

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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MERCK SHARP & DOHME LLC,  
Petitioner

v.

HALOZYME, INC.,  
Patent Owner

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Case PGR2025-00017  
U.S. Patent No. 12,110,520

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**DECLARATION OF BARBARA TRIGGS-RAINE, PH.D. IN SUPPORT OF  
PATENT OWNER DISCRETIONARY DENIAL BRIEF**

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Halozyyme EX2001  
Merck v. Halozyyme  
PGR2025-00017

**TABLE OF CONTENTS**

I.	INTRODUCTION .....	1
II.	MY BACKGROUND AND QUALIFICATIONS .....	4
III.	SUMMARY OF OPINIONS .....	9
IV.	LIST OF DOCUMENTS CONSIDERED.....	10
V.	PERSON OF ORDINARY SKILL IN THE ART.....	18
VI.	LEGAL BASIS FOR MY ANALYSIS.....	28
	A. Claim Construction .....	28
	B. Written Description.....	29
	C. Enablement.....	32
	D. Obviousness.....	33
VII.	CLAIMS 1-2, 6-15, and 17-30 DO NOT REQUIRE HYALURONIDASE ACTIVITY .....	36
	A. A POSA Would Have Understood That a “modified PH20 polypeptide” Means “a PH20 polypeptide that contains at least one amino acid modification compared to a reference unmodified PH20 polypeptide” .....	36
	B. A POSA Would Not Have Interpreted Claims 1-2, 6-15, and 17-30 as Being Limited to Enzymatically Active Mutant PH20 Polypeptides .....	46
VIII.	CLAIMS 1-2, 6-15, and 17-30 WOULD NOT HAVE BEEN OBVIOUS IN VIEW OF CHAO AND THE ’429 PATENT.....	49
	A. Even in Combination, Chao and the ’429 Patent Fail to Disclose or Suggest a E324 Mutation of PH20, Much Less a E324 Mutation to Aspartic acid (D), Asparagine (N), or Arginine (R), as Required by Claims 1-2, 6-15, and 17-30.....	51
	1. Chao and the ’429 Patent Do Not Disclose Any Mutation of E324, Nor Do Chao and the ’429 Patent Disclose Any Mutation of E324 to Aspartic acid (D), Asparagine (N), or Arginine (R), as Required by Claims 1-2, 6-15, and 17-30. ....	52
	2. Dr. Hecht Does Not Adequately Explain Why a POSA Would Have Supplied the Missing E324 Mutation or Modified the	

Missing Mutation of E324 to Aspartic acid (D), Asparagine (N), or Arginine (R) based on Chao and the '429 Patent Using Common Sense or Ordinary Creativity. .... 53

- B. Even in Combination, Chao and the '429 Patent Would Not Have Provided Any Motivation to Make a E324 Mutation of PH20, Much Less a E324 Mutation to Aspartic acid (D), Asparagine (N), or Arginine (R), as Required by Claims 1-2, 6-15, and 17-30.....55
  - 1. Chao and the '429 Patent Would Not Have Motivated a POSA to Make the Claimed E324 Mutation. .... 56
    - a. The '429 Patent Would Not Have Provided Any Reason to Make Single Amino Acid Mutations in “Non-Essential” Regions..... 56
    - b. Chao Would Not Have Identified “Non-Essential” Regions..... 57
    - c. A POSA Would Not Have Been Motivated to Prepare nor Rely on the 88-Sequence Alignment Prepared by Dr. Hecht Because Dr. Park’s Alignment Includes Mostly Non-PH20 Sequences. .... 57
      - i. Different Hyaluronidases Were Known to Have Different Functions and Substrates before December 29, 2011.....59
    - d. Drs. Hecht and Parks’ Stated Reason for Focusing on Position 324 is Not Based on the Prior Art..... 61
    - e. Drs. Hecht and Park Do Not Establish That a POSA Would Have Been Motivated to Mutate E324 to Aspartic acid (D), Asparagine (N), or Arginine (R)..... 63
      - i. Drs. Hecht and Park Do Not Explain Why a POSA Would Have Been Motivated to Undertake Nearly 30 Discrete Steps to Make the E324D Mutation..63
- C. Drs. Hecht and Park Have Not Established That, in the Absence of the Guidance Provided in the Specification, Chao and the '429 Patent Would Have Provided a POSA with a Reasonable Expectation That an E324D Mutation of PH20 Would Have Produced a Polypeptide with “at Least Comparable Hyaluronidase Activity as Unmodified PH20<sub>1-447</sub>” .....67

D.	Dr. Hecht’s Declarations in Related Proceedings Further Reveal that the ’429 Patent and Chao Would Not Have Provided Any Reason to Make an E324D Mutation.....	79
IX.	THE EXAMINER CONSIDERED THE SAME OR SUBSTANTIALLY THE SAME ART AND ARGUMENTS AS NOW PRESENTED BY DRs. HECHT AND PARK.....	89
A.	The Examiner Considered Stern, Zhang, and Arming, Which Include Teachings Cumulative to the Relied-Upon Teachings of Chao. ....	89
1.	Stern was considered by the Examiner.....	92
2.	Zhang was considered by the Examiner. ....	101
3.	Arming was considered by the Examiner. ....	103
X.	CONCLUSION .....	106

I, Barbara Triggs-Raine, 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 \$300 per hour.

3. I understand that this Declaration accompanies a Patent Owner Discretionary Denial Brief filed in this PGR involving U.S. Patent No. 12,110,520 (“the ’520 patent”) (EX1001), which resulted from U.S. Patent Application No. 18/068,418 (“the ’418 application”), filed on December 19, 2022.

4. I understand that the ’520 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 ’520 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 ’520 patent and the ’731 application, I have reviewed a redline comparison of the specifications of the ’520 patent and the ’731 application (EX1045). The ’520 patent has substantively the same specification<sup>1</sup> as the ’731 application filed on December 28, 2012. *Compare* EX1001, *with* EX1026, 14-370. Dr. Hecht agrees that the specifications of the ’520 patent and the ’731 application are substantively the same and share a “common

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<sup>1</sup> I understand that the specification of the ’520 patent includes its Figures and a narrative discussion, EX1001, pp. 15-181, including any documents that are “incorporated by reference,” as well as the patent’s sequence listing, EX2006.

disclosure.” EX1003, ¶18. Like Dr. Hecht, for convenience, I provide citations to the disclosure in the ’520 patent and at times refer to the specification that is substantively shared by the ’520 patent and the ’731 application as the “common disclosure.”<sup>2</sup> 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 ’520 patent and its entire prosecution history, including the entire prosecution histories of the ’568 application, the ’590 application, the ’572 application, the ’489 application, the ’731 application, the ’208 provisional application, and the ’313 provisional application 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 filing date of the ’731 application that is related to the ’520 patent.<sup>3</sup> In assessing obviousness

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<sup>2</sup> Because the specification of the ’520 patent and the specification of the ’731 application share a common disclosure that is substantively the same, I may refer to them interchangeably.

<sup>3</sup> 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

in response to Dr. Hecht's and 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 § V, as of the timeframes discussed herein (e.g., paragraph 7), in light of general knowledge in the art.

## **II. MY BACKGROUND AND QUALIFICATIONS**

9. I am a Professor in the Department of Biochemistry & Medical Genetics at the Rady Faculty of Health Sciences at the University of Manitoba. And I was the Head of the Department of Biochemistry & Medical Genetics from 2018-2025. I hold a Ph.D. in Microbial Genetics and B.Sc. in Microbiology, with First Class Honors from the University of Manitoba. In addition, I am a board

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date (December 28, 2012). I understand that the '520 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, 123-124; EX1004, Appendix A.

member of the International Society for Hyaluronan Sciences, a member of the American Society of Matrix Biology, and a member of the American Society for Biochemistry & Molecular Biology.

10. I have extensive experience in the fields of cellular and molecular biology, biochemistry (including protein biochemistry), glycobiology, and human genetics—including extensive practical experience researching hyaluronidases, which have been a focus of my research since 1995.

11. My *curriculum vitae* is submitted herewith as EX2002.

12. In 1983, I completed my B.Sc. at the University of Manitoba, during which I was awarded the Natural Sciences and Engineering Research Council of Canada (“NSERC”) Undergraduate University Summer Research Award and the NSERC Postgraduate Scholarship. In 1987, I completed my Ph.D. at the University of Manitoba (studying a gene encoding a bacterial enzyme and its regulation). I was also extended awards for the Medical Research Council Postgraduate Scholarship (in 1983)<sup>4</sup> and the Sigma Xi Student Award for Excellence in Research and the NSERC Postdoctoral Fellowship (in 1987).

13. Prior to my postdoctoral work, I was a teaching assistant (sessional) in the Dept. of Microbiology in the Faculty of Science, University of Manitoba from

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<sup>4</sup> I declined these awards.

1987–1988.

14. From 1988–1989, I was a postdoctoral fellow at the Hospital for Sick Children, Toronto (studying the genetics of Tay-Sachs disease), during which I was awarded the Medical Research Council Postdoctoral Fellowship. From 1989–1991, I continued my postdoctoral training at the McGill University-Montreal Children’s Hospital, Montreal (studying the genetics of Tay-Sachs disease).

15. In 1991, I returned to the University of Manitoba, where I have held multiple roles until the present date, including: (i) at the Faculty of Medicine—Assistant Professor, Dept. Biochemistry & Medical Genetics (1991–1997); Assistant Professor, Dept. Human Genetics (1994–1997); Associate Professor, Dept. Biochemistry & Medical Genetics (1997–2003); and Associate Professor, Dept. Pediatrics & Child Health (2003–2006); (ii) at the Manitoba Institute of Child Health—Director of Research, Facilities and Space Development (2008–2010); and (iii) at the Rady Faculty of Health Sciences—Professor, Dept. Biochemistry & Medical Genetics (2004–present); Professor, Dept. Pediatrics & Child Health (2006–2021); Associate Head, Dept. Biochemistry & Medical Genetics (2011–2018); Scientific Director, Central Animal Core Facility (2015–2022); and Head, Dept. Biochemistry & Medical Genetics (2018–February 2025).

16. While at the University of Manitoba, I received multiple awards, including: Manitoba Health Research Council Scholarship (1991–1992), Medical

Research Council of Canada Scholarship (1992–1997), Rh Award in the Health Sciences (1995), Journal of Biological Chemistry: Best of 2012—Glycobiology and Extracellular Matrices (2012), Manitoba Medical Students' Association Nomination for Best Teaching: Small Group Setting—Class of 2020 (2018), Bachelor of Science in Medicine Supervisor Mentorship Award (2018–2019), and Science Co-op Supervisor Recognition Award (2023).

17. Since 1995, a primary focus of my research has been researching and characterizing hyaluronidases. My research has led to over 80 peer-reviewed research publications and six book chapters, over 20 of which specifically relate to hyaluronidases. I have also received over 50 grants, over 10 of which specifically related to the characterization of hyaluronidases. Additionally, I have advised 6 doctoral students and 10 masters students, seven of whom specifically studied hyaluronidases for their dissertation.

18. I have also advised three postdoctoral fellows, two resident research projects, and over forty undergraduate students, and I have served on over 70 graduate student committees. I frequently peer review publications and have been an *ad hoc* reviewer for the publications *Nature Communications*, *American Journal of Human Genetics*, *American Journal of Medical Genetics*, *BMC Genetics*, *BMC Medical Genetics*, *Brain and Behavior*, *Canadian Journal of Physiology and Pharmacology*, *European Journal of Human Genetics*, *Gene*, *Gene*

*Therapy, Glycobiology, Matrix Biology, Molecular Genetics and Metabolism, Molecular and Cellular Biochemistry, Mutation Research, Orphanet Journal of Rare Diseases, Plant Biotechnology Journal, PLOS One, Journal of Biological Chemistry, and Mutation Research.* The publications I reviewed often related to hyaluronan or hyaluronidase biology.

19. In addition to my educational training and my professional and research experience, I have kept abreast of the fields of cellular and molecular biology, biochemistry (including protein biochemistry), glycobiology, and human genetics by reading scientific literature, conferring with colleagues in the field, and attending and presenting lectures at scientific conferences. I have given over 20 presentations and invited lectures, over half of which specifically related to hyaluronidases and/or hyaluronan degradation. I have provided abstracts to 100 scientific conferences, approximately 40 of which specifically related to hyaluronidases and/or hyaluronan. I have also taught undergraduate and graduate courses on genetics in biomedicine, glycobiology, human genetics, environmental microbiology, biological energy transductions, genetic counseling, and medical biochemistry—and I was the Symposium Program Chair at the 2018 Annual Glycomics Symposium. I was also a member of the organizing committee for the International Society of Hyaluronan Sciences biennial meeting in 2023. I currently serve in the same role for the 2025 meeting.

### III. SUMMARY OF OPINIONS

20. I have been asked to consider whether a POSA would have found the subject matter of claims 1-2, 6-15, and 17-30 of the '520 patent (i.e., the claimed invention) to be adequately described in the patent (Ground A), 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 (Ground B). In view of the state of the art as of December 28, 2012, and in light of the teachings in the common disclosure, a POSA would not have found the challenged claims to lack written description support or enablement. In particular, a POSA would have recognized that the claimed modified PH20 polypeptides all include common structural features, which are defined by amino acid sequence identity—not hyaluronidase activity; thus, Dr. Hecht's analysis of written description and enablement, which is predicated on requiring that the claimed modified PH20 polypeptides exhibit hyaluronidase activity, is erroneous.

21. I have also been asked to consider (1) whether all limitations of the challenged claims are found in Dr. Hecht's asserted prior art: Chao (EX1006) and the '429 patent (EX1005); (2) whether a POSA would have had a reason to combine the teachings of Chao (EX1006) and the '429 patent (EX1005) to make the claimed modified PH20 polypeptides that include an E324D, E324N, or E324R

substitution, including whether (i) bridging the gap between Chao, the '429 patent, and the claimed invention would have required more than ordinary creativity; and (ii) whether a POSA exercising ordinary creativity would have had a reasonable expectation of success in combining Chao and the '429 patent to arrive at the claimed invention (Ground C). In view of the state of the art before December 29, 2011, a POSA would not have found the challenged claims to have been obvious.

22. Additionally, I have been asked to consider (i) whether the disclosures in Chao and the '429 patent on which Drs. Park and Hecht rely would have conveyed to a POSA the same or substantially the same information as the references the patent examiner considered during prosecution of the '600 patent; and (ii) whether the pending claims materially differed from the final issued claims of the '600 patent.

#### **IV. LIST OF DOCUMENTS CONSIDERED**

In providing my testimony, I considered the documents cited in this Declaration and the documents listed in the table below.

<b>Exhibit No.</b>	<b>Description</b>
1001	U.S. Patent No. 12,110,520
1002	File History of U.S. Patent No. 12,110,520
1003	Declaration of Dr. Michael Hecht
1004	Declaration of Dr. Sheldon Park

Exhibit No.	Description
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," <i>Biochemistry</i> , 46:6911-6920 (2007)
1007	WO 2010/077297, published 8 July 2010
1008	Stern et al., "The Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action," <i>Chem. Rev.</i> 106:818-839 (2006)
1009	Jedzrejas et al., "Structures of Vertebrate Hyaluronidases and Their Unique Enzymatic Mechanism of Hydrolysis," <i>Proteins: Structure, Function and Bioinformatics</i> , 61:227-238 (2005)
1010	Zhang et al., "Hyaluronidase Activity of Human Hyal1 Requires Active Site Acidic and Tyrosine Residues," <i>J. Biol. Chem.</i> , 284(14):9433-9442 (2009)
1011	Arming et al., "In vitro mutagenesis of PH-20 hyaluronidase from human sperm," <i>Eur. J. Biochem.</i> , 247:810-814 (1997)
1012	Bordoli et al., "Protein structure homology modeling using SWISSMODEL workspace," <i>Nature Protocols</i> , 4(1):1-13 (2008)
1013	Frost, "Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration," <i>Expert Opinion on Drug Delivery</i> , 4(4):427-440 (2007)
1014	Brandon & Tooze, "Introduction to Protein Structure," Second Ed., Chapters 1-6, 11-12, 17-18 (1999)
1015	Table Associating Citations from the '520 patent (EX1001) to Corresponding Citations in the '731 Application (EX1026)
1016	Steipe, "Consensus-Based Engineering of Protein Stability: From Intrabodies to Thermostable Enzymes," <i>Methods in Enzymology</i> , 388:176-186 (2004)

Exhibit No.	Description
1017	Green, "Computer Graphics, Homology Modeling, and Bioinformatics," Protein Eng'g & Design, Ch. 10, 223-237 (2010)
1018	Chica et al., "Semi-rational approaches to engineering enzyme activity: combining the benefits of directed evolution and rational design," Curr. Opin. Biotechnol., (4):378-384 (2005)
1019	Hardy et al., "Assessment of contraceptive vaccines based on recombinant mouse sperm protein PH20," Reprod., 127:325-334 (2004)
1020	Pomering et al., "Restricted Entry of IgG into Male and Female Rabbit Reproductive Ducts Following Immunization with Recombinant Rabbit PH-20," Am. J. Reprod. Immunol., (3):174-82 (2002)
1021	Baba et al., "Mouse Sperm Lacking Cell Surface Hyaluronidase PH-20 Can Pass through the Layer of Cumulus Cells and Fertilize the Egg," J. Biol. Chem., 277(33):30310-4 (2002)
1022	Primakoff et al., "Reversible Contraceptive Effect of PH-20 Immunization in Male Guinea Pigs," Biol Reprod., 56(5):1142-6 (1997)
1023	Tung et al., "Mechanism of Infertility in Male Guinea Pigs Immunized with Sperm PH-20," Biol. Reprod., 56(5):1133-41 (1997)
1024	Rosengren et al., "Recombinant Human PH20: Baseline Analysis of the Reactive Antibody Prevalence in the General Population Using Healthy Subjects," BioDrugs, 32(1):83-89 (2018)
1025	U.S. Patent No. 9,447,401
1026	U.S. Patent Application No. 13/694,731
1029	Gmachl et al., "The human sperm protein PH-20 has hyaluronidase activity," FEBS Letters, 3:545-548 (1993)
1030	Sills, "Retraction," Science, 319:569 (2008)

Exhibit No.	Description
1031	Yue et al., “Loss of Protein Structure Stability as a Major Causative Factor in Monogenic Disease,” J. Mol. Biol., 353:459-473 (2005)
1032	Wang & Moulton, “SNPs, Protein Structure, and Disease,” Hum. Mutation, 17:263-270 (2001)
1033	Marković-Housley et al., “Crystal Structure of Hyaluronidase, a Major Allergen of Bee Venom,” Structure, 8:1025-1035 (2000)
1034	“Negative Results,” Nature: Editorials, 453:258 (2008)
1035	Lins et al., “Analysis of Accessible Surface of Residues in Proteins,” Protein Sci., 12:1406-1417 (2003)
1036	Hayden, “Chemistry: Designer Debacle,” Nature, 453:275-278 (2008)
1037	Benkert et al., “Toward the Estimation of the Absolute Quality of Individual Protein Structure Models,” Bioinformatics, 27:343-350 (2010)
1038	Schwede et al., “SWISS-MODEL: An Automated Protein Homology-Modeling Server,” Nucleic Acids Res., 31:3381-3385 (2003)
1039	Alberts, “Molecular Biology of the Cell,” Fifth Edition, Chapter 3 (2007).
1040	He et al., “NMR Structures of Two Designed Proteins with High Sequence Identity but Different Fold and Function,” PNAS, 105:14412-14417 (2008)
1041	Alexander et al., “A Minimal Sequence Code for Switching Protein Structure and Function,” PNAS, 106:21149-21154 (2009)
1042	Ruan et al., “Design and Characterization of a Protein Fold Switching Network,” Nature Comm., 14 (2023)

