UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MERCK SHARP & DOHME LLC,
Petitioner

v.

HALOZYME, INC.,
Patent Owner

Case PGR2025-00039
U.S. Patent No. 12,104,185

DECLARATION OF MELANIE A. SIMPSON, PH.D. IN SUPPORT OF PATENT OWNER'S RESPONSE

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Halozyme EX2068
Merck v. Halozyme
PGR2025-00039

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I, Melanie A. Simpson, Ph.D., hereby declare as follows.

I. INTRODUCTION

1. I am over the age of 18 and competent to make this declaration.

2. I have been retained as an expert witness on behalf of Patent Owner Halozyme, Inc. ("Patent Owner") for the above-captioned post-grant review proceeding ("PGR"). I am being compensated for my time in connection with this PGR at my standard consulting rate, which is $400 per hour.

3. I understand that this Declaration accompanies a Patent Owner's Response filed in this PGR involving U.S. Patent No. 12,104,185 ("the '185 patent") (EX1001), which resulted from U.S. Patent Application No. 18/599,428 ("the '428 application"), filed on March 8, 2024.

4. I understand that the '185 patent is related to the following U.S. Patent Applications:

- U.S. Patent Application No. 17/327,568 ("the '568 application"), filed on May 21, 2021;
- U.S. Patent Application No. 16/912,590 ("the '590 application"), filed on June 25, 2020; and
- U.S. Patent Application No. 16/824,572 ("the '572 application"), filed on March 19, 2020;
- U.S. Patent Application No. 15/226,489 ("the '489 application"),

filed on August 2, 2016; and

- U.S. Patent Application No. 13/694,731 ("the '731 application"), filed on December 28, 2012. Dr. Hecht provided the '731 application as EX1026.

5. I understand that the '185 patent is also related to the following provisional applications:

- Provisional Application No. 61/796,208 ("the '208 provisional application"), filed on November 1, 2012; and
- Provisional Application No. 61/631,313 ("the '313 provisional application"), filed on December 30, 2011.

6. In addition to reviewing the '185 patent and the '731 application, I have reviewed a redline comparison of the specifications of the '185 patent and the '731 application (EX1045). The '185 patent has substantively the same specification[1] as the '731 application filed on December 28, 2012. *Compare* EX1001, *with* EX1026, 14-370. Dr. Hecht agrees that the specifications of the '185 patent and the '731 application are substantively the same and share a

[1] I understand that the specification of the '185 patent includes its Figures and a narrative discussion, EX1001, pp. 15-184, including any documents that are "incorporated by reference," as well as the patent's sequence listing, EX2006.

"common disclosure." EX1003, ¶18. Like Dr. Hecht, for convenience, I provide citations to the disclosure in the '185 patent and at times refer to the specification that is substantively shared by the '185 patent and the '731 application as the "common disclosure."[2] Below, I often refer to December 28, 2012, the filing date of the '731 application, as I have been asked to consider the '731 application in my analysis of written description and enablement.

7. In preparing this Declaration, I reviewed: (i) the '185 patent and its prosecution history[3] and (ii) each of the documents cited in this declaration, in light of general knowledge in the art in the following timeframes referenced: In assessing written description and enablement, I refer to December 28, 2012—the

[2] Because the specification of the '185 patent and the specification of the '731 application share a common disclosure that is substantively the same, I may refer to them interchangeably.

[3] I understand a patent's prosecution history contains the back-and-forth communications between the patent applicant and the patent examiner, starting with the applicant submitting a patent application and culminating in issuance of the patent.

filing date of the '731 application that is related to the '185 patent.[4] In assessing obviousness in response to Dr. Hecht's and Dr. Park's declarations, I refer to the same timeframe Dr. Hecht considered: before December 29, 2011. EX1003, ¶11.

8. In formulating my opinions, I relied upon my experience, education, and knowledge in the relevant art. In formulating my opinions, I also considered the viewpoint of a person of ordinary skill in the art ("POSA"), as defined below in Section V, as of the timeframes discussed herein (e.g., paragraph 7), in light of general knowledge in the art.

II. BACKGROUND AND QUALIFICATIONS

9. I am the Head of the Department of Molecular and Structural Biochemistry at North Carolina State University; I have been Department Head

[4] I understand that in assessing the '731 application for written description and enablement, the application must be assessed as of the '731 application's filing date (December 28, 2012). I understand that the '185 patent is also related to two earlier-dated patent applications: the '208 provisional application filed on November 1, 2012, and the '313 provisional application filed on December 30, 2011. However, Drs. Hecht and Park did not assess the earlier-dated '208 and '313 provisional applications for written description or enablement, or even include them on their Exhibit Lists. EX1003, 152-154; EX1004, Appendix A.

since 2017, and I have been a professor in the Department of Molecular and Structural Biochemistry since 2017. I am also the Director of Research of the Integrative Sciences Initiative at North Carolina State University, Raleigh, NC, where I have been Director since 2023. And I am the President of the International Society for Hyaluronan Sciences ("ISHAS"), where I have been an invited member since 2008.

10. I hold a Ph.D. in Biochemistry, Molecular Biology, and Biophysics and a B.S. *summa cum laude* in Biochemistry from the University of Minnesota.

11. I have extensive experience in the fields of biochemistry (including protein biochemistry and enzymology), cellular and molecular biology (including structural biology), biophysics, human disease and cancer research, and glycobiology—including extensive practical experience researching hyaluronidases, which have been a focus of my research since 1998.

12. Additionally, I am a member of multiple professional organizations, including: the Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC (since 2017), the American Association for Cancer Research (since 2000), the American Society for Matrix Biology (since 2004), the American Society for Biochemistry and Molecular Biology (since 2007), the Women in Cancer Research (since 2007), and the American Association for the Advancement of Science (since 2015).

13. My *curriculum vitae* is submitted herewith as EX2069.

14. In 1992, I completed my B.S. *summa cum laude* in Biochemistry at the University of Minnesota, during which I was awarded the Department of Biochemistry Graduate Academic Award. And in 1997, I completed my Ph.D. in Biochemistry, Molecular Biology, and Biophysics from the University of Minnesota (studying the physiological and biophysical properties governing the cellular trafficking of fat between the bloodstream and the fat storage depots in adipocytes, *i.e.*, "fat cells," in the context of obesity-induced diabetes in cell culture and mouse models, with complementary mechanistic studies employing protein chemistry and protein structure determination), during which I was awarded the National Institutes of Health Predoctoral Fellowship (in 1994–1996) and Department of Biochemistry Travel Award (in 1997) and served as the President of Biochemistry Graduate Students (in 1995–1996). I was a postdoctoral fellow in the Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN from 1998–2002 (studying the interplay between hyaluronan synthesis, hyaluronan synthase enzyme function, and cell surface hyaluronan receptors in promoting cancer initiation, progression, and bone metastasis of prostate cancer, using cell culture, mouse models, and human specimens), during which I was awarded the National Institutes of Health NRSA Postdoctoral Fellowship (in 2000–2002).

15. In 2002, I went to the University of Nebraska, where I held multiple

roles, including: Assistant Professor of Biochemistry (2002–2008); Tenured Associate Professor of Biochemistry (2008–2011); Susan J. Rosowski Associate Professor of Biochemistry (2011–2013); Rosowski Professor of Biochemistry (2013–2016); Adjunct Faculty at the Department of Oral Biology, College of Dentistry (2002–2017); Member, Fred and Pamela Buffett Cancer Center and University of Nebraska Medical Center (2002–2017); Founding Director of the NIH-funded Molecular Mechanisms of Disease Predoctoral Program (2013–2017) and Complex Biosystems PhD Program (2014–2017); Associate Director, Center for Biotechnology (2014–2017); Willa Cather Professor of Biochemistry (2016–2017); and Adjunct Professor of Biochemistry (2017–2021). I was also a scientific consultant at LI-COR Biosciences (2005–2013).

16. While I was at the University of Nebraska, I received multiple honors and awards including: University of Nebraska Junior Faculty Award for Excellence in Research (2006); Nominee, CASNR Week Outstanding Teaching Award (2009); Beta Theta Pi Fraternity Outstanding Educator Award (2009); Susan J. Rosowski Professorship (2011–2016); CASNR Parents Award (2012); McNair Program Spotlight Mentor (2012); McNair Faculty Appreciation Award (2013); Mortar Board People Who Inspire Award (2013); Elected Member of the Board of Trustees of ISHAS (2015–2020); Willa Cather Professorship (2016–2021); and Dean's Award for Excellence in Graduate Education (2017).

17. In 2017, I went to North Carolina State University, Raleigh, NC where I have held my current roles since 2017 (Head and Professor) and 2023 (Director of Research—as well as additional roles, including: LEAD 21 Class 14 Program Alum (2018–2019); NSF Alliances for Graduate Education and the Professoriate (AGEP-NC), Fellow (2018–2021); Treasurer and President-elect of ISHAS (2020–2023); Food Systems Leadership Institute Cohort 17 Alum (2021–2023); Associate Director of Chemistry of Life Division of the Comparative Medicine Institute at North Carolina State University (2021–2024); NSF Alliances for Graduate Education and the Professoriate (AGEP-NC), co-PI (2022–present); and Gordon Research Conference on Proteoglycans Vice Chair/Chair-Elect (2024–present).

18. Since 1998, a primary focus of my research has been researching and characterizing hyaluronidases. My research has led to publications, including: 45 peer-reviewed research publications; 15 book chapters, review articles, and journal special editions; and one U.S. patent—over 25 of which specifically relate to hyaluronidases and/or their substrate, hyaluronan. I have also received over 30 grants, over 10 of which specifically related to hyaluronidases and/or their substrate, hyaluronan. And I have participated in over 60 invited talks and panels, over 25 of which specifically related to hyaluronidases and/or their substrate, hyaluronan. Additionally, I have advised 18 graduate students, eight of whom specifically

studied hyaluronidases and/or their substrate, hyaluronan, for their dissertation.[5]

19. I am also an associate editor for *Proteoglycan Research* and an editorial board member for *Matrix Biology*. And I frequently peer review publications and have been an *ad hoc* reviewer for many publications, including: *Analytical Biochemistry*, *BMC-Cancer*, *British Journal of Pharmacology*, *Cancer Research*, *Cell Biochemistry and Function*, *Cell Proliferation*, *Clinical and Experimental Metastasis*, *Current Urology*, *European Journal of Biochemistry*, *Experimental Cell Research*, *FASEB Journal*, *FEBS Letters*, *Glycobiology*, *In Vitro Cellular and Developmental Biology*, *Journal of Biological Chemistry*, *Journal of Cellular Physiology*, *Molecular Cancer*, *Oncogene*, *Pathology Research*, *PLoS One*, *Tissue Engineering*, and *Stryer's Biochemistry*. The publications I have reviewed often related to hyaluronidase biology; hyaluronan; and metabolic enzymes, including UDP-Glucose Dehydrogenase, which produces UDP-Glucuronate, a precursor for hyaluronan synthases.

20. In addition to my educational training and my professional and research experience, I have kept abreast of the fields of biochemistry (including protein biochemistry and enzymology), cellular and molecular biology (including structural

[5] I have also advised five postdoctoral fellows and over 35 undergraduate students, and I have served on 37 graduate and 19 undergraduate honors thesis committees.

biology), biophysics, and glycobiology by reading scientific literature, conferring with colleagues in the field, and attending and presenting lectures at scientific conferences. I have also taught undergraduate and graduate courses on Macromolecular Structure and Function, Biochemistry, Metabolic Function and Dysfunction, Nutraceuticals and Functional Foods, Creativity in the Arts and Sciences, Biosystems Research, Scientific Analysis and Technical Writing, Biomolecules and Metabolism, and Recombinant DNA laboratory methods.

III. SUMMARY OF OPINIONS

21. Below I summarize the topics on which I have been asked to opine and provide a brief overview of my conclusions, which are set out in detail in the sections that follow.

22. I have been asked to consider how a POSA would have understood the claimed invention of claims 1-4 and 7-11, and in particular whether a POSA would have understood the claims to require hyaluronidase activity or a particular level of hyaluronidase activity.

23. I have also been asked to consider whether a POSA would have found the specification of the ’185 patent provides adequate written description for the subject matter of claims 1-4 and 7-11 of the ’185 patent, and whether it would have required undue experimentation for a POSA to practice the claimed invention, in light of the teachings in the common disclosure and general knowledge in the field.

24. Finally, I have been asked to consider whether claims 1-4 and 7-11 of the ’185 patent would have been obvious over Chao (EX1006) and the ’429 patent (EX1005).

25. **Claim construction.**

26. I understand that for this patent, the Patentee does not dispute that the modified PH20 polypeptides covered by the claims have at least some hyaluronidase activity.

27. I have been asked to opine as to whether the POSA would have understood that the claims require the claimed modified PH20 polypeptides to have 40% hyaluronidase enzymatic activity. I conclude that they do not.

28. The specification defines “modified PH20 polypeptide” as “a PH20 polypeptide that contains at least one amino acid modification, such as at least one amino acid replacement as described herein, in its sequence of amino acids compared to a reference unmodified PH20 polypeptide.” EX1001, 48:41-46.

29. Additional language in the paragraph in which this definition appears describes some “modified PH20 polypeptides” as exhibiting hyaluronidase activity even with up to 150 modifications. And similar examples in the specification describe “modified PH20 polypeptides” as inactive.

30. The specification does not define the term “modified PH20 polypeptide” to require at least 40% hyaluronidase activity. The claims also do not

include any such language.

31. **Written description.** A POSA would have found that the specification provides adequate written description support demonstrating that the inventors possessed the full scope of the invention.

32. The claimed methods are directed to C-terminally truncated PH20 variants that range in length from 430 to 465 amino acids and that have a modification at position 320: aspartate (D) is changed to either lysine (K), arginine (R), histidine (H) or serine (S). The claimed methods also are directed to 95% identical sub-variants of those PH20 variants.

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 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.

34. POSAs would have understood that the ’185 patent describes both a representative number of species of this narrow genus as well as the common structural features of the narrow set of claimed PH20 variants and sub-variants. The patent disclosure would have allowed a POSA to visualize or recognize the claimed set of modified PH20s.

35. The specification would have conveyed to a POSA that the inventors

were in possession of the full scope of the claimed PH20 polypeptides.

36. A POSA would have understood that the common disclosure provides unprecedented data correlating PH20's primary sequence and the resulting secondary and tertiary structures with its enzymatic activity or loss of activity across the entire sequence of the PH20. The disclosure reports the effect of substitutions at every position in the mature, soluble C-terminal truncated PH20 of SEQ ID NO: 3, thereby mapping how structure relates to function.

37. The patent's teachings and data, along with the knowledge available to a POSA, identify many common structures, including: (1) the common alpha-beta barrel tertiary structure shared by active PH20 variants and sub-variants, (2) a modification at position 320, (3) a common active site shared by active modified PH20 polypeptides, including active site residues that are involved in catalysis or substrate binding, (4) disulfide bridges essential to PH20s tertiary structure, and (5) other areas of the PH20 structure intolerant to change, including previously-unknown substitutions in and outside the active site that, when made, render PH20 inactive.

38. The common disclosure identifies four representative PH20 variants (sequences with histidine (H), lysine (K), arginine (R), or serine (S) at position 320) that a POSA would have understood to show the inventors had possession of the full scope of the claimed 95% identical sub-variants.

39. The patent also provides extensive disclosure of the types of changes that will inactivate a PH20, including substitutions at catalytic site residues essential for hydrolysis, as well as substitutions at positions that destabilize disulfide bridges or other structural elements necessary for maintaining the alpha-beta barrel fold.

40. Although the inactive modified PH20 variants described in the examples do not meet the claims (because they do not have the claimed 320 modification), they still provide valuable information to a POSA because they teach the POSA that additional variation in the structure will be tolerated, where it will be tolerated, and where it will not.

41. **Enablement**. I understand that enablement requires the patent to provide enough information for a POSA to make and use the full scope of the claimed invention without having to engage in undue experimentation.

42. The claimed methods are directed to C-terminally truncated PH20 variants that range in length from 430 to 465 amino acids and that have a modification at position 320: aspartate (D) is changed to either lysine (K), arginine (R), histidine (H) or serine (S). The claimed methods also are directed to 95% identical sub-variants of those PH20 variants.

43. As I understand it, there is no dispute that a POSA could make and use the claimed PH20 ***variants***—meaning the set of C-terminal truncated PH20s ranging in length from 430 to 465 amino acids (SEQ ID Nos: 3 and 32-66) with a

D320K, D320R, D320H or D320S substitution. The POSA could readily generate, for example, SEQ ID NO: 3 with the D320R substitution through routine procedures according to the patent teachings. For example, the POSA would have understood from the teachings of the patent how to use SEQ ID NO: 3 with the D320K mutation as a spreading factor.

44. With respect to the 95% identical sub-variants, a POSA would have readily been able to make and use the full scope of 95% sub-variants using the disclosure of the patent in conjunction with the knowledge in the art and using routine techniques.

45. For example, the patent provides a detailed structure-function correlation across the PH20 sequence, showing which positions generally tolerate substitution and which do not. This information allows a POSA to predictably determine which sub-variants will retain activity and to make and use those sub-variants.

46. **Obviousness.** In view of the state of the art before December 29, 2011, it is my opinion that a POSA would not have considered the claims obvious over Chao (EX1006) and the ’429 patent (EX1005). Chao and the ’429 patent do not teach every limitation of the claims (such as a modification at the claimed position 320), and a POSA would not have had any motivation to combine Chao and the ’429 patent to make the claimed modified PH20 polypeptides that include a D320K

modification.

IV. MATERIALS CONSIDERED

47. The information that I have relied upon in forming my opinions for this declaration, in addition to my professional and academic experience, is set forth throughout the report and in the Table of Exhibits.

V. PERSON OF ORDINARY SKILL IN THE ART

48. As an expert, I have offered opinions throughout this declaration from the perspective of a person of ordinary skill in the art ("POSA").

49. I understand that a POSA is a hypothetical person who is presumed to be aware of all pertinent art, who thinks along conventional wisdom in the art, and is a person of ordinary creativity.

50. I have been asked to apply Merck's definition of a POSA for purposes of my analysis provided here:

> A person of ordinary skill in the art would have had an undergraduate degree, a Ph.D., and post-doctoral experience in scientific fields relevant to study of protein structure and function (*e.g.*, chemistry, biochemistry, biology, biophysics). From training and experience, the person would have been familiar with factors influencing protein structure, folding and activity, production of modified proteins using recombinant DNA techniques, and use of biological assays to characterize protein function, as well [as] with techniques used to analyze protein structure (*i.e.*, sequence searching and alignments, protein modeling software, etc.).

EX1003, ¶13.

51. Further to Merck's definition, I note that a POSA would have been able to work as part of a multidisciplinary team.

52. Likewise, to further support or expand upon my discussion herein, I refer at times to the Declaration of my multidisciplinary team member: Gregory A. Petsko, Ph.D., who provides protein structure analysis (EX2070: Petsko Decl.).

53. Considering my ample practical experience with hyaluronidases, as described in my background and qualifications, I am more experienced than a POSA under Merck's definition and was so before 2011.

54. Throughout my declaration, my opinions are given from the perspective of a POSA as of December 28, 2012, unless I have specified a different date.

VI. LEGAL BASIS FOR MY ANALYSIS

55. My understanding regarding the legal principles relating to the definition of a POSA is provided in Section V above. In formulating my further opinions set forth in this Declaration, I also applied the following legal principles:

A. Claim Construction

56. I understand that in a post-grant review ("PGR"), patent claim terms are given their ordinary and customary meaning as understood by a POSA at the time of the invention, in view of the patent's claims, specification, and prosecution

history—unless the patent explicitly defines the claim term, in which case, I understand that the patent's definition controls.

57. I understand that the phrases "as used herein" and "refers to" convey an intent to define a term.

58. I also understand that, in claim construction, the intrinsic evidence (consisting of the claims, specification, and prosecution history) is accorded greater weight than extrinsic evidence (such as dictionaries, journal articles, or other external sources).

59. Regarding the claims, I understand that no two claims in the same patent should be interpreted to be identical in scope, *i.e.*, I understand that every claim is presumed to be distinct. I also understand that the scope of a dependent claim is presumed to be narrower than the scope of the claim(s) from which it depends.

60. I understand that claim 1 is the sole independent claim in the '185 patent and that claims 2-4 and 7-11 are dependent claims (depending directly or indirectly from claim 1). I understand that a dependent claim contains all limitations (*i.e.*, elements) of the claim(s) from which it depends. Thus, I understand that claims 2-4 and 7-11 contain all limitations of claim 1, as well as those of any additional claims from which they depend. The copy of the '185 patent provided as Exhibit 1001 also lists claims 5-6, but I understand that Patent

Owner, Halozyme, Inc., statutorily disclaimed claims 5-6 on June 13, 2025. EX2003. Thus, I understand that the disclaimed claims 5-6 are not currently part of this post-grant review proceeding.[6]

B. Written Description

61. I understand that the written description provided by a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.[7] I understand this analysis is assessed as of the patent's filing date or as of the filing date of a related earlier-filed application, if the patent owner would like to receive the benefit of that earlier date. Here, I have been asked to assess the '185 patent's written description as of the filing date of the earlier '731 application: December 28, 2012. And I understand that this involves an objective inquiry into the disclosure provided in the specification from the perspective of a POSA as of December 28, 2012.

62. I understand that possession may be shown in a variety of ways

[6] I understand that a statutory disclaimer means that, effectively, the only claims at issue in the PGR are claims 1-4 and 7-11.

[7] I understand "possession" does not mean physical possession and does not require making or testing the invention.

including a description of an actual reduction to practice or a description of distinguishing or identifying characteristics, sufficient to show that the inventor was in possession of the claimed invention.

63. I further understand that this written description requirement must be assessed in view of the state of the knowledge of a POSA. In addition, I understand that a patent specification is written for a POSA and that such a hypothetical person presumably has all of the knowledge of the state of the art as of the relevant date, in addition to the knowledge provided by the patent specification itself.

64. I understand that there is sufficient (*i.e.*, adequate) written description support when the specification's disclosure allows a POSA to visualize or recognize the full scope of the claimed subject matter and that the claimed subject matter need not be provided verbatim in the specification. I also understand that a POSA comes to the patent with knowledge in the art; therefore, it is unnecessary to spell out every detail of the invention in the specification for a POSA to conclude that there is written description support.

65. I understand that assessing whether there is written description

support for a genus[8] claim involves consideration of a number of factors, including the (i) nature and scope of the claims; (ii) existing knowledge in the particular field and extent and content of the prior art; (iii) maturity of the science or technology and scientific and technologic knowledge already in existence; (iv) predictability of the aspect at issue; and (v) scope of the invention at issue.

66. I also understand that some genus claims are referred to as "functional" genus claims because they describe the claimed invention in terms of what it does—rather than defining the claimed invention by its structural components. By contrast, I understand that "structural" genus claims define the claimed invention by its structural components.

67. I further understand that a sufficient description of a genus may be provided by the disclosure of either (i) a representative number of species falling within the scope of the genus or (ii) structural features common to the members of the genus so that a POSA can visualize or recognize the members of the genus. Additionally, I understand 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

[8] I understand that a "genus" is a group that covers multiple "species," and that "species" are sometimes referred to as "embodiments."

claimed genus from other materials.

68. I understand that satisfactory disclosure of a "representative number" of species depends on whether a POSA would recognize that the inventor was in possession of the necessary common attributes or features possessed by the members of the genus in view of the disclosed species.

69. I also understand that written description can be established by disclosure of a reasonable structure-function correlation. I understand such correlations may be established by the inventor, as described in the specification, or they may be known in the art at the time of the filing date.

70. Finally, I understand that 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.

C. Enablement

71. I understand that a patent application specification that provides an enabling disclosure must allow a POSA to practice the full scope of the claimed invention without undue experimentation as of that application's filing date. Here, I have been asked to consider whether the '731 application provides an enabling disclosure for the challenged patent claims of the '185 patent, which I understand is a consideration in determining whether the '185 patent is entitled to be treated as

though it were effectively filed as of the '731 application's December 28, 2012 filing date.

72. I understand that the following factors can be used to determine whether undue experimentation would have been required to make and use the full scope of the claimed genus (the so-called *Wands* factors): (1) the breadth of the claims; (2) the nature of the invention; (3) the state of the prior art; (4) the level of one of ordinary skill; (5) the level of predictability in the art; (6) the amount of direction provided by the inventor; (7) the existence of working examples; and (8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

73. I understand that the determination that "undue experimentation" would have been needed to make and use the claimed invention is a conclusion reached by weighing the above-noted factors.

74. I understand that a patent that discloses only a research plan or a starting point for further iterative research, without guidance or predictions as to which embodiments may be operative, such that a POSA would need to make and screen all candidate embodiments for their function in order to practice the claims, constitutes excessive experimentation that may be undue.

75. I understand that undue experimentation is a matter of degree and that even a considerable amount of experimentation is permissible as long as it is not

undue. I understand, for example, that a considerable amount of experimentation is not undue if the experimentation is merely routine, or the specification provides a reasonable amount of guidance regarding the direction of experimentation. The experimentation must be reasonable.

76. Furthermore, I understand that evidence of a pharmaceutical property in any standard experimental animal is sufficient to establish utility (*i.e.*, usefulness).

D. Obviousness

77. I understand that an obviousness analysis involves comparing a claim with the prior art to determine whether the claimed subject matter would have been obvious to a POSA in view of the asserted prior art and the general knowledge in the background art. I understand that obviousness must be assessed from the viewpoint of a POSA at the time of the invention. In assessing obviousness, I have been asked to consider the timeframe before December 29, 2011, the same timeframe Dr. Hecht considered. EX1003, ¶11.

78. I further understand that to establish obviousness, a party seeking to establish obviousness must perform the following factual inquiries: (1) determining the scope and content of the prior art; (2) ascertaining the differences between the claimed invention and the prior art; and (3) resolving the level of skill in the art. I understand that, in determining the scope and content of the prior art and

ascertaining the differences between the claimed invention and the prior art, a patent challenger may (a) specify where each element of the claim is found in the prior art or (b) explain why a POSA exercising ordinary creativity would bridge any gaps (*i.e.*, missing elements) between the prior art and the claimed invention to produce the claimed invention.

79. I understand that one way of showing obviousness is by establishing that a POSA would have had both (i) a motivation to modify or combine the teachings of the prior art to achieve the claimed invention and (ii) a reasonable expectation of success in doing so. I understand that the motivation to combine prior-art references can come from a variety of sources, not just the prior art itself or the specific problem the patentee was trying to solve. And I understand that the references themselves need not provide the motivation needed to arrive at the claimed invention. Moreover, I understand that evidence showing that lack of motivation to combine may support a conclusion that the claimed invention was nonobvious.

80. I understand that a "reasonable expectation of success" is assessed in view of the prior art and general knowledge in the art from the viewpoint of a POSA before the relevant date (Dr. Hecht considered the timeframe before December 29, 2011, and I have been asked to consider the same timeframe). Furthermore, I understand that the expectation of success need only be reasonable,

not absolute. I understand that this expectation, if not explicitly provided by the prior art, may be implicitly provided by the prior art. Moreover, I understand that evidence showing that there was no reasonable expectation of success may support a conclusion that the claimed invention was nonobvious.

81. I also understand that, before reaching a conclusion that the claimed invention would have been obvious, one must consider any objective evidence of non-obviousness if it is available. The objective evidence of non-obviousness can include evidence of commercial success attributable to the claimed invention, evidence of industry praise for the claimed invention, evidence of a long-felt need that was solved by the claimed subject matter, evidence that others copied the claimed subject matter, or evidence that the claimed subject matter achieved an unexpected, superior result relative to the closest prior art. I understand that such evidence must have a nexus, or causal relationship, to the claimed subject matter beyond what was available in the prior art, and must be commensurate in scope with the patent claim(s) at issue.

VII. TECHNICAL BACKGROUND

82. I summarize the state of the art as of December 28, 2012 regarding certain protein engineering concepts, the structure and function of hyaluronidases, their mechanism of action, and the amino acids known to be important for activity below.

A. Protein Structure

1. Amino Acids

83. Proteins comprise amino acids, which are small carbon chains containing a central carbon atom (C_α) chemically bonded (*i.e.*, chemically connected) to a hydrogen atom (H), an amino group (NH_2), a carboxyl group (COOH), and a variable side chain (R), as depicted below. EX1014, 4.

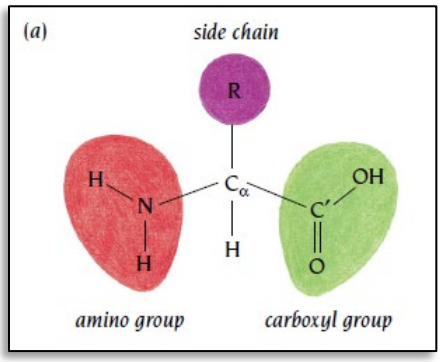
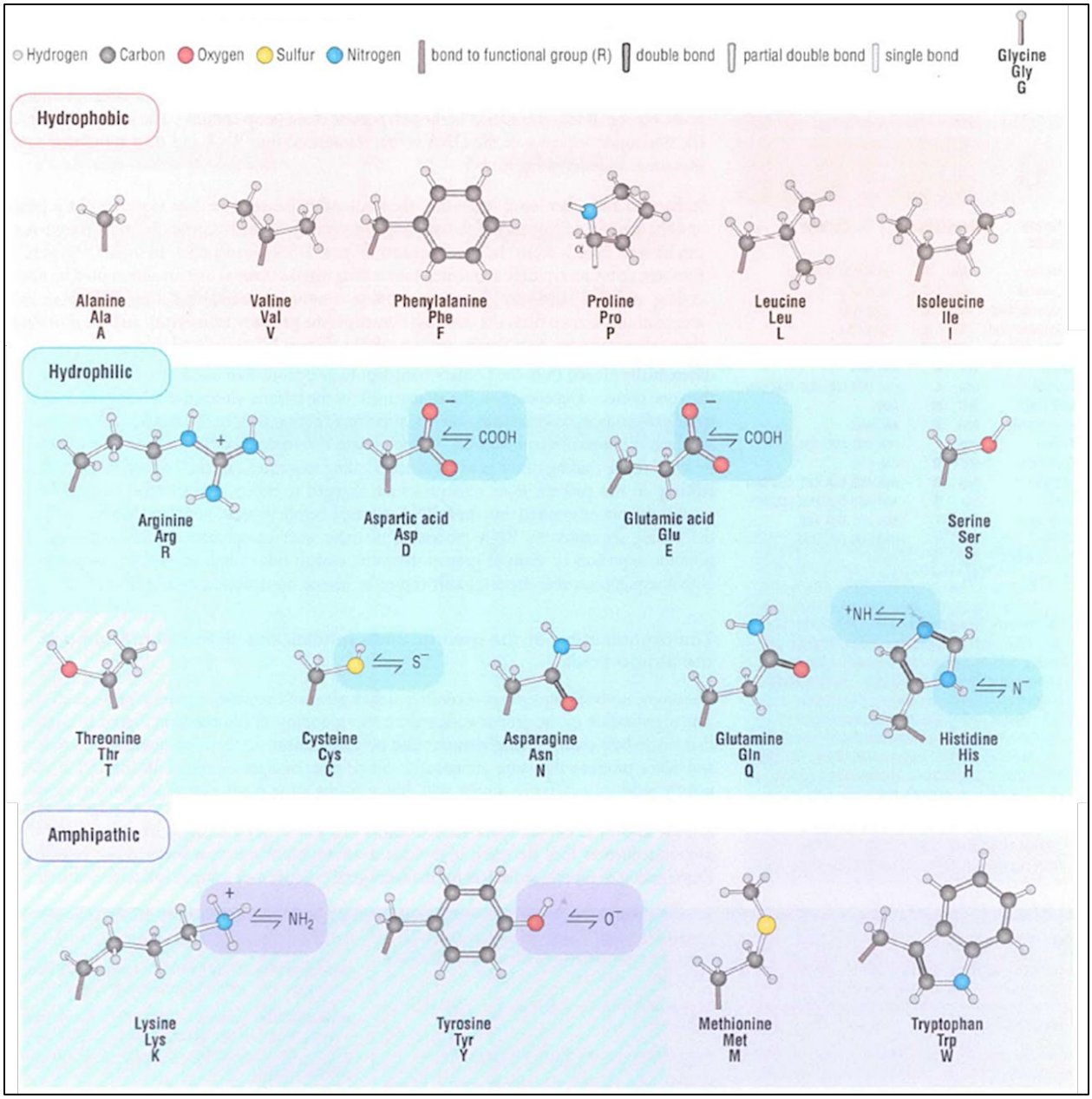


Figure 1.2(a) of EX1014, 4.

