UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

FRESENIUS KABI USA, LLC and FRESENIUS KABI SWISSBIOSIM GmbH
Petitioners,
v.

AMGEN, INC. and AMGEN MANUFACTURING LIMITED
Patent Owner.

IPR2019-00971

Patent No. 9,856,287

Title: REFOLDING PROTEINS USING A CHEMICALLY CONTROLLED REDOX STATE

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,856,287 B1

Mail Stop PATENT BOARD
Patent Trial and Appeal Board
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

TABLE OF CONTENTS

Page

TABLE OF CONTENTS
(Continued)

Page

TABLE OF AUTHORITIES

Page(s)

Cases

PTAB Decisions

Statutes

Regulations

LIST OF EXHIBITS

EXHIBIT NO.	DESCRIPTION
1001	United States Patent No. 9,856,287
1002	Declaration of Professor Paul A. Dalby, Ph.D.
1003	United States Patent No. 4,237,224
1004	United States Patent No. 4,468,464
1005	United States Patent No. 4,740,470
1006	Curriculum Vitae of Professor Paul A. Dalby, Ph.D.
1007	De Bernardez Clark, E. *et al*., "Oxidative Renaturation of Hen Egg-White Lysozyme. Folding vs Aggregation," Biotechnology Progress 14(1):47-54 (1998)
1008	De Bernardez Clark, E., "Refolding of recombinant proteins," Current Opinion in Biotechnology 9:157-163 (April 2001)
1009	Ejima, D. *et al*., "High Yield Refolding and Purification Process for Recombinant Human Interleukin-6 Expressed in *Escherichia coli*," Biotechnology and Bioengineering, 62(3):301-310 (February 1999)
1010	Excerpts of United States Patent No. 9,856,287 File History
1011	Ferrer-Miralles, N. *et al*., "Microbial factories for recombinant pharmaceuticals," Microbial Cell Factories 8:17 (2009)
1012	Georgiou, G. & Valax, P., "Isolating Inclusion Bodies from Bacteria," Methods in Enzymology 309:48-58 (1999)
1013	Gilbert, H., "Molecular and Cellular Aspects of Thiol-Disulfide Exchange," in *Advances in Enzymology and Related Areas of Molecular Biology*, ed. Alton Meister, Vol. 63, pp. 69-172 (John Wiley & Sons 1990)
1014	Gilbert, H., "Thiol/Disulfide Exchange Equilibria and Disulfide Bond Stability," in *Methods in Enzymology*, ed. Lester Packer, Vol. 251, pp. 8-28 (Academic Press 1995)
1015	Graumann, K. & Premstaller, A., "Manufacturing of recombinant therapeutic proteins in microbial systems," Biotechnology Journal 1:164-186 (2006)

EXHIBIT NO.	DESCRIPTION
1016	Hevehan, D, & Clark, E., "Oxidative Renaturation of Lysozyme at High Concentrations," Biotechnology and Bioengineering, 54(3):221-230 (May 1997)
1017	Horton, R. *et al*., *Principles of Biochemistry* (Pearson Education, 4th ed., 2006)
1018	Jungbauer, A. & Kaar, W., "Current status of technical protein refolding," Journal of Biotechnology 128:587-596 (2007)
1019	Keire, D. *et al*., "Kinetics and Equilibria of Thiol/Disulfide Interchange Reactions of Selected Biological Thiols and Related Molecules with Oxidized Glutathione," J. Org. Chem. 57(1):123-127 (1992)
1020	Neubauer, P. *et al*., "Protein Inclusion Bodies in Recombinant Bacteria," in *Inclusions in Prokaryotes*, ed. J.M. Shively, pp. 237-292 (Springer-Verlag Berlin Heidelberg 2006)
1021	Palmer, I. & Wingfield, P., "Preparation and Extraction of Insoluble (Inclusion-Body) Proteins from *Escherichia coli*, Curr Protoc Protein Sci. Chapter: Unit-6.3 (November 2004)
1022	Panda, A., "Bioprocessing of Therapeutic Proteins from the Inclusion Bodies of *Escherichia coli*," Adv Biochem Engin/Biotechnol 85:43-93 (2003)
1023	Patra, A. *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)
1024	Profacgen, "Inclusion body purification & protein refolding," accessed at https://www.profacgen.com/inclusion-body-purification-protein-refolding.htm
1025	International Publication No. WO 95/32216
1026	Ryan, R. *et al*., "Structure-Function Relationships of Gonadotropins," in *Recent Progress in Hormone Research*, Vol. 43, pp. 383-429 (Academic Press 1987)
1027	Schafer, F. & Buettner, G., "Redox Environment of the Cell as Viewed Through the Redox State of the Glutathione Disulfide/Glutathione Couple," Free Radical Biology & Medicine 30(11):1191-1212 (June 2001)
1028	United States Patent Application Publication No. 2007/0238860

EXHIBIT NO.	DESCRIPTION
1029	Singh, S. & Panda, A., "Solubilization and Refolding of Bacterial Inclusion Body Proteins," Journal of Bioscience and Bioengineering 99(4):303-310 (2005)
1030	Vallejo, L. & Rinas, U., "Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins," Microbial Cell Factories 3:11 (2004)
1031	European Patent Application No. 1 449 848 A1, Method for the production of cystine-knot proteins (2004)
1032	Ventura, S. & Villaverde, A., "Protein quality in bacterial inclusion bodies," Trends in Biotechnology 24(4):179-185 (April 2006)
1033	Wetlaufer, D. *et al*., "The oxidative folding of proteins by disulfide plus thiol does not correlate with redox potential," Protein Engineering 1(2):141-146 (1987)
1034	Whitford, D., *Proteins: Structure and Function* (John Wiley & Sons 2005)
1035	Peptides Guide, "What are Proteins?" accessed at http://www.peptidesguide.com/proteins.html
1036	Xie, Y. *et al*., "Recombinant Human Retinol-Binding Protein Refolding, Native Disulfide Formation, and Characterization," Protein Expression and Purification 14:31-37 (1998)
1037	Patent Owner's Preliminary Response Under 37 C.F.R. § 42.207, dated January 23, 2019
1038	Final Written Decision in IPR2016-01542, Patent 8,952,138, dated February 15, 2018
1039	Archer, D. *et al*., "Hen Egg White Lysozyme Expressed In, and Secreted from, *Aspergillus Niger* is Correctly Processed and Folded," Bio/Technology 8:741-745 (August 1990)
1040	United States Patent No. 5,663,304
1041	United States Patent No. 8,952,138
1042	De Bernardez Clark, E., "Protein refolding for industrial processes," Current Opinion in Biotechnology 12(2):202-207 (1998)
1043	Atassi, M.Z., "Chemical Strategy for Studying the Antigenic Structures of Disulfide-Containing Proteins: Hen Egg-White

EXHIBIT NO.	DESCRIPTION
	Lysozyme as a Model," in *Protein Crosslinking*, ed. M. Friedman, Vol. 6, pp. 89-137 (Plenum Press 1977)
1044	Table of categorized claims for United States Patent No. 9,856,287
1045	"Glutathione" in *The Merck Index*, *12th Ed.*, pp. 4483-4484 (Merck Research Laboratories 1996)
1046	Middleberg, A., "Preparative protein folding," TRENDS in Biotechnology 20(10):437-443 (October 2002)

I. INTRODUCTION

Fresenius Kabi USA, LLC and Fresenius Kabi SwissBioSim GmbH, pursuant to 35 U.S.C. §§ 311-319 and 37 C.F.R. § 42, *et seq.*,[1] petition for *Inter Partes* Review ("IPR") of claims 1, 4-6, 8-10, 12, 14-16, 19-21, 23-26, 29-30 of U.S. Patent No. 9,856,287 ("the '287 patent," Ex. 1001). Petitioners' request is supported by the Expert Declaration of Paul Dalby, Ph.D. (Ex. 1002) and the other exhibits submitted herewith.

The challenged claims of the '287 patent are generally directed to methods of refolding proteins expressed in non-mammalian cells. Unfolded proteins are incubated in a buffer containing, among other ingredients, amounts of an oxidant and a reductant that permit the proteins to refold into their native three-dimensional structure. This basic "redox" refolding method was in common use as of June 22, 2009, the earliest possible filing date of the patent, and scientists routinely tailored the compositions of their redox buffers to optimize the yield of properly refolded proteins. In particular, it was understood that for a given protein, the yield could be optimized in part by varying the ratio and strength of the oxidant and reductant (*i.e.*, thiol pair) to determine which combinations produced the highest yield at a

[1] Unless otherwise stated, all statutory and regulatory citations herein are to 35 U.S.C. or 37 C.F.R.

given protein concentration. As explained by Dr. Dalby, this optimization was routine and well within the scope of ordinary skill in 2009.

While the '287 patent purports to disclose novel mathematical equations to calculate thiol pair ratio and buffer strength, it cannot be disputed that the specific ranges of thiol pair ratio and buffer strength that appear in the claims encompass ratios and strengths described in the prior art. Moreover, the equations themselves are not novel. They express basic redox chemistry principles and were expressly disclosed and used in the prior art. Even if the equations had not been written down in the prior art, a mathematical equation does not make a claim patentable where "its 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." *Apotex Inc. v. Amgen Inc.*, IPR 2016-01542, Paper 60 at 29 (P.T.A.B. Feb. 15, 2018) (Ex. 1038).

As described below, each of the challenged claims is anticipated by the prior art. To the extent a single reference does not disclose every element of every claim, every element was disclosed in the prior art and there was a motivation to combine these elements, rendering the claimed subject matter obvious from that art as a whole. Petitioners are not aware of any relevant secondary evidence of non-obviousness.

The Board should institute review because there is at least a reasonable likelihood that Petitioners will prevail with respect to at least one challenged claim. § 314(a). Moreover, there are no persuasive grounds for denying institution under § 314(a) or § 325(d). This is Petitioners' first petition challenging any claim of the '287 patent, and the petition in part raises arguments that have not previously been presented to the Office.

The required fee set forth in § 42.15(a) is paid pursuant to § 42.103, and the Commissioner is hereby authorized to charge all fees due in connection with this matter to Attorney Deposit Account 506989.

II. GROUNDS FOR STANDING

Pursuant to § 42.104(a), Petitioners certify that the '287 patent is available for IPR and that Petitioners are not barred or estopped from requesting IPR on the grounds raised in this petition. In accordance with § 311(c), more than 9 months have passed since issuance of the '287 patent and no post-grant review ("PGR") has been instituted.[2] Moreover, neither Petitioners nor their privies or the real

[2] As disclosed in section III(b), *infra*, the '287 patent is the subject of a petition for PGR filed in PGR2019-00001 by Adello Biologics, LLC and others. Because the Board has not instituted review, Petitioners need not wait until termination of PGR2019-0001 before filing this petition for IPR. *Cf.* § 311(c) ("if a post-grant review ***is instituted*** . . . , [a petition for IPR shall be filed after . . .] the date of the

parties in interest have filed or been served with any complaint alleging infringement or invalidity of the ’287 patent, and therefore are not subject to any bar under § 315(a) or (b).

