UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

APOTEX INC. and APOTEX CORP.
Petitioners

v.

AMGEN INC. and AMGEN MANUFACTURING LIMITED
Patent Owner

Inter Partes Review No.: IPR2016-01542

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 8,952,138 UNDER 35 U.S.C. §§ 311-319 AND 37 C.F.R. §§ 42.1-.80, 42.100-.123

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TABLE OF CONTENTS

Petitioners' Exhibit List

Exhibit	***Description***
1001	Shultz et al., U.S. Patent No. 8,952,138, "Refolding Proteins Using a Chemically Controlled Redox State," issued February 10, 2015.
1002	Declaration of Dr. Anne S. Robinson .
1003	Schlegl, U.S. Patent Publication No. 2007/0238860, "Method for Refolding a Protein," published October 11, 2007 ("*Schlegl*").
1004	Hevehan and Clark, "Oxidative Renaturation of Lysozyme at High Concentrations," Biotechnology and Bioengineering, 1996, 54(3): 221-230 ("*Hevehan*").
1005	Brady et al., U.S. Patent Publication No. 2006/0228329, "Homogenous Preparations of IL-31," published October 12, 2006 ("*Brady*").
1006	Hakim and Benhar, "Inclonals," mAbs, published online May 1, 2009, 1:3, 281-287 ("*Inclonals*").
1007	Whitford, "Proteins: Structure and Function," September 1, 2005, excerpted.
1008	http://chemistry.umeche.maine.edu/CHY431/Ribo-fold.jpg
1009	*Reserved*
1010	Cohen et al., U.S. Patent No. 4,237,224, "Process for Producing Biologically Functional Molecular Chimeras," issued December 2, 1980.
1011	Cohen et al., U.S. Patent No. 4,468,464, "Biologically Functional Molecular Chimeras," issued August 28, 1984.
1012	Cohen et al., U.S. Patent No. 4,740,470, "Biologically Functional Molecular Chimeras," issued April 26, 1988.
1013	Johnson, "Human insulin from recombinant DNA technology". Science (1983) 219 (4585): 632–637.

Exhibit	***Description***
1014	Vallejo et al. "Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins," Microbial Cell Factories (2004) 3, 1-12.
1015	Neubauer et al. "Protein inclusion bodies in recombinant bacteria. Inclusions in Prokaryotes." Microbiology Monographs Edited by: Shively JM. Springer; (2006) 237-292.
1016	Ventura and Villaverde "Protein quality in bacterial inclusion bodies" TRENDS in Biotechnology Vol.24 No.4 April 2006.
1017	https://www.profacgen.com/inclusion-body-purification-protein-refolding.htm
1018	Georgiou and Valax, "Isolating Inclusion Bodies from Bacteria", Chapter 3 in Methods in Enzymology, VOL. 309, p. 48-58 (1999) Academic Press.
1019	Palmer and Wingfield "Preparation and Extraction of Insoluble (Inclusion-Body) Proteins from *Escherichia coli*" Curr Protoc Protein Sci. (2004) November; CHAPTER: Unit–6.3. doi:10.1002/0471140864.ps0603s38
1020	Clark, "Protein Refolding for Industrial Processes," Current Opinion in Biotechnology, (2001) 12:202-207.
1021	Clark, "Refolding of Recombinant Proteins" Current Opinion in Biotechnology, (1998) 9:157-163.
1022	Shortle et al., "Clustering of Low-Energy Conformations Near the Native Structures of Small Proteins," Proc Natl Acad Sci (1998) 95, 11158-62.
1023	Panda, "Bioprocessing of Therapeutic Proteins from the Inclusion Bodies of Escherichia coli" Adv Biochem Engin/Biotechnol (2003) 85: 43–93.
1024	Vincentelli, "High-throughput automated refolding screening of inclusion bodies," Protein Science (2004) 13:2782–2792.

Exhibit	***Description***
1025	Willis et al., "Investigation of protein refolding using a fractional factorial screen: A study of reagent effects and interactions." Protein Science (2005) 14(7), 1818–1826.
1026	Jungbauer and Kaar "Current status of technical protein refolding," Journal of Biotechnology 128 (2007) 587–596.
1027	Ferrer-Miralles et al. "Microbial factories for recombinant pharmaceuticals" Microbial Cell Factories (2009) 8:17
1028	Graumann and Premsaller, "Manufacturing of recombinant therapeutic proteins in microbial systems," Biotech J. (2006) 1:164-186.
1029	Xie and Wetlaufer, "Control of aggregation in protein refolding: The temperature-leap tactic," Protein Science (1996) 5:517-523.
1030	Puri, "Refolding of recombinant porcine growth hormone in a reducing environment limits in vitro aggregate formation," FEBS (1991) vol. 292, no. 1.2, 187-190.
1031	Ejima, "High yield refolding and purification process for recombinant human interleukin-6 expressed in *Escherichia coli*," Biotechnology and Bioengineering (1999) vol. 62, no. 3, 301-310.
1032	Patra et al., "Optimization of inclusion body solubilization and renaturation of recombinant human growth hormone from *Escherichia coli*," Protein Expression and Purification (2000) 18, 182-192.
1033	Builder et al., U.S. Patent No. 5,663,304, "Refolding of misfolded insulin-like growth factor-1," issued September 2, 1997.
1034	Notice of Allowance, U.S. Patent Application No. 12/820,087 (now Patent No. 8,952,138), mailed October 23, 2014.
1035	Information Disclosure Statement, U.S. Patent Application No. 12/820,087 (now Patent No. 8,952,138), filed September 23, 2010.

Exhibit	***Description***
1036	Information Disclosure Statement, U.S. Patent Application No. 12/820,087 (now Patent No. 8,952,138), filed September 20, 2012.
1037	Markman Order, Amgen v. Apotex, Case no. 0:15-cv-61631-JIC, Document 119, entered April 7, 2016.
1038	Amgen Opening Markman Brief, Amgen v. Apotex, Case no. 0:15-cv-61631-JIC, Document 77, filed December 11, 2015.
1039	Information Disclosure Statement, U.S. Patent Application No. 12/820,087 (now Patent No. 8,952,138), filed October 20, 2010.
1040	Acknowledgement of consideration of references, U.S. Patent Application No. 12/820,087 (now Patent No. 8,952,138), January 9, 2012.
1041	Clark et al., "Oxidative renaturation of hen egg-white lysozyme. Folding vs Aggregation," Biotechnol. Prog. (1998) 14, 47-54.
1042	Mannall et al., "Factors Affecting Protein Refolding Yields in a Fed-Batch and Batch-Refolding System," Biotechnology and Bioengineering, (2007) vol. 97, no. 6, 1523-1534.
1043	Fischer et al., "Isolation, Renaturation, and Formation of Disulfide Bonds of Eukaryotic Proteins Expressed in *Escherichia coli* as Inclusion Bodies," Biotechnology and Bioengineering (1993) vol. 41, pp 3-13.
1044	Misawa and Kumagai "Refolding of Therapeutic Proteins Produced in Escherichia coli as Inclusion Bodies" Biopoly (1999) 51: 297–307.
1045	Protein Data Bank, Hen Egg White Lysozyme, http://www.rcsb.org/pdb/explore/explore.do?structureId=193L; http://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=193L.
1046	Gegg et al., U.S. Patent No. 7,442,778, "Modified Fc Molecules," issued October 28, 2008.

Exhibit	***Description***
1047	Enbrel™ (etanercept) label, November 1998.
1048	Bolado, "Amgen Opens Trial in Fight Over Neulasta Generic," Law360, July 11, 2016 (http://www.law360.com/articles/814748/amgen-opens-trial-in-fight-over-neulasta-generic).
1049	Dr. Anne S. Robinson CV
1050	http://pubs.rsc.org/services/images/RSCpubs.ePlatform.Service.FreeContent.ImageService.svc/ImageService/Articleimage/2014/TB/c4tb00168k/c4tb00168k-f2_hi-res.gif

I. INTRODUCTION

Petitioners Apotex Inc. and Apotex Corp. ("Apotex"; "Petitioners") respectfully request inter partes review under 35 U.S.C. § 311 and 37 C.F.R. § 42.101 of claims 1-24 of U.S. Patent No. 8,952,138 ("the '138 Patent"), titled "Refolding Proteins Using a Chemically Controlled Redox State." (EX 1001).

II. OVERVIEW

The challenged claims of the '138 Patent recite methods of refolding proteins expressed in a non-mammalian expression system by a simple three-step process: (1) contacting the desired protein with a refold buffer having certain characteristics, (2) incubating the resulting refold mixture, and (3) isolating the protein from that refold mixture. *See*, *e.g.*, EX1001 at claim 1. This simple process, including all of the characteristics of the refold buffer and the various categories of desired proteins claimed by the '138 Patent, were well-researched, well-tested, and well-known long before June 2009. Claims 1-24 of the '138 Patent are therefore anticipated or at least obvious over the prior art references discussed herein.

III. STANDING (37 C.F.R. § 42.104(a)); PROCEDURAL STATEMENTS

Petitioners certify that (1) the '138 Patent is available for IPR and (2) Petitioners are not barred or estopped from requesting IPR of any claim of the '138 Patent. This Petition is filed in accordance with 37 C.F.R. § 42.106(a). This

Petition is being filed less than one year from the date on which the Petitioners were served with a complaint by the Patent Owner regarding the '138 Patent. A Power of Attorney and an Exhibit List are filed concurrently herewith. The required fee is paid online via credit card. The Office is authorized to charge fee deficiencies and credit overpayments to Deposit Acct. No. 05-1323 (Customer ID No. 23911).

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

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

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

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

The '138 Patent is currently the subject of the following litigation: *Amgen Inc. et al. v. Apotex Inc. et al.*, No. 0:15-CV-61631-JIC/BSS (S.D. Fla.).

U.S. Application Serial Nos. 14/611,037 and 14/793,590 are pending and claim common priority to the '138 Patent.

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

Lead Counsel	Back-Up Counsel
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Notice of Service Information (37 C.F.R. § 42.8(b)(4)):

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

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

Petitioners request IPR and cancellation of claims 1-24. Petitioners' full statement of the reasons for the relief requested is set forth in detail below, and in particular in Section X.

VI. TECHNOLOGY BACKGROUND

The challenged claims of the '138 Patent generally recite methods of refolding proteins that are created ("expressed") in a non-mammalian cell ("non-mammalian expression system"). More specifically, the claims recite the well-known process of refolding a protein expressed in a non-mammalian expression system by starting with a solution containing a desired protein and following the steps of (1) mixing that protein solution with a refold buffer solution; (2) incubating the resulting mixture; and (3) isolating the protein from that mixture. The refolding of proteins by this process was quite familiar to those in the art as of June 2009, and the science behind this process was well known.

