

EP 1 378 520 discloses a cyclic single strand trispecific antibody.

Summary

5 Herein is reported a single circular polypeptide as a novel target binder. In this circular polypeptide the N-terminal portion comprises a first part of a binding site and the C-terminal portion comprises the second part of the binding site. The first part of the binding site and the second part of the binding site associate with each other to form the complete or functional binding site. Thereby the polypeptide is circularized. The association can be non-covalently or covalently. In case the association is covalently it is not by a peptide bond, but, e.g. by a disulfide bond.

10 As reported herein is a (circular) (single chain) fusion polypeptide that (specifically) binds 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 (forming a structural domain after folding),
- 15 - the first part of the binding domain is a polypeptide and is fused (either directly or) via a first (peptidic) linker to the N-terminus of the spacer domain,
- the second part of the binding domain is a polypeptide and is fused (either directly or) via a second (peptidic) linker to the C-terminus of the spacer domain,
- 20 - the first part of the binding domain and the second part of the binding domain (of the same (single chain) fusion polypeptide) (associate/are associated with/covalently or non-covalently bind to each other and) form a (functional) binding site that specifically binds to the target.

25 One aspect as reported herein is 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 of the first fusion polypeptide is a polypeptide and comprises at least 25 amino acid residues,
- 30 - the first part of the binding domain of the first fusion polypeptide is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain of the first fusion polypeptide,

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

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,
10 wherein

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

wherein the first and the second fusion polypeptide are identical or different and
25 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 of the first fusion polypeptide is an antibody heavy chain variable domain and the second part of the binding domain of the first fusion polypeptide is an antibody light chain variable domain or vice versa,

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

10 wherein the spacer domain of the first fusion polypeptide comprises an antibody hinge region or a fragment thereof and an antibody CH2 domain or a fragment thereof, and

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

15 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.

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.

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

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 of the second fusion polypeptide is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the binding domain of the second fusion polypeptide is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain of the second fusion polypeptide,

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

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

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

wherein the spacer domain of the first fusion polypeptide comprises an antibody hinge region or a fragment thereof and an antibody CH2 domain or a fragment thereof, and

wherein the spacer domain of the second fusion polypeptide 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 of the first fusion polypeptide is an antibody heavy chain Fab fragment and the second part of the binding domain of the first fusion polypeptide is an antibody light chain Fab fragment or vice versa, and

wherein the first part of the binding domain of the second fusion polypeptide is an antibody heavy chain Fab fragment and the second part of the binding domain of the second fusion polypeptide 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 of the first fusion polypeptide and the second part of the binding domain of the first fusion polypeptide are associated covalently by a disulfide bond with each other, and

wherein the first part of the binding domain of the second fusion polypeptide and the second part of the binding domain of the second fusion polypeptide 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 of the first fusion polypeptide comprises an antibody hinge region or a fragment thereof, an antibody CH2 domain, and an antibody CH3 domain or a fragment thereof, and

wherein the spacer domain of the second fusion polypeptide 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 of the first fusion polypeptide is/are a peptidic linker, and wherein the first and/or the second linker of the second fusion polypeptide 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 of the first fusion polypeptide is a C-terminal fragment, and wherein the fragment of the antibody hinge region of the second fusion polypeptide 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 of the first fusion polypeptide is a N-terminal fragment, and wherein the fragment of the antibody CH2 domain of the second fusion polypeptide 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.