

CLAIMS

We claim the following:

1. A method for analyzing a macromolecule, comprising the steps of:
 - (a) providing a macromolecule and an associated recording tag joined to a solid support;
 - (b) contacting the macromolecule with a first binding agent capable of binding to the macromolecule, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;
 - (c) transferring the information of the first coding tag to the recording tag to generate a first order extended recording tag;
 - (d) contacting the macromolecule with a second binding agent capable of binding to the macromolecule, wherein the second binding agent comprises a second coding tag with identifying information regarding the second binding agent;
 - (e) transferring the information of the second coding tag to the first order extended recording tag to generate a second order extended recording tag; and
 - (f) analyzing the second order extended recording tag.
2. The method of claim 1, wherein contacting steps (b) and (d) are performed in sequential order.
3. The method of claim 1, where wherein contacting steps (b) and (d) are performed at the same time.
4. The method of claim 1, further comprising, between steps (e) and (f), the following steps:
 - (x) repeating steps (d) and (e) one or more times by replacing the second binding agent with a third (or higher order) binding agent capable of binding to the macromolecule, wherein the third (or higher order) binding agent comprises a third

(or higher order) coding tag with identifying information regarding the third (or higher order) bind agent; and

(y) transferring the information of the third (or higher order) coding tag to the second (or higher order) extended recording tag to generate a third (or higher order) extended recording tag;

and wherein the third (or higher order) extended recording tag is analyzed in step (f).

5. A method for analyzing a macromolecule, comprising the steps of:

(a) providing a macromolecule, an associated first recording tag and an associated second recording tag joined to a solid support;

(b) contacting the macromolecule with a first binding agent capable of binding to the macromolecule, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;

(c) transferring the information of the first coding tag to the first recording tag to generate a first extended recording tag;

(d) contacting the macromolecule with a second binding agent capable of binding to the macromolecule, wherein the second binding agent comprises a second coding tag with identifying information regarding the second binding agent;

(e) transferring the information of the second coding tag to the second recording tag to generate a second extended recording tag; and

(f) analyzing the first and second extended recording tags.

6. The method of claim 5, wherein contacting steps (b) and (d) are performed in sequential order.

7. The method of claim 5, wherein contacting steps (b) and (d) are performed at the same time.

8. The method of claim 5, wherein step (a) further comprises providing an associated third (or higher order) recording tag joined to the solid support.

9. The method of claim 8, further comprising, between steps (e) and (f), the following steps:

(x) repeating steps (d) and (e) one or more times by replacing the second binding agent with a third (or higher order) binding agent capable of binding to the macromolecule, wherein the third (or higher order) binding agent comprises a third (or higher order) coding tag with identifying information regarding the third (or higher order) binding agent; and

(y) transferring the information of the third (or higher order) coding tag to the third (or higher order) recording tag to generate a third (or higher order) extended recording tag;

and wherein the first, second and third (or higher order) extended recording tags are analyzed in step (f).

10. The method of any one of claims 5-9, wherein the first coding tag, second coding tag, and any higher order coding tags comprise a binding cycle specific spacer sequence.

11. A method for analyzing a peptide, comprising the steps of:

(a) providing a peptide and an associated recording tag joined to a solid support;

(b) modifying the N-terminal amino acid (NTAA) of the peptide with a chemical agent;

(c) contacting the peptide with a first binding agent capable of binding to the modified NTAA, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;

(d) transferring the information of the first coding tag to the recording tag to generate an extended recording tag; and

(e) analyzing the extended recording tag.

12. The method of claim 11, wherein step (c) further comprises contacting the peptide with a second (or higher order) binding agent comprising a second (or higher order) coding tag with identifying information regarding the second (or higher order) binding agent, wherein the second (or higher order) binding agent is capable of binding to a modified NTAA other than the modified NTAA of step (b).

13. The method of claim 12, wherein contacting the peptide with the second (or higher order) binding agent occurs in sequential order following the peptide being contacted with the first binding agent.

14. The method of claim 12, wherein contacting the peptide with the second (or higher order) binding agent occurs simultaneously with the peptide being contacted with the first binding agent.

15. The method of any one of claims 11-14, wherein the chemical agent is an isothiocyanate derivative, 2,4-dinitrobenzenesulfonic (DNBS), 4-sulfonyl-2-nitrofluorobenzene (SNFB) 1-fluoro-2,4-dinitrobenzene, dansyl chloride, 7-methoxycoumarin acetic acid, a thioacylation reagent, a thioacetylation reagent, or a thiobenzoylation reagent.

