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 Owners

Case IPR2016-01542
Patent 8,952,138

PATENT OWNERS' PRELIMINARY RESPONSE

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EXHIBIT LIST

Exhibit Number	Exhibit
2001	Declaration of Richard C. Willson, Ph.D.
2002	C.V. of Richard C. Willson, Ph.D.
2003	Findings of Fact and Conclusions of Law, *Amgen Inc. et al. v. Apotex Inc. et al.*, Case no. 0:15-cv-61631-JIC, Dkt. 267, entered September 6, 2016
2004	Partial Findings Regarding Apotex's Assertion of Invalidity of the '138 Patent, *Amgen Inc. et al. v. Apotex Inc. et al.*, Case no. 0:15-cv-61631-JIC, Dkt. 245, ordered July 14, 2016
2005	Lu et al., "Folding and Oxidation of Recombinant Human Granulocyte Colony Stimulating Factor Produced in *Escherichia coli*," The Journal of Biological Chemistry, Vol. 267, No. 13, pp. 8770-8777 (1992)
2006	Lehninger, "Principles of Biochemistry" (1982), excerpted
2007	Declaration of Richard C. Willson, Ph.D. Regarding Claim Construction of Shultz et al., U.S. Patent No.

Exhibit Number	Exhibit
	8,952,138, *Amgen Inc. et al. v. Apotex Inc. et al.*, Case no. 0:15-cv-61631-JIC, Dkt. 77-4, filed December 11, 2015
2008	Rebuttal Declaration of Richard C. Willson, Ph.D. Regarding Claim Construction of Shultz et al., U.S. Patent No. 8,952,138, *Amgen Inc. et al. v. Apotex Inc. et al.*, Case no. 0:15-cv-61631-JIC, Dkt. 83-1, filed January 8, 2016
2009	Kamau et al., "Alpha-Lactalbumin: Its Production Technologies and Bioactive Peptides," Comprehensive Reviews in Food Science and Food Safety, Vol. 9, pp. 197-212 (2010)
2010	Slangen et al., "Use of Mass Spectrometry To Rapidly Characterize the Heterogeneity of Bovine α-Lactalbumin," J. Agric. Food Chem., Vol. 47, pp. 4549-4556 (1999)
2011	Phillips, "The Three-dimensional Structure of an Enzyme Molecule," Scientific American, November 1966, pp. 78-90

Exhibit Number	Exhibit
2012	UniProt Human IL-31, http://www.uniprot.org/uniprot/Q6EBC2 (last visited 11/21/2016)
2013	UniProt Mouse IL-31, http://www.uniprot.org/uniprot/Q6EAL8 (last visited 11/21/2016)
2014	IUPAC, https://goldbook.iupac.org/D01514.html (last visited (11/21/2016)
2015	Berg et al., "Biochemistry," 2002 (5th edition), excerpted
2016	Schrödel et al., "Characterization of the Aggregates Formed During Recombinant Protein Expression in Bacteria," BMC Biochemistry, 6:10 (2005)
2017	European Patent Application No. 0657466

I. Introduction

Pursuant to 35 U.S.C. § 313 and 37 C.F.R. § 42.107(a), Patent Owners, Amgen Inc. and Amgen Manufacturing Limited (together, "Amgen") submit this Preliminary Response to the Petition for *Inter Partes* Review (the "Petition") of U.S. Patent No. 8,952,138 ("the '138 Patent") by Petitioners, Apotex Inc. and Apotex Corp. (together, "Apotex"). The Board should reject all four Grounds of the Petition because Apotex has not demonstrated a reasonable likelihood that claims 1-24 of the '138 Patent are either anticipated, or would have been rendered obvious, by the asserted prior art references.

The '138 Patent is directed to a novel protein refolding method that solves a problem associated with recombinant protein production. Non-mammalian (*e.g.*, bacterial) expression systems frequently produce "misfolded" proteins, associated with inactivity and aggregation; the methods of the '138 Patent ensure proper refolding of proteins at high concentrations with the use of so-called "redox chemicals." These chemical oxidants and reductants facilitate the correct formation of disulfide bonds between residues of the amino acid cysteine that are found in most proteins.[1]

[1] Disulfide bonds between the "right" cysteine residues maintain a protein in its properly folded, three-dimensional shape; disulfide bonds between the "wrong"

Sole independent Claim 1 of the '138 Patent recites two material elements of the refolding method, a "thiol-pair ratio" ("TPR") and a "redox buffer strength" ("RBS"): "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." TPR and RBS are calculated with equations defined in the specification (*see* EX1001 at 6:25-41) based on variables of concentration of reductant ("[reductant]") and oxidant ("[oxidant]"). Various solutions in Claim 1's refolding method, including a "redox component," a "refold buffer," and a "refold mixture," may contain oxidants and reductants. But it is undisputed that TPR and RBS are calculated from the concentrations of reductant and oxidant based on the volume of the redox component. *See* Petition at 24-26[2]. The claim language itself requires that TPR and RBS are based on the redox component: "redox component comprising a . . . thiol-pair ratio . . . and a redox buffer strength . . . ."

All Grounds of Apotex's Petition suffer from a critical deficiency: none of the asserted prior art references disclose or suggest the material TPR and

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cysteine residues make misfolded proteins. Reductants can break incorrect disulfide bonds, whereas oxidants can form desired ones.

[2] Except for patent and patent application Exhibits, EX1002 (Robinson Declaration), and EX2001 (Willson Declaration), all cites herein refer to the page numbers added by Apotex or Amgen at the bottom of each Exhibit.

RBS elements of independent Claim 1. Straining to map the *Schlegl* (EX1001), *Brady* (EX1005), and *Hevehan* (EX1004) prior art references to Claim 1, Apotex (and its expert, Dr. Robinson) miscalculate TPR and RBS with concentrations of reductant and oxidant based on the volume of the refold buffer or refold mixture—not the redox component. There is no justification for Apotex's approach. Apotex conflates the redox component with separate and distinct elements of Claim 1, the refold buffer and the refold mixture. And Apotex does not demonstrate how the claimed TPR based on the volume of a redox component can be extrapolated from its calculation of TPR based on the volume of a refold buffer or a refold mixture. What accounts for Apotex's inability to do so is the fact that TPR is volume-dependent; the volumes of redox component, refold buffer, and refold mixture in Claim 1 are necessarily different. Apotex can make only conclusory arguments that its selected prior art references disclose the requisite TPR and RBS values. Because Apotex cannot demonstrate that the material TPR and RBS elements of Claim 1 are taught by its asserted prior art, no trial should be instituted for that sole independent claim and all 23 claims depending from it.

There are additional reasons why the Board should not institute trial in relation to dependent Claims 9, 10, and 11, which recite that the protein expressed in a non-mammalian expression system to be refolded is "an antibody," "a complex protein," and "a multimeric protein," respectively. *Schlegl* (Ground 3),

Brady (Ground 4), and *Schlegl* in combination with *Hevehan* (Ground 1) do not direct a person of ordinary skill in the art ("POSITA") to a method of refolding a "complex protein" so as to anticipate or render obvious Claim 10. Similarly, *Schlegl* in combination with *Hevehan* do not teach or suggest the refolding of "an antibody" or "a multimeric protein" so as to render obvious Claims 9 and 11.

Apotex's prior art challenges are not new. Apotex already asserted its primary references, *Schlegl* and *Brady*, in *Amgen Inc. et al. v. Apotex Inc. et al.*, No. 0:15-CV-61631-JIC/BSS (S.D. Fla.) (hereinafter, "the related litigation"). Curiously, Apotex's Petition is silent as to what transpired at trial, which ended 18 days before Apotex filed its Petition. After litigating Apotex's prior art invalidity case for a year, Amgen was taken by surprise when Apotex withdrew its *Brady*-based defense on the eve of trial and withdrew its *Schlegl*-based defense mid-trial. The adverse outcome: on July 15, 2016, Judge Cohn entered judgment in Amgen's favor that the '138 Patent is not invalid for anticipation and obviousness.[3] EX2004 at 2-5. By dropping its defenses after having had an opportunity to litigate them in full, Apotex has tacitly acknowledged that it cannot meet the "clear and convincing" evidentiary burden in district court that *Schlegl* or

[3] On September 6, 2016, Judge Cohn issued Findings of Fact and Conclusions of Law in Apotex's favor on the remaining infringement issues. EX2003 at 2. As of December 5, 2016, Amgen's appeal will be pending with the Federal Circuit.

Brady invalidates the ’138 Patent. And with no evidence of the material TPR and RBS elements in its asserted prior art, Apotex cannot meet the “preponderance” standard here either.

In sum, the Board should reject Apotex’s attempt to resuscitate its failed invalidity case. No trial should be instituted on Grounds 1-4 of Apotex’s Petition.

II. The ’138 Patent

The ’138 Patent is entitled “Refolding Proteins Using a Chemically Controlled Redox State.” The ’138 Patent is generally directed to improved methods of refolding proteins expressed in non-mammalian cell culture systems (*e.g.* bacterial expression systems), using redox chemicals and other additives to achieve refolding at high protein concentrations. EX2001 at ¶58.

