UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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

AMGEN, INC.
Patent Owner.

IPR2019-01183

Patent No. 9,643,997

Title: CAPTURE PURIFICATION PROCESSES FOR PROTEINS EXPRESSED IN A NON-MAMMALIAN SYSTEM

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,643,997 B1

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TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

Page

TABLE OF AUTHORITIES

Page(s)

Cases

Statutes

Regulations

LIST OF EXHIBITS

EXHIBIT NO.	DESCRIPTION
1001	U.S. Patent No. 9,643,997, *Capture Purification Processes For Proteins Expressed in a Non-Mammalian System* (filed on January 16, 2015) (issued May 9, 2017)
1002	Declaration of Dr. Peter M. Tessier, Ph.D. in Support of Petition for *Inter Partes* Review of Patent No. 9,643,997
1002A	*Curriculum Vitae* of Dr. Peter M. Tessier, Ph.D.
1002B	Materials Reviewed by Dr. Peter M. Tessier, Ph.D.
1003	Wang, C. *et al*., "Solubilization and Refolding with Simultaneous Purification of Recombinant Human Stem Cell Factor," Appl Biochem Biotechnol (2008) 144:181-189 (2008) ("Wang")
1004	United States Patent Application Publication No. 2006/0172384, *FGF18 Production in Prokaryotic Hosts* (filed on December 12, 2005) (published August 3, 2006) ("Reardon")
1005	United States Patent Application Publication No. 2008/0260684, *Method for the Purification of G-CSF* (filed on July 14, 2006) (published October 23, 2008) ("Dietrich")
1006	International Publication No. WO 2006/097944, *Process for the Purification of Recombinant Garnulocyte-Colony Stimulating Factor* (filed on March 13, 2006) (published September 21, 2006) ("Komath '944")
1007	International Publication No. WO 2004/001056, *Process for Preparing G-CSF* (filed on June 24, 2002) (published December 31, 2003) ("Komath '056")
1008	Creighton, T., *Encyclopedia of Molecular Biology* (John Wiley & Sons, vols. 1-4, 1999)

EXHIBIT NO.	DESCRIPTION
1009	Whitford, D., *Proteins: Structure and Function* (John Wiley & Sons 2005)
1010	Horton, R. *et al.*, *Principles of Biochemistry* (Pearson Prentice Hall, 4th ed. 2006)
1011	Stirling, P. *et al*., "Getting a grip on non-native proteins," European Molecular Biology Organization 4(6):565-570 (2003)
1012	Profacgen, "Inclusion body purification & protein refolding," accessed at https://www.profacgen.com/inclusion-body-purification-protein-refolding.htm
1013	Intentionally Omitted
1014	Georgiou, G. & Valax, P., "Isolating Inclusion Bodies from Bacteria," Methods in Enzymology 309:48-58 (1999)
1015	De Bernardez Clark, E. et al., "Oxidative Renaturation of Hen Egg-White Lysozyme. Folding vs Aggregation," Biotechnology Progress 14(1):47-54 (1998)
1016	De Bernardez Clark, E., "Protein refolding for industrial processes," Current Opinion in Biotechnology 12(2):202-207 (April 2001)
1017	Bollag, D. *et al.*, *Protein Methods*, (John Wiley & Sons, 2nd ed. 1996)
1018	Deutscher, M. *Methods in Enzymology: Volume 182 Guide to Protein Purification* (Academic Press, 1999)
1019	Neubauer, P. *et al*., "Protein Inclusion Bodies in Recombinant Bacteria," in *Inclusions in Prokaryotes*, ed. J.M. Shively, pp. 237-292 (Springer-Verlag Berlin Heidelberg 2006)
1020	Jungbauer, A. & Kaar, W., "Current status of technical protein refolding," Journal of Biotechnology 128:587-596 (2007)
1021	De Bernardez Clark, E., "Refolding of recombinant proteins," Current Opinion in Biotechnology 9:157-163 (1998)

EXHIBIT NO.	DESCRIPTION
1022	United States Patent Application Publication No. 2007/0238860, *Method for Refolding A Protein* (filed on April 3, 2007) (published October 11, 2007)
1023	Intentionally Omitted
1024	Intentionally Omitted
1025	Intentionally Omitted
1026	Intentionally Omitted
1027	*Protein Purification - Handbook* (Amersham Biosciences, ed. AC, 2001)
1028	Cutler, P., *Protein Purification Protocols* (Humana Press, 2nd ed., 2004) ("Cutler")
1029	Intentionally Omitted
1030	Intentionally Omitted
1031	GE Healthcare, *Recombinant Protein Purification Handbook: Principles and Methods* (General Electric Company, 2000)
1032	Intentionally Omitted
1033	File History for U.S. Patent No. 9,643,997
1034	Amgen's Opening Claim Construction Brief, dated June 1, 2018
1035	Amgen's Reply Claim Construction Brief, Dated July 20, 2018
1036	*Ion Exchange Chromatography & Chromatofocusing: Principles and Methods* (Amersham Biosciences, ed. AA, 2004)
1037	GE Healthcare, *Purifying Challenging Proteins: Principles and Methods* (General Electric Company, 2007)

EXHIBIT NO.	DESCRIPTION
1038	Wang, X. *et al*., "Perturbation of the antigen-binding site and staphylococcal Protein A-binding site of IgG before significant changes in global conformation during denaturation: an equilibrium study," Biochem. J. 325:707-710 (1997)

I. INTRODUCTION

Fresenius Kabi USA, LLC and Fresenius Kabi SwissBioSim GmbH, pursuant to 35 U.S.C. §§ 311-319 and 37 C.F.R. § 42, *et seq.*,[1] petition for *Inter Partes* Review ("IPR") of claims 9-10, 13-21, and 23-30 of U.S. Patent 9,643,997 ("the '997 patent," Ex. 1001). Petitioners' request is supported by the Expert Declaration of Peter Tessier, Ph.D. (Ex. 1002) and the other exhibits submitted herewith.

The challenged claims of the '997 patent are broadly directed to the purification of any limited soluble proteins expressed in non-mammalian cells by (a) solubilizing the protein in a solution containing reagents that cause the proteins to disaggregate and unfold, (b) forming a refold solution by diluting the solubilization solution into a buffer containing reagents that allow the proteins to properly refold, (c) applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix, (d) washing the matrix, and (e) eluting the protein. As of the earliest priority date of the '997 patent, this sequence of steps was routinely used to recover and purify recombinant proteins expressed in bacterial cells, and was widely disclosed in prior-art

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

publications. While Patent Owner ("PO") has asserted that the claimed process improved upon the prior art by eliminating the perceived need for removing components of the refold solution before applying the protein to the matrix, the prior art demonstrates that skilled artisans understood that such intervening steps were not necessary for the purification of all proteins using all separation matrices (as encompassed by the challenged claims), and had already identified ways to avoid these intervening steps.

As described below, each of the challenged claims is anticipated by the prior art. To the extent a single reference does not disclose every element of a challenged claim, every element was disclosed in the prior art and there was motivation to combine these elements with a reasonable expectation of success, rendering the claimed subject matter obvious. Petitioners are not aware of any secondary evidence of non-obviousness that are commensurate in scope with the challenged claims. Ex. 1002 at ¶ 263–65.

The Board should institute review because there is at least a reasonable likelihood that Petitioners will prevail with respect to at least one challenged claim. §314(a). Moreover, although an unrelated party, Kashiv BioSciences, LLC, has filed an IPR petition challenging the '997 patent (IPR2019-00797), there are no persuasive grounds for denying institution under §314(a) or §325(d), including the factors outlined in *General Plastic Industrial Co., Ltd., v. Canon Kabushiki*

Kaisha, IPR2015-01954, Paper 19 (P.T.A.B. Sept. 6, 2017). Petitioners drafted this petition independently of Kashiv, consulted their own expert, developed new arguments, and relied on three prior art references not raised in the Kashiv petition or otherwise presented to the Board. Petitioners are filing their petition before the PO files its response to Kashiv's petition, and thus, have not gained any unfair advantage. Petitioners are also amenable to proceeding on a coordinated schedule with Kashiv's IPR to minimize any additional burden on the Board and the PO.

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

II. GROUNDS FOR STANDING

As required by § 42.104(a), Petitioners certify that the '997 patent is available for IPR and that the Petitioners are not barred or estopped from requesting IPR on the grounds identified herein.

III. MANDATORY NOTICES

A. Real Parties-in-Interest (§ 42.8(b)(1))

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

B. Related Matters (§ 42.8(b)(2))

The ’997 patent is currently the subject of the following litigations: *Kashiv Biosciences, LLC v. Amgen, Inc.*, No. IPR2019-00797 (P.T.A.B.); *Sandoz, Inc. v. Amgen Inc.*, No. 19-cv-00977 (N.D. Cal.); *Amgen Inc. v. Adello Biologics LLC*, No. 2:18-cv-03347 (D.N.J.), *Amgen Inc. v. Mylan Inc.*, No. 2:17-cv-01235 (W.D. Pa.); and *Amgen Inc. v. Hospira Inc.*, No. 18-cv-01064 (D. Del.).

In addition, U.S. Patent Application 15/476,691 is pending and claims priority to the ’997 patent.

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

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IV. TECHNICAL BACKGROUND

A. Protein Synthesis

Proteins are molecules made up of subunits known as "amino acids" joined into chains by "peptide bonds." Ex. 1002 at ¶ 42. Proteins can perform their biological function only if this "polypeptide" chain is properly folded into a three-dimensional shape known as its "native" form or structure. Ex. 1002 at ¶ 42. The cells of both mammals (such as mice or humans) and non-mammals (such as yeasts and bacteria), naturally create proteins by "transcribing" information encoded in DNA into an RNA molecule and "translating" that information into a sequence of amino acids. Ex. 1002 at ¶ 43.

