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(54) **APTAMER-BASED COLORIMETRIC SENSOR SYSTEMS**

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(57) **ABSTRACT**

The present invention provides an aptamer-based colorimetric sensor system for determining the presence and optionally the concentration of an analyte in a sample. Methods of utilizing the sensor system and kits that include the sensor also are provided. The sensor utilizes a linker and oligonucleotide functionalized particles to form an aggregate, which disaggregates in response to the analyte.

**30 Claims, 16 Drawing Sheets**

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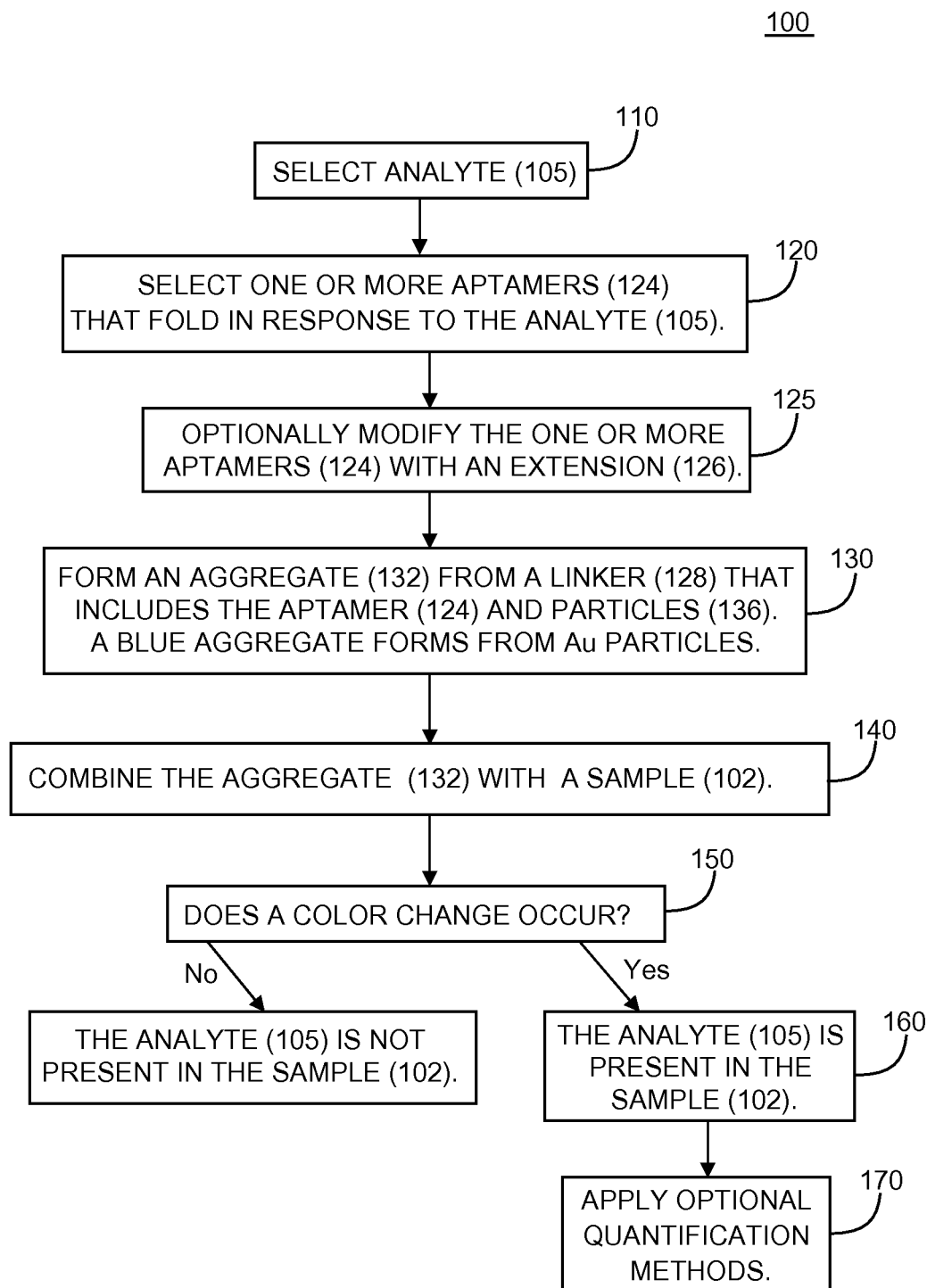
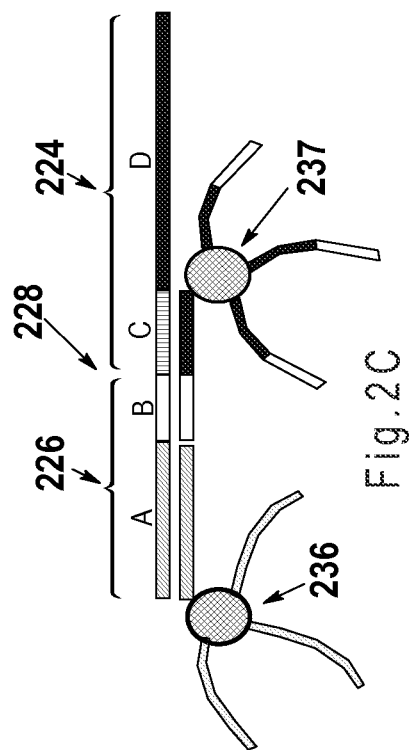
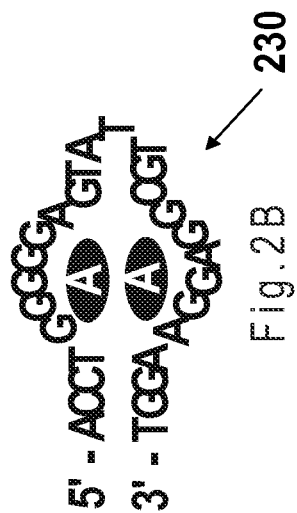
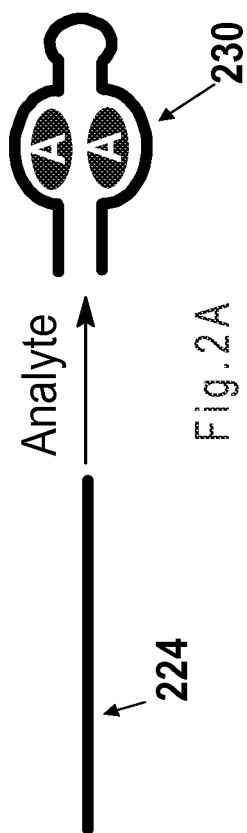
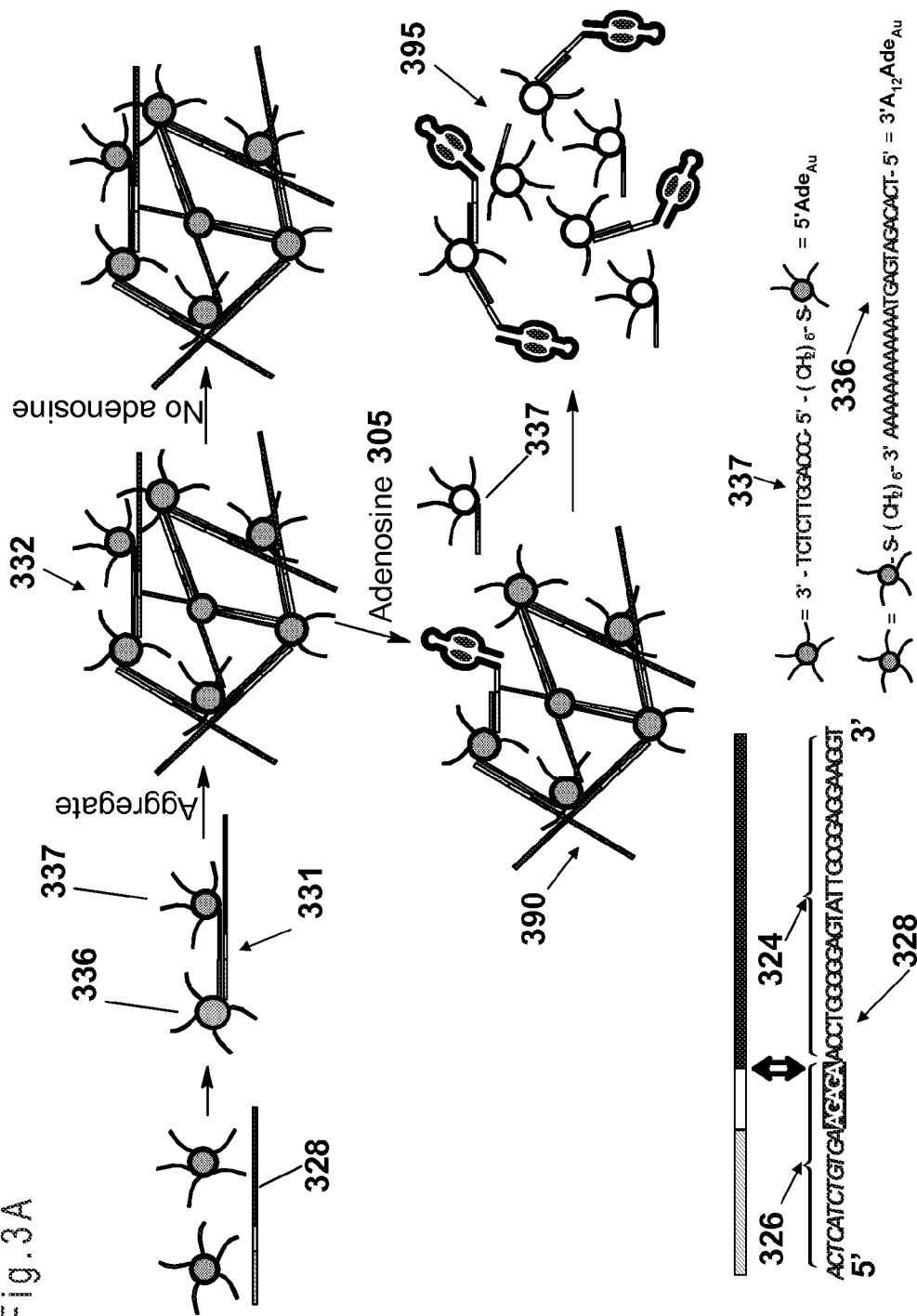
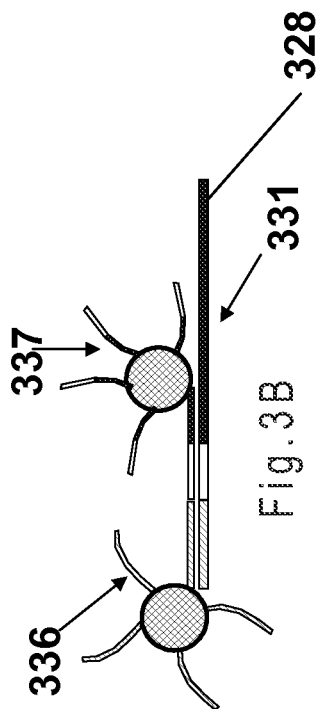


Fig.1

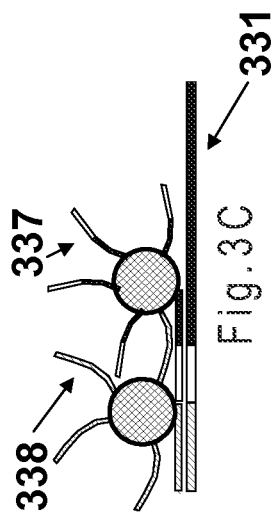


3A  
3  
3  
3  
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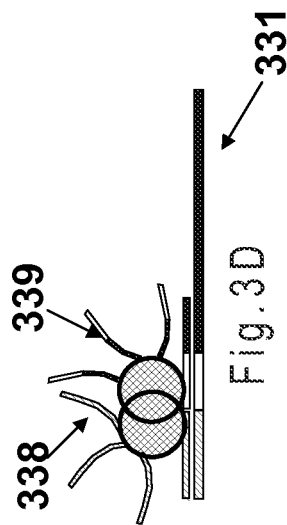




