

CLAIMS

1. A non-natural immunoglobulin (Ig) binding protein comprising one or more non-natural Ig-binding domains, wherein at least one non-natural Ig-binding domain comprises the amino acid sequence
 $X_1X_2X_3X_4X_5X_6X_7X_8QX_{11}AFYX_{15}X_{16}LX_{18}X_{19}PX_{21}LX_{23}X_{24}X_{25}QRX_{28}X_{29}FIQSLKDDPSX_{40}SX_{42}X_{43}X_{44}LX_{46}EAX_{49}KLX_{52}X_{53}X_{54}QX_{56}PX_{58}$ (SEQ ID NO: 1), wherein
 X_1 is A, V, Q, N, or P;
 X_2 is D, A, or Q;
 X_3 is A, S, or N;
 X_4 is K, Q, or N;
 X_5 is H or F;
 X_6 is D, S, or A;
 X_7 is E or K;
 X_8 is D, E or A;
 X_{11} is S or N;
 X_{15} is E, D, or Q;
 X_{16} is V or I;
 X_{18} is H or N;
 X_{19} is L or M;
 X_{21} is N, S, or D;
 X_{23} is T or N;
 X_{24} is E or A;
 X_{25} is D or E;
 X_{28} is N, S, or A;
 X_{29} is G or A;
 X_{40} is V or Q or T;
 X_{42} is K, T, or A;
 X_{43} is E, N, or S;
 X_{44} is V, L, or I;
 X_{46} is G or A;
 X_{49} is K or Q;
 X_{52} is N, S, or D;
 X_{53} is D or E;
 X_{54} is S or A;
 X_{56} is A or P; and
 X_{58} is K or P; wherein the dissociation constant K_D of said non-natural Ig-binding protein to human IgG1 is 1 μ M or less and wherein said non-natural Ig-binding protein is stable under alkaline conditions.

2. The non-natural Ig-binding protein according to claim 1, wherein the dissociation constant is 100 nM or less.
3. The non-natural Ig-binding protein according to claim 1, or 2, wherein at least one non-natural Ig-binding domain comprises the amino acid sequence
 $X_1X_2X_3X_4X_5X_6X_7X_8QX_{11}AFYEILHLPX_{21}LTEX_{25}QRX_{28}AFIQSLKDDPSX_{40}SX_{42}X_{43}X_{44}LX_{46}EAX_{49}KLX_{52}X_{53}X_{54}QAPX_{58}$ (SEQ ID NO: 38), wherein
 X_1 is N, V, P, or A;
 X_2 is D or A;
 X_3 is A, S, or N;
 X_4 is K or Q;
 X_5 is H or F;
 X_6 is D, S, or A;
 X_7 is E or K;
 X_8 is D, E or A;
 X_{11} is S or N;
 X_{21} is N, S, or D;
 X_{25} is D or E;
 X_{28} is N, S, or A;
 X_{40} is V, T, or Q;
 X_{42} is K or A;
 X_{43} is E or S;
 X_{44} is V, L, or I;
 X_{46} is G or A;
 X_{49} is K or Q;
 X_{52} is N, S, or D;
 X_{53} is D or E;
 X_{54} is S or A; and
 X_{58} is K or P.
4. The non-natural Ig-binding protein according to claim 1 or 2, wherein said non-natural Ig-binding protein comprises the amino acid sequence
 $X_1X_2AX_4X_5DX_7X_8QX_{11}AFYEILHLPNLTEX_{25}QRNAFIQSLKDDPSX_{40}SX_{42}X_{43}X_{44}LX_{46}EAX_{49}KLNX_{53}X_{54}QAPK$ (SEQ ID NO: 48), wherein
 X_1 is N or V;
 X_2 is D or A;
 X_4 is K or Q;
 X_5 is H or F;
 X_7 is E or K;
 X_8 is D, E or A;
 X_{11} is S or N;
 X_{25} is D or E;
 X_{40} is V or Q;

X₄₃ is E or S;

X₄₂ is K or A;

X₄₄ is V or I;

X₄₆ is G or A;

X₄₉ is K or Q;

X₅₃ is D or E; and

X₅₄ is S or A.

