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DESCRIPTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/558,537 filed on November 11, 2011.

FIELD OF THE INVENTION

[0002] The present invention is directed to methods for conducting immunoassays. The methods are particularly well suited for reducing cross-reactivity between species employed in immunoassays, e.g., antibodies and antigens.

BACKGROUND OF THE INVENTION

[0003] A substantial body of literature has been developed concerning techniques that employ binding reactions, e.g., antigen-antibody reactions, nucleic acid hybridization and receptor-ligand reactions, for the sensitive measurement of analytes of interest in samples. The high degree of specificity in many biochemical binding systems has led to many assay methods and systems of value in a variety of markets including basic research, human and veterinary diagnostics, environmental monitoring and industrial testing. The presence of an analyte of interest may be measured by directly measuring the participation of the analyte in a binding reaction. In some approaches, this participation may be indicated through the measurement of an observable label attached to one or more of the binding materials.

[0004] One of the challenges with immunoassay measurements is the potential for cross-reactivity among antibodies and analytes. Cross-reactivity can lead to higher backgrounds, reduced sensitivity, and influence the detection of one analyte in a plurality. In a multiplexed immunoassay with N capture antibodies and N detection antibodies, there are NxN potential antibody interactions (including non-specific binding interactions). Therefore, in a 25-plex assay, there are 625 possible interactions. While antibodies are typically highly specific for a particular analyte, undesired interactions are common and have to be screened out when formulating a multiplexed assay panel. Finding antibodies that work well in a multiplexed format becomes increasingly challenging as the degree of multiplexing is increased.

[0005] WO 2010059820 (A1) relates to a method for detecting a target comprising a repeating epitope.
US2010261292 (A1) relates to methods for conducting solid-phase binding assays. One example is an assay method having improved analyte specificity where specificity is limited by the presence of non-specific binding interactions.

[0006] Heyduk et al., in Anal. Chem., 2008, 80 (13), pp 5152-5159 ("Molecular Pincers: Antibody-Based Homogeneous Protein Sensors"), describe a homogeneous antibody-based protein sensor design (molecular pincers) that allows rapid and sensitive detection of a specific protein in solution.

[0007] US2004121382 (A1) concerns methods and compositions which are provided for detection of analytes, such as cell surface moieties, preferably in multiplexed assays, such that multiple analytes can be assayed simultaneously.
WO2009067009 (A1) relates to the detection of among others tumor-specific fusion proteins and protein interactions.

[0008] In a multiplexed sandwich immunoassay format, the analyte specificity is provided by the capture antibody. As shown in Fig. 1(a), if the capture antibody (bound to a surface) and labeled detection antibody each bind to analyte A, A is detected in the assay. However, as shown in Figs. 1(b)-(d), undesired interactions between capture and detection antibodies (1(b)), capture antibody and analyte B (1(c)), or capture and detection antibodies and an additional antibody present in the sample (1(d)) can result. If a capture antibody cross-reacts with another species in the sample, e.g., as shown in Fig. 1(b)-(d), the resulting signal can be misinterpreted as the presence of the target analyte, yielding a false positive result.

[0009] In addition, complex matrices, such as human serum/plasma and cell lysates, may contain molecules or molecular complexes that will crosslink capture antibodies to detection antibodies. This is particularly problematic as the effect is unpredictable (unlike direct antibody-antibody cross reactivity), and it can lead to falsely elevated measurements of a particular analyte. One example of a class of matrix-mediated cross-reactivity is human anti-mouse antibodies (knows as a HAMA effect), or other human anti-animal antibodies. When anti-mouse antibodies are present in human serum, they can bind to mouse-derived antibodies that are typically used in immunoassays, and potentially form a bridge between capture and detection antibodies, falsely mimicking the presence of an analyte. While this problem is relevant in single analyte immunoassays, it is exacerbated by a factor of N2 in multiplexed immunoassays where N detection antibodies can be bridged to N capture antibodies.

SUMMARY OF THE INVENTION

[0010] Accordingly, the present invention provides, according to its first embodiment, a method of conducting a binding assay comprising:

1. (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to saidanalyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence and (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second

binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said first oligonucleotide sequence hybridizes to said second oligonucleotide sequence, wherein said first and second binding reagents are brought into proximity to one another via said first and second linking agents, respectively, bound to each of the binding reagents, wherein a binding energy between the first oligonucleotide sequence and the second oligonucleotide sequence is below a level to stably attach the first oligonucleotide sequence and the second oligonucleotide sequence to each other in the absence of simultaneous binding of the first and second binding regions to the analyte; and

2. (b) measuring the amount of said analyte bound to said solid support.

The first embodiment is set out in claim 1 as herewith attached. The present invention also provides, according to its second embodiment, a kit for the conduct of a binding assay comprising:

1. (a) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; and

   (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence, wherein a binding energy between the first oligonucleotide sequence and the second oligonucleotide sequence is below a level to stably attach the first oligonucleotide sequence and the second oligonucleotide sequence to each other in the absence of simultaneous binding of the first and second binding regions to the analyte. The second embodiment is set out in claim 3 as herewith attached.

Preferred variants of the aforementioned first and second embodiments are set out in the dependent claims as attached.

ADDITIONAL ASPECTS OF THE INSTANT DISCLOSURE

[0011] The instant disclosure also provides a method of conducting a binding assay comprising: (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence and (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said first oligonucleotide sequence hybridizes to said second oligonucleotide sequence; and (b) measuring the amount of said analyte bound to said solid support.

[0012] In an alternative aspect, the instant disclosure further provides a method of conducting a binding assay comprising: (a) contacting a sample comprising an analyte of interest with a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; (b) contacting the mixture formed in step (a) with a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence; wherein said contacting step (b) is conducted under conditions wherein said first and second binding regions bind to said analyte and said first oligonucleotide sequence hybridizes to said second oligonucleotide sequence; and (b) measuring the amount of said analyte bound to said solid support.

[0013] In one aspect, the instant disclsosure includes a method of conducting a binding assay comprising: (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and (iii) a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said bridging oligonucleotide sequence hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and (b) measuring the amount of said analyte bound to said solid support.

[0014] Still further, the instant disclosure includes a method of conducting a binding assay comprising: (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; and (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said contacting step (a) is conducted under conditions wherein said first and second binding regions bind to said analyte; (b) contacting the mixture formed in step (b) with a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step (c) is conducted under conditions wherein said bridging oligonucleotide sequence hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and (d) measuring the amount of said analyte bound to said solid support.

