

CLAIMS

What is claimed is:

1. A biomolecule complex for the detection and/or quantitation of an analyte in a sample, comprising:

- a) an anchor strand attached to a support;
- b) a capture reagent attached to the support; and
- c) a detection reagent releasably attached to the anchor strand, the detection reagent or the anchor strand being optionally attached to a first label, the first label being inactive or undetectable;

wherein:

the capture reagent and the detection reagent can simultaneously bind to the analyte, if present in the sample, forming a tertiary complex;

release of the detection reagent from the anchor strand can release the detection reagent from the support in the absence of the analyte; and

the first label can be activated or detected when the detection reagent is released from the anchor strand.

2. The biomolecule of claim 1, wherein the first label is only detected on the support when the analyte is present.

3. The biomolecule complex of claim 2, wherein the amount of the first label on the support when the detection reagent is released from the anchor strand is proportional to the quantity and/or concentration of the analyte in the sample.

4. The biomolecule complex of any one of claims 1 to 3, wherein the detection reagent is releasably attached to the anchor strand directly via a covalent bond, a biotin-streptavidin bond, hydrogen bonding, a hydrophobic interaction, affinity binding, or a non-covalent interaction.

5. The biomolecule complex of any one of claims 1 to 3, wherein the detection reagent is attached to the anchor strand indirectly via a hook strand, the detection reagent

being linked to the hook strand and the hook strand being releasably attached to the anchor strand,

wherein release of the hook strand from the anchor strand can release the detection reagent from the support in the absence of the analyte, and

the first label can be activated or detected when the hook strand is released from the anchor strand.

6. The biomolecule complex of claim 5, wherein the amount of the first label on the support when the hook strand is released from the anchor strand is proportional to the quantity and/or concentration of the analyte in the sample.

7. The biomolecule complex of claim 5 or 6, wherein the first label is optionally attached to the hook strand.

8. The biomolecule complex of claim 7, wherein at least one of the detection reagent and the hook strand is optionally attached to the first label.

9. The biomolecule complex of claim 8, wherein the detection reagent is attached to the first label and the hook strand is not attached to the first label; wherein the hook strand is attached to the first label and the detection reagent is not attached to the first label; or wherein both the detection reagent and the hook strand are attached to the first label.

10. The biomolecule complex of any one of claims 5 to 9, wherein the hook strand is a polymer, an oligonucleotide, or a polysaccharide.

11. The biomolecule complex of claim 10, wherein the polymer is polyethylene glycol (PEG).

12. The biomolecule complex of claim 10 or 11, wherein the oligonucleotide is a single-stranded DNA oligonucleotide, a single-stranded RNA oligonucleotide, or a double-stranded DNA or RNA oligonucleotide.

13. The biomolecule complex of any one of claims 1 to 12, wherein the capture reagent is attached to the support directly.

14. The biomolecule complex of any one of claims 1 to 13, wherein the capture reagent is attached to the support via a covalent bond, a biotin-streptavidin bond, a DNA oligonucleotide linker, or a polymer linker.
15. The biomolecule complex of any one of claims 1 to 14, wherein the capture reagent is attached to the support indirectly by being attached to the anchor strand.
16. The biomolecule complex of claim 15, wherein the capture reagent is attached to the anchor strand by a covalent bond, a biotin-streptavidin bond, hydrogen bonding, a hydrophobic interaction, affinity binding, or a non-covalent interaction.
17. The biomolecule complex of claim 15, wherein the capture reagent is attached to the anchor strand by a capture strand linked to the capture reagent and the anchor strand.
18. The biomolecule complex of claim 17, wherein the capture strand is an oligonucleotide, a polymer such as PEG, or a polysaccharide.
19. The biomolecule complex of any one of claims 1 to 18, wherein the anchor strand is a polymer such as PEG, an oligonucleotide, or an oligosaccharide.
20. The biomolecule complex of claim 19, wherein the oligonucleotide is a DNA oligonucleotide.
21. The biomolecule complex of claim 19 or 20, wherein the anchor strand is a single-stranded DNA oligonucleotide, a single-stranded RNA oligonucleotide, a double-stranded DNA or RNA oligonucleotide, or a DNA-RNA hybrid.
22. The biomolecule complex of any one of claims 1 to 21, wherein the support is a microparticle, the surface of a multi-well plate, the surface of a glass slide, or a hydrogel matrix.
23. The biomolecule complex of claim 22, wherein the microparticle is a polystyrene bead, optionally barcoded.
24. The biomolecule complex of any one of claims 1 to 23, wherein the anchor strand is attached to the support via a covalent bond, a biotin-streptavidin bond, an oligonucleotide linker, a DNA oligonucleotide linker, a polymer linker, a PEG linker, or another chemical interaction such as hydrogen bonding, a hydrophobic interaction, affinity binding, or a non-covalent interaction.

25. The biomolecule complex of any one of claims 1 to 24, wherein the capture reagent is an antibody, an antibody fragment, an aptamer, a modified aptamer, a somamer, an affimer, an antigen, a protein, a polypeptide, a multi-protein complex, an exosome, an oligonucleotide, or a low molecular weight compound.