The ’138 Patent addresses a problem associated with producing recombinant proteins in non-mammalian cell cultures. While tremendously important from the standpoint of commercial scale operations, bacterial cells engineered by recombinant DNA techniques to express useful but foreign proteins nevertheless present a particular production challenge: they typically are unable to produce such proteins as properly folded molecules, especially at high levels of expression. EX2001 at ¶¶33-35, 50, 57; EX1001 at 1:18-33. The misfolded proteins precipitate within the bacterial cells in limited solubility forms, referred to

as "inclusion bodies." EX2001 at ¶51; EX1001 at 1:18-24. The inclusion bodies must be isolated, and the incorrectly folded protein within them must be solubilized, unfolded into the primary amino sequence (a linear molecule), and subsequently refolded to form the proper three-dimensional conformation associated with the biologically active protein. EX2001 at ¶51; EX1001 at 34-45.

Many, if not most, proteins have cysteine residues. Cysteine is an amino acid that contains a sulfhydryl group bonded to a carbon atom (-C-SH), also known as a thiol group. EX2001 at ¶¶46, 53. For proteins with several cysteine residues, proper folding may involve the formation of disulfide (-S-S-) linkages between the thiols of specific cysteines. *Id.* at ¶¶46-47, 53. The refolding of proteins with two or more disulfide linkages, particularly those with an odd number of cysteine residues, can be especially challenging because there may be mismatched thiols. *Id.* at ¶¶47-49. As the number of cysteine residues increases, the likelihood of incorrect disulfide linkages increases exponentially. *Id.* at ¶47. For complex proteins (defined as "a protein that (a) is larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form" (*see infra*)), the task of refolding them *in vitro* can be especially challenging. EX1001 at 1:18-29.

Prior to the '138 Patent, the art appreciated that, to a certain extent, protein refolding can be influenced by controlling redox chemistry. As noted in

the ’138 Patent, “[w]hen cysteine residues are present in the primary amino acid sequence, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (*e.g.*, a redox system).” EX1001 at 1:47-51. Redox systems provide thiol-reactive chemicals (also called thiol pairs or reductants and oxidants) that facilitate a reversible thiol exchange with another thiol (so-called “shuffling”). EX2001 at ¶¶53-55. These systems can improve the odds of proper disulfide bond formation and folding. *Id.* at ¶56.

However, as also noted in the ’138 Patent, the primary approach in the prior art was to refold proteins, including those containing two or more disulfide bonds, at dilute protein concentrations. EX2001 at ¶57; EX1001 at 1:54. Under such conditions, which allow for the protein molecules to be spatially apart from one another, the risk of protein aggregation decreases, so that a protein can properly fold on itself. EX2001 at ¶¶57, 112. Working at dilute protein concentrations at industrial scale, however, requires huge refolding tanks and the facilities to house them. *Id.* at ¶57; EX1001 at 1:57-60. The approach taught in the ’138 Patent, which permits refolding at high protein concentrations, avoids the limitations imposed by working under the dilute conditions (typically no greater than 0.5 g/l protein) of the prior art. EX2001 at ¶57.

The inventors of the ’138 Patent invented an improved method for refolding cysteine-containing proteins that is based on their unique appreciation of

specific relationships between RBS, TPR, and protein concentration. EX2001 at ¶¶58-59; *see, e.g.*, EX1001 at 4:35-58. They brought a degree of mathematical precision and sophistication to redox chemistry-based protein refolding nowhere reported or appreciated in the prior art. EX2001 at ¶60. Their patent teaches how to create optimal refolding environments by applying defined equations for the redox parameters, TPR and RBS. *Id.* at ¶58; EX1001 at 6:25-38. The equation for TPR ($\frac{[reductant]^2}{[oxidant]}$), in particular, is unique. EX2001 at ¶60. These teachings optimize and enhance the efficiency of refolding proteins at concentrations significantly higher, *i.e.*, significantly less dilute, than those typically employed in the prior art. *Id.* at ¶¶58-60. This, in turn, can reduce practical and economic problems associated with refolding proteins in physically large volumes. *Id.* at ¶57. In short, the invention claimed in the '138 Patent is an improved, redox chemistry-based methodology for refolding proteins at high concentration. *Id.* at ¶60.

III. Claim Construction

Claim 1 of the '138 Patent reads as follows:

> 1. A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:
> (a) contacting the protein with a refold buffer comprising
> a redox component comprising

a final thiol-pair ratio having a range of 0.001 to 100 and

a redox buffer strength of 2 mM or greater

and one or more of:

(i) a denaturant;

(ii) an aggregation suppressor; and

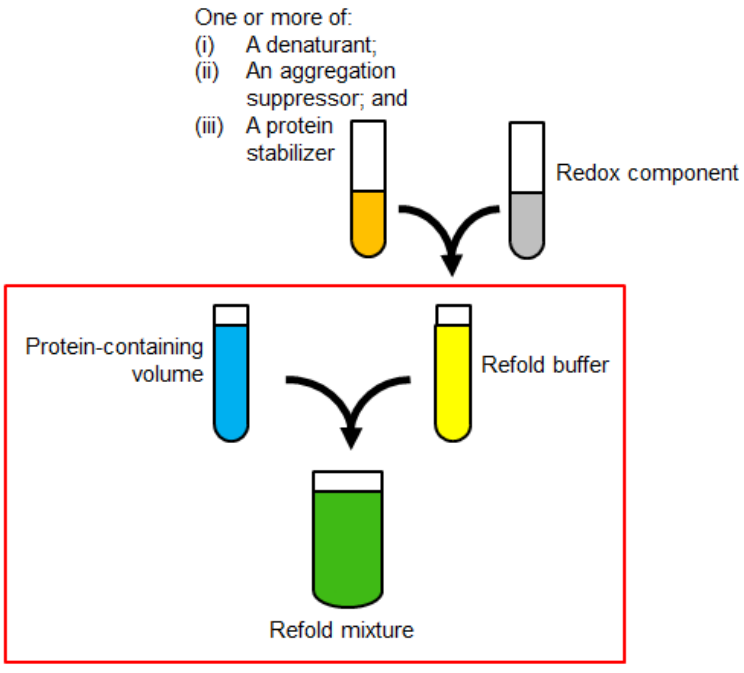
(iii) a protein stabilizer;

to form a refold mixture;

(b) incubating the refold mixture; and

(c) isolating the protein from the refold mixture.

The highlighted terms are color-coded to match the illustration below, which, to assist the Board, illustrates the elements that make up step (a) of Claim 1, the "contacting" step (the "contacting" step is represented by the red box):



The diagram illustrates what is required by the claim: the redox component (gray), refold buffer (yellow) and refold mixture (green) are separate and distinct

elements. EX2001 at ¶63. The refold buffer (yellow) comprises the redox component (gray) and the denaturant/aggregation suppressor/protein stabilizer (orange); the refold mixture (green) comprises the protein-containing volume (blue) and the refold buffer (yellow). *Id*. Accordingly, the claim elements necessarily have different volumes:

- volume of the refold mixture (green) > volume of the refold buffer (yellow) > volume of the redox component (gray); and
- volume of the refold mixture (green) > volume of the "protein-containing volume" (blue).

EX2001 at ¶63.

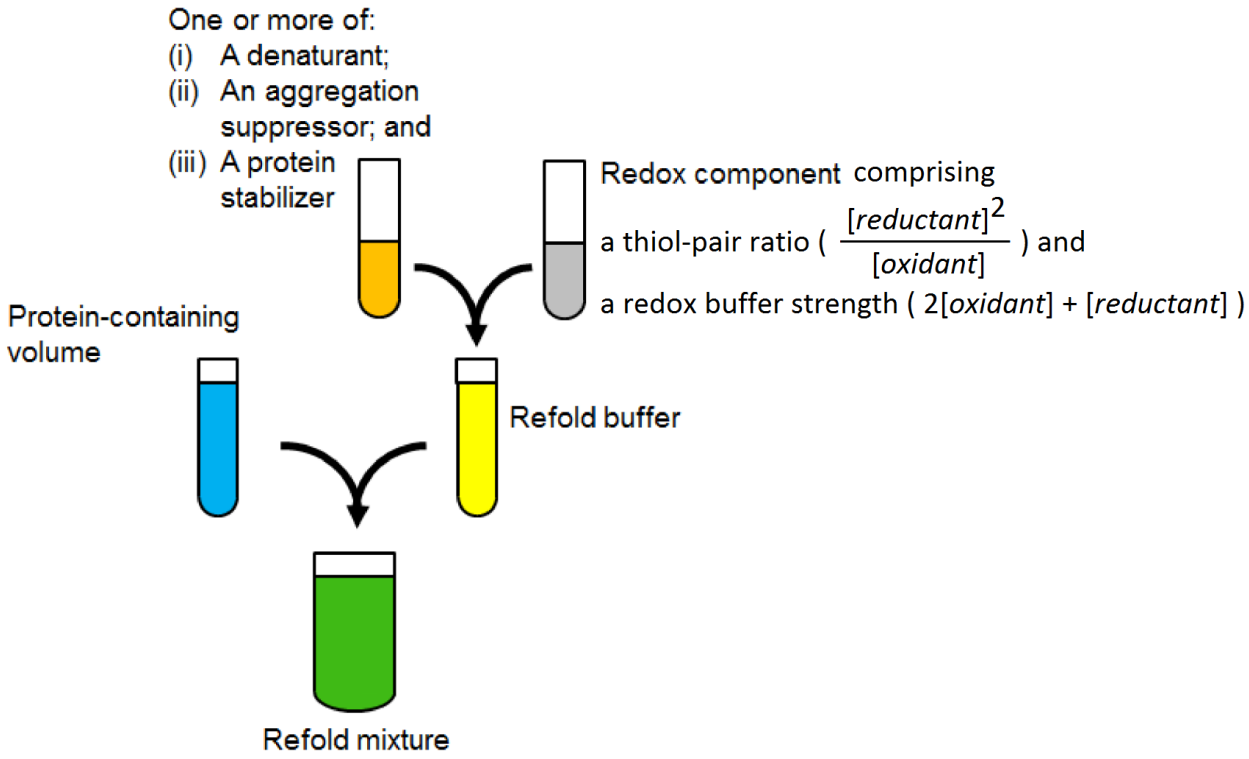
A. Undisputed Claim Terms

1. "a protein . . . present in a volume at a concentration of 2.0 g/L or greater"; final thiol-pair ratio"; and "redox buffer strength"

The construction of three terms in this proceeding is undisputed. In its Petition, Apotex asks for constructions of three claim terms that are "identical to the one[s] proposed by Patent Owner in the Neulasta Litigation under the *Phillips* standard" (*see* Petition at 22, 24, 25) and ultimately adopted by the district court (*see* EX1037 at 5, 8, 9). Those undisputed constructions are summarized in the following table:

Term	Undisputed Construction
"a protein . . . present in a volume at a concentration of 2.0 g/L or greater"	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.
"final thiol-pair ratio"	Defined by the following equation: $$\frac{[reductant]^2}{[oxidant]},$$ where the concentrations are the concentrations in the redox component.
"redox buffer strength"	Also called "buffer thiol strength," "thiol-pair buffer strength," or "thiol-pair strength," defined by the following equation: $$2[oxidant] + [reductant],$$ where the concentrations are the concentrations in the redox component.

Thus, it is undisputed that "final thiol-pair ratio" and "redox buffer strength" are calculated based on reductant and oxidant concentrations in the redox component. An updated diagram of Claim 1 is provided below, reflecting TPR and RBS in the redox component:



2. "2 mM or greater"

For purposes of this proceeding, the parties agree that no construction is necessary for "2 mM or greater." Petition at 28.[4]

B. Disputed Claim Terms

1. "a protein"

Amgen proposes a construction for "a protein" that is taken directly

[4] This represents a shift in claim construction for Apotex. In the related litigation, Apotex had proposed a claim construction that was adopted by the district court under the *Phillips* standard (EX1037 at 9-10 (*viz.*, "2 mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100 mM")).

from the patent specification[5]: "any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bands." The patentees, acting as their own lexicographer, defined "protein":

> As used herein, the terms "protein" and "polypeptide" are used interchangeably and mean any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds.

EX1001 at 5:47-50; EX2001 at ¶67. That construction satisfies the broadest reasonable interpretation in light of the specification, and was also adopted by the district court in the related litigation. *See* EX2001 at ¶68; EX2003 at 5.

2. "refold mixture"

Amgen submits that the broadest reasonable interpretation for "refold mixture" in light of the specification is the construction it proposed in the related litigation, which the district court adopted. EX1037 at 9. "Refold mixture" should be interpreted to mean "a mixture formed from contacting (1) the volume in which the concentration of protein is 2.0 g/L or greater with (2) the refold buffer. The refold mixture has a high protein concentration, where 'high protein concentration' is at or above about 1 g/L protein." EX2001 at ¶¶70, 76.

Amgen's proposed construction is taken directly from the

[5] Petitioner incorrectly speculates that Amgen will seek "a construction that limits the term to 'complex proteins.'" Petition at 20.

specification. The preamble of sole independent Claim 1 reads: "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 . . . ." In the portion of the specification similarly entitled "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," the '138 Patent teaches repeatedly that the lowest concentration of protein in a refold mixture is 1 g/L:

> The isolated soluble protein is often released from non-mammalian cells in a reduced form and therefore can be prepared for refolding by addition of a denaturant, such as a chaotrope. Further combination with protein stabilizers, aggregation suppressors and redox components, at an optimized Thiol-pair ration [sic] and Thiol-pair Buffer Strength, allows for refolding at concentrations of **1**-40 g/L, for example at concentrations of 10-20 g/L.
>
> * * *
>
> In another exemplary refolding operation, inclusion bodies obtained from a non-mammalian expression system are solubilized in the range of 10 to 100 grams of protein per liter and more typically from 20-40 g/L for approximately 10-300 min. The solubilized inclusion bodies are then diluted to achieve reduction of the denaturants and reductants in the solution to a level that allows the protein to refold. The dilution results in protein concentration in the range of **1** to 15 g/L in a refold buffer containing urea, glycerol or sucrose, arginine, and the redox pair (e.g., cysteine and cystamine).

EX1001 at 10:9-16 (emphases added), 12:40-49 (emphases added).

The parties do not dispute that the protein referred to in the preamble at a concentration of 2.0 g/L or greater is the "protein as it exists in a volume before contacting the volume with a refold buffer." *See supra* at 10. Thus, the claim language is structured in such a way that the protein in the initial protein-containing volume (blue), at 2.0 g/L or greater, gets diluted (*i.e.*, so that the protein concentration can be less than 2.0 g/L) upon contact with the refold buffer to form the refold mixture (green). *Id.*; EX2001 at ¶72. The specification makes clear that the resultant protein concentration in the refold mixture is, at minimum, about 1 g/L. *Id.* at ¶74. A floor of about 1 g/L makes sense in view of the '138 Patent's disclosure in the Background of the Invention section that prior art refold concentrations were "typically 0.01-0.5 g/L." EX1001 at 1:54; EX2001 at ¶73.

The '138 Patent specification also teaches that the protein concentration in the refold mixture is "high." In its Petition at 27, Apotex states categorically, "[t]he '138 Patent specification uses the phrase 'high protein concentrations' only once, and describes such concentrations as 'concentrations higher than 2.0 g/L.'" Though the words "high protein concentrations" may literally appear once, the patent pervasively refers to "refolding <u>proteins at high concentrations</u>," including in the Field of the Invention. EX1001 at 1:11-12 (emphasis added); EX2001 at ¶75; *see also*, EX1001 at 2:22, 2:24, 2:28-29, 4:9,

and 4:58. As for the passage cited by Apotex (EX1001 at 4:20-24) in support of the 2.0 g/L cutoff, that passage does not define "high"; it is a mere example of a high protein concentration (". . . such as concentrations higher than 2.0 g/L"). EX2001 at ¶75.

C. Additional Claim Terms to Construe

1. "complex protein"

Amgen's proposed construction for a "complex protein" is taken directly from the definition in the specification:

> The protein can be a complex protein, i.e., a protein that (a) is larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form

EX1001 at 12:58-61 (emphasis added); EX2001 at ¶78. That definition is repeated in relation to "complex molecule," which likewise refers to a "complex protein":

> As used herein, the term "complex molecule" means any protein that is (a) larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form.

EX1001 at 5:64-67 (emphasis added); EX2001 at ¶¶79-80.

Apotex attempts to take advantage of a typographical error in the specification by contending that the "'138 Patent defines 'complex protein' most broadly as a protein 'comprising 2-23 disulfide bonds or greater than 250 amino

acid resides, or having a MW of greater than 20,000 daltons.'" Petition at 22 (emphasis omitted). And yet, Apotex does not rely on a passage taken from the Definitions section of the patent. Instead, Apotex selectively cites from the specification:

> The method can be applied to any type of protein, including simple proteins and complex proteins (e.g., proteins comprising 2-23 disulfide bonds or greater than 250 amino acid residues, or having a MW of greater than 20,000 daltons)

EX1001 at 4:24-27 (emphasis added). Unquestionably, the patent's stated definitions (featuring an "*i.e.*") trumps the passage cited by Apotex with a mere "*e.g.*" EX2001 at ¶81.

2. "non-mammalian expression system"

Amgen's proposed construction for "non-mammalian expression system" is taken directly from the definition in the specification: "a system for expressing proteins in cells derived from an organism other than a mammal, including but not limited to, prokaryotes, including bacteria such as *E. coli*, and yeast." The patentees, acting as their own lexicographer defined "non-mammalian expression system":

> [T]he term "non-mammalian expression system" means a system for expressing proteins in cells derived from an organism other than a mammal, including but not limited to, prokaryotes, including bacteria such as *E. coli* and yeast.

EX1001 at 4:63-67; EX2001 at ¶69. That construction satisfies the broadest reasonable interpretation in light of the specification.

IV. The Person of Ordinary Skill in the Art

The person of ordinary skill in the art of protein refolding in June of 2009, the priority date of the '138 Patent, would have had a Ph.D. degree in biochemistry, biochemical engineering, molecular biology, or a related biological/chemical/engineering discipline, or a master's degree in such disciplines and several years of industrial experience producing proteins in non-mammalian expression systems. *See* EX2001 at ¶16.