5. The non-natural Ig-binding protein according to claim 1 or 2, wherein said non-natural Ig-binding protein comprises the amino acid sequence

X₁X₂X₃X₄X₅X₆X₇X₈QX₁₁AFYEILHLPX₂₁LTEDQRX₂₈AFIQSLKDDPSX₄₀SKX₄₃X₄₄LGEAKKLX₅₂DAQAPP (SEQ ID NO: 49), wherein

X₁ is P, N, or A;

X₂ is A or D;

X₃ is A, S, or N;

X₄ is K or Q;

X₅ is H or F;

X₆ is D, S, or A;

X₇ is K or E;

X₈ is D, E or A;

X₁₁ is S or N;

X₂₁ is N, S, or D;

X₂₈ is S or A;

X₄₀ is V or T;

X₄₃ is E or S;

X₄₄ is I or L; and

X₅₂ is N, S, or D.

6. The non-natural Ig-binding protein according to any one of claims 1 to 5, wherein at least one non-natural Ig-binding domain comprises an amino acid sequence selected from the group consisting of:

NAAQHAKEQQNAFYELHLPNLTEDQRAAFIQSLKDDPSVSKEILGEAKKLNDQAPP (SEQ ID NO: 9),

NAAQHDKEQQNAFYELHLPNLTEDQRAAFIQSLKDDPSVSKEILGEAKKLNDQAPP (SEQ ID NO: 10),

NAAQHSKEQQNAFYELHLPNLTEDQRSFAFIQSLKDDPSVSKEILGEAKKLNDQAPP (SEQ ID NO: 11),

NAAQHSKDQQSAFYELHLPNLTEDQRSFAFIQSLKDDPSVSKEILGEAKKLNDQAPP (SEQ ID NO: 12),

PAAQHDKDQQSAFYELHLPNLTEDQRSFAFIQSLKDDPSVSKEILGEAKKLNDQAPP (SEQ ID NO: 13),

PAAKHDKDQQSAFYELHLPNLTEDQRSFAFIQSLKDDPSVSKEILGEAKKLNDQAPP (SEQ ID NO: 14),

ADNKFDEAQQSAFYELHLPNLTEDQRAAFIQSLKDDPSVSKEILGEAKKLNDQAAPP (SEQ ID NO: 15),
 ADSKFDEAQQSAFYELHLPNLTEDQRAAFIQSLKDDPSVSKEILGEAKKLNDQAAPP (SEQ ID NO: 16),
 ADSKFDEAQQSAFYELHLPNLTEDQRAAFIQSLKDDPSVSKSLLGEAKKLNDQAAPP (SEQ ID NO: 17),
 ADSKFDEAQQSAFYELHLPDLTEDQRAAFIQSLKDDPSVSKSLLGEAKKLNDQAAPP (SEQ ID NO: 18),
 ADSKFDEAQQSAFYELHLPSTLEDQRAAFIQSLKDDPSVSKSLLGEAKKLNDQAAPP (SEQ ID NO: 19),
 ADSKFDEAQQSAFYELHLPSTLEDQRAAFIQSLKDDPSTSKSLLGEAKKLNDQAAPP (SEQ ID NO: 20),
 ADSKFDEAQQSAFYELHLPSTLEDQRAAFIQSLKDDPSTSKSLLGEAKKLDDQAAPP (SEQ ID NO: 21),
 ADSKFDEAQQSAFYELHLPSTLEDQRAAFIQSLKDDPSTSKSLLGEAKKLSDQAAPP (SEQ ID NO: 22),
 PAAKHDKDQQSAFYELHLPSTLEDQRAAFIQSLKDDPSTSKSILGEAKKLNDQAAPP (SEQ ID NO: 23),
 NAAQHDKDQQNAFYELHLPNLTEDQRNAFIQSLKDDPSVSKEILGEAKKLNDQAAPK (SEQ ID NO: 24),
 ADNKFDEAQQSAFYELHLPNLTEDQRNAFIQSLKDDPSVSKEILGEAKKLNDQAAPK (SEQ ID NO: 25),
 NAAKHDKDQQSAFYELHLPNLTEDQRNAFIQSLKDDPSVSKEILGEAKKLNDQAAPP (SEQ ID NO: 26),
 NAAQHDKDQQSAFYELHLPNLTEEQRNAFIQSLKDDPSVSKEILAEAKKLNDQAAPK (SEQ ID NO: 27),
 NAAKFDEAQQSAFYELHLPNLTEEQRNAFIQSLKDDPSVSKEVLGEAQKLNDSQAPK (SEQ ID NO: 28),
 QQAQHDEAQQSAFYQVLHLPNLTAQRNAFIQSLKDDPSQSAEVLGEAQKLNDSQAPK (SEQ ID NO: 29), and
 VDAQHDEDQQSAFYELHLPNLTEEQRNAFIQSLKDDPSQSAEILAEAKKLNESQAPK (SEQ ID NO: 30).

