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(54) Title: DETECTION OF DEMENTIA BIOMARKERS USING APTAMER-MODIFIED GRAPHENE FIELD-EFFECT TRANSISTORS

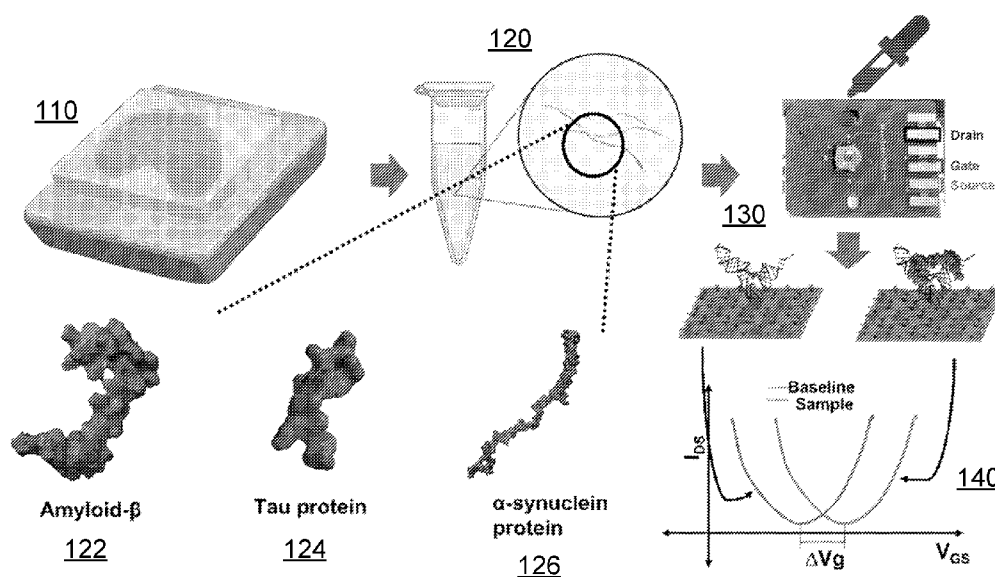


FIG. 1

(57) Abstract: Dementia is a brain disease which results in irreversible and progressive loss of cognition and motor activity. Despite global efforts, there are no simple and reliable diagnosis or treatment options. Embodiments of the disclosed technology provide an aptamer-based graphene field-effect transistor (GFET) biosensor platform that has high sensitivity and precision across a range of epidemiologically significant Alzheimer's disease and Parkinson's disease variants, and enables at-home and point-of-care (POC) testing for neurodegenerative diseases. An example method of detecting for detecting a molecular biomarker for dementia of a subject includes receiving a biological sample comprising the molecular biomarker for dementia, contacting the biological sample with a biosensor device comprising a GFET-based detection chip, and detecting a presence of the molecular biomarker for dementia in the biological sample.

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DETECTION OF DEMENTIA BIOMARKERS USING APTAMER-MODIFIED GRAPHENE FIELD-EFFECT TRANSISTORS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent document claims priority to and benefits of U.S. Provisional Application No. 63/581,533, entitled “APTAMER-MODIFIED GRAPHENE FIELD-EFFECT TRANSISTOR,” and filed on September 8, 2023. The entire contents of the before-mentioned patent application are incorporated by reference as part of the disclosure of this patent document.

REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0002] This patent document contains an ST.26 compliant Sequence Listing, which is submitted concurrently in xml format via Patent Center and is hereby incorporated by reference in its entirety. The .xml copy, created on September 9, 2024, is named Sequence Listing 009062-8501WO00.xml and is 3,846 bytes in size.

TECHNICAL FIELD

[0003] This patent document relates to detection of biomarkers, and in particular, to the detection of biomarkers using aptamer-modified graphene field-effect transistors (GFETs).

BACKGROUND

[0004] A biosensor is a device that can detect a biological substance (e.g., a biochemical species, a compound, or an organism) by using a transducing element to convert a detection event into a signal for processing and/or display. Biosensors can use a biological material as the biologically sensitive component, e.g., such as biomolecules including enzymes, antibodies, nucleic acids, etc., as well as living cells. For example, molecular biosensors can be configured to use specific chemical properties or molecular recognition mechanisms to identify target agents. Biosensors use the transducer element to transform a signal resulting from the detection of an analyte by the biologically sensitive component into a different signal that can be addressed by optical, electronic or other means. For example, the transduction mechanisms can include physicochemical, electrochemical, optical, piezoelectric, and/or other transduction means.

SUMMARY

[0005] Disclosed are methods, devices and systems for a graphene field-effect transistor (GFET)-based biosensor capable of detecting biomarker analytes after functionalization with biomarker specific probes that include aptamers (e.g., ssDNA aptamers).

[0006] In some example aspects, a system, in accordance with the disclosed technology, includes a portable, wireless, readout-based graphene field-effect transistors (GFET) biosensor platform that can detect proteins and small molecules with single-molecule sensitivity and specificity. The biosensor platform is used for the detection of three important amyloids, namely, Amyloid beta ($A\beta$), Tau (τ), and α -Synuclein (αS) using DNA aptamer nanoprobe. These amyloids were isolated, purified, and characterized from autopsied brain tissues of Alzheimer's disease (AD) and Parkinson's disease (PD) patients. The limit of detection (LoD) of the sensors are 10 fM, 1–10 pM, and 10–100 fM for the $A\beta$, τ , and αS amyloids, respectively. Synthetic and autopsied brain-derived amyloids showed a statistically significant sensor response with respect to derived thresholds derived using synthetic $A\beta$, τ , and αS amyloids, confirming the ability to define diseased vs non-diseased states. The detection of each amyloid was specific to their aptamers: $A\beta$, τ , and αS peptides when tested respectively with aptamers non-specific to them showed statistically insignificant cross-reactivity. The aptamer-based GFET biosensor platform has high sensitivity and precision across a range of epidemiologically significant AD and PD variants, and enables at-home and point-of-care (POC) testing for neurodegenerative diseases.

[0007] In other example aspects, a method for producing a biosensor device configured to detect a dementia biomarker of a subject is disclosed. The method includes receiving a GFET-based detection chip, and functionalizing the monolayer graphene by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule. In this example, the GFET-based detection chip comprises (i) monolayer graphene on a silicon-based substrate, (ii) a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene, (iii) a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene, (iv) a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and (v) a well formed using an insulating material and configured to receive a biological sample comprising a target molecular biomarker for dementia such that the biological sample is in contact with the monolayer graphene. Furthermore, the aptamer is configured to specifically bind with the target

molecular biomarker with at least a predetermined specificity, and the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene.

[0008] In yet other example aspects, a biosensor device for detecting a dementia biomarker of a subject is disclosed. The biosensor device includes a GFET-based detection chip and a functionalization layer comprising (a) a heterobifunctional linker molecule and (b) an aptamer coupled to the heterobifunctional linker molecule. In this example, the GFET-based detection chip includes (i) monolayer graphene on a silicon-based substrate, (ii) a source electrode comprising a conducting material and positioned at a first end of the monolayer graphene, (iii) a drain electrode comprising the conducting material and positioned at a second end of the monolayer graphene, (iv) a gate electrode comprising the conducting material and positioned in a same plane as the source electrode and the drain electrode, and (v) a well comprising an insulating material and configured to receive a biological sample comprising a target molecular biomarker for dementia such that the biological sample is in contact with the monolayer graphene. Furthermore, the functionalization layer is attached to the monolayer graphene using the heterobifunctional linker molecule, the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene.

[0009] In yet other example aspects, a method for detecting a neurogenerative disease biomarker of a subject is disclosed. The method includes receiving a biological sample comprising a target molecular biomarker for a neurogenerative disease, contacting the biological sample with a biosensor device comprising a graphene field-effect transistor (GFET)-based detection chip, detecting, using the biosensor device, a presence and a concentration of the target molecular biomarker in the biological sample, and determining, based on comparing the concentration to a first threshold and a second threshold, whether the subject has a first neurogenerative disease or a second neurogenerative disease. In this example, the GFET-based detection chip comprises (i) monolayer graphene on a silicon-based substrate, (ii) a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene, (iii) a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene, (iv) a gate electrode formed using the conducting material and

positioned in a same plane as the source electrode and the drain electrode, and (v) a well formed using an insulating material and configured to receive the biological sample such that the biological sample is in contact with the monolayer graphene. Furthermore, the monolayer graphene is functionalized by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule, the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and the presence of the target molecular biomarker is detected based on a change in a conductance of the monolayer graphene.

[0010] The subject matter described in this patent document can be implemented in specific ways that provide one or more of the following features.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 shows a schematic of testing process that uses an example biosensor platform in accordance with the described technology.

[0012] FIG. 2 shows an example of a graphene FET (GFET) sensor on a chip carrier and a breadboard setup for graphene FET sensors.

[0013] FIG. 3 shows an example drain-source current analysis with respect to gate voltage for the experimental setup shown in FIG. 2.

[0014] FIGS. 4A–4F show stages in an example fabrication of the graphene FET.

[0015] FIG. 5 shows a two-dimensional view of an example graphene FET.

[0016] FIGS. 6–9 show partial cross-sectional views of example embodiments of a GFET-based detection device in an assembled state.

[0017] FIG. 10 shows another example embodiment of a detection device.

[0018] FIG. 11A and 11B show examples of assembled portable, compact devices.

[0019] FIG. 11C shows a diagram of an example embodiment of a biosensor device for detecting a biomarker, in accordance with the present technology.

[0020] FIGS. 12A–12D show example Raman maps and Brightfield images of an unmodified functionalized FET sensor and a PBASE functionalized FET sensor.

[0021] FIGS. 13A–13F shows an example of GFET sensor characterization using Raman spectroscopy and atomic force microscopy (AFM).

[0022] FIGS. 14A–14D show example numerical results for GFET detection sensitivity and aptamer probe-specificity for A β , Tau, and α S proteins.

[0023] FIG. 15 show example numerical results for the specificity of A β aptamer for the detection of A β ₁₋₄₂.

[0024] FIGS. 16A–16C shows example numerical results for detection thresholds for synthetic A β , Tau, and α S proteins using their specific aptamer probes.

[0025] FIGS. 17A–17D show the detection thresholds and specificities of AD patients' autopsied brain-derived A β , Tau, and α S proteins.

[0026] FIG. 18 shows numerical results for example experiments done on amyloid-beta aptamer functionalized GFET biosensors at two concentrations.

[0027] FIG. 19 shows example experimental results of α S oligomers derived from the brain being loaded onto an SDS-PAGE gel.

[0028] FIG. 20 shows example numerical results for the three metrics used in the quality control screening developed for optimizing the GFET-based detection chip functionality.

[0029] FIG. 21 shows a flowchart of an example method for producing a biosensor device configured to detect a dementia biomarker of a subject.

[0030] FIG. 22 shows a flowchart of an example method for detecting a dementia biomarker of a subject.

DETAILED DESCRIPTION

[0031] The disclosed system, devices, and methods use GFET technology that has been engineered for the detection of certain neurodegenerative disease-associated biomarkers (e.g., Amyloid Beta₁₋₄₂, Tau, and Alpha-Synuclein proteins). Example embodiments of the disclosed technology has been demonstrated to show the capacity for detecting fM levels of these example biomarkers in a controlled PBS buffer. Detecting these biomarker proteins can be used to diagnose neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and potentially chronic traumatic encephalopathy (CTE). In some example embodiments, the disclosed technology can be accurately quantify the concentration of these biomarkers in physiological fluids such as cerebrospinal fluid (CSF), blood (plasma or serum), saliva or urine, thus paving the way to track the progression of the above mentioned neurological conditions.

[0032] In the described embodiments, a GFET biosensor design was re-engineered and adapted to various testing capacities by modifying the surface with target probe molecules, for example, specifically ssDNA strands that are capable of folding into a conformation that is

optimized for binding to the biomarker of interest. The example GFET platform was configured to utilize aptamers specific to neurodegenerative disease biomarkers, where the aptamers can increase specificity beyond that of antibodies. The disclosed GFET biosensing technology provides a significant improvement beyond conventional techniques, e.g., liquid chromatography-mass spectrometry (LC-MS) methods, as it is several orders of magnitude cheaper and faster in producing results.

[0033] Conventional techniques focus on neurodegenerative disease related biomarker detection with methods that do not utilize a field effect transistor (FET), such as gold nanoparticle modified electrodes and EIS. Some conventional techniques that utilize FET technology utilize the Dirac point as the signal mechanism, with an example being a graphene-oxide FET with functionalized antibodies specific to $A\beta_{1-42}$. However, these techniques are significantly different than the disclosed GFET technology at least because the present technology utilizes aptamers, e.g., ssDNA aptamers, and not antibodies—which overcome significant challenges and provides additional benefits, e.g., such as a lower working distance from the graphene surface eliminating the influence of bulk ionic concentration changes in the fluid, and usage of monolayer graphene instead of graphene oxide which offers superior electronic transduction capabilities. Other clinically available tests utilize LC-MS to measure blood plasma levels of the ratio $A\beta_{1-42}/A\beta_{1-40}$ via LC-MS, such as the Quest Diagnostics AD-detect test and the Precivity AD test, which are extremely costly, time-consuming and trained personnel intensive process, limiting its availability on a mass-scale, or a luminometric immunoassay by Lumipulse used to quantify $A\beta_{1-42}/A\beta_{1-40}$ ratio in CSF samples. Importantly, the disclosed GFET biosensor system, device, and method is significantly cheaper than the conventional options and produces results in a faster time period without the involvement of trained personnel.

[0034] As used in this patent document, the term “biomarker” is any combination of biological metrics that indicate the presence or absence of a condition, or said another way, a measurable indicators of what is happening in a subject’s body. Before the early 2000s, the only sure way to know whether a person had Alzheimer’s disease or another form of dementia was after death through autopsy. But presently, there are now techniques to identify and characterize biomarkers associated with dementia in a living person. Some biomarkers indicative of dementia (dementia biomarkers) are found in the cerebrospinal fluid (CSF). Some examples of CSF

biomarkers for Alzheimer's disease include $A\beta_{1-42}$ (the major component of amyloid plaques in the brain), Tau, and phospho-Tau (major components of tau tangles in the brain, which are another hallmark of Alzheimer's).

