[0017] The disclosure is directed to methods for determining the presence of or the amount of an analyte in a sample. The methods involve:

(i) providing a sample suspected of containing an analyte;

(ii) contacting the sample with a fluorescent tracer and a binding partner to provide an assay composition;

wherein the binding partner is specific for the analyte and the fluorescent tracer;

(iii) irradiating the assay composition with light at a first wavelength, wherein the light at the first wavelength is not linearly polarized; and

(iv) measuring the intensity of light emitted at a second wavelength,

wherein the intensity of the light emitted at the second wavelength is proportional to the concentration of the analyte in the sample.

[0017a] In one aspect, the disclosure is directed to a method for determining the presence of or the amount of an analyte in a sample comprising:

(i) providing a sample suspected of containing an analyte;

(ii) contacting the sample with a fluorescent tracer and a binding partner to provide an assay composition;

wherein the binding partner is specific for the analyte and the fluorescent tracer;

(iii) irradiating the assay composition with light at a first wavelength; and

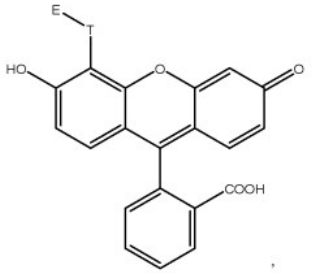
(iv) measuring the intensity of light emitted at a second wavelength,

wherein

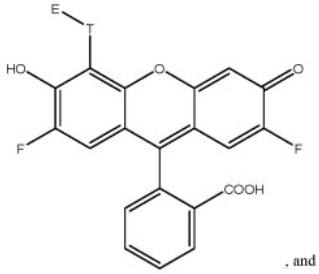
(a) the intensity of the light emitted at the second wavelength is greater if the fluorescent tracer is not bound to the binding partner than if the fluorescent tracer is bound to the binding partner,

(b) the light at the first wavelength is not linearly polarized, and

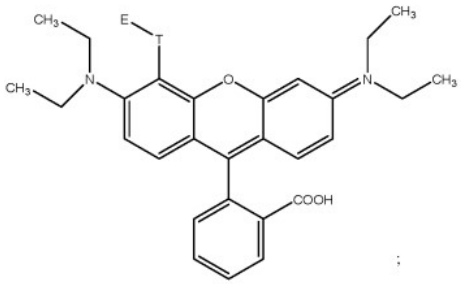
(c) the fluorescent tracer is selected from the group consisting of:



,



, and



;

wherein T is a bond or a linking group and E is an epitopic moiety; and

wherein the intensity of the light emitted at the second wavelength is directly proportional to the concentration of the analyte in the sample.

[0018] The method can be performed in solution or as a dry slide assay. In one example, the analyte is SDMA. In one example, the method does not involve a separation step.

[0019] The disclosure also encompasses fluorescent tracers that can be used in the methods of the disclosure. In one example, the fluorescent tracer is selected from the group consisting of:

[0019a] In another aspect, the disclosure is directed to a method for determining the presence of or the amount of an analyte in a sample comprising:

(i) providing a sample suspected of containing an analyte;

(ii) contacting the sample with a fluorescent tracer and a binding partner to provide an assay composition;

wherein the binding partner is specific for the analyte and the fluorescent tracer;

(iii) irradiating the assay composition with light at a first wavelength; and

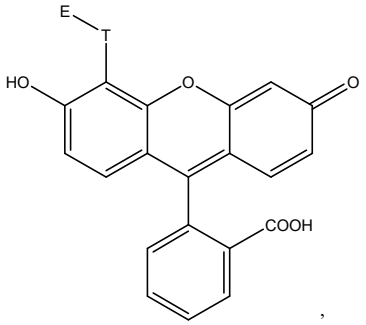
(iv) measuring the intensity of light emitted at a second wavelength,

wherein

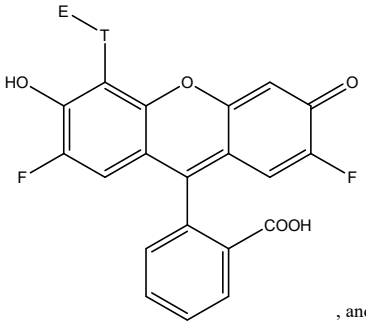
(a) the intensity of the light emitted at the second wavelength is greater if the fluorescent tracer is not bound to the binding partner than if the fluorescent tracer is bound to the binding partner,