16. A method for analyzing a peptide, comprising the steps of:

(a) providing a peptide and an associated recording tag joined to a solid support;

(b) modifying the N-terminal amino acid (NTAA) of the peptide with a chemical agent to yield a modified NTAA;

(c) contacting the peptide with a first binding agent capable of binding to the modified NTAA, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;

- (d) transferring the information of the first coding tag to the recording tag to generate a first extended recording tag;
- (e) removing the modified NTAA to expose a new NTAA;
- (f) modifying the new NTAA of the peptide with a chemical agent to yield a newly modified NTAA;
- (g) contacting the peptide with a second binding agent capable of binding to the newly modified NTAA, wherein the second binding agent comprises a second coding tag with identifying information regarding the second binding agent;
- (h) transferring the information of the second coding tag to the first extended recording tag to generate a second extended recording tag; and
- (i) analyzing the second extended recording tag.

17. A method for analyzing a peptide, comprising the steps of:

- (a) providing a peptide and an associated recording tag joined to a solid support;
- (b) contacting the peptide with a first binding agent capable of binding to the N-terminal amino acid (NTAA) of the peptide, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;
- (c) transferring the information of the first coding tag to the recording tag to generate an extended recording tag; and
- (d) analyzing the extended recording tag.

18. The method of claim 17, wherein step (b) further comprises contacting the peptide with a second (or higher order) binding agent comprising a second (or higher order) coding tag with identifying information regarding the second (or higher order) binding agent, wherein the second (or higher order) binding agent is capable of binding to a NTAA other than the NTAA of the peptide.

19. The method of claim 18, wherein contacting the peptide with the second (or higher order) binding agent occurs in sequential order following the peptide being contacted with the first binding agent.

20. The method of claim 18, wherein contacting the peptide with the second (or higher order) binding agent occurs simultaneously with the peptide being contacted with the first binding agent.

21. A method for analyzing a peptide, comprising the steps of:

- (a) providing a peptide and an associated recording tag joined to a solid support;
- (b) contacting the peptide with a first binding agent capable of binding to the N-terminal amino acid (NTAA) of the peptide, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;
- (c) transferring the information of the first coding tag to the recording tag to generate a first extended recording tag;
- (d) removing the NTAA to expose a new NTAA of the peptide;
- (e) contacting the peptide with a second binding agent capable of binding to the new NTAA, wherein the second binding agent comprises a second coding tag with identifying information regarding the second binding agent;
- (h) transferring the information of the second coding tag to the first extended recording tag to generate a second extended recording tag; and
- (i) analyzing the second extended recording tag.

22. The method of any one of claims 1-10, wherein the macromolecule is a protein, polypeptide or peptide.

23. The method of any one of claims 1-10, wherein the macromolecule is a peptide.

24. The method of any one of claims 11-23, wherein the peptide is obtained by fragmenting a protein from a biological sample.

25. The method of any one of claims 1-10, wherein the macromolecule is a lipid, a carbohydrate, or a macrocycle.

26. The method of any one of claims 1-25, wherein the recording tag is a DNA molecule, DNA with pseudo-complementary bases, an RNA molecule, a BNA molecule, an XNA molecule, a LNA molecule, a PNA molecule, a γ PNA molecule, or a combination thereof.

27. The method of any one of claims 1-26, wherein the recording tag comprises a universal priming site.

28. The method of claim 27, wherein the universal priming site comprises a priming site for amplification, sequencing, or both.

29. The method of claims 1-28, where the recording tag comprises a unique molecule identifier (UMI).

30. The method of any one of claims 1-29, wherein the recording tag comprises a barcode.

31. The method of any one of claims 1-30, wherein the recording tag comprises a spacer at its 3'-terminus.

32. The method of claim any one of claims 1-31, wherein the macromolecule and the associated recording tag are covalently joined to the solid support.

33. The method of any one of claims 1-32, wherein the solid support is a bead, a porous bead, a porous matrix, an array, a glass surface, a silicon surface, a plastic surface, a filter, a membrane, nylon, a silicon wafer chip, a flow through chip, a biochip including signal transducing electronics, a microtitre well, an ELISA plate, a spinning interferometry disc, a nitrocellulose membrane, a nitrocellulose-based polymer surface, a nanoparticle, or a microsphere.

34. The method of claim 33, wherein the solid support is a polystyrene bead, a polymer bead, an agarose bead, an acrylamide bead, a solid core bead, a porous bead, a paramagnetic bead, glass bead, or a controlled pore bead.

35. The method of any one of claims 1-34, wherein a plurality of macromolecules and associated recording tags are joined to a solid support.

36. The method of claim 35, wherein the plurality of macromolecules are spaced apart on the solid support at an average distance > 50 nm.

37. The method of any one of claims 1-36, wherein the binding agent is a polypeptide or protein.

38. The method of claim 37, wherein the binding agent is a modified aminopeptidase, a modified amino acyl tRNA synthetase, a modified anticalin, or a modified ClpS.

