Epistasis Was an Established Phenomenon

252. 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, however, 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.

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.

EX1133 (Naismith), ¶ 252; Reply 20, 23

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.), 89:3-90:24; Reply 20, 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.), 270:9-17; Reply 20, 23

Epistasis Was an Established Phenomenon

125. 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):[138]

> **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.

126. 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."[139] That challenge reveals the shortcomings of the relatively simplistic 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).[140] But because of epistasis, combinations generated in a second or subsequent library may alter the effect of the first mutation.

EX133 (Naismith), ¶¶ 125-126, citing (EX1141, 409); Reply 23

Mutations Can Cause Aggregation

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Aggregation Is a Well-Established Phenomenon

327. 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:[351]

EX1133 (Naismith), ¶ 327; 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

329. I disagree. Protein aggregation is a well-established phenomenon and recognized in the scientific literature as a "fundamental problem in biotechnology."[353] 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."[354] 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."[355] 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"[356] (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), ¶ 329 (citing EX1014 (Branden & Tooze), 91); Reply, 5

Aggregation Is a Well-Established Phenomenon

330. 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."[357] Critically, the effects of multiple mutations on aggregation can be cumulative, arising from combined perturbations to fundamental physicochemical properties of the polypeptide chain.[358] 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.[359]

EX1133 (Naismith), ¶ 330; Reply, 5

333. 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) [363] and that immunogenicity in biopharmaceuticals may arise "largely because misfolded proteins break tolerance."[364]

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), ¶ 333 (Citing EX1152 (Ringe and Petsko), 80.1, 80.3); Reply, 5

Aggregation Is a Well-Established Phenomenon

334. 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), ¶ 334; Reply, 5

diseases but is a generic phenomenon of polypeptide changes."[355] 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"[356] (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), ¶ 329; Reply 5

Legal Authority

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

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

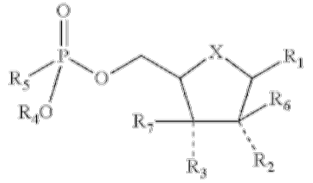
...a sufficient description of a genus instead requires the disclosure of **either a representative number of species** falling within the scope of the genus or **structural features common to the members of the genus** so that one of skill in the art **can "visualize or recognize" the members of the 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]

Juno Therapeutics Inc., v. Kite Pharma, 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)

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.

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., 26-27, 32-34, 44-45, 48-49, 52-60; Reply, 4-7, 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.

PO Response, 56

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

PO Response, 63

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

PO Response, 65

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

PO Response, 52

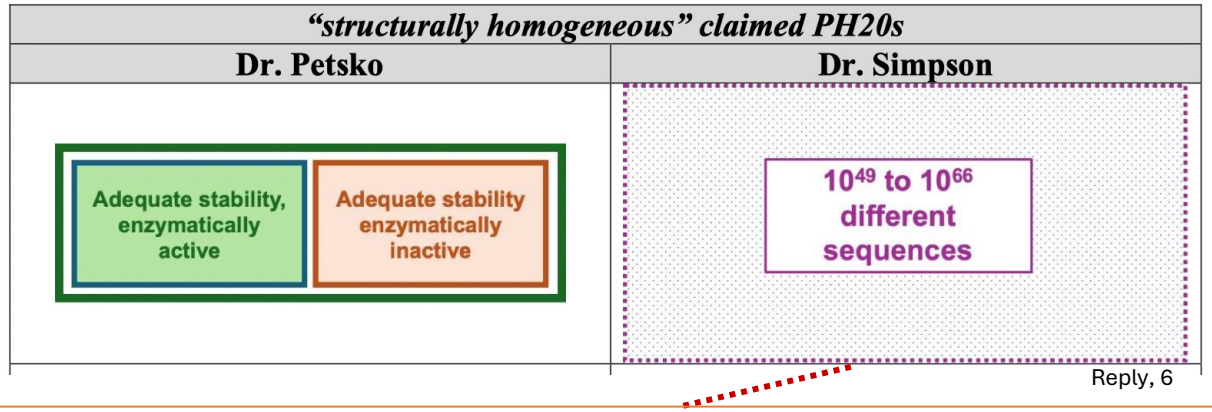
54 (internal quotation marks omitted). Here, the inventors in no way overreached in exchange for their expansive disclosure; they sought and received highly limited claims to a structurally homogeneous set of modified PH20s that require a single amino acid mutation at a single position that is trivially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

PO Response, 52

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 95% identical primary structure, the D320 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, ¶¶389-

