concentration of antigen in the test sample, leads to more of the antibodies being bound to antigen from the test sample and less being bound to fluorescent antigen (*i.e.*, leads to more unbound fluorescent antigen). By maintaining constant the concentration of the fluorescent antigen and the antibody, the ratio of sample antigen-antibody complex to fluorescent antigen-antibody complex is directly proportional to the amount of antigen present in the sample. Similarly, the amount of unbound fluorescent antigen is directly proportional to the amount of antigen present in the sample. Therefore, by exciting the mixture with light and measuring the intensity of the emitted light, one is able to quantitatively determine the amount of antigen in the sample. The concentration of antigen in the test sample is directly proportional to the intensity of the emitted light.

[0012] These techniques are not limited to antibody-antigen binding partners. Similar assays can be performed using a protein (which is not an antibody) to determine the presence or concentration of a substrate that specifically binds to the protein. The tracer in these assays can be, for example, a detectable label linked to the protein or a detectable label linked to a molecule that binds to the protein.

[0013] Immunoassays are advantageous over other analytical methods for measuring the presence or concentration of a substance in a test sample, such as, for example, gas chromatography (GC) and high-performance liquid chromatography (HPLC), because immunoassays avoid the extractions and other complex sample work-up procedures and lengthy assay times that are often associated with these other analytical methods.

[0014] There remains a need in the art, however, for immunoassays that exhibit higher sensitivity, are simpler to perform, and less expensive to perform.

[0015] Citation of any reference in this application is not to be construed that such reference is prior art to the present application.

[0016] These and other features and advantages of the present invention will become apparent from the remainder of the disclosure, in particular the following detailed description of the preferred embodiments, all of which illustrate by way of example the principles of the invention.

SUMMARY

[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;

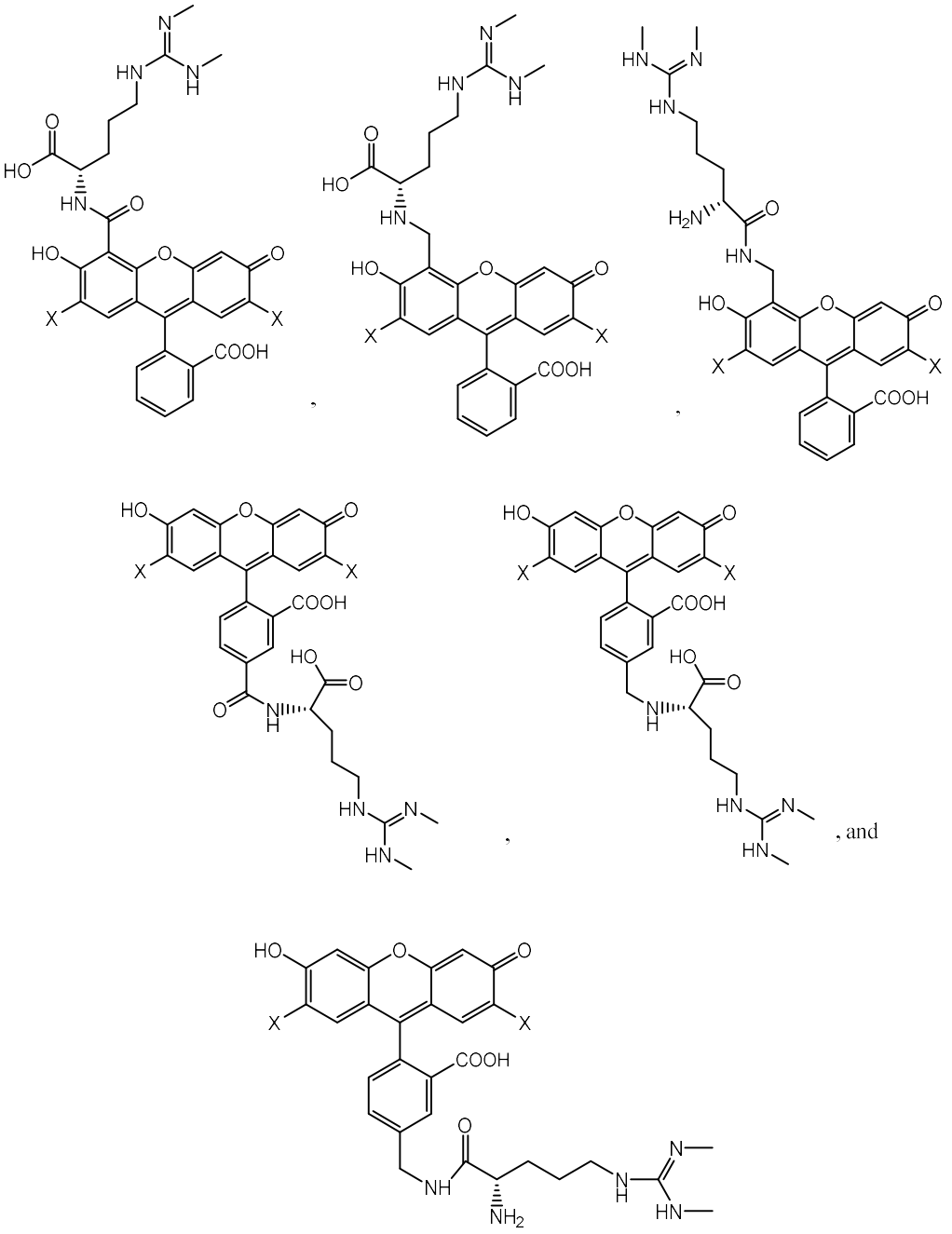
(iii) irradiating the assay composition with light at a first wavelength; and

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

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

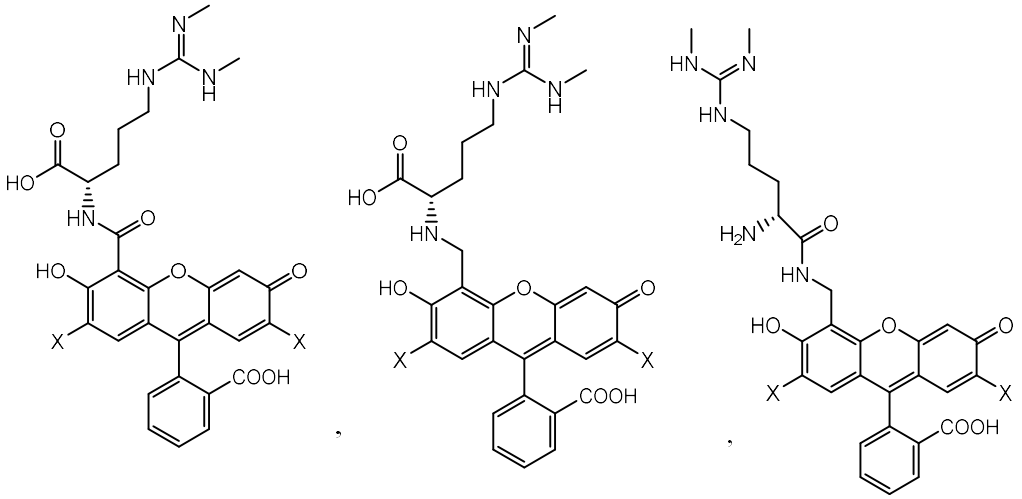
[0018] The method can be performed in solution or as a dry slide assay. In one example, the analyte is SDMA. In one example, the method does not involve a separation step.

[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:



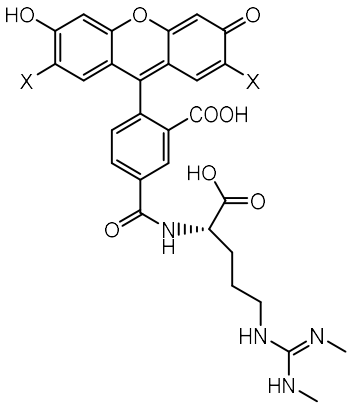
wherein X = -H, -F, -CH_3, -OCH_3, - Cl, -OH, -NO_2, -CN, -COOH, or -SO_3H.

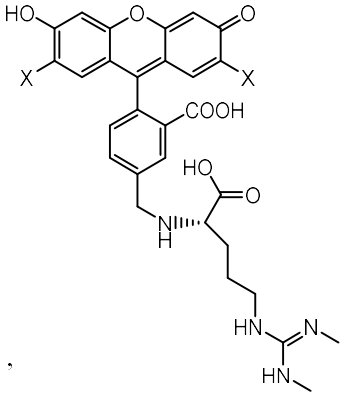
[0019a] In another aspect, the disclosure is directed to a compound selected from the group consisting of:

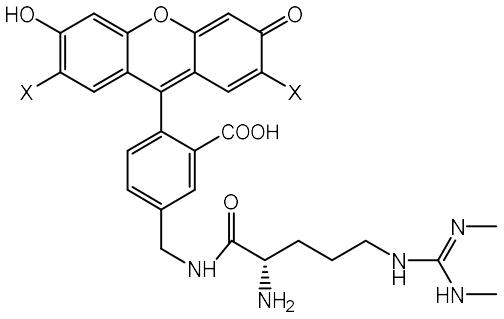

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[0020] The disclosure also encompasses a slide that can be used in the methods.

[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 the intensity of the light emitted at the second wavelength is 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 fluorescent tracer is a fluorescent moiety attached to an amino acid chain, wherein the length of the amino acid chain is shorter than the amino acid length of the protein, and wherein the amino acid sequence of the amino acid 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.

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 9, wherein the amino acid sequence is attached to the 5-position of the fluorescein.

11. The method of claim 10, wherein the amino acid sequence is attached to the 5-position of the fluorescein with a -NH-C(O)- linker.

12. The method of claim 10, wherein the anti-cystatin-B antibody is conjugated to a quencher.

13. The method of claim 9, wherein the amino acid sequence is attached to the 4'-position of the fluorescein.

14. The method of claim 13, wherein the amino acid sequence is attached to the 4'-position of the fluorescein with a -CH_2- linker.

15. The method of claim 13, wherein the anti-cystatin-B antibody is conjugated to a quencher.

16. The method of claim 4, wherein the amino acid sequence is attached to the 5-position of the fluorescein.

17. The method of claim 16, wherein the amino acid sequence is attached to the 5-position of the fluorescein with a -NH-C(O)- linker.

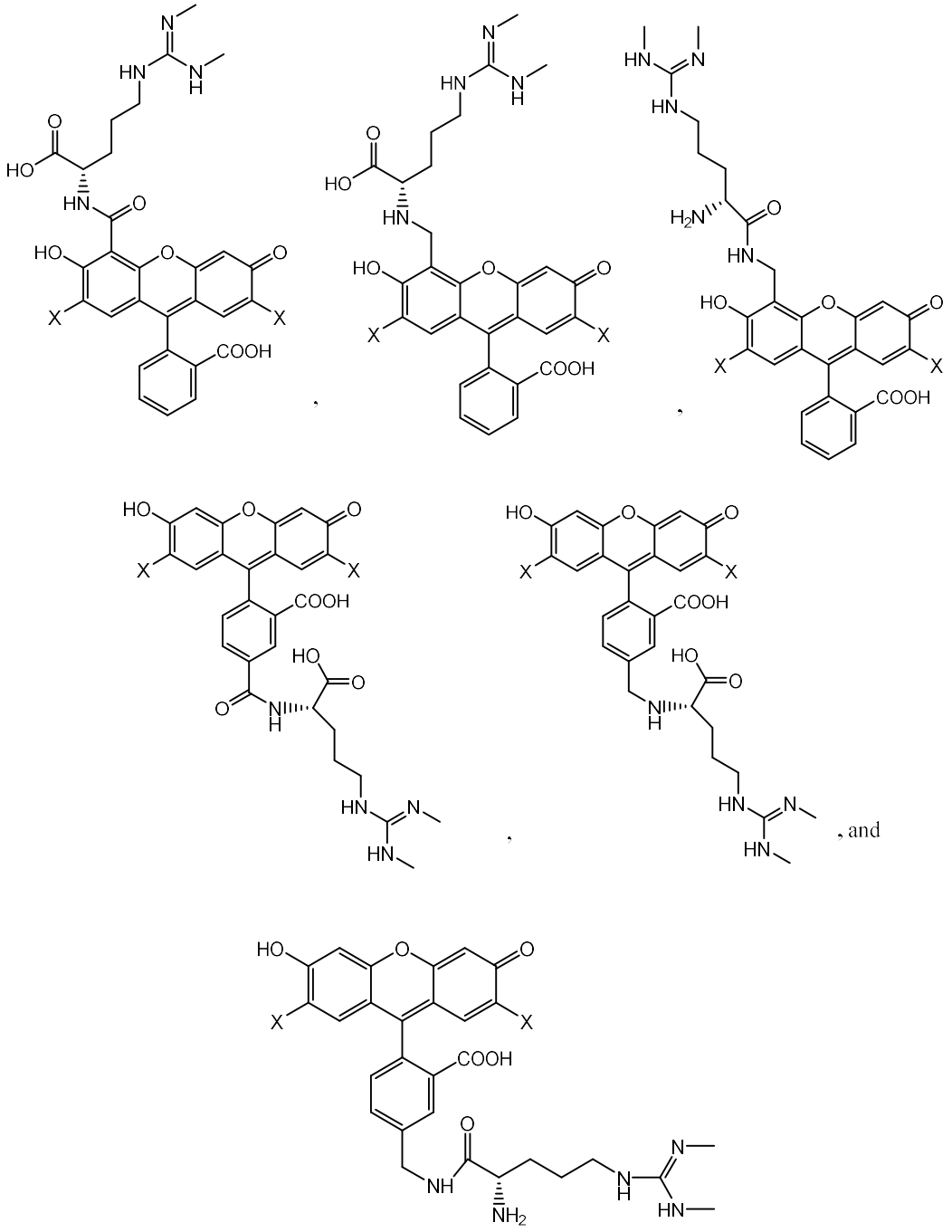
18. The method of claim 16, wherein the anti-cystatin-B antibody is conjugated to a quencher.

