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 ~~OF THE INVENTION~~

[0017] The ~~invention~~ 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;

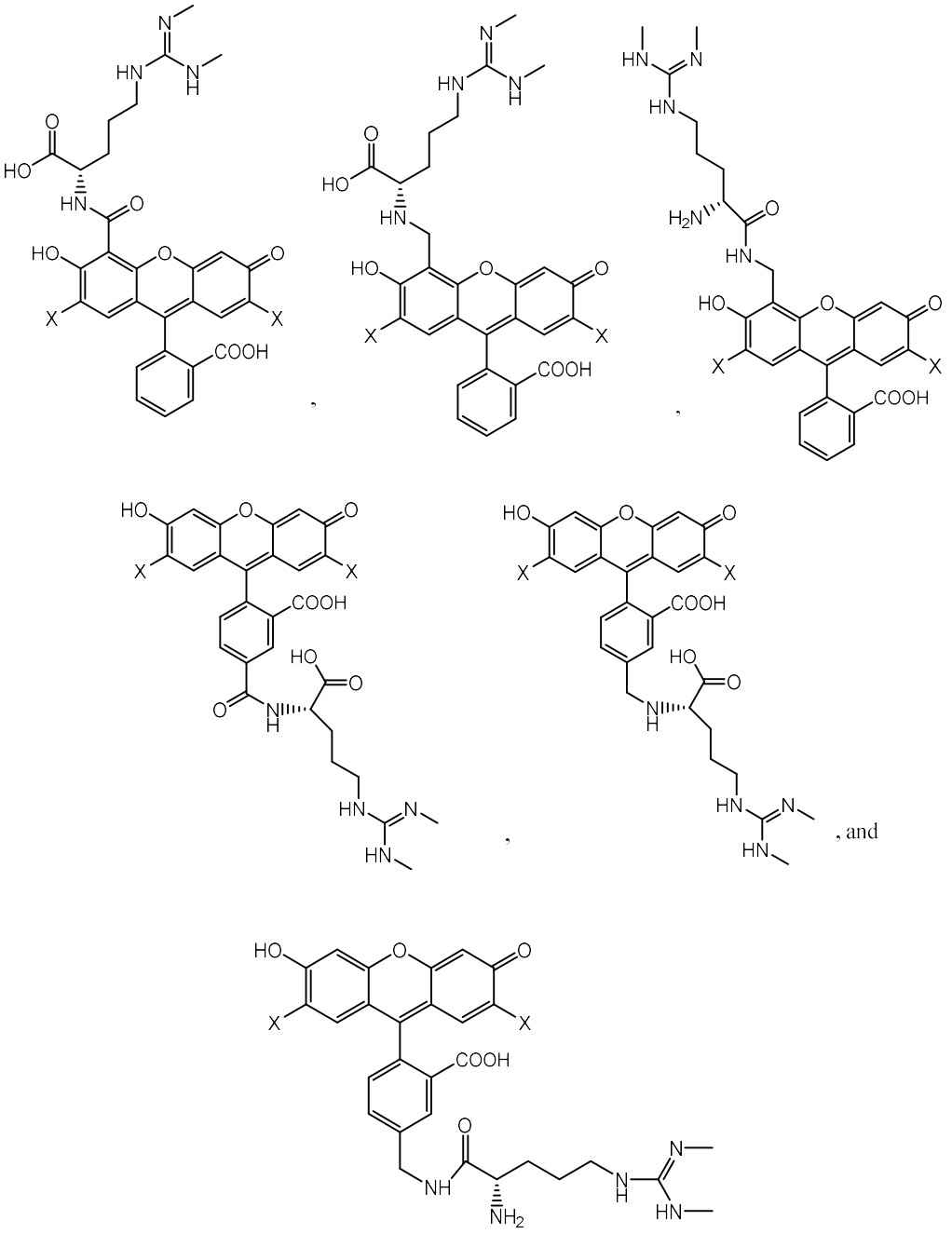
(iii) irradiating the assay composition with light at a first wavelength; and

(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.

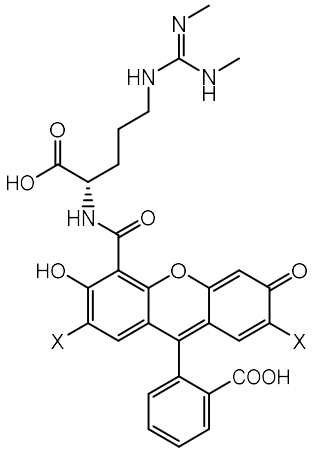
[0018] The method can be performed in solution or as a dry slide assay. In one ~~embodiment~~example, the analyte is SDMA. In one ~~embodiment~~example, the method does not involve a separation step.

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

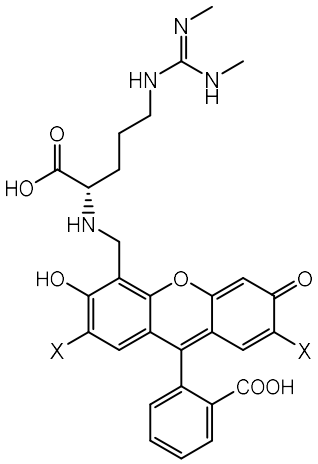


wherein X = -H, -F, -CH_3, -OCH_3, - Cl, -OH, -NO_2, -CN, -COOH, or -SO_3H.

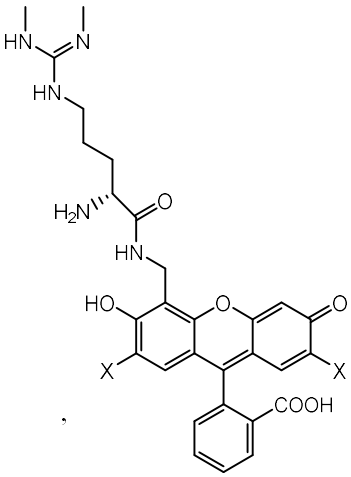
[0019a] In another aspect, the disclosure is directed to a compound selected from the group consisting of:



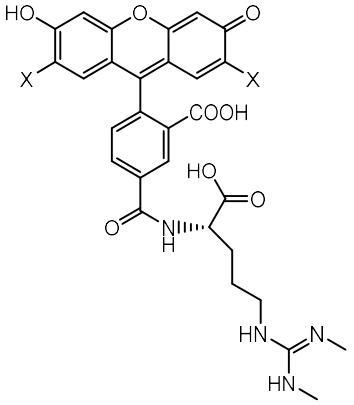
,



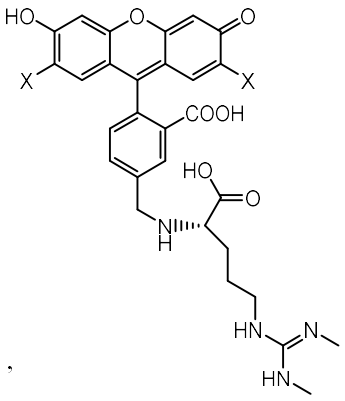
,



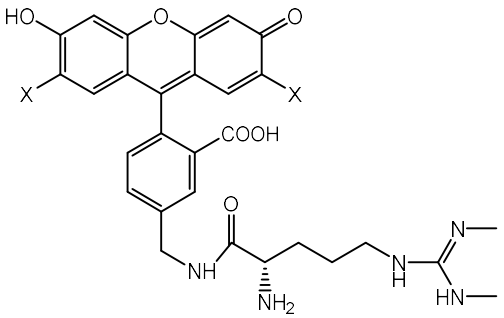
,



,



, and



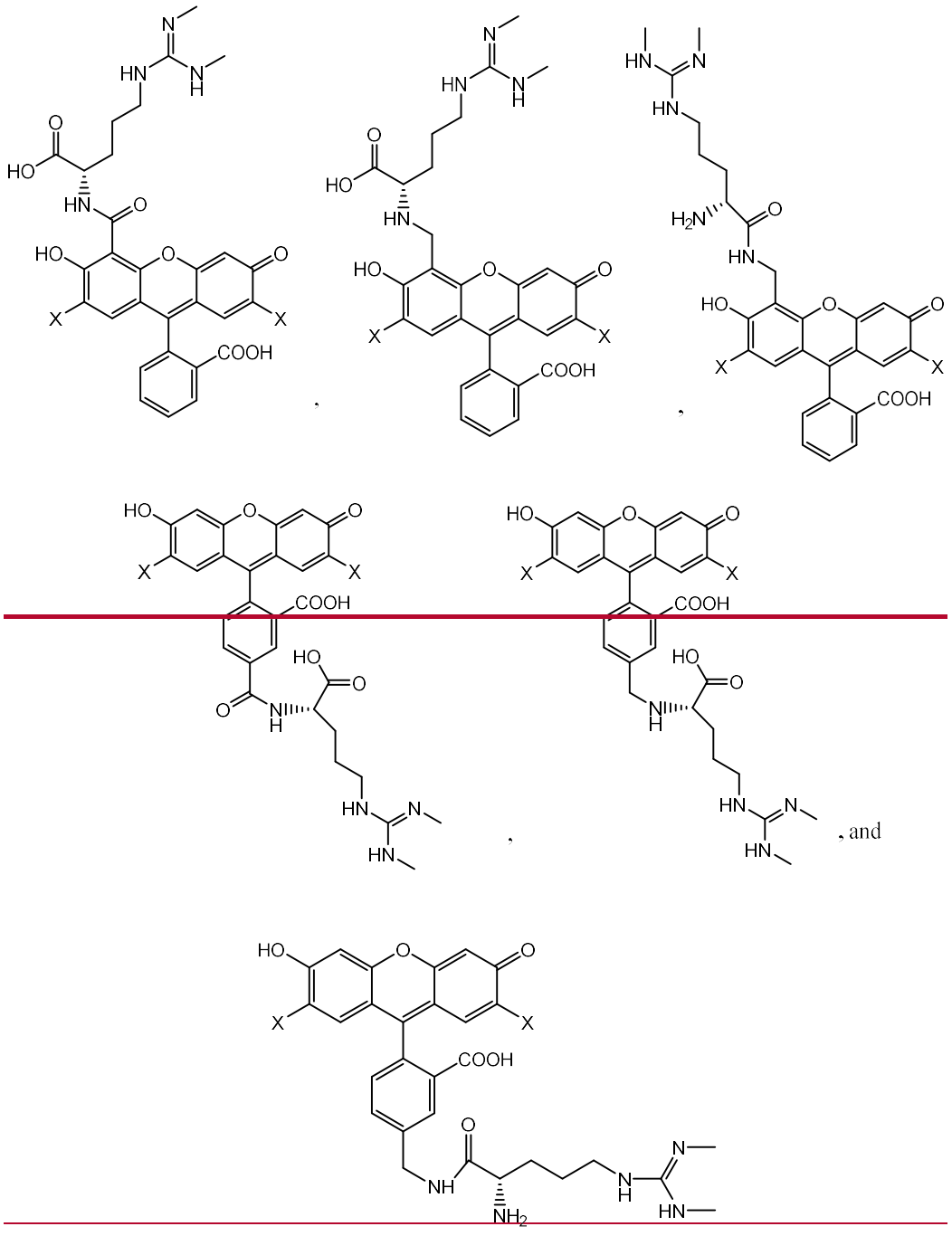
[0020] The ~~invention~~ disclosure also encompasses a slide that can be used in the methods.