Exhibit No.	Description
1043	Sievers et al., “Fast, Scalable Generation of High-Quality Protein Multiple Sequence Alignments Using Clustal Omega,” <i>Molecular Sys. Biology</i> , 7.1 (2011)
1044	Mihel, “PSAIA – Protein Structure and Interaction Analyzer,” <i>BMC Structural Biology</i> , 8:21 (2008)
1045	Redline Comparison of the '731 and '520 Specifications
1046	Beasley & Hecht, “Protein Design: The Choice of de Novo Sequences,” <i>J. Biological Chemistry</i> , 272:2031-2034 (1997)
1047	Xiong et al., “Periodicity of Polar and Nonpolar Amino Acids is the Major Determinant of Secondary Structure in Self-Assembling Oligomeric Peptides,” <i>PNAS</i> , 92: 6349-6353 (1995)
1048	Hayden, “Key Protein-Design Papers Challenged,” <i>Nature</i> , 461:859 (2009)
1049	KEGG, DRUG: Hyaluronidase (human recombinant), available at: <a href="https://www.genome.jp/entry/D06604">https://www.genome.jp/entry/D06604</a>
1050	Pace & Scholtz, “A Helix Propensity Scale Based on Experimental Studies of Peptides and Proteins,” <i>Biophysical J.</i> 75:422-427
1051	U.S. Patent Application No. 61/631,313
1052	U.S. Patent Application No. 61/796,208
1053	Hom_pre2011
1054	Hom_pre2011_header
1055	Hom_pre2011_header_clean
1056	Hom_pre2011.fasta
1057	Ph20_pre2011.aln-clustal_num
1058	Ph20_pre2011 Alignment html

Exhibit No.	Description
1059	Leisola & Turunen, “Protein Engineering: Opportunities and Challenges,” Appl. Microbiol. Biotechnol. 75:1225-1232 (2007)
1060	Hecht et al., “De Novo Proteins from Designed Combinatorial Libraries,” Protein Sci., 13:1711-1723 (2004)
1061	Rosengren et al., “Clinical Immunogenicity of rHuPH20, a Hyaluronidase Enabling Subcutaneous Drug Administration,” AAPS J., 17:1144-1156 (2015)
1064	Collection of BLAST Webpages from the Internet Archive, navigable from: <a href="https://web.archive.org/web/20111022151531/http://www.clustal.org/omega/">https://web.archive.org/web/20111022151531/http://www.clustal.org/omega/</a>
1065	Collection of Clustal Omega Webpages from the Internet Archive, navigable from: <a href="https://web.archive.org/web/20111022151531/http://www.clustal.org/omega/">https://web.archive.org/web/20111022151531/http://www.clustal.org/omega/</a>
1066	Collection of SWISS-MODEL Webpages from the Internet Archive, navigable from: <a href="https://web.archive.org/web/20110519141121/http://swissmodel.expasy.org/?pid=smh01&amp;uid=&amp;token=">https://web.archive.org/web/20110519141121/http://swissmodel.expasy.org/?pid=smh01&amp;uid=&amp;token=</a>
1067	Collection of PyMol Webpages from the Internet Archive, navigable from: <a href="https://web.archive.org/web/20110701072314/http://pymol.org/">https://web.archive.org/web/20110701072314/http://pymol.org/</a>
1068	Declaration of Jeffrey P. Kushan
1069	Swiss Model Printout of PH20 Model
1070	Swiss Model Printout of PH20 Model with E324D Mutation
1071	Swiss Model Printout of PH20 Model with E324N Mutation
1072	Swiss Model Printout of PH20 Model with E324R Mutation
1073	Swiss Model Printout of PH20 Model with E324A Mutation

Exhibit No.	Description
1074	Swiss Model Printout of PH20 Model with E324H Mutation
1075	Swiss Model Printout of PH20 Model with E324S Mutation
2002	<i>Curriculum Vitae</i> of Barbara Triggs-Raine, Ph.D.
2003	Disclaimer in a Patent under 37 C.F.R. § 1.321(a), filed in U.S. Patent Application No. 18/068,418, May 7, 2025
2004	“Halozyme Therapeutics to Present Data on PEGPH20 at the Upcoming 2011 EORTC-NCI-ASCO Annual Meeting,” Halozyme Therapeutics, Inc. Press Release, October 24, 2011
2005	LinkedIn profiles of Michael Shepard, Robert Connor, Ge (Gina) Wei, and Qiping Zhao
2006	Sequence listing of U.S. Patent Application No. 18/068,418
2018	Duterme, C., <i>et al.</i> , “Two Novel Functions of Hyaluronidase-2 (Hyal2) Are Formation of the Glycocalyx and Control of CD44-ERM Interactions,” <i>The Journal of Biological Chemistry</i> , 284(48):33495-33508 (November 27, 2009)
2019	Atmuri, V., <i>et al.</i> , “Hyaluronidase 3 ( <i>HYAL3</i> ) knockout mice do not display evidence of hyaluronan accumulation,” <i>Matrix Biology</i> 27:653-660 (2008)
2020	Hemming, R., <i>et al.</i> , “Mouse Hyal3 encodes a 45- to 56-kDa glycoprotein whose overexpression increases hyaluronidase 1 activity in cultured cells,” <i>Glycobiology</i> 18(4):280-289 (2008)
2021	Miller, A., “Hyaluronidase 2 and its intriguing role as a cell-entry receptor for oncogenic sheep retroviruses,” <i>Seminars in Cancer Biology</i> 18:296-301 (2008)
2022	Kaneiwa, T. <i>et al.</i> , “Identification of human hyaluronidase-4 as a novel chondroitin sulfate hydrolase that preferentially cleaves the galactosaminidic linkage in the trisulfated tetrasaccharide sequence,” <i>Glycobiology</i> 20(3):300-309 (March 2010)

Exhibit No.	Description
2029	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00004 (P.T.A.B.), November 26, 2024
2030	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00006 (P.T.A.B.), December 10, 2024
2031	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00009 (P.T.A.B.), December 27, 2024
2032	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00003 (P.T.A.B.), November 12, 2024
2033	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00030 (P.T.A.B.), February 4, 2025
2034	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00024 (P.T.A.B.), February 21, 2025
2035	Lokeshwar, V., <i>et al.</i> , "Regulation of Hyaluronidase Activity by Alternative mRNA Splicing," <i>The Journal of Biological Chemistry</i> 277(37):33654-33663 (2002)
2036	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00033 (P.T.A.B.), March 7, 2025
2038	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00039 (P.T.A.B.), March 28, 2025
2057	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00042 (P.T.A.B.), April 15, 2025

Exhibit No.	Description
2061	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00046 (P.T.A.B.), April 29, 2025
2063	Declaration of Michael Hecht, Ph.D. (Exhibit 1003), <i>Merck Sharp &amp; Dohme LLC v. Halozyme Inc.</i> , Case No. PGR2025-00050 (P.T.A.B.), May 7, 2025

**V. PERSON OF ORDINARY SKILL IN THE ART**

23. As an expert, I have offered opinions throughout this declaration from the perspective of a person of ordinary skill in the art (“POSA”). I understand that the level of ordinary skill in the art acts as a lens through which the prior art and claimed invention must be viewed. I further understand that an assessment of whether an invention is adequately described, enabled, or nonobvious is conducted from the viewpoint of a POSA.

24. 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. I also understand that the following factors are pertinent to the determination of the level of ordinary skill: (1) the educational level of the inventor(s), (2) the type of problems encountered in the art, (3) the prior art solutions to those problems, (4) the rapidity with which innovations are made, (5) the sophistication of the technology, and (6) the educational level of active workers in the field.

25. Dr. Hecht opined that “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 [the] study of protein structure and function (*e.g.*, chemistry, biochemistry, biology, biophysics) . . . [and] [f]rom 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 with techniques and tools used to analyze protein structure (*i.e.*, sequence searching and alignments, protein modeling software, etc.).” EX1003, ¶13.

26. I disagree with Dr. Hecht’s assessment of the level of skill of a POSA because he omits any practical experience with hyaluronidases and because a Ph.D. and/or post-doctoral experience is not required to make and use the claimed modified PH20 polypeptides. EX1003. As I discuss below, a POSA in the relevant field, or at least a member of their team, would have had experience working with hyaluronidases.

27. The ’520 patent’s specification and its cited references are informative to factors 2-4. The ’520 patent contains significant description regarding hyaluronidases, and the ’520 patent cites numerous references relating to hyaluronidases. For example, more than 80 references cited plainly relate to hyaluronidase according to their title. EX1001, 3-13 (containing more than 80

references mentioning hyaluronidase and/or its substrate hyaluronan in their titles); EX1001, 8 (“Hyaluronidase and its substrate hyaluronan: biochemistry, biological activities and therapeutic uses”).

28. The patent also states that the field of the invention relates to modified PH20 hyaluronidase peptides. EX1001, 4:15-20. And the background of the invention discusses hyaluronan and “hyaluronan-degrading enzymes (e.g., hyaluronidases).” EX1001, 4:21-50. The background of the invention also discusses the therapeutic use of hyaluronidases. For Example: EX1001, 4:41-46 (“Various hyaluronidases have been used therapeutically (e.g., hyaluronidase sold under the trademarks Hydase® (bovine testicular hyaluronidase), Vitrase® (ovine hyaluronidase), and Wydase® (bovine hyaluronidase))”). Throughout, the specification relates to modified PH20 polypeptides, as do the claims.

29. Additionally, the references that Dr. Hecht relies upon for his obviousness analysis (the '429 patent and Chao) both relate to hyaluronidases, as do many of the other references Dr. Hecht cites in his Declaration, including, *e.g.*, EX1001 (the '520 patent), EX1004 (the Declaration of Dr. Park), EX1005, EX1006, EX1007, EX1009, EX1010, EX1011, EX1013, EX1019, EX1020, EX1021, EX1024, EX1026, EX1029, EX1033, EX1049, and EX1061. EX1003.

30. Furthermore, Halozyme, the owner of (and original applicant for) the '520 patent, was known in December 2011 for developing and commercializing

products involving hyaluronidases. EX1002, 20 (listing Halozyme, Inc. as applicant); EX2004, 1.<sup>5</sup> And I understand that each of the inventors, or at least one or more members of the team of inventors, had at least two years of experience working with hyaluronidases in December 2011. EX2005, 1-14.<sup>6</sup>

31. In addition, I understand that each of the inventors was employed by Halozyme, Inc. (the owner of, and original applicant for, the '520 patent) in December 2011; therefore, their experience working with hyaluronidases would

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<sup>5</sup> On February 27, 2025, I retrieved EX2004 online from Fierce Pharma Biopharma News & Insights and generated a PDF from the online webpage. EX2004 is true and accurate to the best of my knowledge. I consider EX2004 to be a reliable source of information containing the type of information upon which an expert in the field would typically rely. Accordingly, I rely on EX2004 here.

<sup>6</sup> On December 18, 2024, I retrieved the LinkedIn profiles of Michael Shepard, Robert Conner, Ge Wei, and Qiping Zhao from LinkedIn and generated PDFs from the corresponding online webpages. I combined these PDFs into a single document to create EX2005. EX2005 is true and accurate to the best of my knowledge. I consider EX2005 to be a reliable source of information containing the type of information upon which an expert in the field would typically rely. Accordingly, I rely on EX2005 here.

have been examples of active workers in the field of the invention at this time.

EX1002, 20; EX2005, 1-14.

32. In view of the above, and in accordance with the first and sixth factors referenced above in paragraph 24, a POSA in this field or a member of a team that includes the POSA would have at least two years of practical experience with hyaluronidases. The practical experience with hyaluronidases would need to come from either the POSA's own experience or through collaborations with a team having experience studying and characterizing hyaluronidases.

33. A POSA for the '520 patent would also typically have a degree such as a B.S., M.S., or a Ph.D., with at least two years of experience and training in cellular and molecular biology and protein biochemistry. This experience and training could come from a POSA's undergraduate or graduate studies or employment.

34. Regarding the educational level of the inventors of the '520 patent, I understand that (i) in December 2011, Michael Shepard had a B.S. in Zoology (Cellular and Developmental Biology) and a Ph.D. in Cellular and Developmental Biology & Genetics; (ii) in December 2011, Robert Conner had a B.A. in Biochemistry and Molecular Biology and a Ph.D. in Biological Chemistry; and (iii) in December 2011, Ge Wei had a B.S. in Biochemistry, an M.S. in Biochemistry, and a Ph.D. in Biochemistry and Molecular Genetics. EX2005, 1-14. Therefore, I

understand that at least 3 of the 4 inventors held a degree such as a B.S., M.S., or a Ph.D., with at least two years of experience and training in (i) cellular and molecular biology and (ii) protein biochemistry from their undergraduate or graduate studies and/or employment. EX2005, 1-14.

35. And, as mentioned above, I understand that each of the inventors was employed by Halozyme, Inc. (the owner of, and original applicant for, the '520 patent) in December 2011; therefore, their educational background and experience and training in cellular and molecular biology and protein biochemistry would have been examples of active workers in the field of the invention at this time. EX1002, 20; EX2005, 1-14.

36. In view of the above, and in accordance with the first and sixth factors referenced above in paragraph 24, a POSA for the '520 patent would typically have a degree such as a B.S., M.S., or a Ph.D., and the POSA or a team member would have at least two years of experience and training in cellular and molecular biology and protein biochemistry.

37. The second through fifth factors referenced above in paragraph 24<sup>7</sup>

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<sup>7</sup> *I.e.*, (ii) the type of problems encountered in the art, (iii) the prior art solutions to those problems, (iv) the rapidity with which innovations are made, and (v) the sophistication of the technology.

also support a POSA definition requiring at least two years of practical experience with hyaluronidases from either the POSA or a member of a multi-disciplinary team that includes the POSA because, as discussed above, the field and background of the invention relate to hyaluronidases. EX1001, 4:15-50.

38. Additionally, the '520 patent discusses various problems encountered in the art and contemplated solutions to those problems, which require familiarity with hyaluronidases. For Example: EX1001, 109:26-29 (“PH20 hyaluronidase, such as rHuPH20, rapidly loses activity in the presence of preservatives . . .”), 109:56-67 (“The modified PH20 polypeptides provided herein that exhibit increased stability in the presence of phenolic preservatives exhibit more than 15% enzymatic activity in the presence of at least one phenolic preservative . . .”); EX1001, 118:37 (“PH20 denatures in the presence of low salt or no salt.”); and 118:47-51 (“Provided herein are modified PH20 polypeptides that exhibit increased stability in the presence of low concentrations of salt . . .”).

39. Regarding the sophistication of the technology, while the hyaluronidase assays described in the common disclosure do not require extensive experience to employ, they do require familiarity with hyaluronidases. For example: EX1001, 134:15-23 (“Provided herein are methods for identifying a modified or variant hyaluronan-degrading enzyme, such as a modified hyaluronidase or modified PH20 polypeptide.”), 130:8-135:26, 171:8-173:5

(describing hyaluronidase assays), 171:12-15 (“assays can be used to assess the hyaluronidase activity of the PH20 polypeptide”), 130:8-135:26, 171:8-173:5 (describing hyaluronidase assays).

40. Furthermore, the '520 patent describes the role of PH20 in contraception, and comprehending the role of PH20 in contraception is relevant to understanding a contraceptive utility (i.e., use) for the claimed modified PH20 polypeptides. EX1001, 188:6-27 (“PH20 plays a role in fertilization by facilitating entry of the sperm through the cumulus layer surrounding the unfertilized egg. PH20 also is able to bind to hyaluronic acid (HA) on the zona pellucida during early phases of fertilization. This binding also initiates intracellular signaling that aids in the acrosome reaction. Immunization with PH20 has been shown to be an effective contraceptive in male guinea pigs.”), 188:6-27 (“Modified PH20 polypeptides provided herein can be used as vaccines in contraceptive applications.”).

41. Accordingly, the second through fifth factors referenced above in paragraph 24 also support requiring at least two years of practical experience with hyaluronidases from either the POSA or a member of a team that includes the POSA.

42. The second through fifth factors referenced above in paragraph 24<sup>8</sup> also support a POSA definition where the POSA would have had at least two years of experience and training in cellular and molecular biology and protein biochemistry from their undergraduate or graduate studies or employment.

43. The '520 patent extensively describes methods for producing the claimed modified PH20 polypeptides. EX1001, 142:59-67 (“Polypeptides of a modified PH20 polypeptide set forth herein can be obtained by methods well known in the art for protein purification and recombinant protein expression. Polypeptides also can be synthesized chemically. Modified or variant, including truncated, forms can be engineered from a wild[-]type polypeptide using standard recombinant DNA methods. For example, modified PH20 polypeptides can be engineered from a wild[-]type polypeptide, such as by site-directed mutagenesis.”), 135:28-149:54, 188:35-225:9 (further detailing methods for producing modified PH20 polypeptides). Recombinant DNA methods, site-directed mutagenesis, recombinant protein expression, chemical protein synthesis, and protein purification methods are routine cellular and molecular biology and protein

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<sup>8</sup> *I.e.*, (ii) the type of problems encountered in the art, (iii) the prior art solutions to those problems, (iv) the rapidity with which innovations are made, and (v) the sophistication of the technology.

biochemistry methods and/or techniques that a POSA equipped with the '520 patent specification's guidance could have used to make the claimed modified PH20 polypeptides. EX1001, 142:59-67.

44. In view of the above, and in accordance with the second through fifth factors referenced above in paragraph 24, a POSA for the '520 patent would typically have a degree such as a B.S., M.S., or a Ph.D., with at least two years of experience and training in cellular and molecular biology and protein biochemistry.

45. Altogether, in view of the six factors referenced above in paragraph 24, a POSA in this field or a member of a multi-disciplinary team that includes the POSA would have also had at least two years of practical experience with hyaluronidases. The practical experience with hyaluronidases would need to come from either the POSA's own experience or through collaboration with a member of a multi-disciplinary team having experience studying and characterizing hyaluronidases. Additionally, a POSA for the '520 patent would also typically have a degree such as a B.S., M.S., or a Ph.D., with at least two years of experience and training in cellular and molecular biology and protein biochemistry. The experience and training in cellular and molecular biology and protein biochemistry could come from the POSA's experience and training during their undergraduate or graduate studies or employment.

