1UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ADELLO BIOLOGICS. LLC, APOTEX INC. and APOTEX CORP.
Petitioners

v.

AMGEN INC. and AMGEN MANUFACTURING LIMITED
Patent Owner

Post-Grant Review No.: PGR2019-00001

PETITION FOR POST-GRANT REVIEW OF U.S. PATENT NO. 9,856,287

Mail Stop **Patent Board**
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

TABLE OF CONTENTS

I. INTRODUCTION 1
II. STANDING (37 C.F.R. § 42.204(a)); PROCEDURAL STATEMENTS 2
III. MANDATORY NOTICES (37 C.F.R. § 42.8(a)(1)) 2
A. Real Party-In-Interest (37 C.F.R. § 42.8(b)(1)) 2
B. Related Matters (37 C.F.R. § 42.8(b)(2)) 3
C. Designation of Lead and Back-Up Counsel (37 C.F.R. § 42.8(b)(3)) 3
D. Notice of Service Information (37 C.F.R. § 42.8(b)(4)) 4
IV. STATEMENT OF THE PRECISE RELIEF REQUESTED AND THE REASONS THEREFOR (37 C.F.R. § 42.22(a)) 4
V. TECHNOLOGY BACKGROUND 4
A. The Basic Science of Proteins 4
1. Protein Structure 4
2. Protein Synthesis in and out of the Lab 5
B. Recovery of Bioactive Protein and Protein Refolding 6
1. Step 1: Isolate the Inclusion Bodies 8
2. Step 2: Solubilize the Inclusion Bodies 9
3. Step 3: Refold the Solubilized Protein 10
C. Additional Considerations in Commercial Production of Recombinant Proteins 11
VI. THE ’287 PATENT, SKILL IN THE ART, PROSECUTION HISTORY, AND THE UNPATENTABLE CLAIMS OF THE ’138 PATENT 13
A. The ’287 Patent 13
B. Person of Ordinary Skill in the Art 14
C. Prosecution History 15
D. The Unpatentable Claims of the ’138 Patent 16

VII. CLAIM CONSTRUCTION 20

A. "protein" 20

B. "wherein the thiol-pair buffer strength maintains the solubility of the preparation" 20

VIII. ELIGIBILITY FOR POST-GRANT REVIEW 23

A. None of the priority applications provide support for "at least about 25% of the proteins are properly refolded" recited in claims 1 and 16 27

B. Ground 1: Claims 1-9 and 16-25 lack written description support for "at least about 25% of the proteins are properly refolded" 32

C. The priority applications fail to provide enablement for "at least about 25% of the proteins are properly refolded" and "about 30-80% of the proteins are properly refolded" 33

D. Ground 2: Claims 1-30 lack enablement for "at least about 25% of the proteins are properly refolded" and "about 30-80% of the proteins are properly refolded" 36

IX. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED 36

A. Ground 1: Claims 1-9 and 16-25 lack written description support for "at least about 25% of the proteins are properly refolded" 37

B. Ground 2: Claims 1-30 lack enablement for "at least about 25% of the proteins are properly refolded" 37

C. Ground 3: Claims 1-4, 7-19, and 22-30 Are Unpatentable Under 35 U.S.C. § 102(a)(1) over Vallejo 38

1. Claims 1, 10, 16, and 26 38

a. The Preamble 38

b. Creating a mixture of components for protein refolding 39

c. Components of the mixture 40

d. Redox Components 42

e. Incubating the refold mixture 45

2. Claims 2, 3, 11, 13, 17, 18, 27 and 28 46
3. Claims 4, 12, 19 and 29 47
4. Claims 7 and 22...48
5. Claims 8, 9, 14, 15, 23, 24, 25 and 30 49
D. Ground 4: Claims 1-4, 8-19, and 23-30 Are Unpatentable Under 35 U.S.C. § 102(a)(1) over Schlegl..50
1. Claims 1, 10, 16, and 26 52
a. The Preamble 53
b. Creating a mixture of components for protein refolding..53
c. Components of the mixture 54
d. Redox Components...55
e. Incubating the refold mixture 56
2. Claims 2, 3, 11, 13, 17, 18, 27 and 28 57
3. Claims 4, 12, 19 and 29 57
4. Claims 8, 9, 14, 15, 23, 24, 25 and 30 58
E. Ground 5: Claims 7 and 22 Are Unpatentable Over the Combination of Schlegl and Vallejo 59
F. Ground 6: Claims 1-4, 7-19, and 22-30 Are Unpatentable Over Ruddon in view of Vallejo 61
1. Claims 1, 10, 16, and 26 62
a. The Preamble 62
b. Creating a mixture of components for protein refolding..63
c. Components of the mixture 65
d. Redox Components...67
e. Incubating the refold mixture 69
2. Claims 2, 3, 11, 13, 17, 18, 27 and 28 71
3. Claims 4, 12, 19 and 29 73

4. Claims 7 and 22 ... 74
5. Claims 8, 9, 14, 15, 23, 24, 25 and 30 ... 75
G. Ground 7: Claims 5, 6, 20, and 21 Are Unpatentable Over Vallejo in view of Hevehan ... 76
H. Objective Indicia of Nonobviousness Do Not Save the '287 Patent ... 79
I. Ground 8: Claims 1-15 are indefinite ... 79
X. CONCLUSION ... 81

Petitioners' Exhibit List

Exhibit	*Description*
1001	Shultz et al., U.S. Patent No. 9,856,287, "Refolding Proteins Using a Chemically Controlled Redox State," issued January 2, 2018.
1002	Declaration of Dr. Anne S. Robinson
1003	Dr. Anne S. Robinson CV
1004	Shultz et al., U.S. Patent No. 8,952,138, "Refolding Proteins Using a Chemically Controlled Redox State," issued February 10, 2015.
1005	*Apotex Inc. et al. v. Amgen Inc. et al.,* IPR2016-01542, Final Written Decision, Paper 60, February 15, 2018.
1006	Whitford, "Proteins: Structure and Function," September 1, 2005, excerpted.
1007	Schlegl, U.S. Patent Publication No. 2007/0238860, "Method for Refolding a Protein," published October 11, 2007.
1008	Builder et al., U.S. Patent No. 5,663,304, "Refolding of Misfolded Insulin-Like Growth Factor-1," issued September 2, 1997.
1009	http://chemistry.umeche.maine.edu/CHY431/Ribo-fold.jpg
1010	Cohen et al., U.S. Patent No. 4,237,224, "Process for Producing Biologically Functional Molecular Chimeras," issued December 2, 1980.
1011	Cohen et al., U.S. Patent No. 4,468,464, "Biologically Functional Molecular Chimeras," issued August 28, 1984.
1012	Cohen et al., U.S. Patent No. 4,740,470, "Biologically Functional Molecular Chimeras," issued April 26, 1988.

Exhibit	***Description***
1013	Johnson, "Human Insulin from Recombinant DNA Technology," 219 Science, Vol. 219, 632–637 (1983).
1014	Vallejo and Rinas, "Strategies for the Recovery of Active Proteins Through Refolding of Bacterial Inclusion Body Proteins," Microbial Cell Factories, 1-12 (2004).
1015	Neubauer et al., "Protein Inclusion Bodies in Recombinant Bacteria, Inclusions in Prokaryotes," Microbiology Monographs, Edited by J.M. Shively, Springer, Berlin, Heidelberg, 237-292 (2006).
1016	Ventura and Villaverde, "Protein Quality in Bacterial Inclusion Bodies," TRENDS in Biotechnology, Vol. 24 No. 4, 179-185 (2006).
1017	Brady et al., U.S. Patent Publication No. 2006/0228329, "Homogenous Preparations of IL-31," published October 12, 2006.
1018	https://www.profacgen.com/inclusion-body-purification-protein-refolding.htm, printed August 5, 2016.
1019	Georgiou and Valax, "Isolating Inclusion Bodies from Bacteria," Methods in Enzymology, Vol. 309, 48-58 (1999).
1020	Clark, "Protein Refolding for Industrial Processes," Current Opinion in Biotechnology, 12:202-207 (2001).
1021	Palmer and Wingfield, "Preparation and Extraction of Insoluble (Inclusion-Body) Proteins from *Escherichia coli,*" Curr Protoc Protein Sci., Chapter: Unit–6.3, 1-25 (2004).
1022	Jungbauer and Kaar, "Current Status of Technical Protein Refolding," Journal of Biotechnology, 128, 587–596 (2007).

Exhibit	***Description***
1023	Panda, "Bioprocessing of Therapeutic Proteins from the Inclusion Bodies of Escherichia coli" Adv Biochem Engin/Biotechnol (2003) 85: 43–93.
1024	Hevehan and Clark, "Oxidative Renaturation of Lysozyme at High Concentrations," Biotechnology and Bioengineering, Vol. 54, No. 3, 221-230 (1997).
1025	Misawa and Kumagai, "Refolding of Therapeutic Proteins Produced in Escherichia coli as Inclusion Bodies," Biopolymers (Peptide Science), Vol. 51, 297–307 (1999).
1026	Ferrer-Miralles et al., "Microbial Factories for Recombinant Pharmaceuticals" Microbial Cell Factories, 1-8 (2009).
1027	Graumann and Premstaller, "Manufacturing of Recombinant Therapeutic Proteins in Microbial Systems," Biotech J. 1:164-186 (2006).
1028	Puri, "Refolding of Recombinant Porcine Growth Hormone in a Reducing Environment Limits In Vitro Aggregate Formation," FEBS Vol. 292, No. 1,2, 187-190 (1991).
1029	Ejima et al., "High Yield Refolding and Purification Process for Recombinant Human Interleukin-6 Expressed in *Escherichia coli*," Biotechnology and Bioengineering, Vol. 62, No. 3, 301-310 (1999).
1030	Patra et al., "Optimization of Inclusion Body Solubilization and Renaturation of Recombinant Human Growth Hormone from *Escherichia coli*," Protein Expression and Purification, 18, 182-192 (2000).

Exhibit	***Description***
1031	Excerpts from file history of U.S. Application No. 15/422,327, filed February 1, 2017.
1032	Chiti et al., "Conformational Stability of Muscle Acylphosphatase: The Role of Temperature, Denaturant Concentration, and pH," Biochemistry, 37:1447-55 (1998)
1033	Righetti et al., "Folding/unfolding/refolding of proteins: present methodologies in comparison with capillary zone electrophoresis," Electrophoresis, 22, 2359-74 (2001).
1034	U.S. Application No. 14/793,590 as filed July 7, 2015.
1035	U.S. Application No. 14/611,037 as filed January 30, 2015.
1036	U.S. Application No. 12/820,087 as filed June 21, 2010.
1037	Table of categorized claims for U.S. Patent No. 9,856,287.
1038	Vallejo et al., European Patent Application No. EP1449848, "Method for the Production of Cystine-Knot Proteins," published August 25, 2004.
1039	Excerpts from file history of U.S. Application No. 14/611,037, filed January 30, 2015.
1040	Ruddon et al, International Publication No. WO 95/32216, "Biologically Active Glycoprotein Hormones Produced in Procaryotic Cells," published November 30, 1995.
1041	Kuwajima, "The molten globule state of α-lactalbumin", FASEB (1996) 10:102-109.

Exhibit	*Description*
1042	Andersson et al., "Assignment of Interchain Disulfide Bonds in Platelet-Derived Growth Factor (PDGF) and Evidence for Agonist Activity of Monomeric PDGF," The Journal of Biological Chemistry, Vol. 267, No. 16, 11260-11266 (1992).
1043	Kliemannel et al., "The Mature Part of proNGF Induces the Structure of its Pro-Peptide," FEBS Letters, 566, 207-212 (2004).
1044	Daopin et al., "Crystal Structure of Transforming Growth Factor-β2: An Unusual Fold for the Superfamily," Science, Vol. 257, 369-373 (1992).
1045	Gaspar et al., "Cysteine 116 Participates in Intermolecular Bonding of the Human VEGF Homodimer," Archives of Biochemistry and Biophysics, 404, 126-135 (2002).
1046	Scher et al., U.S. Patent No. 9,540,429, "Host Cell Lines Expressing Recombinant GDF-5 Protein," issued January 10, 2017.
1047	https://embryology.med.unsw.edu.au/embryology/index.php/Human_Chorionic_Gonadotropin
1048	Anfinsen, "Principles that Govern the Folding of Protein Chains," Science, Vol. 181, No. 4096, 223-230 (1973).
1049	"How to Fold Graciously," Mössbaun Spectroscopy in Biological Systems Proceedings, Univ. of Illinois Bulletin, 67, No. 41, 22-24 (1969).
1050	Jahn and Radford, "Folding Versus Aggregation: Polypeptide Conformations on Competing Pathways," Archives of Biochemistry and Biophysics, 469, 100-117 (2008).
1051	Clark, "Refolding of Recombinant Proteins" Current Opinion in Biotechnology, (1998) 9:157-163.

I. INTRODUCTION

Adello Biologics, LLC, Apotex Inc., and Apotex Corp. (collectively "Petitioners") respectfully request post-grant review under 35 U.S.C. § 311 and 37 C.F.R. § 42.101 of claims 1-30 of U.S. Patent No. 9,856,287 ("the '287 Patent") (Ex. 1001).

U.S. Patent No. 9,856,287 claims priority to U.S. Patent No. 8,952,138 ("the '138 patent"; Ex. 1004). Claims 1-17 and 19-24 of the '138 patent were found unpatentable by the PTAB on February 15, 2018. Ex. 1005, IPR2016-01542, Paper 60. As with the unpatentable claims of the '138 patent, the challenged claims of the '287 patent recite methods of refolding proteins expressed in a non-mammalian expression system by a simple two-step process: (1) contacting the proteins with a buffer having certain characteristics, and (2) incubating the mixture of the proteins and buffer. *E.g.*, Ex. 1001 at claim 1, 10, 16 and 26; Ex. 1004 at claim 1. This petition demonstrates that it is more likely than not that each of claims 1-30 of the '287 Patent is unpatentable for failing to satisfy the written description and enablement requirements, unpatentable as indefinite, and unpatentable as anticipated and rendered obvious by the prior art. Claims 1-30 of the '287 Patent should be canceled.

II. STANDING (37 C.F.R. § 42.204(a)); PROCEDURAL STATEMENTS

Petitioners certify that the ’287 Patent is available for PGR and that Petitioners are not barred or estopped from requesting PGR of any claim of the ’287 Patent. This Petition is being filed less than one year from the date on which the Petitioners were served with complaints by the Patent Owner regarding the ’287 Patent *(see Amgen Inc. v. Adello Biologics LLC*, 2:18-cv-03347 D.N.J, filed March 8, 2018, and *Amgen Inc. v. Apotex Inc.*, 0:18-cv-61828, S.D.Fla, filed August 7, 2018).

