METHODS, COMPOSITONS, AND SYSTEMS FOR ANALYTE DETECTION

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/512,502, filed July 7, 2023, which is entirely incorporated herein by reference.

BACKGROUND

[0002] The proximity of analytes within a sample have been analyzed and used for determining the state of the sample. Methods have been developed for analyzing the proximity of analytes within a sample.

SUMMARY

[0003] The disclosure provides methods of detecting analytes in a sample, the method comprising: a) providing a first probe and a second probe, wherein the first probe comprises: (i) a first binding site configured to couple to a first analyte at a first portion of the first analyte; (ii) a second binding site configured to couple to the first analyte at a second portion of the first analyte, wherein the first portion of the first analyte is adjacent to the second portion of the first analyte; (iii) a third binding site configured to couple to the second probe; (iv) a barcode; (v) a first end; and (vi) a second end; wherein the second probe comprises: (i) a fourth binding site configured to couple to the first probe; and (ii) a fifth binding site configured to couple to the second analyte; b) contacting a sample comprising a plurality of analytes comprising the first analyte and the second analyte with the first probe and the second probe, such that: (i) the first probe is coupled to the first analyte; (ii) the second probe is coupled to the second analyte; and (iii) the first probe is coupled to the second probe; c) ligating the first end and the second end to form a circular oligonucleotide; d) amplifying the circular oligonucleotide to generate an amplicon, wherein the amplicon comprises a complement of the barcode; and e) detecting the complement of the barcode or a derivative thereof using a plurality of detection probes, thereby determining a proximity of the first analyte to the second analyte.

[0003a] In one aspect, there is provided a method comprising: a) providing a first probe and a second probe, wherein said first probe comprises: (i) a first binding site configured to couple to a first analyte at a first portion; (ii) a second binding site configured to couple to said first analyte at a second portion, wherein said first portion is adjacent to said second portion; (iii) a third binding site configured to couple to said second probe; (iv) a first end; and (v) a second end; wherein said second probe comprises: (i) a fourth binding site configured to couple to said first

probe; and (ii) a fifth binding site configured to couple to said second analyte; b) contacting a sample comprising a plurality of analytes comprising said first analyte and said second analyte with said first probe and said second probe, such that: (i) said first probe is coupled to said first analyte; (ii) said second probe is coupled to said second analyte; and (iii) said first probe is coupled to said second probe; c) using said first probe and said second probe to generate an amplicon; and d) detecting said amplicon using a plurality of detection probes, thereby determining a proximity of said first analyte to said second analyte.

[0004] In some examples, the sample is a tissue sample. In some examples, the tissue sample is a fresh-frozen tissue sample. In some examples, the tissue sample is a formalin-fixed paraffin embedded tissue sample. In some examples, the sample is 5-250μm thick. In some examples, the sample is 10-200μm thick. In some examples, the sample is 25-150μm thick.

[0005] In some examples, the first analyte comprises a nucleic acid. In some examples, the nucleic acid is a ribonucleic acid. In some examples, the ribonucleic acid is a messenger ribonucleic acid. In some examples, the ribonucleic acid is a ribosomal ribonucleic acid. In some examples, the nucleic acid is a deoxyribonucleic acid. In some examples, the first analyte comprises a polypeptide. In some examples, the comprises a ribosomal protein. In some examples, the first analyte comprises a chemical modification. In some examples, the second analyte comprises a nucleic acid. In some examples, the nucleic acid is a ribonucleic acid. In some examples, the ribonucleic acid is a messenger ribonucleic acid. In some examples, the ribonucleic acid is a ribosomal ribonucleic acid. In some examples, the nucleic acid is a deoxyribonucleic acid. In some examples, the second analyte comprises a polypeptide. In some examples, the polypeptide comprises a ribosomal protein.

