

What is claimed:

1. A method of purifying a polypeptide comprising a circularly permuted IL-2 fused to the extracellular portion of an IL-2R α chain, the method comprising:
 - a) contacting a clarified cell supernatant comprising the polypeptide and host cell protein (HCP) with a first chromatography matrix under conditions such that the polypeptide binds to the matrix, and selectively eluting the polypeptide from the matrix in a first eluate;
 - b) adjusting the pH of the first eluate from step (a) to at least 10.5 and at most 11.5 to produce a pH-adjusted eluate; and
 - c) contacting the pH-adjusted eluate from step (b) with a second chromatography matrix such that the polypeptide binds to the matrix, and selectively eluting the polypeptide from the matrix in a second eluate, thereby purifying the polypeptide.
2. The method of claim 1, wherein the polypeptide comprises: an amino acid sequence that is at least 85%, 90%, 95%, or 99% identical to SEQ ID NO: 1; or that comprises the amino acid sequence of SEQ ID NO: 1.
3. The method of any one of the preceding claims, wherein the first chromatography matrix comprises an anion exchange chromatography (AEX) matrix.
4. The method of claim 3, wherein prior to contact with the AEX matrix the clarified cell supernatant is buffer-exchanged into a solution having a conductivity of about 1 - 2 mS/cm and a pH of about 8.0 – 8.5.
5. The method of any one of claims 3 and 4, wherein the AEX matrix comprises quaternary amine groups.
6. The method of any one of claims 3-5, wherein the AEX matrix comprises hydroxylated methacrylic polymer beads functionalized with quaternary amine groups.
7. The method of claim 6, wherein the mean diameter of the beads is about 75 μ m.

8. The method of claim 6 or 7, wherein the mean pore size of the beads is about 100 nm.
9. The method of any one of claims 3-8, wherein the polypeptide is eluted from the AEX matrix using a solution having a salt concentration equivalent of conductivity of about 15 to about 25 mS/cm.
10. The method of any one of claims 3-9, wherein the polypeptide is eluted from the AEX matrix using an aqueous solution of about 0.20 – 0.25 M sodium chloride at about pH 8.4 – 8.6.
11. The method of any one of the preceding claims, wherein the pH of the first eluate is adjusted using sodium carbonate and/or sodium hydroxide.
12. The method of any one of the preceding claims, wherein the pH of the first eluate is adjusted using sodium carbonate or sodium hydroxide at a ratio of about 0.1 kg sodium carbonate or sodium hydroxide to about 1kg of the first eluate.
13. The method of any one of the preceding claims, wherein the pH of the pH-adjusted eluate is maintained at or above 10.7 but below 11.0 for at least 1 hour.
14. The method of any one of the preceding claims, wherein the pH of the pH-adjusted eluate is maintained at or above 10.7 but below 11.0 for at least 1 hour, wherein the pH at or above 10.7 but below 11.0 is achieved by adding sodium carbonate or sodium hydroxide at a ratio of about 0.1 kg sodium carbonate or sodium hydroxide to about 1kg of the first eluate.
15. The method of any one of the preceding claims, further comprising lowering the pH of the pH-adjusted eluate by adding citric acid.
16. The method of any one of the preceding claims, further comprising lowering the pH of the pH-adjusted eluate to about pH 8.3-8.7.

17. The method of any one of the preceding claims, wherein prior to contact with the HIC matrix the conductivity of the pH-adjusted eluate is altered to be about 130 to about 160 mS/cm and the pH is altered to be about 8.5 ± 0.2 .
18. The method of claim 16, wherein the conductivity of the pH-adjusted eluate is altered using ammonium sulfate.
19. The method of any one of the preceding claims, wherein the second chromatography matrix comprises a hydrophobic interaction chromatography (HIC) matrix.
20. The method of claim 19, wherein prior to contact with the HIC matrix the pH-adjusted eluate is altered to comprise about 1 – 1.2 M ammonium sulfate.
21. The method of any one of claims 19 and 20, wherein the HIC matrix comprises polypropylene glycol groups.
22. The method of any one of claims 19-21, wherein the HIC matrix comprises hydroxylated methacrylic polymer beads linked to polypropylene glycol groups.
23. The method of claim 22, wherein the mean diameter of the beads is about 40 to about 90 μm .
24. The method of claim 22 or 23, wherein the mean pore size of the beads is about 75 nm.
25. The method of any one of claims 19-24, wherein the polypeptide is eluted from the HIC matrix using a solution having a salt concentration equivalent of conductivity of about 100 to about 140 mS/cm.
26. The method of any one of claims 19-25, wherein the polypeptide is eluted from the HIC matrix using a sequential multistep gradient of decreasing salt concentration.

