

CLAIMS

1. A polypeptide complex comprising:
 - 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 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.
2. The polypeptide complex of claim 1, wherein the non-native interchain bond is formed between a first mutated residue comprised in C1 and a second mutated residue comprised in C2, optionally wherein at least one of the first and the second mutated residues is a cysteine residue.
3. The polypeptide complex of claim 1 or 2, wherein at least one native cysteine residue is absent in C1 and/or C2; and/or wherein at least one native N-glycosylation site is absent in C1 and/or C2.
4. The polypeptide complex of any one of claims 1-3, wherein the dimer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 non-native interchain bonds, optionally wherein at least one of the non-native interchain bonds is a disulphide bond.
5. The polypeptide complex of any one of claims 1-4, wherein:
 - a) C1 comprises an engineered CBeta, and C2 comprises an engineered CAlpha;
 - b) C1 comprises an engineered CAlpha, and C2 comprises an engineered CBeta;
 - c) C1 comprises an engineered CBeta, and C2 comprises an engineered CPre-Alpha;
 - d) C1 comprises an engineered CPre-Alpha, and C2 comprises an engineered

CBeta;

- e) C1 comprises an engineered CGamma, and C2 comprises an engineered CDelta; or
- f) C1 comprises an engineered CDelta, and C2 comprises an engineered CGamma;

optionally, wherein the first VH is operably linked to C1 at a first conjunction domain, and the first VL is operably linked to C2 at a second conjunction domain.

6. The polypeptide complex of claim 5, wherein
the engineered CBeta lacks a FG loop encompassing the amino acid residues 101-107 of the native CBeta and/or a DE loop encompassing the amino acid residues 66-71 of the native CBeta.
7. The polypeptide complex of claim 5 or 6, wherein
 - (a) the engineered CPre-Alpha comprises any one of SEQ ID NO: 82-83, 311, 312, 313, 314, 315, 316, 317, or 318, and/or the engineered CBeta comprises any one of SEQ ID NO: 84, 33-41, 319, 320, 321, 322, 323, or 324,
 - (b) the engineered CAlpha comprises any one of SEQ ID NO: 43-48, and/or the engineered CBeta comprises any one of SEQ ID NO: 33-41, or
 - (c) the engineered CGamma comprises any one of SEQ ID NO: 113, or 114, 333, 334, 335, 336, 337, 338, 339, or 340, and/or the engineered CDelta comprises any one of SEQ ID NO: 115, 116, 325, 326, 327, 328, 329, 330, 331, or 332.
8. The polypeptide complex of any one of claims 5-7, wherein
 - (a) C1 comprises the engineered CBeta, and C2 comprises the engineered CPre-Alpha; and wherein the first conjunction domain comprises SEQ ID NO: 49 or 50, and/or the second conjunction domain comprises SEQ ID NO: 81 or 131,
 - (b) C1 comprises the engineered CPre-Alpha, and C2 comprises the engineered CBeta; and wherein the first conjunction domain comprises SEQ ID NO: 132 or 133, and/or the second conjunction domain comprises SEQ ID NO: 49 or 50,
 - (c) C1 comprises the engineered CBeta, and C2 comprises the engineered CAlpha; and wherein the first conjunction domain comprises SEQ ID NO: 49 or 50, and/or the second conjunction domain comprises or is SEQ ID NO: 51 or 52,

- (d) C1 comprises the engineered CAlpha, and C2 comprises the engineered CBeta; and wherein the first conjunction domain comprises SEQ ID NO: 129 or 130, and/or the second conjunction domain comprises SEQ ID NO: 49 or 50,
- (e) C1 comprises the engineered CGamma, and C2 comprises the engineered CDelta; and wherein the first conjunction domain comprises SEQ ID NO: 117 or 118, and/or the second conjunction domain comprises SEQ ID NO: 119 or 120, or
- (f) C1 comprises the engineered CDelta, and C2 comprises the engineered CGamma; and wherein the first conjunction domain comprises SEQ ID NO: 123 or 124, and/or the second conjunction domain comprises SEQ ID NO: 125 or 126.
9. A bispecific polypeptide complex, comprising a first antigen-binding moiety associated with 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 and C2 are capable of forming a dimer comprising at least one non-native interchain bond between a first mutated residue comprised in C1 and a second mutated residue comprised in C2, and the non-native interchain bond is capable of stabilizing the dimer, and
- the first antibody has a first antigenic specificity,
- the second antigen-binding moiety has a second antigenic specificity which is different from the first antigenic specificity, and
- the first antigen-binding moiety and the second antigen-binding moiety are less prone to mispair than otherwise would have been if both the first and the second antigen-binding moieties are counterparts of natural Fab; optionally

wherein the second antigen-binding moiety comprises a heavy chain variable domain and a light chain variable domain of a second antibody having the second antigenic specificity; further optionally wherein the second antigen-binding moiety comprises a Fab.

