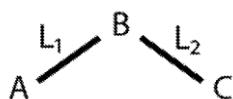


CLAIMS

What is claimed:

1. A method for identifying the terminal amino acid of at least one peptide comprising:
 - (a) contacting the peptide with a ClickP compound, wherein the ClickP compound binds to a terminal amino acid or a chemically modified terminal amino acid of the peptide to form a ClickP-peptide complex, wherein the ClickP compound is of Formula I:



Formula I

wherein:

A is a terminal amino acid reactive group capable of conjugating to the terminal amino acid or the chemically modified terminal amino acid of the peptide, wherein the terminal amino acid reactive group is selected from the group consisting of isothiocyanate, phenyl isothiocyanate (PITC), isocyanate, acyl azide, N-hydroxysuccinimide ester (NHS ester), sulfonyl chloride, an aldehyde, a glyoxal, an epoxide, an oxirane, a carbonate, an aryl halide, an imidoester, a carbodiimide, an anhydride, a fluorophenyl ester, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, acetyl chloride, cyanogen bromide, sodium thiocyanate, ammonium thiocyanate, and a carboxypeptidase;

B is a releasable group providing a mechanism to release a ClickP-amino acid complex from a substrate, wherein the releasable group is the same as C or wherein the releasable group is selected from the group consisting of a disulfide, a peptide, an oligonucleotide, and a carbohydrate;

C is a tetherable group capable of immobilizing the ClickP-peptide complex to the substrate, wherein the tetherable group is selected from the group consisting of isothiocyanate, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, azide, an alkyne, Dibenzocyclooctyne (DBCO), maleimide, succinimide, a thiol-thiol disulfide bond, Tetrazine, TCO, Vinyl, methylcyclopropene, a primary amine, a carboxylic acid, an alkyne, an acryloyl, an allyl, and an aldehyde; and

L₁ and L₂ are independent spacers;

(b) tethering the tetherable group, of the ClickP-peptide complex, to the substrate;
(c) cleaving the terminal amino acid or the chemically modified terminal amino acid from the peptide thereby providing a ClickP-amino acid complex; and
(d) detecting the ClickP-amino acid complex.

2. The method according to claim 1, wherein detecting the ClickP-amino acid complex comprises contacting the ClickP-amino acid complex with one or more ClickP-amino acid complex antibodies, wherein the one or more ClickP-amino acid complex antibodies bind to a ClickP-amino acid complex or a subgroup of ClickP-amino acid complexes.

3. The method according to claim 1, wherein detecting the ClickP-amino acid complex comprises direct detection through wavelengths of light.

4. The method according to any one of the preceding claims, further comprising identifying the amino acid of the ClickP-amino acid complex.

5. The method according to any one of claims 1-4, wherein the terminal amino acid reactive group, of the ClickP compound, is capable of conjugating to an N-terminal amino acid or chemically modified N-terminal amino acid of the peptide to form the ClickP-peptide complex.

6. The method according to any one of claims 1-4, wherein the terminal amino acid reactive group, of the ClickP compound, is capable of conjugating to a C-terminal amino acid or chemically modified C-terminal amino acid of the peptide to form the ClickP-peptide complex.

7. The method according to any one of the preceding claims, further comprising (e) releasing the ClickP-amino acid complex from the substrate.

8. The method according to claim 7, further comprising repeating (a) through (e).

9. The method according to any one of the preceding claims, wherein, prior to (b) or (c), excess and/or unbound ClickP compound is washed away.

10. The method according to any one of the preceding claims, wherein the peptide is affixed to the substrate.
11. The method according to claim 10, wherein the peptide is affixed to the substrate through the C'-terminal carboxyl group or a side chain functional group of the peptide.
12. The method according to claim 10, wherein the peptide is affixed to the substrate through the N'-terminal amino group or a side chain functional group of the peptide.
13. The method according to claim 10, wherein the peptide is covalently affixed to the substrate.
14. The method according to any one of the preceding claims, wherein the substrate is optically transparent.
15. The method according to any one of the preceding claims, wherein the substrate comprises a functionalized surface.
16. The method according to claim 15, wherein the functionalized surface is selected from the group consisting of an azide functionalized surface, a thiol functionalized surface, alkyne, DBCO, maleimide, succinimide, tetrazine, TCO, vinyl, methylcyclopropene, a primary amine surface, a carboxylic surface, a DBCO surface, an alkyne surface, and an aldehyde surface.
17. The method according to claim 2, wherein the one or more ClickP-amino acid complex antibodies comprises:
 - (a) one or more antibodies that bind to a subgroup of natural proteinogenic amino acids complexed with the ClickP compound;
 - (b) one or more antibodies that bind to at least a subgroup of post-translationally modified amino acids complexed with the ClickP compound;
 - (c) one or more antibodies that bind to a chemically modified amino acid of (a) or (b); or

(d) combinations of antibodies of (a), (b) or (c).

18. The method according to claim 17, wherein at least one of the one or more antibodies comprises a detectable label.

19. The method according to claim 18, wherein detecting the ClickP-amino acid complex comprises detecting the detectable label.

20. The method according to claim 1, wherein the ClickP compound further comprises a detectable label.

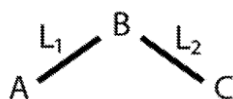
21. The method according to claim 1, wherein the at least one peptide comprises two or more peptides, and wherein the two or more peptides are independently affixed to spatially resolved attachment points on the substrate prior to (a).

22. The method according to claim 1, further comprising:
(e) identifying the amino acid of the ClickP-amino acid complex;
(f) releasing the ClickP-amino acid complex from the substrate; and
(g) repeating (a) through (f).

23. A method for sequencing at least a portion of two or more peptides in a sample comprising:

(a) independently affixing the two or more peptides to spatially resolved attachment points on a substrate;

(b) contacting the two or more peptides with a ClickP compound, wherein the ClickP compound binds to terminal amino acids or chemically modified terminal amino acids of the two or more peptides to form ClickP-peptide complexes, wherein the ClickP compound is of Formula I:



Formula I

wherein:

A is a terminal amino acid reactive group capable of conjugating to the terminal amino acid or chemically modified terminal amino acid of a peptide, wherein the terminal amino acid reactive group is selected from the group consisting of isothiocyanate, phenyl isothiocyanate (PITC), isocyanate, acyl azide, N-hydroxysuccinimide ester (NHS ester), sulfonyl chloride, an aldehyde, a glyoxal, an epoxide, an oxirane, a carbonate, an aryl halide, an imidoester, a carbodiimide, an anhydride, a fluorophenyl ester, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, acetyl chloride, cyanogen bromide, sodium thiocyanate, ammonium thiocyanate, and a carboxypeptidase;

B is a releasable group providing a mechanism to release a ClickP-amino acid complex from the substrate, wherein the releasable group is the same as C or wherein the releasable group is selected from the group consisting of a disulfide, a peptide, an oligonucleotide, and a carbohydrate;

C is a tetherable group capable of immobilizing the ClickP-peptide complex to the substrate, wherein the tetherable group is selected from the group consisting of isothiocyanate, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, azide, an alkyne, Dibenzocyclooctyne (DBCO), maleimide, succinimide, a thiol-thiol disulfide bond, Tetrazine, TCO, Vinyl, methylcyclopropene, a primary amine, a carboxylic acid, an alkyne, an acryloyl, an allyl, and an aldehyde; and

L₁ and L₂ are independent spacers;

(c) tethering the tetherable group, of the ClickP-peptide complexes, to the substrate;

(d) cleaving the terminal amino acids or the chemically modified terminal amino acids from the two or more peptides to form ClickP-amino acid complexes;

(e) detecting the ClickP-amino acid complexes;

(f) identifying the amino acids of the ClickP-amino acid complexes;

(g) releasing the ClickP-amino acid complexes from the substrate; and

(h) repeating (b) through (g), thereby sequencing the at least the portion of two or more peptides in the sample.

24. The method according to claim 23, wherein detecting the ClickP-amino acid complexes comprises independently contacting the ClickP-amino acid complexes with one or more ClickP-

amino acid complex antibodies, wherein the one or more ClickP-amino acid complex antibodies bind to a ClickP-amino acid complex or a subgroup of ClickP-amino acid complexes.

25. The method according to claim 23, wherein detecting the ClickP-amino acid complexes comprises direct detection through wavelengths of light.