84. The genetic code specifies 20 amino acids, each containing a different side chain (R) and identified by a distinct three- or one-letter code: alanine (Ala, A), arginine (Arg, R), asparagine (Asn, N), aspartate (Asp, D), cysteine (Cys, C), glutamine (Gln, Q), glutamate (Glu, E), glycine (Gly, G), histidine (His, H), isoleucine (Ile, I), leucine (Leu, L), lysine (Lys, K), methionine (Met, M), phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), tryptophan (Trp, W), tyrosine (Tyr, Y), and valine (Val, V). EX1014, 4-7.

85. A chart showing the side chains of each amino acid is provided below:



EX2164 (Petsko), 5.

2. Primary Structure

86. The amino acid sequence of a protein's polypeptide chain is referred to as its "primary structure." EX1014, 3. "Primary structure is the amino acid sequence, or, in other words, the arrangement of amino acids along a linear

polypeptide chain." *Id.* at 29. The amino acids in the polypeptide chain are sometimes referred to as "residues."

87. The amino acids of a protein's polypeptide chain are connected via peptide bonds between the carboxyl carbon atom (C') and amino nitrogen atom (N) of adjacent amino acids. EX1014, 4.

88. A protein's amino acid sequence "determines how the protein folds into higher-level structures." EX2164 (Petsko), 3.

3. Secondary Structure

89. Regions of an amino acid sequence (*i.e.*, primary structure) fold to form local, regular secondary structures: alpha (α) helices and beta (β) sheets. EX1014, 3. In proteins, alpha helices form when a region of the chain of amino acids adopts a coil-like shape. EX1014, 15. A depiction of an alpha helix is presented below:

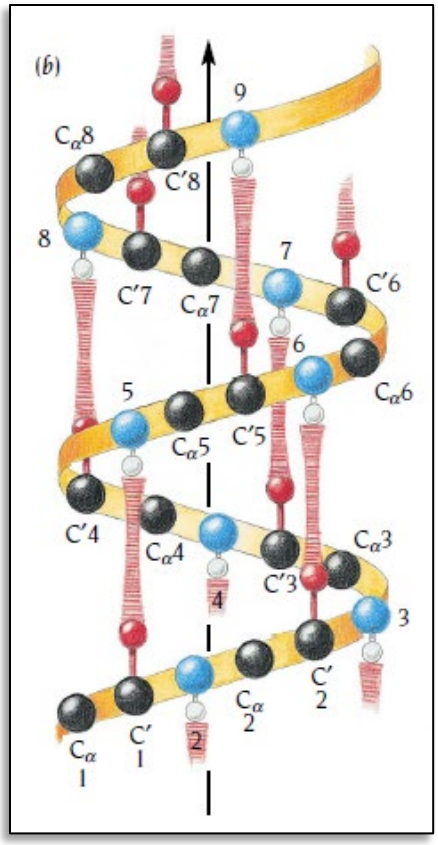


Figure A. Figure 2.2(b) of EX1014, 15. Schematic illustration of an alpha helix. Approximate positions for main-chain atoms and hydrogen bonds are presented. The arrow denotes the direction from the N-terminus to the C-terminus. Carbon atoms are depicted in black; oxygen atoms are depicted in red; and nitrogen atoms are depicted in blue.

90. Beta sheets are structured like pleated sheets formed by hydrogen bonds between beta strands. EX1014, 19. Beta strands refer to regions of approximately five to ten residues within a polypeptide chain; in particular, beta sheets are formed by hydrogen bonds between the carbonyl oxygen atoms and the amide hydrogen atoms between adjacent beta strands. *Id.*

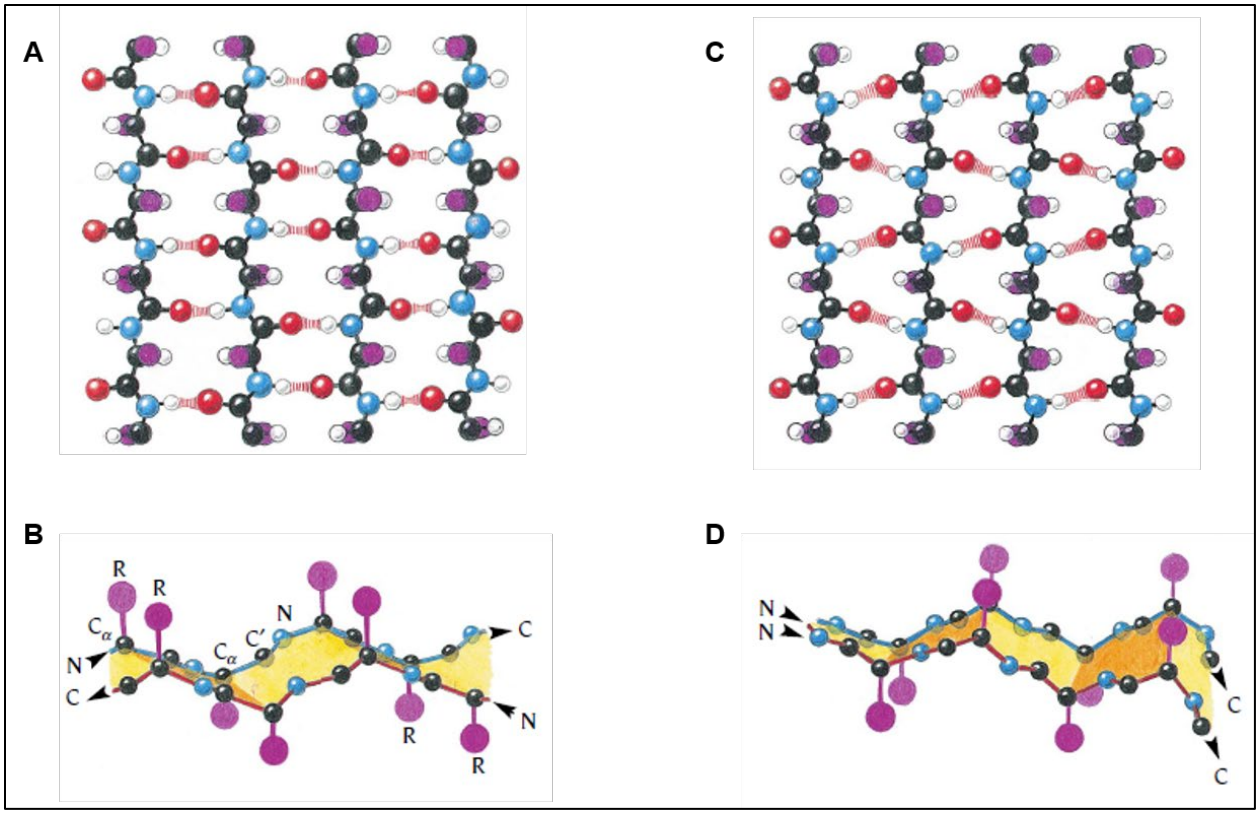
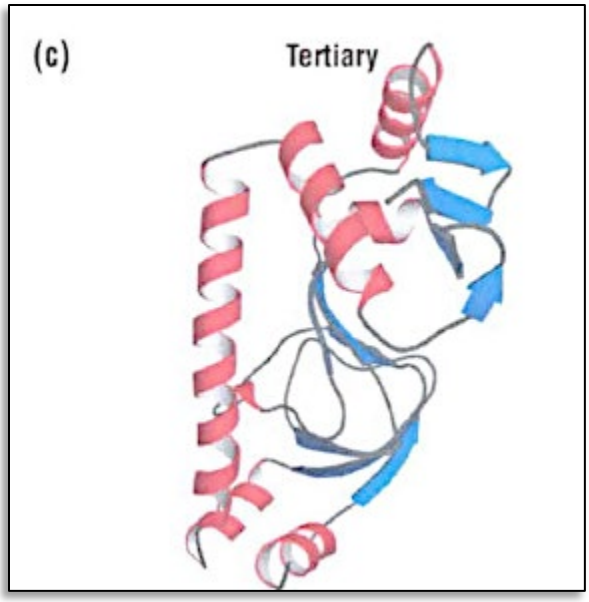


Figure B. Figures (A)-(D) correspond to Figures 2.5(c), 2.5(d), 2.6(b), and 2.6(c), respectively of EX1014, 18-19. Schematic illustrations of (A) an antiparallel beta sheet; (B) a pleat of an antiparallel beta sheet; (C) a parallel beta sheet; and (D) a pleat of a parallel beta sheet. Carbon atoms are depicted in black; oxygen atoms are depicted in red; and nitrogen atoms are depicted in blue.

91. Secondary structures (*i.e.*, alpha helices and/or beta sheets) can be connected by loop regions of variable lengths and shapes. EX1014, 3, 21.

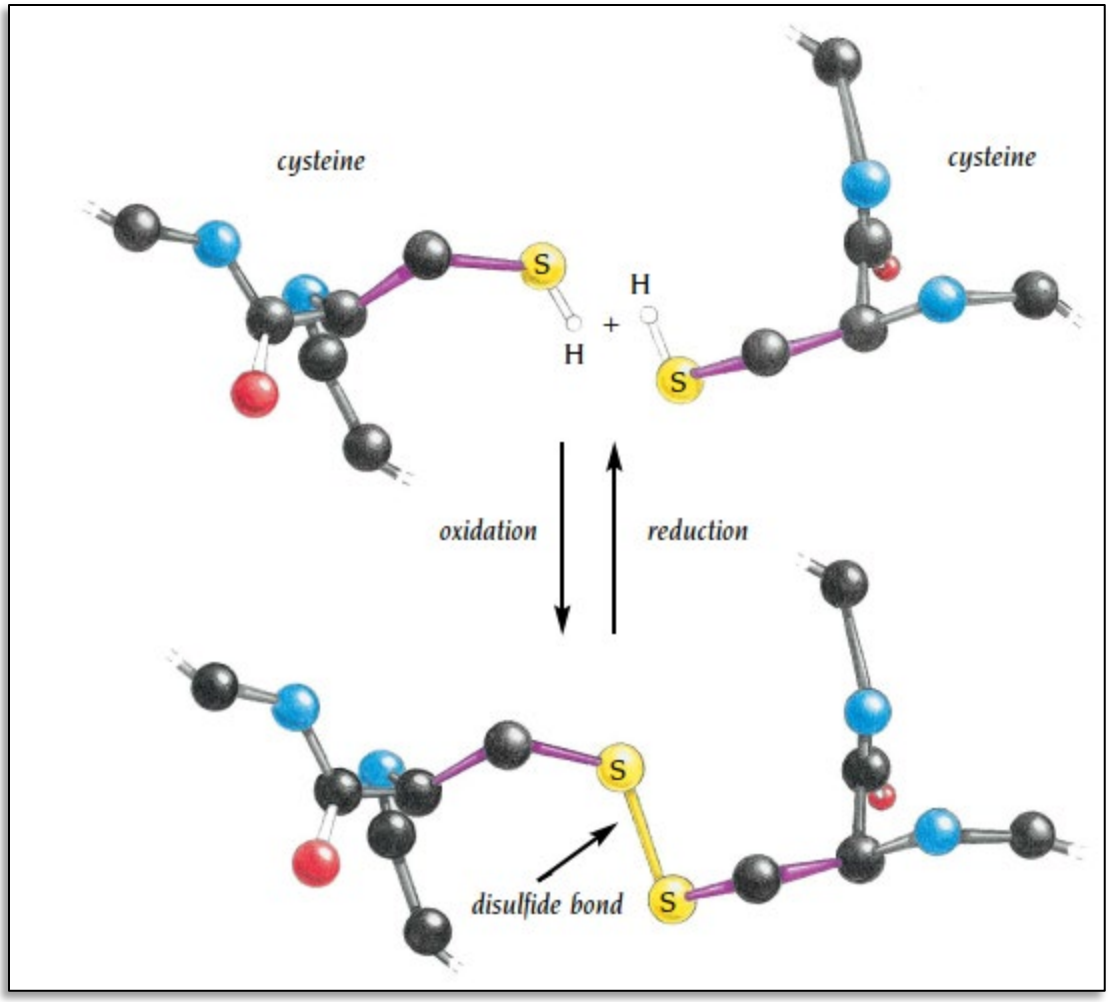
4. Tertiary Structure

92. The "overall fold of the protein" is referred to as the "tertiary structure." EX2164 (Petsko), 3. Secondary structure elements (alpha helices, beta sheets, or both), "as well as loops and links that have no secondary structure, are folded into a tertiary structure." *Id*. A protein's tertiary structure is depicted in the figure below:



EX2164 (Petsko), 3.

93. Folded proteins are "compact and nearly solid object[s] stabilized by weak interactions involving both polar and nonpolar groups." EX2164 (Petsko), 22. A protein's tertiary structure is stabilized primarily by non-covalent interactions. EX2164 (Petsko), 23. Disulfide bridges also "stabilize three-dimensional structure." EX1014, 8. Disulfide bridges are covalent bonds formed between the side chains of cysteine residues. As Branden & Tooze explains, "[t]wo cysteine residues in different parts of the polypeptide chain but adjacent in the three-dimensional structure of a protein can be oxidized to form a disulfide bridge (Figure 1.4)." EX1014, 5. I have reproduced Figure 1.4 from Branden & Tooze below:



Id.

94. PH20 adopts a characteristic alpha-beta barrel tertiary structure, as discussed below.

5. Sequence Alignments and the Concept of Homology

95. Amino acid sequences can be compared by performing an alignment to determine the degree of identity (identical amino acids appear in each position in the sequence) or similarity (not identical but amino acids of a similar chemical class appear in each position in the sequence). *See generally* EX2164 (Petsko),

132. Two naturally occurring amino acid sequences with significant similarity may have been derived from the same ancestral sequence: "Sequences related in this way are called homologous and the evolutionary similarity between them is known as homology." *Id.*

96. A "pairwise" alignment compares two amino acid sequences. Determining the "fraction of identical amino acids or nucleotides gives a similarity measure known as **percent identity."** EX2164 (Petsko), 132.

97. In a multiple sequence alignment, multiple (*i.e.,* more than two) amino acid sequences are compared: "[M]ultiple sequence alignments are arrived at by successively considering all possible pairwise alignments…. From this alignment one can see that certain residues are identical in all the sequences. Any residue, or short stretch of sequence, that is identical in all sequences in a given set (such as that of a protein family) is said to be conserved. Multiple alignments tend to give a better assessment of similarity than pairwise alignments and can identify distantly related members of a gene family that would not be picked up by pairwise alignments alone." EX2164 (Petsko), 133.

98. Pairwise and multiple sequence alignments were well known in the art as of December 28, 2012. At that time, BLAST had a pairwise sequence alignment tool that was commonly used by POSAs. Dr. Park testified at his deposition that a POSA seeking to perform a pairwise sequence alignment in 2011 would have used

BLAST (EX2077 (Park Tr.), 96:13-15), and I agree.

99. With respect to multiple sequence alignments, Clustal Omega was commonly used at that time by POSAs. Dr. Park testified at deposition that a POSA seeking to perform a multiple sequence alignment in 2011 would have used Clustal Omega to do so (EX2077, 96:5-12), and I agree.

6. Using the Concept of Homology and Conservation of Folded Structures Among Homologous Proteins to Generate Models

100. There is a foundational principle in structural biology that, through evolution, "structure changes more slowly than sequence," so "if there is a high degree of sequence identity between two proteins, their overall folds will always be similar." EX2164 (Petsko), 142. As Dr. Park explained in his deposition: "If you ask a practitioner in the field: I have two proteins that are homologous and have high sequence similarity, would I expect them to fold to the same structure, of course I would say yes." EX2077, 60:4-13.

101. As Dr. Park also explained:

> Protein structures, if one compares the folding structure to an entire space of – conformational space that can be adopted by the polypeptide, it's a very small fraction. Now, if you have homologous proteins, meaning they came from the same ancestor, and they have maintained similar sequence similarity, they would be obligated to explore that very small sliver of the conformational space. They

> would not be able to go and randomly find another island of stability in that conformational space that can be occupied by that particular sequence. So that restricts what the protein can do. ***So this is the foundation behind the assumption that homologous highly similar sequences would adopt the same conformational three-dimensional structure.***

EX2077 (Park Tr.), 60:16-61:10 (emphasis added).

102. Similarly, as Dr. Hecht explained:

When you have a family of homologous proteins that share common ancestry and share considerable sequence identity, share evolutionary history, and in most cases share enzymatic activity, then one infers from that that they're going to share structure.EX2076 (Hecht Tr.), 80:9-15.

103. Based on this concept, it is possible to use an experimentally determined structure as a template to generate a homology model for a protein whose structure has not been experimentally determined. For example, as Dr. Park explained, "[t]he absence of an experimentally determined structure for [human PH20] protein does not preclude the use of structure-based modeling methods. That is because the structure of a protein can be modeled in a process called 'homology modeling.'" EX1004 (Park Decl.), ¶36.

104. "Homology modeling was routinely used by 2011 and provided an 'accurate computational method to generate reliable structural models.'" EX1004

(Park Decl.), ¶36. "The technique of **homology modeling** aims to produce a reasonable approximation to the structure of an unknown protein by comparison with the structure of a known sequence homolog (a protein related to it by divergent evolution from a common ancestor)." EX2164 (Petsko), 142 (emphasis added).

105. I have reviewed the work Dr. Petsko performed showing the common tertiary structures of bee venom hyaluronidase, HYAL-1, and PH20, based on the solved crystal structures. EX2070 (Petsko Decl.), ¶¶126-230. I agree with his analysis and note that it would have been within the common knowledge of a POSA that hyaluronidases such as these, despite sharing only 30-40% sequence identity, would share a common tertiary structure—as Dr. Petsko shows, an "alpha-beta barrel fold." EX2070 (Petsko Decl.), ¶¶129-134.

106. I also reviewed Dr. Petsko's analysis and conclusion that a POSA would have expected that a folded modified PH20 that is 95% identical in sequence to wild-type ("WT") PH20 would adopt the same alpha-beta barrel fold as WT PH20. EX2070 (Petsko Decl.), ¶¶128, 206. I agree with Dr. Petsko. I agree with Dr. Petsko's assessment that, if a 95% identical modified PH20 polypeptide folds into a stable structure, it will fold into the same alpha-beta barrel motif as WT PH20. I also note that, as set out below, a POSA would be able to use the teachings in the patent in view of the knowledge in the art to identify changes

to the PH20 primary structure that will or are likely to destabilize the protein such that it does not adopt the alpha-beta barrel fold.

107. For example, the POSA would have understood that changing the cysteine residues that form disulfide bonds in PH20, which are conserved in the primary sequence between PH20 and HYAL-1, would be highly likely to result in a protein that does not fold correctly and is not active.

108. I understand that Drs. Park and Hecht both agree that it would have been common and expected for a POSA to generate a PH20 homology model. EX2077 (Park Tr.), 176:2-5; EX2076 (Hect Tr.), 93:4-22; EX1003, ¶¶79, 81-88; EX1004, ¶¶84, 88-93. For example, as Dr. Park explained,

> Q So what's the purpose of generating a homology model for a protein structure?
>
> A It allows you to look at the molecule, as opposed to imagining it in your head.
>
> Q And it's useful to be able to look at the molecule, right?
>
> A Absolutely.
>
> Q And you can use homology modeling to predict the impact of substitutions in a protein?
>
> A A homology model can help you to evaluate the impact of a substitution. Yes.

EX2077, 53:15-54:5.

109. I agree. In fact, as I explain below, before the HYAL-1 crystal structure was published, I generated a homology model of HYAL-1 using the Swiss Model program and using the bee venom hyaluronidase structure as a template. We (my team and I) used the homology model to guide our mutagenesis experiments in which we sought to identify and experimentally confirm residues important to the HYAL-1 mechanism of action.

B. PH20 and Other Hyaluronidases

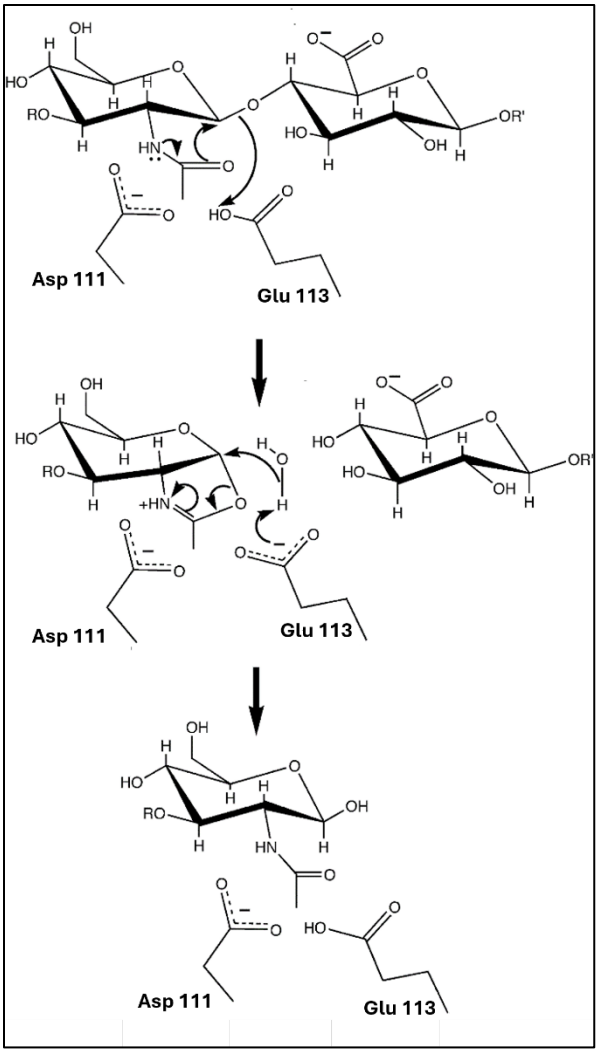
1. PH20

110. PH20 is a member of a class of enzymes called hyaluronidases. EX1001, 45:44-45. PH20 occurs in sperm, where it plays a role in contraception and is active at neutral pH. EX1001, 72:45-62.

111. As a hyaluronidase, PH20 cleaves hyaluronan (also called hyaluronic acid, hyaluronate, or "HA"), which is a linear, negatively charged glycosaminoglycan made of repeating disaccharide units. EX1006 (Chao), 6911; EX2086 (El-Safory), 166-168.

112. PH20 facilitates fertilization. EX1001, 72:45-62. To perform its biological function, PH20 must, among other things, digest HA. EX1001, 72:45-62.

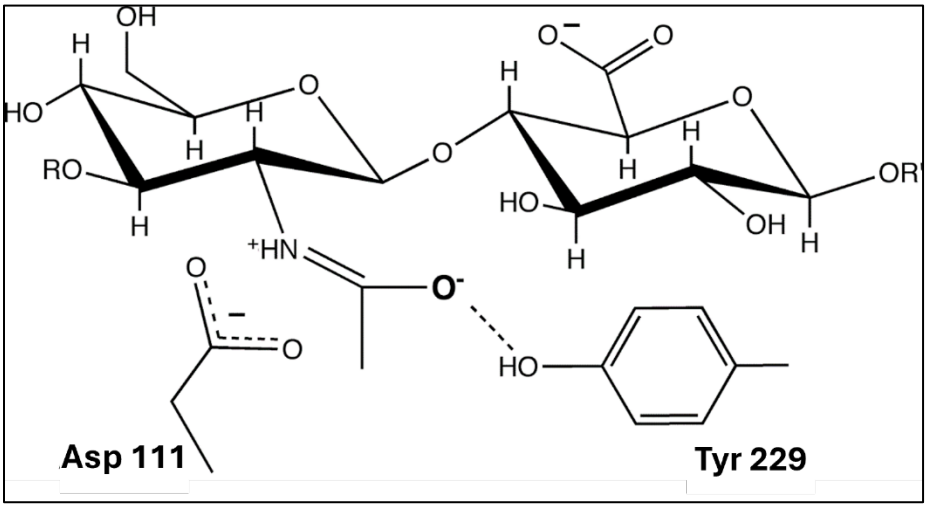
113. PH20 cleaves HA through a substrate-assisted hydrolysis reaction. The mechanism of action is shown below:



114. In the first step of the reaction, HA binds at the active site, with the N-acetyl group of N-acetylglucosamine in the β1-4 glycoside linkage to undergo cleavage positioned within ≈3Å of the essential glutamic acid (Glu113) shown in the figure.

115. In step 2, the reaction is initiated by an adjacent aspartate (Asp111) and a proximal tyrosine (Tyr229, shown in the figure below), which stabilize an alternate conformation of the amide bond in the N-acetyl group to produce the

oxyanion nucleophile that reaches out to form a bond with C1 in a 5-membered ring structure, thus releasing C4 of the formerly linked glucuronate and the remainder of that portion of the HA chain.



116. Step 3 shows the active site glutamic acid (Glu113) donating its proton to the C4 hydroxyl of the leaving group (on the HA chain). Step 4 shows that the deprotonated glutamate (Glu113) now activates a water molecule, which allows the resultant hydroxide ion to act as a nucleophile to form a new hydroxyl linkage at the C1 carbon and resolves the 5-membered ring to regenerate the N-acetylglucosamine end of this remaining portion of the HA chain. In step 5, the protonated glutamic acid (Glu113) is restored, and the rest of the HA chain dissociates from the active site.

2. Other Hyaluronidases

117. Hyaluronidases were first identified as "spreading factors" in extracts from mammalian testes as early as 1928 (EX2170 (Chain & Duthie 1940), 324), and

the term "hyaluronidase" was introduced in 1940 (EX2088 (Stern & Cóska 2000), 2). Since 1940, an entire field of research has developed directed to the study of hyaluronidases. EX2086 (El-Safory), 166.

118. There are five human hyaluronidases, three of which are known (and were known in 2012) to play a role in degrading HA: PH20, HYAL-1 and HYAL-2. EX1006, 6911; EX2086 (El-Safory), 169. HA-degrading enzymes also occur in other organisms, including bees (for instance, bee venom hyaluronidase helps venom from a sting spread). EX1006.

3. Hyaluronidase Crystal Structures and Mutagenesis Work

119. By 2012, the crystal structures for bee venom hyaluronidase (EX1033) and human HYAL-1 (EX1006) had been solved. Solving these structures was an important step in the field. The crystal structures, combined with mutagenesis data disclosed in the literature, allowed POSAs to identify residues likely involved in forming interactions with the HA substrate, including those that facilitate the substrate-assisted catalysis. I performed some of that work in my lab, as I explain below.

a. Bee Venom Hyaluronidase (Marković-Housley)

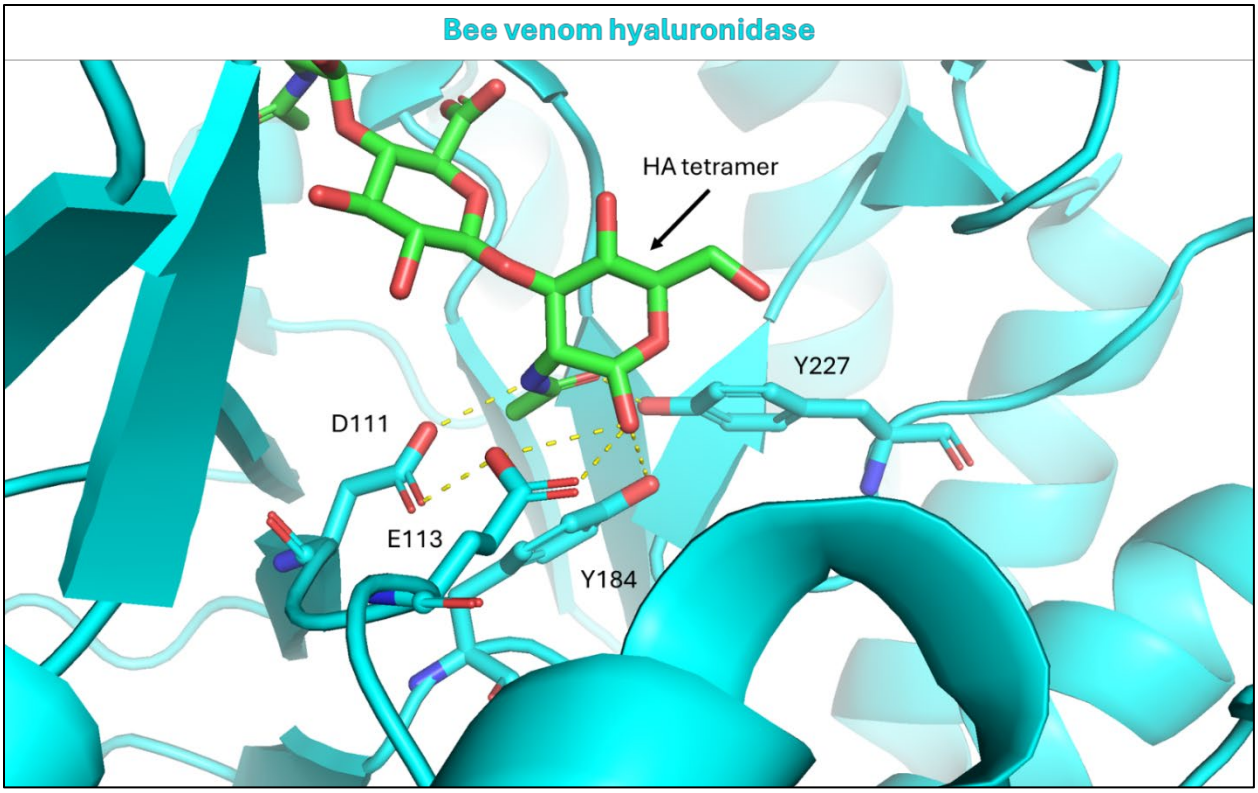
120. The crystal structure for bee venom hyaluronidase was determined by Marković-Housley and described in a paper published in 2000. EX1033.

