UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

KASHIV BIOSCIENCES, LLC
Petitioner

v.

AMGEN INC.
Patent Owner

Case No. IPR2019-00797
U.S. Patent No. 9,643,997

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,643,997

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1002	Declaration of Anne S. Robinson, Ph.D.
1003	*Curriculum vitae* of Anne S. Robinson, Ph.D.
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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 9-10, 13-15, 17-21, 23, and 26-30 of U.S. Patent No. 9,643,997 ("the '997 patent"), which issued on May 9, 2017 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 '997 patent (EX1001) unpatentable.

II. OVERVIEW

The challenged claims of the '997 patent are directed to routine protein purification methods comprising the basic steps of (i) solubilizing the expressed protein, (ii) forming a refold solution, (iii) applying the refold solution to a separation matrix, (iv) washing the separation matrix, and (v) eluting the protein from the separation matrix. *See*, *e.g.*, EX1001, 22:36-55. 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.*, EX1004-1009. Indeed, skilled artisans routinely applied refold solutions to separation matrices long before the '997 patent in various protein purification methods, including directly applying such solutions to separation matrices. *See, e.g.*, EX1004-1009. Moreover, a person of ordinary skill in the art (a "POSA") as of June 2009 would have understood how to apply a refold solution to a separation matrix so that the target

protein could associate successfully with a separation matrix. Thus, the claims recite merely known methods of protein purification with predictable and expected results.

Petitioner thus requests IPR and cancellation of claims 9-10, 13-15, 17-21, 23, and 26-30 of the '997 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. Proteins are naturally produced by the processes of transcription (from DNA to RNA) and translation (from RNA to a protein). EX1002, ¶¶34-37; EX1017, 125-149; EX1012.

Proteins can also be synthesized in the laboratory using "recombinant DNA" technology, which has been available since at least the 1970s. *See* EX1013; EX1014; EX1015; EX1016; EX1002, ¶38. Recombinant DNA combines two or more pieces of DNA, often from different sources. Recombinant DNA can be inserted into a "host" cell to produce a desired, "recombinant protein" that the cell typically does not synthesize. *See* EX1017, 182-183; EX1002, ¶¶38-39.

Both mammalian and non-mammalian host cells (referred to as "expression systems") can be used as "factories" to produce recombinant proteins. 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* 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, ¶¶38-39.

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 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.

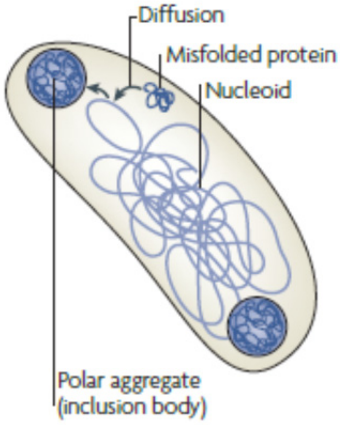

FIG. 1: Inclusion Body in Bacteria (EX1065, Figure 2)

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 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, 181. *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* EX1022, 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 inclusion bodies are isolated 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. Solubilization is used to "denature" the protein into an unfolded state. *Id.*

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. 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.

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

3. Refolding the Solubilized Proteins

After solubilization of inclusion bodies, the denatured proteins are "refolded." EX1024, 2-3; EX1023, 2-5; EX1017, 313. 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 containing the unfolded proteins with a refold buffer. EX1022, 3; EX1024, 6; EX1002, ¶51. Known 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

[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* EX1002, ¶51.

also acts as a protein stabilizers. EX1023, Table 1; EX1075; EX1076; EX1002, ¶52. Sugars and polyols were also known to act as protein stabilizers, and 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 other components present in a refold solution, *e.g.*, unwanted protein, DNA, and the chemicals used for solubilization and refolding. *See, e.g.*, EX1004-1009; EX1002, ¶55. One of the most common and well-understood methods to separate components of a mixture is chromatography. *See* EX1002, ¶¶55-83.

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 Section F below.

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 were a matter of routine optimization, as failing to consider them could adversely impact the binding of the target protein, resulting in lower yield and/or lower purity of target protein. *Id.*, ¶¶57-62.

It was known by 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 skilled artisans 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 combining a denaturant at a low concentration (*e.g.*,

2M urea) with an alkaline pH (pH 11.0 to 12.5) could successfully 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, "[a]nionic, cationic, zwitterionic and non-ionic (neutral) detergents can be used during IEX chromatography." EX1007, 50. 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 by 2009 and commonly used with an affinity-

based separation matrix, *e.g.*, affinity chromatography. EX1031, 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 also were known in the art. *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 proteins, each based upon a particular way of binding the protein with the

separation matrix. *See, e.g.*, EX1004-1009. Accordingly, skilled artisans 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, ¶¶62-83.

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 separates molecules (*e.g.*, proteins) on the basis of net surface charge. *Id.*, 9 13; EX1017, 197-200; EX1002, ¶64. 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). EX1002, ¶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 <0.05M) 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, 44-49; EX1002, ¶¶66-67.

Accordingly, skilled artisans 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, ¶¶69-76.

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 that do not have affinity for the ligand will flow straight through the column. EX1017, 201, 203; EX1032, 19; EX1002, ¶77. 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 denaturant that were commonly used with an affinity-based separation matrix, *e.g.*, affinity chromatography, were well known as of June 2009. EX1031, 135; EX1002, ¶¶77-79.

3. Expanded Bed Adsorption Chromatography (EBA)

Expanded bed adsorption (EBA) chromatography 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 and columns to recover a target protein from a crude mixture. 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) is suspended in an equilibration buffer, creating a stable fluidized bed (b). EX1002, ¶82. A feed (*e.g.*, a refold solution comprising a target protein 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.

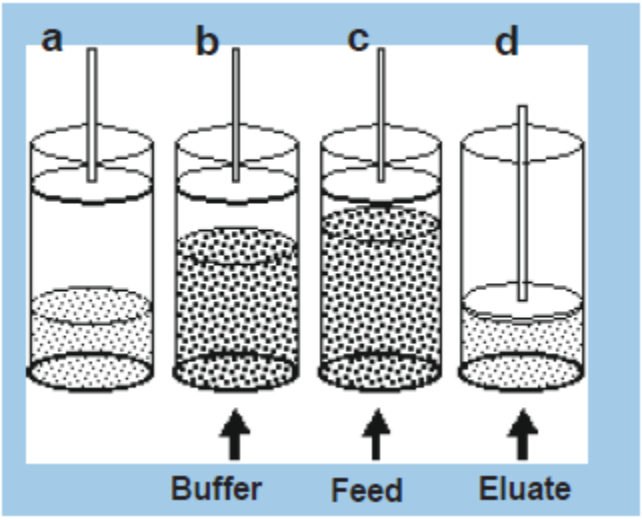

FIG. 2: The principle of operation of EBA (EX1019, Fig. 57)

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. 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 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.

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 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, 2005, 14:2141-2153 ("Ferré") was published in 2005. Ferré is not listed on the face of the '997 patent.

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. Ferré describes using its "continuous protein refolding and direct EBA capture" method for producing and purifying several proteins, including N-terminally-tagged human β2-microglobulin (HAT-hβ2m), interleukin-2, and granulocyte colony-stimulating factor (G-CSF). *Id.*, Abstract, 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, 10. 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, ¶¶86-88.

B. Komath

WO Publication No. 2004/001056 ("Komath") is entitled "Process for preparing G-CSF" and was published December 31, 2003. Komath is not listed on the face of the '997 patent.

Komath discloses "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. Komath first discloses culturing and lysing *E. coli* cells expressing G-CSF. *Id.*, 5, 6, 8-9.

Komath then discloses solubilizing the inclusion bodies using "from 2M to 6M" of urea at a high pH. *Id.*, 6, 10, 12. 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.