27. The method of any one of claims 19-26, wherein the polypeptide is eluted from the HIC matrix using a buffer comprising about 0.85 to about 0.95 M ammonium sulfate at pH 8.5 ± 0.2 .

28. The method of any one of claims 19-26, wherein prior to contact with the HIC matrix the pH-adjusted eluate is filtered through a 0.2 μm filter.

29. The method of any one of the preceding claims, wherein prior to contact with the second chromatography matrix the pH-adjusted eluate is subject to viral inactivation.

30. The method of claim 29, wherein the viral inactivation is achieved by admixture of the pH-adjusted eluate with tri-n-butyl phosphate and polysorbate 20.

31. The method of any one of the preceding claims, wherein the polypeptide is further purified from the second eluate using mixed-mode chromatography (MMC).

32. The method of any one of the preceding claims, wherein the polypeptide is further purified from the second eluate using MMC followed by AEX.

33. The method of any one of the preceding claims, wherein the clarified cell supernatant is from a Chinese hamster ovary (CHO) cell culture.

34. The method of any one of the preceding claims, wherein the polypeptide in the second eluate is at least 90% pure.

35. The method of claim 34, wherein purity of the polypeptide is determined by Reverse Phase (RP) HPLC.

36. A method for reducing host cell protein (HCP) content from a clarified cell supernatant containing a polypeptide comprising a circularly permuted IL-2 fused to the extracellular portion of an IL-2R α chain, the method comprising contacting a partially purified polypeptide with an AEX matrix under conditions such that the polypeptide

binds to the AEX matrix, and selectively eluting the polypeptide from the AEX matrix under gradient elution conditions, thereby reducing HCP content from the polypeptide.

37. The method of claim 36, wherein the polypeptide comprises: an amino acid sequence that is at least 85%, 90%, 95%, or 99% identical to SEQ ID NO: 1; or that comprises the amino acid sequence of SEQ ID NO: 1.

38. The method of any one of claims 36 or 37, further comprising contacting the clarified cell supernatant comprising the polypeptide and HCP with one or more chromatography resins to obtain the partially purified polypeptide.

39. The method of any one of claims 34-36, wherein the HCP content is $\geq$ about 300 ppm prior to contacting the partially purified polypeptide with the AEX matrix.

40. The method of any one of claims 36-39, wherein the HCP content is $\geq$ about 150 ppm prior to contacting the partially purified polypeptide with the AEX matrix.

41. The method of any one of claims 36-40, wherein the HCP content is $\leq$ about 100 ppm after eluting the polypeptide from the AEX matrix under gradient elution conditions.

42. The method of any one of claims 36-41, wherein the HCP content is $\leq$ about 50 ppm after eluting the polypeptide from the AEX matrix under gradient elution conditions.

43. The method of any one of claims 36-42, wherein the gradient elution conditions comprise one or more of:

increasing the conductivity of an elution buffer over time;

increasing the salt concentration of an elution buffer over time; or

decreasing the pH of an elution buffer over time.

44. The method of claim 43, wherein the conductivity of the elution buffer is increased from about ≤ 5 mS/cm to about ≥ 15 mS/cm.

45. The method of claim 43, wherein the conductivity of the elution buffer is increased from about ≤ 2 mS/cm to about ≥ 15 mS/cm.
46. The method of claim 43, wherein the conductivity of the elution buffer is increased from about ≤ 2 mS/cm to about ≥ 20 mS/cm.
47. The method of claim 43, wherein the conductivity of the elution buffer is increased to between about 15 mS/cm to about 100 mS/cm.
48. The method of claim 43, wherein the conductivity of the elution buffer is increased to between about 20 mS/cm to about 50 mS/cm.
49. The method of claim 43, wherein the elution buffer comprises a final conductivity of between about 20 mS/cm to about 50 mS/cm.
50. The method of claim 43, wherein the elution buffer initially comprises a conductivity of about ≤ 5 mS/cm.
51. The method of claim 43, wherein the elution buffer initially comprises a conductivity of about ≤ 2 mS/cm.
52. The method of claim 43, wherein the salt concentration of the elution buffer is increased from about 0 M salt to about 1.5 M salt.
53. The method of claim 43, wherein the salt concentration of the elution buffer is increased from about 0 M salt to about 1.0 M salt.
54. The method of claim 43, wherein the salt concentration of the elution buffer is increased from about 0 M salt to about 0.5 M salt.
55. The method of claim 43, wherein the elution buffer comprises a final salt concentration of between about 0.2 M salt to about 1.0 M salt.

