SPIROPYRAN-BASED COLORIMETRIC DETECTION

(5) Abstract: A composition for use in detecting an analyte in a sample by colorimetric readout comprising a polymer and chromophores contained in the polymer, wherein said chromophores are capable of undergoing a color change when the composition is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout. Related methods of using the composition and devices incorporating the composition are disclosed.
SPIROPYRAN-BASED COLORIMETRIC DETECTION
CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit from U.S. Provisional Application 61/715,994, filed October 19, 2012, and U.S. Provisional Application 61/777,837, filed March 12, 2013, which are incorporated herein by reference for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not Applicable

FIELD OF THE INVENTION

[0003] The present invention generally relates to colorimetric detection. In particular, the invention is related to compositions, devices and methods related to spiropyran-based colorimetric readout detection using molecularly imprinted polymers (MIPs), non-imprinted polymers (NIPs), polymer solutions, or spiropyran solutions.

BACKGROUND

[0004] Over the years, investigators have developed new methods for utilizing polymers, ranging from conducting polymers to matrices for cellular manipulation and development. As the interest in these polymers increased, attention to molecularly imprinted polymers (MIPs) became more of a focus for expanding polymer capabilities by increasing their selectivity to entrap molecules. Researchers have found that polymer manipulation through changes in pH, temperature, and environmental chemical gradients allows for further use of the product, including controlled chemical release from the polymer. MIPs in particular have found great use in industry, where they were traditionally used to filter and purify compounds within a production process.

[0005] The exploration of chromism phenomena has been of interest for quite some time, but the applications of such chromophores have been limited. The most popular and successful application to date are photochromic reading glasses that darken upon ultraviolet (UV) exposure (under sunlight outdoors), and return to a colorless reading frame indoors. Gold (Au) nanoparticles have also been explored as potential agents for a visible readout, but currently, the use of absorbance instrumentation is still required.

[0006] Current commercial methods for urine drug detection in the field, for example, primarily involve antibody staining systems which are attached to Whatman filter paper and targeted for specific analytes. A method of use is to dip an antibody drug detection device in a
sample and to subsequently observe a color change when an analyte is present or absent. A drawback to these systems is that they tend to yield false positive results, giving a color change when the analyte is not present. Additionally, the inability to maintain a permanent color change has been shown to be an issue in the legal system where court proceedings may take months to years. Thus, a technology that can withstand and maintain its integrity complementary to the respective procedural timelines is expected.

[0007] Needed in the art are additional methods, devices and compositions of colorimetric readout for a substance or a molecule of interest.

**SUMMARY OF THE INVENTION**

[0008] In one embodiment, the present invention relates to a composition for use in detecting an analyte in a sample by colorimetric readout, comprising a polymer; and chromophores contained in the polymer, wherein said chromophores are capable of undergoing a color change when the composition is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout.

[0009] In another embodiment, the present invention relates to a device for detection of analyte in a sample by colorimetric readout comprising a chamber for containing a sample that includes an analyte, and a composition for use in detecting an analyte in the chamber by colorimetric readout, comprising a polymer; and chromophores contained in the polymer, wherein the analyte in the sample causes a visible color change of the composition, thereby providing detection of the analyte by colorimetric readout.

[0010] In another embodiment, the present invention relates to a solution for use in detecting an analyte in a sample by colorimetric readout, comprising a solvent selected from the group consisting of water, dimethyl sulfoxide (DMSO), acetone, alcohol, dimethylformamide (DMF), and pyridine, a phosphate buffer, a polymer; and chromophores contained in the polymer, wherein said chromophores are capable of undergoing a color change when the composition is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout.

[0011] In one embodiment, the present invention relates to a spiropyran (SP) solution for use in detecting an analyte in a sample by colorimetric readout, comprising a solvent selected from the group consisting of water, dimethyl sulfoxide (DMSO), acetone, alcohol, dimethylformamide (DMF), and pyridine, a phosphate buffer, acrylic acid or its derivatives thereof; and spiropyran (SP), wherein said spiropyran (SP) is capable of undergoing a color
change when the solution is contacted with an analyte, thereby providing detection of the
analyte by colorimetric readout.

[0012] In one embodiment, the present invention relates to systems of a SP solution consisting of SP and a solvent and methods of using such system for colorimetric detection. Any suitable solvent may be used for the SP solution. In another embodiment, a suitable solvent for the present invention may be a mixture combination of two or more solvents.

DESCRIPTION OF DRAWINGS

[0013] Fig. 1 is a schematic diagram depicting general formulation for imprinted polymers. Polymer was created with the analyte templates, and the templates were later extracted, thus producing binding sites specific to the analytes.

[0014] Fig. 2 is a diagram showing the isomerization between spiropyran (SP) and merocyanine (MC). Theoretically, Spiropyran (SP) is colorless and merocyanine (MC) is colored. In the present invention, however, a SP solution shows a yellow color. It is likely that the yellow color is due to the acrylic acid or the existence of some MC isomers.

[0015] Fig. 3 is an illustration demonstrating a non-imprinted polymer (NIP) and its non complimentary binding ability for rapid tests and results.

[0016] Fig. 4 are photographs showing the set of compared experiments between molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) systems in the presence of the imprinted molecule of disodium succinate. Fig. 4A shows the experiments of MIP (left) and NIP (center and right) systems in the absence of UV polymerization. Fig.4B shows the experiments of MIP (left) and NIP (center and right) systems after UV polymerization.

[0017] Fig. 5 are photographs showing the color evolution after disodium succinate solution (0.25 M in phosphate buffer) was added into one of the NIP systems (NIP2, right). The same amount of phosphate buffer was added into the MIP and the other NIP (NIP1, center) the systems. After 50 minutes, the same color as that in the MIP system was developed in the NIP2 system. Fig. 5A t=5 minutes. Fig. 5B t=10 minutes. Fig. 5C t=20 minutes. Fig. 5D t=50 minutes.

[0018] Fig. 6 is a picture showing cut pieces of solid phase polymers or hydrogels developed from the corresponding solutions shown in Fig. 4a. The MIP polymer or hydrogel (Left) was imprinted with disodium succinate, developed from the corresponding MIP solution (left, Fig. 4a), and incubated in buffer. The NIP polymer or hydrogel (center) was incubated in buffer, developed from the corresponding NIP1 solution (center, Fig. 4a). The NIP polymer or
hydrogel (right) was incubated in 0.25 M disodium succinate solution, developed from the corresponding NIP2 solution (right, Fig. 4a).

[0019] Fig. 7 is a graph showing optical absorption spectra of spiropyran solutions with increasing concentrations of disodium succinate molecules.

[0020] Fig. 8 is a picture showing a comparison of sample color scales of the polymer or hydrogel solutions as the concentration of disodium succinate increases.

[0021] Fig. 9 is a picture showing the non-imprinted polymer or hydrogel solid forms prepared following UV polymerization of the corresponding solutions. [Exposure to UV light does not affect SP molecular photochromic properties, since the samples retain the same color after UV irradiation.

[0022] Fig. 10 is a set of pictures showing the color evolution of the solid phase of non-imprinted polymers or hydrogels in the presence of disodium succinate solutions. Fig. 10A t=0. Fig. 10B t=20 minutes. Fig. 10C t=50 minutes. Fig. 10D t=2 hours.

[0023] Fig. 11 is a picture showing the solid phase of non-imprinted polymers or hydrogels incubated in disodium succinate solutions after 24 hours.

[0024] Fig. 12 is a set of pictures showing a comparison between the solid phase of non-imprinted polymers or hydrogels after being incubated in disodium succinate for 24 hours and a controlled sample. Fig. 12A top view. Fig. 12B side view.

[0025] Fig. 13 is a picture showing the samples of polymer or hydrogel solutions including analyte of disodium succinate (left) and a controlled experiment in the absence of analyte of disodium succinate (right) before UV polymerization.

[0026] Fig. 14 is a graph illustrating the absorbance spectra of a controlled experiment (green line) and the polymer or hydrogel solutions containing 4 mg of disodium succinate (blue line). The lighter blue line shows the absorbance spectra of the polymer or hydrogel solution containing 4 mg of disodium succinate after an additional 6 mg disodium succinate were added.

[0027] Fig. 15 is a picture showing the color changes of the polymer or hydrogel solutions in the absence of disodium succinate by adding different concentrations of acrylic acid.

[0028] Fig. 16 is a picture showing the color changes of the polymer or hydrogel solutions, acrylic acid concentrations consistent with the solutions shown in Fig. 15, in the presence of disodium succinate (2mg).

[0029] Fig. 17 is a set of pictures showing the effect of gold (Au) nanoparticles. Fig. 17A polymer or hydrogel solutions of an MIP system (left) and NIP systems (center and right) with gold (Au) nanoparticles. Fig. 17B polymer or hydrogel of an MIP system (left) and NIP systems
(center and right) containing both SP molecules and gold (Au) nanoparticles initially incubated in buffer (MIP and NIP-center), and disodium succinate solution (NIP-right).

[0030] Fig. 18 is a picture showing the color change over time of the polymer or hydrogel solutions of MIP system (left) and NIP systems (center and right) after disodium succinate solution was added into one of the NIP systems (NIP2, right).

[0031] Fig. 19 are pictures showing Fig. 19A a Puritan® flocked swab and Fig. 19B a Puritan®'s sponge swab.

[0032] Fig. 20 is a set of pictures showing a transport system of a Puritan® sponge swab in colorimetric detection. Fig. 20A a Puritan® sponge swab before colorimetric detection. Fig. 20B the Puritan® sponge swab exhibiting a purple color after exposing to disodium succinate and spiropyran (SP) solutions successively. Fig. 20C the Puritan® sponge swab exhibiting a yellow color after exposing to spiropyran (SP) solutions for overnight. Fig. 20D the Puritan® sponge swab exhibiting a yellow color after exposing to spiropyran (SP) solution for overnight and the remaining spiropyran (SP) solution showing no colors.

[0033] Fig. 21 is a picture showing that neutralizing buffer is not a suitable transport media for colorimetric detection. Fig. 21A Neutralizing buffer and spiropyran (SP) solution. Fig. 21B Neutralizing buffer, spiropyran (SP) and disodium succinate solution. Fig. 21C Water, spiropyran (SP) and disodium succinate solution. Fig. 21 Spiropyran (SP) and disodium succinate solution.

[0034] Fig. 22 is a picture showing that Peptone water is a suitable transport media for colorimetric detection. Fig. 22A Peptone water and spiropyran (SP) solution. Fig. 22B Peptone water, spiropyran (SP) and disodium succinate solution. Fig. 22C Water, spiropyran (SP) and disodium succinate solution. Fig. 22D Spiropyran (SP) and disodium succinate solution. Fig. 22E Peptone water and solid-phase spiropyran (SP).

[0039] Fig. 23 is a picture showing that Ames solution is a suitable transport media for colorimetric detection. Fig. 23A Ames solution and spiropyran (SP) solution. Fig. 23B Ames solution, spiropyran (SP) and disodium succinate solution. Fig. 23C Water, spiropyran (SP) and disodium succinate solution. Fig. 23D Spiropyran (SP) and disodium succinate solution. Fig. 23E Ames solution and solid-phase spiropyran (SP).

[0036] Fig. 24 is a picture showing that Butterfield's solution is a suitable transport media for colorimetric detection. Fig. 24A Butterfield's solution and spiropyran (SP) solution. Fig. 24B Butterfield's solution, spiropyran (SP) and disodium succinate solution. Fig. 24C Water, spiropyran (SP) and disodium succinate solution. Fig. 24D Spiropyran (SP) and disodium succinate solution. Fig. 24E Butterfield's solution and solid-phase spiropyran (SP).
Fig. 25 is a picture showing that Letheen broth is a suitable transport media for colorimetric detection. Fig. 25A Letheen broth and spiropyran (SP) solution. Fig. 25B Letheen broth, spiropyran (SP) and disodium succinate solution. Fig. 25C Water, spiropyran (SP) and disodium succinate solution. Fig. 25D Spiropyran (SP) and disodium succinate solution. Fig. 25E Letheen broth and solid-phase spiropyran (SP).

Fig. 26 is a picture showing that UTM-RT is a suitable transport media for colorimetric detection. Fig. 26A UTM-RT and spiropyran (SP) solution. Fig. 26B UTM-RT, spiropyran (SP) and disodium succinate solution. Fig. 26C Water, spiropyran (SP) and disodium succinate solution. Fig. 26D Spiropyran (SP) and disodium succinate solution. Fig. 26E UTM-RT and solid-phase spiropyran (SP).

Fig. 27 is a set of pictures showing that the present invention of colorimetric detection may be combined with elastic polymers and elastic hydrogels. Fig. 27A-C) An elastic hydrogel in the absence of colorimetric detection system showing colorless. Fig. 27D-F) An elastic hydrogel having spiropyran (SP) showing a yellow color. Fig. 27G-I) An elastic hydrogel having spiropyran (SP) in the present of disodium succinate showing a pink color.

Fig. 28 is a picture showing adenosine triphosphate (ATP) detection in a SP solution. Color change was observed upon the addition of an ATP solution to the SP solution. The primary control sample was purple, the secondary control sample was dark pink, and the test sample shows yellow. The secondary control sample was used to illustrate that the yellow color change observed by ATP interactions was not due to the solvent. The solvent was water (or a PBS buffer) in all the cases. SP solutions were produced by mixing suitable amount of spiropyran (SP) and hydroxyethyl methacrylate (HEMA).

Fig. 29 is a graph showing UV-Vis spectra for ATP detection according to experiments shown in Fig. 28. The yellow line represents the primary control sample of HEMA+SP at 1mg/mL in the absence of water. The pink line represents the secondary control sample of HEMA+SP/DI Water in a 1:1 ratio. The Green line represents the test sample with HEMA+SP/ATP solution in a 1 to 1 ratio. This data corresponds with the proposed merocyanine (MC) isomers associated with the "chromic" properties of the spiropyran (SP) molecule.

Fig. 30 a set of pictures and graphs showing the color changes of SP solutions during ATP detection along with peak shifts and peak intensity changes in UV-Vis spectra of the corresponding SP solutions. Fig. 30A shows the color changes of the polymer solutions before (left) and after the addition of an ATP solution (right). Fig. 30B shows the peak shifts and peak
intensity changes in UV-Vis spectra of the ATP detection solution (yellow) as compared with those in the control experiment (pink).