[0006] In some examples, the first probe comprises a nucleic acid. In some examples, the nucleic acid comprises an oligonucleotide. In some examples, the oligonucleotide comprises one or more modifications. In some examples, the one or more modifications comprise a 5’ phosphate modification. In some examples, the one or more modification comprise an internucleotide linkage. In some examples, the internucleotide linkage is a phosphorothioate. In some examples, the internucleotide linkage is a phosphodiester. In some examples, the first probe recognizes a ribonucleic acid. In some examples, the nucleic acid comprises a single nucleotide polymorphism. In some examples, the first probe recognizes the single nucleotide polymorphism and wherein the ligating in c) does not occur if the first analyte does not comprise the single nucleotide polymorphism. In some examples, the first probe does not recognize the single nucleotide polymorphism and wherein the ligating in c) does not occur if the first analyte comprises the single nucleotide polymorphism. In some examples, the nucleic acid comprises a modification. In some examples, the modification is selected from the group consisting of N 6-methyladenosine, 5-methylcytosine, N 1-methyladenosine, N 7-methylguanosine, N 4-acetylcytosine, pseudouridine, and N1-methylpseudouridine. In some examples, the first probe recognizes the modification and wherein the ligating in c) does not occur if the first analyte does not comprise the modification. In some examples, the first probe does not recognize the modification and wherein the ligating in c) does not occur if the first analyte comprises the modification. In some

examples, the first probe recognizes a deoxyribonucleic acid. In some examples, the first probe recognizes a deoxyribonucleic acid modification. In some examples, the deoxyribonucleic acid modification is a methyl modification. In some examples, the first probe comprises a first reactive chemical moiety at the first end and a second reactive chemical

moiety at the second end. In some examples, the first reactive chemical moiety is selected from the group consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine, carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some examples, the second reactive chemical moiety is selected from the group consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine, carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some examples, the ligating in c) comprises a reaction between the first reactive chemical moiety and the second reactive chemical moiety.

[0007] In some examples, the ligating in c) comprises performing a ligation reaction with a ligase. In some examples, the ligase is a T4 ligase. In some examples, the second probe comprises a nucleic acid. In some examples, the nucleic acid comprises an oligonucleotide. In some examples, the oligonucleotide comprises one or more modifications. In some examples, the one or more modifications comprise a 5' phosphate modification. In some examples, the one or more modification comprise an internucleotide linkage. In some examples, the internucleotide linkage is a phosphorothioate. In some examples, the internucleotide linkage is a phosphodiester. In some examples, the nucleic acid comprises an aptamer. In some examples, the second probe comprises a polypeptide. In some examples, the polypeptide comprises an antibody or antibody fragment. In some examples, the polypeptide comprises an affimer. In some examples, the polypeptide comprises a nanobody. In some examples, the second probe recognizes a ribonucleic acid. In some examples, the second probe recognizes a ribonucleic acid modification. In some examples, the ribonucleic acid modification is selected from the group consisting of N 6-methyladenosine, 5-methylcytosine, N 1-methyladenosine, N 7-methylguanosine, N 4-acetylcytosine, pseudouridine, and N1-methylpseudouridine. In some examples, the second probe recognizes a deoxyribonucleic acid. In some examples, the probe recognizes a deoxyribonucleic acid modification. In some examples, the deoxyribonucleic acid modification is a methyl modification. In some examples, the second probe recognizes a polypeptide. In some examples, the polypeptide is a protein. In some examples, the protein is a transcription factor. In some examples, the protein is a ribosomal protein. In some examples, the protein is a histone. In some examples, the protein is a polymerase. In some examples, the protein is a helicase. In some examples, the protein is a

restriction enzyme. In some examples, the protein is a ribonucleic acid binding protein. In some examples, the second probe recognizes a post-translational modification of the protein.

[0008] In some examples, the barcode comprises a nucleic acid. In some examples, the nucleic acid is deoxyribonucleic acid. In some examples, the nucleic acid is ribonucleic acid. In some examples, the nucleic acid is at least 4 nucleotides in length. In some examples, the nucleic acid is at least 6 nucleotides in length. In some examples, the nucleic acid is at least 8 nucleotides in length. In some examples, the nucleic acid is at least 10 nucleotides in length. In some examples, the barcode corresponds to the first analyte. In some examples, the barcode corresponds to the second analyte. In some examples, the barcode corresponds to the first analyte being proximal to the second analyte. In some examples, the first probe further comprises a second barcode. In some examples, the second barcode comprises a nucleic acid. In some examples, the nucleic acid is deoxyribonucleic acid. In some examples, the nucleic acid is ribonucleic acid. In some examples, the nucleic acid is at least 4 nucleotides in length. In some examples, the nucleic acid is at least 6 nucleotides in length. In some examples, the nucleic acid is at least 8 nucleotides in length. In some examples, the nucleic acid is at least 10 nucleotides in length. In some examples, the second barcode corresponds to the first analyte. In some examples, the second barcode corresponds to the second analyte. In some examples, the second barcode corresponds to the first analyte being proximal to the second analyte.

