CLAIMS

1. A polypeptide comprising an amino acid sequence having at least 80% identity to amino acid residues 1 to 98 of SEQ ID NO: 1;

characterised in that the polypeptide comprises one or more mutations relative to SEQ ID NO: 1 selected from the group consisting of:

N32G, T51L, T51V, M65V, A59I, E29M, T34V, T34R, T45I, T45V, T51F, A59L, L67I, (E29K, K30E, E33K), (A59L, G60N, D61G, N62K), (A59V, D61N, N62K), (G60N, D61G, N62K), (G60N, ΔD61, N62G), ΔD61, (A59L, G60N, ΔD61, N62G), (A59V, G60N, D61G, N62K), (A59I, G60N, D61G, N62K), (A59L, G60N, ΔD61, N62G), (A59V, G60N, ΔD61, N62G), (A59V, ΔD61), L38A, V20I, A40I, L38V, A12I, A12V, I16L, V20L, Q26L, T31K, N32D, N32H, T34D, T34P, A40V, Q42D, V48E, V48G, V48A, T51A, (V20I, L38A), (V20L, L38A), (V20I, L38V), (V20L, L38V), (Y54D, T83D, Q86E), (G60P, ΔD61, N62P), (G60P, D61P, N62K), (G60P, ΔD61, N62G), (G60P, D61G, N62K), (D61N, N62K) and (T83D, Q86E);

in which the polypeptide has a Tm higher than the Tm of the polypeptide with the sequence MIPRGLSEAK PATPEIQEIV DKVKPQLEEK TNETYGKLEA VQYKTQVLAS TNYYIKVRAG DNKYMHLKVF NGPPGQNADR VLTGYQVDKN KDDELTGF.

2. A polypeptide according to Claim 1, in which the polypeptide further comprises at least one heterologous peptide insertion, in which the heterologous peptide insertion comprises a heterologous peptide inserted at one of the following positions relative to SEQ ID NO: 1:

(a) 47-<heterologous peptide>-55

(b) 46-<heterologous peptide>-54

(c) 46-<heterologous peptide>-50

(d) 48-<heterologous peptide>-50

(e) 49-<heterologous peptide>-51

(f) 50-<heterologous peptide>-52

(g) 66-<heterologous peptide>-85

(h) 67-<heterologous peptide>-84

(i) 70-<heterologous peptide>-74

(j) 72-<heterologous peptide>-74

(k) 71-<heterologous peptide>-73

(l) 72-<heterologous peptide>-81

(m) 73-<heterologous peptide>-80

(n) 79-<heterologous peptide>-81

(o) 80-<heterologous peptide>-81

(p) 82-<heterologous peptide>-83

(q) 72-<heterologous peptide>-77

(r) 73-<heterologous peptide>-78

(s) 74-<heterologous peptide>-79

(t) 4-<heterologous peptide>-5

3. A polypeptide according to Claim 2, in which the polypeptide comprises two heterologous peptide insertions, a first heterologous peptide insertion at any of positions (a) to (f), and a second heterologous peptide insertion at any of positions (g) to (s).

4. A polypeptide according to Claim 2, in which the polypeptide comprises two heterologous peptide insertions, a first heterologous peptide insertion at any of positions (a) to (f), and a second heterologous peptide insertion at position (t).

5. A polypeptide according to Claim 2, in which the polypeptide comprises two heterologous peptide insertions, a first heterologous peptide insertion at any of positions (g) to (s), and a second heterologous peptide insertion at position (t).

6. A polypeptide according to Claim 2, in which the polypeptide comprises three heterologous peptide insertions, a first heterologous peptide insertion at any of positions (a) to (f), and a second heterologous peptide insertion at any of positions (g) to (s), and a third heterologous peptide insertion at position (t).

7. A polypeptide according to any of Claims 1 to 8, in which the one or more mutations relative to SEQ ID NO: 1 is: N32G.

8. A polypeptide according to any preceding claim, in which the polypeptide further comprises one or more mutations relative to SEQ ID NO: 1 selected from the group consisting of: G4R, G4W, V48D, V48E, G50 S, Y35W, Y43W, Y53W, Y54W, Y64W, F70W, Y85W, F98W, (K71N S72G L73P), or (E78A L80R).

