

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.