V. Asserted Prior Art

A. Primary References

1. *Schlegl* (EX1003)

At trial in the related litigation, Apotex withdrew its invalidity defense based on *Schlegl*, and judgment was entered in Amgen's favor that the '138 Patent is not invalid for anticipation under 35 U.S.C. § 102 or obviousness under 35 U.S.C. § 103. EX2004 at 2-5.

USPTO has already considered *Schlegl*'s disclosure. The '138 Patent was issued in view of a foreign counterpart of *Schlegl* (EX1003). Specifically, EP 1845103 A1 ("the '103 EP application") is the European counterpart of *Schlegl*, and shares its same disclosure. (The documents differ only in that the '103 EP application contains a list of cited references and has slightly different claim

dependencies.) And the '103 EP application is cited on the face of the '138 Patent. EX1001 at cover page at (56).

Schlegl teaches a fundamentally different approach to protein refolding than the '138 Patent. *Schlegl*'s method is a mechanical approach using "defined mixing conditions" to achieve protein refolding at dilute protein concentrations—even more dilute than that of the prior art. EX2001 at ¶¶93-95; *see* EX1003 at [0023], [0024], [0033], [0039]. In contrast, the method claims of the '138 Patent are a chemical approach using redox chemicals at specified mathematical relationships to achieve protein refolding at high protein concentrations. EX2001 at ¶93.

To achieve "conditions that approximate ideal mixing," *Schlegl* combines a stream containing solubilized (unfolded) protein at a low flow rate with a refolding buffer stream at a very high flow rate. EX2001 at ¶94; *see* EX1003 at [0023], [0024], [0033], [0037]. Under such conditions, refolding takes place at very low concentrations, and not the high protein concentrations taught by the '138 Patent. EX2001 at ¶95. According to *Schlegl*,

> By maintaining a very high flow rate of the refolding buffer and a low flow rate of the feed stream containing the unfolded protein, the method of the invention provides very high local dilution rates; preferred dilution rates range from 1:5 to 1:5000 and from 1:10 to 1:10000.

* * *

> In the process of the invention, the actual protein concentration immediately after mixing is much lower as compared to conventional refolding methods.

EX1003 at [0033] (emphasis added), [0039] (emphasis added).

Because *Schlegl* advocates refolding protein under extremely dilute conditions, there is no focus on the use of redox chemicals to facilitate protein refolding. EX2001 at ¶¶96-98; EX1003 at [0033], [0041] , [0042] , [0045] , [0056], [0061]. Redox chemicals are merely optional to the disclosed method. *Id.* at [0036]; EX2001 at ¶93.

In its sole experimental Example, *Schlegl* uses "renaturation buffer" (*i.e.*, refold buffer, and not redox component) with 2 mM cystine (an oxidant) and 2 mM cysteine (a reductant). EX1003 at [0075]. But *Schlegl* does not provide information necessary to calculate the claimed TPR or RBS values, based on the volume of a redox component. EX2001 at ¶99. Indeed, the terms "thiol-pair ratio," "redox buffer strength," and "redox component" are nowhere to be found in *Schlegl*.

Moreover, *Schlegl* also does not demonstrate refolding of a complex protein, an antibody, or a multimeric protein. The model protein used in the sole example of *Schlegl*, bovine α-lactalbumin, has 123 amino acid residues and a

molecular weight (MW) of 14,178. EX2001 at ¶100 (citing EX1002 at ¶59 (bovine α-lactalbumin "contain[s] 123 amino acid residues . . .")); EX2010 at Abstract, 1 ("the molecular mass of bovine α-lactalbumin is 14[,]178 Da[6]"); EX2009 at 1 ("Bovine α-La [α-lactalbumin] . . . [is] made up of 123 amino acids . . . has 4 disulfide bonds . . . with a molecular mass corresponding to . . . 14[,]178 Da in bovine milk . . ."). In addition, bovine α-lactalbumin is neither an antibody nor a multimeric protein (a protein with two or more polypeptide chains). *Id.* at ¶¶100, 155. Bovine α-lactalbumin is a calcium-binding subunit of the lactose synthase complex comprising a single polypeptide chain. *Id.*; EX2009 at 1 ("Bovine α-La [α-lactalbumin] occurs as an acidic, single-chain Ca^{2+} binding protein . . . . α-La forms the regulatory subunit of lactose synthase complex . . . .").

2. *Brady* (EX1005)

Mere days before trial in the related litigation, Apotex withdrew its invalidity defense based on *Brady* and judgment was entered in Amgen's favor that the '138 Patent is not invalid for anticipation under 35 U.S.C. § 102 or obviousness under 35 U.S.C. § 103. EX2004 at 2-5.

Brady teaches a fundamentally different approach to protein refolding than the '138 Patent. Brady's method is a genetic approach to achieve protein

[6] Daltons (Da) and MW are the same. EX2001 at 75, n.5; EX2014 (defining Dalton as "equal to the unified atomic mass unit [*i.e.*, molecular weight].").

refolding at dilute protein concentrations. EX2001 at ¶127. In contrast, the method claims of the '138 Patent are a chemical approach using redox chemicals with specified mathematical relationships to achieve protein refolding at high protein concentrations. *Id*.

Brady teaches protein refolding of Interleukin 31 (IL-31). EX2001 at ¶127; *see, e.g.*, EX1005 at [0050]. Because murine (mouse) and human forms of IL-31 have an odd number of cysteines, IL-31 production is hampered by the formation of mismatched disulfide bonds (with its attendant misfolded and potentially inactive proteins). EX2001 at ¶128. *Brady* identifies the problem to be solved as follows:

> Expression of recombinant IL-31 can result in a heterologous mixture of proteins composed of intramolecular disulfide binding in multiple conformations. The separation of these forms can be difficult and laborious. It is therefore desirable to provide IL-31 molecules having a single intramolecular disulfide bonding pattern upon expression and methods for refolding and purifying these preparations to maintain homogeneity.

EX1005 at [0045] (emphasis added).

Brady's solution to the problem of mismatched disulfide bonds, and its attendant protein misfolding, is based on genetics, not redox chemistries:

> The present invention provides mutations in the IL-31 wildtype sequences . . . that result in expression of single forms of the IL-31

> molecule. Because the heterogeneity of forms is believed to be a result of multiple intramolecular disulfide bonding patterns, specific embodiments of the present invention includes mutations to the cysteine residues within the wildtype IL-31 sequences.

EX1005 at [0050] (emphasis added). Specifically, *Brady* inserts genetic mutations to ensure a reduction in mismatched disulfide bonds during protein production. EX2001 at ¶129.

Several prophetic examples in *Brady* describe refold buffers with oxidants and reductants. But *Brady* does not provide the information necessary to calculate the claimed TPR or RBS values based on the volume of a redox component. EX2001 at ¶131. Indeed, the terms "thiol-pair ratio," "redox buffer strength," and "redox component" are nowhere to be found in *Brady*.

Brady teaches methods for refolding proteins at dilute concentrations. EX2001 at ¶130. While *Brady* discloses starting concentrations of protein prior to contact with a refold buffer that are 2 g/L or greater, the protein becomes substantially diluted by the refold buffer; the protein concentrations in the refold mixtures reported in, *e.g.*, Examples 6, 7, 8, and 9 of *Brady*, are either 0.10 mg/ml or 0.15 mg/ml. *See* EX1005 at [0241], [0253], [0264], [0276]; EX2001 at ¶130.

Brady does not demonstrate refolding of complex proteins. *Brady* teaches refolding IL-31. EX2001 at ¶127; *see, e.g.*, EX1005 at [0050]. Both human IL-31 and mouse IL-31 are not complex proteins. Human IL-31 has 164

amino acid residues and a MW of 18,205. EX2001 at ¶¶132, 146; EX1005 at SEQ ID NO 2 (showing 164 amino acids), [0044] (SEQ ID NO: 2 is the "native . . . polypeptide sequence[] for human IL-31"); EX2012 at 4 ("Length: 164" amino acids and "Mass (Da): 18,205"). Mouse IL-31 has 163 amino acid residues and a MW of 18,120. EX2001 at ¶¶132, 146; EX1005 at SEQ ID NO 5 (showing 163 amino acids), [0044] (SEQ ID NO: 5 is the "native . . . polypeptide sequence[] for mouse IL-31"); EX2013 at 4 ("Length: 163" amino acids and "Mass (Da): 18,120").

B. Secondary References

1. *Hevehan* (EX1004)

Hevehan is a fundamentally different approach to protein refolding than the '138 Patent. *Hevehan*'s method is a chemical approach to achieve protein refolding focused primarily on denaturant with some consideration of oxidant (without its reductant partner) in the refold buffer. EX2001 at ¶107. In contrast, the method claims of the '138 Patent are a chemical approach to achieve protein refolding focused on oxidant and its reductant partner in a redox component at specified mathematical relationships (to calculate RBS and TPR). *Id*.

