

Filed on behalf of: Halozyme, Inc.

By: Michael T. Rosato (mrosato@wsgr.com)
Sonja R. Gerrard (sgerrard@wsgr.com)
Jad Mills (jmills@wsgr.com)
Aubrey Haddach (ahaddach@halozyme.com)

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HALOZYME, INC.,
Petitioner,

v.

ALTEOGEN, INC.
Patent Owner.

Case No. IPR2026-00176
Patent No. 12,221,638

**PETITION FOR INTER PARTES REVIEW OF
U.S. PATENT NO. 12,221,638**

TABLE OF CONTENTS

	<u>Page</u>
I. Introduction.....	1
II. Background and Overview	4
A. The temperature shift method for recombinant protein culturing was well known at the critical date.	5
B. Culturing conditions and glycosylation were routine and conventional parameters at the critical date.	6
III. The '638 Patent.....	7
A. Prosecution History	9
B. Level of skill in the art	14
IV. The Asserted Art.....	14
A. Wei	15
B. Zmuda.....	17
C. Wei 2013	18
V. Statement of the Precise Relief Requested for Each Claim Challenged	18
VI. Claim Construction.....	18
VII. Detailed Explanation for Grounds of Unpatentability	19
A. [Ground 1] Claims 1-15 are unpatentable as obvious under 35 U.S.C. §103 over Wei and Zmuda.	21
1. Claim 1	27
2. Claim 2	40
3. Claim 3	42
4. Claim 4	42
5. Claim 5	45
6. Claim 6	45
7. Claim 7	46
8. Claim 8	47
9. Claim 9-10.....	48

10.	Claims 11-12	49
11.	Claims 13-15	50
B.	[Ground 2] Claims 4 and 7 are unpatentable as obvious under 35 U.S.C. §103 over Wei, Zmuda, and Wei 2013.	53
1.	Claim 4	54
2.	Claim 7	56
VIII.	Conclusion	57
IX.	Grounds for Standing.....	58
X.	Mandatory Notices under 37 C.F.R. §42.8.....	58
XI.	Certificate of Compliance.....	59
XII.	Payment of Fees under 37 C.F.R. §§42.15(a) and 42.103	60
XIII.	Appendix – List of Exhibits.....	61

LISTING OF CHALLENGED CLAIMS

1. **[PREAMBLE]** A method for producing a recombinant protein that comprises at least one of hyaluronidase PH20 or a variant thereof, the method comprising:

[ELEMENT 1.1] first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL, wherein first culturing is performed within a first temperature range from 35° C. to 38° C.; and

[ELEMENT 1.2] subsequently second culturing at least part of the host animal cells from first culturing for 2 to 18 days, which produces the recombinant protein, wherein second culturing is performed within a second temperature range from 28° C. to 34° C.,

[ELEMENT 1.3] wherein the second culturing maintains (a) a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L and/or (b) pH within a range from 6.8 to 7.2 in a culture medium therefor,

[ELEMENT 1.4] wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and

[ELEMENT 1.5] wherein the produced recombinant protein has a hyaluronidase enzymatic activity at 10,000 units/mL or higher.

2. The method according to claim 1, wherein galactosylation of N-glycan is 1 to 68%, and mannosylation of N-glycan is 40 to 63%.

3. The method according to claim 1, wherein sialylation of N-glycan is 1 to 30%.

4. The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.

5. The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed by one or more methods selected from the

group consisting of batch culture, repeated batch culture, fed-batch culture, repeated fed-batch culture, continuous culture, and perfusion culture.
<p>6. The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed under one or more conditions selected from the group consisting of:</p> <p>(i) a condition in which an ammonia concentration is maintained at 5 mM or higher in a culture medium thereof;</p> <p>(ii) a condition in which one or more substances is added to the culture medium thereof, wherein the one or more substances are selected from the group consisting of glutamine, glucosamine, uridine, glucosamine, and sodium butyrate; and</p> <p>(iii) a condition in which galactose and manNAc are not added to the culture medium thereof.</p>
7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.
8. The method according to claim 1, further comprising separating and purifying the produced recombinant protein.
9. The method according to claim 8, wherein separating and purifying the produced recombinant protein are performed using ionic bond and/or hydrophobic interaction characteristics thereof, rather than affinity binding.
10. The method according to claim 9, wherein separating and purifying the recombinant protein are performed using hydrophobic interaction chromatography and ion exchange chromatography, rather than affinity chromatography.
11. The method according to claim 8, wherein purifying further comprises removing at least part of acidic recombinant protein.

12. The method according to claim 11, wherein removing the acidic recombinant protein uses ion exchange chromatography.

13. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus when compared to an amino acid sequence of a wild human PH20.

14. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

15. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus and at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

Halozyme, Inc., (“Petitioner” or “Halozyme”) hereby requests *inter partes* review of United States Patent No. 12,221,638. This petition demonstrates by a preponderance of the evidence, that each of claims 1-15 of the ’638 patent is unpatentable under 35 U.S.C. §103.

I. INTRODUCTION

The challenged claims are directed to a temperature shift method for recombinant protein production in cultured animal cells. In particular, the claims recite producing hyaluronidase PH20 in animal cell culture using a first culturing temperature in the range of 35-38°C to achieve cell density, and then employing a second culturing temperature in the range of 28-34°C for 2-18 days to produce the recombinant protein. But the temperature shift method was well-known at that time and was thoroughly documented in the literature including for production of PH20. Indeed, the second culturing temperature was recognized at the critical date as a result-effective variable and the prior art disclosed a range overlapping with that claimed.

The chart below exemplifies overlap between the claimed temperature range and the prior art:

Reference	First Temperature	Second Temperature
Wei (EX1006)	37°C	less than 37°C (e.g., 35.5, 36.0, 36.5)
Zmuda (EX1005)	37°C	30 to less than 37°C (e.g., 32°C)
Xu (EX1012)	36.5°C	33 to 35°C
Becerra (EX1014)	37°C	28 to less than 37°C
Challenged Patent	35-38°C	28-34°C (e.g., 32°C)

Zmuda (EX1005), for example, teaches that shifting the temperature down (e.g., to 32°C) improved titers for “most proteins,” with the specific benefit “depending on the nature of the protein.” *See, e.g.*, EX1005, 82. Zmuda thus provides a compelling example of the prior art available at the critical date disclosing a temperature range overlapping with the claimed range, and also disclosing the temperature as a result-effective variable that was subject to routine optimization within the range.

The remaining elements of the challenged claims merely reflect conventional characteristics of PH20 obtained through existing culturing conditions available at the critical date. Wei, for example, demonstrates that prior art temperature shift protocols for PH20 production practiced the glucose and pH limitations of the claims and produced recombinant PH20 with the claimed sialylation and enzymatic activity levels. In short, the challenged patent merely followed prior art guidance to obtain exactly what would have been expected.

Zmuda was not before the Examiner during prosecution. Nor were Xu or Becerra. The Examiner thus appears to have been unaware of the overlapping prior art range, and never asserted any rejection in view of overlapping ranges.

Accordingly, the instant challenge presents art and arguments that are materially different from what was considered during prosecution, and the challenged patent issued in error because these references were not previously considered or applied.

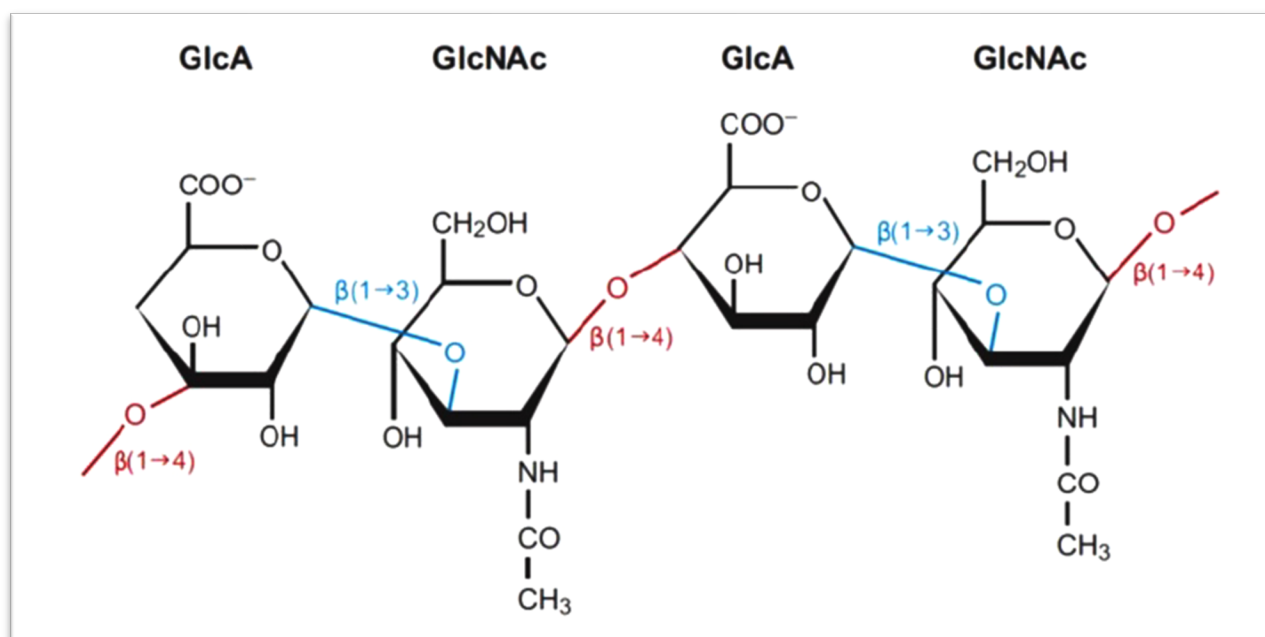
The challenged claims also issued in error because the Examiner was unaware that the prior art disclosed the claimed PH20 enzymatic activity (at or above 10,000 units/mL). The Examiner believed the enzymatic activity limitation was missing from the prior art and arose uniquely from a temperature reduction below 37°C that the Examiner did not understand was already disclosed in the prior art. As referenced above, however, Wei disclosed the enzymatic activity limitation the Examiner believed was missing from the prior art. Wei provides this express disclosure in portions of the reference ***the Examiner did not cite***. Wei also discloses that it obtained these results using a temperature shift below 37°C, a temperature range that overlaps with the claimed range. Wei thus confirms that there was no criticality to culturing in the claimed range as opposed to the temperature used successfully in Wei to achieve the same results.

Together, Zmuda and Wei powerfully demonstrate the challenged claims are obvious. Accordingly, claims 1-15 should be cancelled as unpatentable.

II. BACKGROUND AND OVERVIEW

The present case relates to methods for making an enzyme (hyaluronidase) that catalyzes the breakdown of hyaluronic acid in the body. EX1006, [0002].

