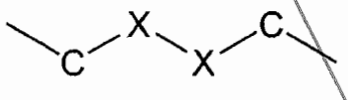
What is claimed is:

1. A compound comprising a first stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which comprises a binding site for a target; and

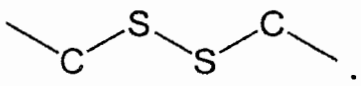
a second stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which is identical to the sequence of the first stretch of consecutive amino acids and which comprises an identical binding site for the target;

wherein each of the first stretch of amino acids and the second stretch of amino acids has at a predefined end thereof a cysteine residue or a selenocysteine residue and such cysteine residues or such selenocysteine residues are joined by a bond having the structure:



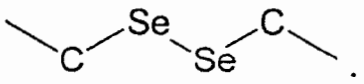
wherein each X is the same and represents a sulfur (S) or a selenium (Se) and each C represents a beta-carbon of one of such cysteine or selenocysteine residues.

2. The compound claim 1, wherein the bond has the structure:



.

3. The compound claim 1, wherein the bond has the structure:



.

4. The compound claim 1, wherein the residue at the predefined end of each of the first stretch and the second stretch of consecutive amino acids is a cysteine residue.

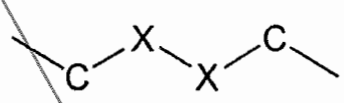
5. The compound claim 1, wherein the residue at the predefined end of each of the first stretch and the second stretch of consecutive amino acids is a selenocysteine residue.

6. The compound of claim 1, wherein the first stretch of consecutive amino acids or second stretch of consecutive amino acids comprises at least 20 amino acids.

7. The compound of claim 1, wherein the first stretch of consecutive amino acids or second stretch of consecutive amino acids comprises at least 100 amino acids.

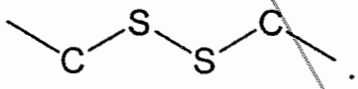
8. A compound comprising a first stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which comprises a binding site for a target; and
a second stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which is different from the sequence of the first stretch of consecutive amino acids and which comprises a binding site for a different moiety;
wherein each of the first stretch of amino acids and the second stretch of amino acids has at a predefined end thereof a cysteine residue or a selenocysteine

residue and such residues are joined by a bond having the structure:

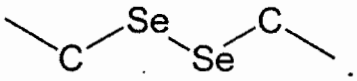


wherein each X may be the same or different and represents a sulfur (S) or a selenium (Se) and each C represents a beta-carbon of one of such cysteine or selenocysteine residues.

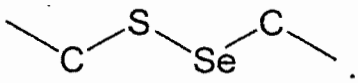
9. The compound claim 8, wherein the bond has the structure:



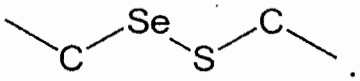
10. The compound claim 8, wherein the bond has the structure:



11. The compound claim 8, wherein the bond has the structure:



12. The compound claim 8, wherein the bond has the structure:



13. The compound claim 8, wherein both of the residues at the predefined ends of each of the first stretch of amino acids and the second stretch of amino acids are cysteine residues.

14. The compound claim 8, wherein both of the residues at the predefined ends of each of the first stretch of

amino acids and the second stretch of amino acids are selenocysteine residues.

15. The compound claim 8, wherein the residue at one predefined end of one of the first stretch or second stretch of consecutive amino acids is a cysteine residue and the residue at the other predefined end is a selenocysteine residue.

16. The compound of claim 8, wherein the first stretch of consecutive amino acids or second stretch of consecutive amino acids comprises at least 20 amino acids.

17. The compound of claim 8, wherein the first stretch of consecutive amino acids or second stretch of consecutive amino acids comprises at least 100 amino acids.

18. A multimer comprising two or more identical compounds according to any one of claims 1 or 8 joined together by at least one bond.

19. The multimer of claim 18, wherein the multimer is a dimer.

20. The multimer of claim 18, wherein the multimer is a trimer.

21. The multimer of claim 18, wherein the multimer is a tetramer.

22. The compound of any of claims 1 or 8, wherein the predefined end of both the first stretch of amino acids

and the second stretch of amino acids is a N-terminal end thereof.

23. The compound of any of claims 1 or 8, wherein the predefined end of both the first stretch of amino acids and the second stretch of amino acids is a C-terminal end thereof.

24. The compound of any of claims 1 or 8, wherein the predefined end of one of the first stretch of amino acids and the second stretch of amino acids is a C-terminal end and the other predefined end is a N-terminal end.

25. The compound of claim 1 or 8, wherein the first stretch of amino acids comprises L-amino acids.

26. The compound of claim 1 or 8, wherein the first stretch of amino acids comprises D-amino acids.

27. The compound of claim 1 or 8, wherein the first stretch of amino acids comprises L-amino acids and D-amino acids

28. The compound of claim 8, wherein the second stretch of amino acids comprises L-amino acids.

29. The compound of claim 8, wherein the second stretch of amino acids comprises D-amino acids.

30. The compound of claim 8, wherein the second stretch of amino acids comprises L-amino acids and D-amino acids.

31. The compound of claim 1 or 8, wherein the first stretch of amino acids comprises at least 50 consecutive amino acids.