9. A polypeptide according to any preceding claim comprising five or fewer mutations relative to SEQ ID NO: 1.

10. A polypeptide according to Claim 9, in which the five or fewer mutations are selected from the group consisting of N32G, Y35W, V48D, M65I, Q42E and T51L.

11. A polypeptide according to Claim 9, in which the five or fewer mutations are selected from the group consisting of N32G, V48D, M65I, Q42E and T51L.

12. A polypeptide according to Claim 9 having each of the mutations in one of the following groups:

(i) N32G V48D

(ii) N32G V48D M65I

(iii) N32G V48D M65I T51L

(iv) N32G V48D M65I Q42E

(v) N32G V48D M65I Q42E T51L

13. A polypeptide according to Claim 12 having each of the mutations iv) N32G, V48D, M65I and Q42E.

14. A polypeptide according to any of Claims 1 to 8 having each of the mutations in one of the following groups:

(a) N32G Y35W V48D M65I Q42E T51L (A59V ΔD61) (E29K K30E E33K)

(b) N32G Y35W V48D M65I Q42E T51L (A59V G60N ΔD61 N62G) (E29K K30E E33K)

15. A polypeptide according to Claim 14 having each of the mutations (b) N32G Y35W V48D M65I Q42E T51L (A59V G60N ΔD61 N62G) (E29K K30E E33K).

16. A polypeptide according to any preceding claim, in which the heterologous peptide is 6 to 36 amino acids in length.

17. A fusion protein comprising:

(a) a polypeptide according to any preceding claim; and

(b) one or more additional amino acid sequences selected from the group consisting of: secretion signal sequences, peptide linker sequences, affinity tags, transmembrane domains, cell surface retention sequence, substrate recognition sequences for post-translational modifications, multimerization domains to create multimeric structures of the protein aggregating through protein-protein interactions, half-life extending polypeptide moieties, polypeptide sequences for altering tissue localization and antigen binding site of an antibody, one or more additional polypeptides according to any preceding claim binding to the same or different targets, and one or more additional Affimer polypeptide sequences binding to the same or different targets.

18. A fusion protein according to Claim 17, comprising one or more half-life extending polypeptide moieties selected from the group consisting of an Fc domain or portion thereof, an albumin protein or portion thereof, an albumin-binding polypeptide moiety, transferrin or portion thereof, a transferrin-binding polypeptide moiety, fibronectin or portion thereof, or a fibronectin-binding polypeptide moiety.

19. A polypeptide according to Claim 18, in which the Fc domain or a portion thereof retains FcN binding.

20. A polypeptide according to Claim 18, in which the Fc domain or a portion thereof is from IgA, IgD, IgE, IgG, and IgM or a subclass (isotype) thereof such as IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2.

21. A polypeptide according to Claim 18, in which the Fc domain or a portion thereof retains effector function selected from C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of B cell receptor, or a combination thereof.

22. A polypeptide according to Claim 18, in which the half-life extending polypeptide moiety increases the serum half-life of the protein by at least 5-fold relative to its absence from the protein.

23. A polypeptide according to any preceding claim which is an affimer polypeptide.

24. A nucleic acid comprising a nucleotide sequence encoding a polypeptide according to any preceding claim.

25. A vector comprising a nucleic acid according to Claim 24.

26. A library comprising a population of polypeptides according to any of Claims 1 to 23, in which at least two individual polypeptides within the population comprise different heterologous peptide insertions.

27. A library comprising a population of nucleic acids, the nucleic acids comprising nucleotide sequences encoding a population of polypeptides according to Claim 26.

28. A host cell comprising a polypeptide according to any of Claims 1 to 23, a nucleic acid according to Claim 24, or a library according to Claim 26 or 27.

29. A polypeptide according to any of Claims 1 to 23 for use in medicine.

30. A polypeptide according to any of Claims 1 to 23 for use in the manufacture of a medicament.

31. A method for identifying a peptide capable of binding a structure of interest, the method comprising:

(i) providing a polypeptide according to any of Claims 1 to 23 comprising a heterologous peptide insertion;

(ii) contacting the polypeptide with the structure of interest; and

(iii) monitoring the association between the polypeptide and the structure of interest;

in which association of the polypeptide with the structure of interest identifies the peptide as a candidate peptide capable of binding the structure.