[0015] Moreover, the instant disclosure provides a method of conducting a binding assay comprising: (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence, wherein said contacting step (a) is conducted under conditions wherein said first binding region that binds to said analyte; (b) contacting the mixture formed in step (a) with a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said contacting step (b) is conducted under conditions wherein said second binding region that binds to said analyte; (c) contacting the mixture formed in step (b) with a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step (c) is conducted under conditions wherein said bridging oligonucleotide sequence hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and (d) measuring the amount of said analyte bound to said solid support.

[0016] Also contemplated by the instant disclosure is a method of conducting a binding assay for a plurality of analytes comprising: (a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to one or more of said analytes and each of said one or more first binding reagents are linked to one or more first linking agents each comprising a first oligonucleotide sequence; and (ii) one or more second binding reagents each comprising a second binding region that binds to one or more of said analytes and each of said one or more second binding reagents are linked to one or more second linking agents each comprising a second oligonucleotide sequence, wherein said second oligonucleotides sequence comprises a sequence complementary to at least a portion of said first oligonucleotides sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions that binds to said analytes and said first oligonucleotides sequence hybridizes to said second oligonucleotides sequence; and (c) measuring the amount of said analytes bound to said solid support.

[0017] The instant disclosure further provides a method of conducting a binding assay for a plurality of analytes comprising: (a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to one or more of said analytes and each of said one or more first binding reagents are linked to one or more first linking agents each comprising a first oligonucleotide sequence; (b) contacting the mixture formed in step (a) with one or more second binding reagents each comprising a second binding region that binds to one or more of said analytes, wherein each of said one or more second binding reagents are linked to one or more a second linking agents each comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence; wherein said contacting step (b) is conducted under conditions wherein said first and second binding regions bind to said analyte and said first oligonucleotide sequence hybridizes to said second oligonucleotide sequence; and (c) measuring the amount of said analyte bound to said solid support.

[0018] Alternatively, the methods of the instant disclosure include (a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to said analytes and each of said one or more first binding reagents are linked to a first linking agent comprising a first oligonucleotide sequence; (ii) one or more second binding reagents each comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and (iii) one or more bridging oligonucleotide sequences each comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analytes and said bridging oligonucleotide sequences hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and (b) measuring the amount of said analytes bound to said solid support.

[0019] Also provided by the instant disclosure is a method of conducting a binding assay for a plurality of analytes comprising: (a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to said analytes, wherein each of said first binding reagents are linked to a first linking agent comprising a first oligonucleotide sequence; and (ii) one or more second binding reagents each comprising a second binding region that binds to said analyte of interest, wherein said each of said one or more second binding reagents is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said contacting step (a) is conducted under conditions wherein said first and second binding regions bind to said analytes; (b) contacting the mixture formed in step (b) with one or more bridging oligonucleotide sequences each comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step (c) is conducted under conditions wherein said bridging oligonucleotide sequences hybridize to said first oligonucleotide sequence and said second oligonucleotide sequence; and (c) measuring the amount of said analytes bound to said solid support.

[0020] Further included by the instant disclosure is a method of conducting a binding assay for a plurality of analytes comprising: (a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to said analytes, wherein each of said first binding reagents is linked to a first linking agent comprising a first oligonucleotide sequence, wherein said contacting step (a) is conducted under conditions wherein said first binding region that binds to said analytes; (b) contacting the mixture formed in step (a) with one or more second binding reagents each comprising a second binding region that binds to said analytes, wherein said each of said one or more second binding reagents is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said contacting step (b) is conducted under conditions wherein said second binding region binds to said analytes; (c) contacting the mixture formed in step (b) with one or more bridging oligonucleotide sequences comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step (c) is conducted under conditions wherein said one or more bridging oligonucleotide sequences hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and (d) measuring the amount of said analytes bound to said solid support.

[0021] The present disclosure also contemplates kits for the conduct of a binding assay comprising: (a) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; and (b) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence.

[0022] Further, the kits of the instant disclosure may also comprise a solid surface including

1. (i) a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence;
2. (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and
3. (iii) a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence.

[0023] Moreover, the kits of the instant disclosure are configured to conduct a binding assay for a plurality of analytes and comprise (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to one or more of said analytes and each of said one or more first binding reagents are linked to one or more first linking agents each comprising a first oligonucleotide sequence; and (ii) one or more second binding reagents each comprising a second binding region that binds to one or more of said analytes and each of said one or more second binding reagents are linked to one or more second linking agents each comprising a second oligonucleotide sequence, wherein said second oligonucleotides sequence comprises a sequence complementary to at least a portion of said first oligonucleotides sequence.

[0024] Another aspect of a kit configured to conduct a binding assay for a plurality of analytes according to the instant disclosure includes (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to said analytes and each of said one or more first binding reagents are linked to a first linking agent comprising a first oligonucleotide sequence;

(ii) one or more second binding reagents each comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and

(iii) one or more bridging oligonucleotide sequences each comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence.

BRIEF DESCRIPTION OF DRAWINGS

[0025]

Figs. 1(a)-(d) show various interactions between a capture antibody bound to a solid support and one or more species that may be present in a biological sample that may lead to undesired binding interactions and high background signals in an immunoassay. Fig. 1(a) shows a desired binding interaction between a capture antibody bound to a surface, bound to analyte A, which is bound to a labeled detection antibody. Figs. 1(b)-(d) show undesired binding interactions. Fig. 1(b) shows a binding event between a capture antibody and a labeled detection antibody, Fig. 1(c) shows a binding event between a capture antibody and an extraneous analyte B, that may be present in a sample, and Fig. 1(d0 shows a binding event between a capture antibody and an additional antibody that may be present in the sample.

