

Designed ankyrin repeat domains with altered surface residues

Cross-reference to related applications

The present application claims the benefit of priority to EP19215433.4, filed on December 11, 2019; EP19215434.2, filed on December 11, 2019; EP19215435.9, filed on December 11, 2019; EP19215436.7, filed on December 11, 2019; EP20161059.9, filed on March 4, 2020; and EP20181234.4, filed on June 19, 2020. The disclosures of these six patent applications are incorporated herein for all purposes by reference in their entirety.

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Field of the invention

The present invention relates to designed ankyrin repeat domains with altered surface residues, as well as to proteins comprising such a designed ankyrin repeat domain, nucleic acids encoding such domains or proteins, methods of preparing such proteins, pharmaceutical compositions comprising such proteins or nucleic acids, and the use of such proteins, nucleic acids or pharmaceutical compositions in the treatment of diseases.

Background of the invention

Designed ankyrin repeat domains are useful for the creation of drug candidates (WO2002020565; WO2011135067; WO2016156596; WO2018054971) for the treatment of disease. The designed ankyrin repeat domains comprised in such drug candidates typically bind target molecules with high affinity, thereby acting pharmacodynamically on the target, e.g. antagonizing target activity. To achieve a long systemic half-life of drug candidates based on designed ankyrin repeat domains, the drug candidate typically needs to comprise a moiety conferring long half-life, which can be achieved, e.g., by chemical modification with polyethylene glycol (PEG; WO2011135067) or by including one or more genetically fused designed ankyrin repeat domains with binding specificity for serum albumin (WO2012069654). Using the latter approach, drug candidates having long terminal half-life have been generated and described (WO2016156596; WO2018054971). Such designed ankyrin repeat domains with binding specificity for serum albumin can prolong the terminal half-life of proteins, e.g., to a terminal half-life similar to that of serum albumin (Steiner et al., *Protein Eng Des Sel.* 30(9):583-591 (2017)). As described in more detail below, Applicant has observed, however, that some designed ankyrin repeat domains exhibit fast

clearance and thus short terminal half-life despite the fact that they are genetically fused to a designed ankyrin repeat domain with binding specificity for serum albumin. The importance of good pharmacokinetic properties of biologic drugs is well known in the art (see, e.g., Strohl, *BioDrugs* 29:215–239 (2015)).

- 5 Thus, there still remains a need for new methods or approaches of improving the pharmacokinetic properties (including prolonging the terminal half-life) of proteins, such as designed ankyrin repeat domains and proteins comprising one or more designed ankyrin repeat domain(s).

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Summary of the Invention

- Applicant has unexpectedly observed that some designed ankyrin repeat domains exhibit fast clearance and thus short terminal half-life despite the fact that they are genetically fused to a designed ankyrin repeat domain with binding specificity for serum albumin (See e.g. Example 6 and Figure 5 for Proteins #24, #27, #31, and #35). In efforts to understand this observation, the inventors have surprisingly discovered that altering certain amino acid residues in the N-terminal capping module and/or the C-terminal capping module of the designed ankyrin repeat domain results in improved pharmacokinetic properties, including a prolonged terminal half-life, of the designed ankyrin repeat domain and of proteins comprising the designed ankyrin repeat domain. The altered amino acid residues are mostly surface exposed residues. Thus, the present invention provides amino acid sequences that lead to improved pharmacokinetic properties of a designed ankyrin repeat domain and of proteins comprising the designed ankyrin repeat domain.
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- The invention provides designed ankyrin repeat domains comprising novel amino acid sequences. In one embodiment said designed ankyrin repeat domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81, 88 to 94, and 107 to 111, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81 and 107 to 111, wherein X represents any amino acid, and (ii) an amino acid sequence selected from the group consisting of SEQ ID NOs: 88 to 94, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 78, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NOs: 91, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 95 to 102, wherein X represents any amino acid. The invention further provides a protein comprising (i) at least
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one designed ankyrin repeat domain of the invention, and (ii) at least one moiety for half-life extension. In one embodiment, said moiety for half-life extension is a designed ankyrin repeat domain with binding specificity for serum albumin. In one embodiment, said ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 80% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8. In one embodiment, said protein further comprises at least one polypeptide linker. In one embodiment, said polypeptide linker is a glycine-serine (GS)-rich linker or a proline-threonine (PT)-rich linker, wherein preferably said polypeptide linker has the amino acid sequence of SEQ ID NO: 2 or 3, or of variants thereof. In one embodiment, said protein is a recombinant binding protein. The invention also provides a protein comprising (i) at least one, two, or three designed ankyrin repeat domains of the invention, and (ii) at least one or two designed ankyrin repeat domains with binding specificity for serum albumin, wherein preferably each of said designed ankyrin repeat domain(s) with binding specificity for serum albumin independently comprises an amino acid sequence with at least 80% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8. In one embodiment, said protein is a recombinant binding protein. Figure 1 further illustrates the invention schematically. The invention further provides nucleic acids encoding a designed ankyrin repeat of the invention or a protein of the invention. The invention further provides a pharmaceutical composition comprising a designed ankyrin repeat domain of the invention, a protein of the invention, or a nucleic acid of the invention. The invention further provides a method of treating a medical condition, the method comprising the step of administering, to a patient in need of such treatment, a therapeutically effective amount of a pharmaceutical composition of the invention. The invention further provides a method for preparing a protein, the method comprising the steps of (A) preparing a nucleic acid that encodes in one open reading frame (i) at least one designed ankyrin repeat domain of the invention, and (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin, and (B) transferring said nucleic acid into an expression host. In one embodiment, the expression host is *E. coli*.

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Brief Description of the Figures

Figure 1. Schematic illustration of the invention. (a) Schematic representation of a designed ankyrin repeat domain of the invention. The designed ankyrin repeat domain (rectangle) comprises certain amino acids at defined positions, illustrated by the vertical black lines. (b) Schematic representation of a comparator designed ankyrin repeat domain. The

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comparator designed ankyrin repeat domain comprises different amino acids than (a) in the corresponding positions. For example, (a) may include SEQ ID NO: 75 (of the present invention), whereas (b) may include SEQ ID NO: 69 (i.e. a sequence known in the art). (c) Schematic illustration of a designed ankyrin repeat domain with binding specificity for serum albumin. A designed ankyrin repeat domain consisting of SEQ ID NO: 4 is an example of such a designed ankyrin repeat domain with binding specificity for serum albumin. (d) Schematic illustration of a polypeptide linker. SEQ ID NOs: 2 and 3 are examples of such polypeptide linkers. (e) Schematic illustration of the pharmacokinetic profile of a designed ankyrin repeat domain (covalently bound to a designed ankyrin repeat domain with binding specificity for serum albumin via a polypeptide linker) in comparison to a comparator designed ankyrin repeat domain (covalently bound to an identical designed ankyrin repeat domain with binding specificity for serum albumin via an identical polypeptide linker). The pharmacokinetic traces are schematically shown on the left, the respective construct is shown schematically on the right, with arrows indicating which curve is observed with which protein. C: Concentration, t: time. (f) Schematic representation of an example of a protein of the invention. The protein comprises, from N terminus (left) to C terminus (right), a first designed ankyrin repeat domain with binding specificity for serum albumin, a first polypeptide linker, a first designed ankyrin repeat domain of the invention, a second polypeptide linker, a second designed ankyrin repeat domain of the invention, a third polypeptide linker, and a second designed ankyrin repeat domain with binding specificity for serum albumin. The designed ankyrin repeat domains of the invention may be binding domains with specificity for one target or for different targets. The order and number of the different domains may vary in the proteins of the invention.

Figure 2. Sequence alignment of amino acid sequences present in N-terminal capping modules. SEQ ID NOs: 69 to 74 correspond to amino acid sequences found in N-terminal capping modules of designed ankyrin repeat domains known in the art (see, e.g., WO2012069655). SEQ ID NOs: 75 to 81 and 107 to 111 correspond to amino acid sequences of the present invention. Residue numbers are indicated above the sequences.

Figure 3. Sequence alignment of amino acid sequences present in C-terminal capping modules. SEQ ID NOs: 82 to 87 correspond to amino acid sequences found in C-terminal capping modules of designed ankyrin repeat domains known in the art (see, e.g., WO2014001442 or WO2016156596). SEQ ID NOs: 88 to 94 correspond to amino acid sequences of the present invention. Residue numbers are indicated above the sequences.

Figure 4. SDS-PAGE of Proteins #9 to #23 (a) and Proteins #24 to #38 (b). Proteins #9 to 38 (corresponding to SEQ ID NOs: 9 to 38, additionally having a His-tag (SEQ ID NO: 1) at the N-terminus) were expressed and purified as described in Example 2, subjected to a stability study as described in Example 3, and subjected to SDS-PAGE analysis as described in Example 4. All proteins were highly pure. M: molecular weight marker, molecular weight is indicated at the left of each figure; -: -80°C control; +: Protein sample incubated at 60°C for 1 week.

Figure 5. Pharmacokinetic profiles in mouse of variants of designed ankyrin repeat domains (each genetically linked to an identical designed ankyrin repeat domain with binding specificity for serum albumin via an identical polypeptide linker). (a) Pharmacokinetic profile in mouse of Protein #24, and variant Proteins #25 and #26. (b) Pharmacokinetic profile in mouse of Protein #27, and variant Proteins #28, #29, and #30. (c) Pharmacokinetic profile in mouse of Protein #31, and variant Proteins #32 #33, and #34. (d) Pharmacokinetic profile in mouse of Protein #35, and variant Proteins #36, #37, and #38. The experiment was performed as described in Example 6 using Balb/c mice and 1 mg/kg intravenous dosing. Proteins #24 to #38 (comprising SEQ ID NOs: 24 to 38, respectively, with each having a His-tag (SEQ ID NO: 1) at the N terminus; symbol indicated in the figure) were produced and purified as described in Example 2. C: concentration in [nM]; t: time in [hours].

Detailed description of the invention

In a first aspect, the present invention provides a designed ankyrin repeat domain comprising novel amino acid sequence motifs in the N-terminal capping module and/or the C-terminal capping module.

- 5 In one embodiment, the designed ankyrin repeat domain of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81, 88 to 94, and 107 to 111, wherein X represents any amino acid. Amino acid sequences SEQ ID NOs: 75 to 81 and 107 to 111 are examples of sequences of the invention present in N-terminal capping modules of designed ankyrin repeat domains of the invention. These
- 10 sequences are further illustrated in Figure 2. Amino acid sequences SEQ ID NOs: 69 to 74 are examples of sequences present in N-terminal capping modules of designed ankyrin repeat domains known in the art (see also Figure 2). Amino acid sequences SEQ ID NOs: 88 to 94 are examples of sequences of the invention present in C-terminal capping modules of designed ankyrin repeat domains of the invention. These sequences are further
- 15 illustrated in Figure 3. Amino acid sequences SEQ ID NOs: 82 to 87 are examples of sequences present in C-terminal capping modules of designed ankyrin repeat domains known in the art (see also Figure 3). In one embodiment, any of the amino acid sequences selected from SEQ ID NOs: 75 to 81 and 107 to 111 is present in the N-terminal capping module of a designed ankyrin repeat domain. In one embodiment, any of the amino acid
- 20 sequences selected from SEQ ID NOs: 88 to 94 is present in the C-terminal capping module of a designed ankyrin repeat domain.

- In one embodiment, said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81, more preferably SEQ ID NOs: 77 to 81, more preferably SEQ ID NOs: 78 to 81, more preferably SEQ ID
- 25 NOs: 79 to 81, more preferably SEQ ID NOs: 80 to 81, most preferably SEQ ID NO: 81, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 81, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 80, wherein X represents any amino acid. In one
- 30 embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 79, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 78, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 77, wherein X represents any amino
- 35 acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 75 or 76. In one embodiment, said designed ankyrin repeat

domain comprises the amino acid sequence of SEQ ID NO: 75. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 76.

In another embodiment, said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 107 to 111, more preferably

5 108 to 111, more preferably 109 to 111, more preferably 110 to 111, most preferably 111, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 111, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 110, wherein X represents any amino acid. In one

10 embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 109, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 107 or 108. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 107. In one embodiment, said designed ankyrin repeat domain comprises

15 the amino acid sequence of SEQ ID NO: 108.

In a further embodiment, said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 88 to 94, more preferably 90 to 94, more preferably 91 to 94, more preferably 92 to 94, more preferably 93 to 94, most preferably 94, wherein X represents any amino acid. In one embodiment, said designed

20 ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 94, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 93, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 92, wherein X represents any amino acid. In one embodiment,

25 said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 91, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 90, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NOs: 88 or 89. In one embodiment, said designed ankyrin repeat

30 domain comprises the amino acid sequence of SEQ ID NO: 88. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 89.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81 and 107 to 111, wherein X represents any amino acid, and (ii) an amino acid sequence selected

35 from the group consisting of SEQ ID NOs: 88 to 94, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid

sequence of SEQ ID NO: 81, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 93, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 81, wherein X represents any amino acid, and (ii) the amino acid
5 sequence of SEQ ID NO: 94, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 80, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 93, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid
10 sequence of SEQ ID NO: 80, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 94, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 79, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 92, wherein X represents any amino acid.

15 In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 78, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 91, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 77, wherein X represents any amino acid, and (ii) the amino acid
20 sequence of SEQ ID NO: 90, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 76, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 89, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid
25 sequence of SEQ ID NO: 75, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 88, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 111, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 94, wherein X represents any amino acid.

30 In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 111, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 93, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 111, wherein X represents any amino acid, and (ii) the amino acid
35 sequence of SEQ ID NO: 92, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 110, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 91, wherein X represents any amino acid.

5 In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 109, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 90, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 108, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 89, wherein X represents any amino acid.

10 In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 108, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 88, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 107, wherein X represents any amino acid, and (ii) the amino acid
15 sequence of SEQ ID NO: 88, wherein X represents any amino acid.

In one embodiment, said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 107, wherein X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 89, wherein X represents any amino acid.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises an
20 N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q and/or the amino acid at position 15 is L. In one embodiment, said designed ankyrin repeat domain comprises a N-terminal capping module having an amino acid sequence wherein the amino acid at position 4 is S, the amino acid at position 8 is Q, the amino acid at position 15 is L, the amino acid at position 17 is T, the amino acid at position
25 20 is T, and/or the amino acid at position 23 is Q. In a preferred embodiment, said N-terminal capping module comprises an amino acid sequence of 30 amino acids. In a further preferred embodiment, said N-terminal capping module consists of an amino acid sequence of 30 amino acids. Preferably, said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ
30 ID NO: 69. Preferably, said alignment comprises no amino acid gaps. Sequence alignment generation is a procedure well known in the art.

In one embodiment, the designed ankyrin repeat domain of the invention comprises a C-terminal capping module having an amino acid sequence wherein the amino acid at position
35 14 is R and/or the amino acid at position 18 is Q. In one embodiment, said designed ankyrin repeat domain comprises a C-terminal capping module having an amino acid sequence wherein the amino acid at position 3 is T, the amino acid at position 4 is Q, the amino acid

at position 6 is T, the amino acid at position 14 is R, the amino acid at position 18 is Q, the amino acid at position 19 is Q, the amino acid at position 22 is S, and/or the amino acid at position 26 is Q. In a preferred embodiment, said C-terminal capping module comprises an amino acid sequence of 28 amino acids. In a further preferred embodiment, said C-terminal capping module consists of an amino acid sequence of 28 amino acids. Preferably, said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82. Preferably, said alignment comprises no amino acid gaps.