10. The bispecific polypeptide complex of claim 9, 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; optionally wherein one of the first and the second antigenic specificities is directed to CD3, and the other is directed to a tumor associated antigen or CD19.
11. The bispecific polypeptide complex of claim 9 or 10, wherein the first antigen-binding moiety further comprises a first dimerization domain, and the second antigen-binding moiety further comprises a second dimerization domain, wherein the first and the second dimerization domains are associated; optionally wherein the association is via a connector, a disulphide bond, a hydrogen bond, electrostatic interaction, a salt bridge, or hydrophobic-hydrophilic interaction, or the combination thereof; further optionally wherein the first and/or the second dimerization domain comprises at least a portion of an antibody hinge region, optionally derived from IgG1, IgG2 or IgG4; further optionally wherein the first and/or the second dimerization domain further comprises an antibody CH2 domain, and/or an antibody CH3 domain, or wherein the first dimerization domain is operably linked to the first TCR constant region (C1) at a third conjunction domain.
12. The bispecific polypeptide complex of claim 11, wherein:
 - a) C1 comprises an engineered CBeta, and the third conjunction domain is comprised in SEQ ID NO: 53 or 54;
 - b) C1 comprises an engineered CAlpha, and the third conjunction domain is comprised in SEQ ID NO: 134, 135, 140 or 141;
 - c) C1 comprises an engineered CPre-Alpha, and the third conjunction domain is comprised in SEQ ID NO: 134, 135, 140 or 141;
 - d) C1 comprises an engineered CGamma, and the third conjunction domain is comprised in SEQ ID NO: 121 or 122; or
 - e) C1 comprises an engineered CDelta, and the third conjunction domain is

comprised in SEQ ID NO: 127 or 128.

13. The bispecific polypeptide complex of claim 11 or 12, wherein the first and the second dimerization domains are different and associate in a way that discourages homodimerization and/or favors heterodimerization, optionally wherein the first and the second dimerization domains are capable of associating into heterodimers via knobs-into-holes, hydrophobic interaction, electrostatic interaction, hydrophilic interaction, or increased flexibility.
14. The bispecific polypeptide complex of any one of claims 9-13, wherein the first antigen binding moiety comprises the first polypeptide comprising VH operably linked to a chimeric constant region, and the second polypeptide comprises VL operably linked to C2, wherein the chimeric constant region and C2 comprise a pair of sequences selected from the group consisting of: SEQ ID NOs: 177/176, 179/178, 184/183, 185/183, 180/176, 181/178, 182/178, 184/186, 185/186, 188/187, 196/187, 190/189, 192/191, 192/193, 195/194, 198/197, 200/199, 202/201, 203/201, 203/204, 205/204, 206/204, 208/207, 208/209, 211/210, 213/212, 213/151, 214/212, 214/151, 234/233, 232/231, 216/215, 218/217, 220/219, 222/221, 224/223, 226/225, 227/223, 229/228, 229/230, 236/235 and 238/237.
15. The bispecific polypeptide complex of any one of claims 9-14, wherein the first antigenicity is directed to CD3, and the first polypeptide and the second polypeptide comprise a pair of sequences selected from the group consisting of: SEQ ID NOs: 2/1, 4/3, 5/1, 6/3, 7/3, 9/8, 10/8, 9/11, 10/11, 13/12, 15/14, 17/16, 17/18, 20/19, 21/12, 65/64, 67/66, 69/68, 70/68, 70/71, 72/71, 73/71, 75/74, 75/76, 78/77, 86/85, 90/89, 91/92, 94/93, 96/95, 98/97, 99/95, 101/100, 101/102, 106/105, 108/107, 110/109, 112/111, 137/136, 138/136, 137/139 and 138/139.
16. The bispecific polypeptide complex of any one of claims 9-15, wherein the first antigen binding moiety is capable of binding to CD3, and the second antigen binding moiety is capable of binding to CD19, and the bispecific polypeptide complex comprises a combination of four polypeptide sequences selected from the group consisting of: SEQ ID NOs: 22/12/24/23, 25/12/26/23, and 25/12/27/23.
17. A conjugate comprising the polypeptide complex of any one of claims 1-8, or the bispecific polypeptide complex of any one of claims 9-16, conjugated to a moiety.
18. A method of producing the polypeptide complex of any one of claims 1-8 or the bispecific

polypeptide complex of any one of claims 9-16, 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, and

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

the method optionally comprising

c) 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, and

d) allowing the host cell to express the bispecific polypeptide complex;

wherein the method optionally further comprises isolating the polypeptide complex or the bispecific polypeptide complex.

