CLAIMS

1. A method of analyzing a sample for an analyte, comprising:

a) combining:

i) a solid support comprising:

a capture moiety attached to the solid support, wherein the capture moiety specifically binds an analyte; and

a capture oligonucleotide attached to the solid support independently of the capture moiety, wherein the capture oligonucleotide comprises a 3' hybridizing region;

ii) a detection conjugate comprising:

a detection moiety that specifically binds the analyte; and

a detection oligonucleotide attached to the detection moiety, wherein the detection oligonucleotide comprises a 3' hybridizing region complementary to the 3' hybridizing region of the capture oligonucleotide; and

iii) a sample;

wherein, if the analyte is present in the sample, it is bound to the solid support by the capture moiety and to the detection conjugate by the detection moiety to form a capture complex in which the capture oligonucleotide and the detection oligonucleotide are in proximity;

b) permitting the 3' hybridizing region of the capture oligonucleotide and the 3' hybridizing region of the detection oligonucleotide that are in proximity to hybridize to each other;

c) extending the hybridized capture oligonucleotide and/or the hybridized detection oligonucleotide to generate an on-target extension product;

d) releasing the on-target extension product from the solid support; and

e) determining the presence or the absence of the released on-target extension product to determine the presence or the absence of the analyte in the sample.

2. The method of claim 1, wherein step a) comprises first combining the solid support and the sample such that the capture moiety of the solid support is bound to the analyte, if present in the sample, and then combining the detection conjugate.

3. The method of claim 2, further comprising removing unbound components of the sample after the capture moiety of the solid support is bound to the analyte, if present in the sample, and prior to combining the detection conjugate with the analyte-bound solid support, wherein removing unbound components of the sample comprises washing the analyte-bound solid support.

4. The method of claim 1, wherein the capture oligonucleotide comprises a first 5' tethering region and is attached to the solid support via hybridization to a first tether oligonucleotide attached to the solid support, and the detection oligonucleotide comprises a second 5' tethering region and is attached to the detection moiety via hybridization to a second tether oligonucleotide attached to the detection moiety.

5. The method of claim 4, wherein the hybridized capture oligonucleotide and the hybridized detection oligonucleotide are extended by a strand-displacing DNA polymerase, and the on-target extension product is released from the solid support by the strand-displacing DNA polymerase, and wherein the strand-displacing DNA polymerase comprises a Klenow fragment.

6. The method of claim 1, wherein the capture moiety is covalently attached to a first member of a binding pair that binds to a second member of the binding pair, wherein the second member is attached to the solid support, wherein the first member of the binding pair comprises biotin and the second member of the binding pair comprises streptavidin.

7. The method of claim 4, wherein the first tether oligonucleotide is covalently attached to a first member of a binding pair that binds to a second member of the binding pair, wherein the second member is attached to the solid support, wherein the first member of the binding pair comprises biotin and the second member of the binding pair comprises streptavidin.

8. The method of claim 1, wherein the capture oligonucleotide comprises a first barcode sequence that identifies a binding target of the capture moiety, and wherein the detection oligonucleotide comprises a second barcode sequence that identifies a binding target of the detection moiety.

9. The method of claim 1, wherein the capture oligonucleotide and detection oligonucleotide each comprise, from 5' to 3': a tethering region, a primer binding region configured to bind a primer for amplifying the released on-target extension product, a barcode sequence, and the 3' hybridizing region.

10. The method of claim 1, wherein the solid support is a magnetically responsive bead.
11. The method of claim 1, wherein the capture moiety and the detection moiety are independently an antibody or an antibody fragment.
12. The method of claim 1, wherein the combining in step a) further comprises combining one or more blocker oligonucleotides, wherein each blocker oligonucleotide specifically hybridizes to a subpart of one or both of the capture oligonucleotide and/or the detection oligonucleotide, wherein the subpart to which a first blocker oligonucleotide of the one or more blocker oligonucleotides hybridizes to the 3' hybridizing region or a portion thereof of the capture oligonucleotide or the detection oligonucleotide, and wherein the first blocker oligonucleotide competes with: the 3' hybridizing region of the detection oligonucleotide for binding to the 3' hybridizing region of the capture oligonucleotide; or the 3' hybridizing region of the capture oligonucleotide for binding to the 3' hybridizing region of the detection oligonucleotide.
13. The method of claim 12, comprising removing the first blocker oligonucleotide after the combining in step a) and before the extending in step c).
14. The method of claim 1, wherein the capture and/or detection oligonucleotide(s) comprises a unique molecular identifier (UMI) at least 4 nucleotides long between the 5' tethering region and the 3' hybridizing region.
15. The method of claim 1, wherein determining the presence or the absence of the released on-target extension product comprises performing qPCR, or sequencing the released on-target extension product.
16. The method of claim 1, comprising providing a plurality of paired combinations of the solid support and the detection conjugate, wherein a binding target of the capture moiety and the detection moiety of each paired combination is the same, and wherein different paired combinations of the plurality of paired combinations have different binding targets.
17. The method of claim 16, wherein the different binding targets are different analytes, or different epitopes on the same analyte.
18. The method of claim 16, wherein the 3' hybridizing region of the capture oligonucleotide and detection oligonucleotide of a first paired combination of the plurality of paired combinations is not complementary to the 3' hybridizing region of the detection