In one embodiment, the designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q and/or the amino acid at position 15 is L, and/or (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 14 is R and/or the amino acid at position 18 is Q. In one embodiment, the designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q and/or the amino acid at position 15 is L, and (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 14 is R and/or the amino acid at position 18 is Q. In one embodiment, said designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q, and (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 14 is R and/or the amino acid at position 18 is Q. In one embodiment, said designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 15 is L, and (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 14 is R and/or the amino acid at position 18 is Q. In one embodiment, said designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q and/or the amino acid at position 15 is L, and (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 14 is R. In one embodiment, said designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q and/or the amino acid at position 15 is L, and (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 18 is Q. In one embodiment, said designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence wherein the amino acid at position 8 is Q and the amino acid at position 15 is L, and (ii) a C-terminal capping module having an amino acid sequence wherein the amino acid at position 14 is R and the amino

- acid at position 18 is Q. In a preferred embodiment, said N-terminal capping module comprises an amino acid sequence of 30 amino acids and said C-terminal capping module comprises an amino acid sequence of 28 amino acids. In a further preferred embodiment, said N-terminal capping module consists of an amino acid sequence of 30 amino acids and
- 5 said C-terminal capping module consists of an amino acid sequence of 28 amino acids. Preferably, said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ ID NO: 69, and said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82. Preferably, said
- 10 alignments comprise no amino acid gaps.
- In a further embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module having an amino acid sequence DLGKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids,
- 15 up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids.
- In a further embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module having an amino acid sequence
- 20 DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids.
- 25 In one embodiment, the designed ankyrin repeat domain of the invention comprises a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one
- 30 amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids.
- In one embodiment, the designed ankyrin repeat domain of the invention comprises a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQA (SEQ ID NO: 89), wherein up to 10 amino acids,
- 35 up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one

amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids.

- In one embodiment, the designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence
- 5 DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an
- 10 amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids.
- 15 In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 10 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino
- 20 acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said
- 25 designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 9 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO:
- 30 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping
- 35 module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 8 amino acids of SEQ ID NO: 75 in positions other than

position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 7 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 6 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 5 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence

DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 4 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 3 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to 2 amino acids of SEQ ID NO: 75 in positions other than position 8 and position 15 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGKKLLQAARAGQLDEVRELLKAGADVNA (SEQ ID NO: 75), wherein up to one amino acid of SEQ ID NO: 75 in a position other than position 8 and position 15 is optionally exchanged by another amino acid, and (ii) a C-terminal capping module having an amino acid sequence QDKSGKTPADLAARAGHQDIAEVLQKAA (SEQ ID NO: 88), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 88 in positions other than position 14 and position 18 are optionally exchanged by other amino acids.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVRTLLQAGADVNA (SEQ ID NO: 76), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 75 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids.

In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVRTLLQAGADVNA (SEQ ID NO: 76), wherein up to 10 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVRTLLQAGADVNA (SEQ ID NO: 76), wherein up to 9 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVRTLLQAGADVNA (SEQ ID NO: 76), wherein up to 8 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an

amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 7 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 6 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 5 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 4 amino

acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 3 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to 2 amino acids of SEQ ID NO: 76 in positions other than positions 4, 8, 15, 17, 20 and 23 are optionally exchanged by other amino acids, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids. In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having an amino acid sequence DLGSKLLQAARAGQLDVTLLQAGADVNA (SEQ ID NO: 76), wherein up to one amino acid of SEQ ID NO: 76 in a position other than positions 4, 8, 15, 17, 20 and 23 is optionally exchanged by another amino acid, and (ii) a C-terminal capping module having an amino acid sequence QDTQGTTTPADLAARAGHQQIASVLQQAA (SEQ ID NO: 89), wherein up to 10 amino acids, up to 9 amino acids, up to 8 amino acids, up to 7 amino acids, up to 6 amino acids, up to 5 amino acids, up to 4 amino acids, up to 3 amino acids, up to 2 amino acids, or up to one amino acid of SEQ ID NO: 89 in positions other than positions 3, 4, 6, 14, 18, 19, 22 and 26 are optionally exchanged by other amino acids.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises (i) an N-terminal capping module having an amino acid sequence selected from SEQ ID NOs: 69 to 74, preferably SEQ ID NOs: 70 to 74, more preferably SEQ ID NOs: 71 to 74, more preferably SEQ ID NOs: 72 to 74, more preferably SEQ ID NOs: 73 to 74, most preferably SEQ ID NO: 74, and (ii) a C-terminal capping module having an amino acid sequence selected from SEQ ID NOs: 82 to 87, preferably SEQ ID NOs: 83 to 87, more preferably SEQ ID NOs: 84 to 87, more preferably SEQ ID NOs: 85 to 87, more preferably SEQ ID NOs: 86 to 87, most preferably SEQ ID NO: 87, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X. Amino acid residues comprising a negatively charged side chain include D and E.

In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 74 and (ii) a C-terminal capping module having the amino acid sequence of SEQ ID NO: 87, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 73 and (ii) a C-terminal capping module having the amino acid sequence of SEQ ID NO: 86, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 72 and (ii) a C-terminal capping module having the amino acid sequence of SEQ ID NO: 85, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 71 and (ii) a C-terminal

capping module having the amino acid sequence of SEQ ID NO: 84, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

5 In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 70 and (ii) a C-terminal capping module having the amino acid sequence of SEQ ID NO: 83, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

10 In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 71 and (ii) a C-terminal capping module having the amino acid sequence of SEQ ID NO: 83, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

15 In one embodiment, said designed ankyrin repeat domain comprises (i) an N-terminal capping module having the amino acid sequence of SEQ ID NO: 72 and (ii) a C-terminal capping module having the amino acid sequence of SEQ ID NO: 84, wherein 3 or 4, preferably 4, amino acid residues comprising a negatively charged side chain in said N- and C-terminal capping modules of said designed ankyrin repeat domain are exchanged by amino acids selected from the group consisting of L, Q, R, S, and T, more preferably L, Q, and R, in positions other than X.

20 In a further embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module consisting of the amino acid sequence of SEQ ID NO: 75. In one embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module consisting of the amino acid sequence of SEQ ID NO: 76. In one embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module consisting of the amino acid sequence of SEQ ID NO: 107. In one embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module consisting of the amino acid sequence of SEQ ID NO: 108. In one embodiment, the designed ankyrin repeat domain of the invention comprises a C-terminal capping module consisting of the amino acid sequence of SEQ ID NO: 88. In one

embodiment, the designed ankyrin repeat domain of the invention comprises a C-terminal capping module consisting of the amino acid sequence of SEQ ID NO: 89.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 95 to 102, wherein

5 X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 95 to 98, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 99 to 102, wherein X represents any amino acid. In one embodiment, said

10 designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 96 and 97, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 96, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 97, wherein X

15 represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 95, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 98, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO:

20 99, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 100, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of SEQ ID NO: 101, wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain comprises the amino acid sequence of

25 SEQ ID NO: 102, wherein X represents any amino acid.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises the amino acid sequence

DLGX₁KLLQAAX₂X₃GQLDEVX₄LX₅X₆X₇GADVNA₈DX₉X₁₀GX₁₁TPLHX₁₂AAX₁₃X₁₄GHLE
IVEVLLKX₁₅GADVNA₁₆DX₁₇X₁₈GX₁₉TPLHX₂₀AAX₂₁X₂₂GHLEIVEVLLKX₂₃GADVNA₂₄DX
30 25X₂₆GX₂₇TPADX₂₈AARX₂₉GHEDIAEVLQKX₃₀X₃₁ (SEQ ID NO: 96), wherein

X₁ represents any amino acid, preferably K or S;

X₂ represents any amino acid, preferably R;

X₃ represents any amino acid, preferably A;

X₄ represents any amino acid, preferably E or I;

35 X₅ represents any amino acid, preferably M, L, I, more preferably L;

X₆ represents any amino acid, preferably A or K; more preferably K;

- X₇ represents any amino acid, preferably A or N; more preferably A;
 X₈ and X₁₆ represent any amino acid, preferably K;
 X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₇, X₁₈, X₁₉, X₂₀, X₂₁, and X₂₂ represent any amino acid,
 preferably any amino acid selected from the group consisting of A, D, E, F, H, I, K,
 5 L, N, P, Q, R, S, T, V, W, Y;
 X₁₅, and X₂₃ represent any amino acid, preferably A, N, H, or Y; more preferably A;
 X₂₄ represents any amino acid, preferably K or Q, more preferably Q;
 X₂₅ represents any amino acid, preferably K or T, more preferably K;
 X₂₆ represents any amino acid, preferably S or Q, more preferably S;
 10 X₂₇ represents any amino acid, preferably K or T, more preferably K;
 X₂₈ represents any amino acid, preferably L or I, more preferably L;
 X₂₉ represents any amino acid, preferably A or N, more preferably A;
 X₃₀ represents any amino acid, L or A, more preferably A; and
 X₃₁ represents any amino acid, N or A, more preferably A.

15

In one embodiment, the designed ankyrin repeat domain of the invention comprises the amino acid sequence

DLGX₁KLLQAAX₂X₃GQLDEVX₄LX₅X₆X₇GADVX₈DX₉X₁₀GX₁₁TPHX₁₂AAX₁₃X₁₄GHLE
 IVEVLLKX₁₅GADVX₁₆DX₁₇X₁₈GX₁₉TPHX₂₀AAX₂₁X₂₂GHLEIVEVLLKX₂₃GADVX₂₄DX
 20 ₂₅X₂₆GX₂₇TPHX₂₈AAX₂₉X₃₀GHLEIVEVLLKX₃₁GADVX₃₂DX₃₃X₃₄GX₃₅TPADX₃₆AARX₃₇G
 HEDIAEVLQKX₃₈X₃₉ (SEQ ID NO: 97), wherein

- X₁ represents any amino acid, preferably K or S;
 X₂ represents any amino acid, preferably R;
 X₃ represents any amino acid, preferably A;
 25 X₄ represents any amino acid, preferably E or I;
 X₅ represents any amino acid, preferably M, L, I, more preferably L;
 X₆ represents any amino acid, preferably A or K; more preferably K;
 X₇ represents any amino acid, preferably A or N; more preferably A;
 X₈, X₁₆, and X₂₄ represent any amino acid, preferably K;
 30 X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₇, X₁₈, X₁₉, X₂₀, X₂₁, X₂₂, X₂₅, X₂₆, X₂₇, X₂₈, X₂₉, and X₃₀
 represent any amino acid, preferably any amino acid selected from the group
 consisting of A, D, E, F, H, I, K, L, N, P, Q, R, S, T, V, W, Y;
 X₁₅, X₂₃, and X₃₁ represent any amino acid, preferably A, N, H, or Y; more preferably
 A;
 35 X₃₂ represents any amino acid, preferably K or Q, more preferably Q;
 X₃₃ represents any amino acid, preferably K or T, more preferably K;

- X₃₄ represents any amino acid, preferably S or Q, more preferably S;
X₃₅ represents any amino acid, preferably K or T, more preferably K;
X₃₆ represents any amino acid, preferably L or I, more preferably L;
X₃₇ represents any amino acid, preferably A or N, more preferably A;
5 X₃₈ represents any amino acid, L or A, more preferably A; and
X₃₉ represents any amino acid, N or A, more preferably A.

In a further embodiment, the designed ankyrin repeat domain of the invention consists of an amino acid sequence wherein the amino acid at position 8 is Q, the amino acid at position 10 15 is L, the amino acid at position 110 is R, and the amino acid at position 114 is Q. In a preferred embodiment, said designed ankyrin repeat domain consists of an amino acid sequence with a length of 124 amino acids. Preferably, said position numbers are determined by alignment to SEQ ID NO: 96 using the position numbers of SEQ ID NO: 96. Preferably, said alignment comprises no amino acid gaps. Sequence alignment generation 15 is a procedure well known in the art.

In one embodiment, the designed ankyrin repeat domain of the invention consists of an amino acid sequence wherein the amino acid at position 8 is Q, the amino acid at position 15 is L, the amino acid at position 143 is R, and the amino acid at position 147 is Q. In a preferred embodiment, said designed ankyrin repeat domain consists of an amino acid 20 sequence of 157 amino acids. Preferably, said position numbers are determined by alignment to SEQ ID NO: 97 using the position numbers of SEQ ID NO: 97. Preferably, said alignment comprises no amino acid gaps. Sequence alignment generation is a procedure well known in the art.

In one embodiment, the designed ankyrin repeat domain of the invention consists of an amino acid sequence wherein the amino acid at position 8 is Q, the amino acid at position 25 15 is L, the amino acid at position 77 is R, and the amino acid at position 81 is Q. In a preferred embodiment, said designed ankyrin repeat domain consists of an amino acid sequence of 91 amino acids. Preferably, said position numbers are determined by alignment to SEQ ID NO: 95 using the position numbers of SEQ ID NO: 95. Preferably, said 30 alignment comprises no amino acid gaps. Sequence alignment generation is a procedure well known in the art.

In one embodiment, the designed ankyrin repeat domain of the invention consists of an amino acid sequence wherein the amino acid at position 8 is Q, the amino acid at position 15 is L, the amino acid at position 176 is R, and the amino acid at position 180 is Q. In a 35 preferred embodiment, said designed ankyrin repeat domain consists of an amino acid sequence of 190 amino acids. Preferably, said position numbers are determined by

alignment to SEQ ID NO: 98 using the position numbers of SEQ ID NO: 98. Preferably, said alignment comprises no amino acid gaps. Sequence alignment generation is a procedure well known in the art.

- In a further embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which differs from SEQ ID NO: 104 by at least one, preferably at least two, more preferably at least three amino acids, and by up to 30, up to 25, up to 20, up to 15, up to 14, up to 13, up to 12, up to 11, up to ten, up to 9, up to 8, up to 7, up to 6, up to 5, preferably up to 4 amino acids, outside the positions comprising X, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 104 by the amino acids at positions 15 and 114, more preferably positions 8, 15, and 114, more preferably positions 8, 15, 110, and 114, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 104 by the amino acids at positions 15 and 114, more preferably positions 8, 15, and 114, more preferably positions 8, 15, 110, and 114, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 110 is R, and said amino acid at position 114 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 104 by the amino acids at positions 4, 8, 15, 17, 20, 23, 99, 100, 102, 110, 114, 115, 118, and 122, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 104 by the amino acids at positions 4, 8, 15, 17, 20, 23, 99, 100, 102, 110, 114, 115, 118, and 122, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 99 is T, said amino acid at position 100 is Q, said amino acid at position 102 is T, said amino acid at position 110 is R, said amino acid at position 114 is Q, said amino acid at position 115 is Q, said amino acid at position 118 is S, and said amino acid at position 122 is Q, and wherein X represents any amino acid.
- In one embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which differs from SEQ ID NO: 105 by at least one, preferably at least two, more preferably at least three amino acids, and by up to 30, up to 25, up to 20, up to 15, up to 14, up to 13, up to 12, up to 11, up to ten, up to 9, up to 8, up to 7, up to 6, up to 5, preferably up to 4 amino acids, outside the positions comprising X, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 105 by the amino acids at positions

15 and 147, more preferably positions 8, 15, and 147, more preferably positions 8, 15, 143, and 147, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 105 by the amino acids at positions 15 and 147, more preferably positions 8, 14, and 147, more preferably positions 8, 15, 143, and 147, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 143 is R, and said amino acid at position 147 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 105 by the amino acids at positions 4, 8, 15, 17, 20, 23, 132, 133, 135, 143, 147, 148, 151, and 155, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 105 by the amino acids at positions 4, 8, 15, 17, 20, 23, 132, 133, 135, 143, 147, 148, 151, and 155, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 132 is T, said amino acid at position 133 is Q, said amino acid at position 135 is T, said amino acid at position 143 is R, said amino acid at position 147 is Q, said amino acid at position 148 is Q, said amino acid at position 151 is S, and said amino acid at position 155 is Q, and wherein X represents any amino acid.