26. The biomolecule complex of any one of claims 1 to 25, wherein the detection reagent is an antibody, an antibody fragment, an aptamer, an affimer, a modified aptamer, a somamer, an antigen, a protein, a polypeptide, a multi-protein complex, an exosome, an oligonucleotide, or a low molecular weight compound.

27. The biomolecule complex of any one of claims 1 to 26, wherein the capture reagent and the detection reagent are different.

28. The biomolecule complex of any one of claims 1 to 26, wherein the capture reagent and the detection reagent are the same.

29. The biomolecule complex of any one of claims 1 to 28, wherein the capture reagent and the detection reagent are both antibodies.

30. The biomolecule complex of claim 28 or 29, wherein the capture reagent and the detection reagent are the same antibody.

31. The biomolecule complex of claim 27 or 29, wherein the capture reagent and the detection reagent are different antibodies and bind to different epitopes on the analyte.

32. The biomolecule complex of any one of claims 1 to 30, wherein the capture reagent and the detection reagent bind to the same epitope on the analyte, the analyte having two or more repeats of the epitope, and the capture reagent and the detection reagent binding to different repeats of the epitope on the analyte.

33. The biomolecule complex of any one of the preceding claims, wherein the capture reagent and the detection reagent are the same, structurally similar, or bind to structurally similar portions or epitopes of the analyte.

34. The biomolecule complex of any one of claims 1 to 33, wherein the analyte is an antigen, an antibody, an affimer, an aptamer, a modified aptamer, a somamer, an antibody fragment, a protein, a polypeptide, a multi-protein complex, an exosome, an oligonucleotide, a hormone, a modified oligonucleotide, or a low molecular weight compound.

35. The biomolecule complex of any one of the preceding claims, wherein the capture reagent and the detection reagent are both antigens, and the analyte is an antibody.
36. The biomolecule complex of any one of claims 5 to 35, wherein the releasable link between the hook strand and the anchor strand comprises a double-stranded DNA hybrid.
37. The biomolecule complex of any one of claims 1 to 36, wherein the sample is a biological sample.
38. The biomolecule complex of any one of claims 1 to 37, wherein the sample is a bodily fluid, an extract, a solution containing proteins and/or DNA, a cell extract, a cell lysate, or a tissue lysate.
39. The biomolecule complex of claim 38, wherein the bodily fluid is urine, saliva, blood, serum, plasma, cerebrospinal fluid, tears, semen, or sweat.
40. The biomolecule complex of any one of claims 1 to 39, wherein the first label is a fluorophore, a specific DNA sequence, an enzyme, or a biotin moiety.
41. The biomolecule complex of any one of claims 1 to 40, wherein the detection reagent can be released from the anchor strand by an enzymatic reaction, by cleavage, or by photocleavage.
42. The biomolecule complex of any one of claims 5 to 40, wherein the hook strand can be released from the anchor strand by an enzymatic reaction, by cleavage, by photocleavage, or by oligonucleotide displacement.
43. The biomolecule complex of any one of claims 5 to 40 and 42, further comprising a displacer agent capable of releasing the hook strand from the anchor strand, thereby releasing the detection agent from the support in the absence of the analyte.
44. The biomolecule complex of claim 43, wherein the hook strand and the anchor strand both comprise DNA oligonucleotides, and the releasable link between the hook strand and the anchor strand is a double-stranded DNA hybrid.
45. The biomolecule complex of claim 43 or 44, wherein the displacer agent is an enzyme, a DNA oligonucleotide, light or DTT.

46. The biomolecule complex of any one of claims 43 to 45, wherein the displacer agent can release the hook strand from the anchor strand by an enzymatic reaction, by cleavage, by photocleavage, or by oligonucleotide displacement.

47. The biomolecule complex of any one of claims 43 to 46, wherein the releasable link between the hook strand and the anchor strand is a double-stranded DNA hybrid; and the displacer agent is a DNA oligonucleotide complementary to at least a portion of the hook strand and capable of hybridizing to the hook strand, thereby releasing the hook strand from the anchor strand via a DNA displacement reaction.

48. The biomolecule complex of any one of claims 43 to 46, wherein the releasable link between the hook strand and the anchor strand is a double-stranded DNA hybrid; and the displacer agent is a DNA oligonucleotide complementary to at least a portion of the anchor strand and capable of hybridizing to the anchor strand, thereby releasing the hook strand from the anchor strand via a DNA displacement reaction.

49. The biomolecule complex of any one of claims 43 to 48, wherein the displacer agent is attached to a second label.

50. The biomolecule complex of claim 49, wherein the first label is absent, neither the detection reagent nor the hook strand being attached to the first label.

51. The biomolecule complex of claim 49 or 50, wherein the second label is a fluorophore, a specific DNA sequence, a biotin moiety, or an enzyme.