32. The compound of claim 8, wherein the second stretch of amino acids comprises at least 50 consecutive amino acids.

33. The compound of claim 1 or 8, wherein the first stretch of amino acids comprises more than one type of amino acid.

34. The compound of claim 1 or 8, wherein the second stretch of amino acids comprises more than one type of amino acid.

35. The multimer of claim 18, wherein the one or more bonds comprises a disulfide bond.

36. The compound of claim 8, wherein the sequence of the second stretch of amino acids corresponds to the sequence of a constant region of an immunoglobulin.

37. The compound of claim 8, wherein the immunoglobulin is a human immunoglobulin.

38. The compound of claim 8, wherein the constant region of the immunoglobulin is a constant region of an IgG, an IgA, an IgE, an IgD, or an IgM immunoglobulin.

39. The compound of claim 8, wherein the constant region of the immunoglobulin is a constant region of an IgG-1, IgG-2, IgG-3 or IgG-4 immunoglobulin.

40. The compound of claim 8, wherein the constant region of the immunoglobulin is a constant region of an IgG immunoglobulin and comprises a hinge region, a CH6 region and a CH3 region.

41. The compound of claim 8, wherein the different moiety is an immunoeffector or immunoregulator.

42. The compound of claim 1 or 8, wherein the target is protein.

43. The compound of claim 1 or 8, wherein the target is an EGF receptor, a HER2, a VEGF receptor, a CD20 antigen, a CD11a, an IgE immunoglobulin, a glycoprotein IIa receptor, a glycoprotein IIIa receptor, a TNF alpha, or a TNF receptor, a gp120.

44. The compound of claim 1, wherein each of the first and second stretch of consecutive amino acids comprises the amino acid sequence of any one of TNFRSF1a, TNFRSF1b, VEGFR1, VEGFR6, VEGFR3, human Erb1, human Erb2, human Erb6, human Erb3, or human Erb4.

45. The compound of claim 8, wherein the first or second stretch of consecutive amino acids comprises the amino acid sequence set forth of any one of TNFRSF1a, TNFRSF1b, VEGFR1, VEGFR6, VEGFR3, human Erb1, human Erb2, human Erb6, human Erb3, or human Erb4.

46. A composition comprising the compound of claim 1 in an amount effective to bind the target, and a carrier.

47. A composition comprising the compound of claim 8 in an amount effective to bind the target, and a carrier.

48. A composition comprising the multimer of claim 18 in an amount effective to bind the target, and a carrier.

49. The composition of claim 48, wherein the multimer is also present in an amount effective to bind the different moiety.

50. The composition of any one of claims 46, 47, 48, or 49, wherein the carrier is a pharmaceutically acceptable carrier.

51. A method of affecting the activity of a target comprising contacting the target with the composition of claim 46, 47, 48, or 49, under conditions such that the compound binds to and affects the activity of the target.

52. The method of claim 51, wherein the binding of the composition to the target increases the activity of the target.

53. The method of claim 51, wherein the binding of the composition to the target decreases the activity of the target.

54. The method of claim 51, wherein the target is an EGF receptor, a HER2 protein, a VEGF receptor, a CD20 antigen, a CD11a, an IgE immunoglobulin, a glycoprotein IIa receptor, a glycoprotein IIIa receptor, a TNF alpha, or a TNF receptor.

55. A complex comprising the composition of any of claim 1, 8, or 18, and a third stretch of consecutive amino

acids, wherein the third stretch of consecutive amino acids is bound to the one of the first or second stretch of consecutive amino acids by one or more bonds.

56. The complex of claim 55, wherein the one or more bonds comprise van der Waals forces.

57. The complex of claim 55, wherein the one or more bonds comprise a hydrogen bond.

58. The complex of claim 55, wherein the one or bonds comprise a covalent bond.

59. The complex of claim 55, wherein the one or bonds comprise a disulfide bond.

60. The multimer of claim 18, wherein the at least one bond is a disulfide bond.

61. The multimer of claim 18, wherein the disulfide bond is between two non-terminal amino acid residues.

62. The multimer of claim 18, wherein the disulfide bond is between two amino acid residues, at least one of which is a non-terminal amino acid residue.

63. A process of making the compound of claim 1, comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which is a N-terminal signal sequence, contiguous with (ii) a second portion, the sequence of which encodes a

stretch of consecutive amino acids contiguous with (iii) a third portion, the sequence of which encodes a C-terminal intein-containing binding domain, under conditions permitting synthesis of a chimeric polypeptide comprising the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain;

(b) isolating the chimeric polypeptide produced in step (a);

(c) treating the chimeric polypeptide so as to cause thio-mediated cleavage of the C-terminal intein-containing binding domain from the stretch of consecutive amino acids and its replacement with a C-terminal thioester;

(d) treating the product of step (c) to permit the attachment of a cysteine residue to the product so as to form product with a C-terminal cysteine; and

(e) oxidizing the product of step (e) in the presence of another product of step (e) under conditions permitting formation of the compound.

64. The method of claim 63, wherein the recombinant nucleic acid has the sequence set forth in any one of SEQ ID NOs. 1-8.

65. The method of claim 63, wherein the C-terminal intein-containing binding domain is an intein-chitin binding domain.

66. The method of claim 63, wherein the C-terminal intein-containing binding domain is an Mth RIR1 intein-chitin binding domain.

67. The method of claim 63, wherein the chimeric polypeptide is isolated in step b) by affinity chromatography.

68. The method of claim 63, wherein the chimeric polypeptide is isolated in step b) by exposure of the product to a chitin-derviatized resin.