A Power of Attorney and an Exhibit List are filed concurrently herewith. The required fee is paid online via deposit account. The Office is authorized to charge fee deficiencies and credit overpayments to Deposit Acct. No. 05-1323 (Customer No. 23911).

III. MANDATORY NOTICES (37 C.F.R. § 42.8(a)(1))

A. Real Party-In-Interest (37 C.F.R. § 42.8(b)(1))

Adello Biologics, LLC, Apotex Inc., and Apotex Corp. are the real parties-in-interest. Additional real parties-in-interest are Amneal Pharmaceuticals, Inc., Apotex Pharmaceuticals Holdings Inc., Apotex Holdings, Inc., ApoPharma USA, Inc., and Intas Pharmaceuticals Limited.

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

The ’287 Patent is currently the subject of the following litigations: *Amgen Inc. et al. v. Adello Biologics LLC*, 2:18-cv-03347 D.N.J., and *Amgen Inc. et al. v. Apotex Inc. et al*., 0:18-cv-61828, S.D.Fla.

U.S. Patent Application No. 15/889,559 is pending and claims priority to the ’287 Patent.

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

Lead Counsel	Back-Up Counsel
Teresa Stanek Rea (Reg. No. 30,427) CROWELL & MORING LLP Intellectual Property Group 1001 Pennsylvania Avenue, N.W. Washington, DC 20004-2595 Telephone No.: (202) 624-2620 Facsimile No.: (202) 628-5116 TRea@Crowell.com	Deborah H. Yellin (Reg. No. 45,904) CROWELL & MORING LLP Intellectual Property Group 1001 Pennsylvania Avenue, N.W. Washington, DC 20004-2595 Telephone No.: (202) 624-2947 Facsimile No.: (202) 628-5116 DYellin@Crowell.com Shannon Lentz (Reg. No. 65,382) CROWELL & MORING LLP Intellectual Property Group 1001 Pennsylvania Avenue, N.W. Washington, DC 20004-2595 Telephone No.: (202) 624-2897 Facsimile No.: (202) 628-5116 SLentz@Crowell.com

D. Notice of Service Information (37 C.F.R. § 42.8(b)(4))

Please direct all correspondence regarding this Petition to lead counsel at the above address. Petitioners consent to service by email at: TRea@Crowell.com, DYellin@Crowell.com, and SLentz@Crowell.com.

IV. STATEMENT OF THE PRECISE RELIEF REQUESTED AND THE REASONS THEREFOR (37 C.F.R. § 42.22(a))

Adello Biologics, LLC, Apotex Inc., and Apotex Corp. (collectively "Petitioners") respectfully request post-grant review under 37 C.F.R. § 42.201 of claims 1-30 of U.S. Patent No. 9,856,287.

Petitioners' full statement of the reasons for the relief requested is set forth below, in particular in Sections VIIIB and D; IX.

V. TECHNOLOGY BACKGROUND

A. The Basic Science of Proteins

1. Protein Structure

Proteins are a three-dimensional arrangement of atoms defined by multiple levels of structure: (i) primary structure, (ii) secondary structure, (iii) tertiary structure, and (iv) quaternary structure. Ex. 1006 at 43-67. The first three levels of structure are known as the protein's native structure and confer the protein's biological function. *Id.* at 44-67; Ex. 1007 at [0030]; Ex. 1002 at ¶ 39.

A protein's primary structure simply refers to the amino acid sequence along the linear polypeptide chain. Ex. 1006 at 19, 43. Secondary structure refers to the

local conformation of the polypeptide chain, generally characterized by α-helices and β-sheets, which are caused by intramolecular forces (*i.e.*, hydrogen bonding). *Id.* at 43-44. Tertiary structure refers to the compact three-dimensional structure of the entire protein. *Id.* at 54-57, 63; Ex. 1002 at ¶ 40.

Certain chemical bonds in proteins known as "disulfide bonds" can be very important to a protein's native structure. Ex. 1006 at 33; Ex. 1002 at ¶41. Disulfide bonds stabilize the protein's three-dimensional structure, which form between particular amino acids that are close in proximity. Ex. 1006 at 32-33. When these disulfide bonds are misformed, however, a protein can misfold, *i.e.*, take an undesirable structure that differs from its native structure. Ex. 1008 at 2:8-14; Ex. 1002 at ¶37.

2. Protein Synthesis in and out of the Lab

In general, organisms naturally create proteins by the processes of transcription (from DNA to RNA) and translation (from RNA to a protein) Ex. 1006 at 114-115.

Proteins can also be synthesized in the laboratory using recombinant DNA technology, which has been known in the art since at least the mid to late 1970s. Ex. 1002 at ¶39. The use of this technology was patented by Cohen and Boyer in

1974, and the first commercial production using this technology was human insulin by Eli Lilly in 1981. *Generally* Ex. 1010; Ex. 1011; Ex. 1012; Ex. 1013.

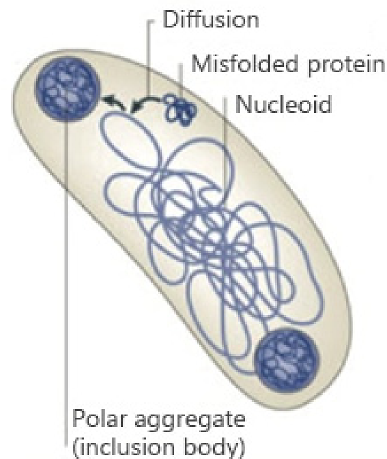
Recombinant DNA combines two or more pieces of DNA. Ex. 1002 at ¶39. The recombinant DNA is then inserted into a cell and enables the cell, among other things, to produce a desired protein that the cell typically does not naturally synthesize. Ex. 1006 at 179. In essence, use of recombinant DNA technology can turn a host cell into a "factory" that creates a large amount of the desired protein in a highly efficient manner. Ex. 1013 at 5; Ex. 1002 at ¶¶41-44.

Recombinant DNA technology can be used in both mammalian and non-mammalian expression systems, but low-yield mammalian expression systems are generally cost prohibitive. Ex. 1014 at 1. Scientists have turned to high-yield bacterial expression systems to express recombinant proteins. *Id.*; Ex. 1002 at ¶40. One well-established host organism in the field of recombinant technology is *Escherichia coli*, commonly referred to as *E. coli*. Ex. 1002 at ¶45. *E. coli* is easily manipulated; thus it is the organism of choice for many researchers. Ex. 1015 at 2, 5-6; Ex. 1016 at 179; Ex. 1017 at [0002]; Ex. 1002 at ¶45.

B. Recovery of Bioactive Protein and Protein Refolding

A host cell expressing recombinant proteins produces two types of proteins: (1) correctly folded proteins in their native structure and (2) misfolded proteins that

group together in the cell in what are known as "inclusion bodies," as shown in the example below:



Ex. 1018; *also* Ex. 1016 at 3-4; Ex. 1002 at ¶46. Inclusion bodies contain between 35-95% of the overexpressed desired protein, as well as DNA, ribosomal RNA, lipids, and other proteins. Ex. 1019 at 2, 4; Ex. 1015 at 11; Ex. 1021 at 10.

Scientists generally believe that inclusion bodies are the result of using a non-mammalian expression system. Ex. 1002 at ¶47. Proteins have a tendency to aggregate because of the growth conditions used for the bacterial cells, including media, growth temperature, and how the protein is expressed. Ex. 1015 at 4, 9; Ex. 1016 at 1. Bacterial cells provide for a more rapid production of protein than the natural process of protein generation in mammalian cells. Ex. 1002 at ¶47. The bacterial cells have trouble "keeping up" with this rapid rate of protein generation and, as a result, the proteins misform and group together. *Id.* In

addition, the chemical environment of a bacterial cell does not promote the formation of disulfide bonds, and promotes aggregation and inclusion body formation for proteins that contain disulfide bonds in their native structure. *Id.*; Ex. 1015 at 6.

Recombinant proteins expressed in *E. coli* were known to have this exact problem with inclusion bodies. *Generally* Ex. 1020; Ex. 1016 at 2. Accordingly, techniques for recovering native, folded proteins in a bioactive and stable form from those inclusion bodies were developed. As early as 1998, there were "over 300 reports of mammalian, plant, and microbial proteins obtained and renatured from inclusion bodies formed in *E. coli*." Ex. 1019 at 1; Ex. 1002 at ¶ 48.

One of those techniques follows a three-step process: (1) isolation and purification of the inclusion bodies; (2) solubilization of the inclusion bodies; and (3) refolding of the solubilized protein. *Generally* Ex. 1021; Ex. 1002 at ¶49.

1. Step 1: Isolate the Inclusion Bodies

To isolate inclusion bodies, host cells undergo disruption of their cell membrane, known as "lysing" the cell. Ex. 1006 at 183-84; *also* Ex. 1020 at 1. Then the contents of the cell are released, and the resulting suspension is centrifuged to separate the lighter soluble portion (containing the soluble proteins)

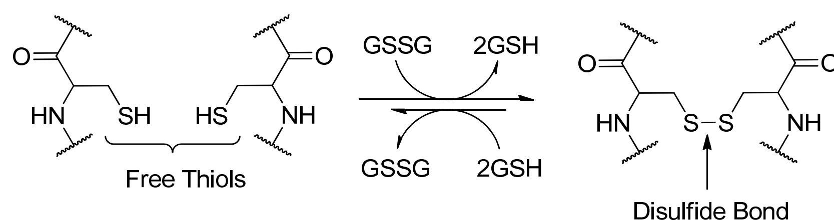
from the heavier insoluble portion (containing inclusion bodies and cellular debris). Ex. 1006 at 185-87; *also* Ex. 1020 at 1; Ex. 1002 at ¶ 50.

2. Step 2: Solubilize the Inclusion Bodies

Next, the inclusion bodies are isolated from the cellular debris in the insoluble portion. Ex. 1002 at ¶51. The isolated inclusion bodies are washed to remove surface-absorbed material, and solubilized with detergents or high concentrations of denaturants (*e.g.*, guanidinium chloride or urea) to release the desired protein from the inclusion bodies. Ex. 1020 at 2; *also* Ex. 1004 at Example 4 (15:34-40). These chemicals disrupt the hydrogen bonding network in the misfolded protein structure of the inclusion bodies to bring the protein back to an unfolded state. Ex. 1015 at 31-32; Ex. 1021 at 10, 12-13; Ex. 1002 at ¶ 51.

Other linkages present in the protein, such as disulfide bonds, are typically reduced to free thiols using a reducing agent, as misformed disulfide bonds support the misfolded conformation, rather than the native protein structure. Ex. 1015 at 267-268; Ex. 1021 at 12-13; Ex. 1002 at ¶52. For example, disulfide bonds can be reduced via a redox reaction with two molecules of reduced glutathione (“GSH”) to give two free thiols and a molecule of oxidized glutathione (“GSSG”), as shown in the reaction from right to left in the below diagram. The GSSG can then oxidize

the thiols again and reform the disulfide bond, as shown in the reaction from left to right in the below diagram:



Ex. 1002 at ¶52.

3. Step 3: Refold the Solubilized Protein

After solubilization of inclusion bodies, the protein must be "refolded." Ex. 1022 at 2-3; Ex. 1021 at 2-5. This "refolding" process causes an unstructured protein to fold into its native three-dimensional structure necessary to its bioactivity. Ex. 1006 at 43; Ex. 1007 at [0030]; Ex. 1002 at ¶53.

A refold buffer has a number of components, such as denaturants, aggregate suppressors, and protein stabilizers that must be optimized, including the type and relative concentrations of the components and redox systems. Ex. 1015 at 33-36; Ex. 1023 at 31-32; Ex. 1014 at 7. Other variables that can be optimized include pH, temperature and timing of the process, and purification methods to complement the procedure. Ex. 1023 at 4; Ex. 1014 at 7; Ex. 1002 at ¶ 54.

It was known before 2009 that the protein released from the inclusion bodies by the solubilizing step must be placed in an environment that facilitates the

formation of the desired native protein structure (*e.g.*, low denaturant concentration). Ex. 1024 at 2. When the desired protein contained disulfide bonds in a native state, the solubilized inclusion bodies must be placed in appropriate redox conditions to reform those disulfide bonds. *Id.* at 5. Scientists used a redox system that favored oxidation consisting of a mixture of reduced and oxidized thiols to refold the protein. *Id.*

The desired equilibrium of reduction and oxidization was well known to be controlled by the ratio and relative concentration of the thiol pairs in the redox mixture. *Id.* at 5-6. While the example on page 12, *supra*, uses GSH/GSSG, other thiol pairs of choice included cysteine/cystine and cysteamine/cystamine. Ex. 1020 at 4. By using the appropriate redox system in the refolding buffer, the disulfide-bond-forming reaction could be balanced to achieve an optimal redox state. Ex. 1025 at 5. This allowed the protein to fold to its native structure. Ex. 1002 at ¶¶55-57.

C. Additional Considerations in Commercial Production of Recombinant Proteins

Many therapeutic proteins are difficult to obtain from natural sources and need to be produced by recombinant DNA technologies. Ex. 1002 at ¶58. As of 2009, 30% of the 151 recombinantly produced approved pharmaceuticals were produced by bacteria. Ex. 1026 at 1-2. In part, the use of *E. coli* is attributed to its

advantages in meeting "[t]he ultimate goal of recombinant fermentation research," which is "to obtain the highest amount of protein in a given volume in the least amount of time." Ex. 1023 at 4; *also* Ex. 1027 at 2.

Production of commercial therapeutic proteins also required large refolding vessels that were a necessary consequence of the common (and inexpensive) method of refolding protein through the three-step process discussed above. Ex. 1024 at 1-2; Ex. 1002 at ¶59. Those skilled in the art were able to decrease the size of these refolding vessels by increasing the concentration of protein before and during refolding. *Id*. Unfortunately, with higher concentrations came another issue.

During refolding at higher concentrations, intermediates in the refolding process are more prone to associate in unproductive ways, leading to misfolded proteins called "aggregates." Ex. 1014 at 3; Ex. 1007 at [0008]. This process of "aggregation" competed with the desired folding pathway, lowering the yield of properly folded proteins. Ex. 1007 at [0008]-[0009]; Ex. 1002 at ¶ 60.

Those skilled in the art had numerous solutions at their disposal to deal with aggregation prior to 2009. Ex. 1002 at ¶61. One of those solutions was to add an aggregation suppressor, with arginine being the most commonly used. Ex. 1014 at 6. By adding arginine to the refold buffer, the yield of refolded protein increased

by almost 50%, due to the suppression of aggregation. Ex. 1024 at 4. Another solution was to add the protein to the refold buffer in batches, known as "pulse renaturation," where the protein concentration was increased gradually to allow the protein to properly refold. Ex. 1002 at ¶61. These and other similar pre-2009 solutions made it possible to refold proteins of varying complexity at a high protein concentrations. *See Id.;* Ex. 1024 at 1; Ex. 1028 at 1, 3; Ex. 1029 at 1-2; Ex. 1030 at 5-6; Ex. 1008 at Abstract, 6:45-47, 18:40-42.