52. The biomolecule complex of any one of claims 43 to 51, wherein the displacer agent is an enzyme or light or DTT and is capable of breaking the link between the hook strand and the anchor strand via an enzymatic reaction or by photocleavage.

53. The biomolecule complex of any one of claims 1 to 52, wherein the detection reagent and the capture reagent are both antibodies, the analyte is an antigen, and the detection reagent and the capture reagent can bind different epitopes on the antigen.

54. The biomolecule complex of any one of claims 1 to 52, wherein the detection reagent and the capture reagent are both antibodies, the analyte is an antigen, and the detection reagent and the capture reagent can bind the same epitope on the antigen.

55. The biomolecule complex of claim 54, wherein the antigen has two or more repeats of the epitope, and the detection reagent and the capture reagent can bind simultaneously to different repeats of the epitope on the antigen.

56. The biomolecule complex of any one of claims 1 to 55, further comprising a stem strand complementary to the surface-proximate sequence of the anchor strand, the anchor strand being a single-stranded oligonucleotide, wherein the stem strand is capable of binding to the anchor strand to form a double-stranded oligonucleotide anchor strand.

57. The biomolecule complex of any one of claims 1 to 56, wherein the relative density of the anchor strand and the capture reagent on the support can be adjusted to control the effective affinity of the assay.

58. The biomolecule complex of any one of claims 5 to 57, wherein the length of the hook strand and/or the anchor strand can be adjusted to control the effective affinity of the assay; and/or wherein the surface densities of the capture reagent and/or the detection reagent can be adjusted to control the effective affinity of the assay.

59. The biomolecule complex of any one of claims 5 to 58, wherein the conjugation between the detection reagent and the hook strand is monovalent.

60. The biomolecule complex of any one of claims 5 to 59, wherein at least 90% of the detection reagent is linked to the support via only one hook strand.

61. The biomolecule complex of any one of claims 1 to 60, wherein, when the capture reagent is linked to the anchor strand via a capture strand, the conjugation between the capture reagent and the capture strand is monovalent.

62. The biomolecule complex of any one of claims 1 to 53, wherein, when the capture reagent is linked to the anchor strand via a capture strand, at least 90% of the capture reagent is linked to the anchor strand via only one capture strand.

63. The biomolecule complex of any one of claims 1 to 62, wherein the anchor strand is stochastically distributed on the support.

64. The biomolecule complex of any one of claims 1 to 63, wherein the capture reagent is stochastically distributed on the support.

65. The biomolecule complex of any one of claims 5 to 64, wherein the length and/or the flexibility of the hook strand can be selected to be sufficient to allow binding of the detection reagent to the analyte in the presence of the capture reagent.

66. The biomolecule complex of any one of claims 5 to 65, wherein, when the link between the hook strand and the anchor strand is a double-stranded DNA hybrid, the melting temperature (T_m) of the double-stranded DNA hybrid is from about 50 to about 80 degrees Celsius.

67. The biomolecule complex of any one of claims 1 to 66, wherein the concentration of the detection reagent after release or displacement is less than about 10 picomolar.

67a. A biomolecule complex for the detection and/or quantitation of an analyte in a sample, comprising:

- a) an anchor strand attached to a support;
- b) a capture reagent attached to the support;
- c) a detection reagent linked to a hook strand, the hook strand being releasably attached to the anchor strand, the hook strand and the anchor strand being linked together by a double-stranded DNA hybrid; and
- d) a displacer agent comprising a DNA oligonucleotide complementary to at least a portion of the hook strand and capable of hybridizing to the hook strand, thereby releasing the hook strand from the anchor strand via a DNA displacement reaction, the displacer agent being detectably labeled;

wherein:

the capture reagent and the detection reagent can simultaneously bind to the analyte, if present in the sample, forming a tertiary complex; and

release of the hook strand from the anchor strand by the displacer agent can release the detection reagent from the support in the absence of the analyte.

68. The biomolecule complex of any one of claims 1 to 67a, further comprising:

- a) a second anchor strand attached to the support;
- b) a second detection reagent releasably attached to the second anchor strand, wherein the second detection reagent is optionally attached to a third label, wherein the third label is inactive or undetectable;

wherein:

the capture reagent, the detection reagent and the second detection reagent can simultaneously bind to the analyte, if present in the sample, forming a quaternary complex; and

release of the second detection reagent from the second anchor strand can release the second detection reagent from the support in the absence of the analyte; and

the third label can be activated or detected when the second detection reagent is released from the second anchor strand.

69. The biomolecule complex of claim 68, wherein the second detection reagent is linked to a second hook strand, the second hook strand being releasably linked to the second anchor strand, wherein at least one of the second detection reagent and the second hook strand is optionally attached to the third label, the third label being inactive or undetectable;

wherein release of the second hook strand from the second anchor strand can release the second detection reagent from the support in the absence of the analyte; and

the third label can be activated or detected when the second hook strand is released from the second anchor strand.

70. The biomolecule complex of claim 68 or 69, wherein the second detection reagent is attached to the third label; the second hook strand is attached to the third label; or both the second detection reagent and the second hook strand are attached to the third label.

