CLAIMS

WHAT IS CLAIMED IS:

~~1. A delivery construct consisting of an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 13.~~

~~2. The delivery construct of claim 1, wherein the delivery construct consists of the amino acid sequence set forth in SEQ ID NO: 5.~~

~~3. The delivery construct of claim 1, wherein the delivery construct consists of the amino acid sequence set forth in SEQ ID NO: 13.~~

~~4. The delivery construct of any one of claims 1-3, wherein the delivery construct is part of a homodimer.~~

~~5. The delivery construct of any one of claims 1-4, wherein the delivery construct is disposed within a composition that is formulated for oral administration, wherein the composition for oral administration comprises a plurality of delivery constructs identical to the delivery construct, and wherein at least 80% of the delivery constructs are in a dimer form.~~

~~6. A method of treating an inflammatory disease in a subject, the method comprising administering to the subject an effective amount of a delivery construct of any one of claims 1-5.~~

~~7. The method of claim 6, wherein the inflammatory disease is ulcerative colitis, proctitis, pouchitis, Crohn's disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft versus host disease (GVHD), rheumatoid arthritis, inflammatory bowel disease (IBD), Celiac disease, psoriatic arthritis, or psoriasis.~~

~~8. The method of claim 6, wherein the inflammatory disease is ulcerative colitis.~~

~~9. The method of claim 6, wherein the inflammatory disease is Crohn's disease.~~

~~10. The method of claim 6, wherein the inflammatory disease is celiac disease.~~

~~11. A method of refolding an IL-10 delivery construct, the method comprising:~~

(i) contacting inclusion bodies comprising the IL-10 delivery construct with a solubilization solution comprising a chaotropic agent to produce a soluble IL-10 delivery construct;

(ii) contacting the soluble IL-10 delivery construct with a refolding solution comprising reduced glutathione and oxidized glutathione to produce a refolded IL-10 delivery construct; wherein the method does not comprise contacting the soluble IL-10 delivery construct with a sulfitolysis agent or a reducing agent prior to the contacting of step (ii).

12. The method of claim 11, wherein the IL-10 delivery construct comprises a carrier.

13. The method of claim 12, wherein the carrier is derived from a polypeptide secreted by a bacterium.

14. The method of claim 13, wherein the bacterium is *Vibrio cholerae*.

15. The method of claim 14, wherein the polypeptide secreted by *Vibrio cholerae* is a cholix polypeptide.

16. The method of any one of claims 11-15, wherein the IL-10 delivery construct has at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 5.

17. The method of claim 16, wherein the IL-10 delivery construct has a V1L substitution at amino acid position 1 of the carrier.

18. The method of any one of claims 11-17, wherein the refolding solution comprises a ratio of the reduced glutathione to the oxidized glutathione from 0.8:1 to 1.2:1.

19. The method of any one of claims 11-18, wherein the refolding solution comprises from 0.75 mM to 1.5 mM reduced glutathione.

20. The method of any one of claims 11-19, wherein the refolding solution comprises from 0.25 mM to 0.75 mM oxidized glutathione.

21. The method of any one of claims 11-20, wherein the refolding solution has a pH from 7.5 to 8.5.

22. The method of any one of claims 11-21, wherein the refolding solution comprises arginine, Tris, and EDTA.

~~23. The method of claim 22, wherein the refolding solution comprises sucrose.~~

~~24. The method of any one of claims 11-21, wherein the refolding solution comprises arginine, sucrose, Tris, EDTA, or a combination thereof~~

~~25. The method of claim 22, wherein arginine is present in the refolding solution at a concentration of between 900 mM and 1.1 M.~~

~~26. The method of any one of claims 22-25, wherein sucrose is present in the refolding solution at a concentration of between 200 mM and 300 mM.~~

~~27. The method of any one of claims 22-26, wherein Tris is present in the refolding solution at a concentration of from 75 mM to 125 mM.~~

~~28. The method of any one of claims 22-27, wherein EDTA is present in the refolding solution at a concentration of from 1.75 mM to 2.25 mM.~~

~~29. The method of any one of claims 11-28, further comprising lysing a cell comprising the inclusion bodies.~~

~~30. The method of claim 29, wherein the cell is a bacterium.~~

~~31. The method of claim 30, wherein the bacterium is *Escherichia coli*.~~

~~32. The method of any one of claims 29-31, wherein the lysing comprises high-pressure homogenization.~~

~~33. The method of any one of claims 11-32, further comprising isolating the inclusion bodies.~~

~~34. The method of any one of claims 11-33, wherein the chaotropic agent comprises guanidine hydrochloride or urea.~~

~~35. The method of any one of claims 11-34, wherein the solubilization solution further comprises Tris.~~

~~36. The method of any one of claims 11-35, wherein the contacting the soluble IL-10 delivery construct with a refolding solution occurs for at least 16 hours.~~

~~37. The method of any one of claims 11-36, wherein the contacting the soluble IL-10 delivery construct with a refolding solution occurs from 12 hours to 18 hours.~~

38. The method of any one of claims 11-37, wherein the contacting the IL-10 delivery construct with a refolding solution occurs from 2°C to 8°C.

39. The method of any one of claims 11-38, further comprising a first sterile filtration of the refolded IL-10 delivery construct.

40. The method of claim 39, wherein the first sterile filtration occurs after the contacting with the refolding solution.

41. The method of claim 40, further comprising performing a tangential flow filtration of the refolded IL-10 delivery construct.

42. The method of claim 41, wherein the tangential flow filtration comprises diafiltration.

43. The method of claim 42, wherein the diafiltration comprises a first diavolume, a second diavolume, a third diavolume, and a fourth diavolume.

44. The method of claim 43, wherein the first diavolume and the second diavolume comprise a cold buffer.

45. The method of claim 44, wherein the third diavolume and the fourth diavolume comprise a room temperature buffer.

46. The method of claim 44 or 45, wherein the cold buffer and the room temperature buffer comprise Tris and NaCl.

47. A method of enriching for IL-10 delivery construct dimers from a pool comprising IL-10 delivery constructs in a dimer form, a monomer form, and an aggregate form, the method comprising:

(i) performing anion exchange (AEX) chromatography on the pool by binding the IL-10 delivery construct dimers to an anion exchange column and subsequently eluting the IL-10 delivery construct dimers from the anion exchange column, thereby creating a first plurality of fractions, one of which is a first fraction enriched in IL-10 delivery constructs in the dimer form; and

(ii) performing ceramic hydroxyapatite (CHT) chromatography on the fraction enriched in IL-10 delivery constructs in the dimer form, thereby creating a second plurality of fractions, one of which is a second fraction further enriched in IL-10 delivery constructs in the dimer form.

48. The method of claim 47, wherein the IL-10 delivery construct comprises a carrier.

49. The method of claim 48, wherein the carrier is derived from a polypeptide secreted by a bacterium.

50. The method of claim 49, wherein the bacterium is *Vibrio cholerae*.

51. The method of claim 50, wherein the polypeptide secreted by *Vibrio cholerae* is a cholix polypeptide.

52. The method of any one of claims 47-51, wherein the IL-10 delivery construct has at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 5.

53. The method of claim 52, wherein the IL-10 delivery construct has a V1L substation at amino acid position 1 of the carrier.

54. The method of any one of claims 47-53, further comprising determining a percentage of IL-10 delivery constructs in the dimer form in each fraction of the first plurality of fractions.

55. The method of claim 54, wherein the determining is by size exclusion chromatography.

56. The method of claim 55, wherein the size exclusion chromatography is size exclusion high performance liquid chromatography (SE-HPLC).

57. The method of claim 47, further comprising determining a percentage of IL-10 delivery constructs in the dimer form in each fraction of the second plurality of fractions.

58. The method of claim 57, wherein the determining is by size exclusion chromatography.

59. The method of claim 58, wherein the size exclusion chromatography is size exclusion high performance liquid chromatography (SE-HPLC).

60. The method of any one of claims 47-59, wherein at least 75% of the IL-10 delivery constructs in the first fraction are IL-10 delivery constructs in the dimer form.

61. The method of any one of claims 47-60, wherein at least 80% of the IL-10 delivery constructs in the second fraction are IL-10 delivery constructs in the dimer form.

62. The method of any one of claims 47-61, further comprising performing tangential flow filtration of the second fraction.

63. The method of claim 62, wherein the tangential flow filtration comprises ultrafiltration.

64. The method of claim 63, further comprising diafiltration.

65. The method of claim 64, further comprising performing sterile filtration the second fraction.

66. The method of any one of claims 47-65, wherein the method does not comprise cation exchange chromatography.

67. The method of any one of claims 47-66, wherein the pool comprises refolded IL-10 delivery constructs obtained from the method of any one of claims 11-46.