19. The method of claim 1, wherein the light at the first wavelength is not linearly polarized.

20. The method of claim 19, wherein the analyte is an antigen and the binding partner is an antibody.

21. The method of claim 20, wherein the antigen is SDMA and the antibody is an antibody for SDMA.

22. The method of claim 21, wherein the fluorescent tracer is selected from the group consisting of:

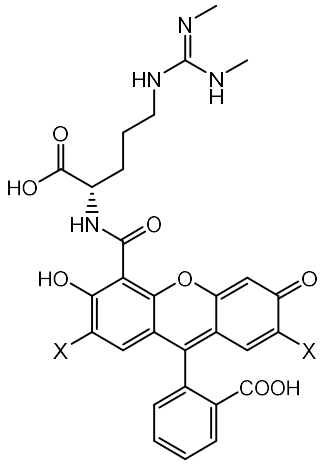
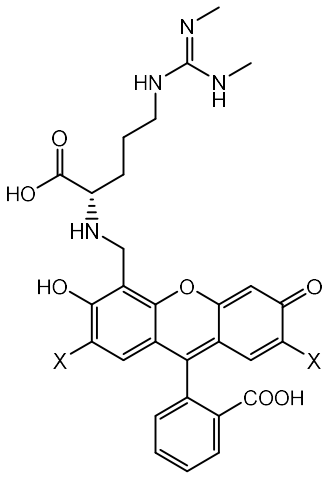
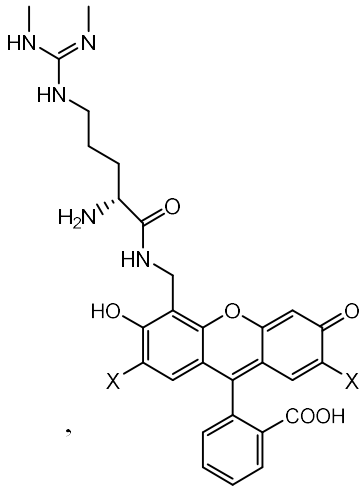
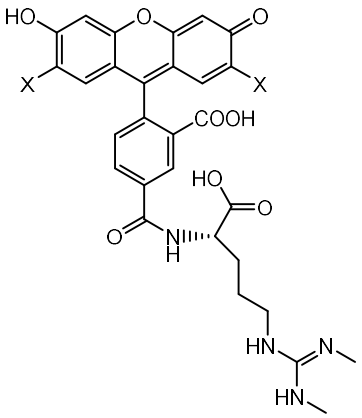
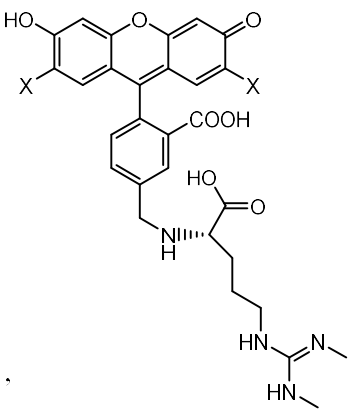
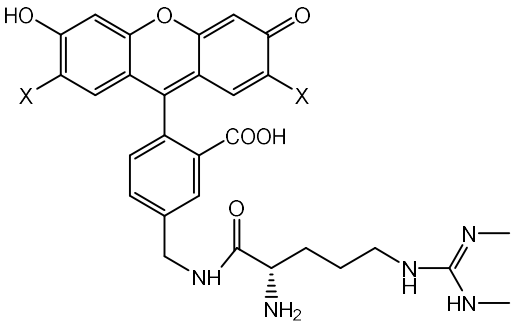


wherein X is selected from the group consisting of -H, -F, -CH_3, -OCH_3, -OH, -NO_2, -CN, -COOH, and -SO_3H.

23. The method of claim 22, wherein the antibody for SDMA is conjugated to a quencher.

24. The method of claim 23, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

25. A compound selected from the group consisting of:


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26. The method of claim 19, 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) irradiating the support layer of the slide with the light that is not polarized at a first wavelength; and

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

27. The method of claim 26, wherein the antigen is SDMA and the antibody is an antibody for SDMA.