39. The method of any one of claims 1-38, wherein the binding agent is capable of selectively binding to the macromolecule.

40. The method of any one of claims 1-39, wherein the coding tag is DNA molecule, an RNA molecule, a BNA molecule, an XNA molecule, a LNA molecule, a PNA molecule, a γ PNA molecule, or a combination thereof.

41. The method of any one of claims 1-40, wherein the coding tag comprises an encoder sequence.

42. The method of any one of claims 1-41, wherein the coding tag further comprises a spacer, a binding cycle specific sequence, a unique molecular identifier, a universal priming site, or any combination thereof.

43. The method of any one of claims 1-42, wherein the binding agent and the coding tag are joined by a linker.

44. The method of claims 1-42, wherein the binding agent and the coding tag are joined by a Spy Tag/SpyCatcher or SnoopTag/SnoopCatcher peptide-protein pair.

45. The method of any one of claims 1-44, wherein transferring the information of the coding tag to the recording tag is mediated by a DNA ligase.

46. The method of any one of claims 1-44, wherein transferring the information of the coding tag to the recording tag is mediated by a DNA polymerase.

47. The method of any one of claims 1-44, wherein transferring the information of the coding tag to the recording tag is mediated by chemical ligation.

48. The method of any one of claims 1-47, wherein analyzing the extended recording tag comprises a nucleic acid sequencing method.

49. The method of claim 48, wherein the nucleic acid sequencing method is sequencing by synthesis, sequencing by ligation, sequencing by hybridization, polony sequencing, ion semiconductor sequencing, or pyrosequencing.

50. The method of claim 48, wherein the nucleic acid sequencing method is single molecule real-time sequencing, nanopore-based sequencing, or direct imaging of DNA using advanced microscopy.

51. The method of any one of claims 1-50, wherein the extended recording tag is amplified prior to analysis.

52. The method of claim 1-51, wherein the order of coding tag information contained on the extended recording tag provides information regarding the order of binding by the binding agents to the macromolecule.

53. The method of claim 1-52, wherein frequency of the coding tag information contained on the extended recording tag provides information regarding the frequency of binding by the binding agents to the macromolecule.

54. The method of claim 1-53, wherein a plurality of extended recording tags representing a plurality of macromolecules are analyzed in parallel.

55. The method of claim 54, wherein the plurality of extended recording tags representing a plurality of macromolecules is analyzed in a multiplexed assay.

56. The method of any one of claims 1-55, wherein the plurality of extended recording tags undergoes a target enrichment assay prior to analysis.

57. The method of any one of claims 1-56, wherein the plurality of extended recording tags undergoes a subtraction assay prior to analysis.

58. The method of any one of claims 1-57, wherein the plurality of extended recording tags undergoes a normalization assay to reduce highly abundant species prior to analysis.

59. The method of any one of claims 1-58, wherein the NTAA is removed by a modified aminopeptidase, a modified amino acid tRNA synthetase, mild Edman degradation, Edmanase enzyme, or anhydrous TFA.

60. The method of any one of claims 1-59, wherein at least one binding agent binds to a terminal amino acid residue.

61. The method of any one of claims 1-60, wherein at least one binding agent binds to a post-translationally modified amino acid.

62. A method for analyzing one or more peptides from a sample comprising a plurality of protein complexes, proteins, or polypeptides, the method comprising:

- (a) partitioning the plurality of protein complexes, proteins, or polypeptides within the sample into a plurality of compartments, wherein each compartment comprises a plurality of compartment tags optionally joined to a solid support, wherein the plurality of compartment tags are the same within an individual compartment and are different from the compartment tags of other compartments;
- (b) fragmenting the plurality of protein complexes, proteins, and/or polypeptides into a plurality of peptides;

(c) contacting the plurality of peptides to the plurality of compartment tags under conditions sufficient to permit annealing or joining of the plurality of peptides with the plurality of compartment tags within the plurality of compartments, thereby generating a plurality of compartment tagged peptides;

(d) collecting the compartment tagged peptides from the plurality of compartments; and

(e) analyzing one or more compartment tagged peptide according to a method of any one of claims 1-21 and 26-61.