Figs. 2(a)-(c) show the use of a bridging oligonucleotides sequence to facilitate a binding interaction in an immunoassay. Fig. 2(a) shows a bridge between a capture antibody, an analyte, A, and a detection antibody, wherein the bridging oligonucleotide includes a detectable label. Fig. 2(b) shows a bridge between a capture antibody, and an additional analyte in a sample, B, and Fig. 2(c) shows a binding interaction between a capture antibody, analyte B, and a detection antibody in the absence of a bridging oligonucleotide sequence.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0027] The present invention improves the specificity of immunoassays by introducing a linking reagent that binds to a first binding reagent, e.g., a capture antibody, if the correct second binding reagent, e.g., a detection antibody, is present. Therefore, according to its first embodiment, the present invention provides a method of conducting a binding assay comprising:

1. (a) contacting a sample comprising an analvte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence and (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said first oligonucleotide sequence hybridizes to said second oligonucleotide sequence, wherein said first and second binding reagents are brought into proximity to one another via said first and second linking agents, respectively, bound to each of the binding reagents, wherein a binding energy between the first oligonucleotide sequence and the second oligonucleotide sequence is below a level to stably attach the first oligonucleotide sequence and the second oligonucleotide sequence to each other in the absence of simultaneous binding of the first and second binding regions to the analyte; and
2. (b) measuring the amount of said analyte bound to said solid support.

[0028] Moreover, the instant disclosure teaches a method of conducting a binding assay comprising contacting a sample including an analyte of interest with

1. (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to the analyte of interest, wherein the first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence, and
2. (ii) a second binding reagent comprising a second binding region that binds to the

analyte of interest, wherein the second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein the second oligonucleotide sequence comprises a sequence complementary to at least a portion of the first oligonucleotide sequence. The contacting step is preferably conducted under conditions wherein the first and second binding regions bind to the analyte and the first oligonucleotide sequence hybridizes to the second oligonucleotide sequence. Therefore, in this aspect of the instant disclosure, the first and second binding reagents are brought into proximity to one another via a first and second linking agent, respectively, bound to each of the binding reagents, wherein the linking agents include complementary oligonucleotides sequences.

[0029] In an alternative aspect of the instant disclosure, the first and second binding reagents are brought into proximity via a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of the first linking agent and the second linking agent. Therefore, the instant disclosure provides a method of conducting a binding assay comprising

(a) contacting a sample comprising an analyte of interest with

1. (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence;
2. (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and
3. (iii) a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said bridging oligonucleotide sequence hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence;

and (b) measuring the amount of said analyte bound to said solid support.

[0030] In each aspect of the instant disclosure, the linking and/or bridging oligonucleotide sequences are designed so that the binding interactions, i.e., between the first and second binding reagents and the linking or bridging sequence, are required for a stable attachment. Therefore, in the first aspect of the instant disclosure in which the first and second binding reagents are held in proximity via an interaction between the first and second linking agents, a stable attachment forms when the first and second binding reagents bind to one another and the first linking agent binds to the complementary sequence of the second linking agent. If a bridging oligonucleotide sequence is employed, a stable attachment forms when the first and second binding reagents bind to one another and the bridging oligonucleotide sequence binds to its complement on the first and second linking agents.

[0031] In a preferred aspect of the instant disclosure, the complementary sequence employed in the linking agent and/or bridging sequence is designed to have a relatively low binding energy such that a single interaction is insufficient to stably attach the linking agent to either of the binding reagents alone, but the combined avidity of simultaneous binding to the binding reagents is designed to be sufficient to stably bind the linking and/or bridging sequence. DNA oligonucleotides offer a conveniently adjustable and specific choice for the linking and/or bridging sequences. The binding energy can be adjusted by the length of the binding sequence, the temperature, and the salt concentration. Oligonucleotides also offer a convenient flexible tether between binding reagents and the length may be selected so that the linker or bridging sequence can bind the first and second binding reagents, e.g., capture and detection antibodies, as shown in Figure 2.

[0032] The bridging/linking oligonucleotide sequence should be selected to be unique and as orthogonal as possible to other sequences in order to minimize unintended binding events. In one aspect of the instant disclosure, the bridging/linking oligonucleotide sequence is about 5-20 bases in length, preferably 8-15 bases in length, more preferably about 8-12 bases in length and most preferably about 4-8 bases in length. The sequence and length will depend on the intended incubation temperature, salt concentration, and G/C content. In a preferred aspect of the instant disclosure, the G/C content is between about 40-60%. The binding energy of individual pairings should be selected to be weak enough so that single oligonucleotides pairings is not stable on its own. Moreover, the linking/bridging sequence should be sufficiently long to allow for linkage/bridging between the first and second binding reagents. The oligonucleotide sequence may comprise a DNA sequence, e.g., poly-A or poly-T, or it could include additional moieties, e.g., polymer units such as ethylene glycol. In addition, the oligonucleotide sequences employed in the linking/bridging moieties need not be identical in length and in certain aspects of the instant disclosure it may be beneficial to provide one oligonucleotide sequence that is longer than its binding partner, e.g., by up to 25 bases, or up to 15 bases, or up to 10 bases.

[0033] The methods of the present disclosure may involve one or more steps. For example, the binding assay may comprise:

1. (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence and (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said first oligonucleotide sequence hybridizes to said

second oligonucleotide sequence; and

2. (b) measuring the amount of said analyte bound to said solid support.

[0034] Alternatively, the method of the instant disclosure may involve a bridging oligonucleotide sequence, as follows:

1. (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and (iii) a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analyte and said bridging oligonucleotide sequence hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and
2. (b) measuring the amount of said analyte bound to said solid support.

[0035] Still further, if a bridging oligonucleotides sequence is employed, the binding assay according to the instant disclosure may involve a number of discrete steps, as follows:

1. (a) contacting a sample comprising an analyte of interest with (i) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence, wherein said contacting step (a) is conducted under conditions wherein said first binding region binds to said analyte;
2. (b) contacting the mixture formed in step (a) with a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said contacting step (b) is conducted under conditions wherein said second binding region binds to said analyte;
3. (c) contacting the mixture formed in step (b) with a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step (c) is conducted under conditions wherein said bridging oligonucleotide sequence hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and
4. (d) measuring the amount of said analyte bound to said solid support.

[0036] Still further, the method of the present disclosure may be applied to singleplex or multiplex formats where multiple assay measurements are performed on a single sample. Multiplex measurements may comprise the acts of contacting at least a portion of a sample with one or more binding surfaces comprising a plurality of binding domains, immobilizing one or more analytes on the domains and measuring the analytes immobilized on the domains. In certain aspects of the instant disclosure, at least two of the binding domains differ in their specificity for analytes of interest. In one example of such an aspect, the binding domains are prepared by immobilizing, on one or more surfaces, discrete domains of binding reagents that bind analytes of interest. Optionally, the sample is exposed to a binding surface that comprises an array of binding reagents. Optionally, the surface(s) may define, in part, one or more boundaries of a container (e.g., a flow cell, well, cuvette, etc.) which holds the sample or through which the sample is passed. The method may also comprise generating assay signals that are indicative of the amount of the analytes in the different binding domains, e.g., changes in optical absorbance, changes in fluorescence, the generation of chemiluminescence or electrochemiluminescence, changes in reflectivity, refractive index or light scattering, the accumulation or release of detectable labels from the domains, oxidation or reduction or redox species, electrical currents or potentials, changes in magnetic fields, etc.

