mutants (Table 9)		
<40%	160	2.7%
Inactive Mutants (Tables 5 and 10)		
Table 5 "inactive mutants"	3,368	56.9%
Table 10 'inactive mutants"	3,380	57.1%

EX1003 (Hecht), ¶¶ 104-105; Pet. 42-43; Reply, 27



d. There are approximately 50 errors in the classifications of the mutants (*e.g.*, due to double counting, errors in counts, etc.)[266];

e. Approximately 896 of the mutants made (Table 8) but were not classified as an "active" or "inactive" mutant. No reason is provided for the lack of classification of these mutants;

f. Approximately 1,740 substitutions were not made.

EX1133 (Naismith), ¶ 236; Reply 27

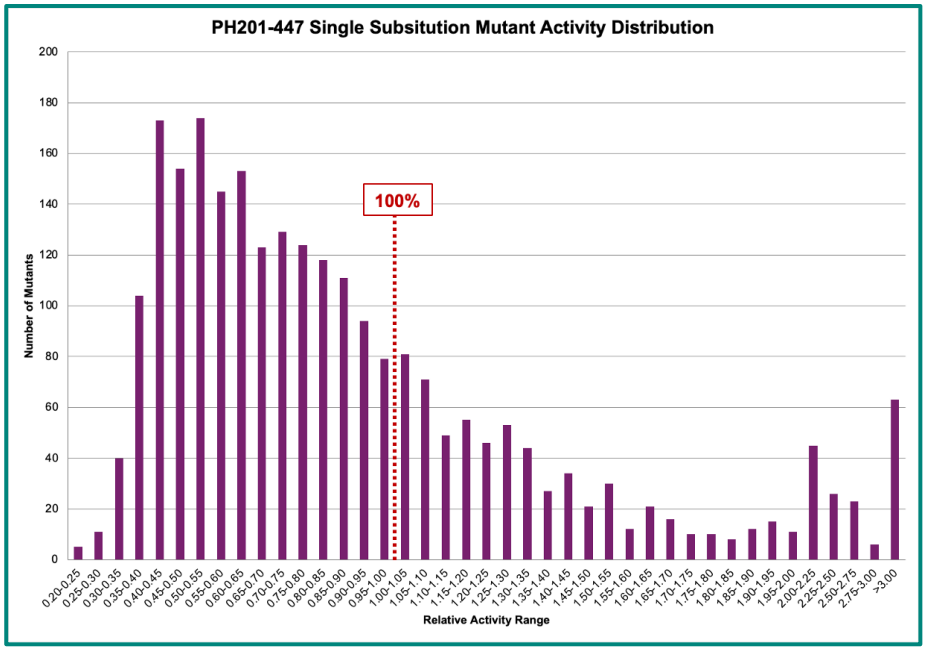
Most Single Mutations Changed PH20 Activity

268. The bar chart in ¶ 175 shows that only ~160 substitutions retain near-native activity (0.95 to 1.05), and in no position do all changes result in native activity. Seeing such a complex picture of structure-function effects for even single mutants, would also alert a POSA to the likelihood of epistasis. Neither the common disclosure nor PH20 homology models provide a basis for predicting or managing such long-range effects.

EX1133 (Naismith), ¶ 268; Reply, 20, 28

269. Since even a single amino acid substitution can induce long-range effects on packing, electrostatics, dynamics, or folding pathways, substitutions deemed "tolerated" at a given position do not place the protein into the single native functional state, but rather into a range of states. This dispersion underscores the limits of predicting function from single-mutant tolerance data: neither the magnitude nor the direction of functional effects for even untested single substitutions can be reliably inferred from the behavior of the tested subset, even when all tested variants exceed an activity cutoff. What is true for single substitutions is only more so true for combinations of mutants.