A. The Basic Science of Proteins

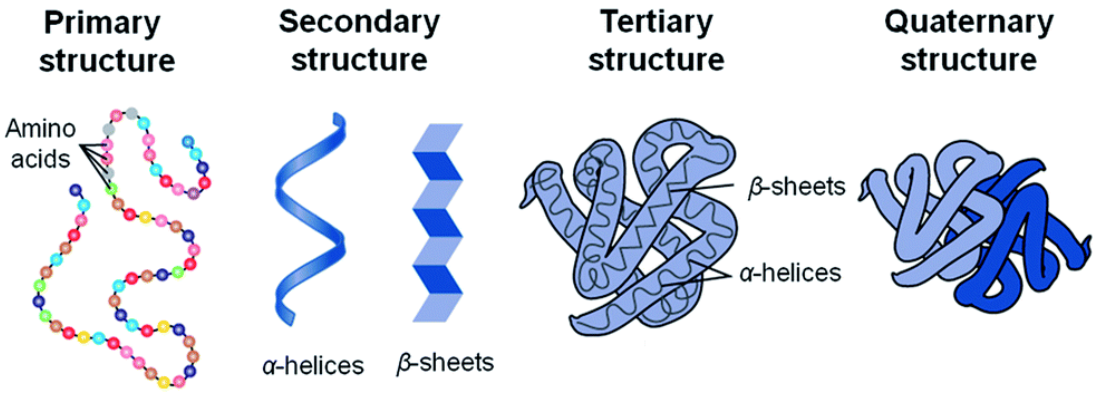
1. Protein Structure in General

Proteins are a three-dimensional arrangement of atoms defined by "levels" of structure: (i) primary structure, (ii) secondary structure, (iii) tertiary structure, and (iv) quaternary structure. EX1007 at 43-67.[1] The first three levels of structure are known as the protein's native structure and confer the protein's biological

[1] Except for patent and patent publication Exhibits and EX1002, this Petition cites to the page numbers added by Petitioners at the bottom of each Exhibit.

function. *Id.* at 44-67; EX1003 at [0030]; EX1002 at ¶ 35. Each of these structural levels is important. *Id.* If something is incorrect at any of these levels, the protein will not perform its function. *Id.*

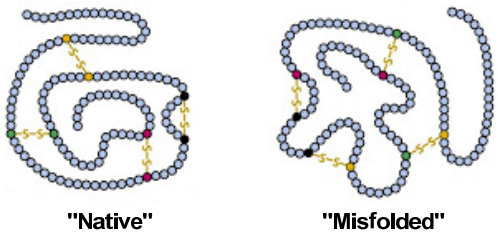
A protein's primary structure simply refers to the amino acid sequence along the linear polypeptide chain, shown below. EX1007 at 19, 43. Secondary structure refers to the local conformation of the polypeptide chain, generally characterized by α-helices and β-sheets, as shown below, which are caused by intramolecular forces (*i.e.*, hydrogen bonding). *Id.* at 43-44. Tertiary structure refers to the compact three-dimensional structure of the entire protein; an example shown below. *Id.* at 54-57, 63. Quaternary structure refers to the number and arrangement of multiple folded protein subunits in a multi-subunit complex.[2] *Id.* at 66-67.



[2] Refolding of multiple folded proteins in a multi-subunit complex is not at issue.

EX1050.

Certain chemical bonds in the protein known as "disulfide bonds" are equally important to a protein's native structure. EX1007 at 33; EX1002 at ¶ 37. Disulfide bonds stabilize the protein's three-dimensional structure, which form between particular amino acids that are close in proximity. EX1007 at 32-33. When these disulfide bonds are misformed, however, the protein could misfold, *i.e.*, take an undesirable structure other than its native structure. EX1033 at 2:8-14.



EX1008; EX1002 at ¶ 37. A protein in a conformation other than its native structure may not be bioactive. EX1002 at ¶ 37.

2. Protein Synthesis in and out of the Lab

Generally, organisms naturally create proteins by the following process. EX1007 at 114. Information encoded in DNA is copied to generate an RNA molecule, which serves as a template for the synthesis of a protein. *Id.* Then, the genetic information stored in RNA is "read" by the ribosomes of the cell, and is converted into protein sequences. *Id.* This process of transcription (from DNA to

RNA) and translation (from RNA to a protein) is known as biosynthesis, shown below:

Id. at 114-15.

Proteins can also be synthesized in the lab using recombinant DNA technology, which has been known in the art since at least the mid to late 1970s. EX1002 at ¶ 39. For example, the use of this technology was patented by Cohen and Boyer in 1974, and the first commercial production was human insulin by Eli Lilly in 1981. *See generally* EX1010; EX1011; EX1012; EX1013.

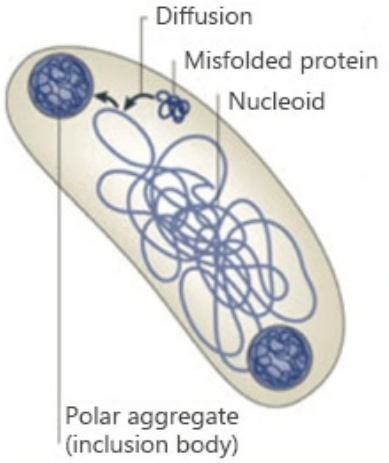
Recombinant DNA combines two or more pieces of DNA. EX1002 at ¶ 39. The recombinant DNA is then inserted into a cell and enables the cell to, among other things, produce a desired protein that the cell typically does not synthesize. EX1007 at 179. In essence, recombinant DNA technology turns the host cell into a "factory" that creates a large amount of the desired protein in a highly efficient manner. *See* EX1013 at 5; EX1002 at ¶ 39. Proteins that are expressed using recombinant DNA technology are called recombinant proteins. EX1007 at 178.

Recombinant DNA technology can be used in both mammalian and non-mammalian cells ("expression systems"), but low-yield mammalian expression systems are generally cost prohibitive. EX1014 at 1. Scientists thus turned to

very-high-yield bacterial expression systems to express recombinant proteins. *Id.*; EX1002 at ¶ 40. One well-established host organism in the field of recombinant technology is *Escherichia coli*, more commonly referred to as *E. coli*. EX1002 at ¶ 40. The biochemistry and genetics of *E. coli* are very well known and *E. coli* is easily manipulated; thus it is the organism of choice for many researchers. EX1015 at 2, 5-6; EX1016 at 179; EX1005 at [0002]; EX1002 at ¶ 40.

B. Recovery of Bioactive Protein and Protein Refolding

A host cell expressing recombinant proteins produces two types of proteins: (1) correctly folded proteins in their native structure and (2) misfolded proteins that group together in the cell in what are known as "inclusion bodies," as shown in the example below of a bacteria cell expressing protein:



EX1017; *see also* EX1016 at 3-4; EX1002 at ¶ 41. Inclusion bodies are known for several decades to contain between 35-95% of the overexpressed (recombinant)

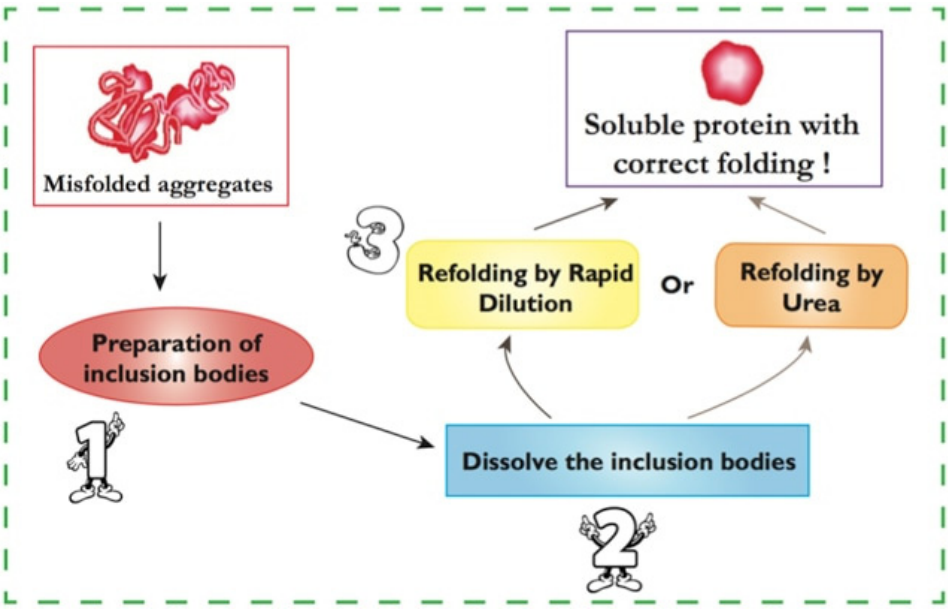
desired protein, as well as DNA, ribosomal RNA, lipids, and other proteins. EX1018 at 2, 4; EX1015 at 11; EX1019 at 10.

Scientists generally believe that inclusion bodies are the result of various consequences of using a non-mammalian expression system for creating the proteins. EX1002 at ¶ 41. Proteins have a tendency to aggregate because of the growth conditions used for the bacterial cells, including media, growth temperature, and how the protein is expressed. EX1015 at 4, 9; EX1016 at 1. Bacterial cells provide for a more rapid production of protein than the natural process of protein generation in mammalian cells. EX1002 at ¶ 41. In essence, the bacterial cells have trouble "keeping up" with this rapid rate of protein generation and as a result, the proteins misform and group together, forming inclusion bodies. *Id.* In addition, the chemical environment of the bacterial cell does not promote the formation of disulfide bonds, as is often termed as a "reducing redox environment", which promotes aggregation and inclusion body formation for proteins that contain disulfide bonds in their native structure. *Id.*; EX1015 at 6.

Recombinant proteins expressed in *E. coli* were known to have this exact problem with inclusion bodies. *See generally* EX1020; *see* EX1016 at 2. Accordingly, techniques for recovering native, folded proteins in a bioactive and stable form from those inclusion bodies were developed. As early as 1998, there

were "over 300 reports of mammalian, plant, and microbial proteins obtained and renatured from inclusion bodies formed in *E. coli*." EX1018 at 1.

One of those techniques follows a three-step process known as "direct dilution": (1) isolation and purification of the inclusion bodies; (2) solubilization of the inclusion bodies; and (3) refolding of the solubilized protein. *See generally* EX1021; EX1002 at ¶ 43. An example of this three-step process is shown below:



EX1017.

1. Step 1: Isolate the Inclusion Bodies

To isolate inclusion bodies, host cells producing protein and thus containing the inclusion bodies must undergo disruption of their cell membrane through chemical or physical methods, known as "lysing" the cell. EX1007 at 183-84; *see also* EX1020 at 1. Once the host cells are lysed, the contents of the cell are

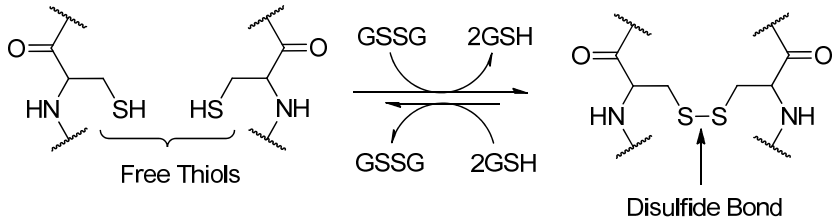
released, and the resulting suspension is centrifuged to separate the lighter soluble portion (that contains the soluble proteins) from the heavier insoluble portion (that contains the inclusion bodies and cellular debris). EX1007 at 185-87; *see also* EX1020 at 1.

2. Step 2: Solubilize the Inclusion Bodies

Next, the inclusion bodies are isolated from the cellular debris in the insoluble fraction. EX1002 at ¶ 45. The isolated inclusion bodies are then washed to remove surface-absorbed material, and solubilized with detergents (*e.g.*, sodium dodecyl sulfate) or high concentrations of denaturants (*e.g.*, guanidinium chloride, urea) to release the desired protein from the inclusion bodies. EX1020 at 2; *see also* EX1004 at 4. These chemicals disrupt the hydrogen bonding network in the misfolded protein structure of the inclusion bodies to bring the protein back to an unfolded state before restarting the folding process. EX1015 at 31-32; EX1019 at 10, 12-13.

Other linkages present in the protein, such as disulfide bonds between sulfur residues, are typically reduced to free thiols using a reducing agent (*e.g.*, beta-mercaptoethanol), as misformed disulfide bonds, common in misfolded protein, support the misfolded conformation, rather than the native, correctly formed protein structure. EX1015 at 267-268; EX1019 at 12-13; EX1002 at ¶ 45. For

example, disulfide bonds can be reduced via a redox reaction with two molecules of reduced glutathione ("GSH") to give two free thiols and a molecule of oxidized glutathione ("GSSG"), as shown in the reaction from right to left in the below diagram. The GSSG can then oxidize the thiols again and reform the disulfide bond, as shown in the reaction from left to right in the below diagram:



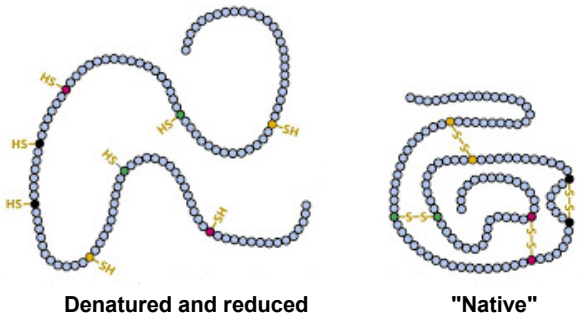
EX1002 at ¶ 45.

