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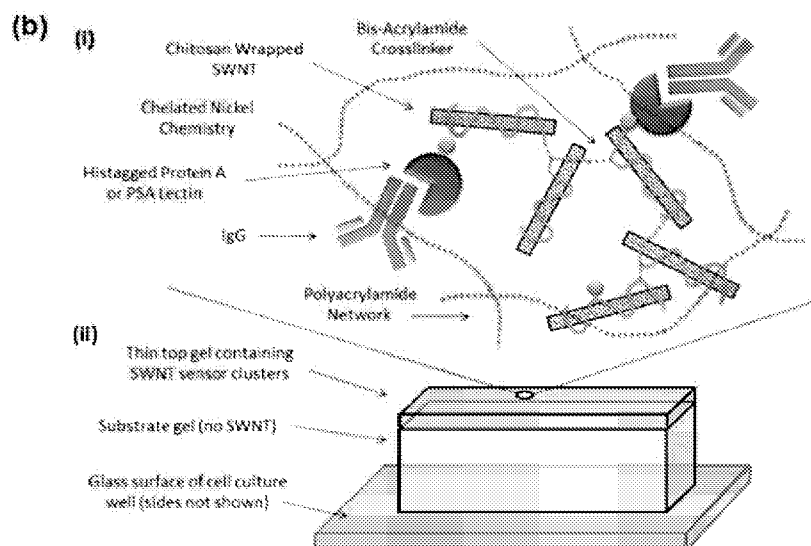
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(54) Title: METHOD OF ANALYSIS USING ARRAY SENSOR

**FIG. 1B**

(57) Abstract: Arrays can be used to assay binding heterogeneity and other characteristics of biomanufactured products.

METHOD OF ANALYSIS USING ARRAY SENSOR

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant No. DGE-1122374 awarded by the National Science Foundation. The government has certain rights in the invention.

RELATED APPLICATIONS

This application claims priority to U.S. Ser. No. 61/767,513 filed February 21, 2013, which is hereby incorporated by reference in its entirety into this application.

TECHNICAL FIELD

The present invention relates to a method of analyzing a product using an array sensor.

BACKGROUND

Improved analytical technology for increasing production of recombinant proteins is an area of great interest. Cell line generation and selecting culture parameters typically take over a year with current assays and often cell candidates are only picked based on static measurements of productivity. Some post-translational modifications, such as glycosylation patterns, can change in response to changes in process conditions (e.g., media components, temperature, pH, pCO₂, dissolved oxygen, cell density, duration, and others) and can have a dramatic effect on the properties of the protein product (e.g., pharmacokinetics and immunogenicity of a protein drug). Current analytical technologies for determining titer and glycosylation, such as ELISA and tandem LC/MS systems, respectively, can deliver detailed information but are costly in terms of of time, reagents, and multiplexing capabilities. Furthermore, these methods are incompatible with on-line process use.

SUMMARY

In general, a method of detecting an analyte can include using an array to provide a plurality of data points that can be used to assess the analyte.

In one aspect, a method of detecting an analyte can include contacting a sample including a biologic analyte with a sensor array, the sensor array including a plurality of photoluminescent

nanostructures, collecting emissive data from each of the photoluminescent nanostructures, and analyzing the emissive data from each nanostructure of the plurality of photoluminescent nanostructures to measure a distribution of binding affinities. In some embodiments, the method of detecting an analyte can include calculating a calibration curve for the sensor.

5 In some embodiments, analyzing the emissive data can include measuring a variance of the emissive data from the plurality of photoluminescent nanostructures, measuring a skew of the emissive data from the plurality of photoluminescent nanostructures, or measuring a standard deviation of a plume angle of the emissive data from the plurality of photoluminescent nanostructures.

10 In some embodiments, the method of detecting an analyte can include evaluating a binding heterogeneity of the biologic analyte of the sample from the analyzed emissive data. In some embodiments, the method of detecting an analyte can include evaluating a cell line for production of the biologic analyte of the sample from the analyzed emissive data. In some embodiments, the method of detecting an analyte can include evaluating a glycosylation pattern
15 of the biologic analyte of the sample from the analyzed emissive data.

In some embodiments, the array can include at least 500 of the photoluminescent nanostructures. In some embodiments, the array can include at least 1,000 of the photoluminescent nanostructures. In some embodiments, the array can include at least 2,000 of the photoluminescent nanostructures. In some embodiments, the array can include at least 5,000
20 of the photoluminescent nanostructures.

In some embodiments, the sensor can include an analyte-binding compound associated with the photoluminescent nanostructure. In some embodiments, the sensor can include a linker, wherein the analyte-binding compound is associated with the photoluminescent nanostructure via the linker. In some embodiments, the sensor can include a polymer including clusters of
25 photoluminescent nanostructures.

In some embodiments, the analyte can be a biologic analyte. In some embodiments, the biologic analyte can be an antibody. In some embodiments, the biologic analyte can be a protein. In some embodiments, the antibody can be IgG. In some embodiments, the analyte binding compound can be a capture protein comprising Protein A or PSA-lectin.

30 Other aspects, embodiments, and features will be apparent from the following description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-D illustrate nanosensor array fabrication and detection methods. FIG. 1A illustrates a nIR micrograph of nanosensor array – each SWNT-illuminated pixel records changes in intensity upon analyte binding, such as nickel quenching or signal return from IgG adsorption shown. FIG. 1B illustrates a multi-layer, poly-acrylamide gel platform used to immobilize the SWNT sensors and provide a porous network for IgG diffusion. SWNT sensors are wrapped in chitosan, nickel-chelated, and functionalized with a capture protein (Protein A or PSA – Lectin). FIG. 1C illustrates ensemble (i) and all-points histogram (ii) response to 100mM nickel (1), 1mg/ml Protein A (2), 1.5mg/ml Human IgG (3) and 2mg/ml BSA (4) additions. FIG. 1D illustrates selectivity of the sensor array (nIR micrograph of starting intensities shown) as demonstrated by a heat map reporting the percent modulation response of the 10,000 top responding pixels after analyte addition.

FIGS. 2(a)-(f) illustrate simulation frames. 2a) Starting K_D distribution from 10,000 simulated points. 2b) Distributions of Langmuir coverage fraction coefficients (θ_L). Resulting response of all simulated points in cartesian (2c) and polar coordinates (2d). 2e) Bivariate Gaussian fit of plume angle mean and standard deviation (θ_μ and θ_σ). Simulation sensor points at many different concentrations results in calibration curve (2f).

FIGS. 3A-D illustrate modeling a nanosensor array for measuring K_D distributions. FIG. 3A illustrates a graph depicting the effect of averaging number of molecules (N) on a single sensor site on measured variance (σ_m^2) assuming a Gaussian shaped K_D distribution with known variance (σ_t^2). FIG. 3B illustrates a design regime where σ_t^2 can be reconstructed showing two spectrum limits (ensemble and single molecule detection) and where the nanosensor platform operates. FIG. 3C illustrates modeling nanotube array response – (i) assuming a Weibull (skewed) K_D distribution, (ii) Langmuir coverage fraction to determine extent of nanosensor modulation, (iii) resulting simulated response ‘plume’ normalized by average sensor intensity (I_{avg}) where each point represents a sensor site, and (iv) calibration curve from fitting the plume angle mean and standard deviation (θ_μ and θ_σ) with a bivariate Gaussian distribution. FIG. 3D illustrates the effect of changing K_D distribution skewness (β) on resulting θ_μ and θ_σ calibration curves as well as fit parameters ‘B’ (steepness of calibration curve) and ‘C’ (K_D mean).

FIGS. 4A-E illustrate experimental results from lyophilized IgG in PBS, murine IgG (TA99) from HEK cells, and human IgG from CHO cells screened on sensor arrays at various

concentrations. FIG. 4A are graphs depicting all point sensor response ‘plumes’ and fit calibration curves. FIG. 4B are graphs depicting the yielding K_D 95% confidence intervals, B-fit parameters, and angle saturation values (θ_{SAT}) calculated from the starting intensity distribution of each sensor batch (Eq. 5). FIG. 4C is a graph depicting relation of B-fit parameter and K_D skewness parameter (β) as found by simulation results. FIG. 4D is a graph depicting measured K_D distributions using assumed Weibull PDF. FIG. 4E is a graph depicting K_D histograms directly calculated from sensor response assuming a constant functionalization value – revealing the approximate shape of the true PDF.

FIGS. 5A-H illustrate hypermannosylation detection on PSA-lectin conjugated sensor arrays. FIG. 5A depicts a diagram showing that weaker mannose-PSA lectin interactions can be transduced on independent nanosensors. FIG. 5B are graphs depicting results of testing concept with chicken IgG which has > 40% high mannose glycoforms where human and mouse IgG do not contain these isoforms. FIGS. 5C-E are graphs depicting an all point ‘plume’ responses and calibration curve (FIG. 5D) as before with corresponding K_D distribution (FIG. 5E). FIG. 5F is a graph depicting media compositions used to elicit hypermannosylation in CHO cell culture. Culture was sampled for eight days showing expected changes in IgG titer (ELISA) and mannose content (SWNT sensor array). FIG. 5G shows IgG titer for each of the culture days. FIG. 5H shows general trends of mannosylation derived from the distribution averages in FIG. 5D.

FIGS. 6(a)-(b) illustrate a surface staining experiment to confirm the presence of mannose in the CHO culture samples. 6a) A surface is treated to bind the IgG in the CHO samples and a FITC-labeled PSA lectin is used to stain the mannose content. 6b) Representative FITC fluorescent images of the stained surface showing a marked increase in mannose content as found by the SWNT nanosensors.

FIGS. 7A-E illustrate sensor response to local cell production. FIG. 7A illustrates qualitative images of control and IgG producing cells showing colocalization of SWNT response. FIG. 7B is a graph depicting location of top 1000 SWNT in IgG and control cell images, presented as coverage percentage of cell area (specific) and gel area (non-specific) in each image (FIG. 7C). FIG. 7D are graphs depicting dynamic response of SWNT sensors to IgG producing cells plated on gel presented as distributions of top 1000 SWNT from images and micrographs of scaled SWNT intensity. FIG. 7E are micrographs depicting mapping visible IgG

producing cell islands, the colocalized SWNT signal underneath, and ranking the islands' productivity based on intensity normalized by island area.

FIG. 8 illustrates the experimental domain addressed by arrayed nanosensors in comparison to existing technologies. Also mapped are approximate assay times of each technique.

FIG. 9 illustrates that His-tagged sensor protein needed to reduce nonspecific binding - data presented as distribution of all SWNT sensor sites.

FIG. 10 illustrates the role of nickel in SWNT sensor modulation. 10a) Testing the current hypothesis of nickel being the crucial transduction component by swapping it out with another small molecule binding site – biotin. Histograms show sensor responses of the biotinylated chitosan-wrapped SWNT to neutravidin and BSA. 10b) Two proposed quenching mechanisms at work – 1) the nickel acts as a quencher providing electronic states for non-radiative decay of the SWNT exciton, 2) the bound macromolecule displaces aqueous quenching species such as nickel and water from the SWNT surface.

DETAILED DESCRIPTION

Methods of using sensors including plurality of photoluminescent nanostructures are described. The photoluminescent nanostructures are associated with analyte-binding compounds. Binding of the analyte to the analyte-binding compound changes the photoluminescent properties of the photoluminescent nanostructures. Thus, the presence or absence of the analyte can be determined based on the observed photoluminescent properties of the photoluminescent nanostructures. The change in the photoluminescence can include a change in photoluminescence intensity, a change in peak wavelength, a Raman shift, or a combination thereof. Importantly, the plurality of photoluminescent nanostructures in the sensor forms an array of nanosensors, each of which provides important data about the analyte, the statistics of which are significant in interpreting the population of analyte species in a sample. Specifically, the statistical analysis based on a calibration, such as variance, skew, standard deviation and other parameters, can reflect different attributes of the analyte, such as a biologic analyte, for example an antibody or a protein product. The statistical analysis provides information about analytes in the sample, for example, binding heterogeneity of a biologic analyte, cell line for production of a biologic analyte, or glycosylation patterns of a biologic analyte.

The array can include more than 100, more than 200, more than 500, more than 1,000, more than 2,000, more than 5,000 nanosensors, or more, each of which has a photoproperty that can be monitored.

In some cases, detection of analytes with photoluminescent nanostructures can be limited
5 by the presence of non-specific background photoluminescence. Background photoluminescence can arise from, among other sources, a support that underlies the photoluminescent nanostructures, e.g., supports including glass or plastic. One way to minimize background photoluminescence is to use a support composed of highly purified materials that are free of contaminants that may be responsible for the background photoluminescence. However, the use
10 of such materials can increase the cost and complexity of analyte detection.

Another method to avoid background photoluminescence is to use a detection system that does not detect the background photoluminescence. In such a system, substrates that suffer from background photoluminescence can nonetheless be used, because the detection system detects only photoluminescence arising from the background photoluminescence. In some cases, this
15 might be achieved with appropriate sets of wavelength filters. However, if the background photoluminescence and the photoluminescence arising from the photoluminescent nanostructures occur at overlapping wavelengths, filters may not be able to reject a sufficient amount of the background photoluminescence while still allowing a sufficient amount of the photoluminescence arising from the photoluminescent nanostructures to pass.

It can be advantageous to use a detection system that rejects background
20 photoluminescence on the basis of spatial configuration rather than on the basis of wavelength. One way to accomplish this is to use an optical detection system that provides a distinct focal plane, where the detected photoluminescence is substantially from sources in the focal plane. Photoluminescent sources outside the focal plane are not detected by the detector. In this way,
25 the detector does not suffer from issues associated with background photoluminescence.

Thus, a sensor for detecting an analyte can be arranged on a support. Photoluminescence from the sensor is detected by an optical system that detects photoluminescence substantially only from sources in the focal plane. Sources outside the focal plane do not contribute substantially to the detected photoluminescence. When the sensor is arranged directly on the
30 support, the focal plane may extend outside the boundaries of the sensor and include a portion of the support. In this situation, background photoluminescence will be detected. Rather than being

arranged directly on the support, the sensor can be arranged on an intermediate substrate. The intermediate substrate, in turn, is in contact with the support. The intermediate substrate separates the sensor from the support, such that the focal plane does not include any portion of the support. The intermediate substrate can be advantageously composed of materials that are substantially free of background photoluminescence. The intermediate substrate can be advantageously composed of materials that are compatible with the sensor. For example, the sensor can include a hydrogel, and the intermediate substrate can also include a polymer matrix or a gel.

With reference to **FIG. 1B**, sensor includes (illustrated in lower panel (ii)) support which supports substrate gel, which in turn supports sensor gel. Sensor gel (detailed in upper panel (i)) includes a gel network, a plurality of photoluminescent nanostructures and associated linking polymers, such as a polysaccharide, for example, chitosan. Optional crosslinks, for example bis-acrylamide crosslinkers can be present between linking polymers, and an analyte-binding compound, such as a nickel chelate. In **FIG. 1B**, sensor gel is illustrated with analyte being associated with analyte-binding compound, though it will be understood that a sensor gel will typically be formed in the absence of analyte, and analyte may be contacted with sensor gel after formation. Thus in some states, sensor gel is free of analyte and in other states includes analyte (e.g., associated with analyte-binding compound). The presence of the analyte alters the photoluminescent properties of the photoluminescent nanostructures. As also illustrated in **FIG. 1B**, the sensor array may be arranged upon one or more support materials. In some embodiments, the solid support may comprise a first gel lacking photoluminescent nanostructures and a second gel comprising photoluminescent nanostructures upon the first gel (**FIG. 1B**). In some embodiments, the one or more support material may comprises a solid material (e.g. glass), a first gel lacking photoluminescent nanostructures, and a second gel comprising photoluminescent nanostructures upon the first gel.

The concept of vastly multiplexing analyte detection using arrays of independently addressable sensors is ubiquitous in the literature, following successful demonstrations of the DNA and protein microarrays. See, for example, Kingsmore, S.F. *Nature Reviews Drug Discovery* **5**, 310-320 (2006), Nielsen, U.B. & Geierstanger, B.H. *Journal of Immunological Methods* **290**, 107-120 (2004), Epstein, J.R., *et al. Analytica Chimica Acta* **469**, 3-36 (2002), Cheng, M.M.C. *et al. Current Opinion in Chemical Biology* **10**, 11-19 (2006), Schweitzer, B. &

Kingsmore, S.F. *Current Opinion in Biotechnology* **13**, 14-19 (2002), and Wilson, D.S. & Nock, S. *Angewandte Chemie-International Edition* **42**, 494-500 (2003). This approach has been a prevailing motivation for further reduction in array size to nanometer dimensions. See, for example, Niemeyer, C.M. *Nano Today* **2**, 42-52 (2007), Tam, J.M., *et al. Biosensors & Bioelectronics* **24**, 2488-2493 (2009), and Tan, C.P., *et al. Nano Letters* **10**, 719-725 (2010). Such nanosensor arrays, however, have other important capabilities that are not as well recognized, even when functionalized for just a single analyte. For example, many biological analytes, including antibodies, demonstrate a distribution of dissociation constants even in relatively purified form. See, for example, Werblin, T.P. & Siskind, G.W. *Immunochemistry* **9**, 987-& (1972), Pierson, L., *et al. Journal of Immunological Methods* **211**, 97-109 (1998), and Steensgaard, J., *et al. Molecular Immunology* **17**, 689-698 (1980). Surprisingly, an array of sensors can reconstruct this important distribution via sampling a large number of independent interactions. Such arrays can also quantify weakly-affined interactions by recording a large number of rare binding events. Notably, a nanosensor array can also characterize and differentiate biosynthesis around single cells and colonies, enabling the label-free selection of more productive strains. These properties have the potential to greatly enhance process analytics for biomanufacturing applications.

Improved analytical technology for the rapidly increasing production of clinical recombinant antibodies is an area of great interest. Three apparent needs are better tools for (1) clonal selection, (2) glycan analysis, and (3) determining the affinity distribution or heterogeneity of the expressed product. See, for example, Hacker, D.L. *et al. Biotechnology Advances* **27**, 1023-1027 (2009), Browne, S.M., *et al. Trends in Biotechnology* **25**, 425-432 (2007), Burke, J.F. *et al. Genetic Engineering & Biotechnology News* **29**, 38-39 (2009), Elliott, S. *et al. Nature Biotechnology* **21**, 414-421 (2003), Hermeling, S. *et al. Pharmaceutical Research* **21**, 897-903 (2004), Jefferis, R. *Biotechnology Progress* **21**, 11-16 (2005), Jefferis, R. *Nature Reviews Drug Discovery* **8**, 226-234 (2009), Raju, T.S., *et al. Glycobiology* **10**, 477-486 (2000), van Berkel, P.H.C. *et al. Biotechnology Progress* **25**, 244-251 (2009), Werblin, T.P. & Siskind, G.W. *Immunochemistry* **9**, 987-& (1972), Pierson, L., *et al. Journal of Immunological Methods* **211**, 97-109 (1998), and Steensgaard, J., *et al. Molecular Immunology* **17**, 689-698 (1980). Cell line generation and selecting culture parameters typically take over a year with current assays and often cell candidates are only picked based on static measurements of

productivity. See, for example, Wurm, F.M. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nature Biotechnology* 22, 1393-1398 (2004), and Browne, S.M. & Al-Rubeai, M. *Trends in Biotechnology* 25, 425-432 (2007), and Burke, J.F. *et al. Genetic Engineering & Biotechnology News* 29, 38-39 (2009). Glycosylation patterns can easily change
5 due to processing conditions (media components, temperature, pH, pCO₂, dissolved oxygen, cell density, duration, etc.) and the patterns can have a dramatic effect on the pharmacokinetics and immunogenicity of the resulting drug. See, for example, Pacis, E., *et al. Biotechnology and Bioengineering* 108, 2348-2358 (2011), Gramer, M.J. *et al. Biotechnology and Bioengineering* 108, 1591-1602 (2011), Lee, S.Y. *et al. Process Biochemistry* 47, 1411-1418 (2012), Ahn, W.S.,
10 *et al. Biotechnology and Bioengineering* 101, 1234-1244 (2008), Muthing, J. *et al. Biotechnology and Bioengineering* 83, 321-334 (2003), Schmelzer, A.E. & Miller, W.M. Hyperosmotic stress and elevated pCO₂ alter monoclonal antibody charge distribution and monosaccharide content. *Biotechnology Progress* 18, 346-353 (2002), Serrato, J.A., *et al. Biotechnology and Bioengineering* 88, 176-188 (2004), Majid, F.A.A., *et al. Biotechnology and Bioengineering* 97, 156-169 (2007), Bumbaca, D., *et al. Aaps Journal* 14, 554-558 (2012) and
15 Goetze, A.M. *et al. Glycobiology* 21, 949-959 (2011). Current titer and glycosylation analytical technologies such as ELISA and tandem LC/MS systems respectively can deliver exquisite detail at the expense of much time and reagent. Furthermore their processing steps are prohibitive to any on-line process use. The trend to milliliter sized bioreactors for upstream process
20 optimization will also require platforms that can accurately assay much lower protein and glycan quantities. See, for example, Lee, K.S., *et al. Lab on a Chip* 11, 1730-1739 (2011). Finally, harsh biomanufacturing process conditions (pH, temperature, mixing) and variability in cell production result in heterogeneous products with a distribution of binding affinities and currently there is no convenient platform on which to measure the dissociation constant (K_D) distribution.
25 Emerging nanoengineered sensors fabricated in massive (10,000+ sites) arrays, of up to 10,000 sites or more, could provide solutions to these three areas of biomanufacturing analytics. See, for example, Reuel, N.F., *et al. Chemical Society Reviews* 41, 5744-5779 (2012). Carbon-nanotube based optical sensors can be used for single protein and single glycan detection. See, for example, Ahn, J.H. *et al. Nano Letters* 11, 2743-2752 (2011), and Reuel, N.F. *et al. Journal of the American Chemical Society* 133, 17923-17933 (2011). By creating an array of sensors in a
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hydrogel platform, binding heterogeneity, weakly-affined hypermannosylation detection, and local cell productivity of biomanufactured products can be assessed.

For example, the gel can be a polymer including, but are not limited to, collagen, silicon-containing polymers, polyacrylamides, crosslinked polymers (e.g., polyethylene oxide, polyAMPS and polyvinylpyrrolidone), polyvinyl alcohol, acrylate polymers (e.g., sodium polyacrylate), or copolymers with an abundance of hydrophilic groups. The gel can have a porous structure can be determined by factors including the concentration of polymers and crosslinking agent. The pore size can be in the range of, for example, 10 nm to 1,000 nm, 20 nm to 500 nm, 50 nm to 250 nm, or 10 nm to 100 nm. When the analyte is a macromolecule (e.g., a protein, such as an immunoglobulin), a pore size greater than 10 nm, greater than 20 nm, greater than 30 nm, greater than 40 nm, greater than 50 nm, greater than 60 nm, greater than 70 nm, greater than 80 nm, greater than 90 nm, or 100 nm or greater can be desirable. As described above and illustrated in **FIG. 1B**, a nanostructure may comprise different types of gels. For instance, in some embodiments, the nanostructure may comprise a first gel lacking photoluminescent nanostructures and a second gel comprising photoluminescent nanostructures positioned upon the first gel.

As used herein, the term “nanostructure” refers to articles having at least one cross-sectional dimension of less than about 1 μm , less than about 500 nm, less than about 250 nm, less than about 100 nm, less than about 75 nm, less than about 50 nm, less than about 25 nm, less than about 10 nm, or, in some cases, less than about 1 nm. Examples of nanostructures include nanotubes (e.g., carbon nanotubes), nanowires (e.g., carbon nanowires), graphene, and quantum dots, among others. In some embodiments, the nanostructures include a fused network of atomic rings.

A “photoluminescent nanostructure,” as used herein, refers to a class of nanostructures that are capable of exhibiting photoluminescence. Examples of photoluminescent nanostructures include, but are not limited to, single-walled carbon nanotubes (“SWNT”), double-walled carbon nanotubes, semi-conductor quantum dots, semi-conductor nanowires, and graphene, among others. In some embodiments, photoluminescent nanostructures exhibit fluorescence. In some instances, photoluminescent nanostructures exhibit phosphorescence.

If the nanostructure is a carbon nanotube, the carbon nanotube can be classified by its chiral vector (n,m) , which can indicate the orientation of the carbon hexagons. The orientation of

carbon hexagons can affect interactions of the nanotube with other molecules, which in turn, can affect a property of the nanostructure.

A linker can be associated with the nanostructure. The association can be a bond, for example, a covalent, ionic, van der Waals, dipolar or hydrogen bond. The association can be a physical association. For example, at least a portion of the nanostructure can be embedded in the polymer or a portion of the polymer can encompass the nanostructure.

A linker can include a polymer. A polymer can include a polypeptide, a polynucleotide or a polysaccharide. Examples of polysaccharides include dextran and chitosan. A polymer can include a plastic, for example, polystyrene, polyamide, polyvinyl chloride, polyethylene, polyester, polypropylene, polycarbonate, polyacrylamide or polyvinyl alcohol.

A polymer can be biocompatible, which can mean that the polymer is well tolerated by an organism. More specifically, biocompatibility can mean that a polymer does not elicit an immune response when it is brought in contact with an organism. It can also mean that a polymer can integrate into cell structures, cells, tissues or organs of an organism. The organism can be mammal, in particular, a human.

An exemplary polymer can exhibit minimal binding with other molecules. In certain circumstances, a polymer can have a protein adsorption of less than $5 \mu\text{g}/\text{cm}^2$, less than $1 \mu\text{g}/\text{cm}^2$, less than $0.5 \mu\text{g}/\text{cm}^2$, less than $0.1 \mu\text{g}/\text{cm}^2$, less than $0.05 \mu\text{g}/\text{cm}^2$, or less than $0.01 \mu\text{g}/\text{cm}^2$.

The association of a linker with a nanostructure can change a property of the nanostructure. The property can be conductivity, polarity, or resonance. The property can be photoluminescence, including fluorescence or phosphorescence. More specifically, the property can be fluorescence with a wavelength in the near infrared spectrum. The property can be an emission wavelength, an emission intensity, a conductance, an electromagnetic absorbance or an emittance.

A linker can be configured to interact with an analyte-binding compound. The analyte-binding compound undergoes a specific and typically reversible binding with an analyte. One class of suitable analyte binding compounds are proteins. In particular, proteins that undergo specific and typically reversible binding with an analyte (referred to herein as “capture proteins”) can be suitable.

A capture protein can include a protein, a polypeptide or a peptide. In some cases, a capture protein can be a complex of proteins. A capture protein can also include a full length

protein, a fragment of a protein or a protein domain. A capture protein can be a fusion protein, which can include portions originating from one protein or portions originating from more than one protein. A capture protein can include a protein tag or marker. A capture protein can also be modified, for example, by glycosylation, ubiquitination, PEGylation, SUMOylation or
5 biotinylation. A capture protein can be synthesized from a nucleic acid sequence that was amplified from a cDNA library, genomic DNA, a DNA vector or plasmid, or a DNA fragment.