[0043] Fig. 31 is a set of pictures and graphs showing the color changes of polymer solutions during ATP detection along with peak shifts and peak intensity changes in UV-Vis spectra of the corresponding polymer solutions. Fig. 31A shows the color changes of the polymer solutions before (left) and after the addition of an ATP solution into the polymer solutions (right). Fig. 31B shows the peak shifts and peak intensity changes in UV-Vis spectra of the ATP detection solution (yellow) as compared with those in the control experiment (pink). A polymer solution formed by mixing suitable amount of acrylamide, N,N-Methylene Bisacrylamide, and 4,4-Azobis(4-Cyanovaleric acid). Suitable amounts of SP and HEMA were later added into the polymer solution.

[0044] Fig. 32 is a set of pictures showing the color changes of solid NIP polymers during ATP detection. The NIP polymer solutions formed by mixing suitable amount of acrylamide, N,N-Methylene Bisacrylamide, and 4,4-Azobis(4-Cyanovaleric acid). Suitable amounts of SP and HEMA were later added into the NIP polymer solutions. The NIP polymer solutions were polymerized to form solid NIP polymers following UV irradiation. The solid NIP polymer appears to be pink (Fig. 32A, left) after a PBS solution was added. After the addition of an ATP solution, the solid NIP polymer appears to be yellow (Fig. 32B, right).

[0045] Fig. 33 is a set of pictures showing time-dependent color changes of solid NIP polymers as shown in Fig. 32. For each of the picture, the samples in the left lane are MIP solid polymers, the samples in the center lane are NIP solid polymers in the absence of ATP, and the samples in the right lane are NIP solid polymers in the presence of ATP. For each lane of the samples, the top samples are corresponding polymer solutions before polymerization. The NIP solid polymers or corresponding solutions turn yellow within 1 minutes after the addition of ATP solutions.

[0046] Fig. 34 is a picture showing the color changes of NIP polymer solutions in the presence of different concentration of ATP. The ATP concentrations were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mg/mL for the NIP polymer solutions from left to right. Color of the NIP polymer solutions shifts from purple/pink to lighter pink to yellow as the ATP concentrations increase.

[0047] Fig. 35 is a graph showing UV-Vis spectra of the corresponding NIP polymer solutions as shown in Fig. 34. Peak shift and intensity change can be associated with isomerization of SP.
Fig. 36 is a picture showing the color changes of SP solutions in dimethyl sulfoxide (DMSO) after addition of water or ammonium nitrate solutions. SP solutions in DMSO show a blue color (left). After addition of DI water into the SP solution in DMSO, the solutions turn pink (center). After addition of an ammonium nitrate solution into the SP solution in DMSO, the solution turns yellow (right). A SP solution in DMSO was produced by dissolving suitable amount of SP in DMSO. Ammonium nitrate solutions used in the experiments have a concentration of 10 mg/mL in DI water.

Fig. 37 is a graph showing UV-Vis spectra of the solutions as shown in Fig. 36. The light blue/blue-green (aqua) line represents a primary control sample of SP solution in DMSO at 1 mg/mL in the absence of water. The green line represents a secondary control sample of SP solution in DMSO and DI water in a 1:1 ratio. The dark blue line represents the sample of a SP solution in DMSO and an ammonium nitrate solution in a 1:1 ratio. Peak shift and intensity change can be associated with isomerization of SP.

Fig. 38 is a set of pictures and graphs showing ammonium nitrate detection by using SP solutions. After addition of DI water into the SP solution in DMSO, the solutions turn dark pink (left, Fig. 38A). After addition of an ammonium nitrate solution into the SP solution in DMSO, the solution turns yellow (right, Fig. 38A). Fig. 38B shows UV-Vis spectra of the SP solution in DMSO after addition of DI water (purple) and after addition of an ammonium nitrate solution (yellow).

Fig. 39 is a set of pictures and graphs showing ammonium nitrate detection by using polymer and SP solutions. Polymer and SP solutions were produced using similar protocols as discussed above. The polymer and SP solution in the absence of ammonium nitrate shows a pink color (left, Fig. 39A). After addition of an ammonium nitrate solution, the polymer and SP solution turns yellow (right, Fig. 39A). Fig. 39B shows UV-Vis spectra of solutions as shown in Fig. 39A. The pink line represents a control sample of polymer and SP solution. The yellow line represents the sample of polymer and SP solution after addition of an ammonium nitrate solution.

Fig. 40 is a set of pictures showing the color changes of solid NIP polymers during ammonium nitrate detection. The samples in the left lane (pink) are NIP solid polymers in the absence of ammonium nitrate, and the samples in the right lane (yellow) are NIP solid polymers in the presence of ammonium nitrate. Solid NIP polymers were produced following a similar protocol as discussed above.

Fig. 41 is a set of pictures showing time-dependent color changes of solid NIP polymers as shown in Fig. 40. For each of the picture, the samples in the left lane are MIP solid
polymers, the samples in the center lane are NIP solid polymers in the absence of ammonium nitrate, and the samples in the right lane are NIP solid polymers in the presence of ammonium nitrate. The NIP solid polymers can start turning yellow within 1 minute after the addition of ammonium nitrate solutions.

[0054] Fig. 42 is a picture showing the color changes of SP solutions in DMSO in the presence of different concentration of ammonium nitrate. In the back row from left to right, the ammonium nitrate concentration changes from 0 to 14 µL. In the front row from left to right, the ammonium nitrate concentration changes from 18 to 500 µL. The solutions in the front row shows a concentration-dependent color change, and the color changes from purple/pink to yellow as the concentration of ammonium nitrate increases.

[0055] Fig. 43 is a graph showing UV-Vis spectra of the solutions as shown in Fig. 42. Peak shift and intensity change can be associated with isomerization of SP. After addition of ammonium nitrate solutions, a new peak was observed at 470 nm.

[0056] Fig. 44 is a graph showing a Gram positive identification flow chart.

DESCRIPTION OF THE INVENTION

[0057] Before the present materials and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, materials, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by any later-filed non-provisional applications.

[0058] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. As well, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. The terms "comprising" and variations thereof do not have a limiting meaning where these terms appear in the description and claims. Accordingly, the terms "comprising", "including", and "having" can be used interchangeably.

[0059] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications and patents specifically mentioned herein are incorporated by reference for all purposes including describing and disclosing the chemicals,
instruments, statistical analysis and methodologies which are reported in the publications which might be used in connection with the invention. All references cited in this specification are to be taken as indicative of the level of skill in the art. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0060] As used herein, the term "analyte" refers to a substance, atom or molecule of interest such as a chemical, which may be detected by a color readout system.

[0061] As used herein, the term "color system" refers to a solution phase or a solid phase of a Polymer, or a SP solution, which changes color in the presence of an analyte.

[0062] As used herein, the term "chromophore" refers to a molecule, which undergoes conformation or isomerization change under a condition, leading to a visible color change of the solution having the molecule.

[0063] As used herein, the term "photochromism" refers to reversible transformation of a chemical species between two forms by the absorption of electromagnetic radiation, where the two forms have different absorption spectra. It may also be described as a reversible change of colour upon exposure to light. As photochromism is just a special case of a photochemical reaction, almost any photochemical reaction type may be used to produce photochromism with appropriate molecular design. Specifically in the present invention, photochromism may be conserved in any suitable forms, such as hydrogel.

[0064] As used herein, the term "polymer or hydrogel solution" refers to a solution containing at least some of the polymer or hydrogel components, which is soluble in the solution. It may include both MIP and NIP color systems.

[0065] As used herein, the terms "polymer" and "hydrogel", referring to the same or similar meaning in the invention, are often interchangeable throughout the invention. The terms "polymer" and "hydrogel" include both solid phases of polymers or hydrogels and polymer or hydrogel solutions.

[0066] As used herein, the terms "imprinting molecule" and "analyte", referring to the same or similar meaning in the invention, are interchangeable throughout the invention unless indicated otherwise.

[0067] As used herein, the terms "colorimetric readout" and "colorimetric detection", referring to the same or similar meaning in the invention, are interchangeable throughout the invention unless indicated otherwise.

[0068] As used herein, smart polymers, stimuli-responsive polymers or stimuli-responsive hydrogels are high-performance polymers or hydrogels that change according to the
environment they are in. Such materials can be sensitive to a number of factors, such as temperature, humidity, pH, the intensity of light or an electrical or magnetic field and can respond in various ways, like altering color or transparency, becoming conductive or permeable to water or changing shape (shape memory polymers). Usually, slight changes in the environment are sufficient to induce greater change in the polymer's properties.

Smart polymers and hydrogels may appear in highly specialized applications and everyday products alike. They may be used for the production of hydrogels, biodegradable packaging, and to a great extent in biomedical engineering. One example is a polymer that undergoes conformational change in response to pH change, which may be used in drug delivery. In one embodiment the present invention relates to smart polymers, stimuli-responsive polymers or stimuli-responsive hydrogels for colorimetric detection.

As used herein, the term "kit", refers to a device for detection of an analyte in a sample by colorimetric readout. Specifically, in the present invention, a kit for detection of an analyte in a sample by colorimetric readout may comprise any composition as discussed in the specification, a solid support, and a means for detecting a visible color change of the composition, thereby providing detection of the analyte by colorimetric readout.

Molecularly Imprinted Polymers (MIPs) and Molecular Imprinting Technique: Molecularly imprinted polymers (MIPs) are the polymers which have been processed by a molecular imprinting technique, and these polymers thus possess cavities in polymer matrix with affinity to a chosen template molecule. A molecular imprinting technique is a laboratory technique commonly adapted by many scientists in the field. Briefly, molecular imprinting is a technique that creates specific recognition sites for a target molecule within a synthetic polymer, and the goal of a molecular imprinting technique is to make an artificial lock for a specific molecule which serves as the key.

Referring now to Fig. 1, a general formulation and procedure to make molecularly imprinted polymers (MIPs) is depicted. The formulation generally includes a cross-linking monomer (not shown), and a template (the imprint molecule). Functional and cross-linking monomers are copolymerized in the presence of a template (the imprint molecule) in a suitable solvent. The template may be the target molecule or any structural derivatives of the target molecule. MIPs show specific binding to the imprint molecules.

As shown in Fig.1, the functional monomers generally are monomers crossing link with each other. Prior to polymerization, the functional monomers initially form a complex with the template molecules usually by intermolecular interactions such as van der Waals force, electrostatic force, hydrogen bond, ionic bond, etc. After polymerization, the polymer matrix
forms around the complex of the functional monomers and the template molecules, so that the monomer-template assembly is held in position by the highly cross-linked three-dimensional rigid structures. After subsequent removal of the imprinted molecules, cavities are produced within the polymer matrix showing specific sizes, shapes, and chemical functionalities complementary to those of the template molecules. Consequently, the resulting MIPs show specific affinity with the template molecules (the imprint molecules). The resulting MIP contains recognition sites, with shape and functional groups complementary to the imprint molecule. There exists a significant difference between a hydrogel MIP of the present invention and a traditional MIP. Traditional MIPs require a specific linker in order to accommodate the geometrical inter molecular distances for which the prior interactive forces may be operative. Hydrogel MIPs of the present invention do not require a specific linker within the imprint to ensure the stability of a MIP specifically for the analyte being used. This is critical as the complication of discovering the specific linker is the rate limiting step in the traditional MIP paradigm. Further, the stabilization of analytes is the restriction factor in non-hydrogel polymers, thus limiting the utility of the traditional methodology of MIPs.

By using the above molecular imprinting technique, a molecular memory is introduced into the resulting MIPs, which is capable of selectively binding specific target molecules. Thus, the techniques and the MIPs may be used to fabricate sensors with heightened sensitivity and selectivity. One benefit of the imprinting technique and the MIPs is the capability of controlling the process of uptaking and releasing target molecules simply by varying the experimental conditions such as temperature, pH, or solvents, etc.

A variety of monomers may be used in molecular imprinting. For example, different monomers capable of different interactions with imprint molecules may be used. Further, molecular imprinting can be implemented in many systems, such as bulk polymers, beads, membranes, polymer films, polymer sprays and other forms where the proposed device would be useful. In one embodiment, the present invention relates to smart polymers, stimuli-responsive polymers or stimuli-responsive hydrogels for colorimetric detection.

The present invention may utilize a polymer or co-polymer comprising one or more polymerizable monomers. A suitable monomer may include acrylamide, 2-ethylphenoxy acrylate, 2-ethylphenoxy methacrylate, 2-ethylthiophenyl acrylate, 2-ethylthiophenyl methacrylate, 2-ethylaminophenyl acrylate, 2-ethylaminophenyl methacrylate, phenyl acrylate, phenyl methacrylate, benzyl acrylate, benzyl methacrylate, 2-phenylethyl acrylate, 2-phenylethyl methacrylate, 3-phenylpropyl acrylate, 3-phenylpropyl methacrylate, 3-propylphenoxy acrylate, 3-propylphenoxy methacrylate, 4-butylphenoxy acrylate, 4-
butylphenoxy methacrylate, 4-phenylbutyl acrylate, 4-phenylbutyl methacrylate, 4-methylphenyl acrylate, 4-methylphenyl methacrylate, 4-methylbenzyl acrylate, 4-methylbenzyl methacrylate, 2-2-methylenylethyl acrylate, 2-2-methylenylethyl methacrylate, 2-3-methylenylethyl acrylate, 2-3-methylenylethyl methacrylate, 2-4-methylenylethyl acrylate, 2-4-methylenylethyl methacrylate, 2-(4-proplyphenyl)acrylate, 2-(4-proplyphenyl)methacrylate, 2-[(4-methyl)phenyl]ethylacrylate, 2-[(4-methyl)phenyl]ethylmethacrylate, 2-(4-methoxyphenyl)acrylate, 2-(4-methoxyphenyl)methacrylate, 2-[(4-(1-methyl)phenyl)ethyl]acrylate, 2-[(4-(1-methyl)phenyl)ethyl]methacrylate, 2-(2-chlorophenyl)acrylate, 2-(2-chlorophenyl)methacrylate, 2-(3-chlorophenyl)acrylate, 2-(3-chlorophenyl)methacrylate, 2-(4-chlorophenyl)acrylate, 2-(4-chlorophenyl)methacrylate, 2-[(4-bromophenyl)ethyl]acrylate, 2-[(4-bromophenyl)ethyl]methacrylate, 2-(3-phenylphenyl)acrylate, 2-(3-phenylphenyl)methacrylate, 2-(4-phenylphenyl)acrylate, 2-(4-phenylphenyl)methacrylate, 2-(4-phenylphenyl)ethyl acrylate, and 2-(4-phenylphenyl)ethyl methacrylate.

