

WHAT IS CLAIMED:

Claims

1. An isolated single chain variable fragment (scFv) comprising:

a) a heavy chain variable region (VH), a linker (L) and a light chain variable region (VL), wherein the scFv comprises: a)

(i) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;

b) (ii) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or

c) (iii) 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; or

2. An isolated scFv comprising b) a VH, a L and a VL, wherein:

a) (i) 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) (ii) 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) (iii) 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.

2. The scFv of claim 1 or 2, wherein:

a) 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 b) the VH Cys is at H3, H5, H40, H43, H46 or H105, wherein residue numbering is according to Chothia; or

5. The scFv of any one of claims 1-4, wherein c) the VL Cys is at L3, L5, L39, L42, L45, L100 or L102, wherein residue numbering is according to Chothia.

6.

3. The scFv of any ~~one of claims~~ claim 1-5 or claim 2, wherein:

- a) ~~a)~~ the VH Cys is at H105 and the VL Cys is at L42; ~~b)~~
- b) ~~b)~~ the VH Cys is at H43 and the VL Cys is at ~~a~~ ~~L100~~; ~~c)~~ L100;
- c) ~~c)~~ the VH Cys is at H3 and the VL Cys is at L3; ~~d)~~
- d) ~~d)~~ the VH Cys is at H3 and the VL Cys is at L5; ~~e)~~
- e) ~~e)~~ the VH Cys is at H3 and the VL Cys is at L39; ~~f)~~
- f) ~~f)~~ the VH Cys is at H3 and the VL Cys is at L42; ~~g)~~
- g) ~~g)~~ the VH Cys is at H3 and the VL Cys is at L45; ~~h)~~
- h) ~~h)~~ the VH Cys is at H3 and the VL Cys is at ~~L100~~; ~~i)~~ L100;
- i) ~~i)~~ the VH Cys is at H3 and the VL Cys is at ~~L102~~; ~~j)~~ L102;
- j) ~~j)~~ the VH Cys is at H5 and the VL Cys is at L3; ~~k)~~
- k) ~~k)~~ the VH Cys is at H5 and the VL Cys is at L5; ~~l)~~
- l) ~~l)~~ the VH Cys is at H5 and the VL Cys is at L39; ~~m)~~
- m) ~~m)~~ the VH Cys is at H5 and the VL Cys is at L42; ~~n)~~
- n) ~~n)~~ the VH Cys is at H5 and the VL Cys is at L45; ~~o)~~
- o) ~~o)~~ the VH Cys is at H5 and the VL Cys is at ~~L100~~; ~~p)~~ L100;
- p) ~~p)~~ the VH Cys is at H5 and the VL Cys is at ~~L102~~; ~~q)~~ L102;
- q) ~~q)~~ the VH Cys is at H40 and the VL Cys is at L3; ~~r)~~
- r) ~~r)~~ the VH Cys is at H40 and the VL Cys is at L5; ~~s)~~
- s) ~~s)~~ the VH Cys is at H40 and the VL Cys is at L39; ~~t)~~
- t) ~~t)~~ the VH Cys is at H40 and the VL Cys is at L42; ~~u)~~
- u) ~~u)~~ the VH Cys is at H40 and the VL Cys is at L45; ~~v)~~
- v) ~~v)~~ the VH Cys is at H40 and the VL Cys is at ~~L100~~; ~~w)~~ L100;
- w) ~~w)~~ the VH Cys is at H40 and the VL Cys is at ~~L102~~; ~~x)~~ L102;
- x) ~~x)~~ the VH Cys is at H43 and the VL Cys is at L3; ~~y)~~
- y) ~~y)~~ the VH Cys is at H43 and the VL Cys is at L5; ~~z)~~
- z) ~~z)~~ the VH Cys is at H43 and the VL Cys is at L39; ~~aa)~~
- aa) ~~aa)~~ the VH Cys is at H43 and the VL Cys is at L42; ~~bb)~~
- bb) ~~bb)~~ the VH Cys is at H43 and the VL Cys is at L45;
- cc) ~~cc)~~ the VH Cys is at H43 and the VL Cys is at ~~L102~~ L102;
- dd) ~~dd)~~ the VH Cys is at H46 and the VL Cys is at L3;
- ee) ~~ee)~~ the VH Cys is at H46 and the VL Cys is at L5;
- ff) ~~ff)~~ the VH Cys is at H46 and the VL Cys is at L39;

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

7.