[0037] In a preferred aspect of the instant disclosure, a multiplexed assay method comprises:

(a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to one or more of said analytes and each of said one or more first binding reagents are linked to one or more first linking agents each comprising a first oligonucleotide sequence; and (ii) one or more second binding reagents each comprising a second binding region that binds to one or more of said analytes and each of said one or more second binding reagents are linked to one or more second linking agents each comprising a second oligonucleotide sequence, wherein said second oligonucleotides sequence comprises a sequence complementary to at least a portion of said first oligonucleotides sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analytes and said first oligonucleotides sequence hybridizes to said second oligonucleotides sequence; and

(c) measuring the amount of said analytes bound to said solid support.

[0038] Alternatively, a multiplexed assay method according to the instant disclosure may involve a bridging oligonucleotide sequence, as follows:

1. (a) contacting a sample comprising a plurality of analytes with (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to said analytes and each of said one or more first binding reagents are linked to a first linking agent comprising a first oligonucleotide sequence; (ii) one or more second binding

reagents each comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and (iii) one or more bridging oligonucleotide sequences each comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence; wherein said contacting step is conducted under conditions wherein said first and second binding regions bind to said analytes and said bridging oligonucleotide sequences hybridizes to said first oligonucleotide sequence and said second oligonucleotide sequence; and

2. (b) measuring the amount of said analytes bound to said solid support.

[0039] Moreover, the binding assay may include one or more washing steps. For example, once the sample and the first binding reagent are combined, the solid support may be washed prior to contacting the mixture with the second binding reagent and/or prior to introducing a bridging oligonucleotide.

[0040] According to its second embodiment, the invention also contemplates a kit for the conduct of a binding assay comprising:

1. (a) a solid surface including a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence; and

   (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, wherein said second oligonucleotide sequence comprises a sequence complementary to at least a portion of said first oligonucleotide sequence, wherein a binding energy between the first oligonucleotide sequence and the second oligonucleotide sequence is below a level to stably attach the first oligonucleotide sequence and the second oligonucleotide sequence to each other in the absence of simultaneous binding of the first and second binding regions to the analyte.

[0041] Alternatively, the kit of the present disclosure may comprise a solid surface including

1. (i) a first binding reagent immobilized thereto comprising a first binding region that binds to said analyte of interest, wherein said first binding reagent is linked to a first linking agent comprising a first oligonucleotide sequence;
2. (ii) a second binding reagent comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and
3. (iii) a bridging oligonucleotide sequence comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide

sequence.

[0042] Still further, the instant disclosure provides a kit for a multiplexed assay measurement for a plurality of analytes comprising

1. (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to one or more of said analytes and each of said one or more first binding reagents are linked to one or more first linking agents each comprising a first oligonucleotide sequence; and
2. (ii) one or more second binding reagents each comprising a second binding region that binds to one or more of said analytes and each of said one or more second binding reagents are linked to one or more second linking agents each comprising a second oligonucleotide sequence, wherein said second oligonucleotides sequence comprises a sequence complementary to at least a portion of said first oligonucleotides sequence.

[0043] Alternatively, the kit according to the instant disclosure may employ a bridging oligonucleotide to facilitate a multiplexed assay comprising:

1. (i) a solid surface including one or more first binding reagents immobilized thereto, wherein said one or more first binding reagents each comprise a first binding region that binds to said analytes and each of said one or more first binding reagents are linked to a first linking agent comprising a first oligonucleotide sequence;
2. (ii) one or more second binding reagents each comprising a second binding region that binds to said analyte of interest, wherein said second binding reagent is linked to a second linking agent comprising a second oligonucleotide sequence, and
3. (iii) one or more bridging oligonucleotide sequences each comprising a sequence complementary to at least a portion of said first oligonucleotide sequence and said second oligonucleotide sequence.

[0044] Examples of samples that may be analyzed by the methods of the present invention include, but are not limited to food samples (including food extracts, food homogenates, beverages, etc.), environmental samples (e.g., soil samples, environmental sludges, collected environmental aerosols, environmental wipes, water filtrates, etc.), industrial samples (e.g., starting materials, products or intermediates from an industrial production process), human clinical samples, veterinary samples and other samples of biological origin. Biological samples that may be analyzed include, but are not limited to, feces, mucosal swabs, physiological fluids and/or samples containing suspensions of cells. Specific examples of biological samples include blood, serum, plasma, feces, mucosal swabs, tissue aspirates, tissue homogenates, cell cultures and cell culture supernatants (including cultures of eukaryotic and prokaryotic cells), urine, saliva, sputum, and cerebrospinal fluid.

[0045] Analytes that may be measured using the methods of the invention include, but are not limited to proteins, toxins, nucleic acids, microorganisms, viruses, cells, fungi, spores, carbohydrates, lipids, glycoproteins, lipoproteins, polysaccharides, drugs, hormones, steroids, nutrients, metabolites and any modified derivative of the above molecules, or any complex comprising one or more of the above molecules or combinations thereof. The level of an analyte of interest in a sample may be indicative of a disease or disease condition or it may simply indicate whether the patient was exposed to that analyte.

