

CLAIMS

1. A hetero-dimeric protein comprising a first and a second single-chain protein,
wherein said first single-chain protein comprises a first amino acid sequence consisting of (from the N- to the C-terminus):
 - (ia) a first VL domain,
 - (iia) a first polypeptide linker, and
 - (iiaa) a second VL domain, andwherein said second single-chain protein comprises a second amino acid sequence consisting of (from the N- to the C-terminus):
 - (ib) a first VH domain,
 - (iib) a second polypeptide linker, and
 - (iiib) a second VH domain, andwherein said first VL domain forms a first cognate pair of variable domains with specificity to a first target antigen with either said first or said second VH domain, and said second VL domain forms a second cognate pair of variable domains with specificity to a second target antigen with the other of said VH domains, wherein one of said target antigens is human serum albumin and the other said target antigen is human CD3,
and wherein at least one of said first or said second single-chain protein further comprises
 - (iv) at least one additional domain as third functional domain that is fused via a third polypeptide linker to said first or said second amino acid sequence.
2. The hetero-dimeric protein of claim 1, wherein said hetero-dimeric protein does not comprise a cognate pair of a first and a second immunoglobulin constant domain, wherein said first immunoglobulin constant domain is comprised in said first single-chain protein and wherein said second immunoglobulin constant domain is comprised in said second single-chain protein.
3. The hetero-dimeric protein of claim 1 or 2, further comprising

- (v) a fourth functional domain that is fused via a fourth polypeptide linker to said first or said second amino acid sequence;
 - (vi) a fourth and a fifth functional domain that are fused via a fourth and a fifth polypeptide linker, respectively, to said first and said second amino acid sequence; or
 - (vi) a fourth, a fifth and a sixth functional domain that are fused via a fourth, a fifth and a sixth polypeptide linker, respectively, to said first and said second amino acid sequence.
4. The hetero-dimeric protein of any one of claims 1 to 3, wherein said first polypeptide linker consists of from 5 to 20 amino acid residues, particularly from of 6 to 15 amino acid residues.
 5. The hetero-dimeric protein of any one of claims 1 to 4, wherein said first single-chain protein and said second single-chain protein hetero-dimerize in a parallel orientation.
 6. The hetero-dimeric protein of any one of claims 1 to 4, wherein said first single-chain protein and said second single-chain protein hetero-dimerize in an anti-parallel orientation
 7. The hetero-dimeric protein of claim 6, wherein said first and said second polypeptide linker each consists of from 10 to 20 amino acid residues comprising between 40 and 60% charged residues, particularly from of 12 to 16 amino acid residues comprising 50% charged residues, in each case, wherein the two linkers are able to interact by forming interchain pairs of positively and negatively charged residues; particularly wherein the charged residues on one of said first and second linkers are exclusively positively charged residues, and the charged residues on the other of said first and second linkers are exclusively negatively charged residues, particularly wherein said first and second linkers are selected from SEQ ID NOs. 16 and 17.
 8. The hetero-dimeric protein of any one of claims 1 to 7, wherein (a) said first VL domain (ia) and said first VH domain (ib) form a first cognate pair of variable domains with specificity to human serum albumin, and said second VL domain (iia) and said second VH domain (iib) form a second cognate pair of variable domains

with specificity to human CD3; or (b) said first VL domain (ia) and said second VH domain (iib) form a first cognate pair of variable domains with specificity to human CD3, and said second VL domain (iia) and said first VH domain (ib) form a second cognate pair of variable domains with specificity to human serum albumin.

9. The hetero-dimeric protein of any one of claims 1 to 8, wherein said third, fourth, fifth and/or sixth functional domains are independently selected from the list of: binding domains, toxins, enzymes, hormones, and signaling proteins; particularly wherein said third, fourth, fifth and/or sixth functional domains are independently selected from binding domains; particularly wherein said binding domains are independently selected from the list of: antibody-based binding domains, particularly scFv fragments, Fab fragments and single antibody variable domains, and binding domains based on alternative scaffolds, particularly ankyrin-based domains, fynomers, avimers, anticalins and binding sites being built into constant regions of antibodies.
10. The hetero-dimeric protein of any one of claims 1 to 9, wherein at least one of said antibody variable domains comprises CDR regions derived from a parental rabbit antibody.
11. The hetero-dimeric protein of any one of claims 1 to 10, wherein at least one of said antibody variable domains comprises human framework regions.
12. The hetero-dimeric protein of any one of claims 1 to 11, wherein said first single-chain protein and said second single-chain protein are cross-linked by at least one disulfide bond.
13. The hetero-dimeric protein of claim 12, wherein said disulfide bond is formed between a first cysteine residue flanking said first or said second VL domain and a second cysteine residue flanking said first or said second VH domain.
14. The hetero-dimeric protein of claim 12, wherein said disulfide bond is formed between a first cysteine residue comprised in a framework region of said first or said second VL domain and a second cysteine residue comprised in a framework region of said first or said second VH domain.
15. The hetero-dimeric protein of claim 14, wherein said first cysteine residue is located at position 141 of said first or said second VL domain and said second cysteine residue is located at position 51 of said first or said second VH domain.

