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(54) **FREE-SOLUTION RESPONSE FUNCTION INTERFEROMETRY**

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G01N 21/19	(2006.01)

(52) **U.S. Cl.**

CPC ***G01N 33/536*** (2013.01); ***G01N 21/19*** (2013.01); ***G01N 21/4133*** (2013.01)

(57) **ABSTRACT**

Disclosed are methods for the free solution measurement of molecular interactions by refractive index sensing other than backscattering interferometry. The disclosed methods can have very low detection limits and/or very low sample volume requirements. Also disclosed are various biosensor applications of the disclosed techniques. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present invention.

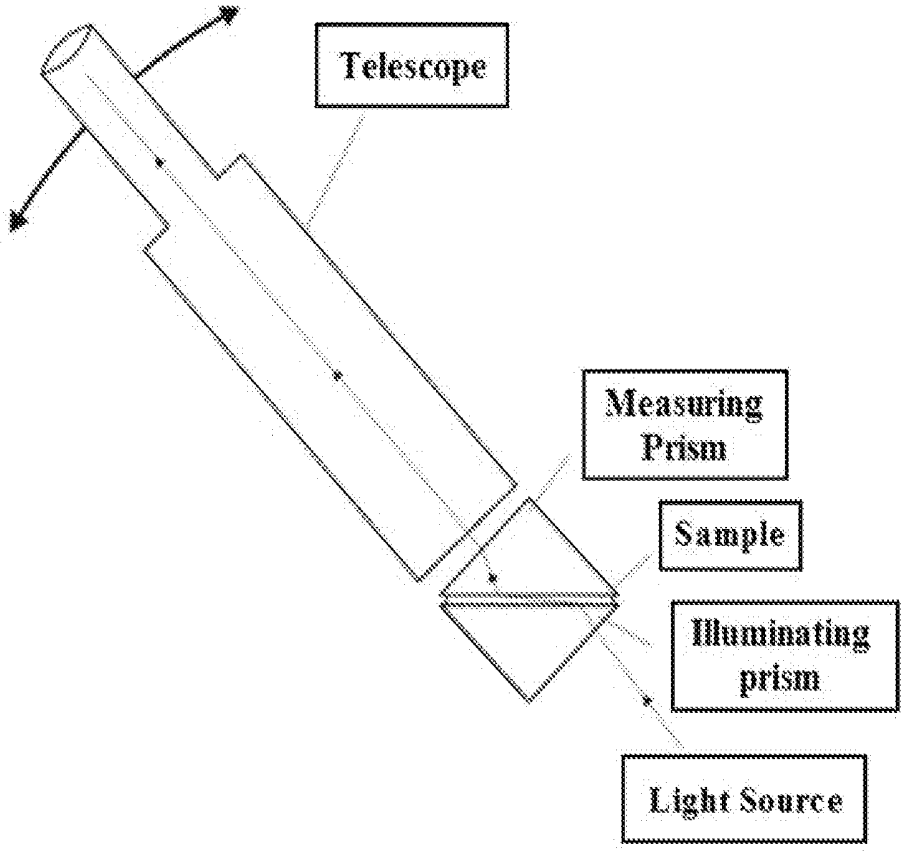

Telescope
Measuring Prism
Sample
Illuminating prism
Light Source

FIG. 1A

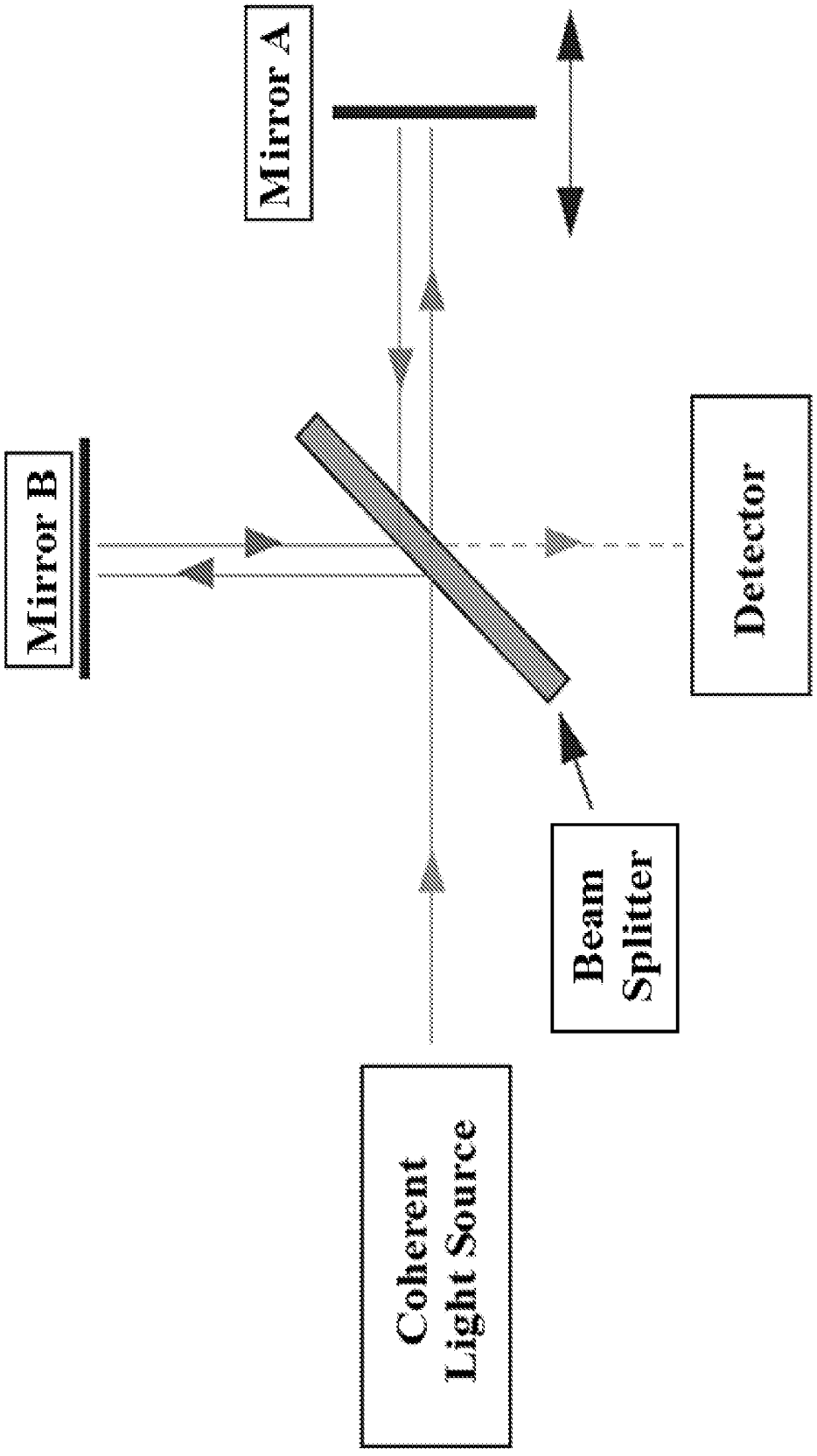

Mirror A
Mirror B
Detector
Beam Splitter
Coherent Light Source

FIG. 1B

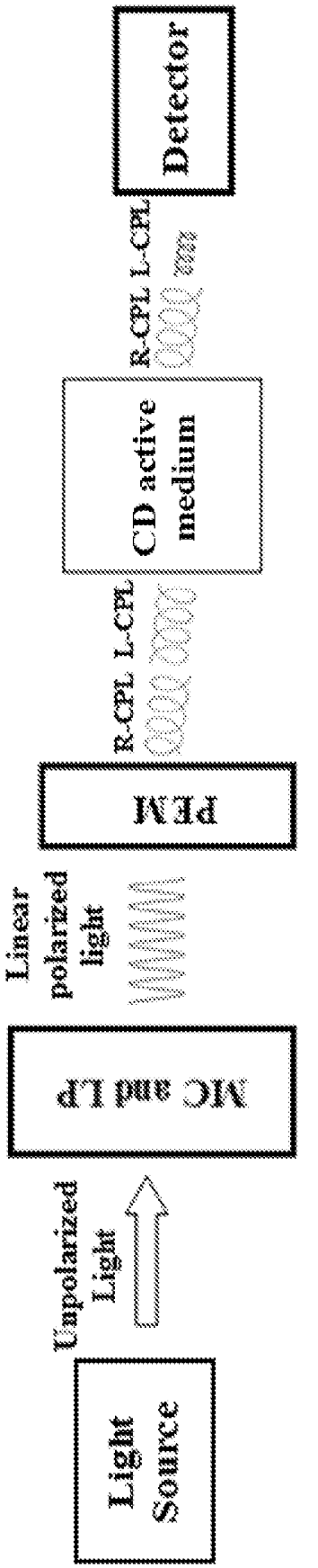

Light Source
Unpolarized Light
MC and LP
Linear polarized light
PEM
R-CPL L-CPL
CD active medium
R-CPL L-CPL
Detector

FIG. 2

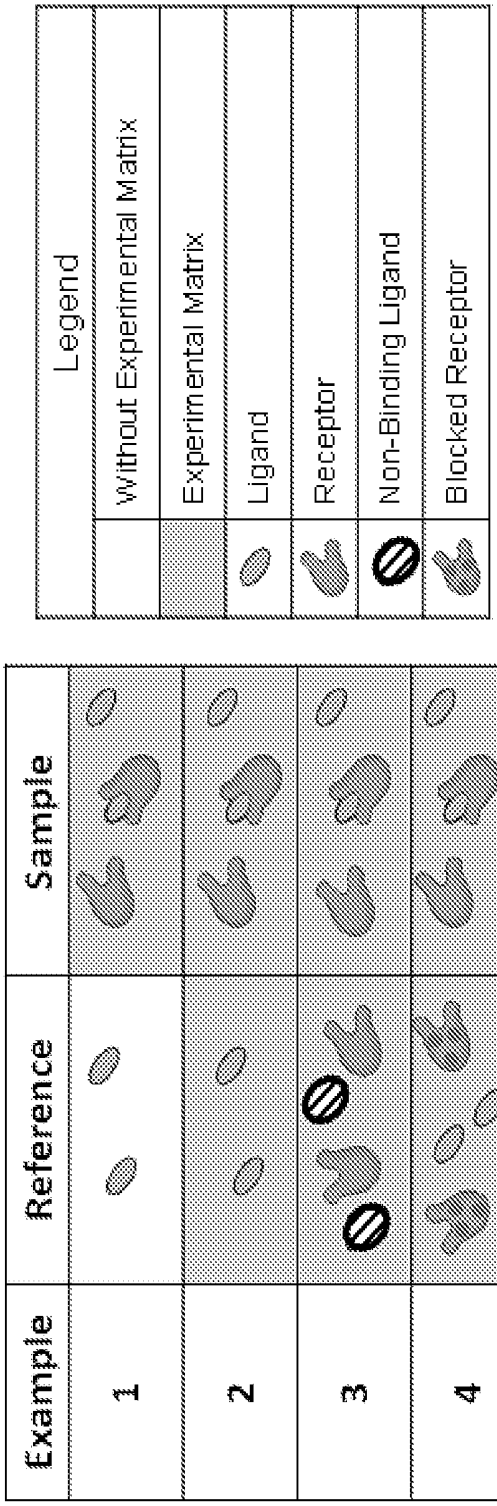

Example
Reference
Sample
1
2
3
4
Legend
Without Experimental Matrix
Experimental Matrix
Ligand
Receptor
Non-Binding Ligand
Blocked Receptor

FIG. 3

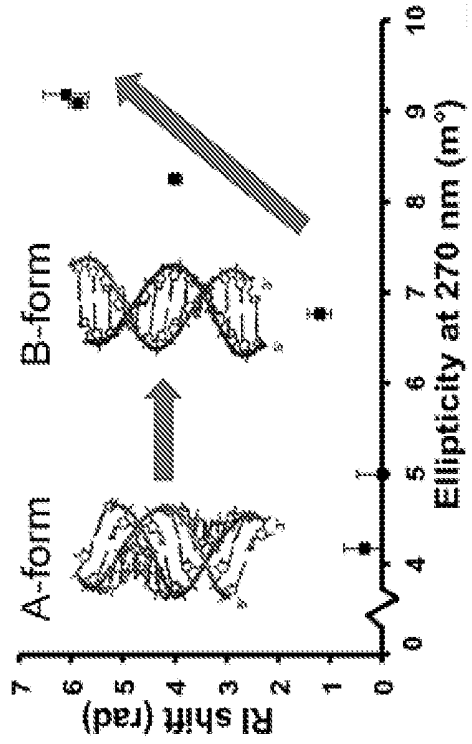

A-form
B-form
RI shift (rad)
Ellipticity at 270 nm (m°)

FIG. 4B

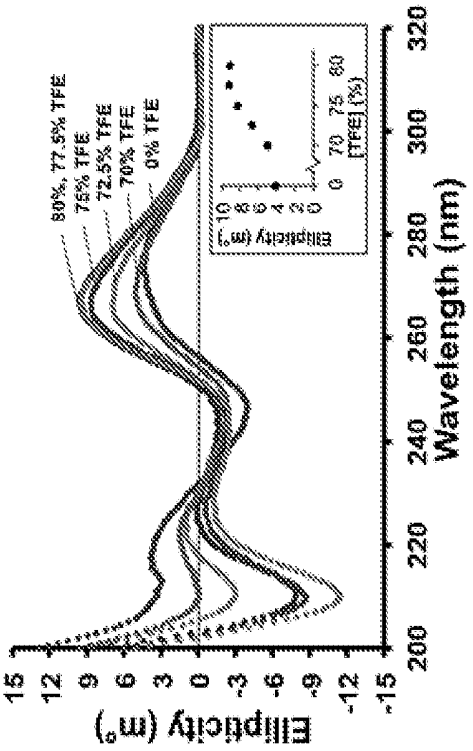

80%, 77.5% TFE
75% TFE
72.5% TFE
70% TFE
0% TFE
Ellipticity (m°)
Wavelength (nm)
[TFE] (%)

FIG. 4A

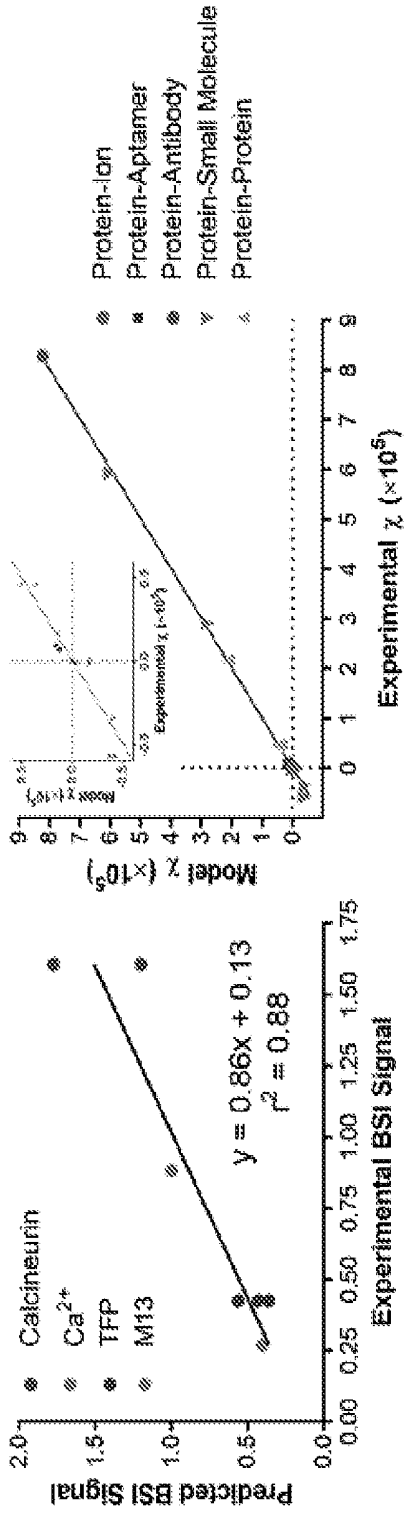

Predicted BSI Signal
Experimental BSI Signal
Calcineurin
Ca^{2+}
TFP
M13
y = 0.86x + 0.13
r^2 = 0.88
Model χ ($\times 10^5$)
Experimental χ ($\times 10^5$)
Protein-Ion
Protein-Aptamer
Protein-Antibody
Protein-Small Molecule
Protein-Protein

FIG. 5A

FIG. 5B

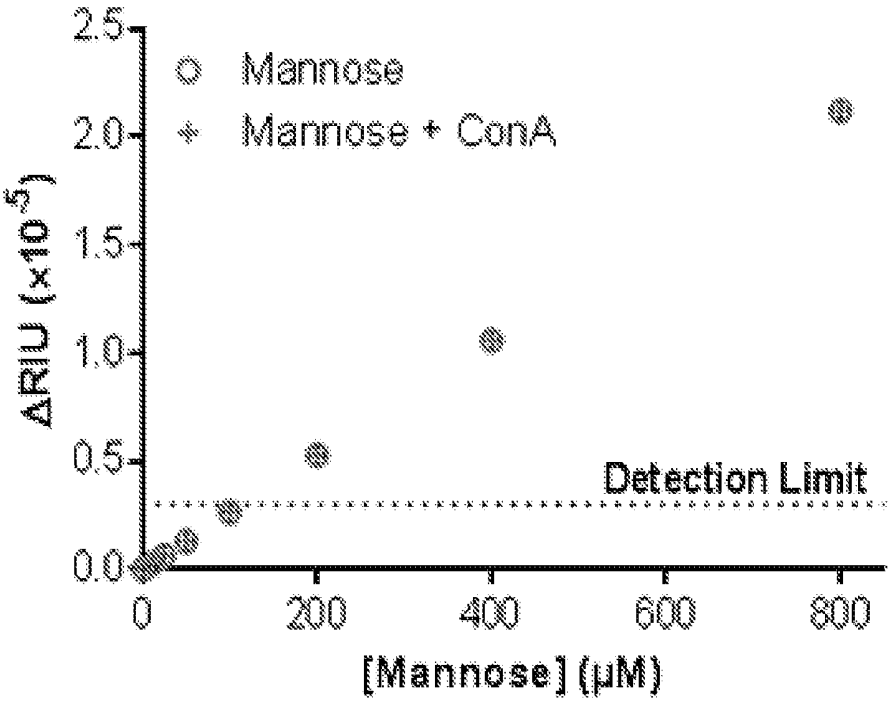

2.5
2.0
1.5
1.0
0.5
0.0
ΔRIU (x10^-5)
Mannose
Mannose + ConA
Detection Limit
0
200
400
600
800
[Mannose] (μM)

FIG. 6A

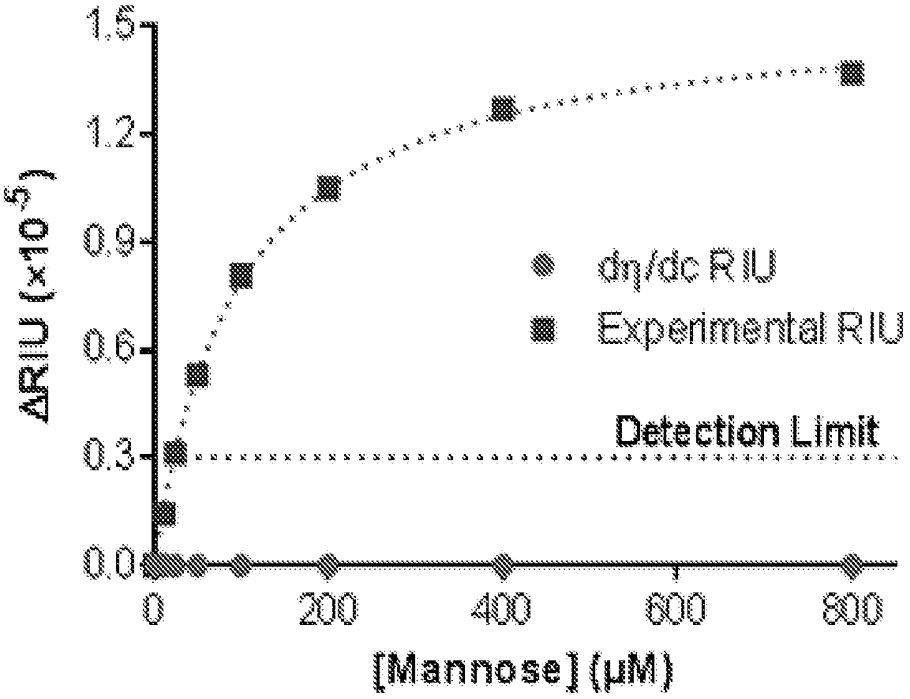

1.5
1.2
0.9
0.6
0.3
0.0
ΔRIU (x10^-5)
dη/dc RIU
Experimental RIU
Detection Limit
0
200
400
600
800
[Mannose] (μM)

