# 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]

EX1133 (Naismith), ¶¶ 64-65 (citing EX1136 (Dobson 2004a), 11, Figure 5); Reply, 25-26

Time sensitive

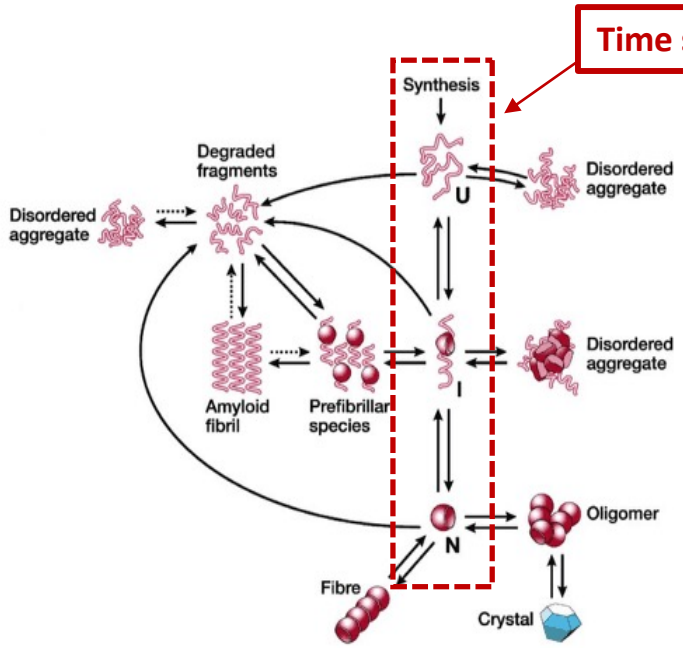



Fig. 5. Schematic representation of some of the states accessible to a polypeptide chain following its synthesis on a ribosome. The relative populations of the different states depend on the kinetics and thermodynamics of the various equilibria shown in the diagram. In living systems the fate of a given 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].

# Challenges in Protein Folding – Limited Time to Fold

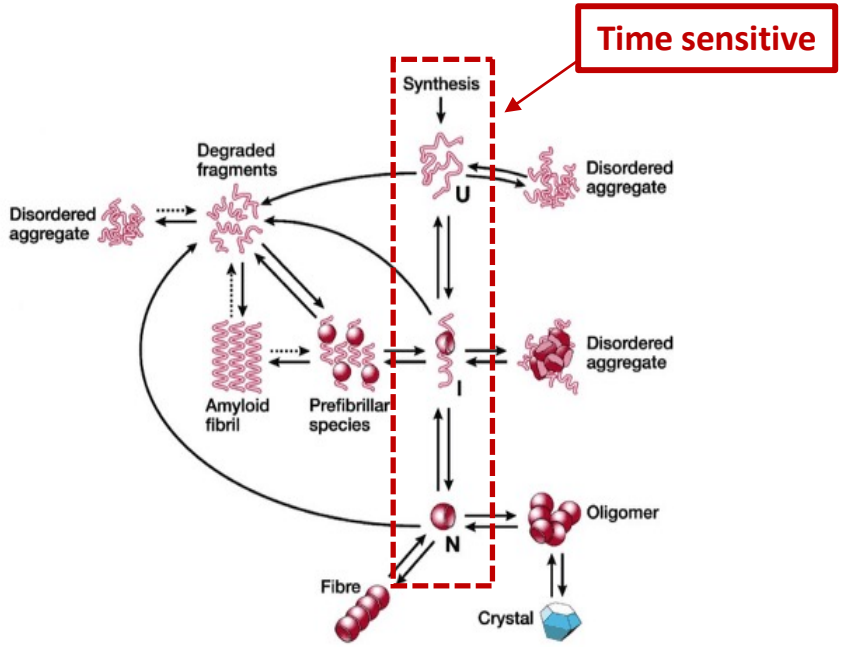



Fig. 5. Schematic representation of some of the states accessible to a polypeptide chain following its synthesis on a ribosome. The relative populations of the different states depend on the kinetics and thermodynamics of the various equilibria shown in the diagram. In living systems the fate of a given 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 (Dobson 2004a), 11, Figure 5), Reply, 25-26

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

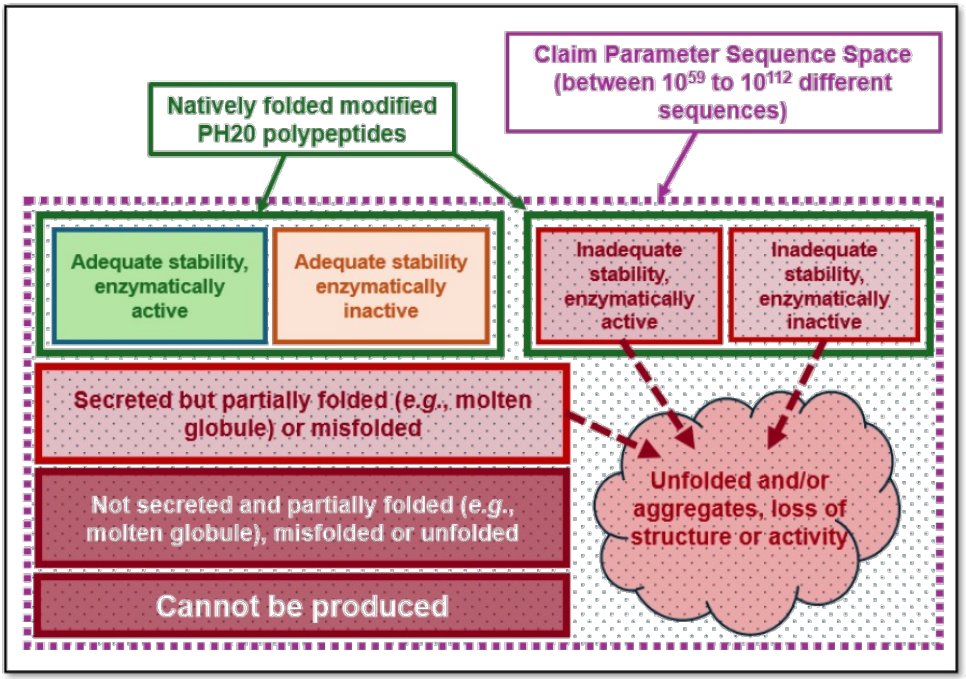
```
15          Proteins have to fold within a defined
16    time period, otherwise a partially folded protein
17    might accidentally get degraded while it hasn't
18    got enough time to complete the folding. There
19    are proteins, for example, that if they -- if
20    they weren't protected from 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

245. Dr. Hecht expressed concerns over use of the mutants classified as inactive to draw conclusions about PH20 folding,[278] 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), ¶ 245; Reply, 21, 33




EX1133 (Naismith), ¶¶ 77-79; Reply, 4-6, 21, 27-28

# Unrestricted Mutations in PH20 Sequence Prevent Folding of Billions of PH20s

347. A POSA seeking to make a modified PH20 with the '590 patent in hand would not make a randomly generated combinatorial library of billions of sequences that happen to be at least 91% 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 '590 patent, the POSA has a much more sensible strategy.

EX2070 (Petsko), ¶ 347; Reply, 7-8, 35-36

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 21 to 42 substitutions at any positions in PH20 (and, except at position 307, 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), ¶ 142; Pet. 42-43, 45, 53, 56-57; Reply 20, 30

# 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]

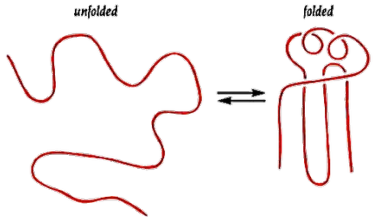



**Figure 6.1** A polypeptide chain is extended and flexible in the unfolded, denatured state whereas it is globular and compact in the folded, native state.

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 (citing EX1014 (Branden), 90); Pet., 52; 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

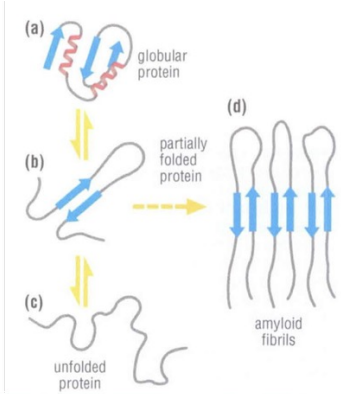


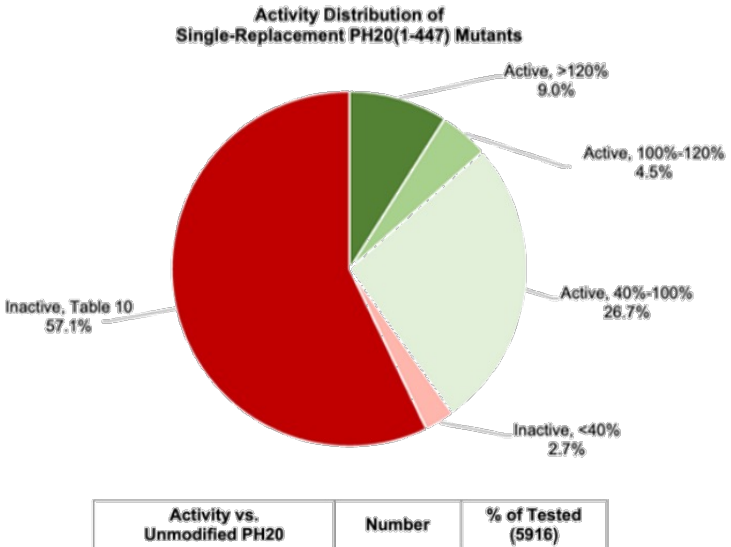

**Figure 4-53 A possible mechanism for the formation of amyloid fibrils by a globular protein** The correctly folded protein **(a)** is secreted from the cell. Under certain conditions, or because it contains a mutation, the protein unfolds partially **(b)** or completely **(c)**; the unfolded forms 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]

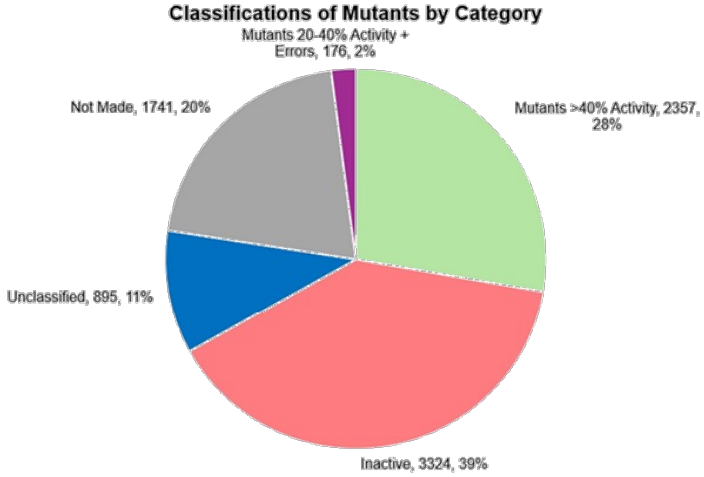
EX1133 (Naismith), ¶ 76; Reply, 26-27

# 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. 41-43; Reply, 27-28




d. There are approximately 50 errors in the classifications of the mutants (*e.g.*, due to double counting, errors in counts, etc.)[268];

e. Approximately 897 of the mutants made (Table 8) were not classified as an "active" or "inactive" mutant. No reason is provided for the lack of classification of these mutants; and

f. Approximately 1,741 substitutions were not made.

