

CLAIMS

1. A multispecific antigen-binding protein comprising at least two VL regions respectively paired with at least two VH regions to form at least two antigen-binding sites and at least two CH1 regions respectively paired with two CL regions,

wherein at least one CH1/CL pair comprises CH1/CL mutations to facilitate pairing, wherein the CH1/CL mutations comprise a T192E (CH1) mutation and N137K and S114A (CL) mutations,

wherein when two CH1/CL pairs comprise mutations to facilitate pairing for two different VH/VL pairs, the two CH1/CL pairs do not comprise same mutations, and

wherein at least one VH/VL pair comprise opposite charged mutations to facilitate pairing, said opposite charged mutations comprising (1) a mutated residue in the VH region at Kabat position 39 selected from E, D, K, R, or H, and (2) a mutated residue in the VL region at Kabat position 38 selected from E, D, K, R, or H, and

wherein the mutated residue in the VH region has an opposite charge from the mutated residue in the VL region.

2. A multispecific antigen-binding protein of claim 1, wherein at least one CH1 region is operably linked to a heterodimerization domain.
3. The multispecific antigen-binding protein or the antigen-binding protein according to claim 1 or 2, further comprising one or more cysteine residues engineered into one or several VH/VL pairs to form one or more disulfide bonds, wherein one or both VH regions comprise one or both of 44C and 105C mutations, and one or both VL regions comprise one or both of 100C and 43C mutations.
4. A kit comprising one or more isolated nucleic acid molecules comprising one or more nucleotide sequences encoding the multispecific antigen-binding protein or the antigen-binding protein of any one of claims 1-3.
5. A kit comprising one or more expression vectors comprising the one or more nucleic acid molecules of claim 4.
6. An isolated host cell comprising the one or more nucleic acid molecules of claim 4 or the one or more expression vectors of claim 5.
7. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the multispecific antigen-binding protein or the antigen-binding protein of any one of claims 1-3.
8. The multispecific antigen-binding protein or the antigen-binding protein of any one of claims 1-3 for use as a medicament.

9. The multispecific antigen-binding protein or the antigen-binding protein of any of claims 1 to 3, comprising three HCDRs for each VH region and three LCDRs for each VL region, and further comprising binding specificity to one or more target antigens or one or more target epitopes.