[0046] The assays of the present invention may be used to determine the concentration of one or more, e.g., two or more analytes in a sample. Thus, two or more analytes may be measured in the same sample. Panels of analytes that can be measured in the same sample include, for example, panels of assays for analytes or activities associated with a disease state or physiological conditions. Certain such panels include panels of cytokines and/or their receptors (e.g., one or more of TNF-alpha, TNF-beta, ILl-alpha, ILl-beta, IL2, IL4, IL6, IL-10, IL-12, IFN-y, etc.), growth factors and/or their receptors (e.g., one or more of EGF, VGF, TGF, VEGF, etc.), drugs of abuse, therapeutic drugs, vitamins, pathogen specific antibodies, auto-antibodies (e.g., one or more antibodies directed against the Sm, RNP, SS-A, SS-alpha, J0-1, and Scl-70 antigens), allergen-specific antibodies, tumor markers (e.g., one or more of CEA, PSA, CA-125 II, CA 15-3, CA 19-9, CA 72-4, CYFRA 21-1, NSE, AFP, etc.), markers of cardiac disease including congestive heart disease and/or acute myocardial infarction (e.g., one or more of Troponin T, Troponin I, myoglobin, CKMB, myeloperoxidase, glutathione peroxidase, β-natriuretic protein (BNP), alpha-natriuretic protein (ANP), endothelin, aldosterone, C-reactive protein (CRP), etc.), markers associated with hemostasis (e.g., one or more of Fibrin monomer, D-dimer, thrombin-antithrombin complex, prothrombin fragments 1 & 2, anti-Factor Xa, etc.), markers of acute viral hepatitis infection (e.g., one or more of IgM antibody to hepatitis A virus, IgM antibody to hepatitis B core antigen, hepatitis B surface antigen, antibody to hepatitis C virus, etc.), markers of Alzheimers Disease (alpha-amyloid, beta-amyloid, Aβ 42, Aβ 40, Aβ 38, Aβ 39, Aβ 37, Aβ 34, tau-protein, etc.), markers of osteoporosis (e.g., one or more of cross-linked Nor C-telopeptides, total deoxypyridinoline, free deoxypyridinoline, osteocalcin, alkaline phosphatase, C-terminal propeptide of type I collagen, bone-specific alkaline phosphatase, etc.), markers of fertility state or fertility associated disorders (e.g., one or more of Estradiol, progesterone, follicle stimulating hormone (FSH), lutenizing hormone (LH), prolactin, hCG, testosterone, etc.), markers of thyroid disorders (e.g., one or more of thyroid stimulating hormone (TSH), Total T3, Free T3, Total T4, Free T4, and reverse T3), and markers of prostrate cancer (e.g., one or more of total PSA, free PSA, complexed PSA, prostatic acid phosphatase, creatine kinase, etc.). Certain embodiments of invention include measuring, e.g., one or more, two or more, four or more or 10 or more analytes associated with a specific disease state or physiological condition (e.g., analytes grouped together in a panel, such as those listed above; e.g., a panel useful for the diagnosis of thyroid disorders may include e.g., one or more of thyroid stimulating hormone (TSH), Total T3, Free T3, Total T4, Free T4, and reverse T3).

[0047] The methods of the present invention are designed to allow detection of a wide variety of biological and biochemical agents, as described above. In one embodiment, the methods may be used to detect pathogenic and/or potentially pathogenic virus, bacteria and toxins including biological warfare agents ("BWAs") in a variety of relevant clinical and environmental matrices, including and without limitation, blood, sputum, stool, filters, swabs, etc. A non-limiting list of pathogens and toxins that may be analyzed (alone or in combination) using the methods of the present invention is *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Vibrio cholerae* (cholera), *Francisella tularensis* (tularemia), *Brucella* spp. (Brucellosis), *Coxiella burnetii* (Q fever), orthopox viruses including variola virus (smallpox), viral encephalitis, Venezuelan equine encephalitis virus (VEE), western equine encephalitis virus (WEE), eastern equine encephalitis virus (EEE), Alphavirus, viral hemorrhagic fevers, Arenaviridae, Bunyaviridae, Filoviridae, Flaviviridae, Ebola virus, staphylococcal enterotoxins, ricin, botulinum toxins, *Clostridium botulinum,* mycotoxin, *Fusarium, Myrotecium, Cephalosporium, Trichoderma, Verticimonosporium, Stachybotrys,* glanders, wheat fungus, *Bacillus globigii, Serratia marcescens,* yellow rain, trichothecene mycotoxins, *Salmonella typhimurium,* aflatoxin, *Xenopsylla cheopis, Diamanus montanus,* alastrim, monkeypox, Arenavirus, Hantavirus, Lassa fever, Argentine hemorrhagic fevers, Bolivian hemorrhagic fevers, Rift Valley fever virus, Crimean-Congo virus, Hanta virus, Marburg hemorrhagic fevers, yellow fever virus, dengue fever viruses, influenza (including human and animal strains including H5N1 avian influenza), human immunodeficiency viruses I and II (HIV I and II), hepatitis A, hepatitis B, hepatitis C, hepatitis (non-A, B or C), Enterovirus, Epstein-Barr virus, Cytomegalovirus, herpes simplex viruses, *Chlamydia trachomatis, Neisseria gonorrheae, Trichomonas vaginalis,* human papilloma virus, *Treponema pallidum, Streptococcus pneumonia, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila, Staphylococcus aureus, Moraxella catarrhalis, Streptococcus pyogenes, Clostridium difficile, Neisseria meningitidis, Klebsiella pneumoniae, Mycobacterium tuberculosis,* coronavirus, Coxsackie A virus, rhinovirus, parainfluenza virus, respiratory syncytial virus (RSV), metapneumovirus, and adenovirus.

[0048] The skilled artisan in the field of binding assays will readily appreciate the scope of binding agents and companion binding partners that may be used in the present methods. A non-limiting list of such pairs include (in either order) receptor/ligand pairs, antibodies/antigens, natural or synthetic receptor/ligand pairs, amines and carbonyl compounds (i.e., binding through the formation of a Schiff's base), hapten/antibody pairs, antigen/antibody pairs, epitope/antibody pairs, mimitope/antibody pairs, aptamer/target molecule pairs, hybridization partners, and intercalater/target molecule pairs.

[0049] The binding assays of the methods of the present invention may employ antibodies or other receptor proteins as binding reagents. The term "antibody" includes intact antibody molecules (including hybrid antibodies assembled by in vitro reassociation of antibody subunits), antibody fragments and recombinant protein constructs comprising an antigen binding domain of an antibody (as described, e.g., in Porter, R. R. and Weir, R. C. J. Cell Physiol., 67 (Suppl); 51-64 (1966) and Hochman, 1. Inbar, D. and Givol, D. Biochemistry 12: 1130 (1973)), as well as antibody constructs that have been chemically modified, e.g., by the introduction of a detectable label.

