COMPOSITIONS AND METHODS FOR ANALYTE DETECTION USING BIOLUMINESCENCE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to and the benefit of U.S. Provisional Patent Application No. 62/832,052, filed April 10, 2019, which is incorporated herein by reference in its entirety and for all purposes.

FIELD

[0002] Provided herein are systems and methods for the detection of an analyte or analytes in a sample. In particular, the present disclosure provides compositions, assays, and methods for detecting and/or quantifying a target analyte using a bioluminescent complex comprising substrates, peptides, and/or polypeptides capable of generating a bioluminescent signal that correlates to the presence, absence, or amount of the target analyte.

BACKGROUND

[0003] Biological processes rely on covalent and non-covalent interactions between molecules, macromolecules, and molecular complexes. In order to understand such processes, and to develop techniques and compounds to manipulate them for research and clinical and other practical applications, it is necessary to have tools available to detect and monitor these interactions and/or components involved in such interactions. The study of these interactions, particularly under physiological conditions (e.g., at normal expression levels for monitoring protein interactions), requires high sensitivity.

[0004] Creation of better assays for use in the field and in clinical settings is an ongoing area of urgent need. Speed, sensitivity, selectivity, robustness, simplicity, quantitative versus qualitative capabilities, and cost are all critical factors affecting the relevance of a diagnostic bioassays, and thus their utility to and adoption by the relevant community. Rapid diagnostic tests are not only relevant to clinical settings, but also can be applied to environmental, industrial, and direct to consumer contexts.

SUMMARY

[0005] Disclosed herein are compositions and formulations comprising a luminogenic substrate and a target analyte binding agent comprising a target analyte binding element and one

of a polypeptide component of a bioluminescent complex, or a peptide component of a bioluminescent complex. In one aspect, the present disclosure refers to a composition comprising a dried formulation of: a luminogenic substrate; and a target analyte binding agent comprising a first target analyte binding element and a polypeptide component of a bioluminescent complex comprising at least 60% sequence identity with SEQ ID NO: 5, wherein the composition is capable of being reconstituted in a solutions, and wherein when reconstituted, the polypeptide component is capable of forming a bioluminescent analyte detection complex when the polypeptide component associates with a peptide component of the bioluminescent complex bound to a second target analyte binding element in the presence of the target analyte.

[0006] In accordance with these examples, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 5; at least 60% sequence identity with SEQ ID NO: 9; or at least 60% sequence identity with SEQ ID NO: 12.

[0007] In some examples, the peptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 10; at least 60% sequence identity with SEQ ID NO: 11; at least 60% sequence identity with SEQ ID NO: 13; or at least 60% sequence identity with SEQ ID NO: 14.

[0008] In some examples, the composition comprises a complementary peptide or polypeptide component of the bioluminescent complex, wherein the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0009] In some examples, the composition that comprises the luminogenic substrate and the target analyte binding agent are combined in a dried formulation, and the complementary peptide or polypeptide component of the bioluminescent complex comprises a liquid formulation, wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0010] In some examples, the composition comprising the luminogenic substrate, the target analyte binding agent, and the complementary peptide or polypeptide component of the bioluminescent complex are combined in a dried formulation, wherein the dried formulation forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0011] In some examples, the complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte.

[0012] In some examples, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 10.

[0013] In some examples, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 14.

[0014] Examples of the present disclosure also include a composition comprising a dried formulation comprising (a) a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9, and (b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10.

[0015] In some examples, the dried formulation further comprises a luminogenic substrate.

[0016] In some examples, the composition further comprises a liquid formulation comprising the target analyte.

[0017] Examples of the present disclosure also include a composition comprising a dried formulation comprising (a) a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and (b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14.

[0018] In some examples, the dried formulation further comprises a luminogenic substrate.

[0019] In some examples, the composition further comprises a liquid formulation comprising the target analyte.

[0020] Examples of the present disclosure also include a composition comprising a dried formulation comprising (a) a first target analyte binding agent comprising a first target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, (b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and (c) a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12.

[0021] In some examples, the dried formulation further comprises a luminogenic substrate.

[0022] In some examples, the composition further comprises a liquid formulation comprising the target analyte.

[0023] Examples of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11.

[0024] Examples of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 9.

[0025] Examples of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14.

[0026] In some examples, the dried formulation further comprises a luminogenic substrate.

[0027] In some examples, the liquid formulation further comprises a luminogenic substrate.