32. Use of a polypeptide according to any of Claims 1 to 23 as a scaffold protein.

CLAIMS

1. A polypeptide that is suitable for use as a scaffold protein and that comprises an amino acid sequence having at least 80% identity to amino acid residues 1 to 98 of SEQ ID NO: 1;

characterised in that the polypeptide comprises one or more mutations relative to SEQ ID NO: 1 selected from the group consisting of:

N32G, T51L, T51V, M65V, A59I, E29M, T34V, T34R, T45I, T45V, T51F, A59L, L67I, (E29K, K30E, E33K), (A59L, G60N, D61G, N62K), (A59V, D61N, N62K), (G60N, D61G, N62K), (G60N, ΔD61, N62G), ΔD61, (A59L, G60N, ΔD61, N62G), (A59V, G60N, D61G, N62K), (A59I, G60N, D61G, N62K), (A59I, G60N, ΔD61, N62G), (A59V, G60N, ΔD61, N62G), (A59V, ΔD61), L38A, V20I, A40I, L38V, A12I, A12V, I16L, V20L, Q26E, T31K, N32D, N32H, T34D, T34P, A40V, Q42D, V48E, V48G, V48A, T51A, (V20I, L38A), (V20L, L38A), (V20I, L38V), (V20L, L38V), (Y54D, T83D, Q86E), (G60P, ΔD61, N62P), (G60P, D61P, N62K), (G60P, ΔD61, N62G), (G60P, D61G, N62K), (D61N, N62K) and (T83D, Q86E);

in which the polypeptide has a Tm higher than the Tm of the polypeptide with the sequence MIPRGLSEAK PATPEIQEIV DKVKPQLEEK TNETYGKLEA VQYKTQVLAS TNYYIKVRAG DNKYMHLKVF NGPPGQNADR VLTGYQVDKN KDDELTGF.

2. A polypeptide according to Claim 1, in which the polypeptide further comprises at least one heterologous peptide insertion, in which the heterologous peptide insertion comprises a heterologous peptide inserted at one of the following positions relative to SEQ ID NO: 1:

(a) 47-<heterologous peptide>-55

(b) 46-<heterologous peptide>-54

(c) 46-<heterologous peptide>-50

(d) 48-<heterologous peptide>-50

(e) 49-<heterologous peptide>-51

(f) 50-<heterologous peptide>-52

(g) 66-<heterologous peptide>-85

(h) 67-<heterologous peptide>-84

(i) 70-<heterologous peptide>-74

(j) 72-<heterologous peptide>-74

(k) 71-<heterologous peptide>-73

(l) 72-<heterologous peptide>-81

(m) 73-<heterologous peptide>-80

(n) 79-<heterologous peptide>-81

(o) 80-<heterologous peptide>-81

(p) 82-<heterologous peptide>-83

(q) 72-<heterologous peptide>-77

(r) 73-<heterologous peptide>-78

(s) 74-<heterologous peptide>-79

(t) 4-<heterologous peptide>-5

3. A polypeptide according to Claim 2, in which the polypeptide comprises two heterologous peptide insertions, a first heterologous peptide insertion at any of positions (a) to (f), and a second heterologous peptide insertion at any of positions (g) to (s).

4. A polypeptide according to Claim 2, in which the polypeptide comprises two heterologous peptide insertions, a first heterologous peptide insertion at any of positions (a) to (f), and a second heterologous peptide insertion at position (t).

5. A polypeptide according to Claim 2, in which the polypeptide comprises two heterologous peptide insertions, a first heterologous peptide insertion at any of positions (g) to (s), and a second heterologous peptide insertion at position (t).

6. A polypeptide according to Claim 2, in which the polypeptide comprises three heterologous peptide insertions, a first heterologous peptide insertion at any of positions (a) to (f), and a second heterologous peptide insertion at any of positions (g) to (s), and a third heterologous peptide insertion at position (t).

7. A polypeptide according to any preceding claim, in which the one or more mutations relative to SEQ ID NO: 1 is: N32G.

8. A polypeptide according to any preceding claim, in which the polypeptide further comprises one or more mutations relative to SEQ ID NO: 1 selected from the group consisting of: G4R, G4W, V48D, V48E, G50 S, Y35W, Y43W, Y53W, Y54W, Y64W, F70W, Y85W, F98W, (K71N S72G L73P), or (E78A L80R).

