UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

KASHIV BIOSCIENCES, LLC
Petitioner

v.

AMGEN INC.
Patent Owner

Case No. IPR2019-00791
U.S. Patent No. 8,940,878

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 8,940,878

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Petitioner's Exhibit List

Exhibit No.	Description
1001	U.S. Patent No. 8,940,878
1002	Declaration of Anne S. Robinson, Ph.D.
1003	*Curriculum vitae* of Anne S. Robinson, Ph.D.
1004	Henrik Ferré *et al.*, "A novel system for continuous protein refolding and on-line capture by expanded bed absorption," *Protein Science*, 14:2141-2153 (2005) ("Ferré")
1005	WO 2004/001056 ("Komath")
1006	U.S. Application Publication No. 2004/0018586 ("Rosendahl")
1007	Amersham Biosciences, *Ion Exchange Chromatography & Chromatofocusing: Principle and Methods*, No. 11-0004-21, Amersham Biosciences Limited (GE Healthcare) (Ed. AA) (2004) ("GE Handbook")
1008	U.S. Application Publication No. 2008/0260684 ("Dietrich")
1009	Hahm *et al.*, "Refolding and Purification of Yeast Carboxypeptidase Y Expressed as Inclusion Bodies in *Escherichia coli*," *Protein Expression and Purification*, 22:101-107 (2001) ("Hahm")
1010	Peter Neubauer *et al.*, "Protein Inclusion Bodies in Recombinant Bacteria," in Shively J.M. (eds) Inclusions in Prokaryotes. *Microbiology Monographs*, vol 1. Springer, Berlin, Heidelberg (2006)
1011	Luis Felipe Vallejo *et al.*, "Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins," *Microbial Cell Factories*, 3:11 (2004)

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1014	U.S. Patent No. 4,468,464
1015	U.S. Patent No. 4,740,470
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1036	U.S. Patent Application No. 12/822,990, August 28, 2012 Non-Final Rejection
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1038	U.S. Patent Application No. 12/822,990, September 9, 2013 Final Rejection
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1041	U.S. Patent Application No. 12/822,990, September 16, 2014 Notice of Allowance
1042	August 4, 2016 Order Construing Claims, in *Amgen Inc. et al. v. Sandoz Inc. et al*., 14-cv-04741-RS (N.D. Cal.)
1043	April 1, 2016, Amgen's Opening Claim Construction Brief, in *Amgen Inc. et al. v. Sandoz Inc. et al*., 14-cv-04741-RS (N.D. Cal.)
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1046	July 13, 2016 Transcript of Claim Construction Hearing, held on July 1, 2016, in *Amgen Inc. et al. v. Sandoz Inc. et al*., 14-cv-04741-RS (N.D. Cal.)
1047	November 13, 2017 Amgen's Opposition to Sandoz's Motion for Summary Judgement on Damages, in *Amgen Inc. et al. v. Sandoz Inc. et al*., 14-cv-04741-RS (N.D. Cal.)
1048	November 20, 2018 Order Regarding Claim Construction, in *Amgen Inc., et al. v. Mylan Inc. et al.*, 2:17-cv-01235 (W.D. Pa.)
1049	June 1, 2018 Declaration of Richard C. Willson in support of Amgen's Opening Claim Construction Brief, in *Amgen Inc., et al. v. Mylan Inc. et al.*, 2:17-cv-01235 (W.D. Pa.)
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1073	Tara M. Mezzasalma *et al*. "Enhancing Recombinant Protein Quality and Yield by Protein Stability Profiling," *Journal of Biomolecular Screening*, 12:418-428 (2007)

I. INTRODUCTION

Pursuant to 35 U.S.C. §§ 311-319 and 37 C.F.R. § 42, Kashiv BioSciences, LLC ("Petitioner") respectfully requests *Inter Partes* Review ("IPR") of claims 7-8, 11-13, 15-19, and 21 of U.S. Patent No. 8,940,878 ("the '878 patent"), which issued on January 27, 2015 and is assigned to Amgen Inc. ("Patent Owner"). This Petition demonstrates by a preponderance of the evidence that the asserted prior art renders all challenged claims of the '878 patent (EX1001) unpatentable.

II. OVERVIEW

The challenged claims of the '878 patent are directed to routine methods for purifying proteins expressed in non-mammalian expression systems, including (i) expressing a protein, (ii) lysing the cell, (iii) solubilizing the expressed protein, (iv) forming a refold solution, (v) directly applying that refold solution to a separation matrix, (vi) washing the matrix, and (vii) eluting the protein from the matrix. *E.g.*, EX1001, 22:3-28. Each of these steps, and the use of them together in the recited sequence, was well known in the art as of the earliest alleged priority date, June 25, 2009. *See, e.g.*, EX1004-1007; EX1002, ¶32.

As one of its purported inventive concepts, the '878 patent touts that proteins may be purified by "***directly*** applying a protein of interest, which is present in a refold mixture, to a separation matrix" without any intervening processing steps. EX1001, 4:47-49 (emphasis added); 15:25-28; EX1046, 161-163. As

demonstrated in this Petition, however, this was a routine practice in various protein purification methods long before the '878 patent. *See, e.g.*, EX1004-1007. Additionally, the characteristics of the various separation matrices described in the '878 patent, as well as the components of the solutions recited in the '878 patent, were well understood at the time of the invention. Thus, a person of ordinary skill ("POSA") as of June 2009 would have understood how to directly apply a refold solution to a separation matrix so that the target protein could associate successfully with the separation matrix. The claims recite nothing more than known protein purification methods with predictable and expected results.

Petitioner thus requests IPR and cancellation of claims 7-8, 11-13, 15-19, and 21 of the '878 patent under 35 U.S.C. § 311. Petitioner's request is supported by the Declaration of Dr. Anne Robinson (EX1002) and exhibits submitted herewith.

III. TECHNOLOGY BACKGROUND

A. Protein Synthesis

Proteins are large, complex molecules comprising one or more long chains of amino acids. EX1002, ¶¶34-35. In the human body, proteins are naturally produced by the processes of transcription (from DNA to RNA) and translation (from RNA to a protein). *Id.*, ¶¶36-37; EX1017, 125-149; EX1012.

Proteins can also be synthesized in the laboratory using "recombinant DNA" technology, which has been known in the art since at least the 1970s. *See* EX1013; EX1014; EX1015; EX1016; EX1002, ¶38. Recombinant DNA refers to DNA that is formed by combining two or more pieces of DNA, often from different sources, which is then inserted into a "host" cell to produce a desired, "recombinant protein" that the cell naturally does not synthesize. *See* EX1017, 182-183; EX1002, ¶38.

Both mammalian and non-mammalian host cells (referred to as "expression systems") can be used as "factories" to produce proteins using recombinant DNA technology. EX1002, ¶¶38-39. However, because mammalian expression systems are costly and can be associated with low yield and cultivation challenges, non-mammalian expression systems are typically preferred for many proteins for ease of production and reduced cost. EX1011, 1. As of 2009, expression systems using bacteria such as *Escherichia coli* ("*E. coli*") were widely used to express recombinant proteins; the biochemistry and genetics of *E. coli* were well known, and *E. coli* could be readily grown to produce high yields of desired proteins. EX1018, 1; EX1017, 182-183; EX1023, 1; EX1002, ¶39.

B. Recovering Bioactive Protein and Protein Refolding

A protein of interest produced by an expression system needs to adopt its native, 3D structure in order to perform its biological function and thus be therapeutically useful. EX1017, 44-68; EX1002, ¶40. Non-mammalian expression systems such as *E. coli*, however, sometimes produce recombinant proteins in non-native forms -- that is, having a structure that is different from the protein's native 3D structure. These proteins can accumulate in host cells as insoluble, intracellular aggregates to form what are called "inclusion bodies," as shown below in FIG. 1 (reproduced from EX1065). *See also* EX1011, 1; EX1023, 1; EX1021; EX1002, ¶40.

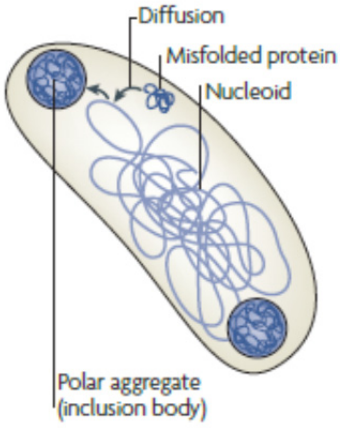

FIG. 1: Inclusion Body in Bacteria (EX1065, Figure 2)

Inclusion bodies typically contain between 35-95% of the overexpressed recombinant protein of interest, as well as DNA, ribosomal RNA, lipids, other proteins, and water. EX1020, 2, 4. EX1018, 2; EX1021, 9; EX1002, ¶41.

Recombinant proteins made in bacterial cells tend to aggregate because of the conditions used to generate high protein expression levels in the bacterial cells (*e.g.*, rapid growth conditions). EX1010, 4, 9; EX1018, 1. Bacterial host cells provide for quicker intracellular production of recombinant proteins than the natural protein generation process in mammalian cells. EX1002, ¶42. As a result, the bacterial host cells have difficulty "keeping up" with this rapid rate of recombinant protein production to allow proper folding of the produced recombinant proteins. Thus, the proteins can become misfolded and aggregate to form inclusion bodies. EX1010, 6; EX1002, ¶¶42-43.

It was well known by June 2009 that recombinant proteins expressed in *E. coli* had the specific problem of forming inclusion bodies. *See* EX1022; EX1018, 2. To remedy the problem, techniques were developed to recover proteins successfully in a bioactive and stable form from inclusion bodies. EX1002, ¶44. It was reported that "[a]s of 1998, there have been over 300 reports of mammalian, plant, and microbial proteins obtained and renatured from inclusion bodies formed in *E. coli*." EX1020, 1; EX1002, ¶44. The "general strategy" for recovering proteins from inclusion bodies included "three steps: firstly, inclusion body isolation and washing; secondly, solubilization of the aggregated protein, which causes denaturation; and finally, refolding of the solubilized protein." EX1023, 1;

EX1002, ¶¶44, 176. *See also* EX1052, 2; EX1020. These routine steps are described below.

1. Isolating Inclusion Bodies

To isolate inclusion bodies, bacterial host cells (*e.g.*, *E. coli*) containing the inclusion bodies undergo disruption of their cell membrane through cell "lysis". EX1017, 187-188; *see also* EX1025, 1; EX1002, ¶45. Upon cell lysis, cell contents are released, and the resulting suspension is further processed (*e.g.*, by centrifugation) to separate the lighter soluble portion (containing the soluble proteins) from the heavier insoluble portion (containing the inclusion bodies and cellular debris). EX1017, 189-192; EX1022, 1; EX1002, ¶45.

2. Solubilizing Inclusion Bodies

After isolating inclusion bodies from the insoluble fraction, the inclusion bodies are washed to remove surface-absorbed materials and solubilized with chemicals that disrupt the interactions between protein molecules of the inclusion bodies (*e.g.*, decrease non-covalent interactions between protein molecules, and/or reduce undesirable inter- and/or intra-molecular disulfide bonds). EX1002, ¶¶46-47. This solubilization step is used to "denature" the protein into an unfolded state. *Id.*, ¶47.

Inclusion body proteins are often solubilized with denaturants, reductants (reducing agents), and/or surfactants[1] (*e.g.*, detergents). *See, e.g.*, EX1022, 2-3; EX1002, ¶¶48-49. Common denaturants include urea and guanidine chloride. *See, e.g.*, EX1017, 217; EX1023, 5. Typical reductants include dithiothreitol (DTT), dithioerythritol (DTE), and 2-mercaptoethanol. *See, e.g.*, EX1022, 3. Common detergents include sodium dodecyl sulfate (SDS) and n-cetyl trimethylammonium bromide (CTAB). *See, e.g.*, EX1022, 2; EX1002, ¶49.