EX1133 (Naismith), ¶ 235; Reply 27

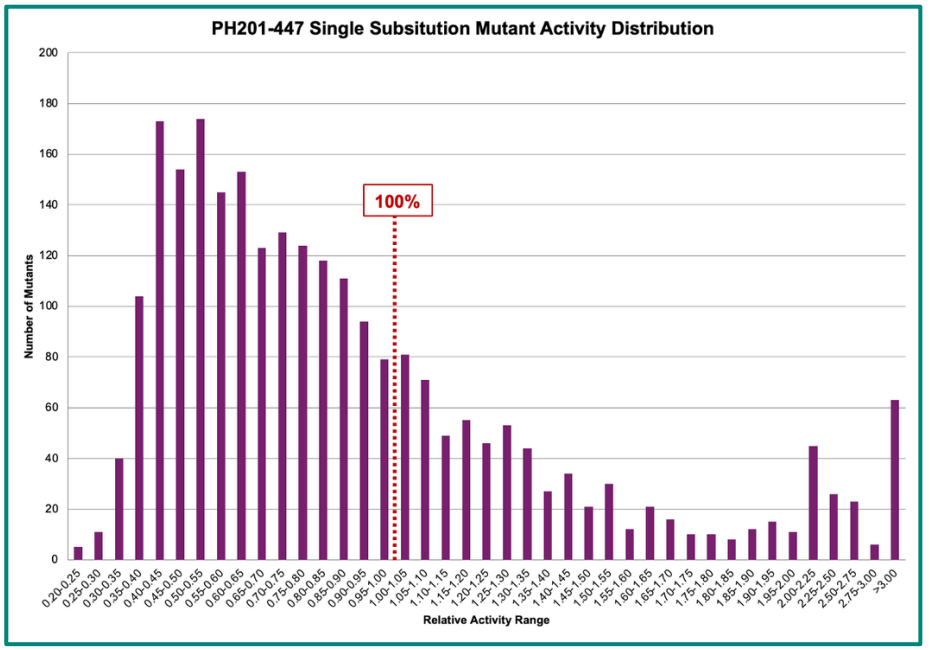
# Most Single Mutations Changed PH20 Activity

267. The bar chart in ¶ 174 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 only increase a POSA's concerns about epistasis. Neither the common disclosure nor PH20 homology models provide a basis for predicting or managing such long-range effects.

EX1133 (Naismith), ¶ 267; Reply, 20, 29

268. 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), ¶ 268; Reply, 20, 29

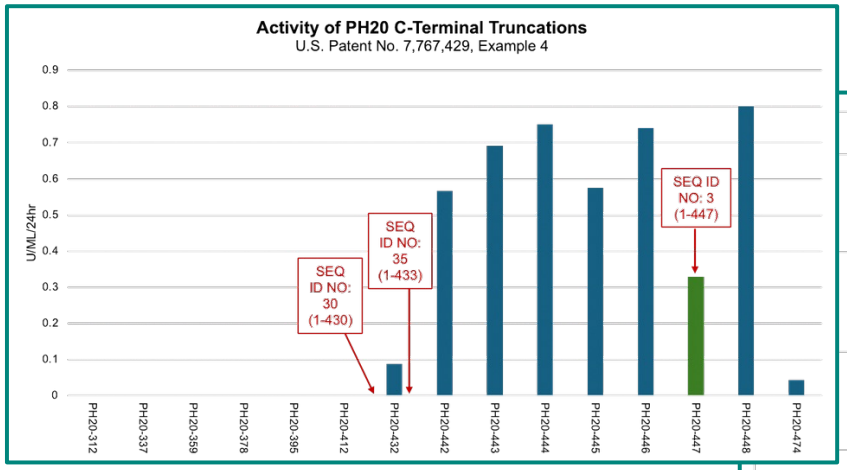



EX1133 (Naismith), ¶ 174; Reply, 34

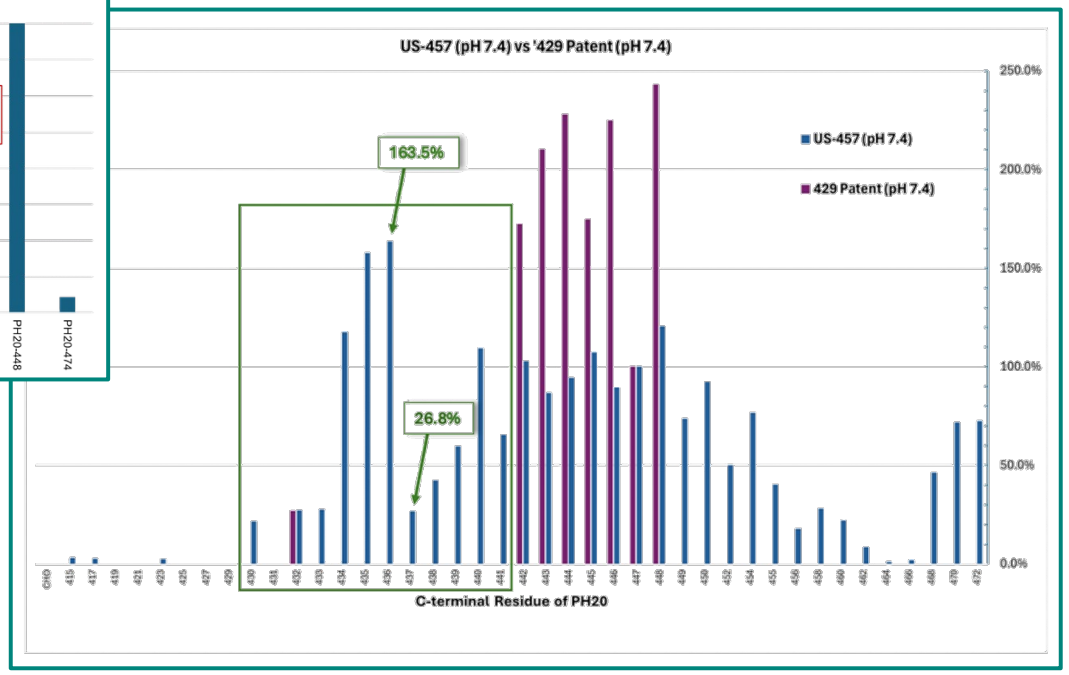
# C-Terminal Deletions Cause Unpredictable Effects in Native PH20

> 167. The common disclosure describes no multiply-modified "active mutant" PH20 polypeptides, including those having fewer than 447 residues 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. 33, 37, 57, 71; Reply, 7




EX1003 (Hecht), ¶ 91; Pet. 38

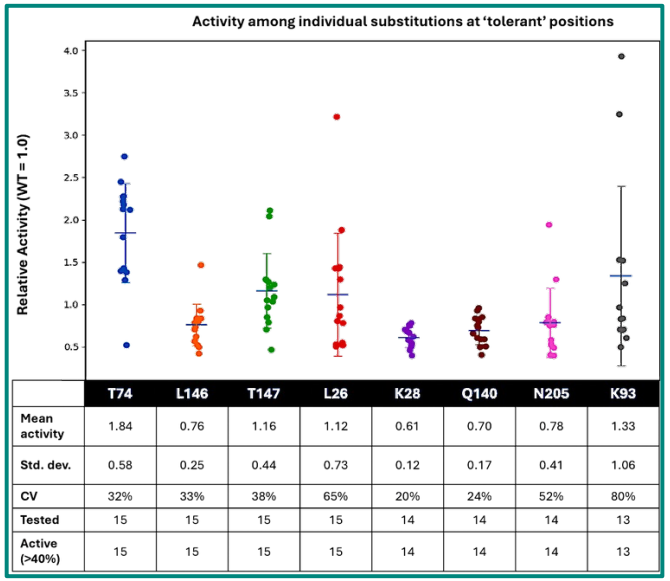



EX1133 (Naismith), ¶ 220; Reply, 36

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

266. 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), ¶ 266; Reply, 20, 29

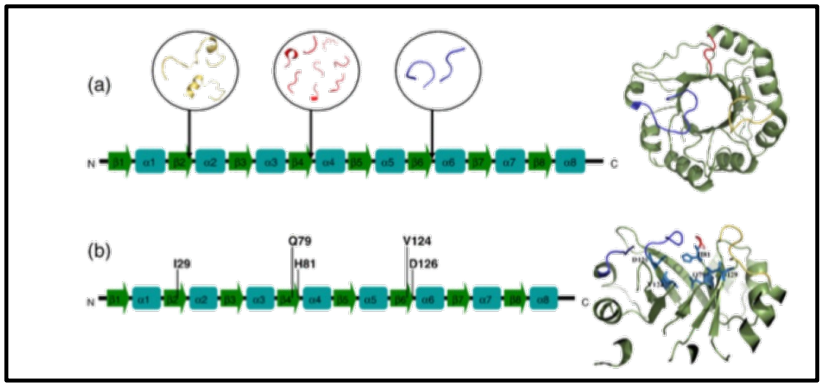



| | T74 | L146 | T147 | L26 | K28 | Q140 | N205 | K93 |
|---|---|---|---|---|---|---|---|---|
| Mean activity | 1.84 | 0.76 | 1.16 | 1.12 | 0.61 | 0.70 | 0.78 | 1.33 |
| Std. dev. | 0.58 | 0.25 | 0.44 | 0.73 | 0.12 | 0.17 | 0.41 | 1.06 |
| CV | 32% | 33% | 38% | 65% | 20% | 24% | 52% | 80% |
| Tested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



EX1133 (Naismith), ¶¶ 266-268; Reply, 28-29

# Unpredictable Effects of Multiple Mutations in α-β Barrel Enzyme




EX1144 (Ochoa-Levya), 951, Figure 1; Reply, 31-32

**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, 31-32

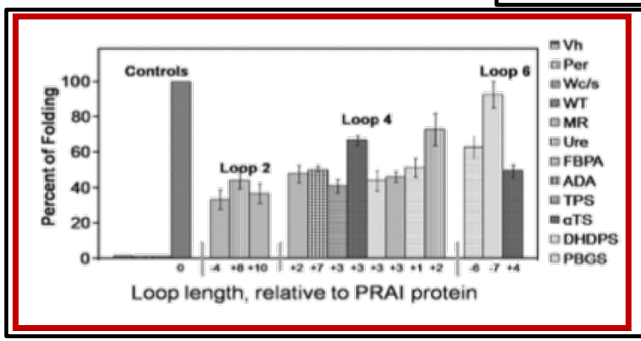
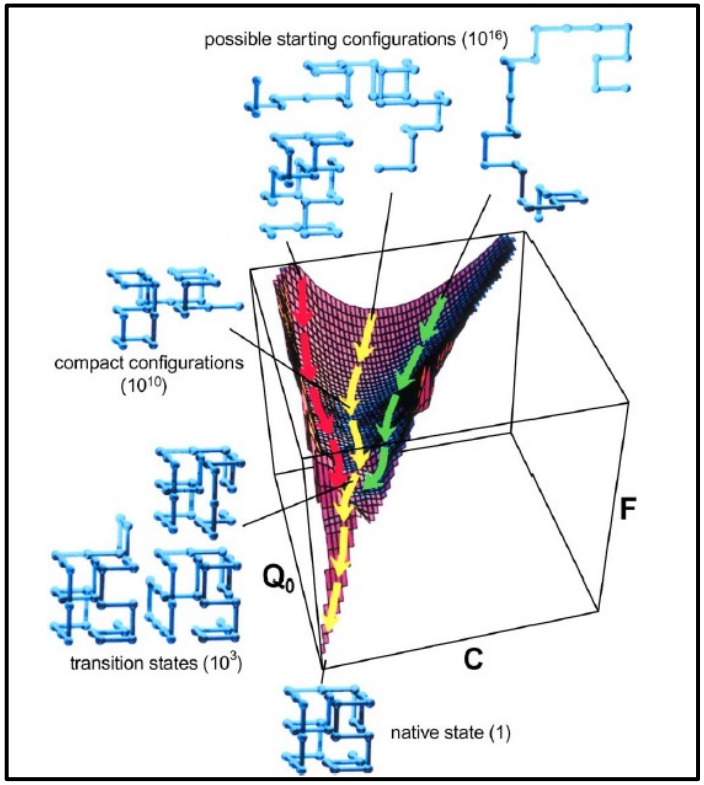


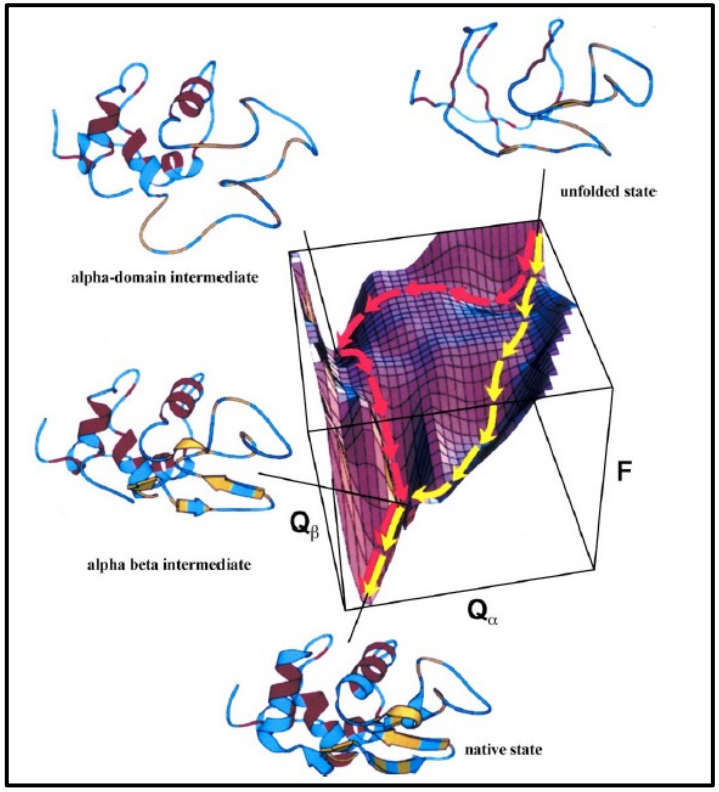

**Fig. 4.** Percentage of folded proteins fused to CAT was calculated as described in Materials and Methods. Values and error bars represent the average and standard deviation for each library analyzed. Controls are designated as follows: Vh, antibody; Per, circular permutation of PRAI; Wc/s, PRAI-LoxP 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, 32-33

# 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., 28

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, 75; Reply, 21, 23

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), ¶ 57; Pet. 11, 54, 56; Reply 21, 23

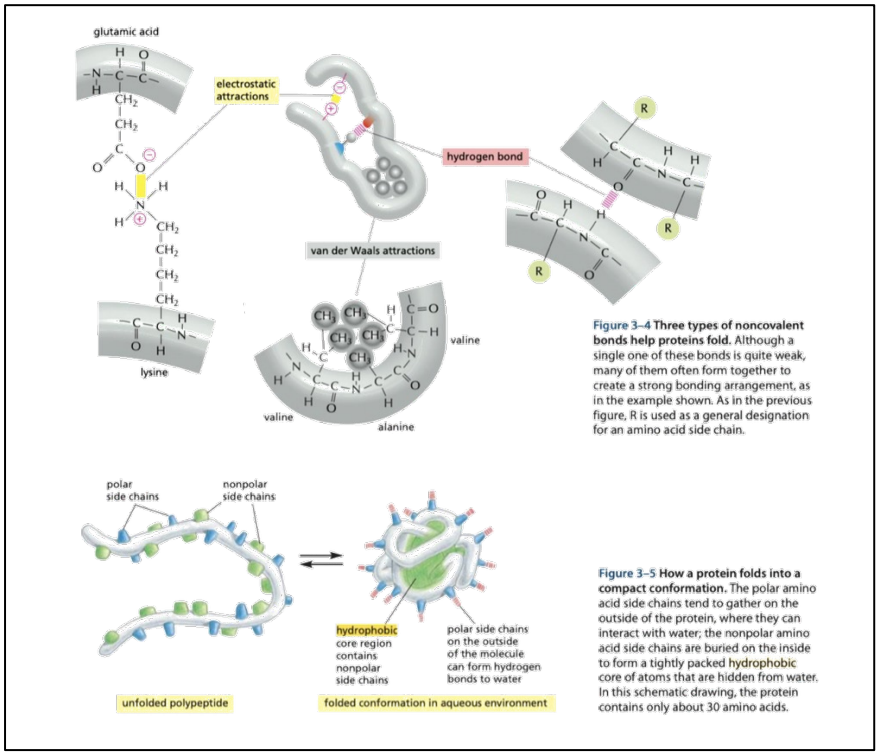
58. Introducing one substitution in a region of a protein can create a new set of interactions with neighboring amino acids in that position that are favorable to the stability of the protein. Introducing nine more substitutions in that region along with the first one, where some of the other nine causes unfavorable interactions, may collectively destabilize that region of the protein structure, despite the positive contribution of the first substitution. There are approximately $6 \times 10^{12}$ 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), ¶ 58; Pet. 11, 55, 56, 75; Reply 21, 23

# 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:




EX1003 (Hecht), ¶¶ 55-56; EX1039 (Alberts), 126-127, 130; Pet. 10, 53-56; Reply 21, 23

# 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, 73; Reply, 20-21, 23

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 41 additional substitutions.

EX1003 (Hecht), ¶ 141; Pet. 23; Reply, 20-21

# 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 1 to 41 *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., 61

# Single Substitutions Not Representative of Multiple Substitutions

115. These limited examples do not meaningfully probe the combinatorial sequence space covered by the claims. They are also not representative of the breadth of modified PH20 polypeptides within the scope of the claims. Dr. Hecht 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, 13

# 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, 49, 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 42 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 1 to 41 *additional* substitutions that are within the scope of the claims.

EX1003 (Hecht), ¶ 143; Pet. 43, 45, 53-56, 59, 62, 80; Reply, 20

# Limited Single Mutant Examples Do Not Make Multiple Mutations Predictable

231. These statements are based on experimental work discussed in the common disclosure that investigated the effects of single substitutions within one PH20 sequence ($PH20_{1-447}$).[262] 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 known to inactivate 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), ¶ 231; Reply 20, 23

# Epistasis Was an Established Phenomenon

**D. Petitioner's Theoretical Concerns About Unpredictability Contradict the Record and Are Unsupported**

PO Sur-Reply, 13-14

251. Neither Dr. Simpson nor Dr. Petsko directly address the well-known phenomenon of epistasis in discussing the predictability of making PH20 polypeptides with multiple amino acid changes. Epistasis was widely recognized by POSAs in 2011-2012 and has profound implications for introducing multiple mutations into a polypeptide. Dr. Petsko made observations in his declaration and at his deposition reflecting his awareness of the phenomenon of epistasis.

a. In ¶¶ 323-324, he discusses making combinations of changes using surface residues that are tolerant to change. He contrasts making substitutions "in different parts of the protein" where "they would be independent of each other" from "making several changes close together on the protein." In the latter situation, he says the POSA would need "an assessment of the local environment of the residues" and would need to determine if "the desired changes are likely to preserve the interactions formed by the WT residues" or if they "are likely to have an adverse impact on, for example, packing." His use of the term "independent" reflects an awareness that combinations of mutations can interact unpredictably through epistatic effects. However, in the 2012 timeframe, a POSA could not reliably predict whether mutations would behave independently; that could only be determined experimentally.