4. ~~The scFv of any one of claims 1-6 to 3, wherein:~~

~~a)~~ ~~the L comprises a contiguous amino acid sequence derived from an
 immunoglobulin (Ig) hinge region;~~ optionally wherein:

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

~~9. The scFv of claim 8, wherein ii)~~ ~~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 ~~IgG~~IgG1,
 IgG2, IgG3 or IgG4 isotype;~~

~~11. The scFv of any one of claims 1-10, wherein b)~~ ~~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~~Gln), histidine (His), isoleucine (~~Ile~~Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe),
 threonine (Thr), tryptophan (Trp) or tyrosine (Tyr), and y is an integer from 1 to 3;~~

optionally

~~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 c)~~ ~~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 d)~~ 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 e)~~ 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~~Gln, His, ~~Ile~~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; optionally wherein:

~~16. The scFv of claim 15, wherein i)~~ 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~~Gln, His, ~~Ile~~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; or

~~17. The scFv of claim 16, wherein ii)~~ 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 f)~~ 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 or~~
g) 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.~~

5. _____

~~21. A scFv comprising a VH, a L and a VL, wherein:~~

~~a) a) a VH, a L and a VL, wherein:~~

~~i) the VH comprises Cys at H105;~~

~~b) ii) 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. iii)~~

~~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. Aiv) the scFv comprising is in the VL-L-VH orientation;~~

b) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H105;~~

~~b) ii) the VL comprises Cys at L39;~~

~~c) iii) 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. Aiv) the scFv comprising is in the VL-L-VH orientation;~~

d) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H5;~~

~~b) ii) the VL comprises Cys at L42;~~

~~c) iii) 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. Aiv) the scFv comprising is in the VL-L-VH orientation;~~

e) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H5;~~

~~b) ii) the VL comprises Cys at L45;~~

~~c) iii) 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.~~

~~26. Aiv) the scFv comprising is in the VL-L-VH orientation;~~

f) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H5;~~

~~b) ii) the VL comprises Cys at L39;~~

~~c) iii) 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.~~

~~27. Aiv) the scFv comprising is in the VL-L-VH orientation;~~

g) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H3;~~

~~b) ii) the VL comprises Cys at L42;~~

~~c) iii) 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.~~

~~28. Aiv) the scFv comprising is in the VL-L-VH orientation;~~

- h) _____ a VH, a L and a VL, wherein:
- a) ~~i)~~ _____ the VH comprises Cys at H3;
 - b) ~~ii)~~ _____ the VL comprises Cys at L45;
 - c) ~~iii)~~ _____ 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.~~
- ~~29. Aiv)~~ _____ the scFv comprising is in the VL-L-VH orientation;
- i) _____ a VH, a L and a VL, wherein:
- a) ~~i)~~ _____ the VH comprises Cys at H3;
 - b) ~~ii)~~ _____ the VL comprises Cys at L39;
 - c) ~~iii)~~ _____ 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.~~
- ~~30. Aiv)~~ _____ the scFv comprising is in the VL-L-VH orientation;
- j) _____ a VH, a L and a VL, wherein:
- a) ~~i)~~ _____ the VH comprises Cys at H43;
 - b) ~~ii)~~ _____ the VL comprises Cys at L100;
 - c) ~~iii)~~ _____ 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.~~
- ~~31. Aiv)~~ _____ the scFv comprising is in the VH-L-VL orientation;
- k) _____ a VH, a L and a VL, wherein:
- a) ~~i)~~ _____ the VH comprises Cys at H43;
 - b) ~~ii)~~ _____ the VL comprises Cys at L102;
 - c) ~~iii)~~ _____ 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.~~
- ~~32. Aiv)~~ _____ the scFv comprising is in the VH-L-VL orientation;
- l) _____ a VH, a L and a VL, wherein:
- a) ~~i)~~ _____ the VH comprises Cys at H43;
 - b) ~~ii)~~ _____ the VL comprises Cys at L5;
 - c) ~~iii)~~ _____ 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.~~
- ~~33. Aiv)~~ _____ the scFv comprising is in the VH-L-VL orientation;
- m) _____ a VH, a L and a VL, wherein:
- a) ~~i)~~ _____ the VH comprises Cys at H43;
 - b) ~~ii)~~ _____ the VL comprises Cys at L3;
 - c) ~~iii)~~ _____ 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.~~