121. The bee venom structure was a critical step forward in hyaluronidase

research because it was the first solved crystal structure of a mammalian type hyaluronidase. Prior to that time, structures had been solved for bacterial hyaluronidases, but those enzymes function through a different mechanism of action. The bacterial hyaluronidases are lyases, not hydrolases.

122. The bee venom hyaluronidase structure shows that the protein adopts an alpha-beta barrel fold, as Dr. Petsko explains in his declaration. EX2070 (Petsko Decl.), ¶¶129-134. I agree with Dr. Petsko that the bee venom hyaluronidase adopts an alpha-beta barrel fold.

123. And the bee venom hyaluronidase was crystallized with an HA tetramer, thereby allowing POSAs to see where exactly the HA substrate binds to bee venom hyaluronidase. Dr. Petsko's figure below illustrates this:



EX2070 (Petsko Decl.), ¶151

124. Now, with this crystal structure in hand, POSAs could begin to understand the features of the active site for proteins like HYAL-1 and PH20. And, as I explain below, I used the bee venom crystal structure to investigate the HYAL-1 mechanism of action and identify important residues for HYAL-1 function.

125. However, the bee venom hyaluronidase lacks the EGF-like motif present in HYAL-1 and PH20, so the bee venom structure did not provide a complete picture of the structure for those proteins.

b. Simpson Lab Work Based on the Bee Venom Hyaluronidase Structure

126. Following publication of the bee venom hyaluronidase crystal

structure, and before the HYAL-1 structure was published, my lab sought to use the bee venom crystal structure to investigate the HYAL-1 mechanism of action and identify important residues in HYAL-1. We knew that HYAL-1 and bee venom hyaluronidase are both hydrolases that cleave HA. And because a portion of the HYAL-1 sequence (the N-terminal portion without the EGF-like motif) is homologous to bee venom hyaluronidase, we expected that portion of HYAL-1 would adopt the same tertiary structure as bee venom hyaluronidase.

127. We designed mutagenesis experiments in which we mutated HYAL-1 residues we expected may be important for enzymatic activity and tested the resulting protein. In order to identify the residues to mutate, we generated a homology model of HYAL-1 using the bee venom hyaluronidase crystal structure as a template. We used the Swiss Model program to generate our homology model.

128. This work was initiated before the HYAL-1 crystal structure was published and before the Chao paper that describes the HYAL-1 crystal structure (EX1006) published in 2007.

129. When we generated the homology model, we omitted the EGF-like motif portion of the HYAL-1 sequence because the bee venom hyaluronidase does not have that EGF-like motif. We only modeled the portion of HYAL-1 that is homologous to the bee venom hyaluronidase.

130. We used our resulting HYAL-1 homology model to identify residues to mutate in our mutagenesis study. We superimposed the HA tetramer from the bee venom structure onto our homology model, and we then identified residues within 5 Angstroms (Å) of the tetramer as potential candidates for binding to HA and as residues likely facilitate the substrate-assisted catalysis. Our choice of residues was further informed by the sequence alignment of human hyaluronidases in Jedrzejas and Stern (2005) (EX1009), since two critical catalytic residues (Asp111 and Glu113) that had been reported in PH20 by Arming et al. in 1997 (EX1011) were also among the fully conserved residues in the hyaluronidase family.

131. We performed mutagenesis experiments and recorded our data, and, while we were performing this work, Chao et al. published the HYAL-1 crystal structure. EX1006.

132. We continued our experiments following publication of the HYAL-1 crystal structure, and going forward we used the HYAL-1 crystal structure instead of our homology model. In particular, we were interested in the EGF-like motif, because the HYAL-1 crystal structure shows the structure for this motif and how it interacts with the rest of the protein.

133. Results from the mutagenesis work I describe above eventually were published in the Zhang paper (EX1010), which I describe in detail below.

c. HYAL-1 Crystal Structure (Chao)

134. The crystal structure for human HYAL-1 was determined by Chao et al. and published in 2007. EX1006, 6911. Now, for the first time, there was a published structure of a human hyaluronidase, and we could see the structure of the EGF-like motif that was missing from the bee venom structure.

135. The HYAL-1 structure confirms, as we expected, that the homologous HYAL-1 and bee venom hyaluronidase proteins adopt the same alpha-beta barrel fold.

136. And, just as I had done with my homology model, Chao superimposed the HA tetramer from the bee venom hyaluronidase structure onto the HYAL-1 structure to see which residues are likely involved in binding HA and facilitating catalysis. EX1006, 6917.

137. I note that, in his analysis, Dr. Petsko also superimposed the HA tetramer from the bee venom structure on the HYAL-1 structure and the PH20 homology model (*see* EX2070 (Petsko Decl.), ¶129), which again is consistent with this common practice.

4. Mutagenesis Studies of PH20

138. As I described above, in my lab we performed a mutagenesis study to identify and experimentally define residues that were a) critical for HYAL-1 enzymatic activity and b) important for other aspects of HYAL-1 function. Results

from that study were published in the Zhang paper. I am the senior co-author of the Zhang paper, EX1010 (Zhang), and I was directly involved in its experimental design, execution, and interpretation.

139. The Zhang paper presents a detailed biochemical characterization of human HYAL-1 through site-directed mutagenesis and quantitative kinetic analysis. The study aimed to "probe the molecular interactions that specify hyaluronidase activity of human HYAL-1" by examining how specific amino acid residues contribute to enzymatic activity. EX1010 (Zhang), 9435.

140. Below, I describe what the POSA would have understood from the teachings of the Zhang paper as of December 28, 2012.

141. In the Zhang paper, we describe the results obtained for point mutations "at residues Asp129, Glu131, Tyr202, Asn216, Tyr247, Ser245, and Arg265 to investigate their individual contributions to the enzyme mechanism." EX1010 (Zhang), 9435.

142. Additionally, we investigated glycosylation effects on HYAL-1 activity. Specifically, we mutated two N-glycosylation sites on HYAL-1: Asn216 and Asn350. EX1010 (Zhang), 9438.

143. Also, based on our analysis of the HYAL-1 crystal structure, we decided to investigate the extent to which the EGF-like motif is required for enzymatic activity. The HYAL-1 structure shows that the EGF-like motif is not a

separate domain (as some had thought it would be); rather, this region adopts a folded structure that packs tightly against the rest of the protein as a single domain. To investigate the importance of the EGF-like motif, we removed it and tested the resulting protein for activity.

144. Specifically, we generated a truncated version of HYAL-1 in which we removed the EGF-like region. One variant included the N350 glycosylation site (we introduced a stop codon at position 356 so the C-terminal end of the protein was residue 355) and the other variant did not (we introduced a stop codon at position 350 so the C-terminal end of the protein was residue 349). EX1010 (Zhang), 9439.

145. In our assay, we used the Morgan-Elson color reaction to measure activity. This is a common method of measuring the activity of enzymes like HYAL-1 (and PH20) which repeatedly cleave a polymer substrate. Hyaluronidases do not have a single substrate or produce a single product. They successively bind HA, cleave HA, release HA, then re-bind and re-cleave the HA. Thus, each HA substrate and product molecule has the potential to be a differently sized polymer, rather than a homogeneous population. The Morgan-Elson color reaction allows POSAs to quantify the number of cleavage events and thereby study the kinetics of the enzyme. Each HA molecule has an N-acetylglucosamine at the end and each cleavage event produces two shorter polymers, each of which has N-acetylglucosamine at the end. The Morgan-Elson reaction chemically labels these ends, so they can be visualized

and quantified spectrophotometrically. In the assay, we incubated the HA with the enzyme and then heat inactivated the reaction after a set amount of time. We then decorated the HA "ends" by adding potassium tetrahydroborate and acidified dimethylamino benzaldehyde, which reacts with the N-acetylglucosamine to produce a color. The intensity of the color is measured and interpreted using a standard curve to quantify the number of cleavage events.

146. Table 1 reports the results of the assays and is reproduced below. We interpolated Km and Vmax values from "the curve fit where indicated." EX1010 (Zhang), 9435.

TABLE 1
Summary of Hyal1 wild-type (WT) and mutant kinetic constants

Enzyme	K_m	V_{max}	% WT activity at 50 μM HA
	μM	μmol/min/mg	
Hyal1 wild-type	38.1 ± 4.8	12.5 ± 0.7	100.0 ± 1.8
Catalytic mutants			
E131Q	NA[a]	NA	0.08 ± 0.01
Y247F	NA	NA	0.04 ± 0.01
D129N	181 ± 19[b]	1.9 ± 0.1[b]	5.10 ± 0.09
Substrate binding mutants			
Y202F	367 ± 37[b]	6.7 ± 0.5[b]	11.1 ± 0.0
S245A	110 ± 19	10.7 ± 1.1	41.1 ± 0.4
Putative structural mutants			
R265L	NS[c]	NS	4.18 ± 0.09
N216A	103 ± 14	10.0 ± 0.8	44.6 ± 1.3
N350A	NS	NS	0.12 ± 0.01
N350tr	NS	NS	2.49 ± 0.17[d]
L356tr	NS	NS	6.29 ± 1.50[d]

[a] NA indicates no measurable activity at any HA concentration.
[b] Indicates extrapolated value from saturable curve fit.
[c] NS, not saturable, indicates data do not fit a saturation curve.
[d] Values measured at 125 μM HA.

147. I note that the assay setup we used in the Zhang work was designed to generate saturation curves that we could use to calculate kinetic constants for each

substitution. We did this because we wanted to be able to determine not just whether a particular substitution impacts activity, but to measure specifically ***how*** the substitution impacts activity. I explain what the kinetic constants we describe in the chart mean and how changes in those constants provide information about the importance of particular residues in the paragraphs that follow.

148. Km represents the substrate concentration needed for the enzyme to achieve half of its maximum rate of catalysis. A lower Km indicates the enzyme has higher substrate affinity, meaning that the enzyme requires less substrate to reach the half-maximum rate. A higher Km indicates the enzyme has lower substrate affinity, meaning that more substrate is required to reach the half-maximum rate. The Km was reported in μM in the Zhang paper.

149. Vmax represents the maximum catalytic rate achievable when the enzyme is saturated with substrate. The reaction rate here refers to the number of cleavage events per unit time. As substrate is added, the reaction rate will increase (*i.e.,* the number of HAs cleaved per unit time will increase) until saturation is reached, at which point adding additional substrate no longer increases the reaction rate. A higher Vmax indicates a higher catalytic rate and a lower Vmax indicates a lower catalytic rate at saturation. Vmax was measured in μmol/min/mg of HYAL-1 enzyme in the Zhang paper.

150. We also reported the percent activity as compared to the wild-type

enzyme at a substrate concentration of 50 μM. We did this because we were not able to obtain saturation curves for all of the variants we tested, so we could not perform the calculation to obtain Km and Vmax for those variants, but we wanted to report a comparison of activity for those variants. Also, even for those variants that did achieve saturation, the % WT activity value provides useful information because it demonstrates the extent to which activity is impaired at a particular substrate concentration.

151. When I observed increased Km values compared to the baseline value, I interpreted this as reduced substrate binding affinity in the context of the reaction. For example, the mutant S245A has "lower affinity for HA as indicated by the ≈3-fold higher Km," indicating "the importance of Ser245 at the enzyme active site is to ensure substrate binding." EX1010 (Zhang), 9437. Consistent with the conclusion that S245A is an important residue, the S245A mutant only achieves around 41% activity relative to wild-type HYAL-1 at 50 μM HA. *Id.*, 9438. Notably, there was not a reduction in Vmax for Ser245, *id.*, indicating that it is still possible for the enzyme to achieve maximum rate with S245A, but more substrate is required to achieve that maximum rate. In other words, the S245A substitution impairs activity at lower concentrations of HA but does not abrogate intrinsic catalytic potential of the enzyme.

152. Similarly, the HYAL-1 mutant Y202F had a 10-fold increase in the Km

compared to wild-type HYAL-1, indicating Y202 is "a substrate binding determinant." EX1010 (Zhang), 9437-38.

153. When I observed reduced Vmax values compared to the baseline value, I interpreted this as impaired catalytic function. For example, Y202F reduced Vmax by about 50%, which suggested partial loss of catalytic function. EX1010 (Zhang), 9438. In other words, the Y202F variant was only able to achieve half of the maximum cleavage rate achieved by the wild type enzyme at saturation.

154. Finally, the result of "no measurable activity at any HA concentration" means complete loss of catalytic function for active site residues E131Q and Y247F. EX1010 (Zhang), 9438.

155. I note that the mutagenesis experiments that I and others performed were more limited in scope than the mutagenesis experiments described in the '185 patent. For that reason, we used different methods to, for instance, measure activity. But the fundamental idea behind both my work in the Zhang paper and the work conducted in the '185 patent is that one can illuminate common structural features of hyaluronidases (and other enzymes) through mutagenesis studies, by modifying the primary structure and determining the impact of the change on activity.

156. In particular, as a conceptual matter, POSAs can experimentally assess potential common structural features of the three-dimensional protein by introducing mutations to its primary structure and seeing what abolishes activity, and then

evaluating the results in the context of an experimental structure (*e.g.,* a crystal structure) or a homology model. This is a core tenet of this type of work. The mutagenesis data generated by such studies allows the POSA to understand common structural features and structure-function correlations. A POSA understand that is one of the primary benefits of generating such data.

157. I note that in Zhang, we tested single point mutations, but we only changed certain positions to one other amino acid. By contrast, in the experiments in the patent, the inventors changed each position in the protein to, on average, 13 other amino acids. As I explain below, and as Dr. Petsko explains in his declaration, these data provide highly significant information regarding the tolerance of each position in the protein, to substitution, such that a POSA can determine where to make changes to maintain or abolish enzymatic activity, or to reduce or improve enzymatic activity. The patent data provide a POSA with an extremely powerful tool to engage in further modification of the protein in a reasonably predictable manner.

VIII. THE ’185 PATENT

A. Overview

158. The ’185 patent discloses a comprehensive and systematic analysis of each and every position within the primary structure of PH20, which the inventors performed through large-scale mutagenesis and enzymatic activity testing of over

5800 variants.

159. Based on the structure-function data provided via the assays described in Examples 2-4, the '185 patent teaches "modified PH20 polypeptides" that "exhibit altered activities or properties compared to a wild-type, native or reference PH20 polypeptide." EX1001, 75:45-47.

160. In particular, the patent teaches that the disclosed modified PH20 polypeptides include those that are active variants, inactive variants, and variants with increased stability. *Id.*, 75:44-58. Among these variants, the inventors identified numerous substitutions that increase enzymatic activity, including the four substitutions at position 320 recited in the claims. EX1001, Table 9.

161. Considered as a whole, the inventors' work provides a detailed description of the structure-function correlations in PH20 that identifies, for the POSA, the extent to which each position in the sequence tolerates substitutions and which do not, which types of residues at each position are likely to be successful in terms of maintaining activity, and which positions in particular could be targeted to maintain or improve activity.

162. As the '185 patent states, the "[d]etailed structure/function of virtually each amino acid in a PH20 polypeptide is provided herein" along with "the identification of residues and loci that contribute to alteration of a property, such as stability in particular conditions." EX1001, 4:58-62.

163. This level of structure/function detail for an individual protein of this size was virtually unheard of as of 2012. I am not aware of a comparable project.

164. The data are extraordinarily useful to a POSA seeking to make and use modified PH20 polypeptides.

B. PH20-Related Terminology and SEQ ID NOs.

165. Before describing the examples and claims, I first address how the POSA would understand the various PH20-related terminology (*e.g.,* "wild-type PH20," "signal sequence," "mature sequence," "C-terminal truncations," etc.) used in the specification, and that I use in this declaration, including the SEQ ID NOs.

166. The patent teaches that "'native' or 'wildtype' with reference to a PH20 polypeptide refers to a PH20 polypeptide encoded by a native or naturally occurring PH20 gene, including allelic variants" present in nature in organisms encompassing humans and other animals. EX1001, 47:22-26. The patent also teaches that "[t]he amino acid sequences of exemplary wild-type human PH20 are set forth in SEQ ID NOs: 6 and 7 and those of allelic variants, including mature forms thereof, are set forth in SEQ ID NOs: 68-72." *Id.*, 47:36-40.

167. The wild-type human PH20 amino acid sequence, which is provided in SEQ ID NO: 6, includes an N-terminal 35 amino acid signal sequence. The patent refers to the full-length PH20 sequence with the 35 amino acid signal sequence as a "PH20 precursor polypeptide." EX1001, 69:5-16. As the patent

explains, "[t]he mRNA transcript is typically translated to generate a precursor protein containing a 35 amino acid signal sequence at the N-terminus. Following transport to the ER, the signal peptide is removed to yield a mature PH20 polypeptide." *Id.*, 69:13-17.

168. SEQ ID NO: 6, which is the full-length, wild-type "precursor" PH20 with its signal sequence, contains 509 amino acids. EX1001, 69:26-30.

169. The "mature" wild-type PH20 polypeptide lacks the 35 amino acid signal sequence; the sequence for full-length "mature" wild-type PH20 is provided in SEQ ID NO: 7. EX1001, 69:17-19. Mature, wild-type PH20 has 474 amino acids. *Id.*, 69:30-33. POSAs would have understood that mature, wild-type PH20 (*i.e.,* SEQ ID NO: 7) is enzymatically active.

170. The various references, including the patent, alternate between the "mature" PH20 sequence and the "precursor" PH20 sequence when identifying amino acid positions in the protein. Position 36 in the "precursor" protein corresponds to position 1 in the "mature" protein. It is possible to obtain the "mature" position from the "precursor" position by subtracting 35. The chart below can be used to convert positions in the precursor sequence to the mature sequence and vice versa.

Precursor position	Mature position	Precursor position	Mature position	Precursor position	Mature position	Precursor position	Mature position
36	1	67	32	98	63	129	94
37	2	68	33	99	64	130	95
38	3	69	34	100	65	131	96
39	4	70	35	101	66	132	97
40	5	71	36	102	67	133	98
41	6	72	37	103	68	134	99
42	7	73	38	104	69	135	100
43	8	74	39	105	70	136	101
44	9	75	40	106	71	137	102
45	10	76	41	107	72	138	103
46	11	77	42	108	73	139	104
47	12	78	43	109	74	140	105
48	13	79	44	110	75	141	106
49	14	80	45	111	76	142	107
50	15	81	46	112	77	143	108
51	16	82	47	113	78	144	109
52	17	83	48	114	79	145	110
53	18	84	49	115	80	146	111
54	19	85	50	116	81	147	112
55	20	86	51	117	82	148	113
56	21	87	52	118	83	149	114
57	22	88	53	119	84	150	115
58	23	89	54	120	85	151	116
59	24	90	55	121	86	152	117
60	25	91	56	122	87	153	118
61	26	92	57	123	88	154	119
62	27	93	58	124	89	155	120
63	28	94	59	125	90	156	121
64	29	95	60	126	91	157	122
65	30	96	61	127	92	158	123
66	31	97	62	128	93	159	124

Precursor position	Mature position	Precursor position	Mature position	Precursor position	Mature position	Precursor position	Mature position
160	125	191	156	222	187	253	218
161	126	192	157	223	188	254	219
162	127	193	158	224	189	255	220
163	128	194	159	225	190	256	221
164	129	195	160	226	191	257	222
165	130	196	161	227	192	258	223
166	131	197	162	228	193	259	224
167	132	198	163	229	194	260	225
168	133	199	164	230	195	261	226
169	134	200	165	231	196	262	227
170	135	201	166	232	197	263	228
171	136	202	167	233	198	264	229
172	137	203	168	234	199	265	230
173	138	204	169	235	200	266	231
174	139	205	170	236	201	267	232
175	140	206	171	237	202	268	233
176	141	207	172	238	203	269	234
177	142	208	173	239	204	270	235
178	143	209	174	240	205	271	236
179	144	210	175	241	206	272	237
180	145	211	176	242	207	273	238
181	146	212	177	243	208	274	239
182	147	213	178	244	209	275	240
183	148	214	179	245	210	276	241
184	149	215	180	246	211	277	242
185	150	216	181	247	212	278	243
186	151	217	182	248	213	279	244
187	152	218	183	249	214	280	245
188	153	219	184	250	215	281	246
189	154	220	185	251	216	282	247
190	155	221	186	252	217	283	248

Precursor position	Mature position	Precursor position	Mature position	Precursor position	Mature position	Precursor position	Mature position
284	249	315	280	346	311	377	342
285	250	316	281	347	312	378	343
286	251	317	282	348	313	379	344
287	252	318	283	349	314	380	345
288	253	319	284	350	315	381	346
289	254	320	285	351	316	382	347
290	255	321	286	352	317	383	348
291	256	322	287	353	318	384	349
292	257	323	288	354	319	385	350
293	258	324	289	355	320	386	351
294	259	325	290	356	321	387	352
295	260	326	291	357	322	388	353
296	261	327	292	358	323	389	354
297	262	328	293	359	324	390	355
298	263	329	294	360	325	391	356
299	264	330	295	361	326	392	357
300	265	331	296	362	327	393	358
301	266	332	297	363	328	394	359
302	267	333	298	364	329	395	360
303	268	334	299	365	330	396	361
304	269	335	300	366	331	397	362
305	270	336	301	367	332	398	363
306	271	337	302	368	333	399	364
307	272	338	303	369	334	400	365
308	273	339	304	370	335	401	366
309	274	340	305	371	336	402	367
310	275	341	306	372	337	403	368
311	276	342	307	373	338	404	369
312	277	343	308	374	339	405	370
313	278	344	309	375	340	406	371
314	279	345	310	376	341	407	372

Precursor position	Mature position
408	373
409	374
410	375
411	376
412	377
413	378
414	379
415	380
416	381
417	382
418	383
419	384
420	385
421	386
422	387
423	388
424	389
425	390
426	391
427	392
428	393
429	394
430	395
431	396
432	397
433	398
434	399
435	400
436	401
437	402
438	403

Precursor position	Mature position
439	404
440	405
441	406
442	407
443	408
444	409
445	410
446	411
447	412
448	413
449	414
450	415
451	416
452	417
453	418
454	419
455	420
456	421
457	422
458	423
459	424
460	425
461	426
462	427
463	428
464	429
465	430
466	431
467	432
468	433
469	434

Precursor position	Mature position
470	435
471	436
472	437
473	438
474	439
475	440
476	441
477	442
478	443
479	444
480	445
481	446
482	447

171. But wild-type PH20 is expressed on the surface of the cell and has a GPI anchor. In order to make a soluble, secreted PH20, some portion of the end of the sequence must be removed (*i.e.*, truncated). Protein sequences begin at the "N-terminus" and stop at the "C-terminus." Using that terminology, some portion of the C-terminus of wild-type PH20 must be removed to obtain a secreted, soluble PH20. As I explain in detail below, the '429 patent, the Frost paper, and the '457 publication teach which C-terminal truncations produce a soluble, secreted protein.

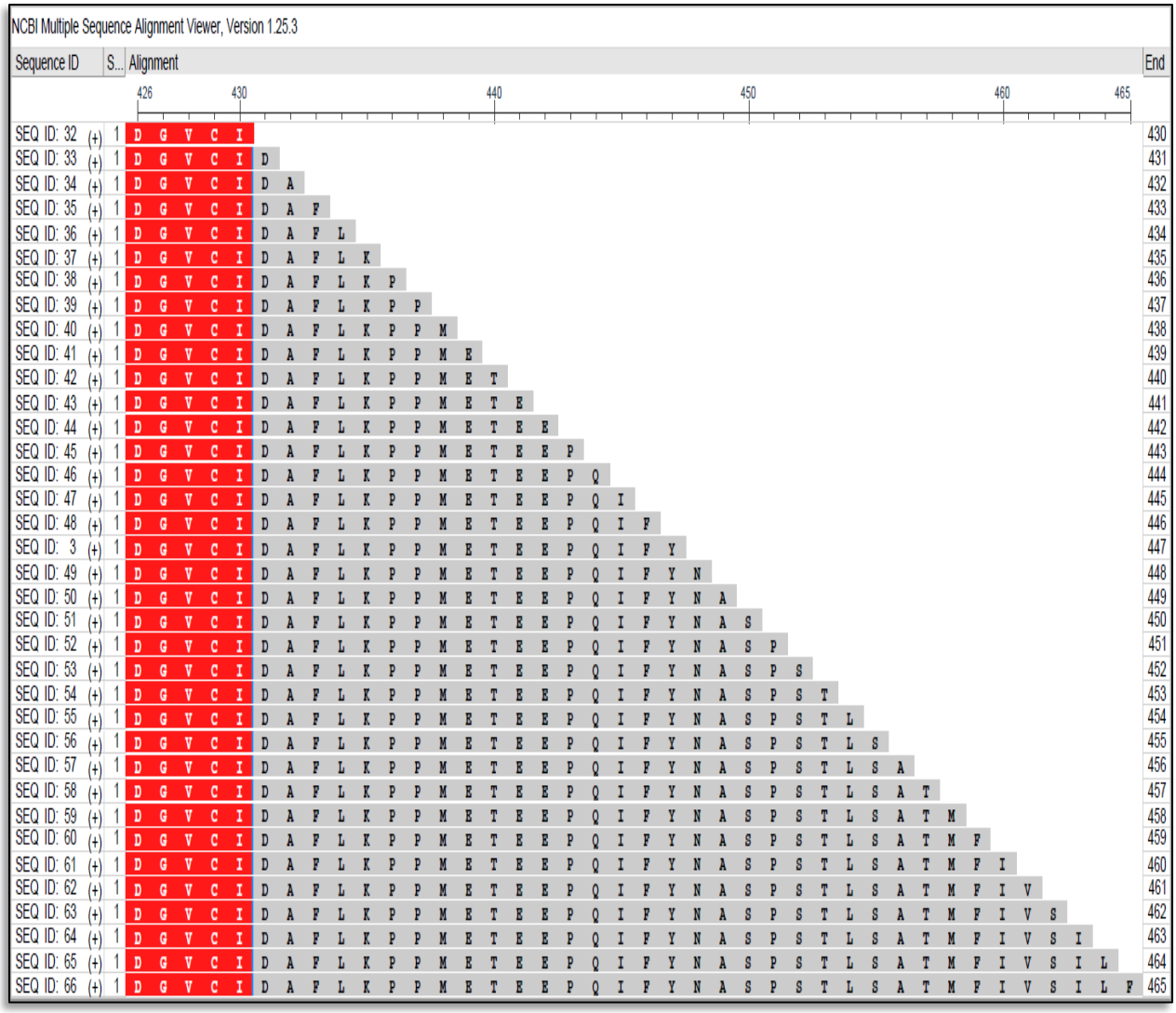
172. The inventors used one particular C-terminal truncated PH20 as the control in the '185 patent—SEQ ID NO: 3. EX1001, 5:2-6. The PH20 in SEQ ID NO: 3 lacks the signal sequence and includes 447 amino acids. *Id.*, 75:23-25. If SEQ ID NO: 3 included the 35 amino acid signal sequence, it would include 482 amino acids.

173. The inventors refer to the SEQ ID NO: 3 polypeptide as "wild-type rHuPH20" or sometimes simply as "wildtype" (or "wild type"). *See, e.g.*, EX1001, 226:8-14, 226:61-64, and 227:9-12. Throughout this declaration, for simplicity, I may refer to SEQ ID NO: 3 as wild-type PH20 or "wild type" instead of rHuPH20. SEQ ID NO: 3 is not a naturally occurring protein; it is a C-terminal truncated variant of native PH20. However, it does not have any substitutions—it is unmodified in that sense—so I refer to it as "wild type" in the context of this discussion, just as the inventors did in the patent.

174. To summarize, precursor wild-type human PH20 has 509 amino acids, contains a 35 amino acid signal peptide, and has the sequence in SEQ ID NO: 6. Mature wild-type human PH20 has 474 amino acids, does not have the 35 amino acid signal peptide, and has the sequence in SEQ ID NO: 7. rHuPH20, or SEQ ID NO: 3, is a C-terminal truncation variant of human PH20 that has 447 amino acids and does not have the signal sequence. And, to reiterate, I refer to SEQ ID NO: 3 as "wild type" for purposes of the discussion in this declaration, like the inventors did in the patent.

175. Now, there are other C-terminal truncation variants of PH20 disclosed in the specification and recited in the claims. Those variants are disclosed in SEQ ID NOs. 32-66. In combination with SEQ ID NO: 3, those sequences encompass PH20s ending at (mature sequence) positions 430 through 465, as illustrated in the figure below. The figure[9] below shows a sequence alignment I prepared aligning the C-terminal portions of SEQ ID NOs. 3 and 32-66 and showing that each successive sequence adds one amino acid at the C-terminal end.

[9] An enlarged copy of this Figure is presented in Appendix A.



C. Incorporated by Reference Teachings Regarding C-terminal Truncations and Glycosylation Sites

176. The ’429 patent (EX1005) and U.S. Patent Publication No. 2010/0143457 (“the ’457 publication”) (EX2165) describe foundational teachings regarding how to make soluble, active PH20.

177. I note that the ’429 patent and the ’457 publication are incorporated by reference into the ’185 patent, which I understand means that they are treated as if they are part of the disclosure of the ’185 patent.

1. The ’429 Patent

178. PH20, as it is expressed *in vivo*, is a membrane-bound protein due to its modification with a GPI anchor. The GPI anchor is attached at position 490 in the full sequence. POSAs seeking to work with PH20 before the ’429 patent had to contend with the fact that PH20 is expressed as a membrane-bound protein. That posed serious obstacles to using PH20 as a spreading factor; as the ’429 patent recognized, previous attempts to generate truncated PH20s (*i.e.,* to express a PH20 that would not have the GPI anchor) resulted in protein that did not secrete and was not active. EX1005, 4:1-7.

179. The ’429 patent solved this problem. The ’429 patent teaches POSAs what portion of the PH20 sequence is required to generate a protein that will be secreted from cells (without a GPI anchor), will remain soluble in the extracellular space, and will be enzymatically active.

180. The ’429 patent Example 4 describes using ELISA assays to measure hyaluronidase activity for C-terminal truncation mutants. EX1005, 86:4-88:24. The results are reproduced below:

AMINO ACID 1 TO:	U/ML/24 HRS PH 7.4
347	0.000
372	0.000
394	0.000
413	0.000
430	0.000
447	0.000
467	0.089
477	0.567
478	0.692
479	0.750
480	0.575
481	0.740
482	0.329
483	0.800
509	0.044

Id., 87:57-88:10.