For several decades, scientists have artificially synthesized "recombinant proteins" in laboratory and commercial settings by constructing recombinant DNA molecules, inserting the recombinant DNA into host cells, and incubating the cells to allow them to replicate and express the protein of interest on a large scale. Ex. 1002 at ¶ 44-45. Recombinant proteins can be expressed in mammalian or non-mammalian cells, and the bacteria *Escherichia coli* ("*E. coli*") has been among the

most widely used host systems for large-scale production of proteins. Ex. 1002 at ¶ 46. However, bacterial host cells like *E. coli* often have difficulty producing properly refolded recombinant proteins, and the misfolded proteins clump together within the cells into insoluble, biologically inactive aggregates known as "inclusion bodies." Ex. 1002 at ¶ 48. By 2009, scientists had devised various techniques for recovering functional and therapeutically useful proteins from such inclusion bodies. Ex. 1002 at ¶ 49–50.

B. Recovery and Purification of Bioactive Recombinant Protein

As of 2009, a typical procedure for recovering functional proteins from inclusion bodies involved (1) isolating the inclusion bodies from host cells; (2) solubilizing the inclusion bodies to unfold the aggregated proteins; and (3) refolding the proteins into their native shape. Ex. 1002 at ¶ 51; Clark 1998 (Ex. 1015) at 47. More specifically, first, the host cells were "lysed" to release their contents, the resulting suspension was centrifuged to separate the inclusion bodies from other cell contents, and the inclusion bodies were washed to remove contaminants. Ex. 1002 at ¶ 53. Second, the isolated inclusion bodies were solubilized in a solution containing reagents, such as surfactants, denaturants, and reductants, that released the desired proteins from the inclusion bodies by disrupting bonding networks among the misfolded proteins. Ex. 1002 at ¶ 54–55. Third, the unfolded proteins were allowed to refold into their native bioactive

structure by changing the components or properties of their surrounding solution. Ex. 1002 at ¶ 56. One of the simplest refolding methods was directly diluting the solubilization solution containing the protein into a "refold solution," which contained components that promoted formation of native hydrogen and di-sulfide bonds while minimizing protein aggregation. Ex. 1002 at ¶ 57–58.

Proteins that were recovered using these steps were often purified using a number of common chromatography methods. Ex. 1002 at ¶ 52, 59. One such chromatography method relied on three basic processes: (1) "capture" of the protein onto the "separation matrix" of the chromatography column based on certain interactions between the matrix and the protein, (2) "washing" of the matrix using one or more solutions that promoted dissociation of contaminants from the matrix, and (3) "elution" of the protein using one or more solutions that promoted dissociation of the protein from the matrix. Ex. 1002 at ¶ 59, 64–65. Chromatography methods were characterized based on the type of association between the protein and the matrix: "affinity" chromatography methods, such as Protein A chromatography, captured proteins based on their interactions with a specific ligand, while "non-affinity" chromatography methods, such as ion-exchange chromatography and hydrophobic interaction chromatography, captured proteins based on general properties of the molecule, such as size, isoelectric point, or hydrophobicity. Ex. 1002 at ¶ 60–61.

After refolding but before purifying a protein solution by chromatography, the solution was sometimes processed via intervening steps to remove either insoluble particulates or components of the solution. Techniques such as depth filtration were used to remove insoluble debris that would clog the matrix. Ex. 1002 at ¶ 62. At times, methods such as dialysis, precipitation, and ultrafiltration were used to remove components of the solution that sometimes interfered with the association of the proteins with certain chromatography matrices. Ex. 1002 at ¶ 62. However, scientists did not believe that removal of reagents from the refold solution was always necessary for all forms of chromatography, and had developed protein purification protocols that did not include such intervening steps. Ex. 1002 at ¶ 63; Exs. 1003-1007.

V. THE '997 PATENT

The '997 patent is titled "Capture Purification Processes for Proteins Expressed in a Non-Mammalian System," and claims the earliest possible priority date of June 25, 2009.

The '997 patent is generally directed to the purification of proteins expressed in both soluble and limited-solubility forms in non-mammalian systems. The specification focuses on problems observed during the purification of proteins containing "Fc" regions (Fc-containing proteins) using affinity chromatography methods such as Protein A chromatography. Ex. 1001 at 1:20-60. Specifically, the

patent asserts that "a drawback to the use of Protein A, Protein G and other chemistries is that in order for a protein comprising an Fc region to associate with the Protein A or Protein G molecule, the protein needs to have a minimum amount of structure." *Id.* at 1:34–36. The patent further states that "[i]n the case of a protein expressed in an insoluble non-native form," proteins must typically be both refolded and diluted out of its refold solution before purification by Protein A chromatography because

> it was believed that after a protein has been refolded it was necessary to dilute or remove the components of the refold mixture in a wash step, due to the tendency of the components that typically make up a refold solution to disrupt interactions between the target protein and the Protein A molecules (Wang et al., (1997). . . .)

Id. at 1:41-52. The patent purports to "address[] these issues by providing simplified methods of purifying proteins comprising Fc regions . . . ." *Id.* at 1:56–58. In particular, the patent purports to disclose a method that "omits the need for removing any components of the refold mixture before the refold mixture is applied to a separation matrix," thereby "saving steps, time and resources that are typically expended on removing the protein from refolding and dilution buffers in purification processes." *Id*. at 4:66-5:4; Ex. 1002 ¶ 69.

Examples 2 and 3 of the '997 patent describe methods of recovering and purifying proteins Fc-containing proteins expressed in limited-solubility forms.

Example 2 describes a procedure for purifying proteins using affinity chromatography (specifically, Protein A chromatography), and Example 3 describes procedures for purifying proteins using non-affinity chromatography (specifically, cation exchange chromatography). Ex. 1002 at ¶ 72–74; Ex. 1001 at 18:58-21:14.

The challenged claims, 9–10, 13–21, and 23–30, are each directed to purification of proteins expressed in limited-solubility forms using either affinity or non-affinity chromatography; they are not limited to the purification of Fc fusion proteins or to purification using affinity chromatography. Ex. 1002 at ¶ 76.

Claim 9, the only independent claim challenged, recites a "method of purifying a protein" expressed "in a non-native limited solubility form" in a "non-mammalian expression system" comprising the following step: (a) "solubilizing the expressed protein in a solubilization solution," (b) "forming a refold solution comprising the solubilization solution and a refold buffer," (c) "applying the refold solution to a separation matrix[2] under conditions suitable for the protein to

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[2] Defined in the '997 patent as "any media by which the protein of interest can be separated from the components of the resuspension and/or lysis buffer, including impurities such as host cell proteins, DNA and chemical impurities introduced by the components of the solubilization and/or lysis buffer." Ex. 1001 at 15:25-30.

associate with the matrix," (d) "washing the separation matrix," and (e) "eluting the protein" from the matrix. Ex. 1001 at 22:36-55. The claim further recites the following components of the "solubilization solution" and "refold buffer," respectively:

Solubilization Solution	Refold Buffer
". . . one or more of the following: (i) a denaturant; (ii) a reductant; and (iii) a surfactant"	". . . one or more of the following: (i) a denaturant; (ii) an aggregation suppressor; (iii) a protein stabilizer; and (iv) a redox component"

Examples of these components are provided in the specification at 5:45–53; 5:35-44; 5:54–63; 14:10-16; 14:10-16; and 15:25-30, and in the dependent claims listed below. Ex. 1002 ¶ 96. The components of each solution are listed in the alternative, and do not contain any limitations as to relative or absolute concentration (nor does any of the challenged dependent claims discussed below).

The remaining challenged claims depend either directly or indirectly from claim 9 and include the following additional limitations:

Claim(s)	Depends from	Additional Limitation
10	claim 9	wherein the non-native limited solubility form is a component of an inclusion body.
13	any one of claims 9-12	wherein the non-mammalian expression system comprises bacteria or yeast cells.

Claim(s)	Depends from	Additional Limitation
14	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.
15, 23	claims 9, 14, respectively	wherein the reductant comprises one or more of cysteine, dithiothreitol, beta-mercaptoethanol and glutathione.
16, 24	claims 9, 15, respectively	wherein the surfactant it comprises one or more of sarcosyl and sodium dodecylsulfate.
17, 25	claims 9, 16, respectively	wherein the aggregation suppressor is selected from the group consisting of arginine, profile, polyethylene glycols, nonionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.
18, 26	claims 9, 17, respectively	wherein the protein stabilizer comprises one or more of arginine, profile, polyethylene glycols, nonionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.
19, 27,	claims 9, 18, respectively	wherein the redox component comprises one or more of glutathione-reduced, glutathione-oxidized, cysteine, cysteine, cysteamine, cystamine and beta-mercaptoethanol.
20, 28	claims 9, 19, respectively	wherein the separation matrix is either (i) an affinity resin, selected from the group consisting of Protein A, Protein G, and synthetic mimetic affinity resin; or (ii) a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin

Claim(s)	Depends from	Additional Limitation
21, 29, 30	any one of claims 1 or 9–12; claim 13; and claim 20, respectively	wherein the protein is isolated after elution from the separation matrix

Notably, because claim 9 recites the components of the solubilization solution and refold buffer in the alternative, and the additional claim limitations recited in dependent claims 14–19 and 23–27 merely limit the scope of one of these components to certain reagents, under a plain reading, these dependent claims do not require use of one of the recited chemicals, so long as one of the alternative components recited in claim 9 is present in the solubilization solution or refold buffer.