TAIL-TO-TAIL



HEAD-TO-TAIL



HEAD-TO-HEAD

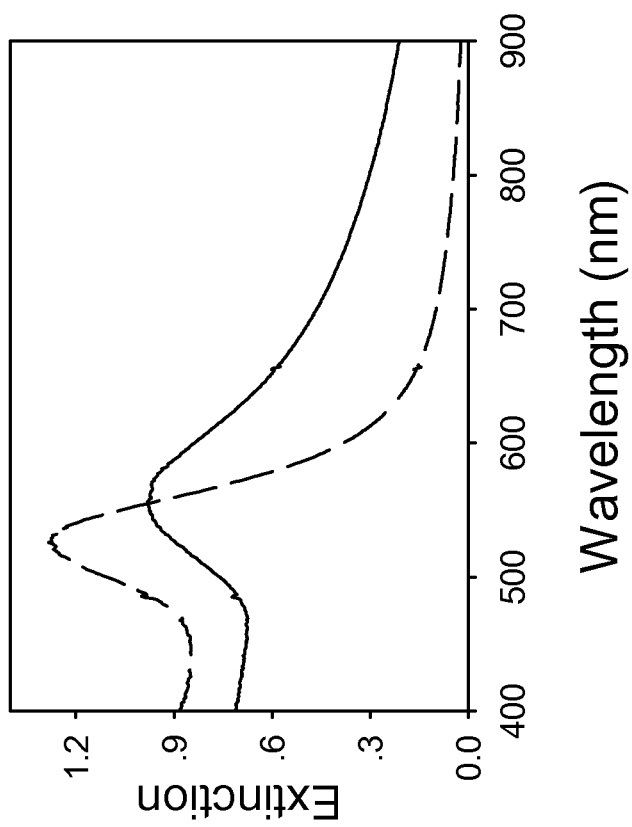


Fig. 4

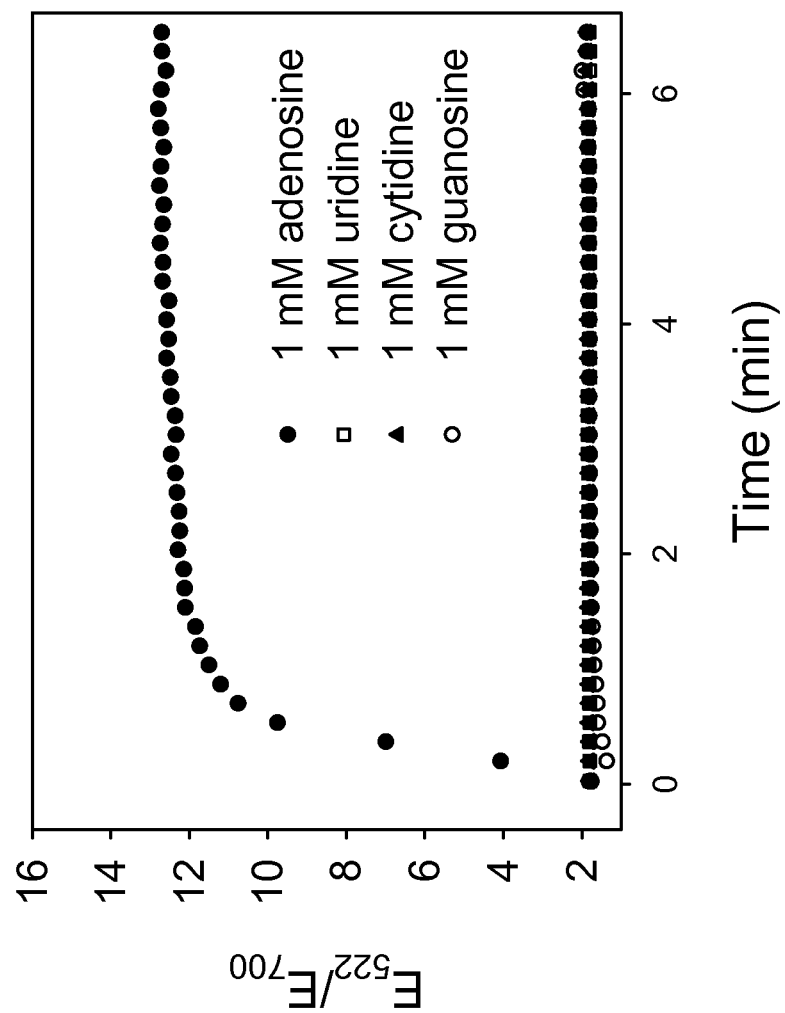


Fig. 5A

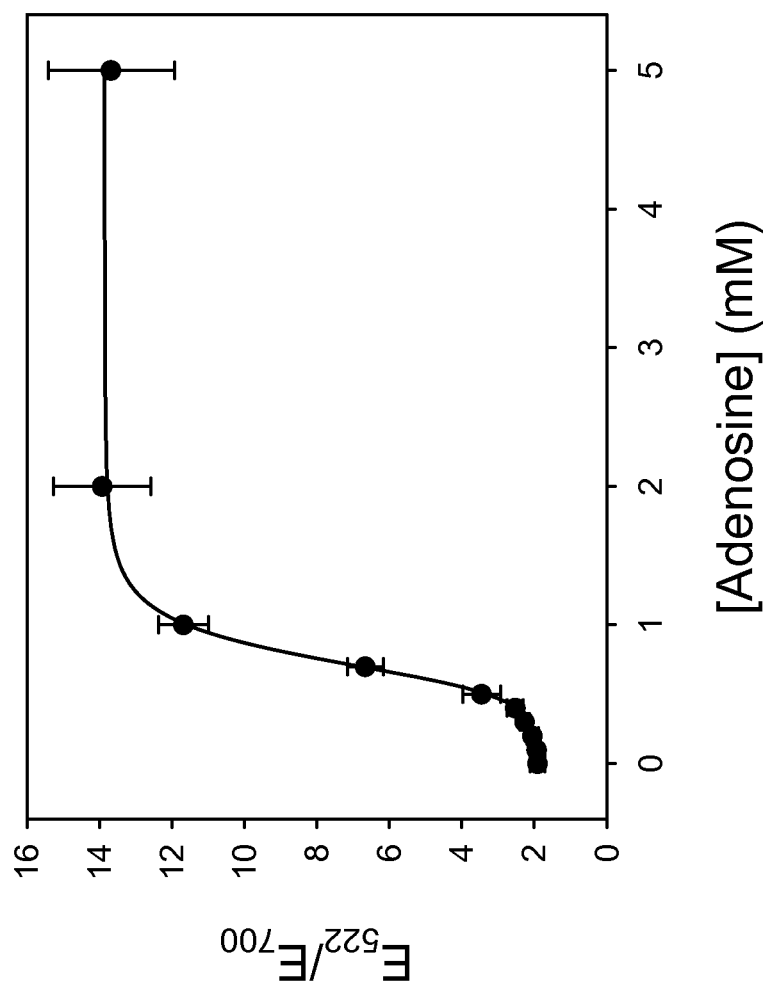


Fig. 5B



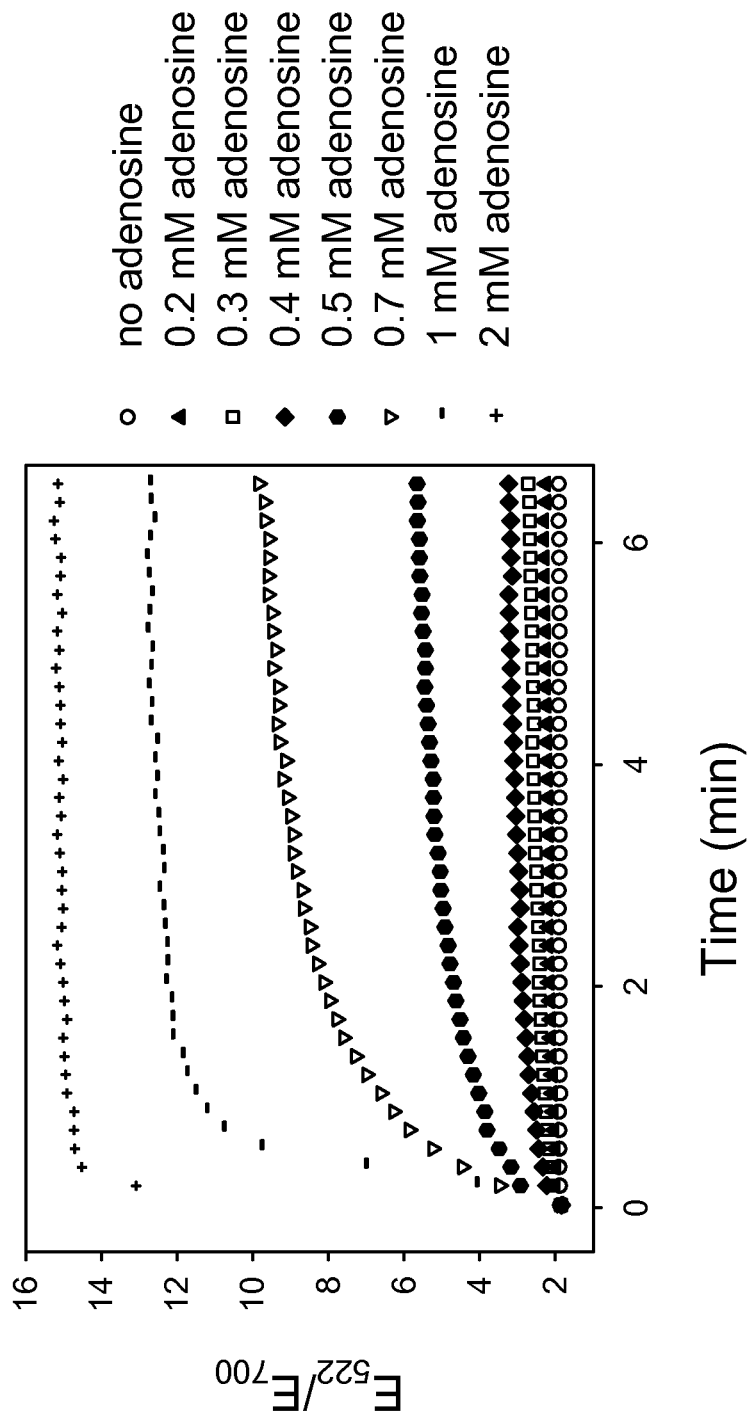


Fig. 5C

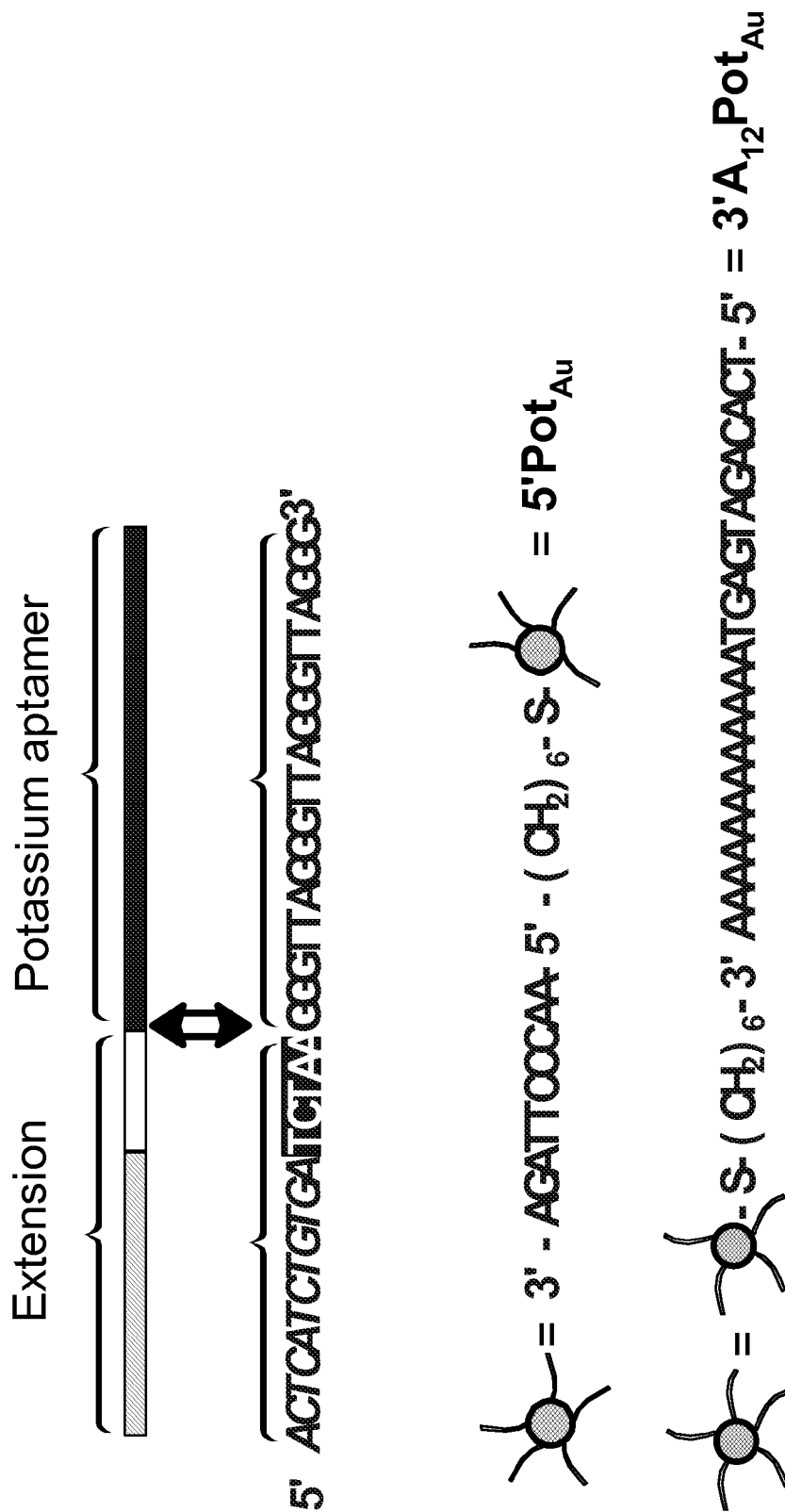
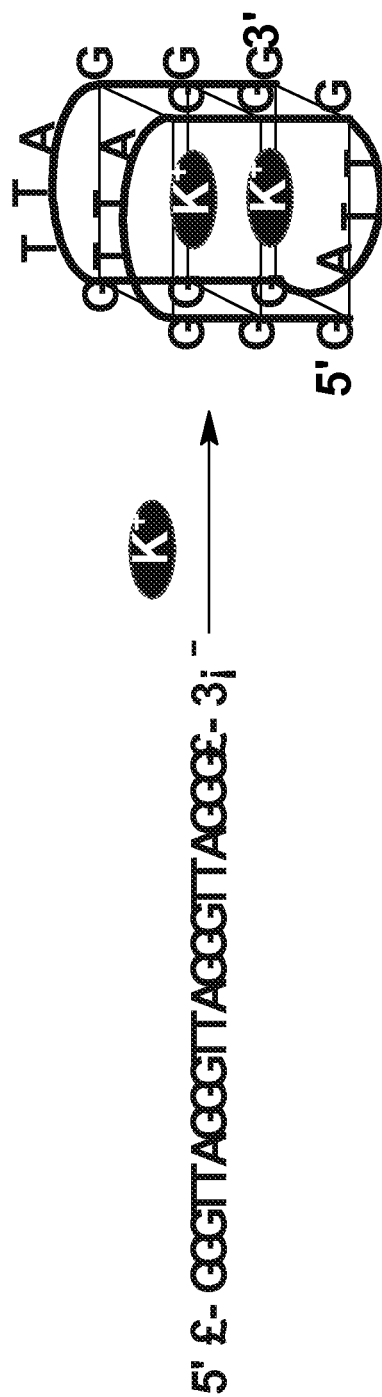


