Claims

1. A peptide ligand specific for human Kallikrein comprising a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold, wherein the loops of the peptide ligand comprise three, four or five, but less than six, amino acids.

2. A peptide ligand according to claim 1, wherein the loops of the peptide ligand comprise three amino acids and the polypeptide has the consensus sequence $G_rFxxG_rRVxG_r$, wherein G_r is a reactive group.

3 A peptide ligand according to claim 2, which comprises one of the polypeptides set forth in **Table 3**.

4. A peptide ligand according to claim 1, wherein the loops of the peptide ligand comprise five amino acids and a first loop comprises the consensus sequence $G_rGGxxNG_r$, wherein G_r is a reactive group.

5. A peptide ligand according to claim 4, wherein two adjacent loops comprise the consensus sequence $G_rGGxxNG_rRxxxxG_r$.

6. A peptide according to claim 4 or claim 5, which comprises one of the peptides set forth in **Table 4**.

7. A peptide ligand according to claim 1, wherein the loops of the peptide ligand comprise five amino acids and a first loop comprises the motif $G_rx^W/_FPx^K/_RG_r$, wherein G_r is a reactive group.

8. A peptide ligand according to claim 7, further comprising a second loop which comprises the motif $G_r^T/_LH^Q/_TxLG_r$.

9. A peptide ligand according to claim 1, wherein the loops of the peptide ligand comprise five amino acids and a first loop comprises the motif $G_rxHxDLG_r$, wherein G_r is a reactive group.

10. A peptide ligand according to claim 1, wherein the loops of the peptide ligand comprise five amino acids and a first loop comprises the motif $G_rTHxxLG_r$, wherein G_r is a reactive group.

11. A peptide ligand according to any one of claims 8 to 10, wherein two adjacent loops comprise the motif $G_rx^W/_FPx^K/_RG_r^T/_LH^Q/_TDLG_r$.

12. A peptide ligand according to any one of claims 7 to 11, which comprises one of the polypeptides set forth in **Table 4, Table 5 or Table 6**.

13. A peptide ligand according to any one of claims 7 to 11, wherein the first loop comprises the sequence $G_rxWPARG_r$.

14. A peptide ligand according to any one of claims 7 to 11, wherein the first loop comprises the sequence $G_rxWPSRG_r$.

15. A peptide ligand according to any one of claims 7 to 11, wherein the first loop comprises the sequence $G_rxFPFRG_r$.

16. A peptide ligand according to any one of claims 7 to 11, wherein the first loop comprises the sequence $G_rxFPYRG_r$.

17. A peptide ligand according to any one of claims 13 to 16, wherein x is S or R.

18. A peptide ligand according to any preceding claim, wherein the reactive group is cysteine.

19. A polypeptide ligand according to any preceding claim which comprises one or more non-natural amino acid substituents and is resistant to protease degradation.

20. A polypeptide ligand according to claim 19, wherein the modified amino acid is selected from N-methyl Arginine, homo-arginine, hydroxyproline, and guanidyl-phenylalanine, and azetidine carboxylic acid.

21. A polypeptide ligand according to claim 20, wherein the polypeptide comprises a first loop which comprises the motif $G_rxWPARG_r$, wherein Pro is replaced with azetidine carboxylic acid; and/or R is replaced with N-methyl arginine; and/or R is replaced with homoarginine; and/or R is replaced with guanidylphenylalanine.

22. A polypeptide ligand according to claim 20, wherein the polypeptide comprises a first loop which comprises the motif $G_rxFPYRG_r$, wherein R is replaced with N-methyl arginine; and/or R is replaced with homoarginine, and/or R is replaced with guanidylphenylalanine, and wherein Pro is replaced with azetidine carboxylic acid.

23. A method for producing a mutant polypeptide ligand to produce an improved level of binding activity for a target over that of a parent polypeptide ligand, wherein the parent polypeptide ligand comprises a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold, comprising the steps of:

(a) for each of two or more amino acid positions in each of the loop sequences, producing n different libraries of mutants, each library consisting of parent polypeptides in which one of said amino acid positions in the loop sequence has been mutated by replacement with one of n different non-parental amino acids;

(b) screening each library for binding to the parental target, and scoring each mutation;

(c) identifying the amino acid positions at which mutations are tolerated; and

(d) producing one or more mutant polypeptides comprising one or more mutations located at the amino acid positions identified in step (c).

24. A method according to claim 23, wherein step (d) comprises preparing a library comprising polypeptides which incorporate mutations at two or more of the amino acid positions identified in step (c), and screening the library for polypeptides with an improved level of binding activity for the target.