A suitable monomer may also include benzyl acrylate, phenyl acrylate, naphthyl acrylate, pentabromophenyl acrylate, 2-phenoxyethyl acrylate, 2-phenoxyethyl methacrylate, and 2,3-dibromopropyl acrylate, n-butyl acrylate, n-hexyl acrylate, 2-ethylhexyl acrylate, 2-ethoxyethyl acrylate, 2,3-dibromopropyl acrylate, 1-dihydroperfluorobutyl acrylate, 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylate, 3-hydroxypropyl acrylate, 3-hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, 4-hydroxybutyl methacrylate, 2,3-dihydroxypropyl acrylate, 2,3-dihydroxypropyl methacrylate, N-methyl acrylamide, N-ethyl acrylamide, N-propyl acrylamide, N-isopropylacrylamide, N-butyl acrylamide, methacrylic acid, 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylate, 2-N-ethylacrylate pyrrolidone, 2-hydroxy-3-phenoxypropyl acrylate, 2,3-dihydroxypropyl acrylate, 2,3-dihydroxypropyl methacrylate, 2-N-vinyl pyrrolidone, polyethylene oxide, hydroxyethylmethacrylate and methyl methacrylate, vinyl pyrrolidone and hydroxyethylmethacrylate, vinyl pyrrolidone and methyl methacrylate, glycerol methacrylate and methyl methacrylate, glyceryl-methacrylate and 2-hydroxyethylmethacrylate, hydroxyethylmethacrylate or diacetone acyl amide and hydroxyalkyl methacrylates, hydroxyethylmethacrylate or diacetone acyl amide and acrylates with the alkyl groups having from 2 to 6 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and vinyl hydroxyl acetate, hydroxyethylmethacrylate or diacetone acyl amide and vinyl hydroxy propionate, hydroxyethylmethacrylate or diacetone acyl amide and vinyl hydrox butyrate, hydroxyethylmethacrylate or diacetone acyl amide and N-vinylactams namely N-vinyl pyrrolidone, N-vinyl caprolactam and N-vinyl piperidone, hydroxyethylmethacrylate or diacetone...
acyl amide and N,N dialkyl amino ethyl methacrylates and acrylates with the alkyl groups having from 0 to 2 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and hydroxyalkyl vinyl ethers with the alkyl groups having 2 to 4 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and 1-vinylxoy 2-hydroxyethylene, hydroxyethyl methacrylate or diacetone acyl amide and 1-vinylxoy 5-hydroxy 3-oxapentane, hydroxyethylmethacrylate or diacetone acyl amide and 1-vinylxoy 8-hydroxy 3,6-dioxapentane, hydroxyethylmethacrylate or diacetone acyl amide and 1-vinylxoy 14-hydroxy 3,6,9,12 tetraoxatetradectane, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl morpholine; hydroxyethylmethacrylate or diacetone acyl amide and N,N dialkyl acrylamide with the alkyl groups having from 0 to 2 carbons atoms, hydroxyethylmethacrylate or diacetone acyl amide and alkyl vinyl ketone with the alkyl group having 1 to 2 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl succinimide or N-vinyl glutarimide, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl imidazole, and hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl 3-morpholinone.

[0078] Imprinting using acrylate-based polymers containing methacrylic or acrylic acid or acrylamide is common in the art. These polymers may form either a noncovalent hydrogen bond or ionic interactions with the imprint molecule. The polymerization may be performed in a dry organic solvent. The suitable organic solvents may include supercritical carbon dioxide, acetone or any other ketones, ethanol and any other alcohols, methyl acetate or any other acetates, tetrachloroethylene, toluene, turpentine, hexane, petrol ether, or any other alkanes, 1,4-Dioxane or any its derivatives, cyclopentane or any other cycloalkanes, Benzene or any of its derivatives, chloroform, dichloromethane, diethyl ether or any other ethers, tetrahydrofuran, dimethylformamide, acetonitrile, dimethyl sulfoxide, propylene carbonate, formic acid or any other organic acid, and any other organic compounds. The polymerization may also be performed in water or another inorganic solvent. The suitable inorganic solvents may include a solvent other than water that is not an organic compound. Common examples may include liquid ammonia, liquid sulfur dioxide, sulfuryl chloride and sulfuryl chloride fluoride, phosphoryl chloride, dinitrogen tetroxide, antimony trichloride, bromine pentafluoride, hydrogen fluoride, pure sulfuric acid and any other inorganic acids. Polymers may be prepared with impressive selectivity towards low molecular weight or stereochemistry. The molecules may include, but not limited to, small or low molecular weight organic molecules, inorganic ions, biologic pathway molecules, industrial solvents, explosive or environmentally toxic chemicals. Polymers may be prepared with impressive selectivity towards small molecules including physiologically active drugs. Pharmacological structures imprinted for include classes of drugs (imprint antigens),
such as Theophylline, Diazepam, Morphine, S-Propranolol and Cortisol. Many other molecules can be imprinted and thus, this application is not limited to the molecules named here.

The indicator mechanism of the present invention may be operated via any suitable process resulting in a permanent and detectable change in the template or in the indicator (sensor) mechanism. For example, a subsequent polymerization in the presence of a cross-linker, a cross-linking reaction or other process, results in the formation on an insoluble matrix in which the template sites reside. In one embodiment of the invention, the permanent and detectable change may be the color change of the polymers matrix solution and the resulting polymers, which is caused by the shift of UV-Vis absorption bands of the indicator compounds under certain conditions.

Hydrogels and Molecularily Imprinted Hydrogels: Hydrogels are insoluble networks of crosslinked polymer structures comprising hydrophilic homo- or hetero-copolymers. Hydrogels are highly absorbent natural or synthetic polymers and they can contain over 99.9% water. Hydrogels may also include macroporous structures. Hydrogel MIPs of the present invention do not require a specific linker within the imprint to ensure the stability of a MIP specifically for the analyte being used. This is critical as the complication of discovering the specific linker is the rate limiting step in the traditional MIP paradigm. Further, the stabilization of analytes is the restriction factor in non-hydrogel polymers, thus limiting the utility of the traditional methodology of MIPs.

In one embodiment of the invention, molecularily imprinted polymers (MIPs) may be molecularily imprinted hydrogels. Molecularily imprinted polymers (MIPs) or hydrogels may be developed from a polymer or hydrogel solution following the additional procedures. The additional procedures may include UV light irradiation, temperature change, solvent change, evaporation of solvents, pH change, addition of chemicals, change of salt concentration, etc.

In one embodiment, the present invention is about a solution phase of the polymer or hydrogel, including the polymer or hydrogel-forming solution, which changes colors upon addition of a chemical or an analyte.

In another embodiment, the present invention is about solid phases of the polymer or hydrogel, which changes colors upon addition of a chemical or an analyte. The solid phases of polymers or hydrogels may be films or any other solid form.

In one embodiment, the present invention is about a SP solution, which changes colors upon addition of a chemical or an analyte.

Photochromism of Spiropyrans and Merocyanines Isomerization: Researchers have recently examined the ability of a chromophore to adhere a cation, and release it upon UV
exposure. Photochromism and solvatochromism are the two key fields on which the intense researches have been focused. Photochromism is the reversible transformation of a chemical species between two forms by the absorption of electromagnetic irradiation, where the two forms have different absorption spectra. Photochromism usually refers to compounds that undergo a photochemical reaction where an absorption band in visible region of the electromagnetic spectrum changes dramatically in strength or wavelength. Solvatochromism refers to the ability of a chemical substance to change color due to a change in solvent polarity.

Referring now to Fig. 2, a generic diagram of photochromism of spiropyran and merocyanine isomerization is depicted. As shown in Fig. 2, spiropyran (SP, left), as the spiro form of a benzopyran ring where the aromatic parts of the molecule is separated by a sp^3- hybridized carbon. Upon change of environments such as UV light irradiation, solvent polarity, pH, or chemicals, the isomerization takes place where the bond between the oxygen of the benzopyran and the spiro-carbon breaks, leading to the opening of the pyran ring. Consequently, the spiro-carbon has a sp^2 hybridization, showing a planar structure, and the rotation of aromatic group leads to the alignment of π-orbitals of the spiro-carbon with the rest of the molecule. The resulting form of merocyanine (MC, right), a zwitterion form, possesses a highly conjugated double-bond system, with a capability of absorbing photons in the visible light region of the electromagnetic spectrum. Therefore, merocyanine (MC) appears to be colored.

In one embodiment of the present invention, photochromism of spiropyran and merocyanine isomerization may be used as the indicator mechanism of color readout. It is found that the interaction of certain monomers and molecules with the two forms of SP or MC may drive the equilibrium towards the colorless or colored states, forming the fundamentals of optical readout system in the present invention. It is believed by some researchers that different MC isomers yield different colors. Thus, photochromism of SP and MC isomerization may enable a color readout system showing different colors.

In one embodiment, the invention is a non-imprinted polymer (NIP) capable of color readout. Fig. 3 shows a simple illustration of a non-imprinted polymer (NIP). As depicted in Fig. 3, there are no specific binding sites forming in the process of the NIP. Thus, unlike MIPs, NIPs do not possess specific binding ability to a pre-designed analyte or chemical. However, NIPs may be highly desired under a simple environment where specificity is not required and a fast result is highly expected. These types of environments may be seen mostly in production settings where the molecule to be detected is very specific and delays in this detection could be very costly, thus making the rapid detection a top priority. Field testing would be another beneficial application of the product as some companies require validation testing
before importation. While the non-imprinted polymer lacks specificity, it does still offer a certain level of retention.

[0089] In one embodiment of the invention, an MIP color system including a polymer or hydrogel forming solution, disodium succinate as the analyte, and spiropyran as the chromophore, may be created. As a comparison, two identical non-imprinted Polymer color systems (NIPs) in the absence of sodium succinate molecules were included to contrast the effects of disodium succinate on the chromophore Spiropyran (SP) in the MIP color system. The use of disodium succinate may be served as an analog to the Gamma Hydroxy Butyrate metabolite, succinic acid. The results are illustrated in Fig. 4.

[0090] As shown in Fig. 4A, the MIP color system of the polymer or hydrogel solution in the presence of disodium succinate (left) exhibits a distinguishably red color as compared with the other two NIP color systems (NIP1 and NIP2), showing a yellow-orange color. Further, during polymerization, UV light does not affect SP molecular photochromic properties. It is desired for a color readout system that a color change occurs only through the interaction of the analyte with the SP molecule, and other environmental parameters such as UV light or sunlight, may not affect the interaction. As shown in Fig. 4B, following UV irradiation, the colors of the MIP color system and the NIP color systems remained unchanged, confirming that exposure to UV light does not affect SP molecule photochromic properties after polymerization.

[0091] Further, to demonstrate that the color change is only due to the presence of disodium succinate, a similar amount of phosphate buffer solution (PBS) was added to the vials of the MIP color system (left) and one of the NIP color system (NIP1, center). The amount of 0.25M disodium succinate was added to the vial of the other NIP color systems (NIP2, right). The resulting polymer or hydrogel solid forms produced from UV irradiation of the corresponding solutions were depicted in Fig. 5. As shown in Fig. 5, after the addition of disodium succinate, the NIP2 color system appeared to change toward (Fig. 5A-5C) and eventually reach the same red color (Fig. 5D) as that of the MIP color system. These observations confirmed that color change in the MIP system was indeed due to the presence of the analyte of disodium succinate.

In one embodiment, these observations also demonstrated that a NIP color system may be created with a late addition of disodium succinate.

[0092] In one embodiment of the invention, the solid forms of polymers or hydrogels may be produced for color readout systems. Referring now to Fig. 6, the cutting pieces of polymers or hydrogels formed from the corresponding polymer or hydrogel solid forms in buffer and disodium succinate solutions described in Fig. 5D are depicted. As shown in Fig. 6, polymers or hydrogels produced from the corresponding MIP color system and the NIP2 color
system with later addition of disodium succinate show the same red color as those of the solutions. The NIP1 color system in the absence of disodium succinate produced the polymer or hydrogel showing the original yellow-orange color. These observations further demonstrated that the presence of the imprinting molecule of disodium succinate causes the color changes, and the color readout systems may be created by late addition of the analyte into the NIP color system and by producing the solid forms of polymers or hydrogels.

Further, due to the reversible reaction nature of the spiropyran isomerization, it is expected that an MIP color system, where the disodium succinate was extracted, would show the yellow-orange color, as seen in the NIP1 color system (Fig. 6, center). After exposure to disodium succinate, the color of the MIP color system would again change to the red color.

Further, the color differences of the color readout systems may also be evaluated using optical absorption spectroscopy. Fig. 7 shows optical absorption spectra of spiropyran (SP) solutions comprising phosphate buffer solution (PBS), acrylic acid, spiropyran (SP) with different concentrations of disodium succinate measured by an UV-Vis spectrometer. While Fig. 8 illustrates sample color scales of the polymer and hydrogel solutions having SP and different concentrations of disodium succinate, which were measured by an UV-Vis spectrometer, the polymer and hydrogel solutions with different concentrations of disodium succinate were made and analyzed in the visible color range to further confirm that the color change of the color readout systems observed in the previous experiments was due to the interaction between the analyte (disodium succinate) and the chromophore (SP and MC).

