

- the second part of the binding domain is a polypeptide that is fused either directly or via a second linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain of the same single chain fusion polypeptide associate and form a functional binding site that specifically binds to the target,

and a second fusion polypeptide specifically binding to a target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the binding domain is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain,
- the second part of the binding domain is a polypeptide that is fused either directly or via a second linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain of the same single chain fusion polypeptide associate and form a functional binding site that specifically binds to the target,

wherein the first and the second fusion polypeptide are identical or different and wherein the spacer domain of the first fusion polypeptide is covalently conjugated to the spacer domain of the second fusion polypeptide,

wherein the first part of the binding domain is an antibody heavy chain variable domain and the second part of the binding domain is an antibody light chain variable domain or vice versa, and

wherein the spacer domain comprises an antibody hinge region or a fragment thereof and an antibody CH2 domain or a fragment thereof.

In one embodiment the (circular) (single chain) fusion polypeptide comprises exactly one part of the binding domain N-terminal to the spacer domain and exactly one, but different, part of the same binding domain C-terminal to the spacer domain.

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In one embodiment the first part of the binding domain is an antibody heavy chain variable domain and the second part of the binding domain is an antibody light chain variable domain or vice versa.

One aspect as reported herein is the (circular) fusion polypeptide as reported herein or the dimeric (circular) fusion polypeptide as reported herein for use as a medicament.

5 As reported herein is the use of the (circular) fusion polypeptide as reported herein or the dimeric (circular) fusion polypeptide as reported herein in the manufacture of a medicament.

Detailed Description of the Invention

I. Definitions

10 The knobs into holes dimerization modules and their use in antibody engineering are described in Carter P.; Ridgway J.B.B.; Presta L.G.: Immunotechnology, Volume 2, Number 1, February 1996 , pp. 73-73(1).

15 General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

20 As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and is referred to as “numbering according to Kabat” herein. Specifically, the Kabat numbering system (see pages 647-660) of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) is used for the light chain constant domain CL of kappa and lambda isotype, and the Kabat EU index numbering system (see pages 25 661-723) is used for the constant heavy chain domains (CH1, Hinge, CH2 and CH3, which is herein further clarified by referring to “numbering according to Kabat EU index” in this case).

30 Useful methods and techniques for carrying out the current invention are described in e.g. Ausubel, F.M. (ed.), Current Protocols in Molecular Biology, Volumes I to III (1997); Glover, N.D., and Hames, B.D., ed., DNA Cloning: A Practical Approach, Volumes I and II (1985), Oxford University Press; Freshney, R.I. (ed.), Animal Cell Culture – a practical approach, IRL Press Limited (1986); Watson,

Claims

1. A dimeric fusion polypeptide

comprising a first fusion polypeptide specifically binding to a target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the binding domain is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain,
- the second part of the binding domain is a polypeptide that is fused either directly or via a second linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain of the same single chain fusion polypeptide associate and form a functional binding site that specifically binds to the target,

and

a second fusion polypeptide specifically binding to a target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the binding domain is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain,
- the second part of the binding domain is a polypeptide that is fused either directly or via a second linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain of the same single chain fusion polypeptide associate and form a functional binding site that specifically binds to the target,

wherein the first and the second fusion polypeptide are identical or different and wherein the spacer domain of the first fusion polypeptide is covalently conjugated to the spacer domain of the second fusion polypeptide,

wherein the first part of the binding domain is an antibody heavy chain variable domain and the second part of the binding domain is an antibody light chain variable domain or vice versa, and

wherein the spacer domain comprises an antibody hinge region or a fragment thereof and an antibody CH2 domain or a fragment thereof.

2. The dimeric fusion polypeptide according to claim 1, wherein the first part of the binding domain is an antibody heavy chain Fab fragment and the second part of the binding domain is an antibody light chain Fab fragment or vice versa.
3. The dimeric fusion polypeptide according to any one of claims 1 to 2, wherein the first part of the binding domain and the second part of the binding domain are associated covalently by a disulfide bond with each other.
4. The dimeric fusion polypeptide according to any one of claims 1 to 3, wherein the spacer domain comprises an antibody hinge region or a fragment thereof, an antibody CH2 domain, and an antibody CH3 domain or a fragment thereof.
5. The dimeric fusion polypeptide according to any one of claims 1 to 4, wherein the first and/or the second linker is/are a peptidic linker.
6. The dimeric fusion polypeptide according to any one of claims 1 to 5, wherein the fragment of the antibody hinge region is a C-terminal fragment.
7. The dimeric fusion polypeptide according to any one of claims 1 to 6, wherein the fragment of the antibody CH2 domain is a N-terminal fragment.
8. A pair of isolated nucleic acids together encoding the dimeric fusion polypeptide according to claim 1.
9. A host cell comprising the pair of nucleic acids according to claim 8.
10. A method of producing a fusion polypeptide comprising culturing the host cell of claim 9 so that the fusion polypeptide or the dimeric fusion

polypeptide is produced and recovering the fusion polypeptide or the dimeric fusion polypeptide from the cell or the cultivation medium.

11. A pharmaceutical formulation comprising the dimeric fusion polypeptide according to any one of claims 1 to 7 and a pharmaceutically acceptable carrier.
12. The dimeric fusion polypeptide according to any one of claims 1 to 7 for use as a medicament.