CLAIMS

What is claimed is:

1. A multispecific antigen binding protein comprising:
   (a) a first antigen binding portion comprising a single-chain variable fragment (scFv) comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the scFv specifically binds a first epitope,
   (b) a second antigen binding portion comprising a single-domain antibody (sdAb) that specifically binds a second epitope, and
   (c) an Fc region,
   wherein the first antigen binding portion and the second antigen binding portion are fused to each other, and wherein the N-terminus of the sdAb is fused to the C-terminus of the scFv and the C-terminus of the sdAb is fused to the N-terminus of the Fc region.
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 Fc region is an IgG1 Fc, or an IgG4 Fc comprising an S228P mutation.
5. The multispecific antigen binding protein of any one of claims 1-4, wherein the sdAb is a camelid, humanized, or human sdAb.
6. The multispecific antigen binding protein of any one of claims 1-5, wherein the scFv is a human, humanized, or chimeric scFv.
7. The multispecific antigen binding protein of any one of claims 1-6, wherein the multispecific antigen binding protein is bispecific.
8. The multispecific antigen binding protein of any one of claims 1-7, wherein the multispecific antigen binding protein comprises two identical polypeptides each comprising from the N-terminus to the C-terminus: scFv-an optional peptide linker-sdAb-CH2-CH3.
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 8 or 9, wherein the peptide linker is no more than 30 amino acids long.
11. The multispecific antigen binding protein of any one of claims 8-10, 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 12 or 13, wherein the scFv is an anti-PD-1 scFv.
15. The multispecific antigen binding protein of claim 14, wherein the anti-PD-1 scFv is derived from pembrolizumab or nivolumab.
16. The multispecific antigen binding protein of claim 12 or 13, wherein the scFv is an anti-PD-L1 scFv.
17. The multispecific antigen binding protein of claim 16, wherein the anti-PD-L1 scFv is derived from durvalumab or atezolizumab.
18. The multispecific antigen binding protein of any one of claims 1-13, wherein the sdAb 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 sdAb is an anti-CTLA-4 sdAb.
21. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from a tumor antigen, such as any of HER2, BRAF, EGFR, VEGFR2, CD20, RANKL, CD38, or CD52.
22. The multispecific antigen binding protein of any one of claims 1-11, wherein the scFv is an anti-Ang2 scFv.
23. The multispecific antigen binding protein of any one of claims 1-11, wherein the sdAb is an anti-VEGF sdAb.

24. The multispecific antigen binding protein of claim 1, wherein
    (i) the scFv is an anti-Ang2 scFv, and the sdAb is an anti-VEGF sdAb; or
    (ii) the scFv is an anti-PD-1 scFv or an anti-PD-L1 scFv, and the sdAb is an anti-CTLA-4 sdAb.
25. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from a pro-inflammatory molecule, such as any of IL-1β, TNF-α, IL-5, IL-6, IL-6R, or eotaxin-1.
26. The multispecific antigen binding protein of any one of claims 1-25, wherein the multispecific antigen binding protein can be produced recombinantly in mammalian cells at an expression level of at least 10 mg/L.
27. The multispecific antigen binding protein of any one of claims 1-26, wherein the multispecific antigen binding protein has an aggregation onset temperature of at least 65ºC measured by Capillary Differential Scanning Calorimetry (DSC) or Dynamic Light Scattering (DLS).
28. A pharmaceutical composition comprising the multispecific antigen binding protein of any one of claims 1-27, and a pharmaceutically acceptable carrier.
29. Use of an effective amount of the multispecific antigen binding protein of any one of claims 1-27 in the manufacture of a medicament for treating a disease in an individual, wherein the disease is a cancer, an inflammatory disease, or an autoimmune disease.
30. The use of claim 29, wherein:
    (i) 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; or
    (ii) the inflammatory or autoimmune disease is selected from the group consisting of arthritis, colitis, psoriasis, severe asthma, and moderate to severe Crohn’s disease.