[0035] Furthermore, the terminology of detecting a molecular biomarker using an aptamer with a high-affinity for that molecular biomarker is used for the sake of clarity of exposition, but the techniques disclosed in this patent document are not limited thereto, and may be used for other biomarkers. In some examples, one or more methods or devices may be described for the detection of a specific molecular biomarker, e.g., $A\beta_{1-42}$ (a CSF biomarker for Alzheimer's disease), but they are equally applicable to the detection of biomarkers that include ratios of various molecular biomarkers, e.g., $A\beta_{1-42}/A\beta_{1-40}$, pTau/tTau, $A\beta_{1-42}/p\text{Tau}$, and the like, which are also relevant biomarkers that indicate the progression of Alzheimer's disease. Similarly, the disclosed technology is equally applicable to the detection of other biomarkers that are correlated with other neurodegenerative diseases (and which is discussed at the end of Section 3).

[0036] Section headings are used in the present document to improve readability of the description and do not in any way limit the discussion or the embodiments (and/or examples, implementations) to the respective sections only.

1. Introduction

[0037] One of the greatest modern challenges is an effective prevention and treatment of degenerative brain disorders such as Alzheimer's (AD) and Parkinson's disease (PD). Although there has been a concerted effort to understand, diagnose, treat, and cure neurodegenerative diseases, the progress for early and simple AD and PD diagnosis is abysmal.

[0038] The progression of neurodegenerative diseases, especially AD, is historically associated with Amyloid- β ($A\beta$) protein plaque formation within the extracellular space and Tau neurofibrillary tangles inside neurons of the brain. The two major isoforms, $A\beta_{1-40}$ and $A\beta_{1-42}$ are formed via the successive proteolytic cleavage of amyloid precursor protein. Due to their high aggregation propensity, $A\beta$ proteins oligomerize and eventually form insoluble amyloid fibrils present in the core of senile plaques, characteristic of AD. The current prevailing view in the AD community is that the soluble $A\beta$ oligomers are connected to early AD symptoms and disease onset. Upon hyperphosphorylation, microtubule-associated protein Tau (τ) can form helical filaments called the neurofibrillary tangles (NFTs). These NFTs follow a characteristic spatiotemporal progression in AD-diagnosed individuals. As the NFTs and plaque concentrations

grow there is an increase in neurite cell death and eventual decline in cognitive ability and death. Together NFTs and A β plaques form the core of AD pathophysiology and progression.

[0039] Parkinson disease (PD) is identified by a distinct α -Synuclein(α S)-linked pathophysiology and histological hallmarks, specifically, the presence of Lewy bodies (LBs) that occur in dopaminergic neurons of the substantia nigra pars compacta (SNpc) neurons. The death of the dopaminergic neurons has been linked to loss of autonomic, motor control, and cognitive ability leading to dementia. Recent work has shown that alpha-synuclein (α S) protein is the primary fibrillar component of LB and that α S overexpression can cause dopaminergic neuron cell death. The precise pathophysiology between α S and PD diagnosis is not clearly understood, but many studies point to some disruption of dopamine function (i.e., storage, efflux, interaction with SNARE complex).

[0040] Alzheimer's, Parkinson's, and other neurodegenerative diseases often have biological onset decades prior to any clinical diagnosis or identifiable traits. Clinicians often use amnesic phenotypes, and visual/auditory or vocal impairment as key features of dementia caused by AD, but studies have indicated that many patients diagnosed with AD via autopsy never showed clinically diagnosable levels of impairment. The depletion of soluble A β_{1-42} and the reduction in the ratio of A β_{1-42} /A β_{1-40} levels in bodily fluids, such as cerebrospinal fluid (CSF), have been shown to be reliable biomarkers in the diagnosis of AD. In the case of PD, the protein α -synuclein (α S) is involved in various stages of disease progression and is a promising biomarker for the diagnosis of PD since aggregates are closely correlated with PD pathogenesis.

[0041] The early detection of PD and AD prior to the onset of phenotypic changes is critical for effective prevention and treatment. Although some commercial diagnostic tests are being marketed to test for AD and PD biomarkers from blood, they are mostly designed to be "sample collection" from the user, with the actual test being run by qualified professionals with expensive equipment and procedures. The goal is early detection and enhanced longitudinal studies in the preclinical stages. Some promising approaches for a point-of-care (POC) test for rapid and accurate detection of A β , Tau, and α S concentrations using CSF and/or brain blood have also been demonstrated. However, there is no POC or at-home testing of commonly accessible biofluids, such as blood (plasma or serum), saliva, urine containing A β , Tau, and α S with single molecule sensitivity and specificity to correlate with predictive value.

[0042] Embodiments of the disclosed technology address the above-described problems by

using, for example, a graphene field-effect transistor (GFET)-based biosensor platform with an aptasensor (e.g., a sensor that uses aptamers as recognition elements for the detection of proteins) that includes a single-atomic layer of graphene in between a source and drain electrodes with a liquid-gated electrode for the generation of the field effect at the graphene surface. This biosensor platform is adapted for the detection of specific protein biomarkers for AD and PD. The graphene surface electric charge transfer is modulated with aptamers specific to A β , Tau, and α S. The amyloid-aptamer binding-induced change is detected as the shift in the Dirac point—the minimum value (i.e., charge neutrality point)—in the I-V curve.

[0043] The described embodiments further include (1) characterizing the functionality of the GFET platform using Raman spectroscopy, atomic force microscopy (AFM), and electrical measurements, (2) functionalizing the graphene surface with identified high-affinity aptamers (as shown in Table 1) specific to various neurodegenerative disease-associated proteins, specifically, A β 1-42, Tau441, and α S, (3) quantifying the aptasensor's specificity and the limit of detection (LoD) for these proteins using the synthetic isoforms of the proteins in controlled buffer environment, and (4) testing the biosensor platform against brain-derived amyloid proteins, thereby developing a reliable sensor for amyloid protein biomarker detection in AD patient samples. Appropriate control experiments are used to demonstrate high specificity and low cross-reactivity. Thus, embodiments of the disclosed technology provide an aptamer-GFET sensor that can specifically detect protein biomarkers for AD and PD with high fidelity.

[0044] FIG. 1 shows a schematic of testing process that uses an example biosensor platform in accordance with the described technology. As shown therein, the overall procedure includes sample preparation, and dementia biomarker detection and analysis using the biosensor device. Example applications of the described aptamer-modified GFET device can utilize more easily accessible fluids (e.g., saliva, urine and, to a lesser extent, CSF).

[0045] As shown in FIG. 1, the device testing procedure starts by isolating the biomarker proteins from autopsied brain tissues of diseased patients via homogenization and immunoprecipitation, as shown in operations 110 and 120. The three-dimensional models of neurodegenerative amyloid proteins, A β , Tau, and α S (which were generated using ChimeraX) are shown as 122, 124 and 126, respectively (and where the Protein Data Bank (PDB) IDs for A β , Tau, and α S are 6cvj, 1xq8, and 2mxu, respectively).

[0046] The purified samples are then added at various dilutions to the silicone well in the

GFET-based biosensor chip at operation 130. The aptamers bind to the target of interest and bring the target analyte in close proximity to the graphene sensing surface, as shown in the upper portion of operation 140. This creates a change in the charge density on the surface of the graphene chip. For example, in a typical testing workflow, the gate voltage is swept across a preset voltage range and then the drain-source current is measured. This generates a shift in the minima of the plot which is known as the Dirac shift (with respect to the baseline reading, and without the presence of analytes), as shown in the lower portion of operation 140. A greater Dirac shift is indicative of the biomarker being more presently bound to the aptamers and the greater the concentration of biomarker in the tested fluid. The testing procedure in FIG. 1 is performed on brain tissue homogenized samples from autopsied patients; however, the GFET technology described here can also be used with more commonly accessible physiological fluids (e.g., CSF, blood, saliva, urine, etc.).

[0047] In some example embodiments, an aptamer-modified GFET biosensor device includes a graphene field-effect transistor including a graphene monolayer that transduces signal based upon changes to the charge distribution on the surface of graphene produced by a swept voltage gate electrode in the presence/absence of an analyte of interest. By sweeping the gate voltage, the device is able to produce a distinct drain-source current versus gate voltage plot that has a distinct Dirac point. This is a characteristic that is particularly unique to graphene over some other semiconductor materials. The Dirac point (the minima of the current across the gate voltage sweep) will shift based upon changes to the charge density and this can be tuned by immobilizing specific probes to the graphene surface (antibodies or aptamers). The example aptamer-modified GFET biosensor device can include ssDNA aptamers specific to Amyloid beta ($A\beta_{1-42}$), Tau (τ), and α -Synuclein (αS), which, for example, can be done through the use of an intermediary linker such as PBASE (pyrenebutyric acid NHS ester or pyrene maleimide). In some embodiments, for example, the device can be configured for detecting neurodegenerative disease, e.g., since $A\beta_{1-42}$ is a biomarker for AD, Tau protein is a biomarker for AD chronic traumatic encephalopathy (CTE), and alpha-synuclein is a biomarker for PD.

[0048] Example implementations of the disclosed aptamer-modified GFET technology include a working graphene field-effect transistor device that allows us to modify the surface with an aptamer of interest. To run a test, an electronic reader is utilized, to which the GFET chip can be plugged into. The electronic reader can apply a fixed drain-source voltage and sweep the

gate voltage to obtain the characteristic Dirac voltage and Dirac shift. In addition, the example implementations have tested example aptamer-modified GFET devices that have been functionalized with the aptamers specific to the neurodegenerative disease biomarkers of interest; and example results of such implementations have shown an ability to detect the presence of clinically significant concentrations of the target biomarker in a controlled PBS buffer solution.

[0049] Various aspects and advantages of the above-described examples and embodiments for detecting dementia biomarkers using the GFET-based biosensor platform are further detailed and described in the following sections.

2. Examples of the GFET-based biosensor device

[0050] In some embodiments, the biosensor device comprises a housing unit having a first opening, a second opening, and a third opening. A cartridge is adapted to be removably fixed within the housing to facilitate measurements. The cartridge comprises a detection chip that is in electrical communication with a surface of the cartridge during detection of a target. A cap is removably affixed over the second opening, and the cap includes circuitry and a visual indicator. When the cartridge is disposed within the housing with the cap disposed over the second opening, the target-sensing detection chip is disposed in fluid communication with the first opening and the cap is disposed in electrical communication with the surface of the cartridge.

[0051] FIGS. 2 and 3 show a diagram and data plot, respectively. The diagram in FIG. 2 depicts an example GFET sensor on a chip carrier and a breadboard setup for graphene FET sensors; and the data plot in FIG. 3 shows an example drain-source current analysis with respect to gate voltage for the experimental setup shown in FIG. 2.

[0052] In some embodiments, the detection chip (which can be mounted on a breadboard as shown in FIG. 2) utilizes electron mapping or electron density mapping to distinguish a change in energy between a single nucleotide pair. Such identification of the change in energy determines the proteins that comprise the nucleotide pair. The example setup shown in FIG. 2 results in the drain-source current analysis with respect to gate voltage, as shown in FIG. 3. In this example, the gate voltage was scanned in the range of +1 V to -1 V with a step size of 2 mV, the drain-source voltage (V_{ds}) was 30 mV (which was optimized in the 0-100 mV range in increments of 10 mV), the drain-source current (I_{ds}) was on the order of μA , and the Dirac voltage was analyzed at the I_{ds} minima.

[0053] In some embodiments, the detection chip, upon detection of one or more targets, for example, dementia biomarkers, can transmit that information in any of a number of ways. In some embodiments, the detection chip can be connected via circuitry to one or more colored lights, for example LEDs, and signals the illumination of a different color or colors preselected to represent a detection event. The color or colors, or the intensity thereof, or the number of individual LEDs illuminated could also be an indication of the concentration of the detection event. In other embodiments, the detection chip can be electrically connected with circuitry that includes a wireless transmitter that transmits data regarding the detection event to a computer or tablet or other portable or non-portable data storage device for analysis and/or later display.

[0054] FIGS. 4A–4F illustrate the stages in an example fabrication of the graphene FET. As shown therein, the source and drain electrodes (formed from gold (Au) or chromium (Cr) with a thickness of ~100nm) are deposited on an SiO₂ substrate (FIG. 4A) using sputtering deposition, which is followed by depositing a passivation layer (SiO₂ or Al₂O₃ ~80nm) on the source drain electrodes (FIG. 4B). In FIG. 4C, the graphene is wet transferred onto the patterned substrate and the Poly(methyl methacrylate) (PMMA) is removed by dissolving with acetone. In the next step, as shown in FIG. 4D, the (polymer, polymethyl glutarimide) (PMGI) photoresist is applied to protect the sensor area, and the extra graphene layer is removed by O₂ plasma etching. The PGMI photoresist is lifted off and the graphene FET is annealed in forming gas, e.g., a hydrogen-nitrogen atmosphere (FIG. 4E), and finally polydimethylsiloxane (PDMS) or epoxy is applied to form a well for containing the sample liquid (FIG. 4F).

[0055] In some embodiments, the graphene FET may be configured to detect multiple distinct biomarkers by attaching multiple probes (e.g., aptamers) on non-overlapping portions of the graphene FET. Each of the multiple probes attached is selected to bind to different specific proteins. In other embodiments, an array of detection chips can be used to detect multiple distinct biomarkers. In this example, each of the multiple probes is attached to a corresponding one of the array of detection chips. Thus, embodiments of the disclosed technology provide alternate ways of detecting multiple biomarkers.

[0056] FIG. 5 illustrates a two-dimensional schematic of an example GFET, wherein the source, drain and gate electrodes are fabricated using gold (Au) pads, upon which there are passivating layers, and then the graphene layer. Alternatively, or additionally, chromium (Cr) can be used as the conducting material for the electrodes. In some examples, the GFET includes a

500µm microfluidic channel in between the passivating layers adjacent to the gate and the drain source electrodes. In other examples, the source, drain and gate electrodes are in the same horizontal plane. In yet other examples, the liquid-gated electrode corresponds to the voltage at the gate electrode being controlled by the conductance of the graphene surface in the silicone well, which includes the biological sample in the buffer contacting with the aptamers and the functionalized graphene surface of the GFET.

