preferred form of multispecific antibody proteins, and methods for eliminating disulfide heterogeneity of such recombinant antibody proteins.

Solution to Problem

[0014]

Antigen-binding molecules which are capable of binding to multiple different antigens (e.g., CD3 on T cells, and CD137 on T cells, NK cells, DC cells, and/or the like), but do not non-specifically crosslink two or more immune cells such as T cells are provided. Such multispecific antigen-binding molecules are capable of modulating and/or activating an immune response while circumventing the cross-linking between different cells (e.g., different T cells) resulting from the binding of a conventional multispecific antigen-binding molecule to antigens expressed on the different cells, which is considered to be responsible for adverse reactions when the multispecific antigen-binding molecule is used as a drug.

[0014a]

In one aspect, the present disclosure provides a method for producing a preparation of a multispecific antigen binding molecule, wherein the multispecific antigen binding molecule comprises:

(a) a first antigen-binding moiety and a second antigen-binding moiety, each of the first antigen-binding moiety and the second antigen-binding moiety is capable of binding to CD3 and CD137 but does not bind both CD3 and CD137 at the same time; and

(b) a third antigen-binding moiety capable of binding to a third antigen different from the first and the second antigen, preferably an antigen expressed on a cancer cell/tissue,wherein each of the first antigen-binding moiety and the second antigen-binding moiety comprises at least one cysteine residue (via mutation, substitution or insertion) in a CH1 region, said at least one cysteine residue is capable of forming at least one disulfide bond between the first antigen-binding moiety and the second antigen-binding moiety in the CH1 region;

wherein said method comprises contacting the preparation with a reducing agent, wherein the reducing agent is TCEP,

wherein the first antigen-binding moiety and the second antigen-binding moiety each comprises an antibody variable region comprising any one of (a1) to (a15) below:

(a1) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 17, the heavy chain CDR 2 of SEQ ID NO: 31, the heavy chain CDR 3 of SEQ ID NO: 45, the light chain CDR 1 of SEQ ID NO: 64, the light chain CDR 2 of SEQ ID NO: 69 and the light chain CDR 3 of SEQ ID NO: 74;
(a2) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 18, the heavy chain CDR 2 of SEQ ID NO: 32, the heavy chain CDR 3 of SEQ ID NO: 46, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a3) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 19, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy chain CDR 3 of SEQ ID NO: 47, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a4) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 19, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy chain CDR 3 of SEQ ID NO: 47, the light chain CDR 1 of SEQ ID NO: 65, the light chain CDR 2 of SEQ ID NO: 70 and the light chain CDR 3 of SEQ ID NO: 75;
(a5) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 20, the heavy chain CDR 2 of SEQ ID NO: 34, the heavy chain CDR 3 of SEQ ID NO: 48, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a6) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 22, the heavy chain CDR 2 of SEQ ID NO: 36, the heavy chain CDR 3 of SEQ ID NO: 50, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a7) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 23, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 51, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a8) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 23, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 51, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;

(a9) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 24, the heavy chain CDR 2 of SEQ ID NO: 38, the heavy chain CDR 3 of SEQ ID NO: 52, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a10) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 25, the heavy chain CDR 2 of SEQ ID NO: 39, the heavy chain CDR 3 of SEQ ID NO: 53, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;
(a11) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 26, the heavy chain CDR 2 of SEQ ID NO: 40, the heavy chain CDR 3 of SEQ ID NO: 54, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;
(a12) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 26, the heavy chain CDR 2 of SEQ ID NO: 40, the heavy chain CDR 3 of SEQ ID NO: 54, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a13) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 27, the heavy chain CDR 2 of SEQ ID NO: 41, the heavy chain CDR 3 of SEQ ID NO: 55, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a14) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 28, the heavy chain CDR 2 of SEQ ID NO: 42, the heavy chain CDR 3 of SEQ ID NO: 56, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73; and
(a15) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 82, the heavy chain CDR 2 of SEQ ID NO: 83, the heavy chain CDR 3 of SEQ ID NO: 84, the light chain CDR 1 of SEQ ID NO: 65, the light chain CDR 2 of SEQ ID NO: 70 and the light chain CDR 3 of SEQ ID NO: 75.

