**Claims**

1. An isolated protein comprising an immunoglobulin variable region comprising:

(i) at least two cysteine residues positioned within framework region (FR) 2, wherein if at least two of the cysteine residues in FR2 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR2; and/or

(ii) at least two cysteine residues positioned within framework region (FR) 3, wherein if at least two of the cysteine residues in FR3 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR3.

2. An isolated protein comprising an immunoglobulin heavy chain variable region ($V_H$) and an immunoglobulin light chain variable region ($V_L$), wherein at least one of the variable regions comprises:

(i) at least two cysteine residues positioned within framework region (FR) 2, wherein if at least two of the cysteine residues in FR2 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR2; and/or

(ii) at least two cysteine residues positioned within framework region (FR) 3, wherein if at least two of the cysteine residues in FR3 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR3.

3. The protein according to claim 1 or 2, wherein the disulphide bond is present under non-reducing conditions.

4. The protein according to any one of claims 1 to 3, wherein the cysteine residues within FR2 are positioned between CDR1 and CDR2, and/or the cysteine residues within FR3 are positioned between CDR2 and CDR3.

5. The protein according to any one of claims 2 to 4, wherein the cysteine residues are within a $V_H$ and the cysteine residues within FR2 are positioned between residues 36 to 49 numbered according to the Kabat numbering system, and/or and the cysteine residues within FR3 are positioned between residues 66 to 94 numbered according to the Kabat numbering system.

6. The protein according to claim 5, wherein the cysteine residues within FR2 are positioned between residues 39 to 45 numbered according to the Kabat numbering system, and/or the cysteine residues within FR3 are positioned between residues 68 to 86 numbered according to the Kabat numbering system.

7. The protein according to claim 5 or 6, wherein the cysteine residues within FR2 are positioned between residues 39 to 45 numbered according to the Kabat numbering system, and/or wherein the cysteine residues within FR3 are positioned between residues 68 to 81 numbered according to the Kabat numbering system.

8. The protein according to any one of claims 5 to 7, wherein the cysteine residues are positioned within FR3 between residues 68 to 81 numbered according to the Kabat numbering system.

9. The protein according to any one of claims 5 to 8, wherein the cysteine resides are positioned at:

(i) positions 39 and 43 of FR2 numbered according to the Kabat numbering system;

(ii) positions 39 and 45 of FR2 numbered according to the Kabat numbering system;

(iii) positions 70 and 79 of FR3 numbered according to the Kabat numbering system; and/or

(iv) positions 72 and 75 of FR3 numbered according to the Kabat numbering system.

10. The protein according to any one of claims 2 to 4, wherein the cysteine residues are within a $V_L$ and the cysteine residues within FR2 are positioned between residues 35 to 49 numbered according to the Kabat numbering system, and/or the cysteine residues positioned within FR3 are positioned between residues 57 to 88 numbered according to the Kabat numbering system.

11. The protein according to claim 10, wherein the cysteine residues within FR2 are positioned between residues 38 to 44 numbered according to the Kabat numbering system, and/or the cysteine residues within FR3 are positioned between residues 63 to 82 numbered according to the Kabat numbering system.

12. The protein according to claim 10 or 11, wherein the cysteine residues within FR3 are positioned between residues 63 to 74 numbered according to the Kabat numbering system.

13. The protein according to any one of claims 10 to 12, wherein the cysteine residues are positioned within FR3 between residues 63 to 74 numbered according to the Kabat numbering system.

14. The protein according to any one of claims 10 to 13 wherein the cysteine resides are positioned at:

(i) positions 38 and 42 of FR2 numbered according to the Kabat numbering system;

(ii) positions 38 and 44 of FR2 numbered according to the Kabat numbering system; and/or

(iii) positions 65 and 72 of FR3 numbered according to the Kabat numbering system.

15. The protein according to any one of claims 1 to 14 that specifically binds to human epidermal growth factor (Her) 2, tumor associated glycoprotein TAG72, MUC1 or prostate specific membrane antigen (PSMA).

16. The protein according to any one of claims 1 to 15, wherein the protein comprises a $V_H$ and a $V_L$ comprising sequences at least about 80% identical to a $V_H$ and a $V_L$ sequence set forth in any one or more of SEQ ID NOs: 59, 61, 63 or 65, modified to include the two or more cysteine residues positioned within FR2 and/or FR3.

17. The protein according to claim 16 comprising a sequence at least about 80% identical to a sequence set forth in any one or more of SEQ ID NO: 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149, optionally comprising a N-terminal serine residue.