III. MANDATORY NOTICES

A. Real Parties In Interest (§ 42.8(b)(1))

The real parties in interest are Fresenius Kabi USA, LLC, Fresenius Kabi SwissBioSim GmbH, Fresenius Kabi AG, Fresenius Kabi Pharmaceuticals Holding, Inc., Fresenius Kabi Deutschland GmbH, Fresenius SE & Co. KGaA, Dr. Reddy’s Laboratories, Ltd., Dr. Reddy’s Laboratories, S.A., and Dr. Reddy Laboratories Inc.

B. Related Matters (§ 42.8(b)(2))

The ’287 Patent is currently the subject of the following litigations and post-grant proceedings: *Amgen Inc. et al. v. Adello Biologics LLC*, 2:18-cv-03347 D.N.J.; *Amgen Inc. et al. v. Apotex Inc. et al.*, 19-cv-61828, S.D. Fla; and *Adello Biologics, LLC. et al. v. Amgen Inc.*, PGR-2019-00001 (P.T.A.B.).

---

termination of such post-grant review”); *see also* § 42.101. Moreover, if the ’287 patent does not fall under section 2(n)(1) of the Leahy-Smith America Invents Act, as the Board must determine in PGR2019-0001, then an IPR may be filed at any time after the date of the grant of the patent. *See* Pub. L. 112–274, § 1(d)(1), Jan. 14, 2013, 126 Stat. 2456; 37 C.F.R. § 42.102.

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

C. Identification of Counsel (§ 42.8(b)(3)) and Service Information (§ 42.8(b)(4))

Lead Counsel	Back-Up Counsel	Back-Up Counsel
Huiya Wu (Reg. No. 44,411) Goodwin Procter LLP, 620 Eighth Avenue, New York, NY 10018, T: (212) 813-7295 Fax: (212) 355-3333 hwu@goodwinlaw.com	Robert V. Cerwinski (to seek *pro hac vice* admission) Goodwin Procter LLP, 620 Eighth Avenue, New York, NY 10018, T: (212) 813-8800 Fax: (212) 355-3333 rcerwinski@goodwinlaw.com	Linnea Cipriano (Reg. No. 67,729) Goodwin Procter LLP, 620 Eighth Avenue, New York, NY 10018, T: (212) 813-7295 Fax: (212) 355-3333 lcipriano@goodwinlaw.com

Please direct all correspondence to lead counsel and back-up counsel at the contact information above. Petitioners consent to electronic mail service at the following addresses: hwu@goodwinlaw.com; rcerwinski@goodwinlaw.com; lcipriano@goodwinlaw.com.

IV. TECHNOLOGY BACKGROUND

A. The Basic Science of Proteins

1. Protein Structure

Protein molecules must fold into precise three-dimensional shapes in order to be biologically active. Ex. 1002 ¶ 42. The biologically-active form of a protein is known as the "native" form. *Id*. Usually the native form is the most thermodynamically stable way of folding the particular sequence of amino acids

that make up the protein. *Id*. Thus, under appropriate conditions, proteins will automatically fold into their native forms. *Id*.

For many proteins, their native three-dimensional structure is stabilized by "disulfide" bonds that cross-link different parts of the folded polypeptide chain. Disulfide bonds form between particular amino acids called "cysteines" when they come into close proximity during refolding and help lock the protein into its native shape . Ex. 1002 ¶ 43; Ex. 1006 at 32-33. However, if disulfide bonds form in improper locations, the proteins can misfold. Misfolded proteins can be inactiv

2. Protein Synthesis in and out of the Lab

In nature, organisms create proteins through the processes of transcription (DNA is used to make RNA) and translation (RNA is used to make the protein) Ex. 1002 ¶ 44; Horton (Ex. 1017) at 683-711. This natural machinery can be harnessed to make commercial amounts of protein using "recombinant" DNA technology, which has been known in the art since at least the 1970s. Ex. 1002 ¶ 45. In these methods, host cells in a cell culture are turned into "factories" for manufacturing proteins of interest by inserting a segment of recombinant DNA that encodes the protein into the host cells. Ex. 1002 ¶ 46; Ex. 1017 at 719-23.

Recombinant DNA technology can be used with both mammalian and non-mammalian cell cultures (often referred to as "expression systems"), but scientists have generally turned to high-yield bacterial expression systems to express

recombinant proteins at a lower cost. *Id.*; Ex. 1002 ¶ 47. One well-established bacterial expression system is *Escherichia coli,* commonly referred to as *E. coli*. Ex. 1002 ¶ 48.

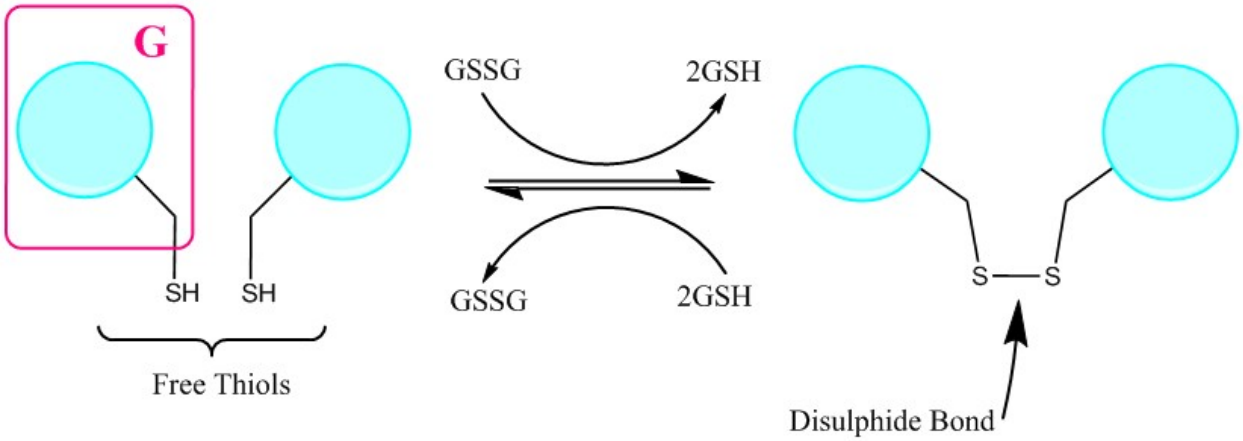
B. Recovery and Refolding of Expressed Protein

A bacterial host cell expressing recombinant proteins often produces misfolded proteins that aggregate together into "inclusion bodies" in the cell. In particular, recombinant proteins expressed in *E. coli* were known to form inclusion bodies. Neubauer (Ex. 1020) at 244-47; Georgious (Ex. 1012) at 57-58. To make the proteins more usable, techniques for recovering native, folded proteins in a bioactive and stable form from those inclusion bodies were developed. The most common techniques follow a process in which proteins that are isolated and purified from inclusion bodies are (1) solubilized, causing the proteins to unfold; and (2) refolded in a refolding buffer. *See* Ex. 1002 ¶ 52.

1. Unfolding and Refolding of Recombinant Proteins

During solubilization, the inclusion bodies are "denatured." The bonds and other forces holding the aggregated proteins together are disrupted by chemical denaturants, which causes the proteins to unfold into single strands of polypeptide. Ex. 1002 ¶ 53; Ex. 1015 at 267-68; Ex. 1021 at 12-13. Disulfide bonds are often broken or "reduced" via a redox reaction. For example, two molecules of reduced glutathione ("GSH") will reduce a disulfide bond 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.



Ex. 1002 ¶ 54. Importantly, this process can be reversed during refolding of the protein: the GSSG can oxidize the free thiols and re-form the disulfide bond, as shown in the reaction from left to right in the above diagram.

Refolding of the denatured solubilized proteins is generally accomplished with a "refold buffer." Ex. 1002 ¶ 57. A refold buffer generally includes a number of components, including denaturants, aggregate suppressors, protein stabilizers, oxidants, and reductants, each of which may be adjusted to optimize the efficiency of the refolding. Ex. 1002 ¶ 57. This includes the relative and total concentrations of the components and redox systems. Ex. 1002 ¶¶ 62-70; Ex. 1020 at 269-73; Panda (Ex. 1022) at 73-76; Vallejo 2004 (Ex. 1030) at 7-8.

2. Optimizing Redox Conditions

It was known before 2009 that proteins solubilized from inclusion bodies must be placed in an environment that facilitates the formation of the desired

native protein structure. Ex. 1002 ¶¶ 55-58; Ex. 1007. In particular, if the desired protein in its native state contains disulfide bonds, the unfolded protein must be placed in appropriate redox conditions that favor the formation of the correct disulfide bonds. Ex. 1002 ¶ 61. The redox conditions must be balanced so that bond formation (oxidation) is favored, but not so favored that mis-formed bonds cannot "reshuffle"—break and reform—as thermodynamics drive the protein chains to fold into their native, most-stable conformation over time. Ex. 1002 ¶ 62; Ex. 1042 at 158-59. Scientists generally used redox systems consisting of a mixture of reduced and oxidized thiols to refold the protein. *Id.* This mixture is sometimes called a "thiol pair."

The "equilibrium" of oxidation and reduction that permitted optimal "reshuffling" and refolding was known to be controlled by the ratio and relative concentrations of the thiol pair oxidant and reducant in the buffer. Ex. 1008 at 205. While the example in Ex. 1002 ¶ 64, uses GSH/GSSG, other thiol pairs of choice included cysteine/cystine and cysteamine/cystamine. Ex. 1002 ¶ 71; Ex. 1007. The refolding reaction was often optimized by selecting an appropriate redox system and adjusting the ratios and concentrations of the oxidant and reductant, until the yield of properly-refolded proteins was maximized. Ex. 1020 at 270-72.

It was also well known that the optimal redox conditions had to be identified experimentally, and differed for each protein. Ex. 1002 at 68. The yield of properly-refolded protein can vary greatly depending on the particular protein being refolded, the concentration of the protein, the ingredients of the refold buffer, and other parameters such as pH, temperature, incubation time, and purification method. Ex. 1002 at 70; Ex. 1008; Ex. 1030. Thus, scientists routinely used multifactorial matrix screens to test various physical and chemical parameters to optimize the yield of properly-refolded proteins. Ex. 1002 at 57; Ex. 1046 at 441. Moreover, refolding is never 100% efficient; even optimized yields were always less than 100% and often much lower. Ex. 1002 at 59.

C. Additional Considerations in Commercial Production of Recombinant Proteins

As of 2009, a POSA would have recognized that "[t]he ultimate goal of recombinant fermentation research," was "to obtain the highest amount of protein in a given volume in the least amount of time." Ex. 1002 ¶ 59; Ex. 1023 at 182. POSAs also sought to decrease the size of refolding vessels by increasing the concentration of protein before and during refolding. Ex. 1028 at [0019]. However, it was recognized that at higher concentrations, refolding proteins were more prone to associate in unproductive ways, leading to misfolded proteins called "aggregates." *Id*. at [0008]. This process of "aggregation" competed with the

desired native folding pathway, lowering the yield of properly folded proteins. *Id*. at [0008]-[0009]; Ex. 1002 ¶¶ 58-59.

