

WHAT IS CLAIMED

1. A polypeptide complex comprising a first antigen-binding moiety and a second antigen-binding moiety, wherein:

the first antigen-binding moiety comprises

a first polypeptide comprising, from N-terminus to C-terminus, a first heavy chain variable domain (VH) of a first antibody operably linked to a first T cell receptor (TCR) constant region (C1), and

a second polypeptide comprising, from N-terminus to C-terminus, a first light chain variable domain (VL) of the first antibody operably linked to a second TCR constant region (C2),

wherein:

C1 comprises an engineered CBeta and C2 comprises ~~or~~ an engineered CAlpha, or

~~C2-C1~~ comprises an engineered CAlpha and C2 comprises ~~or~~ an engineered CBeta,

C1 and C2 are capable of forming a dimer comprising at least one non-native interchain bond between C1 and C2, and the non-native interchain bond is capable of stabilizing the polypeptide complex,

the engineered CAlpha and/or engineered CBeta further comprises at least one mutation at the c-terminus region of the engineered CAlpha and/or engineered CBeta, or at one or more amino acid positions that are spatially close to the c-terminus of engineered CAlpha and/or engineered CBeta, wherein the at least one mutation improves the stability of engineered CAlpha, the stability of engineered CBeta, and/or the interfacial stability of the engineered CAlpha-CBeta,

and

the first antibody has a first antigenic specificity; and

the second antigen-binding moiety has a second antigenic specificity.

2. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises at least one mutated residue selected from P92S, E93D, S94V, and S95P, and/or the engineered CBeta comprises at least one mutated residue selected from E17K and S21A.
3. The polypeptide complex of any of claims 1-2, wherein the engineered CAlpha and/or CBeta comprise one or more mutated residues to form one or more non-native disulfide bonds,

selected from: P8C on CAlpha, A9C on CAlpha, V10C on CAlpha, F26C on CAlpha, F29C on CAlpha, T33C on CAlpha, Q34C on CAlpha, V35C on CAlpha, S36C on CAlpha, S38C on CAlpha, K39C on CAlpha, F78C on CAlpha, N80C on CAlpha, S81C on CAlpha, I82C on CAlpha, P84C on CAlpha, D86C on CAlpha, T87C on CAlpha, F88C on CAlpha, F89C on CAlpha, P90C on CAlpha, and A18C on CBeta.

4. The polypeptide complex of any of claims 1-3, wherein the engineered CAlpha comprises at its C-terminus a deletion of "FFPSPESS" (SEQ ID NO: 9) or "PESS" (SEQ ID NO: 6).
5. The polypeptide complex of any of claims 1-4, wherein the engineered CAlpha comprises mutations at the C-terminus of CAlpha with "VEPKS" (SEQ ID NO: 5) in place of "PESS" (SEQ ID NO: 6).
6. The polypeptide complex of any of claims 1-4, wherein the engineered CAlpha comprises mutations at the C-terminus of CAlpha with "NRGE" (SEQ ID NO: 7) in place of "PESS" (SEQ ID NO: 6).
7. The polypeptide complex of any of claims 1-6, wherein the engineered CAlpha and the engineered CBeta comprise mutations S22F, T33I, and A73T on CAlpha and E17K, H22R, D38P, and S53D on CBeta.
8. The polypeptide complex of any of claims 1, 3, and 4, wherein the engineered CAlpha comprises mutated residues P8C and D86C on CAlpha, and a deletion of 4 amino acid residues at the C terminus of CAlpha (amino acid residues 92-95).
9. The polypeptide complex of any of claims 1, 3, and 4, wherein the engineered CAlpha and the engineered CBeta comprise mutated residues P90C on CAlpha and A18C on CBeta, and a deletion of 4 amino acid residues at the C terminus of CAlpha (amino acid residues 92-95).
10. The polypeptide complex of claim 1, wherein the engineered C2 comprises any one of SEQ ID NOs: 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 79, and 80, and/or the engineered C1 comprises any one of SEQ ID NOs: 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, and 65.
11. The polypeptide complex of claim 1, wherein the engineered C1 comprises any one of SEQ ID NOs: 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 79, and 80, and/or the engineered C2 comprises any one of SEQ ID NOs: 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, and 65.

12. The polypeptide complex of claim 1, wherein the engineered C1 and the engineered C2 respectively comprise a pair of sequences selected from the group consisting of SEQ ID NOs: 10/11, 12/13, 14/15, 16/17, 18/19, 20/21, 22/23, 24/25, 26/27, 28/29, 30/31, 32/33, 34/35, 36/37, 38/39, 40/41, 42/43, 44/45, 46/47, 48/49, 50/51, 52/53, 54/55, 56/57, 58/59, 60/61, 62/63, 64/65, 79/43 and 80/51.
13. The polypeptide complex of claim 1, wherein the engineered C2 and the engineered C1 respectively comprise a pair of sequences selected from the group consisting of SEQ ID NOs: 12/13, 14/15, 16/17, 18/19, 20/21, 22/23, 24/25, 26/27, 28/29, 30/31, 32/33, 34/35, 36/37, 38/39, 40/41, 42/43, 44/45, 46/47, 48/49, 50/51, 52/53, 54/55, 56/57, 58/59, 60/61, 62/63, 64/65, 79/43, and 80/51.
14. The polypeptide complex of claim 1, wherein the engineered C2 and the engineered C1 respectively comprise a pair of sequences of SEQ ID NOs: 42 and 43, or the engineered C1 and the engineered C2 respectively comprise a pair of sequences of SEQ ID NOs: 42 and 43.
15. The polypeptide complex of claim 1, wherein the engineered C2 and the engineered C1 respectively comprise a pair of sequences of SEQ ID NOs: 50 and 51, or the engineered C1 and the engineered C2 respectively comprise a pair of sequences of SEQ ID NOs: 50 and 51.
16. The polypeptide complex of claim 1, wherein the engineered C2 and the engineered C1 respectively comprise a pair of sequences of SEQ ID NOs: 79 and 43, or the engineered C1 and the engineered C2 respectively comprise a pair of sequences of SEQ ID NOs: 79 and 43.
17. The polypeptide complex of claim 1, wherein the engineered C2 and the engineered C1 respectively comprise a pair of sequences of SEQ ID NOs: 80 and 51, or the engineered C1 and the engineered C2 respectively comprise a pair of sequences of SEQ ID NOs: 80 and 51.
18. The polypeptide complex of any of claims 1-17, wherein one of the first and the second antigenic specificities is directed to an exogenous antigen, an endogenous antigen, an autoantigen, a neoantigen, a viral antigen or a tumor antigen.
19. The polypeptide complex of any of claims 1-17, wherein one of the first and the second antigenic specificities is directed to a T-cell specific receptor molecule and/or a natural killer cell (NK cell) specific receptor molecule, and the other is directed to a tumor associated

antigen.

20. The polypeptide complex of any of claims 1-17, wherein one of the first and the second antigenic specificities is directed to PD-L1, and the other is directed to 4-1BB.
21. The polypeptide complex of any of claims 1-17, wherein one of the first and the second antigenic specificities is directed to HER2 D2, and the other is directed to HER2 D4.
22. The polypeptide complex of any of claims 1-17, wherein one of the first and the second antigenic specificities is directed to IL-17, and the other is directed to IL-20.
23. The polypeptide complex of any of claims 1-17, wherein one of the first and the second antigenic specificities is directed to IL-4, and the other is directed to IL-13.
24. A conjugate comprising the polypeptide complex of any of claims 1-23, conjugated to a moiety.
25. An isolated polynucleotide encoding the polypeptide complex of any of claims 1-23.
26. An isolated vector comprising the polynucleotide of claim 25.
27. A host cell comprising the isolated polynucleotide of claim 25 or the isolated vector of claim 26.
28. A method of expressing the polypeptide complex of any of claims 1-23, comprising culturing a host cell under the condition at which the polypeptide complex is expressed, wherein the host cell comprises a polynucleotide encoding the polypeptide complex.
29. A method of producing the polypeptide complex of any of claims 1-23, comprising:
 - a) introducing to a host cell:
 - a first polynucleotide encoding a first polypeptide comprising, from N-terminus to C-terminus, a first heavy chain variable region (VH) of a first antibody operably linked to a first TCR constant region (C1), and
 - a second polynucleotide encoding a second polypeptide comprising, from N-terminus to C-terminus, a first light chain variable domain (VL) of the first antibody operably linked to a second TCR constant region (C2),
 wherein:
 - C1 and C2 are capable of forming a dimer comprising at least one non-native interchain bond between C1 and C2, and the non-native interchain bond is capable of stabilizing the dimer, and

the first antibody has a first antigenic specificity,

b) allowing the host cell to express the polypeptide complex.

30. The method of claim 29, further comprising:

introducing to the host cell

one or more additional polynucleotides encoding a second antigen-binding moiety,

wherein the second antigen-binding moiety has a second antigenic specificity different from the first antigenic specificity.

31. The method of any of claims 29-30, further comprising isolating the polypeptide complex.

32. A composition comprising the polypeptide complex of any of claims 1-23.

33. A pharmaceutical composition comprising the polypeptide complex of any of claims 1-23 and a pharmaceutically acceptable carrier.

34. ~~A method of treating a condition in a subject in need thereof, comprising administering to the subject a therapeutically effective amount~~ Use of the polypeptide complex of any of claims 1-23 in the manufacture of a medicament for treating a condition in a subject in need thereof.

~~35. The method of claim 34, wherein the condition can be alleviated, eliminated, treated, or prevented when the first antigen and the second antigen are both modulated.~~

~~35~~36. A kit comprising one or more containers comprising the polypeptide complex of any of claims 1-23.