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(54) **APTAMER- AND NUCLEIC ACID ENZYME-BASED SYSTEMS FOR SIMULTANEOUS DETECTION OF MULTIPLE ANALYTES**

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See application file for complete search history.

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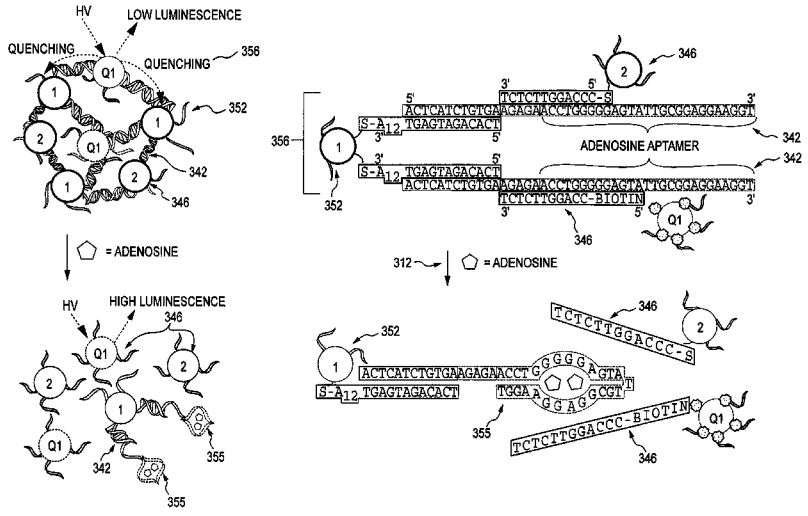
(57) **ABSTRACT**

The present invention provides aptamer- and nucleic acid enzyme-based systems for simultaneously determining the presence and optionally the concentration of multiple analytes in a sample. Methods of utilizing the system and kits that include the sensor components are also provided. The system includes a first reactive polynucleotide that reacts to a first analyte; a second reactive polynucleotide that reacts to a second analyte; a third polynucleotide; a fourth polynucleotide; a first particle, coupled to the third polynucleotide; a second particle, coupled to the fourth polynucleotide; and at least one quencher, for quenching emissions of the first and second quantum dots, coupled to the first and second reactive polynucleotides. The first particle includes a quantum dot having a first emission wavelength. The second particle includes a second quantum dot having a second emission wavelength different from the first emission wavelength. The third polynucleotide and the fourth polynucleotide are different.

20 Claims, 11 Drawing Sheets



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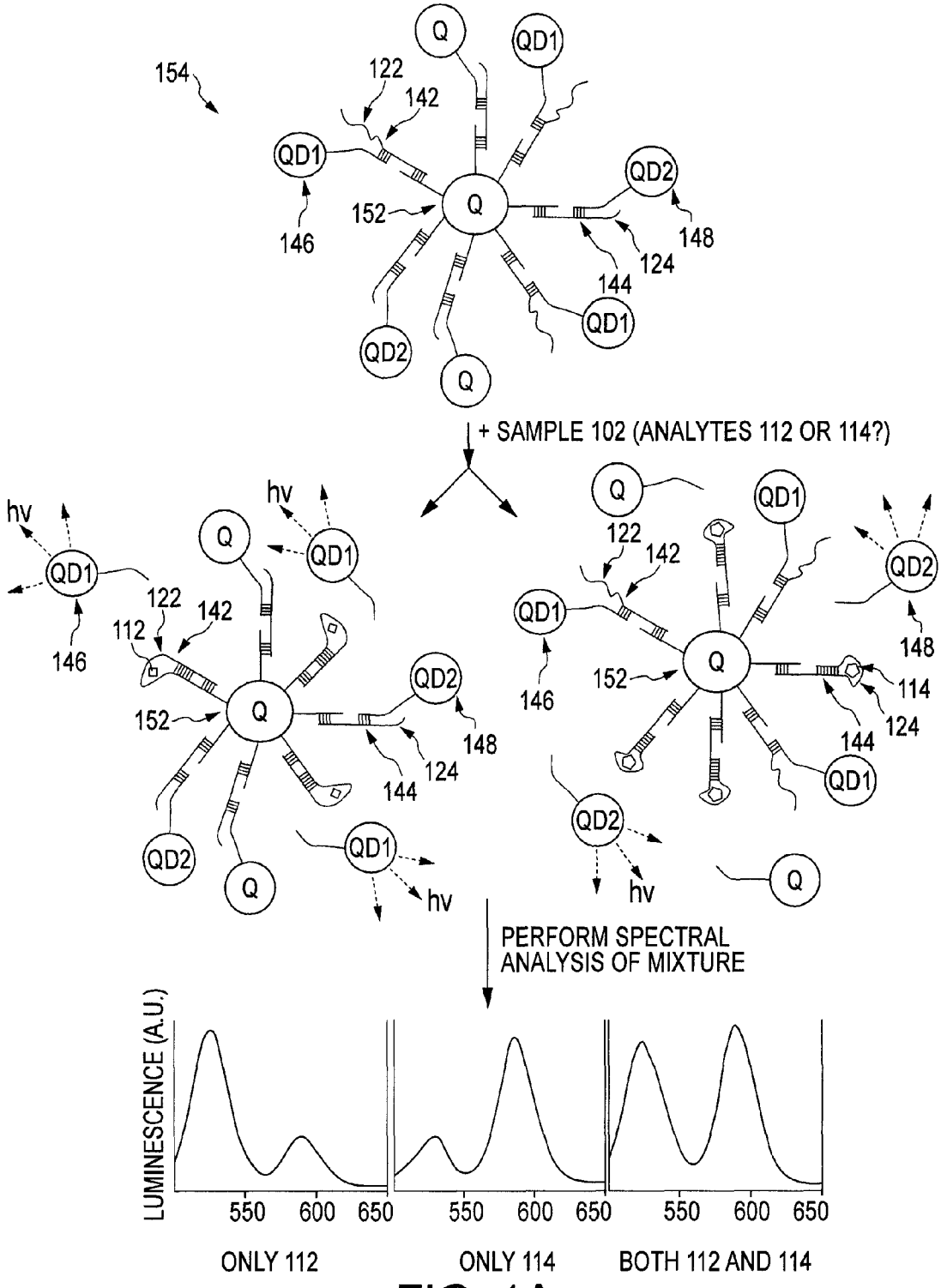

154
Q
QD1
122
142
QD1
QD2
152
Q
146
148
124
144
QD1
QD2
Q
+ SAMPLE 102 (ANALYTES 112 OR 114?)
hv
hv
Q
QD1
QD1
122
142
112
146
QD2
152
Q
148
124
144
QD2
Q
QD1
hv
Q
QD1
122
142
QD2
QD1
148
146
152
Q
114
124
144
QD1
QD2
hv
Q
PERFORM SPECTRAL ANALYSIS OF MIXTURE
LUMINESCENCE (A.U.)
550 600 650
550 600 650
550 600 650
ONLY 112
ONLY 114
BOTH 112 AND 114

FIG. 1A

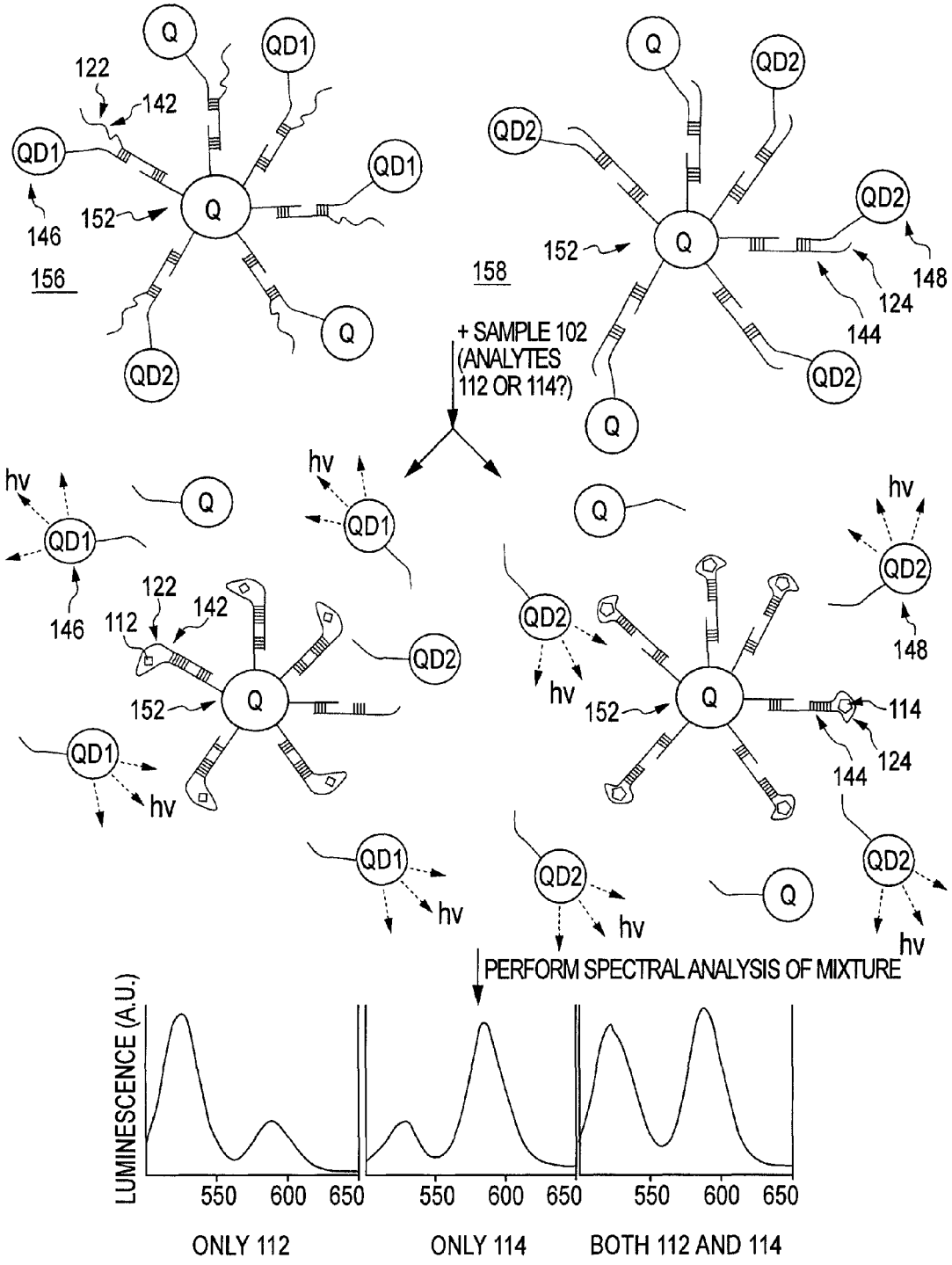

Q
QD1
122
142
QD1
QD1
152
Q
146
156
Q
QD2
Q
QD2
QD2
152
Q
158
QD2
148
124
144
+ SAMPLE 102
(ANALYTES
112 OR 114?)
QD2
Q
hv
QD1
Q
hv
QD1
146
122
112
142
Q
QD2
152
Q
QD1
hv
QD2
hv
Q
hv
QD2
148
152
Q
114
124
144
QD1
hv
QD2
hv
Q
QD2
hv
PERFORM SPECTRAL ANALYSIS OF MIXTURE
LUMINESCENCE (A.U.)
550
600
650
550
600
650
550
600
650
ONLY 112
ONLY 114
BOTH 112 AND 114