VI. THE '287 PATENT, SKILL IN THE ART, PROSECUTION HISTORY, AND THE UNPATENTABLE CLAIMS OF THE '138 PATENT

A. The '287 Patent

The '287 Patent is entitled: "Refolding Proteins Using a Chemically Controlled Redox State." The '287 Patent issued on January 2, 2018 from U.S. Patent Application No. 15/422,327, filed February 1, 2017, which is a continuation of U.S. Application No. 14/793,590, filed July 7, 2015, which is a continuation of U.S. Application No. 14/611,037, filed January 30, 2015, which is a divisional of U.S. Application No. 12/820,087, filed June 21, 2010, now the '138 patent, which claims priority to Provisional Application No. 61/219,257, filed on June 22, 2009. As discussed below, claims 1-17 and 19-24 of the '138 patent were found to be unpatentable by the Board on February 15, 2018. Ex. 1005.

The ’287 Patent has four independent claims: 1, 10, 16 and 26, each of which recites a “method of refolding proteins.” Ex. 1001 at claims 1, 10, 16 and 26. These independent claims very broadly set forth the steps of refolding by (1) contacting the proteins with a buffer having certain characteristics, and (2) incubating the mixture of the proteins and buffer. *Id.* Not only are these claims very broad – clearly overlapping the prior art – they also substantially overlap the subject matter of the claims of the ‘138 Patent that the Board has held to be unpatentable. Sections VID and IX.C-G below.

B. Person of Ordinary Skill in the Art

The person of ordinary skill in the art (“POSA”) to which the ’287 Patent is directed would have at least a Bachelor’s degree (or the equivalent) in biochemistry or chemical engineering with several years’ experience in biochemical manufacturing, protein purification, and protein refolding, or alternatively, an advanced degree (Masters or Ph.D.) in biochemistry or chemical engineering with emphasis in these same areas. Ex. 1002 at ¶¶18-19. This person may also work in collaboration with other scientists and/or clinicians who have experience in protein refolding or related disciplines. *Id.* A POSA would have easily understood the prior art references referred to herein and would have had the capacity to draw inferences from them. *Id.*

C. Prosecution History

On February 2, 2017, the day after filing the application, the applicants submitted new claims, which included the element "wherein the thiol-pair ratio and the thiol-pair-buffer strength yield at least about 25% properly refolded protein". Ex. 1031 at 4,6.

Along with addressing rejections of their claims based on obviousness-type double patenting ("OTDP") over the claims of the '138 patent, and a number of prior art rejections, the applicants amended the claims to recite "wherein the thiol-pair ratio and the thiol-pair-buffer strength are such that incubating the refold mixture achieves consistent yields of at least about 25% properly refolded proteins". *Id*. at 20.

In the Office Action of August 22, 2018, referring back to the amendment of February 2, 2017, and specifically to the language reciting "about at least 25% properly refolded protein" the Examiner stated that the "specification or original claims do not support such phrases and the claims are new matter." *Id.* at 41.

Applicants amended the claims again, to remove the rejected subject matter and replaced it with "incubating the refold mixture so that at least about 25% of the proteins are properly refolded." Ex. 1031 at 45. Applicants further argued that the specification provided support, referring to Figures 1a-f and Example 3, as

disclosing "wherein approximately 30-80% properly refolded protein was obtained." The Examiner allowed the claims.

However, as discussed *infra* at VIII.A, the Examiner misapprehended the level of support present in the specification for "at least about 25% properly refolded protein" and did not address applicant's failure to provide support for the claimed range.

D. The Unpatentable Claims of the '138 Patent

Like the claims at issue here, the claims of the '138 patent are directed to a method of refolding a protein expressed in a non-mammalian expression system. As with the '287 Patent claims, the claims of the '138 patent included the steps of (1) contacting the protein with a buffer having certain characteristics and (2) incubating the mixture of the proteins and the buffer. Ex. 1004 at claim 1. Not only are the steps the same between the claims of the '287 Patent and '138 patents, the components of the refold buffer are the same: both include a reductant and an oxidant (a redox component), and both include at least one of a denaturant, an aggregation suppressor, and a protein stabilizer. The table below provides a comparison between unpatentable claim 1 of the '138 patent and claim 1 of the '287 Patent:\

Unpatentable Claim 1 of the '138 Patent	Claim 1 of the '287 Patent
1. A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:	1. A method of refolding proteins expressed in a non-mammalian expression system, the method comprising:
(a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater[1] and one or more of: (i) a denaturant; (ii) an aggregation suppressor; and (iii) a protein stabilizer; to form a refold mixture;	contacting the proteins with a preparation that supports the renaturation of at least one of the proteins to a biologically active form, to form a refold mixture, the preparation comprising: at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer; an amount of oxidant; and an amount of reductant, wherein the amounts of the oxidant and the reductant are related through a thiol-pair ratio and a thiol-pair buffer strength, wherein the thiol-pair ratio is in the range of 0.001-100

[1] Claim 4 of the '287 patent depends on claim 1 and further recites that "the thiol-pair buffer strength is 2 mM or greater."

	wherein the thiol-pair buffer strength maintains the solubility of the preparation; and
(b) incubating the refold mixture; and	incubating the refold mixture so that at least about 25% of the proteins are properly refolded.
(c) isolating the protein from the refold mixture.	

The claims of the '138 and the '287 Patents both contain an element relating to the "thiol-pair ratio" and the "thiol-pair buffer strength" (also referred to the "redox buffer strength)." Ex. 1001 at 6:63-7:7 and Ex. 1004 at 6:35-46 (referring to "Buffer Thiol-Pair Buffer Strength/Thiol Buffer Strength" as "(BS)" and also referring to "Redox Buffer Strength" as "(BS)"). As these two patents share the same specification, these terms are defined the same. The Board defined these terms in the '138 patent as follows:

- "final thiol-pair ratio" or "TPR" is "the relationship of the reduced and oxidized species used in the redox component of the refold buffer as defined by the equation $\frac{[\text{reductant}]^2}{[\text{oxidant}]}$. Ex. 1005 at 10.
- "redox buffer strength" (also referred to as "thiol-pair buffer strength" in the specification) means 2[oxidant] + [reductant]. Ex. 1005 at 11.

Although none of the prior art publications relied on by Petitioner in that proceeding directly disclosed the equations for either the thiol-pair ratio or the redox buffer strength, the Board nonetheless found these claims unpatentable. Because "[t]o hold otherwise would eviscerate long-standing legal precedent and simply allow for the patenting of inventions whose only contribution was to quantify into a previously unwritten equation relationships that were discernible to one of ordinary skill in the art from the prior art." Ex. 1005 at 29.[2] The same analysis applies here. The equations merely define ratios and concentrations of oxidant and reductant. Ex. 1005 at 28. As the Board explained, "[i]n order to discern whether the claims are obvious, [the Board] of necessity must determine whether the prior art ratios and concentrations render the claimed range obvious." *Id.* Likewise, in the case of anticipation, the Board must determine that the prior art ratios and concentrations anticipate the claimed range. And it is an established principle that "when, as by a recitation of ranges or otherwise, a claim covers several compositions, the claim is 'anticipated' if *one* of them is in the prior art." *Titanium Metals Corp.v. Banner,* 778 F.2d 775, 227 USPQ 773 (Fed. Cir. 1985)

---

[2] Ruddon, cited in this Petition as Ex. 1040, discloses the TPR equation. Section VI.D.4.b below.

(citing *In re Petering,* 301 F.2d 676, 682, 133 USPQ 275, 280 (CCPA 1962)) (emphasis in original). As discussed below, the prior art teaches ratios and concentrations that anticipate and render obvious the ranges required by the claims.

VII. CLAIM CONSTRUCTION

In a post-grant review proceeding, claim terms in an unexpired patent are interpreted according to their broadest reasonable construction in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,764 (Aug. 14, 2012); *Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 2131, 2144-46 (2016). To be clear, any claim terms not included in the following discussion should be given their broadest reasonable construction in light of the specification.

A. "protein"

The term "protein" should be interpreted to mean "any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds." This definition is set forth in the '287 specification. Ex. 1001 at 6:4-7. The claims of the '287 Patent recite "proteins," i.e., more than one protein. Ex. 1002 at ¶63.

B. "wherein the thiol-pair buffer strength maintains the solubility of the preparation"

The term "wherein the thiol-pair buffer strength maintains the solubility of the preparation" has two constructions:

(1) Read in light of the specification, "wherein the thiol-pair buffer strength maintains the solubility of the preparation" should be interpreted to mean that the thiol-pair buffer strength maintains the solubility of the proteins when the proteins contact the preparation, forming the refold mixture.

(2) Under the plain language of claims 1 and 10, the term "wherein the thiol-pair buffer strength maintains the solubility of the preparation" would be interpreted to mean that the thiol-pair buffer strength maintains the solubility of the preparation itself, with the "preparation" defined in claims 1 and 10 as comprising "at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer," "an amount of oxidant," and "an amount of reductant," but where the preparation does not contain the proteins. Ex. 1001 at claims 1 and 10.

The first construction is the broadest reasonable interpretation in light of the specification. The specification refers to the solubility and solubilization of the proteins at multiple locations. The '287 Patent defines solubilization as "a process in which salts, ions, denaturants, detergents, reductants and/or other organic molecules are added to a solution comprising a protein of interest, thereby removing some or all of a protein's secondary and/or tertiary structure and dissolving the protein into the solvent." EX1001 at 7:28-33. The '287 Patent also

states that "[a]n optimal balance of species was attainable. As shown in Figures 1a-f, there is a clear relationship between thiol-pair buffer strength and thiol-pair ratio that can be identified to maintain the optimal species balance and thus facilitate efficient refolding of low solubility proteins." *Id*. at 17:32-36. The specification also states that "[o]ptimization of the refold buffer can be performed for each protein and each final protein concentration level using the novel method provided herein." *Id.* at 11:8-10. In other words, the protein is the solute, and the preparation is the solvent.

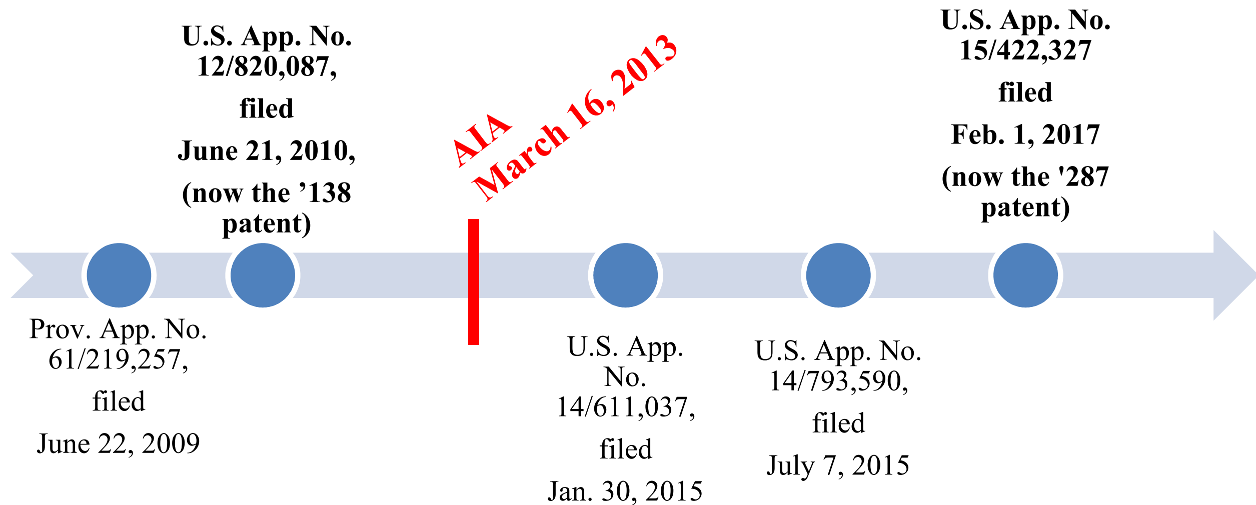
However, this construction is not consistent with the plain language used in claims 1 and 10. Claims 1 and 10 recite that when the proteins are contacted with the preparation, a refold mixture is formed. *Id.* The preparation cannot contain the proteins, because once the proteins are contacted with the preparation, the preparation becomes something different, i.e., the refold mixture. Claims are to be construed to preserve their validity. *Ruckus Wireless, Inc. v. Innovative Wireless Solutions*, 824 F.3d 999, 1004 (Fed. Cir. 2016). The specification does not provide support or enablement for the claims if the second construction is applied, rendering the claims unpatentable under 35 U.S.C. § 112. The term "preparation" as used in claims 1 and 10 does not appear in the specification, and the specification does not disclose any information regarding its "solubility." It is

unclear which ingredients of the preparation are the solvent and which are the solute. Ex. 1002 at ¶¶64-67.

VIII. ELIGIBILITY FOR POST-GRANT REVIEW

The ‘287 Patent claims priority to a chain of applications, each one being a continuation or a divisional of the previous application. The ‘287 Patent is a continuation of U.S. Application No. 14/793,590, filed July 7, 2015 (Ex. 1034), which is itself a continuation of U.S. Application No. 14/611,037, filed January 30, 2015 (Ex. 1035), which is a divisional of U.S. Application No. 12/820,087, filed June 21, 2010 (the ‘138 patent) (Ex. 1036),[3] which claims priority to U.S. Provisional Application No. 61/219,257. As illustrated in the timeline below, the ‘287 Patent is considered a transitional application because it was filed after March 16, 2013—the effective date of the AIA—but it claims priority to an application filed prior to March 16, 2013.

[3] The non-provisional applications share a common specification, varying only in the claims, assertions of priority, and non-material corrections. The ‘138 Patent, filed prior to March 16, 2013, is cited to as representative of the priority applications.



Where a patent claims priority to a series of continuations and divisionals – all sharing specifications that are substantively identical – the presumption is often that applicant is entitled to the priority date of the earliest filing. However, this is only true where no unsupported subject matter is added to the claims. The consequence of an applicant adding unsupported matter to the claims of a transition application is that the patent becomes subject to the Leahy-Smith America Invents Act, Pub. L. No. 112-29, 125 Stat. 284 (2011) ("AIA"), and eligible for post-grant review.

Claims 1-30 are not enabled and/or do not have adequate written description in the priority applications, as required by 35 U.S.C. § 112(a). Therefore, the claims are not entitled to claim priority to any application with a filing date prior to

March 16, 2013, the critical date for the AIA, and the ‘287 Patent is eligible for post-grant review.