[0057] In some embodiments, a schematic representation of a biosensor device 100 according to the present technology is illustrated in partial cross-section in an assembled state in FIG. 6 and shown in partial cross-section with the major components exploded in FIG. 7. As shown therein, the major components include a housing 110 that can, for example, serve as a handle and a chassis for supporting the other major components. In some embodiments, the housing 110 and the major components are arranged along a longitudinal centerline 105. In other embodiments, the components can be arranged in any geometry as aesthetically or functionally may be desirable, for example as illustrated in FIGS. 8 and 9 described further hereinbelow.

[0058] Referring to FIGS. 6 and 7, in one embodiment the housing 110 includes a first opening 120, a second opening 130 and at least one third opening 140. An insert or cartridge 150 is adapted to be removably fixed within the housing 110. For example, the cartridge 150 in one embodiment could be removably fixed by a threaded connection through the second opening 130. In another embodiment the cartridge 150 includes a shoulder 160 that extends laterally from an end of the cartridge 150 so that when the cartridge 150 is disposed within the housing 110, the shoulder 160 overhangs an edge of the second opening 130 and is compressively held against the edge of the second opening 130 by a cap 170 that attaches, for example by threads, over the shoulder 160 of the cartridge 150 at the second opening 130. In other embodiments the cartridge 150 could be removably fixed within the housing 110 by either of the above disclosed mechanisms and/or by a press fit or a magnetic attachment or any single attachment mechanism or combination of attachment mechanisms as known in the art.

[0059] Referring to FIGS. 8 and 9, in another embodiment of a device 200, a housing 210 includes a first opening 220, a second opening 230 and at least one third opening 240. In this embodiment an insert or cartridge 250 is adapted to be removably fixed within the housing 250. For example, the cartridge 250 in one embodiment could be removably fixed by a threaded connection through the second opening 230. In another embodiment the cartridge 250 includes a

shoulder 260 that extends laterally from an end of the cartridge 250 so that when the cartridge 250 is disposed within the housing 210, the shoulder 260 overhangs an edge of the second opening 230 and is compressively held against the edge of the second opening 230 by a cap 270 that attaches, for example by threads, over the shoulder 260 of the cartridge 250 at the second opening 230. In other embodiments the cartridge 250 could be removably fixed within the housing 210 by either of the above disclosed mechanisms and/or by a press fit or a magnetic attachment or any single attachment mechanism or combination thereof as known in the art.

[0060] Regardless of the geometry of the housing 110, 210 in regard to how the major components fit together, whether as shown in FIGS. 6-9 or using other geometries as are known in the art for a housing with an insertable and removable insert or cartridge, all embodiments of the cartridge 150, 250 include a detection chip 300 that is in electrical communication with a surface 310 of the cartridge 150, 250.

[0061] In some embodiments the detection chip 300 is reusable through a cleansing process so that the cartridge 150, 250 on which it is disposed is also reusable. In other embodiments the detection chip 300 is a single-use chip so that the cartridge 150, 250 is a disposable cartridge 150, 250. The detection chip 300 is electrically communicative to the surface 310 for example, wirelessly, via wires 320, or traces or an internal circuit board having wires or traces.

[0062] Still referring to FIGS. 6-9, in some embodiments a cap 170, 270 attaches, for example by threads, over the second opening 130, 230 so that circuitry 330 within the cap 170, 270 is in electrical communication with the surface 310, and therefore also in electrical communication with the detection chip 300. The cap 170, 270 in other embodiments attaches over the second opening 130, 230 by a press fit, a snap fit, a magnetic attachment, a latch mechanism or by any other mechanism for removable attachment as may be known in the art.

[0063] The circuitry 330 is of the type as known in the art that can interface with a signal from the detection chip 300 and relay or send an independent signal to a visual indicator 340 disposed on an outside of the cap 170, 270. The visual indicator 340 in one embodiment is one or more LEDs but in other embodiments can be one or more incandescent bulbs, an LED or LCD digital display, or other sorts of visual indicators as may be known in the art. Like the description hereinabove for the detection chip 30, the visual indicator 340 signals the illumination of a different color or colors preselected to represent a detection event. The color or colors, or the intensity thereof, or the number of individual LEDs illuminated could also be an indication of the

concentration of the detection event. In another embodiment the visual indicator 340 is electrically connected with the circuitry 330 that includes a wireless transmitter that transmits data regarding the detection event to a computer or tablet or other portable or non-portable data storage device for analysis and/or later display. As illustrated in FIGS. 6 and 8, when the cartridge 150, 250 is removably fixed within the housing 110, 210 with the cap 170, 270 disposed over the second opening 130, 230, the detection chip 300 is disposed in fluid communication with the first opening 120, 220.

[0064] Referring now to FIG. 10, in another embodiment of a device 400, a housing 410 includes a port for insertion of a detection chip 420 having all the structural and functional features of the detection chips. In this embodiment the housing 410 further includes circuitry 430, and a visual indicator 440, both of which function the same as the circuitry 330 and visual indicator 340 described hereinabove. The detection chip 420 when inserted into the housing 410 functions in the same way as the detection chip 300 by having electrical connections on a side that communicate electrically with the circuitry 430. In this embodiment, the detection chip 420 can be exposed to sample molecules, for example, by applying saliva to the chip 420 or by breathing or coughing onto the chip 420.

[0065] Referring to any of the embodiments in FIGS. 6-10, a power source 350, for example one or more batteries or a battery pack is schematically shown as disposed within the device 100, 200, 400 and is in electrical communication with the circuitry of that embodiment. For example, in the embodiments shown in FIGS. 6-9 the power source 350 is disposed within the cap 170, 270 and is in electrical communication with the circuitry 330. Therefore, when the cap 170, 270 is installed on the housing 110, 210, the power source 350 is also in electrical communication with the surface 310, and therefore is further in electrical communication with the detection chip 300. Therefore, the power source 350 can provide electrical power not only to the internal circuitry 330 within the cap 170, 270, but can also provide electrical power to the detection chip 300 when the device 100, 200 is assembled.

[0066] Still referring to FIGS. 6-9, in some embodiments a disposable mouthpiece 360 is removably attachable over the first opening 120, 220. In some embodiments the disposable mouthpiece 360 attaches, for example by threads, over the first opening 120, 220, whereas in other embodiments the disposable mouthpiece 360 attaches over the first opening 120, 220 by a press fit, a snap fit, a magnetic attachment, a latch mechanism or by any other mechanism for

removable attachment as may be known in the art. For the neurogenerative disease biomarkers targeted in the described embodiments, the disposable mouthpiece 360 can be used when the biological sample is saliva or breath, whereas this component can be removed when assessing biological fluids such as blood, CSF, etc.

[0067] In some embodiments prior to operation a fresh unused cartridge 150, 250 is removably inserted into a housing 110, 210 and the cap 170, 270 is affixed to the housing 110, 210. When the device 100, 200 is so assembled, for example, as illustrated in FIGS. 6 and 8, the detection chip 300 on the cartridge 150, 250 is in fluid communication with the first opening 120, 220.

[0068] As noted above, a person's breath can carry a biomarker or tiny aerosolized droplets containing a biomarker and/or molecular components of the biomarker, and certain biomarkers can be identified by a target protein (or amyloid) that makes up the biomarker. The detection chip 300, 420 can indicate the presence of the target molecule, protein or amyloid associated with the particular biomarker, for example the A β protein, the τ protein, and/or the α S protein. If the target molecule, and therefore the particular biomarker, is detected the detection chip 300, 420 in association with the attached circuitry 330, 430 sends a signal to the visual indicator 340, 440 disposed on the outside of the cap 170, 270 or housing 410. The visual indicator 340, 440 signals the illumination of a different color or colors preselected to represent a detection event. The color or colors, or the intensity thereof, or the number of individual LEDs illuminated could also be an indication of the concentration of the detection event. In another embodiment the visual indicator 340, 440 is electrically connected with the circuitry 330, 430 that includes a wireless transmitter that transmits data regarding the detection event to a computer or tablet or other portable or non-portable data storage device for analysis and/or immediate or delayed detection event display. In some embodiments the visual indicator 340, 440 can additionally flash and/or illuminate to signal a malfunction, low battery, or other error or problem.

[0069] Returning now to FIGS. 6-9, third openings 140, 240 allow for the user's breath to exit the housing 110, 210 without causing a pressure buildup therein. Making the mouthpiece 360 disposable allows for a fresh mouthpiece 360 to be installed on the device 100, 200 prior to each use thus lowering the risk of contamination between those persons tested.

[0070] In other embodiments illustrated for example as portions of FIGS. 6 and 8, the cartridge 150, 250 and cap 170, 270 can be attached to one another directly by any of the method

of attachment as described hereinabove or as otherwise known in the art and without the housing 110, 210. In these embodiments a user need only breathe onto the detection chip 300 to be tested for the presence of a target protein and therefore the associated biomarker.

[0071] In any of these embodiments, the biosensor device can be embedded in different assemblies and products, or appended, affixed, or removably affixed to clothing or hats via a clip, hook and loop fastener, or other fastening mechanisms known in the art that can accommodate a detection chip of this invention and hook or append the same to a target surface, such as, for instance, a hat or a mask. Moreover, the detection chip can be replaceable or disposable. Furthermore, the detection chip can be used to detect more than one biomarkers' presence by including an aptamer specifically created to detect the presence of each of a plurality of different biomarkers' nucleic acid or protein with particularity, each being identifiable by having a different color or colors, or illumination pattern coordinated with a detection event.

[0072] FIGS. 11A and 11B show diagrams illustrating an example of the final assembled portable and compact device, with electronics integrated with the sensor chip in accordance with the present technology. For example, in FIGS. 11A and 11B, a handheld device is shown that can be configured to receive the exemplary biosensor device comprising the sensor chip. In some examples, the handheld device is configured to perform a detection of the one or more neurogenerative disease biomarker(s) based on the one or more molecular probes (e.g., aptamer(s)) specifically binding to the one or more biomarker(s). In some examples, the handheld device comprises a wireless transceiver that is configured to transmit a result of the detection. The wireless transceiver may support at least one of a Bluetooth protocol, a Wi-Fi protocol, or a cellular protocol. In some embodiments, the handheld device includes a power source, one or more visual indicators, coupled to the power source, configured to indicate a start and a completion of the detection of the one or more neurogenerative disease biomarker(s), and a display, coupled to the power source, to present a result of the detection for each of the one or more neurogenerative disease biomarker(s). In some examples, the one or more visual indicators comprise LEDs, the display comprises an LCD, and the power source comprises one or more batteries.

[0073] FIG. 11C shows a diagram illustrating an example embodiment of a biosensor device 1100 for detecting a biomarker of a subject. Notably, for example, the Biomarker Sensor shown in FIG. 11A is an embodiment of the biosensor device 1100 shown in FIG. 11C. The biosensor

device 1100 includes a GFET-based detection chip 1110 and a functionalization layer 1120. The GFET-based detection chip 1110 includes a monolayer graphene 1111 on a substrate 1112 (e.g., silicon-based substrate); a source electrode (not shown in FIG. 11C) that comprises a conducting material and positioned at a first end of the monolayer graphene 1111; a drain electrode (not shown in FIG. 11C) that comprises the conducting material and positioned at a second end of the monolayer graphene 1111, a gate electrode (not shown in FIG. 11C) that comprises the conducting material and positioned in a same plane as the source electrode and the drain electrode, and a well (not shown in FIG. 11C) that comprises an insulating material and configured to receive a biological sample comprising a target molecular biomarker 1199 (e.g., a biomarker for dementia) when the biological sample is in contact with the monolayer graphene 1111. The GFET-based detection chip 1110 can include various embodiments disclosed herein, including but not limited to the GFET-based biosensor chip shown at operation 130 of FIG. 1 or the GFET shown in FIG. 5, among others. The functionalization layer 1120 includes a heterobifunctional linker molecule 1122 (e.g., 1-pyrene butanoic acid N hydroxysuccinimide (NHS) ester (PBASE), or pyrene maleimide, or other) and an aptamer 1124 coupled to the heterobifunctional linker molecule 1122. The functionalization layer 1120 is attached to the monolayer graphene 1111 of the GFET-based detection chip 1110 using the heterobifunctional linker molecule 1122. The aptamer 1124 is configured to specifically bind with the target molecular biomarker 1199 with at least a predetermined specificity, where the aptamer 1124 specifically binding with the target molecular biomarker 1199 causes a change in a conductance of the monolayer graphene 1111.

[0074] In some embodiments where the heterobifunctional linker molecule 1122 includes PBASE, for example, this allows the pyrene group to bond with the graphene carbon atom lattice through the pi stacking interactions between the pi bonds of the graphene and pyrene group. The pyrene molecule also is attached to another functional group on the aptamer 1124 that allows for binding of the aptamer 1124 (e.g., target probe molecule) to the interlinking pyrene functional molecule. In the case of PBASE, for example, this is an NHS ester group, which under the proper conditions, will form covalent peptide bonds with free tertiary amine groups. This allows modification of the aptamers of interest with a free amine on the 3' end of the DNA strand. In some embodiments where the heterobifunctional linker molecule 1122 includes pyrene maleimide, for example, this allows for the same pi-stacking interaction between the graphene

and linker molecule but focuses on a different reactive group on the probe, in this case a thiol group.

[0075] There are several unique features of the provided biosensor device. For example, first, the biosensor device possesses multi-target diagnostic capabilities, including (i) detection of biomarker particles with a resolution of less than 7 particles/sample; (ii) detection of molecular components of said biomarker, with detection limits in low nanomolar range; and (iii) detection of nucleic acids with single nucleotide resolution and femtomolar sensitivity. Second, the sensor surface is specifically processed and tuned to be charge sensitive to a higher degree of specificity. Third, electrical recording and electronic data analysis algorithm are designed to increase S/N ratio to distinguish smallest change in the Dirac potential minima. This allows for recording interaction of sample (e.g., biomarker proteins) to probe (e.g., aptamer) at the highest resolution (lowest number) with low power consumption. These features allow miniaturization and portability of the device.