56. The method of claim 43, wherein the elution buffer comprises a final salt concentration of about 0.2 M salt.
57. The method of any one of claims 43-56, wherein the salt comprises sodium chloride.
58. The method of any one of claims 43-57, wherein the elution buffer further comprises a pH of about 7.0 to about 9.0.
59. The method of any one of claims 43-58, wherein the elution buffer further comprises a pH of about 8.0.
60. The method of any one of claims 43-59, wherein the one or more chromatography resins to obtain the partially purified polypeptide are selected from the group consisting of AEX, HIC, and MMC.
61. The method of claim 60, wherein obtaining the partially purified polypeptide comprises the steps of:
- a1) contacting the clarified cell supernatant comprising the polypeptide and HCP with a first AEX matrix under conditions such that the polypeptide binds to the AEX matrix, and selectively eluting the polypeptide from the AEX matrix in a first eluate;
 - a2) contacting the first eluate from step (a1) with a HIC matrix such that the polypeptide binds to the HIC matrix, and selectively eluting the polypeptide from the HIC matrix in a second eluate; and
 - a3) contacting the second eluate from step (a2) with a MMC matrix such that the polypeptide binds to the MMC matrix, and selectively eluting the polypeptide from the MMC matrix in a third eluate, thereby obtaining the partially purified polypeptide.
62. The method of claim 61, wherein prior to contact with the first AEX matrix the clarified cell supernatant is buffer-exchanged into a solution having a conductivity of about 1 - 2 mS/cm and a pH of about 8.0 - 8.5.

63. The method of any one of claims 61 or 62, wherein the first AEX matrix comprises quaternary amine groups.
64. The method of any one of claims 61-63, wherein the first AEX matrix comprises hydroxylated methacrylic polymer beads functionalized with quaternary amine groups.
65. The method of claim 64, wherein the mean diameter of the beads is about 75 μm .
66. The method of claim 64 or 65, wherein the mean pore size of the beads is about 100 nm.
67. The method of any one of claims 61-66, wherein the polypeptide is eluted from the first AEX matrix using a solution having a salt concentration equivalent of conductivity of about 15 to about 25 mS/cm.
68. The method of any one of claims 61-67, wherein the polypeptide is eluted from the first AEX matrix using an aqueous solution of about 0.20 – 0.25 M sodium chloride at about pH 8.4 – 8.6.
69. The method of any one of claims 61-68, wherein the pH of the first eluate is adjusted using sodium carbonate and/or sodium hydroxide to produce a pH-adjusted first eluate.
70. The method of any one of claims 61-69, wherein the pH of the first eluate is adjusted using sodium carbonate or sodium hydroxide at a ratio of about 0.1 kg sodium carbonate or sodium hydroxide to about 1kg of the first eluate.
71. The method claim 69 or 70, wherein the pH of the pH-adjusted first eluate is maintained at or above 10.7 but below 11.0 for at least 1 hour.
72. The method of any one of claims 61-71, wherein the pH of the pH-adjusted eluate is maintained at or above 10.7 but below 11.0 for at least 1 hour, wherein the pH at or above 10.7 but below 11.0 is achieved by adding sodium carbonate or sodium hydroxide

at a ratio of about 0.1 kg sodium carbonate or sodium hydroxide to about 1kg of the first eluate.

73. The method of claim 71 or 72, further comprising lowering the pH of the pH-adjusted first eluate by adding citric acid.

74. The method of any one of claims 69-73, further comprising lowering the pH of the pH-adjusted first eluate to about pH 8.3-8.7.