71. The biomolecule complex of any one of claims 68 to 70, wherein the third label is a fluorophore, a specific DNA sequence, a biotin moiety or an enzyme.

72. The biomolecule complex of any one of claims 68 to 71, wherein the third label is detected on the support only when the analyte is present.

73. The biomolecule complex of any one of claims 69 to 72, further comprising a second displacer agent capable of releasing the second hook strand from the second anchor strand, such that the second detection agent is released from the support in the absence of the analyte.

74. The biomolecule complex of claim 73, wherein the second hook strand and the second anchor strand are DNA oligonucleotides and the link between the second hook strand and the second anchor strand is a double-stranded DNA hybrid.

75. The biomolecule complex of claim 73 or 74, wherein the second displacer agent can release the second hook strand from the second anchor strand by an enzymatic reaction, by cleavage, by photocleavage, or by oligonucleotide displacement.

76. The biomolecule complex of any one of claims 73 to 75, wherein the link between the second hook strand and the second anchor strand comprises a double-stranded DNA hybrid, and the second displacer agent is capable of breaking the link between the second hook strand and the second anchor strand via a DNA strand displacement reaction.

77. The biomolecule complex of any one of claims 73 to 76, wherein the second displacer agent is detectably labeled.

78. The biomolecule complex of claim 77, wherein the second displacer agent is labeled with a fluorophore, a specific DNA sequence, an enzyme, or a biotin moiety.

79. The biomolecule complex of claim 77 or 78, wherein the third label is absent, neither the second detection reagent nor the second hook strand being attached to the third label.

80. A multiplex sandwich assay system for the simultaneous detection and/or quantitation of two or more analytes in a sample, the system comprising two or more biomolecule complexes as defined in any one of claims 1 to 79, wherein the two or more biomolecule complexes are attached to the same support, and wherein each biomolecule complex is for the detection and/or quantitation of a different analyte in the sample.

81. The multiplex sandwich assay system of claim 80, wherein the support is a microparticle such as a polystyrene bead, a multi-well plate, the surface of a glass slide, or a hydrogel matrix.

82. A multiplex sandwich assay system for the simultaneous detection and/or quantitation of two or more analytes in a sample, the system comprising two or more biomolecule complexes as defined in any one of claims 1 to 79, wherein the two or more biomolecule complexes are for the detection and/or quantitation of a respective first analyte and a respective second analyte in the sample,

wherein the two or more biomolecule complexes are attached to a respective first support and a respective second support, and the sample contacts the first support and the second support simultaneously.

83. The multiplex sandwich assay system of claim 82, wherein the first support and the second support are the same support.

84. The multiplex sandwich assay system of claim 83, wherein said same support is a planar surface, and the two or more biomolecule complexes are positioned at separate locations on the planar surface.

85. The multiplex sandwich assay system of claim 84, wherein the planar surface is the surface of a multi-well plate, the surface of a glass slide, or a hydrogel matrix.

86. The multiplex sandwich assay system of claim 82, wherein the first support and the second support are different supports.

87. The multiplex sandwich assay system of claim 86, wherein the first support and the second support are both microparticles.

88. The multiplex sandwich assay system of claim 87, wherein the microparticle is a polystyrene bead.

89. The multiplex sandwich assay system of claim 87 or 88, wherein the microparticle is barcoded.

90. The multiplex sandwich assay system of any one of claims 82 to 89, wherein the first support is a first bead encoded with a first barcode and the second support is a second bead encoded with a second barcode, allowing identification of the respective beads.

91. The multiplex sandwich assay system of any one of claims 82 to 90, wherein the two or more biomolecule complexes comprise a respective first hook strand and a respective second hook strand, and a respective first anchor strand and a respective

second anchor strand, and the first hook strand, the second hook strand, the first anchor strand, and the second anchor strand are all DNA oligonucleotides, the links between the first hook strand and the first anchor strand, and the second hook strand and the second anchor strand, being double-stranded DNA hybrids;

wherein the two or more biomolecule complexes further comprise a respective first displacer agent and a respective second displacer agent, wherein the first displacer agent is capable of releasing the first hook strand from the first anchor strand and the second displacer agent is capable of releasing the second hook strand from the second anchor strand;

wherein the first displacer agent and the second displacer agent are the same.

92. The multiplex sandwich assay of claim 91, wherein the first displacer agent and the second displacer agent are a displacer DNA oligonucleotide that can bind to both the first hook strand and the second hook strand, thereby displacing the first hook strand and the second hook strand from their respective anchor strands via a DNA displacement reaction.

93. The multiplex sandwich assay of claim 92, wherein the displacer DNA oligonucleotide is detectably labeled.

94. The multiplex sandwich assay of claim 93, wherein the label is a fluorophore, a specific DNA sequence, an enzyme, or a biotin moiety.

95. The multiplex sandwich assay of any one of claims 82 to 94, wherein the first hook strand and the second hook strand are attached to a first label and a third label respectively, the first label and the third label being inactive or undetectable, wherein the first label and the third label can be activated or detected when the first hook strand is released from the first anchor strand and the second hook strand is released from the second anchor strand, respectively.