68. An oral formulation comprising:

(a) IL-10 delivery constructs;

(b) one or more pharmaceutically acceptable excipients; and

(c) a first coat comprising two or more copolymers each having a different nominal dissolution pH;

wherein the oral formulation is configured to release substantially none of the IL-10 delivery construct after 1 h exposure to a solution having a pH of 1.0 in a Type 4 dissolution apparatus in open mode.

69. The oral formulation of claim 68, wherein the solution having the pH of 1.0 is a dissolution media containing hydrochloric acid.

70. The oral formulation of claim 68 or claim 69, wherein the oral formulation is configured to release at least 40% of the IL-10 delivery construct after 2 hours of exposure to a solution having a pH of 7.0 in a Type 4 dissolution apparatus in open mode.

71. The oral formulation of any one of claims 68-70, wherein at least 5%, at least 10%, at least 20%, or at least 25% of the IL-10 delivery constructs released following 2 hours of exposure to the solution having the pH of 7.0 are in a dimer form.

72. The oral formulation of claim 70 or claim 71, wherein the solution having the pH of 7.0 is a citrate/phosphate buffer.

73. The oral formulation of any one of claims 68-72, wherein the IL-10 delivery construct comprises a carrier.

74. The method of claim 73, wherein the carrier is derived from a polypeptide secreted by a bacterium.

75. The method of claim 74, wherein the bacterium is *Vibrio cholerae*.

76. The method of claim 75, wherein the polypeptide secreted by *Vibrio cholerae* is a cholix polypeptide.

77. The oral formulation of any one of claims 73-76, wherein the IL-10 delivery constructs have at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 5.

78. The oral formulation of claim 77, wherein the IL-10 delivery constructs have a V1L substitution at amino acid position 1 of the carrier.

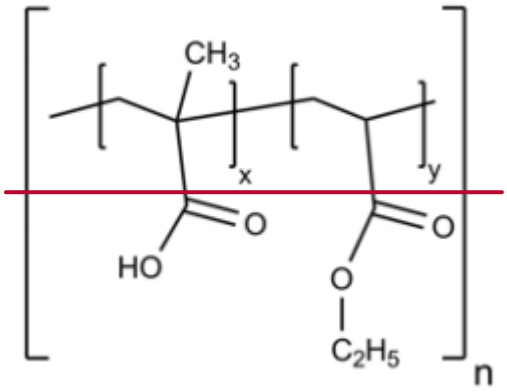
79. The oral formulation of any one of claims 68-78, wherein the oral formulation is in a capsule or a tablet.

80. The oral formulation of any one of claims 68-79, wherein a first copolymer has at least 50% nominal dissolution at pH > 5.5 and a second copolymer has at least 50% nominal dissolution at pH > 7.0.

81. The oral formulation of claim 80, wherein the first copolymer comprises methacrylic acid and ethyl acrylate.

82. The oral formulation of claim 80 or claim 81, wherein the first polymer has a weight average molecular mass of from 200,000 g/mol to 450,000 g/mol, or from 250,000 g/mol to 400,000 g/mol, or from 280,000 g/mol to 370,000 g/mol, or from 300,000 g/mol to 340,000 g/mol.

83. The oral formulation of any one of claims 80-82, wherein the first copolymer comprises the polymer of formula I:



Formula I;

wherein x, y, and n are each greater than or equal to one.

84. The oral formulation of any one of claims 80-83, wherein a ratio of free carboxyl groups to ester groups in the first copolymer is from 0.8:1 and 1.2:1.

85. The oral formulation of any one of claims 80-84, wherein the second copolymer is different from the first copolymer.

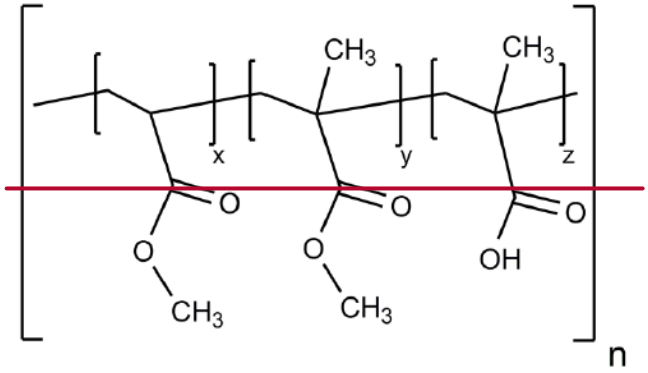
86. The oral formulation of any one of claims 80-85, wherein a ratio of the first copolymer to the second copolymer in the first coat is from 15:85 to 55:45.

87. The oral formulation of any one of claims 80-85, wherein a ratio of the first copolymer to the second copolymer in the first coat is 20:80, 30:70, 40:60, or 50:50.

88. The oral formulation of any one of claims 80-87, wherein the second copolymer comprises methacrylic acid, methyl methacrylate, and methyl acrylate.

89. The oral formulation of any one of claims 80-88, wherein the second polymer has a weight average molecular mass of from 160,000 g/mol to 400,000 g/mol or from 200,000 g/mol to 360,000 g/mol, or from 240,000 g/mol to 320,000 g/mol, or from 260,000 g/mol to 300,000 g/mol.

90. The oral formulation of any one of claims 80-89, wherein the second copolymer comprises the polymer of formula II:



Formula II;

wherein x, y, z, and n are each greater than or equal to one.

91. The oral formulation of any one of claims 80-90, wherein a ratio of free carboxyl groups to ester groups in the second copolymer is from 0.8:1 to 1.2:1.

92. The oral formulation of any one of claims 80-91, wherein the first coat further comprises an anti-tacking agent, a plasticizer, a surfactant, or a combination thereof.

93. The oral formulation of claim 92, wherein the first coat comprises an anti-tacking agent, wherein the anti-tacking agent comprises glycerol monostearate.

94. The oral formulation of claim 92 or claim 93, wherein the first coat comprises a plasticizer, wherein the plasticizer is triethyl citrate.

95. The oral formulation of any one of claims 92-94, wherein the first coat comprises a surfactant, wherein the surfactant is polysorbate 80.

96. The oral formulation of any one of claims 92-95, wherein from 5% to 15% (w/w) of the first coat is a mixture of glycerol monostearate, triethyl citrate, and polysorbate 80.

97. The oral formulation of any one of claims 68-96, wherein the first coat has a thickness substantially equivalent to the thickness of a 60 mg coat on a size 1 capsule.

98. The oral formulation of any one of claims 68-96, wherein the first coat is disposed around an interior portion in an amount from 0.1 mg/mm^2 to 0.2 mg/mm^2.

99. The oral formulation of any one of claims 68-96, wherein the first coat has a mass from 30 mg to 60 mg.

~~100. The oral formulation of any one of claims 68-99, further comprising a second coat exterior of the first coat.~~

~~101. The oral formulation of claim 100, wherein the second coat comprises hydroxypropyl methylcellulose (HPMC).~~

~~102. The oral formulation of claim 100 or claim 101, further comprising a third coat interior to the first coat and exterior of the IL-10 delivery constructs and the one or more pharmaceutically acceptable excipients.~~

~~103. The oral formulation of claim 102, wherein the third coat comprises HPMC.~~

~~104. The oral formulation of any one of claims 68-103, wherein the IL-10 delivery constructs are present in the oral formulation in an amount from 1 mg to 20 mg.~~

~~105. The oral formulation of any one of claims 68-103, wherein the IL-10 delivery constructs are present in the oral formulation in an amount of 1 mg, 5 mg, or 20 mg.~~

~~106. The oral formulation of any one of claims 68-105, wherein the one or more pharmaceutically acceptable excipients comprise a surfactant, an osmolyte, a salt, and a bulking agent.~~

~~107. The oral formulation of claim 106, wherein the salt comprises potassium phosphate, the bulking agent comprises glycine, the osmolyte comprises sucrose, and the surfactant comprises poloxamer 188.~~

~~108. The oral formulation of claim 106 or claim 107, wherein the oral formulation comprises a weight ratio of the osmolyte to the IL-10 delivery construct of from 0.45:1 to 0.55:1, preferably about 0.5:1.~~

~~109. The oral formulation of any one of claims 106-108, wherein the oral formulation comprises a weight ratio of the surfactant to the IL-10 delivery construct of from 0.12:1 to 0.18:1, preferably about 0.15:1.~~

~~110. The oral formulation of any one of claim 106-109, wherein the oral formulation comprises a weight ratio of the salt to the IL-10 delivery construct of from 0.05:1 to 0.09:1, preferably about 0.07:1.~~

111. The oral formulation of any one of claim 106-110, wherein the oral formulation comprises a weight ratio of the bulking agent to the IL-10 delivery construct of from 0.8:1 to 1.2:1, preferably about 1:1.

112. The oral formulation of any one of claims 68-111, wherein the oral formulation is a solid.

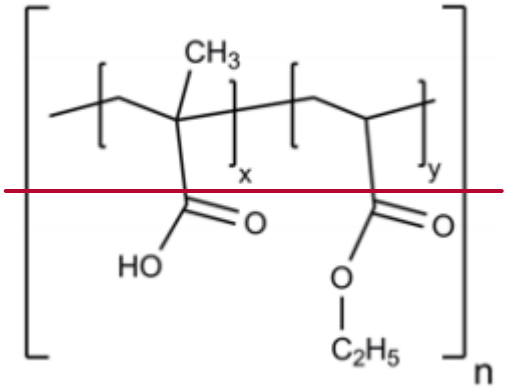
113. The oral formulation of any one of claims 68-112, wherein the oral formulation is in a unit dose form.

114. The oral formulation of any one of claims 68-113, wherein the oral formulation has a shelf-life of at least 3 months, at least 6 months, at least 12 months, at least 18 months, or at least 24 months.

115. The oral formulation of claim 68, wherein the one or more pharmaceutically acceptable excipients comprise a potassium salt, glycine, sucrose or trehalose, and a poloxamer, wherein the poloxamer has a weight average molecular mass of from 15,000 to 25,000 daltons and a polyoxythylene content of from 70% to 90% by weight; and

wherein the oral formulation further comprises:

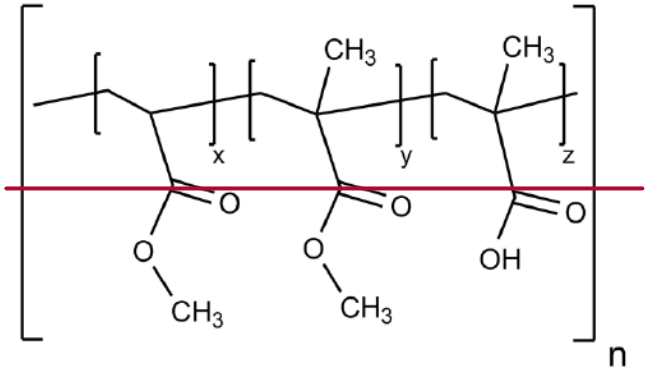
(c) a first coat comprising a first copolymer, wherein the first copolymer comprises a polymer of formula I:



Formula I;

wherein x, y, and n are each greater than or equal to one;

and further comprises a second copolymer, wherein the second copolymer comprises a polymer of formula II:



Formula II;

wherein x, y, z, and n are each greater than or equal to one;

wherein a ratio of the first copolymer to the second copolymer is 30:70; and

wherein the first coat further comprises from 5% to 15% (w/w) of a mixture of glycerol monostearate, triethyl citrate, and polysorbate 80;

(d) a second coat comprising HPMC positioned exterior of the first coat; and

(e) a third coat comprising HPMC positioned interior of the first coat and exterior of the therapeutic payload and the one or more pharmaceutically acceptable excipients.

116. A solid composition comprising:

IL-10 delivery constructs; and

one or more excipients;

wherein each of the IL-10 delivery constructs comprises IL-10 coupled to a carrier that promotes transcytosis of the IL-10 delivery construct across a polarized gut epithelial cell; and

wherein greater than 80% of the IL-10 delivery constructs are in a dimer form.

117. The solid composition of claim 116, wherein the solid composition is lyophilized or spray dried.

118. The solid composition of claim 116 or claim 117, wherein the solid composition is a tablet or a capsule.

119. The solid composition of any one of claims 116-118, wherein the one or more pharmaceutically acceptable excipients comprise a surfactant.

120. The solid composition of claim 119, wherein the surfactant is a poloxamer.

121. The solid composition of claim 120, wherein the poloxamer is poloxamer 188.

122. The solid composition of claim 119, wherein the surfactant does not include a polysorbate.

123. The solid composition of any one of claims 116-122, wherein the one or more pharmaceutically acceptable excipients comprise an osmolyte.

124. The solid composition of claim 123, wherein the osmolyte is sucrose.

125. The solid composition of any one of claims 116-124, wherein the one or more pharmaceutically acceptable excipients comprise a salt.

126. The solid composition of claim 125, wherein the salt is potassium phosphate.

127. The solid composition of any one of claims 116-126, wherein the one or more pharmaceutically acceptable excipients comprise a bulking agent.

128. The solid composition of claim 127, wherein the bulking agent is glycine.

129. The solid composition of any one of claims 116-128, wherein the IL-10 has at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 2.

130. The solid composition of any one of claims 116-129, wherein the IL-10 is coupled to the carrier via a linker.

131. The solid composition of claim 130, wherein the linker comprises an amino acid sequence set forth in SEQ ID NO: 6.

132. The solid composition of any one of claims 116-131, wherein the carrier comprises an amino acid sequence set forth in SEQ ID NO: 4.

133. The solid composition of any one of claims 116-131, wherein the carrier comprises an amino acid sequence set forth in SEQ ID NO: 12.

134. The solid composition of any one of claims 116-132, wherein the IL-10 delivery construct has at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 5.

135. The solid composition of claim 134, wherein the IL-10 delivery construct has a V1L substitution at amino acid position 1 of the carrier.

136. The solid composition of any one of claims 116-135, wherein greater than 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90% of the IL-10 delivery constructs are in a dimer form.

137. The solid composition of any one of claims 116-135, wherein from 85% to 92% of the IL-10 delivery constructs are in a dimer form.

138. The solid composition of any one of claims 116-137, wherein the solid composition comprises a first coat.

139. The solid composition of claim 138, wherein the first coat comprises a first copolymer and a second copolymer, wherein the first coat is external of the IL-10 delivery constructs and one or more excipients.

140. The solid composition of claim 139, wherein the first copolymer comprises methacrylic acid and ethyl acrylate.

141. The solid composition of claim 139 or claim 140, wherein the second copolymer comprises methacrylic acid, methyl methacrylate, and methyl acrylate.

142. The solid composition of any one of claims 139-141, wherein a ratio of the first copolymer to the second copolymer in the first coat is from about 15:85 to about 55:45.

143. The solid composition of any one of claims 139-141, wherein a ratio of the first copolymer to the second copolymer in the first coat is 20:80, 30:70, 40:60, or 50:50.

144. The solid composition of any one of claims 138-143, further comprising a second coat exterior of the first coat.

145. The solid composition of claim 144, wherein the second coat comprises hydroxypropyl methylcellulose (HPMC).

146. The solid composition of any one of claims 138-145, further comprising a third coat interior to the first coat and exterior of the IL-10 delivery constructs and the one or more excipients.

147. The solid composition of claim 146, wherein the third coat comprises HPMC.

148. A solid oral formulation comprising:

(a) an IL-10 delivery construct comprising IL-10 coupled to a carrier that promotes transcytosis of IL-10 delivery construct across a polarized gut epithelial cell; and

(b) one or more pharmaceutically acceptable excipients, wherein the one or more pharmaceutically acceptable excipients comprise a non-ionic lubricant; and

(c) a first coat surrounding the IL-10 delivery construct and the one or more pharmaceutically acceptable excipients.

149. The solid oral formulation of claim 148, wherein the non-ionic lubricant is glyceryl behenate.

150. The solid oral formulation of claim 148 or claim 149, wherein the oral formulation lacks magnesium stearate.

151. The solid oral formulation of any one of claims 148-150, wherein the oral formulation is in a tablet form.

152. The solid oral formulation of any one of claims 148-151, wherein the oral formulation is configured such that substantially none of the IL-10 delivery construct is released from the oral formulation after 1 h exposure to a solution at pH 1.0 in a Type 4 dissolution apparatus in open mode.

153. The solid oral formulation of any one of claims 148-152, wherein the oral formulation is configured to release at least 40% of the IL-10 delivery construct after 2 hours of exposure to a solution at pH 7.0 in a Type 4 dissolution apparatus in open mode.

154. The solid oral formulation of any one of claims 148-153, wherein the oral formulation further comprises a first coat comprising two or more copolymers each having a different nominal dissolution pH.

155. The solid oral formulation of any one of claims 148-154, wherein at least 45% of the IL-10 delivery construct is in a dimer form.

156. The solid oral formulation of any one of claims 148-155, wherein the one or more pharmaceutically acceptable excipients comprise a bulking agent, a disintegrant, or a combination thereof

157. The solid oral formulation of claim 156, wherein the bulking agent is silicified microcrystalline cellulose (SMCC).

158. The solid oral formulation of claim 156, wherein the disintegrant is crospovidone (crosslinked polyvinylpyrrolidone).

