heterologous peptides. 3t4$(GGS)_3$ contains the heterologous peptide sequence GGSGGSGGS (SEQ ID NO: 92) in place of A49 (loop 2) and in place of P74-G75-Q76-N77 (loop 4).

Thus it can be seen that over the wavelengths measured, the shape of the spectra for the scaffolds bearing heterologous peptide insertions are essentially no different from the scaffolds without insertions. This shows the structure has been maintained with heterologous peptide insertions. This is shown for different exemplary scaffolds of the invention.

Example 5: Immunogenicity

The immunogenicity of polypeptides according to the present invention was modelled.

%rank is the %rank of predicted affinity compared with a set of 200000 random natural human peptides, e.g. a value of 1% means the 15mer peptide is predicted to bind as strongly as the top 1% of the 200000 random peptides. Any rank below 10% (i.e. 10%-100%) is not considered immunogenic. These calculations were carried out using the NetMHCIIpan (v3.1) server. Forty-one MHC alleles were tested in total for each sequence. This plot shows the result for one allele (the DRB1 1502 allele) as a representative example.

Referring to Figure 9, it can concluded that the mutations taught herein produce only small movements up or down on immunogenicity. The plots are largely similar to hSteA. No large immunogenic region has been introduced. No large changes/deviations from wild type have been introduced.

Example 6: Serum Stability

Stability such as serum stability was tested.

Each protein was incubated in human serum (Sigma) at 37 °C for up to 16 days. Samples were withdrawn at days 0, 1, 2, 4, 8 and 16 and kept frozen until measurement. All samples were analysed using the Wes simple western system (Protein Simple) to detect the presence of monomer and any other species present. Detection was *via* an anti-(His6) monoclonal. Over the course of this experiment, SQT showed a steady decrease in the area of the monomer peak (Figure 10a), whereas the other proteins remained steady. The decrease in the amount of SQT monomer was matched by an increase in SQT dimer (Figure 10b). This is expected as SQT has V48L and so is still able to dimerise.

There was no evidence of any material appearing at lower molecular weights, for example from protease digestion.

The same results were seen in buffer (rather than serum).

Thus it is demonstrated that polypeptides according to the present invention are stable and do not significantly degrade in human serum over the time course shown, or indeed in standard storage buffer.

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), (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 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.