Hyaluronic acid is a sugar polymer comprising repeating glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc) units. EX1029, Figure 1 (reproduced in part below). Mammalian hyaluronidases break down the β -1,4 glycosidic bond that links GlcNAc to GlcA (bond depicted in red below). EX1006, [0111]; *see also* EX1002, ¶¶21-22.



PH20 is a specific kind of hyaluronidase made in mammals and used in medical applications. EX1006, [0108]-[0109], [0255]; EX1030, 230-231. But using hyaluronidase extracts derived from animals had a tendency to induce allergic reactions. EX1030, 239 (in as much as 10% of population). Accordingly,

skilled artisans developed methods for producing and purifying recombinant human PH20 hyaluronidase (rHuPH20) from cultured animal cells (e.g., Chinese Hamster Ovary cells, abbreviated as “CHO cells”). EX1031, 432-433; EX1006, [0255]; EX1007, 1113-1114, Tables 1-4; EX1008, 1; EX1011, 8. In 2005, for example, Halozyme’s rHuPH20 product (Hylenex™) was approved by the FDA for several applications. EX1031, 435-436. The approved indications for Hylenex™ have since expanded. EX1032, 98, 100; *see also* EX1002, ¶¶23-26.

A. The temperature shift method for recombinant protein culturing was well known at the critical date.

At the critical date, skilled artisans were familiar with optimal culturing practices for high levels of recombinant protein production in CHO cells. EX1024, 942; EX1019, 57; EX1011, 8; EX1014, Table 2. In particular, it was well known to culture at physiologic temperature of 37°C for optimum cell proliferation but then to “temperature downshift” to “improve protein productivity.” EX1012, 191; EX1006, [0360], [0373]; EX1014, 2; EX1016, 11378; EX1017, 21; EX1018, 2. After the cultured cells reached desired density, the temperature was routinely reduced to a mildly hypothermic temperature of about 28-35°C, especially at 32°C, to improve protein productivity. EX1005, [0021]; EX1014, 2, Table 2; EX1006, Examples 13 & 15. These prior art culturing conditions are precisely what the challenged claims recite. *See also* EX1002, ¶¶27-37.

B. Culturing conditions and glycosylation were routine and conventional parameters at the critical date.

At the critical date, skilled artisans also knew to feed the CHO cell culture with glucose (sometimes called dextrose) using glucose feedstock of concentration up to 6.5 g/L during cell proliferation, but to maintain a lower glucose concentration (e.g., to 1-4 g/L) during the protein production culture. EX1006, [0354], [0360], [0373]; EX1005, [0041], [0233], [0236], Table 1, Figs. 5-6; EX1009, 772, Fig. 1; EX1012, 200, Fig. 1; EX1024, Fig. 3, Table II. It was similarly well known at the critical date that the optimal pH for CHO culture was in the normal physiological range of 7.0-7.2. EX1006, [0360]; EX1019, 57; EX1020, 4; EX1021, 1036; EX1022, 1052; *see also* EX1002, ¶¶38-43.

Another known parameter for recombinant protein production in CHO cells at the critical date was the type of N-linked glycosylation: high mannose, hybrid, or complex. EX1006, [0018], [0130], Fig. 2; EX1011, 12; EX1024, Fig. 1A, C. High mannose glycans, as their name implies, are made up mostly of mannose and do not contain sialic acid. Sialic acid was found only in hybrid and complex N-glycans where it is attached to galactose. EX1002, ¶¶44-47.

It was further known that N-linked glycosylation may be removed from proteins enzymatically. For example, skilled artisans appreciated that sialidase—an enzyme that cleaves sialic acid from N-glycans— accumulates in bioreactors due

to its release from dead cells. EX1024, 943-944. Accordingly, the level of sialylation on recombinant proteins was known to relate not just to the enzymatic addition of sialic acid within the producing cells but also to the build-up of sialidase outside the cells. *See also* EX1002, ¶¶48-50.

III. THE '638 PATENT

The '638 patent claims methods for producing and purifying recombinant PH20 hyaluronidase in cultured animal cells (e.g., CHO cells). EX1001, claims. It issued on February 11, 2025, based on a 2022 application, and claims earliest priority to August 2020. EX1001, cover; EX1002, ¶62.

The '638 patent reports results of what already was a prior art method of rHuPH20 production in CHO cells at the critical date. EX1001, 25:2-3 (CHO cells). In particular, the working examples of the '638 patent culture the cells at 37°C during the cell proliferation phase and then perform a routine temperature shift to 32°C during the protein production phase. EX1001, 17:41-42 (“shifted the culture temperature to 32°C”), 17:48-49 (same), 17:56-57 (same), 17:64-65 (“followed by shifting the culture temperature to 32°C”), 18:8-9 (same), 18:60-61 (“cultured at a decreased temperature of 32°C”), 19:28-30 (“temperature was shifted to 32°C”), 19:60-62 (same), 20:66-67 (same), 25:10-12 (“a lowered temperature of 32°C”). The '638 patent asserts that this temperature shift improves enzymatic activity of the produced PH20 to at least 10,000 units/mL. EX1001,

4:49-64 (improved enzymatic activity “can be remarkably improved by performing culture for a certain time period after changing the culture temperature,” which controls “the sialylation, galactosylation and/or mannosylation levels” of the N-glycans). But none of these characteristics distinguish the prior art. EX1002, ¶¶63-79.

The text of the sole independent claim is below, with elements demarcated in brackets:

1. [Preamble] A method for producing a recombinant protein that comprises at least one of hyaluronidase PH20 or a variant thereof, the method comprising:

[1.1] first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL, wherein first culturing is performed within a first temperature range from 35° C. to 38° C.; and

[1.2] subsequently second culturing at least part of the host animal cells from first culturing for 2 to 18 days, which produces the recombinant protein, wherein second culturing is performed within a second temperature range from 28° C. to 34° C.,

[1.3] wherein the second culturing maintains (a) a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L and/or (b) pH within a range from 6.8 to 7.2 in a culture medium therefor,

[1.4] wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and

[1.5] wherein the produced recombinant protein has a hyaluronidase enzymatic activity at 10,000 units/mL or higher.

A. Prosecution History

The '638 patent was filed as U.S. Application No. 17/907,538 (“the '538 application”) on September 27, 2022, claiming priority to a PCT application (PCT/KR2021/010368) filed on August 6, 2021, and a Korean application (10-2020-0099100) filed on August 7, 2020.

In the only substantive Office action, original claims 1-13 were rejected in relevant part as obvious under 35 U.S.C. §103 over EP3037529 (“Wei,” EX1006) in view of Chen and Frost (EX1031). The Examiner correctly stated that Wei taught a method for producing recombinant PH20 with 25% sialylation (element [1.4]) but overlooked disclosure of the claimed enzymatic activity limitation (element [1.5]):

EP3037529 teaches host cells expressing human-derived pH20 hyaluronidase are cultured in a medium at 37°C and pH 7.2 for 14 days; an integral cell density increases with the culture period; 25% of sialic acid is contained; and an enzymatic activity is 1600 units/mL (see entire publication and abstract especially paragraphs [0322], [0327], [0328] and [0358] and Tables 10, 11, 12 and 28). The teachings of the reference differ from the claims in that the reference does not teach the recited method steps of lowering the culture temperature to 28-34°C and the activity in a culture solution of the enzyme, is 10,000 unit/mL or more.

EX1004, 47. Patent Owner did not challenge the Examiner's finding that Wei discloses recombinant PH20 hyaluronidase with a 25% sialylation level (satisfying element **[1.4]**). Nor did Patent Owner bring Wei's disclosure of element **[1.5]** to the Examiner's attention. *See* VII.A.1.vi below.

Patent Owner instead argued that Wei does not disclose decreasing the culture temperature limitation of element **[1.2]**, or the residual glucose concentration/pH maintenance range alternatives of element **[1.3]**:

EP529 fails to disclose or provide basis for deriving the step of “(2) decreasing the culture temperature to 28°C to 34°C and then culturing the host cells for 2 to 18 days” and **EP529** fails to disclose or provide basis for deriving the limitations (a) and (b) specified in amended claim **1**, viz., “(a) wherein the residual glucose concentration in the medium is maintained at 0.001 g/L to 4.5 g/L during the culture period” and “(b) wherein the pH of the culture medium is maintained at 6.8-7.2” in such step (2).

EX1004, 38-39.¹

As discussed in detail herein, however, reduction of the culture temperature to 34°C or below was routine in the art at the critical date. *See, e.g.*, Section II.A

¹ By its plain language, element [1.4] can be satisfied *either* via glucose concentration *or* pH.

above. For example, Zmuda (EX1005), which is relied on herein in Ground 1, exemplifies the temperature shift method for protein production in CHO cells at 32°C and discloses an overlapping range between 30°C and less than 37°C for recombinant protein production. *See, e.g.*, VII.A.1.iii below. Patent Owner did not submit any of Zmuda (EX1005), Xu (EX1012), or Becerra (EX1014), or identify any prior art disclosure of the temperature shift method or overlapping range. Accordingly, the Examiner did not have the benefit of any of these references during prosecution and never made any rejection based on prior art disclosure of the temperature shift in an overlapping range with what is claimed. *But see In re Peterson*, 315 F.3d 1325, 1329 (Fed. Cir. 2003) (“A prima facie case of obviousness typically exists when the ranges of a claimed composition overlap the ranges disclosed in the prior art.”). The present art and arguments are thus materially distinct from those considered and applied during prosecution, and the claims issued in error for want of these salient references.

Compounding this error, the Examiner identified as the reason for allowance a supposed failure of the prior art to disclose hyaluronidase enzymatic activity of at least 10,000 units/mL in combination with the claimed sialylation percentage:

The prior art neither teaches nor suggests the claimed method for producing a recombinant protein wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and wherein the produced

recombinant protein has hyaluronidase enzymatic activity at 10,000 units/mL or higher.

EX1004, 8. But the Examiner was incorrect because Wei expressly teaches—in portions of the reference not cited by the Examiner—the produced recombinant protein has hyaluronidase enzymatic activity at 10,000 units/mL or higher.

Wei's disclosure of the claimed enzymatic activity is found at least in two examples it provides of producing recombinant hyaluronidase—e.g., Examples 13 & 15. Both Examples describe growing cells at 37°C then decreasing the culture temperature to 35.5°C and maintaining the culture at that temperature for 3 days. EX1006, [0360], [0373]. Example 15 produces crude soluble rHuPH20 with an enzymatic activity of 17,000 U/mL, satisfying element **[1.5]**. EX1006, [0373]. Example 13 produces soluble rHuPH20 with an enzymatic activity exceeding 10,000 U/mL, likewise satisfying element **[1.5]**. EX1006, [0362], Table 20 (reporting enzymatic activity of four independent preparations of 24,493; 26,683; 18,321; and 21,052 U/mL); *see also id.*, Tables 21 & 23, [0361]-[0367]. Neither the Examiner nor the Patent Owner ever cited any of this disclosure. The Examiner thus did not account for the PH20 enzymatic activity in Examples 13 and 15, each of which provide the disclosure of element **[1.5]** the Examiner erroneously believed was missing. This again demonstrates that the challenged claims issued in error.