The number of linkers associated with the nanostructure present in the analysis region can exceed the number of capture proteins. More specifically, the number of capture protein binding sites on linkers associated with a nanostructure can exceed the number of capture
10 proteins. The ratio of capture protein binding sites on linkers associated with a nanostructure to capture proteins can be greater than 1.1 to 1, greater than 1.5 to 1, greater than 2 to 1, greater than 5 to 1, or greater than 10 to 1. Having an excess of capture protein binding sites on linkers associated with a nanostructure can minimize the amount of unbound capture protein in a sample. Unbound capture proteins within the sample can compete with capture proteins bound
15 to the composition for binding to the analyte. This can affect the accuracy and/or precision of the analyte detection. Having an excess of capture protein binding sites on linkers associated with a nanostructure can also increase the analyte concentration range over which analyte can be accurately detected because the saturation limit of the binding sites is increased.

The interaction between the linker and the capture protein can be binding to a capture
20 protein. The linker can be configured to interact with a capture protein by including a first binding partner in the linker that can interact with the capture protein. The first binding partner can be known binding partner of the capture protein or a portion thereof. The first binding partner can include an ion. The ion can be a metal ion. The metal ion can be a nickel, iron, cadmium, copper, magnesium, calcium, arsenic, lead, mercury or cobalt ion (e.g. Ni^{2+} , Fe^{2+} ,
25 Cd^{2+} , Cu^{2+} , Mg^{2+} , Ca^{2+} , As^{2+} , Pb^{2+} , Hg^{2+} or Co^{2+}). The first binding partner can include a protein, a nucleotide, a saccharide, a lipid or combinations thereof. See, for example, U.S. Pat. Pub. 2012/0178640A1 (WO 2012/030961), which is incorporated by reference in its entirety.

A linker can further include a chelating region. A chelating region can include a chelator, which can be a polydentate ligand capable of forming two or more bonds with a single central
30 atom. A chelator can include one or more carboxylate ions. For example, a linker can include

N_{α},N_{α} -bis(carboxymethyl)-L-lysine. A chelator can bind to a first binding partner (e.g. a metal ion) in order to incorporate the first binding partner into a linker.

The ion can act a proximity quencher of photoluminescent nanostructure. In particular, the ion can quench near infrared fluorescence. The quenching can be reversible. The quenching
5 can also depend on the distance between the nanostructure and the ion. In other words, as the distance between the nanostructure and the ion changes, the photoluminescence from the nanostructure can also change. Generally, as the distance between the ion and the nanostructure decreases, the amount of photoluminescence quenching can increase.

In some embodiments, the capture protein can include a second binding partner, such that
10 the first binding partner and second binding partner can bind together. The second binding partner can be an endogenous motif or endogenous domain within a capture protein. Alternatively, the second binding partner can be added to a capture protein. In some embodiments, the second binding partner can be a protein tag. A protein tag can be a peptide sequence grafted onto a protein, which can be used for separating (e.g. using tag affinity
15 techniques), increasing solubility, immobilizing, localizing or detecting a protein. The protein tag can be a histidine tag, chitin binding protein tag, maltose binding protein tag, glutathione-S-transferase tag, c-myc tag, FLAG-tag, V5-tag or HA-tag. One method for adding a second binding partner to a capture protein can include using primers including the sequence encoding for the second binding partner to PCR amplify DNA encoding for the capture protein. A second
20 method can include cloning DNA encoding for the capture protein into an expression vector designed to produce a fusion of the capture protein and the second binding partner.

Binding of a first and a second binding partner can be selective binding, which can provide the selectivity needed to bind to the corresponding binding partner (or relatively small group of related molecules or proteins) in a complex mixture. The degree of binding can be less
25 than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of a second binding partner present binding to a first binding partner. The degree of binding can be more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% or more than 90% of a second binding partner present binding to a first binding partner. A first
30 binding partner and a second binding partner can bind with a dissociation constant less than 1 mM, less than 0.1 mM, less than 0.01 mM, less than 1 μ M, less than 0.1 μ M, or less than 0.01

μM . A first binding partner and a second binding partner can bind with a dissociation constant greater than 1 nM , greater than $0.01\ \mu\text{M}$, greater than $0.1\ \mu\text{M}$, greater than $1\ \mu\text{M}$, greater than 0.01 mM , or greater than 0.1 mM .

The linker can also be configured to interact with a capture protein by including a region
5 capable of chemically reacting with the capture protein. The chemical reaction can form a covalent, ionic, van der Waals, dipolar or hydrogen bond between the linker and the capture protein.

The interaction of a capture protein with a linker associated with a nanostructure can change a property of the nanostructure. The property can be conductivity, polarity, or resonance.
10 The property can be photoluminescence, including fluorescence or phosphorescence. The photoluminescence can be fluorescence with a wavelength within the near infrared spectrum. The property can be an emission wavelength, an emission intensity, a conductance, an electromagnetic absorbance or an emittance.

Without intending to be bound by any particular mechanism, the change in the property
15 can be caused by a change in the distance between an ion in the first binding partner and the nanostructure. As the distance between the nanostructure and the ion changes, a nanostructure property can also change. For example, as the distance between the nanostructure and the ion changes, nanostructure photoluminescence can also change. When the capture protein binds to the linker, the distance between the ion and nanostructure can change, which can alter the
20 nanostructure photoluminescence. Generally, as the distance between the ion and the nanostructure decreases, the amount of photoluminescence quenching can increase.

In some embodiments, a composition can further include a capture protein, which can be configured to specifically interact with at least one analyte. In particular, the capture protein can be configured to specifically bind to at least one analyte. Specific binding can be more limited
25 than selective binding. Specific binding can be used to distinguish a binding partner from most other chemical species except optical isomers, isotopic variants and perhaps certain structural isomers. The degree of binding can be less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of an analyte present binding to a capture protein. The degree of binding can be more than 10%,
30 more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% or more than 90% of an analyte present binding to a capture protein. An

analyte and a capture protein can bind with a dissociation constant less than 1 mM, less than 0.1 mM, less than 0.01 mM, less than 1 μ M, less than 0.1 μ M, or less than 0.01 μ M. An analyte and a capture protein can bind with a dissociation constant greater than 1nM, greater than 0.01 μ M, greater than 0.1 μ M, greater than 1 μ M, greater than 0.01 mM, or greater than 0.1 mM.

5 The analyte can be a small molecule, protein, biomolecule, drug, biologic, or a metabolite thereof. For example, the analyte can be a monosaccharide, a polysaccharide, an amino acid, peptide, polypeptide, protein, a nucleotide, an oligonucleotide, a lipid, a poly lipid, or a combination thereof. As one example, the analyte can be immunoglobulin G (IgG) and the capture protein can be selected to specifically bind IgG. In this case, the capture protein can be, 10 for example, protein A or *Pisum sativum* agglutinin (PSA). Thus, exemplary capture proteins may include, for instance, a protein that binds an analyte (e.g., Protein A which binds antibody and / or PSA-Lectin which binds mannose). As shown in **FIG. 1B**, such capture proteins may be physically associated with a photoluminescent nanostructure and the analyte of interest (e.g., antibody). The physical association may be accomplished using one or more linkers such as a 15 moiety on the capture protein and a second moiety that physically associates with the photoluminescent nanostructure or another linker moiety physically associated with the the photoluminescent nanostructure. In some embodiments (e.g., as illustrated in **FIG. 1B**), the capture protein may be Protein A comprising a histidine tag that physically associates with nickel which is or becomes physically associated with the photoluminescent nanostructure or another 20 linker which is or becomes physically associated with the photoluminescent nanostructure (e.g., chitosan). Other embodiments are also contemplated by this disclosure as would be understood by those of ordinary skill in the art.

25 The interaction of an analyte with a capture protein that is interacting with a linker associated with a nanostructure can change a property of the nanostructure. The property can be conductivity, polarity, or resonance. The property can be photoluminescence, including fluorescence or phosphorescence. More specifically, the property can be a fluorescent emission within the near infrared spectrum. The property can be an emission wavelength, an emission intensity, a conductance, an electromagnetic absorbance or an emittance.

30 The interaction of an analyte with a capture protein can be reversible, meaning that the analyte can bind to the capture protein and then release and be free of binding. The change in a property of the nanostructure due to the interaction of an analyte with a capture protein can also

be reversible. For example, the property of a nanostructure can have a first value, the analyte can bind to the capture protein and alter the property to a second value, then the analyte can release from the capture protein and the property can return to the first value.

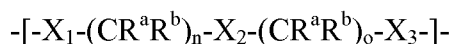
A method of detecting an analyte can include determining the presence of an analyte in the sample based on the monitored property. Determining the presence of an analyte can include determining the absence of the analyte. In some embodiments, determining the presence of an analyte can include determining the concentration of the analyte, determining the purity of the analyte or determining the quantity of the analyte. In some embodiments, relatively low concentrations or quantities of an analyte can be determined. The ability to determine low concentrations of an analyte may be useful, for example, in detecting trace pollutants or trace amounts of toxins within a subject. In some embodiments, analyte concentrations of less than about 100 micromolar, less than about 10 micromolar, less than about 1 micromolar, less than about 100 nanomolar, less than about 10 nanomolar, or less than about 1 nanomolar can be determined. The quantity of the analyte that can be determined can be less than 1 mole, less than 1 millimole, less than 1 micromole, less than 1 nanomole, less than 1 picomole, less than 1 femtomole, less than 1 attomole or less than 1 zeptomole. In some cases, a single molecule of an analyte can be determined. The purity of the analyte can be greater than 25% pure, greater than 50%, greater than 75% pure, greater than 80%, greater than 85% pure, greater than 90% pure, greater than 95% pure, greater than 99% pure or greater than 99.9% pure.

A linker can have a formula: A-L-C, where A can include a polymer, where at least a portion of the nanostructure is embedded in the polymer, L can be a linking moiety including a saturated or unsaturated C₄₋₁₀ hydrocarbon chain optionally containing at least two conjugated double bonds, at least one triple bond, or at least one double bond and one triple bond; said hydrocarbon chain being optionally substituted with C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₁₋₄ alkoxy, hydroxyl, halo, carboxyl, amino, nitro, cyano, C₃₋₆ cycloalkyl, 3-6 membered heterocycloalkyl, unsubstituted monocyclic aryl, 5-6 membered heteroaryl, C₁₋₄ alkylcarbonyloxy, C₁₋₄ alkyloxycarbonyl, C₁₋₄ alkylcarbonyl, or formyl and said hydrocarbon chain being optionally interrupted by O, S, N(R^a), C(O), N(R^a)C(O)O, OC(O)N(R^a), N(R^a)C(O)N(R^b), C(O)O, or OC(O)O; each of R^a and R^b, independently, being hydrogen, alkyl, alkenyl, alkynyl, alkoxy, hydroxylalkyl, hydroxyl, or haloalkyl, or L can be a bond, and C can be a metal ion complexing moiety.

The composition can include a chelator-containing compound, which can include a chelator region and a non-chelator region. C can be the chelator region. L can include the non-chelator region.

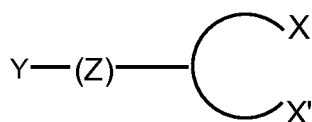
In some circumstances, A can include a polymer $[(M)_x(N)_y(Q)_z]_q$, where each of M, N and Q, independently, can be selected from the group consisting of a linear or cyclic C₃-C₈ hydrocarbyl, heterocyclyl, cyclyl, or aryl including one or more amine, alcohol or carboxylic acid group, where each M-N, M-Q or N-Q can include O, S, N(R^a), C(O), N(R^a)C(O)O, OC(O)N(R^a), N(R^a)C(O)N(R^b), C(O)O, or OC(O)O, each of R^a and R^b, independently, can be hydrogen, alkyl, alkenyl, alkynyl, alkoxy, hydroxylalkyl, hydroxyl, or haloalkyl, and where each of x, y and z can be integers between 0 and 50, 0 and 20 or 0 and 10 and q can be an integer between 1 and 1000, 5 and 500, or 10 and 100.

In some circumstances, L can have the formula:



where each X₁, X₂ and X₃, can be O, S, N(R^a), C(O), N(R^a)C(O)O, OC(O)N(R^a), N(R^a)C(O)N(R^b), C(O)O, or OC(O)O, each of R^a and R^b, independently, can be hydrogen, alkyl, alkenyl, alkynyl, alkoxy, hydroxylalkyl, hydroxyl, or haloalkyl, and where the value of n added to o can be 4 to 10.

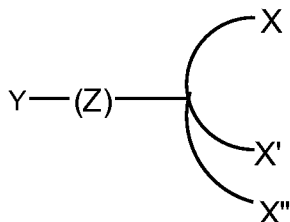
In some circumstances, C can have the formula, H_zG((CH₂)_nCO₂H)_y and salts thereof, where G can be a bond, C, O, S, P, P=O or N; n is 0-6; and z and y can be selected to satisfy the valence requirements of G. In other preferred embodiments, the compound can have the formula,



where X and X' can be the same or different and can be metal binding groups including atoms selected from the group of O, S, N, P or O=P, including carboxyl; Y can be bond, C, O, S, P, P=O or N; and Z can be a hydrocarbon having a backbone of one to six atoms, such as an alkyl group or alkenyl group. Each of X and X' can include other substituents in order to satisfy the valence requirements, such as for example, amine, thiol, phosphine or phosphine oxide, substituted by hydrogen or other organic moieties. In addition, the atoms bridging X and X' can be selected to form a 5-membered to 8-membered ring upon coordination to the metal ion. The

bridging atoms can typically be carbon, but may be other elements, such as oxygen, nitrogen, or sulfur.

In yet another preferred embodiment, the compound can have the formula,



5

where X, X' and X'' can be the same or different and can be metal binding groups including atoms selected from the group of O, S, N, P or O=P, including carboxyl; Y can be a bond, C, O, S, P, P=O or N; and Z can be a hydrocarbon having a backbone of one to six atoms, such as an alkyl group or alkenyl group. Each of X, X' and X'' can include other substituents in order to satisfy the valence requirements, such as for example, amine, thiol, phosphine or phosphine oxide, substituted by hydrogen or other organic moieties. In addition, the atoms bridging X and X', X and X'' or X' and X'' can be selected to form a 5-membered to 8-membered ring upon coordination to the metal ion. The bridging atoms can typically be carbon, but may be other elements, such as oxygen, nitrogen, or sulfur. For example, C can be derived from

10 HSCH₂CH₂CH(SH)(CH₂)_nCOOH, H₂CH₂H₂CH(NH₂)(CH₂)_nCOOH, (HOOC(CH₂)_n)HNCH₂CH₂NH((CH₂)_nCOOH), (HOOC(CH₂)_n)₂PCH₂CH₂P((CH₂)_n COOH)₂, (HOOC(CH₂)_n)₂P(O)CH₂CH₂ P(O)((CH₂)_n COOH)₂, HSCH₂CH₂CH(SH)(CH₂)₄CONH(CH₂)_nCOOH, where n is an integer between 1 and 10, or

15 20 N_a,N_a-bis(carboxymethyl)-L-lysine.

In some embodiments, the composition can include a nanostructure and a linker having a formula: A-L-C, where A can include the polymer covalently bonded to a portion of the nanostructure, L can be a linking moiety including a saturated or unsaturated C₄₋₁₀ hydrocarbon chain optionally containing at least two conjugated double bonds, at least one triple bond, or at least one double bond and one triple bond; said hydrocarbon chain being optionally substituted

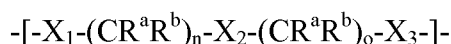
25 with C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₁₋₄ alkoxy, hydroxyl, halo, carboxyl, amino, nitro, cyano, C₃₋₆ cycloalkyl, 3-6 membered heterocycloalkyl, unsubstituted monocyclic aryl, 5-6

membered heteroaryl, C₁₋₄ alkylcarbonyloxy, C₁₋₄ alkyloxycarbonyl, C₁₋₄ alkylcarbonyl, or formyl and said hydrocarbon chain being optionally interrupted by O, S, N(R^a), C(O), N(R^a)C(O)O, OC(O)N(R^a), N(R^a)C(O)N(R^b), C(O)O, or OC(O)O; each of R^a and R^b, independently, being hydrogen, alkyl, alkenyl, alkynyl, alkoxy, hydroxylalkyl, hydroxyl, or haloalkyl, or L can be a bond, and C can be a metal ion complexing moiety.

The composition can include a chelator-containing compound, which can include a chelator region and a non-chelator region. C can be the chelator region. L can include the non-chelator region.

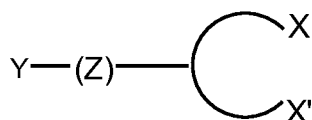
In some circumstances, A can include a polymer [(M)_x(N)_y(Q)_z]_q, where each of M, N and Q, independently, can be selected from the group consisting of a linear or cyclic C₃-C₈ hydrocarbyl, heterocyclyl, cyclyl, or aryl including one or more amine, alcohol or carboxylic acid group, where each M-N, M-Q or N-Q can include O, S, N(R^a), C(O), N(R^a)C(O)O, OC(O)N(R^a), N(R^a)C(O)N(R^b), C(O)O, or OC(O)O, each of R^a and R^b, independently, can be hydrogen, alkyl, alkenyl, alkynyl, alkoxy, hydroxylalkyl, hydroxyl, or haloalkyl, and where each of x, y and z can be integers between 0 and 50, 0 and 20 or 0 and 10 and q can be an integer between 1 and 1000, 5 and 500, or 10 and 100.

In some circumstances, L can have the formula:



where each X₁, X₂ and X₃, can be O, S, N(R^a), C(O), N(R^a)C(O)O, OC(O)N(R^a), N(R^a)C(O)N(R^b), C(O)O, or OC(O)O, each of R^a and R^b, independently, can be hydrogen, alkyl, alkenyl, alkynyl, alkoxy, hydroxylalkyl, hydroxyl, or haloalkyl, and where the value of n added to o can be 4 to 10.

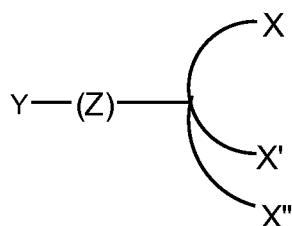
In some circumstances, C can have the formula, H_zG((CH₂)_nCO₂H)_y and salts thereof, where G can be a bond, C, O, S, P, P=O or N; n is 0-6; and z and y can be selected to satisfy the valence requirements of G. In other preferred embodiments, the compound can have the formula,



where X and X' can be the same or different and can be metal binding groups including atoms selected from the group of O, S, N, P or O=P, including carboxyl; Y can be bond, C, O, S, P, P=O or N; and Z can be a hydrocarbon having a backbone of one to six atoms, such as an

alkyl group or alkenyl group. Each of X and X' can include other substituents in order to satisfy the valence requirements, such as for example, amine, thiol, phosphine or phosphine oxide, substituted by hydrogen or other organic moieties. In addition, the atoms bridging X and X' can be selected to form a 5-membered to 8-membered ring upon coordination to the metal ion. The bridging atoms can typically be carbon, but may be other elements, such as oxygen, nitrogen, or sulfur.

In yet another preferred embodiment, the compound can have the formula,



where X, X' and X'' can be the same or different and can be metal binding groups including atoms selected from the group of O, S, N, P or O=P, including carboxyl; Y can be a bond, C, O, S, P, P=O or N; and Z can be a hydrocarbon having a backbone of one to six atoms, such as an alkyl group or alkenyl group. Each of X, X' and X'' can include other substituents in order to satisfy the valence requirements, such as for example, amine, thiol, phosphine or phosphine oxide, substituted by hydrogen or other organic moieties. In addition, the atoms bridging X and X', X and X'' or X' and X'' can be selected to form a 5-membered to 8-membered ring upon coordination to the metal ion. The bridging atoms can typically be carbon, but may be other elements, such as oxygen, nitrogen, or sulfur. For example, C can be derived from

$\text{HSCH}_2\text{CH}_2\text{CH}(\text{SH})(\text{CH}_2)_n\text{COOH}$, $\text{H}_2\text{CH}_2\text{H}_2\text{CH}(\text{NH}_2)(\text{CH}_2)_n\text{COOH}$,
 $(\text{HOOC}(\text{CH}_2)_n)\text{HNCH}_2\text{CH}_2\text{NH}((\text{CH}_2)_n\text{COOH})$, $(\text{HOOC}(\text{CH}_2)_n)_2\text{PCH}_2\text{CH}_2\text{P}((\text{CH}_2)_n\text{COOH})_2$,
 $(\text{HOOC}(\text{CH}_2)_n)_2\text{P}(\text{O})\text{CH}_2\text{CH}_2\text{P}(\text{O})((\text{CH}_2)_n\text{COOH})_2$,
 $\text{HSCH}_2\text{CH}_2\text{CH}(\text{SH})(\text{CH}_2)_4\text{CONH}(\text{CH}_2)_n\text{COOH}$, where n is an integer between 1 and 10, or N_α, N_α -bis(carboxymethyl)-L-lysine.

Compounds can be prepared according to published procedures such as those described, for example, in Parameswara et al., Synthesis, 815-818 (1980) and Denny et al., J. Org. Chem., 27, 3404 (1962).

A sensor array can include a plurality of analysis regions on a support. A support can be glass or plastic. A support may also be any material suitable for supporting the nanostructure, as would be understood by those of ordinary skill in the art. An analysis region can be a divot, a tube, a tray, a well, or a similar compartment for suitable for containing a liquid sample. In some cases, an analysis region can include a droplet or spot on the surface of a support (e.g., a flat support). In those cases, an analysis region can be formed by spotting the composition on a support. A plurality of analysis regions can be arranged in a pattern on a support. A pattern can include concentric circles, a spiral, a row, a column or a grid. In some embodiments, the plurality of analysis regions can include two or more subsets of analysis regions. For example, a first subset of analysis regions can differ from a second subset of analysis regions by including a different nanostructure, a different linker, a different binding partner, a different capture protein, a different analyte or a different sample. Additionally, a first subset of analysis regions can differ from a second subset of analysis regions by including a different environmental factor including a buffer, a reagent, a nutrient, a serum, an exposure to light, an oxygen concentration, a temperature or a pH. As described above and illustrated in **FIG. 1B**, a nanostructure may comprise, for instance, different types of materials. For instance, in some embodiments, the nanostructure may comprise a first gel lacking photoluminescent nanostructures and a second gel comprising photoluminescent nanostructures positioned upon the first gel. In some embodiments, the first and second gel may be positioned upon a solid support material such as, for instance, glass and/or plastic. Any combination of materials may be used as a support material and are contemplated by this disclosure as would be understood by those of ordinary skill in the art.

It is widely recognized that an array of addressable sensors can be multiplexed for the label-free detection of a library of analytes, however such arrays, even when monofunctionalized, have lesser appreciated capabilities that emerge from the ensemble. For example, an array of nanosensors can estimate the mean and variance of the observed dissociation constant (K_D), using three different examples of binding IgG with Protein-A as the recognition site, including polyclonal human IgG ($K_{D\mu} = 11 - 27 \mu\text{M}$), murine IgG ($K_{D\mu} = 3.3 - 5.3 \text{ nM}$), and human IgG from CHO cells ($K_{D\mu} = 0.6 - 16 \text{ nM}$). Secondly, an array of nanosensors can uniquely monitor weakly-affined analyte interactions *via* the increased number of observed interactions. One application involves monitoring the metabolically-induced

hypermannosylation of human IgG from CHO using PSA-lectin conjugated sensor arrays where temporal glycosylation patterns are measured and compared. Lastly, the array of sensors can also spatially map the local production of an analyte from cellular biosynthesis, thus providing a powerful approach to clonal selection. As an example, productivity of IgG-producing HEK colonies cultured can be ranked directly on the array of nanosensors itself. This study opens new avenues for the use of nanosensor arrays for biomanufacturing applications.

Thus, in some embodiments, this disclosure provides methods for detecting an analyte, comprising: contacting a sample including a biologic analyte with a sensor array, the sensor array including a plurality of photoluminescent nanostructures; collecting emissive data from each of the photoluminescent nanostructures; and, analyzing the emissive data from each nanostructure of the plurality of photoluminescent nanostructures to measure a distribution of binding affinities. In some embodiments, the photoluminescent nanostructures may be physically associated with a linker (e.g., “wrapped” in chitosan); directly or indirectly “chelated” with a metal ion (e.g., nickel); and / or “functionalized” with a capture protein (e.g., Protein A, PSA-Lectin) (e.g., “functionalized” refers to a direct or indirect physical association providing a functional relationship between the photoluminescent nanostructure and the capture protein) (see, e.g., FIGS. 1B and 5A). In some embodiments, the photoluminescent nanostructures may be selected from the group consisting of carbon nanotubes, single-walled carbon nanotubes, double-walled carbon nanotubes, semi-conductor quantum dots, semi-conductor nanowires, and graphene. In some embodiments, the sensor array is arranged upon one or more support materials which may be at least one gel. In some embodiments, the one or more support materials comprises a first gel lacking photoluminescent nanostructures and a second gel comprising photoluminescent nanostructures upon the first gel. Any number of layers of gels or other support material may be included in the nanostructures. The one or more support materials may also include a solid material (e.g. glass) supporting the photoluminescent nanostructures. Thus, in some embodiments, the one or more support materials comprises a solid material (e.g. glass), a first gel lacking photoluminescent nanostructures, and a second gel comprising photoluminescent nanostructures upon the first gel. In some embodiments, the capture protein is Protein A and/or a derivative thereof (e.g., where the analyte is antibody) or PSA-lectin and/or a derivative thereof (e.g., where the analyte is mannose). In some embodiments, the sample may comprises cell that colonize the photoluminescent nanostructures and the methods provide for

the detection of one or more analytes produced by the cells is detected. In some embodiments, the methods described herein may also comprise: calculating a calibration curve for the sensor; analyzing the emissive data by measuring a variance of the emissive data from the plurality of photoluminescent nanostructures (where the emissive data may include measuring a skew of the emissive data from the plurality of photoluminescent nanostructures and/or measuring a standard deviation of a plume angle of the emissive data from the plurality of photoluminescent nanostructures); evaluating a binding heterogeneity of the biologic analyte of the sample from the analyzed emissive data; evaluating a cell line for production of the biologic analyte of the sample from the analyzed emissive data; and/or evaluating a glycosylation pattern of the biologic analyte of the sample from the analyzed emissive data. The arrays described here may include, for instance, at least about any of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, or 5000 photoluminescent nanostructures. These methods may also comprise sensors that include an analyte-binding compound associated with the photoluminescent nanostructure. In some embodiments, the sensor may comprise a linker, wherein the analyte-binding compound is associated with the photoluminescent nanostructure via the linker. In some embodiments, the sensor may comprise a polymer including clusters of photoluminescent nanostructures. In some embodiments, the analyte is a biologic analyte such as a protein (e.g., an antibody such as IgG). In some embodiments, the analyte binding compound is a capture protein comprising Protein A or a derivative thereof or PSA-lectin or a derivative thereof. Thus, in such embodiments, the analyte may be any that specifically interacts with Protein A or PSA-lectin, respectively. In some embodiments, the capture protein (e.g., Protein A or a derivative thereof or PSA-lectin or a derivative thereof) comprises a tag having specificity for a binding partner associated with the photoluminescent nanostructure. In some embodiments, the tag is a histidine tag and the binding partner is nickel. Other embodiments are also contemplated by this disclosure as would be understood by those of ordinary skill in the art. A better understanding of the present invention and of its many advantages will be had from the following examples, given by way of illustration.