FIG. 6B

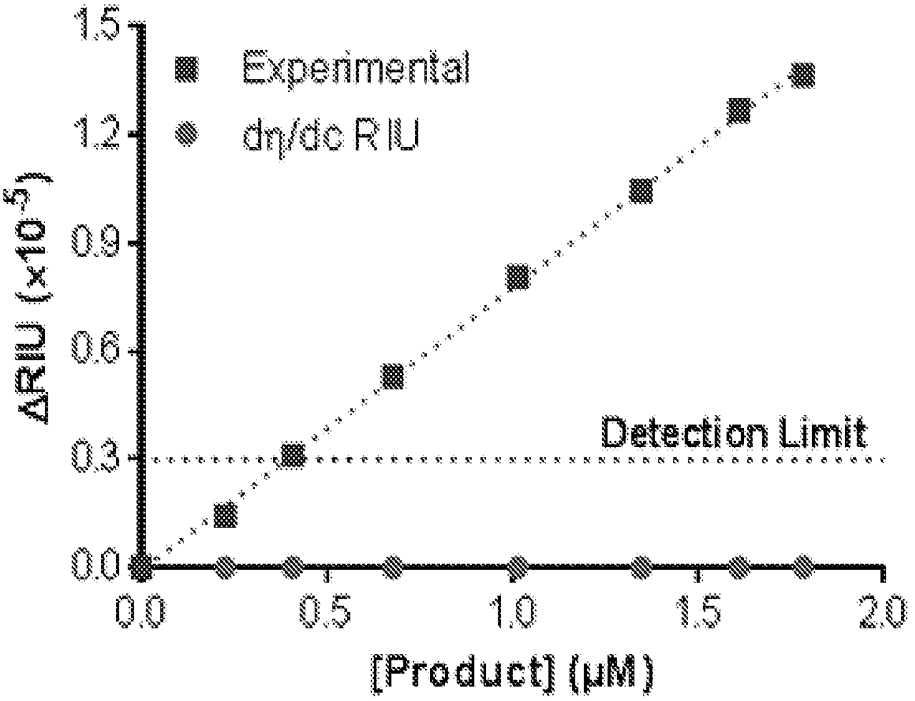

1.5
1.2
0.9
0.6
0.3
0.0
ΔRIU (x10-5)
Experimental
dn/dc RIU
Detection Limit
0.0
0.5
1.0
1.5
2.0
[Product] (μM)

FIG. 6C

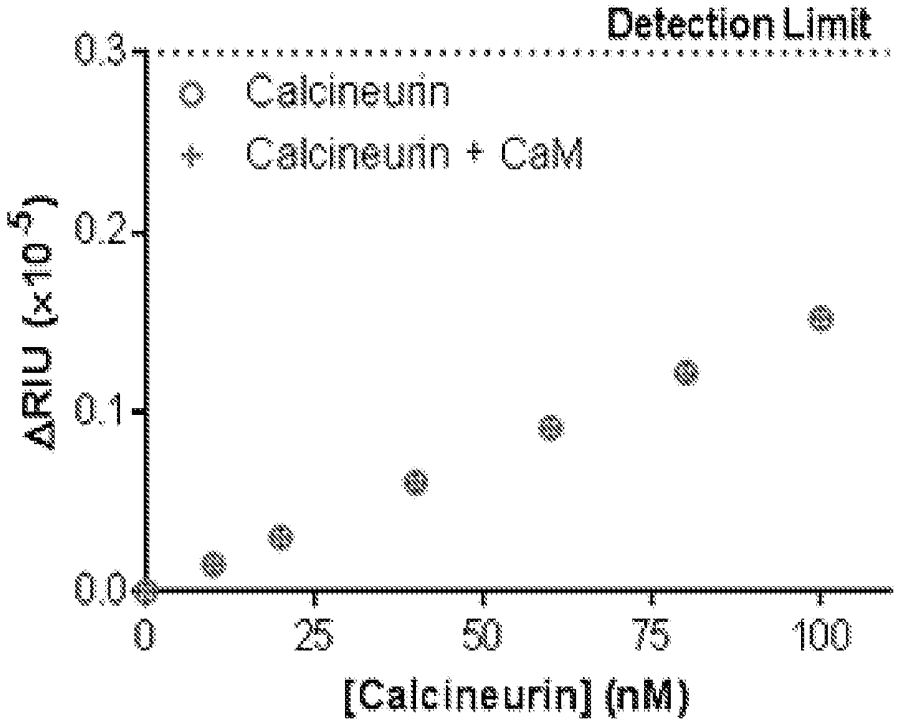

Detection Limit
0.3
0.2
0.1
0.0
ΔRIU (x10-5)
Calcineurin
Calcineurin + CaM
0
25
50
75
100
[Calcineurin] (nM)

FIG. 6D

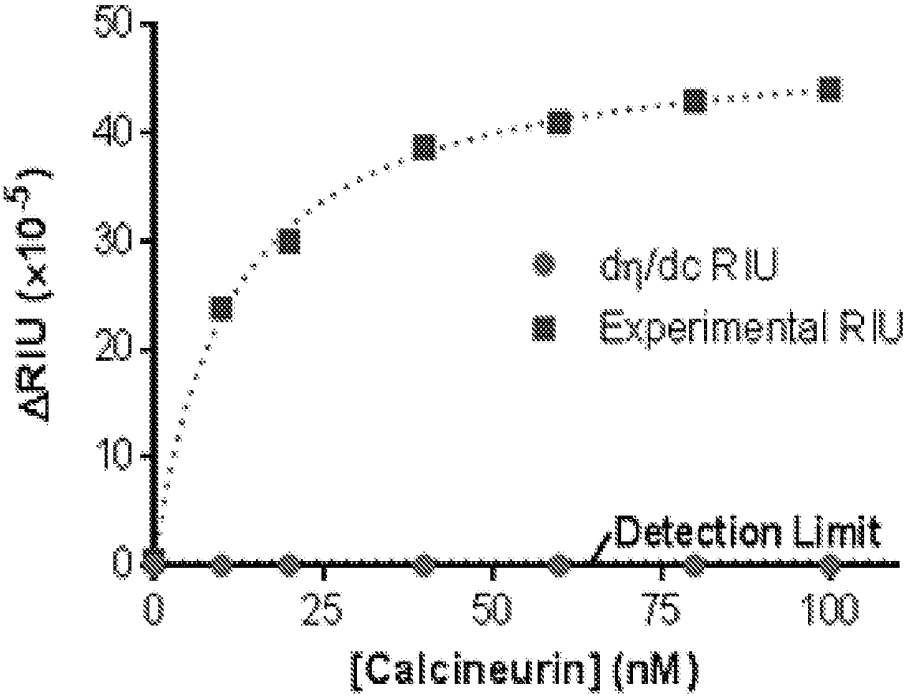

ΔRIU (x10-5)
50
40
30
20
10
0
dη/dc RIU
Experimental RIU
Detection Limit
0
25
50
75
100
[Calcineurin] (nM)

FIG. 6E

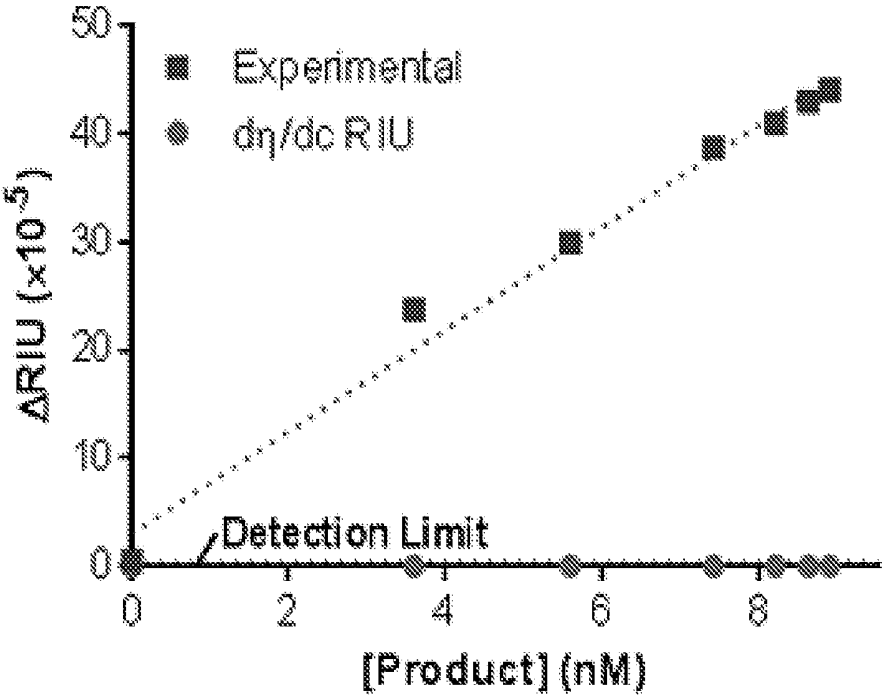

ΔRIU (x10-5)
50
40
30
20
10
0
Experimental
dη/dc RIU
Detection Limit
0
2
4
6
8
[Product] (nM)

FIG. 6F

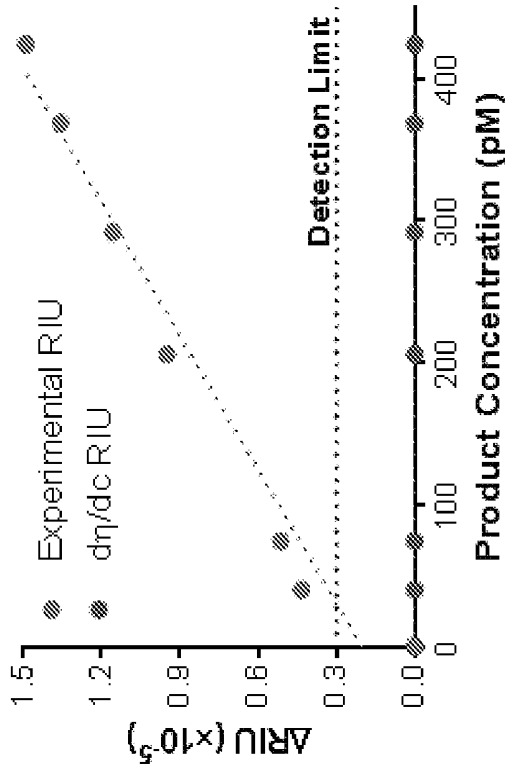

ΔRIU (×10⁻⁵)
Experimental RIU
dη/dc RIU
Detection Limit
Product Concentration (pM)
0.0
0.3
0.6
0.9
1.2
1.5
0
100
200
300
400

FIG. 7B

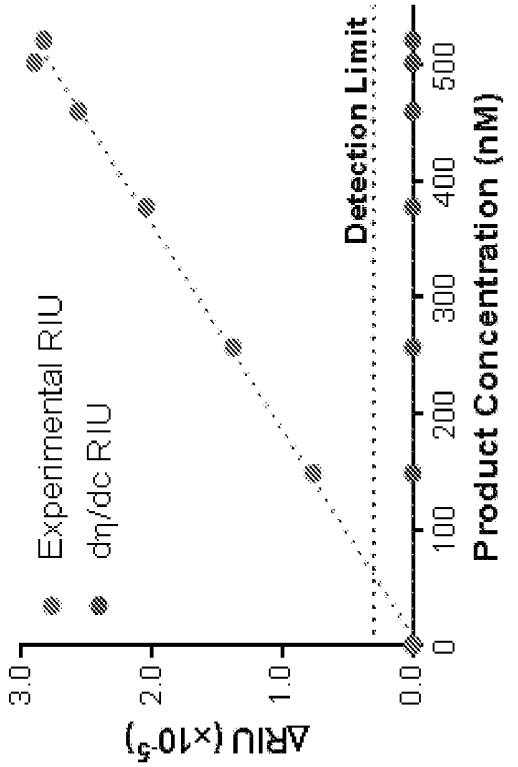

ΔRIU (×10⁻⁵)
Experimental RIU
dη/dc RIU
Detection Limit
Product Concentration (nM)
0.0
1.0
2.0
3.0
0
100
200
300
400
500

FIG. 7A

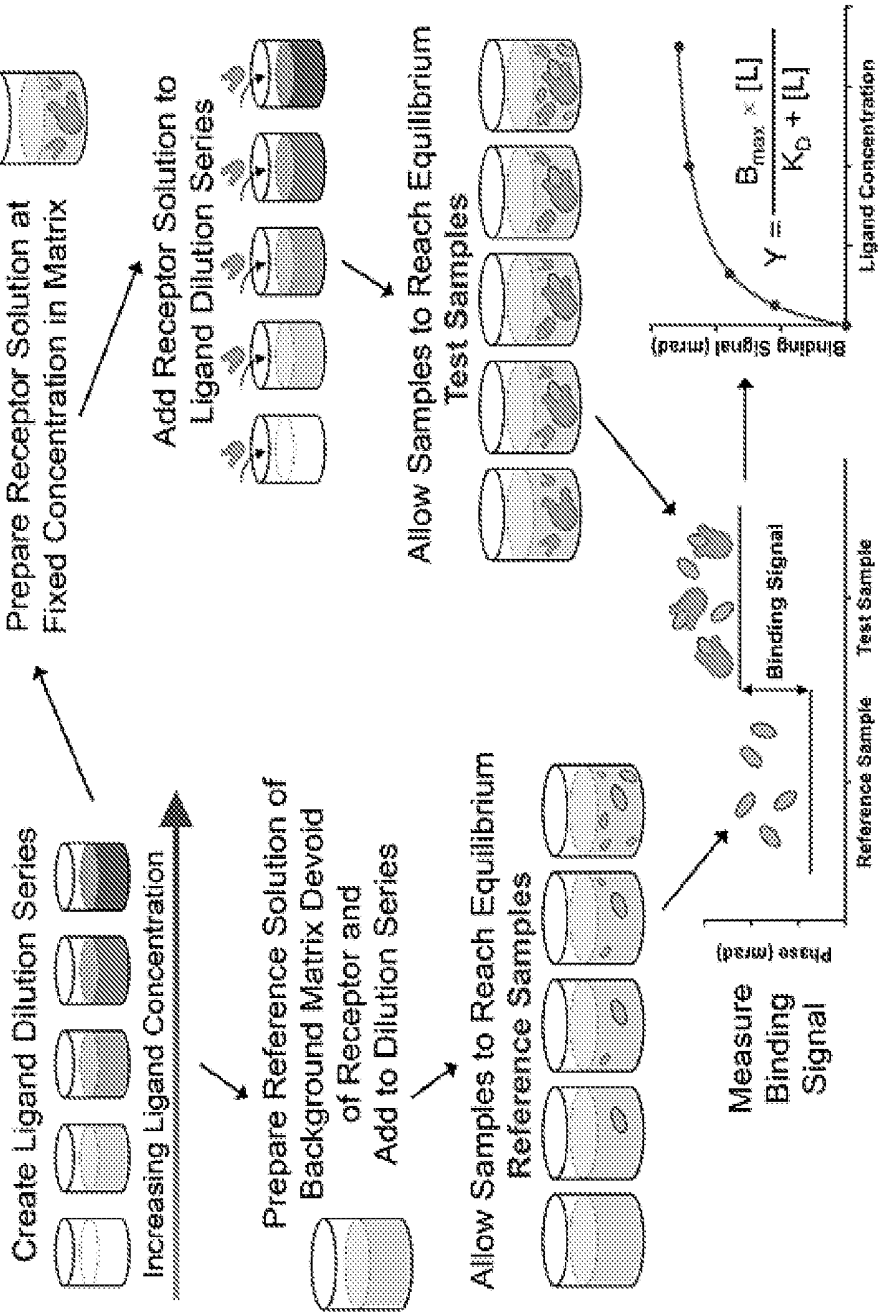

Create Ligand Dilution Series
Increasing Ligand Concentration
Prepare Receptor Solution at Fixed Concentration in Matrix
Add Receptor Solution to Ligand Dilution Series
Prepare Reference Solution of Background Matrix Devoid of Receptor and Add to Dilution Series
Allow Samples to Reach Equilibrium Reference Samples
Allow Samples to Reach Equilibrium Test Samples
Measure Binding Signal
Phase (mrad)
Reference Sample
Test Sample
Binding Signal
Binding Signal (mrad)
Ligand Concentration
$Y = \frac{B_{max} \times [L]}{K_D + [L]}$

FIG. 8

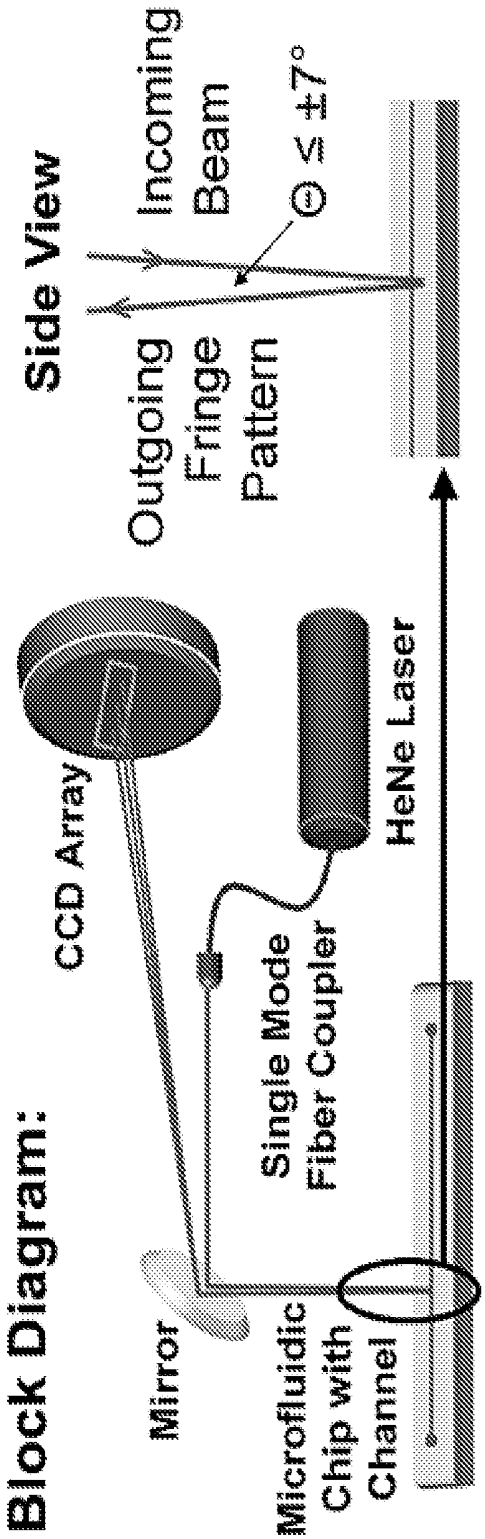

Block Diagram:
Side View
Mirror
Microfluidic Chip with Channel
Single Mode Fiber Coupler
CCD Array
HeNe Laser
Outgoing Fringe Pattern
Incoming Beam
Θ ≤ ±7°