69. The method of claim 63, wherein the oxidizing conditions permit formation of a disulfide bond between the C-terminal cysteine of each of the products.

70. A compound comprising an independently folding protein domain fused to a second independently folding protein domain by non-peptide bond.

71. A compound comprising a first polypeptide chain, comprising a terminal cysteine residue, fused at its S-terminus to a S-terminus of a second polypeptide chain comprising a terminal cysteine residue.

72. A compound comprising a first polypeptide chain, comprising a terminal selenocysteine residue, fused at its Se-terminus to a S-terminus of a second polypeptide chain comprising a terminal cysteine residue.

73. A compound comprising a first polypeptide chain, comprising a terminal selenocysteine residue, fused at its Se-terminus to a Se-terminus of a second

polypeptide chain comprising a terminal selenocysteine residue.

74. A multimer comprising two or more identical compounds according to any one of claims 70-73 joined together by at least one bond.

75. A method of making a stretch of consecutive amino acids comprising an N-terminal cysteine comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which encodes a N-terminal signal sequence contiguous with (ii) a second portion, the sequence of which encodes a stretch of consecutive amino acids comprising a N-terminal cysteine residue, under conditions permitting (i) synthesis of a chimeric polypeptide which comprises the N-terminal signal sequence joined by a peptide bond at its C-terminus to the N-terminal cysteine of the stretch of consecutive amino acids and (ii) cleavage of the N-terminal signal sequence from the chimeric polypeptide within the cell so as to produce a stretch of consecutive amino acids comprising an N-terminal cysteine;

b) recovering the stretch of consecutive amino acids produced in step (a).

76. The method of claim 75, wherein the stretch of consecutive amino acids comprises an immunoglobulin Fc polypeptide.

77. The method of claim 76, wherein the immunoglobulin Fc polypeptide is a human immunoglobulin Fc polypeptide.

78. The method of claim 77, wherein the N-terminal cysteine residue is a cys-5 residue of the human immunoglobulin Fc polypeptide.

79. The method of claim 75, wherein the cell is a 293 human embryonic cell or a CHO-K1 hamster ovary cell.

80. The method of claim 75, wherein the transfection is performed with a plasmid pSA.

81. The method of claim 75, wherein the N-terminal signal sequence is selected from a protein having a N-terminal cysteine.

82. The method of claim 75, wherein the signal peptide is sonic hedgehog, interferon alpha-2 or cholesterol ester transferase.

83. The method of claim 75, wherein the stretch of consecutive amino acids is recovered by affinity chromatography.

84. The method of claim 75, wherein the cleavage of the chimeric polypeptide within the cell is effected by a cellular signal peptidase.

85. A method of making a stretch of consecutive amino acids comprising an N-terminal cysteine or selenocysteine comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which encodes a N-terminal signal sequence contiguous with (ii) a second portion,

the sequence of which encodes a stretch of consecutive amino acids comprising a N-terminal cysteine residue, under conditions permitting (i) synthesis of a chimeric polypeptide comprising the N-terminal signal sequence joined at its C-terminus to the N-terminal cysteine of the Fc polypeptide and (ii) cleavage of the N-terminal signal sequence from the chimeric polypeptide within the cell so as to produce a stretch of consecutive amino acids comprising an N-terminal cysteine;

(b) ligating the N-terminal of the stretch of consecutive amino acids produced in step (a) with a C-terminal of a peptide comprising the amino acid sequence cys-asp-lys-thr-his-thr or with a peptide comprising the amino acid sequence sec-asp-lys-thr-his-thr so as to thereby produce the stretch of consecutive amino acids comprising an N-terminal cysteine or selenocysteine; and

(c) recovering the stretch of consecutive amino acids produced in step (b).

86. The method of claim 85, wherein the stretch of consecutive amino acids comprises an immunoglobulin Fc polypeptide.

87. The method of claim 85, wherein the immunoglobulin Fc polypeptide is a human immunoglobulin Fc polypeptide.

88. The method of claim 87, wherein the N-terminal cysteine residue is a cys-5 residue of the human immunoglobulin Fc polypeptide.

89. The method of claim 85, wherein the N-terminal cysteine residue is a cys-11 residue of the human immunoglobulin Fc polypeptide.

90. The method of claim 85, wherein peptide in step (b) comprises the amino acid sequence cys-asp-lys-thr-his-thr and the stretch of consecutive amino acids produced comprises an N-terminal cysteine.

91. The method of claim 85, wherein peptide in step (b) comprises the amino acid sequence sec-asp-lys-thr-his-thr and the stretch of consecutive amino acids produced comprises an N-terminal selenocysteine.

92. The method of claim 85, wherein the peptide in step (b) is protected with a N-terminal Msc protecting group prior to ligation.

93. The method of claim 85, wherein the cell is a 293 human embryonic cell or a CHO-K1 hamster ovary cell.

94. The method of claim 85, wherein the transfection is performed with a plasmid pSA.

95. The method of claim 85, wherein the N-terminal signal sequence is selected from a protein having a N-terminal cysteine.

96. The method of claim 85, wherein the signal peptide is sonic hedgehog, interferon alpha-2 or cholesterol ester transferase.

97. The method of claim 85, wherein the stretch of consecutive amino acids is recovered by affinity chromatography.

98. The method of claim 85, wherein the cleavage of the chimeric polypeptide within the cell is effected by a cellular signal peptidase.

