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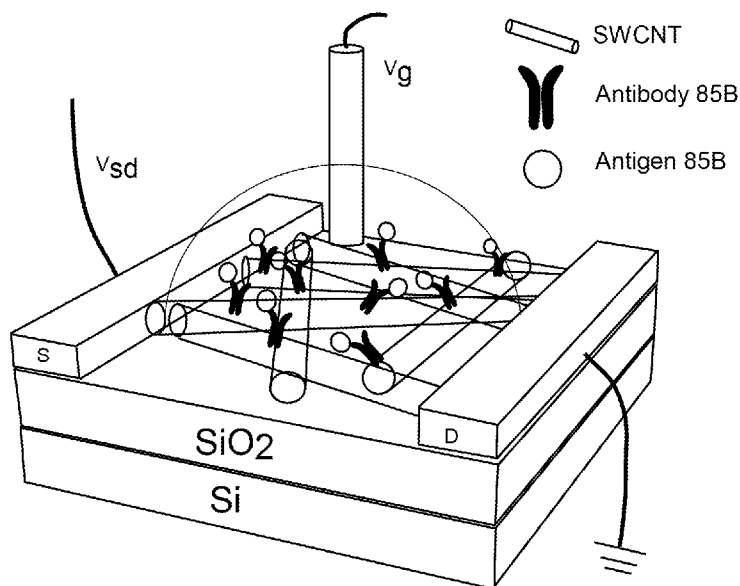


FIG. 1A

(57) Abstract: A sensor device for diagnosis of tuberculosis includes a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode. The sensor medium includes nanostructures and at least one of one or more recognition entities including at least one active binding site for Ag85 antigen. A liquid is deposited over the sensor medium. An ionic strength of the liquid is selected at which binding of the Ag85 antigen to the one or more recognition entities occurs. The ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid.



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NANOSTRUCTURE-BASED DIAGNOSIS OF TUBERCULOSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Patent Application Serial No. 63/520,728, filed August 21, 2023, the disclosure of which is incorporated herein by reference.

BACKGROUND

[0002] The following information is provided to assist the reader in understanding technologies disclosed below and the environment in which such technologies may typically be used. The terms used herein are not intended to be limited to any particular narrow interpretation unless clearly stated otherwise in this document. References set forth herein may facilitate understanding of the technologies or the background thereof. The disclosure of all references cited herein are incorporated by reference.

[0003] Tuberculosis (TB), caused by *mycobacterium tuberculosis* (MTB), is a highly contagious and airborne disease spread through the air when a patient coughs, speaks, or sneezes. According to the World Health Organization (WHO), MTB is one of infectious agents that kill the most people in the world, and TB remains a leading cause of morbidity and mortality in many developing countries. TB is clinically dichotomized into active TB and latent TB forms. Latent TB is the case where the concentration of MTB is too low to show symptoms. The latest data from the Centers for Disease Control and Prevention (CDC) show that there are 13 million people with latent TB in the USA. If left untreated, latent TB can turn into active TB. Thus, the early diagnosis of TB is essential in preventing its burden.

[0004] Currently, TB diagnosis focuses on screening tools (that is, chest X-ray), the detection of bacilli by microscopic techniques (i.e., smear microscopy) and bacterial growth cultures, detection of host immune response to the pathogen (that is, Mantoux test), and bacterial nucleic acid amplification methods. The interferon- γ release assays like QuantiFERON-TB Gold Plus, widely used in the USA and Europe, can also diagnose TB by detecting IFN- γ secretion from the collected blood lymphocytes after their stimulation with ESAT-6 and CFP-10 antigens that are quite specific for MTB. A chest X-ray shows low sensitivity to latent TB. The Mantoux test requires several days before result reading, and the accuracy of it depends on previous vaccinations. Although the molecular and functional immunological assays have high

specificity, sensitivity, and accuracy, they are time-consuming, and require specialized equipment, reagents, and operators, making them inappropriate for developing regions and limiting their widespread use.

[0005] At the forefront of digital-health technology, there is an urgent demand for a rapid, simple, and effective TB diagnostic method. Nanobiosensors, which are analytical detection tools based on functional nanomaterials that transduce biological responses into measurable signals, may have significant potential to satisfy above requirements. A number of nanobiosensors have been developed for TB diagnosis through the detection of MTB specific DNA, cells or antigens. For example, carbon nanotube- and nanowire polypyrrole (nw-Ppy)-based biosensors have been developed. Both sensors were functionalized with PAMAM dendrimers, ferrocenyl group, and DNA probe and successfully detected the MTB DNA in real samples by cyclic voltammetry (CV) and square wave voltammetry (SWV). Although DNA detection provided high specificity in TB diagnosis, the real TB samples needed pretreatment such as amplification by polymerase chain reaction (PCR) before sensing, which is complex and time-consuming. There have also been attempts to develop an immune-resistive biosensor to screen the MTB cells and the MTB antigen MPT64 through the functionalization of carbon nanotubes with specific antibodies. The limits of detection (LODs) were 10 CFU/mL for cells and 100 ng/mL for MPT64 in tongue swab samples within 30 min. Such work sought to achieve the application of cells or antigens as the biomarker in TB screening but showed low sensitivity. Furthermore, when antibodies are used as receptors, antigens are more favorable than cells as biomarkers due to antigen-antibody specific interactions, which can decrease the LOD and increase the specificity, motivating their use in TB diagnostics. For instance, a nanobiosensor was developed which was fabricated with antibody and CdSe-ZnS quantum dots/silica nanoparticles/screen-printed carbon electrode-modified electrode to detect CFP10-ESAT6 antigen complex. The LOD was improved to 0.15 ng/mL by linking enzyme catalase to the electrode and measuring generated differential pulse voltammetry (DPV) currents.

[0006] Field-effect transistor (FET)-based biosensors are increasingly recognized as a promising type of biosensor for their ability to provide rapid, sensitive, label-free, and highly-specific detection of analytes. An FET-based immunosensor for the detection of antigen 85 complex B (Ag85B) has been developed which is the major MTB-secreted product. That immunosensor has a high potential for binding anti-TB antibodies. Through the integration of the silicon nitride layer and glutaraldehyde, the monoclonal antibody against recombinant

Ag85B protein was immobilized onto the biosensor for antigen detection and the LOD was determined as 0.12 $\mu\text{g/mL}$. Recently, a silicon nanowire-based field-effect transistor (SiNW-FET) biosensing platform was developed to detect the MTB. Based on the binding between Ag85B and anti-Ag85B antibody, that biosensor demonstrated sensitivity and obtained responses from sputum samples of TB patients. Such studies demonstrated the feasibility of TB diagnosis through the detection of MTB antigen-antibody interactions using nanomaterial-based FET biosensors.

[0007] Since 1998, single-walled carbon nanotubes (SWCNTs) have been utilized to create FETs, showcasing outstanding performance in biosensing as a result of their unique physical properties. With an average diameter of approximately 1 nm, SWCNTs are comparable in size to biomolecules. They also exhibit relatively low charge-carrier density and high intrinsic carrier mobility that are preferred in detecting electrostatic interactions and charge transfer during biological processes. In contrast to other FET functional nanomaterials, such as graphene, silicon nitride, and silicon nanowires, SWCNTs with their extremely small diameter can reduce gate leakage and demonstrate high conductivity, biocompatibility, charge-mobility, and stability. Additionally, a variety of proteins have been reported to be attached onto the sidewalls of SWCNTs through noncovalent (e.g., π - π stacking or polymer wrapping) or covalent functionalization (e.g., fluorination of SWCNTs or protein coupling via carboxyl groups), making SWCNTs well-suited for biosensing. Several researchers have fabricated SWCNT FET biosensors for medical applications, including the detection of SARS-CoV-2 antigens, cancer exosomal miRNA, and Alzheimer's disease biomarkers, demonstrating LOD comparable to that of sophisticated methods like nucleic acid amplification tests (NAATs) and enzyme-linked immunosorbent assay (ELISA).

SUMMARY

[0008] In one aspect, a sensor device for diagnosis of tuberculosis includes a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode. The sensor medium includes a plurality of nanostructures and at least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site for Ag85 antigen. A liquid is deposited over the sensor medium,

wherein an ionic strength of the liquid is selected at which binding of the Ag85 antigen to the recognition entities occurs. The ionic strength and mass loading of the recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid (for example, to achieve a determined limit of detection). The sensor device further includes electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the Ag85 antigen. The one or more antibodies may, for example, be selected from the group consisting of a human or nonhuman Ag85A, Ag85B, Ag85C, and Ag85G antibody or the one or more antibody fragments are selected from the group consisting of fragments of human or nonhuman Ag85A, Ag85B, Ag85C, and Ag85G antibody. In a number of embodiments, the one or more antibodies are nonhuman Ag85B antibodies or the one or more antibody fragments are fragments of nonhuman Ag85B antibodies. In a number of embodiments, the sensor device is a field-effect transistor device and the liquid functions as a gating liquid.

[0009] A blocking buffer which is interactive with non-selective binding sites may be applied to the sensor before the liquid is deposited thereon. The blocking buffer may, for example, include a protein. The protein may, for example, be covalently attached to the nanostructures. The protein may be crosslinked via a crosslinking agent. In a number of embodiments, the protein is bovine serum albumin. In a number of embodiments, the protein is bovine serum albumin and the crosslinking agent is glutaraldehyde.

[0010] The average height of immobilized recognition entities may, for example, be within 0.2 to 2.0 times of the maximum mass loading at which the average height remains within a Debye screening length of the liquid, within 0.5 to 1.5 times of the maximum mass loading at which the average height remains within a Debye screening length of the liquid, or within 0.75 to 1.25 times of the maximum mass loading at which the average height remains within a Debye screening length of the liquid. The maximum mass loading at the selected ionic strength may, for example, be determined by experimentally determining a maximum response for a determined Ag85 antigen concentration over varying mass loading. The selected ionic strength may, for example, be determined experimentally by determining response to the Ag85 antigen at each of a plurality of ionic strengths.

[0011] In a number of embodiments, the nanostructures are carbon nanotubes. The carbon nanotubes may, for example, be single walled carbon nanotubes. The single-walled carbon nanotubes may, for example, be semiconductor enriched single-walled carbon nanotubes. In a number of embodiments, a semiconducting content of the semiconductor enriched single-walled carbon nanotubes is at least 90%, at least 95%, at least 99%, or at least 99.9%.

[0012] The ionic strength of the liquid may be experimentally optimized based on the limit of detection for a given sensor configuration. The mass loading of the recognition entities may be experimentally optimized based upon a limit of detection for a given sensor configuration.

[0013] In a number of embodiments, the one or more recognition entities are immobilized upon a metal nanoparticles immobilized upon the plurality of nanostructures. In a number of embodiments, the metal nanoparticles are gold nanoparticles.

[0014] In a number of embodiments, the plurality of nanostructures comprise a plurality of semiconductor enriched single-walled carbon nanotubes, and a plurality of rabbit polyclonal Ag85B antibodies are covalently attached to the plurality of semiconductor enriched single-walled carbon nanotubes. The liquid may include phosphate buffered saline in a concentration range of approximately 0.1x to 0.001x. A blocking buffer may be applied to the sensor before the liquid is deposited thereon, wherein the blocking buffer comprising bovine serum albumin, a non-ionic surfactant and polyethylene glycol. The mass loading of rabbit polyclonal Ag85B antibodies may, for example, be in the range of approximately 0.5 -10 μg for a SWCNT-device with an $I-V_g$ curve off-current (at approximately +0.6 V) between approximately -1 and 1 μA and an on-current (at approximately -0.6 V) larger than 10 μA . In a number of embodiments, the concentration of the phosphate buffered saline is approximately 0.01x and the mass loading is approximately 5 μg .

[0015] In another aspect, a method of detecting tuberculosis includes applying a body fluid sample to a sensor device and measuring a variable which provides a measure of change in at least one property of a sensor medium which is dependent upon the presence of the Ag85 antigen. The sensor device includes a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and the sensor medium on the substrate between the first electrode and the second electrode. The sensor medium includes a plurality of nanostructures. At least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of

nanostructures. Each of the one or more recognition entities includes at least one active binding site for Ag85 antigen. A liquid is deposited over the sensor medium. An ionic strength of the liquid is selected at which binding of the Ag85 antigen to the one or more recognition entities occurs. The ionic strength and mass loading of the recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized one or more recognition entities is within a determined of a maximum mass loading at which the average height remains within a Debye screening length of the liquid (for example, to achieve a determined limit of detection). The sensor device further includes at least one measurement system in operative connection with the sensor. The sensor device may be otherwise characterized as described above and elsewhere herein. The body fluid sample may, for example, be sputum or a blood fluid. The body fluid sample may, for example, be a blood fluid.

[0016] In another aspect, a sensor device for detecting an antigen includes a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode. The sensor medium includes a plurality of nanostructures. At least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments are immobilized on the plurality of nanostructures. Each of the one or more recognition entities includes at least one active binding site for the antigen. A liquid is deposited over the sensor medium. An ionic strength of the liquid is selected at which binding of the antigen to the recognition entities occurs. The ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid (for example, to achieve a determined limit of detection). The sensor device further includes electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the antigen.

[0017] In a number of embodiments, the sensor device is a field-effect transistor device and the liquid functions as a gating liquid. A blocking buffer which is interactive with non-selective binding sites may be applied to the sensor before the liquid is deposited thereon. The protein may be covalently attached to the nanostructures. In a number of embodiments, the protein is crosslinked via a crosslinking agent. The protein may, for example, be bovine serum albumin.

In a number of embodiments, the protein is bovine serum albumin and the crosslinking agent is glutaraldehyde.

[0018] The average height of the one or more immobilized recognition entities may, for example, be within 0.2 to 2.0 times of the maximum mass loading at which the average height remains within a Debye screening length of the liquid, within 0.5 to 1.5 times of the maximum mass loading at which the average height remains within a Debye screening length of the liquid, or within 0.75 to 1.25 times of the maximum mass loading at which the average height remains within a Debye screening length of the liquid. The maximum mass loading at the selected ionic strength may, for example, be determined by experimentally determining a maximum response for a determined Ag85 antigen concentration over varying mass loading. The selected ionic strength may, for example, be determined experimentally by determining response to the antigen at each of a plurality of ionic strengths.

[0019] The maximum mass loading at the selected ionic strength may be determined by experimentally determining a maximum response for a determined antigen concentration over varying mass loading. The selected ionic strength may be determined experimentally by determining response to the antigen at each of a plurality of ionic strengths.

[0020] In a number of embodiments, the nanostructures are carbon nanotubes. The carbon nanotubes may, for example, be single walled carbon nanotubes. In a number of embodiments, the single-walled carbon nanotubes are semiconductor enriched single-walled carbon nanotubes. The semiconducting content of the semiconductor enriched single-walled carbon nanotubes may be at least 90%, at least 95%, at least 99%, or at least 99.9%.

[0021] As described above, the ionic strength of the liquid may experimentally optimized based on the limit of detection for a given sensor configuration. The mass loading of the recognition entities may be experimentally optimized based upon limit of detection for a given sensor configuration.

[0022] As also described above, the one or more recognition entities may be immobilized upon a metal nanoparticles immobilized upon the plurality of nanostructures. In a number of embodiments, the metal nanoparticles include or are gold nanoparticles.

[0023] In a number of embodiments, the plurality of nanostructures includes a plurality of semiconductor enriched single-walled carbon nanotubes, and a plurality of the antibodies or antibody fragments are covalently attached to the plurality of semiconductor enriched single-walled carbon nanotubes.

[0024] In a further aspect, a method of detecting an antigen includes applying a body fluid sample to a sensor device and measuring a variable which provides a measure of change in at least one property of a sensor medium which is dependent upon the presence of the antigen. The sensor device includes a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode. The sensor medium includes a plurality of nanostructures. At least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of nanostructures. Each of the one or more recognition entities includes at least one active binding site for the antigen. A liquid is deposited over the sensor medium. An ionic strength of the liquid is selected at which binding of the antigen to the one or more recognition entities occurs. The ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized recognition entities is within a determined of a maximum mass loading at which the average height remains within a Debye screening length of the liquid (for example, to achieve a determined limit of detection). The sensor device further includes at least one measurement system in operative connection with the sensor. The sensor device may be otherwise characterized as described above and elsewhere herein. The body fluid sample may, for example, be sputum or a blood fluid. The body fluid sample may, for example, be a blood fluid.

[0025] In still a further aspect, a method of fabricating a sensor device for detecting an antigen wherein the sensor device includes a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures, at least one or one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of nanostructures, each of the one or more recognition entities includes at least one active binding site for the antigen, a liquid being deposited over the sensor medium, and electronic circuitry including at least one measurement system in operative connection with the sensor to measure

a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the antigen. The method includes determining an ionic strength of the liquid at which binding of the antigen to the one or more recognition entities occurs and determining a mass loading of the one or more recognition entities immobilized on the plurality of nanostructures so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid (for example, to achieve a determined limit of detection).