18. An isolated protein comprising a Fv comprising at least one protein according to any one of claims 2 to 17 in which at least one $V_L$ binds to at least one $V_H$ to form an antigen binding site.

19. The protein according to claim 18, wherein the $V_L$ and the $V_H$ which form the antigen binding site are in a single polypeptide chain.

20. The protein according to claim 19, which is:

(i) a single chain Fv fragment (scFv);

(ii) a dimeric scFv (di-scFv); or

(iii) at least one of (i) and/or (ii) linked to a Fc or a heavy chain constant domain ($C_H$) 2 and/or $C_H3$.

21. The protein according to claim 18, wherein the $V_L$ and $V_H$ which form the antigen binding site are in different polypeptide chains.

22. The protein according to claim 21, which is:
(i) a diabody;
(ii) a triabody; or
(iii) a tetrabody.

23. The protein according to claim 21, which is an immunoglobulin.

24. The protein according to any one of claims 1 to 23, wherein the cysteine residues are linked by a disulphide bond.

25. The protein according to any one of claims 1 to 23 comprising a compound conjugated to at least one of the cysteine residues, wherein conjugation of the compound does not prevent binding of the protein to an antigen.

26. The protein according to claim 25, wherein the compound is selected from the group consisting of a radioisotope, a detectable label, a therapeutic compound, a colloid, a toxin, a nucleic acid, a peptide, a protein, a compound that increases the half life of the protein in a subject and mixtures thereof.

27. The protein of any one of claims 1 to 26 additionally comprising at least one N-terminal threonine or serine residue.

28. The protein according to claim 27 comprising a compound conjugated to the threonine or serine residue, wherein conjugation of the compound does not prevent binding of the protein to an antigen.

29. The protein according to claim 28, wherein the compound is selected from the group consisting of a radioisotope, a detectable label, a therapeutic compound, a colloid, a toxin, a nucleic acid, a peptide, a protein, a compound that increases the half life of the protein in a subject and mixtures thereof.

30. The protein according to claim 28 or 29 comprising a first compound conjugated to at least one of the cysteine residues in FR2 and/or FR3 and a second compound conjugated to the threonine or serine residue, wherein the second compound is different to the first compound.

31. The protein of any one of claims 25, 26 or 28 to 30, wherein the compound is polyethylene glycol (PEG).

32. The protein of claim 31, wherein the PEG is monodispersed PEG.

33. The protein of claim 32, wherein the monodispersed PEG has no more than 48 ethylene glycol units.

34. The protein of claim 32 or 33, wherein the monodispersed PEG has about 24 ethylene glycol units.

35. A composition comprising the protein according to any one of claims 1 to 34 and a pharmaceutically acceptable carrier.

36. An isolated nucleic acid encoding the protein of any one of claims 1 to 23 or 27.

37. An expression construct comprising the nucleic acid according to claim 38 operably linked to a promoter.

38. An isolated cell comprising an exogenous nucleic acid according to claim 36 and/or the expression construct of claim 37 wherein the cell expresses the protein.

39. The cell according to claim 38, wherein the cell is a bacterial cell, a yeast cell, a mammalian cell or an insect cell.

40. A method for producing the protein according to any one of claims 1 to 23 or 27 the method comprising maintaining the expression construct according to claim 37 under conditions sufficient for the encoded protein to be produced.

41. The method according to claim 40 comprising culturing the cell according to claim 38 or 39 under conditions sufficient the encoded for the protein to be produced.

42. The method according to claim 40 or 41 comprising isolating the protein.

43. A method for producing the protein according to any one of claims 25, 26 or 28 to 34, the method comprising:

(i) obtaining the protein according to any one of claims 1 to 23 or 27; and

(ii) conjugating a compound to at least one of the cysteine residues in the FR2 and/or FR3 of the protein to thereby produce the protein.

44. The method according to claim 43, wherein the cysteine residues in FR2 and/or FR3 of the protein obtained at (i) are linked by a disulphide bond and the method additionally comprises reducing or otherwise breaking the disulphide bond prior to conjugating the compound to the cysteine residue(s).

45. The method according to claim 44, wherein reducing or otherwise breaking the disulphide bond generates a free thiol group in the protein and the compound has a thiol reactive group permitting conjugation of the compound to the protein.

46. The method according to any one of claims 43 to 45, wherein the protein comprises at least one N-terminal serine or threonine residue and the method additionally comprises conjugating a compound to the serine or threonine residue.

47. Use of the protein according to any one of claims 1 to 34 or the composition according to claim 35 in medicine.

48. A method of treating or preventing a condition in a subject, the method comprising administering the protein according to any one of claims 1 to 34 or the composition according to claim 35 to a subject in need thereof.

49. A method for delivering a compound to a cell, the method comprising contacting the cell with the protein according to any one of claims 25, 26 or 28 to 34 or the composition according to claim 35.

50. The method according to claim 49, wherein the cell is contacted by administering the protein or composition to a subject.

51. A method for localising or detecting an antigen in a subject, said method comprising:

(i) administering to a subject the protein according to any one of claims 25, 26 or 28 to 34 or the composition according to claim 35 for a time and under conditions sufficient for the protein to bind to an antigen, wherein the protein is conjugated to a detectable label; and

(ii) detecting or localising the detectable label *in vivo*.

52. A method for diagnosing or prognosing a condition in a subject, the method comprising contacting a sample from the subject with the protein according to any one of claims 1 to 34 or the composition according to claim 35 for a time and under conditions sufficient for the protein to bind to an antigen and form a complex and detecting the complex, wherein detection of the complex is diagnostic or prognostic of the condition in the subject.

53. The method according to claim 52, comprising determining the level of the complex, wherein an enhanced or reduced level of said complex is diagnostic or prognostic of the condition in the subject.

**Claims**

1. An isolated protein comprising an immunoglobulin variable region comprising:

(i) at least two cysteine residues positioned within framework region (FR) 2, wherein if at least two of the cysteine residues in FR2 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR2; and/or

(ii) at least two cysteine residues positioned within framework region (FR) 3, wherein if at least two of the cysteine residues in FR3 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR3.

2. An isolated protein comprising an immunoglobulin heavy chain variable region ($V_H$) and an immunoglobulin light chain variable region ($V_L$), wherein at least one of the variable regions comprises:

(i) at least two cysteine residues positioned within framework region (FR) 2, wherein if at least two of the cysteine residues in FR2 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR2; and/or

(ii) at least two cysteine residues positioned within framework region (FR) 3, wherein if at least two of the cysteine residues in FR3 are not conjugated to a compound then an intra-framework region disulphide bond is capable of forming between the cysteine residues in FR3.

3. The protein according to claim 1 or 2, wherein the disulphide bond is present under non-reducing conditions.

4. The protein according to claim 2 or 3, wherein the cysteine residues are within a $V_H$ and the cysteine residues within FR2 are positioned within one or more of the following regions:

(i) between residues 36 to 49 numbered according to the Kabat numbering system; and/or

(ii) between residues 39 to 45 numbered according to the Kabat numbering system, and/or and the cysteine residues within FR3 are positioned within one or more of the following regions:

(ii) between residues 66 to 94 numbered according to the Kabat numbering system;

(iv) between residues 68 to 86 numbered according to the Kabat numbering system; and/or

(v) between residues 68 to 81 numbered according to the Kabat numbering system.

5. The protein according to claim 4, wherein the cysteine resides are positioned at:

(i) positions 39 and 43 of FR2 numbered according to the Kabat numbering system;

(ii) positions 39 and 45 of FR2 numbered according to the Kabat numbering system;

(iii) positions 70 and 79 of FR3 numbered according to the Kabat numbering system; and/or

(iv) positions 72 and 75 of FR3 numbered according to the Kabat numbering system.

6. The protein according to claim 2 or 3, wherein the cysteine residues are within a $V_L$ and the cysteine residues within FR2 are positioned within one or more of the following regions

(i) between residues 35 to 49 numbered according to the Kabat numbering system; and/or

(ii) between residues 38 to 44 numbered according to the Kabat numbering system, and/or the cysteine residues positioned within FR3 are positioned within one or more of the following regions:

(iii) between residues 57 to 88 numbered according to the Kabat numbering system;

(iv) between residues 63 to 82 numbered according to the Kabat numbering system; and/or

(v) between residues 63 to 74 numbered according to the Kabat numbering system.

7. The protein according to claim 6 wherein the cysteine resides are positioned at:

(i) positions 38 and 42 of FR2 numbered according to the Kabat numbering system;

(ii) positions 38 and 44 of FR2 numbered according to the Kabat numbering system; and/or

(iii) positions 65 and 72 of FR3 numbered according to the Kabat numbering system.

8. The protein according to any one of claims 1 to 7 that specifically binds to human epidermal growth factor (Her) 2, tumor associated glycoprotein TAG72, MUC1 or prostate specific membrane antigen (PSMA).

9. The protein according to any one of claims 1 to 8, wherein the protein comprises:

(i) a $V_H$ and a $V_L$ comprising sequences at least about 80% identical to a $V_H$ and a $V_L$ sequence set forth in any one or more of SEQ ID NOs: 59, 61, 63 or 65, modified to include the two or more cysteine residues positioned within FR2 and/or FR3; or

(ii) a sequence at least about 80% identical to a sequence set forth in any one or more of SEQ ID NO: 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149, optionally comprising a N-terminal serine residue, wherein the protein comprises the two or more cysteine residues positioned within FR2 and/or FR3.

10. An isolated protein comprising a Fv comprising at least one protein according to any one of claims 2 to 9 in which at least one $V_L$ binds to at least one $V_H$ to form an antigen binding site.

11. The protein according to claim 10, wherein the $V_L$ and the $V_H$ which form the antigen binding site are in a single polypeptide chain, or wherein the $V_L$ and $V_H$ which form the antigen binding site are in different polypeptide chains.

12. The protein according to claim 11, wherein if the $V_L$ and the $V_H$ are in a single polypeptide chain, the protein is:

(i) a single chain Fv fragment (scFv);

(ii) a dimeric scFv (di-scFv); or

(iii) at least one of (i) and/or (ii) linked to a Fc or a heavy chain constant domain ($C_H$) 2 and/or $C_H$3,

or if $V_L$ and $V_H$ are in different polypeptide chains, the protein is:

(iv) a diabody;

(v) a triabody;

(vi) a tetrabody; or

(vii) an immunoglobulin.

13. The protein according to any one of claims 1 to 12, wherein the cysteine residues are linked by a disulphide bond.

14. The protein according to any one of claims 1 to 12 comprising a compound conjugated to at least one of the cysteine residues, wherein conjugation of the compound does not prevent binding of the protein to an antigen.

15. The protein of any one of claims 1 to 14 additionally comprising at least one N-terminal threonine or serine residue.

16. The protein according to claim 15 comprising a compound conjugated to the threonine or serine residue, wherein conjugation of the compound does not prevent binding of the protein to an antigen.

17. The protein according to claim 14 or 16, wherein the compound is selected from the group consisting of a radioisotope, a detectable label, a therapeutic compound, a colloid, a toxin, a nucleic acid, a peptide, a protein, a compound that increases the half life of the protein in a subject and mixtures thereof.

18. The protein of claim 14 or 16, wherein the compound is a physiologically acceptable polymer.

19. The protein of claim 18, wherein the physiologically acceptable polymer is polyethylene glycol (PEG).

20. The protein of claim 19, wherein the PEG is monodispersed PEG.

21. The protein according to claim 16 or 17 comprising a first compound conjugated to at least one of the cysteine residues in FR2 and/or FR3 and a second compound conjugated to the threonine or serine residue, wherein the second compound is different to the first compound.

22. A composition comprising the protein according to any one of claims 1 to 21 and a pharmaceutically acceptable carrier.

23. An isolated nucleic acid encoding the protein of any one of claims 1 to 12 or 15.

24. An expression construct comprising the nucleic acid according to claim 23 operably linked to a promoter.

25. An isolated cell comprising an exogenous nucleic acid according to claim 23 and/or the expression construct of claim 24 wherein the cell expresses the protein.

26. A method for producing the protein according to any one of claims 1 to 13 or 15 the method comprising maintaining the expression construct according to claim 23 under conditions sufficient for the encoded protein to be produced and, optionally, isolating the protein.

27. The method according to claim 26 comprising culturing the cell according to claim 24 under conditions sufficient the encoded for the protein to be produced.

28. A method for producing the protein according to any one of claims 14 or 16 to 19, the method comprising:

(i) obtaining the protein according to any one of claims 1 to 12 or 15; and

(ii) conjugating a compound to at least one of the cysteine residues in the FR2 and/or FR3 of the protein to thereby produce the protein.

29. The method according to claim 28, wherein the protein comprises at least one N-terminal serine or threonine residue and the method additionally comprises conjugating a compound to the serine or threonine residue.

30. Use of the protein according to any one of claims 1 to 21 or the composition according to claim 22 in medicine.

31. A method for delivering a compound to a cell, the method comprising contacting the cell with the protein according to any one of claims 14 or 16 to 21 or the composition according to claim 22.

32. The method according to claim 31, wherein the cell is contacted by administering the protein or composition to a subject.

33. A method for localising or detecting an antigen in a subject, said method comprising:

(i) administering to a subject the protein according to any one of claims 14 or 16 to 21 or the composition according to claim 22 for a time and under conditions sufficient for the protein to bind to an antigen, wherein the protein is conjugated to a detectable label; and

(ii) detecting or localising the detectable label *in vivo*.