159. The solid oral formulation of any one of claims 148-158, wherein the IL-10 delivery construct has at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 5.

160. The solid oral formulation of claim 159, wherein the IL-10 delivery construct has a V1L substitution at amino acid position 1 of the carrier.

161. The solid oral formulation of any one of claims 148-160, wherein the oral formulation is created by compression of the IL-10 delivery construct and the one or more pharmaceutically acceptable excipients.

162. The solid oral formulation of claim 161, wherein the compression occurs with a compression force of from about 2000 pound-force (lbf) to about 3500 lbf.

163. The solid oral formulation of any one of claims 148-162, wherein the first coat comprises a first copolymer and a second copolymer, wherein the first coat is external of the IL-10 delivery constructs and one or more pharmaceutically acceptable excipients.

164. The solid oral formulation of claim 163, wherein the first copolymer comprises methacrylic acid and ethyl acrylate.

165. The solid oral formulation of claim 163 or claim 164, wherein the second copolymer comprises methacrylic acid, methyl methacrylate, and methyl acrylate.

166. The solid oral formulation of any one of claims 163-165, wherein a ratio of the first copolymer to the second copolymer in the first coat is from about 15:85 to about 55:45.

167. The solid oral formulation of any one of claims 163-165, wherein a ratio of the first copolymer to the second copolymer in the first coat is 20:80, 30:70, 40:60, or 50:50.

168. The solid oral formulation of any one of claims 163-167, further comprising a second coat exterior of the first coat.

169. The solid oral formulation of claim 168, wherein the second coat comprises hydroxypropyl methylcellulose (HPMC).

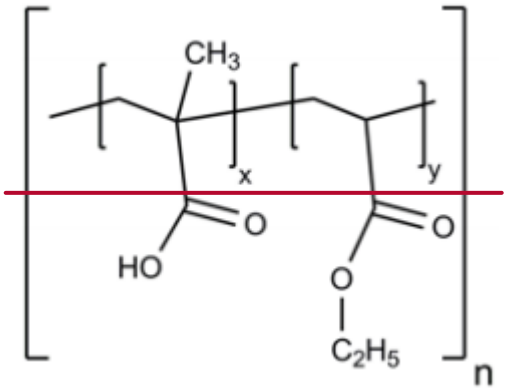
170. The solid oral formulation of any one of claims 163-169, further comprising a third coat interior to the first coat and exterior of the IL-10 delivery constructs and the one or more pharmaceutically acceptable excipients.

171. The solid oral formulation of claim 170, wherein the third coat comprises HPMC.

172. The solid oral formulation of claim 148, wherein the one or more pharmaceutically acceptable excipients further comprise a potassium salt, glycine, sucrose or trehalose, and a poloxamer, wherein the poloxamer has a weight average molecular mass of from 15,000 to 25,000 daltons and a polyoxythylene content of from 70% to 90% by weight; and

wherein the oral formulation further comprises:

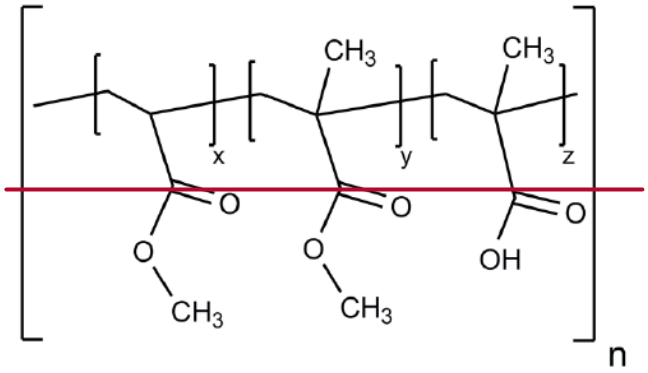
(c) a first coat comprising a first copolymer, wherein the first copolymer comprises a polymer of formula I:



Formula I;

wherein x, y, and n are each greater than or equal to one;

and further comprises a second copolymer, wherein the second copolymer comprises a polymer of formula II:



Formula II;

wherein x, y, z, and n are each greater than or equal to one;

wherein a ratio of the first copolymer to the second copolymer is 30:70; and

wherein the first coat further comprises from 5% to 15% (w/w) of a mixture of glycerol monostearate, triethyl citrate, and polysorbate 80;

(d) a second coat comprising HPMC positioned exterior of the first coat; and

(e) a third coat comprising HPMC positioned interior of the first coat and exterior of the therapeutic payload and the one or more pharmaceutically acceptable excipients.

173. An oral formulation comprising: (a) an IL-10 and (b) one or more pharmaceutically acceptable excipients, wherein administration of a dose of the oral formulation to an individual results in an immunomodulatory response selected from the group consisting of:

(i) a decrease in a concentration of fecal calprotectin (FCP) relative to an FCP baseline,

(ii) a decrease in a concentration of C-Reactive Protein (CRP) relative to a CRP baseline,

(iii) a decrease in a Geboes score relative to a Geboes score baseline, and

(iv) a combination of (i)-(iii).

174. The oral formulation of claim 173, wherein the immunomodulatory response comprises the decrease in FCP relative to the FCP baseline.

175. The oral formulation of claim 173 or claim 174, wherein the concentration of FCP is determined from a fecal sample or a colonic biopsy.

176. The oral formulation of any one of claims 173-175, wherein the decrease in the concentration of FCP is a decrease of at least 20%, 30%, 40%, or 50% relative to the FCP baseline.

177. The oral formulation of any one of claims 173-176, wherein the FCP baseline is an initial concentration of FCP in the individual prior to the administration.

178. The oral formulation of claim 177, wherein the initial concentration of FCP can be indicative of a gastrointestinal indication of the individual.

179. The oral formulation of claim 178, wherein the initial concentration of FCP is greater than 150 µg/g.

~~180. The oral formulation of claim 178 or claim 179, wherein the gastrointestinal indication is ulcerative colitis (UC) or Crohn's disease.~~

~~181. The oral formulation of any one of claims 177-180, wherein the concentration of FCP is decreased at least 50% relative to the initial concentration of FCP, and the dose of the oral formulation is from about 1 mg to about 3 mg.~~

~~182. The oral formulation of any one of claims 174-176, wherein the FCP baseline is a placebo-adjusted FCP baseline.~~

~~183. The oral formulation of claim 182, wherein the concentration of FCP is decreased at least 20% relative to the placebo-adjusted FCP baseline and the dose of the oral formulation is from about 1 mg to about 3 mg.~~

~~184. The oral formulation of any one of claims 174-183, wherein the concentration of FCP is decreased to 50 µg/g or less.~~

~~185. The oral formulation of any one of claims 173-184, wherein the immunomodulatory response comprises the decrease in the concentration of CRP relative to the CRP baseline.~~

~~186. The oral formulation of claim 185, wherein the concentration of CRP is a systemic concentration of CRP.~~

~~187. The oral formulation of claim 185 or claim 186, wherein the concentration of CRP is determined from a blood sample.~~

~~188. The oral formulation of any one of claims 185-187, wherein the decrease in the concentration of CRP is a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% relative to the CRP baseline.~~

~~189. The oral formulation of any one of claims 185-188, wherein the CRP baseline is an initial concentration of CRP in the individual prior to the administration.~~

~~190. The oral formulation of claim 189, wherein the initial concentration of CRP is greater than 5 mg/L.~~

~~191. The oral formulation of claim 189 or claim 190, wherein the initial concentration of CRP is indicative of a gastrointestinal indication of the individual.~~

192. The oral formulation of claim 191, wherein the gastrointestinal indication is irritable bowel disease (IBD).

193. The oral formulation of any one of claims 189-192, wherein the concentration of CRP is decreased at least 40% relative to the initial concentration CRP and the dose of the oral formulation is from about 1 mg to about 3 mg.

194. The oral formulation of any one of claims 185-188, wherein the CRP baseline is a placebo-adjusted CRP baseline.

195. The oral formulation of claim 194, wherein the concentration of CRP is decreased at least 10% relative to the placebo-adjusted CRP baseline and the dose of the oral formulation is about 3 mg.

196. The oral formulation of claim 194, wherein the concentration of CRP is decreased at least 40% relative to placebo-adjusted CRP baseline and the dose of the oral formulation is about 1 mg.

197. The oral formulation of any one of claims 185-196, wherein the concentration of CRP is decreased to less than 5 mg/L.

198. The oral formulation of any one of claims 173-197, wherein the immunomodulatory response comprises the decrease in the Geboes score relative to the Geboes score baseline.

199. The oral formulation of claim 198, wherein the Geboes score baseline is an initial Geboes score prior to the administration.