If the Examiner had adopted Patent Owner’s argument regarding the recited pH and glucose requirements of element **[1.3]** (the Examiner did not), that too would have been error. As an initial matter, the claims require satisfaction of either the pH *or* glucose requirement—not both. As for pH, the claim merely recites physiological pH. Of course, physiologic pH was conventionally used for cell culture. Indeed, the scientific literature available at the critical date is replete with discussion that cells are typically cultured at physiological pH. EX1002, ¶¶42-43. For example, Sellick discloses that the “optimum pH was in the region of 7.0 to 7.2” for protein production in CHO cells. EX1019, 57. This was confirmed throughout the literature. EX1006, [0360] (“Parameters were temperature setpoint, 37°C; pH: 7.2”); EX1012, 200 (“the pH was controlled at pH 7.1”); EX1020, 4 (“Typical pH set points for CHO cells are neutral to slightly alkaline (7.0–7.2).”); EX1021, 1036 (“At reduced pH levels (6.90 and 6.80) the cultivation time could be prolonged to 9 and 12 days.”); *see also* Section II.B above. Indeed, the ’638 patent itself describes a pH range of 7.2±0.4—which overlaps with the claimed range — as “conventional.” EX1001, 21:5-11, Table 2; *see also Qualcomm Inc. v. Apple Inc.*, 24 F.4th 1367, 1376 (Fed. Cir. 2022) (“[A] patentee’s admissions about the scope and content of the prior art provide a factual foundation as to what a skilled artisan would have known at the time of invention....”). Accordingly, Patent Owner’s prosecution arguments cannot save its deficient claims.

B. Level of skill in the art

A person of ordinary skill in art (POSA) as of August 7, 2020, would typically have an M.S. or Ph.D. in cell biology, biochemistry or related life sciences discipline, or could have less education but significant professional experience in one or more of these fields. EX1002, ¶¶80-81.

This petition is supported by the Declaration of Dr. Michael Butler, the Principal Investigator in Cell Technology at NIBRT in Ireland and a Distinguished Professor Emeritus in the Department of Microbiology at the University of Manitoba, Canada. EX1002, ¶¶1-9; EX1003. Dr. Butler is well qualified to opine from the perspective of a POSA in this case. *Id.* As Dr. Butler explains, and as addressed in further detail below, the challenged claims would not have been considered non-obvious to a POSA at the relevant time.

IV. THE ASSERTED ART

In one sense, the claims are rendered unpatentable by one simple modification to Wei (EX1006). That is, simply extending Wei's temperature shift from the exemplified 35.5°C to 34°C or below. This simple modification alone is sufficient to meet all aspects of the challenged claim. Moreover, this simple modification is well supported in the prior art literature available at the critical date. But the obviousness of the challenged claims is more comprehensive than even that.

The challenged patent simply takes what Wei did, and applies it in a commercially available ExpiCHO cell line, essentially following the manufacturer's protocols that were also prior art. Zmuda (EX1005), for example, applies a temperature shift method for cell culture protein production directly to the ExpiCHO cell line. *See also* EX1036 (describing protocols for ExpiCHO cell line). As explained in further detail below, Zmuda specifically discloses the temperature range of 30°C to less than 37°C, and exemplifies operation specifically at 32°C, squarely within the claimed range. Zmuda, filed years after Wei, powerfully corroborates that the claimed temperature range is merely a routine subset of the temperature range disclosed in the prior art at the critical date.

A. Wei

European Patent 3037529 to Ge Wei et al. (“Wei,” EX1006) discloses making rHuPH20. EX1002, ¶51; EX1006, Title. It issued on March 27, 2019, based on a December 9, 2009, application, and was first published on June 26, 2016. EX1006, cover. It is prior art to the challenged claims at least under 35 U.S.C. §102(a)(1)-(2).

Wei describes temperature shift culturing methods for production of recombinant PH20 hyaluronidase (“rHuPH20”). Among other recombinant PH20 variants, Wei describes production and purification of “rHuPH20”—also known as SEQ ID NO:122—which is a recombinant human PH20 protein. The rHuPH20

protein differs from the 509 amino acid human PH20 protein in that it has 447 amino acids and lacks the 35 amino acid signal peptide at the N-terminus and 27 amino acids at the C-terminus of the molecule. EX1006, [0029], [0205], [0321] (“rHuPH20 (SEQ ID NO:122)”), [0386], Tables 29-30, pp. 292-293 (SEQ ID NO:122); *see also* EX1031, 430 (“509 amino acid human PH20 sequence”), 432 (describing rHuPH0 as “a 447 amino acid 61 kDa glycoprotein with a properly processed amino terminus and 6 N-linked glycosylation sites.”); EX1002, ¶¶52.

Wei provides Examples 13 and 15 which are methods for producing rHuPH20 by initially culturing the cells at 37°C and then shifting the temperature below 37°C (e.g., to 35.5°C) and maintaining the culture at that lower temperature for 3 days for protein production while controlling both the solution pH and the glucose concentration. EX1006, [0360], [0373]; EX1002, ¶53.

In Example 6, Wei describes the N-linked glycosylation characteristics of the rHuPH20 (produced according to Example 15) and disclosed that the sialylation of the N-glycan of the produced recombinant protein is 25%. EX1006, [0324], [0322]. Wei discloses producing crude soluble rHuPH20 with an enzymatic activity of 17,000 U/mL and purified soluble rHuPH20 with an enzymatic activity of ~18,000-26,000 U/mL. EX1006, [0373] (crude), [0362] (purified), Table 20 (24,493, 26,683, 18,321, 21,052 U/mL), Tables 21 & 23, [0361]-[367] (describing concentration and purification); EX1002, ¶¶54-55.

B. Zmuda

International Patent Publication WO2017/011598 (“Zmuda,” EX1005) provides a “System and Method for Improved Transient Protein Expression in CHO Cells.” EX1002, ¶56; EX1005, Title. It published on January 19, 2017. EX1005, cover. It is prior art to the challenged claims at least under 35 U.S.C. §102(a)(1).

Zmuda describes an improved CHO cell line—referred to as “ExpiCHO”—and explains that the line is capable of generating “gram per liter or greater levels of protein expression.” EX1005, [0012], [0058], [0241]. Zmuda discloses improving the production of recombinant proteins in CHO cells by performing a temperature shift for protein production. In particular, Zmuda discloses culturing CHO cells at the physiologic temperature of 37°C for cell proliferation and decreasing the temperature to sub-physiologic temperatures “less than 37°C and greater than 30°C, less than 35°C and greater than 31°C, or at a temperature of about 32°C” to improve yields of recombinant protein. EX1005, [0021]; *see also id.*, Fig. 5, p. 82. Zmuda exemplifies the temperature shift to 32°C for improved protein yield in both its “high titer” and “max titer” protocols. EX1005, 82; *see also* EX1036, 3-4; EX1002, ¶¶57-59.

C. Wei 2013

International Patent Publication WO2013/102144 (“Wei 2013,” EX1037) provides “PH20 Polypeptide Variants, Formulations and Uses Thereof.” EX1002, ¶60; EX1037, Title. It published on July 4, 2013. EX1037, cover. It is prior art to the challenged claims at least under 35 U.S.C. §102(a)(1)-(2). Wei 2013 discloses recombinant PH20 hyaluronidase variants that have “increased stability and/or increased activity.” EX1037, Abstract; EX1002, ¶¶60-61.

V. STATEMENT OF THE PRECISE RELIEF REQUESTED FOR EACH CLAIM CHALLENGED

Petitioner requests review of claims 1-15 of the ’638 patent under 35 U.S.C. §311 and AIA §6. The grounds for relief are as follows:

Ground	Claims	Description
1	1-15	Obvious under 35 U.S.C. §103 over Wei and Zmuda.
2	4, 7	Obvious under 35 U.S.C. §103 over Wei, Zmuda, and Wei 2013

VI. CLAIM CONSTRUCTION

Claims are construed to the extent necessary to find the claims unpatentable in view of the prior art. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). In mapping the prior art to the claims, Petitioner has applied the plain “meaning that [a] term would have to a person of

ordinary skill in the art in question at the time of the invention.” *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc). EX1002, ¶82.

VII. DETAILED EXPLANATION FOR GROUNDS OF UNPATENTABILITY

“[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.” *Janssen Pharms., Inc. v. Teva Pharms. USA, Inc.*, Slip Op. 16 (Fed. Cir. July 8, 2025) (quoting *E.I. DuPont de Nemours & Co. v. Synvina C.V.*, 904 F.3d 996, 1006–08 (Fed. Cir. 2018)). It generally is thus sufficient to establish *prima facie* obviousness and generate a presumption of obviousness where, as here, the challenged claim requires a feature in a numerical amount, and a prior art reference teaches that feature in amounts that overlap with the claimed numerical amount. *Id.* at 12.

Indeed, “[t]he normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages.” *In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003); *id.* at 1329 (“A *prima facie* case of obviousness typically exists when the ranges of a claimed composition overlap the ranges disclosed in the prior art.”); *id.* at 1329 (numerical amounts can be “close enough such that one skilled in the art would have expected to them to have the same properties”); *see also Ormco Corp. v. Align Technology, Inc.*, 463 F.3d

1299, 1310–11 (Fed. Cir. 2006); *Galderma Laboratories, L.P. v. Tolmar, Inc.*, 737 F.3d 731, 736–38 (Fed. Cir. 2013); *Allergan, Inc. v. Sandoz Inc.*, 796 F.3d 1293, 1304–05 (Fed. Cir. 2015); *Valeant Pharmaceuticals International, Inc. v. Mylan Pharmaceuticals Inc.*, 955 F.3d 25, 31–33 (Fed. Cir. 2020); *Almirall, LLC v. Amneal Pharmaceuticals LLC*, 28 F.4th 265, 272–73 (Fed. Cir. 2022); *Pfizer Inc. v. Sanofi Pasteur Inc.*, 94 F.4th 1341, 1347–48 (Fed. Cir. 2024). Accordingly, overlapping ranges or numerical disclosure close enough to warrant an inference of having similar properties establish a motivation to combine or modify with a reasonable expectation of success sufficient to presume obviousness. *Janssen*, Slip Op. 16.

This presumption applies “even where there is no strict overlap with a teaching of a single piece of prior art, if the ranges are ‘close enough that one skilled in the art would have expected them to have the same properties,’” and when a plurality of prior art references together teach a range. *Janssen*, Slip Op. 13-14 (quoting *Peterson*, 315 F.3d at 1329); *Iron Grip Barbell Co., Inc. v. USA Sports, Inc.*, 392 F.3d 1317, 1322 (Fed. Cir. 2004) (range from plurality of references affirmatively suggested for combination). Accordingly, each of the challenged claims is obvious over the prior art discussed herein, as explained with particularity below.