96. The multiplex sandwich assay of any one of claims 82 to 94, wherein the first label and the third label are absent, and only the displacer DNA oligonucleotide is labeled.

97. A multiplex sandwich assay system for the simultaneous detection and/or quantitation of two or more analytes in a sample, the system comprising:

a) a first biomolecule complex for detection and/or quantitation of a first analyte in the sample, the first biomolecule complex comprising a first anchor strand, a first capture reagent, and a first detection reagent optionally linked to a first hook strand, the first detection reagent or the first hook strand being releasably linked to the first anchor strand, at least one of the first detection reagent and the first hook strand being optionally attached to a first label, wherein the first capture reagent and the first detection reagent can simultaneously bind to the first analyte, if present in the sample, forming a first tertiary complex; and

b) a second biomolecule complex for detection and/or quantitation of a second analyte in the sample, the second biomolecule complex comprising a second anchor strand, a second capture reagent, and a second detection reagent optionally linked to a second hook strand, the second detection reagent or the second hook strand being releasably linked to the second anchor strand, and at least one of the second detection reagent and the second hook strand being optionally attached to a second label, wherein the second capture reagent and the second detection reagent can simultaneously bind to the second analyte, if present in the sample, forming a second tertiary complex;

wherein the first anchor strand, the first capture reagent, the second anchor strand, and the second capture reagent are all attached to a support;

wherein the first label and the second label are inactive or undetectable and can be activated or detected when their respective detection reagents or hook strands are released from their respective anchor strands.

98. The multiplex sandwich assay system of claim 97, wherein the first label and the second label are only detected on the support when the first analyte and the second analyte respectively are present.

99. The multiplex sandwich assay system of claim 97 or 98, further comprising a displacer agent capable of releasing the first detection reagent or the first hook strand from the first anchor strand and the second detection reagent or the second hook strand from the second anchor strand.

100. The multiplex sandwich assay system of any one of claims 97 to 99, wherein: the displacer agent is a DNA oligonucleotide; the first hook strand, the second hook strand, the first anchor strand, and the second anchor strand comprise DNA oligonucleotides

and the links between the hook strands and their respective anchor strands are double-stranded DNA hybrids; and the displacer agent is capable of hybridizing to the first hook strand and the second hook strand and breaking said links between the hook strands and their respective anchor strands via a DNA strand displacement reaction.

101. The multiplex sandwich assay system of claim 99 or 100, wherein the displacer agent is detectably labeled.

102. The multiplex sandwich assay system of claim 101, wherein the first label and the second label are absent, only the displacer agent being labeled.

103. The multiplex sandwich assay system of any one of claims 99 to 102, wherein the displacer agent is labeled with a fluorophore, a specific DNA sequence, an enzyme, or a biotin moiety.

104. The multiplex sandwich assay system of any one of claims 97 to 103, wherein the support is a planar surface or the surface of a multi-well plate, the surface of a glass plate, or a hydrogel, and the first and second biomolecule complexes are positioned at a first location and a second location respectively on the support.

105. The multiplex sandwich assay system of any one of claims 97 to 104, wherein the first biomolecule complex is attached to a first support, and the second biomolecule complex is attached to a second support.

106. The multiplex sandwich assay system of claim 105, wherein the first support is a microparticle such as a polystyrene bead.

107. The multiplex sandwich assay system of claim 105 or 106, wherein the second support is a microparticle such as a polystyrene bead.

108. The multiplex sandwich assay system of any one of claims 105 to 107, wherein the first support and the second support are mixed together.

109. The multiplex sandwich assay system of any one of claim 108, wherein the first support and the second support are barcoded.

110. The multiplex sandwich assay system of any one of claims 97 to 109, wherein the first biomolecule complex system is a first biomolecule complex system as defined in any one of claims 1 to 79, and wherein the second biomolecule complex system is a second biomolecule complex system as defined in any one of claims 1 to 79.

111. A method for detecting and/or quantitating an analyte in a sample, the method comprising:

- a) providing a support, a capture reagent attached to the support, an anchor strand attached to the support, and a detection reagent optionally linked to a hook strand, wherein the detection reagent or the hook strand is releasably linked to the anchor strand, and wherein at least one of the detection reagent and the hook strand is optionally attached to a first label, said first label being inactive or undetectable;
- b) contacting the support with the sample under conditions that allow simultaneous binding of the capture reagent and the detection reagent to the analyte, to form a tertiary complex; and
- c) adding a displacer agent optionally attached to a second label, wherein the displacer agent releases the detection reagent or the hook strand from the anchor strand, such that the detection reagent optionally linked to the hook strand is released from the support in the absence of the analyte, and wherein said release of the detection strand or the hook strand from the anchor stand activates said first label or makes said first label detectable.

112. The method of claim 111, further comprising the following step (d):

- d) determining the presence and/or the amount of the first label and/or the second label on the support, wherein the presence of the first and/or the second label on the support indicates the presence of the analyte in the sample, and the amount of the first and/or the second label is proportional to the quantity and/or concentration of the analyte in the sample.