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. *Id.*, 12. The column is then washed and G-CSF is successfully eluted. *Id.* *See* EX1002, ¶¶89-94.

C. Hahm

Hahm et al., "Refolding and Purification of Yeast Carboxypeptidase Y Expressed as Inclusion Bodies in *Escherichia coli*," Protein Expression and Purification (2001), 22:101-107 ("Hahm") was published May 7, 2001. Hahm is not listed on the face of the '997 patent.

Hahm describes refolding and purification of two proteins, including carboxypeptidase Y (CPY). EX1009, Abstract. cDNA encoding CPY was inserted into *E. coli* cells and expressed as inclusion bodies. EX1009, Abstract, 1-4. The *E. coli* cells were harvested and lysed. *Id.*, 2.

Hahm discloses solubilizing CPY inclusion bodies in a buffer containing Tris-HCl/EDTA, and guanidinium chloride (GdmCl). EX1009, 2, 4. The denatured CPY was refolded by dilution in Tris-HCl, EDTA, and NaCl. *Id.* A CPY propeptide (CPYPR)-His_6 was also added to the refold buffer to promote in vitro refolding of CPY. *Id.*, 2, 4-5.

Hahm further discloses that the refolded CPY was purified on a *p*-aminobenzylsuccinic acid affinity chromatography column packed with Amino Spherilose resin. EX1009, 2-3. *See* EX1002, ¶¶95-98.

D. Rosendahl

U.S. Application Publication No. 2004/0018586 ("Rosendahl") is entitled "Method for Refolding Proteins Containing Free Cysteine Residues" and was published January 29, 2004. Rosendahl is not listed on the face of the '997 patent.

Rosendahl teaches methods for refolding proteins that are expressed in an insoluble or aggregated form by *E. coli.* EX1006, ¶¶[0014], [0015], [0021]. Rosendahl discloses a solubilization solution that includes "a disulfide reducing agent" (*i.e.*, a reductant) such as cysteine and reduced glutathione. *Id.*, ¶[0038]. Rosendahl also discloses 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]. *See* EX1002, ¶¶99-102.

E. Dietrich

U.S. Application Publication No. 2008/0260684 ("Dietrich") is entitled "Method for the Purification of G-CSF" and was published October 23, 2008.

Dietrich is listed on the face of the '997 patent and in a March 1, 2016 Information Disclosure Statement, but the Examiner did not rely on Dietrich during prosecution. *See* EX1040; EX1036; EX1038.

Dietrich discloses methods for purifying recombinant G-CSF, expressed in *E. coli* cells as inclusion bodies, using cation exchange chromatography and hydrophobic interaction chromatography. EX1008, ¶¶[0001], [0016], [0058], [0063]-[0067]. Dietrich discloses solubilizing the inclusion bodies using a solution containing Tris, EDTA, guanidine-HCl, and Glutathione (GSH). *Id.*, ¶[0068].

Dietrich next discloses forming a refolding solution comprising the solubilization solution and a refold buffer containing Tris, Glutathione Disulfide (GSSG), GSH, and urea. EX1008, ¶[0069]. Dietrich discloses filtering the refold solution before applying the refold solution to a separation matrix. *Id.*, ¶¶[0032]-[0034], [0070]. Dietrich also discloses applying the filtered solution to a cation exchange chromatography column packed with SP Sepharose XL matrix, washing the column, and subsequently eluting G-CSF from the column. *Id.*, ¶¶[0035]-[0036], [0071]-[0072]. *See* EX1002, ¶¶103-106.

V. THE '997 PATENT AND A PERSON OF SKILL IN THE ART

A. The '997 Patent

The '997 patent is entitled "Capture Purification Processes for Proteins Expressed in a Non-mammalian System." EX1001. The only independent claim

challenged, claim 9, recites generally a "method of purifying a protein" that involves only the basic steps of purifying a low solubility protein: (1) solubilizing, (2) refolding, and (3) purifying via applying the refold solution to a separation matrix, washing the matrix, and eluting the protein from the matrix. *Id.*, 22:36-55. The remaining challenged claims depend either directly or indirectly from claim 9.

B. Prosecution History

The '997 patent issued on May 9, 2017 from U.S. Patent Application No. 14/559,336, filed January 16, 2015 (the "'336 application"). The '997 patent is a divisional of U.S. Application No. 12/822,990, filed June 24, 2010, now U.S. Patent No. 8,940,878 (the "'878 patent"), which claims priority to provisional application No. 61/220,477, filed June 25, 2009. EX1001.

Original claim 9 of the '336 application was nearly identical to issued claim 9 of the '997 patent, including the limitation "***applying*** the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix." EX1035, 2 (emphasis added).

The Examiner rejected claim 9 and its dependent claims over U.S. Patent No. 7,138,370 ("Oliner") (EX1034). EX1036, 7-9. In response to the rejection, Patent Owner stated:

> Claim 9 recites, *inter alia,* (b) forming a refold solution; and (c) applying the refold solution to a separation matrix under conditions

> suitable for the protein to associate with the matrix. In contrast, the '370 patent recites that the refolded protein is subject to dialysis, precipitation, and centrifugation. *See*, the '370 patent, col 76, lns 51-59. The supernatant of the '370 patent is then pH adjusted and loaded onto a column. Because the '370 patent does not recite forming a refold solution and applying the refold solution to a separation matrix, the '370 patent fails to teach each and every element of claim 1 [sic].

EX1037, 11. Patent Owner did not amend claim 9 to overcome the rejection over Oliner. Nevertheless, the Examiner withdrew the rejection of claim 9, "in light of applicant's arguments thereto." EX1038, 4; EX1039.

C. Person of Ordinary Skill in the Art

A POSA to which the '997 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. This person may also work in collaboration with other scientists and/or clinicians who have experience in protein purification, protein refolding, or related disciplines. EX1002, ¶¶19, 110.

VI. CLAIM CONSTRUCTION

In IPR, the terms of challenged claims are to "be construed using the same claim construction standard" used in district court litigation, "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 terms not expressly discussed 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 '997 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

[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.

> 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, 7:60-8:4. EX1002, ¶112.

B. "aggregation suppressor"

The '997 patent defines "aggregation suppressor" as "any compound having the ability to disrupt and decrease or eliminate interactions between two or more proteins." *Id.*, 5:45-47. 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.*, 14:34-37.

The patent provides that "[e]xamples of aggregation suppressors can include, but are not limited to…surfactants such as, polysorbate-20… and combinations thereof." *Id.*, 5:48-53. *See also id.*, 2:48-53 ("the aggregation suppressor can be selected from the group consisting of … non-ionic surfactants, … tris… and osmolytes"); 14:50-54 ("In various embodiments, the aggregation suppressor can be selected from the group consisting of … non-ionic surfactants, … Tris…and osmolytes"). Neither the claims nor the specification requires that the aggregation suppressor have a particular concentration. EX1002, ¶¶113-114.

C. "protein stabilizer"

The '997 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, 5:54-57. 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.*, 14:38-40. 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." EX1001, 5:57-63. *See also id.*, 2:53-57, 14:44-49 ("…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, ¶¶115-116.

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

This term should be given its plain and ordinary meaning, regardless of whether there are any intermediate steps, as would be understood by a POSA.

1. Plain and Ordinary Meaning

As another court has recognized, the term "applying the refold solution" of the '997 patent is "plainly broader than the corresponding" term, "directly applying the refold solution" from the '997 patent's parent, the '878 patent. EX1048, 23 (emphasis in original). Thus, "[b]oth applying a solution without removing

components or performing intermediate processing steps as well as applying a solution after some processing steps would fall within the plain meaning of the term." EX1048, 23-24. As evidenced by the specification and the prosecution history, this is precisely how a POSA would have understood the term and what the applicants of the '997 patent intended. EX1002, ¶¶117-125.

a. The Specification

The specification provides various embodiments of purification methods that involve "applying the refold solution to a separation matrix," including embodiments where the refold solution is ***directly*** applied, as well as embodiments where the refold solution is subjected to an intervening or intermediate process before it is applied to a separation matrix.