A. [Ground 1] Claims 1-15 are unpatentable as obvious under 35 U.S.C. §103 over Wei and Zmuda.

The obviousness of the challenged claims, as well as rationale to arrive at what is claimed with reasonable expectation of success, is addressed further below and throughout the petition. *See, e.g.*, Introduction (chart exemplifying overlapping temperature range), Section II (discussing prior art disclosing conventional temperature shift and culturing methodologies), Section III (level of skill in the art), Section IV (summarizing combined teachings of the cited prior art), Section VII above (summarizing overlapping range and routine optimization case law).

As discussed in detail below, the combination of Wei and Zmuda renders each of claims 1-15 obvious. Wei specifically employs a temperature shift to enhance production of rHuPH20 in CHO cells, achieving enzymatic activity greater than 10,000 U/mL with 25% sialylation. *See also* Section IV.A. above. For example, Wei's examples 13 and 15 each produced rHuPH20 by first culturing the CHO cells at 37°C and then shifting to a lower second temperature for enhanced enzyme production. EX1006, [0360], [0373]. Indeed, Wei exemplifies culturing the cells for 3 days at the lower 35.5°C temperature prior to harvesting. EX1006, [0360], [0373]. Wei teaches maintaining the pH at 7.2. EX1006, [0360], [0373], & Table 27. Additionally, Wei maintains glucose levels below 4.5 g/mL subsequent to the temperature shift. EX1006, [0360], [0373], Table 27. Wei discloses

sialylation of N-glycan of the rHuPH20 is 25%. EX1006, [0324]. In at least one example, the enzymatic activity of the crude rHuPH20 was 17,000 U/mL. EX1006, [0373]. In at least one further example, the enzymatic activity of the purified rHuPH20 ranged from ~18,000-26,000 U/mL. EX1006, [0362] (purified), Table 20 (24,493, 26,683, 18,321, 21,052 U/mL), Tables 21 & 23, [0361]-[0367] (describing concentration and purification). Accordingly, the rHuPH20 disclosed in Wei satisfies the protein and enzymatic characteristics recited in the challenged claims. *See also* EX1002, ¶¶83-96.

As confirmed by Dr. Butler, and the guidance in the literature, a POSA would have well understood at the relevant time period that there was no criticality associated with the claimed temperature range. Indeed, Wei discloses a temperature shift to the range below 37°C to optimize PH20 production, and specifically exemplifies a shift to 35.5°C. Moreover, a POSA would have understood at the critical date that the favorable results obtained in Wei were likely to continue or even improve at a temperature slightly lower than that exemplified (e.g., 30-34°C). *See also* EX1002, ¶¶30-37.

Indeed, such a temperature shift was a matter of routine optimization and was conventionally practiced in the art at the critical date. EX1005, [0021], [00245], Figs. 5, 6, 8; EX1014, 2, Table 2; EX1012, 191; EX1022, 1055; *see also* Section II.A above. Prior to the critical date, references explain that the

temperature shift was understood to operate by balancing out the longevity and productivity of the cells after cell density had been achieved. EX1012, 191 (“In response to sub-physiological temperatures, the cell doubling time increases and the death rate is reduced with cells arrested in the G1 phase of the cell cycle. ...”); EX1014, 4 (“The apoptosis delay in CHO cell cultures cultivated under mild hypothermia conditions is a phenomenon that has been reported by several authors...”). At the lower temperature, proliferation was not as rapid (but was unneeded because cell density had already been achieved), but the cells lived longer, thereby optimizing protein production. A POSA thus had good reason at the critical date to combine the teachings of Wei with references teaching temperature shifts in CHO cells, such as Zmuda. EX1002, ¶¶92-95.

Zmuda describes an optimized CHO cell line (ExpiCHO) to produce high titers of recombinant glycoproteins, and specifically applies a temperature shift after cell proliferation to a lower temperature (as low as 30°C) for enhanced protein production. EX1005, [0013], [0021], [00245], Figs. 5, 6, 8; *see also* Section IV.B above. Zmuda specifically teaches lowering the culture temperature within a range higher than 30°C but lower than 37°C. EX1005, [0021] (“then further cultured at a temperature of less than 37°C and greater than 30°C, less than 35°C and greater than 31°C, or at a temperature of about 32°C.”). Zmuda teaches that decreasing the temperature from 37°C (e.g., preferably to 32°C) may yield 2-3

times greater recombinant protein titers than cultures grown at a constant 37°C.

EX1005, 82. A POSA thus reasonably would have expected at the critical date that the desirable enzyme production obtained in Wei at 35.5°C would continue using a temperature slightly lower than what is exemplified (e.g., decreasing to 34°C or below), including in the overlapping range of 30-34°C expressly disclosed in the prior art Zmuda reference. EX1002, ¶¶56-59, 91-93.

A POSA further would have understood at the critical date that there was no criticality in the temperature range claimed, and no patentable distinction between what is claimed and the prior art overlapping range of less than 37°C (e.g., including the prior art range of 30-34°C specifically encompassed by the challenged claims). Indeed, it would have been routine at the critical date for a POSA to apply Zmuda's overlapping range temperature shift (e.g., including 30-34°C, which overlaps with the claimed range) in combination with Wei. A POSA thereby reasonably would have expected at the critical date to produce rHuPH20 as recited in the challenged claims. This reasonable expectation of success was further supported at the critical date by additional literature describing optimization of the lower second culturing temperature to improve yields. EX1012, 191 ("Since temperature downshifts have been reported to improve protein productivity by many different CHO cell lines, it is widely used as an effective platform strategy for cell culture process development and manufacturing in the biopharmaceutical

industry.”); EX1014, 2 (“... a decrease of a few degrees in temperature from 37°C, the usual culture temperature, until 28°C, ..., allows an incremental increase in the specific productivity of r-proteins in CHO cell culture (Table 2).”), Table 2 (collecting examples of mild hypothermia); EX1022, 1055 (“shifts to lower incubation temperatures are commonly used in industry to increase culture duration and titer”). A POSA at the critical date thus would have presumptively been motivated with a reasonable expectation of success to arrive at what is claimed in view of overlapping prior art disclosure—with the obviousness of the claimed subject matter at the time further corroborated by disclosure and guidance in the scientific literature. EX1002, ¶¶90-95.

Moreover, the recitation of conventional culturing parameters does not elevate the obvious subject matter claimed to a condition of patentability. The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results. *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 416 (2007); *see also* Section II.B above. For example, it was routine and conventional at the critical date to maintain a lower glucose concentration (e.g., to 1-4 g/L) during the protein production phase of culturing. EX1009, 772, Fig. 1; EX1024, Fig. 3, Table II; EX1012, 200, Fig. 1; EX1006, [0354], [0360], [0373]; EX1005, [0041], [0233], [0236], Table 1, Figs. 5-6. It was similarly well known at the critical date that the optimal pH for CHO culture was

in the normal physiological range of 7.0-7.2. EX1019, 57; EX1006, [0360]; EX1020, 4; EX1021, 1036; EX1022, 1052.

Moreover, no detrimental change in sialylation would have been expected at the critical date from the temperature shift. EX1027, 196 (“These results suggest that the commonly used temperature shift strategy to enhance productivity is generally a safe practice from a protein glycosylation perspective...”); EX1028, Figure 6A (showing only minor differences in sialylation in cultures grown at 30°C as opposed to 37°C); EX1014, 2 (“With regard to the quality of the r-protein, it seems that in most cases, there are no significant changes in the glycosylation profiles, including the degree of terminal sialylation.”); EX1020, 8 (“no decrease in Epo-Fc sialylation is anticipated at low cultivation temperature (30°C)”). That expected result is precisely what the challenged patent reported. EX1001, 17:24-25 (“Cell line clone test culture #1 is a 2 L batch culture performed at 37°C.”), 17:36-42 (“Cell line test culture #1 was obtained by culturing at a culture temperature of 37°C ... then shifted the culture temperature to 32°C.”), Table 1. In view of the foregoing, a POSA at the critical date had good reason to combine Wei and Zmuda with a reasonable expectation of successfully obtaining thereby precisely what is recited by the challenged claims. *See also* Section II; EX1002, ¶¶95-96.

1. Claim 1

i. Preamble

A method for producing a recombinant protein that comprises at least one of hyaluronidase PH20 or a variant thereof, the method comprising

To the extent the preamble is limiting, Wei and Zmuda teach the preamble of claim 1. For example, Wei discloses methods for producing recombinant hyaluronidase PH20 proteins in CHO cells. EX1006, [0197] (describing CHO cell lines for expressing mammalian proteins), [0186] (use of expression hosts for production of PH20 polypeptides). In particular, Wei identifies rHuPH20, a species of hyaluronidase PH20 (EX1006, [0026]), and provides working examples using CHO cells to produce rHuPH20. EX1006, Examples 11, 13-15; *see also id.*, [0012], [0013], [0025]-[0034], [0072], [0123]-[0130], [0135]-[0157]. Accordingly, Wei discloses the preamble. *See also* EX1002, ¶¶97-101;

Zmuda also discloses a method for producing recombinant protein. For example, Zmuda describes an improved CHO cell line—referred to as “ExpiCHO”—and explains that the line is capable of generating “gram per liter or greater levels of protein expression.” EX1005, [0012], [0058], [0241]. EX1002, ¶59. Zmuda discloses improving the production of recombinant proteins in CHO cells by performing a temperature shift for the protein production phase after the cell proliferation stage. EX1005, [0021]; *see also id.*, Fig. 5, p. 82; To the extent

Zmuda does not mention hyaluronidase PH20, a POSA at the time would have understood that ExpiCHO cells were useful for recombinant hyaluronidase PH20 production. *See also* EX1002, ¶102. Accordingly, in view of Zmuda and Wei, the preamble of claim 1 would have been obvious. *See also* EX1002, ¶103.

ii. Element [1.1].

first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL, wherein first culturing is performed within a first temperature range from 35°C to 38°C; and

The cell density recited in the claims (Element [1.1]) is a conventional density a skilled artisan would expect to employ when culturing CHO cells for protein expression. Accordingly, this element is disclosed in the prior art and also is obvious. EX1002, ¶¶104-122.

And, in fact, the density limitation is taught by Wei. For example, Wei teaches or suggests first culturing host animal cells expressing the recombinant protein at 37°C (within a first temperature range of 35°C to 38°C). Wei employs 3D35M and 2B2 CHO cells to produce rHuPH20 (Examples 11 & 14), using the Gen1 and Gen2 methods, respectively. EX1006, Examples 13 & 15. In each case, Wei cultures the CHO cells at a first temperature of 37°C through day six. EX1006, [0360], [0373]. Accordingly, Wei discloses the first culturing temperature within the claimed range. EX1002, ¶¶106-109.