[0009] In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 6 nucleotides in length. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 14 nucleotides in length. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 20 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 14 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 20 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some

examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid

is at least 12 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 12 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 6 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 12 nucleotides in length.

[0010] In some examples, (c) involves performing rolling circle amplification. In some examples, (d) comprises hybridizing a detection probe and an anchor probe of the plurality of detection probes to the amplicon. In some examples, the detection probe and the section detection probe are ligated. In some examples, the detection probe comprises a label. In some examples, the label comprises a fluorescent molecule. In some examples, the label comprises a quantum dot. In some examples, the label comprises an enzyme. In some examples, the enzyme generates a signal indicative of the label. In some examples, (e) comprises detecting the label. In some examples, (e) comprises in situ sequencing using the plurality of detection probes. In some examples, (e) comprises imaging the sample. In some examples, the first probe recognizes a messenger ribonucleic acid, and the second probe recognizes a ribosomal protein. In some examples, the second probe comprises and antibody or antibody fragment. In some examples, the first probe recognizes a messenger ribonucleic acid, and the second probe recognizes a ribosomal ribonucleic acid. In some examples, the first probe recognizes a messenger ribonucleic acid, and the second probe recognizes a messenger ribonucleic acid modification. In some examples, the second probe comprises a reactive chemical moiety. In some examples, the second probe comprises an antibody or antibody fragment. In some examples, the first probe recognizes a deoxyribonucleic acid, and the second probe recognizes a deoxyribonucleic acid modification. In some examples, the second probe comprises a reactive chemical moiety. In some examples, the second probe

comprises an antibody or antibody fragment. In some examples, the sample is embedded in a hydrogel. In some examples, the ligating in c) comprises ligating the first end to the second end. In some examples, the first end and the second end are separated by at least one

nucleotide when coupled after (b). In some examples, the method further comprises performing a gap filling reaction after (b) and prior to (c), such that the first end and the third end are directly adjacent to each other.

[0011] The disclosure provides methods of detecting analytes in a sample, the method comprising: a) providing a first probe, wherein the first probe comprises: (i) a first binding site configured to couple to a first analyte at a first portion of the first analyte; (ii) a second binding site configured to couple to the first analyte at a second portion, wherein the first portion of the first analyte is adjacent to the second portion of the first analyte; (iii) a third binding site configured to couple to the second probe; (iv) a barcode; (v) a first end; and (vi) a second end b) contacting a sample comprising a plurality of analytes comprising the first analyte and the second analyte with the first probe, such that the first probe couples to the first analyte, wherein the first end and the second end are separated by a gap; c) performing a gap-filling reaction to fill the gap; d) ligating the first end and the second end to form a circular oligonucleotide, e) contacting the circular oligonucleotide with a second probe, wherein the second probe comprises: (i) a fourth binding site that couples to the first probe; and (ii) a fifth binding site that couples to the second analyte; f) amplifying the circular oligonucleotide to generate an amplicon, wherein the amplicon comprises a complement of the barcode; and g) detecting the complement of the barcode or a derivative thereof using at least one detection probe, thereby determining a proximity of the first analyte to the second analyte.

[0012] The disclosure provides methods of detecting analytes in a sample, the method comprising: a) Providing a first probe, a second probe, and a third probe, wherein the first probe comprises: (i) a first binding site configured to couple to a first analyte; (ii) a second binding site configured to couple to the second probe; (iii) a barcode; (iv) a first end; and (v) a second end; wherein the second probe comprises: (i) a third binding site configured to couple to the first probe; (ii) a fourth binding site configured to couple to the third probe; and (iii) a fifth binding site configured to couple to a second analyte; and wherein the third probe comprises: (i) a sixth binding site configured to couple to the second probe; (ii) a seventh binding site configured to couple to the first analyte; (iii) a third end, wherein the third end is adjacent to the first end; and (iv) a fourth end, wherein the fourth end is adjacent to the second end; b) contacting a sample comprising a plurality of analytes comprising the first analyte and the second analyte with the first probe, the second probe, and the third probe such that: (i) the first probe is coupled to the first analyte; (ii) the second probe is coupled to the second analyte; (iii) the third probe is coupled to the first analyte; (iv) the first probe is coupled to the second probe; and (v) the third probe is coupled to the second probe; c) ligating the first end and

the third end and ligating the

second end the fourth end to form a circular oligonucleotide; d) amplifying the circular oligonucleotide to generate an amplicon, wherein the amplicon comprises a complement of the barcode; and e) detecting the complement of the barcode or a derivative thereof using a plurality of detection probes, thereby determining a proximity of the first analyte to the second analyte.

