

WHAT IS CLAIMED:

1. An isolated single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a linker (L) and a light chain variable region (VL), wherein the scFv comprises:
 - a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
 - b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
 - c) the first disulfide bond between the structurally conserved surface exposed VH Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.
2. An isolated scFv comprising a VH, a L and a VL, wherein
 - a) the VH comprises a VH Cys at a structurally conserved surface exposed VH framework residue position and the L comprises a first L Cys;
 - b) the VL comprises a VL Cys at a structurally conserved surface exposed VL framework residue position and the L comprises a second L Cys; or
 - c) the VH comprises the VH Cys at a structurally conserved surface exposed VH framework residue position, the VL comprises the VL Cys at a structurally conserved surface exposed VL framework residue position and the L comprises the first L Cys and the second L Cys, wherein the VH Cys and the first L Cys are capable of forming a disulfide bond and the VL Cys and the second L Cys are capable of forming a disulfide bond.
3. The scFv of claim 1 or 2, wherein the distance between the VH Cys and the VL Cys is from about 7 Å to about 9 Å.
4. The scFv of any one of claims 1-3, wherein the VH Cys is at H3, H5, H40, H43, H46 or H105, wherein residue numbering is according to Chothia.
5. The scFv of any one of claims 1-4, wherein the VL Cys is at L3, L5, L39, L42, L45,

L100 or L102, wherein residue numbering is according to Chothia.

6. The scFv of any one of claims 1-5, wherein
- a) the VH Cys is at H105 and the VL Cys is at L42;
 - b) the VH Cys is at H43 and the VL Cys is at a L100;
 - c) the VH Cys is at H3 and the VL Cys is at L3;
 - d) the VH Cys is at H3 and the VL Cys is at L5;
 - e) the VH Cys is at H3 and the VL Cys is at L39;
 - f) the VH Cys is at H3 and the VL Cys is at L42;
 - g) the VH Cys is at H3 and the VL Cys is at L45;
 - h) the VH Cys is at H3 and the VL Cys is at L100;
 - i) the VH Cys is at H3 and the VL Cys is at L102;
 - j) the VH Cys is at H5 and the VL Cys is at L3;
 - k) the VH Cys is at H5 and the VL Cys is at L5;
 - l) the VH Cys is at H5 and the VL Cys is at L39;
 - m) the VH Cys is at H5 and the VL Cys is at L42;
 - n) the VH Cys is at H5 and the VL Cys is at L45;
 - o) the VH Cys is at H5 and the VL Cys is at L100;
 - p) the VH Cys is at H5 and the VL Cys is at L102;
 - q) the VH Cys is at H40 and the VL Cys is at L3;
 - r) the VH Cys is at H40 and the VL Cys is at L5;
 - s) the VH Cys is at H40 and the VL Cys is at L39;
 - t) the VH Cys is at H40 and the VL Cys is at L42;
 - u) the VH Cys is at H40 and the VL Cys is at L45;
 - v) the VH Cys is at H40 and the VL Cys is at L100;
 - w) the VH Cys is at H40 and the VL Cys is at L102;
 - x) the VH Cys is at H43 and the VL Cys is at L3;
 - y) the VH Cys is at H43 and the VL Cys is at L5;
 - z) the VH Cys is at H43 and the VL Cys is at L39;
 - aa) the VH Cys is at H43 and the VL Cys is at L42;
 - bb) the VH Cys is at H43 and the VL Cys is at L45;

- cc) the VH Cys is at H43 and the VL Cys is at L102;
- dd) the VH Cys is at H46 and the VL Cys is at L3;
- ee) the VH Cys is at H46 and the VL Cys is at L5;
- ff) the VH Cys is at H46 and the VL Cys is at L39;
- gg) the VH Cys is at H46 and the VL Cys is at L42;
- hh) the VH Cys is at H46 and the VL Cys is at L45;
- ii) the VH Cys is at H46 and the VL Cys is at L100;
- jj) the VH Cys is at H46 and the VL Cys is at L102;
- kk) the VH Cys is at H105 and the VL Cys is at L3;
- ll) the VH Cys is at H105 and the VL Cys is at L5;
- mm) the VH Cys is at H105 and the VL Cys is at L39;
- nn) the VH Cys is at H105 and the VL Cys is at L45;
- oo) the VH Cys is at H105 and the VL Cys is at L100; or
- pp) the VH Cys is at H105 and the VL Cys is at L102, wherein residue numbering is according to Chothia.