Fig. 6A





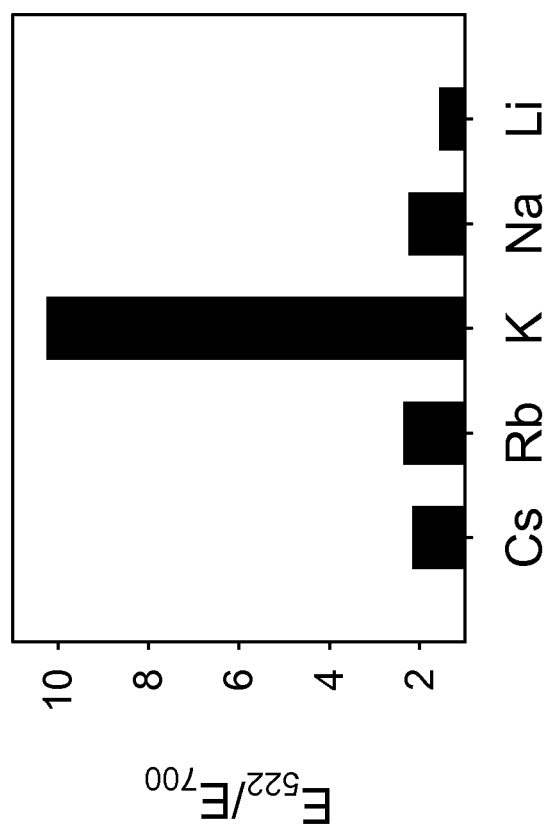


Fig. 6C

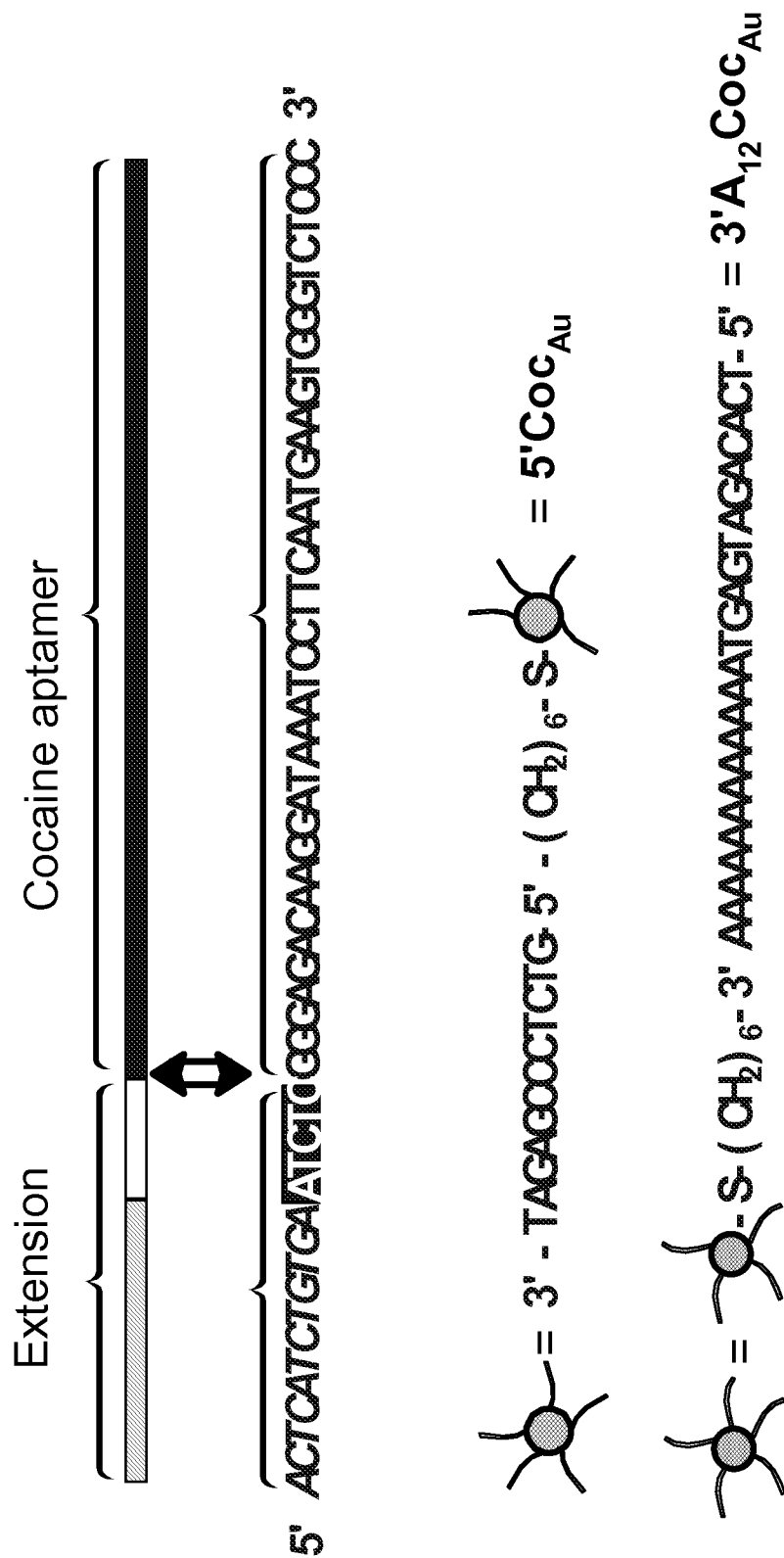
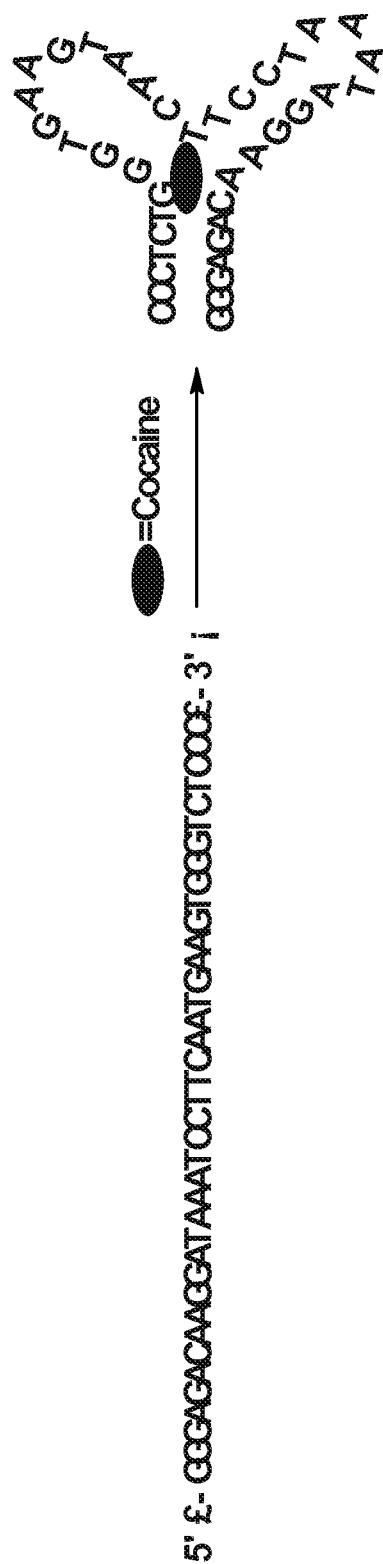