Specifically, the specification describes the invention as relating "generally to processes for purifying proteins expressed in non-mammalian systems in both non-native soluble and non-native insoluble forms, and ***more particularly*** to the direct capture of such proteins from a refold mixture…." EX1001, 1:13-18 (emphasis added). In one embodiment, "the present invention relates to a method of isolating a protein of interest…[where] it is necessary to isolate or dilute the protein from these components for further processing, particularly before applying the protein to a separation matrix." *Id.*, 4:41-45, 4:54-57. Indeed, in most of the

examples of the '997 patent, there is an intermediate step before the refold solution is applied to a separation matrix. *See id.*, 20:56-62 (Example 3) (the refold solution "was diluted 3-fold with water, titrated with 50% hydrocholoric acid to ~pH 4.5 and was filtered through a series of depth and/or membrane filter…" before applying to a separation matrix); 19:34-40 (Example 2) (the refold solution is "conditioned and filtered" before applying to a separation matrix); 21:45-50 (Example 4) (same).

Separately, the specification also describes a different embodiment of the invention that "omits the need for removing any components of the refold mixture before the refold mixture is applied to a separation matrix":

> In ***one embodiment*** of the disclosed method, purification is achieved by ***directly*** applying a protein of interest, which is present in a refold mixture, to a separation matrix. ***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….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., 4:58-5:4 (emphasis added); *see id.*, 3:53-57, 15:23-230, 16:1-4. As such, a POSA would understand the term "applying the refold solution to a separation matrix" recited in claim 9 to refer to any application, regardless of whether there are any intermediate steps. EX1002, ¶125.

b. The Prosecution History

The prosecution histories of the '997 patent and its parent '878 patent support the plain meaning construction of this term. Original claim 9 of the '997 patent and the '878 patent were identical, both including the same term, "applying the refold solution to a separation matrix." *Compare* EX1035 *with* EX1067. During prosecution of the parent '878 patent application, the Examiner rejected claim 9 over Oliner et al. (EX1034). In response, Patent Owner sought to distinguish Oliner by arguing that "Oliner et al. teaches a method ***that differs markedly*** from the ***direct application*** of refold solution to the separation matrix." EX1068, 8 (emphasis added). The Examiner disagreed, 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." EX1069, 8 (emphasis in bold added). In response, Patent Owner

expressly ***amended*** the claim language from "applying" to "***directly*** applying" to capture unequivocally, in the parent '878 patent, the embodiment where no intermediate processing steps were performed. EX1070, 3, 7-8.

In contrast, during prosecution of the '997 patent application, Patent Owner did ***not*** amend the language of claim 9 to overcome the rejection over Oliner. Instead, Patent Owner distinguished Oliner on several grounds, stating that Oliner (1) "recites that the refolded protein is subject to dialysis, precipitation, and centrifugation;" (2) that "[t]he supernatant of [Oliner] is then pH adjusted and loaded onto a column;" and (3) that Oliner "does not recite ***forming a refold solution*** and applying the refold solution to a separation matrix." EX1037, 11 (emphasis added). The Examiner summarily withdrew the rejection. EX1038, 4; EX1039. It is thus unclear whether Patent Owner successfully distinguished Oliner's *combination* of multiple intermediate processes, or each individual process on its own, from the claimed invention.[4] EX1002, ¶120. As such, because the specification describes multiple embodiments of the invention, including direct

[4] The claims are also directed to a method "comprising" the listed steps, which leaves open additional, unidentified processing steps. *See* MPEP § 2111.03; *Solvay S.A. v. Honeywell Int'l Inc.*, 742 F.3d 998, 1005 (Fed. Cir. 2014).

and indirect application of the refold solution, and because Patent Owner did not make a clear and unmistakable disclaimer of particular intermediate processes, a plain meaning construction is appropriate. *See* EX1002, ¶¶117-125.

2. "Without Intervening Steps of Dilution, Centrifugation, Dialysis, or Precipitation"

In a separate litigation, *Amgen Inc., et al. v. Mylan Inc. et al.*, No. 2:17-cv-01235 (W.D. Pa.), Patent Owner asserted that this term should be construed as applying the refold solution to a separation matrix[5] "***without intervening steps of dilution***[6]***, centrifugation, dialysis, or precipitation***." EX1041, 16-21 (emphasis

[5] Patent Owner previously attempted to limit the term to column chromatography, but conceded that the limiting language was not appropriate. EX1074, 25; EX1048, 23 n.10. *See also* 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, 17:1-13.

[6] Patent Owner defines the excluded "dilution" as prior-art, "***significant*** dilutions, *i.e.*, ***multifold or nearly multifold*** dilutions." EX1047, 20 (emphasis added); EX1001, 12:33-50. According to Patent Owner, a POSA "would understand that (Continued...)

added). The Mylan court adopted this alternative construction, distinguishing it from the term "***directly*** applying" from the parent '878 patent. EX1048, 8-14, 23-29. Patent Owner asserted that it "narrowly surrendered" these specific intervening steps -- centrifugation, dialysis, and precipitation -- during prosecution to overcome the rejection over Oliner. EX1041, 18.; *see also* EX1037, 11. While Petitioner does not agree[7] with this construction, in this proceeding, the challenged claims are unpatentable over the prior art under either construction, as described in detail below.

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the patent specification ***is not referring to diluting a refold solution by adding a minor amount of liquid.***" EX1047, 20. (emphasis added). EX1002, ¶119.

[7] A POSA would not understand "applying the refold solution" to exclude ***only*** the precise steps of centrifugation, dialysis, and precipitation. Indeed, the specification does not discuss centrifugation, dialysis, or precipitation ***at all***, let alone as excluded intervening processing steps performed prior to "applying." Centrifugation is only mentioned with respect to the separation of non-mammalian cells from growth media before lysing to release the protein (EX1001, 9:35-49, 13:21-25, 13:48-55, 18:23-24, 19:8-10, 19:14-21, 20:37-46) or as a suitable means of separation via a batch process (*id.*, 11:45-49, 17:7-10). EX1002, ¶125.

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, 22:45-50. 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, ¶126.

VII. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED

Petitioners request cancellation of claims 9-10, 13-15, 17-21, 23, and 26-30 of the '997 patent on the following specific grounds:

Ground	Reference(s)	Basis	Challenged Claims
1	Ferré	§ 102	9-10, 13-14, 17-18, 20-21, 26, 29-30
2	Komath	§ 102	9-10, 13-14, 17-18, 20-21, 26, 29-30
3	Komath	§ 103	9-10, 13-14, 17-18, 20-21, 26, 29-30
4	Hahm	§ 102	9-10, 13-15, 17-18, 21, 23, 26, 29
5	Dietrich	§ 102	9-10, 13-15, 17-21, 23, 26-30
6	Ferré or Komath or Dietrich in view of Rosendahl	§ 103	15, 19, 23, 27-28

A. Ground 1: Ferré Anticipates Claims 9-10, 13-14, 17-18, 20-21, 26, 29, and 30

1. Ferré discloses every limitation of claim 9

a. "A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system comprising:"[8]

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*. *See* EX1004, Abstract, 2, 10. Ferré discloses a method where "HAT-hβ_2m was produced as insoluble inclusion bodies by *Escherichia coli* fermentations." *Id.*, 2. 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, ¶128.

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

b. "(a) 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 '997 patent acknowledges that urea is a denaturant. EX1001, 2:43-45, 4:46, 5:41-42, 14:4-7, 19:22-23, 22:66-23:1; EX1002, ¶129.

c. "(b) 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, ¶¶130-131.