[0021] The ~~invention~~ 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 ~~invention~~disclosure.

THE CLAIMS

What is claimed is:

~~1. A compound selected from the group consisting of:~~



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

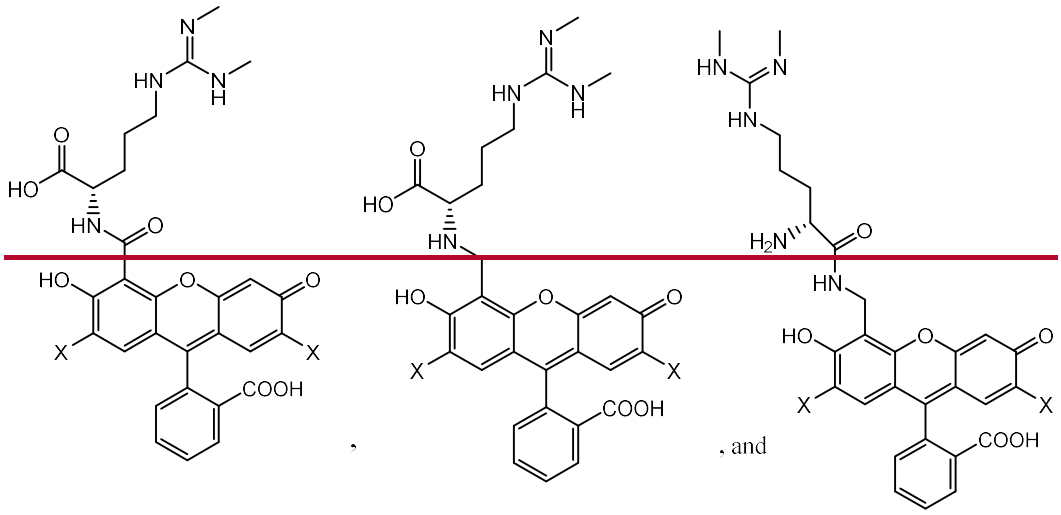
2. A complex comprising a compound of claim 1 and an antibody for symmetrical dimethyl arginine (SDMA).

3. The complex of claim 2, wherein the antibody is conjugated to a quencher.

4. The complex of claim 3, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

5. The complex of claim 4, wherein the quencher is BHQ10.

6. A compound of claim 1, selected from the group consisting of:



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

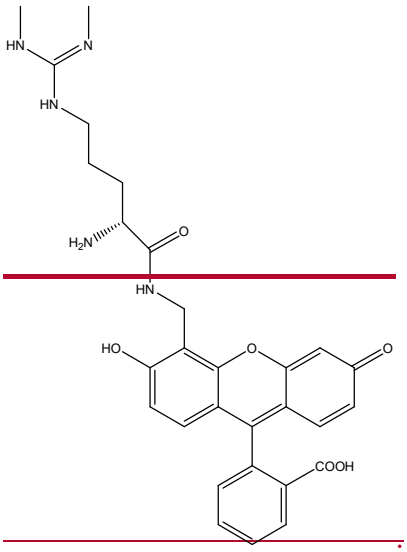
7. A complex comprising a compound of claim 6 and an antibody for symmetrical dimethyl arginine (SDMA).

8. The complex of claim 7, wherein the antibody is conjugated to a quencher.

9. The complex of claim 8, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

10. The complex of claim 9, wherein the quencher is BHQ10.

11. A compound of claim 6, having the structure:



12. A complex comprising the compound of claim 11 and an antibody for SDMA.

13. The complex of claim 12, wherein the antibody is conjugated to a quencher.

14. The complex of claim 13, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

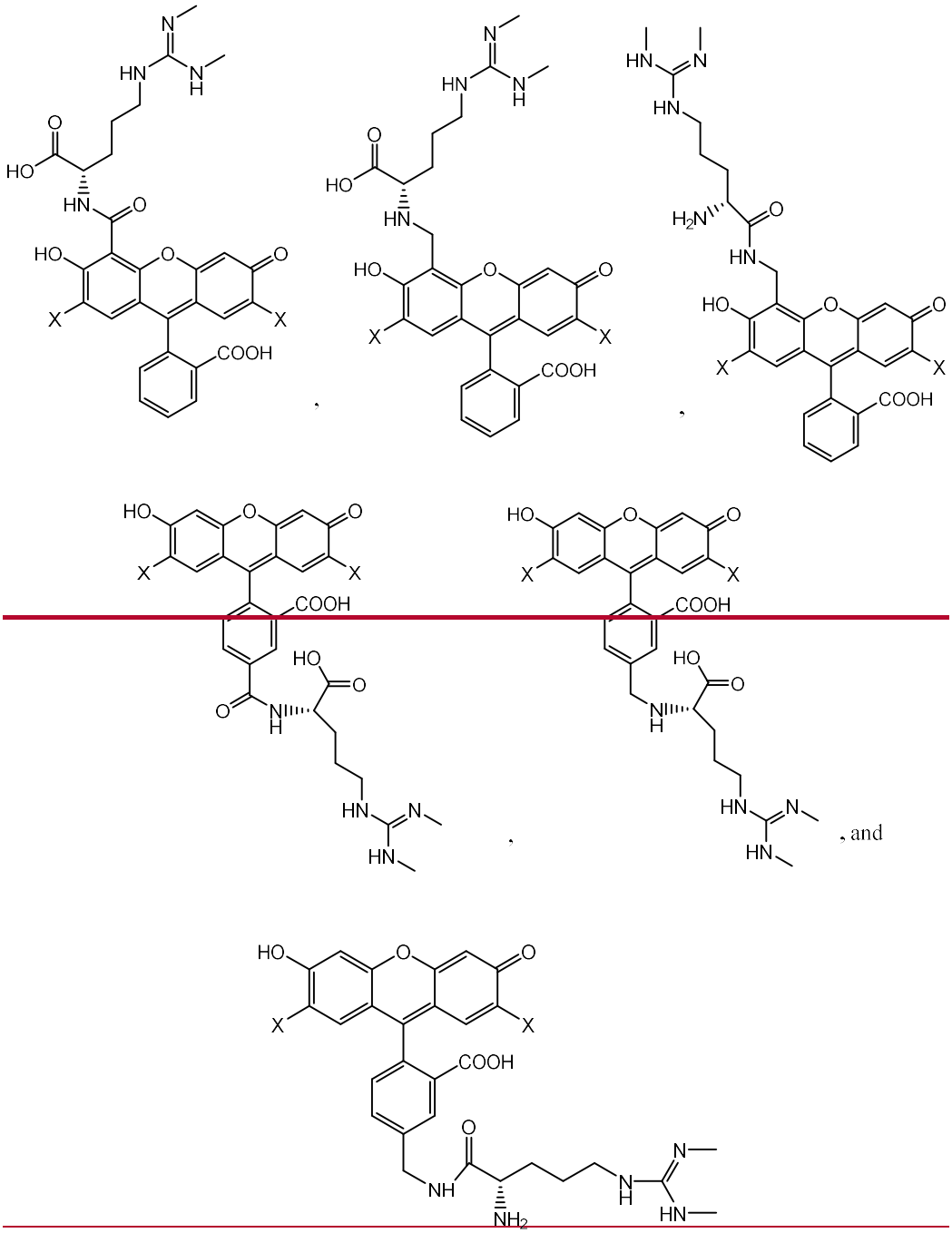
15. The complex of claim 14, wherein the quencher is BHQ10.

16. A method for determining the presence of or the amount of symmetrical dimethyl arginine (SDMA) in a sample comprising:

(i) providing a sample suspected of containing SDMA;

(ii) intermixing the sample with

(a) a fluorescent tracer 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, and

(b) an antibody for SDMA,

to provide an assay solution;

(iii) irradiating the assay solution with light that is not polarized 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 SDMA in the sample.~~

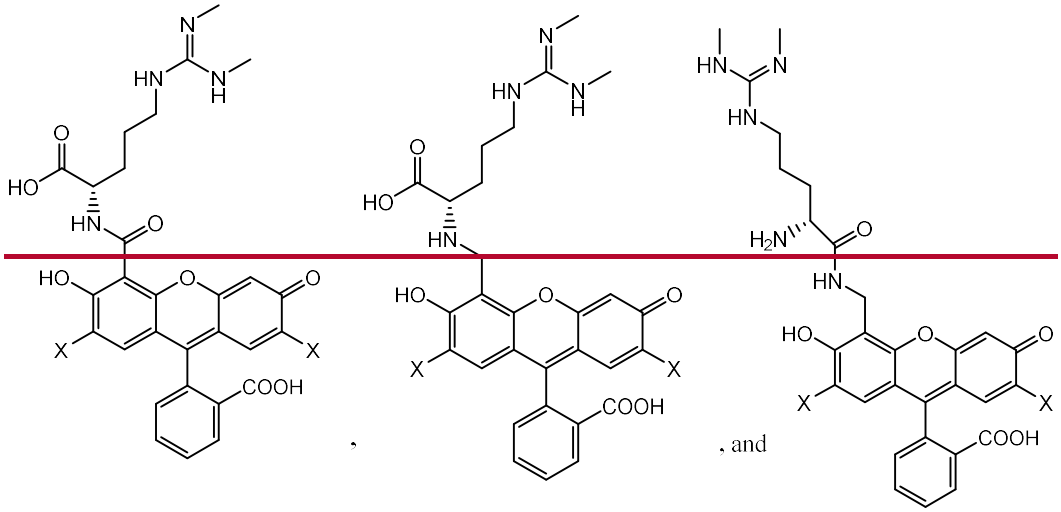
~~17. The method of claim 16, wherein the method does not involve a separation step.~~

~~18. The method of claim 16, wherein the antibody for SDMA is conjugated to a quencher.~~

~~19. The method of claim 18, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~20. The method of claim 19, wherein the quencher is BHQ10.~~

~~21. The method of claim 16, 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.~~

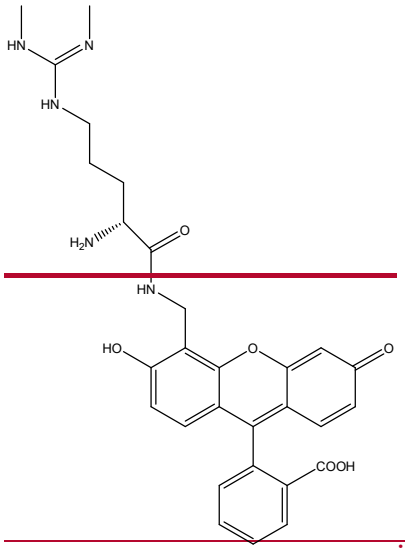
~~22. The method of claim 21, wherein the method does not involve a separation step.~~

~~23. The method of claim 21, wherein the antibody for SDMA is conjugated to a quencher.~~

24. The method of claim 21, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

25. The method of claim 24, wherein the quencher is BHQ10.

26. The method of claim 21, wherein the fluorescent tracer is:



.