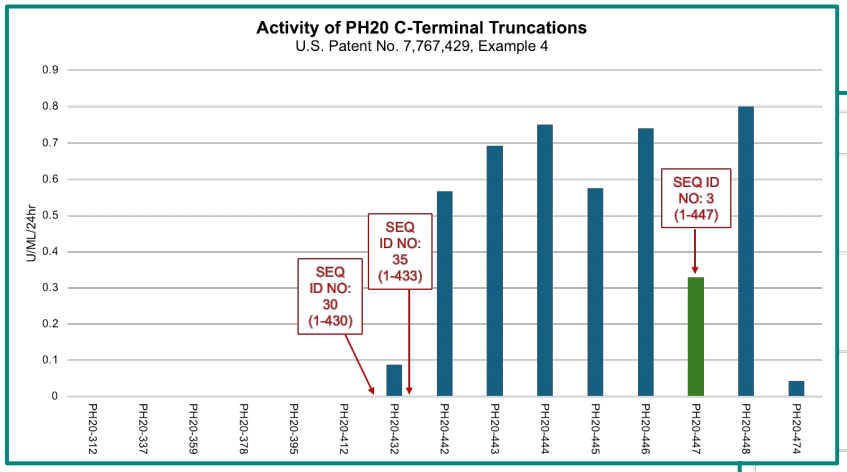
EX1133 (Naismith), ¶ 269; Reply, 20, 28



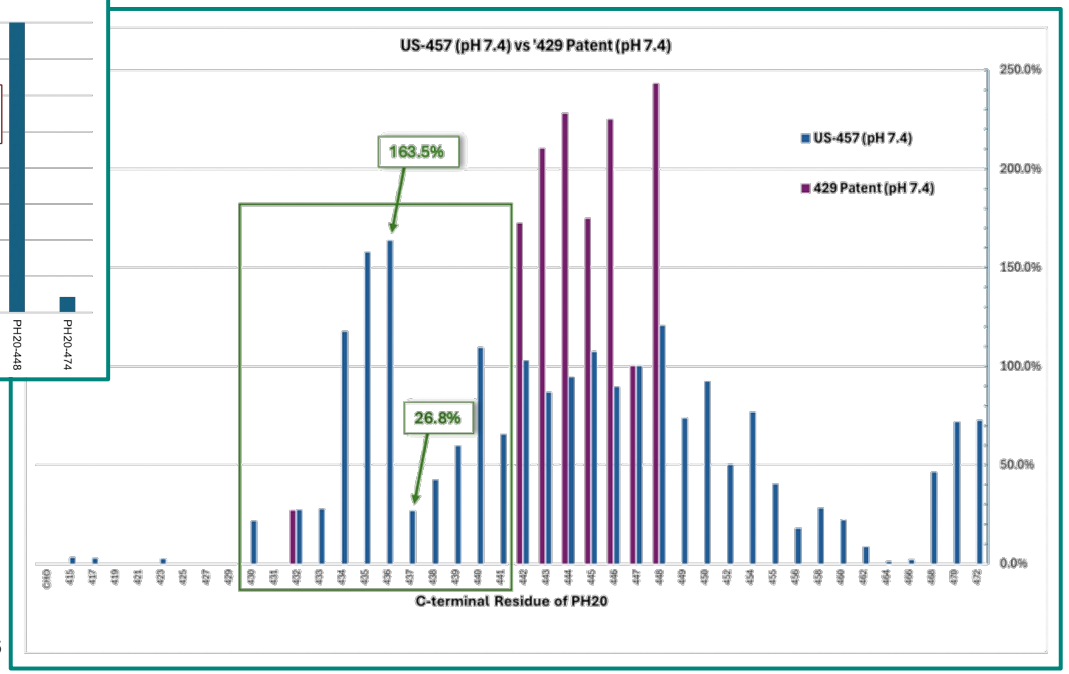
EX1133 (Naismith), ¶ 175; Reply, 33

C-Terminal Deletions Cause Unpredictable Effects in Native PH20

> 167. The common disclosure describes no multiply-modified "active mutant" PH20 polypeptides having fewer than 447 residues (or even an unmodified PH20 with such lengths) and provides no guidance about making enzymatically active mutants based on PH20 sequences ending before position 447 and containing 2 or more substitutions.
>
> EX1003 (Hecht), ¶ 167; Pet. 34, 41, 57, 71; Reply, 7