99. A process of making a stretch of consecutive amino acids comprising a C-terminal cysteine or a C-terminal selenocysteine, comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which encodes a N-terminal signal sequence, contiguous with (ii) a second portion, the sequence of which encodes a stretch of consecutive amino acids contiguous with (iii) a third portion, the sequence of which encodes a C-terminal intein-containing binding domain, under conditions permitting (i) synthesis of a chimeric polypeptide comprising the N-terminal signal sequence contiguous with the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain and (ii) cleavage of the N-terminal signal sequence from the chimeric polypeptide so as to produce a second chimeric polypeptide having a N-terminal lysine residue and comprising the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain;

(b) isolating the second chimeric polypeptide produced in step (a);

(c) treating the second chimeric polypeptide so as to cause thio-mediated cleavage of the C-terminal intein-containing binding domain from the stretch of consecutive amino acids so as to form a C-terminal thioester;

(d) ligating the product of step (c) with a cysteine residue or selenocysteine residue at its C-terminal so as to form product with a C-terminal cysteine or a C-terminal selenocysteine; and

(e) recovering the product of step (d).

100. The method of claim 99, wherein the stretch of consecutive amino acids contiguous comprises an IgG immunoglobulin Fc polypeptide and an IgG M1 exon.

101. The method of claim 100, wherein the IgG immunoglobulin is a human IgG immunoglobulin.

102. The method of claim 99, wherein the stretch of consecutive amino acids contiguous comprises a CD4 extracellular domain.

103. The method of claim 99, wherein the N-terminal signal sequence is selected from a protein having a N-terminal lysine.

104. The method of claim 99, wherein the N-terminal signal sequence is a CD2 T-cell surface glycoprotein or a CD4 T-cell surface glycoprotein.

105. The method of claim 99, wherein the cell is a 293 human embryonic cell or a CHO-K1 hamster ovary cell.

106. The method of claim 99, wherein the transfection is performed with a plasmid pSA.

107. The method of claim 99, wherein the C-terminal intein-containing binding domain is an intein-chitin binding domain.

108. The method of claim 99, wherein the C-terminal intein-containing binding domain is an Mth RIR1 intein-chitin binding domain.

109. The method of claim 99, wherein the C-terminal intein-containing binding domain is a self-splicing intein-containing binding domain.

110. The method of claim 99, wherein the chimeric polypeptide is isolated in step b) by exposure of the product to a chitin-derivatized resin.

111. The method of claim 99, wherein the cleavage of the second chimeric polypeptide within the cell is effected by a cellular signal peptidase.

112. The method of claim 99, wherein the product of step (c) is ligated with a cysteine residue.

113. The method of claim 99, wherein the product of step (c) is ligated with a selenocysteine residue.

114. A process of making a stretch of consecutive amino acids comprising a N-terminal cysteine and a C-terminal cysteine or selenocysteine comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the

sequence of which encodes a N-terminal signal sequence, contiguous with (ii) a second portion, the sequence of which encodes a stretch of consecutive amino acids contiguous with (iii) a third portion, the sequence of which encodes a C-terminal intein-containing binding domain, under conditions permitting (i) synthesis of a chimeric polypeptide comprising the N-terminal signal sequence contiguous with the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain and (ii) cleavage of the N-terminal signal sequence from the chimeric polypeptide so as to produce a second chimeric polypeptide having a N-terminal cysteine residue and comprising the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain;

(b) isolating the second chimeric polypeptide produced in step (a);

(c) treating the second chimeric polypeptide so as to cause thio-mediated cleavage of the C-terminal intein-containing binding domain from the stretch of consecutive amino acids so as to form a C-terminal thioester;

(d) ligating the product of step (c) with a cysteine residue or selenocysteine residue at its C-terminal so as to form product with a C-terminal cysteine or a C-terminal selenocysteine; and

(e) recovering the product of step (d).

115. The method of claim 114, wherein the stretch of consecutive amino acids contiguous comprises an IgG immunoglobulin Fc polypeptide and an IgG M1 exon.

116. The method of claim 115, wherein the IgG immunoglobulin is a human IgG immunoglobulin.

117. The method of claim 114, wherein the N-terminal signal sequence is selected from a protein having a N-terminal cysteine.

118. The method of claim 114, wherein the N-terminal cysteine residue is a cys-5 residue of the human immunoglobulin Fc polypeptide.

119. The method of claim 114, wherein the N-terminal cysteine residue is a cys-11 residue of the human immunoglobulin Fc polypeptide.

120. The method of claim 114, wherein the N-terminal signal is a sonic hedgehog, interferon alpha-2 or cholesterol ester transferase.

121. The method of claim 114, wherein the cell is a 293 human embryonic cell or a CHO-K1 hamster ovary cell.

122. The method of claim 114, wherein the transfection is performed with a plasmid pSA.

123. The method of claim 114, wherein the C-terminal intein-containing binding domain is an intein-chitin binding domain.

124. The method of claim 114, wherein the C-terminal intein-containing binding domain is an Mth RIR1 intein-chitin binding domain.

125. The method of claim 114, wherein the C-terminal intein-containing binding domain is a self-splicing intein-containing binding domain

126. The method of claim 114, wherein the second chimeric polypeptide is isolated in step b) by exposure of the product to a chitin-derivatized resin.

127. The method of claim 114, wherein the product is recovered in step e) by affinity chromatography.

128. The method of claim 114, wherein the cleavage of the chimeric polypeptide within the cell is effected by a cellular signal peptidase.