27. The method of claim 26, wherein the method does not involve a separation step.

28. The method of claim 26, wherein the antibody for SDMA is conjugated to a quencher.

29. The method of claim 28, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

30. The method of claim 29, wherein the quencher is BHQ10.

31. 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) intermixing the sample with

(a) a fluorescent tracer and

~~(b) a binding partner,~~

~~wherein the binding partner is specific for the analyte and the fluorescent tracer to provide an assay solution;~~

~~(iii) irradiating the assay solution with light that is not polarized at a first wavelength; and~~

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

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

~~32. 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) intermixing the sample with~~

~~(a) a fluorescent tracer and~~

~~(b) a binding partner,~~

~~wherein the binding partner is specific for the analyte and the fluorescent tracer to provide an assay solution;~~

~~(iii) determining the amount of fluorescent tracer bound to the binding partner by irradiating the assay solution with light that has not been polarized at a first wavelength and measuring the intensity of light emitted at a second wavelength.~~

~~33. The method of claim 31 or 32, wherein the analyte is selected from the group consisting of melamine, antibiotics, T3, T4, penicillins, sulfa drugs, cephalosporins, progesterone, cortisol, and cystatin B.~~

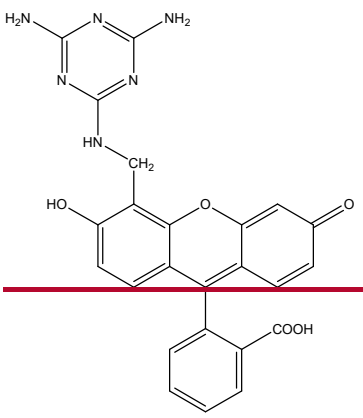
~~34. The method of claim 31 or 32, wherein the binding partner is an antibody against the analyte.~~

~~35. The method of claim 31 or 32, wherein the method does not involve a separation step.~~

~~36. The method of claim 31 or 32, wherein the method uses a dry slide assay.~~

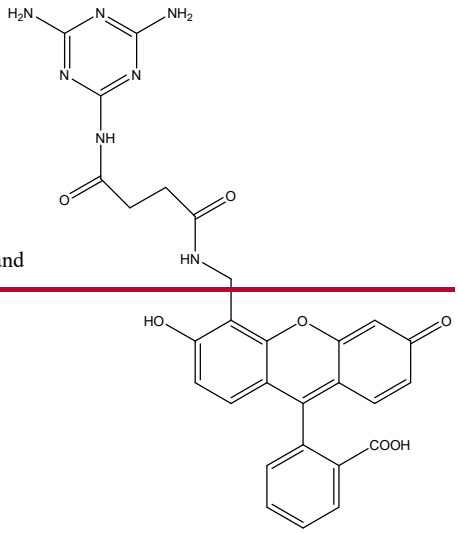
~~37. The method of claim 31 or 32, wherein the fluorescent tracer is selected from the group consisting of a 4'-substituted fluorescein tracer, a 4'- substituted fluorescein tracer derivative, a 5- substituted fluorescein tracer, and a 5- substituted fluorescein tracer derivative.~~

~~38. The method of claim 31 or 32, wherein the analyte is melamine, the fluorescent tracer is selected from the group consisting of:~~



Mel-F

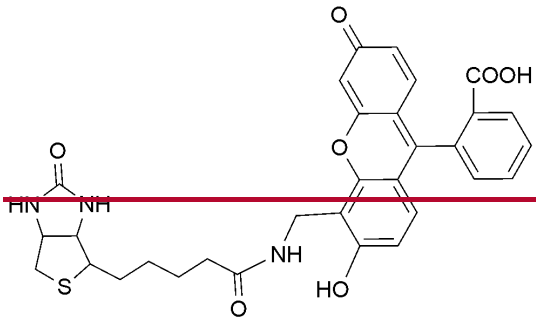
and



Mel-Su-F

~~and the binding partner is an antibody against melamine.~~

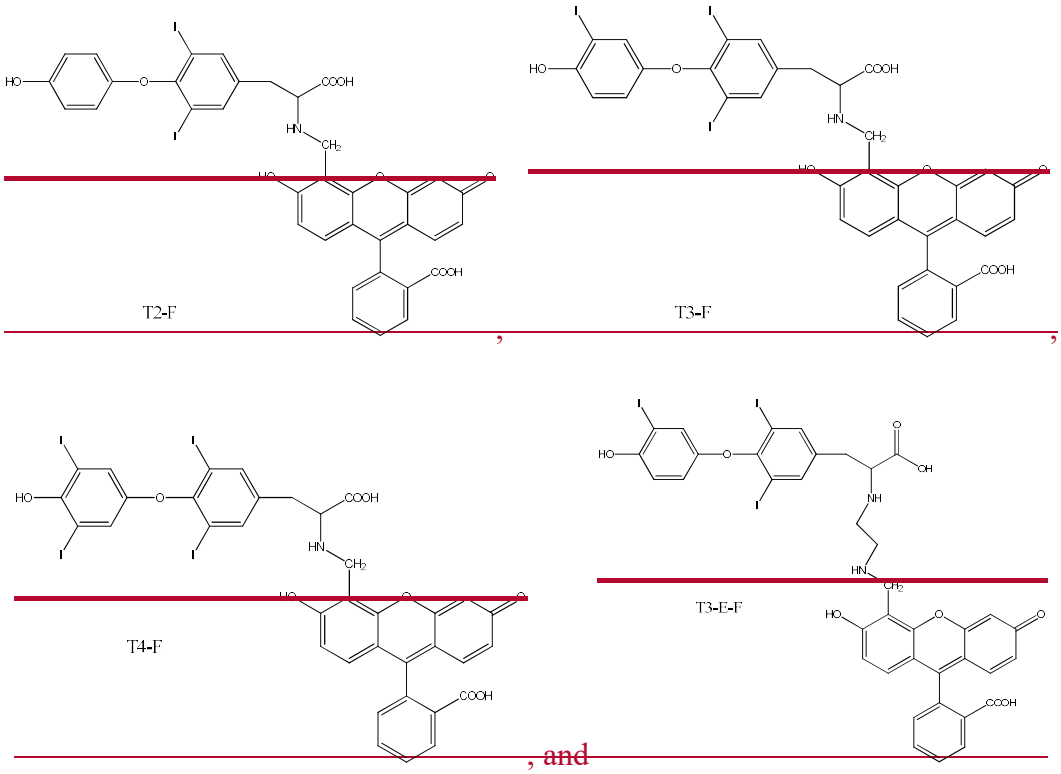
~~39. The method of claim 31 or 32, wherein the analyte is biotin, the fluorescent tracer is:~~



Biotin-F

and the binding partner is an antibody against biotin.

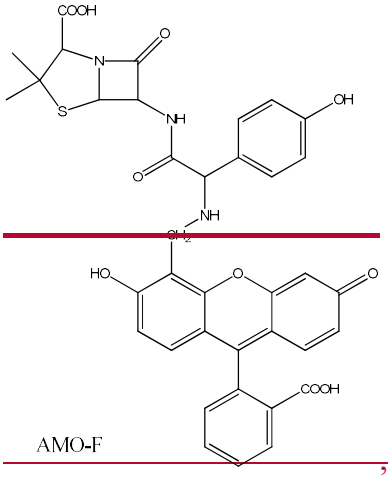
40. The method of claim 31 or 32, wherein the analyte is thyroxine, the fluorescent tracer is selected from the group consisting of:



T2-F, T3-F, T4-F, T3-E-F, and

and the binding partner is an antibody against thyroxine.

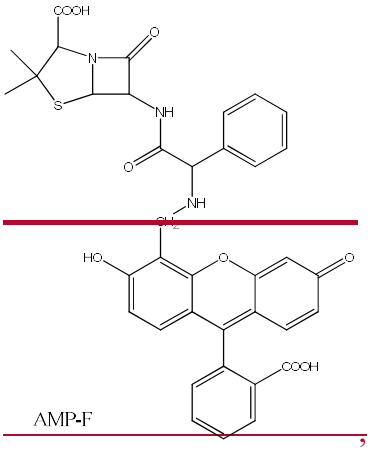
41. The method of claim 31 or 32, wherein the analyte is amoxicillin, the fluorescent tracer is:



AMO-F,

and the binding partner is an antibody against amoxicillin.

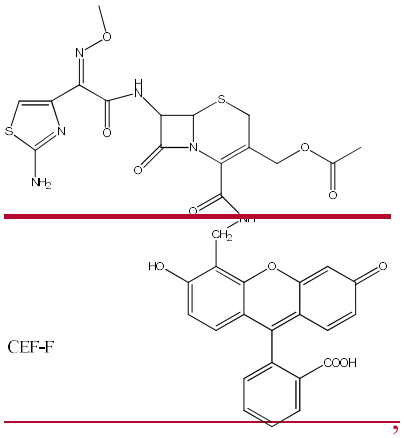
42. The method of claim 31 or 32, wherein the analyte is ampicillin, the fluorescent tracer is:



AMP-F,

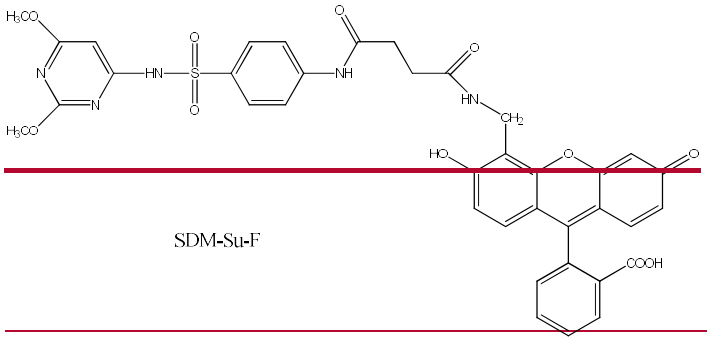
and the binding partner is an antibody against ampicillin.

43. The method of claim 31 or 32, wherein the analyte is cefotaxime, the fluorescent tracer is:

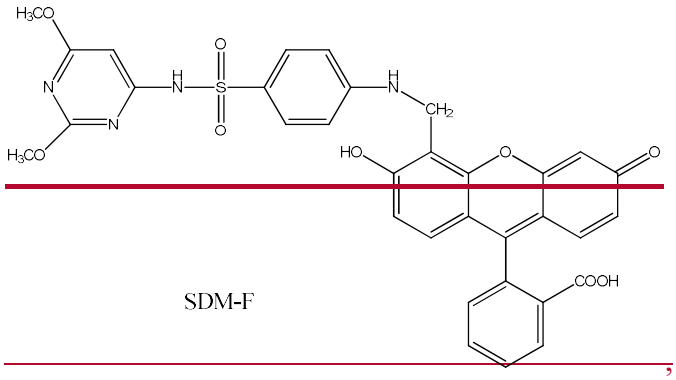


and the binding partner is an antibody against cefotaxime.