[0026] The present devices, systems, and methods, along with the attributes and attendant advantages thereof, will best be appreciated and understood in view of the following detailed description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] FIG. 1A illustrates a schematic illustration of a Ab85B-SWCNT FET for detection of MTB antigen Ag85B including interdigitated gold electrodes (illustrated as blocks), contacting a network of SWCNTs (illustrated as transparent), which are configured as the source (S) and drain (D) electrodes, wherein in a number of embodiments, the source-drain voltage (V_{sd}) was 50 mV, and the gate voltage (V_g) was applied through an Ag/AgCl reference electrode inserted into the gating electrolyte.

[0028] FIG. 1B illustrates a scanning electron microscopy (SEM) image of a bare-SWCNT FET device.

[0029] FIG. 1C illustrates an SEM image of SWCNT network deposited between channels.

[0030] FIG. 1D illustrates an AFM image of an Ab85B-SWCNT FET device.

[0031] FIG. 1E illustrates schematic representation of an embodiment of an FET sensor device hereof.

[0032] FIG. 1F illustrates schematic representation of an embodiment of a chemiresistor sensor device.

[0033] FIG 2A illustrates relative responses after adding 10 μL (100 $\mu\text{g/mL}$) of Ag85B solutions onto the SWCNT devices functionalized with different amount of Ab85B; relative response = $\frac{I-I_0}{I_0}$, wherein all data points plotted in the histogram are mean $\pm$ standard error of the mean based on 3 devices.

[0034] FIG. 2B illustrates calibration plots showing the effect of the gating electrolyte on the Ag85B detection, wherein all data points plotted in the calibration plots are mean $\pm$ standard error of the mean based on 5 devices.

[0035] FIG 3A illustrates FET characteristic curves of an Ab85B-SWCNT FET device upon exposure to increasing concentrations of Ag85B in PBS, wherein response is shown to increase with increasing concentration as represented by arrow A from blank to 100 $\mu\text{g/mL}$ samples.

[0036] FIG. 3B illustrates calibration plot for Ag85B detection, effect from solvent and non-specific binding detection, wherein all data points plotted in the calibration plots are mean $\pm$ standard error of the mean and the number of devices (n) used is indicated in the parenthesis.

[0037] FIG. 4A illustrates FET characteristic curves of Ab85B-SWCNT FET devices upon exposure to increasing concentrations of Ag85B in artificial sputum.

[0038] FIG. 4B illustrates a calibration plot showing the detection of Ag85B in artificial sputum and the corresponding control experiment studying the effect of artificial sputum, wherein all data points plotted in the calibration plots are mean $\pm$ standard error of the mean based on 4 devices.

[0039] FIG. 4C illustrates FET characteristic curves of BSA-blocked-Ab85B-SWCNT FET devices upon exposure to increasing concentrations of Ag85B in human peripheral blood serum.

[0040] FIG. 4D illustrates a calibration plot showing the detection of Ag85B in serum and the corresponding control experiment studying the nonspecific interactions.

[0041] FIG. 4E illustrates a specificity test of BSA-blocked-Ab85B-SWCNT FET devices, wherein the control was pure serum (10 μL), experimental was Ag85B, SARS-CoV-2 NAg, and SARS-CoV-2 SAg (and wherein all proteins are 10 μL , 1 ng/ mL in serum, all data points

plotted in the calibration plots are mean $\pm$ standard error of the mean, and the number of devices (n) used is indicated in the parenthesis in FIGS. 4A through 4E).

[0042] FIG. 4F illustrates the relative responses after adding 10 μ L TB clinical blood samples onto the SWCNT FET devices, wherein all data plotted are mean $\pm$ standard error of the mean and the number of devices is six for the positive sample and seven for the negative sample.

[0043] FIG 5A illustrates schematically a surface functionalization process of an FET device for detection of Ag85B protein in human peripheral blood serum with Ab85B-cBSA-SWCNT FET

[0044] FIG. 5B illustrates FET characteristic curves of a Ab85B-cBSA-SWCNT FET device upon exposure to increasing concentrations of Ag85B in human peripheral blood serum.

[0045] FIG. 5C illustrates a calibration plot for Ag85B detection, effect from human peripheral blood serum and non-specific binding detection, wherein all data points plotted in the calibration plots are mean $\pm$ standard error of the mean, and the number of devices (n) used is indicated in the parenthesis.

DETAILED DESCRIPTION

[0046] It will be readily understood that the components of the embodiments, as generally described and illustrated in the figures herein, may be arranged and designed in a wide variety of different configurations in addition to the described representative embodiments. Thus, the following more detailed description of the representative embodiments, as illustrated in the figures, is not intended to limit the scope of the embodiments, as claimed, but is merely illustrative of representative embodiments.

[0047] Reference throughout this specification to “one embodiment” or “an embodiment” (or the like) means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearance of the phrases “in one embodiment” or “in an embodiment” or the like in various places throughout this specification are not necessarily all referring to the same embodiment.

[0048] Furthermore, described features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. In the following description, numerous specific

details are provided to give a thorough understanding of embodiments. One skilled in the relevant art will recognize, however, that the various embodiments can be practiced without one or more of the specific details, or with other methods, components, materials, et cetera. In other instances, well known structures, materials, or operations are not shown or described in detail to avoid obfuscation.

[0049] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, reference to “an antibody” includes a plurality of such antibodies and equivalents thereof known to those skilled in the art, and so forth, and reference to “the antibody” is a reference to one or more such antibodies and equivalents thereof known to those skilled in the art, and so forth. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each separate value, as well as intermediate ranges, are incorporated into the specification as if individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contraindicated by the text. As used herein with respect to a value, the term “approximately,” means within 10% (or typically more desirably within 5%) of the stated value (or of each value in a state range).

[0050] The terms “electronic circuitry,” “circuitry” or “circuit,” as used herein include, but are not limited to, hardware, firmware, software, or combinations of each to perform a function(s) or an action(s). For example, based on a desired feature or need, a circuit may include a software controlled microprocessor, discrete logic such as an application specific integrated circuit (ASIC), or other programmed logic device. A circuit may also be fully embodied as software. As used herein, “circuit” is considered synonymous with “logic.” The term “logic,” as used herein includes, but is not limited to, hardware, firmware, software, or combinations of each to perform a function(s) or an action(s), or to cause a function or action from another component. For example, based on a desired application or need, logic may include a software controlled microprocessor, discrete logic such as an application specific integrated circuit (ASIC), or other programmed logic device. Logic may also be fully embodied as software.

[0051] The term “processor,” as used herein includes, but is not limited to, one or more of virtually any number of processor systems or stand-alone processors, such as microprocessors, microcontrollers, central processing units (CPUs), and digital signal processors (DSPs), in any

combination. The processor may be associated with various other circuits that support operation of the processor, such as random access memory (RAM), read-only memory (ROM), programmable read-only memory (PROM), erasable programmable read only memory (EPROM), clocks, decoders, memory controllers, or interrupt controllers, etc. These support circuits may be internal or external to the processor or its associated electronic packaging. The support circuits are in operative communication with the processor. The support circuits are not necessarily shown separate from the processor in block diagrams or other drawings.

[0052] The term “memory system” refers to a collection of electronic components that store data and instructions. In computerized systems, a processor system can quickly access information stored in a memory system. Memory allows storage and retrieval of information and may, for example, include primary memory and secondary memory. Primary memory includes, for example, RAM, cache memory, etc. Secondary memory includes, for example, hard drives, hard disk drives etc.

[0053] The term “controller,” as used herein includes, but is not limited to, any circuit or device that coordinates and controls the operation of one or more input and/or output devices. A controller may, for example, include a device having one or more processors, microprocessors, or central processing units capable of being programmed to perform functions.

[0054] The term “software,” as used herein includes, but is not limited to, one or more computer readable or executable instructions that cause a computer or other electronic device to perform functions, actions, or behave in a desired manner. The instructions may be embodied in various forms such as routines, algorithms, modules, or programs including separate applications or code from dynamically linked libraries. Software may also be implemented in various forms such as a stand-alone program, a function call, a servlet, an applet, instructions stored in a memory, part of an operating system or other types of executable instructions. It will be appreciated by one of ordinary skill in the art that the form of software is dependent on, for example, requirements of a desired application, the environment it runs on, or the desires of a designer/programmer or the like.

[0055] As used herein, an “antigen” (sometimes abbreviated Ag) is any substance that can be specifically recognized by an antibody. Certain antigens induce a body to make an immune response against the antigen. Antigens may include toxins, chemicals, viruses, bacteria, and other substances having an origin outside the body. Antigens may include a molecule or

molecular structure which, for example, may be present on the outside of a pathogen. Antigens may also have an origin within the body. Body tissues and cells (for example, cancer cells) may also include antigens that cause an immune response. Further, hormones may be antigens. Antigens can be bound by antigen-specific antibodies or by a B-cell antigen receptor. Antigens may be proteins, peptides (which are chains of amino acids) or polysaccharides (which are chains of monosaccharides or simple sugars). Lipids and nucleic acids may become antigens when combined with proteins and polysaccharides. An “antibody” (sometimes abbreviated as Ab and sometimes referred to as an immunoglobulin or Ig), is a relatively large, Y-shaped protein that is used by the immune system to identify and neutralize foreign objects (including pathogenic bacteria and viruses). As used herein, an “antigen receptor” is a protein which selectively binds/interacts with an antigen.

[0056] In general, nanostructures are structures of intermediate size between microscopic and molecular structures. Nanostructures may, for example, have at least one dimension in the range of 0.1 to hundreds of nanometers. Many nanostructures have at least one dimension in the range of 1 to 100 nm. Nanotubes are, for example, considered two-dimensional nanostructures and may have a diameter in the range of, for example, 0.1 nm to hundreds of nm and a length that may be significantly greater than the diameter.

[0057] Chemically sensitive solid-state resistors (chemiresistors) and field-effect transistors (FETs) hereof may, for example, exhibit room temperature liquid phase sensitivity to antigens (or antibodies) in, for example, a clinical sample such as a saliva, nasopharyngeal swabs, serum/plasma, bronchoalveolar lavage (BAL), or endotracheal aspirate (ETA). In general, any biological fluid/fluid sample may be analyzed to determine if the fluid sample includes an analyte above the detection level of a device hereof. In certain embodiments, it is also possible to analyze breath (for example, droplets of fluid from breath). In a nanostructure-based FET device, one, for example, measures electrical current through nanostructures such as semiconductor-enriched SWCNTs (sc-SWCNT) under an applied gate voltage. In chemiresistor devices, a gate voltage is not applied. In both types of devices, an electrical property (for example, conductance or resistance) of nanostructures such as nanotubes changes upon exposure to an analyte, thereby providing a sensor signal. Depending on the semiconducting nature of the nanostructures, application of a gate voltage can provide amplification of the sensor signal. Nanotubes such as single-walled carbon nanotubes or SWCNTs and, particularly, semiconductor-enriched (sc-)-SWCNTs provide an ideal candidate

for incorporation into extremely small and low power devices hereof because they demonstrate extreme environmental sensitivity, high electrical conductivity, and inherent compatibility with existing microelectronic fabrication techniques. In a number of representative embodiment of devices, systems, and methods hereof an sc-SWCNT FET device was studied for the detection of an anti-MTB antigen in, for example, serum/plasma.

[0058] In that regard, in a number of embodiments hereof an anti-MTB antigen 85B antibody-functionalized SWCNT (Ab85B-SWCNT) FET device was studied to detect the major MTB-secreted antigen 85B (Ag85B) in biological fluids. Fabricated from commercial semiconductor-enriched SWCNTs (sc-SWCNTs), the studied FET devices hereof had a high on/off ratio of $\sim 10^4$. In a number of embodiments, taking advantage of the presence of carboxyl groups on the sidewalls of commercial SWCNTs, Ab85B was functionalized onto SWCNTs through EDC/Sulfo-NHS coupling. To obtain the optimal sensing result, the effects of mass/mass loading of antibody to decoration and the ion concentration of the gating electrolyte were investigated with calibration samples. Calibration plots demonstrated that the Ab85B-SWCNT FET device could successfully detect Ag85B spiked in PBS with a LOD of 0.05 fg/mL. As current TB diagnostic methods concentrate on detecting MTB in sputum and serum matrices, the performance of embodiments of Ab85B-SWCNT FET devices hereof were studied in artificial sputum and serum samples spiked with varying concentrations of Ag85B. The devices hereof can identify the presence of Ag85B spiked in artificial sputum and serum. Moreover, AB85B-SWCNT FET devices hereof with blocking buffer (for example, bovine-serum-albumin- or BSA-blocked devices hereof) can detect Ag85B spiked in serum and can distinguish TB positive clinical samples from negative samples in approximately 10 min with, for example, a portable potentiostat, highlighting the potential practicality of the biosensor devices, systems, and methods hereof for TB diagnosis. The robustness of a number of embodiments of the Ab85B-SWCNT devices to biofluids was further enhanced by integrating a BSA cross-linking blocking layer onto SWCNT networks. Representative Ab85B-cBSA-SWCNT FET devices hereof underwent 12 serum tests without the blocking layer being washed away. The devices, systems, and methods hereof provide opportunities for the development of robust biosensors for medical diagnostics

[0059] Studied representative embodiments of anti-MTB Ag85B antibody-functionalized SWCNT (Ab85B-SWCNT) FET devices for specific detection of Ag85B proteins were fabricated on silicon chips with gold interdigitated electrodes (IDEs) (illustrated schematically

in FIG. 1A). The full name for Ab85B antibody is anti-mycobacterium tuberculosis Ag85B antibody. In the present studies, rabbit polyclonal to mycobacterium tuberculosis Ag85B (antigen) was used as a representative example. The antibody is a full-length IgG-type antibody with a typical weight of 150 kDa. Ab85B can be covalently bound to the carboxyl groups on semiconducting-SWCNT through EDC/sulfo-NHS coupling as described below. SWCNTs were deposited using dielectrophoresis (DEP) between the interdigitated gold electrodes (see FIG. 1B). Scanning electron microscopy (SEM) imaging revealed the formation of interconnected networks of carbon nanotubes between the fingers of interdigitated gold electrodes (FIG. 1C). Through 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide/N-hydroxysulfosuccinimide (EDC/sulfo-NHS) coupling, carboxyl groups on SWCNTs were activated into amine reactive O-acylisourea intermediates, where Ab85B was conjugated. Raman spectroscopy tracked the activation of carboxyl groups and functionalization of Ab85B proteins. Several peaks were observed in the radial breathing mode (RBM) region (150 – 250 cm^{-1}), indicating the diameter distribution of SWCNTs. The decrease of peak intensity in the RBM region indicated the successful EDC/sulfo-NHS coupling and antibody conjugation because the functionalization broke the symmetry of SWCNT. The Raman spectra also featured the D peak (1302 cm^{-1}) and G peak (1569 cm^{-1}), and the ratio between the intensities of D and G peaks (I_D/I_G) reflected the defects on SWCNTs. D and G peak regions of Raman spectra of the SWCNTs during functionalization, wherein the RBM region was recorded using a 785 nm excitation laser, all spectra were normalized to the Si peak at 520 cm^{-1} . D and G peak regions were recorded using a 638 nm excitation laser, and all spectra were normalized to the G peak at 1569 cm^{-1} . The observed increase of I_D/I_G corresponded to the increase in defect degree caused by the antibody functionalization. The antibody decoration was also characterized by atomic force microscopy (AFM) (FIG. 1D). Based on the calculation of 20 AFM height profiles, the surface height increased by 4.05 nm after the decoration of Ab85B, indicating the successful conjugation of Ab85B to carbon nanotube sidewalls. X-ray photoelectron spectroscopy (XPS) has provided further evidence of Ab85B conjugation onto SWCNTs. After the immobilization of Ab85B onto SWCNTs, the O=C–N (399.4 eV) peak appeared in XPS spectra of N 1s. Before incorporating Ab85B onto SWCNT, the C–O (532.3 eV) peak in O 1s XPS spectra indicated the presence of defects on SWCNTs. After it, N=C–O at 533.3 eV, C–O at 532.3 eV, and COO⁻ at 531.4 eV can be identified in the Ab85B-SWCNT O 1s XPS spectra by deconvolution of a single broad peak, demonstrating the successful functionalization of

carbon nanotubes with antibodies. The C–N (285.5 eV) peak was also observed in the deconvoluted C 1s XPS spectra due to the introduction of Ab85B.

[0060] Schematic representation of embodiment of an FET sensor device 10 hereof is set forth in FIG. 1E, while an embodiment of a chemiresistor sensor device 10a hereof is illustrated in FIG. 1F. The illustrated sensor devices 10, 10a include a sensing medium material including one or more representative nanostructures. Such nanostructures include, for example, sc-SWCNTs 20, 20a. In a number of embodiments, the nanostructures are a network of sc-SWCNTs). Single-walled carbon nanotubes are classified based on their electrical properties. Nanotubes may, for example, be considered to be either semiconducting or metallic. The nanotube synthesis process typically yields a mix of both metallic and semiconducting nanotubes. Purification steps are required to enrich the samples to be either mostly metallic or mostly semiconducting. Either mixed or purified nanostructures may be used in the sensor systems hereof. However, purified semiconducting nanostructures may provide improved, lower levels of detection and a wider dynamic range in devices, systems, and methods hereof. As used herein, the term “semiconductor enriched” (in reference to nanostructures such as sc-SWCNTs) indicates that there is a semiconducting content of at least 66%. In a number of embodiments, the semiconducting content is at least 90%, at least 95%, at least 99%, or at least 99.9%. In general, a greater semiconducting content will result in a better output signal.