The refold buffer in Ferré contains 20mM Tris-HCl (*Id.*, 10), which the '997 patent acknowledges is both a protein stabilizer and aggregation suppressor. EX1001, 2:48-56, 5:57-62, 14:44-48, 14:50-54, 23:8-18, 24:7-17. 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. EX1075, 2, 6-7, 11; EX1076, 5, 7; EX1002, ¶132. Ferré thus discloses this limitation.

d. "(c) 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, ¶134. As discussed in Section III(C)(3) above, a POSA as of June 2009 could have utilized a variety of separation

matrices with EBA in order to separate a target protein from its environment. *See* EX1002, ¶¶80-83. Because Ferré expressly discloses that the folded protein is "***directly captured***" following refolding by the separation matrix, the method of Ferré does not include any intervening steps (*e.g.*, dilution, centrifugation, dialysis, or precipitation). Ferré thus discloses applying the refold solution to a separation matrix under either construction of this limitation. *See, e.g.*, EX1004, 2; EX1002, ¶¶134-135. *See* Section V(D) *supra*.

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 denatured protein in solubilization solution. EX1004, 3-4, 10. The denatured protein suspension (DP) and the refold "aqueous buffer" (AB) are constantly 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.*, Abstract, 3-4, 10. The refold solution containing the refolded protein then enters the separation column through valve 1 (V1). *Id.*; EX1002, ¶135. Using this method, "the nascently folded product is directly recovered on the EBA column after the refolding event." EX1004, 9.

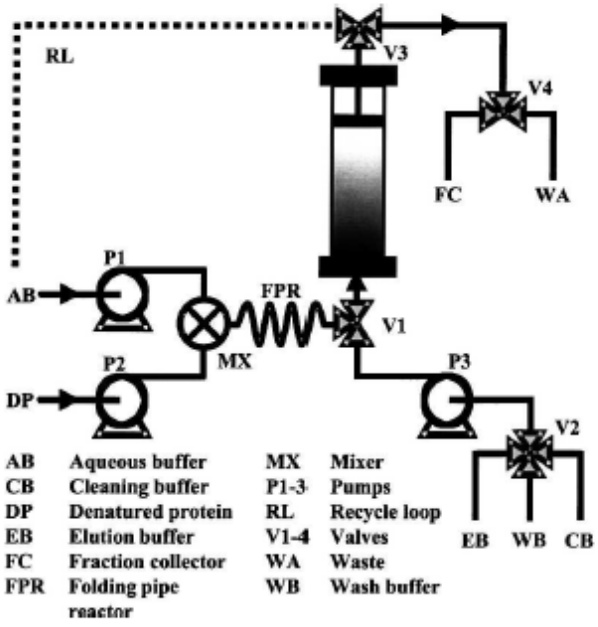


Figure 3. Schematic representation of the system for continuous protein refolding and on-line EBA capture. All EBA operations—i.e., equilibration, loading, washing, elution, and cleaning—were performed in expanded mode in Fastline10 and 50 columns. The current system allows the refolding buffer to be 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).

Ferré uses Fastline10 and 50 columns, which are expanded bed adsorption (EBA) columns. *Id.* These columns use a "STREAMLINE DEAE medium." *Id.*, 4, 11. A POSA would have understood that DEAE refers to diethylaminoethanol and is an ion exchange resin, specifically, an anion exchange resin. EX1019, 53; EX1002, ¶136. The '997 patent expressly contemplates these columns and resins as examples of a "separation matrix." EX1001, 11:41-45, 11:28-40, 15:42-49.

Ferré also discloses that the refold solution is applied to a separation matrix under conditions suitable for the protein to associate with the matrix. Ferré discloses that biologically active protein was successfully captured by the EBA column. EX1004, Abstract, 4, 10, 11, Table 1; EX1002, ¶137.

e. "(d) washing the separation matrix; and (e) 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, ¶¶138-140.

2. Ferré discloses every limitation of claims 10, 13-14, 17-18, 20-21, 26, 29, and 30

Claim 10 requires the method of claim 9, "wherein the non-native limited solubility form is a component of an inclusion body." Ferré discloses a method of purifying a protein that is expressed in *E. coli* cells as insoluble inclusion bodies. EX1004, 2; EX1002, ¶141.

Claim 13 requires the method of any one of claims 9-12, "wherein the non-mammalian expression system comprises bacteria or yeast cells." Ferré uses *E. coli* cells to express HAT-hβ2m . EX1004, 2; EX1002, ¶141.

Claim 14 requires the method of of any one of claims 9-12, "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 discloses that the released inclusion bodies were washed and solubilized "in 8 M ***urea***." EX1004, 2 (emphasis added). *See also id.*, 10; EX1002, ¶142.

Claims 17 and 18 require the method of claim 9, and claim 26 requires the method of claim 17, wherein the aggregation suppressor (claim 17) and the protein stabilizer (claims 18 and 26) that comprise the refold buffer of claim 9 is selected from a group consisting of, or comprises, Tris. Ferré's refold buffer contains Tris-HCl (EX1004, 10), which is a protein stabilizer and aggregation suppressor. EX1001, 2:48-56, 5:57-62, 14:44-48, 14:50-54, 23:8-18, 24:7-17. It was also well known in the art that Tris is used as a protein stabilizer and aggregation suppressor. *See* EX1075, 2, 6, 7, 11; EX1076, 5, 7, Fig. 4(A,B); EX1002, ¶¶132, 143.

Claim 20 requires the method of claim 9, "wherein the separation matrix is … (ii) a non-affinity resin selected from the group consisting of ion exchange … resin." Ferré uses an ion exchange non-affinity resin, "STREAMLINE DEAE." EX1004, 4, 11; EX1019, 53; EX1002, ¶144.

Claims 21, 29, and 30, require the method of any one of claim 1 or 9-12 (claim 21), claim 13 (claim 29), or claim 20 (claim 30), "wherein the protein is isolated after elution from the separation matrix." The method of Ferré includes a further step of collecting or isolating the target protein from the separation matrix

after elution. For example, Table 1 shows the percentage of protein that is actually recovered after purification. EX1004, Table 1. *See also id.*, 5 (further teaching that "[a]pproximately 43% of the total amount of the denatured monomeric HAT-hβ2m offered to the refolding reaction could be recovered as monomeric HAT-hβ2m under native elution conditions"). Additionally, Ferré discloses that "[f]ractions from continuous refolding and EBA capture containing HAT-hβ2m were pooled and concentrated on a 3-kDa NMWL filter." *Id.*, 11; EX1002, ¶145.

Accordingly, Ferré anticipates the above challenged claims. *See* EX1002, ¶¶127-146.

B. Ground 2: Komath Anticipates Claims 9-10, 13-14, 17-18, 20-21, 26, 29, and 30

1. Komath discloses every limitation of claim 9

As described in detail below, Komath expressly discloses every limitation of claim 9. 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," (5) subjecting the hG-CSF to "ion exchange chromatography," and (6) "recovering purified hG-CSF." EX1005, 6. A POSA as

of June 2009 would have understood Komath to teach the use of its disclosed techniques in the recited sequence in order to refold and purify G-CSF. EX1002, ¶¶147-149. *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…comprising:"

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 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, ¶150.

b. "(a) solubilizing the expressed protein in a solubilization solution comprising…"

Komath discloses solubilizing the expressed protein in a solubilization solution that contains urea, a denaturant. EX1001, 2:43-45, 4:46, 5:41-42, 14:4-7, 19:22-23, 22:66-23:1. Komath expressly states that the expressed protein "is solubilized with urea at concentrations ranging from 2M to 6M." EX1005, 12. *See also id.*, 10 (teaching solubilization "using a combination of a denaturant and high alkaline pH" and "a sub-denaturing concentration of urea is chosen (2M)"); EX1002, ¶151.

c. "(b) forming a refold solution comprising…"

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 6-8 hours. EX1005, 12. *See also id.*, 6, 10; EX1002, ¶¶152-154. The '997 patent teaches that polysorbate 20 is an aggregation suppressor. EX1001, 5:48-53. Moreover, a POSA would have understood that polysorbate 20 may also act as a protein stabilizer. *See* EX1023, 4-5; EX1029, Abstract. Indeed, as a non-ionic detergent, it was a common formulation additive. EX1029; EX1002, ¶152.