44. The method of claim 31 or 32, wherein the analyte is sulfadimethoxine and the fluorescent tracer is selected from the group consisting of:

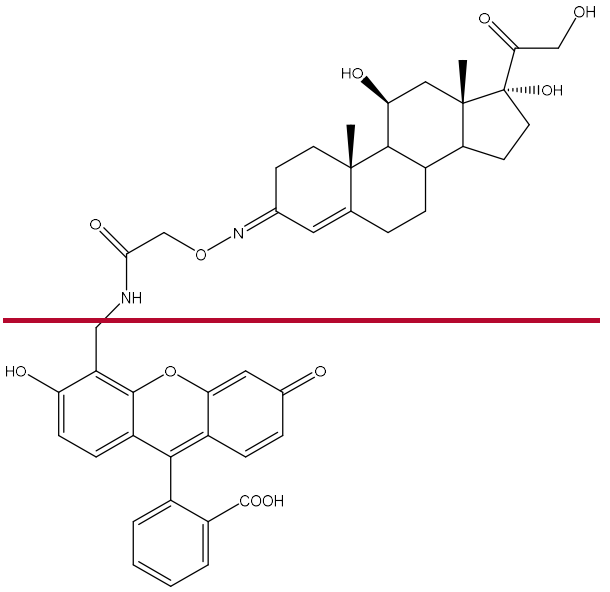


and



and the binding partner is an antibody against msulfadimethoxine.

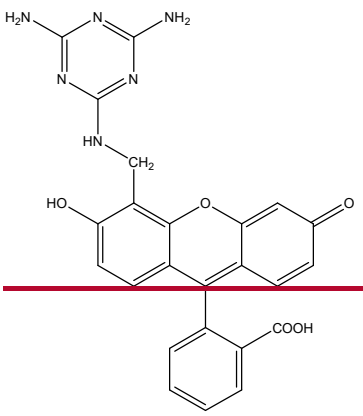
45. The method of claim 31 or 32, wherein the analyte is cortisol, the fluorescent tracer is:



Cortisol-4-FI,

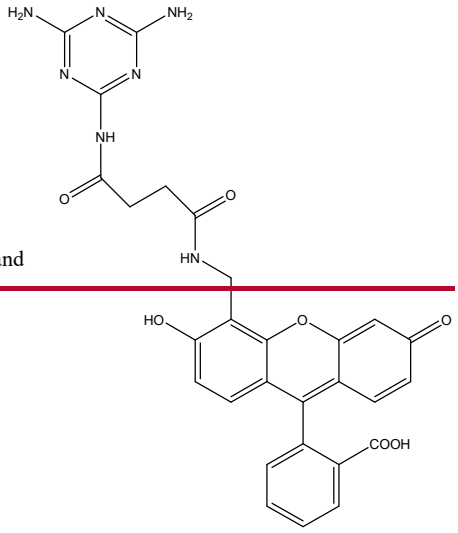
and the binding partner is an antibody against cortisol.

46. A compound selected from the group consisting of:



Mel-F

and



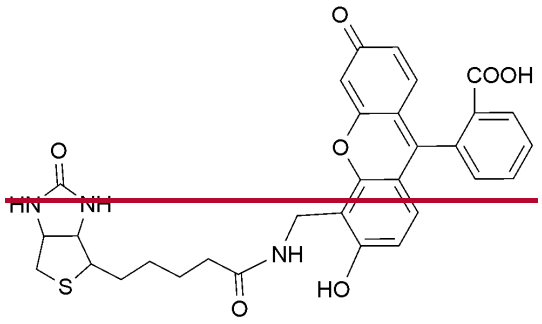
Mel-Su-F.

47. A complex comprising the compound of claim 46 and an antibody against melamine.

48. The complex of claim 47, wherein the antibody is conjugated to a quencher.

49. The complex of claim 48, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

50. A compound of formula:



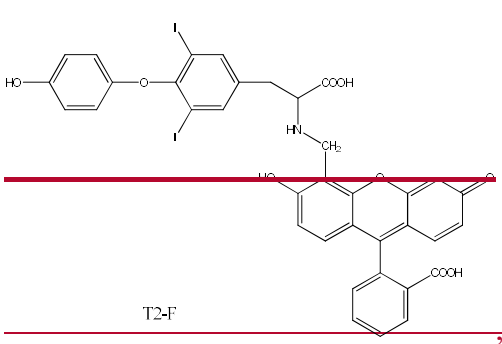
Biotin-F .

51. A complex comprising the compound of claim 50 and an antibody against biotin.

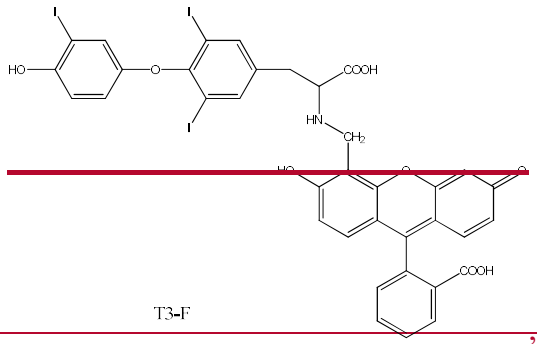
52. The complex of claim 51, wherein the antibody is conjugated to a quencher.

53. The complex of claim 52, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

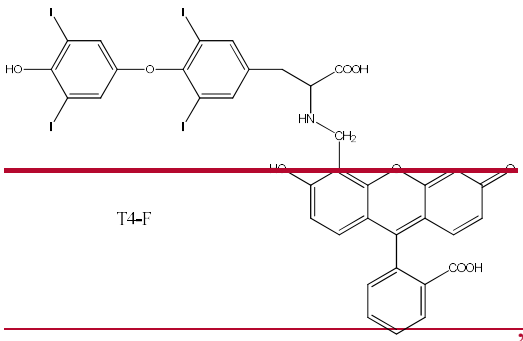
54. A compound selected from the group consisting of:



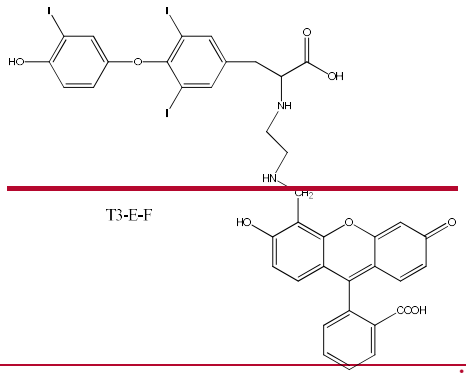
T2-F ,



T3-F ,



T4-F



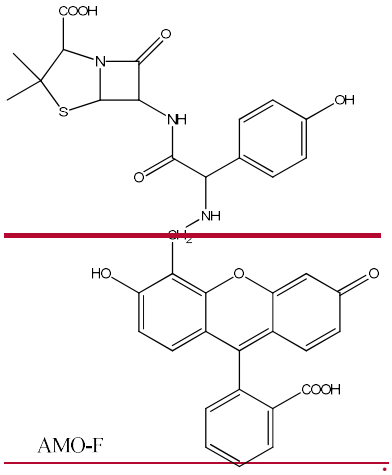
T3-E-F , and .

~~55. A complex comprising a compound of claim 54 and an antibody against thyroxine.~~

~~56. The complex of claim 55, wherein the antibody is conjugated to a quencher.~~

~~57. The complex of claim 56, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~58. A compound of formula:~~



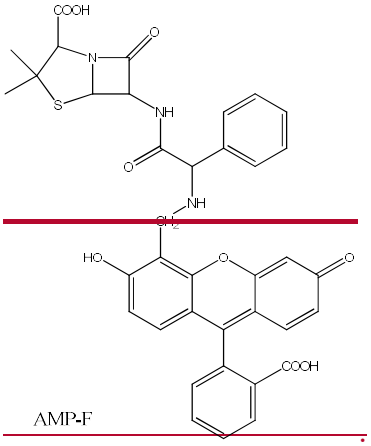
AMO-F.

~~59. A complex comprising the compound of claim 58 and an antibody against amoxicillin.~~

~~60. The complex of claim 59, wherein the antibody is conjugated to a quencher.~~

~~61. The complex of claim 60, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~62. A compound of formula:~~



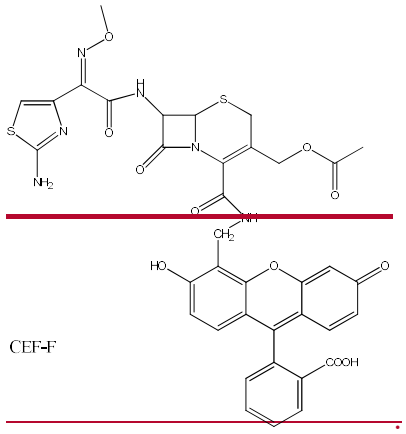
AMP-F

~~63. A complex comprising the compound of claim 62 and an antibody against ampicillin.~~

~~64. The complex of claim 63, wherein the antibody is conjugated to a quencher.~~

~~65. The complex of claim 64, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~66. A compound of formula:~~



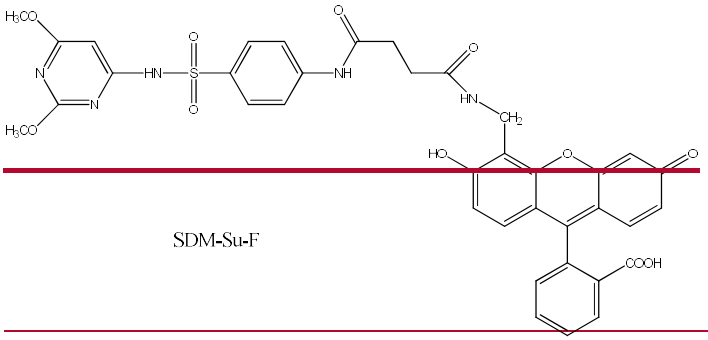
CEF-F

~~67. A complex comprising the compound of claim 66 and an antibody against cefotaxime.~~

~~68. The complex of claim 67, wherein the antibody is conjugated to a quencher.~~