[0076] Because of the design features, the biosensor device can achieve the following: (i) read-out in 10 minutes; (ii) sample can be from saliva, aerosols, and body fluids, e.g., blood or CSF; (iii) high accuracy (~95%); (iv) portability with low power (9V battery) requirement and cell phone-comparable dimension; (v) inexpensive mass production (\$10/test) capability; and (vi) non-technical operation requirement, i.e., easy-to-use with layman's training without any medical professional help.

3. Examples of using the biosensor device for detecting dementia

[0077] GFET characterization. A heterobifunctional linker molecule, e.g., 1-pyrene butanoic acid NHS ester (PBASE), was used as a linker between graphene and aptamer. The chemical functionalization of PBASE on graphene was examined by Raman spectroscopy on the bare graphene surface (with a surface area of $150 \times 250 \mu\text{m}^2$) and compared to a PBASE-modified graphene surface, using a 532 nm laser with 20x magnification (see brightfield images in FIGS. 12C and 12D, respectively). The Raman map of the 2D/G peak intensity ratios on 96 consecutive points on the brightfield image with $20 \mu\text{m}$ pitch indicates an average ratio of 1.9 corresponding to a uniform graphene monolayer on our sensor (see FIG. 12A). After PBASE conjugation, the ratio lowered to around 1.29 (see FIG. 12B), along with the formation of significant D and D' peaks. This indicates pyrene group binding and enhanced sp^2 bonding.

[0078] FIGS. 13A-13F shows an example of GFET sensor characterization using Raman

spectroscopy and atomic force microscopy (AFM). As shown therein, FIG. 13A shows an AFM height image of a bare GFET sensor with its section profile (denoted by the white line), FIG. 13B shows an AFM height image of a PBASE functionalized sensor, FIG. 13C shows an AFM height image of sensor post fully functionalizing and adding $A\beta_{1-42}$, and FIG. 13D shows an AFM height image of $A\beta_{1-42}$ on freshly cleaved mica showing a distribution of lower order oligomers and fibrils. In these plots, R_q is the root mean square (RMS) roughness of the entire AFM image, and all heights in AFM images are between 0-30 nm. FIG. 13E shows a Raman spectroscopy plot of bare graphene chip on a single $20\mu\text{m} \times 20\mu\text{m}$ area, whereas FIG. 13F shows a Raman spectroscopy plot of PBASE functionalized graphene on a single $20\mu\text{m} \times 20\mu\text{m}$ area.

[0079] As noted above, the morphology of the graphene surface was examined by atomic force microscopy (AFM). The R_q of bare graphene was 0.733 ± 0.20 nm, and PBASE functionalization increased surface roughness to 1.4 ± 0.6 nm. The additional roughness after the PBASE addition phase indicates the successful binding of pyrene and graphene pi-pi stacking interaction. An increase in the roughness was also observed when $A\beta_{1-42}$ was added to the fully functionalized chip, as seen in FIG. 13C.

[0080] *GFET biosensor validation.* To validate the aptamer-GFET biosensor platform as a quantitative and precise diagnostic tool, the lower limits of detection were determined for each neurodegenerative disease-associated biomarker ($A\beta$, Tau, αS) by measuring Dirac shifts after the application of various concentrations of these amyloids suspended in 0.1x PBS buffer (FIGS. 14A-14D). In FIGS. 14A-14C, the dotted black line represents the sensing threshold, which indicates a signal-to-noise ratio (SNR) of 3 (e.g., $3 \times$ the PBS control experiments Dirac shift) and corresponds to 60 mV, 60 mV, and 70 mV for $A\beta$, Tau, αS respectively. As seen therein, there is a precipitous drop in signal at concentrations < 10 fM indicating the possibility to detect concentrations of a biomarker in the femtomolar range but a significant reduction in signal below the 10-100 fM concentration range. Thus, the $A\beta$ and αS aptamer-GFET biosensor can detect proteins at concentrations with the lower limit of detection (LOD) of 10 fM (FIGS. 14A and 14B, respectively). Tau protein, likewise, can be detected at concentrations approaching 100 fM (FIG. 14C).

[0081] FIG. 14D shows the summary histograms of experimental results supporting the specificity of aptamer probes for their cognate proteins ($A\beta_{1-42}$, Tau, and αS). GFET response of synthetic $A\beta$, Tau, and αS peptides to their specific aptamers ($A\beta$ aptamer, Tau aptamer, αS

aptamer), are represented in the first three left bars. The next three bars show the significantly lower, non-specific response for amyloids tested against their non-specific aptamer. The last bar from the left indicates an average of the results of all three aptamers tested with their non-specific cognate proteins. The x-axis denotes the analyte protein tested using the aptamers. The positive controls had the correct protein added to the sample (bars to the left of the vertical dotted line). The negative controls are an average of both other proteins added to the incorrect aptamer chip (bars to the right of the vertical dotted line). The result labeled as Avg is an average of each of the negative control nonspecific protein experiments. Significant p-values between the cross-protein controls and correct protein-aptamer Dirac shift results are illustrated.

[0082] *Aptamer-specificity for A β , Tau and α S by cross-control experiments.* The efficacy of any sensor is reliant on the ability to detect a specific protein or analyte of interest without noise or interference from other non-target molecules in each sample. This was verified for the described GFET-based sensors by testing how the sensor, when functionalized with a specific aptamer, reacts with other proteins associated with neurodegenerative diseases. The aptamers discussed above were used and the synthetic proteins were at a set concentration of 50 nM. The Dirac voltage shifts for each of the combinations was measured, and the results of the non-specific, non-target protein on each aptamer GFET combination was averaged. The results indicate there was no statistically significant change in signal from adding non-target proteins to the sample and conclude that our GFET aptamers are specific to the target protein and not to non-specific adsorption at the surface of the graphene layer (see FIG. 14D). As shown in FIG. 14D, for a specific aptamer there is a Dirac shift of ~20-30 mV difference between the specific protein and a non-specific protein. Significant specificity of the A β aptamer to A β ₄₂ is seen, but there is a weaker significant response to A β ₄₀, and a no specificity (cross-reactivity) to a nonspecific viral protein in the supplementary information (e.g., as shown in FIG. 15).

[0083] More specifically, as seen in FIG. 15, A β ₁₋₄₀, when tested with an A β ₁₋₄₂ aptamer-functionalized GFET biosensors showed significantly non-specific response compared to testing A β ₁₋₄₂ proteins. Similarly, a non-amyloid protein (SARS-CoV2 spike protein) when tested using the A β aptamer shows significantly non-specific response. The reduced response to A β ₁₋₄₀ compared to that of A β ₁₋₄₂ indicates that with an aptamer specific to A β ₁₋₄₀, the quantification of the ratio of A β ₄₀/A β ₄₂ for diagnostic purposes could be possible. Additional controls with non-specific proteins from SARS-CoV2 are also shown against the A β ₁₋₄₂ aptamer. There is

functionality of SARS-CoV2 Spike protein detection using aptamers specific to the Spike protein with the GFET biosensors.

[0084] Detection threshold of GFET sensors for A β , Tau and α S. The sensitivity of the biosensor was measured using synthetically derived proteins in a controlled PBS (phosphate-buffered saline) buffer solution to define the detection threshold. In FIGS. 16A, 16B and 16C, A β ₁₋₄₂ at varying concentrations as well as PBS alone (control) were tested using A β aptamer functionalized GFET, Tau protein at varying concentrations as well as PBS alone (control) were tested using Tau aptamer, and α S protein at varying concentrations as well as PBS alone (control) were tested using α S aptamer, respectively. Each plot in FIGS. 16A-16C illustrates the significant p-values between the synthetic protein and PBS buffer control experiments. The Dirac voltage shift threshold line is shown as a reference for a theoretical cutoff signifying positive from negative results. In the case of A β , where higher soluble A β ₁₋₄₂ levels correlate more with normal cognition rather than an AD state, the greater Dirac shift will relate to less probable AD diagnosis.

[0085] Detection of brain-derived A β , Tau and α S proteins. The described embodiments can detect or diagnose physiological A β , tau, and α S from saliva, urine and other biofluids. The example biosensor platform was tested on brain-derived A β , tau, and α S oligomers at various sample dilutions on these amyloids. Herein, brain-derived A β shows a dose dependent response. The lower limit appears to be below physiological level at a concentration of $\sim$ 10fM, which was significantly resolvable with respect to PBS control. The A β -aptamer appears to have higher sensitivity than the other aptamers. Tau protein-specific ssDNA aptamer can detect a relatively low concentration of brain-derived Tau (10-100fM), and α S protein-specific ssDNA aptamer was able to detect as low as 10-100 nM concentration of brain-derived α S (as shown in FIGS. 17A-17C, wherein the solid ΔV_g Threshold line is the detection threshold for the Dirac shift in positive samples). FIG. 17D shows a comparison of the different brain-derived proteins against each other. The p-value of each result is shown with respect to a PBS buffer control.

[0086] The clinically significant levels of A β ₁₋₄₂ in CSF from AD-patients are $\sim$ 900 pg/mL (225 pM) and 600 pg/mL (150 pM) in healthy adults. A detection limit was calculated based upon a Dirac shift greater than $3 \times$ SNR ($3 \times$ the Dirac shift of PBS control experiment on specific aptamer). The GFET sensor's detection limit of 10 fM for A β indicates that the sensor is capable of detecting an even a lower A β concentration present in later stage AD patients (see FIG. 14A).

With the detection limit of 1-10 pM for Tau protein (see FIG. 14B), the GFET sensors can detect Tau in both healthy individuals (300 pg/mL or 5.5 pM) as well as in unhealthy patients (600 pg/mL or 11 pM). With a detection limit of 10-100 fM for synthetic α S (see FIG. 14C), the GFET sensors can detect α S in patients diagnosed with PD since these patients have higher levels of blood-plasma α -synuclein (3 pg/mL or 200 fM) as compared to healthy control patients (20 fg/mL or 1.33 fM).

[0087] *Aptamer selection and preparation.* An extensive literature review enabled shortlisting an aptamer for every protein based on the lowest disassociation constant (K_D). The A β , tau, and α S aptamers that were selected are listed in Table 1.

Table 1. Different aptamers used in detection of A β , Tau and α S proteins

Analyte (Aptamer name)	K_D (nM)	Sequence (5'-3')
Amyloid- β (A β 7-92-1H1)	63.4	CCG GTG GGG GAC CAG TAC AAA AGT GGG TAG GGC GGG TTG GAA AA (SEQ ID NO:1)
α -synuclein (F5R1)	2.4	ATC GAG TGT GTA CGG GGT CCG GTA GGG TGG CGA GGT CTT CCT GTC GTA GCA GGA TCC A (SEQ ID NO:2)
Tau441 (IT2)	5.5	CAG CAC CGT CAA CTG AAT AAG GAC TGC TTA GGA TTG CGA TGA TTC AGG GTG ATG CGA TGG AGA TGT (SEQ ID NO:3)

[0088] The A β aptamer was selected against A β ₁₋₄₂ monomers, the α S aptamer was specifically selected to bind to monomers, and the Tau aptamer was specifically designed for phosphorylated-Tau (pTau) and/or total-Tau (tTau). In some examples, the Tau aptamer was specifically designed for plasma p-tau217 or p-tau181. The aptamer sequences had an amino modification on the 3' end and manufactured such that they would come pre-diluted in IDTE buffer at pH 8.0 with a 100 μ M concentration. This would be further dissolved using 1 $\times$ PBS and 0.5 mM MgCl₂ to 1 μ M. The aptamers are then stored at -20 °C until further use. The specificity of the biosensors was tested with scrambled amino acid sequence amyloid-beta protein (see FIG. 18). Finally, the cross-reactivity of each aptamer with their non-specific amyloid proteins (e.g., aptamer for A β tested for τ and α S, aptamer for τ tested for A β and α S, and aptamer for α S tested for A β and τ , as shown in FIG. 14D) were also tested.

[0089] The A β aptamer was chosen as it is specific to the A β ₁₋₄₂ strand monomer, and the affinity of the aptamer to a specific 3D structure of A β was tested and compared the performance to a scrambled amino acid variant A β ₁₋₄₂ (see FIG. 18). The secondary and tertiary structures of the scrambled A β were different enough to indicate that the aptamer/sensor does not simply rely on electrostatic interactions but is also reliant on the secondary/tertiary structure of the target A β ₁₋₄₂. The results in FIG. 18 indicate the amino-acid sequence specific shift in the Dirac point significantly above the detection threshold when using the A β aptamer-functionalized GFET biosensors. The results in FIG. 18, for example, highlight the specificity of the aptamer biosensor to the specific amyloid-beta sequence.

[0090] *Reagents.* Synthetic A β ₁₋₄₂, Tau441, and α S were purchased from [Anaspec] Standard desalted quality 3' amino functionalized oligonucleotide aptamer probes for A β ₁₋₄₂, Tau441, and α S were used. The aptamers were obtained from IDT [Integrated DNA Technologies]. The nucleotide sequences are shown in Table 1. Molecular biology grade 1 $\times$ PBS [Gibco], MgCl₂ [Invitrogen], DMF [Sigma-Aldrich], Isopropyl Alcohol [Acros], Acetone [Fischer], and Ethanol [Decon Labs] were used throughout the study. Analytical-grade PBASE [Invitrogen] and ethanolamine [Alfa Aesar] were used without further processing.

[0091] *Brain Derived A β ₁₋₄₂.* Brain-derived samples of A β ₁₋₄₂ were collected with informed consent and according to prevalent institutional regulations. Briefly, postmortem brain tissue from AD patients were homogenized in PBS buffer with protease inhibitors. Purified A β ₁₋₄₂ was extracted from the PBS fraction via immunoprecipitation (using antibodies 4G8 and 6E10). The purified A β samples were then aliquoted and stored in -80° C until further use. Is it noted that the purified brain-derived A β samples are monomeric.

[0092] *Brain Derived Tau 441 and α S.* Postmortem brain tissue of AD patients was homogenized in phosphate-buffered saline (PBS) containing a protease inhibitor cocktail (Roche; 11836145001) using a brain-to-PBS dilution ratio of 1:3 (w/v). The samples were subsequently subjected to centrifugation at 10,000 rpm for 10 minutes at 4 $^{\circ}$ C. The resulting supernatants were aliquoted, rapidly frozen, and preserved at -80° C until further use.