(b) the light at the first wavelength is not linearly polarized,

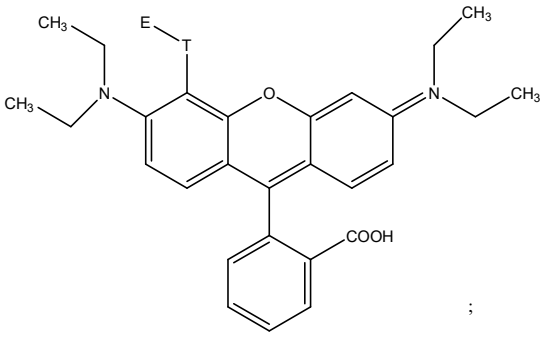
(c) the fluorescent tracer is selected from the group consisting of:



,



, and



;

wherein T is a bond or a linking group and E is an epitopic moiety capable of specifically binding to the binding partner;

(d) the intensity of the light emitted at the second wavelength is directly proportional to the concentration of the analyte in the sample; and

[0020] (e) wherein the analyte is selected from the group consisting of SDMA, melamine, T4, bile acid, biotin, thyroxine, cystatin-B, NT-proBNP, antibiotics, sulfadimethoxine, cortisol, and progesterone.The disclosure also encompasses a slide that can be used in the methods.

[0021] The disclosure also encompasses derivatized fluorescein molecules that are useful as intermediates for synthesizing the substituted fluorescent tracers that can be used in the methods of the disclosure.

THE CLAIMS

What is claimed is:

1. A method for determining the presence of or the amount of an analyte in a sample comprising:

(i) providing a sample suspected of containing an analyte;

(ii) contacting the sample with a fluorescent tracer and a binding partner to provide an assay composition;

wherein the binding partner is specific for the analyte and the fluorescent tracer;

(iii) irradiating the assay composition with light at a first wavelength; and

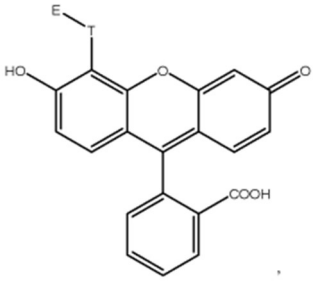
(iv) measuring the intensity of light emitted at a second wavelength,

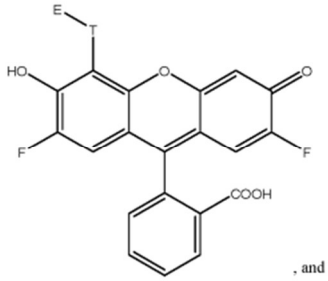
wherein

(a) the intensity of the light emitted at the second wavelength is greater if the fluorescent tracer is not bound to the binding partner than if the fluorescent tracer is bound to the binding partner,

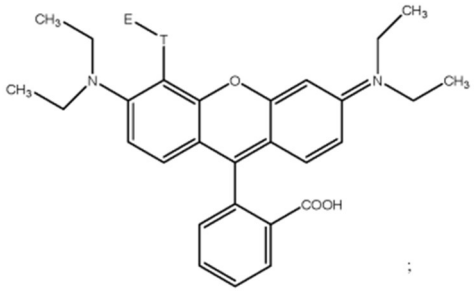
(b) the light at the first wavelength is not linearly polarized, and

(c) the fluorescent tracer is selected from the group consisting of:





, and



;

wherein T is a bond or a linking group and E is an epitopic moiety; and

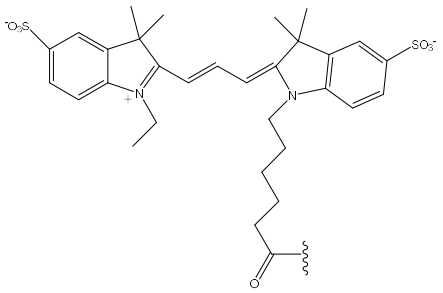
wherein the intensity of the light emitted at the second wavelength is directly proportional to the concentration of the analyte in the sample.

2. The method of claim 1, wherein the analyte is a macromolecule.

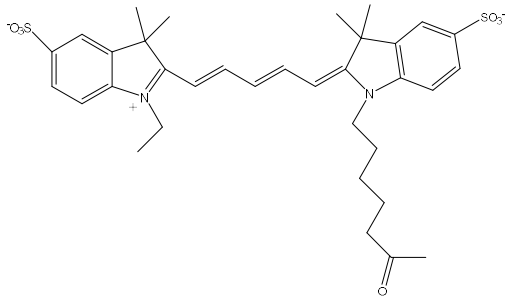
3. The method of claim 2, wherein the analyte is a protein.