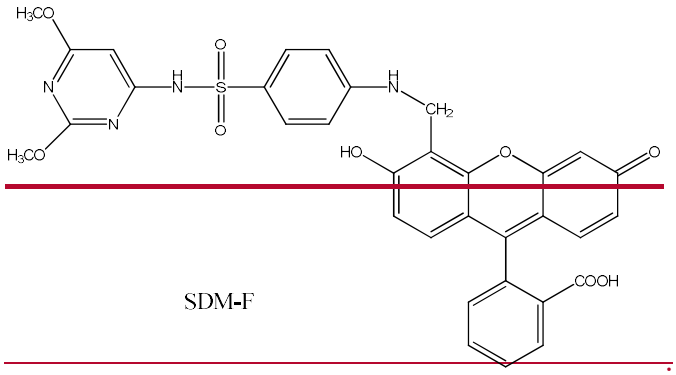
~~69. The complex of claim 68, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~70. A compound selected from the group consisting of:~~



SDM-Su-F

and



SDM-F

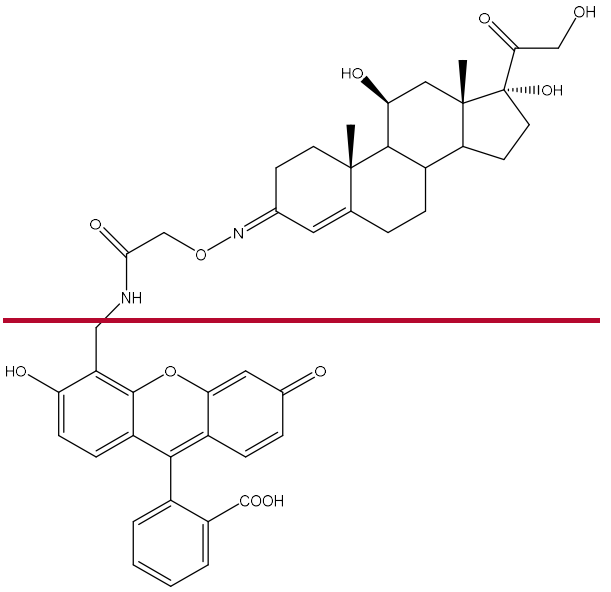
.

71. A complex comprising a compound of claim 70 and an antibody against sulfadimethoxine.

72. The complex of claim 71, wherein the antibody is conjugated to a quencher.

73. The complex of claim 72, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

74. A compound of formula:



Cortisol-4-Fl.

75. A complex comprising the compound of claim 74 and an antibody against cortisol.

76. The complex of claim 75, wherein the antibody is conjugated to a quencher.

77. The complex of claim 76, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

78. The method of claim 31 or 32, wherein the binding partner is conjugated to a quencher.

79. The method of claim 78, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

80. A slide comprising a first layer and a second layer, wherein

the first layer comprises a first polymer selected from the group consisting of polystyrene, polyesters, polycarbonates, cellulose derivatives, polyethylene terephthalate, and mixtures thereof, and

the second layer comprises a second polymer with a fluorescent tracer and a binding partner dispersed therein,

wherein the binding partner is specific for the fluorescent tracer.

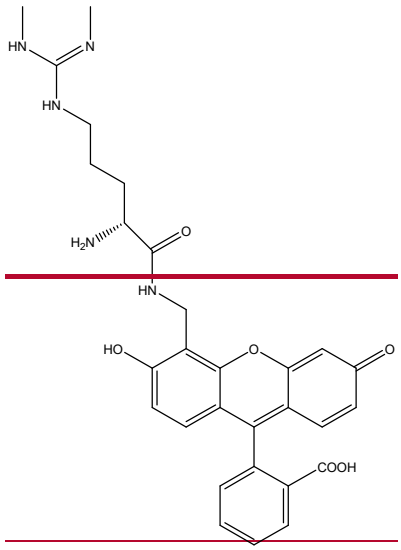
81. The slide of claim 80, wherein the second layer comprises pullulan.

82. The slide of claim 80, wherein the second layer comprises cellulose in an amount ranging from about 2% by wt. to about 40% by wt. and pullulan in an amount ranging from about 0.5% by wt. to about 40% by wt. of the second layer.

83. The slide of claim 80, wherein the thickness of the first layer ranges from about 15 µm to about 200 µm and the thickness of the second layer ranges from about 30 µm to about 200 µm.

84. The slide of claim 80, wherein binding partner is an antibody, and the concentration of the fluorescent tracer in the second layer ranges from about 1 x 10^{-5} % by wt. to about 1 x 10^{-1} % by wt. of the second layer, and the concentration of antibody ranges from about 1 x 10^{-4} % by wt. to about 1 % by wt. of the second layer.

85. The slide of claim 80, wherein the fluorescent tracer is:



and the binding partner is an antibody against SDMA.

86. The slide of claim 80, further comprising a third layer comprising a third polymer selected from the group consisting of polyurethane, cellulose, and mixtures thereof, wherein the third layer is coated on top of the second layer.

87. The slide of claim 86, wherein the polyurethane is present in an amount ranging from about 40% by wt. to about 95% by wt. and the cellulose is present in an amount ranging from about 5% by wt. to about 60% by wt. of the third layer.

~~88. The slide of claim 86, wherein the thickness of the third layer ranges from about 5 µm to about 50 µm.~~

~~89. The slide of claim 86, further comprising a fourth layer comprising cellulose and a hydrophilic polymer, wherein the fourth layer is coated on top of the third layer.~~

~~90. The slide of claim 89, wherein the hydrophilic polymer is selected from the group consisting of polyacrylic acid, polyvinylpyrrolidone, polythylene glycol, polyethylene oxide, polyvinyl alcohol, polyacrylamides, polethylenimines, and mixtures thereof.~~

~~91. The slide of claim 90, wherein the fourth layer further comprises polyvinylpyrrolidone.~~

~~92. The slide of claim 89, wherein the cellulose is present in an amount ranging from about 50% by wt. to about 98% by wt.~~

~~93. The slide of claim 91, wherein the cellulose is present in an amount ranging from about 50% by wt. to about 98% by wt. and the polyvinylpyrrolidone is present in an amount ranging from about 1% by wt. to about 30% by wt. of the fourth layer.~~

~~94. The slide of claim 86, wherein the thickness of the fourth layer ranges from about 25 µm to about 250 µm~~

~~95. The slide of claim 80, 86, and 89, further comprising a fifth layer comprising polyurethane, wherein the fifth layer is positioned between the first layer and the second layer.~~

~~96. The slide of claim 95, wherein the thickness of the fifth layer ranges from about 1 µm to about 20 µm.~~

~~97. The slide of claim 95, further comprising a sixth layer comprising carbon black dispersed in a sixth polymer selected from the group consisting of polyurethane, polyethylene oxide, silicones, polyvinyl alcohol, polyacrylamides, and mixtures thereof, wherein the sixth layer is positioned between the third layer and the fourth layer.~~

~~98. The slide of claim 97, wherein the amount of carbon black ranges from about 10% by wt. to about 40% by wt. of the sixth layer.~~

~~99. The slide of claim 97, wherein the sixth layer has a thickness ranging from about 5 µm to about 30 µm.~~

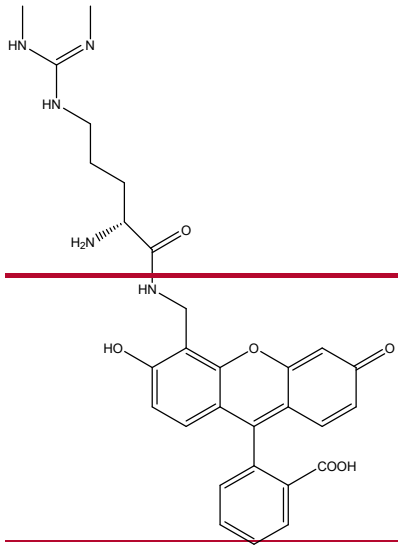
~~100. The slide of claim 95, further comprising a seventh layer comprising titanium oxide dispersed in a seventh polymer selected from the group consisting of polyurethane, polyethylene oxide, silicones, polyvinyl alcohol, polyacrylamides, and mixtures thereof, wherein the seventh layer is positioned between the second layer and the third layer.~~

~~101. The slide of claim 100, wherein the titanium oxide is present in an amount ranging from about 20% by wt. to about 60% by wt. of the seventh layer.~~

~~102. The slide of claim 100, wherein the thickness of the seventh layer ranges from about 50 µm to about 250 µm.~~

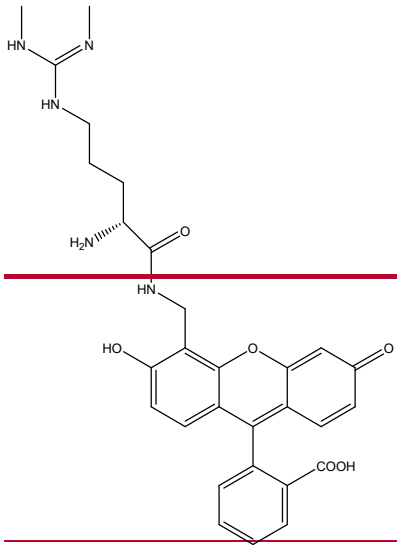
~~103. The slide of claim 100, wherein the titanium oxide is provided as particles having an average particle size of less than about 20 µm.~~

~~104. The slide of claim 86, wherein the fluorescent tracer is:~~



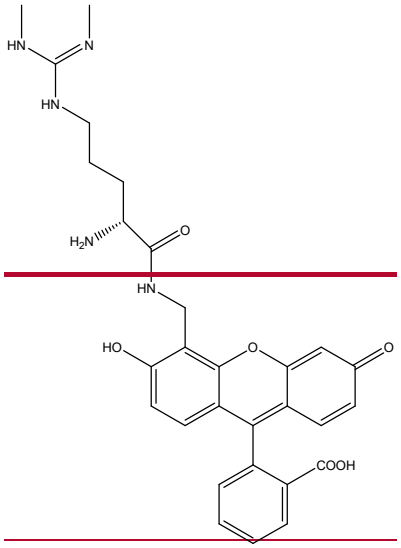
~~and the binding partner is an antibody against SDMA.~~

~~105. The slide of claim 89, wherein the fluorescent tracer is:~~



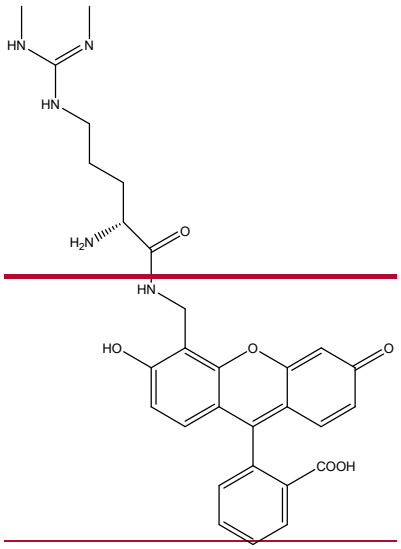
and the binding partner is an antibody against SDMA.