A common way of solubilizing inclusion bodies was by diluting the solution containing the isolated inclusion bodies with a refold buffer (also called refolding buffer or renaturation buffer). EX1002 at ¶ 47. The refold buffer had a number of components, such as but not limited to denaturants, aggregate suppressors, and protein stabilizers. *Id.* The refold buffer thus had a number of variables to optimize, including the type and relative concentrations of the components and redox systems. EX1015 at 33-36; EX1023 at 31-32; EX1014 at 7. Other variables that could be optimized included pH, temperature and timing of the process, and purification methods to complement the procedure. EX1023 at 4; EX1014 at 7.

These variables were well understood and could be easily selected from a variety of well-developed parameters and techniques. EX1004 at 4-6; EX1033 Tables IV-VII; EX1002 at ¶ 47. In fact, those skilled in the art even used robots to screen a large number of conditions for protein refolding and optimized those conditions for a given desired protein, with a success rate of about 70%. *See* EX1024 at 1-2; EX1025 at 1; EX1002 at ¶ 47.

3. Step 3: Refold the Solubilized Protein

After solubilization of inclusion bodies through denaturation and reduction, the released protein must be "refolded" to regain the protein's native bioactive structure. EX1026 at 2-3; EX1021 at 2-5. This "refolding" process causes an unstructured (or denatured) protein to fold into its unique and native three-dimensional structure necessary to its bioactivity. EX1007 at 43; EX1003 at [0030]; EX1002 at ¶ 46. Representations of these three protein structure states are shown below:



EX1008.

It was known before 2009 that the protein released from the inclusion bodies by the solubilizing step must be placed in an environment that facilitates the formation of the desired native protein structure (*e.g.*, low denaturant concentration). EX1004 at 2. When the desired protein contained disulfide bonds in a native state, the solubilized inclusion bodies must be placed in appropriate redox conditions to reform those necessary disulfide bonds. *Id.* at 5. Scientists thus used a redox system that favored oxidation consisting of a mixture of reduced and oxidized thiols to refold the desired protein. *Id.*

The desired equilibrium of reduction and oxidization, discussed on pages 11-12, *supra*, was well known to be controlled by the ratio and relative concentration of the thiol pairs in the redox mixture. *Id.* at 5-6. While the example on page 12, *supra*, uses GSH/GSSG, other thiol pairs of choice included cysteine/cystine and cysteamine/cystamine. EX1020 at 4. By using the appropriate redox system in the refolding buffer, the disulfide-bond-forming reaction could be made balanced to achieve an optimal redox state. EX1044 at 5. This allowed the protein to fold to its native structure. EX1002 at ¶ 49.

C. Additional Considerations in Commercial Production of Recombinant Proteins

Many therapeutic proteins are difficult to obtain from natural sources and need to be produced by recombinant DNA technologies. EX1002 at ¶ 50. As of

2009, 30% of the 151 recombinantly-produced approved pharmaceuticals were produced by bacteria. EX1027 at 1-2. In part, the use of *E. coli* is attributed to its advantages in meeting "[t]he ultimate goal of recombinant fermentation research," which is "to obtain the highest amount of protein in a given volume in the least amount of time." EX1023 at 4. For example, as of 2005, tissue plasminogen activator, human insulin, human growth hormone, human parathyroid hormone, granulocyte-colony stimulating factor, interferon alfacon-1, interferon β-1b, among others were produced and FDA-approved as commercial therapeutics by refolding from inclusion bodies produced in *E. coli*. EX1028 at 2.

Production of commercial therapeutic proteins also required huge refolding vessels that were a necessary consequence of the common (and inexpensive) method of refolding protein through the three-step process of direct dilution discussed above. EX1004 at 1-2; EX1002 at ¶ 51. Those skilled in the art were able to decrease the size of these refolding vessels by increasing the concentration of protein before and during refolding, all before 2009. EX1002 at ¶ 51. Unfortunately, with higher concentrations came another issue.

During refolding at a higher concentration, intermediates in the refolding process are more prone to associate in unproductive ways, leading to misfolded proteins called "aggregates." EX1014 at 3; EX1003 at ¶ [0008]. This process of

"aggregation" competed with the desired folding pathway, lowering the yield of properly folded proteins. EX1003 at ¶¶ [0008]-[0009]. Those skilled in the art had numerous solutions at their disposal to deal with aggregation prior to 2009, however. EX1002 at ¶ 51.

One of those solutions was to add an aggregation suppressor, with arginine being the most commonly used aggregate suppressor. EX1014 at 6. By adding arginine to the refold buffer, the yield of refolded protein increased by almost 50%, due to the suppression of aggregation. EX1004 at 4. Another solution was to incubate the refold mixture at a low temperature to slow down the rate of aggregation and promote the refolding process. EX1029 at 3. These and other similar pre-2009 solutions made it possible to refold proteins of varying complexity at a high protein concentration, including 1-5 mg/mL of protein or greater, by the process of direct dilution. *See* EX1004 at 1; EX1030 1, 3; EX1031 at 1-2; EX1032 at 5-6; EX1033 at Abstract, 6:45-47, 18:40-42.

VII. THE '138 PATENT, SKILL IN THE ART, AND PROSECUTION HISTORY

A. The '138 Patent

The '138 Patent is entitled: "Refolding Proteins Using a Chemically Controlled Redox State." The '138 Patent issued on February 10, 2015 from U.S. Application No. 12/820,087 ("the '087 application"), which was filed on June 21,

2010. The '138 Patent claims priority to Provisional Application No. 61/219,257, filed on June 22, 2009.

The '138 Patent has one independent claim, which recites a "method of refolding a protein." EX1001 at claim 1. Claim 1 places no restriction on the desired protein to be refolded or on the characteristics of the protein, other than that the protein must be "expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater." *Id.* No specific size or complexity of protein is required. Claim 1 then sets forth the very basic and well-known steps of refolding by direct dilution: (1) "contacting the protein with a refold buffer" of certain characteristics "to form a refold mixture;" (2) "incubating the refold mixture;" and (3) "isolating the protein from the refold mixture." *Id.*

The '138 Patent specification does not provide a single example of refolding any *specific* protein with the claimed methods. Instead, the '138 Patent specification broadly applies to "any type of protein." *Id.* at 4:23-29. The few examples given are directed to general categories of proteins that can be refolded using the claimed method. *See*, *e.g.*, *id.* at 3:21-25, 4:23-29, 13:41-45, 13:66-67, 14:53-55. The Figures of the '138 Patent likewise fail to further specify applicable proteins and shed no light on the optimum selection of refolding process variables such as thiol-pair ratio and redox buffer strength. *See*, *e.g.*, *id.* at 8:44-56, Figs. 1a-

1f. This omission is surprising, given the '138 Patent specification's repeated guidance to optimize the refold reaction based on the specific desired protein and its concentration. *See id.* at 8:19-36, 9:20-33, 10:45-47, 14:6-10.

The '138 Patent also uses the term "protein" twice in claim 1, once before contacting the refold buffer and once after isolation from the refold buffer. *Id.* at claim 1. It is unclear whether the "protein" recited before contacting the refold buffer and recited after isolation from the refold buffer are identical in terms of structure, biological function, and other characteristics. Claims 4-12 depend on claim and focus on further characterizing "protein" but shed no more light on this issue. Claim 8 even adds additional confusion to what "endogenous"—a relative term, *i.e.*, endogenous to what species?—means. *Id.* at claim 8.

B. Person of Ordinary Skill in the Art

The person of ordinary skill in the art ("POSA") to which the '138 Patent is directed would have had at least a Bachelor's degree (or the equivalent) in Biochemistry or Chemical Engineering with several years' experience in biochemical manufacturing, protein purification, and protein refolding, or alternatively, an advanced degree (Masters or Ph.D.) in Biochemistry or Chemical Engineering with emphasis in these same areas. EX1002 at ¶ 17. This person may also work in collaboration with other scientists and/or clinicians who have

experience in protein refolding or related disciplines. *Id.* A POSA would have easily understood the prior art references referred to herein and would have had the capacity to draw inferences from them. *Id.*

C. Prosecution History

The '087 Application that led to the '138 Patent was allowed because "[t]he most pertinent prior art U.S. Pat. 7,138,370 does not teach or suggest the final thiol-pair ratio of 0.001-100 as required by the claimed invention" and because the applicants filed a terminal disclaimer over copending U.S. Patent Application 12/822,990, which "obviates the non-statutory obviousness type double patenting rejection." EX1034 at 6.

During prosecution, the Applicants filed a number of Information Disclosure Statements. A number of the references that the Applicants disclosed in these Information Disclosure Statements were articles written by Eliana De Bernardez Clark. *See* EX1035 at 4 (Cite No. 2); EX1036 at 4 (Cite Nos. 2, 3). But the Applicants curiously did not disclose another article written by Clark and an additional author, Hevehan, ("*Hevehan*", EX1004) to the USPTO. Petitioners rely on *Hevehan* here to invalidate the '138 Patent as part of Ground 1.

VIII. CLAIM CONSTRUCTION

In an *inter partes* review, claim terms in an unexpired patent are interpreted according to their broadest reasonable construction in light of the specification of

the patent in which they appear. 37 C.F.R. § 42.100(b); Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,764 (Aug. 14, 2012); *Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 2131, 2144-46 (2016).

The claims of the '138 Patent were previously construed by the U.S. District Court for the Southern District of Florida in *Amgen Inc. v. Apotex Inc.*, No. 15-cv-61631 (S.D. Fla.) ("the Neulasta Litigation"). *See* EX1037. That claim construction was based on the different legal standard utilized by Federal District Courts, known as the "*Phillips* standard" from the seminal case *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005) (en banc). EX1037 at 2-4.

In accordance with 37 C.F.R. § 42.100(b), the challenged claims must be given their broadest reasonable interpretations in light of the specification of the '138 Patent. To be clear, any claim terms not included in the following discussion should be given their broadest reasonable construction in light of the specification.

A. "a protein"

The term "a protein" does not need a specialized construction apart from its broadest reasonable interpretation in light of the specification. Petitioners address the term, however, because Petitioners anticipate that Patent Owner will attempt to propose a construction that limits the term to "complex proteins."

Limiting the term "a protein" in such a way does not comport with the broadest reasonable interpretation of the claims in light of the specification. There is no language in claim 1 that requires a "complex" protein, as claim 1 covers *any* protein that is "expressed in a non-mammalian expression system" and is "present in a volume at a concentration of 2.0 g/L or greater." Because "protein" is recited broadly in claim 1, the simple language of the claim should prevail.

Limiting the claims to "complex" proteins would also be improper because claim 10 depends from claim 1 and recites, "wherein the protein is a complex protein." The principle of claim differentiation presumes that construing "a protein" to be a "complex protein" is improper. *See Enzo Biochem Inc. v. Applera Corp.*, 780 F.3d 1149, 1156-57 (Fed. Cir. 2015); *Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d 898, 910 (Fed. Cir. 2004); *see also HM Elecs., Inc. v. 3M Innovative Properties Co.*, IPR2015-00491, Paper 35, at 19 (April 18, 2016). This presumption is not overcome here, because there is no contrary construction "dictated" by the specification or prosecution history, and the proposed construction is reasonable. *See Seachange Int'l, Inc. v. C-COR, Inc.*, 413 F.3d 1361, 1368-69 (Fed. Cir. 2005).

Nevertheless, if the Board intends to limit the term "protein" to a "complex protein," the '138 Patent specification provides guidance on the interpretation of

"complex." The '138 Patent defines "complex protein" most broadly as a protein "comprising 2-23 disulfide bonds **or** greater than 250 amino acids, **or** having a MW [molecular weight] of greater than 20,000 daltons." EX1001 at 4:25-27 (emphases added); *see also id.* at 2:13-5, 5:64-67, 12:58-61. Therefore, the broadest reasonable interpretation of "complex protein" in light of the specification is "a protein that has 2 to 23 disulfide bonds or more than 250 amino acids or a molecular weight of more than 20,000 daltons."

