What is claimed is:

1. A method of purifying a chimeric cytokine protein preparation comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, the method comprising using ~~at least two of~~ a cation exchange column, an anion exchange column, ~~or~~ and a hydroxyapatite column to purify the protein preparation.

~~2. The method of claim 1, comprising using all three of the cation exchange column, the anion exchange column, and the hydroxyapatite column to purify the protein preparation.~~

~~3~~2. The method of claim 1 ~~or claim 2~~, wherein the chimeric cytokine protein preparation is harvested from *E. coli* cells cultured in at least 1 liter of medium, wherein the *E. coli* cells contain a plasmid comprising a nucleic acid sequence expressing the chimeric cytokine protein under an inducible promoter.

~~4. The method of any of claims 1 to 3, wherein the cells are cultured in the presence of at 0.05 mM to 0.8mM EDTA.~~

~~5~~3. The method of ~~any of claims 1 to 4~~ claim 1 or 2, wherein the cells are lysed in the presence of at least 2.5mM EDTA.

~~6~~4. The method of any of the preceding claims, wherein:

(a) the chimeric cytokine protein preparation is applied to a cation exchange column (CEX);

(b) the cation exchange column is washed with a wash buffer that does not elute the chimeric cytokine protein, and the bound protein is eluted from the cation exchange column with an elution buffer to provide a CEX eluate that contains the chimeric cytokine protein;

(c) the CEX eluate is applied to an anion exchange surface;

(d) the flow through from the anion exchange column (AEX flowthrough) is collected and loading onto a ceramic hydroxyapatite column (CHA);

(f) the bound protein is eluted from the CHA column;

thereby purifying the chimeric cytokine protein preparation.

~~7~~5. The method of any of the preceding claims, wherein the ratio of intact protein to des-Ala protein isoforms~~, methionated protein isoforms and acetylated protein isoforms~~ is increased after the purification.

86. The method of any of the preceding claims, wherein the purified preparation comprises less than 10% of des-Ala form of the protein, less than 10% of acetylated form of the protein~~, and less than 10% of methionated form of the protein~~.

97. The method of any of the preceding claims, wherein the purified preparation contains less than 50 parts per million of host cell protein.

~~10. The method of any of the preceding claims, wherein the purified protein preparation comprises~~

~~70% to 100% of intact protein;~~
~~0% to 15% of Des-Ala protein isoform;~~
~~0% to 10% of methionated protein isoform; and~~
~~0% to 15% of acetylated protein isoform.~~

~~11. The method of any of the preceding claims, wherein the purified protein preparation comprises:~~

~~80% to 99% of intact protein;~~
~~2% to 7% of Des-Ala protein isoform;~~
~~0.1% to 4% of methionated protein isoform; and~~
~~0% to 5% of acetylated protein isoform.~~

~~12~~8. A method of purifying a chimeric cytokine protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, the method comprising:

(a) applying a load preparation containing intact and optionally des-Ala forms of the chimeric cytokine protein to a cation exchange surface;

(b) washing the cation exchange surface with a wash buffer that does not elute the chimeric cytokine protein, and eluting the cation exchange surface with an elution buffer to provide a CEX eluate that contains the intact form of the chimeric cytokine protein;

(c) applying the CEX eluate to an anion exchange surface;

(d) collecting the flow through from the anion exchange surface (AEX flowthrough);

(e) loading the AEX flowthrough onto a ceramic hydroxyapatite column (CHA);

(f) eluting the CHA column;

(g) collecting the eluate containing purified chimeric cytokine protein;

(h) optionally subjecting the eluate to an ultrafiltration and/or a diafilatration step and collecting the retentate containing purified chimeric cytokine protein.