106. The slide of claim 95, wherein the fluorescent tracer is:



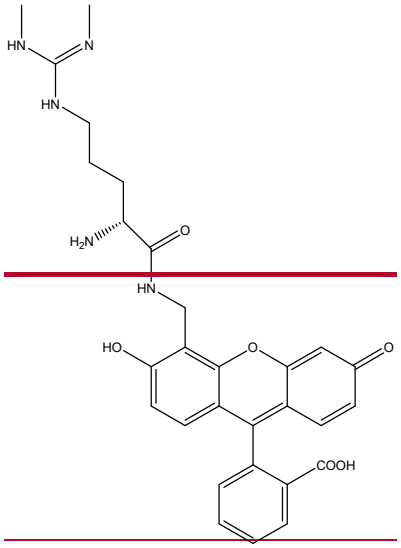
and the binding partner is an antibody against SDMA.

107. The slide of claim 97, wherein the fluorescent tracer is:



and the binding partner is an antibody against SDMA.

108. The slide of claim 100, wherein the fluorescent tracer is:



and the binding partner is an antibody against SDMA.

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

depositing a sample on a top layer of a slide of anyone of claims 80-108, wherein the top layer of the slide is the layer farthest from the first layer;

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

~~measuring the intensity of the light emitted at the second wavelength from the first layer of the slide,~~

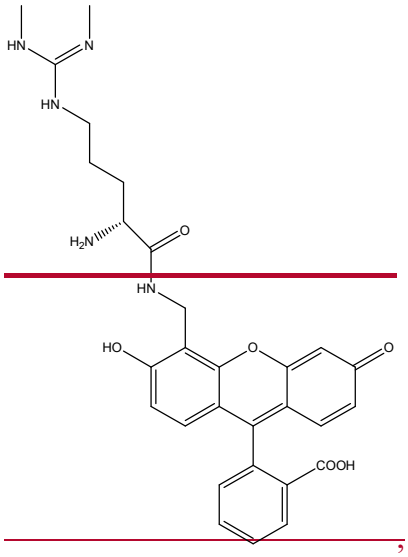
~~wherein the intensity of the light emitted at a second wavelength is proportional to the concentration of the analyte in the sample,~~

~~wherein the first layer of the slide is optically clear to the first wavelength and the second wavelength, and~~

~~wherein the binding partner is specific for the analyte.~~

~~110. The method of claim 109, wherein the method does not involve a separation step.~~

~~111. The method of claim 109, wherein the analyte is SDMA, the fluorescent tracer is:~~



~~and the binding partner is an antibody against SDMA.~~

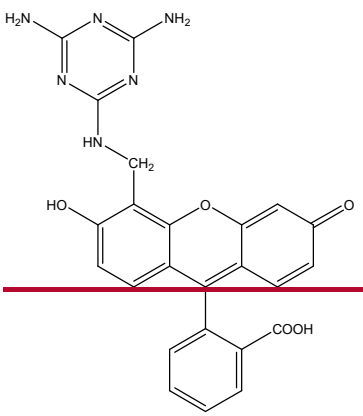
~~112. The method of claim 111, wherein the antibody against SDMA is conjugated to a quencher.~~

~~113. The method of claim 112, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~114. The method of claim 113, wherein the quencher is BHQ10.~~

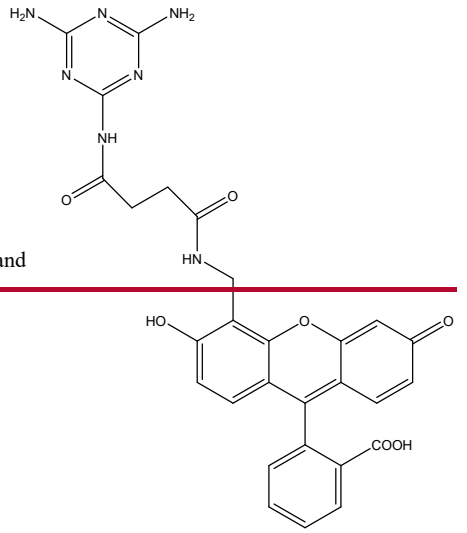
115. The method of claim 111, wherein the first wavelength is about 490 nm and the second wavelength is about 520 nm.

116. The method of claim 109, wherein the analyte is melamine, the fluorescent tracer is selected from the group consisting of:



Mel-F

and



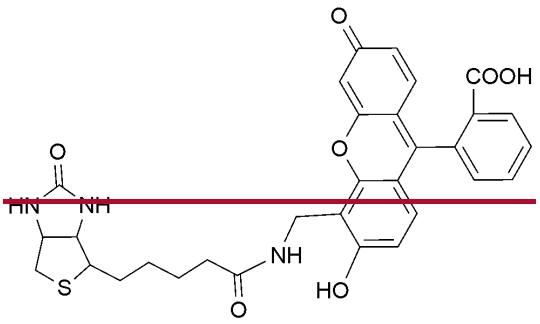
Mel-Su-F,

and the binding partner is an antibody against melamine.

117. The method of claim 116, wherein the antibody against melamine is conjugated to a quencher.

118. The method of claim 117, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

119. The method of claim 109, wherein the analyte is biotin, the fluorescent tracer is:



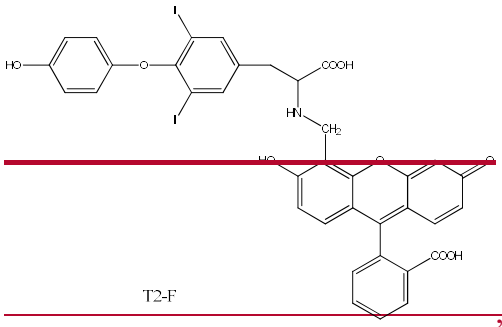
Biotin-F

and the binding partner is an antibody against biotin.

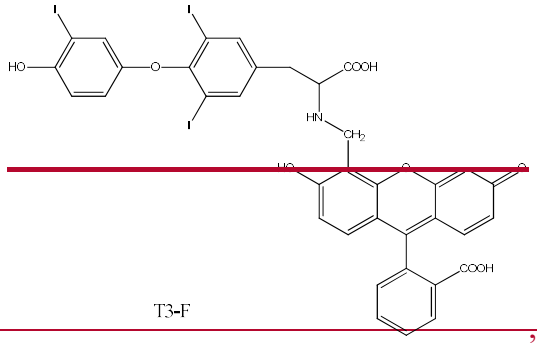
120. The method of claim 119, wherein the antibody against biotin is conjugated to a quencher.

121. The method of claim 120, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

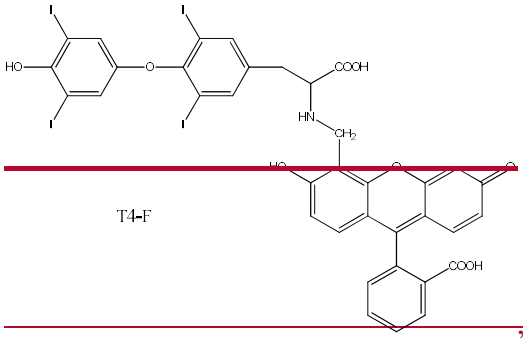
122. The method of claim 109, wherein the analyte is thyroxine, the fluorescent tracer is selected from the group consisting of:



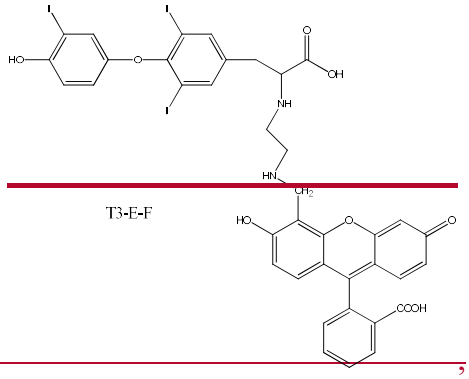
,



,



, and



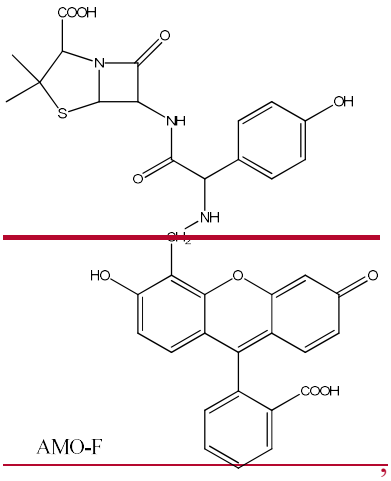
,

and the binding partner is an antibody against thyroxine.

123. The method of claim 122, wherein the antibody against thyroxine is conjugated to a quencher.

124. The method of claim 123, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

125. The method of claim 109, wherein the analyte is amoxicillin, the fluorescent tracer is:



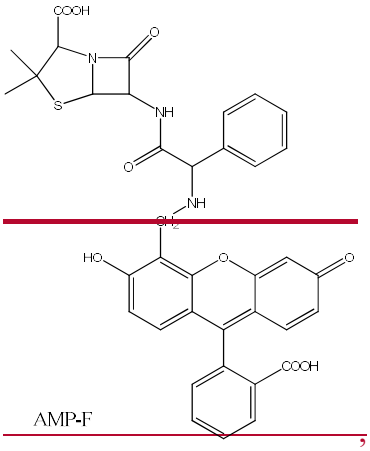
AMO-F,

~~and the binding partner is an antibody against amoxicillin.~~

~~126. The method of claim 125, wherein the antibody against amoxicillin is conjugated to a quencher.~~

~~127. The method of claim 126, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~128. The method of claim 109, wherein the analyte is ampicillin, the fluorescent tracer is:~~



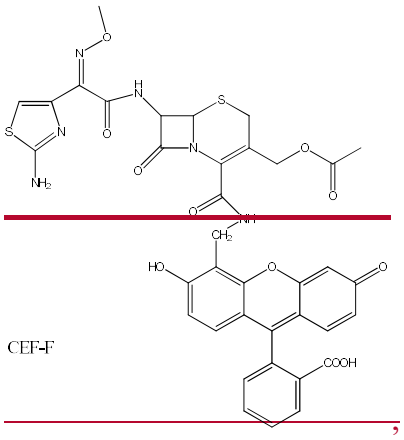
AMP-F,

~~and the binding partner is an antibody against ampicillin.~~

~~129. The method of claim 128, wherein the antibody against ampicillin is conjugated to a quencher.~~

~~130. The method of claim 129, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~131. The method of claim 109, wherein the analyte is cefotaxime, the fluorescent tracer is:~~



