Claims

We claim:

1. A fibronectin type III (FnIII) domain-based cradle polypeptide comprising one or more amino acid substitutions in at least a loop region and at least a non-loop region.

2. The cradle polypeptide of claim 1, wherein the non-loop region is selected from the group consisting of a beta strand C, a beta strand D, a beta strand F, and a beta strand G.

3. The cradle polypeptide according to claim 1 or 2, wherein the loop region is selected from the group consisting of an AB loop, a BC loop, a CD loop, a DE loop, an EF loop and an FG loop.

4. The cradle polypeptide according to any one of claims 1-3, comprising one or more amino acid substitutions in two loop regions and/or two non-loop regions.

5. The cradle polypeptide of claim 4, wherein the non-loop regions are the beta strands C and F, and the loop regions are the CD and FG loops.

6. The cradle polypeptide according to any one of claims 1-5, wherein the one or more amino acid substitutions are introduced to the cradle residues in the beta strands.

7. The cradle polypeptide according to any one of claims 1-6, further comprising an insertion and/or deletion of at least one amino acid in at least one loop and/or non-loop region.

8. The cradle polypeptide of claim 7, which comprises an insertion and/or deletion of at least one amino acid in two loop regions and/or two non-loop regions.

9. The cradle polypeptide of claim 8, wherein the non-loop regions are the beta strands C and F, and the loop regions are the CD and FG loops.

10. The cradle polypeptide according to any one of claims 1-9, wherein the one or more amino acid substitutions in the non-loop region do not change the structure of the FnIII domain scaffold and/or the shape of the loop regions.

11. The cradle polypeptide according to any one of claims 1-10, wherein the one or more amino acid substitutions in the non-loop region exclude the non-cradle residues.

12. The cradle polypeptide according to any one of claims 1-11, wherein the FnIII domain is the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th, 15th or 16th FnIII domain of human fibronectin.

13. The cradle polypeptide of claim 12, wherein the FnIII domain is the 7th, 10th or 14th FnIII domain of human fibronectin.

14. The cradle polypeptide of claim 13, wherein the FnIII domain is the 7th FnIII domain of human fibronectin ($FnIII^{07}$).

15. The cradle polypeptide of claim 13, wherein the FnIII domain is the 10th FnIII domain of human fibronectin ($FnIII^{10}$).

16. The cradle polypeptide of claim 13, wherein the FnIII domain is the 14th FnIII domain of human fibronectin ($FnIII^{14}$).

17. The cradle polypeptide according to any one of claims 1-16, wherein the CD loop is about 3-11 residues in length.

18. The cradle polypeptide of claim 17, wherein the CD loop is about 4-9 residues in length.

19. The cradle polypeptide of claim 18, wherein the CD loop is 5 residues in length.

20. The cradle polypeptide according to any one of claims 1-19, wherein the FG loop is about 1-10 residues in length.

21. The cradle polypeptide of claim 20, wherein the FG loop is about 5 or 6 residues in length.

22. The cradle polypeptide of claim 21, wherein the FG loop is 5 residues in length.

23. The cradle polypeptide of claim 21, wherein the FG loop is 6 residues in length.

24. The cradle polypeptide of claim 23, wherein position 1 of the FG loop is a Gly residue, position 2 of the FG loop is a Leu, Val or Ile residue, position 3 of the FG loop is a charged or polar residue, position 4 of the FG loop is a Pro residue, position 5 of the FG loop is a Gly residue, and position 6 of the FG loop is a polar residue.

25. The cradle polypeptide of claim 22, wherein positions 3 and/or 5 of the FG loop are a Gly residue.

26. The cradle polypeptide according to any one of claims 1-25, wherein the beta strand C is about 6-14 residues in length and the beta strand F is about 8-13 residues in length.

27. The cradle polypeptide of claim 26, wherein the beta strand C is about 8-11 residues in length.

28. The cradle polypeptide of claim 27, wherein the beta strand C is 9 residues in length.

29. The cradle polypeptide according to any one of claims 26-28, wherein positions 2, 4, and 6 of the beta strand C are a hydrophobic residue.