~~34. Aiv) the scFv comprising is in the VH-L-VL orientation;~~

n) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H40;~~

~~b) ii) the VL comprises Cys at L100;~~

~~c) iii) 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.~~

~~35. Aiv) the scFv comprising is in the VH-L-VL orientation;~~

o) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H40;~~

~~b) ii) the VL comprises Cys at L102;~~

~~c) iii) 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. Aiv) the scFv comprising is in the VH-L-VL orientation;~~

p) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H40;~~

~~b) ii) the VL comprises Cys at L5;~~

~~c) iii) 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. Aiv) the scFv comprising is in the VH-L-VL orientation;~~

q) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H40;~~

~~b) ii) the VL comprises Cys at L3;~~

~~c) iii) 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. Aiv) the scFv comprising is in the VH-L-VL orientation;~~

r) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H46;~~

~~b) ii) the VL comprises Cys at L100;~~

~~c) iii) 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. Aiv) the scFv comprising is in the VH-L-VL orientation;~~

s) a VH, a L and a VL, wherein:

~~a) i) the VH comprises Cys at H46;~~

~~b)-ii)~~ the VL comprises Cys at L102;

~~c)-iii)~~ 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. Aiv)~~ the scFv comprising is in the VH-L-VL orientation;

t) a VH, a L and a VL, wherein:

~~a)-i)~~ the VH comprises Cys at H46;

~~b)-ii)~~ the VL comprises Cys at L5;

~~c)-iii)~~ 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. Aiv)~~ the scFv comprising is in the VH-L-VL orientation;

or

u) a VH, a L and a VL, wherein:

~~a)-i)~~ the VH comprises Cys at H46;

~~b)-ii)~~ the VL comprises Cys at L3;

~~c)-iii)~~ the L comprises an amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6 or 7; and ~~d)~~

~~iv)~~ the scFv is in the VH-L-VL orientation.

~~42.~~

~~6.~~ The scFv of any one of claims ~~21-41~~ to 5, wherein:

~~a)~~ the L comprises the amino acid sequence of SEQ ID NO: ~~3~~;

~~43. The scFv of any one of claims 21-41, wherein b)~~ the L comprises the amino acid sequence of SEQ ID NO: ~~6~~; or

~~44. The scFv of any one of claims 21-41, wherein c)~~ the L comprises the amino acid sequence of SEQ ID NO: 7.

~~45.~~

7. The scFv of any one claims ~~1-44~~ to 6, wherein the scFv is conjugated to a second molecule; optionally wherein:

~~46. The scFv of claim 45, wherein a)~~ the second molecule is a half-life extending moiety;

~~47. The scFv of claim 46; optionally 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 b)~~ the second molecule is:

~~i)~~ a cytotoxic agent or;

ii) a detectable label;

~~49. The scFv of claim 48, wherein the second molecule is iii) an antibody or a fragment thereof.~~

~~50. The scFv of claim 49; optionally wherein, the scFv and the antibody or the fragment thereof bind distinct antigens; or~~

~~51. The scFv of claim 45, wherein the second molecule is a iv) chimeric antigen receptor (CAR).~~

~~52.~~

8. A pharmaceutical composition comprising the scFv of any one of claims 1-~~51~~ to 7 and a pharmaceutically acceptable carrier.

~~53.~~

9. A polynucleotide encoding the scFv of any one of claims 1-~~44~~ to 7.

~~54.~~

10. A vector comprising the polynucleotide of claim ~~53~~ 9.

~~55.~~

11. A host cell comprising the vector of claim ~~54~~ 10.

~~12. ~~56.~~ A method of producing the scFv of any one of claims 1-~~44~~ to 7, comprising culturing the host cell of claim ~~55~~ 11 in conditions that the scFv is produced, and purifying the scFv.~~

~~57. The method of claim 56; optionally wherein, the host cell is a prokaryotic cell.~~

~~58. The method of claim 56, wherein the host cell is an or a eukaryotic cell.~~

~~59.~~

13. An anti-idiotypic antibody that binds to the scFv of any one of claims 1-~~44~~ to 7.

~~60.~~

14. A kit comprising the scFv of any one of claims 1-~~44~~ to 7.

~~61.~~

15. A multispecific molecule comprising the scFv of any one of claims 1 to ~~44~~ 7.

~~62.~~

16. The multispecific molecule of claim ~~61~~ 15, wherein the multispecific molecule comprises:

a) an antibody or an antibody fragment; or

~~63. The multispecific molecule of claim 61, wherein the multispecific protein comprises b) an Ig constant region or a fragment of the Ig constant region.~~