16. The hetero-dimeric protein of any one of claims 1 to 15, wherein said cognate pair of variable domains with specificity for human serum albumin comprises the three VL CDRs present in one of the VL protein sequence selected from SEQ ID NOs: 10, 12, and 14 in a human antibody VL framework, wherein the VL framework comprises V κ frameworks FR1, FR2 and FR3, particularly V κ 1 frameworks, and a framework FR4, which is selected from a V κ FR4, particularly V κ 1 FR4, and a V λ framework 4, and the three VH CDRs present in one of the VH protein sequences selected from SEQ ID NOs: 11, 13, and 15 in a human antibody VH framework, particularly a VH3 framework.
17. The hetero-dimeric protein of claim 16, wherein said cognate pair of variable domains with specificity for human serum albumin comprises a VL domain comprising at least positions 5 to 140, particularly at least positions 3 to 145, of a protein sequence selected from SEQ ID NOs: 10, 12, and 14, and a VH domain comprising at least positions 5 to 140, particularly at least positions 3 to 145, of a protein sequence selected from SEQ ID NOs: 11, 13, and 15, particularly wherein said cognate pair of variable domains with specificity for human serum albumin comprises a VL domain selected from SEQ ID NOs: 10, 12, and 14, and a VH domain selected from SEQ ID NOs: 10, 12, and 14.
18. The hetero-dimeric protein of any one of claims 1 to 17, wherein said cognate pair of variable domains with specificity for human CD3 comprises the three VL CDRs present in one of the VL protein sequence selected from SEQ ID NOs: 2, 4, 6 and 8 in a human antibody VL framework, wherein the VL framework comprises V κ frameworks FR1, FR2 and FR3, particularly V κ 1 frameworks, and a framework FR4, which is selected from a V κ FR4, particularly V κ 1 FR4, and a V λ framework 4, and the three VH CDRs present in one of the VH protein sequences selected from SEQ ID NOs: 3, 5, 7 and 9 in a human antibody VH framework, particularly a VH3 framework.
19. The hetero-dimeric protein of claim 18, wherein said cognate pair of variable domains with specificity for human CD3 comprises a VL domain comprising at least positions 5 to 140, particularly at least positions 3 to 145, of a protein sequence selected from SEQ ID NOs: 2, 4, 6 and 8, and a VH domain comprising at least positions 5 to 140, particularly at least positions 3 to 145, of a protein

sequence selected from SEQ ID NOs: 3, 5, 7 and 9, particularly wherein said cognate pair of variable domains with specificity for human serum albumin comprises a VL domain selected from SEQ ID NOs: 2, 4, 6 and 8, and a VH domain selected from SEQ ID NOs: 3, 5, 7 and 9.

20. A nucleic acid sequence or two nucleic acid sequences encoding the first and the second single-chain proteins of the hetero-dimeric protein of any one of claims 1 to 19.
21. A vector or two vectors comprising the nucleic acid sequence or the two nucleic acid sequences of claim 20.
22. A host cell or host cells comprising the vector or the two vectors of claim 21.
23. A method for producing the hetero-dimeric protein of any one of claims 1 to 19, or the first and the second single-chain proteins of said hetero-dimeric protein, comprising (i) providing the nucleic acid sequence or the two nucleic acid sequences of claim 20, or the vector or the two vectors of claim 21, expressing said nucleic acid sequence or nucleic acid sequences, or said vector or vectors, and collecting said hetero-dimeric protein from the expression system, or (ii) providing a host cell or host cells according to claim 22, culturing said host cell or said host cells; and collecting said first and second single-chain proteins, or said hetero-dimeric protein, from the cell culture.
24. A pharmaceutical composition comprising the hetero-dimeric protein of any one of claims 1 to 19 and a pharmaceutically acceptable carrier.
25. The hetero-dimeric protein of any one of claims 1 to 19 for use in the treatment of a disease, particularly a human disease, more particularly a human disease selected from cancer, an inflammatory and an autoimmune disease, wherein at least one of said third, fourth, fifth, or sixth functional domains is able to specifically interact with a target of therapeutic relevance in the corresponding disease.
26. A method for the treatment of a disease, particularly a human disease, more particularly a human disease selected from cancer, an inflammatory and an autoimmune disease, comprising the step of administering the hetero-dimeric protein of any one of claims 1 to 19, wherein at least one of said third, fourth, fifth, or sixth functional domains is able to specifically interact with a target of therapeutic relevance in the corresponding disease.