The post-grant provisions of the AIA only apply to any patent containing at least one claim with an effective date after March 16, 2013. AIA, §§3(n)(1) and 6(f)(2)(A). The definition of “effective filing date” referenced in §3(n)(1) provides that:

> (A) if subparagraph (B) does not apply, the actual filing date of the patent or the application for the patent containing a claim to the invention; or
>
> (B) the filing date of the earliest application for which the patent or application is entitled, as to such invention, to a right of priority under section 119, 365(a), or 365(b) or to the benefit of an earlier filing date under section 120, 121, or 365(c).

35 U.S.C. §100(i)(l).

Entitlement to the benefit of an earlier date under Sections 119, 120, 121, and 365 is premised on disclosure of the claimed invention "in the manner provided by §112(a)" in the earlier application. 35 U.S.C. §§119(e), 120. Accordingly, a patent issuing from an application filed after March 16, 2013 is available for post-grant review, notwithstanding any priority claim to an

application filed before March 16, 2013,[4] if the patent includes "at least one claim that was not disclosed in compliance with the written description and enablement requirements of § 112(a) in the earlier application." *Inguran, LLC v. Premium Genetics (UK) Ltd.,* PGR2015-00017, Paper 8 at 11 (Dec. 22, 2015). *Schul International Company, LLC v. Emseal Joint Systems, Ltd.*, PGR2017-00053, Paper No. 10 (April 2018) is on point. In *Schul*, the Board instituted a PGR based upon lack of written description support in a priority application for claimed subject matter that had been added by amendment after prosecution on the merits had commenced. The Board accorded the patent the effective filing date of its application, not the filing date of the priority application. *Id*. at 13.

In the present case, at least claims 1-9 and 16-25 were not fully disclosed until added via amendments to the claims of the '287 patent application, and claims 1-30 were not enabled. Ex. 1002 at ¶ 71.

---

[4] The mere fact that an applicant made a priority claim to an earlier filed application filed prior to March 16, 2013 is not probative of the effective filing date. Rather, patent priority for determining PGR eligibility hinges on the substance of the parent application. *Minerva Surgical, Inc. v. Hologic, Inc.*, PGR2017-00002, Paper No. 10 (Dec 14, 2017).

A. None of the priority applications provide support for "at least about 25% of the proteins are properly refolded" recited in claims 1 and 16[5]

None of the priority applications provide information sufficient to describe the range of "at least about 25% of the proteins are properly refolded." During the prosecution of the '287 Patent, applicants added "at least about 25% properly refolded protein" to the claims. Ex. 1031 at 4, 6. When the Examiner rejected the subject matter directed to this subject matter as new matter, Applicants merely rephrased the claim element, though maintaining "at least about 25% properly refolded protein" and argued that Figures 1a-f and Example 3 provided support. *Id.* at 41, 45; Ex. 1002 at ¶ 72.

---

[5] The elements "incubating the refold mixture so that at least about 25% of the proteins are properly refolded" or "incubating the refold mixture so that about 30-80% of the proteins are properly refolded" do not appear in the claims or prosecution histories of the '138 patent or U.S. App. No. 14/793,590. During the prosecution of U.S. App No. 14/611,037, applicants added new dependent claims 26, 27 and 31 in an Amendment filed on July 20, 2017, directed to wherein the "desired yield of the properly refolded protein is at least about 25%" or "desired yield of the properly refolded protein is at least about 30%'". Ex. 1039 at 2-3. However, the Office then issued a Notice of Non-Compliant Amendment. Applicants responded by canceling all claims and submitted new claims 41-60, which did not include subject matter directed to "desired yield of the properly refolded protein is at least about 25%" or "desired yield of the properly refolded protein is at least about 30%." *Id.* at 14.Thus, these elements have not been examined, at least so far, during the prosecution of the '590 application.

The Examiner's initial rejection was correct, as allowing this subject matter was based on a misapprehension of the level of support provided by the specification, as well as the priority applications. Moreover, additional evidence not before the Examiner is submitted here by way of Dr. Robinson's declaration. *See Guardian Indus. Corp. v. Pilkington Deutschland AG*, IPR2016-01635, Paper 9 at 9-10 (P.T.A.B. 2017); *Hospira, Inc. v. Genentech, Inc.*, IPR2017-00804, Paper 13 at 11-13 (P.T.A.B. 2017); *Apotex Inc. v. Novartis AG*, IPR2017-00854, Paper 11 at 13-14 (P.T.A.B. 2017).

The specification does not provide support for "at least about 25% of the proteins are properly refolded," i.e., 25%-100%. This claim language, or even the range of "at least about 25%," does not appear anywhere in the '138 patent specification, or the specifications of the intervening priority applications. And the few instances in the disclosure regarding yields of folded proteins fail to provide support for the full scope of 25-100%. Ex. 1002 at ¶73.

The figures fail to provide adequate support for the full scope of claims 1 and 16 for several reasons. The solid lines of Figures 1a-f purportedly disclose the

percentage of properly folded species.[6] However, it is also clear the percentage of species that are properly folded never rises above about 35%, and in fact is often lower than 25%. Ex. 1002 at ¶73.

Moreover, the data presented in Figures 1a-f is not commensurate in scope with the claims. Figures 1a-f purportedly "depict the effect of thiol-pair ratio and thiol-pair buffer strength on the distribution for product-related species…for a complex dimeric protein." Ex. 1001 at 9:6-8. Thus, the data presented relates to a particular protein, though that protein is not identified. In contrast, the scope of the claims cover the yields resulting from the refolding of any protein that happens to be present, whether that protein is a protein of interest or a protein considered to be an impurity. Ex. 1002 at ¶74.

Example 3 of the '138 patent discloses that "yields of approximately 30-80% were obtained, depending on the redox condition evaluated." Ex. 1004 at 15:8-10.[7] Example 4 of the '287 Patent discloses that "[y]ields of desired product of approximately 27-35% were obtained at both scales." *Id.* at 15:64-65. However,

---

[6] Notably, the percentages of the properly refolded and not properly refolded species do not add up to 100%, casting doubt on the particulars of the experiment. Ex. 1002 at ¶73.

[7] Patent Owner has captured this subject matter in claims 10 and 26, which recite that "about 30-80% of the proteins are properly refolded."

even these examples fail to show that Patent Owner had possession of the range because examples 3 and 4 do not provide the particular protein tested, or which "redox condition" resulted in the respective 30-80% and 27-35% ranges. Ex. 1002 at ¶75.

Finally, and fatally, the priority applications provide no disclosure for any percentages of properly refolded protein over 80%. As explained by Dr. Robinson, the higher yields of refolded protein are more difficult to achieve and thus the complete absence of support in the specification is especially compelling. Ex. 1002 at ¶¶76-77. Thus, the specification cannot provide support for the full scope of "at least 25% or more."

Also fatally, the specification fails to demonstrate any criticality behind the claimed range. Ex. 1002 at ¶ 78. In *Grünenthal GmbH v. Antecip Bioventures II LLC*, No. PGR2017-00008, Paper No. 7 (P.T.A.B. July 7, 2017), the PTAB instituted post-grant review of claims reciting a range of "about 80 to about 500 mg of zoledronic acid." *Id.* at 4. The Board found that the specification failed to demonstrate any criticality behind the particular range stating "neither the text accompanying the examples, nor the data, nor anything else in the specification in any way emphasizes the [recited limitation]." *Id.* (quoting *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1326 (Fed. Cir. 2000). The Board concluded that

the specification contained no description suggesting the dosing regimen limitation was significant and as such, a POSA upon reading the specification would not have been directed to create a dosing range of "about 80 mg to about 500 mg." Here, there is no criticality or significance imparted to "at least about 25% properly refolded proteins." Indeed, the specification does not even disclose this range.

The priority applications also fail to provide inherent support for the full scope of "at least about 25%". With respect to numerical range limitations, the analysis must take into account which ranges one skilled in the art would consider inherently supported by the discussion in the original disclosure. In *In re Wertheim*, the claim at issue recited "at least 35%," but the ranges described in the original specification included a range of "25%-60%" and specific examples of "36%" and "50%." 541 F.2d 257 (CCPA 1976). The Court found that the claims reciting "at least 35%" did not meet the written description requirement because the phrase "at least" had no upper limit and caused the claim to read literally on embodiments outside the "25%-60%" range. *Id.* at 262. Here, "at least 25%" reads on embodiments outside values purportedly taught in the specification of the '138 patent. The specification provides no indication that the inventors had possession of any percentages of properly refolded proteins at the higher end of the range.

B. Ground 1: Claims 1-9 and 16-25 lack written description support for "at least about 25% of the proteins are properly refolded"

For the same reasons set forth in Section VIII.A. above, the '287 specification fails to support the recitation of "at least about 25% of the proteins are properly refolded." The '287 Patent is a continuation of a patent application which is a continuation of a patent application which is a divisional of the '138 patent. The content of the '287 specification is substantively identical to the content of the '138 patent discussed above. Thus, as the '138 patent fails to provide written description support for claims 1-9 and 16-25 of the '287 Patent, the '287 specification also fails. Ex. 1002 at ¶ 79.

In this instance, the Board should not exercise its discretion under 35 U.S.C. § 325(d) to deny institution simply because the issue of support for "at least about 25% of the proteins are properly refolded" was before the examiner during prosecution of the '287 Patent.[8] In this case, the Examiner misapprehended the lack of support provided in the specification. Moreover, this Petition is supported by the Expert Declaration of Dr. Anne Robinson, providing new evidence not

---

[8] In *MCM Portfolio LLC v. Hewlett-Packard Co.*, the Federal Circuit affirmed the constitutionality of *inter partes* review (IPR) proceedings. 812 F.3d 1284, 1291-92 (Fed. Cir. 2015). In doing so, the Federal Circuit explained that Congress created IPRs to serve "an important public purpose—to correct the agency's own errors in issuing patents in the first place." *Id*. at 1290.

before the Examiner, which weighs against applying 325(d). *Guardian Indus. Corp. v. Pilkington Deutschland AG*, IPR2016-01635, Paper 9 at 9-10 (P.T.A.B. 2017); *Hospira, Inc. v. Genentech, Inc.*, IPR2017-00804, Paper 13 at 11-13 (P.T.A.B. 2017); *Apotex Inc. v. Novartis AG*, IPR2017-00854, Paper 11 at 13-14 (P.T.A.B. 2017). Dr. Robinson's testimony regarding the insufficiencies in the support provided by the specification for the full scope of "at least about 25% of the proteins are properly refolded," at paragraphs 73-78 of her declaration, was not before the Examiner during prosecution.

C. The priority applications fail to provide enablement for "at least about 25% of the proteins are properly refolded" and "about 30-80% of the proteins are properly refolded"

The priority applications fail to provide enablement for "at least about 25% of the proteins are properly refolded" and "about 30-80% of the proteins are properly refolded". The specification must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation.'" *In re Wright*, 999 F.2d 1557, 1561 (Fed. Cir. 1993). Undue experimentation factors include "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the

art, and (8) the breadth of the claims." *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). As explained by Dr. Robinson, an analysis of the *Wands* factors demonstrates that it would have required undue experimentation to make the full scope of the claimed invention. Ex. 1002 at ¶ 80.

The challenged claims have a broad scope. Claims 1 and 16 cover a range of 25% to 100% of properly refolded proteins and claims 10 and 26 cover a range of 30% to 80% of properly refolded proteins. The claims recite that the thiol-pair ratio is in the range of 0.001-100, and do not place a numerical limit on the thiol-pair buffer strength, resulting in a vast number of possible redox conditions. Nor do claims 1, 10, 16 and 26 place any limitation on the proteins to be refolded. The overly broad scope of the claims adds to the level of undue experimentation that would be required for a POSA to perform the claimed method, based at least upon the vast number of proteins and redox conditions covered by the claims which could ostensibly result in a range of at least 25% of properly refolded protein or 30-80% of properly refolded protein. Ex. 1002 at ¶80.

To determine if the full scope of the claimed methods is enabled, the skilled artisan would look to the direction provided by the inventors in the specification. Here, the teaching of the specification is insufficient, as it does not teach how to achieve a range of at least 25% properly refolded protein. Nor do the examples

provide sufficient guidance. A POSA could not replicate the percentages of properly refolded species in Figure 1a-f without undue experimentation, as neither the specific protein nor its concentration is provided. Ex. 1002 at ¶¶81-82. Example 3 of the priority specifications broadly states that "[y]ields of desired product of approximately 30-80% were obtained depending on the redox condition evaluated." Ex. 1004 at 15:8-10. Similarly, Example 4 states that "yields of desired product of approximately 27-35% were obtained at both scales." Id. at 64-65. Again, these examples fail to name the protein used. A POSA would necessarily need to resort to undue experimentation to determine what protein was tested and what redox conditions would provide these yields for that protein. Ex. 1002 at ¶82.

Even assuming that the '138 patent specification provides sufficient enablement for the lower ends of the range of at least 25% of the proteins are properly refolded, which it does not, the specification provides no guidance for the higher ends of this range. As Dr. Robinson explains, the higher the percentage of properly folded protein sought, the more difficult that percentage is to achieve. Ex. 1002 at ¶83. The driving forces for aggregation and misfolding are the same as refolding, which makes promotion of refolding a challenging task even at lower yields of properly refolded proteins, and far more difficult at higher yields. Ex.

1002 at ¶46; Ex. 1048; Ex. 1049. There is no showing in the priority applications that the patentees were able to overcome the extreme difficulty in achieving the higher levels of properly refolded protein such as 85, 90, 95 or 100%. Given that the specification teaches nothing on this point, undue experimentation would be required to achieve the higher ends of the range of "at least about 25% of the proteins are properly refolded." Ex. 1002 at ¶83.

D. Ground 2: Claims 1-30 lack enablement for "at least about 25% of the proteins are properly refolded" and "about 30-80% of the proteins are properly refolded"

For the same reasons set forth in Section VIII.C. above, the '287 specification fails to enable the claims limitation "at least about 25% of the proteins are properly refolded" and "at least about 30-80% of the proteins are properly refolded" requiring the POSA to resort to undue experimentation. The content of the '287 specification is substantively identical to the content of the '138 patent discussed above. Thus, as the '138 patent fails to enable claims 1-9 and 16-25 of the '287 Patent, the '287 specification also fails. Ex. 1002 at ¶84.

IX. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED

It is more likely than not that at least one of the challenged claims of the '287 Patent is unpatentable. Claims 1, 10, 16 and 26 and all claims dependent therefrom (claims 1-30), are unpatentable under 35 U.S.C. §112 (Grounds 1, 2, and 8), §102(a)(1) (Grounds 3-4), and § 103 (Grounds 5-7). The sections below, as

confirmed by the cited evidence, demonstrate how the claims are rendered unpatentable through their failure to comply with the requirements of sections §§ 112, 102, and 103.