[0028] In some examples, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0029] In some examples, the composition further comprises a second complementary peptide or polypeptide component of the bioluminescent complex, wherein the target analyte

binding agent, the first complementary peptide or polypeptide component of the bioluminescent complex, and the second complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0030] In some examples, the composition comprising the target analyte binding agent comprises a dried formulation, and wherein the first complementary peptide or polypeptide component and the second complementary peptide or polypeptide of the bioluminescent complex comprise a liquid formulation; wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0031] In some examples, the composition comprising the target analyte binding agent, and either the first or the second complementary peptide or polypeptide component are combined in a dried formulation, and wherein the first or the second complementary peptide or polypeptide component that is not present in the dried formulation comprises a liquid formulation; wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0032] In some examples, the target analyte binding agent, the first complementary peptide or polypeptide component, and the second complementary peptide or polypeptide component are combined in a dried formulation that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0033] In some examples, the dried formulation further comprises a luminogenic substrate.

[0034] In some examples, the liquid formulation further comprises a luminogenic substrate.

[0035] In some examples, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0036] In some examples, either the first or the second complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0037] In some examples, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein either the first or the second complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with either SEQ ID NO: 13 or SEQ ID NO: 15.

[0038] Examples of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0039] Examples of the present disclosure also include (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and (b) a liquid formulation comprising a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0040] Examples of the present disclosure also include (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0041] Examples of the present disclosure also include (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and (b) a liquid

formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 6.

[0042] Examples of the present disclosure also include (a) a dried formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and (b) a liquid formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15.

[0043] Examples of the present disclosure also include a composition comprising a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 6.

[0044] In some examples, the dried formulation further comprises a luminogenic substrate.

[0045] In some examples, the liquid formulation further comprises a luminogenic substrate.

[0046] In some examples, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0047] In some examples, a bioluminescent signal produced in the presence of the luminogenic substrate is substantially increased when the target analyte binding agent contacts one or more of the complementary peptide or polypeptide components of the bioluminescent complex, as compared to a bioluminescent signal produced by the target analyte binding agent and the luminogenic substrate alone.

[0048] In some examples, the target analyte is a target antibody.

[0049] In some examples, the target analyte binding agent comprises an element that binds non-specifically to antibodies.

[0050] In some examples, the target analyte binding agent comprises an element that binds specifically to an antibody.

[0051] In some examples, the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

[0052] In some examples, a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0053] In some examples, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW, 1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives.

[0054] In some examples, the composition further comprises a polymer.

[0055] In some examples, the polymer is a naturally-occurring biopolymer. In some examples, the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof. In some examples, the naturally-occurring biopolymer is pullulan.

[0056] In some examples, the polymer is a cyclic saccharide polymer or a derivative thereof. In some examples, the polymer is hydroxypropyl β-cyclodextrin.

[0057] In some examples, the polymer is a synthetic polymer. In some examples, the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block. In some examples, the synthetic polymer is poloxamer 188.

[0058] In some examples, the composition further comprises a substance to reduce autoluminescence.

[0059] In some examples, the substance to reduce autoluminescence is ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0060] In some examples, the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof. In some examples, the is surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80.

[0061] In some examples, the composition is used in conjunction with an analyte detection platform to detect an analyte in a sample.

[0062] In some examples, sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

[0063] Examples of the present disclosure also include a method of detecting an analyte in a sample comprising combining any of the compositions described above with a sample comprising a target analyte.

[0064] In some examples, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex.

[0065] In some examples, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex.

[0066] In some examples, the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte.

[0067] In some examples, one or more of the components of the composition exhibits enhanced stability within the composition compared to the component in solution alone.

[0068] Examples of the present disclosure also include systems and methods for the detection of an analyte or analytes in a sample. In particular, the present disclosure provides compositions, assays, and methods for detecting and/or quantifying a target analyte using a bioluminescent complex comprising substrates, peptides, and/or polypeptides capable of generating a bioluminescent signal that correlates to the presence, absence, or amount of the target analyte.

[0069] Examples of the present disclosure include a lateral flow detection system. In accordance with these examples, the system includes an analytical membrane that includes a detection region and a control region. In some examples, the detection region includes a first target analyte binding agent immobilized to the detection region, a conjugate pad comprising a second target analyte binding agent, and a sample pad. In some examples, the first target

analyte binding agent and the second target analyte binding agent form a bioluminescent analyte detection complex in the at least one detection region when a target analyte is detected in a sample.

[0070] In some examples, the first target analyte binding agent includes a target analyte binding element and is non-luminescent. In some examples, the second target analyte binding agent includes a target analyte binding element and a bioluminescent polypeptide. In some examples, the bioluminescent polypeptide has at least 60% sequence identity with SEQ ID NO: 5.