Fig. 7A



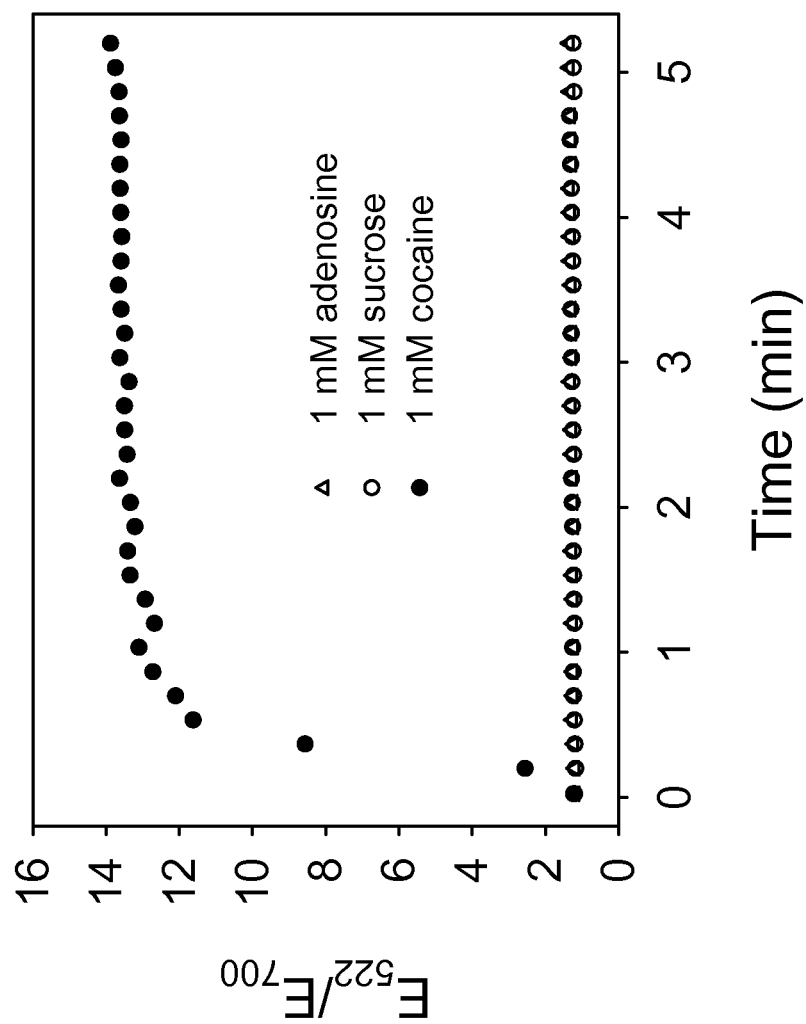


Fig. 8A

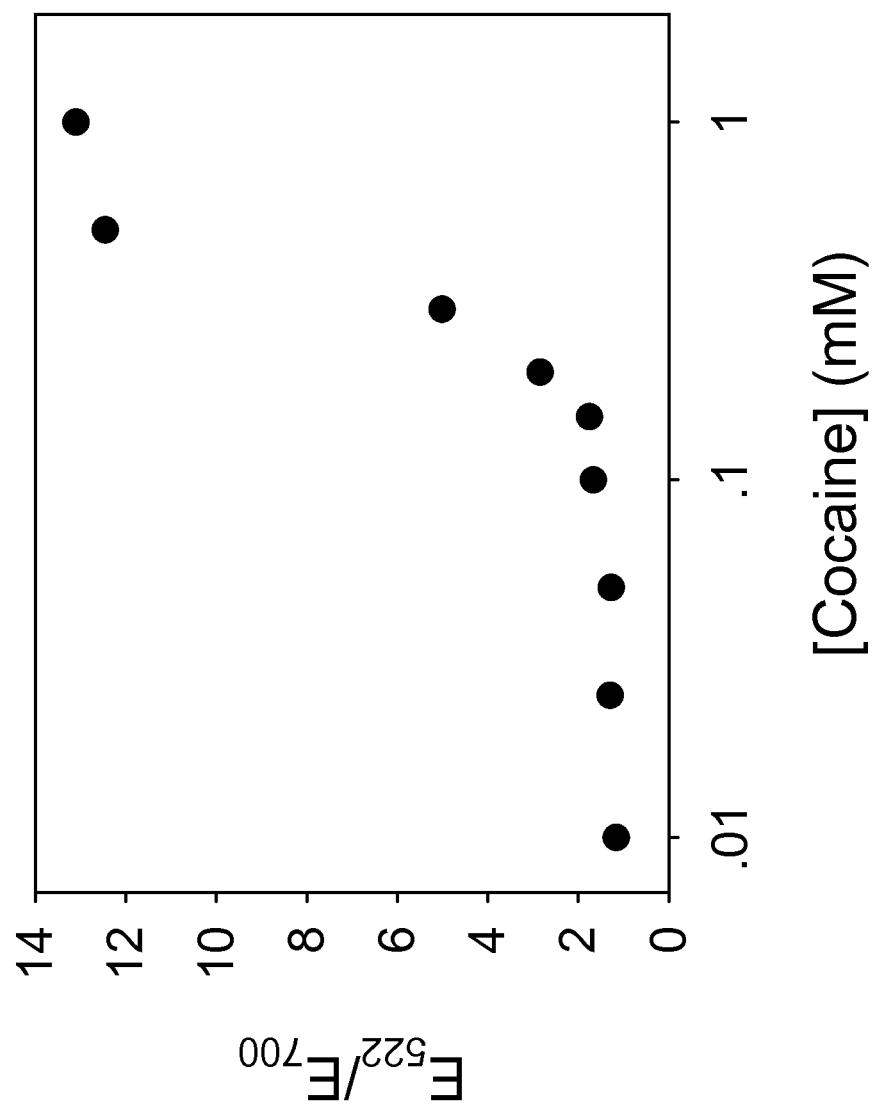


Fig. 8B



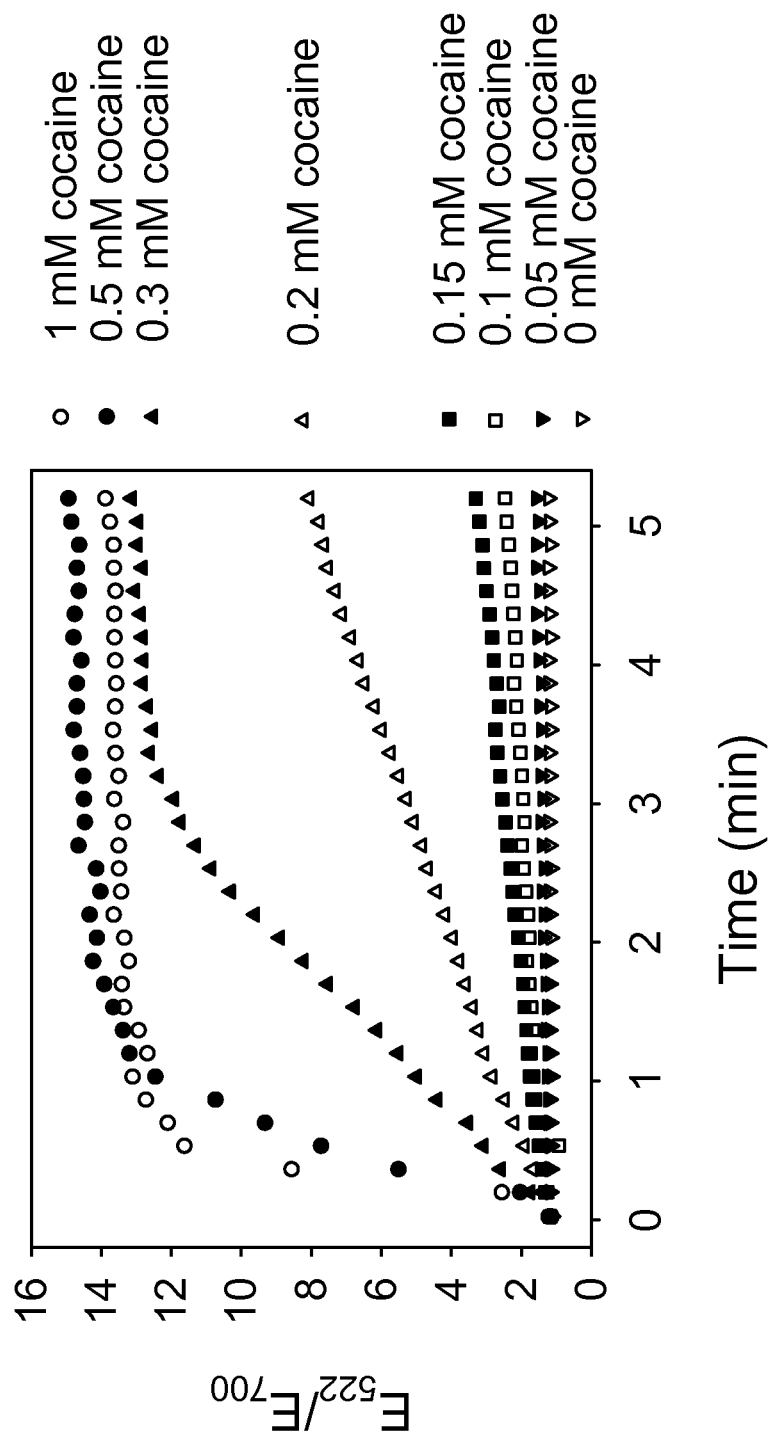


Fig. 8C

1

**APTAMER-BASED COLORIMETRIC SENSOR SYSTEMS****CROSS REFERENCE TO RELATED APPLICATION**

This application is a continuation application of U.S. patent application Ser. No. 11/202,380, filed Aug. 11, 2005, now U.S. Pat. No. 7,892,734 entitled "APTAMER-BASED COLORIMETRIC SENSOR SYSTEMS."

**FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT**

This subject matter of this application may have been funded in part under the following research grants and contracts: National Science Foundation Contract Numbers CTS-0120978 and DMR-0117792. The U.S. Government may have rights in this invention.

**SEQUENCE LISTING**

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 26, 2011, is named ILL76CON.txt and is 14,947 bytes in size.

**BACKGROUND**

The ability to determine the presence of an analyte in a sample is of significant benefit. For example, many metals and metal ions, such as lead, mercury, cadmium, chromium, and arsenic, pose significant health risks when present in drinking water supplies. To prevent the contamination of drinking and other water supplies, it is common to test industrial waste-streams before their release to the water treatment plant. Biological fluids, such as blood and those originating from body tissues, also may be tested for a variety of analytes to determine if the body has been exposed to harmful agents or if a disease state exists. For example, the need to detect trace amounts of anthrax in a variety of samples has recently emerged.

Colorimetric methods are commonly used for the detection of metals and ions in soil, water, waste-streams, biological samples, body fluids, and the like. In relation to instrument based methods of analysis, such as atomic absorption spectroscopy, colorimetric methods tend to be rapid and require little in the way of equipment or user sophistication. For example, colorimetric tests are available to aquarists that turn darker shades of pink when added to aqueous samples containing increasing concentrations of the nitrate ( $\text{NO}_3^-$ ) ion. In this manner, colorimetric tests show that the analyte of interest, such as nitrate, is present in the sample and also may provide an indicator of the amount of analyte in the sample through the specific hue of color generated. While conventional colorimetric tests are extremely useful, they only exist for a limited set of analytes, and often cannot detect very small or trace amounts of the analyte.

As can be seen from the above description, there is an ongoing need for colorimetric sensor systems that can identify trace amounts of a broader scope of analytes and that increase the reliability of the analysis.

**SUMMARY**

A sensor system for detecting an analyte includes a linker comprising an aptamer that folds in response to the analyte

2

and second particles coupled to a second oligonucleotide that is complementary to at least a portion of the aptamer. The linker may include an extension where a first oligonucleotide coupled to first particles is complementary to at least a portion of the extension.

A method of detecting an analyte includes combining an aggregate with a sample to detect a color change responsive to the analyte. The aggregate may include a linker and second particles. The aggregate also may include first particles and the linker may include an extension.

A kit for detecting an analyte includes a first container containing a system for forming aggregates that includes second particles and a linker including an aptamer, which folds in response to the analyte. The second particles are coupled to second oligonucleotides that are complementary to at least a portion of the aptamer.

A method for determining the sensitivity and selectivity of an aptamer to an analyte includes combining an aggregate with the analyte, detecting a color change responsive to the analyte, and determining if the DNA strand folded to provide the color change. The aggregate includes second particles and a linker including a DNA strand. The aggregate also may include first particles. The linker may include an extension.

In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided.

The term "sample" is defined as a composition that will be subjected to analysis that is suspected of containing the analyte of interest. Typically, a sample for analysis is in a liquid form, and preferably the sample is an aqueous mixture. A sample may be from any source, such as an industrial sample from a waste-stream or a biological sample, such as blood, urine, or saliva. A sample may be a derivative of an industrial or biological sample, such as an extract, a dilution, a filtrate, or a reconstituted precipitate.

The term "analyte" is defined as one or more substance potentially present in the sample. The analysis determines the presence, quantity, or concentration of the analyte present in the sample.

The term "colorimetric" is defined as an analysis where the reagent or reagents constituting the sensor system produce a color change in the presence or absence of an analyte.

The term "light-up" refers to a colorimetric sensor system that undergoes a desired color change in response to an analyte present in a sample.

The term "light-down" refers to a colorimetric sensor system that does not undergo a color change when an analyte is present in a sample, but does undergo a desired color change in the absence of the analyte.

The term "sensitivity" refers to the smallest increase in an analyte concentration that is detectable by the sensor system (resolution) or to the lowest concentration limit at which a sensor system can differentiate a signal responsive to the analyte from a background signal (detection limit). Thus, the more sensitive a sensor system is to an analyte, the better the system is at detecting lower concentrations of the analyte.

The term "selectivity" refers to the ability of the sensor system to detect a desired analyte in the presence of other species.

The term "hybridization" refers to the ability of a first polynucleotide to form at least one hydrogen bond with at least one second nucleotide under low stringency conditions.

The term "aptamer" refers to a strand of nucleic acids that undergoes a conformational change in response to an analyte.

The term “conformational change” refers to the process by which an aptamer adopts a tertiary structure from another state. For simplicity, the term “fold” may be substituted for conformational change.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention can be better understood with reference to the following drawings and description. The components in the figures are not necessarily to scale and are not intended to accurately represent molecules or their interactions, emphasis instead being placed upon illustrating the principles of the invention.

FIG. 1 represents a colorimetric analysis for determining the presence and optionally the concentration of an analyte in a sample.

FIG. 2A depicts an aptamer that depends on two analyte molecules to fold.

FIG. 2B depicts the base pairs for an aptamer depending on two adenosine molecules to fold (SEQ ID NO: 57).

FIG. 2C depicts an aptamer joined to an extension to form a linker.

FIG. 3A represents the disaggregation of an aggregate in the presence of an adenosine analyte (SEQ ID NOS: 46, 45, 44).

FIG. 3B represents the tail-to-tail hybridization of oligonucleotide functionalized particles with a linker.

FIG. 3C represents the head-to-tail hybridization of oligonucleotide functionalized particles with a linker.

FIG. 3D represents the head-to-head hybridization of oligonucleotide functionalized particles with a linker.

FIG. 4 is a graph relating extinction ratios to the wavelengths of light emitted from a sample by aggregated (solid line) and disaggregated (dashed line) gold nanoparticles.

FIG. 5A is a graph showing the change in extinction ratios over time for samples containing guanosine (○), cytidine (▲), uridine (□), and adenosine (●).

FIG. 5B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the adenosine analyte after one minute.

FIG. 5C is a graph depicting the extinction ratios for multiple adenosine concentrations over a 6 minute time period.

FIG. 6A provides the sequences of the extension and aptamer portions of a linker and of the oligonucleotide functionalized particles for a potassium ion sensor system (SEQ ID NOS: 52, 53, and 44, respectively in order of appearance).

FIG. 6B depicts the aptamer folding in the presence of the K(I) analyte (SEQ ID NO: 1).