63. The method of claim 62, wherein the compartment is a microfluidic droplet.

64. The method of claim 62, wherein the compartment is a microwell.

65. The method of claim 62, wherein the compartment is a separated region on a surface.

66. The method of any one of claims 62-65, wherein each compartment comprises on average a single cell.

67. A method for analyzing one or more peptides from a sample comprising a plurality of protein complexes, proteins, or polypeptides, the method comprising:

(a) labeling of the plurality of protein complexes, proteins, or polypeptides with a plurality of universal DNA tags;

(b) partitioning the plurality of labeled protein complexes, proteins, or polypeptides within the sample into a plurality of compartments, wherein each compartment comprises a plurality of compartment tags, wherein the plurality of

compartment tags are the same within an individual compartment and are different from the compartment tags of other compartments;

(c) contacting the plurality of protein complexes, proteins, or polypeptides to the plurality of compartment tags under conditions sufficient to permit annealing or joining of the plurality of protein complexes, proteins, or polypeptides with the plurality of compartment tags within the plurality of compartments, thereby generating a plurality of compartment tagged protein complexes, proteins or polypeptides;

(d) collecting the compartment tagged protein complexes, proteins, or polypeptides from the plurality of compartments;

(e) optionally fragmenting the compartment tagged protein complexes, proteins, or polypeptides into a compartment tagged peptides; and

(f) analyzing one or more compartment tagged peptide according to a method of any one of claims 1-21 and 26-61.

68. The method of any one of claims 62-67, wherein compartment tag information is transferred to a recording tag associated with a peptide via primer extension or ligation.

69. The method of any one of claims 62-68, wherein the solid support comprises a bead.

70. The method of claim 69, wherein the bead is a polystyrene bead, a polymer bead, an agarose bead, an acrylamide bead, a solid core bead, a porous bead, a paramagnetic bead, glass bead, or a controlled pore bead.

71. The method of any one of claims 62-70, wherein the compartment tag comprises a single stranded or double stranded nucleic acid molecule.

72. The method of any one of claims 62-71, wherein the compartment tag comprises a barcode and optionally a UMI.

73. The method of claim 72, wherein the solid support is a bead and the compartment tag comprises a barcode, further wherein beads comprising the plurality of compartment tags joined thereto are formed by split-and-pool synthesis.

74. The method of claim 72, wherein the solid support is a bead and the compartment tag comprises a barcode, further wherein beads comprising a plurality of compartment tags joined thereto are formed by individual synthesis or immobilization.

75. The method of any one of claims 62-74, wherein the compartment tag is a component within a recording tag, wherein the recording tag optionally further comprises a spacer, a unique molecular identifier, a universal priming site, or any combination thereof.

76. The method of any one of claims 62-75, wherein the compartment tags further comprise a functional moiety capable of reacting with an internal amino acid or N-terminal amino acid on the plurality of protein complexes, proteins, or polypeptides.

77. The method of claim 76, wherein the functional moiety is an NHS group.

78. The method of claim 76, wherein the functional moiety is an aldehyde group.

79. The method of any one of claims 62-78, wherein the plurality of compartment tags is formed by: printing, spotting, ink-jetting the compartment tags into the compartment, or a combination thereof.

80. The method of any one of claims 62-79, wherein the compartment tag further comprises a peptide.

81. The method of claim 80, wherein the compartment tag peptide comprises a protein ligase recognition sequence.

82. The method of claim 81, wherein the protein ligase is butelase I or a homolog thereof.

83. The method of any one of claims 62-82, wherein the plurality of polypeptides is fragmented with a protease.

84. The method of claim 83, wherein the protease is a metalloprotease.

85. The method of claim 84, wherein the activity of the metalloprotease is modulated by photo-activated release of metallic cations.

86. The method of any one of claims 62-85, further comprising subtraction of one or more abundant proteins from the sample prior to partitioning the plurality of polypeptides into the plurality of compartments.

87. The method of claim 62-86, further comprising releasing the compartment tags from the solid support prior to joining of the plurality of peptides with the compartment tags.

88. The method of claim 62, further comprising following step (d), joining the compartment tagged peptides to a solid support in association with recording tags.

89. The method of claim 88, further comprising transferring information of the compartment tag on the compartment tagged peptide to the associated recording tag.

90. The method of claim 89, further comprising removing the compartment tags from the compartment tagged peptides prior to step (e).

91. The method of any one of claims 62-90, further comprising determining the identity of the single cell from which the analyzed peptide derived based on the analyzed peptide's compartment tag sequence.

92. The method of any one of claims 62-90, further comprising determining the identity of the protein or protein complex from which the analyzed peptide derived based on the analyzed peptide's compartment tag sequence.

93. A method for analyzing a plurality of macromolecules, comprising the steps of:

(a) providing a plurality macromolecules and associated recording tags joined to a solid support;

(b) contacting the plurality of macromolecules with a plurality of binding agents capable of binding to the plurality of macromolecules, wherein each binding agent comprises a coding tag with identifying information regarding the binding agent;

(c) (i) transferring the information of the macromolecule associated recording tags to the coding tags of the binding agents that are bound to the macromolecules to generate extended coding tags; or (ii) transferring the information

of macromolecule associated recording tags and coding tags of the binding agents that are bound to the macromolecules to a di-tag construct;

- (d) collecting the extended coding tags or di-tag constructs;
- (e) optionally repeating steps (b) – (d) for one or more binding cycles;
- (f) analyzing the collection of extended coding tags or di-tag constructs.