3. Refolding the Solubilized Proteins

After solubilization of inclusion bodies, the denatured proteins are "refolded." EX1024, 2-3; EX1023, 2-5. This "refolding" or "renaturation" process causes a denatured (unfolded/unstructured) protein to fold into its unique and native 3D structure necessary for its bioactivity. EX1017, 44; EX1002, ¶50.

As of June 2009, it was well known that refolding proteins from solubilized inclusion bodies could be accomplished by diluting[2] the solubilization solution

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[1] "Surfactant" is short for of "**sur**face **act**ive **a**ge**nt**." EX1002, ¶49; EX1050, 16.

[2] While "dilution" refers to the process of reducing the concentration of a solute in solution, usually by adding more solvent, the term is also broadly understood in the art and refers to simply adding one solution to another (*i.e.*, mixing solutions). *See*

(Continued...)

containing the unfolded proteins with a refold buffer. EX1022, 3; EX1024, 6; EX1002, ¶51. At the time, known suitable refold buffers included, among others, denaturants, aggregation suppressors, protein stabilizers, and/or redox components. *See, e.g.*, EX1023, Table 1; EX1002, ¶52. Common aggregation suppressors included sugars and polyols such as glycerol and sucrose, amino acids such as arginine, detergents/surfactants such as Tween (polysorbate), and salts such as Tris, which also acts as a protein stabilizers. EX1023, Table 1; EX1072; EX1073; EX1002, ¶52. Sugars and polyols were also known to act as protein stabilizers, and known redox components included, *e.g.*, cysteine/cysteine or cysteamine/cystamine. EX1023, 2, Table 1; EX1024; EX1002, ¶¶52-53.

Additionally, a POSA as of June 2009 well understood that there were other refolding variables that could also be optimized such as pH, temperature, and timing of the process. EX1002, ¶54.

C. Applying a Refold Solution to a Separation Matrix

After refolding, the desired protein needs to be separated from any other components present in a refold solution, *e.g.*, unwanted protein, DNA, and the chemicals used for solubilization and refolding. *See, e.g.*, EX1004-1007; EX1002,

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EX1002, ¶51.

¶55. One of the most common and well-understood methods of purification to separate components of a mixture is chromatography. *Id*.

In a typical chromatographic method, a target protein is present in a "mobile phase," normally an aqueous buffered solution. The mobile phase containing the target protein is applied to a separation matrix or resin that is often packed in a column ("stationary phase"). The separation matrix preferentially binds or adsorbs the target protein based upon interactions between the resin and the target protein. Other undesirable proteins and impurities do not interact with the resin and flow through the column as additional buffered solution is added. The target protein can then be eluted from the resin by changing the conditions using an elution buffer. EX1017, 195; EX1002, ¶56. Examples of chromatographic methods are discussed in Sections III(C)(1)-(3) *infra*.

By June 2009, a POSA would have understood that certain solutions may need to be adjusted to provide conditions suitable for the target protein to associate with a separation matrix. Such adjustments may include adjusting a solution's pH or conductivity. EX1002, ¶57. These adjustments and considerations were a matter of routine optimization, as failing to consider them could adversely impact the association of the target protein with the separation matrix, resulting in lower yield and/or lower purity of target protein. *Id*.

It was known in the art in 2009 that certain detergents and denaturants may impact the interaction between the target protein and the particular separation matrix used. Thus, selecting types and conditions (*e.g.*, pH, concentrations) of detergents and/or denaturants for solubilizing and/or refolding proteins that are compatible with varying chromatographic methods would have been ordinary practice for POSAs at the time. EX1002, ¶58. For example, instead of using a high concentration of a denaturant to solubilize and denature inclusion body proteins, a POSA would have known that a combination of a denaturant at a low concentration (*e.g.*, 2M urea) and an alkaline pH (pH 11.0 to 12.5) could be used successfully to solubilize inclusion body proteins. EX1005, 10; EX1002, ¶58.

Further, numerous detergents and/or denaturants that are compatible with particular separation matrices were well known as of June 2009. For example, "[d]etergents are useful as solubilizing agents for proteins with low aqueous solubility such as membrane components. Anionic, cationic, zwitterionic and non-ionic (neutral) detergents can be used during IEX chromatography." EX1007, 50-51. Table 4, reproduced below, shows a representative list of detergents and denaturants that were well known in the art by 2009 as being commonly used with a non-affinity separation matrix, *e.g.*, ion exchange resin. *Id*., 51; EX1002, ¶59.

Table 4. Commonly used detergents and denaturing agents.

Detergent	Type	Typical conditions for use	Compatibility
Urea		2 M–8 M	anion or cation ion exchangers
Guanidine hydrochloride		3 M–6 M	anion or cation ion exchangers
Triton™ X-100	non-ionic	2%	anion or cation ion exchangers
N-octylglucoside	non-ionic	2%	anion or cation ion exchangers
Sodium dodecyl sulfate	ionic	0.1%–0.5%	exchange for non-ionic detergent during first chromatography step, avoid anion exchangers
Sarcosyl	non-ionic	1.5%	anion or cation ion exchangers
Nonidet P40	non-ionic		anion or cation ion exchangers
Polyoxyethylene ethers (e.g. Brij 35)	non-ionic		anion or cation ion exchangers
Polyoxyethylene sorbitans (e.g. Tween™ 80)	non-ionic		anion or cation ion exchangers
CHAPS	zwitterionic, derivative of cholic acid		anion or cation ion exchangers (pH dependent)
CHAPSO	zwitterionic, derivative of cholic acid		anion or cation ion exchangers (pH dependent)
Deoxycholate	cation		anion ion exchangers

Table 15, reproduced below, provides a representative list of detergents and denaturants that were well known in the art by 2009 and commonly used with an affinity-based separation matrix, *e.g.*, affinity chromatography. EX1015, 135; EX1002, ¶60.

Table 15.

Denaturing agent	Typical conditions for use	Removal/comment
Urea	2 M–8 M	Remove using Sephadex G-25.
Guanidine hydrochloride	3 M–6 M	Remove using Sephadex G-25 or during IEX.
Triton X-100	2%	Remove using Sephadex G-25 or during IEX.
Sarcosyl	1.5%	Remove using Sephadex G-25 or during IEX.
N-octyl glucoside	2%	Remove using Sephadex G-25 or during IEX.
Sodium dodecyl sulphate	0.1%–0.5%	Exchange for non-ionic detergent during first chromatographic step, avoid anion exchange chromatography.
Alkaline pH	> pH 9, NaOH	May need to adjust pH during chromatography to maintain solubility.

Details taken from:
Scopes R.K., Protein Purification, Principles and Practice, Springer, (1994), J.C. Janson and L. Rydén, Protein Purification, Principles, High Resolution Methods and Applications, 2nd ed. Wiley Inc, (1998) and other sources.

Methods for developing or optimizing a chromatographic separation using solutions containing detergents were also known. *See* EX1007, 51-52. For example, blank gradients with additives included in a refold solution could be run in order to determine their effect on the chromatographic profile. *Id.*, 50-51; EX1002, ¶61. Additionally, empirical testing to determine the effects of various detergents and/or denaturants on separation had been established well before June 2009, thus allowing a POSA to determine whether and how the components used in solubilization and/or refolding would actually affect separation. EX1030; EX1002, ¶61.

By June 2009, many types of chromatography had been used to separate different types of proteins, each based upon a particular way of binding the protein with the separation matrix. *See, e.g.*, EX1004-1007. Accordingly, POSAs were well versed in how various components of different solutions could impact the ability of a protein to associate with a given separation matrix. EX1002, ¶¶62-63.

1. Ion exchange chromatography (IEX)

Ion exchange chromatography ("IEX"), a form of non-affinity chromatography, has been used to separate biomolecules since the 1960s. EX1007, 9. IEX refers to any chromatographic process that separates molecules (*e.g.*, proteins) on the basis of net surface charge. *Id.*, 9, 13; EX1017, 197-200;

EX1002, ¶¶64-76. The net surface charge of a protein will vary with pH; at a pH at which a protein carries no net charge (*i.e.*, its isoelectric point (pI)), the protein will not interact with a charged separation matrix. However, at a pH above its pI, a protein will bind to a positively-charged separation matrix (anion exchanger); at a pH below its pI, a protein will bind to a negatively-charged separation matrix (cation exchanger). *Id.*, ¶65; EX1007, 13. Interactions between charged proteins and an oppositely-charged IEX matrix can be modulated (*e.g.*, by adjusting the solution's pH) to favor either binding of proteins to the separation matrix or eluting of bound proteins from the separation matrix, thereby achieving separation of proteins from other contaminants or impurities. EX1002, ¶65.

In addition to performing IEX at an appropriate pH, solutions are typically applied to separation matrices at low ionic strength (normally I <0.05 M) to maximize the interactions between the protein and the matrix. EX1017, 198. The separation matrix can then be washed with solution(s) of constant pH and low ionic strength to remove unbound molecules (*e.g.*, impurities). *Id.* Afterwards, the target protein may be eluted by increasing the ionic strength of the buffer or by changing the pH. *Id.*; *see* EX1007, 45; EX1002, ¶¶66-67.

Accordingly, POSAs as of June 2009 understood that the particular pH and ionic strength of a refold solution during IEX were important to achieve effective

protein separation. EX1002, ¶68. The optimum pH for purification was known to be a range where the target protein remains stable and retains its biological activity during the performance of IEX. *Id.* It was also known that the working pH may influence the charge characteristics of certain components of a refold solution, *e.g.*, detergent(s), denaturant(s), and/or salts. Thus, purification procedures routinely required that "[a]ny additives used for dissociation, solubilization, metal chelation, enzyme inhibition, etc., should always be checked for their charge characteristics at the working pH" to make sure that undesired molecules, *e.g.*, detergent(s) and/or denaturant(s), will not interact with the separation matrix at the working pH. EX1007, 50; EX1002, ¶69.

2. Affinity Chromatography

Affinity chromatography refers to any chromatographic process that separates proteins on the basis of a reversible non-covalent interaction between a protein and a specific ligand immobilized to a separation matrix. EX1031, 9; EX1017, 201-202. Proteins showing a high affinity for the ligand will bind to the matrix, while compounds or molecules that do not have affinity for the ligand will flow straight through the column. EX1017201, 203; EX1032, 19; EX1002, ¶¶77-78. The bound protein then can be eluted specifically by using a competitive ligand, or non-specifically by changing the pH, ionic strength, or polarity.

EX1031, 17, 20-23; EX1002, ¶77. As discussed above, detergents and denaturants that were commonly used with an affinity-based separation matrix, *e.g.*, affinity chromatography, were well known as of June 2009. EX1031, 135; EX1002, ¶79.

3. Expanded Bed Adsorption Chromatography (EBA)

Expanded bed adsorption (EBA) chromatography refers to a chromatographic technique that utilizes a fluidized "bed" containing a separation matrix to separate desired proteins from a crude mixture "without the need for prior clarification." EX1019, 99; EX1002, ¶81. EBA typically involves a unit operation that uses a variety of adsorbents to recover a target protein from a crude mixture. Notably, both IEX and affinity chromatography resins can be used in EBA. EX1002, ¶80.

As shown in FIG. 2 below, unlike a traditional packed column, a sedimented bed of adsorbent particles (a) are suspended in an equilibration buffer, creating a stable fluidized bed (b). EX1002, ¶82. A feed (*e.g.*, a refold solution comprising a protein of interest and other contaminants) is passed upward through the expanded bed (c). The expansion of the adsorbent bed creates a distance between the adsorbent particles, *i.e.*, increased void volume fraction in the bed, which allows for unrestricted passage of particulates and contaminants through the expanded bed while the target protein is bound to the adsorbent particles. Unbound material is

washed out with the upward flow of the buffer (c). The bound proteins are then eluted from the adsorbent in a sedimented bed mode (d). EX1019, 100-101; EX1025, 4; EX1033, 20; EX1002, ¶82.