129. The method of claim 114, wherein the product of step (c) is ligated with a cysteine residue.

130. The method of claim 114, wherein the product of step (c) is ligated with a selenocysteine residue.

131. A process of making a stretch of consecutive amino acids comprising a N-terminal cysteine or selenocysteine and a C-terminal cysteine or selenocysteine comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which encodes a N-terminal signal sequence, contiguous with (ii) a second portion, the sequence of which encodes a

stretch of consecutive amino acids contiguous with (iii) a third portion, the sequence of which encodes a C-terminal intein-containing binding domain, under conditions permitting (i) synthesis of a chimeric polypeptide comprising the N-terminal signal sequence contiguous with the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain and (ii) cleavage of the N-terminal signal sequence from the chimeric polypeptide so as to produce a second chimeric polypeptide having a N-terminal cysteine residue and comprising the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain;

(b) isolating the second chimeric polypeptide produced in step (a);

(c) (i) ligating the N-terminal of the stretch of consecutive amino acids produced in step (a) with a C-terminal of a peptide comprising the amino acid sequence cys-asp-lys-thr-his-thr or with a peptide comprising the amino acid sequence sec-asp-lys-thr-his-thr so as to thereby produce the stretch of consecutive amino acids comprising a N-terminal cysteine or a N-terminal selenocysteine, respectively; (ii) treating the chimeric polypeptide so as to cause thio-mediated cleavage of the C-terminal intein-containing binding domain from the stretch of consecutive amino acids and its replacement with a C-terminal thioester; (iii) ligating the product of step (c) with a cysteine residue or selenocysteine residue at its C-terminal so as to form product with a C-

terminal cysteine pr with a C-terminal selenocysteine; and

(d) recovering the product of step (c)(iii).

132. The method of claim 131, wherein the stretch of consecutive amino acids contiguous comprises an IgG immunoglobulin Fc polypeptide and an IgG M1 exon.

133. The method of claim 132, wherein the IgG immunoglobulin is a human IgG immunoglobulin.

134. The method of claim 131, wherein the N-terminal signal sequence is selected from a protein having a N-terminal cysteine.

135. The method of claim 131, wherein the N-terminal cysteine residue is a cys-11 residue of the human immunoglobulin Fc polypeptide.

136. The method of claim 131, wherein the N-terminal signal sequence is selected from a protein having a N-terminal cysteine.

137. The method of claim 131, wherein the N-terminal signal is a sonic hedgehog, interferon alpha-2 or cholesterol ester transferase.

138. The method of claim 131, wherein the cell is a 293 human embryonic cell or a CHO-K1 hamster ovary cell.

139. The method of claim 131, wherein the transfection is performed with a plasmid pSA.

140. The method of claim 131, wherein the C-terminal intein-containing binding domain is an intein-chitin binding domain.

141. The method of claim 131, wherein the C-terminal intein-containing binding domain is an Mth RIR1 intein-chitin binding domain.

142. The method of claim 131, wherein the chimeric polypeptide is isolated in step (b) by exposure of the product to a chitin-derivatized resin.

143. The method of claim 131, wherein the immunoglobulin Fc polypeptide is a human immunoglobulin Fc polypeptide.

144. The method of claim 131, wherein the N-terminal cysteine residue of the Fc polypeptide is a cys-11 residue.

145. The method of claim 131, wherein peptide in step (c)(i) comprises the amino acid sequence cys-asp-lys-thr-his-thr and the stretch of consecutive amino acids produced comprises an N-terminal cysteine.

146. The method of claim 131, wherein peptide in step (c)(i) comprises the amino acid sequence sec-asp-lys-thr-his-thr and the stretch of consecutive amino acids produced comprises an N-terminal selenocysteine.

147. The method of claim 131, wherein the peptide in step (c)(i) is protected with a N-terminal Msc protecting group prior to ligation.

148. The method of claim 131, wherein the cell is a 293 human embryonic cell or a CHO-K1 hamster ovary cell.

149. The method of claim 131, wherein the transfection is performed with a plasmid pSA.

150. The method of claim 131, wherein the N-terminal signal sequence is selected from a protein having a N-terminal cysteine.

151. The method of claim 131, wherein the signal peptide is sonic hedgehog, interferon alpha-2 or cholesterol ester transferase.

152. The method of claim 131, wherein the stretch of consecutive amino acids is recovered by affinity chromatography.

153. The method of claim 131, wherein step (c) is performed in the order step (c)(i); step (c)(ii); step (c)(iii).

154. The method of claim 131, wherein step (c) is performed in the order step (c)(ii); step (c)(iii); step (c)(i).

155. A process for making a compound comprising contacting a stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which comprises a binding site for a target with a second stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which is identical to the sequence of the first stretch of consecutive amino acids and which comprises an identical binding site for the target, wherein each of

the first stretch of amino acids and the second stretch of amino acids has at a predefined end thereof a cysteine residue or a selenocysteine residue, under reducing conditions so as to make the compound.

156. A process for making a compound comprising contacting a stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which comprises a binding site for a target with a second stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which is different to the sequence of the first stretch of consecutive amino acids and which comprises an identical binding site for the target, wherein each of the first stretch of amino acids and the second stretch of amino acids has at a predefined end thereof a cysteine residue or a selenocysteine residue, under reducing conditions so as to make the compound.

157. The method of claim 155 or 156, wherein the reducing conditions do not denature the stretches of consecutive amino acids.