FIG. 9A

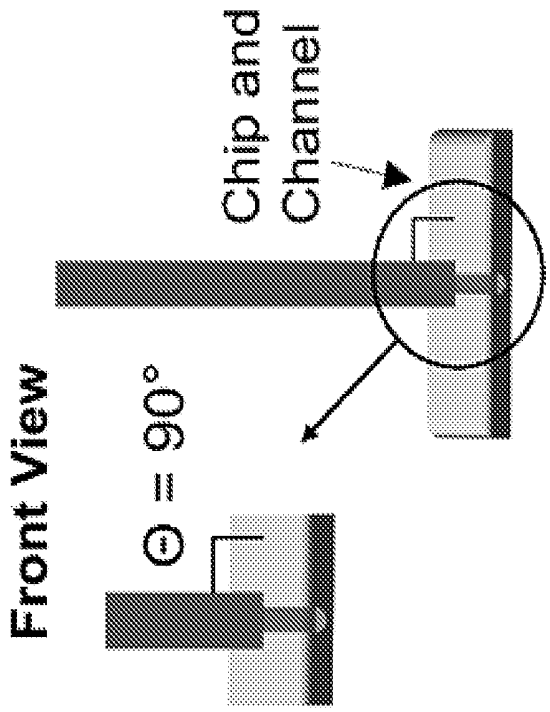

Front View
Θ = 90°
Chip and Channel

FIG. 9C

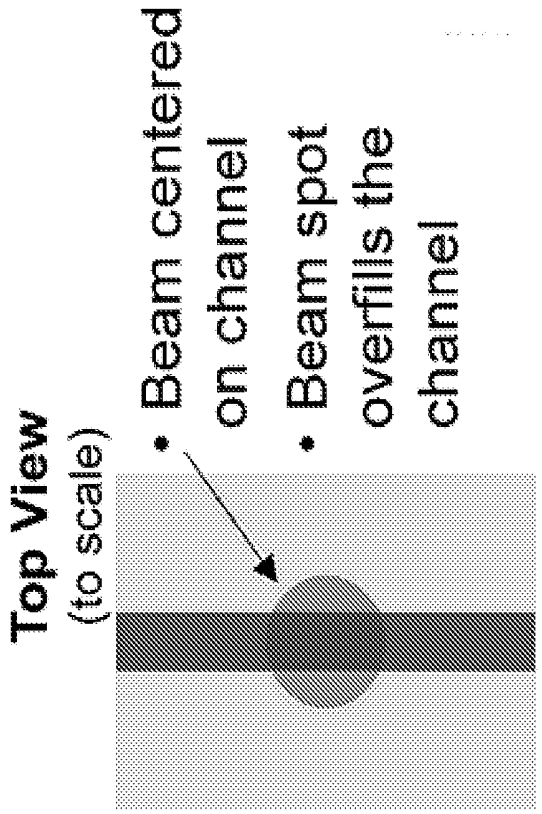

Top View
(to scale)
• Beam centered on channel
• Beam spot overfills the channel

FIG. 9B

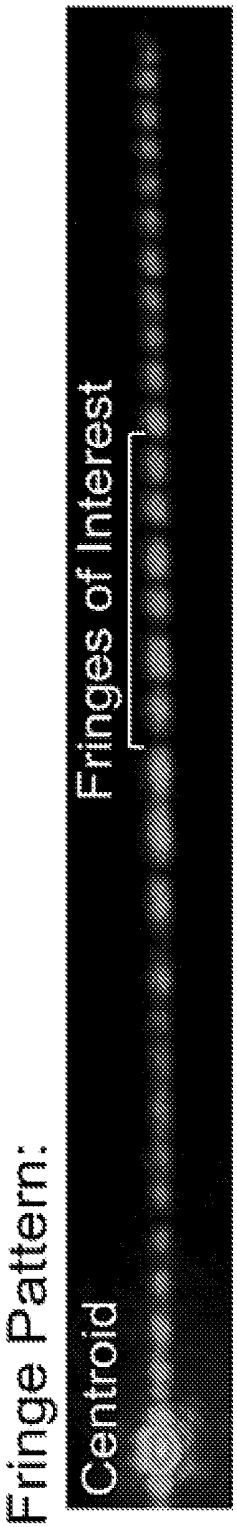


FIG. 9D

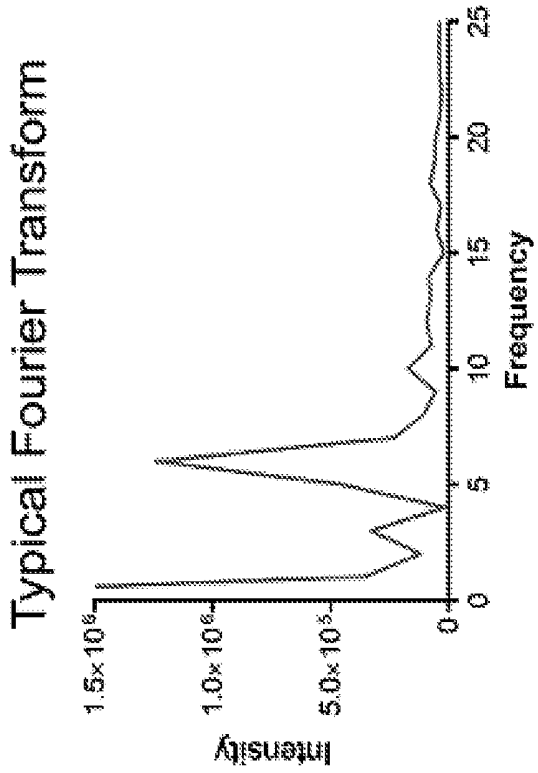

Typical Fourier Transform
Intensity
1.5×10^6
1.0×10^6
5.0×10^5
0
0
5
10
15
20
25
Frequency

FIG. 9F

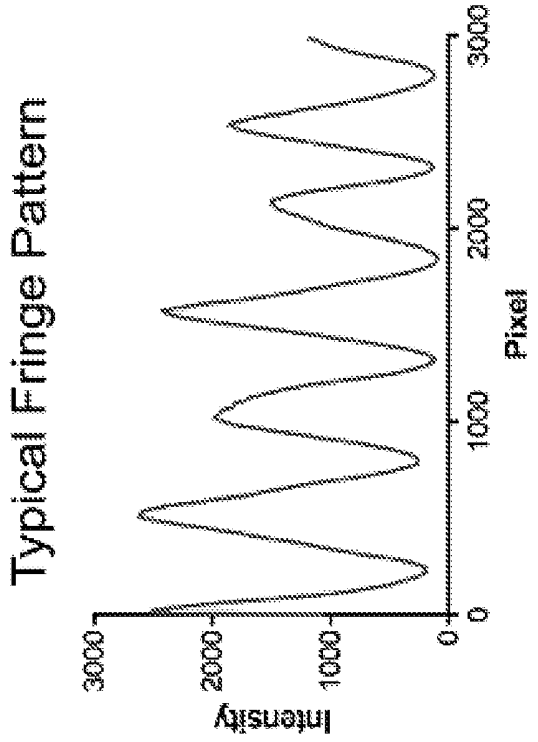

Typical Fringe Pattern
Intensity
3000
2000
1000
0
0
1000
2000
3000
Pixel

FIG. 9E

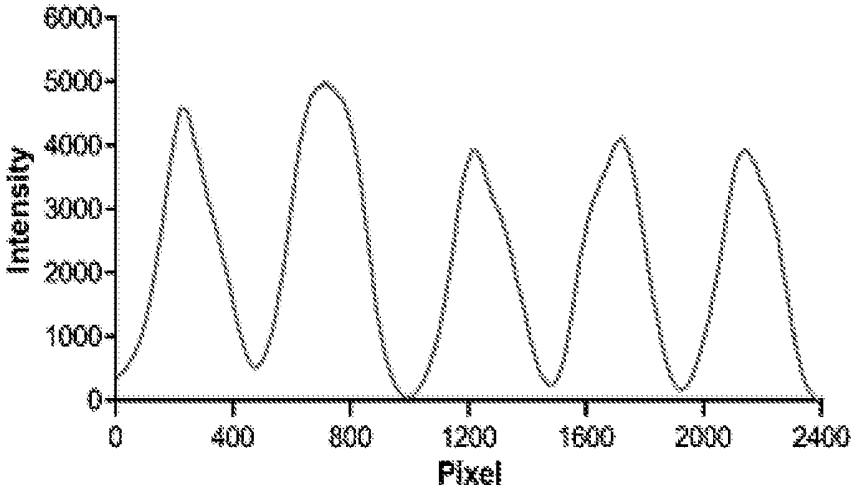


FIG. 10A

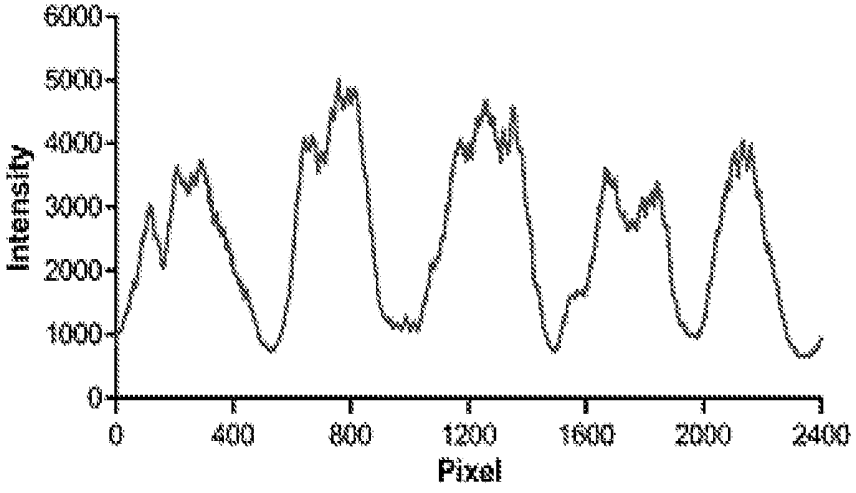


FIG. 10B

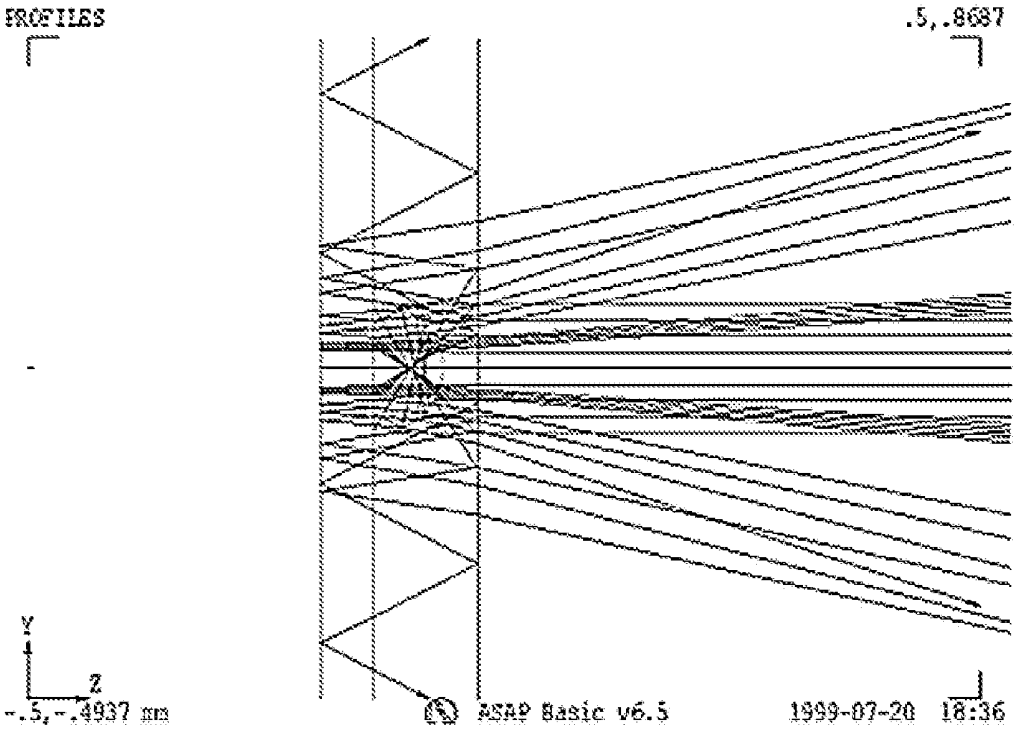


FIG. 11A

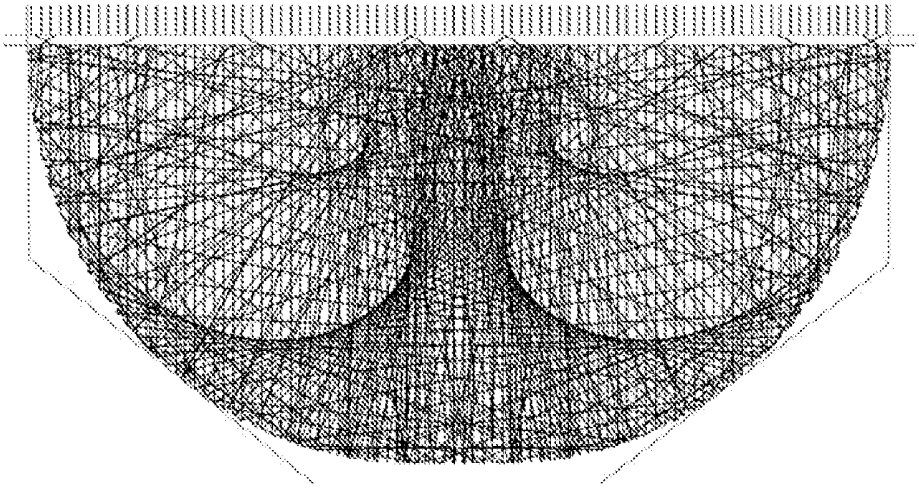


FIG. 11B

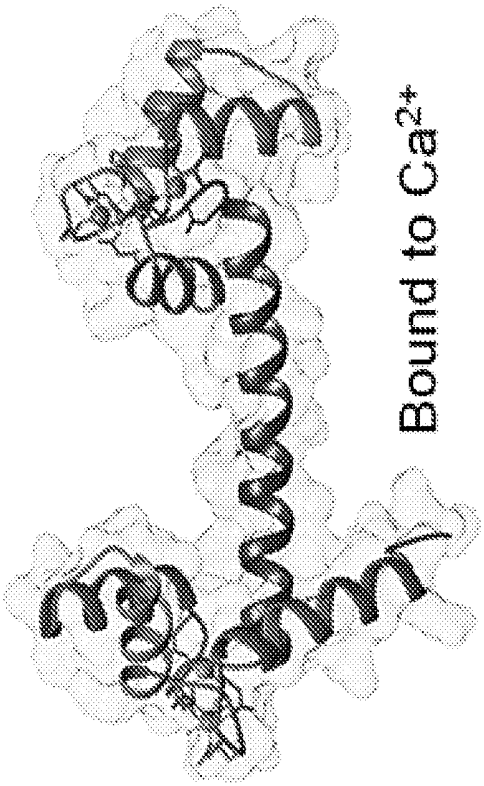


FIG. 12B

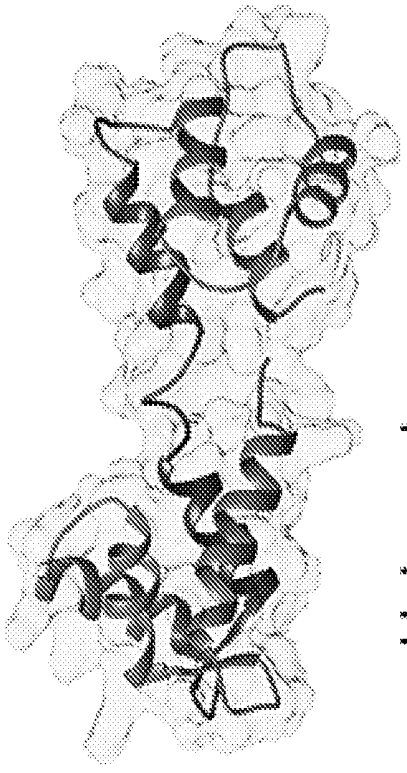
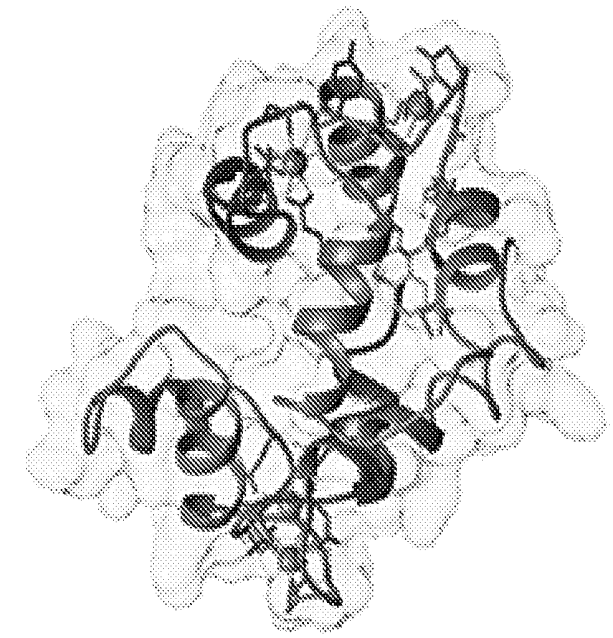
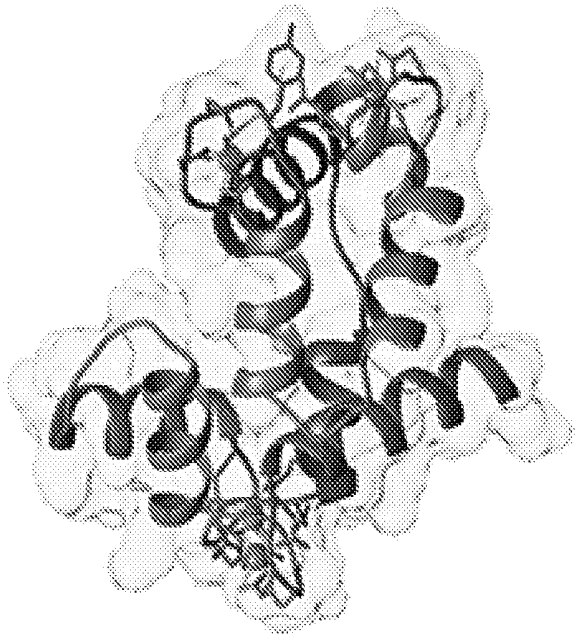