113. The method of claim 111 or 112, further comprising a step of washing the support to remove any unbound reagents or materials after step (c).

114. The method of any one of claims 111 to 113, further comprising a step of storing the support after step (c).

115. The method of any one of claims 111 to 114, wherein the first label is attached to the detection reagent.

116. The method of any one of claims 111 to 115, wherein the first label is attached to the hook strand.

117. The method of any one of claims 111 to 116, wherein the second label is attached to the displacer agent.

118. The method of any one of claims 111 to 117, wherein the first label and/or the second label is a fluorophore, a specific DNA sequence, an enzyme, or a biotin moiety.

119. The method of any one of claims 111 to 118, wherein the first label is absent, and the displacer agent is attached to the second label.

120. The method of any one of claims 111 to 119, wherein the capture reagent is attached to the support directly.

121. The method of any one of claims 111 to 120, wherein the capture reagent is attached to the support via a covalent bond, a biotin-streptavidin bond, a DNA oligonucleotide linker, or a polymer linker.

122. The method of any one of claims 111 to 119, wherein the capture reagent is attached to the support indirectly by being attached to the anchor strand.

123. The method of claim 122, wherein the capture reagent is attached to the anchor strand by a covalent bond, a biotin-streptavidin bond, hydrogen bonding, a hydrophobic interaction, affinity binding, or a non-covalent interaction.

124. The method of claim 122, wherein the capture reagent is attached to the anchor strand by a capture strand linked to the capture reagent and the anchor strand.

125. The method of claim 124, wherein the capture strand is an oligonucleotide, a polymer such as PEG, or a polysaccharide.

126. The method of any one of claims 111 to 125, wherein the anchor strand is a polymer such as PEG, an oligonucleotide, or an oligosaccharide.

127. The method of claim 126, wherein the oligonucleotide is a single-stranded DNA oligonucleotide, a single-stranded RNA oligonucleotide, a double-stranded DNA or RNA oligonucleotide, or a DNA-RNA hybrid.

128. The method of any one of claims 111 to 127, wherein the support is a microparticle, the surface of a multi-well plate, the surface of a glass slide, or a hydrogel matrix.

129. The method of claim 128, wherein the microparticle is a polystyrene bead.

130. The method of claims 128 or 129, wherein the microparticle is barcoded.

131. The method of any one of claims 111 to 130, wherein the hook strand is a polymer such as PEG, a DNA oligonucleotide such as a single-stranded DNA oligonucleotide, a single-stranded RNA oligonucleotide, or a double-stranded DNA or RNA oligonucleotide, or an oligosaccharide.

132. The method of any one of claims 111 to 131, wherein the hook strand is absent and the detection reagent is linked to the anchor strand directly via a covalent bond or a biotin-streptavidin bond.

133. The method of any one of claims 111 to 113, wherein the anchor strand is attached to the support via a covalent bond, a biotin-streptavidin bond, a DNA oligonucleotide linker, a polymer linker, or another chemical interaction such as hydrogen bonding, a hydrophobic interaction, affinity binding, or a non-covalent interaction.

134. The method of any one of claims 111 to 133, wherein the capture reagent and/or the detection reagent is an antibody, an antibody fragment, an aptamer, a modified aptamer, a somamer, an affimer, an antigen, a protein, a polypeptide, a multi-protein complex, an exosome, an oligonucleotide, or a low molecular weight compound.

135. The method of any one of claims 111 to 134, wherein the capture reagent and the detection reagent are different.

136. The method of any one of claims 111 to 134, wherein the capture reagent and the detection reagent are the same, structurally similar, or bind to structurally similar portions or epitopes of the analyte.

137. The method of claim 136, wherein the analyte has two or more repeats of the portion to which the capture reagent and the detection reagent bind, and the capture reagent and the detection reagent bind to different repeats.

138. The method of claim 136, wherein the capture reagent and the detection reagent bind to the same epitope, and the analyte has two or more repeats of the epitope, the capture reagent and the detection reagent binding to different repeats of the epitope on the analyte.

139. The method of any one of claims 111 to 138, wherein the capture reagent and the detection reagent bind to different portions or epitopes of the analyte.

140. The method of any one of claims 111 to 139, wherein the capture reagent and the detection reagent are both antibodies.

141. The method of claim 140, wherein the analyte is an antigen, a protein, a polypeptide, a multi-protein complex, a hormone, or an exosome.

142. The method of claim 141, wherein the analyte is an antigen.

143. The method of any one of claims 111 to 139, wherein the capture reagent and the detection reagent are both antigens, proteins, or peptides, and the analyte is an antibody.

144. The method of any one of claims 111 to 143, wherein the displacer agent releases the detection reagent or the hook strand from the anchor strand by an enzymatic reaction, by cleavage, by photocleavage, or by oligonucleotide displacement.

