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(54) **Title**: DETECTION OF AN ANALYTE IN A SAMPLE

(57) **Abstract**: Disclosed herein are methods of detecting an analyte in a sample. The methods can provide detection of an analyte in small volume samples (up to 12 µl). Further provided herein are devices for detecting analytes, wherein the device is selected based upon an absorbance spectrum of a chromophore resulting from the association of the analyte and a precursor agent.

DETECTION OF AN ANALYTE IN A SAMPLE

BACKGROUND

[0001] Many types of assays have been used to detect the presence of various analytes in samples. These assays typically involve antigen antibody reactions, ligand, anti-ligand, ligand receptor and utilize, synthetic conjugates comprising radioactive, enzymatic, fluorescent, or visually observable metal soluble tags, and specially designed reactor chambers. Most current tests are designed to make a quantitative determination, but in many circumstances all that is required is qualitative or positive/negative indication. Many times, such assays fail to provide a versatile assay for a wide range of analytes at a wide range of concentrations or sample volumes.

[0002] Thus, a need exists for devices and methods of detecting an analyte in a sample that can be tailored for a specific analyte or a specific set of analytes, which can also be useful in detecting analytes at low concentration or in low volumes.

SUMMARY

[0003] Disclosed herein are methods of detecting an analyte in a sample and/or measuring the concentration of an analyte in a sample, and further disclosed are devices for use in detecting an analyte.

[0004] In one aspect, provided herein are methods for detecting an analyte in a sample comprising exciting a detection agent in the sample to produce a detectable signal, and measuring the detectable signal; wherein the detection agent is dissolved, at least partially dissolved, or suspended in the sample; the sample further comprises a precursor agent that has the ability to form a chromophore in the presence of the analyte, the chromophore having an absorbance spectrum that overlaps with the emission spectrum of the detection agent, and the detectable signal is modulated when the analyte is present in the sample. In various cases, the detectable signal is modulated in proportion to the concentration of the analyte. In various cases, the detectable signal decreases in the presence of the analyte. In some or any cases, the chromophore is formed by a covalent interaction between the analyte and the precursor agent. In various cases, the chromophore is formed by a non-covalent interaction between the analyte and the precursor agent. In various cases, the chromophore is formed by a reaction between the analyte and the precursor agent.

[0005] In another aspect, provided herein are methods of measuring the concentration of an analyte in a sample comprising admixing the sample, a precursor agent, and a detection agent in a container to form a mixture, wherein the detection agent is a fluorophore; exciting

the fluorophore to produce a detectable signal; and measuring the detectable signal to measure the concentration of the analyte in the sample, wherein the fluorophore is dissolved, at least partially dissolved, or suspended in the mixture; the precursor agent, in the presence of the analyte, forms a chromophore that has an absorbance spectrum that overlaps with the emission and/or excitation spectrum of the fluorophore, and the detectable signal of the mixture decreases in the presence of the analyte. In various embodiments, the detectable signal decreases in proportion to the concentration of the analyte in the mixture. In various cases, the method further comprises comparing the fluorescence of the mixture to a standard curve. In various embodiments, the chromophore has an absorbance spectrum that overlaps with the emission spectrum of the fluorophore.

[0006] In various embodiments, the analyte is a protein, a polypeptide, an antibody, an oligonucleotide, a polysaccharide, inorganic phosphate, ATP, GTP, UTP, CTP, ADP, GDP, cGMP, cAMP, CoA, FAD, FMN, FADP, NADH, NADPH, a thiol, glutathione, creatinine, creatine, hydrogen peroxide, a reactive oxygen species, or nitric oxide. In some or any cases, the detection agent comprises one or more of a fluorophore and a luminescent agent. In some specific embodiments, the luminescent agent is a chemiluminescent agent or a bioluminescent agent. In various cases, the detectable signal comprises one or more of a detectable color, fluorescence, or luminescence. In various cases, the detection agent is at a concentration of 0.1 μM to 100 μM, or 1 μM to 10 μM. In various cases, the detection agent, precursor agent, or both is selected from the group consisting of bicinchoninic acid (BCA), fluorescein, rhodamine, quinaldine red, malachite green, Alexa 488, coomassie brilliant blue-R, coomassie brilliant blue-G, a silver stain, a gold stain, a zinc stain, a copper stain, a periodic acid-Schiff (PAS) stain, a quantum dot, a cyanine dye or derivative thereof, epicoccone, Fast Green, bromocresol green, amido black, a reuthenium metal chelate, a pyrene compound, a benzopyrillium compound, a monobromobimane, Ponceau Red, and Alcian Blue.

[0007] In various embodiments, the sample has a volume of up to 12 μL. In some or any cases, the sample has a volume of up to 8 μL, or a volume of 2 μL to 5 μL. In various embodiments, the analyte is present in the sample in an amount of 0.1 μg/mL to 1 μg/mL, or in an amount less than 0.5 μg/mL. In some or any cases, the sample is from a human, such as from a biopsy.

[0008] In various cases, the exciting occurs in a container that is a multi-well plate. In some or any cases, the exciting occurs in a container (e.g., a multi-well plate) that does not have an inherent emission and/or excitation spectrum that overlaps with the absorbance spectrum of the chromophore. In various cases, the container exhibits little or no fluorescence. In various embodiments, the container, e.g., the multi-well plate, is black. In various embodiments, the container does not have an emission spectrum that overlaps with the absorbance spectrum of the chromophore.

[0009] In yet another aspect, provided herein are devices for detecting an analyte in a sample comprising a container having a detection agent associated therewith, wherein the detection agent has an emission and/or excitation spectrum that overlaps with an absorption spectrum of chromophore that results from an association of the analyte with a precursor agent. In various embodiments, the container, in the absence of the associated detection agent, does not have an emission and/or excitation spectrum that overlaps with the absorption spectrum of a chromophore resulting from the association of the analyte and the precursor agent. In various cases, the detection agent comprises a fluorophore or a luminescent agent. In some or any cases, the luminescent agent is a chemiluminescent agent or a bioluminescent agent. In various cases, the chromophore forms from a covalent interaction between the analyte and the precursor agent. In some or any cases, the chromophore forms from a non-covalent interaction between the analyte and the precursor agent. In various embodiments, the detection agent has an emission spectrum that overlaps with an absorption spectrum of chromophore.

[0010] In various cases, the chromophore forms from a reaction between the analyte and the precursor agent. In various embodiments, the detection agent is embedded in the container. In various embodiments, the detection agent is coated on a surface of the container. In various embodiments, the detection agent is covalently attached to a material of the container. In some or any embodiments, upon contact with a sample comprising the analyte, the detection agent is released from the container into the sample. In various cases, the detection agent is selected from the group consisting of fluorescein, cascade blue, Pacific blue, Dylight 405, Dylight 594, and Dylight 549. In some or any cases, the device further comprises a second detection agent associated with the container. In various cases, the container is a multifluidic chamber, a multi-well plate, or a microscope slide.

[0011] In still another aspect, provided herein are kits comprising a device as disclosed herein and a precursor agent. In various embodiments, the precursor agent comprises quinaldine red, malachite green, 2,2-azino-bis(3-ethylbenzothiazoine-6-sulfonate (ABTS), tetramethylbenzidine (TMB), Coomassie Brilliant Blue G-250 (Bradford Reagent), or bicinchoninic acid (BCA). In some or any cases, the detection agent is fluorescein and the precursor agent is quinaldine red; the detection agent is Dylight 594 and the precursor agent is malachite green; the detection agent is cascade blue and the precursor agent is ABTS; the detection agent is Pacific blue and the precursor agent is TMB; the detection agent is Dylight 594 and the precursor agent is Bradford Reagent; or the detection agent is Dylight 549 and the precursor agent is BCA.