FIG. 1B

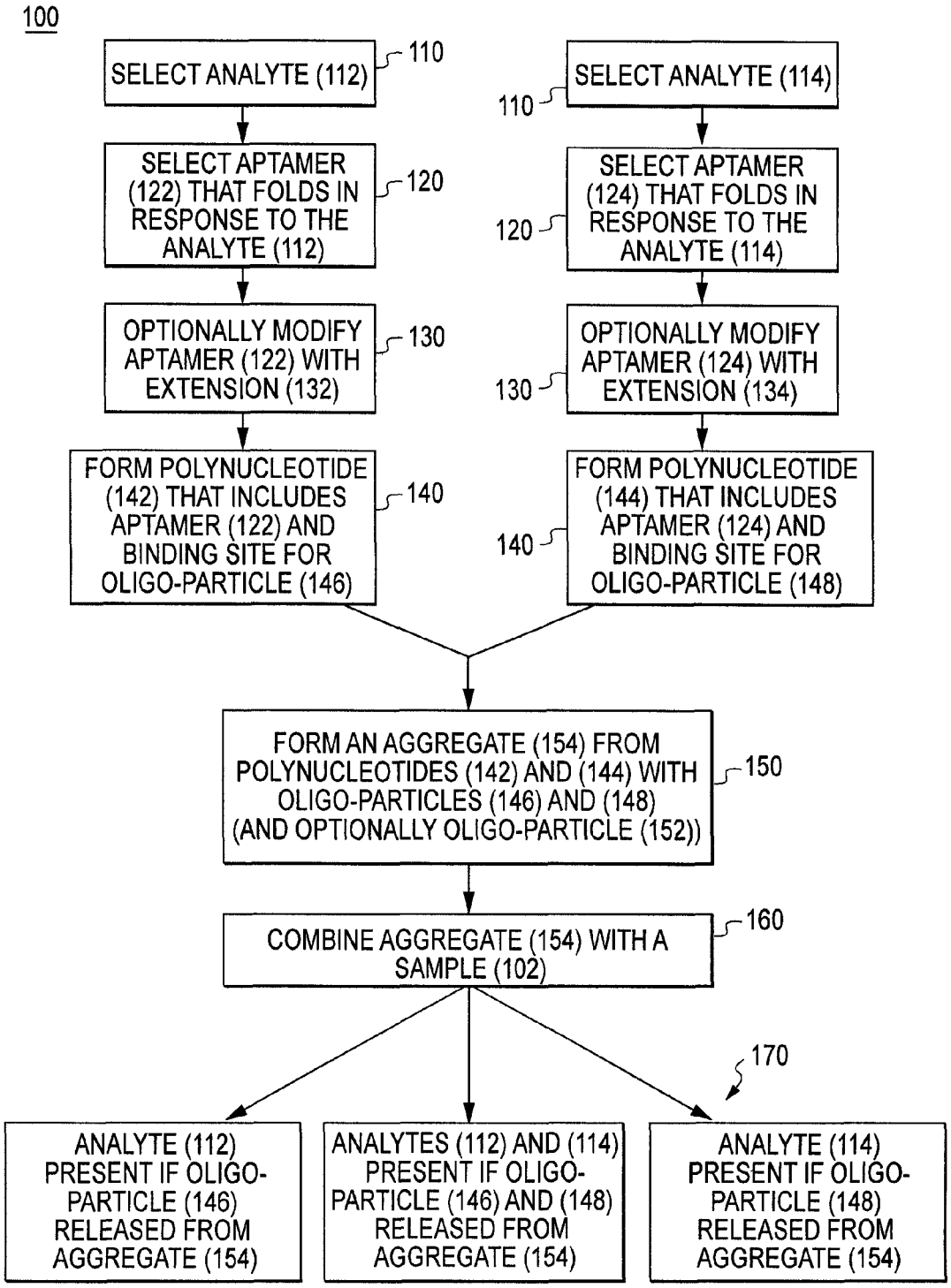

100
SELECT ANALYTE (112)
110
SELECT APTAMER (122) THAT FOLDS IN RESPONSE TO THE ANALYTE (112)
120
OPTIONALLY MODIFY APTAMER (122) WITH EXTENSION (132)
130
FORM POLYNUCLEOTIDE (142) THAT INCLUDES APTAMER (122) AND BINDING SITE FOR OLIGO-PARTICLE (146)
140
SELECT ANALYTE (114)
110
SELECT APTAMER (124) THAT FOLDS IN RESPONSE TO THE ANALYTE (114)
120
OPTIONALLY MODIFY APTAMER (124) WITH EXTENSION (134)
130
FORM POLYNUCLEOTIDE (144) THAT INCLUDES APTAMER (124) AND BINDING SITE FOR OLIGO-PARTICLE (148)
140
FORM AN AGGREGATE (154) FROM POLYNUCLEOTIDES (142) AND (144) WITH OLIGO-PARTICLES (146) AND (148) (AND OPTIONALLY OLIGO-PARTICLE (152))
150
COMBINE AGGREGATE (154) WITH A SAMPLE (102)
160
170
ANALYTE (112) PRESENT IF OLIGO-PARTICLE (146) RELEASED FROM AGGREGATE (154)
ANALYTES (112) AND (114) PRESENT IF OLIGO-PARTICLE (146) AND (148) RELEASED FROM AGGREGATE (154)
ANALYTE (114) PRESENT IF OLIGO-PARTICLE (148) RELEASED FROM AGGREGATE (154)

FIG. 2

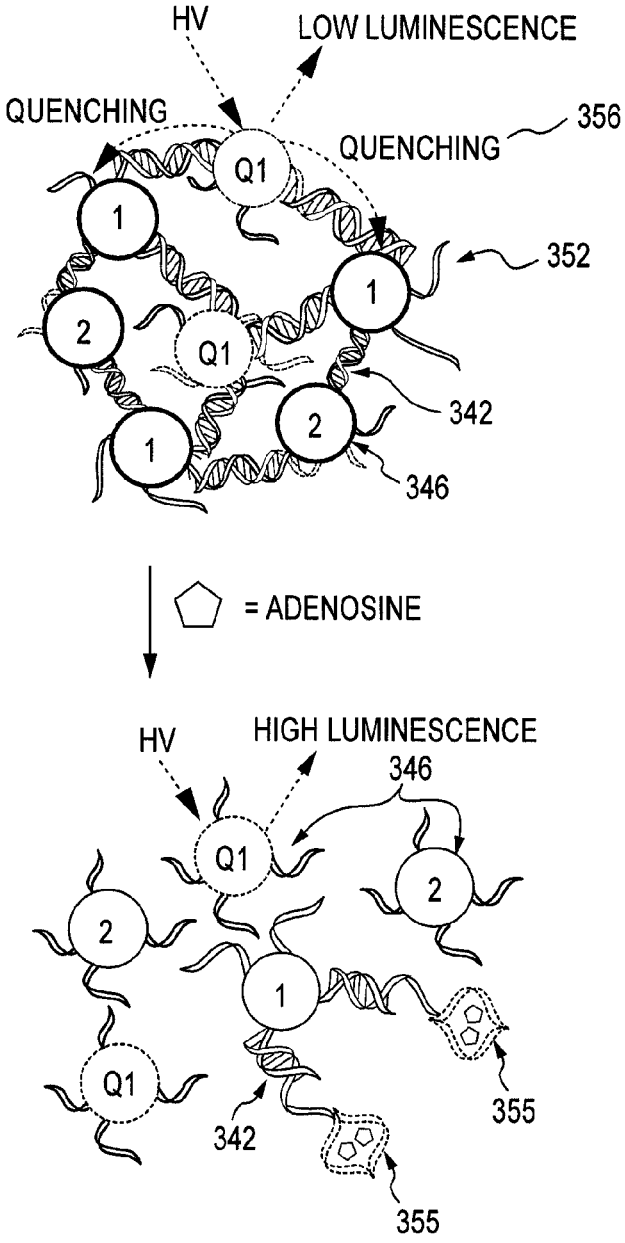

HV
LOW LUMINESCENCE
QUENCHING
QUENCHING
356
Q1
1
1
352
2
Q1
342
1
2
346
= ADENOSINE
HV
HIGH LUMINESCENCE
346
Q1
2
2
1
Q1
342
355
355

FIG. 3A

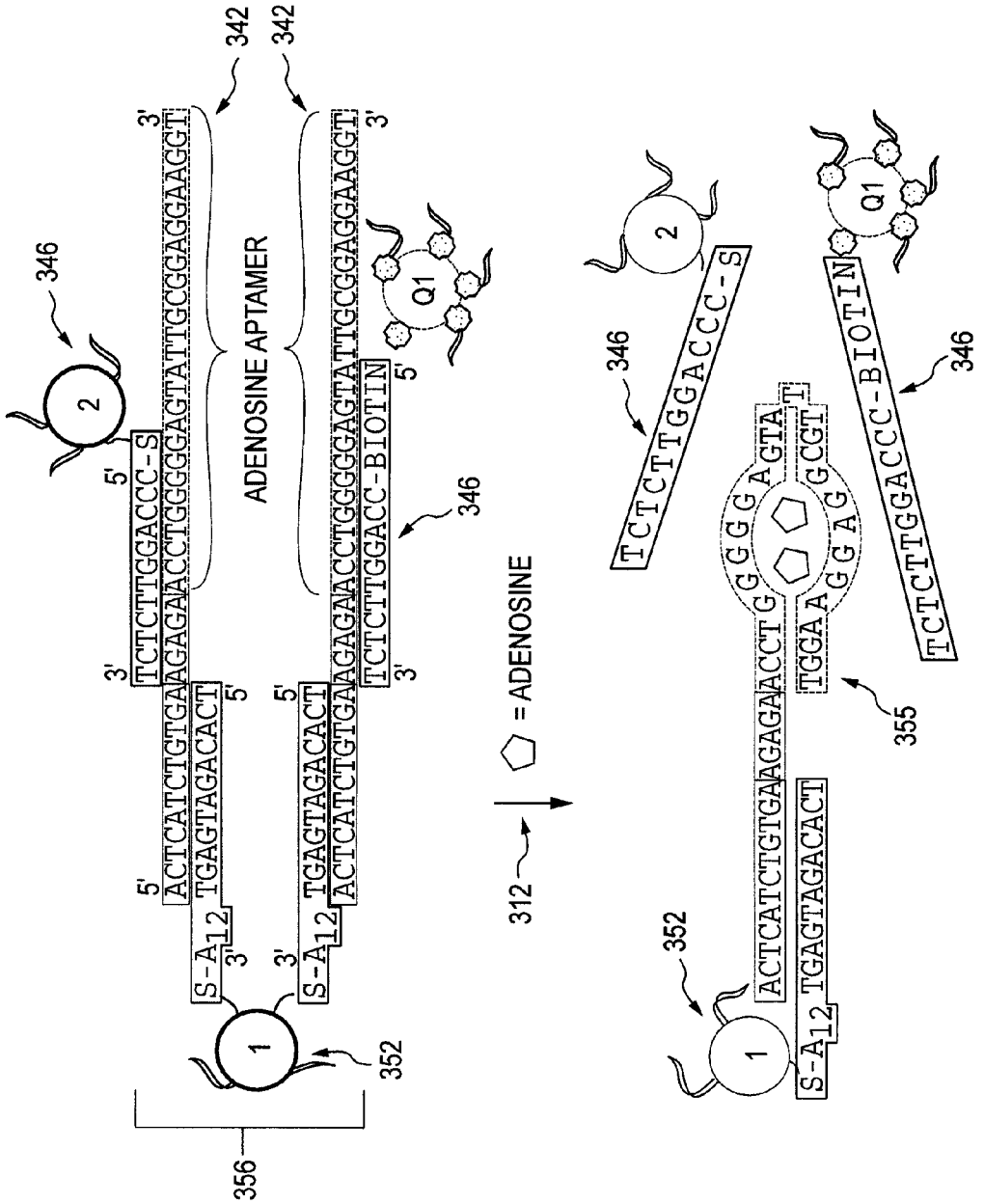

342
342
346
ADENOSINE APTAMER
Q1
346
= ADENOSINE
312
352
356
355
346
346
Q1

FIG. 3B

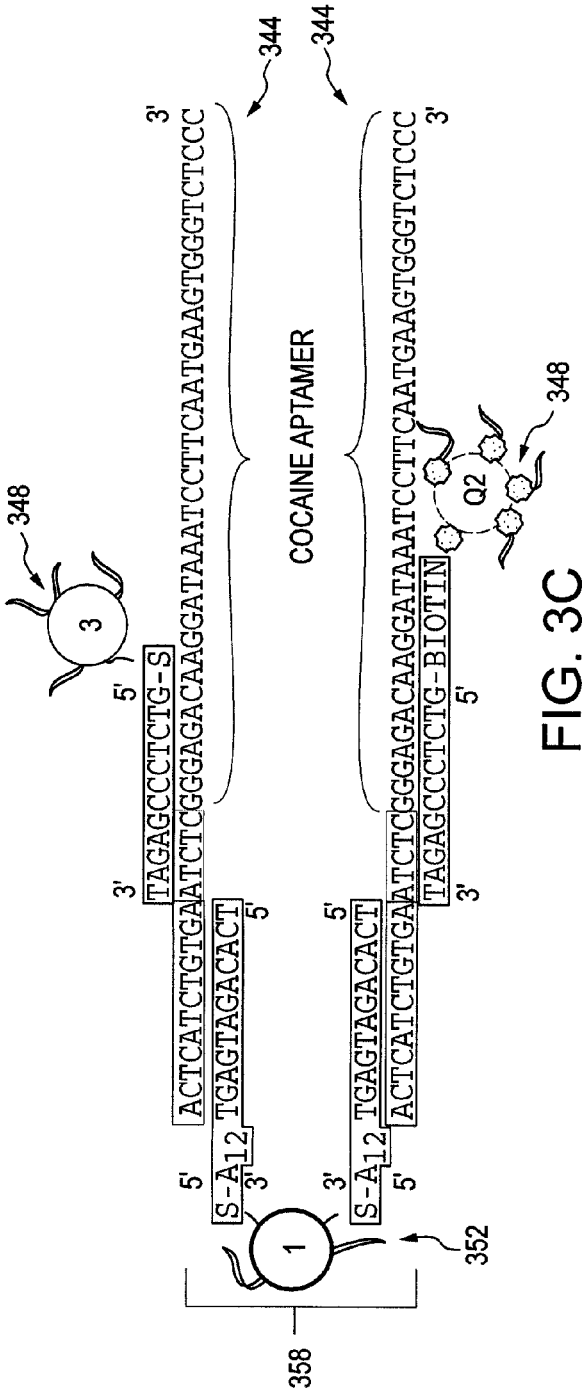

348
3
5'
TAGAGCCCTCTG-S
3'
344
3'
ACTCATCTGTGAATCTCGGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCC
COCAINE APTAMER
5'
S-A12
3'
TGAGTAGACACT
5'
1
3'
S-A12
5'
TGAGTAGACACT
ACTCATCTGTGAATCTCGGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCC
344
3'
TAGAGCCCTCTG-BIOTIN
5'
Q2
348
352
358