181. I note that these results show the hyaluronidase activity in units per mL per 24 hours (in the right-hand column above) for a set of PH20 C-terminal truncation variants (listed in the left-hand column above). The PH20 C-terminal truncation variants tested stopped at (precursor sequence) positions 347, 372, 394, 413 and so on. The results show that PH20s that end at (precursor sequence position) 477-483 are soluble and active.

182. Dr. Hecht criticizes these results because there is variation between, for example, the 0.329 result for PH20 ending at position 482 and the 0.800 result for the PH20 ending at position 483. EX1003, ¶¶92-100. But that degree of variability is expected in hyaluronidase activity ELISA assays like this and is in line with what a POSA would expect. I note that Dr. Hecht admitted at deposition that he had not performed a hyaluronidase activity assay, nor has he performed any

assays with PH20. EX2076, 67:5-8, 64:12-13.

183. Consistent with what a POSA would expect for this type of assay, there is some variability in the numbers reported for activity. That variability would not concern a POSA. POSAs with experience running these types of assays expect there to be variability. That is because, in part, the reaction being measured is not a single substrate/single product reaction. Instead, HA is repeatedly cleaved to generate product. And the assay is measuring the HA remaining on the plate following the reaction. This assay set up has inherent variability. As a result, like anything in science with a large standard error, more replicates and/or greater absolute value changes are required to identify significant differences. Accordingly, POSAs would consider the difference between, for example, 0.800 and 0.044 to be significant because they differ by an order of magnitude, whereas POSAs would not consider the difference between 0.800 and 0.329 to be significant because they are in the same order of magnitude as truncation variants ending at positions 477-483.

2. The ’457 Publication

184. The inventors of the ’457 publication built further upon the findings of the ’429 patent by presenting data for additional C-terminal truncation mutants. EX2165.

185. The ’457 patent examples also used an ELISA assay to measure HA

activity. The results were reported in Tables 6 and 6A. EX2165, ¶362. I have combined Tables 6 and 6A into one table below:

Deletion Mutant	**Precursor (AA)**	**Mature (AA)**	**pH 7.4 Activity (Units/ml)**	**pH 5.5 Activity (Units/ml)**
VASL	509	474	1.8045	0.891
VASL + PLC	509	474	3.96	2.313
SPAM1-SSVA	507	472	1.4715	1.125
SPAM1-SSVA	507	472	1.782	1.256
SPAM1-IISS	505	470	1.458	0.837
SPAM1-IISS	505	470	1.863	0.932
SPAM1-FLII	503	468	0.9405	0.6345
SPAM1-FLII	503	468	1.094	0.648
SPAM1-LFLI	502	467	0.608	0.324
SPAM1-ILFL	501	466	0.0405	0.0405
SPAM1-ILFL	501	466	0.446	0.122
SPAM1-SILF	500	465	0.365	0.162
SPAM1-VSIL	499	464	0.02025	0.045
SPAM1-VSIL	499	464	0.486	0.122
SPAM1-IVSI	498	463	0.527	0.203
SPAM1-FIVS	497	462	0.1755	0.216
SPAM1-FIVS	497	462	0.365	0.162
SPAM1-TMFI	495	460	0.45	0.612
SPAM1-TMFI	495	460	0.689	0.77

Deletion Mutant	Precursor (AA)	Mature (AA)	pH 7.4 Activity (Units/ml)	pH 5.5 Activity (Units/ml)
SPAM1-SATM	493	458	0.5715	0.7335
SPAM1-SATM	493	458	0.689	0.851
SPAM1-TLSA	491	456	0.3645	0.5625
SPAM1-TLSA	491	456	0.851	0.729
SPAM1-STLS	490	455	0.819	1.2375
SPAM1-PSTL	489	454	1.557	1.089
SPAM1-PSTL	489	454	1.985	3.321
SPAM1-ASPS	487	452	1.017	0.9225
SPAM1-ASPS	487	452	1.134	1.58
SPAM1-YNAS	485	450	1.8765	1.74825
SPAM1-FYNA	484	449	1.4985	1.26225
SPAM1-IFYN	483	448	2.45025	2.3085
SPAM1-QIFY	482	447	2.03175	1.647
SPAM1-PQIF	481	446	1.818	1.701
SPAM1-EPQI	480	445	2.1825	1.6425
SPAM1-EEPQ	479	444	1.917	2.0745
SPAM1-TEEP	478	443	1.764	1.584
SPAM1-ETEE	477	442	2.088	2.0475

Deletion Mutant	Precursor (AA)	Mature (AA)	pH 7.4 Activity (Units/ml)	pH 5.5 Activity (Units/ml)
SPAM1-METE	476	441	1.332	1.278
SPAM1-PMET	475	440	2.223	2.0925
SPAM1-PPME	474	439	1.2105	1.341
SPAM1-KPPM	473	438	0.8595	0.91575
SPAM1-LKPP	472	437	0.5445	0.9
SPAM1-FLKP	471	436	3.321	2.79
SPAM1-AFLK	470	435	3.204	2.925
SPAM1-DAFL	469	434	2.3895	2.2365
SPAM1-IDAF	468	433	0.5625	0.62775
SPAM1-CIDA	467	432	0.5535	0.4725
SPAM1-VCID	466	431	0	0.2115
SPAM1-GVCI	465	430	0.441	0.468
SPAM1-DGVC	464	429	0	0.045
SPAM1-IADG	462	427	0	0.00225
SPAM1-VCIA	460	425	0	0.0135
SPAM1-VDVC	458	423	0.0495	0.0585
SPAM1-DAVD	456	421	0	0.0675
SPAM1-DTDA	454	419	0	0.054

Deletion Mutant	**Precursor (AA)**	**Mature (AA)**	**pH 7.4 Activity (Units/ml)**	**pH 5.5 Activity (Units/ml)**
SPAM1-VKDT	452	417	0.054	0.0225
SPAM1-ADVK	450	415	0.063	0.0405
HZ24-PH20	482	447	0.499	0.726188
CHO-S	n/a	n/a	0	0.012375

186. I note that, as was the case with the '429 patent data, there is variability in the results reported. Again, a POSA with hyaluronidase assay experience would expect that type of variability in this type of assay, and as a result would look for larger numerical differences and consistent trends among truncation positions to identify significant changes in activity.

187. In other words, in the table above, POSAs would not consider values of 2 and 3 to be meaningfully different, particularly between adjacent residues if not maintained as a trend. POSAs would consider values of 0.05 and 2 to be meaningfully different.

188. From the data in the table above, I created the table below showing what C-terminal truncation variants a POSA would have understood to be inactive, active, or have reduced or limited activity. To be clear, when I say "C-terminal position" in the chart below, I am referring to the position of the last amino acid in the sequence (*i.e.,* C-terminal position 430 in the mature sequence has 430 amino acids).

C-terminal Position (Precursor sequence)	**C-terminal Position (Mature sequence)**	**Expected Activity**
465	430	Inactive
466	431	Inactive
467	432	Reduced activity
468	433	Reduced activity
469	434	Active
470	435	Active
471	436	Active
472	437	Active
473	438	Active
474	439	Active
475	440	Active
476	441	Active
477	442	Active
478	443	Active
479	444	Active
480	445	Active
481	446	Active
482	447	Active
483	448	Active
484	449	Active

C-terminal Position (Precursor sequence)	**C-terminal Position (Mature sequence)**	**Expected Activity**
485	450	Active
486	451	Active
487	452	Active
488	453	Active
489	454	Active
490	455	Reduced activity
491	456	Reduced activity
492	457	Reduced activity
493	458	Reduced activity
494	459	Reduced activity
495	460	Reduced activity
496	461	Reduced activity
497	462	Reduced activity
498	463	Inactive/little activity
499	464	Inactive/little activity
500	465	Inactive/little activity

a. Mutation of Glycosylation Sites

189. The ’457 publication Example 10 described site-directed mutagenesis

of PH20 N-glycosylation sites. EX2165, ¶¶376-381.

190. Six mutants were generated, one for each of the N-glycosylation sites. EX2165, ¶377. In addition, three double mutants and a triple mutant were generated for asparagine residues N82, N166 and N254, and a double mutant N368A/N393A was also generated. *Id.* The secretion and activity were assessed and presented in Table 16 at pH 5.5 and pH 7.4. *Id.*, ¶378. I note that the residue numbers above refer to the precursor sequence, not the mature sequence.

191. Table 16 is reproduced below:

TABLE 16

Human PH20 Hyaluronidase Deglycosylation Mutants

Mutant	SEQ ID NO	Glycan Type	Secretion	Activity pH 5.5	Activity pH 7.4
PH20sHis (parent)	187	Both	YES	YES	YES
N82A	202	Complex	YES	YES	YES
N166A	203	Complex	YES	YES	YES
N235A	204	High Mannose (80%) Complex (20%)	YES	NO	NO
N254A	205	Complex	YES	YES	YES
N368A	188	High Mannose	NO	NO	NO
N393A	189	High Mannose	YES (WEAK)	NO	NO
N82A/N166A	206	Complex	YES	YES	YES
N82A/N254A	207	Complex	YES	YES	YES
N166A/N254A	208	Complex	YES	YES	YES
N82A/N166A/ N254A	209	Complex	YES	YES	YES
N368A/N393A	190	High Mannose	NO	NO	NO

EX2165, ¶378.

192. The results in Table 16 show which mutants were secreted and which

were active at neutral and acidic pH (here, pH of 5.5). These data show that the following PH20s with multiple mutations are active: N82A/N166A, N82A/N254A, N166A/N254A, N82A/N166A/N254A. Using the mature sequence positions, those variants are as follows: N47A/131A, N47A/N219A, N131A/N219A and N47A/N131A/N219A.

D. '185 patent Examples

193. The inventors designed elegant experiments that comprehensively assayed the activity of PH20 sequence variations, amassing carefully catalogued data that chronicle the effect of the mutations made at each of the 447 residues of the mature, soluble PH20 sequence.

1. Example 1—rHuPH20 (wild type) Production

194. Example 1 teaches, in detail, how the inventors made the rHuPH20 (SEQ ID NO: 3) that was used as a control (*i.e.,* wild type) in the rest of the examples. EX1001, 188:7-193:35. Example 1 describes first generating a cell line that expresses rHuPH20, then teaches how to generate rHuPH20 in cell culture in a 300 L bioreactor, and how to purify the rHuPH20 product. *Id.*

195. Example 1 teaches that the rHuPh20 produced had a hyaluronidase activity of 145 USP U/mL. EX1001, 193:25-31. I note that the USP (U.S. Pharmacopeia) has a standard hyaluronidase assay, and a POSA would have understood that the "145 USP U/mL" result refers to the result achieved in the USP

assay as reported in the standard "units per mL" format.

2. Example 2—Library Generation

196. The inventors first generated an extensive PH20 mutant library, as described in detail in Example 2 of the '185 patent. EX1001, 193:37-224:52. Example 2 of the patent describes the creation of a mutagenesis library in which the mature PH20 polypeptide was systematically mutated at each of its 447 residues by substituting at least one and up to 18 different amino acids. *Id.* Results are provided for, on average, 13 substitutions at each position. EX2166. Table 8 provides the resulting set of 6753 modified PH20 polypeptides. EX1001, 194:47-223:46.

3. Example 3—Hyaluronidase Assay Description

197. The inventors assessed hyaluronidase activity of the PH20 variants using an ELISA assay in which biotinylated hyaluronic acid ("bHA") substrate is bound to a microtiter plate. EX1001, 224:55-227:2. I am familiar with this type of assay and have performed it myself.

198. In the assay, at a high level, first, the bHA substrate is bound to plastic microtiter plates. EX1001, 225:44-45. Next, the transfected variant or wild-type PH20 supernatants were added to the plates and incubated. *Id.*, 226:8-21. Then, after washing, Streptavidin-HRP (horse radish peroxidase) was added to the plate. *Id.*, 226:22-29. The streptavidin binds the biotin on the HA (that is bound to the plate). TMB is added, which reacts with the HRP to generate a color. *Id.*, 226:39-

43. Stop solution is added to stop the color reaction, and the amount of color in each well is quantified using an ELISA reader. *Id.*, 226:43-47.

199. If the PH20 was highly active, it would have cleaved the plate-bound HA, and the resulting product would have been washed off the plate during the washing step. So, relatively speaking, little HA will remain on the plate at the end of the reaction, so there will be little biotin (on the HA) available to bind the streptavidin-HRP, and there will be little HRP on the plate available to react with the TMB and produce a color. As a result, highly active PH20s will produce wells with less color and lower absorbance values. *See* EX1001, 226:47-51.

200. Conversely, if the PH20 is not very active, relatively speaking, more HA will be on the plate, so more biotin will be available to bind the streptavidin, and more HRP will be present to react with TMB, generating a darker color. As a result, less active PH20s will produce darker colored wells with higher absorbance values. *See* EX1001, 226:47-51.

201. In addition to measuring hyaluronidase activity, SEAP activity in the supernatant was also measured. EX1001, 226:52-227:2. The inventors subcloned DNA that encoded PH20 into an expression vector that also expressed the gene for secreted alkaline phosphatase ("SEAP") under the same promoter. This is significant because with a single CMV promoter that drives expression of PH20 and SEAP in the construct, this expression vector allows for the co-expression of the

coding sequences of both genes, allowing the inventors to track and normalize the levels of PH20 expression across all of the variants. SEAP activity can be measured using ELISA to normalize and account for the amount of protein in the assay and the number of cells that received the DNA construct. In this way, the inventors were able to account for differences in both transfection and expression levels (and thus different amounts of PH20 in the supernatants that were tested).

202. In other words, the inventors (and POSAs) expect that, in assays like this that are testing supernatants, the amount of PH20 in each well will vary because some cells may receive more of the genetic construct than others and/or some cells will produce more protein than others and/or there could be slight differences in absolute cell numbers between wells. By normalizing to SEAP expression, the inventors were able to account for the amount of PH20 in each well when reporting their results.

203. The inventors accounted for variability between wells by restricting the reported active proteins to those with results within certain variability limits. Specifically, Example 3 states that for the high throughput ("HTP") screening, the transfected supernatant cutoff required a SEAP signal of ≥0.1 (meaning that a significant and reproducible number of cells in the well received and expressed the transfected construct) and the signal for the rHuPH20 wild-type control corresponding to the SEAP signals that met this cutoff requirement then needed to

produce a signal of ≥1 U/mL (which would be the theoretical minimum PH20 signal for the assay). EX1001, 226:61-64. The criteria for each screen were that the standard curves had a signal to noise ratio ("S/N") of ≥5 at 405 nm, defined as the difference between the absorbance value at 405 nm for the 3 U/mL standard versus the 0 U/mL standard. *Id.*, 226:64-227:2. In addition, variability of the results was restricted such that there were less than three standards with a coefficient of variation ("CV") of at least three was ≥10%, and at least four of the standards needed to be in the linear range of the standard curve for the assay. *Id.*, 226:64-227:2.

4. Example 4—Hyaluronidase Assay Results and Tables 9 and 10

204. Table 9 reports the results for "active" mutants. Specifically, Table 9 provides "the average hyaluronidase activity of tested duplicates normalized by SEAP values compared to average of wildtype PH20 activities in each plate, which were also normalized to their own SEAP values." EX1001, 227:22-26. "For example, a value of 0.40 indicates that the variant exhibits 40% of the hyaluronidase activity of wildtype PH20, a value of 1 indicates that the variant exhibits a similar hyaluronidase activity of wildtype and a value of 3.00 indicates that the variant exhibits 300% of the hyaluronidase activity of wildtype PH20 or 3-fold increased activity compared to wildtype." *Id.*, 227:26-32. The patent also lists the residues at each position that resulted in active PH20s in Table 3.

205. "The results in Table 9 show that over 600 tested mutants exhibit

activity that is increased compared to wildtype. For example, about 536 mutants exhibit 120% or >120% of the hyaluronidase activity of wildtype PH20 and about 75 of the mutants exhibit 300% or >300% of the hyaluronidase activity of wildtype PH20." *Id*., 227:33-38. The data show that at least one amino acid change made in 352 different positions in PH20 generated active polypeptides. EX1001, Table 9.

206. The data in Table 9 show that some substitutions substantially increased activity—by more than 2-fold, more than 3-fold, more than 5-fold or even more than 10-fold.

207. Of particular relevance to my opinions here, the inventors disclosed sixteen modified amino acids at amino acid residue number D320, including histidine (H), lysine (K), arginine (R), and serine (S). *See* EX1001, Table 8, Table 9. Thirteen of the modifications at residue D320 produced enzymatically active PH20, and four resulted in increased activity—H, K, R, and S. *See* EX1001, Table 3, Table 9.

208. A variant using lysine (K) provided the greatest improvement in activity—642% compared to wild-type—and is one of the highest reported increases in the patent. *Id*.

209. The inventors also identified the inactive mutants through initial screening as provided in Table 5. The patent provides the residues at each position that resulted in less than 20% activity in Table 5.

210. The inventors rescreened the inactive mutants in a "modified assay" involving a longer incubation time that was "intended to detect PH20 activities below 0.2 U/mL." *Id*., 262:24-54. Specifically, mutants that "exhibited less than 20% hyaluronidase activity of wildtype PH20, in at least one of the duplicates, were rescreened to confirm that the dead mutants are inactive." *Id*., 262:25-28. Table 10 provides the "reconfirmed inactive mutants," which again are those that achieved less than 20% of WT activity. *Id*., 262:51-52. The patent also provides the residues at each position that resulted in less than 20% activity in Table 5.

211. The Table 10 data show that at least one amino acid change made in 407 different positions in PH20 generated inactive polypeptides. EX1001, Table 10.

212. Using the methods described above, the inventors meticulously collected and presented comprehensive activity data for up to 18, and on average 13, substitutions at each of the 447 positions in the mature PH20 sequence.

5. Patent Example ELISA Assay Data Are Extremely Useful To POSAs

213. I note that the ELISA assays used in the patent are different from the assay used in the Zhang paper. The ELISA assays used in the patent cannot be used to, for example, calculate kinetic constants. But that does not mean they are not useful, as Dr. Hecht suggests. The inventors were not attempting to measure kinetic constants. The inventors sought to assess activity of mutants as compared to wild-type.

214. The data show how the activity and soluble expression of the thousands of tested variants compared to those properties of the wild-type enzyme and identified many beneficial substitutions, including some that dramatically increase activity. The data identify positions that do not tolerate substitutions and those that tolerate limited substitutions as compared to those that are more tolerant to substitution. The data identify changes that abolish activity, those that maintain activity, and those that significantly improve activity.

215. In the context of an assay like this, the POSA is not concerned with small changes in activity. The POSA uses these data to understand big picture concepts in terms of what changes to make and where to retain, abolish, reduce or improve activity. To reiterate, these data are extremely useful to POSAs.

6. Example 5

216. Example 5 in the '185 patent describes an "Assay for Hyaluronidase Activity Under Temperature and Phenophilic Conditions." EX1001, 273:27-28. In the example, "[s]upernatants from PH20 activity variants set forth in Table 9, as identified in Example 4, were tested for stability under thermophilic and/or phenophilic conditions." *Id.*, 273:31-33. POSAs would have understood from this statement that the modified PH20s tested in the primary screen in Example 5 are from the set of active modified PH20s identified in Table 9 through the testing described in Example 4.

217. The '185 patent explains that "phenolic compounds" like metacresol (m-cresol) are "the most common preservatives" for "parenteral drug products" and that "[m]ultidose formulations must contain antimicrobial preservatives to protect them from microbial contamination." *Id.*, 104:61-65. The patent also explains that "PH20 hyaluronidase, such as rHuPH20, rapidly loses activity in the presence of preservatives, likely due to unfolding of the protein and subsequent aggregate formation," and specifically, "following incubation with 0.4% m-cresol for 4 hours, PH20 (e.g., rHuPH20) retains only about 10% of its activity (see e.g., Example 5)." *Id.*, 105:57-60, 64-66. POSAs thus would have understood that inventors sought to make modified PH20s with improved stability in the presence of common phenolic preservatives (hence the term "phenophile").

218. The Example 5 assay is a modified version of the Example 3 hyaluronidase activity assay in that it also included a four-hour incubation of samples with and without m-cresol at 37° C. EX1001, 273:32-38. The Example 5 assay "was used to identify PH20 mutants with thermophilic properties (activity greater at 37° C. condition than at 4° C.) and/or with phenolphilic properties (greater activity in the presence of m-cresol than wildtype PH20)." *Id.*, 273:40-43.

219. As noted above, Example 5 describes a primary screen on the Table 9 modified PH20. Example 5 teaches that, for the primary screen, the "results were depicted as the percent (%) activity remaining under each of the following

parameters: ratio of activity at 1) 37° C. preincubation without m-cresol/4° C.; 2) 37° C. after preincubation with m-cresol/4° C.; and 3) 37° C. after preincubation with m-cresol/after preincubation at 37° C. without m-cresol." *Id.*, 274:61-66.

220. The inventors then identified those modified PH20s with ≥20% of the original activity at 37° C for rescreening. *Id.*, 274:66-275:2. For the rescreen, the inventors performed a new round of transfection and expression in CHO-S cells and then tested for "hyaluronidase activity under various conditions as described above: 1) non-preincubated transfected variant supernatant (no incubation; 4° C).; 2) preincubated transfected variant supernatants preincubated at 37° C for 4 hours with 0.4% m-cresol (Cresol; 37° C.); or 3) preincubated transfected variant supernatant preincubated at 37° C. for 4 hours without 0.4% m-cresol (no cresol; 37° C.)." *Id.*, 275:3-32. The inventors then determined the hyaluronidase activity for each modified PH20 and the "percent activity was determined as a ratio of activity at 37° C./4° C., 37° C. plus m-cresol/4° C., and at 37° C. plus m-cresol/37° C." *Id.*, 275:33-38. The results of the experiment are provided in Tables 11 and 12 in the patent. Table 11 provides the "absolute hyaluronidase activity" for each tested PH20, expressed in U/mL (units per mL). *Id.*, 275:33-38. Table 12 provides the percent activity of duplicates 1 and 2.

221. The inventors highlighted the results for one particular mutant, F204P, in that the "assay showed that the F204P mutant protein was highly resistant" to m-

cresol because it retained "approximately equal" activity with and without m-cresol. *Id.*, 293:26-50. By contrast, "[t]he positive control (WT PH20-OHO) showed a reduction in activity of 75% and 83% on the day of the assay (as assayed from two different OHO transfections)." *Id.*, 293:50-53.

222. The patent includes additional examples involving F204P and another variant, V58R.

7. Responses to Dr. Hecht's Comments Regarding Stability Assays

223. In paragraph 62, Dr. Hecht states "[m]any 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." EX1003, ¶62. It is not clear why Dr. Hecht makes this point. To the extent Dr. Hecht suggests that POSAs would not understand the conditions to use when using PH20s to ensure that the PH20 retains its fold and activity, I disagree. POSAs with hyaluronidase experience know what conditions to use. Also, the '185 patent (and the '429 patent and the '457 publication) teach the conditions used in the various assays.

224. Dr. Hecht criticizes the stability testing in the patent because the patent measured hyaluronidase activity in the presence of, for example, m-cresol, and then referred to the results in terms of impacts on stability. *E.g.*, EX1003, ¶¶67-69. Dr. Hecht explains, for example, that the example 5 testing does not

include a "thermodynamic assessment" that would "indicate that the measured activity under the tested conditions is due to increased stability, or is due to others factors, such as more efficient catalysis within an equivalently stable protein." *Id.*, ¶69. But the inventors are not evaluating the impacts of mutations on thermodynamics. The inventors are evaluating the impacts of mutations on protein activity in the presence of m-cresol. In view of the patent's teaching that m-cresol causes wild-type PH20 to lose activity (which the inventors attribute to likely unfolding), it makes sense that the inventors characterized modified PH20s having improved activity in the presence of m-cresol as having improved stability.

225. Dr. Hecht also criticizes Example 5 because there was "significant variability" in the results obtained for the controls. EX1003, ¶70. But as I have explained repeatedly, POSAs expect variability for these types of assays. And for this assay in particular, which involves incubating PH20 for four hours with a reagent known to denature PH20 (*i.e.,* m-cresol), a POSA would expect even greater levels of variability. POSAs would not have been concerned by the variability reported.

226. Dr. Hecht concludes that the reported variability "raises serious doubts about how probative or instructive the values for individual tested mutants are that fall within the range of variability observed for the control." EX1003, ¶72. It is not clear what Dr. Hecht means by this statement, but to the extent Dr. Hecht

is opining that there are serious doubts about the reliability of data with this level of variability, I strongly disagree. POSAs with hyaluronidase assay experience would not reach that conclusion.

E. Alignments with Primate PH20s

227. The patent provides "exemplary alignments of human soluble PH20 set forth in SEQ ID NO:3 with other PH20 polypeptides," including those of several primate PH20s, in Figure 2A-2L. EX1001, 43:9-11. In each alignment, an asterisk "*" means that the aligned residues are identical, a colon ":" means that aligned residues are not identical, but are similar, and contain conservative amino acid changes at the aligned position, and a period "." means that the aligned residues are similar and contain semi-conservative amino acid changes at the aligned position. *Id.*, 43:11-16. The patent teaches that "[e]xemplary, non-limiting, corresponding positions for amino acid replacements are indicated by highlighting" in the figures. *Id.*, 43:16-18.

228. Specifically, "FIG. 2A depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with chimpanzee PH20 set forth in SEQ ID NO:10." *Id.*, 43:18-20. Figure 2A is reproduced below:



229. Similarly, FIG. 2J depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Gibbon PH20 set forth in SEQ ID NO:857, and FIG. 2L depicts the alignment of a human soluble PH20 set forth in SEQ ID NO:3 with Orangutan PH20 set forth in SEQ ID NO:861. *Id.*, 43:39-40, 44-46.

230. Notably, the patent teaches that "the modifications provided herein also can be made in a PH20 polypeptide set forth as SEQ ID NO: 10, 12, 14, 24, 857, 859, 861 or 870." *Id.*, 76:8-11. The POSA would have understood from this teaching that the substitutions described in the patent could be made in, for example, chimpanzee, gibbon, and orangutan PH20 (SEQ ID NOs: 10, 857 and 861, respectively).

231. As I explained above in detail, SEQ ID NO. 3 is a C-terminal truncated version of the mature PH20 that terminates at position 447. The chimpanzee sequence shown in the figure and disclosed in the patent is a full-length sequence (*i.e.*, it has no C-terminal truncation). Accordingly, it ends at position 474 (as does the mature human PH20 that is not C-terminally truncated, as described above). I note from reviewing the alignments in the patent that the C-terminal portion of the chimpanzee sequence is similar to that for the orangutan and gibbon sequences.

232. I generated a multiple sequence alignment using CLUSTAL OMEGA for the full-length mature human PH20 (SEQ ID NO: 7), chimpanzee PH20, gibbon PH20 and orangutan PH20. In the figure below, I drew a red box around the C-terminal end of the PH20s showing the portion of the sequence after position 447 (which is where SEQ ID NO: 3 ends). The sequences are similar (and in fact, are identical, with the exception of the orangutan sequence).

```
orangutan       LNFRAPPIIPNMPFLWAWNAPSEFCLGKFDEPLDMSLFSLIGSPRINVTGQAVTIFYVDR    60
gibbon          LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSLTGSPRINVTGQGVTIFYVDR    60
Human           LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR    60
Chimpanzee      LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINVTGQDVTIFYVDR    60
                *******:***:**************************: ******.*** ********

orangutan       LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDILFYMPVDNLGMAVIDWEEWRPTWA    120
gibbon          LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKQDITFYMPVDNLGMAVIDWEEWRPTWA    120
Human           LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA    120
Chimpanzee      LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA    120
                ********************************:** **********************

orangutan       RNWKPKDVYKNRSIELVQQQNVQLNLTEATEKAKQEFEKAGKDFMVETIKLGKLLRPNHL    180
gibbon          RNWKPKDVYKNRSIELVQQQNVQLSLAEATEKAKQEFEKAGKDFMVETIKLGKLLRPNHL    180
Human           RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL    180
Chimpanzee      RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL    180
                ************************.*:*****************:***************

orangutan       WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT    240
gibbon          WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT    240
Human           WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT    240
Chimpanzee      WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT    240
                ************************************************************

orangutan       LYVRNRVREAIRVSKIPDAKSPLPVFVYARIVFTDQVLKFLSQDELVYTFGETVALGASG    300
gibbon          LYVRNRVREAIRVSKIPDAKSPLPVFVYARIVFTDQVLKFLSRDELVYTLGETVALGASG    300
Human           LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG    300
Chimpanzee      LYVRNRVQEAIRVSKIPDAKSPLPVFVYTRIVFTDQVLKFLSQDELVYTFGETVALGASG    300
                *******:*****************.*:*************:******:**********

orangutan       IVIWGSLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSS    360
gibbon          IVIWGSLSIVRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKDWNSS    360
Human           IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS    360
Chimpanzee      IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS    360
                *****:***:**********************************************:****

orangutan       DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV    420
gibbon          DYLHLNPDNFAIQLEKGGKFTVRGKPTPEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV    420
Human           DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV    420
Chimpanzee      DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV    420
                *************************** ********************************

orangutan       DVCIADGVCIDAFLKPPMETEESQIFYNASPSTLSATMFIWRLEVWDQG-ISRMGFF       476
gibbon          DVCIADGVCIDAFLKPPKETEESQIFYNASPSTLSATMFIVSILFLIISSVVSL---       474
Human           DVCIADGVCIDAFLKPPMETEEPQIFYNASPSTLSATMFIVSILFLIISSVASL---       474
Chimpanzee      DVCIADGVCIDAFLKPPMETEESQIFYNASPSTLSATMFIVSILFLIISSVASL---       474
                *************** **** ***************  : .   . :  :
```

233. The POSA would have understood that the portions of the sequence in the red box above can be truncated with the same goal of removing the GPI anchor

and producing a soluble, active PH20 for each of the orangutan, gibbon and chimpanzee sequences. In other words, POSAs would have understood that chimpanzee, orangutan, and gibbon PH20s ending at position 447 would be soluble and active.

234. As noted above, the patent teaches that substitutions can be made in, for example, chimpanzee, gibbon, and orangutan PH20s. To evaluate the differences between chimpanzee, gibbon, and orangutan PH20s, and SEQ ID NO. 3, I generated a series of pairwise alignments using BLAST.

235. I generated a pairwise alignment using BLAST of the chimpanzee PH20 sequence disclosed in the patent (SEQ ID NO: 10) truncated at position 447 and SEQ ID NO: 3. As shown in the alignment below, the truncated chimpanzee PH20 differs from SEQ ID NO: 3 by five residues.