EXAMPLES

Example 1

A. Sensor Fabrication and Detection Method

Single-walled carbon nanotubes (SWNT) are arrayed (**FIG. 1A**) in a multi-layer, highly-porous (60-90nm) polyacrylamide hydrogel to reduce nonspecific binding and allow for fast diffusion of the IgG analytes to the SWNT sensors (**FIG. 1A**). See, for example, Ruchel, R., *et al. Journal of Chromatography* 166, 563-575 (1978), and Wang, J., *et al. Advanced Materials* 20, 4482-4489 (2008). A basal substrate gel with no SWNT is used on a glass substrate to position the thin sensor layer in a separate focal plane and eliminate the background fluorescence caused by glass impurities. For example, the platform is excited by a 660nm laser (Crystal Laser) on an inverted microscope (Zeiss D.1) and the nIR emission is collected as an image stack on a 256x320 pixel InGaAs array (Princeton Instruments Acton Array). The SWNT are suspended in chitosan and have been chemically modified as before to display chelated nickel groups that act as both the docking site for a His-tagged capture proteins (Protein A and PSA Lectin in these examples) and as the signal transducer (proximity quencher). See, for example, Reuel, N.F. *et al. Journal of the American Chemical Society* 133, 17923-17933 (2011), which is incorporated by reference in its entirety. In brief, the nanotube acts as an optical switch, brightening as antibodies bind to the capture protein. The ensemble response of the array is created by averaging the intensity values over the entire array for each time point and is analogous to other bioassay techniques like ELISA and Biacore® SPR measurements. Divalent nickel, protein A, and IgG addition (100mM, 1 mg/ml, 1.5 mg/ml) cause a decrease, increase, and additional increase respectively (**FIG. 1C(i)**). Subsequent washing of the gel surface shows negligible effects on the ensemble signal, which can be interpreted as absence of unbinding. Adding a BSA control (2mg/ml) does not elicit a sensor response (**FIG. 1C(i)**). Alternatively, the responses of all SWNT pixels can be monitored as histograms of percent modulation ($(I_o - I_{Final})/I_o$) (**Fig. 1C(ii)**). Sensor specificity can also be observed qualitatively with a nIR heatmap filtered to the 10,000 most responsive sites (**FIG. 1D**). To render the sensor array specific, the sensor protein (Protein A or PSA lectin) must first be docked to the chelated nickel; otherwise BSA would elicit a response. Although the ensemble response can be efficiently used to construct calibration curves and monitor titer and glycosylation trends, the averaging loses the valuable information about the affinity distribution recorded by each of the individual sensor elements.

B. Modeling a Nanosensor Array to Measure K_D Distributions

A numerical simulation was also used to determine the analytical relation between K_D variance (β – skewness parameter in φ_1) and the fit nanosensor response (B-fit parameter). In a single iteration of the program, 10000 sensor sites are generated with random K_D , I_o , and PF values generated from φ_1 , φ_2 , and φ_3 respectively. The K_D values, centered at 100nM (**FIG. 2(a)**), determine the coverage fraction coefficients (θ_L) (**FIG. 2(b)**) as specified in the paper (Eq. 2). The response of each sensor is determined for 29 concentration points between 1nM and 10 μ M (**Fig. 2(c)**). The responses are then fit in polar coordinates (**FIG. 2(d)**) with a bivariate Gaussian distribution (**FIG. 2(e)**). The plume angle mean and standard deviation (θ_μ and θ_σ) are then recorded for each concentration. The program completes 6 iterations to create a smooth calibration curve (**FIG. 2(f)**). The initial K_D skewness parameter (β) is then changed and the program is run again. This was done for 200 different β values spanning 2 to 45 and the results are shown in **FIG. 3D**, as described in more detail below. The full code for this simulation can be found in Example 2(K)(1) (Code 3a).

In addition to the more obvious uses of macroscale sensor arrays (efficient replicates, multiplexed response, spatial mapping of response, etc.), nanoscale sensor arrays could be used to measure intrinsic properties of the sample such as affinity distributions. To mathematically study the emergent properties of monofunctionalized sensor arrays, one can consider an analyte to have a Gaussian distribution of K_D with a set variance (σ_t^2) which is then assayed on a 1 mm² array of sensors. If this sensor area is divided in enough independent sensor regions, so that each molecule is assayed independently, the full distribution can be recovered. If each sensor element, however, reports an average of multiple molecule signal transductions the measured sample variance (σ_m^2) becomes much smaller than the actual value (**FIG. 3A** insert). For a Gaussian distribution of K_D , this decay of (σ_m / σ_t) appears to be proportional to $(N)^{-1/2}$ where N is the number of molecules averaged on each sensor site (**FIG. 3A**). This relation informs the effect of concentration and sensor area and identifies the regime where nanosensor arrays can effectively report the variance of K_D (**FIG. 3B** – assumes 10nm² area for antibodies). The platform uses nanotubes arranged in 1.4 μ m² pixels in the concentration ranges of nM to μ M, thus determining distributions of K_D among samples is feasible when the intrinsic variance of the sensor response is low.

A model is first needed to map our experimental array response back to an initial variance in binding. To simulate the array response, a more realistic distribution of K_D for an antibody is used. The Weibull distribution has been proposed for antibody affinity due to its ability to effectively present skewness in the probability distribution function using two shape parameters (α and β). See, for example, Steensgaard, J., *et al. Molecular Immunology* 17, 689-698 (1980). Antibody affinity is intuitively non-symmetric as changes in the optimal protein will more often result in reduced binding and rarely enhance the product. Thus an antibody with positive skew in $\log_{10}(K_D)$ is modeled (tail with higher K_D values or analytes with less affinity) and the probability density function (PDF) is expressed as:

$$\phi_1(x; \alpha, \beta) = \frac{\beta}{\alpha} \left(\frac{x}{\alpha}\right)^{\beta-1} e^{-\left(\frac{x}{\alpha}\right)^\beta} \text{ where } x = \log_{10}\left(\frac{1}{K_D}\right)$$

Eq. 1

This distribution is initially centered at $K_D = 100$ nM (can be translated for other values) such that $\alpha = 7$ and β can vary from 2 to 45+ with 2 being a very large tail (high variance) and 45+ being an equally monodisperse distribution (**FIG. 3C(i)**). The sensor response is modeled by the Langmuir equation (Eq 2) where the coverage fraction (θ_L) is determined by $1/K_D$ and sample concentration (C) (**FIG. 3C(ii)**). In this case, θ_L represents the extent to which a nanotube sensor is turned on by a small number of local interacting molecules during the time of light acquisition (1s per frame).

$$\theta_L(\phi_1, C) = \frac{\left(\frac{1}{K_D}\right)C}{1 + \left(\frac{1}{K_D}\right)C} \text{ where } \left(\frac{1}{K_D}\right) \text{ is sampled from } \phi_1(x; \alpha, \beta)$$

Eq. 2

The extent to which a nanotube sensor responds to adsorption is dictated by its starting intensity (I_0) and extent of functionalization (PF) which can both be experimentally measured and fit with another Weibull (ϕ_2) and Gaussian distribution(ϕ_3) respectively. It is important to note that the number of binding sites on the sensor protein is also imbedded in the functionalization variance (if all nanotubes were uniformly wrapped, functionalized, and had the same number of binding sites, this variable would be a constant). To stochastically simulate the response of an ensemble,

for example, individual sensor responses, R , can be obtained as a coverage fraction, intensity, and percent functionalization value (θ , I_o , and PF ,) randomly generated from their respective PDFs (ϕ_1 , ϕ_2 , ϕ_3) and subsequently evaluated as:

$$R(\theta_L(\phi_1, C), \phi_2(I_o), \phi_3(PF)) = \frac{I_o \left(\frac{1}{1 - PF} - 1 \right) \theta_L}{I_{avg}}$$

Eq. 3

The response is normalized by the average intensity (I_{avg}) as each experimental platform's overall intensity may change due to variance in the experimental setup or quality of SWNT. This allows for clear comparison of distinct arrays assayed at different concentrations. By plotting against the normalized starting intensity ($(I_o - I_{min}) / (I_{max} - I_{min})$) characteristic response 'plumes' are observed (**FIG. 3C(iii)**). The 'plume' is the distribution of data points shown in the plot. The experimental 'plumes,' are fit well by a bivariate Gaussian distribution in polar coordinates (θ, R) and the mean 'plume' angle (θ_μ) is used to create the calibration curve (**FIG. 2C(iv)**). The standard deviation in the plume angle (θ_σ) also has an interesting dependence on starting K_D skew (β) and is reported (**FIG. 3C(iv)**). The (θ_μ) calibration curve is fit by a four parameter logistics curve (used for many other bioassays that exhibit a signal saturation – i.e. ELISA) however in this case two parameters are known from experimental conditions ($A = \theta_{min} = 0$ and $D = \theta_{SAT} = \text{Eq. 5}$).

$$Plume \theta_\mu(x) = \left(\frac{A - D}{1 + \left(\left(\frac{X}{C} \right)^B \right)} \right) + D \quad \text{where } x = \log_{10}(C)$$

Eq. 4

$$D = \theta_{SAT} = \tan \left(\frac{1}{\frac{1 - PF}{I_{AvgNorm}} - 1} \right)$$

Eq. 5

The parameter B can be thought of as a measure of 'steepness' and C is equal to the inflection point at K_D . By analyzing the model at many different extents of K_D skewness ($2 < \beta < 45$), the effect of K_D variance on the fit parameters were found (**FIG. 3D**). As the distribution of antibody affinities becomes tighter (β increases) the nanosensor array yields a steeper

calibration curve (FIG. 3C fits this relation). This model can be used to analyze experimental data.

C. K_D Distributions and Calibration Curves from Experimental Data

The array of nanotube sensors conjugated to His-tagged Protein A (Abcam) was used to assay three different samples of IgG with expected differences in affinity distributions: (1) commercial, lyophilized, polyclonal Human IgG reconstituted in PBS, (2) murine IgG (TA99) from an engineered human embryonic kidney (HEK) cell line, and (3) human IgG (b12) cultured from Chinese hamster ovary (CHO) cells. The data ‘plumes’ predicted by the model were observed for each of the systems (FIG. 4A) and the resulting calibration curves (FIG. 4B) yielded K_D mean values (95% confidence intervals for each: 11 – 27 μ M, 3.3 – 5.3 nM, and 0.6 – 16 nM) comparable to those found in literature for IgG-Protein A interactions (2-50 nM from SPR, 34 nM from acoustic device (see, for example, Nohldén, S. in Department of Physics, Chemistry and Biology, Vol. Masters 68 (Linköpings universitet, Linköping; 2008), and Saha, K., *et al. Analytical Chemistry* 75, 835-842 (2003)). The calibration curve also provided ‘B’ fit parameters (Eq. 4) that are related to the starting distribution skew parameter (β) as solved from the model simulation (FIG. 4C). With K_D mean (α) and skewness (β), the affinity PDF of each system (Eq. 1) can be determined (FIG. 4D). As expected, the freshly expressed Human IgG has the greatest affinity for Protein A with the least amount of variance. The murine antibody has a comparable K_D average, but much more predicted variance. This difference could be due to less efficient binding of murine IgG to Protein A as observed in literature. See, for example, Richman, D.D., *et al. Journal of Immunology* 128, 2300-2305 (1982). Finally the lyophilized, polyclonal human IgG shows a 1000x reduction in K_D and a much broader distribution of affinities likely due to denaturation damage (see, for example, Schersch, K. *et al. Journal of Pharmaceutical Sciences* 99, 2256-2278 (2010), which is incorporated by reference in its entirety) or freeze-thaw cycles (*vs.* the freshly expressed CHO product). One limitation of the modeling approach is the *a priori* assumption of PDF form (in this case Weibull). The array of nanosensors can be used to report distributions directly, without any assumption of PDF form by calculating the Langmuir coverage from the response of each individual sensor (Eq. 2-3) and creating a histogram of K_D values (FIG. 4E). To do this now, however, one assumes a constant percent functionalization value ($PF = 0.14$, mean of ϕ_3) and chooses a concentration value away

from saturation (these assumptions are an approximation as a distribution of functionalization (ϕ_3) exists and each concentration does not align perfectly on the calibration curve, so each ‘plume’ will yield a slightly different K_D histogram). This direct technique for measurement can be improved by better fabrication methods, reducing the intrinsic variance of length and functionalization. The direct reporting approach also presents K_D distributions with similar shape and positive skew as the model assumed.

D. Hypermannosylation Detection – Weakly affined interactions on nanosensor arrays

Another advantage of nanosensor arrays is their ability to report weak binding events – a greater number of individual sensor sites increases the probability of a detection event and this event is not averaged to null with other non-responsive sites, as in the case for an ensemble sensor. The label-free nature of the platform is also beneficial to detecting weakly-affined ligands since it requires no washing steps. By swapping out the His-tagged Protein A with a His-tagged, mannose-specific plant lectin, *Pisum sativum* agglutinin (PSA) the sensor platform can detect specifically high mannose content IgG (**FIG. 5A**). Different species of IgG were initially used to test this concept. Chicken IgG contains an appreciable amount of glycoforms with high mannose content (>40% of population) whereas these are virtually absent in human and mouse IgG. See, for example, Raju, T.S., *et al. Glycobiology* 10, 477-486 (2000), which is incorporated by reference in its entirety. The SWNT sensor responses to human, mouse, and chicken IgG in PBS align with these findings and confirm that the platform can detect mannose species with the PSA lectin specifically (**FIG. 5B**). A well characterized, high-mannose content IgG sample from a microbial source was then used to further validate detection and obtain a calibration curve and K_D distribution as before (**Figs. 5C-E**). The mannose IgG – PSA affinity ($K_D = 1.3 - 55 \mu\text{M}$ 95% confidence interval) is comparable to literature values ($\mu\text{M} > K_D > \text{mM}$ for lectin-glycan interactions) with a broad distribution (**FIG. 5E**). See, for example, Hirabayashi, J. *Journal of Biochemistry* 144, 139-147 (2008). This is expected as 60% of the IgG sample is aglycosylated (verified by MS analysis) and the remaining 40% bear differing lengths and structures of high mannose-type glycans (verified by released glycan analysis).

It has also been shown that changing culture conditions such as media components can affect the onset and extent of IgG hypermannosylation. In order to further validate the platform, an experiment was designed for CHO cultures in which levels of NaCl were increased and a

MnCl₂ additive was used while monitoring mannose content over time using traditional Peptide-N-Glycosidase F release and capillary electrophoresis. See, for example, Pacis, E., *et al. Biotechnology and Bioengineering* 108, 2348-2358 (2011). Four identical dishes of CHO cells were cultured and fed media compositions derived from this study (**FIG. 5F**). The supernatants were collected after each 24 hour period, diluted to a standardized 10ng/ml IgG concentration and assayed on our PSA rendered sensor gels. The IgG concentrations were determined with ELISA (**FIG. 5G**) and if below 10ng/ml, the sample was run at stock concentration. The resulting trends (**FIG. 5H**) determined by the mean percent modulation from the nanosensor array distributions match those found in the previous study: 1) increased mannose content as culture time increases, 2) increased mannose from higher NaCl osmolality, and 3) delayed onset of hypermannosylation from MnCl₂ additive. The presence of mannose in these samples was confirmed by surface staining with fluorescently tagged PSA and the sensitivity demonstrated by the array exceeded the capabilities of the current protein A capture followed by LC/MS technology. Due to the low volumes of sample and the low level of antibody expression, the antibody capture step was unsuccessful.

An alternative method was therefore used to confirm the presence of mannose in the CHO cultures. A surface staining approach was used (**FIG. 6(a)**) in which Protein A conjugated to a porous resin bead (Pierce 53139) was immobilized to a poly-lysine coated glass surface *via* glutaraldehyde in 2.5mm diameter Teflon patterned wells. The daily CHO samples diluted to 10ng/ml IgG (used in Manuscript Figure 5 assay) were then spotted on the glass slide at 20μl. The IgG was allowed to interact for 2 hours and the chip was then washed in PBS. The chip was then spotted with 300μg/ml BSA to block non-specific sites and washed with PBS again. Finally FITC-conjugated PSA (Vector Labs FL-1051) was spotted on the sample at 25ug/ml and allowed to interact overnight. The samples were then washed with PBS and assayed on an inverted microscope reading the visible FITC emission. Qualitatively, the observation of more conjugated FITC-PSA corresponded with the days peak mannose was observed using the nanosensor platform (**FIG. 6(b)**). This supports the finding of mannose in the IgG samples.

E. Nanosensor Arrays for Monitoring Local Cell Colony Production

A gel with an imbedded array of nanosensors can be used to screen local production of cells. Unfortunately single cells are very difficult to culture for long periods of time on the

current porous platform (little indications of healthy, single cell adherence). The single cells that did culture well, however, displayed colocalization of IgG production on a Protein A-incubated gel (**FIG. 7A**). By seeding a greater number of cells, larger colonies of cells formed on the porous gel surface and were able to produce for longer time periods (24 hours). By analyzing
5 images of control and IgG-producing HEK cells islands after 24 hours of production, a statistical difference between the two production profiles can be seen (see Code 3c presented in Example 2(K)(3)). By ranking the brightest 1000 SWNT and then querying their location, there is a greater localization of the bright SWNT under IgG producing islands where as they are evenly or randomly distributed within and outside of the control cell islands (**FIGS. 7B,C**). HEK cells
10 producing IgG were plated on a gel and acquired multiple images of the nIR intensities at 0, 1, 2, and 3 hours. Histograms of the 1000 brightest SWNT pixels in these images show a ‘turn-on’ trend that is likely due to IgG production (**FIG. 7D**). Finally, large islands of HEK cells were allowed to grow overnight on a Protein A gel. The nIR response was clearly co-localized under each of the islands, and the response was summed, averaged over the island area and each island
15 was ranked based on productivity (**FIG. 7E**).

Carbon nanotube-based fluorescent sensor arrays can be used for monitoring distributions of K_D , hypermannosylation, and local cellular production of IgG with clear implications in biomanufacturing. The platform was demonstrated with lyophilized IgG in PBS as well as characterizing freshly-expressed IgG in complex media from three different cellular expression
20 systems: HEK, CHO, and a fungal cell line. The sensor array was rendered specific to mannose with PSA-lectin and trends in metabolically induced hypermannosylation from a previous study were confirmed. Finally, local production of IgG from HEK cell colonies cultured on sensor arrays was monitored. Better upstream colony selection could be performed with a sensor gel optimized for healthy cell culture. The cell colonies could be exposed to various culture and
25 media conditions and their productivity and glycosylation patterns could be monitored in real time. This platform could lead to more rapid and informed selection of master cell lines and culture conditions based on multiple parameters rather than picking colonies based on static snapshots of productivity provided by current assays. See, for example, Burke, J.F. *et al. Genetic Engineering & Biotechnology News* 29, 38-39 (2009). During production, sensor arrays
30 in a microfluidic platform can be used to monitor product titer, K_D distribution, and glycosylation by periodically sampling the bioreactor, filtering cellular components, diluting to a

set level depending on the cell line's average productivity read the fluorescent signal, and then regenerate for the next sample. Protein A gels can be regenerated using a pH 3.0 release wash, similar to regeneration of Protein A purification columns with little loss of sensitivity. Detection of mannose has been validated here but other glycans of interest (galactose, fucose, sialic acids, and non-human, immunogenic glycans like gal- α 1,3-gal (see, for example, Chung, C.H. *et al. New England Journal of Medicine* 358, 1109-1117 (2008)) could also be detected by multiplexing portions of the nanosensor array with different His-tag lectins.

His-tag lectins can be made by chemical conjugation of a His-tag and a target lectin. A kit, such as a his-tag linkage kit (Solulink) can be used to form a polyhistidine tagged lectin. Alternatively, for example, suitable starting materials can include a native lectin (Vector Labs), a linker such as SMCC linker (Pierce), an activating agent such as Traut's reagent (Pierce), and a polyhistidine such as hexahis peptide (Abbiotec). An example protocol can include the following steps:

Prepping SMCC stock

1) Dissolve 6 mg SMCC in 1 mL DMF (the official protocol called for 1.5 mg/mL, but we increased it to avoid adding too much DMF to the protein solution)

Prepping Protein, Adding SMCC

1) Make a 1 mg/mL PBS solution of each lectin you want to tag
2) 25 μ L of the SMCC stock was added per mL protein solution (this is assuming the protein is on the order of ~50-75 kDa)
3) Incubate this reaction mixture at room temp for 30 min

Prepping Traut stock, Use Traut on His-tag Peptide

1) Make a 14 mM solution of Traut's reagent in H₂O (e.g. 2 mg/mL)
2) For a 1 mg/mL solution of HexaHis, add 4.6 μ L Traut stock per mL HexaHis solution
3) Incubate at room temp for 1 hour

Tagging the Protein

1) Again assuming the protein is on the order of ~50-75 kDa, add 16.8 μ L of Traut+His solution per mL of Protein solution (1 mg Protein per mL PBS)

Suitable glycans and lectins can be selected based on the properties to be detected. Examples of glycans or lectins can be identified in the literature. See, for example, van Berkel, P. H., *et al. Biotechnology Progress*, Volume: 25, Issue: 1, pages: 244-251 (2009), Hirabayashi,

J., *J. of Biochemistry* Volume: 144, Issue: 2, pages: 139-147 (2008), Gabius, H.-J. *et al.*, *Trends in Biochem. Sci.* Volume: 36, Issue: 6, pages: 298-313 (2011), Dam, T.K.; *et al.* *Glycobio.* Volume: 20, Issue: 3, pages: 270-279 (2010),): Tateno, H. *et al.* *Methods in Enzymology*, Volume: 478, pages: 181-195 (2010), and Hsu, K.-L. *et al.* *Current Opinion in Chem. Bio.* Volume: 13, Issue: 4, pages: 427-432 (2009), and Krishnamoorthy, L., *et al.* *ACS Chem. Bio.* Volume: 4, Issue: 9, pages: 715-732 (2009).

The longstanding goal of nanosensor arrays is to preserve the sensitivity and analytical advantages of single-molecule nanosensors with the multiplexing ability of macroscale techniques, thus filling an untapped analytical regime (**FIG. 8**). The fast assay time (< 5 min) of nanosensor arrays could also provide a disruptive alternative to the more time intensive ELISA and LC/MS analytics that are currently used (**FIG. 8**). The current limitations of these arrays are the intrinsic variances caused by non-automated production in small batches (16-32 gels per batch). Small variations in polymer casting time, initiator concentration, and washing procedures result in gels with varying levels of functionalization and sensitivity. A standardized gel from an automated printing/production system could reduce this variance and provide a robust tool for biomanufacturing analytics and beyond.

Example 2

Methods and Materials

A. SWNT Sensor and Gel Platform Fabrication

SWNT were suspended in chitosan according to Reuel, N.F. *et al.* *J. Am. Chem. Soc.* **133**, 17923-17933 (2011). In brief, 3 mg of purified HiPCO SWNT (Unidym) were added to 20ml of chitosan suspension (0.25 wt% in water containing 1 vol% acetic acid – Sigma). The resulting mixture was tip sonicated (1/4" tip Cole Parmer, Model CV18) at 10W for 45 minutes in an ice bath and table-top centrifuged three times at 13.2 RPM for 90 min each, while collecting the suspended SWNT supernatant and discarding the aggregate pellet after each cycle. The SWNT was then mixed at a 50:50 volume ratio with the polyacrylamide mixture for casting as the top layer. The amount of monomer (acrylamide) and cross-linker (N,N'-Methylenebisacrylamide – both Sigma) are specified using standard %T %C nomenclature, where %T refers to the overall weight % of polymer (monomer and crosslinker) in the solution and %C refers to the wt% of the total polymer that is cross linker. Surface gels with a 3%C and 1%T composition performed

well. A substrate gel (6%T, 1%C) was also prepared. TEMED (Tetramethylethylenediamine) was added in at 0.7 vol% in both the top and bottom gel solutions to stabilize the radical reaction. A fresh initiator solution of 1 wt% Ammonium persulfate (APS) was made immediately prior to each gel batch. The APS, bottom and top gel solutions, and substrate chips (8 chamber Lab-Tek
5 by Nunc) were degassed in the glove box antechamber to remove absorbed and dissolved oxygen. Within a nitrogen controlled glove box (MBraun LABstar), 1 vol% of the APS solution was added to the substrate gel to initiate the polymerization and it was immediately cast (100ul to each well) and then allowed to cure for one hour. The top gel was then initiated with 1 vol% APS and immediately spotted at 20 ul to each gel surface and allowed to cure for 1 hour.

10 The functionalization steps of the chitosan wrapped SWNT is also similar to that described in Reuel, N.F. et al. *J. Am. Chem. Soc.* **133**, 17923-17933 (2011). In brief, the amine groups of the chitosan were reacted with succinic anhydride (133 mM in PBS 7.4 buffer - Sigma) overnight and then washed thoroughly with water. The carboxylic acid functional groups were then activated with 100mM EDC and 520mM NHS (Sigma) in MES Buffer pH 4.7 (Pierce) for 2
15 hours. After washing with water thoroughly, the gels were then reacted with 34mM Na₂Bis(carboxymethyl)-L-lysine hydrate (Sigma) in PBS 7.4 buffer overnight. The gels were then washed and incubated with a 100mM nickel sulfate solution for 20 minutes. These chips were then washed thoroughly in water and stored in water.

As explained in section C, the sensors were manufactured for use with a his-tag sensor
20 protein ((Protein A (Abcam) or PSA lectin). The original SWNT sensors for protein and glycan recognition from our group were cast in a chitosan hydrogel. Not only were the desired attributes of large pore size and local SWNT functionalization hard to control in this gel, but the chitosan proved to exacerbate the non-specific binding of the more 'sticky' IgG and Protein A species. Adding Protein A-Histag followed by Human IgG (Fig. S1a in U.S. Ser. No.
25 61/767,513) resulted in the expected loading curves but when other proteins were swapped for Protein A, like EFGP-Histag, we still observed a loading curve from the IgG (Fig. S1b in U.S. Ser. No. 61/767,513). Furthermore, Protein A would also non-specifically bind to the gel, as Protein A without a Histag also resulted in binding (Fig. S1c of U.S. Ser. No. 61/767,513). A set of control experiments show that the docked sensor protein (Protein A) is needed to render the
30 gel response specific, i.e., suppress a response from BSA (**FIG. 9**).

B. Poroelastic Relaxation Indentation and Dextran Release Curves

The top acrylamide gel layer containing the SWNT sensors was tuned to a maximum pore size to ensure that large antibodies could diffuse to the sensor sites. The effect of crosslinker concentration on pore size has been established qualitatively in literature with TEM
5 imaging (Ruchel, et al. Transmission-Electron Microscopic Observations Of Freeze-Etched Polyacrylamide Gels. *Journal of Chromatography* **166**, 563-575 (1978)). It is noted that these experiments were performed while protecting the radical polymerization from quenching oxygen species in a nitrogen-filled glove box. It was found that solutions with as low as 3 wt% polymer (97% water) were able to crosslink in this environment. It was determined from FITC dextran
10 release profiles (Fig. S2d of U.S. Ser. No. 61/767,513) that the largest dextran probe (31.4nm in diameter) was able to freely diffuse into the 3%T 1%C optimized gel (%C is standard nomenclature for the crosslinker weight percent of the total polymer weight percent (%T); thus, in this case, it would be 0.03 wt% of the total mixture).