VI. PERSON OF ORDINARY SKILL IN THE ART

The person of ordinary skill in the art ("POSA") to which the '997 patent is directed would have had a Ph.D. in biochemistry, biology, chemical engineering, biomedical engineering or bioengineering and several years' experience in the recovery and purification of recombinant proteins from non-mammalian expression systems. In the alternative, the POSA would have had an equivalent level of education and experience, including a Bachelor's or Master's degree with more practical work experience in the above fields. Ex. 1002 at ¶¶ 66–67. This person would have worked in collaboration with other scientists and/or clinicians

with experience in the design and expression of recombinant proteins, biochemical manufacturing, pharmaceutical development of biologics, therapeutic use of biologics, or related areas. *Id.*

VII. CLAIM CONSTRUCTION

Each term of challenged claims should be construed in accordance with its ordinary and customary meaning as understood by a POSA in light of the patent specification and the prosecution history. §42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005) (*en banc*). Accordingly, any claim term not expressly defined in the specification or discussed below should be given its ordinary and customary meaning to a POSA as of the filing date of the '997 patent.[3]

A. Terms for Construction

1. "applying the refold solution to the separation matrix"

In district court litigation, PO asserted that the term "applying the refold solution to the separation matrix," means "applying the refold solution to a column that contains the separation matrix without intervening steps of dilution,

[3] Petitioners adopt the identified claim construction positions for purposes of this Petition based on currently available information and reserve the right to change or modify their positions in response to statements made by Amgen in this or other proceedings, court rulings, or other information that becomes available. Petitioners do not waive any argument concerning invalidity under §112.

centrifugation, dialysis, or precipitation." The district court adopted this construction, although the parties did not dispute whether an intervening "dilution step" was excluded from the scope of the claims. *Amgen v. Mylan*, No. 17-cv-01235-MRH, (W.D. Pa.), Amgen's Opening Claim Construction Brief (Ex. 1034) at 10-11.

As Dr. Tessier explains, this construction is inconsistent with the plain meaning of the term to the POSA when read in light of the claim as a whole, the specification, and file history, at least with respect to the exclusion of any pre-chromatography dilution of the refold solution. First, the preamble of claim 9 recites "a method . . . comprising," and the word "comprising," when used in transitioning from the preamble to the body of a claim "signals that the entire claim is presumptively open-ended," and allows for additional steps. *Gillette Co. v. Energizer Holdings Inc.*, 405 F.3d 1367, 1371-73 (Fed. Cir. 2005). There is no intrinsic evidence sufficient to overcome this presumption, at least with respect to dilution of the refold solution.

Second, as explained by Dr. Tessier, a plain reading of the words "applying the refold solution to the separation matrix" would not exclude dilution of the refold solution by, for example adding water, prior to chromatography, as all components of the refold solution are ultimately applied to the matrix. Ex. 1002 at ¶ 86–87.

Third, Example 3 of the ’997 patent, which is the only example of the ’997 patent describing purification of a protein expressed in limited-solubility form using non-affinity chromatography, describes a 3-fold dilution of the refold solution (among other intervening steps) prior to chromatography. Specifically, Example 3 states “an aliquot of protein” was “sampled directly from a refold solution, was diluted 3-fold with water, titrated with 50% hydrochloric acid to ~pH 4.5 and was filtered through a series of depth and/or membrane filter to remove particulates” before loading onto a cation exchange resin. Ex. 1001 at 20:56-62. While elsewhere, the specification states that an advantage of the claimed methods is elimination of the “need” to perform an intervening dilution step, (e.g. Ex. 1001 at 3:53–57), the patent as a whole does not clearly convey that omission of such a step is a limitation of the claimed methods. Ex. 1002 at ¶ 88.

Fourth, during prosecution, PO distinguished prior-art methods based on the inclusion of certain intervening steps, i.e., “dialysis, precipitation, and centrifugation,” but did not discuss dilution. Ex. 1033 at 102-103 (October 2, 2015 Non-Final Office Action). Specifically, in a non-final office action, the Examiner rejected claim 9 and its dependent claims as anticipated by U.S. Patent 7,138,370 (“Oliner”) and as obvious over a combination of Oliner and a second prior art reference disclosing components of a solubilization solution. *Id.* In response to

the rejection, PO contrasted the process disclosed in Oliner with the claimed process as follows:

> 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 centrifugatio***n. *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*].

Id. at 102[4]. In light of PO's arguments, the Examiner withdrew the rejection of claim 9 and its dependent claims. There is no such disclaimer distinguishing the claimed invention from the prior art on the basis of a dilution step.

Accordingly, while for the purposes of this Petition, Petitioners take no position on whether the challenged claims allow other intervening processes

[4] Unless otherwise stated, all emphases in this Petition are added.

between forming the refold solution and applying the solution to the separation matrix, a POSA would not construe the term "applying the refold solution to the separation matrix" to exclude an intervening step of dilution, at least on the scale of a 3-fold water dilution described in Example 3 of the '997 patent. Ex. 1002 ¶ 90.

2. "washing" and "eluting/elution"

In district court, PO has asserted that the term "washing the separation matrix" which appears in challenged claim 9, means "applying a solution to the column that contains the separation matrix, which application has the effect of removing unbound protein, lysate, impurities, and unwanted components of the refold solution from the separation matrix while preserving interactions between the protein and the separation matrix." Ex. 1034 at 18–19.

PO has also asserted that the terms "eluting/elution," which appear in challenged claims 9, 21, and 29, means "applying a solution to the column that contains the separation matrix, which application has the effect of reversing the interactions between the protein and the separation matrix." *Id.* at 19–20.

For the purposes of this Petition, Petitioners adopt these constructions of the term "washing" and "eluting/elution."

3. "isolated after elution"

As explained by Dr. Tessier, a POSA would understand the term "isolated after elution" to refer to the protein separated from other components in the eluate collected after the chromatography, which has been purified in comparison to the refold solution loaded onto the column. Ex. 1002 ¶ 95. The term should not be construed to require a separate and subsequent purification step (although as discussed below, at least Komath '944 (Ex. 1006) and Reardon (Ex. 1004) disclose this limitation if so construed). Ex. 1002 ¶ 95.

VIII. IDENTIFICATION OF CHALLENGE AND RELIEF REQUESTED

Petitioners request review and cancellation of claims 9–10, 13–21, and 23–30 of the '997 patent under sections 102 and 103 for the reasons explained in this petition, which may be summarized as follows:

Ground No.	Claims and Bases
1	The challenged claims are anticipated by Wang (Ex. 1003).
2	The challenged claims are obvious over Wang in view of Cutler (Ex. 1028).
3	The challenged claims are anticipated by Reardon (Ex. 1004).
4	The challenged claims are anticipated by Dietrich (Ex. 1005).
5	The challenged claims are obvious over Komath '944 (Ex. 1006) in view of Komath '056 (Ex. 1007).

A. Ground 1: The Challenged Claims Are Anticipated by Wang (Ex. 1003)

Wang et al., "*Solubilization and Refolding with Simultaneous Purification of Recombinant Human Stem Cell Factor*," was published in print in February 2008 and online in January 2008. Wang is a prior-art printed publication to the '997 patent under § 102(b) (pre-AIA). Wang was not cited during the prosecution of the application that issued as the '997 patent.

Wang teaches that "Recombinant human SCF (rhSCF) has been expressed in Escherichia coli by many laboratories," but the protein "often forms insoluble and inactive inclusion bodies in *E. coli*." Ex. 1003 at 182. Wang applied the well-known steps of recovering rhSCH from inclusion bodies, including "solubilization of inclusion bodies, and refolding into its native conformation." However, "in previous literature," rhSCF renatured protein was "concentrated by ultrafiltration and buffer exchanged by acid precipitation" prior to purification by chromatography, resulting in limited final yield. Specifically, Wang notes that "high concentrations of urea or guanidine hydrochloride" led to aggregation during protein refolding, which affects the ultimate yield, but that a high pH buffer can effectively solubilize proteins in inclusion proteins with improved refolding. Wang thus describes an experiment studying the effect of pH and urea in the solubilization solution on the efficiency of recovery and purification of the protein rhSCF. Ex. 1002 at ¶ 98–101.

The experiment in Wang also compares the results of a "***Refolding with Simultaneous Purification***" method with two more traditional methods involving sequential solubilization, refolding, and purification steps. Ex. 1002 at ¶ 102–105. In particular, one of the traditional methods involves ***refolding by "dilution,"*** in which "Four hundred microliters of sample solution containing the denatured rhSCF was diluted 100-fold" with a buffer containing Tris, EDTA, GSH, and GSSG, in order to generate a re-fold solution and subsequently "purified by IEC." This method describes the formation of a refold solution by combining the solubilization solution with a refold buffer containing additional components. Ex. 1002 at ¶ 104.

Wang states in the section entitled "***Refolding of rhSCF by Dilution***" that "after refolding, the rhSCF was purified by IEC. *Id*. at 184. The general procedure for purification of rhSCF by IEC is described in the section "***Refolding with Simultaneous Purification of rhSCF by IEC***" as follows:

> Four hundred microliters of sample solution containing the solubilized and denatured rhSCF was ***directly injected into the column***. After ***washing the column with 10 ml of the solution A***, the refolding with simultaneous purification of rhSCF was accomplished after a ***linear gradient elution from 100% A to 100% B*** (solution B consisted of solution A plus 1.0 $mol \cdot l^{-1}$ NaCl) in 30 min with a delay of 10 min at a flow rate of 2.0 $ml \cdot min^{-1}$.

Id.

Table 1 of Wang illustrates the results of the methods studied, showing the successful recovery and purification of active protein, as reflected by a positive mass recovery (MR) and specific bioactivity (SB), for all of the methods studied:

Table 1 Comparison of results for rhSCF solubilized and refolded by using a different method.

rhSCF sample	$SB_{dilution}$ ($IU \cdot mg^{-1}$)	$MR_{dilution}$ (%)	$SB_{dialysis}$ ($IU \cdot mg^{-1}$)	$MR_{dialysis}$ (%)	SB_{IEC} ($IU \cdot mg^{-1}$)	MR_{IEC} (%)
$rhSCF_{urea}$ [a]	$(3.3 \pm 0.94) \times 10^5$	18.8±1.53	$(4.5 \pm 1.1) \times 10^5$	16.8±1.07	$(7.6 \pm 1.8) \times 10^5$	36.4±3.10
$rhSCF_{pH}$ [b]	$(4.7 \pm 0.86) \times 10^5$	25.4±2.16	$(5.4 \pm 1.4) \times 10^5$	26.2±1.79	$(7.8 \pm 1.5) \times 10^5$	43.0±2.93

[a] $rhSCF_{urea}$ presents the rhSCF solubilized by 8.0 $mol \cdot l^{-1}$ urea.