As shown in Fig. 7, with the concentration increase of disodium succinate, the UV-Vis spectra of the SP solutions demonstrate that an absorption band centered at 370 nm, due to SP, shows a decreasing intensity, and the other absorption band centered at 530 nm, due to MC, has an increasing intensity. The UV-Vis spectra were further visualized by sample color scales of the polymer and hydrogel solutions having SP and different concentrations of disodium succinate shown in Fig. 8. The concentrations of disodium succinate increase from left to right. These observations of UV-Vis spectra and sample color scales further confirm that the interaction between the analyte (disodium succinate) and the chromophore (SP and MC) is indeed the cause of the color change in the SP solutions. The increasing intensity of the absorption band at 530 nm corresponds to the increased color intensity of the solutions.

In another embodiment of the invention, a NIP color system including a polymer or hydrogel solution in the absence of analyte (disodium succinate), and with spiropyran as the chromophore, may be created. Similar to the MIP color system, a NIP color system demonstrates novel utilities in detecting an analyte, such as disodium succinate. The NIP color
systems of the polymer or hydrogel solutions including the chromophore (SP and MC) were made following a similar procedure to the above experiments. The initially prepared polymer or hydrogel solutions showed a yellow-orange color. To demonstrate the stability of the solutions, ten samples having the same concentrations of the polymer or hydrogel and the chromophore were irradiated by UV light, and the resulting polymer or hydrogel solutions were depicted in Fig. 9. As shown in Fig. 9, upon UV irradiation, the polymer or hydrogel solutions remained consistent in color (yellow-orange), illustrating no additional attribution to the photochromic properties of the SP-MC system.

Further, as shown in Figs 4-5 and 7-8, after the addition of an analyte such as disodium succinate, the polymer or hydrogel solutions demonstrate clearly color changes. These observations confirm that a NIP color system, including a polymer or hydrogel solution in the absence of analyte (disodium succinate), and with spiropyran as the chromophore, may also be used to detect an analyte.

In one embodiment of the invention, a solid phase of a NIP color system having a polymer or hydrogel and a chromophore (SP and MC) in the absence of analyte (disodium succinate) may be produced. The polymer or hydrogel in a solid phase may demonstrate the same characteristic behavior of color change in the presence of an analyte. The solid phase of a NIP color system may be produced from the corresponding polymer or hydrogel solutions as shown in Fig. 9 by a suitable means. The suitable means may include solvent evaporation, heating, or any other methods known to those skilled in the art.

As shown in Fig. 10, the polymer or hydrogel of a NIP color system in a solid phase was made, and three of the samples were each put into a solution of disodium succinate (the first three samples from left), and another sample was put into a phosphate buffer solution as a controlled experiment (the right). As shown in Figs 10B-10D, the polymer or hydrogel in a solid phase in the solution of disodium succinate demonstrates a clear color change from yellow-orange to red. As a comparison, in the absence of disodium succinate, the polymer or hydrogel in a solid phase remained the yellow-orange color.

Further, as shown in Fig. 11, after 24 hours, the samples of the polymer or hydrogel in a solid phase in the solution of disodium succinate (the first three samples from left) show the same red color, while the sample of the polymer or hydrogel in a solid phase in the absence of disodium succinate remained the yellow-orange color. Fig. 12 shows the samples of the polymer or hydrogel in a solid phase after taken out from the solutions. As shown in Fig. 12, the samples of the polymer or hydrogel in a solid phase which were in the solution of disodium succinate (the first three samples from left) show the same red color, while the sample of the
polymer or hydrogel in a solid phase which was in phosphate buffer (the right) remained the yellow-orange color. Since the color changes are due to the interaction between the analyte (disodium succinate) and the chromophore (SP and MC), showing the penetration of analyte into the solid phase of the polymer or hydrogel, these observations demonstrate that the polymer or hydrogel in a solid phase may also be used as a color readout system to detect an analyte.

[00101] To further confirm the color change in a NIP system was due to the interaction between the chromophore and the analyte, the color systems of the NIP systems were evaluated using an UV-Vis spectrometer. One sample of polymer or hydrogel solutions and one sample of a controlled experiment were used for the experiments. As shown in Fig. 13, the sample of polymer or hydrogel solution shows a red color due to the interaction between an analyte (disodium succinate) and the chromophore (SP and MC), and the sample of a controlled experiment shows a yellow-orange color in the absence of the analyte of disodium succinate.

[00102] Fig. 14 shows the UV-Vis spectra observed on the polymer or hydrogel solution of the NIP system and on the sample of a controlled experiment. As shown in Fig. 14, an absorption band centered at 530 nm appears, which was due to MC in the presence of disodium succinate (4 mg in 10ml; blue line). In the absence of disodium succinate, the same band was negligible. Further, after the addition of 6mg more disodium succinate into the polymer or hydrogel solution, the intensity of the absorption band at 530 nm increases (lighter blue line), demonstrating an increasing interaction between the analyte of disodium succinate and the chromophore (SP and MC). These results and observations illustrate that the chromophore system of SP-MC may be used as a suitable detection system. This advancement by Applicants validates the use of molecular polymers wherein a detected molecular species result in an optical readout (visible to the "untrained" eye).

[00103] To further demonstrate the sensitivity of the MIP and NIP color systems, some of the parameters including the concentrations of acrylic acid were changed. Particularly, Applicants found that by changing the concentrations of acrylic acid, the color readout of the MIP and NIP systems varied accordingly. Fig. 15 shows a color scale of the polymer or hydrogel solutions of a NIP system including the chromophore (SP and MC) in the absence of the analyte of disodium succinate with a different concentration of acrylic acid. As shown in Fig. 15, in the absence of acrylic acid, the polymer or hydrogel solution appears to be red (the left). With the addition and the Increasing concentrations of the acrylic acid (from left to right), the polymer or hydrogel solutions initially change to colorless, and slowly turn into a yellow-orange color, which is typical for SP. These observations demonstrate that in the absence of
acrylic acid, the isomerization equilibrium of SP and MC (Fig. 2) favors MC, and the addition of acrylic acid turns the isomerization equilibrium of SP and MC to favor SP. Thus, in one embodiment, a NIP color system may be built by including a polymer or hydrogel solution, a chromophore (SP and MC), and a different concentration of acrylic acid in the absence of the analyte of disodium succinate.

[00104] In one embodiment, acrylic acid derivatives may substitute acrylic acid in NIP color systems. The acrylic acid derivatives may include acrylic acid esters, such as ethyl acrylate, butyl acrylate, 2-ethylhexyl acrylate, sorbyl acrylate, 2-(dimethylamino)-ethyl acrylate, 3,3-dimethoxypropyl acrylate, 2,2,3,3,4,4,4-heptafluorobutyl acrylate, 2-cyanoethyl acrylate, 4-fluorophenyl acrylate, chloroethyl acrylate, 2-(propen-1-ylxy)-ethyl acrylate, phenyl acrylate, allyl acrylate, hydroxyethylmethacrylate (HEMA), acrylamides, such as N,N-dimethylacrylamide, and acrylonitrile.

[00105] Further, the addition of acrylic acid demonstrates a similar effect on the MIP color systems. As shown in Fig. 16, eight samples of polymer or hydrogel solutions were made including the chromophore (SP and MC), the analyte of disodium succinate (2mg in 1mL), and the increasing concentrations of acrylic acid (from left to right). In absence of acrylic acid (the left, Fig. 16), the polymer or hydrogel solution appears to be red, similar to the above previous systems (Figs 5 and 8). With the increase of the acrylic acid concentration, however, the colors of the polymer or hydrogel solutions turn from red to yellow-orange. While the red color is due to MC, the yellow-orange color is attributed to SP. Thus, these observations demonstrate that in the presence of disodium succinate, the isomerization equilibrium of SP and MC (Fig. 2) favors MC, and the addition of acrylic acid slowly turns the isomerization equilibrium of SP and MC to favor SP. These experiments provide a strategy to fine tune the color readout scale for the detection of an analyte.

[00106] Applicants previously demonstrated a method of analyte detection using gold (Au) nanoparticles. In one embodiment of the invention, it is found that the present MIP and NIP systems may be applied as color readout systems in the presence of Au nanoparticles. Fig. 17 shows the color changes of both MIP (left) and NIP systems (center and right) in the presence of Au nanoparticles. As shown in Fig. 17A, the polymer or hydrogel solutions of an MIP system (left) and NIP systems (center and right) in the absence of chromophore (SP and MC) were made. Even in the absence of a chromophore, the interaction between the analyte of disodium succinate and Au nanoparticles led to a red color of the resulting solution for the MIP system (left). The NIP systems (center and right), having no analyte of disodium succinate,
exhibit a different color of purple-black. Thus, a color readout system may be created using Au nanoparticles instead of the chromophore of SP and MC.

[00107] Further, Fig. 17B shows the color changes of both MIP (left) and NIP systems (center and right) in the presence of Au nanoparticles and the chromophore (SP and MC). As shown in Fig. 17B, the polymer or hydrogel solid forms in solutions for the MIP system illustrates a red color (left), while the solutions for the NIP systems appear to be brown (center and right). Thus, Au nanoparticles have an effect on the NIP systems by changing the color from yellow-orange to brown. The polymers here are representative of the initial time period of solution with buffer and disodium succinate solutions being added respectively.

[00108] Fig. 18 shows an additional experiment following those in Fig. 17B. The analyte of disodium succinate was added into one of the NIP systems (right) while the other NIP system (center) and the MIP system (left) remained the same as those in Fig. 17B. As shown in Fig. 18, after the addition of disodium succinate, the polymer or hydrogel solid forms in solutions (right) changes to a same red color as that in the MIP system (left) after 50 minutes. These results and observations demonstrate that Au nanoparticles and the chromophore (SP and MC) may be used as a suitable detection method or a device, as the combination of Au nanoparticles and the chromophore (SP and MC) yields a colorimetric readout upon exposure to the analyte of disodium succinate.

[00109] A molecularly imprinted polymer (MIP) system may be suited for an environment requiring specificity, and a non-imprinted polymer (NIP) system, possessing sufficient analyte retention levels, may offer prescreening and early detection capabilities. Both systems may offer a visible readout, which is yet to be accomplished by others in the art. A NIP system may serve well as a prescreening device or a technology to be utilized under an analyte-specific environment. For example, if the goal were to detect bacterial metabolites in a food production setting, as a prescreening device, the NIP system would yield a color based on the overall concentration of metabolites. If no color change were detected, no further testing would be required. If there was a color change, further testing, which may be performed using an MIP system, would be required. The purpose of imprinting and employing a polymer (MIP) is to confer specificity of detection. Very large amounts of analyte would cause a color change in both the MIP and NIP systems. However, at lower concentrations of analyte, it is expected that an MIP system would show a much higher sensitivity over a NIP system. Thus, it is desired that at certain levels of concentrations the MIP would change color on a greater scale due to the imprinting.
The present invention of the color readout systems of MIPs and NIPs may be very well suited for detection of metabolites the field of metabolomics, whereas other technologies were more geared towards proteomics and genomics. The present invention of the color readout systems of MIPs and NIPs may be very well suited for many sectors in industry that desire faster detections in the field of detection. For example, USDA consistently researches improvements in health and safety in the food industry, and the detection represents a large part of advancement in the field. Future areas of interest for the invention may include the fields of E.Coli, toxins (water safety), and food packaging safety.

In one embodiment, the present invention relates to a spiropyran (SP) solution, which is capable of undergoing a color change when the solution is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout. The SP solution comprises phosphate buffer solution (PBS), acrylic acid, spiropyran (SP), and disodium succinate as the analyte. The SP solution may be made in any suitable solvent. The suitable solvents may include water, DMSO, acetone, alcohol, DMF, pyridine, or any other solvents capable of dissolving SP, acrylic acid, and disodium succinate. Preferably, the solvent is water. SP is initially dissolved in acrylic acid, and the resulting solution is then diluted with PBS. In the absence of disodium succinate, the SP solution appears to be yellow. As sodium succinate is added continuously, the color of the SP solution changes from yellow to gold, pink, and eventually to red. The further addition of disodium succinate will lead to an increasing intensity of the red SP solution, indicating an increase conversion of SP to MC. Further, UV-Vis spectroscopy demonstrates that there is a linear correlation between disodium succinate concentrations and the intensity of absorbance peak centered at 522 nm.

In another embodiment, the present invention relates to a transport system, on which a colorimetric detection may be visualized. The term "transport system", as used herein, refers to a system which can carry at least a certain amount of the substrates after it contacts a solution having substrates. By "substrates", we mean chemical compounds in a solution. Specifically, in the present invention, the substrates may include spiropyran (SP), disodium succinate, phosphate buffer, acrylic acid, MIPs, NIPs, nanoparticles, and others. A transport system may generally have sponge-like or any other macroporous structures, providing a means to hold substrates. Preferably, the transport system may be flocked or sponge swabs, and more preferably, the transport system may be flocked or sponge swabs having white or other light colors. In one embodiment, the transport system may be Puritan® flocked or sponge swabs.
The Examples describe transport systems for colorimetric detection. Although
flocked and sponge swabs were used for demonstrations in Example, a person having ordinary
skill in the art will appreciate that the present invention may be generally applied to any suitable
transport systems. In one embodiment, the substrates may be transported by inserting the
transport system into a solution of disodium succinate and an SP solution successively. The
transport system may be first inserted into the solution of disodium succinate, and it may be
then inserted into the SP solution. In one embodiment, the successive insertion into the solution
of disodium succinate and the SP solution may be performed once. In another embodiment, the
successive insertion into the solution of disodium succinate and the SP solution may be
performed twice or multiple times.

In another embodiment, the present invention relates to transport medias as
suitable platforms for colorimetric detection. As used herein, “transport media” refers to a media
selected from the group consisting of temporary storage of specimens being transported to the
laboratory for cultivation, those maintaining the viability of all organisms in the specimen without
altering their concentration, those containing only buffers and salt, those lack of carbon,
nitrogen, and organic growth factors so as to prevent microbial multiplication, and those medias
used in the isolation of anaerobes free of molecular oxygen. Examples of transport media may
include, but not limited to, thioglycolate broth for strict anaerobes, Stuart Transport medium (a
non-nutrient soft agar gel containing a reducing agent to prevent oxidation, and charcoal to
neutralise), certain bacterial inhibitors (for gonococci, and buffered glycerol saline for enteric
bacilli), or Venkat-Ramakrishnan (VR) medium for v. cholerae.