B. "a protein … present in a volume at a concentration of 2.0 g/L or greater"

The phrase "a protein … present in a volume at a concentration of 2.0 g/L or greater" should be interpreted to mean "a protein as it exists in a volume before contacting the volume with a refold buffer. The protein concentration in the volume is 2.0 g/L or greater." This construction is identical to the one proposed by Patent Owner in the Neulasta Litigation under the *Phillips* standard. EX1037 at 4; EX1038 at 9. Having argued for that construction in the Neulasta Litigation, Patent Owner cannot now argue for a narrower claim construction. The broadest reasonable interpretation of this phrase cannot be narrower than the construction of the phrase under the *Phillips* standard. *See Facebook, Inc. v. Pragmatus AV, LLC*, 582 F. App'x 864, 869 (Fed. Cir. 2014).

One such narrower construction is that proposed by Petitioners in the Neulasta Litigation, wherein the recited 2.0 g/L concentration is measured "after dilution in a refold buffer." *See* EX1038 at 9. This construction is narrower than the one proposed by Patent Owner in the Neulasta Litigation because Patent Owner's Neulasta Litigation proposal measures concentration of an initial volume ("before contacting the volume with a refold buffer") rather than a resultant volume ("after dilution in a refold buffer"). *Id.* Patent Owner's Neulasta Litigation proposal necessarily limits only the protein concentration in the initial protein volume whereas Petitioners' Neulasta Litigation proposal additionally limits the volume of the refold buffer. *See id.* at 9-10. This is because the combination of those two volumes must yield a certain concentration of protein. *See id.*

Patent Owner submitted the following argument in support for its claim construction in the Neulasta Litigation: "Prior to formation of the refold mixture, the volume containing the protein at 2.0 g/L or greater is necessarily separate from the refold buffer, otherwise it would not need to be "contacted" with the refold buffer." *Id.* at 10. Patent Owner also cited to portions of the '138 Patent specification as support for that claim construction. *See id.* at 10-11 (citing EX1001 at 10:12-23, 11:9, 11:64-67, 12:40-53, 14:66-15:8, 15:47-62).

C. "final thiol-pair ratio"

The term "final thiol-pair ratio" should be interpreted to mean "the relationship of the reduced and oxidized redox species used in the refold buffer as defined by the equation $\frac{[\text{reductant}]^2}{[\text{oxidant}]}$." This definition comes straight from the '138 Patent specification. EX1001 at 6:20-27.

This term should also be interpreted in relation to the concentrations in the redox component, so that the construction is identical to the one proposed by Patent Owner in the Neulasta Litigation under the *Phillips* standard. EX1037 at 7; EX1038 at 12-13. Having argued for that construction in the Neulasta Litigation, Patent Owner cannot now argue for a narrower claim construction, as the broadest reasonable interpretation of this phrase cannot be narrower than the construction of the phrase under the *Phillips* standard. *See Facebook*, 582 F. App'x at 869.

Patent Owner submitted the following argument in support for its claim construction in the Neulasta Litigation: "The plain language of claim 1 recites a redox component that is not initially part of the refold mixture, and in that context, the claim recites the final thiol-pair ratio as being an element of the redox component. EX1038 at 13 (citation omitted). Patent Owner also cited to portions of the '138 Patent specification as support for that claim construction. *See id.* (citing EX1001 at 10:24-30, 11:11-17, 11:40-46, 11:64-67).

D. "redox buffer strength"

The term "redox buffer strength" should be interpreted to mean "2[oxidant] + [reductant]." This definition comes straight from the '138 Patent specification. EX1001 at 6:29-38.

This term should also be interpreted in relation to the concentrations in the redox component, so that the construction is identical to the one proposed by Patent Owner in the Neulasta Litigation under the *Phillips* standard. EX1037 at 8; EX1038 at 13-14. Having argued for that construction in the Neulasta Litigation, Patent Owner cannot now argue for a narrower claim construction, as the broadest reasonable interpretation of this phrase cannot be narrower than the construction of the phrase under the *Phillips* standard. *See Facebook*, 582 F. App'x at 869.

One such narrower construction was proposed by Petitioners in the Neulasta Litigation. Petitioners suggested that "redox buffer strength" is in relation to "the concentrations in the refold mixture." *See* EX1038 at 13. This construction is narrower than the one proposed by Patent Owner in the Neulasta Litigation because Patent Owner's Neulasta Litigation proposal relates to the concentration of a component of the refold mixture ("the redox component") rather than the entire refold mixture, which includes other components. Patent Owner's Neulasta Litigation proposal necessarily limits only the concentration in the refold mixture

whereas Petitioners' Neulasta Litigation proposal additionally limits the concentration of the protein volume, the denaturant, the aggregation suppressor, and the protein stabilizer. *See* EX1001 at claim 1.

Patent Owner submitted the following argument in support for its claim construction in the Neulasta Litigation: "The plain language of claim 1 recites a redox component that is not initially part of the refold mixture, and in that context, the claim recites the redox buffer strength as being an element of the redox component." EX1038 at 13 (citation omitted). Patent Owner also cited to portions of the '138 Patent specification as support for that claim construction. *Id.* at 14 (citing EX1001 at 10:24-30, 11:11-17, 11:40-46).

E. "refold mixture"

The term "refold mixture" does not need a specialized construction apart from its broadest reasonable interpretation in light of the specification. Petitioners addresses the claim term, however, due to Patent Owner's proposed construction for this term in the Neulasta Litigation. There, Patent Owner argued, and the District Court agreed, that construction of the term under the *Phillips* standard required that the refold mixture have "a high protein concentration, where 'high protein concentration' is at or above about 1 g/L protein." EX1037 at 9; EX1038

at 15-16. Petitioners anticipate that Patent Owner may propose that improper construction again here.

This additional limitation does not comport with the broadest reasonable interpretation of the claims in light of the specification. There is no language in claim 1 that requires a "high protein concentration" and there is no language that sets such a concentration "at or above about 1 g/L protein." There is simply no limitation related to the concentration of the "refold mixture," in light of the proposed construction of "a protein … present in a volume at a concentration of 2.0 g/L or greater" here and in the Neulasta Litigation by Patent Owner.

What is more, a construction of "refold mixture" that requires "high protein concentrations" that are "at or above about 1 g/L protein" is inconsistent with the '138 Patent specification. The '138 Patent specification uses the phrase "high protein concentrations" only once, and describes such concentrations as "concentrations higher than 2.0 g/L." EX1001 at 4:20-24. A construction of "refold mixture" that includes "high protein concentrations" that are between "about" 1.0 g/L and 2.0 g/L is simply not supported by the specification. Here, where the *Phillips* standard does not apply, the simple language of the claim should prevail.

F. "2 mM or greater"

The term "2 mM or greater" does not need a specialized construction apart from its broadest reasonable interpretation in light of the specification. Petitioners address the claim term, however, due to the District Court's interpretation of the term in the Neulasta Litigation. There the District Court construed the term under the *Phillips* standard as "2 mM or greater, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM." EX1037 at 9-10. Petitioners anticipate that Patent Owner may propose that narrow construction here.

The additional limitation that "the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM" does not comport with the broadest reasonable interpretation of the claims in light of the specification. There is no language in claim 1 that places an upper boundary on the term "2 mM or greater"; claim 1 provides only the 2 mM lower boundary for the redox buffer strength. Here, where the *Phillips* standard does not apply, the simple language of the claim should prevail.

IX. Printed Publications Relied On

Petitioners rely on the following publications:

1. *Schlegl* (EX1003)
2. *Hevehan* (EX1004)
3. *Brady* (EX1005)

4. *Inclonals* (EX1006)

A. Overview of *Schlegl* (EX1003)

U.S. Patent Application No. 11/695,950 is entitled "Method for Refolding a Protein," was filed on April 3, 2007, and was published as US 2007/0238860 ("*Schlegl*") on October 11, 2007. Thus, *Schlegl* is prior art to the '138 Patent under 35 U.S.C. § 102(b). *Schlegl* was not considered by the Examiner during prosecution of the '138 Patent.[3]

Schlegl discloses methods for protein refolding, including the use of non-mammalian expression systems for the refolding and production of recombinant proteins. EX1003 at Abstract, ¶ [0004]. *Schlegl* solves the problem of protein accumulation in a denatured inactive form by a dilution method of protein refolding that results in a protein concentration up to 10 mg/ml. *Id.* at ¶¶ [0004]-[0008], [0016].

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[3] On October 20, 2010, the '138 Patent applicant filed an Information Disclosure Statement, which disclosed a European counterpart of *Schlegl*. EX1039 at 1 (Cite No. 2). The Examiner acknowledged receipt and consideration on January 9, 2012, but did not cite to that European counterpart during prosecution of the '138 Patent. EX1040 at 1.

The dilution method of *Schlegl* follows the "simpl[e] methodology" commonly used in industrial scale applications in 2007. *Id.* at ¶ [0016]. It is carried out by mixing/diluting the solution containing solubilized protein with a diluent refolding buffer containing a solubilizing agent in an amount necessary to reach the optimal level of dilution. *Id.* The protein in *Schlegl* can come in a protein solution feed, which can be "obtained from solubilization of the inclusion bodies." *Id.* at ¶ [0063].

Schlegl is primarily directed to optimizing the flow rate of the protein solution feed for "ideal" mixing conditions with the refolding buffer. *Id.* at ¶¶ [0023]-[0024], [0032], [0037]. *Schlegl* optimizes this flow rate by keeping the concentration of *unfolded* proteins low and adding the protein solution at a flow rate that gives the unfolded protein time to properly fold. *See id.* at ¶¶ [0033], [0037]-[0038], [0041]-[0042], [0045], [0056], [0061]. Before mixing, however, *Schlegl* starts with a "high concentration of unfolded protein." *Id.* at ¶ [0040].

Moreover, the flow rate optimization of *Schlegl* does not obviate the need for a redox system to refold the proteins. EX1002 at ¶ 58. Indeed, *Schlegl* teaches a refolding buffer with a redox system having a defined thiol-pair ratio and redox buffer strength. EX1003 at ¶¶ [0036], [0041], [0075]; EX1002 at ¶ 60 & nn.3-4. The refolding buffer also contains a denaturant, an aggregation suppressor, and/or

a protein stabilizer. EX1003 at ¶¶[0036], [0041], [0074]-[0075]. The method of *Schlegl* finally incubates the protein with the refolding buffer, *id.* at ¶ [0060], and isolates and purifies the refolded protein, *id.* at ¶ [0065]. The methods of *Schlegl* are applicable to "any protein, protein fragment or peptide that requires refolding upon recombinant expression in order to obtain such protein in its biologically active form." *Id.* at ¶ [0031].

The Example in *Schlegl* describes the refolding of a particular protein, bovine α-lactalbumin, which is a complex protein containing 123 amino acid residues and four disulfide bonds. *Id.* at ¶ [0073]. The concentration of bovine α-lactalbumin in solubilized inclusion bodies (the protein-containing volume) is 16.5 mg/ml (16 g/L) before dilution. EX1003 at ¶ [0075]. While the particular Example in *Schlegl* discloses a final protein concentration of "approx. 0.5 mg/ml," *id.* at ¶ [0082], *Schlegl* discloses that the final protein concentration can be as high as 10 mg/ml (10 g/L). *Id.* at ¶ [0035] ("The concentration of the protein after dilution with refolding buffer is in the range of ca. 1 ng/ml to 10 mg/ml, for example ca. 100 ng/ml to ca. 5 mg/ml or ca. 1 ug/ml to ca. 1 mg/ml."). This is confirmed by claim 6 of *Schlegl*, which recites the same 10 mg/ml final concentration. *Id.* at claim 6.

B. Overview of *Hevehan* (EX1004)

Hevehan and Clark, Oxidative Renaturation of Lysozyme at High Concentrations, *Biotechnology and Bioengineering*, 1996, 54(3): 221-230 ("*Hevehan*") was published in 1996. Thus, *Hevehan* is prior art to the '138 Patent under 35 U.S.C. § 102(b). *Hevehan* was not considered by the Examiner during prosecution of the '138 Patent.