FIG. 3C

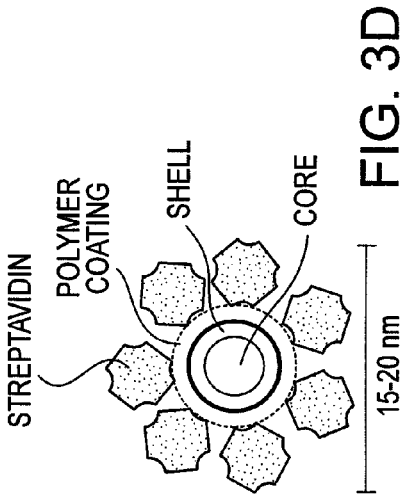

STREPTAVIDIN
POLYMER COATING
SHELL
CORE
15-20 nm

FIG. 3D

FIG. 4A

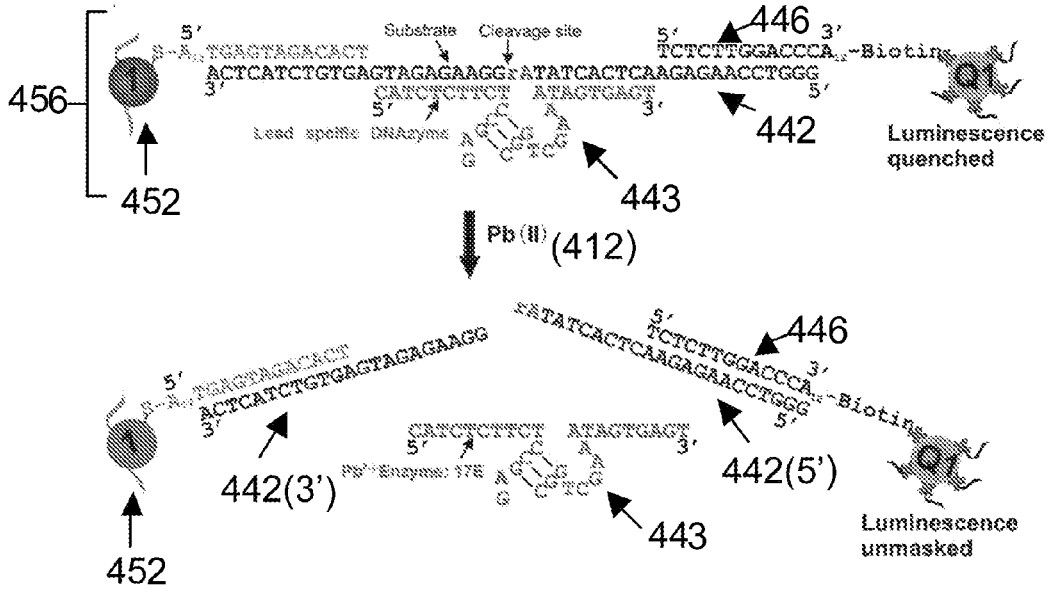
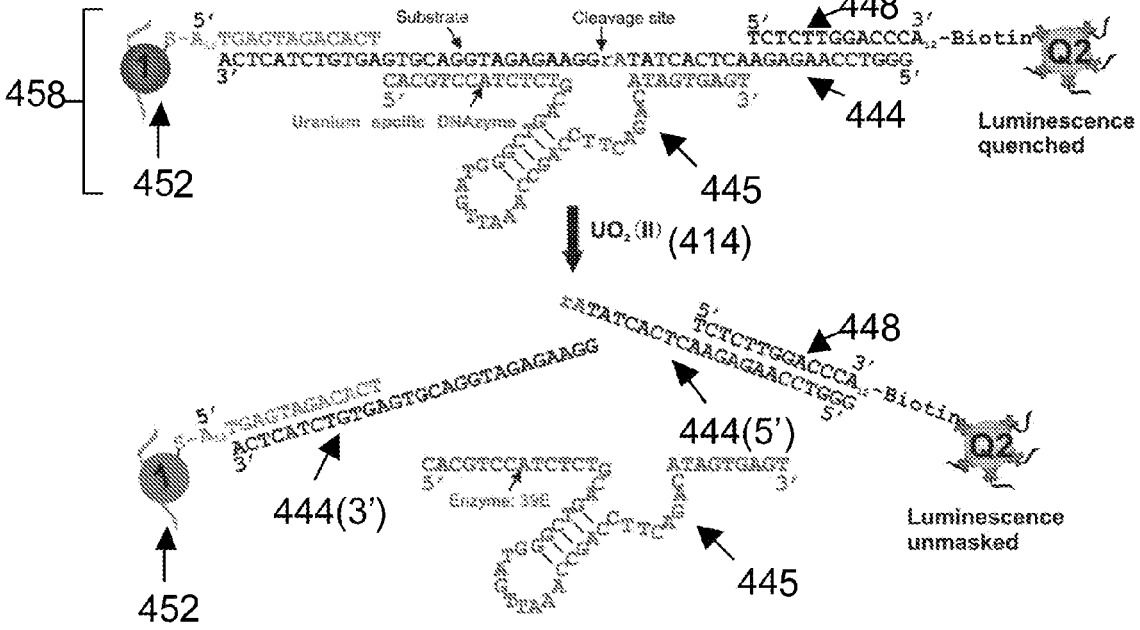

456
452
Substrate
Cleavage site
446
442
443
Q1
Luminescence quenched
Pb(II) (412)
442(3')
442(5')
Luminescence unmasked

FIG. 4B


458
452
Substrate
Cleavage site
448
444
445
Q2
Luminescence quenched
UO2(II) (414)
444(3')
444(5')
Luminescence unmasked

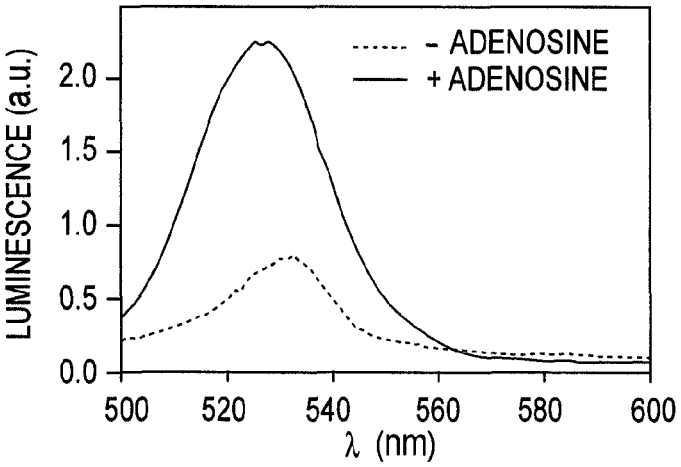


FIG. 5A

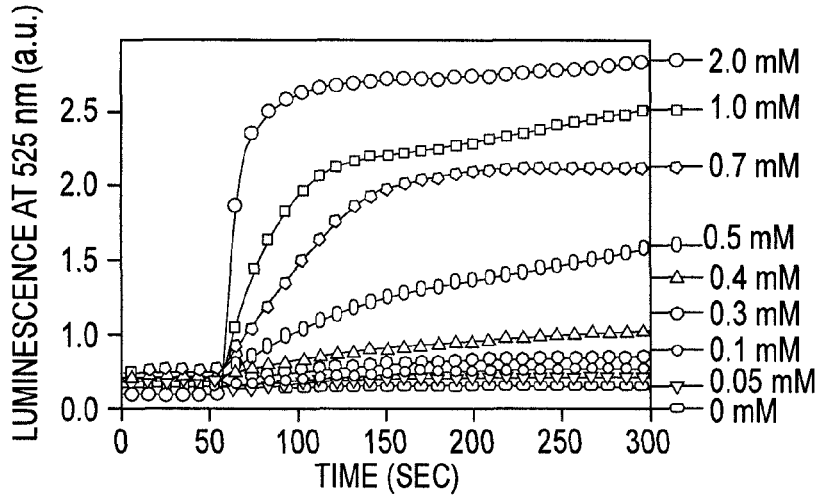


FIG. 5B

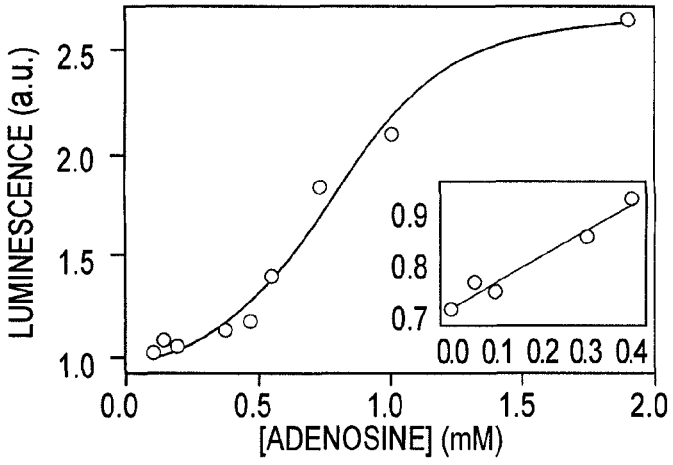


FIG. 5C

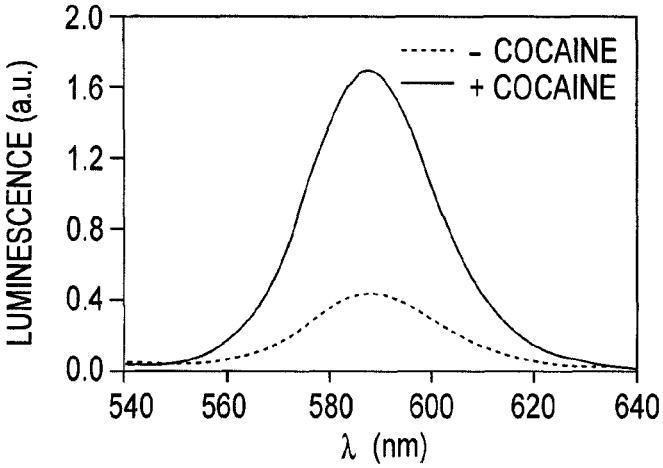


FIG. 5D

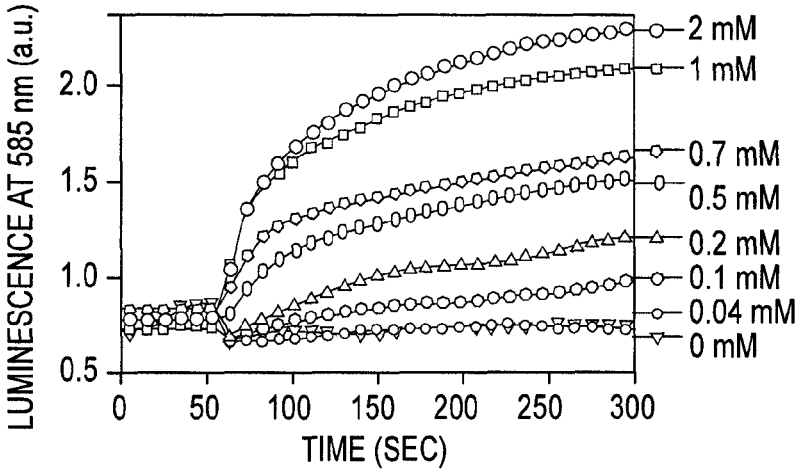


FIG. 5E

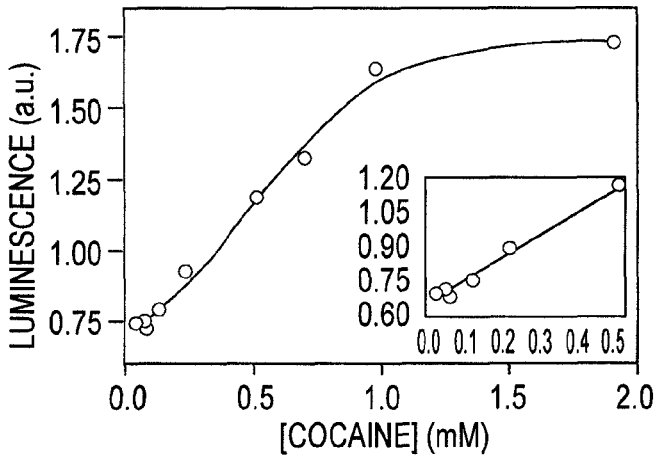


FIG. 5F

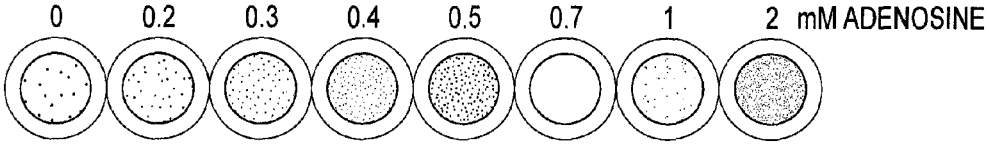


FIG. 6A

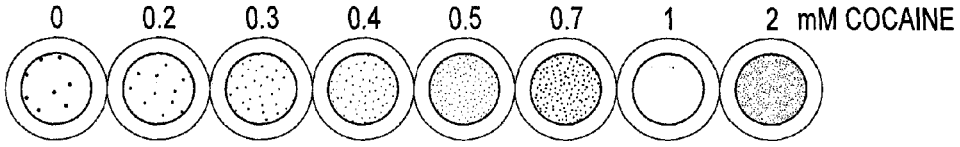


FIG. 6B

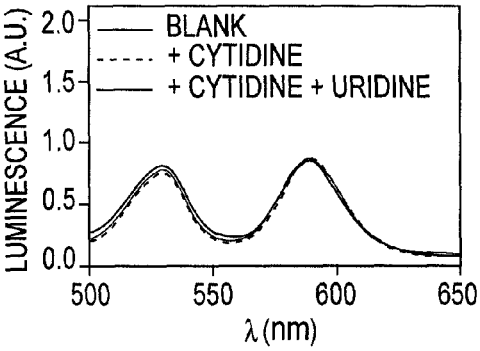


FIG. 7A

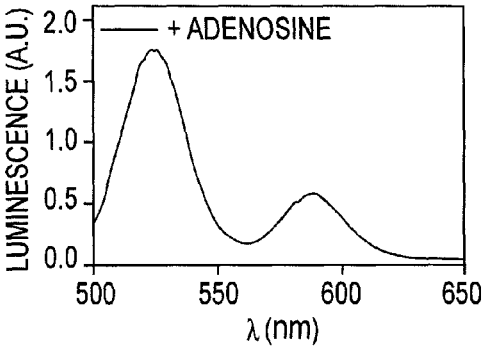


FIG. 7B

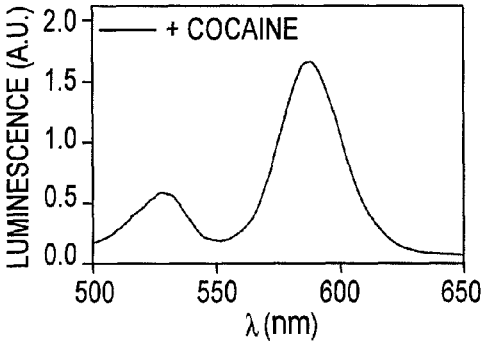


FIG. 7C

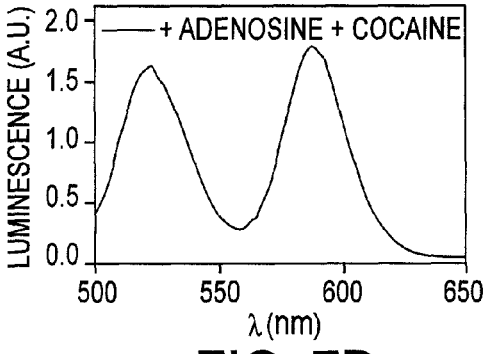


FIG. 7D

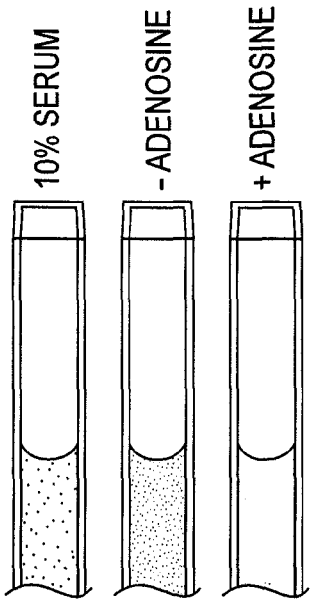


FIG. 8A

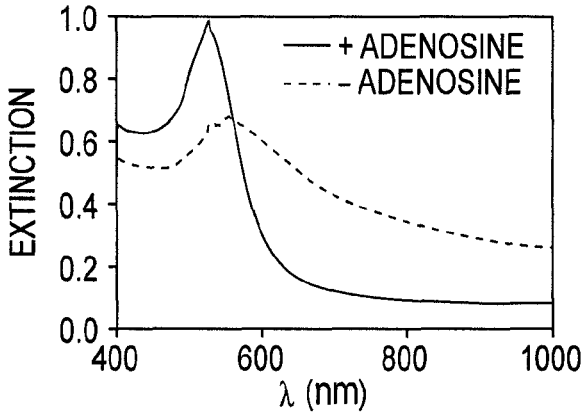


FIG. 8B

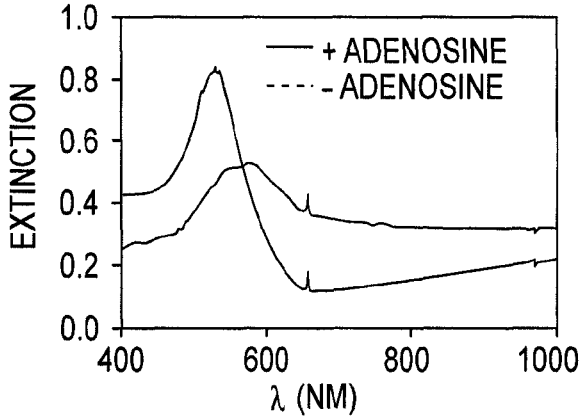


FIG. 8C

APTAMER- AND NUCLEIC ACID ENZYME-BASED SYSTEMS FOR SIMULTANEOUS DETECTION OF MULTIPLE ANALYTES

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/913,757 entitled "Aptamer- and Nucleic Acid Enzyme-Based Systems for Simultaneous Detection of Multiple Analytes" filed Apr. 24, 2007, which is incorporated by reference in its entirety.