158. The method of claim 155 or 156, wherein the reducing conditions comprise exposing the stretches of consecutive amino acids to a buffer comprising Tris-HCL and mercaptoethanol.

159. The method of claim 158, wherein the buffer is between pH 7.6 and 8.4.

160. The method of claim 158, wherein the buffer is pH 8.

161. The method of claim 158, wherein the method further comprises exchanging the product into oxidation buffer.

162. The method of claim 155 or 156, wherein the stretches of consecutive amino acids comprises a CD4 extracellular domain.

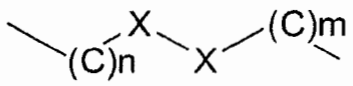
163. The method of claim 155 or 156, wherein the stretches of consecutive amino acids comprises a sequence of an immunoglobulin Fc polypeptide.

164. The method of claim 163, wherein the immunoglobulin is a human immunoglobulin.

165. A compound comprising a first stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which comprises a binding site for a target; and

a second stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which is identical to the sequence of the first stretch of consecutive amino acids and which comprises an identical binding site for the target;

wherein each of the first stretch of amino acids and the second stretch of amino acids has at a predefined end thereof, independently, a natural amino acid or non-natural amino having a linear aliphatic side-chain acid comprising a sulfur (S) or a selenium (Se) and wherein such sulfur (S) or a selenium (Se) are joined by a bond having the structure:



wherein each X is a sulfur (S) or a selenium (Se) and each (C) represents a carbon of the linear aliphatic side-chain of one of such natural or non-natural amino acid and wherein n and m are, independently, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

166. The compound of claim 165, wherein the natural amino acid is homocysteine of homoselenocysteine.

167. The compound of claim 165, wherein the first stretch and second stretch of amino acids have a homocysteine at the predefined end thereof.

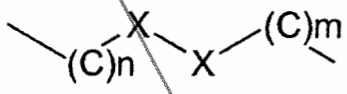
168. The compound of claim 165, wherein the first stretch and second stretch of amino acids have a homoselenocysteine at the predefined end thereof.

169. The compound of claim 165, wherein the first stretch and second stretch of amino acids have a homocysteine at the predefined end thereof.

170. The compound of claim 168 or 169, wherein the predefined end is a C-terminus.

171. A compound comprising a first stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which comprises a binding site for a target; and
a second stretch of consecutive amino acids, each of which is joined to the preceding amino acid by a peptide bond and the sequence of which is different from the sequence of the first stretch of consecutive amino acids and which comprises a binding site for a different moiety;

wherein each of the first stretch of amino acids and the second stretch of amino acids has at a predefined end thereof, independently, a natural amino acid or non-natural amino having a linear aliphatic side-chain acid comprising a sulfur (S) or a selenium (Se) and wherein such sulfur (S) or a selenium (Se) are joined by a bond having the structure:



wherein each X may be the same or different and represents a sulfur (S) or a selenium (Se) and each (C) represents a carbon of the linear aliphatic side-chain of one of such natural or non-natural amino acid and wherein n and m are, independently, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

172. The compound of claim 171, wherein the natural amino acid is homocysteine of homoselenocysteine.

173. The compound of claim 171, wherein the first stretch and second stretch of amino acids have a homocysteine at the predefined end thereof.

174. The compound of claim 171, wherein the first stretch and second stretch of amino acids have a homoselenocysteine at the predefined end thereof.

175. The compound of claim 171, wherein the first stretch and second stretch of amino acids have a homocysteine at the predefined end thereof.

176. The compound of claim 174 or 175, wherein the predefined end is a C-terminus.

177. A method of producing a protein which comprises a first polypeptide contiguous with an intein, which intein is contiguous with a second polypeptide comprising a binding domain, the method comprising transfecting an animal cell with a nucleic acid, which nucleic acid comprises (i) a first portion which encodes the polypeptide contiguous with (ii) a second portion which encodes the intein, contiguous with a and the third portion of which encodes the binding domain, under conditions such that the animal cell expresses and secretes the protein.

178. The method of claim 177, wherein the animal cell is a derived from mammal.

179. The method of claim 177, wherein the binding domain is a chitin-binding domain.

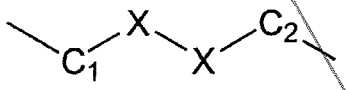
180. A composition comprising a polypeptide attached to a solid surface through a terminal disulfide bond.

181. The composition of claim 180, wherein the solid surface is a chip or a bead.

182. A compound comprising:
a first stretch of consecutive amino acids each of which is joined to the preceding amino acid by a peptide bond and which first stretch of consecutive amino acids comprises an amino acid residue having a chalcogen functional group-containing side chain; and
a second stretch of consecutive amino acids, comprising at least 100 amino acids, each of which is joined to the preceding amino acid by a peptide bond, wherein at least 90 consecutive amino acids of the second stretch

of consecutive amino acids have a sequence identical to portion of a human immunoglobulin constant region polypeptide, and wherein the second stretch of consecutive amino acids comprises an amino acid residue having a chalcogen functional group-containing side chain at a predefined terminus thereof,

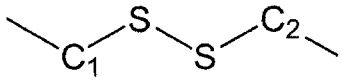
wherein said amino acid residue having a chalcogen functional group-containing side chain of the first stretch of consecutive amino acids and said amino acid residue having a chalcogen functional group-containing side chain of the second stretch of consecutive amino acids are joined by a bond having the structure:



wherein each X represents, independently, a chalcogen, and wherein C_1 represents a side chain carbon of the amino acid residue having a chalcogen functional group-containing side chain of the first stretch of consecutive amino acids and C_2 represents a side chain carbon of the second stretch of consecutive amino acids.