[0012] In another aspect, provided herein are methods of detecting the presence of an analyte in a sample comprising providing the sample in the container of a device as disclosed herein; exciting the detection agent to produce a detectable signal, and measuring the detectable signal to detect the presence of the analyte in the sample, wherein the detectable signal of the mixture decreases in the presence of the analyte. In various embodiments, the method further comprises adding the precursor agent to the sample in the container. In various cases, the detection agent is a fluorophore or a luminescent agent, such as a chemiluminescent agent or a bioluminescent agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1. Development of a fluorescence-based modification of the BCA assay. (A) Results of an excitation wavelength scan in white, opaque 384-well plates, in which the emission was held constant at 562 nm. Peak intensity was observed at Ex = 435 nm. (B) Spectral overlap between the absorbance of the $Cu^{1+}(BCA)_2$ complex (dotted line) and the fluorescence emission of white 384-well plates (solid line). Plates were irradiated at Ex = 435 nm. (C) Schematic of the absorption of fluorescence by the $Cu^{+1}(BCA)_2$ complex.

[0014] Figure 2. The optimized fluorescence-based BCA assay is more sensitive than the absorbance-based method. (A) The apparent fluorescence of a white plate was reduced by increasing concentrations of the model protein, BSA. (B) Using optimized ratios of WR and protein, the white plate-based BCA assay provided larger signal and improved linearity. Addition of free fluorescein (1 μM) to the WR and reading the fluorescence in black plates also provided enhanced sensitivity at the same volumes. Fluorescence was transformed using the equation $OD_{562} = -\log(F/F_0)$, where F = fluorescence of the sample and F_0 is the

fluorescence of WR with buffer. (C) The fluorescence methods were able to accurately detect a recombinant, purified analyte, *E. coli* DnaJ, in low volume. As in (B), the three platforms were compared using the optimized conditions. (D) The white plates could accurately detect total protein content in a crude, bacterial cell lysate. All results are the average of triplicates and the error bars represent standard deviations. Note that some bars are smaller than the symbols.

DETAILED DESCRIPTION

[0015] Provided herein are methods of detecting an analyte in a sample, and devices for carrying out the same. More particularly, in some aspects, provided herein are methods of detecting an analyte in a sample comprising exciting a detection agent in a sample to produce a detectable signal and measuring that detectable signal. In the presence of the analyte, the detectable signal is modulated. In some cases, the signal decreases in the presence of the analyte.

[0016] The methods disclosed herein provide the ability to detect an analyte in a sample at low concentrations and a low sample volumes, which has proved difficult in the past for other methods. In some cases, the analyte is present in the sample at a concentration of up to 1 μg/mL, 0.1 μg/mL to 1 μg/mL, less than 0.5 μg/mL, or 0.1 to 0.5 μg/mL.

[0017] As used herein, "sample" means any solution, fluid, homogenate or liquid having or suspected of having an analyte of interest. In some cases, the sample is from a biological source, e.g., from serum, blood, plasma, urine, or other bodily fluid, or a filtrate of a bodily fluid, from a test subject. The test subject is an animal, e.g., mammal, and more specifically human. In one specific case, the sample is from a biopsy.

[0018] The methods and devices provided herein offer an advantage of being able to detect an analyte in a small sample, an advantage that is of importance when, for example, the sample is from a scarce source or the sample is costly. Examples of such samples include from a biopsy, but also include other sources where large test volumes are not easily obtainable. Contemplated sample volumes for use in the disclosed methods include a volume of up to 12 μL, up to 10 μL, up to 8 μL, less than 6 μL, less than 5 μL, or about 2 μL to about 5 μL. In general, the sample has a lower volume limit, for example, of about 0.5 μL is, that is restricted only by the mechanical ability to measure and/or deliver the volume, and/or the ability to maintain the sample during the course of an assay.

[0019] In various embodiments, the analyte is a protein, a polypeptide, an antibody, an oligonucleotide, a polysaccharide, inorganic phosphate, ATP, GTP, UTP, CTP, ADP, GDP, cGMP, cAMP, CoA, FAD, FMN, FADP, NADH, NADPH, a thiol, glutathione, creatinine, creatine, hydrogen peroxide, a reactive oxygen species, a drug of abuse (e.g., cocaine, heroin, marijuana), ions (e.g., Ca^{2+}), one or more amino acid, or nitric oxide.

[0020] The analyte in the sample interacts with a precursor agent, which is added to the sample, to form a chromophore. The precursor agent, in the absence of the analyte, does not modulate the detectable signal. Interaction, or association, of the precursor agent with the analyte produces a chromophore that modulates the detectable signal. For example, the chromophore absorbs at least a portion of the detectable signal, resulting in a decrease in detectable signal.

[0021] Interaction of the precursor agent and the analyte can be, for example, formation of a covalent bond between the analyte and the precursor agent, formation of a non-covalent interaction (e.g., chelating, hydrogen bonding, van der Waals interaction), formation of the chromophore through a chemical reaction allowable due to the presence of both the precursor agent and the analyte.

[0022] Precursor agents contemplated include, but are not limited to, an enzyme substrate, a pH indicator, an ion chelator, and other molecules used in various biological detection assays.

[0023] The precursor agent is selected in view of the analyte to be detected, as a chromophore is formed by the association of the analyte and the precursor agent. For example, for detection of an acidic analyte, a precursor agent that produces a detectable color when protonated is a suitable choice. Precursor agents of this type include, without limitation, a triarylmethane dye or an acidic pH indicator. In other examples for detection of an oxidizing analyte, a precursor agent that produces a detectable color when oxidized is a suitable choice. Precursor agents of this type include, without limitation, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) which becomes colored when oxidized by, e.g., hydrogen peroxide or other oxidative species. In still other examples, for detecting reducing analyte, a precursor agent that produces a detectable color when reduced or in the presence of a reduced species is a suitable choice. For example, peptide bonds (e.g., as in peptides, polypeptides, proteins, and glycoproteins) can reduce copper (II) to copper (I), and copper (I) can chelate to two molecules of bicinchoninic acid to form a purple-colored

chromophore. Other suitable pairings of precursor agent and analyte are readily determined by the ordinarily skilled artisan, using common or known chromophore detection assays.

[0024] Non-limiting examples of enzyme substrates include substrates of horse radish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase (GAL), glucose-6-phosphate dehydrogenase, beta-N-acetylglucosaminidase, .beta.-glucuronidase, invertase, xanthine oxidase, luciferase (e.g., firefly, renilla, gaussia, cypridina, rail worm), and glucose oxidase (GO).

[0025] Examples of substrates of HRP include 3-amino-9-ethylcarbazole (AEC), Benzidine dihydrochloride (BDHC), Hanker-Yates reagent (HYR), Indophane blue (IB), tetramethylbenzidine (TMB), 4-chloro-1-naphtol (CN), .alpha.-naphtol pyronin (.alpha.-NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosphate (BCIP), Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT), tetranitro blue tetrazolium (TNBT), 5-bromo-4-chloro-3-indoxyl-beta-D-galactoside/ferro-ferricyanide (BCIG/FF), 5-amino-2-[3-[5-amino-1,3-dihydro-3,3-dimethyl-1-(4sulfobutyl)-2H indol-2-ylidene]-1-propenyl-3,3-dimethyl-1-(4sulfobutyl)-3H-Indolium. In one preferred embodiment the small molecule may be 5-amino-2-[3-[5-amino-1,3-dihydro-3,3-dimethyl-1-(4sulfobutyl)-2H-indol-2- -ylidene]-1-propenyl]-3,3-dimethyl-1-(4sulfobutyl)-3H-Indolium .

[0026] Examples of substrates of AP include Naphthol-AS-B1-phosphate/fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/fast red TR(NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/new fuschin (NABP/NF), bromochloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT), and 5-Bromo-4-chloro-3-indolyl-b-d-galactopyranoside (BCIG).