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 Grant Numbers CTS-0120978 and DMR-0117792, U.S. Department of Defense Contract Number DAAD19-03-1-0227, and U.S. Department of Energy Contract Number DE-FG02-01 ER63179. The U.S. Government may have rights in this invention.

BACKGROUND

The ability to determine the presence of an analyte in a sample is of significant benefit. For example, analytes composed of certain ions and metals, such as those toxic elements belonging to the RCRA-8 metal group (lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), cadmium (Cd), barium (Ba), silver (Ag), and selenium (Se)), pose significant health risks when present in water supplies. It is common to perform sample analysis on drinking water, ground water, and waste water to monitor and safeguard water quality used for human consumption and agricultural purposes, as well as to preserve the environment.

Sample analysis is equally important for medical reasons and for homeland security. 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. In a similar vein, the detection of harmful agents, such as bioterrorist materials (for example, poisons like anthrax), minute quantities of highly explosive materials (for example, C4 plastic explosive and Trinitrotoluene (TNT)), and illegal drug substances and related contraband (for example, cocaine) is important for the safety of both individuals and society at large.

Colorimetric methods are commonly used for the detection of analytes in soil, water, or waste-stream samples, biological samples, body fluids, and the like. In relation to instrument-based methods of analysis, such as atomic absorption spectroscopy, calorimetric methods tend to be rapid and require little in the way of equipment or user sophistication. While conventional calorimetric 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.

Recently, colorimetric sensors based upon aptamers have been developed. Aptamers are nucleic acids (such as DNA or RNA) that recognize target effector molecules with high affinity and specificity (Ellington and Szostak 1990, Jayasena 1999). Aptamers have several unique properties that make them an ideal platform for designing highly sensitive and selective analyte sensors. First, in vitro selection methods can be used to obtain aptamers for a wide range of target effector molecules with exceptionally high affinity, having dissociation constants in the picomolar range (Brody and Gold 2000, Jayasena 1999, Wilson and Szostak 1999). Second, aptamers are easier to obtain and less expensive to produce than antibodies, because aptamers can be generated in vitro in short time periods (for example, within days) and at economical cost. Third, aptamers display remarkable structural durability and can be denatured and renatured many times without losing their ability to recognize their targets.

One particularly advantageous calorimetric sensor is an aptamer design that directs assembly or disassembly of metallic particle aggregates in response to an analyte. Metallic particles are exquisitely sensitive calorimetric reagents, having extinction coefficients three orders of magnitude higher than those of organic dyes (Link et al. 1999). Aptamer systems may be designed to bind two or more oligonucleotides that are coupled to particles (oligo-particles), thereby resulting in formation of an aggregate of particles (particle aggregate). Upon exposure to a sample containing the effector molecule (analyte), the aptamer binds to the effector molecule by undergoing a conformational change that precludes or weakens binding of the oligo-particles to each other, and the particle aggregate dissociates. Because particle aggregates display spectral attributes dependent upon the distance between the particles, the aggregation status of the oligo-particles is reflected by the appearance of distinct calorimetric properties. Since aptamers are designed to recognize a specific analyte, the presence of the specific analyte in a sample is reported calorimetrically as the particle aggregates dissociate. An example of this technology is described in U.S. Patent Application Publication No. 20070037171 A1, entitled Aptamer-Based Colorimetric Sensor Systems to Y. Lu et al., published Feb. 15, 2007.

Other types of sensors based upon nucleic acid enzymes (for example, aptazymes, DNAzymes, and RNAzymes) have been described. Nucleic acid enzymes are well known in the art, and have been used in sensor applications designed to detect single analyte species (see, for examples, U.S. Patent Application Publication No. 20030215810 A1, entitled Simple Catalytic DNA Biosensors For Ions Based On Color Changes to Y. Lu et al., published Nov. 20, 2003; U.S. Patent Application Publication No. 20040175693 A1, entitled Nucleic Acid Biosensors to Y. Lu et al., published Sep. 9, 2004).

Because aptamers and nucleic acid enzymes are selected for their ability to bind to specific target effector molecules, colorimetric sensors based on these conventional designs are limited to detecting a single analyte species in a sample. However, there is often a need to detect more than one type of analyte species in a given sample. For example, for a complete environmental analysis of mercury contaminants in a given sample, it is important to analyze the sample for the presence of both organic and inorganic mercury species. Even if aptamer and nucleic acid enzyme-based sensor system designs were available that recognize two or more analyte species, calorimetric sensor designs have not been implemented to permit selective detection of the different analyte species. Thus, sensors capable of simultaneously detecting multiple analytes present in a sample have not been described.

SUMMARY

In a first aspect, the invention is a system for simultaneously detecting multiple analytes in a sample that includes a first reactive polynucleotide that reacts to a first analyte; a second reactive polynucleotide that reacts to a second analyte; a third polynucleotide; a fourth polynucleotide; a first particle, coupled to the third polynucleotide; a second par-

ticle, coupled to the fourth polynucleotide; and at least one quencher, for quenching emissions of the first and second quantum dots, coupled to the first and second reactive polynucleotides. The first particle includes a quantum dot having a first emission wavelength. The second particle includes a second quantum dot having a second emission wavelength different from the first emission wavelength. The third polynucleotide and the fourth polynucleotide are different.

In a second aspect, the invention is a method for simultaneously detecting multiple analytes in a sample that includes combining at least one aggregate with a sample; and detecting a first and second emission responsive to the first and second analytes, respectively. The at least one aggregate includes a first reactive polynucleotide that reacts to a first analyte; a second reactive polynucleotide that reacts to a second analyte; a third polynucleotide; a fourth polynucleotide; a first particle, coupled to the third polynucleotide; a second particle, coupled to the fourth polynucleotide; and at least one quencher, for quenching emissions of the first and second quantum dots, coupled to the first and second reactive polynucleotides. The first particle includes a quantum dot having a first emission wavelength. The second particle includes a second quantum dot having a second emission wavelength different from the first emission wavelength. The third polynucleotide and the fourth polynucleotide are different.

In a third aspect, the invention is a kit for the simultaneous detection of multiple analytes in a sample that includes an aggregate forming system and a first container. The aggregate forming system includes a first reactive polynucleotide that reacts to a first analyte; a second reactive polynucleotide that reacts to a second analyte; a third polynucleotide; a fourth polynucleotide; a first particle, coupled to the third polynucleotide; a second particle, coupled to the fourth polynucleotide; and at least one quencher, for quenching emissions of the first and second quantum dots, coupled to the first and second reactive polynucleotides. The first particle includes a quantum dot having a first emission wavelength. The second particle includes a second quantum dot having a second emission wavelength different from the first emission wavelength. The third polynucleotide and the fourth polynucleotide are different. The first container contains the aggregate forming system, where a sample may be added to a container selected from the group including the first container and a second container.

In a fourth aspect, the invention is an indicator for a system for simultaneously detecting multiple analytes in a sample that includes a third polynucleotide; a fourth polynucleotide; a first particle, coupled to the third polynucleotide; and a second particle, coupled to the fourth polynucleotide. The first particle comprises a quantum dot having a first emission wavelength. The second particle comprises a second quantum dot having a second emission wavelength different from the first emission wavelength. The third polynucleotide and the fourth polynucleotide are different.

DEFINITIONS

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 substances potentially present in a sample. The analysis determines the presence, quantity, and/or concentration of the analyte present in a sample.

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 a first polynucleotide forming a complex with a second nucleotide through hydrogen bonding.

The term "complementary" refers to the ability to form base-pairing relationships between nucleobases, such as the ability to form a base-pairing between guanosine and cytosine or a base-pairing between adenine and thymine (or uridine). A polynucleotide may be partially or fully complementary with another polynucleotide. For example, a first polynucleotide having the sequence 5'-GATTCTAAGC-'3 (SEQ ID NO: 61) is partially complementary to a second polynucleotide having the sequence 5'-GAATCGCCCGAT-'3 (SEQ ID NO: 62) (the underlined sequences represent the possible base-pairing relationships between the two sequences). A first polynucleotide having the sequence 5'-GATTCTAAGC-'3 (SEQ ID NO: 61) is fully complementary to a second polynucleotide having the sequence 5'-GCTTAGAATC-3' (SEQ ID NO: 63).

The term "coupled" refers to attachment by either a covalent bond or a non-covalent bond. An example of a non-covalent bond is a hydrogen bond.

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

The term "nucleic acid enzyme" means an enzyme composed of a nucleic acid. Examples of nucleic acid enzyme include ribozymes (RNAzymes), deoxyribozymes (DNAzymes), and aptazymes.

The term "aptazyme", also referred to as "allosteric nucleic acid enzyme" or "allosteric (deoxy)ribozyme," is a nucleic acid enzyme in which the enzymatic activity is regulated by an effector. An aptazyme typically contains an aptamer domain, which recognizes an effector, and a catalytic domain. See, for example, Hesselberth et al. (2000); Soukup et al. (2000); and Tang et al. (1997).

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.

The term "reactive polynucleotide" is a generic term that includes aptamers, aptazymes, and nucleic acid enzymes.

The term "react," as related to the term "reactive polynucleotide," refers to the reactive polynucleotide responding to the analyte by undergoing a conformational change or by causing or catalyzing a reaction (for example, a cleavage of a substrate).

The terms "oligo," "oligonucleotide," and "polynucleotide" are used interchangeably.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates preferred embodiments for the simultaneous detection of multiple analytes in a single sample **102**

using aggregate system **154** that includes a single aggregate structure (A) or a mixture of separate aggregate structures (B);

FIG. **2** represents an analysis **100** for simultaneously determining the presence of multiple analytes in a sample;

FIG. **3**A depicts an aggregate **356** that contains quantum dots (Q**1**) that are quenched by quenching particles (**1** and **2**) and the disaggregation of aggregate **356** in the presence of the analyte adenosine to release Q**1**, which is accompanied by high luminescence;

FIG. **3**B depicts the composition of aggregate **356** that includes a polynucleotide **342**, oligo-particles **346** (which may contain a quantum dot (Q**1**) or a quenching particle (**2**)), and oligo-particle **352** (which may contain a quenching particle (**1**)), and the resultant formation of structure **355** and the release of oligo-particles **346** in response to an analyte **312** (adenosine);

FIG. **3**C depicts the composition of aggregate **358** that includes a polynucleotide **344**, oligo-particles **348** (which may contain a quantum dot (Q**2**) or a quenching particle (**3**)), and oligo-particle **352** (which may contain a quenching particle (**1**)), where the oligo-particles **348** react in response to cocaine as the analyte;

FIG. **3**D depicts the structure of one preferred quantum dot of oligo-particle **346** of FIG. **3**A;

FIG. **4** represents FIGS. **4**A and **4**B. FIG. **4**A depicts the composition of aggregate **456** that contains a polynucleotide **442** that includes a substrate for a DNAzyme **443**, oligo-particles **446** (which contain a quantum dot (Q**2**)), and oligo-particle **452** (which may contain a quenching particle (**1**)), and the resultant release of oligo-particles **446** from aggregate **456** by activation of a DNAzyme **443** in response to an analyte **412** (Pb(II)) and cleavage of the substrate in polynucleotide **442** to form products **442**(**5'**) and **442**(**3'**);

FIG. **4**B depicts the composition of aggregate **458** that contains a polynucleotide **444** that includes a substrate for a DNAzyme **445**, oligo-particles **448** (which contain a quantum dot (Q**2**)), and oligo-particle **452** (which may contain a quenching particle (**1**)), and the resultant release of oligo-particles **448** from aggregate **458** by activation of a DNAzyme in response to an analyte **414** (UO_2(II)) and cleavage of the substrate in polynucleotide **444** to form products **444**(**5'**) and **444**(**3'**);

FIGS. **5**A, B, and C depict the spectral characteristics of aggregate **456**, a kinetic time course for spectral luminescence, and the dependence of the spectral emission as a function of analyte concentration (adenosine), respectively;

FIGS. **5**D, E, and F depict the spectral characteristics of aggregate **458**, a kinetic time course for spectral luminescence, and the dependence of the spectral emission as a function of analyte concentration (cocaine), respectively;

FIGS. **6**A and **6**B illustrate the colorimetric assay of the concentration dependence of sensors as a function of analyte concentration (FIG. **6**A, adenosine as the analyte; FIG. **6**B, cocaine as the analyte);

FIGS. **7**A-D illustrate the spectral characteristics of quantum dot emission as a function of different analytes present in a sample;

FIG. **8**A illustrates the image of 10% human blood serum alone (left), and in the presence of the adenosine aptamer-coupled gold nanoparticles without (middle) or with 2 mM adenosine (right).

FIG. **8**B depicts the extinction of particle aggregates in 10% serum in the presence or absence of 2 mM adenosine when the particle aggregates were used immediately before use; and

FIG. **8**C depicts the extinction of the particle aggregates in 10% serum in the presence or absence of 2 mM adenosine after the particle aggregates were soaked in serum for 17 hours at room temperature.