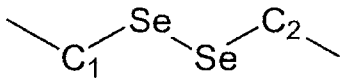
183. The compound of claim 182, wherein at least one of C_1 and C_2 is a beta carbon of amino acid.

184. The compound claim 182, wherein the bond has the structure:

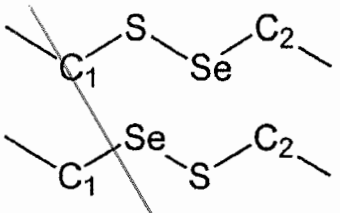


, wherein S is sulfur.

185. The compound claim 182, wherein the bond has the structure:



,



, or

,

wherein S is sulfur and Se is selenium.

186. The compound claim 182, wherein the amino acid residue having a chalcogen functional group-containing side chain at the predefined terminus of the second stretch of amino acids is a cysteine.

187. The compound claim 182, wherein the amino acid residue having a chalcogen functional group-containing side chain at the predefined terminus of the second stretch of amino acids is a selenocysteine, homocysteine or homoseleneocysteine.

188. The compound claim 182, wherein the amino acid residue having a chalcogen functional group-containing side chain of the first stretch of consecutive amino acids is a cysteine.

189. The compound claim 182, wherein the amino acid residue having a chalcogen functional group-containing side chain of the first stretch of amino acids is a selenocysteine, homocysteine or homoseleneocysteine

190. The compound claim 182, wherein the amino acid residue having a chalcogen functional group-containing side chain of the first stretch of consecutive amino acids is a terminal residue.

191. The compound claim 182, wherein the amino acid residue having a chalcogen functional group-containing side chain of the first stretch of consecutive amino acids is a penultimate, antepenultimate, or pre-antepenultimate terminal residue.

192. The compound of claim 182, wherein the second stretch of consecutive amino acids has a sequence identical to a human immunoglobulin constant region.

193. The compound of claim 192, wherein the second stretch of consecutive amino acids is a portion of a human immunoglobulin constant region.

194. The compound of claim 182, wherein the first stretch of consecutive amino acids has a sequence identical to a human immunoglobulin constant region.

195. The compound of claim 182, wherein the human immunoglobulin constant region polypeptide is a human IgG1, human IgG2, human IgG3, or human IgG4.

196. The compound of claim 195, wherein the side chain of at least one of amino acid residues having a chalcogen functional group-containing side chain comprises a C1-C10 alkylene.

197. A composition comprising two of the compounds of claim 182 bonded together via at least one disulfide bond between the second stretch of consecutive amino acids of each of the compounds.

198. A polypeptide consisting of consecutive amino acids having the sequence set forth in one of SEQ ID NOS:35

through 46, or having the sequence set forth in one of SEQ ID NOS:53 through 67, or having the sequence set forth in one of SEQ ID NOS:74 through 82, or having the sequence set forth in one of SEQ ID NOS:89 through 97.

199. A composition comprising the polypeptide of claim 198 and a carrier.

200. The composition of claim 199, wherein the carrier is phosphate-buffered saline.

201. A composition comprising two of the polypeptides of claim 198 bonded together via at least one disulfide bond between the two polypeptides.

202. A polypeptide consisting of consecutive amino acids having a sequence identical to a portion of the sequence set forth in SEQ ID NO:44, SEQ ID NO:64, SEQ ID NO:81 or SEQ ID NO:96, wherein at least one of the terminal residues of the polypeptide has a chalcogen functional group-containing side chain.

203. The polypeptide of claim 202, wherein the terminal residue having a chalcogen functional group-containing side chain is a cysteine or analog thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of producing a desired protein, said method comprising:

(a) producing an initial protein which comprises a first polypeptide contiguous with an intein, which intein is contiguous with a second polypeptide comprising a binding domain,

wherein said first polypeptide is a fusion polypeptide comprising an N-terminal signal sequence which can direct secretion of the initial protein, wherein production of the initial protein comprises transfecting a cell with a nucleic acid comprising (i) a first portion which encodes the first polypeptide, contiguous with (ii) a second portion which encodes the intein, contiguous with (iii) a third portion which encodes the second polypeptide comprising the binding domain, under conditions such that the cell expresses and secretes the initial protein; and

(b) generating a thioester intermediate of the initial protein using an intein autocleavage reaction and adding a cysteine, homocysteine, selenocysteine, homoselenocysteine or a functional analogue of cysteine, homocysteine, selenocysteine, or homoselenocysteine to the C-terminus of the thioester intermediate of the initial protein by native chemical ligation to thereby produce the desired protein.

2. The method of claim 1, wherein in step (b) a cysteine, homocysteine, selenocysteine or homoselenocysteine is added to the C-terminus of the thioester intermediate of the initial protein by native chemical ligation.

3. The method of claim 1 or 2, wherein the cell is a prokaryotic cell.

4. The method of claim 1 or 2, wherein the cell is a eukaryotic cell.

5. The method of claim 4, wherein the eukaryotic cell is a mammalian cell.

6. The method of claim 5, wherein the eukaryotic cell is a Chinese Hamster Ovary (CHO) cell or a 293 human embryonic kidney cell.