7. The scFv of any one of claims 1-6, wherein the L comprises a contiguous amino acid sequence derived from an immunoglobulin (Ig) hinge region.

8. The scFv of claim 7, wherein the Ig hinge region is derived from a human or a non-human Ig hinge region.

9. The scFv of claim 8, wherein the Ig hinge region is derived from the human Ig hinge region.

10. The scFv of claim 9, wherein the human Ig hinge region is an IgG1, IgG2, IgG3 or IgG4 isotype.

11. The scFv of any one of claims 1-10, wherein the L comprises an amino acid sequence C(X)_yC (**SEQ ID NO: 23**), wherein X is glycine (Gly), serine (Ser), proline (Pro), alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln),

histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe), threonine (Thr), tryptophan (Trp) or tyrosine (Tyr), and y is an integer from 1 to 3.

12. The scFv of claim 11, wherein the L comprises an amino acid sequence C(X)_yC (**SEQ ID NO: 24**), wherein X is Gly, Ser or Pro, and y is an integer from 1 to 3.

13. The scFv of any one of claims 1-12, wherein the L comprises the amino acid sequence CPC, CGC, CSC, CPPC (SEQ ID NO: 1), CGPC (SEQ ID NO: 28), CPGC (SEQ ID NO: 29), CGGC (SEQ ID NO: 30), CSPG (SEQ ID NO: 31), CPSC (SEQ ID NO: 32), CSSC (SEQ ID NO: 33), CGSC (SEQ ID NO: 34), CSGC (SEQ ID NO: 35), CPPPC (SEQ ID NO: 36), CGPPC (SEQ ID NO: 37), CPGPC (SEQ ID NO: 38), CPPGC (SEQ ID NO: 39), CGGPC (SEQ ID NO: 40), CPGGC (SEQ ID NO: 41), CGGGC (SEQ ID NO: 42), CSPPC (SEQ ID NO: 43), CPSPC (SEQ ID NO: 44), CPPSC (SEQ ID NO: 45), CSSPC (SEQ ID NO: 46), CPSSC (SEQ ID NO: 47), CSSSC (SEQ ID NO: 48), CGSPC (SEQ ID NO: 49), CPGSC (SEQ ID NO: 50), CSGPC (SEQ ID NO: 51) or CPSGC (SEQ ID NO: 52).

14. The scFv of any one of claims 1-13, wherein the L comprises from about 14 to about 19 amino acids, such as about 14, about 15, about 16, about 17, about 18 or about 19 amino acids.

15. The scFv of any one of claims 1-14 wherein the L comprises the amino acid sequence (X)_mC(X)_yC(X)_n (**SEQ ID NO: 25**); wherein X is Gly, Ser, Pro, Ala, Arg, Asn, Asp, Glu, Gln, His, Ile, leu, Lys, Phe, Thr, Trp or Tyr, m is an integer from 6 to 9, y is an integer from 1 to 3 and n is an integer from 4 to 6.

16. The scFv of claim 15, wherein the L comprises the amino acid sequence (X)_mC(X)_yC(X)_n (**SEQ ID NO: 26**); wherein X is Gly, Ser, Pro, Ala, Arg, Asn, Asp, Glu, Gln, His, Ile, Leu, Lys, Thr or Tyr, m is an integer from 6 to 9, y is an integer from 1 to 3 and n is an integer from 4 to 6.

17. The scFv of claim 16, wherein the L comprises the amino acid sequence (X)_mC(X)_yC(X)_n (**SEQ ID NO: 27**); wherein X is Gly or Pro, m is an integer from 6 to 9, y is an integer from 1 to 3 and n is an integer from 4 to 6.

18. The scFv of any one of claims 1-17, wherein the L comprises the amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7.
19. The scFv of any one of claims 1-18, wherein the scFv is in the VL-L-VH orientation.
20. The scFv of any one of claims 1-18, wherein the scFv is in the VH-L-VL orientation.
21. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H105;
 - b) the VL comprises Cys at L42;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VL-L-VH orientation.
22. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H105;
 - b) the VL comprises Cys at L45;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VL-L-VH orientation.
23. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H105;
 - b) the VL comprises Cys at L39;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VL-L-VH orientation.
24. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H5;
 - b) the VL comprises Cys at L42;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VL-L-VH orientation.

25. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H5;
 - the VL comprises Cys at L45;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VL-L-VH orientation.
26. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H5;
 - the VL comprises Cys at L39;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VL-L-VH orientation.
27. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H3;
 - the VL comprises Cys at L42;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VL-L-VH orientation.
28. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H3;
 - the VL comprises Cys at L45;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VL-L-VH orientation.
29. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H3;
 - the VL comprises Cys at L39;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VL-L-VH orientation.

30. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H43;
 - the VL comprises Cys at L100;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VH-L-VL orientation.
31. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H43;
 - the VL comprises Cys at L102;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VH-L-VL orientation.
32. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H43;
 - the VL comprises Cys at L5;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VH-L-VL orientation.
33. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H43;
 - the VL comprises Cys at L3;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VH-L-VL orientation.
34. A scFv comprising a VH, a L and a VL, wherein
- the VH comprises Cys at H40;
 - the VL comprises Cys at L100;
 - the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - the scFv is in the VH-L-VL orientation.
35. A scFv comprising a VH, a L and a VL, wherein

- a) the VH comprises Cys at H40;
 - b) the VL comprises Cys at L102;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
36. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H40;
 - b) the VL comprises Cys at L5;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
37. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H40;
 - b) the VL comprises Cys at L3;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
38. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H46;
 - b) the VL comprises Cys at L100;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
39. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H46;
 - b) the VL comprises Cys at L102;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
40. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H46;

- b) the VL comprises Cys at L5;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
41. A scFv comprising a VH, a L and a VL, wherein
- a) the VH comprises Cys at H46;
 - b) the VL comprises Cys at L3;
 - c) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
 - d) the scFv is in the VH-L-VL orientation.
42. The scFv of any one of claims 21-41, wherein the L comprises the amino acid sequence of SEQ ID NO: 3.
43. The scFv of any one of claims 21-41, wherein the L comprises the amino acid sequence of SEQ ID NO: 6.
44. The scFv of any one of claims 21-41, wherein the L comprises the amino acid sequence of SEQ ID NO: 7.
45. The scFv of any one claims 1-44, wherein the scFv is conjugated to a second molecule.
46. The scFv of claim 45, wherein the second molecule is a half-life extending moiety.
47. The scFv of claim 46, wherein the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol.
48. The scFv of claim 45, wherein the second molecule is a cytotoxic agent or a detectable label.
49. The scFv of claim 48, wherein the second molecule is an antibody or a fragment thereof.

50. The scFv of claim 49, wherein the scFv and the antibody or the fragment thereof bind distinct antigens.
51. The scFv of claim 45, wherein the second molecule is a chimeric antigen receptor (CAR).
52. A pharmaceutical composition comprising the scFv of any one of claims 1-51 and a pharmaceutically acceptable carrier.
53. A polynucleotide encoding the scFv of any one of claims 1-44.
54. A vector comprising the polynucleotide of claim 53.
55. A host cell comprising the vector of claim 54.
56. A method of producing the scFv of any one of claims 1-44, comprising culturing the host cell of claim 55 in conditions that the scFv is produced, and purifying the scFv.
57. The method of claim 56, wherein the host cell is a prokaryotic cell.
58. The method of claim 56, wherein the host cell is an eukaryotic cell.
59. An anti-idiotypic antibody that binds to the scFv of any one of claims 1-44.
60. A kit comprising the scFv of any one of claims 1-44.
61. A multispecific molecule comprising the scFv of any one of claims 1 to 44.
62. The multispecific molecule of claim 61, wherein the multispecific molecule comprises an antibody or an antibody fragment.

63. The multispecific molecule of claim 61, wherein the multispecific protein comprises an Ig constant region or a fragment of the Ig constant region.
64. The multispecific protein of claim 63, wherein the fragment of the Ig constant region comprises a Fc region.
65. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises a CH2 domain.
66. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises a CH3 domain.
67. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain.
68. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises at least a portion of a hinge, the CH2 domain and the CH3 domain.
69. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises the hinge, the CH2 domain and the CH3 domain.
70. The multispecific molecule of any one of claims 63-69, wherein the scFv is conjugated to the N-terminus of the Ig constant region or to the N-terminus of the fragment of the Ig constant region.
71. The multispecific molecule of any one of claims 63-69, wherein the scFv is conjugated to the C-terminus of the Ig constant region or to the N-terminus of the fragment of the Ig constant region.
72. The multispecific molecule of any one of claims 63-71, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

73. The multispecific molecule of any one of claims 63-72, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the multispecific molecule to Fc γ R.