Hevehan teaches the problems associated with folding proteins at higher concentrations, many of which are discussed in Section VI, *supra*, and proposes solutions to those problems. EX1002 at ¶ 64. The main problem identified by *Hevehan* is the low recovery of correctly folded proteins often due to aggregation, of which protein concentration is believed to be a "predominant factor." EX1004 at 1. The authors sought, and found, a solution to that problem. *Hevehan* proposes a modification to the standard dilution method to successfully refold proteins at high concentrations. *Id.*

The *Hevehan* authors tested hen egg white lysozyme, a complex protein that is known to aggregate at high concentrations, *id.* at 2, and that has 129 amino acids, a MW of 14389.68, and four disulfide bonds, EX1004 at 2; *see also* EX1045. The authors of *Hevehan* sought conditions that minimized aggregation of the hen egg white lysozyme at 5 mg/mL (5 g/L) while using the same approach

used for refolding at a concentration of 1 mg/mL (1 g/L). EX1004 at 6. The *Hevehan* authors thus diluted the protein with a refold buffer and used an experimental matrix to change the parameters of the refold buffer in a systematic way. *Id.* at 5-6. In all dilution tests, though, the final protein concentration was 1–5 mg/mL following dilution. *Id.* at 3.

The matrix approach helped the authors to study the relationship between the thiol-pair ratio and the thiol-pair buffer strength and to optimize yields at higher concentrations, arriving at concentrations higher than 2 g/L. *Id.* at 5-6, Figure 4. By varying the concentrations of reducing agent dithiothreitol ("DTT") and oxidizing agent GSSG in the redox mixture, the *Hevehan* authors concluded that yields were "strongly dependent" on thiol concentrations in the renaturation buffer. *Id.* at 5.

The highest yields were obtained after 3 hours of refolding using 2 mM DTT to 4–7 mM GSSG and 4 mM DTT to 7 mM GSSG. *Id.* at 5. The *Hevehan* authors also found that as DTT concentration increased from 1-6 mM, higher GSSG concentrations were needed to optimize yields, resulting in optimum GSSG concentrations of 4-7 mM in the presence of 2 mM DTT, or 7 mM GSSG with 4 mM DTT. *See id.* at Figure 4. Tested redox compositions, including GSSG values to 13 mM, equate to a calculated thiol-pair ratio of 0.3 to 9 ($[\text{reductant}]^2/[\text{oxidant}]$)

and a calculated redox buffer strength of 5 to 19 mM (optimum 10 to 16 mM). *Id*.; EX1002 at ¶ 68 & n.5.

The refold buffer used in *Hevehan* also included two folding aids, GdmCl (a denaturant) and L-arginine (a protein stabilizer and aggregation suppressor). *Id.* at Abstract The authors found that such folding aids present in low concentrations during refolding can limit "aggregation resulting in reactivation yields as high as 95%." EX1003 at Figure 4. Finally, the authors of *Hevehan* incubated the refold mixture. *Id.* at 3.

C. Overview of *Brady* (EX1005)

U.S. Patent Application No. 11/344,451 is entitled "Homogeneous Preparations of IL-31," was filed on January 30, 2006, and was published as US 2006/0228329 ("*Brady*") on October 12, 2006. Thus, *Brady* is prior art to the '138 Patent under 35 U.S.C. § 102(b). *Brady* was not considered by the PTO during prosecution of the '138 Patent.

Brady is primarily directed to using upstream genetic methods to reduce the number of disulfide bonds in a desired recombinant protein for greater ease of downstream refolding. EX1005 at ¶¶ [0045], [0050]. But *Brady* also discloses the refolding techniques that were standard in the art at that time as proof of success of *Brady*'s unique genetic technique. EX1002 at ¶ 70. Example 8 of *Brady* discloses

the refolding and purification of a murine IL-31 ligand following expression in *E. coli*. EX1005 at ¶¶ [0258]-[0268]. Murine (mice) IL-31 is a complex protein having amino acid sequences between 164 and 904 amino acids in length, and multiple disulfide bonds. *Id.* at ¶ [0052].

The refolding in *Brady* follows the then-standard dilution method and starts with a protein concentration of 15.4 mg/ml (15.4 g/L) prior to dilution. *Id.* at ¶ [0264]; EX1002 at ¶ 71 & n.6. *Brady* then dilutes that protein concentration with a refold buffer containing a calculated thiol-pair ratio of 3.125 and a calculated redox buffer strength of 2.25 mM. EX1005 at ¶ [0264]; EX1002 at ¶ 71 & n.7. The refold buffer of *Brady* further contains "0.75 M Arginine" (an aggregation suppressor and protein stabilizer), "PEG 3350 0.055% (w/v)" (an aggregation suppressor and protein stabilizer), and "glycerol" (an aggregation suppressor and protein stabilizer). *Id.* Other examples in Brady follow a similar methodology. (*See* Example 6, ¶¶[0235]-[0244] and Example 7, ¶¶[0245]-[0257]).

After diluting the protein concentration with the refold buffer, *Brady* incubates the resulting mixture by, in Example 8, capping the vessel and allowing it to "gently stir at room temperature for 16 hours." *Id.* at [0253]. Finally, the method of *Brady* captures the renatured protein from the dilute mixture by cation exchange chromatography and purifies the refolded protein by hydrophobic

interaction chromatography. *Id.* at ¶¶ [0049], [0080]-[0081], [0268]. *Brady* also discloses further purification using anion exchange in binding assays. *Id.*

D. Overview of *Inclonals* (EX1006)

Hakim and Benhar, Inclonals, mAbs, 2009, 1:3, 281-287 ("*Inclonals*") was published online on May 1, 2009. Thus, *Inclonals* is prior art to the '138 Patent under 35 U.S.C. § 102(a). *Inclonals* was not considered by the PTO during prosecution of the '138 Patent.

Inclonals teaches the production of antibodies and fusion proteins in *E. coli*. *Inclonals* uses the term "fusion protein" as a general term referring to the linking of two proteins together, such as an antibody and another protein. EX1002 at ¶ 74. Specifically, in *Inclonals*, anti-CD30 and anti-EGFR antibodies, the antibody heavy and light chains or toxin fusions thereof, were expressed in separate bacterial cultures and isolated as insoluble inclusion bodies. EX1006 at 2. The inclusion bodies were solubilized, refolded and purified to give high yields of full-length antibodies and antibody-toxin fusions. *Id.*

In addition to production of full-length antibodies, *Inclonals* teaches the production of fusion proteins. *Id.* at 4. Specifically, the *Inclonals* authors produced PE38 fusions of the heavy chain or the light chain. *Id.* This allowed for the delivery of the toxic PE38 to the binding site of the CD30 antibody. *Id.*

The bacterial expression system developed by *Inclonals* allowed the production of antibodies in 8-9 days, instead of the eight weeks required when expressed in mammalian cells. *Id.*

X. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED

Petitioners request cancellation of claims 1-24 of the '138 Patent in view of the following prior art references:

Reference	Date of Issuance/ Publication	Exhibit No.
U.S. Patent Application Publication No. 2007/0238860 ("*Schlegl*")	October 11, 2007	EX1003
Hevehan et al., Oxidative Renaturation of Lysozyme at High Concentrations, Biotechnology and Bioengineering,1996, 54(3): 221-230 ("*Hevehan*")	1996	EX1004
U.S. Patent Application Publication No. 2006/0228329 ("*Brady*")	October 12, 2006	EX1005
Hakim and Benhar, Inclonals, mAbs, 2009, 1:3, 281-287 ("*Inclonals*")	May 1, 2009	EX1006

Petitioners assert the following specific grounds of rejection:

Ground No.	Claim No(s).	Proposed Statutory Rejections for the '138 Patent
1	1-11, 13-24	Is obvious under 35 U.S.C. § 103(a) over *Schlegl* in view of *Hevehan*

Ground No.	Claim No(s).	Proposed Statutory Rejections for the '138 Patent
2	12	Is obvious under 35 U.S.C. § 103(a) over *Schlegl* and *Hevehan* in view of *Inclonals*
3	1-7, 10, 13-17, 23	Is anticipated under 35 U.S.C. § 102(b) by *Schlegl*
4	1-7, 10, 12-17, 19, 22, 23	Is anticipated under 35 U.S.C. § 102(b) by *Brady*

A. Ground 1: Claims 1-11 and 13-24 Are Unpatentable Under 35 U.S.C. § 103(a) over *Schlegl* and *Hevehan*

Petitioners address independent claim 1 of the '138 Patent first before turning to the dependent claims.

1. A POSA Would Have Been Motivated To Combine *Schlegl* and *Hevehan* in 2009

In 2009, a POSA would have been motivated to combine the teachings of *Schlegl* and *Hevehan* related to protein refolding methods and would have had a reasonable expectation of success in refolding desired proteins into active, native form at concentrations of 2 g/L or higher. EX1002 at ¶ 111.

Hevehan adeptly explains the viewpoint of a POSA looking to tackle the problems known in the art in 2009 and allegedly solved by the '138 Patent. EX1004 at 2; EX1002 at ¶ 112. *Hevehan* also shows the systematic approach that those skilled in the art would take to refold a desired protein. EX1004 at 1-2;

EX1002 at ¶ 112. Specifically, the authors of *Hevehan* considered conditions already known to successfully refold proteins at low concentrations, minimizing aggregation. EX1004 at 2; EX1002 at ¶ 112. The *Hevehan* authors then applied these conditions to refolding of proteins at higher concentrations. EX1004 at 2; EX1002 at ¶ 112

What they found was that optimal refolding of proteins expressed in a non-mammalian expression system at higher concentrations is related to the thiol-pair ratio and redox buffer strength. EX1004 at 5; EX1002 at ¶ 113. By varying the conditions of a reductant (DTT) and an oxidant (GSSG) and recording the outcomes, the authors of *Hevehan* concluded that yields are "strongly dependent" on thiol concentrations in the renaturation buffer. EX1004 at 5; EX1002 at ¶ 113. The optimum thiol-pair ratio was between 0.57 and 2.3 (DTT/GSSG). *See* EX1004 at Fig. 4; EX1002 at ¶¶ 67 & n.5, 113. And in fact, other references prior to 2009 taught the importance of finding the optimal thiol-pair ratio and buffer concentration. *See* EX1041 at 2; EX1042 at 5; EX1002 at ¶ 113.

Hevehan tested hen egg white lysozyme, which was known to aggregate at high concentrations. EX1004 at 2; EX1002 at ¶ 114. As recognized in *Hevehan*, hen egg white lysozyme is a standard model protein. EX1004 at 2; EX1002 at ¶ 114. A POSA would understand this to mean that the results for the standard

model protein, and thus the results disclosed in *Hevehan* of the various refolding tests, would be transferrable to other desired proteins. EX1002 at ¶ 114. It follows that a POSA in 2009 would have known that the teachings of *Hevehan* apply to the dilution refolding methods for refolding taught in *Schlegl*, including the refolding methods disclosed for the particular protein bovine α-lactalbumin discussed in the Example of *Schlegl*. *Id.* at ¶ 114.

A POSA would also have known in 2009 that the refolding methods of *Hevehan* and *Schlegl* would be just as applicable to refolding of proteins in inclusion bodies as they are to the refolding of native proteins that had been denatured. *Id.* at ¶ 115. It was known as early as 1992 that methods originally developed for studying the folding of naturally occurring proteins "have been successfully adapted for reactivation of recombinant" proteins. EX1043 at Abstract, 8; EX1002 at ¶ 115. This includes application of such studies to the "manufacturing scale," including a number of therapeutic proteins that "were solubilized from inclusion bodies" and refolded successfully. EX1043 at 8; EX1002 at ¶ 115. In fact, one reference states that "[a]fter careful optimization, in vitro folding of any recombinant protein deposited in IBs [inclusion bodies] will likely be successful." EX1015 at 36; *see also* EX1028 at 7; EX1002 at ¶ 115.

Moreover, dilution refolding has been conducted since the early 1980s, and examples for lysozyme have been in the literature throughout the decades up to 2009. EX1002 at ¶ 116; *see also* EX1041 at 1, 3. Even dilution refolding using a redox system that provided high protein concentrations was well understood. *See* EX1033 at 6:45-47, 7:10-13; 11:55-64; 12:35-51; 18:40-42. Thus, a POSA would understand the relevance and applicability of hen egg white lysozyme to other systems. EX1002 at ¶ 116; *see also* EX1041 at 1, 3.