[0027] Contemplated pH indicators include, but are not limited to, phenolphthalein, methyl red, gentian violet (Methyl violet 10B), leucomalachite green, thymol blue, methyl yellow, bromophenol blue, Congo red, methyl orange, bromocresol green, azolitmin, bromocresol purple, bromothymol blue, phenol red, neutral red, naphtholphthalein, cresol red, thymolphthalein, alizarine, and litmus.

[0028] In various embodiments, the precursor agent is present at a concentration of 0.001 μM to 1000 μM, 0.01 μM to 500 μM, 0.1 μM to 100 μM, 0.5 μM to 50 μM, 0.0.7 μM to 25 μM, 1 μM to 25 μM, 5 μM to 25 μM, 10 μM to 25 μM, greater than 10 μM to 100 μM, 11 μM to 50 μM, 11 μM to 25 μM, or 11 μM to 20 μM. Specific concentrations of the

precursor agent contemplated include 0.001 μM, 0.005 μM, 0.01 μM, 0.05μM, 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 6 μM, 7 μM, 8 μM, 9 μM, 10 μM, 11 μM, 12 μM, 13 μM, 14 μM, 15 μM, 16 μM, 17 μM, 18 μM, 19 μM, 20 μM, 21 μM, 22 μM, 23 μM, 24 μM, 25 μM, 26 μM, 27 μM, 28 μM, 29 μM, 30 μM, 35 μM, 40 μM, 45 μM, 50 μM, 55 μM, 60 μM, 65 μM, 70 μM, 75 μM, 80 μM, 85 μM, 90 μM, 95 μM, 100 μM, 105 μM, 110 μM, 115 μM, 120 μM, 125 μM, 130 μM, 135 μM, 140 μM, 145 μM, 150 μM, 155 μM, 160 μM, 165 μM, 170 μM, 175 μM, 180 μM, 185 μM, 190 μM, 195 μM, 200 μM, 210 μM, 220 μM, 230 μM, 240 μM, 250 μM, 260 μM, 270 μM, 280 μM, 290 μM, 300 μM, 325 μM, 350 μM, 375 μM, 400 μM, 450 μM, 500 μM, 550 μM, 600 μM, 650 μM, 700 μM, 750 μM, 800 μM, 850 μM, 900 μM, 950 μM, and 1000 μM.

[0029] The detection agent, which is dissolved, partially dissolved, or suspended in the sample, is excited and produces a detectable signal. When the analyte is present, the signal is absorbed by a chromophore in the sample, which is formed from an association of the precursor agent and the analyte. By monitoring the detectable signal in the sample, one can determine whether an analyte is present, by noting whether the detectable signal changes, compared to a control detectable signal, where no analyte is present. In some cases, the detectable signal is a fluorophore and the detectable signal is fluorescence. In various cases, the detection agent is a luminescent agent, such as a chemiluminescent agent or a bioluminescent agent, and the detectable signal is luminescence. In some cases, the detectable signal is one or more of a detectable color, fluorescence, or luminescence.

[0030] In the disclosed methods, the detection agent is excited to produce a detectable signal. In some cases, excitation comprises exposing the detection agent to light that excites the detection agent. The light can be at a selected wavelength or narrow band of wavelengths, e.g., using a wavelength filter. The light can be a broad spectrum of light (e.g., UV light, infrared, a white lightbulb, a fluorescent light bulb, a xenon flash lamp, solid state laser, LED, mercury arc lamp, tungsten-halogen lamp).

[0031] A number of fluorescent or luminescent agents are contemplated. Many of them are commercially available, for example fluorescent stains Alexa fluorophores (Molecular Probes) and DyLight fluorophores (Thermo Fisher Scientific). Other examples include 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate, rhodamine, tetramethylrhodamine,

Cy2, Cy3, Cy5, AMCA, PerCP, R-phycoerythrin (RPE) allophycoerythrin (APC), Texas Red, Princeton Red, Green fluorescent protein (GFP) coated CdSe nanocrystallites, DNP, digoxiginin, ruthenium derivatives, luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines.

[0032] Specific luminescent agents contemplated include luciferase substrates, luminol, luciferin, and ruthenium complexes (e.g., $Ru(Bpy)_3^{2+}$).

[0033] Other fluorophore agents contemplated include without limitation 1,8-ANS (1-Anilinonaphthalene-8-sulfonic acid), 1-Anilinonaphthalene-8-sulfonic acid (1,8-ANS), 5-(and-6)-Carboxy-2', 7'-dichlorofluorescein pH 9.0, 5-FAM pH 9.0, 5-ROX (5-Carboxy-X-rhodamine, triethylammonium salt), 5-ROX pH 7.0, 5-TAMRA, 5-TAMRA pH 7.0, 5-TAMRA-MeOH, 6 JOE, 6,8-Difluoro-7-hydroxy-4-methylcoumarin pH 9.0, 6-Carboxyrhodamine 6G pH 7.0, 6-Carboxyrhodamine 6G, hydrochloride, 6-HEX, SE pH 9.0, 6-TET, SE pH 9.0, 7-Amino-4-methylcoumarin pH 7.0, 7-Hydroxy-4-methylcoumarin, 7-Hydroxy-4-methylcoumarin pH 9.0, Alexa 350, Alexa 405, Alexa 430, Alexa 488, Alexa 532, Alexa 546, Alexa 555, Alexa 568, Alexa 594, Alexa 647, Alexa 660, Alexa 680, Alexa 700, Alexa Fluor 430 antibody conjugate pH 7.2, Alexa Fluor 488 antibody conjugate pH 8.0, Alexa Fluor 488 hydrazide-water, Alexa Fluor 532 antibody conjugate pH 7.2, Alexa Fluor 555 antibody conjugate pH 7.2, Alexa Fluor 568 antibody conjugate pH 7.2, Alexa Fluor 610 R-phycoerythrin streptavidin pH 7.2, Alexa Fluor 647 antibody conjugate pH 7.2, Alexa Fluor 647 R-phycoerythrin streptavidin pH 7.2, Alexa Fluor 660 antibody conjugate pH 7.2, Alexa Fluor 680 antibody conjugate pH 7.2, Alexa Fluor 700 antibody conjugate pH 7.2, Allophycocyanin pH 7.5, AMCA conjugate, Amino Coumarin, APC (allophycocyanin) ,Atto 647, BCECF pH 5.5, BCECF pH 9.0, BFP (Blue Fluorescent Protein), BO-PRO-1-DNA, BO-PRO-3-DNA, BOBO-1-DNA, BOBO-3-DNA, BODIPY 650/665-X, MeOH, BODIPY FL conjugate, BODIPY FL, MeOH, Bodipy R6G SE, BODIPY R6G, MeOH, BODIPY TMR-X antibody conjugate pH 7.2, Bodipy TMR-X conjugate, BODIPY TMR-X, MeOH, BODIPY TMR-X, SE, BODIPY TR-X phallacidin pH 7.0, BODIPY TR-X, MeOH, BODIPY TR-X, SE, BOPRO-1, BOPRO-3, Calcein, Calcein pH 9.0, Calcium Crimson, Calcium Crimson Ca2+, Calcium Green, Calcium Green-1 Ca2+, Calcium Orange, Calcium Orange Ca2+, Carboxynaphthofluorescein pH 10.0, Cascade Blue, Cascade Blue BSA pH 7.0, Cascade Yellow, Cascade Yellow antibody conjugate pH 8.0, CFDA, CFP (Cyan Fluorescent Protein), CI-NERF pH 2.5, CI-NERF pH 6.0, Citrine, Coumarin, Cy 2, Cy 3, Cy 3.5, Cy 5, Cy 5.5, CyQUANT GR-DNA, Dansyl Cadaverine, Dansyl Cadaverine, MeOH,