[0013] In some examples, the sample is a tissue sample. In some examples, the tissue sample is a fresh-frozen tissue sample. In some examples, the tissue sample is a formalin-fixed paraffin embedded tissue sample. In some examples, the sample is 5-250μm thick. In some examples, the sample is 10-200μm thick. In some examples, the sample is 25-150μm thick. In some examples, the first analyte comprises a nucleic acid. In some examples, the nucleic acid is a ribonucleic acid. In some examples, the messenger ribonucleic acid is a messenger ribonucleic acid. In some examples, the messenger ribonucleic acid is a ribosomal messenger ribonucleic acid. In some examples, the nucleic acid is a deoxyribonucleic acid. In some examples, the nucleic acid comprises a single nucleotide polymorphism. In some examples, the first probe recognizes the single nucleotide polymorphism and wherein the ligating in c) does not occur if the first analyte does not comprise the single nucleotide polymorphism. In some examples, the first probe does not recognize the single nucleotide polymorphism and wherein the ligating in c) does not occur if the first analyte comprises the single nucleotide polymorphism. In some examples, the third probe recognizes the single nucleotide polymorphism and wherein the ligating in c) does not occur if the first analyte does not comprise the single nucleotide polymorphism. In some examples, the third probe does not recognize the single nucleotide polymorphism and wherein the ligating in c) does not occur if the first analyte comprises the single nucleotide polymorphism. In some examples, the nucleic acid comprises a modification. In some examples, the modification is selected from the group consisting of N 6-methyladenosine, 5-methylcytosine, N 1-methyladenosine, N 7-methylguanosine, N 4-acetylcytosine, pseudouridine, and N1-methylpseudouridine. In some examples, the first probe recognizes the modification and wherein the ligating in c) does not occur if the first analyte does not comprise the modification. In some examples, the first probe does not recognize the modification and wherein the ligating in c) does not occur if the first analyte comprises the modification. In some examples, the third probe recognizes the modification and wherein the ligating in c) does not occur if the first analyte does not comprise the modification. In some examples, the third probe does not recognize the

modification and wherein the ligating in c) does not occur if the first analyte comprises the modification. In some examples, the first analyte comprises a

polypeptide. In some examples, the polypeptide comprises a ribosomal protein. In some examples, the first analyte comprises a chemical modification.

[0014] In some examples, the second analyte comprises a nucleic acid. In some examples, the nucleic acid is a ribonucleic acid. In some examples, the ribonucleic acid is a messenger ribonucleic acid. In some examples, the ribonucleic acid is a ribosomal ribonucleic acid. In some examples, the nucleic acid is a deoxyribonucleic acid. In some examples, the second analyte comprises a polypeptide. In some examples, the polypeptide comprises a ribosomal protein.

[0015] In some examples, the first probe comprises a nucleic acid. In some examples, the nucleic acid comprises an oligonucleotide. In some examples, the oligonucleotide comprises one or more modifications. In some examples, the one or more modifications comprise a 5' phosphate modification. In some examples, the one or more modification comprise an internucleotide linkage. In some examples, the internucleotide linkage is a phosphorothioate. In some examples, the internucleotide linkage is a phosphodiester. In some examples, the first probe recognizes a ribonucleic acid. In some examples, the first probe recognizes a ribonucleic acid modification. In some examples, the ribonucleic acid modification is selected from the group consisting of N 6-methyladenosine, 5-methylcytosine, N 1-methyladenosine, N 7-methylguanosine, N 4-acetylcytosine, pseudouridine, and N1-methylpseudouridine. In some examples, the first probe recognizes a deoxyribonucleic acid. In some examples, the first probe recognizes a deoxyribonucleic acid modification. In some examples, the deoxyribonucleic acid modification is a methyl modification. In some examples, the first probe comprises a first reactive chemical moiety at the first end and the third probe comprises a second reactive chemical moiety at the third end. In some examples, the first reactive chemical moiety is selected from the group consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine, carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some examples, the second reactive chemical moiety is selected from the group consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine, carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some examples, the ligating in c) comprises a reaction between the first reactive chemical moiety and the second reactive chemical moiety. In some examples, the first probe comprises a third reactive chemical moiety at the second end and the third probe comprises a fourth reactive chemical moiety at the