A POSA would also be motivated to use the teachings of *Schlegl* and *Hevehan* to refold a "complex" protein, and would have a reasonable expectation of success in doing so. EX1002 at ¶ 117. Both references teach the refolding of "complex" proteins by a dilution refolding method. *Id.* *Schlegl* teaches the refolding of bovine α-lactalbumin, a protein containing 123 amino acid residues and four disulfide bonds. EX1003 at ¶ [0073]. *Hevehan* teaches the refolding of hen egg white lysozyme, a protein that has 129 amino acids, a MW of 14389.68, and four disulfide bonds. EX1004 at 2; see also EX1045.

Such teachings of refolding of complex proteins at high concentration were replete in the art. *See* EX1030 at Abstract; 3 (folding rPGH at 5 g/L and up to 7.5 g/L); EX1031 at Abstract; 2 (folding hIL-6 at 1 g/L); EX1032 at 5 (2 g/L); EX1042 at 5 (refolding lysozyme at 15.9 mg/ml at a 1:15 dilution yielding 1.07

g/L); *see also* EX1015 at 37 (providing an industrial process for the refolding of *t*-PA, which has 527 amino acids and 17 disulfide bonds, thus exemplifying "all the challenges for protein renaturation" which allowed "high-yield production of *t*-PA from IBs [inclusion bodies])".

The demonstrated success of industrial refolding of therapeutic proteins would have been an additional motivation to a POSA. EX1002 at ¶ 118. As of 2009, 30% of the 151 recombinantly-produced approved pharmaceuticals were produced by bacteria. EX 1027 at 2. This list includes tissue plasminogen activator, human insulin, human growth hormone, human parathyroid hormone, granulocyte-colony stimulating factor, interferon alfacon-1, interferon β-1b, and others, all of which were produced and FDA-approved as commercial therapeutics by refolding from inclusion bodies produced in *E. coli*. EX1028 at 2.

2. Claim 1 Is Obvious over *Schlegl* and *Hevehan*

Claim 1 is obvious over *Schlegl* and *Hevehan*. *Schlegl* discloses each step of the process of claim 1, refolding a protein expressed in a non-mammalian expression system.

a. *"a method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater"*

Schlegl discloses the refolding of recombinant proteins expressed using non-mammalian expression systems such as bacterial and yeast expression systems. EX1003 at ¶ [0004]. *Schlegel* also discloses protein present at a volume of 16.5 mg/mL (16.5 g/L) before being diluted by the refold buffer. *Id.* at ¶ [0075]. Moreover, *Schlegl* discloses a protein concentration after dilution with refolding buffer in the range of 1 ng/ml to 10 mg/ml (10 g/L). *Id.* at ¶ [0035]. If the final concentrations following dilution are up to 10 mg/ml, the protein concentration before dilution necessarily must have been higher than 10 mg/ml. EX1002 at ¶ 120. Typical dilutions of solubilized inclusion bodies into refolding buffer range from 1/10 to 1/250, so one would expect that protein concentrations prior to dilution would be at least 100 g/L. *Id.*

Likewise, *Hevehan* discloses the refolding of hen egg white lysozyme, a protein expressed in a non-mammalian expression system, using the dilution method with a protein concentration greater than 2.0 g/L prior to dilution with a refold buffer. EX1004 at 5-6.

To the extent a narrow interpretation of the term "a protein" is taken to require a "complex protein," *i.e.*, a protein that has 2 to 23 disulfide bonds or more

than 250 amino acids or a molecular weight of more than 20,000 daltons, *see* Section VIII(A), *supra*, *Schlegl* and *Hevehan* still disclose this limitation. EX1002 at ¶ 122. *Schlegl* discloses the refolding of bovine α-lactalbumin, a protein containing 123 amino acids residues and four disulfide bonds. EX1003 at ¶ [0073]. *Hevehan* tested hen egg white lysozyme, a protein having 129 amino acids, a MW of 14389.68, and four disulfide bonds. EX1004 at 2; *see also* EX1045.

Further, to the extent a narrow interpretation of the phrase "a protein … present in a volume at a concentration of 2.0 g/L or greater" is taken, such that the concentration is measured after dilution of the protein with the refold buffer, *Schlegl* and *Hevehan* still disclose this limitation. EX1002 at ¶ 123. *Schlegl* discloses a protein concentration after dilution with refolding buffer of up to 10 mg/ml (10 g/L). EX1003 at ¶ [0035]. And *Hevehan* discloses a final protein concentration of up to 5 mg/ml (5 g/L). EX1004 at 3.

b. ***"contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater … to form a refold mixture"***

The Example in *Schlegl* discloses contacting the bovine α-lactalbumin with a refold buffer comprising a redox component as part of the dilution refold method

of *Schlegl* to form a refold mixture. EX1003 at ¶ [0075]. As indicated in *Schlegl*, the refolding buffer may contain "a redox system (e.g reduced glutathione GSH/oxidized glutathione GSSG)," EX1003 at ¶ [0036], and a POSA would understand that the addition of cysteine and cysteine here serve as the redox system or redox component for bovine α-lactalbumin. EX1002 at ¶ 124. That redox component has a thiol-pair ratio of 2 and a redox buffer strength of 6 mM. *See* EX1003 at ¶¶ [0036], [0075]; EX1002 at ¶ 124. Likewise, *Hevehan* discloses contacting the hen egg white lysozyme with a refold buffer comprising a redox component to form a refold mixture. EX1004 at 6. That redox component has a thiol-pair ratio of between 0.3 and 9 and a redox buffer strength of 5 mM to 19 mM, the optimum being between 10-16 mM. *Id.* at 5.; EX1002 at ¶ 124.

To the extent a narrow interpretation of the term "final thiol-pair ratio" is taken to interpret the term in relation to the concentrations of the refold mixture, *i.e.*, after contacting the protein with the refold buffer, *see* Section VIII(C), *supra*, *Schlegl* and *Hevehan* still inherently disclose this limitation. EX1002 at ¶ 125. A POSA in 2009 would have known that the thiol-pair ratio of a refold buffer in a dilution refolding method is the same as the thiol-pair ratio of a refold mixture after contact with a protein. *Id.* Therefore, *Schlegl* inherently discloses a thiol-pair ratio of 2 in the refold mixture. EX1003 at ¶ [0075]; EX1002 at ¶ 125. Likewise,

Hevehan inherently discloses a thiol-pair ratio of between 0.3 and 9 in the refold mixture. EX1004 at 5; EX1002 at ¶ 125.

Further, to the extent a narrow interpretation of the term "redox buffer strength" is taken to interpret the term in relation to the concentrations of the refold mixture, *i.e.*, after contacting the protein with the refold buffer, *see* Section VIII(D), *supra*, *Schlegl* and *Hevehan* still inherently disclose this limitation. EX1002 at ¶ 126. A POSA in 2009 would have known that the redox buffer strength of a refold buffer in a dilution refolding method is necessarily higher (stronger) than the redox buffer strength of a refold mixture after contact with a protein. *Id.* This is because the refold buffer can only become more diluted after contact with the protein and, as a result, the redox buffer strength can only be reduced (weakened) by contact with the protein. *Id.* Therefore, *Schlegl* inherently discloses a redox buffer strength of greater than 6 mM in the refold mixture. *See* EX1003 at ¶ [0075]; EX1002 at ¶ 126. Likewise, *Hevehan* inherently discloses a redox buffer strength of greater than 5 mM to 19 mM in the refold mixture. *See* EX1004 at 5; EX1002 at ¶ 126.

c. *"a refold buffer comprising … one or more of: (i) a denaturant; (ii) an aggregation suppressor; and (iii) a protein stabilizer"*

Schlegl discloses a refold buffer containing guadinium chloride, DTT and optionally a redox system (*e.g.*, GSH/GSSG), EDTA, detergents, salts, and refolding additives like L-arginine. EX1003 at ¶ [0036]. These are "typical buffer components." *Id.* *Schlegl* also discloses that compounds may be added to the refolding buffer to "suppress or completely prevent unfolding/ aggregation" that were "known in the art," including "L-arginine, Tris, [and] detergents." *Id.* at ¶ [0041]. *Schlegl* further discloses a refold buffer containing "0.1 M Tris-HCl" (a protein stabilizer and aggregation suppressor) and "6 M GdmHCl" (a denaturant). *Id.* at ¶ [0074].

Likewise, *Hevehan* discloses using "[s]olubilizing agents such as guanidinium chloride (GdmCl) and folding aids such as L-arginine present in low concentrations during refolding" to "effectively enhance[] renaturation yields by suppressing aggregation" and to achieve "reactivation yields as high as 95%." EX1004 at Abstract. A POSA would be well-versed with protein refolding aids like L-arginine and GdmCl. *See* EX1015 at 35-37; EX1044 at 6; EX1029 at 2, 6; EX1026 at 5; EX1002 at ¶ 128.

d. *"incubating the refold mixture"*

Schlegl discloses: "[c]omplete refolding, including formation of disulfide bonds, proline isomerization and domain pairing may take hours and up to several days." EX1003 at ¶ [0016]. *Schlegl* also discloses further incubation in the refolding tank to allow complete refolding of the protein. *Id.* at ¶ [0060]. Likewise, *Hevehan* discloses incubating the refold mixture for at least 24 hours. EX1004 at 6.

e. *"isolating the protein from the refold mixture"*

Schlegl discloses isolation of the protein from the refold mixture as a final step in the disclosed refold method. EX1003 at ¶¶ [0039], [0065]. *Schlegl* discloses that the protein can be separated and purified through methods including dialysis, filtration, extraction, precipitation and chromatography. *Id.* at ¶ [0065].

3. Claims 2, 3, and 14-17 Are Obvious over *Schlegl* and *Hevehan*

Claims 2, 3, and 14-17 depend directly on claim 1 and recite particular limitations relating to the refold mixture. As explained above, the combination of *Schlegl* and *Hevehan* would have rendered obvious the method of claim 1. Furthermore, the combination of *Schlegl* and *Hevehan* taught the limitations described in claims 2, 3, and 14-17.

a. Claim 2: *"the final thiol-pair ratio is selected from the group consisting of …."*

The Example in *Schlegl* discloses contacting the protein with a refold buffer with a thiol-pair ratio of 2. EX1003 at ¶ [0075]. *Hevehan* discloses a thiol pair ratio of 0.3 to 9. EX1004 at 5. Both disclosures fall within the scope of claim 2. EX1002 at ¶ 133.

b. Claim 3: *"the thiol-pair buffer strength is selected from the group consisting of …."*

The Example in *Schlegl* discloses a redox buffer strength of 6 mM. EX1003 at ¶ [0075]. *Hevehan* discloses a redox buffer strength of 5 to 19 mM (optimum 10 to 16 mM). EX1004 at 5. Both disclosures fall within the scope of claim 3. EX1002 at ¶ 134.

c. Claim 14: *"wherein the denaturant is selected from the group consisting of …."*

Schlegl teaches the use of "components that promote the solubilization of inclusion bodies, e.g. chaotropic agents such as urea, guanidinium chloride (GdmCl), sodium and/or potassium thiocyanate." EX1003 at ¶ [0064]. Guanidinium chloride is a guanidinium salt, one of the denaturants listed in claim 14 and urea is another of the denaturants listed in claim 14. EX1002 at ¶ 135.

d. Claims 15 and 16: *"wherein the protein stabilizer is selected from the group consisting of …."* and *"wherein the aggregation suppressor is selected from the group consisting of …."*

Claims 15 and 16 are directed to the same group. The only difference is that claim 15 recites that the group acts as a "protein stabilizer," while claim 16 recites that the group acts as an "aggregation suppressor." EX1002 at ¶ 136.

Schlegl discloses that "[r]efolding buffers are known in the art and [are] commercially available; typical buffer components are guadinium chloride, dithiothreitol (DTT) and optionally a redox system (e.g. reduced glutathione GSH/oxidized glutathione GSSG), EDTA, detergents, salts, and refolding additives like L-arginine." EX1003 at ¶¶ [0036], [0041]. L-arginine is one enantiomer of arginine (the other being D-arginine, which is not produced or consumed by cells), one of the protein stabilizers listed in claim 15. EX1002 at ¶ 137. Detergents are generally synonymous with surfactants, and represent a group of chemicals that are partly hydrophobic and act as protein stabilizers as listed in claim 15. *Id.* "Salts" is a general term that encompasses Tris, sodium sulfate, and potassium sulfate—three salts listed as protein stabilizers in claim 15. *Id.* A POSA would recognize that at least L-arginine, detergents, and salts as taught by *Schlegl* could be used as protein stabilizers in a refolding buffer, especially given that it was well

established that these particular additives could act in this capacity. *Id.*; EX1003 at ¶ [0041].