[0015] In one example, the antigen-binding molecule of the present disclosure provides new antigen-binding molecules which have very unique structure format(s), which improve or enhance the efficacy of the multispecific antigen-binding molecules. The new antigen-binding molecules with unique structure formats provide the increased number of antigen-binding domains to give the increased valency and/or specificities to respective antigens on effector cells and target cells with the reduced unwanted adverse effects.

[0016] In a further example, one of the antigen-binding molecules having such new unique structure format of the present disclosure comprises at least two first and second antigen-binding moieties (e.g., Fab domains) which are linked together (e.g., via Fc, disulfide bond, linker, or the like), each of which binds to a first and/or second antigen on effector cells (e.g., immune cells such as T cells, NK cells, DC cells, or the like), and further comprises a third (and optionally a fourth) antigen-binding domain(s) which is linked to any one of the first or second antigen-binding moieties, which bind(s) to the third antigen on target cells (e.g., tumor cells).

[0017] In a further example, one of the antigen-binding molecules having such new unique structure format of the present disclosure comprises at least a first antigen-binding moiety and a second antigen-binding moiety (e.g., Fab domains) which are linked together (e.g., via Fc, disulfide bond, linker, or the like), each of which binds to a first and/or second antigen on effector cells (e.g., immune cells such as T cells, NK cells, DC cells, or the like), and further comprises a third (and optionally the fourth) antigen-binding moiety(s) which is linked to any one of the first or second antigen-binding moiety, which bind(s) to the third antigen on target cells (e.g., tumor cells), wherein the first and second antigen-binding moieties (e.g. Fab domains) capable of binding to the first antigen and/or a second antigen comprise at least one amino acid mutation(s)

[CLAIMS]
[Claim 1]
A method for producing a preparation of a multispecific antigen binding molecule, wherein the multispecific antigen binding molecule comprises:
(a) a first antigen-binding moiety and a second antigen-binding moiety, each of the first antigen-binding moiety and the second antigen-binding moiety is capable of binding to CD3 and CD137 but does not bind both CD3 and CD137 at the same time; and
(b) a third antigen-binding moiety capable of binding to a third antigen different from the first and the second antigen, preferably an antigen expressed on a cancer cell/tissue,wherein each of the first antigen-binding moiety and the second antigen-binding moiety comprises at least one cysteine residue (via mutation, substitution or insertion)in a CH1 region,; said at least one cysteine residue is capable of forming at least one disulfide bond between the first antigen-binding moiety and the second antigen-binding moiety in the CH1 region;
wherein said method comprises contacting the preparation with a reducing agent, wherein the reducing agent is TCEP,
wherein the first antigen-binding moiety and the second antigen-binding moiety each comprises an antibody variable region comprising any one of (a1) to (a15) below:
(a1) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 17, the heavy chain CDR 2 of SEQ ID NO: 31, the heavy chain CDR 3 of SEQ ID NO: 45, the light chain CDR 1 of SEQ ID NO: 64, the light chain CDR 2 of SEQ ID NO: 69 and the light chain CDR 3 of SEQ ID NO: 74;
(a2) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 18, the heavy chain CDR 2 of SEQ ID NO: 32, the heavy chain CDR 3 of SEQ ID NO: 46, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a3) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 19, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy chain CDR 3 of SEQ ID NO: 47, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a4) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 19, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy chain CDR 3 of SEQ ID NO: 47, the light chain CDR 1 of SEQ ID NO: 65, the light chain CDR 2 of SEQ ID NO: 70 and the light chain CDR 3 of SEQ ID NO: 75;
(a5) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 20, the heavy chain CDR 2 of SEQ ID NO: 34, the heavy chain CDR 3 of SEQ ID NO: 48, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a6) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 22, the heavy chain CDR 2 of SEQ ID NO: 36, the heavy chain CDR 3 of SEQ ID NO: 50, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a7) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 23, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 51, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a8) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 23, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 51, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;