26. The method according to any one of claims 23-25, wherein the terminal amino acid reactive group, of the ClickP compound, is capable of conjugating to an N-terminal amino acid or chemically modified N-terminal amino acid of a peptide to form a ClickP-peptide complex.

27. The method according to any one of claims 23-25, wherein the terminal amino acid reactive group, of the ClickP compound, is capable of conjugating to a C-terminal amino acid or chemically modified C-terminal amino acid of a peptide to form a ClickP-peptide complex.

28. The method according to any one of claims 23-27, wherein, prior to (c) or (d), excess ClickP and/or unbound ClickP compound is washed away.

29. The method according to claim 23, wherein a plurality of the two or more peptides are affixed to the substrate through the C'-terminal carboxyl group or a side chain functional group of the plurality of the two or more peptides.

30. The method according to claim 23, wherein a plurality of the two or more peptides are affixed to the substrate through the N'-terminal amino group or a side chain functional group of the plurality of the two or more peptides.

31. The method according to any one of claims 23-30, wherein the substrate comprises a functionalized surface selected from the group consisting of an azide functionalized surface, a thiol functionalized surface, alkyne, DBCO, maleimide, succinimide, tetrazine, TCO, vinyl, methylcyclopropene, a primary amine surface, a carboxylic surface, a DBCO surface, an alkyne surface, and an aldehyde surface.

32. The method according to claim 24, wherein the one or more ClickP-amino acid complex antibodies comprise:

- (a) one or more antibodies that bind to a subgroup of natural proteinogenic amino acids complexed with the ClickP compound;
- (b) one or more antibodies that bind to at least a subgroup of post-translationally modified amino acids complexed with the ClickP compound;
- (c) one or more antibodies that bind to a chemically modified amino acid of (a) or (b); or
- (d) combinations of the one or more antibodies of (a), (b) or (c).

33. The method according to claim 24, wherein the one or more ClickP-amino acid complex antibodies comprise:

- (a) one or more antibodies that bind to one of the natural proteinogenic amino acids complexed with the ClickP compound;
- (b) one or more antibodies that bind to a post-translationally modified amino acid complexed with the ClickP compound;
- (c) one or more antibodies that bind to a chemically modified amino acid of (a) or (b); or
- (d) a combination of the one or more antibodies of (a), (b) or (c).

34. The method according to any one of claims 23-33, wherein the sample comprises a biological fluid, cell extract, tissue extract, or a mixture of synthetically synthesized peptides.

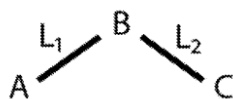
35. The method according to any one of claims 23-34, further comprising comparing the sequence of at least one peptide determined in (h) to a reference protein sequence database.

36. The method according to any one of claims 23-34, further comprising comparing the sequences of each peptide determined in (h), grouping similar peptide sequences and counting the number of instances of each similar peptide sequence.

37. A ClickP-amino acid complex comprising:

- (a) a ClickP compound bound to a natural proteinogenic amino acid;
- (b) a ClickP compound bound to a post-translationally modified amino acid; or

(c) a ClickP compound bound to a chemically modified amino acid of (a) or (b), wherein the ClickP compound is of Formula I:



Formula I

wherein:

A is a terminal amino acid reactive group capable of conjugating to a terminal amino acid or a chemically modified terminal amino acid of a peptide, wherein the terminal amino acid reactive group is selected from the group consisting of isothiocyanate, phenyl isothiocyanate (PITC), isocyanate, acyl azide, N-hydroxysuccinimide ester (NHS ester), sulfonyl chloride, an aldehyde, a glyoxal, an epoxide, an oxirane, a carbonate, an aryl halide, an imidoester, a carbodiimide, an anhydride, a fluorophenyl ester, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, acetyl chloride, cyanogen bromide, sodium thiocyanate, ammonium thiocyanate, and a carboxypeptidase;

B is a releasable group providing a mechanism to release the ClickP-amino acid complex from a substrate, wherein the releasable group is the same as C or wherein the releasable group is selected from the group consisting of a disulfide, a peptide, an oligonucleotide, and a carbohydrate;

C is a tetherable group capable of immobilizing a ClickP-peptide complex to the substrate, wherein the tetherable group is selected from the group consisting of isothiocyanate, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, azide, an alkyne, Dibenzocyclooctyne (DBCO), maleimide, succinimide, a thiol-thiol disulfide bond, Tetrazine, TCO, Vinyl, methylcyclopropene, a primary amine, a carboxylic acid, an alkyne, an acryloyl, an allyl, and an aldehyde; and

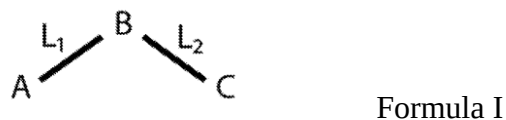
L₁ and L₂ are independent spacers.