EX1003 (Hecht), ¶ 91; Pet. 40

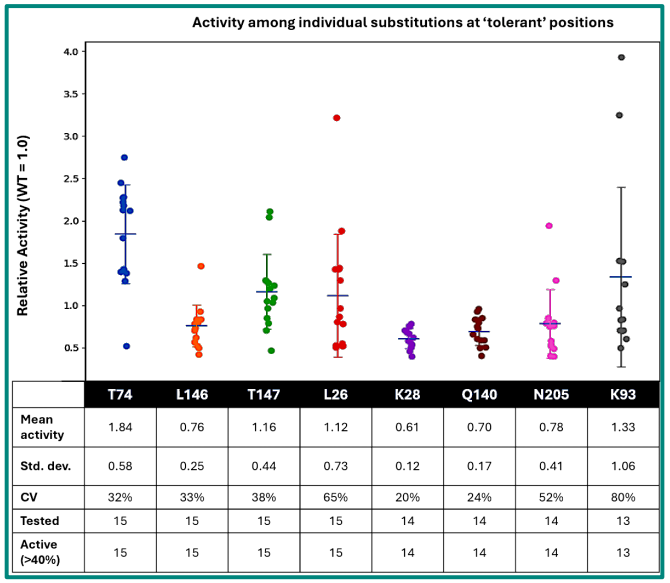


EX1133 (Naismith), ¶ 221; Reply, 36

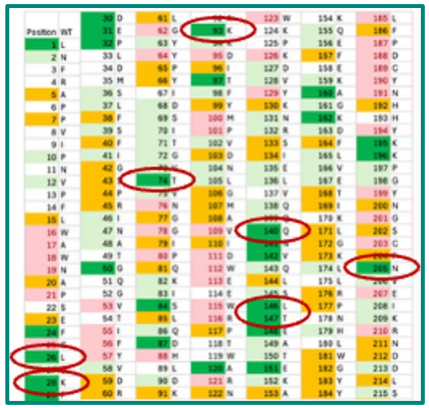
Dr. Petsko's 100% Tolerated Residues Show Unpredictable Activities

267. The experimental data associated with "active mutant" classifications would alert a POSA to the high potential for epistasis when multiple mutations are introduced. Even at positions Dr. Petsko labels as "100% tolerated", PH20 activity of each mutant varies, reflecting each mutant's unique profile of perturbations to local and long-range interaction networks and, thus, to the protein's structure-function relationship. A position with no influence on PH20 activity would show no activity change across substitutions. The data instead show that different mutations at the same position--even far from the active site--produce different activity outcomes, demonstrating sensitivity to distal perturbations. When multiple such mutations are combined particularly outside the active site, a POSA in 2011-2012 could not predict how their "effects at a distance" would interact to alter activity.

EX1133 (Naismith), ¶ 267; Reply, 20, 28

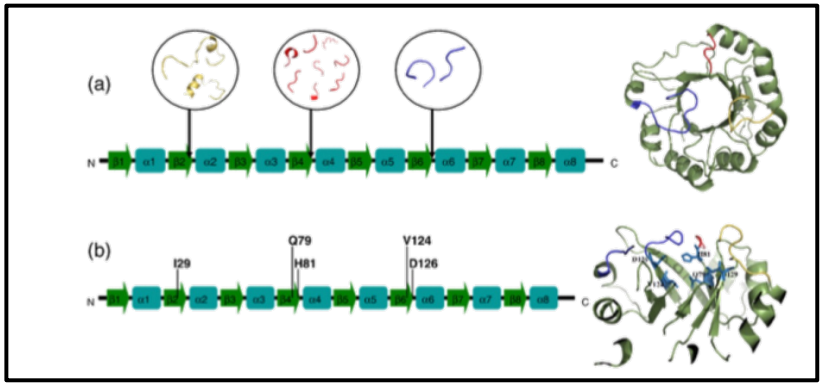


	T74	L146	T147	L26	K28	Q140	N205	K93
Mean activity	1.84	0.76	1.16	1.12	0.61	0.70	0.78	1.33
Std. dev.	0.58	0.25	0.44	0.73	0.12	0.17	0.41	1.06
CV	32%	33%	38%	65%	20%	24%	52%	80%
Tested	15	15	15	15	14	14	14	13
Active (>40%)	15	15	15	15	14	14	14	13