FIG. 12A



Bound to Ca^{2+} and TFP

FIG. 12D



Bound to Ca^{2+} and M13

FIG. 12C

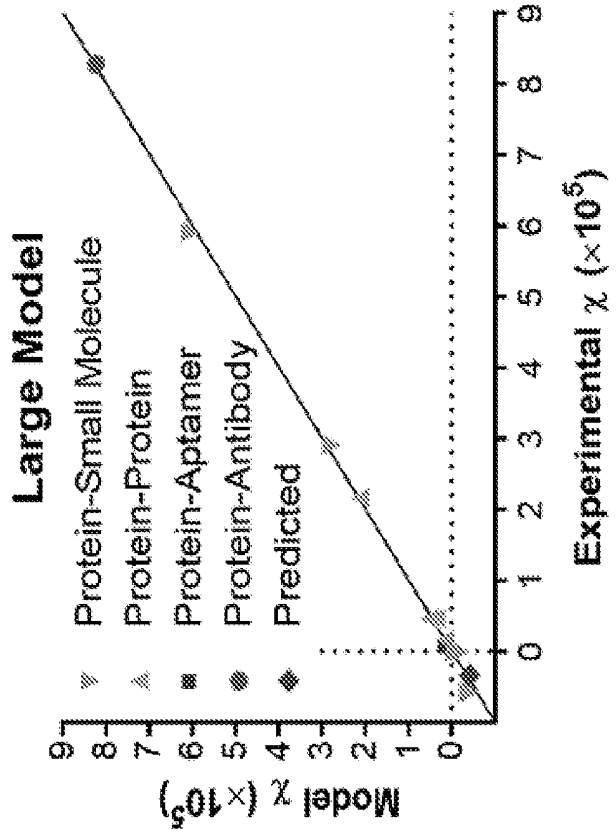


FIG. 13B

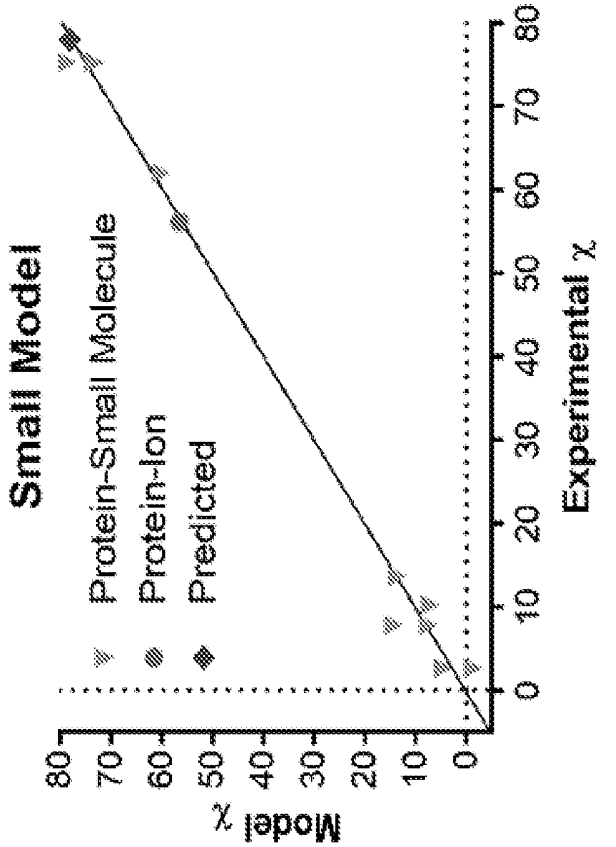


FIG. 13A

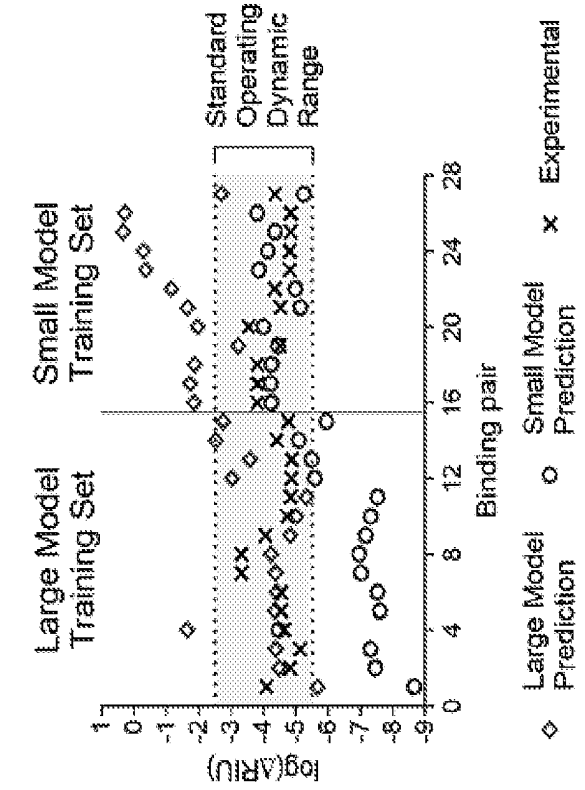


FIG. 14B

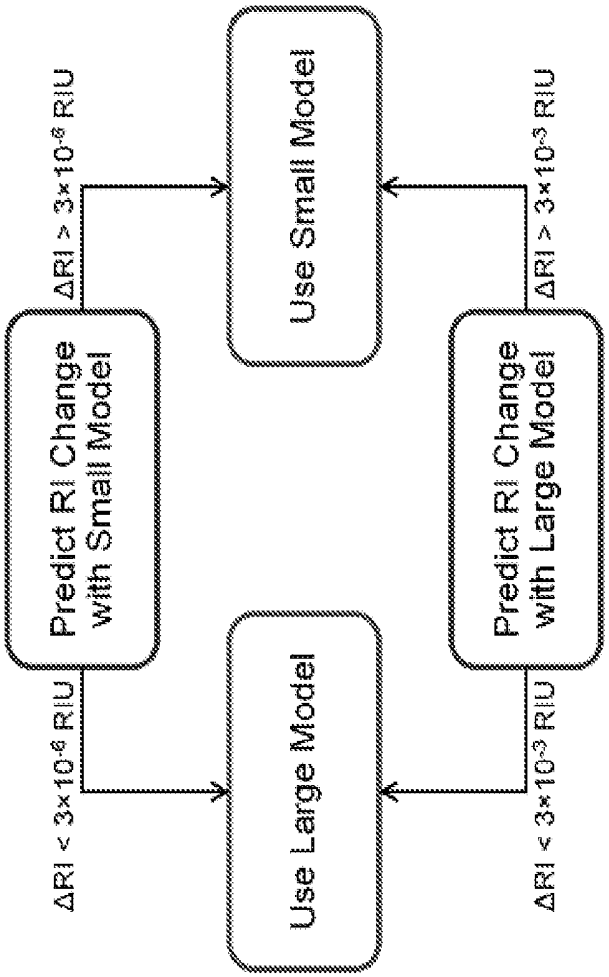


FIG. 14A

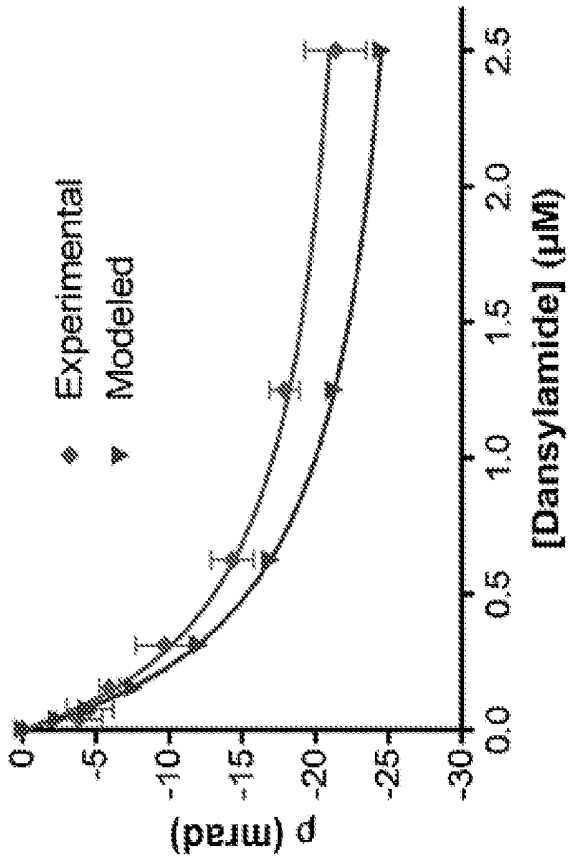


FIG. 15B

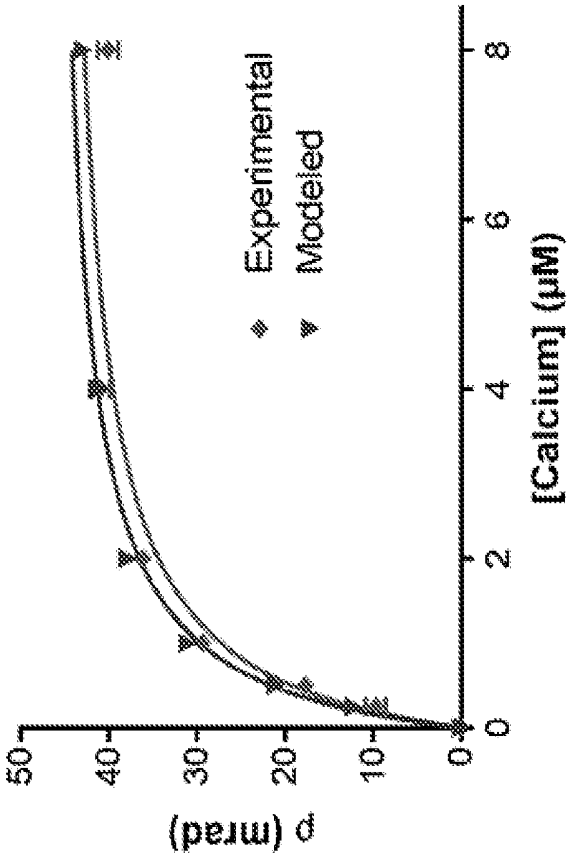


FIG. 15A

FIG. 16A

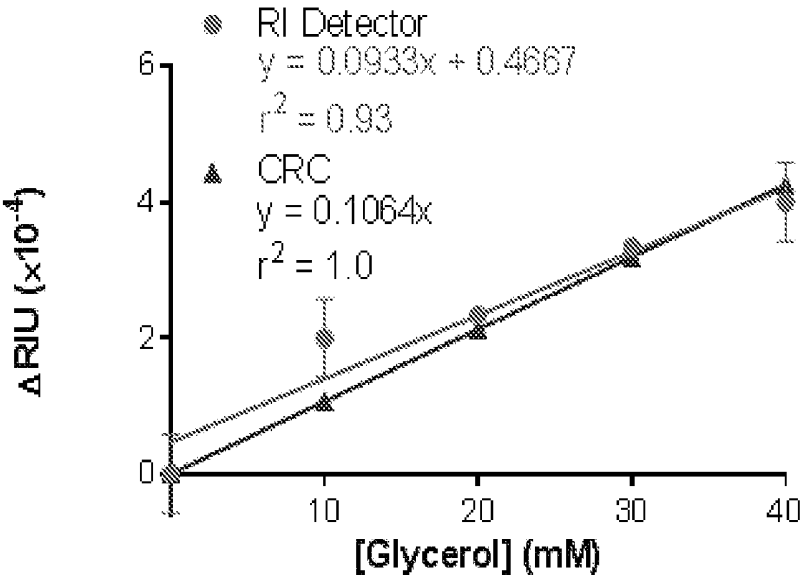


FIG. 16B

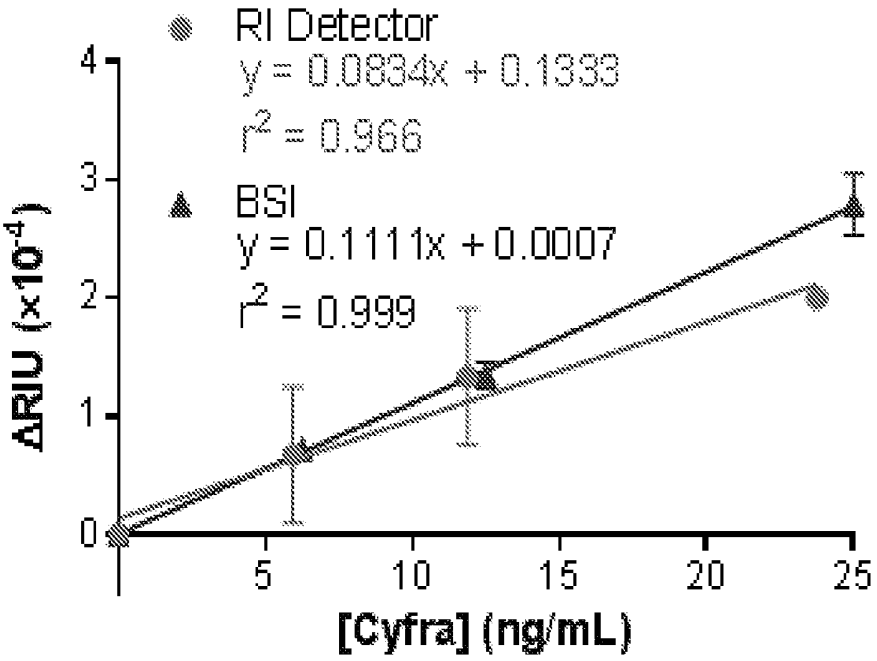


FIG. 16C

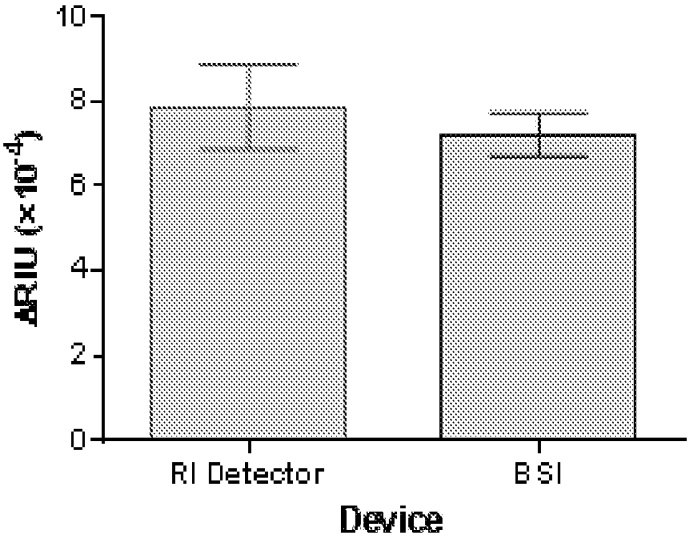


FIG. 16D

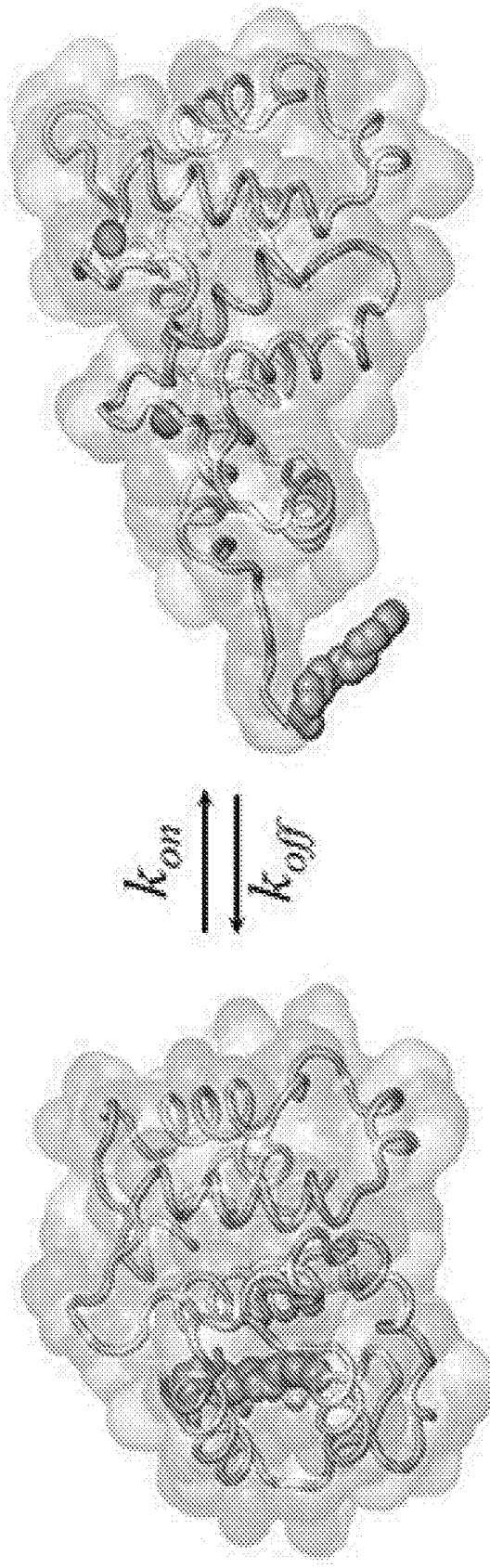


FIG. 17A

FIG. 17B

FIG. 18A

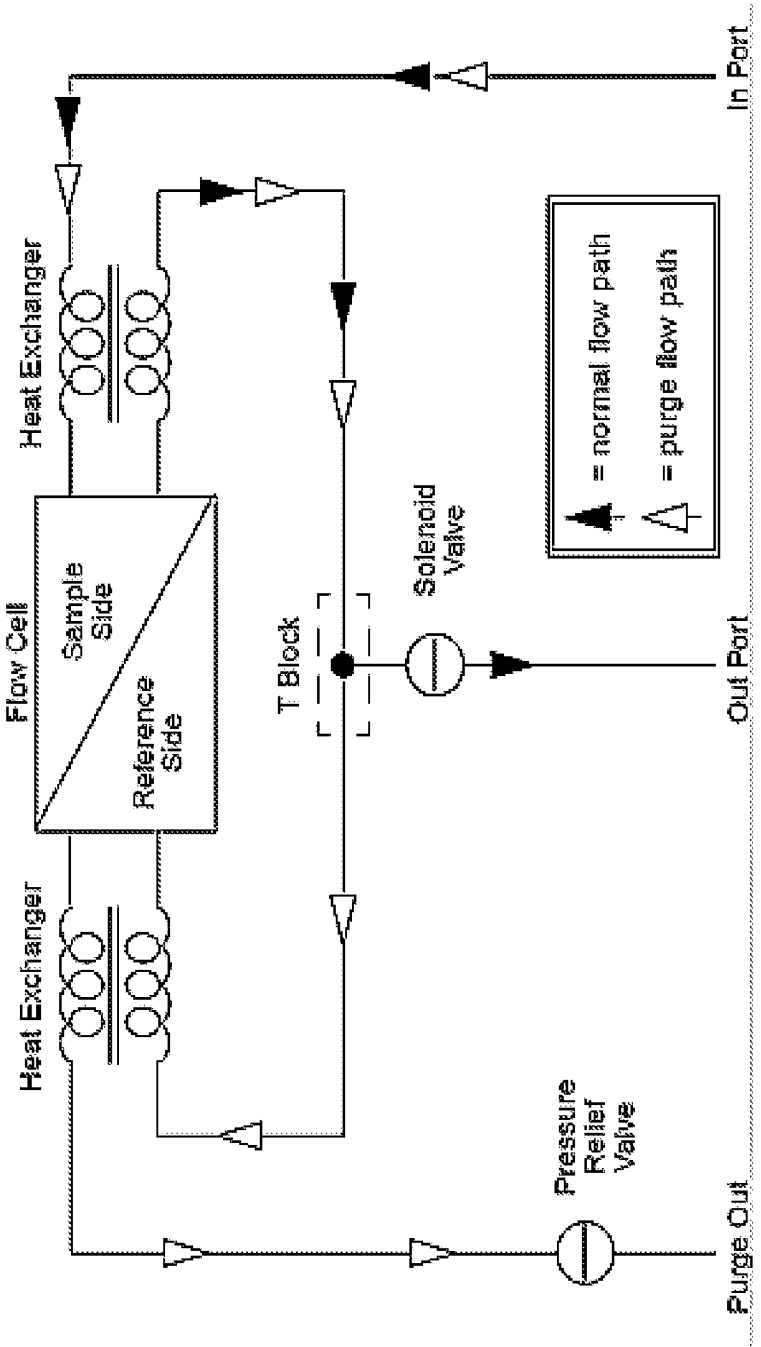

In Port
Heat Exchanger
Flow Cell
Sample Side
Reference Side
T Block
Solenoid Valve
= normal flow path
= purge flow path
Out Port
Heat Exchanger
Pressure Relief Valve
Purge Out

FIG. 18B

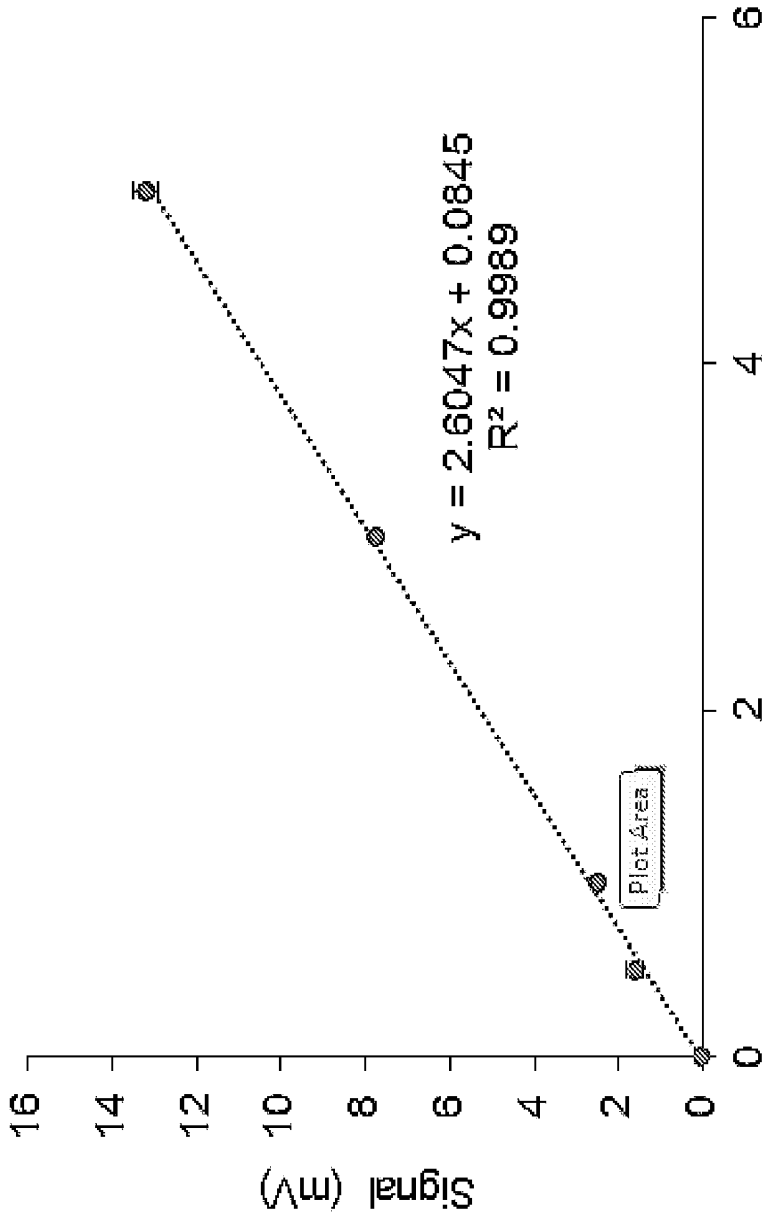

Signal (mV)
0
2
4
6
8
10
12
14
16
0
2
4
6
glycerol, mM
y = 2.6047x + 0.0845
R² = 0.9989
Plot Area