[0071] In some examples, the first target analyte binding agent includes a target analyte binding element and a polypeptide component of a bioluminescent complex, and the second target analyte binding agent includes a target analyte binding element and a peptide component of a bioluminescent complex. In some examples, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the first target analyte binding agent and the luminogenic substrate alone.

[0072] In some examples, the first target analyte binding agent includes a target analyte binding element and a peptide component of a bioluminescent complex, and the second target analyte binding agent includes a target analyte binding element and a polypeptide component of a bioluminescent complex. In some examples, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the first target analyte binding agent and the luminogenic substrate alone.

[0073] In some examples, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 6. In some examples, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 10. In some examples, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 12. In some examples, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 14.

[0074] In some examples, the first target analyte binding agent includes a target analyte binding element and a first peptide component of a tripartite bioluminescent complex, and the second target analyte binding agent includes a target analyte binding element and a second

peptide component of the tripartite bioluminescent complex. In some examples, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent and a polypeptide component of the tripartite bioluminescent complex as compared to a bioluminescent signal produced by (i) the first target analyte binding agent, the second target analyte binding agent, and/or the polypeptide component and (ii) the luminogenic substrate alone.

[0075] In some examples, the first peptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 11. In some examples, the second first peptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 13. In some examples, the polypeptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 12.

[0076] In some examples, the target analyte is a target antibody. In some examples, the first target analyte binding element includes an agent that binds non-specifically to antibodies. In some examples, the second target analyte binding element comprises an agent that binds specifically to the target antibody. In some examples, the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

[0077] In some examples, a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0078] In some examples, the system further includes a luminogenic substrate. In some examples, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some examples, the luminogenic substrate is applied to the system as part of a composition that includes the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some

examples, the luminogenic substrate is applied to the system as part of a composition that includes the luminogenic substrate and a substance to reduce autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0079] In some examples, the composition is applied to at least one of the sample pad, the conjugation pad, the detection region, and the control region.

[0080] In some examples, the analytical membrane includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements.

[0081] In some examples, the system further includes a device for detecting or quantifying bioluminescent signals from the analyte detection complex.

[0082] Examples of the present disclosure also include a conjugate pad comprising at least one target analyte binding agent. In accordance with these examples, the at least one target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0083] In some examples, the target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0084] In some examples, the conjugate pad further includes a luminogenic substrate. In some examples, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some examples, the luminogenic substrate contained on or within the conjugate pad as part of a composition that includes the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose,

cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the luminogenic substrate is applied to the system as part of a composition that includes the luminogenic substrate and a substance to reduce autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0085] Examples of the present disclosure also include an analytical membrane that includes a detection region and a control region. In accordance with these examples, the detection region includes at least one target analyte binding agent immobilized to the detection region.

[0086] In some examples, the at least one target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0087] In some examples, the target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0088] In some examples, the analytical membrane further includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements. In some examples, the analytical membrane further includes a luminogenic substrate. In some examples, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives.

[0089] In some examples, the luminogenic substrate is reversibly conjugated to the conjugate pad as part of a composition including the luminogenic substrate and a polymer

selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the luminogenic substrate is part of a composition that includes the luminogenic substrate and a substance that reduces autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0090] Examples of the present disclosure also include a solid phase detection platform comprising a detection region. In accordance with these examples, the detection region includes at least one target analyte binding agent conjugated to the detection region. In some examples, the at least one target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0091] In some examples, the target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0092] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 10 applied to the detection region.

[0093] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 10 conjugated to the detection region; and a second target

analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 6 applied to the detection region.

[0094] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 11 conjugated to the detection region; a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 13 applied to the detection region; and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12 applied to the detection region.

[0095] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with ID NO: 14 applied to the detection region.

[0096] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 14 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 applied to the detection region.

[0097] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide applied to the detection region.

[0098] In some examples, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5 applied to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide conjugated to the detection region.

[0099] In some examples, the detection platform further includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements. In some examples, the detection platform further includes a control region. In some examples, the detection platform further includes a luminogenic substrate. In some examples, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some examples, the luminogenic substrate is reversibly conjugated to the conjugate pad as part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the luminogenic substrate is part of a composition comprising the luminogenic substrate and a substance that reduces autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0100] Examples of the present disclosure also include a solution phase detection platform that includes at least one detection receptacle and a lyophilized tablet (lyocake). In accordance with these examples, the lyocake comprises a target analyte binding agent comprising a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0101] In some examples, the target analyte binding agent comprises a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0102] In some examples, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6; and a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 10.