fourth end. In some examples, the third reactive chemical moiety is selected from the group consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine,

carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some examples, the fourth reactive chemical moiety is selected from the group consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine, carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some examples, the ligating in c) comprises a reaction between the first reactive chemical moiety and the second reactive chemical moiety. In some examples, the ligating in c) comprises performing a ligation reaction with a ligase. In some examples, the ligase is a T4 ligase.

[0016] In some examples, the second probe comprises a nucleic acid. In some examples, the nucleic acid comprises an oligonucleotide. In some examples, the oligonucleotide comprises one or more modifications. In some examples, the one or more modifications comprise a 5' phosphate modification. In some examples, the one or more modification comprise an internucleotide linkage. In some examples, the internucleotide linkage is a phosphorothioate. In some examples, the internucleotide linkage is a phosphodiester. In some examples, the nucleic acid comprises an aptamer. In some examples, the second probe comprises a polypeptide. In some examples, the polypeptide comprises an antibody or antibody fragment. In some examples, the polypeptide comprises an affimer. In some examples, the polypeptide comprises a nanobody. In some examples, the second probe recognizes a ribonucleic acid. In some examples, the second probe recognizes a ribonucleic acid modification. In some examples, the ribonucleic acid modification is selected from the group consisting of N 6-methyladenosine, 5-methylcytosine, N 1-methyladenosine, N 7-methylguanosine, N 4-acetylcytosine, pseudouridine, and N1-methylpseudouridine. In some examples, the second probe recognizes a deoxyribonucleic acid. In some examples, the second probe recognizes a deoxyribonucleic acid modification. In some examples, the deoxyribonucleic acid modification is a methyl modification. In some examples, the second probe recognizes a polypeptide. In some examples, the polypeptide is a protein. In some examples, the protein is a transcription factor. In some examples, the protein is a ribosomal protein. In some examples, the protein is a histone. In some examples, the protein is a polymerase. In some examples, the protein is a helicase. In some examples, the protein is a restriction enzyme. In some examples, the protein is a ribonucleic acid binding protein. In some examples, the second probe recognizes a post-translational

modification of the protein. In some examples, the second probe comprises a reactive chemical moiety. In some examples, the third reactive chemical moiety is selected from the list consisting of tetrazine, alkyne, azide, trans-cyclooctene, maleimide, N-hydroxysuccinimide ester, amine, carboxylic acid, hydroxyl, cyclopropenone, and thiol, norbornene. In some

examples, the reactive chemical moiety reacts with the second analyte. In some examples, the reactive chemical moiety reacts with the first analyte.

[0017] In some examples, the third probe comprises a nucleic acid. In some examples, the nucleic acid comprises an oligonucleotide. In some examples, the oligonucleotide comprises one or more modifications. In some examples, the one or more modifications comprise a 5' phosphate modification. In some examples, the one or more modification comprise an internucleotide linkage. In some examples, the internucleotide linkage is a phosphorothioate. In some examples, the internucleotide linkage is a phosphodiester. In some examples, the third probe recognizes a ribonucleic acid. In some examples, the third probe recognizes a ribonucleic acid modification. In some examples, the ribonucleic acid modification is selected from the group consisting of N 6-methyladenosine, 5-methylcytosine, N 1-methyladenosine, N 7-methylguanosine, N 4-acetylcytosine, pseudouridine, and N1-methylpseudouridine. In some examples, the third probe recognizes a deoxyribonucleic acid. In some examples, the third probe recognizes a deoxyribonucleic acid modification. In some examples, the deoxyribonucleic acid modification is a methyl modification. In some examples, the third probe comprises a reactive chemical moiety. In some examples, the reactive chemical moiety reacts with the third probe.