DETAILED DESCRIPTION

The present invention makes use of the discovery of sensor systems that include polynucleotides coupled to quantum dots (oligo-particles, where the particles are QDs) having at least two different types of QDs with distinct emissions to permit simultaneous detection of multiple analytes in a single sample. Because QD's display sharp emission peaks, it is possible to have over ten distinct emission wavelengths in the visible range. By using QDs of different emission wavelengths, the identity of the analytes can be distinguished. In this manner, sensors are provided that are capable of reporting the presence of different analytes in a given sample, thus providing an advantage over previous sensor systems. Furthermore, the sensor systems may include aptazymes, RNAzymes, and DNAzymes, thereby broadening the range of analyte which may be detected. Finally, the sensor systems display remarkable stability under conditions that would normally degrade nucleic acids. This unexpected property affords the advantage of using the sensor systems which can detect multiple analytes in a sample obtained from biological sources, such as blood serum.

FIG. **1**A illustrates one preferred embodiment of simultaneous detection of multiple analytes in a single sample **102** with the described sensor systems. Aggregate system **154** contains first and second polynucleotides **142** and **144**; first and second particles coupled to third and fourth polynucleotides (oligo-particles) **146** and **148**, respectively; and optionally, a third oligo-particle **152** that includes a fifth polynucleotide. The first and second polynucleotides **142** and **144** may include first and second reactive polynucleotides **122** and **124**, respectively. The first oligo-particle **146** includes a third polynucleotide that may be partially complementary to a portion of the first reactive polynucleotide **122**, while the second oligo-particle **148** includes a fourth polynucleotide that may be partially complementary to the second reactive polynucleotide **124**. Oligo-particles **146** and **148** each may contain a particle encoding a unique QD (QD**1** and QD**2**, respectively) having a distinct spectral property. A representative portion of the first and second oligo-particles **146** and **148** may also encode a quencher, Q, that may serve to quench the spectral property of the QD. When included, the third oligo-particle **152** includes a fifth polynucleotide that may be partially complementary to a portion of the first and second polynucleotides **142** and **144**. The oligo-particle **152** may also encode a quencher, Q, that quenches the spectral property of the QDs.

The aggregate system **154** may be combined with a sample **102** suspected of containing analytes **112** and/or **114**. In the presence of **112**, reactive polynucleotide **122** becomes reactive and causes partial disaggregation of aggregate **154** to release oligo-particles **146** from aggregate **154**. As an oligo-particle **146** floats away from aggregate **154**, the QD**1** of the oligo-particle **146** is no longer quenched, and spectral property of the QD**1** becomes evident at a distinct wavelength (for example, increased luminescence emission at 585 nm). Similarly, in the presence of **114**, reactive polynucleotide **124** becomes reactive and causes partial disaggregation of aggregate **154** to release oligo-particles **148** from aggregate **154**. As an oligo-particle **148** floats away from aggregate **154**, the

QD2 of the oligo-particle **148** is no longer quenched, and spectral property of the QD2 becomes evident at a wavelength different from that of QD1.

FIG. **1**B illustrates a second preferred embodiment for the simultaneous detection of multiple analytes in a single sample **102** with the described sensor systems. Aggregate system **154** represents a mixture of separate aggregates **156** and **158**. Aggregate **156** includes reactive polynucleotide **122** that specifically binds to analyte **112**, oligo-particle **146**, and optionally, oligo-particle **152**. Aggregate **158** includes reactive polynucleotide **124** that specifically binds to analyte **114**, oligo-particle **148**, and optionally, oligo-particle **152**. Oligo-particles **146** and **148** each may contain a particle encoding a unique QD (QD1 and QD2, respectively) having a distinct spectral property. A representative portion of the first and second oligo-particles **146** and **148** may also encode a quencher, Q, that may serve to quench the spectral property of the QD. When included, the third oligo-particle **152** includes a fifth polynucleotide that may be partially complementary to a portion of the first and second polynucleotides **142** and **144**. The oligo-particle **152** may also encode a quencher, Q, that quenches the spectral property of the QDs.

The aggregate system **154** may be combined with a sample **102** suspected of containing analytes **112** and/or **114**. In the presence of **112**, reactive polynucleotide **122** becomes reactive and causes disaggregation of aggregate **156** to release oligo-particles **146** from aggregate **156**. As an oligo-particle **146** floats away from aggregate **156**, the QD1 of the oligo-particle **146** is no longer quenched, and spectral property of the QD1 becomes evident at a distinct wavelength (for example, increased luminescence emission at 585 nm). Similarly, in the presence of **114**, reactive polynucleotide **124** becomes reactive and causes disaggregation of aggregate **158** to release oligo-particles **148** from aggregate **158**. As an oligo-particle **148** floats away from aggregate **158**, the QD2 of the oligo-particle **148** is no longer quenched, and spectral property of the QD2 becomes evident at a wavelength different from that of QD1.

Examples of reactive polynucleotides include aptamers, aptazymes, RNAzymes, and DNAzymes. Aptamers become reactive upon binding an analyte by undergoing a conformational change. Aptazymes, RNAzymes, and DNAzymes become reactive upon binding an analyte by undergoing a chemical reaction (for example, cleaving a substrate). In each instance, the outcome of the reactive polynucleotide becoming reactive is to cause disaggregation of the aggregate and the release of at least one oligo-particle having a distinct spectral property.

FIG. **2** represents in greater detail an analysis **100** for simultaneously determining the presence and optionally the concentration of two or more different analytes **112** and **114** in a sample **102**. Analysis **100** includes processes **110**, **120**, **140**, **150**, **160**, and **170**. Optionally, analysis **100** includes process **130**. Though aptamers have been selected as the exemplified reactive polynucleotides in **100**, one skilled in the art will appreciate that the same principles can be applied to make and use nucleic acid enzymes (for example, aptazymes, RNAzymes, and DNAzymes) as the reactive polynucleotides.

In **110**, the desired analytes **112** and **114** for which the method **100** will determine the presence/concentration of are selected. If additional analytes are to be detected, a plurality of **110** may be performed, where each **110** is specific for a particular analyte.

In one aspect, the analytes **112** and **114** may be any ions that cause aptamers **122** and **124** to fold. In another aspect, the analyte **112** and **114** may be any metal ions that cause aptamers **122** and **124** to fold. Preferable monovalent ions having a 1+ formal oxidation state (I) include 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, including elements belonging to the RCRA-8 metal group (lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), cadmium (Cd), barium (Ba), silver (Ag), and selenium (Se)).

Preferred ions also include those compounds that share a common metal element, but differ only in their formal oxidation state. For example, inorganic mercury species possess an oxidation state of 2+, whereas organic mercury species possess an oxidation state of 1+. Samples that contain both inorganic and organic mercury species would be amenable to simultaneous detection with the present invention by using two different aptamers that recognize selectively the different oxidation states of the mercury species.

In another aspect, analytes **112** and **114** may be any biomolecules that causes aptamers **122** and **124** to fold. Preferable biomolecules include large biomolecules, such as proteins (for example, proteins related to viruses, such as human immunodeficiency virus (HIV), and cellular proteins, such as insulin), antibodies, growth factors, enzymes, viruses (for example, HIV, influenza virus, small pox virus, etc.), viral derived components (for example, HIV-derived molecules), bacteria (for example, *Bacillus anthracis* (cause of anthrax)), bacteria derived molecules and components (for example, molecules derived from *Bacillus anthracis*), fertility or pregnancy markers (for example, Luteinizing Hormone (LH) and Human chorionic gonadotropin (hCG), respectively), cancer markers (for example, carcinoembryonic antigen (CEA), prostate specific antigen (PSA)) or cells. Preferable biomolecules also may include small biomolecules, such as amino acids (for example, arginine), nucleotides (for example, ATP, GTP), neurotransmitters (for example, dopamine), cofactors (for example, biotin), peptides, or amino-glycosides.

In another aspect, analytes **112** and **114** may be any organic molecules that cause the aptamers **122** and **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).

Following section of multiple analytes in **110**, multiple aptamers, each specific for a given analyte, are selected in **120**. 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 analytes (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). Since aptamers are intended to permit detection of a specific analyte (for example, aptamer **122** being specific for analyte **112** and aptamer **124** being specific for analyte **114**), selection **120** should be performed

with each aptamer to exclude binding to different analytes, which might be subject to simultaneous detection in a given sample.

For example, an aptamer may be selected that specifically binds Pb while not significantly binding Hg, As, Cr, Cd, Ba, Ag, Se, or other competing metal ions. In one aspect, this may be achieved by isolating aptamers that bind Pb, then removing any aptamers that bind Hg, As, Cr, Cd, Ba, Ag, or Se. In another aspect, aptamers that bind Hg, As, Cr, Cd, Ba, Ag, or Se are first discarded and then those that bind Pb are isolated. In this manner, the selectivity of a particular aptamer may be increased.

In a similar manner, a pair of different aptamers **122** and **124** may be selected, in which each aptamer specifically binds to individual analyte species **112** and **114** that share a common element, but which differ in their formal oxidation state. For example, an aptamer **122** may be selected that specifically binds to analyte **112** that is an organic mercury species having an oxidation state of 1+ while not binding to analyte **114** that is an inorganic mercury species having an oxidation state of 2+. In one aspect, this may be achieved by isolating aptamers that bind mercury species having an oxidation state of 1+, then removing any aptamers that bind mercury species having an oxidation state of 2+. In another aspect, aptamers that bind mercury species having an oxidation state of 2+ are first discarded and then those that bind mercury species having an oxidation state of 1+ are isolated. In this manner, the selectivity of a particular aptamer **122** for a given analyte **112** may be increased.

Aptamers **122** and **124** include a nucleic acid strand that folds in the presence of specific analytes **112** and **114**, respectively. 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 polynucleotide coupled to a particle (oligo-particle). In this aspect, at least portions of the base sequence of the aptamer **122** and **124** may be complementary to at least one portion of another polynucleotide, such as oligo-particles **146** and **148**, respectively.

Aptamers **122** and **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.

Numerous examples of analytes and aptamers that bind with and fold in response to that analyte are well known in the art. Examples of each are described in U.S. patent application Ser. No. 11/202,380, entitled APTAMER-BASED COLORIMETRIC SENSOR SYSTEMS to Y. Lu et al., filed Aug. 11, 2005 and in Lee et al. (2004). Some of these examples are shown in Table I.

TABLE I

Examples of Aptamers, Nucleic Acid Enzymes, and Analytes

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO.)	Ref
Metal ions	K(I)	GGGTTAGGGTTAGGGTTAGGG (SEQ ID NO. 1)	33.
	Zn(II)	AGGCGAGGUGAAAUGAGCGGUAAUA GCCU (SEQ ID NO. 2)	8.
	Ni(II)	GGGAGAGGAUACUACACGUGAUAGU CAGGGAACAUGACAAACACAGGGAC UUGCGAAAAUCAGUGUUUUGCCAUU GCAUGUAGCAG AAGCUUCCG (SEQ ID NO. 3)	16.
Organic dyes	Cibacron blue	GGGAGAATTCCCGCGGCAGAAGCCC ACCTGGCTTTGAACTCTATGTTATTG GGTGGGGGAAACTTAAGAAAACTAC CACCCTTCAACATTACCGCCCTTCAG CCTGCCAGCGCCCTGCAGCCCGGGA AGCTT (SEQ ID NO. 4)	13.
	Malachite green	GGAUCCCGACUGGCGAGAGCCAGG UAACGA AUGGAUCC (SEQ ID NO. 5)	15.
	Sulforhodamine B	CCGGCCAAGGGTGGGAGGGAGGGG GCCGG (SEQ ID NO. 6)	39.
Small organic molecules	Biotin	AUGGCACCGACCAUAGGCUCGGGUU GCCAGAGGUUCCACACUUUCAUCGA AAAGCCUAUGC (SEQ ID NO. 7)	40.
	Theophylline	GGCGAUACCAGCCGAAAGGCCCUUG GCAGCGUC (SEQ ID NO. 8)	42.
	Adenine	GAUAGGACGAUUAUCGAAAAUCACC AGAUUGGACCCUGGUUAACGAUCCA UU (SEQ ID NO. 9)	23.

TABLE I-continued

Examples of Aptamers, Nucleic Acid Enzymes, and Analytes

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO.)	Ref
	Cocaine	GGGAGACAAGGATAAATCCTTCAATG AAGTGGGTCGACA (SEQ ID NO. 10)	31.
	Dopamine	GGGAAUUCCGCGUGUGCGCCGCGG AAGAGGGAAUAUAGAGGCCAGCACA UAGUGAGGCCCUCCUCCC (SEQ ID NO. 11)	22.
Amino acids	Arginine	GGGAGCUCAGAAUAAACGCUCAAGG AGGACCGUGCACUCCUCGAACAUUU CGAGAUGAGACACGGAUCCUGC (SEQ ID NO. 12)	10.
	Citrulline	GACGAGAAGGAGUGCUGGUUAUACU AGCGGUUAGGUCACUCGUC (SEQ ID NO. 13)	14.
Nucleosides & nucleotides	ATP	ACCTGGGGGAGTATTGCGGAGGAAG GT (SEQ ID NO. 14)	29.
	cAMP	GGAAGAGAUGGCGACUAAAACGACU UGUCGC (SEQ ID NO. 15)	18.
	GTP	UCUAGCAGUUCAGGUAACCACGUAA GAUACGGGUCUAGA (SEQ ID NO. 16)	11.
	Guanosine	GGGAGCUCAGAAUAAACGCUCAACC CGACAGAUCGGCAACGCCNUGUUUU CGACANGAGACACCGAUCCUGCACC AAAGCUUCC (SEQ ID NO. 17)	9.
	Adenosine	ACCTGGGGGAGTATTGCGGAGGAAG GT (SEQ ID NO. 18)	17.
RNA	TAR-RNA	GCAGTCTCGTCGACACCCAGCAGCG CATGTAACTCCCATACATGTGTGTGC TGGATCCGACGCAG (SEQ ID NO. 19)	4.
Biological cofactors	CoA	GGGCACGAGCGAAGGGCAUAAGCU GACGAAAGUCAGACAAGACAUGGUG CCC (SEQ ID NO. 20)	6.
	NMN	GGAACCCAACUAGGCGUUUGAGGG GAUUCGGCCACGGUAACAACCCCUC (SEQ ID NO. 21)	19.
	FAD	GGGCAUAAGGUAUUUAAUUCCAUAC AAGUUUACAAGAAAGAUGCA (SEQ ID NO. 22)	27.
	Porphyrin	TAAACTAAATGTGGAGGGTGGGACG GGAAGAAGTTTA (SEQ ID NO. 23)	7.
	Vitamin B12	CCGGUGCGCAUAACCACCUCAGUGC GAGCAA (SEQ ID NO. 24)	21.
Amino-glycosides	Tobramycin	GGGAGAAUUCCGACCAGAAGCUUUG GUUGUCUUGUACGUUCACUGUUACG AUUGUGUUAGGUUUAACUACACUUU GCAAUCGCAUAUGUGCGUCUACAUG GAUCCUCA (SEQ ID NO. 25)	36.
Oligo-	Cellobiose	GCGGGGTTGGGCGGGTGGGTTCGCT	41.