The Examples describe transport medias as suitable platforms for colorimetric
detection. In one embodiment the present invention may be applied to any transport medias of
non-neutralizing buffer. Specifically, a suitable transport media may be chosen from, but not
limited to, the group consisting of peptone water, Ames solution, Butterfield's solution, Letheen
Broth, and UTM-RT.

In another embodiment, such incorporation of the colorimetric detection into
transport medias may provide a broad color spectrum for a readout scale.

In yet another embodiment, the present invention relates to elastic polymers and
elastic hydrogels for colorimetric detection. The present invention of colorimetric detection
may be combined with elastic polymers and elastic hydrogels. Elastic polymers and elastic
hydrogels, sometimes referred to as elastomers, are materials with viscoelasticity (colloquially
"elasticity"), generally having low Young's modulus (E) and high yield strain compared with other
materials. Elastic polymers may be amorphous polymers existing above their glass transition
temperature, so that considerable segmental motion is possible. At ambient temperatures, elastic polymer may thus be relatively soft (E~3MPa) and deformable but they recover their shape readily when the applied external stress is removed. Their primary uses may be for, but not limited to, seals, adhesives and molded flexible parts. In the proposed embodiment, they could add sensing capabilities for detection of molecules.

[00118] Colorimetric detection of the present invention may be suitable for any elastic polymers and elastic hydrogels. The Examples describe elastic polymers and elastic hydrogels for colorimetric detection. An elastic polymer and elastic hydrogel for colorimetric detection may provide additional functions of colorimetric readout while the elastic properties are maintained. In one embodiment, an elastic polymer and elastic hydrogel for colorimetric detection may be made by combining all the chemicals of colorimetric detection and those of elastic polymers and elastic hydrogels before polymerization. In another embodiment an elastic polymer and elastic hydrogel for colorimetric detection may be made by adding all the chemicals of colorimetric detection into the elastic polymer and elastic hydrogel.

[00119] In another embodiment, the present invention relates to a kit for detection of an analyte in a sample by colorimetric readout. The kit may comprise any chemical compositions for colorimetric detection as discussed above. The kit may comprise a solid support. A solid support may comprise any suitable means of support. In one embodiment, the solid support may be a transport system as discussed above. The kit may further comprise a transport media. A transport media may comprise any suitable mediae. In one embodiment, the transport media may be any media as discussed above. The kit may further comprise an elastic polymer. An elastic polymer may comprise any suitable polymers. In one embodiment, the elastic polymer may be any polymer as discussed above.

[00120] In one embodiment, the present invention relates to systems of a SP solution consisting of SP and a solvent and methods of using such system for colorimetric detection. Any suitable solvent may be used for the SP solution. For example, a suitable solvent may be selected from the group consisting of water, DMSO, acetone, alcohol, DMF, hydroxyethyl methacrylate (HEMA), and pyridine. Preferably, a suitable solvent may be selected from the group consisting of DMSO, acetone, alcohol and hydroxyethyl methacrylate (HEMA). In another embodiment, a suitable solvent for the present invention may be a mixture combination of two or more solvents. The two or more solvents may be mixed initially before they are used to dissolve SP. Alternatively, the two or more solvents may also be used in a subsequent manner, in which one or more of the solvents are used to dissolve SP to make a SP solution, and other solvents are subsequently added into the solution. In some embodiments, the present methods of
colorimetric detection using a SP solution consisting of SP and a solvent may show different color changes depending on the manners in which two or more solvents are added, e.g., simultaneously, subsequently, and the sequence of the solvents.

[00121] SP solution consisting of SP and a solvent may be applicable to any of the above methods, devices, and kits. Preferably, the present SP solution consisting of SP and a solvent may be used in a kit or a transport system as discussed above.

[00122] In one embodiment, the present SP solution consisting of SP and a solvent may be used to detect a bio-molecule such as a nucleoside triphosphate. Preferably, the nucleoside triphosphate to be detected may be Adenosine-5'-triphosphate (ATP). Figs. 28-30 and the Example show colorimetric detection of ATP by using a SP solution consisting of SP and a solvent.

[00123] In another embodiment, the present SP solution consisting of SP and a solvent may further comprise polymers for colorimetric detection of a bio-molecule such as ATP. Any polymers may be applicable for the present invention. In one specific embodiment, the present SP solution may be applicable to a solution-phase detection. For example, Fig. 31 and the Example show a solution-phase colorimetric detection of ATP by using a SP solution comprising SP, a solvent and polymers.

[00124] In another specific embodiment, the present SP solution may be applicable to a solid-phase colorimetric detection. For example, Figs. 32-33 and the Example show a solid-phase colorimetric detection of ATP by using a SP solution comprising SP, a solvent and polymers.

[00125] The present colorimetric detection by using a SP solution may be sensitive to the concentration of the analyte. For example, as shown in Figs. 34-35, visually color changes may be detectable in the presence of 2 mg/mL ATP.

[00126] In another embodiments, the present SP solution and methods of using such solution may be applicable to detect explosives. The present invention may be suitable for detecting any substances related to explosives. For example, the chemical compound ammonium nitrate, the nitrate of ammonia with the chemical formula NH₄NO₃, is a white crystalline solid at room temperature and standard pressure. It is commonly used in agriculture as a high-nitrogen fertilizer, and it has also been used as an oxidizing agent in explosives, including improvised explosive devices. It is the main component of ANFO, a popular explosive, which accounts for 80% explosives used in North America. Ammonium-nitrate-based explosives were used in the Oklahoma City bombing and in the 2011 Delhi bombing, 2013
Hyderabad blasts and the bombing in Oslo 2011. A simple method for detecting such chemical compounds would be of great value for anti-terrorism.

Figs. 36-39 and the Example show solution-phase colorimetric detection of ammonium nitrate by using a SP solution. In some embodiments, the present colorimetric detection of ammonium nitrate may be also applicable to solid-phase detections. Figs. 40-41 and the Examples show solid-phase colorimetric detection of ammonium nitrate by using a SP solution.

The present colorimetric detection of ammonium nitrate by using a SP solution may be sensitive to the concentration of ammonium nitrate. For example, as shown in Figs. 42-43, visually color changes may be detectable in the presence of tens µL ammonium nitrate.

In some embodiments, Applicants envision that the present colorimetric detection may be applicable to detect bacteria. For example, the above methods for detecting ATPs may be used to detect bacteria which contains ATPs.

Specifically, the present invention may be used as a substitute method for Gram staining. As shown in Fig. 44, Gram staining (or Gram's method) is a method of differentiating bacterial species into two large groups (gram-positive and gram-negative). Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram-positive bacteria. In Gram staining, dyes are generally used. A gram-positive results in a purple-blue color while a gram-negative results in a pink-red color.

The Gram stain is almost always the first step in the identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. No real development has been found in this front line method of immediate detection.

The present SP solution may provide an easy way to differentiate Gram negative bacteria and positive bacteria by colorimetric detection. For example, a Gram negative bacterium may exhibit different color from that of a Gram positive bacterium. Gram negative bacteria may comprise Salmonella sp., Campylobacter jejuni, Yersinia enterocolitis, Shigella sp., Vibrio parahaemolyticus, Coxiella burnetii, Mycobacterium bovis, Brucella sp., Vibrio cholerae serogroup 01 and 0139, Vibrio cholerae serogroup non-01 and non-0139, Vibrio vulnificus, Cronobacter (Enterobacter sakazakii) sp., Aeromonas hydrophila and other sp., Plesiomonas shigelloides, Miscellaneous bacterial enterics, Franciscella tularensis, Enterotoxigenic Escherichia coli (ETEC), Enteropathogenic Escherichia coli (EPEC),...
Enterohemorrhagic *Escherichia coli* (EHEC), and Enteroinvasive *Escherichia coli* (EIEC). Gram positive bacteria may comprise *Clostridium perfringens*, *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus cereus* and other *Bacillus* sp., *Listeria monocytogenes*, *Streptococcus* sp., and *Enterococcus*.

**EXAMPLES**

Example 1

[00133] **Molecularly Imprinted Hydrogels and Detections via Spiropyran Molecules.**
Molecularly imprinted polymers (MIPs) were produced in 20 mL glass vials with the compositions of 5M acrylamide, 0.5 M acrylic acid, 0.1 M N,N'-methylene-bisacrylamide, 0.25 M disodium succinate, and 0.0178 g 4,4'-azobis(4-cyanovaleric acid). The copolymerization reactions of acrylamide and bisacrylamide were initiated by 4,4'-azobis(4-cyanovaleric acid). The high ratio of acrylamide to bisacrylamide (20/1) leads to the formation of the polymer networks where bisacrylamide serves as the bridge junctions. The above compounds were dissolved in a phosphate buffer with a pH of 7.4 with a total volume of 5 mL. 1.0 mg of SP (chromophore) was then added into the each vial and the resulting solutions were sonicated for at least 5 minutes.

[00134] As shown in Figs 4-6, the MIP color system of the polymer or hydrogel solid forms in solutions in the presence of disodium succinate (left) exhibits a distinguishably red color as compared with the other two NIP color systems (NIP1 and NIP2), showing a yellow-orange color. Further, during polymerization, UV light does not affect SP molecular photochromic properties. It is desired for a color readout system that a color change occurs only through the interaction of the analyte with the SP molecule, and other environmental parameters such as UV light or sunlight, may not affect the interaction. As shown in Fig. 4B, a colorimetric system was initially include in the MIP polymer system. Following UV irradiation, the colors of the MIP color system and the NIP color systems remained unchanged, confirming that exposure to UV light does not affect SP molecule photochromic properties after polymerization.

Example 2

[00135] **Non-Imprinted Hydrogels and Detections via Spiropyran Molecules.**
Non-imprinted polymers (NIPs) were made in 20 mL glass vials with the compositions of 5M
acrylamide, 0.5 M acrylic acid, 0.1 M N,N'-methylene-bisacrylamide, and 0.0355g 4,4'-azobis(4-cyanovaleric acid). The copolymerization reactions of acrylamide and bisacrylamide were initiated by 4,4'-azobis(4-cyanovaleric acid). The high ratio of acrylamide to bisacrylamide (20/1) leads to the formation of the polymer networks where bisacrylamide serves as the bridge junctions. The above compounds were dissolved in a phosphate buffer with a pH of 7.4 with a total volume of 5 mL. 0.5 mg of SP (chromophore) was then added into the each vial and the resulting solutions were sonicated for at least 5 minutes. 4mg/ml of disodium succinate in a PBS buffer was also made to be later added to the polymer or hydrogel solutions.

[00136] As shown in Fig. 9, the initially prepared polymer or hydrogel solutions showed a yellow-orange color. To demonstrate the stability of the solutions, ten samples having the same concentrations of the polymer or hydrogel and the chromophore were irradiated by UV light, and the resulting polymer or hydrogel solid forms in solutions were depicted in Fig. 9. As shown in Fig. 9, upon UV irradiation, the polymer or hydrogel solid forms in solutions remained consistent in color (yellow-orange), illustrating no additional attribution to the photochromic properties of the SP-MC system. Further, as shown in Figs 4-5 and 7-8, after the addition of an analyte such as disodium succinate, the polymer or hydrogel solutions and the solid forms in solutions demonstrate clearly color changes. These observations confirm that a NIP color system, including a polymer or hydrogel solution or a solid form in solutions in the absence of analyte (disodium succinate), and with spiropyran as the chromophore, may also be used to detect an analyte.

[Example 3]

[00137] Molecularly Imprinted Hydropels. Non-Imprinted Hydropels. Au Nanoparticles and detections via spiropyran molecules or Au nanoparticles. The systems of Molecularly imprinted hydrogels or non-imprinted hydrogels and Au Nanoparticles were made following a similar procedure as discussed above. For example, an MIP system using Au nanoparticles instead of SP was made in 20 mL glass vials with the compositions of 5M acrylamide, 0.5 M acrylic acid, 0.1 M N,N'-methylene-bisacrylamide, 0.25 M disodium succinate, and 0.0178 g 4,4'-azobis(4-cyanovaleric acid). The above compounds were dissolved in a phosphate buffer with a pH of 7.4 with a total volume of 5 mL.

[00138] A NIP system using Au nanoparticles instead of SP was made in 20 mL glass vials with the compositions of 5M acrylamide, 0.5 M acrylic acid, 0.1 M N,N'-methylene-bisacrylamide, and 0.0178 g 4,4'-azobis(4-cyanovaleric acid). The above compounds were dissolved in a phosphate buffer with a pH of 7.4 with a total volume of 5 mL. Corresponding
MIP and NIP systems were also made in the presence of SP, and similar procedures were used by later addition of 1.0 mg SP molecule to each vial and the solutions were sonicated for at least 5 minutes.

[00139] Fig. 17 shows the color changes of both MIP (left) and NIP systems (center and right) in the presence of Au nanoparticles. As shown in Fig. 17A, the polymer or hydrogel solutions of an MIP system (left) and NIP systems (center and right) in the absence of chromophore (SP and MC) were made. Even in the absence of a chromophore, the interaction between the analyte of disodium succinate and Au nanoparticles led to a red color of the resulting solution for the MIP system (left). The NIP systems (center and right), having no analyte of disodium succinate, exhibit a different color of purple-black. Thus, a color readout system may be created using Au nanoparticles instead of the chromophore of SP and MC.

[00140] Fig. 17B shows the color changes of both MIP (left) and NIP systems (center and right) in the presence of Au nanoparticles and the chromophore (SP and MC). As shown in Fig. 17B, the polymer or hydrogel solid forms in solutions for the MIP system illustrates a red color (left), while the solutions for the NIP systems appear to be brown (center and right). Thus, Au nanoparticles have an effect the NIP systems by changing the color from yellow-orange to brown.

[00141] Fig. 18 shows an additional experiment following those in Fig. 17B. The analyte of disodium succinate was added into one of the NIP systems (right) while the other NIP system (center) and the MIP system (left) remained the same as those in Fig. 17B. As shown in Fig. 18, after the addition of disodium succinate, the polymer or hydrogel solid forms in solutions (right) changes to a same red color as that in the MIP system (left) after 50 minutes. These results and observations demonstrate that Au nanoparticles and the chromophore (SP and MC) may be used as a suitable detection method or a device, as the combination of Au nanoparticles and the chromophore (SP and MC) yields a colorimetric readout upon exposure to the analyte of disodium succinate.