Komath broadly discloses that a refold solution may also include a surfactant. EX1005, 6. The '997 patent teaches that surfactants may be used as both aggregation suppressors and protein stabilizers. *See* EX1001, 2:48-55, 5:48-

51, 14:44-47 ("the protein stabilizer in the refold buffer can be selected from the group consisting of…non-ionic surfactants, ionic surfactants…"), 14:50-53 ("the aggregation suppressor can be selected from the group consisting of…non-ionic surfactants, ionic surfactants…"); EX1002, ¶153. Komath thus discloses this limitation.

d. "(c) applying the refold solution…"

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 include any intermediate or intervening steps (*e.g.*, dilution[9], centrifugation, dialysis, or precipitation. EX1005, 10, 12. Rather, Komath states that "[t]he refolded protein solution is loaded on [the] column" (*id.*, 10) and that "[a]ll the

[9] In one embodiment, Komath discloses 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 understand this pH shift as a significant dilution of the refold solution, as contemplated by the specification of the '997 patent and as understood by a POSA. *See* EX1047, 20; EX1002, ¶156.

contaminants like endotoxins and host DNA are removed by an ion exchange column." *Id.*, 6-7. *Compare* EX1001, 15:25-29 (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 applying the refold solution to a separation matrix under either construction of this limitation. EX1002, ¶155; *see supra* Section V(D) *supra*.

Komath further discloses 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, 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." EX1002, ¶¶156-157. 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, ¶157.

e. "(d) washing the separation matrix; and (e) 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 280nm 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 "[t]he recovery of G-CSF under these elution conditions was found to be maximal." *Id.*, 10, 12; EX1002, ¶158.

2. Komath discloses every limitation of claims 10, 13-14, 17-18, 20-21, 26, 29, and 30

Komath discloses claims 10 and 13. 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, ¶160.

Komath discloses claim 14. Komath discloses solubilizing the expressed protein using a solubilization solution that contains urea, a denaturant. *Id.*, 10, 12; EX1002, ¶161.

Komath discloses claims 17, 18, and 26. As discussed above, 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 '997 patent teaches is an aggregation suppressor. EX1001, 5:48-53. 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, ¶162.

Komath discloses claim 20. As discussed above, Komath uses a non-affinity ion exchange resin – a radial flow cation exchange column packed with SP – Sepharose (Pharmacia) matrix. EX1005, 6, 10, 12; EX1019, 46; EX1002, ¶163.

Finally, Komath discloses claims 21, 29, and 30. Komath describes "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. Komath expressly includes a step *after* chromatography of "recovering purified hG-CSF" and discloses that "recovery of G-CSF" was "maximal." *Id.*, 6, 10. A POSA would have further understood that because Komath is directed to producing G-CSF "for therapeutic applications," it would have been necessarily to isolate the purified protein after elution from the

separation matrix. *Id.*, 2; EX1002, ¶164. Thus, Komath discloses isolating the protein after elution from the separation matrix.

For all of these reasons, Komath anticipates the above challenged claims. *See* EX1002, ¶¶147-165.

C. Ground 3: Claims 9-10, 13-14, 17-18, 20-21, 26, 29, and 30 Are Obvious over Komath

As discussed in Section VII(B), Komath anticipates claims 9-10, 13-14, 17-18, 20-21, 26, 29, and 30. 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. EX1002, ¶¶166-202.

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 9 of the '997 patent. A POSA would have been motivated to purify a target protein using the steps of Komath and would have understood that these steps could be practiced together as

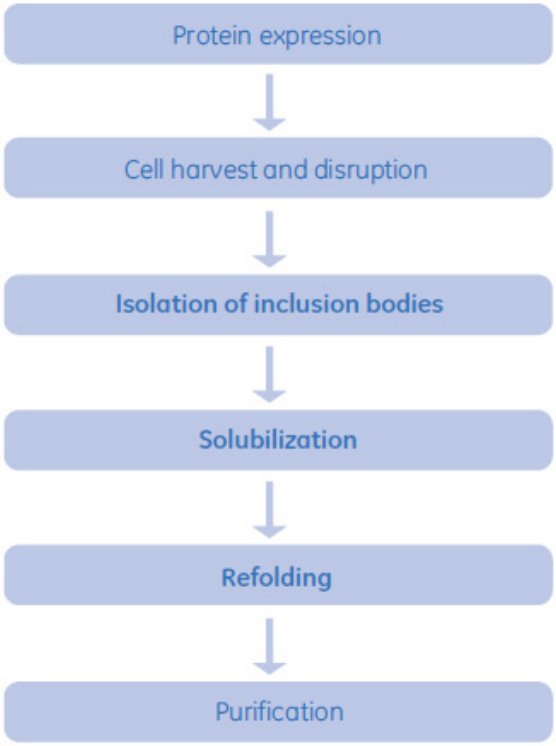
arranged in claim 9 with a reasonable expectation of success. *See* EX1005, Abstract; EX1002, ¶¶181-196.

1. Komath discloses every limitation of claim 9

As discussed in Section VII(B)(1) *supra*, Komath expressly discloses each and every limitation of claim 9. *See* EX1005; EX1002, ¶¶147-159, 169-172.

2. A POSA would have been motivated to combine the steps of Komath in the order recited in claim 9

A POSA as of June 2009 would have understood that the steps in Komath are designed to work together precisely as arranged in claim 9. In particular, a POSA would have recognized that claim 9 merely outlines known, standard steps of purifying a protein from inclusion bodies expressed in a non-mammalian expression system. EX1001, 22:36-55; EX1020, 1; EX1002, ¶¶173-181. As a 2005 handbook for protein purification provides, the "general workflow for handing inclusion bodies" had the exact steps recited as arranged in claim 9:



EX1073, 69; EX1023, 1; EX1052; EX1020; EX1077 (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 solubilizing the expressed protein before refolding, and refolding before further purification steps, *e.g.*, chromatography. EX1063, 2:66-3:2; EX1002, ¶181.

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. For example, dilution was known to be time-consuming and resource intensive. *See* EX1001, 12:14-20, 45-50; EX1052, 2; EX1005, 5 ("a simplified procedure with fewer steps will give higher yields in a shorter time, besides being economical."). Komath discloses a "simple and cost effective process" with such "fewer steps." EX1005, 5. EX1002, ¶182

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 how those components may interact with the particular separation matrix used. EX1002, ¶¶183-185; *see* Section III(C) *supra*. Thus, optimizing purification conditions for proteins expressed in non-mammalian expression systems were routine. *Id.* Moreover, a POSA could readily arrive – and 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. *See* EX1004-1009; EX1002, ¶185. In particular, by June 2009, many types of chromatography had been used successfully to separate different types of proteins. *See, e.g.*, EX1004-1009; EX1002, ¶¶57-83, 184-186.