[0018] In some examples, one or more barcodes comprises a nucleic acid. In some examples, the nucleic acid is deoxyribonucleic acid. In some examples, the nucleic acid is ribonucleic acid. In some examples, the nucleic acid is at least 4 nucleotides in length. In some examples, the nucleic acid is at least 6 nucleotides in length. In some examples, the nucleic acid is at least 8 nucleotides in length. In some examples, the nucleic acid is at least 10 nucleotides in length. In some examples, the barcode corresponds to the first analyte. In some examples, the barcode corresponds to the second analyte. In some examples, the barcode corresponds to the first analyte being proximal to the second analyte. In some examples, the first probe further comprises a second barcode. In some examples, the second barcode comprises a nucleic acid. In some examples, the nucleic acid is deoxyribonucleic acid. In some examples, the nucleic acid is ribonucleic acid. In some examples, the nucleic acid is at least 4 nucleotides in length. In some examples, the nucleic acid is at least 6 nucleotides in length. In some examples, the nucleic acid is at least 8 nucleotides in length. In

some examples, the nucleic acid is at least 10 nucleotides in length. In some examples, the second barcode corresponds to the first analyte. In some examples, the second barcode corresponds to the second analyte. In some examples, the second barcode corresponds to the first analyte being proximal to the second analyte. In some

examples, the third probe comprises a third barcode. In some examples, the third barcode comprises a nucleic acid. In some examples, the nucleic acid is deoxyribonucleic acid. In some examples, the nucleic acid is ribonucleic acid. In some examples, the nucleic acid is at least 4 nucleotides in length. In some examples, the nucleic acid is at least 6 nucleotides in length. In some examples, the nucleic acid is at least 8 nucleotides in length. In some examples, the nucleic acid is at least 10 nucleotides in length. In some examples, the third barcode corresponds to the first analyte. In some examples, the third barcode corresponds to the second analyte. In some examples, the third barcode corresponds to the first analyte being proximal to the second analyte. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 6 nucleotides in length. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 14 nucleotides in length. In some examples, the first binding site comprises a nucleic acid and wherein the nucleic acid is at least 20 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 2 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the second binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 2 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the third binding site comprises a nucleic acid and wherein the nucleic acid is at least 12 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 2 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the fourth binding site comprises a nucleic acid and wherein the nucleic

acid is at least 12 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 6 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some

examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 14 nucleotides in length. In some examples, the fifth binding site comprises a nucleic acid and wherein the nucleic acid is at least 20 nucleotides in length. In some examples, the sixth binding site comprises a nucleic acid and wherein the nucleic acid is at least 2 nucleotides in length. In some examples, the sixth binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the sixth binding site comprises a nucleic acid and wherein the nucleic acid is at least 8 nucleotides in length. In some examples, the sixth binding site comprises a nucleic acid and wherein the nucleic acid is at least 12 nucleotides in length. In some examples, the seventh binding site comprises a nucleic acid and wherein the nucleic acid is at least 4 nucleotides in length. In some examples, the seventh binding site comprises a nucleic acid and wherein the nucleic acid is at least 10 nucleotides in length. In some examples, the seventh binding site comprises a nucleic acid and wherein the nucleic acid is at least 14 nucleotides in length. In some examples, the seventh binding site comprises a nucleic acid and wherein the nucleic acid is at least 20 nucleotides in length.

[0019] In some examples, (c) involves performing rolling circle amplification. In some examples, (d) comprises hybridizing a detection probe and an anchor probe of the plurality of detection probes to the amplicon. In some examples, the detection probe and the section detection probe are ligated. In some examples, the detection probe comprises a label. In some examples, the label comprises a fluorescent molecule. In some examples, the label comprises a quantum dot. In some examples, the label comprises an enzyme. In some examples, the enzyme generates a signal indicative of the label. In some examples, (e) comprises detecting the label. In some examples, (e) comprises in situ sequencing using the plurality of detection probes. In some examples, (e) comprises imaging the sample. In some examples, the first probe and third probe recognize a messenger ribonucleic acid and the second probe recognizes a ribosomal ribonucleic acid. In some examples, the first probe and third probe recognize a messenger ribonucleic acid and the second probe recognizes a messenger ribonucleic acid modification. In some examples, the second probe comprises a reactive chemical moiety. In some examples, the second probe comprises an antibody or antibody fragment. In some examples, the first probe and third probe recognize a deoxyribonucleic acid and the second probe recognizes a deoxyribonucleic acid modification. In some examples,