46. Considering my ample practical experience with hyaluronidases as

described in my background and qualifications, I am more experienced than a POSA under both my own definition and Dr. Hecht's.<sup>9</sup>

## VI. LEGAL BASIS FOR MY ANALYSIS

47. 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

48. 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 specification and its prosecution history—unless the patent explicitly defines the claim term. I also understand that when a patent explicitly defines a claim term in the specification, the patent's

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<sup>9</sup> I note that Dr. Hecht, according to his *curriculum vitae* and Background and Qualifications, has no practical experience with hyaluronidases, PH20, or contraceptives, whereas a POSA or a member of the POSA's multidisciplinary team would have had practical experience with hyaluronidases. EX1019-EX1021; Section V; EX1003, ¶¶1-9, Appendix B. I also note that Dr. Hecht's declaration does not mention consulting with anyone having practical experience with hyaluronidases. EX1003.

definition controls. I also understand that no two claims in the same patent should be interpreted to be the same invention, *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/they depends.

49. I understand that claim 1 is the sole independent claim in the '520 patent and that claims 2, 6-15, and 17-30 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, 6-15, and 17-30 contain all limitations of claim 1, as well as those of any additional claims from which they depend. The copy of the '520 patent provided as Exhibit 1001 also lists claims 3-5, 16, and 31-35, but I understand that Patent Owner Halozyme, Inc. statutorily disclaimed claims 3-5, 16, and 31-35 on May 7, 2025. EX2003. Thus, I understand that the disclaimed claims 3-5, 16, and 31-35 are not currently part of this post-grant review proceeding.<sup>10</sup>

**B. Written Description**

50. I understand that the written description provided by a patent

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<sup>10</sup> I understand that a statutory disclaimer means that, effectively, the only challenged claims are claims 1-2, 6-15, and 17-30.

specification must convey clearly to a POSA that the applicant was in possession<sup>11</sup> of the claimed invention 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 '520 patent's written description as of the filing date of the earlier '731 related 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.

51. I further understand that this written description requirement must be assessed in view of the state of the knowledge of a POSA in the art. 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 patent's filing date, in addition to the knowledge provided by the patent specification itself.

52. I understand that there is sufficient (*i.e.*, adequate) written description support when a POSA can visualize or recognize the full scope of the claimed subject matter and that the claimed subject matter need not be provided verbatim in

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<sup>11</sup> I understand possession does not mean physical possession and does not require making or testing the invention.

a specification. I also understand that a skilled artisan 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 skilled artisan to conclude that there is written description support.

53. I understand that assessing whether there is written description support for a genus<sup>12</sup> claim involves consideration of a number of factors, including (i) the 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 of technology and scientific and technologic knowledge already in existence; (iv) predictability of the aspect at issue; and (v) scope of the invention at issue.

54. 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.

55. 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

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<sup>12</sup> I understand a genus to be a group that covers multiple “species” and that “species” are sometimes referred to as “embodiments.”

within the scope of the genus or (ii) 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. 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 claimed genus from other materials.

**C. Enablement**

56. I understand that a patent application specification that provides an enabling disclosure must allow a POSA to make and use 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 '520 patent, which I understand is a consideration in determining whether the '520 patent is entitled to be treated as though it were effectively filed as of the '731 application's December 28, 2012 filing date. I understand that the following factors can be used to determine whether undue experimentation would have been needed (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.

57. 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 factual considerations. I understand that whether some experimentation is necessary does not necessarily make such experimentation undue and that even a considerable amount of experimentation is not undue, if it is merely routine. Furthermore, I understand that evidence of a pharmaceutical property in any standard experimental animal is sufficient to establish utility (*i.e.*, usefulness).

**D. Obviousness**

58. 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, 194. I further understand that to establish obviousness, a party seeking to establish obviousness must perform the following factual inquiries: (a) determining the scope and content of the prior art; (b) ascertaining the differences between the claimed invention and the prior art;

and (c) 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, a patent challenger may (1) specify where each element of the claim is found in the prior art or (2) 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.

59. I understand that one way of showing obviousness is by establishing that a POSA would have had both (i) a reason 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 reason 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 a specific hint or suggestion of the alteration needed to arrive at the claimed invention; the analysis may include recourse to logic, judgment, and common sense available to a person of ordinary skill that does not need to be explicit in any reference. Moreover, I understand that evidence showing that lack of motivation to combine may support a conclusion that the claimed invention was nonobvious.

60. 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 rationale, 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.

61. 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.

62. Finally, I understand that the patent examiner is charged with assessing all pending claims for compliance with certain requirements under the

patent laws, including the requirement that the specification provide an adequate written description for the invention and that the claims be non-obvious.

**VII. CLAIMS 1-2, 6-15, and 17-30 DO NOT REQUIRE HYALURONIDASE ACTIVITY**

63. Although Dr. Hecht omits any mention of the concept of claim construction<sup>13</sup>, I have considered how a POSA would have construed the claimed invention: claims 1-2, 6-15, and 17-30—in view of the principles of claim construction I summarized in Section VI.A.

**A. A POSA Would Have Understood That a “modified PH20 polypeptide” Means “a PH20 polypeptide that contains at least one amino acid modification compared to a reference unmodified PH20 polypeptide”**

64. A POSA interpreting claims 1-2, 6-15, and 17-30 of the '520 patent would have deemed it necessary to interpret the meaning of “modified PH20 polypeptide” because the term “modified PH20 polypeptide” appears either

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<sup>13</sup> I note that Dr. Hecht nonetheless argues that “[a] skilled artisan would have understood the claims to necessarily cover modified PH20 polypeptides that are active mutants, and would not view them as including inactive mutants.” EX1003, ¶134 (emphasis added). I note also that Dr. Park omits any mention of the concept of claim construction in his declaration; in particular, Dr. Park does not cite the '520 patent in his declaration. EX1004.

explicitly or via incorporation in each of claims 1-2, 6-15, and 17-30 and is fundamental to interpreting the claims. EX1001, Claims 1-2, 6-15, and 17-30.

65. Claim 1, for example, states:

*A<sup>14</sup> modified PH20 polypeptide*, comprising one or more amino acid modifications in an unmodified PH20 polypeptide, wherein: the unmodified PH20 polypeptide consists of the amino acid sequence selected from the group consisting of *SEQ ID NO: 3, 7 and 32-66*; amino acid modifications are selected from the group consisting of amino acid replacements(s), deletion(s), and/or insertion(s); the modified PH20 polypeptide comprises an amino acid replacement at a position corresponding to residue 324, with reference to amino acid positions set forth in SEQ ID NO: 3; the replacement at the position corresponding to residue 324 is selected from the group consisting of *A, D, H, M, N, R and S*; corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide having the amino acid sequence of SEQ ID NO: 3; and the modified PH20 polypeptide has *at least 91% sequence identity* to a polypeptide having the amino acid sequence selected from the group consisting of *SEQ ID NO: 3, 7 and 32-66*.

EX1001, Claim 1 (emphasis added).<sup>15</sup> The modified PH20 polypeptide of Claim 1

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<sup>14</sup> I understand the term “a” to mean one or more.

<sup>15</sup> Claim 21 likewise explicitly recites the term “modified PH20 polypeptide.” EX1001, Claim 21.

is thus defined by the following common features: (i) the modified PH20 polypeptide of claim 1 shares “at least 91%” of the structure of the disclosed sequences (SEQ ID NO: 3, 7 and 32-66), implicitly limiting any sequence variation to 5%, and (ii) that the modified PH20 polypeptide of claim 1 contains one amino acid modification (selected from A, D, H, M, N, R, and S) at position 324 (with reference to amino acid positions set forth in SEQ ID NO: 3). EX1001, Claim 1. Claim 1, therefore, is defined purely by structure, and not by any function. EX1001, Claim 1.

66. Specifically, I understand that claims 1 and 21, which both explicitly recite the term “modified PH20 polypeptide,” are the only independent claims in the ’520 patent. And I understand that dependent claims contains all limitations (*i.e.*, all distinct claim components) of the claim(s) from which they depend.

Section VII.A. Claims 2, 6-15, and 17-30 depend from and thus incorporate every element of claim 1; therefore, the term “modified PH20 polypeptide” also appears via incorporation in each of claims 2, 6-15, and 17-30. EX1001, Claims 2, 6-15, and 17-30. Thus, I explain the meaning of “modified PH20 polypeptide” below.

67. **First**, I understand that when a patent explicitly defines a claim term in the specification, the patent’s definition controls. Section VII.A. The term “modified PH20 polypeptide” is expressly defined in the specification of the ’520 patent, 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.” EX1001, 48:38-43 (emphasis added). The express definition of “modified PH20 polypeptide” does not encompass any function because it describes only structural components: (i) a sequence of amino acids (ii) with at least one amino acid modification (iii) relative to a reference sequence. EX1001, 48:38-43.

68. The term “modified PH20 polypeptide,” therefore, has a purely structural meaning in the context of the specification,<sup>16</sup> and a POSA applying its express definition would have understood that the term “modified PH20 polypeptide” is defined by a sequence of amino acids and not by any particular function. EX1001, 48:38-43. This structural definition—explicitly and solely described by a sequence of amino acids—does not *require* hyaluronidase activity. EX1001, 48:38-43.

69. **Second**, I understand that claim terms are given their ordinary and

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<sup>16</sup> The modified PH20 polypeptide of independent claim 21 is also defined purely by its structure and not any function; therefore, my analysis of claim 1 also applies to claim 21.

customary meaning as understood by a POSA at the time of the invention in view of the patent’s specification and its prosecution history—unless the patent explicitly defines the claim term. Section VII.A, above. As discussed above, “modified PH20 polypeptide” is explicitly defined; I do not separately analyze any ordinary and customary meaning of “modified PH20 polypeptide.”

70. **Third**, I understand per Section VII.A that claims are to be interpreted in view of the entirety of the patent’s specification, including the entirety of the claims, and the entirety of the ’520 patent’s specification clearly explains that “modified PH20 polypeptide” also refers to polypeptides (including hyaluronidases) that do not exhibit hyaluronidase activity.

71. A POSA interpreting claim 1 in view of the other dependent claims would find further support indicating that the term “modified PH20 polypeptide” does not require hyaluronidase activity. Dependent claims 8-10, for example, specify further modifications to the modified PH20 polypeptide of claim 1, including glycosylation. Claim 18 specifically recites, “The modified PH20 polypeptide of claim 17, wherein the post-translational modification is *glycosylation*.” EX1001, Claim 18 (emphasis added).

72. Dr. Hecht states, “PH20 enzymes must be glycosylated to exhibit their catalytic activity.” EX1003, ¶197. The ’520 patent also states that glycosylation “is required for PH20 hyaluronidase activity” and that “at least N-linked glycosylation

sites corresponding to amino acid residues N200, N333 and N358 are required for secretion and/or *activity* of the enzyme.” EX1001, 70:67-71:4 (emphasis added).

73. Because the modified PH20 polypeptide of dependent claim 18 must be glycosylated, claim 1 must cover *both* glycosylated and unglycosylated modified PH20 polypeptides; otherwise, the scope of claims 1 and 18 would be identical. EX1001, Claims 1 and 18. As discussed above, I understand that the scope of dependent claims are presumed to be distinct because the scope of a dependent claim should be narrower than the independent and dependent claims preceding it. Section VI.A.

74. Accordingly, a POSA would have understood that claim 1 necessarily encompasses unglycosylated modified PH20 polypeptides—and because the ’520 patent states that glycosylation “is required for PH20 hyaluronidase activity,” a POSA would have understood claim 1 to encompass modified PH20 polypeptides that *lack* PH20 hyaluronidase activity.<sup>17</sup>

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<sup>17</sup> Dependent claims 2-35 are likewise consistent with the understanding that claim 1 does not require hyaluronidase activity. I understand that claims 3-5, 16, and 31-35 have been disclaimed; however, I have assessed them insofar as they would have informed the meaning of claim 1 to a POSA and do not interpret them as suggesting that claim 1 requires hyaluronidase activity. For example, I do not

75. Reviewing the claims in view of other descriptions in the common disclosure further supports the understanding that the claims do not require hyaluronidase activity. EX1001, 115:40-123:22, 251:1-6, 75:58-60, 115:59-62, 116:51-59, 188:21-25, 251:1-256:67, Tables 5 and 10. For example, the specification explicitly explains that some modified PH20 polypeptides are *inactive*. In particular, the specification defines inactive mutants as modified PH20 polypeptides:

*Inactive Mutants:* Provided herein are *modified PH20 polypeptides* that contain one or more amino acid replacements in a PH20 polypeptide and that are *inactive, whereby the polypeptides do not exhibit hyaluronidase activity* or exhibit low or diminished hyaluronidase activity...The *modified PH20 polypeptides* provided herein that are inactive *generally exhibit less than 20%*, such as less than 10%, of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the polypeptide set forth in SEQ ID NO: 3 or 7....exhibit less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, ... 0.05% or less of the hyaluronidase activity....

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interpret the term “increased” to support the notion that claim 1 *requires* hyaluronidase activity because this term relates to the activity of the modified PH20 polypeptides of claims 3-4 relative to the hyaluronidase activity of the polypeptide set forth in SEQ ID NO: 3, an *unmodified* PH20 polypeptide. EX1001, Claims 3-4.

EX1001, 115:40-123:22 (emphasis added). Numerous additional descriptions in the specification describe that modified PH20 polypeptides can be *inactive*.

EX1001, 251:1-6 (emphasis added) (“The other mutants that exhibited less than [20%] hyaluronidase activity of wildtype PH-20, in at least one of the duplicates, were rescreened to confirm that the dead mutants are *inactive*”); EX1001, 75:58-60 (emphasis added) (“Also provided are modified PH20 polypeptides that are *inactive*, and that can be used, for example, as antigens in contraception vaccines”); EX1001, 115:59-62 (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, 116:51-59 (emphasis added) (“The amino acid replacement(s) can be at the corresponding position in a PH20 polypeptide as set forth in any of SEQ ID NOs: ... 3, 6-66 ... or a variant thereof having at least ... 91% or more sequence identity thereto, so long as the resulting modified PH20 polypeptide is *inactive*.”); EX1001, 188:21-25 (emphasis added) (“the modified PH20 polypeptides can be *inactive* enzymes”); EX1001, 251:1-256:67 (describing a modified assay that is intended to specifically detect inactive mutants); EX1001, Table 5 (providing replacements identified as corresponding to inactive mutants); EX1001, Table 10 (providing numerous examples of inactive mutants).

76. The specification also states that “[i]ncluded among the modified

PH20 polypeptides provided herein are PH20 polypeptide that are active mutants....” EX1001, 75:49-54 (emphasis added). Because the specification states that active mutants are “included among” the “the modified PH20 polypeptides provided herein,” the specification clearly contemplates that the modified PH20 polypeptides are not limited to active mutants even beyond the explicit descriptions of inactive mutants referenced above. EX1001, 75:49-54.

77. I note that the specification states that a modified PH20 polypeptide can have “150 amino acid replacements, so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity”; however, this description is not part of the express definition of “modified PH20 polypeptide”; therefore a POSA would not have interpreted this statement to mean that the term “modified PH20 polypeptide” requires hyaluronidase activity. EX1001, 48:38-53.

78. A POSA would also have understood that this statement merely describes an *upper limit* for the number of modifications possibly allowing a modified PH20 polypeptide to exhibit enzymatic activity. EX1001, 48:38-53.

79. And moreover, a POSA would have understood that the claims do not encompass a modified PH20 polypeptide having 150 amino acid replacements because a modified PH20 polypeptide having 150 amino acid replacements would exhibit a lower percent identity than the “at least 91%” structural identity required by the claims. EX1001, 48:38-53 and Claims 1-2, 6-15, and 17-30; EX2006. SEQ

ID No. 3, for example, contains 447 amino acids. EX2006. If 150 amino acid replacements were made to SEQ ID NO. 3, the resulting polypeptide would have only ~66% structural identity to SEQ ID NO. 3. EX2006. Likewise, the shortest amino acid sequence of SEQ ID Nos. 32-66—SEQ ID No. 32, has 430 amino acid residues; and the longest amino acid sequence of SEQ ID Nos. 32-66—SEQ ID No. 66, has 465 amino acid residues. EX2006. If 150 amino acid replacements were made to SEQ ID Nos. 32 and 66, the resulting polypeptides would have only ~65% and ~68% structural identity to SEQ ID Nos. 32 and 66, respectively.<sup>18</sup> In any event, a 150 amino acid replacement would result in significantly less than the “at least 91%” structural identity required by the claims. EX1001, 48:38-53, Claims 1-4, 8-21; EX2006.

80. In view of the above, therefore, the specification clearly does not restrict “modified PH20 polypeptide” to active enzymes nor contradict nor modify the specification’s explicit definition of modified PH20 polypeptide.

81. **Finally**, I reviewed the prosecution history for the ’520 patent (EX1002), but I did not find in it any discussion that contradicts or modifies the

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<sup>18</sup> These values were calculated as follows:  $[(\text{total amino acid residues} - 150) / \text{total amino acid residues}] * 100$ , *i.e.*:  $[(447 - 150) / 447] * 100 = 66$ ;  $[(430 - 150) / 430] * 100 = 65$ ;  $[(465 - 150) / 465] * 100 = 68$ .

specification's explicit definition of modified PH20 polypeptide.

82. In sum, a POSA interpreting “modified PH20 polypeptide” according to the express definition in view of the specification and prosecution history would have understood that “modified PH20 polypeptide” simply means “a PH20 polypeptide that contains at least one amino acid modification, such as at least one amino acid replacement as described [in the patent], in its sequence of amino acids compared to a reference unmodified PH20 polypeptide.” In other words, “modified PH20 polypeptide” is a purely *structural* term (defined by an amino acid sequence) that does not require hyaluronidase activity (i.e., a particular function).

**B. A POSA Would Not Have Interpreted Claims 1-2, 6-15, and 17-30 as Being Limited to Enzymatically Active Mutant PH20 Polypeptides**

83. As discussed, a POSA would not have interpreted “modified PH20 polypeptide” to require any hyaluronidase activity. Section VII.A. Rather, as discussed, a POSA would have understood that “modified PH20 polypeptide” is a purely structural term defined by an amino acid sequence. Section VII.A.

84. Furthermore, because nothing in the remainder of the claims, specification, or prosecution history would have otherwise indicated to a POSA that the claims should be limited to modified PH20 polypeptides having hyaluronidase activity, *e.g.*, “active mutants,” a POSA would not have interpreted Claims 1-2, 6-15, and 17-30 as requiring hyaluronidase activity.

85. Dr. Hecht acknowledges that the '520 patent contemplates both active and inactive mutants. EX1003, III.A, ¶98. Nevertheless, he argues that the claims would be understood to concern active mutant PH20 modified polypeptides. EX1003, IV.B. Dr. Hecht provides two primary reasons for this assertion—(1) that “a skilled artisan reading the common disclosure would have understood it to be describing two, mutually exclusive types of modified PH20 polypeptides: (i) active mutants are those with significant levels of hyaluronidase activity (i.e., above 40% of the activity of unmodified PH20), and (ii) inactive mutants, which do not exhibit significant hyaluronidase activity (i.e., less than 20% of the activity of the unmodified PH20),” EX1003, ¶107—and (2) that “[t]he brief suggestion in the common disclosure about possibly using inactive mutant forms of PH20 as the immunogen of a contraceptive vaccine does not seem credible....” EX1003, ¶112.