TABLE I-continued

Examples of Aptamers, Nucleic Acid Enzymes, and Analytes

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO.)	Ref
saccharides		GGGCAGGGGGCGAGTG (SEQ ID NO. 26)	
Poly-saccharides	Sephadex	UACAGAAUGGGUUGGUAGGCAUACC UAAUCGAGAAUGAUA (SEQ ID NO. 27)	30.
Antibiotics	Viomycin	GGAGCUCAGCCUUCACUGCAAUGGG CCGCUAGGUUGAUGUGCAGUGAAG UCAGCUGAGGCCCAGGGCUGAAAG GAUCGCCCUCCUCGACUCGUGGCAC CACGGUCGGAUCCAC (SEQ ID NO. 28)	35.
	Streptomycin	GGAUCGCAUUUGGACUUCUGCCCAG GGGGCACCACGGUCGGAUCC (SEQ ID NO. 29)	34.
	Tetracycline	GGCCUAAAACAUACCAGAUUUCGAU CUGGAGAGGUGAAGAAUUCGACCAC CUAGGCCGGU (SEQ ID NO. 30)	1.
	Vasopressin	ACGTGAATGATAGACGTATGTCGAGT TGCTGTGTGCGGATGAACGT (SEQ ID NO. 31)	38.
Peptides	Substance P	GGGAGCUGAGAAUAAACGCUCAAGG GCAACGCGGGCACCCCGACAGGUG CAAAAACGCACCGACGCCCGGCCGA AGAAGGGGAUUCGACAUGAGGCCCG GAUCCGGC (SEQ ID NO. 32)	25.
Enzymes	HIV Rev Transcriptase	UCCGUUUUCAGUCGGGAAAAACUG (SEQ ID NO. 33)	32.
	Human thrombin	GGTTGGTGTGGTTGG (SEQ ID NO. 34)	3.
Growth factors	$VEGF_{165}$	GCGGUAGGAAGAAUUGGAAGCGC (SEQ ID NO. 35)	28.
Transcription factors	NF-κB	GGGAUAUCCUCGAGACAUAAGAAAC AAGAUAGAUCCUGAAACUGUUUUAA GGUUGGCCGAUCUUCUGCUCGAGA AUGCAUGAAGCGUUCCAUAUUUUU (SEQ ID NO. 36)	20.
Antibodies	Human IgE	GGGGCACGTTTATCCGTCCCTCCTAG TGGCGTGCCCC (SEQ ID NO. 37)	37.
Gene Regulatory factors	Elongation factor Tu	GGGGCUAUUGUGACUCAGCGGUUC GACCCCGCUUAGCUCCACCA (SEQ ID NO. 38)	24.
Cell adhesion molecules	Human CD4	UGACGUCCUUAGAAUUGCGCAUUCC UCACACAGGAUCUU (SEQ ID NO. 39)	12.
cells	YPEN-1 endothelial	ATACCAGCTTATTCAATTAGGCGGTG CATTGTGGTTGGTAGTATACATGAGG TTTGGTTGAGACTAGTCGCAAGATAT AGATAGTAAGTGCAATCT (SEQ ID NO. 40)	2.
Viral/bacterial components	Anthrax spores Rous sarcoma virus	Sequences are not given AGGACCCUCGAGGGAGGUUGCGCA GGGU (SEQ ID NO. 42)	5. 26.

Reference Listing for Table I.

TABLE I-continued

Examples of Aptamers, Nucleic Acid Enzymes, and Analytes

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO.)	Ref

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TABLE I-continued

Examples of Aptamers, Nucleic Acid Enzymes, and Analytes

Analyte class	Example	Aptamer Motif Sequence (SEQ ID NO.)	Ref

40. Wilson, C., Nix, J. & Szostak, J. Functional Requirements for Specific Ligand Recognition by a Biotin-Binding RNA Pseudoknot. Biochemistry 37, 14410-14419 (1998).
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Referring again to FIG. **2**, analysis **100** for the simultaneous detection of multiple analytes in a sample is performed with the following system components. A first aptamer **122** that contains a binding region for a first analyte **112** may be adapted for use in a first polynucleotide **142**. For example, the non-analyte binding region of an aptamer specific for adenosine may be modified to provide the aptamer and the included polynucleotide **142**.

In a similar manner, a second aptamer **124** that contains a binding region for a second analyte **114** may be adapted for use in a second polynucleotide **144**. For example, the non-analyte binding region of aptamer specific for another analyte unrelated to adenosine (for example, cocaine) may be modified to provide the aptamer and the included polynucleotide **148**.

After selecting an appropriate aptamer or aptamers in **120**, the polynucleotides **142** and **144** are formed that includes the aptamers **122** and **124**, respectively. In one aspect (process **140**), the aptamers **122** and **124** may serve directly as the polynucleotides **142** and **144**, respectively. In another aspect (process **130**), the polynucleotides **142** and **144** may be formed by joining one or more extensions **132** and **134** with the aptamers **122** and **124**, respectively.

In **130**, extensions **132** and **134** may be any nucleic acid sequence that may be joined with aptamers **122** and **124**, that may undergo at least partial hybridization with at least one oligo-particle, and that is compatible with the analysis **100**. In this aspect, at least a portion of the base sequence of the extension **132** and **134** may be complementary to at least one portion of one or more oligo-particles. In one aspect, solid phase synthesis may be used to join aptamers **122** and **124** to extensions **124** and **134** to form polynucleotides **142** and **144**, respectively. In another aspect, after the aptamer **122** portion of the polynucleotide **142** is synthesized, the synthesis is continued to form the extension **132**. Similarly, the polynucleotide **144** containing aptamer **124** may be extended to include the extension **134**. In these latter aspects, any method commonly employed in the art may be used, such as chemical methods (for example, solid phase-based procedures) or enzymatic methods (for example, PCR-based procedures).

Preferably, extensions **132** and **134** include from 1 to 100 bases. In one aspect, preferably at least 25, 50, 70, or 90% of the bases present in extension **132** are capable of hybridizing with a complementary portion of an oligo-particle, such as the 5'-TCACAGATGAGT (SEQ ID NO. 56) portion of oligo-particle **352** in FIG. **3**B, while at least 50, 35, 25, or 10% of the bases present in the extension **332** are capable of hybridizing with another polynucleotide coupled to a particle, such as particle **346** in FIG. **3**B.

Referring to **140** of FIG. **2**, the polynucleotide **142** hybridizes with the oligo-particles **146** and includes the aptamer **122** and may include the extension **132**. For example, if the polynucleotide portions of the oligo-particles **346** and **352** have base sequences of 5'-CCCAGGTTCTCT-3' (SEQ ID NO. 45) and 5'-TCACAGATGAGT$(A)_{12}$-3' (SEQ ID NO. 44), respectively, an appropriate sequence for the polynucleotide **342** that includes the aptamer **322** that folds in the presence of an adenosine analyte and the extension **332** may be

(SEQ ID NO. 43)
5'-ACTCATCTGTGAAGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3'.

For the adenosine analyte, the extension **332** portion of the polynucleotide **342** is the 5'-ACTCATCTGTGAAGAGA-3' (SEQ ID NO. 57) portion of the sequence, which allows the extension **332** to hybridize with five bases of oligo-particle **346** and twelve bases of oligo-particle **352** (FIG. **3**B) Similarly, the aptamer **322** portion of the polynucleotide **342** is the 5'-ACCTGGGGGAGTATTGCGGAGAAGGT-3' (SEQ ID NO. 58) portion of the sequence, which allows the 5'-ACCTGGG-3' (SEQ ID NO. 59) portion of the aptamer **322** to hybridize with the 5'-CCCAGGT-3' (SEQ ID NO. 60) portion of the oligo-particle **346** (FIG. **3**B).

Referring to FIGS. **1** and **2**, the extensions **132** and **134** need not be the identical sequence for the polynucleotides **142** and **144**. Preferably, oligo-particles **146** and **148** include a plurality of sequences that are complementary to a portion of extensions **132** and **134**, respectively. Thus, a plurality of polynucleotides **142** and **144** can hybridize to oligo-particles **146** and **148** (FIGS. **1** and **2**). The formation of unique complexes **142**:**146** and **144**:**148** are possible where the sequence complementarities differ in the hybridized portions of the complexes.

The oligo-particle **152** includes a sequence complementary to an identical portion of extensions **132** and **134** such that oligo-particle **152** can hybridize to both polynucleotides **142** and **144**. Preferably, the oligo-particle **152** contains a plurality of such sequence complementarities, thereby permitting a plurality of polynucleotides **142** or **144** to bind to the single oligo-particle **152**, to form an aggregate system **154** containing both polynucleotides **142** and **144** (FIG. **1**A; corresponding to FIG. **2**, process **150**). Preferably, the aggregate system **154** includes the polynucleotides **142** and **144**, as well as oligo-particles **146**, **148** and **152**. Considering the physical size of its components, the aggregate system **154** may be quite large.

Aggregate system **154** also may be composed of separate aggregates **156** and **158**, which are prepared by separately mixing oligo-particles **152** with mixtures of polynucleotides **142** and oligo-particles **146** and mixtures of polynucleotides **144** and oligo-particles **148**, respectively (FIG. **1**B; corresponding to FIG. **2**, process **150**). The resultant aggregates **156** and **158** are responsive to separate analytes **112** and **114**, respectively. Thus, aggregate system **154** may include a mixture of separate aggregates **156** and **158** for the simultaneous detection of analytes **112** and **114** in sample **102**.

Because the oligo-particles **146** and **148** demonstrate distance-dependent optical properties, the particles are

quenched when closely held in the aggregate system **154** and undergo an increase in emission (for example, increased fluorescence) as the distance between the particles increases. For example, when the oligo-particles **146** and **148** include quantum dots, the aggregate system **154** displays a distinct emission spectrum characteristic of each quantum dot as disaggregation proceeds (FIG. **1**).

Referring to FIG. **2**, process for simultaneous detection of analytes **112** and **114** in sample **102** is performed in process **100** in the following manner. In **160**, aggregate system **154** is combined with sample **102**. In **170**, one of several fates may be possible for aggregate system **154**. If neither analyte **112** and **114** is present in sample **102**, then aggregate system **154** may not undergo any disaggregation. Under these circumstances, there may not be any discernible change in the spectral properties of aggregate system **154**.

Disaggregation of aggregate system **154** may occur under one of three scenarios. In the presence of analyte **112**, disaggregation may occur when the aptamer **122** portion of the polynucleotide **142** binds with and folds in response to the analyte **112**. When the aptamer **122** folds, a portion of the hybridization with the oligo-particles **146** is lost. This hybridization loss may allow the oligo-particles **146** to separate from the aggregate system **154**. Thus, as the oligo-particles **146** diffuse away from the aggregate system **154**, the solution luminescence at a specific wavelength may increase.

In the presence of analyte **114**, disaggregation also may occur when the aptamer **124** portion of the polynucleotide **144** binds with and folds in response to the analyte **114**. When the aptamer **124** folds, a portion of the hybridization with the oligo-particles **148** may be lost. This hybridization loss may allow the oligo-particles **148** to separate from the aggregate system **154**. Thus, as the oligo-particles **148** diffuse away from the aggregate system **154**, the solution luminescence at a wavelength different from that associated with oligo-particles **346** may increase.

In the presence of both analytes **112** and **114**, the aptamer **122** and **124** portions of the polynucleotides **142** and **144** bind with and fold in response to the analytes **112** and **114**, respectively. When these aptamers fold, portions of the hybridization with the oligo-particles **146** and **148** may be lost, which permits their separation from the aggregate system **154**. Thus, as both particles **146** and **148** diffuse away from the aggregate system **154**, the solution luminescence emission at two different wavelengths may increase.

In process **170** of FIG. **2**, the sample **102** may monitored for distinct emissions, such as an increase in a specific luminescence emission. Thus, the analysis **100** may provide a discriminatory sensor system because distinct emissions occur in the presence of the analytes **112** and **114**.

The oligo-particles **146**, **148**, and **152** may be composed of any particle species that demonstrate distance-dependent optical properties and are compatible with the operation of the sensor system. Quantum dots are preferred particles, because each type of quantum dot displays a unique emission wavelength. Preferred quantum dot particles include quantum dot semiconductors, such as CdS, CdSe, CdTe, ZnS, ZnSe, ZnTe, Pln, and PbSe. Additional preferred quantum dots may include ternary quantum dots, such as $Cd_xZn_{1-x}Se$ or CdS_xSe_{1-x}. Additional preferred quantum dots may include core-shell quantum dots, such as those having a CdSe core and ZnS shell. The quantum dots can also have different morphologies, including dots, rods, tetrapods, and the like. In a preferred aspect, the particles are quantum dot semiconductors having average diameter from 2 to 50 nanometers.

Other particles may be used in conjunction with quantum dots that may quench the spectral properties (for example, emission) of the quantum dots in aggregate system **154**. Preferred quenchers include those selected from the family of noble metal elements (Au, Ag, Pt, and Pd) and their alloys. Other preferred quenchers include organic quenchers, such as Dabycl, Black hole quenchers, Iowa black quenchers. These quenchers may be attached to other nanoparticles such as polystyrene or silica nanoparticles for use in oligo-particles **146**, **148**, and **152**. An especially preferred quencher is a gold nanoparticle.

Because energy transfer occurs to the quenching particles instead of the quantum dots, an increase in luminescence emission may realized by the inclusion of quenching oligo-particles in the aggregate mixture **154** (for example, a 200% increase), thereby improving the sensitivity of the sensor system. A portion of oligo-particles **146** and **148** may represent quenching particles, such as Au particles, while the remaining portion of the oligo-particles **146** and **148** may represent specific types of quantum dots. In a preferred aspect, the ratio of Au particles to quantum dots in oligo-particles **146** and **148**, ranges from 1:10 to 3:1, including 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, and 2:1. An especially preferred aspect, the ratio of Au particles to quantum dots in oligo-particles **146** and **148** is 1:1.

The rate at which a substantially complete spectral change occurs in response to analytes **112** and **114** may be considered the response time of the sensor system. In one aspect, the emission may be considered substantially complete when the extinction coefficient at 522 nm over 700 nm increases by 200% for quantum dots. 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. FIGS. **5** A, B, and C provide the spectral characteristics of aggregate **456**, a kinetic time course, and the dependence of emission of a QD as a function of the concentration of an adenosine analyte. FIGS. **5** D, E, and F provide the spectral characteristics of aggregate **458**, a kinetic time course, and the dependence of emission of a QD as a function of the concentration of a cocaine analyte.