25. A library of polypeptide ligands, wherein the polypeptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold, said library consisting of m different mutants of a polypeptide ligand in which a defined amino acid position in the loop sequences has been mutated by replacement with one of m different amino acids, wherein m is at least 2.

26. A set of libraries of polypeptide ligands, wherein the polypeptide ligands comprise a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold, which set comprises two or more libraries of polypeptide ligands, each of said libraries of polypeptide ligands consisting of m different mutants of a polypeptide ligand in

which a defined amino acid position in the loop sequences has been mutated by replacement with one of m different amino acids.

27. A set of libraries according to claim 26, wherein m is between 2 and 20.

CLAIMS

1. A peptide ligand specific for human Kallikrein comprising a polypeptide comprising at least three reactive groups, separated by at least two loop sequences, and a molecular scaffold which forms covalent bonds with the reactive groups of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold, in which the loops of the peptide ligand comprise three, four or five, but less than six, amino acids.

2. A peptide ligand according to Claim 1, in which the loops of the peptide ligand comprise three amino acids and the polypeptide has the consensus sequence $G_rFxxG_rRVxG_r$, in which G_r is a reactive group.

3 A peptide ligand according to Claim 2, which comprises one of the polypeptides set forth in **Table 3**.

4. A peptide ligand according to Claim 1, in which the loops of the peptide ligand comprise five amino acids and a first loop comprises the consensus sequence $G_rGGxxNG_r$, in which G_r is a reactive group.

5. A peptide ligand according to Claim 4, in which two adjacent loops comprise the consensus sequence $G_rGGxxNG_rRxxxxG_r$.

6. A peptide according to Claim 4 or 5, which comprises one of the peptides set forth in **Table 4**.

7. A peptide ligand according to Claim 1, in which the loops of the peptide ligand comprise five amino acids and a first loop comprises the motif $G_rx^{W}/_{F}Px^{K}/_{R}G_r$, in which G_r is a reactive group.

8. A peptide ligand according to Claim 7, further comprising a second loop which comprises the motif $G_r{}^{T}/_{L}H^{Q}/_{T}xLG_r$.

9. A peptide ligand according to Claim 1, in which the loops of the peptide ligand comprise five amino acids and a first loop comprises the motif $G_rxHxDLG_r$, in which G_r is a reactive group.

10. A peptide ligand according to Claim 1, in which the loops of the peptide ligand comprise five amino acids and a first loop comprises the motif $G_rTHxxLG_r$, in which G_r is a reactive group.

11. A peptide ligand according to Claim 8, 9 or 10, in which two adjacent loops comprise the motif $G_rx^{W}/_{F}Px^{K}/_{R}G_r^{T}/_{L}H^{Q}/_{T}DLG_r$.

12. A peptide ligand according to any of Claims 7 to 11, which comprises one of the polypeptides set forth in **Table 4, Table 5 or Table 6**.

13. A peptide ligand according to any of Claims 7 to 11, in which the first loop comprises the sequence $G_rxWPARG_r$**.**

14. A peptide ligand according to any of Claims 7 to 11, in which the first loop comprises the sequence $G_rxWPSRG_r$**.**

15. A peptide ligand according to any of Claims 7 to 11, in which the first loop comprises the sequence $G_rxFPFRG_r$**.**

16. A peptide ligand according to any of Claims 7 to 11, in which the first loop comprises the sequence $G_rxFPYRG_r$**.**

17. A peptide ligand according to any of Claims 13 to 16, in which x is S or R.

18. A peptide ligand according to any preceding claim, in which the reactive group is cysteine.

19. A polypeptide ligand according to any preceding claim which comprises one or more non-natural amino acid substituents and is resistant to protease degradation.

20. A polypeptide ligand according to Claim 19, in which the modified amino acid is selected from N-methyl Arginine, homo-arginine, hydroxyproline, and guanidyl-phenylalanine, and azetidine carboxylic acid.

21. A polypeptide ligand according to Claim 20, in which the polypeptide comprises a first loop which comprises the motif $G_rxWPARG_r$, in which Pro is replaced with azetidine carboxylic acid; and/or R is replaced with N-methyl arginine; and/or R is replaced with homoarginine; and/or R is replaced with guanidylphenylalanine.

22. A polypeptide ligand according to Claim 20, in which the polypeptide comprises a first loop which comprises the motif $G_rxFPYRG_r$, in which R is replaced with N-methyl arginine; and/or R is replaced with homoarginine, and/or R is replaced with guanidylphenylalanine, and in which Pro is replaced with azetidine carboxylic acid.