86. **First**, a POSA would not have understood the claims to encompass only “active mutants.” As Dr. Hecht acknowledges, both active and inactive mutants are contemplated in the specification. EX1003, III.A, ¶98. Dr. Hecht argues that “the common disclosure identifies each of these substitutions [i.e., each of E324D, E324N, or E324R] as causing PH20<sub>1-447</sub> to exhibit increased hyaluronidase activity.” EX1003, ¶¶126-128. However, a POSA interpreting claim 1 in view of the other dependent claims (together with the specification and prosecution history) would have found further support indicating that the term

“modified PH20 polypeptide” still does not require hyaluronidase activity.

Dependent claims 17-18, for example, specify further modifications to the modified PH20 polypeptide of claim 1, including glycosylation. EX1001, Claims 17-18. Claim 18 recites, “The modified PH20 polypeptide of claim 17, wherein the post-translational modification is *glycosylation*.” EX1001, Claim 18 (emphasis added). EX1001, Claim 18. Dr. Hecht states, “PH20 enzymes must be glycosylated to exhibit their catalytic activity.” EX1003, ¶197. And the common disclosure also states that glycosylation “is required for PH20 hyaluronidase activity.” EX1001, 70:67-71:4. Therefore, a POSA would have understood that claim 1 encompasses both active and inactive mutants. Moreover, the term “active” is a functional term not found in the claims; the claimed modified PH20 polypeptides are *not* defined by any function. EX1001, Claims 1-4, 8-21; EX1003, ¶107. And Dr. Hecht does not identify any claim term appearing in any of claims 1-4 or 8-21 that imposes a requirement for hyaluronidase activity.

87. Additionally, Dr. Hecht argues that the statement in the specification that modifications “can be in any PH20 polypeptide ... so long as the modified form exhibits hyaluronidase activity” suggests that the claimed modified PH20 polypeptides are limited to “active mutant[s]”; however, the specification only explains that modifications can be made to create active modified PH20 polypeptides, not that all claimed modified PH20 polypeptides must have

hyaluronidase activity. EX1003, ¶¶128-129. Furthermore, the specification is not limited to two, mutually exclusive groups of mutants defined by either (i) above 40% or (ii) less than 20% activity. Table 9 of the specification, for example, provides 536 modified PH20 polypeptides that exhibit 120% or greater hyaluronidase activity of wild-type PH20, approximately 75 mutants that exhibited 300% or greater activity than wild-type, and 192 polypeptides that exhibit between 20-40% activity. EX1001, Table 9. Therefore, the claimed modified PH20 polypeptides are not limited to polypeptides falling into the two categories Dr. Hecht delineated in paragraph 98 of his declaration. EX1001, Table 9.

88. Thus, altogether, a POSA would not have interpreted Claims 1-2, 6-15, and 17-30 as being limited to active mutant PH20 polypeptides.

#### **VIII. CLAIMS 1-2, 6-15, and 17-30 WOULD NOT HAVE BEEN OBVIOUS IN VIEW OF CHAO AND THE '429 PATENT**

89. In Section VI.D, I summarize the legal principles I applied in assessing obviousness in view of the asserted prior art and the general knowledge in the background art from the viewpoint of a POSA at the time of the invention. I apply those principles in my analysis here. In considering obviousness, I address the timeframe of “before December 29, 2011,” in order to respond to Dr. Hecht’s and Dr. Park’s analysis regarding that same timeframe—a timeframe that is before the December 30, 2011 filing date of Halozyme’s ’313 provisional application to which the ’520 patent is related. EX1003, ¶11; EX1004, ¶10.

90. Dr. Hecht's declaration states that Dr. Hecht reviewed Dr. Park's declaration, and Dr. Hecht repeatedly agrees with Dr. Park and/or bases his analysis (at least in part) on Dr. Park's analysis. EX1003, ¶¶20-22, 85, 122-125, 158, 215-220, and 224-237. For convenience, however, I refer to Dr. Park's and Dr. Hecht's arguments collectively as Dr. Hecht's arguments.

91. Briefly, Dr. Hecht argues: (1) that the '429 patent would have motivated a POSA to make single amino acid substitutions in "non-essential regions"; and (2) that the multiple sequence alignment of five different hyaluronidases and the structural information regarding Hyal-1 ("Hyal-1") in Chao would have indicated where such "non-essential regions" would have been located in PH20. EX1003, ¶¶209-216.

92. Dr. Hecht concludes that the combination of the '429 patent and Chao would have motivated a POSA to specifically mutate position 324 of PH20. EX1003, ¶220. Specifically, he concludes that a POSA "would have expected that the E324D, E324N, and E324R substitutions in PH20<sub>1-447</sub> to be tolerated."<sup>19</sup> EX1003, ¶221. He also concludes that a POSA would have had reasonably

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<sup>19</sup> D is an abbreviation for the amino acid aspartic acid; E is an abbreviation for glutamic acid; N is an abbreviation for asparagine; and R is an abbreviation for arginine. EX1003, ¶115.

expected that mutating E324 to aspartic acid (D), asparagine (N), or arginine (R) “would exhibit comparable activity to the unmodified PH20<sub>1-447</sub> enzyme.” EX1003, ¶221. And Dr. Hecht further concludes that a “skilled artisan also would have expected that the PH20<sub>1-447</sub> protein incorporating a single amino acid substitution in a non-essential region would generally have the same therapeutic uses and utilities as described with respect to the PH20<sub>1-447</sub> protein.” EX1003, ¶207.

**A. Even in Combination, Chao and the '429 Patent Fail to Disclose or Suggest a E324 Mutation of PH20, Much Less a E324 Mutation to Aspartic acid (D), Asparagine (N), or Arginine (R), as Required by Claims 1-2, 6-15, and 17-30.**

93. As I summarized in Section VI.C above, 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 exercising common sense or ordinary creativity would have bridged any gaps (*i.e.*, missing elements) between the prior art and the claimed invention to produce the claimed invention.

94. First, I assess whether all elements of claims 1-2, 6-15, and 17-30 can be found in Chao and the '429 patent. Second, I assess whether a POSA would have nonetheless supplied the missing elements from Chao and the '429 patent using common sense or ordinary creativity.

**1. Chao and the '429 Patent Do Not Disclose Any Mutation of E324, Nor Do Chao and the '429 Patent Disclose Any Mutation of E324 to Aspartic acid (D), Asparagine (N), or Arginine (R), as Required by Claims 1-2, 6-15, and 17-30.**

95. Claim 1 recites:

*A<sup>20</sup> modified PH20 polypeptide*, comprising one or more amino acid modifications in an unmodified PH20 polypeptide, wherein: the unmodified PH20 polypeptide consists of the amino acid sequence selected from the group consisting of *SEQ ID NO: 3, 7 and 32-66*; amino acid modifications are selected from the group consisting of amino acid replacements(s), deletion(s), and/or insertion(s); the modified PH20 polypeptide comprises an amino acid replacement at a position corresponding to residue 324, with reference to amino acid positions set forth in *SEQ ID NO: 3*; the replacement at the position corresponding to residue 324 is selected from the group consisting of *A, D, H, M, N, R and S*; corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide having the amino acid sequence of *SEQ ID NO: 3*; and the modified PH20 polypeptide has *at least 91% sequence identity* to a polypeptide having the amino acid sequence selected from the group consisting of *SEQ ID NO: 3, 7 and 32-66*.

EX1001, Claim 1 (emphasis added).

96. Neither Chao nor the '429 patent disclose “an amino acid modification at a position corresponding to position 324” as required by claims 1-

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<sup>20</sup> I understand the term “a” to mean one or more.

2, 6-15, and 17-30. EX1001, Claim 1; EX1005-EX1006. And neither Chao nor the '429 patent disclose replacing E324 with an amino acid "selected from the group consisting of A, D, H, M, N, R, and S." EX1001, Claim 1; EX1005; EX1006. Instead, Chao focuses on the three-dimensional structure of human hyaluronidase-1 ("hHyal-1") by describing its resolved crystal structure, and the '429 patent focuses on soluble neutral hyaluronidase glycoproteins ("sHASEGPs"). EX1005-EX1006. Neither a mutation at E324, much less a mutation of E324 to aspartic acid (D), asparagine (N), or arginine (R), is mentioned whatsoever in Chao or the '429 patent. EX1005; EX1006. Accordingly, these claim elements are missing from the prior art Dr. Hecht cites.

97. Moreover, Dr. Hecht does not provide a reasonable explanation as to how or *why* a POSA would have nonetheless supplied these missing elements, as I explain below. EX1003; EX1005; EX1006.

**2. Dr. Hecht Does Not Adequately Explain Why a POSA Would Have Supplied the Missing E324 Mutation or Modified the Missing Mutation of E324 to Aspartic acid (D), Asparagine (N), or Arginine (R) based on Chao and the '429 Patent Using Common Sense or Ordinary Creativity.**

98. Dr. Hecht does not explicitly argue that either common sense or ordinary creativity supplies the missing E324 mutation, much less the mutation of E324 to aspartic acid (D), asparagine (N), or arginine (R). EX1003. Rather, he summarized Dr. Park's methodology as follows:

[Dr. Park's] methodology included (i) using a multiple-sequence alignment to identify non-essential regions of PH20 (including position 324), (ii) *identifying the amino acids that occur at those non-essential regions* in the proteins in the set used for the alignment, and (iii) *assessing whether amino acid substitutions appearing in nature at position 324 would be tolerated by PH20.*

EX1003, ¶215 (emphasis added). This summary of Dr. Park's methodology jumps from identifying amino acid residues within "non-essential regions" to assessing whether substitutions at position 324 would be "tolerated by PH20." EX1003, ¶215. Dr. Hecht does not explain why a POSA would have arrived at the specific E324 locus. Nor does he explain why a POSA would have jumped to mutating E324 to aspartic acid (D), asparagine (N), or arginine (R). EX1003, ¶215.

99. Dr. Hecht states: "Position 324 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park's analysis." EX1003, ¶217.<sup>21</sup>

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<sup>21</sup> Dr. Park alleges that, based on his 88-sequence alignment, "a skilled artisan would have deemed 'essential residues'" to be located where "non-identical amino acids appear[] in less than ~5% of the proteins in the data set." EX1004, ¶30. But this statement is unsupported by any reference, and Dr. Park cites only to his own declaration and appendices for support. EX1004, ¶¶30-32. And Dr. Hecht relies on this determination by Dr. Park without any additional further support. EX1003, ¶215; EX1004, ¶¶30-32.

Notwithstanding whether position 324 is in a “non-essential region,” which I will discuss in Section VIII.B below, Dr. Hecht does not explain *why* a POSA would have selected position 324 from any other residue within the purported “non-essential region” surrounding E324 or any other “non-essential” region purportedly identified by Dr. Park. EX1003, ¶215.

100. Instead of providing a reason to focus on position 324 that is based on common sense or ordinary creativity of a POSA or any teaching or suggestion in the art, as will be discussed further below, Dr. Park states that he was “asked by counsel to report [his] conclusions with respect to position 324.” EX1004, ¶103. Dr. Hecht then relied on Dr. Park’s declaration without providing any further reason based on the common sense or ordinary creativity of a POSA or any teaching or suggestion in the art. EX1004, ¶103; EX1003, ¶¶20-22, 85, 122-125, 158, 215-220, and 224-237. But a POSA reading the ’429 patent in combination with Chao would not have had a reason to mutate position 324.

**B. Even in Combination, Chao and the ’429 Patent Would Not Have Provided Any Motivation to Make a E324 Mutation of PH20, Much Less a E324 Mutation to Aspartic acid (D), Asparagine (N), or Arginine (R), as Required by Claims 1-2, 6-15, and 17-30.**

101. As I summarized in Section VI.D, I understand that one way of showing obviousness is by establishing that a POSA would have had both (i) a reason 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 address both of

these inquiries in turn.

**1. Chao and the '429 Patent Would Not Have Motivated a POSA to Make the Claimed E324 Mutation.**

**a. The '429 Patent Would Not Have Provided Any Reason to Make Single Amino Acid Mutations in “Non-Essential” Regions.**

102. Dr. Hecht argues that the '429 patent describes “that making a single amino acid substitution within a non-essential region of PH20 would be tolerated by the enzymatically active forms of PH20 being described in the '429 Patent”—and states that “‘in general, single amino acid substitutions in non-essential regions of polypeptides’ ... ‘do not substantially alter biological activity.’” EX1003, ¶206.

103. However, as explained below, the '429 patent does not identify any regions of PH20 as being non-essential. EX1005. And contrary to Dr. Hecht’s testimony, the '429 patent does not provide any *reason* to make a single amino acid substitution in “non-essential” regions. Instead, the '429 patent merely states that “[s]uitable conservative substitutions of amino acids are known to those of skill in this art and *can be* made generally without altering the biological activity” and states that making modifications at non-essential positions generally will not “alter[] the biological activity.” EX1005, 16:14-22 (emphasis added). These statements in the '429 patent would not have provided a POSA with any *reason* to make an E324D, E324N, or E324R mutation of PH20. Indeed, a POSA would have been disinclined to expend resources (e.g., time or materials) to make a mutation

that was expected not to “alter[] the biological activity,” and Drs. Hecht and Park have not identified any reasons to make such a mutation.

104. And, as explained below, Chao does not fill in any of these shortcomings of the '429 patent's teachings.

**b. Chao Would Not Have Identified “Non-Essential” Regions.**

105. Dr. Hecht states that “Chao provided an annotated alignment of the five human hyaluronidase enzymes which identified *conserved residues* among the set of five related proteins.” EX1003, ¶83 (emphasis added). He argues that the conserved residues identified by Chao are “essential” and argues that a POSA would have combined Chao and the '429 patent because “the '429 Patent would have encouraged a skilled artisan to make modified PH20 proteins having single amino acid substitutions in non-essential regions.” EX1003, ¶212.

106. However, Chao never identified any residues as “essential” in its annotated alignment, and the word “essential” is not even mentioned in Chao. EX1006, 6916, FIG. 3. Accordingly, a POSA would not have interpreted Chao as identifying essential or non-essential residues in its annotated alignment. EX1006, 6916, FIG. 3. And as explained in Section VIII.B.1.c below, a POSA would not have interpreted Chao as identifying non-conserved residues as “non-essential.”

**c. A POSA Would Not Have Been Motivated to Prepare nor Rely on the 88-Sequence Alignment Prepared by**

**Dr. Hecht Because Dr. Park's Alignment Includes  
Mostly Non-PH20 Sequences.**

107. Dr. Park argued that the non-conserved regions he purportedly identified “align with what [he] considers to be the ‘non-essential regions’ referred to by the ’429 patent.” EX1004, ¶32. He identified non-conserved regions by: (1) identifying “largely invariant residues that a skilled artisan would have deemed ‘essential’ in PH20<sub>1-447</sub>” from a multiple sequence alignment of 88 hyaluronidases and (2) “identif[ying] important residues in hyaluronidase proteins or which reported experimental results showing that modifying single residues impaired or eliminated activity of the enzymes.” EX1004, ¶¶26, 30.

108. Of the 88 sequences that Dr. Park aligned, only 18 are PH20 sequences. EX1004, ¶27; EX1056. The remaining 70 sequences are for other hyaluronidases, including, *e.g.*, Hyal-1, Hyal-2, Hyal-3, Hyal-4, Hyal-5, and Hyal-6. EX1004, ¶27; EX1056.<sup>22</sup> Dr. Park contends that his 88-sequence alignment is more informative than Chao’s alignment because he included “homologous

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<sup>22</sup> Hyal is an abbreviation for hyaluronidase. I note that the proper nomenclature for these proteins is HYAL1, HYAL2, HYAL3, HYAL4, HYAL5, and HYAL6; however, because Hyal-1 is not capitalized in Chao, I use the above nomenclature throughout (*i.e.*, Hyal-1, Hyal-2, Hyal-3, Hyal-4, Hyal-5, and Hyal-6) for consistency.

proteins from human and non-human species.” EX1004, ¶93.

109. However, a POSA would not have been motivated to prepare the 88-sequence alignment prepared by Dr. Park because he includes so many disparate, evolutionarily distant sequences—many of which are not even from humans, such as a tunicate (a marine invertebrate animal). EX1056. And furthermore, a POSA would not have been motivated to rely on such an 88-sequence alignment because different hyaluronidases were known to have different enzymatic functions and substrates before December 29, 2011. EX1056; EX2018, 33495, 33507; EX2019, 653, 658; EX2020, 286; EX2021, 296; EX1008, 825; EX2022, 6-7. EX1001, 72:48-51 and 160:24-29; EX1006, 6911, 6915-6916.

**i. Different Hyaluronidases Were Known to Have Different Functions and Substrates before December 29, 2011<sup>23</sup>.**

110. For example, before December 29, 2011, it was known that Hyal-3 does not exhibit *any* enzymatic activity and that Hyal-2 is only weakly enzymatically active or in many cases, *inactive*. EX2018, 33495, 33507; EX2019, 653, 658; EX2020, 286. Hyal-2 was known to be a receptor for a retrovirus in

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<sup>23</sup> In assessing written description and enablement, I refer to December 28, 2012—the filing date of the ’731 application that is related to the ’520 patent.

sheep before December 29, 2011. EX2021, 296. And Hyal-4 was known to catalyze different substrates than PH20. EX1008, 825; EX2022, 6-7. EX1001, 72:45-48, 167:25-30. Hyal-4 was identified specifically as a chondroitinase, meaning it catabolizes (*i.e.*, breaks down) chondroitin sulfate of proteoglycans. EX1008, 825; EX2022, 6-7.

111. By contrast, PH20 is a sperm-associated protein involved in fertilization, and its natural substrate is hyaluronan. EX1001, 72:45-48, 167:25-30. And Chao explained that Hyal-1 does not “contain glycosylphosphatidylinositol-signal sequences” and is not “membrane-bound upon maturation”—unlike PH20, which *does* contain a glycosylphosphatidylinositol-signal sequence and *is* membrane bound. EX1006, 6911; EX1004, ¶36.

112. Chao also acknowledged “different catalytic properties” and “differ[ences] in their catalytic efficiencies and pH profiles” between different human hyaluronidases. EX1006, 6914, 6916. Chao notes that for Hyal-1, the “activity optimum is at pH 3.8.” EX1006, 6915. I note that, by contrast, the activity of PH20 was measured at a pH of 7.4 in the specification. EX1001, 226:36-38.

113. A POSA, therefore, would have known that different hyaluronidases were known to have different functions and substrates. Thus, a POSA would not have been motivated to prepare the 88-sequence alignment prepared by Dr. Park nor rely on such an alignment considering the wide variety of hyaluronidases



(outlined in red) below. EX1006, 6916, FIG 3.