The pore size was also more rigorously probed using a recent hydrogel characterization
15 technique called microscale poroelastic indentation by AFM as explained in the Kalcioğlu, Z.I., et al. (From macro- to microscale poroelastic characterization of polymeric hydrogels via indentation. *Soft Matter* **8**, 3393-3398 (2012)) *Soft Matter* **8**, 3393-3398 (2012); see also , which is incorporated by reference in its entirety. In brief, a short silicon tip with a 45 μ m polystyrene sphere (Novascan) was fitted on an AFM (Asylum Research – MFP3D) and the IgorPro software
20 indentation panel was used to drive the tip into the gel at a specified distance and record the force over time. This was done in replicates for multiple sites on each gel type. A custom Matlab algorithm was then used to analyze the force relaxation curves and determine the average pore size. FITC-conjugated dextran particles (Invitrogen) of various sizes (10, 40, 70, and 500 kD) were also absorbed into 150 μ l cylindrical gel plugs over 48 hours. The impregnated gels were
25 then removed, washed, and inserted into clean water. The release of the FITC particles was observed by sampling the exterior fluid and assaying the FITC content with a plate reader. Using standard curves, the release was then determined in terms of cumulative mass release over time.

In more detail, a microsphere that was orders of magnitude larger than the average pore size ($R = 22.5 \mu$ m) is indented into the gel at a specified distance (h) and held while the displaced
30 solvent leaks into surrounding regions. The resulting force *versus* time curve (Fig. S2b of U.S. Ser. No. 61/767,513) exhibited an initial spike (F_0) that relates to the shear modulus of the gel (G

in Eq 6) and then relaxed to an equilibrium state (F_∞) which relates to the diffusion constant of the displaced fluid. The force relaxation curve was obtained for multiple penetration depths ($h = 8, 6, \text{ and } 4 \mu\text{m}$), normalized (Fig S2c of U.S. Ser. No. 61/767,513) and fit by a numerically solved model for an indented sphere geometry³ to determine the diffusion constant (D in Eq 7) and Poisson ratio (ν_s in Eq 8). These parameters and the viscosity of water (η) were then used to solve for the average pore size of the network (ξ_p in Eq 9) (Chan, et al. Spherical indentation testing of poroelastic relaxations in thin hydrogel layers. *Soft Matter* **8**, 1492-1498 (2012)). These measurements yielded an average pore size of 57-93 nm (95% confidence interval) which is 5-9 times the calculated size ($\sim 10\text{-}11\text{nm}$) of a hydrated IgG antibody (Armstrong, et al. The hydrodynamic radii of macromolecules and their effect on red blood cell aggregation. *Biophysical Journal* **87**, 4259-4270 (2004)) (see Code 3b preset in Example 2(K)(2)).

$$F_0 = \frac{16}{3} G(\sqrt{Rh})h \quad \text{Eq. 6}$$

$$\frac{F(t) - F_\infty}{F_0 - F_\infty} = 0.491 \exp\left(-0.908 \sqrt{\frac{Dt}{Rh}}\right) + 0.509 \exp\left(-1.679 \frac{Dt}{Rh}\right) \quad \text{Eq. 7}$$

$$\frac{F_0}{F_\infty} = 2(1 - \nu_s) \quad \text{Eq. 8}$$

$$\xi_p = 2 \left(\frac{\eta D(1 - 2\nu_s)}{2 G(1 - \nu_s)} \right)^{\frac{1}{2}} \quad \text{Eq. 9}$$

C. Analyzing SWNT Clusters with SEM, Raman Mapping, and TEM

In an attempt to understand the variation between sensor gel batches, substantial characterization of the polyacrylamide hydrogel network was performed. In these experiments, the thin, top polyacrylamide gel entraps the SWNT sensors in a sparsely cross-linked network. If the chitosan wrapped SWNT are added to the solution containing acrylamide monomers and bis-acrylamide crosslinker immediately prior to radical polymerization initiated by ammonium

persulfate, the resulting gel has a homogenously distributed SWNT pattern. Conversely, if the SWNT are able to interact with the monomer and crosslinker solution during the bottom gel curing time, the amine groups on the chitosan-wrapped SWNT undergo linear step growth Michael Addition Polymerization with the bisacrylamide cross-linker (forming poly amido amines) leading to larger fluorescent SWNT clusters. The cluster sizes are dependent on the interaction time of these two constituents before starting the radical polymerization that consumes the remaining crosslinkers and immobilizes the clusters. These clusters can be clearly seen in the nIR micrographs (Fig. S2a of U.S. Ser. No. 61/767,513), raman maps, visible micrographs (Fig. S4 of U.S. Ser. No. 61/767,513), SEM (Figs. S2a and S3 of U.S. Ser. No. 61/767,513), and TEM imaging (Fig S5) and were found to be more responsive to IgG than the gels with sparse, evenly distributed SWNT. This is likely due to binding events affecting a greater population of SWNT when in cluster formations *versus* interacting with only a few SWNT when patterned as more sparse elements. The clusters visible in the top gel nIR micrographs were characterized with SEM (Fig. S3 of U.S. Ser. No. 61/767,513) and the presence of SWNT was confirmed with Raman mapping (Fig. S4 of U.S. Ser. No. 61/767,513) and TEM (Fig. S5 of U.S. Ser. No. 61/767,513). The SEM images were created from flash dried samples as this was found to preserve the structural integrity of the underlying gel and some of the clusters in the top gel.

D. Data collection on nIR Inverted Microscope

SWNT sensor data was collected on a custom inverted microscope (Zeiss D.1 Observer) that was fitted with a 660 nm laser (Crystal Laser, 100 mW). A 20x planar objective (Zeiss) was used and the emission intensities were recorded by a nitrogen-cooled InGaAs array (Princeton Instruments). Win Spec software (Princeton Instruments) was used to collect the SWNT emission and saved as an image stack TIF file. This file was then analyzed using Matlab. Analyte samples were added to the sensor gels by hand, applying the 100µl sample to the lower right corner of the well, so as not to place the plastic pipette tip in the laser beam path. To prepare a sensor gel for testing, it was first thoroughly washed with PBS to exchange the buffer and then allowed to incubate with the his-tag sensor protein (Protein A (Abcam) or PSA lectin (Vector Labs – conjugated to His-tag peptide (Abbiotec) via Traut's reagent and SMCC linker

(Pierce)) at 500µg/ml overnight. The gel was again washed thoroughly with PBS and then fitted on the microscope for testing.

E. Quenching and Signal Transduction Studies

5 Based on previous work (Reuel, N.F. et al. Transduction of Glycan-Lectin Binding Using Near-Infrared Fluorescent Single-Walled Carbon Nanotubes for Glycan Profiling. *Journal of the American Chemical Society* **133**, 17923-17933 (2011); Ahn, J.H. et al. Label-Free, Single Protein Detection on a Near-Infrared Fluorescent Single-Walled Carbon Nanotube/Protein Microarray Fabricated by Cell-Free Synthesis. *Nano Letters* **11**, 2743-2752 (2011)), it was postulated that
10 the chelated nickel used to bind the His-tag sensor protein was responsible for the modulation of the SWNT fluorescence. Experiments clearly showed that nickel quenches chitosan-wrapped SWNT in solution and immobilized on a surface (**Fig. 10**). Divalent nickel cations have been shown to quench anionic surfactant wrapped SWNT⁸ and quantum dots (Wu, et al. Ni(2+)-modulated homocysteine-capped CdTe quantum dots as a turn-on photoluminescent sensor for
15 detecting histidine in biological fluids. *Biosensors & Bioelectronics* **26**, 485-490 (2010)). The proximity of the chelated nickel groups alter the local electronic environment and offer non-radiative decay pathways for the SWNT exciton. To explore if this is the only mechanism at work, we conceived of an experiment to replace the nickel with another small molecule proxy – biotin. The chitosan wrapped SWNT are biotinylated with a commercially available NHS linker
20 (Pierce EZ-Link NHS-Biotin) and then exposed to neutravidin and a BSA control (500µg/ml). The specific response is again a positive modulation and the control is null (**FIG. 10(a)**) although the turn-on response is approximately 20% of what is typically seen when nickel is present. This supports two interoperating mechanisms (**FIG. 10(b)**): (1) the sensor proteins are originally quenched by chelated nickel groups and the return of signal occurs when the nickel is displaced
25 upon binding of the sensor protein or IgG, and (2) the chitosan wrapped SWNT is originally quenched by aqueous quenching species (water molecules (Strano, M.S. et al. The Role of Surfactant Adsorption during Ultrasonication in the Dispersion of Single-Walled Carbon Nanotubes. *Journal of Nanoscience and Nanotechnology* **3**, 81-86 (2003)), dissolved oxygen, and protons (Blackburn, J.L. et al. Protonation effects on the branching ratio in photoexcited
30 single-walled carbon nanotube dispersions. *Nano Letters* **8**, 1047-1054 (2008)) have all been shown to quench exposed portions of suspended SWNT) and the bound macromolecule displaces

these species to cause a return in signal. A macroscopic swelling mechanism (as in previous work with PVA hydrogels (Barone, P.W. et al. Modulation of Single-Walled Carbon Nanotube Photoluminescence by Hydrogel Swelling. *ACS Nano* 3, 3869-3877 (2009)) was ruled out as the recorded movies of SWNT arrays during testing show that the SWNT sensors are immobilized and do not move on the pixel resolution recorded (1.2 μm per pixel).

F. Gaussian distribution (σ_m/σ_t) decay is proportional to (Number Sites Averaged)^{-1/2}

To verify Gaussian distribution (σ_m/σ_t) decay is proportional to (Number Sites Averaged)^{-1/2}, a simulation in which a Gaussian distribution with σ_t is assumed and then a new distribution is created by selecting a number of points (N_{avg}) from the original distribution and then measure this distribution's new standard deviation (σ_m), where:

N = number of sites in full distribution

N_{avg} = number of sites averaged together from full distribution

$$\sigma_t^2 = \frac{1}{N} [(x_1 - \mu)^2 + (x_2 - \mu)^2 + \dots + (x_N - \mu)^2] \text{ where } \mu = \frac{1}{N} (x_1 + \dots + x_N)$$

$$\sigma_m^2 = \frac{1}{N/N_{avg}} [(x_1 - \mu)^2 + (x_2 - \mu)^2 + \dots + (x_N - \mu)^2] \text{ where } \mu = \frac{1}{N} (x_1 + \dots + x_N)$$

$$\frac{\sigma_m^2}{\sigma_t^2} = \frac{\frac{1}{N/N_{avg}}}{\frac{1}{N}} = \frac{1}{N_{avg}}$$

$$\frac{\sigma_m}{\sigma_t} = \frac{1}{\sqrt{N_{avg}}}$$

By running the simulation multiple times and averaging the simulation results, the relationship found above clearly emerges. Using the simulation of averaging a number of points (N_{avg}) from a given distribution with standard deviation σ_t and determining the ratio of the new standard deviation (σ_m) with the original, the -1/2 power law determined above emerges (as illustrated by FIG. S8 of U.S. Ser. No. 61/767,513).

G. Starting Intensity of the SWNT (I_o) and the Percent Functionalization (PF)

Two additional inputs to the sensor response are the starting intensity of the SWNT (I_o) and the percent functionalization (PF). Both will determine the extent to which a given sensor can modulate. Both can be fit with probability density functions (PDF) from experimental data. An all points histogram of pixel intensity (Fig S9a of U.S. Ser. No. 61/767,513) reveals two distinct populations of pixels – those that are not illuminated by the laser spot and the pixels that contain SWNT emission. The starting SWNT intensities for each sensor gel can be fit by a Weibull distribution (Fig S9b of U.S. Ser. No. 61/767,513) and vary in their mean values (α) and skewness of bright SWNT (β) depending on processing conditions, age of SWNT suspension etc. However, this does not affect the simulation results as variation in the SWNT I_o distribution translates to shifting of the center of the plume distribution in the radial distance when performing the Gaussian bivariate fit in polar coordinates. The plume angle is used to report the calibration curve, and not the radial position, thus any positively skewed Weibull distribution can be used. For the simulation (see Code 3a presented in Example 2(K)(1)), the following PDF fit (Fig S9b of U.S. Ser. No. 61/767,513) to the first data set (nickel quench in **FIG. 1a,d**):

$$\phi_2(x; \alpha, \beta) = \frac{\beta}{\alpha} \left(\frac{x}{\alpha}\right)^{\beta-1} e^{-\left(\frac{x}{\alpha}\right)^{\beta}} \text{ where } x = I_o, \alpha = 6872, \text{ and } \beta = 2.2$$

The extent of sensor functionalization was determined by monitoring the extent of quenching caused by nickel addition. This provides a reasonable measure to the accessibility and functionalization of each nanotube. The percent quenching was found to be invariant in SWNT length (banded when plotted vs. I_o) and approximated well by a Gaussian distribution (Fig S9c-d of U.S. Ser. No. 61/767,513) with the following PDF (Fig S9d of U.S. Ser. No. 61/767,513):

$$\phi_3(x; \mu, \sigma) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \text{ where } x = PF, \mu = -14.2, \text{ and } \sigma = 13.9$$

It was found that the population is fit with a Weibull PDF (Fig. S9b of U.S. Ser. No. 61/767,513) and can be created for each experiment. The population was found to be conserved amongst separated gels and is fit with a Gaussian PDF (Fig. S9d of U.S. Ser. No. 61/767,513) (histogram of quenching percent after nickel addition).

H. HEK Cell Line Generation and CHO Origin

A tricistronic expression cassette pLB2-CMV-GFP-TA99 was created using 2A skip peptides (see Hu, T., et al. *Biotechnology Letters* **31**, 353-359 (2009), which is incorporated by reference in its entirety). The light and heavy chain sequences of TA99, a murine IgG2a antibody (Thomson, T.M., et al. *J. of Investigative Dermatology* **85**, 169-174 (1985)), were linked by a T2A sequence. The expression cassette was cloned into the lentiviral vector, pLB2 (Stern, P. et al. *PNAS* **105**, 13895-13900 (2008), which is incorporated by reference in its entirety), modified with a CMV promoter driving GFP-F2A expression, creating the complete plasmid sequence of pLB2-CMV-GFP-F2A-LC-T2A-HC. All cloning was performed using overlap extension PCR.

HEK-GFP-TA99 cells were generated using a modified version of a previously described protocol (see Kuroda, H., et al. *J. of Virological Methods* **157**, 113-121 (2009)). Briefly, HEK-293FT cells (Invitrogen) were transfected with the following plasmids: pLB2-CMV-GFP-TA99, pCMV-dR8.91, and pCMV-VSV-G at a mass ratio of 2:1:1 using PEI (see Zufferey, R., et al. *Nature Biotechnology* **15**, 871-875 (1997); and Dull, T. et al. *J. of Virology* **72**, 8463-8471 (1998)). After 24 hours, fresh media was exchanged. 48 and 72 hours later, supernatant containing lentiviral particles was harvested. HEK-293 cells were transduced twice for 24 hours by incubation with freshly harvested supernatant supplemented with protamine sulfate at 5 µg/mL. GFP positive cells were selected to a purity of greater than 95% using flow fluorescence activated cell sorting. Details on the generation of the CHO cell line can be found in Hezareh, M., et al. *J. Virology* **75**, 12161-12168 (2001). Additional data is presented in Supplement 2e of of U.S. Ser. No. 61/767,513.

I. Cell Passaging and Culture

For standard culture the medias used were DMEM (with 4.5 g/L glucose, 10% heat inactivated FBS (Invitrogen), 2 mM L-glutamine, 100 U/ml Penicillin, 100 U/ml Streptomycin – rest Sigma), and GMEM (same additives - Sigma) for the HEK and CHO cultures, respectively. For cultures used in experiments, a serum free media was used for growth and as a buffer in the sensor gel (Invitrogen Freestyle 293). To passage the cells, they were allowed to grow to confluence, washed with PBS, and then released with Trypsin (0.05% w/0.53 mM EDTA). The cells were then pelleted, resuspended in fresh media and diluted at a 1:5 ratio. The cells were passaged every 2-3 days and discarded after the 20th passage.

J. Hypermannosylation CHO Culture Experiment

CHO cells were seeded at equal density in small culture flasks (25cm² – Sarstedt) and allowed to grow to confluence with regular GMEM media (overnight). The growth media was then exchanged with 3ml serum free media (Freestlye 293 Invitrogen) and the cells were allowed to produce for 24 hours. The media was then saved and a fresh 3ml of serum-free media was added for the next 24 cycle. This was repeated for 10 days. The IgG content was measured by ELISA (ICL Lab, Inc.) and the samples were diluted to 10ng/ml in Freestyle to run on the PSA-incubated SWNT gels.

K. Codes Utilized in the Examples

1. Code 3a: Modeling a Nanosensor Array

The following code was used to simulate the effect of the K_D distribution skewness parameter (β) on the calibration fit parameter:

```

function Simulation_Effect_of_Beta
% Coded by Nigel F. Reuel on 1.8.12
% Used to explain:
% 1. How going from KD --> K --> LangTheta --> Plume of Data
% 2. How to fit the plume of data
% 3. Effect of changing KD distribution skewness parameter (Beta) on fit
%
% Number of sensor points to simulate:
Npoints = 10000;
% Number of concentration points between those that are graphically
represented!
BP = 4;
Nconc = BP*6+5;
logC = linspace(-9,-5,Nconc); % ## <----- Change Conc Range here!!
Lt = zeros(Npoints,Nconc);
Kvec = zeros(Npoints,1);
KDGlobalMean = 7;
%
% ##### KD Beta Parameter Loop Start
BetaMin = 2;
BetaMax = 45;
nB = 200; % ## <-----Change number of Beta parameters
here
BetaSpan = linspace(BetaMin,BetaMax,nB);
ThetaSAVE = zeros(nB,Nconc);
SigmaSAVE = zeros(nB,Nconc);
ThetaSAVEstd = zeros(nB,Nconc);
SigmaSAVEstd = zeros(nB,Nconc);
for Bloop = 1:nB

```

```

Beta = BetaSpan(1,Bloop);

% ##### Replicate Loop START #####

5  NR = 6; % <----- Number of Replicates here
   MuTheta = zeros(NR,Nconc);
   S22 = zeros(NR,Nconc);
   for Rep = 1:NR

10  for i = 1:Nconc
      for j = 1:Npoints
          K = 10^(wblrnd(KDGlobalMean,Beta));
          C = 10^(logC(1,i));
          Lt(j,i) = K*C/(1+K*C);
15  Kvec(j,1) = K;
      end
      if Rep == NR
          if i == 1
              bins = linspace(-11.1,-2.9,200);
20  KDL = log10(1./Kvec);
              [n x] = hist(KDL,bins);
              subplot(2,3,1) % <-----Plot 1
              bar(x,n)
              xlim([-11 -3])
25  xlabel('log10(K_D)')
              ylabel('Frequency')
              title('K_D Distribution')
              text(-7,max(n)*.95,[' \leftarrow \beta = ',int2str(Beta)])
          end
          subplot(2,3,2) % <----- Plot 2
          [n x] = hist(Lt(:,i),200);
          if i == 1+BP
              plot(x,n,'b.')
          elseif i == 2*BP+2
35  plot(x,n,'r.')
          elseif i == 3*BP+3
              plot(x,n,'g.')
          elseif i == 4*BP+4
              plot(x,n,'m.')
          elseif i == 5*BP+5
40  plot(x,n,'k.')
          end
          xlim([0 1])
          hold on
45  end
      end
      %Cspan = 10.^(logC)*10^9; % Reported in nM
      if Rep == NR
          %hleg1 = legend([int2str(Cspan(1,1+BP)), '
50  nM'],[int2str(Cspan(1,2*BP+2)), ' nM'],[int2str(Cspan(1,3*BP+3)), '
nM'],[int2str(Cspan(1,4*BP+4)), ' nM'],[int2str(Cspan(1,5*BP+5)), ' nM']);
          %set(hleg1,'Location','NorthWest')
          title('Langmuir Coverage')
          ylabel('Frequency')
55  xlabel('\theta - Coverage Fraction')
          hold off

```

```

end
%
NSWNT = Npoints;
Length = zeros(NSWNT,Nconc);
5 Response = zeros(NSWNT,Nconc);
RespNorm = zeros(NSWNT,Nconc);
INorm = zeros(NSWNT,Nconc);
% Create matrix of SWNT lengths, then do the calculation:
for i = 1:Nconc
10     for j = 1:NSWNT
        Length(j,i) = wblrnd(6871.7,2.2);
    end
end
% Values to Normalize SWNT Length
15 MaxL = max(Length);
MinL = min(Length);
MeanL = mean(Length);
%MeanLNorm = (MeanL - MinL) ./ (MaxL - MinL)
for i = 1:Nconc
20     for j = 1:NSWNT
        Theta = Lt(j,i);
        L = Length(j,i);
        Response(j,i) = Theta*L;
        RespNorm(j,i) = Theta*L/MeanL(1,i);
25     INorm(j,i) = (L - MinL(1,i)) / (MaxL(1,i) - MinL(1,i));
    end
end

end
% Plot the expected response at different concentrations
30 %subplot(3,3,6) %
%plot(INorm(:,1),RespNorm(:,1),'.b',INorm(:,2),RespNorm(:,2),'.r',INorm(:,3),
RespNorm(:,3),'.g',INorm(:,4),RespNorm(:,4),'.m',INorm(:,5),RespNorm(:,5),'.k
')
%legend([int2str(Cspan(1,1)),' nM'],[int2str(Cspan(1,2)),'
35 nM'],[int2str(Cspan(1,3)),' nM'],[int2str(Cspan(1,4)),'
nM'],[int2str(Cspan(1,5)),' nM'])
%title('Simulated Sensor Response - No Functionalization Parameter')
%ylabel('(Io*\theta)/I_a_v_g')
%xlabel('(Io - I_m_i_n)/(I_m_a_x-I_m_i_n)')
40 % But the SWNT have been functionalized to a certain extent
PM_Ni = csvread('PM_Nickel.csv').*100;
%[n x] = hist(PM_Ni,200);
%subplot(3,3,7) %
%bar(x,n)
45 %xlim([-35 0])
%title('Nickel Functionalization')
%ylabel('Frequency')
%xlabel('% Quench')
%
50 % Read in the nickel quenching data to create your Gaussian fit:
%
Gfit = gmdistribution.fit(PM_Ni,1);
%GMean = Gfit.mu
%GSigma = Gfit.Sigma;
55 %Gm = mean(PM_Ni);
%Gs = std(PM_Ni);

```

```

%Gfit2 = gmdistribution(Gm,Gs,1);
%subplot(3,3,8) %
%X = linspace(-50,10,100)';
%Y = pdf(Gfit,X);
5 %plot(X,Y)
%xlim([-35 0])
%xlabel('% Quench')
%ylabel('f(x;\mu,\sigma)')
%title('Gaussian Fit of Functionalization Extent')
10 % Now plot what the experimental output would look like with
% functionalization distribution
FuncResponse = zeros(NSWNT,Nconc);
FuncRespNorm = zeros(NSWNT,Nconc);
for i = 1:Nconc
15     for j = 1:NSWNT
        Theta = Lt(j,i);
        Io = Length(j,i);
        FuncRange = Io*(1/(1-random(Gfit)/-100) - 1);
        FuncResponse(j,i) = Theta*FuncRange;
20         FuncRespNorm(j,i) = Theta*FuncRange/MeanL(1,i);
    end
end
if Rep == NR
    subplot(2,3,3) % <-----Plot 3
25 plot(INorm(:,1+BP),FuncRespNorm(:,1+BP),'.b',INorm(:,2*BP+2),FuncRespNorm(:,2
*BP+2),'.r',INorm(:,3*BP+3),FuncRespNorm(:,3*BP+3),'.g',INorm(:,4*BP+4),FuncR
espNorm(:,4*BP+4),'.m',INorm(:,5*BP+5),FuncRespNorm(:,5*BP+5),'.k')
    %hleg2 = legend([int2str(Cspan(1,1+BP)), '
30 nM'],[int2str(Cspan(1,2*BP+2)), ' nM'],[int2str(Cspan(1,3*BP+3)), '
nM'],[int2str(Cspan(1,4*BP+4)), ' nM'],[int2str(Cspan(1,5*BP+5)), ' nM']);
    %set(hleg2,'Location','NorthWest')
    title('Sensor Response - Cartesian')
    ylabel(' (Io*(1/(1-PF))-1)*\theta/I_a_v_g')
35 xlabel(' (Io-I_m_i_n)/(I_m_a_x-I_m_i_n)')
    ylim([0 1.2])
end
%
% Now plot what this plume reduces to with your radial analysis
40 %{
ThetaMean = zeros(1,Nconc);
L = zeros(1,Nconc);
U = zeros(1,Nconc);
ThetaSig = zeros(1,Nconc);
45 Lsig = zeros(1,Nconc);
Usig = zeros(1,Nconc);
%}
MixGausData = zeros(3,2,Nconc);
for i = 1:Nconc
50     Xn = INorm(:,i);
     Yn = FuncRespNorm(:,i);
     [THETA1 RHO1] = cart2pol(Xn,Yn);
     % Plot according to the point density (to see what this looks like)...
     %n1 = 100;
55     %n2 = 100;
     %[z,C] = hist3([RHO1 THETA1],[n1 n2]);

```

```

%xb = C{1,1}; % RHO Values
%yb = C{1,2}; % THETA Values
clear X
X(:,1) = THETA1;
5 X(:,2) = RHO1;
if Rep == NR
    subplot(2,3,4) %<----- Plot 4
    if i == 1+BP
        plot(THETA1, RHO1, '.b')
10         hold on
            Cloud1 = gmdistribution.fit(X,1);
            MU = Cloud1.mu;
            SIG = Cloud1.Sigma;
            MixGausData(1,1:2,i) = MU;
15            MixGausData(2:3,1:2,i) = SIG(:, :, 1);
            %ezcontourf(@(x,y)pdf(Cloud1,[x y]),[0 .8],[0 1]);

    elseif i == 2*BP+2
        plot(THETA1, RHO1, '.r')
20         Cloud2 = gmdistribution.fit(X,1);
            MU = Cloud2.mu;
            SIG = Cloud2.Sigma;
            MixGausData(1,1:2,i) = MU;
            MixGausData(2:3,1:2,i) = SIG(:, :, 1);
25            %ezcontourf(@(x,y)pdf(Cloud2,[x y]),[0 .8],[0 1]);

    elseif i == 3*BP+3
        plot(THETA1, RHO1, '.g')
30         Cloud3 = gmdistribution.fit(X,1);
            MU = Cloud3.mu;
            SIG = Cloud3.Sigma;
            MixGausData(1,1:2,i) = MU;
            MixGausData(2:3,1:2,i) = SIG(:, :, 1);
35            %ezcontourf(@(x,y)pdf(Cloud3,[x y]),[0 .8],[0 1]);

    elseif i == 4*BP+4
        plot(THETA1, RHO1, '.m')
40         Cloud4 = gmdistribution.fit(X,1);
            MU = Cloud4.mu;
            SIG = Cloud4.Sigma;
            MixGausData(1,1:2,i) = MU;
            MixGausData(2:3,1:2,i) = SIG(:, :, 1);
45            %ezcontourf(@(x,y)pdf(Cloud4,[x y]),[0 .8],[0 1]);

    elseif i == 5*BP+5
        plot(THETA1, RHO1, '.k')
50         Cloud5 = gmdistribution.fit(X,1);
            MU = Cloud5.mu;
            SIG = Cloud5.Sigma;
            MixGausData(1,1:2,i) = MU;
            MixGausData(2:3,1:2,i) = SIG(:, :, 1);
55            %ezcontourf(@(x,y)pdf(Cloud5,[x y]),[0 .8],[0 1]);

    else
        CloudNull = gmdistribution.fit(X,1);