[0061] In single-walled carbon nanotubes, all carbon atoms are located on the surface where current flows, making a stable conduction channel that is extremely sensitive to a surrounding chemical environment. Nanotubes and other nanostructures, including single-walled nanotubes (SWNTs) such as SWCNT's, have the ability to change conductance in response to interaction with analytes. This characteristic is, for example, implemented in a number of embodiments of systems 10 and 10a (see FIGS. 1E and 1F).

[0062] Various nanostructures other than SWCNTs are suitable for use in the present invention. Such nanostructures include, but are not limited to, multi-walled carbon nanotubes, graphene nanosheets and their derivatives (for example, reduced graphene oxide and holey graphene), nanowires, nanofibers, nanorods, nanospheres, nanoribbons (for example, interconnected nanoribbons of holey reduced graphene oxide) or the like, or mixtures of such nanostructures. Moreover, in addition to carbon, those skilled in the art will appreciate that the nanostructures of the present invention can be formed of boron, boron nitride, and carbon boron

nitride, silicon, germanium, gallium nitride, zinc oxide, indium phosphide, molybdenum disulfide, silver, and/or other suitable materials. The formation and/or function of reduced graphene oxide and holey graphene compositions are, for example, discussed in U.S. Patent Nos. 8,920,764, 9,482,638, and 10,801,982, and U.S. Patent Application Publication No. 2021/0122638, the disclosures of which are incorporated herein by reference.

[0063] As illustrated in FIGS. 1E and 1F, the sensing medium or material, including semiconducting sc-SWCNTs or a network of sc-SWCNTs 20 (or other nanostructures), may, for example, be disposed upon a substrate 30 (for example, silicon dioxide or quartz) and contacted by two conductive (for example, metallic - such as Au and/or Ti) electrodes representing a source (S) (a conductive electrode or terminal) and a drain (D) (a conductive electrode or terminal). In the operation of an FET circuit such as illustrated in FIG. 1E, changes in electrical conductivity may, for example, be measured for an applied gate voltage. One may, for example, measure current flow between source (S) and drain (D) as a function of a swept/varied gate voltage range. Liquid gating was used in the studied sensors hereof.

[0064] As described above, a chemiresistor sensor device such as device 10a need not include an applied gate voltage. In chemiresistor 10a the sensing medium or material, including nanostructures 20a, bridges the gap between two conductive electrodes 40a and 40a' (for example, gold electrodes), which may be referred to a source and a drain. The sensing medium or material may alternatively be immobilized upon a set of interdigitated electrodes. The resistance/conductance between electrodes 40a and 40a' can be readily measured. The sensing medium or material has an inherent resistance/conductance that is changed by the presence of the analyte. In a chemiresistor, a source-drain bias voltage may, for example, be swept through a range of voltages, and drain current may be measured. As known in the art, sensor devices such as sensor devices 10 and 10a may be controlled, and responses thereof measured, using electronic circuitry 100 and 100a, respectively. As known in the art, electronic circuitry 100, 100a may include a processor system in communicative connection with a memory system in which one or more software algorithms executable by the processor system (for example, to achieve biosensor control and response measurement) are stored.

[0065] In a number of embodiments, antibodies 24, 24a are covalently attached to nanostructures 20, 20a or to a blocking layer formed thereon to provide detection of antigens. By immobilizing the antibodies on the nanostructures or a blocking layer, more robust sensors

may be achieved compared to various a number of other immobilization techniques. One may, for example, readily remove (for example, via simple rinsing or washing) unbound molecular species. The immobilization methodologies hereof may allow the achievement of increased sensitivities.

[0066] An important factor that contributes to the sensitivity of SWCNT-based FET biosensors is the Debye screening length. In principle, as the negative gate voltage is applied, there is a charge separation resulting in two electrical double layers (EDLs). Specifically, cations accumulate at the interface between the gate electrode and the electrolyte while anions accumulate at the interface between the SWCNTs and the electrolyte. The width of each EDL is referred as Debye screening length (λ_D), which can be calculated by equation (1):

$$\lambda_D = \sqrt{\frac{\epsilon_0 \epsilon_r k_B T}{2 N_A q^2 I}} \quad (1)$$

where ϵ_0 is the vacuum permittivity, ϵ_r is the relative permittivity of the medium, k_B is the Boltzmann constant, T is the temperature, N_A is the Avogadro number, q is the elementary charge, and I is the ionic strength. Both EDLs will screen the rest of the ions from them. For example, for biomolecules functionalized on SWCNTs, the charged species that are beyond the Debye length will be screened. Therefore, one strategy toward mitigating the Debye screening effect is to optimize the loading of Ab85B on the SWCNT surfaces so that the available binding sites can be maximized while keeping the height of the biorecognition layer within the Debye screening length. To study such optimization, activated bare-SWCNT devices were incubated with 0 (control), 0.5 μg , 2.5 μg , 5 μg , 7.5 μg and 10 μg of Ab85B (with a concentration of 500 $\mu\text{g}/\text{mL}$) for 12 h for surface functionalization to find to an optimized mass. After blocking, the devices were immersed in 10 μL Ag85B solution (100 $\mu\text{g}/\text{mL}$ in PBS) before FET measurements. The histogram of the relative response vs. mass of Ab85B used is illustrated in FIG. 2A. The response showed a rising trend when Ab85B was added from 0 to 5 μg . However, the response decreased from 534.1% to 27.88% when the mass of Ab85B was further added to 10 μg , indicating that the antibody-antigen interactions cannot be detected by SWCNT devices, which may be due to the formation of a new antibody configuration where the excess antibody proteins stayed nonspecifically on the device, leading to the biorecognition layer away from the Debye length. FET measurements were performed on SWCNT devices before and after the antibody functionalization. The calibration plot showed a decreasing trend

first (0-5 μg), which can be explained as the change of electronic properties of the metal-nanotube contact due to protein adsorption. Then, the response became relatively stable, proving the antibody loading saturation happened when 5 μg of Ab85B was introduced onto SWCNT devices. SWCNT devices were further characterized with different amounts of antibody by fluorescence microscopy. The fluorescence intensity of 10 μg -Ab85B SWCNT devices was higher than 5 μg -Ab85B SWCNT devices, indicating the existence of excess antibodies on the biosensor. Therefore, 5 μg Ab85B was chosen in the subsequent antibody functionalization studies hereof.

[0067] Liquid gating was used as it is a favorable technique for biosensing, generating higher transconductance than back gating, and reducing noise. The gating electrolytes of different concentrations have different ionic strength I , as shown in equation (2):

$$I = \frac{1}{2} \sum C_i Z_i^2 \quad (2)$$

where C_i is the concentration of the electrolyte and Z_i is the charge of ion. Gating electrolytes of different concentrations have different ionic strength and thus provide different Debye screening lengths. The representative gating media used in studies hereof were PBS solutions of varying concentrations, resulting in different ionic strengths and Debye screening length (i.e., 1 $\times$ PBS: 0.7 nm, 0.1 $\times$ PBS: 2.4 nm, 0.01 $\times$ PBS: 7.4 nm, and 0.001 $\times$ PBS: 20 nm), which may have an impact on the detection outcomes. Therefore, the effect of a series of PBS solutions (1 $\times$ to 0.001 $\times$ PBS) as the gating electrolyte for the detection of Ag85B by Ab85B-SWCNT FET devices was studied. The calibration plot showed that when using 0.01 $\times$ PBS as the gating liquid, the relative response was significantly larger than other gating electrolytes (FIG. 2B). Previous studies have concluded that the sensing responses optimized when the distance of the bound charges in receptor-ligand complexes to the SWCNT surface was within the Debye screening length. After the introduction of Ag85B, it would bind specifically to Ab85B. Therefore, one could assume the height of bound charged species as the average height of the antibody (4.05 nm), which is within the Debye screening length of 0.01 $\times$ PBS (7.4 nm) and 0.001 $\times$ PBS (20 nm). However, the relative response of 0.001 $\times$ PBS was not satisfactory in comparison to 0.01 $\times$ PBS, possibly (and without limitation to any mechanism) because the low ion concentration is not favorable to the protein binding. PBS concentration, ionic strength and Debye screening length for the various PBS concentrations are set forth in Table 1 below.

Table 1

PBS Concentration	Ionic Strength (mM)	Debye Screening length (nm)
1×PBS	162.7	0.7
0.1×PBS	16.27	2.4
0.01×PBS	1.627	7.4
0.001×PBS	0.1627	20

[0068] In a number of embodiments, the amount of antibody immobilized in the studied devices hereof may, for example, be in the range of approximately 0.5-10 μg . An optimized amount is approximately 5 μg as described above. In a number of embodiments, for the test conditions of the studies hereof, ionic strength can range from approximately 0.1× to 0.001× PBS (Debye screening length between 2.4 and 20 nm), with approximately 0.01× PBS being optimal. 1× PBS (phosphate buffered saline) is a ready-to-use, sterile, pH adjusted blend of phosphate buffer and saline solution of 10 mM NaH_2PO_4 , 137 mM NaCl, 2.7 mM KCl, and 1.8 mM KH_2PO_4 at pH 7.4. Composition examples. 1×: 10 mM NaH_2PO_4 , 137 mM NaCl, 2.7 mM KCl, 1.8 mM KH_2PO_4 (pH 7.4) 0.1×: 1 mM NaH_2PO_4 , 13.7 mM NaCl, 0.27 mM KCl, 0.18 mM KH_2PO_4 .

[0069] Such ranges and optimal values are applicable for the particular antibody studied, the attachment scheme thereof, and the blocking strategy used. One can reasonably anticipate that such values will vary for different antibodies (for example, goat antibodies instead of rabbit, antibody fragments, antibodies for different antigens, etc.), attachments schemes (for example, via gold nanoparticles rather than direct coupling), and blocking strategy (for example, crosslinking BSA, different proteins, polymers, surfactants etc.) -- that is, for a particular sensor configuration. Thus, ion concentration and amount/mass of a particular recognition entity (antibody/antibody fragments) immobilized may be optimized using theory and experimentation (based, for example, on achieving a determined lower limit of detection) for a give sensor/sensor device configuration. In general, for a liquid deposited over the sensor medium, the ionic strength/ion concentration of the liquid may be selected to provide a determined Debye screening length while being within a range in which binding of the antigen to the recognition element occurs. The ionic strength/ion concentration may be optimized on

the basis of response. Further the mass loading of recognition elements immobilized on the plurality of nanostructures may be determined so that an average height of immobilized recognition entities is within determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection. For the selected ionic strength, for example, the mass loading of the recognition entities immobilized on the plurality of nanostructures may be selected to be within predetermined range of a value at which relative response is a maximum. The present studies describe the design and optimization of a representative sensor device including Ab95b antibodies in the sensor medium thereof for binding with Ag85B proteins in the detection of TB. However, one skilled in the art appreciates that the devices, systems and methods hereof are applicable to any recognition entity/antigen pair to provide limits of detection lower than previously attainable.

[0070] After identifying improved or optimized factors for device fabrication as described above, the performance and sensing mechanism of the Ab85B-SWCNT FET devices for the detection of Ag85B proteins spiked in PBS as a calibration was investigated. FIG. 3A shows the FET transfer characteristics, that is, $I - V_g$ curves, of an Ab85B-SWCNT FET device after exposure to Ag85B solutions (1 fg/mL to 100 μ g/mL). With increasing antigen concentration, the characteristic curves shifted to more positive gate voltages, and both the threshold voltage and the absolute value of the curve's linear region slope showed an increasing trend. Without limitation to any mechanism, all these changes can be explained by the adsorption of negatively charged Ag85B ($pI = 5.5$) onto SWCNTs inducing positive charges that p-doping SWCNTs and the Schottky barrier effect. The calibration curve for the detection of Ag85B proteins spiked in PBS is depicted in FIG. 3B. The calibration curve was linearly fit ($y = 0.4066 \times \log(x) + 6.6724$) and a linear correlation coefficient of 0.9804 was obtained, showing a large dynamic range of this Ab85B-SWCNT device. The calibration sensitivity, defined as the slope of the calibration curve, is 0.4066. Furthermore, non-specific binding was investigated by examining the responses of bare-SWCNT FET devices to Ag85B spiked in PBS. The influence of solvent was also explored by incubating Ab85B-SWCNT FET devices with pure PBS 12 times. The extremely low relative responses of these two control experiments shown in FIG. 3B showed that relative responses of the Ab85B-SWCNT FET devices upon exposure to Ag85B solution came from the specific binding between Ab85B and Ag85B. To determine the LOD of Ab85B-SWCNT FET devices, measuring the signal of a blank device

was repeated 20 times. Based on a signal that exceeds 3-times the noise response, the log scale of the LOD was $\log(x) = -16.3$. Therefore, the LOD was $\text{antilog}(-16.3) = 5 \times 10^{-17}$ or 0.05 fg/mL, which is lower than the immune-polymerase chain reaction (I-PCR) method.

[0071] Representative Ab85B-SWCNT FET devices hereof were further studied to assess the detection of Ag85B proteins spiked in artificial sputum. The artificial sputum was prepared with 1 wt.% methyl cellulose. See Banik, S.; Mahony, J.; Selvaganapathy, P. R. Elution of Artificial Sputum from Swab by Rotating Magnetic Field-Induced Mechanical Impingement. *Appl. Sci.* **2017**, 7 (12), 1255. FIG. 4A shows the $I - V_g$ curves of the Ab85B-SWCNT FET device after exposure to Ag85B solutions in artificial sputum. Similar to the detection of Ag85B in PBS, a shift of the curves toward more positive gate voltage was observed, indicating the successful detection of Ag85B proteins in artificial sputum. The calibration curve for the detection of Ag85B proteins in artificial sputum was plotted (FIG. 4B) and further linearly fitted. Compared with the calibration plot of calibration samples, relatively larger error bars and smaller response change, calibration sensitivity (0.1197) and linear correlation coefficient (0.9683) were observed due to the effect of artificial sputum. Therefore, the performance of our Ab85B-SWCNT FET devices with the control experiment was further evaluated by exposing them to pure artificial sputum 12 times, and the calibration curve is plotted in FIG. 4B. The difference of the relative responses between the experimental and control groups was observed from 1 fg/mL, indicating that the devices hereof could detect Ag85B at relatively low concentrations in artificial sputum samples. Despite the difference in responses, the increasing response from pure artificial sputum could not be ignored. The possible reason for the increased response may be that the negatively charged methyl cellulose ($pI = 4.6$) brought additional hole carriers to the SWCNTs and thus the devices were p-doped due to electrostatic gating effect.

[0072] Ab85B-SWCNT FET devices hereof were further evaluated for detection of Ag85B spiked in human peripheral blood serum. A control experiment examining the interference from serum to the biosensor was also performed. The response trend for detecting Ag85B in serum with Ab85B-SWCNT FET devices deviated from the trend for detecting Ag85B in PBS and overlapped with the control group trend, indicative of a compromised sensing capability toward Ag85B in human serum. This can be attributed to the nonspecific binding of serum components, such as albumin and globulin, to the device surface, causing interfering responses

in the device, even though a blocking buffer with Tween 20 and PEG was applied. The enhancement of the blocking buffer is thus important for testing in the human serum.

[0073] To eliminate the influence from serum, serum, non-fat dry milk (NFDM), bovine serum albumin (BSA), and BSA & NFDM were introduced into the Tween 20-polyethylene glycol (PEG) blocking buffer. In general, a blocking buffer is a solution of blocking component such as a foreign protein, a combination of proteins, or another compound that absorbs all remaining (non-selective) binding surfaces. The blocking buffer binds to potential sites of nonspecific interaction and eliminates background noise without altering the epitope for antibody binding. The blocking agent improves sensitivity by reducing background interference and improving signal-to-noise ratio. Proteins, polymers, serum (for example, mouse serum) and surfactants may, for example, be used as non-specific blocking agent. To compare with the detection of Ag85B in PBS, Ag85B in serum was tested from 1 fg/mL to 100 µg/mL for each order of magnitude with Ab85B-SWCNT FET devices blocked by the three kinds of blocking buffer described above. The resulting calibration plot for detecting Ag85B proteins in serum demonstrated a negative relative response produced by serum blocked Ab85B-SWCNT FET devices (demonstrating the failure of Ag85B detection) which may be a result of excessive blocking by serum. The calibration plot for BSA-, NFDM-, and BSA&NFDM-blocked Ab85B-SWCNT FET devices exhibited the same increasing trend in the few concentration measurements at the beginning. However, then the calibration curves for all three kinds of devices showed a decreasing trend. This phenomenon could be attributed to the number of tests performed or other experimental factors. To obtain a calibration curve and explore the dynamic range, antigen solutions were typically tested from 1 fg/mL to 100 µg/mL, covering each order of magnitude from low to high. In this experiment, 12 tests were performed to detect 12 different concentrations of Ag85B solutions. However, due to the incubation and washing process, which may cause the dissociation of NFDM or BSA and the recovery of serum influence, a reduction in relative response after a certain number of tests was observed. To test this hypothesis, Ag85B detection was performed from 1 fg/mL while reducing the number of tests to 6, 4, and 3 by adjusting the concentrations used in the detection with BSA blocked-Ab85B-SWCNT FET devices. A control experiment testing the non-specific bindings between the devices and serum was also performed. During the six-test experiment, a decrease in relative response was observed starting from the fifth test (100 ng/mL). Additionally, a difference in relative responses between the experimental and the control group was observed

as early as 1 fg/mL. On the other hand, when four tests were performed, only an increasing trend of the relative response was observed. Based on this evidence, one can conclude that the decrease in relative response was not dependent on a specific concentration, but rather related to the number of tests performed. To mitigate this influence, the performance of devices hereof was tested in detecting Ag85B in serum by evaluating three different concentrations (1 fg/mL, 1 ng/mL, and 100 µg/mL) using the BSA-blocked-Ag85B-SWCNT FET devices. As shown in FIG. 4C, the $I - V_g$ curves of the device after exposure to Ag85B in serum shifted toward a more positive gate voltage, similar to the detection of Ag85B in PBS, demonstrating successful detection of Ag85B proteins in serum. FIG. 4D shows the calibration curve for the detection of Ag85B in serum along with the calibration curve depicting the effect of serum on devices hereof and the nonspecific binding between the antigen and electrode. The different trends in the experimental and control groups further demonstrated the feasibility of the BSA-blocked-Ag85B-SWCNT FET devices in detecting Ag85B in serum.