[0103] In some examples, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12.

[0104] In some examples, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6; and a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with ID NO: 14.

[0105] In some examples, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5; and a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide.

[0106] In some examples, the detection platform comprises a 96-well microtiter plate comprising a plurality of detection receptacles, and at least two distinct target analyte binding agents comprising distinct target analyte binding elements.

[0107] In some examples, the lyocake comprises a luminogenic substrate. In some examples, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives.

[0108] In some examples, the lyocake comprises a luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

[0109] In some examples, the lyocake comprises a luminogenic substrate and a substance to reduce autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0110] In some examples, the detection platform further comprises at least one sample. In some examples, the sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

[0111] Examples of the present disclosure also include a method of detecting an analyte in a sample using the lateral flow assay systems described above. In accordance with these examples, the method includes applying a sample to the sample pad, facilitating flow of the sample from the sample pad to the conjugate pad, and then from the conjugate pad to the detection region and the control region on the analytical membrane. In some examples, the first target analyte binding agent, the second target analyte binding agent, and the target analyte form the analyte detection complex in the at least one detection region when the target analyte is detected in the sample.

[0112] In some examples, the sample is a sample from a subject selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, tissue, and saliva. In some examples, the sample is selected from a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample. In some examples, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from the analyte detection complex.

[0113] In some examples, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex. In some examples, the method further comprises diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

[0114] Examples of the present disclosure also include a method of detecting an analyte in a sample using the solid phase detection platform described above. In accordance with these examples, the method includes exposing a sample to the detection region and control region. In some examples, the at least one target analyte binding agent and the at least one target analyte form an analyte detection complex in the at least one detection region when the target analyte is detected in the sample.

[0115] In some examples, the sample is a sample from a subject selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, tissue, and saliva. In some examples, the sample is selected from a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample. In some examples, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from the analyte detection complex.

[0116] In some examples, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex. In some examples, the method further comprises diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

[0117] Examples of the present disclosure also include a method of producing a substrate for use in a bioluminescent assay. In accordance with these examples, the method includes applying a solution onto a substrate. In some examples, the solution contains at least one target analyte binding agent comprising a target analyte binding element and one of a polypeptide component of a bioluminescent complex or a peptide component of a bioluminescent complex. In some examples, the method includes drying the substrate containing the solution.

[0118] In some examples, the solution further includes a complementary peptide or polypeptide component of the bioluminescent complex. In some examples, the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0119] In some examples, the solution comprises a protein buffer and at least one excipient. In some examples, the solution comprises a luminogenic substrate.

[0120] In some examples, the substrate comprising the dried solution is W-903 paper, FTA paper, FTA Elute paper, FTA DMPK paper, Ahlstrom A-226 paper, M-TFN paper, FTA paper, FP705 paper, Bode DNA collection paper, nitrocellulose paper, nylon paper, cellulose paper, Dacron paper, cotton paper, and polyester papers, or combinations thereof. In some examples, the substrate is a mesh comprising plastic, nylon, metal, or combinations thereof.

[0121] In some examples, drying the substrate containing the solution comprises drying at a temperature from about 30℃ to 40℃ for a period of time between about 30 mins and 2 hours.

In some examples, drying the substrate containing the solution comprises lyophilizing and/or freezing the substrate.

[0122] In some examples, the method further comprises drying the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex onto a first substrate, and drying the luminogenic substrate onto a second substrate.

[0123] In accordance with these examples, a bioluminescent signal is generated upon exposure of the substrate containing the solution to the target analyte, and in some examples, the bioluminescent signal is proportional to the concentration of the target analyte.

[0124] In some examples, the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex exhibit(s) enhanced stability when dried on the substrate.

[0125] Examples of the present disclosure include a composition comprising a luminogenic substrate, a target analyte binding agent comprising a target analyte binding element and a polypeptide component of a bioluminescent complex, and a complementary polypeptide component of the bioluminescent complex. In accordance with these examples, the target analyte binding agent and the complementary polypeptide component of the bioluminescent complex are capable of forming a bioluminescent analyte detection complex in the presence of a target analyte.

[0126] In some examples, the composition further comprises a second target analyte binding agent comprising a second target analyte binding element and a second polypeptide component of a bioluminescent complex.

[0127] In some examples, the first and second target analyte binding agents bind separate portions of the same target analyte.