200. The oral formulation of claim 198, wherein the Geboes score baseline is a placebo-adjusted Geboes score baseline.

201. The oral formulation of claim 200, wherein the Geboes score is decreased a least 2 units relative to the placebo-adjusted Geboes score baseline and the dose of the oral formulation is from about 1 mg to about 30 mg.

202. The oral formulation of any one of claims 173-201, wherein less than 5% of the administered IL-10 enters the bloodstream of the individual.

~~203. The oral formulation of any one of claims 173-202, wherein the immunomodulatory response is observed after daily administration of the dose of the oral formulation for 14 days.~~

~~204. The oral formulation of any one of claims 173-203, wherein the dose of the oral formulation is 10 mg or less.~~

~~205. The oral formulation of claim 204, wherein the dose of the oral formulation is from 1 mg to 10 mg, from 3 mg to 10 mg, or from 1 mg to 3mg.~~

~~206. The oral formulation of claim 204, wherein the dose of the oral formulation is 1 mg, 3 mg, or 10 mg.~~

~~207. The oral formulation of any one of claims 173-206, wherein the oral formulation is a capsule.~~

~~208. The oral formulation of any one of claims 173-207, wherein the oral formulation is biodegradable.~~

~~209. The oral formulation of any one of claims 173-208, wherein the one or more pharmaceutically acceptable excipients comprise a surfactant.~~

~~210. The oral formulation of claim 209, wherein the surfactant is poloxamer 188.~~

~~211. The oral formulation of any one of claims 173-210, wherein the one or more pharmaceutically acceptable excipients comprise an osmolyte.~~

~~212. The oral formulation of claim 211, wherein the osmolyte is sucrose.~~

~~213. The oral formulation of any one of claims 173-212, wherein the one or more pharmaceutically acceptable excipients comprise a salt.~~

~~214. The oral formulation of claim 213, wherein the salt is potassium phosphate.~~

~~215. The oral formulation of any one of claims 173-214, wherein the one or more pharmaceutically acceptable excipients comprise a bulking agent.~~

~~216. The oral formulation of claim 215, wherein the bulking agent is glycine.~~

~~217. The oral formulation of any one of claims 173-216, wherein the oral formulation comprises a first coat, wherein the first coat is external of the IL-10 and one or more pharmaceutically acceptable excipients.~~

~~218. The oral formulation of claim 217, wherein the first coat comprises a first copolymer comprising methacrylic acid and ethyl acrylate and a second copolymer comprising methacrylic acid, methyl methacrylate, and methyl acrylate.~~

~~219. The oral formulation of claim 218, wherein a ratio of the first copolymer to the second copolymer in the first coat is from about 15:85 to 55:45.~~

~~220. The oral formulation of claim 218, wherein a ratio of the first copolymer to the second copolymer in the first coat is 20:80, 30:70, 40:60, or 50:50.~~

~~221. The oral formulation of any one of claims 217-219, further comprising a second coat located interior of the first coat and external of the IL-10 and one or more pharmaceutically acceptable excipients.~~

~~222. The oral formulation of claim 221, wherein the second coat comprises hydroxypropyl methylcellulose (HPMC).~~

~~223. The oral formulation of any one of claims 217-222, further comprising a third coat interior to the first coat and exterior of the IL-10 and the one or more pharmaceutically acceptable excipients.~~

~~224. The oral formulation of claim 223, wherein the third coat comprises HPMC.~~

~~225. The oral formulation of any one of claims 173-224, wherein the IL-10 is part of an IL-10 delivery construct having at least 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence set forth in SEQ ID NO: 5.~~

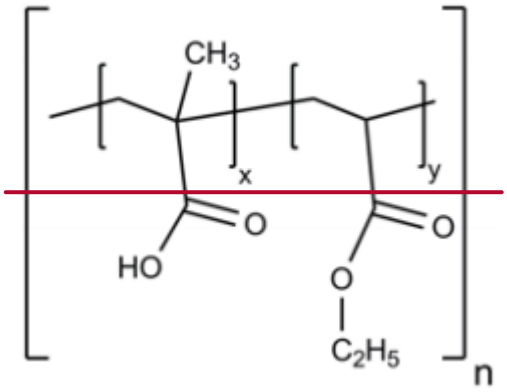
~~226. The oral formulation of claim 225, wherein the IL-10 delivery construct comprises a carrier.~~

~~227. The oral formulation of claim 226, wherein the IL-10 delivery construct has a V1L substitution at amino acid position 1 of the carrier.~~

~~228. The oral formulation of claim 173, wherein the one or more pharmaceutically acceptable excipients comprise a potassium salt, glycine, sucrose or trehalose, and a poloxamer, wherein the poloxamer has a weight average molecular mass of from 15,000 to 25,000 daltons and a polyoxythylene content of from 70% to 90% by weight; and~~

~~wherein the oral formulation further comprises:~~

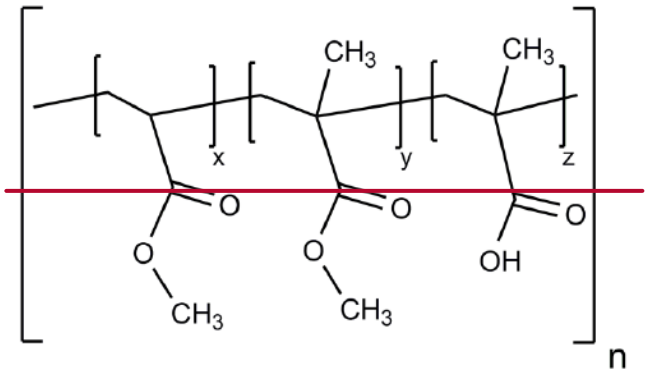
~~(c) a first coat comprising a first copolymer, wherein the first copolymer comprises a polymer of formula I:~~



Formula I;

wherein x, y, and n are each greater than or equal to one;

and further comprises a second copolymer, wherein the second copolymer comprises a polymer of formula II:



Formula II;

wherein x, y, z, and n are each greater than or equal to one;

wherein a ratio of the first copolymer to the second copolymer is 30:70; and

wherein the first coat further comprises from 5% to 15% (w/w) of a mixture of glycerol monostearate, triethyl citrate, and polysorbate 80;

(d) a second coat comprising HPMC positioned exterior of the first coat; and

(e) a third coat comprising HPMC positioned interior of the first coat and exterior of the therapeutic payload and the one or more pharmaceutically acceptable excipients.

229. A method of treating an inflammatory disorder in an individual, the method comprising administering to the individual an oral formulation comprising IL-10 and one or more pharmaceutically acceptable excipients to the individual, wherein the administering results in an immunomodulatory response selected from the group consisting of:

(i) a decrease in a concentration of fecal calprotectin (FCP) relative to an FCP baseline,

(ii) a decrease in a concentration of C-Reactive Protein (CRP) relative to a CRP baseline,

(iii) a decrease in a Geboes score relative to a baseline Geboes score, and

(iv) a combination of (i)-(iii).

230. The method of claim 229, wherein the inflammatory disorder is selected from the group consisting of ulcerative colitis, inflammatory bowel disease (IBD), proctitis, pouchitis, Crohn's disease, Celiac disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft versus host disease (GVHD), rheumatoid arthritis, psoriatic arthritis, or psoriasis.

231. The method of claim 229 or claim 230, wherein the oral formulation is the oral formulation of any one of claims 68-115 or 148-228.

232. A method of modulating a biomarker in an individual with an inflammatory disorder, the method comprising administering to the individual an oral formulation comprising IL-10 and one or more pharmaceutically acceptable excipients to the individual, wherein the biomarker is selected from the group consisting of: fecal calprotectin (FCP), C-Reactive Protein (CRP), and a combination thereof.

233. The method of claim 232, wherein the administering results in a decrease in a concentration of the FCP relative to an FCP baseline.

234. The method of claim 232 or claim 233, wherein the administering results in a decrease in a concentration of the CRP relative to a CRP baseline.

235. The method of any one of claims 232-234, wherein the administering further results in a decrease in a Geboes score relative to a baseline Geboes score.