20 In one embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which differs from SEQ ID NO: 103 by at least one, preferably at least two, more preferably at least three amino acids, and by up to 30, up to 25, up to 20, up to 15, up to 14, up to 13, up to 12, up to 11, up to ten, up to 9, up to 8, up to 7, up to 6, up to 5, preferably up to 4 amino acids, outside the positions comprising X, and wherein X represents any amino acid.

25 In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 103 by the amino acids at positions 15 and 81, more preferably positions 8, 15, and 81, more preferably positions 8, 15, 77, and 81, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 103 by the amino acids at positions 15 and 81, more preferably positions 8, 15, and 81, more preferably positions 8, 15, 77, and 81, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 77 is R, and said amino acid at position 81 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 103 by the amino acids at positions 4, 8, 15, 17, 20, 23, 66, 67, 69, 77, 81, 82, 85, and 89, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino

acid sequence which differs from SEQ ID NO: 103 by the amino acids at 4, 8, 15, 17, 20, 23, 66, 67, 69, 77, 81, 82, 85, and 89, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 66 is T, said amino acid at position 67 is Q, said amino acid at position 69 is T, said amino acid at position 77 is R, said amino acid at position 81 is Q, said amino acid at position 82 is Q, said amino acid at position 85 is S, and said amino acid at position 89 is Q, and wherein X represents any amino acid.

In one embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which differs from SEQ ID NO: 106 by at least one, preferably at least two, more preferably at least three amino acids, and by up to 30, up to 25, up to 20, up to 15, up to 14, up to 13, up to 12, up to 11, up to ten, up to 9, up to 8, up to 7, up to 6, up to 5, preferably up to 4 amino acids, outside the positions comprising X, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 106 by the amino acids at positions 15 and 180, more preferably positions 8, 15, and 180, more preferably positions 8, 15, 176, and 180, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 106 by the amino acids at positions 15 and 180, more preferably positions 8, 15, and 180, more preferably positions 8, 15, 176, and 180, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 176 is R, and said amino acid at position 180 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 106 by the amino acids at positions 4, 8, 15, 17, 20, 23, 66, 67, 69, 77, 81, 82, 85, and 89, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which differs from SEQ ID NO: 106 by the amino acids at 4, 8, 15, 17, 20, 23, 165, 166, 168, 176, 180, 181, 184, and 188, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 165 is T, said amino acid at position 166 is Q, said amino acid at position 168 is T, said amino acid at position 176 is R, said amino acid at position 180 is Q, said amino acid at position 181 is Q, said amino acid at position 184 is S, and said amino acid at position 188 is Q, and wherein X represents any amino acid.

In a further embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids

at positions 15 and 114. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 8, 15, and 114. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 8, 15, 110 and 114. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 99, 100, 102, 110, 114, 115, 118, and 122. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 99, 100, 102, 110, 114, 115, 118, and 122, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 99 is T, said amino acid at position 100 is Q, said amino acid at position 102 is T, said amino acid at position 110 is R, said amino acid at position 114 is Q, said amino acid at position 115 is Q, said amino acid at position 118 is S, and said amino acid at position 122 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 8, 15, 110 and 114, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 110 is R, and said amino acid at position 114 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 8, 15, and 114, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, and said amino acid at position 114 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 104, with the exception of the amino acids at positions 15 and 114, and wherein said amino acid at position 15 is L, and said amino acid at position 114 is Q, and wherein X represents any amino acid.

In one embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 15 and 147. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 8, 15 and 147. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 8, 15, 143 and 147. In one embodiment, said designed ankyrin

repeat domain has an amino acid sequence which is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 132, 133, 135, 143, 147, 148, 151, and 155. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 132, 133, 135, 143, 147, 148, 151, and 155, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 132 is T, said amino acid at position 133 is Q, said amino acid at position 135 is T, said amino acid at position 143 is R, said amino acid at position 147 is Q, said amino acid at position 148 is Q, said amino acid at position 151 is S, and said amino acid at position 155 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 8, 15, 143 and 147, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 143 is R, and said amino acid at position 147 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 8, 15 and 147 and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, and said amino acid at position 147 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain is identical to SEQ ID NO: 105, with the exception of the amino acids at positions 15 and 147, and wherein said amino acid at position 15 is L, and said amino acid at position 147 is Q, and wherein X represents any amino acid.

In one embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 15 and 81. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 8, 15 and 81. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 8, 15, 77 and 81. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 66, 67, 69, 77, 81, 82, 85, and 89. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 66, 67, 69, 77, 81, 82, 85, and 89, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino

acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 66 is T, said amino acid at position 67 is Q, said amino acid at position 69 is T, said amino acid at position 77 is R, said amino acid at position 81 is Q, said amino acid at position 82 is Q, said amino acid at position 85 is S, and said amino acid at position 89 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 8, 15, 77 and 81, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 77 is R, and said amino acid at position 81 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 8, 15 and 81, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, and said amino acid at position 81 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain is identical to SEQ ID NO: 103, with the exception of the amino acids at positions 15 and 81, and wherein said amino acid at position 15 is L, and said amino acid at position 81 is Q, and wherein X represents any amino acid.

In one embodiment, the designed ankyrin repeat domain of the invention has an amino acid sequence which is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 15 and 180. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 8, 15 and 180. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 8, 15, 176 and 180. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 165, 166, 168, 176, 180, 181, 184, and 188. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 4, 8, 15, 17, 20, 23, 165, 166, 168, 176, 180, 181, 184, and 188, and wherein said amino acid at position 4 is S, said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 17 is T, said amino acid at position 20 is T, said amino acid at position 23 is Q, said amino acid at position 165 is T, said amino acid at position 166 is Q, said amino acid at position 168 is T, said amino acid at position 176 is R, said amino acid at position 180 is Q, said amino acid at position 181 is Q, said amino acid at position 184 is S, and said amino acid at position 188 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain has an amino acid sequence which is identical to SEQ ID NO: 106, with the exception of the amino acids at

positions 8, 15, 176 and 180, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, said amino acid at position 176 is R, and said amino acid at position 180 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 8, 15 and 180, and wherein said amino acid at position 8 is Q, said amino acid at position 15 is L, and said amino acid at position 180 is Q, and wherein X represents any amino acid. In one embodiment, said designed ankyrin repeat domain is identical to SEQ ID NO: 106, with the exception of the amino acids at positions 15 and 180, and wherein said amino acid at position 15 is L, and said amino acid at position 180 is Q, and wherein X represents any amino acid.

In a further embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module having the amino acid sequence

X₁X₂X₃X₄X₅X₆X₇QX₈X₉X₁₀X₁₁X₁₂X₁₃LX₁₄X₁₅X₁₆X₁₇X₁₈X₁₉X₂₀X₂₁X₂₂GADVNA (SEQ ID NO: 81), wherein

- 15 X₁ represents any amino acid, preferably D;
X₂ represents any amino acid, preferably L;
X₃ represents any amino acid, preferably G;
X₄ represents any amino acid, preferably K or S;
X₅ represents any amino acid, preferably K;
- 20 X₆ represents any amino acid, preferably L;
X₇ represents any amino acid, preferably L;
X₈ represents any amino acid, preferably A;
X₉ represents any amino acid, preferably A;
X₁₀ represents any amino acid, preferably R;
- 25 X₁₁ represents any amino acid, preferably A;
X₁₂ represents any amino acid, preferably G;
X₁₃ represents any amino acid, preferably Q;
X₁₄ represents any amino acid, preferably D;
X₁₅ represents any amino acid, preferably E or T;
- 30 X₁₆ represents any amino acid, preferably V;
X₁₇ represents any amino acid, preferably R;
X₁₈ represents any amino acid, preferably E or T;
X₁₉ represents any amino acid, preferably L;
X₂₀ represents any amino acid, preferably L;
- 35 X₂₁ represents any amino acid, preferably K or Q; and
X₂₂ represents any amino acid, preferably A.

In one embodiment, X₄ is S. In one embodiment, X₁₅ is T. In one embodiment, X₁₈ is T. In one embodiment, X₂₁ is Q. In one embodiment, X₄ is S, X₁₅ is T, X₁₈ is T, and X₂₁ is Q. In a preferred embodiment, X₆ is L, X₇ is L, X₈ is A, X₉ is A, and X₁₂ is G (SEQ ID NO: 80). In a more preferred embodiment, X₁ is D, X₂ is L, X₃ is G, X₆ is L, X₇ is L, X₈ is A, X₉ is A, X₁₂ is G, X₁₃ is Q, X₁₄ is D, X₁₆ is V, X₁₇ is R and X₁₉ is L (SEQ ID NO: 79). In an even more preferred embodiment X₁ is D, X₂ is L, X₃ is G, X₅ is K, X₆ is L, X₇ is L, X₈ is A, X₉ is A, X₁₂ is G, X₁₃ is Q, X₁₄ is D, X₁₆ is V, X₁₇ is R, X₁₉ is L, X₂₀ is L and X₂₂ is A (SEQ ID NO: 78). In another even more preferred embodiment X₁ is D, X₂ is L, X₃ is G, X₅ is K, X₆ is L, X₇ is L, X₈ is A, X₉ is A, X₁₂ is G, X₁₃ is Q, X₁₄ is D, X₁₅ is E, X₁₆ is V, X₁₇ is R, X₁₈ is E, X₁₉ is L, X₂₀ is L, X₂₁ is K and X₂₂ is A (SEQ ID NO: 77). In a most preferred embodiment, X₁ is D, X₂ is L, X₃ is G, X₄ is S, X₅ is K, X₆ is L, X₇ is L, X₈ is A, X₉ is A, X₁₀ is R, X₁₁ is A, X₁₂ is G, X₁₃ is Q, X₁₄ is D, X₁₅ is T, X₁₆ is V, X₁₇ is R, X₁₈ is T, X₁₉ is L, X₂₀ is L, X₂₁ is Q, and X₂₂ is A (SEQ ID NO: 76). In an alternative most preferred embodiment, X₁ is D, X₂ is L, X₃ is G, X₄ is K, X₅ is K, X₆ is L, X₇ is L, X₈ is A, X₉ is A, X₁₀ is R, X₁₁ is A, X₁₂ is G, X₁₃ is Q, X₁₄ is D, X₁₅ is E, X₁₆ is V, X₁₇ is R, X₁₈ is E, X₁₉ is L, X₂₀ is L, X₂₁ is K, and X₂₂ is A (SEQ ID NO: 75).

In a further embodiment, the designed ankyrin repeat domain of the invention comprises an N-terminal capping module comprising the amino acid sequence

DLGX₁X₂LLQAAX₃X₄GQLDX₅VRX₆LX₇X₈X₉ (SEQ ID NO: 111), wherein

- X₁ represents any amino acid, preferably K or S;
- X₂ represents any amino acid, preferably K;
- X₃ represents any amino acid, preferably R;
- X₄ represents any amino acid, preferably A;
- X₅ represents any amino acid, preferably E or T;
- X₆ represents any amino acid, preferably E or T;
- X₇ represents any amino acid, preferably L;
- X₈ represents any amino acid, preferably K or Q; and
- X₉ represents any amino acid, preferably A.

In one embodiment, X₁ is S. In one embodiment, X₅ is T. In one embodiment, X₆ is T. In one embodiment, X₈ is Q. In one embodiment, X₁ is S, X₅ is T, X₆ is T, and X₈ is Q. In a preferred embodiment, X₂ is K, X₇ is L, and X₉ is A (SEQ ID NO: 110). In a more preferred embodiment, X₂ is K, X₅ is E, X₆ is E, X₇ is L, and X₉ is A (SEQ ID NO: 109). In a most preferred embodiment X₁ is S, X₂ is K, X₃ is R, X₄ is A, X₅ is T, X₆ is T, X₇ is L, X₈ is Q, and X₉ is A (SEQ ID NO: 108). In an alternative most preferred embodiment, X₁ is K, X₂ is K, X₃ is R, X₄ is A, X₅ is E, X₆ is E, X₇ is L, X₈ is K, and X₉ is A (SEQ ID NO: 107).

In a further embodiment, the designed ankyrin repeat domain of the invention comprises a C-terminal capping module having the amino acid sequence

$X_1DX_2X_3GX_4TPX_5X_6X_7X_8X_9RX_{10}X_{11}X_{12}QX_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}$, wherein

- X_1 represents any amino acid, preferably Q;
- X_2 represents any amino acid, preferably K or T;
- X_3 represents any amino acid, preferably S or Q;
- 5 X_4 represents any amino acid, preferably K or T;
- X_5 represents any amino acid, preferably A;
- X_6 represents any amino acid, preferably D;
- X_7 represents any amino acid, preferably L;
- X_8 represents any amino acid, preferably A;
- 10 X_9 represents any amino acid, preferably A;
- X_{10} represents any amino acid, preferably A;
- X_{11} represents any amino acid, preferably G;
- X_{12} represents any amino acid, preferably H;
- X_{13} represents any amino acid, preferably D or Q;
- 15 X_{14} represents any amino acid, preferably I;
- X_{15} represents any amino acid, preferably A;
- X_{16} represents any amino acid, preferably E or S;
- X_{17} represents any amino acid, preferably V;
- X_{18} represents any amino acid, preferably L;
- 20 X_{19} represents any amino acid, preferably Q;
- X_{20} represents any amino acid, preferably K or Q;
- X_{21} represents any amino acid, preferably A; and
- X_{22} represents any amino acid, preferably A.

In one embodiment, X_2 is T. In one embodiment, X_3 is Q. In one embodiment, X_4 is T. In
 25 one embodiment, X_{13} is Q. In one embodiment, X_{16} is S. In one embodiment, X_{20} is Q. In
 one embodiment, X_2 is T, X_3 is Q, X_4 is T, X_{13} is Q, X_{16} is S, and X_{20} is Q. In a preferred
 embodiment, X_{21} is A and X_{22} is A (SEQ ID NO: 94). In another preferred embodiment, X_9
 is A (SEQ ID NO: 93). In a more preferred embodiment, X_8 is A, X_9 is A, X_{11} is G, X_{21} is A
 and X_{22} is A (SEQ ID NO: 92). In an even more preferred embodiment X_5 is A, X_6 is D, X_8
 30 is A, X_9 is A, X_{11} is G, X_{12} is H, X_{14} is I, X_{15} is A, X_{17} is V, X_{18} is L, X_{19} is Q, X_{21} is A and X_{22}
 is A (SEQ ID NO: 91). In another even more preferred embodiment X_5 is A, X_6 is D, X_8 is A,
 X_9 is A, X_{11} is G, X_{12} is H, X_{13} is D, X_{14} is I, X_{15} is A, X_{16} is E, X_{17} is V, X_{18} is L, X_{19} is Q, X_{20}
 is K, X_{21} is A and X_{22} is A (SEQ ID NO: 90). In a most preferred embodiment, X_1 is Q, X_2 is
 35 T, X_3 is Q, X_4 is T, X_5 is A, X_6 is D, X_7 is L, X_8 is A, X_9 is A, X_{10} is A, X_{11} is G, X_{12} is H, X_{13} is
 Q, X_{14} is I, X_{15} is A, X_{16} is S, X_{17} is V, X_{18} is L, X_{19} is Q, X_{20} is Q, X_{21} is A and X_{22} is A (SEQ
 ID NO: 89). In an alternative most preferred embodiment, X_1 is Q, X_2 is K, X_3 is S, X_4 is K,

X₅ is A, X₆ is D, X₇ is L, X₈ is A, X₉ is A, X₁₀ is A, X₁₁ is G, X₁₂ is H, X₁₃ is D, X₁₄ is I, X₁₅ is A, X₁₆ is E, X₁₇ is V, X₁₈ is L, X₁₉ is Q, X₂₀ is K, X₂₁ is A and X₂₂ is A (SEQ ID NO: 88).

The designed ankyrin repeat domains consisting of SEQ ID NOs: 10, 11, 13, 14, 15, 17, 18,
5 19, 21, 22, and 23 are examples of such designed ankyrin repeat domains of the invention.

In a second aspect, the invention provides a protein comprising one or more designed ankyrin repeat domains of the invention. In a preferred embodiment, said protein is a recombinant binding protein. In one embodiment, said protein comprises one, two, three,
10 four or five designed ankyrin repeat domains of the invention. In the context of the present invention, when the protein of the invention comprises more than one designed ankyrin repeat domain of the invention, each of said designed ankyrin repeat domains may be independently selected among any one of the designed ankyrin repeat domains of the invention described herein. In one embodiment, said protein further comprises at least one
15 moiety for half-life extension. In one preferred embodiment, said moiety for half-life extension is a designed ankyrin repeat domain with binding specificity for serum albumin. In one embodiment, said protein further comprises one, two or three designed ankyrin repeat domains with binding specificity for serum albumin. In the context of the present invention, when the protein of the invention comprises more than one designed ankyrin
20 repeat domains with binding specificity for serum albumin, each of said designed ankyrin repeat domains may be independently selected among any one of the designed ankyrin repeat domains with binding specificity for serum albumin described herein.

In one embodiment, said protein comprises (i) at least one designed ankyrin repeat domain of the invention, and (ii) at least one moiety for half-life extension. Such moieties for half-life
25 extension are well-known in the art and comprise, amongst others, polyethylene-glycol (PEG), serum albumin-binding polypeptides, serum albumin-binding proteins, serum albumin, and immunoglobulin Fc fragments. In one preferred embodiment, a moiety for half-life extension is a designed ankyrin repeat domain with binding specificity for serum albumin.

30 In one embodiment, said protein comprises (i) at least one designed ankyrin repeat domain of the invention, and (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin.

In one embodiment, said protein comprises (i) at least one designed ankyrin repeat domain of the invention, and (ii) at least one designed ankyrin repeat domain with binding specificity
35 for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 80%, 81%, 82%, 83%,

84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 80% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 85% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 90% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 92% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 94% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 96% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 98% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, preferably SEQ ID NOs: 4 to 7, more preferably SEQ ID NOs: 4 to 6. In one embodiment, said designed ankyrin repeat domain with binding specificity for serum albumin does not comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81, and 88 to 94, wherein X represents any amino acid.

In one embodiment, said protein further comprises at least one peptide linker. In one particular embodiment, the protein of the invention comprises (i) at least one designed ankyrin repeat domain of the invention, (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin as described herein, and (iii) at least one peptide linker.

In one embodiment, said peptide linker is a proline-threonine rich peptide linker or a glycine-serine rich peptide linker. In one embodiment, said peptide linker is a proline-threonine rich peptide linker. In one embodiment, said peptide linker is a glycine-serine rich peptide linker. In one preferred embodiment, said peptide linker has the amino acid sequence of SEQ ID NO: 2 or 3. In one embodiment, said peptide linker has the amino acid sequence of SEQ ID NO: 2. In one embodiment, said peptide linker has the amino acid sequence of SEQ ID NO: 3.

The proteins consisting of SEQ ID NOs: 25, 26, 28, 29, 30, 32, 33, 34, 36, 37, 38, 40, 41, 43, 44, 45, 47, 48, 49, 51, 52, 53, 55, 56, 58, 59, 60, 62, 63, 64, 66, 67, and 68 are examples of such proteins of the invention.

In one embodiment, said protein comprises at least two designed ankyrin repeat domains of the invention. In one embodiment, said protein comprises (i) at least two designed ankyrin repeat domains of the invention, and (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin. In a preferred embodiment, said protein comprises (i) at least two designed ankyrin repeat domains of the invention, and (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin as described herein. In a preferred embodiment, said protein comprises (i) at least two designed ankyrin repeat domains of the invention, and (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin has an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, more preferably 4 to 6. In one embodiment, said protein comprises (i) at least two designed ankyrin repeat domains of the invention, and (ii) at least two designed ankyrin repeat domains with binding specificity for serum albumin. In a preferred embodiment, said recombinant binding protein comprises (i) at least two designed ankyrin repeat domains of the invention, and (ii) at least two designed ankyrin repeat domains with binding specificity for serum albumin as described herein. In a preferred embodiment, said recombinant binding protein comprises (i) at least two designed ankyrin repeat domains of the invention, and (ii) at least two designed ankyrin repeat domains with binding specificity for serum albumin, wherein each of said designed ankyrin repeat domains with binding specificity for serum albumin independently has an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, more preferably 4 to 6. In one preferred embodiment, the protein of the invention is a recombinant binding protein.

The designed ankyrin repeat domain provided by the present invention and described herein comprises sequence modifications that lead to improved pharmacokinetic properties of said designed ankyrin repeat domain compared to the designed ankyrin repeat domain not comprising said sequence modifications. Moreover, said sequences modifications lead to improved pharmacokinetic properties of a protein comprising said designed ankyrin repeat domain, compared to a comparator protein which comprises the designed ankyrin repeat domain not comprising said sequence modifications and which is otherwise identical to said protein. The designed ankyrin repeat domains consisting of SEQ ID NOs: 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, and 23 are examples of such designed ankyrin repeat domains with improved pharmacokinetic properties. The proteins consisting of SEQ ID NOs: 25, 26, 28, 29, 30, 32, 33, 34, 36, 37, 38, 40, 41, 43, 44, 45, 47, 48, 49, 51, 52, 53, 55, 56, 58, 59, 60, 62, 63, 64, 66, 67, and 68 are examples of proteins comprising such designed ankyrin repeat domains with improved pharmacokinetic properties.

The designed ankyrin repeat domains consisting of SEQ ID NOs: 10 and 11 are examples of designed ankyrin repeat domains comprising such sequence modifications (compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 9, which does not comprise such sequence modifications), which exhibit improved pharmacokinetic properties, as shown by the pharmacokinetic profiles of Proteins #25 and #26 (comprising SEQ ID NOs: 10 and 11, respectively) in comparison to Protein #24 (comprising SEQ ID NO: 9). Likewise, the designed ankyrin repeat domains consisting of SEQ ID NOs: 13 to 15 are examples of designed ankyrin repeat domains comprising such sequence modifications (compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 12, which does not comprise such sequence modifications), which exhibit improved pharmacokinetic properties, as shown by the pharmacokinetic profiles of Proteins #28, #29 and #30 (comprising SEQ ID NOs: 13, 14 and 15, respectively) in comparison to Protein #27 (comprising SEQ ID NO: 12). Likewise, the designed ankyrin repeat domains consisting of SEQ ID NOs: 17 to 19 are examples of designed ankyrin repeat domains comprising such sequence modifications (compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 16, which does not comprise such sequence modifications), which exhibit improved pharmacokinetic properties, as shown by the pharmacokinetic profiles of Proteins #32, #33 and #34 (comprising SEQ ID NOs: 17, 18 and 19, respectively) in comparison to Protein #31 (comprising SEQ ID NO: 16). Likewise, the designed ankyrin repeat domains consisting of SEQ ID NOs: 21 to 23 are examples of designed ankyrin repeat domains comprising such sequence modifications (compared to the designed ankyrin repeat domain consisting of SEQ ID NO: 20, which does not comprise such sequence modifications), which exhibit

improved pharmacokinetic properties, as shown by the pharmacokinetic profiles of Proteins #36, #37 and #38 (comprising SEQ ID NOs: 21, 22 and 23, respectively) in comparison to Protein #35 (comprising SEQ ID NO: 20). These examples are described in detail in the Examples.

5 In one embodiment, the term improved pharmacokinetic properties refers to an increased area under the curve, a reduced clearance, or an increased terminal half-life.

In one embodiment, the term improved pharmacokinetic properties refers to an increased area under the curve. In one embodiment, said increase in area under the curve is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%,

10 most preferably 85%.

In one embodiment, the term improved pharmacokinetic properties refers to a reduced clearance. In one embodiment, said reduction in clearance is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, most preferably 45%.

15 In one embodiment, the term improved pharmacokinetic properties refers to an increased terminal half-life. In one embodiment, said increase in terminal half-life is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, most preferably 85%.

20 In one embodiment the pharmacokinetic parameters are determined in mouse. Preferably, said pharmacokinetic parameters in mouse are determined by applying a protein at a dose of 1 mg/kg by intravenous injection into the tail vein of Balb/c mice. This procedure is described in Example 6.

25 In one embodiment the pharmacokinetic parameters are determined in cynomolgus monkey. Preferably, said pharmacokinetic parameters in cynomolgus monkey are determined by applying a protein at a dose of 1 mg/kg by 30 min intravenous injection. This procedure is described in Example 7.

30 In one embodiment, the pharmacokinetic properties of a designed ankyrin repeat domain of the invention are assessed by measuring the pharmacokinetic properties of a protein comprising (i) said designed ankyrin repeat domain of the invention, and (ii) a moiety for half-life extension, and by comparing the pharmacokinetic properties of said protein with a comparator protein as described herein. Preferably, said moiety for half-life extension is a designed ankyrin repeat domain with binding specificity for serum albumin. Proteins #25, #26, #28, #29, #30, #32, #33, #34, #36, #37, #38, #40, #41, #43, #44, #45, #47, #48, #49, #51, #52, #53, #55, #56, #58, #59, #60, #62, #63, #64, #66, #67, and #68 are examples of such proteins. Proteins #24, #27, #31, #35, #39, #42, #46, #46, #50, #54, #57, #61, and
35 #65 are examples of such comparator proteins. Examples of designed ankyrin repeat domains with binding specificity for serum albumin are the designed ankyrin repeat domains

consisting of SEQ ID NOs: 4 to 8. In one embodiment, the designed ankyrin repeat domain with binding specificity for serum albumin is N-terminal of the designed ankyrin repeat domain of the invention. In one embodiment, said improved pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising (i) a
5 designed ankyrin repeat domain of the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin consists of an amino acid sequence with at least 80% amino acid sequence identify with any of SEQ ID NOs: 4 to 8, preferably 4 to 6, more preferably 4, and by comparing the pharmacokinetic properties of said protein with a
10 comparator protein as described herein.

In one embodiment, said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising (i) a designed ankyrin repeat domain of the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum
15 albumin consists of the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identify with SEQ ID NO: 4, and by comparing the pharmacokinetic properties of said protein with a comparator protein as described herein. In one embodiment, said pharmacokinetic properties are
20 assessed by measuring the pharmacokinetic properties of a protein comprising (i) a designed ankyrin repeat domain of the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin consists of the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%,
25 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identify with SEQ ID NO: 4, and (iii) a polypeptide linker consisting of the amino acid sequence of SEQ ID NO: 3, and by comparing the pharmacokinetic properties of said protein with a comparator protein as described herein. In one embodiment, said
30 pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising from N-terminus to C-terminus (i) SEQ ID NO: 4, (ii) SEQ ID NO: 3, and (iii) a designed ankyrin repeat domain of the invention, and by comparing the pharmacokinetic properties of said protein with a comparator protein as described herein.

In one embodiment, said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising (i) a designed ankyrin repeat domain of
35 the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum

albumin consists of the amino acid sequence of SEQ ID NO: 5 or an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identify with SEQ ID NO: 5, and by comparing the pharmacokinetic properties of said protein with a comparator

5 protein as described herein. In one embodiment, said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising (i) a designed ankyrin repeat domain of the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin consists of the amino acid sequence of SEQ ID

10 NO: 5 or an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identify with SEQ ID NO: 5, and (iii) a polypeptide linker consisting of the amino acid sequence of SEQ ID NO: 3, and by comparing the pharmacokinetic properties of said protein with a comparator protein as described herein. In one embodiment, said

15 pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising from N to C terminus (i) SEQ ID NO: 5, (ii) SEQ ID NO: 3, (iii) a designed ankyrin repeat domain of the invention.

In one embodiment, said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising (i) a designed ankyrin repeat domain of

20 the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin consists of the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identify with SEQ ID NO:

25 6, and by comparing the pharmacokinetic properties of said protein with a comparator protein as described herein. In one embodiment, said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein comprising (i) a designed ankyrin repeat domain of the invention, and (ii) a designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain

30 with binding specificity for serum albumin consists of the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identify with SEQ ID NO: 6, and (iii) a polypeptide linker consisting of the amino acid sequence of SEQ ID NO: 3, and by comparing the pharmacokinetic properties of said

35 protein with a comparator protein as described herein. In one embodiment, said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of

a protein comprising from N to C terminus (i) SEQ ID NO: 6, (ii) SEQ ID NO: 3, (iii) a designed ankyrin repeat domain of the invention.