74. The multispecific molecule of claim 73, wherein the at least one mutation that results in reduced binding of the multispecific molecule to Fc γ R is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/L235A, N297A, V234A/G237A, K214T/E233P/L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index.

75. The multispecific molecule of any one of claims 63-72, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific molecule to Fc γ R.

76. The multispecific molecule of claim 75, wherein the at least one mutation that results in enhanced binding of the multispecific molecule to Fc γ R is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

77. The multispecific molecule of any one of claims 73-76, wherein Fc γ R is Fc γ RI, Fc γ RIIA, Fc γ RIIB or Fc γ RIII, or any combination thereof.

78. The multispecific molecule of any one of claims 63-72, wherein the Ig constant region or fragment of the Ig constant region comprises at least one mutation that modulates a half-life of the multispecific molecule.

79. The multispecific molecule of claim 78, wherein the at least one mutation that modulates the half-life of the multispecific molecule is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

80. The multispecific molecule of any one of claims 63-72, wherein the Ig constant region or fragment of the Ig constant region comprises at least one mutation in the CH3 domain.

81. The multispecific molecule of claim 80, wherein the at least one mutation in the CH3 domain is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

82. The multispecific molecule of any one of claims 61-81, wherein the multispecific molecule is bispecific.

83. The multispecific molecule of any one of claims 61-81, wherein the multispecific molecule is trispecific.

84. The multispecific molecule of any one of claims 61-81, wherein the multispecific molecule is tetraspecific.

85. A pharmaceutical composition comprising the multispecific molecule of any one of claims 61-84 and a pharmaceutically acceptable carrier.

86. A heterologous molecule comprising the scFv of any one of claims 1-44.

87. The heterologous molecule of claim 86, wherein the scFv is conjugated to a second protein, a polynucleotide, a therapeutic agent, a cytotoxic agent or a detectable label.
88. The heterologous molecule of claim 87, wherein the second protein is an antibody or a fragment thereof.
89. The heterologous molecule of claim 87, wherein the second protein is an alternative scaffold.
90. The heterologous molecule of claim 87, wherein the second protein is a chimeric antigen receptor (CAR) or a fragment thereof.
91. The heterologous molecule of any one of claims 86-90, wherein the heterologous molecule is monospecific.
92. The heterologous molecule of any one of claims 86-90, wherein the heterologous molecule is multispecific.
93. The heterologous molecule of claim 92, wherein the heterologous molecule is bispecific.
94. The heterologous molecule of claim 92, wherein the heterologous molecule is trispecific.
95. The heterologous molecule of claim 92, wherein the heterologous molecule is tetraspecific.
96. A pharmaceutical composition comprising the heterologous molecule of any one of claims 86-95 and a pharmaceutically acceptable carrier.
97. A process for preparing a stabilized scFv, comprising:
providing a heavy chain variable region (VH) and a light chain variable region (VL) that form an antigen binding domain;

providing a linker (L) that comprises or is engineered to comprise a first L Cys;
engineering the VH to comprise a VH Cys at a structurally conserved surface exposed
VH framework residue position; and
forming a disulfide bond between the VH Cys and the first L Cys to prepare the stabilized
scFv.

98. A process for preparing a stabilized scFv, comprising:
providing a VH and a VL that form an antigen binding domain;
providing a L that comprises or is engineered to comprise a second L Cys;
engineering the VL to comprise a VL Cys at a structurally conserved surface exposed
VL framework residue position; and
forming a disulfide bond between the VL Cys and the second L Cys to prepare the
stabilized scFv.

99. A process for preparing a stabilized scFv, comprising:
providing a VH and a VL that form an antigen binding domain;
providing a L that comprises or is engineered to comprise a first L Cys and a second L
Cys;
engineering the VH to comprise a VH Cys at a structurally conserved surface exposed
VH framework residue position;
engineering the VL to comprise a VL Cys at a structurally conserved surface exposed VL
framework residue position; and
forming a disulfide bond between the VH Cys and the first L Cys and a disulfide bond
between the VL Cys and the second L Cys to prepare the stabilized scFv.