[0050] The methods of the present invention may be used in a variety of assay devices and/or formats. The assay devices may include, e.g., assay modules, such as assay plates, cartridges, multi-well assay plates, reaction vessels, test tubes, cuvettes, flow cells, assay chips, lateral flow devices, etc., having assay reagents (which may include targeting agents or other binding reagents) added as the assay progresses or pre-loaded in the wells, chambers, or assay regions of the assay module. These devices may employ a variety of assay formats for specific binding assays, e.g., immunoassay or immunochromatographic assays. Illustrative assay devices and formats are described herein below. In certain embodiments, the methods of the present invention may employ assay reagents that are stored in a dry state and the assay devices/kits may further comprise or be supplied with desiccant materials for maintaining the assay reagents in a dry state. The assay devices preloaded with the assay reagents can greatly improve the speed and reduce the complexity of assay measurements while maintaining excellent stability during storage. The dried assay reagents may be any assay reagent that can be dried and then reconstituted prior to use in an assay. These include, but are not limited to, binding reagents useful in binding assays, enzymes, enzyme substrates, indicator dyes and other reactive compounds that may be used to detect an analyte of interest. The assay reagents may also include substances that are not directly involved in the mechanism of detection but play an auxiliary role in an assay including, but not limited to, blocking agents, stabilizing agents, detergents, salts, pH buffers, preservatives, etc. Reagents may be present in free form or supported on solid phases including the surfaces of compartments (e.g., chambers, channels, flow cells, wells, etc.) in the assay modules or the surfaces of colloids, beads, or other particulate supports.

[0051] A wide variety of solid phases are suitable for use in the methods of the present invention including conventional solid phases from the art of binding assays. Solid phases may be made from a variety of different materials including polymers (e.g., polystyrene and polypropylene), ceramics, glass, composite materials (e.g., carbon-polymer composites such as carbon-based inks). Suitable solid phases include the surfaces of macroscopic objects such as an interior surface of an assay container (e.g., test tubes, cuvettes, flow cells, cartridges, wells in a multi-well plate, etc.), slides, assay chips (such as those used in gene or protein chip measurements), pins or probes, beads, filtration media, lateral flow media (for example, filtration membranes used in lateral flow test strips), etc.

[0052] Suitable solid phases also include particles (including but not limited to colloids or beads) commonly used in other types of particle-based assays e.g., magnetic, polypropylene, and latex particles, materials typically used in solid-phase synthesis e.g., polystyrene and polyacrylamide particles, and materials typically used in chromatographic applications e.g., silica, alumina, polyacrylamide, polystyrene. The materials may also be a fiber such as a carbon fibril. Microparticles may be inanimate or alternatively, may include animate biological entities such as cells, viruses, bacterium and the like.

[0053] The particles used in the present method may be comprised of any material suitable for attachment to one or more binding partners and/or labels, and that may be collected via, e.g., centrifugation, gravity, filtration or magnetic collection. A wide variety of different types of particles that may be attached to binding reagents are sold commercially for use in binding assays. These include non-magnetic particles as well as particles comprising magnetizable materials which allow the particles to be collected with a magnetic field. In one embodiment, the particles are comprised of a conductive and/or semiconductive material, e.g., colloidal gold particles.

[0054] The microparticles may have a wide variety of sizes and shapes. By way of example and not limitation, microparticles may be between 5 nanometers and 100 micrometers. Preferably microparticles have sizes between 20 nm and 10 micrometers. The particles may be spherical, oblong, rod-like, etc., or they may be irregular in shape.

[0055] The particles used in the present method may be coded to allow for the identification of specific particles or subpopulations of particles in a mixture of particles. The use of such coded particles has been used to enable multiplexing of assays employing particles as solid phase supports for binding assays. In one approach, particles are manufactured to include one or more fluorescent dyes and specific populations of particles are identified based on the intensity and/or relative intensity of fluorescence emissions at one or more wave lengths. This approach has been used in the Luminex xMAP systems (see, e.g., US Patent No. 6,939,720) and the Becton Dickinson Cytometric Bead Array systems. Alternatively, particles may be coded through differences in other physical properties such as size, shape, imbedded optical patterns and the like.

[0056] The methods of the invention can be used with a variety of methods for measuring the amount of an analyte and, in particular, measuring the amount of an analyte bound to a solid phase. Techniques that may be used include, but are not limited to, techniques known in the art such as cell culture-based assays, binding assays (including agglutination tests, immunoassays, nucleic acid hybridization assays, etc.), enzymatic assays, colorometric assays, etc. Other suitable techniques will be readily apparent to one of average skill in the art. Some measurement techniques allow for measurements to be made by visual inspection, others may require or benefit from the use of an instrument to conduct the measurement.

[0057] Methods for measuring the amount of an analyte include label free techniques, which include but are not limited to i) techniques that measure changes in mass or refractive index at a surface after binding of an analyte to a surface (e.g., surface acoustic wave techniques, surface plasmon resonance sensors, ellipsometric techniques, etc.), ii) mass spectrometric techniques (including techniques like MALDI, SELDI, etc. that can measure analytes on a surface), iii) chromatographic or electrophoretic techniques, iv) fluorescence techniques (which may be based on the inherent fluorescence of an analyte), etc.

[0058] Methods for measuring the amount of an analyte also include techniques that measure analytes through the detection of labels which may be attached directly or indirectly (e.g., through the use of labeled binding partners of an analyte) to an analyte. Suitable labels include labels that can be directly visualized (e.g., particles that may be seen visually and labels that generate an measurable signal such as light scattering, optical absorbance, fluorescence, chemiluminescence, electrochemiluminescence, radioactivity, magnetic fields, etc). Labels that may be used also include enzymes or other chemically reactive species that have a chemical activity that leads to a measurable signal such as light scattering, absorbance, fluorescence, etc. The use of enzymes as labels has been well established in in Enzyme-Linked ImmunoSorbent Assays, also called ELISAs, Enzyme ImmunoAssays or EIAs. In the ELISA format, an unknown amount of antigen is affixed to a surface and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme converts to a product that provides a change in a detectable signal. The formation of product may be detectable, e.g., due a difference, relative to the substrate, in a measurable property such as absorbance, fluorescence, chemiluminescence, light scattering, etc. Certain (but not all) measurement methods that may be used with solid phase binding methods according to the invention may benefit from or require a wash step to remove unbound components (e.g., labels) from the solid phase Accordingly, the methods of the invention may comprise such a wash step.