DAPI, DAPI-DNA, Dapoxyl (2-aminoethyl) sulfonamide, DDAO pH 9.0, Di-8 ANEPPS, Di-8-ANEPPS-lipid, DiI, DiO, DM-NERF pH 4.0, DM-NERF pH 7.0, DsRed, DTAF, dTomato, eCFP (Enhanced Cyan Fluorescent Protein), eGFP (Enhanced Green Fluorescent Protein), Eosin, Eosin antibody conjugate pH 8.0, Erythrosin-5-isothiocyanate pH 9.0, Ethidium Bromide, Ethidium homodimer, Ethidium homodimer-1-DNA, eYFP (Enhanced Yellow Fluorescent Protein), FDA, FITC, FITC antibody conjugate pH 8.0, FlAsH, Fluo-3, Fluo-3 Ca2+, Fluo-4, Fluor-Ruby, Fluorescein, Fluorescein 0.1 M NaOH, Fluorescein antibody conjugate pH 8.0, Fluorescein dextran pH 8.0, Fluorescein pH 9.0, Fluoro-Emerald, FM 1-43, FM 1-43 lipid, FM 4-64, FM 4-64, 2% CHAPS, Fura Red Ca2+, Fura Red, high Ca, Fura Red, low Ca, Fura-2 Ca2+, Fura-2, high Ca, Fura-2, no Ca, GFP (S65T), HcRed, Hoechst 33258, Hoechst 33258-DNA, Hoechst 33342, Indo-1 Ca2+, Indo-1, Ca free, Indo-1, Ca saturated, JC-1, JC-1 pH 8.2, Lissamine rhodamine, LOLO-1-DNA, Lucifer Yellow, CH, LysoSensor Blue, LysoSensor Blue pH 5.0, LysoSensor Green, LysoSensor Green pH 5.0, LysoSensor Yellow pH 3.0, LysoSensor Yellow pH 9.0, LysoTracker Blue, LysoTracker Green, LysoTracker Red, Magnesium Green, Magnesium Green Mg2+, Magnesium Orange, Marina Blue, mBanana, mCherry, mHoneydew, MitoTracker Green, MitoTracker Green FM, MeOH, MitoTracker Orange, MitoTracker Orange, MeOH, MitoTracker Red, MitoTracker Red, MeOH, mOrange, mPlum, mRFP, mStrawberry, mTangerine, NBD-X, NBD-X, MeOH, NeuroTrace 500/525, green fluorescent Nissl stain-RNA, Nile Blue, EtOH, Nile Red, Nile Red-lipid, Nissl, Oregon Green 488, Oregon Green 488 antibody conjugate pH 8.0, Oregon Green 514, Oregon Green 514 antibody conjugate pH 8.0, Pacific Blue, Pacific Blue antibody conjugate pH 8.0, Phycoerythrin, PicoGreen dsDNA quantitation reagent, PO-PRO-1, PO-PRO-1-DNA, PO-PRO-3, PO-PRO-3-DNA, POPO-1, POPO-1-DNA, POPO-3, Propidium Iodide, Propidium Iodide-DNA, R-Phycoerythrin pH 7.5, ReAsH, Resorufin, Resorufin pH 9.0, Rhod-2, Rhod-2 Ca2+, Rhodamine, Rhodamine 110, Rhodamine 110 pH 7.0, Rhodamine 123, MeOH, Rhodamine Green, Rhodamine phalloidin pH 7.0, Rhodamine Red-X antibody conjugate pH 8.0, Rhodaminen Green pH 7.0, Rhodol Green antibody conjugate pH 8.0, Sapphire, SBFI-Na+, Sodium Green Na+, Sulforhodamine 101, EtOH, SYBR Green I, SYPRO Ruby, SYTO 13-DNA, SYTO 45-DNA, SYTOX Blue-DNA, Tetramethylrhodamine antibody conjugate pH 8.0, Tetramethylrhodamine dextran pH 7.0, Texas Red-X antibody conjugate pH 7.2, TO-PRO-1-DNA, TO-PRO-3-DNA, TOTO-1-DNA, TOTO-3-DNA, TRITC, X-Rhod-1 Ca2+, YO-PRO-1-DNA, YO-PRO-3-DNA, YOYO-1-DNA, and YOYO-3-DNA.

List of fluorescent polypeptides

Green Proteins	
EGFP	Emerald
CoralHue® Azami Green	CoralHue® Monomeric Azami Green
CopGFP	AceGFP
ZsGreen1	TagGFP
TurboGFP	mUKG
Blue/UV Proteins	
EBFP	TagBFP
Azurite	EBFP2
mKalama1	GFPuv
Sapphire	T-Sapphire
Cyan Proteins	
ECFP	Cerulean
AmCyan1	CoralHue® Midoriishi-Cyan
TagCFP	mTFP1
Yellow Proteins	
EYFP	Citrine
Venus	PhiYFP
TagYFP	TurboYFP
ZsYellow1	
Orange Proteins	
CoralHue® Kusabira-Orange	CoralHue® Monomeric Kusabira-Orange
mOrange	mKOκ
Red Proteins	
TurboFP602	tdimer2(12)
mRFP1	DsRed-Express
DsRed2	DsRed-Monomer
HcRed1	AsRed2
eqFP611	mRaspberry
mCherry	mStrawberry
mTangerine	tdTomato
TagRFP	JRed
Far Red Proteins	
TurboFP635	mPlum
AQ143	TagFP635
HcRed-Tandem	
Large Stokes Shift Proteins	
CoralHue® mKeima Red	CoralHue® dKeima Red
CoralHue® dKeima570	
Photoconvertible Proteins	
CoralHue® Dronpa	CoralHue® Kaede (green)
CoralHue® Kaede (red)	CoralHue® KikGR1 (green)
CoralHue® KikGR1 (red)	KFP-Red
PA-GFP	PS-CFP
PS-CFP	mEosFP

[0034] In various embodiments, the detection agent is present in a concentration of 0.001 μM to 1000 μM or 0.1 μM to 100 μM. In some specific cases, the concentration of the detection agent is 1 to 10 μM. Specific concentrations contemplated include 0.001 μM, 0.005 μM, 0.01 μM, 0.05μM, 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 6 μM, 7 μM, 8 μM, 9 μM, 10 μM, 11 μM, 12 μM, 13 μM, 14 μM, 15 μM, 16 μM, 17 μM, 18 μM, 19 μM, 20 μM, 21 μM, 22 μM, 23 μM, 24 μM, 25 μM, 26 μM, 27 μM, 28 μM, 29 μM, 30 μM, 35 μM, 40 μM, 45 μM, 50 μM, 55 μM, 60 μM, 65 μM, 70 μM, 75 μM, 80 μM, 85 μM, 90 μM, 95 μM, and 100 μM.

[0035] In various embodiments disclosed herein, the detectable signal decreases in the presence of the analyte and decreases in proportion to the concentration of the analyte. Thus, in some cases, the method provides a measurement of the concentration of the analyte in the sample. The detectable signal is compared to a standard curve prepared from detectable signals measured from samples of known concentrations of analyte. Such techniques are well understood by the person of skill in the art.

[0036] In some embodiments, the sample, precursor agent, and detecting agent are admixed together to form a mixture in a container. Some specific examples of containers include a well of a multi-well plate, a microfluidic chamber, or a microscope slide. In various cases, the container does not have an inherent emission or excitation spectrum that overlaps with an absorbance spectrum of a resulting chromophore (i.e., resulting from the association of the precursor agent and analyte). In various embodiments, the container comprises a detection agent having an emission or excitation spectrum that overlaps with an absorbance of a chromophore. In these embodiments, such a container is not considered a container having an "inherent" emission or excitation spectrum. In some specific cases, the container is a well in a black multi-well plate.

[0037] In various embodiments, the mixture of sample, precursor agent, and detecting agent has a volume of up to 50 μL, up to 40 μL, up to 30 μL, up to 25 μL, up to 20 μL, up to 15 μL, up to 14 μL, up to 13 μL, up to 12 μL, up to 11 μL, up to 10 μL, up to 9 μL, up to 8 μL, up to 7 μL, up to 6 μL, up to 5 μL, up to 4 μL, or up to 3 μL. . In general, the mixutre has a lower volume limit, for example, of about 0.5μL is, that is restricted only by the mechanical ability to measure and/or deliver the volume, and/or the ability to maintain the mixture during the course of an assay.