Example 4

[00142] Puritan® Flock or Sponge Swabs As Transport Systems for Colorimetric Detection. Both Puritan® flocked or sponge swabs may be used as transport systems for colorimetric detection. Both a disodium succinate solution and a SP solution were made according to the methods discussed above. Specifically, a disodium succinate (DS) Solution was made after a suitable amount of disodium succinate dissolved in phosphate buffer solution
or dewater with a concentration depending on the experiment. A polymer solution refers to the mixture of polymer components (buffer, monomers, crosslinker, initiator, analyte when applicable, SP when applicable) before polymerization. A polymer/solid polymer refers to the mixture of polymer components (buffer, monomers, crosslinker, initiator, analyte when applicable, SP when applicable) AFTER polymerization. A SP solution refers to a mixture consisting of only 1mg SP dissolved in 685uL acrylic acid after the addition of 20mL of PBS. A 20mL SP solution was thus produced.

Both flocked and sponge swabs were inserted into the disodium succinate solution and the SP solution successively. For the flocked swab, the successive insertion into disodium succinate and SP solutions were repeated three times, and the flocked swab was rinsed with water each time between each trial. As shown in Fig. 19, both flocked and sponge swabs showed a white color initially.

During and after the insertion of the flocked swab, the SP solution remains its original color of dark yellow, while the disodium succinate solution remain colorless. During the first two round of insertions, the flocked swab's color changed from white to faint yellow. This process was repeated three times. The only difference in the three trials was the time period the swab was left in each solution. During the first trial it was less than 5 seconds. During the 2nd, it was about 10 seconds and during the 3rd, it was about 20 seconds.

After the third insertion into the disodium succinate solution, the flocked swab turned red-pink (not shown), indicating that a colorimetric detection is accomplished and visualized on the flocked swab. Upon a further insertion into the SP solution, the red-pink flocked swab turned back to yellow. These observations indicate that both on the flocked swab and in the SP solution, after the insertions, the equilibrium of the equation in Fig. 2 favors the non-colored form of the molecule (SP; left). The resulting SP solution was yellow.

As shown in Fig. 20, a sponge swab appeared to be white initially (Fig. 20a). After a first insertion into disodium succinate and a second insertion into SP solutions, the sponge swab turned purple (Fig. 20b) and the SP solution remained yellow (not shown). These observations indicate that on the sponge swab after the successive insertions, a colorimetric detection is accomplished and is visualized. It appears that on the sponge swab after the successive insertions, the equilibrium of the equation in Fig. 2 favors the colored form of the molecule (MC, right).

Still in Fig. 20, if the purple sponge swab was kept in the SP solution for overnight, the sponge swab turned dark yellow (Fig. 20c and Fig. 20d, left). The SP solution turned colorless (Fig. 20d, right), while the color of the SP solution was initially yellow. These
observations indicate that on the sponge swab after it was kept in the SP solution for overnight, and in the SP solution, the equilibrium of the equation in Fig. 2 favors the non-colored form of the molecule (SP; left). Theoretically, Spiropyran (SP) is colorless and merocyanine (MC) is colored. In the present invention, however, a SP solution shows a yellow color. It is likely that the yellow color is due to the acrylic acid or the existence of some MC isomers.

[Example 5

[00148] Transport Medias as Suitable Platforms for Colorimetric Detection. Various transport medias was tested for suitable platforms for colorimetric detection. It appeared that most of the transport medias except for the neutralizing buffer are suitable for colorimetric detection in the present invention.

[00149] As shown in Fig. 21, a neutralizing buffer was tested for colorimetric detection. A neutralizing buffer comprises monopotassium phosphate (0.0425g/L), sodium thiosulfate (0.16g/L), and aryl sulfonate complex (5.0g/L). A solution comprising neutralizing buffer and SP solutions showed a yellow color (Fig. 21A). A solution comprising neutralizing buffer, SP and disodium succinate solutions showed a yellow color (Fig. 21B). As comparison, solutions comprising SP and disodium succinate solutions with (Fig. 21C) or without (Fig. 21D) additional water show red-pink colors. Thus, a neutralizing buffer is not a suitable platform for colorimetric detection.

[00150] As shown in Fig. 22, Peptone water was tested for colorimetric detection. Peptone water may include any kind of various water-soluble protein derivatives obtained by partial hydrolysis of a protein by an acid or enzyme during digestion and used in culture media in bacteriology. Peptones may be derived from animal milk or meat digested by proteolytic digestion. In addition to containing small peptides, Peptone water may further include fats, metals, salts, vitamins and many other biological compounds. Peptone water may be used in nutrient media for growing bacteria and fungi. A buffered Peptone water comprises Peptone (10.0 g/L), sodium chloride (5.0 g/L), disodium phosphate (3.5 g/L), monopotassium phosphate (1.5 g/L), at final pH of 7.2 ± 0.2 at 25°C. A solution comprising peptone water and SP solutions showed a yellow color (Fig. 22A). Contrastingly, a solution comprising peptone water SP and disodium succinate solutions showed a red-pink color (Fig. 22B). This observation, consistent with those standard colorimetric detections (Fig. 22 C-D), indicates that peptone water may be used as a suitable transport media for colorimetric detection. As shown in Fig. 22E, an
experiment of directly mixing of a solid phase of SP and peptone water showed that the solid phase of SP is not very soluble in the peptone water and the solution remained yellow.

[00151] As shown in Fig. 23, Ames solution was tested for colorimetric detection. Ames solution comprises inorganic salts of calcium chloride (0.1275 g/L), magnesium sulfate (0.1488 g/L), potassium chloride (0.231 g/L), potassium phosphate monobasic (anhydrous) (0.068 g/L), sodium chloride (7.01 g/L), amino acids of L-Alanine (0.0024 g/L), L-Arginine · HCl (0.00421 g/L), L-Asparagine (anhydrous) (0.00084 g/L), L-Aspartic Acid (0.00012 g/L), L-Cystine · 2HCl (0.000065 g/L), L-Glutamine (0.073 g/L), L-Glutamic Acid (sodium) (0.001183 g/L), Glycine (0.00045 g/L), L-Histidine · HCl · H2O (0.002513 g/L), L-Isoleucine (0.00058 g/L), L-Leucine (0.00144 g/L), L-Lysine · HCl (0.003648 g/L), L-Methionine (0.00039 g/L), L-Phenylalanine (0.00132 g/L), L-Proline (0.00007 g/L), L-Serine (0.00252 g/L), L-Taurine (0.00075 g/L), L-Threonine (0.00333 g/L), L-Tryptophan (0.00049 g/L), L-Tyrosine · 2Na · 2H2O (0.00211 g/L), and L-Valine (0.00176 g/L), and Vitamins of Ascorbic Acid · Na (0.01796 g/L), D-Biotin (0.0001 g/L), Choline Chloride (0.0007 g/L), Folic Acid (0.0001 g/L), myo-Inositol (0.0272 g/L), Niacinamide (0.0001 g/L), D-Pantothenic Acid (hemicalcium)(0.0001 g/L), Pyridoxal · HCl (0.0001 g/L), Riboflavin (0.00001 g/L), Thiamine · HCl (0.0001 g/L), and Others of Cytidine (0.00073 g/L), D-Glucose (1.081 g/L), Hypoxanthine (0.00082 g/L), Pyruvic Acid (sodium) (0.01333 g/L), Thymidine (0.00024 g/L), Uridine (0.00073 g/L) and added sodium bicarbonate (1.9 g/L).

[00152] A solution comprising Ames and SP solutions showed a yellow color (Fig. 23A). Contrastingly, a solution comprising Ames, SP and disodium succinate solutions showed a red-pink color (Fig. 23B). This observation, consistent with those standard colorimetric detections (Fig. 23C-D), indicates that Ames solution may be used as a suitable transport media for colorimetric detection. As shown in Fig. 23E, an experiment of directly mixing of a solid phase of SP and Ames solution showed that the solid phase of SP is not very soluble in the Ames solution and the solution remained yellow.

[00153] As shown in Fig. 24, Butterfield's solution was tested for colorimetric detection. Butterfield's solution comprises enzymatic digest of casein (1.00g/L), monopotassium phosphate (0.04g/L), Polysorbate 80 (20mL/L), and the pH value of the solution was adjusted to 7.3±0.3. A solution comprising Butterfield's solution and SP solutions showed a yellow color (Fig. 24A). Contrastingly, a solution comprising Butterfield's solution, SP and disodium succinate solutions showed a red-purple color (Fig. 24B). This observation, consistent with those standard colorimetric detections (Fig. 24C-D), indicates that Butterfield's solution may be used as a suitable transport media for colorimetric detection. As shown in Fig. 24E, a
Butterfield’s solution comprising dissolved solid phase SP appeared to be purple. The solution of “Butterfields+SP solution” includes a butter-fields media and a SP solution (SP+acrylic acid+PBS). The solution of “Butterfields+SP” includes a butteriields media and a solid SP from SIGMA.

[00154] As shown in Fig. 25, Letheen Broth was tested for colorimetric detection. Letheen broths are highly nutritious medias containing Lecithin and Tween® 80 for neutralizing quaternary ammonium compounds. These media may be modifications of the AOAC formulae. Letheen Broth may be used for determining the phenol coefficient of quaternary compounds. A typical composition of Letheen Broth may include peptone from meat 20.0 (g/liter); peptone from casein 5.0 (g/liter); meat extract 5.0 (g/liter) yeast extract 2.0 (g/liter) sodium chloride 5.0 (g/liter) lecithin 0.7 (g/liter); sodium bisulfite 0.1 (g/liter). Letheen Broth may be prepared as the following procedure: Suspend 37.8 g and 5 ml of Tween® 80 in 1 liter of distilled or purified water until evenly dispersed; heat, if necessary, with repeated stirring to dissolve completely and autoclave at 121 °C for 15 min; maintain pH of 7.2 ± 0.2 at 25 °C. The as-prepared broth is turbid and yellowish-brown following incubation for 18 - 48 hours at 35 - 37 °C. In the specific experiment, the Letheen Broth includes enzymatic digest of animal tissue (10 g/l), beef extract (5 g/L), sodium chloride (5 g/L), Tween 80 (Polysorbate 80)(5 mL), Lecithin (0.7 g), with a final pH of 7.0 ± 0.2 at 25°C.

[00155] A solution comprising Letheen Broth and SP solutions showed a yellow color (Fig. 25A). Contrastingly, a solution comprising Letheen Broth, SP and disodium succinate solutions showed a red color (Fig. 25B). This observation, consistent with those standard colorimetric detections (Fig. 25C-D), indicates that Letheen Broth may be used as a suitable transport media for colorimetric detection. As shown in Fig. 25E, an experiment of directly mixing of a solid phase of SP and Letheen Broth showed that the solid phase of SP is not very soluble in the Letheen Broth.

[00156] As shown in Fig. 26, UTM-RT was tested for colorimetric detection. UTM-RT comprises modified Hank’s balanced salt solution, gelatin and bovine serum albumin as stabilizers, sucrose, glutamic acid and 4-(2-hydroxyethyl)-1piperazineethanesulfonic acid (HEPES). The presence of buffered salts in the medium protects pathogens that are sensitive to pH changes. Gelatin and bovine serum albumin are source of nutrition to support viability of fastidious bacteria during storage and transport. Sucrose aids in the preservation of viruses and chlamydiae when specimens are frozen for prolonged storage. Antimicrobial agents are incorporated to minimize commensal bacterial and fungal contamination. Phenol red is added to act as a pH indicator.
A solution comprising UTM-RT and SP solutions showed a yellow color (Fig. 26A). Contrastingly, a solution comprising UTM-RT, SP and disodium succinate solutions showed a red-peach color (Fig. 26B). This observation, consistent with those standard colorimetric detections (Fig. 26C-D), indicates that UTM-RT may be used as a suitable transport media for colorimetric detection. As shown in Fig. 26E, an experiment of directly mixing of a solid phase of SP and UTM-RT showed that the solid phase of SP is not very soluble in the UTM-RT.

These observations demonstrate that it may be possible to incorporate the colorimetric detection and the SP technology to different transport medias. Such incorporation implies a broad color spectrum for a readout scale, as a variety of color changes were observed. Further testing, including cell viability, may be performed in order to validate the use of this system for detection as well as preservation of cell life during transport.

[Example 6]

Elastic Polymers For Colorimetric Detection. To make elastic polymers for colorimetric detection, the chemicals for colorimetric detection include 3.556g Acrylamide, 0.0786g Calcium Chloride, 0.00213g N,N Methylene bisacrylamide, 6mg 4,4 Azobis(4cyanovaleric acid), 10ml DI Water, A separate polyacrylic acid solution includes 3ml DI Water, 0.350ml Acrylic Acid and 6 mg initiator. Both the polymer and polyacrylic acid solution were sonicated for mixing. The polyacrylic acid solution was then polymerized with UV. The resulting polyacrylic acid mixture (linearly polymerized) was added to the polymer mixture to form the elastic polymer solution. Three stock samples were made (S1, S2, S3). From each stock sample, three 2ml samples of elastic polymers were polymerized. MIP elastic polymers were also created by formulating the elastic polymer solution, with the addition of disodium succinate and acrylic acid (about 10mg and 90uL respectively, in a 5ml polymer solution). NIP samples were prepared using the above polymer composition. The polymer was incubated in a SP solution. After the polymer color became consistent with the SP solution color, the excess solution was discarded. A disodium succinate solution was then added to the polymers. The polymers were left to incubate in the DS solution for at least 1hr. Detection was indicated by the polymer turning from yellow to pink upon interaction with the disodium succinate solution.
As comparison, three sets of elastic polymers were made. The first set of elastic polymers were made in the absence of colorimetric detection (Fig. 27A-C). The second set of elastic polymers comprising all the chemicals of NIPs in the absence of the analyte of disodium succinate were made (Fig. 27D-F). The last set of elastic polymers comprising all the chemicals of NIPs in the presence of the analyte of disodium succinate were made (Fig. 27G-I).