A POSA also would have understood Wei to teach that the first culturing continued until an integral viable cell density (IVCD) of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL. As Dr. Butler explains, IVCD is typically measured by the area under the growth curve. EX1002, ¶1110; EX1034, 887. Wei discloses cell density and growth rates from which the IVCD is calculated to satisfy the claimed range beginning at day 6. EX1006, Table 19 (0.33 and 0.46×10^6 cells/mL at T_0 , doubling during growth phase every 23.5-29.8 h, maximum cell density 5.65 - 9.70×10^6 cells/mL; HUA0420C: 0.46×10^6 cells/mL at T_0 , doubling every 23.5 h, maximum cell density of 9.70×10^6 cells/mL). Dr. Butler explains that these calculations indicate IVCD of 28.62×10^6 on day 6, rising to 73.51×10^6 on day 13, all of which fall within the claimed range of 20×10^6 to 120×10^6 cells $\times$ day/mL. Dr. Butler's IVCD calculations are consistent with documentary evidence describing the viable cell density over time of rHuPH20 produced using the same Gen2 method in the same CHO cells. EX1035, Example 7, Table 24. Accordingly, Wei teaches culturing cells at a first temperature (37°C) until an IVCD in a range of 20×10^6 to 120×10^6 cells $\times$ day/mL is achieved, thereby satisfying element [1.1]. EX1002, ¶¶1110-122.

iii. Element [1.2]

subsequently second culturing at least part of the host animal cells from first culturing for 2 to 18 days, which produces the

recombinant protein, wherein second culturing is performed within a second temperature range from 28°C to 34°C

As an initial matter, there was nothing critical about the recited 2-to-18-day range for the second culturing temperature limitation, as this was routine and conventional in the art at the critical date. See Section II.A above. As discussed herein, both Wei and Zmuda disclose temperature shift protocols that render the challenged claims obvious. Indeed, Wei and Zmuda both teach ranges overlapping with Element [1.2]. Accordingly, this element is disclosed in the prior art and also is obvious. EX1002, ¶¶123-130.

For example, Wei teaches subsequently culturing at least part of the host animal cells from the first culturing for 2 to 18 days, which produces the recombinant protein, wherein the second culturing is performed within a second temperature range lower than the 37°C first culturing temperature. EX1006, [0360], [0373]. The second temperature range of lower than 37°C is an overlapping range with the recited range of 28°C-34°C. *Peterson*, 315 F.3d at 1325, 1330. Wei exemplifies the temperature shift method for producing rHuPH20 in which the temperature of the culture is reduced from 37°C to a second culturing temperature below 37°C. Specifically, Wei discloses decreasing the temperature in a stepwise fashion to 35.5°C and cultures the cells for an additional 3 days. EX1006, [0360], [0373]. Wei's teaching to use a second culturing temperature

lower than 37°C, e.g., for an additional 3 days, provides an overlapping range with the recited temperature reduction and renders the claimed range obvious. EX1002, ¶124.

Zmuda also expressly discloses a second culturing temperature range that overlaps with the claimed range, rendering this limitation obvious. For example, Zmuda discloses the second culturing temperature range “of less than 37°C and greater than 30°C, less than 35°C and greater than 31°C, or at a temperature of about 32°C.” EX1005, [0021]. Zmuda exemplifies shifting from 37°C to a culturing temperature of 32°C in its “high titer” and “max titer” protocols.

EX1005, 82, 88. Like Wei, Zmuda discloses culturing at the lower temperature for 2 to 18 days. EX1005, 81-82, 88. Both Wei and Zmuda thus provide good reason to optimize recombinant protein yield through routine operation of the lower second culturing temperature within known ranges at the critical date. EX1005, [0021], [00245], Figs. 5, 6, 8; EX1014, 2, Table 2; EX1012, 191; EX1022, 1055; *see also* Section II; EX1002, ¶¶125-129. Wei and Zmuda thus teach or suggest, and at a minimum render obvious, Element [1.2]. EX1002, ¶130.

iv. Element [1.3]

wherein the second culturing maintains (a) a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L and/or (b) pH within a range from 6.8 to 7.2 in a culture medium therefor,

As an initial matter, there was nothing critical about the recited glucose or pH ranges, all of which were routine and conventional in the art at the critical date. *See* Section II.B. above. Additionally, Wei teaches Element **[1.3]** under each individually-satisfactory alternative. EX1002, ¶¶131-144. Accordingly, this element is disclosed in the prior art and also is obvious.

Wei teaches the first alternative of element **[1.3]**, specifically that the second culturing maintains a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L. For example, Wei teaches maintaining the residual glucose concentration during the second culturing by feeding the cells with a supplement that comprises glucose on the day of the temperature shift. EX1006, [0360], Example 13. Based on the starting glucose concentration of the CHO media (4.5-6.5 g/L glucose), the total glucose concentration of Feed #3, and the volume of Feed #3, Dr. Butler explains that the glucose concentration after each feed is about 2.1 g/L. In Example 15, the same calculations indicate the glucose concentration after each feed is about 1.4 g/L. EX1006, [0373]. Each of these glucose concentrations falls within the claimed range, and a POSA at the critical date would have expected the glucose concentration of the media to be maintained thereby within the claimed range (not falling below 0.001 g/L and not rising above 4.5 g/L. EX1012, Fig. 1B; *see also* EX1002, ¶¶133-139.

Even if Patent Owner were to argue (incorrectly) Wei does not expressly teach maintaining the residual glucose concentration in a range from 0.001 to 4.5 g/L, it was obvious to do so. As Dr. Butler explains, a key objective of feeding the cells was to maintain nutrients within an optimal range. EX1002, ¶140. It was conventional at the critical date to maintain residual glucose concentration in a range from 1.0 to 4.0 g/L through sequential feeding of the culture. EX1012, Fig. 1B (exemplifying maintaining the glucose concentration in a range of 1-4 g/L), 200 (“Additional glucose was supplied to maintain the concentration range”); EX1009, Abstract (“where 4.4 mM represents a common target concentration in the biopharmaceutical industry for controlled fed-batch cultures”), 772 (“...Other researchers have focused directly on maintaining low glucose concentrations (~5 mM²) via controlled fed-batch conditions...”); EX1006, [0354], [0360], [0373]; *see also* Section II.B. Accordingly, as Dr. Butler explains, a POSA would have found it obvious at the critical date to maintain the concentration of the essential nutrient (glucose) within the overlapping range of concentrations known to promote viability of the cultured cells (e.g., 1.0 to 4.0 g/L). EX1002, ¶140.

² 4.4 mM and 5 mM glucose is about 0.8 g/L and 0.9 g/L, respectively.

EX1002, ¶140n.1.

Wei also teaches the second alternative of element **[1.3]**, specifically that second culturing maintains the pH of the culture medium within a range of 6.8 to 7.2. For example, Wei teaches culturing cells in a bioreactor at a pH set to 7.2 and further teaches sampling daily for “pH verification.” EX1006, [0360], [0373]. In addition, Wei teaches equipping bioreactors with instrumentation that allows for monitoring and control of pH. EX1006, Table 27 (“Equipped with instrumentation that monitors and controls pH”). This disclosure falls within the pH range of the claim. EX1002, ¶¶141.

Moreover, the recited pH range was conventional and routine in the art at the critical date. EX1019, 57 (“The optimum pH was in the region of 7.0 to 7.2”); EX1012, 11 (“the pH was controlled at pH 7.1”); EX1020, 4 (“Typical pH set points for CHO cells are neutral to slightly alkaline (7.0–7.2).”); EX1021, 1036 (“At reduced pH levels (6.90 and 6.80) the cultivation time could be prolonged to 9 and 12 days.”). The ’638 patent admits as much. EX1001, 21:9-11 (“the conventional pH 7.2 ± 0.4 ”), Table 2. Wei’s disclosure of the claimed pH is thus consistent with the literature, including the disclosure of Zmuda to use “buffering agents suitable for use in cell culture applications,” such as MOPS, HEPES, and phosphate which are optimal for buffering at physiological pH levels. EX1005, [0147]; *see also* EX1002, ¶¶142-143.

In view of the forgoing, Wei teaches or suggests Element [1.3]. EX1002, ¶144.

v. Element [1.4]

wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and

As an initial matter, there was nothing critical about recombinant PH20 comprising N-glycan sialylation of 1-38%, as this was routine and conventional in the art at the critical date. See Section II.B above. Additionally, Wei teaches element [1.4]. EX1002, ¶¶145-149. Accordingly, this element is disclosed in the prior art and also is obvious.

For example, Wei discloses performing a “glycan analysis study” on the rHuPH20 made according to Example 15, and reports 25% sialylation of the N-glycans. EX1006, [0322] (“Briefly, rHuPH20 (as produced in Example 15C)...”), [0324] (“About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.”). This falls squarely within the claimed range. As Dr. Butler explains, multiple prior art references evaluated the correspondence between temperature and sialylation, demonstrating that small differences in temperature do not meaningfully alter the amount of sialylation. EX1002, ¶148; EX1020, 8 (“Product sialylation did not change between cultivation temperatures of 39–35°C,

but decreased by 20% at 33°C and 40% at 30°C. However, Trummer et al. (2006b) later showed that reduced sialylation at cell culture temperature of 33°C and 30°C was prevented when used in combination with slightly acidic pH levels (pH set point 6.90).”); EX1027, 196 (“Reducing the culture temperature from 37 C to 33 C in a CHO cell line expressing human granulocyte macrophage colony stimulating factor saw little change in protein glycosylation and terminal sialylation, but did enhance specific productivity [43].”); EX1028, Figure 6A; EX1014, 2 (“With regard to the quality of the r-protein, it seems that in most cases, there are no significant changes in the glycosylation profiles, including the degree of terminal sialylation.”); *see also* Section II above; EX1002, ¶¶147-148.

Accordingly, viewing the prior art here, a POSA would understand and expect the small difference in temperature for the second culturing (35.5°C vs. 34°C) would not take Wei’s sialylation outside the claimed range. EX1002, ¶¶148-149. Dr. Butler confirms that this is corroborated by the Patent Owner’s own experimental data, which reported sialylation levels at different temperatures and shows that culturing temperature has no material impact on sialylation levels. EX1002, ¶149; EX1001, 17:24-25 17:36-42, Table 1. Wei thus teaches element [1.4]. EX1002, ¶150.

vi. Element [1.5]

wherein the produced recombinant protein has a hyaluronidase enzymatic activity at 10,000 units/mL or higher

Wei teaches Element [1.5]. For example, Wei discloses the Gen2 method produces crude rHuPH20 with “a final productivity of 17,000 Units per ml.” EX1006, [0373]. As Dr. Butler explains, a POSA would not have expected a modest reduction in culture temperature, such as 35.5°C to 34°C, to result in a final productivity of hyaluronidase enzymatic activity below 10,000 units per mL for multiple reasons. First, the Gen2 method produces hyaluronidase enzymatic activity in vast excess of what is claimed and such a modest reduction in temperature would not be expected to dramatically alter productivity. *See also* EX1002, ¶¶151-158.