oligonucleotide and capture oligonucleotide, respectively, of at least one other paired combination of the plurality of paired combinations.

19. The method of claim 16, wherein each capture and/or detection oligonucleotide(s) attached to a solid support of the plurality of the solid supports comprises a barcode sequence that identifies a binding target of the capture moiety attached to the respective solid support.

20. The method of claim 16, wherein the sample is divided into a plurality of subpools comprising at least a first subpool and a second subpool.

21. A method of analyzing a sample for an analyte, comprising:

a) combining:

i) a first conjugate comprising:

a first moiety that specifically binds an analyte;

a first tether region attached to the first moiety; and

a first splint oligonucleotide comprising a 5' tethering region, a first moiety barcode, and a 3' hybridizing region, wherein the 5' tethering region of the first splint oligonucleotide is hybridized to the first tether region attached to the first moiety;

ii) a second conjugate comprising:

a second moiety that specifically binds the analyte;

a second tether region attached to the second moiety; and

a second splint oligonucleotide comprising a 5' tethering region, a second moiety barcode, and a 3' hybridizing region, wherein the 5' tethering region of the second splint oligonucleotide is hybridized to the second tether region attached to the second moiety, and wherein the 3' hybridizing region of the second splint oligonucleotide complementary to the 3' hybridizing region of the first splint oligonucleotide; and

iii) a sample;

d) permitting the 3' hybridizing region of the first splint oligonucleotide and the 3' hybridizing region of the second splint oligonucleotide that are in proximity to hybridize to each other if the analyte is present in the sample;

e) extending the hybridized first splint oligonucleotide and the hybridized second splint oligonucleotide using a strand-displacing DNA polymerase to generate an on-target

extension product that comprises the extended first splint oligonucleotide and/or the extended second splint oligonucleotide and release by strand displacement the on-target extension product from the first moiety and the second moiety; and

f) determining the presence or the absence of the on-target extension product to thereby determine the presence or the absence of the analyte in the sample.

22. The method of claim 21, wherein the first moiety and the second moiety specifically bind different epitopes on the same analyte.

23. The method of claim 21, wherein the first moiety specifically binds with a first analyte and the second moiety specifically binds with a second analyte, wherein the first analyte and the second analyte interact, wherein the first analyte is an enzyme and the second analyte is a substrate for the enzyme.

24. The method of claim 21, wherein the combining in a) comprises combining the first conjugate and the sample, thereby allowing the first moiety of the first conjugate to be bound to the analyte if present in the sample, and then combining the second conjugate with the first conjugate and the sample.

25. The method of claim 21, comprising providing: a plurality of paired combinations of the first conjugates and the second conjugates, wherein the analyte bound by the first moiety and second moiety of each paired combination is the same, and wherein different paired combinations of the plurality of paired combinations bind different analytes.

26. A method of determining a pairwise combination of binding moieties that can simultaneously bind to a binding target, the method comprising:

a) combining

i) a plurality of different populations of solid supports, each solid support comprising:

a first binding moiety attached to the solid support; and

a capture oligonucleotide independently attached to the solid support, wherein the capture oligonucleotide comprises a 3’ hybridizing region and a capture barcode region, wherein the 3’ hybridizing regions of the capture oligonucleotides are the same in the plurality of different populations of solid supports, and wherein the capture barcode regions of the capture oligonucleotides are different for each different population of solid supports

and the capture barcode region identifies the binding moiety attached to the same solid support, and;

ii) a plurality of different populations of detection conjugates comprising:

a second binding moiety; and

a detection oligonucleotide attached to the second binding moiety, wherein the detection oligonucleotide comprises a 3' hybridizing region complementary to the 3' hybridizing region of the capture oligonucleotide and a detector barcode region, wherein the detector barcode regions of the detection oligonucleotide are different for each different population of detection conjugates and identifies the second binding moiety to which it is attached; and

iii) one or more binding targets;