4. The method of claim 3, wherein (i) the epitopic moiety is an amino acid side chain and wherein the sequence of the amino acid side chain comprises the amino acid sequence of an epitope on the protein that is responsible for complexing with the binding partner, and (ii) the binding partner is optionally conjugated to a quencher.

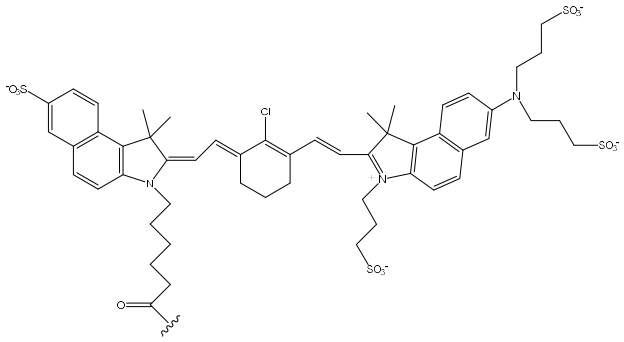
5. The method of claim 4, wherein the quencher is selected from the group consisting of



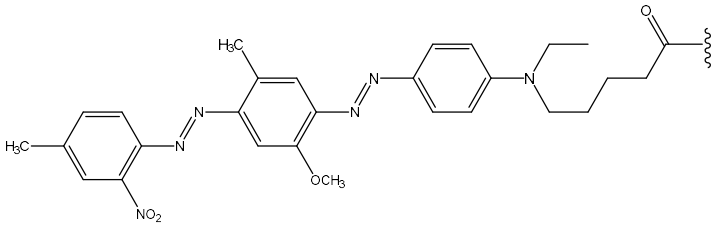
CY3



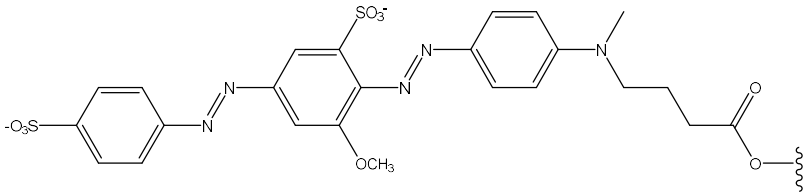
CY5



IRDyeQC1



BHQ1, and



BHQ10

wherein the wavy line indicates a linkage point to the binding partner.

6. The method of claim 4, wherein the binding partner is an antibody.

7. The method of claim 4, wherein the protein is selected from the group consisting of canine CysB protein and canine NT-proBNP.

8. The method of claim 7, wherein the protein is canine CysB protein.

9. The method of claim 8, wherein the binding partner is an anti-cystatin-B antibody and the fluorescent tracer is fluorescein attached to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

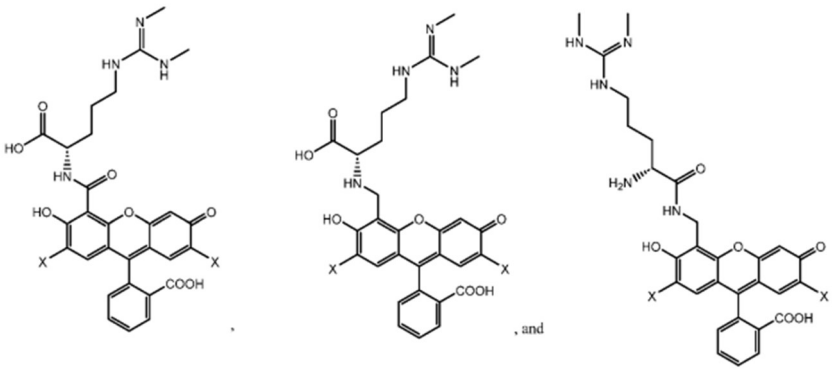
10. The method of claim 1, wherein the amino acid sequence is attached to the 4'-position of the fluorescein with a $-CH_2-$ linker.

11. The method of claim 9, wherein the anti-cystatin-B antibody is conjugated to the quencher.

12. The method of claim 1, wherein the analyte is an antigen and the binding partner is an antibody.

13. The method of claim 12, wherein the antigen is SDMA and the antibody is an antibody for SDMA.

14. The method of claim 13, wherein the fluorescent tracer is selected from the group consisting of:



wherein each X is -H or each X is -F.