~~64. The multispecific protein of claim 63; optionally wherein, the fragment of the Ig constant region comprises a Fe region.~~

~~65. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises:~~
~~i) a Fc region;~~
~~ii) 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:~~
~~iii) a CH3 domain;~~

~~iv) a CH2 domain and a CH3 domain;~~

~~68. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises:~~
~~v) at least a portion of a hinge, the CH2 domain and the CH3 domain; or~~

~~69. The multispecific molecule of claim 63, wherein the fragment of the Ig constant region comprises:~~
~~vi) the hinge, the CH2 domain and the CH3 domain.~~

~~70.~~

~~17. The multispecific molecule of any one of claims 63-69,~~ claim 16, wherein the scFv:
a) is conjugated to the N-terminus of the Ig constant region or to the N-terminus of the fragment of the Ig constant region; or

~~71. The multispecific molecule of any one of claims 63-69, wherein the scFv~~ b) 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.~~

~~18. The multispecific molecule of any one of claims 63-71,~~ claim 16 or claim 17, wherein:
a) 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~~ b) 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 FcγR.

~~74. The multispecific molecule of claim 73,~~ FcγR; optionally wherein, the at least one mutation that results in reduced binding of the multispecific molecule to FcγR FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/

P238 S/H268 A/V 309L/A330 S/P331 S P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331 A/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 c) 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 FcγR.~~

~~76. The multispecific molecule of claim 75, FcγR; optionally wherein, the at least one mutation that results in enhanced binding of the multispecific molecule to FcγR FcγR 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 FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.~~

~~78. The multispecific molecule of any one of claims 63-72, wherein d) 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; optionally 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; or~~

~~80. The multispecific molecule of any one of claims 63-72, wherein e) 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; optionally 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.~~

~~19. The multispecific molecule of any one of claims 61-81 claim 18, wherein the multispecific molecule is bispecific FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.~~

~~83.~~

~~20. The multispecific molecule of any one of claims 61-81 15 to 19, wherein:~~

~~a) the multispecific molecule is bispecific;~~

b) _____ the multispecific molecule is trispecific; or

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

~~21.85.~~ A pharmaceutical composition comprising the multispecific molecule of any one of claims ~~61-84~~15 to 20 and a pharmaceutically acceptable carrier.

~~22.86.~~ A heterologous molecule comprising the scFv of any one of claims ~~1-44.~~ to 7.
~~87.~~

23. _____ The heterologous molecule of claim ~~86~~22, wherein the scFv is conjugated to a second protein, a polynucleotide, a therapeutic agent, a cytotoxic agent or a detectable label; optionally wherein, the second protein is:

~~88. The heterologous molecule of claim 87, wherein the second protein is a) an antibody or a fragment thereof;~~

~~89. The heterologous molecule of claim 87, wherein the second protein is b) an alternative scaffold;~~ or

~~90. The heterologous molecule of claim 87, wherein the second protein is c) a chimeric antigen receptor (CAR) or a fragment thereof.~~

~~91.~~

24. _____ The heterologous molecule of ~~any one of claims 86-90~~claim 22 or claim 23, wherein the heterologous molecule is:

a) _____ monospecific;

~~92. The heterologous molecule of any one of claims 86-90, wherein the heterologous molecule is multi-specific.~~

~~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 b)~~

_____ multispecific;

c) _____ bispecific;

d) _____ trispecific; or

e) _____ tetraspecific.

~~96.~~

25. _____ A pharmaceutical composition comprising the heterologous molecule of any one of claims ~~86-95~~22 to 24 and a pharmaceutically acceptable carrier.

97.

26. A process for preparing a stabilized scFv, comprising:

a)

- (i) providing a heavy chain variable region (VH) and a light chain variable region (VL) that form an antigen binding domain;
- (ii) providing a linker (L) that comprises or is engineered to comprise a first L Cys;
- (iii) engineering the VH to comprise a VH Cys at a structurally conserved surface exposed VH framework residue position; and
- (iv) 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:~~

~~b)~~

- ~~(i) providing a VH and a VL that form an antigen binding domain;~~
- ~~(ii) providing a L that comprises or is engineered to comprise a second L Cys;~~
- ~~(iii) engineering the VL to comprise a VL Cys at a structurally conserved surface exposed VL framework residue position; and~~
- ~~(iv) forming a disulfide bond between the VL Cys and the second L Cys to prepare the stabilized scFv; or~~

~~99. A process for preparing a stabilized scFv, comprising:~~

~~providing a VH and a VL that form an antigen binding domain; or~~

~~c)~~

- ~~(i) providing a L that comprises or is engineered to comprise a first L Cys and a second L Cys;~~
- ~~(ii) engineering the VH to comprise a VH Cys at a structurally conserved surface exposed VH framework residue position;~~
- ~~(iii) engineering the VL to comprise a VL Cys at a structurally conserved surface exposed VL framework residue position; and~~
- ~~(iv) 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.