Komath discloses using IEX to recover purified G-CSF protein. EX1005, 6; EX1007, 9; EX1002, ¶186. The principles of IEX, having been used since the 1960s, were well understood by June 2009. For example, in deciding on the particular conditions for using an IEX matrix to recover a target protein, a POSA would have known that it is important to consider the protein's charge characteristics at the relevant pH. EX1007, 50; EX1002, ¶187. 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. It was also understood that detergents that bind to the matrix may affect protein loading capacity, pH, conductivity, and resolution. *Id.*, 51. Likewise, a non-ionic (neutral) substance would not affect how the protein associated with the matrix. EX1002, ¶188. Furthermore, if a component of a refold solution *was* ionic, a POSA could have determined if it would 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 9

The art as of June 2009 also provided substantial guidance as to suitable solution components for different separation matrices. *See, e.g.*, EX1007, 50, Table 4 & Appendix 2 (providing list of buffers compatible with anionic and

cationic exchange columns). As such, a POSA would have reasonably expected to achieve successful protein purification using the particular solution components of Komath, in the recited sequence of claim 9. EX1002, ¶190.

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, ¶191. The GE Handbook teaches generally that this concentration of urea is appropriate to use with IEX. EX1007, 51 (teaching 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.[10] EX1005, 10. This concentration is further reduced upon the dilution of the solubilization solution with the refold

[10] Komath also discloses that the denaturant may be 6M guanidine hydrochloride, if additional steps to "reduce the conductivity of the GdnHCl" are included "***before*** refolding the denatured protein." EX1005, 10 (emphasis added). These conditions, likewise, would not have prevented the protein from binding to the IEX matrix. EX1002, ¶191 n.1.

buffer. Thus, a POSA would not expect the urea present in the refold solution to prevent successful binding to the separation matrix. EX1002, ¶191.

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, ¶192. As calculated by Dr. Robinson, the small amount of NaOH present (0.00001M) 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 for refolding to affect the ability of G-CSF to associate with the IEX matrix. EX1023, 4-5; EX1007, 50; EX1002, ¶193. 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 matrix. EX1005, 12; EX1002, ¶194. 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 that G-CSF protein was successfully recovered under these conditions – in fact, recovery was "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 reasonably expected success in using the particular solutions disclosed by Komath for protein purification. EX1002, ¶¶195-196.

For these reasons, it would have been obvious to a POSA to perform the steps disclosed by Komath as arranged in claim 9 for protein purification. *Id.*, ¶¶166-196.

5. Claims 10, 13-14, 17-18, 20-21, 26, 29, and 30 Are Obvious over Komath

Claims 10, 13-14, 17-18, 20-21, 26, 29, and 30 are also obvious over Komath. *See* Section VII(B)(2) *supra*.

Claims 10 and 13 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, ¶197.

Claim 14 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, ¶198.

Claims 17, 18, and 26 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 '997 patent expressly teaches is an aggregation suppressor. EX1001, 5:48-51. Further, it was known that polysorbate 20 (*a.k.a*, "Tween®" or "Tween® 20") is a non-ionic surfactant (EX1051, Abstract) that has been successfully used as an aggregation suppressor and may also act as a protein stabilizer. *See* EX1023, 4-5; EX1029, Abstract; EX1002, ¶199. 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, ¶199.

Claim 20 is obvious because Komath uses a non-affinity ion exchange resin (radial flow cation exchange column packed with SP – Sepharose (Pharmacia) matrix). EX1005, 6, 10, 12; EX1019, 46. A POSA would have been motivated to use such a resin with a reasonable expectation of success because by 2009, such resins were known to be part of successful protein purification techniques. *See* EX1002, ¶200.

Finally, claims 21, 29, and 30 are obvious because Komath expressly includes a step *after* chromatography of "recovering purified hG-CSF" and discloses that "recovery of G-CSF" was "maximal." EX1005, 6, 10. Because

Komath is directed to producing G-CSF "for therapeutic applications," it would have been necessarily to isolate the purified protein for further processing. *Id.*, 2. Thus, it would have been obvious to a POSA to further isolate the protein after elution from the separation matrix. EX1002, ¶201.

D. Ground 4: Hahm Anticipates Claims 9-10, 13-15, 17-18, 21, 23, 26, and 29

1. Hahm discloses every limitation of claim 9

a. "A method of purifying a protein…comprising:"

Hahm expressly discloses the purification of proteins, including carboxypeptidase Y (CPY), that were expressed as inclusion bodies in *E. coli*. EX1009, Abstract, 1-4. EX1002, ¶104.

b. "(a) solubilizing the expressed protein in a solubilization solution comprising…"

Hahm discloses solubilizing CPY inclusion bodies in a buffer containing Tris-HCl/ EDTA, and guanidinium chloride (GdmCl). EX1009, 2, 4. The CPY inclusion bodies were solubilized in a 50mM Tris-HCl/3mM EDTA buffer (pH 8.0) containing 6 MGdmCl, pH 8.0. *Id.* GdmCl is a well-known denaturant. EX1001, 2:43-44, 5:41-42, 14:5-6, 21:31-32, 22:67-23:1; EX1002, ¶205.

CPY inclusion bodies were also solubilized in a "solubilization buffer containing the reducing agents such as 100 mM β-mercaptoethanol and 300 mM dithiothreitol [DTT], and then refolding was carried out as described above."

EX1009, 5-6. DTT and β-mercaptoethanol are well-known reductants. EX1001, 2:43-46, 3:8-9, 14:13-15, 21:32-33, 23:3-5, 24:1-3; EX1002, ¶206.

c. "(b) forming a refold solution comprising…"

Hahm discloses refolding of CPY in a renaturation buffer by adding a refold buffer containing 50mM Tris-HCl, 3mM EDTA, and 0.5M NaCl to the solubilization solution. EX1009, Abstract, 2, 4-5. In addition, CPY propeptide (CPYPR)-His_6 was added to the renaturation buffer to promote in vitro folding of CPY. *Id.*, 5. EX1002, ¶207.

NaCl was known to have the ability to "destabilize protein-protein interactions" or "enhanc[e] native protein stability", *e.g.*, at a concentration of 0.5M. EX1071, 1-2, Table 1; EX1072, 5. NaCl was also known as an osmolyte, which the '997 patent acknowledges is an aggregation suppressor and/or a protein stabilizer. *See* EX1001, 2:48-57, 5:45-63; EX1002, ¶208.

Tris-HCl is also listed by the '997 as a protein stabilizer and aggregation suppressor. EX1001, 2:48-56, 5:57-62, 14:44-48, 14:50-54, 23:8-18, 24:7-17; EX1002, ¶209. 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. EX1075, 2, 6, 7; EX1076, 5, Fig. 4(A,B); EX1002, ¶¶132, 209; EX1001, 5:54-57.

Indeed, it was established by 2009 that Tris-HCl as a sole component in a refold buffer, when added to a solubilization solution comprising a denatured protein, can be used for refolding. EX1004.

Finally, a POSA in June 2009 would have understood that CPYPR-His_6 acts as a protein stabilizer for the refolding of CPY, as Hahm discloses that increasing the molar ratio of CPYPR-His_6 to CPY resulted in an increase in the CPY refolding yield. EX1009, Abstract; EX1002, ¶209.

d. "(c) applying the refold solution…"

Hahm discloses applying the refold solution comprising refolded CPY to a *p*-aminobenzylsuccinic acid affinity chromatography column packed with Amino Spherilose resin. EX1009, 102-103, 106 ("After refolding of the denatured CPY in the presence of 10 molar eq CPYPR-His6, the refolded CPY was purified using p-aminobenzylsuccinic acid affinity chromatography."); EX1002, ¶210. Hahm does not disclose any intermediate steps before applying the refold solution to the separation matrix. EX1009; EX1002, ¶211. Further, a POSA would have understood that 3mM EDTA in the presence of a refold buffer would not significantly affect the performance of p-aminobenzylsuccinic acid affinity chromatography, and thus a POSA would not have had a reason to remove EDTA

by any intermediate steps (*e.g.*, centrifugation, precipitation, or dialysis). EX-1002, ¶211.