PO Response, 3

Halozyme's Experts Contradict Each Other on Structural Homogeneity



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 subvariants all share at least 95% sequence identity with the variant D320 PH20s, 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 Dec.), ¶ 33, Reply, 6

Q. You portray this as a narrow set of PH20 variants.

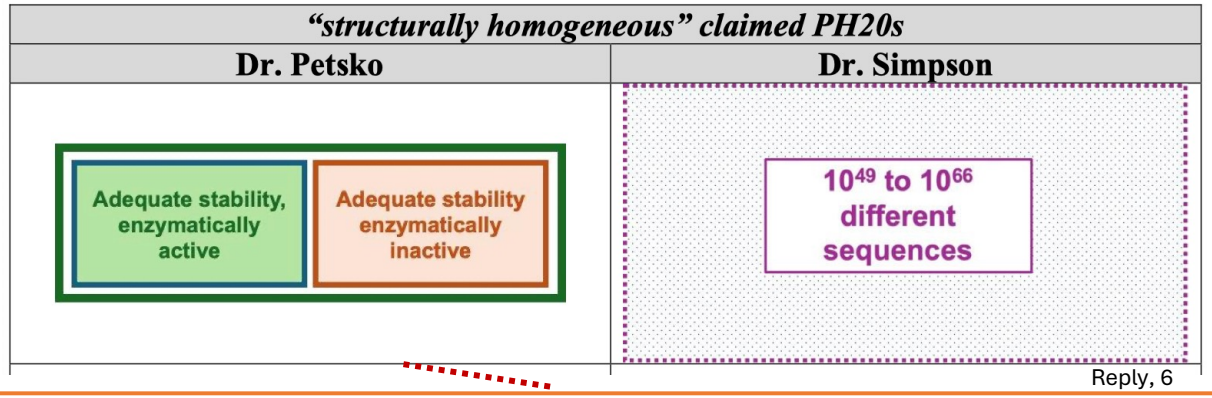
Do you mean that the number of different polypeptide sequences that are in that set is a narrow set of sequences?

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

391. POSAs think of the class of modified PH20 proteins sharing 95% of their sequence (including the recited change at position 320) 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 Dec.), ¶¶ 51, 391, 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.), 72:7-19, Reply, 6

Enzymatically Active PH20s Are in a Portion of Claim Sequence Space

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

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

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

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

EX1001 (PGR2025-00003)('600 Patent)



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 2 to 17, 21, 22 or 23 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 (all of which have the D320K mutation) 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, 50, 71, Reply, 7-8

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 22 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 22 substitutions that result in the PH20 polypeptide having greater than 100% of the activity of the parent unmodified PH20, or greater than 120% 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, 62, 67, 71; Reply, 7-8, 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 an amino acid sequence, wherein:

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

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

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

EX1001 (PGR2025-00003)('600 Patent)

83. I do not agree that the 95% 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.*, 22 changes divided by 447 total residues = .0492, which equates to .9508, or 95.08% 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, 7, 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), ¶ 83; Reply, 7, 11

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

The Patents thus provide a very narrow set of working examples relative to the diversity of modified PH20 polypeptides being claimed.[150] The examples are restricted to ***one type of change*** (a single amino acid replacement) in ***one type of PH20 polypeptide*** (SEQ ID NO:3).[151] By contrast, the claims encompass changes in 35 different unmodified PH20 sequences, and include, in addition to one identified replacement, anywhere from 1 to 21 (claim 1), 1-16 (claim 3) or 1-20 (claim 4) additional changes.[152] A simple illustration demonstrates how ***non-representative*** the examples are: all of the Patents' examples of single-replacement $PH20_{1-447}$ mutants fit into one box of the array below.

Pet., 58-59

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

Reply, 8

	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
3																						
32																						
33																						
34																						
35																						
36																						
37																						
38																						
39																						
40																						
41																						
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66																						

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 sets of between 2 and 22 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., 54, 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. 36-37, 56; 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, 52-53, 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 22 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, 53, 56, 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., 36-37

161. First, the sequence identity language, read literally, would capture multiply-modified PH20 polypeptides that include substitutions listed in Tables 5 and 10. These are the substitutions that, when made as a single substitution in $PH20_{1-447}$, rendered the $PH20_{1-447}$ protein inactive. Also, there are no examples of any multiply-modified PH20 polypeptides in the specification, much less ones that combine substitutions that rendered the protein inactive from Tables 5 and 10 with other substitutions that together cause $PH20_{1-447}$ to have hyaluronidase activity (or increased levels of that activity).

EX1003 (Hecht), ¶ 161, Pet. 37, 57, 61

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. 37, 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

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. 51-52

α-β Barrel Motif Is Not the Tertiary Structure of PH20

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

153. 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.[171] That is not the case for PH20.

EX1133 (Naismith), ¶ 153; 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, 12

149. Another important structural insight reported in Stern is that the helices 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).[170] 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), ¶ 149; 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, 68

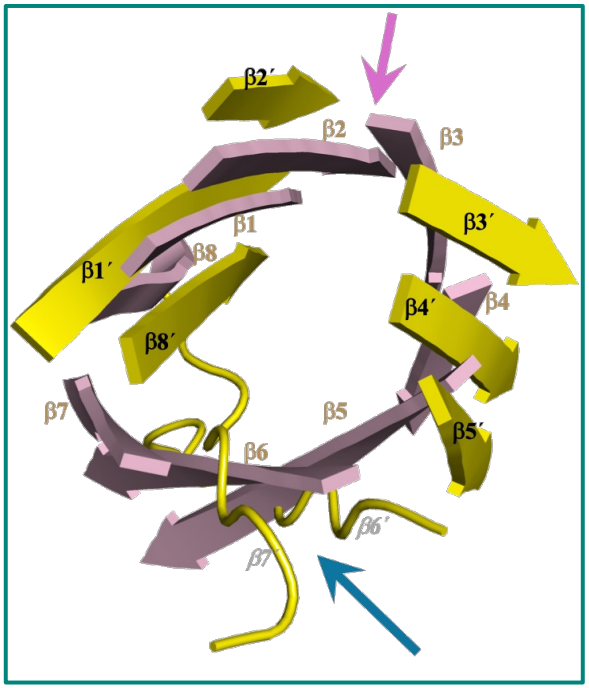
142. Chao (EX1006) also reported that the human Hyal-1 structure (2PE4) had a distorted barrel fold and that the beta barrel is open despite the presence of all eight β-strands (below). The bee venom hyaluronidase lacks the β2 strand.[161] In addition, the α-helix following the β2 strand is missing in human Hyal-1 (but not in the bee venom hyaluronidase). These are meaningful structural distinctions from the canonical α/β barrel motif, and between the bee venom and these mammalian hyaluronidases.

EX1133 (Naismith), ¶ 142; 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 β2-strand is missing in bvHyal. However, this β2 does not form β-sheet interactions with β3, hence the β-barrel is open despite the presence of all eight strands. The barrel imperfection extends to the α-helix following β2, which is absent in hHyal-1. A number of secondary structure units are inserted in loops on the C-terminal side of the β-barrel: an α-helix (labeled α4′ following the numbering of the primary β/α motifs), two 3_{10} helices (η4′ and η8′), and a β-hairpin (β3′-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).

EX1133 (Naismith), ¶ 162 (citing EX006 (Chao), 6913); Reply, 13-14

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



EX1133 (Naismith), ¶ 144; 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), ¶ 141 (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. 53-54, 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 Dec.), ¶ 52; Reply, 30

152. 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 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), ¶ 152; Reply, 14

Comparison of α-β Barrel Motif Description in Halozyme Response and Sur-Reply

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, 12

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

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

PO Sur-Reply, 21

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, ¶¶149-160, 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, 22-23

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

Park Testimony Cited by Halozyme

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, ¶¶149-160, 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, 22-23

Q So in the 2011 time frame, if a POSA is trying to determine the impact of a potential substitution, would they have done the similar analysis where they look at -- where they look at the substitution and determine if it's potentially going to favorable, neutral or unfavorable?

A In the year 2011, to consider a mutation, it would have been very common and expected for a POSA to generate a model and consider the substitution in that structural context.

EX2077 (Park Dep.), 175:18-176:5; PO Sur-Reply, 22-23

Q And you can use homology modeling to predict the impact of substitutions in a protein?

ATTORNEY KUSHAN: Objection to foundation. Also to form.

A A homology model can help you to evaluate the impact of a substitution. Yes.

EX2077 (Park Dep.), 53:22-54:5; PO Sure-Reply, 22-23

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

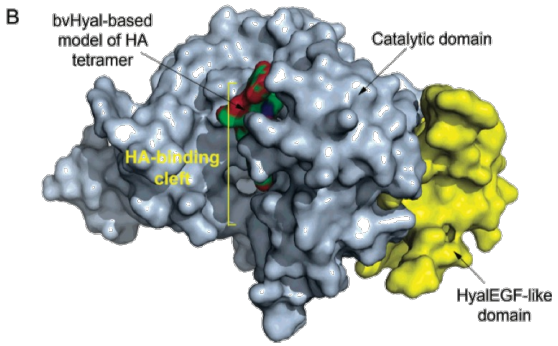
167. The common disclosure does not include or reference a structural model of PH20 protein. It also does not identify a particular three-dimensional structural feature within the tertiary structure that corresponds to the α/β barrel motif in PH20, or portray that as being a common structural feature 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.[185] 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), ¶ 167; Reply 13-14.

168. 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), ¶ 168; Reply 13-14.

Hyal-EGF Domain Essential for PH20 Enzymatic Activity



EX006 (Chao), 6917, Figure 4B; Pet. 89

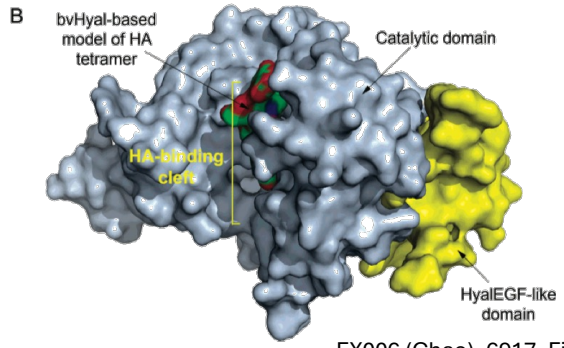
88. Thus, before 2011, a number of residues within the region of the catalytic site in PH20 or HYAL1 had been experimentally shown to be necessary or important to the catalytic activity of hyaluronidases. For example, Arming (EX1011) identified positions D111, E113, R176, E249, and R252. Zhang (EX1010), 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; Pet., 89; Reply, 13,17

189. Drs. Hecht and Park discussed the characterization of the Hyal-EGF domain in Chao and Zhang,[214] 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), ¶ 189; Reply, 18-19

Hyal-EGF Domain Essential for PH20 Enzymatic Activity



EX006 (Chao), 6917, Figure 4B; Pet. 89

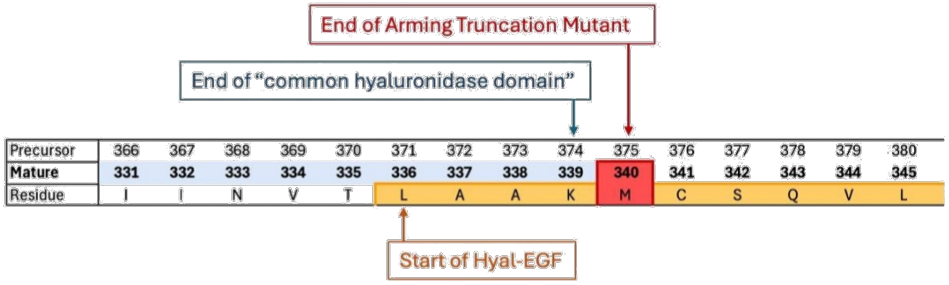
84. A notable finding in Chao was its identification of the "Hyal-EGF" domain in the C-terminal region of human hyaluronidases. Chao observed that the C-terminal region of mammalian hyaluronidases does not exhibit significant homology but does contain a cysteine-rich pattern of residues recognized by sequence analysis tools as an epidermal growth factor (EGF)-like motif (below). Chao identified the pattern of sequences that generates this Hyal-EGF structural motif (*i.e.*, "x4Cx0-48Cx3-12Cx1-70Cx1-6Cx2Gax0-21Gx2C, where "a" denotes a hydrophobic residue, "x" denotes any residue, and the gaps between cysteine residues vary in length as indicated by the subscripts.").[41] This is a good

EX1003 (Hecht), ¶ 84; Pet., 40, 89; Reply, 13

Deletion of the EGF-like Domain Abolishes Hyal1 Activity Independently of Asparagine 350—The C-terminal EGF-like domain is not found in the bacterial or bee venom hyaluronidases, so previous models of Hyal1 using coordinates for the bee venom ortholog depicted this region as an independently folded domain with minimal contacts to the active site containing domain. Inspection of the human Hyal1 model reveals a single overall folded domain, with extensive contacts between the EGF-like region, the sugar moieties of the Asn^{350} glycosyl adduct housed within that region, and the remainder of the protein. We introduced stop codons at position 350 (N350tr), which deleted 86 amino acids, including the Asn^{350} glycosylation site, and at position 356 (L356tr), which deleted the 80-amino acid EGF-like domain but retained Asn^{350}. Neither deletion mutant was secreted to the extracellular space but both were expressed well in the soluble lysate (Fig. 5*C*). Western blot showed the N350tr lysates contained a major band of ≈38 kDa, the predicted molecular mass for the polypeptide, whereas the major band in L356tr lysates was ≈42 kDa, consistent with the 4-kDa shift observed for the fully glycosylated wild-type enzyme. The lysates showed ≈2.5 and ≈6.3% of wild-type activity for N350tr and L356tr, respectively, at the 125 μM HA amount. Thus, loss of this domain is deleterious to both secretion and activity of Hyal1, although the residual activity suggests the effect is indirect.

EX1010 (Zhang), 9439; Pet., 13; Reply, 14, 17

Disclosure's Common Hyaluronidase Domain Does Not Identify Hyal-EGF Domain



Precursor	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380
Mature	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345
Residue	I	I	N	V	T	L	A	A	K	M	C	S	Q	V	L

Reply, 18-19

among PH20 hyaluronidase polypeptides. PH20 hyaluronidases contain a common core hyaluronidase domain region of about 340 amino acids in length that corresponds to amino acid residues 38-374 of the precursor human PH20 sequence set forth in SEQ ID NO:6. A mature PH20 polypeptide lacking the signal sequence and containing a contiguous sequence of amino acids having a C-terminal amino acid residue corresponding to amino acid residue 464 of SEQ ID NO:6 (*e.g.*, amino acid residues corresponding to positions 36-464 of the amino acid sequence set forth in SEQ ID NO:6) is the minimal sequence required for hyaluronidase activity (see *e.g.*, U.S. Patent Application No. 10/795,095,which is issued as U.S. Patent No. 7,767,429; see also U.S. Publication No. US20100143457).

EX1026 ('731 App.), 79:22-31; Reply, 18

162. If Dr. Petsko is suggesting that positions 3 to 339 correspond to the α/β barrel motif in PH20, that would be misleading. That is because several loops in PH20 are much more extensive and contain additional and elongated secondary structural elements when compared to the canonical α/β barrel (which comprises 248 residues in 1TIM). Some examples are the strand loop strand (Tyr 64 – Ser 84), a helix (Lys126-Gln140), a sixteen residue longer helix (Leu146-Leu175), a longer loop (Pro187-Asn205), and a twenty residue longer helix (Met313 – Leu345). Similar observations were made by Chao (EX1006) in the paragraph discussed in ¶ 142.[181]

EX1133 (Naismith), ¶ 162; Reply 18-19

163. The "common core hyaluronidase domain" of PH20 referred to in the common disclosure (quoted by Dr. Petsko) when produced has no enzymatic activity as Dr. Hecht noted.[182] The model of PH20 also shows that position 339 is part of an elongated alpha helix. The "common core hyaluronidase domain" also excludes the EGF domain, an indispensable feature of the structure-function relationship of PH20.

EX1133 (Naismith), ¶ 163; Reply 18-19

Hyal-EGF Domain Not Characterized in Disclosure

Finally, Petitioner makes much of a "hyal-EGF" domain," which Petitioner acknowledges is common structural feature shared by actives. Petitioner contends the hyal-EGF domain is not identified by name in the specification. But the patent need not identify common structural features using Petitioner's preferred terminology (particularly given the Hyal-EGF is a misnomer). The patent expressly reports the import of this region ("amino acid residues corresponding to positions 36-464… is the minimal sequence required for hyaluronidase activity") and incorporates by reference Arming, which Petitioner itself says "proves" the region was essential based on its teaching that a C-terminal truncation mutant ending at position 340 was not secreted or active. EX1001, 69:66-70:14.

PO Sur-Reply, 23

96. The data in the patent (particularly Tables 3 and 5 for "active mutants" and "inactive mutants," respectively) shows that making single amino acid substitutions in the region from 337-409 resulted in both active mutants and inactive mutants for most of the range of positions. See Appendix A-9. In the aggregate, there appear to be somewhat more single mutations that result in inactive mutants than those that result in active mutants, but that is a qualitative assessment. Unsurprisingly, mutations that changed the cysteine residues (which are also highly conserved) were uniformly inactive. The varying effects of changing residues in the Hyal-EGF region of PH20 show that that a skilled artisan's belief that changes in this region would be unpredictable were warranted, and would be more so if multiple changes were made concurrently.

EX1003 (Hecht), ¶ 96; Pet. 40

Lack of Enablement

- **The disclosure must enable the full scope of the claims**
  - The claims encompass multi-modified PH20 polypeptides that are not predictable from the disclosure
- **Neither iterative mutagenesis nor homology modeling can enable the full scope of the claims**
  - Scale of experimentation is impossible
  - Dr. Petsko's tolerance analysis is flawed
- **Halozyme's claim construction encompasses mutants with no credible utility and inoperative species**

See, e.g., Pet., 66-76, 79-83; Reply, 22-29, 32-34, 37-42

Limitations of Structural Homology Models

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Drs. Hecht and Park Explain Limitations of Homology Models

158. The effects of these myriad sets of combinations of multiple substitutions within PH20 could not have been predicted by a skilled artisan in the 2011 timeframe using the tools that were available then. For example, while the PH20 protein structure models Dr. Park used provided reliable insights when modeling the change of a single residue at a position where the model was, they cannot provide reliable insights when the modeled sequence incorporates many (*e.g.*, more than ~5) substitutions not found in a naturally occurring protein. That is because (i) if the modeled sequence incorporates multiple changes, it no longer has validity as a naturally occurring sequence, and (ii) the changes significantly diminish the reliability of other positions of the model used to assess the change because they are no longer based on the structural positioning of residues within the template structure used to generate the model. Thus, a skilled artisan would have had to discover which combinations of substitutions to the PH20 protein would result in mutants that do exhibit hyaluronidase activity by making and testing all of them, an impossibly large undertaking.

EX1003 (Hecht), ¶ 158, Pet. 69, 75, Reply, 7, 23

160. Protein structure models generated by SWISS-MODEL and similar tools in the 2011 time frame would have been reliable for evaluating single amino acid substitutions that are in regions of the PH20 model where there is a sufficiently reliable local QMEAN score. This is possible because only one residue is being changed and the remainder of the protein is still based on a reliable experimentally-determined structure (here, HYAL1).

161. In 2011, a second or third substitution could possibly be considered, but modeling multiple substitutions is more challenging and predictions can quickly become unreliable. This is especially true if the mutated residues are close to each other, either in sequence or in space, so that they interact with each other. That is because one needs to model interaction between substituted residues as well as interaction between a substituted residue with constant neighbors. Because this is difficult to visualize, it becomes necessary to model individual combinations through homology modeling, which becomes combinatorial intractable.

162. Consequently, a skilled artisan in 2011 could not have used SWISS-MODEL to reliably predict the effects on the structure of a modified PH20 polypeptide of many substitutions (*e.g.*, 5 or more) in the PH20 sequence, if each position is allowed to vary independently to one of several amino acids. In addition, a skilled artisan could not have used a SWISS-MODEL of PH20 to reliably predict the effects of substitutions within portions of the PH20 sequence where the model is not reliable (*i.e.*, outside of the PH20 sequence between positions 7 and 403).

EX1004 (Park), ¶¶ 160-162, Pet., 11, 14-15; Reply, 9

Homology Models Could Not Accurately Predict Structure from Sequence in 2011-2012

133. The 2024 Chemistry Nobel Price Background paper that Dr. Hecht references in his declaration provides an accurate historical perspective on the capabilities of computational tools to accurately predict a protein's tertiary structure from its amino acid sequence. As that paper shows, those computational tools did not reach predictive accuracy levels comparable to experimental determination of structure until around 2019 (below).[150] While experimental techniques were known by 2011-2012 that could investigate the preservation of secondary structures and generally characterize the folded state of proteins, it would not have been possible for a POSA to use them to assess even a tiny fraction of the sequence space defined by the claim parameters that include modified PH20 polypeptides with 5, 10, 15 or 20 changes.

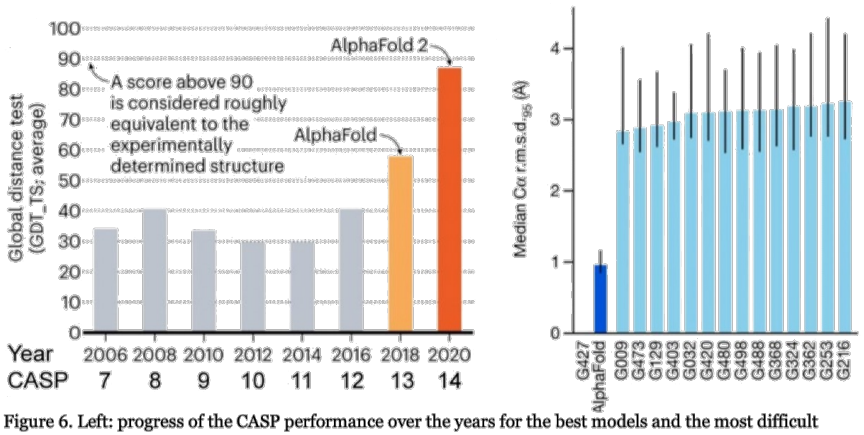


Figure 6. Left: progress of the CASP performance over the years for the best models and the most difficult targets.[38] Right: performance of AlphaFold2 relative to the top 15 entries by other groups in CASP14. Data are the median coordinate error and the 95% confidence interval of the median, estimated from 10 000 bootstrap samples.[41]

EX1133 (Naismith), ¶ 133 (citing EX1027 (Nobel Chemistry Background), 8, Figure 6), Reply, 9

Homology Model Cannot Model C-Terminal Region of PH20 Past Position 403

Additionally, all parties agree that an accurate homology model of PH20's structure, showing the distorted PH20 alpha-beta barrel fold and location of each amino acid in the structure, was available to a POSA. Pet., 11-15, 74, 101-107;

PO Sur-Reply, 5

152. Portions of a structural model become less reliable in regions where there are differences in the target-template alignment.[126] The HYAL1 and PH20 sequences have different lengths: PH20 has 474 residues in its mature sequence, while HYAL1 has only 414. Also, the HYAL1 template structure cannot provide structural information for PH20 amino acids past position 403, because the sequence is no longer homologous after position 421 in HYAL.

EX1004 (Park), ¶ 152; Pet., 75



EX2070 (Petsko), ¶ 131; POR, 12

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SEQ ID NO: 3            moltype = AA  length = 447
FEATURE                 Location/Qualifiers
REGION                  1..447
                        note = Mature PH20 36-482
source                  1..447
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 3
LNFRAPPVIP NVPFLWAWNA PSEFCLGKFD EPLDMSLFSF IGSPRINATG QGVTIFYVDR  60
LGYYPYIDSI TGVTVNGGIP QKISLQDHLD KAKKDITFYM PVDNLGMAVI DWEEWRPTWA  120
RNWKPKDVYK NRSIELVQQQ NVQLSLTEAT EKAKQEFEKA GKDFLVETIK LGKLLRPNHL  180
WGYYLFPDCY NHHYKKPGYN GSCFNVEIKR NDDLSWLWNE STALYPSIYL NTQQSPVAAT  240
LYVRNRVREA IRVSKIPDAK SPLPVFAYTR IVFTDQVLKF LSQDELVYTF GETVALGASG  300
IVIWGTLSIM RSMKSCLLLD NYMETILNPY IINVTLAAKM CSQVLCQEQG VCIRKNWNSS  360
DYLHLNPDNF AIQLEKGGKF TVRGKPTLED LEQFSEKFYC SCYSTLSCKE KADVKDTDAV  420
DVCIADGVCI DAFLKPPMET EEPQIFY                                      447
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EX1026 ('731 App.), SEQ ID NO:3

Prior Art PH20 Model Did Not Accurately Describe PH20 Structure

Additionally, all parties agree that an accurate homology model of PH20's structure, showing the distorted PH20 alpha-beta barrel fold and location of each amino acid in the structure, was available to a POSA. Pet., 11-15, 74, 101-107;

PO Sur-Reply, 5

The HyalEGF-like fold does not resemble the Hyal-1 C-terminal domain fold predicted by ab initio approaches (*33*). The close interaction between the two domains is also very different from the earlier prediction that proposed a flexible linker and lack of interactions between the two domains. Moreover, the modeling of the five human hyaluronidases C-terminal domains yielded a different fold in each case, whereas we expect that these domains adopt the HyalEGF-like fold because the amino acid sequences contain the characteristic disulfide bond pattern.