~~13. A purified preparation of chimeric cytokine protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, produced using the method of claim 9.~~

~~14. The purified preparation of claim 13, wherein the protein is greater than 90% pure, wherein the preparation comprises less than 10% of des-Ala form, less than 10% of acetylated form, and less than 10% of methionated form.~~

~~15. The purified preparation of claim 13 or claim 14, wherein the preparation contains less than 50 parts per million of host cell protein.~~

~~16~~9. A method comprising applying a load preparation containing intact and ~~optionally~~ des-Ala, ~~acetylated or methionated~~ forms of a chimeric cytokine protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21 to a cation exchange surface;

washing the cation exchange surface with a wash buffer that does not elute the chimeric cytokine protein, and eluting the cation exchange surface with an elution buffer to provide a CEX eluate that contains the intact form of the chimeric cytokine protein, wherein the method enriches the intact form relative to the des-Ala form by decreasing the percentage of the des-Ala form in the CEX eluate by at least 1, 3, 5, 7, or 8% relative to the load preparation.

~~17~~10. The method of claim ~~16~~ 9 wherein the load preparation has a conductivity less than 5, 4, 3.5, 3.3 mS/cm.

~~18~~11. The method of ~~any of claims 16 or 17~~claim 9 wherein the load preparation is detergent free.

~~19~~12. The method of ~~any of claims 16 to 18~~claim 9 wherein the load preparation is prepared from lysate that has not been previously chromatographed.

~~20~~13. The method of claim ~~16~~ 9 wherein the load preparation is clarified by centrifugation and/or filtration.

~~21~~14. The method of claim ~~16~~ 9 wherein the load preparation has a pH of less than 6.0, 5.8, 5.6, or 5.4, or 5.3.

~~22. The method of claim 16 wherein the load preparation contains less than 30%, 25%, or 20% of the des-Ala form of the chimeric cytokine protein.~~

~~23. The method of claim 16 wherein the load preparation contains less than 30%, 25%, or 20% of the acetylated form of the chimeric cytokine protein.~~

~~24. The method of claim 16 wherein the load preparation contains less than 30%, 25%, or 20% of the methionated form of the chimeric cytokine protein.~~

2515. The method of claim ~~16~~ 9 further comprising adjusting the lysate to a pH less than 6.0 to prepare the load preparation.

2616. The method of claim ~~16~~ 9 wherein the cation exchange surface is a strong cation exchange (CEX) surface.

2717. The method of claim ~~16~~ 9 wherein the surface comprises a CEX matrix that can be packed into a column.

2818. The method of claim ~~27~~ 17 wherein the CEX matrix comprises a crosslinked poly(styrene-divinylbenzene) bead.

2919. The method of claim ~~27~~ 17 wherein the surface comprises sulfopropyl functional groups.

~~30. The method of claim 27 wherein the CEX matrix is Poros®XS, CMC-cellulose, SP-Sephadex® and SP-Sepharose® FF.~~

3120. The method of claim ~~27~~ 17 wherein the CEX matrix is in a column greater than 20 ml volume.

3221. The method of claim ~~27~~ 17 wherein the column is washed with greater than 1, 5, or 10 column volumes.

3322. The method of claim ~~27~~ 17 wherein the column is washed with a buffer with conductivity less than 5, 4, 3.5, or 3.3 mS/cm.

3423. The method of claim ~~27~~ 17 wherein the wash buffer has less than 40, 30, or 25 mM NaCl and the wash buffer has at least 5, 10, or 15 mM NaCl.

~~35. The method of claim 27 wherein the cation exchange surface is washed with two different buffers.~~

~~36. The method of claim 35, wherein one wash buffer has pH 5.3, and the other wash buffer has pH 6.2.~~

3724. The method of claim ~~27~~ 17 wherein the eluting comprises a step elution.

3825. The method of claim ~~37~~ 17 wherein the step elution comprises applying an elution buffer with a higher pH.

3926. The method of claim ~~37~~ 17 wherein the step elution is from a pH less than 5.5 to a pH above 5.9.

4027. The method of claim ~~37~~ 24 wherein the step elution comprises applying an elution buffer with increased salt concentration and/or increased conductivity.

