

not appropriate as a basis for easily developing recombinant, tri-or tetraspecific antibodies against three or four antigens starting from two antibodies against the first and the second antigen, as either the heavy chains of these antibodies and/or the identical light chains have to be optimized first and then further antigen binding peptides against the third and fourth antigen have to be added.

WO 2006/093794 relates to heterodimeric protein binding compositions. WO 99/37791 describes multipurpose antibody derivatives. Morrison, S.L., et al., J. Immunol. 160 (1998) 2802-2808 refers to the influence of variable region domain exchange on the functional properties of IgG.

WO 2013/02362 relates to heterodimerized polypeptides. WO 2013/12733 relates to polypeptides comprising heterodimeric Fc regions. WO 2012/131555 relates to engineered hetero-dimeric immunoglobulins. EP 2647707 relates to engineered hetero-dimeric immunoglobulins.

WO 2013/026835 relates to bispecific, Fc free antibodies with a domain crossover. WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254 and Schaefer, W. et al, PNAS, 108 (2011) 11187-1191 relate to bivalent, bispecific IgG antibodies with a domain crossover.

The multispecific antibodies with VH/VL replacement/exchange in one binding to prevent light chain mispairing (CrossMab^{VH-VL}) which are described in WO2009/080252, (see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191) clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange). However their preparation is not completely free of side products. The main side product is based on a Bence-Jones -type interaction -see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191; in Fig. S1I of the Supplement).

Therefore there is still a need for further reduction of such side products improve e.g. the yield of such bispecific antibodies.

Summary

The disclosure relates to a multispecific antibody, comprising:

wherein

5 in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147, numbering according to Kabat EU index, or the amino acid at position 213, numbering according to Kabat EU index, is substituted independently by glutamic acid (E) or aspartic acid (D).

10 The disclosure, in another aspect, relates to a multispecific antibody according to any one of the preceding claims that specifically binds to human TWEAK and that specifically binds to human IL17, wherein

A) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:24, and a variable light chain domain (VL) of SEQ ID NO:25; and

B) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:26, and a variable light chain domain (VL) of SEQ ID NO:27.

15 A further aspect of the disclosure is a method for the preparation of a multispecific antibody according to the disclosure

comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

20 a) the first light chain and the first heavy chain of the multispecific antibody of the disclosure; and

b) the second light chain and the second heavy chain of the multispecific antibody of the disclosure;

B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and

5 C) recovering said antibody molecule from said culture.

A further aspect of the disclosure is a nucleic acid encoding the amino acid sequences of a multispecific antibody according to the disclosure.

10 A further aspect of the disclosure are expression vectors containing the nucleic acid according to the disclosure capable of expressing said nucleic acid in a host cell.

Patent Claims

1. A multispecific antibody, comprising:
 - a) a first light chain and a first heavy chain of a first antibody which specifically binds to a first antigen; and
 - b) a second light chain and a second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147, numbering according to Kabat EU index, or the amino acid at position 213, numbering according to Kabat EU index, is substituted independently by glutamic acid (E) or aspartic acid (D).

2. The multispecific antibody according to claim 1,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147, numbering according to Kabat EU index, is substituted independently by glutamic acid (E) or aspartic acid (D).

3. The multispecific antibody according to claim 1,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted independently by lysine (K) or arginine (R), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 213, numbering according to Kabat EU index, is substituted independently by glutamic acid (E) or aspartic acid (D).

4. The multispecific antibody according to one of claims 1 to 3,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted independently by lysine (K), arginine (R) or histidine (H) and the amino acid at position 123, numbering according to Kabat, is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147, numbering according to Kabat EU index, is substituted independently by glutamic acid (E) or aspartic acid (D)

and the amino acid at position 213, numbering according to Kabat EU index, is substituted independently by glutamic acid (E) or aspartic acid (D).

5. The multispecific antibody according to claim 4, wherein in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted independently by lysine (K) or arginine (R).

6. The multispecific antibody according to one of claims 1 to 5,

wherein in the constant domain CL of the first light chain under a) the amino acid at position 124, numbering according to Kabat, is substituted by lysine (K) and the amino acid at position 123, numbering according to Kabat, is substituted by lysine (K),

and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147, numbering according to Kabat EU index, is substituted by glutamic acid (E) and the amino acid at position 213, numbering according to Kabat EU index, is substituted by glutamic acid (E).

7. The antibody according to any one of the preceding claims, characterized in that

a first CH3 domain of the first heavy chain of the antibody under a) and a second CH3 domain of the second heavy chain of the antibody under b) each meet at an interface which comprises an original interface between the antibody CH3 domains,

wherein said interface is altered to promote the formation of the multispecific antibody, wherein the alteration is characterized in that:

- i) the CH3 domain of one heavy chain is altered,
so that within the original interface of the CH3 domain of the one heavy chain that meets the original interface of the CH3 domain of the other heavy chain within the multispecific antibody,
an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the interface of the CH3 domain of the one heavy chain which is positionable in a cavity within the interface of the CH3 domain of the other heavy chain;

11. A method for the preparation of the multispecific antibody according to any one of claims 1 to 10,

comprising the steps of

- A) transforming a host cell with vectors comprising nucleic acid molecules encoding
 - a) the first light chain and the first heavy chain of the multispecific antibody according to any one of claims 1 to 10; and
 - b) the second light chain and the second heavy chain of the multispecific antibody according to any one of claims 1 to 10;
- B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and
- C) recovering said antibody molecule from said culture.