The degree the spectral change in response to the analytes **112** and **114** may be quantified in **170** by quantification methods known to those skilled in the art. Various fluorimeters 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 fluorimeters may be used to plot a calibration curve in the desired concentration range. The emission of the sample may then be compared with the curve and the concentration of the analyte present in the sample determined.

FIG. **3**A depicts an aggregate **356** that contains a polynucleotide **342**, oligo-particles **346** and **352**. The polynucleotide **342** includes an aptamer **322** and optionally an extension **332**. The oligo-particle **346** includes a polynucleotide that is complementary to a portion of the extension and a particle that may be either a quantum dot (Q**1**) or a quenching agent (**2**). The oligo-particle **352** includes a polynucleotide that is complementary to a portion of the extension and a particle that may be a quenching agent (**1**). The quantum dots of oligo-particles **346** are quenched by quenching particles (**1** and **2**) when present in aggregate **356**. As the oligo-particles

346 are released, the aggregate 356, which has low luminescence, begins to disaggregate. This partial disaggregation displays enhanced luminescence as the oligo-particles 346 that contain a specific quantum dot diffuse away from the aggregate 356. If enough of the adenosine analyte 312 is present in the sample, the reaction will continue until all of the oligo-particles 346 are released from aggregate 356. Complete disaggregation of oligo-particles 346 from aggregate 356 results in high luminescence intensity of the spectral emission wavelength characteristic of the quantum dot of particle 346 due to the greater distance between the particles 346 (that is, those particles containing a quantum dot Q1) and 352 that contain a quencher (or other particles, such as oligo-particles 346, that include a quencher)).

FIG. 3B provides greater detail of the structure of the aggregate 356 and its disaggregation in the presence of adenosine analyte 312. The aggregate 356 is formed from multiple aggregate subunits. Some of the aggregate units may be formed from a first polynucleotide 342, which is hybridized to polynucleotide-coupled particles (oligo-particles) 346 and 352. The polynucleotide 342 includes an aptamer portion 322 and an extension portion 332. The polynucleotide portion of oligo-particle 352 (3'-$A_{12}Ade_{Au}$) (A_{12} disclosed as SEQ ID NO: 72) hybridizes with the extension 332, while the polynucleotide portion of particle 346 (5'-Ade_{Q1}) hybridizes with the extension 332 and the aptamer 322 to from the aggregate unit. In the presence of the analyte 312 (adenosine), the aptamer 322 undergoes a conformation change to form folded conformation 355 to release of oligo-particles 346.

FIG. 3C depicts the detailed structure of aggregate 358 that undergoes disaggregation in the presence of a cocaine analyte 314. Similar to that described above for the adenosine sensor, the aggregate 358 is formed from multiple aggregate subunits. Some of the aggregate units may be formed from a first polynucleotide 344, which is hybridized to a portion of the polynucleotides of oligo-particles 348 and 352. The polynucleotide 344 includes an aptamer portion 324 and an extension portion 334. The 3'-$A_{12}Ade_{Au}$ particle 352 hybridizes with the extension 334, while the 5'-Ade_{Q1} particle 348 hybridizes with the extension 334 and the aptamer 324 to from the aggregate unit. In the presence of the analyte 314 (cocaine), the aptamer 324 undergoes a conformation change to form a folded conformation (not shown) to release oligo-particles 348 from the particle aggregate 358, and an increase in the emission of the QD's (labeled as Q2 in FIG. 3B) associated with oligo-particles 348 results as the oligo-particles 348 float away from the aggregate 358.

TABLE IA

Polynucleotides and corresponding SEQ ID Nos.

Sequence	SEQ ID NO.
GTCTCCCGAGAT	64
ACTCATCTGTGAATCTCGGGAGACAAGGATAAATCCTTCAAT GAAGTGGGTCTCCC	65
TCTCTTGGACCCAAAAAAAAAAAA	66
GGAAGAGATGAGTGTCTACTCA	67
GGGTCCAAGAGAACTCACTATA	68
CACGTCCATCTCTGCAGTCGGGTAGTTAAACCGACCTTCAG ACATAGTGAGT	69
GGAAGAGATGGACGTGAGTGTCTACTCA	70

TABLE IA-continued

Polynucleotides and corresponding SEQ ID Nos.

Sequence	SEQ ID NO.
GGGTCCAAGAGAACTCACTATAGGAAGAGATGGACGTGAGT GTCTACTCA	71

The oligo-particles 346, 348, and 352 are designed with the particle moiety coupled to either the 5'- or 3'-terminus of the respective polynucleotides. Other particle attachment locations are possible within the polynucleotide, including site-specific attachment locations internal to the polynucleotide. Furthermore, different types of coupling linkers are possible for attaching different types of particles to oligonucleotides. For example, the oligo-particle 352 may contain a sulfur linker between a gold particle and the 3' terminus of the polynucleotide, whereas, the oligo-particle 346 may include a biotin linker between the quantum dot particle and the 5' terminus of the polynucleotide (FIGS. 3B and C). The structure of the quantum dot of oligo-particle 346 that is shown in FIG. 3D, which includes an internal core, an outer shell, a polymer coating attached to the outer shell, and streptavidin attached to the polymeric coating, would be amenable for coupling to a polynucleotide containing a biotin moiety. The particles may include nanoparticles, such as particles having an average outer diameter of 10-100 nm, including 15, 20, 25, 50, and 75 nm. The preparation of these and other polynucleotide-coupled particles is described in U.S. Provisional Patent Application Ser. No. 60/865,744, entitled Alignment of Nanomaterials and Micromaterials to Lu et al., filed Nov. 14, 2006.

Referring to FIGS. 3B and C, preferred oligo-particles 346 (and 348) and 352 may include quantum dots and gold particles, respectively. Optionally, a proportion of the oligo-particles 346 (and 348) may be substituted with quenching particles. Preferred proportions of particles 346 and 348 that may be substituted with quenching particles include 0 to 90% of the total population of particles 346 and 348, including 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, and 90%. Even more preferably, the quenching particles may be present at 30-70% of the total population of particles 346 and 348. Most preferably the quenching particles may be present at 40-60%, such as 50%, of the total population of particles 346 and 348. By substituting some of the quantum dot particles of particles 346 and 348 with quenching particles, such as gold particles, the basal luminescence of the quantum dot may be reduced further, thereby improving the signal to noise ratio of the sensor system upon exposure to bona fide analytes.

While one base sequence for the polynucleotide 342 (and 344) and the particles 346 (and 348) and 352 are shown in FIGS. 3 and 4, the bases may be changed on the opposing strands to maintain the complementary relationships (that is, base-pairings). For example, any cytosine in portions of the extension 332 (and 334) may be changed to thymine, as long as the corresponding base pairing partner of the oligo-particle is changed from guanine to adenine.

The oligo-particles 346 and 348 may be composed of quantum dots having different spectral emission properties. This feature is especially advantageous to enable simultaneous detection of multiple analytes in a sample. For example, if an aggregate subunit disaggregates in response to a first analyte, such as adenosine, then only the spectral emission property of the quantum dot particle associated only with the aptamer

specific for adenosine will be affected. If an aggregate unit disaggregates in response to a second analyte, such as cocaine, then only the spectral emission property of the quantum dot particle associated only with the aptamer specific for cocaine will be affected. If both types of analytes are present in a sample, then it will be possible to simultaneously detect the luminescence associated with the unique spectral emission properties of both types of quantum dot particles.

Aggregate system **154** may include other types of nucleic acid-based sensors, such as nucleic acid enzymes (aptazymes, DNAzymes, and RNAzymes). Aggregate system **154** may include two or more aptamers, aptazymes, DNAzymes, RNAzymes, or mixtures thereof. Rather than promoting disaggregation through a conformational change in their structure, however, nucleic acid enzymes may promote disaggregation of aggregate system **154** by cleaving a substrate in a polynucleotide that forms a linking part of the aggregate system **154**. Rather than selecting for aptamers in process **120** of FIG. **2**, nucleic acid enzymes are instead selected (see for example, Lu et al. 2003 and 2004).

In FIG. **1**, polynucleotides **142** and **144** include the reactive polynucleotides **122** and **124**, respectively, and optionally, extensions **132** and **134**, respectively. Thus, in one preferred embodiment, polynucleotides **142** and **144** represent a single nucleic acid, wherein the reactive polynucleotide is covalently connected to the extension. In another preferred embodiment, however, the reactive polypeptide may be separated from the extension to provide a polynucleotide **142** that includes two separate nucleic acids. According to this embodiment, oligo-particles **146**, **148**, and **152** may be available to hybridize to the polynucleotide containing the extension. Preferably, the polynucleotide containing the extension may also include a substrate for the reactive polynucleotide. Preferably, this substrate may be located in a region of the polynucleotide **142** that lies between the hybridization sites for different oligo-particles (for example oligo-particles **146** and **152**). Thus, the reactive polynucleotide will not be available to simultaneously hybridize to two different oligo-particles. Upon binding the desired analyte, the reactive polynucleotide becomes reactive, hybridizes to the substrate portion of the polynucleotide **142**, and catalyzes cleavage of the substrate. The result of substrate cleavage is the release of specific oligo-particles from the particle aggregate.

Examples of this preferred embodiment are illustrated in FIG. **4**. An aggregate **456** may be designed to disaggregate in response to an analyte acting as an effector for an aptazyme or as a cofactor for a DNAzyme. Referring to FIG. **4**A, aggregate **456** includes a polynucleotide **442** that contains a substrate for a nucleic acid enzyme **443** and oligo-particles **446** and **452**. Portions of the polynucleotides present in oligo-particles **446** and **452** are complementary to portions of polynucleotide **442**. The oligo-particles **446** and **452** preferably contain quantum dots and quenching particles (for example, gold particles), respectively. The nucleic acid enzyme **443** may be selected to react as an endonuclease to cleave the substrate of polynucleotide **442** in the presence of a cofactor, such as a metal ion (for example, Pb(II)). Upon exposure to the Pb(II) analyte **412**, the nucleic acid enzyme **443** becomes active and cleaves the substrate of polynucleotide **442**. Once cleaved, the oligo-particles containing the QD are released from the aggregate **456**, resulting in an increase of luminescence emission. FIG. **4**B depicts an analogous system (that is, aggregate **458**, which includes a polynucleotide **444** that contains a substrate for a nucleic acid enzyme **445** and oligo-particles **448** and **452**) that uses a nucleic acid enzyme that is reactive to a different analyte (UO_2(II)).

The aggregates display remarkable stability in human blood serum (FIG. **8**). This result suggests that the aggregate structure, itself, may afford some protection from nucleolytic degradation caused by serum-borne nucleases. This feature is an unexpected, surprising result, because most nucleic acids exposed to serum would be degraded quickly under normal circumstances. The robustness of the aggregates to withstand nucleolytic degradation suggests their advantageous use in sample analysis from body fluids, such as blood, and from other samples that may contain nucleases.

The methodology of FIG. **2** may be applied to other analytes (for example, analytes such as those listed in Table 1), besides those described for adenosine, cocaine, Pb(II), and UO_2(II). Table II gives the base sequences of the linkers and particles for adenosine, K(I), UO_2(II), Pb(II), 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

Polynucleotides and corresponding SEQ ID Nos. (A_{12} disclosed as SEQ ID NO: 72).

Name	Sequence	SEQ ID NO.
Adenosine Linker	5'-actcatctgtgaagagaACCTGGGGGAGTATTGCGGAGGAAGGT	43
3'-A_{12}Ade$_{Au}$	3'-AAAAAAAAAAAATGAGTAGACACT	44
5'-Ade$_{Au}$	5'-CCCAGGTTCTCT	45
Potassium Linker	5'-actcatctgtgatctaaGGGTTAGGGTTAGGGTTAGGG	46
3'-A_{12}K(I)$_{Au}$	3'-AAAAAAAAAAAATGAGTAGACACT	47
5'-K(I)$_{Au}$	5'-AACCCTTAGA	48
Cocaine Linker	5'-actcatctgtgaatctc GGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCCC	49
3'-A_{12}Coc$_{Au}$	3'-AAAAAAAAAAAATGAGTAGACACT	50

TABLE II-continued

Polynucleotides and corresponding SEQ ID Nos. (A_{12} disclosed as SEQ ID NO: 72).

Name	Sequence	SEQ ID NO.
5'-Coc_{Au}	5'-GTCTCCCGAGA	51
Pb(II) substrate Linker	5'-gggtccaagagaACTCACTATArGGAAGAGATGagtgtctactca	52
P(II)Enzyme	5'-CATCTCTTCTCCGAGCGGTCGAAATAGTGAGT	53
UO_2(II) substrate Linker	5'-gggtccaagagaACTCACTATArGGAAGAGATGGACGTG agtgtctactca	54
UO_2(II) Enzyme	5'-CACGTCCATCTCTGCAGTCGGGTAGTTAAACCGACCTTCA GACATAGTGAGT	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^+ ions, for example. In a preferred aspect, the monovalent metal ion concentration of the sample, which contains the aggregate, is from 30 mM to 1 M. 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. Chemical denaturants, such as urea and formamide, also may influence the aggregate binding, possibly attributable to the formation of hydrogen bonds of the polynucleotide base pairs with the chemical moieties of the chemical denaturants.

Thus, the performance of the sensor may be improved by adjusting the ionic strength and pH of the sample, or the inclusion of chemical denaturants in 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, pH, or presence of chemical denaturant may be controlled).

The sensor system, including the aptamers, an extension, and oligo-particles may be provided in the form of a kit. In one aspect, the kit includes the aptamer and the extension joined to form polynucleotide. In yet another 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 polynucleotide and oligo-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. Fluorimeters; standard analyte solutions, such as a 10 μm 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.

EXAMPLES

All DNA samples were purchased from Integrated DNA Technologies, Inc. (Coralville, Iowa). The aptamer DNA molecules were purified by denaturing polyacrylamide gel electrophoresis. Thiol-modified and biotinylated DNA were purified by standard desalting. Quantum dots may be obtained from commercial sources. For example, streptavidin coated QDs were purchased from Invitrogen (Carlsbad, Calif.). Adenosine, cytidine, uridine, Tris(2-carboxyethyl)-phosphine hydrochloride (TCEP), and cocaine hydrochloride were purchased from Aldrich (St. Louis, Mo.). Gold nanoparticles (AuNPs) (13 nm diameter) were prepared by literature procedures, and the extinction of the nanoparticle at 522 nm peak was about 2.4.

