CLAIMS

1. A genetically encoded peptide construct comprising a bicyclic structure resulting from attaching a linker having two-fold symmetry, a first end and a second end comprising two reactive groups to a polypeptide, wherein the first end is ligated to a terminus of the polypeptide by a covalent bond, and wherein both reactive groups of the linker second end are each reacted to side chain residues of the polypeptide, wherein the polypeptide consists of unprotected, natural, proteogenic amino acids and does not comprise a free N-terminus.

2. The construct of claim 1 wherein the polypeptide terminus is an N-terminus and the side chain residues are cysteine, lysine or tyrosine.

3. The construct of any one of claims 1-2 wherein the linker reactive groups are electrophilic groups reactive to thiol, amine or phenol.

4. The construct of any one of claims 1-3 wherein the linker first end comprises an aldehyde reactive group such as oxime, hydrazine, 2-amino benzamidoxime, phosphonium ylide, sulphur ylide, nitrogen ylides or any other carbon nucleophile and carbenoid reagents known to be reactive with aldehydes and stable in aqueous environment.

5. The construct of any one of claims 1-4 wherein the polypeptide comprises two cysteine residues and an N-terminal serine or threonine residue, wherein the N-terminal serine or threonine is first converted to an aldehyde by selective oxidation and wherein the second end reactive groups are covalently bonded to the cysteine residues.

6. The construct of any one of claims 1-5 which is attached to a carrier such as a phage particle bearing the polypeptide externally and including a nucleic acid encoding the polypeptide.

7. The construct of any one of claims 1-5 which is a RNA display compound, said RNA display bearing the polypeptide; and including an RNA sequence encoding the polypeptide, and linked to the polypeptide.

8. The construct of any one of claims 1-5 which is a DNA display compound, said DNA display bearing the polypeptide; and including a DNA encoding the polypeptide, linked to the polypeptide.

9. The construct of claim 1 wherein the polypeptide terminus is a C-terminus and the side chain residues are cysteine or lysine, and wherein the linker first end comprises a reactive group with reactivity to C-terminus in acidic pH and the linker second end comprises two electrophilic groups that react specifically with thiol or amine residues.

10. The construct of claim 9 wherein the reactive group with reactivity to C-terminus in acidic pH is a C-terminal-selective photoredoxdecarboxylative conjugate addition.

11. The construct of claim 3 or 9 wherein the linker electrophilic groups are reactive with thiol in water, such as haloketone, haloacetamide, halobenzyl, a Michael acceptor comprising a conjugated C-C double bond including maleimides, actylates, carbonylacrylic reagents, 3-arylpropiolonitriles, allenamides fluoroarene, chlorotetrazines, Julia–Kocieński-like reagents,

2-azidoacrylates, organometallic palladium reagents, organo gold(I) reagents, conversion of thiols to dehydroalanines (Dha) followed by conjugate addition to Dha.

12. The construct of claim 3 or 9 wherein the linker electrophilic groups are reactive with amine in water, such as N-hydroxysuccinimide esters, aryl esters, perfluoroaryl esters, perfluoroarenes, ketenes, ortho-phthalaldehydes, or strain-release amine modifying agents.

13. The construct of claim 3 or 9 wherein the linker electrophilic groups are reactive with phenol in water, such as allylpaladium, diazodicarboxylate, diazonium salts, aniline-formaldehyde hemiaminal, rhodium carbenoids, dirhodium metallopeptide catalysts, Manganese-Catalyzed C-H Alkynylation, Waser's reagent, 1-[(triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1 H)-one (TIPS-EBX), under gold(I) catalysis, selective ruthenium-(II)-catalyzed C–H activation, palladium (II) acetate catalyzed C-H activation with aryl iodides in water.

14. The construct of claim 1 wherein the polypeptide comprises a terminal serine residue and two cysteine residues separated by at least 2, 3, 4 or 5 amino acid residues.

15. The construct of any one of claims 1-14 wherein the linker comprises an alkyl chain having between 1-6 carbon atoms.

16. The construct of any one of claims 1-15 wherein the linker comprises TSL1, TSL3 or TSL6.

17. A method for making a phage display complex, said method comprising (i) providing a phage particle comprising a polypeptide having a terminus (ii) providing a linker having a first end and a second end comprising two reactive groups, and forming a linear intermediate complex by ligating the linker first end with the polypeptide terminus to form a covalent bond, and (iii) forming a bicyclic structure from the intermediate complex by reacting both reactive groups of the linker second end to side chain residues of the polypeptide, wherein steps (ii) and (iii) are independent and sequential.

18. The method of claim 17 wherein steps (ii) and (iii) are performed at different pHs.

19. The method of any one of claims 17-18 wherein the terminus of the polypeptide is an N-terminus and is oxidized to aldehyde before the ligation step, and wherein the ligation step comprises mixing the polypeptide comprising N-terminal aldehyde with the linker in aqueous buffer of acidic pH.

20. The method of any one of claims 17-19 wherein the intermediate complex is purified, such as by size-exclusion purification, such as by gel filtration or dialysis in aqueous buffer of acidic pH, such as below about pH 5.

21. The method of claim 20 wherein the purified intermediate complex is reduced with a reducing agent such as TCEP (“reduction step”), prior to the bicyclization step.

22. The method of claim 21 wherein the reduced intermediate complex is exposed to pH of 7 or above to induce bicyclization, wherein the second end reactive groups each comprise a thiol-reactive group and the polypeptide comprises two cysteine residues.

23. The method of any one of claims 17-22, wherein the linker comprises TSL1, TSL3 or TSL6.

24. A method for measuring the yield of a reaction after at least one step described in one of claims 17-22 comprising the steps of exposing the polypeptide to a capture reagent which is reactive to unreacted polypeptide but not reacted polypeptide and measuring the incorporation of capture agent with an affinity reagent.

25. A genetically encoded library of polypeptides comprising a plurality of different peptide sequences forming a bicyclic structure as described in any one of claims 1-16, where each peptide sequence is separately encoded by DNA sequences, and each separately modified with at least two different linkers L1 and L2, forming at least four distinct constructs.

26. The mixed library of claim 25 where linker L1 is reacted with two peptides that are associated with pre-defined nucleic acid code B1 and linker L2 is reacted with two peptides that are associated with pre-defined nucleic acid code B2.

27. The mixed library of claim 26 wherein the nucleic acid codes are silent genetic barcodes.

28. A method for identifying a bicyclic polypeptide which binds to a ligand, the method comprising (i) contacting a mixed library of complexes as claimed in claim 25 with the ligand, and (ii) selecting those complexes which bind to the ligand (iii) identifying the structure of a binding complex by sequencing a nucleic acid that encodes the structure of peptide and/or linker.