EX006 (Chao), 6913; Pet., 13, 89

7.1. Description of the Model Structures

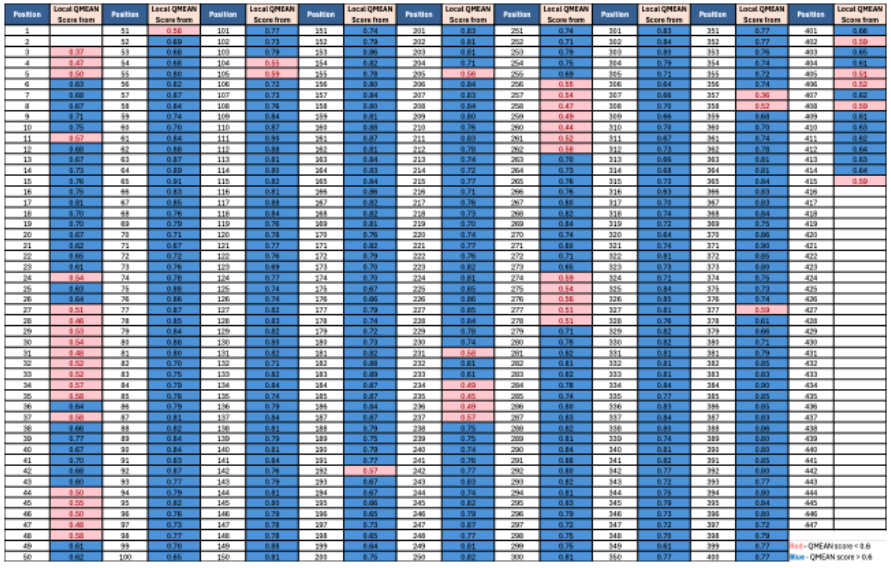
For all five human Hyal structure models, the major catalytic domain (Figures 4 and 5) closely resembles the $(\beta/\alpha)_8$ TIM barrel of the parent BVHyal structure. The number of sheets constituting the central part of the TIM barrel varies, however, but is similar to those of various forms of the BVHyal structures. The wider end of the TIM barrel assumes a conformation that creates a large and elongated cleft. Its sides are built primarily from aa residues of loops formed between sheets and helices of the TIM barrel. Based on similarity to BVHyal[11] and mutation analysis of HPH-20,[89] this region is responsible for binding the HA substrate and for catalysis, and it is large enough to bind at least an 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.

EX008 (Stern), 829; Reply, 13-16

Regions of PH20 Have Insufficient Quality in the PH20 Homology Model

Additionally, all parties agree that an accurate homology model of PH20's structure, showing the distorted PH20 alpha-beta barrel fold and location of each amino acid in the structure, was available to a POSA. Pet., 11-15, 74, 101-107;

PO Sur-Reply, 5



EX1133 (Naismith), App'x B; Reply, 33.

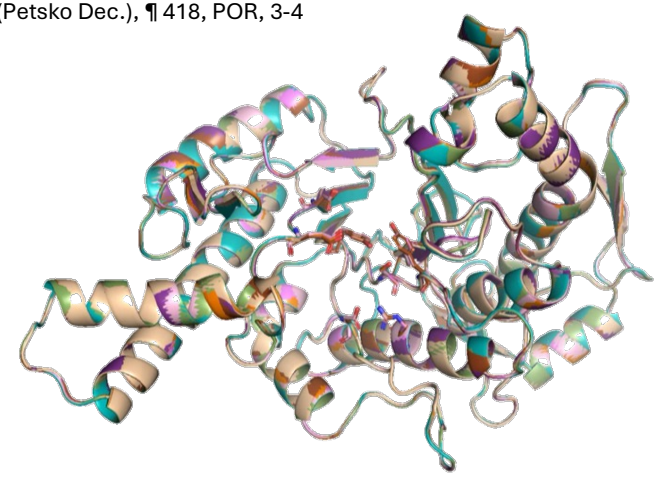
313. A POSA can classify residues as buried or surface residues using information in a PH20 homology model file ("pdb" file) that measures the solvent-accessible surface area ("SASA") for each modeled residue. For PH20, that information is only available for positions 4-403 because that is the portion of the PH20 protein that is supported by the HYAL1 template structure. A POSA would not rely on portions of a PH20 homology model that fall outside of the template structure used to generate it, or for which in insufficient quality in that part of the model exists. A POSA thus would not have included residues 1-3 and 404-447 in the set of positions Dr. Petsko labeled "generally tolerant" because there is no structural context available for those positions. Likewise, the POSA would have considered positions 3–5, 27–35, 44–48, 104–105, 234–237, 256–262, 274–278, 357–358, 405–406 to have unreliable local QMEAN values.

EX1133 (Naismith), ¶ 313; Reply, 33.

Homology Models Do Not Identify Structural Differences of Functionally Distinct Proteins

418. Finally, in the figures below, the human D320K, chimpanzee D320K, gibbon D320K, orangutan D320K, N47A/N131A/D320K, N47A//N219A/D320K, N131A/N219A/D320K and N47A/N131A/N219A/D320K variant models are shown on top of each other. The figures shows that the key active region residues I described above adopt essentially the same position in all variant models, illustrating how the variants adopt a common structure in the active site:

EX2070 (Petsko Dec.), ¶ 418, POR, 3-4



184. When modeled on the human HYAL-1 structure as the template, the overlaid structures have only subtle visual differences (below).[201] This is a similar overlay of the homology models Dr. Petsko used in his declaration.[202] Using Dr. Petsko's high level perspective, all appear have the same "alpha-beta barrel" motif. Despite this similarity, the five proteins exhibit subtly different substrate preferences and activity.

EX1133 (Naismith), ¶ 184, Reply, 22