[0074] To further evaluate the specificity of BSA-blocked-Ag85B-SWCNT FET devices to Ag85B, an interference test was performed. The pure serum (10 µL) was incubated onto sensor chips for 10 min as the control group. Due to the Coronavirus disease 2019 (COVID-19) pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid protein (N antigen, NAg) and SARS-CoV-2 spike protein (S antigen, SAg) (10 µL, 10 ng/mL in serum) were chosen as related proteins for this specificity test. As illustrated in FIG. 4E, the relative responses from NAg and SAg were significantly lower than those from Ag85B, indicating the high specificity of the BSA-blocked-Ag85B-SWCNT devices hereof to Ag85B.

[0075] The BSA-blocked-Ag85B-SWCNT FET devices were further tested with TB clinical samples. One positive sample and one negative sample were tested separately with a portable TB detection device including SWCNT FET devices hereof and a Metrohm potentiostat (available from Metrohm USA, Inc. of Riverview, FL, USA). After the 10 min-sample incubation, the device can give the result in several seconds. FIG. 4F shows the relative response of the biosensor for the detection of TB positive and negative clinical samples. The histogram demonstrates that portable biosensors hereof can distinguish between the TB-positive and TB-negative samples successfully with a p -value of 7.0446×10^{-4} ($\alpha = 0.05$) and have significant potential in TB POC diagnosis.

[0076] The robustness of the devices to biofluids and the stability of the blocking layer may be improved by, for example, binding the blocking layer to the devices hereof such that the blocking layer is difficult to remove during washing. A mixture of nanostructures/BSA/glutaraldehyde (GA), for example, can form a stable and antifouling biosensor coating layer as a result of the formation of a thick and porous BSA matrix around the nanostructures/nanotubes. The matrix can significantly reduce the non-specific binding at the same time.

[0077] In a number of studied embodiments of devices hereof, BSA was bound directly onto the SWCNT and was crosslinked with GA. SEM imaging characterized the process of BSA binding and crosslinking. It has been demonstrated that streptavidin proteins could adsorb on CNTs through non-covalent hydrophobic interactions. Therefore, the SWCNT FET device was first blocked with a BSA cross-linking layer noncovalently for Ag85B detection. The results demonstrated that the variation in response trend could still be seen after a few tests, indicating the weak affinity between SWCNTs and BSA proteins. Thus, other studies were conducted in which the BSA cross-linking layer was bound to SWCNTs covalently through EDC/ Sulfo-NHS coupling. The surface functionalization process of the Ab85B functionalized-cross-linked BSA-blocked-SWCNT (Ab85B-cBSA-SWCNT) FET devices is shown in FIG. 5A. SEM imaging characterized the process of BSA binding and cross-linking and showed the BSA proteins binding to SWCNT devices. After the introduction of GA to BSA, a dense layer covering SWCNT and aggregated particles was observed on the device surface, which was further characterized by fluorescence images. The devices exhibited green and red fluorescence, confirming the existence of a BSA cross-linking layer and some aggregated BSA protein.

[0078] To evaluate the performance of our Ab85B-cBSA-SWCNT FET devices, Ag85B in serum form was tested 1 fg/mL to 100 μ g/mL for each order of magnitude. Although the addition of the BSA cross-linking layer between SWCNT and Ab85B increases the height of the biorecognition layer, it also helps mitigate the Debye screening effect. Due to the high cost of entropy for ion partition from the solution to the cross-linking layer, the concentration of ions is significantly smaller at the interface between the cross-linking layer and the solution. Therefore, the Debye screening length is increased and is higher than the biorecognition layer. Thus, 0.01 $\times$ PBS was still chosen as the gating liquid. FIG. 5B shows the $I - V_g$ curves of an Ab85B-cBSA-SWCNT FET device after exposure to Ag85B solutions. A shift of the curves

toward more positive gate voltage was observed, indicating the successful detection of Ag85B in serum. The corresponding calibration curve is plotted in FIG. 5C and presents a single increasing trend similar to the detection in PBS. Compared with BSA-blocked-Ab85B-SWCNT devices, the Ab85B-cBSA-SWCNT FET device can withstand 12 tests without the blocking layer being washed away, demonstrating the improved robustness of SWCNT-based biosensors and stronger evidence of the large dynamic range of SWCNT FET devices in detecting Ag85B in human serum. The effect of serum on the devices and nonspecific bindings between the cBSA-SWCNT device and Ag85B were also evaluated as control experiments and are plotted in FIG. 5C. The deviation in the relative responses between the experimental and control groups can be observed from 1 fg/mL, indicating the increasing response trend coming from the antigen-antibody interactions and good sensitivity of the Ab85B-cBSA-SWCNT FET device. Further improvement can be achieved via optimization of the ratio between SWCNT, GA, and BSA to maintain good robustness while minimizing the blocking layer thickness and maximizing the sensor response. Additionally, antibody fragments could be used instead of the whole antibody to minimize the distance between SWCNTs and the antigen binding site.

[0079] Although the sensitivity of devices hereof in detecting Ag85B are slightly less in the case of use of a crosslinked blocking layer, the sensitivity is still significantly better than, for example, ELISA. If greater sensitivity is desired (for example, in early diagnosis), a device hereof in which the antibody is covalently attached to the nanostructures and covered by a mixture of, for example, BSA, PEG and Tween 20, as described herein may be used. Such devices may, for example, be useful for two to four tests with intervening washing. If repeated uses greater than two to four tests are desired and slightly less sensitivity is suitable, a crosslinked blocking layer may be used.

[0080] As described above, one or more antibody fragments which include an active binding site for the antigen can be used in device hereof. The one or more antibody fragments may, for example, include reduced antibody fragments as described in U.S. Patent Application Serial No. 18/631,488. The one or more antibodies or one or more antibody fragments hereof may, for example, be covalently attached to at least a portion of the nanostructures of the devices hereof. Alternatively, one or more antibodies may be attached to at least a portion of a plurality of metal (for example, gold) nanoparticles immobilized upon the nanostructures (for example, single-walled carbon nanotubes). See, for example, U.S. Patent Application Serial No. 18/631,488.

[0081] In summary of the studies of representative devices, systems, and methods hereof, anti-mycobacterium tuberculosis antigen 85B antibody-functionalized SWCNT FET devices were fabricated for potential TB diagnostics. Through investigating the influence of the mass loading of antibody and gating electrolyte, the biosensor demonstrated high specificity and large dynamic range toward the detection of MTB-secreted Ag85B with an LOD of 0.05 fg/mL in calibration samples. The performance of the Ab85B-SWCNT FET devices hereof were also studied by detecting Ag85B spiked in complex matrices. The difference in relative responses between the experimental and control groups indicates that the devices hereof have the ability to identify Ag85B in artificial sputum. The addition of a blocking protein (BSA) into a Tween20-PEG blocking buffer demonstrated that the Ab85B-SWCNT FET devices hereof can detect Ag85B in serum and distinguish TB positive clinical samples. If required for a particular use, the robustness (for example, to repeated use/intervening washing) of devices hereof in TB diagnosis in biofluids can be improved by crosslinking the BSA layer. In a number of studies, a GA-crosslinked BSA layer was attached directly to the SWCNT device. Furthermore, the fabrication of each sensor is economical, and the test time is around 10 min, which indicates the possibility of developing a complete integrated point-of-care (POC) device with the antibody-functionalized SWCNT FET for the diagnosis of TB.

[0082] Tuberculosis (TB), a common infectious disease caused by mycobacterium tuberculosis (MTB), is a major cause of death worldwide. However, current TB diagnostic strategies focusing on the detection of bacilli by microscopy methods, bacterial growth cultures, and host immune response to the pathogen present less accuracy. While immunological assays are specific and sensitive, they are expensive, time-consuming, and operator-dependent, making them inappropriate for use in resource-poor settings and limiting their widespread use. Therefore, the need for rapid, affordable, and reliable TB diagnostic methods has emerged. Representative devices hereof provide a quick, economical, sensitive and specific diagnostic test for TB based on rapid detection of MTB 30 kDa secretory antigen protein (Ag85B), which is the most abundant protein expressed by MTB serving as a feasible target for developing innovative approaches for TB diagnosis. A representative field-effect transistor (FET) nanobiosensor by employing semiconducting single-walled carbon nanotube (SWCNT)-functionalized with antibody-antigen 85B (Ab85B) was developed and Ag85B detection was demonstrated in PBS, artificial sputum, and human blood serum.

[0083] The devices, system, and methods hereof may provide, for example, a point-of-care device for the fast, inexpensive but sensitive and reliable detection of MTB Ag85B in different biological fluids. The FET-based biosensing devices hereof exhibit the advantages of high sensitivity, small size, label-free, and real-time detection. In a number of embodiments, the use of semiconducting SWCNT in FET devices hereof yields high on-state conductance and high on/off ratio for FETs, which in turn achieve higher analytical sensitivity toward the target analytes compared to other FET devices. Based on results of a number of studies thereof, the biosensor devices hereof exhibit a limit of detection (LOD) of 0.05 fg/mL in PBS and have the ability to identify the Ag85B in sputum and serum in 10 min.

[0084] A comparison between, for example, a SWCNT FET biosensor device hereof and previously reported sensor technologies for TB diagnosis indicates that the SWCNT-based biosensors hereof are more sensitive than most existing TB diagnostic methods. Moreover, biosensor devices, systems, and methods hereof can be used with both artificial sputum and blood serum and can identify the existence of Ag85B at extremely low concentrations. Blocking layer-enhanced SWCNT FET devices hereof can also recognize Ag85B in blood serum above 1 fg/mL. The large dynamic range is maintained even when exposed to biofluids. Additionally, the total test cost, including the sensor chip fabrication and antibody functionalization, is less expensive than present commercially available TB tests.

[0085] Experimental

[0086] Device Fabrication. By using photolithography, the interdigitated gold electrodes were patterned onto the 2.6×2.6 mm Si/SiO₂ wafers to form 6 μ m channels. The chips were wire-bonded onto the standard 40-pin ceramic dual inline package and secured with polydimethylsiloxane (PDMS) by heating at 200 °C for 20 min. For the anti-MTB Ag85B antibody-functionalized SWCNT (Ab85B-SWCNT) FET devices, commercial semiconducting single-walled carbon nanotubes (SWCNTs) (IsoSol-S100, NanoIntegris) were used for the fabrication of sensors. 2 μ L IsoSol-SWCNT (0.02 mg/mL in toluene) were deposited between gold electrodes by dielectrophoresis (DEP) with an applied bias voltage of 10 V and AC frequency of 100 kHz for 2 min. Isopropyl alcohol was used to wash excess IsoSol-SWCNT and the devices were annealed at 200 °C for 12 h. SWCNT-devices were further sent to FET measurement for screening before use. Specially, devices with an off-current (at approximately +0.6 V) between approximately -1 and 1 μ A and an on-current (at

approximately -0.6 V) larger than 10 μ A will be used for further sensing experiments. To functionalize anti- mycobacterium tuberculosis Ag85B antibody (Ab85B) onto the device, 1-ethyl-3-(3- dimethylaminopropyl)carbodiimide (EDC)/N-hydroxysulfosuccinimide (sulfo-NHS) coupling was used. Specifically, the devices were incubated with 100 μ L of EDC/sulfo-NHS solution [50 mM/50 mM in phosphate-buffered saline (PBS)] for 30 min to activate the carboxyl groups on IsoSol- SWCNT into an amine reactive O-acylisourea intermediate. The excess EDC/sulfo-NHS solution was washed away with nanopure water. Then the devices were incubated with 10 μ L Ab85B (500 μ g/mL in PBS) (ab43019; Abcam, USA) for 12 h at 4 $^{\circ}$ C. After a thorough washing with nanopure water, the devices were covered by a blocking buffer (0.1% TWEEN[®] 20 (poly(oxyethylene)_n-sorbitan-monolaurate, a non-ionic surfactant) and 4% polyethylene glycol in PBS) for 30 min to prevent unspecific interactions. The devices were washed with nanopure water again to remove any excess chemicals before the antigen 85B (Ag85B) incubation and FET measurements.

[0087] For the Ab85B functionalized-crosslinked BSA blocked-SWCNT (Ab85B-cBSA-SWCNT) FET devices, the deposition of SWCNT was the same as stated above. To link BSA onto the SWCNT, the devices were incubated with 100 μ L of EDC/sulfo-NHS solution (50 mM/50 mM in PBS) for 30 min to activate the carboxyl groups on IsoSol-SWCNT. The excess EDC/sulfo-NHS solution was washed away with nanopure water. Then the devices were covered by a Tween20/PEG/BSA buffer (0.1% Tween 20, 4% polyethylene glycol and 1% BSA in PBS) for 1 hour. The excess buffer was washed away with nanopure water. Then the devices were incubated with 1% glutaraldehyde (GA) solution (in PBS) for 2 hours for crosslinking. The excess GA solution was washed away with nanopure water. After that, the devices were incubated with 100 μ L of EDC/sulfo-NHS solution (50 mM/50 mM in PBS) for 30 min to activate the carboxyl groups on BSA crosslinking layer. The excess EDC/sulfo- NHS solution was washed away with nanopure water. Then the devices were incubated with 10 μ L Ab85B (500 μ g/mL in PBS) for 12 h at 4 $^{\circ}$ C for the antibody functionalization.

[0088] FET Measurements. FET devices for the detection of Ag85B were investigated through liquid-gated FET device configuration by Keithley Source Meter Unit 2400. A 1 M Ag/AgCl electrode (CH Instruments, Inc.) was used as the gate electrode and PBS was used as the gating electrolyte. In FET measurements, the gate voltage (V_g) was swept from +0.6 to -0.6 V with a source-drain voltage (V_{sd}) of 50 mV.

[0089] A series of recombinant mycobacterium tuberculosis Ag85B protein (ab83471; Abcam, USA) solutions from 1 fg/mL to 100 µg/mL were prepared with PBS. For each experiment, Ag85B solutions were tested from the lowest to the highest concentration. Specifically, the devices were incubated with 10 µL of the Ag85B solution for 10 min, which was then washed away with nanopure water thoroughly. Then the FET measurements were done with PBS solution as gating electrolyte.

[0090] The relative response of each FET device was calculated as $\Delta I/I_0$ at $V_g = -0.3$ V, where $\Delta I = I_d - I_0$. I_d is the drain current in PBS after antigen exposure and I_0 is the drain current in PBS before antigen exposure at $V_g = -0.3$ V. The figures were plotted with averaged relative responses of several devices with standard error of the mean as error bars. The number of devices (n) tested for each experiment was specified in the caption and figure.

[0091] For control experiments studying the effect of solvent, the FET devices were incubated with 10 µL of the solvent for 10 min, which was then washed away with nanopure water thoroughly. Then the FET measurements were done with PBS solution as gating electrolyte. This step was repeated 12 times since we studied antigen solutions with 12 orders of magnitude. The relative response of each FET device is set forth above.

[0092] Scanning Electron Microscopy Imaging. Scanning electron microscopy (SEM) was performed with Zeiss Sigma VP Scanning Electron Microscope.

[0093] Atomic Force Microscopy. Atomic force microscopy (AFM) data were collected by a Bruker Multimode 8 AFM system with a Veeco Nanoscope III a controller in the tapping mode. The AFM image and height profiles were processed and obtained by Gwyddion.

[0094] X-ray Photoelectron Spectroscopy. X-ray photoelectron spectroscopy (XPS) data were generated on a Thermo ESCALAB 250 Xi XPS using monochromated Al K α X-rays as the source. A 650 µm spot size was used, and the samples were charge-compensated using an electron flood gun.