Furthermore, to the extent a modest reduction in temperature does alter productivity, Dr. Butler explains that a POSA would have expected it to be improved. For example, POSAs appreciated that lower culture temperatures tend to correlate with increased productivity of the cells and improvements to the quality of the produced recombinant protein. EX1012, 191 (“improve fermentation and cell culture performance for a prolonged period with better overall productivity and quality”); EX1014, 2 (“the temperature is decreased to increase r-protein productivity and extend culture viability”); *see also* Section II. In particular,

Zmuda explains that dropping the temperature from 37°C to 32°C improved recombinant protein yields 2-3 fold. EX1005, [0021], [00245] (“The Max Titer protocol generated approximately 3-fold higher yields than the Standard protocol, while the High Titer protocol generated approximately 2-fold higher yields.”), Figs. 5, 6, 8; *see also* EX1002, ¶153.

Dr. Butler further explains that increasing cell productivity routinely results in increased protein yield. Moreover, temperature reduction also tends to improve protein quality resulting in increased levels of active protein due to, for instance, reducing the amount of damaged protein (e.g., denatured or aggregated forms). *E.g.*, EX1012, 191 (discussing improved quality attributes including reductions to “protein aggregation” at lower culture temperature). Such improvements to cellular productivity and quality would have been expected to increase the final productivity of the culture. *See also* EX1002, ¶154.

In addition, while a POSA would have expected increases to the productivity of the culture (e.g., yield, quality), the claims are not limited to just crude hyaluronidase enzymatic activity production levels. The claims encompass hyaluronidase enzymatic activity levels obtained following concentration or purification of the recombinant hyaluronidase such as described by Wei. Wei specifically teaches that hyaluronidase enzymatic activity may be increased using concentration and purification. Wei discloses that the Gen1 method produces “a

final productivity of 1600 Units per ml.” EX1006, [0354]. However, Wei further discloses concentrating and purifying the rHuPH20 produced with the Gen1 method such that the hyaluronidase enzymatic activity levels exceed 10,000 units/mL. Wei describes concentrating the rHuPH20 produced using MWCO (molecular weight cut off) filters. EX1006, [0361], Table 19 (reproduced in part below with highlighting). The concentration step increases the harvest hyaluronidase enzymatic activity levels approximately 10-fold. *See also* EX1002, ¶155.

Table 19. Monitoring data for cell culture, harvest, concentration and buffer exchange steps.				
Parameter	HUA0406C	HUA04010C	HUA0415C	HUA0420C
Time from thaw to inoculate 100 L bioreactor (days)	21	19	17	18
100 L inoculation density ($\times 10^6$ cells/mL)	0.45	0.33	0.44	0.46
Doubling time in logarithmic growth (hr)	29.8	27.3	29.2	23.5
Max. cell density ($\times 10^6$ cells/mL)	5.65	8.70	6.07	9.70
Harvest viability (%)	41	48	41	41
Harvest titer (U/ml)	1964	1670	991	1319
Time in 100-L bioreactor (days)	13	13	12	13
Clarified harvest volume (mL)	81800	93300	91800	89100
Clarified harvest enzyme assay (U/mL)	2385	1768	1039	1425
Concentrate enzyme assay (U/mL)	22954	17091	8561	17785

Wei further describes partially purifying the concentrated rHuPH20 on a Q Sepharose column. EX1006, [0362], Table 20 (reproduced below with highlighting). This column chromatography step further increased the

hyaluronidase enzymatic activity levels such that all lots exceed the claimed 10,000 units/mL. *See also* EX1002, ¶156.

Table 20. Q sepharose column data				
Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Load volume (mL)	10647	13524	12852	20418
Load Volume/Resin Volume ratio	3.1	4.9	4.5	7.3
Column Volume (mL)	2770	3840	2850	2880
Eluate volume (mL)	6108	5923	5759	6284
Protein Conc. of Eluate (mg/mL)	2.8	3.05	2.80	2.86
Eluate Enzyme Assay (U/mL)	24493	26683	18321	21052
Enzyme Yield (%)	65	107	87	76

Accordingly, a skilled artisan would have expected a modest reduction in culture temperature (e.g., 35.5°C to 34°C) to produce rHuPH20 with a final productivity in excess of 10,000 units/mL. Such a skilled artisan would have also expected, based on Wei's teachings, to produce concentrated or purified rHuPH20 in excess of 10,000 units/mL. *See also* EX1002, ¶157.

In view of the forgoing, claim 1 as a whole is obvious over Wei and Zmuda. EX1002, ¶¶158-159.

2. Claim 2

The method according to claim 1, wherein galactosylation of N-glycan is 1 to 68%, and mannosylation of N-glycan is 40 to 63%.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda. Wei also teaches the additional limitation of claim 2. EX1002, ¶¶160-167. Wei

discloses the N-linked glycosylation of its rHuPH20, including galactosylated and mannosylated N-glycans in amounts falling within the ranges recited in the claim.

For example, Wei states:

rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results show that N254 is approximately 75% occupied, N393 is approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, ***rHuPH20 contains about 45% high mannose glycans***, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.

EX1006, [0324]. Wei's disclosure of 45% high mannose glycans satisfies the broad claimed range of 40 to 63% mannosylation. Moreover, as Dr. Butler explains, the percentage of galactosylated N-glycans is implicitly disclosed because none of the high mannose N-glycans contain galactose—setting a ceiling of 55% for galactosylated N-glycans, and sialic acid is added only to N-glycans with galactose—setting a floor of 25%. EX1002, ¶163. Wei thus discloses the galactosylated N-glycans is between 25 and 55%, which is well within the claimed range and would remain therein even if the second culturing temperature were 32-

34°C rather than 35.5°C. EX1020, 8; EX1027, 196; EX1028, Figure 6A; EX1014, 2; *see also* Section II above; EX1001, 17:24-25 17:36-42, Table 1 (showing that culturing temperature does not materially affect N-glycosylation). Accordingly claim 2 is obvious over Wei and Zmuda. EX1002, ¶¶160-167.

3. Claim 3

The method according to claim 1, wherein sialylation of N-glycan is 1 to 30%.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda. Wei also teaches the additional limitation of claim 3, because the 25% sialylation disclosed in Wei satisfies the range recited in claim 3. EX1002, ¶¶168-170; *see also* VII.A.1.v above. Accordingly, claim 3 is obvious over Wei and Zmuda.

4. Claim 4

The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda. Wei also teaches the additional limitation of claim 4. EX1002, ¶¶171-175. For example, Wei specifically discloses the rHuPH20 has enzymatic activity at least 10% higher than wild human PH20:

The human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is increased or decreased compared to the wild-type GPI-anchored form of PH20. For example, the human

esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, **10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form.** In some examples, human esPH20 polypeptides exhibit increased hyaluronidase activity compared to the wildtype GPI-anchored form. The hyaluronidase activity of human esPH20 polypeptides can be increased by 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more compared to the hyaluronidase activity of the wildtype GPI-anchored form.

EX1006, [0128].

While Wei describes hyaluronidase enzymatic activity (e.g., units/mL), a person of ordinary skill in the art would have appreciated that these variants would also have increased specific enzymatic activity (e.g., units/mg) relative to wild human PH20. *See, e.g.*, EX1006, [0353] (“Specific activity (Units/mg) was calculated by dividing the hyaluronidase activity (U/ml) by the protein concentration (mg/mL).”). This is because a more enzymatically active variant will have more units of activity on a milligram-by-milligram basis. That a more active PH20 variant will have both higher activity and specific activity is supported by the ’638 patent:

As described above, in the hyaluronidase PH20 or variant thereof produced by the production method according to the present invention, the specific activity of the enzyme can be increased by 10% or more compared to that produced by a general method, in particular, compared to the specific activity of wild-type human PH20. This increase in enzymatic activity is due to changes in N-glycosylation properties and/or charge modification of the hyaluronidase PH20 or variant thereof produced by the method according to the present invention.

EX1001, 10:66-11:8; *see also* EX1002, ¶¶171-173.

Furthermore, a POSA would have known at the critical date that specific activity is a function of purity of the recombinant protein. *See, e.g.*, EX1006, [0071] (“A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.”); *see also* EX1002, ¶174.

Purified wild-type human PH20 has higher specific activity than a corresponding unpurified wild-type human PH20. This is because impurities increase the weight (mg) but do not increase hyaluronidase activity. EX1006, [0071] (“As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived,”). Accordingly, a POSA would have known at the critical date that a

preparation of purified wild-type recombinant PH20 would have had at least 10% higher specific activity than the corresponding unpurified wild-type recombinant PH20. Accordingly, claim 4 is obvious over Wei and Zmuda. EX1002, ¶¶174-175.

5. Claim 5

The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed by one or more methods selected from the group consisting of batch culture, repeated batch culture, fed-batch culture, repeated fed-batch culture, continuous culture, and perfusion culture.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda. Wei also teaches the additional limitation of claim 5. EX1002, ¶¶176-179. For example, Wei's Gen1 and Gen2 culturing methods are fed-batch cultures. EX1006, [0360] ("The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Nutrient feeds were added during the run."), [0373] (same); EX1011, 8-10 (discussing routine nature of batch, fed-batch, continuous and perfusion culture modes); *see also* Section II above. Accordingly, claim 5 is obvious over Wei and Zmuda. EX1002, ¶179.

6. Claim 6

The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed under one or more conditions selected from the group consisting of:

(i) a condition in which an ammonia concentration is maintained at 5 mM or higher in a culture medium thereof;

(ii) a condition in which one or more substances is added to the culture medium thereof, wherein the one or more substances

are selected from the group consisting of glutamine, glucosamine, uridine, glucosamine, and sodium butyrate; and

(iii) a condition in which galactose and manNAc are not added to the culture medium thereof.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda.

Wei also teaches the additional limitation of claim 6. EX1002, ¶¶180-184.

Beginning with additional claim element 6(ii), Wei discloses feed medium supplemented with sodium butyrate, satisfying this element. EX1006, [0360], [0373]. Addressing claim element 6(iii), Wei discloses the media components used for culturing in the Gen1 and Gen2 methods do not include galactose or manNAc, as was typical as of the critical date. *See, e.g., id.*, Examples 13 & 15. Accordingly, claim 6 is obvious over Wei and Zmuda. EX1002, ¶184.

7. Claim 7

7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda.

Wei also teaches the additional limitation of claim 7. EX1002, ¶¶185-189. For example, Wei teaches producing soluble recombinant human PH20 hyaluronidase (“rHuPH20”) that differs from wild-type by one or more amino acid substitution. EX1006, [0023] (“polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence

identity to the precursor polypeptides set forth in SEQ ID NO:107 and 109, or the mature forms thereof”), [0026] & [0127] (similar); *see also id.*, [0023] (“PH20 includes those of any origin including, but not limited to, human, ...”), [0029], [0024] (“any form of PH20 that is soluble under physiologic conditions”); [0018], Fig. 1 (SEQ ID NO: 107 is full-length wild-type human PH20). Accordingly, claim 7 is obvious over Wei and Zmuda. EX100, ¶189.