Hahm further discloses successful recovery of CPY from the column, thus demonstrating conditions suitable for the protein to associate with the matrix. *See* EX1009, 6 ("The CPY, specifically bound with *p*-aminobenzylsuccinic acid, eluted with 0.1 M succinic acid solution (pH 6.0) containing 0.5 M NaCl (Fig. 6).") and Table I; EX1002, ¶212.

e. "(d) washing the separation matrix; and (e) eluting the protein from the separation matrix"

Hahm discloses that the *p*-aminobenzylsuccinic acid affinity chromatography column "was washed with 0.01 M Mes buffer (pH 6.0) containing 0.5 M NaCl, and the bound CPY eluted with 0.1 M succinic acid solution (pH 6.0) containing 0.5 M NaCl." EX1009, 3; *see also id.*, (FIG. 6) (disclosing a washing step and an elution step followed by refolded CPY loading into the column); EX1002, ¶213.

2. Hahm discloses every limitation of claims 10, 13-15, 17-18, 21, 23, 26, and 29

Hahm discloses claims 10 and 13. Hahm expressly discloses the purification of a protein, CPY, that has been expressed as inclusion bodies in *E. coli*. EX1009, Abstract, 1-4. EX1002, ¶215.

Hahm discloses claims 14, 15, and 23. Claim 14 requires the method of any one of claims 9-12, "wherein the denaturant of the solubilization solution or the refold buffer comprises one or more of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea," and claims 15 and 23 require that the reductant of the solubilization solution comprises "one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione." Hahm discloses solubilizing CPY inclusion bodies in a buffer containing 50mM Tris-HCl/3mM EDTA buffer (pH 8.0), and 6M GdmCl. EX1009, Abstract, 2. Further, Hahm discloses that the CPY inclusion bodies were solubilized in a "solubilization buffer containing the reducing agents such as 100 mM β-mercaptoethanol and 300 mM dithiothreitol [DTT], and then refolding was carried out as described above." *Id.*, 5-6. GdmCl, a guanidinium salt, is a denaturant identified in claim 14, and DTT and β-mercaptoethanol are reductants identified in claims 15 and 23. EX1001, 22:67-23:2, 23:3-5, 24:1-3; EX1002, ¶216.

Hahm discloses claims 17, 18, and 26. Hahm discloses refolding of CPY in a renaturation buffer that includes NaCl and Tris-HCl. EX1009, Abstract, 2, 4-5. NaCl (an osmolyte) and Tris-HCl are well-known protein stabilizers and/or aggregation suppressors and are identified in claims 17, 18, and 26. EX1001, 2:48-

57, 5:57-62, 14:44-49, 14:50-54, 23:8-18, 24:7-17; EX1023, 5; EX1024; EX1002, ¶217.

Hahm discloses claims 21 and 29. Hahm describes a method including isolating the protein after elution from the separation matrix. For example, Hahm discloses that "the refolded CPY was purified using *p*-aminobenzylsuccinic acid affinity chromatography" and that "[t]he CPY fraction collected was dialyzed against 50 mM Mes buffer (pH 6.0) containing 1 mM EDTA and then stored at 4ºC." EX1009, 6. Hahm further details the results of the purification and "recovery yield" in Table 1. *Id.*; EX1002, ¶218.

Accordingly, Hahm anticipates the above-challenged claims. *See* EX1002, ¶¶203-219.

E. Ground 5: Dietrich Anticipates Claims 9-10, 13-15, 17-21, 23, and 26-30

1. Dietrich discloses every limitation of claim 9

a. "A method of purifying a protein…comprising:"

Dietrich discloses methods for purifying recombinant G-CSF expressed in a non-native limited solubility form using non-mammalian expression systems such as *E. coli.* EX1008, ¶¶[0016], [0058], [0063]-[0067]. Dietrich discloses that using *E. coli* expression systems for expressing G-CSF, and the resulting inclusion bodies, was known in the art. *Id.*, ¶[0060]; EX1002, ¶220.

b. "(a) solubilizing the expressed protein in a solubilization solution comprising…"

Dietrich discloses solubilizing the expressed G-CSF using a solubilization buffer containing 30mM Tris, 1mM EDTA, 6.0M guanidine-HCl, and 100mM GSH at pH 8.0. EX1008, ¶[0068]. Guanidine-HCl (otherwise known as guanidinium chloride) is the hydrochloride salt of guanidine, which is a strong chaotrope and denaturant. EX1001, 2:43-45, 4:46-47, 5:41-42, 14:4-7, 19:22-23, 22:66-23:1. GSH (glutathione) is a reductant. EX1001, 3:8-10; 14:13-15; 23:3-5. EX1002, ¶221.

c. "(b) forming a refold solution comprising…"

Following solubilization, Dietrich discloses forming a refold solution comprising the solubilization solution and a refold buffer containing 30mM Tris, 2mM GSSG, 2mM GSH, and 3M urea at pH 7.5. EX1008, ¶[0069]. It was known that GSSG (glutathione disulfide) and GSH are redox components of glutathione, corresponding to the oxidized and reduced states, respectively. EX1001, 2:57-60, 4: 50-51, 14: 55-58, 23:19-22, 24:18-21. Urea is a well-known denaturant. *Id.*, 2:43-45, 4:46-47, 5:41-42, 14:4-7, 19:22-23, 22:66-23:1. EX1002, ¶222.

Further, the '997 patent teaches, and it was well known in the art, that Tris is a protein stabilizer and aggregation suppressor. *Id.*, 2:48-56, 5:54-62, 14:44-48; 14:50-54; EX1004; EX1002, ¶132. Dietrich thus discloses this limitation.

d. "(c) applying the refold solution…"

Dietrich discloses filtering the refold solution using depth filtration and then applying the refold solution to a separation matrix. EX1008, ¶¶[0032]-[0034], [0070]. Dietrich discloses that "[s]ubsequently to refolding, the refolding step is filtrated before the first chromatographic step is conducted." *Id.*, ¶[0070]. Dietrich further discloses loading the filtered refold solution onto a cation exchange chromatography column packed with a non-affinity resin, SP Sepharose XL matrix. *Id.*, ¶¶[0035]-[0036], [0071]-[0072]; EX1019, 46; EX1002, ¶223.

Dietrich thus discloses "applying the refold solution to a separation matrix," including without intervening steps of dilution, centrifugation, dialysis, or precipitation. Accordingly, Dietrich discloses this limitation under either construction of the term. EX1002, ¶223; *see* Section V(D) *supra*.

Dietrich further discloses applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Specifically, Dietrich discloses adjusting the pH of the refold solution to pH 3.2 prior to applying the refold solution to a cation exchange column. EX1008, ¶[0070]. A POSA would have understood that the purpose of such pH adjustment was to optimize the condition for proteins to bind to a negatively-charged separation matrix in cation exchangers, because the net charge of most proteins at low pH

values is positive. EX1002, ¶224. Dietrich also discloses equilibrating the SP Sepharose XL matrix with 1.5 column volumes of 20mM sodium acetate, pH 5.0, followed by loading the refold solution to the column, and successfully eluting G-CSF from the column. EX1008, ¶[0072]. Dietrich further discloses that "[t]he purity of the eluted G-CSF was determined by means of rpHPLC; it was higher than 80%" and that "[t]he activity of the G-CSF obtained by the method according to the present invention was determined." *Id.*, ¶¶[0072], [0082]; EX1002, ¶224.

e. "(d) washing the separation matrix; and (e) eluting the protein from the separation matrix"

Dietrich discloses that washing the separation matrix "with 1.5 column volumes washing buffer (20 mM sodium acetate, pH 5.0)." EX1008, ¶[0072]. Dietrich further discloses that "[s]ubsequently, the G-CSF was eluted from the column with 3 column volumes elution buffer (20 mM sodium acetate, 200 mM NaCl, pH 5.0)." *Id.*; *see also id.*, ¶[0088]; EX1002, ¶225.