```

```

        MU = CloudNull.mu;
        SIG = CloudNull.Sigma;
        MixGausData(1,1:2,i) = MU;
        MixGausData(2:3,1:2,i) = SIG(:, :, 1);
5
    end
    hold on
else
10    CloudNull = gmdistribution.fit(X,1);
    MU = CloudNull.mu;
    SIG = CloudNull.Sigma;
    MixGausData(1,1:2,i) = MU;
    MixGausData(2:3,1:2,i) = SIG(:, :, 1);
15    end

end
if Rep == NR
20    xlim([0 .75])
    ylim([0 1])
    title('Sensor Response - Polar')
    hold off
    xlabel('\theta')
25    ylabel('R')
    % Represent polar fits as bivariate distributions
    subplot(2,3,5)
    h = ezcontourf(@(x,y)pdf(Cloud1,[x y]),[0 .8],[0 .8],100);
    set(h,'LineStyle','none');
30    hold on
    h = ezcontourf(@(x,y)pdf(Cloud3,[x y]),[0 .8],[0 .8],100);
    set(h,'LineStyle','none');
    hold on
    h = ezcontourf(@(x,y)pdf(Cloud5,[x y]),[0 .8],[0 .8],100);
35    set(h,'LineStyle','none');
    hold on
    colormap(hot)
    title('Bivariate Gaussian PDF Fit')
    xlabel('\theta')
40    ylabel('R')
    hold off
end
%
% Now in your last plot, plot the parameters of interest (Rho-Mu and Rho
45 % sigm11)
%

for i = 1:Nconc
    MuTheta(Rep,i) = MixGausData(1,1,i);
50    S22(Rep,i) = MixGausData(2,1,i);
end

Rep
% ##### End for Replicate Loop #####
55    end

```

```

X = logC;
subplot(2,3,6)
[AX,H1,H2] = plotyy(X,mean(MuTheta),X,mean(S22),'plot');
set(get(AX(1),'Ylabel'),'String','\theta_\mu')
5 set(get(AX(2),'Ylabel'),'String','\theta_\sigma')
set(AX(1),'YLim',[0 .6])
set(AX(2),'YLim',[0 .1])
xlabel('log10(Conc)')
title('Calibration Curve')
10 set(H1,'LineStyle','--')
set(H2,'LineStyle',':')
%
% Set up the picture, save and close!

15 set(gcf, 'Position', [5 5 1000 1000])
figure_name_fig = [int2str(Bloop),'.png'];
print(gcf,'-dpng',figure_name_fig);
close
ThetaSAVE(Bloop,:) = mean(MuTheta);
20 SigmaSAVE(Bloop,:) = mean(S22);
ThetaSAVEstd(Bloop,:) = std(MuTheta);
SigmaSAVEstd(Bloop,:) = std(S22);
Bloop
% ##### End for BetaLoop
25 end
% Save the calibration curve data:
csvwrite('ThetaMu_mean.csv',ThetaSAVE);
csvwrite('ThetaMu_std.csv',ThetaSAVEstd);
csvwrite('ThetaSig_mean.csv',SigmaSAVE);
30 csvwrite('ThetaSig_std.csv',SigmaSAVEstd);

end

```

2. Code 3b: Poroelastic Indentation Analysis Code

35 This code was used to analyze the force relaxation curves presented above to identify the hydrogel pore size:

```

function AFM_PRI_Analysis_v4_8um
% Coded by Nigel F. Reuel on 8.7.2012
40 % Used to analyze the PRI curves derived from AFM as the Van Vliet group
% has demonstrated - Analyzes the curves to find the following:
% 1. G = shear modulus
% 2. D = diffusion rate
% 3. Vs = Poisson's ratio
45 % 4. PS = Pore Size
%
% 8.8.2012 v2 provides 95% error limits on the pore calculation
% 8.8.2012 v3 corrects for drift present in low crosslink gels
% 8.27.12 v4 adjusted for new data set (8.23.12 experiments)
50 %
% ----- 8um Indentation Step ----- 30s indentation
%

```

```

% (1) Read in the raw data:
%
% Number of files, time of indentation, and data rate
NF = 18;
5  TI = 20; % In seconds
% Determine the recording rate:
Record_temp = dlmread('3p5p_8um_1.txt');
Time1 = Record_temp(5,1);
Time2 = Record_temp(4,1);
10  DR = 1/(Time1-Time2); % In Hertz
%
RD_8um = zeros(TI*DR,NF);
Nrows = floor(TI*DR);
for i = 1:NF
15  Data_temp = dlmread(['3p5p_8um_',int2str(i),'.txt']);
    % Check and correct for drift, using last 5000 time points:
    L = floor(TI*DR);
    L2 = 5000;
    yp = Data_temp(L-L2+1:L,2);
20  xp = (1:L2)';
    p = polyfit(xp,yp,1);
    plot(xp,yp)
    %p1 = slope and p2 = intercept
    % Correct for slope:
25  p2 = 0;
    Data_Temp_Cor = zeros(L,1);
    for j = 1:L
        Data_Temp_Cor(j,1) = Data_temp(j,2) - p2*(j-1);
    end
30  RD_8um(:,i) = Data_Temp_Cor(1:Nrows,1);
end

% Center the values so the initial Force is zero
CenterD_8um = zeros(Nrows,NF);
35  for i = 1:NF
    for j = 1:Nrows
        CenterD_8um(j,i) = RD_8um(j,i) - RD_8um(1,i);
    end
end

40  % Try plotting together to see what they look like:
X = (1:Nrows)';
plot(X,CenterD_8um)
%
% Good replication in 1-14
45  Avg8umCurve = zeros(Nrows,1);
    ConfPosCurve = zeros(Nrows,1);
    ConfNegCurve = zeros(Nrows,1);
    SR = 1;
    ER = 14;
50  for i = 1:Nrows
        Avg8umCurve(i,1) = mean(CenterD_8um(i,SR:ER));
        ConfPosCurve(i,1) = mean(CenterD_8um(i,SR:ER)) +
2*std(CenterD_8um(i,SR:ER));
        ConfNegCurve(i,1) = mean(CenterD_8um(i,SR:ER)) -
55  2*std(CenterD_8um(i,SR:ER));
    end
end

```

```

% Plot the averaged curve with error bars
plot(X,Avg8umCurve,X,ConfPosCurve,'r--',X,ConfNegCurve,'r--')
csvwrite('AvgF_8um.csv',Avg8umCurve)
csvwrite('AvgFP_8um.csv',ConfPosCurve)
5 csvwrite('AvgFN_8um.csv',ConfNegCurve)
%
% Plot the force relax curve (w/ error bars)
[Fzero index] = max(Avg8umCurve);
Finf = mean(Avg8umCurve(Nrows-100:Nrows,1));
10 F = zeros(1:Nrows-index+1,1);
for i = index:Nrows
    Ft = Avg8umCurve(i,1);
    F(i-index+1,1) = (Ft - Finf)/(Fzero-Finf);
end
15 Xtime = ((1:Nrows-index+1)*1/DR)';
% ---- High error
[FzeroH indexH] = max(ConfPosCurve);
FinfH = mean(ConfPosCurve(Nrows-100:Nrows,1));
FH = zeros(1:Nrows-indexH+1,1);
20 for i = indexH:Nrows
    FtH = ConfPosCurve(i,1);
    FH(i-indexH+1,1) = (FtH - FinfH)/(FzeroH-FinfH);
end
XtimeH = ((1:Nrows-indexH+1)*1/DR)';
25 % ---- Low error
[FzeroL indexL] = max(ConfNegCurve);
FinfL = mean(ConfNegCurve(Nrows-100:Nrows,1));
FL = zeros(1:Nrows-indexL+1,1);
for i = indexL:Nrows
30    FtL = ConfNegCurve(i,1);
    FL(i-indexL+1,1) = (FtL - FinfL)/(FzeroL-FinfL);
end
XtimeL = ((1:Nrows-indexL+1)*1/DR)';
% Plot together...
35 plot(Xtime,F,XtimeH,FH,'r--',XtimeL,FL,'r--')
% Save the F and X file
csvwrite('Force_8um.csv',F);
csvwrite('Time_8um.csv',Xtime);
% Solve for tau
40 R = 22.5*10^-6; %Radius of the bead
h = 8*10^-6; % Depth of the indentation
a = (R*h)^(1/2);
eta = 1*10^-3; % (N s/m2)
D0 = .000000002;
45 % ----- Calculated value ----
D = fminsearch(@ForceFunc,D0,[],F,a,Xtime);
G = Fzero*3/16/a/h;
Vs = 1-Fzero/Finf/2;
PS = 2*(eta/2*D*(1-2*Vs)/G/(1-Vs))^(1/2)*10^9 %Reported in nanometers!
50 % ----- High error value ----
DH = fminsearch(@ForceFunc,D0,[],FH,a,XtimeH);
GH = FzeroH*3/16/a/h;
VsH = 1-FzeroH/FinfH/2;
PSH = 2*(eta/2*D*(1-2*VsH)/GH/(1-VsH))^(1/2)*10^9 %Reported in nanometers!
55 % ----- Low error value ----
DL = fminsearch(@ForceFunc,D0,[],FL,a,XtimeL);

```

```

GL = FzeroL*3/16/a/h;
VsL = 1-FzeroL/FinfL/2;
PSL = 2*(eta/2*DL*(1-2*VsL)/GL/(1-VsL))^(1/2)*10^9 %Reported in nanometers!
end

```

5

```

function FZet = ForceFunc(D0,F,a,Xtime)
rows = length(F);
ErrorSQR = 0;
10 for i = 1:rows
    tau = D0*Xtime(i,1)/a^2;
    ErrorSQR = (F(i,1) - (0.491*exp(-.908*(tau)^(1/2)) + 0.509*exp(-
1.679*tau)))^2 + ErrorSQR;
end
15 FZet = ErrorSQR;
end

```

3. Code 3c: Local Production Analysis of Cells on Gel Images

The following code was used to analyze the local cell production images. First the
20 images of control cells and IgG producing cells were analyzed to find the top 10000 SWNT and
the cell regions were recorded using the ROI matlab function. These locations were then used to
determine the coverage of the SWNT in the cell regions (specific) and outside the cell regions
(un-specific) using the CellAnalysis Function (below). The time response of the IgG producing
cells was determined again using the top 10,000 SWNT pixels and plotting their absolute
25 intensity distributions over time for the series of images collected using the CellTime function
(below). Finally, the AnalyzeCellPic function was used to plot the average intensity of cell
islands as a contour plot as presented in **FIG. 7**.

```

function CellAnalysis
30 % Coded by Nigel F. Reuel on 9.19.2012
% This program does the basic comparison analysis of our control and IgG
% producing cells
%
NFiles = (1:21);
35 PSpec = zeros(1:21,1);
PNSpec = zeros(1:21,1);
for i = NFiles
    CellLoc = csvread(['K',int2str(i),'_CellLoc.csv']);
    SWNTLoc = csvread(['SLoc_Rank10000_',int2str(i),'.csv']);
40    CellCount = sum(sum(CellLoc));
    OutCellCount = 320*256 - CellCount;
    SWNTinCell = 0;
    SWNToutCell = 0;
    for j = 1:320
45        for k = 1:256
            if SWNTLoc(j,k) > 0 && CellLoc(j,k) > 0;
                SWNTinCell = SWNTinCell + 1;
            end
        end
    end
end

```

```

elseif SWNTLoc(j,k) > 0 && CellLoc(j,k) == 0;
    SWNToutCell = SWNToutCell + 1;
end
end
5   end
    PSpec(i,1) = SWNTinCell/CellCount*100; % Percent Coverage
    PNSpec(i,1) = SWNToutCell/OutCellCount*100; % Percent Coverage
end
X = (1:21)';
10  plot(X,PSpec, X,PNSpec)
    Data = zeros(4,1); % 1 = IgG Cell specific, 2 = IgG Cell non specific, 3 = Control
    Cell Specific, 4 = Control Cell non specific
    Error = zeros(4,1);
    Data(1,1) = mean(PSpec([13:16 18:21],1));
15  Data(2,1) = mean(PNSpec([13:16 18:21],1));
    Data(3,1) = mean(PSpec(1:7,1));
    Data(4,1) = mean(PNSpec(1:7,1));
    Error(1,1) = std(PSpec([13:16 18:21],1));
    Error(2,1) = std(PNSpec([13:16 18:21],1));
20  Error(3,1) = std(PSpec(1:7,1));
    Error(4,1) = std(PNSpec(1:7,1));
    bar(Data)
    hold on
    errorbar(Data,Error,'or')
25  hold off
    ylabel('% Coverage')

end

30  function CellTime
    % Coded by Nigel Reuel on 9.19.2012
    % This code analyzes the time traces of the cells
    %
    PL_0 = 0;
35  PL_1 = 0;
    PL_2 = 0;
    PL_3 = 0;
    count1 = 1;
    count2 = 1;
40  count3 = 1;
    count4 = 1;
    for i = 1:19
        PLData = imread(['HS',int2str(i),'.tif']);
        SWNTLoc = csvread(['SLoc_Rank1000_',int2str(i),'.csv']);
45
        for j = 1:320
            for k = 1:256
                if SWNTLoc(j,k) > 0
                    if i < 3 % Time Zero
50  PL_0(count1,1) = PLData(j,k);
                        count1 = count1 + 1;
                    elseif i < 10 % Time 1-1.5 hr
                        PL_1(count2,1) = PLData(j,k);
                        count2 = count2 + 1;
55  elseif i < 18 % Time 2-2.5 hr
                        PL_2(count3,1) = PLData(j,k);

```

```

        count3 = count3 + 1;
    else % Time 3-3.5 hr
        PL_3(count4,1) = PLData(j,k);
        count4 = count4 + 1;
5      end
    end
  end
end
10 % Construct histograms for these time points
X = linspace(5000,30000,1000);
%
subplot(1,4,1)
n = hist(PL_0,X);
15 bar(X,n)
xlim([7500 15000])
%
subplot(1,4,2)
n = hist(PL_1,X);
20 bar(X,n)
xlim([7500 15000])
%
subplot(1,4,3)
n = hist(PL_2,X);
25 bar(X,n)
xlim([7500 15000])
%
subplot(1,4,4)
n = hist(PL_3,X);
30 bar(X,n)
xlim([7500 15000])

function AnalyzeCellPic
35 % Coded by Nigel F. Reuel on 9.12.2012
% This code will analyze a single cell picture (*.tif)
% For Figure 6 of biomanufacturing paper
%
%}
40 % Plot the Data together!!
NC = 10;
% Data file number
FN = 3;
45 %
CData = zeros(320,256);
PLData = imread(['S',int2str(FN),'.tif'],1);
Cell = imread(['C',int2str(FN),'.tif'],1);
for i = 1:NC
50   Ccount = 0;
   Stotal = 0;
   Cloc = csvread(['C',int2str(FN),'_CellIsland',int2str(i),'Loc.csv']);

   for j = 1:320
55     for k = 1:256
       if Cloc(j,k) > 0

```

```

        Ccount = Ccount + 1;
        Stotal = Stotal + PLData(j,k);
    end
    end
5    end
    AvgSig = Stotal/Ccount;
    for j = 1:320
        for k = 1:256
            if Cloc(j,k) > 0
10         CData(j,k) = AvgSig;
            end
        end
    end
    end
15    contourf(CData)
    set(gca, 'Clim', [0 8000])
    colormap(hot)
    m = 10;
    imagesc(Cell)
20    colormap(gray)
    m = 10;
    imagesc(PLData)
    m = 10;
    end
25

```

For the purposes of interpreting this specification, the following definitions apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with the usage of that word in any other document, including any document incorporated herein by reference, the definition set forth

herein and below shall always control for purposes of interpreting this specification and its associated claims unless a contrary meaning is clearly intended. It is noted that, as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless expressly and unequivocally limited to one referent. The use of “or” means “and/or” unless stated otherwise. The use of “comprise”, “comprises”, “comprising”, “include”, “includes”, and “including” are interchangeable and not intended to be limiting. Furthermore, where the description of one or more embodiments uses the term “comprising” those skilled in the art would understand that, in some instances, the embodiment or embodiments can be alternatively described using the language “consisting essentially of” and/or “consisting of.” The section headings used herein are for organizational purposes only and are not to be construed as

limiting the subject matter described. All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises are hereby expressly incorporated by reference in their entirety for any purpose. In the event that

one or more of the incorporated documents defines a term that contradicts that term's definition in this application, this application controls. While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such
5 equivalent variations that come within the scope of the invention as claimed. Other embodiments are within the scope of the following claims.

CLAIMS

What is claimed is:

1. A method of detecting an analyte, comprising:
contacting a sample including a biologic analyte with a sensor array, the sensor array
5 including a plurality of photoluminescent nanostructures;
collecting emissive data from each of the photoluminescent nanostructures; and
analyzing the emissive data from each nanostructure of the plurality of photoluminescent
nanostructures to measure a distribution of binding affinities.
2. A method of detecting an analyte, comprising:
10 contacting a sample including a biologic analyte with a sensor array, the sensor array
including a plurality of photoluminescent nanostructures;
collecting emissive data from each of the photoluminescent nanostructures; and
analyzing the emissive data from each nanostructure of the plurality of photoluminescent
nanostructures to measure a distribution of binding affinities;
15 wherein the photoluminescent nanostructures are:
physically associated with a linker;
directly or indirectly chelated with a metal ion; and / or
functionalized with a capture protein.
3. The method of claim 1 or 2 wherein the photoluminescent nanostructures are selected from
20 the group consisting of carbon nanotubes, single-walled carbon nanotubes, double-walled
carbon nanotubes, semi-conductor quantum dots, semi-conductor nanowires, and graphene.
4. The method of any one of claims 1-3 wherein the sensor array is arranged upon one or more
support materials.
5. The method of claim 4 wherein the one or more support materials comprises a gel.
- 25 6. The method of claim 4 or 5 wherein the one or more support materials comprises a first gel
lacking photoluminescent nanostructures and a second gel comprising photoluminescent
nanostructures upon the first gel.
7. The method of any one of claims 4-6 wherein one or more support materials comprises a
solid material (e.g. glass) supporting the photoluminescent nanostructures.

8. The method of any one of claims 4-7 wherein the one or more support materials comprises a solid material (e.g. glass), a first gel lacking photoluminescent nanostructures, and a second gel comprising photoluminescent nanostructures upon the first gel.
9. The method of any one of claims 1-8 wherein the analyte is an antibody.
- 5 10. The method of claim 2 wherein the capture protein is Protein A or a derivative thereof.
11. The method of claim 2 wherein the capture protein is PSA-lectin or a derivative thereof.
12. The method of claim 11 wherein the analyte is mannose.
13. The method of any one of claims 1-12 wherein the sample comprises cells that colonize the photoluminescent nanostructures.
- 10 14. The method of claim 13 wherein one or more analytes produced by the cells is detected.
15. The method of any one of claims 1-14, further comprising calculating a calibration curve for the sensor.
16. The method of any one of claims 1-15, wherein analyzing the emissive data includes measuring a variance of the emissive data from the plurality of photoluminescent nanostructures.
- 15 17. The method of any one of claims 1-16, wherein analyzing the emissive data includes measuring a skew of the emissive data from the plurality of photoluminescent nanostructures.
18. The method of any one of claims 1-17, wherein analyzing the emissive data includes measuring a standard deviation of a plume angle of the emissive data from the plurality of photoluminescent nanostructures.
- 20 19. The method of any one of claims 1-18, further comprising evaluating a binding heterogeneity of the biologic analyte of the sample from the analyzed emissive data.
20. The method of any one of claims 1-19, further comprising evaluating a cell line for production of the biologic analyte of the sample from the analyzed emissive data.
- 25 21. The method of any one of claims 1-20, further comprising evaluating a glycosylation pattern of the biologic analyte of the sample from the analyzed emissive data.
22. The method of any one of claims 1-21, wherein the array includes at least 500 of the photoluminescent nanostructures.
23. The method any one of claims 1-22, wherein the array includes at least 1,000 of the photoluminescent nanostructures.
- 30

24. The method of any one of claims 1-23, wherein the array includes at least 2,000 of the photoluminescent nanostructures.
25. The method of any one of claims 1-24, wherein the array includes at least 5,000 of the photoluminescent nanostructures.
- 5 26. The method of any one of claims 1-25, wherein the sensor includes an analyte-binding compound associated with the photoluminescent nanostructure.
27. The method of any one of claims 1-26, wherein the sensor further comprises a linker, wherein the analyte-binding compound is associated with the photoluminescent nanostructure via the linker.
- 10 28. The method of any one of claims 1-27, wherein the sensor includes a polymer including clusters of photoluminescent nanostructures.
29. The method of any one of claims 1-28, wherein the analyte is a biologic analyte.
30. The method of any one of claims 1-29, wherein the biologic analyte is an antibody.
31. The method of any one of claims 1-29, wherein the biologic analyte is a protein.
- 15 32. The method of claim 30, wherein the antibody is IgG.
33. The method of any one of claims 1-29, wherein the analyte binding compound is a capture protein comprising Protein A or PSA-lectin.
34. The method of any one of claims 1-33 wherein the capture protein comprises a tag having specificity for a binding partner associated with the photoluminescent nanostructure.
- 20 35. The method of claim 34 wherein the tag is a histidine tag and the binding partner is nickel.