8. Claim 8

8. The method according to claim 1, further comprising separating and purifying the produced recombinant protein.

As discussed above in VII.A.1, claim 1 is obvious over Wei and Zmuda. Wei also teaches the additional limitation of claim 8. EX1002, ¶¶190-193. For example, Wei teaches separating and purifying the produced recombinant protein from the culture media. EX1006, [0355] (“Conditioned media from the 3D35M clone was clarified by depth filtration and tangential flow diafiltration.”), [0361], Table 27 (“Filtration of bioreactor cell culture” & “Concentration and buffer exchange prior to chromatography”). Wei specifically teaches purifying the produced recombinant protein using SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange. EX1006, [0202]. Accordingly, claim 8 is obvious over Wei and Zmuda. EX1002, ¶193.

9. Claim 9-10

9. The method according to claim 8, wherein separating and purifying the produced recombinant protein are performed using ionic bond and/or hydrophobic interaction characteristics thereof, rather than affinity binding.

10. The method according to claim 9, wherein separating and purifying the recombinant protein are performed using hydrophobic interaction chromatography and ion exchange chromatography, rather than affinity chromatography.

As discussed above in Section VII.A.8, claim 8 is obvious over Wei and Zmuda. Wei also teaches the additional limitation of claims 9 and 10. EX1002, ¶¶194-198. For example, Wei teaches the produced recombinant protein may be purified using ionic bond chromatography.

Proteins, such as soluble PH20 polypeptides, including esPH20 polypeptides, or other C-terminal truncated PH20 polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange.

EX1006, [0202]; Wei further teaches the use of hydrophobic bond chromatography. EX1006, [0363] (“Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed.”), [0377] (same); *see also* EX1002, ¶¶195-196.

While Wei discloses purification of rHuPH20 using a combination of ion exchange, hydrophobic, and affinity chromatography, claims 9 and 10 do not require any particular purification level for the produced recombinant protein. Thus, to the extent either claim is construed to exclude use of “affinity chromatography,” it would have been obvious to perform only the ion exchange and reverse-phase chromatography steps of Wei’s method. EX1002, ¶197. Accordingly, each of claims 9 and 10 is obvious over Wei and Zmuda. EX1002, ¶198.

10. Claims 11-12

11. The method according to claim 8, wherein purifying further comprises removing at least part of acidic recombinant protein.

12. The method according to claim 11, wherein removing the acidic recombinant protein uses ion exchange chromatography.

As discussed above in Section VII.A.8, claim 8 is obvious over Wei and Zmuda. Wei also teaches the additional limitations of claims 11 and 12. EX1002, ¶¶199-204. For example, Wei teaches separating and purifying the produced recombinant protein from the culture media. EX1006, [0355] (“Conditioned media from the 3D35M clone was clarified by depth filtration and tangential flow diafiltration.”), [0361], Table 27 (“Filtration of bioreactor cell culture” & “Concentration and buffer exchange prior to chromatography”). Wei teaches purifying the produced recombinant protein using ionic exchange chromatography,

including anion exchange. EX1006, [0202]. Wei further teaches using a Q Sepharose ion exchange column to purify rHuPH20. EX1006, [0355] (“Soluble rHuPH20 was then purified by sequential chromatography on Q Sepharose (Pharmacia) ion exchange, ...”). As Dr. Butler explains, Q Sepharose is a positively charged ion exchange column that binds negatively charged moieties such as acidic groups (e.g., sialic acid). EX1002, ¶203. A POSA would have understood that column chromatography, such as Q Sepharose, does not offer quantitative recovery of the desired protein. *E.g.*, EX1006, Table 20 (reporting yields from Q Sepharose column as low as 65%). Accordingly, a POSA would have expected that at least a portion of the acidic recombinant protein will not bind the Q Sepharose column and thus will be removed by the Q Sepharose column, as disclosed by Wei. Accordingly, each of claims 11 and 12 is obvious over Wei and Zmuda. EX1002, ¶204.

11. Claims 13-15

13. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus when compared to an amino acid sequence of a wild human PH20.

14. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

15. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid

residue of N-terminus and at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

As discussed above in Section VII.A.7, claim 7 is obvious over Wei and Zmuda.

The '638 patent does not disclose the sequence of wild human PH20 but refers to a Korean PCT application as providing the sequence. EX1001, 16:65-67 (“The sequences of wild-type human PH20 and HM46 are disclosed in International Patent Publication No. 2020/022791.”); *see also* EX1013 (WO2020/022791 which is a publication of PCT/KR2019/009215). The sequence of wild human PH20 is also disclosed in a published U.S. patent application—US2021/0155913 (“the '913 application”)—claiming priority to the PCT application. EX1015 (US2021/0155913 claiming priority to PCT/KR2019/009215). According to the '913 application, wild-type human PH20 is identified as SEQ ID NO: 1. EX1015, Abstract (“wild-type PH20 having the amino acid sequence of SEQ ID NO: 1”), pp. 36-37 (SEQ ID NO: 1). The wild-type human PH20 of SEQ ID NO: 1 has 509 amino acids. EX1015, pp. 36-37; *see also* EX1002, ¶¶206-207.

Wei teaches the additional limitations of claims 13-15. EX1002, ¶¶205-213. For example, Wei describes production and purification of “rHuPH20”—also known as SEQ ID NO:122—which is a recombinant human PH20 protein. The

rHuPH20 protein differs from the 509 amino acid wild human PH20 protein in that it has 447 amino acids and lacks the 35 amino acid signal peptide at the N-terminus and 27 amino acids at the C-terminus of the molecule. EX1006, [0029], [0205], [0321] (“rHuPH20 (SEQ ID NO:122)”), [0386], Tables 29-30, pp. 292-293 (SEQ ID NO:122); *see also* EX1031, 430 (“509 amino acid human PH20 sequence”), 432 (describing rHuPH0 as “a 447 amino acid 61 kDa glycoprotein with a properly processed amino terminus and 6 N-linked glycosylation sites.”). Accordingly, because rHuPH20 has both an N- and C-terminal truncation of at least one amino acid, it satisfies the requirements of claims 13-15. EX1002, ¶208.

Wei further discloses soluble human PH20 hyaluronidases including variants that comprise truncations at the C-terminus relative to the wild-type human PH20, satisfying claim 14:

The PH20 polypeptides provided herein are soluble proteins that are truncated at the C-terminus and include those that lack all of the GPI-anchor attachment signal sequence (e.g. are truncated at amino acid positions 450 to 490).

EX1006, [0006]; *see also id.*, [0023], [0025], [0026], [0041], [0108], [0109], [0119]-[0128]; EX1002, ¶¶209-211.

Wei similarly discloses the soluble human PH20 hyaluronidases include variants that comprise truncations at the C-terminus and N-terminus relative to the

wild-type human PH20, satisfying claims 13-15. For example, Wei describes soluble human PH20 hyaluronidases (i.e., those that lack the C-terminus) that also lack the N-terminal signal peptide and thus begin at amino acid 36. EX1006, [0009], [0012], [0013], [0026], [0030], [0124]-[0127]; *see also id.*, [0171] (explaining that the PH20 signal peptide sequence may be replaced with a heterologous sequence). Accordingly, each one of claims 13-15 is obvious over Wei and Zmuda. EX1002, ¶¶212-213.

B. [Ground 2] Claims 4 and 7 are unpatentable as obvious under 35 U.S.C. §103 over Wei, Zmuda, and Wei 2013.

Ground 2 builds upon and incorporates by reference the disclosures of Zmuda (EX1005) and Wei (EX1006) discussed in Ground 1 above (Section VII.A), their application to claim 1 and its dependent claims, which render these claims obvious. *See, e.g.*, Sections VII.A.1, VII.A.4, and VII.A.7 above. To the extent additional prior art support is needed to render claim 4 and/or claim 7 obvious, the obviousness of these claims is further demonstrated by Wei 2013, which teaches recombinant PH20 hyaluronidase variants with “increased stability and/or increased activity.” EX1037, Abstract; EX1037, 13:22-25 (“modified PH20 polypeptides that contain at least one amino acid replacement in a PH20 polypeptide...exhibits increased hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement”), 265:4-22 (“536 mutants

exhibits 120% or greater than 120% of the hyaluronidase activity of wildtype PH20 and about 75 of the mutants exhibit 300% or greater than 300%[] of the hyaluronidase activity of wildtype PH20”), Table 9 (data). A skilled artisan at the critical date had good reason with reasonable expectation of success to combine one or more of these variants, including those with specific enzymatic activity 10% higher than wild-type hyaluronidase, with the disclosures of Wei and Zmuda discussed above, at least because Wei explains that its disclosed methods apply to making PH20 variants and Wei 2013 discloses the same method as Wei for production of “Gen2 soluble rHuPH20.” EX1037, 228:17-229:19; EX1006, [0008]-[0010], [0023], [0027], [0029], [0065]-[0067], [0121], [0124], [0126], [0135]-[0137], SEQ ID NO: 187, 202-205, 208, 189, 206-209, 190; *see also* EX1002, ¶¶214-221.

1. Claim 4

The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.

As discussed above in Sections VII.A.1 and VII.A.4, claims 1 and 4 are obvious over Wei and Zmuda. As discussed above in Section VII.A.4, Wei teaches the additional limitation of claim 4. *See, e.g.*, EX1002, ¶¶222-227; EX1006, [0128] (“the human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, **10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%**,

110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form.”),

[0353] (specific enzymatic activity conversion).

Relying on the methodology disclosed in Wei, Wei 2013 teaches recombinant PH20 hyaluronidase having a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20. *See, e.g.*, EX1037, 13:22-25 (“modified PH20 polypeptides that contain at least one amino acid replacement in a PH20 polypeptide...exhibits increased hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement”), 265:4-22 (“536 mutants exhibits 120% or greater than 120% of the hyaluronidase activity of wildtype PH20 and about 75 of the mutants exhibit 300% or greater than 300%[] of the hyaluronidase activity of wildtype PH20”), Table 9 (data); A skilled artisan would have understood at the critical date that Wei 2013’s disclosure that the PH20 variants exhibited 120-300% or more hyaluronidase activity as compared to wildtype disclosed a specific enzymatic activity as hyaluronidase at least 10% higher than wild human PH20, including after purification. EX1006, [0353] (specific activity conversion), [0071] (purification enhances specific activity); EX1037, 74:2-8 (same); EX1001, 10:66-11:8 (acknowledging higher specific activity naturally flows from higher activity).

Accordingly, claim 4 is obvious over Wei, Zmuda, and Wei 2013. EX1002, ¶¶222-227.

2. Claim 7

7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.