FIG. 19

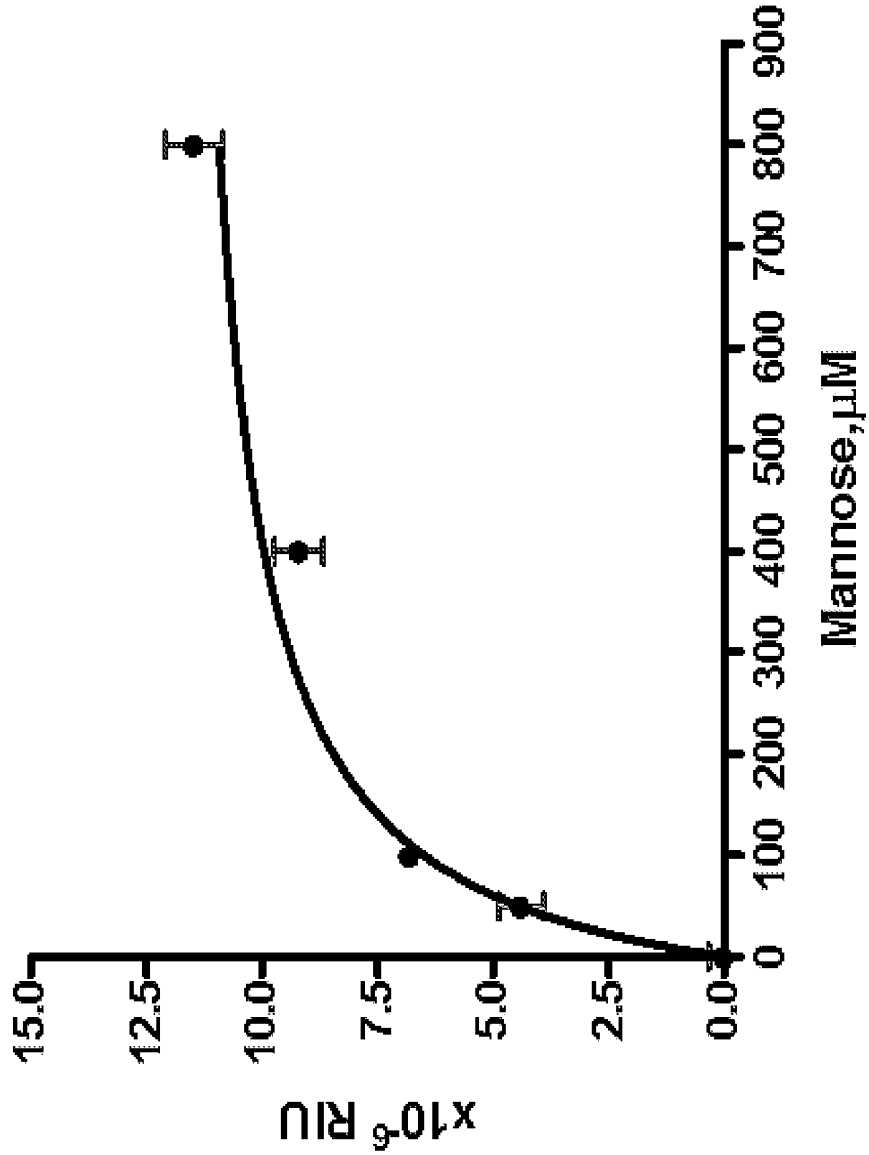

x10^6 RLU
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
100
200
300
400
500
600
700
800
900
Mannose,μM

FIG. 20

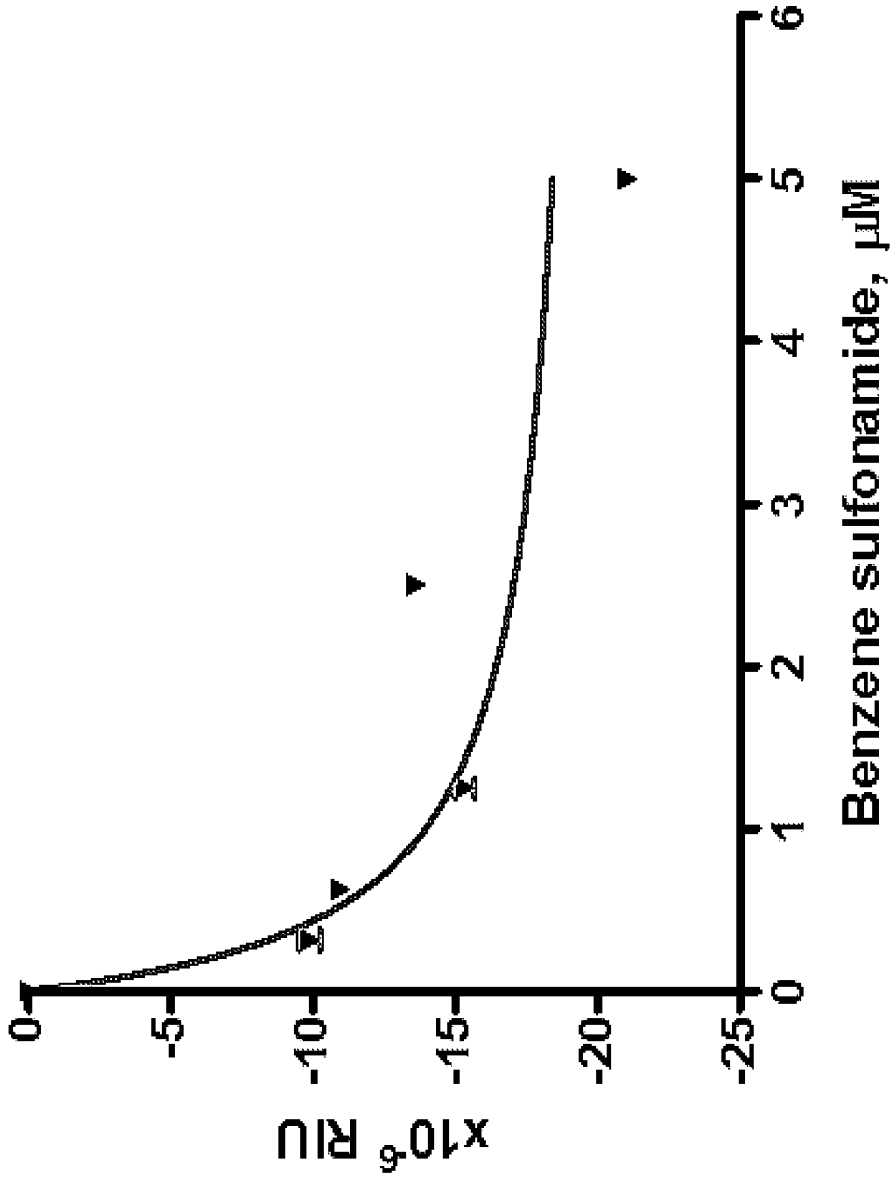


FIG. 21A

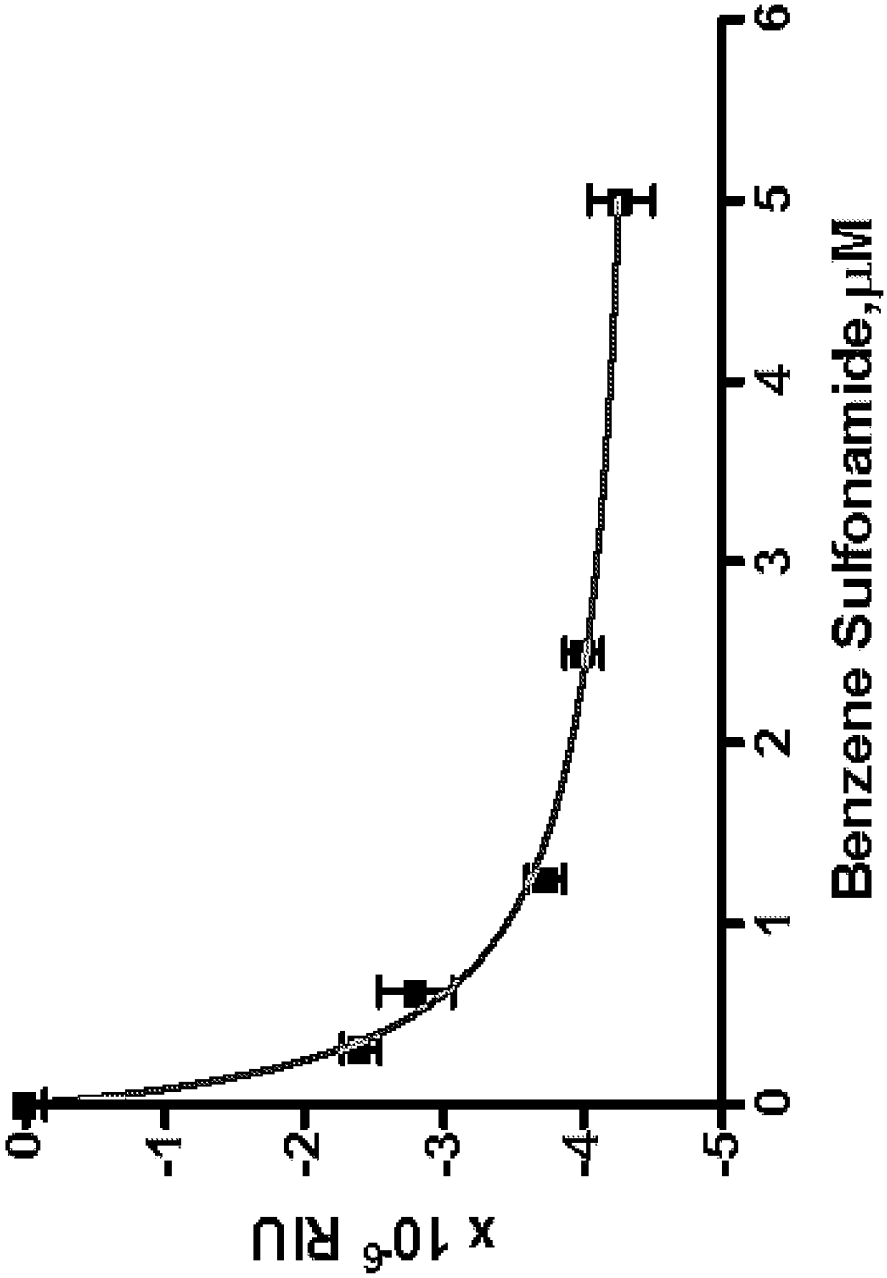


FIG. 21B

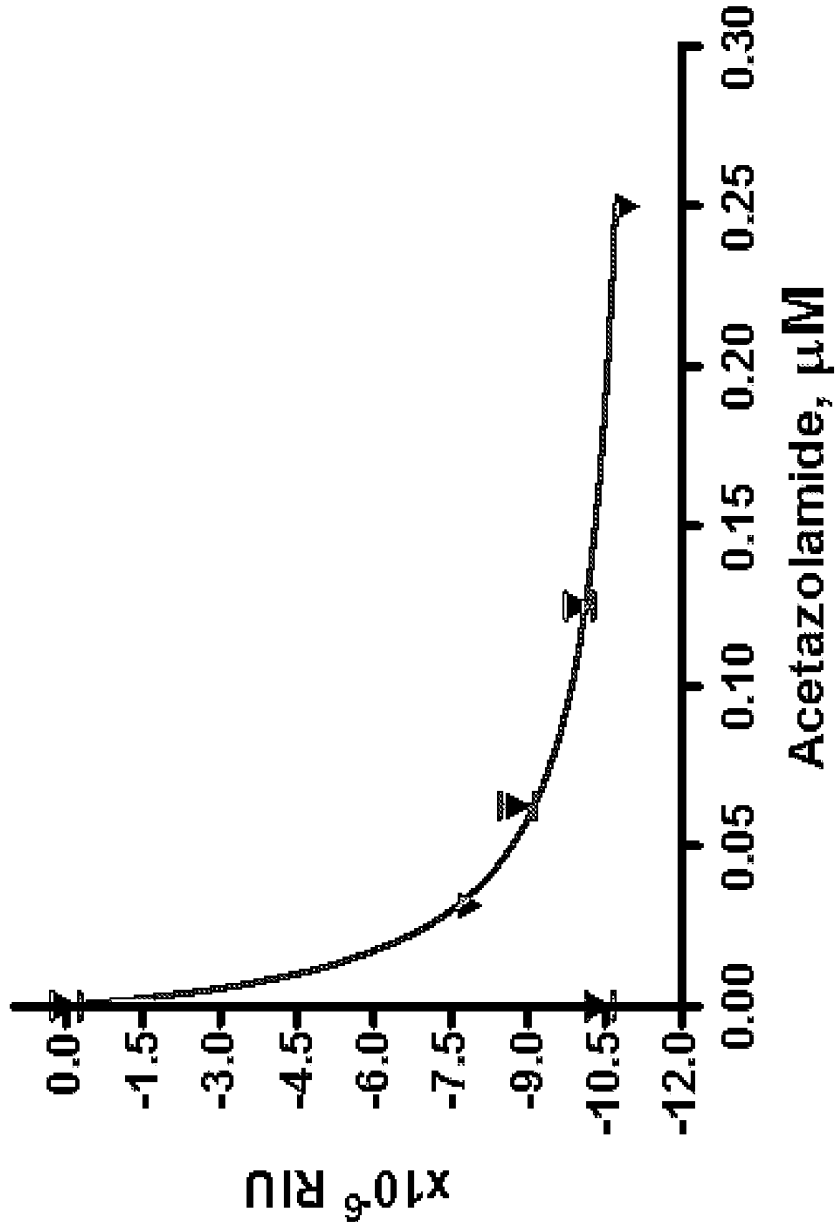

x10⁶ RIU
0.0
-1.5
-3.0
-4.5
-6.0
-7.5
-9.0
-10.5
-12.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Acetazolamide, μM

FIG. 22A

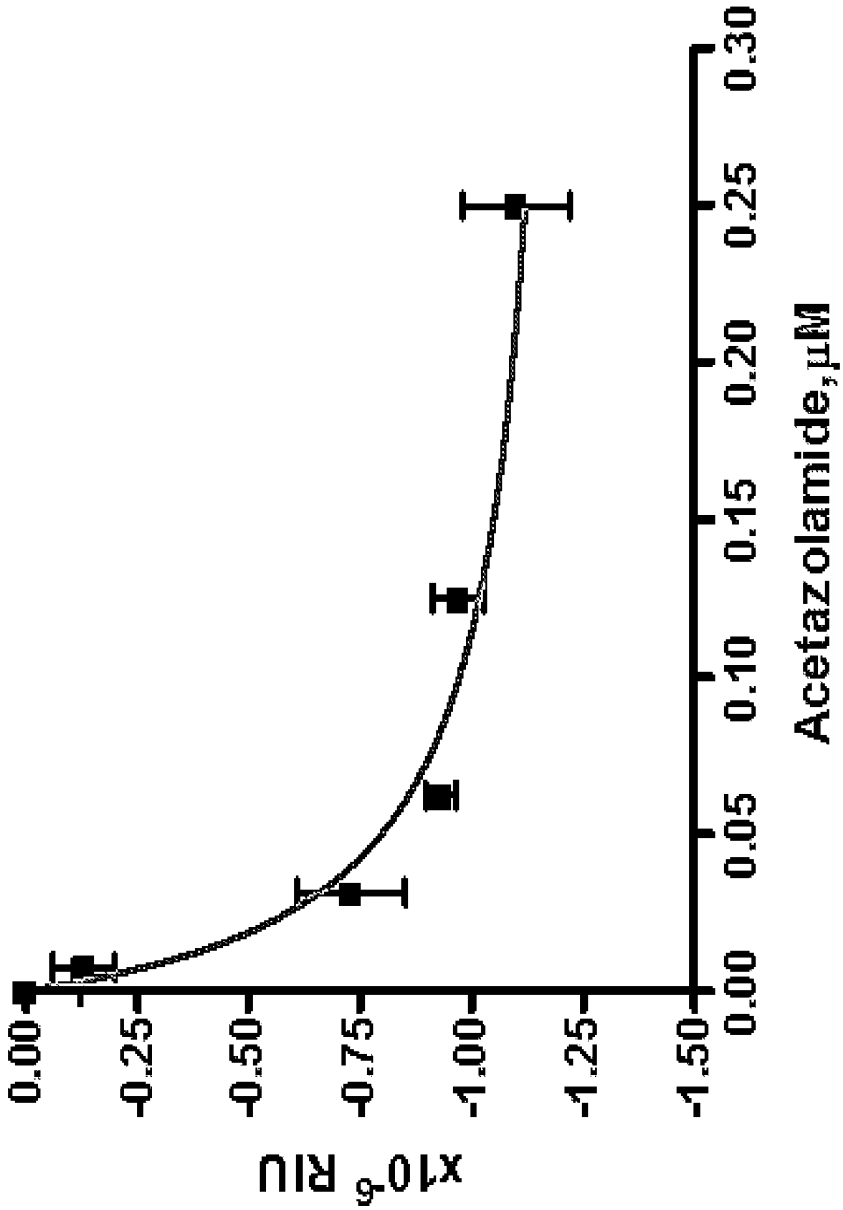

0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
x10⁻⁶ RIU
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Acetazolamide, μM

FIG. 22B

FREE-SOLUTION RESPONSE FUNCTION INTERFEROMETRY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims the benefit of U.S. Provisional Application No. 62/288,926, filed on Jan. 29, 2016, which is incorporated herein by reference in its entirety.

ACKNOWLEDGEMENT

[0002] This invention was made with government support under Grant No. CHE 1307899 awarded by the National Science Foundation. The government has certain rights in the invention.

BACKGROUND

[0003] Contemporary assays have enabled single molecule detection (Betzig and Chichester (1993) Single Molecules Observed by near-Field Scanning Optical Microscopy. *Science* 262(5138):1422-1425; Levene et al. (2003) Zero-mode waveguides for single-molecule analysis at high concentrations. *Science* 299(5607):682-686) have accelerated the sequencing of the human genome (Anonymous (2001) Unsung Heroes. *Science* 291(5507):1207) and facilitated imaging with extraordinary resolution without labels (Hell SW & Wichmann J (1994) Breaking the Diffraction Resolution Limit by Stimulated-Emission—Stimulated-Emission-Depletion Fluorescence Microscopy. *Opt Lett* 19(11):780-782). To most closely study an interaction in the natural state, an assay would interrogate the processes (reaction, molecular interaction, protein folding event, etc.) without perturbation. Label-free chemical and biochemical investigations (Liedberg et al. (1995) Biosensing with Surface-Plasmon Resonance—How It All Started. *Biosens Bioelectron* 10(8):R1-R9; Yu et al. (2014) Shedding new light on lipid functions with CARS and SRS microscopy. *Bba-Mol Cell Biol L* 1841(8):1120-1129) transduce the desired signal without an exogenous label (fluorescent, radioactive, or otherwise) representing an essential step toward this goal. Many label-free methods require one of the interacting species to be either tethered or immobilized to the sensor surface, introducing a potential perturbation to the natural state of the species (Moreira et al. (2005).