94. The method of claim 93, wherein the macromolecule is a protein.

95. The method of claim 93, wherein the macromolecule is a peptide.

96. The method of claim 95, wherein the peptide is obtained by fragmenting a protein from a biological sample.

97. The method of any one of claims 93-96, wherein the recording tag is a DNA molecule, an RNA molecule, a PNA molecule, a BNA molecule, an XNA, molecule, an LNA molecule, a γ PNA molecule, or a combination thereof.

98. The method of any one of claims 93-97, wherein the recording tag comprises a unique molecular identifier (UMI).

99. The method of claims 93-98, wherein the recording tag comprises a compartment tag.

100. The method of any one of claims 93-99, wherein the recording tag comprises a universal priming site.

101. The method of any one of claims 93-100, wherein the recording tag comprises a spacer at its 3'-terminus.

102. The method of any one of claims 93-101, wherein the 3'-terminus of the recording tag is blocked to prevent extension of the recording tag by a polymerase and the information of macromolecule associated recording tag and coding tag of the binding agent that is bound to the macromolecule is transferred to a di-tag construct.

103. The method of any one of claims 93-102, wherein the coding tag comprises an encoder sequence.

104. The method of any one of claims 93-103, wherein the coding tag comprises a UMI.

105. The method of any one of claims 93-104, wherein the coding tag comprises a universal priming site.

106. The method of any one of claims 93-105, wherein the coding tag comprises a spacer at its 3'-terminus.

107. The method of any one of claims 93-106, wherein the coding tag comprises a binding cycle specific sequence.

108. The method of any one of claims 93-107, wherein the binding agent and the coding tag are joined by a linker.

109. The method of any one of claims 93-108, wherein transferring information of the recording tag to the coding tag is effected by primer extension.

110. The method of any one of claims 93-108, wherein transferring information of the recording tag to the coding tag is effected by ligation.

111. The method of any one of claims 93-108, wherein the di-tag construct is generated by gap fill, primer extension, or both.

112. The method of any one of claims 93-97, 107, 108, and 111, wherein the di-tag molecule comprises a universal priming site derived from the recording tag, a compartment tag derived from the recording tag, a unique molecular identifier derived from the recording tag, an optional spacer derived from the recording tag, an encoder sequence derived from the coding tag, a unique molecular identifier derived from the coding tag, an optional spacer derived from the coding tag, and a universal priming site derived from the coding tag.

113. The method of any one of claims 93-112, wherein the macromolecule and the associated recording tag are covalently joined to the solid support.

114. The method of claim 113, wherein the solid support is a bead, a porous bead, a porous matrix, an array, a glass surface, a silicon surface, a plastic surface, a filter, a membrane, nylon, a silicon wafer chip, a flow through chip, a biochip including signal transducing electronics, a microtitre well, an ELISA plate, a spinning interferometry disc, a nitrocellulose membrane, a nitrocellulose-based polymer surface, a nanoparticle, or a microsphere.

115. The method of claim 114, wherein the solid support is a polystyrene bead, a polymer bead, an agarose bead, an acrylamide bead, a solid core bead, a porous bead, a paramagnetic bead, glass bead, or a controlled pore bead.

116. The method of any one of claims 93-115, wherein the binding agent is a polypeptide or protein.

117. The method of claim 116, wherein the binding agent is a modified aminopeptidase, a modified amino acyl tRNA synthetase, a modified anticalin, or an antibody or binding fragment thereof.

118. The method of any one of claims 95-117 wherein the binding agent binds to a single amino acid residue, a dipeptide, a tripeptide or a post-translational modification of the peptide.

119. The method of claim 118, wherein the binding agent binds to an N-terminal amino acid residue, a C-terminal amino acid residue, or an internal amino acid residue.

120. The method of claim 118, wherein the binding agent binds to an N-terminal peptide, a C-terminal peptide, or an internal peptide.

121. The method of claim 119, wherein the binding agent binds to the N-terminal amino acid residue and the N-terminal amino acid residue is cleaved after each binding cycle.

122. The method of claim 119, wherein the binding agent binds to the C-terminal amino acid residue and the C-terminal amino acid residue is cleaved after each binding cycle.

123. The method of claim 121, wherein the N-terminal amino acid residue is cleaved via Edman degradation.

124. The method of claim 93, wherein the binding agent is a site-specific covalent label of an amino acid or post-translational modification.