NW Score	Identities	Positives	Gaps
2376	442/447(99%)	443/447(99%)	0/447(0%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    ..............................................V...D.........  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ............................................................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ............................................................  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  .......Q..................V.............................  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  ............................................................  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ............................................................  420

Query  421  DVCIADGVCIDAFLKPPMETEEPQIFY  447
Sbjct  421  ......................S....  447
```

236. Introducing a D320K substitution into C-terminally truncated chimpanzee PH20 results in a protein that is 99% identical to SEQ ID NO: 3, as shown in the below alignments (generated using BLAST). A POSA would have expected this protein to be enzymatically active.

Score	Expect	Method	Identities	Positives	Gaps
922 bits(2384)	0.0	Compositional matrix adjust.	441/447(99%)	442/447(98%)	0/447(0%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    ............................................V...D.........  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ............................................................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ............................................................  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  .......Q...................V..............................  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  ...................K........................................  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ............................................................  420

Query  421  DVCIADGVCIDAFLKPPMETEEPQIFY  447
Sbjct  421  ......................S....  447
```

237. I generated a pairwise alignment using BLAST of the gibbon PH20 sequence disclosed in the patent (SEQ ID NO: 857) truncated at position 447 and SEQ ID NO: 3. As shown in alignment below, the truncated gibbon PH20 differs from SEQ ID NO: 3 at 16 positions.

Score	Expect	Method	Identities	Positives	Gaps
902 bits(2331)	0.0	Compositional matrix adjust.	431/447(96%)	437/447(97%)	0/447(0%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    ....................................LT......V.............  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ................................Q.........................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ........................A..................M...............  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  ........................V.A.............R......L..........  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  .....S...V.............................................D....  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ........................P...............................  420

Query  421  DVCIADGVCIDAFLKPPMETEEPQIFY  447
Sbjct  421  ................K....S....  447
```

238. Introducing a D320K substitution into C-terminally truncated gibbon PH20 results in a protein that is 96% identical to SEQ ID NO: 3, as shown in the below alignments (generated using BLAST). A POSA would have expected this protein to be enzymatically active.

Score	Expect	Method	Identities	Positives	Gaps
903 bits(2334)	0.0	Compositional matrix adjust.	430/447(96%)	436/447(97%)	0/447(0%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    ....................................LT......V.............  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ................................Q.........................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ........................A.................M...............  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  ........................V.A.............R......L..........  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  .....S...V.........K.................................D....  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ........................P...........................P.....  420

Query  421  DVCIADGVCIDAFLKPPMETEEPQIFY  447
Sbjct  421  ................K....S....  447
```

239. I generated a pairwise alignment using BLAST of the orangutan PH20 sequence disclosed in the patent (SEQ ID NO: 861) truncated at position 447 and SEQ ID NO: 3. As shown in the alignment below, the truncated orangutan PH20 differs from SEQ ID NO: 3 at 13 positions.

Score	Expect	Method	Identities	Positives	Gaps
910 bits(2353)	0.0	Compositional matrix adjust.	434/447(97%)	440/447(98%)	0/447(0%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    .......I...M...........................L.......V...A........  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ....................................L.......................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ........................N...................M...............  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

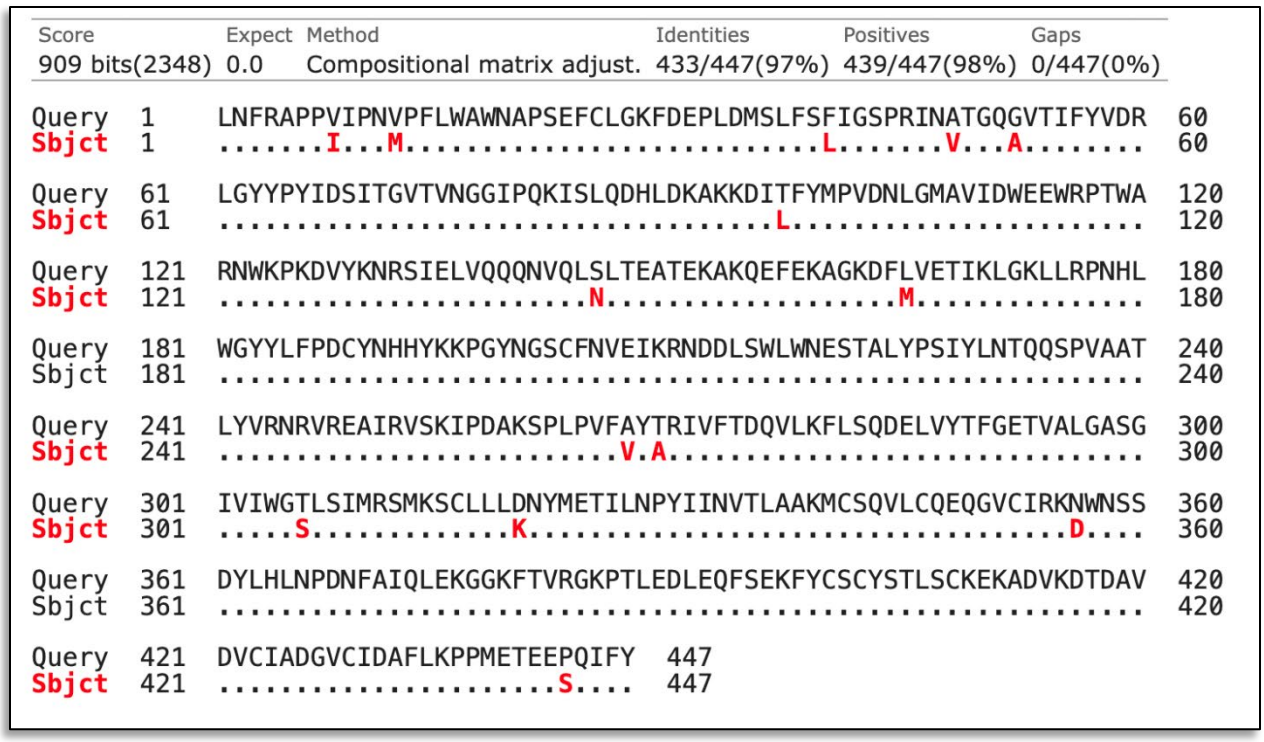
Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  ............................V.A...............................  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  .....S.....................................................D....  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ............................................................  420

Query  421  DVCIADGVCIDAFLKPPMETEEPQIFY  447
Sbjct  421  ......................S....  447
```

240. Introducing a D320K substitution into C-terminally truncated orangutan PH20 results in a protein that is 97% identical to SEQ ID NO: 3, as shown in the below alignments (generated using BLAST). A POSA would have expected this protein to be enzymatically active.



Score	Expect	Method	Identities	Positives	Gaps
909 bits(2348)	0.0	Compositional matrix adjust.	433/447(97%)	439/447(98%)	0/447(0%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    .......I...M.............................L.......V...A.........  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ....................................L.......................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ........................N...................M...............  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  ............................V.A.............................  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  .....S.............K.....................................D....  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ............................................................  420

Query  421  DVCIADGVCIDAFLKPPMETEEPQIFY  447
Sbjct  421  .......................S....  447
```

241. I generated a pairwise alignment using BLAST of the gibbon PH20 sequence disclosed in the patent (SEQ ID NO: 857) truncated at position 447 and SEQ ID NO: 44. As shown in alignment below, the truncated gibbon PH20 differs from SEQ ID NO: 44 at 20 positions.

NW Score	Identities	Positives	Gaps
2287	427/447(96%)	433/447(96%)	5/447(1%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    ....................................LT......V.............  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ................................Q.............................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ........................A.................M................  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  ........................V.A.............R......L..........  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  .....S...V.............................................D....  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ........................P...............................  420

Query  421  DVCIADGVCIDAFLKPPMETEE-----  442
Sbjct  421  ................K....SQIFY  447
```

242. Introducing a D320K substitution into C-terminally truncated gibbon PH20 results in a protein that is 95% identical to SEQ ID NO: 44, as shown in the below alignment (generated using BLAST). A POSA would have expected this protein to be enzymatically active.

NW Score	Identities	Positives	Gaps
2280	426/447(95%)	432/447(96%)	5/447(1%)

```
Query  1    LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDR  60
Sbjct  1    ....................................LT......V.............  60

Query  61   LGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWA  120
Sbjct  61   ................................Q.........................  120

Query  121  RNWKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL  180
Sbjct  121  ........................A..................M...............  180

Query  181  WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAAT  240
Sbjct  181  ............................................................  240

Query  241  LYVRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASG  300
Sbjct  241  ........................V.A.............R......L..........  300

Query  301  IVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS  360
Sbjct  301  .....S...V.........K.................................D....  360

Query  361  DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAV  420
Sbjct  361  ........................P...........................------  420