38. A ClickP-amino acid complex antibody comprising:

(a) an antibody that binds to a subgroup of natural proteinogenic amino acids complexed with a ClickP compound;

(b) an antibody that binds to a subgroup of post-translationally modified amino acids complexed with the ClickP compound; or

(c) an antibody that binds to a chemically modified amino acid of (a) or (b), wherein the ClickP compound is of Formula I:



wherein:

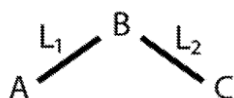
A is a terminal amino acid reactive group capable of conjugating to a terminal amino acid or a chemically modified terminal amino acid of a peptide, wherein the terminal amino acid reactive group is selected from the group consisting of isothiocyanate, phenyl isothiocyanate (PITC), isocyanate, acyl azide, N-hydroxysuccinimide ester (NHS ester), sulfonyl chloride, an aldehyde, a glyoxal, an epoxide, an oxirane, a carbonate, an aryl halide, an imidoester, a carbodiimide, an anhydride, a fluorophenyl ester, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, acetyl chloride, cyanogen bromide, sodium thiocyanate, ammonium thiocyanate, and a carboxypeptidase;

B is a releasable group providing a mechanism to release the ClickP-amino acid complex from a substrate, wherein the releasable group is the same as C or wherein the releasable group is selected from the group consisting of a disulfide, a peptide, an oligonucleotide, and a carbohydrate;

C is a tetherable group capable of immobilizing a ClickP-peptide complex to the substrate, wherein the tetherable group is selected from the group consisting of isothiocyanate, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, azide, an alkyne, Dibenzocyclooctyne (DBCO), maleimide, succinimide, a thiol-thiol disulfide bond, Tetrazine, TCO, Vinyl, methylcyclopropene, a primary amine, a carboxylic acid, an alkyne, an acryloyl, an allyl, and an aldehyde; and

L₁ and L₂ are independent spacers.

39. A ClickP-amino acid complex antibody comprising:
- (a) an antibody that binds to a natural proteinogenic amino acid complexed with a ClickP compound;
 - (b) an antibody that binds to a post-translationally modified amino acid complexed with the ClickP compound; or
 - (c) an antibody that binds to a chemically modified amino acid of (a) or (b),
- wherein the ClickP compound is of Formula I:



Formula I

wherein:

A is a terminal amino acid reactive group capable of conjugating to a terminal amino acid or a chemically modified terminal amino acid of a peptide, wherein the terminal amino acid reactive group is selected from the group consisting of isothiocyanate, phenyl isothiocyanate (PITC), isocyanate, acyl azide, N-hydroxysuccinimide ester (NHS ester), sulfonyl chloride, an aldehyde, a glyoxal, an epoxide, an oxirane, a carbonate, an aryl halide, an imidoester, a carbodiimide, an anhydride, a fluorophenyl ester, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, acetyl chloride, cyanogen bromide, sodium thiocyanate, ammonium thiocyanate, and a carboxypeptidase;

B is a releasable group providing a mechanism to release the ClickP-amino acid complex from a substrate, wherein the releasable group is the same as C or wherein the releasable group is selected from the group consisting of a disulfide, a peptide, an oligonucleotide, and a carbohydrate;

C is a tetherable group capable of immobilizing a ClickP-peptide complex to the substrate, wherein the tetherable group is selected from the group consisting of isothiocyanate, tetrabutylammonium isothiocyanate, diphenylphosphoryl isothiocyanate, azide, an alkyne, Dibenzocyclooctyne (DBCO), maleimide, succinimide, a thiol-thiol disulfide bond, Tetrazine, TCO, Vinyl, methylcyclopropene, a primary amine, a carboxylic acid, an alkyne, an acryloyl, an allyl, and an aldehyde; and

L₁ and L₂ are independent spacers.