9. A polypeptide according to any preceding claim comprising five or fewer mutations relative to SEQ ID NO: 1.

10. A polypeptide according to Claim 9, in which the five or fewer mutations are selected from the group consisting of N32G, Y35W, V48D, M65I, Q42E and T51L.

11. A polypeptide according to Claim 9, in which the five or fewer mutations are selected from the group consisting of N32G, V48D, M65I, Q42E and T51L.

12. A polypeptide according to Claim 9 having each of the mutations in one of the following groups:

(i) N32G V48D

(ii) N32G V48D M65I

(iii) N32G V48D M65I T51L

(iv) N32G V48D M65I Q42E

(v) N32G V48D M65I Q42E T51L

13. A polypeptide according to Claim 12 having each of the mutations iv) N32G, V48D, M65I and Q42E.

14. A polypeptide according to any of Claims 1 to 8 having each of the mutations in one of the following groups:

(a) N32G Y35W V48D M65I Q42E T51L (A59V ΔD61) (E29K K30E E33K)

(b) N32G Y35W V48D M65I Q42E T51L (A59V G60N ΔD61 N62G) (E29K K30E E33K)

15. A polypeptide according to Claim 14 having each of the mutations (b) N32G Y35W V48D M65I Q42E T51L (A59V G60N ΔD61 N62G) (E29K K30E E33K).

16. A polypeptide according to any preceding claim, in which the peptide is 6 to 36 amino acids in length.

17. A fusion protein comprising:

(a) a polypeptide according to any preceding claim; and

(b) one or more additional amino acid sequences selected from the group consisting of: secretion signal sequences, peptide linker sequences, affinity tags, transmembrane domains, cell surface retention sequence, substrate recognition sequences for post-translational modifications, multimerization domains to create multimeric structures of the protein aggregating through protein-protein interactions, half-life extending polypeptide moieties, polypeptide sequences for altering tissue localization and antigen binding site of an antibody, one or more additional polypeptides according to any preceding claim binding to the same or different targets, and one or more additional Affimer polypeptide sequences binding to the same or different targets.

18. A fusion protein according to Claim 17, comprising one or more half-life extending polypeptide moieties selected from the group consisting of an Fc domain or portion thereof, an albumin protein or portion thereof, an albumin-binding polypeptide moiety, transferrin or portion thereof, a transferrin-binding polypeptide moiety, fibronectin or portion thereof, or a fibronectin-binding polypeptide moiety.

19. A polypeptide according to Claim 18, in which the Fc domain or a portion thereof retains FcN binding.

20. A polypeptide according to Claim 18, in which the Fc domain or a portion thereof is from IgA, IgD, IgE, IgG, and IgM or a subclass (isotype) thereof such as IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2.

21. A polypeptide according to Claim 18, in which the Fc domain or a portion thereof retains effector function selected from C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of B cell receptor, or a combination thereof.

22. A polypeptide according to Claim 18, in which the half-life extending polypeptide moiety increases the serum half-life of the protein by at least 5-fold relative to its absence from the protein.

23. A polypeptide according to any preceding claim which is an affimer polypeptide.

24. A nucleic acid comprising a nucleotide sequence encoding a polypeptide according to any preceding claim.

25. A vector comprising a nucleic acid according to Claim 24.

26. A library comprising a population of polypeptides according to any of Claims 1 to 23, in which at least two individual polypeptides within the population comprise different heterologous peptide insertions.

27. A library comprising a population of nucleic acids, the nucleic acids comprising nucleotide sequences encoding a population of polypeptides according to Claim 26.

28. A host cell comprising a polypeptide according to any of Claims 1 to 23, a nucleic acid according to Claim 24, or a library according to Claim 26 or 27.

29. A polypeptide according to any of Claims 1 to 23 for use in medicine.

30. A polypeptide according to any of Claims 1 to 23 for use in the manufacture of a medicament.

31. A method for identifying a peptide capable of binding a structure of interest, the method comprising:

(i) providing a polypeptide according to any of Claims 1 to 23 comprising a heterologous peptide insertion;

(ii) contacting the polypeptide with the structure of interest; and

(iii) monitoring the association between the polypeptide and the structure of interest;

in which association of the polypeptide with the structure of interest identifies the peptide as a candidate peptide capable of binding the structure.

32. Use of a polypeptide according to any of Claims 1 to 23 as a scaffold protein.