In an example, the present disclosure provides a method of determining (defining) the amino acid sequence of HRI of a, amino acid chain I and/or of HRII of an amino acid sequence II comprising the steps of:

(i) selecting a 1st CRI, a 2nd CRI, a 3rd CRI, and a 4th CRI;

(ii) determining (defining) the seven beta strands A, B, C, D, E, F and G of the 1st CRI, 2nd CRI, 3rd CRI, and 4th CRI, intervening sequences b, c, d, e, f, and g of the 1st CRI, 2nd CRI, 3rd CRI, and 4th CRI, N- and C-terminal sequences a and h, respectively of the 1st CRI, 2nd CRI, 3rd CRI, and 4th CRI;

(iii) replacing at least 1 amino acid of a of the 1st CRI with at least 1 amino acid of a of the 2nd (Replacement 1); replacing at least 1 amino acid of c of the 1st CRI with at least 1 amino acids of c of the 2nd CRI (Replacement 2); replacing at least 1 amino acid of g of the 1st CRI with at least 1 amino acid of g of the 2nd CRI (Replacement 3); replacing at least 1 amino acid of a of the 3rd CRI with at least 1 amino acid of a of the 4th CRI (Replacement 4); replacing at least 1 amino acid of c of the 3rd with at least 1 amino acid of c of the 4th CRI (Replacement 5); and replacing at least 1 amino acid of g of the 3 with at least 1 amino acid g of the 4th CRI (Replacement 6),

wherein the 1st CRI and the 3rd CRI are different from each other and specifically bind to each other under physiological conditions.

In yet another aspect, the disclosure provides a method of producing amino acid chain I comprising the HRI sequence as described herein and/or amino acid chain II with the HRII sequence as described herein, said method comprising the step of introducing a nucleic acid encoding the amino acid chain I and/or amino acid chain II into a host cell and expressing amino acid chain I and/or II.

In an example, the disclosure provides a method of producing amino acid chain I comprising the HRI sequence determined (defined) as described herein and/or amino acid chain II comprising the HRII sequence determined (defined) as described herein.

In yet another aspect, the disclosure provides the protein complex as described herein or of amino acid chain I and/or II as described herein for use as a medicament.

Claims

1. A protein complex comprising at least two amino acid chains I and II, which are non-covalently bound to each other through a heterodimerization region I (HRI) comprised in amino acid chain I and a heterodimerization region II (HRII) comprised in amino acid chain II, wherein:
HRI consists of the amino acid sequence of SEQ ID NO: 20 or a heterodimerizing variant thereof having at least 97% sequence identity to SEQ ID NO: 20, and wherein HRII consists of the amino acid sequence of SEQ ID NO: 21 or a heterodimerizing variant thereof having at least 97% sequence identity to SEQ ID NO: 21.

2. The protein complex of claim 1, wherein amino acid chain I and/or amino acid chain II further comprise one or more amino acid elements selected from the group consisting of a CH2 or CH3 domain of an antibody; one or more antigen specific ligand (ASL), preferably selected from the group consisting of a Fv, a single-chain Fv (scFv), a disulfide-stabilized Fv, a disulfide-stabilized scFv, a Fab, a single-chain Fab, a single domain antibody, a variable heavy chain domain (V_H), a variable light chain domain (VL), T-cell receptor or antigen binding fragments thereof, a Nanobody, a VHH, one or more antibody-like binding proteins; an antibody hinge region (HR), one or more linker sequences (L), one or more cytokines, interleukines, interferons, growth factors, hormones, ligands, peptides, receptor fragments with ligand-binding activity, chelators, enzymes, coagulation factors, and anti-coagulants, and derivatives thereof.

3. The protein complex of claim 2, wherein amino acid chain I comprises one or more antigen specific ligands (ASL) and/or one or more effector molecules, and wherein amino acid chain II comprises one or more antigen specific ligands (ASL) and/or one or more effector molecules,
wherein the ASL modules are selected from a group of molecules, specifically binding to cell surface proteins, hormones, growth factors, cytokines, ligands, serum proteins, coagulation factors, fibrinolytic factors, chemokines, and enzymes, and
wherein the effector molecules are selected from a group consisting of cell surface proteins, hormones, growth factors, cytokines, ligands, serum proteins, coagulation factors, fibrinolytic factors, chemokines, and enzymes.

4. The protein complex according to any one of claims 1 to 3, wherein chain I and chain II have the amino acid sequence according to
SEQ ID NO: 41 and SEQ ID NO: 42,
SEQ ID NO: 34 and SEQ ID NO: 35,
SEQ ID NO: 43 and SEQ ID NO: 44,
SEQ ID NO: 36 and SEQ ID NO: 37 (scFv13.7-Fc1k),
SEQ ID NO: 36 and SEQ ID NO: 38 (scFv13.7-CD3-Hinge-Fc1k),
SEQ ID NO: 39 and SEQ ID NO: 38 (scFv3-43-CD3-Hinge-Fc1k), or
SEQ ID NO: 40 and SEQ ID NO: 38 (scFvhuMCSP-CD3-Hinge-Fc1k).

5. A nucleic acid encoding the amino acid chain I and/or II of claim 4.

6. A vector comprising a nucleic acid of claim 5.

7. Method of producing amino acid chain I comprising the HRI sequence defined in any one of claims 1 to 4 and/or amino acid chain II with the HRII sequence defined in any one of claims 1 to 4, said method comprising the step of introducing a nucleic acid encoding the amino acid chain I and/or amino acid chain II into a host cell and expressing amino acid chain I and/or II.

8. The protein complex of claim 4 for use as a medicament.