

hKd and the cKd of the single domain serum albumin binding protein are between about 1 nM and about 5 nM. In some embodiments, the single domain serum albumin binding protein comprises an amino acid sequence wherein amino acid position 6 of CDR 1 (SEQ ID NO:11) is mutated to lysine, amino acid position 7 of CDR2 (SEQ ID NO: 12) is mutated to threonine, and wherein the hKd and the cKd of the single domain serum albumin binding protein are between about 1 nM and about 5 nM. In some embodiments, the single domain serum albumin binding protein comprises an amino acid sequence wherein amino acid position 6 of CDR 1 (SEQ ID NO:11) is mutated to lysine, amino acid positions 7 and 13 of CDR2 (SEQ ID NO: 12) are mutated to threonine and glutamic acid, respectively, and wherein the hKd and the cKd of the single domain serum albumin binding protein are between about 1 nM and about 5 nM. In some embodiments, the single domain serum albumin binding protein comprises an amino acid sequence wherein amino acid position 6 of CDR1 (SEQ ID NO:11) is mutated to arginine, amino acid position 6 of CDR3 (SEQ ID NO: 12) is mutated to lysine, and wherein the hKd and the cKd of the single domain serum albumin binding protein are between about 5 nM and about 10 nM. In some embodiments, the single domain serum albumin binding protein comprises an amino acid sequence wherein amino acid position 6 of CDR 1 (SEQ ID NO:11) is mutated to lysine, amino acid position 7 of CDR2 (SEQ ID NO: 12) is mutated to arginine, amino acid position 6 of CDR3 (SEQ ID NO: 13) is mutated to valine, and wherein the hKd and the cKd of the single domain serum albumin binding protein are between about 1 nM and about 5 nM. In some embodiments, the single domain serum albumin binding protein comprises an amino acid sequence wherein amino acid position 6 of CDR 1 (SEQ ID NO:11) is mutated to lysine, amino acid positions 7 and 13 of CDR2 (SEQ ID NO: 12) is mutated to arginine and glutamic acid, respectively, and amino acid position 6 of CDR3 (SEQ ID NO: 13) is mutated to valine, and wherein the hKd and the cKd of the single domain serum albumin binding protein are between about 1 nM and about 5 nM.

[0010a] Provided herein in another embodiment is a single domain antibody that binds to a serum albumin, wherein said single domain antibody comprises complementarity determining regions CDR1, CDR2, and CDR3, and wherein

- (a) the amino acid sequence of CDR1 is GFTFSRFGMS (SEQ ID NO. 14);
- (b) the amino acid sequence of CDR2 is SISGSGSDTLYADSVK (SEQ ID NO. 12);
- and
- (c) the amino acid sequence of CDR3 is GGSLSR (SEQ ID NO. 13).

[0010b] Provided herein in another embodiment is a polynucleotide encoding the antibody as described herein or the multispecific binding protein as described herein.

[0011] Provided herein in another embodiment, a polynucleotide encoding a single domain serum albumin binding protein according to the present disclosure. A further embodiment describes a vector comprising the polynucleotide as disclosed herein. Another embodiment describes a host cell transformed with the vector according to the present disclosure. In one embodiment is provided a pharmaceutical composition comprising (i) a single domain serum albumin binding protein according to the present disclosure, a polynucleotide according to the present disclosure, a vector according to the present disclosure or a host cell according to the present disclosure, and (ii) a pharmaceutically acceptable carrier.

[0011a] Provided herein in another embodiment, pharmaceutical composition that comprises (i) a single domain antibody as described herein, the multispecific binding protein as described herein, the polynucleotide as described herein, the vector as described herein, or the host cell as described herein, and (ii) a pharmaceutically acceptable carrier.

[0011b] Provided herein in another embodiment is a process for the production of the single domain antibody as described herein or the multispecific binding protein as described herein, said process comprising culturing a host transformed or transfected with a vector comprising a polynucleotide as described herein under conditions allowing the expression of the single domain antibody or the multispecific binding protein, and recovering and purifying the produced single domain antibody or the produced multispecific binding protein from the culture.

[0012] Described herein in another embodiment, is a process for the production of a single domain serum albumin binding protein according to the present disclosure, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a single domain serum albumin binding protein as described herein under conditions allowing the expression of the serum albumin binding protein and recovering and purifying the produced protein from the culture.

[0013] Further described is a method for the treatment or amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease comprising the administration of the single domain serum albumin binding protein according to the present disclosure, to a subject in need thereof. In some embodiments, the subject is human. In some embodiments, the method further comprises administration of an agent in combination with the single domain serum albumin binding protein according to the present disclosure.

[0014] In another embodiment is described a multispecific binding protein comprising the single domain serum albumin binding protein according to the present disclosure. In another embodiment is described an antibody comprising the single domain serum albumin binding protein according to the present disclosure.

