

Claims

1. A designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain comprises an amino acid sequence that has at least
5 90% sequence identity with the amino acid sequence of SEQ ID NO: 3 or 4, and wherein said designed ankyrin repeat domain does not have an asparagine (N) at the position corresponding to position 77 of SEQ ID NO: 3 or 4.
2. The designed ankyrin repeat domain of claim 1, wherein said designed ankyrin repeat
10 domain does not have a glutamic acid (E) at the position corresponding to position 78 of SEQ ID NO: 3 or 4.
3. The designed ankyrin repeat domain of claim 1 or 2, further comprising at its N-terminus a G, an S, or a GS.
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4. The designed ankyrin repeat domain of any one of claims 1 to 3, wherein A at the second last position is substituted with L and/or A at the last position is substituted with N.
5. The designed ankyrin repeat domain of any one of claims 1 to 4, wherein said designed
20 ankyrin repeat domain comprises the amino acid sequence
KDFAGKTPLHLAAX₁X₂G (SEQ ID NO: 13)
wherein
X₁ represents an amino acid residue selected from the group consisting of A, D and I; and
X₂ represents an amino acid residue selected from the group consisting of A and D.
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6. The designed ankyrin repeat domain of any one of claims 1 to 5, wherein said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 to 6.
7. The designed ankyrin repeat domain of any one of claims 1 to 6, wherein said designed
30 ankyrin repeat domain comprises SEQ ID NO: 3 or SEQ ID NO: 4.
8. The designed ankyrin repeat domain of any one of claims 1 to 7, wherein said designed ankyrin repeat domain binds human serum albumin in PBS with a dissociation constant (K_D)
35 of or below 100 nM.

9. The designed ankyrin repeat domain of any one of claims 1 to 8, wherein said designed ankyrin repeat domain has an improved stability compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 2.

5 10. The designed ankyrin repeat domain of any one of claims 1 to 9, wherein said designed ankyrin repeat domain exhibits less degradation bands on SDS-PAGE compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 2, wherein said SDS-PAGE is performed following one week incubation at 100 micromolar at 60°C at pH 5.0.

10 11. The designed ankyrin repeat domain of any one of claims 1 to 10, wherein said designed ankyrin repeat domain (i) exhibits less higher molecular weight bands on SDS-PAGE compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 2, wherein said SDS-PAGE is performed following one week incubation at 100 micromolar at 60°C at pH 5.0, and/or (ii) exhibits less higher molecular weight peak in size-exclusion chromatography
15 compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 2, wherein said size-exclusion chromatography is performed following one week incubation at 100 micromolar at 60°C at pH 5.0.

20 12. The designed ankyrin repeat domain of any one of claims 1 to 11, wherein said designed ankyrin repeat domain exhibits at least 10% less higher molecular weight bands in LabChip analysis compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 2, wherein said LabChip analysis is performed following incubation at 100 micromolar at 60°C at pH 6.0 or 7.4.

25 13. A recombinant binding protein comprising one or two designed ankyrin repeat domains of any one of claims 1 to 12.

30 14. The recombinant binding protein of claim 13, wherein said recombinant binding protein has an improved stability compared to a recombinant binding protein with identical amino acid sequence except that each of said designed ankyrin repeat domains with binding specificity for serum albumin is substituted with a designed ankyrin repeat domain consisting of SEQ ID NO: 2.

35 15. A nucleic acid encoding the designed ankyrin repeat domain or the recombinant binding protein of any one of claims 1 to 14.

16. A pharmaceutical composition comprising the designed ankyrin repeat domain or the recombinant binding protein of any one of claims 1 to 14, or the nucleic acid of claim 15, and a pharmaceutically acceptable carrier and/or diluent.