EX1133 (Naismith), ¶ 251; Reply 20, 23

b. At his deposition he referred to a mutation involving "action at a distance," and described it as common, complicated and subtle (two or more distant mutations affecting the active site would raise concerns about epistatic effects):[281]

c. He referenced "long-range" effects, where mutations could cause effects even though two residues were not directly interacting with each other (below).[282] For example, he identified "long range interactions" as one of the types of effects that a mutation could have that would result in loss of activity in a secreted mutant.

15 But the most common things are more
16 complicated and more subtle. They involve, for
17 example, action at a distance. You could have a
18 mutation that changes the electrostatic potential
19 of the protein at the active site even though
20 it's not in the active site, for example. But it
21 would still have exactly the same fold. We see
22 that sort of thing often.

EX1131 (Petsko Dep.), 79:15-22; Reply 20, 23

22 A. For example, I mentioned earlier
23 long-range interactions. Usually of the
24 electrostatic type. Those are often -- it is
25 often possible to determine that that's likely
1 what happened by the nature of a substitution in
2 its location.
9 A. We'd still have the alpha-beta barrel
10 fold, but it would have an effect at the active
11 site that would perturb it's catalytic activity
12 without directly modifying the amino acids in the
13 active site.

EX1131 (Petsko Dep.), 89:22-90:13; Reply, 23

11 A. They might be. But the patent doesn't
12 form that conclusion. They say they could be.
13 All right? They could contribute in other ways.
14 These are charged residues. All right? And
15 charged residues can exert long-range
16 electrostatic effects, for example, that have
17 nothing to do with direct binding.

EX1131 (Petsko Dep.), 270:11-17; Reply, 20, 23

# Epistasis Was an Established Phenomenon

124. There also were significant challenges in designing and carrying out large scale screening and selection experiments in the 2011-2012 timeframe. These challenges were summarized concisely in a 2012 review by Goldsmith (EX1141):[139]

> **Screening and selection methodologies**
> **To screen or select?**
> The main dilemma facing those designing directed enzyme evolution experiments is whether to apply a high, or even ultrahigh throughput selection methodology ($10^6$ to $>10^{12}$ variants processed in parallel), or a screen that might be of much lower throughput ($10^2$–$10^4$ variants tested individually). Ultra or high throughput screens or selections become advantageous with libraries of variants generated by extensive random mutagenesis or by simultaneous saturation mutagenesis of many positions. Such libraries need to be applied when: firstly, the available phylogenetic and/or structural information is insufficient for the design of small and focused libraries. Secondly, the identity and position of the mutations required to induce the desired property are hard to predict. Thirdly, the target change in activity depends on highly epistatic mutations that demand the simultaneous exploration of more than one position. In these, and possibly in other cases, large libraries might be the method of choice.

**D. Petitioner's Theoretical Concerns About Unpredictability Contradict the Record and Are Unsupported** PO Sur-Reply, 13-14

125. One challenge Goldsmith notes is that a "target change in activity depends on highly epistatic mutations that demand the simultaneous exploration of more than one position."[140] That challenge reveals the shortcomings of the limited guidance in the common disclosure about possible designs for an iterative mutagenesis strategy. For example, the common disclosure proposes to target positions identified in a first library that were identified as being deleterious in a first library (below).[141] But because of epistasis, combinations generated in a second or subsequent library may alter the effect of the first mutation.

EX1133 (Naismith), ¶¶ 124-125, (citing EX1141 (Goldsmith), 409); Reply 23

# Mutations Can Cause Aggregation

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

# Aggregation Is a Common Phenomenon in Mutated Proteins

326. At his deposition, Dr. Petsko agreed—and I agree—that aggregation is a common phenomenon that can occur when substitutions are made to a protein:[355]

EX1133 (Naismith), ¶ 326; Reply, 5

Q. Can aggregation occur during the process of protein folding if there has been a mutation to the protein?
A. It can occur even if there hasn't been a mutation in the protein depending on the external conditions. Okay. But usually, that's concentration dependent. And it typically requires nonphysiological concentrations.
Q. Can aggregation occur within the cell during transcription/translation?
A. Yes. It can. And often, that's the result of overexpression that occurs because of, say, a promoter mutation.
Q. So those -- those interactions, those are hydrophobic patches that become exposed on the surface of the protein?
A. Or that are created on the surface, yes. Those are the most common ways that you would see that kind of aggregation, would be hydrophobic.
Q. And when those interactions occur, those are basically random interactions between the locations on the proteins that are interacting with each other, right?
A. No. It can be, but in the case of sickle cell hemoglobin it is an extremely precise interaction.

EX1131 (Petsko Dep.), 42:20-43:7, 47:7-19; Reply, 5

328. I disagree. Protein aggregation is a well-established phenomenon and recognized in the scientific literature as a "fundamental problem in biotechnology."[357] For example, "the *de novo* design of novel proteins or the rational modification of existing proteins, is frequently frustrated by the fact that the polypeptide chains aggregate into large assemblies."[358] The ability to aggregate is not "restricted to a relatively few amino acid sequences associated with specific diseases but is a generic phenomenon of polypeptide changes."[359] As the Branden & Tooze textbook explains, aggregation is a phenomenon that occurs commonly when a protein is not folded into its native state, which can occur inside or outside the cell, and is one of "[t]he most common obstacles to correct folding"[360] (below).

> even if such calculations could be made with great accuracy. One important question therefore is how a living cell can prevent the folding pathway from becoming blocked at an intermediate stage. The most common obstacles to correct folding seem to be (1) aggregation of the intermediates through exposed hydrophobic groups, (2) formation of incorrect disulfide bonds, and (3) isomerization of proline residues. To circumvent these three obstacles cells produce special proteins that assist the folding process, as we shall discuss later in this chapter.

EX1133 (Naismith), ¶ 328 (citing EX1014 (Branden & Tooze), 91); Reply, 5

# Aggregation Is a Well-Established Phenomenon

329. Individual mutations can directly impact the likelihood of protein aggregation by, for example, increasing "the hydrophobicity of the polypeptide chain", increasing "its propensity to convert from α-helical to β-sheet structure" or decreasing "the overall net charge on the protein molecule."[361] Critically, the effects of multiple mutations on aggregation can be cumulative, arising from combined perturbations to fundamental physicochemical properties of the polypeptide chain.[362] As a result, substitutions that do not individually produce detectable aggregation can, when combined, increase the rate or likelihood of aggregation sufficiently to yield an aggregated protein.[363]

EX1133 (Naismith), ¶ 329; Reply, 5

332. Dr. Petsko has written extensively about the relationship between protein misfolding, aggregation, and immunogenicity. For example, he correctly observes that "[p]roteins are only marginally stable under physiological conditions," that misfolded proteins can aggregate with "deleterious consequences," (top, below) [367] and that immunogenicity in biopharmaceuticals may arise "largely because misfolded proteins break tolerance."[368]

The term chaperone is borrowed from the name of a class of proteins that function in living cells [1]. Protein molecules are usually only marginally stable under physiological conditions, so some percent of them are often unfolded or misfolded. Such molecules can aggregate with one another, or with properly functioning proteins, with deleterious consequences to the cell. Protein chaperones prevent these unwanted associations by sequestering unfolded and misfolded proteins and providing them with an environment in which they have the opportunity to

(iii) ***Understanding why most biopharmaceuticals are immunogenic, even though they are human (or humanized) proteins.*** My own guess is that it is largely because misfolded proteins break tolerance (*10*); if that is true, research on improving the folding and stability of expressed proteins would be worthwhile.

EX1133 (Naismith), ¶ 332 (Citing EX1152 (Ringe and Petsko), 80.1, 80.3); Reply, 5

# Aggregation Is a Well-Established Phenomenon

333. There is no scientific basis to treat PH20 variants containing numerous concurrent substitutions as exempt from the principles governing aggregation that is observed. While aggregation may be uncommon for the wild-type PH20, that observation does not permit reliable prediction across the broad genus of modified PH20 polypeptides claimed here. A POSA would have recognized aggregation as being a meaningful and unpredictable risk, especially for multiply modified PH20 variants. As Dr. Petsko has opined, the presence of such aggregates is suspected to be a problem in biopharmaceuticals.

EX1133 (Naismith), ¶ 333; Reply, 5

diseases but is a generic phenomenon of polypeptide changes."[359] As the Branden & Tooze textbook explains, aggregation is a phenomenon that occurs commonly when a protein is not folded into its native state, which can occur inside or outside the cell, and is one of "[t]he most common obstacles to correct folding"[360] (below).

***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 **molecular chaperones**, ubiquitous and abundant families of proteins that assist the folding of both nascent polypeptides still attached to ribosomes and released completed polypeptide chains. Several chaperones are induced

The cytoplasm of all cells contains folded proteins and folding polypeptides at high concentrations. Unfolded proteins with exposed hydrophobic patches aggregate easily by non-specific hydrophobic interactions. To circumvent this problem a class of proteins called chaperones have evolved to sequester unfolded polypeptides. The complex structure of one class of mul-

EX1014 (Branden & Tooze ), 99-100, 118; EX1133 (Naismith), ¶ 328; Reply 5

# Legal Authority

## *Ariad Pharms. v. Eli Lilly*, 598 F.3d 1336, 1349-50 (Fed. Cir. 2010) (citing *Regents of Cal v. Eli Lilly*)

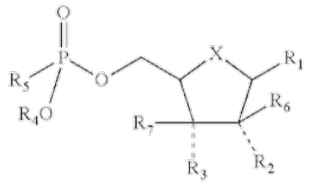
...a sufficient description of a genus instead requires the disclosure of **either a representative number of species** falling within the scope of the genus or **structural features common to the members of the genus** so that one of skill in the art **can "visualize or recognize" the members of the genus.** Id. at 1568-69. We explained that an adequate written description **requires a precise definition**, such as by **structure, formula**, chemical name, physical properties, or other properties, of species falling within the genus sufficient to distinguish the genus from other materials. []. We have also held that functional claim language can meet the written description requirement when the art has established a correlation between structure and function. [] **But merely drawing a fence around the outer limits of a purported genus is not an adequate substitute for describing a variety of materials constituting the genus and showing that one has invented a genus and not just a species.**

...For example, a generic claim may define the boundaries of a vast genus of chemical compounds, and yet the question may still remain **whether the specification, including original claim language, demonstrates that the applicant has invented species sufficient to support a claim to a genus**. The problem is **especially acute** with genus claims that use functional language to define the boundaries of a claimed genus....

## *Regents of Univ. of Minnesota v. Gilead Sciences*, 61 F.4th 1350, 1356 (Fed. Cir. 2023)

Written description of an invention claimed as a genus of chemical compounds, as here, raises particular issues because, as we have held, written description of a broad genus requires **description not only of the outer limits of the genus but also of either a representative number of members** of the genus or structural features common to the members of the genus, **in either case with enough precision that a relevant artisan can visualize or recognize the members of the genus**. *See Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1350-52 (Fed. Cir. 2010) (en banc). **A broad outline of a genus's perimeter is insufficient.** *See id.*

1. A compound of formula I:



wherein:

$R_1$ is guanine, cytosine, thymine,

[remaining parameters omitted]

I

## *Juno Therapeutics Inc. v. Kite Pharma*,10 F.4th 1330, 1339 (Fed. Cir. 2021)

It is undisputed that **scFvs generally have a common structure**, as described by Dr. Brocker. But, as Dr. Brocker acknowledged, **an scFv with the same general common structure but with a different amino acid sequence would recognize a different antigen**. J.A. 33938. Dr. Brocker also testified that all scFvs have a common structure, regardless of whether they bind. J.A. 33959. The '190 patent not only fails to disclose structural features common to scFvs capable of binding specific targets, **it also fails to disclose a way to distinguish those scFvs capable of binding from scFvs incapable of binding those targets**. The '190 patent provides no amino acid sequences or other distinguishing characteristics of the scFvs that bind. Simply put, the '190 patent claims a "problem to be solved while claiming all solutions to it… cover[ing] any compound later actually invented and determined to fall within the claim's functional boundaries," *Ariad*, 598 F.3d at 1353, which fails to satisfy the written description requirement.

## *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1567-68 (Fed. Cir. 1997)

UC also argues that the district court erred in holding claims 1 and 2, which ***generically*** **recite cDNA** encoding vertebrate insulin, and claim 4, which is directed *generically* to cDNA encoding mammalian insulin, invalid. Dependent claims 6 and 7 similarly recite cDNA encoding vertebrate insulin. In support of this argument, UC cites the disclosure of a species (the rat insulin-encoding cDNA) within the scope of those generic claims.

We agree with Lilly that the claims are invalid. Contrary to UC's argument, **a description of rat insulin cDNA is not a description of the broad classes of vertebrate or mammalian insulin cDNA.** A written description of an invention involving a chemical genus, like a description of a chemical species, "requires a precise definition, such as by structure, formula, [or] chemical name," of the claimed subject matter sufficient to distinguish it from other material.

## *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571 (Fed. Cir. 1996); *Regents of Univ. of Minn. v. Gilead Sciences Inc.*, 61 F.4th 1350, 1357 (Fed. Cir. 2023)

> ...The basis for this contention seems to be that Wattanasin lists cyclopropyl as one possible moiety for R in his disclosure of the genus. Clearly, however, **just because a moiety is listed as one possible choice for one position does not mean there is *ipsis verbis* support for every species or sub-genus that chooses that moiety**. **Were this the case, a "laundry list" disclosure of every possible moiety for every possible position would constitute a written description of every species in the genus**. This cannot be because **such a disclosure would not "reasonably lead" those skilled in the art to any particular species**. We therefore reject Fujikawa's argument on this point. *Fujikawa*, 93 F.3d at 1571

> ... The claims of P1 recite a compendium of common organic chemical functional groups, yielding a laundry list disclosure of different moieties for every possible side chain or functional group. Indeed, the listings of possibilities are so long, and so interwoven, that **it is quite unclear how many compounds actually fall within the described genera and subgenera**.
> ...
> After failing to establish that P1 claim 47 constitutes an *ipsis verbis* disclosure, Minnesota attempts to recast this claim as a blaze mark. But again, similar to Fujikawa, even if P1 claim 47 "blaze[s] a trail through the forest" that runs close by the later-claimed tree, the priority applications "do[ ] not direct one to the proposed tree in particular, and do[ ] not teach the point at which one should leave the trail to find it." [] We conclude that the Board's finding that NP2-P1 failed to provide sufficient blaze marks to support the '830 patent's priority claims was supported by substantial evidence. *Regents*, 61 F.4th 1357

## *Novozymes A/S v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1348, 1350 (Fed. Cir. 2013)

Novozymes nonetheless maintains that one of ordinary skill in the art directed to position 239 **would have known how to test every possible variant at that position and thus would have found the claimed variants as a matter of course. That argument misses the point**, however. The question before us is not whether one of ordinary skill in the art presented with the 2000 application would have been enabled to take those final steps, but whether the 2000 application "discloses the [variants] to him, specifically, as something appellants actually invented." []

In this case, to **actually possess the variant enzymes claimed in the '23 patent would have required Novozymes to confirm its predictions by actually making and testing individual variants or at least identifying subclasses of variants that could be expected to possess the claimed properties**, which it did not do before filing the 2000 application**. At best, the 2000 application describes a roadmap** for producing candidate alpha-amylase variants and then determining **which might exhibit enhanced thermostability**. A patent, however, "is not a reward for the search, but compensation for its successful conclusion." [] For that reason, the written description requirement prohibits a patentee from "leaving it to the ... industry to complete an unfinished invention." *Id*.

## *Abbvie Deutschland GMBH & CO v. Janssen Biotech Inc.*, 759 F.3d 1285, 1299-1300 (Fed. Cir. 2014)

On the other hand, analogizing the genus to a plot of land, **if the disclosed species only abide in a corner of the genus, one has not described the genus sufficiently to show that the inventor invented, or had possession of, the genus**. He only described a portion of it. That is the case here. It is important not to take the analogy of a plot of land too far in thinking of written description issues because, even if one builds a house only in one corner of the plot, one may still own the whole plot. One describes a plot of land by its furthest coordinates, in effect drawing a perimeter fence around it. That may be akin to the function of patent claims to particularly point out and distinctly circumscribe the outer boundaries of a claimed invention. **With the written description of a genus, however, merely drawing a fence around a perceived genus is not a description of the genus. One needs to show that one has truly invented the genus, i.e., that one has conceived and described sufficient representative species encompassing the breadth of the genus**. Otherwise, one has only a research plan, leaving it to others to explore the unknown contours of the claimed genus.

## *Idenix Pharmaceuticals v. Gilead Sciences*, 941 F.3d 1149, 1164-1165 (Fed. Cir. 2019)

In this case, we hold that the '597 patent is invalid for lack of written description, as it fails to provide sufficient blaze marks to direct a POSA to the specific subset of 2'-methyl-up nucleosides that are effective in treating HCV. The patent provides eighteen position-by-position formulas describing "principal embodiments" of compounds that may treat HCV. [] However, other than generic language regarding "pharmaceutically acceptable salts and prodrugs thereof" (a category not at issue here), the specification provides no indication that any nucleosides outside of those disclosed in its formulas could be effective to treat HCV—much less any indication as to which of those undisclosed nucleosides would be effective.

Idenix argues that it provides "abundant traditional blazemarks for the claims— working examples, formulas, data, synthesis routes, and the target." [] Each of these suffer from the same flaw. **They provide lists or examples of supposedly effective nucleosides, but do not explain what makes them effective, or why**. As a result, a **POSA is deprived of any meaningful guidance into what compounds beyond the examples and formulas, if any, would provide the same result.** In the absence of that guidance, the listed examples and formulas cannot provide adequate written description support for undisclosed nucleosides that also happens to treat HCV. The written description requirement specifically defends against such attempts to "cover any compound later actually invented and determined to fall within the claim's functional boundaries."

## *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); see also 35 U.S.C. § 112(a) ("The specification shall contain a written description of the invention ...."); Carnegie Mellon Univ. v. Hoffmann-La Roche Inc., 541 F.3d 1115, 1124 (Fed. Cir. 2008) ("[T]o satisfy the written description requirement for a claimed genus, a specification must describe the claimed invention in such a way that a person of skill in the art would understand that the genus that is being claimed has been invented, not just a species of the genus.").

## *Amgen v. Sanofi*, 598 U.S. 594, 613-614 (2023)

... For if our cases teach anything, it is that **the more a party claims**, the broader the monopoly it demands, **the more it must enable.** ... To be fair, Amgen does not dispute this much. It freely admits that it seeks to claim for itself an entire universe of antibodies. Still, it says, its broad claims are enabled because scientists can make and use every undisclosed but functional antibody if they simply follow the company's "roadmap" or its proposal for "conservative substitution."

We cannot agree. These two approaches amount to **little more than two research assignments**. The first merely describes step-by-step Amgen's own trial-and-error method for finding functional antibodies—calling on scientists to **create a wide range of candidate antibodies and then screen each to see which happen to bind to PCSK9 in the right place** and block it from binding to LDL receptors. [] The second isn't much different. It requires scientists **to make substitutions to the amino acid sequences of antibodies known to work and then test the resulting antibodies to see if they do too**—an uncertain prospect given the state of the art. Whether methods like a "roadmap" or "conservative substitution" might suffice to enable other claims in other patents—perhaps because, as this Court suggested in *Incandescent Lamp*, the inventor identifies a quality common to every functional embodiment, *supra*, at 13—they do not here. They leave a scientist about where Sawyer and Man left Edison: **forced to engage in "painstaking experimentation" to see what works.** 159 U. S., at 475. That is not enablement. More nearly, it is "a hunting license." *Brenner* v. *Manson*, 383 U. S. 519, 536 (1966)

***Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 750 F.2d 1569, 1576-77 (Fed. Cir. 1984)**

Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. "It is not a function of the claims to specifically exclude ... possible inoperative substances...." [citations omitted] **Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid**. *See, e.g.*, *In re Cook*, 439 F.2d 730, 735, 58 CCPA 1049, 169 USPQ 298, 302 (1971). ...

## *Wyeth v. Abbott Laboratories*, 720 F. 3d 1380, 1385-86 (Fed. Cir. 2013)

... there is no genuine dispute that it would be necessary to first synthesize and then screen *each* candidate compound using the assays disclosed in the specification to determine whether it has immunosuppressive and antirestenotic effects. There is no evidence in the record that any particular substitutions outside of the macrocyclic ring are preferable. Indeed, a Wyeth scientist confirmed the unpredictability of the art and the ensuing need to assay each candidate by testifying that, "until you test [compounds], you really can't tell whether they work or not [*i.e.*, have antirestenotic effects]." J.A. 6929. In sum, there is no genuine dispute that practicing the full scope of the claims would require synthesizing and screening *each* of at least tens of thousands of compounds. ...

Here, the specification similarly discloses only a starting point for further iterative research in an unpredictable and poorly understood field. ... Even putting the challenges of synthesis aside, one of ordinary skill would need to assay each of at least tens of thousands of candidates. ... The specification offers no guidance or predictions about particular substitutions that might preserve the immunosuppressive and antirestenotic effects observed in sirolimus. The resulting need to engage in a systematic screening process for each of the many rapamycin candidate compounds is excessive experimentation. ...

## *Boston Sci. Corp. v. Johnson & Johnson*, 647 F.3d 1353, 1366-67 (Fed. Cir. 2011)

When determining whether a specification contains adequate written description, one must make an "objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art." [] Because the specification is viewed from the perspective of one of skill, in some circumstances, a patentee may rely on information that is "well-known in the art" for purposes of meeting the written description requirement. [] However, **when the four corners of the specification directly contradict information that the patentee alleges is "well-known" to a person of skill at the effective filing date**, no reasonable jury could conclude that the patentee possessed the invention. Here, the specification of the 1997 patents itself refutes any conclusion that "the structural elements of rapamycin and its mechanism of action and biological activity was known." ...

**Given the absence of information regarding structural characteristics of macrocyclic lactone analogs or examples of macrocyclic lactone analogs in the specification**, the unpredictability of the art and the nascent state of using drug-eluting stents to inhibit restenosis, we affirm the district court's grant of summary judgment. The patent laws do not reward an inventor's invitation to other researchers to discover which of the thousands of macrocyclic lactone analogs of rapamycin could conceivably work in a drug-eluting stent.

## *Teva Pharms. Int'l GmbH v. Lilly*, 172 F.4th 1367, 1378-79 (Fed. Cir. 2026)

Here, in contrast, the asserted claims are not to "**methods of antagonizing CGRP using humanized antibodies that antagonize CGRP"**; they are to **methods of treating headache using such antibodies—something different from the function that characterizes these antibodies.**

Simply put, neither [*Rochester* nor *Ariad*] presented the circumstances of . . . this case—i.e., **a well-known genus used as part of a different invention.**

The asserted claims here are unlike the claims in *Amgen* and *Baxalta* . . . because they **do not claim humanized anti-CGRP antagonist antibodies themselves**; instead, they claim only the use of such antibodies for the different, limited purpose of treating headache. In light of the **well-known status of anti-CGRP antagonist antibodies** and the **routine nature of humanization**, the more relevant "research assignment" . . . would have been determining which humanized anti-CGRP antagonist antibodies treat headache. **That assignment was completed; the specification disclosed that <u>all such antibodies work</u> for that purpose.** . . . [U]ndertaking to find or make all of them would . . . be more akin to **extra credit than a necessary research assignment left to others to complete.**

## *Wyeth LLC v. AstraZeneca Pharms. LP*, No. 2024-2325, 2026 WL 1981095, at *6-7 (Fed. Cir. July 9, 2026)

[T]he specification must ***enable the full scope of the invention as defined by its claims***." *Amgen Inc. v. Sanofi*, 598 U.S. 594, 610, 143 S.Ct. 1243, 215 L.Ed.2d 537 (2023) (emphasis added). ... [T]he claims require administering an irreversible EGFR inhibitor daily in a functionally-defined "unit dosage"—an amount calculated to produce a therapeutic effect "in a patient." [(citation omitted)]. Thus, to enable the claims, the specification must provide guidance to allow a skilled artisan to determine a daily unit dosage calculated to produce a therapeutic effect in a patient across the full scope of claimed compounds without undue experimentation. *See Baxalta*, 81 F.4th at 1365. ...[T]he specification must provide some basis for translating information about the disclosed compounds' *in vitro* activity into the claimed daily dosing regimen "in a patient," [], rather than leaving that determination entirely to a skilled artisan's knowledge and experimentation. *See Idenix Pharms. LLC v. Gilead Scis. Inc.*, 941 F.3d 1149, 1159 (Fed. Cir. 2019). ... The issue is not whether skilled artisans knew that some irreversible EGFR inhibitors could be administered to patients, but ***whether the specification teaches how to determine the claimed therapeutically effective daily "unit dosage" for the claimed compounds***. *See Idenix*, 941 F.3d at 1159. ... The patent thus identifies ranges of "projected" daily unit dosage for administration to a patient, while ***leaving the skilled artisan to supply the critical teachings necessary to achieve or validate that projection***. ... Thus, where, as here, the specification omits critical guidance needed to practice the claimed invention, requiring skilled artisans to fill those gaps through their own experimentation, undue experimentation is required. ... [W]ithout teaching how to distinguish operative from inoperative embodiments, the specification leaves that task to the skilled artisan. [(citation omitted)]. ***That is precisely the type of undue experimentation that the enablement requirement forbids***. *See ALZA Corp.*, 603 F.3d at 941.

## Lack of Written Description

- **Full scope of the claims must be described**
  - Full scope of claimed polypeptides is structurally heterogeneous
- **No disclosure of representative species**
  - Single-substitution $PH20_{1\text{-}447}$ mutants are not representative of the claimed genus
- **No identification of common structural features**
  - Percentage sequence identity is not a common structural feature
  - The α-β Barrel motif is not an inherent common structural feature
  - The disclosure does not identify a structure-function relationship for the claimed genus

*See e.g.*, Pet., 27-28, 32-33, 44-45, 48-49, 52-59; Reply, 4-6, 11-16, 20-22

# Halozyme Contends Claimed Genus is Structurally Homogeneous

But as explained above, Dr. Petsko's and Dr. Simpsons' analyses show that in fact the claimed subvariants are structurally homogeneous. And the large number of inactivating mutations are not evidence of unpredictability. EX2068, ¶¶379-380.

POR, 58

They would. As an initial matter, the challenged claims do not encompass an "immense genus" of unrelated polypeptides, as Petitioner contends (Pet., 67), but instead nine variants with the L307 mutation and a narrowly defined and structurally homogeneous set of PH20 subvariants with 91% sequence identity. *See* Sections

POR, 65

As discussed in Section V.A-C, the challenged claims recite a narrow and structurally homogeneous genus of nine PH20 variants with one of nine substitutions at position L307, as well as subvariants of each that must maintain at least 91% sequence identity. The scope of the claims is extremely narrow. While Petitioner

POR, 68

This detailed structure-function correlation of a narrowly defined, structurally homogeneous genus demonstrates the inventors were in possession of the full scope of the invention. These data collectively would have provided more than sufficient

POR, 53

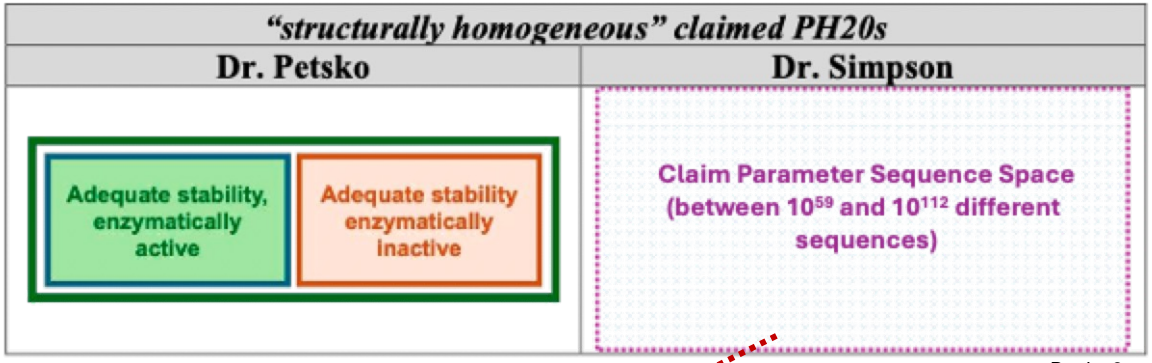
F.3d at 1353–54 (internal quotation marks omitted). Here, the inventors in no way overreached in exchange for their expansive disclosure; they sought and received highly limited claims to a structurally homogeneous set of modified PH20s that require a single amino acid mutation at a single position that is trivially avoided unless one is looking over the inventor's shoulders, copying their work. They

POR, 7

Additionally, as Patentee's experts, Drs. Simpson and Petsko, explain, within this narrow genus, the claimed proteins are structurally homogeneous in that they share at least 91% identical primary structure, the L307 mutation, and for those that are active, the same PH20 tertiary structure, including several common structural features (including a common active site).[4] EX2068, ¶¶33, 207-304; EX2070,

POR, 3-4

# Halozyme's Experts Contradict Each Other on Structural Homogeneity




Reply, 6

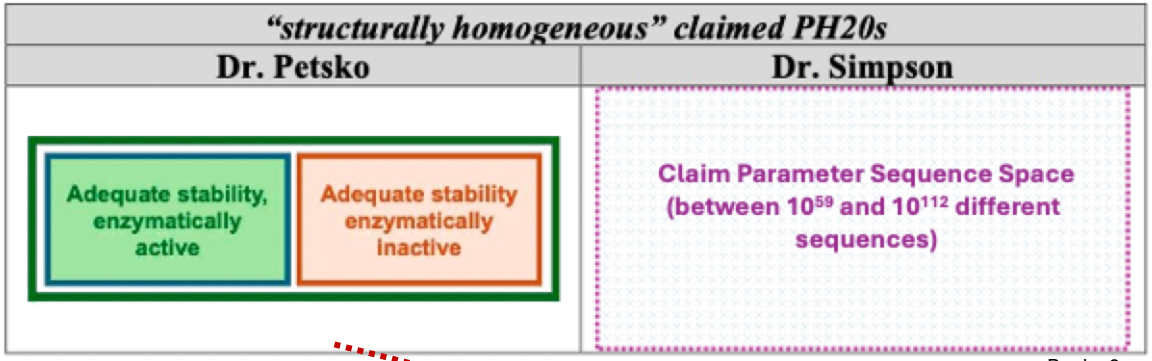
33. POSAs would have understood that the claimed genus is a narrow set of PH20 variants and sub-variants that are structurally homogeneous. Because the sub-variants all share at least 91% sequence identity with the L307 PH20 variants, there is almost no structural diversity within the claimed genus. POSAs would expect the active sub-variants to retain, for instance, the same alpha-beta barrel fold.

EX2068 (Simpson), ¶ 33; Reply, 6

Q. You portray this as a narrow set of PH20 variants.
Do you mean that the number of different polypeptide sequences that are in that set is a narrow set of sequences?
MS. MARTIN: Object to form.
A. I maintain that -- or have expressed that POSAs would have understood the claimed genus to be a narrow set of PH20 variants and subvariants, structurally homogeneous.

EX1130 (Simpson Dep.), 89:16-25; Reply, 6

# Halozyme's Experts Contradict Each Other on Structural Homogeneity




Reply, 6

393. POSAs think of the class of modified PH20 proteins sharing 91% of their sequence (including the recited change at position 307) as an extremely limited set of proteins. Those that fold are a limited set of structurally homogeneous proteins.

51. The POSA would have understood that properly folded PH20s adopt the same tertiary structure-an alpha-beta barrel fold. The claimed modified PH20s (that fold) thus share this fundamental common structural feature (the PH20 alpha-beta barrel fold).

EX2070 (Petsko), ¶¶ 51, 393; Reply, 6

Q. -- you say one of the common structural features is this alpha-beta barrel fold, right?
A. Yes.
Q. And is that shared by all of the mem- -- species in the genus?
A. It's shared by all of the species in the genus that give a stable protein fold.

EX1131 (Petsko Dep.), 72:13-19; Reply, 6

# Halozyme: "Inactive Mutants" Do Not Have Common Structural Features

patent itself, mutate the primary structure slightly more than 9%. Given the lack of structural diversity in the genus (EX2068, ¶¶354-359), a patent monopoly could hardly be more limited.

POR, 5

[4] To the extent inactives fall within the genus under Patentee's construction (*see* Section IV), the lack of one or more of these common structural features (e.g. disulfide bridges or catalytic residues critical to activity) distinguishes them from actives. EX2068, ¶¶303-304; EX2070, ¶¶325-342.

POR, 4 (FN4)

# Enzymatically Active PH20s Are in a Portion of Claim Sequence Space

**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)




EX1133 (Naismith), ¶ 78 ; Reply, 5-6

# Impossible to Identify Enzymatically Active PH20s in Sequence Space

189. Given the massive number of possible distinct PH20 polypeptides having between 2 and 42 substitutions that the sequence identity parameters of the claims capture, one would have to repeat this iterative process innumerable times to identify all of the enzymatically active multiply-mutated PH20 polypeptides in the scope of the claims. As I noted earlier, the aggregate mass of the collection of individual modified PH20 polypeptides covered by the claims (all of which have one of the three specified position 307 mutations of the narrowest claims) that would have to be produced and screened is greater than the mass of the earth. This task is simply impossible!

EX1003 (Hecht), ¶ 189; Pet., 27, 51, 73; Reply, 7, 9

190. The common disclosure does not provide guidance that a skilled artisan could have used to identify which of the trillions of possible combinations of 2 to 42 substitutions in PH20 would retain 40% of the activity of the unmodified parent PH20 polypeptide. It also does not provide any guidance that would enable a skilled artisan to identify the subset of that massive (and unknowable) set of enzymatically active PH20 polypeptides having the myriad possible combinations of between 2 and 42 substitutions that result in the PH20 polypeptide having greater than 100% of the activity of the parent unmodified PH20. A skilled artisan, for example, could not, as a practical matter, use rational design techniques to assess all these possible variant sequences of PH20. There is not enough time in a person's lifetime to do that. Also, protein structural models available in the 2011 timeframe would not be able to reliably assess sequences that incorporated more than a few variant residues in one region of the protein.

EX1003 (Hecht), ¶ 190; Pet., 50-51, 62, 67, 72; Reply, 7, 9, 23

# Impossible to Identify Enzymatically Active PH20s in Sequence Space

If someone were asked in 2011 or 2012 and they were a person of ordinary skill in the art, and they were asked to determine the number of PH20 polypeptides in that genus of claim 1, that were properly folded and enzymatically active, how would they go about doing that?
A. They wouldn't even go about doing that.
BY MR. KUSHAN:
Q. Can you -- again, my question is, I am asking that person to do that. And I am asking you to tell me how they would do that.
A. What I'm saying is that would be their answer. They would say, I am not -- I won't do that because there isn't a simple way to do that.

Q. Is it possible to calculate that number, in 2011, 2012?
A. I don't believe so.
Q. Is it possible to experimentally determine which ones are properly folded and enzymatically active in 2011 or 2012?
MS. MARTIN: Object to form.
A. Not from experimenting on the entire ensemble.

EX1131 (Petsko Dep.), 63:19-64:17; Reply, 7

# Sequence Identity Percentage Is Not a Structural Feature of a PH20 Molecule

**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)

83. I do not agree that the 91% sequence identity requirement is a "structural feature" of a modified PH20 polypeptide. It is a claim requirement that is determined mathematically: one determines the number of changes between two amino acid sequences and then divides that number by the number of amino acids in the reference PH20 sequence (*e.g.*, 40 changes divided by 447 total residues = .0895, which equates to .9105, or 91.05% identity).[65] It does not define any particular physical characteristic of a modified PH20 molecule. It functions solely to limit the size of the sequence space of the modified PH20 polypeptides.

EX1133 (Naismith), ¶ 83; Reply, 11

85. A POSA would consider "structural features" of a protein to be the physical characteristics of the protein molecule. For example, a particular amino acid located at a particular position in the amino acid sequence of the protein is a structural feature. Structural features of folded proteins are discrete three-dimensional structures within the tertiary structure of those proteins: secondary structures, higher order motifs (*e.g.*, a catalytic site or substrate binding site), a domain, or the protein's overall tertiary structure. The phrase "structure of a protein" would, unless prefixed by "primary" or "secondary," be taken by a POSA to refer only to the three-dimensional structure of a protein.[67]

EX1133 (Naismith), ¶ 85; Reply, 6, 11

# All Modified PH20 Examples Have One Change in One Length of One PH20

The common disclosure thus provides a very narrow set of working examples relative to the diversity of modified PH20 polypeptides being claimed.[156] The examples are restricted to ***one type of change*** (a single amino acid replacement) in ***one type of PH20 polypeptide*** (SEQ ID NO: 3).[157] By contrast, the claims encompass changes in 37 different unmodified PH20 sequences, and include, in addition to one identified replacement at position 307, anywhere from 1 to 41 (claim 1) to 20 (claims 25-26) additional changes.[158] A simple illustration demonstrates how ***non-representative*** the examples are: all of the examples of single-replacement $PH20_{1-447}$ mutants fit into one box of the array below (claim 2).

Pet., 57-58

| Total Change | Total Sequences | # |
|---|---|---|
| 1 | 1 | 1.00E+00 |
| 2 | 8,152 | 8.15E+03 |
| 3 | 33,150,118 | 3.32E+07 |
| 4 | 89,660,073,504 | 8.97E+10 |
| 5 | 181,449,739,545,075 | 1.81E+14 |
| 6 | 293,077,978,086,132,000 | 2.93E+17 |
| 7 | 393,555,483,371,437,000,000 | 3.94E+20 |
| 8 | 451,914,977,218,566,000,000,000 | 4.52E+23 |
| 9 | 452,989,259,911,317,000,000,000,000 | 4.53E+26 |
| 10 | 402,658,125,051,895,000,000,000,000,000 | 4.03E+29 |
| 11 | 321,362,356,487,055,000,000,000,000,000,000 | 3.21E+32 |
| 12 | 232,608,649,507,388,000,000,000,000,000,000,000 | 2.33E+35 |
| 13 | 153,968,078,195,545,000,000,000,000,000,000,000,000 | 1.54E+38 |
| 14 | 93,849,823,863,830,800,000,000,000,000,000,000,000,000 | 9.38E+40 |
| 15 | 52,991,852,975,982,400,000,000,000,000,000,000,000,000,000 | 5.30E+43 |
| 16 | 27,859,708,843,273,000,000,000,000,000,000,000,000,000,000,000 | 2.79E+46 |
| 17 | 13,698,336,949,198,000,000,000,000,000,000,000,000,000,000,000,000 | 1.37E+49 |
| 18 | 6,323,829,801,952,950,000,000,000,000,000,000,000,000,000,000,000,000 | 6.32E+51 |
| 19 | 2,750,529,891,360,250,000,000,000,000,000,000,000,000,000,000,000,000,000 | 2.75E+54 |
| 20 | 1,130,619,221,191,820,000,000,000,000,000,000,000,000,000,000,000,000,000,000 | 1.13E+57 |
| 21 | 440,435,474,482,722,000,000,000,000,000,000,000,000,000,000,000,000,000,000,000 | 4.40E+59 |

Reply, 8

| | Number of Changes | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SEQ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| 3 | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | | | | | | | | | | | | |
| 32 | | | | | | | | | | | | | | | | | | | | | | | |
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| 66 | | | | | | | | | | | | | | | | | | | | | | | |

Pet., 58

# Disclosure's Single-Substitution Examples Are Not Representative

159. Because of the complicated interactions multiple amino acid substitutions may have within a protein, the activity of multi-substituted polypeptides having sets of 5, 10, 15, or more changes could not generally be predicted from data regarding the activity of mutants containing only a single amino acid substitution. Put differently, the single-replacement $PH20_{1-447}$ polypeptides reported in the common disclosure are not representative of all the types of mutated $PH20_{1-447}$ polypeptides that have a particular substitution at position 307 and sets of between 1 and 41 *additional* substitutions at any of hundreds of positions within the PH20 protein.

EX1003 (Hecht), ¶ 159; Pet. 55, 56; Reply, 7, 23

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, 62; Reply, 21

151. The absence of any explanation why single amino acid substitutions of non-conserved residues rendered the $PH20_{1-447}$ inactive limits the insights one can draw from the patent's disclosure. For example, there is no discussion of the effect on any secondary or other structure within the PH20 protein of the substitution. The insights one can draw from this set of data is even more limited relative to PH20 proteins that incorporate multiple substitutions. It is what the common disclosure says, which is to not include any of the substitutions in Tables 5 and 10 that rendered $PH20_{1-447}$ inactive as part of a set of substitutions in a multiply modified PH20 polypeptides that is intended to have activity, regardless of the length of the PH20 polypeptide or the number of additional amino acid replacements.

EX1003 (Hecht), ¶ 151; Pet. 36, 52; Reply, 10

# No Reporting of Effects of Single Mutations on PH20 Structural Features

139. Random mutagenesis experiments like the one reported in the common disclosure provide empirical results. Typically, they are followed by additional analyses and experimentation to understand why the results were observed, and to determine what changes influenced discrete structures within the protein. This work, if it was performed, is not documented or discussed in the common disclosure beyond experiments concerning two specific mutants (F204P and V58R). See ¶ 74-75. There are no observations from the experimental results on any specific secondary structures or structural motifs within the PH20 protein that were influenced (positively or negatively) by individual mutations. There also is no guidance regarding additional mutations that could be made to further enhance or alter the characteristics of these mutants.

EX1003 (Hecht), ¶ 139; Pet. 44-45, 52, 71

140. The common disclosure does not provide any information that a skilled artisan could use to predict the effect of incorporating into a PH20 protein the myriad different sets of between 2 and 42 substitutions drawn from the thousands of individual mutations in $PH20_{1-447}$ listed in the common disclosure. It does not, for example, suggest that incorporating one of the specific single substitutions that caused that $PH20_{1-447}$ mutant to exhibit increased activity will cause a similar increase in the activity of any other PH20 polypeptide that contains additional substitutions, regardless of their number, location, or identity. That also would not be scientifically plausible. In other words, the functional and other characteristics of a PH20 protein that incorporates a first single amino acid substitution cannot be extrapolated to modified PH20 polypeptides that incorporate the myriad sets of combinations of multiple substitutions in addition to the first.

EX1003 (Hecht), ¶ 140; Pet. 45, 52, 62; Reply, 20-21

# Combinations Disclosure Says to Avoid

148. The common disclosure says that substitutions that rendered $PH20_{1-447}$ an inactive mutant as a single amino acid replacement should be avoided in modified PH20 polypeptides intended to have hyaluronidase activity. It also makes these statements without regard to how many additional substitutions or other changes might be incorporated into the mutant. As it states:

> To retain hyaluronidase activity, modifications ***typically are not made*** at those positions that are less tolerant to change or required for hyaluronidase activity. For example, generally modifications are not made at a position corresponding to position … *[ 96 positions ]* … with reference to amino acid positions set forth in SEQ ID NO:3. Also, in examples where modifications are made at any of *[… 313 positions … ]* with reference to amino acid positions set forth in SEQ ID NO:3, the modification(s) is/are not the corresponding amino acid replacement(s) set forth in Table 5 or 10 herein, which are amino acid replacements that result in an inactive polypeptide.[71]

EX1003 (Hecht), ¶ 148; Pet., 36

140. The common disclosure does not provide any examples of a modified PH20 polypeptide (regardless of its length) that (i) incorporates one amino acid substitution that causes it to have hyaluronidase activity or increased hyaluronidase activity, and then (ii) introduces additional changes that render that multiply-modified PH20 protein an "inactive mutant." I can identify no scientific or practical reason why a skilled artisan would pursue such a path. It makes no sense for a skilled artisan to go to the effort of making a modified PH20 with hyaluronidase activity (or greater activity than the unmutated protein), and then intentionally introduce additional mutations to make that modified protein inactive. If the goal were to make inactive mutants, the skilled artisan would have started by selecting one of the thousands of examples of PH20 polypeptides reported as having one amino acid replacement that produced inactive mutants.

EX1003 (Hecht)(PGR2025-00046), ¶ 140; Reply, 3

162. In fact, the common disclosure instructs skilled artisans to not include substitutions from Table 5/10 in any PH20 polypeptides with hyaluronidase activity, including those with multiple substitutions. See § V.A.4. To make all the multiply-modified PH20 polypeptides covered by the claims' sequence identity parameters, the skilled artisan would have to disregard the guidance in the common disclosure indicating that substitutions listed in Tables 5/10 should not be included in PH20 polypeptides intended to have hyaluronidase activity.

EX1003 (Hecht), ¶ 162; Pet. 36, 57

# Disclosure's Iterative Mutagenesis Process is a Research Plan

**4. Iterative Methods**

**The method provided herein also is iterative. In one example, after the method is performed, any modified hyaluronan-degrading enzymes identified as exhibiting stability, such as increased stability, under a denaturation condition can be modified or further modified to increase or optimize the stability. A secondary library can be created by introducing additional modifications in a first identified modified hyaluronan-degrading enzyme. For example, modifications that were identified as conferring stability, such as increasing stability, can be combined to generate a combinatorial library. The secondary library can be tested using the assays and methods described herein.**

**In another example of an iterative aspect of the method, modified hyaluronan-degrading enzymes that are identified as not exhibiting stability such as increased stability (*e.g.*, such that they are not active or do not have increased activity under the a denaturation condition), can be further modified and retested for stability under a denaturation condition. The further modifications can be targeted near particular regions (*e.g.*, particular amino acid residues) associated with activity and/or stability of the molecule. For example, residues that are associated with activity and/or stability of the molecule generally are critical residues that are involved in the structural folding or other activities of the molecule. Hence, such residues are required for activity, generally under any condition. Critical residues can be identified**

**A further library of modified proteins can be generated with amino acid mutations targeted at or near to the identified critical amino acid residues, such as adjacent to the identified critical amino acid residues. In some examples, the mutations can be amino acid replacement to any other of up to 19 other amino acid residues. The secondary library can be tested using the assays and methods described herein.**

EX1026 ('731 App.), 150:1-26; Pet. 48-52; Reply, 35-36

180. The common disclosure's theoretical research plan is also largely meaningless when it is applied to the data in the common disclosure. The plan proposes to introduce modifications "near particular regions (e.g., particular amino acid residues) associated with activity and/or stability of the molecule." The data reported in Tables 5 and 10 show that at least one substitution at each of 405 different positions between positions 1 and 444 of the sequence rendered the 447 amino acid PH20 polypeptide inactive.[87] In other words, under the logic of the common disclosure's research plan, the skilled artisan should "target" mutations to positions "near" 90% of the protein sequence. Thus, the research plan provides little to no guidance on how to design and/or choose mutations in this protein.

EX1003 (Hecht), ¶ 180; Pet. 50-51

# Halozyme Mischaracterizes the Alpha/Beta Barrel Motif

In sum, these hyaluronidases all share a tertiary structure a POSA would recognize as an "alpha-beta barrel fold." Their nearly identical tertiary structures are depicted below:




POR, 12

152. Dr. Petsko's use of the "alpha-beta barrel fold" terminology in his declaration where it discusses PH20 is imprecise. This is a problem because he is attempting to use this term to define a particular structural feature in the tertiary structure of a modified PH20 polypeptide. To do that, the POSA would refer to the "alpha-beta barrel" as a motif or sub-domain within the tertiary structure of the protein, which makes clear there are other structural features in the tertiary structure. The POSA also would describe how the α-helix and β-strand elements of the α/β barrel relate to other elements of the protein structure. The phrase α/β barrel ***fold***, by contrast, conveys that the α/β barrel motif ***is*** the tertiary structure of the protein.[172] That is not the case for PH20.

EX1133 (Naismith), ¶ 152; Reply, 12, 14

```
24    Is the alpha-beta barrel fold of the
25    human PH20 polypeptide, is that also called the
1     tertiary structure of the human PH20 polypeptide?
2     A. It's part of the tertiary structure
3     because there are other elements in the protein
4     besides that particular subdomain, if you will.
5     But yes, it is part of the tertiary structure.
```

EX1131 (Petsko Dep.), 68:24-69:5; Reply, 6, 12

# PH20 Active Site Is Not Within the α-β Barrel Motif

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.*

POR, 45-46

148. Another important structural insight reported in Stern is that the residues involved in catalysis in hyaluronidases are located ***outside*** the α/β barrel core, rather than being integral to the α/β barrel motif structure (below).[171] While enzymatically active hyaluronidases do preserve the spatial positioning of many residues involved in substrate binding and catalysis, Stern explicitly excludes attributing those conserved structural features to the α/β barrel motif.

EX1133 (Naismith), ¶ 148; Reply, 13, 16

octasaccharide segment of HA. The extreme N-terminal segment of the catalytic domain, ranging in length from 28 aa for human Hyal-1 to 41 aa for HPH-20, always assumes a helical conformation. It takes on a structure of a single helix for Hyal-1 and Hyal-2 (the Hyals with the shortest segment at their N-terminus) or a set of two helices connected by a short loop for Hyal-3, Hyal-4, and HPH-20. Hyal-4 and HPH-20 have the longest aa segments at their N-termini (Figure 3). In all five cases, however, the helices are located on the outside of the TIM barrel core structure, are not incorporated into the distorted TIM barrel fold, and are primarily associated with the catalytic domain only; the interactions with the C-terminal domain are minimal, if any (Figures 4 and 5). The sequence similarity in this region of all human Hyals is very low, compared to the remaining portion of these Hyals, and therefore allows for some variations in structure.

EX1008 (Stern), 829; Reply, 13-14, 16

# Prior Art Distinguished α-β Barrel Motifs in Bee Venom and HYAL1 Proteins

The art also recognized that hyaluronidases, despite only 30-40% sequence identity, share a conserved alpha-beta barrel fold, a common active site, and a conserved set of catalytic residues positioned similarly across Hyal-1, bee venom hyaluronidase, and PH20. EX1006, 6912, 6914; EX1008, 826, 833; EX1033, 1028; EX2070 ¶¶126-27. Mutagenesis studies in Hyal-1 and PH20 had further confirmed

POR, 70-71

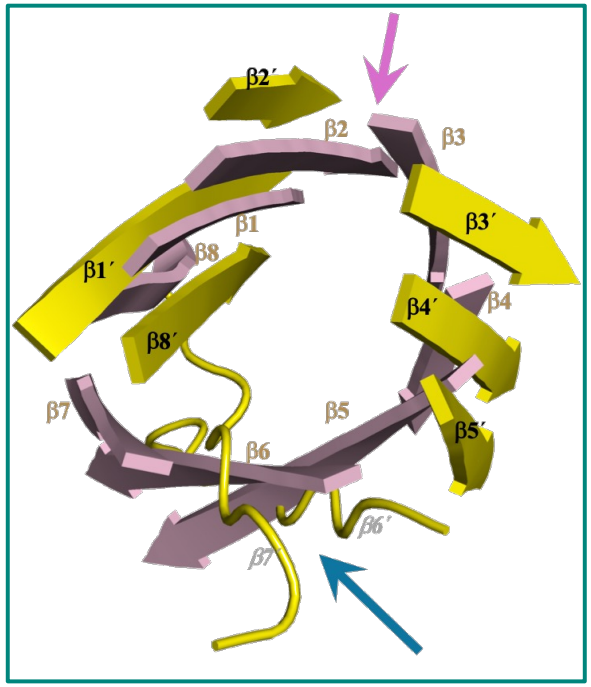
141. Chao (EX1006) also reported that the human Hyal-1 structure (2PE4) had a distorted barrel fold and that the beta barrel is open despite the presence of all eight β-strands (below). The bee venom hyaluronidase lacks the β2 strand.[162] In addition, the α-helix following the β2 strand is missing in human Hyal-1 (but not in the bee venom hyaluronidase). To a POSA these represent important and meaningful structural distinctions from the canonical α/β barrel motif, and between the bee venom and these mammalian hyaluronidases.