Query  421  DVCIADGVCIDAFLKPPMETEE-----  442
Sbjct  421  ................K....SQIFY  447
```

F. Dr. Petsko's Patent Data Analysis

243. I have reviewed the Declaration of Dr. Petsko, EX2070 (Petsko Decl.), including the spreadsheet (EX2166) containing data from Tables 3, 5, 9 and 10 of the '185 patent. EX2070, ¶¶263-296.

244. Dr. Petsko's spreadsheet (EX2166) shows the number (and percentage) of positions for which all tested[10] substitutions retain activity. Those data show the tolerance of each position to substitution and show how a POSA would have used the patent data. I have pasted an excerpt of the highlighted spreadsheet below, in

[10] I understand that Dr. Petsko uses the term "tested" to refer to substitutions that are identified as active or inactive in Tables 9 and 10.

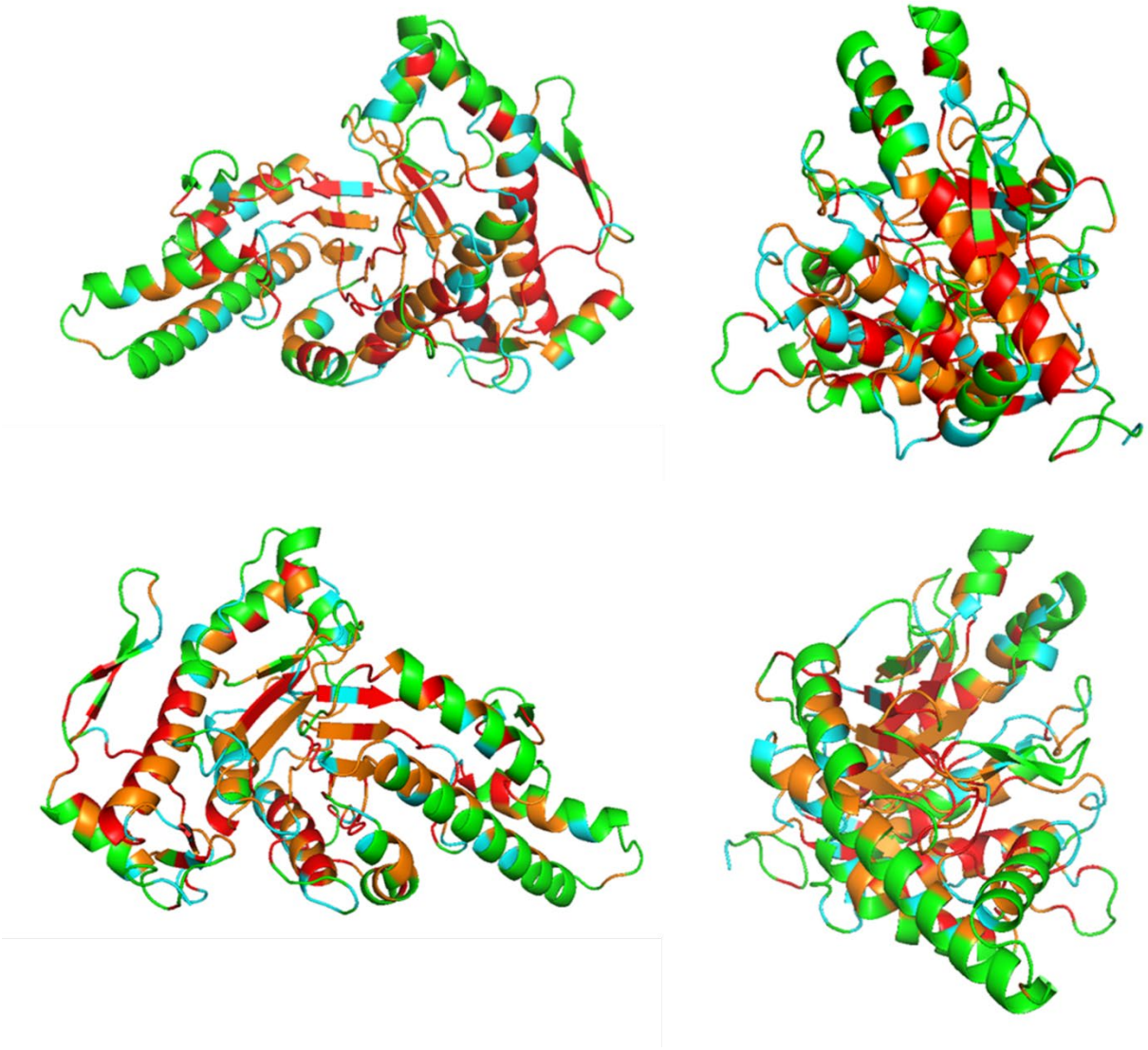
which positions for which all substitutions were tolerated are colored green, and those for which no substitutions were tolerated are colored red. Positions for which 60% or more of the substitutions tested were tolerated are colored light green, and positions for which 30% or less of the substitutions were tolerated are colored orange. Finally, those residues that are not colored were shown to tolerate more than 30% but less than 60% of the tested substitutions.

Position	WT	30	D	61	L	92	A	123	W	154	K	185	L
1	L	31	E	62	G	93	K	124	K	155	Q	186	F
2	N	32	P	63	Y	94	K	125	P	156	E	187	P
3	F	33	L	64	Y	95	D	126	K	157	F	188	D
4	R	34	D	65	P	96	I	127	D	158	E	189	C
5	A	35	M	66	Y	97	T	128	V	159	K	190	Y
6	P	36	S	67	I	98	F	129	Y	160	A	191	N
7	P	37	L	68	D	99	Y	130	K	161	G	192	H
8	V	38	F	69	S	100	M	131	N	162	K	193	H
9	I	39	S	70	I	101	P	132	R	163	D	194	Y
10	P	40	F	71	T	102	V	133	S	164	F	195	K
11	N	41	I	72	G	103	D	134	I	165	L	196	K
12	V	42	G	73	V	104	N	135	E	166	V	197	P
13	P	43	S	74	T	105	L	136	L	167	E	198	G
14	F	44	P	75	V	106	G	137	V	168	T	199	Y
15	L	45	R	76	N	107	M	138	Q	169	I	200	N
16	W	46	I	77	G	108	A	139	Q	170	K	201	G
17	A	47	N	78	G	109	V	140	Q	171	L	202	S
18	W	48	A	79	I	110	I	141	N	172	G	203	C
19	N	49	T	80	P	111	D	142	V	173	K	204	F
20	A	50	G	81	Q	112	W	143	Q	174	L	205	N
21	P	51	Q	82	K	113	E	144	L	175	L	206	V
22	S	52	G	83	I	114	E	145	S	176	R	207	E
23	E	53	V	84	S	115	W	146	L	177	P	208	I
24	F	54	T	85	L	116	R	147	T	178	N	209	K
25	C	55	I	86	Q	117	P	148	E	179	H	210	R
26	L	56	F	87	D	118	T	149	A	180	L	211	N
27	G	57	Y	88	H	119	W	150	T	181	W	212	D
28	K	58	V	89	L	120	A	151	E	182	G	213	D
29	F	59	D	90	D	121	R	152	K	183	Y	214	L
		60	R	91	K	122	N	153	A	184	Y	215	S

216	W	247	V	278	L	309	I	340	M	371	A	402	C	433	F
217	L	248	R	279	K	310	M	341	C	372	I	403	Y	434	L
218	W	249	E	280	F	311	R	342	S	373	Q	404	S	435	K
219	N	250	A	281	L	312	S	343	Q	374	L	405	T	436	P
220	E	251	I	282	S	313	M	344	V	375	E	406	L	437	P
221	S	252	R	283	Q	314	K	345	L	376	K	407	S	438	M
222	T	253	V	284	D	315	S	346	C	377	G	408	C	439	E
223	A	254	S	285	E	316	C	347	Q	378	G	409	K	440	T
224	L	255	K	286	L	317	L	348	E	379	K	410	E	441	E
225	Y	256	I	287	V	318	L	349	Q	380	F	411	K	442	E
226	P	257	P	288	Y	319	L	350	G	381	T	412	A	443	P
227	S	258	D	289	T	320	D	351	V	382	V	413	D	444	Q
228	I	259	A	290	F	321	N	352	C	383	R	414	V	445	I
229	Y	260	K	291	G	322	Y	353	I	384	G	415	K	446	F
230	L	261	S	292	E	323	M	354	R	385	K	416	D	447	Y
231	N	262	P	293	T	324	E	355	K	386	P	417	T		
232	T	263	L	294	V	325	T	356	N	387	T	418	D		
233	Q	264	P	295	A	326	I	357	W	388	L	419	A		
234	Q	265	V	296	L	327	L	358	N	389	E	420	V		
235	S	266	F	297	G	328	N	359	S	390	D	421	D		
236	P	267	A	298	A	329	P	360	S	391	L	422	V		
237	V	268	Y	299	S	330	Y	361	D	392	E	423	C		
238	A	269	T	300	G	331	I	362	Y	393	Q	424	I		
239	A	270	R	301	I	332	I	363	L	394	F	425	A		
240	T	271	I	302	V	333	N	364	H	395	S	426	D		
241	L	272	V	303	I	334	V	365	L	396	E	427	G		
242	Y	273	F	304	W	335	T	366	N	397	K	428	V		
243	V	274	T	305	G	336	L	367	P	398	F	429	C		
244	R	275	D	306	T	337	A	368	D	399	Y	430	I		
245	N	276	Q	307	L	338	A	369	N	400	C	431	D		
246	R	277	V	308	S	339	K	370	F	401	S	432	A		

245. I understand that Dr. Petsko also generated a homology model for PH20 using SwissModel. EX2070, ¶¶297-342. Dr. Petsko then highlighted the PH20 homology model he generated to correspond with the spreadsheet. Specifically, residues for which no tested substitutions were tolerated were colored red, residues for which 30% or fewer changes were tolerated were colored orange, and residues for which 60% or more of the substitutions were tolerated were colored green. Dr. Petsko generated the figures below of the highlighted model. In these figures, the

model is rotated around the Y-axis in 90-degree increments.



EX2070, ¶299.

246. The ’185 patent provides a detailed functional map of PH20 through its comprehensive and systematic analysis of PH20, achieved through large-scale mutagenesis and enzymatic activity testing of thousands of variants, with up to 18 mutations per position. The results show positions that a POSA would understand are critical and tolerate minimal modification or no modification, as well as residues

that do tolerate substitution, and particular substitutions that substantially increase activity.

247. The ’185 patent thus provides considerable guidance regarding whether particular mutations are likely to eliminate or retain activity.

G. Claims

248. I understand that the ’185 patent, as issued, contains 11 total claims. As noted above, I understand that claims 5-6 have been disclaimed and are not, therefore, part of this post-grant proceeding, leaving a total of 9 claims.

249. I understand that claim 1 is the sole independent claim in the ’185 patent. I understand that claims 2-4 and 7-11 are dependent claims and contain all elements of claim 1, as well as any additional claims from which they depend.

250. I have reproduced claim 1 below:

> 1. A method for increasing delivery of a therapeutic agent to a subject, comprising administering to a subject a pharmaceutical composition comprising:
>
> (a) a therapeutic agent; and
>
> (b) a modified PH20 polypeptide comprising an amino acid sequence, wherein:
>
> i. 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

ii. 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

iii. the modification at position 320 is a replacement selected from the group consisting of H, K, R and S.

251. The modified PH20 polypeptide of claim 1 is thus defined by the recited structure: the amino acid sequences, the mutation at D320 in reference to SEQ ID No. NO: 3, and the 95% sequence identification.

1. Response to Dr. Hecht's Characterization of the 36 SEQ ID NOs. Recited in the Claims

252. Dr. Hecht states that the "focus of the patent claims, as I explain below, are modified PH20 polypeptides that have incorporated at least one amino acid substitution at position 320, and optionally may incorporate 16 to 22 additional substitutions at any other position in the wild-type sequence of 36 different PH20 polypeptides of varying length…." EX1003, ¶34.

253. Dr. Hecht's characterization that the claims recite "36 different PH20

polypeptides of varying lengths" could suggest that the claimed modified PH20s are more structurally diverse than what a POSA would understand to be the case, so I will clarify.

254. The claims require (1) one of four substitutions at position 320 and (2) at least 95% sequence identity to one of SEQ ID NOs. 3 or 32-66. From (mature) positions 1-430, SEQ ID NOs. 3 and 32-66 are identical. From position 430 and on, they differ successively by one amino acid, as shown in the alignment figure in paragraph 175 above. Together, SEQ ID NOs. 3 and 32-66 cover PH20s that end at (mature sequence) positions 430 through 465, with the C-terminal residue at (mature sequence) position 430-465.

IX. CLAIM CONSTRUCTION

255. I understand that the term "modified PH20 polypeptide" recited in the claims has been preliminarily construed to require at least some hyaluronidase activity. I understand that the Patentee does not dispute that the modified PH20 polypeptides covered by the '185 patent claims have at least some hyaluronidase activity.

256. I have been asked to consider whether the POSA would have understood the term "modified PH20 polypeptide" as used in the claims to require at least 40% activity. In my opinion, a POSA would have understood that the claims do not require ≥ 40% hyaluronidase enzymatic activity, as suggested by Dr. Hecht.

EX1003, ¶¶126-135.

257. In particular, I understand that Dr. Hecht testified at deposition that the claims require ≥ 40% hyaluronidase activity compared to wildtype, such that a modified PH20 polypeptide that otherwise was covered by the claims would not be covered if it only had 39% (or less) activity. EX2076 (Hecht Tr.), 165:1-13. I do not agree.

258. As I discussed in Section VI.A., I understand that when a patent explicitly defines a claim term in the specification, the patent's definition controls.

259. The '185 patent explicitly defines "modified PH20 polypeptide," in the first sentence of the following paragraph, as follows:

> As used herein, "modified PH20 polypeptide" or "variant PH20 polypeptide" refers to a PH20 polypeptide that contains at least one amino acid modification, such as at least one amino acid replacement as described herein, in its sequence of amino acids compared to a reference unmodified PH20 polypeptide. A modified PH20 polypeptide can have up to 150 amino acid replacements, so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity. Typically, a modified PH20 polypeptide contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acid

> replacements. It is understood that a modified PH20 polypeptide also can include any one or more other modifications, in addition to at least one amino acid replacement as described herein.

EX1001, 48:38-53.

260. The first sentence in the passage above defines "modified PH20 polypeptide" by reference to an amino acid sequence (its primary structure), not a particular function. It describes only structural features: (i) a sequence of amino acids (ii) with at least one amino acid modification (iii) relative to a reference sequence. EX1001, 48:41-46. These structural features are similar to the structural features listed in claim 1, and this description does not imply or specify importing the 40% activity threshold.

261. I understand that Dr. Hecht suggested that the second sentence in the passage above also defines a "modified PH20 polypeptide" and requires hyaluronidase activity for all modified PH20 polypeptides.

262. In my opinion, this would be inconsistent with how the specification uses the term "modified PH20 polypeptides" because it says "modified PH20 polypeptides" can be inactive throughout.

263. A POSA would see that multiple places in the specification describe "modified PH20 polypeptides" as inactive, which is inconsistent with treating the second sentence as a definition.

264. The outline at the beginning of the Detailed Description, for instance, lists both "Active Mutants" and "Inactive Mutants" under the section "Modified PH20 Polypeptides." EX1001, 43:57-63.

265. This is consistent with the use of the term throughout the specification based on my review.

266. The specification explains, for example, that some modified PH20 polypeptides are inactive:

> Inactive Mutants: Provided herein are modified PH20 polypeptides that contain one or more amino acid replacements in a PH20 polypeptide and that are inactive, whereby the polypeptides do not exhibit hyaluronidase activity or exhibit low or diminished hyaluronidase activity.

EX1001, 115:40-45.

267. Similarly, the specification states that "modified PH20 polypeptides" that are "inactive" can have low or no discernable activity:

> The modified PH20 polypeptides provided herein that are inactive generally exhibit less than 20%, such as less than 10%, of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the polypeptide set forth in SEQ ID NO: 3 or 7. For example … less than 9%, 8%, 7%, 6%, 4%, 3%, 2%, 1%, … 0.05% or less … hyaluronidase activity….

EX1001, 115:45-57.

268. Other examples of the specification describing that "modified PH20 polypeptides" can be inactive are below, with bolded language to ease review:

- "Also provided are **modified PH20 polypeptides** that are **inactive**, and that can be used, for example, as antigens in contraception vaccines" EX1001, 75:56-58 (emphasis added);
- "For example, provided herein are **PH20 polypeptides** that are **inactive** and **that are modified**, for example by amino acid replacement or substitution, compared to a wildtype or reference PH20 polypeptide" EX1001, 115:58-61 (emphasis added);
- "For example, a **modified PH20 polypeptide** provided herein that is **inactive** contains one or more amino acid replacements at position(s) corresponding to … **320** … with reference to with reference to amino acid positions set forth in SEQ ID NO:3" EX1001, 115:61-116:36 (emphasis added);
- "[T]he **modified PH20 polypeptides** can be **inactive** enzymes" EX1001, 187:64-65 (emphasis added).

269. The '185 patent specification also describes an assay in which the incubation time was increased (and the standard curve was adjusted) to detect a lower amount of activity in order to identify those mutants with residual activity: "The modified assay is intended to detect PH20 activities below 0.2 U/mL."

EX1001, 262:24-54. And Tables 5 and 10, both titled "Inactive Mutants," specifically identify replacements corresponding to inactive mutants. EX1001, 117:8-124:33 (Table 5), 262:26-267:55 (Table 10).

270. I also understand that Dr. Hecht argues that a POSA would have interpreted claim 1 as requiring ≥ 40% hyaluronidase activity because "the common disclosure identifies each of these substitutions [i.e., each of D320H, D320K, D320R or D320S] as causing $PH20_{1-447}$ to exhibit increased hyaluronidase activity." EX1003, ¶¶126-128. I do not agree.

271. Based on the teaching of the specification, a POSA would understand that modified PH20 polypeptides with the D320H, D320K, D320R or D320S are not always active.

272. As I discuss in Sections X.A and X.D, the specification describes to a POSA exactly how to inactivate PH20s with those specific mutations because, for instance, it identifies mutations that always inactivate PH20.

273. I also understand that Merck and Dr. Hecht rely on statements in the specification relating to 40% or more activity to argue the claims are limited to active mutants with, specifically, 40% or more activity. However, these passages describe examples of active mutants. A POSA would not have interpreted them as requiring the claimed modified PH20 polypeptides to have "at least 40%" or "≥40% hyaluronidase activity."

274. I have reproduced below these passages with bolded language that, in my opinion, shows these are merely examples:

- "**Included among the modified PH20 polypeptides** provided herein are PH20 polypeptide[s] that are active mutants, whereby the polypeptides exhibit at least 40% of the hyaluronidase activity of the corresponding PH20 polypeptide not containing the amino acid modification" EX1001, 75:47-52 (emphasis added);
- "The modified PH20 polypeptides **can** exhibit 40% to 5000% of the hyaluronidase activity of a wildtype or reference PH20 polypeptide" EX1001, 79:29-32 (emphasis added);
- "Activity retained can be, **for example**, hyaluronidase activity that is a[t] least about 40% or more of the PH20 polypeptide that does not include the replacement" EX1001, 4:66-5:1 (emphasis added);
- "**In one example**, any one or more of the replacements are in SEQ ID NO:3, so long as the resulting modified PH20 polypeptide exhibits at least 40% of the hyaluronidase activity of the PH20 polypeptide set forth in SEQ ID NO:3" EX1001, 82:6-10 (emphasis added).

275. Finally, I reviewed the prosecution history for the '185 patent (EX1002), but I did not find in it any discussion that requires importation of the 40% activity limitation.

276. In view of the above, in my opinion, the specification does not clearly show the inventors intended to restrict "modified PH20 polypeptide" to active enzymes with greater than 40% activity.

X. THE SPECIFICATION PROVIDES WRITTEN DESCRIPTION SUPPORT FOR THE CLAIMED INVENTION

277. As noted in Section VI.B, I understand that written description of a genus can be met by 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.

278. As explained above, the claimed methods are directed to C-terminally truncated PH20 variants (*i.e.,* PH20s of different lengths ranging from 430 amino acids to 465 amino acids) with a modification at position 320: aspartate (D) is changed to either lysine (K), arginine (R), histidine (H) or serine (S). The claimed methods also are directed to 95% identical sub-variants of those PH20 variants.

279. As I will explain below, POSAs would have understood that the '185 patent describes both a representative number of species and the common structural features of the narrow set of claimed PH20 variants and sub-variants.

280. Also, the patent teaches common structural features shared by the claimed set of modified PH20s. The patent disclosure would have allowed a POSA to visualize or recognize the claimed set of modified PH20s.

281. The specification would have conveyed to a POSA that the inventors were in possession of the full scope of the claimed PH20 polypeptides.

A. The Claims Cover a Narrow Set of PH20 Variants and Sub-Variants

282. The claims cover a narrow set of PH20 variants and sub-variants; they share at least 95% of their amino acid sequence.

283. To understand how POSAs view the scope and variability of this genus, it is helpful to compare and contrast the claimed proteins from those that fall outside the scope of the claims.

284. For example, consider the genus of all proteins that cleave hyaluronan. That genus comprises a large number of different sequences from different organisms that adopt different tertiary structures and that cleave hyaluronan through different mechanisms of action.

285. The claims do not cover the entire vast, unknowably large set of proteins that cleave hyaluronan. The claims do not cover, for example, HYAL-1 or HYAL-1 variants. Nor do the claims cover bacterial hyaluronidases, which cleave hyaluronan through a different mechanism of action.

286. The claims also do not cover all PH20s. The claims are limited to only the specified C-terminally truncated modified PH20s with the recited D320 mutation and their 95% identical sub-variants.

B. The Specification Describes Common Structural Features of the Claimed PH20 Variants and Sub-Variants

287. First, the claimed PH20 variants and sub-variants share at least 95% of their amino acid sequence (*i.e.,* their primary structure).

288. Second, the claimed PH20 variants and sub-variants share a common structural feature in that they all share a substitution at position 320.

289. Third, the claimed active PH20s share the same tertiary structure. Specifically, the claimed active PH20s share the same characteristic alpha-beta barrel fold tertiary structure. EX2070, ¶¶345, 397, 401-434.

290. POSAs understand that certain structural features, like disulfide bridges in specific positions, are required to achieve that characteristic tertiary structure.

291. Fourth, the claimed active PH20s also share common secondary structural features, like alpha helices, beta sheets and loops, that pack together to form the alpha-beta barrel tertiary structure.

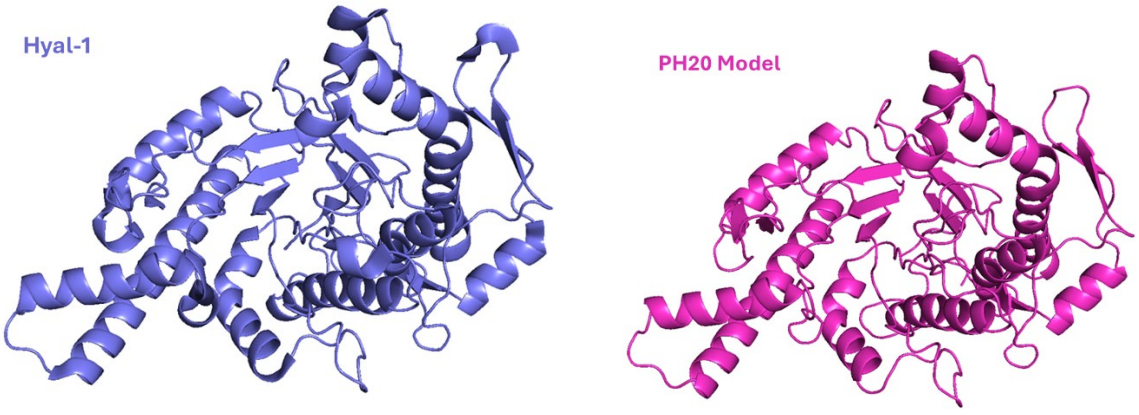
292. Fifth, the claimed active PH20s share a common active site groove, which contains conserved residues that are positioned in similar ways in order to bind HA and facilitate catalysis.

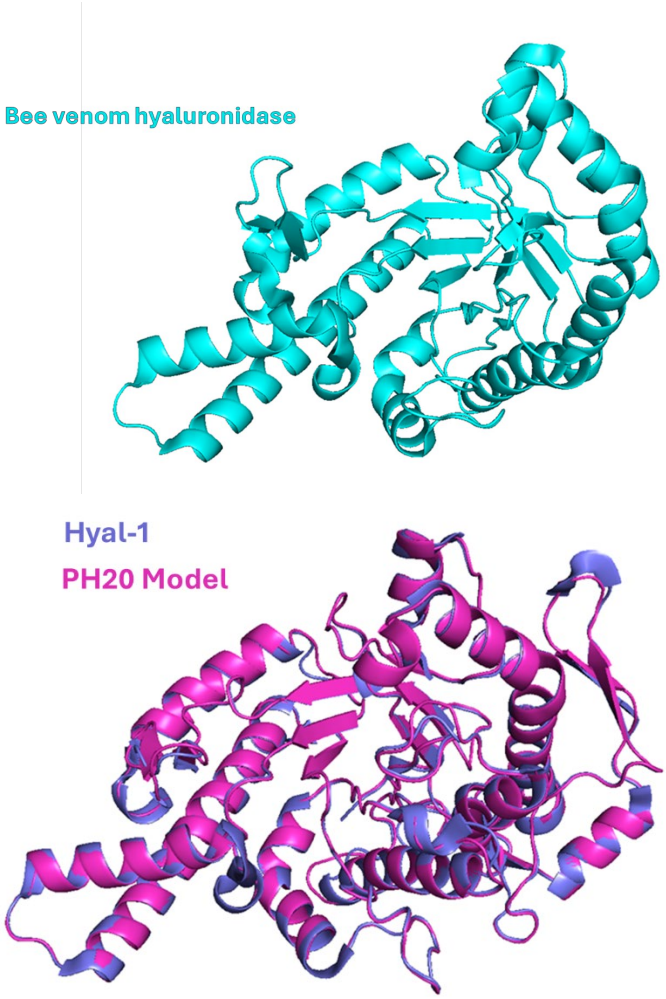
293. Sixth, the claimed active PH20s share a common glycosylation site in that glycosylation at position N333 is required for activity.

294. Dr. Petsko's homology model analysis illustrates some of these common structural features.

295. But first, for context, consider the common structural features shared by homologous proteins that differ by 60-70% of their primary structure (as opposed to 5% or less). As Dr. Petsko illustrates, HYAL-1, PH20, and bee venom hyaluronidase, despite having sequence identities of 30-40%, share fundamental structural features, including the characteristic alpha-beta barrel fold and conserved residues essential for activity and stability that occupy comparable positions and form analogous chemical interactions.

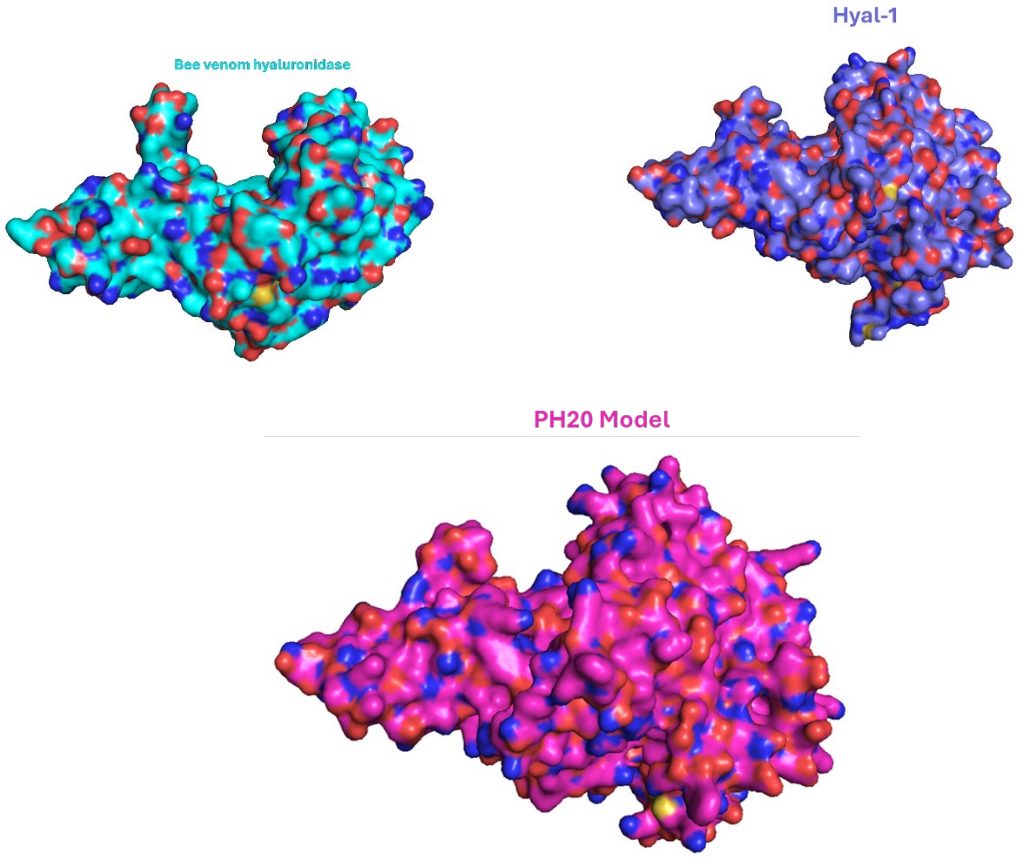
296. Dr. Petsko shows this by overlaying the PH20 homology model on the HYAL-1 crystal structure and comparing both to the bee venom hyaluronidase structure. EX2070, ¶¶129-134. The figures reproduced below from his analysis confirm that all three enzymes also share the same alpha-beta barrel tertiary structure:



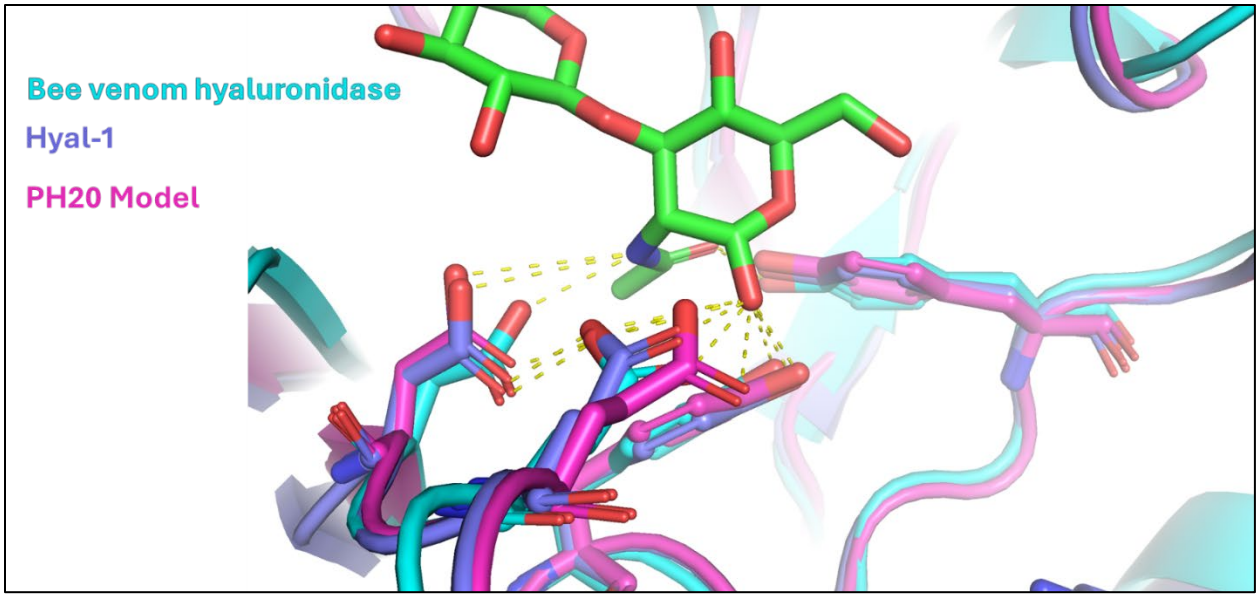


Id.

297. A similar observation can be made for the active site. PH20, HYAL-1, and bee venom hyaluronidase each contain an elongated groove that accommodates hyaluronic acid, a feature described in the literature as characteristic of hyaluronidases. Consistent with that observation, Dr. Petsko's figures, reproduced below, show that the PH20 model also contains the same elongated cleft, indicating that this structural feature is preserved across these related enzymes. EX2070, ¶¶140-144.



298. In the active site groove, key catalytic residues adopt almost the same positions across three proteins and make similar contacts with the hyaluronic acid substrate. EX2070, ¶¶153-160.



EX2070, ¶157.

299. Given the extent to which 30-40% identical proteins share common structural features, a POSA certainly would expect folded proteins that are more than 95% identical to share significant common structural features, including a common tertiary structure and common active site residues positioned in approximately the same way to achieve the same mechanism of action.

300. For example, Dr. Petsko generated homology models for chimpanzee, gibbon, and orangutan PH20s with the D320K substitution and compared their structures to SEQ ID NO:3 with the D320K substitution.[11] As discussed in Section

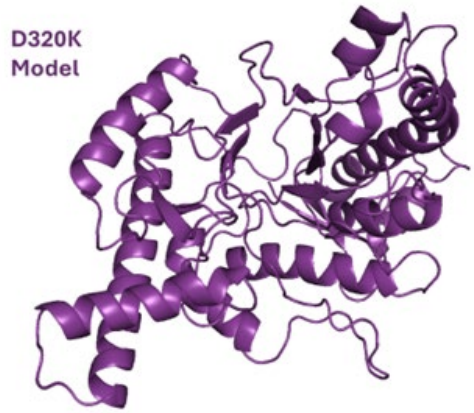
[11] As I explained in Section VIII.E, the modified D320K chimpanzee PH20 that has been C-terminally truncated differs from SEQ ID NO: 3 by 5 residues. The

VIII.E, these D320K primate sequences have at least 95% identity to SEQ ID NO: 3 and fall within the scope of the claims.

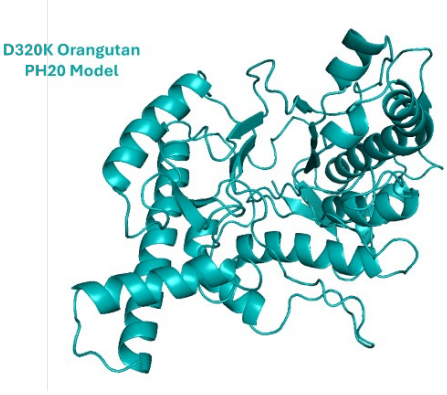
301. Dr. Petsko's figures below show that, despite the D320K substitution and other sequence differences, the primate PH20 variants all adopt the same alpha-beta barrel tertiary structure.

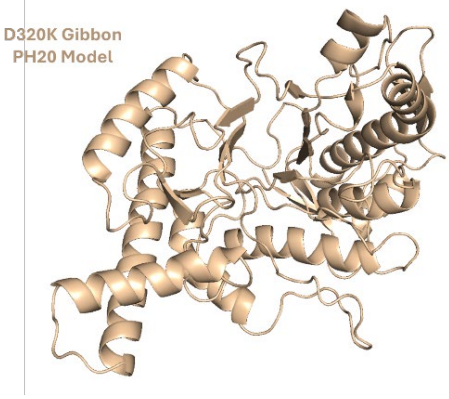
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modified D320K orangutan PH20 that has been C-terminally truncated differs from SEQ ID NO: 3 at 13 positions. The modified D320K C-terminally truncated gibbon PH20 differs from SEQ ID NO: 3 at 16 positions. It has 21 changes as compared to SEQ ID NO: 44, which I noted above the '457 patent publication teaches as being active. Also as discussed in Section VIII.E, a POSA would have expected truncating these primate PH20s at the same position as SEQ ID NO: 3 would yield a soluble, active PH20. A POSA would also have expected that introducing the D320K substitution into those truncated proteins would result in an active sub-variant, since the patent shows that the D320K substitution preserves enzymatic activity in PH20.



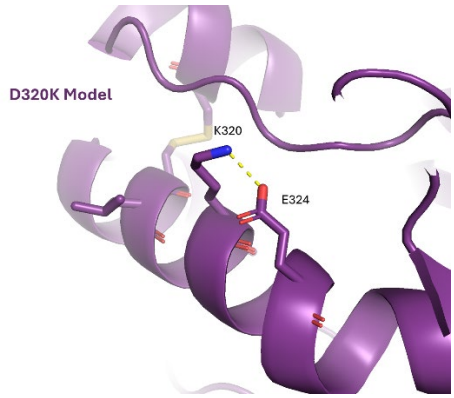


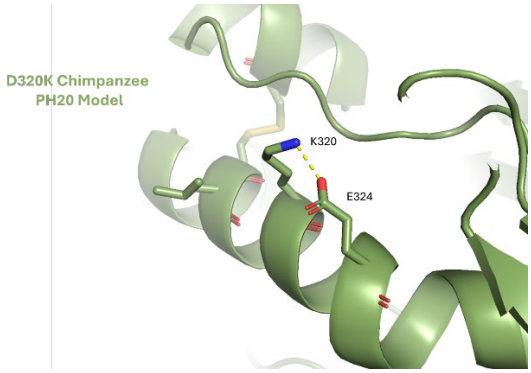


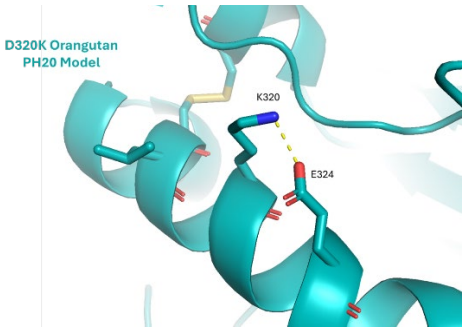


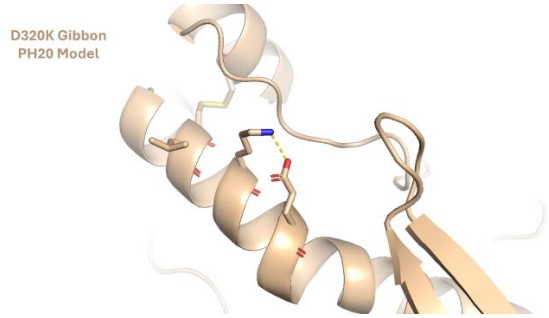
EX2070, ¶¶417, 413-416.

302. And each of these D320K PH20 sub-variants share common interactions at position 320, as shown in the figures below:









EX2070, ¶¶418-426.

303. In my opinion, a POSA would understand which variants are inactive because, for example, they incorporate substitutions the patent identifies as inactivating, which disrupt the common PH20 fold. The patent provides representative examples of such inactivating changes, including substitutions at catalytic residues essential for hydrolysis and substitutions at positions that destabilize disulfide bonds or other elements required to maintain the alpha-beta barrel structure. For example, the patent teaches as follows:

- **Specific amino acid positions in PH20 that are necessary to enzymatic activity** such that they should not be disturbed, such as "positions 25, 111, 113, 176, 189, 203, 246, 249, 252, 316, 341, 346, 352, 400, 402, 408, 423 and 429 of … SEQ ID NO: 3 or 7 … since mutagenesis of these residues results in an enzyme that is not active…." EX1001, 70:46-54.
- The patent further characterizes the roles of these specific amino acid positions in detail, **including**:

- **Disulfide bridges -** 12 cysteine residues "corresponding to amino acid residue 25, 189, 203, 316, 341, 346, 352, 400, 402, 408, 423 and 429 of … SEQ ID NO: 3 or 7" that "form disulfide bridges." EX1001, 70:14-23.
- **Sites contributing to substrate binding / activity -** The patent also identifies amino acids at positions 111, 113, 249, 176, 246, and 252 of SEQ ID NO: 3 or 7 as "contribut[ing] to substrate binding and/or hyaluronidase activity." EX1001, 70:34-41.

304. Because these features are required for enzymatic activity, in my opinion, a POSA would have also regarded them as structural elements common to the claimed D320 PH20 variants and their sub-variants.

C. The Specification Provides Structure-Function Correlations Allowing for Single and Multiple Mutations

305. A POSA would also understand that these common structural features translate into structure-function correlations. For example, the active PH20 variants and sub-variants within the scope of the claims share a common alpha-beta barrel fold that includes an active site groove and critical conserved residues that facilitate catalysis. The common structural features result in the function of cleaving HA.

306. POSAs would understand that there is a correlation between having the PH20 tertiary structure and the active site residues positioned appropriately and the

function of cleaving HA.

307. The patent data show other structure-function correlations. Specifically, the patent teaches the types of changes to the PH20 structure that result in loss of function, maintaining function, or increasing function.

308. The patent teaches that making changes at particular residues always or almost always abrogates activity. The POSA understands from those data that there is a correlation between structure (a particular residue or type of residue capable of forming the required interactions at a given position) and function (enzymatic activity).

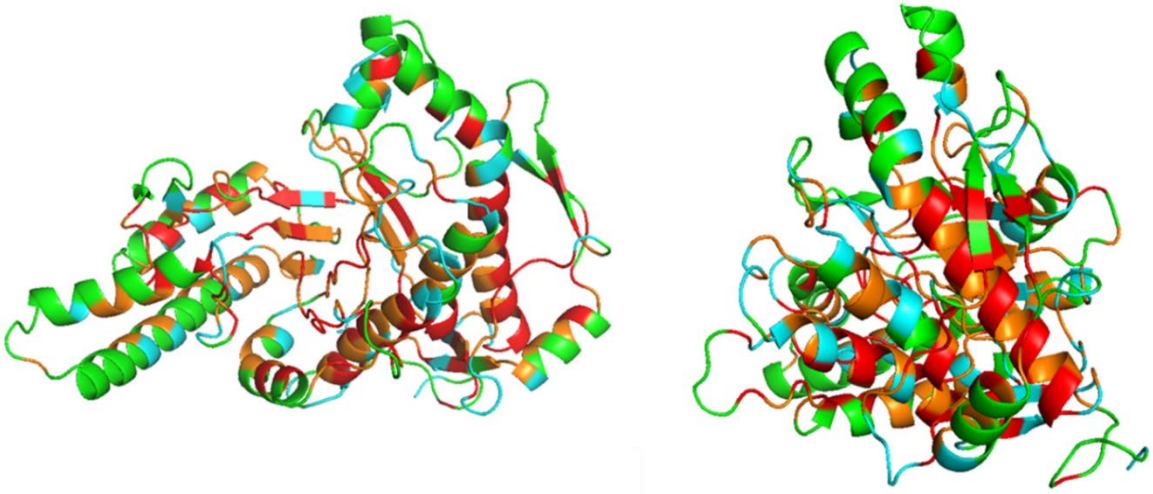
309. The patent's data set out clear structure–function relationships that a POSA could use to determine which 95% identical D320 sub-variants would retain activity and which would be inactive.

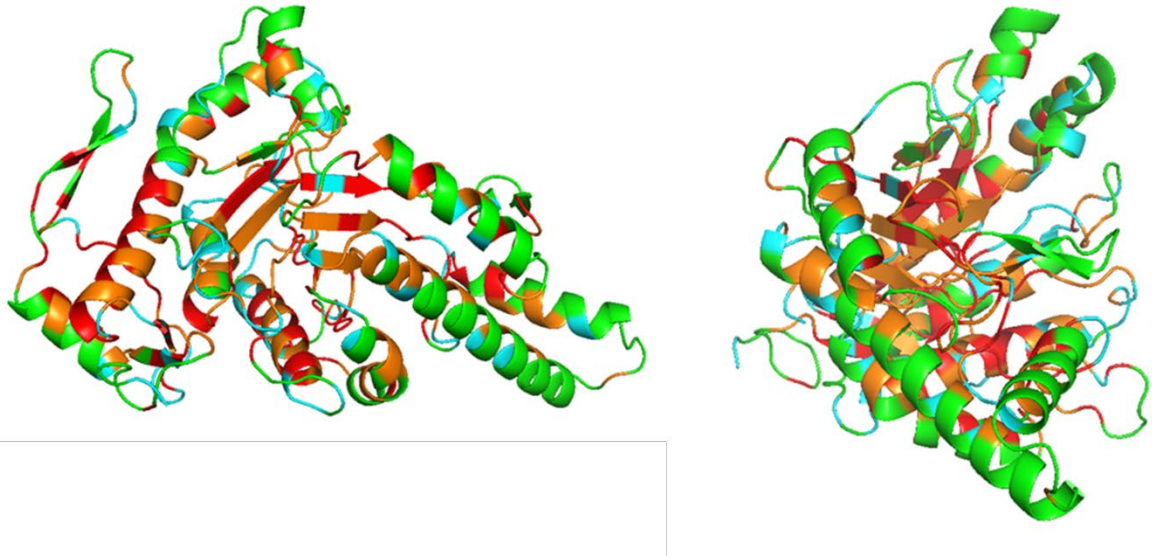
310. The patent data provide a framework for a POSA to identify positions that are: (1) critical to activity, (2) generally intolerant and allow only limited substitutions, and (3) generally tolerant and accommodate a broad range of substitutions. EX2070, ¶¶305-42. The data identify tolerant positions that retain activity even when residues with very different physicochemical properties, such as negatively charged, positively charged, large, small, hydrophobic, or hydrophilic, are substituted. *Id.*