19. A polypeptide complex comprising:

1) a first antigen-binding moiety comprising:

a heavy chain variable domain (VH) of a first antibody operably linked to a first T cell receptor (TCR) constant region (C1), and

a 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 a first mutated residue comprised in C1 and a

second mutated residue comprised in C2, and the non-native interchain bond is capable of stabilizing the dimer, and

wherein

a) C1 comprises an engineered CBeta, and C2 comprises an engineered CAlpha;

b) C1 comprises an engineered CAlpha, and C2 comprises an engineered CBeta;

c) C1 comprises an engineered CBeta, and C2 comprises an engineered CPre-Alpha;

d) C1 comprises an engineered CPre-Alpha, and C2 comprises an engineered CBeta;

e) C1 comprises an engineered CGamma, and C2 comprises an engineered CDelta; or

f) C1 comprises an engineered CDelta, and C2 comprises an engineered CGamma; and

2) a second antigen-binding moiety comprising:

a VH of a second antibody operably linked to an antibody heavy chain CH1 domain, and

a VL of the second antibody operably linked to an antibody light chain constant (CL) domain,

wherein the first antigen-binding moiety and the second antigen-binding moiety are less prone to mispair than otherwise would have been if both the first and the second antigen-binding moieties are counterparts of natural Fab,

wherein the polypeptide complex optionally comprises a third antigen-binding moiety comprising a VH of a third antibody operably linked to an antibody heavy chain CH1 domain, and a VL of the third antibody operably linked to an antibody light chain CL domain, wherein the CH1 of the third antigen-binding moiety is operably linked to the VH of the second antigen-binding moiety.

20. A polypeptide complex comprising:

1) a first antigen-binding moiety comprising:

a heavy chain variable domain (VH) of a first antibody operably linked to a first T cell receptor (TCR) constant region (C1), and

a 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 a first mutated residue comprised in C1 and a second mutated residue comprised in C2, and the non-native interchain bond is capable of stabilizing the dimer, and

wherein

a) C1 comprises an engineered CBeta, and C2 comprises an engineered CAlpha;

b) C1 comprises an engineered CAlpha, and C2 comprises an engineered CBeta;

c) C1 comprises an engineered CBeta, and C2 comprises an engineered CPre-Alpha;

d) C1 comprises an engineered CPre-Alpha, and C2 comprises an engineered CBeta;

e) C1 comprises an engineered CGamma, and C2 comprises an engineered CDelta; or

f) C1 comprises an engineered CDelta, and C2 comprises an engineered CGamma; and

2) a second antigen-binding moiety comprising:

a VH of a second antibody operably linked to C1, and a VL of the second antibody operably linked to C2,

wherein C1 and C2 are capable of forming a dimer comprising at least one non-native interchain bond between a first mutated residue comprised in C1 and a second mutated residue comprised in C2, and the non-native interchain bond is capable of stabilizing the dimer, and

wherein

a) C1 comprises an engineered CBeta, and C2 comprises an engineered CAlpha;

b) C1 comprises an engineered CAlpha, and C2 comprises an engineered CBeta;

- c) C1 comprises an engineered CBeta, and C2 comprises an engineered CPre-Alpha;
 - d) C1 comprises an engineered CPre-Alpha, and C2 comprises an engineered CBeta;
 - e) C1 comprises an engineered CGamma, and C2 comprises an engineered CDelta; or
 - f) C1 comprises an engineered CDelta, and C2 comprises an engineered CGamma;
- 3) a third antigen-binding moiety comprising:
- a VH of a third antibody operably linked to an antibody heavy chain CH1 domain, and
 - a VL of the third antibody operably linked to an antibody light chain CL domain;
- 4) a fourth antigen-binding moiety comprising:
- a VH of a fourth antibody operably linked to an antibody heavy chain CH1 domain, and
 - a VL of the fourth antibody operably linked to an antibody light chain CL domain;

the polypeptide complex further comprising a first and second antibody CH2 domain and optionally a first and second antibody CH3 domain, and

wherein

- (i) the VH from the first antigen-binding moiety and the VH from the second antigen-binding moiety are operably linked to the first and second antibody CH3 domains, respectively, the CH1 from the third antigen-binding moiety and the CH1 from the fourth antigen-binding moiety are operably linked to the first and second antibody CH2 domains, respectively, and the third antigen-binding moiety and the fourth antigen-binding moiety are capable of forming a dimer,
- (ii) the C1 from the first antigen-binding moiety and the C1 from the second antigen-binding moiety are operably linked to the first and second antibody CH2 domains, respectively, the VH from the third antigen-binding moiety and the VH from the fourth antigen-binding moiety are operably linked to the first and second antibody

CH3 domains, respectively, and the first antigen-binding moiety and the second antigen-binding moiety are capable of forming a dimer,

(iii) the CH1 from the third antigen-binding moiety and the CH1 from the fourth antigen-binding moiety are operably linked to the first and second antibody CH2 domains, respectively, the C1 from the first antigen-binding moiety is operably linked to the VH from the first antigen-binding moiety, the C1 from the second antigen-binding moiety is operably linked to the VH from the second antigen-binding moiety, and the third antigen-binding moiety and the fourth antigen-binding moiety are capable of forming a dimer, or

(iv) the C1 from the first antigen-binding moiety and the C1 from the second antigen-binding moiety are operably linked to the first and second antibody CH2 domains, respectively, the CH1 from the third antigen-binding moiety is operably linked to the VH from the first antigen-binding moiety, the CH1 from the fourth antigen-binding moiety is operably linked to the VH from the second antigen-binding moiety, and the first antigen-binding moiety and the second antigen-binding moiety are capable of forming a dimer.

21. The polypeptide complex of any one of claims 5-8, 19, and 20, or the bispecific polypeptide complex of any one of claims 12-16, wherein:

(a) the engineered CBeta comprises a mutated cysteine residue within a contact interface selected from the group consisting of amino acid residues 9-35, 52-66, 71-86 and 122-127; and/or

the engineered CAlpha comprises a mutated cysteine residue within a contact interface selected from a group consisting of amino acid residues 6-29, 37-67, and 86-95;

(b) the engineered CBeta comprises a mutated cysteine residue within a contact interface selected from the group consisting of amino acid residues 9-35, 52-66, 71-86 and 122-127; and/or

the engineered CPre-Alpha comprises a mutated cysteine residue within a contact interface selected from a group consisting of amino acid residues 7-19, 26-34, 56-75 and 103-106; or

(c) the engineered CDelta comprises a mutated cysteine residue within a contact

interface selected from the group consisting of amino acid residues 8-26, 43-64, and 84-88; and/or

the engineered CGamma comprises a mutated cysteine residue within a contact interface selected from a group consisting of amino acid residues 11-35, and 55-76.

22. The polypeptide complex of any one of claims 5-8, 19, 20, and 21 or the bispecific polypeptide complex of any one of claims 12-16 and 21, wherein

(a) the engineered CBeta comprises a mutated cysteine residue that substitutes for an amino acid residue at a position selected from: S56C, S16C, F13C, V12C, E14C, F13C, L62C, D58C, S76C, and R78C, and/or the engineered CALpha comprises a mutated cysteine residue that substitutes for an amino acid residue at a position selected from: T49C, Y11C, L13C, S16C, V23C, Y44C, T46C, L51C, and S62C, optionally wherein the engineered CBeta and the engineered CALpha comprise a pair of mutated cysteine residues that substitute for a pair of amino acid residues selected from the group consisting of: S56C in CBeta and T49C in CALpha, S16C in CBeta and Y11C in CALpha, F13C in CBeta and L13C in CALpha, S16C in CBeta and L13C in CALpha, V12C in CBeta and S16C in CALpha, E14C in CBeta and S16C in CALpha, F13C in CBeta and V23C in CALpha, L62C in CBeta and Y44C in CALpha, D58C in CBeta and T46C in CALpha, S76C in CBeta and T46C in CALpha, S56C in CBeta and L51C in CALpha, S56C in CBeta and S62C in CALpha, and R78C in CBeta and S62C in CALpha, and wherein the pair of cysteine residues are capable of forming a non-native interchain disulphide bond, further optionally wherein the native cysteine residue at position C74 of the engineered CBeta is absent;

(b) the engineered CBeta comprises a mutated cysteine residue that substitutes for an amino acid residue at a position selected from: S16C, A18C, E19C, F13C, A11C, S56C, and S76C, and/or the engineered CPre-Alpha comprises a mutated cysteine residue that substitutes for an amino acid residue at a position selected from S11C, A13C, I16C, S62C, T65C, and Y59, optionally wherein the engineered CBeta and the engineered CPre-Alpha comprise a pair of mutated cysteine residues that substitute for a pair of amino acid residues selected from the group consisting of: S16C in CBeta and S11C in CPre-alpha, A18C in CBeta and S11C in CPre-alpha, E19C in CBeta and S11C in CPre-alpha, F13C in CBeta and A13C in CPre-alpha, S16C in CBeta and A13C in CPre-alpha, A11C in CBeta and I16C in CPre-alpha, S56C in CBeta and S62C in CPre-alpha, S56C in CBeta and T65C in CPre-alpha, and S76C in CBeta, and Y59C in CPre-alpha, and wherein the

pair of mutated cysteine residues are capable of forming a non-native interchain disulphide bond; or

23. (c) the engineered CGamma comprises a mutated cysteine residue that substitutes for an amino acid residue at a position selected from: S17C, E20C, F14C, T12C, M62C, Q57C, and A19C, and/or the engineered CDelta comprises a mutated cysteine residue that substitutes for an amino acid residue at a position selected from: F12C, M14C, N16C, D46C, V50C, F87C, and E88C, optionally wherein the engineered CGamma and the engineered CDelta comprise a pair of mutated cysteine residues that substitute for a pair of amino acid residues selected from the group consisting of: Q57C in CGamma and V50C in CDelta, A19C in CGamma and E88C in CDelta, S17C in CGamma and F12C in CDelta, E20C in CGamma and F12C in CDelta, F14C in CGamma and M14C in CDelta, T12C in CGamma and N16C in CDelta, M62C in CGamma and D46C in CDelta, and A19C in CGamma and F87C in CDelta, and wherein the pair of mutated cysteine residues are capable of forming an interchain disulphide bond. The polypeptide complex of any one of claims 5-8, and 19-22 or the bispecific polypeptide complex of any one of claims 12-16, 21 and 22, wherein

(a) at least one native glycosylation site is absent in the engineered CBeta and/or in the engineered CAlpha, optionally wherein the native glycosylation site in the engineered CBeta is N69, and/or the native glycosylation site(s) in the engineered CAlpha is/are selected from N34, N68, N79, and any combination thereof;

(b) at least one native glycosylation site is absent in the engineered CBeta and/or in the engineered CPre-Alpha, optionally wherein the absent glycosylation site in the engineered CBeta is N69, and/or the absent glycosylation site in the engineered CPre-Alpha is N50; or

(c) at least one native glycosylation site is absent in the engineered CGamma and/or in the engineered CDelta, optionally wherein the native glycosylation site in the engineered CGamma is N65, and/or the native glycosylation site(s) in the engineered CDelta is/are one or both of N16 and N79.

24. An isolated polynucleotide encoding the polypeptide complex of any one of claims 1-8 and 19-23 or the bispecific polypeptide complex of any one of claims 9-16 and 21-23, optionally wherein the isolated polynucleotide is comprised within an isolated vector.
25. A host cell comprising the isolated polynucleotide or the isolated vector of claim 24.

26. A pharmaceutical composition comprising the polypeptide complex of any one of claims 1-8 and 19-23 or the bispecific polypeptide complex of any one of claims 9-16 and 21-23, and a pharmaceutically acceptable carrier.
27. Use of the polypeptide complex of any one of claims 1-8 and 19-23 or the bispecific polypeptide complex of any one of claims 9-16 and 21-23 for the manufacture of a medicament for treating a condition in a subject in need thereof, wherein the medicament is to be administered in a therapeutically effective amount.
28. The polypeptide complex of any one of claims 1-8, and 19-23 or the bispecific polypeptide complex of any one of claims 9-16 and 21-23, wherein C1 or C2 comprises an engineered CAlpha, and wherein at least one native Ser residue in the engineered CAlpha is mutated to reduce O-glycosylation, optionally wherein the mutated amino acid residue is selected from S19, S36, S41, S91 and S94.