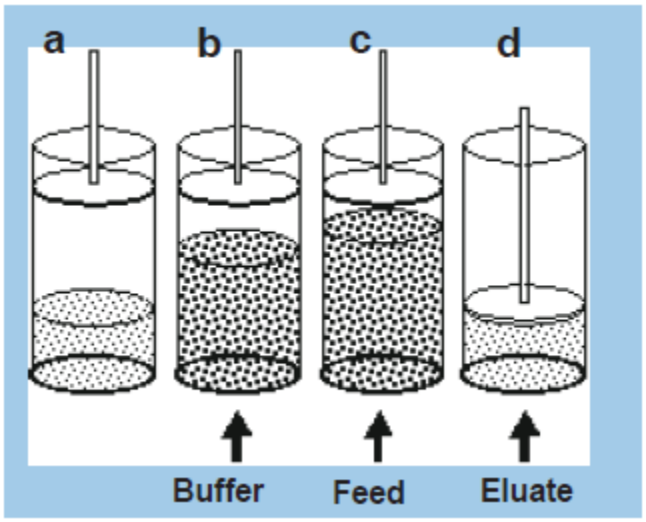

FIG. 2: The principle of operation of EBA (EX1019, Fig. 57)

As of June 2009, EBA technology had been successfully used to recover inclusion body protein from bacterial cells such as *E. coli*. *See* EX1004, 2; EX1002, ¶83.

D. Washing and Eluting the Protein

Washing a separation matrix to remove unbound materials such as impurities and eluting the bound target protein from the separation matrix for collection were well-known, logical steps in chromatography processes as of June 2009. *See* EX1007, 13-14; EX1031, 11-12; EX1002, ¶84. Wash buffers were generally used to wash the separation matrix such that unbound materials (*e.g.*,

impurities) were removed from the matrix while the target protein remained bound. Following the wash, an elution buffer was generally applied to the separation matrix to reverse the interaction between the target protein and the functional groups of the matrix, such that the bound target protein could be eluted from the separation matrix and collected. EX1007, 13-14; EX1019, 11; EX1031, 11-12, 17. For example, for an affinity separation matrix, elution could be performed specifically (using a competitive ligand) or non-specifically (by changing the pH, ionic strength, or polarity). EX1031, 20-21. For a non-affinity separation matrix, such as IEX, bound proteins could be eluted from the matrix by changes in ionic strength or pH. EX1007, 42; EX1002, ¶85.

E. Regenerating a Separation Matrix

It was also well known in the art that separation matrices that have become contaminated with detergents or other contaminants could be "regenerated" – that is, cleaned, or otherwise decontaminated to restore the original properties of a separation matrix for future use. EX1007, 67; EX1056; EX1002, ¶86.

Methods for regenerating a separation matrix were well known for various types of matrices. For example, it was known that one could clean an IEX column to remove "precipitated proteins, lipids, hydrophobically bound proteins or lipoproteins." EX1007, 67, Appendix 10. The GE Handbook specifically

discloses standard ways of washing columns with NaCl and NaOH to remove common contaminants. EX1007, 50, 66-67. It was also known that a column could be used without regeneration after each and every run. EX1002, ¶87.

IV. PRIOR ART RELIED UPON

Each of the art relied upon in this Petition is a patent or printed publication published before June 25, 2009, is not listed on the face of the '878 patent, and was not considered during prosecution. *See* 35 U.S.C. §§ 102, 311, 325(d).

A. Ferré

Ferré et al., "A novel system for continuous protein refolding and on-line capture by expanded bed absorption," Protein Science, 14:2141-2153 ("Ferré") was published in 2005.

Ferré discloses a method for protein purification where "continuous renaturation-by-dilution is followed by direct capture on an expanded bed absorption (EBA) column." EX1004, Abstract, Figure 3. The performance of the method is tested using N-terminally-tagged human β2-microglobulin (HAT-hβ2m). *Id.*, Abstract. Ferré further states that the described "continuous protein refolding and direct EBA capture" method has been used to produce and purify other proteins, including interleukin-2 and granulocyte colony-stimulating factor (G-CSF). *Id.*, 10.

Ferré discloses that HAT-hβ2m is expressed in *E-coli*, and the resulting inclusion bodies are released by lysis and solubilized with urea and Tris-HCl. *Id.*, 2, 100. Ferré discloses that the solubilization solution containing the denatured protein is diluted with a refold buffer containing Tris-HCl in a flowthrough mixing chamber and pipe reactor for a defined refolding time. *Id.*, 2, 3, 11. The mixed refold solution is "then fed directly to an EBA column, where the protein was captured, washed, and finally eluted as soluble folded protein." *Id.*, Abstract; EX1002, ¶¶88-90.

B. Komath

WO Publication No. 2004/001056 ("Komath") is entitled "Process for preparing G-CSF" and was published on December 31, 2003.

Komath is directed to "a simple and cost effective process for purifying large quantities of recombinant human G-CSF from *E. coli* and other cells in which inclusion bodies of G-CSF are formed." EX1005, 5; EX1002, ¶91. Komath first discloses culturing *E. coli* cells expressing G-CSF and then lysing the cells. EX1005, 5, 6, 8-9; EX1002, ¶92.

Komath then discloses solubilizing the inclusion bodies using "from 2M to 6M" of urea at a high pH. *Id.*, 6, 10, 12. In particular, Example 3 discloses that the inclusion body pellet is solubilized using 2M urea in water, in which the pH

has been shifted briefly to between 11 and 12.5 using 1N NaOH and then shifted back to 8.0 using acetic acid. EX1005, 12; EX1002, ¶¶93-94.

Following solubilization, Komath discloses refolding the G-CSF protein for a total of 12-16 hours. EX1005, 12. *See also id.*, 6. Komath discloses that surface active agents may be used during refolding, including polysorbate 20, which Komath discloses is a "non-ionic detergent." *Id.*, 6, 10-12. EX1002, ¶95.

Komath discloses subsequent purification of refolded G-CSF using cation or anion exchange chromatography involving the standard steps of loading, washing, and eluting. EX1005, 6-7, 12. Komath uses a column packed with SP-Sepharose matrix and discloses that the "refolded protein solution is loaded on this column." *Id.*, 10. Komath further discloses conditions that allow the refolded G-CSF to bind to the column, in a pH range between 4.0-5.0, preferably 4.5. *Id.*, 12. The column is then washed and G-CSF is successfully eluted. *Id.*; EX1002, ¶96.

C. Rosendahl

U.S. Application Publication No. 2004/0018586 ("Rosendahl") is entitled "Method for Refolding Proteins Containing Free Cysteine Residues" and was published on January 29, 2004.

Rosendahl discloses methods for refolding proteins that are expressed in an insoluble or aggregated form by *E. coli.* EX1006, ¶¶[0014], [0015], [0021];

EX1002, ¶97. Rosendahl discloses a solubilization step using a solubilization buffer that includes "a disulfide reducing agent" (*i.e.*, a reductant) such as cysteine and reduced glutathione. *Id.*, ¶[0038]; EX1002, ¶98.

Rosendahl also discloses a refolding step following solubilization using a refold buffer that includes "an oxidizing agent," (*i.e.*, a redox component) such as cysteine, oxidized glutathione, and cystamine, or a "a redox mixture of an oxidizing agent and a reducing agent," such as "cysteine/cystine, cysteine/cystamine, cysteamine/cystamine, reduced glutathione/oxidized glutathione, and the like." *Id*., ¶[0039]; EX1002, ¶¶99-100.

D. GE Handbook

Ion Exchange Chromatography & Chromatofocusing, Principles and Methods, was published by Amersham Biosciences as part of GE Healthcare in 2004. EX1007 (the "GE Handbook").

The GE Handbook provides a general overview of the principles of IEX and provides specific examples of media and other components and solutions that are typically used in performing IEX. EX1002, ¶¶101-102. The GE Handbook specifically discloses common detergents, denaturants, and other additives that were well known to a POSA as of 2004, as well as common methods for washing,

eluting, and cleaning and regenerating separation matrices. *See, e.g.*, EX1007, 48-50, 103; EX1002, ¶102.

V. THE '878 PATENT AND A PERSON OF SKILL IN THE ART

A. The '878 Patent

The '878 patent is entitled "Capture Purification Processes for Proteins Expressed in a Non-Mammalian System." EX1001. The only independent claim challenged, claim 7, recites a "method of purifying a protein" that involves only basic, well-known steps for purifying a low solubility protein: (1) expressing a protein in a non-native limited solubility form in a non-mammalian cell, (2) lysing the cell, (3) solubilizing the protein, (4) forming a refold solution comprising the solubilization solution and the refold buffer, and (5) purifying the protein using chromatography by directly applying the refold solution to a separation matrix, washing the matrix, and eluting the protein from the matrix. *Id.* All of the remaining challenged claims (8, 11-13, 15-19, and 21) depend either directly or indirectly from claim 7.

B. Prosecution History

The '878 patent issued on January 27, 2015 from U.S. Patent Application No. 12/822,990, filed June 24, 2010 (the "'990 application"). The '878 patent claims priority to provisional application No. 61/220,477, filed June 25, 2009. EX1001.

Original claim 9 of the '990 application, which issued as claim 7 of the '878 patent, was directed to a "method of purifying a protein expressed in a non-native limited solubility form in a non mammalian expression system comprising" the same steps recited in claim 7 of the '878 patent, ***except*** that original claim 9 required only "***(e) applying*** the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix" and not "***directly applying***." EX1035, 2 (emphasis added).

During prosecution, the Examiner rejected original claim 9 and related claims as anticipated by and obvious over U.S. Patent No. 7,138,370 ("Oliner") (EX1034). EX1036, 5-6. In response to the Examiner's rejection, Patent Owner stated:

> The claimed invention at step (e) indicates that the refold solution is applied to a separation matrix. ***In contrast,*** the patent of Oliner et al. teaches at column 76, lines 51-59 that ***the refolded protein is subject to dialysis, precipitation, and centrifugation***. The supernatant is then pH adjusted and loaded onto a column. Thus, Oliner et al. teaches a method ***that differs markedly from the direct application of refold solution to the separation matrix***.

EX1037, 7-8 (emphasis added). The Examiner again rejected Patent Owner's arguments regarding Oliner, stating:

> ***The claims are not limited to a method requiring direct application*** of refold solution to the separation matrix. The claim clearly states that the method *comprises* the listed steps. Therefore, additional steps may be added. ***There is nothing in the claim which precludes additional purification steps***. ***In fact, as can be seen on pages 28-31 of the specification, in all of the examples in the specification of the claimed method, the refolded protein was filtered through "a series of depth and/or membrane filter to remove particulates" before applying*** the "conditioned and filtered protein mixture" to the column.

EX1038, 8 (emphasis added). *See, e.g.*, EX1001, 19:34-41, 20:56-64.

In response to the Examiner's rejection, Patent Owner amended the language of the claim to add "directly" before "applying." EX1039, 7. Patent Owner explained:

> ***Claim 9 as amended recites at step (e) "directly applying the refold solution to a separation matrix." (emphasis added).*** As stated on page 23 of the specification, "[i]t is noted that when performing the method, the refold solution comprising the refolded protein of interested [sic] is applied directly to the separation matrix, ***without the need for diluting or removing the components of the solution required for refolding the protein.*** This is an advantage of the disclosed method." Lines 3-6. ***Oliner et al., in contrast, discloses that the solution comprising the refolded protein is subject to dialysis, precipitation, and centrifugation before being pH adjusted***

> ***and loaded on the column. Therefore, Oliner et al. fails to teach each element of the claimed invention.***

Id. (emphasis added). *See also id.*, 8. The Examiner subsequently allowed the claims. EX1040, at 3-4; EX1041.

C. Person of Ordinary Skill in the Art

A POSA to which the '878 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, ¶¶19, 106. This person may also work in collaboration with other scientists and/or clinicians who have experience in protein purification, protein refolding, or related disciplines. *Id*.

VI. CLAIM CONSTRUCTION

In IPR, the terms of challenged claims are to "be construed using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b), including construing the claim in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." 37 C.F.R. § 42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005) (*en*

banc). For the purpose of this proceeding[3], any claim terms not included in the following discussion should be given their ordinary and customary meaning as understood by a POSA at the time of the invention. This section addresses the meaning of certain terms appearing the challenged claims.