30. The cradle polypeptide according to any one of claims 26-29, wherein positions 1, 3, 5 and 7-9 of the beta strand C are altered relative to the wild type sequence.

31. The cradle polypeptide according to any one of claims 26-30, wherein position 1 of the beta strand C is selected from the group consisting of Ala, Gly, Pro, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys and Arg.

32. The cradle polypeptide according to any one of claims 26-31, wherein position 3 of the beta strand C is a hydrophobic residue.

33. The cradle polypeptide according to any one of claims 26-32, wherein position 3 of the beta strand C is selected from the group consisting of Ile, Val, Arg, Leu, Thr, Glu, Lys, Ser, Gln and His.

34. The cradle polypeptide according to any one of claims 26-33, wherein positions 5 and 7-9 of the beta strand C are selected from the group consisting of Ala, Gly, Pro, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys and Arg.

35. The cradle polypeptide according to any one of claims 26-34, wherein the beta strand F is about 9-11 residues in length.

36. The cradle polypeptide of claim 35, wherein the beta strand F is 10 residues in length.

37. The cradle polypeptide according to claim 35 or 36, wherein positions 1, 3, 5 and 10 of the beta strand F are altered relative to the wild type sequence.

38. The cradle polypeptide according to any one of claims 35-37, wherein positions 1, 3, 5 and 10 of the beta strand F are selected from the group consisting of Ala, Gly, Pro, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys and Arg.

39. The cradle polypeptide according to any one of claims 35-38, wherein positions 2, 4 and 6 of the beta strand F are a hydrophobic residue.

40. The cradle polypeptide according to any one of claims 35-39, wherein position 7 of the beta strand F is a hydrophobic residue.

41. The cradle polypeptide according to any one of claims 35-40, wherein position 7 of the beta strand F is selected from the group consisting of Arg, Tyr, Ala, Thr and Val.

42. The cradle polypeptide according to any one of claims 35-41, wherein position 8 of the beta strand F is selected from the group consisting of Ala, Gly, Ser, Val and Pro.

43. The cradle polypeptide according to any one of claims 35-42, wherein position 9 of the beta strand F is selected from the group consisting of Val, Leu, Glu, Arg and Ile.

44. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 30, 31, 32, 33, 34, 35, 36, 37, 38 and/or 39 of SEQ ID NO:1.

45. The cradle polypeptide of claim 44, wherein the amino acid substitutions comprise positions 31, 33, 35, 37, 38 and/or 39 of SEQ ID NO:1.

46. The cradle polypeptide of claim 45, wherein the amino acid substitutions comprise positions 31 and/or 33 of SEQ ID NO:1.

47. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 44, 45, 46, 47, 48, 49, 50 and/or 51 of SEQ ID NO:1.

48. The cradle polypeptide of claim 47, wherein the amino acid substitutions comprise positions 44, 45, 47 and/or 49 of SEQ ID NO:1.

49. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 40, 41, 42, 43, 44 and/or 45 of SEQ ID NO:1.

50. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 67, 68, 69, 70, 71, 72, 73, 74, 75 and/or 76 of SEQ ID NO:1.

51. The cradle polypeptide of claim 50, wherein the amino acid substitutions comprise positions 67, 69, 71, 73 and/or 76 of SEQ ID NO:1.

52. The cradle polypeptide of claim 50, wherein the amino acid substitutions comprise positions 71, 73, 75 and/or 76 of SEQ ID NO:1.

53. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 and/or 86 of SEQ ID NO:1.

54. The cradle polypeptide of claim 53, wherein the amino acid substitutions comprise positions 84 and/or 85 of SEQ ID NO:1.

55. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 85, 86, 87, 88, 89, 90, 91, 92, 93 and/or 94 of SEQ ID NO:1.

56. The cradle polypeptide of claim 15, wherein the amino acid substitutions comprise positions 31, 33, 47, 49, 73 and/or 75 of SEQ ID NO:1.