[0059] In one embodiment, the detection antibody includes a detectable label attached thereto. Alternatively, a bridging oligonucleotide is employed and the bridging oligonucleotide includes a detectable label. As shown in Figs. 2(a)-(c), the desired binding interaction is detected if the bridging oligonucleotide is in position between the first and second linking agents and the bridging sequence also bears the detectable label.

[0060] In one embodiment, an analyte(s) of interest in the sample may be measured using electrochemiluminescence-based assay formats, e.g. electrochemiluminescence (ECL) based immunoassays. The high sensitivity, broad dynamic range and selectivity of ECL are important factors for medical diagnostics. Commercially available ECL instruments have demonstrated exceptional performance and they have become widely used for reasons including their excellent sensitivity, dynamic range, precision, and tolerance of complex sample matrices. Species that can be induced to emit ECL (ECL-active species) have been used as ECL labels, e.g., i) organometallic compounds where the metal is from, for example, the noble metals of group VIII, including Ru-containing and Os-containing organometallic compounds such as the tris-bipyridyl-ruthenium (RuBpy) moiety and ii) luminol and related compounds. Species that participate with the ECL label in the ECL process are referred to herein as ECL coreactants. Commonly used coreactants include tertiary amines (e.g., see U.S. Patent No. 5,846,485), oxalate, and persulfate for ECL from RuBpy and hydrogen peroxide for ECL from luminol (see, e.g., U.S. Patent No. 5,240,863). The light generated by ECL labels can be used as a reporter signal in diagnostic procedures (Bard et al., U.S. Patent No. 5,238,808). For instance, an ECL label can be covalently coupled to a binding agent such as an antibody, nucleic acid probe, receptor or ligand; the participation of the binding reagent in a binding interaction can be monitored by measuring ECL emitted from the ECL label. Alternatively, the ECL signal from an ECL-active compound may be indicative of the chemical environment (see, e.g., U.S. Patent No. 5,641,623 which describes ECL assays that monitor the formation or destruction of ECL coreactants). For more background on ECL, ECL labels, ECL assays and instrumentation for conducting ECL assays see U.S. Patents Nos. 5,093,268; 5,147,806; 5,324,457; 5,591,581; 5,597,910; 5,641,623; 5,643,713; 5,679,519; 5,705,402; 5,846,485; 5,866,434; 5,786,141; 5,731,147; 6,066,448; 6,136,268; 5,776,672; 5,308,754; 5,240,863; 6,207,369; 6,214,552 and 5,589,136 and Published PCT Nos. W099/63347; WOOO/03233; W099/58962; W099/32662; W099/14599; W098/12539; W097/36931 and W098/57154.

[0061] The methods of the invention may be applied to singleplex or multiplex formats where multiple assay measurements are performed on a single sample. Multiplex measurements that can be used with the invention include, but are not limited to, multiplex measurements i) that involve the use of multiple sensors; ii) that use discrete assay domains on a surface (e.g., an array) that are distinguishable based on location on the surface; iii) that involve the use of reagents coated on particles that are distinguishable based on a particle property such as size, shape, color, etc.; iv) that produce assay signals that are distinguishable based on optical properties (e.g., absorbance or emission spectrum) or v) that are based on temporal properties of assay signal (e.g., time, frequency or phase of a signal).

[0062] One embodiment of the present invention employs a specific binding assay, e.g., an immunoassay, immunochromatographic assay or other assay that uses a binding reagent. The immunoassay or specific binding assay according to one embodiment of the invention can involve a number of formats available in the art. The binding reagents can be labeled with a label or immobilized on a surface. Thus, in one embodiment, the detection method is a binding assay, e.g., an immunoassay, receptor-ligand binding assay or hybridization assay, and the detection is performed by contacting an assay composition with one or more detection molecules capable of specifically binding with an analyte(s) of interest in the sample.

[0063] In one embodiment, the assay uses a direct binding assay format. An analyte is bound to a binding partner of the analyte, which may be immobilized on a solid phase. The bound analyte is measured by direct detection of the analyte or through a label attached to the analyte (e.g., by the measurements described above).

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Patentkrav

1. Fremgangsmåde til at udføre en bindingsassay, omfattende:
(a) at bringe en prøve, som omfatter en analyt af interesse, med (i) en fast overflade, indeholdende en første bindingsreagens, der er immobiliseret dertil, omfattende et første bindingsområde, som binder til analyten af interesse,
hvor den første bindingsreagens er bundet til et første bindingsmiddel, som omfatter en første oligonukleotidsekvens, og (ii) en anden bindingsreagens, som omfatter et andet bindingsområde, der binder til analyten af interesse,
hvor den anden bindingsreagens er bundet til et andet bindingsmiddel, omfattende en anden oligonukleotidsekvens,
hvor den anden oligonukleotidsekvens omfatter en sekvens, der er komplementær til mindst en del af den første oligonukleotidsekvens;
hvor trinnet med at bringe i kontakt udføres under forhold, hvor det første og andet bindingsområde binder til analyten, og den første oligonukleotidsekvens hybridiserer til den anden oligonukleotidsekvens,
hvor den første og anden bindingsreagens bringes i nærheden af hinanden via henholdsvis det første og andet bindingsmiddel, som er bundet til enhver af bindingsreagenserne,
hvor en bindingsenergi mellem den første oligonukleotidsekvens og den anden oligonukleotidsekvens er under et niveau til at fastgøre den første oligonukleotidsekvens og den anden oligonukleotidsekvens stabilt til hinanden i fraværet af samtidig binding af det første og andet bindingsområde til analyten; og
(b) at måle mængden af den analyt, der er bundet til den faste bærer.

2. Fremgangsmåde ifølge krav 1, hvor den faste bærer er en elektrode, og måletrinnet yderligere omfatter påføring af en spændingsbølgeform til elektroden for at generere ECL.

3. Kit til at udføre en bindingsassay, omfattende:

(a) en fast overflade, indeholdende en første bindingsreagens, der er immobiliseret dertil, omfattende et første bindingsområde, som binder til analyten af interesse, hvor den første bindingsreagens er bundet til et første bindingsmiddel, omfattende en første oligonukleotidsekvens; og
(ii) en anden bindingsreagens, omfattende et andet bindingsområde, som binder til analyten af interesse,
hvor den anden bindingsreagens er bundet til et andet bindingsmiddel, omfattende en anden oligonukleotidsekvens,
hvor den anden oligonukleotidsekvens omfatter en sekvens, der er komplementær til mindst en del af den første oligonukleotidsekvens,
hvor en bindingsenergi mellem den første oligonukleotidsekvens og den anden oligonukleotidsekvens er under et niveau til at fastgøre den første oligonukleotidsekvens og den anden oligonukleotidsekvens stabilt til hinanden i fraværet af samtidig binding af det første og andet bindingsområde til analyten.