CEF-F

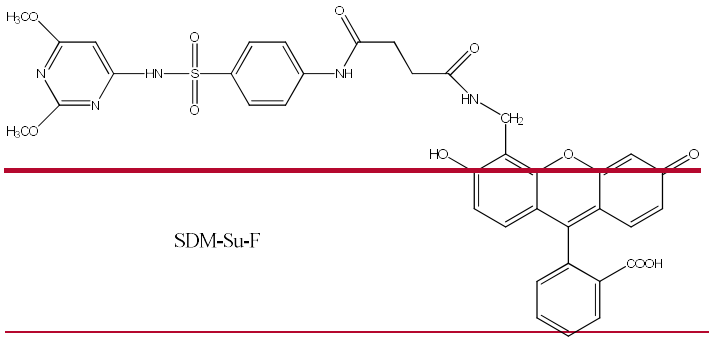
~~,~~

~~and the binding partner is an antibody against cefotaxime.~~

~~132. The method of claim 131, wherein the antibody against cefotaxime is conjugated to a quencher.~~

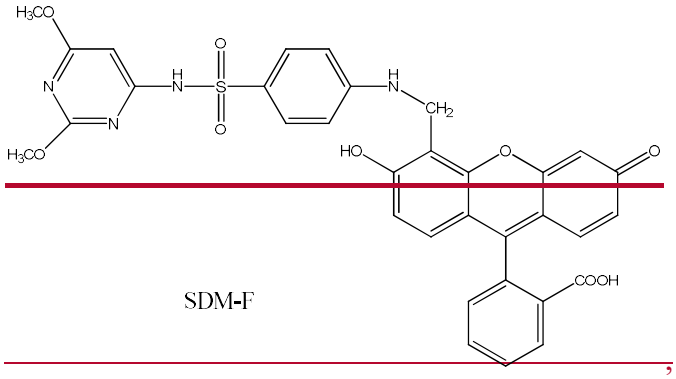
~~133. The method of claim 132, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~134. The method of claim 109, wherein the analyte is sulfadimethoxine, the fluorescent tracer is selected from the group consisting of:~~



SDM-Su-F

~~and~~



SDM-F

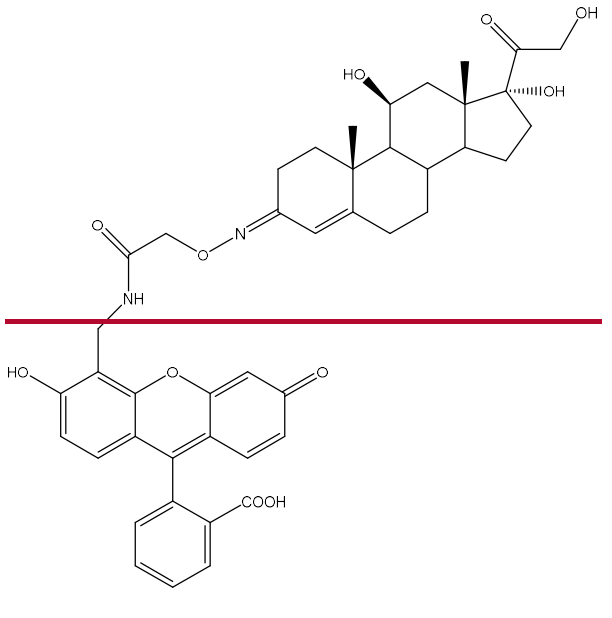
,

and the binding partner is an antibody against sulfadimethoxine.

135. The method of claim 134, wherein the antibody against sulfadimethoxine is conjugated to a quencher.

136. The method of claim 135, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

137. The method of claim 109, wherein the analyte is cortisol, the fluorescent tracer is:



Cortisol-4-Fl

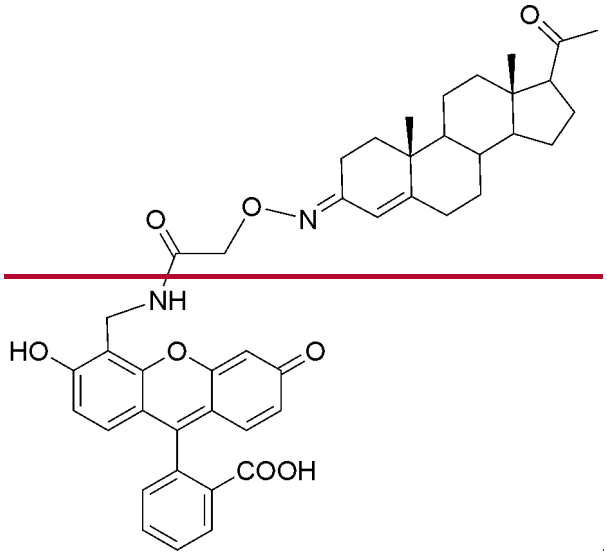
,

and the binding partner is an antibody against cortisol.

138. The method of claim 137, wherein the antibody against cortisol is conjugated to a quencher.

~~139. The method of claim 138, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~140. The method of claim 109, wherein the analyte is progesterone, the fluorescent tracer is:~~



~~and the binding partner is an antibody against progesterone.~~

~~141. The method of claim 140, wherein the antibody against progesterone is conjugated to a quencher.~~

~~142. The method of claim 141, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.~~

~~143. A slide comprising in sequential order:~~

~~(i) a first layer comprising polyester,~~

~~(ii) a second layer comprising a polyurethane,~~

~~(ii) a third layer comprising:~~

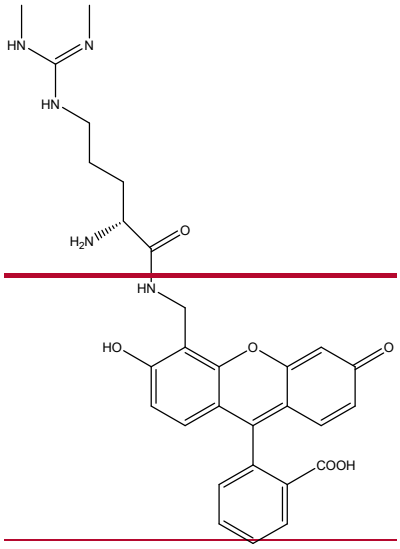
~~(a) cellulose in an amount of about 57% by wt. of the second layer,~~

~~(b) pullulan in an amount of about 28% by wt. of the second layer,~~

(c) a HEPES buffer in an amount of about 13% by wt. of the second layer, wherein an aqueous solution of the HEPES buffer has a pH of about 8,

(d) a non-ionic surfactant in an amount of about 1.17% by wt. of the second layer,

(e) a fluorescent tracer having the structure:



in an amount of about 1.0×10^{-3}% by wt. of the second layer, and

(f) an antibody against SDMA in an amount of about 1.4×10^{-2}% by wt. of the second layer.

(iv) a fourth layer comprising cellulose in an amount of about 20% by wt. of the fourth layer, polyurethane in an amount of about 38% by wt. of the fourth layer, and titanium dioxide in an amount of about 42% by wt. of the fourth layer

(v) a fifth layer comprising carbon black in an amount of about 24% by wt. of the fifth layer dispersed in polyurethane in an amount of about 76% by wt. of the fifth layer, and

(vi) a sixth layer comprising cellulose in an amount of about 83% by wt. of the sixth layer, tetramethyl ammonium hydroxide in an amount of about 0.4% by wt. of the sixth layer, polyacrylic acid in an amount of about 0.5% by wt. of the sixth layer, and polyvinyl pyrrolidone in an amount of about 16% by wt. of the sixth layer.

~~144. The slide of claim 143, wherein the thickness of the first layer is about 75 µm, the thickness of the second layer is about 4 µm, the thickness of the third layer is about 35 µm, the thickness of the fourth layer is about 20 µm, the thickness of the fifth layer is about 14 µm, and the thickness of the sixth layer is about 100 µm.~~

~~145. A method for determining the presence of or the amount of symmetrical dimethyl arginine (SDMA) in a sample comprising:~~

~~(i) providing a sample suspected of containing SDMA,~~

~~(ii) contacting the sample with the sixth layer of the slide of claim 144 or 145,~~

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

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

~~146. The method of claim 145, wherein the first wavelength is about 490 nm and the second wavelength is about 520 nm.~~

~~147. The slide of claim 80, wherein the fluorescent tracer and a binding partner are present as complex comprising the fluorescent tracer and a binding partner.~~

~~148. The slide of anyone of claims 80, 86, and 89, 95, or 100 wherein the fluorescent tracer is a 4'-substituted fluorescein tracer.~~

~~149. The method of claim 109, wherein the fluorescent tracer s a 4'-substituted fluorescein tracer.~~

~~150. The method of claim 31 or 32, wherein the fluorescent tracer s a 4'-substituted fluorescein tracer.~~

~~151. A matrix comprising (i) a complex comprising a fluorescent tracer and a binding partner and (ii) a polymer comprising pullulan, wherein the complex is dispersed in the polymer.~~

~~152. The matrix of claim 151, wherein the polymer further comprises cellulose.~~

~~153. The matrix of claim 151, wherein the matrix comprises pullulan in an amount of about 1% by wt. to about 60% by wt. of the matrix.~~

~~154. The matrix of claim 152 or 153, wherein the cellulose is present in an amount of about 30% by wt. to about 90% by wt.~~

~~155. The matrix of anyone of claims 151-154, wherein the matrix is substantially free of water.~~

~~156. The method of claim 109, wherein the analyte is a protein, the fluorescent tracer is a fluorescent moiety attached to the protein; and the binding partner is an antibody against the protein.~~

~~157. The method of claim 156, wherein the antibody against the protein is conjugated to a quencher.~~

~~158. The method of claim 157, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, BHQ10, and BODIPY.~~

~~159. The method of claim 158, wherein the quencher is selected from the group consisting of BHQ10 and BODIPY.~~

~~160. The method of claim 109, wherein the analyte is cystatin-B, the fluorescent tracer is a fluorescent moiety attached to the cystatin-B; and the binding partner is an anti-cystatin-B antibody.~~

~~161. The method of claim 160, wherein the anti-cystatin-B antibody is conjugated to a quencher.~~

~~162. The method of claim 161, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, BHQ10, and BODIPY.~~

~~163. The method of claim 162, wherein the quencher is selected from the group consisting of BHQ10 and BODIPY.~~

~~164. The method of claim 160, wherein the fluorescent moiety is fluorescein.~~

~~165. The method of claim 164, wherein the fluorescein is conjugated to the cystatin-B through one or more lysine residues.~~

~~166. The method of claim 164, wherein the fluorescein is conjugated to the cystatin-B through one or more cysteine residues.~~

~~167. The method of claim 160, wherein the fluorescent moiety is CysB-Peptide 9 conjugated to fluorescein.~~

~~168. The method of claim 167, wherein the fluorescein is conjugated to the CysB-Peptide 9 through one or more lysine residues.~~

~~169. The method of claim 164 , wherein the binding partner is an anti-cystatin-B antibody that is conjugated to one or more BHQ10 molecules or to one or more boron-dipyrromethene (BODIPY) molecules.~~

~~170. The method of claim 165 , wherein the binding partner is an anti-cystatin-B antibody that is conjugated to one or more BHQ10 molecules or to one or more boron-dipyrromethene (BODIPY) molecules.~~

~~171. The method of claim 166 , wherein the binding partner is an anti-cystatin-B antibody that is conjugated to one or more BHQ10 molecules or to one or more boron-dipyrromethene (BODIPY) molecules.~~

