

monoclonal antibodies having two heavy chains and two light chains, sdAbs can bind antigens with similar affinity and specificity as mAbs. Used as building blocks, the sdAbs can be fused to IgG Fc domains to create IgG-like antibodies, including bivalent and bispecific antibodies (see, for example, Hmila I. *et al. Mol. Immunol.* 2008; 45: 3847-3856).

[0007] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[0008] The present application provides a multispecific antigen binding protein (MABP) comprising one or more single-domain antibodies (sdAbs) fused to a full-length four-chain antibody or an antigen binding fragment derived therefrom.

[0009] Accordingly, disclosed is a MABP comprising: (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds a first epitope, and (b) a second antigen binding portion comprising a single-domain antibody (sdAb) that specifically binds a second epitope, wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, the first epitope and the second epitope are from the same antigen. In some embodiments, the first epitope and the second epitope are from different antigens. In some embodiments, the MABP is bispecific.

[0009a] In one aspect, there is provided a multispecific antigen binding protein comprising: (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds a first epitope, and (b) a second antigen binding portion comprising a single-domain antibody that specifically binds a second epitope, wherein the first antigen binding portion and the second antigen binding portion are fused to each other.

[0010] In some embodiments according to any one of the MABPs described above, the first antigen binding portion is a full-length antibody consisting of two heavy chains and two light chains. In some embodiments, the first antigen binding portion is an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L . In some embodiments, the second antigen binding portion comprises a single polypeptide chain. In some embodiments, the C terminus of the second antigen binding portion is fused to the N-terminus of at least one heavy chain of the first antigen binding portion. In some embodiments, the C terminus of the second antigen binding portion is fused to the N-terminus of at least one light chain of the first antigen binding portion. In some embodiments, the N terminus of the second antigen binding portion is fused to the C-terminus of at least one heavy chain of the first antigen binding portion. In some embodiments, the N terminus of the second antigen binding portion is fused to the C-terminus of at least one light chain of the first antigen binding portion. In some

MABP is stable for at least about one week at 37 °C at a concentration of at least about 50 mg/mL. In some embodiments, the MABP is stable after at least about 5 freeze-thaw cycles at a concentration of at least 50 mg/mL.

[0020] Disclosed is a pharmaceutical composition comprising any one of the MABPs described above and a pharmaceutically acceptable carrier. In some embodiments, the concentration of the MABP is at least about 100 mg/mL, such as at least about 150 mg/mL, 200 mg/mL or higher.

[0020a] In another aspect, there is provided a pharmaceutical composition comprising the multispecific antigen binding protein as described herein, and a pharmaceutically acceptable carrier.

[0021] Disclosed is a method of treating a disease in an individual, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some embodiments, the disease is a cancer. In some embodiments, the cancer is selected from the group consisting of breast cancer, renal cancer, melanoma, lung cancer, glioblastoma, head and neck cancer, prostate cancer, ovarian carcinoma, bladder carcinoma, and lymphoma. In some embodiments, the disease is an inflammatory or autoimmune disease. In some embodiments, the inflammatory or autoimmune disease is selected from the group consisting of arthritis (such as rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, and arthritic ulcerative colitis), colitis, psoriasis, severe asthma, and moderate to severe Crohn's disease.

[0021a] In another aspect, there is provided use of an effective amount of the multispecific antigen binding protein as described herein in the manufacture of a medicament for treating a disease in an individual.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 depicts a schematic structure of an exemplary bispecific antigen binding protein (also referred herein as "BABP") comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and an sdAb, wherein the N-terminus of the sdAb is fused to the C-terminus of one heavy chain via an optional peptide linker. The full-length antibody has two antigen binding sites that specifically bind the first epitope. The sdAb specifically binds the second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_H-C_H1-C_H2-C_H3-V_HH; (3) V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the first epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the first epitope, and V_HH specifically binds the second epitope.