2. Dietrich discloses every limitation of claims 10, 13-15, 17-21, 23, and 26-30

Dietrich discloses claims 10 and 13. Dietrich discloses the purification of recombinant G-CSF expressed in *E. coli* as inclusion bodies. EX1008, ¶¶[0058], [0060], [0063]-[0067]; EX1002, ¶227.

Dietrich discloses claims 14, 15, and 23. Dietrich discloses solubilizing the expressed protein using a solubilization solution that contains a denaturant,

guanidine-HCl (a guanidinium salt), and a reductant, glutathione (GSH). EX1008, ¶[0068]. Dietrich also discloses a refold buffer containing a denaturant, urea. *Id*., ¶[0069]; EX1002, ¶228.

Dietrich discloses claims 17, 18, 19, 26, and 27. Dietrich discloses a refold buffer that includes Tris, which the '997 patent lists as a protein stabilizer and aggregation suppressor. EX1008, ¶[0069]; EX1001, 2:48-56, 5:54-62, 14:44-48; 14:50-54; EX1002, ¶229. Tris was known in the art as a protein stabilizer and aggregation suppressor. *See* EX1023, 5; EX1075; EX1076; EX1002, ¶132. Dietrich also discloses redox components of glutathione, *i.e.*, GSH (reduced state) and GSSG (oxidized state), present in a refold buffer. EX1008, ¶[0069]; EX-1002, ¶230.

Dietrich discloses claims 20 and 28. Dietrich discloses purification of refolded G-CSF by non-affinity ion exchange resins, specifically, a cation exchange column packed with SP Sepharose XL matrix, and a hydrophobic interaction column packed with Phenyl Sepharose HP resin. EX1008, Abstract, ¶¶[0035]-[0038], [0050], [0071]-[0076]; EX1019, 46; EX1002, ¶231.

Finally, Dietrich discloses claims 21, 29, and 30. Dietrich discloses that the eluted G-CSF was further subjected to analysis by rpHPLC to determine its purity at "higher than 80%," and that "the yield was also higher than 80%." EX1008,

¶[0072]. Thus, Dietrich discloses isolating the protein after elution from the separation matrix. EX1002, ¶232.

Accordingly, Dietrich anticipates the above challenged claims. *See* EX1002, ¶¶220-223.

F. Ground 6: Claims 15, 19, 23, 27, and 28 Are Obvious over Ferré, Komath, or Dietrich in View of Rosendahl

Claims 15, 19, 23, 27, and 28 are unpatentable as obvious over Ferré, Komath, or Dietrich in view of Rosendahl. As discussed in Sections VII(A)(1), VII(B)(1), and VII(C)(1) *supra*, each of Ferré, Komath, and Dietrich expressly discloses every limitation of claim 9. A POSA would have further understood in view of Rosendahl that the methods of Ferré, Komath, or Dietrich could be performed using the particular reductant and redox components described in or required by claims 15, 19, 23, 27, and 28.

Claims 15 and 23 require that the reductant of the solubilization solution comprise one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol, and glutathione. EX1001, 23:3-5, 24:1-3. Claims 19, 27, and 28 require 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.*, 23:19-22, 24:18-21. EX1002, ¶235.

Rosendahl teaches 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, ¶237.

Rosendahl also 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 teaches 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, ¶240.

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 teaching of particular reductants/redox components that are successfully able to solubilize and refold aggregated proteins. EX1002, ¶¶238-239, 241-242. Indeed, Rosendahl teaches 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]. The advantageous components disclosed by Rosendahl would have been particularly attractive to a POSA interested in producing biologically active proteins "in high yield," as described in Ferré, Komath, and Dietrich. *Id.*, ¶[0013]; EX1004, 10; EX1005, 5; EX1008, ¶[0013]; EX1002, ¶238. Thus, a POSA would have been motivated to use the solubilization solution and refold buffer components of Rosendahl in the methods taught by Ferré, Komath, or Dietrich to a create a more efficient and cost effective method of solubilizing, refolding, and purifying proteins. *See* EX1004, 2, 10; EX1005, 5; EX1008; EX1002, ¶239. A POSA would have reasonably expected that using these 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, ¶239.

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, ¶241. Ferré, Komath, and Dietrich each describes refolding of proteins having disulfide bonds. EX1004, Abstract; EX1005, 2; EX1008, ¶[0009]; EX1053; EX1054; EX1006, ¶[0009]; EX1002, ¶242. 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 components of Rosendahl in the methods of Ferré, Komath, or Dietrich to allow for proper formation of the disulfide bond(s) of each method's protein of interest. EX1002, ¶242. A POSA would have reasonably expected such use of the components of Rosendahl would result in successful formation of the protein's native disulfide bonds. *Id.* Accordingly, claims 15, 19, 23, and 27, are obvious over each of Ferré, Komath, or Dietrich, in view of Rosendahl. *Id.*, ¶¶234-246.

Notably, Rosendahl does not teach away from using its disclosed components with a method that involves "applying the refold solution to a separation matrix," under either construction of this term. While Rosendahl discloses an example where a refold solution is clarified using centrifugation before it is loaded onto a chromatography column (*e.g.*, EX1006, ¶[0060]), Rosendahl does not teach that this step is necessary when using the particular reductants/redox components in the refold buffer in order for the target protein to associate successfully with the separation matrix. EX1002, ¶243. *See Bayer Pharma AG v. Watson Labs., Inc.*, 874 F.3d 1316, 1327 (Fed. Cir. 2017).

Moreover, this step is expressly permitted under the plain meaning construction of this term.

Finally, claim 28 depends from claim 19 and requires that the method be performed with an affinity or non-affinity separation matrix. Ferré, Komath, and Dietrich each discloses the use of a non-affinity separation matrix. *See supra* pp. 40, 47, 56, 64, and 66. Further, Rosendahl teaches using known methods to purify the refolded protein, including using IEX. EX1006, ¶[0042]. Thus, claim 28 is also obvious over the asserted combination. EX1002, ¶246.

G. No Objective Indicia of Nonobviousness

The prosecution history of the '997 patent and its parent '878 patent are devoid of any evidence of objective indicia of nonobviousness. Instead, the specification of the '997 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." EX1001, 15:58-67. These unsupported assertions, however, are not probative of nonobviousness because it was well-understood in the art that a refold solution could be applied to a separation matrix and result in successful protein separation.

See Section III(C) *supra*; EX1002, ¶¶247-250. *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). Where a strong obviousness showing exists—such as is the case here—the Federal Circuit has held that even 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 secondary considerations, the challenged claims would have been obvious over the art cited herein.

VIII. CONCLUSION

For the foregoing reasons, Petitioner requests IPR and cancellation of claims 9-10, 13-15, 17-21, 23, and 26-30 of the '997 patent.[11]

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 '997 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.), *Amgen Inc. et al. v. Mylan Inc. et al.*, No. 2:17-cv-01235-MRH (W.D. Pa.), and *Amgen Inc. et al. v. Hospira Inc. and Pfizer Inc.*, No. 1:18-cv-01064-CFC (D. Del.).

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[11] 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 is pending and claims priority to the '997 patent.

The '997 patent claims priority to the following applications: (1) U.S. Application No. 12/822,990 (issued as U.S. Patent No. 8,940,878); and (2) U.S. Provisional Application No. 61,220,477.

U.S. Patent No. 8,940,878 is also subject to IPR2019-00791, 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 '997 patent is available for IPR and that Petitioner is not barred or estopped from requesting IPR of any claim of the '997 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 '997 patent.

Respectfully submitted,

Date: March 7, 2019

By: / Rolando Medina /

Rolando Medina, Reg. No. 54,756
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CERTIFICATE OF COMPLIANCE

I hereby certify that the foregoing Petition contains **13,971** 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. 9,643,997**, 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 '997 Patent:

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

Respectfully submitted,

Date: March 7, 2019

By: / Rolando Medina /

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