[0004] However, back-scattering interferometry (BSI) is a free-solution label-free technique with the added benefit of sensitivity that rivals fluorescence (Bornhop et al. (2007) Free-solution, label-free molecular interactions studied by back-scattering interferometry. *Science* 317(5845):1732-1736). There are other techniques performed in free solution, such as mass spectrometry (MS) (Cubrilovic et al. (2014) Quantifying Protein-Ligand Binding Constants Using Electrospray Ionization Mass Spectrometry: A Systematic Binding Affinity Study of a Series of Hydrophobically Modified Trypsin Inhibitors. *J Am Soc Mass Spectr* 23(10):1768-1777; Kaltashov et al. (2012) Advances and challenges in analytical characterization of biotechnology products: Mass spectrometry-based approaches to study properties and behavior of protein therapeutics. *Biotechnol Adv* 30(1):210-222) and nuclear magnetic resonance (NMR) (Hu et al. (2004) The mode of action of centrin—Binding of Ca2+ and a peptide fragment of Karlp to the C-terminal domain. *J Biol Chem* 279(49):50895-50903; Tzeng and Kalodimos (2011) Protein dynamics and allostery: an NMR view. *Curr Opin Struc Biol* 21(1):62-67) and the widely used isothermal titration calorimetry (ITC) (Ababou and Ladbury (2007) Survey of the year 2005: literature on applications of isothermal titration calorimetry. *Journal of Molecular Recognition* 20(1):4-14; Liang, Y. (2006) Applications of isothermal titration calorimetry in protein folding and molecular recognition. *J Iran Chem Soc* 3(3):209-219). As with NMR, ITC has many advantages, but exhibits modest sensitivity and often requires large sample quantities. Another increasingly popular free-solution approach is micro-scale thermophoresis (MST). Yet, for MST to operate label-free, one of the binding partners must have a significant absorption/fluorescence cross-section (Wienken et al. (2010) Protein-binding assays in biological liquids using microscale thermophoresis. *Nat Commun* 1; Zhang et al. (2014) Microscale thermophoresis for the assessment of nuclear protein-binding affinities. *Methods Mol Biol* 1094:269-276). BSI represents an attractive alternative to these methods because of its high sensitivity, small sample volume requirement, optical simplicity and broad applicability (Baksh et al. (2011) Label-free quantification of membrane-ligand interactions using backscattering interferometry. Nat *Biotechnol* 29(4):357-360; Kussrow et al. (2012) Interferometric Methods for Label-Free Molecular Interaction Studies. *Anal Chem* 84(2):779-792; Olmsted et al. (2014) Toward Rapid, High-Sensitivity, Volume-Constrained Biomarker Quantification and Validation using Backscattering Interferometry. *Anal Chem* 86(15):7566-7574; Saetear et al. (2015) Quantification of *Plasmodium*-host protein interactions on intact, unmodified erythrocytes by back-scattering interferometry. *Malaria J* 14). Whereas ITC and MST have well known or established theoretical descriptions, the fundamental mechanistic basis for the signal observed in BSI is less well understood.

[0005] Accordingly, there remains a need in the art for systems and methods for free-solution, label-free detection of intermolecular interactions between analytes, preferably with low detection limits and/or low sample volume requirements.

SUMMARY

[0006] As embodied and broadly described herein, the invention, in one aspect, relates to free-solution analytical methods for use in the detection of molecular interactions between non-immobilized analytes and/or characteristic properties of a sample.

[0007] Disclosed are free-solution analytical methods comprising detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the detection is performed by refractive index sensing other than backscattering interferometry or by circular dichroism.

[0008] Also disclosed are free-solution analytical methods comprising the steps of: (a) providing a refractive index sensor for reception of a fluid sample to be analyzed; (b) introducing a first sample comprising a first non-immobilized analyte to be analyzed and a second sample comprising a second non-immobilized analyte to be analyzed onto the sensor, wherein the first analyte is allowed to interact with the second analyte; (c) interrogating the fluid sample with light; (d) detecting the light after interaction with the fluid sample, wherein the detected light is not backscattered; and (e) detecting a molecular interaction between the first and second analyte.

[0009] Also disclosed are free-solution analytical methods comprising detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the determination comprises determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion chanel or membrane protein, and wherein the determination is performed by refractive index sensing.

[0010] Also disclosed are free-solution analytical methods comprising determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion chanel or membrane protein, and wherein the determination is performed by refractive index sensing.

[0011] Also disclosed are systems comprising a refractive index sensor for detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, and a pressure change compensator.

[0012] Also disclosed are free-solution analytical methods comprising detecting a molecular change, wherein the detection is performed by refractive index sensing other than backscattering interferometry.

[0013] Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a disclosed method or system does not specifically state that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

BRIEF DESCRIPTION OF THE FIGURES

[0014] The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects and together with the description serve to explain the principles of the invention.

[0015] FIG. **1**A and FIG. **1**B show representative block diagrams of a refractometer (**1**A) and a forward scattering interferometer (**1**B).

[0016] FIG. **2** shows a representative block diagram of a circular dichroism spectrometer.

[0017] FIG. **3** shows a representative diagram of exemplary reference and sample contents.

[0018] FIG. **4**A and FIG. **4**B show representative data illustrating the A-form to B-form transition of the DNA duplex. Specifically, FIG. **4**A shows a representative CD spectra of the DNA duplex (inset shows the A-form to B-form transition monitored at 270 nm by ellipticity). FIG. **4**B shows a representative correlation for BSI signal and ellipticity.

[0019] FIG. **5**A and FIG. **5**B show representative data illustrating the predicted versus BSI experimental values for the CaM binding system (**5**A) and the correlation of χ_{exp} and Xmodel of the unsegregated learning set (**5**B).

[0020] FIG. **6**A-F show representative data related to the predicted dry/dc ARIU. Plots show the calculated dry/dc for the reference (○) and test (+) samples for the Concanavalin A-Mannose system (**6**A) the Calmodulin-Calcineurin system (**6**D), the predicted $d\eta/dc_{complex}$ signal compared with the experimentally observed signal for ConA-mannose (**6**B) and c=Calmodulin-calcineurin (**6**E), and the predicted $d\eta/dc_{complex}$ signal compared with the experimentally observed signal versus product concentration for ConA-mannose (**5**C) and Calmodulin-calcineurin (**6**F).

[0021] FIG. **7**A and FIG. **7**B show representative comparisons of experimental and modeled dry/dc signal. Specifically, plots showing the experimental BSI signal in RIU with the calculated dry/dc signal for recoverin binding Ca^{2+} (**7**A) and carbonic anhydrase II binding dansylamide (**7**B).

[0022] FIG. **8** shows a representative illustration of the procedure for using BSI to measure a binding affinity.

[0023] FIG. **9**A-F show representative BSI block diagrams showing the orientation of the beam relative to the chip (**7**A-C), a representative image of the fringe pattern (**7**D), a representative line profile of the region of interest for a good fringe pattern (**7**E), and a representative FFT spectrum for that region of interest (ROI) (**7**F).

[0024] FIG. **10**A and FIG. **10**B show representative fringe patterns with good (**7**A) and bad (**7**B) alignment.

[0025] FIG. **11**A and FIG. **11**B show representative images illustrating the optical modeling of the beam path for BSI. Specifically, FIG. **11**A shows a representative image of ten parallel rays impinged on a chip from the right that are allowed to refract and reflect and exit to the right and interfering. FIG. **11**B shows a representative image of a many beam optical ray trace of a semicircular channel in a microfluidic chip.

[0026] FIG. **12**A-D show representative ribbon drawings for Calmodulin unbound (PDB: 1CFD) (**12**A), bound to Calcium (PDB: 1OSA) (**12**B), bound to M13 (PDB: 1CDL) (**12**C), and bound to TFP (PDB: 1CTR) (**12**D).

[0027] FIG. **13**A and FIG. **13**B show representative plots showing the correlation of χ_{exp} and χ_{model} when the learning sets are split into small (**13**A) and large (**13**B) χ values.

[0028] FIG. **14**A and FIG. **14**B show representative flow diagrams for predicting the suitable model (small or large) for a binding pair (**14**A) and for predicting the model for the entire learning set (**14**B).

[0029] FIG. **15**A and FIG. **15**B show representative experimental and modeled FreeSRF binding curves for Recoverin-Ca^{2+} (**15**A) and carbonic anhydrase II-Dansylamide (**15**B).

[0030] FIG. **16**A-D show representative data illustrating Cyfra 21-1 binding CK19 as measured via a hand-held refractometer. Specifically, FIG. **16**A shows a representative image of a hand-held Reichert RI Detector. FIG. **16**B shows representative data illustrating the response of a hand-held RI detector for glycerol calibration standards. FIG. **16**C shows representative data illustrating label-free, free-solution detection of Cyfra 21-1 in PBS using a hand-held RI detector. FIG. **16**D shows representative data illustrating the comparison of signal at 50 ng/mL using a hand-held RI detector and a BSI detector.

[0031] FIG. **17**A and FIG. **17**B show representative images of recoverin before (**17**A) and after (**17**B) Ca^{2+} binding.

[0032] FIG. **18**A and FIG. **18**B show representative images of a RI detector (**18**A) and the flow path within a RI detector (**18**B).

[0033] FIG. **19** shows representative data illustrating the response of a RI detector for glycerol calibration standards.

[0034] FIG. **20** shows representative data illustrating label-free, free-solution detection of mannose in buffer using a RI detector.

[0035] FIG. **21**A and FIG. **21**B show representative data illustrating label-free, free-solution detection of benzene sulfonamide in buffer binding to 50 nM CAII (**21**A) and 10 nM CAII (**21**B) using a RI detector.

[0036] FIG. **22**A and FIG. **22**B show representative data illustrating label-free, free-solution detection of acetazolamide in buffer using a RI detector.

[0037] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or can be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

DETAILED DESCRIPTION

[0038] The present invention can be understood more readily by reference to the following detailed description of the invention and the Examples included therein.

[0039] Before the present compounds, compositions, articles, systems, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[0040] All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein can be different from the actual publication dates, which may need to be independently confirmed.

A. Definitions

[0041] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a substrate," "a polymer," or "a sample" includes mixtures of two or more such substrates, polymers, or samples, and the like.

[0042] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0043] As used herein, the terms "optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0044] As used herein, the term "polymer" refers to a relatively high molecular weight organic compound, natural or synthetic (e.g., polyethylene, rubber, cellulose), whose structure can be represented by a repeated small unit, the monomer (e.g., ethane, isoprene, β-glucose). Synthetic polymers are typically formed by addition or condensation polymerization of monomers.

[0045] As used herein, the term "copolymer" refers to a polymer formed from two or more different repeating units (monomer residues). By way of example and without limitation, a copolymer can be an alternating copolymer, a random copolymer, a block copolymer, or a graft copolymer.

[0046] As used herein, the term "bioassay" refers to a procedure for determining the concentration, purity, and/or biological activity of a substance.

[0047] As used herein, the term "chemical event" refers to a change in a physical or chemical property of an analyte in a sample that can be detected by the disclosed systems and methods. For example, a change in refractive index (RI), solute concentration and/or temperature can be a chemical event. As a further example, a biochemical binding or association (e.g., DNA hybridization) between two chemical or biological species can be a chemical event. That is, a chemical event can be the formation of one or more interaction products of the interaction of a first analyte with a second analyte. As a further example, a disassociation of a complex or molecule can also be detected as an RI change. As a further example, a change in temperature, concentration, and association/dissociation can be observed as a function of time. As a further example, bioassays can be performed and can be used to observe a chemical event.

[0048] As used herein, the terms "equilibrium constant" and "Kc" and "Keq" refer to the ratio of concentrations when equilibrium is reached in a reversible reaction. For example, for a general reaction given by the equation:

$$aA+bB \leftrightharpoons cC+dD,$$

the equilibrium constant can be expressed by:

$$K_c = \frac{[C]^c[D]^d}{[A]^a[B]^b}.$$

[0049] An equilibrium constant can be temperature- and pressure-dependent but has the same value, irrespective of the amounts of A, B, C, and D. A specific type of equilibrium constant that measures the propensity of a larger object to separate (dissociate) reversibly into smaller components is a

"dissociation constant" or "Kd." A dissociation constant is the inverse of an "affinity constant."

[0050] As used herein, the term "dissociation rate" is a concentration dependent quantity and involves the "dissociation rate constant" or "K_D." The dissociation rate constant relates the rate at which molecules dissociate to the concentration of the molecules. A dissociation can be described as AB→A+B, and the rate of dissociation (dissociation rate) is equal to K_D[AB]. In general, the larger the value of K_D, the faster the inherent rate of dissociation.

[0051] As used herein, the term "association rate" is a concentration dependent quantity and involves the "association rate constant" or "K_A." The association rate constant relates the rate at which molecules associate to the concentration of the molecules. An association can be described as A+B→AB, and the rate of association (association rate) is equal to K_A[A][B]. In general, the larger the value of K_A, the faster the inherent rate of association.

[0052] As used herein, the term "free-solution" refers to a lack of surface immobilization. The term is not meant to exclude the possibility that one or more molecules or atoms of analyte may associate with a surface. Rather, the term can describe the detection of an analyte without the requirement for surface immobilization during analysis.

[0053] As used herein, the terms "label-free" and "unlabeled" describe a detection method wherein the detectability of an analyte is not dependent upon the presence or absence of a detectable label. For example, "label-free" can refer to the lack of a detectable label. It is understood that the ability of a label to be detected can be dependent upon the detection method. That is, an analyte having a moiety capable of serving as a detectable label for a first detection method can be considered "label-free" or "unlabeled" when a second detection method (wherein the label is not detectable) is employed. In a further aspect, the analytes employed in the disclosed systems and methods can lack detectable labels.

[0054] As used herein, the term "detectable label" refers to any moiety that can be selectively detected in a screening assay. Examples include without limitation, radiolabels (e.g., ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{131}I) affinity tags (e.g. biotin/avidin or streptavidin), metal binding domains, epitope tags, FLASH binding domains (see U.S. Pat. Nos. 6,451,569; 6,054,271; 6,008,378 and 5,932,474), glutathione or maltose binding domains, photometric absorbing moieties, fluorescent or luminescent moieties (e.g. fluorescein and derivatives, GFP, rhodamine and derivatives, lanthanides etc.), and enzymatic moieties (e.g. horseradish peroxidase, β-galactosidase, β-lactamase, luciferase, alkaline phosphatase). Such detectable labels can be formed in situ, for example, through use of an unlabeled primary antibody which can be detected by a secondary antibody having an attached detectable label. Further examples include imaging agents such as radioconjugate, cytotoxin, cytokine, Gadolinium-DTPA, a quantum dot, iron oxide, and manganese oxide.

[0055] Disclosed are the components to be used to prepare the compositions of the invention as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc., of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular compound is disclosed and discussed and a number of modifications that can be made to a number of molecules including the compound are discussed, specifically contemplated is each and every combination and permutation of the compound and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the compositions of the invention. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific aspect or combination of aspects of the methods of the invention.

[0056] It is understood that the compositions disclosed herein have certain functions. Disclosed herein are certain structural requirements for performing the disclosed functions, and it is understood that there are a variety of structures that can perform the same function that are related to the disclosed structures, and that these structures will typically achieve the same result.

B. Refractive Sensing

[0057] In one aspect, disclosed are systems comprising a refractive index sensor for detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, and a pressure change compensator. In a further aspect, both analytes are unlabeled. In a still further aspect, at least one of the analytes is present in an amount of less than about 1.0×10^{-3} M.

[0058] In various aspects, refractive sensing refers to the measurement of the refractive index of a sample, for example, a fluid sample. The refractive index or change in refractive index of a sample can be used to determine a variety of biologically important measurements including, but not limited to, an equilibrium constant, a dissociation constant, a dissociation rate, an association rate, a concentration of an analyte, and the presence of an analyte. Refractive index measurements are also used in other applications such as, for example, process control and the detection of explosives (Bowen et al. (2003) "Gas phase detection of trinitrotoluene utilizing a solid-phase antibody immobilized on a gold film by means of surface plasmon resonance spectroscopy" Appl. Spectrosc. 57(8): 906-914).

[0059] Various devices and techniques for measuring refractive index are known. These include the Abbe-type refractometer (see FIG. **1**A), and sensors based on surface plasmon resonance. Optical waveguides can also be used. The presence of a liquid adjacent to an optical waveguide can alter the effective modal index of light propagating within the waveguide. This modification of index can be measured using techniques that are sensitive to changes in optical path length. For example, interferometer structures have been used to measure index changes and hence to sense the presence of proteins (Heideman et al. (1993) "Performance of a highly sensitive optical wave-guide Mach-

Zehnder interferometer immunosensor" *Sensors and Actuators B-Chemical* 10(3): 209-217) (see FIG. **1**B).

[0060] Referring to FIG. **1**A, a block diagram of an exemplary Abbe-type refractometer is illustrated. As shown, the sample is contained between two prisms, the illuminating prism and the measuring prism. The light source generates light, which enters the sample from the illuminating prism. The surface of the illuminating prism is matted, so that light enters the sample at all possible angles, including those almost parallel to the surface. The light is then refracted at the critical angle at the bottom surface of the measuring prism and directed into the telescope. Additionally, two Amici prisms that can be rotated are located within the telescope (not shown), which can be used to correct the dispersion. The telescope is used to measure the position of the border between dark and light areas. Knowing the angle and refractive index of the measurement prism allows for the refractive index of the sample to be calculated.

[0061] Referring to FIG. **1**B, a block diagram of an exemplary Michelson interferometer is illustrated. As shown, a coherent light source emits light that hits a beam splitter. A portion of the light is transmitted directly through beam splitter to mirror A, while some is reflected in the direction of mirror B. Both beams are then reflected back onto the beam splitter to produce an interference pattern incident on the detector. If an angle is observed between the two returning beams the detector will record a sinusoidal fringe pattern. Alternatively, if there is perfect spatial alignment between the two returning beams, the detector will record a constant intensity over the beam dependent on the differential path length.

[0062] Accordingly, the disclosed invention provides a method for detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the detection is performed by refractive index sensing other than backscattering interferometry. Examples of refractive index (RI) detectors include, but are not limited to, RI detectors based on the angle of deviation method of measurement, RI detectors based on the Fresnel method of RI measurement, a Christiansen effect detector, an interferometer detector, or a differential refractometer detector. Additional examples include a refractomax 521 RI detector, a RID-20A RI detector, a RID-10A RI detector, a Waters RI detector, a Waters RI detector, a Wyatt RI detector, a HPLC, an Acquity RI detector, a 1260 Infinity RI detector, an Optilab RI detector, a Knauer RI detector, a Shimadzu RI detector, a Shodex RI detector, a LC-4000 Series RI detector, or other suitably sensitive RI detectors.

[0063] In a further aspect, detection is performed by refractive index sensing other than forward scattering or side scattering interferometry.

[0064] In various aspects, the first and/or second analyte may be contained in, e.g. flowed through, a capillary dimensioned flow channel such as a capillary tube. The cross-sectional depth of the channel is limited only by the coherence length of the light and its breadth is limited only by the width of the light beam. Preferably, the depth of the channel is from 1 to 10 μm, but it may be from 1 to 20 μm or up to 50 μm or more, e.g. up to 1 mm or more. However, sizes of up to 5 mm or 10 mm or more are possible. Suitably, the breadth of the channel is from 0.5 to 2 times its depth, e.g., equal to its depth. In various aspects, the channel may comprise a substantially circular, generally semi-circular, or rectangular cross-section.

[0065] The sample is typically a liquid, and can be flowing or stationary. However, the sample can also be a solid or a gas in various aspects of the present invention. The first and/or further materials will normally be solid but in principle can be liquid, e.g., can be formed by a sheathing flow of guidance liquid(s) in a microfluidic device, with the sample being sheathed flow of liquid between such guidance flows. The sample may also be contained in a flow channel of appropriate dimensions in substrate such as a microfluidic chip. The method may therefore be employed to obtain a read out of the result of a reaction conducted on a "lab on a chip" type of device.

[0066] The invention includes apparatus for use in performing a method as described, which apparatus comprises a source of spatially coherent light, a sample holder for receiving a sample upon which to perform the method positioned in a light path from the light source, a detector for detecting light, and data processing means for receiving measurements of light intensity from the detector and for conducting an analysis thereon, wherein the analysis comprised determining an equilibrium constant, a dissociation constant, a dissociation, rate an association rate, calculating a change in hydrodynamic volume, entropy, or enthalpy, the concentration of the first and/or second analyte, identifying the presence of the first and/or second analyte, or identifying the presence of a third analyte. In various aspects, the analysis comprises quantification of the sample.