7. The non-natural Ig-binding protein according to any one of claims 1 to 6, wherein 2, 3, 4, 5, 6, 7, or 8 non-natural Ig-binding domains are linked to each other.
8. The non-natural Ig-binding protein according to any one of claims 1 to 7, wherein said non-natural Ig-binding protein comprises a specific attachment site for site-specific covalent attachment to a solid phase.
9. A composition comprising the non-natural Ig binding protein as defined in any one of claims 1 to 8.
10. The composition of claim 9 wherein the composition is an affinity separation matrix.

11. Use of the non-natural Ig-binding protein of any one of claims 1 to 8 or use of the composition of claim 9 for affinity purification of immunoglobulins.
12. A method of affinity purification of immunoglobulins comprising the steps of
 - a. providing a liquid containing an immunoglobulin;
 - b. providing an affinity separation matrix comprising an immobilized non-natural Ig-binding protein of any one of claims 1 to 8;
 - c. contacting said liquid and said affinity separation matrix, wherein said immunoglobulin binds to said immobilized protein; and
 - d. eluting said immunoglobulin from said matrix, thereby obtaining an eluate containing said immunoglobulin.
13. The method of affinity purification of immunoglobulins of claim 12, further comprising one or more washing steps carried out between steps (c) and (d).
14. The method of affinity purification of immunoglobulins of claim 12 or 13 wherein in step d, the immunoglobulin is eluted by a change in pH or a change in salt concentration.
15. The method according to any one of claims 12 to 14, wherein the immunoglobulin is selected from the group consisting of human IgG1, human IgG2, human IgG4, human IgM, human IgA, mouse IgG1, mouse IgG2A, mouse IgG2B, mouse IgG3, rat IgG1, rat IgG2C, goat IgG1, goat IgG2, bovine IgG2, guinea pig IgG, rabbit IgG, immunoglobulin fragments comprising the Fc region, fusion proteins comprising an Fc region of an immunoglobulin, and conjugates comprising an Fc region of an immunoglobulin.
16. A method of generation of a non-natural Ig binding protein according to any one of claims 1 to 8, wherein at least one Ig-binding domain is obtainable by a shuffling process of at least two naturally occurring Ig-binding domains from a naturally occurring Ig-binding protein.
17. A nucleic acid molecule encoding a non-natural Ig binding protein as defined in any one of claims 1 to 8.
18. A vector comprising the nucleic acid molecule of claim 17.
19. A host cell or a non-human host comprising the non-natural Ig binding protein as defined in any one of claims 1 to 8, a nucleic acid as defined in claim 17, or a vector of claim 18.
20. A method for the production of a non-natural Ig binding protein of any one of the claims 1 to 8, comprising the step(s):
 - (a) culturing the host cell of claim 19 under suitable conditions for the expression of the binding protein in order to obtain said non-natural Ig binding protein.
21. The method according to claim 20, further comprising the step of (b) isolating said non-natural Ig binding protein.