A. "non-native limited solubility form"

The '878 patent defines "non-native limited solubility form" as

> 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 in an appropriate in vivo or in vitro assay designed to assess the protein's biological activity and/or (b) forms aggregates that require treatment, such as chemical treatment, to become soluble. The term specifically includes proteins existing in inclusion bodies, such as those sometimes found when a recombinant protein is expressed in a non-mammalian expression system.

EX1001, 7:44-54. EX1002, ¶108.

[3] Petitioner's constructions are offered solely for the purpose of this proceeding and are not admissions as to the scope or definiteness of any claim term in any other proceeding.

B. "aggregation suppressor"

The '878 patent defines "aggregation suppressor" as "any compound having the ability to disrupt and decrease or eliminate interactions between two or more proteins." *Id.*, 5:33-35. The patent explains that the "aggregation suppressor can be included as a means of preventing non-specific association of one protein with another, or with one region of a protein with another region of the same protein." *Id.*, 14:12-15.

The patent provides that "[e]xamples of aggregation suppressors can include, but are not limited to, amino acids…; polyols and sugars…; surfactants such as, polysorbate-20… and combinations thereof." *Id.*, 5:36-40. *See also id.*, 2:43-47 ("the aggregation suppressor can be selected from the group consisting of … non-ionic surfactants, … tris…"); 14:27-31 ("In various embodiments, the aggregation suppressor can be selected from the group consisting of … non-ionic surfactants, … Tris…"). Neither the claims nor the specification requires that the aggregation suppressor have a particular concentration. EX1002, ¶¶109-110.

C. "protein stabilizer"

The '878 patent defines "protein stabilizer" as "any compound having the ability to change a protein's reaction equilibrium state, such that the native state of the protein is improved or favored." EX1001, 5:41-44. The patent explains that

the "protein stabilizer can be included as a means of promoting stable native protein structure and may also suppress aggregation." *Id.*, 14:15-17. The patent also states, that "[e]xamples of protein stabilizers can include, but are not limited to, ...osmolytes and certain Hoffmeister salts such as Tris…; and combinations thereof." *Id.*, 5:44-50. *See also id.*, 2:47-51; 14:21-26 ("In various embodiments, the protein stabilizer in the refold buffer can be selected from the group consisting of … non-ionic surfactants, … Tris…"). Neither the claims nor the specification requires that the protein stabilizer have a particular concentration. EX1002, ¶¶111-112.

D. "directly applying the refold solution to a separation matrix"

This term should be construed as "applying the refold solution to a separation matrix without removing any components of or diluting the refold solution," as supported by the specification and as understood by a POSA as of June 2009. EX1002, ¶¶113-119.

1. The Specification

The specification states that the prior art taught that "after a protein has been refolded ***it was necessary to dilute or remove the components of the refold mixture*** in a wash step." EX1001, 1:44-46 (emphasis added). The specification explains that this was because "it was expected that the highly ionic and/or chaotropic compounds ***and various other components of the refold solution***

would inhibit the association of the protein with the separation matrix." *Id.*, 15:30-33 (emphasis added). Since this "dilution step can consume time and resources which, when working at a manufacturing scale of thousands of liters of culture, can be costly" (*id.*, 1:50-52), the specification repeatedly touts the alleged advantage of the claimed methods as the absence of any intermediate steps to remove any components from the refold solution or to meaningfully dilute the solution prior to applying it to the separation matrix. *See, e.g.*, EX1001, 3:46-49 ("the advantages of the present invention over typical processes ***include the elimination of the need to dilute the protein out of a refold solution prior to capturing it on a separation matrix***") (emphasis added).

Indeed, the specification makes clear that, as a result of "directly applying the refold solution to a separation matrix," it is the separation matrix (and not any other intermediate process) that allows for "the protein of interest [to] . . . be separated from the components of the resuspension and/or lysis buffer, including impurities such as host cell proteins, DNA and chemical impurities introduced by the components of the solubilization and/or lysis buffer." EX1001, 15:1-5. *See also id.*, 15:43-46 ("the separation matrix is washed to ***remove unbound protein, lysate, impurities and unwanted components of the refold solution***") (emphasis added).

The claimed invention thus involves "the ***direct*** capture of a protein expressed in a non-native limited solubility form." *Id.*, 3:45-46 (emphasis added). In particular, the specification states:

> In this approach, following a refold step ***the entire refold mixture, including the protein of interest, is applied directly to a separation matrix***, such as a Protein A or G resin. The protein of interest associates with the matrix in the presence of the components of refold buffer, ***impurities are washed away*** and the protein is eluted. Since ***the method omits the need for removing any components of the refold mixture before the refold mixture is applied to a separation matrix***, the method can have the effect of saving steps, time and resources that are typically expended on removing the protein from refolding and dilution buffers in purification processes.

Id., 4:49-4:60 (emphases added).

This construction is also consistent with the claim language of "***directly*** applying ***the*** refold solution." Claim 7 recites first "forming **a** refold solution" and then applying "***the*** refold solution" to a separation matrix. The Federal Circuit has explained "[s]ubsequent use of the definite articles 'the' or 'said' in a claim refers back to the same term recited earlier in the claim." *Wi-Lan, Inc. v. Apple, Inc.*, 811 F.3d 455, 462 (Fed. Cir. 2016). Thus, "directly applying the refold solution" must not include any intermediate step that removes any components of the solution or dilutes the solution before applying the refold solution to the separation matrix.

2. The Prosecution History

The prosecution history likewise supports a construction of "directly applying" that does not involve any intermediate processing steps. The Examiner originally rejected the claims over Oliner by finding that "[t]here is nothing in the claim which precludes additional purification steps" and cited, as support, the fact that "in all of the examples in the specification of the claimed method, the refolded protein ***was filtered through 'a series of depth and/or membrane filter to remove particulates'*** before applying the 'conditioned and filtered protein mixture' to the column." EX1038, 8 (emphasis added). *See, e.g.*, EX1001, 19:9-15, 20:30-33. In order to overcome this rejection, Patent Owner amended the claim to add the word "directly," asserting:

> Oliner et al. does not teach applying the solution comprising the refolded protein ***without the need for diluting or removing components of the solution*** required for refolding the protein. ***Rather,*** Oliner et al. discloses that the solution comprising the refolded protein is subject to dialysis, precipitation, and centrifugation before being pH adjusted and loaded on the column.

EX1039, 8 (emphasis added). Patent Owner thus agreed that "directly applying" does not include intermediate processing steps such as, but not limited to, dilution, filtration, centrifugation, dialysis, or precipitation. EX1002, ¶115.

3. Other Proceedings

Patent Owner itself previously asserted, and a court adopted, that "directly applying" means "applying the refold solution to a separation matrix[4] without removing components of or diluting the refold solution" in *Amgen Inc. et al. v. Sandoz Inc. et al*., No. 14-cv-04741-RS (N.D. Cal.) (the "Sandoz Litigation"). EX1042, 33; EX1043, 24. Citing the specification (EX1001, 15:25-29, 1:44-57, 3:45-49, 4:49-60, 12:14-25), Patent Owner emphasized that "direct application means that processing steps are not performed prior to the application of the solution to the separation matrix, such as dilution or removal of components from the solution." EX1043, 24-25.

Patent Owner agreed that "directly applying" does not involve removing *any* components of the solution. *Id.*, 20 ("Nothing in the specification identifies

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[4] Patent Owner previously attempted to limit this term to column chromatography, but a district court disagreed. *See* EX1042, 23 ("the word "column" does not appear in the claim, and thus there is no reasonable argument for the proposition "column" is a synonym for any word appearing therein"); EX1001, 16:42-54. Patent Owner has since conceded that the limiting language was not appropriate for the same term in the child '997 patent. *See* EX1067, 20; EX1048, 23 n.10.

components that can be removed within the scope of 'direct application.' This is not an accident."). Patent Owner explained:

> [T]he claim makes clear that ***there are other components in the refold solution*** beyond a denaturant, a reductant, a surfactant, an aggregation suppressor, and a protein stabilizer. …Furthermore the specification makes clear that the inventors had discovered that ***it was not necessary to remove <u>any</u> component of the refold solution*** prior to directly applying it to a separation matrix, ***and that "any component" would include host protein and DNA.***

EX1044, 15 (bold and italics emphasis added, underline in original); *see also id.* (the specification "provides that the refold solution encompasses other components such as 'impurities such as host cell proteins, DNA and chemical impurities introduced by components of the solubilization and/or lysis buffer'"); EX1045, 5-7; *id.*, 8-9 ("[T]he refold solution…also does not undergo steps such as Oliner et al.'s ***precipitation and filtration that could remove, e.g., host cell DNA from the refold solution***.") (emphasis added); EX1046, 162 ("The components are not limited to a certain subset. The material that we're talking about being directly applied can include lots of different things. ***It does not just include the materials that were added for purposes of refolding.***") (emphasis added); EX1002, ¶119.

Likewise, Patent Owner has previously acknowledged that the "prior-art dilutions contemplated by the inventors were ***significant*** dilutions, ***i.e., multifold***

or nearly multifold dilutions." EX1047, 20 (emphasis added); *see* EX1001, 12:22-26. As such, "a person skilled in the art would understand that the patent specification ***is not referring to diluting a refold solution by adding a minor amount of liquid.***" EX1047, 20 (emphasis added); EX1002, ¶114. Rather, the specification makes clear that the claimed methods do not include any steps to ***meaningfully*** or ***significantly*** dilute the refold solution prior to applying it to the separation matrix. *See, e.g.*, EX1001, 3:46-49; EX1002, ¶114.

The court in the Sandoz Litigation adopted Patent Owner's construction of "directly applying." EX1042, 21. The court noted that Patent Owner "contends the word 'directly' means ***there are no intermediary steps of any kind*** between refolding and purification." *Id.* (emphasis added). Citing the prosecution history and the specification, the court found:

> The six components listed in the claim ***are not necessarily the only components of the refold solution.*** Moreover, the patentee's attempt to distinguish the claimed method from the prior art, and the '370 Patent [Oliner], in particular, clarify that ***the patentee believed there should not be any intermediary steps between the refolding process and application of such solution to the separation matrix***.

Id., 23 (emphasis added). As such, the proper construction of "directly applying the refold solution to a separation matrix," as supported by the specification, prosecution history, and understanding of a POSA, is "applying the refold solution

to a separation matrix without removing any components of or diluting the refold solution." EX1002, ¶¶113-119.

E. "refold buffer"

The claims define a "refold buffer" as a solution "comprising one or more of the following: (i) a denaturant; (ii) an aggregation suppressor; (iii) a protein stabilizer, and (iv) a redox component." EX1001, 22:15-20. A POSA would understand that the "refold buffer" of the claim does not need to be a pH-buffered solution, but rather that the term "buffer" is "commonly used in the art to refer to liquid preparations in biochemistry generally, regardless of whether such a preparation resists pH changes." EX1049, ¶44; EX1048, 17-20; EX1002, ¶120.

VII. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED

Petitioner requests cancellation of claims 7-8, 11-13, 15-19, and 21 of the '878 patent on the following specific grounds:

Ground	Reference(s)	Basis	Challenged Claims
1	Ferré	§ 102	7-8, 11-12, 15-16, 18-19, 21
2	Komath	§ 102	7-8, 11-12, 15-16
3	Komath	§ 103	7-8, 11-12, 15-16
4	Ferré or Komath in view of Rosendahl	§ 103	13, 17
5	Ferré or Komath in view of GE Handbook	§ 103	18-19, 21

A. Ground 1: Ferré Anticipates Claims 7-8, 11-12, 15-16, 18-19, and 21

1. Ferré discloses every limitation of claim 7

a. "A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system comprising: (a) expressing a protein in a non-native limited solubility form in a non-mammalian cell"[5]

Ferré discloses a method of purifying a protein, tagged human β_2-microglobulin (HAT-hβ_2m), expressed in a non-native limited solubility form in a non-mammalian expression system, *E. coli*. EX1004, Abstract, 2. Ferré discloses a method where "HAT-hβ_2m was produced as insoluble inclusion bodies by *Escherichia coli* fermentations." *Id.*, 10. Ferré also discloses that the disclosed "continuous protein refolding and direct EBA capture" method was used to produce and purify other proteins such as interleukin-2 and granulocyte-colony stimulating factor (G-CSF). *Id.*, 10; EX1002, ¶122.

b. "(b) lysing a non-mammalian cell"

Ferré discloses lysing *E. coli* cells and releasing inclusion bodies through enzymatic (chemical) or mechanical disruption of the cells. EX1004, 2.