FIG. 6C depicts the extinction ratio of the potassium ion sensor for multiple metal ions.

FIG. 7A provides the sequences of the extension and aptamer portions of a linker and of the oligonucleotide functionalized particles for a cocaine sensor system (SEQ ID NOS: 54, 58 and 44, respectively in order of appearance).

FIG. 7B depicts the aptamer folding in the presence of the cocaine analyte (SEQ ID NO: 59).

FIG. 8A is a graph showing the change in extinction ratios over time for samples containing adenosine (Δ), sucrose (○), and cocaine (●).

FIG. 8B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the cocaine analyte after 1 minute of aggregation.

FIG. 8C is a graph depicting the extinction ratios for multiple cocaine analyte concentrations over a 5 minute time period.

#### DETAILED DESCRIPTION

Aptamers may be easier to isolate than nucleic acid based catalysts. The simpler structure of aptamers in relation to nucleic acid enzymes also may allow for the design of analyte sensor systems for which nucleic acid enzymes are not available. The present invention makes use of the discovery that by selecting the hybridization strength between the folded and unfolded conformations of an aptamer and an oligonucleotide functionalized particle, the particle may be released in response to an analyte. In this manner, a light-up colorimetric sensor is provided that undergoes a desired color change in response to a selected analyte at room temperature, thus overcoming a disadvantage of the sensor system disclosed in U.S. Ser. No. 10/144,679.

FIG. 1 represents a colorimetric analysis 100 for determining the presence and optionally the concentration of an analyte 105 in a sample 102. In 110, the analyte 105 for which the method 100 will determine the presence/concentration of is selected.

In one aspect, the analyte 105 may be any ion that causes an aptamer 124 to fold. In another aspect, the analyte 105 may be any metal ion that causes an aptamer 124 to fold. Preferable monovalent ions having a +1 formal oxidation state (I) include  $\text{NH}_4^+$ , K(I), Li(I), Tl(I), and Ag(I). Preferable divalent metal ions having a +2 formal oxidation state (II) include Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Zn(II), Cd(II), Cu(II), Pb(II), Hg(II), Pt(II), Ra(II), Sr(II), Ni(II), and Ba(II). Preferable trivalent and higher metal ions having +3 (III), +4 (IV), +5 (V), or +6 (VI) formal oxidation states include Co(III), Cr(III), Ce(IV), As(V), U(VI), Cr(VI), and lanthanide ions. More preferred analyte ions include monovalent metal ions and metal ions that are toxic to living organisms, such as Ag(I), Pb(II), Hg(II), U(VI), and Cr(VI).

In another aspect, the analyte 105 may be any biomolecule that causes the aptamer 124 to fold. Preferable biomolecules include large biomolecules, such as proteins (e.g. proteins related to HIV, hCG-hormone, insulin), antibodies, growth factors, enzymes, virus (e.g. HIV, small pox), viral derived components (e.g. HIV-derived molecules), bacteria (e.g. anthrax), bacteria derived molecules and components (e.g. anthrax derived molecules), or cells. Preferable biomolecules also may include small biomolecules, such as amino acids (e.g. arginine), nucleotides (e.g. ATP, GTP), neurotransmitters (e.g. dopamine), cofactors (e.g. biotin), peptides, or amino-glycosides.

In another aspect, the analyte 105 may be any organic molecule that causes the aptamer 124 to fold. Preferable organic molecules include drugs, such as antibiotics and theophylline, or controlled substances, such as cocaine, dyes, oligosaccharides, polysaccharides, glucose, nitrogen fertilizers, pesticides, dioxins, phenols, 2,4-dichlorophenoxyacetic acid, nerve gases, trinitrotoluene (TNT), or dinitrotoluene (DNT).

Once the analyte 105 is selected, the one or more aptamer 124 is selected that folds in response to the analyte 105. The aptamer selection 120 may be performed by in vitro selection, directed evolution, or other method known to those of ordinary skill in the art. The aptamer selection 120 may provide one or more aptamers that demonstrate enhanced folding in the presence of the selected analyte 105 (thereby providing sensor sensitivity). The selection 120 also may exclude aptamers that fold in the presence of selected analytes, but

that do not fold in the presence of non-selected analytes and/or other species present in the sample **102** (thereby providing sensor selectivity).

For example, an aptamer may be selected that specifically binds K(I), while not significantly binding Na(I), Li(I), Cs(I), Rb(I), or other competing metal ions. In one aspect, this may be achieved by isolating aptamers that bind K(I), then removing any aptamers that bind Na(I), Li(I), Cs(I), or Rb(I). In another aspect, aptamers that bind Na(I), Li(I), Cs(I), or Rb(I) are first discarded and then those that bind K(I) are isolated. In this manner, the selectivity of the aptamer may be increased.

The aptamer **124** includes a nucleic acid strand that folds in the presence of the analyte **105**. In one aspect, the folding may be considered the conversion of a primary or duplex structure to a tertiary structure. The base sequence of the aptamer may be designed so that the aptamer may undergo at least partial hybridization with at least one oligonucleotide functionalized particle. In this aspect, at least a portion of the base sequence

of the aptamer **124** may be complementary to at least one oligonucleotide of the oligonucleotide functionalized particle.

The aptamer **124** may be formed from deoxyribonucleotides, which may be natural, unnatural, or modified nucleic acids. Peptide nucleic acids (PNAs), which include a polyamide backbone and nucleoside bases (available from Biosearch, Inc., Bedford, Mass., for example), also may be useful.

Table I below lists analytes, the aptamer or aptamers that bind with and fold in response to that analyte, and the reference or references where the sequence of each aptamer is described. The analyte binding region of these, and other, aptamers may be adapted for use in a linker **128**. For example, the non-analyte binding region of the cocaine aptamer, given as SEQ ID NO: 10 in Table I below, may be modified to provide the aptamer GGGAGACAAGGATAAAATCCT-TCAATGAAGTGGGTCTCCC (SEQ ID NO: 56) and included in the linker **128**.

TABLE I

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO:)	Ref
Metal ions	K(I)	GGGTTAGGGTTAGGGTTAGGG (SEQ ID NO: 1)	1
	Zn(II)	AGGCGAGGUGAAAUGAGCGUAAUAGCCU (SEQ ID NO: 2)	2
	Ni(II)	GGGAGAGGAUACUACACGUGAUAGUCAGGGAAC AUGACAAACACAGGACUUGCGAAAUCAGUGU UUUGCCAUUGCAUGUAGCAG AAGCUUCCG (SEQ ID NO: 3)	3
	Cibacron blue	GGGAGAATTCCCGCGGAGAGCCACCTGGCT TTGAACTCTATGTTATTGGGTGGGGAACTTA AGAAAATACCACCTTCAACATTACCGCCCTT CAGCCTGCCAGCGCCTGCAGCCCGGAAGCTT (SEQ ID NO: 4)	4
	Malachite green	GGAUCCCGACUGGCGAGAGCCAGGUA ACGAU GGAUCC (SEQ ID NO: 5)	5
	Sulforhodamine B	CCGGCCAAGGGTGGGAGGGGGCCGG (SEQ ID NO: 6)	6
Small organic molecules	Biotin	AUGGCACCGACCAUAGGUCGGGUUGCCAGAGG UUCCACACUUUCAUGGAAAGCCUAUGC (SEQ ID NO: 7)	7
	Theophylline	GGCGAUACCGAGCCGAAAGGCCUUGGCAGCGUC (SEQ ID NO: 8)	8
	Adenine	GAUAGGACGAUUAUCGAAAACACAGAUUGGA CCUGGUUAACGAUCCAU (SEQ ID NO: 9)	9
	Cocaine	GGGAGACAAGGATAAAATCCTTCAATGAAGTGGG TCGACA (SEQ ID NO: 10)	10
	Dopamine	GGGAAUUCGCGUGUGCGCGGAGAGGGAA UAUAGAGGCCAGCACAUAGUGAGGCCUCCUCC C (SEQ ID NO: 11)	11
	Arginine	GGGAGCUCAGAAUAAACGCUCAGGAGGACCGU GCACUCCUCGAACAUAUUCGAGAUAGACACGGA UCCUGC (SEQ ID NO: 12)	12
Amino acids	Citrulline	GACGAGAAGGAGUGCGUUAUACUAGCGGUUA GGUCACUCGUC (SEQ ID NO: 13)	13
	ATP	ACCTGGGGAGTATTGCGGAGGAAGGT (SEQ ID NO: 14)	14
Nucleosides & nucleotides	cAMP	GGAAGAGAUGGCGACUAAAACGACUUGUCGC (SEQ ID NO: 15)	15
	GTP	UCUAGCAGUUCAGGUAACCACGUAAGAUACGGG UCUAGA (SEQ ID NO: 16)	16
	Guanosine	GGGAGCUCAGAAUAAACGCUCACCCGACAGAU CGGCAACGCCNUGUUUUCGACANGAGACACCGA UCCUGCACCAAAGCUCC (SEQ ID NO: 17)	17

TABLE I-continued

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO:)	Ref
RNA	Adenosine	ACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID NO: 18)	18
	TAR-RNA	GCAGTCTCGTCGACACCCAGCAGCGCATGTAAC TCCCATACATGTGTGTGCTGGATCCGACGCAG (SEQ ID NO: 19)	19
Biological cofactors	CoA	GGGCACGAGCGAAGGGCAUAAGCUGACGAAAGU CAGACAAGACAUGGUGCCC (SEQ ID NO: 20)	20
	NMN	GGAAACCAACUAGGCGUUUGAGGGGAUUCGGCC ACGGUACAACCCUC (SEQ ID NO: 21)	21
	FAD	GGGCAUAAGGUUUUAAUCCAUACAAGUUUAC AAGAAAGAUCA (SEQ ID NO: 22)	22
	Porphyrin	TAAACTAAATGTGAGGGTGGGACGGGAAGAAG TTTA (SEQ ID NO: 23)	23
	Vitamin B12	CCGGUGCGCAUAACCACCUCAGUGCGAGCAA (SEQ ID NO: 24)	24
Amino-glycosides	Tobramycin	GGGAGAAUCCGACCAGAAGCUUUGGUUGUCUU GUACGUUCACUGUUACGAUUGUUGUUAGUUUAA CUACACUUUGCAAUCGCAUAUGUGCGUCUACAU GGAUCCUCA (SEQ ID NO: 25)	25
Oligo-saccharides	Cellobiose	GCGGGGTTGGGCGGGTGGGTTGCTGGGCAGGG GGCGAGTG (SEQ ID NO: 26)	26
Poly-saccharides	Sephadex	UACAGAAUGGGUUGGUAGGCAUACCUAAUCGAG AAUGAUA (SEQ ID NO: 27)	27
Antibiotics	Viomycin	GGAGCUCAGCCUUCACUGCAAUGGGCCGCUAGG UUGAUGUGCAGUGAAGUCAGCUGAGGCCAGGG CUGAAAGGAUCGCCUCCUCGACUCGUGGCACC ACGGUCGGAUCCAC (SEQ ID NO: 28)	28
	Streptomycin	GGAUCCGAUUUGGACUUCUGCCCAGGGGCACC ACGGUCGGAUCC (SEQ ID NO: 29)	29
	Tetracycline	GGCCUAAAACAUACCAGAUUUCGAUCUGGAGAG GUGAAGAAUUCGACCACCUAGGCCGGU (SEQ ID NO: 30)	30
	Vasopressin	ACGTGAATGATAGACGTATGTGAGTTGCTGTG TGCGGATGAACGT (SEQ ID NO: 31)	31
Peptides	Substance P	GGGAGCUGAGAAUAAACGCUAAGGGCAACGCG GGCACCCGACAGGUGCAAAAACGCACCGACGC CCGGCCGAAGAAGGGGAUUCGACAUGAGCCCG GAUCCGGC (SEQ ID NO: 32)	32
Enzymes	HIV Rev Transscriptase	UCCGUUUUCAGUCGGGAAAAACUG (SEQ ID NO: 33)	33
	Human thrombin	GGTTGGTGTGGTTGG (SEQ ID NO: 34)	34
Growth factors	VEGF <sub>165</sub>	GCGGUAGGAAGAAUUGGAAGCGC (SEQ ID NO: 35)	35
Transcription factors	NF-KB	GGGAUAUCCUCGAGACAUAAGAAACAAGAUAGA UCCUGAAACUGUUUUAGGUUGGCCGAUCUUCU GCUCGAGAAUGCAUGAAGCGUCCAUUUUUU (SEQ ID NO: 36)	36
Antibodies	Human IgE	GGGGCACGTTTATCCGTCCCTCCTAGTGGCGTG CCCC (SEQ ID NO: 37)	37
Gene Regulatory factors	Elongation factor Tu	GGGGCUAUUGACUCAGCGGUUCGACCCCGCU UAGCUCCACCA (SEQ ID NO: 38)	38

TABLE I-continued

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO:)	Ref
Cell adhesion molecules	Human CD4	UGACGUCCUUAGAAUUGCGCAUUCUCAC ACAGGAUCUU (SEQ ID NO: 39)	39
cells	YPEN-1 endothelial	ATACCAGCTTATTCAATTAGGCGGTGCATTGTG GTTGGTAGTATACATGAGGTTTGGTTGAGACTA GTCGCAAGATATAGATAGTAAGTGCAATCT (SEQ ID NO: 40)	40
Viral/bacterial components	Anthrax spores	Sequences are not given	41
	Rous sarcoma virus	AGGACCCUCGAGGGAGGUUGCGCAGGGU (SEQ ID NO: 42)	42

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After selecting an appropriate aptamer or aptamers in **120**, a linker **128** is formed that includes the aptamer **124**. In one aspect, the aptamer **124** may serve directly as the linker **128**. In another aspect, the linker **128** may be formed by joining the aptamer **124** with one or more extensions **126**.