In a further embodiment, the designed ankyrin repeat domain of the invention, exhibits improved pharmacokinetic properties compared to a comparator designed ankyrin repeat domain,

wherein said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain have the identical amino acid sequence with the exception that

said designed ankyrin repeat domain comprises (i) in the N-terminal capping module the amino acid Q at position 8 and the amino acid L at position 15, and (ii) in the C-terminal capping module the amino acid R at position 14 and the amino acid Q at position 18,

and said comparator designed ankyrin repeat domain comprises (i) in the N-terminal capping module amino acids different from Q at position 8 and D at position 15, and (ii) in the C-terminal capping module amino acids different from R at position 14 and E at position 18,

wherein said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ ID NO: 69, wherein said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82, and wherein said alignment comprises no amino acid gaps, and

wherein said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein and a comparator protein,

wherein said protein from N to C terminus consists of (i) a designed ankyrin repeat domain with binding specificity for serum albumin selected from the group consisting of SEQ ID NOs: 4 to 8, (ii) a polypeptide linker selected from the group consisting of SEQ ID NOs: 2 to 3, and (iii) said designed ankyrin repeat domain, and

wherein said comparator protein from N to C terminus consists of (i) a designed ankyrin repeat domain with binding specificity for serum albumin selected from the group consisting of SEQ ID NOs: 4 to 8, (ii) a polypeptide linker selected from the group consisting of SEQ ID NOs: 2 to 3, and (iii) said comparator designed ankyrin repeat domain, and

wherein the amino acid sequences of said designed ankyrin repeat domains with binding specificity for serum albumin of said protein and said comparator

protein are identical, and wherein the amino acid sequences of said polypeptide linkers of said protein and said comparator protein are identical. In one embodiment, the designed ankyrin repeat domain of the invention exhibits improved pharmacokinetic properties compared to a comparator designed ankyrin repeat domain,

5 wherein said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain have the identical amino acid sequence with the exception that said designed ankyrin repeat domain comprises (i) in the N-terminal capping module the amino acid Q at position 8 and the amino acid L at position 15, and (ii) in the C-terminal capping module the amino acid R at position 14 and

10 the amino acid Q at position 18, and said comparator designed ankyrin repeat domain comprises (i) in the N-terminal capping module the amino acid E at position 8 and the amino acid D at position 15, and (ii) in the C-terminal capping module the amino acid D at position 14 and the amino acid E at position 18,

15 wherein said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ ID NO: 69, wherein said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82, and wherein said alignment

20 comprises no amino acid gaps, and wherein said pharmacokinetic properties are assessed by measuring the pharmacokinetic properties of a protein and a comparator protein,

wherein said protein from N to C terminus consists of (i) a designed ankyrin repeat domain with binding specificity for serum albumin selected from the

25 group consisting of SEQ ID NOs: 4 to 8, (ii) a polypeptide linker selected from the group consisting of SEQ ID NOs: 2 to 3, and (iii) said designed ankyrin repeat domain, and wherein said comparator protein from N to C terminus consists of (i) a designed ankyrin repeat domain with binding specificity for serum albumin selected from the group consisting of SEQ ID NOs: 4 to 8, (ii) a polypeptide linker selected from the group consisting of SEQ ID NOs: 2 to 3, and (iii) said

30 comparator designed ankyrin repeat domain, and wherein the amino acid sequences of said designed ankyrin repeat domains with binding specificity for serum albumin of said protein and said comparator protein are identical, and wherein the amino acid sequences of said

35 polypeptide linkers of said protein and said comparator protein are identical.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety has (i) in the N-terminal capping module the amino acid E at position 8 and the amino acid D at position 15, and (ii) in the C-terminal capping module the amino acid D at position 14 and the amino acid E at position 18,

15 wherein said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ ID NO: 69, wherein said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82, and wherein said alignment comprises no amino acid gaps.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

25 wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

30 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

35 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety has (i) in the N-terminal

capping module the amino acid E at position 8 and the amino acid D at position 15, and (ii) in the C-terminal capping module the amino acid D at position 14 and the amino acid E at position 18,

5 wherein said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ ID NO: 69, wherein said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82, and wherein said alignment comprises no amino acid gaps.

10 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

15 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

20 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety has (i) in the N-terminal capping module the amino acid E at position 8 and the amino acid D at position 15, and (ii) in the C-terminal capping module the amino acid D at position 14 and the amino acid E at position 18,

25 wherein said position numbers of positions of the N-terminal capping module are determined by alignment to SEQ ID NO: 69 using the position numbers of SEQ ID NO: 69, wherein said position numbers of positions of the C-terminal capping module are determined by alignment to SEQ ID NO: 82 using the position numbers of SEQ ID NO: 82, and wherein said alignment comprises no amino acid gaps.

30 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

35 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with any one of SEQ ID NOs: 4 to 8, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises (i) any one of SEQ ID NOs: 75 to 81 and 107 to 111, preferably SEQ ID NOs: 75 to 80 and 107 to 111, more preferably SEQ ID NOs: 75 to 79 and 107 to 111, more preferably SEQ ID NOs: 75 to 78 and 107 to 110, more preferably SEQ ID NOs: 75 to 77 and 107 to 109, more preferably SEQ ID NOs: 75 to 76 and 107 to 108, more preferably SEQ ID NOs: 75 and 107, and (ii) any one of SEQ ID NOs: 88 or 94, preferably SEQ ID NOs: 88 to 93, more preferably SEQ ID NOs: 88 to 92, more preferably SEQ ID NOs: 88 to 91, more preferably SEQ ID NOs: 88 to 90, more preferably SEQ ID NOs: 88 to 89, more preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and 107 to 111 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 94.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises (i) any one of SEQ ID NOs: 75 to 81 and 107 to 111, preferably SEQ ID NOs: 75 to 80 and 107 to 111, more preferably SEQ ID NOs: 75 to 79 and 107 to 111, more preferably SEQ ID NOs: 75 to 78 and 107 to 110, more preferably SEQ ID NOs: 75 to 77 and 107 to 109, more preferably SEQ ID NOs: 75 to 76 and 107 to 108, more preferably SEQ ID NO: 75 and 107, and (ii) any one of SEQ ID

NOs: 88 or 94, preferably SEQ ID NOs: 88 to 93, more preferably SEQ ID NOs: 88 to 92, more preferably SEQ ID NOs: 88 to 91, more preferably SEQ ID NOs: 88 to 90, more preferably SEQ ID NOs: 88 to 89, more preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 94.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO:

5, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises (i) any one of SEQ ID NOs: 75 to 81 and 107 to 111, preferably SEQ ID NOs: 75 to 80 and 107 to 111, more preferably SEQ ID NOs: 75 to 79 and 107 to 111, more preferably SEQ ID NOs: 75 to 78 and 107 to 110, more preferably SEQ ID NOs: 75 to 77 and 107 to 109, more preferably SEQ ID NOs: 75 to 76 and 107 to 108, more preferably SEQ ID NO: 75 and 107, and (ii) any one of SEQ ID NOs: 88 or 94, preferably SEQ ID NOs: 88 to 93, more preferably SEQ ID NOs: 88 to 92, more preferably SEQ ID NOs: 88 to 91, more preferably SEQ ID NOs: 88 to 90, more preferably SEQ ID NOs: 88 to 89, more preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 94.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

5 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises (i) any one of SEQ ID NOs: 75 to 81 and 107 to 111, preferably SEQ ID NOs: 75 to 80 and 107 to 111, more preferably SEQ ID NOs: 75 to 79 and 107 to 111, more preferably SEQ ID NOs: 75 to 78 and 107 to 110, more preferably SEQ ID NOs: 75 to 77 and 107 to 109, more preferably SEQ ID NOs: 75 to 76 and 107 to 108, more preferably SEQ ID NO: 75 and 107, and (ii) any one of SEQ ID NOs: 88 or 94, preferably SEQ ID NOs: 88 to 93, more preferably SEQ ID NOs: 88 to 92, more preferably SEQ ID NOs: 88 to 91, more preferably SEQ ID NOs: 88 to 90, more preferably SEQ ID NOs: 88 to 89, more preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 94.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NOs: 75 or 76, preferably SEQ ID NO: 75, and SEQ ID NOs: 88 or 89, preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 75 or 76 and SEQ ID NO: 82 instead of SEQ ID NO: 88 or 89.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NOs: 75 or 76, preferably SEQ ID NO: 75, and SEQ ID
15 NOs: 88 or 89, preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 75 or 76 and SEQ ID NO: 82 instead of SEQ ID NO: 88 or 89.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NOs: 75 or 76, preferably SEQ ID NO: 75, and SEQ ID NOs: 88 or 89, preferably SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ
35 ID NO: 69 instead of SEQ ID NO: 75 or 76 and SEQ ID NO: 82 instead of SEQ ID NO: 88 or 89.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 81 and any one of SEQ ID NOs: 15 88 to 94, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 94.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 80 and any one of SEQ ID NOs: 88 to 93, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of 35 SEQ ID NOs: 75 to 80 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 93.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 79 and any one of SEQ ID NOs: 15 88 to 92, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 79 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 92.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 78 and any one of SEQ ID NOs: 88 to 91, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 78 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 35 91.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 77 and any one of SEQ ID NOs: 15 88 to 90, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 77 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 90.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 76 and any one of SEQ ID NOs: 88 to 89, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of 35 SEQ ID NOs: 75 to 76 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 89.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 81 and any one of SEQ ID NOs: 15 88 to 94, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 94.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 80 and any one of SEQ ID NOs: 88 to 93, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of 35 SEQ ID NOs: 75 to 80 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 93.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 79 and any one of SEQ ID NOs: 15 88 to 92, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 79 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 92.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 78 and any one of SEQ ID NOs: 88 to 91, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 78 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 35 91.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 77 and any one of SEQ ID NOs: 15 88 to 90, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 77 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 90.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 76 and any one of SEQ ID NOs: 88 to 89, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of 35 SEQ ID NOs: 75 to 76 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 89.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 5, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 75 and SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 75 and SEQ ID NO: 82 instead of SEQ ID NO: 88.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

20 wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 25 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 81 and any one of SEQ ID NOs: 88 to 94, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 81 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 35 94.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 80 and any one of SEQ ID NOs: 15 88 to 93, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 80 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 93.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 79 and any one of SEQ ID NOs: 88 to 92, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 79 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 35 92.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 78 and any one of SEQ ID NOs: 15 88 to 91, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 78 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 91.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 77 and any one of SEQ ID NOs: 88 to 90, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 77 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 35 90.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises any one of SEQ ID NOs: 75 to 76 and any one of SEQ ID NOs: 15 88 to 89, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of any one of SEQ ID NOs: 75 to 76 and SEQ ID NO: 82 instead of any one of SEQ ID NOs: 88 to 89.

20 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

25 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 6, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 75 and SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 75 and SEQ ID NO: 82 instead of SEQ ID NO: 35 88.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

5 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

10 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 75 and SEQ ID NO: 88, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 75 and SEQ ID NO: 82 instead of SEQ ID NO: 88.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

20 wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 25 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

30 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 76 and SEQ ID NO: 89, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 76 and SEQ ID NO: 82 instead of SEQ ID NO: 89.

35 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%,
5 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

10 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 77 and SEQ ID NO: 90, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 77 and SEQ ID NO: 82 instead of
15 SEQ ID NO: 90.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

20 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

25 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 78 and SEQ ID NO: 91, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 78 and SEQ ID NO: 82 instead of
30 SEQ ID NO: 91.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

35 wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO:

5 4, and

wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 79 and SEQ ID NO: 92, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 79 and SEQ ID NO: 82 instead of SEQ ID NO: 92.

10 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

20 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

25 wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 79 and SEQ ID NO: 92, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 79 and SEQ ID NO: 82 instead of SEQ ID NO: 92.

30 In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

35 wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

5 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 80 and SEQ ID NO: 93, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 80 and SEQ ID NO: 82 instead of SEQ ID NO: 93.

In one embodiment, the protein of the invention comprises at least a first moiety and a second moiety,

15 wherein said first moiety is a designed ankyrin repeat domain of the present invention, and

wherein said second moiety is a designed ankyrin repeat domain with binding specificity for serum albumin comprising an amino acid sequence with at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity with SEQ ID NO: 4, and

20 wherein said protein exhibits improved pharmacokinetic properties compared to a comparator protein,

wherein said comparator protein consists of an identical amino acid sequence as said protein, with the exception that said designed ankyrin repeat domain of said first moiety comprises SEQ ID NO: 81 and SEQ ID NO: 94, whereas the designed ankyrin repeat domain of said comparator protein corresponding to said first moiety comprises SEQ ID NO: 69 instead of SEQ ID NO: 81 and SEQ ID NO: 82 instead of SEQ ID NO: 94.

30 In one embodiment, the designed ankyrin repeat domain of the invention, when fused C-terminally to SEQ ID NO: 4 via a polypeptide linker consisting of SEQ ID NO: 3, exhibits improved pharmacokinetic properties compared to a comparator designed ankyrin repeat domain fused C-terminally to SEQ ID NO: 4 via a polypeptide linker consisting of SEQ ID NO: 3, and the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues, other than X, that differ between SEQ ID NO: 81 and SEQ ID NO: 74 and between SEQ ID NO: 94 and 87. In one

35

embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues, other than X, that differ between SEQ ID NO: 81 and SEQ ID NO: 74 and between SEQ ID NO: 93 and 86. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues, other than X, that differ between SEQ ID NO: 80 and SEQ ID NO: 73 and between SEQ ID NO: 92 and 85. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues, other than X, that differ between SEQ ID NO: 79 and SEQ ID NO: 72 and between SEQ ID NO: 91 and 84. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues, other than X, that differ between SEQ ID NO: 78 and SEQ ID NO: 71 and between SEQ ID NO: 90 and 83. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues, other than X, that differ between SEQ ID NO: 77 and SEQ ID NO: 70 and between SEQ ID NO: 90 and 83. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues that differ between SEQ ID NO: 76 and SEQ ID NO: 69 and between SEQ ID NO: 89 and 82. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in residues that differ between SEQ ID NO: 75 and SEQ ID NO: 69 and between SEQ ID NO: 88 and 82. In one embodiment, the amino acid sequences of said designed ankyrin repeat domain and said comparator designed ankyrin repeat domain only differ in position 8 and 15 of the N-terminal capping module and in position 14 and 18 of the C-terminal capping module, wherein the position numbers of the N-terminal capping module correspond to the positions in SEQ ID NO: 69 and the position numbers of the C-terminal capping module correspond to the positions in SEQ ID NO: 82.

In a third aspect, the invention provides nucleic acids encoding any designed ankyrin repeat domain and/or protein of the present invention. Furthermore, the invention provides vectors comprising any of said nucleic acids. In one preferred embodiment, said vector is an expression vector. Vectors and expression vectors are known to the person skilled in the art.

In a fourth aspect, the invention provides a pharmaceutical composition comprising a designed ankyrin repeat domain and/or a protein of the present invention, or a nucleic acid encoding a designed ankyrin repeat domain and/or a protein of the present invention, and

optionally a pharmaceutically acceptable carrier and/or diluent. Pharmaceutically acceptable carriers and/or diluents are known to the person skilled in the art, and are explained in more detail below. Even further, a diagnostic composition is provided comprising one or more of the herein described designed ankyrin repeat domains and/or
5 proteins of the invention.

In one embodiment, said pharmaceutical composition comprises proteins as described above and a pharmaceutically acceptable carrier, excipient or stabilizer, for example as described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]. Suitable carriers, excipients or stabilizers known to the skilled man are saline, Ringer's
10 solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids and amino acid copolymers. A
15 pharmaceutical composition may also be a combination formulation, comprising an additional active agent, such as an anti-cancer agent or an anti-angiogenic agent.

The formulations to be used for *in vivo* administration must be aseptic or sterile. This is readily accomplished by filtration through sterile filtration membranes.

The pharmaceutical composition of the invention may be administered by any suitable
20 method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parenteral administration, the pharmaceutical composition of the invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. The dosage and mode of administration will depend on the individual to be treated and the
25 particular disease.

In one embodiment, said pharmaceutical composition is for use in the treatment of a disorder. Also provided is the use of the designed ankyrin repeat domain of the invention or the protein of the invention for the manufacture of a medicament for the treatment of a
30 disorder.

In a fifth aspect, the invention provides methods of treatment. In one particular aspect, the invention provides a method of treating a medical condition, the method comprising the step of administering, to a patient in need of such treatment, a therapeutically effective amount
35 of a designed ankyrin repeat domain of the invention, a protein of the invention, a nucleic acid of the invention, or a pharmaceutical composition of the invention. In one embodiment,

said protein is a recombinant binding protein. In one preferred embodiment, the patient is a mammal, including human. In one preferred embodiment, said medical condition is a cancer, an infectious disease, preferably a viral infectious disease, a metabolic disease, a neurological disease, an eye disease, an immunological disease, an inflammatory disease, or an autoimmune disease. In one embodiment, said medical condition is a cancer. In one embodiment, such cancer is selected from the group consisting of epithelial malignancies (primary and metastatic), including but not limited to lung, colorectal, gastric, bladder, ovarian and breast carcinomas, blood cell malignancies, including but not limited to leukemia, lymphoma, and myeloma, sarcomas, including but not limited to bone and soft tissue sarcomas, and melanoma. In one preferred embodiment, such cancer is selected from the group consisting of liposarcoma, neuroblastoma, synovial sarcoma, melanoma and ovarian cancer. In another preferred embodiment, such cancer is selected from the group consisting of melanoma, lung cancer, liver cancer, stomach cancer, skin cancer, neuroblastoma, soft tissue sarcoma, bladder cancer, testicular cancer and ovarian cancer. In one embodiment, said medical condition is an infectious disease, preferably a viral infectious disease. In one preferred embodiment, such infectious disease is a viral infection caused by hepatitis B virus (HBV). In another preferred embodiment such infectious disease is a viral infection caused by Epstein–Barr virus (EBV). In one embodiment, said medical condition is an autoimmune disease. In one preferred embodiment, such autoimmune disease is selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis and type I diabetes.