~~27.~~ The process of ~~any one of claims 97-99~~ claim 26, wherein the stabilized scFv:

a) is the scFv of any one of claims 1-44, to 7; or

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

~~102.~~

28. A process for preparing a stabilized scFv, comprising:

a)- providing a polynucleotide encoding a VH, a L and a VL, wherein i-

i. the VH comprises Cys at H105 and the VL comprises Cys at L42; ~~ii.~~

ii. the VH comprises Cys at H43 and the VL comprises Cys at a L100; ~~iii.~~

iii. the VH comprises Cys at H3 and the VL comprises Cys at L3; ~~iv.~~

iv. the VH comprises Cys at H3 and the VL comprises Cys at L5; ~~v.~~

v. the VH comprises Cys at H3 and the VL comprises Cys at L39; ~~vi.~~

vi. the VH comprises Cys at H3 and the VL comprises Cys at L42; ~~vii.~~

vii. the VH comprises Cys at H3 and the VL comprises Cys at L45; ~~viii.~~

viii. the VH comprises Cys at H3 and the VL comprises Cys at L100; ~~ix.~~

ix. the VH comprises Cys at H3 and the VL comprises Cys at L102; ~~x.~~

x. the VH comprises Cys at H5 and the VL comprises Cys at L3; ~~xi.~~

xi. the VH comprises Cys at H5 and the VL comprises Cys at L5; ~~xii.~~

xii. the VH comprises Cys at H5 and the VL comprises Cys at L39; ~~xiii.~~

xiii. the VH comprises Cys at H5 and the VL comprises Cys at L42; ~~xiv.~~

xiv. the VH comprises Cys at H5 and the VL comprises Cys at L45; ~~xv.~~

xv. the VH comprises Cys at H5 and the VL comprises Cys at L100; ~~xvi.~~

xvi. the VH comprises Cys at H5 and the VL comprises Cys at L102; ~~xvii.~~

xvii. the VH comprises Cys at H40 and the VL comprises Cys at L3; ~~xviii.~~

xviii. the VH comprises Cys at H40 and the VL comprises Cys at L5; ~~xix.~~

xix. the VH comprises Cys at H40 and the VL comprises Cys at L39; ~~xx.~~

xx. the VH comprises Cys at H40 and the VL comprises Cys at L42; ~~xxi.~~

xxi. the VH comprises Cys at H40 and the VL comprises Cys at L45; ~~xxii.~~

xxii. the VH comprises Cys at H40 and the VL comprises Cys at L100; ~~xxiii.~~

xxiii. the VH comprises Cys at H40 and the VL comprises Cys at L102; ~~xxiv.~~

xxiv. the VH comprises Cys at H43 and the VL comprises Cys at L3; ~~xxv.~~

xxv. the VH comprises Cys at H43 and the VL comprises Cys at L5; ~~xxvi.~~

xxvi. the VH comprises Cys at H43 and the VL comprises Cys at L39; ~~xxvii.~~

~~xxvii.~~ the VH comprises Cys at H43 and the VL comprises Cys at L42; ~~xxviii.~~
~~xxviii.~~ the VH comprises Cys at H43 and the VL comprises Cys at L45; ~~xxix.~~
~~xxix.~~ the VH comprises Cys at H43 and the VL comprises Cys at L102; ~~xxx.~~
~~xxx.~~ the VH comprises Cys at H46 and the VL comprises Cys at L3; ~~xxxi.~~
~~xxxi.~~ the VH comprises Cys at H46 and the VL comprises Cys at L5;
~~xxxii.~~ ~~xxxii.~~ the VH comprises Cys at H46 and the VL comprises Cys at L39;
~~xxxiii.~~ ~~xxxiii.~~ the VH comprises Cys at H46 and the VL comprises Cys at L42;
~~xxxiv.~~ ~~xxxiv.~~ the VH comprises Cys at H46 and the VL comprises Cys at L45;
~~xxxv.~~ ~~xxxv.~~ the VH comprises Cys at H46 and the VL comprises Cys at L100;
~~xxxvi.~~
~~xxxvi.~~ the VH comprises Cys at H46 and the VL comprises Cys at L102; ~~xxxvii.~~
~~xxxvii.~~ the VH comprises Cys at H105 and the VL comprises Cys at L3; ~~xxxviii.~~
~~xxxviii.~~ the VH comprises Cys at H105 and the VL comprises Cys at L5;
~~xxxix.~~ ~~xxxix.~~ the VH comprises Cys at H105 and the VL comprises Cys at L39;
~~xl.~~ ~~xl.~~ the VH comprises Cys at H105 and the VL comprises Cys at L45; ~~xli.~~
~~xli.~~ the VH comprises Cys at H105 and the VL comprises Cys at L100; or ~~xlii.~~
~~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.~~