75. The method of any one of claims 61-74, wherein prior to contact with the HIC matrix the conductivity of the pH-adjusted first eluate is altered to be about 130 to about 160 mS/cm and the pH is altered to be about 8.5 ± 0.2 .

76. The method of claim 75, wherein the conductivity of the pH-adjusted first eluate is altered using ammonium sulfate.

77. The method of claim 76, wherein prior to contact with the HIC matrix the pH-adjusted first eluate is altered to comprise about 1 – 1.2 M ammonium sulfate.

78. The method of any one of claims 61-77, wherein the HIC matrix comprises polypropylene glycol groups.

79. The method of any one of claims 61-78, wherein the HIC matrix comprises hydroxylated methacrylic polymer beads linked to polypropylene glycol groups.

80. The method of claim 79, wherein the mean diameter of the beads is about 40 to about 90 μm .

81. The method of claim 79 or 80, wherein the mean pore size of the beads is about 75 nm.

82. The method of any one of claims 61-81, wherein the polypeptide is eluted from the HIC matrix using a solution having a salt concentration equivalent of conductivity of about 100 to about 140 mS/cm.

83. The method of any one of claims 61-82, wherein the polypeptide is eluted from the HIC matrix using a sequential multistep gradient of decreasing salt concentration.

84. The method of any one of claims 61-83, wherein the polypeptide is eluted from the HIC matrix using a buffer comprising about 0.85 to about 0.95 M ammonium sulfate at pH 8.5±0.2.

85. The method of any one of claims 61-84, wherein prior to contact with the HIC matrix the pH-adjusted eluate is filtered through a 0.2 µm filter.

86. The method of any one of claims 61-85, wherein prior to contact with the HIC matrix the pH-adjusted first eluate is subject to viral inactivation.

87. The method of claim 86, wherein the viral inactivation is achieved by admixture of the pH-adjusted eluate with tri-n-butyl phosphate and polysorbate 20.

88. The method of any one of claims 61-87, wherein the clarified cell supernatant is from a Chinese hamster ovary (CHO) cell culture.

89. A method for reducing host cell protein (HCP) content from a clarified cell supernatant to containing a polypeptide comprising a circularly permuted IL-2 fused to the extracellular portion of an IL-2Rα chain, the method comprising the steps of:

- a. contacting the clarified cell supernatant comprising the polypeptide and HCP with a first AEX matrix under conditions such that the polypeptide binds to the first AEX matrix, and selectively eluting the polypeptide from the first AEX matrix in a first eluate;
- b. contacting the first eluate with a HIC matrix such that the polypeptide binds to the HIC matrix, and selectively eluting the polypeptide from the HIC matrix in a second eluate;

- c. contacting the second eluate with a MMC matrix such that the polypeptide binds to the MMC matrix, and selectively eluting the polypeptide from the MMC matrix in a third eluate; and
- d. contacting the third eluate with a second AEX matrix under conditions such that the polypeptide binds to the second AEX matrix, and selectively eluting the polypeptide from the second AEX matrix under gradient elution conditions, thereby reducing HCP content from the polypeptide, wherein the HCP content is $\leq$ about 50 ppm after step (d).

90. The method of claim 89, wherein the polypeptide comprises: an amino acid sequence that is at least 85%, 90%, 95%, or 99% identical to SEQ ID NO: 1; or that comprises the amino acid sequence of SEQ ID NO: 1.

91. The method of claims 36-90, wherein an affinity purification step is not used.

92. A composition comprising a polypeptide comprising a circularly permuted IL-2 fused to the extracellular portion of an IL-2R α chain, wherein the HCP content of the composition comprises $\leq$ about 100 ppm.

93. The composition of claim 92, wherein the HCP content of the composition comprises $\leq$ about 50 ppm.

94. The composition of claim 92, wherein the polypeptide comprises: an amino acid sequence that is at least 85%, 90%, 95%, or 99% identical to SEQ ID NO: 1; or that comprises the amino acid sequence of SEQ ID NO: 1.