~~172. The method of claim 167 , wherein the binding partner is an anti-cystatin-B antibody that is conjugated to one or more BHQ10 molecules or to one or more boron-dipyrromethene (BODIPY) molecules.~~

~~173. The method of claim 168 , wherein the binding partner is an anti-cystatin-B antibody that is conjugated to one or more BHQ10 molecules or to one or more boron-dipyrromethene (BODIPY) molecules.~~

~~174. The method of claim 109, wherein the analyte is a protein, the fluorescent tracer is a fluorescent moiety attached to an anti-protein antibody; and the binding partner is the protein conjugated to one or more quenchers.~~

~~175. The method of claim 174, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, BHQ10, and BODIPY.~~

~~176. The method of claim 175, wherein the quencher is selected from the group consisting of BHQ10 and BODIPY.~~

~~177. The method of claim 109, wherein the analyte is cystatin-B, the fluorescent tracer is a fluorescent moiety attached to an anti-C cystatin-B antibody; and the binding partner is cystatin-B that is conjugated to one or more quenchers.~~

~~178. The method of claim 177, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, BHQ10, and BODIPY.~~

~~179. The method of claim 178, wherein the quencher is selected from the group consisting of BHQ10 and BODIPY.~~

~~180. The method of claim 177, wherein the fluorescent tracer is obtained by reacting an anti-CysB antibody with fluorescein-NHS or fluorescein aldehyde.~~

~~181. The method of claim 178, wherein the fluorescent tracer is obtained by reacting an anti-CysB antibody with fluorescein-NHS or fluorescein aldehyde.~~

~~182. The method of claim 181, wherein the fluorescent tracer is obtained by reacting an anti-CysB antibody with fluorescein-NHS or fluorescein aldehyde.~~

~~183~~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.

~~184~~2. The method of claim ~~183~~1, wherein the analyte is a macromolecule.

~~185~~3. The method of claim ~~184~~2, wherein the analyte is a protein.

~~1864~~. The method of claim ~~1853~~, 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.

~~1875~~. The method of claim ~~1864~~, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

~~1886~~. The method of claim ~~1864~~, wherein the binding partner is an antibody.

~~1897~~. The method of claim ~~1864~~, wherein the protein is selected from the group consisting of canine CysB protein and canine NT-proBNP.

~~1908~~. The method of claim ~~1897~~, wherein the protein is canine CysB protein.

~~1919~~. The method of claim ~~1908~~, 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.

~~19210~~. The method of claim ~~1919~~, wherein the amino acid sequence is attached to the 5-position of the fluorescein.

~~19311~~. The method of claim ~~19210~~, wherein the amino acid sequence is attached to the 5-position of the fluorescein with a -NH-C(O)- linker.

~~19412~~. The method of claim ~~19210~~, wherein the anti-cystatin-B antibody is conjugated to a quencher.

~~19513~~. The method of claim ~~1919~~, wherein the amino acid sequence is attached to the 4’-position of the fluorescein.

~~19614~~. The method of claim ~~19513~~, wherein the amino acid sequence is attached to the 4’-position of the fluorescein with a -CH_2- linker.

~~197~~15. The method of claim ~~195~~13, wherein the anti-cystatin-B antibody is conjugated to a quencher.

~~198~~16. The method of claim ~~186~~4, wherein the amino acid sequence is attached to the 5-position of the fluorescein.

~~199~~17. The method of claim ~~198~~16, wherein the amino acid sequence is attached to the 5-position of the fluorescein with a -NH-C(O)- linker.

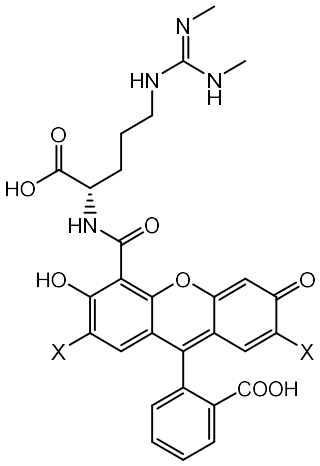
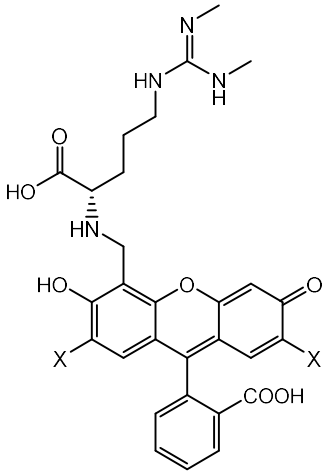
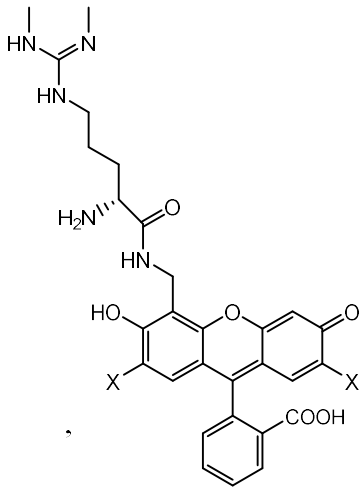
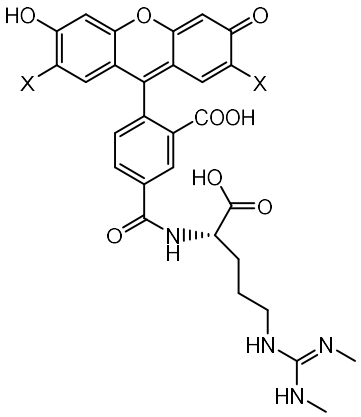
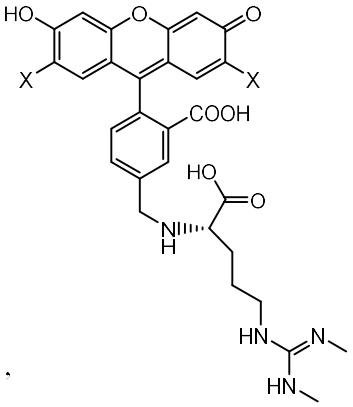
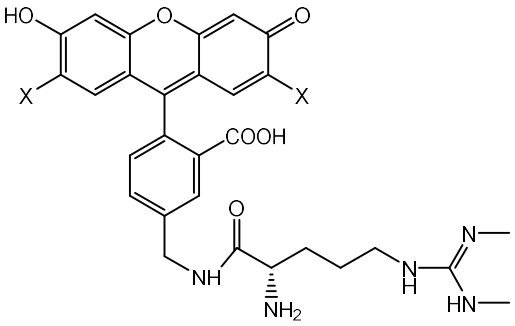
~~200~~18. The method of claim ~~198~~16, wherein the anti-cystatin-B antibody is conjugated to a quencher.

~~201~~19. The method of claim ~~183~~1, wherein the light at the first wavelength is not linearly polarized.

~~202~~20. The method of claim ~~201~~19, wherein the analyte is an antigen and the binding partner is an antibody.

~~203~~21. The method of claim ~~202~~20, wherein the antigen is SDMA and the antibody is an antibody for SDMA.

~~204~~22. The method of claim ~~203~~21, wherein the fluorescent tracer is selected from the group consisting of:

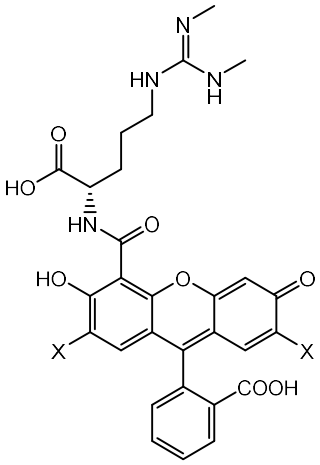
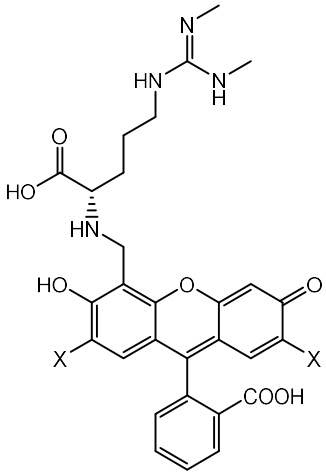
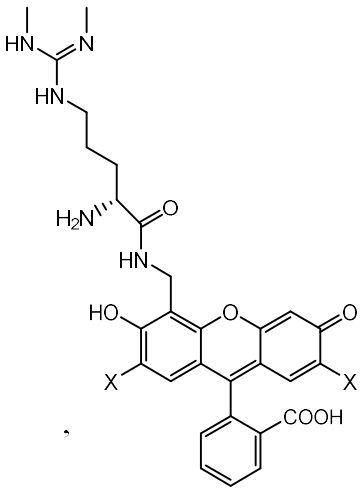
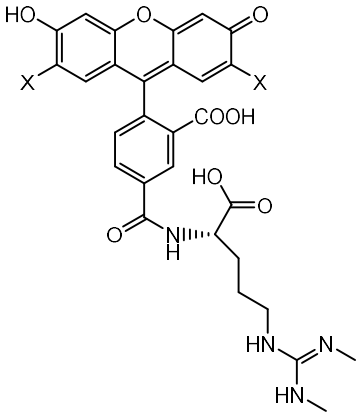
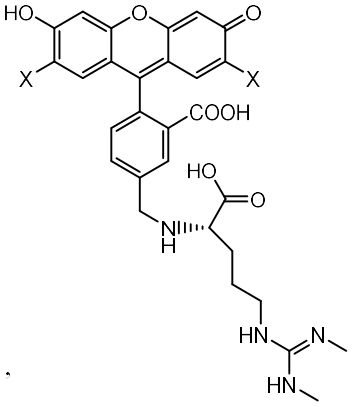
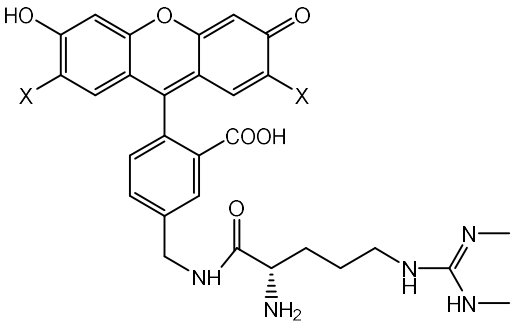





, and


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

~~205~~23. The method of claim ~~204~~22, wherein the antibody for SDMA is conjugated to a quencher.

~~206~~24. The method of claim ~~205~~23, wherein the quencher is selected from the group consisting of CY3, CY5, IRDyeQC1, BHQ1, and BHQ10.

~~207~~25. A compound selected from the group consisting of:






, and


~~208~~26. The method of claim ~~201~~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.

~~209~~27. The method of claim ~~28~~26, wherein the antigen is SDMA and the antibody is an antibody for SDMA.