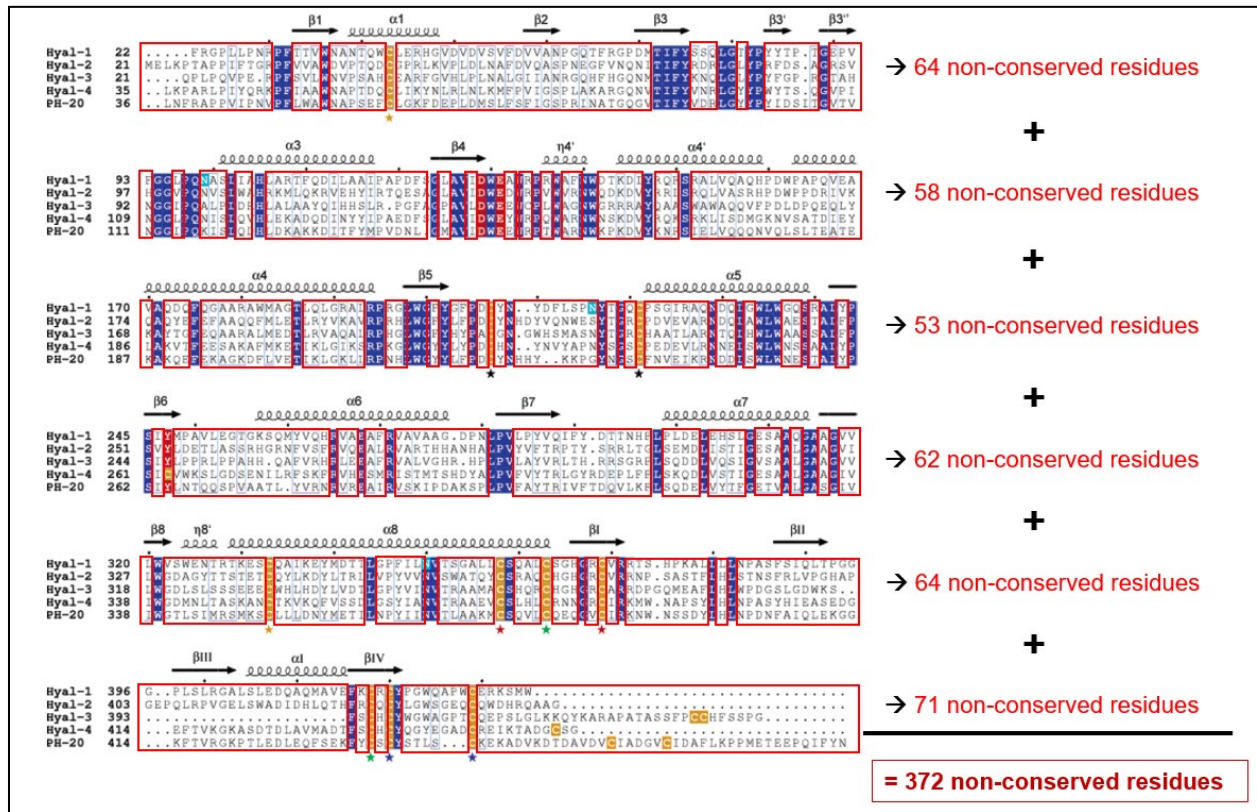


Figure B. My annotation of Figure 3 of Chao. The non-conserved regions are outlined in red, and the non-conserved residues are uncolored, identifying a total of 372 non-conserved residues. EX1006, FIG. 3.

116. Drs. Hecht and Park do not establish why a POSA would have mutated E324 rather than any of the other 371 non-conserved amino acids. EX1004; EX1005. And, similarly, Dr. Park’s alignment, which is not a prior art reference, identifies “379 positions in PH20<sub>1-447</sub>” that he considers “non-essential.” EX1004, ¶¶26-29, 31-32, Appendix D-1. And Dr. Park does not explain why a POSA would have selected position 324 from among the other 378 options in his alignment. EX1004, ¶¶26-29, 31-32, Appendix D-1.

117. In total, in view of my discussions in Sections VIII.A and VIII.B.1a-e, a POSA would not have been motivated to mutate E324 in view of Chao and the '429 patent. Notwithstanding all of the above, I also explain here why a POSA would not have been motivated to mutate E324 to aspartic acid (D), asparagine (N), or arginine (R).

**e. Drs. Hecht and Park Do Not Establish That a POSA Would Have Been Motivated to Mutate E324 to Aspartic acid (D), Asparagine (N), or Arginine (R).**

**i. Drs. Hecht and Park Do Not Explain Why a POSA Would Have Been Motivated to Undertake Nearly 30 Discrete Steps to Make the E324D Mutation.**

118. Unable to identify any disclosure in the '429 patent or Chao that suggests making an E324D substitution, Drs. Hecht and Park argue that a POSA would have found it obvious to engage in a lengthy series of steps set forth in Drs. Hecht and Parks' declarations. EX1003, ¶¶83, 195, 217-222; EX1004, ¶¶20-159, Appendix C, Appendix D-1.

119. Indeed, Drs. Hecht and Park argue that a POSA would have engaged in nearly 30 discrete steps to arrive at an E324D mutation as follows:

- 1) Review Halozyme's Hylenex biological product. EX1003, ¶195.
- 2) Superimpose the HYAL1 and bee venom hyaluronidase structures.  
EX1004, ¶¶89-91.
- 3) Align five human hyaluronidases of Chao. EX1003, ¶83.

- 4) Identify a characteristic pattern for the Hyal-EGF domain of PH20.  
EX1004, ¶¶97-98.
- 5) Identify invariant residues by analyzing a multiple sequence alignment of a set of published hyaluronidase sequences that was available in December of 2011. EX1004, ¶26.
- 6) Review scientific literature that identified important residues in hyaluronidase proteins or which reported experimental results showing that modifying single residues impaired or eliminated activity of the enzymes. EX1004, ¶¶26, 88.
- 7) Generate a dataset of sequences that were homologous to PH20 and that were publicly available by 2011 by performing a BLAST search using the human PH20 sequence in FASTA format (Uniprot P38567). EX1004, ¶¶28, 156-157.
- 8) Perform a search against the “reference proteins” database. EX1004, ¶¶156-157.
- 9) Download the search results as a text file. EX1004, ¶¶156-157.
- 10) Copy the header section of the text file into a separate file to decrease the amount of text so the data would have been easier to manipulate.  
EX1004, ¶157.
- 11) Extract the accession numbers of the retrieved sequences from the last

column and save them as a list of alphanumeric codes in a temporary file.

EX1004, ¶157.

- 12) Write and run another perl script to identify any duplicates. EX1004, ¶157.
- 13) For any duplicates, keep only the longest isoform of each unique enzyme from each organism to yield a final set of 88 unique sequences which were homologous to human PH20 and available by December 2012. EX1004, ¶157.
- 14) Save this list as a text file. EX1004, ¶157.
- 15) Write and run a third perl script to retrieve the FASTA format for each sequence from the file with the original BLASTP results and save the results as a new file. EX1004, ¶158.
- 16) Use Clustal Omega to generate a multiple sequence alignment. EX1004, ¶159.
- 17) Upload 88 sequences in FASTA format. EX1004, ¶159.
- 18) Save the MSA generated to a local file. EX1004, ¶160.
- 19) Use Clustal Omega to determine whether a residue is “conserved” or “semi-conserved,” which takes into account how similar a residue is across all of the sequences. EX1004, ¶29.
- 20) Use alignment to identify 68 largely invariant residues that a skilled

- artisan would have deemed “essential residues” in PH20<sub>1-447</sub>. EX1004, ¶¶30-32, 41-43, Appendix D-1.
- 21) Identify the 379<sup>24</sup> positions other than the 68 essential residues deemed “non-essential” residues. EX1004, ¶31.
  - 22) Determine whether position 324 is within a non-essential region of PH20<sub>1-447</sub>. EX1004, ¶¶32, 31, Appendix D-2; EX1003, ¶217.
  - 23) Identify the frequencies of amino acids that occur in sequences homologous to PH20. EX1003, ¶¶214-216; EX1004, ¶21.
  - 24) Determine the variability in the amino acids at non-conserved positions. EX1004, ¶¶21, 31, 41-42; EX1003, ¶218.
  - 25) Determine that the residue at position 324 in PH20 is glutamic acid (E). EX1004, ¶¶30-21, 41-43, 105, Appendix D-1; EX1003, ¶218.
  - 26) Determine the most prevalent amino acid at position 324 of the 88 sequence alignment is aspartic acid (D), which appears at position 324 in ~25% of the 88 proteins. EX1003, ¶218.
  - 27) Determine the second most-frequently occurring amino acid at position

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<sup>24</sup> Dr. Park purports to have identified 379 non-essential amino acid positions in PH20<sub>1-447</sub> based on his alignment of 88 sequences. EX1004, ¶31, Appendix D-2.

324 of the 88-sequence alignment is threonine (T), which appears at position 324 in ~13.6% of the 88 proteins. EX1004, ¶43; EX1003, ¶218.

28) Determine that aspartic acid, asparagine, and arginine are obvious choices for substitutions for glutamic acid at position 324. EX1003, ¶¶220-221.

120. But Drs. Hecht and Park do not explain why a POSA would have been motivated to undertake all of these steps. In particular, Drs. Hecht and Park do not explain why a POSA would have been motivated to undertake these steps to prepare an E324D-containing PH20 polypeptide with “at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>.” EX1003, ¶236. And furthermore Drs. Hecht and Park do not sufficiently explain all of these steps.

121. Dr. Park also does not adequately explain the perl scripts he wrote, how he used them, or how he assessed their reliability. EX1004, ¶¶157-158. For example, Dr. Park states: “To generate a file with these 88 sequences in FASTA format, I wrote and ran another perl script.” EX1004, ¶158. But he does not cite or describe what perl script he used, nor explain how he determined whether his further custom perl script produced the intended results. EX1004, ¶¶157-158.

122. Therefore Dr. Park did not sufficiently explain all of the steps he undertook, and Dr. Hecht did not supply sufficient additional explanation.

**C. Drs. Hecht and Park Have Not Established That, in the Absence of the Guidance Provided in the Specification, Chao and the '429**

**Patent Would Have Provided a POSA with a Reasonable Expectation That an E324D Mutation of PH20 Would Have Produced a Polypeptide with “at Least Comparable Hyaluronidase Activity as Unmodified PH20<sub>1-447</sub>”**

123. As I summarized in Section VI.D, I understand that one way of showing obviousness is by establishing that a POSA would have had both (i) a reason 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. As I explained above, a POSA would not have had a motivation to leap from the teachings of Chao and the '429 patent to make an E324D mutation of PH20 as required by claims 1-2, 6-15, and 17-30.

124. In view of the legal principles summarized in Section VI.D., and as explained by my analysis below, Drs. Hecht and Park also have not shown that Chao and the '429 patent would have provided a POSA with a reasonable expectation of successfully producing a protein exhibiting “at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>,” without the guidance in the common disclosure. EX1003, ¶236. I note again that the claims *do not require enzymatic activity*, but Dr. Hecht argues that a POSA “would reasonably expect that the E324D, E324N, and E324R substitutions in PH20 would each be tolerated, yielding a protein that exhibits at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>.” EX1003, ¶236.

125. In fact, Dr. Hecht goes as far as saying that “based on modeling

techniques available in 2011, the E324D and E324N substitutions would each be expected to be a neutral change, while the E324R substitution would be expected to be a beneficial change.” EX1003, ¶229.

126. To support this assertion, Drs. Hecht and Park propose that a POSA would have engaged in over fifty steps that are not outlined in Chao and/or the '429 patent, as follows:

- 1) Prepare PH20 structural model to visualize amino acid substitutions in PH20. EX1004, ¶¶33-36, 162-165.
- 2) Use SWISS-MODEL to generate a model of the PH20 structure using the HYAL1 structure. EX1004, ¶¶39-40.
- 3) Determine that the PH20 model was reliable by assessing the QMEAN value for the model. EX1004, ¶163.
- 4) Analyze the C-terminus region's local QMEAN reliability using B-factor scores in the modeled PH20 structure. EX1004, ¶165.
- 5) Use HYAL1 as the template structure for PH20 in SWISS-MODEL. EX1004, ¶167.
- 6) Devise a consistent, objective methodology for assessing substitutions using the PH20 model. EX1004, ¶¶102-103.
- 7) Assess possible interactions between the wild-type residue and its neighboring amino acids. EX1004, ¶¶44-47, 53-60, 65-85.

- 8) Assess possible interactions between an amino acid at a particular position with solvent. EX1004, ¶45.
- 9) Determine hydrophobicity and sterics of each neighboring amino acid. EX1004, ¶46.
- 10) Consider the varying characteristics of side chains of each amino acid and their interactions, including hydrophobic residues with other hydrophobic residues, van der Waals attractions between residues, hydrogen bonding among polar residues. EX1004, ¶¶48-52.
- 11) Consider factors within the context of the specific location of the substitution, including whether the residue being substituted is on the surface of the protein's structure or buried within protein's structure. EX1004, ¶¶53-54.
- 12) Define a classification system for assessing single amino acid substitutions in PH20. EX1004, ¶55.
  - a. Changes that were likely to stabilize the protein.
  - b. Changes that were either neutral or mildly positive or negative for the local protein structure.
  - c. Changes that were likely to be significantly destabilizing.
- 13) Inspect the PH20 structure produced using the PyMol viewer to identify the neighbors of the residues to assess in the wild-type PH20 sequence. EX1004,

¶56.

- 14) Note “neighbors” that were within  $\sim 5$  Å of the side chain of the amino acid being evaluated, with greater number of neighbors indicative of the residue being buried and fewer neighbors indicative of a solvent accessible surface residue. EX1004, ¶¶56-57.
- 15) Determine the fractional solvent accessible surface area (“SASA”) to quantitatively measure whether a residue is solvent accessible. EX1004, ¶58.
- 16) Inspect the distance between neighbors and a particular residue to determine whether there was likely a van der Waals interaction. EX1004, ¶59.
- 17) Periodically use a custom script, which runs within the PyMol environment to show all of the neighboring amino acids encompassed in a shell, allowing one to visualize the chemical moieties surrounding an amino acid at a given position EX1004, ¶60.
- 18) Use the “mutagenesis” feature of PyMol, which replaces an amino acid at a defined position with another amino acid, to evaluate whether a mutation would have been likely be tolerated. EX1004, ¶61.
- 19) Consider the chemical similarity of the substitution to the wild-type amino acid at the position being evaluated. EX1004, ¶62.
- 20) Assess interactions with neighboring residues to determine impact of

substitution, including: EX1004, ¶¶63, 85.

- 21) For each substitution, consider whether the change introduces a hydrophobic residue into a hydrophilic environment EX1004, ¶¶64-68.
- 22) For each substitution, evaluate its compatibility with the predicted secondary structures at that position. EX1004, ¶¶69-73.
- 23) For each substitution, consider how the substitution would have altered steric interactions compared to the wild-type residue. EX1004, ¶¶74-79.
- 24) For each substitution, consider tertiary interactions that might be influenced by a substitution. EX1004, ¶¶80-83.
- 25) Consult the structure of human HYAL1 and/or bee venom hyaluronidase to consider how a particular substitution might influence the structure of PH20 (reflects the evolutionary influences of the protein's structure). EX1004, ¶84.
- 26) Balance the type of impact of the substitutions based on the magnitude of each interaction may have on the protein's structure. EX1004, ¶85.
- 27) Assign a score for each substitution reflecting the aggregate effect. EX1004, ¶¶86-87.
  - a. A score of 1 (reduce protein stability), 2 (no effect or slightly positive or negative effect), or 3 (improve overall stability).
- 28) Consider the biochemical and structural data reported in the scientific

literature, including Chao, Zhang, Stern, and Arming. EX1004, ¶¶88-101.

29) Evaluate and assign scores to many different substitutions to develop a consistent and unbiased methodology to evaluate potential substitutions.

EX1004, ¶¶102-103.

**Analysis of Position 324:**

30) Assess the local environment near position 324 in PH20, which is glutamic acid (E) in the wild-type form of human PH20. EX1004, ¶105.

31) Visualize E324 within the PH20 model using PyMol. EX1004, ¶107.

32) Confirm that E324 was not near the active site. EX1004, ¶107.

33) Note that E324 has six neighbors. EX1004, ¶107.

34) Inspect placement of neighboring amino acids and their interactions with E324. EX1004, ¶107.

35) Confirm more distant, non-neighboring amino acids do not interact with E324. EX1004, ¶107.

36) Determine that residue E324 is located in the middle of helix 8 ( $\alpha 8$ ). EX1004, ¶108.

37) Determine that a proline residue at position 329 causes a kink causing the  $\alpha 8$  helix to be partially unwound around position 329. EX1004, ¶109.

38) Determine that E324 is located at a solvent-exposed position. EX1004, ¶110.

- 39) Determine that E324 has a fractional SASA (fSASA) of 0.48, which is similar to the median fSASA value for glutamic acid, which is 0.45. EX1004, ¶110.
- 40) Determine that E324's side chain is pointed toward the solvent and is not restricted by E324's six neighbors. EX1004, ¶110.
- 41) Create custom scripts that run within the PyMol environment. EX1004, ¶¶60, 177.
- a. Inspect positions.
  - b. Identify each residue, its neighbors, the distance between the residue and each neighbor, as well as the surface environment.
  - c. Display the pockets of a particular location in the structure using a script that invokes display options built into PyMol.
- 42) Use a built-in function in PyMol that replaces the amino acid in the structure at a position with a different amino acid ("mutagenesis"). EX1004, ¶¶61, 107, 120, 178.
- 43) Assess numerous substitutions representing diverse interactions, including E324D, E324N, E324R, E324A, E324H, and E324S. EX1004, ¶¶113-153.
- 44) Evaluate the E324D substitution in PH20<sub>1-447</sub>. EX1004, ¶¶113-121.
- 45) For E324D, determine that aspartic acid (D) is found at position 324 in about 25% of the 88 proteins reviewed. EX1004, ¶113.

- 46) Determine that aspartic acid can tolerate a solvent-accessible environment because it is a hydrophilic amino acid. EX1004, ¶114.
- 47) Use the PyMol protein mutagenesis feature, which suggested rotamer 3 as the best fit for aspartic acid at position 324. EX1004, ¶115.
- 48) Determine that, when aspartic acid was substituted in position 324:
- a) The terminal carboxyl group of D324 will point toward and interact with solvent;
  - b) D324 would be expected to contribute to the creation of a solvent shielded hydrophobic environment around F380;
  - c) The positioning of D324 will sterically impede the movement of solvent molecules around F380 (like E324); and
  - d) The van der Waals and/or hydrophobic interactions may be reduced because:
    - i. D324's carboxyl group may interfere with the adjacent hydrophobic interaction involving the side chains of F380; and
    - ii. The proximity of the polar hydroxyl of D324 and the hydrophobic side chain of L374 may be destabilizing. EX1004, ¶¶116-118.
- 49) Conclude that the magnitude of any reduction of van der Waals and/or hydrophobic interactions that may result from the E324D substitution would

not be expected to significantly impact the stability of the protein. EX1004, ¶119.

- 50) Determine that, although aspartic acid has a low helix propensity, its substitution with E324 would not be expected to significantly impact the secondary helical structure around position 324 because the helical structure around position 324 has been disrupted due to the proline at position 329. EX1004, ¶119.
- 51) Confirm that the modeled structure with E324D, which incorporates energy minimization, supported the evaluation based on PyMol's protein mutagenesis feature. EX1004, ¶120.
- 52) Assign the E324D mutation a score of 2 in view of the expected effects of the E324D mutation. EX1004, ¶121.
- 53) Assess whether asparagine (N) would have been tolerated at position 324. EX1004, ¶¶122-129.
- 54) Conclude that N324 would be tolerated. EX1004, ¶129.
- 55) Assess whether arginine (R) would have been tolerated at position 324. EX1004, ¶¶130-137.
- 56) Conclude that R324 would be tolerated. EX1004, ¶137.
- 57) Assess whether alanine (A) would have been tolerated at position 324. EX1004, ¶¶138-142.

58) Conclude that A324 would be tolerated. EX1004, ¶142.

59) Assess whether histine (H) would have been tolerated at position 324.

EX1004, ¶¶143-147.

60) Conclude that H324 would be tolerated. EX1004, ¶147.

61) Assess whether serine (S) would have been tolerated at position 324.

EX1004, ¶¶148-153.

62) Conclude that S324 would be tolerated.<sup>25</sup> EX1004, ¶153.

127. Drs. Hecht and Park provide no explanation demonstrating that a POSA would have been motivated to carry out these more than fifty steps to form a reasonable expectation that making the E324D mutation of PH20 would have yielded a PH20 polypeptide with “at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>,” absent the guidance in the specification. EX1003, ¶236.

128. Additionally, similarly to the nearly 30 steps previously described above, Dr. Park also does not fully explain the above more than fifty steps here although they, too, include creating and using custom scripts. EX1004, ¶¶60, 177.

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<sup>25</sup> I note that Dr. Park provides no assessment regarding whether methionine (M) would have been tolerated at position 324, nor does he conclude that a POSA would have determined that methionine (M) would have been tolerated at position 324.

129. Dr. Park also states that he “periodically used a custom script that [he] wrote which runs within the PyMol environment” that “shows all of the neighboring amino acids encompassed in a shell, allowing one to visualize the chemical moieties surrounding an amino acid at a given position.” EX1004, ¶60. But he does not explain what he means by “periodically,” nor does he explain how these scripts work to “visualize the chemical moieties surrounding an amino acid at a given position.” EX1004, ¶60.

130. In sum, the combination of Chao and the '429 patent do not provide any guidance suggesting that mutating E324 to aspartic acid (D) would have yielded a PH20 polypeptide with “at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>.” EX1003, ¶236; EX1005; EX1006.

131. Furthermore, the '429 patent's general guidance that amino acid substitutions in non-essential regions of a polypeptide “do not *substantially* alter biological activity” would not have provided a POSA with sufficient guidance to reasonably expect that making the E324D mutation would have yielded a PH20 polypeptide with “at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>.” EX1003, ¶236; EX1005, 9:47-50 (emphasis added). Rather, a POSA reading the '429 patent's general guidance that amino acid substitutions in non-essential regions of a polypeptide “do not *substantially* alter biological activity” could also have concluded that such substitutions would not *substantially decrease* biological

activity. EX1005, 16:14-22 (emphasis added). The word “alter” does not specifically indicate an increase or decrease in “biological activity.” EX1005, 16:14-22.

132. Thus Drs. Hecht and Park have failed to show that a POSA would have had a reasonable expectation that making the E324D mutation would have yielded a PH20 polypeptide with “at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>” before December 29, 2011 and absent the guidance in the specification. EX1003, ¶236.

**D. Dr. Hecht’s Declarations in Related Proceedings Further Reveal that the ’429 Patent and Chao Would Not Have Provided Any Reason to Make an E324D Mutation.**

133. Dr. Hecht has submitted declarations in eleven other related proceedings.<sup>26</sup> In each case, he applies essentially the same references and reasoning as explained above, but to different claimed modifications:

- 1) U.S. Patent No. 11,952,600 requires an amino acid modification as position 320;
- 2) U.S. Patent No. 12,018,298 requires an amino acid modification at position 313;

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<sup>26</sup> Obviousness was not challenged in the proceeding related to U.S. Patent No. 12,077,791. EX2063.

- 3) U.S. Patent No. 12,152,262 requires an amino acid modification at position 317;
- 4) U.S. Patent No. 12,123,035 requires an amino acid modification at position 312;
- 5) U.S. Patent No. 12,054,758 requires an amino acid modification at position 317;
- 6) U.S. Patent No. 12,060,590 requires an amino acid modification at position 371;
- 7) U.S. Patent No. 12,049,652 requires an amino acid modification at position 324;
- 8) U.S. Patent No. 12,104,185 requires an amino acid modification at position 320;
- 9) U.S. Patent No. 12,037,618 requires an amino acid modification at position 309; and
- 10) U.S. Patent No. 12,091,692 requires an amino acid modification at position 313.

EX1003; EX2029-2034, EX2029-2034, EX2036, EX2038, EX2057, and EX2061.

134. I illustrate this point with exemplary excerpts of Dr. Hecht's Declarations related to the these U.S. Patents, below. The text that is deleted relative to Dr. Hecht's '600 patent declaration (EX2030) is depicted in red strike

through, and the text that is new relative to Dr. Hecht’s ’600 patent declaration is depicted in blue underline text:

<b>Exemplary Excerpt 1</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
US Patent No. 12,018,298 <b>PGR2025-00004</b>  EX2029, ¶200; EX2030, ¶200	I note that these conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution ( <i>e.g.</i> , the <del>D320K</del> <u>M313K</u> substitution I discuss below) with little effort.
US Patent No. 12,152,262 <b>PGR2025-00006</b>  EX2032, ¶200; EX2030, ¶200	I note that these conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution ( <i>e.g.</i> , the <del>D320K</del> <u>L317Q</u> substitution I discuss below) with little effort.
US Patent No. 12,123,035 <b>PGR2025-00009</b>  EX2031, ¶203; EX2030, ¶200	I note that these conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein <u>that are described in the patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution ( <i>e.g.</i> , the <del>D320K</del> <u>S312T or S312N</u> substitution I discuss below) with little effort.
U.S. Patent No. 12,110,520 <b>PGR2025-00017</b>  EX1003, ¶203; EX2030, ¶200	I note that <del>these</del> conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein <u>that are described in the patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution ( <i>e.g.</i> , the <del>D320K-substitution</del> <u>E324D, E324N, or E324R substitutions</u> I discuss below) with little effort.
U.S. Patent No. 12,054,758 <b>PGR2025-00030</b>	I note that <del>these</del> conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein <u>that are described in the ’429 patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution

<b>Exemplary Excerpt 1</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
EX2033, ¶203; EX2030, ¶200	(e.g., the <del>D320K</del> <u>L317Q</u> substitution I discuss below) with little effort.
U.S. Patent No. 12,060,590 <b>PGR2025-00024</b>  EX2034, ¶203; EX2030, ¶200	I note that <del>these</del> conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein <u>that are described in the '429 patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution (e.g., the <del>D320K</del> <u>L307W, L307T, or L307S</u> substitution I discuss below) with little effort.
U.S. Patent No. 12,049,652 <b>PGR2025-00033</b>  EX2036, ¶213; EX2030, ¶200	I note that <del>these</del> conventional procedures relating to production of the wild-type PH20 <sub>1-447</sub> protein <u>that are described in the '429 patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution (e.g., the E324D <u>or E324S</u> substitution I discuss below) with little effort.
U.S. Patent No. 12,104,185 <b>PGR2025-00039</b>  EX2038, ¶216; EX2030, ¶200	I note that <del>these</del> conventional procedures relating to production of the wild- type PH20 <sub>1-447</sub> protein <u>that are described in the '429 patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution (e.g., the D320K substitution I discuss below) with little effort.
U.S. Patent No. 12,037,618 <b>PGR2025-00042</b>  EX2057, ¶216; EX2030, ¶200	I note that <del>these</del> conventional procedures relating to production of the wild- type PH20 <sub>1-447</sub> protein <u>that are described in the '429 patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution (e.g., the <del>D320K</del> <u>I309N</u> substitution I discuss below) with little effort.
U.S. Patent No. 12,091,692 <b>PGR2025-00046</b>  EX2061, ¶203; EX2030, ¶200	I note that <del>these</del> conventional procedures relating to production of the wild- type PH20 <sub>1-447</sub> protein <u>that are described in the '429 patent</u> could be applied to produce forms of PH20 <sub>1-447</sub> that incorporate a single amino acid substitution

<b>Exemplary Excerpt 1</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
	(e.g., the <del>D320K</del> <a href="#">E324D</a> , <a href="#">E324N</a> , or <a href="#">E324R</a> substitutions I discuss below) with little effort.

<b>Exemplary Excerpt 2</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
US Patent No. 12,018,298 <b>PGR2025-00004</b>  EX2029, ¶201; EX2030, ¶201	The '429 Patent reports that expressing the <del>D320K</del> <a href="#">M313K</a> PH20 <sub>1-447</sub> mutant in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
US Patent No. 12,152,262 <b>PGR2025-00006</b>  EX2032, ¶201; EX2030, ¶201	The '429 Patent reports that expressing the <del>D320K</del> <a href="#">L317Q</a> PH20 <sub>1-447</sub> mutant in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
US Patent No. 12,123,035 <b>PGR2025-00009</b>  EX2031, ¶204; EX2030, ¶201	The '429 Patent reports that expressing the <del>D320K</del> <a href="#">S312T</a> or <a href="#">S312N</a> PH20 <sub>1-447</sub> mutant in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
U.S. Patent No. 12,110,520 <b>PGR2025-00017</b>  EX1003, ¶204; EX2030, ¶201	The '429 Patent reports that expressing <del>the D320K PH20<sub>1-447</sub>-mutant</del> <a href="#">PH20<sub>1-447</sub> mutants</a> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
U.S. Patent No. 12,054,758 <b>PGR2025-00030</b>	The '429 Patent reports that expressing <del>the D320K PH20<sub>1-447</sub>-mutant</del> <a href="#">PH20<sub>1-447</sub> mutants</a> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.

<b>Exemplary Excerpt 2</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
EX2033, ¶204; EX2030, ¶201	
U.S. Patent No. 12,060,590 <b>PGR2025-00024</b>  EX2034, ¶204; EX2030, ¶201	The '429 Patent reports that expressing <del>the D320K PH20<sub>1-447</sub>-mutant</del> <u>PH20<sub>1-447</sub> mutants</u> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
U.S. Patent No. 12,049,652 <b>PGR2025-00033</b>  EX2036, ¶214; EX2030, ¶201	The '429 Patent reports that expressing <del>the E324D PH20<sub>1-447</sub>-mutant</del> <u>PH20<sub>1-447</sub> mutants</u> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
U.S. Patent No. 12,104,185 <b>PGR2025-00039</b>  EX2038, ¶217; EX2030, ¶200	The '429 Patent reports that expressing <del>the D320K PH20<sub>1-447</sub>-mutant</del> <u>PH20<sub>1-447</sub> mutants</u> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
U.S. Patent No. 12,037,618 <b>PGR2025-00042</b>  EX2057, ¶217; EX2030, ¶200	The '429 Patent reports that expressing <del>the D320K PH20<sub>1-447</sub>-mutant</del> <u>PH20<sub>1-447</sub> mutants</u> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.
U.S. Patent No. 12,091,692 <b>PGR2025-00046</b>  EX2061, ¶204; EX2030, ¶200	The '429 Patent reports that expressing <del>the D320K PH20<sub>1-447</sub>-mutant</del> <u>PH20<sub>1-447</sub> mutants</u> in a CHO cell yields a glycosylated form of the protein that is enzymatically active.

<b>Exemplary Excerpt 3</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
US Patent No. 12,018,298 <b>PGR2025-00004</b>  EX2029, ¶213; EX2030, ¶213	Position <del>320</del> 313 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.
US Patent No. 12,152,262 <b>PGR2025-00006</b>  EX2032, ¶213; EX2030, ¶213	Position <del>320</del> 317 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.
US Patent No. 12,123,035 <b>PGR2025-00009</b>  EX2031, ¶217; EX2030, ¶213	Position <del>320</del> 312 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.
U.S. Patent No. 12,110,520 <b>PGR2025-00017</b>  EX1003, ¶217; EX2030, ¶213	Position <del>320</del> 324 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.
U.S. Patent No. 12,054,758 <b>PGR2025-00030</b>  EX2033, ¶217; EX2030, ¶213	Position <del>320</del> 317 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.
U.S. Patent No. 12,060,590 <b>PGR2025-00024</b>  EX2034, ¶217; EX2030, ¶213	Position <del>320</del> 307 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.

<b>Exemplary Excerpt 3</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
U.S. Patent No. 12,049,652 <b>PGR2025-00033</b>  EX2036, ¶227; EX2030, ¶213	Position 320 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment of Chao.
U.S. Patent No. 12,104,185 <b>PGR2025-00039</b>  EX2038, ¶230; EX2030, ¶200	Position 320 is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment in Chao.
U.S. Patent No. 12,037,618 <b>PGR2025-00042</b>  EX2057, ¶231; EX2030, ¶200	Position <del>320</del> <u>309</u> is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment in Chao.
U.S. Patent No. 12,091,692 <b>PGR2025-00046</b>  EX2061, ¶217; EX2030, ¶200	Position <del>320</del> <u>324</u> is within a non-essential region of the PH20 sequence, based on my review of Dr. Park’s analysis and the sequence alignment in Chao.

<b>Exemplary Excerpt 4</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
US Patent No. 12,018,298 <b>PGR2025-00004</b>  EX2029, ¶216; EX2030, ¶216	Given the explanations above, a skilled artisan, in 2011, would have readily identified position <del>320</del> <u>313</u> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the ’429 Patent.

<b>Exemplary Excerpt 4</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
US Patent No. 12,152,262 <b>PGR2025-00006</b>  EX2032, ¶216; EX2030, ¶216	Given the explanations above, a skilled artisan, in 2011, would have readily identified position <del>320</del> <u>317</u> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
US Patent No. 12,123,035 <b>PGR2025-00009</b>  EX2031, ¶220; EX2030, ¶216	Given the explanations above, a skilled artisan, in 2011, would have readily identified position <del>320</del> <u>312</u> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
U.S. Patent No. 12,110,520 <b>PGR2025-00017</b>  EX1003, ¶220; EX2030, ¶216	<del>Given the explanations above, a</del> <u>A</u> skilled artisan, in 2011, would have readily identified position <del>320</del> <u>324</u> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
U.S. Patent No. 12,054,758 <b>PGR2025-00030</b>  EX2033, ¶221; EX2030, ¶216	<del>Given the explanations above, a</del> <u>A</u> skilled artisan, in 2011, would have readily identified position <del>320</del> <u>317</u> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
U.S. Patent No. 12,060,590 <b>PGR2025-00024</b>  EX2034, ¶220; EX2030, ¶216	<u>First</u> <del>Given the explanations above,</del> a skilled artisan, in 2011, would have readily identified position <del>320</del> <u>307</u> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
U.S. Patent No. 12,049,652 <b>PGR2025-00033</b>  EX2036, ¶231; EX2030, ¶216	<del>Given the explanations above, a</del> <u>A</u> skilled artisan, in 2011, would have readily identified position 320 as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.

<b>Exemplary Excerpt 4</b>	
<b>Comparative Patent</b>	<b>Deleted Text Is Depicted in Red; New Text Is Depicted in Blue</b>
U.S. Patent No. 12,104,185 <b>PGR2025-00039</b>  EX2038, ¶234; EX2030, ¶200	<del>Given the explanations above, a</del> <b>A</b> skilled artisan, in 2011, would have readily identified position 320 as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
U.S. Patent No. 12,037,618 <b>PGR2025-00042</b>  EX2057, ¶231; EX2030, ¶200	<del>Given the explanations above, a</del> <b>A</b> skilled artisan, in 2011, would have readily identified position <del>320</del> <b>309</b> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.
U.S. Patent No. 12,091,692 <b>PGR2025-00046</b>  EX2061, ¶220; EX2030, ¶200	<del>Given the explanations above, a</del> <b>A</b> skilled artisan, in 2011, would have readily identified position <del>320</del> <b>324</b> as being in one of the non-essential regions of PH20 <sub>1-447</sub> contemplated by the '429 Patent.

135. As can be seen from the above excerpts, Dr. Hecht’s analysis is substantially the same in his declarations submitted in support of PGR Petitions filed against the above ten U.S. patents. EX2029-2034, EX2036, EX2038, EX2057, and EX2061.

136. In closing, Drs. Hecht and Park appear to have relied on hindsight, based on the direction of counsel, rather than any teachings or suggestions stemming from the prior art that would have motivated a POSA to make an E324D mutation. Drs. Hecht and Park fail to identify any disclosure of a mutation of E324

(or an E324 mutation to aspartic acid (D), asparagine (N), or arginine (R)) in Chao and the '429 patent, whether explicitly or as would have been supplied by the common sense or ordinary creativity of a POSA. And absent the guidance of the specification, Drs. Hecht and Park further fail to establish that a POSA would have had a motivation to combine Chao and the '429 patent to make a E324 mutation of PH20 or establish that a POSA would have reasonably expected that mutating E324 to aspartic acid (D) would have resulted in a modified PH20 polypeptide with at least comparable hyaluronidase activity as unmodified PH20<sub>1-447</sub>. As depicted above, Drs. Hecht and Parks' analysis is, in essence, based on cherry-picking using hindsight rather than reflecting the view of a POSA reading the '429 patent in combination with Chao.

**IX. THE EXAMINER CONSIDERED THE SAME OR SUBSTANTIALLY THE SAME ART AND ARGUMENTS AS NOW PRESENTED BY DRS. HECHT AND PARK**

137. Dr. Hecht concludes that the claims are obvious over the '429 patent in combination with Chao. However, neither Dr. Hecht nor Dr. Park (on whom Dr. Hecht relies), explain whether or how their arguments differ from what the Examiner already considered during prosecution.

**A. The Examiner Considered Stern, Zhang, and Arming, Which Include Teachings Cumulative to the Relied-Upon Teachings of Chao.**

138. Drs. Park and Hecht rely on the '429 patent (EX1005) and Chao

(EX1006) to contend that claims 1-2, 6-15, and 17-30<sup>27</sup> would have been obvious over the '429 patent in view of Chao. The teachings that Drs. Park and Hecht rely on in Chao are cumulative (i.e., the same or substantially the same as) of the teachings in Stern (EX1008), Zhang (EX1010), and Arming (EX1011), each of which were cited during prosecution and considered by the Examiner. EX1002, 500, 504, 513, 514.

139. As I discussed above, I have reviewed the prosecution history of the '520 patent and I understand that patent examiners are charged with determining whether the patent application complies with the patent laws, including the requirements for written description and obviousness. I also understand that applicants are obligated to submit to the United States Patent and Trademark Office (USPTO) any known information that may be material to patentability, and such information may be provided in the form of an Information Disclosure Statement (IDS). I also understand the Examiner is expected to consider the information submitted and indicate that they have done so by initialing or signing the IDS.

140. In my review of the prosecution history of the '520 patent (EX1002), I

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<sup>27</sup> Claims 3-5, 16, and 31-35 of the '520 patent have been statutorily disclaimed.

note that the '429 patent, which is a U.S. Patent granted August 3, 2010, was submitted to the Examiner in an IDS as reference description (Ref. Des. CK). EX1002, 380.<sup>28</sup> I note that Stern, which is non-patent literature, was submitted to the Examiner in the same IDS as Ref. Des. OB\*\*. EX1002, 393. I note that Zhang (EX1010), which is non-patent literature, was submitted to the Examiner in the same IDS as Ref. Des. PK\*\*. EX1002, 394. And, I note that Arming (EX1011), which is non-patent literature, was submitted to the Examiner in the same IDS as Ref. Des. HE\*\*. EX1002, 384.

141. I have compared the prior art identified by Dr. Hecht's obviousness ground to the information provided in the prosecution history. Upon my review, I conclude that the Examiner of the '520 Patent considered each of the Stern, Zhang, and Arming references in the same manner as Dr. Hecht and Dr. Park apply Chao. In other words, to a POSA, the relied-upon portions of Chao are substantially similar and cumulative to teachings considered by the Examiner in Stern alone, and further supported by a combination of Stern, Zhang, and Arming. I will address each of these considered references in turn.

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<sup>28</sup> I understand that the Examiner's initials on Form 1449 indicate that they considered the '429 Patent, Stern, Zhang, and Arming. EX1002, 500, 504, 513, 514.

**1. Stern was considered by the Examiner.**

142. Stern is a review paper entitled “Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action.” EX1008.

143. Below, I provide a comparison between the information Drs. Hecht and Park extract from Chao with the disclosure in Stern. Drs. Hecht and Park rely on disclosure in Chao that is substantially the same as the disclosure in Stern.

144. Dr. Hecht alleges that:

The Chao paper provided new, highly relevant information for evaluating structural features of PH20, particularly by someone interested in modifying the structure of PH20. For example its sequence alignment identified secondary structures and sites of conserved amino acids in both PH20 and HYAL1. Its structure of HYAL1 provided important insights, such as the existence of the Hyal-EGF domain, and provided a template to use in more accurate modeling of PH20.

EX1003, ¶86. I disagree with Drs. Hecht and Park. This information is neither new nor relevant, much less highly relevant. Instead, as I detail below, it was already disclosed in, for example, Stern, Zhang, and Arming and was considered by the Examiner.

145. The sequences of the human hyaluronidase enzymes like PH20 were already in the art before Chao, including in the references considered by the Examiner. Stern, like Chao, align the five human hyaluronidase sequences and

predict secondary structure(s). EX1006, 6916-6918; EX1008, 826, 830-832.

146. Below, I provide a comparison between the information the Petition extracts from Chao with the disclosure in Stern. I did not find such a comparison in Dr. Hecht's or Dr. Park's Declaration or a comparison of Chao to any other reference cited during prosecution. A majority of Chao's disclosure relied on by Drs. Hecht and Park is found in Stern. EX1008.

147. Chao provided a multiple sequence alignment of the five hyaluronidase enzymes. EX1006, 6916 (FIG. 3, see below). Stern provided a multiple sequence alignment of the very same five hyaluronidase enzymes. EX1008, 826 (FIG. 3, see below).<sup>29</sup> In fact, Chao relies on Stern to establish that there are five homologous hyaluronidase enzymes encoded in the human genome:

There are five homologous hyaluronidases encoded in the human genome: hHyal-1 through -4 and the sperm adhesion molecule 1 (termed PH-20). In addition, the human genome contains a related pseudo gene, PHYAL1. These genes exhibit different tissue distribution profiles. Human Hyal-1 and Hyal-2 are expressed in most tissues and are responsible for the catabolism of intracellular and extracellular HA, respectively.

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<sup>29</sup> The "Supplementary Data" in Stern also includes a sequence alignment of the human and mouse hyaluronidases Hyal-1 – Hyal-4, PH-20, and Phyal1. EX1008, 840. The PH-20 mouse sequence has an "Q" at position 324. EX1008, 841.

EX1006, 6911.

148. Chao did not purport to have discovered any new sequences. Rather, Chao repeated substantially the same sequence alignment of the five human hyaluronidases that was already performed by Stern. EX1006, 6916, FIG. 3; EX1008, 826, FIG. 3. The sequence alignment in Chao identifies the region between C316 and L327 as being allegedly “non-essential,” but the same portion of the molecule is identified in the sequence alignment in Stern, as shown below annotated by the green lines in both graphics (Figures J and K). EX1006, 6916, FIG. 3; EX1008, 826, FIG. 3.

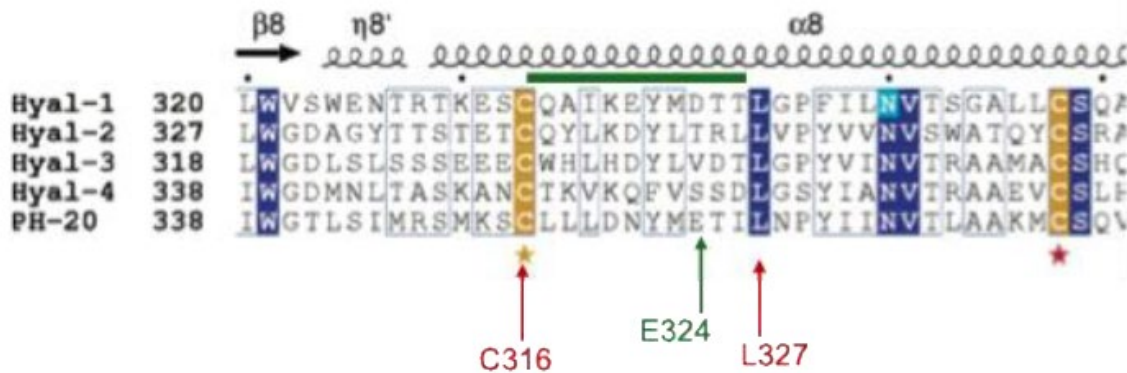


Figure C. EX1003, ¶217 (excerpted and annotated in the Hecht declaration from EX1006, 6916, FIG. 3 with green line and arrows identifying residues C316, E324, L327).



Appendix B. Reference amino acid sequences equivalent to those aligned in Stern and Chao were identified in NCBI GenPept for human PH20 (NP\_694859.1), human Hyal-1 (NP\_149349.2), human Hyal-2 (NP\_003764.3), human Hyal-4 (NP\_036401.2) and bee venom hyaluronidase (NP\_001011619.1). The human Hyal-3 reference sequence (NP\_003540.2) that was selected differed from AAC709152 previously used by Stern and Chao, which has an incorrect C-terminal sequence. Multiple sequence alignments were performed using web-based platforms for CLUSTAL W 2.1 and CLUSTAL O (1.2.4) using default parameters.

151. For the CLUSTAL 2.1 alignment of the sequences presented by Chao, the alignment was manually adjusted in three locations (G107, N126, and C443, numbering based on NP\_694859.1) for optimal alignment. These alignments that I have performed with Clustal W and Omega show similarity among the sequences and conservation of the same residues shown in the alignments in the counterpart alignments from the Stern and Chao papers.<sup>31</sup> However, contrary to Dr. Park's

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<sup>31</sup> The human hyaluronidase alignments in Chao and Stern are nearly identical. This fact is further supported by Chao's use of the same Hyal-3 sequence as Stern, which has the incorrect C terminus. To illustrate this point, I note that the human Hyal-3 sequence that was used in both the Stern and Chao alignments matches that of AAC709152 from 1998 and the C-terminus of that particular

allegations, there is no indication as to why a POSA would have been motivated to modify position 324 based on the conserved residues shared between the human hyaluronidases. EX1004, ¶¶104, 129.

152. Dr. Hecht identifies the region between the C316 and L327 residues as being allegedly “non-essential.” EX1003, ¶217. As indicated above in the green annotated line, Stern identifies the same region between the C316 and L327 residues. EX1008, 826 (FIG. 3 legend, “The conserved residues are marked as follows: \* = identical in entire column”). Chao does not, in any manner, provide a motivation to make single amino acid substitutions in non-essential regions of PH20. Chao only identifies *invariant conserved residues* in their alignment of hyaluronidases. EX1006, FIG. 3, 6916. Whether residues are “conserved” or “not conserved” does not mean they are “essential” or “non-essential.”

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sequence is not represented in any of the other reference sequences now available in GenBank. Even before 2007 there were examples of the correct sequence such as AF502912 in Lokeshwar. EX2035, 33659-33660. I ran a BLAST of AAC709152 against non-redundant protein sequences and only the same sequence came up as perfectly matching. This indicates that the alignments are indeed very similar as Chao used the same “unusual” Hyal-3 sequence as Stern which has the wrong C-terminus. This did not affect the alignment of conserved residues.

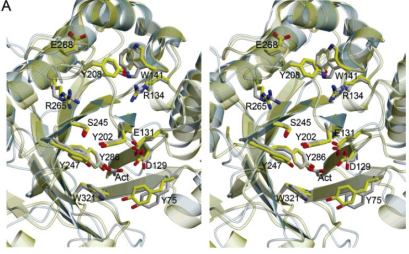
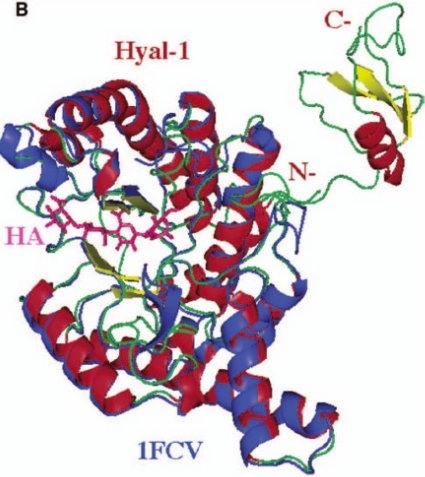
153. I note that Chao crystallized Hyal-1 (not a PH20) to predict the Hyal-1 structure. EX1006, 6915-6918. Chao concluded that the N-terminus folds into a distorted ( $\beta/\alpha$ ) barrel and the C-terminus folds into an EGF-like domain. Then Chao used sequence alignments to speculate that other hyaluronidases would have folded like Hyal-1 does. Moreover, one does not need to know the crystal structure of Hyal-1 to know that E324 is a non-conserved amino acid in some hyaluronidases. The amino acid substitution at position 324 is not within the EGF-like domain, so Chao's disclosure of an EGF-like domain (which was already disclosed in other art that the Examiner previously considered) does not represent a new insight that would have motivated a POSA to make a E324 substitution. EX1003, 224-225, Appendix A-9; EX1002, 504, 513, 514. As a result, Chao's discussion of the HyalEGF-like domain is irrelevant to the existence of a HyalEGF-like domain in Hyal-1 and the speculation that such a domain exists in PH20 would not have given a POSA any reason to make a E324D, E324N, or E324R mutation in PH20 or provided a POSA with a reasonable expectation of success. And neither Drs. Park nor Hecht explain why Chao's discussion of the HyalEGF-like domain would have provided any reasonable expectation of success. To the contrary, Dr. Hecht discloses a table of amino acid residues comprising the HYAL-EGF region and the impact of residue substitution on activity, which includes positions 337-412. EX1003, 224-225, Appendix A-9. Therefore, since the

HYAL-EGF region does not contain position 324, a POSA would not have had any reason to make a E324D, E324N, or E324R mutation in PH20.

154. Additionally, with respect to structure, Stern, like Chao, recognized that human hyaluronidases are composed of two domains: a major catalytic domain followed by a C-terminal domain of unknown function. EX1008, 829; EX1006, 6912. Dr. Park himself points out that “in December of 2011 (and even *today*), the structure of human PH20 was not solved.” EX1004, ¶36 (emphasis added). It is therefore, unsupported to allege that Chao provided “*highly relevant* information” as it pertains to the structure of human PH20, because Chao did not determine the structure of human PH20. EX1003, ¶86 (emphasis added). Chao, even when viewed in combination with the ’429 patent, certainly provides no motivation to modify position 324. And Chao does not identify any purported function of the EGF-like domain. EX1006, 6916. Therefore, the information that Dr. Park relies on from Chao is either irrelevant or previously considered by the Examiner.

155. Aspects of Chao cited by Drs. Hecht and Park are likewise also included in Stern, as shown in the chart below.

Relied-upon Teaching of Chao	Same Teaching in Stern
“There are <b>five homologous hyaluronidases</b> encoded in the <b>human genome: hHyal-1 through -4</b> and the sperm adhesion molecule 1 (termed <b>PH-20</b> ).” EX1006, 6911.	“All models of <b>human Hyals-1—4</b> as well as <b>HPH-20</b> are of high quality and <b>are essentially identical to one another in the structure of their main domain</b> (Figures 4 and 5). These five models, therefore, represent reliable structural

Relied-upon Teaching of Chao	Same Teaching in Stern
	models for <b>all five human Hyal enzymes.</b> ” EX1008, 828.
 <p>“(A) <b>Stereoscopic representation of the active site region of hHyal-1 (gray ribbon) superimposed on that of bvHyal (yellow ribbon; (22)).</b> Selected amino acids are colored in the atomic color scheme: red, oxygen; blue, nitrogen; gray (hHyal-1) and yellow (bvHyal), carbon.” EX1006, 6917, FIG. 4.</p>	 <p>“(B) <b>Comparison of the 3D structures of Hyal-1 and BVHyl enzymes. ... The positions of the catalytic Glu and carbonyl positioning residues are essentially identical in the two structures (data not shown). The BVHyl does not have a C-terminal domain.</b>” EX1008, 830.</p>

156. In sum, Drs. Park and Dr. Hecht rely on Chao for substantially the same teachings that are disclosed in Stern 1) to align the five homologous human hyaluronidase enzymes encoded in the human genome and to establish that E324 is a non-conserved amino acid in PH20 and 2) for the HyalEGF-like domain, which is not pertinent to making a E324D, E324N, or E324R mutation.

**2. Zhang was considered by the Examiner.**

157. Zhang et al. “Zhang” is a paper published in 2009 entitled “Hyaluronidase Activity of Human Hyal1<sup>32</sup> Requires Active Site Acidic and Tyrosine Residues.” EX1010. Drs. Hecht and Park rely on hyaluronidase biochemical and structural disclosure in Chao that is substantially the same as the disclosure in Zhang in combination with Stern, which were both considered by the Examiner. EX1002, 513, 514.

158. Dr. Park and Dr. Hecht themselves rely upon Zhang for hyaluronidase biochemical and structural data, including for identifying “residues expected to be important in Hyal-1’s active site using the Chao Hyal structure.” EX1004, ¶¶88, 94. Like in Chao, Zhang overlaid the bee venom hyaluronidase structure in complex with HA onto the Hyal-1 structure (Figure L). EX1004, ¶94.

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<sup>32</sup> I note that Zhang uses “Hyal1” (lower case letters). Except where quoted, I will use “Hyal-1” to be consistent with the previous nomenclature used in this declaration.

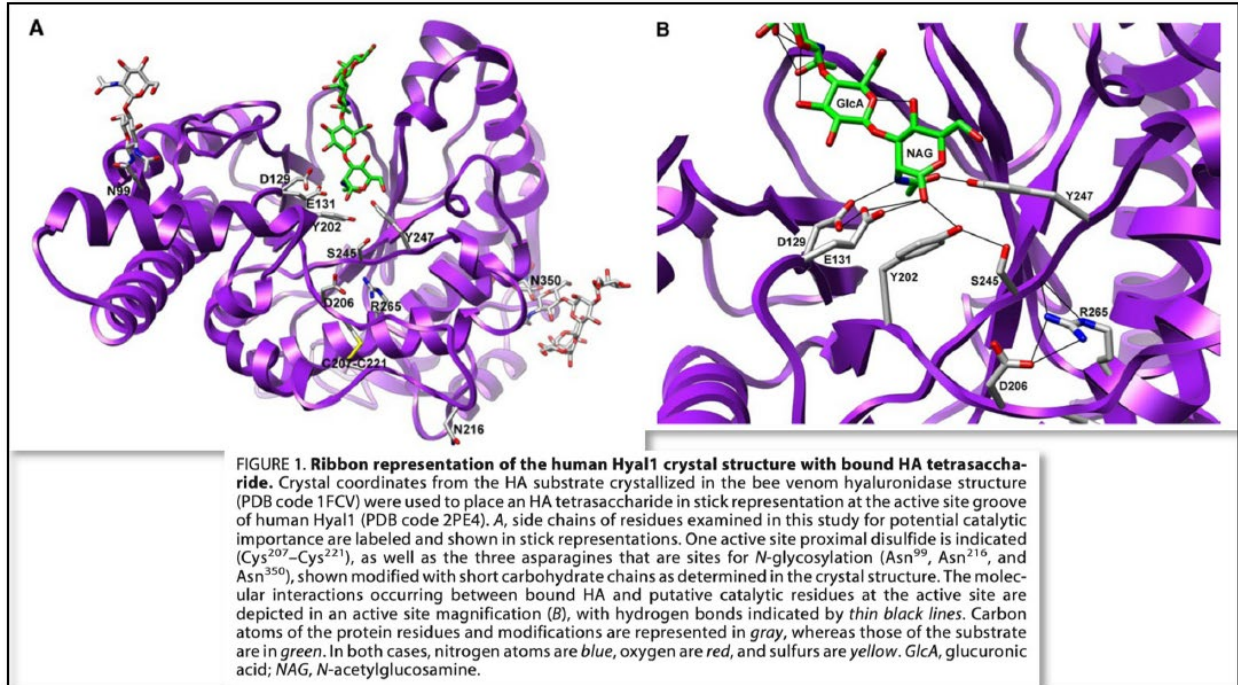


Figure E. EX1004, ¶94.

159. Zhang explicitly cites Chao's crystal structure, including the EGF-like domain, for human Hyal-1. EX1010, 9434. Zhang expressed a truncated form of the Hyal-1 protein in which residues that form the HYAL-EGF domain, which starts at position 356 of Hyal-1 and at position 374 in PH20. EX1004, ¶97. The HYAL-EGF domain is a unique domain found in mammalian hyaluronidases. The structure is characterized by a particular pattern of cysteine and glycine residues that was explained in Chao (Figure D). EX1004, ¶97.

as that of bvHyal, comprising a distorted  $(\beta/\alpha)_8$  barrel. No sequence homology has been reported in the scientific literature for the C-terminal domains of the mammalian hyaluronidases. However, this region contains a cysteine-rich pattern,  $x_4Cx_{0-48}Cx_{3-12}Cx_{1-70}Cx_{1-6}Cx_2Ga_{x_{0-21}}Gx_2C$ , where “a” denotes a hydrophobic residue, “x” denotes any residue, and the gaps between cysteine residues vary in length as indicated by the subscripts. This pattern is identified in the SMART (23) and PROSITE (24) databases as an epidermal growth factor (EGF)-like motif.

Figure F. EX1004, ¶97.

160. Further, Park admits the HyalEGF-like domain was described in Zhang. EX1004, ¶99 (acknowledging Zhang “found a mutation at Asn<sup>350</sup> in the ‘c-terminal EGF-like domain’”); EX1010, 9438 (noting residue Asn 350 “was located in the C-terminal EGF-like domain.”).

161. Drs. Park and Hecht rely on Chao for substantially the same teachings that are disclosed in Zhang—that human hyaluronidases contain an EGF-like domain. EX1004, ¶97. Accordingly, substantially the same teachings were in the art that was before the Examiner.