311. In particular, as Dr. Petsko's analysis reflects, a POSA has many

alternative ways of selecting positions to change. POSAs could change those positions that tolerate all tested substitutions, or POSAs could change positions that tolerated 90% or more of the tested substitutions. POSAs seeking to consider more options to change could consider positions that tolerated 60% or more of the tested substitutions. EX2070, ¶358. In my opinion, the patent's data allow the POSA to predictably obtain PH20s with the desired activity profile because the teachings allow POSAs to distinguish actives from inactives.

312. As I explained above, Dr. Petsko mapped the patent's functional data on the PH20 homology model. I reproduce some of Dr. Petsko's figures below, which show PH20 rotated around the Y-axis. The model is color-coded as follows: generally tolerant (green), intolerant (red), generally intolerant (orange), and in between (cyan).





EX2070, ¶299.

313. Dr. Petsko's figures show that the tolerant and intolerant residues are not scattered randomly throughout the protein. EX2070, ¶¶299-303. Many of the residues identified in red and orange correspond to residues involved in substrate binding or catalysis, are buried and are thus important for protein structure, are involved in positioning the active site residues, or are otherwise important in packing the secondary structural elements together to form the tertiary structure. However, many of the red and orange residues are on the surface of the protein, and the POSA would not necessarily have expected those residues to be intolerant to substitution. But now, with the patent in hand, POSAs know to avoid making changes or to proceed with caution in making changes at those positions. By contrast, the residues identified in green tend to be located on the surface and are not involved in packing or in the active site. EX2070, ¶303. The data provide clear guideposts to a POSA seeking to make modified PH20s.

314. Accordingly, in my opinion, the '185 patent provides a POSA the information needed to predictably identify both single and multiple substitutions capable of producing hyaluronidase activity, and distinguish them from inactive variants. Considered as a whole, and consistent with Dr. Petsko's analysis, the patent's data allow a POSA to evaluate each position in the sequence to determine the degree of tolerance to substitution, the types of residues that are most likely to preserve activity, and the positions that could be selected to maintain or enhance enzymatic function.

315. Particularly in view of the narrowly defined and structurally homogeneous set of claimed variants and sub-variants, the structure-function data disclosed in the patent show that the inventors had possession of the full scope of the claimed invention. The patent also provides sufficient guidance for a POSA to visualize and recognize the members of the claimed modified PH20s. EX2070, ¶¶50, 343-389.

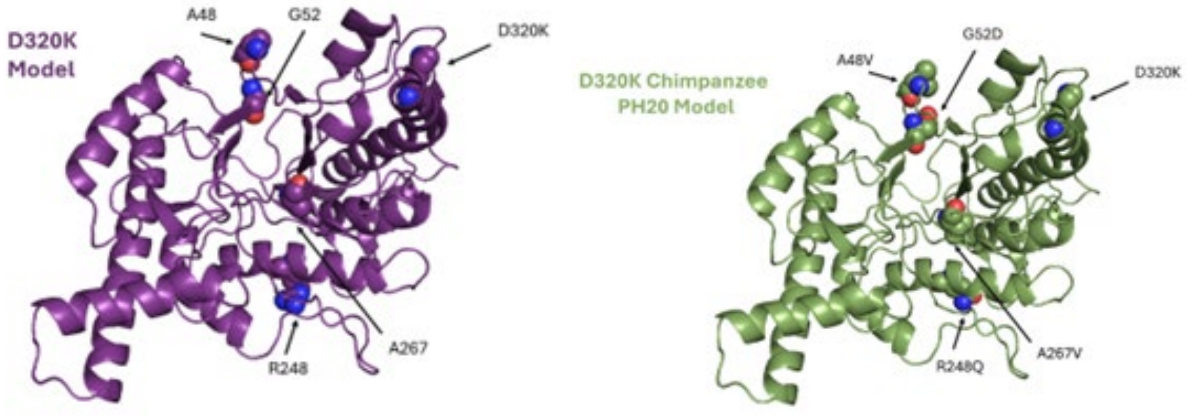
D. The Example D320 Variants Are Representative of the Claimed 95% Identical Sub-Variants

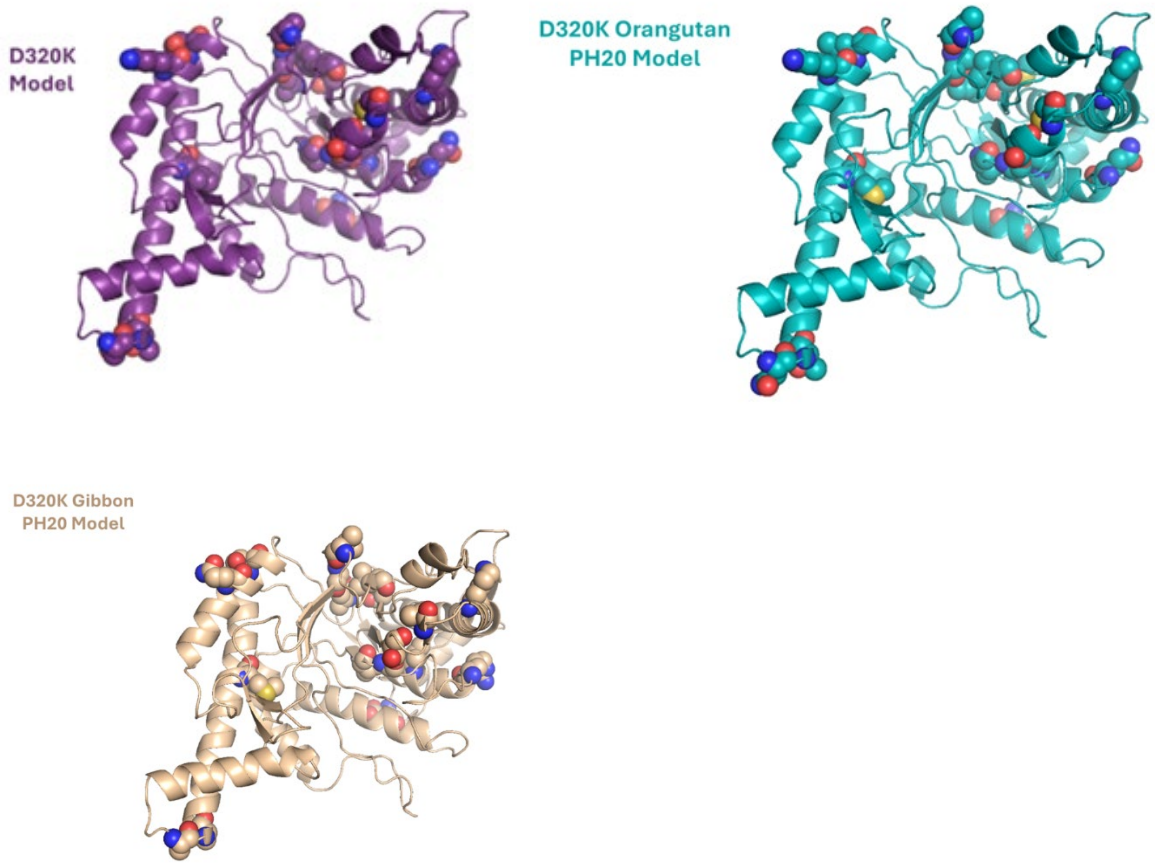
316. In my opinion, a POSA would have understood that the four D320 variants disclosed in the patent are representative of their 95% identical sub-variants, which retain nearly the entire sequence of the disclosed variants, and therefore there is little structural diversity among them. As discussed above in Section X.B, the active PH20s with the substitution at position 320 share a common structural

scaffold, with only minor variations on the surface of the tertiary structure, none of which alters the overall fold.

317. As I explained in Section X.A, a POSA would have understood that this level of similarity reflects a homogeneous and narrowly defined set of PH20 proteins, and the four disclosed D320 active variants are representative of their 95% identical sub-variants. EX2070, ¶¶58, 390-434.

318. To contextualize, Dr. Petsko illustrates this point using examples of multiply mutated PH20s that, based on the disclosure of the patent, a POSA would have expected to be active. EX2070, ¶¶413-434. As shown in the figures below, these multiply modified primate PH20s, which differ from one another by 5 to 17 positions, share the same tertiary structure and scaffold, with only minor variation. The only changes are in the amino acid side chains that decorate the protein scaffold.





EX2070, ¶¶429-430.

319. As discussed in Section X.B, these analyses reflect that the four D320 variants disclosed in the patent retain the characteristic PH20 alpha-beta barrel fold, and that this same fold is preserved across the active multiply substituted PH20 sub-variants having at least 95% sequence identity to SEQ ID NO: 3.

320. A POSA would therefore recognize the active D320 variants disclosed in the patent as representative of all active modified PH20 sub-variants within the scope of the claims. EX2070, ¶¶427-434.

321. Dr. Hecht states that the "common disclosure has no examples of any

PH20 with multiple substitutions and …, a skilled artisan could not realistically predict" the effects of multiply substituted mutants. EX1003 (Hecht Decl.), ¶148. First, I note that in fact the '185 patent does provide the results of multiple mutants in that it incorporated by reference the '457 publication, and the '457 publication teaches several multiply modified and active PH20s (*e.g.,* N47A/N131A).

322. As discussed in Section VIII.D, by systematically substituting, on average, 13 different amino acids at each of the 447 positions in the PH20 sequence, the inventors generated data that map the tolerance of each position to change. These data allow a POSA to identify positions that generally tolerate substitutions along with the types of substitutions likely to be tolerated, as well as those that generally do not.

323. Consistent with Dr. Petsko's analysis, in my opinion, a POSA can use this information to make single and multiple substitutions in PH20. EX2070, ¶¶54-58, 262-389.

E. The Patent Teaches Which C-terminal Truncation Mutants Are Active, Which Are Inactive, and Which Are Minimally Active

324. Dr. Hecht states that "[b]ecause there are no examples in the common disclosure testing the effects of introducing one or more substitutions into a PH20 polypeptide truncated to a position below position 447 (*e.g.*, 433, 430 or within the Hyal-EGF region at 337 to 409), there is no basis for predicting what might happen if one made such mutants, especially if that truncated form of the wild-type sequence

was inactive." EX1003, ¶100. I disagree.

325. First, I disagree that there is no basis for predicting what will happen when POSAs make changes at positions 337-409 in the mature sequence. The specification teaches POSAs what will happen when mutations are made in that region. For example, the image from Dr. Petsko's spreadsheet below shows what happens when changes are made at positions 337-367. EX2166. Generally speaking, substitutions are not tolerated in that region, with some limited exceptions.

Position		WT	Inactive subs (<20%)	Active subs (20%<x<40%)	Active subs (>40%)	% active subs (>20%)
337	always inactive	A	CFGIKLMRTW			0.00%
338	depends	A	CDEFGHIKLPRTV		Q	7.14%
339	depends	K	DEFGHLNPSTVWY		M	7.14%
340	always inactive	M	ACDEFGHKPRSTVW			0.00%
341	always inactive	C	AEGHKLMNQRSTVY			0.00%
342	depends	S	DEFHKLMPQRTY		A	7.69%
343	depends	Q	CDFIPW		TV	25.00%
344	always inactive	V	FGHLMNPQRSTWY			0.00%
345	always inactive	L	ACEHKNQRTVY			0.00%
346	always inactive	C	ADFGIKLMPRSTVW			0.00%
347	depends	Q	CFIPTVW	L	AEGMRS	50.00%
348	depends	E	CHILPQRTVWY		DGS	21.43%
349	depends	Q	DFGPVWY		AEKMNRT	50.00%
350	always inactive	G	ADEFHKLMNPRSTVY			0.00%
351	depends	V	DEFHNRWY	CIQ	AS	38.46%
352	always inactive	C	ADEFGKMPQRSTVWY			0.00%
353	depends	I	CFGHKLMQRSW		TV	15.38%
354	always inactive	R	CDEGHIKLMPQSVWY			0.00%
355	always inactive	K	DFGHLMNPQRSTVWY			0.00%
356	depends	N	CGKLPRTVW		ADHS	30.77%
357	depends	W	DEFGLMQR	K	ACST	38.46%
358	depends	N	EHIKPQRW	L	CGT	33.33%
359	depends	S	AFGLPW		DEHKMTV	53.85%
360	depends	S	ACEFGIKLMPQRV		T	7.14%
361	depends	D	ACEGMNPQRSVW	H		7.69%
362	always inactive	Y	ACEGHKLMNPRSTVW			0.00%
363	always inactive	L	ACDEFGHIPQRSTVW			0.00%
364	always inactive	H	ACDEFGKLMPRSTVY			0.00%
365	always inactive	L	ACDEGMNPQRSTWY			0.00%
366	always inactive	N	ACEFGKMPQRTW			0.00%
367	depends	P	EFILMQV		ACGKRS	46.15%

326. The image from Dr. Petsko's spreadsheet below shows what happens when changes are made at positions 368-398. EX2166. This region is more tolerant to substitution in that, while there are still some residues that tolerate little change or no change, some residues will tolerate some degree of variance.

Position		WT	Inactive subs (<20%)	Active subs (20%<x<40%)	Active subs (>40%)	% active subs (>20%)
367	depends	P	EFILMQV		ACGKRS	46.15%
368	depends	D	CPW		AEGHKLMRSTV	78.57%
369	depends	N	CEFIKLPQVW		HRS	23.08%
370	always inactive	F	ADEGHKLNPQRSVY			0.00%
371	depends	A	P	G	EFHIKLMRSV	91.67%
372	always inactive	I	ADEFGHKLNPRSTVW			0.00%
373	depends	Q	CPW		AEFKLMRSV	75.00%
374	depends	L	DE	W	AHIMNPRSTVY	85.71%
375	depends	E	CFPVY	I	AGKLMNRST	66.67%
376	depends	K	IPW	L	ADEMQRSTVY	78.57%
377	depends	G	CILV		DEHKPRST	66.67%
378	depends	G	DEFILMQTWY		KNR	23.08%
379	depends	K	ACEFILMW		GHRST	38.46%
380	depends	F	CDEGQRS	VT	ILPWY	50.00%
381	depends	T	GLPWY	E	HKNQRSV	61.54%
382	always inactive	V	EGHKLMNPQRSTWY			0.00%
383	depends	R	GP		AEHIKLMNSTV	84.62%
384	always inactive	G	CFMQST			0.00%
385	depends	K	CLMPWY		AGHNQRSTV	60.00%
386	always inactive	P	ACFGHILMNQRSTVY			0.00%
387	depends	T	CEFGHILMNVWY		S	7.69%
388	depends	L	CGPQ		FHIMRTVWY	69.23%
389	depends	E	FV		AGHKLMPQRSTY	85.71%
390	always inactive	D	ACEFGHLNPRSTVWY			0.00%
391	depends	L	ADGHKNPQRSTVWY		C	6.67%
392	depends	E	CP	W	AFGKLMQRSTVY	86.67%
393	depends	Q	CP		ADFHKLMNRST	84.62%
394	depends	F	ADEGIKNPQRSTV		LW	13.33%
395	depends	S	CLMP	T	AGHKRW	63.64%
396	depends	E	CFGIPY	L	ADHQRST	57.14%
397	always inactive	K	ACEFGILMPQTV		R	7.69%
398	depends	F	ACEGHINPRSTVWY	L		6.67%

327. Progressing further along the sequence, as shown below, the region from 399-409 includes both tolerant and invariant or generally invariant positions. EX2166.

Position		WT	Inactive subs (<20%)	Active subs (20%<x<40%)	Active subs (>40%)	% active subs (>20%)
399	depends	Y	DP		ACEKMNQRSTVW	85.71%
400	always inactive	C	ADEFGILMPQRSTVY			0.00%
401	depends	S	CFHKRWY	GQ	AEN	41.67%
402	always inactive	C	ADEFLMPQRSTVWY			0.00%
403	depends	Y	ACEGHKLMNPQRT		F	7.14%
404	depends	S	CDFGHLMNRVWY	T	AP	20.00%
405	depends	T	CIV	F	AGKMPQRSWY	78.57%
406	depends	L	PR		ACEFGINQSTVY	85.71%
407	always active	S			ADEFGHLMNPQRVW	100.00%
408	always inactive	C	AEFGIKLPRSTVWY			0.00%
409	always active	K			ADEGHIPQRSTV	100.00%

328. The above data provide substantial guidance to the POSA seeking to make modifications in this region of the protein. I disagree with Dr. Hecht that there is "no basis for predicting what might happen" if a POSA were to make changes in the 337-409 region of PH20.

329. Second, I disagree with Dr. Hecht that a POSA would not be able to predict what would happen if the POSA were to make changes in a PH20 sequence that was truncated to the point of being inactive (*i.e.,* that ends at position 431 or before position 431). The POSA would expect any such modified PH20 to be inactive because it lacks the minimum primary structure required for activity. There is nothing unpredictable about this.

330. Dr. Hecht states that "changes terminating at positions between 430 and 442 were not characterized, other than at position 432 (which exhibited ~27% of the activity of PH201-447)." EX1003, ¶161. That is incorrect. As I have explained

above, the ’457 publication teaches the results of successive residue-by-residue C-terminal truncations, including the range of positions from 430 and 442. I described the ’457 patent results in paragraphs 184-188 above, and I summarized how a POSA would have understood the results for each truncation in a chart, which I have reproduced below for convenience:

C-terminal Position (Precursor sequence)	**C-terminal Position (Mature sequence)**	**Expected Effect on Activity**
465	430	Inactive
466	431	Inactive
467	432	Reduced activity
468	433	Reduced activity
469	434	Active
470	435	Active
471	436	Active
472	437	Active
473	438	Active
474	439	Active
475	440	Active
476	441	Active
477	442	Active
478	443	Active

C-terminal Position (Precursor sequence)	**C-terminal Position (Mature sequence)**	**Expected Effect on Activity**
479	444	Active
480	445	Active
481	446	Active
482	447	Active
483	448	Active
484	449	Active
485	450	Active
486	451	Active
487	452	Active
488	453	Active
489	454	Active
490	455	Reduced activity
491	456	Reduced activity
492	457	Reduced activity
493	458	Reduced activity
494	459	Reduced activity
495	460	Reduced activity
496	461	Reduced activity
497	462	Reduced activity

C-terminal Position (Precursor sequence)	**C-terminal Position (Mature sequence)**	**Expected Effect on Activity**
498	463	Inactive/little activity
499	464	Inactive/little activity
500	465	Inactive/little activity

See also, EX2070, ¶¶218-230, 385-389.

331. Dr. Hecht states that "the examples of single substitutions in PH20 1-447 are not analogous to substitutions in PH20 proteins truncated below 433, given the latter's reduced or ablated activity." EX1003, ¶100. I disagree.

332. The patent teaches a set of active C-terminally truncated PH20s that end at positions 432-462. Within that set, POSAs would have understood that PH20s that end at, for example, positions 431 and 432 would have been active. POSAs would not have expected them to have as strong of activity as, for example, a PH20 that ends at position 447. However, the POSA would have expected them to be active. The POSA also would have expected that making a change that the patent teaches would improve activity, like D320K, could improve the activity of, for example, a PH20 that ends at position 433. However, because the unmodified 433 amino acids long variant is expected to have less activity than the unmodified 447 amino acids long PH20 variant, the D320K variant that is 433 amino acids

long also would be expected to have less activity than the D320K variant that is 447 amino acids long.

333. As a general matter, POSAs would expect that substitutions in (mature) PH20s that are between 434-454 amino acids long (*i.e.*, that end at position 434 up through 454) would have the same activity as that exhibited for the exemplary 447 amino acid long PH20 tested in the example (SEQ ID NO: 3).

334. As evidence of this, for example, the patent teaches that there is heterogeneity in the C-terminus when PH20s are expressed. For example, when PH20 of SEQ ID NO: 3 is expressed using the expression vector taught in the patent, it contains a "mixture" of polypeptides that "can include any one or more of" PH20s that end at positions 442-447 "in various abundance." EX1001, 75:25-32. In view of this disclosure, POSAs would expect to obtain substantially the same result when introducing substitutions into any of the PH20 ending at positions 434 up through 454.

335. Thus, the exemplified D320 substituted SEQ ID NO: 3 PH20s (which end at position 447) are representative of other C-terminal truncation variants recited in the claims, which also have the D320 substitution.

F. Modified PH20s Dr. Hecht Says Are Excluded From Patent

336. I understand that Dr. Hecht asserts the claims encompass PH20 polypeptides that, according to Dr. Hecht, "are combinations of substitutions that the

disclosure[] say[s] to ***not*** include in modified PH20 polypeptides." EX1003, ¶153. Dr. Hecht points to six specific combinations of amino acid substitutions, which are listed below.

- P13A/L464W, N47A/N131A, N47A/N219A, N131A/N219A, N333A/N358A or N47A/N131A/N219A

337. I disagree with Dr. Hecht's analysis. First, none of the six combinations identified by Dr. Hecht fall within the scope of the asserted claims. EX1001, 77:45-58, claim 1. Each of the combinations is limited to two or three substitutions at positions other than 320. But the claims require a modification at position 320. As a result, the claims do not encompass any of the combinations Dr. Hecht suggests.

338. I note that the '457 publication referenced in the '185 patent disclosed that some of these combinations of substitutions do in fact result in active mutants as shown below:

TABLE 16

Human PH20 Hyaluronidase Deglycosylation Mutants

Mutant	SEQ ID NO	Glycan Type	Secretion	Activity pH 5.5	Activity pH 7.4
PH20sHis (parent)	187	Both	YES	YES	YES
N82A	202	Complex	YES	YES	YES
N166A	203	Complex	YES	YES	YES
N235A	204	High Mannose (80%) Complex (20%)	YES	NO	NO
N254A	205	Complex	YES	YES	YES
N368A	188	High Mannose	NO	NO	NO
N393A	189	High Mannose	YES (WEAK)	NO	NO
N82A/N166A	206	Complex	YES	YES	YES
N82A/N254A	207	Complex	YES	YES	YES
N166A/N254A	208	Complex	YES	YES	YES
N82A/N166A/ N254A	209	Complex	YES	YES	YES
N368A/N393A	190	High Mannose	NO	NO	NO

EX2165, Table 16.

339. The ’457 patent shows that PH20 variants with multiple substitutions at (precursor sequence positions 82, 166 and 254), which correspond to (mature) positions 47, 131, and 219 (e.g., N47A/N131A, N47A/N219A, N131A/N219A, and N47A/N131A/N219A) had enzymatic activity.

340. Given that disclosure, a POSA would have reasonably expected that introducing the following substitutions into, for example, SEQ ID NO: 3 would result in an active PH20: N47A/N131A/D320K, N47A/N219A/D320K, N131A/N219A/D320K, and N47A/N131A/N219A/D320K.

341. To the extent Dr. Hecht interprets the specification to instruct POSAs

not to make the N47A, N131A and N219A substitutions as a general matter, I disagree. The patent simply states that, when ***only two changes*** are made the changes are not P13A/L464W, N47A/N131A, N47A/N219A, N131A/N219A or N333A/N358A, and when ***only three changes*** are made, the changes are not N47A/N131A/N219A.

G. The Specification Provides Written Description Support For Claims 2-4 and 7-11

342. Dr. Hecht argues that "claims 2 to 4 add additional requirements." EX1003, ¶118. However, in my opinion, claim 2 merely limits the substitution at position 320 to lysine (K). And claims 3 and 4 merely further limit the modified PH20 polypeptide of claim 2 by requiring, for claim 3, at least 96% identity to the amino acid sequence of SEQ ID NO: 35, and for claim 4, at least 95% identity to the amino acid sequence of SEQ ID NO: 32. And Dr. Hecht acknowledges that claims 2-4 encompass a smaller set of modified PH20 polypeptides. *See* EX1003, ¶124. Therefore, for the reasons already discussed with respect to claim 1 in Sections X.A-F, the specification provides adequate written description support for claims 2-4. I understand that Dr. Hecht does not contest that the additional claim elements recited in claims 7-11 have sufficient written description support. I agree. In other words, I understand that Dr. Hecht does not challenge written description for claims 7-11 on any other grounds independent of the arguments Dr. Hecht raised for claim 1. Thus, for the reasons already discussed with respect to claim 1 in Sections X.A-F,

the specification provides adequate written description support for claims 2-4 and 7-11. My written description analysis for claim 1 applies equally to these claims.

XI. THE CLAIMS ARE ENABLED

343. I understand that enablement requires the patent to provide enough information for a POSA to make and use the full scope of the claimed invention without having to engage in undue experimentation.

344. As explained above, the claimed methods are directed to C-terminally truncated PH20 variants with a modification at position 320: aspartate (D) is changed to either lysine (K), arginine (R), histidine (H) or serine (S). The claimed methods also are directed to 95% identical sub-variants of those PH20 variants.

345. As I understand it, there is no dispute that a POSA could make the claimed PH20 ***variants***—meaning the set of C-terminal truncated PH20s (SEQ ID NOs: 3 and 32-66) with a D320K, D320R, D320H or D320S substitution. The POSA could readily generate, for example, SEQ ID NO: 3 with the D320R substitution through routine procedures. I do not understand Dr. Hecht to contend otherwise. As I also understand it, Dr. Hecht does not dispute that a POSA would have been enabled to use *active* modified PH20 polypeptides in a method for increasing delivery. I do not understand him to contend otherwise.

346. A POSA also can readily make and use the claimed PH20 variants—meaning the set of C-terminal truncated PH20s (SEQ ID NOs: 3 and 32-66) with a

D320K, D320R, D320H or D320S substitution—using routine techniques according to the patent teachings. For example, the POSA would have understood from the teachings of the patent how to, for example, use SEQ ID NO: 3 with the D320K mutation as a spreading factor.

347. With respect to the 95% identical sub-variants, a POSA would have readily been able to make and use the full scope of 95% sub-variants using the disclosure of the patent in conjunction with the knowledge in the art and using routine techniques.

348. For example, the patent provides a detailed structure-function correlation across the PH20 sequence, showing which positions generally tolerate substitution and which do not. This information allows a POSA to predictably determine which sub-variants will retain activity and to make and use those sub-variants.

349. As I will explain, the specification in conjunction with the knowledge in the art would have enabled a POSA to make and use the full scope of the claimed polypeptides using routine techniques. Undue experimentation would not have been required to practice the full scope of the claims.

350. Additionally, as discussed, I conclude that a POSA can reasonably distinguish between active and inactive modified PH20 variants and predictably make and use the claimed modified PH20 variants.

A. Undue Experimentation Is Not Required to Practice the Claims

351. As I explained in Section VI.C, I understand that the *Wands* factors are used to assess whether the undue experimentation is required to practice the claims. I understand the *Wands* factors are as follows: (1) the breadth of the claims; (2) the nature of the invention; (3) the state of the prior art; (4) the level of one of ordinary skill; (5) the level of predictability in the art; (6) the amount of direction provided by the inventor; (7) the existence of working examples; and (8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. In the following paragraphs, I address these factors.

1. Scope of Claims and Nature of the Invention

352. As I described in Section X.A, the claims cover a narrow set of modified PH20 variants and sub-variants. They consist of a set of C-terminally truncated PH20s with one of four substitutions at position 320 and their 95% identical sub-variants.

353. Dr. Hecht emphasizes the absolute number of possible sequences that meet the 95% sequence identity limitation in the claims. But POSAs would not evaluate the scope of the claimed genus by calculating the total number of amino acid sequences that satisfy the 95% identity limitation. POSAs would evaluate the scope of the claims by considering the variation across the set of claimed proteins. And there is little variation across this set.

354. The set of claimed sequences share more than 95% of their primary structure. That is a very high degree of identity. The claimed PH20s vary only slightly (by at most 5% of their primary sequence) with respect to one another.

355. As explained above in Section VII.A.6, the enzymatically active claimed variants and sub-variants share the same alpha-beta barrel tertiary structure. They share common secondary structures (e.g., alpha helices, beta sheets, and loops) that pack into a folded protein and form the PH20 conserved globular tertiary active structure.

356. The enzymatically active claimed variants and sub-variants share a common scaffold with common structures (*e.g.,* disulfide bonds) that enable them to fold into this common tertiary structure.

357. The enzymatically active claimed variants and sub-variants share a common active site and common residues involved in facilitating the catalysis reaction. They share a glycosylation site at (mature) position 333, because glycosylation at that site is required for activity.

358. All enzymatically active claimed variants and sub-variants share a common structural scaffold. The only differences between the claimed variants and sub-variants are (1) variations in their C-terminal position (*i.e.,* variations in their length) and (2) variations (5% or less) of the amino acid side chains that decorate the scaffold.

359. As a whole, and viewed in context, that is a tiny amount of diversity. As a whole, POSAs would consider the claimed genus to be structurally homogeneous with very little variation.

360. As to the nature of the invention, the claims cover modified PH20 variants and sub-variants. A POSA would have understood that predictably making and using the claimed PH20 variants and sub-variants required only routine molecular biology and protein biochemistry techniques, particularly in view of extensive structure-function guidance provided in the '185 patent and the knowledge in the art.

361. POSAs could readily generate the claimed variants and sub-variants by introducing the mutation at position 320 and any other desired modifications and then use, for example, the techniques taught in the patent to express, purify and characterize the protein. EX1001, 135:44-149:57.

362. Accordingly, in my opinion, both the nature of the invention and the limited scope of the claims show that undue experimentation is not required to make and use the claimed polypeptides.

2. Level of Skill in the Art and State of the Art

363. As set forth in Section V, I was asked to apply Merck's definition of a POSA.

364. I also note that such a POSA would have had the ability to consult with

colleagues experienced in hyaluronidase biology as part of a multidisciplinary team. I understand that Dr. Hecht agrees. EX2076 (Hecht Tr.), 63:8-64:2.

365. As also explained in Section VII.B, much was known regarding the structure and mechanism of action of hyaluronidases HYAL-1 and PH20 as of December 28, 2012.

366. By that time, the crystal structures of bee venom hyaluronidase and human HYAL-1 had been solved. EX1033; EX1006.

367. The HYAL-1 crystal structure made it possible to generate accurate homology models of PH20's tertiary structure, and I understand Drs. Hecht and Park confirmed that by 2011 a POSA could readily model PH20 and evaluate the effect of amino acid substitutions in that structural framework. EX2077 (Park Tr.), 94:4-22, 176:2-5. I agree, and as evidence that a POSA would have done this, as I explained above, before 2012 (in fact before 2007), I used the bee venom hyaluronidase crystal structure to generate a HYAL-1 homology model using Swiss Model.

368. As discussed in Section VII.A.6, it was known that bee venom hyaluronidase and HYAL-1, even with only 30% sequence identity, share a common tertiary structure (the alpha-beta barrel fold), a common active site groove, and common, conserved residues that facilitate catalysis. EX1006, 6912, 6914; EX1008, 826, 833; EX1033, 1028; EX2070 (Petsko Decl.), ¶¶126-206. And

the PH20 homology model shows that PH20, which shares 40% sequence identity with HYAL-1, also shares these features. EX2070 (Petsko Decl.), ¶¶126-206.