Schlegl further teaches the use of a refold buffer containing "refolding additives … to suppress or completely prevent unfolding/aggregation," where "compounds useful as refolding additives are known the art, examples are L-arginine, Tris, detergents, redox systems like GSH/GSSG, ionic liquids like N'-alkyl and N'-(omega-hydroxy-alkyl)-N-methylimidazolium chlorides etc." *Id.* at ¶¶ [0036], [0041]. L-arginine is one enantiomer of arginine (the other being D-arginine, which is not produced or consumed by cells), one of the aggregation suppressors listed in claim 16. EX1002 at ¶ 138. Detergents are generally synonymous with surfactants, and represent a group of chemicals that are partly hydrophobic and act as aggregation suppressors as listed in claim 16. *Id.* "Salts" is a general term that encompasses Tris, sodium sulfate, and potassium sulfate—three salts listed as aggregation suppressors in claim 16. *Id.* It was also well established by 2009 that these particular additives could act in this capacity. *Id.*

e. Claim 17: *"wherein the thiol-pairs comprise at least one component selected from the group consisting of …."*

Schlegl teaches the use of a refold buffer containing "refolding additives," where "compounds useful as refolding additives are known the art, examples are

L-arginine, Tris, detergents, redox systems like GSH/GSSG, ionic liquids like N'-alkyl and N'-(omega-hydroxy-alkyl)-N-methylimidazolium chlorides etc." *Id.* at ¶ [0041]. GSH is glutathione-reduced and GSSG is glutathione-oxidized, two of the thiol-pair components listed in claim 17. EX1002 at ¶ 139.

4. Claims 4-11 Are Obvious over *Schlegl* and *Hevehan*

Claims 4-11 depend directly, or indirectly, on claim 1 and include limitations relating to the protein that is refolded using the claimed method.

a. Claim 4: *"wherein the protein is present in the volume in a non-native limited solubility form"*

The specification of the '138 Patent discloses that the term "non-native limited solubility form" means "any form or state in which the protein lacks at least one formed structural feature found in a form of the protein that (a) is biologically active … and/or (b) forms aggregrates that require treatment, such as chemical treatment, to become soluble." EX1001 at 7:51-59. The specification further states that "[t]he term [non-native limited solubility form] specifically includes proteins existing in inclusion bodies." *Id.* at 7:59-60.

Schlegl discloses that the protein is deposited in the cells in a paracrystalline form, in so-called "inclusion bodies," also termed "refractile bodies." EX1003 at ¶ [0006]. *Hevehan* discloses that the "[a]ctive protein can be recovered by

solubilization of inclusion bodies followed by renaturation of the solubilized (unfolded) protein." EX1004 at Abstract.

b. Claim 5: *"wherein the non-native limited solubility form is an inclusion body"*

Schlegl discloses that the protein is deposited in the cells in a "paracrystalline form," or "inclusion bodies." EX1003 at ¶ [0006]. *Hevehan* discloses that the "[a]ctive protein can be recovered by solubilization of inclusion bodies followed by renaturation of the solubilized (unfolded) protein." EX1004 at Abstract.

c. Claim 6: *"wherein the protein is present in the volume in a soluble form"*

Schlegl discloses a method of refolding a protein, where that protein before refolding is a "solubilized protein" contained in solution. EX1003 at ¶ [0016]. *Schlegl* also discloses that "[t]he feed is a protein solution, usually obtained from solubilization of the inclusion bodies." *Id.* at ¶ [0063]. Furthermore, *Schlegl* discloses that after inclusion bodies are "harvested by centrifugation," they can be "dissolved" in a buffer. *Id.* at ¶ [0008].

d. Claims 7-11: *"wherein the protein is recombinant", "an endogenous protein", "an antibody", "a complex protein", "a multimeric protein"*

Schlegl discloses a method of refolding the various proteins identified in claims 7-11. In particular, *Schlegl's* methods can be applied to "any protein,

protein fragment or peptide that requires refolding upon recombinant expression in order to obtain such protein in its biologically active form." *Id.* at ¶ [0031]. *Schlegl* also "relates to the field of recombinant protein production." *Id.* at ¶ [0002]. *Schlegl* further discloses the refolding of bovine α-lactalbumin, a protein containing 123 amino acids residues and four disulfide bonds. EX1003 at ¶ [0073]; see also EX1004 at 2 (refolding hen egg white lysozyme having 129 amino acids and four disulfide bonds).

Additionally, though the terms "endogenous," "antibody," and "multimeric protein" are not explicitly used in *Schlegl*, the methods disclosed in *Schlegl* are broad enough to cover each of these specific types of proteins. EX1002 at ¶ 145. Furthermore, a POSA would immediately recognize that the methods taught by *Schlegl* could be applied to each of these types of proteins. *Id.* For example, a POSA would know from a number of references known in the art that dilution refolding methods like those in *Schlegl* could be applied to endogenous proteins, antibodies, and multimeric proteins. *Id.*

5. Claim 13 Is Obvious over *Schlegl* and *Hevehan*

Claim 13 is directed to a method of protein refolding "wherein the non-mammalian expression system is one of a bacterial expression system and a yeast expression system." *Schlegl* discloses this limitation:

> To produce a recombinant protein, the cDNA encoding the protein of interest is inserted into an expression vector and the recombinant vector is transformed into host cells, which are grown to express the protein. The host cells may be selected from microorganisms such as bacteria, yeast or fungi, or from animal or plant cells.

EX1003 at ¶ [0004]. Further, *Schlegl* discloses a protein feed "obtained from fermentation of bacterial, yeast, fungal, plant or animal cells carrying an expression vector to produce a heterologous protein of interest." *Id.* at ¶ [0063].

6. Claims 18-24 Are Obvious over *Schlegl* and *Hevehan*

Claims 18-24 are directed to various conditions or methods relating to the incubation or isolation step of claim 1. These various conditions or methods were standard methods well known in the art.

a. Claim 18: *"wherein the incubation is performed under non-aerobic conditions"*

It was well known at the time of the invention that aerobic conditions could impact the redox chemistry of the refolding reaction. EX1001 at 8:3-17; EX1002 at ¶ 148. For example, *Hevehan* discloses that "[s]olutions of reduced DTT were prepared immediately prior to each experiment to minimize air oxidation." EX1004 at 2; *see also* EX1028; EX1020.

b. Claims 19-24: particular isolation methods

Claims 19-24 are directed to particular isolation methods including an affinity separation matrix and filtration. As described in the specification of the

'138 Patent, each of these methods are well known in the art at the time of the invention. *See* EX1001 at 12:26-27 ("The isolation can be achieved using any known protein purification method."); EX1002 at ¶ 149.

These standard methods and their usage are the result of routine optimization, and thus are not patentably distinguishing claim elements. EX1002 at ¶ 149. Further, neither the prosecution history nor the specification indicate any specific affinity separation matrices or filtration methods that are unexpectedly superior to the standard methods known in the art, or that there is any criticality assigned to the particular affinity separation matrices or filtration methods used. *Id.* Furthermore, *Schlegl* discloses that "the protein is separated and purified according to methods known in the art, including, but not limited to, dialysis, filtration, extraction, precipitation and chromatography techniques." EX1003 at ¶ [0065].

B. Ground 2: Claim 12 Is Unpatentable Under 35 U.S.C. § 103(a) over *Schlegl*, *Hevehan*, and *Inclonals*

Claim 12 depends from claim 1 and further requires that "the protein is an Fc-protein conjugate." As discussed in Section X(A)(2), *supra*, each and every element of claim 1 is taught by *Schlegl* and *Hevehan*. A POSA would have further understood in view of the teaching of *Inclonals* that the methods of *Schlegl* and *Hevehan* could be applied to an Fc-protein conjugate. EX1002 at ¶ 151.

As defined by the specification of the '138 Patent, the terms "Fc" and "Fc region" "mean a fragment of an antibody that comprises human or non-human (e.g. murine) C_{H2} and C_{H3} immunoglobulin domains, or which comprises two contiguous regions which are at least 90% identical to human or non-human C_{H2} and C_{H3} immunoglobulin domains." EX1001 at 5:36-41; EX1002 at ¶ 152. *Inclonals* describes a method for producing a full-length antibody fusion protein using an *E. coli* expression system. EX1006 at Abstract; EX1002 at ¶ 152.

Because *Inclonals* was able to successfully obtain a full-length antibody fusion protein using an *E. coli* expression system, a POSA would have had a reasonable expectation of success in using the method described by *Schlegl* and *Hevehan* to produce a fusion protein with an antibody fragment (*i.e.*, the Fc region) because the Fc region is a smaller portion of a heavy chain, and an Fc-conjugate represents a polypeptide linkage between the Fc region and another protein. EX1002 at ¶ 152.

A POSA also would have been motivated to produce an Fc-protein conjugate because of the well-established effectiveness of Fc-fusion proteins against a range of pathologies. *Id.* at ¶ 153; *see also*, *e.g.*, EX1046 at 1:9-22, Table 1. For example, Enbrel® (etanercept) is an Fc-fusion protein that was first

approved by the FDA in 1998 for the treatment of moderate to severe rheumatoid arthritis. EX1002 at ¶ 153; EX1047 at 1.

C. Ground 3: Claims 1-7, 10, 13-17, and 23 Are Unpatentable Under 35 U.S.C. § 102(b) over *Schlegl*

Each element of claims 1-7, 10, 13-17, and 23 of the '138 Patent comes from a general teaching in *Schlegl* or from the Example at pages 5-6. Thus, the elements of the claims of the '138 Patent do not come from different embodiments disclosed in *Schlegl*.

A discussion of how *Schlegl* discloses each and every element of claim 1 is provided in Section X(A)(2), *supra*. A discussion of how *Schlegl* discloses each and every element of claims 2, 3, and 14-17 is provided in Section X(A)(3), *supra*. A discussion of how *Schlegl* discloses each and every element of claims 4-7 and 10 is provided in Section X(A)(4), *supra*. A discussion of how *Schlegl* discloses each and every element of claim 13 is provided in Section X(A)(5), *supra*. A discussion of how *Schlegl* discloses each and every element of claim 23 is provided in Section X(A)(6)(b), *supra*.

D. Ground 4: Claims 1-7, 10, 12-17, 19, 22, and 23 Are Unpatentable Under 35 U.S.C. § 102(b) over *Brady*

Petitioners assert that claims 1-24 are unpatentable under 35 U.S.C. § 102(b) over *Brady* **only if** the phrase "a protein … present in a volume at a concentration of 2.0 g/L or greater" is interpreted to mean "a protein as it exists in a volume

before contacting the volume with a refold buffer. The protein concentration in the volume is 2.0 g/L or greater" as requested by Patent Owner in the Neulasta Litigation and as construed there by the District Court. *See* Section VIII(B), *supra*.

A discussion of how *Brady* discloses each and every element of claim 1 is provided below. Each element comes from a general teaching in *Brady* or from Example 8. Thus, the elements of the claims of the '138 Patent do not come from different embodiments disclosed by *Brady*.

1. Claim 1 Is Unpatentable over *Brady*

Claim 1 is unpatentable over *Brady*. *Brady* discloses each and every step of the process of claim 1, refolding a protein expressed in a non-mammalian expression system.

a. *"a method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater"*

Brady discloses the same non-mammalian expression systems as recited in the claims of the '138 Patent. *Brady* discloses expression in bacteria, yeast, fungi and other non-mammalian cells. EX1005 at ¶¶ [0002], [0046]. Example 8 of *Brady* discloses a protein concentration of 15.4 mg/ml (15 g/L) prior to dilution. *Id.* at ¶ [0264]; EX1002 at ¶ 157.

b. *"contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater … to form a refold mixture"*

Brady teaches contacting the protein with a refold buffer comprising a redox component comprising a thiol-pair ratio of 3.125. EX1005 at ¶ [0264]; EX1002 at ¶ 158. *Brady* also discloses a redox buffer strength of 2.25 mM. EX1005 at ¶ [0264]; EX1002 at ¶ 158.