[0093] Immunoprecipitation of toxic tau was performed. Briefly, tosyl-activated magnetic Dynabeads (Dynal Biotech, Lafayette Hill, PA) were coated with 20 μ g of T18 antibody (1.0 mg/ml) diluted in 0.1 M borate, pH 9.5, overnight at 37 $^{\circ}$ C. The beads were washed and exposed with PBS soluble AD post-mortem brain homogenate. The homogenate and bead

mixture were incubated at RT for 1 h. The beads were washed three times with PBS and eluted using 0.1M glycine, pH 2.8. The pH was then neutralized using 1M Tris base. The samples were then quantified using bicinchoninic acid protein assay and stored at -80°C until further use.

[0094] The human tau-441 isoform (2N4R) was expressed as a recombinant in E. coli BL21 (DE3) cells and purified. The monomer was seeded with brain derived tau at a ratio of 1:100 (w/w) with a rotation of 48h at 37°C . The samples were characterized using SDS-PAGE followed by western blotting and atomic force microscopy (AFM). The samples were then flash frozen until further use. αS were also expressed in E. Coli BL21(DE3) cells as described above. The purified tau proteins show both monomeric and dimeric forms.

[0095] αS oligomers were immunoprecipitated using F8H7 (a-synuclein) antibodies. Brain tissue of PD patients was homogenized in phosphate-buffered saline (PBS) with protease inhibitor cocktail (Cat. 11836145001, Roche Diagnostic). The samples were centrifuged at 10,000 rpm for 10 minutes at 4°C . The αS brain-derived samples when characterized by gel electrophoresis and silver staining show that post immunoprecipitation, the oligomers are mainly monomers, dimers, and trimers (see FIG. 19). Specifically, the gel revealed the presence of dimers and trimers (with disassociation constant (K_D) values of $\sim 30\text{kDa}$ and $\sim 45\text{kDa}$, respectively), in addition to monomeric species (with a K_D value of $\sim 15\text{kDa}$).

[0096] GFET sample preparation. The synthetic proteins, which are in lyophilized form, were diluted in 0.1 M sodium phosphate buffer and stored in 5 μL aliquots capable of serial dilution of the sample to the desired concentrations prior to testing. Brain-derived amyloid protein stock was received in buffer form with a concentration of 0.3-0.5 mg/mL and stored in 5 μL aliquots. The serial dilutions to the required concentrations were made in $0.1 \times \text{PBS}$ with 0.5 mM MgCl_2 which acts as the standard buffer throughout the described embodiments. These monomer dilutions would be prepared fresh (stored on ice) and used immediately or within 3-6 hours in order to prevent oligomerization.

[0097] GFET fabrication and characterization. The fabrication process included the following operations. The graphene was synthesized by low-pressure chemical vapor deposition (LPCVD) on 25 μm thick copper foil (MTI Corp.), then it was spin-coated at 3000 rpm for 45 sec by 120 K molecular weight poly methyl methacrylate (PMMA) for a PMMA assisted wet transfer process. Oxygen plasma etching was applied to remove the graphene on the backside of the copper foil. Ferric chloride solution was used to etch copper foil and subsequently rinsed

with deionized (DI) water. The large-sized PMMA/graphene film was transferred on a 4-inch SiO₂/Si substrate with 100 nm thick Au/Cr electrodes. For 1 hour, the PMMA was dissolved via acetone treatment, which was subsequently, followed by an application of isopropyl alcohol (IPA) rinse and nitrogen blow-dried. To protect the graphene channels and define a 500 μ m graphene channel length, photolithographic micro-patterning methods with PMGI photoresist were utilized. Excess graphene was removed via oxygen plasma etching. Followed by the removal of photoresist, the surface of graphene was further annealed at 200 °C for 2 hours under forming gas atmosphere to anneal impurities. Raman spectroscopies were performed on 96 consecutive points on the given brightfield image with 20 μ m pitch, which allowed the intensities to be mapped (see FIGS. 12A and 12B), and resistance measurements confirmed graphene monolayer quality of the GFET chips.

[0098] After dicing the patterned wafer, the GFET chips were glued to a PCB board/chip carrier and the gate, source and drain terminals were wire bonded to the contact pads. The Au/Cr electric pads and wire bonds were shielded from direct contact with the electrolyte solution with silicone paste, and a well (3-5 mm internal diameter), made of silicone tubing, was glued onto the chip to serve as a reservoir during derivatization and sample incubation. This process was automated, and additional characterization data was obtained using Raman spectroscopy and atomic force microscopy (AFM), see supplementary information.

[0099] In some embodiments, and different from previous techniques and implementations, the transfer of the graphene wafer to the silicon substrate is automated, which allows for the scaling up of production as and when necessary.

[00100] *Chip functionalization.* In order for the chips to specifically bind and target the proteins of interest, the graphene surface is modified with the aptamers specifically developed for binding said target proteins. This was achieved by first applying a layer of PBASE (1-Pyrenebutyric acid N-hydroxysuccinimide ester) to the graphene surface. The pyrene ring binds to the graphene surface by pi-bond interactions, known as pi-stacking. N-hydroxysuccinimide (NHS) modifications of the PBASE molecule bind to the amine group at the 3' end of the amine-modified ssDNA and allow the aptamer to fold freely on its own and bind to the target protein. The free NHS groups are passivated by adding ethanolamine after the aptamer addition step. The amine group binds to the NHS ester and eliminates the potential for reactivity with free-floating amine groups on non-target proteins and molecules.

[00101] *Experimental procedures.* Once the chips are functionalized, the buffer is added to the chip well and allowed to sit in moist conditions for a day to avoid potentially significant drift in the Dirac voltage and bring some stability. After 24 hours, the well is washed with a buffer to remove any salt deposits. Finally, 10 μL of the buffer is added to the chip well and measured using the biosensor device. This PBS Dirac point acts as the baseline measurement. Anywhere between 3 to 4 such baseline measurements in 10-minute intervals are taken to reach a relatively consistent characteristic Dirac voltage. Following this, 10 μL of the required sample is added and incubated for 10 minutes. This new addition is then measured on the biosensor device, where 3 to 4 consecutive measurements are taken in 10-minute intervals, and acts as the sample Dirac voltage. The difference between the final baseline measurement and sample Dirac voltage constitutes the characteristic Dirac shift for a given chip, and this procedure is repeated on many such chips. In some embodiments, every chip has its own characteristic measurements and will be different from the others therefore, and the use of chip-specific baseline calculations is critical. The biosensor device can be connected to a computer remotely for data transmission which would enable clinicians to easily access patient data.

[00102] *Quality control metrics.* For the GFET biosensors, the sensitivity of graphene as a biosensor template is optimized when a uniform monolayer of graphene is used. GFET mass production introduced challenges with consistent data and results from the variation of the surface layer. These challenges were overcome by first controlling for the drain-source resistance and limited the analysis to biosensors that were within the range of 1-10 $\text{k}\Omega$. The disclosed technology includes an algorithmic approach to identify the non-functioning chips automatically, e.g., they are easily distinguishable by their characteristic low signal-to-noise ratio (SNR) and flattened I-V curve shape, as opposed to the characteristic graphene U-shaped IDS vs VGS curve (ambipolar I-V curve). A hyperbolic curve is first fitted to the IDS-VGS plot of the graphene voltage sweep for 100 good and bad data sets, which yielded the relative distribution of parameter values (a, b, c, d) of the hyperbolic equation shown below.

$$\frac{(x - c)^2}{a^2} + \frac{(y - d)^2}{b^2} = 1$$

[00103] A threshold value was set to 3 times the standard deviation beyond the minimum and maximum values for each of the parameters. This fit was then applied to each new dataset and a score was calculated based on how many of these parameters were outside the established range

of good data parameter values. This same methodology was also used for a basic parabolic graph fit and the same parameter range and curve scoring method was used for determining the likelihood of a poor graphene chip. Additional scores were calculated from the peak-to-peak variation by finding the difference from the low-pass filtered data and test fitting straight lines to both linear regions on either side of the Dirac point. The results of these quality control experiments are summarized in FIG. 20. In this figure, the first (left-side) column shows metrics for a good chip, whereas the second (right-side) column shows metrics for a bad chip. The top graphs are from a hyperbolic fit with the raw data, the middle graphs are from a parabolic fit, and the bottom graphs are from a linear fit to either side of the linear regime of the Dirac curve.

[00104] *Statistical analysis.* Certain datasets were excluded depending on the validity of the data generated from the GFET biosensor chips. Multiple baseline measurements were taken, and then those results that showed a greater than 8% variation in Dirac point among each I-V sweep were excluded to eliminate those chips that have significant drift in a control buffer solution and improve the sensitivity of the biosensor results. Additional quality checks were conducted by measuring the drift in consecutive sweeps on a single chip. If a baseline buffer sample showed a significant coefficient of variation greater than 0.08, those chip results were also excluded as this could be indicative of a faulty GFET chip and surface impurities. The p values were calculated using a standard heteroscedastic two-tailed student t-test for all results presented. The standard deviation of multiple Dirac sweeps would indicate the error in the Dirac point for a given chip. The average error of multiple chips would be the standard deviation of the Dirac points from the multiple chips. The error bars in each graph are indicative of the average plus the standard deviation across multiple chips (3-5). The LOD was calculated as the concentration at which signal reaches $3 \times \text{SNR}$ (signal to noise ratio). The baseline signal was determined as the Dirac shift of 4-5 chips averaged together in PBS buffer (20 mV $\text{A}\beta$, 20 mV Tau, 25 mV αS).

3.1 Examples of using the biosensor device for detecting neurogenerative diseases

[00105] As discussed earlier, the described methods, systems, and devices can be configured to detect any misfolded protein (or amyloid) biomarker using the corresponding aptamer that has a high-affinity for that specific misfolded protein or amyloid. Table 2 shows some example protein biomarkers and concentrations that the disclosed methods, systems, and devices can be configured to detect and corresponding disease or degenerative conditions that could be diagnosed accordingly.

Table 2: Correlations between diseases and protein biomarkers for diagnosis

Protein Biomarker	Disease concentration	Biomarker combination criteria	Diagnosis
Amyloid- β A β 1-42, A β 1-40	- Ab142/p-Tau ratio > 6.5 - Ab142/Ab140 < 15-20% * Threshold (longitudinal) - Ab142 < 530ng/l	- Ab142/Ab140 (concentration ratio) - p-Tau/t-Tau (concentration ratio) - p – Tau181/Ab142 (concentration ratio) - Ab142 < Threshold conc + t-Tau > Threshold conc	- Alzheimer's Disease (AD) - Mild Cognitive Impairment (MCI)
Total Tau (t-Tau)	T-Tau > 350ng/l	t-Tau > Threshold, p-Tau = normal	- Rapid neurodegeneration - Creutzfeldt-Jakob disease - autoimmune encephalitis - FTD
Phospho Tau (p-Tau) p-Tau 181, p-Tau 217, p-Tau 231	- P-Tau > 300-500% * Threshold - P-Tau > 60ng/l		AD
alpha-Synuclein (α S)	αS < 3000 pg/ml	αS < Threshold conc	- Parkinson's Disease (PD) - Dementia with Lewy Bodies
Neurofilament Light Chain (NfL)		NfL > Threshold	- AD - Amyotrophic Lateral Sclerosis (ALS) - Frontotemporal Dementia (FTD) - Biomarker for severity of neuronal degradation
Glial Fibrillary Acidic Protein (GFAP)		GFAP > Threshold	- Early AD - Cognitive Decline

[00106] As shown above, various protein biomarkers in certain concentrations are indicative of the presence (or absence) of a neurogenerative disease in the subject. The disclosed

technology can be advantageously used to determine the concentration of a misfolded protein for which a corresponding aptamer that has high-affinity for that biomarker is available (e.g., determined using an extensive literature search and SELEX (systematic evolution of ligands by exponential enrichment) and/or protein data bank (PDB)).

[00107] In some embodiments, the threshold corresponds to an average concentration of the biomarker in age-matched healthy individuals population. For example, if a particular subject has a concentration of a biomarker (or a ratio of biomarkers) that is significantly higher than the average of their cohort (e.g., age-matched healthy individuals), then it is likely that the subject suffers from the corresponding neurogenerative disease.

[00108] In other embodiments, the threshold corresponds to the concentration of a biomarker at a first time ($t=0$) for a subject, which can serve as a baseline measurement for that subject. In some examples, the baseline measurement can be adjusted based on the biographic information of the subject or their cohort, e.g., the baseline measurement can be adjusted lower if there is a family history of the neurogenerative disease, which may result in being able to provide preventative treatment to the subject because a particular neurogenerative disease is identified at an earlier stage of its progression. The biomarker concentration of the subject is then retested at a second time ($t=1$) that is after the first time, and the relative concentrations between the first and second times can be indicative of whether the neurogenerative disease has progressed. Similarly, the biomarker concentration of the subject is then retested at a third time ($t=2$) or subsequent time(s) ($t=\dots n$) that is after the second time, and the relative concentrations between the third or subsequent time and a previous time (e.g., which can be the baseline first time, second time or other) can be indicative of whether the neurogenerative disease has progressed or further progressed.

4. Additional examples and embodiments of the disclosed technology

[00109] The world is continuing to face an increasingly aging population, which is exacerbated by declining birth rates and increased life expectancy. This brings to the forefront of modern medicine the need to better understand, prevent and treat elderly patients who are at risk of developing AD, PD, and other neurodegenerative diseases. In addition to age-related risk factors, traumatic brain injury (TBI) is being studied as a potential risk factor for the development of neurodegenerative disease such as AD or encephalopathy.

[00110] The disclosed aptamer-modified GFET biosensor systems, devices, and methods are

envisioned to be used to diagnose patients with various neurodegenerative diseases using readily available biofluids such as saliva, urine etc. Older individuals or those at risk for AD and PD could take these tests at a point-of-care location on a routine basis and track the progression of the disease over time. In some embodiments, for example, an aptamer-modified GFET device can be configured with an electronic reader and GFET chips, which can be one-time use disposable components, for point-of-care facilities, clinics or businesses of interest. In some embodiments, for example, an aptamer-modified GFET device can be configured into an at-home test for diagnosing patients and measuring disease progression over months and weeks. Other example applications can include detecting severe head trauma in those who are at risk for developing CTE, including but not limited to athletes in high-impact sports (e.g., wrestling, football, boxing, etc.) and could be a means of monitoring the status of the patient's brain health over time. For example, a multiplexed GFET device and/or system (e.g., single chip with multiple GFET transistors) could utilize a combination of these neurodegenerative proteins and others to improve robustness of results and diagnose more complex neurodegenerative diseases which require parallel detection of multiple biomarkers.