[0038] In some cases, the method comprises detecting multiple analytes by exciting multiple detection agents in the presence of multiple precursor agents. In such cases, each precursor agent forms a chromophore with single analyte, and each chromophore is discernible from other chromophores formed. By "multiple" analytes is meant more than one, e.g., two or more, three or more, four or more, five or more, six or more. In some embodiments, each detection agent is excited under different conditions from the other detection agents (e.g., exposure to different selected wavelengths). In some embodiments, two or more detection agents are excited under the same conditions but produce detectable signals (e.g., emission or excitation spectra) that are discernible so that each detectable signal is absorbed by a distinct chromophore (which in turn is formed by distinct precursor agent/analyte associations) and results in detection of a different analyte.

Devices for Detecting An Analytes in a Sample

[0039] Another aspect disclosed herein is a device that is used to detect an analyte in a sample. The device comprises a container having a detection agent associated therewith, wherein the detection agent has an emission or excitation spectrum that overlaps with an absorption spectrum of chromophore that results from an association of the analyte with a precursor agent. In some cases, the device, in the absence of the associated detection agent does not have an emission or excitation spectrum that overlaps with the absorption spectrum of the resulting chromophore. The container is, e.g., a well of a multi-well plate, a micro-fluidic chamber, or a microscope slide.

[0040] In various cases, the detection agent is a fluorophore or a luminescent agent (e.g., a bioluminescent agent or a chemiluminescent agent). In some cases, the detection agent can be any agent as disclosed herein.

[0041] The detection agent is embedded, coated, deposited, covalently attached, or otherwise associated with the container of the device. In some specific cases, upon contact with a sample, the detection agent is released from the container into the sample.

[0042] The agent is embedded in the device by, e.g., adding the agent during formation or production of the device. For example, the detection agent can be added prior to, e.g., injection molding of the device.

[0043] In some cases, the detection agent is coated or deposited onto the surface of the container of the device. Spraying the container with a solution comprising the detection agent allows for a coating of the detection agent on the container. The detection agent can be

deposited on the container by vapor deposition, solution coating, stamping, electrochemical vaporization, spray deposition, or the like. In various cases, the container surface is modified such that the detection agent is covalently attached to the surface, e.g., a glass container modified with a silane than is then modified with the detection agent. In some cases, the detection agent is covalently attached to the material of the container, such as by co-polymerization of the container material with the detection agent.

[0044] In some cases, the device comprises a second, third, or fourth detection agent having a different emission or excitation spectrum than that of the first detection agent. In such cases, the device is suitable for detection of multiple analytes, e.g., one analyte that can form a chromophore (e.g., in the presence of the proper precursor agent) that absorbs at a compatible wavelength as the emission/excitation of the first detection agent and a second analyte that can form a second chromophore (e.g., in the presence of the proper precursor agent) that absorbs at a compatible wavelength as the emission/excitation of the second detection agent.

[0045] Another aspect disclosed herein are kits for practicing the disclosed methods or comprising the disclosed devices. Such kits include, for example and without limitation, (1) a device as disclosed herein, and (2) an appropriate precursor agent. The device and precursor agent are appropriately paired such that the chromophore (which results from an association or interaction between the precursor agent and the analyte of interest) has an absorbance spectrum that overlaps with the detection agent in or of the device. In cases where the device comprises more than one detection agent, the kit further comprises a second precursor agent that is appropriately paired with the detection agent in order to provide the spectral overlap of (1) the absorbance spectrum of a second resulting chromophore and (2) the emission/excitation spectrum of the second detection agent.

[0046] Specific combinations of detection agent and precursor agent contemplated for devices disclosed herein include: fluorescein and quinaldine red; Dylight 594 and malachite green; fluroescein and malachite green; fluorescein and BCA; cascade blue and ABTS; Pacific blue and TMB; Dylight 594 and Bradford reagent; Dylight 549 and BCA.

[0047] The devices disclosed herein is used in various methods for detecting an analyte of interest, similar to as described above, except the detecting agent is, at least initially, associated with the container.

[0048] The invention will be more fully understood by reference to the following examples which detail exemplary embodiments of the invention. They should not, however, be construed as limiting the scope of the invention. All citations throughout the disclosure are hereby expressly incorporated by reference.

EXAMPLES

[0049] BCA assay protocol. Stock solutions of bovine serine albumin (BSA) were 1:2 serially diluted in Tris buffer (100 mM Tris, 20 mM KCl, 6 mM MgCl2, pH = 7.4) to create standards with concentrations of 8 μg/μL to 0.125 μg/μL. The Pierce BCA protein assay kit was purchased from Thermo Scientific (Waltham, MA) and the "working reagent" (WR) was prepared as instructed in the user's manual. The working reagent has the precursor agent (BCA). The 384-well, flat bottom, non-treated, transparent, sterile polystyrene plates (cat #3680) were purchased from Corning (Lowell, MA). The 384-well, low-volume, opaque white plates (cat #784075) were purchased from Greiner Bio-One (Frickenhausen, Germany). To perform the absorbance-based BCA assay, WR was added into the wells of the transparent 384-well plate, followed by the indicated amount of the BSA standard. After incubation at the indicated temperature for 30 minutes, the absorbance at 562 nm was measured on a SpectraMax M5 multi-mode microplate reader (Molecular Devices). The white plate-based BCA assay was performed similarly, except that the working reagent and BSA solution were added into the wells of white plates. After incubation for 30 minutes, the fluorescence signal (Ex = 435 nm, Em = 562 nm, cut-off = 550 nm) was measured on the SpectraMax M5. Note that we haven't exhaustively tested the influence of varying the emission wavelength. All liquid handling was performed with a Matrix Electronic Multichannel Pipette (Thermo Fisher). During the 30-minute incubations, the plates were covered with adhesive covers. For the experiments involving addition of soluble fluorophore (e.g. Figure 2B and Figure 2C), fluorescein (final concentration of 1 μM) was added to the WR, all incubations were carried out in the dark and the experiments were performed in black, opaque, non-treated, low volume 384-well plates (ThermoFisher Sci.). The DnaJ was purified essentially as previously described [7] and the E. coli lysate was prepared in Tris-HCl by sonication and clarification (13,2000 rpm, 15 min. 4 °C).

[0050] *Optimization of the spectral overlap*: This method is expected to rely on spectral overlap between the emitting fluorophore and the detecting chromophore [9]. Because the $Cu^{+1}(BCA)_2$ chromophore has an absorbance maxima at 562 nm, we white 384-well plates

were excited at wavelengths from 300 to 525 nm and it was found that 435 nm was optimal for producing a strong emission in this region (Figure 1A). Using these settings, the fluorescence from the plates was confirmed to exhibit a partial overlap with the BCA chromophore (Figure 1B). Based on these findings, it was expected that some of the fluorescence might be absorbed by the $Cu^{+1}(BCA)_2$ complex (Figure 1C). Similar models have been proposed for molybdate-malachite green and molybdate-quinaldine red chromophores [7; 9]. To test this idea for the BCA assay, known concentrations of a model analyte, bovine serum albumin (BSA), were added and the resulting fluorescence measured. Consistent with the model, increasing BSA levels quenched the apparent fluorescence signal (Figure 2A).

[0051] *Optimization of the BCA assay in low volume.* Based on these results, the performance of the fluorescence-based assay was optimized. Briefly, both the incubation temperature and the ratio between the WR and BSA solutions was varied, because these parameters have been previously found to impact performance of the BCA assay [1; 2; 4]. These experiments were carried out using BSA stock solutions between 0.125 and 8 μg/μL to explore a relatively wide dynamic range and the results were fit to linear regression in GraphPad PRISM. Further, the WR and BSA solutions were delivered side-by-side into either transparent or white plates, which it was anticipated would allow direct comparison of the platforms. In comparing the assays, both the goodness of fit (r2 value) and the relative signal intensity change (slope) was assessed.