[0023] FIG. 2 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two

CLAIMS

What is claimed is:

1. A multispecific antigen binding protein comprising:
 - (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds a first epitope, and
 - (b) a second antigen binding portion comprising a single-domain antibody that specifically binds a second epitope,wherein the first antigen binding portion and the second antigen binding portion are fused to each other.
2. The multispecific antigen binding protein of claim 1, wherein the first epitope and the second epitope are from the same antigen.
3. The multispecific antigen binding protein of claim 1, wherein the first epitope and the second epitope are from different antigens.
4. The multispecific antigen binding protein of any one of claims 1-3, wherein the first antigen binding portion is a full-length antibody consisting of two heavy chains and two light chains.
5. The multispecific antigen binding protein of any one of claims 1-4, wherein the second antigen binding portion comprises a single polypeptide chain, and wherein:
 - (i) the C terminus of the second antigen binding portion is fused to the N-terminus of at least one heavy chain of the first antigen binding portion;
 - (ii) the C terminus of the second antigen binding portion is fused to the N-terminus of at least one light chain of the first antigen binding portion;
 - (iii) the N terminus of the second antigen binding portion is fused to the C-terminus of at least one heavy chain of the first antigen binding portion; or
 - (iv) the N terminus of the second antigen binding portion is fused to the C-terminus of at least one light chain of the first antigen binding portion.
6. The multispecific antigen binding protein of any one of claims 1-4, wherein the second antigen binding portion is a Fab-like domain comprising a first polypeptide chain comprising a first single-domain antibody fused to a first heavy chain constant (C_{H1})

domain, and a second polypeptide chain comprising a second single-domain antibody fused to a light chain constant (C_L) domain.

7. The multispecific antigen binding protein of any one of claims 1-3 and 6, wherein the first antigen binding portion comprises an Fc region.
8. The multispecific antigen binding protein of claim 7, wherein the second antigen binding portion is fused to the N-terminus of the Fc region.
9. The multispecific antigen binding protein of any one of claims 1-8, wherein the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker.
10. The multispecific antigen binding protein of claim 9, wherein the peptide linker is no more than 30 amino acids long.
11. The multispecific antigen binding protein of claim 10, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 1, 8 or 13.
12. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from an immune checkpoint molecule.
13. The multispecific antigen binding protein of claim 12, wherein the immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3, LAG-3, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40.
14. The multispecific antigen binding protein of claim 13, wherein the first antigen binding portion is an anti-PD-1 antibody or antigen binding fragment thereof.
15. The multispecific antigen binding protein of claim 14, wherein the anti-PD-1 antibody is pembrolizumab or nivolumab.
16. The multispecific antigen binding protein of claim 13, wherein the first antigen binding portion is an anti-PD-L1 antibody or antigen binding fragment thereof.
17. The multispecific antigen binding protein of claim 16, wherein the anti-PD-L1 antibody is durvalumab or atezolizumab.
18. The multispecific antigen binding protein of any one of claims 12-17, wherein the single-domain antibody specifically binds an immune checkpoint molecule.
19. The multispecific antigen binding protein of claim 18, wherein the immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3, LAG-3, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40.

20. The multispecific antigen binding protein of claim 19, wherein the second antigen binding portion comprises an anti-CTLA-4 single-domain antibody.
21. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from a tumor antigen.
22. The multispecific antigen binding protein of any one of claims 1-11, wherein the first antigen binding portion is an anti-Ang2 antibody or antigen binding fragment thereof.
23. The multispecific antigen binding protein of claim 22, wherein the second antigen binding portion is an anti-VEGF single-domain antibody.
24. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from a pro-inflammatory molecule.
25. The multispecific antigen binding protein of any one of claims 1-24, wherein the multispecific antigen binding protein can be produced recombinantly at an expression level of at least 10 mg/L.
26. The multispecific antigen binding protein of any one of claims 1-25, wherein the multispecific antigen binding protein has an aggregation onset temperature of at least 65°C.
27. A pharmaceutical composition comprising the multispecific antigen binding protein of any one of claims 1-26, and a pharmaceutically acceptable carrier.
28. Use of an effective amount of the multispecific antigen binding protein of any one of claims 1-26 in the manufacture of a medicament for treating a disease in an individual.
29. The use of claim 28, wherein the disease is a cancer, an inflammatory disease, or an autoimmune disease.