[5] To the extent that the preamble is limiting, each of the art relied upon in this Petition discloses the preamble.

Specifically, Ferré discloses that the "inclusion bodies were released with lysozyme," an enzymatic method for cell disruption. *Id.*, 10; *see also id.*, 2; EX1002, ¶123.

c. "(c) solubilizing the expressed protein in a solubilization solution comprising one or more of the following: (i) a denaturant; (ii) a reductant; and (iii) a surfactant"

Ferré expressly discloses that the released inclusion bodies were washed and solubilized "in 8 M urea under non-reducing conditions, yielding denatured and oxidized HAT-hβ_2m." EX1004, 2. Ferré further states that the inclusion bodies were "solubilized in 8 M urea, 20 mM Tris-HCl (pH 8.0)." *Id.*, 10. The '878 patent acknowledges that urea is a denaturant. EX1001, 2:38-39, 4:35-37, 5:29-30, 13:49-51, 22:38-41; EX1002, ¶124 Thus, Ferré discloses solubilizing the expressed protein in a solubilization solution comprising at least "(i) a denaturant," as required by this limitation.

d. "(d) forming a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of the following: (i) a denaturant; (ii) an aggregation suppressor; (iii) a protein stabilizer; and (iv) a redox component"

Ferré discloses a process of forming a refold solution by diluting the solubilization solution containing the denatured protein with an aqueous, "refolding" buffer in a small flow-through mixing chamber and a folding pipe

reactor to allow refolding. EX1004, Abstract, 3-4, 9 ("Continuous refolding-by dilution is achieved by pumping the denatured protein suspension and the aqueous buffer through a very small flowthrough mixing chamber"), Figure 3. Ferré's approach "uncouples the events of protein refolding and capture, thereby allowing each event to be optimized individually." *Id.*, 2. *See also id.*, 3, 9. Because "the folding reaction [is] initiated instantaneously" and "the system ensures that all denatured protein molecules that enter the pipe reactor experience the same refolding environment and time before capture on the EBA support," Ferré discloses the step of "forming a refold solution." *Id.*, 9; EX1002, ¶¶125-126.

The refold buffer in Ferré contains 20 mM Tris-HCl (*Id.*, 10), which the '878 patent acknowledges is both a protein stabilizer and an aggregation suppressor. EX1001, 2:43-50, 5:44-49, 14:21-25, 14:27-30, 22:47-51, 22:23-56. As Dr. Robinson explains, Tris has the ability to maintain a stable pH despite influences that might otherwise shift the pH, thereby promoting stable native protein structure and suppressing further association between proteins. *Id.*; EX1072, 6-7, 11; EX1073, 5, 7; EX1002, ¶¶127-128. Thus, Ferré expressly discloses forming a refold solution comprising the solubilization solution and a refold buffer comprising an aggregation suppressor or a protein stabilizer, as recited in this limitation.

e. "(e) directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix"

Ferré discloses directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Ferré discloses that

> the proteins pass through a folding pipe reactor with sufficient retention time to allow folding. Finally, ***the nascently folded protein is directly captured*** by expanded bed adsorption (EBA)—a special type of fluidized bed chromatography.

EX1004, 2 (emphasis added). *See id.*, 9; EX1002, ¶129. Because Ferré expressly discloses that, following refolding, the folded protein is "***directly captured***" by the separation matrix, the method of Ferré does not remove any components of the refold solution or significantly dilute the refold solution before applying the refold solution to a separation matrix; indeed, the method does not include any intermediate processing steps. Ferré thus discloses directly applying the refold solution to a separation matrix. *See, e.g.*, EX1004, 2; EX1002, ¶129. *See supra* pp. 28-35.

Figure 3 of Ferré (reproduced below) provides a "[s]chematic representation of the system for continuous protein refolding and on-line EBA capture." The denatured protein suspension (DP) contains the solubilization solution and the

denatured protein. EX1004, 3, 10. The aqueous buffer (AB) is the refold buffer. *Id.*, Abstract, 10. The denatured protein suspension (DP) and the refold buffer (AB) are pumped into a mixer (MX) to form a refold solution, and the refold solution then flows into a folding pipe reactor (FPR) to allow the protein to refold. *Id.*, 3. The refold solution containing the refolded protein then directly enters the separation column through valve 1 (V1). *Id.*; EX1002, ¶130. Ferré discloses that as a result of this method, "the nascently folded product is directly recovered on the EBA column after the refolding event." EX1004, 9.

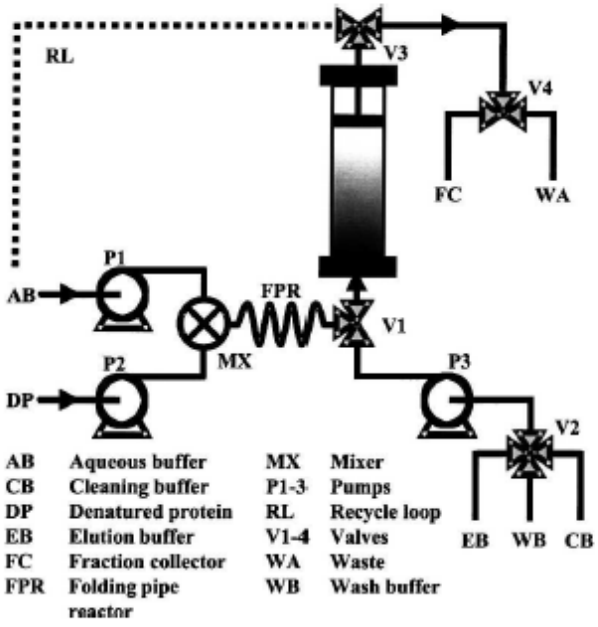

Figure 3. Schematic representation of the system for continuous protein refolding and on-line EBA capture. All EBA operations—i.e., equilibration, loading, washing, elution, and cleaning—were performed in expanded mode in Fastline10 and 50 columns. The current system allows the refolding buffer to be recycled through the system. Closed arrows indicate the direction of liquid flow during the folding/capture step, and open arrows indicate manual valves.

Id., 4 (Figure 3).

The Fastline10 and 50 columns used in Ferré (Figure 3) are EBA columns. *Id.* These columns use a "STREAMLINE DEAE medium" for protein adsorption. *Id.*, 4, 11. DEAE refers to diethylaminoethanol, which is an ion exchange resin, specifically, an anion exchange resin. EX1019, 53; EX1002, ¶131. The '878 patent expressly contemplates these columns and resins as examples of a "separation matrix." *See, e.g.*, EX1001, 7:10-15, 11:19-23, 11:7-18.

Ferré also discloses that the refold solution is directly applied to a separation matrix under conditions suitable for the protein to associate with the matrix. In particular, Ferré discloses that biologically active protein was successfully captured by the EBA column. EX1004, Abstract, 4, 10, 11, Table 1; EX1002, ¶132.

f. "(f) washing the separation matrix; and (g) eluting the protein from the separation matrix"

Ferré discloses that once the refold solution is fed into the EBA column, the protein in the refold solution is "captured, washed, and finally eluted as soluble folded protein." EX1004, Abstract. *See id.*, 4, 5, 11, Figure 3; EX1002, ¶¶133-134.

g. "wherein the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and hydrophobic interaction resin."

As discussed above, Ferré use a "STREAMLINE DEAE medium" for protein adsorption, which is an ion (specifically, anion) exchange resin. EX1004, 4, 11; EX1019, 53; EX1002, ¶135.

For these reasons, claim 7 is unpatentable as anticipated by Ferré. EX1002, ¶¶121-136.

2. Ferré discloses every limitation of claims 8, 11-12, 15-16, 18-19, and 21

Claim 8 requires the method of claim 7, "wherein the non-native limited solubility form is a component of an inclusion body" and claim 11 requires the method of claim 7, "wherein the non-mammalian expression system is bacteria or yeast cells." As discussed above, Ferré discloses a method of purifying a protein that is expressed in *E. coli* cells as insoluble inclusion bodies. EX1004, 2 ("HAT-hβ2m was produced as insoluble inclusion bodies by *Escherichia coli* fermentations"); EX1002, ¶137.

Claim 12 requires the method of claim 7, "wherein the denaturant of the solubilization solution or the refold buffer comprises one or more of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea." Ferré expressly

discloses that the released inclusion bodies were washed and solubilized "in 8 M ***urea***." EX1004, 2 (emphasis added). *See also id.*, 10; EX1002, ¶138.

Claims 15 and 16 require the method of claim 7, wherein the aggregation suppressor (claim 15) and the protein stabilizer (claim 16) that comprise the refold buffer is selected from a group consisting of, or comprises, Tris. As discussed above, the refold buffer in Ferré contains 20 mM Tris-HCl. EX1004, 10. The '878 patent lists Tris as a protein stabilizer and an aggregation suppressor. EX1001, 2:43-50, 5:44-49, 14:21-25, 14:27-30, 22:47-51, 22:23-56; EX1002, ¶139. It was also well known in the art that Tris is used as a protein stabilizer and an aggregation suppressor. *See* EX1023, 5; EX1024; EX1002, ¶139.

Claim 18 requires the method of claims 1 or 7, "further comprising the step of washing the separation matrix with a regeneration agent," while claim 19 requires that the regeneration reagent be a strong base or acid, and claim 21 requires that such strong base be sodium hydroxide. The specification expressly states that a "regeneration reagent can be one of a strong base, ***such as sodium hydroxide*** or a strong acid, such as phosphoric acid." EX1001, 2:63-5 (emphasis added). A POSA would have well understood that the purpose of further "washing" the separation matrix with a "regeneration agent" such as sodium hydroxide is to clean and prepare the separation matrix for further use. EX1007,

65 (using a washing procedure "to remove common contaminants"); EX1056; EX1002, ¶140.

Ferré discloses that after purifying and eluting the protein, the column was cleaned by "pumping 1-2 expanded CVs of 1 M NaOH through the bed at a flow rate of 50 mL/min (150 cm/h), followed by recycling of the remaining NaOH solution (8 L) overnight (~16 h)." EX1004, 11. A POSA would have known at the time of the invention that NaOH (sodium hydroxide) is a regeneration agent and also a strong base. EX1007, 67; EX1056; EX1064; EX1002, ¶140. As such, Ferré expressly discloses each and every limitation of claims 18, 19, and 21.

Accordingly, Ferré anticipates the above challenge claims. *See* EX1002, ¶¶121-141.