[0095] Raman Spectroscopy. Raman spectrum was recorded with the XplorA Raman-AFM/TERS system. The radial breathing mode (RBM) region was recorded using a 785 nm (100 mW) excitation laser operating at 1% power. D and G peaks region was recorded using a 638 nm (24 mW) excitation laser operating at 1% power.

[0096] Fluorescence Imaging. Fluorescence images were taken with an Olympus 1X81/1X2-UCB microscope. Fluorescence image of Ab85B-cBSA-SWCNT devices taken under excitation of 532 nm and 633 nm respectively.

[0097] Preparation of Artificial Sputum and Detection of Ag85B in Artificial Sputum. Artificial Sputum was prepared from methyl cellulose (Sigma-Aldrich, USA; Viscosity: 400 cP). Specifically, 20 mL water was preheated to 80 °C. The methyl cellulose (1 wt.%) was put into the hot water and the mixture was stirred until the particles were well dispersed. Then 40 mL water was poured into the beaker and the mixture was stirred for another half an hour to get transparent artificial sputum. The sputum could be stored at 4°C for 1 month. The artificial sputum was incubated onto Ab85B-SWCNT FET devices 12 times to examine the effect from artificial sputum.

[0098] A series of Ag85B protein solutions from 1 fg/mL to 100 µg/mL were prepared with artificial sputum for antigen detection. Ag85B solutions were tested from the lowest to the highest concentration and the FET measurement operation was the same as stated above.

[0099] Detection of Ag85B in Serum. Human serum (Fisher Scientific, USA, Cat# BP2657100) was used for this work. To reduce the influence from serum, four kinds of blocking buffer were prepared to block the non-specific sites on the Ab85B-SWCNT devices respectively. Specifically, the serum blocking buffer was prepared with 0.1% Tween 20, 4% polyethylene glycol and 5% serum in PBS; the non-fat dry milk blocking buffer was prepared with 0.1% Tween 20, 4% polyethylene glycol and 5% NFDM in PBS; the BSA blocking buffer was prepared with 0.1% Tween 20, 4% polyethylene glycol and 1% BSA in PBS and the BSA&NFDM blocking buffer was prepared with 0.1% Tween 20, 4% polyethylene glycol, 5% NFDM and 1% BSA in PBS. The devices were blocked by a buffer for 60 min. A series of Ag85B protein solutions from 1 fg/mL to 100 µg/mL were prepared in blood serum for antigen detection. To prove the number of tests affecting the sensing result, detections for Ag85B in serum with different number of tests were performed. When testing twelve times, a series of Ag85B protein solutions in serum from 1 fg/mL to 100 µg/mL were tested by each order of magnitude; when testing six times, a series of Ag85B protein solutions in serum (1 fg/mL, 100 fg/mL, 10 pg/mL, 1 ng/mL, 100 ng/mL and 10 µg/mL) were tested; when testing four times, a series of Ag85B protein solutions in serum (1 fg/mL, 1 pg/mL, 1 ng/mL and 1 µg/mL) were tested; when testing three times, a series of Ag85B protein solutions in serum (1 fg/mL, 1

ng/mL and 1 μ g/mL) were tested. Each test was performed from the low to high concentration of Ag85B solution. For non-specific interactions test, the serum was incubated onto the BSA blocked Ab85B-SWCNT FET devices 12 times.

[00100] When using Ab85B-cBSA-SWCNT FET devices for detecting Ag85B in serum, a series of Ag85B protein solutions from 1 fg/mL to 100 μ g/mL were prepared in blood serum for antigen detection. The FET measurement process was the same as stated above.

[00101] Detection of TB Clinical Samples. Remnant samples from QuantiFeron test, which were mainly plasma, were used for this clinical detection. Clinical samples were kept frozen at -30°C before test. 10 μ L sample was incubated onto the BSA-blocked Ab85B-SWCNT devices for 10 min. Then the sample was washed away thoroughly. The FET measurement operation was the same as stated above.

[00102] The foregoing description and accompanying drawings set forth a number of representative embodiments at the present time. Various modifications, additions and alternative designs will, of course, become apparent to those skilled in the art in light of the foregoing teachings without departing from the scope hereof, which is indicated by the following claims rather than by the foregoing description. All changes and variations that fall within the meaning and range of equivalency of the claims are to be embraced within their scope.

WHAT IS CLAIMED IS:

1. A sensor device for diagnosis of tuberculosis, comprising:

a sensor comprising a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures and at least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site for Ag85 antigen,

a liquid deposited over the sensor medium, wherein an ionic strength of the liquid is selected at which binding of the Ag85 antigen to the one or more recognition entities occurs, and the ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized one or more recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection, and

electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the Ag85 antigen.
2. The sensor device of claim 1 wherein the antibodies are selected from the group consisting of a human or nonhuman Ag85A, Ag85B, Ag85C, and Ag85G antibody or the antibody fragments are selected from the group consisting of fragments of human or nonhuman Ag85A, Ag85B, Ag85C, and Ag85G antibody.
3. The sensor device of claim 1 wherein the antibodies are nonhuman Ag85B antibodies or the antibody fragments are fragments of nonhuman Ag85B antibodies.
4. The sensor device of claim 2 wherein the sensor device is a field-effect transistor device and the liquid functions as a gating liquid.
5. The sensor device of claim 4 wherein a blocking buffer which is interactive with non-selective binding sites is applied to the sensor before the liquid is deposited thereon.

6. The sensor device of claim 5 wherein the blocking buffer comprises a protein.
7. The sensor device of claim 6 wherein the protein is bovine serum albumin.
8. The sensor device of claim 6 wherein the protein is covalently attached to the nanostructures.
9. The sensor device of claim 8 wherein the protein is crosslinked via a crosslinking agent.
10. The sensor device of claim 9 wherein the protein is bovine serum albumin and the crosslinking agent is glutaraldehyde.
11. The sensor device of claim 1 wherein the average height of immobilized recognition entities is within 0.2 to 2.0 times of the maximum mass loading at which the average height remains within the Debye screening length of the liquid.
12. The sensor device of claim 1 wherein the average height of immobilized recognition entities is within 0.5 to 1.5 times of the maximum mass loading at which the average height remains within the Debye screening length of the liquid.
13. The sensor device of claim 1 wherein the average height of immobilized recognition entities is within 0.75 to 1.25 times of the maximum mass loading at which the average height remains within the Debye screening length of the liquid.
14. The sensor device of claim 1 wherein the maximum mass loading at the selected ionic strength is determined by experimentally determining a maximum response for a determined Ag85 antigen concentration over varying mass loading.
15. The sensor device of claim 12 wherein the maximum mass loading at the selected ionic strength is determined by experimentally determining a maximum response for a determined Ag85 antigen concentration over varying mass loading.
16. The sensor device of claim 1 wherein the selected ionic strength of the liquid is determined experimentally by determining response to the Ag85 antigen at each of a plurality of ionic strengths.

17. The sensor device of any one of claims 1 through 16 wherein the nanostructures are carbon nanotubes.

18. The sensor device of claim 17 wherein the carbon nanotubes are single walled carbon nanotubes.

19. The sensor device of claim 18 wherein the single-walled carbon nanotubes are semiconductor enriched single-walled carbon nanotubes.

20. The sensor device of claim 19 wherein a semiconducting content of the semiconductor enriched single-walled carbon nanotubes is at least 90%, at least 95%, at least 99%, or at least 99.9%.

21. The sensor device of claim 1 wherein the ionic strength of the liquid is experimentally optimized based on the limit of detection for a given sensor configuration.

22. The sensor device of claim 1 wherein the mass loading of the recognition entities is experimentally optimized based upon the limit of detection for a given sensor configuration.

23. The sensor device of claim 1 wherein the one or more recognition entities are immobilized upon metal nanoparticles immobilized upon the plurality of nanostructures.

24. The sensor device of claim 23 wherein the metal nanoparticles comprise gold nanoparticles.

25. The sensor device of claim 1 wherein the plurality of nanostructures comprise a plurality of semiconductor enriched single-walled carbon nanotubes, and a plurality of rabbit polyclonal Ag85B antibodies or a plurality of rabbit polyclonal Ag85B antibody fragments are covalently attached to the plurality of semiconductor enriched single-walled carbon nanotubes.

26. The sensor device of claim 25 wherein the liquid comprises phosphate buffered saline in a concentration range of approximately 0.1x to 0.001x, a blocking buffer is applied to the sensor before the liquid is deposited thereon, the blocking buffer comprising bovine serum albumin, a non-ionic surfactant and polyethylene glycol, and the mass loading of rabbit polyclonal Ag85B antibodies is in the range of approximately 0.5-10 μg for sensor device

having an I - V_g curve off-current, at approximately +0.6 V, between approximately -1 and 1 μ A and an on-current, at approximately -0.6 V, larger than 10 μ A.

27. The sensor device of claim 26 wherein the concentration of the phosphate buffered saline is approximately 0.01x and the mass loading is approximately 5 μ g.

28. A method of detecting tuberculosis, comprising:

applying a body fluid sample to a sensor device comprising

a sensor comprising a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures, at least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site for Ag85 antigen,

a liquid deposited over the sensor medium, wherein an ionic strength of the liquid is selected at which binding of the Ag85 antigen to the one or more recognition entities occurs, and the ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized one or more recognition entities is within a determined of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection, and

electronic circuitry including at least one measurement system in operative connection with the sensor; and

measuring a variable which provides a measure of change in at least one property of the sensor medium which is dependent upon the presence of the Ag85 antigen.

29. The method of claim 28 wherein the body fluid sample is sputum or a blood fluid.

30. The method of claim 28 wherein the body fluid sample is a blood fluid.

31. A sensor device for detecting an antigen, comprising:

a sensor comprising a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures, at least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site for the antigen,

a liquid deposited over the sensor medium, wherein an ionic strength of the liquid is selected at which binding of the antigen to the one or more recognition entities occurs, and the ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection, and

electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the antigen.

32. The sensor device of claim 31 wherein the sensor device is a field-effect transistor device and the liquid functions as a gating liquid.

33. The sensor device of claim 32 wherein a blocking buffer which is interactive with non-selective binding sites is applied to the sensor before the liquid is deposited thereon.

34. The sensor device of claim 33 wherein the blocking buffer comprises a protein.

35. The sensor device of claim 34 wherein the protein is bovine serum albumin.

36. The sensor device of claim 34 wherein the protein is covalently attached to the nanostructures.

37. The sensor device of claim 36 wherein the protein is crosslinked via a crosslinking agent.

38. The sensor device of claim 37 wherein the protein is bovine serum albumin and the crosslinking agent is glutaraldehyde.

39. The sensor device of claim 31 wherein the average height of immobilized recognition entities is within 0.2 to 2.0 times of the maximum mass loading at which the average height remains within the Debye screening length of the liquid.

40. The sensor device of claim 31 wherein the average height of immobilized recognition entities is within 0.5 to 1.5 times of the maximum mass loading at which the average height remains within the Debye screening length of the liquid

41. The sensor device of claim 31 wherein the average height of immobilized recognition entities is within 0.75 to 1.25 times of the maximum mass loading at which the average height remains within the Debye screening length of the liquid.

42. The sensor device of claim 31 wherein the maximum mass loading at the selected ionic strength is determined by experimentally determining a maximum response for a determined antigen concentration over varying mass loading.

43. The sensor device of claim 39 wherein the maximum mass loading at the selected ionic strength is determined by experimentally determining a maximum response for a determined antigen concentration over varying mass loading.

44. The sensor device of claim 31 wherein the selected ionic strength of the liquid is determined experimentally by determining response to the antigen at each of a plurality of ionic strengths.

45. The sensor device of any one of claims 31 through 44 wherein the nanostructures are carbon nanotubes.

46. The sensor device of claim 45 wherein the carbon nanotubes are single-walled carbon nanotubes.

47. The sensor device of claim 46 wherein the single-walled carbon nanotubes are semiconductor enriched single-walled carbon nanotubes.

48. The sensor device of claim 47 wherein a semiconducting content of the semiconductor enriched single-walled carbon nanotubes is at least 90%, at least 95%, at least 99%, or at least 99.9%.

49. The sensor device of claim 31 wherein the ionic strength of the liquid is experimentally optimized based on the limit of detection for a given sensor configuration.

50. The sensor device of claim 31 wherein the mass loading of the recognition entities is experimentally optimized based upon the limit of detection for a given sensor configuration.

51. The sensor device of claim 31 wherein the one or more recognition entities are immobilized upon metal nanoparticles immobilized upon the plurality of nanostructures.

52. The sensor device of claim 51 wherein the metal nanoparticles comprise gold nanoparticles.

53. The sensor device of claim 31 wherein the plurality of nanostructures comprise a plurality of semiconductor enriched single-walled carbon nanotubes, and a plurality of the antibodies or antibody fragments are covalently attached to the plurality of semiconductor enriched single-walled carbon nanotubes.

54. A method of fabricating a sensor device for detecting an antigen, the sensor device including a sensor including a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures, at least one or one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site for the antigen, a liquid deposited over the sensor medium, and electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the antigen, the method comprising: determining an ionic strength of the liquid at which binding of the antigen to the one or more recognition entities occurs, and determining a mass loading of the one or more recognition entities immobilized on the plurality of nanostructures so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection.

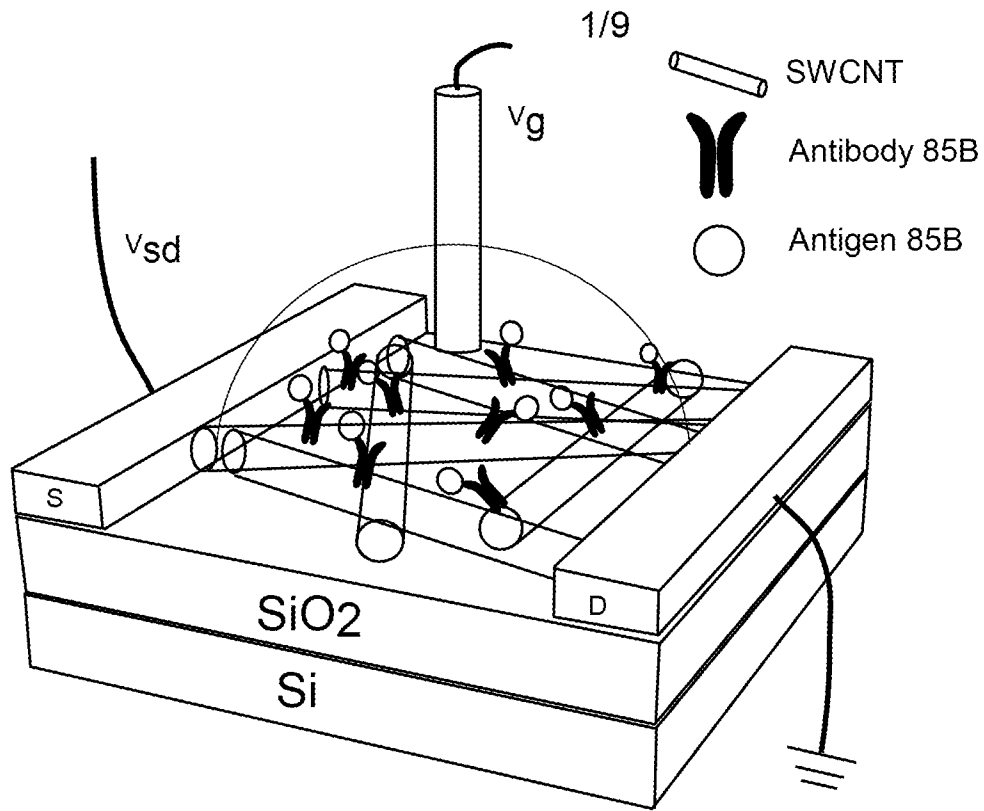


FIG. 1A

FIG. 1B

FIG. 1C

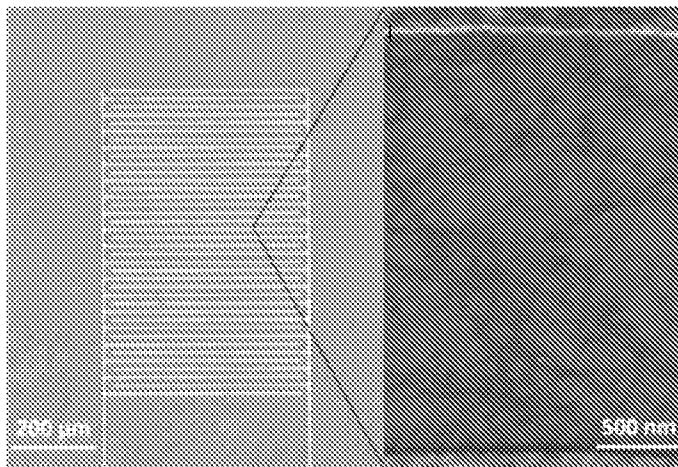
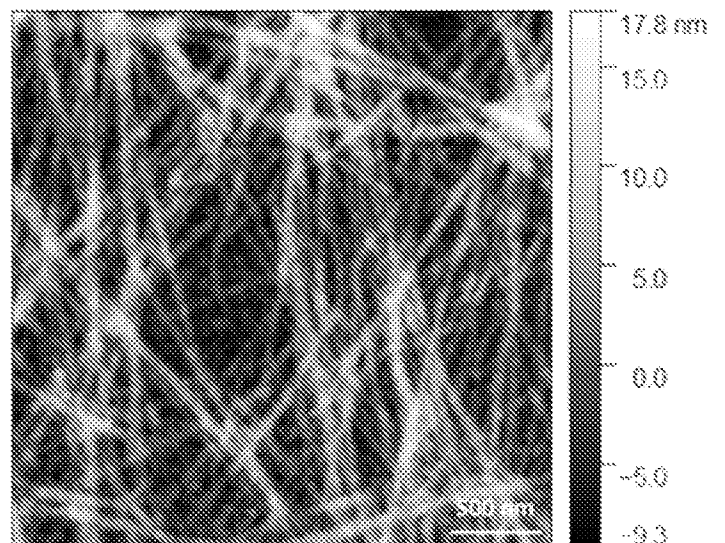