Example 1

Coupling Polynucleotides to Particles to Form Oligo-Particles

Thiol-modified DNA molecules (1 mM) were activated with two equivalents of TCEP at pH 5.5 for one hour at room temperature. After mixing TCEP activated thiol-modified DNA and AuNPs at room temperature for 16 hours or longer, the solution was brought to 100 mM NaCl and 5 mM Tris acetate, pH 8.2. The solution was allowed to sit at room temperature for another day. DNA-coupled AuNPs were purified by centrifugation at 13,200 rpm for 15 minutes followed by careful removal of the supernatant. Buffer (100 mM NaCl, 25 mM Tris acetate pH 8.2) was added to re-disperse the nanoparticles. The centrifugation process was repeated to completely remove free DNA. Streptavidin coated QDs (1 μM) were mixed with 5 equivalents of biotinylated DNA at 4° C. for at least 30 minutes and the mixture was directly used without further treatments.

Example 2

Preparation of Aptamer-Coupled Nanoparticles

To prepare adenosine aptamer-coupled nanoparticles (see FIG. **1**A), 1 mL of particle **1** (12 nM) and 1 mL of particle **2** (12 nM) were purified by centrifugation as described above. The two kinds of nanoparticles were mixed in a buffer (300 mM NaCl, 25 mM Tris acetate, pH 8.2) with a final volume of 1.4 mL. 10 μL of biotinylated DNA-coupled QDs (1 μM, emission peak at 525 nm) and a final concentration of 100 nM of the adenosine aptamer DNA was added. The mixture was incubated at 4° C. overnight to form aggregates, which were harvested by centrifugation and removal of supernatant. Finally, the nanoparticles were suspended in 1 mL of 200 mM NaCl, 25 mM Tris acetate, pH 8.2. The supernatant was almost colorless, suggesting that all gold nanoparticles were aggregated. Comparing the luminescence intensity of the supernatant with that of the aggregates (after disassembly) suggested that only 40% of the QDs were aggregated (data not shown). As a result, the molar ratio of particles 1:2:Q**1** was estimated to be 3:3:1. To prepare cocaine-responsive aptamer-containing aggregates, the procedures were the same except that 5 μL of 1 μM QDs (emission peak at 585 nm) was added and therefore the ratio of 1:3:Q**2** was around 6:6:1.

Example 3

Detection with Individual Sensors Based on Emission

The luminescence of QDs was monitored on a fluorometer (FluoroMax-P, Jobin Yvon Inc.). The excitation wavelength was set at 450 nm and emission at 525 nm and 585 nm was monitored for the adenosine and cocaine sensors, respectively. In a 0.5×0.5 cm quartz cuvette, 225 μL of 100 mM NaCl 25 mM Tris acetate, pH 8.2 buffer, 175 μL of 200 mM NaCl 25 mM Tris acetate buffer and 50 μL of the above nanoparticle aggregates so that final NaCl concentration was 150 mM and the final volume was 450 μL. The cuvette was vortexed before measurement to assure a homogenous suspension. After monitoring emission for 50 seconds, the cuvette was quickly taken out and a small volume of concentrated adenosine or cocaine solution was added. The cuvette was vortexed again and placed back into the fluorometer to continue the emission monitoring. FIG. **5** summarizes the spectral emission characteristics of these systems; the kinetic time course for emission production; and the dependence of the emission yield upon the concentration of the analyte.

Example 4

Detection with Individual Sensors Based on Color

In a 96 well plate (flat bottom), 80 μL of 100 mM NaCl solution was first added and then varying concentrations of adenosine or cocaine were added to each well. The reaction was initiated by addition of 80 μL of adenosine or cocaine sensor aggregates (dispersed in 200 mM NaCl). The plate was scanned at 5 min after addition of mixing. FIG. **6** shows the colorimetric results for aggregates containing aptamers that bind to adenosine or cocaine analytes.

Example 5

Detection with Mixed Sensors

The adenosine and cocaine sensors were mixed at a 2:1 ratio so that the emission intensities at the 525 and 585 peaks were roughly the same. The buffer condition was the same as in the individual sensors (150 mM NaCl, 25 mM Tris acetate, pH 8.2). The mixed sensors were added with varying analytes or combination of analytes. After 1 min the emission spectra were collected with excitation at 450 nm. FIG. **7** illustrates the spectra of aggregate systems upon disaggregation in the presence of control analytes (cytidine or cytidine and uridine; see FIG. **7**A), adenosine alone (FIG. **7**B), cocaine alone (FIG. **7**C), or both adenosine and cocaine (FIG. **7**D).

Example 6

Stability and Performance of Aptamer-Coupled Aggregates in Serum

Human blood serum (10% vol/vol) was prepared by diluting 50 μL of serum (Sigma) into 450 μL of buffer (300 mM NaCl, 25 mM Tris acetate, pH 8.2). Aggregates made from AuNPs **1** and **2** in FIG. **3**B were dispersed in the serum. Adenosine (2 mM) was added into one of the tubes and a photo was taken 20 seconds after adenosine addition (FIG. **8**A). The absorption spectra of the nanoparticles were also recorded on a UV-vis spectrometer (Hewlett-Packard Model No. 8453) by using freshly prepared 10% serum as the blank (FIGS. **8**B and C).

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:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 36

gggauauccu cgagacauaa gaaacaagau agauccugaa acuguuuuaa gguuggccga 60

ucuucugcuc gagaaugcau gaagcguucc auauuuuu 98

<210> SEQ ID NO 37
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

oligonucleotide

<400> SEQUENCE: 37

ggggcacgtt tatccgtccc tcctagtggc gtgcccc 37

<210> SEQ ID NO 38
<211> LENGTH: 44
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 38

ggggcuauug ugacucagcg guucgacccc gcuuagcucc acca 44

<210> SEQ ID NO 39
<211> LENGTH: 39
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 39

ugacguccuu agaauugcgc auuccucaca caggaucuu 39

<210> SEQ ID NO 40
<211> LENGTH: 96
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 40

ataccagctt attcaattag gcggtgcatt gtggttggta gtatacatga ggtttggttg 60

agactagtcg caagatatag atagtaagtg caatct 96

<210> SEQ ID NO 41

<400> SEQUENCE: 41

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<210> SEQ ID NO 42
<211> LENGTH: 28
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 42

aggacccucg agggagguug cgcagggu 28

<210> SEQ ID NO 43
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 43

actcatctgt gaagagaacc tgggggagta ttgcggagga aggt 44

<210> SEQ ID NO 44
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 44

tcacagatga gtaaaaaaaa aaaa 24

<210> SEQ ID NO 45
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 45

cccaggttct ct 12

<210> SEQ ID NO 46
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 46

actcatctgt gatctaaggg ttagggttag ggttaggg 38

<210> SEQ ID NO 47
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 47

tcacagatga gtaaaaaaaa aaaa 24

<210> SEQ ID NO 48
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 48

aacccttaga 10

<210> SEQ ID NO 49
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 49

actcatctgt gaatctcggg agacaaggat aaatccttca atgaagtggg tctccc 56

<210> SEQ ID NO 50

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<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 50

tcacagatga gtaaaaaaaa aaaa 24

<210> SEQ ID NO 51
<211> LENGTH: 11
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 51

gtctcccgag a 11

<210> SEQ ID NO 52
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 52

gggtccaaga gaactcacta taggaagaga tgagtgtcta ctca 44

<210> SEQ ID NO 53
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 53

catctcttct ccgagcggtc gaaatagtga gt 32

<210> SEQ ID NO 54
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide
<220> FEATURE:
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<400> SEQUENCE: 54

gggtccaaga gaactcacta taggaagaga tggacgtgag tgtctactca 50

<210> SEQ ID NO 55
<211> LENGTH: 52
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 55

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cacgtccatc tctgcagtcg ggtagttaaa ccgaccttca gacatagtga gt 52

<210> SEQ ID NO 56
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 56

tcacagatga gt 12

<210> SEQ ID NO 57
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 57

actcatctgt gaagaga 17

<210> SEQ ID NO 58
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 58

acctggggga gtattgcgga ggaaggt 27

<210> SEQ ID NO 59
<211> LENGTH: 7
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 59

acctggg 7

<210> SEQ ID NO 60
<211> LENGTH: 7
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 60

cccaggt 7

<210> SEQ ID NO 61
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 61

gattctaagc 10

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<210> SEQ ID NO 62
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 62

gaatcgcccg at 12

<210> SEQ ID NO 63
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 63

gcttagaatc 10

<210> SEQ ID NO 64
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 64

gtctcccgag at 12

<210> SEQ ID NO 65
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 65

actcatctgt gaatctcggg agacaaggat aaatccttca atgaagtggg tctccc 56

<210> SEQ ID NO 66
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 66

tctcttggac ccaaaaaaaa aaaa 24

<210> SEQ ID NO 67
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 67

ggaagagatg agtgtctact ca 22

<210> SEQ ID NO 68

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<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 68

gggtccaaga gaactcacta ta 22

<210> SEQ ID NO 69
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 69

cacgtccatc tctgcagtcg ggtagttaaa ccgaccttca gacatagtga gt 52

<210> SEQ ID NO 70
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 70

ggaagagatg gacgtgagtg tctactca 28

<210> SEQ ID NO 71
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 71

gggtccaaga gaactcacta taggaagaga tggacgtgag tgtctactca 50

<210> SEQ ID NO 72
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 72

aaaaaaaaaa aa 12

The invention claimed is:

1. A system for simultaneously detecting multiple nucleic acid enzyme cofactors in a sample, comprising:

a first nucleic acid enzyme, that reacts to a first nucleic acid enzyme cofactor;

a second nucleic acid enzyme, that reacts to a second nucleic acid enzyme cofactor;

a first polynucleotide, coupled to a first quantum dot having a first emission wavelength;

a second polynucleotide, coupled to a second quantum dot having a second emission wavelength different from the first emission wavelength;

at least one quencher, for quenching emissions of the first quantum dot and the second quantum dot, coupled to the first and second nucleic acid enzymes, respectively;

a first substrate of the first nucleic acid enzyme;

a second substrate of the second nucleic acid enzyme;

wherein each of the first substrate and the second substrate is a nucleic acid, and the first polynucleotide and the second polynucleotide are different nucleic acid sequences;

the first polynucleotide is hybridized to the first substrate;

the second polynucleotide is hybridized to the second substrate;

the first nucleic acid enzyme is hybridized to the first substrate and cleaves the first substrate in the presence of the first nucleic acid enzyme cofactor;

the second nucleic acid enzyme is hybridized to the second substrate and cleaves the second substrate in the presence of the second nucleic acid enzyme cofactor,

the first and second cofactors are different and the first and second nucleic acid enzymes are different.

2. The system of claim **1**, wherein the first and second quantum dots comprise at least one member selected from the group consisting of CdS, CdSe, CdTe, ZnS, ZnSe, ZnTe, PIn, and PbSe.

3. The system of claim **1**, wherein the at least one quencher comprises at least one member selected from the group consisting of gold, silver, platinum, and palladium.

4. The system of claim **1**, wherein at least one quencher comprises gold.

5. The system of claim **1**, wherein the first and second nucleic acid cofactors comprise metal ions.

6. The system of claim **5**, wherein the metal ions comprise at least one member selected from the group consisting of lead, mercury, arsenic, chromium, cadmium, barium, silver, and selenium.

7. The system of claim **5**, wherein the first and second nucleic acid cofactors comprise identical elemental metals differing only in formal oxidation state.

8. The system of claim **1**, wherein the first and second nucleic acid cofactors comprise inorganic and organic mercury species.

9. The system of claim **1**, wherein the first and second nucleic acid enzymes further comprise a nucleic acid sequence extension, wherein the extension comprises 1 to 100 bases, at least 25% of the bases of the extension complementary to the first or second nucleic acid enzyme.

10. A kit for simultaneously detecting multiple nucleic acid enzyme cofactors in a sample, comprising:

an aggregate forming system, and

a first container containing the aggregate forming system, where the sample can be added to a container selected from the group consisting of the first container and a second container;

wherein the aggregate forming system comprises:

a first nucleic acid enzyme, that reacts to a first nucleic acid enzyme cofactor;

a second nucleic acid enzyme, that reacts to a second nucleic acid enzyme cofactor;

a first polynucleotide, coupled to a first quantum dot having a first emission wavelength;

a second polynucleotide, coupled to a second quantum dot having a second emission wavelength different from the first emission wavelength;

at least one quencher, for quenching emissions of the first quantum dot and the second quantum dot, coupled to the first and second nucleic acid enzymes, respectively;

a first substrate of the first nucleic acid enzyme;

a second substrate of the second nucleic acid enzyme;

wherein each of the first substrate and the second substrate is a nucleic acid, and the first polynucleotide and the second polynucleotide are different nucleic acid sequences;

the first polynucleotide is hybridized to the first substrate;

the second polynucleotide is hybridized to the second substrate;

the first nucleic acid enzyme is hybridized to the first substrate and cleaves the first substrate in the presence of the first nucleic acid enzyme cofactor;

the second nucleic acid enzyme is hybridized to the second substrate and cleaves the second substrate in the presence of the second nucleic acid enzyme cofactor,

the first and second cofactors are different and the first and second nucleic acid enzymes are different.

11. The kit of claim **10**, wherein the aggregate forming system forms at least one aggregate having a potential to disaggregate in response to the first or second cofactors when the first nucleic acid enzyme and the second nucleic acid enzyme are DNAzymes and the first polynucleotide and the second polynucleotide are DNA.

12. The kit of claim **10**, further comprising a means for quantifying luminescence intensities of the first quantum dot and the second quantum dot at the first and second emission wavelengths.

13. The kit of claim **10**, further comprising a fluorimeter.

14. The kit of claim **10**, wherein intensities at the first and second emission wavelengths are proportional to the quantity of the first and second nucleic acid cofactors.

15. The kit of claim **10**, wherein the sample is from a source selected from the group consisting of an environmental source, a biological source and a chemical source.

16. The kit of claim **10**, wherein the sample is from an environmental source.

17. The kit of claim **10**, wherein said first and second nucleic acid cofactors comprise metal ions.

18. The kit of claim **17**, wherein the metal ions comprise at least one member selected from the group consisting of lead, mercury, arsenic, chromium, cadmium, barium, silver, and selenium.

19. The kit of claim **17**, wherein the metal ions comprise identical elemental metals differing only in formal oxidation state.

20. The kit of claim **10**, wherein the nucleic acid cofactors comprise inorganic and organic mercury species.

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