Petitioners assert the following specific grounds of rejection:

Ground No.	**Claim No(s).**	**Proposed Statutory Rejections for the ’287 Patent**	**Prior Art**
1	1-9 and 16-25	35 U.S.C. § 112	n/a
2	1-9 and 16-25	35 U.S.C. § 112	n/a
3	1-4, 7-19, and 22-30	35 U.S.C. § 102(a)(1)	Vallejo
4	1-4, 8-19, and 23-30	35 U.S.C. § 102(a)(1)	Schlegl
5	7 and 22	35 U.S.C. § 103	Schlegl and Vallejo
6	1-4, 7-19, and 22-30	35 U.S.C. § 103	Ruddon and Vallejo
7	5, 6, 20 and 21	35 U.S.C. § 103	Vallejo and Hevehan
8	1-15	35 U.S.C. § 112	n/a

A. Ground 1: Claims 1-9 and 16-25 lack written description support for “at least about 25% of the proteins are properly refolded”

Ground 1 is found in Section VIII.B. above.

B. Ground 2: Claims 1-30 lack enablement for “at least about 25% of the proteins are properly refolded”

Ground 2 is found in Section VIII.C. above.

C. Ground 3: Claims 1-4, 7-19, and 22-30 Are Unpatentable Under 35 U.S.C. § 102(a)(1) over Vallejo

1. Claims 1, 10, 16, and 26

For this prior art ground, and the further prior art grounds discussed below, Petitioners treat the limitation "wherein the thiol-pair buffer strength maintains the solubility of the preparation" of claim 1 and 10 to mean the same as the limitation "wherein the thiol-pair buffer strength maintains the solubility of the solution" of claim 16 and 26. Thus, claims 1 and 10 are identical except for the last limitation relating to the percentage of properly refolded protein, and claims 16 and 26 are identical except for the last limitation relating to the percentage of properly refolded protein. Ex. 1037.

a. The Preamble[9]

Claims 1, 10, 16, and 26
"A method of refolding proteins expressed in a non-mammalian expression system"

[9] Throughout the rest of this Petition, the independent claims are broken down and addressed in the same way as in this ground. A claim listing, as broken down in these grounds can be found in Exhibit 1037.

Vallejo discloses a "method of producing a biologically active recombinant cystine-knot protein comprising (a) solubilisation of inclusion bodies comprising said cystine-knot protein produced in a bacterium in the presence of a chaotropic agent; (b) renaturation of the solubilized cystine-knot protein in batch or by pulse addition of said solubilized cystine-knot protein to a refolding buffer*…."* Ex. 1038 at [0001]. Vallejo also discloses that "[a]ny suitable bacterium can be employed for carrying the method of the invention… [and] a more preferred embodiment of the method of the present invention said bacterium is E.coli." Ex. 1038 at [0018]-[0019]; Ex. 1002 at ¶¶89-90.

b. Creating a mixture of components for protein refolding

Claims 1 and 10	Claims 16 and 26
"contacting the proteins with a preparation that supports the renaturation of at least one of the proteins to a biologically active form, to form a refold mixture	*preparing a solution comprising:* *the proteins;* *at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer;* *an amount of oxidant; and* *an amount of reductant,*

> Vallejo's method includes:
>
> (a) solubilisation of inclusion bodies comprising said cystine-knot protein…;
> (b) renaturation of the solubilized cystine-knot protein in batch or by pulse addition of said solubilized cystine-knot protein to a refolding buffer….comprising
> (ba) an aggregation suppressor…;
> (bb) a mixture of reduced and oxidized glutathione wherein the ratio of reduced to oxidized glutathione is equal or above 1:10; and
> (bc) a solubilizing chaotropic agent in a non-denaturing concentration…*"*

*Id.*at [0001].

Further, Vallejo discloses that the "[s]tandard renaturation conditions were as follows: Dilution of unfolded and reduced rhBMP-2 with a final concentration of 0.1 mg mL^{-1} rhBMP-2 in standard renaturation buffer." *Id.*at [0054]. Reduced and oxidized glutathione are a reductant and an oxidant, respectively. Ex. 1001 at 7:20-25. Therefore, Vallejo discloses contacting the protein with a preparation that supports the renaturation of a protein to a biologically active form, to form a refold mixture as described in claims 1 and 10. And Vallejo discloses preparing a solution comprising the proteins and the other components described in claims 16 and 26. Ex. 1002 at ¶¶91-92.

c. Components of the mixture

Claims 1 and 10	Claims 16 and 26
"*the preparation comprising:*	*"preparing a solution comprising:*

at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer…”	*the proteins;* *at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer…”*

Vallejo discloses a method of refolding a protein using a refolding buffer comprising:

> “(ba) an aggregation suppressor…;
> (bb) a mixture of reduced and oxidized glutathione wherein the ratio of reduced to oxidized glutathione is equal or above 1:10; and
> (bc) a solubilizing chaotropic agent in a non-denaturing concentration…*”*

Ex. 1038 at [0001].

Vallejo discloses “The standard renaturation buffer contains 0.5 mol L^{-1} Gdn-HCl, 0.75 mol L^{-1} CHES and 1 mol L^{-1} NaCl (#3233). These additives are known to effect protein stability and the aggregation propensity during refolding.” Id. at [0047]. Vallejo further teaches that the aggregation suppressor can be arginine. Id. at [0021]. The ‘287 Patent describes arginine as both an aggregation suppressor and a protein stabilizer. Ex. 1001 at 5:41-58. Therefore, Vallejo teaches using an aggregation suppressor and a protein stabilizer in the preparation. Ex. 1002 at ¶¶93-95.

Further, Vallejo describes a chaotropic agent as "any substance that disturbs the three-dimensional structure of the hydrogen bonds in water." Ex. 1038 at [0047]; Ex. 1002 at ¶96.

The '287 Patent describes a "denaturant" as: "any compound having the ability to remove some or all of a protein's secondary and tertiary structure when placed in contact with the protein." Ex. 1001 at 5:31-34. A POSA would understand the definition of a chaotropic agent as described by Vallejo to be consistent with a "denaturant" as defined by the '287 Patent. Ex. 1002 at ¶97.

Therefore, Vallejo teaches a refold buffer containing an aggregation suppressor, protein stabilizer, and a denaturant. Ex. 1002 at ¶ 98.

d. Redox Components

Claims 1 and 10	Claims 16 and 26
"an amount of oxidant; and an amount of reductant, *wherein the amounts of the oxidant and the reductant are related through a thiol-pair ratio and a thiol-pair buffer strength,* *wherein the thiol-pair ratio is in the range of 0.001-100,* *wherein the thiol-pair buffer strength maintains the solubility of the* ***preparation****"*	*"an amount of oxidant; and an amount of reductant,* *wherein the amounts of the oxidant and the reductant are related through a thiol-pair ratio and a thiol-pair buffer strength,* *wherein the thiol-pair ratio is in the range of 0.001-100,* *wherein the thiol-pair buffer strength maintains the solubility of the* ***solution****"*

Vallejo discloses that "[f]or renaturation of disulfide-bonded proteins, mixtures of reduced and oxidized glutathione are employed to allow disulfide-bond reshuffling until the most stable disulfide-bond structures are obtained, in general the native state of the protein." Ex. 1038 at [0045]. In other words, Vallejo appreciates that it is the balance or relationship between the amount of reductant and oxidant that allow for the disulfide-bonds to reshuffle until the protein is properly refolded. Further, Vallejo discloses that standard renaturation conditions contained a final concentration of "3 mmol L^{-1} total glutathione in a 2:1 ratio of glutathione reduced to glutathione oxidized (GSH:GSSG)." Ex. 1038 at [0054]. Reduced glutathione (GSH) and oxidized glutathione (GSSG) are a reductant and an oxidant, respectively. Ex. 1001 at 7:20-25; Ex. 1002 at ¶99.

Vallejo includes examples where rhBMP-2 is refolded using varied concentrations of reductant and oxidant. Ex. 1038 at [0045] and Fig. 2. In particular, Vallejo varies the ratio of GSH to GSSG from 40:1 to 1:20. Ex. 1038 at Fig. 2b, [0042] and [0045]. A POSA would understand these ratios to be molar ratios and would understand that this is a simple ratio of [reductant]/[oxidant]. The '287 Patent defines the thiol-pair ratio as $[reductant]^2/[oxidant]$. Therefore,

Vallejo teaches a calculated thiol-pair ratio of 0.05 to 1600.[10] Therefore, Vallejo discloses multiple examples of refolding a protein using a thiol-pair ratio within the range of 0.001-100. Ex. 1002 at ¶100.

Vallejo discloses that standard renaturation conditions contained a final concentration of "3 mmol L^{-1} total glutathione." Ex. 1038 at [0054]. A POSA in 2009, would understand that mmol L^{-1} is equivalent to mM. A POSA would also understand that when Vallejo describes the final concentration of glutathione, it is describing what the '287 Patent identifies as the thiol-pair buffer strength. Ex. 1002 at ¶101. To calculate the total glutathione ([GSH]) the concentration of GSSG ([GSSG]) must be doubled because when reduced, one GSSG forms two GSH molecules. The '287 Patent defines the thiol-pair buffer strength as 2[oxidant] + [reductant] which in Vallejo equals 2[GSSG] + [GSH]. A POSA would understand that the thiol-pair buffer strength is simply the total glutathione concentration because the term 2[GSSG] is equal to the concentration of reduced GSH. Therefore, when Vallejo teaches a final concentration of glutathione of

---

[10] The '287 patent teaches the thiol-pair ratio as $[reductant]^2/[oxidant] = [GSH]^2/[GSSG] = [40]^2/[1] = 1600$ and $[1]^2/[20] = 0.05$.

3mmol L^{-1} (i.e. 3 mM), it is also disclosing a thiol-pair buffer strength greater than 2 mM. Ex. 1002 at ¶101.

Finally, Vallejo teaches that the thiol-pair buffer strength maintains the solubility of the preparation (claims 1 and 10) and the solution (claims 16 and 26). Vallejo teaches that its method results in properly refolded proteins. Ex. 1038 at [0012]. This result would not be possible unless the redox components maintained the solubility of the protein while the protein refolded. Ex. 1002 at ¶102.

e. Incubating the refold mixture

Claims 1 and 16	Claims 10 and 26
"incubating the refold mixture so that at least about 25% of the proteins are properly refolded."	*"incubating the refold mixture so that about 30-80% of the proteins are properly refolded."*

Vallejo teaches that the optimization of the refolding conditions allowed for a refolding yield of 44%. Ex. 1038 at [0012]. Vallejo also teaches that the concentration of protein could be increased in the refolding mixture and the yield of active protein would stay in the range of 33-38%. *Id.* Vallejo concludes that its method could be used for commercial production: "the protein concentration during renaturation could be increased 12 to 13 fold as compared to the previously published procedure making the *E. coli* expression system even more acceptable for commercial production." Ex. 1038 at [0012]; Ex. 1002 at ¶103. A POSA in

2009, would understand that the "renaturation yield" would mean the yield of properly refolded protein. Ex. 1002 at ¶104.

One way a POSA would know whether a protein was properly refolded to its native form would be to determine if it retained the biological activity of the native form of the protein. Ex. 1002 at ¶105. The '287 Patent confirms this understanding, teaching that the non-native form of the protein lacks structural features making it biologically inactive. Ex. 1001 at 7:66-8:10. Vallejo teaches that its refolded recombinant rhBMP-2 is biologically active: "[t]he final conentration [sic] of *dimerized active rhBMP-2* reached 0.7 to 0.8 mg/ml corresonding [sic] to a final yield of 32 to 38%." Ex. 1038 at [0049]; *also id.* at [0056] ("Biological activity of rhBMP-2 was analyzed by alkaline phosphatase induction in C2C12 cells (ATCC-1772) as described previously");Ex. 1002 at ¶105.

2. Claims 2, 3, 11, 13, 17, 18, 27 and 28

Claims 2 and 3; 11 and 13; 17 and 18; and 27 and 28 depend directly on claims 1, 10, 16 and 26, respectively, and recite particular limitations relating to the concentration of protein in the refold mixture or solution. Ex. 1037.

As explained above, Vallejo anticipates the method of claim 1, 10, 16 and 26. Vallejo also discloses the limitations described in claims 2, 3, 11, 13, 17, 18, 27 and 28.

Vallejo discloses a pulsed refolding process that results in a final protein concentration of 2.1 mg/mL. Ex. 1038 at [0012]. The pulsed refolding process of Vallejo involves adding the protein to the refolding buffer in several steps. Because the claims of the '287 Patent do not require that the proteins be added to a refold buffer in one step, this pulsed refolding process would satisfy the element of claims 1 and 10 ("contacting the proteins with a preparation… to form a refold mixture") and also the element of claims 16 and 26 ("preparing a solution comprising: the proteins;…at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer, an amount of an oxidant, and an amount of a reductant…"). Ex. 1002 at ¶107. A POSA in 2009 would understand that mg/mL is equivalent to g/L. *Id.* Therefore, Vallejo discloses a refold mixture with a protein concentration of 2.0 g/L or greater, and also in a range of 1-40 g/L.

3. Claims 4, 12, 19 and 29

Claims 4, 12, 19 and 29 depend directly on claims 1, 10, 16 and 26 respectively and recite that "the thiol-pair buffer strength is 2 mM or greater." As

explained above, Vallejo anticipates claims 1, 10, 16 and 26. Vallejo also discloses the limitations described in claims 4, 12, 19 and 29.

As discussed above, Vallejo discloses that standard renaturation conditions contained a final concentration of "3 mmol L^{-1} total glutathione." *Id.* at [0054]. A POSA would understand that the thiol-pair buffer strength is simply the total glutathione concentration because the term 2[GSSG] is equal to the concentration of reduced GSH. Therefore, when Vallejo teaches a final concentration of glutathione of 3mmol L^{-1} (i.e. 3 mM), it is also disclosing a thiol-pair buffer strength greater than 2 mM. Ex. 1002 at ¶109.

4. Claims 7 and 22

Claims 7 and 22 depend directly on claim 1 and 16, respectively, and recite that "at least one of the proteins is a complex protein." As explained above, Vallejo anticipates claims 1 and 16. Vallejo also discloses the limitations described in claim 7 and 22.

Vallejo discloses a method of refolding dimeric rhBMP-2. *Id.* at [0049]. Dimeric rhBMP-2 is ~26 kDa and has 7 disulfide bonds. *Id.* at [0003] and Fig. 1; Ex. 1002 at ¶111; Ex. 1038 at [0003] and Fig. 1. Vallejo further teaches that its method can be used to refold other cystine-knot proteins such as "BMPs, PDGFs, human nerve growth factors, TGF-β, VEGF, GDF-5." Ex. 1038 at [0023]. These

proteins are all cystine knot proteins and share characteristics with rhBMP-2. They are also all complex proteins as defined by the '287 Patent:

Protein	Size	# of disulfide bonds
PDGF	30 kDa	8
human nerve growth factors (hNGF)	~32 kDa	7
TGF-β	~25 kDa	9
VEGF	~27 kDa	9
GDF-5	~27 kDa	7

Ex. 1042; Ex. 1043; Ex. 1044 ; Ex. 1045 ; Ex. 1046 ; Ex. 1002 at ¶111. Therefore, Vallejo teaches a method of refolding a complex protein as defined by the '287 Patent.

