

CLAIMS

We claim:

1. A single domain antibody (sdAb) or antigen-binding fragment thereof that specifically bind human and cynomolgus monkey serum transferrin protein and protein A resin, wherein the sdAb can be purified by a protein A column and used for half-life extension fragments for short half-life proteins or fragments, and wherein the sdAb comprises:
 - (a) a complementarity determining region (CDR)1 having an amino acid sequence selected from the group consisting of
 - a. SEQ ID NO: 46-SEQ ID NO: 90; and
 - b. amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequences of SEQ ID NOs: 46- SEQ ID NO: 90; and/or
 - (b) a CDR2 having an amino acid sequence selected from the group consisting of
 - a. SEQ ID NO: 136-SEQ ID NO: 180; and
 - b. amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequences of SEQ ID NOs: 136- SEQ ID NO: 180; and/or
 - (c) a CDR3 having an amino acid sequence selected from the group consisting of
 - a. SEQ ID NO: 226-SEQ ID NO: 270; and
 - b. amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequences of SEQ ID NOs: 226- SEQ ID NO: 270.
2. The sdAb or antigen-binding fragment thereof of claim 1, comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of SEQ ID NO: 355, 353, 316, 356, 358, 357, 359, 360, 332, 317 to 331, 333 to 352, and 354.
3. The sdAb or antigen-binding fragment thereof of claim 2, comprising an amino acid sequence at least 95% identical to the amino acid sequence of AS01274
(QVQLVESGGGLVQPGGSLRLSCVASGSIASATMAWYRQAPGQQREL VAGITRGGSTK
YADSVKGRFTISRDN AKNTLYLQMNSLKPDDTAVYYCTDYSRKYYQDYWGQGTQVTV
SS (SEQ ID NO: 316)) or AS01299
(QVQLVESGGGLVQPGGSLRLSCAASGSIFSISATMAWYRQAPGKQREL VAGITRSGSTNY
RDSVKGRFTISRDN AKNTMYLQMNSLKPEDTAVYYCTDYSSRYHHDYWGQGTQVTVS
S (SEQ ID NO: 332)) or AS01299VH3a

(EVQLVESGGGLVQPGGSLRLSCAASGSIFSIAATMAWYRQAPGKGLELVAGITRSGSTNY
RDSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCTDYSSRYHHDYWGQGTLVTVS
S (SEQ ID NO: 354)) or AS01299VH3a-A49

(EVQLVESGGGLVQPGGSLRLSCAASGSIFSIAATMAWYRQAPGKGLELVSGITRSGSTNY
RDSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCTDYSSRYHHDYWGQGTLVTVS
S (SEQ ID NO: 356)) or AS01299VH3a-L47

(EVQLVESGGGLVQPGGSLRLSCAASGSIFSIAATMAWYRQAPGKGLEWVAGITRSGSTN
YRDSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCTDYSSRYHHDYWGQGTLVTV
SS (SEQ ID NO: 357)) or AS01299VH3a-M78

(EVQLVESGGGLVQPGGSLRLSCAASGSIFSIAATMAWYRQAPGKGLELVAGITRSGSTNY
RDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTDYSSRYHHDYWGQGTLVTVSS
(SEQ ID NO: 358)) or AS01299VH4

(EVQLVESGGGLVQPGGSLRLSCAASGSIFSIAATMAWYRQAPGKGLELVSGITRSGSTNY
RDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTDYSSRYHHDYWGQGTLVTVSS
(SEQ ID NO: 359)) or AS01299VH4-L47

(EVQLVESGGGLVQPGGSLRLSCAASGSIFSIAATMAWYRQAPGKGLEWVSGITRSGSTNY
RDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTDYSSRYHHDYWGQGTLVTVSS
(SEQ ID NO: 360)).

4. The sdAb or antigen-binding fragment thereof of any of claims 1-2, having a dissociation constant (K_D) of 10^{-7} M or less.
5. The sdAb or antigen-binding fragment thereof of any of claims 1-4, wherein the sdAb or antigen-binding fragment thereof is produced recombinantly.
6. An isolated nucleic acid sequence encoding the sdAb or antigen-binding fragment thereof of any one of claims 1-5.
7. A fusion protein comprising the sdAb or antigen-binding fragment thereof according to any of claims 1-5, a target protein, and optionally a linker, wherein the sdAb or antigen-binding fragment thereof is fused to the carboxyl-terminus or amino-terminus of the target protein, and the linker optionally separates the sdAb and the carboxyl-terminus or amino-terminus of the target protein.
8. A composition comprising an effective amount of the fusion protein of claim 7 and transferrin.

9. The fusion protein according to claim 8, wherein the fusion protein is capable of being purified by protein A resin.
10. A method of increasing the half-life of a target protein in the fusion protein of claim 7 or 9, the method comprising exposing the fusion protein to transferrin in cynomolgus monkey serum to thereby increase the half-life of the target protein in the fusion protein.
11. A method of producing the fusion protein of claim 7 or 9, comprising:
- (a) obtaining an expression vector encoding the fusion protein;
 - (b) introducing the expression vector into a cell to obtain a recombinant cell;
 - (c) growing the recombinant cell under conditions to allow expression of the fusion protein; and
 - (d) obtaining the fusion protein from the recombinant cell or its supernatant.
12. A method for increasing the half-life of a target protein, the method comprising:
- (1) obtaining a fusion protein, wherein the fusion protein comprises the sdAb or antigen-binding fragment thereof according to any of claims 1-5, a target protein, and optionally a linker, wherein the sdAb or antigen-binding fragment thereof is fused to the carboxyl-terminus or amino-terminus of the target protein, and the linker optionally separates the sdAb and the carboxyl-terminus or amino-terminus of the target protein, and
 - (2) exposing the fusion protein to transferrin,
wherein the transferrin increases the half-life of the target protein in the fusion protein compared to the target protein alone.
13. The method of claim 12, wherein the exposing step comprises administering the fusion protein to a serum comprising the transferrin of cynomolgus monkey.
14. A system for increasing the half-life of a target protein, the system comprising:
- (a) a first nucleotide sequence encoding the sdAb or antigen-binding fragment thereof according to any of claims 1-5, and optionally a second nucleotide sequence encoding a linker, wherein the first and second nucleotide sequences are operably linked;
 - (b) a host cell; and
 - (c) the transferrin.