4128. The method of claim ~~37~~ 24 wherein the salt concentration is increased from a concentration less than that of 30 mM NaCl to a concentration greater than that of 40 mM NaCl.

4229. The method of claim ~~37~~ 24 wherein the primary salt in the elution buffer is NaCl.

4330. The method of claim ~~37~~ 24 wherein the elution buffer has a conductivity greater than 4.

4431. The method of claim ~~37~~ 24 wherein the elution buffer comprises a buffer with buffering capacity in at least the range of pH 5.9-6.1.

4532. The method of claim ~~27~~ 9 wherein the intact form of the chimeric cytokine protein and the des-Ala form elute with different kinetics.

4633. The method of claim ~~27~~ 9 wherein the des-Ala form elutes subsequent to the intact form.

~~47. The method of claim 27 wherein the method enriches the intact form relative to the des-Ala form by decreasing the percentage of the des-Ala form in the CEX eluate by at least 1, 3, 5, 7, or 8% relative to the load preparation.~~

4834. The method of claim ~~27~~ 9 wherein the des-Ala form in the CEX elute is less than 20, 15, 12, or 10% of the total chimeric cytokine protein.

~~49. The method of claim 27 wherein the acetylated and methionated forms elute before the intact form.~~

~~50. The method of claim 27 wherein the method enriches the intact form relative to the acetylated and methionated forms by decreasing the percentage of the acetylated and methionated forms in the CEX eluate by at least 1, 3, 5, 7, or 8% relative to the load preparation.~~

~~51~~35. The method of claim ~~27~~ 9 wherein the ~~acetylated and~~ methionated form~~s~~ in the CEX elute ~~are each~~ is less than 20, 15, 12, or 10% of the total chimeric cytokine protein.

~~52~~36. The method of claim ~~27~~ 9 wherein the chimeric cytokine protein is at least 50, 60, 70, 80, 85, 90, or 95% pure in the CEX eluate.

~~53~~37. The method of claim ~~27~~ 9 wherein the CEX eluate has less HCP levels than the load preparation.

~~54~~38. The method of claim ~~27~~ 9 wherein the CEX eluate is applied to an anion exchange surface under conditions in which the chimeric cytokine protein does not substantially bind to the anion exchange surface.

~~55. A preparation of a chimeric cytokine protein comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21, wherein the preparation comprises the CEX eluate obtained by the method of claim 16.~~

~~56~~39. A method comprising applying a preparation containing a chimeric cytokine protein comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21 to an anion exchange (AEX) surface; and

collecting the AEX flow through from the anion exchange surface, wherein the AEX flowthrough contains fewer host cell proteins than the preparation applied to the anion exchange surface.

~~57. The method of claim 56 wherein the AEX flowthrough contains fewer host cell proteins than the preparation applied to the anion exchange surface.~~

~~58~~40. The method of claim ~~56 or 57~~39 wherein the anion exchange surface has an ionic capacity of 100-300 micromol Cl-/ml.

5941. The method of claim 56-39 wherein the anion exchange surface is a strong quaternary ammonium anion exchanger.

~~60. The method of claim 56 wherein the anion exchange surface comprises a Poros® HQ or Capto™ Q anion exchange resin.~~

6142. The method of claim 56-39 wherein the anion exchange surface comprises a membrane.

~~62. The method of claim 52 wherein endotoxin in the AEX flowthrough is reduced relative to the preparation applied to the anion exchange surface.~~

~~63. A method of producing a batch preparation of a protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, the method comprising the steps of:~~

~~providing a fraction from a batch preparation of the protein purified over a hydroxyapatite column;~~

~~evaluating the fraction by wCEX chromatography to determine the presence of one or more of des-Ala form, acetylated form, and methionated form of the protein; and~~

~~based upon the determination, further processing the batch protein preparation by a method including one or more steps selected from the group consisting of classifying, selecting, accepting, discarding, releasing, withholding, processing into a drug product, shipping, moving to a different location, formulating, labeling, packaging, releasing into commerce, selling and offering for sale the batch.~~