5. Claims 8, 9, 14, 15, 23, 24, 25 and 30

Dependent claims 8, 9, 14, 15, 23, 24, 25, and 30 recite either the equation for the thiol-pair ratio, the thiol-pair buffer strength, or both. Ex. 1037.

As discussed above in section VI.D.a, Vallejo discloses examples where a protein is refolded using a balance of concentrations of reductant and oxidant falling within the ranges of the claims. These dependent claims specifying that the thiol-pair ratio and the thiol-pair buffer strength are calculated according to the equations listed in the specification are similarly anticipated for the same reasons

described above for claims 1, 10, 16, and 26. Ex. 1002 at ¶113. That Patent Owner decided to explicitly identify the equations in the dependent claims does not change the way in which a determination is made as to whether a prior art reference reads on the claim—by simply determining whether the prior art teaches a thiol-pair ratio and a thiol-pair buffer strength within the range of the claims. Ex. 1005 at 28.

D. Ground 4: Claims 1-4, 8-19, and 23-30 Are Unpatentable Under 35 U.S.C. § 102(a)(1) over Schlegl

Section 325(d) is not applicable here. While the combination of Schlegl and another reference (Hevehan, Ex. 1024) was considered by the Examiner during prosecution, the arguments put forth by the Patent Owner during the prosecution of the '287 Patent application were the same arguments that were rejected by the Board in the Final Written Decision of the '138 patent. Ex. 1005. And, the Examiner did not have the benefit of having the Final Written Decision when examining the claims of the '287 Patent – a piece of evidence that directly contradicts the findings of the Examiner. For this reason alone, section 325(d) does not apply.

In arguing the rejection over Schlegl and Hevehan during the prosecution of the '287 Patent application, the Patent Owner stated: "The cited specific example of Schlegl discloses refolding of the purified model protein bovine a-LA

(paragraph [0075]). Significantly, Schlegl discloses that redox chemicals are optional for refolding of a-LA. This is because a-LA is capable of refolding without any redox chemicals." Ex. 1031 at 32. Based on this distinction, the Patent Owner concludes that Schlegl fails to teach the claims.[11] In view of this argument, the Examiner withdrew the rejection over the combination of Schlegl and Hevehan.

In the IPR relating to the '138 patent (the parent of the '287 Patent), the Board expressly rejected the very same arguments relating to Schlegl.

> Dr. Willson, on the other hand states that Schlegl "does not focus on" the use of redox chemicals. Ex. 2001, ¶ 93. The Response then asserts that because Schlegl's example was a well-known model protein and easy to refold, that "redox chemicals do not play a role in Schlegl's refolding method." Resp. 36. Focusing on the sole example, the Response notes that protein was simple to refold and uses calcium. *Id.*
>
> This testimony of Dr. Willson, while literally true, cannot in our view be reconciled with Schlegl's express teaching of a customizable refolding buffer with a redox buffer option.

---

[11] At this stage in the prosecution, the pending claims were amended to require that the method "***achieve[d] consistent yields*** of at least about 25% properly refolded proteins." Ex. 1031 at 22. This amendment was ultimately rejected as reflected in the final claims. Compare claim 25 from Ex. 1031 (pros history) to claim 1 of the '287 patent.

Ex. 1005 at 19.

Even further, as discussed below, this Petition cites to portions of the Schlegl reference that do not appear to have been considered by the Examiner – and were ignored by the Patent Owner. In particular, paragraph [0082] of Schlegl discusses the yield of properly refolded protein using its described method – that yield exceeding the required at least about 25% of the claims. Ex. 1007 at [0082] ("The final yield of refolded protein at equilibrium is 63% for the batch system and 81% for the fed-batch system. Using the fast mix refolding method of the present invention, the yield of refolded protein is 90%.")[12]

1. Claims 1, 10, 16, and 26

As discussed above, claims 1 and 10 are identical except for the last limitation relating to the amount of properly refolded protein, and claims 16 and 26 are identical except for the last limitation relating to the amount of properly refolded protein. Ex. 1037.

---

[12] Furthermore, the present Petition is supported by the Expert Declaration of Dr. Anne Robinson. This further evidence also weighs against applying 325(d). *Guardian Indus. Corp. v. Pilkington Deutschland AG*, IPR2016-01635, Paper 9 at 9-10 (P.T.A.B. 2017); *Hospira, Inc. v. Genentech, Inc.*, IPR2017-00804, Paper 13 at 11-13 (P.T.A.B. 2017); *Apotex Inc. v. Novartis AG*, IPR2017-00854, Paper 11 at 13-14 (P.T.A.B. 2017).

a. The Preamble

Schlegl discloses the refolding of recombinant proteins expressed using non-mammalian expression systems such as bacterial and yeast expression systems. Ex. 1005 at 13 and 25; Ex. 1007 at [0004]. For example, Schlegl teaches a method for refolding bovine α-lactalbumin. Ex. 1007 at [0073]; Ex. 1002 at ¶114.

b. Creating a mixture of components for protein refolding

The Example in Schlegl discloses contacting the bovine α-lactalbumin with a buffer containing Tris-HCl, cysteine, and cystine. Ex. 1005 at 26; Ex. 1007 at [0075]. The example in Schlegl yields properly refolded protein. Ex. 1007 at [0082]; Ex. 1002 at ¶115.

Schlegl provides further teaching regarding the various ingredients that can be used in the refold buffer. For example, Schlegl discloses a refold buffer containing guadinium chloride, DTT and optionally a redox system (*e.g.*, GSH/GSSG), EDTA, detergents, salts, and refolding additives like L-arginine. Ex. 1007 at [0036]. These are "typical buffer components." Ex. 1007 at [0036]. Schlegl also discloses that compounds may be added to the refolding buffer to "suppress or completely prevent unfolding/ aggregation" that were "known in the art," including "L-arginine, Tris, [and] detergents." Ex. 1005 at 40; Ex. 1007 at [0041]. Schlegl further discloses a refold buffer containing "0.1 M Tris-HCl" (a

protein stabilizer and aggregation suppressor) and "6 M GdmHCl" (a denaturant). Ex. 1007 at [0074]; Ex. 1002 at ¶116.

Schlegl further indicates that the refold buffer should be customized for the protein of interest and may contain "a redox system (e.g., reduced glutathione GSH/oxidized glutathione GSSG)." Ex. 1005 at 19; Ex. 1007 at [0036]; Ex. 1002 at ¶117.

Therefore, Schlegl discloses contacting the protein with a preparation that supports the renaturation of a protein to a biologically active form, to form a refold mixture as described in claims 1 and 10. And Schlegl discloses preparing a solution comprising the proteins and the other components described in claims 16 and 26. Ex. 1002 at ¶118.

c. Components of the mixture

Schlegl discloses a refold buffer containing guadinium chloride, DTT and optionally a redox system (*e.g.*, GSH/GSSG), EDTA, detergents, salts, and refolding additives like L-arginine. Ex. 1007 at [0036]. These are "typical buffer components." Ex. 1007 at [0036]. Schlegl also discloses that compounds may be added to the refolding buffer to "suppress or completely prevent unfolding/aggregation" that were "known in the art," including "L-arginine, Tris, [and] detergents." Ex. 1005 at 40; Ex. 1007 at [0041]. Schlegl further discloses a

refold buffer containing "0.1 M Tris-HCl" (a protein stabilizer and aggregation suppressor) and "6 M GdmHCl" (a denaturant). Ex. 1005 at 38; Ex. 1007 at [0074]; Ex. 1002 at ¶119.

Therefore, Schlegl teaches a refold buffer containing an aggregation suppressor, protein stabilizer, and a denaturant. Ex. 1002 at ¶120.

d. Redox Components

Schlegl teaches optimizing the refold buffer for the particular protein to be refolded. Ex. 1007 at [0036]. This optimized refold buffer will include a redox component when refolding a protein containing disulfide bonds. Ex. 1007 at [0073]-[0082], and Ex. 1002 at ¶121; Ex. 1005 at 18. The optimized refold buffer containing a redox component will contain an amount of a reductant and an amount of an oxidant that allow for the disulfide bonds to reshuffle. Ex. 1007 at [0073]-[0082], and Ex. 1002 at ¶121.

The Example in Schlegl discloses contacting the bovine α-lactalbumin with a refold buffer comprising a redox component to form a refold mixture. Ex. 1005 at 26; Ex. 1007 at [0075]. As indicated in Schlegl, the refolding buffer may contain "a redox system (e.g., reduced glutathione GSH/oxidized glutathione GSSG)," Ex. 1007 at [0036], and a POSA would understand that the addition of cysteine and cystine here serve as the redox system or redox component for bovine

α-lactalbumin. Ex. 1005 at 26; Ex. 1002 at ¶122. That redox component has a thiol-pair ratio of 2. Ex. 1005 at 13 ("Schlegl futher describes a refolding buffer with a redox system having a defined thiol-pair ratio and redox buffer strength."), also at 26-27; Ex. 1007 at [0036], [0075]; Ex. 1002 at ¶122. Therefore, Schlegl discloses a thiol-pair ratio within the range of 0.001-100. Ex. 1005 at 27.

Finally, Schlegl teaches that the thiol-pair buffer strength maintains the solubility of the preparation (claims 1 and 10) and the solution (claims 16 and 26). Schlegl teaches that its method results in properly refolded proteins. Ex. 1007 at [0082]. This result would not be possible unless the redox components maintained the solubility of the protein while the protein refolded. Ex. 1002 at ¶123.

e. Incubating the refold mixture

Schlegl discloses: "[c]omplete refolding, including formation of disulfide bonds, proline isomerization and domain pairing may take hours and up to several days." Ex. 1007 at [0016]. Schlegl also discloses further incubation in the refolding tank to allow complete refolding of the protein. *Id*. at [0060]. Schlegl further teaches that its method yields "refolded protein at equilibrium [of] 63% for the batch system and 81% for the fed-batch system." *Id.* at [0082]. Schlegl confirmed that its results represented properly refolded protein:

> Native conformation of refolded protein is also confirmed by circular dichroism (see FIG. 6). The spectra

> of the refolded and native protein are identical, whereas the unfolded protein shows a completely different spectrum.

Ex. 1007 at [0083]; Ex. 1002 at ¶¶124-126.

2. Claims 2, 3, 11, 13, 17, 18, 27 and 28

Claims 2 and 3; 11 and 13; 17 and 18; and 27 and 28 depend directly on claims 1, 10, 16 and 26, respectively, and recite particular limitations relating to the concentration of protein in the refold mixture or solution. Ex. 1037.

Schlegel discloses a protein concentration after dilution with refolding buffer in the range of 1 ng/ml to 10 mg/ml (10 g/L). Ex. 1007 at [0035] and claim 6; Ex. 1005 at 13 ("Schlegl utilizes a dilution method of protein refolding that results in a protein concentration up to 10 mg/ml"); Ex. 1002 at ¶128.

3. Claims 4, 12, 19 and 29

Claims 4, 12, 19 and 29 depend directly on claims 1, 10, 16 and 26 respectively and recite that "the thiol-pair buffer strength is 2 mM or greater." As explained above, Schlegl anticipates claims 1, 10, 16 and 26. Schlegl also discloses the limitations described in claims 4, 12, 19 and 29.

The Example in Schlegl discloses contacting the bovine α-lactalbumin with a refold buffer comprising a redox component to form a refold mixture. Ex. 1007 at [0075] (Denatured and reduced aliquots at 16.5 mg/ml are rapidly diluted (batch-dilution) 32 fold into renaturation buffer consisting of 100 mM Tris-HCl, 5 mM

CaCl2, 2 mM cysteine and 2 mM cystine…"). A POSA would understand that the addition of cysteine and cystine here serve as the redox system or redox component for bovine α-lactalbumin. Ex. 1002 at ¶124. That redox component has a thiol-pair buffer strength of 6 mM. Ex. 1007 at [0036], [0075]; Ex. 1002 at ¶130; Ex. 1005 at 13 ("Schlegl further describes a refolding buffer with a redox system having a defined thiol-pair ratio and redox buffer strength."). Therefore, Schlegl discloses a thiol-pair buffer strength greater than 2 mM. Ex. 1005 at 26-27; Ex. 1002 at ¶130.

4. Claims 8, 9, 14, 15, 23, 24, 25 and 30

Dependent claims 8, 9, 14, 15, 23, 24, 25, and 30 recite either the equation for the thiol-pair ratio, the thiol-pair buffer strength, or both. Ex. 1037.

As discussed above in section VI.D.3.b., Schlegl teaches the refolding of a protein with concentrations of reductant and oxidant falling within the ranges of the claims. The dependent claims specifying that the thiol-pair ratio and the thiol-pair buffer strength are calculated according to the equations listed in the specification are similarly anticipated for the same reasons described above for claims 1, 10, 16, and 26. That Patent Owner decided to explicitly identify the equations in the dependent claims does not change the way in which a determination is made as to whether a prior art reference reads on the claim—by

simply determining whether the prior art teaches a thiol-pair ratio and a thiol-pair buffer strength within the range of the claims. Ex. 1005 at 28; Ex. 1002 at ¶¶131-132.

E. Ground 5: Claims 7 and 22 Are Unpatentable Over the Combination of Schlegl and Vallejo

A POSA would have combined the teachings of Schlegl and Vallejo and would have had a reasonable expectation of successfully refolding a complex protein, at least because (1) Schlegl teaches that its method can be used to refold any protein; (2) Schlegl teaches a batch wise and fed-batch mode for protein refolding; (3) Vallejo teaches a method of refolding utilizing either a batch wise or fed-batch mode; and (4) Vallejo successfully refolds a complex protein.

As discussed above in section VI.D.3.b., Schlegl anticipates claims 1-6, 8-21, and 23-30. Patent Owner may argue that Schlegl does not explicitly disclose that its method can be used to refold a complex protein as defined by the '287 Patent. The Board has already expressly rejected this argument in the '138 Patent proceeding. Ex. 1005 at 37. However, even considering this argument, a POSA would understand that Schlegl's method could be used to refold a complex protein, at least as evidenced by the teaching of Vallejo. Ex. 1002 at ¶¶133-134.

Schlegl teaches a method for refolding a protein that has been expressed in non-mammalian expression system by mixing a protein solution with a refold

buffer using a batch wise, a fed-batch mode, or by continuously adding the protein solution to the refold buffer. Ex. 1007 at Abstract and [0004]. Schlegl teaches that "batch wise" mixing or "batch dilution" means that "the diluent is added in a defined volume, the 'batch', to the unfolded protein solution." Ex. 1007 at [0017]. Schlegl further teaches that "fed-batch" processes means that "the unfolded protein is added to the refolding tank in a semi-continuous or *pulse wise* manner." In the Example provided in Schlegl, the α-lactalbumin is refolded both using a batch and a pulse wise method. Ex. 1007 at [0075]-[0076]; Ex. 1002 at ¶135.