The extension **126** may be any nucleic acid sequence that may be joined with the aptamer **124**, that may undergo at least partial hybridization with one or more oligonucleotide functionalized particles, and that is compatible with the analysis **100**. In this aspect, at least a portion of the base sequence of the extension **126** may be complementary to at least one oligonucleotide of one or more oligonucleotide functionalized particle. In one aspect, solid phase synthesis may be used to join the aptamer **124** with the extension **126** to form the linker **128**. In another aspect, after the aptamer **124** portion of the linker **128** is synthesized, the synthesis is continued to form the extension **126**. Similarly, the linker **128** may be extended with the aptamer **124** sequence.

Preferably, the extension **126** includes from 1 to 100 bases. In one aspect, at least 50, 70, or 90% of the bases present in the

extension **126** are capable of hybridizing with a complementary portion of a first oligonucleotide functionalized particle, such as the TGAGTAGACACT-5' (SEQ ID NO: 43) portion of particle **336** in FIG. 3A, while at least 50, 35, 25, or 10% of the bases present in the extension are capable of hybridizing with a second oligonucleotide functionalized particle, such as particle **337** in FIG. 3A.

After selecting or synthesizing the linker **128**, an aggregate **132** may be formed in **130**. The aggregate **132** includes the linker **128** and oligonucleotide functionalized particles **136**. Considering the physical size of its components, the aggregate **132** may be quite large.

The linker **128** hybridizes with the oligonucleotide functionalized particles **136** and includes the aptamer **124** and may include the extension **126**. For example, if first and second oligonucleotide functionalized particles have base sequences of 3'-AAAAAAAAAAAAATGAGTAGACACT (SEQ ID NO: 44) and 5'-CCCAGGTTCTCT (SEQ ID NO: 45), respectively, an appropriate sequence for the linker **128** that includes the aptamer **124** that folds in the presence of an

13

adenosine analyte and the extension **126** may be 5'-ACTCATCTGTGAAGAGAACCTGGGGGAGTAT-TGCGGAGGAAGGT (SEQ ID NO. 46).

For the adenosine analyte, the extension **126** portion of the linker **128** is the ACTCATCTGTGAAGAGA (SEQ ID NO: 47) portion of the sequence, which allows the extension **126** to hybridize with twelve bases of the first functionalized particle and five bases of the second functionalized particle. Similarly, the aptamer **124** portion of the linker **128** is the ACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID NO: 18) portion of the sequence, which allows the ACCTGGG (SEQ ID NO: 48) portion of the aptamer **124** to hybridize with the TGGACCC (SEQ ID NO: 49) portion of the second functionalized particle.

Because the particles **136** demonstrate distance-dependent optical properties, the particles are one color when closely held in the aggregate **132** and undergo a color change as the distance between the particles increases. For example, when the particles **136** are gold nanoparticles, the aggregate **132** displays a blue color in aqueous solution that turns red as disaggregation proceeds.

Disaggregation occurs when the aptamer **124** portion of the linker **128** binds with and folds in response to the analyte **105**. When the aptamer **124** folds, a portion of the hybridization with the second oligonucleotide functionalized particles is lost. This hybridization loss allows the second oligonucleotide functionalized particles to separate from the aggregate **132**. Thus, as the particles **136** diffuse away from the aggregate **132**, the solution changes from blue to red.

The particles **136** may be any species that demonstrate distance-dependent optical properties and are compatible with the operation of the sensor system. Suitable particles may include metals, such as gold, silver, copper, and platinum; semiconductors, such as CdSe, CdS, and CdS or CdSe coated with ZnS; and magnetic colloidal materials, such as those described in Josephson, Lee, et al., *Angewandte Chemie*, International Edition (2001), 40(17), 3204-3206. Specific useful particles may include ZnS, ZnO, TiO<sub>2</sub>, AgI, AgBr, HgI<sub>2</sub>, PbS, PbSe, ZnTe, CdTe, In<sub>2</sub>S<sub>3</sub>, In<sub>2</sub>Se<sub>3</sub>, Cd<sub>3</sub>P<sub>2</sub>, Cd<sub>3</sub>As<sub>2</sub>, InAs, and GaAs.

In a preferred aspect, the particles are gold (Au) nanoparticles and have an average diameter from 5 to 70 nanometers (nm) or from 10 to 50 nm. In a more preferred aspect, gold nanoparticles having an average diameter of from 10 to 15 nm are functionalized to the oligonucleotides.

For a more detailed treatment of how to prepare oligonucleotide functionalized gold particles, See U.S. Pat. No. 6,361,944; Mirkin, et al., *Nature* (London) 1996, 382, 607-609; Storhoff, et al., *J. Am. Chem. Soc.* 1998, 120, 1959-1064; and Storhoff, et al., *Chem. Rev.* (Washington, D.C.) 1999, 99, 1849-1862. While gold nanoparticles are presently preferred, other species that undergo a distance-dependent color change, such as inorganic crystals, quantum dots, and the like also may be attached to oligonucleotides.

In **140** the aggregate **132** may be combined with the sample **102**. In **150** the sample **102** is monitored for a color change. If a color change does not occur, then the analyte **105** is not present in the sample **102**. If a color change does occur in **160**, the analyte **105** is present in the sample **102**. The color change signifies that the analyte **105** caused the aptamer **124** to fold, thus allowing the particles **136** to diffuse away from the aggregate **132** and into the solution of the sample **102**. Thus, the analysis **100** provides a "light-up" sensor system because a color change occurs in the presence of the analyte **105**.

The rate at which a substantially complete color change occurs in response to the analyte **105** may be considered the response time of the sensor system. In one aspect, the color

14

change may be considered substantially complete when the absorption peak in the visible region increases by 20%. In another aspect, the color change may be considered substantially complete when the extinction coefficient at 522 nm over 700 nm increases by 100% for gold particles. Preferable response times for the sensor system are from 1 second to 60 minutes or from 2 seconds to 10 minutes. More preferable response times for the sensor system are from 5 seconds to 2 minutes or from 8 to 12 seconds. Preferable temperature ranges for operation of the sensor system are from 0° to 60° or from 15° to 40° C. More preferable ranges for operation of the sensor system are from 23° to 37° or from 25° to 30° C. In another aspect, when the analysis **100** is conducted from 23° to 37° C., a preferable response time may be less than 2 minutes or from 1 to 12 seconds.

The degree the color changes in response to the analyte **105** may be quantified by colorimetric quantification methods known to those of ordinary skill in the art in **170**. Various color comparator wheels, such as those available from Hach Co., Loveland, Colo. or LaMotte Co., Chestertown, Md. may be adapted for use with the present invention. Standards containing known amounts of the selected analyte may be analyzed in addition to the sample to increase the accuracy of the comparison. If higher precision is desired, various types of spectrophotometers may be used to plot a Beer's curve in the desired concentration range. The color of the sample may then be compared with the curve and the concentration of the analyte present in the sample determined. Suitable spectrophotometers include the Hewlett-Packard 8453 and the Bausch & Lomb Spec-20.

In yet another aspect, the method **100** may be modified to determine the sensitivity and selectivity of an aptamer to the analyte **105**. In this aspect, one or more DNA strand suspected of being an aptamer responsive to the analyte **105** is selected in **120**. The DNA strand may or may not be modified with the extension **126** in **125**. In **130**, an aggregate is formed from the DNA strands and appropriate particles. In **140**, the aggregates are combined with the analyte **105**. If the aggregate undergoes a color change, then the DNA strand is an appropriate aptamer sequence for the target **105**. In this manner, multiple aptamers selected in **120** may be tested for use in a colorimetric sensor system.

FIG. 2A depicts an aptamer **224** that depends on two analyte molecules to undergo a conformational change to adopt a folded structure **230**. FIG. 2B depicts the specific base pairs of the aptamer **224** that forms the folded structure **230** in response to two adenosine molecules. While this specific aptamer sequence relies on two molecules of the adenosine analyte to fold, other aptamers may fold in response to a single analyte molecule.

FIG. 2C depicts the aptamer **224** joined to an extension **226** to form a linker **228**. The extension **226** includes A and B portions, where the A portion includes enough complementary bases to form stable hybridization with the oligonucleotide of a first particle **236**. For example, a non-complementary poly-adenine chain (A<sub>12</sub>) including 12 adenine bases may be appended to the complementary sequence of the first particle **236** to enhance hybridization stability. The sequence for the B portion of the extension **226** may be selected to eliminate linkers that inherently form stable secondary structures. A computer program, such as M-fold available at ([www.bioinfospa.edu/applications/mfold/](http://www.bioinfospa.edu/applications/mfold/)) (M. Zuker. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31 (13), 3406-15, (2003)) or others, may be used to predict stable linkers for elimination. For example if a stable sequence for a linker is ACTCATCTGTGAAGATGACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID



15

NO: 50), by substituting the underlined TG bases for GA bases to give the sequence ACTCATCTGTGAAGA GAACCTGGGGGAGTATTGCGGAGGAAGGT (SEQ ID NO: 51), the linker may be rendered inherently unstable.

The aptamer **224** includes C and D portions. In one aspect, the hybridization stability of the combined B portion of the extension **226** and the C portion of the aptamer **224** with the second particle **237** may be less than that for A and the first particle **236**. In a preferred aspect, the melting temperature of this C+B/second particle hybridization is higher than the temperature at which the sensor system is to be used. In another preferred aspect, the melting temperature of a B portion hybridized to the oligonucleotide sequence of the second particle **237** is less than the temperature at which the sensor system is to be used. In another preferred aspect, the stability of a C+D+analyte complex should be greater than the hybridization stability of C with the second particle **237**. In another aspect, the sequences of B and C are as short as is compatible with the operation of the sensor system.

FIG. 3A depicts the disaggregation of an aggregate **332** in the presence of an adenosine analyte **305**. The aggregate **332** is formed from multiple aggregate units **331**. Each of the aggregate units **331** is formed from a linker **328**, which is hybridized to the 3' and 5' thiol-oligonucleotide functionalized particles **336** and **337**, respectively. The linker **328** includes an aptamer portion **324** and an extension portion **326**. The 3'-A<sub>12</sub>Ade<sub>Au</sub> (SEQ ID NO: 44) particle **336** hybridizes with the extension **326**, while the 5'-Ade<sub>Au</sub> particle **337** hybridizes with the extension **326** and the aptamer **324** to from the aggregate unit **331**.

While one base sequence for the linker and the particles are shown, the bases may be changed on the opposing strands to maintain the pairings. For example, any cytosine in the A or B portions of the linker **228** may be changed to thymine, as long as the paired base of the particle oligonucleotide is changed from guanine to adenine.

In the presence of the adenosine analyte **305**, the aptamer portion **324** of the linker **328** folds, thus eliminating at least a portion of the hybridization between the aptamer portion **324** of the linker **328** and the 5'-Ade<sub>Au</sub> particle **337**. This loss of hybridization between the aptamer **324** and the 5'-Ade<sub>Au</sub> particle **337** releases the 5'-Ade<sub>Au</sub> particle to the solution.

As the 5'-Ade<sub>Au</sub> particles **337** are released, the blue aggregate **332** begins to disaggregate to form partial aggregate **390**. This partial disaggregation adds red color to the blue solution as the particles **337** diffuse away from the aggregate **332**, thus giving a purple solution. If enough of the adenosine analyte **305** is present in the sample, the reaction will continue until the aggregate **332** is completely disaggregated, to give **395**. Complete disaggregation results in a red solution due to the greater distance between the particles **336**, **337**.

The alignment of the particles **336**, **337** (tail-to-tail, head-to-tail, or head-to-head) with respect to each other may influence how tightly the aggregate units **331** bind. FIG. 3B depicts the aggregate unit **331** formed when the functionalized particles, such as **336** and **337**, hybridize to the linker **328** in a tail-to-tail arrangement. Head-to-tail (FIG. 3C) or head-to-head (FIG. 3D) hybridization may be selected by reversing one or both ends of the oligonucleotide to which the particle is attached, respectively. Thus, by reversing the 3' attachment of functionalized particle **336** to 5', particle **338** may hybridize to give the head-to-tail alignment of FIG. 3C. Similarly by reversing the 3' attachment of the functionalized particle **336** to the 5' attachment of the particle **338** and by reversing the 5' attachment of the particle **337** to the 3' attachment of particle **339**, the particles **338**, **339** may hybridize to give the head-to-head alignment of FIG. 3D.