EX1133 (Naismith), ¶¶ 267-269; Reply, 28-29

Unpredictable Effects of Multiple Mutations in α-β Barrel Enzyme



EX1144 (Ochoa-Levya), 951, Figure 1; Reply, 30-31

Table 1. Characteristics of the exchanged loops in the PRAI-LoxP scaffold

Enzyme (EC number)	Position of the exchanged loop[a]	Sequence of the loop	Lengths
MR (EC 5.1.2.2)	β/α loop 2	GYPAL	5
FBPA (EC 4.1.2.13)	β/α loop 2	SNGGASFIAGKGVKSDVPQ	19
Ure (EC 3.5.1.5)	β/α loop 2	GGTGPAAGTHATTCTPG	17
PRAI WT (5.3.1.24)	β/α loop 2	VATSPRCVN[b]	9
DHDPS (EC 4.2.1.52)	β/α loop 4	PYYNRPS	7
TPS (EC 2.5.1.3)	β/α loop 4	LGQEDLH	7
MR (EC 5.1.2.2)	β/α loop 4	EPTLEHD	7
FBPA (EC 4.1.2.13)	β/α loop 4	DLSEES	6
αTS (EC 4.2.1.20)	β/α loop 4	DVPVQQS	7
Ure (EC 3.5.1.5)	β/α loop 4	EDWGAT	6
ADA (EC 3.5.4.4)	β/α loop 4	GDELGFPGSLF	11
PBGS (EC 4.2.1.24)	β/α loop 4	AAMDG	5
PRAI WT (5.3.1.24)	β/α loop 4	GNEE[b]	4
DHDPS (EC 4.2.1.52)	β/α loop 6	TGNL	4
PBGS (EC 4.2.1.24)	β/α loop 6	PAGAY	5
αTS (EC 4.2.1.20)	β/α loop 6	SRAGVTGAENRAALP	15
PRAI WT (5.3.1.24)	β/α loop 6	NGQGGSGQRFD[b]	11

[a] Structural position in which the WT loop was replaced for the new loop in the PRAI-LoxP scaffold.
[b] Sequence corresponding to the WT loops from PRAI-LoxP.

EX1144 (Ochoa-Levya), 951, Table 1, Figure 1; Reply, 30-31

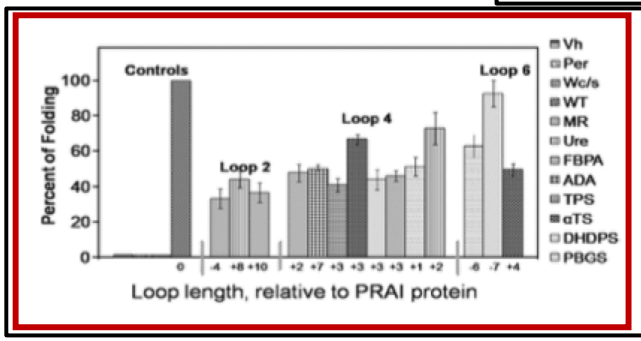
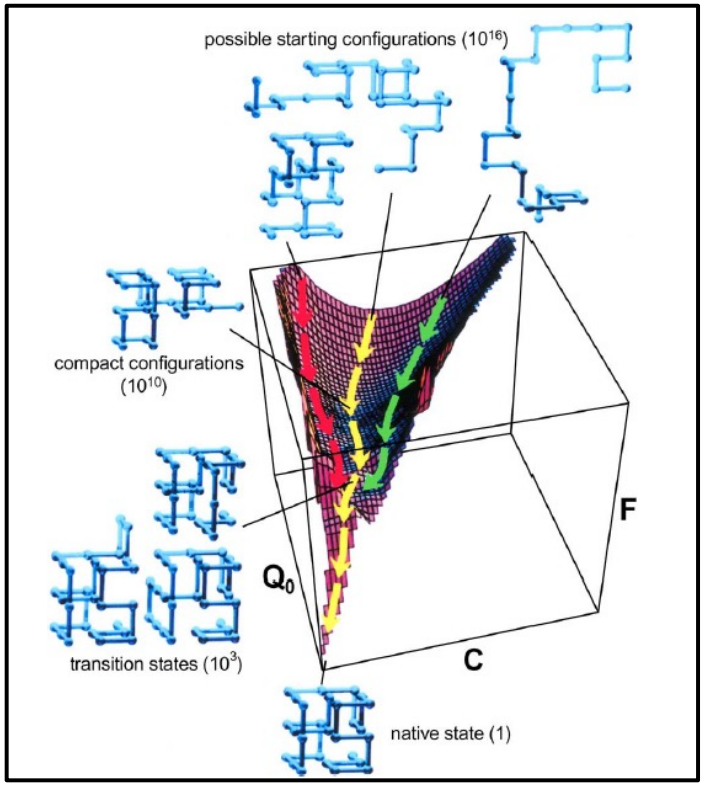