[b] $rhSCF_{pH}$ presents the rhSCF solubilized by 0.05 $mol \cdot l^{-1}$ Tris (pH 13.0) containing 0.05 $mol \cdot l^{-1}$ Na_2HPO_4 and 2.0 $mol \cdot l^{-1}$ urea.

As described below, each and every feature of claims 9–10, 13–21, and 23–30 is disclosed in the teachings of Wang, as would have been understood by a POSA as of 2009.

1. Independent Claim 9 Is Anticipated by Wang

a. The Preamble

Wang discloses a method for purifying a protein, *i.e.* "[r]ecombinant human stem cell factor (rhSCF)" expressed in a non-native limited-solubility form, *i.e.* inclusion bodies, in a non-mammalian expression system, *i.e.* "*Escherichia coli*." Ex. 1002 at ¶ 98, 141; Ex. 1003 at 181. Therefore, Wang teaches a method of purifying a protein expressed in a non-native limited-solubility form in a non-mammalian expression system.

b. The Solubilization Step

Wang states that purified rhSCF inclusion bodies were solubilized in various solutions, including solution II that contains, *inter alia*, urea. *Id*. at 183-84. The 997 patent discloses urea as a denaturant within the definition of the 997 patent. Ex. 1002 at ¶ 101, 142. Therefore, Wang teaches the use of a solubilization solution comprising at least a denaturant.

c. The Refold Step

Wang describes a process of refolding by diluting the solution from the solubilization step using a refold buffer that contains, *inter alia*, Tris, GSH, and GSSG. Ex. 1002 at ¶ 103–05, 144. Tris is an aggregation suppressor and a protein stabilizer, as defined in the '997 patent; GSH and GSSG are redox components. Ex. 1001 at 2:48–60; 14:44–58; Ex. 1002 at ¶ 144. Thus, Wang teaches the formation of a refold solution comprising the solubilization solution and a refold buffer comprising at least a protein stabilizer and a redox component. Ex. 1002 ¶¶ 143-44.

d. The Capture or "Applying" Step

Wang teaches that, for the refold by dilution method, "after refolding, the rhSCF was purified by IEC." Ex. 1003 ¶ 184. Ion exchange resins, which IEC uses, are disclosed in the '997 patent as examples of separation matrices. Ex. 1001 at 2:60–65; 4:10–15.

Moreover, a POSA would have understood that the refold solution generated in the refold by dilution method was purified by IEC without intervening steps both because no intervening steps are disclosed and because the protocol for this arm of the experiment would have been kept as close as possible to the protocol for "Refolding with Simultaneous purification," in which the protein solution is "directly injected into the column." Ex. 1002 at ¶ 146; Ex. 1003 at 184.

Further, a POSA would have understood from Wang's mass recovery results that conditions were appropriate for the protein to associate with the matrix. Ex. 1002 at ¶ 147; Ex. 1003 at 187 (Table 1). Thus, Wang teaches application of a refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Ex. 1002 ¶ 145-48.

e. The Wash Step

Wang discloses that after rhSCF is refolded by dilution, it was "purified by IEC" and that a packed strong anion exchange chromatography column was used for chromatography. Ex. 1002 at ¶ 149; Ex. 1003 at 184, 183. A POSA would have understood that purification by IEC entailed washing the column. Ex. 1002 at ¶ 150. Moreover, in the "refolding with simultaneous purification" protocol, Wang expressly describes purification by IEC as including "washing the column with 10 mL of the solution A." Ex. 1002 at ¶ 149; Ex. 1003 at 184. Even absent this express disclosure, a POSA would have understood that purification refers to the

removal of impurities from the mixture containing the protein. Ex. 1002 at ¶ 150. In particular, a POSA would have understood that the normal and usual operation of an IEC column involves washing the column to remove impurities after the protein solution is loaded. Ex. 1002 at ¶ 150. The Federal Circuit has held that a device in the prior art can be used to support an anticipation rejection of a method claim when the method claim is simply directed to a function which the prior art device "in its normal and usual operation, will perform." *In re King*, 801 F.2d 1324, 1326-27 (Fed. Cir. 1986). Thus, Wang expressly, implicitly, and inherently teaches washing the separation matrix.

f. The Elution Step

Similarly, as Dr. Tessier explains, a POSA would have understood that Wang discloses elution of the protein—after washing—from the IEC column. Ex. 1002 at ¶ 151. Wang describes purification by IEC to include "a linear gradient elution" following a wash step. Ex. 1003 at 184. Moreover, even without this express disclosure, a POSA would have understood that purification by IEC entails elution of the protein of interest and occurs after the washing step. In addition, Wang provides the mass recovery of the rhSCF obtained from the ***refolding by "dilution"***. Ex. 1003 at 187 and Table 1. A POSA would have understood that the protein could have been recovered from the IEC chromatography column only if it

was eluted from the column. Ex. 1002 at ¶ 152. Thus, Wang expressly, implicitly, and inherently teaches eluting the protein from the separation matrix.

For these reasons, Wang anticipates claim 9 of the '997 patent. Ex. 1002 ¶¶ 141-154, 165.

2. Claims 10, 13-21, and 23-30 Are Anticipated by Wang

Claim 10 is directed to "[t]he method of claim 9, wherein the nonnative limited solubility form is a component of an inclusion body." Claim 13 is directed to "[t]he method of any one of claims 9-12, wherein the non-mammalian expression system comprises bacteria or yeast cells." Wang discloses a method of purifying a protein, rhSCF, that was expressed in a non-native limited-solubility form—i.e., in inclusion bodies—in a non-mammalian bacterial system, "*Escherichia coli*." Ex. 1002 at ¶ 155. For these reasons, and the reasons discussed with respect to claim 9, Wang discloses each limitation of claims 10 and 13.

Claim 14 is directed to "[t]he 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 . . . ." As Dr. Tessier explains, Wang teaches solubilization solutions that comprise the denaturant urea. Ex. 1002 at ¶ 156. For these reasons, and the reasons discussed above with respect to claims 9 and 10, Wang discloses each limitation of claim 14.

Claims 15 and 23 depend from claims 9 and 14, respectively, and require that, if there is a reductant, "the reductant comprises one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione." Under a plain reading of the claim language, claims 15 and 23 do not require the use of any reductant at all because claim 9 recites a solubilization solution comprising "one or more of" a denaturant, a reductant, and a surfactant. Ex. 1002 at ¶ 157. Wang teaches a process that satisfies claims 15 and 23 because it discloses a solubilization solution comprising the denaturant urea and otherwise discloses the remaining limitations of claim 9 and 14. Moreover, even assuming that claims 15 and 23 require a reductant in addition to a denaturant, Wang teaches a solubilization solution that comprises the reductant beta-mercaptoethanol: "[s]everal batches of 1.0 g of purified rhSCF inclusion bodies were solubilized in 20 mL of . . . solution III (8.0 $mol \cdot l^{-1}$ urea containing 0.1 $mol \cdot l^{-1}$Tris, pH 8.0; 0.02 $mol \cdot l^{-1}$ EDTA; and 0.1 $mol \cdot l^{-1}$ ***β-mercaptolethanol***)." *Id*. at 183-84. For these reasons, and the reasons discussed with respect to claims 9 and 14, Wang teaches each limitation of claims 15 and 23.

Claims 16 and 24 depend from claims 9 and 15, respectively, and require that, if there is a surfactant, "the surfactant comprises one or more of sarcosyl and sodium dodecylsulfate." Under a plain reading, claims 16 and 24 do not require the use of any surfactant at all because claim 9 recites a solubilization solution

comprising "one or more of" a denaturant, a reductant, and a surfactant. As Dr. Tessier explains, Wang teaches a process that satisfies claims 16 and 24 because it discloses a solubilization solution comprising the denaturant urea and otherwise satisfies the remaining limitations of claims 9 and 15. Ex. 1002 at ¶ 158. Thus, Wang teaches each limitation of anticipates claims 16 and 24.

Claims 17 and 25 depend from claims 9 and 16, respectively, and require that, if there is an aggregation suppressor, "the aggregation suppressor is selected from the group consisting of arginine, proline, polyethylene glycols, nonionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes." Under a plain reading, claims 17 and 25 do not require the use of any aggregation suppressor at all because claim 9 recites a refold buffer comprising "one or more of" a denaturant, an aggregation suppressor, a protein stabilizer, and a redox component. As Dr. Tessier explains, Wang teaches a process that satisfies claims 17 and 25 because it discloses the redox components, GSH and GSSG, and otherwise satisfies the limitations of claims 9 and 16. Ex. 1002 at ¶ 159. For these reasons, and the reasons discussed with respect to claims 9 and 16, Wang teaches each limitation of claims 17 and 25.

Claims 18 and 26 depend from claims 9 and 17, respectively, and require that, if there is a protein stabilizer, "the protein stabilizer comprises one or more of

arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, tris, sodium sulfate, potassium sulfate and osmolytes." Under a plain reading, claims 18 and 26 do not require the use of any protein stabilizer at all because claim 9 recites a refold buffer comprising "one or more of" a denaturant, an aggregation suppressor, a protein stabilizer, and a redox component. As Dr. Tessier explains, Wang teaches a process that satisfies claims 18 and 26 because it discloses a refold buffer containing the redox components GSH and GSSG, and otherwise discloses the remaining limitations of claim 9. Ex. 1002 at ¶ 160. For these reasons, and the reasons discussed with respect to claims 9 and 17, Wang teaches each limitation of claim 18 and 26.

Claims 19 and 27 depend from claims 9 and 18, respectively, and require that, "the redox component comprises one or more of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol." As Dr. Tessier explains, Wang discloses a refold buffer containing the redox components GSH and GSSG, and otherwise discloses the remaining limitations of claim 9. Ex. 1002 at ¶ 161. For these reasons, and the reasons discussed with respect to claims 9 and 18, Wang discloses each limitation of claims 19 and 27.

