

CLAIMS

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1. A multispecific antigen-binding fragment comprising at least two Fab fragments with different CH1 and CL domains, wherein each Fab fragment recognizes a different epitope of interest, and said Fab fragments are tandemly arranged in any order, the C-terminal end of the CH1 domain of a first Fab fragment being linked to the N-terminal end of the VH domain
 10 of the following Fab fragment through a polypeptide linker,
 characterized in that the polypeptide linker sequence comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1),
 wherein X₁, X₂ and X₃, identical or different, are Threonine (T) or Serine (S); X₄ and X₅,
 identical or different, are any amino acid selected from the group consisting of Cysteine (C),
 15 Alanine (A), and Glycine (G); and X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from
 the group consisting of Alanine (A), Glycine (G), Valine (V), Asparagine (N), Aspartic acid (D)
 and Isoleucine (I).

2. A multispecific antibody having two identical antigen-binding arms, each consisting of
 20 the multispecific antigen-binding fragment of claim 1.

3. The multispecific antibody of claim 2, which has an immunoglobulin-like structure, comprising:
 - two identical antigen-binding arms each consisting of the multispecific antigen-binding
 25 fragment as defined in claim 1;
 - the dimerized CH2 and CH3 domains of an immunoglobulin;
 - the hinge region of an IgA, IgG, or IgD, linking the C-terminal ends of CH1 domains of the antigen-binding arms to the N-terminal ends of the CH2 domains.

30 4. A multispecific antibody, preferably a bispecific antibody, comprising two heavy chains and four light chains,
 wherein each heavy chain comprises
 a. a Fc region of an immunoglobulin comprising Hinge-CH2-CH3 domains,
 b. which Fc region is linked to Fab heavy chain CH1-VH of antibody 1 (Ab1) by said
 35 Hinge domain,

- c. which in turn is linked to Fab heavy chain CH1-VH of antibody 2 (Ab2), by a polypeptide linker sequence, wherein the polypeptide linker sequence links the N-terminus of said Fab heavy chain VH domain of Ab1 with the C-terminus of said CH1 domain of Ab2, and the four light chains comprise light chains of Ab1 and light chains of Ab2 associated with their cognate heavy chain domains;
- wherein the polypeptide linker sequence comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1), wherein X₁, X₂ and X₃, identical or different, are Threonine (T) or Serine (S); X₄ and X₅, identical or different, are any amino acid selected from the group consisting of Cysteine (C), Alanine (A), and Glycine (G); and X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Alanine (A), Glycine (G), Valine (V), Asparagine (N), Aspartic acid (D) and Isoleucine (I).
5. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 4, wherein X₄ is Cysteine (C).
6. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 5, wherein X₅ is Alanine (A) or Cysteine (C).
7. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 6, wherein X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Alanine (A) and Glycine (G).
8. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 7, wherein X₆ and X₇ are identical and are preferably selected from the group consisting of Alanine (A) and Glycine (G).
9. The multispecific antigen-binding fragment, or multispecific antibody of claim 7, wherein the polypeptide linker sequence comprises or consists of a sequence selected from the group consisting of
EPKSCDKTHTSPAPAPPELLGGPAAPPAPAPAGG (SEQ ID NO: 3);
EPKSCDKTHTSPAPAPPELLGGPGGPPGPGPGGG (SEQ ID NO: 2);
and
EPKSCDKTHTSPAPAPPELLGGPAAPPGPAPGGG (SEQ ID NO: 4).
10. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 9, which is a bispecific antigen-binding fragment, or bispecific antibody.

11. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 10, which recognizes both EGFR and HER2/neu.
12. The multispecific antibody of claim 4, wherein Ab1 and Ab2, being different,
5 independently are cetuximab on the one hand, and trastuzumab on the other hand.
13. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 10, which recognizes both CD38 and PD-L1.
- 10 14. A polypeptide which comprises, preferably consists of, a heavy chain of the antigen-binding fragment or a heavy chain of the multispecific antibody as defined in any of claims 1 to 13.
15. A polynucleotide comprising a sequence encoding the polypeptide of claim 14.
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16. A host cell transfected with an expression vector comprising the polynucleotide of claim 15.
17. The host cell of claim 16, further transformed with at least two polynucleotides
20 encoding two different light chains: a first light chain pairing specifically with a first VH/CH1 region of said heavy chain; a second light chain pairing specifically with a second VH/CH1 region of said heavy chain.
18. The host cell of claim 17, which is additionally transformed with a third polynucleotide
25 encoding a third light chain which is different from the first and second light chains, and which pairs specifically with a third VH/CH1 region of said heavy chain.
19. A method for preparing an antigen-binding fragment or a multispecific antibody as defined in any of claims 1 to 13, wherein said method comprising culturing a host cell of any
30 of claims 16 to 18 and recovering said antigen-binding fragment or antibody from said culture.
20. A polypeptide which comprises or consists of amino acid sequence
EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1),
35 wherein X₁, X₂ and X₃, identical or different, are Threonine (T) or Serine (S); X₄ and X₅, identical or different, are any amino acid selected from the group consisting of Cysteine (C), Alanine (A), and Glycine (G); and X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from

the group consisting of Alanine (A), Glycine (G), Valine (V), Asparagine (N), Aspartic acid (D) and Isoleucine (I).

21. The polypeptide of claim 20, which sequence comprises or consists of a sequence

5 selected from the group consisting of

EPKSCDKTHTSPPAPAPPELLGGPAAPPAPAPAGG (SEQ ID NO: 3);

EPKSCDKTHTSPPAPAPPELLGGPGGPPGPGPGGG (SEQ ID NO: 2);

and

EPKSCDKTHTSPPAPAPPELLGGPAAPPGPAPGGG (SEQ ID NO: 4).

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