Similarly, Vallejo teaches a method for refolding a protein that has been expressed in a non-mammalian expressions system using both a "batch" mixing and a "pulse addition." Ex. 1038 at [0010]-[0011]. Vallejo teaches the refolding of a complex protein, rhBMP-2, and also indicates that its method can be used to refold a number of other complex molecules (*e.g.,* PDGFs, human nerve growth factors, TGF-β, VEGF, and GDF-5). Ex. 1038 at [0012], [0023], and [0055], *also* Section VI.D.3.a. Each of these proteins described by Vallejo are cystine-knot proteins, meaning that they have a "knot-like arrangement of three intramolecular disulfide bridges where one disulfide bond threads through a loop formed by the two other disulfide bonds." Ex. 1038 at [0008]. Vallejo teaches that its method

was the result of "[o]ptimization of the renaturation conditions for [cystine-knot proteins]." Ex. 1038 at [0012]; Ex. 1002 at ¶136.

At least because of the similarities of the methods described by both Schlegl and Vallejo, the teaching that both methods utilize a refold buffer that has been optimized for the particular protein to be refolded, and the successful refolding of a complex protein in Vallejo, a POSA would reasonably expect that the method of Schlegl could be used to refold a complex protein, as defined by the '287 Patent. Ex. 1002 at ¶137.

F. Ground 6: Claims 1-4, 7-19, and 22-30 Are Unpatentable Over Ruddon in view of Vallejo

A POSA would have been motivated to combine the teachings of Ruddon and Vallejo for at least the reasons described below, including (1) the fact that both Ruddon and Vallejo teach methods for refolding proteins expressed in non-mammalian expression systems; (2) the proteins described in both Ruddon and Vallejo share a cystine-knot motif; (3) Ruddon indicates that a need exists for a method to produce its proteins in amounts sufficient for clinical applications; and (4) Vallejo describes ways in which higher concentrations of cystine-knot proteins can be refolded. Ex. 1002 at ¶138.

Ruddon discloses a method of producing and refolding biologically active glycoprotein hormones. Ex. 1040 at 1:7-15. The proteins produced and refolded

in Ruddon are made up of α and β subunits. Ex. 1040 at 2:25-33. And each subunit "possess[es] a high degree of disulfide bridging," with the α subunit containing five disulfide bonds, and the β subunit containing six disulfide bonds. Ex. 1040 at 2:34-3:2. These proteins all share a cysteine-knot motif. Ex. 1040 at 26:15-18. Ruddon indicates that "a need exists for a low-cost, simple and reliable method to produce glycoprotein hormones in amounts sufficient for clinical applications." Ex. 1040 at 6:28-31; Ex. 1002 at ¶¶139-140.

Vallejo teaches a method for producing and refolding a biologically active cystine-knot protein. Ex. 1038 at Abstract. Vallejo teaches that by using its method, in particular the pulse refolding procedure, "the protein concentration during renaturation could be increased 12 to 13 fold as compared to the previously published procedure making the *E.coli* expression system even more acceptable for commercial production." Ex. 1038 at [0012]; Ex. 1002 at ¶141.

Thus, a POSA would have combined the teaching of Ruddon and Vallejo, and in doing so, would have arrived at the claims of the '287 Patent. Ex. 1002 at ¶142.

1. Claims 1, 10, 16, and 26

a. The Preamble

Ruddon discloses a method of producing and refolding biologically active glycoprotein hormones. Ex. 1040 at 1:7-15. Ruddon further teaches that

"[e]xpression systems developed in *E.coli* are preferred for practice of the present invention." Ex. 1040 at 18:11-13; Ex. 1002 at ¶143.

Vallejo discloses a method of producing and refolding a biologically active recombinant cystine-knot protein. Ex. 1038 at [0001]. Vallejo also discloses that "[a]ny suitable bacterium can be employed for carrying the method of the invention… [and] a more preferred embodiment of the method of the present invention said bacterium is E.coli." Ex. 1038 at [0018]-[0019]; Ex. 1002 at ¶¶144-145.

b. Creating a mixture of components for protein refolding

Ruddon teaches:

> As desribed [sic] herein below and in Examples 1 and 2, we have formulated redox buffers capable of enabling disulfide bond formation without interfering with the normal folding process of glycoprotein hormone subunit proteins. These buffers support *in vitro* folding of glycoprotein hormone subunits at rates approaching *in vivo* folding rates. In addition to being excellent folding buffers for glycoprotein hormone subunits, these redox buffers may be used to advantage as general reagents for *in vitro* folding for disulfide-containing proteins, since any disulfide-containing protein is subject to the same constraints as those described above for glycoprotein hormone subunits.

Ex. 1040 at 25:10-23; Ex. 1002 at ¶146.

Ruddon further describes a preferred refold buffer that allows for the proteins to refold into its biologically active form containing: "6.4 mM cysteamine and 3.6 mM cystamine in 50 mM Tris-HCL, pH 8.7." Ex. 1040 at 26:34-27:1. In the redox buffer of Ruddon, cysteamine is the reductant, cystamine is the oxidant, and Tris-HCl is a protein stabilizer. Ex. 1002 at ¶147; *also* Ex. 1001 at 5:50-59 (identifying Tris as a protein stabilizer), and 7:20-27 (listing cysteamine and cystamine as redox components).

Ruddon teaches contacting a protein with a redox buffer, such as the one described above:

> Procaryotically [sic] expressed glycoprotein hormone subunits are folded into assembly-competent conformations and assembled into biologically active glycoprotein hormones in the above-described thiol redox buffers. To accomplish this, an appropriate concentration of unfolded subunit is added to an aliquot of buffer, and incubated at a suitable temperature (i.e. 22-28°C) for a pre-determined amount of time to enable the subunits to fold.

Ex. 1040 at 28:24-32; Ex. 1002 at ¶148.

Vallejo discloses a method of refolding a cystine-knot protein comprising the steps of:

> (a) solubilisation of inclusion bodies comprising said cystine-knot protein produced in a bacterium in the presence of a chaotropic agent;

> (b) renaturation of the solubilized cystine-knot protein in batch or by pulse addition of said solubilized cystine-knot protein to a refolding buffer….comprising
>
> (ba) an aggregation suppressor…;
>
> (bb) a mixture of reduced and oxidized glutathione wherein the ratio of reduced to oxidized glutathione is equal or above 1:10; and
>
> (bc) a solubilizing chaotropic agent in a non-denaturing concentration…*"*

Ex. 1038 at [0001]; Ex. 1002 at ¶149.

Further, Vallejo discloses that the "[s]tandard renaturation conditions were as follows: Dilution of unfolded and reduced rhBMP-2 with a final concentration of 0.1 mg mL^{-1} rhBMP-2 in standard renaturation buffer." *Id.*at [0054]; Ex. 1002 at ¶150. Reduced and oxidized glutathione are a reductant and an oxidant, respectively. Ex. 1001 at 7:20-25.

Therefore, both Ruddon and Vallejo disclose contacting a protein with a preparation that supports the renaturation of a protein to a biologically active form, to form a refold mixture as described in claims 1 and 10. And both Ruddon and Vallejo disclose preparing a solution comprising the proteins and the other buffer components described in claims 16 and 26; Ex. 1002 at ¶151.

c. Components of the mixture

Ruddon further teaches "a preferred thiol redox buffer for use in refolding bacterially expressed glycoprotein hormone subunits comprises, e.g., 6.4 mM cysteamine and 3.6 mM cystamine in 50 mM Tris-HCl, pH 8.7." Ex. 1040 at

26:34-27:1. In the redox buffer of Ruddon, Tris-HCl is a protein stabilizer. Ex. 1002 at ¶152; *also* Ex. 1001 at 5:50-59 (identifying Tris as a protein stabilizer). Ruddon teaches contacting a protein with a redox buffer, such as the "preferred thiol redox buffer" described above. Ex. 1040 at 28:24-32.

Vallejo discloses a method of refolding a protein using a refolding buffer comprising an aggregation suppressor, a mixture of reduced and oxidized glutathione and a solubilizing chaotropic agent. Ex. 1038 at [0001]; Ex. 1002 at ¶153.

Vallejo discloses "The standard renaturation buffer contains 0.5 mol L^{-1} Gdn-HCl, 0.75 mol L^{-1} CHES and 1 mol L^{-1} NaCl (#3233). These additives are known to effect protein stability and the aggregation propensity during refolding." Id. at [0047]. Vallejo further teaches that the aggregation suppressor can be arginine. Id. at [0021]. The '287 Patent describes arginine as both an aggregation suppressor and a protein stabilizer. Ex. 1001 at 5:41-58. Therefore, Vallejo teaches using an aggregation suppressor and a protein stabilizer in the preparation. Ex. 1002 at ¶154. Further, Vallejo describes a chaotropic agent as "any substance that disturbs the three-dimensional structure of the hydrogen bonds in water." Ex. 1038 at [0047]. The '287 Patent describes a "denaturant" as: "any compound having the ability to remove some or all of a protein's secondary and tertiary

structure when placed in contact with the protein." Ex. 1001 at 5:31-34. A POSA would understand the definition of a chaotropic agent as described by Vallejo to be consistent with a "denaturant" as defined by the '287 Patent. Ex. 1002 at ¶¶154-156.

Therefore, Ruddon teaches a refold buffer containing at least a protein stabilizer, and Vallejo teaches a refold buffer containing an aggregation suppressor, protein stabilizer, and a denaturant. Ex. 1002 at ¶157.

d. Redox Components

Ruddon teaches "a preferred thiol redox buffer for use in refolding bacterially expressed glycoprotein hormone subunits comprises, e.g., 6.4 mM cysteamine and 3.6 mM cystamine in 50 mM Tris-HCL, pH 8.7." Ex. 1040 at 26:34-27:1. Ruddon describes creating stock solutions containing the redox components, and Ruddon indicates that they have varied the amounts of redox components to find the optimum conditions. Ex. 1040 at 34:18-20. Ex. 1002 at ¶158.

Ruddon expressly discloses the equation of the thiol-pair ratio described in the '287 Patent, and teaches that its redox buffers have ratios within the range required by the claims:

> 10X stock solutions consisted of 17.3 mM cysteamine and 2.7 mM cystamine, or 63.7 mM cysteamine and 36.3 mM

> cystamine. All 10X redox buffers were prepared in 10 mM HCl to slow the rate of air oxidation. After dilution to 1X, *the value of [reductant]^2/[oxidant] was maintained at 11.1 mM* in both the 2 mM and 10 mM buffers (final concentration)…*We have varied the value of [reductant]^2/(oxidant] and found optimum folding of hCG-β to occur between values of 2 and 40 mM.*

Ex. 1040 at 33:32-34:21 (emphasis added); Ex. 1002 at ¶159.

Vallejo discloses that "[f]or renaturation of disulfide-bonded proteins, mixtures of reduced and oxidized glutathione are employed to allow disulfide-bond reshuffling until the most stable disulfide-bond structures are obtained, in general the native state of the protein." Ex. 1038 at [0045]. Further, Vallejo discloses that standard renaturation conditions contained a final concentration of "3 mmol L^{-1} total glutathione in a 2:1 ratio of glutathione reduced to glutathione oxidized (GSH:GSSG)." Id. at [0054]. Reduced glutathione (GSH) and oxidized glutathione (GSSG) are a reductant and an oxidant, respectively. Ex. 1001 at 7:20-25. Ex. 1002 at ¶¶160-161.

Vallejo discloses a GSH to GSSG ratio of 40:1 to 1:20. Ex. 1038 at Fig. 2b. A POSA would understand these ratios to be molar ratios and would understand that this is a simple ratio of [reductant]/[oxidant]. Ex. 1002 at ¶162. The '287 Patent defines the thiol-pair ratio as [reductant]^2/[oxidant]. Therefore, the

calculated thiol-pair ratio of Vallejo is 0.05 to 1600.[13] *Id*. Therefore, Vallejo discloses a thiol-pair ratio within the range of 0.001-100.

Both Ruddon and Vallejo appreciate that it is the balance or relationship between the amount of reductant and the amount of the oxidant that allow for the disulfide-bonds to reshuffle until the protein is properly refolded. Ex. 1002 at ¶163.

And both Ruddon and Vallejo teach that the thiol-pair buffer strength maintains the solubility of the preparation (claims 1 and 10) and the solution (claims 16 and 26). Both Ruddon and Vallejo teach that its methods results in properly refolded proteins. Ex. 1040 at 52:22-25; Ex. 1038 at [0012]. This result would not be possible unless the redox components maintained the solubility of the protein while the protein refolded. Ex. 1002 at ¶164.

e. Incubating the refold mixture

Ruddon teaches incubating the refold mixture to allow the protein to refold:

> …an appropriate concentration of unfolded subunit is added to an aliquot of buffer, and incubated at a suitable temperature (i.e. 22-28°C) for a pre-

---

[13] The ‘287 patent teaches the thiol-pair ratio as $[\text{reductant}]^2/[\text{oxidant}] = [\text{GSH}]^2/[\text{GSSG}] = [40]^2/[1] = 1600$ and $[1]^2/[20] = 0.05$.

> determined amount of time to enable the subunits to fold.

Ex. 1040 at 28:24-32; Ex. 1002 at ¶165.

Ruddon further teaches adding urea to the refolding buffer, and the resulting "folding efficiency was found to be 40-60% in the presence of 2M urea." Ex. 1040 at 51:2-3. A POSA would understand that the "folding efficiency" would mean the yield of properly refolded protein. This is consistent with the description of Fig. 5, lanes 8-12 at 50: 32-36, where the "percent folded protein" was determined by the refolded protein band intensity on non-reducing SDS gel electrophoresis. Ex. 1002 at ¶166.

Vallejo teaches that the optimization of the refolding conditions allowed for a refolding yield of 44%. Vallejo also teaches that the concentration of protein could be increased in the refolding mixture and the yield of active protein would stay in the range of 33-38%. Vallejo concludes that its method could be used for commercial production. Ex. 1038 at [0012]; Ex. 1002 at ¶167. A POSA would understand that the "renaturation yield" would mean the yield of properly refolded protein. Ex. 1002 at ¶168.

One way a POSA would know whether a protein was properly refolded to its native form would be to determine if it regained the biological activity of the native form of the protein. Ex. 1002 at ¶169. The '287 Patent confirms this

understanding, teaching that the non-native form of the protein lacks structural features making it biologically inactive. Ex. 1001 at 7:66-8:10.

Ruddon teaches that its refolded rehCG-β is biologically active. Ex. 1040 at 52:22-25 ("These *in vitro* and *in vivo* results indicate that rehCG-β folded and assembled with hCG-α in a conformation very similar to that of glycosylated hCG-β that is made in human cells.") Ex. 1002 at ¶170.

Vallejo teaches that its refolded recombinant rhBMP-2 is biologically active: "[t]he final conentration [sic] of *dimerized active rhBMP-2* reached 0.7 to 0.8 mg/ml corresonding [sic] to a final yield of 32 to 38%." Ex. 1038 at [0049]; *also Id.* at [0056] ("Biological activity of rhBMP-2 was analyzed by alkaline phosphatase induction in C2C12 cells (ATCC-1772) as described previously"); Ex. 1002 at ¶171.

2. Claims 2, 3, 11, 13, 17, 18, 27 and 28

Claims 2 and 3; 11 and 13; 17 and 18; and 27 and 28 depend directly on claims 1, 10, 16 and 26, respectively, and recite particular limitations relating to the concentration of protein in the refold mixture or solution. Ex. 1037.

The method of Ruddon is equivalent to what Vallejo describes as a "batch" addition of the protein to the refold buffer. Ex. 1040 at 28:24-32 (explaining that the protein is added at one time to the redox buffer). The method further describes

the amount of protein present in the refold mixture to be at a relatively low concentration to allow the protein to refold. Ex. 1040 at 28:32-29:1 ("*in vitro* folding of glycoprotein hormone subunits is facilitated by adding unfolded subunit to redox buffer at dilute concentrations of the protein (e.g., 0.02 to 0.05 mg/ml)"). Ex. 1002 at ¶173.

A POSA looking to refold the protein of Ruddon at higher concentrations would look to the method of Vallejo, and in particular, the pulsed refolding process described by Vallejo. Ex. 1002 at ¶174. Vallejo teaches that its pulsed refolding process allows for refolding of the protein present in the refold mixture at a concentration of 2.1 mg/mL. Ex. 1038 at [0012]. Like Vallejo, Ruddon is refolding a large protein that has a cystine-knot and numerous disulfide bonds. Ex. 1040 at 26:14-18; and Ex. 1038 at [0008]. A POSA would expect that the pulsed refolding process described by Vallejo used on a cystine-knot protein, would allow for refolding of the cystine-knot protein of Ruddon at higher protein concentrations – because Vallejo explicitly teaches so. Ex. 1002 at ¶174; *also* Ex. 1038 at 3:54-4:1 ("A further increase in the concentration of rhBMP-2 was achieved by a pulsed refolding process").

As described above, the pulsed refolding process of Vallejo involves adding the protein to the refolding buffer in several steps. Because the claims of the '287

Patent do not require that the proteins be added to a refold buffer in one step, this pulsed refolding process would satisfy the element of claims 1 and 10 ("contacting the proteins with a preparation… to form a refold mixture") and also the element of claims 16 and 26 ("preparing a solution comprising: the proteins; …at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer, an amount of an oxidant, and an amount of a reductant…"). Ex. 1002 at ¶175.

3. Claims 4, 12, 19 and 29

Claims 4, 12, 19 and 29 depend directly on claims 1, 10, 16 and 26 respectively and recite that "the thiol-pair buffer strength is 2 mM or greater."

Ruddon teaches "a preferred thiol redox buffer for use in refolding bacterially expressed glycoprotein hormone subunits comprises, e.g., 6.4 mM cysteamine and 3.6 mM cystamine in 50 mM Tris-HCL, pH 8.7." Ex. 1040 at 26:34-27:1. A POSA would understand the cysteamine to be the reductant and the cystamine to be the oxidant in the redox buffer. Thus, the thiol-pair buffer strength of the redox buffer of Ruddon is 2[oxidant]+[reductant] = 2[3.6] + [6.4] = 13.6 mM. Ex. 1002 at ¶177.

Vallejo discloses that standard renaturation conditions contained a final concentration of "3 mmol L^{-1} total glutathione." *Id.* at [0054]. A POSA in 2009,

would understand that mmol L^{-1} is equivalent to mM. A POSA would also understand that when Vallejo describes the final concentration of glutathione, it is describing what the '287 Patent identifies as the thiol-pair buffer strength. Ex. 1002 at ¶178. To calculate the total glutathione ([GSH]) the concentration of GSSG ([GSSG]) must be doubled because when reduced, one GSSG forms two GSH molecules. The '287 Patent defines the thiol-pair buffer strength as 2[oxidant] + [reductant] which in Vallejo equals 2[GSSG] + [GSH]. A POSA would understand that the thiol-pair buffer strength is simply the total glutathione concentration because the term 2[GSSG] is equal to the concentration of reduced GSH. Therefore, when Vallejo teaches a final concentration of glutathione of 3mmol L^{-1} (i.e. 3 mM), it is also disclosing a thiol-pair buffer strength greater than 2 mM. Ex. 1002 at ¶178.

4. Claims 7 and 22

Claims 7 and 22 depend directly on claim 1 and 16, respectively, and recite that "at least one of the proteins is a complex protein."

Ruddon discloses a method of refolding a glycoprotein hormone that contains two subunits (α and β). The α subunit (~15 kDa) contains five disulfide bonds, and the β subunit (~22 kDa) contains six disulfide bonds. As an example, Ruddon teaches the refolding of glycoprotein hormone chorionic gonadotropin

which is 36 kDa. Ex. 1047. The glycoprotein hormones described in Ruddon all have a cystine-knot, like the proteins described in Vallejo. Ex. 1040 at 26:15-18. Ex. 1002 at ¶180.

Vallejo discloses a method of refolding dimeric rhBMP-2. *Id.* at [0049]. Dimeric rhBMP-2 is ~26 kDa and has 7 disulfide bonds. Ex. 1038 at [0003] and Fig. 1; Ex. 1002 at ¶181. Vallejo further teaches that its method ca be used to refold other cysteine-knot proteins such as "BMPs, PDGFs, human nerve growth factors, TGF-β, VEGF, GDF-5." These proteins are all cysteine-knot proteins and share characteristics with rhBMP-2. Ex. 1002 at ¶181. They are also all complex proteins as defined by the '287 Patent. Section VI.D.3.a. above.

Therefore, both Ruddon and Vallejo teach a method of refolding a complex protein as defined by the '287 Patent.

5. Claims 8, 9, 14, 15, 23, 24, 25 and 30

Dependent claims 8, 9, 14, 15, 23, 24, 25, and 30 recite either the equation for the thiol-pair ratio, the thiol-pair buffer strength, or both. Ex. 1037.

Both Ruddon and Vallejo teach the refolding of a protein with concentrations of reductant and oxidant falling within the ranges of the claims. Ruddon further teaches the equation for the thiol-pair ratio explicitly claimed in claims 8, 14, 15, 23, 25, and 30. The dependent claims specifying that the thiol-

pair ratio and the thiol-pair buffer strength are calculated according to the equations listed in the specification are similarly rendered obvious for the same reasons described above for claims 1, 10, 16, and 26. That Patent Owner decided to explicitly identify the equations in the dependent claims does not change the way in which a determination is made as to whether a prior art reference reads on the claim—by simply determining whether the prior art teaches a thiol-pair ratio and a thiol-pair buffer strength within the range of the claims. Ex. 1005 at 28; Ex. 1002 at ¶184.

G. Ground 7: Claims 5, 6, 20, and 21 Are Unpatentable Over Vallejo in view of Hevehan

Claims 5 and 20 depend directly on claims 1 and 16, respectively, and recite that "the thiol-pair buffer strength is increased proportionally to an increase in a total protein concentration in the refold mixture." Claims 6 and 21 depend directly on claims 1 and 16, respectively, and recite that "the thiol-pair buffer strength is decreased proportionally to a decrease in a total protein concentration in the refold mixture." These claims are obvious in view of the teachings of Vallejo and Hevehan.

As discussed above in section VI.D.3.a., Vallejo anticipates claims 1-4, 7-19, and 22-30. Vallejo describes a method for refolding a cystine-knot protein containing disulfide bonds. Vallejo recognizes that in order to refold proteins

containing disulfide bonds an appropriate redox system must be used. Ex. 1002 at ¶186; Ex. 1038 at [0045].

A POSA would have understood, based on the teaching of Hevehan, that the buffer used for refolding needs to be adjusted depending on the concentration of protein to be refolded. Ex. 1024 at 1. Hevehan describes the impact of protein concentration on refolding, and the increase in aggregation as protein concentration increases. Ex. 1002 at ¶187; Ex. 1024 at 1. Further, Hevehan recognizes the importance of a redox system when refolding a protein containing disulfide bonds, teaching that it is important to choose the "right mixture of low molecular weight thiol components in oxidized and reduced forms… to allow disulfide bond formation and shuffling." Ex. 1002 at ¶187; Ex. 1024 at 5.

In its investigation to identify the optimal thiol concentration for the proper refolding of proteins containing disulfide bonds, Hevehan states that "[p]revious studies have indicated that optimum thiol concentrations in the renaturation buffer are 0.8–8 mM reduced glutathione (GSH) and 0.04–0.4 mM oxidized glutathione (GSSG) (Saxena and Wetlaufer, 1970)." Ex. 1002 at ¶188; Ex. 1024 at 5. These redox conditions correspond to a thiol-pair buffer strength of: 2[0.4] + 8 = 8.8 mM. Ex. 1002 at ¶189.

Appreciating that the previously described redox conditions may not be optimal at higher protein concentrations, Hevehan sought to identify the optimal redox conditions for higher protein concentrations. Ex. 1002 at ¶190; Ex. 1024 at 5. Hevehan found that the highest yields for refolding hen egg-white lysozyme at a concentration of 1 g/L was obtained using 8 mM GSH and in the presence of 4 mM DTT. As Dr. Robinson explains, this would rapidly convert to 4 mM GSSG, 8 mM GSH, and 4 mM DTT oxidized yielding an equivalent thiol-pair buffer strength of 2[4] + 8 = 16 mM. Ex. 1002 at ¶190; Ex. 1024 at 5; Ex. 1005 at 27. Thus, Hevehan found that the optimal thiol-pair buffer strength for refolding hen egg-white lysozyme at higher concentrations was higher than what was previously described in the literature. Ex. 1002 at ¶190.

Based on the teaching of Hevehan, a POSA would reasonably expect that the thiol-pair buffer strength would need to be increased when refolding higher concentrations of a protein containing one or more disulfide bonds. Likewise, when refolding a protein at lower concentrations, a POSA would recognize that the optimal thiol-pair buffer strength would be lower. Ex. 1002 at ¶191. When the protein concentration is increased, the concentration of disulfide bonds also increases, thus a POSA would understand that the thiol-buffer strength would need to be increased to compensate for these changes. And a POSA would appreciate

that these teachings of Hevehan would be applicable to the refolding method taught by Vallejo, also a method for refolding a protein containing disulfide bonds. Ex. 1002 at ¶191. Thus, claims 5, 6, 20, and 21 are unpatentable over the combination of Vallejo and Hevehan.

H. Objective Indicia of Nonobviousness Do Not Save the '287 Patent

Patent Owner did not allege any secondary considerations during prosecution. Patent Owner may attempt to avoid a finding of obviousness by asserting the secondary considerations now. However, any assertions of secondary considerations that Patent Owner could make would not support patentability. Moreover, although secondary considerations must be taken into account, they do not necessarily control the obviousness conclusion. *Newell Cos., Inc. v. Kenney Mfg. Co.*, 864 F.2d 757, 768 (Fed. Cir. 1988). And in cases where a strong obviousness showing exists—such as is the case here—the Federal Circuit has repeatedly held that even relevant secondary considerations supported by substantial evidence may not dislodge the primary conclusion of obviousness. *E.g.*, *Leapfrog Enterprises Inc. v. Fisher-Price Inc.*, 485 F.3d 1157, 1162 (Fed. Cir. 2007).

I. Ground 8: Claims 1-15 are indefinite

Should the Board find that the term "wherein the thiol-pair buffer strength maintains the solubility of the preparation" be interpreted to mean anything other

than that the thiol-pair buffer strength maintains the solubility of the proteins, then claims 1-15 are indefinite.

As set forth above in Section VII.B, when read in light of the specification, the term "wherein the thiol-pair buffer strength maintains the solubility of the preparation" should properly be interpreted to mean that the thiol-pair buffer strength maintains the solubility of the *proteins* when the proteins contact the preparation, forming the refold mixture. However, the plain language of the claim makes clear that the preparation itself does not contain the proteins because it expressly defines the preparation in claims 1 and 10, as comprising "at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer," "an amount of oxidant," and "an amount of reductant." The claim further requires "contacting the proteins" with that "preparation" to form a "refold mixture." Read in this light, the meaning of the phrase "wherein the thiol-pair buffer strength maintains the solubility of the preparation" is unclear. It is not clear to one of ordinary skill which of the ingredients of the preparation is the solvent and which is the solute.[14] It is not

[14] The PTAB applies the *Packard* indefiniteness standard in post-grant proceedings. *Telebrands Corp. v. Tinnus Enterprise, LLC*, PGR2015-00018, paper (Continued...)

clear from the specification how the thiol-pair buffer strength maintains such solubility. Ex. 1002 at ¶¶192-193.

X. CONCLUSION

For the foregoing reasons, challenged claims 1-30 of the '287 Patent recite subject matter that is unpatentable. Therefore, Petitioners respectfully request institution of this post-grant review to cancel these claims.

Respectfully submitted,

October 1, 2018

/Teresa Stanek Rea/

Teresa Stanek Rea
Reg. No. 30,427
Deborah H. Yellin
Reg. No. 45,904
Shannon Lentz
Reg. No. 65,382
CROWELL & MORING LLP
Intellectual Property Group
P.O. Box 14300
Washington, DC 20044-4300

75, at 16-18. (December 30, 2016), referencing *In Re Packard*, 751 F.3d 1307, 1310 (Fed. Cir. 2014).

CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24(d), I hereby certify that this Petition complies with the type-volume limitation of 37 C.F.R. § 42.24(a)(1)(ii) because it contains 16,902 words as determined by the Microsoft® Office Word 2010 word-processing system used to prepare the brief, excluding the parts of the brief exempted by 37 C.F.R. § 42.24(a)(1).

/Teresa Stanek Rea/
Teresa Stanek Rea

CERTIFICATION OF SERVICE ON PATENT OWNER

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.205, the undersigned certifies that on October 1, 2018, a complete copy of the foregoing Petitioners' Petition for Post-Grant Review of U.S. Patent No. 9,856,287, Power of Attorney, and all supporting exhibits were served via FedEx® on the Patent Owner by serving the correspondence address of record for the '287 Patent:

AMGEN INC.
Law – Patent Operations, M/S 35-1-B
One Amgen Center Drive
Thousand Oaks, CA 91320-1799

/Shannon Lentz/
Shannon Lentz