the second probe comprises a reactive chemical moiety. In some examples, the second probe comprises an antibody or antibody fragment. In some examples, the sample is embedded in a hydrogel. In some examples, the ligating in c) comprises ligating the first

end to the third end. In some examples, the ligating in c) comprises ligating the second end to the fourth end. In some examples, the first end and the third end are separated by at least one nucleotide when coupled after (b). In some examples, the method further comprises performing a gap filling reaction after (b) and prior to (c), such that the first end and the third end are directly adjacent to each other. In some examples, the second end and the fourth end are separated by at least one nucleotide when coupled after (b). In some examples, the method further comprises performing a gap filling reaction after (b) and prior to (c), such that the second end and the fourth end are directly adjacent to each other.

[0020] The present disclosure also provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0021] The present disclosure also provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0022] Additional aspects, or examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments or examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, or examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0023] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained

by reference to the following detailed description that sets forth illustrative embodiments, in

CLAIMS

WHAT IS CLAIMED IS:

1. A method comprising:

a) providing a first probe and a second probe,

wherein said first probe comprises:

(i) a first binding site configured to couple to a first analyte at a first portion;

(ii) a second binding site configured to couple to said first analyte at a second portion, wherein said first portion is adjacent to said second portion;

(iii) a third binding site configured to couple to said second probe;

(iv) a first end; and

(v) a second end;

wherein said second probe comprises:

(i) a fourth binding site configured to couple to said first probe; and

(ii) a fifth binding site configured to couple to said second analyte;

b) contacting a sample comprising a plurality of analytes comprising said first analyte and said second analyte with said first probe and said second probe, such that:

(i) said first probe is coupled to said first analyte;

(ii) said second probe is coupled to said second analyte; and

(iii) said first probe is coupled to said second probe;

c) using said first probe and said second probe to generate an amplicon; and

d) detecting said amplicon using a plurality of detection probes, thereby determining a proximity of said first analyte to said second analyte.

2. The method of claim 1, wherein said first analyte comprises a first nucleic acid.

3. The method of claim 2, wherein said first nucleic acid comprises a ribonucleic acid.

4. The method of claim 3, wherein said first nucleic acid comprises a single nucleotide polymorphism.

5. The method of claim 0, wherein said first probe recognizes said single nucleotide polymorphism.

6. The method of claim 1, wherein said second analyte comprises a second nucleic acid.

7. The method of claim 1, wherein said first probe comprises a third nucleic acid.

8. The method of claim 7, wherein said third nucleic acid comprises one or more modifications.

9. The method of claim 1, wherein said second probe comprises a fourth nucleic acid.

10. The method of claim 9, wherein said fourth nucleic acid comprises one or more modifications.
11. The method of claim 1, wherein said sample is embedded in a hydrogel.
12. The method of claim 1, wherein said sample is a tissue sample.
13. The method of claim 1, wherein said first analyte comprises a first polypeptide.
14. The method of claim 1, wherein said second analyte comprises a second polypeptide.
15. The method of claim 1, wherein said second probe comprises a third polypeptide.
16. The method of claim 15, wherein said third polypeptide comprises an antibody or antibody fragment.
17. The method of claim 14, wherein said second probe recognizes said second polypeptide.
18. The method of claim 17, wherein said second polypeptide is a protein.
19. The method of claim 18, wherein said protein is a transcription factor.
20. The method of claim 18, wherein said protein is a ribosomal protein.
21. The method of claim 1, further comprising ligating said first end and said second end to form a circular oligonucleotide.
22. The method of claim 21, wherein said ligating comprises contacting said sample with a ligase.
23. The method of claim 21, wherein (c) comprises amplifying said circular oligonucleotide to generate an amplicon.
24. The method of claim 23, wherein said amplifying comprises performing a rolling circle amplification reaction.
25. The method of claim 1, wherein (d) comprises hybridizing an anchor probe and a detection probe of said plurality of detection probes to said amplicon.
26. The method of claim 25, wherein said detection probe comprises a label.
27. The method of claim 26, wherein said label comprises a fluorescent molecule.
28. The method of claim 1, wherein (d) comprises imaging said sample.