236. The method of any one of claims 232-235, further comprising treating the individual with the inflammatory disorder.

237. The method of any one of claims 232-236, wherein the inflammatory disorder is selected from the group consisting of ulcerative colitis, inflammatory bowel disease (IBD), proctitis, pouchitis, Crohn's disease, Celiac disease, multiple sclerosis (MS),

~~systemic lupus erythematosus (SLE), graft versus host disease (GVHD), rheumatoid arthritis, psoriatic arthritis, or psoriasis.~~

~~238. The method of any one of claims 232-237, wherein the oral formulation is the oral formulation of any one of claims 68-115 or 148-228.~~

~~239. A method of treating an inflammatory disorder in an individual refractory or resistant to at least one anti-inflammatory agent, the method comprising administering a formulation comprising IL-10 to the individual.~~

~~240. The method of claim 239, wherein the anti-inflammatory agent is an aminosalicylate.~~

~~241. The method of claim 240, wherein the aminosalicylate is selected from the group consisting of 5-aminosalicylic acid (5-ASA; mesalazine), 4-amino salacylic acid (4-ASA), balsalazide, olsalazine, and sulfasalazine.~~

~~242. The method of any one of claims 239-241, wherein the anti-inflammatory agent is a corticosteroid.~~

~~243. The method of claim 242, wherein the corticosteroid is prednisone.~~

~~244. The method of claim 242 or claim 243, wherein the corticosteroid is an orally administered corticosteroid or an intravenously (IV) administered corticosteroid.~~

~~245. The method of any one of claims 239-244, wherein the anti-inflammatory agent is an immunosuppressive agent.~~

~~246. The method of claim 245, wherein the immunosuppressive agent is selected from the group consisting of azathioprine, 6-mercaptopurine, and a combination thereof.~~

~~247. The method of any one of claims 239-246, wherein the anti-inflammatory agent is a TNFα inhibitor.~~

~~248. The method of claim 247, wherein the TNFα inhibitor is selected from the group consisting of adalimumab, certolizumab, etanercept, golimumab, and infliximab.~~

~~249. The method of any one of claims 239-248, wherein the at least one anti-inflammatory agent is a Janus kinase (JAK) inhibitor.~~

~~250. The method of claim 249, wherein the JAK inhibitor is selected from the group consisting of filgotinib, upadacitinib, peficitinib, and tofacitinib.~~

~~251. The method of any one of claims 239-250, wherein the at least one anti-inflammatory agent is a sphingosine-1-phosphate (S1P) receptor antagonist.~~

~~252. The method of claim 251, wherein the S1P receptor antagonist is selected from the group consisting of ozanimod, amiselimod, and etrasimod.~~

~~253. The method of any one of claims 239-252, wherein the at least one anti-inflammatory agent is an integrin blocker.~~

~~254. The method of claim 253, wherein the integrin blocker is selected from the group consisting of etrolizumab, natalizumab, vedolizumab, abrilumab, and carotegrast methyl.~~

~~255. The method of any one of claims 239-254, wherein the at least one anti-inflammatory agent is an IL-23 inhibitor.~~

~~256. The method of claim 255, wherein the IL-23 inhibitor is selected from the group consisting of ustekinumab. mirikizumab, brazikumab, guselkumab, and risankizumab.~~

~~257. The method of any one of claims 239-256, wherein the at least one anti-inflammatory agent is a phosphodiesterase 4 (PDE4) inhibitor.~~

~~258. The method of claim 257, wherein the at least one PDE4 inhibitor is selected from the group consisting of apremilast, cilomilast, roflumilast, tetomilast, and rolipram.~~

~~259. The method of any one of claims 239-258, wherein the at least one anti-inflammatory agent is laquinimod.~~

~~260. A method of treating an inflammatory disease in a subject in need thereof, the method comprising orally administering an IL-10 therapeutic to the subject and administering a non-IL-10 immunosuppressor to the subject.~~

~~261. A method of treating an inflammatory disease in a subject in need thereof, the method comprising orally administering an IL-10 therapeutic to the subject, wherein the subject concomitantly receives a non-IL-10 immunosuppressor.~~

~~262. A method of treating an inflammatory disease in a subject, wherein the subject had an inadequate response to a non-IL-10 immunosuppressor, the method comprising orally administering an IL-10 therapeutic to the subject.~~

~~263. The method of claim 262, further comprising administering the non-IL-10 immunosuppressor with the IL-10 therapeutic.~~

~~264. The method of claim 262, wherein the subject was treated with the non-IL-10 immunosuppressor for at least 6 weeks prior to determining the inadequate response.~~

~~265. The method of claim 262, wherein the subject was treated with the non-IL-10 immunosuppressor for at least 12 weeks prior to determining the inadequate response.~~

~~266. The method of claim 262, wherein the inadequate response is a partial response.~~

~~267. The method of any one of claims 260-266, wherein the inflammatory disease is selected from the group consisting of: inflammatory bowel disease, psoriasis, plaque psoriasis, hidradenitis suppurativa, psoriatic arthritis, rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, bacterial sepsis, Crohn's disease, fistulizing Crohn's disease, moderate-to-severe ulcerative colitis, mild-to-moderate ulcerative colitis, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's syndrome, indeterminate colitis, pancreatitis, liver inflammation, pouchitis, proctitis, uveitis, graft vs host disease, and epithelial cell injury.~~

~~268. The method of any one of claims 260-267, wherein the inflammatory disease is an inflammatory bowel disease.~~

~~269. The method of any one of claims 260-267, wherein the inflammatory disease is selected from the group consisting of: rheumatoid arthritis, ulcerative colitis, and Crohn's disease.~~

~~270. The method of any one of claims 262-266, wherein the inflammatory disease is rheumatoid arthritis and the subject with an inadequate response has one or more joints with active disease.~~

~~271. The method of claim 270, wherein the one or more joints with active disease are identified by fluorescent optical imaging or magnetic resonance imaging.~~

~~272. The method of any one of claims 270 or 271, wherein the subject with an inadequate response additionally has two or more joints which are tender.~~

~~273. The method of any one of claims 270-272, wherein the subject with an inadequate response additionally has two or more joints which are swollen.~~

~~274. The method of claim 262, wherein the inflammatory disease is ulcerative colitis, and the subject with an inadequate response has moderate to severe ulcerative colitis.~~

~~275. The method of claim 274, wherein the subject with an inadequate response has a modified Mayo Clinic Score (MMS) of between about 4 points and about 9 points.~~

~~276. The method of claim 274 or 275, wherein the subject with an inadequate response has a centrally read MCS endoscopic sub score of grade 2 or higher.~~

~~277. The method of any one of claims 274-276, wherein the subject with an inadequate response has a MMS rectal bleeding sub score of 1 point or higher.~~

~~278. The method of any one of claims 274-277, wherein the subject with an inadequate response has disease extending 15 cm or more from the anal verge.~~

~~279. The method of any one of claims 260-278, wherein the IL-10 therapeutic is an IL-10 delivery construct.~~

~~280. The method of claim 279, wherein the IL-10 delivery construct comprises a carrier consisting of an amino acid sequence set forth in SEQ ID NO: 4~~

~~281. The method of claim 279, wherein the IL-10 delivery construct comprises a carrier consisting of an amino acid sequence at least 90% identical to SEQ ID NO: 4~~

~~282. The method of claim 279, wherein the IL-10 delivery construct comprises an amino acid sequence set forth in SEQ ID NO: 5.~~

~~283. The method of any one claims 260-282, wherein the non-IL-10 immunosuppressor is a TNF alpha inhibitor.~~

~~284. The method of claim 283, wherein the TNF alpha inhibitor is a monoclonal antibody.~~

~~285. The method of claim 283, wherein the TNF alpha inhibitor is selected from the group consisting of infliximab (Remicade), adalimumab (Humira) and golimumab (Simponi).~~

~~286. The method of claim 283, wherein the TNF alpha inhibitor comprises SEQ ID NO: 151 and SEQ ID NO: 152.~~

~~287. The method of claim 283, wherein the TNF alpha inhibitor comprises SEQ ID NO: 153 and SEQ ID NO: 154.~~

~~288. The method of any one of claims 260-287, wherein the TNF alpha inhibitor is not etanercept.~~

1. A solid pharmaceutical composition suitable for oral administration to a subject in need thereof, the pharmaceutical composition comprising:

a plurality of delivery constructs; and

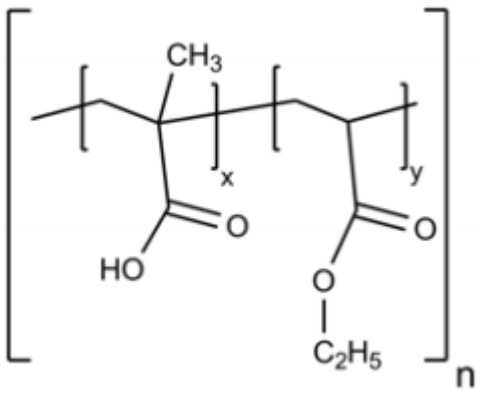
one or more pharmaceutically acceptable excipients;

wherein each of the delivery constructs comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 13;

wherein at least 80% of the delivery constructs are in a dimer form;

wherein the pharmaceutical composition is formulated as an oral dosage form, the oral dosage form comprising (1) a first coat comprising two or more copolymers each having a different dissolution pH and (2) another coat interior to the first coat and exterior to the delivery constructs and the one or more pharmaceutically acceptable excipients; and

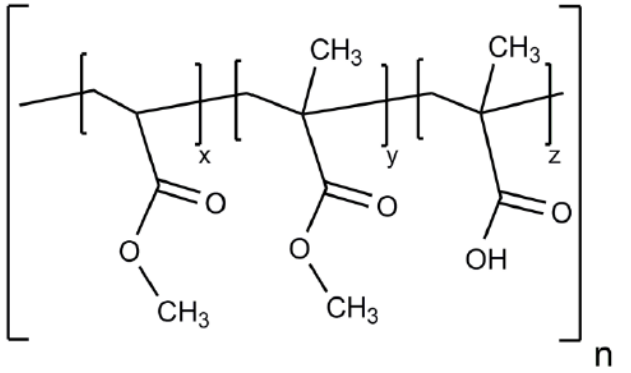
wherein a first copolymer of the first coat comprises a polymer of formula I:



Formula I;

wherein x, y, and n in Formula I are each greater than or equal to one; and

wherein a second copolymer of the first coat comprises a copolymer of formula II:



Formula II;

wherein x, y, z, and n in Formula II are each greater than or equal to one.

2. The pharmaceutical composition of claim 1 wherein in at least one of the one or more pharmaceutically acceptable excipients is a surfactant, wherein the surfactant comprises a poloxamer.

3. The pharmaceutical composition of claim 2, wherein the poloxamer is a poloxamer 188.

4. The pharmaceutical composition of any one of claims 1-3, wherein the one or more pharmaceutically acceptable excipients does not include a polysorbate.

5. The pharmaceutical composition of any one of claims 1-4, wherein the one or more pharmaceutically acceptable excipients comprises a non-ionic lubricant.

6. The pharmaceutical composition of claim 5, wherein the non-ionic lubricant is glyceryl behenate.

7. The pharmaceutical composition of any one of claims 1-6, wherein the one or more pharmaceutically acceptable excipients does not include a magnesium stearate.

8. The pharmaceutical composition of any one of claims 1-7, wherein the pharmaceutical composition is configured to release substantially none of the plurality of delivery constructs after a 1-hour exposure to a solution having a pH of 1.0 in a Type 4 dissolution apparatus in open mode, wherein the solution having the pH of 1.0 is a dissolution media containing hydrochloric acid.

9. The pharmaceutical composition of claim 8, wherein the pharmaceutical composition is configured to release at least 40% of the delivery construct after 2 hours of exposure to a solution having a pH of 7.0 in a Type 4 dissolution apparatus in open mode, wherein the solution having the pH of 7.0 is a citrate/phosphate buffer.

10. The pharmaceutical composition of any one of claims 1-9, wherein a ratio of free carboxyl groups to ester groups in the first copolymer is from 0.8:1 and 1.2:1 and wherein a ratio of free carboxyl groups to ester groups in the second copolymer is from 0.8:1 to 1.2:1.

11. The pharmaceutical composition of any one of claims 1-10, wherein a ratio of the first copolymer of the first coat to the second copolymer of the first coat is from 15:85 to 55:45.

12. The pharmaceutical composition of any one of claims 1-11, wherein the ratio of the first copolymer to the second copolymer is to 30:70.

13. The pharmaceutical composition of any one of claims 1-12, wherein the first coat further comprises an anti-tacking agent, a plasticizer, a surfactant, or a combination thereof.

14. The pharmaceutical composition of any one of claims 1-13, further comprising an additional coat exterior to the first coat.

15. The pharmaceutical composition of claim 14, wherein the additional coat exterior to the first coat comprises hydroxypropylmethylcellulose (HPMC).

16. The pharmaceutical composition of any one of claims 1-15, wherein the another coat comprises HPMC.

17. The pharmaceutical composition of any one of claims 1-16, wherein the oral formulation is in a tablet or a capsule form.

18. The pharmaceutical composition of any one of claims 1-17, wherein the oral formulation is in a unit dose form.

19. The pharmaceutical composition of claim 18, wherein the first coat has a mass from 10 mg to 100 mg per unit dose form.

20. The pharmaceutical composition of claim 18, wherein the plurality of delivery constructs are present in an amount from 1 mg to 20 mg per unit dose of the formulation.

21. The pharmaceutical composition of any one of claims 1-20, wherein each of the delivery constructs comprises the amino acid sequence set forth in SEQ ID NO: 5.

22. The pharmaceutical composition of any one of claims 1-20, wherein each of the delivery constructs comprises the amino acid sequence set forth in SEQ ID NO: 13.

23. A method for producing a solid pharmaceutical composition for oral administration that comprises a plurality of coats according to any one of claims 1-22, the method comprising:

obtaining the delivery constructs that comprise an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 13;

applying a plurality of coats around the delivery construct, wherein the plurality of coats comprises (1) the first coat comprising two or more copolymers each having a different dissolution pH and (2) the another coat interior to the first coat and exterior to the delivery constructs.

24. The method of claim 23, wherein the method further comprises lyophilizing or spray-drying of the delivery constructs.

25. The method of claim 23 or claim 24, wherein the method further comprises adding of one or more pharmaceutically suitable excipients to the pharmaceutical composition.

26. The method of claim 25, wherein the method further comprises compacting the delivery constructs with the one or more pharmaceutically suitable excipients.

27. Use of the pharmaceutical composition of any one of claims 1-22 in the manufacture of a medicament for the treatment of an inflammatory disease in a subject, wherein the medicament is to be administered orally.

28. The use of claim 27, wherein the inflammatory disease is ulcerative colitis, proctitis, pouchitis, Crohn's disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft

versus host disease (GVHD), rheumatoid arthritis, inflammatory bowel disease (IBD), celiac disease, psoriatic arthritis, or psoriasis.

29. The use of claim 27 or claim 28, wherein the administration of the medicament to the subject results in an immunomodulatory response selected from the group consisting of: (i) a decrease in a concentration of fecal calprotectin (FCP) relative to an FCP baseline concentration level present in the subject prior to administration, (ii) a decrease in a concentration of C-reactive protein (CRP) relative to a CRP baseline concentration level present in the subject prior to administration, (iii) a decrease in a Geboes score relative a Geboes score baseline present in the subject prior to administration, or (iv) a combination thereof.

30. The use of claim 28, wherein the inflammatory disease is ulcerative colitis.

31. The use of claim 28, wherein the inflammatory disease is rheumatoid arthritis.

32. The use of claim 28, wherein the inflammatory disease is pouchitis.

33. A method of refolding an IL-10 delivery construct comprising an amino acid sequence with at least 90% sequence similarity to the sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 13, the method comprising:

(i) contacting inclusion bodies comprising the IL-10 delivery construct with a solubilization solution comprising a chaotropic agent to produce a soluble IL-10 delivery construct; and

(ii) contacting the soluble IL-10 delivery construct with a refolding solution comprising reduced glutathione and oxidized glutathione to produce a refolded IL-10 delivery construct, wherein the method does not comprise contacting the soluble IL-10 delivery construct with a sulfitolysis agent or a reducing agent prior to the contacting of step (ii).

34. The method of claim 33, wherein the IL-10 delivery construct consists of an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO:13.

35. The method of claim 33 or claim 34, wherein the refolding solution comprises a weight/weight ratio of the reduced glutathione to the oxidized glutathione from 0.8:1 to 1.2:1.

36. The method of any one of claims 33–35, wherein the refolding solution comprises from 0.75 mM to 1.5 mM reduced glutathione.

37. The method of any one of claims 33–36, wherein the refolding solution comprises from 0.25 mM to 0.75 mM oxidized glutathione.

38. The method of any one of claims 33–37, wherein the refolding solution has a pH from 7.5 to 8.5.

39. The method of any one of claims 33–38, wherein the refolding solution comprises arginine, Tris, EDTA, sucrose, or a combination thereof.

40. The method of claim 39, wherein the arginine is present in the refolding solution at a concentration of between 900 mM and 1.1M, wherein the sucrose is present in the refolding solution at a concentration of between 200 mM and 300 mM, wherein the Tris is present in the refolding solution at a concentration of from 75 mM to 125 mM, and wherein the EDTA is present in the refolding solution at a concentration of from 1.75 mM to 2.25 mM.

41. The method of any one of claims 33–40, further comprising lysing a cell comprising the inclusion bodies.

42. The method of claim 41, wherein the cell is an E. coli bacterium.

43. The method of any one of claims 33–42, wherein the chaotropic agent comprises guanidine hydrochloride or urea.

44. The method of any one of claims 33–43, wherein the solubilization solution further comprises Tris.

45. The method of any one of claims 33–44, wherein the contacting the soluble IL-10 delivery construct with the refolding solution occurs for a duration of 12 hours to 18 hours.

46. The method of any one of claims 33–45, wherein the contacting the soluble IL-10 delivery construct with the refolding solution occurs for a duration of at least 16 hours.

47. The method of any one of claims 33–46, wherein the contacting the IL-10 delivery construct with the refolding solution occurs from 2ºC to 8 ºC.

48. The method of any one of claims 33–47, further comprising performing a first sterile filtration of the refolded IL-10 delivery construct.

49. The method of any one of claims 33–48, further comprising performing a tangential flow filtration of the refolded IL-10 delivery construct.

50. The method of claim 49, wherein the tangential flow filtration comprises diafiltration.

51. A method of enriching for IL-10 delivery construct dimers from a pool comprising IL-10 delivery constructs in a dimer form, a monomer form, and an aggregate form, wherein the IL-10 delivery construct comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 13, the method comprising:

(i) performing anion exchange (AEX) chromatography on the pool by binding the IL-10 delivery construct dimers to an anion exchange column and subsequently eluting the IL-10 delivery construct dimers from the anion exchange column, thereby creating a first plurality of fractions one of which is a first fraction enriched in IL-10 delivery constructs in the dimer form; and

(ii) performing ceramic hydroxyapatite (CHT) chromatography on the fraction enriched in IL-10 delivery constructs in the dimer form, thereby creating a second plurality of fractions, one of which is a second fraction further enriched in IL-10 delivery constructs in the dimer form;

wherein the method does not comprise cation exchange chromatography.

52. The method of claim 51, wherein the IL-10 delivery construct comprises SEQ ID NO: 13.

53. The method of claim 51, wherein the IL-10 delivery construct consists of the amino acid sequence set forth in SEQ ID NO: 5.

54. The method of claim 52, wherein the IL-10 delivery construct consists of the amino acid sequence set forth in SEQ ID NO: 13.

55. The method of any one of claims 51–54, further comprising determining a percentage of the IL-10 delivery constructs in the dimer form in each fraction of the first plurality of fractions.

56. The method of any one of claims 51–55, further comprising determining a percentage of IL-10 delivery constructs in the dimer form in each fraction of the second plurality of fractions.

57. The method of claim 55, wherein the determining is by size exclusion chromatography.

58. The method of claim 57, wherein the size exclusion chromatography is size exclusion high performance liquid chromatography (SE-HPLC).

59. The method of claim 56, wherein the determining is by size exclusion chromatography.

60. The method of claim 59, wherein the size exclusion chromatography is size exclusion high performance liquid chromatography (SE-HPLC).

61. The method of any one of claims 51–60, wherein at least 75% of the IL-10 delivery constructs in the first fraction are IL-10 delivery constructs in the dimer form.

62. The method of any one of claims 51–61, wherein at least 80% of the IL-10 delivery constructs in the second fraction are IL-10 delivery constructs in the dimer form.

63. The method of claim 62, wherein at least 90% of the IL-10 delivery constructs in the second fraction are IL-10 delivery constructs in the dimer form.

64. The method of any one of claims 51–63, further comprising performing tangential flow filtration of the second fraction.

65. The method of claim 64, wherein performing tangential flow filtration of the second fraction comprises performing ultrafiltration.

66. The method of any one of claims 64–65, wherein performing tangential flow filtration comprises performing diafiltration.

67. The method of any one of claim 51–66, wherein the pool comprises refolded IL-10 delivery constructs that have been refolded via contact with a refolding solution comprising reduced glutathione and oxidized glutathione.

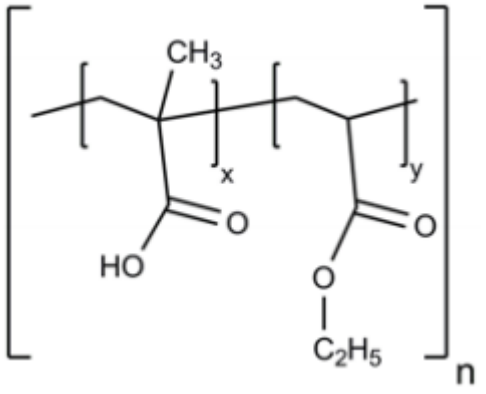
68. A solid oral formulation comprising:

a) a delivery construct consisting of an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 13, wherein the delivery construct comprises a carrier that promotes transcytosis of the delivery construct across a polarized gut epithelial cell;

b) one or more excipients; wherein the one or more excipients comprise a non-ionic lubricant; and

c) a first coat comprising two or more copolymers each having a different nominal dissolution pH

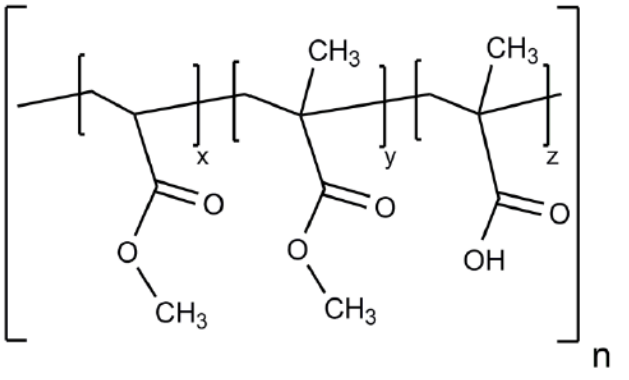
wherein the oral formulation is configured to release substantially none of the IL-10 delivery construct after 1 h exposure to a solution having a pH of 1.0 in a Type 4 dissolution apparatus in open mode, wherein the first copolymer comprises a polymer of formula I:



Formula I;

wherein x, y, and n of Formula I are each greater than or equal to one; and

wherein the second copolymer comprises a polymer of formula II:



Formula II;

wherein x, y, z, and n of Formula II are each greater than or equal to one.

69. The solid oral formulation of claim 68, wherein:

(a) the solution having the pH of 1.0 is a dissolution media containing 0.1M hydrochloric acid; and/or

(b) the formulation is configured to release at least 40% of the delivery construct after 2 hours of exposure to a solution having a pH of 7.0 in a Type 4 dissolution apparatus in open mode, wherein the solution having the pH of 7.0 is a citrate/phosphate buffer, optionally wherein at least 5%, at least 10%, at least 20%, or at least 25% of the delivery construct released following 2 hours of exposure to the solution having the pH of 7.0 is in a dimer form.

70. The solid oral formulation of any one of claims 68–69, wherein:

(a) a ratio of free carboxyl groups to ester groups in the first copolymer is from 0.8:1 and 1.2:1 and wherein a ratio of free carboxyl groups to ester groups in the second copolymer is from 0.8:1 to 1.2:1; and/or

(b) the first copolymer comprises methacrylic acid and ethyl acrylate; and/or

(c) the second copolymer comprises methacrylic acid, methyl methacrylate, and methyl acrylate; and/or

(d) a ratio of the first copolymer to the second copolymer in the first coat is from 15:85 to 55:45; optionally the ratio of the first copolymer to the second copolymer is 20:80, 30:70, 40:60, or 50:50; and/or

(e) the first coat further comprises an anti-tacking agent, a plasticizer, a surfactant, or a combination thereof.

71. The solid oral formulation of any one of claims 68–70, wherein the oral formulation further comprises a second coat located interior of the first coat and external of the IL-10 and one or more pharmaceutically acceptable excipients, wherein the second coat comprises hydroxypropyl methylcellulose.

72. The solid oral formulation of claim 71, further comprising a third coat interior to the first coat and exterior of the IL-10 delivery constructs and the one or more pharmaceutically acceptable excipients, optionally wherein the third coat comprises HPMC.

73. The solid oral formulation of any one of claims 68–72, wherein:

(a) the IL-10 delivery constructs are present in the formulation in an amount from 1 mg to 20 mg; and/or

(b) the one or more pharmaceutically acceptable excipients comprise a surfactant, optionally wherein the surfactant is a poloxamer, and optionally wherein the poloxamer is a poloxamer 188.

74. The solid oral formulation of claim 68, wherein:

(a) the non-ionic lubricant is glyceryl behenate; and/or

(b) the formulation lacks magnesium stearate.

75. The solid oral formulation of claim any one of claims 68–74, wherein:

(a) the formulation is lyophilized or spray dried; and/or

(b) the formulation is in a tablet or a capsule form.

76. Use of the solid oral formulation of any one of claims 68–75, in the manufacture of a medicament for the treatment of an inflammatory disease in a subject, optionally wherein the inflammatory disease is ulcerative colitis, proctitis, pouchitis, Crohn's disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft versus host disease (GVHD), rheumatoid arthritis, psoriatic arthritis, or psoriasis.

77. The pharmaceutical composition of any one of claims 1-22, or the solid oral formulation of any one of claims 68-75, for use in therapy.