100. The process of any one of claims 97-99, wherein the stabilized scFv is the scFv of any
one of claims 1-44.

101. The process of any one of claims 97-100, wherein the stabilized scFv binds an antigen
with comparable affinity when compared to a control scFv that is devoid of the disulfide bonds.

102. A process for preparing a stabilized scFv, comprising:
- a) providing a polynucleotide encoding a VH, a L and a VL, wherein
- i. the VH comprises Cys at H105 and the VL comprises Cys at L42;
 - ii. the VH comprises Cys at H43 and the VL comprises Cys at a L100;
 - iii. the VH comprises Cys at H3 and the VL comprises Cys at L3;
 - iv. the VH comprises Cys at H3 and the VL comprises Cys at L5;
 - v. the VH comprises Cys at H3 and the VL comprises Cys at L39;
 - vi. the VH comprises Cys at H3 and the VL comprises Cys at L42;
 - vii. the VH comprises Cys at H3 and the VL comprises Cys at L45;
 - viii. the VH comprises Cys at H3 and the VL comprises Cys at L100;
 - ix. the VH comprises Cys at H3 and the VL comprises Cys at L102;
 - x. the VH comprises Cys at H5 and the VL comprises Cys at L3;
 - xi. the VH comprises Cys at H5 and the VL comprises Cys at L5;
 - xii. the VH comprises Cys at H5 and the VL comprises Cys at L39;
 - xiii. the VH comprises Cys at H5 and the VL comprises Cys at L42;
 - xiv. the VH comprises Cys at H5 and the VL comprises Cys at L45;
 - xv. the VH comprises Cys at H5 and the VL comprises Cys at L100;
 - xvi. the VH comprises Cys at H5 and the VL comprises Cys at L102;
 - xvii. the VH comprises Cys at H40 and the VL comprises Cys at L3;
 - xviii. the VH comprises Cys at H40 and the VL comprises Cys at L5;
 - xix. the VH comprises Cys at H40 and the VL comprises Cys at L39;
 - xx. the VH comprises Cys at H40 and the VL comprises Cys at L42;
 - xxi. the VH comprises Cys at H40 and the VL comprises Cys at L45;
 - xxii. the VH comprises Cys at H40 and the VL comprises Cys at L100;
 - xxiii. the VH comprises Cys at H40 and the VL comprises Cys at L102;
 - xxiv. the VH comprises Cys at H43 and the VL comprises Cys at L3;
 - xxv. the VH comprises Cys at H43 and the VL comprises Cys at L5;
 - xxvi. the VH comprises Cys at H43 and the VL comprises Cys at L39;
 - xxvii. the VH comprises Cys at H43 and the VL comprises Cys at L42;
 - xxviii. the VH comprises Cys at H43 and the VL comprises Cys at L45;
 - xxix. the VH comprises Cys at H43 and the VL comprises Cys at L102;

- xxx. the VH comprises Cys at H46 and the VL comprises Cys at L3;
- xxxi. the VH comprises Cys at H46 and the VL comprises Cys at L5;
- xxxii. the VH comprises Cys at H46 and the VL comprises Cys at L39;
- xxxiii. the VH comprises Cys at H46 and the VL comprises Cys at L42;
- xxxiv. the VH comprises Cys at H46 and the VL comprises Cys at L45;
- xxxv. the VH comprises Cys at H46 and the VL comprises Cys at L100;
- xxxvi. the VH comprises Cys at H46 and the VL comprises Cys at L102;
- xxxvii. the VH comprises Cys at H105 and the VL comprises Cys at L3;
- xxxviii. the VH comprises Cys at H105 and the VL comprises Cys at L5;
- xxxix. the VH comprises Cys at H105 and the VL comprises Cys at L39;
- xl. the VH comprises Cys at H105 and the VL comprises Cys at L45;
- xli. the VH comprises Cys at H105 and the VL comprises Cys at L100; or
- xlii. the VH comprises Cys at H105 and the VL comprises Cys at L102,

wherein residue numbering is according to Chothia;

- b) the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and
- c) expressing the polynucleotide in a host cell to produce the stabilized scFv.

