

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 terminal amino acid derivative of the peptide to form a ClickP-peptide complex,
 - (b) tethering the ClickP-peptide complex to a substrate;
 - (c) cleaving the ClickP-peptide complex 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 binders, wherein the one or more ClickP-amino acid complex binders 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 any 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 ClickP compound binds to an N-terminal amino acid or N-terminal amino acid derivative of the peptide to form a ClickP-amino acid complex.

6. The method according to any one of claims 1-4, wherein the ClickP compound binds to a C-terminal amino acid or C-terminal amino acid derivative of the peptide to form a ClickP-amino acid complex.
7. The method according any 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 any of the preceding claims, wherein prior to step (b) or (c) excess and/or unbound ClickP compound is washed away.
10. The method according any 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 polypeptide.
13. The method according to claim 10, wherein the peptide is covalently affixed to the substrate.
14. The method according any of the preceding claims, wherein the substrate is optically transparent.
15. The method according any 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 binders comprises:
- (a) one or more binders that bind to a subgroup of natural proteinogenic amino acids complexed with ClickP;
 - (b) one or more binders that bind to at least a subgroup of post-translationally modified amino acids complexed with ClickP;
 - (c) one or more binders that bind to a derivative of (a) or (b); or
 - (d) combinations of binders of (a), (b) or (c).
18. The method according to claim 17, wherein at least one of the one or more binders comprises a detectable label.
19. The method according to claim 18, wherein the step of detecting the binder bound to 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 an attachment point on the substrate prior to (a).
22. The method of claim 1, further comprising identifying at least a portion of a peptide, wherein the method further comprises:
- (d) detecting the ClickP-amino acid complex;
 - (e) identifying the amino acid of the ClickP-amino acid complex

- (f) releasing the ClickP-amino acid complex from the substrate; and
- (g) repeating steps (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 an attachment point on a substrate;
- (b) contacting the two or more peptides with at least one of a ClickP compound, wherein the ClickP compound binds to a terminal amino acid or terminal amino acid derivative to form a ClickP-peptide complex,
- (c) tethering the ClickP-peptide complex to the substrate;
- (d) cleaving the ClickP-peptide complex from the two or more peptides to form a ClickP-amino acid complex;
- (e) detecting the ClickP-amino acid complex;
- (f) identifying the amino acid of the ClickP-amino acid complex;
- (g) releasing the ClickP-amino acid complex from the substrate; and
- (h) repeating steps (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 complex comprises contacting the ClickP-amino acid complex with one or more ClickP-amino acid complex binders, wherein the one or more ClickP-amino acid complex binders bind to the ClickP-amino acid complex or a subgroup of the ClickP-amino acid complex.

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

26. The method according to any one of claims 23-25, wherein the ClickP compound binds to an N-terminal amino acid or N-terminal amino acid derivative of the peptide to form a ClickP-amino acid complex.

27. The method according to any one of claims 23-25, wherein the ClickP compound binds to a C-terminal amino acid or C-terminal amino acid derivative of the peptide to form a ClickP-amino acid complex.
28. The method according to any one of claims 23-27, wherein prior to step (c) or (d) excess ClickP compounds, unbound ClickP compounds, or a combination thereof are 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 peptide.
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 carboxyl group or a side chain functional group of the polypeptide.
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 binders comprise:
- (a) one or more binders that bind to a subgroup of natural proteinogenic amino acids complexed with ClickP;
 - (b) one or more binders that bind to a subgroup of post-translationally modified amino acids complexed with ClickP;
 - (c) one or more binders that bind to a derivative of (a) or (b); or
 - (d) combinations of the one or more binders of (a), (b) or (c).

33. The method according to claim 24, wherein the ClickP-amino acid complex binders comprise:
- (a) one or more binders that bind to a natural proteinogenic amino acids complexed with ClickP;
 - (b) one or more binders that bind to a post-translationally modified amino acid complexed with ClickP;
 - (c) one or more binders that bind to a derivative of (a) or (b); or
 - (d) a combination of the one or more binders 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 step (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 step (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 derivative of (a) or (b).
38. A ClickP-amino acid complex binder comprising:
- (a) a binder that binds to a subgroup of natural proteinogenic amino acids complexed with ClickP;
 - (b) a binder that binds to a subgroup of post-translationally modified amino acids complexed with ClickP; or
 - (c) a binder that binds to a derivative of (a) or (b).

39. A ClickP-amino acid complex binder comprising:
- (a) a binder that binds to a natural proteinogenic amino acid complexed with ClickP;
 - (b) a binder that binds to a post-translationally modified amino acid complexed with ClickP; or
 - (c) a binder that binds to a derivative of (a) or (b).