Hevehan investigates "conditions that can allow oxidative renaturation of proteins at high concentrations," with the refold buffer (not the redox component) as its focal point. EX1004 at 2; EX2001 at ¶108. Optimization

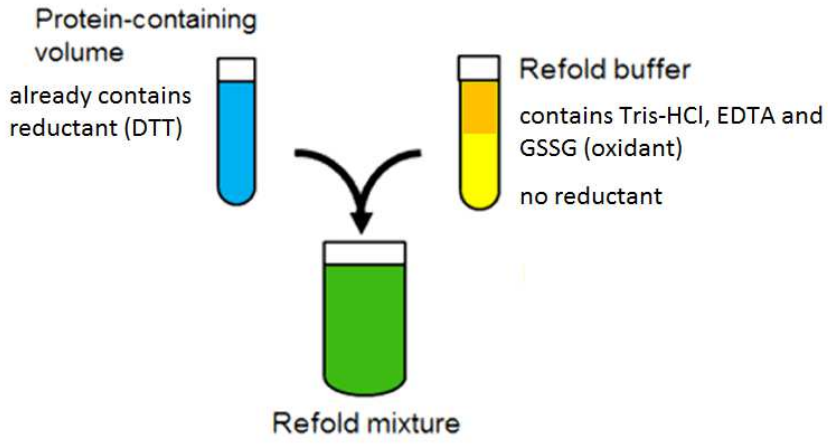
studies in *Hevehan* focus on denaturant (or "solubilizing agent") concentration in the refold buffer. EX2001 at ¶108. The Abstract discloses optimal conditions with "[s]olubilizing agents such as guanidinium chloride (GdmCl) and folding aides such as L-arginine present in low concentrations[. Use of such conditions] during refolding effectively enhanced renaturation yields by suppressing aggregation resulting in reactivation yields as high as 95%." EX1004 at Abstract; *id.* at 2 (". . . [A]ddition of solubilizing agents in non-denaturing concentrations to the renaturation [*i.e.*, refold] buffer seemed to be most effective at decelerating the rate of aggregation"); EX2001 at ¶108. *Hevehan* concludes that "[a]ll of the results lead to a consensus view of the ability of increasing amounts of GdmCl [denaturant] and L-arginine at low concentrations in the refolding solution to enhance renaturation yields but slow renaturation rates." EX1004 at 9; EX2001 at ¶108. For *Hevehan*, increased denaturant in the refold buffer (not the redox component) leads to enhanced protein refolding. *Hevehan*, to a lesser extent, also investigated thiol concentrations (which allow disulfide bond formation and shuffling) in the refold mixture.

Notably, *Hevehan* specifically teaches that addition of a reductant is not necessary to its refolding method:

> Addition of GSSG's reducing partner, GSH, to the renaturation system was <u>not necessary</u> due to the DTT carried over from the denatured solution.

EX1004 at 3 (emphasis added); EX2001 at ¶109. "GSSG" stands for oxidized glutathione (an oxidant). EX2001 at ¶109. "GSH" stands for reduced glutathione (a reductant). *Id.* "DTT" stands for dithiothreitol (a reductant). *Id.* The "denatured solution" refers to a volume containing solubilized protein to be refolded. *Id.* Thus, in *Hevehan*, a protein-containing volume containing a reductant (DTT) is contacted with a refold buffer containing only an oxidant, GSSG, but not its partner, GSH, a reductant. EX2001 at ¶109.

A diagram of *Hevehan* is depicted below (*id.*):



Hevehan does not provide the information necessary to calculate TPR or RBS values based on the volume of a redox component. EX2001 at ¶109. Indeed, the terms "thiol-pair ratio," "redox buffer strength," and "redox component" are nowhere to be found in *Hevehan*. Without the addition of reductant in a given volume, TPR ($\frac{[reductant]^2}{[oxidant]}$) values are necessarily zero irrespective of the oxidant concentration (*i.e.*, $\left(\frac{[reductant]^2}{[oxidant]}\right) = \left(\frac{[0]^2}{[oxidant]}\right) = 0$).

The model protein in *Hevehan* is hen egg white lysozyme, obtained from commercial sources in a high degree of purity. EX1004 at 2 ("Hen egg white lysozyme . . ., three times crystallized, dialyzed and lyophilized . . ."); EX2001 at ¶110. Hen egg white lysozyme is not an antibody nor a multimeric protein. EX2001 at ¶¶110, 154. It is an enzyme found in chicken eggs. *Id.*; EX2011 at 2 ("The enzyme is lysozyme," which is "obtained from egg white consist[ing] of a single polypeptide chain of 129 amino acid subunits of 20 different kinds"), 3. And it is also not a complex protein; it has 129 amino acid residues and a MW of 14,389.68. EX2001 at ¶110 (citing EX1002 at ¶66 (hen egg white lysozyme "has 129 amino acids [and] a molecular weight (MW) of 14389.68")).

2. *Inclonals* (EX1006)

Inclonals reports on the expression of antibodies and antibody-enzyme fusion proteins in bacterial (*E. coli*) expression systems. EX2001 at ¶123. That paper provides no details as to protein concentration and how such proteins are refolded. *Id*. *Inclonals* merely notes that "Refolding was initiated after mixing 50 mg of heavy chain and 50 mg of light chain inclusion bodies protein and reducing the mixture with 1,4-dithioerythritol (DTE)." EX1006 at 3. *Inclonals* does not provide information necessary to calculate the claimed TPR or RBS values, based on the volume of a redox component. EX2001 at ¶124. Indeed, the

terms “thiol-pair ratio,” “redox buffer strength,” and “redox component” are nowhere to be found in *Inclonals*.

VI. Argument

No doubt recognizing the inherent weakness of its anticipation case, Apotex raises anticipation (Grounds 3 and 4) after obviousness (Grounds 1 and 2). Since Apotex’s obviousness challenges (Grounds 1 and 2) and anticipation challenge (Ground 3) share the same primary reference (*Schlegl*), Amgen will not address the Grounds in the same order as raised by Apotex. Instead, Amgen will first address the missing material elements in *Schlegl* individually, before turning to the deficiencies of *Schlegl* in combination with *Hevehan* (Ground 1) and *Hevehan* and *Inclonals* (Ground 2).

Claim 1 of the ’138 Patent is its only independent claim. If a prior art reference lacks one or more elements of a claim, it cannot anticipate that claim or any of its dependent claims. *Endo Pharmaceuticals Inc. v. Depomed, Inc.*, IPR2014-00653, Paper 12 (PTAB 2014) (denying institution of trial because each of the six alleged anticipatory prior art references did not teach every limitation of the challenged claims). As detailed below, Apotex’s primary references, *Schlegl* and *Brady*, do not teach, either expressly or inherently, the claimed TPR and RBS elements relative to the volume of the redox component, as required by Claim 1. Thus, the Board should reject Apotex’s anticipation challenges (Grounds 3 and 4).

And since Apotex's secondary references, *Hevehan* and *Inclonals*, cannot supply these missing material elements nor render them obvious, the Board should likewise reject Apotex's obviousness challenges (Grounds 1 and 2).[7]

A. The Board Should Reject Ground 3: *Schlegl* Does Not Anticipate Claims 1-7, 10, 13-17, and 23 of the '138 Patent Because It Does Not Disclose the TPR and RBS Elements of Claim 1, Either Expressly or Inherently

Given *Schlegl*'s disclosure, Apotex has not demonstrated that *Schlegl* discloses, either inherently or expressly, a method of refolding a 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," as required by Claim 1. The parties agree that Claim 1 requires that TPR and RBS are based on the volume of the redox component. Petition at 24-25; EX1002 at 28, n.3 ("Based on the '138 patent, the thiol-pair (TPR) is defined by the equation, $TPR = \frac{[reductant]^2}{[oxidant]}$, where the TPR is calculated in the redox component") (emphasis added); EX2001 at ¶¶64-65, 85, 101. But Apotex submits no evidence from *Schlegl*'s disclosure reflecting a calculation of TPR and RBS based on a volume of a redox component. EX2001 at ¶¶101-102.

[7] Amgen reserves the right, should trial be instituted, to demonstrate that the prior art references asserted by Apotex lack additional material elements of the claims, and otherwise fail to render any of the claims obvious, alone or in combination.

Straining to argue that *Schlegl* meets the TPR and RBS elements of claim 1, Apotex conflates the refold buffer with the redox component. Apotex cites to the sole Example of *Schlegl*, which discloses denatured protein contacted with "renaturation buffer consisting of . . . 2 mM cystine and 2 mM cysteine[.]" EX1003 at [0075]; Petition at 45; EX2001 at ¶103. Cystine is an oxidant and cysteine is a reductant; renaturation buffer is refold buffer. EX2001 at ¶103. *Schlegl* clearly teaches concentrations of redox chemicals in the refold buffer ("renaturation buffer") with no mention of their concentrations in a redox component. *Id.*

Apotex and its expert can make only the conclusory argument: "That redox component has a thiol-pair ratio of 2 and a redox buffer strength of 6 mM". EX1002 at ¶124 (emphasis added); Petition at 45. But Dr. Robinson readily admits that her math is based on the volume of the refold buffer ("the refolding buffer in the Example has a calculated thiol-pair ratio of 2 and a redox buffer strength of 6 mM"). EX1002 at ¶60 (emphasis added); EX2001 at ¶103. "Thus, Dr. Robinson and Apotex never properly calculate the claimed TPR and RBS using the volume of a redox component (gray) in accordance with the agreed definition of TPR and RBS." EX2001 at ¶103 (emphasis in original).