As shown in Fig. 27A-C, in the absence of colorimetric detection, the elastic hydrogel appears to be colorless. After the addition of all the chemicals of NIPs, the elastic hydrogel shows a yellow color (Fig. 27D-F). Upon further addition of an analyte of disodium succinate, the elastic hydrogel appears to be pink. Further, it appears that the elastic properties of the hydrogel were maintained after the addition of NIP chemicals (Fig. 27E-F) and the analyte of disodium succinate (Fig. 27H-I). These observations demonstrate that colorimetric detection of the present invention may be easily combined with elastic polymers and elastic hydrogels. The pink elastic hydrogel after the addition of the SP solution indicates the formation of MIPs as discussed above.

[Example 7]

SP solutions Consisting of SP and a Solvent for Colorimetric Detection of ATP. Solution comprises 1mg Spiropyran (SP) and 20mL Dimethyl Sulfoxide (DMSO). 1mg of SP was dissolved in DMSO and sonicated for about 10 minutes. The resultant solution was blue. SP solution samples were divided into control and test samples. 0.5ml_ of the solutions was added to plastic cups for illustration purposes. 0.5ml_ of water was added to the control samples. 0.5ml_ of ATP solution was added to the test samples. Note that SP and HEMA were used as a primary control sample where no water or ATP solution was added.

As shown in Figure 28, a color change was observed upon the addition of ATP solution to the SP solution. The primary control sample was purple, the secondary control sample was dark pink, and the test sample was yellow. As seen in previous experiments, the secondary control sample was used to illustrate that the yellow color change observed by ATP interactions was not due to the solvent. In this case, the solvent was water (or PBS).

UV-Vis data also supports the color changes. As shown in Fig. 29, the yellow line represents the primary control sample of HEMA and SP at 1mg/ml_ in the absence of water. The pink line represents the secondary control sample of HEMA and SP and DI Water in a 1 to
1 ratio. The Green line represents the test sample with HEMA and SP and ATP solution in a 1 to 1 ratio. This observation corresponds with the proposed merocyanine (MC) isomers associated with the "chromic" properties of the spiropyran (SP) molecule.

[00165] Figure 30 highlights the transition of the peaks after ATP was added. The shift in the peaks are consistent with a color change driven by the isomerization of SP<->MC<->MC. Previously Applicants associated the yellow color observed with the SP form of the molecule, and the Red color with that of the Merocyanine (MC) form of the molecule. It is possible that we are also looking at multiple forms of the MC isomer.

Example 8

[00166] SP solutions Comprising SP, a Solvent and polymers for Colorimetric Detection of ATP. Polymer Solution: In one 20ml vial, the following was added.

3.556g Acrylamide
0.0077g N,N-Methylene Bisacrylamide
0.0035g 4,4-Azobis(4-Cyanovaleric acid)
In a second 20ml vial, the following was added.
1mg of SP
20mL of HEMA

SP and HEMA is first sonicated for 10min. 20mL the SP+HEMA solution was added to the polymer components weighed out in the first vial, and then shaken and sonicated. The resultant solution was purple.

Solid Polymers: In one 20mL vial, the following was added.
3.556g Acrylamide
0.0077g N,N-Methylene Bisacrylamide
0.0035g 4,4-Azobis(4-Cyanovaleric acid)
In a second 20ml vial, the following was added.
1mg of SP
0.5 M HEMA (for 20ml solution)
20mL Phosphate buffer solution (PBS)
SP and HEMA is first sonicated for 10min. 20mL of PBS was added to the SP+HEMA solution, and then shaken. Vial 2 was then added to Vial 1. The resultant solution was pink.
2mL of the respective polymer solution samples were polymerized by UV and cut into smaller pieces. ATP solution is used for testing is at a concentration of 20mg/ml_ DI Water

[00167] Polymer solution testing was performed utilizing the same procedure stated above and the experiments yielded the results as shown in Fig. 31.

[00168] A change in color was observed after water was added. The polymer solution, in comparison to the SP solution samples, described a much stronger red color than the pink color shown in the SP solution.

[00169] Solid polymer samples were divided into test and control samples. From the pre-polymer solution made (reference "Solid Polymers" above), 2ml_ of control (pink) was added to weigh boats and polymerized under the UV light. This was repeated for use as test samples". 1ml_ of control sample was mixed with 1ml_ of ATP solution (second control sample- yellow), and then polymerized.

[00170] In Figure 32, color changes of the polymer pieces were observed before and after PBS was added to the polymers on the left (NIP), and after an ATP solution was added to the polymers on the right (NIP-tested with ATP). The picture on the left was observed at t=1, and the picture on the right was observed after 24 hours.

[00171] Fig. 33 is a set of pictures showing time-dependent color changes of solid NIP polymers as shown in Fig. 32. For each of the picture, the samples in the left lane are MIP solid polymers, the samples in the center lane are NIP solid polymers in the absence of ATP, and the samples in the right lane are NIP solid polymers in the presence of ATP. For each lane of the samples, the top samples are corresponding polymer solutions before polymerization. The NIP solid polymers or corresponding solutions turn yellow within 1 minute after the addition of ATP solutions.

[00172] Fig. 34 is a picture showing the color changes of NIP polymer solutions in the presence of different concentration of ATP. The ATP concentrations were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mg/mL for the NIP polymer solutions from left to right. Color of the NIP polymer solutions shifts from purple/pink to lighter pink to yellow as ATP concentration increases.

[00173] Fig. 35 is a graph showing UV-Vis spectra of the corresponding NIP polymer solutions as shown in Fig. 34. Peak shift and intensity change can be associated with isomerization of SP.

Example 9
Ammonium Nitrate Detection. Fig. 36 is a picture showing the color changes of SP solutions in dimethyl sulfoxide (DMSO) after addition of water or ammonium nitrate solutions. SP solutions in DMSO show a blue color (left). After addition of DI water into the SP solution in DMSO, the solutions turn pink (center). After addition of an ammonium nitrate solution into the SP solution in DMSO, the solution turns yellow (right). A SP solution in DMSO was produced by dissolving suitable amount of SP in DMSO. Ammonium nitrate solutions used in the experiments have a concentration of 10 mg/mL in DI water.

Fig. 37 is a graph showing UV-Vis spectra of the solutions as shown in Fig. 36. The light blue line represents a primary control sample of SP solution in DMSO at 1mg/mL in the absence of water. The green line represents a secondary control sample of SP solution in DMSO and DI Water in a 1 to 1 ratio. The dark blue line represents the sample of a SP solution in DMSO and an ammonium nitrate solution in a 1 to 1 ratio. Peak shift and intensity change can be associated with isomerization of SP.

Fig. 38 is a set of pictures and graphs showing ammonium nitrate detection by using SP solutions. After addition of DI water into the SP solution in DMSO, the solutions turn pink (left, Fig. 38A). After addition of an ammonium nitrate solution into the SP solution in DMSO, the solution turns yellow (right, Fig. 38A). Fig. 38B shows UV-Vis spectra of the SP solution in DMSO after addition of DI water (purple) and after addition of an ammonium nitrate solution (yellow).

Fig. 39 is set of pictures and graphs showing ammonium nitrate detection by using polymer and SP solutions. Polymer and SP solutions were produced using similar protocols as discussed above. The polymer and SP solution in the absence of ammonium nitrate shows a pink color (left, Fig. 39A). After addition of an ammonium nitrate solution, the polymer and SP solution turns yellow (right, Fig. 39A). Fig. 39B shows UV-Vis spectra of solutions as shown in Fig. 39A. The pink line represents a control sample of polymer and SP solution. The yellow line represents the sample of polymer and SP solution after addition of an ammonium nitrate solution.

Fig. 40 is a set of pictures showing the color changes of solid NIP polymers during ammonium nitrate detection. The samples in the left lane (pink) are NIP solid polymers in the absence of ammonium nitrate, and the samples in the right lane (yellow) are NIP solid polymers in the presence of ammonium nitrate. Solid NIP polymers were produced following a similar protocol as discussed above.

Fig. 41 is a set of pictures showing time-dependent color changes of solid NIP polymers as shown in Fig. 40. For each of the picture, the samples in the left lane are MIP solid
polymers, the samples in the center lane are NIP solid polymers in the absence of ammonium nitrate, and the samples in the right lane are NIP solid polymers in the presence of ammonium nitrate. The NIP solid polymers can start turning yellow within 1 minutes after the addition of ammonium nitrate solutions.

[00180] Fig. 42 is a picture showing the color changes of SP solutions in DMSO in the presence of different concentration of ammonium nitrate. In the back row from left to right, the ammonium nitrate concentration changes from 0 to 14 µL. In the front row from left to right, the ammonium nitrate concentration changes from 18 to 500 µL. The solutions in the front row shows a concentration-dependent color change, and the color changes from purple/pink to yellow as the concentration of ammonium nitrate increases.

[00181] Fig. 43 is a graph showing UV-Vis spectra of the solutions as shown in Fig. 42. Peak shift and intensity change can be associated with isomerization of SP. After addition of ammonium nitrate solutions, a new peak was observed at 470 nm.

Example 10

[00182] Minimal Solvent Requirements and Fine Tuning Colorimetric Detection. Applicants found that the minimal solution required for detection is SP molecule and a specific solvent(s). The specific solvent(s) may be any suitable solvent. The specific solvent(s) may also be a combination of two more suitable solvents and compounds. Some of the exemplified solvent requirements for colorimetric detection of a few different analytes are listed in Table 1.

[00183] The minimum solution required for detection of an analyte is a solvent and spiropyran molecule. In some examples, Applicants have added water/PBS to the solvents. For example, for the detection of ATP (see Figure 30), the solvents may include Hydroxyethyl Methacrylate (HEMA) and SP. The solution of SP in HEMA was purple in color. Upon the addition of ATP in water, the color changed to yellow. As a control, water was added to HEMA and SP solution. The final color was dark pink/purple.

[00184] Another example is the detection of ammonium nitrate (see Figures 38-38). Ammonium nitrate was adding directly into a solution of acetone and SP or acrylic acid and SP. The resultant color changed from blue to purple and red to yellow, respectively.

[00185] Fine tuning requires the selection of a solvent that is specific for the detection of a particular analyte. Applicants found that one particular solvent alone may
not be sufficient for the detection of all analytes. A specific solvent may be found to
detect a specific analyte.

[00186] Fine tuning may also be concentration-dependent, which may be specific
to a particular solvent. Each solvent may yield a different color in the presence of SP, and the color may change with the various concentrations of SP in the solution.

[00187] Fine tuning may also include SP and solvent interaction with the actual analyte. The color readout in the presence of the analyte may vary with the type and concentrations of the solvent. Generally, a specific solvent or a solvent combination, concentration-dependency of the solvent and the interaction between SP and the solvent may be required to optimize a color readout.

Table 1. The exemplified solvent requirements for colorimetric detection of different analytes.

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<tr>
<th>Analyte</th>
<th>Disodium Succinate</th>
<th>ATP</th>
<th>ADP</th>
<th>Ammonium Nitrate</th>
<th>Acetic Anhydride</th>
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<td>Solvent(s)</td>
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[00188] The idea of fine tuning the technology for specific analyte detection as it pertains to solvents being used may refer to two specific dependencies. One may be the actual solvent being used. The second may be changes in solution concentration.

[00189] Applicants found that a specific solvent may be required to optimize the color readout of a desired, or a specific analyte. Table 1 lists some of the solvents as they have been used for detection with the associated analyte. Table 1 is not all inclusive, but serves as an example of "fine tuning" solvents for analyte detection.

[00190] Applicants found that the color readout of a solution may be heavily concentration-dependent. The concentration of the SP molecule within solution has an effect on the color observed within the solution, and therefore the color readout observed during the detection of an analyte. This phenomenon may also be solvent-dependent. For example, SP in Acetone at very low concentrations yields a colorless solution. At higher concentrations of SP, the solution is blue. Another example is SP in DMF at low concentrations yielding a green color. At higher concentrations, a blue color is observed. Applicants noticed that this dependency also affects the time required for
the color change. At higher concentrations, the solution may take longer to change color in the presence of the analyte. At low concentrations, the readout may be faster, but the color may be washed out as it is too light. Due to this dynamic phenomenon, a specific solvent and SP concentration may be determined for each analyte to optimize the readout observed. It should also be mentioned that the color scale previously observed as analyte concentration increases may also be determined by the solvents used in the solutions.

A second part to fine tuning may include the addition of a second solvent, e.g., water. Due to the solvatochromic properties of the SP molecule, the addition of water to each of the solvents may have a major effect on the color of the final solution, as well as the color readout during detection. Taking these components and parameters into consideration, Applicants determine how a molecule can be detected by establishing the solution parameters needed to optimize the color change and color readout.

REFERENCES

1. European patent application (No. 10771306.7)
We claim:

1. A composition for use in detecting an analyte in a sample by colorimetric readout, comprising:

   (a) a polymer; and

   (b) chromophores contained in the polymer, wherein said chromophores are capable of undergoing a color change when the composition is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout.

2. The composition of claim 1, wherein the polymer is a molecularly-imprinted polymer (MIP).

3. The composition of claim 1, wherein the polymer is a non-imprinted polymer (NIP).

4. The composition of claim 1, 2 or 3, wherein said chromophores comprise spiropyran.

5. The composition of claim 1, 2 or 3, wherein said chromophores comprise gold nanoparticles.

6. The composition of claim 1, 2 or 3, wherein the composition further comprises acrylic acid or its derivatives thereof.

7. The composition of claim 6, wherein the acrylic acid derivatives include acrylic acid esters, such as ethyl acrylate, butyl acrylate, 2-ethylhexyl acrylate, sorbyl acrylate, 2-(dimethylamino)-ethyl acrylate, 3,3-dimethoxypropyl acrylate, 2,2,3,3,4,4,4-heptafluorobutyl acrylate, 2-cyanoethyl acrylate, 4-fluorophenyl acrylate, chloroethyl acrylate, 2-(propen-1-yloxy)-ethyl acrylate, phenyl acrylate, allyl acrylate,
hydroxyethylmethacrylate (HEMA), acrylamides, such as N,N-dimethylacrylamide, and acrylonitrile.

8. The composition of claim 6, wherein acrylic acid and its derivatives enable colorimetric readout by driving the isomerization equilibrium of the chromophores.