wherein, if a binding target is present in the sample, and the binding target is specifically bound by the first binding moiety and the second binding moiety, a capture complex is formed in which the capture oligonucleotide and the detection oligonucleotide are in proximity;

b) permitting the 3' hybridizing region of the capture oligonucleotide and the 3' hybridizing region of the detection oligonucleotide to hybridize to each other;

e) extending at least one of the hybridized capture oligonucleotide and the hybridized detection oligonucleotide to generate an extension product that comprises i) an extended capture oligonucleotide and a complement of at least a part of the detection oligonucleotide, and/or ii) an extended detection oligonucleotide and a complement of at least a part of the capture oligonucleotide;

f) releasing the extension product from the solid support; and

g) determining the capture barcode region and the detector barcode region of the released extension product to thereby determine a combination of binding moieties that can simultaneously bind to the binding target.

27. A method of determining interaction between two moieties, the method comprising:

a) combining a plurality of different populations of moieties, each moiety comprising a splint oligonucleotide attached to the moiety, wherein the splint oligonucleotide comprises a 3' hybridizing region and a barcode region, wherein the 3' hybridizing

regions of the splint oligonucleotides are the same in the plurality of different populations of moieties, and wherein the barcode regions of the splint oligonucleotides are different for each different population of moieties and the barcode region identifies the moiety, and wherein, if a first moiety of the plurality of different populations of moieties interacts with a second moiety of the plurality of different populations of moieties, a capture complex is formed in which the splint oligonucleotide of the first moiety and the splint oligonucleotide of the second moiety are in proximity;

b) permitting the 3' hybridizing region of the splint oligonucleotide of the first moiety and the 3' hybridizing region of the splint oligonucleotide of the second moiety to hybridize to each other;

e) extending at least one of the splint oligonucleotide of the first moiety and the splint oligonucleotide of the second moiety to generate an extension product that comprises i) an extended splint oligonucleotide of the first moiety and a complement of at least a part of the splint oligonucleotide of the second moiety, and/or ii) an extended splint oligonucleotide of the second moiety and a complement of at least a part of the splint oligonucleotide of the first moiety;

f) releasing the extension product from the solid support; and

g) determining the barcode region of the splint oligonucleotide of the first moiety and the barcode region of the splint oligonucleotide of the second moiety of the released extension product to thereby determine an interaction between two moieties.

28. The method of claim 27, wherein the first moiety and/or the second moiety is/are protein(s), enzyme(s), substrate(s), and/or aptamer(s).

29. A composition comprising at least 30 different populations of solid supports, each solid support comprising:

i) a capture moiety attached to the solid support, wherein the capture moiety specifically binds an analyte;

ii) a tether oligonucleotide attached to the solid support independent of the capture moiety; and

iii) a capture oligonucleotide comprising from 5' to 3': a tethering region, a capture barcode region, and a 3' hybridizing region, wherein the capture oligonucleotide is attached

to the solid support via hybridization of the tethering region to the tether oligonucleotide attached to the solid support.

30. The composition of claim 29, further comprising at least 30 different populations of detection conjugates, each detection conjugate comprising:

i) a detection moiety that specifically binds an analyte;

ii) a tether oligonucleotide attached to the detection moiety; and

ii) a detection oligonucleotide comprising from 5' to 3': a tethering region, a detection barcode region, and a 3' hybridizing region, wherein the detection oligonucleotide is attached to the detection moiety via hybridization of the tethering region to the tether oligonucleotide attached to the detection moiety,

wherein each of the at least 30 different populations of solid supports forms a paired combination with a corresponding detection conjugate of the at least 30 different populations of detection conjugates, wherein the analyte bound by the capture moiety and detection moiety of each paired combination is the same, wherein the analytes bound by different paired combinations are different, wherein the 3' hybridizing region of the capture oligonucleotide and detection oligonucleotide of each paired combination is not complementary to the 3' hybridizing region of the detection oligonucleotide and the capture oligonucleotide, respectively, of any other paired combination, and wherein each paired combination can be distinguished from any other paired combination by one or both of the sequence of the capture barcode region and/or the sequence of the detection barcode region.

31. The composition of claim 30, further comprising at least 30 different populations of blocker oligonucleotides, wherein each of the different populations of blocker oligonucleotides specifically hybridizes to the 3' hybridizing region or a portion thereof of the capture oligonucleotide or the detection oligonucleotide of each paired combination.

32. The composition of claim 30, wherein the solid support is a magnetically responsive bead.

33. The composition of claim 30, wherein the capture moiety and the detection moiety are independently an antibody or an antibody fragment.

34. The composition of claim 30, further comprising a strand-displacing DNA polymerase.