[0067] In a further aspect, the RI sensor comprises a channel formed in a substrate, the channel has a longitudinal direction and a transverse direction, and a light source for generating a light, wherein the light is elongated in the longitudinal direction of the channel

[0068] In various aspects, the channel of the present invention can be formed from a substrate such as a piece of silica or other suitable optically transmissive material. In various aspects, the material of composition of the substrate has a different index of refraction than that of the sample to be analyzed. In a further aspect, as refractive index can vary significantly with temperature, the substrate can optionally be mounted and/or connected to a temperature control device. In a still further aspect, the substrate can be tilted, for example, about 7°, such that scattered light from channel can be directed to a detector.

[0069] In a further aspect, the channel has a generally semi-circular cross-sectional shape. A unique multi-pass optical configuration is inherently created by the channel characteristics, and is based on the interaction of the unfocused laser beam and the curved surface of the channel that allows interferometric measurements in small volumes at high sensitivity. Alternatively, the channel can have a substantially circular or generally rectangular cross-sectional shape. In a still further aspect, the substrate and channel together comprise a capillary tube. In yet a further aspect, the substrate and channel together comprise a microfluidic device, for example, a silica substrate, or a polymeric substrate [e.g., polydimethylsiloxane (PDMS) or polymethyl methacrylate (PMMA)], and an etched channel formed in the substrate for reception of a sample, the channel having a cross sectional shape. In an even further aspect, the cross sectional shape of a channel is semi-circular. In a still further aspect, the cross sectional shape of a channel is square, rectangular, or elliptical. In yet a further aspect, the cross sectional shape of a channel can comprise any shape suitable for use in a BSI technique. In an even further aspect, a

substrate can comprise one or multiple channels of the same or varying dimensions. In various aspects, the channel can have a radius of from about 5 to about 250 micrometers, for example, about 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, or 250 micrometers. In still other aspects, the channel can have a radius of up to about 1 millimeter or larger, such as, for example, 0.5 millimeters, 0.75 millimeters, 1 millimeter, 1.25 millimeters, 1.5 millimeters, 1.75 millimeters, 2 millimeters, or more.

[0070] In various aspects, the source of coherent light is a laser, suitably a He—Ne laser or a diode laser or VCSEL. The laser light may be coupled to the site of measurement by known wave-guiding techniques or may be conventionally directed to the measurement site by free space transmission.

[0071] In various aspects, the detected light is representative of the refractive index of the sample. The measured refractive index can be indicative of a number of properties of the sample including, but not limited to, the presence or concentration of a solute substance, e.g., a reaction product, pressure, temperature, or flow rate (e.g., by determining when a thermal perturbation in a liquid flow reaches a detector).

[0072] In one aspect, the detector is a CCD array of suitable resolution.

[0073] The apparatus can comprise means for controlling the temperature of the sample, e.g., a heater and/or a Peltier cooler and a temperature measuring device.

[0074] The invention includes apparatus as described herein, wherein the sample holder is configured to allow a sample to flow there through and wherein the sample holder is connected to receive a separated sample from a sample separation device in which components of a mixed sample are separated, e.g., by capillary electrophoresis, capillary electrochromatography, or HPLC. Accordingly, viewed from another perspective, the invention provides chromatography apparatus having a refractive index measuring unit as described herein as a detector.

[0075] More generally, the sample holder of the apparatus described above can be a flow through passage so that the contents of the channel may be continuously monitored to observe changes in the content thereof. These changes may include the temporary presence of cells and the out flow from the sample holder may be diverted to a selected one of two or more outlet channels according to the measurements of refractive index observed in the sample holder, e.g., to achieve sorting of cells in response to such measurements. The sample holder can contain a stationary analytical reagent (e.g., a coating of an antibody, oligonucleotide, or other selective binding agent) and changes in the refractive index caused by the binding of a binding partner to the reagent may be observed. In view of the small sample size which it is possible to observe, the sample holder can contain a biological cell and metabolic changes therein may be observed as changes in the refractive index of the cell.

[0076] Thus, in various aspects, the sample solution and the reference solution may be picked up individually into a cell such as, for example, a capillary tube. Each sample is then loaded into a tray for reading. In a further aspect, the tray can have several cells (e.g., capillaries) integrated into it. In this way, the samples are delivered to the individual cells for introduction into the sensor for analysis.

[0077] In one aspect, the invention relates to a method for detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the detection is performed by refractive index sensing other than backscattering interferometry, wherein the detection comprises determining refractive index variations in the intensity of reflections of light which has passed through the first and second analyte. In a further aspect, detection is performed by refractive index sensing other than forward scattering or side scattering interferometry.

[0078] Refractive index can be negatively affected by changes in pressure. Moreover, as the concentration of the analyte decreases, even smaller changes in pressure can have a significantly greater impact. Thus, in various aspects, the system may comprise a pressure change compensator. A pressure change compensator can balance the pressure inside and outside of the detection system by compensating for variations in the volume of the liquid within the system, which may be due to variations in the ambient pressure and/or temperature. Examples of pressure compensators include, but are not limited to, a back-pressure restrictor and a capillary restrictor.

[0079] A change in environmental temperature can also negatively impact a RI detector. Thus, in various aspects, the system may comprise a temperature change compensator. A temperature change compensator can balance the temperature inside and outside of the detection system by compensating for variations in the temperature of the liquid within the system, which may be due to, for example, variations in the ambient pressure and/or temperature. Alternatively, the temperature change compensator can change the temperature of the incoming mobile phase to match that of the solvent in the detector. Examples of temperature compensators include, but are not limited to, a thermostat cabinet, a thermoelectric temperature controller (e.g., Peltier) and a heat exchanger.

C. Circular Dichroism

[0080] Circular dichroism (CD) is the difference in the absorption of left-handed circularly polarized light (L-CPL) and right-handed circularly polarized light (R-CPL) and occurs when a molecule contains one or more chiral chromophores (light-absorbing groups). CD has a wide range of applications including, but not limited to, analyzing the structure of small molecules, DNA, peptides, nucleic acids, carbohydrates, and proteins, identifying charge-transfer transitions, determining geometric and electronic structure, and analyzing molecular interactions. CD spectra are measured using a circular dichroism spectrometer (see FIG. **2**).

[0081] Accordingly, the disclosed invention provides a method for detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the detection is performed by circular dichroism.

[0082] Referring to FIG. **2**, a block diagram of an exemplary CD spectrometer is illustrated. As shown, the light source emits light that hits the monochromator (MC). A narrow band of wavelengths then pass through the linear polarizer (LP), which splits the unpolarized monochromatic beam into two linearly polarized beams. Next, one of the two linearly polarized beams passes through the photoelastic modulator (PEM), which consists of a plate made of a transparent, optically isotropic material bonded to a piezoelectric quartz crystal. When an alternating electric field is applied, the light emerging from the PEM switches from L-CPL to R-CPL and back with the frequency of the applied electric field. If the sample exhibits CD, the amount of light

absorbed varies periodically with the polarization of the incident light. This, in turn, causes the intensity of the light that reaches the detector to exhibit sinusoidal intensity variations at the frequency of the field applied. Thus, the detector output consists of a signal with a small alternating current (AC) component superimposed on a direct current (DC) component. The AC component is filtered out and amplified. The ratio of the AC to the DC component is directly proportional to the circular dichroism of the sample, and this quantity is recorded as a function of wavelength to provide a CD spectrum.

D. Methods for Free-Solution Determination of Molecular Interactions

[0083] In contrast to conventional techniques that observe immobilized analytes—which necessarily limit conformational and translational freedom for analytes and are, thus, in vitro measurements—free-solution analysis techniques mimic in vivo measurements, because analytes enjoy unrestricted freedom in all three dimensions during measurement.

[0084] In one aspect, disclosed are free-solution analytical methods comprising detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the detection is performed by refractive index sensing other than backscattering interferometry or by circular dichroism. In a further aspect, detection is performed by refractive index sensing other than forward scattering or side scattering interferometry.

[0085] In one aspect, disclosed are free-solution analytical methods comprising the steps of: (a) providing a refractive index sensor for reception of a fluid sample to be analyzed; (b) introducing a first sample comprising a first non-immobilized analyte to be analyzed and a second sample comprising a second non-immobilized analyte to be analyzed onto the sensor, wherein the first analyte is allowed to interact with the second analyte; (c) interrogating the fluid sample with light; (d) detecting the light after interaction with the fluid sample, wherein the detected light is not backscattered; and (e) detecting a molecular interaction between the first and second analyte.

[0086] In one aspect, disclosed are free-solution analytical methods comprising detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the determination comprises determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion chanel or membrane protein, and wherein the determination is performed by refractive index sensing. In a further aspect, the method comprises the steps of: (a) providing a refractive index sensor for reception of a fluid sample to be analyzed; (b) introducing a first sample comprising a first non-immobilized analyte to be analyzed and a second sample comprising a second non-immobilized analyte to be analyzed onto the sensor, wherein the first analyte is allowed to interact with the second analyte to form one or more interaction products; (c) interrogating the fluid sample with light; (d) detecting the deflected and/or refracted light after interaction with the fluid sample, wherein the light is not backscattered; and (e) detecting a molecular interaction between the first and second analyte.

[0087] In one aspect, disclosed are free-solution analytical methods comprising determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion chanel or membrane protein, and wherein the determination is performed by refractive index sensing. In a further asepct, refractive index sensing is not via backscattering interferometry.

[0088] In a further aspect, the light is not scattered.

[0089] In a further aspect, refractive index sensing is via a refractometer. In a still further aspect, refractive index sensing is via interferometry. In yet a further aspect, refractive index sensing is via forward scattering interferometry. In an even further aspect, refractive index sensing is via backscattering interferometry. In a still further aspect, refractive index sensing is via a hand-held refractive index sensing device.

[0090] It is contemplated that the method can be used to determine, for example, one or more of an equilibrium constant, a dissociation constant, a dissociation rate, a dissociation rate constant, an association rate, and/or an association rate constant of the interaction. In a further aspect, the method can be used to determine, for example, the concentration of the first and/or second analyte. In a still further aspect, the method can be used to determine, for example, the presence of the first and/or second analyte. In yet a further aspect, the method can be used to determine, for example, the presence of a third analyte.

[0091] Each of the one or more analytes can be introduced onto the sensor in a sample. Two or more analytes can be present in the same or in different samples. Each of the one or more analytes can independently be present in a suitable concentration, for example, a concentration of less than about 5.0×10^{-4}M, of less than about 1.0×10^{-4}M, of less than about 5.0×10^{-5}M, of less than about 1.0×10^{-5}M, of less than about 5.0×10^{-6}M, of less than about 1.0×10^{-6}M, of less than about 5.0×10^{-7}M, of less than about 1.0×10^{-7}M, a concentration of less than about 5.0×10^{-8}M, of less than about 1.0×10^{-8}M, of less than about 5.0×10^{-9}M, of less than about 1.0×10^{-9}M, of less than about 1.0×10^{-1o} M, of less than about 5.0×10^{-10} M of less than about 5.0×10^{-11}M, of less than about 1.0×10^{-11}M, of less than about 5.0×10^{-12}M, of less than about 1.0×10^{-12}M, of less than about 5.0×10^{-13}M, of less than about 1.0×10^{-13}M, of less than about 5.0×10^{-14}M, of less than about 1.0×10^{-14}M, of less than about 5.0×10^{-15}M, or of less than about 1.0×10^{-15}M.

[0092] In one aspect, the interaction can be a biomolecular interaction. For example, two analytes can associate to provide an interaction product (e.g., adduct, complex, or new compound). In a still further aspect, an analyte can dissociate to provide two or more interaction products. In yet a further aspect, more than two analytes can be involved in the interaction.

[0093] In a further aspect, the first and/or second analyte is a complex. In a still further aspect, the complex is a chemical or biochemical complex. In yet a further aspect, the complex was formed prior to the introducing step. In an even further aspect, the complex was formed subsequent to the introducing step.

[0094] The disclosed techniques can determine the interaction between one or more analytes by monitoring, measuring, and/or detecting the formation and/or steady state relative abundance of one or more analyte interaction products from the interaction of the one or more analytes. The determination can be performed qualitatively or quantita-

tively. Interaction rate information can be derived from various measurements of the interaction.

[0095] In a further aspect, the first sample is combined with the second sample prior to introduction. That is, the analytes are combined (and potentially interacting) prior to performing the disclosed methods. In this aspect, the step of introducing the first sample and the step of introducing the second sample are performed simultaneously.

[0096] In a further aspect, the first sample is combined with the second sample after introduction. That is, the analytes can be combined at a point before the sensor, or at a point on the sensor, when performing the disclosed methods. In this aspect, the step of introducing the first analyte and the step of introducing the second analyte are performed either simultaneously or sequentially. In a further aspect, the detecting step is performed during the interaction of the first analyte with the second analyte.

[0097] Thus, in various aspects, the first and second samples are introduced simultaneously. In a further aspect, the first and second samples are introduced sequentially. In a still further aspect, the first analyte is allowed to interact with the second analyte prior to introducing the first and/or second sample onto the sensor. In yet a further aspect, the first analyte is allowed to interact with the second analyte after introducing the first and/or second sample onto the sensor. In an even further aspect, the first analyte is allowed to interact with the second act while introducing the first and/or second sample onto the sensor.

[0098] A first sample (e.g., a solution including a first non-immobilized analyte to be analyzed) can be introduced onto the refractive index sensor. The first sample can be provided having a known concentration of the first analyte. A baseline response can then be established by directing light onto the first sample.

[0099] A second sample (e.g., a solution including a second non-immobilized analyte to be analyzed) can then be introduced onto the refractive index sensor. In various aspects, the second sample can be provided as a pre-mixed sample of the first non-immobilized analyte and the second non-immobilized analyte or provided by adding a sample comprising the second non-immobilized analyte to the first sample. In one aspect, the first sample is a solution of the first analyte, which is displaced on the sensor by the introduction of the second sample, which is a solution of both the first analyte and the second analyte. The second sample can be provided having a known concentration of the first analyte, which can be the same as the concentration of the first analyte in the first solution. The second sample can also be provided having a known concentration of the second analyte. Light can then be directed onto the sensor.

[0100] In various aspects, a reference sample can be introduced onto the refractive index sensor. The reference sample can be introduced onto the refractive index sensor prior to or subsequent to introduction of the first and/or second sample. In a further aspect, the reference sample comprises a first non-immobilized analyte to be analyzed. In a still further aspect, the method further comprises the steps of: (a) interrogating the reference sample with light; (b) detecting the light after interaction with the reference sample, wherein the detected light is not backscattered; (c) determining a characteristic of the reference sample; and (e) employing the characteristic of the reference sample to compensate for background interference effects in the determination of the molecular interaction between the first and second analyte. Exemplary reference samples are illustrated in FIG. **3**.

[0101] In various aspects, the reference sample can be a ligand alone in the absence of the matrix, more often referred to as a blank. Reference samples are typically comprised of the ligand in the experimental matrix (cell vesicle, cell lysate, serum, urine, other biofluids, etc.) that does not contain the receptor. Examples include, but are not limited to, cell-based matrices that do not contain the receptor, null lipoparticles (devoid of the target receptor), empty viral particle/bacteriophage scaffolds, and an experimental matrix stripped of receptor.

[0102] In various aspects, the reference sample can be the experimental matrix containing the receptor with a non-binding analyte molecule that is similar to the ligand. Examples include, but are not limited to, a denatured ligand, an isotype matched antibody for a different compound, a compound with a similar structural backbone as the ligand that has additional or removed functional groups (e.g., phenol versus 2,4,6-trinitrophenol, dopamine versus 3-methoxytyramine, tyrosine versus 3-nitrotyrosine, serotonin versus tryptophan, histamine versus histidine), a nonsense nucleic acid strand of the same length, a nucleic acid stand with >3 base-pair mismatch, a compound of similar size as the ligand known to not bind the receptor, and other molecules known to non-specifically bind to the matrix (e.g., cholesterol for membrane based experiments).

[0103] In various aspects, the reference sample can be the ligand with the experimental matrix containing the receptor (cell-based matrices, tissues-based matrices, serum, urine, other biofluids, etc.) that has been treated to inhibit binding. Examples include, but are not limited to, enzyme treatment of the receptor, blocking of the receptor with an antibody, blocking of the receptor with a known binding compound, blocking of the receptor with an inhibitor, denaturation of the receptor, receptor without the cofactors necessary for binding, (e.g., calmodulin without Ca^{2+}, concanavalin A without Mn^{2+} and Ca^{2+}, recoverin without Ca^{2+}, etc.).

[0104] In a further aspect, the first analyte and/or the second analyte is/are unlabeled. While the disclosed methods can be used in connection with unlabeled analytes, it is contemplated that the analytes can be optionally labeled. Such labeling can be convenient for preceding, subsequent, or simultaneous analysis by other analytical methods. In a still further aspect, at least one of the analytes is unlabeled. In yet a further aspect, both analytes are unlabeled.

[0105] In a further aspect, the the first and/or second analyte is one or more of an antibody, an antigen, a protein, a small molecule, a drug, a receptor, a cell, an oligonucleotide, a carbohydrate, an enzyme, a substrate, a DNA, an aptamer, a RNA, a nucleic acid, a biomolecule, a molecular imprint, a protein mimetic, an antibody derivative, a lectin, a cell membrane, an ion, a virus particle, a bacteria, and a micro-RNA.

[0106] In a further aspect, detecting a molecular interaction comprises determining a change in a physical or chemical property of the first and/or second sample. In a still further aspect, the change in a physical or chemical property of the first and/or second sample corresponds to the formation of one or more interaction products.

[0107] In a further aspect, detecting a molecular interaction comprises determining an equilibrium constant, a dissociation constant, a dissociation rate, or an association rate.

In a still further aspect, detecting comprises determining the concentration of the first and/or second analyte. In yet a further aspect, detecting comprises identifying the presence of a third analyte. In an even further aspect, calculating a change in hydrodynamic volume, entropy, or enthalpy.

[0108] In a further aspect, detecting a molecular interaction comprises determining a change in a physical or chemical property of the fluid sample. In a still further aspect, the change in physical or chemical property of the fluid sample corresponds to the formation of one or more interaction products.

[0109] In a further aspect, the molecular interaction is the formation of one or more covalent bonds, electrostatic bonds, hydrogen bonds, or hydrophobic interactions. In a further aspect, the molecular interaction is a binding event between one or more of antibody-antigen, protein-protein, small molecule-small molecule, small molecule-protein, drug-receptor, antibody-cell, virus-cell, virus-protein, bacteria-cell, bacteria-protein, virus-DNA, virus-RNA, bacteria-DNA, bacteria-RNA, protein-cell, oligonucleotide-cell, carbohydrate-cell, cell-cell, enzyme-substrate, protein-DNA, protein-aptamer, DNA-DNA, RNA-DNA, DNA-RNA, protein-RNA, small molecule-nucleic acid, biomolecule-molecular imprint, biomolecule-protein mimetic, biomolecule-antibody derivatives, lectin-carbohydrate, biomolecule-carbohydrate, small molecule-cell membrane, ion-protein, and protein-protein.

[0110] In a further aspect, the molecular interaction comprises one or more of a change in conformational structure of the first and/or second analyte and a change in hydration of the first and/or second analyte. In a still further aspect, the molecular interaction comprises a change in conformational structure of the first and/or second analyte. In yet a further aspect, the molecular interaction comprises a change in hydration of the first and/or second analyte. In an even further aspect, the molecular interaction comprises a change in conformational structure of the first and/or second analyte and a change in hydration of the first and/or second analyte.

[0111] In a further aspect, the molecular interaction lacks a change in mass. In a still further aspect, the molecular interaction is a chemical reaction.

[0112] In various aspects, the first and/or second sample is a fluid sample. In a further aspect, a fluid sample can comprise at least one of a liquid or a gas. In particular aspects, a fluid sample comprises a solution of one or more analytes and one or more liquid solvents. A solution can be provided in an organic solvent or in water. In certain aspects, the solution can comprise man-made preparations or naturally occurring substances. In certain aspects, the solution can comprise a body fluid (e.g., peripheral blood, urine, cerebrospinal fluid, pulmonary lavage, gastric lavage, bile, vaginal secretions, seminal fluid, aqueous humor, vitreous humor, serum, and saliva) from a human, a mammal, another animal, or a plant.