In short, Apotex is unable to calculate TPR and RBS values from *Schlegl*'s disclosure based on the volume of a redox component, as required by

sole independent Claim 1. Indeed, the terms "thiol-pair ratio," "redox buffer strength," and "redox component" are nowhere to be found in *Schlegl*. Nor is there any disclosure in *Schlegl* of the inventor's novel equation for TPR, the interrelatedness of TPR and RBS or, more specifically, that when the RBS is 2 mM or greater, the TPR should fall within the range of 0.001 to 100.

Apotex nevertheless attempts to extrapolate a TPR value based on the volume of a redox component, as required by Claim 1, from its expert's calculation of a TPR value based on the volume of refold buffer. Dr. Robinson incorrectly reasons that TPR calculated using the volume of the refold buffer will be the same number if calculated using the volume of a redox component. EX2001 at ¶104; EX1002 at 28, n.3. Not so. The '138 Patent at 6:25-28 defines TPR as: $\frac{[reductant]^2}{[oxidant]}$. Contrary to Dr. Robinson's assertion, calculating TPR in accordance with the teachings of the '138 Patent does not result in a constant ratio of reductant to oxidant. EX2001 at ¶103. TPR is volume-dependent: it changes depending on the volume of the solution used to compute the concentration of reductant and oxidant. EX2001 at ¶¶82, 84-90

$$\left(\frac{[reductant]^2}{[oxidant]} = \frac{\left(\frac{amount\ reductant}{\cancel{total\ volume}}\right)\times\left(\frac{amount\ reductant}{total\ volume}\right)}{\left(\frac{amount\ oxidant}{\cancel{total\ volume}}\right)} = \frac{(amount\ reductant)^2}{(amount\ oxidant)(total\ volume)}\right).$$

Since the volumes of redox component and refold buffer are necessarily different, TPR calculated using the volume of a refold buffer will <u>not</u> result in the same

numerical value if calculated using the volume of a redox component[8]. *Id.* at ¶103. Therefore, "[c]alculating the TPR in a volume other than that of a redox component, as Dr. Robinson and Apotex have, is insufficient to show that the 'final thiol-pair ratio having a range of 0.001 to 100' is met by *Schlegl*." *Id.* at ¶104 (emphasis in original).

Amgen respectfully submits that trial should not be instituted with *Schlegl* as an anticipatory reference (Ground 3). Without disclosure either expressly or inherently of the material TPR and RBS elements of Claim 1 based on a redox component, *Schlegl* is not anticipatory. EX2001 at ¶106. Since Claims 2-7, 10, 13-17, and 23 depend from Claim 1 and share the same material limitations, those dependent claims are also not anticipated by *Schlegl*. *Id.*

[8] Apotex argues that, in the event TPR is construed to be based on the volume of the refold mixture, the value of such TPR would be the same as the value based on the volume of the refold buffer. Petition at 45. Apotex is wrong. EX2001 at ¶105. Calculating TPR in accordance with the teachings of the '138 Patent does not result in a constant ratio of reductant to oxidant. TPR is volume-dependent. TPR calculated according to Equation 1 of the '138 Patent will change depending on the volume of the solution used to compute the concentration of reductant and oxidant. *Id.* at ¶¶82, 84-90. And the volumes of refold mixture and refold buffer are necessarily different. *Id.* at ¶¶63, 86.

B. The Board Should Reject Ground 1; The Combination of *Schlegl* in View Of *Hevehan* Does Not Render Obvious Claims 1-11 and 13-24 of the '138 Patent

1. There Would Have Been No Motivation For a POSITA To Combine The Teachings of *Schlegl* and *Hevehan*

Schlegl and *Hevehan* are fundamentally different and incompatible approaches to protein refolding. *Schlegl*'s method is a mechanical approach to achieve protein refolding at dilute protein concentrations. EX2001 at ¶¶93, 111. *Hevehan*'s method is a chemical approach (focused on denaturant and oxidant, but not reductant, in the refold buffer) to achieve protein refolding at high protein concentrations. *Id.* at ¶111. In *Schlegl*, protein aggregation is avoided by physically separating the protein molecules by dilution. *Id.* at ¶112. In *Hevehan*, refolding proteins at high concentrations necessarily reduces or eliminates such physical separation; chemicals are necessary to avoid aggregation and to achieve proper refolding. *Id.*; EX1004 at 1 (". . . low recovery of correctly folded protein is often due to aggregation . . . The most direct means of minimizing aggregation is by decreasing protein concentration."); EX1003 at [0008] ("The higher the protein concentration, the higher the risk of intermolecular misfolding, and vice versa."). *Hevehan* primarily relies on controlling the amount of denaturant (GdmCl) in the refold buffer in order to minimize protein aggregation. EX2001 at ¶112; EX1004 at 2 ("In particular, addition of solubilizing agents [denaturant] in nondenaturing

concentrations to the renaturation buffer seemed to be most effective at decelerating the rate of aggregation.")

A POSITA would see no benefit to combining *Schlegl* and *Hevehan*'s fundamentally different teachings. Indeed, adding *Hevehan*'s denaturant and oxidant chemicals to *Schlegl*'s dilute refolding method would have been viewed as making *Schlegl*'s process more costly and complicated. *Schlegl* requires a large volume of refolding buffer, which is costly to prepare; adding denaturant and oxidant chemicals to that refolding buffer solution would only exacerbate costs—and be simply unnecessary, given *Schlegl*'s teaching that the use of redox chemicals is "optional[]." EX2001 at ¶113; *see, e.g.*, EX1003 at [0040] ("In its simplest embodiment, the method of the invention is a batch process that comprises, as its essential step, the above-defined mixing operation, in which a feed stream having a high concentration of unfolded protein and a low flow rate is combined with a refolding buffer solution having a high flow rate."), [0036].

2. The Combined Teachings of *Schlegl* and *Hevehan* Cannot Supply the Material TPR and RBS Elements of Claim 1 or Render It and Its Dependent Claims Obvious

Apotex combines *Schlegl* as its primary reference with *Hevehan* as its secondary reference without identifying the specific element(s) lacking in *Schlegl* that *Hevehan* supplies to render Claim 1 of the '138 Patent obvious. No doubt Apotex's simultaneous reliance on *Schlegl* as an allegedly anticipatory reference,

which necessarily must disclose each and every element of the claimed invention, accounts for its vagueness. At a minimum, Apotex's reliance on *Schlegl* in an obviousness combination undermines its position that *Schlegl* is anticipatory.

Nevertheless, *Schlegl* and *Hevehan*, individually and in combination, do not teach or suggest 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," as required by Claim 1.

Like *Schlegl*, *Hevehan* does not teach or suggest concentrations of reductant or oxidant in a redox component. EX2001 at ¶114. Apotex conclusorily asserts that *Hevehan* teaches the claimed RBS and TPR based on a redox component:

> Likewise, *Hevehan* discloses contacting the hen egg white lysozyme with a refold buffer comprising a redox component to form a refold mixture . . . . That redox component has a thiol-pair ratio between 0.3 and 9 and redox buffer strength of 5 mM to 19 mM, the optimum being between 10-16 mM.

Petition at 45 (citations omitted and emphasis added); EX1002 at ¶124. But Apotex and Dr. Robinson yet again conflate two separate and distinct elements of Claim 1—this time, a redox component with a refold mixture. EX2001 at ¶115. *Hevehan* reports that "DTT and GSSG concentrations were varied between 1 and 6 mM and 4 and 13 mM, respectively." EX1004 at 5. DTT is a reductant and GSSG

is an oxidant. EX2001 at ¶116. Those reported concentrations are "final conditions" in the final refold mixture. *Id.* In fact, all of the reductant and oxidant concentrations disclosed in *Hevehan* are based on the volume of the refold mixture. *Id.*; *see, e.g.*, EX1004 at 3 ("the refolding solution [*i.e.*, the refold mixture] contained 5 mM GSSG and 2 mM DTT . . ."), Figs. 3-5 ("Renaturation was initiated by rapidly diluting reduced denatured lysozyme into renaturation buffer" resulting in the refold mixture wherein the "[f]inal conditions" include concentrations of GSSG and DTT in the refold mixture). Given that disclosure, Dr. Robinson's *Hevehan*-related calculations are based only on concentrations in a refold mixture and not concentrations in a redox component as required by Claim 1. EX2001 at ¶¶115, 117. Dr. Robinson's declaration makes this clear:

> Tested redox compositions, including GSSG values to 13 mM equate to a calculated thiol-pair ratio of 0.3 to 9 ([reductant]^2/[oxidant]) and a calculated redox buffer strength of 5 to 19 mM (optimum 10 to 16 mM).

EX1002 at ¶68 and n.5 (emphasis added). Such "compositions" are not redox components but refold mixtures. EX2001 at ¶117.