16

At present, the tail-to-tail hybridization arrangement of FIG. 3B is preferred because the head-to-tail and head-to-head hybridization arrangements of FIGS. 3C and 3D may produce aggregates that sterically hinder aptamer binding and/or aggregate formation. However, this steric hindrance may be reduced through a reduction in the average diameter of the particles or by increasing the number of bases functionalized to the particles and the length of the extension, for example.

While not shown, the methodology of FIG. 3A may be applied to other analytes, including potassium ions, cocaine, and the analytes listed above in Table I. Table II, below, gives the base sequences of the linkers and particles for adenosine, K(I), and cocaine sensor systems. The aptamer portion of each linker is presented in uppercase, while the extension portion of each linker is presented in lowercase.

TABLE II

Name	Sequence	SEQ ID NO:
Adenosine Linker	5' - actcatctgtgaagagaACCTGGGGGAG TATTGCGGAGGAAGGT	46
3' - A <sub>12</sub> Ade <sub>Au</sub>	3' - AAAAAAAAAAATGAGTAGACACT	44
5' - Ade <sub>Au</sub>	5' - CCCAGGTTCTCT	45
Potassium Linker	5' - actcatctgtgatctaAGGGTTAGGGT AGGGTTAGGG	52
3' - A <sub>12</sub> K(I) <sub>Au</sub>	3' - AAAAAAAAAAATGAGTAGACACT	44
5' - K(I) <sub>Au</sub>	5' - AACCCCTTAGA	53
Cocaine Linker	5' - actcatctgtgaatctcGGGAGACAAGG ATAAATCCTTCAATGAAGTGGGTCTCCC	54
3' - A <sub>12</sub> Coc <sub>Au</sub>	3' - AAAAAAAAAAATGAGTAGACACT	44
5' - Coc <sub>Au</sub>	5' - GTCTCCCGAGA	55

The ionic strength of the sample may influence how tightly the moieties that form the aggregate bind together. Higher salt concentrations favor aggregation, thus slowing sensor response, while lower salt concentrations may lack the ionic strength necessary to maintain the aggregates. In one aspect, the sample may include or be modified with a reagent to include a monovalent metal ion concentration of 30 mM and greater. The ionic strength of the sample may be modified with Na<sup>+</sup> ions, for example. In a preferred aspect, the monovalent metal ion concentration of the sample, which contains the aggregate, is from 150 to 400 mM. At present, especially preferred monovalent metal ion concentrations are about 300 mM for adenosine and potassium analytes and about 150 mM for cocaine as an analyte. pH also may influence the aggregate binding, possibly attributable to the protonation of the polynucleotide base pairs at lower pH. In one aspect, a pH from 5 to 9 is preferred, with an approximately neutral pH being more preferred.

Thus, the performance of the sensor may be improved by adjusting the ionic strength and pH of the sample prior to combining it with the aggregate. Depending on the sample, it may be preferable to add the sample or analyte to a solution containing the aggregate (where the ionic strength and pH may be controlled) or the reverse.

The sensor system, including the aptamer, extension, and oligonucleotide functionalized particles may be provided in the form of a kit. In one aspect, the kit includes the aptamer and the extension joined to form the linker. In yet another

17

aspect, the kit includes the extension, but excludes the aptamer, which is then provided by the user or provided separately. In this aspect, the kit also may include the reagents required to link the supplied extension with an aptamer. In this aspect, the kit also may be used to determine the specificity and/or selectivity of various aptamers to a selected analyte. Thus, the kit may be used to select an appropriate aptamer in addition to detecting the analyte. In yet another aspect, the kit includes an exterior package that encloses a linker and oligonucleotide functionalized particles.

One or more of these kit components may be separated into individual containers, or they may be provided in their aggregated state. If separated, the aggregate may be formed before introducing the sample. Additional buffers and/or pH modifiers may be provided in the kit to adjust the ionic strength and/or pH of the sample.

The containers may take the form of bottles, tubs, sachets, envelopes, tubes, ampoules, and the like, which may be formed in part or in whole from plastic, glass, paper, foil, MYLAR®, wax, and the like. The containers may be equipped with fully or partially detachable lids that may initially be part of the containers or may be affixed to the containers by mechanical, adhesive, or other means. The containers also may be equipped with stoppers, allowing access to the contents by syringe needle. In one aspect, the exterior package may be made of paper or plastic, while the containers are glass ampoules.

The exterior package may include instructions regarding the use of the components. Color comparators; standard analyte solutions, such as a 10  $\mu$ m solution of the analyte; and visualization aids, such as thin layer chromatography (TLC) plates, test tubes, and cuvettes, also may be included. Containers having two or more compartments separated by a membrane that may be removed to allow mixing may be included. The exterior package also may include filters and dilution reagents that allow preparation of the sample for analysis.

In another aspect, in addition to the sensor system of the present invention, the kit also may include multiple sensor systems to further increase the reliability of analyte determination and reduce the probability of user error. In one aspect, multiple light-up sensor systems in accord with the present invention may be included. In another aspect, a "light-down" sensor system may be included with the light-up sensor system of the present invention. Suitable light-down sensors for inclusion in the presently claimed kit may rely on DNAzyme/Substrate/particle aggregates that are not formed in the presence of the selected analyte. A more detailed description of suitable light-down sensor systems for inclusion in the presently claimed kit may be found, for example, in U.S. patent application Ser. No. 10/756,825, filed Jan. 13, 2004, entitled "Biosensors Based on Directed Assembly of Particles," which is hereby incorporated by reference.

The preceding description is not intended to limit the scope of the invention to the described embodiments, but rather to enable a person of ordinary skill in the art to make and use the invention. Similarly, the examples below are not to be construed as limiting the scope of the appended claims or their equivalents, and are provided solely for illustration. It is to be understood that numerous variations can be made to the procedures below, which lie within the scope of the appended claims and their equivalents.

## EXAMPLES

All DNA samples were purchased from Integrated DNA Technology Inc., Coralville, Iowa. The DNA samples that

18

formed the extensions were purified by gel electrophoresis, while the thiol-modified DNA samples for forming the oligonucleotide functionalized particles were purified by standard desalting. Adenosine, cytidine, uridine, guanosine and cocaine were purchased from Aldrich. Gold nanoparticles having an average diameter of 13 nm were prepared and functionalized with 12-mer thiol-modified DNA following literature procedures, such as those disclosed in Storhoff, J., et al., "One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold particle probes," *JACS* 120: 1959-1964 (1998), for example. The average diameter of the gold nanoparticles was verified by transmission electronic microscope (JEOL 2010).

### Example 1

#### Preparation of a Colorimetric Adenosine Sensor

Five-hundred microliters of 5'-Ade<sub>Au</sub> (extinction at 522 nm equals 2.2) and 500  $\mu$ L of 3'-A<sub>12</sub>Ade<sub>Au</sub> (SEQ ID NO: 44) (extinction at 522 nm equals 2.2) were mixed in the presence of 300 mM NaCl, 25 mM Tris acetate buffer, pH 8.2, and 100 nM of the adenosine aptamer/extension (Adenosine Linker). The sample was warmed to 65° C. for one minute and then allowed to cool slowly to 4° C. The nanoparticles changed color from red to dark purple during this process. The sample was centrifuged and the precipitates were collected and then dispersed in 2000  $\mu$ L of the same buffer (300 mM NaCl, 25 mM Tris acetate, pH 8.2). The suspension was used for detection of adenosine.

### Example 2

#### Colorimetric Detection of Adenosine

One-hundred microliters of the sensor suspension from Example 1 was added to a small volume of concentrated adenosine solution. For example, 2  $\mu$ L of 50 mM adenosine was added to give a final concentration of 1 mM. The color change was monitored with a UV-vis spectrometer or by the naked eye.

### Example 3

#### Monitoring the Performance of the Adenosine Sensor

The color change of the sample from Example 2 was monitored by UV-vis extinction spectroscopy. FIG. 4 is a graph relating the extinction ratios provided at specific wavelengths from a sample during disaggregation. The dashed line in FIG. 4 shows the strong extinction peak at 522 nm exhibited by separated 13 nm nanoparticles, which provide a deep red color. As may be seen from the solid line in FIG. 4, upon aggregation, the 522 nm peak decreases in intensity and shifts to longer wavelength, while the extinction at 700 nm region increases, resulting in a red-to-blue color transition. Therefore a higher extinction ratio at 522 to 700 nm is associated with the red color of separated nanoparticles, while a low extinction ratio is associated with the blue color of aggregated nanoparticles. This extinction ratio was used to monitor the aggregation state of the particles.

### Example 4

#### Selectivity and Sensitivity of the Adenosine Sensor

To a quartz UV-vis spectrophotometer cell (Hellma, Germany) was added 100  $\mu$ L of the adenosine sensor system

## 19

prepared in Example 1. A small volume (1-5  $\mu\text{L}$ ) of solutions including adenosine, uridine, cytidine or guanosine was added to the spectrophotometer cell to bring the analyte concentration to the desired level in the cell.

The dispersion kinetics for each cell was monitored as a function of time using a Hewlett-Packard 8453 spectrophotometer. FIG. 5A is a graph depicting the ratios of extinction at 522 and 700 nm plotted as a function of time. As may be seen from the plots, only adenosine gave significant increase in the extinction ratio as a function of time, while uridine, cytidine, and guanosine provided a color change consistent with the background. Therefore, the high selectivity of the sensor was confirmed.

FIG. 5B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the adenosine analyte after five minutes of aggregation. The exceptional linearity of the sensor system was evident from about 0.1 to about 1.5 mM.

FIG. 5C is a graph depicting the extinction ratios for multiple adenosine analyte concentrations over a 6 minute time period. The graph demonstrates the ability of the sensor system to effectively differentiate between different analyte concentrations within a few minutes. Thus, the ability of the sensor system to provide accurate quantitative information was established.

In addition to the instrumental method of FIG. 5B, the color developed by the sensor was conveniently observed visually. A color progression from blue to red was seen as the adenosine concentration increased from 0 to 2 mM. Uridine, cytidine, and guanosine provided a color similar to the background.

## Example 5

## Preparation of a Colorimetric Potassium Ion Sensor

FIGS. 6A-6B represent an analyte sensor system that includes an aptamer that folds in the presence of K(I). FIG. 6A provides the sequences of the extension and aptamer portions of a linker and of the oligonucleotide functionalized particles. FIG. 6B depicts the aptamer folding in the presence of the K(I) analyte. To prepare the K(I) sensor system, 500  $\mu\text{L}$  of 5'-K(I)<sub>Au</sub> (extinction at 522 nm equals 2.2) and 500  $\mu\text{L}$  of 3'-A<sub>12</sub>K(I)<sub>Au</sub> (SEQ ID NO: 44) (extinction at 522 nm equals 2.2) were mixed in the presence of 300 mM NaCl, 25 mM Tris acetate buffer, pH 8.2, and 100 nM of the potassium ion aptamer/extension (Potassium Linker). The sample was warmed to 65° C. for one minute and then allowed to cool slowly to 4° C. The nanoparticles changed color from red to dark purple during this process. The sample was centrifuged and the precipitates were collected and dispersed in 10  $\mu\text{L}$  of the same buffer (300 mM NaCl, 25 mM Tris acetate, pH 8.2). The suspension was used for detection of K<sup>+</sup>.

## Example 6

## Colorimetric Detection of Potassium

Metal ion solutions of Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup>, or Cs<sup>+</sup> ions were made by dissolving LiCl, NaCl, KCl, RbCl or CsCl salt, respectively, in deionized water to obtain an ion concentration of 3 M. From these metal ion stock solutions were prepared solutions containing 25 mM of Tris acetate buffer, pH 8.2, and 300 mM of Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup>, or Cs<sup>+</sup> ions. To each of these five solutions was added 1  $\mu\text{L}$  of the K(I) sensor system from Example 5 for each 99  $\mu\text{L}$  of the metal ion containing solution. Therefore, each solution contained ~300 mM of Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup>, or Cs<sup>+</sup> metal ions and an additional 3 mM of

## 20

Na<sup>+</sup> ions as background. Each sample was heated to 65° C. and then cooled slowly to 4° C. in 1 hour. The color change was monitored with a UV-vis spectrometer or by the naked eye. FIG. 6C depicts the extinction ratio of the potassium ion sensor system in the presence of Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Rb<sup>+</sup>, or Cs<sup>+</sup> ions, thus confirming the selectivity of the sensor system to K(I).