[00111] FIG. 21 shows a flowchart of an example method 2100 for producing a biosensor device configured to detect a neurodegenerative disease biomarker of a subject. The method 2100 includes, at operation 2110, receiving a GFET-based detection chip. In example method 2100, the GFET-based detection chip comprises (i) monolayer graphene on a silicon-based substrate, (ii) a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene surface, (iii) a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene surface, (iv) a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and (v) a well formed using an insulating material and configured to receive a biological sample comprising a target molecular biomarker such that the biological sample is in contact with the monolayer graphene surface.

[00112] In some embodiments, a different conducting material is used for each of the source, drain, and gate electrodes (e.g., gold, silver, aluminum). In other configurations, two of the three electrodes (e.g., the source and drain electrodes) are made from a first conducting material (e.g., gold and chromium) and the third electrode (e.g., the gate electrode) is made from a second conducting material (e.g., aluminum and platinum).

[00113] The method 2100 includes, at operation 2120, functionalizing the monolayer graphene by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule. In some embodiments, the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene (e.g., which corresponds to the shift in the IV-curve).

[00114] In general, the heterobifunctional linker molecule has two regions that achieve two different functionalities — a first region (that includes one or more benzene ring structures) attaches to the monolayer graphene via pi-pi interactions (with the carbon rings in the graphene) and a second region that includes a functional group that can be used for covalent bonding. An example of a heterobifunctional linker molecule is PBASE, which undergoes the most energetically favorable interaction with graphene while allowing a biomolecule to be attached to the other end (the ester group). Alternatively, a different class of heterobifunctional linker molecules, such as pyrene maleimide that rely on a thiol derivative (instead of the pyrene unit) to support the pi-pi interactions, can be used in the functionalization of the monolayer graphene.

[00115] FIG. 22 shows a flowchart of an example method 2200 for detecting a molecular biomarker of a subject. The method 2200 includes, at operation 2210, receiving a biological sample comprising a target molecular biomarker for a neurogenerative disease.

[00116] The method 2200 includes, at operation 2220, contacting the biological sample with a biosensor device comprising a GFET-based detection chip. In example method 2200, the GFET-based detection chip comprises (i) monolayer graphene on a silicon-based substrate, (ii) a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene, (iii) a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene surface, (iv) a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and (v) a well formed using an insulating material and configured to receive the biological sample comprising a target molecular biomarker such that the biological sample is in contact with the monolayer graphene on the silicon-based substrate.

[00117] The method 2200 includes, at operation 2230, detecting, using the biosensor device, a presence and a concentration of the target molecular biomarker in the biological sample. In some embodiments, detecting the presence of the target molecular biomarker for the neurogenerative

disease is based on the monolayer graphene being functionalized by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule, the aptamer being configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and the presence of the target neurogenerative disease biomarker being detected based on a change in a conductance of the monolayer graphene (which corresponds to a shift in the IV-curve as previously discussed).

[00118] The method 2200 includes, at operation 2240, determining, based on comparing the concentration to a first threshold and a second threshold, whether the subject has a first neurogenerative disease or a second neurogenerative disease. As previously described in Section 3.1, the first and second neurogenerative diseases are detectable based on the specific aptamers having a high-affinity for the misfolded proteins associated with the first and second neurogenerative diseases. In some examples, the concentration corresponds to the presence of a target molecular biomarker for a neurogenerative disease. In other examples, the concentration corresponds to a ratio of a first target molecular biomarker to a second target molecular biomarker for the same neurogenerative disease.

5. Example Technical Solutions

[00119] Embodiments of the disclosed technology support the following example technical solutions that solve the technical problem of accurately, cheaply, and quickly detecting a dementia biomarker in biological samples.

[00120] Solution A1. A method for producing a biosensor device configured to detect a dementia biomarker of a subject, the method comprising: receiving a graphene field-effect transistor (GFET)-based detection chip, wherein the GFET-based detection chip comprises: monolayer graphene on a silicon-based substrate, a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene, a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene, a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and a well formed using an insulating material and configured to receive a biological sample comprising a target molecular biomarker for dementia such that the biological sample is in contact with the monolayer graphene; and functionalizing the monolayer graphene by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule, wherein the aptamer is configured to specifically bind with

the target molecular biomarker with at least a predetermined specificity, and wherein the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene.

[00121] Solution A2. The method of solution A1 or any of solutions A1 to A10, wherein the target molecular biomarker for dementia comprises an Amyloid beta ($A\beta$) protein, a Tau (τ) protein, or an α -Synuclein (αS) protein.

[00122] Solution A3. The method of solution A2 or any of solutions A1 to A10, further comprising: configuring the aptamer to be an $A\beta$ -aptamer that is selected against $A\beta$ monomers or oligomers when the target molecular biomarker comprises the $A\beta$ protein, configuring the aptamer to be a τ -aptamer that is designed for phosphorylated-Tau (pTau) or total-Tau (tTau) when the target molecular biomarker comprises the τ protein, or configuring the aptamer to be an αS -aptamer that is selected to bind with monomers when the target molecular biomarker comprises the αS protein.

[00123] Solution A3a1. The method of solution A3 or any of solutions A1 to A10, wherein the $A\beta$ -aptamer is $A\beta 7-92-1H1$.

[00124] Solution A3a2. The method of solution A3 or any of solutions A1 to A10, wherein the $A\beta$ monomers or oligomers include $A\beta 1-42$ monomers or oligomers.

[00125] Solution A3b. The method of solution A3 or any of solutions A1 to A10, wherein the τ -aptamer is IT2.

[00126] Solution A3c. The method of solution A3 or any of solutions A1 to A10, wherein the αS -aptamer is F5R1.

[00127] Solution A4. The method of any of solutions A1 to A3 or any of solutions A1 to A10, further comprising: selecting the aptamer from a plurality of aptamers, wherein each selected aptamer of the plurality of aptamers is configured to bind with a particular target molecular biomarker with a corresponding specificity associated with a dissociation constant, and wherein the predetermined specificity is the corresponding specificity of a selected aptamer that has a minimum value of the dissociation constant amongst the plurality of aptamers. In some embodiments, selecting the aptamers is based on an extensive literature review and using the SELEX (systematic evolution of ligands by exponential enrichment) process.

[00128] Solution A5. The method of any of solutions A1 to A4 or any of solutions A1 to A10, wherein the heterobifunctional linker molecule comprises a 1-pyrene butanoic acid N

hydroxysuccinimide (NHS) ester (PBASE).

[00129] Solution A6. The method of any of solutions A1 to A5 or any of solutions A1 to A10, wherein the change in the conductance causes a shift in a Dirac point of a current–voltage curve associated with the GFET-based detection chip, the aptamer, and the target molecular biomarker.

[00130] Solution A7. The method of any of solutions A1 to A6 or any of solutions A1 to A10, wherein the conducting material comprises gold (Au) and chromium (Cr).

[00131] Solution A8. The method of any of solutions A1 to A7 or any of solutions A1 to A10, wherein the conducting material comprises aluminum (Al) and platinum (Pt).

[00132] Solution A9. The method of solution A7 or A8 or any of solutions A1 to A10, wherein the insulating material comprises silicone.

[00133] Solution A10. The method of any of solutions A1 to A9, wherein the biological sample comprises blood, saliva, urine, or cerebrospinal fluid (CSF) from the subject.

[00134] Solution A11. A biosensor device for detecting a dementia biomarker of a subject, the biosensor device comprising: a graphene field-effect transistor (GFET)-based detection chip, comprising: monolayer graphene on a silicon-based substrate, a source electrode comprising a conducting material and positioned at a first end of the monolayer graphene, a drain electrode comprising the conducting material and positioned at a second end of the monolayer graphene, a gate electrode comprising the conducting material and positioned in a same plane as the source electrode and the drain electrode, and a well comprising an insulating material and configured to receive a biological sample comprising a target molecular biomarker for dementia such that the biological sample is in contact with the monolayer graphene; and a functionalization layer comprising (a) a heterobifunctional linker molecule and (b) an aptamer coupled to the heterobifunctional linker molecule, wherein the functionalization layer is attached to the monolayer graphene using the heterobifunctional linker molecule, and wherein the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and wherein the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene.

[00135] Solution A12. The biosensor device of solution A11 or any of solutions A11 to A19, wherein the target molecular biomarker comprises an Amyloid beta (A β) protein, a Tau (τ) protein, or an α -Synuclein (α S) protein.

[00136] Solution A13. The biosensor device of solution A12 or any of solutions A11 to A19,

wherein: when the target molecular biomarker comprises the A β protein, the aptamer comprises an A β -aptamer that is selected against A β monomers or oligomers, when the target molecular biomarker comprises the τ protein, the aptamer comprises a τ -aptamer that is designed for phosphorylated-Tau (pTau) or total-Tau (tTau), or when the target molecular biomarker comprises the α S protein, the aptamer comprises a α S-aptamer that is selected to bind with monomers.

[00137] Solution A13a1. The biosensor device of solution A13 or any of solutions A11 to A19, wherein the A β -aptamer is A β 7-92-1H1.

[00138] Solution A13a2. The biosensor device of solution A3 or any of solutions A1 to A10, wherein the A β monomers or oligomers include A β 1-42 monomers or oligomers.

[00139] Solution A13b. The biosensor device of solution A13 or any of solutions A11 to A19, wherein the τ -aptamer is IT2.

[00140] Solution A13c. The biosensor device of solution A13 or any of solutions A11 to A19, wherein the α S-aptamer is F5R1.

[00141] Solution A14. The biosensor device of any of solutions A11 to A13 or any of solutions A11 to A19, wherein the heterobifunctional linker molecule comprises a 1-pyrene butanoic acid N hydroxysuccinimide (NHS) ester (PBASE).

[00142] Solution A15. The biosensor device of any of solutions A11 to A14 or any of solutions A11 to A19, wherein the change in the conductance causes a shift in a Dirac point of a current-voltage curve associated with the GFET-based detection chip, the aptamer, and the target molecular biomarker.

[00143] Solution A16. The biosensor device of any of solutions A11 to A15 or any of solutions A11 to A19, wherein the conducting material comprises gold (Au) and chromium (Cr).

[00144] Solution A17. The biosensor device of any of solutions A11 to A16 or any of solutions A11 to A19, wherein the conducting material comprises aluminum (Al) and platinum (Pt).

[00145] Solution A18. The biosensor device of solution A16 or A17 or any of solutions A11 to A19, wherein the insulating material comprises silicone.

[00146] Solution A19. The biosensor device of any of solutions A11 to A18, wherein the biological sample comprises blood, saliva, urine, or cerebrospinal fluid (CSF) from the subject.

[00147] Solution A20. A method for detecting a neurogenerative disease biomarker of a

subject, the method comprising: receiving a biological sample comprising a target molecular biomarker for a neurogenerative disease; contacting the biological sample with a biosensor device comprising a graphene field-effect transistor (GFET)-based detection chip; detecting, using the biosensor device, a presence and a concentration of the target molecular biomarker in the biological sample; and determining, based on comparing the concentration to a first threshold and a second threshold, whether the subject has a first neurogenerative disease or a second neurogenerative disease, wherein the GFET-based detection chip comprises: monolayer graphene on a silicon-based substrate, a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene, a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene, a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and a well formed using an insulating material and configured to receive the biological sample such that the biological sample is in contact with the monolayer graphene, wherein the monolayer graphene is functionalized by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule, wherein the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and wherein the presence of the target molecular biomarker is detected based on a change in a conductance of the monolayer graphene.

[00148] Solution A21. The method of solution A20 or any of solutions A20 to A32, wherein the target molecular biomarker for the neurogenerative disease comprises an Amyloid beta ($A\beta$) protein, a Tau (τ) protein, or an α -Synuclein (αS) protein.

[00149] Solution A22. The method of solution A21 or any of solutions A20 to A32, further comprising: configuring the aptamer to be an $A\beta$ -aptamer that is selected against $A\beta$ monomers or oligomers when the target molecular biomarker comprises the $A\beta$ protein, configuring the aptamer to be a τ -aptamer that is designed for phosphorylated-Tau (pTau) or total-Tau (tTau) when the target molecular biomarker comprises the τ protein, or configuring the aptamer to be an αS -aptamer that is selected to bind with monomers when the target molecular biomarker comprises the αS protein.

[00150] Solution A22a1. The method of solution A22 or any of solutions A20 to A32, wherein the $A\beta$ -aptamer is A β 7-92-1H1.

[00151] Solution A22a2. The method of solution A22 or any of solutions A20 to A32,

wherein the A β monomers or oligomers include A β 1-42 monomers or oligomers.

[00152] Solution A22b. The method of solution A22 or any of solutions A20 to A32, wherein the τ -aptamer is IT2.

[00153] Solution A22c. The method of solution A22 or any of solutions A20 to A32, wherein the α S-aptamer is F5R1.

[00154] Solution A23. The method of solution A21 or any of solutions A20 to A32, wherein the first neurogenerative disease is Alzheimer's disease and the second neurogenerative disease is mild cognitive impairment, wherein the first threshold is greater than the second threshold, and wherein the first threshold and the second threshold are greater than zero.

[00155] Solution A24. The method of solution A23 or any of solutions A20 to A32, further comprising: determining, based on the concentration being greater than the first threshold and the second threshold, that the subject has the first neurogenerative disease.

[00156] Solution A25. The method of solution A23 or any of solutions A20 to A32, further comprising: determining, based on the concentration being greater the second threshold and less than the first threshold, that the subject has the second neurogenerative disease.

[00157] Solution A26. The method of solution A23 or any of solutions A20 to A32, further comprising: determining, based on the concentration being less than the first threshold and the second threshold, that the subject is free from both the first neurogenerative disease and the second neurogenerative disease.