### **3. Arming was considered by the Examiner.**

162. Arming et al. “Arming” is a paper published in 1997 entitled “In vitro mutagenesis of PH-20 hyaluronidase from human sperm.” The Examiner considered Arming. EX1002, 504. Drs. Hecht and Park rely on disclosure in Chao that is substantially the same as the disclosure in Arming, when considered in

combination with Stern and Zhang, which were each considered by the Examiner.

EX1002, 504, 513, 514.

163. Dr. Park and Dr. Hecht rely upon Arming for hyaluronidase biochemical and structural data, including for reporting a number of conserved residues between human PH20 and bee venom hyaluronidase. EX1004, ¶¶88, 101. Specifically, Arming identified four conserved cysteine residues that form disulfide bonds. EX1004, ¶101.

164. Arming called out many of the same conserved residues that Dr. Hecht flagged in Chao. EX1011, FIG. 1; EX1003, ¶217 For example, the lines depicted as “( )” above the alignment in EX1011, FIG. 1, reproduced below as Figure E indicates conserved cysteine residues. And amino acids that are conserved between mammalian and insect venom hyaluronidases are marked with the small black square. This includes C316 and L327 that Dr. Hecht also flagged. EX1011, 811 (FIG. 1 legend, “Amino acids conserved between mammalian and insect venom hyaluronidases are marked (small black square), the four conserved cysteine residues are marked ( ).”); EX1003, ¶217.

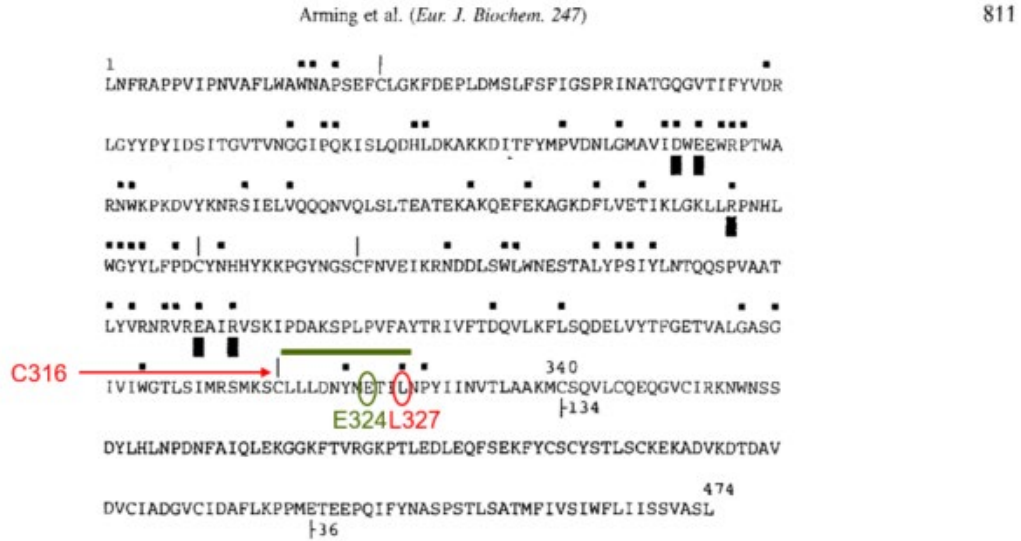


Fig. 1. Amino acid sequence of human PH-20 hyaluronidase. Amino acids conserved between mammalian and insect venom hyaluronidases are marked (■), the four conserved cysteine residues are marked (()). The five mutated amino acids are indicated by (◼). The deletions are marked (-).

Figure G EX1011, 811 (annotated with green line and arrows/circles identifying residues C316, E324, L327).

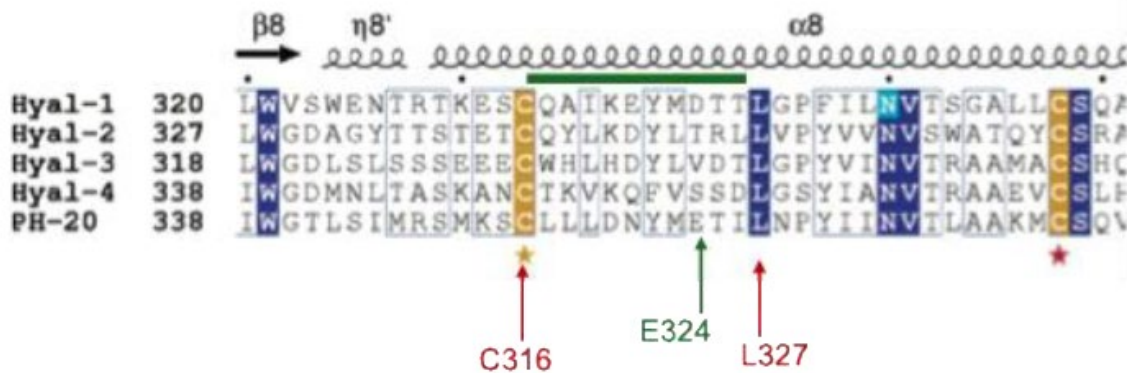


Figure H. EX1003, ¶217 (excerpted and annotated in the Hecht declaration from Chao, 6916, FIG. 3 with green line and arrows identifying residues C316, E324, L327).

165. Drs. Park and Hecht rely on Chao for substantially the same teachings that are disclosed in Arming—identifying conserved residues in the alignment of hyaluronidases (Figures N, O). EX1004, ¶101. Substantially the same propositions were in the art that was before the Examiner.

## **X. CONCLUSION**

166. In summary, from the perspective of a POSA, (i) and in accordance with the legal principles described in Section VI, a POSA would have found that claims 1-2, 6-15, and 17-30 would not have been obvious to a POSA before December 29, 2011 (the date Dr. Hecht used in his analysis); (ii) Dr. Hecht's analysis appears to have relied on hindsight, rather than any teachings or suggestions stemming from the prior art that would have motivated a POSA to make a E324D mutation (or a E324D mutation to asparagine (N), or arginine (R)); and (iii) the teachings that Drs. Park and Hecht rely on in Chao are the same or substantially the same as the teachings in Stern, Zhang, and/or Arming, which were cited during prosecution and considered by the Examiner.

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 12th day of May, 2025.

  
Barbara Triggs-Raine, Ph.D.

## APPENDIX A

Sequences used for Clustal alignment as in the Chao paper. Note that the Chao alignment was based on structure.

>NP\_149349.2 hyaluronidase-1 isoform 1 precursor [Homo sapiens]  
MAAHLLPICALFLTLDDMAQQGFRGPLLPNRPFTTVWNANTQWCLERHGVDVDSVFDVVDVANPGQTFRGPDM  
MTIFYSSQLGTYPPYPTGEPVFGGLPQNASLIAHLARTFQDILAAIPAPDFSGLAVIDWEAWRPRWAFN  
WDTKDIYRQSRALVQAQHPDWPAPQVEAVAQDQFQGAARAWMAGTLQLGRALRPRGLWGFYGFPCDYN  
DFLSPNYTGQCPSGIRAQNDQLGWLWQSRALYPSIYMPAVLEGTGKSQMYVQHRVAEAFRVAVAAGDPN  
LPVLPYVQIFDYDTTNHFLPLDELEHSLGESAAQGAAGVVLWVSWENTRTKESCQAIKEYMDTTLGPFILN  
VTSGALLCSQALCSGHGRCVRRTSHPKALLLLNPASFISIQLTPGGGPLSLRGALSLEDQAQMAVEFKCRC  
YPGWQAPWCERKSMW

>NP\_003764.3 hyaluronidase-2 precursor [Homo sapiens]  
MRAGPGPTVTLALVLAVSWAMELKPTAPPIFTGRPFVVAWDVPTQDCGPRKLVPLDLNAFDVQASPNEG  
VNQNTITIFYRDLGLYPRFDSAGRSVHGGVPPQNVSLWAHRKMLQKRVEHYIRTQESAGLAVIDWEDWRPV  
WVRNWQDKDVYRRLSRQLVASRHPDWPDRIVKQAQYEFEEFAAQFMLETLRYVKAVRPRHLWGFYLF  
CYNHDYVQNWESYTGRCPDVEVARNDQLAWLWAEALFSPVYLDLASSRHGRNFVSVFRVQEAALRVAR  
THHANHALPVYVFTTRPTYSRRLTGLSEMDLISTIGESAALGAAGVILWGDAGYTTSTETCQYLKDYLTR  
LVYVYVNVSWATQYCSRAQCHGHGRCVRRNPSASTFLHLSTNSFRLVPGHAPGEPQLRPVGGELSWADIDH  
LQTHFRCQCYLGWSGEQCQWDHRQAAGGASEAWAGSHLTSLLALAAALFTWTL

>NP\_003540.2 hyaluronidase-3 isoform 1 precursor [Homo sapiens]  
MTTQLGPALVLGVALCLGCGQPLPQVPERPFSVLWNVPSAHCEARFGVHLPLNALGI IANRGQHFHGQNM  
TIFYKNQLGLYPYFGPRGTAHNGGIPQALPLDRHLALAAAYQIHHSRLRPGFAGPAVLWEEWCPLWAGNW  
RRRAYQAASWAWAQVFPDLDPQEQLYKAYTGFEQAARALMEDTLRVAQALRPHGLWGFYHYHYPACGNGWH  
SMASNYTGRCHAATLARNTQLHHLWAASSALFPSIYLPRLPPAHHQAFVRRHLEEAFRVALVGHHRHPLP  
VLAYVRLTHRRSGRFLSQDDLVSIGVSAALGAAGVVLWGDLSLSSSEECWHLHDYLVDTLGPYVINVT  
RAAMACSHQRCHGHGRCARRDPGQMEAFHLHLPDGSGLGDWKSFSCHCYWGAGPTCQEP RPPGKEAV

>NP\_036401.2 hyaluronidase-4 [Homo sapiens]  
MKVLSEGQLKLCVVPVHLTWSLLIFFILKSISCLKPARLPIYQRKPFIAAWNAPTDQCLIKYNLRLNLK  
MFPVIGSPLAKARGQNVTFYVNRLLGYYPWYTSQGVPIINGGLPQNISLQVHLEKADQDINYYI PAEDFSG  
LAVIDWEYWRPQWARNNWNSKDVYRQKSRKLISDMGKNVSATDIEYLAKVTFEESAKAFMKETIKLGIKSR  
PKGLWGYYLYPDCHNYNVYAPNYSGSCPEDEVLRNNELSWLWNSSAALYPSIGVWKSGLDSENILRFSKF  
RVHESMRISTMTSHDYALPVFVYTRLGYRDEPLFFLSKQDLVSTIGESAALGAAGIVIWGDMLNTASKAN  
CTKVKQFVSSDLGSYIANVTRAAEVC SLHLCRNNGRCIRKMWNAPSYLHLNPASYHIEASEDEGFTVKGK  
ASDTDLAVMADTFSCHCYQGYEGADCREIKTADGCSGVSPSPGSLMTLCLLLLASYRSIQL

>NP\_694859.1 hyaluronidase PH-20 isoform 2 [Homo sapiens]  
MGVLKFKHIFFRSFKSSGVSIQIVFTFLIPCCLTNFRAPPVIPNVFPLWAWNAPSEFC LGKFDEPLDM  
SLFSFIGSPRINATGQVTFIFYVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDKAKKDITFYMPVDNL  
GMAVIDWEEWRPTWARNWPKPKDVYKNRSIELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGLLL  
RPNHLWGYLYFPDCYNHYYKPGYNGSCFNVEIKRNDL SWLWNESALYPSIYLNQQSPVAATLYVRN  
RVREAIRVSKI PDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTL SIMRSMKS  
CLLLDNYMETILNPYI INVTLAAKMCSQVLCQEQGVCIRKNWNSSDYHLHLPDNFAIQLEKGGKFTVRGK  
PTLEDLEQFSEKFCYSCYSTLSCKEKADVKD TDAVDVCIADGVCIDAF LKPPMETEEPQIFYNASPSTLS  
ATMFIVSILFLIISSVASL

CLUSTAL 2.1 multiple sequence alignment (<https://www.genome.jp/tools-bin/clustalw>, prepared on February 8, 2025)

```
HYAL2 human -----MRAGPGPTVTTLALVLAVSWAMELKPTAPPIFTGRPFVVAWVDVPTQDC
HYAL3 human -----MTTQLGPALVLGVALCLGCGQPLPQVPE-----RPFVSLWNVPSAHC
HYAL1 human -----MAAHLPLICALFLTLLDMAQGFRGPLLP---NRPFTTVWNANTQWC
HYAL4 human MKVLSSEGQLKLCVVPVHLTWSLLIFFILKSI SCLKPARLP-IYQRKPFITAAWNAPTQDC
PH20 human  MGVLKFKHIFFRSFKSSGVSQIVFTFLLI PCCLTLNFRAPPVIPNV PFLWAWNAPSEFC
          .           :           :                               **  *:. : *

HYAL2 human  GPRLKVPLDLNADFVQASPNEGFVNQNTIFFYRDRLLGLYPRFDS-AGRSVHGCVQNVSL
HYAL3 human  EARFGVHLPLNALGI IANRGQHFHGQNM TIFYKNQLGLYBYFGP-RGTAHNGGIPQALPL
HYAL1 human  LERHGVDVDVSVFDDVANPQGQTFRGPDM TIFYSSQLGTYBYTTP-TGEPVFGGLPONASL
HYAL4 human  LIKYNLRLNLKMFVIGSPLAKARGQNV TIFYVNRLLGYYPWYTS-QGVPINGGLPQNI SL
PH20 human  LGKFDEPLDMSLFSFVIGSPRINATGQGV TIFYVDRLLGYYPYIDSITGVTVNGGIPQKISL
          :   : : . . . . . . . : : * * * * . : * * * . * . * * : * * . *

HYAL2 human  WAHRKMLQKRVEHYIRTQESAGLAVI DWE DWRPVVWRNWQDKDVYRRLSRQLVASRHPDW
HYAL3 human  DRHLALAAAYQIHHSRLR-PGFAGPAVLDWE EWCP LWAGNWGRRRAYQAASWAWAQQVFPDL
HYAL1 human  IAHLARTFQDILAAIPAPDFSGLAVI DWEAWRPRWAFNWDTKDIYRQRSRALVQAQHPDW
HYAL4 human  QVHLEKADQDINYYI PAEDFSGLAVI DWEYWRPQWARNWN SKDVYRQKSRKLISDMGKNV
PH20 human  QDHLDKAKKDI TFYMPV-DNLGMAVI DWE EWRPTWARNWKPKDVYKNRSIELVQQQNVQL
          *   :   :   .   * * : * * * * * * * * * * * * * * * * * * * *

HYAL2 human  PPDRIVKQAQYEFEEFAAQFMLETLRYVKAVRPRHLWGFYLF PDCYNHDYVQNWESYTR
HYAL3 human  DPQEQLYKAYTGFEQAARALMEDTLRVAQALRPHGLWGFYHY PACGN-GWHSMASNYTR
HYAL1 human  PAPQVEAVAQDQFQGAARAWMAGTLQLGRALRPRGLWGFYGF PDCYNDFLS--PNYTCQ
HYAL4 human  SATDIEYLAKVTFEESAKAFMKETIKLGLKSRPKGLWGYLYL PDCNHNVYA--PNYSGS
PH20 human  SLTEATEKAKQEFKAGKDFLVE TIKLGLKLLRPNHLWGYLYL PDCYNHHYK--PGYNGS
          *   * : : : : : * : : * * * * * * * * * * * * * * * * *

HYAL2 human  CPDVEVARNDQLAWLWAE STALF PPSVYLDET LASSRHGRNFVSRVQEA LRVARTHANH
HYAL3 human  CHAATLARNTQLHHLWAAS S ALFPSIYLPRLPPAHH-QAFVRRHLEEA FRVALVGHHR-H
HYAL1 human  CPSGIRAQNDQLGWLWGS RALYPSIYMPAVLEGTGKSQMYVQHRVAE AFRVAVAAGD-P
HYAL4 human  CPEDEVLRNNE LSWLWNSAALYPSIGVWKS LGDSENILRF SKFRVHESMRI STMTSHDY
PH20 human  CFNVEIKRND LSWLWNE STALYPSIYLN TQQSP-VAATLYVRNRVREAIRVSKI PDAKS
          *   * : * : * * * * * * * * * * * * * * * * * * * * * * *

HYAL2 human  ALPVYVTRPTYSRR-LTGLSEMDLISTTIGESAALGAA G VILWGDAGYTTSTETCQYLKD
HYAL3 human  PLPVLAYVRLTHRRS-GRFLSQDDL VQSTIGVSAALGAA G VVLWGDLSLSSEEECWHLHD
HYAL1 human  NLPVLPYVQIFYD TT-NHFLPLDELEHSLGESAAQGA G VVLWVSWENTRTKESCQAIKE
HYAL4 human  ALPVFVYTRLGYRDEPLFFLSKQDLVSTIGESAALGAA G I VIWGD MNLTASKANCTKVQK
PH20 human  PLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGAS G I VIWGTLSIMRSMKSCLLLDN
          * * * * : : . * . : * : : * * * * * * * * * * * * * * * *

HYAL2 human  YLTRLVVPYVNVSWATQYCSRAQCHGHGRCVRRNPSASTFLHLSTNSFRLVPGHAPGEP
HYAL3 human  YLVDTLGPYVINVTRAMACSHQRCHGHGRCARRDP-----G
HYAL1 human  YMDTTLGPFILNVTSGALLCSQALCSGHGRCVRRTSHPKALLLN--PASFSIQLTPGGG
HYAL4 human  FVSSDLGSYIANVTRAAEVCSLHLCRNNGRCIRKMNWNP SYLHLN---PASYHIEASEDG
PH20 human  YMETILNPYIINVT LAAKMC SQVLCQE QV CIRKNWNSSDYLHLN---PDNFAIQLEKGG
          : : * : : * * * : : * * * * * * * * * * * * * * * * *

HYAL2 human  QLRPVGELSWADIDHLQTHFRCCQYLGWSGEQCQWDHRQAAGGASEAWAG-----
HYAL3 human  QMEAFHLWPDGSLGDWKSFSCHCYWG WAGPTCQEPRPGPKEAV-----3' end
doesn't match
HYAL1 human  PLSLRGALSLEDQAQMAVEFKCRCY PGWQAPWCERKSMW-----
HYAL4 human  EFTVKGKASD TDLAVMADTFSCHCYQGYEGADCREIKTADGC SGVSPS-----
PH20 human  KFTVRGKPTLEDLEQFSEKFCYSCYSTLS---CKEKADV KDTDAVDVC IADGVCIDAF LK
          :   .   .   * * * *

HYAL2 human  -----SHLTSLLALAALFTWTL
HYAL3 human  -----
```

```
HYAL1 human -----  
HYAL4 human -----PGSLMTLCLLLLASYRSIQL  
PH20 human PPMETEEPQIFYNASPSTLSATMFIVSILFLIISSVASL
```

Three manual adjustments were made to optimize alignment (and residues to be aligned are indicated in the alignment below which has not been optimized) and regions not in Chao alignment are in grey. Note that the human HYAL3 sequence does not match at the C-terminus. Alignments were performed at <https://www.genome.jp/tools-bin/clustalw> on February 8, 2025 with default parameters.