## Example 7

## Preparation of a Colorimetric Cocaine Sensor

FIGS. 7A-7B represent an analyte sensor system that includes an aptamer that folds in the presence of cocaine. FIG. 7A provides the sequences of the extension and aptamer portions of the linker and of the oligonucleotide functionalized particles. FIG. 7B depicts the aptamer folding in the presence of the cocaine analyte. To prepare the cocaine sensor system, 500  $\mu\text{L}$  of 5'-CocAu (extinction at 522 nm equals 2.2) and 500  $\mu\text{L}$  of 3'-A<sub>12</sub>CocAu (SEQ ID NO: 44) (extinction at 522 nm equals 2.2) were mixed in the presence of 300 mM NaCl, 25 mM Tris acetate buffer, pH 8.2, and 100 nM of the cocaine aptamer/extension (Cocaine Linker). The sample was warmed to 65° C. for one minute and then allowed to cool slowly to 4° C. The nanoparticles changed color from red to dark purple during this process. The sample was centrifuged and the precipitates collected. The collected precipitates were then dispersed in 2000  $\mu\text{L}$  of another buffer (150 mM NaCl, 25 mM Tris acetate, pH 8.2). The suspension was used for detection of cocaine.

## Example 8

## Colorimetric Detection of Cocaine

One-hundred microliters of the above prepared cocaine sensor suspension were combined with a small volume of concentrated cocaine solution. For example, 1  $\mu\text{L}$  of 100 mM cocaine was added to the suspension to give a final concentration of 1 mM. The color change was monitored with a UV-vis spectrometer or by the naked eye.

## Example 9

## Selectivity and Sensitivity of the Cocaine Sensor

To a quartz UV-vis spectrophotometer cell (Hellma, Germany) was added 100  $\mu\text{L}$  of the cocaine sensor system prepared in Example 7. A small volume (0.5-2  $\mu\text{L}$ ) of solutions including cocaine, adenosine, or sucrose was added to the spectrophotometer cell to bring the analyte concentration to the desired level in the cell.

The dispersion kinetics for each cell was monitored as a function of time using a Hewlett-Packard 8453 spectrophotometer. FIG. 8A is a graph depicting the ratios of extinction at 522 and 700 nm plotted as a function of time. As may be seen from the plots, only cocaine gave a significant increase in the extinction ratio as a function of time, while adenosine and sucrose provided a color change consistent with the background. Therefore, the high selectivity of the sensor was confirmed.

FIG. 8B is a graph depicting the correlation between the observed extinction ratios for the color change of the sensor system and the concentration of the cocaine analyte after one minute of aggregation. The exceptional linearity of the sensor system was evident from about 0.1 to about 1 mM.

## 21

FIG. 8C is a graph depicting the extinction ratios for multiple cocaine analyte concentrations over a 5 minute time period. The graph demonstrates the ability of the sensor system to effectively differentiate between different analyte concentrations within a few minutes. Thus, the ability of the sensor system to provide accurate quantitative information was established.

In addition to the instrumental method of FIG. 8B, the color developed by the sensor was conveniently observed visually.

## 22

A color progression from blue to red was seen as the cocaine concentration increased from 0 to 1 mM. Adenosine and sucrose provided a color similar to the background.

As any person of ordinary skill in the art will recognize from the provided description, figures, and examples, that modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of the invention defined by the following claims and their equivalents.

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<220> FEATURE:

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<211> LENGTH: 29

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic aptamer motif sequence

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<211> LENGTH: 95

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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caguguuuug ccauugcaug uagcagaagc uuccg 95

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<211> LENGTH: 132

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<213> ORGANISM: Artificial Sequence

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cccgggaagc tt 132

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<212> TYPE: RNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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c                                                                    61

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uccucucc                                           67

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<210> SEQ ID NO 17
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<212> TYPE: RNA

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<223> OTHER INFORMATION: a, c, u, g, unknown or other
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<222> LOCATION: (56)..(56)
<223> OTHER INFORMATION: a, c, u, g, unknown or other

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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cgcag                                             65

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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 20

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 21

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<210> SEQ ID NO 22
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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&lt;211&gt; LENGTH: 37

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;400&gt; SEQUENCE: 24

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&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 108

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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guuuuacuc acuuugcaau cgcauugug cgucuacaug gauccuca 108

&lt;210&gt; SEQ ID NO 26

&lt;211&gt; LENGTH: 41

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 40

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;400&gt; SEQUENCE: 27

uacagaaugg guugguaggc auaccuauc gagaaugaua 40

&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 113

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence: Synthetic



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 aptamer motif sequence

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&lt;210&gt; SEQ ID NO 29

&lt;211&gt; LENGTH: 45

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;211&gt; LENGTH: 60

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;210&gt; SEQ ID NO 31

&lt;211&gt; LENGTH: 46

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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&lt;400&gt; SEQUENCE: 31

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&lt;211&gt; LENGTH: 107

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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cgacgcccgg ccgaagaagg ggauucgaca ugaggcccgg auccggc 107

&lt;210&gt; SEQ ID NO 33

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: RNA

&lt;213&gt; ORGANISM: Human immunodeficiency virus

&lt;400&gt; SEQUENCE: 33

uccguuuuca gucgggaaaa acug 24

&lt;210&gt; SEQ ID NO 34

&lt;211&gt; LENGTH: 15

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 34

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 <223> OTHER INFORMATION: Description of Unknown: VEGF motif sequence

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 <220> FEATURE:  
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ucuucugcuc gagaaugcau gaagcguucc auauuuuu 98

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 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

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 <213> ORGANISM: Unknown  
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 <211> LENGTH: 39  
 <212> TYPE: RNA  
 <213> ORGANISM: Homo sapiens

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 <223> OTHER INFORMATION: Description of Unknown: YPEN-1 endothelial sequence

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agactagtcg caagatatag atagtaagtg caatct 96

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<400> SEQUENCE: 41

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<210> SEQ ID NO 42  
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<212> TYPE: RNA  
<213> ORGANISM: Rous sarcoma virus

<400> SEQUENCE: 42

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<213> ORGANISM: Artificial Sequence  
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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
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<400> SEQUENCE: 44

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<210> SEQ ID NO 45  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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<400> SEQUENCE: 45

cccagggttct ct 12

<210> SEQ ID NO 46  
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<212> TYPE: DNA  
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<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
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actcatctgt gaagaga 17

<210> SEQ ID NO 48

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41

What is claimed is:

1. A method of detecting an analyte, comprising:  
combining an aggregate with a sample; and  
detecting a color change responsive to the analyte in the  
sample, the aggregate comprising:  
a linker, wherein the linker is a single oligonucleotide  
comprising an aptamer that is capable of folding in  
response to the analyte; and  
first particles coupled to a first oligonucleotide, the first  
oligonucleotide complementary to at least a portion of  
the aptamer, wherein binding of the analyte to the  
aptamer causes disaggregation of the aggregate the  
disaggregated aggregate is detectable as a color  
change.
2. A method for determining the sensitivity and selectivity  
of an aptamer to an analyte, comprising:  
combining an aggregate with the analyte;  
detecting a color change responsive to the analyte, the  
aggregate comprising:  
a linker, wherein the linker is a single oligonucleotide  
comprising the aptamer; and  
first particles coupled to a first oligonucleotide, the first  
oligonucleotide complementary to at least a portion of  
the aptamer, wherein binding of the analyte to the  
aptamer causes disaggregation of the aggregate and  
the disaggregated aggregate are detectable as a color  
change; and  
determining if the aptamer folded to provide the color  
change, thereby determining the sensitivity and selec-  
tivity of the aptamer.
3. The method of claim 2, the aggregate further comprising  
second particles coupled to a second oligonucleotide, the  
second oligonucleotide complementary to at least a portion of  
an extension that forms a portion of the linker, wherein the  
extension comprises bases.
4. The method of claim 3, where the extension comprises  
from 1 to 100 bases, at least 50% of the bases complementary  
to the first or second oligonucleotide.
5. The method of claim 2, where the linker further com-  
prises an extension.
6. The method of claim 3, where at least a portion of the  
bases forming the extension inhibit the linker from forming  
an inherently stable secondary structure without the analyte.
7. The method of claim 3, where a portion of the second  
oligonucleotide is not complementary to the extension.
8. The method of claim 3, where the hybridization stability  
of the aptamer in combination with the analyte is greater than  
the hybridization stability of a portion of the aptamer with the  
first oligonucleotide.
9. The method of claim 3, where the first and second  
particles comprise a material selected from the group consist-  
ing of metals, semiconductors, magnetizable materials, and  
combinations thereof.
10. The method of claim 3, where the first and second  
particles comprise gold.
11. The method of claim 2, where the analyte is a metal ion.
12. The method of claim 2, where the analyte is selected  
from the group consisting of large biomolecules, small bio-  
molecules, and organic molecules.
13. The method of claim 2, where the linker oligonucle-  
otide comprises a sequence selected from the group consist-  
ing of SEQ ID NOS: 46, 52, and 54.
14. The method of claim 2, where the linker oligonucle-  
otide comprises a sequence selected from the group consist-  
ing of SEQ ID NOS: 1-42.
15. The method of claim 2 having a response time of less  
than 60 minutes.

42

16. The method of claim 2 having a response time of less  
than 10 minutes at 23° to 37° C.
17. The method of claim 1, wherein the first oligonucle-  
otide is further complementary to at least a portion of an  
extension that forms a portion of the linker.
18. The method of claim 1, the aggregate further compris-  
ing second particles coupled to a second oligonucleotide, the  
second oligonucleotide complementary to at least a portion of  
an extension that forms a portion of the linker.
19. The method of claim 18, where the hybridization sta-  
bility of the aptamer in combination with the analyte is greater  
than the hybridization stability of a portion of the aptamer  
with the second oligonucleotide.
20. The method of claim 18, where the extension comprises  
from 1 to 100 bases, and at least 50% of the bases are comple-  
mentary to the first or second oligonucleotide.
21. The method of claim 1, where the linker further com-  
prises an extension.
22. The method of claim 18, where the first and second  
particles comprise a material selected from the group consist-  
ing of metals, semiconductors, magnetizable materials, and  
combinations thereof.
23. The method of claim 1, where the analyte is a metal ion.
24. The method of claim 1, where the analyte is selected  
from the group consisting of large biomolecules, small bio-  
molecules, and organic molecules.
25. The method of claim 1, where the linker oligonucle-  
otide comprises a sequence selected from the group consist-  
ing of SEQ ID NOS: 46, 52, and 54.
26. The method of claim 1, where the linker oligonucle-  
otide comprises a sequence selected from the group consist-  
ing of SEQ ID NOS: 1-42.
27. The method of claim 1, where the linker oligonucle-  
otide comprises a variant of a sequence selected from the  
group consisting SEQ ID NOS: 1-42, 46, 52, 54, and wherein  
the variant has one or more bases of that portion of the  
sequence that hybridizes to the first oligonucleotide, and the  
corresponding bases on the first oligonucleotide are also  
modified to maintain hybridization pairings C-G and A-T.
28. The method of claim 2, where the linker oligonucle-  
otide comprises a variant of a sequence selected from the  
group consisting SEQ ID NOS: 1-42, 46, 52, 54, and wherein  
the variant has one or more bases of that portion of the  
sequence that hybridizes to the first oligonucleotide, and the  
corresponding bases on the first oligonucleotide are also  
modified to maintain hybridization pairings C-G and A-T.
29. The method of claim 18, where the first and second  
particles comprise gold.
30. A method of detecting an analyte, comprising:  
combining an aggregate with a sample; and  
detecting a color change responsive to the analyte in the  
sample, the aggregate comprising:  
a linker, wherein the linker is a single oligonucleotide  
comprising an extension and an aptamer that is  
capable of folding in response to the analyte; and  
first particles coupled to a first oligonucleotide, the first  
oligonucleotide complementary to at least a portion of  
the aptamer,  
second particles coupled to a second oligonucleotide,  
the second oligonucleotide complementary to at least  
a portion of the extension,  
wherein binding of the analyte to the aptamer causes dis-  
aggregation of the aggregate and the disaggregated  
aggregate is detectable as a color change.

\* \* \* \* \*

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 8,470,532 B2  
APPLICATION NO. : 13/008568  
DATED : June 25, 2013  
INVENTOR(S) : Lu and Liu

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page, Item (56):

On page 13, 2<sup>nd</sup> column, in the Ellington *et al.* reference, “Opitical” should be --Optical--.

In the Specification:

Column 20, line 30, “pH 8.2.” should be --pH 8.2).--.

In the Claims:

Column 41, line 12, claim 1, “aggregate the” should be --aggregate and the--.

Column 41, line 26, claim 2, “aggregate are” should be --aggregate is--.

Column 42, line 36, claim 27, “consisting SEQ” should be --consisting of SEQ--.

Column 42, line 43, claim 28, “consisting SEQ” should be --consisting of SEQ--.

Signed and Sealed this  
Twenty-eighth Day of January, 2014



Michelle K. Lee  
Deputy Director of the United States Patent and Trademark Office