125. The method of any one of claims 93-124, wherein following step (b), complexes comprising the macromolecule and associated binding agents are dissociated from the solid support and partitioned into an emulsion of droplets or microfluidic droplets.

126. The method of claim 125, wherein each microfluidic droplet, on average, comprises one complex comprising the macromolecule and the binding agents.

127. The method of claim 125 or 126, wherein the recording tag is amplified prior to generating an extended coding tag or di-tag construct.

128. The method of claim 125-127, wherein emulsion fusion PCR is used to transfer the recording tag information to the coding tag or to create a population of di-tag constructs.

129. The method of any one of claims 93-128, wherein the collection of extended coding tags or di-tag constructs are amplified prior to analysis

130. The method of any one of claims 93-129, wherein analyzing the collection of extended coding tags or di-tag constructs comprises a nucleic acid sequencing method.

131. The method of claim 130, wherein the nucleic acid sequencing method is sequencing by synthesis, sequencing by ligation, sequencing by hybridization, polony sequencing, ion semiconductor sequencing, or pyrosequencing.

132. The method of claim 130, wherein the nucleic acid sequencing method is single molecule real-time sequencing, nanopore-based sequencing, or direct imaging of DNA using advanced microscopy.

133. The method of claim 130, wherein a partial composition of the macromolecule is determined by analysis of a plurality of extended coding tags or di-tag constructs using unique compartment tags and optionally UMIs.

134. The method of any one of claims 1-133, wherein the analysis step is performed with a sequencing method having a per base error rate of $> 5\%$, $> 10\%$, $> 15\%$, $> 20\%$, $> 25\%$, or $> 30\%$.

135. The method of any one of claims 1-134, wherein the identifying components of a coding tag, recording tag, or both comprise error correcting codes.

136. The method of claim 135, wherein the identifying components are selected from an encoder sequence, barcode, UMI, compartment tag, cycle specific sequence, or any combination thereof.

137. The method of claim 135 or 136, wherein the error correcting code is selected from Hamming code, Lee distance code, asymmetric Lee distance code, Reed-Solomon code, and Levenshtein-Tenengolts code.

138. The method of any one of claims 1-134, wherein the identifying components of a coding tag, recording tag, or both are capable of generating a unique current or ionic flux or optical signature, wherein the analysis step comprises detection of the unique current or ionic flux or optical signature in order to identify the identifying components.

139. The method of claim 138, wherein the identifying components are selected from an encoder sequence, barcode, UMI, compartment tag, cycle specific sequence, or any combination thereof.

140. A method for analyzing a plurality of macromolecules, comprising the steps of:

- (a) providing a plurality macromolecules and associated recording tags joined to a solid support;
- (b) contacting the plurality of macromolecules with a plurality of binding agents capable of binding to cognate macromolecules, wherein each binding agent comprises a coding tag with identifying information regarding the binding agent;
- (c) transferring the information of a first coding tag of a first binding agent to a first recording tag associated with the first macromolecule to generate a first order extended recording tag, wherein the first binding agent binds to the first macromolecule,;
- (d) contacting the plurality of macromolecules with the plurality of binding agents capable of binding to cognate macromolecules;
- (e) transferring the information of a second coding tag of a second binding agent to the first order extended recording tag to generate a second order extended recording tag, wherein the second binding agent binds to the first macromolecule;
- (f) optionally repeating steps (d) – (e) for “n” binding cycles, wherein the information of each coding tag of each binding agent that binds to the first macromolecule is transferred to the extended recording tag generated from the previous binding cycle to generate an nth order extended recording tag that represents the first macromolecule;
- (g) analyzing the nth order extended recording tag.

141. The method of claim 140, wherein a plurality of nth order extended recording tags that represent a plurality of macromolecules are generated and analyzed.

142. The method of claim 140 or 141, wherein the macromolecule is a protein.

143. The method of claim 142, wherein the macromolecule is a peptide.

144. The method of claim 143, wherein the peptide is obtained by fragmenting proteins from a biological sample.

145. The method of any one of claims 140-144, wherein the plurality of macromolecules comprises macromolecules from multiple, pooled samples.

146. The method of any one of claims 140-145, wherein the recording tag is a DNA molecule, an RNA molecule, a PNA molecule, a BNA molecule, an XNA, molecule, an LNA molecule, a γ PNA molecule, or a combination thereof.