15. The method of claim 14, wherein the antibody for SDMA is conjugated to a quencher.

16. The method of claim 15, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

17. The method of claim 1, comprising:

(A) providing a slide, wherein the slide comprises sequentially:

(i) a support layer selected from the group consisting of glass, polystyrene, polyesters, polycarbonates, cellulose derivatives, polyethylene terephthalate, and mixtures thereof,

wherein the support layer is optically clear to the first wavelength and the second wavelength;

(ii) a primer layer comprising polyurethane;

(iii) an indicator layer comprising the fluorescent tracer and the binding partner dispersed in a second polymer, wherein the second polymer is selected from the group consisting of cellulose, cellulose derivatives, polysaccharides, gelatin, gelatin derivatives, polyvinyl alcohol, polyvinyl pyrrolidone, acrylamide polymers, polyurethane, alginate, xantham, and mixtures thereof,

(iv) a filtering layer comprising polyurethane and cellulose; and

(v) a spreading layer comprising a mixture of cellulose and a hydrophilic polymer, wherein the hydrophilic polymer is selected from the group consisting of polyacrylic acid, polyvinylpyrrolidone, polythylene glycol, polyethylene oxide, polyvinyl alcohol, polyacrylamides, and polethylenimines.

(B) depositing the sample on the spreading layer of the slide;

(C) allowing the sample to diffuse from the spreading layer into the indicator layer so that the sample can contact the fluorescent tracer and the binding partner;

(D) irradiating the support layer of the slide with the light that is not polarized at a first wavelength; and

(E) measuring the intensity of the light emitted at the second wavelength from the support layer of the slide.

18. The method of claim 17, wherein the analyte is SDMA and the antibody is an antibody for SDMA.

19. A method for determining the presence of or the amount of an analyte in a sample comprising:

(i) providing a sample suspected of containing an analyte;

(ii) contacting the sample with a fluorescent tracer and a binding partner to provide an assay composition;

wherein the binding partner is specific for the analyte and the fluorescent tracer;

(iii) irradiating the assay composition with light at a first wavelength; and

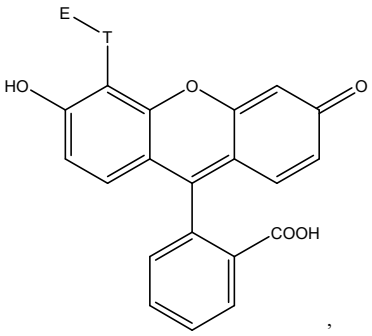
(iv) measuring the intensity of light emitted at a second wavelength,

wherein

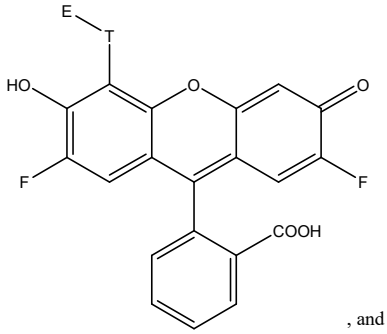
(a) the intensity of the light emitted at the second wavelength is greater if the fluorescent tracer is not bound to the binding partner than if the fluorescent tracer is bound to the binding partner,

(b) the light at the first wavelength is not linearly polarized,

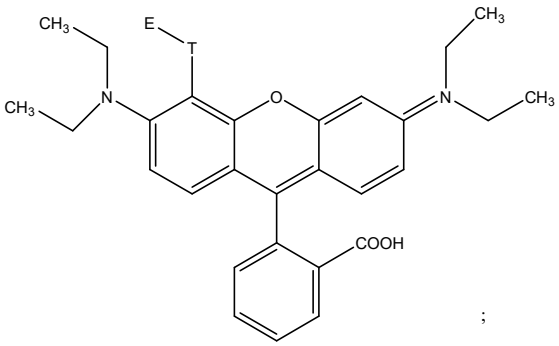
(c) the fluorescent tracer is selected from the group consisting of:



,



, and



;

wherein T is a bond or a linking group and E is an epitopic moiety capable of specifically binding to the binding partner;

(d) the intensity of the light emitted at the second wavelength is directly proportional to the concentration of the analyte in the sample; and

(e) wherein the analyte is selected from the group consisting of SDMA, melamine, T4, bile acid, biotin, thyroxine, cystatin-B, NT-proBNP, antibiotics, sulfadimethoxine, cortisol, and progesterone.