[0052] In the first set of experiments, the performance of the absorbance-based protocol at routine volumes was confirmed. Consistent with the Pierce BCA kit protocol, mixing 60 μL of WR with 20 μL of BSA in transparent, normal volume 384-well plates and incubating at 37 °C produced results with good linearity (r2 = 0.992) and robust signal (slope = 0.167 ± 0.003). However, decreasing the total volume (to between 8 and 20 μL) significantly decreased sensitivity. Moreover, varying the assay parameters, such as temperature and volume ratios, failed to overcome this problem (Table 1A). This finding is consistent with previous reports of absorbance assays uniformly losing sensitivity in low volumes [6; 7]. Based on these studies, the next goal was to determine if the white plate-based approach could overcome the apparent volume limitations. It was found that, when the ratio of the WR to BSA solution was in the range of 5:1 or higher, the performance was generally improved. For example, using only 2 μL of BSA solution with 10 μL of WR at room temperature, the r2 = 0.993 and the slope = 0.089 ± 0.002. Under these conditions, the results were plotted from

both the transparent and white plates side-by-side, illustrating that the fluorescence method produced more favorable relationships between protein content and signal (Figure 2B). To test whether this approach would also work on a more realistic analyte, the 40 kDa DnaJ protein was purified from Escherichia coli, determined its concentration using a traditional BCA approach in a cuvette, and then compared the accuracy of the low volume methods under the optimized conditions (2 μL protein solution, 10 μL WR). These results mirrored those observed using BSA and confirmed that the fluorescence method was superior (Figure 2C). Finally, it was tested whether the total protein content of a crude cell lysate could be determined, so lysates of E. coli were prepated by sonication and added 2 μL of diluted sample to 10 μL of WR. These results confirmed that the fluorescence version of the BCA assay could be used on this complex mixture (Figure 2D). Thus, this simple method permitted accurate detection of protein concentration at approximately 5-fold lower sample volume.

[0053] Together, these studies suggest that the performance of the BCA assay in low volume is improved by taking advantage of the intrinsic fluorescence of white, polystyrene plates. However, the proposed mechanism was further tested (see Figure 1C), by adding 1.0 μM of fluorescein to the combination of BSA and WR solutions and analyzed its fluorescence in black, opaque, 384-well microtiter plates. When excited at 430 nm, fluorescein emits strongly at 520 nm; thus, we reasoned that it might also be adsorbed by the $Cu^{+1}(BCA)_2$ complex . Consistent with this model and similar to what was found with the white plates, addition of fluorescein (1 μM) improved detection of both BSA (Figure 2B) and DnaJ (Figure 2C) in low volume (r2 = 0.987 to 0.999). These findings suggest that free, soluble fluorophore can also overlap with the BCA chromophore, leading to improved sensitivity in low volume.

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What is Claimed is:

1. A method of detecting an analyte in a sample comprising

exciting a detection agent in the sample to produce a detectable signal, and

measuring the detectable signal;

wherein the detection agent is dissolved, at least partially dissolved, or suspended in the sample; the sample further comprises a precursor agent that has the ability to form a chromophore in the presence of the analyte, the chromophore having an absorbance spectrum that overlaps with the emission spectrum of the detection agent, and the detectable signal is modulated when the analyte is present in the sample.

2. The method of claim 1, wherein the detectable signal is modulated in proportion to the concentration of the analyte in the sample.

3. The method of claim 1 or 2, wherein the detectable signal decreases in the presence of the analyte.

4. The method of any one of claims 1-3, wherein the chromophore is formed by a covalent interaction between the analyte and the precursor agent.

5. The method of any one of claims 1-3, wherein the chromophore is formed by a non-covalent interaction between the analyte and the precursor agent.

6. The method of any one of claims 1-3, wherein the chromophore is formed by a reaction between the precursor agent and the analyte.

7. The method of any one of claims 1-6, wherein the sample has a volume of up to 12 μL.

8. The method of claim 7, wherein the sample has a volume of up to 8 μL.

9. The method of claim 8, wherein the sample has a volume of 2 μL to 5 μL.

10. The method of any one of claims 1-9, wherein the analyte is present in the sample in an amount of 0.1 μg/mL to 1 μg/mL.

11. The method of claim 10, wherein the analyte is present in the sample in an amount of less than 0.5 μg/mL.

12. The method of any one of claims 1-11, wherein the detection agent comprises one or more of a fluorophore and a luminescent agent.

13. The method of claim 12, wherein the luminescent agent is a chemiluminescent agent.

14. The method of claim 12, wherein the luminescent agent is a bioluminescent agent.

15. The method of claim 12, wherein the detection agent is a fluorophore.

16. The method of any one of claims 1-15, wherein the detectable signal comprises one or more of a detectable color, fluorescence, or luminescence.

17. The method of any one of claims 1-15, wherein the detection agent, precursor agent, or both is selected from the group consisting of bicinchoninic acid (BCA), fluorescein, rhodamine, quinaldine red, malachite green, Alexa 488, coomassie brilliant blue-R, coomassie brilliant blue-G, a silver stain, a gold stain, a zinc stain, a copper stain, a periodic acid-Schiff (PAS) stain, a quantum dot, a cyanine dye or derivative thereof, epicoccone, Fast Green, bromocresol green, amido black, a reuthenium metal chelate, a pyrene compound, a benzopyrillium compound, a monobromobimane, Ponceau Red, and Alcian Blue.

18. The method of any one of claims 1-17, wherein the detection agent is at a concentration of 0.1 μM to 100 μM.

19. The method of claim 18, wherein the detection agent is at a concentration of 1 μM to 10 μM.

20. The method of any one of claims 1-19, wherein the analyte is a protein, a polypeptide, an antibody, an oligonucleotide, a polysaccharide, inorganic phosphate, ATP, GTP, UTP, CTP, ADP, GDP, cGMP, cAMP, CoA, FAD, FMN, FADP, NADH, NADPH, a thiol, glutathione, creatinine, creatine, hydrogen peroxide, a reactive oxygen species, or nitric oxide.

21. The method of any one of claims 1-20, wherein exciting the detection agent occurs in a multi-well plate.

22. The method of claim 21, wherein the multi-well plate foes not have an inherent emission and/or excitation spectrum that overlaps with the absorbance spectrum of the chromophore.

23. The method of claim 21 or 22, wherein the multi-well plate is black.

24. The method of any one of claims 1-23, wherein the sample is from a human.

25. The method of claim 24, wherein the sample is a from a biopsy.

26. A method of measuring the concentration of an analyte in a sample comprising

admixing the sample, a precursor agent, and a detection agent in a container to form a mixture, wherein the detection agent is a fluorophore;

exciting the fluorophore to produce a detectable signal; and

measuring the detectable signal to measure the concentration of the analyte in the sample,

wherein

the fluorophore is dissolved, at least partially dissolved, or suspended in the mixture;

the precursor agent, in the presence of the analyte, forms a chromophore that has an absorbance spectrum that overlaps with the emission spectrum of the fluorophore, and

the detectable signal of the mixture decreases in the presence of the analyte.

27. The method of claim 26, wherein the detectable signal decreases in proportion to the concentration of the analyte in the mixture.

28. The method of claim 26 or 27, wherein the mixture has a volume of up to 12 μL.

29. The method of claim 28, wherein the mixture has a volume of less than 8 μL.

30. The method of claim 29, wherein the mixture has a volume of 2 μL to 5 μL.

31. The method of any one of claims 26-30, wherein the analyte has a concentration of 0.1 μg/mL to 1 μg/mL.

32. The method of claim 31, wherein the analyte has a concentration of less than 0.5 μg/mL.

33. The method of any one of claims 26-32, wherein the chromophore is produced by a covalent interaction between the analyte and the precursor agent.