B. Ground 2: Komath Anticipates Claims 7-8, 11-12, 15, and 16

1. Komath discloses every limitation of claim 7

As described in detail below, Komath expressly discloses every limitation of claim 7. Komath provides "[a] method for expression, isolation and purification of human granulocyte colony stimulating factor (hG-CSF)," and states the method comprises the ordered steps of (1) "culturing hG-CSF producing recombinant cells in which over-expressed hG-CSF accumulates as inclusion bodies," (2) "lysing said cells" and "isolating the inclusion bodies," (3) "solubilizing and denaturing hG-CSF," (4) "refolding hG-CSF," and (5) subjecting the hG-CSF to "ion

exchange chromatography," and "recovering purified hG-CSF." EX1005, 6. A POSA as of June 2009 would have understood Komath to disclose the use of its disclosed techniques in the recited sequence in order to refold and purify G-CSF. EX1002, ¶¶142-144. *See also* EX1005, Abstract ("The steps include lysing the microorganism, separating the inclusion bodies containing G-CSF, a multi step washing procedure for inclusion bodies to remove protein, LPS, and other host cell impurities, refolding at basic pH and chromatography."); *Finisar Corp. v. DirecTV Grp., Inc.*, 523 F.3d 1323, 1338 (Fed. Cir. 2008) (where the reference "clearly indicated a linkage between" two passages, and a POSA "would read" those passages "in concert," the reference was found to disclose the limitations of the claim "as arranged in those claims").

a. "A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system comprising: (a) expressing a protein in a non-native limited solubility form in a non-mammalian cell"

Komath discloses the refolding and purification of recombinant granulocyte-colony stimulating factor (G-CSF) proteins expressed in a non-native limited solubility form using non-mammalian expression systems, *e.g.*, *E. coli.* EX1005, Abstract, 3, 5, 7, 9. Komath discloses that "hG-CSF is produced by recombinant methods using microbial (fungal or bacterial) expression systems." *Id.*, 7. Komath

states that "[t]he present invention provides a simple and cost effective process for purifying large quantities of recombinant G-CSF from *E. coli* and other cells in which inclusion bodies of G-CSF are formed." *Id.*, 5. *See also id.*, 14; EX1002, ¶145.

b. "(b) lysing a non-mammalian cell"

Komath next discloses lysing a non-mammalian cell to recover the inclusion bodies. EX1005, 5 ("The process involves culturing *E. coli* or other suitable cells and…isolating inclusion bodies containing hG-CSF ***by lysing the cells***.") (emphasis added); *id.* , 6 ("b) lysing said cells"). Specifically, Komath discloses that, "[p]referably, the inclusion bodies containing hG-CSF are recovered from the cells ***by lysing them*** by high pressure homogenization or sonication." *Id.*, 6 (emphasis added); EX1002, ¶146.

c. "(c) solubilizing the expressed protein in a solubilization solution comprising one or more of the following: (i) a denaturant; (ii) a reductant; and (iii) a surfactant"

Komath discloses solubilizing the expressed protein in a solubilization solution that contains urea, a denaturant. EX1001, 2:38-39, 4:35-37, 5:29-30, 13:49-51, 22:38-41; EX1002, ¶147. Komath states that the expressed protein "is solubilized with urea at concentrations ranging from 2M to 6M." EX1005, 12. *See also id.*, 10 ("The IB pellet is solubilized using a combination of a denaturant and

high alkaline pH. The uniqueness of this method is that a sub-denaturing concentration of urea is chosen (2M)…"); EX1002, ¶147.

d. "(d) forming a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of the following: (i) a denaturant; (ii) an aggregation suppressor; (iii) a protein stabilizer; and (iv) a redox component"

Komath discloses refolding G-CSF by forming a refold solution comprising the solubilization solution and a refold buffer comprising an aggregation suppressor. EX1005, 10, 12. Specifically, Komath discloses forming a refold solution by diluting the solubilization solution with 0.1% polysorbate 20 in water at pH 8.0-8.5 for 6 hours and then at pH 4.0-5.0 for 6 to 8 hours. *Id.*, 11. *See also id.*, 9; EX1002, ¶148. *See also* EX1005, 6. The '878 patent discloses that polysorbate 20 is an aggregation suppressor. EX1001, 5:36-39. However, a POSA would have also understood that polysorbate 20 may also act as a protein stabilizer. *See* EX1023, 4-5; EX1029, Abstract; EX1002, ¶148. Indeed, as a non-ionic detergent, it was a common formulation additive. EX1029; EX1002, ¶148.

Komath broadly discloses that a refold solution may also include a surfactant. EX1005, 6 ("If desired, surface active agents may be used during the refolding."). The '878 patent discloses that surfactants may be used as both aggregation suppressors and protein stabilizers. *See* EX1001, 2:43-50, 5:36-40,

14:21-24 ("the protein stabilizer in the refold buffer can be selected from the group consisting of…non-ionic surfactants, ionic surfactants…"), 14:27-29 ("the aggregation suppressor can be selected from the group consisting of…non-ionic surfactants, ionic surfactants…"); EX1002, ¶149. Komath thus discloses this limitation. *Id.*, ¶¶148-150.

e. "(e) directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix"

Komath discloses that "[a] radial flow column is packed with SP-Sepharose (Pharmacia) matrix), which is equilibrated with 25mM sodium acetate buffer, pH 4.5" and that "[t]he refolded protein solution is loaded on this column." EX1005, 10. A POSA would understand that the method of Komath does not remove any components of the refold solution before applying the refold solution to a separation matrix, nor does it include any significant dilution[6] of the refold

[6] In one embodiment, Komath teaches that "[t]he pH of the refolded protein solution is shifted to 4.5 with sodium acetate buffer for loading on an ion exchange column." EX1005, 10. A POSA would not have understood this pH shift to mean a significant dilution of the refold solution, which is consistent with the (Continued...)

solution. EX1005, 10, 12. Rather, Komath states that "[t]he refolded protein solution is loaded on [the] column" (*id.*, 9) and that "[a]ll the contaminants like endotoxins and host DNA are removed by an ion exchange column." *Id.*, 7. *Compare* EX1001, 15:1-5 (the separation matrix separates "the protein of interest" from "the components of the resuspension and/or lysis buffer, including impurities such as host cell proteins, DNA and chemical impurities…"). Komath thus discloses directly applying the refold solution to a separation matrix. EX1002, ¶¶151-152; *see supra* pp. 28-35.

Komath further discloses directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Komath discloses adjusting the pH and conductivity both in forming the refold solution and in equilibrating the column with sodium acetate buffer at pH 4.5 prior to applying the refold solution to the separation matrix, and that refolded G-CSF "binds to the cation exchange column in pH range 4.0 to 5.0, preferably at 4.5." EX1005, 10, 12. A POSA would have understood that these adjustments are used to provide "conditions suitable for the protein to associate with the matrix."

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specification of the '878 patent and as understood by a POSA. *See* EX1047, 20; EX1002, ¶152.

EX1002, ¶153. Komath also discloses that "[i]n the present invention the chromatography procedure has been optimized for maximum recovery" (EX1005, 12), and that, in fact, "the recovery of G-CSF under these elution conditions was found to be maximal, 3 to 5 times more than with NaCl at pH4.5." *Id.*, 10, Table 1; EX1002, ¶153.

f. "(f) washing the separation matrix; and (g) eluting the protein from the separation matrix"

Komath discloses that "[t]he refolded protein solution is loaded on this [separation] column and washed with equilibrium buffer till [sic] the optical density value at 280 nm returns to baseline." EX1005, 10. *See also id.*, 12 ("Washing of the column is done with the same buffer without the detergent till [sic] the optical density at 280nm comes to baseline."). Komath then discloses that "G-CSF is eluted from this column using 0.1M Tris HCl buffer at pH 8.0" and that "the recovery of G-CSF under these elution conditions was found to be maximal." *Id.*, 10, 12; EX1002, ¶154.

g. "wherein the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and hydrophobic interaction resin."

As discussed above, Komath uses a non-affinity ion exchange resin – specifically, a radial flow cation exchange column packed with SP- Sepharose (Pharmacia) matrix. EX1005, 6, 10, 12; EX1019, 46; EX1002, ¶155.

For these reasons, claim 7 is unpatentable as anticipated by Komath. EX1002, ¶¶142-156.

2. Komath discloses every limitation of claims 8, 11, 12, 15, and 16

Komath discloses every limitation of claims 8 and 11. Komath discloses the purification of recombinant G-CSF proteins expressed using non-mammalian expression systems, *e.g.*, *E. coli*, where G-CSF inclusion bodies are formed. EX1005, 3, 5, 7, 9; EX1002, ¶157.

Komath discloses every limitation of claim 12. Komath discloses solubilizing the expressed protein using a solubilization solution that contains urea, a denaturant. EX1005, 10, 12; EX1002, ¶158; EX1001, 2:38-39, 4:35-37, 5:29-30, 13:49-51, 22:38-41.

Komath discloses every limitation of claims 15 and 16. Komath discloses refolding G-CSF using a refold solution comprising the solubilization solution and a refold buffer comprising polysorbate 20, which Komath describes is a "non-ionic detergent" (EX1005, 6, 11, 12), and the '878 patent discloses is an aggregation suppressor. EX1001, 5:36-39. Further, a POSA would have known that polysorbate 20 (otherwise known as "Tween®" or "Tween® 20") is a non-ionic surfactant (EX1051, Abstract), which is used as an aggregation suppressor and

may also act as a protein stabilizer. *See* EX1023, 4-5; EX1029, Abstract; EX1002, ¶159.

For all of these reasons, Komath anticipates the above challenged claims. *See* EX1002, ¶¶142-160.

C. Ground 3: Claims 7-8, 11-12, 15, and 16 Are Obvious over Komath

As discussed in Section VII(B), *supra*, Komath anticipates claims 7, 8, 11, 12, 15, and 16. Patent Owner has asserted in related litigation, however, that Komath does not anticipate the challenged claims because "the techniques disclosed in Komath are discussed individually in different sections." EX1066, 2. Patent Owner is incorrect. *See supra* Section VII(B). However, to the extent the Board disagrees that Komath anticipates these claims, the claims remain unpatentable as obvious over Komath under 35 U.S.C. § 103.

As discussed above, Komath discloses a "simple and economical process involving fewer steps" for purifying "large quantities of recombinant hG-CSF." EX1005, 5. Komath discloses all of the steps of claim 7 of the '878 patent. A POSA would have been motivated to purify a target protein using the steps of Komath and would have further understood that these steps could be practiced together in the order recited in claim 7 with a reasonable expectation of success. *See* EX1005, Abstract; EX1002, ¶¶161-163.

1. Komath discloses every limitation of claim 7.

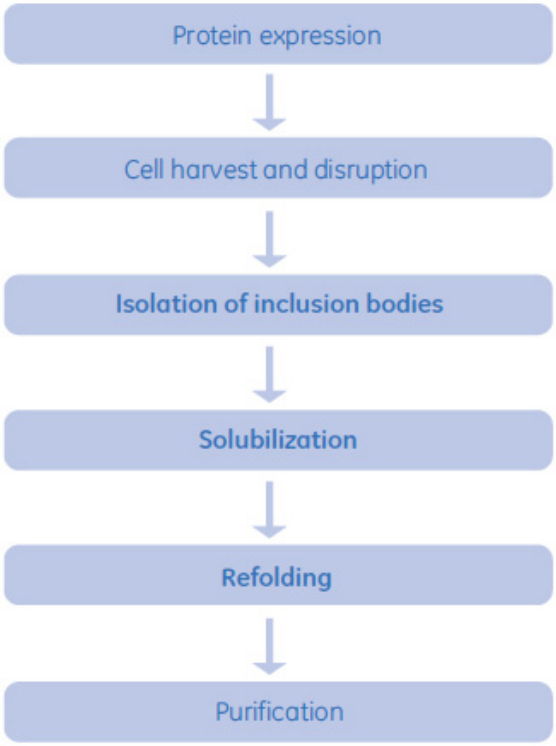
As discussed in Section VII(B)(1), *supra*, Komath expressly discloses every limitation of claim 7. Specifically, Komath discloses the refolding and purification of a protein, G-CSF, that is expressed in a non-native limited solubility form using a non-mammalian expression system, *E. coli.* EX1005, 3; EX1002, ¶¶142-145. Komath discloses lysing the *E. coli* host cells to recover the inclusion bodies and solubilizing the expressed protein using a solubilization solution that contains urea, a denaturant. EX1005, 10; EX1002, ¶¶146-147. Komath further discloses refolding G-CSF by forming a refold solution comprising the solubilization solution and a refold buffer comprising polysorbate 20 as well as buffer to reduce the pH. EX1005, 10, 12; EX1002, ¶148. A POSA would understand that polysorbate 20 is used as an aggregation suppressor, as taught by the '878 patent. EX1001, 5:36-39; EX1002, ¶¶148-149.