Claims 20 and 28 depend from claims 9 and 19, respectively, and require that "the separation matrix is: (i) an affinity resin, selected from the group consisting of Protein A, Protein G, and synthetic mimetic affinity resin; or (ii) a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin." As Dr. Tessier explains, Wang teaches that following solubilization and refold, protein samples are purified by ion exchange chromatography (IEC). Ex. 1002 at ¶ 162, Ex. 1003 at 184. Ion exchange chromatography utilizes a non-affinity resin. Ex. 1001 at 2:14-16. For this reason, and the reasons discussed with respect to claims 9 and 19, Wang discloses each limitation of claims 20 and 28.

Claims 21, 29, and 30 depend from any one of claims 1 or 9-12, claim 13, and claim 20, respectively, and require that "the protein is isolated after elution from the separation matrix." The '997 patent defines the term "isolate" to be synonymous with "purify" and to mean to reduce by any 1% or more, "the amount of heterogenous elements … that may be present in a sample containing a protein of interest." Ex. 1002 at ¶ 94; Ex. 1001 at 7:11-17. As Dr. Tessier explains, a POSA would have understood "isolated after elution" to refer to collection of the purified eluate containing the protein. As Dr. Tessier further explained, IEC involves isolation of the protein (typically eluted in a single peak) from other impurities (typically eluted as one or more additional peaks) as the ionic strength

of the elution buffer steadily increases. Ex. 1002 at ¶ 163. This gradient system of elution results allows a POSA to isolate the eluates containing the protein fractions, which can ultimately be pooled together to capture the final yield of the protein of interest. Ex. 1002 at ¶ 163. For these reasons, and the reasons discussed with respect to claims 9, 13, and 20, Wang discloses each limitation of claims 21, 29, and 30. Ex. 1002 at ¶ 163.

For all of these reasons, Wang discloses a process that satisfies each and every limitation of claims 10, 13-21, and 23-30, of the '997 patent, and thus each of these claims are anticipated by Wang. Ex. 1002 ¶ 140-165.

B. Ground 2: The Challenged Claims Are Obvious over Wang in View of Cutler (Ex. 1028)

As described above, Wang expressly or inherently discloses each element of each of the asserted claims. However, should PO contend that a POSA reading Wang would not have understood each of the steps of purifying a protein by IEC to be disclosed, such a POSA would have looked to a standard reference on protein purification such as Cutler. Cutler is a textbook titled "Protein Purification Protocols," which was published in 2004. Thus, Cutler is a prior-art printed publication to the '997 patent under § 102(b). Cutler describes ion exchange chromatography and specifically outlines the stepwise protocol for carrying out IEC protein purification:

> Once the sample has been applied, ***the column is washed*** with several column volumes of binding buffer (around 5 column volumes, depending on sample and on column packing) to ensure that all nonbound proteins are washed out of the column. . . . ***Elute bound proteins*** by washing the column with an increasing salt gradient of 0–500 mM NaCl in binding buffer over 10–15 column volumes. . . determine in which fractions [of eluted protein] ***the protein of interest has been isolated***.

Ex. 1028 at 129 (emphases added); Ex. 1002 at ¶ 167–68. Cutler thus explicitly teaches the applying protein to the separation matrix such that the protein binds to the matrix, washing the separation matrix, eluting the protein, and isolating it. Ex. 1002 at ¶ 168.

As Dr. Tessier explains, a POSA as of 2009 would have been motivated to combine the protocol for IEC purification in a textbook reference such as Cutler with the protein-recovery-and-purification protocol in Wang, including the refolding by dilution protocol. Ex. 1002 at ¶ 167-68. And a POSA would have had a reasonable expectation of success given the successful recovery of protein disclosed in Wang. Ex. 1002 at ¶ 168. For these reasons, and the absence of any objective indicia of non-obviousness commensurate in scope with the asserted claims, as discussed *infra* Section VIII.E.4, the combined teachings of Wang and Cutler render obvious the subject matter of claims 9–10, 13–21, and 23–30 of the '997 patent. Ex. 1002 at ¶ 166-69.

C. Ground 3: The Challenged Claims Are Anticipated by Reardon (Ex. 1004)

U.S. Patent Publication 2006/0172384, entitled "FGF18 Production in Prokaryotic Hosts," was filed on December 12, 2005, and published on August 3, 2006, to Reardon and others ("Reardon"). Thus Reardon is a prior-art printed publication to the '997 patent under § 102(b). Reardon was not cited during the prosecution of the '997 patent.

Reardon discloses and claims a number of processes for expressing, recovering, and purifying the FGF18 protein and a truncated trFGF18 protein. Ex. 1002 ¶ 171. In particular, Reardon discloses that "expanded bed chromatography can be used for [protein] capture following refolding," and that this procedure avoids the need for concentrating the refold solution and precipitating unfolded and aggregated proteins before chromatography. Ex. 1002 ¶ 180-184; Ex. 1004 at [0086], [0172]. As described below, Reardon discloses every limitation of claims 9-10, 13-21, and 23-30.

1. Independent Claim 9 Is Anticipated by Reardon

a. The Preamble

Reardon discloses and claims a method of purifying a non-glycosylated recombinant FGF18 protein (fibroblast growth factor) produced in a prokaryotic non-mammalian expression system (*E. coli*) in an aggregated state. Ex. 1004 at [0004], [0046], [0078], and [102]-[104]; *see also,* claim 23; Ex. 1002 ¶ 171.

Reardon discloses a protein that was expressed in a non-native limited-solubility form, i.e. inclusion bodies. Ex. 1002 ¶ 171; Ex. 1004 at [0078]. Reardon thus teaches a method of purifying a protein expressed in a non-native limited-solubility form in a non-mammalian expression system. Ex. 1002 ¶ 171.

b. The Solubilization Step

Claim 23 of Reardon recites a process for isolating insoluble FGF18, including "dissolving the insoluble FGF18 protein in a chaotropic solvent comprising about 6M guanidine hydrochloride, 40 mM dithiothreitol (DTT)." Ex. 1002 ¶ 172; Ex. 1004 at claim 23; *see also* claims 20 and 47.

Reardon also teaches that the "[s]olubilization of [i]nclusion [b]odies" can be performed as follows:

> [t]he washed inclusion body prep can be solubilized using ***guanidine hydrochloride*** (5-8 M), guanidine thiocyanate (5-6 M), or ***urea*** (7-8 M) containing ***a reducing agent such as beta mercaptoethanol*** (10-100 mM), or ***dithiothreitol*** (5-50 mM).

Id. at [0075] (emphases added).

Guanidinium hydrochloride (GuHCl) and urea are identified in the '997 patent as denaturants, and β-mercaptoethanol is defined as a reductant. Ex. 1001 at 2:43-48; Ex. 1002 ¶ 174. Therefore, Reardon teaches use of a solubilization

solution comprising one or more of a denaturant, a reductant, and a surfactant. Ex. 1002 ¶ 173-74.

c. The Refold Step

In claim 23, Reardon recites "refolding the dissolved inclusion bodies in a solution by diluting into refolding buffer comprising 50 mM Tris." *Id*. at claim 23; *see also* claims 20 and 47. The specification of Reardon also teaches a refold step: following solubilization, "[t]he reduced FGF18 or trFGF18 is then oxidized in a controlled renaturation step," which involves "dilution in a refold buffer comprising 50 mM ***Tris*** and 120 mM NaCL." *Id*. at [0079]; Ex. 1002 ¶ 177. Reardon teaches that the refold buffer "can also comprise ***arginine hydrochloride***, additional salts, and ***an oxido-shuffling system*** . . . ***based on mixtures of reduced and oxidized molecules such as cysteine and cystine, DTT and cystine, reduced glutathione and oxidized glutathione, and DTT and oxidized glutathione***." *Id*. at [0079]; *see also* [0080]; Ex. 1002 ¶ 177. Reardon teaches that in this dilution refolding process, "the solute containing FGF18 or trFGF18 is added . . . to the refolding buffer with mixing." Ex. 1004 at [0080]; Ex. 1002 ¶ 177.

According to the '997 patent, Tris, arginine, and glycerol are all protein stabilizers and aggregation suppressors; and cysteine and cystine, DTT and cystine, reduced glutathione and oxidized glutathione, and DTT and oxidized glutathione, are redox components. Ex. 1001 at 2:38-60. Therefore, Reardon teaches forming

a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of a denaturant, an aggregation suppressor, a protein stabilizer, and a redox component. Ex. 1002 ¶ 175-78.

d. The Capture or "Applying" Step

Reardon claims and describes the application of a refold solution to a separation matrix. Ex. 1002 ¶ 179-84. Claim 23 of Reardon recites a process for isolating insoluble FGF18, including "loading solution on resin column equilibrated to pH 8.0 using sodium acetate buffer." *Id*. at claim 23; Ex. 1002 ¶ 180. In Example 12, under the heading of "Capture of Refolded FGF18," Reardon describes a process in which "FGF18 is not concentrated by tangential flow filtration prior to capture by cation exchange chromatography." *Id*. at [0172]. Specifically, Reardon teaches that "following refolding, the pH is adjusted to 5.5 and the material is filtered through a 1.2 µm nominal cut off filter." *Id*. Then, "[a]n Amersham Biosciences Streamline column packed with Amersham Biosciences Streamline SP XL" is equilibrated and "refolded FGF18 is loaded onto the column using inline dilution, i.e. 30% filtered, pH-adjusted, refolded FGF18 and 70% water is loaded using the chromatography system to generate the correct ratio." *Id*. at [0172]. A POSA would have understood that the Amersham Biosciences Streamline SP XL column is a non-affinity ion exchange

chromatography column comprising a separation matrix suitable for a protein to associate. Ex. 1036 at 22; Ex. 1002 ¶ 182.