103. The method of claim 102, wherein the host cell is a prokaryotic cell.

104. The method of claim 102, wherein the host cell is an eukaryotic cell.

105. An isolated single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a means for linking (L) and a light chain variable region (VL), wherein the scFv comprises:

- a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- c) the first disulfide bond between the structurally conserved surface exposed VH Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.

106. An isolated single chain variable fragment (scFv) comprising a means for antigen binding, a linker (L) and a light chain variable region (VL), wherein the scFv comprises
- a) a first disulfide bond between a structurally conserved surface exposed antigen binding means cysteine (Cys) and a first L Cys;
 - b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
 - c) the first disulfide bond between the structurally conserved surface exposed antigen binding means Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.
107. An isolated single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a linker (L) and a means for antigen binding, wherein the scFv comprises
- a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
 - b) a second disulfide bond between a structurally conserved surface exposed antigen binding means Cys and a second L Cys; or
 - c) the first disulfide bond between the structurally conserved surface exposed VH Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed antigen binding means Cys and the second L Cys.
108. A multispecific molecule comprising a single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a a means for linking (L) and a light chain variable region (VL), wherein the scFv comprises
- a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
 - b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
 - c) the first disulfide bond between the structurally conserved surface exposed VH Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.

109. A multispecific molecule comprising a single chain variable fragment (scFv) comprising a means for antigen binding, a linker (L) and a light chain variable region (VL), wherein the scFv comprises

- a) a first disulfide bond between a structurally conserved surface exposed antigen binding means cysteine (Cys) and a first L Cys;
- b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- c) the first disulfide bond between the structurally conserved surface exposed antigen binding means Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.

110. A multispecific molecule comprising a single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a linker (L) and a means for antigen binding (VL), wherein the scFv comprises

- a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- b) a second disulfide bond between a structurally conserved surface exposed antigen binding means Cys and a second L Cys; or
- c) the first disulfide bond between the structurally conserved surface exposed VH Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed antigen binding means Cys and the second L Cys.

111. A heterologous molecule comprising a single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a means for linking (L) and a light chain variable region (VL), wherein the scFv comprises

- a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- c) the first disulfide bond between the structurally conserved surface exposed VH

Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.

112. A heterologous molecule comprising a single chain variable fragment (scFv) comprising a means for antigen binding, a linker (L) and a light chain variable region (VL), wherein the scFv comprises

- a) a first disulfide bond between a structurally conserved surface exposed antigen binding means cysteine (Cys) and a first L Cys;
- b) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- c) the first disulfide bond between the structurally conserved surface exposed antigen binding means Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed VL Cys and the second L Cys.

113. A heterologous molecule comprising a single chain variable fragment (scFv) comprising a heavy chain variable region (VH), a linker (L) and a means for antigen binding, wherein the scFv comprises

- a) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- b) a second disulfide bond between a structurally conserved surface exposed antigen binding means Cys and a second L Cys; or
- c) the first disulfide bond between the structurally conserved surface exposed VH Cys and the first L Cys and the second disulfide bond between the structurally conserved surface exposed antigen binding means Cys and the second L Cys.

114. A means for encoding the scFv of any one of claims 105-113.

115. A means for replicating the vector of the 114.

116. A composition comprising a means for stabilizing a scFv.

117. A composition comprising a means for increasing thermostability of a scFv.
118. The composition of claim 116 or 117, wherein the means includes forming a disulfide bond between a VH and a L, between a VL and the L, or between the VH and the L and between the VL and the L.
119. A multispecific molecule comprising a means for stabilizing a scFv.
120. A multispecific molecule comprising a means for increasing thermostability of a scFv.
121. The multispecific molecule of claim 119 or 120, wherein the means includes forming a disulfide bond between a VH and a L, between a VL and the L, or between the VH and the L and between the VL and the L.
122. A heterologous molecule comprising a means for stabilizing a scFv.
123. A heterologous molecule comprising a means for increasing thermostability of a scFv.
124. The heterologous molecule of claim 122 or 123, wherein the means includes forming a disulfide bond between a VH and a L, between a VL and the L, or between the VH and the L and between the VL and the L.
125. A means for producing the composition of any one of claims 116-118.
126. A means for producing the multispecific molecule of any one of claims 119-121.
127. A means for producing the heterologous molecule of any one of claims 122-124.