FIG. 1D



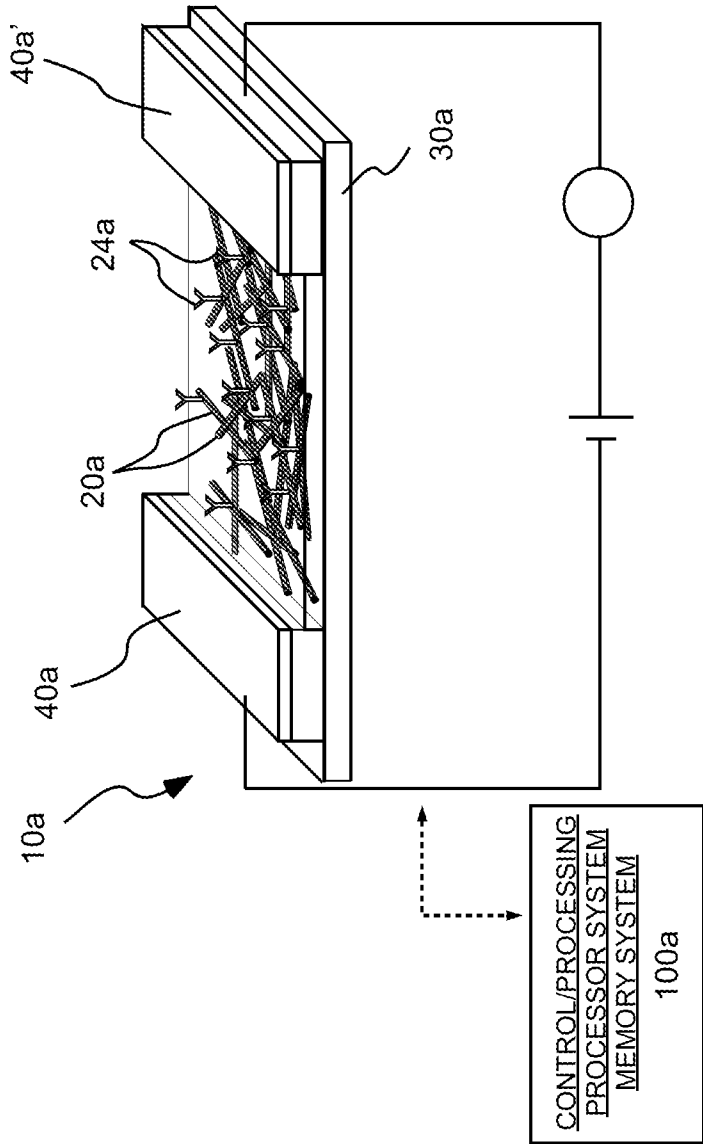


FIG. 1F

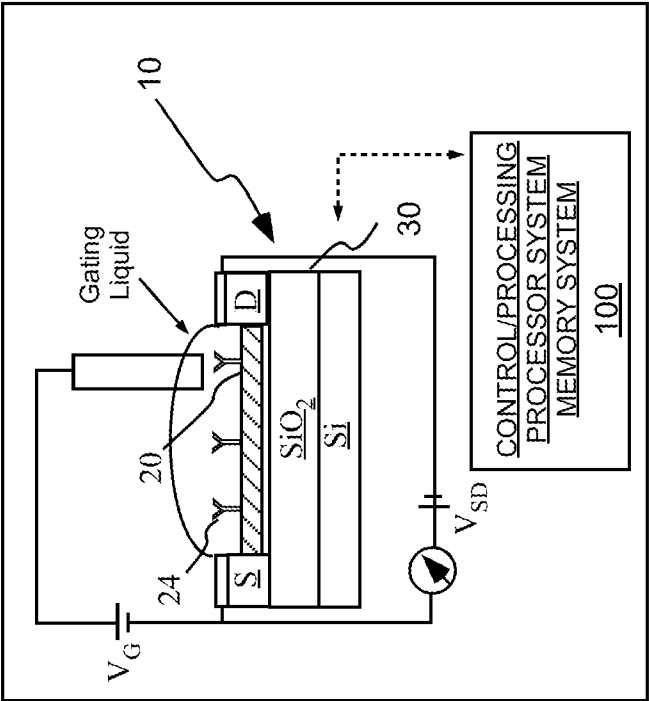


FIG. 1E

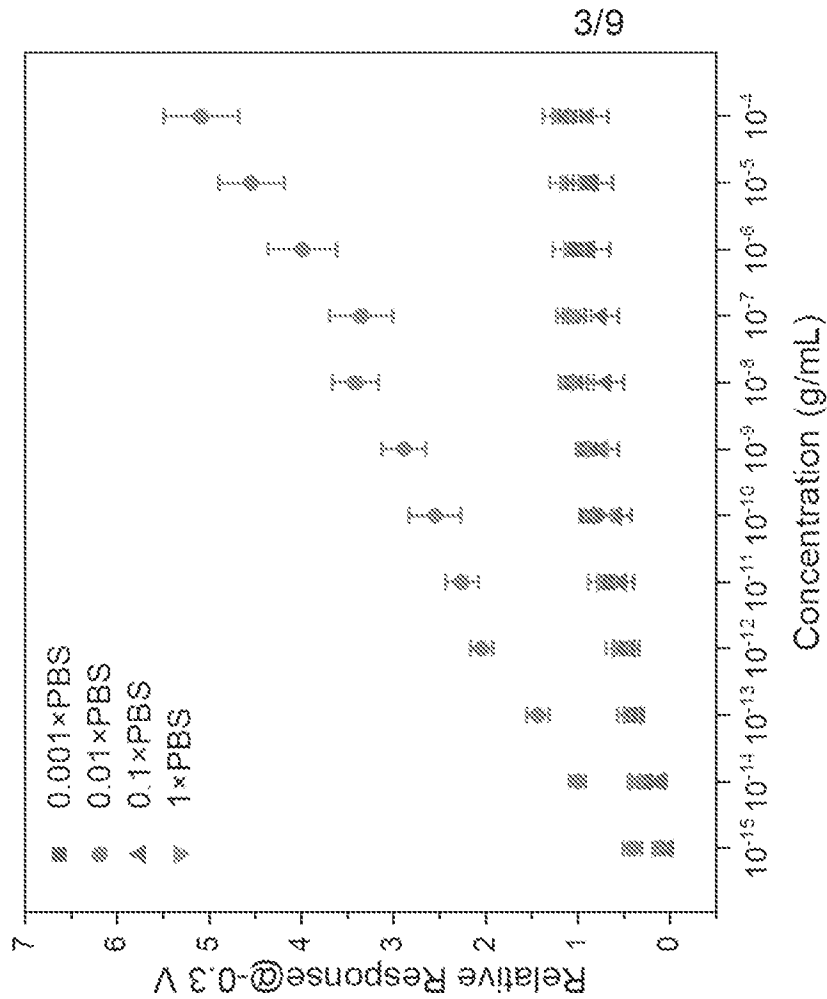


FIG. 2B

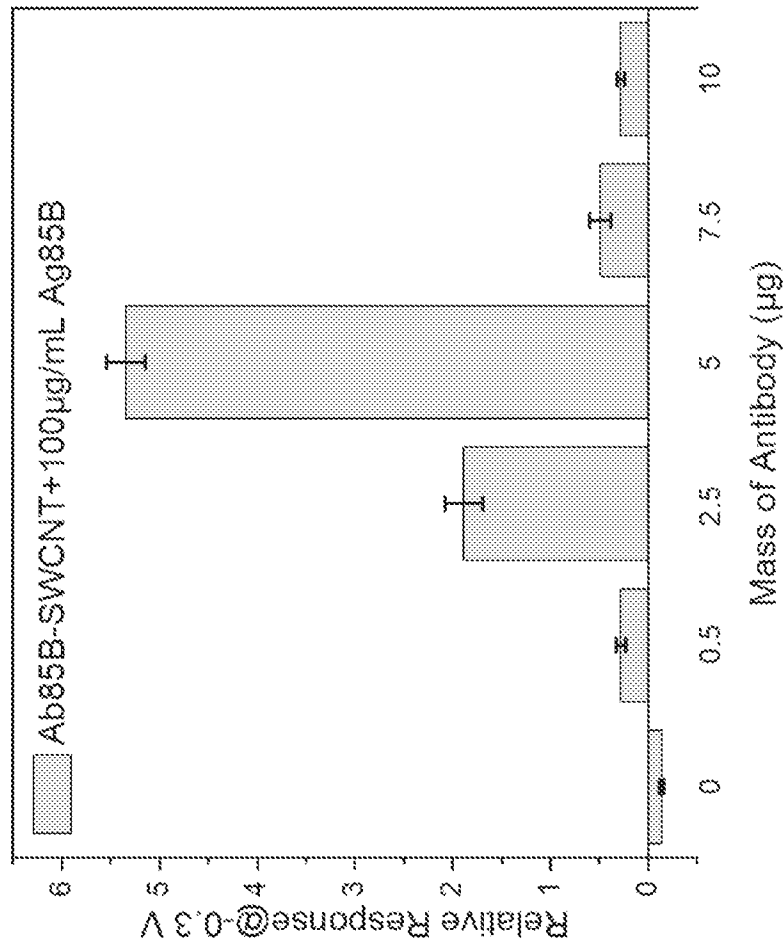


FIG. 2A

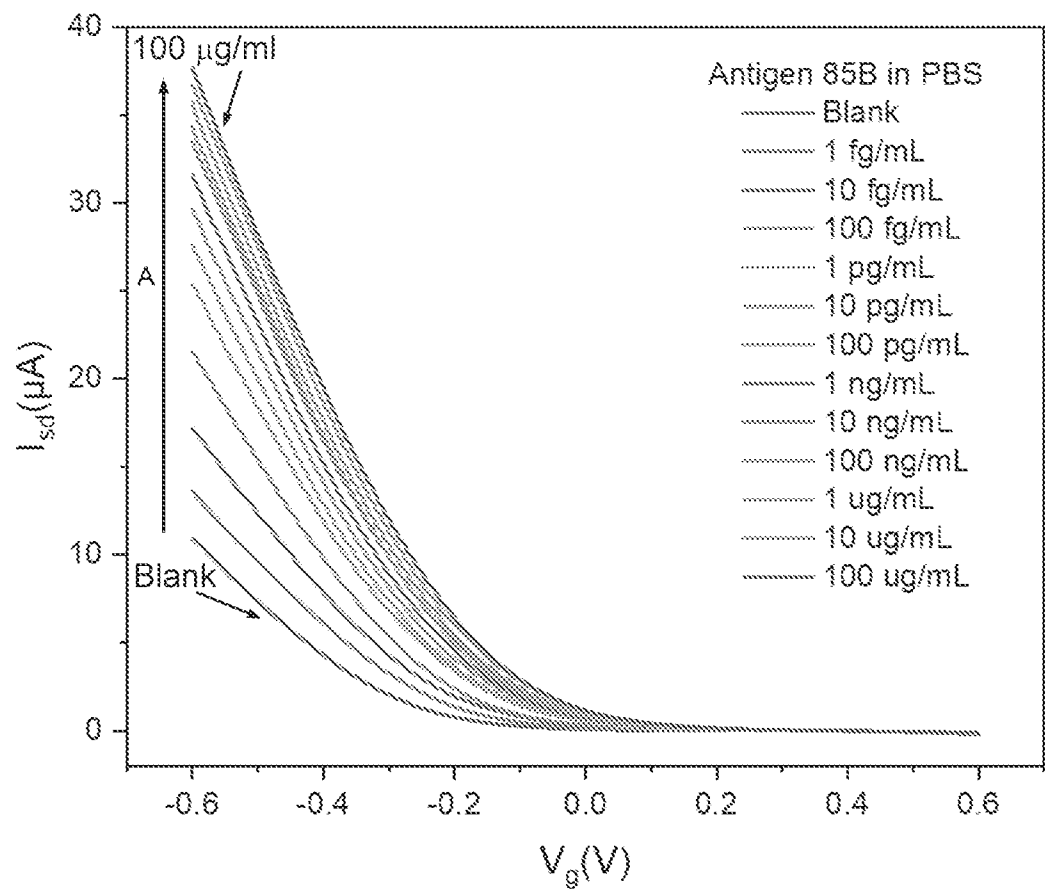


FIG. 3A

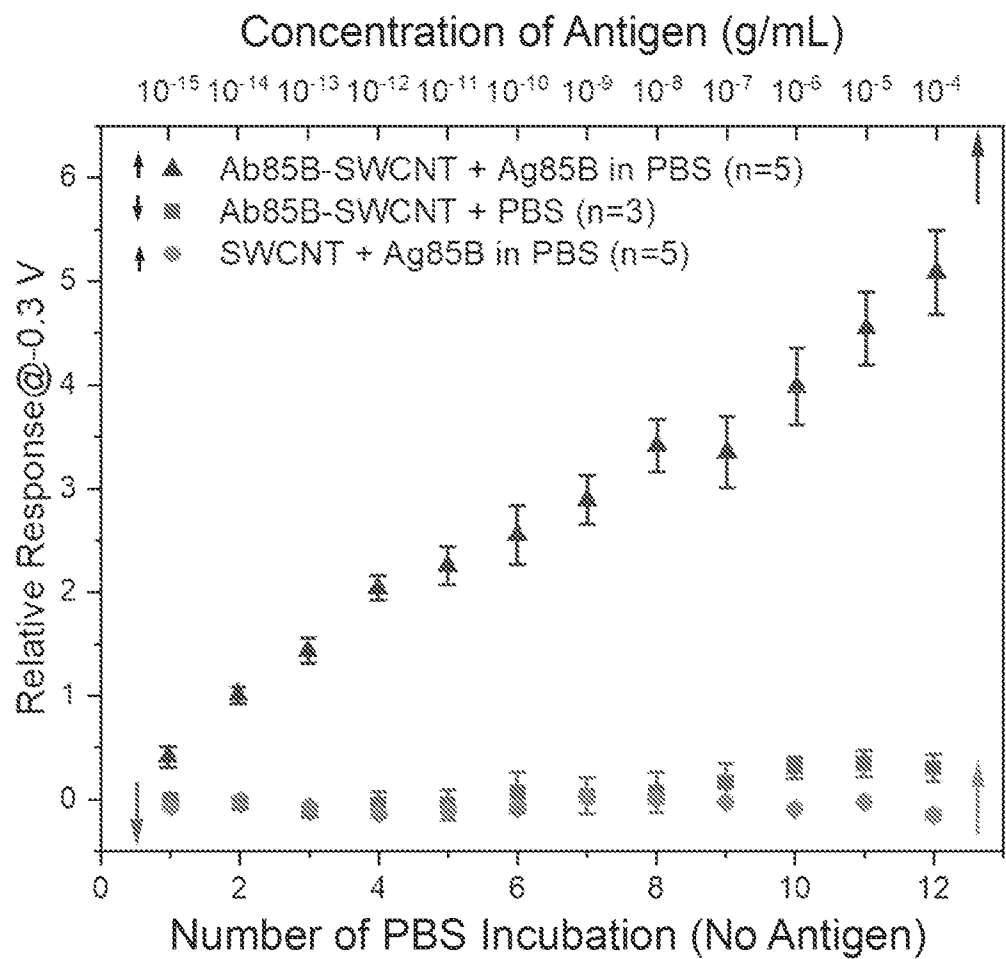