~~64. The method of claim 63, wherein one or more of des-Ala form, acetylated form, and methionated form of the protein is determined to be present in the batch at levels less than 10%.~~

~~65. The method of claim 63, wherein the step of further processing comprises accepting the batch for production of the protein preparation.~~

~~66. The method of claim 63, wherein evaluating comprises determining if the batch meets a predetermined reference value based on levels of one or more of des-Ala form, acetylated form, and methionated form of the protein.~~

~~67. The method of claim 66, wherein evaluating additionally comprises memorializing the determination.~~

~~68. The method of claim 67, wherein memorializing comprises memorializing in a computer readable record.~~

~~69. The method of claim 66, wherein the reference value is a value determined from a commercially available protein sample, or from a previous batch.~~

~~70. The method of claim 66, wherein the reference value is or comprises a production standard imposed by a regulatory agency.~~

~~71. The method of claim 66, wherein the reference value is or comprises a release standard.~~

~~72. The method of claim 63, further comprising altering a step in the production of the protein preparation based upon the determination.~~

~~73. The method of claim 63, wherein the step of determining comprises determining the presence or amount that is correlated with a desired property of the protein preparation.~~

~~74. The method of claim 73, wherein the property is listed on a protein product insert.~~

~~75. The method of claim 73, wherein the property appears in the U.S. PHARMACOPEIA for the protein.~~

~~76. A method of providing a chimeric cytokine protein, the method comprising culturing *E. coli* cells containing a nucleic acid sequence that encodes a protein comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21, to provide said chimeric cytokine protein.~~

~~77. The method of claim 76, wherein said nucleic acid sequence is comprised in a plasmid.~~

~~78. The method of claim 76, wherein said nucleic acid sequence is under the control of an inducible promoter system.~~

~~79. The method of claim 76, wherein said cells are cultured in at least 1 liter, 10L, 100L, 1000L or 5000L of medium.~~

~~80. The method of claim 76 wherein the nucleic acid sequence comprises the nucleotide sequence of SEQ ID NO:29.~~

~~81. The method of claim 76 wherein the cells are grown in media with a richer nutrient content than LB media.~~

~~82. The method of claim 76 wherein the cells are grown in media containing at least 0.5% or 1% or 1.2% tryptone, at least 1%, 2%, 2.2% or 2.4% yeast extract, and/or at least 0.1, 0.2, or 0.4% glycerol.~~

~~83. The method of claim 76 wherein transcription of the nucleic acid sequence is inducible with IPTG.~~

~~84. The method of claim 76 wherein the cells are induced at an OD greater than 5, 10, 20, 30, 35, or 40.~~

~~85. The method of claim 76 further comprising lysing the *E. coli* cells in the presence of a metal chelator to provide a preparation containing protein.~~

~~86. The method of claim 85 wherein the metal chelator is EDTA and is present at a concentration greater than 2.5 mM.~~

~~87. The method of claim 85 wherein the lysis buffer is at pH less than 7.5.~~

~~88. The method of claim 85 wherein the lysis buffer is detergent free.~~

~~89. The method of claim 85 wherein the lysis buffer has less than 200 mM NaCl or conductivity less than a salt solution with such concentrations of NaCl.~~

~~90. The method of claim 85 further comprising purifying the protein from the lysate by no greater than three or two column chromatography steps.~~

~~91. The method of claim 90 wherein the chimeric cytokine protein is at least 50, 60, 70, 80, 90, or 95% pure after a first column chromatography step.~~

~~92. A composition comprising a chimeric cytokine protein wherein the composition is obtained by the lysis method of claim 85, wherein the protein comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21.~~

~~93~~43. A purified preparation of chimeric cytokine protein comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21, wherein the chimeric cytokine protein is greater than 90% pure, wherein the preparation comprises less than 10% of des-Ala form~~, less than 10% acetylated form and less than 10% methionated form~~.