147. The method of any one of claims 140-146, wherein the recording tag comprises a unique molecular identifier (UMI).

148. The method of claims 140-147, wherein the recording tag comprises a compartment tag.

149. The method of any one of claims 140-148, wherein the recording tag comprises a universal priming site.

150. The method of any one of claims 140-149, wherein the recording tag comprises a spacer at its 3'-terminus.

151. The method of any one of claims 140-150, wherein the coding tag comprises an encoder sequence.

152. The method of any one of claims 140-151, wherein the coding tag comprises a UMI.

153. The method of any one of claims 140-152, wherein the coding tag comprises a universal priming site.

154. The method of any one of claims 140-153, wherein the coding tag comprises a spacer at its 3'-terminus.

155. The method of any one of claims 140-154, wherein the coding tag comprises a binding cycle specific sequence.

156. The method of any one of claims 140-155, wherein the coding tag comprises a unique molecular identifier.

157. The method of any one of claims 140-156, wherein the binding agent and the coding tag are joined by a linker.

158. The method of any one of claims 140-157, wherein transferring information of the recording tag to the coding tag is mediated by primer extension.

159. The method of any one of claims 140-158, wherein transferring information of the recording tag to the coding tag is mediated by ligation.

160. The method of any one of claims 140-159, wherein the plurality of macromolecules, the associated recording tags, or both are covalently joined to the solid support.

161. The method of any one of claims 140-160, wherein the solid support is a bead, a porous bead, a porous matrix, an array, a glass surface, a silicon

surface, a plastic surface, a filter, a membrane, nylon, a silicon wafer chip, a flow through chip, a biochip including signal transducing electronics, a microtitre well, an ELISA plate, a spinning interferometry disc, a nitrocellulose membrane, a nitrocellulose-based polymer surface, a nanoparticle, or a microsphere.

162. The method of claim 161, wherein the solid support is a polystyrene bead, a polymer bead, an agarose bead, an acrylamide bead, a solid core bead, a porous bead, a paramagnetic bead, glass bead, or a controlled pore bead.

163. The method of any one of claims 140-162, wherein the binding agent is a polypeptide or protein.

164. The method of claim 163, wherein the binding agent is a modified aminopeptidase, a modified amino acyl tRNA synthetase, a modified anticalin, or an antibody or binding fragment thereof.

165. The method of any one of claims 142-164 wherein the binding agent binds to a single amino acid residue, a dipeptide, a tripeptide or a post-translational modification of the peptide.

166. The method of claim 165, wherein the binding agent binds to an N-terminal amino acid residue, a C-terminal amino acid residue, or an internal amino acid residue.

167. The method of claim 165, wherein the binding agent binds to an N-terminal peptide, a C-terminal peptide, or an internal peptide.

168. The method of any one of claims 142-164, wherein the binding agent binds to a chemical label of a modified N-terminal amino acid residue, a modified C-terminal amino acid residue, or a modified internal amino acid residue.

169. The method of claim 166 or 168, wherein the binding agent binds to the N-terminal amino acid residue or the chemical label of the modified N-terminal amino acid residue, and the N-terminal amino acid residue is cleaved after each binding cycle.

170. The method of claim 166 or 168, wherein the binding agent binds to the C-terminal amino acid residue or the chemical label of the modified C-terminal amino acid residue, and the C-terminal amino acid residue is cleaved after each binding cycle.

171. The method of claim 169, wherein the N-terminal amino acid residue is cleaved via Edman degradation, Edmanase, a modified amino peptidase, or a modified acylpeptide hydrolase.

172. The method of claim 163, wherein the binding agent is a site-specific covalent label of an amino acid or post-translational modification.

173. The method of any one of claims 140-172, wherein the plurality of n^{th} order extended recording tags are amplified prior to analysis

174. The method of any one of claims 140-173, wherein analyzing the n^{th} order extended recording tag comprises a nucleic acid sequencing method.

175. The method of claim 174, wherein a plurality of n^{th} order extended recording tags representing a plurality of macromolecules are analyzed in parallel.

176. The method of claim 174 or 175, wherein the nucleic acid sequencing method is sequencing by synthesis, sequencing by ligation, sequencing by hybridization, polony sequencing, ion semiconductor sequencing, or pyrosequencing.

177. The method of claim 174 or 175, wherein the nucleic acid sequencing method is single molecule real-time sequencing, nanopore-based sequencing, or direct imaging of DNA using advanced microscopy.

CLAIMS

1. A method for analyzing a macromolecule, comprising the steps of:
 - (a) providing a macromolecule and an associated recording tag joined to a solid support;
 - (b) contacting the macromolecule with a first binding agent capable of binding to the macromolecule, wherein the first binding agent comprises a first coding tag with identifying information regarding the first binding agent;
 - (c) transferring the information of the first coding tag to the recording tag to generate a first order extended recording tag;
 - (d) contacting the macromolecule with a second binding agent capable of binding to the macromolecule, wherein the second binding agent comprises a second coding tag with identifying information regarding the second binding agent;
 - (e) transferring the information of the second coding tag to the first order extended recording tag to generate a second order extended recording tag; and
 - (f) analyzing the second order extended recording tag, wherein analyzing the macromolecule comprises characterizing, identifying or quantifying all or a portion of components of the macromolecule.
2. The method of claim 1, further comprising, between steps (e) and (f), the following steps:

repeating steps (d) and (e) one or more times by replacing the second binding agent with a third or higher order binding agent capable of binding to the macromolecule, wherein the third or higher order binding agent comprises a third or higher order coding tag with identifying information regarding the third or higher order binding agent; and by

transferring the information of the third or higher order coding tag to the second or higher order extended recording tag to generate a third or higher order extended recording tag;

and wherein the third or higher order extended recording tag is analyzed in step (f).
3. The method of claim 1 or claim 2, wherein:

step (a) comprises providing a plurality of macromolecules and associated recording tags joined to the solid support; and

each of steps (b) and (d) comprises contacting the plurality of macromolecules with a first or second plurality of binding agents capable of binding to the macromolecules, wherein the first or second plurality of binding agents comprise a first or second coding tag(s) with identifying information regarding the first or second plurality of binding agents.

4. The method of claim 1 or claim 2, wherein the macromolecule is a protein, polypeptide or peptide.

5. The method of claim 4, wherein the peptide is obtained by fragmenting a protein from a biological sample.

6. The method of claim 4, further comprising modifying the N-terminal amino acid (NTAA) of the protein, polypeptide or peptide with a chemical moiety to produce a modified NTAA.

7. The method of claim 6, wherein the binding agent is capable of binding to the modified NTAA.

8. The method of claim 4, further comprising removing the NTAA to expose a new NTAA of the protein, polypeptide or peptide.

9. The method of claim 1, wherein:
the recording tag is a DNA molecule, DNA with pseudo-complementary bases, an RNA molecule, a BNA molecule, an XNA molecule, a LNA molecule, a PNA molecule, a γ PNA molecule, or a combination thereof; and/or
the coding tag is a DNA molecule, an RNA molecule, a BNA molecule, an XNA molecule, a LNA molecule, a PNA molecule, a γ PNA molecule, or a combination thereof.

10. The method of claim 1, wherein the recording tag comprises:
a universal priming site;
a unique molecule identifier (UMI);
a barcode;
a spacer at its 3'-terminus; and/or

a 3' blocking group.

11. The method of claim 3, wherein the plurality of macromolecules are spaced apart on the solid support at an average distance > 50 nm.

12. The method of claim 1, wherein transferring the information of the coding tag to the recording tag is mediated by a DNA ligase, by a DNA polymerase, or by chemical ligation.

13. The method of claim 1, wherein the binding agent is capable of selectively binding to the macromolecule.

14. The method of claim 1, wherein the binding agent binds to a single amino acid residue, a dipeptide, a tripeptide or a post-translational modification of the peptide.

15. The method of claim 14, wherein the post-translational modification is selected from the group consisting of acylation, acetylation, alkylation, biotinylation, butyrylation, carbamylation, carbonylation, deamidation, deimination, diphthamide formation, disulfide bridge formation, eliminylation, flavin attachment, formylation, gamma- carboxylation, glutamylation, glycylation, glycosylation, glypiation, heme C attachment, hydroxylation, hypusine formation, iodination, isoprenylation, lipidation, lipoylation, malonylation, methylation, myristoylation, oxidation, palmitoylation, pegylation, phosphopantetheinylation, phosphorylation, prenylation, propionylation, retinylidene Schiff base formation, S-glutathionylation, S-nitrosylation, S-sulfenylation, selenation, succinylation, sulfination, ubiquitination, and C-terminal amidation.

16. The method of claim 14 or claim 15, wherein the method further comprises removing the post-translational modification.

17. The method of claim 1, wherein the coding tag further comprises a spacer, a binding cycle specific sequence, a unique molecular identifier, a universal priming site, a terminator nucleotide, or any combination thereof.

18. The method of claim 1, wherein analyzing the extended recording tag comprises a nucleic acid sequencing method.

19. The method of claim 1, wherein the extended recording tags is amplified prior to analysis.

20. The method of claim 1, wherein the solid support is a bead, a porous bead, a porous matrix, an array, a glass surface, a silicon surface, a plastic surface, a filter, a membrane, nylon, a silicon wafer chip, a flow through chip, a biochip including signal transducing electronics, a microtitre well, an ELISA plate, a spinning interferometry disc, a nitrocellulose membrane, a nitrocellulose-based polymer surface, a nanoparticle, or a microsphere.

21. The method of claim 20, wherein the solid support is a polystyrene bead, a polymer bead, an agarose bead, an acrylamide bead, a solid core bead, a porous bead, a paramagnetic bead, glass bead, a controlled pore bead, or a combination thereof.