56. The cradle polypeptide of claim 14, wherein the amino acid substitutions comprise positions 33, 35, 37, 39, 40 and/or 41 of SEQ ID NO:97.

57. The cradle polypeptide of claim 14, wherein the amino acid substitutions comprise positions 42, 43, 44, 45, 46, 47 and/or 48 of SEQ ID NO:97.

58. The cradle polypeptide of claim 14, wherein the amino acid substitutions comprise positions 70, 72, 74, 76 and/or 79 of SEQ ID NO:97.

59. The cradle polypeptide of claim 14, wherein the amino acid substitutions comprise positions 80, 81, 82, 83, 84 and/or 85 of SEQ ID NO:97.

60. The cradle polypeptide of claim 16, wherein the amino acid substitutions comprise positions 31, 33, 35, 37 and/or 39 of SEQ ID NO:129.

61. The cradle polypeptide of claim 16, wherein the amino acid substitutions comprise positions 40, 41, 42, 43 and/or 44 of SEQ ID NO:129.

62. The cradle polypeptide of claim 16, wherein the amino acid substitutions comprise positions 66, 68, 70, 72 and/or 75 of SEQ ID NO:129.

63. The cradle polypeptide of claim 16, wherein the amino acid substitutions comprise positions 76, 77, 78, 79, 80 and/or 81 of SEQ ID NO:129.

64. The cradle polypeptide of claim 14, comprising an amino acid sequence set forth in SEQ ID NO:468.

65. The cradle polypeptide of claim 15, comprising an amino acid sequence set forth in SEQ ID NO:469.

66. The cradle polypeptide of claim 16, comprising an amino acid sequence set forth in SEQ ID NO:470.

67. A chimeric cradle polypeptide according to any one of claims 1-66, wherein part of the cradle polypeptide is replaced by a non-FnIII domain polypeptide that enhances the binding affinity of the cradle polypeptide for a target molecule.

68. The chimeric cradle polypeptide of claim 67, wherein the non-FnIII domain polypeptide is all or part of a complementarity determining region (CDR) of an antibody or a T-cell receptor.

69. The chimeric cradle polypeptide of claim 68, wherein the CDR is a CDR1, CDR2 or CDR3 of a single domain antibody.

70. The chimeric cradle polypeptide of claim 69, wherein the single domain antibody is a nanobody.

71. The chimeric cradle polypeptide of claim 68, wherein the CDR replaces part or all of the AB, BC, CD, DE, EF or FG loop.

72. A multispecific cradle polypeptide comprising multiple copies of one or more monomer cradle polypeptides according to any one of claims 1-71.

73. The multispecific cradle polypeptide of claim 72, wherein the monomer cradle polypeptides are linked by a linker sequence.

74. The multispecific cradle polypeptide of claim 73, wherein the linker sequence is selected from the group consisting of GGGGSGGGGS (SEQ ID NO: 471), GSGSGSGSGS (SEQ ID NO: 472), PSTSTST (SEQ ID NO: 473) and EIDKPSQ (SEQ ID NO: 474).

75. A cradle library comprising a plurality of cradle polypeptides according to any one of claims 1-74.

76. The cradle library of claim 75, wherein the cradle polypeptides comprise one or more amino acid substitutions corresponding to amino acid positions 30, 41, 42, 43, 44, 45, 76, 77, 78, 79, 80, 81, 82, 83, 84 and/or 85 of SEQ ID NO:1.

77. The cradle library of claim 76, wherein the cradle polypeptides further comprise one or more amino acid substitutions corresponding to amino acid positions 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 32, 34, 35, 36, 37, 38, 39, 40, 46, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 72, 74, 75, 86, 87, 88, 89, 90, 91, 92, 93 and/or 94 of SEQ ID NO:1.

78. The cradle library of claims 75, wherein the cradle polypeptides are at least 50%, 60%, 70%, 80%, or 90% identical to SEQ ID NO:1.

79. The cradle library according to any one of claims 75-78, wherein the cradle polypeptides further comprise an insertion of at least 1 amino acid in at least one loop region.

80. The cradle library of claim 79, wherein the cradle polypeptides comprise an insertion of at least 2 amino acids in at least one loop region.

81. The cradle library of claim 80, wherein the cradle polypeptides comprise an insertion of about 2-25 amino acids in at least one loop region.

82. The cradle library according to any one of claims 79-81, wherein the cradle polypeptides comprise a deletion of at least 1 amino acid in at least one loop region.

83. The cradle library of claim 82, wherein the cradle polypeptides comprise a deletion of at least 2 amino acids in at least one loop region.

84. The cradle library of claim 83, wherein the cradle polypeptides comprise a deletion of about 2-10 amino acids in at least one loop region.

85. The cradle library according to any one of claims 79, 83 and 84, wherein the cradle polypeptides comprise a deletion of at least 2 amino acids in two loop regions.

86. The cradle library according to any one of claims 79-85, wherein the cradle polypeptides comprise at least 1 amino acid insertion and 1 amino acid deletion in at least one loop region.

87. The cradle library of claim 86, wherein the cradle polypeptides comprise an insertion and deletion of at least 1 amino acid in the same loop region.

88. The cradle library according to any one of claims 79-87, which is pre-selected to bind a target molecule.

89. The cradle library of claim 75, wherein the cradle polypeptides comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 79, 86 and 468-470.

90. The cradle library of claim 75, wherein the cradle library contains at least 10, 100, 1000, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} or more different cradle polypeptides.

91. A polynucleotide encoding a cradle polypeptide according to any one of claims 1-74.

92. The polynucleotide of claim 91, wherein the polynucleotide is an expression construct.

93. The polynucleotide of claim 92, wherein the expression construct is capable of expressing the encoded polypeptide in a bacterium, yeast or mammalian system.

94. A polynucleotide library encoding a cradle library according to any one of claims 75-90.

95. A method of producing a cradle polypeptide comprising:

a) expressing a polynucleotide encoding a cradle polypeptide according to any one of claims 91-93 in a host cell; and

b) isolating and/or purifying the expressed cradle polypeptide.

96. A method of forming a cradle library of FnIII domain polypeptides useful in screening for the presence of one or more polypeptides having a selected binding or enzymatic activity, comprising:

(i) aligning beta strands C and F amino acid sequences and loops CD and FG amino acid sequences in a collection of native FnIII domain polypeptides,

(ii) segregating the aligned beta strand and loop sequences according to length,

(iii) for a selected beta strand or loop of the length from step (ii), performing positional amino acid frequency analysis to determine the frequencies of amino acids at each beta strand or loop position,

(iv) for each beta strand, loop, and length analyzed in step (iii), identifying at each position a conserved or selected semi-conserved consensus amino acid and other natural-variant amino acids,

(v) for at least one selected beta strand, loop, and length, forming:

(1) a cradle library of mutagenesis sequences expressed by a library of coding sequences that encode, at each beta strand or loop position, the consensus amino acid, and, if the consensus amino acid has a occurrence frequency equal to or less than a selected threshold frequency of at least 50%, a single common target amino acid and any co-produced amino acids, or

(2) a cradle library of natural-variant combinatorial sequences expressed by a library of coding sequences that encode, at each beta strand or loop position, a consensus amino acid and, if the consensus amino acid has a frequency of occurrence equal to or less than a selected threshold frequency of at least 50%, other natural variant amino acids, including semi-conserved amino acids and variable amino acids whose occurrence rate is above a selected minimum threshold occurrence at that position, or their chemical equivalents,

(vi) incorporating the library of coding sequences into framework FnIII coding sequences to form an FnIII expression library, and

(vi) expressing the FnIII domain polypeptides of the expression library.

97. The method of claim 96, wherein the selected threshold frequency is 100%.

98. The method of claim 96, wherein the selected frequency is about 50-95%.

99. The method according to any one of claims 96-98, wherein step (ii) segregates the loops and loop lengths into the CD loop at about 3-11 residues in length.