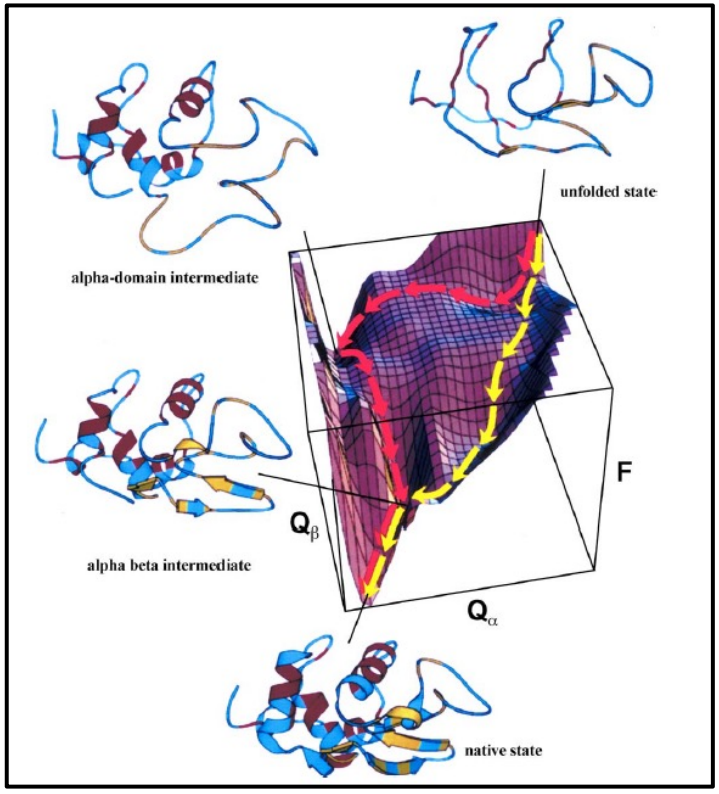
Fig. 4. Percentage of folded proteins fused to CAT was calculated as described in Materials and Methods. Values and error bars represent the average and standard deviation for each library analyzed. Controls are designated as follows: Vh, antibody; Per, circular permutation of PRAI; Wc/s, PRAI-LoxP with stop codons before the fusion to CAT; WT, PRAI-LoxP. The differences in length of loops of the libraries, relative to the PRAI WT protein, are shown in the *x*-axis.

EX1144 (Ochoa-Levya), 954, Figure 4; Reply, 32

Energetic Implications of Conformational Changes During Folding



EX1136 (Dobson), 4, Figure 1; Reply, 24



EX1136 (Dobson), 6, Figure 2; Reply, 24-25

Multiple Mutations in PH20 Are Unpredictable

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Multiple Mutations Can Unpredictably Alter Structural Features of Proteins

On this record, Dr. Hecht persuasively demonstrates that when the full scope of claim 1 is addressed, which includes not just single mutations in the PH20 polypeptide but also multiple mutations, there is no expectation of structural homogeneity, stating that "[i]ntroducing multiple amino acid changes simultaneously . . . could prevent the folding of sequences into secondary structures and structural motifs and can destabilize those structures if they do form." Ex. 1003 ¶ 59. Dr. Hecht notes that claim 1

I.D., 32-33

59. The folding of secondary structures and higher order structures is often sensitive to the correct positioning of parts of the polypeptide chain during the folding process. Multiple substitutions made in a sequence may alter that timing and the presentation of those portions of the polypeptide chain that are necessary to form secondary structures or position them to form higher order structures. Introducing multiple amino acid changes simultaneously in this example could prevent the folding of sequences into secondary structures and structural motifs and can destabilize those structures if they do form.

EX1003 (Hecht), ¶ 59; Pet. 11, 56, 74

57. In any region of a protein structure, there are myriad interactions that occur between residues in the protein that define the structure of the protein in that region. The collective effect of those interactions dictates the stability and thus maintenance of that region of the protein structure.

EX1003 (Hecht), ¶ 59; Pet. 11, 56, 74

58. Introducing one substitution in a region of a protein can create a new set of interactions with neighboring amino acids in that position that are favorable to the stability of the protein. Introducing nine more substitutions in that region along with the first one, where some of the other nine causes unfavorable interactions, may collectively destabilize that region of the protein structure, despite the positive contribution of the first substitution. There are approximately 6×10^{12} different scenarios of 10 substitutions in just this one example (*i.e.*, 10 positions, with 19 different alternative amino acids, or 19^{10}).

EX1003 (Hecht), ¶ 59; Pet. 11, 56, 74

Multiple Mutations Can Unpredictably Alter Structural Features of Proteins

55. Introducing multiple mutations into the amino acid sequence of a protein is a different story. Because so many secondary structures are dependent on particular patterns of amino acids with the correct spacing and character of amino acids at different positions, changing many amino acids simultaneously risks disrupting the pattern necessary to induce formation of the original secondary structure. Eliminating or altering a secondary structure can prevent the protein from folding and be highly destabilizing to the overall protein structure.[21]

56. Introducing multiple substitutions into a protein's amino acid sequence will introduce many new steric and/or chemical interactions between amino acids that can influence the protein's overall structure. There are a variety of such interactions possible depending on each pair of amino acids being considered (illustrated in figure below).[22] Any single substitution may impact numerous interactions, and potentially with competing effects (*e.g.*, some beneficial and some adverse to the protein's stability). Examples of non-covalent interactions that occur between residues within a protein structure are listed below:

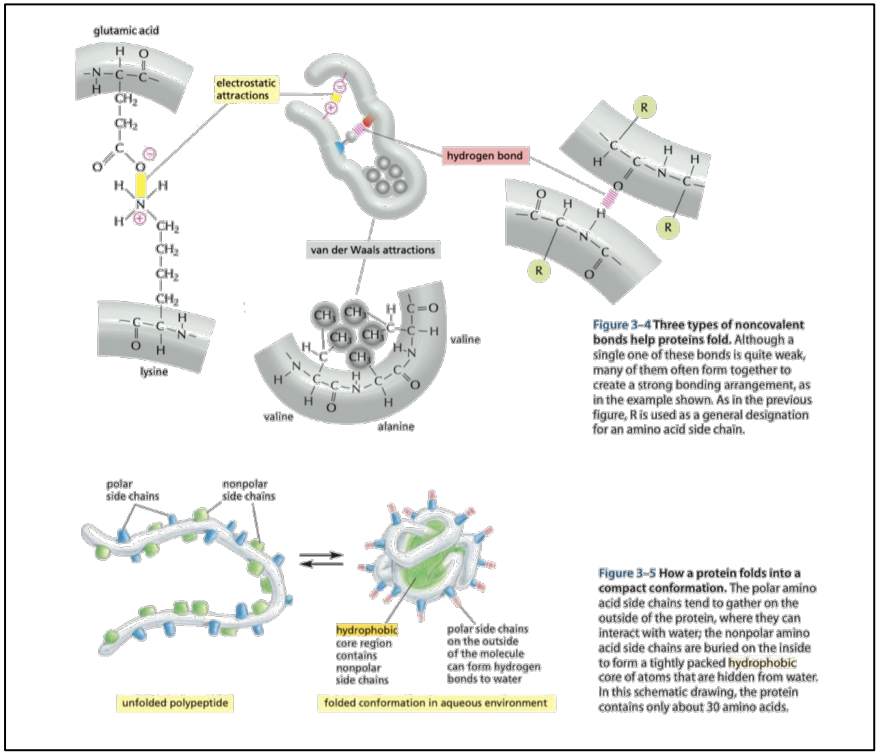


Figure 3–4 Three types of noncovalent bonds help proteins fold. Although a single one of these bonds is quite weak, many of them often form together to create a strong bonding arrangement, as in the example shown. As in the previous figure, R is used as a general designation for an amino acid side chain.

Figure 3–5 How a protein folds into a compact conformation. The polar amino acid side chains tend to gather on the outside of the protein, where they can interact with water; the nonpolar amino acid side chains are buried on the inside to form a tightly packed hydrophobic core of atoms that are hidden from water. In this schematic drawing, the protein contains only about 30 amino acids.

EX1003 (Hecht), ¶ 55, EX1039 (Alberts), 126-127, 130.; Pet. 11, 54, 56

Single Substitutions Not Predictive of Multiple Substitutions

61. An enzyme, like PH20, with one amino acid substitution that exhibits increased enzymatic activity does not prove that the same protein with 9 more changes in addition to the first will also do so. The other 9 substitutions may individually or collective disrupt the structure of the enzyme in a way that causes it to exhibit decreased activity or to be rendered inactive. That effect may occur independently of the effect of the first substitution, or the other 9 changes may collectively disturb the environment of the first substitution in a way that negates the first change's effect. The effects caused by one substitution in a protein like PH20 thus cannot predict the effects on a modified form of that protein that incorporates 5, 10, 15 (or more) substitutions. A skilled artisan would not view the first, single amino acid substituted PH20 to as be representative of all modified PH20 proteins having that one substitution, along with 5, 10 or 15 additional substitutions.

EX1003 (Hecht), ¶ 61; Pet. 53, 55-57, 76; Reply, 3, 16

141. 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. 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, a skilled artisan could not realistically predict whether an effect observed in an active single-modified PH20 polypeptide would be observed the trillions and trillions of modified PH20 polypeptides that incorporate that first mutation plus the myriad other sets of 2 to 22 additional substitutions.

EX1003 (Hecht), ¶ 141; Pet. 23; Reply, 21

Single Substitutions Not Predictive of Multiple Substitutions

157. The common disclosure does not even explain the effects that single amino acid substitutions had on the PH20 protein's native structure, let alone predict the effect additional amino acid substitutions proximate to the previously made single-substitutions would have on protein structure and activity. The common disclosure also does not identify any common structural features shared by the "active mutant" forms of the single-substitution $PH20_{1-447}$ polypeptides, or common structural features that should be shared by "active mutant" forms of multiple-modified PH20 polypeptides generally.

EX1003 (Hecht), ¶ 157; Pet., 53; Reply, 23

191. Also, a skilled artisan would not have assumed that a singly-substituted PH20 polypeptide that exhibited increased activity would retain that activity if 2 to 22 additional substitutions were incorporated into any arbitrary position of its sequence. One can readily imagine scenarios where additional substitutions are made in portions of a sequence responsible for particular secondary structures of the protein, and which would disrupt those structures. See § II.B.2, above. Changes could also be made in the region of the first substitution that would have disruptive effects on secondary structure.