4. Fremgangsmåde ifølge krav 1 eller 2, hvor den komplementære sekvens, som anvendes i bindingsmidlet, har en bindingsenergi, der er utilstrækkelig til at forbinde bindingsmidlet til begge bindingsreagenser stabilt alene i fravær af samtidig binding af bindingsreagenser til analyten.

5. Fremgangsmåde ifølge krav 1 eller 2, hvor den forbindende oligonukleotidsekvens er ca. 5-20 baser i længden, fortrinsvis 8-15 baser i længden, mere foretrukket ca. 8-12 baser i længden.

6. Fremgangsmåde ifølge krav 1 eller 2, hvor en længde af både den første oligonukleotidsekvens og den anden oligonukleotidsekvens hver omfatter en komplementær bindingssekvens på ca. 4-8 baser i længden.

7. Fremgangsmåde ifølge krav 1, hvor den faste overflade er udvalgt blandt assayplader, patroner og partikler.

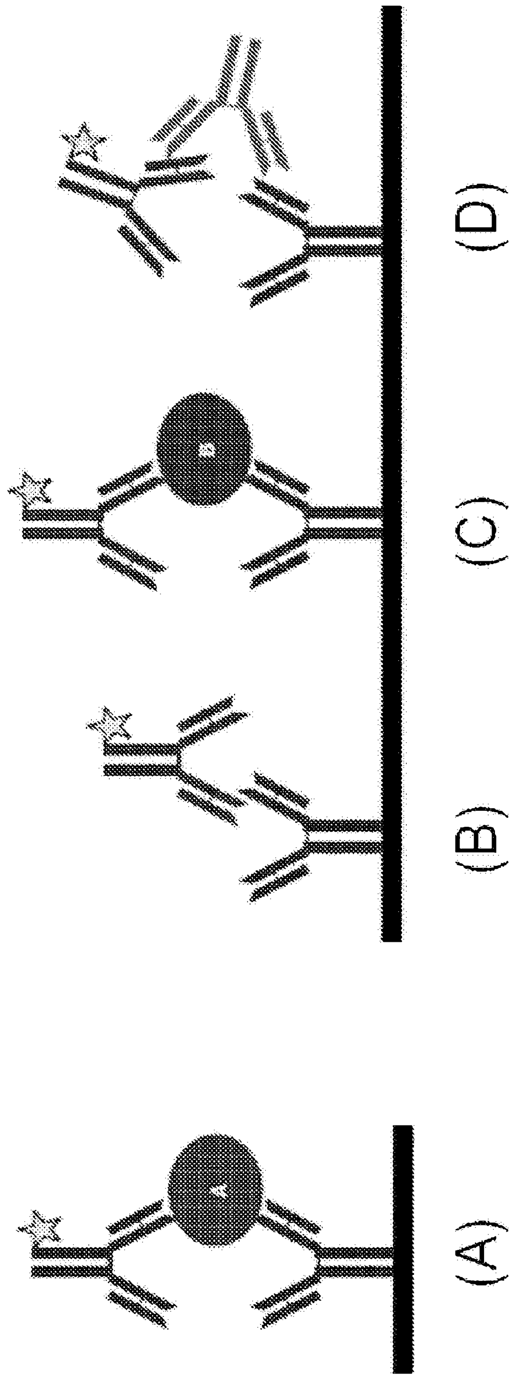
8. Kit ifølge krav 3, hvor den komplementære sekvens, som anvendes i bindingsmidlet, har en bindingsenergi, der er utilstrækkelig til at forbinde bindingsmidlet til begge bindingsreagenser stabilt alene i fravær af samtidig binding af bindingsreagenser til analyten.

9. Kit ifølge krav 3, hvor den forbindende oligonukleotidsekvens er ca. 5-20 baser i længden, fortrinsvis 8-15 baser i længden, mere foretrukket ca. 8-12 baser i længden.

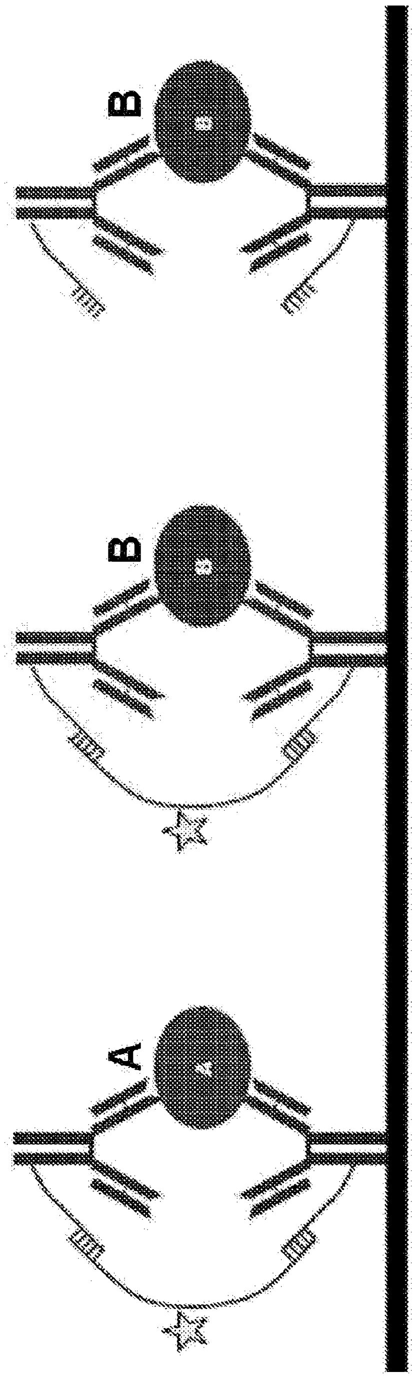
10. Kit ifølge krav 3, hvor en længde af både den første oligonukleotidsekvens og den anden oligonukleotidsekvens er 4-8 baser i længden.

11. Kit ifølge krav 3, hvor den faste overflade er udvalgt blandt assayplader, patroner og partikler.

DRAWINGS



FIGS. 1A-D



(A) (B) (C)

FIGS. 2A-C