[0015] A further embodiment describes a multispecific antibody, a bispecific antibody, an sdAb, a variable heavy domain, a peptide, or a ligand, comprising the single domain serum albumin binding protein according to the present disclosure. In one embodiment is provided an antibody comprising the single domain serum albumin binding protein according to the present disclosure, wherein said antibody is a single domain antibody. In some embodiments, the single domain antibody is derived from a heavy chain variable region of IgG.

CLAIMS

WHAT IS CLAIMED IS:

1. A single domain antibody that binds to a serum albumin, wherein said single domain antibody comprises complementarity determining regions CDR1, CDR2, and CDR3, and wherein

(a) the amino acid sequence of CDR1 is GFTFSRFGMS (SEQ ID NO. 14);

(b) the amino acid sequence of CDR2 is SISGSGSDTLYADSVK (SEQ ID NO. 12);

and

(c) the amino acid sequence of CDR3 is GGSLSR (SEQ ID NO. 13).

2. The single domain antibody according to claim 1, that comprises the following formula:

$$f1-r1-f2-r2-f3-r3-f4$$

wherein, r1 is SEQ ID NO. 14; r2 is SEQ ID NO. 12; and r3 is SEQ ID NO. 13; and wherein f₁, f₂, f₃ and f₄ are framework residues selected so that said single domain antibody is at least eighty percent identical to the amino acid sequence set forth in SEQ ID NO. 10.

3. The single domain antibody according to claim 1, that has an amino acid sequence set forth as SEQ ID NO. 4.

4. The single domain antibody according to any one of claims 1-3, that binds to a serum albumin selected from the group consisting of human serum albumin, cynomolgus serum albumin, and mouse serum albumin.

5. The single domain antibody according to any one of claims 1-4, that binds to human serum albumin and cynomolgus serum albumin with comparable binding affinity (K_d).

6. The single domain antibody according to any one of claims 1-5, that binds to mouse serum albumin with a binding affinity (K_d) of from 1.5 fold to 20 fold weaker than the binding affinity (K_d) of said single domain antibody towards human serum albumin and cynomolgus serum albumin.

7. The single domain antibody according to any one of claims 1-6, that binds to human serum albumin with a human K_d (hK_d) between 1 nM and 100 nM and to cynomolgus serum albumin with a cynomolgus K_d (cK_d) between 1 nM and 100 nM.

8. The single domain antibody according to claim 7, wherein the hK_d and cK_d are between 1 nM and 5 nM, or 5 nM and 10 nM.

9. The single domain antibody according to claim 7, wherein the hKd and the cKd are between 1 nM and 2 nM, 2 nM and 3 nM, 3 nM and 4 nM, 4 nM and 5 nM, 5 nM and 6 nM, 6 nM and 7 nM, 7 nM and 8 nM, 8 nM and 9 nM, or 9 nM and 10 nM.
10. The single domain antibody according to any one of claims 4-9, wherein a ratio between the hKd and the cKd (hKd:cKd) ranges from 20:1 to 1:2.
11. The single domain antibody according to any one of claims 1-10, that comprises the amino acid sequence set forth as SEQ ID NO. 4, wherein the hKd and the cKd are between 1 nM and 5 nM.
12. The single domain antibody according to any one of claims 1-11, that has an elimination half-time of at least 12 hours, at least 20 hours, at least 25 hours, at least 30 hours, at least 35 hours, at least 40 hours, at least 45 hours, at least 50 hours, or at least 100 hours.
13. The single domain antibody according to any one of claims 1-12, that is derived from a heavy chain variable region of IgG.
14. A multispecific binding protein that comprises (i) the single domain antibody according to claim 13, and (ii) a CD3-binding domain.
15. A polynucleotide encoding the antibody according to any one of claims 1-13 or the multispecific binding protein of claim 14.
16. A vector that comprises the polynucleotide according to claim 15.
17. A host cell transformed with the vector according to claim 16.
18. A pharmaceutical composition that comprises (i) a single domain antibody according to any one of claims 1-13, the multispecific binding protein according to claim 14, the polynucleotide according to claim 15, the vector according to claim 16, or the host cell according to claim 17, and (ii) a pharmaceutically acceptable carrier.
19. A process for the production of the single domain antibody according to any one of claims 1-13 or the multispecific binding protein according to claim 14, said process comprising culturing a host transformed or transfected with a vector comprising a polynucleotide according to claim 15 under conditions allowing the expression of the single domain antibody or the multispecific binding protein, and recovering and purifying the produced single domain antibody or the produced multispecific binding protein from the culture.
20. The multispecific binding protein according to claim 14 for use in therapy.
21. Use of the multispecific binding protein according to claim 14 in the manufacture of a medicament for the treatment or amelioration of a proliferative disease, a

tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease, wherein the medicament is to be administered to a subject in need thereof.

22. The use of claim 21, wherein the subject is human.

23. The use of claim 21 or 22, wherein an agent is to be administered in combination with the multispecific binding protein.