34. The method of any one of claims 26-32, wherein the chromophore is produced by a non-covalent interaction between the analyte and the precursor agent.

35. The method of any one of claims 26-32, wherein the chromophore is produced by a reaction between the precursor agent and the analyte.

36. The method of any one of claims 26-35, wherein the container comprises a well in a multi-well plate.

37. The method of any one of claims 26-36, wherein the container exhibits little or no fluorescence.

38. The method of claim 36 or 37, wherein the container is black.

39. The method of any one of claims 26-38, wherein the sample has a volume of less than 6 μL.

40. The method of claim 39, wherein the sample has a volume of 2 μL to 5 μL.

41. The method of any one of claims 26-40, wherein the sample is from a human.

42. The method of claim 41, wherein the sample is from a biopsy.

43. The method of any one of claims 26-42, further comprising comparing the detectable signal of the mixture to a standard curve.

44. A device for detecting an analyte in a sample comprising a container having a detection agent associated therewith, wherein the detection agent has an emission spectrum that overlaps with an absorption spectrum of a chromophore that forms from an association of the analyte with a precursor agent.

45. The device of claim 44, wherein the container, in the absence of the associated detection agent, does not have an emission spectrum that overlaps with the absorption spectrum of a chromophore resulting from the association of the analyte and the precursor agent.

46. The device of claim 44 or 45, wherein the detection agent comprises a fluorophore or a luminescent agent.

47. The device of claim 46, wherein the luminescent agent is a chemiluminescent agent.

48. The device of claim 46, wherein the luminescent agent is a bioluminescent agent.

49. The device of any one of claims 44-48, wherein the chromophore forms from a covalent interaction between the analyte and the precursor agent.

50. The device of any one of claims 44-48, wherein the chromophore forms from a non-covalent interaction between the analyte and the precursor agent.

51. The device of any one of claim 44-48, wherein the chromophore forms from a reaction between the analyte and the precursor agent.

52. The device of any one of claims 44-51, wherein the detection agent is embedded in the container.

53. The device of any one of claims 44-51, wherein the detection agent is coated on a surface of the container.

54. The device of any one of claims 44-51, wherein the detection agent is covalently attached to a material of the container.

55. The device of any one of claims 44-51, wherein, upon contact with a sample comprising the analyte, the detection agent is released from the container into the sample.

56. The device of any one of claims 44-55, wherein the detection agent is selected from the group consisting of fluorescein, cascade blue, Pacific blue, Dylight 405, Dylight 594, and Dylight 549.

57. The device of any one of claims 44-56, further comprising a second detection agent associated with the container.

58. The device of any one of claims 44-57, wherein the container is a microfluidic chamber.

59. The device of any one of claims 44-57, wherein the container is a multi-well plate.

60. The device of any one of claims 44-57, wherein the container is a microscope slide.

61. A kit comprising the device of any one of claims 44-60, and the precursor agent.

62. The kit of claim 40, wherein the precursor agent comprises quinaldine red, malachite green, 2,2-azino-bis(3-ethylbenzothiazoine-6-sulfonate (ABTS), tetramethylbenzidine (TMB), Coomassie Brilliant Blue G-250 (Bradford Reagent), or bicinchoninic acid (BCA).

63. The kit of claim 61 or 62, wherein the detection agent is fluorescein and the precursor agent is quinaldine red.

64. The kit of claim 61 or 62, wherein the detection agent is Dylight 594 and the precursor agent is malachite green.

65. The kit of claim 61 or 62, wherein the detection agent is cascade blue and the precursor agent is ABTS.

66. The kit of claim 61 or 62, wherein the detection agent is Pacific blue and the precursor agent is TMB.

67. The kit of claim 61 or 62, wherein the detection agent is Dylight 594 and the precursor agent is Bradford Reagent.

68. The kit of claim 61 or 62, wherein the detection agent is Dylight 549 and the precursor agent is BCA.

69. A method of detecting the presence of an analyte in a sample comprising

providing the sample in the container of a device of any one of claims 44-60;

exciting the detection agent to produce a detectable signal, and

measuring the detectable signal to detect the presence of the analyte in the sample, wherein the detectable signal of the mixture decreases in the presence of the analyte.

70. The method of claim 69, further comprising adding the precursor agent to the sample in the container.

71. The method of claim 69 or 70, wherein the detection agent is a fluorophore or a luminescent agent.

72. The method of claim 71, wherein the luminescent agent is a chemiluminescent agent.

73. The method of claim 71, wherein the luminescent agent is a bioluminescent agent.

74. The method of claim 71, wherein the detection agent is a fluorophore.

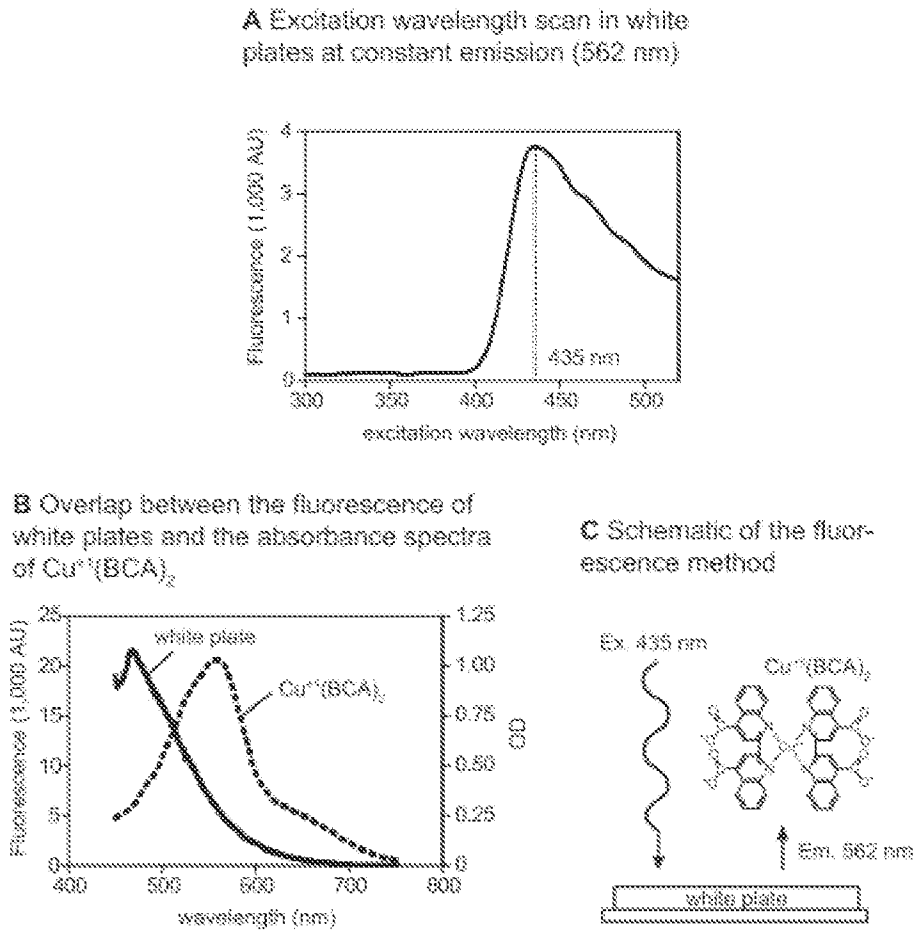

A Excitation wavelength scan in white plates at constant emission (562 nm)
Fluorescence (1,000 AU)
435 nm
excitation wavelength (nm)
B Overlap between the fluorescence of white plates and the absorbance spectra of Cu+1(BCA)2
white plate
Cu+1(BCA)2
Fluorescence (1,000 AU)
OD
wavelength (nm)
C Schematic of the fluorescence method
Ex. 435 nm
Cu+1(BCA)2
Em. 562 nm
white plate