In a sixth aspect, the invention provides a method for preparing a protein, the method comprising the steps of

- (A) preparing a nucleic acid that encodes in one open reading frame
 - (i) at least one designed ankyrin repeat domain of the invention, and
 - (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin, and

(B) transferring said nucleic acid into an expression host.

The term “nucleic acid”, “DNA”, “open reading frame”, and “expression host” are well-known to the practitioner in the art. Examples of expression hosts are, amongst others, *Escherichia coli* (*E. coli*; see examples), chinese hamster ovary cells (CHO cells), HEK293 cells, sf9 insect cells, or yeast (*Saccharomyces cerevisiae*). A preferred expression host is *E. coli*. The term “transferring” refers to procedures such as transformation of bacteria or transfection of eukaryotic cells, procedures well-known to the practitioner in the art. Preferably said nucleic acid further comprises the elements needed for protein expression in the respective expression host. Like this the expression host is able to express said

protein encoded by said open reading frame. The method may additionally comprise the steps of expressing said protein and/or of purifying said protein. Preferably said step of purifying said protein may comprise multiple protein purification methods. Such purification methods like ion-exchange chromatography or hydrophobic-interaction chromatography or
5 diafiltration and alike are well-known to the person skilled in the art. Preferably the protein purity at the end of said step of purifying said protein, is at least 95%, preferably 96%, 97%, 98%, most preferably 99% according to analysis by SDS-PAGE. SDS-PAGE is a method well-known to the person skilled in the art and is described in the examples.

10 The invention is not restricted to the particular embodiments described in the Examples.

This specification refers to a number of amino acid sequences and SEQ ID NOs that are disclosed in the appended Sequence Listing, which is herewith incorporated by reference
15 in its entirety.

Definitions

Unless defined otherwise herein, all technical and scientific terms used herein shall have the meanings that are commonly understood by those of ordinary skill in the art to which the present invention belongs.

20 The term "polypeptide" relates to a molecule consisting of one or more chains of multiple, i.e. two or more, amino acids linked via peptide bonds. Preferably, a polypeptide consists of more than eight amino acids linked via peptide bonds. The term "polypeptide" also includes multiple chains of amino acids, linked together by S-S bridges of cysteines. Polypeptides are well-known to the person skilled in the art.

25 The term "protein" refers to a molecule comprising a polypeptide, wherein at least part of the polypeptide has, or is able to acquire, a defined three-dimensional arrangement by forming secondary, tertiary, and/or quaternary structures within a single polypeptide chain and/or between multiple polypeptide chains. If a protein comprises two or more polypeptide chains, the individual polypeptide chains may be linked non-covalently or covalently, e.g.
30 by a disulfide bond between two polypeptide chains. A part of a protein, which individually has, or is able to acquire, a defined three-dimensional arrangement by forming secondary and/or tertiary structures, is termed "protein domain". Such protein domains are well known to the practitioner skilled in the art.

Patent application WO2002/020565 and Forrer et al., 2003 (Forrer, P., Stumpp, M.T., Binz, H.K., Plückthun, A., 2003. FEBS Letters 539, 2-6), contain a general description of repeat
35 protein, repeat domain and repeat module features, techniques and applications.

- The term "repeat domain" refers to a protein domain comprising two or more consecutive repeat modules as structural units, wherein said repeat modules have structural and sequence homology. Preferably, a repeat domain also comprises an N-terminal and/or a C-terminal capping module. For clarity, a capping module can be a repeat module. Such
- 5 repeat domains, repeat modules, and capping modules, sequence motives, as well as structural homology and sequence homology are well known to the practitioner in the art from examples of ankyrin repeat domains (Binz et al., J. Mol. Biol. 332, 489–503, 2003; Binz et al., 2004, loc. cit.; WO2002/020565; WO2012/069655), leucine-rich repeat domains (WO2002/020565), tetratricopeptide repeat domains (Main, E.R., Xiong, Y., Cocco, M.J.,
- 10 D'Andrea, L., Regan, L., Structure 11(5), 497-508, 2003), and armadillo repeat domains (WO2009/040338). It is further well known to the practitioner in the art, that such repeat domains are different from proteins comprising repeated amino acid sequences, where every repeated amino acid sequence is able to form an individual domain (for example FN3 domains of Fibronectin).
- 15 The term "ankyrin repeat domain" refers to a repeat domain comprising two or more consecutive ankyrin repeat modules as structural units, wherein said ankyrin repeat modules have structural and sequence homology.
- The term "repeat modules" refers to the repeated amino acid sequence and structural units of the designed repeat domains, which are originally derived from the repeat units of
- 20 naturally occurring repeat proteins. Each repeat module comprised in a repeat domain is derived from one or more repeat units of a family or subfamily of naturally occurring repeat proteins, preferably the family of ankyrin repeat proteins.
- Accordingly, the term "ankyrin repeat module" refers to a repeat module, which is originally derived from the repeat units of naturally occurring ankyrin repeat proteins. Ankyrin repeat
- 25 proteins are well known to the person skilled in the art.
- For example, SEQ ID NOs: 4 to 8 and 12 to 23 each comprise one repeat domain comprising an N-terminal capping module (residues 1 to 30 of each of SEQ ID NOs: 4 to 8 and 12 to 23), two repeat modules (residues 31 to 63 and residues 64 to 96, respectively, of each of SEQ ID NOs: 4 to 8 and 12 to 23), and a C-terminal capping module (residues
- 30 97 to 124 of each of SEQ ID NOs: 4 to 8 and 12 to 23). As further examples, SEQ ID NOs: 9 to 11 each comprise one repeat domain comprising an N-terminal capping module (residues 1 to 30 of each of SEQ ID NOs: 9 to 11), three repeat modules (residues 31 to 63, residues 64 to 96, and residues 97 to 129, respectively, of each of SEQ ID NOs: 9 to 11), and a C-terminal capping module (residues 130 to 157 of each of SEQ ID NOs: 9 to
- 35 11). Furthermore, SEQ ID NOs: 69, 75, and 76 are examples of N-terminal capping modules, and SEQ ID NOs: 82, 88, and 89 are examples of C-terminal capping modules.

The term "designed" as used in designed repeat protein, designed repeat domain, designed ankyrin repeat domain, and the like refers to the property that such repeat proteins and repeat domains, respectively, are man-made and do not occur in nature.

The term "recombinant" as used in recombinant protein, recombinant binding protein, 5 recombinant polypeptide, and the like, means that said protein or polypeptide is produced by the use of recombinant DNA technologies well known to the practitioner skilled in the art. For example, a recombinant DNA molecule (e.g. produced by gene synthesis) encoding a polypeptide can be cloned into a bacterial expression plasmid (e.g. pQE30, QIAgen), yeast expression plasmid, mammalian expression plasmid, or plant expression plasmid, or a DNA 10 enabling in vitro expression. If, for example, such a recombinant bacterial expression plasmid is inserted into appropriate bacteria (e.g. *Escherichia coli*), these bacteria can produce the polypeptide(s) encoded by this recombinant DNA. The correspondingly produced polypeptide or protein is called a recombinant polypeptide or recombinant protein. In the context of the present invention, the term "binding protein" refers to a protein 15 comprising a binding domain. A binding protein may also comprise two, three, four, five or more binding domains. Preferably, said binding protein is a recombinant binding protein. The term "binding domain" means a protein domain exhibiting binding specificity for a target. Preferably, said binding domain is a recombinant binding domain.

The term "target" refers to an individual molecule such as a nucleic acid molecule, a peptide, 20 polypeptide or protein, a carbohydrate, or any other naturally occurring molecule, including any part of such individual molecule, or to complexes of two or more of such molecules, or to a whole cell or a tissue sample, or to any non-natural compound. Preferably, a target is a naturally occurring or non-natural polypeptide or protein, or a polypeptide or protein containing chemical modifications, for example, naturally occurring or non-natural 25 phosphorylation, acetylation, or methylation. For example, the target of each of the designed ankyrin repeat domains consisting of SEQ ID NOs: 4 to 8, is serum albumin.

The term "has binding specificity for a target", "specifically binding to a target", "binding to a target with high specificity", "specific for a target" or "target specificity" and the like means that a binding protein or binding domain binds in PBS to a target with a lower dissociation 30 constant (i.e. it binds with higher affinity) than it binds to an unrelated protein such as the *E. coli* maltose binding protein (MBP). Preferably, the dissociation constant ("Kd") in PBS for the target is at least 10^2 ; more preferably, at least 10^3 ; even more preferably, at least 10^4 ; or most preferably, at least 10^5 times lower than the corresponding dissociation constant for MBP. Methods to determine dissociation constants of protein-protein interactions, such as 35 surface plasmon resonance (SPR) based technologies (e.g. SPR equilibrium analysis) or isothermal titration calorimetry (ITC) are well known to the person skilled in the art. The

measured K_D values of a particular protein-protein interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of K_D values are preferably made with standardized solutions of protein and a standardized buffer, such as PBS.

- 5 The term "polypeptide tag" refers to an amino acid sequence attached to a polypeptide/protein, wherein said amino acid sequence is useful for the purification, detection, or targeting of said polypeptide/protein, or wherein said amino acid sequence improves the physicochemical behavior of the polypeptide/protein, or wherein said amino acid sequence possesses an effector function. The individual polypeptide tags, moieties
10 and/or domains of a binding protein may be connected to each other directly or via polypeptide linkers. These polypeptide tags are all well known in the art and are fully available to the person skilled in the art. Examples of polypeptide tags are small polypeptide sequences, for example, His (e.g. the His-tag consisting of SEQ ID NO: 1), myc, FLAG, or Strep-tags or moieties such as enzymes (for example enzymes like alkaline phosphatase),
15 which allow the detection of said polypeptide/protein, or moieties which can be used for targeting (such as immunoglobulins or fragments thereof) and/or as effector molecules. The term "polypeptide linker" refers to an amino acid sequence, which is able to link, for example, two protein domains, a polypeptide tag and a protein domain, a protein domain and a non-polypeptide moiety such as polyethylene glycol or two polypeptide tags tags.
20 Such additional domains, tags, non-polypeptide moieties and linkers are known to the person skilled in the relevant art. Examples of such polypeptide linkers are the linkers consisting of SEQ ID NOs: 2 and 3.

- The terms "nucleic acid" or "nucleic acid molecule" refer to a polynucleotide molecule, which may be a ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) molecule, either single
25 stranded or double stranded, and includes modified and artificial forms of DNA or RNA. A nucleic acid molecule may either be present in isolated form, or be comprised in recombinant nucleic acid molecules or vectors.

- In the context of the invention, the terms "medical condition", "disease" and "disorder" are used interchangeably and include but are not limited to autoimmune disorders, inflammatory
30 disorders, retinopathies (particularly proliferative retinopathies), neurodegenerative disorders, infectious diseases, metabolic diseases, and neoplastic diseases. A "medical condition" may be one that is characterized by inappropriate cell proliferation. A medical condition may be a hyperproliferative condition. A medical condition may be a neoplastic disease. The term "neoplastic disease", as used herein, refers to an abnormal state or
35 condition of cells or tissue characterized by rapidly proliferating cell growth or neoplasm. A medical condition may be a malignant neoplastic disease. A medical condition may be a

cancer. The terms "cancer" and "cancerous" are used herein to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Cancer encompasses solid tumors and liquid tumors, as well as primary tumors and metastases. A "tumor" comprises one or more cancerous cells. Solid tumors typically

5 also comprise tumor stroma. Examples of cancer include, but are not limited to, primary and metastatic carcinoma, lymphoma, blastoma, sarcoma, myeloma, melanoma and leukemia, and any other epithelial and blood cell malignancies. More particular examples of such cancers include brain cancer, bladder cancer, breast cancer, ovarian cancer, kidney cancer, colorectal cancer, gastric cancer, head and neck cancer, lung cancer, pancreatic cancer,

10 prostate cancer, malignant melanoma, osteosarcoma, soft tissue sarcoma, carcinoma, squamous cell carcinoma, clear cell kidney cancer, head/neck squamous cell carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma, small-cell lung cancer (SCLC), triple negative breast cancer, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic

15 leukemia (CLL), chronic myeloid leukemia (CML), diffuse large B- cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma (HL), mantle cell lymphoma (MCL), multiple myeloma (MM), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), Squamous Cell Carcinoma of the Head and Neck (SCCHN), chronic myelogenous leukemia (CML), small lymphocytic lymphoma (SLL), malignant mesothelioma, liposarcoma,

20 neuroblastoma, or synovial sarcoma. The terms "autoimmune disease" and "autoimmune disorder" are used herein to refer to or describe disorders wherein the immune system of a mammal mounts a humoral or cellular immune response to the mammal's own tissue or to antigens that are not intrinsically harmful to the mammal, thereby producing tissue injury in such a mammal. Examples of autoimmune disorders are numerous and include, but are not

25 limited to, systemic lupus erythematosus, rheumatoid arthritis and type I diabetes. Autoimmune diseases also include acute glomerulonephritis, Addison's disease, adult onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, amyotrophic lateral sclerosis, ankylosing spondylitis, autoimmune aplastic anemia, autoimmune hemolytic anemia, Behcet's disease, Celiac disease, chronic active hepatitis, CREST syndrome, Crohn's

30 disease, dermatomyositis, dilated cardiomyopathy, eosinophilia-myalgia syndrome, epidermolysis bullosa acquisita (EBA), giant cell arteritis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, hemochromatosis, Henoch-Schonlein purpura, idiopathic IgA nephropathy, insulin-dependent diabetes mellitus (IDDM), juvenile rheumatoid arthritis, Lambert-Eaton syndrome, linear IgA dermatosis, lupus erythematosus,

35 multiple sclerosis, myasthenia gravis, myocarditis, narcolepsy, necrotizing vasculitis, neonatal lupus syndrome (NLE), nephrotic syndrome, pemphigoid, pemphigus,

polymyositis, primary sclerosing cholangitis, psoriasis, rapidly progressive glomerulonephritis (RPGN), Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjogren's syndrome, stiff-man syndrome, thyroiditis, and ulcerative colitis. The terms "infectious disease" and "infection" are used herein to refer to or describe the invasion and multiplication of microorganisms in body tissues, especially causing pathological symptoms. Examples of infectious diseases include without limitation, viral diseases and bacterial diseases, such as, e.g., HIV infection, West Nile virus infection, hepatitis A, B, and C, small pox, tuberculosis, Vesicular Stomatitis Virus (VSV) infection, Respiratory Syncytial Virus (RSV) infection, human papilloma virus (HPV) infection, SARS, influenza, Ebola, viral meningitis, herpes, anthrax, lyme disease, and E. Coli infections, among others.

The term "treatment" or "treating" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those who have already the disorder as well as those in which the disorder is to be prevented.