29. The method of claim ~~102~~28, wherein the host cell is a prokaryotic cell.

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

~~105.~~

30. An isolated single chain variable fragment (scFv) comprising:

- a) a heavy chain variable region (VH), a means for linking (L) and a light chain variable region (VL), wherein the scFv comprises:
~~a)-(i)~~ (i) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
~~b)-(ii)~~ (ii) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
~~c)-(iii)~~ (iii) 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 b)~~ a means for antigen binding, a linker (L) and a light chain variable region (VL), wherein the scFv comprises:

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

~~107. An isolated single chain variable fragment (scFv) comprising c)~~ a heavy chain variable region (VH), a linker (L) and a means for antigen binding, wherein the scFv comprises:

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

31. A multispecific molecule comprising:

a) 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)-(i)~~ a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- ~~b)-(ii)~~ a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- ~~c)-(iii)~~ 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 b)~~ 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)~~ (i) a first disulfide bond between a structurally conserved surface exposed antigen binding means cysteine (Cys) and a first L Cys;
- ~~b)~~ (ii) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- ~~c)~~ (iii) 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; or

~~110. A multispecific molecule comprising c)~~ 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)~~ (i) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- ~~b)~~ (ii) a second disulfide bond between a structurally conserved surface exposed antigen binding means Cys and a second L Cys; or
- ~~c)~~ (iii) 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.~~

32. A heterologous molecule comprising a single chain variable fragment (scFv) comprising:

a) a heavy chain variable region (VH), a means for linking (L) and a light chain variable region (VL), wherein the scFv comprises:

- ~~a)~~ (i) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;
- ~~b)~~ (ii) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or
- ~~c)~~ (iii) 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 b)~~ a means for antigen binding, a linker (L) and a light chain variable region (VL), wherein the scFv comprises:

a)-(i) a first disulfide bond between a structurally conserved surface exposed antigen binding means cysteine (Cys) and a first L Cys;

b)-(ii) a second disulfide bond between a structurally conserved surface exposed VL Cys and a second L Cys; or

c)-(iii) 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; or

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

a)-(i) a first disulfide bond between a structurally conserved surface exposed VH cysteine (Cys) and a first L Cys;

b)-(ii) a second disulfide bond between a structurally conserved surface exposed antigen binding means Cys and a second L Cys; or

c)-(iii) 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. 33.~~ A means for encoding the scFv of ~~any one of claims 105-113~~ claim 31 or claim 32.

~~115.~~

~~34.~~ A means for replicating the vector of ~~the 114~~ claim 33.

~~116.~~

~~35.~~ A composition comprising a means:

a) for stabilizing a scFv; or

~~117. A composition comprising a means b)~~ for increasing thermostability of a scFv;

~~118. The composition of claim 116 or 117, optionally~~ 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.~~

36. A multispecific molecule comprising:

a) a means for stabilizing a scFv; or

~~120. A multispecific molecule comprising~~ b) a means for increasing thermostability of a scFv;

~~121. The multispecific molecule of claim 119 or 120, wherein~~ optionally 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.~~

37. A heterologous molecule comprising:

a) a means for stabilizing a scFv; or

~~123. A heterologous molecule comprising~~ b) a means for increasing thermostability of a scFv;

~~124. The heterologous molecule of claim 122 or 123, wherein~~ optionally 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.~~

38. A means for producing:

a) the composition of ~~any one of claims 116-118.~~ claim 35;

~~126. A means for producing~~ b) the multispecific molecule of ~~any one of claims 119-121.~~ claim 36; or

~~127. A means for producing~~ c) the heterologous molecule of ~~any one of claims 122-124.~~ claim 37.