1/21

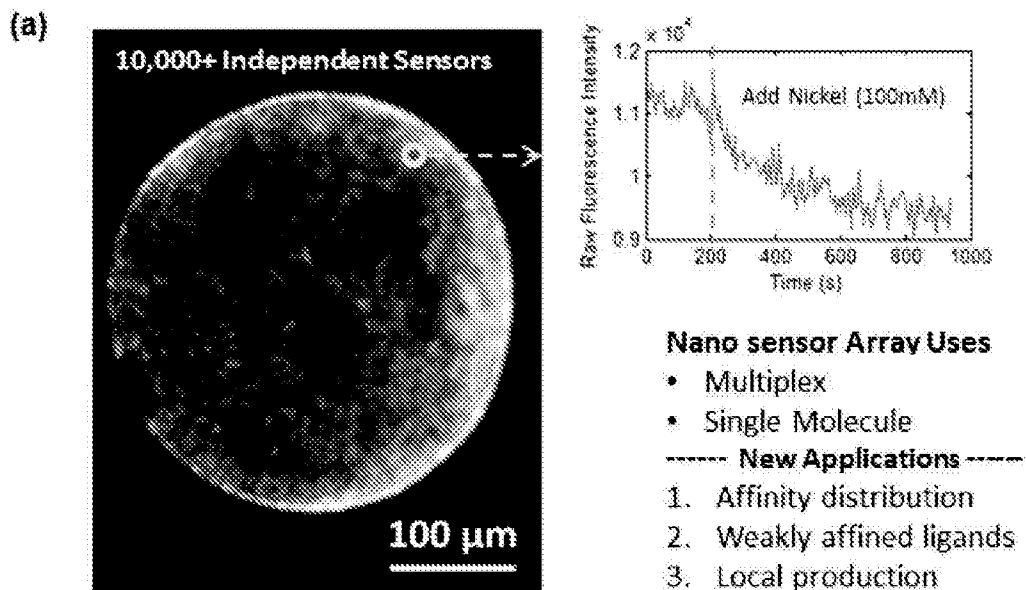


FIG. 1A

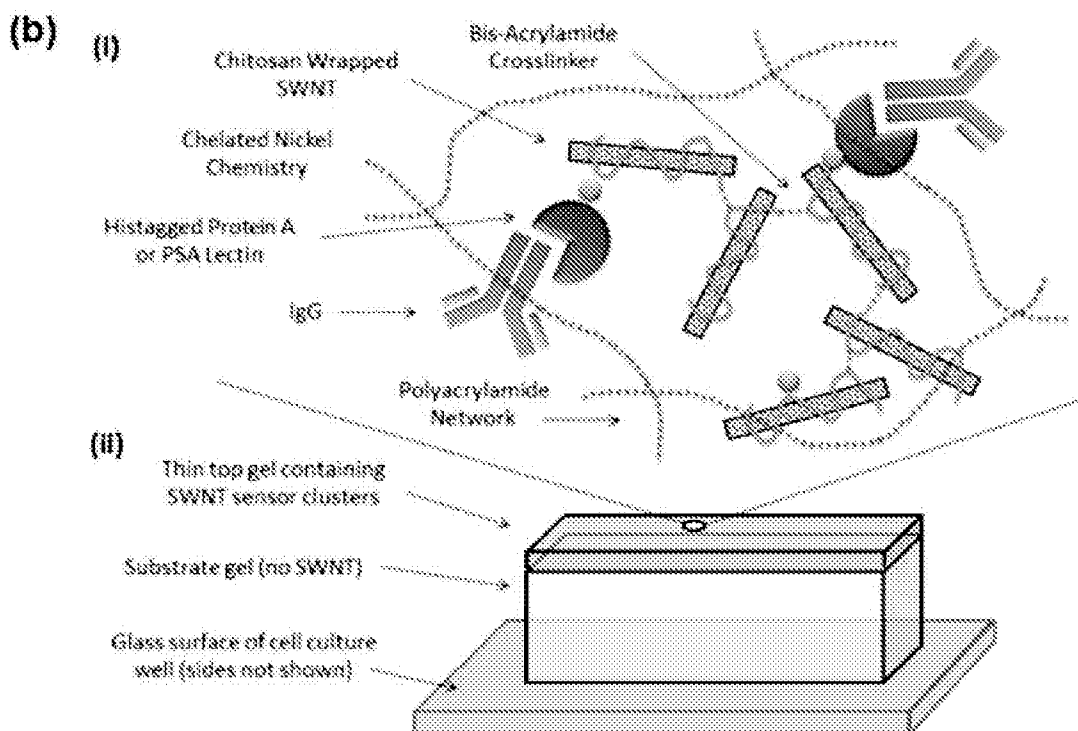


FIG. 1B

2/21

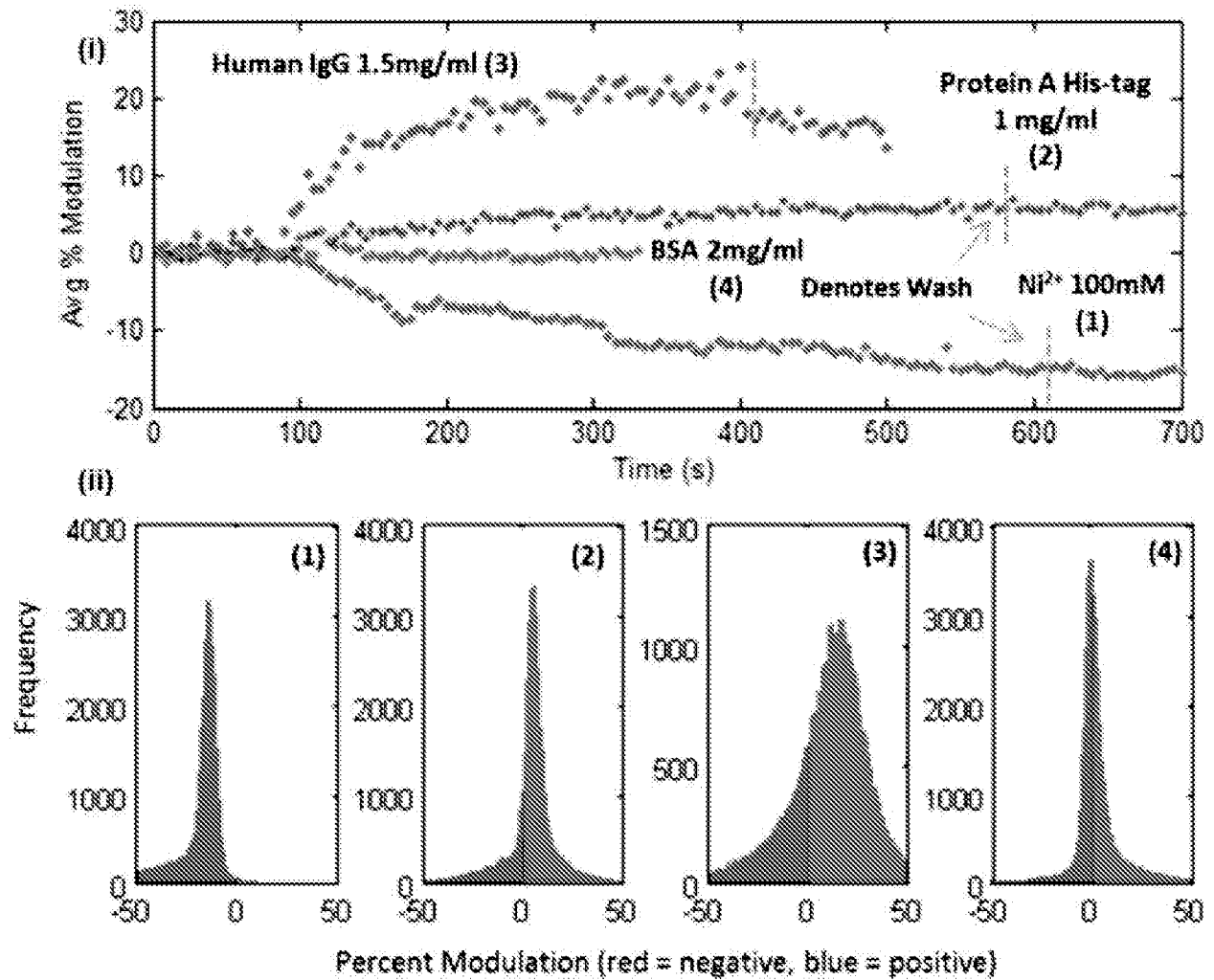


FIG. 1C

3/21

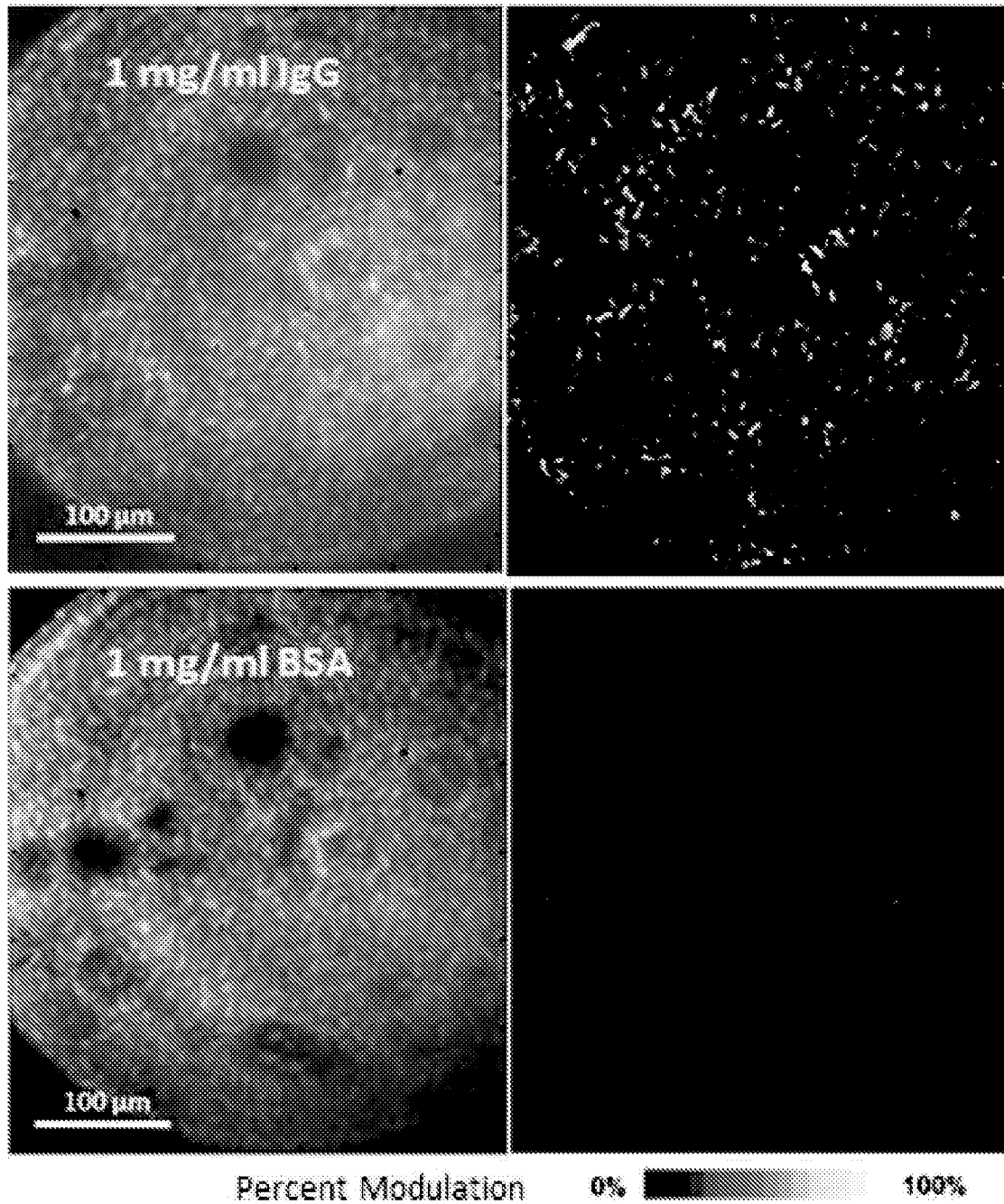


FIG. 1D

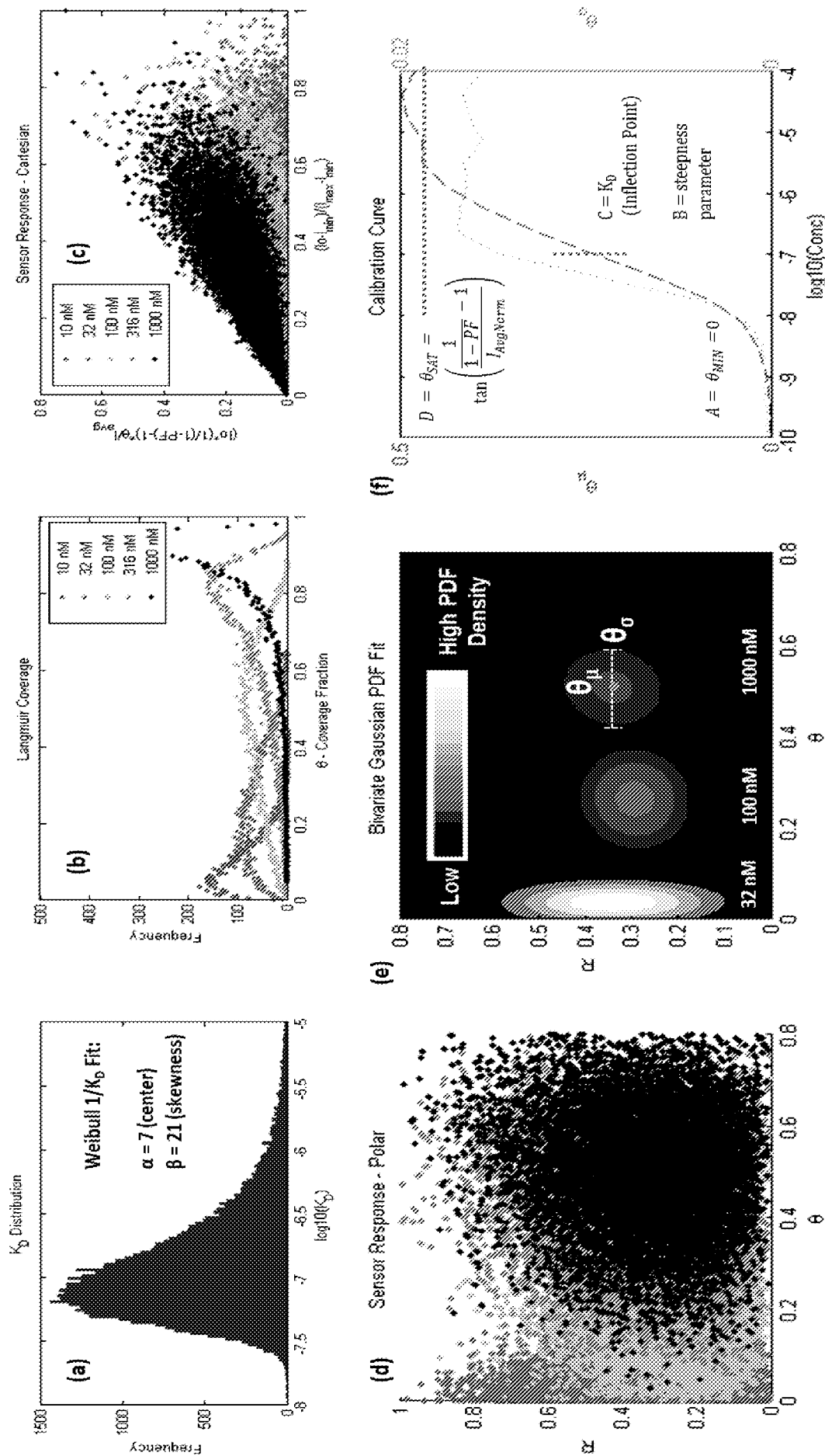


FIG. 2

5/21

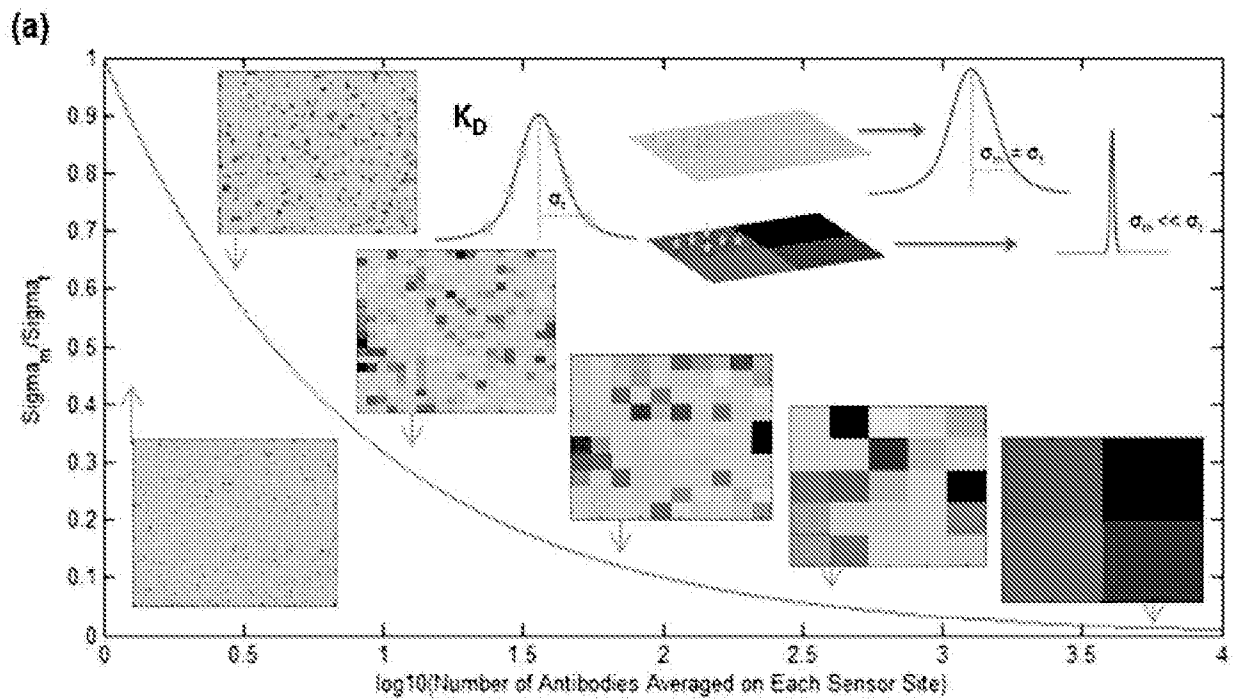


FIG.3A

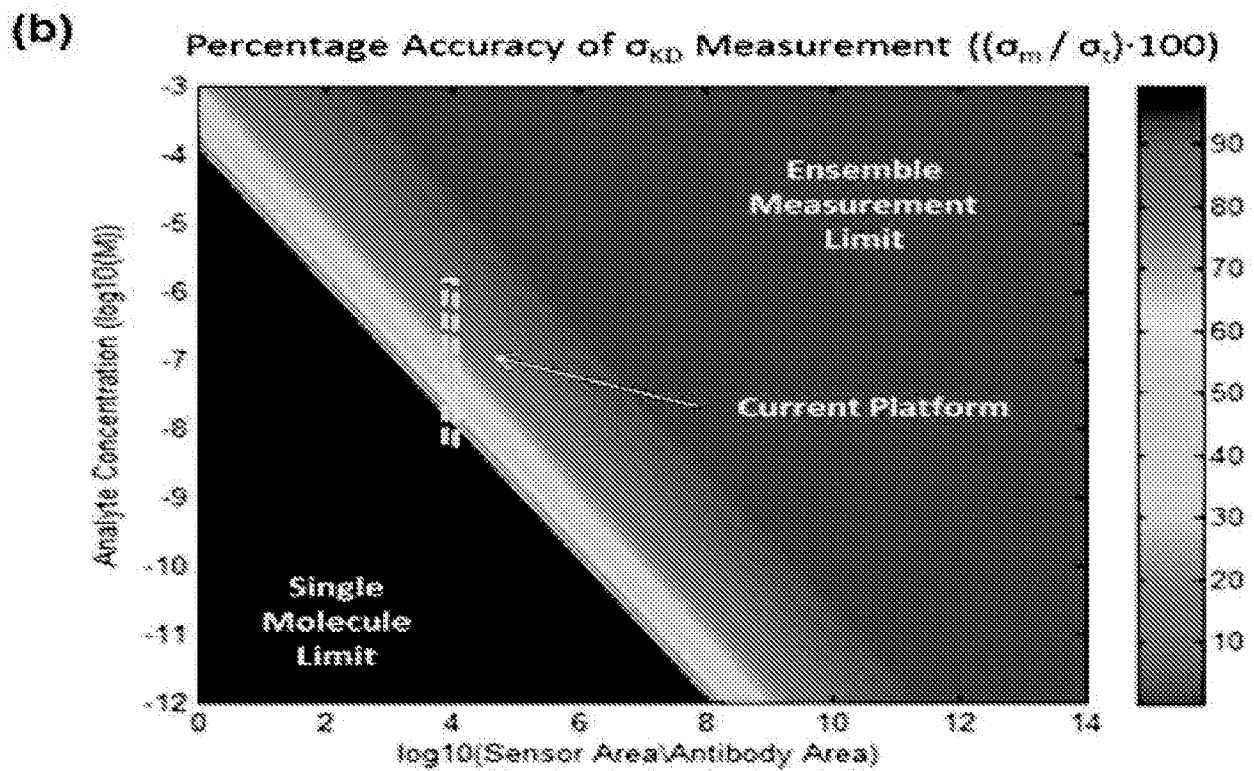


FIG. 3B

6/21

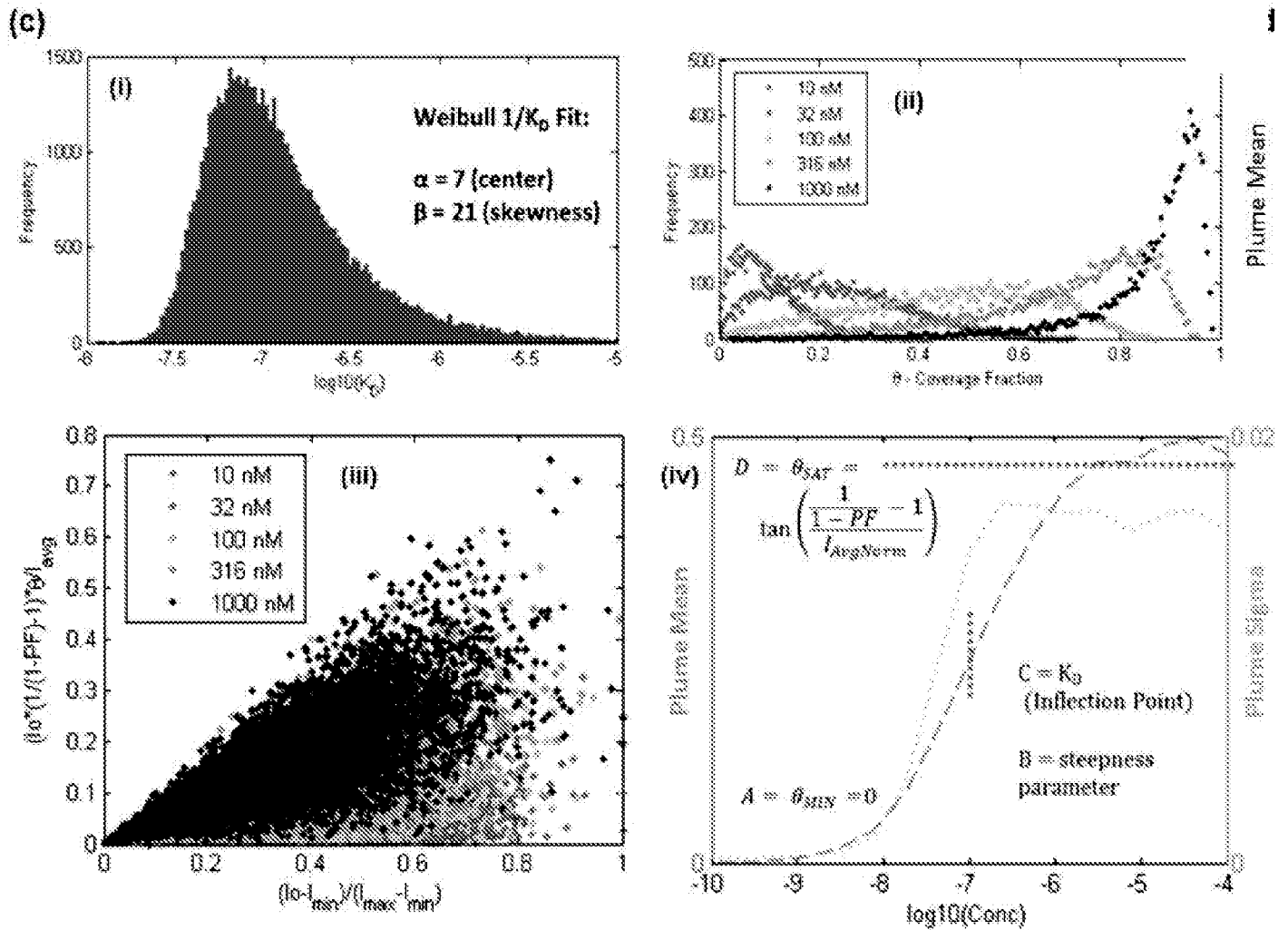


FIG. 3C

7/21

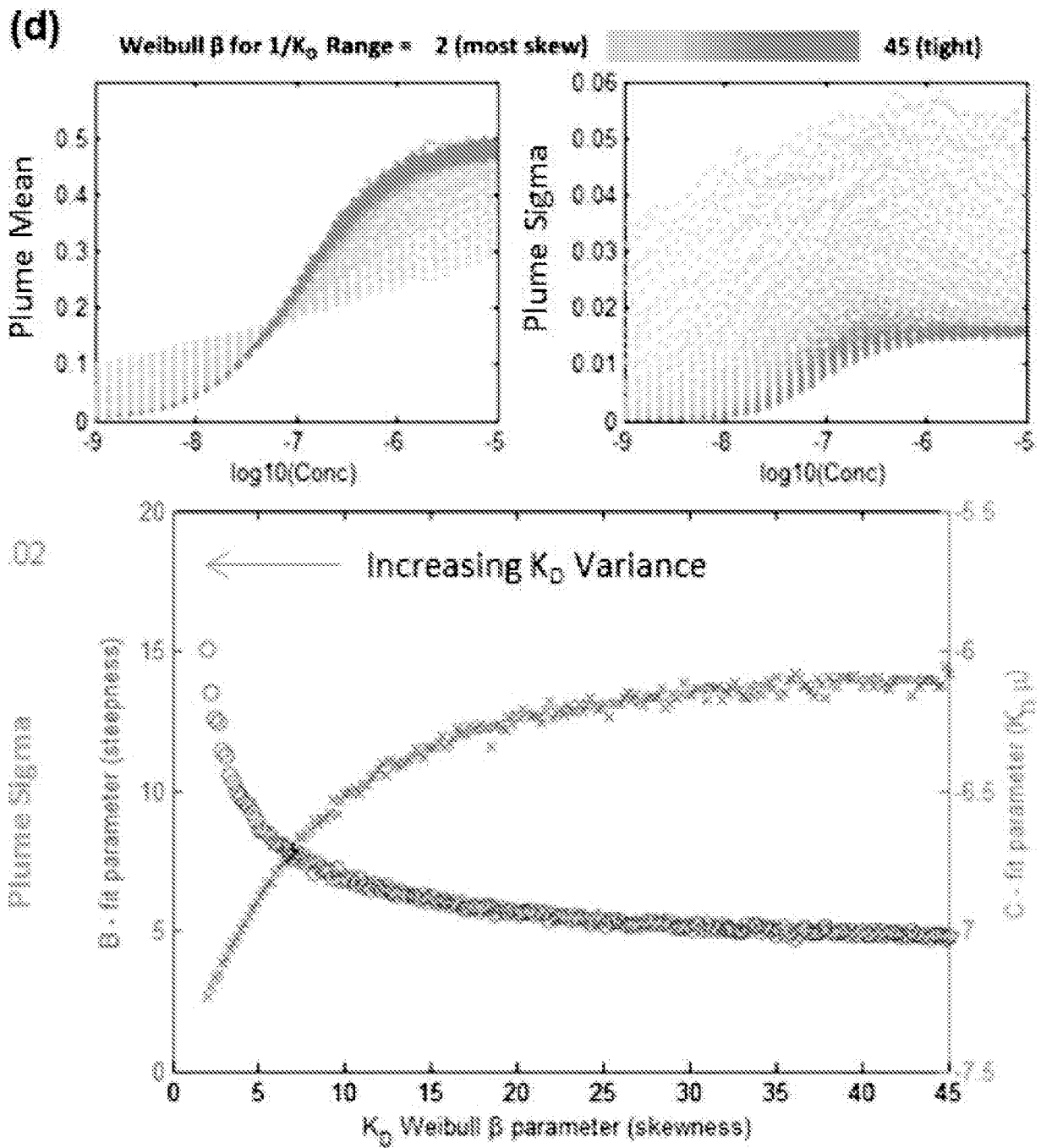


FIG. 3D

8/21

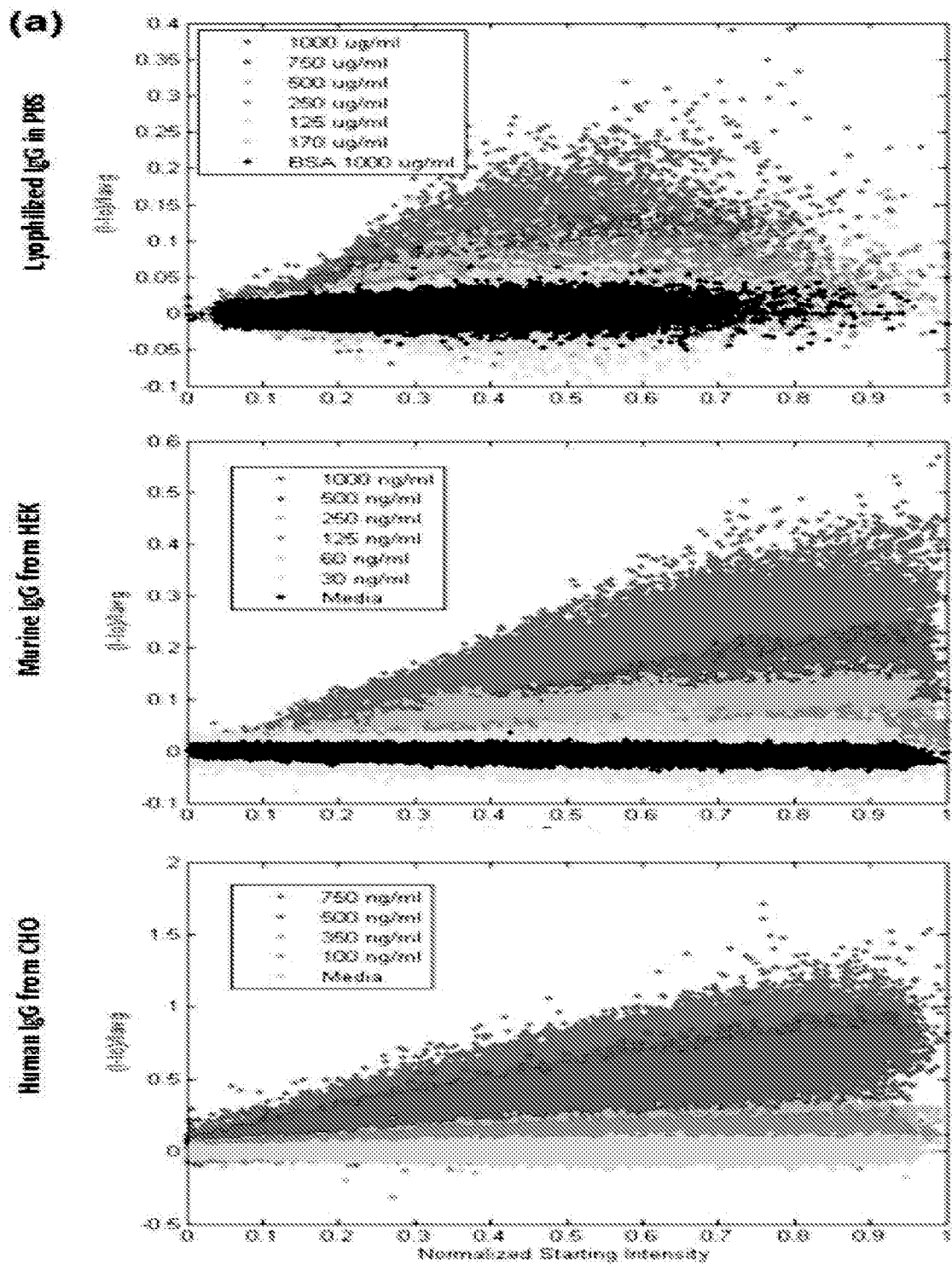


FIG. 4A

9/21

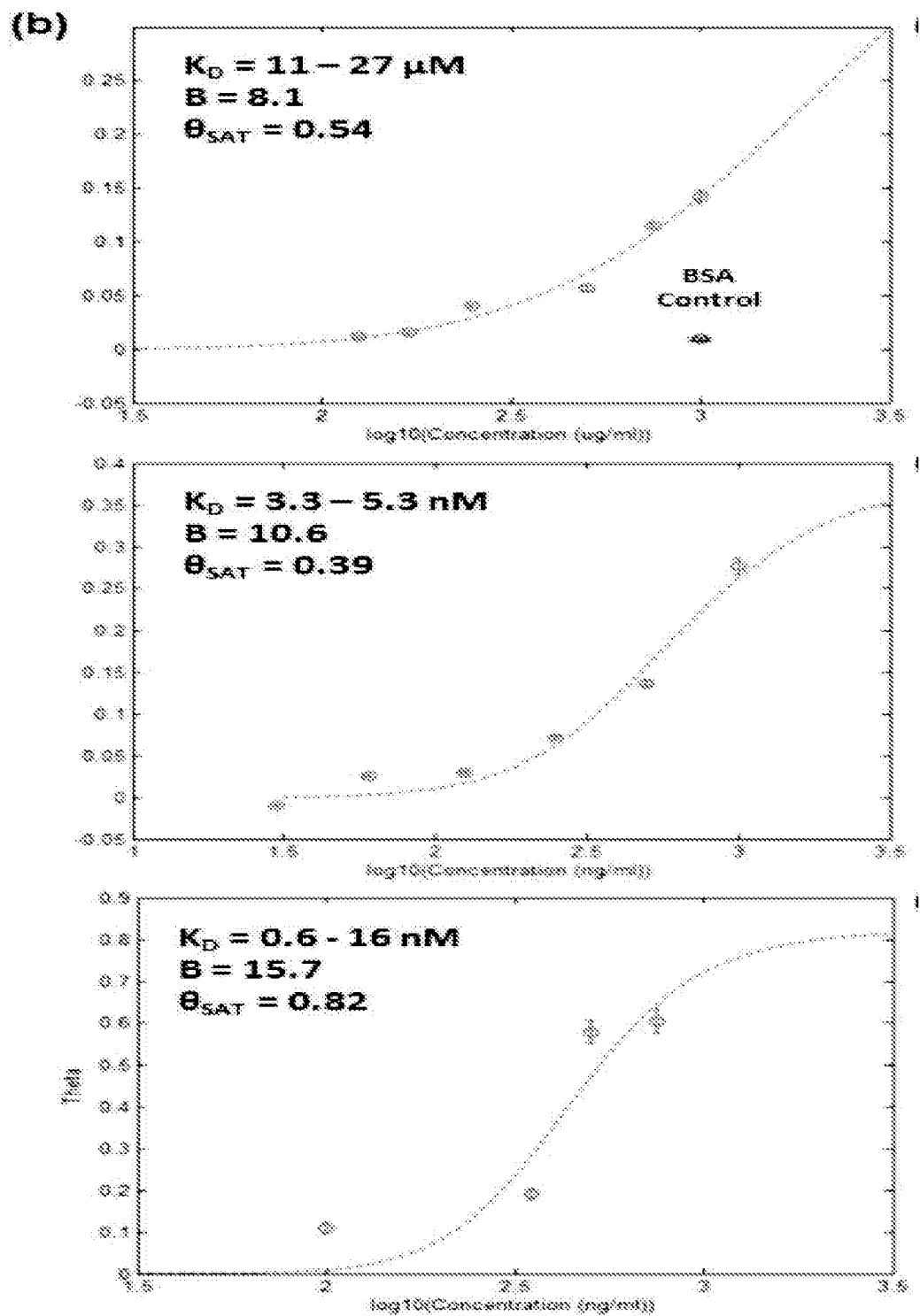


FIG. 4B

10/21