145. The method of any one of claims 111 to 144, wherein the hook strand and the anchor strand both comprise DNA oligonucleotides, and the link between the hook strand and the anchor strand is a double-stranded DNA hybrid.

146. The method of any one of claims 111 to 145, wherein the displacer agent is an enzyme or light or DTT.

147. The method of any one of claims 111 to 145, wherein the displacer agent is a DNA oligonucleotide.

148. The method of claim 147, wherein the displacer agent is complementary to at least a portion of the hook strand and hybridizes to the hook strand, thereby releasing the hook strand from the anchor strand via a DNA strand displacement reaction.

149. The method of claim 147, wherein the displacer agent is complementary to at least a portion of the anchor strand and hybridizes to the anchor strand, thereby

releasing the hook strand from the anchor strand via a DNA strand displacement reaction.

150. The method of any one of claims 111 to 148, wherein the first label is absent, and the displacer agent is attached to the second label.

151. The method of claim 150, wherein the second label is a fluorophore, a specific DNA sequence, an enzyme, or a biotin moiety.

152. The method of any one of claims 111 to 151, wherein the support further comprises a stem strand which is a single-stranded oligonucleotide complementary to the surface-proximate sequence of the anchor strand, the anchor strand being a single-stranded oligonucleotide, wherein the stem strand is capable of binding to the anchor strand to form a double-stranded oligonucleotide anchor strand.

153. The method of any one of claims 111 to 152, wherein the relative density of the anchor strand and the capture reagent on the support is adjusted to control the effective affinity of the assay.

154. The method of any one of claims 111 to 153, wherein the conjugation between the detection reagent and the hook strand is monovalent.

155. The method of any one of claims 111 to 154, wherein at least 90% of the detection reagent is linked to the support via only one hook strand.

156. The method of any one of claims 111 to 155, wherein, when the capture reagent is linked to the anchor strand via the capture strand, the conjugation between the capture reagent and the capture strand is monovalent.

157. The method of any one of claims 111 to 155, wherein, when the capture reagent is linked to the anchor strand via the capture strand, at least 90% of the capture reagent is linked to the anchor strand via only one capture strand.

158. The method of any one of claims 111 to 157, wherein the anchor strand is stochastically distributed on the support.

159. The method of any one of claims 111 to 158, wherein the capture reagent is stochastically distributed on the support.

160. The method of any one of claims 111 to 159, wherein the length and/or the flexibility of the hook strand are selected to be sufficient to allow binding of the detection reagent to the analyte in the presence of the capture reagent.

160a. The method of any one of claims 111 to 160, wherein the length of the hook strand and/or the anchor strand are selected to control the effective affinity of the assay; and/or wherein the surface densities of the capture reagent and/or the detection reagent are selected to control the effective affinity of the assay.

161. The method of any one of claims 111 to 160a, wherein the link between the hook strand and the anchor strand is a double-stranded DNA hybrid having a melting temperature (T_m) of from about 50 to about 80 degrees Celsius.

162. The method of any one of claims 111 to 161, wherein the capture reagent is linked to the anchor strand via an oligonucleotide linker such that the link between the capture reagent and the anchor is a second double-stranded DNA hybrid, the melting temperature (T_m) of the second double-stranded DNA hybrid being from about 50 to about 80 degrees Celsius.

163. The method of any one of claims 111 to 162, wherein the concentration of the detection reagent after release or displacement is less than about 10 picomolar.

163a. A method for the detection and/or quantitation of an analyte in a sample, the method comprising:

- a) providing a support, a capture reagent attached to the support, an anchor strand attached to the support, and a detection reagent linked to a hook strand, wherein the hook strand is releasably linked to the anchor strand by a double-stranded DNA hybrid;
- b) contacting the support with the sample under conditions that allow simultaneous binding of the capture reagent and the detection reagent to the analyte, to form a tertiary complex; and
- c) adding a displacer agent attached to a detectable label, wherein the displacer agent is a DNA oligonucleotide complementary to at least a portion of the hook strand and capable of hybridizing to the hook strand, wherein the displacer agent releases the hook strand from the anchor

strand via a DNA displacement reaction, such that the detection reagent is released from the support in the absence of the analyte; and

- d) optionally determining the presence and/or the amount of the detectable label on the support, wherein the presence of the label on the support indicates the presence of the analyte in the sample, and the amount of the label is proportional to the quantity and/or concentration of the analyte in the sample.

164. The method of any one of claims 111 to 163a, wherein:

- the support further comprises a second anchor strand attached to the support, a second capture reagent attached to the support, and a second detection reagent optionally linked to a second hook strand, wherein the second detection reagent or the second hook strand is linked to the second anchor strand, and wherein at least one of the second detection reagent and the second hook strand is optionally attached to a third label, said third label being inactive or undetectable, wherein the second capture reagent and the second detection reagent can bind simultaneously to a second analyte in the sample, to form a second tertiary complex;

- wherein the displacer agent also releases the second detection reagent or the second hook strand from the second anchor strand, such that the second detection reagent optionally linked to the second hook strand is released from the support in the absence of the second analyte, and wherein said release of the second detection reagent or the second hook strand from the second anchor strand activates said third label or makes said third label detectable;

- wherein the presence and/or the amount of the third label on the support indicates the presence of the second analyte in the sample, and the amount of the third label on the support is proportional to the quantity and/or concentration of the second analyte in the sample;

such that the first analyte and the second analyte can be simultaneously detected and/or quantitated in the sample.