7. The method according to any one of claims 1 to 6, wherein the N-terminal signal sequence is a homologous N-terminal signal sequence.

8. The method according to any one of claims 1 to 6, wherein the N-terminal signal sequence is a heterologous N-terminal signal sequence.

9. The method according to any one of claims 1 to 8, wherein the intein is a Mth RIR1 intein.

10. The method according to any one of claims 1 to 9, wherein the binding domain is a chitin-binding domain.

11. The method according to any one of claims 1 to 10, wherein the initial protein is isolated before step (b) by affinity purification.

12. The method of claim 11, wherein the initial protein is isolated before step (b) by contacting it with a chitin resin.

13. The method according to any one of claims 1 to 12, wherein the cell is in a culture medium, and before step (b) the secreted initial protein is isolated from the culture medium.

14. The method according to any one of claims 1 to 13, wherein the first polypeptide comprises a secreted protein.

15. The method according to any one of claims 1 to 14, wherein the first polypeptide comprises an extracellular domain of a protein.

16. The method according to any one of claims 1 to 15, wherein the desired protein that is produced has biological activity.

17. The method according to claim 16, wherein the biological activity is target-binding activity.

18. The method according to any one of claims 1 to 17, wherein the desired protein that is produced comprises intra-chain disulfide bonds.

19. The method according to any one of claims 1 to 18, wherein the desired protein that is produced is an independently-folding protein.

20. The method according to any one of claims 1 to 19, wherein the desired protein that is produced is a glycosylated protein.

21. The method according to any one of claim 1 or 3 to 20, wherein a cysteine, selenocysteine or an analogue thereof is added to the C-terminus of the thioester intermediate of the initial protein by native chemical ligation.

22. A desired protein when obtained by performing the method according to any one of claims 1 to 21.

23. The desired protein according to claim 22, wherein said desired protein comprises an X-terminal amino acid.

24. The desired protein according to claim 22 or 23, wherein said desired protein comprises intra-chain disulfide bonds.

25. The desired protein according to any one of claims 22 to 24, wherein said desired protein is glycosylated.

26. The desired protein according to any one of claims 22 to 25, wherein said desired protein comprises a C-terminal amino acid which is a functional cysteine derivative or a functional selenocysteine derivative.

27. The desired protein according to claim 26, wherein the functional cysteine derivative or functional selenocysteine derivative comprises a free β-sulfhydryl or β-selenohydryl functional group.

28. The desired protein according to claim 26, wherein the desired protein comprises a C-terminal amino acid which is a functional cysteine derivative.

29. The desired protein according to any one of claims 22 to 28, wherein the desired protein has biological activity.

30. The desired protein according to claim 29, wherein the biological activity is target-binding activity.

31. A process of making a stretch of consecutive amino acids comprising a C-terminal cysteine, homocysteine, selenocysteine, homoselenocysteine or a functional analogue of cysteine, homocysteine, selenocysteine, or homoselenocysteine, said method comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which encodes a N-terminal signal sequence, contiguous with (ii) a second portion, the sequence of which encodes a stretch of consecutive amino acids, contiguous with (iii)a third portion, the sequence of which encodes a C-terminal intein-containing binding domain,

wherein said transfection occurs under conditions sufficient to permit:

(α) synthesis of a chimeric polypeptide comprising the N-terminal signal sequence contiguous with the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain; and

(β) cleavage of the N-terminal signal sequence from the chimeric polypeptide so as to produce a second chimeric polypeptide comprising the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain;

(b) treating the second chimeric polypeptide so as to cause a thio-mediated cleavage of the C-terminal intein-containing binding domain from the stretch of consecutive amino acids so as to form a C-terminal thioester;

(c) ligating the product of step (b) with a cysteine residue, a homocysteine residue, a selenocysteine residue, a homoselenocysteine residue or a functional analogue of a cysteine, homocysteine, selenocysteine, or homoselenocysteine residue so as to form a product with a C-terminal cysteine, homocysteine, selenocysteine, homoselenocysteine or functional analogue of cysteine, homocysteine, selenocysteine, or homoselenocysteine; and

(d) recovering the product of step (c), to thereby produce the stretch of consecutive amino acids comprising the C-terminal cysteine or the C-terminal selenocysteine.

32. A process of making a desired protein, said process comprising:

(a) transfecting a cell with a recombinant nucleic acid which comprises (i) a first portion, the sequence of which encodes a N-terminal signal sequence, contiguous with (ii) a second portion, the sequence of which encodes a stretch of consecutive amino acids, contiguous with (iii) a third portion, the sequence of which encodes a C-terminal intein-containing binding domain,

wherein said transfecting occurs under conditions sufficient to permit synthesis of a chimeric polypeptide comprising the stretch of consecutive amino acids contiguous with the C-terminal intein-containing binding domain;

(b) treating the chimeric polypeptide so as to cause thio-mediated cleavage of the C-terminal intein-containing binding domain from the stretch of consecutive amino acids and its replacement with a C-terminal thioester; and

(c) treating the product of step (b) to permit the attachment of a cysteine residue, a homocysteine residue, a selenocysteine residue, a homoselenocysteine residue or a functional analogue of a cysteine, homocysteine, selenocysteine, or homoselenocysteine residue to the product of step (b), forming a product with a C-terminal cysteine, homocysteine, selenocysteine, homoselenocysteine or functional analogue of cysteine, homocysteine, selenocysteine, or homoselenocysteine, to thereby produce the desired protein.