100. The method of claim 99, wherein the CD loop is about 3-9 residues in length.

101. The method of claim 99, wherein the CD loop is about 4-9 residues in length.

102. The method of claim 99, wherein the CD loop is 5 residues in length.

103. The method according to any one of claims 96-102, wherein step (ii) segregates the loops and loop lengths into the FG loop at about 1-10 residues in length.

104. The method of claim 103, wherein the FG loop is about 5 or 6 residues in length.

105. The method of claim 103, wherein the FG loop is 5 residues in length.

106. The method of claim 103, wherein the FG loop is 6 residues in length.

107. The method of claim 106, wherein position 1 of the FG loop is a Gly residue, position 2 the FG loop is a Leu, Val or Ile residue, position 3 the FG loop is a charged or polar residue, position 4 the FG loop is a Pro residue, position 5 the FG loop is a Gly residue, and position 6 the FG loop is a polar residue.

108. The method according to claim 105, wherein positions 3 and/or 5 of the FG loop are a Gly residue.

109. The method according to any one of claims 96-108, wherein step (ii) segregates the beta strands into the beta strand C at about 6-14 residues in length and the beta strand F at about 8-13 residues in length.

110. The method of claim 109, wherein the beta strand C is about 8-11 residues in length.

111. The method of claim 109, wherein the beta strand C is 9 residues in length.

112. The method of claim 111, wherein positions 2, 4 and 6 of the beta strand C are a hydrophobic residue.

113. The method of claim 111, wherein positions 1, 3, 5 and 7-9 of the beta strand C are altered relative to the wild type sequence.

114. The method of claim 113, wherein position 1 of the beta strand C is selected from the group consisting of Ala, Gly, Pro, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys and Arg.

115. The method of claim 113, wherein position 3 of the beta strand C is a hydrophobic residue.

116. The method of claim 113, wherein position 3 of the beta strand C is selected from the group consisting of Ile, Val, Arg, Leu, Thr, Glu, Lys, Ser, Gln and His.

117. The method of claim 113, wherein positions 5 and 7-9 of the beta strand C are selected from the group consisting of Ala, Gly, Pro, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys and Arg.

118. The method of claim 109, wherein the beta strand F is about 9-11 residues in length.

119. The method of claim 109, wherein the beta strand F is 10 residues in length.

120. The method of claim 119, wherein positions 1, 3, 5 and 10 of the beta strand F are altered relative to the wild type sequence.

121. The method of claim 120, wherein positions 1, 3, 5 and 10 of the beta strand F are selected from the group consisting of Ala, Gly, Pro, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys and Arg.

122. The method of claim 119, wherein positions 2, 4 and 6 of the beta strand F are a hydrophobic residue.

123. The method of claim 119, wherein position 7 of the beta strand F is a hydrophobic residue.

124. The method of claim 119, wherein position 7 of the beta strand F is selected from the group consisting of Arg, Tyr, Ala, Thr and Val.

125. The method of claim 119, wherein position 8 of the beta strand F is selected from the group consisting of Ala, Gly, Ser, Val and Pro.

126. The method of claim 119, wherein position 9 of the beta strand F is selected from the group consisting of Val, Leu, Glu, Arg and Ile.

127. The method according to any one of claims 96-126, wherein the native FnIII domain polypeptides comprise the wild type amino acid sequences of the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th, 15th and/or 16th FnIII domain of human fibronectin.

128. The method of claim 127, wherein the native FnIII domain polypeptides comprise the wild type amino acid sequences of the 7th, 10th and/or 14th FnIII domain of human fibronectin.

129. A cradle library useful in screening for the presence of one or more cradle polypeptides having a selected binding or enzymatic activity designed using a method of forming a cradle library of FnIII domain polypeptides according to any one of claims 96-128.

130. A method of identifying a cradle polypeptide having a desired binding affinity to a target molecule, comprising:

a) reacting a cradle library of FnIII domain polypeptides according to any one of claims 75-90 and 129 with the target molecule, and

b) screening the cradle library of FnIII domain polypeptides to select those having a desired binding affinity to the target molecule.

131. The method of claim 130, wherein the method further comprises the step of identifying the polynucleotides that encode the selected polypeptides.