[00158] Solution A27. The method of any of solutions A20 to A26 or any of solutions A20 to A32, further comprising: selecting the aptamer from a plurality of aptamers, wherein each selected aptamer of the plurality of aptamers is configured to bind with a particular target molecular biomarker with a corresponding specificity associated with a dissociation constant, and wherein the predetermined specificity is the corresponding specificity of a selected aptamer that has a minimum value of the dissociation constant amongst the plurality of aptamers.

[00159] Solution A28. The method of any of solutions A20 to A27 or any of solutions A20 to A32, wherein the heterobifunctional linker molecule comprises a 1-pyrene butanoic acid N hydroxysuccinimide (NHS) ester (PBASE).

[00160] Solution A29. The method of any of solutions A20 to A28 or any of solutions A20 to A32, wherein the change in the conductance causes a shift in a Dirac point of a current-voltage curve associated with the GFET-based detection chip, the aptamer, and the target molecular

biomarker.

[00161] Solution A30. The method of any of solutions A20 to A29 or any of solutions A20 to A32, wherein the conducting material comprises (a) gold (Au) and chromium (Cr) or (b) aluminum (Al) and platinum (Pt).

[00162] Solution A31. The method of solution A30 or any of solutions A20 to A32, wherein the insulating material comprises silicone.

[00163] Solution A32. The method of any of solutions A20 to A31, wherein the biological sample comprises blood, saliva, urine, or cerebrospinal fluid (CSF) from the subject.

[00164] Embodiments of the disclosed technology support the following additional example technical solutions that solve the technical problem of accurately, cheaply, and quickly detecting a dementia biomarker in biological samples.

[00165] Solution B1. A device for detecting a biological marker, comprising: a graphene field effect transistor (GFET) device comprising a graphene surface; and one or more aptamers attached to the graphene surface, wherein the one or more aptamers are configured to specifically bind to one or more target molecules.

[00166] Solution B2. The device of solution B1 or any of solutions B1 to B7, wherein the GFET device includes: a substrate having the graphene surface; a conducting material at a first end and a second end of the graphene surface that form a first electrode and a second electrode, respectively; and an insulating material to insulate the first electrode and the second electrode.

[00167] Solution B3. The device of solution B2 or any of solutions B1 to B7, wherein the insulating material is structured to include a well to receive a biological sample such that the biological sample is in contact with the one or more aptamers.

[00168] Solution B4. The device of any of solutions B1 to B3 or any of solutions B1 to B7, further comprising: an electronic reader operable to apply a fixed drain-source voltage and sweep a gate voltage to obtain a characteristic Dirac voltage value and Dirac shift value.

[00169] Solution B5. The device of any of solutions B1 to B4 or any of solutions B1 to B7, wherein the one or more target molecules include Amyloid Beta₁₋₄₂ protein, Tau protein, or Alpha-Synuclein protein.

[00170] Solution B6. The device of any of solutions B1 to B5 or any of solutions B1 to B7, wherein the biological sample includes at least one of cerebrospinal fluid (CSF), blood plasma, blood serum, saliva, or urine.

[00171] Solution B7. The device of any of solutions B1 to B6, wherein the one or more aptamers includes a ssDNA aptamer.

6. Conclusion

[00172] Implementations of the subject matter and the functional operations described in this patent document can be implemented in various systems, digital electronic circuitry, or in computer software, firmware, or hardware, including the structures disclosed in this specification and their structural equivalents, or in combinations of one or more of them. Implementations of the subject matter described in this specification can be implemented as one or more computer program products, i.e., one or more modules of computer program instructions encoded on a tangible and non-transitory computer readable medium for execution by, or to control the operation of, data processing apparatus. The computer readable medium can be a machine-readable storage device, a machine-readable storage substrate, a memory device, a composition of matter effecting a machine-readable propagated signal, or a combination of one or more of them. The term “data processing unit” or “data processing apparatus” encompasses all apparatus, devices, and machines for processing data, including by way of example a programmable processor, a computer, or multiple processors or computers. The apparatus can include, in addition to hardware, code that creates an execution environment for the computer program in question, e.g., code that constitutes processor firmware, a protocol stack, a database management system, an operating system, or a combination of one or more of them.

[00173] A computer program (also known as a program, software, software application, script, or code) can be written in any form of programming language, including compiled or interpreted languages, and it can be deployed in any form, including as a stand-alone program or as a module, component, subroutine, or other unit suitable for use in a computing environment. A computer program does not necessarily correspond to a file in a file system. A program can be stored in a portion of a file that holds other programs or data (e.g., one or more scripts stored in a markup language document), in a single file dedicated to the program in question, or in multiple coordinated files (e.g., files that store one or more modules, sub programs, or portions of code). A computer program can be deployed to be executed on one computer or on multiple computers that are located at one site or distributed across multiple sites and interconnected by a communication network.

[00174] The processes and logic flows described in this specification can be performed by one

or more programmable processors executing one or more computer programs to perform functions by operating on input data and generating output. The processes and logic flows can be performed by, and apparatus can also be implemented as, special purpose logic circuitry, e.g., an FPGA (field programmable gate array) or an ASIC (application specific integrated circuit).

[00175] Processors suitable for the execution of a computer program include, by way of example, both general and special purpose microprocessors, and any one or more processors of any kind of digital computer. Generally, a processor will receive instructions and data from a read only memory or a random access memory or both. The essential elements of a computer are a processor for performing instructions and one or more memory devices for storing instructions and data. Generally, a computer will also include, or be operatively coupled to receive data from or transfer data to, or both, one or more mass storage devices for storing data, e.g., magnetic, magneto optical disks, or optical disks. However, a computer need not have such devices. Computer readable media suitable for storing computer program instructions and data include all forms of nonvolatile memory, media and memory devices, including by way of example semiconductor memory devices, e.g., EPROM, EEPROM, and flash memory. The processor and the memory can be supplemented by, or incorporated in, special purpose logic circuitry.

[00176] While this patent document contains many specifics, these should not be construed as limitations on the scope of any invention or of what may be claimed, but rather as descriptions of features that may be specific to particular embodiments of particular inventions. Certain features that are described in this patent document in the context of separate embodiments can also be implemented in combination in a single embodiment. Conversely, various features that are described in the context of a single embodiment can also be implemented in multiple embodiments separately or in any suitable subcombination. Moreover, although features may be described above as acting in certain combinations and even initially claimed as such, one or more features from a claimed combination can in some cases be excised from the combination, and the claimed combination may be directed to a subcombination or variation of a subcombination.

[00177] Similarly, while operations are depicted in the drawings in a particular order, this should not be understood as requiring that such operations be performed in the particular order shown or in sequential order, or that all illustrated operations be performed, to achieve desirable results. Moreover, the separation of various system components in the embodiments described in this patent document should not be understood as requiring such separation in all embodiments.

[00178] Only a few implementations and examples are described and other implementations, enhancements and variations can be made based on what is described and illustrated in this patent document.

WHAT IS CLAIMED IS:

1. A method for producing a biosensor device configured to detect a dementia biomarker of a subject, the method comprising:
 - receiving a graphene field-effect transistor (GFET)-based detection chip,
 - wherein the GFET-based detection chip comprises:
 - monolayer graphene on a silicon-based substrate,
 - a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene,
 - a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene,
 - a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and
 - a well formed using an insulating material and configured to receive a biological sample comprising a target molecular biomarker for dementia such that the biological sample is in contact with the monolayer graphene; and
 - functionalizing the monolayer graphene by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule,
 - wherein the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and wherein the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene.
2. The method of claim 1, wherein the target molecular biomarker for dementia comprises an Amyloid beta (A β) protein, a Tau (τ) protein, or an α -Synuclein (α S) protein.
3. The method of claim 2, further comprising:
 - configuring the aptamer to be an A β -aptamer that is selected against A β monomers or oligomers when the target molecular biomarker comprises the A β protein,
 - configuring the aptamer to be a τ -aptamer that is designed for phosphorylated-Tau (pTau) or total-Tau (tTau) when the target molecular biomarker comprises the τ protein,
 - or

- configuring the aptamer to be an α S-aptamer that is selected to bind with monomers when the target molecular biomarker comprises the α S protein.
4. The method of any of claims 1 to 3, further comprising:
selecting the aptamer from a plurality of aptamers,
wherein each selected aptamer of the plurality of aptamers is configured to bind with a particular target molecular biomarker with a corresponding specificity associated with a dissociation constant, and
wherein the predetermined specificity is the corresponding specificity of a selected aptamer that has a minimum value of the dissociation constant amongst the plurality of aptamers.
5. The method of any of claims 1 to 4, wherein the heterobifunctional linker molecule comprises a 1-pyrene butanoic acid N-hydroxysuccinimide (NHS) ester (PBASE).
6. The method of any of claims 1 to 5, wherein the change in the conductance causes a shift in a Dirac point of a current–voltage curve associated with the GFET-based detection chip, the aptamer, and the target molecular biomarker.
7. The method of any of claims 1 to 6, wherein the conducting material comprises gold (Au) and chromium (Cr).
8. The method of any of claims 1 to 7, wherein the conducting material comprises aluminum (Al) and platinum (Pt).
9. The method of claim 7 or 8, wherein the insulating material comprises silicone.
10. The method of any of claims 1 to 9, wherein the biological sample comprises blood, saliva, urine, or cerebrospinal fluid (CSF) from the subject.
11. A biosensor device for detecting a dementia biomarker of a subject, the biosensor device comprising:
a graphene field-effect transistor (GFET)-based detection chip, comprising:
monolayer graphene on a silicon-based substrate,

a source electrode comprising a conducting material and positioned at a first end of the monolayer graphene,
a drain electrode comprising the conducting material and positioned at a second end of the monolayer graphene,
a gate electrode comprising the conducting material and positioned in a same plane as the source electrode and the drain electrode, and
a well comprising an insulating material and configured to receive a biological sample comprising a target molecular biomarker for dementia such that the biological sample is in contact with the monolayer graphene; and
a functionalization layer comprising (a) a heterobifunctional linker molecule and (b) an aptamer coupled to the heterobifunctional linker molecule,
wherein the functionalization layer is attached to the monolayer graphene using the heterobifunctional linker molecule, and
wherein the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and wherein the aptamer specifically binding with the target molecular biomarker causes a change in a conductance of the monolayer graphene.

12. The biosensor device of claim 11, wherein the target molecular biomarker comprises an Amyloid beta ($A\beta$) protein, a Tau (τ) protein, or an α -Synuclein (αS) protein.

13. The biosensor device of claim 12, wherein:
when the target molecular biomarker comprises the $A\beta$ protein, the aptamer comprises an $A\beta$ -aptamer that is selected against $A\beta$ monomers or oligomers,
when the target molecular biomarker comprises the τ protein, the aptamer comprises a τ -aptamer that is designed for phosphorylated-Tau (pTau) or total-Tau (tTau), or
when the target molecular biomarker comprises the αS protein, the aptamer comprises a αS -aptamer that is selected to bind with monomers.

14. The biosensor device of any of claims 11 to 13, wherein the heterobifunctional linker molecule comprises a 1-pyrene butanoic acid N hydroxysuccinimide (NHS) ester (PBASE).

15. The biosensor device of any of claims 11 to 14, wherein the change in the conductance causes a shift in a Dirac point of a current–voltage curve associated with the GFET-based detection chip, the aptamer, and the target molecular biomarker.
16. The biosensor device of any of claims 11 to 15, wherein the conducting material comprises gold (Au) and chromium (Cr).
17. The biosensor device of any of claims 11 to 16, wherein the conducting material comprises aluminum (Al) and platinum (Pt).
18. The biosensor device of claim 16 or 17, wherein the insulating material comprises silicone.
19. The biosensor device of any of claims 11 to 18, wherein the biological sample comprises blood, saliva, urine, or cerebrospinal fluid (CSF) from the subject.
20. A method for detecting a neurogenerative disease biomarker of a subject, the method comprising:
- receiving a biological sample comprising a target molecular biomarker for a neurogenerative disease;
 - contacting the biological sample with a biosensor device comprising a graphene field-effect transistor (GFET)-based detection chip;
 - detecting, using the biosensor device, a presence and a concentration of the target molecular biomarker in the biological sample; and
 - determining, based on comparing the concentration to a first threshold and a second threshold, whether the subject has a first neurogenerative disease or a second neurogenerative disease,
- wherein the GFET-based detection chip comprises:
- monolayer graphene on a silicon-based substrate,
 - a source electrode formed using a conducting material and positioned at a first end of the monolayer graphene,
 - a drain electrode formed using the conducting material and positioned at a second end of the monolayer graphene,

- a gate electrode formed using the conducting material and positioned in a same plane as the source electrode and the drain electrode, and
a well formed using an insulating material and configured to receive the biological sample such that the biological sample is in contact with the monolayer graphene,
wherein the monolayer graphene is functionalized by attaching an aptamer to the monolayer graphene using a heterobifunctional linker molecule,
wherein the aptamer is configured to specifically bind with the target molecular biomarker with at least a predetermined specificity, and
wherein the presence of the target molecular biomarker is detected based on a change in a conductance of the monolayer graphene.
21. The method of claim 20, wherein the target molecular biomarker for the neurogenerative disease comprises an Amyloid beta ($A\beta$) protein, a Tau (τ) protein, or an α -Synuclein (αS) protein.
22. The method of claim 21, further comprising:
configuring the aptamer to be an $A\beta$ -aptamer that is selected against $A\beta$ monomers or oligomers when the target molecular biomarker comprises the $A\beta$ protein,
configuring the aptamer to be a τ -aptamer that is designed for phosphorylated-Tau (pTau) or total-Tau (tTau) when the target molecular biomarker comprises the τ protein,
or
configuring the aptamer to be an αS -aptamer that is selected to bind with monomers when the target molecular biomarker comprises the αS protein.
23. The method of claim 21, wherein the first neurogenerative disease is Alzheimer's disease and the second neurogenerative disease is mild cognitive impairment, wherein the first threshold is greater than the second threshold, and wherein the first threshold and the second threshold are greater than zero.
24. The method of claim 23, further comprising:
determining, based on the concentration being greater than the first threshold and the second threshold, that the subject has the first neurogenerative disease.