While Reardon describes a 30:100 (or 3.33-fold) in-line dilution of the refold solution before loading onto the column, a POSA would understand the term "applying the refold solution" to allow for dilution of the refold solution, especially on a scale comparable to the 3-fold dilution described in Example 3 of the '997 patent—the only example directed to purification of limited solubility proteins using non-affinity chromatography.[5] Ex. 1001 at 20:56-62 ("An aliquot of protein . . . sampled directly from a refold solution, was diluted 3-fold with water."); Ex. 1002 ¶¶ 85-90, 184. The Reardon dilution is of this same magnitude, and is not prohibited by the properly construed terms of the "applying" step. Ex. 1002 ¶ 85-90, 184. Thus, Reardon teaches applying a refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Ex. 1002 ¶ 179-84.

e. The Wash Step

Claim 23 of Reardon recites "washing the resin column with about 0.4 M sodium chloride." Ex. 1003 at 43. Example 12.C of Reardon also teaches that,

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[5] Petitioner reserves the right to assert in subsequent litigation that claim 9 is not enabled or adequately described by the specification as required by §112.

following loading of the FGF18 solution, "the column is then washed with equilibration buffer." *Id*. at [0172]. Reardon thus teaches "washing the separation matrix" as recited in claim 9 of the '997 patent. Ex. 1002 ¶ 185-87.

f. The Elution Step

Claim 23 of Reardon further recites "washing the resin column with about 0.75 M sodium chloride to elute bound FGF18 protein." Example 12.C of Reardon also teaches that, following washing of the column, "FGF18 is then eluted with 50% elution buffer (25 mM sodium acetate, 1.0 M NaCl, pH 5.5)." *Id*. at [0172]. Reardon thus teaches "eluting the protein from the separation matrix" as recited in claim 9 of the '997 patent. Ex. 1002 at ¶ 190.

For all of these reasons, Reardon anticipates claim 9. Ex. 1002 at ¶ 188-91; 202.

2. Claims 10, 13-21, and 23-30 Are Anticipated by Reardon

Claim 10 ("inclusion body") and Claim 13 ("bacteria or yeast cells"): Reardon discloses "methods for recovering recombinant FGF18 or trFGF18 protein from a ***prokaryotic cell*** when the protein is expressed by the host and found within the host cell as an unglycosylated insoluble ***inclusion body***." Ex. 1004 at [0046]. Prokaryotic cells are bacteria cells. Ex. 1002 ¶ 192. For these reasons, and the reasons discussed with respect to claim 9, Reardon anticipates claims 10 and 13. Ex. 1002 ¶ 202.

Claim 14 ("the denaturant of the solubilization solution or the refold buffer comprises one or more of urea, guanidinium salts…"): Reardon discloses the use of two different denaturants for solubilizing and denaturing the FGF18 protein: urea and guanidine hydrochloride. Ex. 1002 ¶ 193; Ex. 1004 at [0079]. Reardon also teaches a "controlled renaturation step" comprising a refold buffer that can comprise guanidine HCl and/or urea. Ex. 1002 ¶ 193; Ex. 1004 at [0079]; *see also* [0080]. For these reasons, and the reasons discussed above with respect to claim 9, Reardon anticipates claim 14. Ex. 1002 ¶ 202.

Claims 15 and 23 ("the reductant comprises one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione"): Reardon teaches solubilizing washed inclusion bodies using a reducing agent such as "beta mercaptoethanol" or "dithiothreitol (DTT)." *Id*. at [0075]; Ex. 1002 ¶ 194. For these reasons, and the reasons discussed with respect to claims 9 and 14, Reardon anticipates claims 15 and 23. Ex. 1002 ¶ 202.

Claims 16 and 24 ("the surfactant comprises one or more of sarcosyl and sodium dodecylsulfate"): As discussed, *supra* 13, claims 16 and 24 do not require the use of any surfactant so long as the solubilization solution contains a denaturant or a reductant. Reardon teaches a solubilization solution comprising denaturants urea and/or guanidine hydrochloride, and reductants beta-mercaptoethanol and/or

DTT. Ex. 1002 ¶ 194. For this reason, and the reasons discussed with respect to claims 9 and 15, Reardon anticipates claims 16 and 24. Ex. 1002 ¶ 202.

Claims 17 and 25 ("the aggregation suppressor is selected from the group consisting of arginine… Tris…"): Reardon discloses a refold buffer comprising aggregation suppressors Tris and arginine hydrochloride. Ex. 1004 at [0080]; Ex. 1002 ¶ 196. For this reason, and the reasons discussed with respect to claims 9 and 16, Reardon discloses each limitation of claims 17 and 25. Ex. 1002 ¶ 202.

Claims 18 and 26 ("the protein stabilizer comprises one or more of arginine… tris…."): Reardon discloses a refold buffer comprising protein stabilizers Tris and arginine hydrochloride. Ex. 1004 at [0080]; Ex. 1002 ¶ 197. For these reasons, and the reasons discussed with respect to claims 9 and 17, Reardon anticipates claims 18 and 26. Ex. 1002 ¶ 202.

Claims 19 and 27 ("the redox component comprises one or more of glutathione-reduced, glutathione-oxidized, cysteine, cystine…"): Reardon discloses a refold buffer comprising "an oxido-shuffling system . . . used to initiate disulfide bonding of the FGF18 or trFGF18 molecule, and is based on mixtures of reduced and oxidized molecules such as cysteine and cystine,… reduced glutathione and oxidized glutathione…." *Id*. at [0079]; Ex. 1002 ¶ 198. For this reason, and the reasons discussed with respect to claims 9 and 18, Reardon discloses each limitation of claims 19 and 27. Ex. 1002 ¶ 202.

Claims 20 and 28 ("… the separation matrix is: (i) an affinity resin… or (ii) a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin."): Reardon discloses the use of an ion exchange column, *i.e*., "[a]n Amersham Biosciences Streamline column packed with Amersham Biosciences Streamline SP XL," which is a non-affinity resin. Ex. 1004 at [0172]; Ex. 1002 ¶ 199. For this reason, and the reasons discussed with respect to claims 9 and 19, Reardon discloses each limitation of claims 20 and 28. Ex. 1002 ¶ 202.

Claims 21, 29, and 30 ("… the protein is isolated after elution from the separation matrix."): Reardon discloses purification of refolded proteins by means of 50% elution buffer (25 mM sodium acetate, 1.0 M NaCl, pH 5.5)." *Id*. at [0172]; Ex. 1002 ¶ 200. Moreover, Example 15 of Reardon (*id*. at [0177]-[0182]), lists additional purification steps, which demonstrates that prior purification steps, as described in earlier examples 12-14, successfully isolated sufficient protein after elution to allow further purification of the product. *Id*. at [0169]-[0176]; Ex. 1002 ¶ 200. For these reasons, and the reasons discussed with respect to claims 9, 13, and 20, Reardon anticipates claims 21, 29 and 30. Ex. 1002 ¶ 202.

D. Ground 4: The Challenged Claims Are Anticipated by Dietrich (Ex. 1005)

U.S. Application Publication 2008/0260684 ("Dietrich"), entitled "Method for the Purification of G-CSF," was published October 23, 2008 (Ex. 1005).

Dietrich is a prior-art printed publication to the '997 patent under § 102(a). 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 the prosecution of the application that issued as the '997 patent. *See* Ex. 1033. As described below, Dietrich discloses every limitation of claims 9-10, 13-21, and 23-30.

1. Independent Claim 9 Is Anticipated by Dietrich

a. The Preamble

Dietrich discloses methods for purifying the recombinant protein, G-CSF, expressed in a non-native limited-solubility form using non-mammalian expression systems such as *E. coli*, as required by the preamble. Ex. 1005 at [0016], [0058], [0063]-[0067]; Ex. 1002 ¶ 204. Dietrich discloses that using *E. coli* expression systems for expressing G-CSF, and the resulting inclusion bodies, was known in the art. *Id*. at [0060]. Dietrich thus teaches a method of purifying a protein expressed in a non-native limited-solubility form in a non-mammalian expression system. Ex. 1002 ¶ 204.

b. The Solubilization Step

Dietrich discloses solubilizing the expressed G-CSF using a solubilization buffer containing 30mM Tris, 1mM EDTA, 6.0M guanidine-HCl, and 100 mM GSH at pH 8.0. Ex. 1005 at [0068]. Guanidine-HCl (otherwise known as guanidinium chloride) is the hydrochloride salt of guanidine, and is a denaturant.

Ex. 1002 ¶ 205. Therefore, Dietrich teaches use of a solubilization solution comprising one or more of a denaturant, a reductant, and a surfactant. Ex. 1002 ¶ 205.

c. The Refold Step

Following solubilization, Dietrich discloses forming a refold solution comprising the solubilization solution and a refold buffer containing 30 mM Tris, 2mM GSSG, 2mM GSH, and 3M urea at pH 7.5. Ex. 1005 at [0069]. GSSG (glutathione disulfide) and GSH are redox components of glutathione, corresponding to the oxidized and reduced states, respectively. Ex. 1002 ¶ 206. Urea is a denaturant as defined in the '997 patent, and Tris is a protein stabilizer and aggregation suppressor. Ex. 1002 ¶ 206. Dietrich thus teaches forming a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of a denaturant, an aggregation suppressor, a protein stabilizer, and a redox component. this limitation. Ex. 1002 ¶ 206.

d. The Capture or "Applying" Step

Dietrich discloses filtering the refold solution using depth filtration and then applying the refold solution to a separation matrix. Ex. 1005 at [0032]-[0034], and [0070]. Dietrich discloses that "[s]ubsequently to refolding, the refolding step is filtrated before the first chromatographic step is conducted." *Id*. at [0070]. Dietrich further discloses loading the filtered refold solution (including both the

protein components of the solution) onto a cation exchange chromatography column packed with a non-affinity resin, SP Sepharose XL matrix. *Id*. at [0035]-[0036], [0071]-[0072] ("the first chromatographic step serves for capturing the target protein and separates refolding agents like urea, GSH, GSSG, as far as those are present in the folding setup, from the target protein"); Ex. 1002 ¶ 207.

In addition to the disclosure that the protein is "captured" by the matrix, Dietrich also discloses adjusting the pH of the refold solution and equilibrating the ion exchange column, before loading the refold solution onto the column. A POSA would have understood that the purpose of such steps was to optimize the condition for proteins to bind to the separation matrix. Ex. 1002 ¶ 207. Finally, Dietrich discloses that "[t]he purity of the eluted G-CSF was determined by means of rpHPLC; it was higher than 80%." *Id*. at [0072], [0076]. A POSA would have understood that G-CSF could not have been eluted if it did not associate with the separation matrix. Ex. 1002 ¶ 209. Dietrich thus teaches applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Ex. 1002 ¶¶ 207-209.

e. The Wash Step

Dietrich discloses that washing the separation matrix "with 1.5 column volumes washing buffer (20 mM sodium acetate, pH 5.0)." Ex. 1005 at [0072]; Ex. 1002 ¶ 210.

f. The Elution Step

Dietrich 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.* at [0078]; Ex. 1002 ¶ 211.