As with *Schlegl*, Apotex has not demonstrated that a TPR value based on the volume of a redox component, as required by Claim 1, will be the same number if based on the volume of refold mixture. EX2001 at ¶117; *see also id.* at ¶¶82, 84-90. Since the volumes of redox component and refold mixture are

necessarily different, TPR calculated using the volume of the refold mixture will not result in the same numerical value if calculated using the volume of a redox component[9]. *Id.* at ¶117. Because the combination of *Schlegl* and *Hevehan* does not teach or suggest the '138 Patent's unique TPR equation, Apotex and its expert must resort to improper hindsight, misapplying that equation to those prior art systems. *Id.* at ¶118.

Critically, *Hevehan* teaches away from a redox component comprising a TPR "having a range of .001 to 100" since *Hevehan* teaches away from adding reductant to a redox component from which TPR is calculated. EX2001 at ¶119. *Hevehan*'s approach with respect to oxidant and reductant chemicals is a fundamentally different approach than that of the '138 Patent. *Id.* at ¶107. The method claims of the '138 Patent rely on the addition of reductant in the redox component in a specified mathematical relationship to its oxidant pair (to calculate

[9] As with *Schlegl*, Apotex argues that, in the event TPR is construed to be based on the volume of the refold mixture, the value of such TPR would be the same as the value based on the volume of the refold buffer. Petition at 45. This argument is nonsensical in the context of *Hevehan*. In *Hevehan*, the concentrations of the reductant and oxidant are based on the volume of the refold mixture, not the refold buffer. In any event, for the same reasons discussed above for *Schlegl* (*supra* at n.8), Apotex is wrong. EX2001 at ¶121.

TPR). EX2001 at ¶107. In contrast, *Hevehan*'s process does not add reductant in a mathematically precise relationship to its oxidant partner (the concentration of reductant in a redox component is zero). *Id.* It merely relies on a reductant (DTT) carried over with the solubilized protein. *Id.* at ¶¶109, 120; EX1004 at 3 ("Addition of GSSG's reducing partner, GSH, to the renaturation system was not necessary due to the DTT carried over from the denatured solution [*i.e.*, protein-containing volume (blue)]") (emphasis added). *Hevehan* discloses a method of refolding protein by (1) denaturing and reducing the protein, (2) diluting the protein "by a rapid 8-fold or 16-fold dilution" (3) "into [a] renaturation buffer" (*i.e.*, the refold buffer). EX1004 at 2-3. The refold buffer contained Tris-HCl, EDTA and GSSG (*i.e.*, the oxidant). EX2001 at ¶120. But the renaturation buffer did not include GSH (*i.e.*, the reductant) because, according to *Hevehan*, "[a]ddition of GSSG's reducing partner, GSH, to the renaturation system was not necessary due to the DTT carried over from the denatured solution." EX1004 at 3 (emphasis added); EX2001 at ¶120.

Assuming, *arguendo*, that there is a volume of a redox component in *Hevehan* on which to base the calculation of TPR, the TPR in such a redox component is necessarily zero, and outside the claimed range of "0.001 to 100." To meet this limitation of Claim 1, the amount of reductant in a redox component must be greater than zero; if there is zero reductant, TPR must be zero (*i.e.*,

$\frac{[reductant]^2}{[oxidant]} = \frac{[0]^2}{[oxidant]} = 0$). EX2001 at ¶119. In *Hevehan*, there is no reductant added to a redox component; instead, reductant is added to the protein-containing volume (blue). *Id*. at ¶¶119-120; EX1004 at 3 ("Addition of GSSG's reducing partner, GSH, to the renaturation system was not necessary due to the DTT carried over from the denatured solution [*i.e.*, protein-containing volume (blue)]") (emphasis added).

Thus, even if there would be motivation to combine *Schlegl* and *Hevehan*, that combination does not teach or suggest the "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." Because *Hevehan* eliminates the material TPR element of Claim 1, that combination necessarily leads to a TPR value of zero, falling outside the claimed TPR range of the 0.001 to 100. EX2001 at ¶¶119, 122. For this reason alone, *Schlegl* and *Hevehan*, in combination, cannot render Claim 1 (and all the challenged claims that depend on it) obvious.

Amgen respectfully submits that trial should not be instituted based on *Schlegl* in view of *Hevehan* (Ground 1). Without disclosure of the material TPR and RBS elements of Claim 1 based on a redox component, that combination does not render obvious Claim 1 of the '138 Patent. *Id.* at ¶122. Since Claims 2-11 and 13-24 depend from Claim 1 and share the same material limitations, those

dependent claims are also not rendered obvious by the combination of *Schlegl* and *Hevehan*. *Id.*

C. The PTAB Should Reject Apotex's Ground 2; The Combination of *Schlegl* and *Hevehan* In View of *Inclonals* Does Not Render Claim 12 of the '138 Patent Obvious

Claim 12 of the '138 Patent depends on Claim 1 and specifies that the protein being refolded is "an Fc-protein conjugate." Building off of its *Schlegl* and *Hevehan* obviousness combination of Ground 1, Apotex uses *Inclonals* solely for its teaching of "IgG-toxin fusion proteins," which it characterizes as Fc-protein conjugates. Petition at 56-58.

The combination of *Inclonals* with *Schlegl* and *Hevehan* does not render claim 12 obvious. Although *Inclonals* discloses refolding of its IgG-toxin fusion proteins that are expressed in non-mammalian *E. coli* expression systems, it provides no details as to how these proteins are refolded. EX1006 at 7; EX2001 at ¶126. Thus, *Inclonals* does not teach or suggest "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," as required by Claim 1 of the '138 Patent, on which Claim 12 depends. EX2001 at ¶126. And as discussed *supra*, *Schlegl* and *Hevehan* also share in that same deficiency. *Id.*

Because Apotex does not establish a reasonable likelihood that Claim 12 is obvious in view of *Schlegl*, *Hevehan*, and *Inclonals*, Amgen respectfully requests that the Board reject Ground 2 of Apotex's Petition. *Id.*

D. The PTAB Should Reject Apotex's Ground 4; *Brady* Does Not Anticipate Claims 1-7, 10, 12-17, 19, 22, and 23 of the '138 Patent Because It Does Not Disclose the TPR and RBS Elements of Sole Independent Claim 1, Either Expressly or Inherently

Apotex has not demonstrated that *Brady* discloses, either inherently or expressly, a method of refolding a 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," as required by Claim 1. The parties agree that Claim 1 requires TPR and RBS to be based on the volume of the redox component. Petition at 24-25; EX1002 at 28, n.3 ("Based on the '138 patent, the thiol-pair (TPR) is defined by the equation, $TPR = \frac{[reductant]^2}{[oxidant]}$, where the <u>TPR is calculated in the redox component</u>") (emphasis added); EX2001 at ¶¶64-65, 85, 133. But Apotex submits no evidence reflecting a calculation of TPR and RBS based on a redox component from *Brady*'s disclosure. EX2001 at ¶¶133-134.

Straining to argue that *Brady* meets the TPR and RBS elements of claim 1, Apotex yet again conflates the redox component with the refold buffer. Apotex cites to Example 8 of *Brady*, which states:

> The redox pair <u>and concentrations in this refold buffer</u> are as follows: [Cysteamine] = 1.25 mM : [Cystamine] = 0.5 mM.

EX1005 at [0264] (emphasis added); EX2001 at ¶135. Cysteamine is a reductant, and cystamine is an oxidant. *Id.* *Brady* clearly teaches concentrations of redox chemicals in the "refold buffer," with no mention of their concentrations in a redox component. *Id.*

Apotex and its expert can make only the conclusory argument that: "*Brady* teaches contacting the protein with a refold buffer comprising a redox component comprising a thiol-pair ratio of 3.125[,]" and further that "*Brady* also discloses a redox buffer strength of 2.25 mM." Petition at 60; *see also* EX1002 at ¶158. But Dr. Robinson readily admits that her calculations are based on oxidant and reductant concentrations in *Brady*'s refold buffer. EX1002 at ¶71 ("*Brady* then dilutes that protein concentration with a refold buffer . . .") (emphasis added); *id.* at 34, n.7; EX2001 at ¶135. "Thus, Dr. Robinson and Apotex never properly calculate the claimed TPR and RBS using the volume of a redox component (gray) in accordance with the agreed definition of TPR and RBS." *Id.* (emphasis in original).

In short, with *Brady*'s disclosure, Apotex is unable to calculate TPR and RBS values based on the volume of a redox component.[10] Nor is there any

[10] As with *Schlegl*, Apotex argues that, in the event TPR is construed to be based on the volume of the refold mixture, the value of such TPR would be the same as the value based on the volume of the refold buffer. Petition at 60. For the same

disclosure in *Brady* of the inventor's novel equation for TPR, the interrelatedness of TPR and RBS or, more specifically, that when the RBS is 2 mM or greater, the TPR should fall within the range of 0.001-100. The terms "thiol-pair ratio," "redox buffer strength," and "redox component" are nowhere to be found.