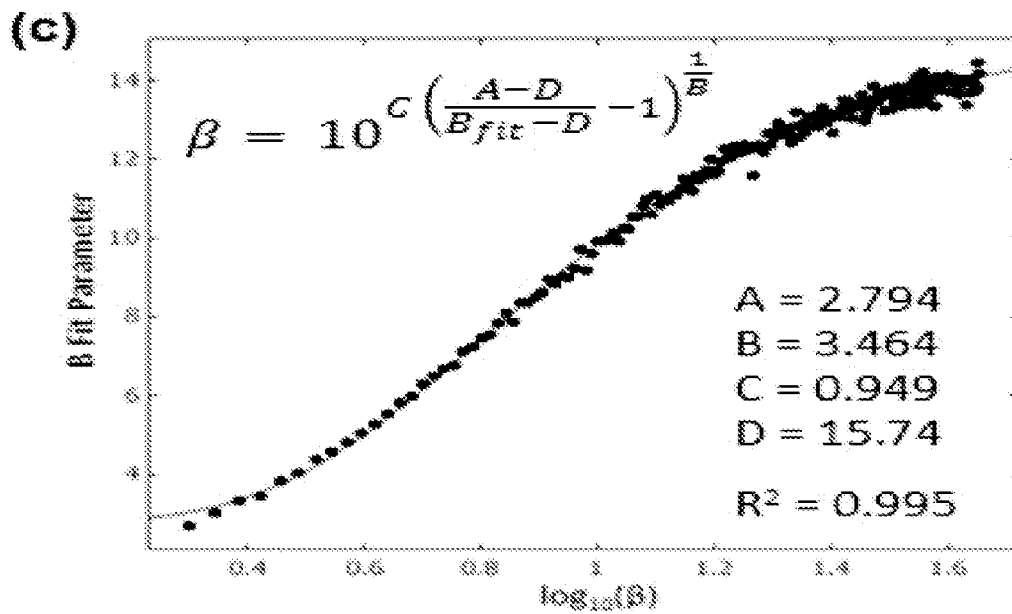


FIG. 4C

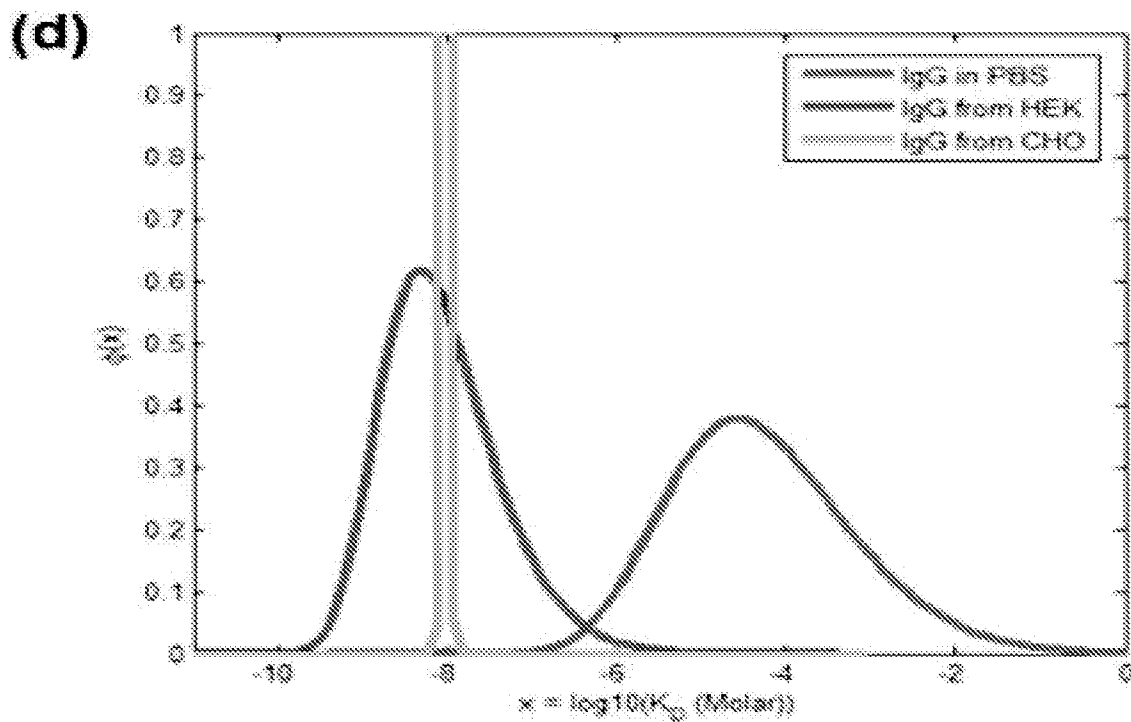


FIG. 4D

11/21

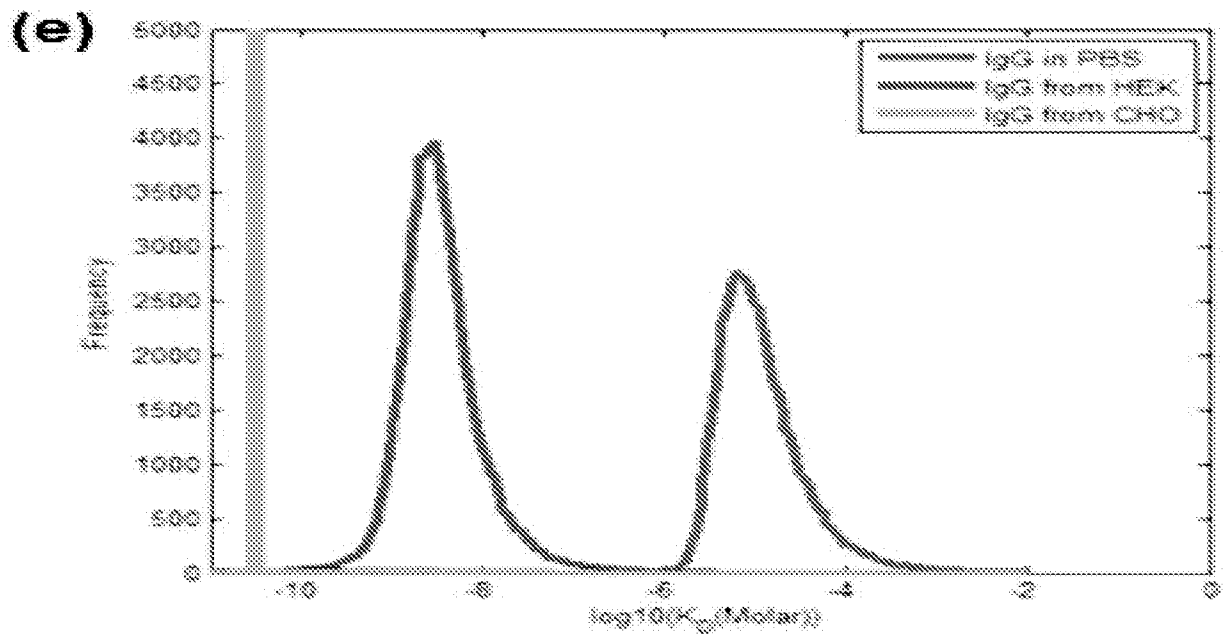


FIG. 4E

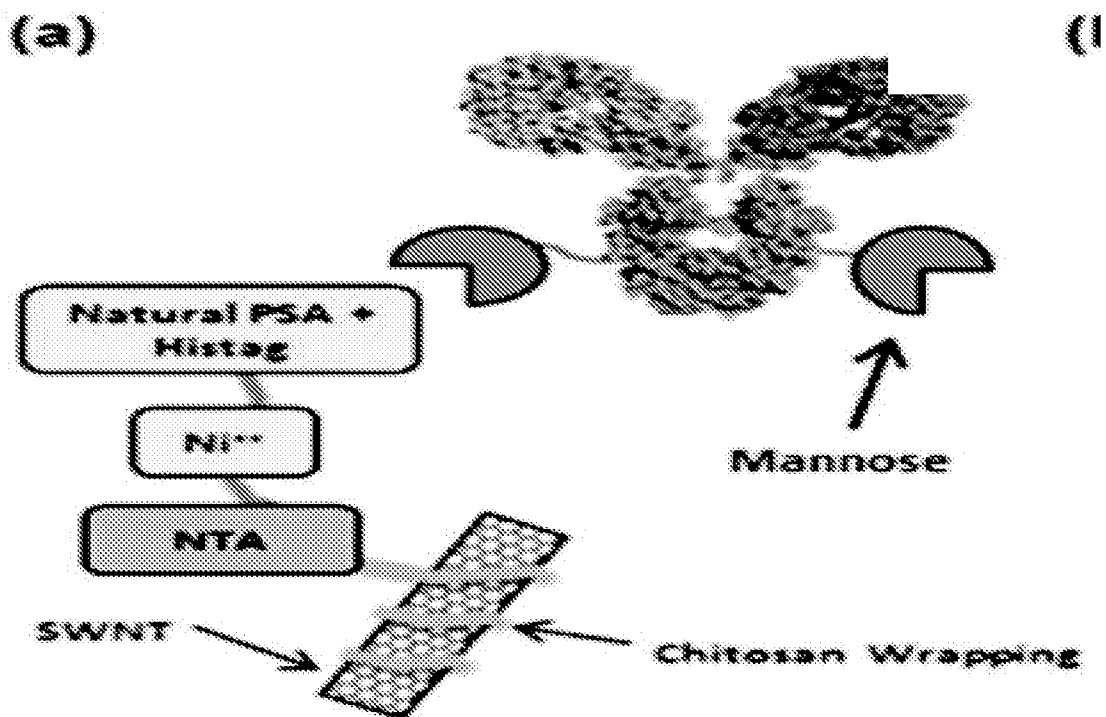


FIG. 5A

12/21

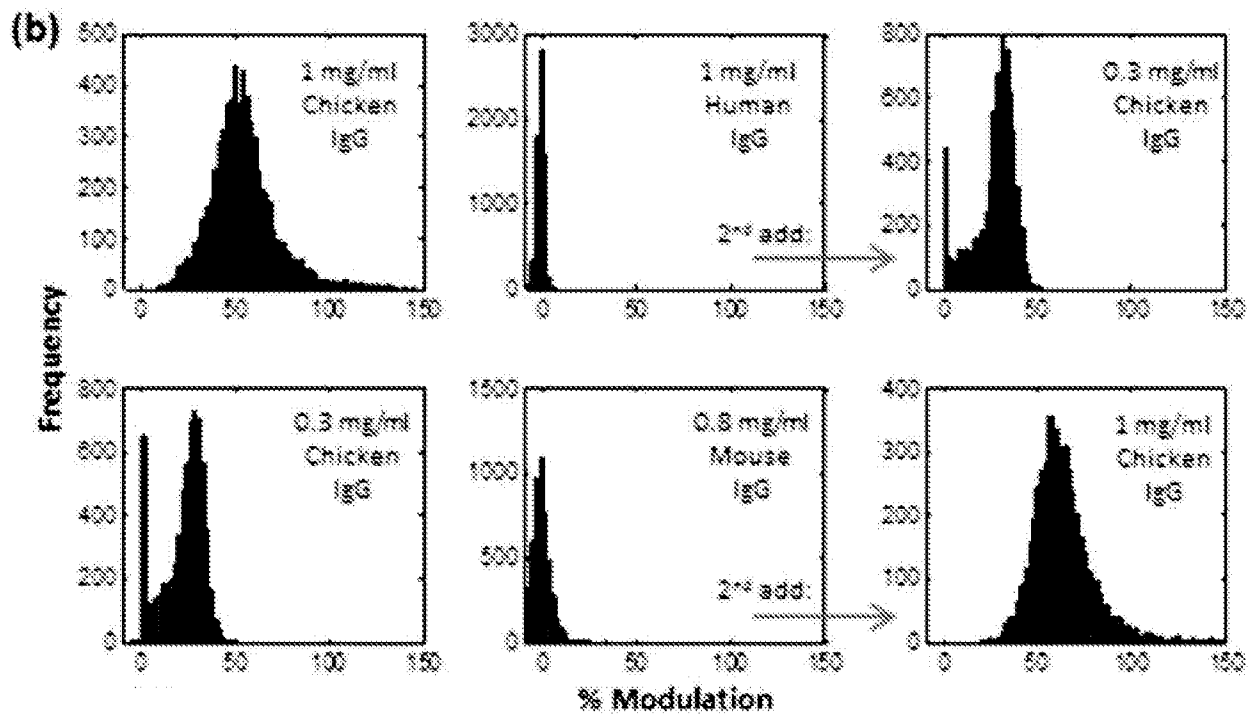


FIG. 5B

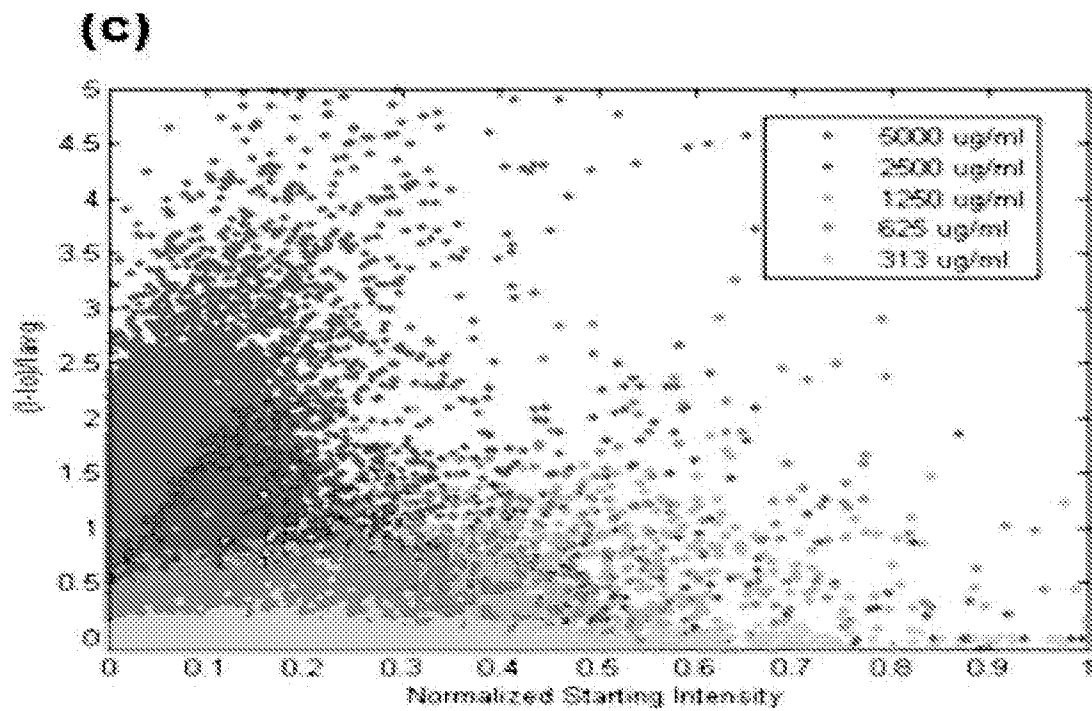


FIG. 5C

13/21

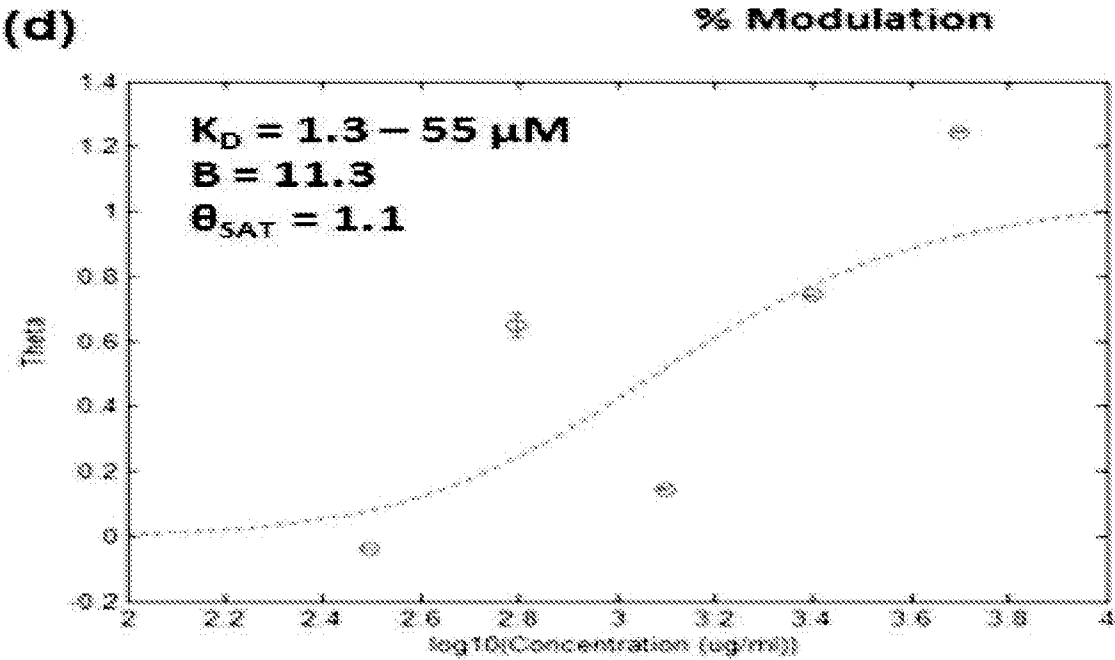


FIG. 5D

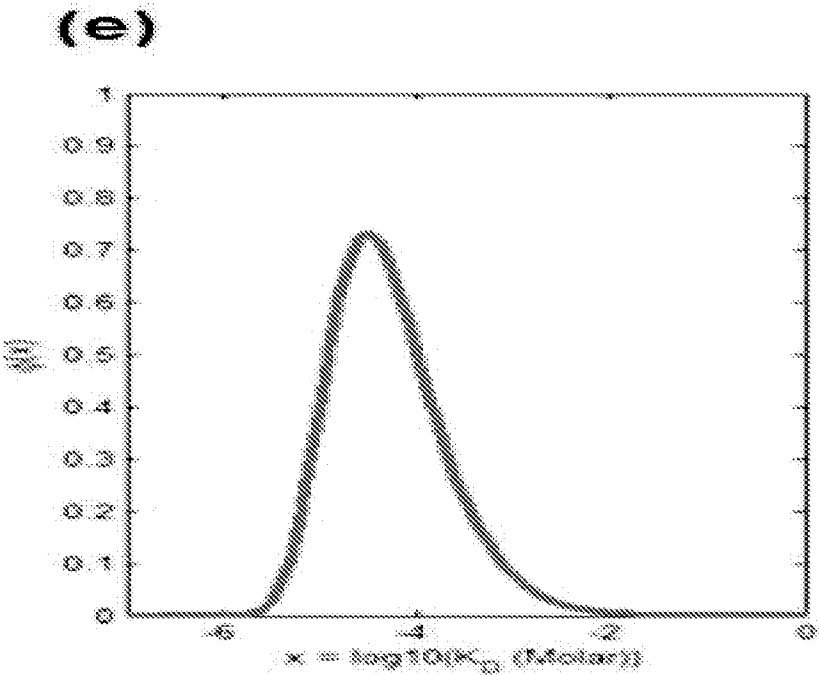


FIG. 5E

14/21

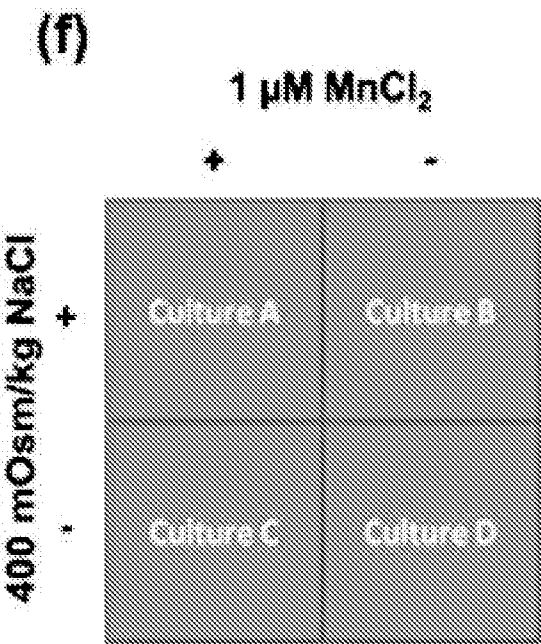


FIG. 5F

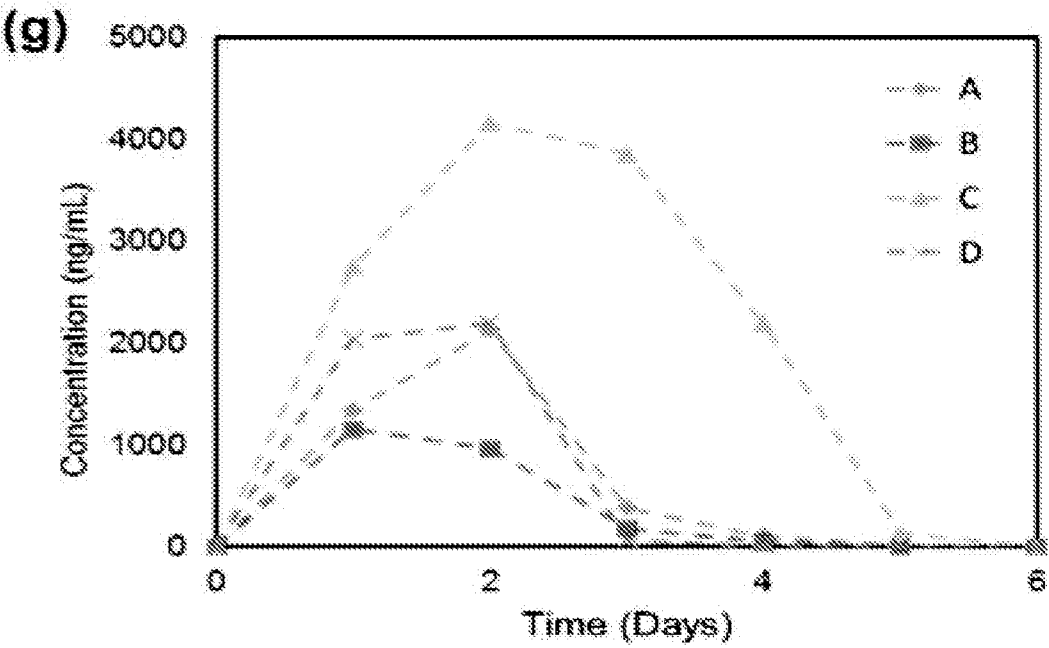


FIG. 5G

15/21

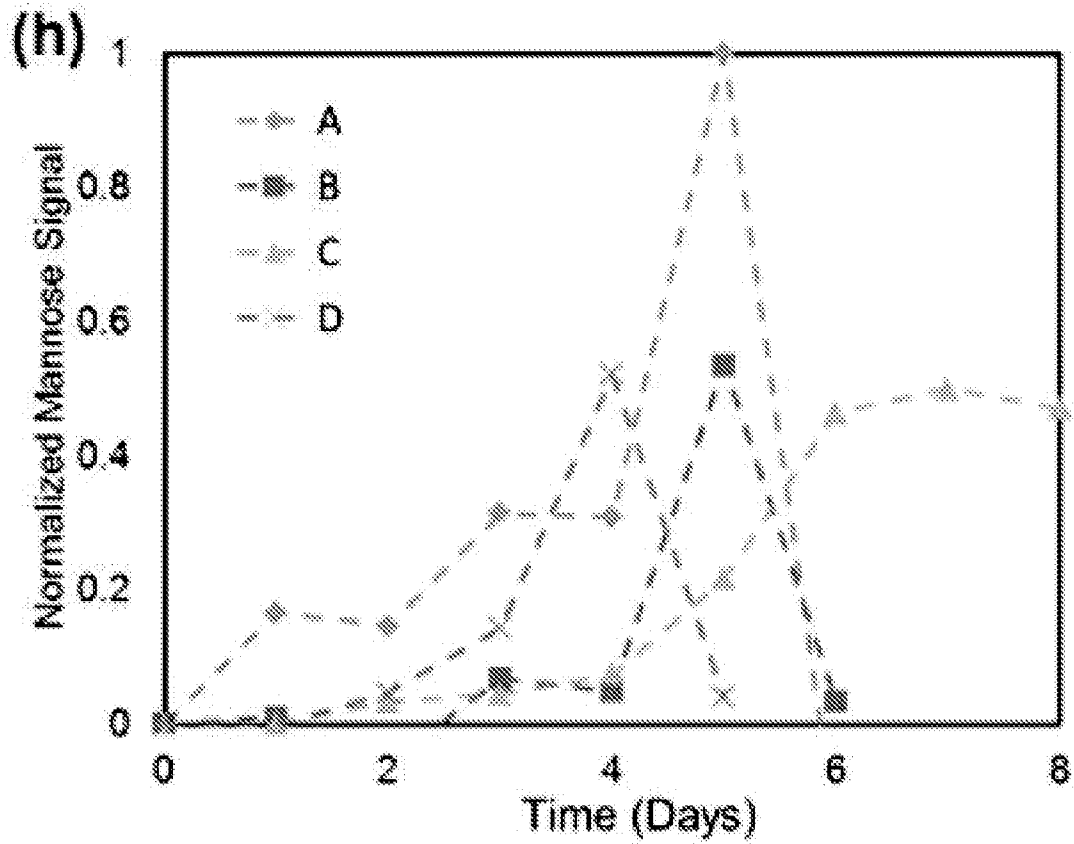
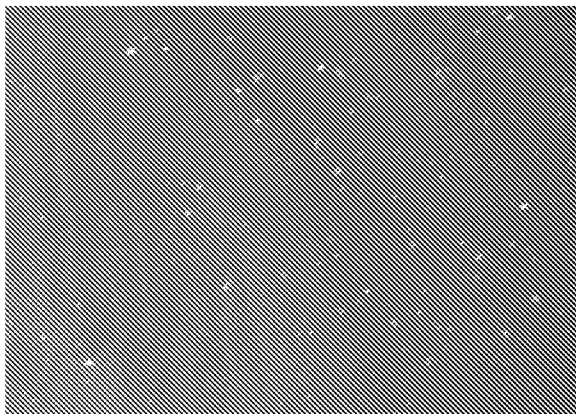