For all of these reasons, Dietrich anticipates claim 9. Ex. 1002 ¶ 212.

2. Dependent Claims 10, 13-21, and 23-30 Are Anticipated by Dietrich

Claim 10 ("… the nonnative limited solubility form is a component of an inclusion body") and Claim 13 ("… the non-mammalian expression system comprises bacteria or yeast cells"): Dietrich discloses the purification of recombinant G-CSF expressed in *E. coli* as inclusion bodies. Ex. 1005 at [0058], [0060], [0063]-[0067]; Ex. 1002 ¶ 213. For these reasons, and the reasons discussed with respect to claim 9, Dietrich anticipates claims 10 and 13.

Claim 14 ("… the denaturant of the solubilization solution or the refold buffer comprises one or more of … guanidinium salts…."): Dietrich discloses solubilizing the expressed protein using a solubilization solution that contains a guanidinium salt. Ex. 1002 ¶ 214; Ex. 1005 at [0068]. For these reasons, and the reasons discussed above with respect to claim 9, Dietrich anticipates claim 14.

Claims 15 and 23 ("the reductant comprises one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione"): Dietrich discloses solubilizing the expressed protein using a solubilization solution that contains the

reductant glutathione (GSH). *Id.*; Ex. 1002 ¶ 215. For these reasons, and the reasons discussed above with respect to claims 9 and 14, Dietrich anticipates claims 15 and 23.

Claims 16 and 24 ("the surfactant comprises one or more of sarcosyl and sodium dodecylsulfate"): As explained, *supra* 13, claims 16 and 24 do not require the use of any surfactant at all so long as the solubilization solution contains a denaturant or a reductant. Dietrich teaches a solubilization solution comprising a guanidinium salt denaturant and the reductant glutathione (GSH). *Id*.; Ex. 1002 ¶ 216. For these reasons, and the reasons discussed with respect to claims 9 and 15, Dietrich anticipates claims 16 and 24.

Claims 17 and 25 ("… the aggregation suppressor is selected from the group consisting of … Tris …."): Dietrich teaches a refold buffer that includes the aggregation suppressor Tris. Ex. 1002 ¶ 217. For this reasons, and the reasons discussed with respect to claims 9 and 16, Dietrich anticipates claims 17 and 25.

Claims 18 and 26 ("… the protein stabilizer comprises one or more of … tris…."): Dietrich discloses a refold buffer that comprises the protein stabilizer Tris. Ex. 1002 ¶ 218; Ex. 1005 at [0069]. For this reason, and the reasons discussed with respect to claims 9 and 17, Dietrich anticipates claims 18 and 26.

Claims 19 and 27 ("… the redox component comprises one or more of glutathione-reduced, glutathione-oxidized…."): Dietrich discloses a refold buffer

that includes redox components of glutathione, i.e., GSH (reduced state) and GSSG (oxidized state). Ex. 1005 at [0069]. For this reason, and the reasons discussed with respect to claims 9 and 18, Dietrich anticipates claims 19 and 27. Ex. 1002 ¶ 219.

Claims 20 and 28 ("… the separation matrix is: (i) an affinity resin, . . . or (ii) a non-affinity resin selected from the group consisting of ion exchange, … and a hydrophobic interaction resin."): 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. Ex. 1002 ¶ 220. For these reasons, and the reasons discussed with respect to claim 9 and 19, Dietrich anticipates claims 20 and 28.

Claims 21, 29, and 30 ("… the protein is isolated after elution from the separation matrix."): Dietrich discloses that the eluted G-CSF was analyzed by reversed phase high-performance liquid chromatography (rpHPLC) to determine its purity at "higher than 80%," and that "the yield was also higher than 80%." Ex. 1005 at [0072]. A POSA would have understood from these results that the protein of interest was collected from the eluate and separated from heterogeneous materials, which corresponds with the meaning of "isolate" as used in the '997 patent. Ex. 1002 ¶ 221; Ex. 1001 at 7:11-17. For these reasons, and the reasons

discussed with respect to claims 9, 13, and 20, Dietrich anticipates claims 21, 29, and 30. Ex. 1002 ¶ 223.

E. Ground 5: The Challenged Claims Are Obvious over Komath '944 (Ex. 1006) in Combination with Komath '056 (Ex. 1007)

PCT Publication 2006/097944 ("Komath '944") entitled "Process for the Purification of Recombinant Granulocyte-Colony Stimulating Factor," published on September 21, 2006, to Uma Devi Komath and others. Komath is a prior-art printed publication to the '997 patent under § 102(b). Komath '944 was not cited during the prosecution of the '997 patent.

Komath '944 teaches a "process for large scale purification of therapeutic grade quality of recombinant human G-CSF from microbial cells, wherein the protein is expressed as inclusion bodies." Ex. 1006 at Abstract. Specifically, Komath '944 teaches the following steps: (1) isolating inclusion bodies containing G-CSF; (2) solubilizing G-CSF protein from inclusion bodies; (3) refolding solubilized G-CSF to obtain active folded protein; and (4) purifying the G-CSF using cation exchange chromatography followed by hydrophobic interaction chromatography. Ex. 1002 ¶ 224.

As discussed in further detail below, the only difference between Komath '944 and the claimed methods is that this reference does not set out the components of the refold buffer. Rather, Komath '944 teaches that following solubilization at a high (alkaline pH), [r]efolding of the protein is carried out at

room temperature for 6-16 hours at acidic pH" and "[t]he pH of the refolded protein solution is maintained in the range of 3.5 to 5.5 ***using any appropriate buffer suitable for maintaining pH in the acidic range***." *Id*. at 8; Ex. 1002 ¶ 227.

Komath '944 cites to PCT Publication 2004/001056 ("Komath '056"), entitled "Process for Preparing G-CSF," which published on December 31, 2003, to Uma Komath and others. Komath is a prior-art printed publication to the '997 patent under § 102(b) (pre-AIA). Komath '056 was not cited during prosecution of the '997 patent. Like Komath '944, Komath '056 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." Ex. 1007 at Abstract. Komath '056 specifically discloses forming a refold solution by diluting the solubilization solution with 0.1% polysorbate 20—an aggregation suppressor. Ex. 1002 ¶ 228; Ex. 1007 at 9, 11.

1. Motivation To Combine and Expectation of Success

A POSA would have been motivated to combine the teachings of Komath '944 and Komath '056 (collectively, the "Komath references") because:

(1) Komath '944 cites to Komath '056 and teaches that it "addressed most of the limitations of lengthy processes described in scientific literature." Ex. 1006 at 1-2.

(2) Both Komath references are concerned with the same protein, G-CSF.

(3) Both Komath references describe methods for refolding G-CSF expressed in inclusion bodies in bacterial expression systems.

(4) Both Komath references seek to address a need for methods to recover properly folded bacterially expressed proteins in amounts sufficient for clinical applications.

(5) Both Komath references set out to investigate improved purification methods to maximize yield of properly folded G-CSF.

Ex. 1002 ¶ 230. Thus, and for the reasons detailed below, a POSA would have been motivated to combine the teachings of the Komath references, and given the results disclosed in Komath '944, would have had a reasonable expectation of success at achieving a method for purifying a protein expressed in limited soluble form in a non-mammalian expression system. Ex. 1002 at 231.

2. Claim 9 Is Obvious over Komath '944 in View of Komath '056

1) The Preamble

Komath '944 discloses methods for purifying "recombinant human G-CSF from microbial cells, wherein the protein is expressed as inclusion bodies." Ex. 1006 at Abstract; *see also*, *id*. at 1. Similarly, Komath '056 discloses a "process for purifying… recombinant human G-CSF from E. coli and other cells in which inclusion bodies of G-CSF are formed." Ex. 1007 at Abstract. G-CSF is a protein, *E. coli* is a non-mammalian expression system, and proteins found in inclusion

bodies are expressed in a non-native limited-solubility form. Ex. 1002 ¶ 232. Thus, the Komath references each teach a method of purifying proteins expressed in a non-native limited-solubility form in a non-mammalian expression system, as required by claim 9.

2) The Solubilization Step

Komath '944 teaches solubilization of G-CSF inclusion bodies with a solution comprising "urea or guanidinium hydrochloride," which are denaturants as defined by the '997 patent. Ex. 1002 ¶ 236; *Id*. at 6, 7-8; Ex. 1001 at 2:43-48. Komath '056 teaches the solubilization of inclusion body pellets using urea at a high (alkaline) pH. Ex. 1002 ¶ 237; Ex. 1007 at 5:22-24. Therefore, the Komath references each teach the use of a solubilization solution comprising at least the denaturant, urea, as required by claim 9. Ex. 1002 ¶ 238.

3) The Refold Step

Following solubilization, Komath '944 describes a refolding step, wherein "[t]he pH of the refolded protein solution is maintained in the range of 3.5 to 5.5 ***using any appropriate buffer suitable for maintaining pH in the acidic range***." Ex. 1006 at 8. As discussed by Dr. Tessier, a POSA would turn to the references cited in the '944 patent, including Komath '056, to determine suitable buffers for refolding. Ex. 1002 ¶¶ 239-40. Komath '056 discloses forming a refold solution by diluting the solubilization solution with 0.1% polysorbate 20 in water at pH 8.0-

8.5 for 6 hours and then at pH 4.0-5.0 for 6 to 8 hours. Ex. 1007 at 11. Polysorbate 20 is an aggregation suppressor as defined by the '997 patent. Ex. 1001 at 5:45-53. Thus, a POSA would have been motivated, with reasonable expectation of success, to form a refold solution comprising the solubilization solution and the aggregation suppressor Polysorbate 20. Ex. 1002 ¶ 240.