Amgen respectfully submits that trial should not be instituted with *Brady* as an anticipatory reference (Ground 4). Without disclosure either expressly or inherently of the material TPR and RBS elements of Claim 1 based on a redox component, *Brady* is not anticipatory. EX2001 at ¶137. Since Claims 2-7, 10, 12-17, 10, 22, and 23 depend from Claim 1 and share the same material limitations, those dependent claims are also not anticipated by *Brady*. *Id.*

E. The Board Should Not Institute Trial on Claims 9, 10 and 11 of the '138 Patent

Claims 9, 10, and 11 depend from independent Claim 1 and further limit the "protein" in Claim 1 to "an antibody," "a complex protein," and "a multimeric protein," respectively. Apotex asserts that Claim 10 of the '138 Patent is invalid because it is (1) anticipated by *Schlegl* (Ground 3), (2) rendered obvious by the combination of *Schlegl* and *Hevehan* (Ground 1), and (3) anticipated by *Brady* (Ground 4). Petition at 37-38. Apotex asserts that Claims 9 and 11 of the

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reasons discussed above for *Schlegl* (*supra* at n.8), Apotex is wrong. EX2001 at ¶136.

’138 Patent are invalid because they are rendered obvious by the combination of *Schlegl* and *Hevehan* (Ground 1). Petition at 38. As discussed above, *Schlegl*, *Hevehan*, and *Brady* do not teach or suggest “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,” as required by independent Claim 1. For that reason alone, the Board should not institute trial on Claims 9, 10, and 11. EX2001 at ¶¶140, 152.

In addition, the Board should not institute trial on Claim 10 because none of the refolded proteins in *Schlegl*, *Hevehan*, and *Brady* are “complex protein[s].” EX2001 at ¶141. The ’138 Patent defines “complex protein” as “any protein that (a) is larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form.” *Id.* *Schlegl*, *Hevehan*, and *Brady* work with proteins that do not meet prong (a) of the definition of “complex protein.” *Id.*

First, *Schlegl*’s sole example was performed on a model protein, bovine α-lactalbumin, which is a small globular protein found in cow’s milk. EX1003 at [0073]; EX2001 at ¶100. Bovine α-lactalbumin is not a “complex protein” because it has a MW of 14,178 daltons (which is less than 20,000 MW) and 123 amino acid residues (which is less than 250). EX2001 at ¶¶100, 142 (citing EX1002 at ¶59 (bovine α-lactalbumin “contain[s] 123 amino acid residues .

. .")); EX2010 at Abstract, 1 ("the molecular mass of bovine α-lactalbumin is 14[,]178 Da"); EX2009 at 1 ("Bovine α-La [α-lactalbumin] . . . [is] made up of 123 amino acids . . . has 4 disulfide bonds . . . with a molecular mass corresponding to . . . 14[,]178 Da in bovine milk . . ."). *Schlegl* contains no teaching that its refolding method has been successfully applied to a "complex protein." EX2001 at ¶143. Second, *Hevehan* uses only hen egg white lysozyme. EX1004 at 2; EX2001 at ¶110. Hen egg white lysozyme is also not a "complex protein" because it has a MW of 14,389.68 daltons (which is less than 20,000 MW) and 129 amino acid residues (which is less than 250). EX2001 at ¶¶110, 144 (citing EX1002 at ¶66 (hen egg white lysozyme "has 129 amino acids [and] a molecular weight (MW) of 14389.68")). *Hevehan* contains no teaching that its refolding method has been successfully applied to a "complex protein." EX2001 at ¶145. Last, *Brady* uses IL-31. EX2001 at ¶¶132, 146. Both human IL-31 and mouse IL-31 are not "complex protein[s]." Human IL-31 has a MW of 18,205 daltons (which is less than 20,000 MW) and 164[11] amino acid residues (which is less than 250). *Id.*;

[11] Dr. Robinson asserts that IL-31 has 904 amino acid residues and for support cites to [0052] in *Brady*. EX1002 at ¶70. Dr. Robinson is wrong. [0052] of *Brady* discloses SEQ ID NOs 20-25—all of which are "mature" or "mutant" mouse IL-31 with 133 amino acid residues. EX2001 at ¶147. It appears that Dr. Robinson is conflating the DNA sequence of SEQ ID NO 1 (showing 904 nucleotides) with

EX1005 at SEQ ID NO 2 (showing 164 amino acids), [0044] (SEQ ID NO: 2 is the "native . . . polypeptide sequence[] for human IL-31"); EX2012 at 4 ("Length: 164" amino acids and "Mass (Da): 18,205"). Mouse IL-31 has a MW of 18,120 daltons (which is less than 20,000 MW) and 163 amino acid residues (which is less than 250). EX2001 at ¶¶132, 146; EX1005 at SEQ ID NO 5 (showing 163 amino acids), [0044] (SEQ ID NO: 5 is the "native . . . polypeptide sequence[] for mouse IL-31"); EX2013 at 4 ("Length: 163" amino acids and "Mass (Da): 18,120"). *Brady* contains no teaching that its refolding method has been successfully applied to a "complex protein." EX2001 at ¶148. In sum, none of the proteins refolded in *Schlegl*, *Hevehan*, and *Brady* are "complex protein[s]." *Id.* at ¶149.

Further, the Board should not institute trial on Claims 9 and 11 because none of the refolded proteins in *Schlegl* and *Hevehan* are "antibod[ies]" (Claim 9) or "multimeric protein[s]" (Claim 11). EX2001 at ¶153. Apotex does not assert that *Hevehan* teaches or suggests "an antibody" or "a multimeric protein." Petition at 53-54; EX2001 at ¶154. Instead, Apotex and Dr. Robinson

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amino acid residue. *Id.* It is well known in the art that three nucleotides encode one amino acid residue. *Id*.; EX2015 at 3 ("Three nucleotides encode an amino acid"). The bottom portion of SEQ ID NO 1 starting with "Met" and ending with "Thr" represents the 164 amino acid residues of SEQ ID NO 1, and not the top portion comprising 904 nucleotides. EX2001 at ¶147.

conclusorily state that "the methods disclosed in *Schlegl* are broad enough to cover [an antibody and a multimeric protein]" and that a POSITA "would immediately recognize that the methods taught by *Schlegl* could be applied to each of these types of proteins." EX1002 at ¶145; Petition at 54. Such unsupported statements are of little probative value and should be rejected. 37 C.F.R. § 42.65(a) (stating opinion testimony that does not disclose underlying facts or data "is entitled to little or no weight"); *Endo Pharmaceuticals Inc. v. Depomed, Inc.*, IPR2014-00655, Paper 12 (PTAB 2014) at 21 (finding petitioner's expert's opinion was "of little probative value" because he "offer[ed] no objective support for [his] assertion"). Apotex has not established a reasonable likelihood that Claims 9 and 11 are obvious.

In any event, none of the refolded proteins in *Schlegl* and *Hevehan* are antibodies or multimeric proteins. EX2001 at ¶153. First, as discussed above, *Schlegl*'s sole example was performed on a model protein, bovine α-lactalbumin. EX1003 at [0073]. Bovine α-lactalbumin is not "an antibody"; it is a calcium-binding subunit of the lactose synthase complex. EX2001 at ¶155; EX2009 at 1 ("Bovine α-La [α-lactalbumin] occurs as an acidic, single-chain Ca^{2+} binding protein . . . . α-La forms the regulatory subunit of lactose synthase complex . . . ."). Bovine α-lactalbumin is also not "a multimeric protein" (a protein with two or more polypeptide chains); it comprises a single polypeptide chain. EX2001 at

¶155; EX2009 at 1 ("Bovine α-La [α-lactalbumin] occurs as an acidic, single-chain Ca^{2+} binding protein . . . . α-La forms the regulatory subunit of lactose synthase complex . . . ."). Second, as discussed above, *Hevehan* uses only hen egg white lysozyme. EX1004 at 2. Hen egg white lysozyme is not "an antibody"; it is an enzyme found in chicken eggs. EX2001 at ¶154; EX2011 at 2 ("The enzyme is lysozyme," which is "obtained from egg white consist[ing] of a single polypeptide chain of 129 amino acid subunits of 20 different kinds"). Hen egg white lysozyme is also not "a multimeric protein" (a protein with two or more polypeptide chains); it comprises a single polypeptide chain. EX2001 at ¶154; EX2011 at 2 (". . . lysozyme obtained from egg white consists of a single polypeptide chain of 129 amino acid subunits of 20 different kinds"), 3. In sum, the refolded proteins in *Schlegl* and *Hevehan* are not "antibod[ies]" or "multimeric protein[s]." EX2001 at ¶156.

Amgen respectfully submits that trial should not be instituted with respect to Claims 9, 10, and 11.

VII. Conclusion

For the reasons set forth above, Amgen requests that the Board deny Apotex's Petition in its entirety.

Dated: November 23, 2016

Respectfully submitted,

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CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24(d), the undersigned certifies that the foregoing PATENT OWNERS' PRELIMINARY RESPONSE complies with the type-volume limitation of 37 C.F.R. § 42.24(b)(1) because it contains 10,789 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)-(b).

Dated: November 23, 2016

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned certifies that on November 23, 2016, a true and correct copy of the foregoing PATENT OWNERS' PRELIMINARY RESPONSE, along with all exhibits supporting and filed with the Preliminary Response, was served by email on the following counsel of record for Apotex Inc. and Apotex Corp.:

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