~~94~~44. The preparation of claim ~~93~~43 wherein HCP levels are less than 1000 ppm.

~~95~~45. The preparation of claim ~~93~~43 that is substantially free of nucleic acids and endotoxin.

~~96~~46. The preparation of claim ~~93~~43 that is lyophilized.

~~97~~47. The preparation of claim ~~93~~43 that is aqueous.

~~98~~48. The preparation of claim ~~93~~43 wherein the protein is between 1 -100 mg/ml.

~~99. A method of evaluating a preparation comprising a protein comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21, comprising:~~
~~acquiring a determination of one or more or all of:~~
~~a) the level of the des-Ala form of said protein;~~
~~b) the level of an acetylated form of said protein; or~~
~~c) the level of a methionated form of said protein;~~
~~thereby evaluating said preparation.~~

~~100. The method of claim 99, wherein the method further comprises comparing a determined level for one or more of all of a, b, and c, with a reference value, wherein each can have its own reference value.~~

~~101. The method of claim 100, wherein said reference value is determined from: a commercially available sample of said protein; another batch of said protein; a value set, recommended, published or required by a regulatory agency; a release specification or standard; a value set, recommended, published or required by a pharmacopeal authority.~~

~~102. The method of claim 100, wherein said determination comprises chromatographic analysis.~~

~~103. The method of claim 102, further comprising determining if said preparation has a preselected relationship to a reference value.~~

104. The method of claim 103, wherein, responsive to said determining, processing the preparation, wherein processing includes one of more of selecting, accepting, processing into drug product, shipping, formulating, labeling, packaging or selling the preparation.

105. The method of claim 103, wherein responsive to said determination, a parameter of a process for producing said protein is altered.

106. The method of claim 99, wherein if the level of one or more of the forms in a, b, or c, is less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% by weight/weight, then processing the preparation, wherein processing includes one of more of selecting, accepting, processing into drug product, shipping, formulating, labeling, packaging or selling the preparation.

107. The method of claim 99, wherein said preparation was made by subjecting a precursor preparation to a purification step.

108. The method of claim 99, wherein said preparation was made by contact of a precursor preparation by one or more or all of a chromatographic substrate.

109. The method of claim 99, wherein said preparation was made by contact of a precursor preparation with, in the following order, a cation exchange, anion exchange, and a hydroxyapatite substrate.

110. The method of claim 99, wherein said preparation is an intermediate in a purification process.

111. The method of claim 99, wherein said preparation comprises eluent from a purification process.

112. The method of claim 99, wherein said preparation comprises eluent from a cation exchange column.

113. The method of claim 99, wherein said preparation comprises eluent from an anion exchange column.

114. The method of claim 99, further comprising, subjecting a precursor preparation to a purification step.

115. The method of claim 99, further comprising contacting a precursor preparation with a chromatographic substrate.

116. The method of claim 99, further comprising contacting a precursor preparation with one or more or all of chromatographic substrate.

117. The method of claim 99, further comprising contacting a precursor preparation with, in the following order, a cation exchange, anion exchange, and a hydroxyapatite, substrate.

118. The method of claim 99, wherein the processing comprises formulating the preparation for administration into an eye.

119. The method of claim 99, further comprising memorializing the evaluation.

120. The method of claim 119, wherein memorializing comprises memorializing in a computer readable record.

121. A method of evaluating a protein preparation, comprising:

providing an isolated fraction from a preparation of a protein purified over a hydroxyapatite column, wherein the protein comprises an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21;

analyzing the fraction with a weak cation exchange (wCEX) chromatography to determine levels of one or more of des-Ala form, acetylated form and methionated form of the protein;

wherein if one or more of des-Ala form, acetylated form and methionated form of the protein is present at less than 10%, then processing the preparation, wherein processing includes one of more of selecting, accepting, processing into drug product, shipping, formulating, labeling, packaging or selling the preparation;

thereby analyzing the protein preparation.