EX1003 (Hecht), ¶ 191; Pet., 62

Single Substitutions Not Representative of Multiple Substitutions

115. These limited examples do not meaningfully probe the combinatorial sequence space covered by the claims and are not representative of the breadth of modified PH20 polypeptides claimed. Dr. Hecht squarely addresses these deficiencies in his declaration. I agree with his criticisms that:

d. Single-substitution $PH20_{1-447}$ mutants are not representative of multiply modified PH20 polypeptides because multiple substitutions can cumulatively disrupt secondary and tertiary structure through local and long-range effects not revealed by any single mutation, and the common disclosure provides no multi-substitution PH20 variants that demonstrate structural preservation across the claimed ranges.[126]

EX1133 (Naismith), ¶ 115; Reply, 20

97. Not all modified PH20 polypeptides that fold will be "properly folded" as Dr. Petsko uses that terminology. A modified PH20 polypeptide may adopt a folded structure that is stable but has differences in its tertiary structure relative to the tertiary structure within the wild-type PH20 protein. As Dr. Hecht accurately explained in his declaration, making multiple changes to an amino acid sequence can alter secondary structures, the positioning of secondary structures, the formation of higher order structural motifs or domains and other features of the protein's three-dimensional shape.[89] Consequently, not all stably folded modified PH20 polypeptides will be "properly folded," as the changes introduced may introduce meaningful distinctions into the tertiary structure of the protein relative to the tertiary structure of the wild-type PH20 protein, including changes that impair the enzymatic activity or stability of the protein.

EX1133 (Naismith), ¶ 97; Reply, 3, 16

Disclosure Does Not Describe Structural Effects of Single or Multiple Mutations

172. 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 $PH20_{1-447}$ mutants, the common disclosure identifies no examples of 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 PH20 polypeptides appear to have actually been made or tested. That seems consistent with the common disclosure's description of a theoretical way of making such multiply-substituted PH20 polypeptides. That description is essentially a research plan that someone might use to discover multiply-modified PH20 polypeptides that retain hyaluronidase activity (through extensive time and

EX1003 (Hecht), ¶ 172; Pet. 23, 62, 72-73; Reply, 7

143. The results of the random mutagenesis study are simply compiled in in the common disclosure. Those results by themselves do not identify any defined correlation between PH20 polypeptides having sets of 2 to 22 amino acid substitutions and PH20 polypeptides that retain 40% or more of the hyaluronidase activity of the unmodified form of the PH20 polypeptide. As the reported data confirms, the individual substitutions did not yield PH20 mutants with consistently observed effects; rather, the effects observed were random and unexplained among the tested mutants. The examples of single-replacement $PH20_{1-447}$ mutants are not representative of the incredible diversity of possible modified PH20 polypeptides having different sets of 2 to 22 additional substitutions that are within the scope of the claims.

EX1003 (Hecht), ¶ 143; Pet. 41, 45, 53, 55-55, 60, 63, 80; Reply, 21

Limited Single Mutant Examples Do Not Make Multiple Mutations Predictable

232. These statements are based on experimental work discussed in the common disclosure that investigated the effects of single substitutions within one PH20 sequence ($PH20_{1-447}$).[260] I do not share these opinions of Dr. Petsko and Dr. Simpson. As I explain further (below). Several of the opinions provided by Drs. Petsko and Simpson also contradict the guidance in the common disclosure. I discussed these in § III.G, above.

a. The well-established phenomenon of non-additivity/epistasis observed when proteins incorporate multiple changes precluded POSAs from being able to predict the effects of multiple changes to the PH20 sequence in the 2011-2012 timeframe.

b. Information on which positions are generally intolerant to change in hyaluronidase proteins was known before 2011 but could not provide insights into the effects of making multiple changes outside of those positions.

c. The common disclosure did not provide a "comprehensive and systematic" functional map establishing the tolerance of each position in PH20 to change. The common disclosure did not present any analysis of the data generated in its mutagenesis study, other than a classification of the mutants into "active mutant" and "inactive mutant" categories.

d. The experimental inputs for Dr. Petsko's positional tolerance analysis have methodological and reporting deficiencies that would have caused a POSA to doubt the scientific credibility, precision, and reproducibility of his tolerability ratings. A POSA would have viewed that data as providing only limited insights into the effects of substitutions at certain positions on the folding and activity of PH20 proteins.

e. Homology models and techniques of using them to assess changes to a protein's structure were incapable of predicting the effects of more than a few concurrent changes. They are incapable of predicting effects of long-range interactions and other phenomena that can prevent folding or retention of the protein's tertiary structure.

EX1133 (Naismith), ¶ 232; Reply 21, 23