As discussed above in Sections VII.A.1 and VII.A.7, claims 1 and 7 are obvious over Wei and Zmuda. As discussed above in Section VII.A.7, Wei teaches the additional limitation of claim 7. *See, e.g.*, EX1002, ¶¶228-233; EX1006, [0023] (“polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NO:107 and 109, or the mature forms thereof”), [0026] & [0127] (similar); *see also id.*, [0023] (“PH20 includes those of any origin including, but not limited to, human, ...”), [0029], [0024] (“any form of PH20 that is soluble under physiologic conditions”); [0018], Fig. 1 (SEQ ID NO: 107 is full-length wild-type human PH20); EX1006, [0355], [0336], & Table 16 (producing PH20 hyaluronidases that include substitutions at positions 82, 166, 235, 254, 368, and 393).

Wei 2013 also teaches the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20. *See, e.g.*, EX1037, 13:22-25 (“modified PH20 polypeptides that

contain at least one amino acid replacement in a PH20 polypeptide...exhibits increased hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement”), Table 3 (substituted PH20 exhibiting hyaluronidase activity). Accordingly, claim 7 is obvious over Wei, Zmuda, and Wei 2013. EX1002, ¶¶228-233.

VIII. CONCLUSION

For the reasons set forth above, each of claims 1-15 of the '638 patent is unpatentable. Petitioner therefore requests that *inter partes* review of these claims be instituted.

Respectfully submitted,

Dated: December 10, 2025

/ Michael T. Rosato/
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

IX. GROUNDS FOR STANDING

Petitioner certifies that, under 37 C.F.R. §42.104(a), the '638 patent is available for *inter partes* review, and Petitioner is not barred or estopped from requesting *inter partes* review of the '638 patent on the grounds identified.

X. MANDATORY NOTICES UNDER 37 C.F.R. §42.8

Real Party-in-Interest (37 C.F.R. §42.8(b)(1)): Halozyme, Inc. is the real party-in-interest.

Related Matters (37 C.F.R. §42.8(b)(2)):

Lead and Back-Up Counsel (37 C.F.R. §42.8(b)(3))

Lead Counsel: Michael T. Rosato (Reg. No. 52,182)

Back-Up Counsel: Sonja R. Gerrard (Reg. No. 72,802); Jad Mills (Reg. No. 63,344); Aubrey Haddach (Reg. No. 48,374)

Service Information – 37 C.F.R. §42.8(b)(4). Petitioner hereby consents to electronic service.

Email: mrosato@wsgr.com; sgerrard@wsgr.com; jmills@wsgr.com; ahaddach@halozyme.com

Post: Wilson Sonsini Goodrich & Rosati, 701 Fifth Avenue, Suite 5100,
Seattle, WA 98104-7036

Tel.: 206-883-2529

Fax: 206-883-2699

XI. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Petition complies with the type-volume limitation of 37 C.F.R. §42.24(a). The word count application of the word processing program used to prepare this Petition indicates that the Petition contains 12,019 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Dated: December 10, 2025

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

XII. PAYMENT OF FEES UNDER 37 C.F.R. §§42.15(A) AND 42.103

The required fees are submitted herewith. If any additional fees are due at any time during this proceeding, the Office is authorized to charge such fees to Deposit Account No. 23-2415.

XIII. APPENDIX – LIST OF EXHIBITS

Exhibit No.	Description
1001	U.S. Patent No. 12,221,638 to Park (“the ’638 patent”)
1002	Declaration of Michael Butler, Ph.D.
1003	Curriculum Vitae of Michael Butler, Ph.D.
1004	Excerpted prosecution history of U.S. Appl. No. 17/907,538
1005	International Patent Publication WO2017/011598 to Jonathan Zmuda and Chao Yan Liu is entitled “System and Method for Improved Transient Protein Expression in CHO Cells”
1006	EP3037529 to Ge Wei, Krishnasamy Paneerselvam, Gregory I. Frost, and Louis Bookbinder is entitled “Extended Soluble PH20 Polypeptides and Uses Thereof” (“Wei”)
1007	“Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives” to Jennifer Dumont, Don Euwart, Baisong Mei, Scott Estes, and Rashmi Kshirsagar (“Dumont”)
1008	“Recombinant Proteins for Industrial versus Pharmaceutical Purposes: A Review of Process and Pricing” to John Puetz and Florian Wurm (“Puetz”)
1009	Gowtham YK, Saski CA, Harcum SW. “Low glucose concentrations within typical industrial operating conditions have minimal effect on the transcriptome of recombinant CHO cells.” Biotechnol Prog. 2017 May;33(3):771-785. (“Gowtham”)
1010	Intentionally left blank
1011	“Mammalian cell culture for production of recombinant proteins: A review of the critical steps in their biomanufacturing” to Róisín O’Flaherty, Adam Bergin, Evangelia Flampouri, Letícia Martins Mota, Ismael Obaidi, Andrew Quigley, Yongjing Xie, Michael Butler (“O’Flaherty”)
1012	“Systematic development of temperature shift strategies for Chinese hamster ovary cells based on short duration cultures and kinetic modeling” to Jianlin Xu, Peifeng Tang, Andrew Yongky, Barry Drew,

	Michael C. Borys, Shijie Liu & Zheng Jian Li (“Xu”)
1013	International Patent Publication No. WO2020/022791 to Park
1014	“Exploring the effect of mild hypothermia on CHO cell productivity” to Silvana Becerra, Julio Berrios, Nelson Osses, Claudia Altamirano (“Becerra”)
1015	U.S. Patent Application Publication US20210155913A1 to Park
1016	“Miniaturized Integrated Platform for Electrical and Optical Monitoring of Cell Cultures” to Carmen Moldovan, Rodica Iosub, Cecilia Codreanu, Bogdan Firtat, Daniel Necula, Costin Brasoveanu and Ion Stan (“Moldovan”)
1017	Invitrogen CELL CULTURE BASICS Handbook
1018	Gibco CD CHO Medium User Guide
1019	“Optimizing CHO Cell Culture Conditions” to Christopher A. Sellick, Arfa R. Maqsood, Alexandra S. Croxford, Alan J. Dickson, Royston Goodacre, and Gill M. Stephens (“Sellick”)
1020	“Biphasic cultivation strategy to avoid Epo-Fc aggregation and optimize protein expression” to Christian Kaisermayer, David Reinhart, Andreas Gili, Martina Chang, Per-Mikael Aberg, Andreas Castan, Renate Kunert (“Kaisermayer”)
1021	“Process Parameter Shifting: Part I. Effect of DOT, pH, and Temperature on the Performance of Epo-Fc Expressing CHO Cells Cultivated in Controlled Batch Bioreactors” to Evelyn Trummer, Katharina Fauland, Silke Seidinger, Kornelia Schriebl, Christine Lattenmayer, Renate Kunert, Karola Vorauer-Uhl, Robert Weik, Nicole Borth, Hermann Katinger, Dethardt Muller (“Trummer”)
1022	“Effects of Culture Conditions on N-Glycolylneuraminic Acid (Neu5Gc) Content of a Recombinant Fusion Protein Produced in CHO Cells” to Michael C. Borys, Nimish G. Dalal, Nicholas R. Abu-Absi, Sarwat F. Khattak, Ying Jing, Zizhuo Xing, Zheng Jian Li (“Borys”)
1023	“Production platforms for biotherapeutic glycoproteins. Occurrence, impact, and challenges of non-human sialylation” to Darius Ghaderi, Mai Zhang, Nancy Hurtado-Ziola & Ajit Varki (“Ghaderi”)
1024	“Optimal and consistent protein glycosylation in mammalian cell culture” to Patrick Hossler, Sarwat F Khattak, and Zheng Jian Li (“Hossler 2009”)

1025	“NIST Interlaboratory Study on Glycosylation Analysis of Monoclonal Antibodies: Comparison of Results from Diverse Analytical Methods” to Maria Lorna A. De Leoz, David L. Duewer, Adam Fung, Lily Liu, Hoi Kei Yau, Oscar Potter, Gregory O. Staples, Kenichiro Furuki, Ruth Frenkel, Yunli Hu, Zoran Susic, Peiqing Zhang, Friedrich Altmann, Clemens Grunwald-Grube, Chun Shao, Joseph Zaia, et al. (“De Leoz”)
1026	Intentionally left blank
1027	“Protein Glycosylation Control in Mammalian Cell Culture: Past Precedents and Contemporary Prospects” to Patrick Hossler (“Hossler 2012”)
1028	“The Combined Effect of Sodium Butyrate and Low Culture Temperature on the Production, Sialylation, and Biological Activity of an Antibody Produced in CHO Cells” to Fei Chen, Tianci Kou, Li Fan, Yan Zhou, Zhaoyang Ye, Liang Zhao, and Wen-Song Tan (“Chen”)
1029	“Hyaluronidase: An overview of its properties, applications, and side effects” to Hyunwook Jung (“Jung”)
1030	“A recombinant human enzyme for enhanced interstitial transport of therapeutics” to L.H. Bookbinder, A. Hofer, M.F. Haller, M.L. Zepeda, G.-A. Keller, J.E. Lim, T.S. Edgington, H.M. Shepard, J.S. Patton, G.I. Frost (“Bookbinder”)
1031	“Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration” to Gregory I Frost (“Frost”)
1032	“ENHANZE® drug delivery technology: a novel approach to subcutaneous administration using recombinant human hyaluronidase PH20” to Kenneth W. Locke, Daniel C. Maneval and Michael J. LaBarre (“Locke”)
1033	“N-Glycosylation and enzymatic activity of the rHuPH20 expressed in Chinese hamster ovary cells” to Liu T, Li Y, Xu J, Guo Q, Zhang D, Song L, Li J, Qian W, Guo H, Zhou X, Hou S. (“Liu”)
1034	Butler M, Meneses-Acosta A. Recent advances in technology supporting biopharmaceutical production from mammalian cells. Appl Microbiol Biotechnol. 2012 Nov;96(4):885-94.
1035	U.S. Patent No. 8,187,855 to David Baker and Louis H. Bookbinder (“Baker”)
1036	Liu, Chao Yan, Virginia Spencer, Shyam Kumar, Jian Liu, Henry Chiou, and Jonathan Zmuda, “Attaining High Transient Titers in CHO Cells”

1037	International Patent Publication WO2013/102144 to Ge Wei, Michael H. Shepard, Qiping Zhao and Robert James Conner is titled “PH20 Polypeptide Variants, Formulations and Uses Thereof” (“Wei 2013”)
------	---

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§42.6(e) and 42.105(a), this is to certify that I caused to be served a true and correct copy of the foregoing Petition for *Inter Partes* Review of U.S. Patent No. 12,221,638 (and accompanying Exhibits 1001-1009, 1011-1025, and 1027-1037) by overnight courier (Federal Express or UPS), on this 10th day of December, on the Patent Owner at the correspondence address of the Patent Owner as follows:

23373 - SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 9000
WASHINGTON, DC
UNITED STATES

Respectfully submitted,

Dated: December 10, 2025

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182