9. The composition of claim 1, 2, or 3, wherein the polymer is a polymer or a co-polymer comprising one or more polymerizable monomers selected from group consisting of 2-ethylphenoxy acrylate, 2-ethylphenoxy methacrylate, 2-ethylthiophenyl acrylate, 2-ethylthiophenyl methacrylate, 2-ethyaminophenyl acrylate, 2-ethyaminophenyl methacrylate, phenyl acrylate, phenyl methacrylate, benzyl acrylate, benzyl methacrylate, 2-phenylethyl acrylate, 2-phenylethyl, methacrylate, 3-phenylpropyl acrylate, 3-phenylpropyl methacrylate, 3-propylphenoxy acrylate, 3-propylphenoxy methacrylate, 4-butylphenoxy acrylate, 4-butylphenoxy methacrylate, 4-phenylbutyl acrylate, 4-phenylbutyl methacrylate, 4-methylphenyl acrylate, 4-methylphenyl methacrylate, 4-methylbenzyl acrylate, 4-methylbenzyl methacrylate, 2-2-methylphenylethyl acrylate, 2-2-methylphenylethyl methacrylate, 2-3-methylphenylethyl acrylate, 2-3-methylphenylethyl methacrylate, 2-4-methylphenylethyl acrylate, 2-4-methylphenylethyl methacrylate, 2-(4-propylphenyl)ethyl acrylate, 2-(4-propylphenyl)ethyl methacrylate, 2-(4-(1-methylethyl)phenyl)ethyl acrylate, 2-(4-(1-methylethyl)phenyl)ethyl methacrylate, 2-(4-methoxyphenyl)ethyl acrylate, 2-(4-methoxyphenyl)ethyl methacrylate, 2-(4-cyclohexylphenyl)ethyl acrylate, 2-(4-cyclohexylphenyl)ethyl methacrylate, 2-(2-chlorophenyl)ethyl acrylate, 2-(2-chlorophenyl)ethyl methacrylate, 2-(3-chlorophenyl)ethyl acrylate, 2-(3-chlorophenyl)ethyl methacrylate, 2-(4-chlorophenyl)ethyl acrylate, 2-(4-chlorophenyl)ethyl methacrylate, 2-(4-bromophenyl)ethyl acrylate, 2-(4-bromophenyl)ethyl methacrylate,
2-(3-phenylphenyl)ethyl acrylate, 2-(3-phenylphenyl)ethyl methacrylate,
2-(4-phenylphenyl)ethyl methacrylate, 2-(4-phenylphenyl)ethyl methacrylate,
2-(4-benzylphenyl)ethyl acrylate, and 2-(4-benzylphenyl)ethyl methacrylate.

10. The composition of claim 1, 2, or 3, wherein the polymer is a polymer or a co-polymer comprising one or more polymerizable monomers selected from group consisting of benzyl acrylate, phenyl acrylate, naphthyl acrylate, pentabromophenyl acrylate, 2-phenoxyethyl acrylate, 2-phenoxyethyl methacrylate, and 2,3-dibromopropyl acrylate, n-butyl acrylate, n-hexyl acrylate, 2-ethylhexyl acrylate, 2-ethoxyethyl acrylate, 2,3-dibromopropyl acrylate, 1-dihydroperfluorobutyl acrylate, 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylate, 3-hydroxypropyl acrylate, 3-hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, 4-hydroxybutyl methacrylate, 2,3-dihydroxypropyl acrylate, 2,3-dihydroxypropyl methacrylate, N-methyl acrylamide, N-ethyl acrylamide, N-propyl acrylamide, N-isopropylacrylamide, N-butyl acrylamide, methacrylic acid, 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylate, 2-N-ethylacrylate pyrrolidone, 2-hydroxy-3-phenoxypropyl acrylate, 2,3-dihydroxypropyl acrylate, 2,3-dihydroxypropyl methacrylate, 2-N-vinyl pyrrolidone, polyethylene oxide, hydroxyethylmethacrylate and methyl methacrylate, vinyl pyrrolidone and hydroxyethylmethacrylate, vinyl pyrrolidone and methyl methacrylate, glyceral methacrylate and methyl methacrylate, glyceryl-methacrylate and 2-hydroxyethylmethacrylate, hydroxyethylmethacrylate or diacetone acyl amide and hydroxyalkyl methacrylates, hydroxyethylmethacrylate or diacetone acyl amide and acrylates with the alkyl groups having from 2 to 6 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and vinyl hydroxyl acetate, hydroxyethylmethacrylate or diacetone acyl amide and vinyl hydroxyl propionate, hydroxyethylmethacrylate or diacetone acyl amide and vinyl hydroxy butyrate, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl lactams namely N-vinyl
pyrrolidone, N-vinyl caprolactam and N-vinyl piperidone, hydroxyethylmethacrylate or diacetone acyl amide and N,N dialkyl amino ethyl methacrylates and acrylates with the alkyl groups having from 0 to 2 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and hydroxyalkyl vinyl ethers with the alkyl groups having 2 to 4 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and 1-vinlyloxy 2-hydroxyethylene, hydroxyethyl methacrylate or diacetone acyl amide and 1-vinlyloxy 5-hydroxy 3-oxapentane, hydroxyethylmethacrylate or diacetone acyl amide and 1-vinlyloxy 8-hydroxy 3,6-dioxaoctane, hydroxyethylmethacrylate or diacetone acyl amide and 1-vinlyloxy 14-hydroxy 3,6,9,12 tetraoxatradecane, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl morpholine; hydroxyethylmethacrylate or diacetone acyl amide and N,N dialkyl acrylamide with the alkyl groups having from 0 to 2 carbons atoms, hydroxyethylmethacrylate or diacetone acyl amide and alkyl vinyl ketone with the alkyl group having 1 to 2 carbon atoms, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl succinimide or N-vinyl glutarimide, hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl imidazole, and hydroxyethylmethacrylate or diacetone acyl amide and N-vinyl 3-morpholinone.

11. A device for detection of analyte in a sample by colorimetric readout, comprising:

(a) a chamber for containing a sample that includes an analyte; and

(b) a composition according to any one of claims 1-10 positioned in the chamber,

wherein the analyte in the sample causes a visible color change of the composition, thereby providing detection of the analyte by colorimetric readout.

12. A method of detecting an analyte in a sample by colorimetric readout, comprising:
(a) contacting a sample that includes an analyte with a composition according to any one of claims 1-10; and

(b) detecting the analyte by visible color change of the composition, thereby providing detection of the analyte by colorimetric readout.

13. An article for detecting an analyte in a sample by colorimetric readout, comprising a composition according to any one of claims 1-10 wherein the analyte in the sample causes a visible color change of the article, thereby providing detection of the analyte by colorimetric readout.

14. A composition of any one of claims 1-10 for use in detecting an analyte by colorimetric readout.

15. Use of a composition of any one of claims 1-10 for the manufacture of a device or article capable of detecting an analyte by colorimetric readout.

16. A device for detection of an analyte in a sample by colorimetric readout, comprising:

(a) a composition according to any one of claims 1-10; and

(b) a means for detecting a visible color change of the composition, thereby providing detection of the analyte by colorimetric readout.

17. A solution for use in detecting an analyte in a sample by colorimetric readout, comprising:

(a) a solvent selected from the group consisting of water, DMSO, acetone, alcohol, DMF, and pyridine;

(b) a phosphate buffer;
(c) a polymer and

(b) chromophores contained in the polymer, wherein said chromophores are capable of undergoing a color change when the composition is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout.

18. The solution of claim 17, wherein the polymer is a molecularly-imprinted polymer (MIP).

19. The solution of claim 17, wherein the polymer is a non-imprinted polymer (NIP).

20. The solution of claim 17, 18 or 19, wherein said chromophores comprise spiropyran.

21. The solution of claim 17, 18 or 19, wherein said chromophores comprise gold nanoparticles.

22. A spiropyran (SP) solution for use in detecting an analyte in a sample by colorimetric readout, comprising:

(a) a solvent selected from the group consisting of water, DMSO, acetone, alcohol, DMF, and pyridine;

(b) a phosphate buffer;

(c) acrylic acid or its derivatives thereof; and

(d) spiropyran (SP), wherein said spiropyran (SP) is capable of undergoing a color change when the solution is contacted with an analyte, thereby providing detection of the analyte by colorimetric readout.

23. A kit for detection of an analyte in a sample by colorimetric readout, comprising:

(a) a composition according to any one of claims 1-22;

(b) a solid support; and
(c) a means for detecting a visible color change of the composition, thereby providing
detection of the analyte by colorimetric readout.

24. The kit according to claim 23, wherein the solid support is a transport system.

25. The kit according to claim 24, wherein the transport system is either a flocked swab or a
sponge swab.

26. The kit according to claim 23, wherein the kit further comprises a transport media.

27. The kit according to claim 26, wherein the transport media is selected from the group
consisting of peptone water, Ames solution, Butterfield’s solution, Letheen Broth, and
UTM-RT.

28. The kit according to claim 23, wherein the kit further comprises an elastic polymer.

29. A spiropyran (SP) solution for use in detecting an analyte in a sample by colorimetric
readout comprising:

(a) a solvent selected from the group consisting of water, DMSO, acetone, alcohol, DMF,
hydroxyethyl methacrylate (HEMA), and pyridine; and

(b) spiropyran (SP), wherein said spiropyran (SP) is capable of undergoing a color
change when the solution is contacted with an analyte, thereby providing detection of the
analyte by colorimetric readout.

30. A composition for use in detecting an analyte in a sample by colorimetric readout,
comprising:

(a) a solvent; and

(b) chromophores contained in the polymer, wherein said chromophores are capable of
undergoing a color change when the composition is contacted with an analyte, thereby
providing detection of the analyte by colorimetric readout.

31. The composition according to claim 30, wherein the chromophore is SP.
32. The composition according to claim 30, wherein the solvent is selected from the group consisting of water, DMSO, acetone, alcohol, DMF, hydroxyethyl methacrylate (HEMA), and pyridine.

33. The composition according to claim 30, wherein the composition further comprises a polymer.

34. The composition according to claim 32, wherein the polymer is a molecularly-imprinted polymer (MIP).

35. The composition according to claim 33, wherein the polymer is a non-imprinted polymer (NIP).

36. A kit for detection of an analyte in a sample by colorimetric readout, comprising:
   a) a composition according to any one of claims 29-35;
   b) a solid support; and
   c) a means for detecting a visible color change of the composition, thereby providing detection of the analyte by colorimetric readout.

37. The kit according to claim 36, wherein the solid support is a transport system.

38. The kit according to claim 37, wherein the transport system is either a flocked swab or a sponge swab.

39. A method for detecting adenosine triphosphate according to any of the claims 1-38.

40. A method for detecting substances related to explosives according to any of claims 1-39.

41. The method according to claim 40, wherein the substance related to explosives is ammonium nitrate.
FIG. 1
FIG. 2
FIG. 3
FIG. 5 A-D
FIG. 11
FIG. 13
Fig. 19 A, B
Fig. 21 A-D
Fig. 24 A-E
Fig. 26 A-E
FIG. 28
FIG. 30 A, B
FIG. 31 A, B
FIG. 33
As [ATP] increases, peaks decrease in absorbance here.

As [ATP] increases, peaks increase in absorbance here.

FIG. 35
FIG. 36

Control    w/ H20    w/ Ammonium Nitrate
FIG. 38 A, B
A

Control Sample
w/o ANS

Test Sample
w/ ANS

B

CONTROL – Pink

TEST – Yellow

FIG. 39 A, B
As [ABS] increases, peaks decrease in absorbance here.

As [ABS] increases, a new peak is observed here.

As [ABS] increases, peaks decrease in absorbance here.

FIG. 43
Gram Positive Identification Flow Chart

Fig. 44
### A. CLASSIFICATION OF SUBJECT MATTER

**INV.** G01N33/532  G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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<th>G01N</th>
<th>C07D</th>
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**EPO-Internal**, **BEI LSTEIN Data**, **BIOSIS**, **CHEM ABS Data**, **EMBASE**, **WPI Data**

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>JINMING HU ET AL: &quot;Responsive Polymers for Detection and Sensing Applications: Current Status and Future Developments&quot;, MACROMOLECULES, vol. 43, no. 20, 26 October 2010 (2010-10-26), pages 8315-8330, XP055096719, ISSN: 0024-9297, DOI: 10.1021/ma1005815 the whole document Schemes 3-4; page 8316</td>
<td>1-41</td>
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**X** Further documents are listed in the continuation of Box C.  
**X** See patent family annex.

* Special categories of cited documents:
  * "A" document defining the general state of the art which is not considered to be of particular relevance
  * "E" earlier application or patent but published on or after the international filing date
  * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  * "O" document referring to an oral disclosure, use, exhibition or other means
  * "P" document published prior to the international filing date but later than the priority date claimed

* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

* "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

* "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

* "A" document member of the same patent family

**Date of the actual completion of the international search**  
16 January 2014

**Date of mailing of the international search report**  
24/01/2014

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2
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**Authorized officer**

Cervigni, S
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<td>MANESIOTIS P ET AL: &quot;An enanti oselecti ve imprinted receptor for Z-gl utamate exhibiting a bind ng induced col or change&quot;, CHEMICAL COMMUNICATIONS CHEM. COMMUN.; [6015D], ROYAL SOCIETY OF CHEMISTRY, GB, 1 January 2004 (2004-01-01), pages 2278-2279, ISSN: 1359-7345, DOI: 10.1039/B407870E the whole document</td>
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<td>X</td>
<td>Wo 2011/053587 AI (MEDTECH DETECT LLC [US]; KN0P RICHARD H [US]) 5 May 2011 (2011-05-05) the whole document paragraph [[0058]]: claims; figures 1-2; examples</td>
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<td>KRISTEN H. FRIES ET AL: &quot;Fabri cati on of Spi ropyran-Contai ning Thin Film Sensors Used for the Simul taneous Identi cati on of Multiple Metal Ions&quot;, LANGMUI R, vol. 27, no. 19, 4 October 2011 (2011-10-04), pages 12253-12260, ISSN: 0743-7463, DOI: 10.1021/la202344w the whole document</td>
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<tr>
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