Claims

1. A method for altering the conformation of a first polypeptide ligand or group of polypeptide ligands, each polypeptide ligand comprising at least two reactive groups separated by a loop sequence covalently linked to a molecular scaffold which forms covalent bonds with said reactive groups, to produce a second polypeptide ligand or group of polypeptide ligands, comprising assembling said second ligand or group of ligands from the polypeptide(s) and structural scaffold of said first ligand or group of ligands, incorporating one of:

   (a) altering at least one reactive group; or

   (b) altering the nature of the molecular scaffold; or

   (c) altering the bond between at least one reactive group and the molecular scaffold; or

   (d) any combination of (a), (b) or (c).

2. A method according to claim 1, wherein the reactive group comprises a sulphur atom.

3. A method according to claim 2, wherein the reactive group is a cysteine or a methionine.

4. A method according to claim 2, wherein the reactive group is altered to cysteine, selenocysteine or methionine.

5. A method according to claim 1, wherein the altered molecular scaffold is asymmetric.

6. A method according to claim 5, wherein the molecular scaffold comprises two or more scaffold reactive groups which are capable of forming covalent bonds with the reactive groups on the polypeptides, and said two or more reactive groups are not identical.

7. A method according to claim 6, wherein one of said scaffold reactive groups is orthogonal.

8. A method any of the above claims wherein the scaffold reactive groups are selected from benzylic halides, α-halocarboxylic acids and acryloyl moieties.

9. A method according to claim 1, wherein at least one bond between the molecular scaffold and the polypeptide are chemically modified after assembly of the polypeptide and the molecular scaffold.

10. A method according to claim 9, wherein at least one thio-ether linkage between the polypeptide and the molecular scaffold is oxidised to form a sulphoxide or a sulphone.

11. A method according to any preceding claim, wherein the first group of polypeptide variants is a first repertoire of polypeptide variants.

12. A method according to any preceding claim, wherein the second group of polypeptide variants is a second repertoire of polypeptide variants.

13. A method for generating a group or repertoire of diverse polypeptide ligands from a first polypeptide ligand comprising at least two reactive groups separated by a loop sequence covalently linked to a molecular scaffold which forms covalent bonds with said reactive groups, comprising assembling a group or repertoire of ligands from the polypeptide and scaffold of said first ligand or group of ligands, and incorporating one of:

a) altering at least one reactive group; or

b) altering the nature of the molecular scaffold; or

c) altering the bond between at least one reactive group and the molecular scaffold; or

d) any combination of (a), (b) or (c); or

e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

14. A method for increasing the conformational diversity of a first repertoire of polypeptide ligands, comprising a plurality of polypeptides comprising at least two reactive groups separated by a loop sequence covalently linked to a molecular scaffold which forms covalent bonds with said reactive groups, comprising assembling a second repertoire of peptide ligands from the polypeptides and scaffold of said first repertoire, incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c).

(e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

15. A method according to claim 14, wherein the second repertoire is assembled from the polypeptides of the first repertoire, and at least two structurally diverse molecular scaffold species.

16. A method for providing a polypeptide ligand comprising a polypeptide covalently linked to a molecular scaffold at three or more amino acid residues, comprising the steps of:

a. providing a first repertoire of polypeptides;

b. conjugating said polypeptides to a molecular scaffold which binds to the polypeptides at two or more amino acid residues, to form a first repertoire of polypeptide conjugates;

c. screening said first repertoire for binding against a target, and selecting members of the first repertoire which bind to the target;

d. introducing further variation into the polypeptide ligands, in accordance with claim 1, yielding a second repertoire of polypeptide conjugates; and

e. screening said second repertoire for improved binding to the target.

17. A method for selecting a peptide ligand having increased protease resistance, comprising the steps of:

a. providing a first repertoire of polypeptides;

b. conjugating said polypeptides to a molecular scaffold which binds to the polypeptides at two or more amino acid residues, to form a first repertoire of polypeptide conjugates;

c. screening said first repertoire for binding against a target, and selecting members of the first repertoire which bind to the target;

d. introducing further variation into the polypeptide ligands, in accordance with the first aspect of the invention set forth above, yielding a second repertoire of polypeptide conjugates;

e. subjecting the second repertoire to selection for protease resistance; and

f. optionally, screening said second repertoire for binding to the target.

18. A method according to any preceding claim, wherein one or more of the groups or repertoires of polypeptides is encoded by one or more libraries of nucleic acid molecules.

19. A method according to any one of claims 1 to 18, wherein the screening of said repertoires is performed using a genetic display system.

20. A method according to claim 22, wherein the genetic display system is phage display.

21. A repertoire of peptide ligands, when produced by a method according to any preceding claim.

22. A method according to any one of claims 1 to 17, wherein any of the groups or repertoires is made from synthetic peptides.

23. A method of claim 22 wherein the second group or repertoire is made from synthetic peptides.

24. A method according to claim 22, wherein said synthetic peptides are arrayed on solid phase.

25. A group of peptide ligands, when produced by a method according to any one of claims 22 to 24

26. A polypeptide conjugate produced by a method according to any one of claims 1 to 20, comprising a polypeptide comprising at least two reactive groups each separated by a loop sequence, covalently linked to a molecular scaffold comprising at least two scaffold reactive groups which form covalent bonds with said reactive groups, wherein at least two of said scaffold reactive groups are different.

27. A polypeptide ligand according to claim 26, which has a molecular weight of less than 3000 Dalton.

28. A polypeptide ligand according to claim 26 or claim 27, wherein the length of the polypeptide loops subtended between any two adjacent points of attachment of the polypeptide to the molecular scaffold is between 0 and 9 amino acids, and the length of the polypeptide sequence between the first and last of said points of attachment is less than 27 amino acids.

CLAIMS

1. A method for altering the conformation of a first polypeptide ligand or group of polypeptide ligands, each polypeptide ligand comprising at least two reactive groups separated by a loop sequence covalently linked to a molecular scaffold which forms covalent bonds with the reactive groups, to produce a second polypeptide ligand or group of polypeptide ligands, comprising assembling the second ligand or group of ligands from the polypeptide(s) and structural scaffold of the first ligand or group of ligands, incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c).

2. A method according to Claim 1, in which the reactive group comprises a sulphur atom.

3. A method according to Claim 2, in which the reactive group is a cysteine or a methionine.

4. A method according to Claim 2, in which the reactive group is altered to cysteine, selenocysteine or methionine.

5. A method according to Claim 1, in which the altered molecular scaffold is asymmetric.

6. A method according to Claim 5, in which the molecular scaffold comprises two or more scaffold reactive groups which are capable of forming covalent bonds with the reactive groups on the polypeptides, and the two or more reactive groups are not identical.

7. A method according to Claim 6, in which one of the scaffold reactive groups is orthogonal.

8. A method any of the above claims in which the scaffold reactive groups are selected from benzylic halides, [alpha]-halocarboxylic acids and acryloyl moieties.

9. A method according to Claim 1, in which at least one bond between the molecular scaffold and the polypeptide are chemically modified after assembly of the polypeptide and the molecular scaffold.

10. A method according to Claim 9, in which at least one thio-ether linkage between the polypeptide and the molecular scaffold is oxidised to form a sulphoxide or a sulphone.

11. A method according to any preceding claim, in which the first group of polypeptide variants is a first repertoire of polypeptide variants.

12. A method according to any preceding claim, in which the second group of polypeptide variants is a second repertoire of polypeptide variants.

13. A method for generating a group or repertoire of diverse polypeptide ligands from a first polypeptide ligand comprising at least two reactive groups separated by a loop sequence covalently linked to a molecular scaffold which forms covalent bonds with the reactive groups, comprising assembling a group or repertoire of ligands from the polypeptide and scaffold of the first ligand or group of ligands, and incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c); or

(e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

14. A method for increasing the conformational diversity of a first repertoire of polypeptide ligands, comprising a plurality of polypeptides comprising at least two reactive groups separated by a loop sequence covalently linked to a molecular scaffold which forms covalent bonds with the reactive groups, comprising assembling a second repertoire of peptide ligands from the polypeptides and scaffold of the first repertoire, incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c).

(e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

15. A method according to Claim 14, in which the second repertoire is assembled from the polypeptides of the first repertoire, and at least two structurally diverse molecular scaffold species.

16. A method for providing a polypeptide ligand comprising a polypeptide covalently linked to a molecular scaffold at three or more amino acid residues, comprising the steps of:

(a) providing a first repertoire of polypeptides;

(b) conjugating the polypeptides to a molecular scaffold which binds to the polypeptides at two or more amino acid residues, to form a first repertoire of polypeptide conjugates;

(c) screening the first repertoire for binding against a target, and selecting members of the first repertoire which bind to the target;

(d) introducing further variation into the polypeptide ligands, in accordance with Claim 1, yielding a second repertoire of polypeptide conjugates; and

(e) screening the second repertoire for improved binding to the target.

17. A method for selecting a peptide ligand having increased protease resistance, comprising the steps of:

(a) providing a first repertoire of polypeptides;

(b) conjugating the polypeptides to a molecular scaffold which binds to the polypeptides at two or more amino acid residues, to form a first repertoire of polypeptide conjugates;

(c) screening the first repertoire for binding against a target, and selecting members of the first repertoire which bind to the target;

(d) introducing further variation into the polypeptide ligands, in accordance with Claim 1, yielding a second repertoire of polypeptide conjugates;

(e) subjecting the second repertoire to selection for protease resistance; and

(f) optionally, screening the second repertoire for binding to the target.

18. A method according to any preceding claim, in which one or more of the groups or repertoires of polypeptides is encoded by one or more libraries of nucleic acid molecules.

19. A method according to any one of claims 1 to 18, in which the screening of the repertoires is performed using a genetic display system.

20. A method according to Claim 22, in which the genetic display system is phage display.

21. A repertoire of peptide ligands, when produced by a method according to any preceding claim, in which the repertoire of peptide ligands is a second repertoire of peptide ligands, as recited in any preceding claim, and is assembled from the polypeptides and structural scaffold of a first group of peptide ligands, as recited in any preceding claim, by incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c); or

(e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

22. A method according to any one of claims 1 to 17, in which any of the groups or repertoires is made from synthetic peptides.

23. A method of Claim 22 in which the second group or repertoire is made from synthetic peptides.

24. A method according to Claim 22, in which the synthetic peptides are arrayed on solid phase.

25. A group of peptide ligands, when produced by a method according to any one of claims 22 to 24, in which the group of peptide ligands is a second group of peptide ligands, as recited in any preceding claim, and is assembled from the polypeptides and structural scaffold of a first group of peptide ligands, as recited in any preceding claim, by incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c); or

(e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

26. A polypeptide conjugate produced by a method according to any one of claims 1 to 20, comprising a polypeptide comprising at least two reactive groups each separated by a loop sequence, covalently linked to a molecular scaffold comprising at least two scaffold reactive groups which form covalent bonds with the reactive groups, in which at least two of the scaffold reactive groups are different, and in which the polypeptide conjugate is a second polypeptide ligand, as recited in any preceding claim, and is assembled from the polypeptide and structural scaffold of a first polypeptide ligand, as recited in any preceding claim, by incorporating one of:

(a) altering at least one reactive group; or

(b) altering the nature of the molecular scaffold; or

(c) altering the bond between at least one reactive group and the molecular scaffold; or

(d) any combination of (a), (b) or (c); or

(e) modifying the sequence of the polypeptide, in combination with any one of (a) to (d).

27. A polypeptide ligand according to Claim 26, which has a molecular weight of less than 3000 Dalton.

28. A polypeptide ligand according to Claim 26 or Claim 27, in which the length of the polypeptide loops subtended between any two adjacent points of attachment of the polypeptide to the molecular scaffold is between 0 and 9 amino acids, and the length of the polypeptide sequence between the first and last of the points of attachment is less than 27 amino acids.