95. The composition of claim 92, produced by the method of any one of claims 30-83.

96. A method of improving the serum half-life of a composition comprising a plurality of polypeptides, each polypeptide of the plurality comprising a circularly permuted IL-2 fused to the extracellular portion of an IL-2R α chain, the method comprising the steps of:

- a. contacting the clarified cell supernatant comprising the polypeptide with a first AEX matrix under conditions such that the polypeptide binds to the first AEX matrix, and selectively eluting the polypeptide from the first AEX matrix in a first eluate;
- b. contacting the first eluate with a HIC matrix such that the polypeptide binds to the HIC matrix, and selectively eluting the polypeptide from the HIC matrix in a second eluate;
- c. contacting the second eluate with a MMC matrix such that the polypeptide binds to the MMC matrix, and selectively eluting the polypeptide from the MMC matrix in a third eluate; and
- d. contacting the third eluate with a second AEX matrix under conditions such that the polypeptide binds to the second AEX matrix, and selectively eluting the polypeptide from the second AEX, thereby improving the serum half-life of the composition.

97. The method of claim 96, wherein the polypeptide comprises: an amino acid sequence that is at least 85%, 90%, 95%, or 99% identical to SEQ ID NO: 1; or that comprises the amino acid sequence of SEQ ID NO: 1.

98. The method of claim 96, further comprising adjusting the pH of the first eluate from step (a) to at least 10.5 and at most 11.5 before contacting the first eluate with the HIC matrix of step (b).

99. A composition comprising a plurality of polypeptides, each polypeptide of the plurality comprising the amino sequence of SEQ ID NO: 1 linked to one or more glycan species, wherein the one or more glycan species are linked to the polypeptide at one or more of amino acid positions N187, N206, and T212 of SEQ ID NO: 1.

100. The composition of claim 99, wherein the glycan species at amino acid position N187 of SEQ ID NO: 1 are selected from the group consisting of:

Hex5HexNAc4FucNeuAc2;

Hex6HexNAc5FucNeuAc2;

Hex5HexNAc4FucNeuAc;

Hex6HexNAc5FucNeuAc3;
Hex4HexNAc4FucNeuAc;
Hex5HexNAc5NeuAc2;
Hex5HexNAc4Fuc;
Hex3HexNAc4Fuc;
Hex4HexNAc4Fuc;
Hex6HexNAc5Fuc; and
Hex5HexNAc5Fuc;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, Fuc represents fucose, and the number represents the number of each glycan structure.

101. The composition of claim 99, wherein the glycan species at amino acid position N206 of SEQ ID NO: 1 are selected from the group consisting of:

Hex6HexNAc5FucNeuAc3;
Hex5HexNAc4FucNeuAc2;
Hex6HexNAc5FucNeuAc2;
Hex7HexNAc6FucNeuAc3;
Hex6HexNAc5FucNeuAc;
Hex5HexNAc4FucNeuAc;
Hex5HexNAc4Fuc; and
Hex4HexNAc4Fuc;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, Fuc represents fucose, and the number represents the number of each glycan structure.

102. The composition of claim 99, wherein the glycan species at amino acid position T212 of SEQ ID NO: 1 are selected from the group consisting of:

HexHexNAc;
HexHexNAcNeuAc; and
HexHexNAcNeuAc2;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, and the number represents the number of each glycan structure.

103. The composition of claim 99, wherein the overall percent of glycan species at amino acid position N187 of SEQ ID NO: 1 of the plurality of polypeptides in the composition comprises:

- about 60% to about 70% Hex5HexNAc4FucNeuAc2;
- about 4% to about 6% Hex6HexNAc5FucNeuAc2;
- about 7% to about 10% Hex5HexNAc4FucNeuAc;
- about 15% to about 17% Hex6HexNAc5FucNeuAc3; and
- about 3% to about 4% Hex5HexNAc5NeuAc2;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, Fuc represents fucose, and the number represents the number of each glycan structure.

104. The composition of claim 99, wherein the overall percent of glycan species at amino acid position N187 of SEQ ID NO: 1 of the plurality of polypeptides in the composition comprises:

- about 60% to about 70% Hex5HexNAc4FucNeuAc2;
- about 4% to about 6% Hex6HexNAc5FucNeuAc2;
- about 7% to about 10% Hex5HexNAc4FucNeuAc;
- about 15% to about 17% Hex6HexNAc5FucNeuAc3;
- about 0.5% to about 1.5% Hex4HexNAc4FucNeuAc;
- about 3% to about 4% Hex5HexNAc5NeuAc2;
- about 0% to about 0.5% Hex5HexNAc4Fuc;
- about 0% to about 0.5% Hex3HexNAc4Fuc;
- about 0% to about 0.5% Hex4HexNAc4Fuc;
- about 0% to about 0.5% Hex6HexNAc5Fuc; and
- about 0% to about 0.5% Hex5HexNAc5Fuc;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, Fuc represents fucose, and the number represents the number of each glycan structure.