132. The method according to claim 130 or 131, wherein the target molecule is selected from the group consisting of lysozyme, human Fc, human serum albumin (HSA), human small ubiquitin-like modifier 1 (SUMO1), human ubiquitin, human Abl SH2 domain, human Scm-like with four mbt domains 2 (SFMBT2), sex comb on midleg homolog 1 (SCMH1) domain and green fluorescent protein (GFP).

133. A cradle polypeptide selected using the methods according to any one of claims 130-132.

134. The cradle polypeptide of claim 134 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:4-78, 80-85, 87-96, 98, 99, 101-128, 130-141, 143, 145-147, 149-159, 161-199, 201-238 and 240-277.

135. A kit comprising, in a container, a cradle polypeptide according to any one of claims 1-74, 133 and 134.

136. A kit comprising, in a container, a cradle library according to any one of claims 75-90 and 129.

137. A kit comprising, in a container, a polynucleotide encoding a cradle polypeptide according to any one of claims 91-93.

138. A kit comprising, in a container, a polynucleotide library encoding a cradle library according to any one of claims 75-90 and 129.

Claims

1. A fibronectin type III (FnIII) domain-based cradle polypeptide comprising i) a non loop region comprising one or more amino acid modifications (e.g., substitutions) in each of a beta strand C and a beta strand F; (ii) a top loop region comprising one or more modifications (e.g., substitutions) in a BC, DE and/or FG loop; and (iii) a bottom loop region comprising one or more modifications (e.g., substitutions) in an AB, CD and/or EF loop.

2. The cradle polypeptide of claim 1, further comprising one or more amino acid substitutions in a beta strand D and a beta strand G.

3. The cradle polypeptide according to claim 1 or 2, wherein: i) the top loop region comprises one or more substitutions in an FG loop; and ii) the bottom loop region comprises one or more substitutions in a CD loop.

4. The cradle polypeptide according to any one of claims 1-3, wherein the one or more amino acid substitutions are introduced to the cradle residues in the beta strands.

5. The cradle polypeptide according to any one of claims 1-4, further comprising an insertion and/or deletion of at least one amino acid in the loop regions and the non-loop regions.

6. The cradle polypeptide of claim 5, wherein the non-loop regions with the insertion and/or deletion of at least one amino are the beta strands C and F, and the loop regions with the insertion and/or deletion of at least one amino are the CD and FG loops.

7. The cradle polypeptide according to any one of claims 1-6, wherein the FnIII domain is the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, 11^{th}, 12^{th}, 13^{th}, 14^{th}, 15^{th} or 16^{th} FnIII domain of human fibronectin.

8. The cradle polypeptide of claim 7, wherein the FnIII domain is the 7^{th} FnIII domain of human fibronectin ($FnIII^{07}$), the 10^{th} FnIII domain of human fibronectin ($FnIII^{10}$), or the 14^{th} FnIII domain of human fibronectin ($FnIII^{14}$).

9. The cradle polypeptide according to any one of claims 1-8, wherein the CD loop is about 3-11 residues in length and the FG loop is about 1-10 residues in length.

10. The cradle polypeptide according to any one of claims 1-9, wherein the beta

strand C is about 6-14 residues in length and the beta strand F is about 8-13 residues in length.

11. The cradle polypeptide of claim 8, wherein the amino acid substitutions comprise positions 31, 33, 35, 37, 38, 39, 67, 69, 71, 73 and/or 76 of SEQ ID NO:1.

12. The cradle polypeptide of claim 8, wherein the amino acid substitutions comprise positions 33, 35, 37, 39, 40, 41, 70, 72, 74, 76 and/or 79 of SEQ ID NO:97.

13. The cradle polypeptide of claim 8, wherein the amino acid substitutions comprise positions 31, 33, 35, 37, 39, 66, 68, 70, 72 and/or 75 of SEQ ID NO:129.