369. Mutagenesis studies in both HYAL-1 and PH20 also had confirmed the importance of certain residues for enzymatic activity and structural stability. EX1010; EX1011; EX1033, 1029; EX2070 (Petsko Decl.), ¶¶167-217.

370. Accordingly, as of December 28, 2012, a POSA would have had in-hand a PH20 homology model to evaluate potential substitutions. And the importance of particular key residues to enzymatic activity and structural stability, including how these features are shared across homologous hyaluronidases, was already known.

371. In light of this developed state of the art, as I explain below, the disclosures in the specification provided ample guidance for a POSA to make and use the full scope of the claimed modified PH20 polypeptides without undue experimentation. EX2070 (Petsko Decl.), ¶¶263-434.

3. Guidance in the Specification and the Presence of Working Examples

372. As set forth in Section X, the specification provides substantial and critical information the POSA requires to make and use the full scope of claimed PH20 variants and sub-variants.

373. The patent provides a structure-function map of PH20 that informs a POSA, position by position, which residues are invariant, which tolerate little

change, and which are amenable to substitution. EX2070 (Petsko Decl.), ¶¶44, 54-56, 233-389. The patent data teach POSAs where to make changes, what changes to make, and where not to make changes as desired in order to (1) maintain activity, (2) abrogate activity or (3) change activity (*i.e.,* reducing or increasing activity). *Id*.

374. Dr. Petsko provides numerous examples of how a POSA could have used the patent data to identify potential single and multiply substituted PH20s, and I agree with his analysis.

375. There is virtually no limit to the ways in which the POSA could use the data in the specification to practice the invention. The specification provides the POSA detailed information for literally every position in the protein, so the POSA can proceed position by position, or region by region, making the desired changes, depending on the POSA's goals.

376. ***As someone with decades of experience studying hyaluronidases, including common structural features and structure-function correlations for hyaluronidases, I cannot emphasize enough the value of these data to a POSA in the field.*** These data, in my view, allow the POSA to practice far more than what has been claimed, and they certainly permit the POSA to make and use the full scope of the claimed proteins.

377. The inventors provide experimental data for the D320K, D320R,

D320H and D320S variants in the patent. As I explained above, the patent also teaches the activity levels for various PH20 polypeptide lengths (*i.e.,* C-terminal truncation variants) recited in the claims. Given the breadth of experimental data establishing a structure-function map of PH20, POSAs would not have required further working examples of every possible sub-variant to make and use the claimed sub-variants. The specification provides a POSA with the guidance needed to make and use the claimed proteins without undue experimentation.

4. Predictability of the Field and Quantity of Experimentation

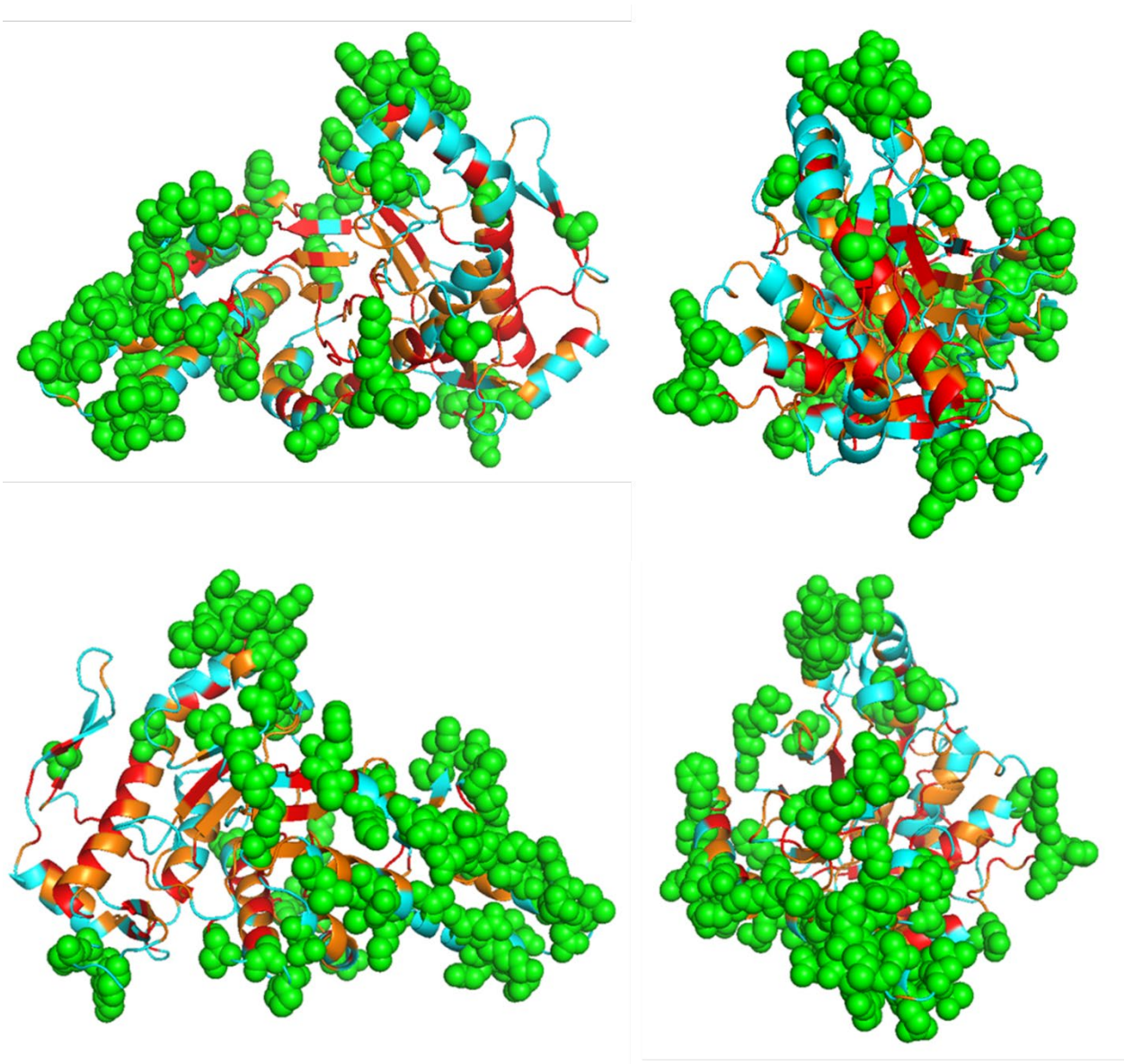
378. As set forth in Section XI.A.1.-3., in my opinion the specification gives a POSA the tools to make and use active (and inactive) PH20 variants and sub-variants and to distinguish between active and inactive PH20 variants using routine techniques.

379. As I have said repeatedly, the patent teaches, on a residue-by-residue basis, which positions are tolerant and intolerant of substitution, including what types of changes are tolerated and not tolerated. For some positions, all tested substitutions, including large residues, small residues, charged residues, and hydrophobic residues were tolerated. For some positions, no or very few residues were tolerated. As Dr. Petsko explains, the POSA would have understood that some of the positions that did not tolerate substitution are invariant and cannot be changed, while others may tolerate change if a "rescue" mutation is introduced to

restore activity. EX2070 (Petsko Decl.), ¶296. The POSA can use the data in the patent to identify such positions and changes, as Dr. Petsko explains. *Id.*, ¶¶374-383, 262-342.

380. The patent data allow POSAs to reasonably predict what changes will maintain activity, abrogate activity, reduce activity, or improve activity. EX2070 (Petsko Decl.), ¶¶44, 54-56, 233-389. That is true for single and multiple substitutions, as Dr. Petsko explains. *Id.*, ¶¶55, 262.

381. For example, as explained above in Section VIII.F., Dr. Petsko mapped the mutagenesis results onto the homology model and highlighted each residue according to its tolerance for substitution. EX2070, ¶¶297-342. In the figures below, the color-coded PH20 homology model is rotated around the Y-axis, and residues that tolerated 90% or more of the substitutions tested are displayed in the "sphere" format.



EX2070, ¶¶356-357.

382. As Dr. Petsko explains, these positions were shown to tolerate a wide variety of substitutions without eliminating activity. A POSA would recognize that such positions are generally permissive to change, and POSAs would have been able to generate singly and multiply modified active PH20s using these data to identify mutation targets. EX2070 (Petsko Decl.), ¶¶262, 322, 353-374.

383. The specification therefore provides substantial teaching and guidance that allows a POSA to make and use the full scope of the claimed PH20 polypeptides without undue experimentation.

384. The immense disclosure in the specification makes generating and using the modified PH20 variants and sub-variants in the claims predictable. That is because the detailed structure-function map provided by the patent allows POSAs to reasonably predict, using their structural biology knowledge, whether substitutions are likely to retain or abolish enzymatic activity.

385. Dr. Hecht opines that "there was uncertainty in the scientific literature about the activity of truncation mutants in the C-terminal region of PH20 (*i.e.*, between positions 337 and 448). Truncations into the C-terminal region of the wild-type PH20 corresponding to the Hyal-EGF domain rendered the protein inactive, and those at its terminus (446-448) caused unpredictable changes in activity." EX1003, ¶100. I disagree. As I explained above in Section X.E., the '457 publication, which I understand is incorporated by reference into the '185 patent, teaches how long the protein must be in order to retain activity. Put another way, the '457 publication teaches POSAs where to truncate the protein and retain activity and where to truncate the protein and lose activity. As a result, I disagree with Dr. Hecht's conclusion that "there is no basis for predicting what might happen if one made" a modified PH20 polypeptide by "introducing one or more

substitutions into a PH20 polypeptide truncated to a position below position 447." EX1003, ¶100. I explained above in Section X.E. what a POSA would expect when making mutations in PH20s of varying lengths, including those that terminate before position 447.

386. Dr. Hecht concludes that the patent data does not "provide[] any meaningful guidance to a skilled artisan that can be used to identify which of the myriad possible combinations of 5, 10, 15 or more substitutions can be incorporated into a PH20 protein to yield an enzymatically active protein." EX1003, ¶182. I disagree with Dr. Hecht.

387. As I have explained and as Dr. Petsko explains, a POSA could have used the patent data to generate multiply modified PH20s with combinations of 5, 10, 15 or more substitutions. In fact, Dr. Petsko provided examples of such modified PH20s, including chimpanzee, gibbon and orangutan PH20s with the D320K substitution. EX2070, ¶¶413-434. The POSA would have expected those proteins to be active in view of the disclosure in the patent.

388. Dr. Hecht appears to suggest that a POSA would need to generate impossibly large libraries of polypeptide sequence and then randomly screen for activity in order to practice the claims. EX1003, ¶¶124-125, 145-146. I disagree.

389. Randomly generating immense PH20 libraries and then randomly screening those libraries is not using the patent disclosure to practice the claims.

390. What Dr. Hecht describes is in fact attempting to practice the claims ***without*** using the disclosure in the patent.

391. A POSA seeking to generate the claimed modified PH20 variants and sub-variants actually using the data in the patent (*i.e.*, without randomly screening large libraries) could have done so, as I have explained.

392. Accordingly, the specification enables a POSA to make and use the claimed polypeptides, including distinguishing actives from inactives, using routine techniques and without undue experimentation.

B. Claims 2-4 and 7-11 Are Enabled

393. I understand that Dr. Hecht does not contest that the additional claim elements recited in claims 2-4 and 7-11 are enabled. I agree. In other words, I understand that Dr. Hecht does not challenge enablement for claims 2-4 and 7-11 on any other grounds independent of the arguments Dr. Hecht raised for claim 1. Accordingly, for the reasons already set forth with respect to claim 1 in Sections XI.A and XI.B, claims 2-4 and 7-11 are enabled. My enablement analysis for claim 1 applies equally to these claims.

XII. THE '429 PATENT AND CHAO DO NOT RENDER CLAIMS 1-4 AND 7-11 OBVIOUS

394. I understand that Dr. Hecht opines that claims 1-4 and 7-11 would have

been obvious in view of Chao and the '429 patent.[12] EX1003, §VI. In particular, Dr. Hecht asserts (i) the '429 patent would have motivated a POSA to introduce single-amino-acid substitutions in non-essential regions of PH20 (EX1003 ¶¶219-221); and (ii) Chao's HYAL-1 crystal structure and its alignment of five human hyaluronidases, together with multiple-sequence alignments of additional homologous hyaluronidases, would have been used to identify non-essential regions of PH20 (EX1003 ¶¶222-228) and to classify position 320 as non-essential with lysine (K) as a tolerated substitution (EX1003 ¶¶230-235).

395. I disagree with Dr. Hecht. As further set out below, the '429 patent and Chao do not teach making a D320 mutation of PH20, much less a D320 mutation to lysine (K), as claimed. In view of the '429 patent and Chao, a POSA would not have been motivated to modify D320 to arrive at the claimed invention and, therefore, would not have found claims 1-4 and 7-11 obvious.[13]

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[12] Dr. Hecht's declaration incorporates and relies on Dr. Park's analysis in several places. For clarity, I refer to their positions collectively as Dr. Hecht's arguments unless otherwise noted.

[13] I apply the legal principles set out in Section VI.D. to assess obviousness in light of the asserted prior art and the general knowledge in the field from the perspective of a POSA at the time of the invention. For purpose of my obviousness

A. The ’429 Patent and Chao Fail to Teach a D320 Mutation of PH20, Much Less a D320 Mutation to Lysine (K), as Claimed

396. I understand that, in determining the scope and content of the prior art and ascertaining the differences between it and the claimed invention, a patent challenger may (1) specify where each element of the claim is found in the prior art or (2) explain why a POSA based on their knowledge in the art would have bridged any gaps (*i.e.*, missing elements) between the prior art and the claimed invention to arrive at the claimed invention. In my opinion, Dr. Hecht has done neither.

1. Overview of the ’429 Patent and Chao

a. The ’429 Patent

397. The ’429 patent, which was filed on March 5, 2004, and issued on August 3, 2010, is prior art to the ’185 patent. EX1005.

398. The ’429 patent discloses “members of the soluble, neutral active Hyaluronidase Glycoprotein family, particularly the human soluble PH-20 Hyaluronidase Glycoproteins (also referred to herein as sHASEGP).” *Id.*, 3:51-54. The ’429 patent teaches that “PH20 is the prototypical neutral-active enzyme” that is “locked to the plasma membrane via a glycosylphosphatidyl inositol [“GPI”]

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analysis, I considered the period before the December 30, 2011 filing date of Halozyme’s ’313 provisional application, to which the ’185 patent claims priority, as Drs. Hecht and Park do. EX1003, ¶11; EX1004, ¶10.

anchor," unlike HYAL-1, which is "soluble" and the "prototypical acid-active enzyme." *Id.*, 2:33-34, 40-46. The '429 patent also teaches that "[a]ttempts to make human PH20" without the GPI anchor "resulted in either a catalytically inactive enzyme, or an insoluble enzyme." *Id.*, 2:57-60.

399. The '429 patent teaches how to make soluble, neutral active PH20 without the GPI anchor. *Id.*, 3:51-53. Specifically, the '429 patent "invention is based upon the discovery that a soluble, neutral-active hyaluronidase activity can be produced with high yield in a mammalian expression system by introducing nucleic acids that lack a narrow region encoding amino acids in the carboxy terminus of the human PH20 cDNA." *Id.*, 3:58-62. The '429 patent states: "single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity." *Id.*, 9:48-52. The '429 patent claims a specific truncated version of PH20 composed of positions 36–482 of SEQ ID NO: 1. *See id*. at 153:39-40.

b. Chao

400. Chao is a publication in the journal Biochemistry that was published in 2007. EX1006 (Chao), 6911. I understand that Chao is prior art to the '185 patent.

401. Chao reports the crystal structure of human hyaluronidase-1 ("hHYAL-1"), a lysosomal enzyme "responsible for the hydrolysis of intracellular HA." *Id.* Chao explains that "[t]here are five homologous hyaluronidases encoded in the human genome: hHYAL-1 through -4 and the sperm adhesion molecule 1 (termed

PH-20)." *Id.*

402. Chao also observes that "[h]uman hyaluronidases exhibit 33–42% sequence identities and even higher conservation of active site residues. Yet, the enzymes differ in their catalytic efficiencies and pH profiles." *Id.*, 6914. Chao also reports that "[t]he sequence homology implies that this hHYAL-1 region adopts the same overall fold as that of bvHyal,[14] comprising a distorted $(\beta/\alpha)_8$ barrel." *Id.*, 6912.

403. Figure 3 of Chao, reproduced below, illustrates conserved versus nonconserved residues across the human hyaluronidases.

[14] "bvHyal" is an abbreviation for bee venom hyaluronidase.

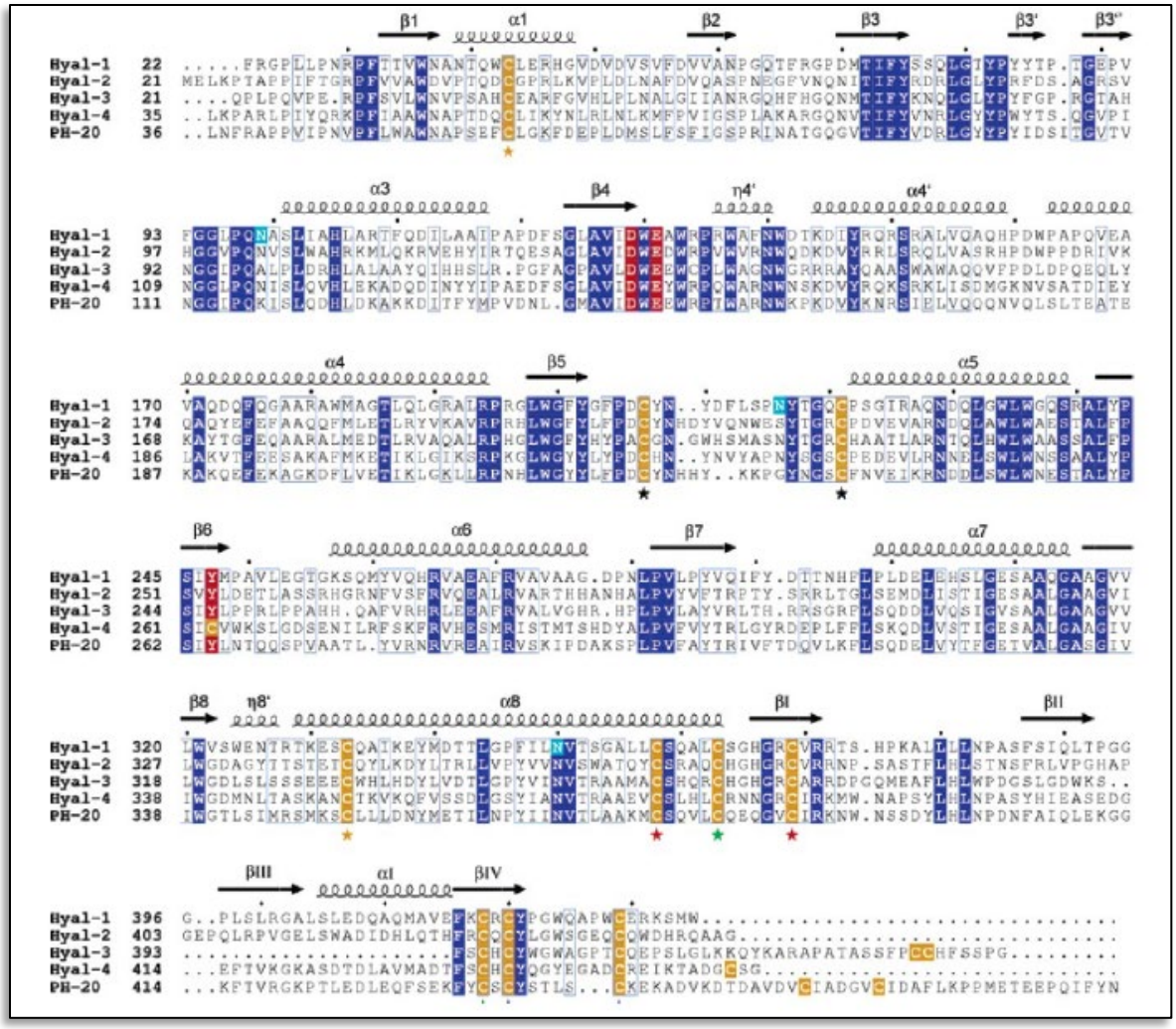


Figure 3 of Chao shows a structure-based sequence alignment of human hyaluronidases. Invariant residues are shown in blue except for three key catalytic residues that are colored red. Cysteine residues are colored yellow. The human HYAL-1 N-glycosylated asparagines residues are colored turquoise. Conserved cysteine residues that form disulfide bonds are indicated by stars with matching colors. Secondary structure units are labeled. *Id.* at 6916.

404. Based on my review of both publications, neither reference discloses "an amino acid modification at a position corresponding to position 320" as required by claims 1-4 and 7-11. EX1001, Claim 1; EX1005; EX1006 (Chao). Nor do they disclose replacing D320 with an amino acid "selected from the group consisting of

H, K, R, and S." EX1001, Claim 1; EX1005; EX1006 (Chao). Chao addresses the three-dimensional structure of human hyaluronidase-1 ("hHYAL-1") by reporting its crystal structure, while the '429 patent addresses soluble neutral hyaluronidase glycoproteins ("sHASEGPs"). EX1005; EX1006 (Chao). Neither mentions a mutation at D320, let alone one to lysine (K). EX1005; EX1006 (Chao). Chao and the '429 patent therefore do not disclose every element required by claims 1-4 and 7-11.

2. A POSA Would Have Had No Motivation to Introduce Substitutions at Position 320

405. I understand that to establish obviousness, a challenger must demonstrate that a POSA would have had a reason to modify or combine the teachings of the prior art to arrive at the claimed invention. In my opinion, neither Chao nor the '429 patent, as relied on by Dr. Hecht, provides any motivation for a POSA to select D320 for mutation as claimed.

406. First, the '429 patent states that a POSA would "recognize that, in general, single amino acid substitutions in non-essential regions of polypeptide do not substantially alter biological activity." EX1005, 9:47-52. Based on this language, Dr. Hecht asserts that a POSA would have been motivated to make substitutions in PH20, would have used multiple sequence alignments and Chao's structural information on HYAL-1 to identify non-essential regions, and would have selected position 320 for substitution with lysine (K). EX1003, §VI.

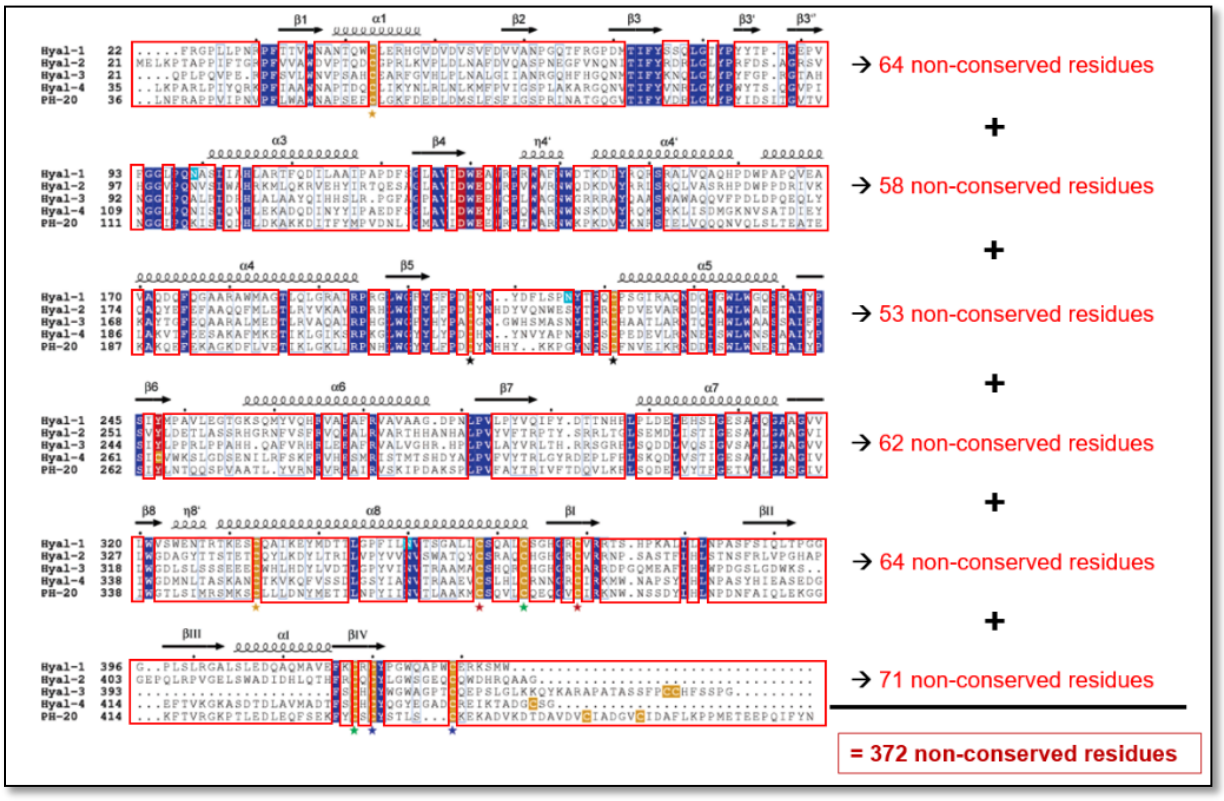
407. I disagree with Dr. Hecht's conclusion that the prior art would have provided a motivation to target D320. As an initial matter, the '429 patent does not identify any region or residue of PH20 as "non-essential," nor does it provide any guidance for selecting which sites to modify. EX1005. Chao likewise does not use the term "non-essential" or designate residues in this way. EX1006 (Chao), 6916, FIG. 3. Therefore, in my opinion, there is no basis in the prior art for Dr. Hecht's assertion that a POSA would have treated D320 as "non-essential" and a target for substitution.

408. Moreover, as I explained above in Section VIII.C., the '429 patent teaches how to make soluble, active C-terminal truncated PH20s. It describes the minimum PH20 sequence needed for activity. EX1005 ('429 patent), 7:3-7. ("However, additional cysteines are required in the carboxy terminus for neutral enzyme catalytic activity such that sHASEGP from amino acids 36 to Cys 464 in SEQ ID No.1 comprise the minimally active human sHASEGP hyaluronidase domain.") But it nowhere identifies position 320 or suggests substituting D320 with lysine (K). EX1005.

409. Likewise, Chao reports the crystal structure of human HYAL-1 and (i) identifies conserved residues, including key catalytic residues shared among various hyaluronidases, and (ii) predicts secondary structures across the human hyaluronidases. EX1006 (Chao), 6912-17. None of these disclosures provide a

reason to modify position 320 in PH20.

410. In fact, Chao's Figure 3 identifies over 372 non-conserved residues in PH20 as set forth below. EX1006 (Chao), FIG. 3.



Annotated Figure 3 of Chao. The non-conserved regions are outlined in red, and the non-conserved residues are uncolored, identifying a total of 372 non-conserved residues. Conserved residues are highlighted in yellow, blue, and red. EX1006 (Chao), FIG. 3.

Chao does not single out D320 among these 372 residues; instead, a POSA would have generally understood the non-conserved positions as potential candidates for substitution, with no reason to treat D320 differently from the hundreds of positions likely tolerant to change.

411. Similarly, Dr. Park's multiple sequence alignment identifies 379

positions in $PH20_{1\text{-}447}$ that he categorizes as "non-essential," which he defined as residues conserved among less than 95% of the 88 homologous sequences he analyzed. EX1004, ¶¶26-29, 31-32, 44, Appendix D-1. But Dr. Hecht and Dr. Park do not explain why a POSA would have singled out D320 from among these hundreds of other candidate amino acid positions that were identified as being able to tolerate substitutions.[15]

412. Dr. Park's further analysis of amino acid substitutions in the "non-essential" positions confirms the absence of motivation. Dr. Park testified that he was tasked with "identifying non-essential positions in PH20" and "evaluat[ing] various substitutions at those positions to see if there are substitutions…that would be tolerated." EX2077 (Park I Tr.), 67:22–68:7. To do so, he generated an alignment

[15] I further note that Dr. Park admitted that his focus on position 320 was not based on an independent scientific analysis, but that he was "asked by counsel to report [his] conclusions with respect to position 320." EX1004 ¶103. I understand that during his deposition, Dr. Park likewise acknowledged that instead of relying on his own scientific analysis, counsel "asked [him] to elaborate on certain positions…." EX2078 (Park II Tr.), 320:14-15. I understand that counsel have agreed that the testimony of Drs. Hecht and Park, from related PGR Nos. PGR2025-00003, -00004, -00006, and -00009, can be applied here.

of 88 hyaluronidase sequences, identified residues conserved more than 95% of the time, and categorized those 68 residues as "essential." EX1004 ¶¶26-29, 31-32, 44; EX2078 (Park II Tr.), 267:13-268:2.

413. Dr. Park categorized the remaining 379 residues as "non-essential" and, using a ≥ 5% frequency cutoff in his multiple sequence alignment, identified substitutions recurring among homologous sequences that he considered worth examining as potentially tolerated. EX1004 ¶¶30-31.

414. In total, Dr. Park identified more than 2,800 possible amino acid substitutions in the so-called "non-essential" positions of PH20. EX2078 (Park II Tr.), 268:15-269:4, 280:3-7. From that pool, Dr. Park evaluated approximately 830 substitutions and concluded that about 90% were predicted to be tolerated. EX2078 (Park II Tr.), 282:19-283:18, 299:3-14. Thus, even within the limited subset of substitutions he analyzed, Dr. Park's results produced hundreds of tolerated substitutions across PH20 that a POSA could have considered. And because Dr. Park left thousands of potential substitutions unevaluated, the number of tolerated options would likely have been even higher. Dr. Park's results offer no basis to single out D320 from the many other substitutions he predicted would have been tolerated.

415. Moreover, the '429 patent's statement that "single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter

biological activity" would not have motivated the POSA to make any substitutions in PH20. The '429 patent merely indicates that modifications to the disclosed truncated PH20s can be made without substantially altering activity. It offers no reason why a POSA, already in possession of a working enzyme, would pursue changes aimed only at preserving the same activity profile. Nothing in the '429 patent would have motivated a POSA to undertake Dr. Park's exercise of identifying "non-essential" regions and evaluating more than 800 potential substitutions in a PH20 polypeptide.

416. Accordingly, in my opinion, neither Dr. Hecht's analysis of the prior art, nor his reliance on Dr. Park's multiple sequence alignment, shows that a POSA would have been motivated to mutate D320.

a. A POSA Would Have Had No Motivation to Replace Aspartic Acid (D) with Lysine (K) at Position 320

417. Even if position 320 were somehow selected, a POSA would have had no reason to substitute aspartic acid (D) with lysine (K). Nothing in Chao or the '429 patent directs a POSA to substitute the aspartic acid at position 320 with lysine. The POSA would have understood that this is a nonconservative substitution. Examining the environment of this residue, the POSA would see it is part of an alpha-helix. The POSA would have had no reason to focus on this position, as explained above, nor was there any reason for the POSA to replace a negatively charged amino acid in an alpha-helix with a positively charged amino acid.

418. Accordingly, in my opinion, Chao, the ’429 patent, and insights from multiple sequence alignment analysis provide no basis for a POSA to select position D320, let alone to substitute D320 with lysine (K). The Challenged Claims 1-4 and 7-11 therefore are not obvious in view of Chao and the ’429 patent.

XIII. CONCLUSION

419. In view of the facts above, and in accordance with the legal principles described in Section VI, a POSA would have found that the common disclosure shared by the ’185 patent and the ’731 application (filed December 28, 2012) adequately described and enabled the subject matter of claims 1-4 and 7-11. Additionally, in view of the facts above, and in accordance with the legal principles described in Section VI, a POSA would have found that claims 1-4 and 7-11 would not have been obvious to a POSA before December 29, 2011 (the date Dr. Hecht used in his analysis).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Executed on this 7th day of January, 2026.



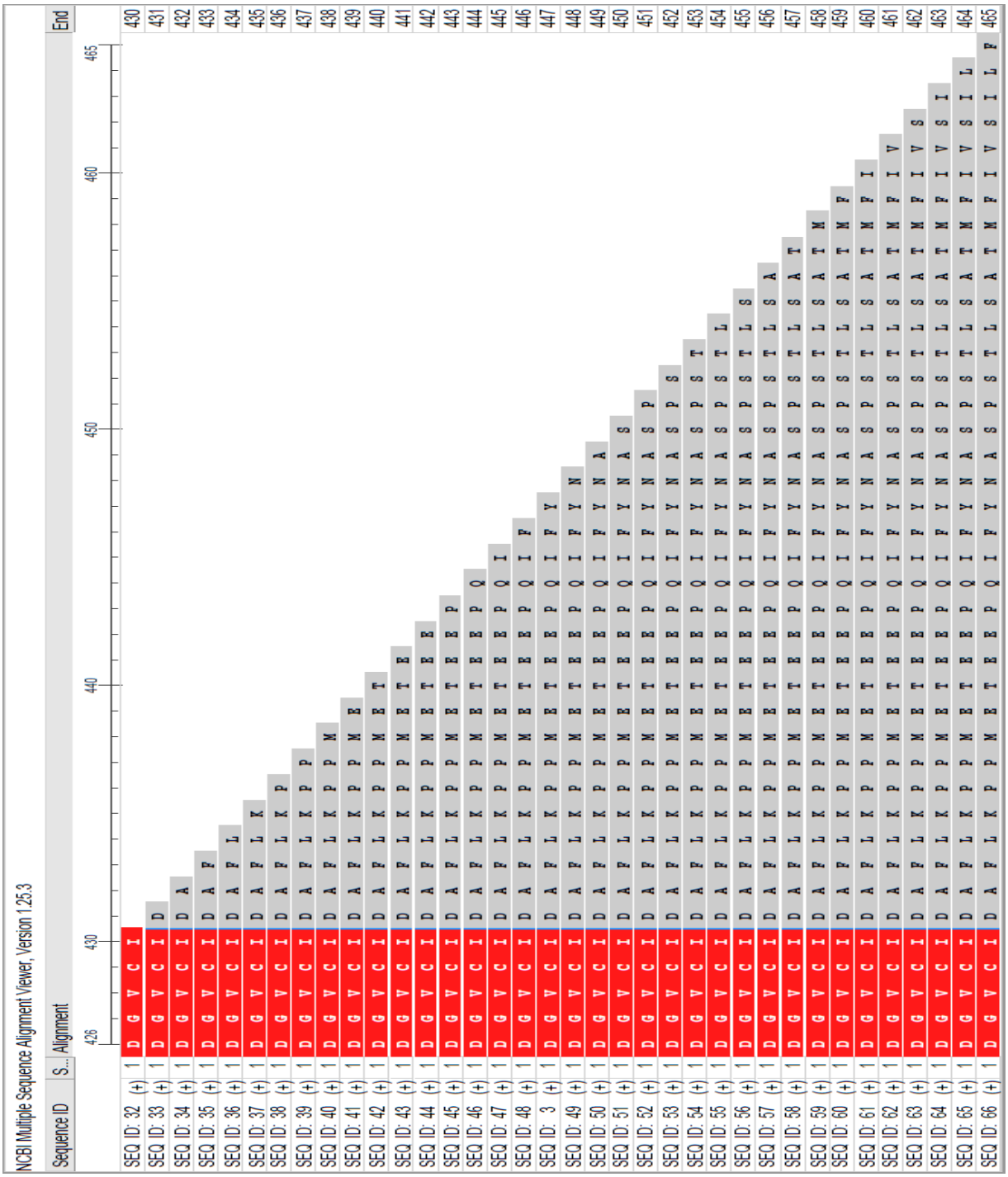
Melanie A. Simpson, Ph.D.

TABLE OF EXHIBITS

Exhibit No.	Description
1001	U.S. Patent No. 12,104,185
1002	File History of U.S. Patent No. 12,104,185
1003	Declaration of Dr. Michael Hecht
1004	Declaration of Dr. Sheldon Park
1005	U.S. Patent No. 7,767,429
1006	Chao et al., "Structure of Human Hyaluronidase-1, a Hyaluronan Hydrolyzing Enzyme Involved in Tumor Growth and Angiogenesis," Biochemistry, 46:6911-6920 (2007)
1008	Stern et al., "The Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action," Chem. Rev. 106:818-839 (2006)
1009	Jedzrejas et al., "Structures of Vertebrate Hyaluronidases and Their Unique Enzymatic Mechanism of Hydrolysis," Proteins: Structure, Function and Bioinformatics, 61:227-238 (2005)
1010	Zhang et al., "Hyaluronidase Activity of Human Hyall Requires Active Site Acidic and Tyrosine Residues," J. Biol. Chem., 284(14):9433-9442 (2009)
1011	Arming et al., "In vitro mutagenesis of PH-20 hyaluronidase from human sperm," Eur. J. Biochem., 247:810-814 (1997)
1014	Brandon & Tooze, "Introduction to Protein Structure," Second Ed., Chapters 1-6, 11-12, 17-18 (1999)
1026	U.S. Patent Application No. 13/694,731
1033	Markovic-Housley et al., "Crystal Structure of Hyaluronidase, a Major Allergen of Bee Venom," Structure, 8:1025-1035 (2000)
1045	Redline Comparison of the '731 and '185 Specifications

Exhibit No.	Description
2003	Disclaimer in a Patent under 37 C.F.R. § 1.321(a), filed in U.S. Patent Application No. 18/599,428, June 13, 2025
2006	Sequence listing of U.S. Patent Application No. 18/599,428
2069	*Curriculum Vitae* of Melanie A. Simpson, Ph.D.
2070	Declaration of Gregory A. Petsko, Ph.D. in Support of Patent Owner's Response
2072	Declaration of Gary N. Cherr, Ph.D. in Support of Patent Owner's Response
2074	Declaration of James J. Moon, Ph.D. in Support of Patent Owner's Response
2076	Transcript of the Deposition of Michael Hecht, Ph.D., August 26, 2025
2077	Transcript of the Deposition of Dr. Sheldon Park, August 7, 2025
2078	Transcript of the Deposition of Dr. Sheldon Park, August 21, 2025
2086	El-Safory, N. et al., "Hyaluronidases, a group of glycosidases: Current and future perspectives," *Carbohydrate Polymers,* 81:165-181 (2010)
2088	Stern, R. et al., "Mammalian Hyaluronidases," Hyaluronan Index (2000)
2164	Petsko, G., et al., *Protein Structure and Function,* New Science Press (2004)
2165	U.S. Patent Application Publication No. 2010/0143457 Al to Wei et al., published June 10, 2010
2166	Petsko Patent Analysis Data
2170	Chain, E. et al., "Identity of Hyaluronidase and Spreading Factor," *British Journal of Experimental Pathology,* 21(6):324-338 (1940)

APPENDIX A



Enlarged view of the Figure at paragraph 175 above. Sequence alignment I prepared aligning the C-terminal portions of SEQ ID NOs. 3 and 32-66 and showing that each sequence differs by only a single amino acid residue at the C-terminus, with respect to the most similar length sequence(s).