EX1133 (Naismith), ¶ 141; Reply, 13, 15

The hHyal-1 molecule comprises two closely associated domains: the N-terminal catalytic domain (Phe22—Thr352) and the smaller C-terminal domain (Ser353—Trp435) (Figure 2A and B). The catalytic domain adapts a distorted $(\beta/\alpha)_8$-barrel fold similar to that of the bvHyal structure (*22*) but with some variations (Figure 2C): The hHyal-1 $\beta$2-strand is missing in bvHyal. However, this $\beta$2 does not form $\beta$-sheet interactions with $\beta$3, hence the $\beta$-barrel is open despite the presence of all eight strands. The barrel imperfection extends to the α-helix following $\beta$2, which is absent in hHyal-1. A number of secondary structure units are inserted in loops on the C-terminal side of the $\beta$-barrel: an α-helix (labeled α4′ following the numbering of the primary $\beta/\alpha$ motifs), two $3_{10}$ helices ($\eta$4′ and $\eta$8′), and a $\beta$-hairpin ($\beta$3′-3″) (Figures 2B and 3). Two of the five hHyal-1 disulfide bonds (Cys43—Cys333 and Cys207—Cys221) reside in the catalytic domain and are also present in bvHyal (Figure 2C).

EX1006 (Chao), 6913; Reply, 13-14

# α-β Barrel Term Cannot Precisely Describe PH20 α-β Barrel Motif Structure




EX1133 (Naismith), ¶ 143; Reply, 15

is common to many hydrolases [28]. However, in Hya only seven strands (β1–β7) form the barrel that is surrounded by α helices A–J. There is a lack of closure between strands β1 and β2, which do not interact through hydrogen bonding, leaving a wide gap (7–8 Å) that could accommodate an additional strand (Figure 3). Loop 34–49 partly fills this gap with two polar mainchain atoms of Leu39 engaged in parallel β sheet hydrogen-bonding interactions with the mainchain atoms of Val13 and Trp15 of β1. Other unusual features, that deviate from the regular $(\beta/\alpha)_8$ barrel, are the presence of two α helices between strands 3 and 4 (helices C and D) and 4 and 5 (helices E and F) and two β hairpins comprising residues 63–75 and 269–279 at the C-terminal end of β2 and β6, respectively. Hya contains two disulfide bridges (Figure 3b). The first, Cys189–Cys201, stabilizes the base of a long loop at the C-terminal end of β4, whereas the Cys22–Cys313 bridge joins distant secondary structure elements, a $3_{10}$ helix near the N terminus and α helix I near the C terminus. The Hya sequence contains

EX1133 (Naismith), ¶ 140 (citing EX1033 (Markovic-Housley), 1027); Reply, 15

# α-β Barrel Motif Does Not Describe Common Structural Features of Active PH20s

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. 53-55, 62

52. The alpha-beta barrel fold structure is a robust tertiary structure that often is resilient to modification without inactivating the protein. However, the patent data show that this is not the case for PH20. The patent data show which positions on PH20 do not tolerate substitutions or tolerate very few substitutions, and the types of substitutions that are tolerated. This extensive, virtually

EX2070 (Petsko), ¶ 52; Reply, 31

151. I have been informed that an adequate written description for a set (or genus) of chemical compounds requires "a precise description, such as by structure, formula, chemical name, physical properties, or other properties of species falling within the genus sufficient to distinguish the genus from other materials." I do not believe the phrase "PH20 alpha-beta barrel fold" as it is being used by Drs. Petsko and Simpson does or is capable of doing that.

a. First, the use of "fold" with "PH20 alpha-beta barrel" is scientifically inaccurate. In PH20, the α/β barrel is a motif, not the fold.

b. Second, the common disclosure does not associate a particular sequence within PH20 to the α/β barrel motif or explain how the active site or substrate binding sites relate to α/β barrel motif.

c. Third, Drs. Petsko and Simpson are using the phrase "alpha-beta barrel" motif at a level of abstraction that cannot differentiate the PH20 protein from other human hyaluronidases, or non-mammalian hyaluronidases. The structures in all these proteins differ (*e.g.*, the bee venom omits the significant portion at the C-terminus of PH20).

EX1133 (Naismith), ¶ 151; Reply, 14, 16

# PH20 Tertiary Structure Not Published Until 2024

Petitioner also argues that the specification does not disclose the PH20 alpha-beta barrel fold structure. But it is an inherent feature of the actives, and Petitioner acknowledges it was known in the art. EX2077, 175:18-176:5, 53:22-54:5; EX1004, ¶¶156-167, 33-52, 88-101. "[A] patentee may rely on information that is 'well-known in the art' for purposes of meeting the written description requirement, because the specification is viewed from the perspective of one of skill" in the relevant art. *Ajinomoto*, 1359; *Zoltek*, 1308. A POSA would use the prior art structure to easily visualize the actives based on the other common features, as Petitioner's experts testified.

PO Sur-Reply, 22

## Cryo-EM Structure of Human Hyaluronidase PH-20

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To date, the empirical three-dimensional information of this enzyme has been unavailable, although crystal structures of human HYAL-1 [34] and bee venom hyaluronidase (bvHyal) [35] have been determined. Herein, we report the cryogenic electron microscopy (cryo-EM) structure of human PH-20 at 3.1 Å resolution, providing a foundation for studying the functional and the catalytic property of PH-20.

EX1126 (Im), 1067; Reply 13

# Halozyme's Assertions on Homology Modeling Not Supported by Park

Petitioner also argues that the specification does not disclose the PH20 alpha-beta barrel fold structure. But it is an inherent feature of the actives, and Petitioner acknowledges it was known in the art. EX2077, 175:18-176:5, 53:22-54:5; EX1004, ¶¶156-167, 33-52, 88-101. "[A] patentee may rely on information that is 'well-known in the art' for purposes of meeting the written description requirement, because the specification is viewed from the perspective of one of skill" in the relevant art. *Ajinomoto*, 1359; *Zoltek*, 1308. A POSA would use the prior art structure to easily visualize the actives based on the other common features, as Petitioner's experts testified.

PO Sur-Reply, 22

```
18      Q So in the 2011 time frame, if a POSA is
19   trying to determine the impact of a potential
20   substitution, would they have done the similar
21   analysis where they look at -- where they look at
22    the substitution and determine if it's potentially
1     going to favorable, neutral or unfavorable?
2         A In the year 2011, to consider a mutation,
3     It would have been very common and expected for a
4     POSA to generate a model and consider the
5     Substitution in that structural context.
```

EX2077 (Park Dep.), 175:18-176:5; PO Sur-Reply, 22

161. The PH20 structural model I generated would be very similar if not identical to the model that SWISS-MODEL would have generated in 2011. As I explained above, SWISS-MODEL works by first identifying a template structure from a library of templates, based on sequence comparisons. If there is more than one suitable template structure, the template structures are superimposed and the atom positions in the structural backbone are averaged to construct the core of the model.[126] If there is only one suitable template structure, like there is here, then no averaging is done and the backbone of the template structure is directly adopted.

EX1004 (Park),¶161;Pet., 108-109

```
22        Q And you can use homology modeling to
1    predict the impact of substitutions in a protein?
2         ATTORNEY KUSHAN: Objection to foundation.
3         Also to form.
4     A A homology model can help you to evaluate
5     the impact of a substitution. Yes.
```

EX2077 (Park Dep.), 53:22-54:5; PO Sur-Reply, 22

# No 3D Structural Information or Information on α-β Barrel Motif in Disclosure

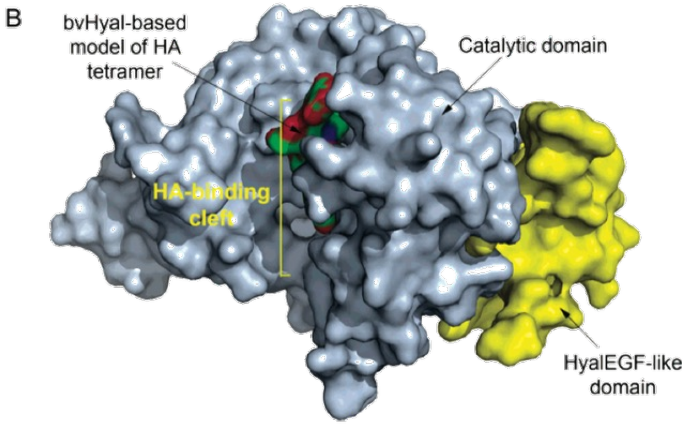
166. The common disclosure does not include or reference a structural model of PH20 protein.[186] It also does not identify a particular three-dimensional structural feature within the tertiary structure that corresponds to the α/β barrel motif in PH20, or portray that as being a common structural feature of all modified PH20 polypeptides that exhibit enzymatic activity. While it does reference the active site residues and substrate binding residues, it does not describe their structural context.[187] It also cites three publications: (i) Arming (EX1011), (ii) Ten Have (EX1149) and (iii) Chowpongpang (EX1150). None of those publications contain an experimentally determined structure or a homology model of a hyaluronidase enzyme. None of these publications discuss a PH20 α/β barrel fold structure or its capacity to be mutated without consequence to folding or activity of the PH20 protein.

EX1133 (Naismith), ¶ 166; Reply 13-14

167. The common disclosure does not associate any particular amino acid sequence(s) within PH20 to an α/β barrel motif in the PH20 protein. It also does not identify or discuss other structural elements present within the tertiary structure of the PH20 protein, including within or outside the α/β barrel region, and does not portray them as being "common structural features" of enzymatically active modified PH20 polypeptides (*e.g.*, the Hyal-EGF domain reported in Chao (EX1006)).

EX1133 (Naismith), ¶ 167; Reply 14, 17

# Hyal-EGF Domain Essential for PH20 Enzymatic Activity




EX1006 (Chao), 6917, Figure 4B; Pet. 89

88. Thus, before 2011, a number of residues within the region of the catalytic site in PH20 or HYAL1 had been experimentally shown to be necessary or important to the catalytic activity of hyaluronidases. For example, Arming (EX1011) identified positions D111, E113, R176, E249, and R252. Zhang (EX1010), identified positions in HYAL1 corresponding to D111, D113, D184, S227, Y229, R246, W304, and N333 in PH20, as well as the Hyal-EGF domain from 337-409 in PH20 identified by Chao.

EX1003 (Hecht), ¶ 88; Reply, 13-14,17-18

188. Drs. Hecht and Park discussed the characterization of the Hyal-EGF domain in Chao and Zhang,[216] and I agree with their opinions, for example, that POSAs would have understood this domain to be necessary for proper folding and enzymatic activity of PH20.

EX1133 (Naismith), ¶ 188; Reply, 13, 17-18, 19