FIGURE 1

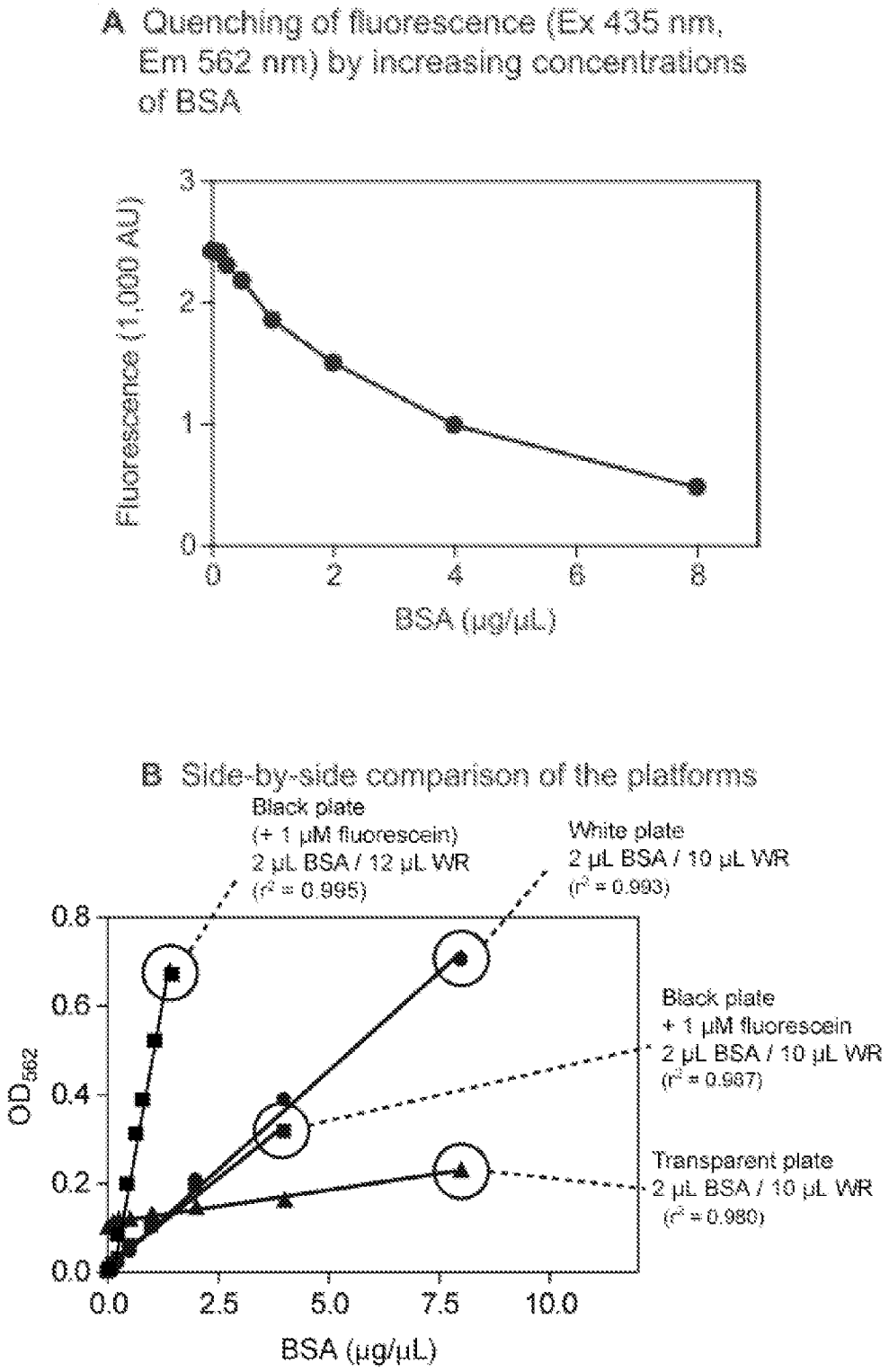
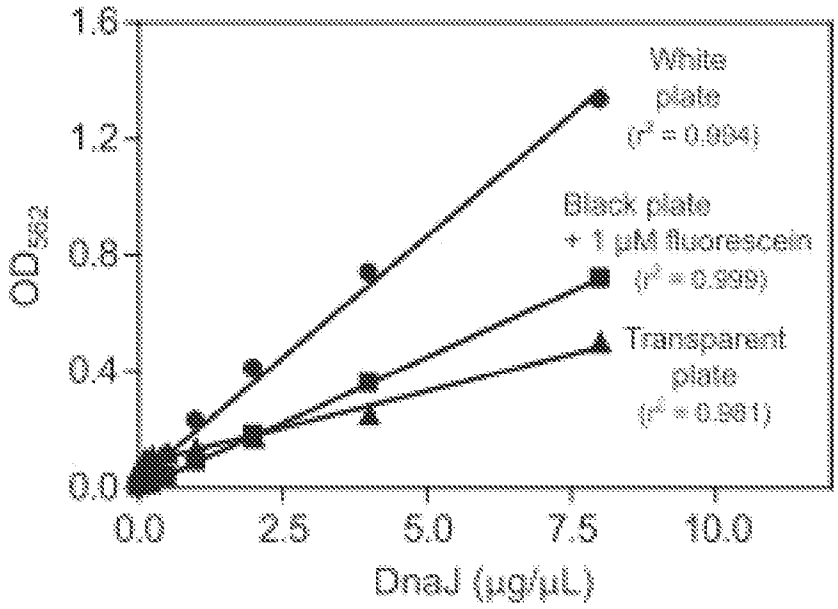

A Quenching of fluorescence (Ex 435 nm, Em 562 nm) by increasing concentrations of BSA
Fluorescence (1,000 AU)
BSA (µg/µL)
B Side-by-side comparison of the platforms
Black plate
(+ 1 µM fluorescein)
2 µL BSA / 12 µL WR
(r² = 0.995)
White plate
2 µL BSA / 10 µL WR
(r² = 0.993)
Black plate
+ 1 µM fluorescein
2 µL BSA / 10 µL WR
(r² = 0.987)
Transparent plate
2 µL BSA / 10 µL WR
(r² = 0.980)
OD562
BSA (µg/µL)

FIGURE 2

C Detection of a model, recombinant, purified protein (*E. coli* DnaJ)



D Detection of protein content in a bacterial cell lysate

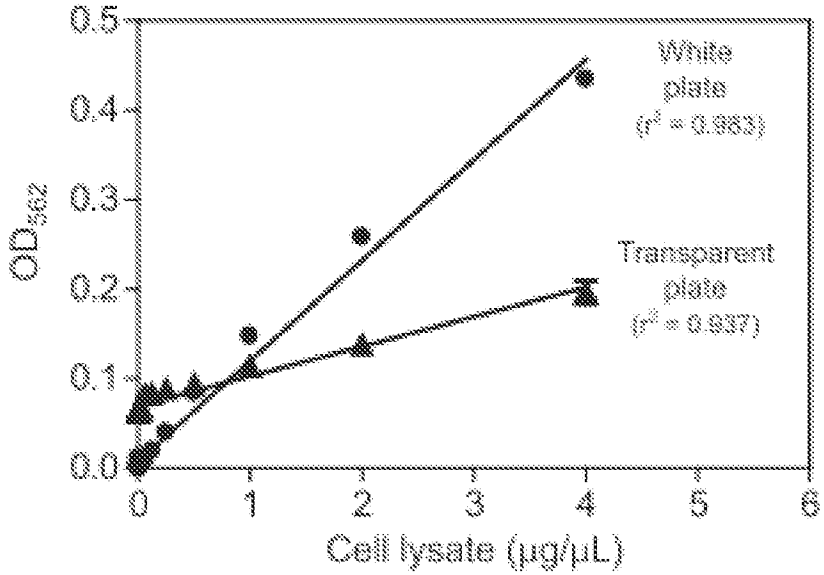


FIGURE 2 (CONT)