Those skilled in the art had various solutions at their disposal to deal with aggregation prior to 2009, including the addition of aggregation suppressors and employing a "pulse renaturation" technique, in which the protein concentration was increased gradually to allow the protein to properly refold. Ex. 1002 ¶ 59; Ex. 1030 at 3-6. While the challenged claims are not limited to commercial production or particular protein concentrations, these and other solutions devised before 2009 allowed POSAs to refold proteins of varying complexities at various concentrations, on a commercial scale. *See e.g.,* Ex. 1016; Ex. 1009; Ex. 1023; Ex. 1040, Ex. 1031, Ex. 1025, and Ex. 1007.

V. THE '287 PATENT, PROSECUTION HISTORY, AND RELATED PROCEEDINGS

A. The '287 Patent

The '287 patent, entitled "Refolding proteins using a chemically controlled redox state," issued on January 2, 2018, and claims priority to a provisional application filed on June 22, 2009. While the priority date of the claims of the '287 patent is contested in PGR2019-00001, Petitioners here rely solely on prior art published before June 22, 2009, the earliest possible priority date.

1. The Known Problem in the Art and the Alleged Innovative Solution

The ’287 patent alleges that prior to the purported invention, practitioners had not understood how to adjust thiol pair ratios and buffer strengths in order to achieve native refolding of complex proteins at concentrations higher than 2.0 g/L. The patent explains that “[u]ntil the present disclosure, specific relationships had not been provided for thiol buffer strength, thiol-pair ratio chemistry, and protein concentration with respect to complex proteins that related to efficiency of protein production.” Ex. 1001 at 4:19-22. “Consequently, the ability to refold proteins in a highly concentrated volume has largely been an inefficient or unachievable goal.” *Id*. 4:22-26. Further, “[p]rior to the present disclosure a specific controlled investigation of the independent effects of thiol-pair ratio and thiol-pair buffer strength had not been disclosed for complex proteins.” *Id*. at 4:27-30.

The ’287 patent alleges that the purported lack of understanding about thiol-pair ratios and buffer strengths for high concentrations of complex proteins was solved by the following alleged invention: “the relationship between thiol pair buffer strength and redox thiol-pair ratio has been investigated and optimized in order to provide a reproducible method of refolding proteins at concentrations of 2.0 g/L and higher.” *Id*. at 4:52-55. The patent explains that “[a] mathematical formula was deduced to allow the precise calculation of the ratios and strengths individual redox couple components to achieve matrices of buffer thiol-pair ratio

and buffer thiol strength." *Id*. at 4:56-59. The mathematical formulas for thiol pair buffer ratio and strength are given as Equations 1 and 2, respectively. *Id*. at 6:51-67. According to the patent, "[o]nce this relationship was established, it was possible to systematically demonstrate that thiol buffer strength and the thiol pair ratio interact to define the distribution of resulting product related species on a refolding reaction." *Id*. at 4:59-63.

The '287 patent then explains that the inventors' solution to achieving refolding of complex proteins at high concentrations is to have the POSA perform an optimization experiment for each protein to be refolded. In that optimization, the POSA varies the thiol pair ratio and buffer strength to determine which ratios and strengths are optimal for a given protein, protein concentration, and incubation time. *Id*. at 9:52-57. According to the patent, "[a]n optimization screen can be set up to systematically evaluate redox chemistries, Thiol-pair ratios, Thiol-pair Buffer Strengths, incubation times, protein concentration and pH in a full or partial factorial matrix . . ." *Id*. at 53-57.

In Example 2, entitled "Identification of Refold Conditions/Redox Components," the inventors performed an "[i]dentification of the refold buffer" for "[m]ultiple complex, microbial-derived proteins" using a "multifactorial matrix or series of multifactorial matrices to identify the refolding reaction for conditions that optimize yield and minimize aggregate formation." *Id*. at 14:35-61.

"Optimum" redox conditions were selected based on "[i]ndividual reactions . . . formed with varying levels of cysteine and cystamine that would allow for a controlled matrix of thiol-pair ratio at various thiol pair buffer strengths . . . " *Id*. at 15:9-20.

In short, the purported innovation described in the '287 patent boils down to a method in which thiol-pair ratio and buffer strength are varied in order to identify the optimal ratio and strength for a given complex protein at concentrations higher than 2.0 g/L.

2. The Scope of the Challenged Claims

Although the '287 patent purports to solve the problem of identifying optimum refolding conditions for high concentrations of complex proteins, the challenged claims are not so limited. The challenged claims plainly cover the refolding of any protein expressed in non-mammalian cells at any concentration and do not recite experimental steps for identifying optimal thiol pair ratios and buffer strengths. Rather, they encompass refolding buffers across a broad range of redox conditions.

Petitioners challenge claims 1, 4-6, 8-10, 12, 14-16, 19-21, 23-26, 29-30 of the '287 patent. The four independent claims: 1, 10, 16 and 26, each recite a "method of refolding proteins expressed in a non-mammalian expression system" comprising the two basic steps of forming and incubating a refold buffer:

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, the preparation comprising : [. . .] incubating the refold mixture so that at least about [25% / 30-80%] of the proteins are properly refolded."	"preparing a solution comprising: the proteins; . . . incubating the solution so that at least about [25% / 30-80%] of the proteins" are properly refolded."

Ex. 1001 at claims 1, 10, 16 and 26. Each independent claim further recites the following buffer components:

> 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,

Id. The claims offer no steps for identifying the optimum amounts of oxidant and reductant for a given protein, except that the thiol-pair ratio fall within a range that spans five orders of magnitude and that the refold preparation or solution remain "soluble":

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

Ex. 1001 at claims 1, 10, 16, and 26.

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

Claims 5-6 and 20-21 depend on claims 1 and 16, respectively, and require that "the thiol-pair buffer strength is increased proportionally to an increase in a total protein concentration" and "decreased proportionally to a decrease in a total protein concentration" in the refold mixture or solution.

Claims 8-9, 14-15, 23-25, and 30, require that the thiol-pair ratio, the thiol-pair buffer strength, or both, be "calculated" according to Equations 1 and 2 provided in the specification.

B. Prosecution History

The '287 patent issued from U.S. Patent Application No. 15/422,327, filed on February 1, 2017, which is a continuation of U.S. Application No. 14/793, 590, which is a continuation of U.S. Application No. 14/611,037. This application is a divisional of an application which issued as U.S. Patent No. 8,952,138 ("the '138 patent"). As discussed below, the Board found most claims of the '138 patent to be unpatentable over the prior art. Ex. 1038 (FWD IPR2016-01542).

The originally-filed claims and specification of the ’287 patent were substantially identical to those of the ’138 patent as issued. Ex. 1010 at 31-33. Shortly after filing, the applicants filed new claims that included, *inter alia*, the language “wherein the thiol-pair ratio and the thiol-pair-buffer strength yield at least about 25% properly refolded protein.” Ex. 1010 at 42.

In response to a Non-Final Rejection anticipation and obviousness over the prior art and double-patenting over the ’138 patent, the applicants amended the claims to add, inter alia, the following language: “wherein the thiol-pair buffer strength maintains the solubility of the preparation and is selected based on a desired yield of refolded protein, and 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 73.

In the next Office Action, the Examiner rejected the new claim language under §112 as unsupported by the specification. *Id*. at 94-95. Following an Applicant-Initiated Interview in which the applicants and the Examiner discussed “support for the new matter rejection and issues with the double patenting rejection,” applicants filed a terminal disclaimer over U.S. Patent No. 8,952,138. Applicants also amended the claims to, among other changes, omit the phrases “is selected based on a desired yield of properly refolded protein” and “consistent yields” *Id*. at 101.

The Examiner allowed the claims, stating, in part, "the claims are allowable because the most pertinent prior art neither teaches nor suggests the final thiol-pair ratio or strength as set forth in claims 34, 35, 56-57, 65-67 and 72." *Id*. at 121.

C. The Adello PGR

In October 2018, Adello and others filed a petition for PGR asserting in part that the '287 patent was not entitled to its asserted priority date and thus was PGR-eligible because the originally-filed specification did not enable or provide written description of the claim language directed to percentages of properly refolded proteins. In response to Adello's Petition, PO asserted that this claim language was non-limiting. Ex. 1037 at 33-36.

D. The Board's Invalidation of Analogous Claims of the '138 Patent

On February 15, 2018, the Board issued a final written decision holding claims 1-17 and 19-24 of the '138 patent unpatentable over the prior art. Ex. 1038. Like the claims of the '287 patent, the claims of the '138 patent are directed to methods of refolding proteins expressed in non-mammalian expression systems by incubating the protein in a refold mixture containing, *inter alia*, redox components (i.e. oxidants and reductants) at certain thiol-pair buffer ratios and strengths. Ex. 1041. These values are defined in the '138 patent using the same equations as those used in the '287 patent, and the values set forth in the claims fall within the ranges set forth in the challenged '287 patent claims. While the '287 patent claims

contain language not found in the ’138 patent claims pertaining to percentages of properly refolded proteins, PO has asserted that this language is not limiting.

VI. 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 had a Ph.D. in biochemistry or chemical engineering and several years’ experience in the fields of biochemical manufacturing, protein purification and/or protein refolding. In the alternative, the POSA would have had an equivalent level of education and experience, including a Bachelor’s or Master’s degree with more practical work experience in the above field(s). Ex. 1002 ¶¶ 39-40. This person would have worked in collaboration with other scientists and/or clinicians with experience in protein refolding, biochemical manufacturing 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.*

VII. CLAIM CONSTRUCTION

In an IPR, the terms of challenged claims are construed “in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent,” just as they are in district court. 37 C.F.R. § 42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005) (*en banc*). For the purpose of this proceeding, any term not

expressly discussed should be given its ordinary and customary meaning to a POSA as of the filing date of the '287 patent, which Petitioners assume for purposes of this IPR only to be June 22, 2009.[3]

A. "Preparation"

In district court litigation and in the POPR to Adello's PGR petition, PO has asserted that the term "preparation," which appears in challenged claims 1, 4-6, 8-10, 12, and 14-15, means "the refold mixture comprising an amount of oxidant, an amount of reductant, and one or more of a denaturant, aggregation suppressor, and a protein stabilizer, prior to contact with the proteins to be refolded." Ex. 1037 at 53. For purposes of this IPR only, Petitioners assume PO's construction.

B. "Is Calculated"

PO has asserted in its POPR to Adello's PGR that the term "calculated," appearing in challenged claims 8-9, 14-15, 23-25, and 30 of the '287 patent, should be construed to include "an active step of determining" and to require a "thiol-pair ratio or thiol-pair buffer strength to actually ***be calculated***." Ex. 1037 at 39. In

[3] Petitioners adopt these claim construction positions for purposes of this IPR only and reserve the right to change or modify their positions in future litigation, for example in response to expert opinions, statements by Amgen, or court rulings. Petitioners do not waive any argument concerning indefiniteness or invalidity under § 112.

district court litigation, PO and Adello asserted that the term "is calculated" means "is determined using an equation as part of practicing the method, rather than using the equation in hindsight." For purposes of this IPR only, Petitioners assume PO's construction.

C. "Maintains Solubility"

As Dr. Dalby explains, the specification and file history do not provide clear guidance as to the meanings of the terms "wherein the thiol-pair buffer strength maintains the solubility of the preparation" (challenged claims 1, 4-6, 8-10, 12 and 14-15) and the "solution" (challenged claims 16, 19-21, 23-26 and 29-30). For purposes of this IPR only, Petitioners will assume the terms mean "maintains the solubility of the protein that properly refolds during incubation."

D. Defined Claim Terms In Specification

(1) "Non-mammalian expression system": "a system for expressing proteins in cells derived from an organism other than a mammal, including but not limited to, prokaryotes, including bacteria such as E. coli, and yeast." Ex. 1001 at 5:16-30.

(2) "Denaturant": "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," including "urea, guanidinium salts, dimethyl urea, methylurea, ethylurea and combinations thereof." Ex. 1001 at 5:31-40.

(3) "Aggregation suppressor": "any compound having the ability to disrupt and decrease or eliminate interactions between two or more proteins," including "amino acids such as arginine, proline, and glycine; polyols and sugars such as glycerol, sorbitol, sucrose, and trehalose; surfactants such as, polysorbate-20, CHAPS, Triton X-100, and dodecyl maltoside; and combinations thereof." Ex. 1001 at 5:41-49.

(4) "Protein stabilizer": "any compound having the ability to change a protein's reaction equilibrium state, such that the native state of the protein is improved or favored," including "sugars and polyhedric alcohols such as glycerol or sorbitol; polymers such as polyethylene glycol (PEG) and α-cyclodextrin; amino acids salts such as arginine, proline, and glycine; osmolytes and certain Hoffmeister salts such as Tris, sodium sulfate and potassium sulfate; and combinations thereof." Ex. 1001 at 5:50-59.

(5) "Protein": "any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds." Ex. 1001 at 6:4-7.

IX. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED

Petitioner requests review and cancellation of claims 1, 4-6, 8-10, 12, 14-16, 19-21, 23-26, 29-30 of the '287 patent under §§ 102 and 103 for the reasons explained in this petition, which may be summarized as follows:

Ground No	Claims and Basis
1	Claims 1, 4, 8-10, 12, 14-16, 19, 23-26, and 29-30 are anticipated by Vallejo
2	Claims 16, 19-21, 23-26, 29-30 are anticipated by Ruddon
3	Claims 1, 4-6, 8-10, 12, 14-16, 19-21, 23-26, 29-30 are obvious over Ruddon in view of Clark 1998 in light of Shafer or Gilbert
4	Claims 8, 9, 14, 15, 23-25 and 30 are obvious over Vallejo in combination with Ruddon and Clark 1998 in light of Schafer or Gilbert

A. Ground 1: Claims 1, 4, 8-10, 12, 14-16, 19, 23-26, 29-30 are anticipated by Vallejo (Ex. 1031)

European Patent Application EP 1449848 A1, titled "*Method for the production of cystine-knot proteins*," and published on August 25, 2004 to authors Luis Felipe Vallejo and Ursula Rinas ("Vallejo") is prior art to the '287 patent under either pre-AIA § 102(b) or post-AIA § 102(a)(1). Vallejo was not cited during examination of the '287 patent.

Vallejo discloses a method of refolding proteins expressed in a non-mammalian expression system, specifically 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. 1031 at [0001].

1. Claims 1, 10, 16, and 26

a. The Preamble[4]

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

Vallejo also discloses refolding proteins expressed in E. coli, a non-mammalian expression system, stating "[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. 1031 at [0018]-[0019]; Ex. 1002 ¶ 119. Examples 2 and 6 of Vallejo disclose refolding rhBMP-2, a polypeptide containing more than five amino acids, and thus a "protein" as defined in the '287 patent. Ex. 1001 at 6:4-7; Ex. 1002 ¶ 119. Thus, Vallejo discloses the claim preamble.

b. The claimed refold mixture

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

[4] An element-by-element list of the claims is attached as Exhibit 1044.

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"	from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer; an amount of oxidant; and an amount of reductant,"

Vallejo discloses a refolding "preparation" and "solution" that contain all of the claimed ingredients, including an aggregation suppressor, protein stabilizer, denaturant, and an amount of oxidant and an amount of reductant. Vallejo describes adding unfolded and reduced rhBMP-2 to a "standard renaturation" refolding buffer "preparation" that contains an aggregation suppressor and a mixture of reduced and oxidized glutathione to form a refolding solution:

> Dilution of unfolded and reduced rhBMP-2 **with a final concentration of 0.1 mg mL^{-1} rhBMP-2** in standard renaturation buffer with a final concentration of **0.5 mol L^{-1} Gdn-HCl, 0.1 mol L^{-1} Tris-HCl (pH 7.8), 0.75 mol L^{-1} 2-(cyclohexylamino)ethanesulfonic acid (CHES)**, 1 mol L^{-1} NaCl, 5 mmol L^{-1} EDTA, **and 3 mmol L^{-1} total glutathione in a 2:1 ratio of glutathione reduced to glutathione oxidized (GSH:GSSG).**

Ex. 1031 at [0054] (emphasis added). Reduced glutathione (GSH) and oxidized glutathione (GSSG) are a reductant and an oxidant, respectively; together they are a thiol pair. Ex. 1002 ¶¶ 120-23; Ex. 1001 at 3:52-54, 7:20-25. Gdn-HCl is guanidinium hydrochloride (a guanidinium salt), which the '287 patent identifies as a denaturant. Ex. 1001 at 5:31-40. Tris is both an aggregation suppressor and a protein stabilizer. *Id.* at 5:41-58. CHES is a protein stabilizer. Ex. 1002 at 122. Examples 2 and 6 of Vallejo refold rhBMP-2 using the same "standard renaturation buffer" Ex. 1031 at [0042] (Captions for Figures 2 and 8), [0045], and [0049]. Therefore, Vallejo teaches using a denaturant, an aggregation suppressor and a protein stabilizer in the "standard renaturation buffer" preparation, as well as the solution formed once protein is added. Ex. 1002 ¶¶ 120-23.

c. Redox Components

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

Vallejo discloses using amounts of oxidant and reductant that form a thiol-pair ratio and a thiol-pair buffer strength to "reshuffle" disulfide bonds and refold rhBMP-2 into its native, soluble form: "[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. 1031 at [0045].

A POSA would understand that when dissolved in a preparation or solution, reduced and oxidized glutathione form a "thiol-pair buffer" with an inherent concentration or "buffer strength" as defined in the '287 patent. Equation 2 of the '287 patent defines "thiol-pair buffer strength" as 2[oxidant] + [reductant] which for glutathione buffers equals 2[GSSG] + [GSH]. While Vallejo does not use the words "thiol pair buffer strength," a POSA would understand that when Vallejo describes the concentration of total glutathione, it is describing thiol-pair buffer strength as used in the patent. Ex. 1002 ¶ 126. A POSA would know that one GSSG molecule contains, and can convert into, two GSH molecules. Therefore, the total glutathione concentration in Vallejo is equal to the concentration of GSH plus twice the concentration of GSSG, i.e. the thiol-pair buffer strength. Ex. 1002 ¶ 126-28; Ex. 1031. Thus, when Vallejo discloses a final concentration of "3 mmol

L^{-1} total glutathione"[5] in its "standard renaturation buffer," it is disclosing a thiol-pair buffer strength of 3 mM. Ex. 1031 at [0055]; Ex. 1002 ¶ 128.

A POSA would also understand that when dissolved in a preparation or solution, reduced and oxidized glutathione form a "thiol-pair ratio" as defined in the '287 patent. Equation 1 of the '287 patent defines "thiol pair ratio" as $[\text{reductant}]^2/[\text{oxidant}]$. A POSA would understand this to be $[GSH]^2/[GSSG]$ for glutathione. Like thiol pair buffer strength, this ratio is also an inherent property of Vallejo's glutathione buffer. For example, Vallejo's "standard denaturation buffer" contains 3 mM total glutathione in a 2:1 ratio of GSH to GSSG. Ex. 1031 at [0055]. A POSA would understand that because each molecule of GSSG contains two molecules of GSH, the standard denaturation buffer contains 1.5 mM GSH and 0.75 mM GSSG. *Id*. ¶ 126. These concentrations of GSH and GSSG in the standard denaturation buffer correspond to a thiol pair ratio of $(1.5)^2/0.75 = 3$ mM. *Id*. ¶ 127.

In Example 6 of Vallejo, "renaturation was carried out in standard renaturation buffer," thus the protein folding mixture in Example 6 has a thiol buffer strength of 3 mM and a thiol pair ratio of 3 mM. In Example 2, rhBMP-2 is refolded using varied concentrations of reductant and oxidant; the total glutathione concentration is held constant at 3 mM and the GSH and GSSG ratio was varied in

[5] A POSA would understand that mmol L^{-1} is equivalent to mM.

a range from a GSH:GSSG ratio of 40:1 to 1:20. Ex. 1031 at [0042] (figure 2 caption), [0045]. A POSA would have easily discerned the concentrations of GSH and GSSG and the thiol-pair ratio from this information. Ex. 1002 ¶ 128. As Dr. Dalby explains, the thiol-pair ratios tested in Example 2 range from 0.004-114 when calculated using Equation 1, and six out of seven are within the claimed range of 0.001-100, indicating that this is a logical, conventional range to choose when optimizing a folding process. *Id*. ¶ 133.

The total glutathione concentrations and GSH:GSSG ratios disclosed in Vallejo are "final concentrations" and ratios, meaning that they refer to the concentrations of GSH and GSSG in the refolding solutions once all of the components, including the protein, have been added. Ex. 1002 ¶ 130; Ex. 1031 at [0055]. Just prior to addition of protein, the concentrations of GSH and GSSG in the renaturation buffer mixture (the "preparation") are slightly higher than the "final concentrations" because the GSH and GSSG are diluted slightly when the relatively small volume of concentrated denatured protein is added. But the thiol buffer strength and thiol-pair ratios of the "preparation" of Vallejo, just before protein is added to the mixture, are equally inherent in the disclosure. Ex. 1002 ¶ 123. A POSA would have immediately recognized that they were not significantly different than the final concentrations and ratios, and would have been able to easily calculate the difference from the data presented in Vallejo. *Id*.

According to Dr. Dalby, the slightly higher concentrations of GSH and GSSG do not affect whether the thiol-pair ratios are within the claimed range. Ex. 1002 at ¶ 130.

Vallejo also teaches that the thiol-pair buffer strength "maintains the solubility" of the "preparation" (before the addition of protein) and the "solution" (after the addition of protein). Vallejo teaches that its method results in properly refolded proteins. *Id*. at [0012]. This would not have occurred unless the redox components maintained the solubility of the protein that properly refolded during incubation. Ex. 1002 ¶ 135. Indeed, it cannot be disputed that the thiol pair buffer strength in Vallejo maintained the solubility of the preparation and solution in accord with the claims, since as explained below the yield of properly-folded protein exceeds the 25% and 30% lower limits of the claims.

d. Incubating the refold mixture

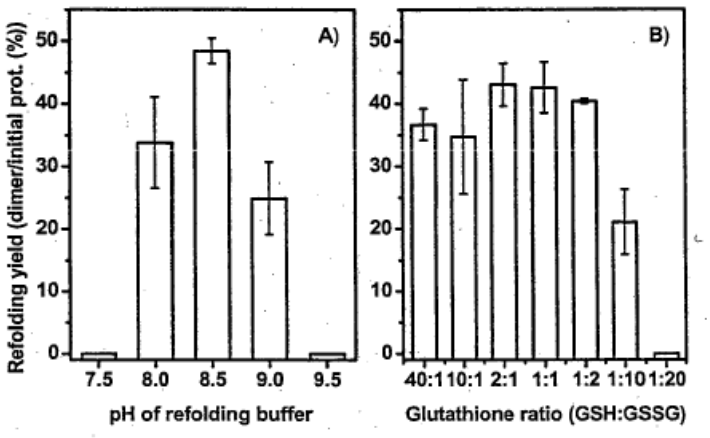
Claims 1 and 16	Claims 10 and 26
"incubating the refold mixture so that at least about 25% of the proteins are properly refolded."	"incubating the refold mixture so that about 30-80% of the proteins are properly refolded."

Vallejo discloses that following establishment of the refolding mixture, the mixture is incubated for 48 hours. Ex. 1002 ¶ 136. Ex. 1031 at [0055] ("After 48 h of incubation, the soluble and aggregated fractions of the renaturation mixture

were separated by centrifugation and analyzed by gel electrophoresis under non-reducing conditions.")

PO has asserted that the claim language directed to the percentage of properly-refolded proteins is not limiting. Ex. 1037 at 33-37. For this IPR only, Petitioners assume that the term is limiting and demonstrate where it is disclosed in the prior art. If it is not limiting, then the claims remain anticipated and obvious on the same grounds and encompass a broader scope of prior art.

Vallejo teaches that optimization of the refolding conditions achieved a "renaturation yield" of 44%. Ex. 1031 at [0012]. A POSA would understand that "renaturation yield" means the yield of properly refolded, biologically active protein. Ex. 1002 ¶ 138. Vallejo systematically varied the [GSH]:[GSSG] ratio from 40:1 to 1:20 (correspond to thiol-pair ratios ranging from 114 to 0.004) and selected total glutathione concentrations (thiol-pair buffer strength) in order to optimize the yield of properly refolded rhBMP-2. Ex. 1031 at Fig. 2b, [0042] and [0045].



Id. at Figure 2. For GSH:GSSG ratios ranging from 40:1 and 1:2, which correspond to thiol-pair ratios ranging from 114 to 0.3 (Ex. 1002 ¶ 139), the refolding yield is about 35%-45%. For Example 6, Vallejo discloses "a final yield of 32 to 38%." Ex. 1031 at [0049].

A POSA would also understand that Vallejo's refolded recombinant rhBMP-2 is biologically active because the monomer protein performed its biological function of assembling into dimers: "[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. 1031 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 ¶ 140.

2. Claims 4, 12, 19 and 29

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

greater." Vallejo anticipates this limitation as well. As discussed above, Vallejo's "standard renaturation conditions" contain a final concentration of total glutathione (i.e. thiol-pair buffer strength) of 3 mM, which is great than 2 mM. Ex. 1002 ¶ 143; Ex. 1031 at [0054]. The refolding experiments disclosed Examples 2 and 6 were also conducted using the standard renaturation buffer. *Id*. at [0042] (captions for figures 2 and 8), [0045], and [0049].

3. 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 ("Equation 1"), the thiol-pair buffer strength ("Equation 2"), or both. Ex. 1001.

As discussed above in section IX.A.1.c, Vallejo inherently discloses examples of refolding using thiol-pair ratios and thiol-pair buffer strengths that fall within the ranges of the other challenged claims and anticipates them. Adding Equations 1 and 2 to claims 8, 9, 14, 15, 23, 24, 25, and 30 does not render them patentable. Assuming the claims require a POSA to calculate the thiol-pair ratio and buffer strength using Equations 1 and 2, these calculations cannot impart novelty to the otherwise anticipated claims. The equations would have been part of the basic redox chemistry knowledge of a POSA in 2009 and their use by a POSA to calculate thiol-pair ratios and buffer strengths would have been trivial and elementary. Ex. 1013, Ex. 1014, Ex. 1027, and Ex. 1025. The calculations

also do not have a "new and nonobvious functional relationship" to the otherwise known method of using thiol pair ratios and buffer strengths within the claimed ranges to effect proper refolding. *See King Pharm., Inc. v. Eon Labs, Inc.*, 616 F.3d 1267, 1278-79 (Fed. Cir. 2010) (relevant inquiry is whether an instructional limitation has a "new and non-obvious functional relationship" to otherwise known method). The mere fact that a POSA informs him or herself of the thiol-pair ratio and buffer strength via Equations 1 and 2 in no way "transforms" the known method of using thiol pair buffers with ratios and strengths that fall within the claimed ranges to properly refold proteins. *Id.* at 1279. Thus, Vallejo anticipates claims 8, 9, 14, 15, 23, 24, 25, and 30.[6]

4. Claim Chart

As charted below, Vallejo discloses each and every limitation of claims 1, 4, 8-10, 12, 14-16, 19, 23-26, 29-30 of the '287 patent.

Claim	Limitation	Support in Vallejo (Ex. 1031)
1, 10	A method of refolding proteins expressed in a non-mammalian expression system,	[Par. 3] ("rhBMP-2 generated by refolding from *E. coli* produced inclusion bodies")

[6] Of course, if claims 8, 9, 14, 15, 23, 24, 25, and 30 do not require a POSA to make the calculation, then Vallejo also inherently anticipates them.

Claim	Limitation	Support in Vallejo (Ex. 1031)
1, 10	the method comprising: 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,	Abstract: ...producing a biologically active recombinant cystine-knot protein [Par. 6, 16] ("a refold buffer . . . comprising...(bc) a solubilizing chaotropic agent in a non-denaturing concentration... guanidinium hydrochloride is used as the chaotropic agent.") [Par. 55] ("Standard 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 with a final concentration of 0.5 mol L^{-1} Gdn-HCl, 0.1 mol L-1 Tris-HCl (pH 7.8), 0.75 mol L^{-1} 2-(cyclohexylamino) ethanesulfonic acid (CHES), 1 mol L^{-1} NaCl, 5 mmol L^{-1} EDTA") [Par 45] ("For renaturation of disulfide-bonded proteins, mixtures of reduced and oxidized glutathione are employed.")
1, 10	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	[Fig. 2] [Par. 42] ("Fig. 2...Renaturation was carried out...in standard renaturation buffer (A) with 3 mmol L-1 total glutathione in a 2:1 ratio (GSH:GSSG)...or (B) at pH 8.5 and 3 mmol L-1 total glutathione at the indicated redox ratios.")

Claim	Limitation	Support in Vallejo (Ex. 1031)
	maintains the solubility of the preparation;	[Par. 55] ("with a final concentration of… 3 mmol L-1 total glutathione in a 2:1 ratio of glutathione reduced to glutathione oxidized (GSH:GSSG)")
1, 10	and incubating the refold mixture so that at least about 25% of the proteins are properly refolded.	[Par. 12] ("renaturation yield of 44%." *See also, e.g.,* [Par. 49] ("an overall renaturation yield of 33 to 38%.")
4, 12, 19, 29	wherein the thiol-pair buffer strength is 2 mM or greater.	[Par. 42] ("Fig. 2...Renaturation was carried out...in standard renaturation buffer (A) with 3 mmol L-1 total glutathione in a 2:1 ratio (GSH:GSSG)...or (B) at pH 8.5 and 3 mmol L-1 total glutathione at the indicated redox ratios.") [Par. 45] ("variation of the total glutathione concentration from 3 to 9 mol L^{-1}")
8, 23	wherein the thiol-pair ratio is calculated according to the following equation: $[\text{the reductant}]^2$ [the oxidant].	[Par. 42] ("Fig. 2...Renaturation was carried out...in standard renaturation buffer (A) with 3 mmol L^{-1} total glutathione in a 2:1 ratio (GSH:GSSG)...or (B) at pH 8.5 and 3 mmol L^{-1} total glutathione at the indicated redox ratios.")
9, 24	wherein the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant]+[the reductant].	[Par. 42] ("Fig. 2...Renaturation was carried out...in standard renaturation buffer (A) with 3 mmol L-1 total glutathione in a 2:1 ratio (GSH:GSSG)...or (B) at pH 8.5 and 3 mmol L-1 total glutathione at the indicated redox ratios.")

Claim	Limitation	Support in Vallejo (Ex. 1031)
14, 15, 25, 30	wherein: the thiol-pair ratio is calculated according to the following equation: [the reductant]2 [the oxidant]; and the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant]+[the reductant].	*See* claims 1, 10.
16, 26	A method of refolding proteins expressed in a non-mammalian expression system	[Par. 3] ("rhBMP-2 generated by refolding from E. coli produced inclusion bodies")
16, 26	the method comprising: 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,	Abstract: ("producing a biologically active recombinant cystine-knot protein.") [Par. 6, 16] ("a refold buffer . . . comprising...(bc) a solubilizing chaotropic agent in a non-denaturing concentration... guanidinium hydrochloride is used as the chaotropic agent.") [Par. 55] ("Standard 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 with a final concentration of 0.5 mol L^{-1} Gdn-HCl, 0.1 mol L-1 Tris-HCl (pH 7.8), 0.75 mol L^{-1} 2-(cyclohexylamino) ethanesulfonic acid (CHES), 1 mol L^{-1} NaCl, 5 mmol L^{-1} EDTA") [Par 45] ("For renaturation of

Claim	Limitation	Support in Vallejo (Ex. 1031)
		disulfide-bonded proteins, mixtures of reduced and oxidized glutathione are employed")
16, 26	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;	[Par. 14] ("the ratio of reduced and oxidized glutathione is equal or above 1:10, ratios of 2:10, 3:10, 4:10, 5:10 etc. such as 10:10, 50:10, 100:10, 200:10 or even 400:10") [Par. 42] ("Fig. 2...Renaturation was carried out...in standard renaturation buffer (A) with 3 mmol L-1 total glutathione in a 2:1 ratio (GSH:GSSG)...or (B) at pH 8.5 and 3 mmol L-1 total glutathione at the indicated redox ratios.")
16, 26	and incubating the solution so that at least about 25% of the proteins are properly refolded.	[Par. 12] ("a renaturation yield of 44%. ") *See also*, *e.g.*, [Par. 49] ("an overall renaturation yield of 33 to 38%.")

B. Ground 2: Claims 16, 19-21, 23-26 and 29-30 are anticipated by Ruddon (Ex. 1025)

1. Claims 16, and 26 are Anticipated by Ruddon

International Patent Application WO 95/32216, titled "*Biologically active glycoprotein hormones produced in prokaryotic cells*," and published on November 30, 1995 to authors Raymond W. Ruddon and Jeffrey R. Huth ("Ruddon") is prior art to the '287 patent under either pre-AIA § 102(b) or post-AIA § 102(a)(1). Ruddon was not cited during examination of the '287 patent.

Ruddon discloses a method of producing and refolding biologically-active glycoprotein hormonesin prokaryotic expression systems. Ruddon (Ex. 1025) at 1:7-15, 8:33-9:19.

a. The Preamble

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

Ruddon teaches the refolding of recombinant hCG-β, a protein hormone subunit, expressed using *E. coli* and other prokaryotic (bacterial) expression systems. Ex. 1002 at 149; Ex. 1025 at 8-9, 16-21. hCG-β is a "protein" as defined by the '287 patent, i.e. a "chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds." Ex. 1001 at 6:4-7; Ex. 1026. Example 2 of Ruddon provides a detailed method for "Folding and Assembly into a Functional αβ Dimer of a Bacterially Expressed hCG-β" Ex. 1025 at 42-52. Thus, Ruddon teaches a method of refolding proteins expressed in a non-mammalian system.

b. The Claimed Refold Mixture

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 oxidant; and an amount of reductant,

Example 2 in Ruddon discloses forming the claimed "solution" to refold hCG-β. Ruddon discloses a refold buffer containing Tris-HCl and varying amounts of urea between 0 and 2 M, cysteamine, and cystamine. *Id.* at 45:30-35. A POSA would have known, and the '287 patent confirms, that urea is a denaturant and Tris is both an aggregation suppressor and a protein stabilizer. Ex. 1001 at 3:39-50. Ruddon teaches that cysteamine/cystamine is a redox pair that can be used "in a simple, inexpensive thiol folding buffer." Ex. 1025 at 26:19-21. A POSA would have known, and the '287 patent confirms, that cysteamine is a reductant and cystamine is an oxidant. Ex. 1002 at 150; Ex. 1001 at 12:23-28. Example 2 of Ruddon discloses that this refold solution is sufficient to support the renaturation of [hCG-β] to a biologically active form because it discloses the production of properly-folded hCG-β protein that is competent for assembly into the full hCG hormone with biological activity. Ex. 1025 at 29-30, 49-52, Figures 5-10.

c. Redox Components

Claims 16 and 26
"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"

Ruddon uses cysteamine/cystamine as the redox thiol pair. As in Vallejo's glutathione redox system, when amounts of cysteamine and cystamine are dissolved in a refold solution, they inherently form a "thiol pair ratio" and "thiol pair buffer." Ruddon expressly uses Equation 1 of the '287 patent to calculate that the cysteamine/cystamine refolding mixture of Examples 1 and 2 has a thiol pair ratio to the amounts of cysteamine/cystamine used in Examples 1 and 2 of Ruddon, Ruddon's refolding mixtures have a thiol-pair ratio of 11.4. Ex. 1002 at 152; Ex. 1025 at 34:34-36; 45:30-35. Therefore, Ruddon discloses a refold solution with a thiol-pair ratio within the range of 0.001-100.

In the same way as Vallejo, *see* section IX.A.1.c, because Ruddon reports that the proteins successfully refolded into native, biologically active form, and as described below did so in yields higher than the 25% and 30% lower bounds of the

claims, a POSA would have understood that Ruddon's thiol-pair buffer strength "maintained the solubility of the preparation" and "solution." Ex. 1002 ¶¶ 150-53.

d. Incubating the refold mixture

Claim 16	Claim 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."

Ruddon discloses incubating the refold buffer to achieve the refold yields recited in the claims. In Example 2, Ruddon discloses that "folding was initiated" by the addition of cysteamine and cystamine, and then the "[r]eactions were incubated for 1 min to 4 h at room temperature." Ex. 1025 at 45-51. In the results section of Example 2, Ruddon discloses that "the folding efficiency was found to be 40-60% in the presence of 2 M urea." *Id*. at 51:2-6. A POSA would have understood "folding efficiency"to refer to the percentage of properly folded hCG-β protein. Ex. 1002 at 159. Proper folding was further confirmed by demonstrating the ability of the folded hCG-β to dimerize with hCG-α. Ex. 1002 at 159, Ex. 1025 at 51.

2. Claims 19 and 29 Are Anticipated by Ruddon

Claims 19 and 29 depend on claims 16 and 26, respectively, and add the limitation "the thiol-pair buffer strength is 2 mM or greater."

Ruddon discloses that the refolding mixture of Example 2 contains final concentrations of 6.4 mM cysteamine and 3.6 mM cystamine. *Id*. at 45. Applying the '287 patent's Equation 2 for calculating thiol-pair buffer strength, the refolding mixture disclosed in Example 2 has a thiol-pair buffer strength of 2*3.6 mM + 6.4 mM = 13.6 mM. Therefore, Ruddon inherently discloses a thiol-pair buffer strength that is 2 mM or greater.

3. Claims 23, 24, 25 and 30 Are Anticipated By Ruddon

Dependent claim 23 requires that "the thiol-pair ratio is calculated according to the following equation: [the reductant]^2/[the oxidant]" (Equation 1 of the 287 patent). Ruddon expressly calculates the thiol-pair ratio of the refolding mixture of Examples 1 and 2 using Equation 1. Ex. 1025 at 32-52. Thus Ruddon anticipates this claim.[7]

[7] Although the equation appears in the discussion of Example 1, the discussion of Example 2 states that authors "have identified optimal redox conditions for the disulfide bond formation that is required for folding of hCG-β (Example 1)" Ex. 1025 at 49:31-34. Ruddon discloses that "this cysteamine/cystamine redox buffer at alkaline pH was used to fold" the hCG-β produced using the bacterial expression system in Example 2. A POSA would have understood that the calculated value of [reductant]^2/[oxidant] for the redox buffer in Example 1 would be the same for the identical buffer used in Example 2. A POSA would

Dependent claims 25 and 30 require that "the thiol-pair ratio is calculated according to the following equation: [the reductant]^2/[the oxidant]" (Equation 1) "and the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant] + [the reductant]" (Equation 2). Dependent claim 24 requires that "the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant] + [the reductant]" (Equation 2). As discussed, Ruddon expressly anticipates the use of Equation 1. In the same way that Equation 2 does not render claims 24, 25 and 30 novel over Vallejo, *see* section IX.A.3, Equation 2 does not render those claims novel over Ruddon. Therefore Ruddon anticipates them as well.

4. Claim Chart

As charted below, Ruddon discloses each and every limitation of claims 16, 19-21, 23-26, 29-30 of the '287 patent.

Claim	Limitation	Support in Ruddon (Ex.__)
16, 26	A method of refolding proteins expressed in a non-mammalian expression system	(Abstract) ("Unfolded glycoprotein hormone subunits are expressed in procaryotic cells, then re-folded in vitro in a thiol redox buffer to form assembly-competent subunits. The subunits are assembled to produce active hormones.")

---

not have expected the authors to repeat the identical calculations for an identical buffer.

Claim	Limitation	Support in Ruddon (Ex.__)
16, 26	the method comprising: 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,	(Pg. 12) ("Fig. 5...Unfolded rehCG-B that had been HPLC purified was diluted to 1.28 μM in the presence of 50 mM Tris-HCl, pH 8.7, 1mM EDTA, 6.4 mM cysteamine, and 3.6 mM cystamine and incubated at room temperature.") (Pg. 50) ("Interestingly, the amount of aggregate formation was reduced when rehCG-B was folded in the presence of 2 M urea (Fig. 5, lanes 8-12).") (Pg. 26) ("The redox buffers of the invention generally comprise a redox pair, such as oxidized and reduced glutathione or cysteamine/cystamine.")
16, 26	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;	(Pg. 26) ("The total concentration of cysteamine/cystamine in such a redox buffer should be between about 2 mM and about 10 mM.") (Pg. 45) ("Folding was initiated by the addition of 6.4 mM cysteamine and 3.6 mM cystamine") (Pg. 34) ("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) … The standard redox potentials of cysteamine and glutathione have been reported to be nearly the same...Given that the standard redox potential of glutathione is between 0.205 v and 0.26 v . . . We have varied the value of $[reductant]^2 / [oxidant]$ and found optimum folding of hCG-B to occur between values of 2 and 40 mM.")

Claim	Limitation	Support in Ruddon (Ex.__)
16, 26	and incubating the solution so that at least about 25% of the proteins are properly refolded.	(Pg. 28) ("incubated at a suitable temperature (i.e. 22-28° C) for a pre-determined amount of time to enable the subunits to fold.") (Pg. 51) ("In this way, the folding efficiency was found to be 40-60% in the presence of 2M urea.")
19, 29	wherein the thiol-pair buffer strength is 2 mM or greater.	(Pg. 26) ("The total concentration of cysteamine/cystamine in such a redox buffer should be between about 2 mM and about 10 mM.") (Pg. 45) ("Folding was initiated by the addition of 6.4 mM cysteamine and 3.6 mM cystamine")
23	wherein the thiol-pair ratio is calculated according to the following equation: [the reductant]2 [the oxidant].	(Pg. 34) ("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-B to occur between values of 2 and 40 mM.")
24	wherein the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant]+[the reductant].	*See* claims 16 and 26.
25, 30	wherein: the thiol-pair ratio is calculated according to the following equation: [the reductant]^2/ [the oxidant]; and the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant]+[the	(Pg. 34) ("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-B to occur between values of 2 and 40 mM.") (Pg. 26) ("The total concentration of

Claim	Limitation	Support in Ruddon (Ex.__)
	reductant].	cysteamine/cystamine in such a redox buffer should be between about 2 mM and about 10 mM.") (Pg. 45) ("Folding was initiated by the addition of 6.4 mM cysteamine and 3.6 mM cystamine") (Pg. 34) ("The standard redox potentials of cysteamine and glutathione have been reported to be nearly the same...Given that the standard redox potential of glutathione is between 0.205 v and 0.26 v, the redox potential of the buffers used in the experiments reported here was calculated to be between -145 and -200 mV.")

C. Ground 3: Claims 1, 4-6, 8-10, 12, 14-16, 19-21, 23-26, 29-30 are obvious over Ruddon in view of Clark 1998 in light of Schafer or Gilbert

Even if the challenged claims are not anticipated, they are unpatentable for obviousness. As explained, Vallejo and Ruddon disclose examples of refolding native proteins expressed in non-mammalian systems using the claimed ingredients in the claimed ranges. Ruddon uses Equation 2 to calculate thiol-pair buffer strength, and while neither Ruddon nor Vallejo expressly disclose the use of Equation 1 to calculate thiol pair ratio, Equation 1 was a well-known means of calculating that parameter and its use would have been trivially obvious to a POSA. Moreover, as discussed below, the concept of optimizing the yield of

natively-refolded proteins by testing various thiol-pair ratios and buffer strengths was both well known in the art and well within the realm of ordinary skill and routine experimentation.

2. Scope and Content of the Prior Art and Differences Between the Prior Art and the Challenged Claims

Clark, *Oxidative Renaturation of Hen Egg-White Lysozyme. Folding vs Aggregation*," Journal Biotechnology Process (1998) ("Clark 1998") was published in a printed publication as of 1998. Ex. 1007. Accordingly, Clark 1998 is prior art to the '287 patent under either pre-AIA § 102(b) or post-AIA § 102(a)(1). Clark 1998 was cited in the '287 patent, which recognized that Clark investigated "a relationship between the thiol pair ratio and the buffer strength" for lysozyme, a protein. Ex. 1001 at 4:6-9.

Schafer[8] and Gilbert[9] were each published in a printed publication before June 2008. Accordingly, each reference is prior art to the '287 patent under either pre-AIA § 102(b) or post-AIA § 102(a)(1).

---

[8] Schafer, *Redox Environment of the Cell as Viewed Through the Redox State of the Glutathione Disulfide/Glutathione Couple*, 30 Free Radical Biology & Medicine 1191 (2001).

[9] Gilbert, *Thiol/Disulfide Exchange Equilibria and Disulfide Bond Stability*, 251 *Methods in Enzymology* 8 (1995)

It was well known prior to June 22, 2009 that when refolding proteins using thiol-pair redox buffers it was important to determine experimentally the particular thiol-pair ratio and buffer strength that optimizes the yield of natively-refolded product and minimizes the formation of misfolded aggregates. For example, in Clark 1998, the authors noted that "[w]hen working with reduced and oxidized glutathione as the thiol/disulfide system, not only must the ratio of reduced to oxidized glutathione be considered but the total glutathione concentration as well." Ex. 1007 at 50. As explained, a POSA would have understood this to mean that both the ratio of thiol-pair oxidant to reductant and the strength of the thiol pair buffer should be evaluated when optimizing a redox refold buffer. *Id*. at 48. Ex. 1002 ¶ 167. Indeed, Clark 1998 uses Equation 2, which the '287 patent states should be used to calculate thiol pair buffer strength, to calculate "total glutathione concentration." Ex. 1007 at 51.

The authors then describe two sets of experiments they conducted to "elucidate how the total glutathione concentration and the ratio of reduced to oxidized glutathione affect the competition between folding and aggregation." *Id*. at 51. In both experiments, hen egg white lysozyme protein[10] was added to a refolding "preparation" containing (1) amounts of oxidized and reduced

[10] Hen egg white lysozyme is a 14.4 kDa protein that has 129 amino acid residues in its primary structure and four disulfide bonds. *See* Atassi (Ex. 1043).

glutathione (listed as a "redox component" in the '287 patent) and (2) guanidinium chloride (listed as a "denaturant" in the '287 patent) to obtain a refolding "solution." *Id*. at 49-50.

In the first set of experiments, the total glutathione concentration (thiol pair buffer strength) was kept constant at 14 mM, the ratio of reduced to oxidized glutathione was varied between 0.33 and 3.67, and the incubation time was 3 hours. *Id*. Both of these sets of values fall within the ranges of the challenged claims when calculated as "thiol pair ratio" using Equation 1 of the '287 patent. Ex. 1002 ¶ 194. As Figure 4a shows, the results showed an optimal refolding yield of >88% at a ratio of 2, with lesser yields at sub-optimal conditions:

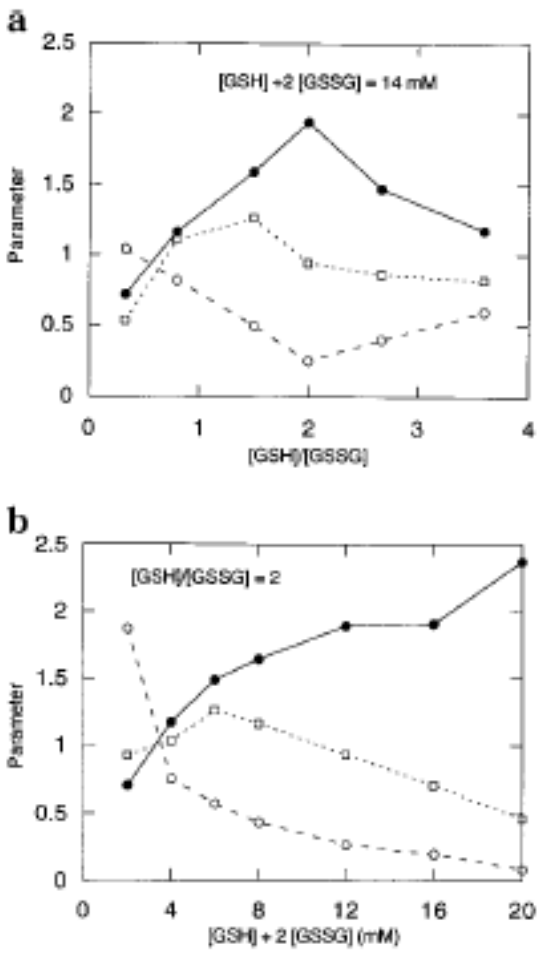


Figure 4. Effect of the ratio of reduced to oxidized glutathione (a) and total glutathione concentration (b) on the competition between folding and aggregation. Folding conditions: 1 mg/mL lysozyme, 1 M GdmCl, 50 mM tris, 1 mM EDTA, pH 8, 22 °C; (●) ψ (defined by eq 2), (□) k_2 (h^{-1}), (○) k_3 ($mL^2\ g^{-2}\ h^{-1}$).

This yield falls above the minimum yields recited in the challenged claims.

In the second set of experiments, the ratio of reduced to oxidized glutathione was kept constant at 2 while the total glutathione concentration (thiol pair buffer strength) was varied between 2 and 20 mM.[11] Again, these values fall within the

[11] The protein concentration in both experiments was 1.0 g/L, which falls within claims 2, 11, 17 and 27, which are not challenged in this IPR.

ranges of the challenged claims. As Figure 4b (above) shows, an optimal refolding yield of >88% was obtained at total glutathione concentrations between 6 and 16 mM. Again, this yield falls within or above the yields recited in the challenged claims.

The only differences between the challenged claims and the experiments in Clark 1998 are that (1) the hen egg white lysozyme was purchased from a supplier instead of obtained from a non-mammalian expression system; and (2) Clark 1998 calculated the thiol pair ratio using the formula [the reductant]/[the oxidant] instead of Equation 1, [the reductant]^2/[the oxidant].

A POSA would not have regarded the source of the hen egg white lysozyme as being material to the experiments reported in Clark 1998. Clark 1998 states that the express purposes of the study are the development of strategies for "the isolation, renaturation, and native disulfide bond formation of proteins produced as insoluble inclusion bodies in *Escherichia coli*" and "optimizing renaturation processes… [that] prevent the formation of off-pathway inactive and aggregated species" for such proteins." Ex. 1007 at 47. Moreover, both the '287 patent and – Clark 1998 fully unfolded their proteins in denaturing solutions prior to their refold experiments, rendering the source of the proteins immaterial. A POSA also would have been aware of the fact that hen egg white lysozyme had been successfully

expressed in *Aspergillus niger*, a non-mammalian expression system. *See e.g.*, Archer (Ex. 1039).

Also, as Ruddon shows, a POSA would have been well aware of the '287 patent's Equation 1 and how to use it to calculate thiol pair ratio. Ex. 1001 at 6:46-55. A POSA would have understood that it made no difference to Clark 1998's method of optimization whether the ratio was calculated using Equation 1 or the one used in Clark 1998. Indeed, all of the formulas disclosed in the '287 patent, Ruddon and Clark 1998 would have been elementary to a POSA knowledgeable about basic redox chemistry and experienced with redox buffer systems. As both Gilbert 1990 and Schafer show, calculating thiol pair ratios and buffer strengths from amounts of oxidant and reductant introduced into solution, and ensuring that one had enough reagent in the refold solution to act as an effective redox buffer for a given concentration of protein, were matters of basic, routine chemistry by 2009. Ex. 1002 at 179; Ex. 1027 at 1197 ("Total glutathione is traditionally considered to be a measurement of the compete pool of GSH.... total glutathione = GSH_i + 2 $GSSG_i$"); Ex. 1013 at 104 (For disulfide bonded proteins, "the applicable redox status of the cellular glutathione redox buffer would be the quantity $[GSH]^2/GSSG]$").

Finally, optimizing the refold yield for a given protein by varying thiol pair ratio and buffer strength required nothing more than routine optimization

experiments. This is clear from the fact that the claims of the ’287 patent cover *any* protein expressed from a non-mammalian system but *do not disclose any refolding conditions for any specific protein*. The patent presumes that a POSA is capable of “tailoring” redox buffers to a particular protein to facilitate refolding, Ex. 1001 at 5:7-10..[12]

3. Motivation To Combine and Expectation of Success

A POSA would have been motivated to combine the teachings of Ruddon and Clark 1998 for at least the following reasons:

- Ruddon and Clark 1998 each report successful methods for refolding proteins expressed as inclusion bodies in bacterial expression systems, as well as successful optimization of those methods, which are the very tasks that the POSA would have been engaged in;
- A POSA would know, and both Ruddon and Clark 1998 confirm,that each protein requires a somewhat different optimization. A POSA would look to related references to inform such optimization;
- The proteins described in both Ruddon and Clark 1998 comprise multiple disulfide bonds;

[12] If it is not, the challenged claims are not enabled.

- Both Ruddon and Clark 1998 disclose results of experiments in which the redox systems in the protein refolding mixtures were systematically varied in order to identify optimal refolding conditions;
- Both Ruddon and Clark 1998 indicate that a need exists for a method to produce bacterially-expressed proteins in amounts sufficient for, e.g., clinical applications; and
- Clark 1998 explores ways in which higher concentrations of disulfide-containing proteins can be refolded.

Both Schafer and Gilbert disclose equations for calculating thiol-pair ratio, thiol-pair buffer strength and other basic equations concerning redox buffers. To the extent a POSA needed to resort to a reference to perform these basic calculations, either Schafer or Gilbert would have been among the references consulted. Schafer concerns how to calculate thiol/disulfide exchange equilibria in the formation of disulfide bonds, which is one (albeit trivial) issue POSA would have faced in designing a refolding buffer for disulfide bond-containing proteins. Similarly, Shafer disclosed how to calculate the redox potential of glutathione redox buffers. The fact that these are the buffers used in Clark 1990 would have provided further motivation.

Thus, a POSA would have combined the teaching of Ruddon and Clark 1998, and if necessary Shafer and Gilbert, and arrived at the claimed method.

Given the success reported in Ruddon and Clark 1998, the POSA also would have had a reasonable expectation of success.

4. Obviousness of the Independent Claims

a. The Preamble

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

Clark 1998 states that the express purposes of its refolding study are the development of strategies for "the isolation, renaturation, and native disulfide bond formation of proteins produced as insoluble inclusion bodies in *Escherichia coli*" and "optimizing renaturation processes… [that] prevent the formation of off-pathway inactive and aggregated species." Ex. 1007 at 47. Ruddon discloses a method of producing and refolding biologically active protein in a non-mammalian expression system. *See* Sections IX.B.1.a-d. Thus, Clark 1998 in combination with Ruddon teach a method of refolding proteins expressed in a non-mammalian expression system.