To the extent a narrow interpretation of the term "final thiol-pair ratio" is taken to interpret the term in relation to the concentrations of the refold mixture, *i.e.*, after contacting the protein with the refold buffer, *see* Section VIII(C), *supra*, *Brady* still inherently discloses this limitation. EX1002 at ¶ 159. A POSA in 2009 would have known that the thiol-pair ratio of a refold buffer in a dilution refolding method is the same as the thiol-pair ratio of a refold mixture after contact with a protein. *Id.* Therefore, *Brady* inherently discloses a thiol-pair ratio of 3.125 in the refold mixture. EX1005 at ¶ [0264].; EX1002 at ¶ 159

Further, to the extent a narrow interpretation of the term "redox buffer strength" is taken to interpret the term in relation to the concentrations of the refold mixture, *i.e.*, after contacting the protein with the refold buffer, *see* Section VIII(D), *supra*, *Brady* still inherently discloses this limitation. EX1002 at ¶ 160.

A POSA in 2009 would have known that the redox buffer strength of a refold buffer in a dilution refolding method is necessarily higher (stronger) than the redox buffer strength of a refold mixture after contact with a protein. *Id.* This is because the refold buffer can only become more diluted after contact with the protein and, as a result, the redox buffer strength can only be reduced (weakened) by contact with the protein. *Id.* Therefore, *Brady* inherently discloses a redox buffer strength of greater than 2.25 mM in the refold mixture. *See* EX1005 at ¶ [0264]; EX1002 at ¶ 160.

c. *"a refold buffer comprising … one or more of: (i) a denaturant; (ii) an aggregation suppressor; and (iii) a protein stabilizer"*

Brady discloses a refold buffer containing "0.75 M Arginine" (an aggregation suppressor and protein stabilizer), "PEG 3350 0.055% (w/v)" (an aggregation suppressor and protein stabilizer), and "glycerol" (an aggregation suppressor and protein stabilizer). *Id.*

d. *"incubating the refold mixture"*

Brady discloses in Example 8 that after the soluble inclusion bodies are diluted in refold buffer, "the vessel was cap[p]ed and allowed to gently stir at room temperature for 16 hours." *Id.*

e. *"isolating the protein from the refold mixture"*

Brady discloses "methods for capturing the renatured [protein] from the dilute refold buffer using cation exchange chromatography, and purifying the refolded [protein] using hydrophobic interaction chromatography. Further purification is achieved using anion exchange in binding assays …." *Id.* at ¶ [0049]; *see also id.* at ¶¶ [0080]-[0081], [0268].

2. Claims 2, 3, and 14-17 Are Unpatentable over *Brady*

Claims 2, 3, and 14-17 depend directly on claim 1 and recite particular limitations relating to the refold mixture. As explained above, *Brady* anticipates the method of claim 1. *Brady* also discloses the limitations described in claims 2, 3, and 14-17.

a. Claim 2: *"the final thiol-pair ratio is selected from the group consisting of …."*

Brady discloses contacting the protein with a refold buffer having a thiol-pair ratio of 3.125, which falls within the scope of claim 2. *Id.* at ¶ [0264].

b. Claim 3: *"the thiol-pair buffer strength is selected from the group consisting of …."*

Brady discloses a redox buffer strength of 2.25 mM, which falls within the scope of claim 3. *Id.*

c. Claim 14: *"wherein the denaturant is selected from the group consisting of …."*

Brady teaches the use of guanidine isothiocyanate or urea for recovering and denaturing proteins. *Id.* at ¶ [0092]. Guanidine isothiocyanate is a guanidinium salt, one of the denaturants listed in claim 14. EX1002 at ¶ 167. Urea is also one of the denaturants listed in claim 14. *Id.*

d. Claims 15 and 16: *"wherein the protein stabilizer is selected from the group consisting of …."* and *"wherein the aggregation suppressor is selected from the group consisting of …."*

Claims 15 and 16 are directed to the same group. The only difference is that claim 15 recites that the group acts as a "protein stabilizer," while claim 16 recites that the group acts as an "aggregation suppressor."

Brady teaches a method where "[t]he inclusion bodies are diluted into the following buffer: 0.75 M Arginine, PEG 3350 0.055% (w/v), 20% glycerol; 10.56 mM NaCl; 0.44 mM KCl; 2.2 mM MgCl2; 2.2 mM CaCl2; 0.055 M Tris at pH 8.2 (room temperature pH)." *Id.* at ¶ [0264]. Arginine, PEG (polyethylene glycol), glycerol, and Tris are protein stabilizers listed in claim 15. EX1002 at ¶ 169. Arginine, PEG, glycerol, and Tris are also aggregation suppressors listed in claim 16. *Id.*

e. Claim 17: *"wherein the thiol-pairs comprise at least one component selected from the group consisting of …."*

Brady teaches a method where "[t]he inclusion bodies are diluted into the following buffer: 0.75 M Arginine, PEG 3350 0.055% (w/v), 20% glycerol; 10.56 mM NaCl; 0.44 mM KCl; 2.2 mM MgCl2; 2.2 mM CaCl2; 0.055 M Tris at pH 8.2 (room temperature pH). The redox pair and concentrations in this refold buffer are as follows: [GSH]=1 mM: [GSSG]=0.1 mM." *Id.* GSH and GSSG are the chemical formulas for glutathione-reduced and glutathione-oxidized, respectively, and serve as two of the thiol-pair components listed in claim 17. EX1002 at ¶ 170.

3. Claims 4-7, 10, and 12 Are Unpatentable over *Brady*

Claims 4-12 depend directly, or indirectly, on claim 1 and include limitations relating to the protein that is refolded using the claimed method.

a. Claims 4 and 5: *"wherein the protein is present in the volume in a non-native limited solubility form"* and *"wherein the non-native limited solubility form is an inclusion body"*

Brady teaches a method "for recovering IL-31 protein from a prokaryotic host when the IL-31 protein is expressed by the host and found within the host cell as an unglycosylated, insoluble inclusion body." *Id.* at ¶ [0049].

b. Claim 6: *"wherein the protein is present in the volume in a soluble form"*

Brady discloses that "the methods of recovering [the protein] can comprise a further step of precipitating, washing, and resolubilizing the [protein.]" *Id.* at ¶ [0076]. *Brady* also discloses:

> The washed inclusion body prep can be solubilized using guanidine hydrochloride (5-8 M) guanidine thiocyanate (5-6 M), or urea (7-8 M) containing a reducing agent such as beta mercaptoethanol (10-100 mM), or dithiothreitol (5-50 mM) …. The supernatant sample containing the solubilized [protein] is decanted and retained, and the concentration of [protein] in the solubilized fraction is determined.

Id. at ¶ [0077].

c. Claims 7 and 10: *"wherein the protein is recombinant"* or *"a complex protein"*

The methods of *Brady* "provide[] expression vectors and methods for producing recombinant IL-31 protein from a prokaryotic host." *Id.* at ¶ [0044]. *Brady* further discloses that its methods can be applied to "a 'protein' [defined as] a macromolecule comprising one or more polypeptides chains." *Id.* at ¶ [0038].

d. Claim 12: *"wherein the protein is an Fc-protein conjugate"*

Claim 12 depends from claim 1 and further requires that "the protein is an Fc-protein conjugate." As discussed in Section X(D)(1), *supra*, each and every element of claim 1 is taught by *Brady*. A POSA would have further understood

that the method taught by *Brady* could be applied to an Fc-protein conjugate. EX1002 at ¶ 175.

As defined by the specification of the '138 Patent, the terms "Fc" and "Fc region" "mean a fragment of an antibody that comprises human or non-human (e.g. murine) C_{H2} and C_{H3} immunoglobulin domains, or which comprises two contiguous regions which are at least 90% identical to human or non-human C_{H2} and C_{H3} immunoglobulin domains." EX1001 at 5:36-41; EX1002 at ¶ 176. *Brady* discloses that "[f]usion proteins can be prepared by methods known to those skilled in the art … and expressed by the methods described herein." EX1005 at ¶ [0098]. Furthermore, *Brady* specifically describes "IL-31 and IL-31Cys mutants cytokine fusion proteins or antibody-cytokine fusion proteins," and "antibody conjugates." EX1005 at ¶¶ [0119], [0120].

Therefore, a POSA would have had a reasonable expectation of successfully using the refolding method described by *Brady* to produce an Fc-protein conjugate as defined by claim 12. EX1002 at ¶ 176. Additionally, a POSA would have been motivated to produce an Fc-protein conjugate because of the well-established effectiveness of Fc-fusion proteins against a range of pathologies. *E.g.*, EX1046 at 1:9-22, Table 1; EX1002 at ¶ 176. For example, Enbrel® (etanercept) is an Fc

fusion protein that was first approved by the FDA in 1998 for the treatment of moderate to severe rheumatoid arthritis. EX1047 at 1.

4. Claim 13 Is Unpatentable over *Brady*

Claim 13 is directed to a method of protein refolding "wherein the non-mammalian expression system is one of a bacterial expression system and a yeast expression system." *Brady* discloses that "[t]he expression of proteins in bacteria is by far the most widely used approach for the production of cloned genes." EX1005 at ¶ [0002]. *Brady* further discloses that "the expression vectors and methods of the present invention comprise an *E. coli* expression system." *Id.* at ¶ [0046]; *see also id.* at ¶¶ [0073], [0092]. And, *Brady* discloses "[a]nother method for constructing expression systems utilizes homologous recombination using a yeast system." *Id.* at ¶ [0069].

5. Claims 19, 22, and 23 Are Unpatentable over *Brady*

Claims 19, 22, and 23 are directed to particular isolation methods including an affinity separation matrix and filtration. *Brady* discloses isolating the protein from the refold mixture using cation and anion exchange chromatography (which utilizes an ion exchange separation matrix). EX1005 at ¶¶ [0049], [0080]-[0081]. *Brady* also discloses using an affinity separation matrix. EX1005 at ¶¶ [0095]-[0096], [0201]-[0202]. *Brady* also discloses isolating the protein from the refold mixture using filtration. EX1005 at ¶ [0264].

E. Objective Indicia of Nonobviousness Do Not Save the '138 Patent

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

In fact, Patent Owner will be unable to make out any case of commercial success of the invention claimed in the '138 Patent. An assertion of commercial success requires Patent Owner to show (a) that a claimed embodiment is successful commercially, *e.g.*, has substantial market share, and (b) there is a nexus between the success and the novel features of the claims. *Tokai Corp. v. Eason Enters., Inc*., 632 F.3d 1358, 1369-70 (Fed. Cir. 2011). Patent Owner cannot show that required nexus, as Patent Owner does not practice the methods of the '138 Patent

claims in refolding the Neupogen and Neulasta proteins. *See* EX1048. Moreover, to date, the prosecution history of the '138 Patent and its related matters is devoid of any such evidence. For similar reasons, Patent Owner will be unable to show that the claimed invention of the '138 Patent was met with any praise in the industry.

Thus, even in view of any alleged objective indicia of nonobviousness, the claims would have been obvious over the prior art discussed above.

XI. CONCLUSION

For the foregoing reasons, challenged claims 1-24 of the '138 Patent recite subject matter that is unpatentable. Therefore, Petitioners respectfully request institution of this *inter partes* review to cancel these claims.

Respectfully submitted,

August 5, 2016

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CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24(d), I hereby certify that this Petition complies with the type-volume limitation of 37 C.F.R. § 42.24(a)(1)(i) because it contains 13,972 words as determined by the Microsoft® Office Word 2010 word-processing system used to prepare the brief, excluding the parts of the brief exempted by 37 C.F.R. § 42.24(a)(1).

/Teresa Stanek Rea/
Teresa Stanek Rea

CERTIFICATION OF SERVICE ON PATENT OWNER

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105, the undersigned certifies that on August 5, 2016, a complete copy of the foregoing Petitioners' Petition for *Inter Partes* Review of U.S. Patent No. 8,952,138, Power of Attorney, and all supporting exhibits were served via FedEx® on the Patent Owner by serving the correspondence address of record for the '138 Patent:

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