4) The Capture or "Applying" Step

Komath '944 teaches that "the refolded protein solution . . . is loaded on an ion exchange column." Ex. 1006 at 8. Komath '944 further teaches that the G-CSF protein is refolded at a high pH "so as to be suitable for direct loading on a cation exchange column." Ex. 1002 ¶ 241; Ex. 1006 at 7. The results in Komath '944 show that G-CSF was successfully captured, purified, and eluted from the column. Ex. 1002 ¶ 241. A POSA would have understood from these results that G-CSF successfully associated with the separation matrix in each of the chromatography columns used, such that the proteins could be collected after washing and elution. Ex. 1002 ¶ 241.

Similarly, Komath '056 teaches purification of refolded G-CSF using cation or anion exchange chromatography. Id. at ¶ 242; Ex. 1007 at 8:4-6.

Thus, Komath '944 and Komath '056 each teach application of the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix. Ex. 1002 ¶¶ 241-243.

5) The Wash Step

Komath '944 teaches that after loading of the refolded protein solution on an ion exchange column, the column is "washed with equilibration buffer." Ex. 1006 at 8. Moreover, Figures 3, 4, and 5 would have taught a POSA that the G-CSF eluate was effectively washed and purified of contaminates during chromatography. *Id*.

Komath '056 similarly discloses a wash step with an equilibration buffer comprised of 25 mM sodium acetate at pH 4.5. Ex. 1007 at 9:17-18; Ex. 1002 ¶ 245.

Thus, the Komath references each disclose washing of the separation matrix. Ex. 1002 ¶ 246.

6) The Elution Step

Komath '944 teaches that following washing of the ion exchange chromatography column, "G-CSF is eluted from this column using a gradient of an ionic salt like chloride, citrate or sulphate in the range of 0.05 M to 0.25 M." Ex. 1006 at 8; Ex. 1002 ¶ 247. Komath '944 also states that "G-CSF protein is recovered with good yields and a minimum amount of aggregated protein." *Id*. at 9; *see also*, Figures 3, 4, and 5. A POSA would have recognized from these results that the G-CSF eluate was succesfully eluted from the ion exchange column. Ex. 1002 ¶ 247.

Komath ’056 also teaches that the refolded and purified G-CSF is eluted from the column using 0.1 M Tris HCl buffer at pH 8.0. Ex. 1002 ¶ 248; Ex. 1007 at 9:20-21. Thus Komath ’944 and Komath ’056 each disclose elution of the protein from the separation matrix.

For all of these reasons, a POSA would have been motivated to combine the overlapping teachings of the Komath references, and in light of the successful result in Komath ’944, would have had a reasonable expectation of success in purifying G-CSF obtained from inclusion bodies. Ex. 1002 ¶ 250. Thus, and in light of the absence of any objective indicia of non-obviousness commensurate with the scope of claim 9, *infra* Section VIII.E.4, claim 9 is obvious over the Komath references.

3. Claims 10, 13-21, And 23-30 Are Obvious over Komath ’944 in View of Komath ’056

Claim 10 (“inclusion body”); Claim 13 (“bacteria or yeast cells”): As Dr. Tessier explains, each of the Komath references teaches purification of G-CSF protein expressed in bacterial cells, i.e. *E. coli*, from inclusion bodies, as required by claims 10 and 13. Ex. 1002 ¶ 251; Ex. 1006 at 6; Ex. 1007 at 4:27-31. Thus, and for the reasons discussed for claim 9, these claims are obvious over the Komath references.

Claim 14 (“…the denaturant comprises one or more of urea, guanidine salts…”): Komath ’944 and Komath ’056 each describes a solubilization buffer

containing the denaturant urea. Ex. 1006 at 6, 7-8; Ex. 1007 at 9:3-7. For this reason, and the reasons discussed above with respect to claim 9, claim 14 would have been obvious over the Komath references. Ex. 1002 ¶ 252.

Claims 15 and 23 ("…the reductant comprises one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione")*:* As explained above, *supra* 13, claims 15 and 23 do not require the use of any reductant at all so long as a denaturant or surfactant are used in the solubilization. Both Komath references disclose a solubilization solution comprising a denaturant – urea – and otherwise discloses the remaining limitations of claim 9. Ex. 1006 at 6, 7-8; Ex. 1007 at 9:3-7. For these reasons, and the reasons discussed above with respect to claims 9 and 14, claims 15 and 23 would have been obvious over the Komath references. Ex. 1002 ¶ 253.

Claims 16 and 24 ("… the surfactant comprises one or more of sarcosyl and sodium dodecylsulfate.")*:* As explained above, *supra* 13, claims 16 and 24 do not require the use of any surfactant at all so long as the solubilization solution contains a denaturant or reductant. The Komath references each teach a solubilization solution containing the denaturant urea. For these reasons, and the reasons discussed with respect to claims 9 and 15, claims 16 and 24 would have been obvious over the Komath references. Ex. 1002 ¶ 254.

Claims 17 and 25 ("… the aggregation suppressor is selected from the group consisting of … nonionic surfactants…."): Komath '056 teaches a refold buffer comprising the nonionic surfactant polysorbate 20. (Ex. 1007 at 9:10-12). Ex. 1002 ¶ 255. As discussed above, a POSA who sought to practice the methods disclosed in Komath '944 would have looked to Komath '056 for an appropriate refold buffer and in light of the results in Komath 944, would have had a reasonable expectation of success in combining the teachings of the two references. Ex. 1002 ¶ 255. For these reasons, and the reasons discussed above with respect to claims 9 and 16, claims 17 and 25 would have been obvious over the Komath references.

Claims 18 and 26 ("… the protein stabilizer comprises one or more of … non-ionic surfactants…."): Komath '056 teaches a refold buffer comprising a nonionic surfactant, *i.e*., polysorbate 20 Ex. 1002 at ¶ 256; Ex. 1007 at 9:10-12; Ex. 1001 at 5:45-53. As described above, a POSA would have been motivated to combine the teachings of Komath '944 with that of Komath '056, and would have had a reasonable expectation of success of using the Komath '056 refold buffer in the Komath '994 process. Ex. 1002 ¶ 256. For these reasons, and the reasons discussed above for claims 9 and 17, claims 18 and 26 would have been obvious over the Komath references.

Claims 19 and 27 ("…the redox component comprises one or more of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol."): As explained above, *supra* 13, claims 19 and 27 do not require the use of any redox component at all so long as the refold buffer contains a denaturant, an aggregation suppressor, or a protein stabilizer. Komath '056 teaches a refold buffer comprising the aggregation suppressor, i.e., polysorbate 20. Ex. 1002 ¶ 257; Ex. 1007 at 9:10-12; Ex. 1001 at 5:51. Moreover, as described above, a POSA would have been motivated to combine the teachings of Komath '944 with that of Komath '056, and would have had a reasonable expectation of success of using the Komath '056 refold buffer in the Komath '994 process. Ex. 1002 ¶ 257. For these reasons, and the reasons discussed above with respect to claims 9 and 18, claims 19 and 27 would have been obvious over the Komath references.

Claims 20 and 28 ("… the separation matrix is: (i) an affinity resin… or (ii) a non-affinity resin selected from the group consisting of ion exchange… and a hydrophobic interaction resin."): Komath '944 teaches the purification of properly refolded G-CSF using, *inter alia*, ion exchange chromatography. Ex. 1002 ¶ 258; Ex. 1007 at 8. Komath '056 also teaches purification of refolded G-CSF using cation or anion exchange chromatography, which are forms of ion exchange chromatography. Ex. 1002 ¶ 258; Ex. 1007 at 6:10-12. For these reasons, and the

reasons discussed with respect to claims 9 and 19, claims 20 and 28 would have been obvious over the Komath references. Ex. 1002 ¶ 258.

Claims 21, 29, and 30 ("…the protein is isolated after elution from the separation matrix"): Komath '944 teaches that after ion exchange chromatography, "the G-CSF protein is recovered with good yields and a minimum amount of aggregated protein." Ex. 1006 at 9. To a POSA, this would have demonstrated that the protein of interest was successfully collected and "heterogeneous elements," in the form of aggregated proteins, were removed following elution, as recited in the definition of "isolate." Ex. 1002 ¶ 259; Ex. 1001 at 7:11-17. Moreover, to the extent the term "is isolated" can be read to require a separate purification step after elution from an initial chromatography step (rather than the mere collection of the eluate containing the protein), Komath '944 describes the use of a second hydrophobic interaction chromatography step following an initial anion exchange step. Ex. 1006 at 7-8; Ex. 1002 ¶ 259.

As described above, POSA would have been motivated to combine the teachings of the Komath references and had a reasonable expectation of success in achieving a process by which a protein is isolated after elution. Ex. 1002 ¶ 260. For these reasons, and for the reasons described with respect to claims 9, 10, 13, and 20, claims 21, 29, and 30 are obvious over the Komath references.

4. No Objective Indicia of Non-Obviousness.

Petitioners are not aware of any secondary considerations that would have a nexus to, and would be commensurate in scope with, the challenged claims. Specifically, full the reasons discussed above, *supra* Section V, and explained by Dr. Tessier, Ex. 1002 ¶ 263-265, the prior art did not teach away from the full scope of the methods recited by the asserted claims; nor were the results of the claimed methods unexpected in light of the prior art. Ex. 1002 ¶ 263-265.

IX. CONCLUSION

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

Dated: June 8, 2019

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105, I certify that I caused to be served a true and correct copy of the foregoing: **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,643,997** and the exhibits cited therein by *U.S.P.S. Priority Mail Express* on this day, June 8, 2019 on the Patent Owner's correspondence address of record for the subject patent as follows:

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CERTIFICATE OF WORD COUNT

The undersigned certifies that the attached Petition for *Inter Partes* Review of U.S. Patent No. 9,643,997 contains 12990 words (as calculated by the word processing system used to prepare this Petition), excluding the parts of the Petition exempted by 37 C.F.R. § 42.24(a)(1).

Dated: June 8, 2019

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