The term "therapeutically effective amount" refers to the amount sufficient to induce a desired biological, pharmacological, or therapeutic outcome in a subject. A therapeutically effective amount in the context of the invention means a sufficient amount of the binding protein to treat or prevent a disease or disorder at a reasonable benefit/risk ratio applicable to any medical treatment.

The term "mammal" for purposes of treatment refers to any animal classified as a mammal, including human, domestic and farm animals, nonhuman primates, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc.

The term "incubation" refers to incubation at pH 7.4. In one embodiment, said incubation at pH 7.4 refers to an incubation in PBS.

The term "PBS" means a phosphate buffered water solution containing 137 mM NaCl, 10 mM phosphate and 2.7 mM KCl and having a pH of 7.4.

The term improved pharmacokinetic properties refers to an increased area under the curve, a reduced clearance, or an increased terminal half-life. These parameters of pharmacokinetic properties and ways to determine them are well known in the art (see, e.g., Mahmood, I., Methods to determine pharmacokinetic profiles of therapeutic proteins, *Drug Discov Today: Technol.*, Volume 5, Issues 2-3, Autumn 2008, Pages e65-e69, doi:10.1016/j.ddtec.2008.12.001).

In the context of the present invention, the term "any amino acids" preferably means any of the 20 most often naturally occurring amino acids, namely alanine (ala; A), arginine (arg; R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L),

lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), valine (val, V).

Examples

5

Proteins used in the examples:

- Protein #4 (SEQ ID NO: 4 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #5 (SEQ ID NO: 5 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #6 (SEQ ID NO: 6 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
10 Protein #7 (SEQ ID NO: 7 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #8 (SEQ ID NO: 8 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #9 (SEQ ID NO: 9 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #10 (SEQ ID NO: 10 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #11 (SEQ ID NO: 11 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
15 Protein #12 (SEQ ID NO: 12 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #13 (SEQ ID NO: 13 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #14 (SEQ ID NO: 14 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #15 (SEQ ID NO: 15 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #16 (SEQ ID NO: 16 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
20 Protein #17 (SEQ ID NO: 17 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #18 (SEQ ID NO: 18 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #19 (SEQ ID NO: 19 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #20 (SEQ ID NO: 20 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #21 (SEQ ID NO: 21 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
25 Protein #22 (SEQ ID NO: 22 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #23 (SEQ ID NO: 23 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #24 (SEQ ID NO: 24 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #25 (SEQ ID NO: 25 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #26 (SEQ ID NO: 26 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
30 Protein #27 (SEQ ID NO: 27 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #28 (SEQ ID NO: 28 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #29 (SEQ ID NO: 29 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #30 (SEQ ID NO: 30 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #31 (SEQ ID NO: 31 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
35 Protein #32 (SEQ ID NO: 32 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);

- Protein #33 (SEQ ID NO: 33 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #34 (SEQ ID NO: 34 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #35 (SEQ ID NO: 35 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #36 (SEQ ID NO: 36 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
5 Protein #37 (SEQ ID NO: 37 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #38 (SEQ ID NO: 38 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #39 (SEQ ID NO: 39 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #40 (SEQ ID NO: 40 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #41 (SEQ ID NO: 41 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
10 Protein #42 (SEQ ID NO: 42 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #43 (SEQ ID NO: 43 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #44 (SEQ ID NO: 44 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #45 (SEQ ID NO: 45 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #46 (SEQ ID NO: 46 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
15 Protein #47 (SEQ ID NO: 47 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #48 (SEQ ID NO: 48 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #49 (SEQ ID NO: 49 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #50 (SEQ ID NO: 50 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #51 (SEQ ID NO: 51 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
20 Protein #52 (SEQ ID NO: 52 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #53 (SEQ ID NO: 53 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #54 (SEQ ID NO: 54 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #55 (SEQ ID NO: 55 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #56 (SEQ ID NO: 56 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
25 Protein #57 (SEQ ID NO: 57 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #58 (SEQ ID NO: 58 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #59 (SEQ ID NO: 59 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #60 (SEQ ID NO: 60 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #61 (SEQ ID NO: 61 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
30 Protein #62 (SEQ ID NO: 62 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #63 (SEQ ID NO: 63 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #64 (SEQ ID NO: 64 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #65 (SEQ ID NO: 65 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
Protein #66 (SEQ ID NO: 66 with a His-tag (SEQ ID NO: 1) fused to its N-terminus);
35 Protein #67 (SEQ ID NO: 67 with a His-tag (SEQ ID NO: 1) fused to its N-terminus); and
Protein #68 (SEQ ID NO: 68 with a His-tag (SEQ ID NO: 1) fused to its N-terminus).

If not described otherwise, experiments were performed according to methods well-known to the person skilled in the art. The experimental conditions for some examples are also further described in WO2012069654 and WO2016156596.

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Example 1: Construction of designed ankyrin repeat domains with improved pharmacokinetic properties

Proteins #9, #12, #16, and #20 are different examples of designed ankyrin repeat domains that, when fused to designed ankyrin repeat domains with binding specificity for serum albumin (resulting e.g. in Proteins #24, #27, #31, and #35, respectively) exhibit fast clearance and short terminal half-life. The pharmacokinetic properties are clearly inferior to the ones observed for designed ankyrin repeat domains with binding specificity for serum albumin (Steiner et al., 2017, loc.cit.). For Proteins #24, #27, #31, and #35, there are no known clearance mechanisms involved such as e.g. target mediated clearance.

15 The present invention provides amino acid sequences for designed ankyrin repeat domains that lead to improved pharmacokinetic profiles (See Figure 1). We surprisingly found that we can modulate the pharmacokinetic properties by applying certain amino acid mutations in the designed ankyrin repeat domains. We mutated the proteins at different positions. Substituting every position of a protein of 124 amino acid lengths by the 19 alternative amino acids and generating the combinations thereof would result in a theoretical diversity of $>10^{158}$ variants. As this is not experimentally feasible, we used rational approaches to refine the process. In the first two rounds we selected mostly surface exposed residues and exchanged them for charged/polar/neutral/hydrophobic amino acids and evaluated the impact on pharmacokinetic properties (as fusion proteins to designed ankyrin repeat domains with binding specificity for serum albumin). We combined multiple amino acid changes per construct and could like this identify variants with favorable amino acid compositions. In further three rounds, we analyzed the multiple amino acid changes in detail to identify the critical amino acid changes that lead to favorable pharmacokinetic properties. In the next two rounds we combined the critical amino acid changes again leading to constructs with minimal changes and improved pharmacokinetic properties. These amino acid changes are described in detail in this application. In summary, through a rationally designed process involving several rounds of amino acid changes at a multitude of positions and characterizing the resulting protein variants in vitro and in vivo, novel sequence patterns and motifs were identified which surprisingly led to improved pharmacokinetic properties when introduced in a designed ankyrin repeat domain. These amino acid sequence motifs are comprised in SEQ ID NOs: 75 to 81, 88 to 94, and 107 to 111. Proteins #10, #11, #13,

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#14, #15, #17, #18, #19, #21, #22, and #23 are examples of designed ankyrin repeat domains comprising such novel amino acid sequence motifs. Similarly, Proteins #25, #26, #28, #29, #30, #32, #33, #34, #36, #37, #38, #40, #41, #43, #44, #45, #47, #48, #49, #51, #52, #53, #55, #56, #58, #59, #60, #62, #63, #64, #66, #67, and #68 are examples of proteins comprising designed ankyrin repeat domains comprising such novel amino acid sequence motifs. The production and characterization as well as the use of these particularly selected sequence motifs is described in the following examples.

Example 2: Expression and purification of proteins

The DNA encoding each of the designed ankyrin repeat domain consisting of SEQ ID NOs: 4 to 23 and the DNA encoding each of the proteins consisting of SEQ ID NOs: 24 to 68 was cloned into a pQE (QIAGEN, Germany) based expression vector providing an N-terminal His-tag to facilitate simple protein purification as described below. Proteins consisting of SEQ ID NOs: 4 to 68, additionally having a His-tag SEQ ID NO: 1 fused to their N termini, were produced in *E. coli*, purified to homogeneity, and stored in PBS buffer. Methods for the production and purification of proteins are well known to the practitioner in the art. For clarity, proteins #4 to #23 are individual designed ankyrin repeat domains, proteins #24 to #68 are proteins consisting two designed ankyrin repeat domains, of which one is a designed ankyrin repeat domain with binding specificity for serum albumin. Proteins expressed and purified as described in this paragraph were used for the experiments of Examples 3 to 11.

Alternatively, proteins consisting of SEQ ID NOs: 4 to 68, additionally having the amino acids GS at the N terminus, are produced in *E. coli*, purified to homogeneity, and stored in PBS buffer. In case the amino acids GS are at the N terminus, the Met residue additionally encoded by the expression vector is efficiently cleaved off in the cytoplasm of *E. coli* from the expressed polypeptide since the start Met is followed by a small Gly residue. The proteins consisting of SEQ ID NOs: 4 to 68, additionally having the amino acids GS at the N terminus, exhibit equivalent results in Examples 3 to 11 as the proteins consisting of SEQ ID NOs: 4 to 68, additionally having a His-tag (SEQ ID NO: 1) fused to the N terminus.

Example 3: Storage stability assessment

Proteins of Example 2 were tested for storage stability by incubating them at 100 micromolar protein concentration at 60°C at pH 7.4 for 1 week (7 days). Buffer used was PBS (pH 7.4; 137 mM NaCl, 10 mM phosphate and 2.7 mM KCl). Upon mixing the protein with the PBS, the resulting pH value was pH 7.4. In parallel to the incubations at 60°C, aliquots of the proteins were incubated at -80°C for 1 week (7 days) as controls.

Example 4: SDS-PAGE of storage stability analysis samples

Samples of proteins of Example 3 (10 microgram protein each lane) were analyzed on NuPAGE 4-12% Bis-Tris sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) gels (Thermo Fisher), stained with instant blue staining (Sigma Aldrich). Results are shown in Figure 4. The SDS-PAGE analysis indicates high purity of all purified proteins. Similar results are obtained when analyzing proteins #39 to #68.

Example 5: Size-exclusion chromatography analysis

Samples of Example 3 were analyzed on a GE Superdex 200 150/5 column on an Agilent 1200 HPLC system in PBS at 0.5 ml/min flow rate. Of each protein, 0.1 ml at 100 micromolar concentration were analyzed. Proteins #9 to #38 all elute as monomeric peaks with at least 95% of the area under the curve corresponding to monomer fraction. Results are shown in Table 1. These results indicate that the proteins are monomeric. Importantly, proteins #24 to #38, which were used for the pharmacokinetic analyses (Example 6), eluted at monomeric peak both after incubation at -80°C and at 60°C as described in Example 3. This indicates that these proteins are stable upon incubation at elevated temperature. Similar results are obtained when analyzing Proteins #39 to #68.

Table 1. Size exclusion chromatography of Proteins #9 to #38

Protein*	Relative area %	Retention time [min]	Protein*	Relative area %	Retention time [min]
#9	100.00	4.31	#24	100.00	4.00
#10	100.00	4.46	#25	100.00	3.98
#11	100.00	4.19	#26	100.00	3.91
#12	100.00	4.05	#27	100.00	3.94
#13	100.00	4.28	#28	100.00	3.99
#14	98.86	4.84	#29	100.00	4.07
#15	100.00	4.02	#30	100.00	3.95
#16	100.00	4.31	#31	100.00	3.92
#17	100.00	4.44	#32	100.00	3.97
#18	100.00	4.45	#33	100.00	3.96
#19	100.00	4.37	#34	100.00	3.94

#20	100.00	4.41	#35	100.00	3.85
#21	100.00	4.59	#36	100.00	3.98
#22	100.00	4.46	#37	100.00	3.97
#23	100.00	4.55	#38	100.00	3.80

* Proteins #9 to #38 in this table represent proteins consisting of the corresponding amino acid sequence of SEQ ID NO: 9 to 38, and additionally an N-terminal His-tag (SEQ ID NO: 1).

Example 6: Mouse pharmacokinetic profiles of protein variants

Pharmacokinetic analyses were performed in female Balb/c mice using Proteins #24 to #38, produced as described in Example 2. Proteins were applied at 1 mg/kg by intravenous injection into the tail vein. Six mice, divided in two groups of 3 mice each, were used for each protein. For every protein, blood was collected from the mice of one group 5 min, 24 h, 72 h, and 168 h post injection, and from the mice of the other group 6 h, 48 h, 96 h, and 168 h post injection. The blood samples were allowed to stand at room temperature and were centrifuged to generate serum using procedures well-known to the person skilled in the art, followed by storage at -80°C pending analyses. Serum concentrations of Proteins #24 to #38 were determined by sandwich ELISA using a rabbit monoclonal anti-DARPin antibody as capture reagent and an anti-RGS-His antibody-HRP conjugate as detection reagent, and using a standard curve. The monoclonal anti-DARPin antibody was generated using conventional rabbit immunization and hybridoma generation techniques well known to the person skilled in the art, and the binding of the monoclonal antibody to Proteins #24 to #38 was verified prior to concentration determination experiments. Briefly, 100 µl of goat-anti-rabbit antibody (10 nM) (Thermo Scientific) in PBS per well were immobilized in a Maxisorp plate (Nunc, Denmark) overnight at 4°C. After washing 5 times with 300 µl PBST (PBS supplemented with 0.1% Tween 20), the wells were blocked with 300 µl PBST-C (PBST supplemented with 0.25% casein) for 1 h at room temperature with shaking at 450 rpm on a Titramax 1000 shaker (Heidolph, Germany). After washing 5 times as described above, 100 µl/well rabbit-anti-DARPin antibody (5 nM) in PBST-C were added for 1 h at room temperature with shaking at 450 rpm. After washing 5 times as described above, different dilutions of serum samples or standard references, diluted in PBST-C, were added for 2 hours at room temperature with shaking at 450 rpm. After washing 5 times as described above, 50 µl mouse anti-RGS-His antibody-HRP conjugate (QIAGEN) (100 ng/ml) in PBST-C was added for 30 min at room temperature with shaking at 450 rpm. After washing 5 times as described above, the ELISA was developed using 50 µl TMB substrate.

- The reaction was stopped after 5 min using 100 μ l 1 M H₂SO₄. The OD (OD 450 nm - OD 620 nm) was then recorded. Pharmacokinetic parameters were determined using standard software such as Phoenix WinNonLin (Certara, Princeton, USA) or GraphPadPrism (GraphPad Software, La Jolla, USA) and standard analyses such as non-compartmental analyses, all well-known to the person skilled in the art. The resulting pharmacokinetic profiles are shown in Figure 5. The pharmacokinetic parameters area under the curve, clearance, volume of distribution, and half-life, derived from the measurements, are listed in Table 2, Table 3, Table 4, and Table 5.

Table 2. Mouse pharmacokinetic parameters of Proteins #24 to #26

Parameter Protein	AUCINF_D_pred h*(nmol/L)	Cl_pred L/(h*kg)	Vss_pred L/kg	HL_Lambda_z h
#24*	4398	0.0071	0.031	5.1
#25*	12221	0.0025	0.06	18.8
#26*	27102	0.0011	0.057	37.3

* Proteins #24 to #26 in this table represent proteins consisting of the corresponding amino acid sequence of SEQ ID NO: 24 to 26, and additionally an N-terminal His-tag (SEQ ID NO: 1).

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Table 3. Mouse pharmacokinetic parameters of Proteins #27 to #30

Parameter Protein	AUCINF_D_pred h*(nmol/L)	Cl_pred L/(h*kg)	Vss_pred L/kg	HL_Lambda_z h
#27*	11332	0.003	0.046	13.1
#28*	21910	0.0016	0.075	36.7
#29*	30314	0.0011	0.051	33.1
#30*	22127	0.0016	0.052	25.4

* Proteins #27 to #30 in this table represent proteins consisting of the corresponding amino acid sequence of SEQ ID NO: 27 to 30, and additionally an N-terminal His-tag (SEQ ID NO: 1).

Table 4. Mouse pharmacokinetic parameters of Proteins #31 to #34

Parameter Protein	AUCINF_D_pred h*(nmol/L)	Cl_pred L/(h*kg)	Vss_pred L/kg	HL_Lambda_z h
#31*	11619	0.003	0.046	13.9

#32*	21758	0.0016	0.057	27.9
#33*	42071	0.0008	0.036	34.5
#34*	23398	0.0015	0.043	25.8

* Proteins #31 to #34 in this table represent proteins consisting of the corresponding amino acid sequence of SEQ ID NO: 31 to 34, and additionally an N-terminal His-tag (SEQ ID NO: 1).

Table 5. Mouse pharmacokinetic parameters of Proteins #35 to #38

Parameter Protein	AUCINF_D_pred h*(nmol/L)	Cl_pred L/(h*kg)	Vss_pred L/kg	HL_Lambda_z h
#35*	4222	0.0082	0.036	4.4
#36*	28590	0.0012	0.057	36.6
#37*	23517	0.0015	0.083	42.7
#38*	30107	0.0011	0.04	27.4

* Proteins #35 to #38 in this table represent proteins consisting of the corresponding amino acid sequence of SEQ ID NO: 35 to 38, and additionally an N-terminal His-tag (SEQ ID NO: 1).

These findings indicate that the sequence modifications described here lead to improved pharmacokinetic properties. In particular, Proteins #25 and #26 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Proteins #24. Also, Proteins #28, #29 and #30 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #27. Similarly, Proteins #32, #33 and #34 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #31. And Proteins #36, #37 and #38 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #35.

Similar results are obtained when comparing the mouse pharmacokinetic parameters of Proteins #39 to #53. In particular, Proteins #40 and #41 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #39. Also, Proteins #43, #44 and #45 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #42. Similarly, Proteins #47, #48 and #49 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #46. And Proteins #51, #52 and #53 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #50. Likewise, similar results are obtained when comparing the mouse

pharmacokinetic parameters of Proteins #54 to #68. In particular, Proteins #55 and #56 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #54. Also, Proteins #58, #59 and #60 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #57. Similarly, Proteins #62, #63 and #64 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #61. And Proteins #66, #67 and #68 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #65. The effect of the sequence modifications on pharmacokinetic properties of the proteins in mouse is thus observed when using different designed ankyrin repeat domains with binding specificity for serum albumin as means for half-life extension. The effect of the sequence modifications on pharmacokinetic properties of the proteins in mouse is thus also observed when using different linker sequences (e.g. Pro-Thr-rich linker instead of Gly-Ser-rich linker).

Example 7: *Cynomolgus monkey pharmacokinetic parameters protein variants*

Pharmacokinetic analyses are performed in two male macaca fascicularis for each protein using Proteins #24 to #38, produced as described in Example 2. Proteins are dosed at 1 mg/kg via 30 min intravenous infusion administration. For every protein, blood is collected from every animal 5 min, 6 h, 24 h, 72 h, 120 h, 168 h, 336 h, 408 h, 504 h, and 672 h post injection. The blood samples are allowed to stand at room temperature and are centrifuged to generate serum using procedures well-known to the person skilled in the art, followed by storage at -80°C pending analyses. Serum concentrations of Proteins #24 to #68 are determined by sandwich ELISA as described in Example 6. Pharmacokinetic parameters are determined using standard software such as Phoenix WinNonLin (Certara, Princeton, USA) or GraphPadPrism (GraphPad Software, La Jolla, USA) and standard analyses such as non-compartmental analyses, all well-known to the person skilled in the art. The pharmacokinetic parameters area under the curve, clearance, volume of distribution, and half-life, are derived from the measurements.

The measurements indicate that the sequence modifications described herein lead to improved pharmacokinetic properties. In particular, Proteins #25 and #26 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #24. Also, Proteins #28, #29 and #30 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #27. Similarly, Proteins #32, #33 and #34 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #31. And Proteins #36, #37 and #38 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #35.

Similar results are obtained when comparing the cynomolgus monkey pharmacokinetic parameters of Proteins #39 to #53. In particular, Protein #40 and #41 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #39. Also, Proteins #43, #44 and #45 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #42. Similarly, Proteins #47, #48 and #49 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #46. And Proteins #51, #52 and #53 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #50. Likewise, similar results are obtained when comparing the cynomolgus monkey pharmacokinetic parameters of Proteins #54 to #68. In particular, Proteins #55 and #56 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #54. Also, Proteins #58, #59 and #60 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #57. Similarly, Proteins #62, #63 and #64 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #61. And Proteins #66, #67 and #68 exhibit slower clearance, larger area under the curve, and longer terminal half-life than Protein #65. The effect of the sequence modifications on pharmacokinetic properties of the proteins in cynomolgus monkey is thus observed when using different designed ankyrin repeat domains with binding specificity for serum albumin as means for half-life extension. The effect of the sequence modifications on pharmacokinetic properties of the proteins in cynomolgus monkey is thus also observed when using different linker sequences (e.g. Pro-Thr-rich linker instead of Gly-Ser-rich linker).

Example 8: Generation of proteins

Proteins of the invention, comprising designed ankyrin repeat domain(s) with sequence modifications described herein, and comparator proteins described herein are generated by recombinant DNA technology or DNA synthesis well-known to the practitioner in the art. One example of such a protein of the invention is a protein comprising from N terminus to C terminus, (i) a designed ankyrin repeat domain consisting of SEQ ID NO: 4, (ii) a ProThr-rich linker consisting of SEQ ID NO: 3, (iii) a designed ankyrin repeat domain consisting of SEQ ID NO: 11, (iv) a ProThr-rich linker consisting of SEQ ID NO: 3, (v) a designed ankyrin repeat domain consisting of SEQ ID NO: 11, (vi) a ProThr-rich linker consisting of SEQ ID NO: 3, and (vii) a designed ankyrin repeat domain consisting of SEQ ID NO: 4 (i.e. SEQ ID NOs: 4-3-11-3-11-3-4). Similarly, one example of such a protein of the invention is a protein comprising from N terminus to C terminus, (i) a designed ankyrin repeat domain consisting of SEQ ID NO: 4, (ii) a ProThr-rich linker consisting of SEQ ID NO: 3, (iii) a designed ankyrin repeat domain consisting of SEQ ID NO: 10, (iv) a ProThr-rich linker consisting of SEQ ID

NO: 3, (v) a designed ankyrin repeat domain consisting of SEQ ID NO: 10, (vi) a ProThr-rich linker consisting of SEQ ID NO: 3, and (vii) a designed ankyrin repeat domain consisting of SEQ ID NO: 4 (i.e. SEQ ID NOs: 4-3-10-3-10-3-4). Similarly, one example of such a comparator protein is a protein comprising from N terminus to C terminus, (i) a designed ankyrin repeat domain consisting of SEQ ID NO: 4, (ii) a ProThr-rich linker consisting of SEQ ID NO: 3, (iii) a designed ankyrin repeat domain consisting of SEQ ID NO: 9, (iv) a ProThr-rich linker consisting of SEQ ID NO: 3, (v) a designed ankyrin repeat domain consisting of SEQ ID NO: 9, (vi) a ProThr-rich linker consisting of SEQ ID NO: 3, and (vii) a designed ankyrin repeat domain consisting of SEQ ID NO: 4 (i.e. SEQ ID NOs: 4-3-9-3-9-3-4). Analogously, proteins of the invention are prepared using designed ankyrin repeat domains with binding specificity for serum albumin consisting of SEQ ID NOs: 4 to 6, polypeptide linkers consisting of SEQ ID NOs: 2 or 3, and designed ankyrin repeat domains consisting of SEQ ID NOs: 10, 11, 13 to 15, 17 to 19 and 21 to 23. Analogously, comparator proteins as described herein are prepared using designed ankyrin repeat domains with binding specificity for serum albumin consisting of SEQ ID NOs: 4 to 6, polypeptide linkers consisting of SEQ ID NOs: 2 or 3, and designed ankyrin repeat domains consisting of SEQ ID NOs: 9, 12, 16, and 20. Examples of such proteins of the inventions are recombinant binding proteins (See Figure 1). An example of a recombinant binding protein of the invention is a variant of the recombinant binding protein consisting of SEQ ID NO: 134 of WO2016156596, wherein position 158 is Q, position 165 is L, position 293 is R, position 297 is Q, position 339 is Q, position 346 is L, position 441 is R, and position 445 is Q. Methods to generate such proteins, for example recombinant binding proteins, are well-known to the practitioner in the art from e.g. WO2016156596 or WO2018054971.

25 Example 9: Production of proteins

Proteins as described in Example 8, additionally having a His-tag (SEQ ID NO: 1) fused to the N terminus are produced as described in Example 2. Similarly, proteins as described in Example 8, additionally carrying the amino acids MGS at the N terminus (wherein the N-terminal methionine is efficiently cleaved off from the expressed polypeptide in the cytoplasm of E. coli since the start Met is followed by a small Gly residue), can be produced in E. coli and be purified using conventional methods. Similarly, a recombinant binding protein as described in Example 8, additionally carrying the amino acids MGS at the N terminus (wherein the N-terminal methionine is efficiently cleaved off from the expressed polypeptide in the cytoplasm of E. coli since the start Met is followed by a small Gly residue), can be produced in E. coli and be purified using conventional methods.

Example 10: Mouse pharmacokinetic profiles of proteins

Proteins or recombinant binding proteins produced as described in Example 9 are tested in mouse to determine pharmacokinetic parameters as described in Example 6. It is observed that a protein consisting of SEQ ID NOs: 4-3-11-3-11-3-4 and a protein consisting of SEQ ID NOs: 4-3-10-3-10-3-4, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 4-3-9-3-9-3-4. Similarly, it is observed that a protein consisting of SEQ ID NOs: 5-3-14-3-14-3-5, a protein consisting of SEQ ID NOs: 5-3-13-3-13-3-5, or a protein consisting of SEQ ID NOs: 5-3-15-3-15-3-5, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 5-3-12-3-12-3-5. Similarly, it is observed that a protein consisting of SEQ ID NOs: 6-2-19-2-19-2-6, a protein consisting of SEQ ID NOs: 6-2-18-2-18-2-6, or a protein consisting of SEQ ID NOs: 6-2-17-2-17-2-6, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 6-2-16-2-16-2-6. Similarly, it is observed that a protein consisting of SEQ ID NOs: 4-3-23-3-23-3-4, a protein consisting of SEQ ID NOs: 4-3-22-3-22-3-4, or a protein consisting of SEQ ID NOs: 4-3-21-3-21-3-4, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 4-3-20-3-20-3-4.

Example 11: Cynomolgus monkey pharmacokinetic profiles of proteins

Proteins or recombinant binding proteins produced as described in Example 9 are tested in cynomolgus monkey to determine pharmacokinetic parameters as described in Example 7. It is observed that a protein consisting of SEQ ID NOs: 4-3-11-3-11-3-4 and a protein consisting of SEQ ID NOs: 4-3-10-3-10-3-4, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 4-3-9-3-9-3-4. Similarly, it is observed that a protein consisting of SEQ ID NOs: 5-3-14-3-14-3-5, a protein consisting of SEQ ID NOs: 5-3-13-3-13-3-5, or a protein consisting of SEQ ID NOs: 5-3-15-3-15-3-5, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 5-3-12-3-12-3-5. Similarly, it is observed that a protein consisting of SEQ ID NOs: 6-2-19-2-19-2-6, a protein consisting of SEQ ID NOs: 6-2-18-2-18-2-6, or a protein consisting of SEQ ID NOs: 6-2-17-2-17-2-6, exhibit slower clearance, larger area under the curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 6-2-16-2-16-2-6. Similarly, it is observed that a protein consisting of SEQ ID NOs: 4-3-23-3-23-3-4, a protein consisting of SEQ ID NOs: 4-3-22-3-22-3-4, or a protein consisting of SEQ ID NOs: 4-3-21-3-21-3-4, exhibit slower clearance, larger area under the

curve, and longer terminal half-life than a protein consisting of SEQ ID NOs: 4-3-20-3-20-3-4.

- Additional examples of N-terminal capping modules and designed ankyrin repeat domains comprising the surface design of the invention are provided in SEQ ID NOs: 112 to 142. These sequences additionally have the amino acids GS at the N terminus.
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Claims

1. A designed ankyrin repeat domain, wherein said designed ankyrin repeat domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 75
5 to 81, 88 to 94, and 107 to 111, wherein X represents any amino acid.
2. The designed ankyrin repeat domain of claim 1, wherein said designed ankyrin repeat domain comprises (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 75 to 81 and 107 to 111, wherein X represents any amino acid, and (ii) an amino
10 acid sequence selected from the group consisting of SEQ ID NOs: 88 to 94, wherein X represents any amino acid.
3. The designed ankyrin repeat domain of any of claims 1 to 2, wherein said designed ankyrin repeat domain comprises (i) the amino acid sequence of SEQ ID NO: 78, wherein
15 X represents any amino acid, and (ii) the amino acid sequence of SEQ ID NO: 91, wherein X represents any amino acid.
4. The designed ankyrin repeat domain of any of claims 1 to 3, wherein said designed ankyrin repeat domain comprises an amino acid sequence selected from the group
20 consisting of SEQ ID NOs: 95 to 102, wherein X represents any amino acid.
5. A protein comprising (i) at least one designed ankyrin repeat domain of any of claims 1 to 4, and (ii) at least one moiety for half-life extension.
- 25 6. The protein of claim 5, wherein said moiety for half-life extension is a designed ankyrin repeat domain with binding specificity for serum albumin.
7. The protein of claim 6, wherein said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 80% amino
30 acid sequence identity with any one of SEQ ID NOs: 4 to 8.
8. The protein of claim 7, further comprising at least one polypeptide linker.
9. The protein of claim 8, wherein said polypeptide linker has the amino acid sequence of
35 SEQ ID NO: 2 or 3.

10. A protein comprising (i) at least two designed ankyrin repeat domains of any of claims 1 to 4, and (ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin, wherein said designed ankyrin repeat domain with binding specificity for serum albumin comprises an amino acid sequence with at least 80% amino acid sequence identity
5 with any one of SEQ ID NOs: 4 to 8.
11. A nucleic acid encoding the designed ankyrin repeat domain of any of claims 1 to 4 or the protein of any of claims 5 to 10.
- 10 12. A pharmaceutical composition comprising the designed ankyrin repeat domain of any of claims 1 to 4, the protein of any of claims 5 to 10, or the nucleic acid of claim 11, and optionally a pharmaceutical acceptable carrier and/or diluent.
- 15 13. A method of treating a medical condition, the method comprising the step of administering, to a patient in need of such treatment, a therapeutically effective amount of the pharmaceutical composition of claim 12.
14. A method for preparing a protein, the method comprising the steps of
(A) preparing a nucleic acid that encodes in one open reading frame
20 (i) at least one designed ankyrin repeat domain of any of claims 1 to 4, and
(ii) at least one designed ankyrin repeat domain with binding specificity for serum albumin, and
(B) transferring said DNA into an expression host.
- 25 15. The method of claim 14, wherein said expression host is *E. coli*.