Komath also discloses directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. The method of Komath does not disclose removing any components of or significantly diluting the refold solution before applying it to the separation matrix. EX1005, 10, 12; EX1002, ¶152. *See supra* pp. 48-50. Rather, Komath discloses that "[t]he refolded protein solution is loaded on [the] column." EX1005, 10. Komath

discloses next washing the column and eluting G-CSF from the column. *Id.*, 10, 12; EX1002, ¶154. Komath finally discloses that the refolded G-CSF was successfully recovered. EX1005, 10 (disclosing that protein recovery was "maximal"), 12; EX1002, ¶154.

2. A POSA would have been motivated to combine the steps of Komath in the order recited in claim 7

A POSA as of June 2009 would have understood that the steps in Komath are designed to work together precisely in the recited order of claim 7. In particular, a POSA would have recognized that claim 7 merely outlines standard steps of purifying a protein from inclusion bodies expressed in a non-mammalian expression system. *See* EX1001, 22:3-28. POSAs were well familiar with these purification steps. EX1020, 1; EX1002, ¶¶168-175. As a 2005 handbook for protein purification provides, the "general workflow for handing inclusion bodies" had the exact steps recited in the order of claim 7:



EX1068, 69. *See also* EX1023, 157; EX1052; EX1020; EX1069 (demonstrating a method of purifying a protein from a non-mammalian expression system using a separation matrix two decades earlier). A POSA would have understood that a successful purification method would necessarily entail expressing the protein before lysing the host cell, lysing before solubilizing the expressed protein, solubilizing before refolding, and refolding before further purification steps, *e.g.*, chromatography. EX1002, ¶176.

Moreover, a POSA would have been highly motivated to avoid extra downstream processing steps in producing therapeutic proteins, as taught by

Komath itself, particularly where fewer steps would result in higher throughput while using fewer resources, making the overall process more economical. EX1005, Abstract, 2, 5; EX1002, ¶177. For example, dilution was known to be time-consuming and resource intensive. *See* EX1001, 12:16-24. *See also* EX1052, 2; EX1005, 5 ("On a commercial scale, yield losses from a multi-step process becomes highly significant. Hence a simplified procedure with fewer steps will give higher yields in a shorter time, besides being economical."); EX1002, ¶177. Komath discloses a "simple and cost effective process" with such "fewer steps." EX1005, 5.

For these reasons, a POSA would have been motivated to combine the standard steps for purifying a protein from inclusion bodies expressed in a non-mammalian expression system in the same order as recited in claim 7. EX1002, ¶184.

3. Optimization of protein purification conditions was well-understood as of June 2009

As Dr. Robinson explains, a POSA as of June 2009 had a deep understanding of the various components commonly used in refold solutions and of how those components may interact with the particular separation matrix to be used for purification. EX1002, ¶¶179-180; *see supra* pp. 9-12. Thus, optimizing purification conditions for a protein expressed in a non-mammalian expression

system were routine. *Id.* Moreover, a POSA could readily arrive – and actually did readily arrive – at methods to purify a protein expressed in a non-mammalian expression system where the refold solution is applied to a separation matrix under conditions suitable for the protein to associate with the matrix, "without removing any components of or diluting the refold solution." *See* EX1004-1007; EX1002, ¶180.

In particular, by June 2009, many types of chromatography had been used successfully to separate different types of proteins. *See, e.g.*, EX1004-1007. There was substantial knowledge in the art as to how various components of solutions could impact the ability of a protein to associate with a particular type of separation matrix. EX1002, ¶179.

Komath discloses using IEX to recover purified G-CSF protein, a common form of chromatography that has been used since the 1960s for separation of biomolecules. EX1005, 6; EX1007, 7. As of June 2009, it was known that "[l]arge volumes of dilute solutions, such as fractions from a desalting step or a cell culture supernatant, can be applied ***directly*** to an IEX medium without prior concentration" and that this allows for a smaller loading band height, which ultimately results in better compound resolution, *i.e.,* a better separation of

impurities from the desired protein. EX1007, 43 (emphasis added); EX1002, ¶181.

The principles of IEX were also well understood by June 2009; thus, in deciding on the particular conditions that would allow the target protein to associate with an IEX matrix, a POSA would have known that it is important to consider the desired protein's charge characteristics at the relevant pH. EX1007, 51; EX1002, ¶182. The art taught that pH and ionic strength should be set to ensure that when the sample is loaded, "proteins of interest bind to the medium and as many impurities as possible do not bind." EX1007, 14. For example, it was understood that detergents that bind to the matrix may affect protein loading capacity, pH, conductivity and resolution. *Id.*, 49; EX1002, ¶182. It was also understood that a non-ionic (neutral) substance would not affect how the protein associated with the matrix. EX1002, ¶183. Furthermore, if a component of a refold solution *was* ionic, a POSA could determine if it would be expected to affect the association of the protein with the separation matrix through routine, empirical testing, which has been available since the mid-1980s. *Id.*; EX1027, 19; EX1030.

4. A POSA would have reasonably expected success in using the steps of Komath in the recited order of claim 7

The art as of June 2009 also provided substantial guidance as to which components would not damage the matrix itself. EX1002, ¶185. For example, the

GE Handbook taught that "[[a]nionic, cationic, zwitterionic and non-ionic (neutral) detergents can be used during IEX [ionic exchange] chromatography," depending on the particular column being used. EX1007, 50, Table 4 & Appendix 2 (providing list of buffers compatible with anionic and cationic exchange columns); EX1002, ¶185. A POSA would have reasonably expected that a method of purifying a protein using the particular solution components of Komath, in the recited sequence of claim 7, would have provided conditions suitable for a protein to associate successfully with a separation matrix. *Id.*.

Komath specifically uses 2M urea to solubilize the G-CSF protein. EX1005, 10. A POSA would not have expected 2M urea to prevent the G-CSF protein from binding to the IEX matrix at the pH of the refold solution when applied to the matrix (pH of 4.5). EX1002, ¶186. The GE Handbook discloses generally this concentration of urea is appropriate to use in IEX. EX1007, 51 (disclosing typical urea concentrations of 2-8M). Komath also discloses that 2M is a low, "sub-denaturing" concentration of urea, which may be used to solubilize the G-CSF protein if the pH is kept high and alkaline.[7] EX1005, 10. This concentration is

[7] Komath also teaches that the denaturant may be 6M guanidine hydrochloride, if additional steps to "reduce the conductivity of the GdnHCl" are included "***before***

(Continued...)

further reduced upon the dilution of the solubilization solution with the refold buffer. Thus, a POSA would not expect the urea present in the refold solution to preclude the G-CSF protein from binding to the separation matrix. EX1002, ¶186.

In Komath, 1N NaOH was added dropwise to raise the pH to 12, and, after solubilization, the pH was reduced to 8 by adding acetic acid. EX1005, 12. A POSA would realize that acetic acid is neutralized by NaOH, producing sodium acetate, and only trace amounts of NaOH would remain. EX1002, ¶187. As calculated by Dr. Robinson, the trace NaOH present (0.00001M) is very small and likewise would not be expected to render the conditions inappropriate for the protein to associate successfully with the IEX matrix. *Id.*

A POSA would not have expected Komath's use of 0.1% polysorbate 20, a non-ionic surfactant, for refolding to affect the ability of G-CSF to associate with the IEX matrix. EX1023, 4-5; EX1007, 50; EX1002, ¶188. Likewise, a POSA would not have expected Komath's use of sodium acetate or sodium phosphate buffers "of low conductivity" to lower the pH to preclude successful binding to the

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refolding the denatured protein." EX1005, 10 (emphasis added). These conditions, likewise, would not have prevented the protein from binding to the IEX matrix. EX1002, ¶186 n.1.

matrix. EX1005, 12; EX1002, ¶189. This is because as long as the concentration is low enough, one can "[u]se a buffer concentration that is sufficient to maintain buffering capacity and constant pH, typically 20-50 mM." EX1007, 39.

Notably, Komath discloses directly applying the refold solution containing these components to the separation matrix and that "the recovery of G-CSF under these elution conditions was found to be maximal, 3 to 5 times more than with NaCl at pH4.5." EX1005, 10. *See also id.*, 12, Table 1. As such, a POSA would have expected with a reasonable expectation of success that using the particular solutions disclosed by Komath creates conditions suitable for the protein to associate with the separation matrix. EX1002, ¶190.

For these reasons, it would have been obvious to a POSA to perform the disclosed steps of Komath in the recited order of claim 7 to purify a protein expressed by a non-mammalian expression system. EX1002, ¶¶161-191.

5. Claims 8, 11-12, 15, and 16 Are Obvious over Komath

Claims 8, 11, 12, 15, and 16 are also obvious over Komath. *See supra* pp. 51-52; EX1002, ¶¶192-195. Claims 8 and 11 are obvious because Komath discloses the purification of recombinant G-CSF proteins expressed using non-mammalian expression systems, including *E. coli*, where inclusion bodies of G-CSF are formed. EX1005, 3, 5; EX1002, ¶192.

Claim 12 is obvious because Komath discloses solubilizing the expressed protein using a solubilization solution that contains urea, a denaturant. EX1005, 10. By 2009, it was well understood that urea is commonly used as a successful denaturant in protein purification processes. EX1002, ¶193.

Claims 15 and 16 are obvious because Komath discloses refolding the G-CSF using a refold solution comprising the solubilization solution and a refold buffer comprising polysorbate 20, which Komath describes as a "non-ionic detergent" (EX1005, 6, 11, 12) and the '878 patent expressly discloses is an aggregation suppressor. EX1001, 5:36-39. Further, a POSA would have known that polysorbate 20 (*a.k.a.*, "Tween®" or "Tween® 20") is a non-ionic surfactant (EX1051, Abstract), which has been successfully used as an aggregation suppressor and may also act as a protein stabilizer. *See* EX1023, 4-5; EX1029, Abstract; EX1002, ¶194. A POSA thus would have been motivated to use polysorbate 20 in a refold solution, and would have reasonably expected, in view of the successful refolding of G-CSF taught by Komath, that using polysorbate 20 would have worked for its intended purpose. EX1005, 10; EX1002, ¶194.

D. Ground 4: Claims 13 and 17 Are Obvious over Ferré or Komath in View of Rosendahl

Claims 13 and 17 are obvious over Ferré or Komath in view of Rosendahl. EX1002, ¶¶196-205. As discussed in Sections VII(A)(1) and VII(B)(1), *supra*,

each of Ferré and Komath expressly discloses every limitation of claim 7. A POSA would have further understood in view of Rosendahl that the methods of Ferré or Komath could be performed using the particular reductant and redox components described in claims 13 and 17. EX1002, ¶197.

Claim 13 requires that the reductant of the solubilization solution comprise "one or more of cysteine, dithiothreitrol (DTT), beta-mercaptoethanol and glutathione." EX1001, 22:42-44. Claim 17 requires that the redox component of the refold buffer comprise "one or more of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol." *Id.*, 22:58-61.

Rosendahl discloses methods for refolding proteins that are expressed in an insoluble or aggregated form by bacterial host cells, preferably *E. coli.* EX1006, ¶¶[0014]. [0015], [0021]. Rosendahl discloses a solubilization step that includes the use of a solubilization buffer, which contains a reductant, "a disulfide reducing agent" such as cysteine and reduced glutathione. *Id.*, ¶[0038]; EX1002, ¶198. Rosendahl discloses a subsequent refolding step using a refold buffer that "allow[s] for regeneration of the protein's native disulfide bond(s)." EX1006, ¶[0039]. Rosendahl discloses that such buffers can include "an oxidizing agent" such as cysteine, oxidized glutathione, and cystamine, or a "a redox mixture of an

oxidizing agent and a reducing agent," such as "cysteine/cystine, cysteine/cystamine, cysteamine/cystamine, reduced glutathione/oxidized glutathione, and the like." *Id.*; EX1002, ¶201.