105. The composition of claim 99, wherein the overall percent of glycan species at amino acid position N206 of SEQ ID NO: 1 of the plurality of polypeptides in the composition comprises:

- about 3% to about 5% Hex6HexNAc5FucNeuAc3;
- about 75% to about 85% Hex5HexNAc4FucNeuAc2;
- about 2% to about 4% Hex6HexNAc5FucNeuAc2;
- about 5% to about 12% Hex5HexNAc4FucNeuAc;
- about 1% to about 3% Hex5HexNAc4Fuc; and

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, Fuc represents fucose, and the number represents the number of each glycan structure.

106. The composition of claim 99, wherein the overall percent of glycan species at amino acid position N206 of SEQ ID NO: 1 of the plurality of polypeptides in the composition comprises:

- about 3% to about 5% Hex6HexNAc5FucNeuAc3;
- about 75% to about 85% Hex5HexNAc4FucNeuAc2;
- about 2% to about 4% Hex6HexNAc5FucNeuAc2;
- about 0.5% to about 1.5% Hex7HexNAc6FucNeuAc3;
- about 0% to about 1% Hex6HexNAc5FucNeuAc;
- about 5% to about 12% Hex5HexNAc4FucNeuAc;
- about 1% to about 3% Hex5HexNAc4Fuc; and
- about 0.5% to about 2% Hex4HexNAc4Fuc;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, Fuc represents fucose, and the number represents the number of each glycan structure.

107. The composition of claim 99, wherein the overall percent of glycan species at amino acid position T212 of SEQ ID NO: 1 of the plurality of polypeptides in the composition comprises:

- about 14% to about 18% HexHexNAcNeuAc; and
- about 8% to about 13% HexHexNAcNeuAc2;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, and the number represents the number of each glycan structure.

108. The composition of claim 99, wherein the overall percent of glycan species at amino acid position T212 of SEQ ID NO: 1 of the plurality of polypeptides in the composition comprises:

- about 0% to about 1% HexHexNAc;
- about 14% to about 18% HexHexNAcNeuAc; and
- about 8% to about 13% HexHexNAcNeuAc₂;

wherein Hex represents hexose, HexNAc represents N-acetylhexosamine, NeuAc represents N-acetylneuraminic acid, and the number represents the number of each glycan structure.

109. A composition comprising a plurality of polypeptides, each polypeptide of the plurality comprising circularly permuted IL-2 fused to the extracellular portion of an IL-2R α chain, wherein the composition comprises a capillary isoelectric focusing (cIEF) profile as depicted in Figure 15.

110. The composition of claim 109, comprising a cIEF profile peak at one or more of about pI 5.73, about pI 5.93, about pI 6.09, about pI 6.28, about pI 6.38, about pI 6.48, about pI 6.53, about pI 6.66, about pI 6.82, and about pI 7.02.

111. The composition of claim 110, comprising a peak area percent of:

- about 8% to about 12% at pI 5.93;
- about 18% to about 26% at pI 6.09;
- about 22% to about 26% at pI 6.38; and
- about 18% to about 28% at pI 6.66.

112. The composition of claim 110, comprising a peak area percent of:

- about 1.5% to about 2.5% at pI 5.73;
- about 8% to about 12% at pI 5.93;
- about 18% to about 26% at pI 6.09;

about 3.5% to about 4.5% at pI 6.28;
about 22% to about 26% at pI 6.38;
about 3% to about 5% at pI 6.48;
about 4% to about 6% at pI 6.53;
about 18% to about 28% at pI 6.66;
about 2% to about 6% at pI 6.82; and
about 0% to about 3% at pI 7.02.

113. The composition of claim 110, wherein the peak at about pI 5.73 comprises the combination of peaks at about pI 5.70 and about pI 5.76.

114. The composition of claim 110, wherein the peak at about pI 5.93 comprises the combination of peaks at about pI 5.89 and about pI 5.97.

115. The composition of any one of claims 99-114, produced by the methods of any one of claims 1-98.