[0128] In some examples, the first and second polypeptide components of the bioluminescent complex bind the complementary polypeptide component of the bioluminescent complex to form a bioluminescent analyte detection complex in the presence of the target analyte.

[0129] In some examples, the first and the second polypeptide components are linked to a modified dehalogenase capable of forming a covalent bond with a haloalkane substrate.

[0130] In some examples, the first and the second target analyte binding elements comprise a haloalkane substrate.

[0131] In some examples, the first or second polypeptide components of the first and second target analyte binding agents comprise: at least 60% sequence identity with SEQ ID NO: 10; at least 60% sequence identity with SEQ ID NO: 11; at least 60% sequence identity with SEQ ID NO: 13; or at least 60% sequence identity with SEQ ID NO: 15.

[0132] In some examples, the complementary polypeptide component comprises: at least 60% sequence identity with SEQ ID NO: 6; at least 60% sequence identity with SEQ ID NO: 9; or at least 60% sequence identity with SEQ ID NO: 12.

[0133] In some examples, the target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0134] In some examples, the target analyte is an antibody, and wherein the target analyte binding element of the first target analyte binding agent comprises antigen recognized by the antibody, and wherein the target analyte binding element of the second target analyte binding agent comprises an Fc binding region.

[0135] In some examples, the first and/or second target analyte binding agents further comprise a fluorophore coupled to the first and/or second polypeptide components of the bioluminescent complex.

[0136] In some examples, one or more components of the composition is in the form of a lyophilized tablet (lyocake) capable of forming a bioluminescent complex when reconstituted in a solution to detect and/or quantify the target analyte.

[0137] In some examples, the composition comprises a solution-phase detection platform capable of detecting and/or quantifying the target analyte.

[0138] In some examples, the polypeptide components and the luminogenic substrate are in the form of a lyophilized tablet (lyocake) capable of forming a bioluminescent complex when reconstituted in a solution to detect and/or quantify the target analyte.

[0139] Examples of the present disclosure also includes a method of detecting an analyte in a sample comprising combining any of the compositions described above with a sample comprising a target analyte.

[0140] In some examples, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex.

[0141] In some examples, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex.

[0142] In some examples, the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte.

BRIEF DESCRIPTION OF THE DRAWINGS

[0143] FIG. 1 shows a representative schematic diagram of a lateral flow assay for detecting and/or quantifying a target analyte(s) in a sample based on bioluminescent complex formation, according to one embodiment of the present disclosure.

[0144] FIG. 2 shows a representative schematic diagram of a solid phase detection platform for detecting and/or quantifying target analytes in a sample based on bioluminescent complex formation, according to one embodiment of the present disclosure.

[0145] FIG. 3 shows representative images demonstrating that components of the bioluminescent complexes produce detectable bioluminescence after being applied to a solid support substrate (e.g., membrane), dried, and stored at room temperature.

[0146] FIG. 4 shows representative images demonstrating that components of the bioluminescent complexes produce detectable bioluminescence after being applied to membrane and paper-based solid support substrates.

[0147] FIG. 5 shows a representative assay schematic (left) and a representative graph (right) demonstrating the ability of components of the bioluminescent complexes to be used as reporters on target analyte binding agents for target analyte detection.

[0148] FIG. 6 shows a representative depiction of an assay platform using components of the bioluminescent complexes as reporters on target analyte binding agents for target analyte detection.

[0149] FIGS. 7A-7E show representative stability tests of an assay platform using components of the bioluminescent complexes as reporters on target analyte binding agents for target analyte detection, according to one embodiment of the present disclosure (FIG. 7A at 4°C;

CLAIMS

What is claimed is:

1. A composition comprising a dried formulation of:
a luminogenic substrate; and
a target analyte binding agent comprising a first target analyte binding element and a polypeptide component of a bioluminescent complex comprising at least 60% sequence identity with SEQ ID NO: 5, wherein the composition is capable of being reconstituted in a solutions, and wherein when reconstituted, the polypeptide component is capable of forming a bioluminescent analyte detection complex when the polypeptide component associates with a peptide component of the bioluminescent complex bound to a second target analyte binding element in the presence of the target analyte.

2. The composition of claim 1, wherein the target analyte is a target antibody.

3. The composition of claim 1, wherein the target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

4. The composition of claim 1, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

5. The composition of claim 1, further comprising a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, hydroxypropyl β-cyclodextrin, a cyclic saccharide polymer, a block copolymer, polystyrene, poly(meth)acrylate, and a combination of any thereof.

6. The composition of claim 1, wherein the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof.