

Claims

1. A multi-specific antibody, comprising:

a polypeptide chain of formula (I):

VH-CH1-(CH2)_s-(CH3)_t-X-(V1)_p; and

a polypeptide chain of formula (II):

(V3)_r-Z-VL-C_L-Y-(V2)_q;

wherein:

VH represents a heavy chain variable domain;

CH1 represents domain 1 of a heavy chain constant region;

CH2 represents domain 2 of a heavy chain constant region;

CH3 represents domain 3 of a heavy chain constant region;

X represents a bond or linker;

V1 represents a dsFcV, a dsFv, a scFv, a VH, a VL or a VHH;

V3 represents a dsFcV, a dsFv, a scFv, a VH, a VL or a VHH;

Z represents a bond or linker;

VL represents a light chain variable domain;

C_L represents a domain from a light chain constant region, such as Ckappa;

Y represents a bond or linker;

V2 represents a dsFcV, a dsFv, a scFv, a VH, a VL or a VHH;

p represents 0 or 1;

q represents 0 or 1;

r represents 0 or 1;

s represents 0;

t represents 0;

wherein when p is 0, X is absent and when q is 0, Y is absent and when r is 0, Z is absent; and

wherein when q is 0, r is 1 and when r is 0, q is 1; and

wherein the polypeptide chain of formula (II) comprises at least one dsFcV, dsFv, scFv, VH or VHH; and

wherein the polypeptide chain of formula (I) comprises a protein A binding domain; and

wherein the polypeptide chain of formula (II) does not bind protein A,

wherein the protein A binding domain comprises a VH3 domain or variant thereof which binds protein A.

2. A multi-specific antibody according to claim 1, wherein the polypeptide chain of formula **(I)** comprises one, two or three protein A binding domains.
3. A multi-specific antibody according to claim 1 or 2, wherein a protein A binding domain is present in VH and/or V1.
4. A multi-specific antibody according to any one of claims 1 to 3, wherein the polypeptide chain of formula **(I)** comprises only one protein A binding domain which is present in VH or V1.
5. A multi-specific antibody according to claim 4, wherein the polypeptide chain of formula **(I)** comprises only one protein A binding domain which is present in VH.
6. A multi-specific antibody according to claim 4, wherein the polypeptide chain of formula **(I)** comprises only one protein A binding domain which is present in V1.
7. A multi-specific antibody according to any one of claims 1 to 6, wherein V2 and/or V3 do/does not comprise a VH3 domain.
8. A multi-specific antibody according to any one of claims 1 to 6, wherein V2 and/or V3, comprise(s) or consist(s) of a VH3 domain or variant thereof which does not bind protein A.
9. A multi-specific antibody according to any one of the preceding claims, wherein p is 1.
10. A multi-specific antibody according to any one of the preceding claims, wherein q is 1.
11. A multi-specific antibody according to any one of the preceding claims, wherein r is 1.

12. A multi-specific antibody according to any one of claims 1 to 9, wherein q is 0 and r is 1.
13. A multi-specific antibody according to any one of claims 1 to 10, wherein s is 0 and t is 0, p is 1, q is 1, r is 0, and wherein V1 and V2 both represent a dsScFv.
14. A multi-specific antibody according to any one of claims 1 to 13, wherein V1 binds albumin and comprises a VH3 of sequence SEQ ID NO: 78.
15. A multi-specific antibody according to any one of the preceding claims, wherein X and/or Y and/or Z is a peptide linker.
16. A multi-specific antibody according to any one of the preceding claims, wherein V1 and/or V2 and/or V3 are/is a dsScFv or a dsFv, and wherein the light chain and heavy chain variable domains of V1 and/or the light chain and heavy chain variable domains of V2 and/or the light chain and heavy chain variable domains of V3 are linked by a disulfide bond between two engineered cysteine residues, wherein the position of the pair of cysteine residues is selected from the group comprising or consisting of: VH37 and VL95, VH44 and VL100, VH44 and VL105, VH45 and VL87, VH100 and VL50, VH100b and VL49, VH98 and VL46, VH101 and VL46, VH105 and VL43 and VH106 and VL57, numbering according to Kabat, wherein the VH and VL values are independently within a given V1 or V2 or V3.
17. A polynucleotide encoding a multi-specific antibody defined in any one of claims 1-16.
18. A vector comprising a polynucleotide defined in claim 16.
19. A host cell comprising a polynucleotide or vector of claim 17 or 18 respectively.
20. A host cell comprising at least two vectors, each vector comprising a polynucleotide encoding a different polypeptide chain of a multi-specific antibody defined in any one of claims 1-16.

21. A pharmaceutical composition comprising a multi-specific antibody according to any one of claims 1-16 and at least one excipient.

22. A multi-specific antibody according to any one of claims 1-16 or a pharmaceutical composition according to claim 21, for use in treatment.

23. A method of producing a multi-specific antibody comprising a polypeptide chain of formula **(I)** and a polypeptide chain of formula **(II)** as defined in claim 1, said method comprising:

- a) Expressing a polypeptide chain of formula **(I)** and a polypeptide chain of formula **(II)** as defined above, in a host cell, wherein the polypeptide chain of formula **(II)** is in excess over the polypeptide chain of formula **(I)**; and
- b) Recovering the composition of polypeptides expressed at step a), said composition comprising a multi-specific antibody and a LC dimer of formula **(II-II)**; and
- c) Purifying the multi-specific antibody, wherein said multi-specific antibody is purified as a dimer with one heavy chain of formula **(I)** and one associated light chain of formula **(II)**; and,
wherein the polypeptide chain of formula **(II)** comprises at least one dsscFv, dsFv, scFv, VH or VHH; and,
wherein the polypeptide chain of formula **(I)** comprises a protein A binding domain; and,
wherein the polypeptide chain of formula **(II)** does not bind protein A; and,
wherein step c) comprises subjecting the composition of polypeptides recovered at step b), following at least one purification step, to a Protein A affinity chromatography column.

24. A method of purifying a multi-specific antibody comprising a polypeptide chain of formula **(I)** and a polypeptide chain of formula **(II)** as defined in claim 1, said method comprising:

- a) Obtaining a composition of polypeptide chains of formula **(I)** and polypeptide chains of formula **(II)** as defined above, said composition comprising a multi-specific antibody, wherein the multi-specific antibody is a dimer with one heavy chain of formula **(I)** and one associated light chain of formula **(II)**; and a dimer of two light chains of formula **(II-II)**, associated together (LC dimer); and, wherein the polypeptide chain of formula **(II)** comprises at least one dsscFv, dsFv, scFv, VH or VHH; and, wherein the polypeptide chain of formula **(I)** comprises a protein A binding domain; and, wherein the polypeptide chain of formula **(II)** does not bind protein A; and
- b) Loading the composition obtained in step a), onto a protein A affinity column, such that the multi-specific antibody is retained on the column whilst the LC dimer does not bind to the column; and
- c) Washing the protein A affinity column; and,
- d) Eluting the multi-specific antibody; and,
- e) Recovering the multi-specific antibody.