165. The method of claim 164, wherein the second anchor strand and the second capture reagent are positioned at a first location and a second location respectively on the support.

166. The method of claim 164, wherein the second anchor strand and the second capture reagent are attached to a second support.

167. The method of claim 166, wherein the second support is a microparticle such as a polystyrene bead.

168. The method of any one of claims 164 to 167, wherein the support is a microparticle such as a polystyrene bead.

169. The method of any one of claims 166 to 168, wherein the first support is a first bead encoded with a first barcode, and the second support is a second bead encoded with a second barcode, allowing identification of the respective beads when the respective barcodes are detected.

170. The method of any one of claims 166 to 169, wherein the first support and the second support are mixed together before being contacted with the sample.

171. The method of any one of claims 166 to 170, wherein the first support and the second support are contacted with the sample simultaneously.

172. The method of any one of claims 111 to 171, wherein the sample is a biological sample.

173. The method of any one of claims 111 to 172, wherein the sample is a bodily fluid, an extract, a solution containing proteins and/or DNA, a cell extract, a cell lysate, or a tissue lysate.

174. The method of claim 173, wherein the bodily fluid is urine, saliva, blood, serum, plasma, cerebrospinal fluid, tears, semen, or sweat.

175. A method for preparing a multiplex sandwich assay system, the method comprising:

(a) providing a first container comprising a first microparticle, the first microparticle being encoded with a first barcode;

(b) attaching the first microparticle to a first capture reagent and a first detection reagent, the first capture reagent and the first detection reagent being capable of binding to a first analyte at the same time;

(c) optionally, storing the first microparticle;

(d) providing a second container comprising a second microparticle, the second microparticle being encoded with a second barcode;

(e) attaching the second microparticle to a second capture reagent and a second detection reagent, the second capture reagent and the second detection reagent being capable of binding to a second analyte at the same time;

(f) optionally, storing the second microparticle; and

(g) mixing the first microparticle and the second microparticle together for use in the multiplex sandwich assay system;

wherein the first capture reagent and the first detection reagent are not mixed with the second capture reagent and the second detection reagent prior to attachment to their respective microparticle.

175a. The method of claim 175, wherein the first barcode and the second barcode are independently a spectral, graphical, or chemical barcode.

176. The method of claim 175 or 175a, wherein the respective capture reagent and the respective detection reagent are attached to their respective microparticle at the same time.

177. The method of claim 175 or 175a, wherein the respective capture reagent and the respective detection reagent are attached to their respective microparticle in a two-step reaction, where either the capture reagent or the detection reagent is attached to the microparticle first, followed by subsequent attachment of the other reagent.

178. The method of any one of claims 175 to 177, further comprising the step of washing the first microparticle and the second microparticle to remove unattached reagents, before mixing them together in step (g).

179. The method of any one of claims 175 to 178, wherein the first microparticle and the second microparticle is a polystyrene bead.

180. The method of any one of claims 175 to 179, wherein the first capture reagent, the first detection reagent, the second capture reagent, and the second detection reagent are antibodies.

181. The method of any one of claims 175 to 180, wherein the first detection reagent is linked to a first hook strand, the second detection reagent is linked to a second hook strand, and the first detection reagent and the second detection reagent are attached to their respective microparticles via linkage of their respective hook strands to a first anchor strand and a second anchor strand respectively, the first anchor strand and the second anchor strand having been attached to their respective microparticles beforehand.

182. The method of any one of claims 175 to 181, wherein the method is for preparing the multiplex sandwich assay system of any one of claims 80 to 110.

183. A method for preparing a multiplex sandwich assay system, the method comprising:

(a) providing a support, the support being a planar surface, the surface of a multi-well plate, the surface of a glass plate, or a hydrogel;

(b) attaching a first capture reagent to the support at a first position;

(c) washing the support to remove unattached first capture reagent;

(d) attaching a second capture reagent to the support at a second position;

(e) washing the support to remove unattached second capture reagent;

(f) attaching a first detection reagent to the support via a first anchor strand attached to the support at the first position;

(g) washing the support to remove unattached first detection reagent;

(h) attaching a second detection reagent to the support via a second anchor strand attached to the support at the second position; and

(i) washing the support to remove unattached second detection reagent;

such that whenever the first capture, second capture, first detection, and/or second detection reagents are mixed together, no more than one reagent at a time is not attached to the support.

184. The method of claim 183, wherein steps (b)-(c), (d)-(e), (f)-(g), and (h)-(i) can be carried out in any order.

185. The method of claim 183 or 184, wherein the first capture reagent, the first detection reagent, the second capture reagent, and the second detection reagent are antibodies.