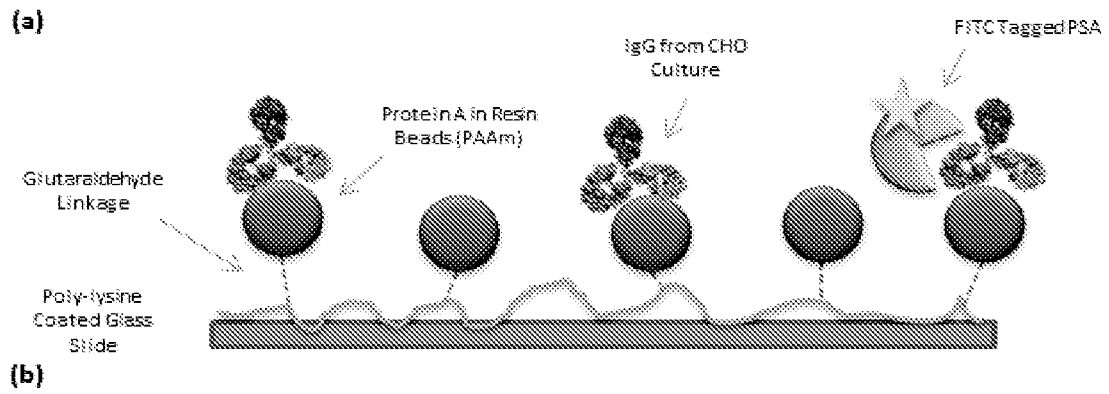
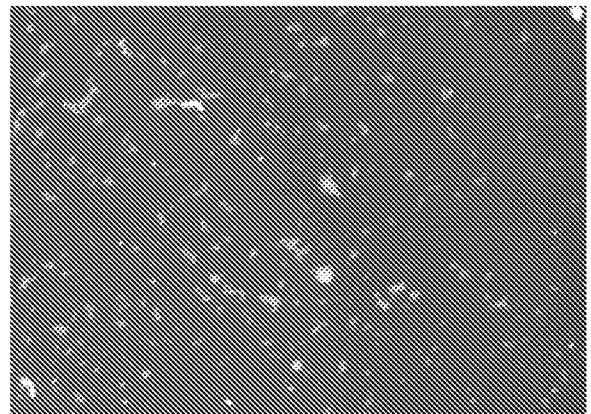


FIG. 5H

16/21

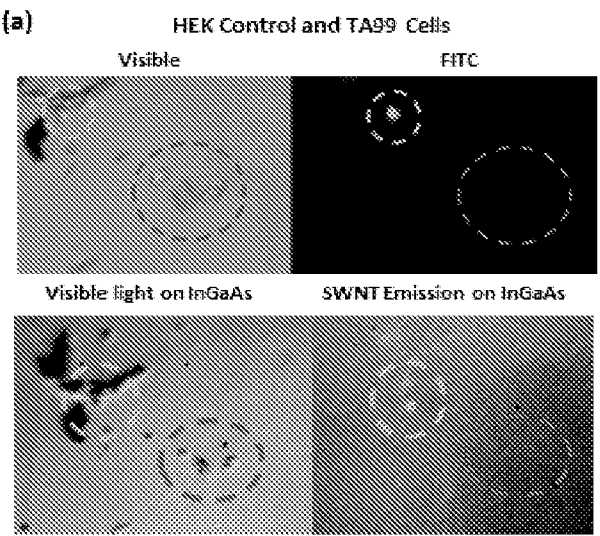


Culture C – Day 1



Culture C – Day 5

FIG. 6



(d) Time Trace of top 10,000 SWNT exposed to HEK-TA99

FIG. 7A

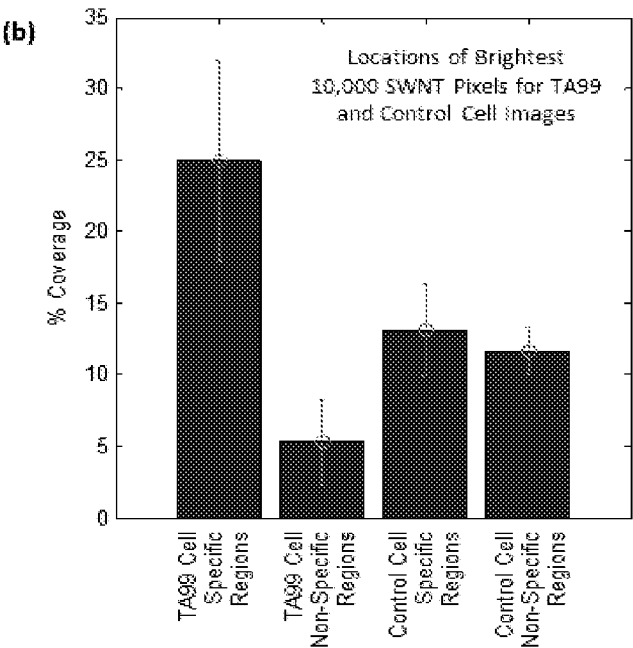


FIG. 7B

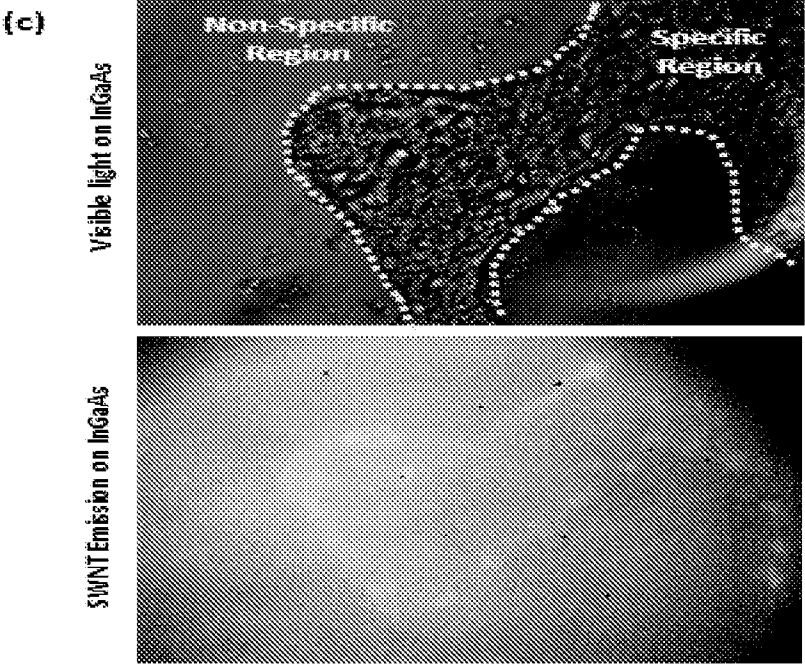


FIG. 7C

18/21

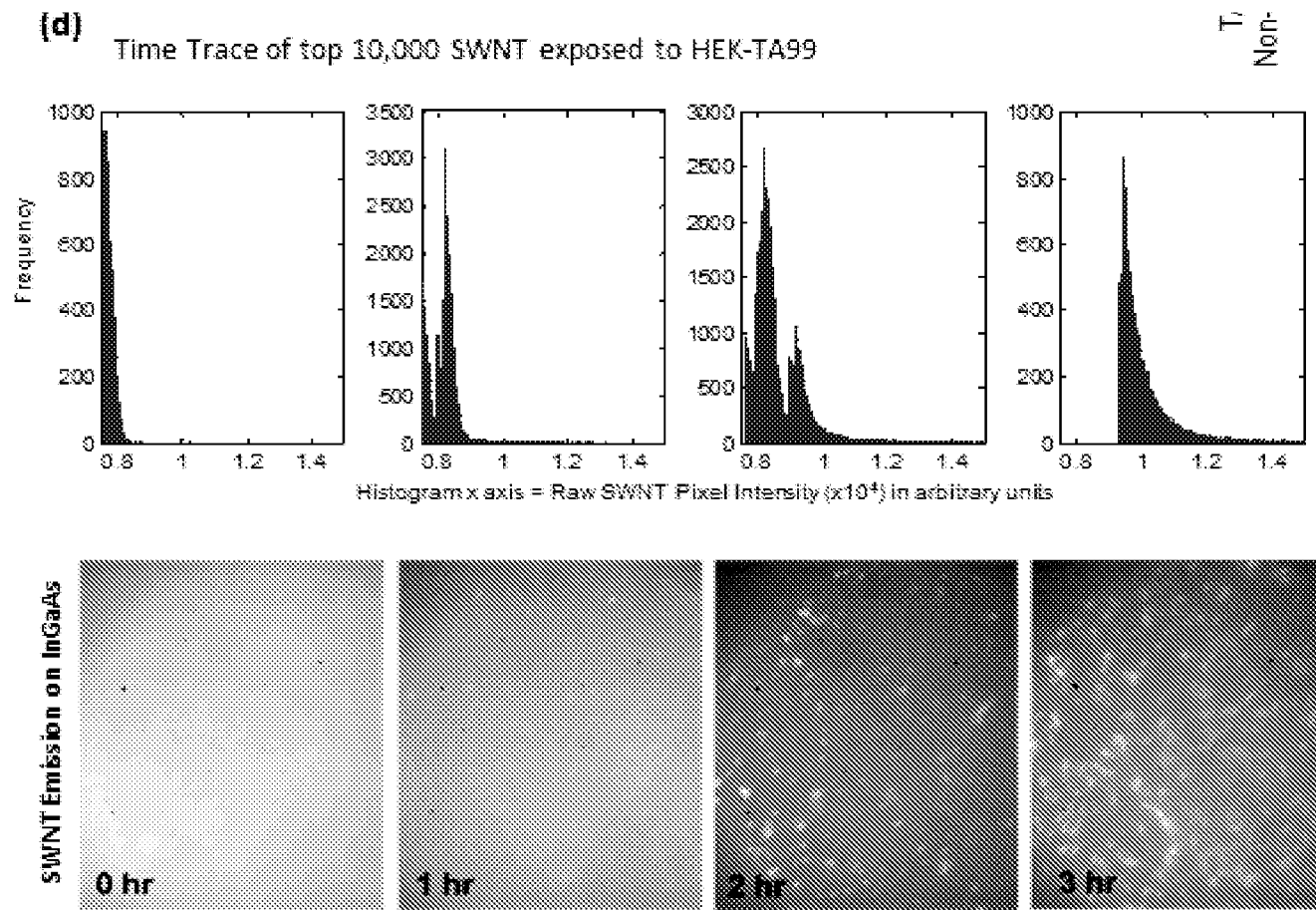


FIG. 7D

(e)

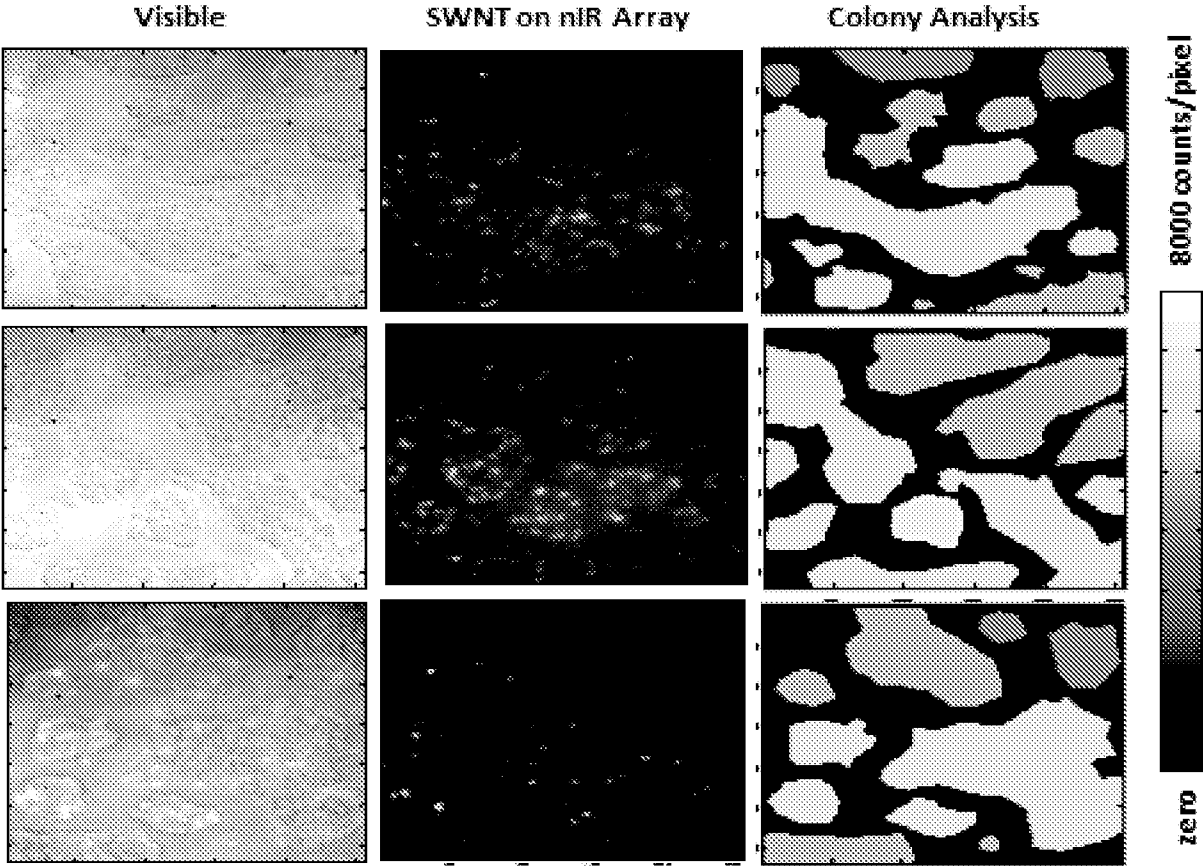


FIG. 7E

20/21

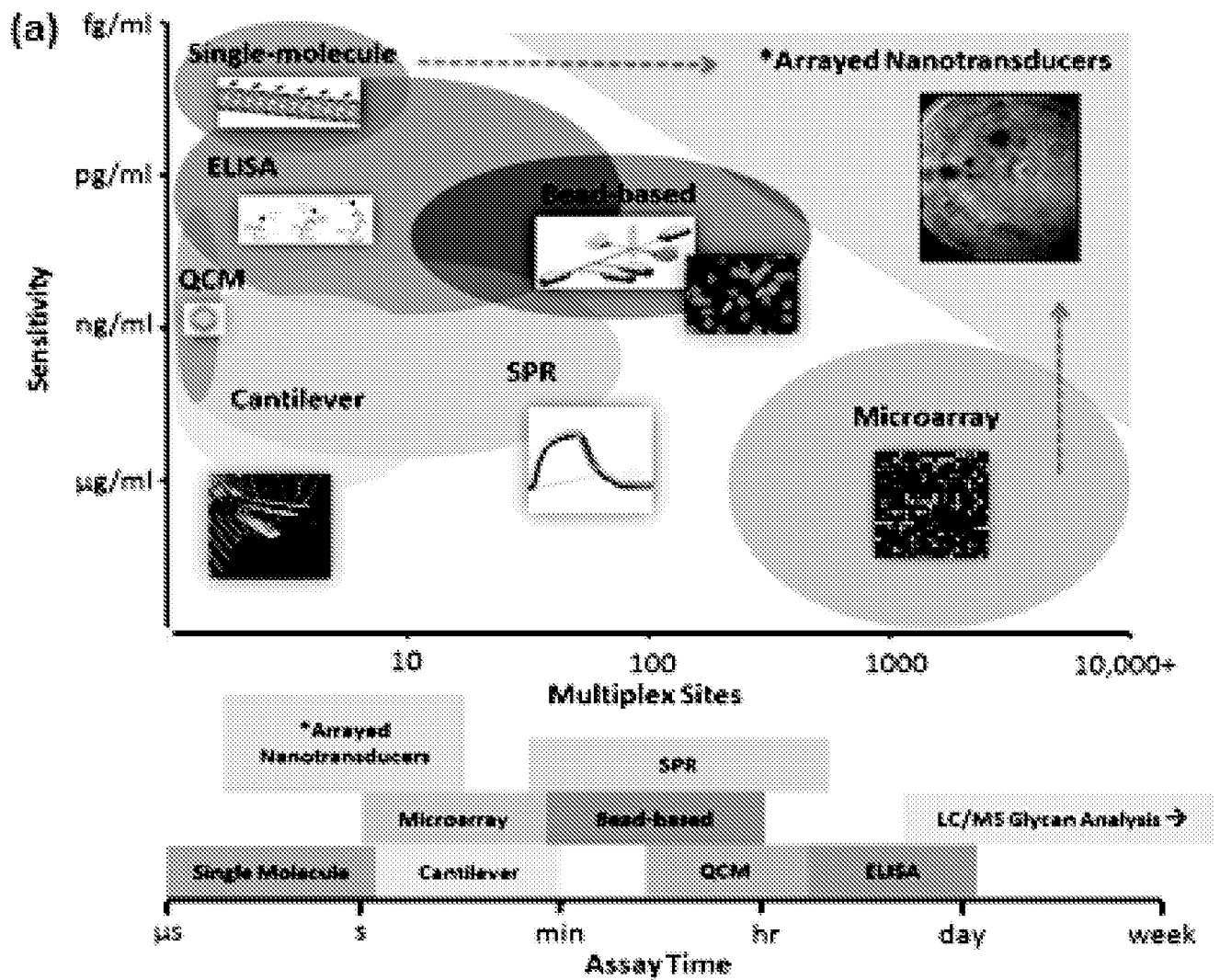


FIG. 8

21/21

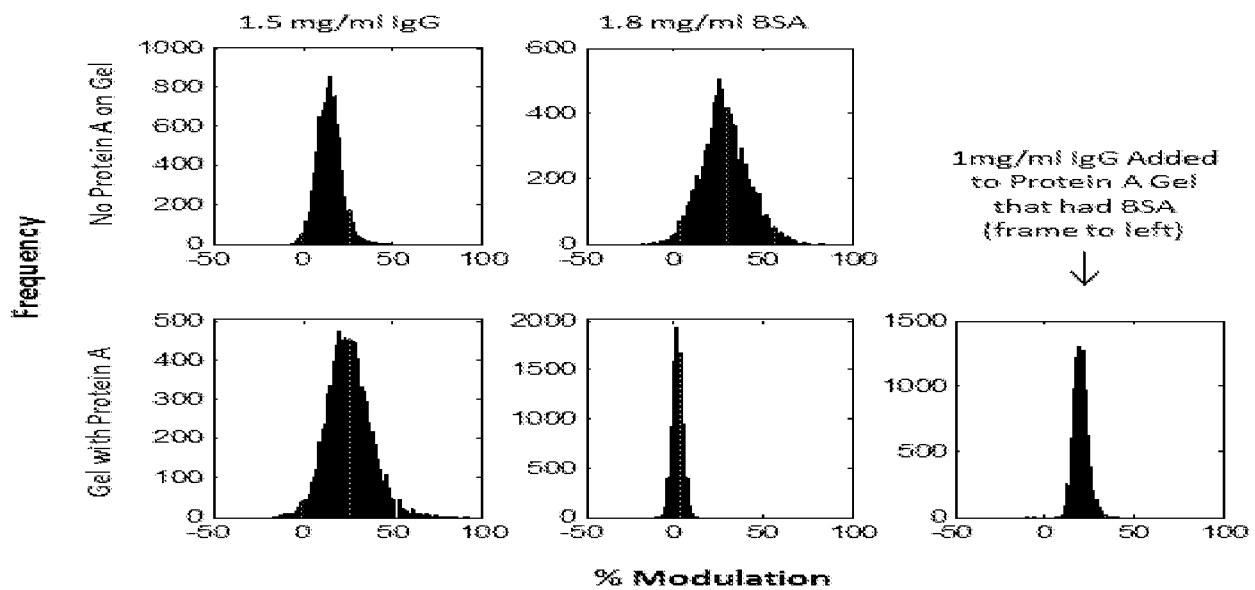


FIG. 9

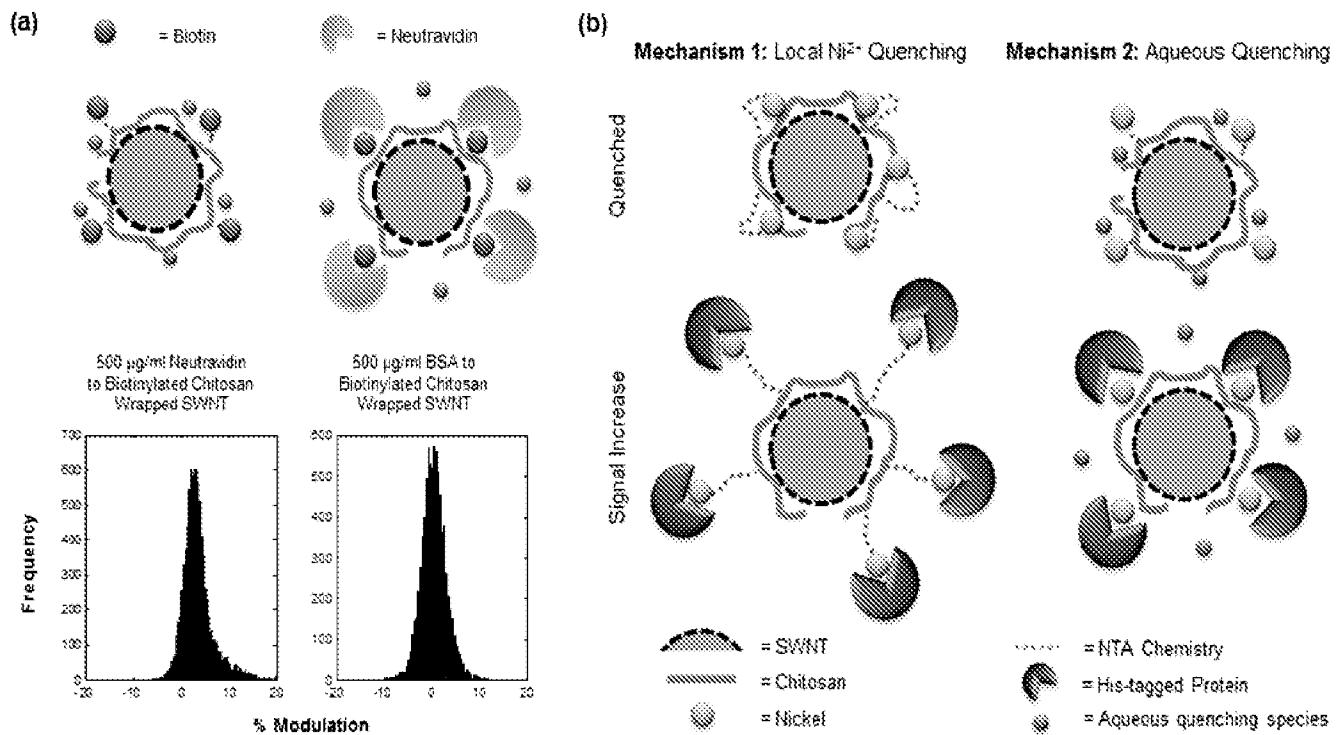


FIG. 10