14. The cradle polypeptide of claim 8, comprising an amino acid sequence set forth in SEQ ID NOs:468-470.

15. A chimeric cradle polypeptide, wherein part of the cradle polypeptide according to any one of claims 1-14 is replaced by a non-FnIII domain polypeptide that enhances the binding affinity of the cradle polypeptide for a target molecule.

16. A multispecific cradle polypeptide comprising multiple copies of one or more monomer cradle polypeptides according to any one of claims 1-15.

17. A cradle library comprising a plurality of cradle polypeptides according to any one of claims 1-16.

18. A method of forming a cradle library of FnIII domain polypeptides, wherein said polypeptides comprise i) a non loop region comprising one or more amino acid modifications (e.g., substitutions) in each of a beta strand C and a beta strand F; (ii) a top loop region comprising one or more modifications (e.g., substitutions) in a BC, DE and/or FG loop; and (iii) a bottom loop region comprising one or more modifications (e.g., substitutions) in an AB, CD and/or EF loop; and wherein said method is useful in screening for the presence of one or more polypeptides having a selected binding or enzymatic activity, comprising:

(i) aligning beta strands C and F amino acid sequences and loops CD and FG amino acid sequences in a collection of native FnIII domain polypeptides,

(ii) segregating the aligned beta strand and loop sequences according to length,

(iii) for a selected beta strand or loop of the length from step (ii), performing positional amino acid frequency analysis to determine the frequencies of amino acids at each

beta strand or loop position,

(iv) for each beta strand, loop, and length analyzed in step (iii), identifying at each position a conserved or selected semi-conserved consensus amino acid and other natural-variant amino acids,

(v) for at least one selected beta strand, loop, and length, forming:

(1) a cradle library of mutagenesis sequences expressed by a library of coding sequences that encode, at each beta strand or loop position, the consensus amino acid, and, if the consensus amino acid has a occurrence frequency equal to or less than a selected threshold frequency of at least 50%, a single common target amino acid and any co-produced amino acids, or

(2) a cradle library of natural-variant combinatorial sequences expressed by a library of coding sequences that encode, at each beta strand or loop position, a consensus amino acid and, if the consensus amino acid has a frequency of occurrence equal to or less than a selected threshold frequency of at least 50%, other natural variant amino acids, including semi-conserved amino acids and variable amino acids whose occurrence rate is above a selected minimum threshold occurrence at that position, or their chemical equivalents,

(vi) incorporating the library of coding sequences into framework FnIII coding sequences to form an FnIII expression library, and

(vi) expressing the FnIII domain polypeptides of the expression library.

19. The FnIII domain-based cradle polypeptide of any one of claims 1-17, wherein the one or more amino acid substitution in the beta strand C correspond to position 30, 31, 32, 33, 34, 35, 36, 37, 38 and/or 39 of SEQ ID NO: 1; position 33, 35, 37, 39, 40 and/or 41 of SEQ ID NO: 97; or position 31, 33, 35, 37 and/or 39 of SEQ ID NO: 129.

20. The FnIII domain-based cradle polypeptide of any one of claims 1-17, wherein the one or more amino acid substitution in beta strand F correspond to position 67, 68, 69, 70, 71, 752, 73, 74, 75 and/or 76 of SEQ ID NO: 1; position 70, 72, 74, 76 and/or 79 of SEQ ID NO: 97; or position 66, 68, 70 and/or 75 of SEQ ID NO: 129.

21. The FnIII domain-based cradle polypeptide of any one of claims 1-17, wherein the one or more amino acid substitution in the CD loop correspond to position 40, 41, 42, 43, 44 and/or 45 of SEQ ID NO: 1; position 42, 43, 44, 45, 46, 47 and/or 48 of SEQ ID NO: 97; or

position 40, 41, 42, 43 and/or 44 of SEQ ID NO: 129.

22. The FnIII domain-based cradle polypeptide of any one of claims 1-17, wherein the one or more amino acid substitution in the FG loop correspond to position 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 and/or 86 of SEQ ID NO: 1; position 80, 81, 82, 83, 84 and/or 85 of SEQ ID NO: 97; or position 76, 77, 78, 79, 80 and/or 81 of SEQ ID NO: 129.