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, the preparation comprising:	"preparing a solution comprising: the proteins; at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein

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,"	stabilizer; an amount of oxidant; and an amount of reductant,"

As discussed above in Section IX.C, Clark 1998 teaches a "preparation" and a solution," and Ruddon teaches a "solution," that supports the renaturation of a protein to a biologically active form. The "preparation" and "solution" of Clark 1998, and the "solution" of Ruddon, contain denaturants, aggregation suppressors, protein stabilizers andan oxidant/reductant. While Ruddon does not contact the protein with a "preparation" containing the redox buffer and other additives, but instead adds the buffer to a solution containing the protein and other additives, this is a trivial difference. and a POSA would have known that the refold mixtures could be assembled either as a "preparation" or a "solution." Thus, Ruddon in combination with Clark 1998 disclose formation of the claimed mixtures of components for protein refolding.

c. Redox Components

Claims 1 and 10	**Claims 16 and 26**
"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 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

wherein the thiol-pair buffer strength maintains the solubility of the preparation"	range of 0.001-100, wherein the thiol-pair buffer strength maintains the solubility of the solution"

As discussed in sections IX.B.1.c and IX.C, Ruddon and Clark 1998 disclose the use of amounts of oxidant and reductant to form buffers of varying strengths and that have thiol pair ratios in the range of 0.001-100. Because Ruddon and Clark 1998 successfully refolded protein into native, biologically active form, at yields that had been optimized, a POSA would have understood that the redox buffer strength they used "maintained the solubility of the preparation" or "solution". A POSA also would have understood the kinetic refolding data in Clark 1998 as disclosing the amount of properly-folded protein produced using various thiol pair ratios and buffer strengths, and demonstrating the relationship between these two redox parameters and the yield of properly refolded native protein versus misfolded proteins and aggregates. Ex. 1007 at 52.

d. 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."

As discussed in section IX.B.1.d, Ruddon teaches refolding methods that include incubation steps and result in amounts of properly refolded protein within the ranges claimed in the claims. Similarly, the methods disclosed in Clark 1998

include incubation steps and produced refolding efficiencies at or above the claimed ranges. *Id*. at 49-52 (reporting "renaturation yields" of ">88%"). Indeed, Clark 1998 discloses kinetic data and data showing the amount of properly folded protein versus incubation time in various refolding conditions. *Id*. at 52.

5. Obviousness of Claims 4, 12, 19 and 29

Claims 4, 12, 19 and 29 require 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. 1025 at 26:34-27:1. A POSA would have understood cysteamine to be the reductant and cystamine to be the oxidant in the redox buffer, and the thiol-pair buffer strength of the redox buffer to be 2[oxidant]+[reductant] = 2[3.6] + [6.4] = 13.6 mM. As discussed above, a POSA would have known this as general knowledge or from, e.g., Clark 1990, Ex. 194. To the extent they did not, Schafer teaches a POSA how to implement basic redox chemistry, including the relationship between thiol ratio and buffer strength that is used in Ruddon. *See* Ex. 1027 at 1197 ("the absolute concentrations of the components of the GSSG/2GSH redox pair have an impact on the reduction potential [i.e. $[GSH]^2/[GSSG]$]").

Clark 1998 also experimentally evaluates the effect of thiol pair buffer strength on protein refolding, and discloses an "optimum range to total glutathione

concentration between 6 and 16 mM." Ex. 1007 at 51. Moreover, Clark 1998 expressly uses the same formula for thiol pair buffer strength that the '287 patent uses.

Therefore, both Ruddon and Clark 1998 disclose the use of refolding buffers with thiol pair buffer strengths of 2 mM or greater.

6. Obviousness of Claims 5, 6, 20, and 21

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 total protein concentration in the solution." 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 total protein concentration in the solution."

As discussed above, a POSA would understand that during refolding of a protein containing disulfide bonds, the reductant and oxidant of a thiol pair must be present in the proper ratio and in an adequate amount to "allow for both formation and reshuffling of disulfide bonds." Ex. 1007 at 48. A POSA would have recognized that at as protein concentration increases, aggregation is favored over folding. Ex. 1002 ¶ 59. A POSA also would have known that the amount of oxidant and reductant present in the buffer must, as a simple matter of stoichiometry, be proportional to the number of disulfide bond-forming cysteine residues present in the protein to be refolded. If the amount of disulfide bond-

forming cysteines is increased but not the amounts of oxidant and reductant, the redox system's capacity to reform and reshuffle disulfide bonds may become overwhelmed. For example, Clark 1998 discloses that increasing total glutathione concentration (i.e. thiol-pair buffer strength) in relation to a static protein concentration increases the renaturation yield (i.e. the percentage of protein properly folded after a lengthy incubation), and that increasing protein concentration in relation to a static glutathione concentration increases the results in lower renaturation yields. *Id.* at 51; 49, 52. It would thus have been obvious to increase or decrease the thiol-pair buffer strength proportionally to an increase or decrease in protein concentration to balance the stoichiometry of the redox refolding system.

7. Obviousness of Claims 8, 9, 14, 15, 23, 24, 25 and 30

Claims 8 and 23 require that "the thiol-pair ratio is calculated according to the following equation: [the reductant]^2/[the oxidant]." Claims 9 and 24 require that "the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant] + [the reductant]." Claims 14, 15, 25 and 30 require that "the thiol-pair ratio is calculated according to the following equation: [the reductant]^2/[the oxidant]; and the thiol-pair buffer strength is calculated according to the following equation: 2[the oxidant] + [the reductant]."

As discussed above, Ruddon teaches the equation for the thiol-pair ratio required by claims 8, 14, 15, 23, 25, and 30 and Clark 1998 teaches the equation for thiol pair buffer strength required by claims 9, 14, 15, and 30. Both of these equations are also explicitly described in Schafer and Gilbert, as discussed previously. The equations, as well as the relationships between reductant concentration, oxidant concentration, and redox power that they represent, were well-known before the priority date of the '287 patent and would have been trivially obvious to a POSA. Ex. 1002 ¶ 147-48; 165-66. *See also*, Ex. 1013 at 85, 104; Ex. 1027 at 1196-98.

D. Ground 4: Claims 8, 9, 14, 15, 23, 24, 25 and 30 Would Have Been Obvious From Vallejo In Combination With Ruddon and Clark 1998, In Light Of Schafer or Gilbert

Dependent claims 8, 9, 14, 15, 23, 24, 25, and 30 recite either the equation for the thiol-pair ratio ("Equation 1"), the thiol-pair buffer strength ("Equation 2"), or both. Ex. 1001. These claims would have been obvious from a combination of Vallejo, Ruddon and Clark 1998, in light of Schafer or Gilbert. As discussed, Ruddon and Clark 1998 disclose the use of these equations to calculate thiol pair ratio and buffer strength. A POSA would have been motivated to combine Vallejo with Ruddon and Clark 1998 for the same reasons a POSA would have been motivated to combine Ruddon and Clark 1998, *e.g.*, these references all deal with how to devise an redox refolding method for optimally refolding inclusion body

proteins, which is what the POSA would be attempting to do. Because of the success reported in these references, a POSA would have had a reasonable expectation of success in achieving the yields of the challenged claims, using the claimed ranges of thiol pair ratio and buffer strength. To the extent a POSA was unfamiliar with any of the basic redox chemistry involved, Schafer or Gilbert would have been instructive. Therefore, claims 8, 9, 14, 15, 23, 24, 25, and 30 would have been obvious.

E. Secondary Considerations

Petitioners are aware of no relevant secondary considerations that have a nexus to, or are commensurate in scope, with any of the challenged claims. While PO has previously asserted that the '287 patent met a long-felt need for "the rational design of refolding proteins using redox chemicals, specifically, an efficient method that could predictably refold proteins, including at high concentrations and for more complex proteins . . . beyond more than just trial and error," as explained, the challenged claims are not directed to the use of any rational method for selecting redox conditions beyond what was well known and widely used in the prior art; nor are they directed specifically to refolding complex proteins at any concentration. Ex. 1037 at 76.

Moreover, the '287 patent has not, as PO asserts, achieved unexpected results of "greater predictability in identifying optimal conditions for refolding

proteins" by "identifying and applying a relationship not known in the prior art" between the TPR and TPBS equations. As discussed, these equations were known and disclosed in the prior art and did not create any practical change to the basic optimization steps required for optimizing refold conditions. Nor has PO established that the methods of the challenged claims led to any unexpected increase in the efficiency of refolding complex proteins at high concentrations beyond methods used in the prior art.

D. CONCLUSION

For the reasons set forth above, Petitioner respectfully submits that it has established a reasonable likelihood of success with respect to the challenged claims and requests that trial be instituted and the challenged claims cancelled.

Dated: April 14, 2019

By: /s/ Huiya Wu
Huiya Wu (Reg. No. 44,411)
Robert V. Cerwinski (to seek *pro hac vice* admission)
Linnea Cipriano (Reg. No. 67,729)

GOODWIN PROCTER LLP
The New York Times Building
620 Eighth Avenue
New York, NY 10018
(212) 813-8800 (telephone)
(212) 355-3333 (facsimile)
hwu@goodwinlaw.com
rcerwinski@goodwinlaw.com
lcipriano@goodwinlaw.com

Counsel for Petitioners

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105, I certify that I caused to be served a true and correct copy of the foregoing: **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,856,287** and the exhibits cited therein by U.S.P.S. *Priority Mail Express* on this day, April 14, 2019 on the Patent Owner's correspondence address of record for the subject patent as follows:

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

Dated: April 14, 2019

By: /s/ Huiya Wu
Huiya Wu (Reg. No. 44,411)
Robert V. Cerwinski (to seek *pro hac vice* admission)
Linnea Cipriano (Reg. No. 67,729)

GOODWIN PROCTER LLP
The New York Times Building
620 Eighth Avenue
New York, NY 10018
(212) 813-8800 (telephone)
(212) 355-3333 (facsimile)
hwu@goodwinlaw.com
rcerwinski@goodwinlaw.com
lcipriano@goodwinlaw.com

Counsel for Petitioners

CERTIFICATE OF WORD COUNT

The undersigned certifies that the attached Petition for *Inter Partes* Review of U.S. Patent No. 9,856,287 contains 13,971 words (as calculated by the word processing system used to prepare this Petition), excluding the parts of the Petition exempted by 37 C.F.R. § 42.24(a)(1).

Dated: April 14, 2019

By: /s/ Huiya Wu
Huiya Wu (Reg. No. 44,411)
Robert V. Cerwinski (to seek *pro hac vice* admission)
Linnea Cipriano (Reg. No. 67,729)

GOODWIN PROCTER LLP
The New York Times Building
620 Eighth Avenue
New York, NY 10018
(212) 813-8800 (telephone)
(212) 355-3333 (facsimile)
hwu@goodwinlaw.com
rcerwinski@goodwinlaw.com
lcipriano@goodwinlaw.com

Counsel for Petitioners