122. A method of analyzing a process of making a preparation of a protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, the method comprising:

providing an isolated fraction of a preparation of the protein,

analyzing the fraction using the method of claim 97, and

if one or more of des-Ala form, acetylated form and methionated form of the protein is present at less than 10%, then maintaining the process based, at least in part, upon the analysis.

~~123. A method of processing a preparation of a protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, the method comprising:~~

~~providing a wCEX determination of the level of one or more of a des-Ala form, acetylated form and methionated form of the protein in a preparation, and~~

~~if one or more of des-Ala form, acetylated form and methionated form of the protein is present at less than 10%, then processing the preparation, wherein processing includes one of more of selecting, accepting, processing into drug product, shipping, formulating, labeling, packaging or selling the preparation.~~

~~124. The method of claim 123, wherein the processing comprises formulating the preparation for administration into an eye.~~

~~125. The method of claim 123, further comprising determining the activity of the protein.~~

~~126~~49. A composition comprising a protein comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21, a salt, a tonicity agent, and a detergent.

~~127~~50. The composition of claim ~~126~~49, wherein the salt is sodium citrate, sodium acetate, or sodium chloride, or Tris acetate.

~~128~~51. The composition of claim ~~126~~49, wherein the detergent is poloxamer 188, polysorbate 20, polysorbate 80, or a polyethoxylate.

~~129~~52. The composition of claim ~~126~~49, wherein the tonicity agent is sorbitol, mannitol, sucrose, trehalose, or glycerol.

~~130~~53. The composition of claim ~~126~~ 49 at pH 5.5, pH 5.6, pH 5.8, pH 5.9, pH 6, pH 6.1, pH 6.3, pH 6.5 or pH 6.6.

~~131. The composition of claim 126, comprising 1 mg/mL to 20mg/mL protein comprising an amino acid sequence of SEQ ID NO:21, 10mM sodium citrate, 5% sorbitol, 0.1% poloxamer 188, and having pH 6.~~

~~132~~54. The composition of claim ~~126~~49, suitable for administration to the eye.

~~133. A preparation comprising a protein comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:21, comprising:~~
~~a) between 0% and 15 % of the des-Ala form of said protein;~~
~~b) between 0% and 15 % of an acetylated form of said protein; and~~
~~c) between 0% and 15% of a methionated form of said protein.~~

~~134. The preparation of claim 133, wherein at least 80, 90, 95, 99% of the protein in said preparation is said protein and forms from a, b, and c.~~

~~135. The preparation of claim 133, comprising at least 6 grams, 60 grams, 600 grams, 6000 grams, 10 kg, 50 kg, 100 kg or 150 kg of said protein.~~

~~136. A preparation comprising a polypeptide, the preparation comprising:~~

~~70% to 100% of the polypeptide;~~
~~0% to 15% of Des-Ala polypeptide;~~
~~0% to 10% of methionated polypeptide; and~~
~~0% to 15% of acetylated polypeptide,~~
~~wherein the polypeptide comprises an amino acid sequence at least 90% identical to the sequence of SEQ ID NO:21.~~

~~137. The preparation of claim 136, the preparation comprising:~~

~~80% to 99% of the polypeptide;~~
~~2% to 7% of Des-Ala polypeptide;~~
~~0.1% to 4% of methionated polypeptide; and~~
~~0% to 5% of acetylated polypeptide.~~

55. The method of any of claims 1-7, wherein the purified preparation comprises less than 10% of methionated form of the protein.

56. The method of claim 8, wherein the eluate containing the purified preparation comprises less than 10% of des-Ala form of the protein.

57. The method of claim 8, wherein the eluate containing the purified preparation comprises less than 50 parts per million of host cell protein.

58. The preparation of claim 43, wherein the preparation contains less than 50 parts per million of host cell protein.

59. The preparation of claim 43, wherein the preparation contains less than 10% of methionated form of the protein.