FIG. 3B

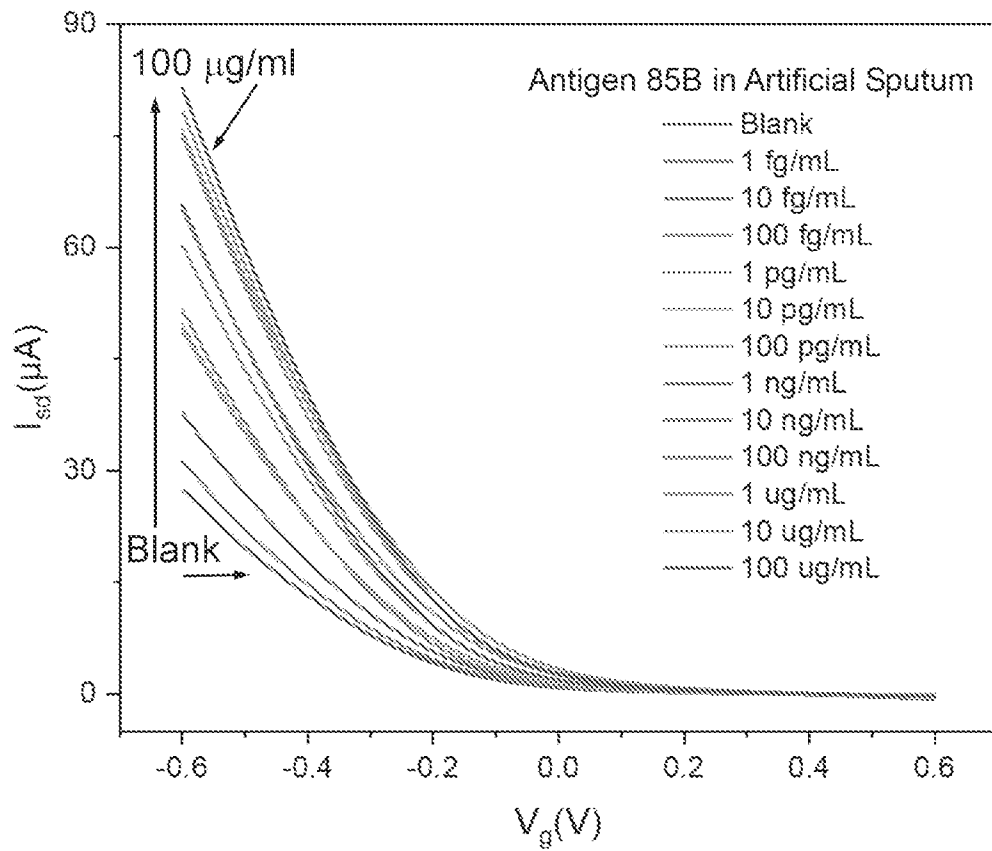


FIG. 4A

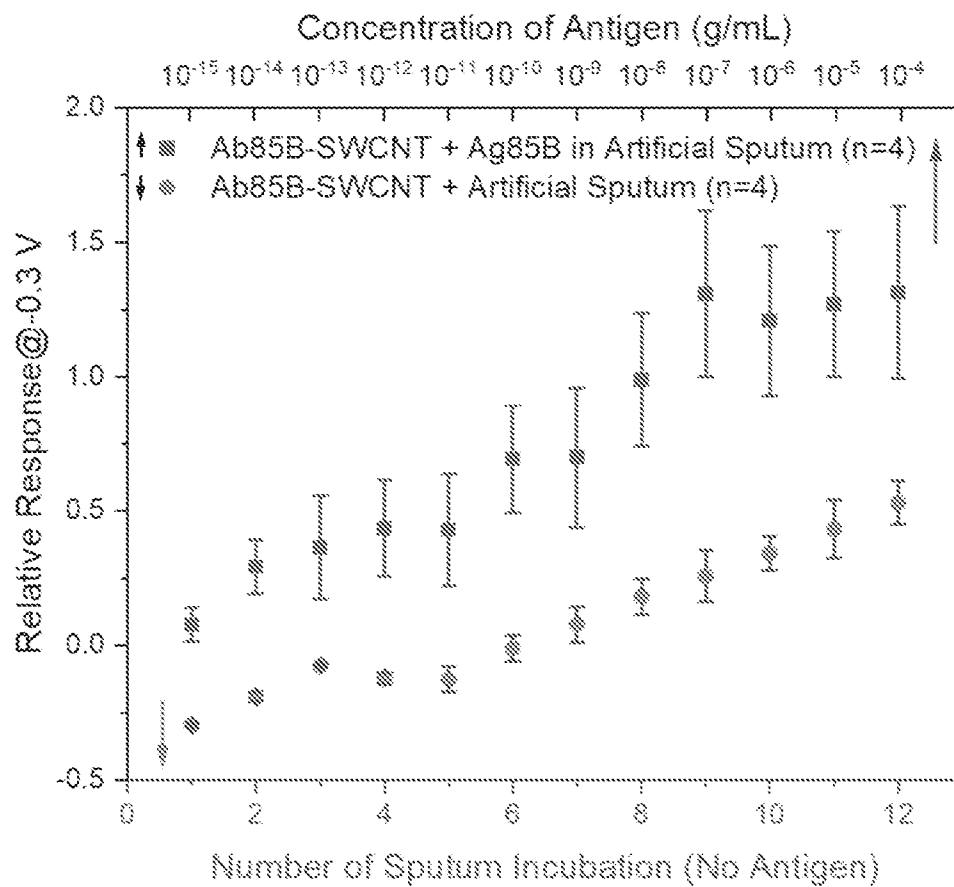


FIG. 4B

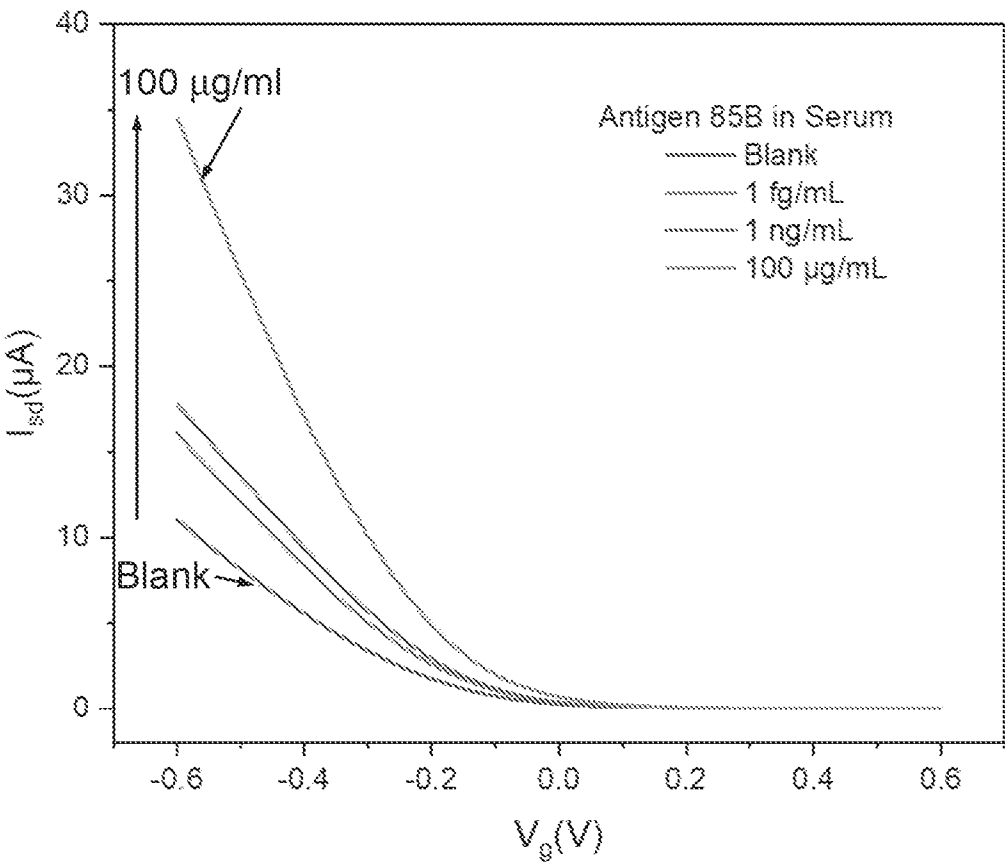


FIG. 4C

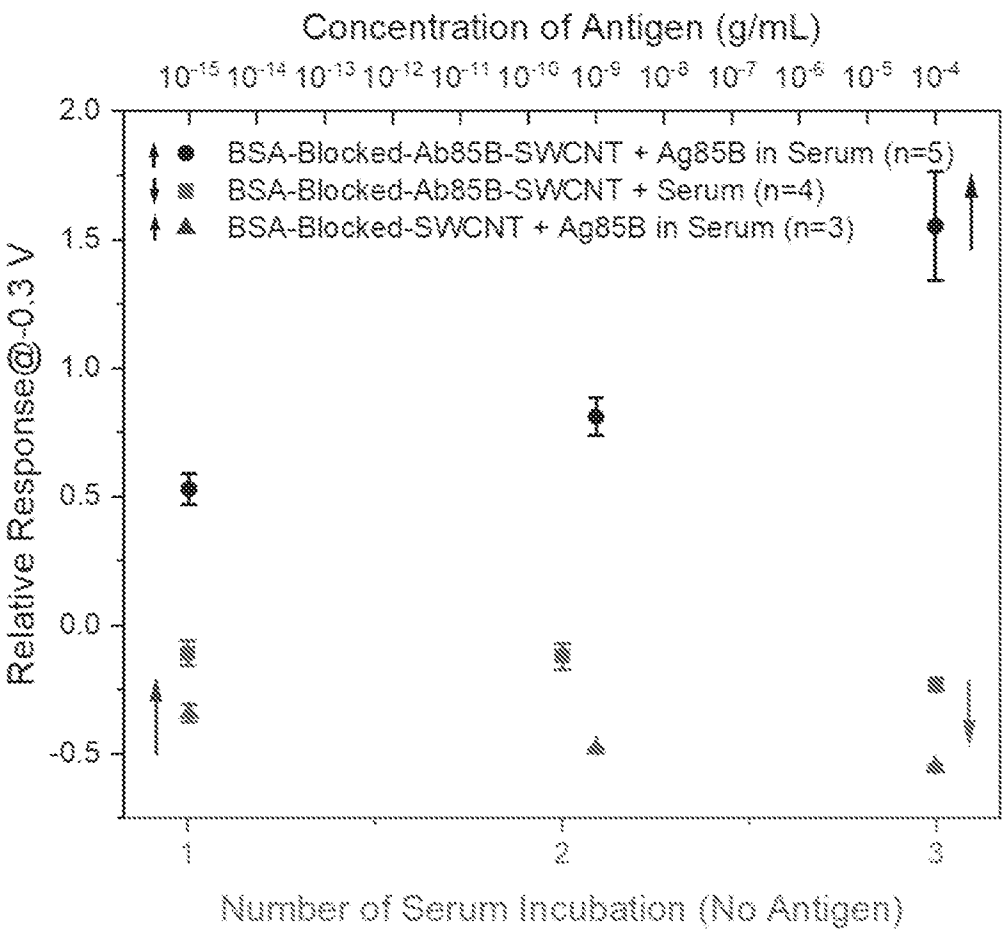


FIG. 4D

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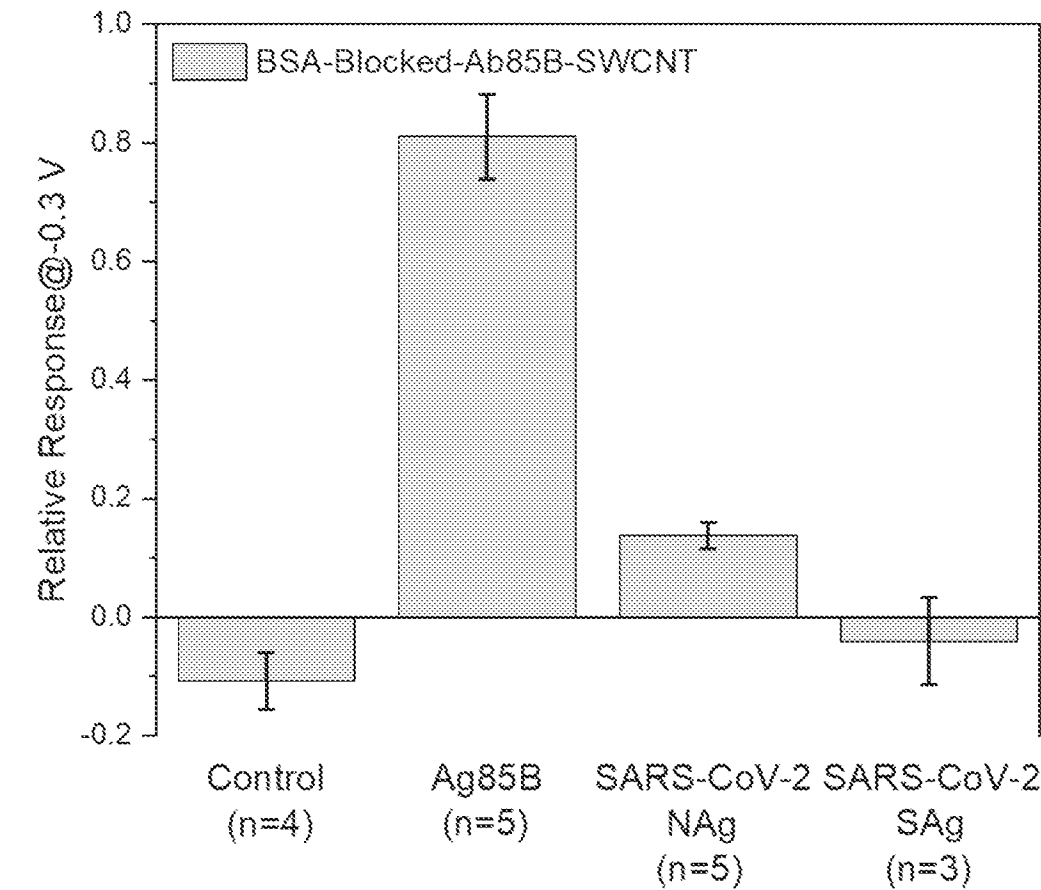