A POSA seeking to solubilize and refold proteins expressed in non-mammalian expression systems (*e.g.*, *E. coli*) in a limited solubility form would have looked to Rosendahl for its disclosure of particular reductants/redox components that are successfully able to solubilize and refold aggregated proteins. EX1002, ¶202. Indeed, Rosendahl discloses that these reductants/redox components are "useful" and "preferred" because they are also cysteine blocking agents, and thus their use "reduces the number of compounds and steps required in the overall process for refolding the insoluble or aggregated protein to a soluble, active form." EX1006, ¶[0038]. These advantages, provided by using the compounds of Rosendahl, would have been particularly attractive to a POSA interested in producing biologically active proteins "in high yield," as described in Ferré and Komath. *Id.*, ¶[0013]; EX1004, 10; EX1005, 5; EX1002, ¶199. Thus, a POSA would have been motivated to use the solubilization solution and refold buffer components of Rosendahl in the methods taught by Ferré or Komath to a create a more efficient and cost effective method of solubilizing, refolding, and purifying proteins. *See* EX1004, 2, 10; EX1005, 5; EX1002, ¶200. A POSA

would have reasonably expected that using the solubilization solution and refold buffer components of Rosendahl would result in successful solubilization and refolding of proteins, such as G-CSF, as disclosed in Rosendahl. *E.g.*, EX1006, ¶[102]; EX1002, ¶200.

Furthermore, a POSA would have understood that if a target protein has native disulfide bonds, including an oxidizing agent or redox mixture in the refold buffer would aid in the correct formation of these disulfide bonds. EX1002, ¶202. Ferré and Komath each describes refolding of proteins that include disulfide bonds. EX1004, Abstract; EX1005, 2; EX1006, ¶[0009]; EX1053; EX1054; EX1002, ¶202. Rosendahl provides examples of oxidizing agents and redox mixtures that are able "to catalyze a disulfide exchange reaction" and "allow for regeneration of the protein's native disulfide bond(s)." EX1006, ¶[0039]. As such, a POSA would have been motivated to use the refold buffer components of Rosendahl in the methods of Ferré or Komath to allow for proper formation of the disulfide bond(s) of each method's target protein. EX1002, ¶204. A POSA would have reasonably expected that use of Rosendahl's refold buffer components would result in successful formation of the protein's native disulfide bonds in a refolding step as taught by Rosendahl, Ferré, and Komath. *Id*.

Notably, Rosendahl does not teach away from using its disclosed components with a method that involves "***directly*** applying" the refold solution to a separation matrix. While Rosendahl discloses that a refold solution is clarified using centrifugation before it is loaded onto a chromatography column (*e.g.*, EX1006, ¶[60]), Rosendahl does not disclose that this step is necessary when using the particular reductants/redox components in the refold buffer in order for the protein of interest to successfully associate with the separation matrix. EX1002, ¶203. *See Bayer Pharma AG v. Watson Labs., Inc.*, 874 F.3d 1316, 1327 (Fed. Cir. 2017).

E. Ground 5: Claims 18, 19, and 21 Are Obvious over Ferré or Komath in View of the GE Handbook

As discussed in Sections VII(A)(1) and VII(B)(1), *supra*, each and every limitation of claim 7 is taught by each of Ferré and Komath. EX1002, ¶¶121-136, 142-156. Ferré further expressly discloses each and every limitation of claims 18, 19, and 21. *See supra* pp. 43-44. As Dr. Robinson explains, a POSA as of June 2009 would have also understood in view of the disclosure of the GE Handbook (EX1007) that the separation matrices used in the methods of Ferré or Komath could be regenerated with a reasonable expectation of success. EX1002, ¶¶206-208. As such, claims 18, 19, and 21 are also obvious over Ferré or Komath in view of the GE Handbook (EX1007).

As discussed above, Ferré discloses cleaning methods for the separation matrix used. For example, Ferré discloses that "[a]fter the elution procedure, the flow rate was decreased to 1.5 mL/min (115 cm/h) and the medium was cleaned with 8 M urea, 1 M NaCl in 20 mM Tris-HCl (pH 8.0), followed by 8 M urea, 1 M NaCl, 5 mM 2-mercaptoethanol (2-ME) in 20 mM Tris-HCl (pH 8.0)." EX1004, 11; EX1002, ¶209. Ferré also discloses that the separation matrix can be cleaned by "pumping 1-2 expanded CVs of 1 M NaOH through the bed at a flow rate of 50 mL/min (150 cm/h), followed by recycling of the remaining NaOH solution (8 L) overnight (~16 h)." EX1004, 1. A POSA as of 2009 would have well understood that NaOH (sodium hydroxide) is a regeneration agent and also a strong base. EX1007, 67; EX1056; EX1002, ¶210.

The GE Handbook further discloses that an IEX matrix can be cleaned after use to remove contaminants so that the column can be used again. In particular, the GE Handbook notes that ion exchange media has "[h]igh chemical stability [that] ensures that the matrix can be cleaned using stringent cleaning solutions if required." EX1007, 23; EX1002, ¶211. The GE Handbook includes "[a] general cleaning procedure for each IEX medium." EX1007, 54 (referencing Chapter 3). For example, the GE Handbook outlines a cleaning procedure for Sepharose Fast Flow, the column used by Komath, as well as a cleaning procedure for Sepharose

Big Beads that are intended to be used to purify crude, viscous samples at large scale. EX1005, 12; EX1007, 115; EX1002, ¶211. Both procedures include a NaOH wash. EX1007, 105, 115-118.

As explained by Dr. Robinson, a POSA would have been motivated to regenerate the matrices taught by each of Ferré and Komath by performing a wash with NaOH, as taught by the GE Handbook, and would have expected the wash to work for its intended purpose. EX1002, ¶212.

The concept of cleaning a separation matrix after it has been used so that it can be used again was not new. *Id.*, ¶213. *See* EX1007, 67. Moreover, a POSA interested in scaling up chromatography systems for production of therapeutic protein products would have been particularly concerned both with reducing cost (by reusing columns) and avoiding contamination (by cleaning the columns before each new use). EX1002, ¶214. Both Ferré and Komath are directed to such preparative or commercial scale protein purification. *See* EX1004, 1-2; EX1005, 5 ("The present invention provides a simple and cost effective process for purifying large quantities of recombinant human G-CSF from *E. coli* . . . "). It was also well known by June 2009 that NaOH was "highly effective" in column regeneration, in part because NaOH "inhibits the growth of and kills many bacteria and microorganisms." EX1056, 1; EX1002, ¶215. Indeed, NaOH was reported to be

"probably the most extensively used cleaning agent" as it was "easy to remove and simple to monitor and is also associated with low cost." EX1064, 3, 37; EX1002, ¶215. Thus, a POSA utilizing the methods of Ferré or Komath to purify a protein by directly applying a refold solution to a separation matrix would have been motivated to clean the matrix before reusing it using a known, effective regeneration agent such as NaOH, as taught by the GE Handbook. *Id*. A POSA would have reasonably expected that such use of NaOH to regenerate a separation matrix would result in successful regeneration of the separation matrix for reuse. *Id.*. Accordingly, claims 18, 19, and 21 are obvious over each of Ferré and Komath, in view of the GE Handbook. *Id.*, ¶¶206-216.

F. No Objective Indicia of Nonobviousness

The prosecution history of the '878 patent and its related matters is devoid of any evidence of secondary considerations. Instead, the specification of the '878 patent makes unsupported assertions that (1) it was "surprising to observe that the protein was in fact able to associate with the separation matrix" and (2) the "unexpected finding that the protein could associate with the separation matrix in the presence of the components of the refold solution facilitates the elimination of a dilution step or buffer exchange operation, providing a savings of time and resources." EX1001, 15:35-42. These unsupported assertions, however, are not

probative of nonobviousness because it was well-understood in the art that a refold solution could be applied directly to a separation matrix, thereby obviating the need for intermediate processing steps, and result in successful protein separation. *See supra* Section VII(A)-(E); EX1002, ¶¶217-220. *See also Tyco Healthcare Grp. LP v. Mut. Pharm. Co.*, 642 F.3d 1370, 1377 (Fed. Cir. 2011) ("Unsupported statements in the specification, however, cannot support a finding of unexpected results."). There is also no evidence of a nexus between any secondary considerations and the purported novel features of the claims. *In re Huai-Hung Kao,* 639 F.3d 1057, 1068 (Fed. Cir. 2011).

Moreover, secondary considerations do not control the obviousness conclusion. *See Newell Cos., Inc. v. Kenney Mfg. Co.*, 864 F.2d 757, 768 (Fed. Cir. 1988). In cases where a strong obviousness showing exists—such as is the case here—the Federal Circuit has repeatedly held that even relevant secondary considerations supported by substantial evidence (which is missing here) 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). Thus, regardless of any alleged objective indicia of nonobviousness, the claims would have been obvious over the art cited herein.

VIII. CONCLUSION

For the foregoing reasons, Petitioner requests IPR and cancellation of claims 7-8, 11-13, 15-19, and 21 of the '878 patent.[8]

IX. MANDATORY NOTICES

A. Real Party-In-Interest

Pursuant to 37 C.F.R. § 42.8(b)(1), the real parties-in-interest are Kashiv BioSciences, LLC, Amneal Pharmaceuticals, Inc., and Amneal Pharmaceuticals LLC.

B. Related Matters

Pursuant to 37 C.F.R. § 42.8(b)(2), Petitioner discloses that the '878 patent is currently the subject of the following litigations: *Amgen Inc. et al. v. Adello Biologics LLC*, No. 2:18-cv-03347-CCC/MF (D.N.J.). The '878 patent was also the subject of the following prior litigations: *Amgen Inc. et al. v. Sandoz Inc. et al.*, No. 3:16-cv-02581-RS (N.D. Cal.) and *Amgen Inc. et al. v. Sandoz Inc. et al.*, No. 3:14-cv-04741-RS (N.D. Cal.).

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[8] The Board should not exercise its discretion under 35 U.S.C. § 325(d) because neither the relied-upon art nor arguments presented herein were previously considered by the Examiner.

U.S. Application No. 15/476,691, which is pending, claims priority to U.S. Patent No. 9,643,997, which claims priority to the '878 patent, which claims priority to U.S. Provisional Application No. 61/220,477.

U.S. Patent No. 9,643,997 is also subject to IPR2019-00797, which is pending.

C. Counsel

Pursuant to 37 C.F.R. §§ 42.8(b)(3) and 42.10(a), Petitioner provides the following designation of counsel:

Lead Counsel	**First Back-Up Counsel**
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D. Service Information

Pursuant to 37 C.F.R. § 42.8(b)(4), Petitioner consents to service by email at: rmedina@choate.com and IPR2019-00791Kashiv@choate.com.

E. Certification of Grounds for Standing

Pursuant to 37 C.F.R. § 42.104(a), Petitioner certifies that the '878 patent is available for IPR and that Petitioner is not barred or estopped from requesting IPR of any claim of the '878 patent. This Petition is being filed less than one year from the date on which the Petitioner was served with a complaint by the Patent Owner regarding the '878 Patent.

Respectfully submitted,

Date: March 7, 2019

By: /Rolando Medina/

Rolando Medina, Reg. No. 54,756
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Attorneys for Petitioner

CERTIFICATE OF COMPLIANCE

I hereby certify that the foregoing Petition contains 13,996 words as measured by the word processing software used to prepare the document, in compliance with 37 C.F.R. § 42.24(a).

Respectfully submitted,

Date: March 7, 2019

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.8(e) and 42.105(b), the undersigned certifies that on March 7, 2019, a complete and entire copy of the **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 8,940,878**, Power of Attorney, and all supporting exhibits, were served via Federal Express, overnight delivery, costs prepaid, to the Patent Owner by serving the correspondence address of record for the '878 patent:

AMGEN INC.
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One Amgen Center Drive
Thousand Oaks, CA 91320-1799

Respectfully submitted,

Date: March 7, 2019

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