[0113] In a further aspect, the refractive index sensor comprises a prism.

[0114] In a further aspect, the refractive index sensor comprises a substrate having a first channel formed therein for reception of a fluid sample to be analyzed and wherein the first and second analyte are introduced into the channel. Generally, the substrate and channel can comprise any material suitable for containing and providing a sample for analysis and capable of being interrogated by light. In one aspect, the substrate and channel together comprise a capillary tube. In a further aspect, wherein the substrate and channel together comprise a microfluidic device.

[0115] In a further aspect, the microfluidic device comprises a polymeric substrate and an etched channel formed in the substrate for reception of a fluid sample, the channel having a cross sectional shape. In a further aspect, the polymeric substrate can be selected from rigid and transparent plastics. In various further aspects, the polymeric substrate comprises one or more polymers selected from polycarbonate, polydimethylsiloxane, fluorosilicone, polytetrafluoroethylene, poly(methyl methacrylate), polyhexamethyldisilazane, polypropylene, starch-based polymers, epoxy, and acrylics.

[0116] In a further aspect, the microfluidic device comprises a silica substrate and an etched channel formed in the substrate for reception of a fluid sample, the channel having a cross sectional shape, which can be substantially circular, substantially semi-circular, or substantially rectangular, as disclosed herein.

[0117] In a further aspect, the first channel is configured for reception of two or more fluid samples by having at least two inlets positioned at opposing locations of the channel, and at least one outlet positioned at a point between the at least two inlets, thereby defining a right side of the channel and a left side of the channel and wherein the first sample is introduced into the right side of the channel and the second sample is introduced into the left side of the channel.

[0118] It is contemplated the substrate can comprise one or more than one channel. Thus, in a further aspect, the substrate further comprises a second channel. In a still further aspect, the first analyte is introduced into the first channel and the second analyte is introduced into the second channel. In yet a further aspect, the first and second analyte are introduced into the first channel.

[0119] In a further aspect, the substrate further comprises a reference channel. In a still further aspect, the method further comprises the steps of: (a) introducing a reference sample; (b) interrogating the reference sample with light; (c) detecting the light after interaction with the reference sample, wherein the detected light is not backscattered; (d) determining a characteristic of the reference sample; and (e) employing the characteristic of the reference sample to compensate for background interference effects in the determination of the molecular interaction between the first and second analyte in the first channel.

[0120] The disclosed methods can provide real-time, free-solution detection of molecular interactions with very low detection limits. That is, in one aspect, the invention relates to a free-solution analytical method for detecting molecular interactions comprising the step of detecting a molecular interaction between two non-immobilized analytes, wherein at least one of the analytes is present during the determination at a concentration of less than about 5.0×10^{-4}M. In various further embodiments, the concentration can be less than about 1.0×10^{-4} M, for example, less than about 5.0×10^{-5}M, less than about 1.0×10^{-5}M, less than about 5.0×10^{-6}M, less than about 1.0×10^{-6}M, less than about 5.0×10^{-7}M, less than about 1.0×10^{-7}M, less than about 5.0×10^{-8}M, less than about 1.0×10^{-8} M, less than about 5.0×10^{-9}M, or less than about 1.0×10^{-9}M. In a further aspect, the concentration can be less than about 5.0×10^{-10} M, for example, less than about 1.0×10^{-10} M, less than about 5.0×10^{-11}M, less than about 1.0×10^{-11}M, less than about 5.0×10^{-12} M, less than about 1.0×10^{-12}M, less than about 5.0×10^{-13}M, less than

about 1.0×10^{-13} M, less than about 5.0×10^{-14}M, less than about 1.0×10^{-14}M, less than about 5.0×10^{-15} M, or less than about 1.0×10^{-15}M.

[0121] The disclosed methods can provide real-time, free-solution detection of molecular interactions with very low sample volume requirements. That is, in one aspect, the invention relates to a free-solution analytical method for detecting molecular interactions comprising the step of detecting a molecular interaction between two non-immobilized analytes, wherein at least one of the analytes is present during the determination in a solution with a volume in the detection zone of less than about 500 μL. In various further embodiments, the sample volume can be less than about 250 μL, for example, less than about 100 μL, less than about 10 μL, less than about 1 μL, less than about 500 nL, less than about 250 nL, less than about 100 nL, less than about 10 nL, less than about 1 nL, less than about 500 pL, less than about 250 pL, or less than about 100 pL.

[0122] In a further aspect, the first and/or second sample comprises an additive. In a still further aspect, the additive is selected from an alcohol, an acid, a base, a high refractive index solvent, a surfactant, and an intercalating agent. In yet a further aspect, the alcohol is deuterated. In an en even further aspect, the alcohol is fluorous.

[0123] In a further aspect, the disclosed methods further comprise the step of determining a change in refractive index. In a still further aspect, the change in refractive index is at least about 10^{-3} RIU. In yet a further aspect, the change in refractive index is at least about 10^{-4} RIU. In an even further aspect, the change in refractive index is at least about 10^{-5} RIU. In a still further aspect, the change in refractive index is at least about 10^{-6} RIU. In yet a further aspect, the change in refractive index is at least about 10^{-7} RIU. In an even further aspect, the change in refractive index is at least about 10^{-8} RIU.

[0124] In a further aspect, the disclosed methods further comprise the step of performing a chromatographic separation and/or an electrophoretic separation on the sample before, during, or after the determining the determining step. In a still further aspect, the method further comprises the step of performing a chromatographic separation or an electrophoretic separation on the sample prior to the determining the determining step. In yet a further aspect, the method further comprises performing a chromatographic separation or an electrophoretic separation on the sample during the determining the determining step. In an even further aspect, the method further comprises performing a chromatographic separation or an electrophoretic separation on the sample after the determining the determining step. In a still further aspect, the method further comprises performing a chromatographic separation and an electrophoretic separation on the sample prior to the determining the determining step. In yet a further aspect, the method further comprises performing a chromatographic separation and an electrophoretic separation on the sample during the determining the determining step. In an even further aspect, the method further comprises performing a chromatographic separation and an electrophoretic separation on the sample after the determining the determining step.

E. Methods for Determining a Characteristic Property of a Sample

[0125] In one aspect, disclosed are methods for determining a characteristic property of a sample, the method comprising the steps of: (a) providing a refractive index sensor for reception of a fluid sample to be analyzed; (b) introducing a fluid sample to be analyzed onto the sensor; (c) interrogating the fluid sample with light; (d) detecting the light after interaction with the fluid sample, wherein the detected light is not backscattered; and (e) determining the characteristic property of the sample. In a further aspect, the fluid sample to be analyzed comprises an analyte. In a still further aspect, the analyte is non-immobilized. In yet a further aspect, the analyte is unlabeled.

[0126] In one aspect, disclosed are free-solution analytical method comprising detecting a molecular change, wherein the detection is performed by refractive index sensing other than backscattering interferometry. In a further aspect, the detection is performed by refractive index sensing other than forward scattering or side scattering interferometry. In a still further aspect, detecting a molecular change comprises determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion Chanel or membrane protein.

[0127] In a further aspect, the light is not scattered.

[0128] In a further aspect, the refractive index sensor comprises a substrate having a first channel formed therein for reception of a fluid sample to be analyzed and wherein the sample is introduced into the channel. Generally, the substrate and channel can comprise any material suitable for containing and providing a sample for analysis and capable of being interrogated by light. In one aspect, the substrate and channel together comprise a capillary tube. In a further aspect, wherein the substrate and channel together comprise a microfluidic device.

[0129] In a further aspect, a channel is formed in the substrate and the method further comprises the steps of: (a) introducing a reference sample in the second channel; (b) determining a characteristic of the reference sample; and (c) employing the characteristic of the reference sample to compensate for background interference effects in the determination of the characteristic of the sample in the first channel.

[0130] In a further aspect, the substrate has a channel formed therein with a generally hemispherical cross sectional shape. In a still further aspect, the channel is formed with first and second curved potions, each curved portion defining a 90° arc, and a first flat portion connecting the first and second curved portions.

[0131] In a further aspect, the sample is positioned inside a channel formed in a substrate, the channel has a longitudinal direction and a transverse direction, and the light is elongated in the longitudinal direction of the channel. In a still further aspect, the light is incident on at least a portion of the channel greater than 4 mm in length along the longitudinal direction.

[0132] In various aspects, the light source generates an easy to align optical beam that is incident on the etched channel for generating scattered light. In a further aspect, the light source generates an optical beam that is collimated, such as, for example, the light emitted from a HeNe laser. In a still further aspect, the light source generates an optical beam that is not well collimated and disperses in, for example, a Gaussian profile, such as that generated by a diode laser.

[0133] Typically, two types of lasers can be employed. In various aspects, one laser (the diode) creates a laser beam

that is elongated in the longitudinal direction of the channel. In further aspects, the other (HeNe) creates a laser beam that is not elongated longitudinally along the length of the channel, but can be later elongated longitudinally along the length of the channel by beam-stretching optics. These methods can both achieve the same end of an elongated beam impinging upon the channel, but do so through different means. It can be noted that, in certain aspects, when the diameter of the laser beam is the same as the thickness of the glass chip, new interference phenomena can arise. This can be avoided by selecting the width of the beam to be smaller than the thickness of the glass chip (0.8 mm width laser and 1.7 mm thickness glass chip).

[0134] In a further aspect, a single light beam is incident upon the substrate.

[0135] In a further aspect, the light beam has a substantially uniform intensity profile across at least a portion of the plurality of discrete zones. In a yet further aspect, the light beam has a substantially Gaussian intensity profile in the axis perpendicular to the zones. In a still further aspect, the portion of the light beam impinging the channel has an elongated intensity profile.

[0136] In various aspects, the light beam is incident on at least a portion of the channel greater than 4 mm in length along the longitudinal direction. In a further aspect, the light beam is incident on at least a portion of the channel greater than 5 mm of length of the channel in the longitudinal direction. In a still further aspect, the light beam is incident on at least a portion of the channel greater than 6 mm of length of the channel in the longitudinal direction. In yet a further aspect, the light beam is incident on at least a portion of the channel greater than 7 mm of length of the channel in the longitudinal direction. In an even further aspect, the light beam is incident on at least a portion of the channel greater than 8 mm of length of the channel in the longitudinal direction. In a still further aspect, the light beam is incident on at least a portion of the channel greater than 9 mm of length of the channel in the longitudinal direction. In yet a further aspect, the light beam is incident on at least a portion of the channel greater than 10 mm, 12 mm, 14 mm, 16 mm, 18 mm, or 20 mm of length of the channel in the longitudinal direction.

[0137] In a further aspect, at least a portion of the light beam incident on the channel covers at least two discrete zones. In a still further aspect, at least a portion of the light beam is incident on the channel such that the intensity of the light on each of at least two zones is the same or substantially the same. In yet a further aspect, at least a portion of the light beam is incident on the channel such that the each of the zones along the channel receive the same or substantially the same intensity of light. For example, a light beam having a Gaussian intensity profile can be incident on a channel such that at least two zones along the channel are within the peak of the intensity profile, receiving the same or substantially the same intensity of light. In an even further aspect, the portion of the light beam incident on the channel can have a non-Gaussian profile, such as, for example, a plateau (e.g., top-hat). The portion of the light beam in the wings of the Gaussian intensity profile can be incident upon other portions of the channel or can be directed elsewhere.

[0138] In a further aspect, variations in light intensity across zones of interest can result in measurement errors. In a still further aspect, if portions of a light beam having varying intensity are incident upon multiple zones of a channel, a calibration can be performed wherein the expected intensity of light, resulting interaction, and scattering is determined for correlation of future measurements.

[0139] The light source can comprise any suitable equipment and/or means for generating light, provided that the frequency and intensity of the generated light are sufficient to interact with a sample and/or a marker compound and provide elongated fringe patterns as described herein. Light sources, such as HeNe lasers and diode lasers, are commercially available and one of skill in the art could readily select an appropriate light source for use with the systems and methods of the present invention.

[0140] In a further aspect, the light beam is directed from a laser formed integrally on the substrate. In a still further aspect, the photodetector is formed integrally on the substrate.

[0141] In a further aspect, the characteristic property comprises the index of refraction of the sample. In a still further aspect, the characteristic property comprises the temperature of the sample.

[0142] In a further aspect, the characteristic property to be determined is whether first and second biochemical functional species bind with one another, and the step of introducing a sample to be analyzed onto the sensor comprises introducing the first biochemical functional species into the channel and introducing the second biochemical functional species into the channel to facilitate a binding reaction between the first and second biochemical species.

[0143] In a further aspect, the first and second biochemical functional species are introduced sequentially. In a still further aspect, the first and second biochemical functional species are introduced simultaneously.

[0144] In a further aspect, the first biochemical functional species is allowed to interact with the second biochemical functional species prior to introducing the first and/or second biochemical species onto the sensor. In a still further aspect, the first biochemical functional species is allowed to interact with the second biochemical functional species after introducing the first and/or second biochemical species onto the sensor.

[0145] In a further aspect, the first and second biochemical functional species are selected from the group comprising complimentary strands of DNA, complimentary proteins, and antibody antigen pairs.

[0146] In a further aspect, the substrate is selected to be formed from PDMS.

[0147] In a further aspect, the laser beam is selected to have a diameter of 2 mm or less. In a still further aspect, the channel, when present, is selected to have a width that is no larger than the diameter of the laser beam.

[0148] In a further aspect, the refractive index sensor comprises a prism.

What is claimed is:

1. A free-solution analytical method comprising detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, wherein the detection is performed by refractive index sensing other than backscattering interferometry, or by circular dichroism.

2. The method of claim **1**, wherein the detection is performed by refractive index sensing other than forward scattering or side scattering interferometry.

3. The method of claim **1**, wherein both analytes are unlabeled.

4. The method of claim 1, wherein at least one of the analytes is present in an amount of less than about 1.0×10^{-3} M.

5. The method of claim 1, wherein detecting comprises one or more of:

(a) determining an equilibrium constant, a dissociation constant, a dissociation rate, or an association rate;

(b) calculating a change in hydrodynamic volume, entropy, or enthalpy;

(c) determining the concentration of the first and/or second analyte;

(d) identifying the presence of the first and/or second analyte; and

(e) identifying the presence of a third analyte.

6. The method of claim 1, wherein refractive index sensing is via a hand-held refractive index sensing device.

7. The method of claim 1, wherein refractive index sensing is via a RI detectors based on the angle of deviation method of measurement, a RI detectors based on the Fresnel method of RI measurement, a Christiansen effect detector, an interferometer detector, or a differential refractometer detector.

8. A free-solution analytical method comprising the steps of:

(a) providing a refractive index sensor for reception of a fluid sample to be analyzed;

(b) introducing a first sample comprising a first non-immobilized analyte to be analyzed and a second sample comprising a second non-immobilized analyte to be analyzed onto the sensor, wherein the first analyte is allowed to interact with the second analyte;

(c) interrogating the fluid sample with light;

(d) detecting the light after interaction with the fluid sample, wherein the detected light is not backscattered; and

(e) detecting a molecular interaction between the first and second analyte.

9. The method of claim 8, wherein the sample is positioned inside a channel formed in a substrate, the channel has a longitudinal direction and a transverse direction, and the light is elongated in the longitudinal direction of the channel.

10. The method of claim 9, wherein the light is incident on at least a portion of the channel greater than 4 mm in length along the longitudinal direction.

11. The method of claim 8, wherein the light is not scattered.

12. The method of claim 8, wherein the first and/or second analyte is a complex.

13. The method of claim 8, wherein the molecular interaction is the formation of one or more covalent bonds, electrostatic bonds, hydrogen bonds, or hydrophobic interactions.

14. The method of claim 8, wherein the first and/or second analyte is one or more of an antibody, an antigen, a protein, a small molecule, a drug, a receptor, a cell, an oligonucleotide, a carbohydrate, an enzyme, a substrate, a DNA, an aptamer, a RNA, a nucleic acid, a biomolecule, a molecular imprint, a protein mimetic, an antibody derivative, a lectin, a cell membrane, an ion, a virus particle, a bacteria, and a micro-RNA.

15. The method of claim 8, wherein the molecular interaction is a binding event between one or more of antibody-antigen, protein-protein, small molecule-small molecule, small molecule-protein, drug-receptor, antibody-cell, virus-cell, virus-protein, bacteria-cell, bacteria-protein, virus-DNA, virus-RNA, bacteria-DNA, bacteria-RNA, protein-cell, oligonucleotide-cell, carbohydrate-cell, cell-cell, enzyme-substrate, protein-DNA, protein-aptamer, DNA-DNA, RNA-DNA, DNA-RNA, protein-RNA, small molecule-nucleic acid, biomolecule-molecular imprint, biomolecule-protein mimetic, biomolecule-antibody derivatives, lectin-carbohydrate, biomolecule-carbohydrate, small molecule-cell membrane, ion-protein, and protein-protein.

16. The method of claim 8, wherein the molecular interaction lacks a change in mass.

17. The method of claim 8, wherein the first and second sample are introduced simultaneously.

18. The method of claim 8, wherein the first and second sample are introduced sequentially.

19. A free-solution analytical method comprising determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion Chanel or membrane protein, and wherein the determination is performed by refractive index sensing.

20. The method of claim 19, wherein at least one of the analytes is unlabeled.

21. A system comprising a refractive index sensor for detecting molecular interactions between a first non-immobilized analyte and a second non-immobilized analyte, and a pressure change compensator.

22. The system of claim 21, wherein the sensor comprises a channel formed in a substrate, the channel has a longitudinal direction and a transverse direction, and a light source for generating a light, wherein the light is elongated in the longitudinal direction of the channel.

23. The system of claim 21, wherein both analytes are unlabeled.

24. The system of claim 21, wherein at least one of the analytes is present in an amount of less than about 1.0×10^{-3} M.

25. A free-solution analytical method comprising detecting a molecular change, wherein the detection is performed by refractive index sensing other than backscattering interferometry.

26. The method of claim 25, wherein detecting a molecular change comprises determining the degree of polymerization, protein folding, protein aggregation, blood oxygenation, the conformational state of an ion channel or membrane protein, or the hydration state of an ion chanel or membrane protein.

27. The method of claim 25, wherein the detection is performed by refractive index sensing other than forward scattering or side scattering interferometry.

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专利名称(译)	自由溶液响应函数干涉测量		
公开(公告)号	US20190033301A1	公开(公告)日	2019-01-31
申请号	US16/073630	申请日	2017-01-27
[标]申请(专利权)人(译)	凡德比特大学		
申请(专利权)人(译)	范德比尔特大学		
当前申请(专利权)人(译)	范德比尔特大学		
[标]发明人	BORNHOP DARRYL J KUSSROW AMANDA KAMMER MICHAEL		
发明人	BORNHOP, DARRYL J. KUSSROW, AMANDA KAMMER, MICHAEL		
IPC分类号	G01N33/536 G01N21/41 G01N21/19		
CPC分类号	G01N33/536 G01N21/4133 G01N21/19 G01N21/41 G01N21/45 G01N2201/0221		
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摘要(译)

公开了通过除了后向散射干涉测量之外的折射率感测来自由溶液测量分子相互作用的方法。所公开的方法可具有非常低的检测限和/或非常低的样品体积要求。还公开了所公开技术的各种生物传感器应用。该摘要旨在作为用于在特定领域中进行搜索的扫描工具，并且不旨在限制本发明。

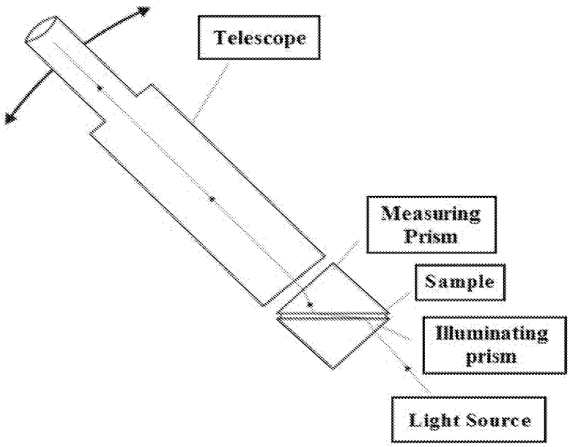

FIG. 1A