FIG. 4E

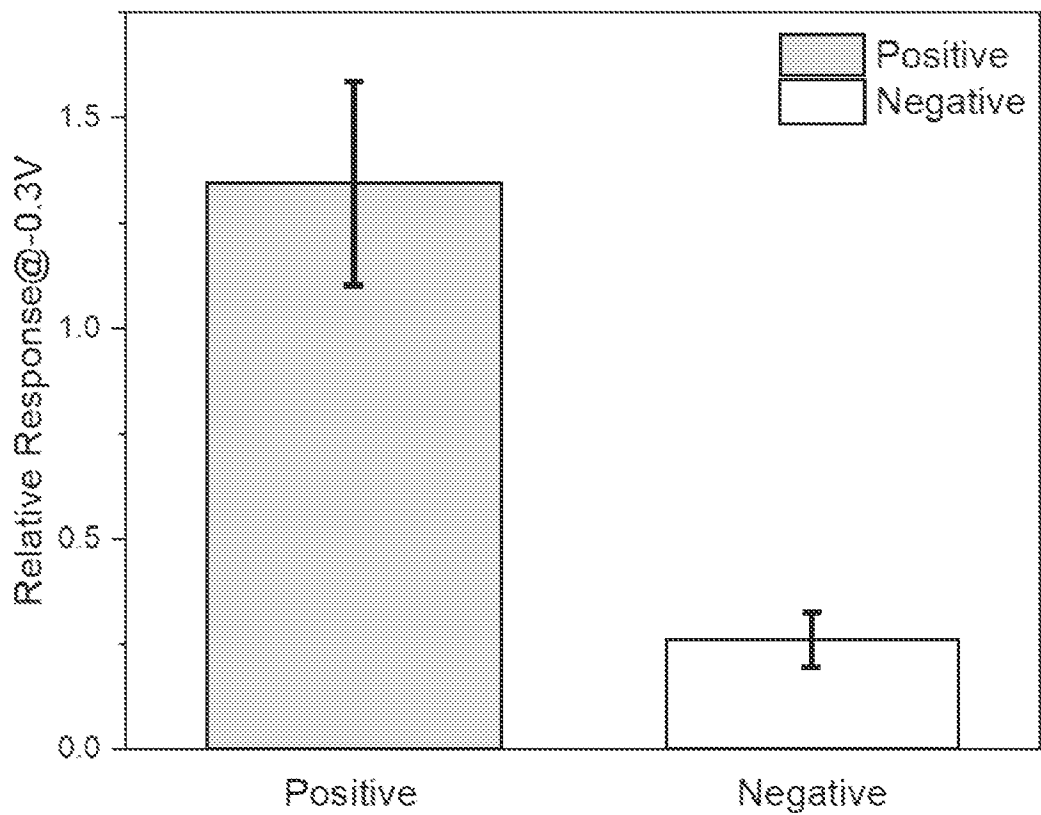


FIG. 4F

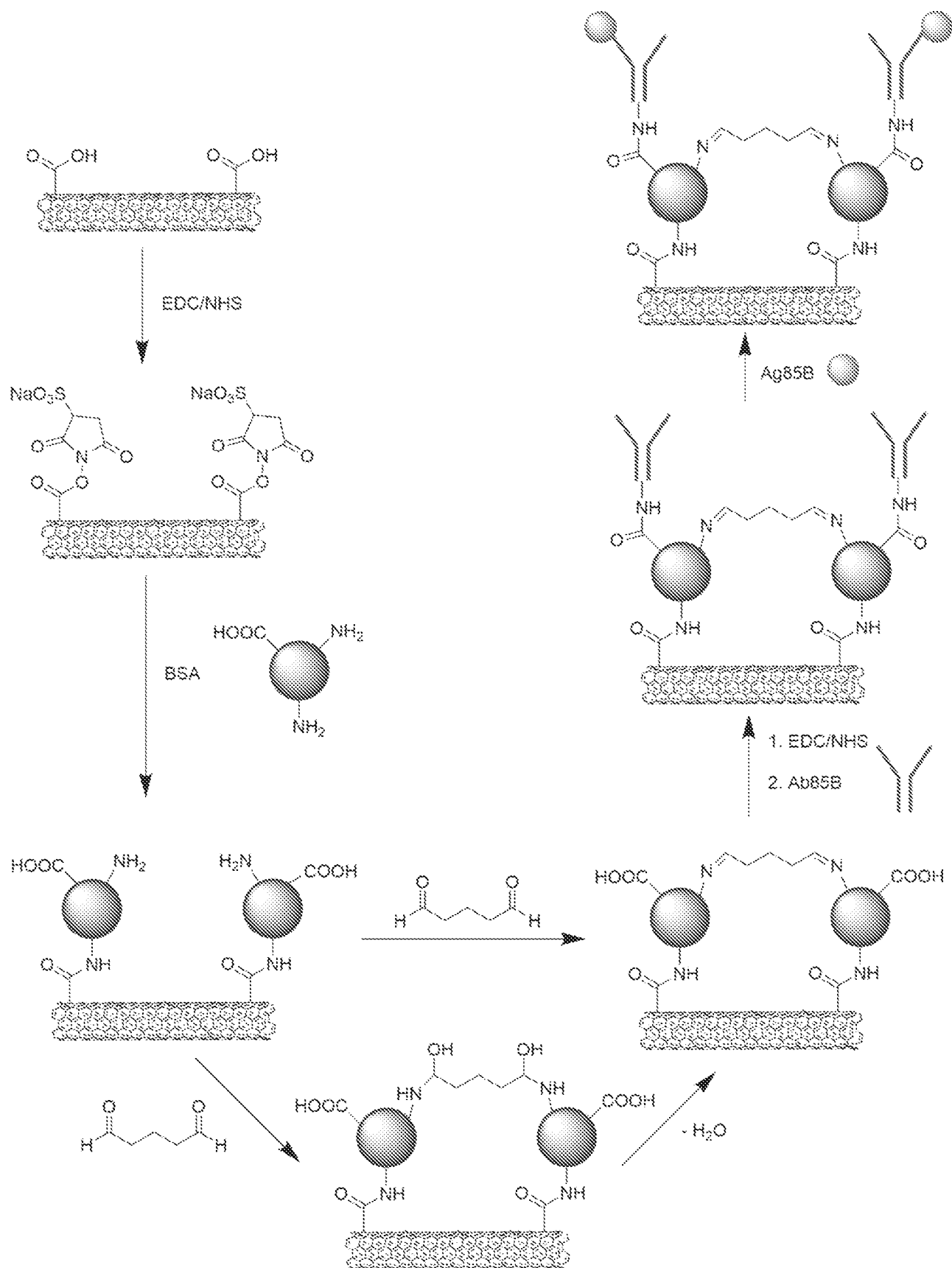


FIG. 5A

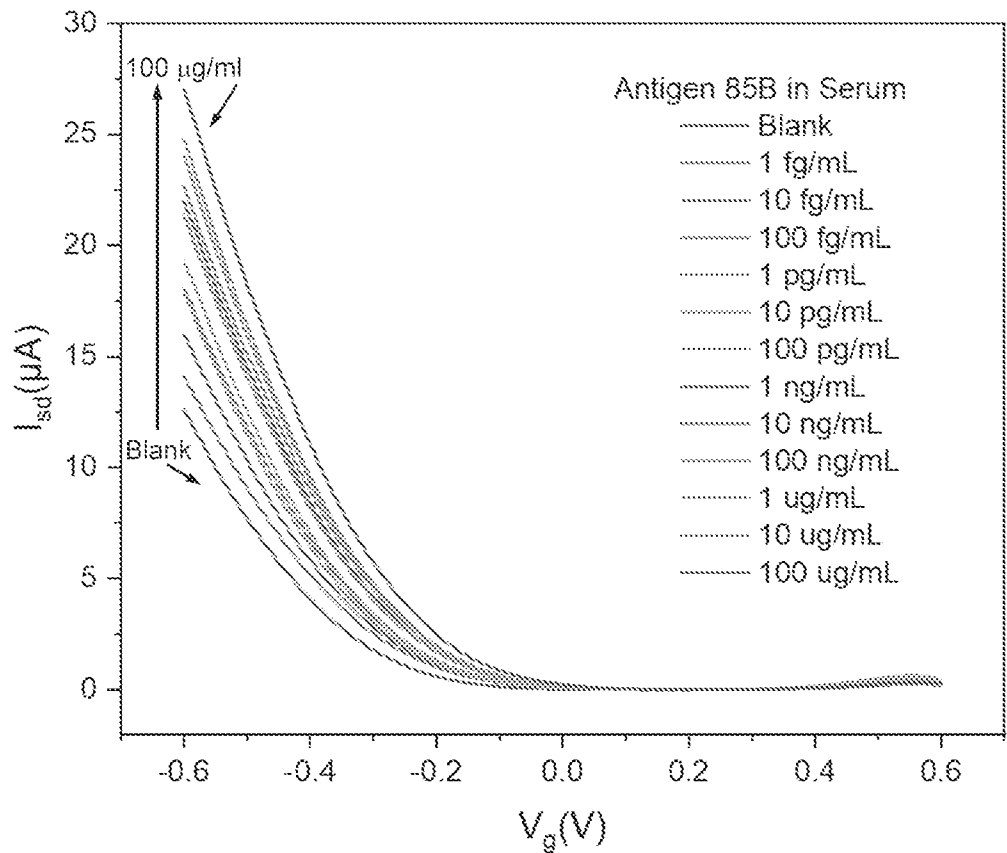


FIG. 5B

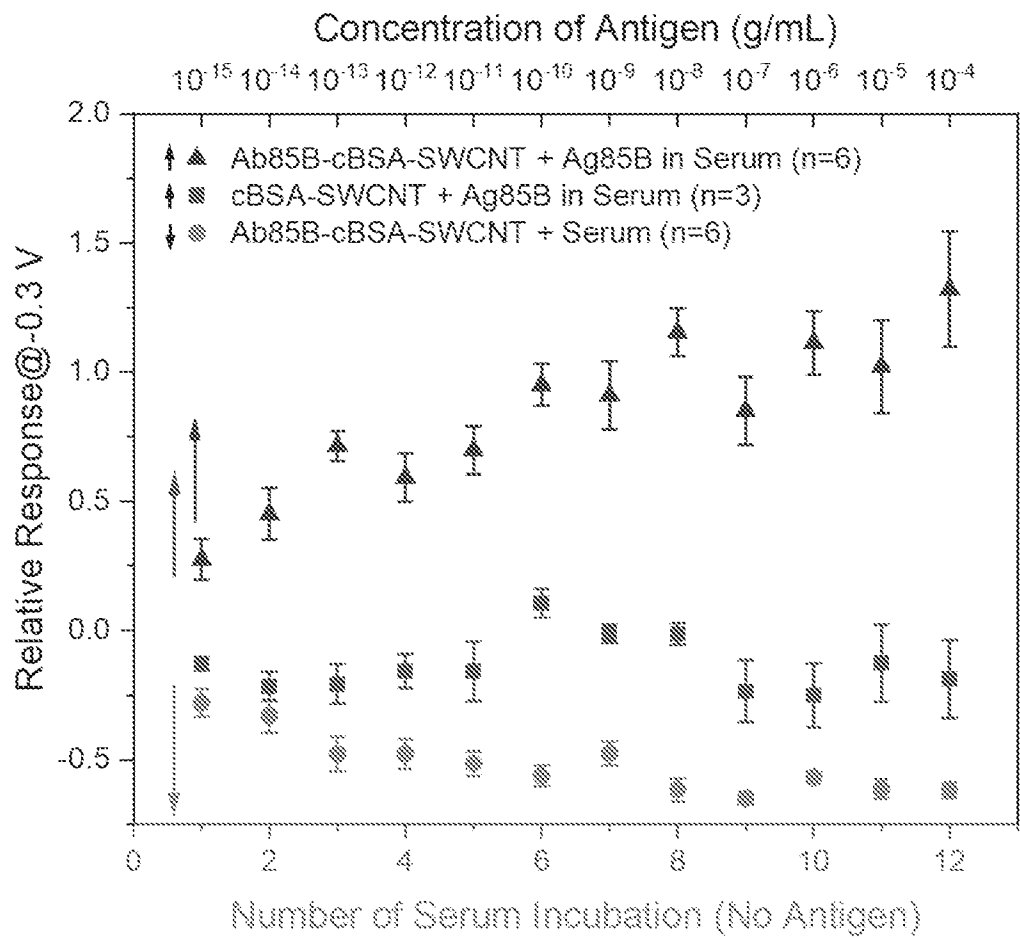


FIG. 5C

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I, claims 1-27, 31-53, directed to a sensor device for detecting an antigen (for diagnosis of tuberculosis).

Group II, claims 28-30, directed to a method of detecting tuberculosis, comprising applying body fluid sample to a sensor device.

Group III, claim 54, directed to a method of fabricating a sensor device for detecting an antigen.

The inventions listed as Groups I-III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features:

Group I has the special technical feature of an article consisting of a sensor device, that is not required by Group II or III.

Group II has the special technical feature of a method of detecting tuberculosis, comprising applying body fluid sample to a sensor device, that is not required by Group I or III.

Group III has the special technical feature of a method of fabricating a sensor device, that is not required by Group I or II.

Common technical features:

Groups I-III share the common technical feature of (use of) a sensor device for diagnosis of tuberculosis (by antigen detection), comprising:

a sensor comprising a substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures and at least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site for Ag85 antigen,

a liquid deposited over the sensor medium, wherein an ionic strength of the liquid is selected at which binding of the Ag85 antigen to the one or more recognition entities occurs, and the ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized one or more recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection, and

electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the Ag85 antigen.

However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is previously made obvious by US 2022/0365078 A1 to University of Pittsburgh - of the Commonwealth System of Higher Education (hereinafter 'Pittsburgh'), in view of the article entitled "Ultrahigh Frequency Surface Acoustic Wave Sensor with Giant Mass-loading Effect on Electrodes" to Chen et al., (ACS Sens. 11 May 2020, Vol 5, No 6, p. 1657-1664), (hereinafter 'Chen'), and US 2021/0072235 A1 to Sugentech, Inc., (hereinafter "Sugentech").

Pittsburgh teaches a sensor device for detecting an antigen (para [0007] - "... detecting an analyte which is an antigen... in a fluid sample includes providing a sensor device") comprising:

a sensor comprising as substrate, a first electrode, a second electrode spaced from the first electrode, and a sensor medium on the substrate between the first electrode and the second electrode, the sensor medium including a plurality of nanostructures, at least one of one or more recognition entities selected from the group consisting of antibodies and antibody fragments is immobilized on the plurality of nanostructures, each of the one or more recognition entities comprising at least one active binding site to the antigen (para [0007] "including a sensor including a substrate and a sensor medium on the substrate (such that the fluid sample contacts the sensor medium). The sensor medium includes a plurality of nanostructures having an

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

enriched semiconducting content and one or more of at least one agent selected from the group consisting of an antibody... immobilized upon at least a portion of the plurality of nanostructures. The sensor device further includes electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the analyte. The at least one agent is an antibody..."; para [0023] - "FIG. 1b illustrated schematically a liquid-gated SWCNT FET for detection of SARS-CoV-2 SAg and NAg. Interdigitated gold electrodes are configured as the source (So) and drain (Dr)."; Figure 1b; Note, Figure 1b depicts a first electrode separated from a second electrode (i.e., source (So) and drain (Dr) electrodes), wherein between the first and second electrodes is a sensor medium covering a nanotube-based sensor medium with immobilized antibodies.),

a liquid deposited over the sensor medium, wherein an ionic strength of the liquid is selected (para [0007] "the liquid of known ionic strength over or covering the sensor medium/sensor. The liquid having a known ionic strength may be chosen to have an ionic strength that is less than the fluid sample to increase sensitivity"; Figure 1b; para [0074]-[0077] - "Sensitivity can be increased in sensor hereof through use of a low - ionic strength fluid through reducing or eliminating the Debye screening effect. As known in the art, Debye screening length (D)... Because of the limitation of Debye screening length, only the interactions within the Debye screening length can be probed... For example, the length of a typical immunoglobulin 0 or IgG type antibody is around 10-15 nm. Therefore, to sense the binding between the antigen and the antibody, it is desirable to use a liquid that has a Debye screening length longer than 10 nm for the measurements. In that regard, it is desirable that the screening length exceed the length of the immobilized antibody, an antigen receptor or an antigen hereof. According to the above equation for the Debye screening length, the ionic strength of the liquid should be lower than 0.94 nmol / L to provide a Debye screening length longer than 10 nm. One may thus, readily determine a desirable ionic strength for liquids used in measurement methodologies of sensors hereof..."), and

electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the antigen (para [0007] "The sensor device further includes electronic circuitry including at least one measurement system in operative connection with the sensor to measure a variable providing a measure of change in at least one property of the sensor medium which is dependent upon the presence of the analyte"), but does not explicitly teach wherein an ionic strength of the liquid is selected at which binding of the antigen to the one or more recognition entities occurs, and the ionic strength and mass loading of the one or more recognition entities immobilized on the plurality of nanostructures are selected so that an average height of immobilized recognition entities is within a determined range of a maximum mass loading at which the average height remains within a Debye screening length of the liquid to achieve a determined limit of detection.

Chen teaches the impact of altering mass-loading on the results obtained from a biosensor (Figure 2; Note, Figure 2 depicts a plurality of states of mass loading which results in an altered result.). Since Pittsburgh teaches selecting a liquid with a desirable ionic strength to limit the Debye screening effect as well as increase sensor sensitivity, which in practice results in selecting a liquid with a longer Debye screening length than that of the antibody used (para [0007]; para [0074]-[0077]), it is well known in the art that that altering the mass load altering the sensitivity to detect a signal, it is well known in the art to take an average of a plurality of measurements to increase confidence in the observed result, and it would have obvious through the course of routine experimentation to optimize the parameters to obtain the best results and/or sensitivity of the instrumentation used; it would have been obvious to one of ordinary skill in the art that the selection of a liquid with a desirable ionic strength used with the biosensor taught by Pittsburgh as well as the mass loading be optimized as taught by Chen to obtain a desirable and/or the highest sensitivity such the optimization results in the average measurement height remains within a Debye screening length of the liquid, so that the sensor can lower concentrations of samples better.

Pittsburgh further teaches the sensor device comprising antibodies specific for tuberculosis antigens (para [0010] "the at least one agent is an antibody and the analyte is an antigen. The antigen may, for example, be an antigen of a pathogen selected from the group of a viral pathogen and a bacterial pathogen...tuberculosis"), yet does not specifically teach detection of an Ag85A antigen.

Sugentech teaches detection of an Ag85A antigen in a patient sample for diagnosis of tuberculosis (claim 1 "A method for diagnosing tuberculosis..."; claim 3 "wherein the antigen is selected from...an antigen 85 complex

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/043037

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

(Ag85), Ag85A"). It would have been obvious to one of ordinary skill in the art to use antibodies specific to detect an Ag85A antigen in a patient sample for diagnosis of tuberculosis using the sensors of Pittsburgh.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I-III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-27, 31-53**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/043037

A. CLASSIFICATION OF SUBJECT MATTERIPC: **G01N 27/30** (2024.01); **G01N 27/327** (2024.01); **G01N 27/416** (2024.01); **G01N 27/48** (2024.01); **G01N 33/483** (2024.01); **G01N 33/53** (2024.01); **G01N 33/543** (2024.01)CPC: **G01N 27/327**; **G01N 27/3275**; **G01N 33/483**; **G01N 33/53**; **G01N 33/54353**; **G01N 33/5438**; **G01N 33/543**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2022/0365078 A1 (UNIVERSITY OF PITTSBURGH – OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 17 November 2022 (17.11.2022) para [0007]; [0010]; [0023]; [0060]; [0072]; [0073]; [0074]-[0077]; [0090]; [0119]	1-27, 31-53
Y	US 2021/0072235 A1 (SUGENTECH, INC.) 11 March 2021 (11.03.2021) para [0025]; [0047]; claim 1; claim 3	1-27
Y	US 2006/0029926 A1 (PAWLAK et al.) 09 February 2006 (09.02.2006) abstract; para [0024]	8-10, (17-20)/(8-10), 34-38, (45-48)/(34-38)
Y	CHEN et al. Ultrahigh Frequency Surface Acoustic Wave Sensor with Giant Mass-loading Effect on Electrodes. ACS Sens. 11 May 2020, Vol 5, No 6, p. 1657-1664 Figure 2	1-27, 31-53



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“D” document cited by the applicant in the international application

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

12 December 2024 (12.12.2024)

Date of mailing of the international search report

26 December 2024 (26.12.2024)

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