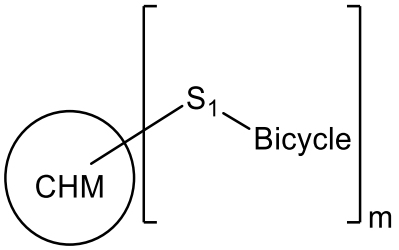
CLAIMS

1. A multimeric binding complex or a pharmaceutically acceptable salt thereof which comprises at least two bicyclic peptide ligands, wherein said peptide ligands may be the same or different, each of which comprises a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold, wherein said bicyclic peptide ligands are specific for the same epitope;
wherein each reactive group is a thiol-containing amino acid;
wherein the molecular scaffold is an organic molecule comprising at least three thiol-reactive groups selected from alkyl halides, maleimides, αβ-unsaturated carbonyl groups and α-halomethylcarbonyl groups;
wherein each loop sequence comprises 5 or 6 amino acids; and
wherein each of said peptide ligands is specific for an epitope present on a T cell or a cancer cell.

2. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 1, wherein each bicyclic peptide ligand is connected to a central hinge moiety by a spacer group.

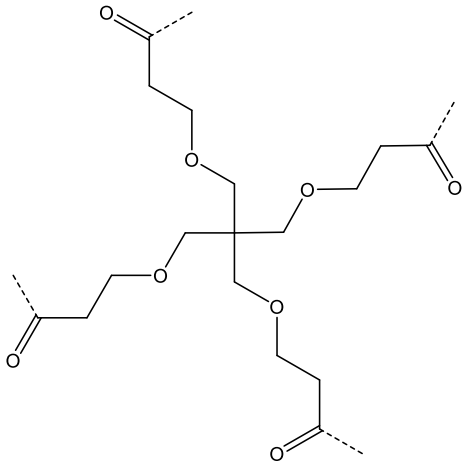
3. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 1 or claim 2, which comprises a compound of formula (I):



(I)

wherein CHM represents a central hinge moiety;
S_1 represents a spacer group;
Bicycle represents a bicyclic peptide ligand as defined in claim 1; and
m represents an integer selected from 2 to 10.

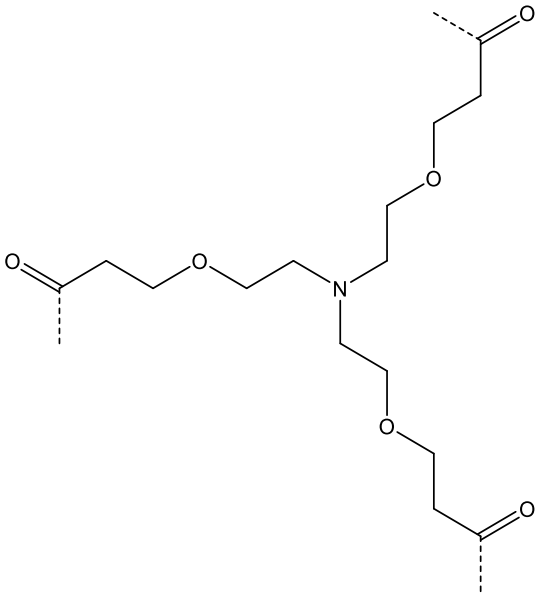
4. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 3, wherein m represents 4 and CHM is a motif of formula (A):



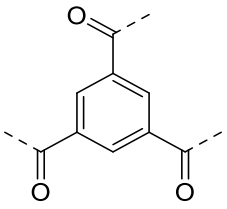
(A)

wherein “-----" represents the point of attachment to each S_1 group.

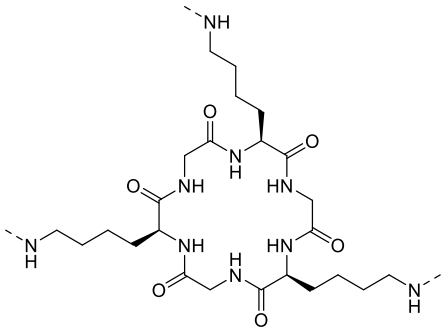
5. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 3, wherein m represents 3 and CHM is selected from a motif of formula (B), (C) or (D):



(B);



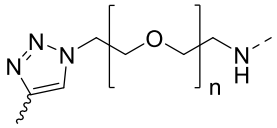
(C); or



(D);

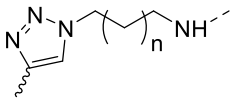
wherein “-----" represents the point of attachment to each S_1 group.

6. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 3 to 5, wherein the spacer (S_1) is selected from any one of spacers S_1A, S_1B, S_1C, S_1D, S_1E, S_1F, S_1G and S_1H:



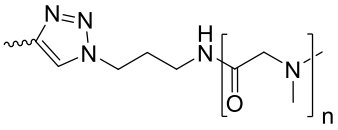
S_1A

n = 5, 10 and 23



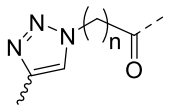
S_1E

n =1



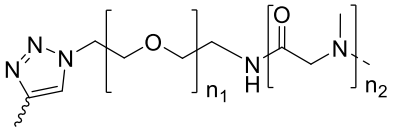
S_1B

n = 5, 10



S_1F

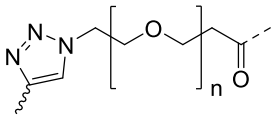
n =1



S_1C

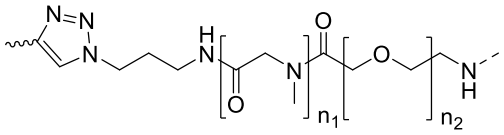
n_1 =5, n_2 =5

n_1 =10, n_2 =10



S_1G

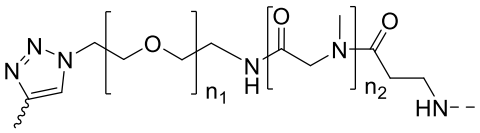
n = 5 and 10



S_1D

n_1 =5, n_2 =5

n_1 =10, n_2 =10



S_1H

n_1 =5, n_2 =5

n_1 =10, n_2 =10

wherein "-----" represents the point of attachment to the CHM group; and

" ~~~~ " represents the point of attachment to the Bicycle group.

7. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 6, wherein the spacer (S_1) is S_1A.

8. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 7, wherein the multimeric binding complex comprises at least two identical bicyclic peptide ligands.

9. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8, wherein the multimeric binding complex comprises at least two differing bicyclic peptide ligands.

10. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 7, wherein the complex comprises bicyclic peptide ligands specific for differing targets.

11. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 10, wherein the bicyclic peptide ligand is conjugated to one half of a binding pair and said other half of said binding pair links each of the bicyclic peptides to the spacer.

12. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 11, wherein said binding pair comprises biotin and streptavidin.

13. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 12, wherein at least one of said peptide ligands are specific for CD137, such as each of said peptide ligands are specific for CD137.

14. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 13, wherein said loop sequences both consist of 6 amino acids.

15. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 13 or claim 14, wherein said peptide ligand comprises a core amino acid sequence selected from:

$C_iIEEGQYC_{ii}FADPY(Nle)C_{iii}$ (SEQ ID NO: 23);
$C_iIKEGQYC_{ii}FADPY(Nle)C_{iii}$ (SEQ ID NO: 24);
$C_iIEKGQYC_{ii}FADPY(Nle)C_{iii}$ (SEQ ID NO: 25);
$C_iIEE(D\text{-}K)QYC_{ii}FADPY(Nle)C_{iii}$ (SEQ ID NO: 26);
$C_iIEEGKYC_{ii}FADPY(Nle)C_{iii}$ (SEQ ID NO: 27);
$C_iIEEGQYC_{ii}KADPY(Nle)C_{iii}$ (SEQ ID NO: 28);
$C_iIEEGQYC_{ii}FADKY(Nle)C_{iii}$ (SEQ ID NO: 29); and
$C_iIEEGQYC_{ii}FADPYKC_{iii}$ (SEQ ID NO: 30);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively and Nle represents norleucine, or a pharmaceutically acceptable salt thereof.

16. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 15, wherein said peptide ligand comprises N and C terminal modifications and comprises an amino acid sequence selected from:

A-C_iIEEGQYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 31; herein referred to as Monomer 1 and BCY3814);

Ac-A-C_iIEEGQYC$_{ii}$FADPY(Nle)C$_{iii}$-Dap (SEQ ID NO: 32; herein referred to as Monomer 2 and BCY7732);

Ac-A-C_iIKEGQYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 33; herein referred to as Monomer 3 and BCY7733);

Ac-A-C_iIEKGQYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 34; herein referred to as Monomer 4 and BCY7734);

Ac-A-C_iIEE(D-K)QYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 35; herein referred to as Monomer 5 and BCY7735);

Ac-A-C_iIEEGKYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 36; herein referred to as Monomer 6 and BCY7736);

Ac-A-C_iIEEGQYC$_{ii}$KADPY(Nle)C$_{iii}$-A (SEQ ID NO: 37; herein referred to as Monomer 7 and BCY7737);

Ac-A-C_iIEEGQYC$_{ii}$FADKY(Nle)C$_{iii}$-A (SEQ ID NO: 38; herein referred to as Monomer 8 and BCY7738);

Ac-A-C_iIEEGQYC$_{ii}$FADPYKC$_{iii}$-A (SEQ ID NO: 39; herein referred to as Monomer 9 and BCY7739);

A-C_iIEEGQYC$_{ii}$F[D-A]DPY[Nle]C$_{iii}$-A (SEQ ID NO: 58; herein referred to as Monomer 10 and BCY8217);

Ac-C_i[tBuAla]PK[D-A]PYC$_{ii}$FADPY[Nle]C$_{iii}$-A (SEQ ID NO: 59; herein referred to as Monomer 11 and BCY8919);

Ac-C_i[tBuAla]PE[D-K]PYC$_{ii}$FADPY[Nle]C$_{iii}$-A (SEQ ID NO: 60; herein referred to as Monomer 12 and BCY8920);

Ac-A-C_iIE[D-K]GQYC$_{ii}$F[D-A]DPY[Nle]C$_{iii}$-A (SEQ ID NO: 61; herein referred to as Monomer 13 and BCY8914);

Ac-A-C_iIE[D-K]GQYC$_{ii}$F[D-A]DPY[Nle]C$_{iii}$-A (SEQ ID NO: 62; herein referred to as Monomer 14 and BCY8915); and

[Ac]-[D-A]-[D-C_i][D-I][D-E][D-E]K[D-Q][D-Y][D-C$_{ii}$][D-F][D-A][D-D][D-P][D-Y][D-Nle][D-C$_{iii}$]-[D-A] (SEQ ID NO: 63; herein referred to as Monomer 15 and BCY11072);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, Ac represents an N-terminal acetyl group, Dap represents diaminopropionic acid, tBuAla represents t-butyl-alanine and Nle represents norleucine, or a pharmaceutically acceptable salt thereof.

17. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 15 or claim 16, wherein said peptide ligand comprises attachment of a PYA moiety at the N-terminus, C-terminus or Lysine residues within said sequence and comprises an amino acid sequence selected from:

(PYA)-A-C_iIEEGQYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 40; herein referred to as Monomer 1A and BCY7740);

Ac-A-C_iIEEGQYC$_{ii}$FADPY(Nle)C$_{iii}$-Dap(PYA) (SEQ ID NO: 41; herein referred to as Monomer 2A and BCY7741);

Ac-A-C_iIK(PYA)EGQYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 42; herein referred to as Monomer 3A and BCY7742);

Ac-A-C_iIEK(PYA)GQYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 43; herein referred to as Monomer 4A and BCY7743);

Ac-A-C_iIEE(D-K)(PYA)QYC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 44; herein referred to as Monomer 5A and BCY7744);

Ac-A-C_iIEEGK(PYA)YC$_{ii}$FADPY(Nle)C$_{iii}$-A (SEQ ID NO: 45; herein referred to as Monomer 6A and BCY7745);

Ac-A-C_iIEEGQYC$_{ii}$K(PYA)ADPY(Nle)C$_{iii}$-A (SEQ ID NO: 46; herein referred to as Monomer 7A and BCY7746);

Ac-A-C_iIEEGQYC$_{ii}$FADK(PYA)Y(Nle)C$_{iii}$-A (SEQ ID NO: 47; herein referred to as Monomer 8A and BCY7747);

Ac-A-C_iIEEGQYC$_{ii}$FADPYK(PYA)C$_{iii}$-A (SEQ ID NO: 48; herein referred to as Monomer 9A and BCY7748);

(PYA)-A-C_iIEEGQYC$_{ii}$F[D-A]DPY[Nle]C$_{iii}$-A (SEQ ID NO: 64; herein referred to as Monomer 10A and BCY8935);

Ac-C_i[tBuAla]PK(PYA)[D-A]PYC$_{ii}$FADPY[Nle]C$_{iii}$-A (SEQ ID NO: 65; herein referred to as Monomer 11A and BCY8927);

Ac-C_i[tBuAla]PE[D-K(PYA)]PYC$_{ii}$FADPY[Nle]C$_{iii}$-A (SEQ ID NO: 66; herein referred to as Monomer 12A and BCY8929);

Ac-A-C_iIE[D-K(PYA)]GQYC$_{ii}$F[D-A]DPY[Nle]C$_{iii}$-A (SEQ ID NO: 67; herein referred to as Monomer 13A and BCY8925);

Ac-A-C_iIE[K(PYA)]GQYC_{ii}F[D-A]DPY[Nle]C_{iii}-A (SEQ ID NO: 68; herein referred to as Monomer 14A and BCY8926); and

[Ac]-[D-A]-[D-C_i][D-I][D-E][D-E][K(PYA)][D-Q][D-Y][D-C_{ii}][D-F][D-A][D-D][D-P][D-Y][D-Nle][D-C_{iii}]-[D-A] (SEQ ID NO: 69; herein referred to as Monomer 15A and BCY11506);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, Ac represents an N-terminal acetyl group, Dap represents diaminopropionic acid, PYA represents propargyl-acid, tBuAla represents t-butyl-alanine and Nle represents norleucine, or a pharmaceutically acceptable salt thereof.

18. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 15 to 17, wherein said peptide ligand comprises attachment of a BCN moiety at the N-terminus or Lysine residues within said sequence and comprises an amino acid sequence selected from:

(BCN)-A-C_iIEEGQYC_{ii}FADPY(Nle)C_{iii}-A (SEQ ID NO: 49; herein referred to as Monomer 1-BCN and BCY8141);

Ac-A-C_iIK(BCN)EGQYC_{ii}FADPY(Nle)C_{iii}-A (SEQ ID NO: 50; herein referred to as Monomer 3-BCN and BCY8095);

Ac-A-C_iIEK(BCN)GQYC_{ii}FADPY(Nle)C_{iii}-A (SEQ ID NO: 51; herein referred to as Monomer 4-BCN and BCY8142);

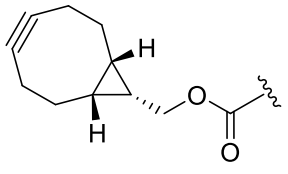
Ac-A-C_iIEE[(D-K)(BCN)]QYC_{ii}FADPY(Nle)C_{iii}-A (SEQ ID NO: 52; herein referred to as Monomer 5-BCN and BCY8096);

Ac-A-C_iIEEGK(BCN)YC_{ii}FADPY(Nle)C_{iii}-A (SEQ ID NO: 53; herein referred to as Monomer 6-BCN and BCY8143);

Ac-A-C_iIEEGQYC_{ii}K(BCN)ADPY(Nle)C_{iii}-A (SEQ ID NO: 54; herein referred to as Monomer 7-BCN and BCY8144); and

Ac-A-C_iIEEGQYC_{ii}FADPYK(BCN)C_{iii}-A (SEQ ID NO: 55; herein referred to as Monomer 9-BCN and BCY8097);

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively, Ac represents an N-terminal acetyl group, Nle represents norleucine and BCN represents:



,

or a pharmaceutically acceptable salt thereof.

19. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 18, wherein said reactive groups comprise cysteine.

20. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 19, wherein said molecular scaffold is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA).

21. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 3 which is a trimer listed in Table 1 or a tetramer listed in Table 2.

22. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in claim 21, which is a trimer selected from BCY7749, BCY7750, BCY7835 and BCY7839 or a tetramer selected from BCY7751, BCY7752, BCY7845, BCY7846, BCY7829, BCY7838, BCY7842, BCY8945 and BCY8947, such as a trimer which is BCY7839 or a tetramer selected from BCY7842, BCY8945 and BCY8947.

23. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 15 to 18, wherein the pharmaceutically acceptable salt is selected from the free acid or the sodium, potassium, calcium, ammonium salt.

24. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 13 to 22, wherein the CD137 is human CD137.

25. A drug conjugate comprising the multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 24, conjugated to one or more effector and/or functional groups.

26. The drug conjugate or the pharmaceutically acceptable salt thereof according to claim 25, wherein the one or more effector and/or functional groups are one or more groups selected from a cytotoxic agent, a radiochelator, a chromophore, and a metal chelator.

27. A pharmaceutical composition which comprises the multimeric binding complex or the pharmaceutically acceptable salt thereof of any one of claims 1 to 24 or the drug conjugate of claim 25 or claim 26, in combination with one or more pharmaceutically acceptable excipients.

28. The multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 24 or the drug conjugate as defined in claim 25 or claim 26, for use in preventing, suppressing or treating a disease or disorder mediated by CD137.

29. Use of the multimeric binding complex or the pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 24 or the drug conjugate as defined in claim 25 or claim 26 in the manufacture of medicament for the prevention, suppression or treatment of a disease or disorder mediated by CD137.