(a9) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 24, the heavy chain CDR 2 of SEQ ID NO: 38, the heavy chain CDR 3 of SEQ ID NO: 52, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a10) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 25, the heavy chain CDR 2 of SEQ ID NO: 39, the heavy chain CDR 3 of SEQ ID NO: 53, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;
(a11) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 26, the heavy chain CDR 2 of SEQ ID NO: 40, the heavy chain CDR 3 of SEQ ID NO: 54, the light chain CDR 1 of SEQ ID NO: 66, the light chain CDR 2 of SEQ ID NO: 71 and the light chain CDR 3 of SEQ ID NO: 76;
(a12) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 26, the heavy chain CDR 2 of SEQ ID NO: 40, the heavy chain CDR 3 of SEQ ID NO: 54, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a13) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 27, the heavy chain CDR 2 of SEQ ID NO: 41, the heavy chain CDR 3 of SEQ ID NO: 55, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73;
(a14) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 28, the heavy chain CDR 2 of SEQ ID NO: 42, the heavy chain CDR 3 of SEQ ID NO: 56, the light chain CDR 1 of SEQ ID NO: 63, the light chain CDR 2 of SEQ ID NO: 68 and the light chain CDR 3 of SEQ ID NO: 73; and
(a15) the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 82, the heavy chain CDR 2 of SEQ ID NO: 83, the heavy chain CDR 3 of SEQ ID NO: 84, the light chain CDR 1 of SEQ ID NO: 65, the light chain CDR 2 of SEQ ID NO: 70 and the light chain CDR 3 of SEQ ID NO: 75.
[Claim 2]
The method of claim 1, wherein each of the first antigen-binding moiety and the second antigen-binding moiety comprises one cysteine residue (via mutation, substitution or insertion) at position 191 according to EU numbering in the CH1 region which is capable of forming one disulfide bond between the CH1 region of the first antigen-binding moiety and the CH1 region of the second antigen-binding moiety.
[Claim 3]
The method of claim 2, wherein said multispecific antigen binding molecule preparation (before contacting with the reducing agent) comprises two or more structural isoforms which differ by at least one disulfide bond formed between amino acid residues located at the position 191 in the CH1 region (EU numbering), and wherein the contacting with reducing agent preferentially enriches or increases the population of a structural isoform having at least one disulfide bond formed between amino acid residues at the position 191 in the CH1 region (EU numbering).
[Claim 4]
The method of any one of claims 1 to 3, wherein the pH of said reducing agent contacting with the multispecific antigen binding molecule is from 3 to 10, preferably pH 6-8.
[Claim 5]
The method of any one of claims 1 to 4, wherein the concentration of the reducing agent is from 0.01 mM to 100 mM.
[Claim 6]

The method of any one of claims 1 to 5, wherein the concentration of the multispecific antigen binding molecule is from 0.1 mg/ml to 50 mg/ml, preferably 10 mg/ml.
[Claim 7]
The method of any one of claims 1 to 6, further comprising a step of promoting re-oxidization of cysteine disulfide bonds, preferably by removing the reducing agent, preferably by dialysis or buffer exchange.
[Claim 8]
The method of any one of claims 1 to 7, wherein the first antigen-binding moiety and the second antigen-binding moiety each comprises an antibody variable region comprising any one of (a1) to (a15) below:
(a1) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 3, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 59;
(a2) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 4, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a3) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a4) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 60;
(a5) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a6) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 8, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a7) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 9, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a8) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 9, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 61;
(a9) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a10) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 11, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 61;
(a11) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 12, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 61;
(a12) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 12, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a13) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 13, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58;
(a14) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 14, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 58; and
(a15) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 81, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 60.
[Claim 9]
The method of any one of claims 1 to 8, wherein the third antigen-binding moiety is capable of binding to DLL3, preferably human DLL3.
[Claim 10]
The method of claim 9, wherein the third antigen-binding moiety capable of binding to DLL3 comprises an antibody variable region comprising the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 233, the heavy chain CDR 2 of SEQ ID NO: 234, the heavy chain CDR 3 of SEQ ID NO: 235, the light chain CDR 1 of SEQ ID NO: 237, the light chain CDR 2 of SEQ ID NO: 238 and the light chain CDR 3 of SEQ ID NO: 239
[Claim 11]

The method of claim 10, wherein the third antigen-binding moiety capable of binding to DLL3 comprises an antibody variable region comprising: a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 232, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 236.

[Claim 12]

The method of any one of claims 1 to 11, wherein the multispecific antigen binding molecule further comprises a Fc domain.