25. The method of claim 23, further comprising:
determining, based on the concentration being greater the second threshold and less than the first threshold, that the subject has the second neurogenerative disease.
26. The method of claim 23, further comprising:
determining, based on the concentration being less than the first threshold and the second threshold, that the subject is free from both the first neurogenerative disease and the second neurogenerative disease.
27. The method of any of claims 20 to 26, further comprising:
selecting the aptamer from a plurality of aptamers,
wherein each selected aptamer of the plurality of aptamers is configured to bind with a particular target molecular biomarker with a corresponding specificity associated with a dissociation constant, and
wherein the predetermined specificity is the corresponding specificity of a selected aptamer that has a minimum value of the dissociation constant amongst the plurality of aptamers.
28. The method of any of claims 20 to 27, wherein the heterobifunctional linker molecule comprises a 1-pyrene butanoic acid N-hydroxysuccinimide (NHS) ester (PBASE).
29. The method of any of claims 20 to 28, wherein the change in the conductance causes a shift in a Dirac point of a current–voltage curve associated with the GFET-based detection chip, the aptamer, and the target molecular biomarker.
30. The method of any of claims 20 to 29, wherein the conducting material comprises (a) gold (Au) and chromium (Cr) or (b) aluminum (Al) and platinum (Pt).
31. The method of claim 30, wherein the insulating material comprises silicone.
32. The method of any of claims 20 to 31, wherein the biological sample comprises blood, saliva, urine, or cerebrospinal fluid (CSF) from the subject.

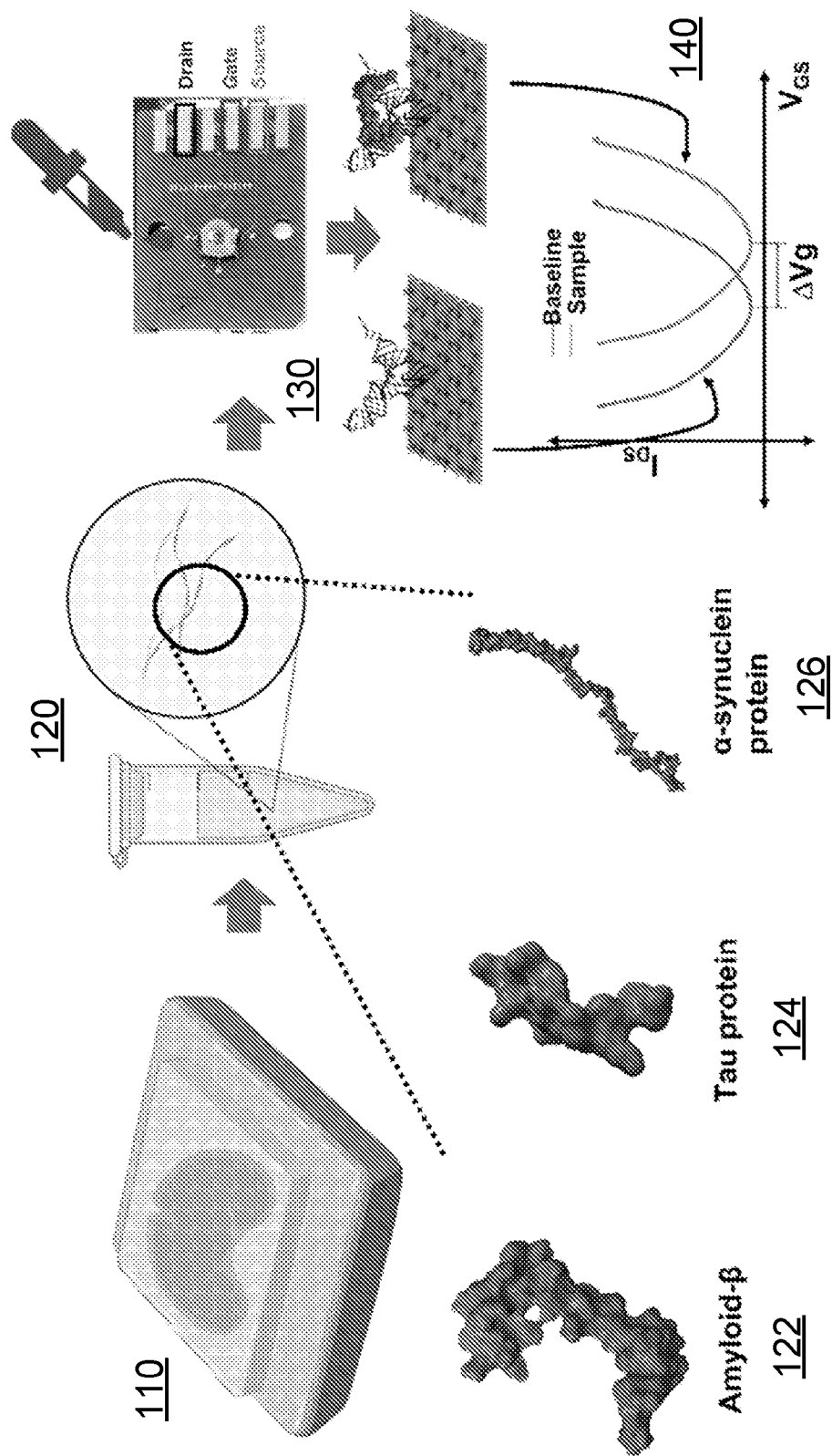


FIG. 1

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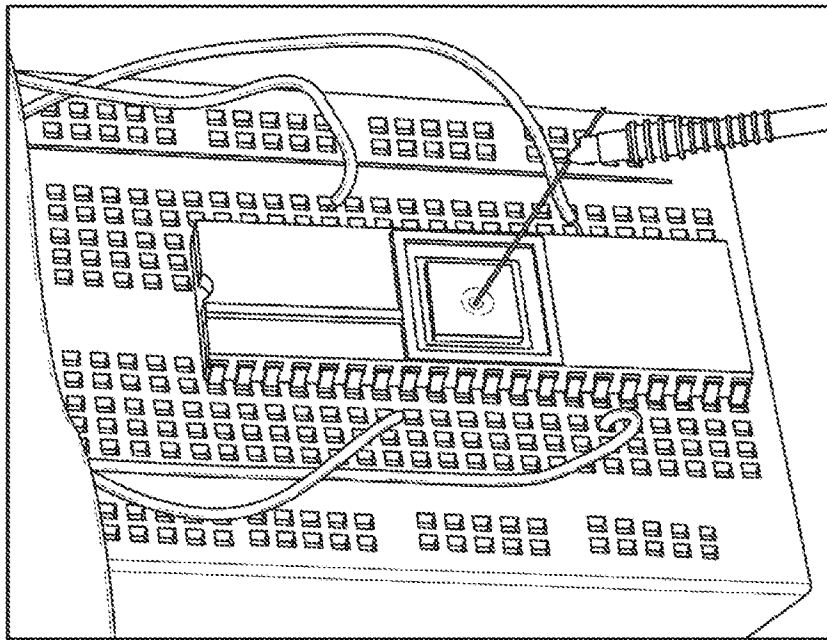


FIG. 2

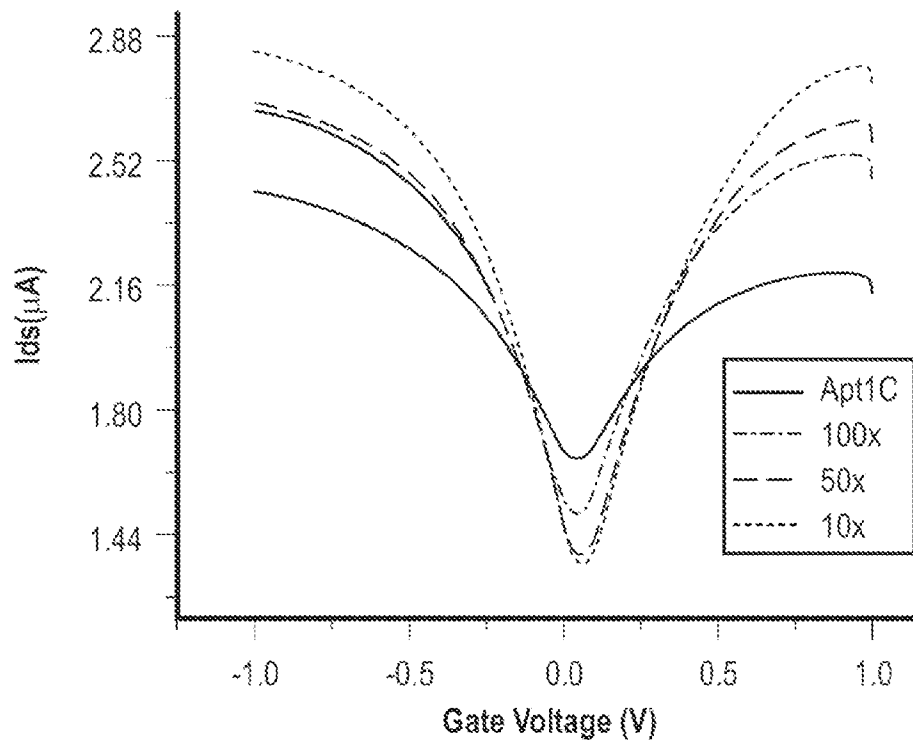


FIG. 3



FIG. 4A



FIG. 4B



FIG. 4C

SiO₂ Au/Cr Passivation layer PMGI photoresist PDMS (or epoxy)

O₂ plasma

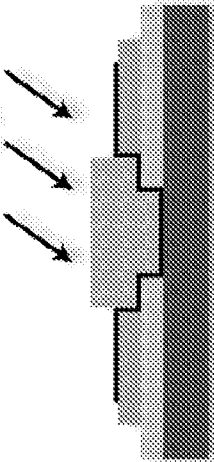


FIG. 4D

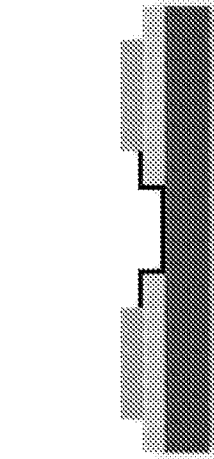


FIG. 4E

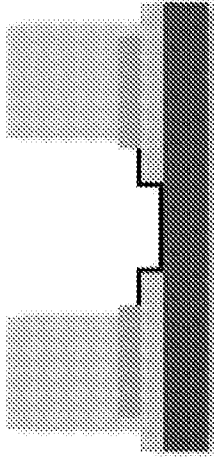


FIG. 4F

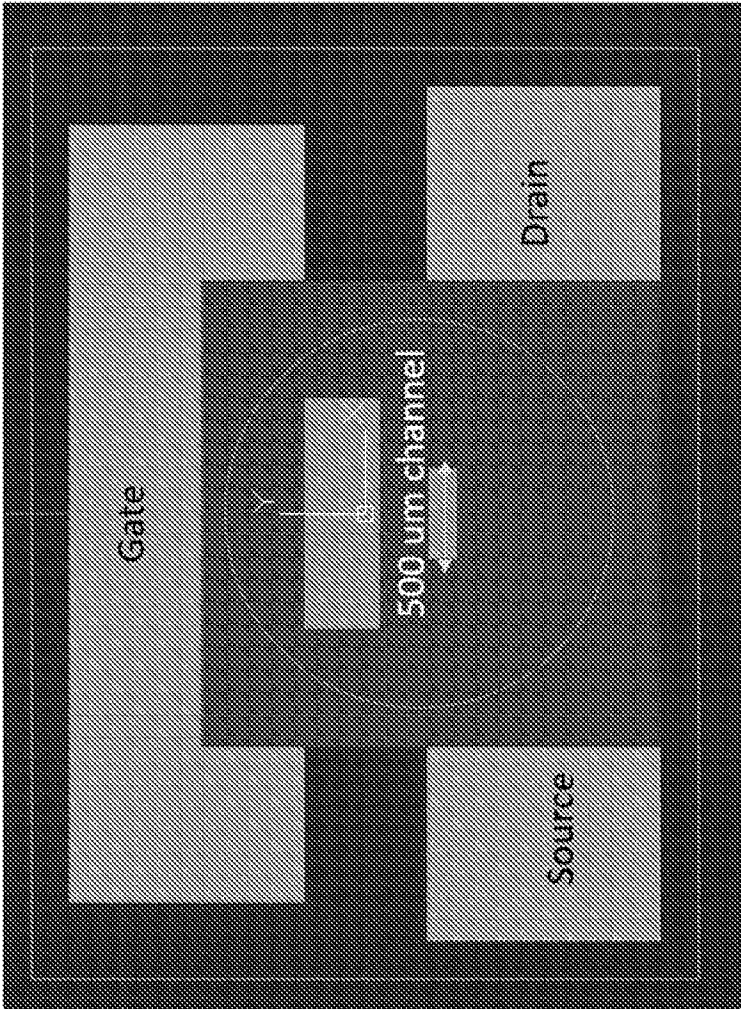
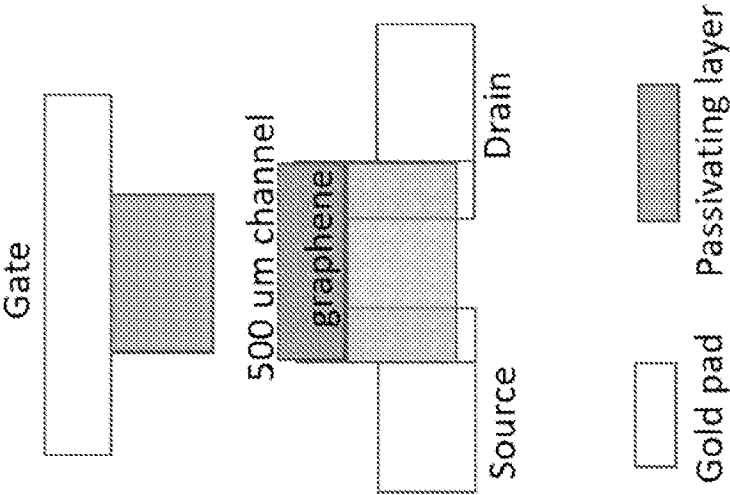


FIG. 5



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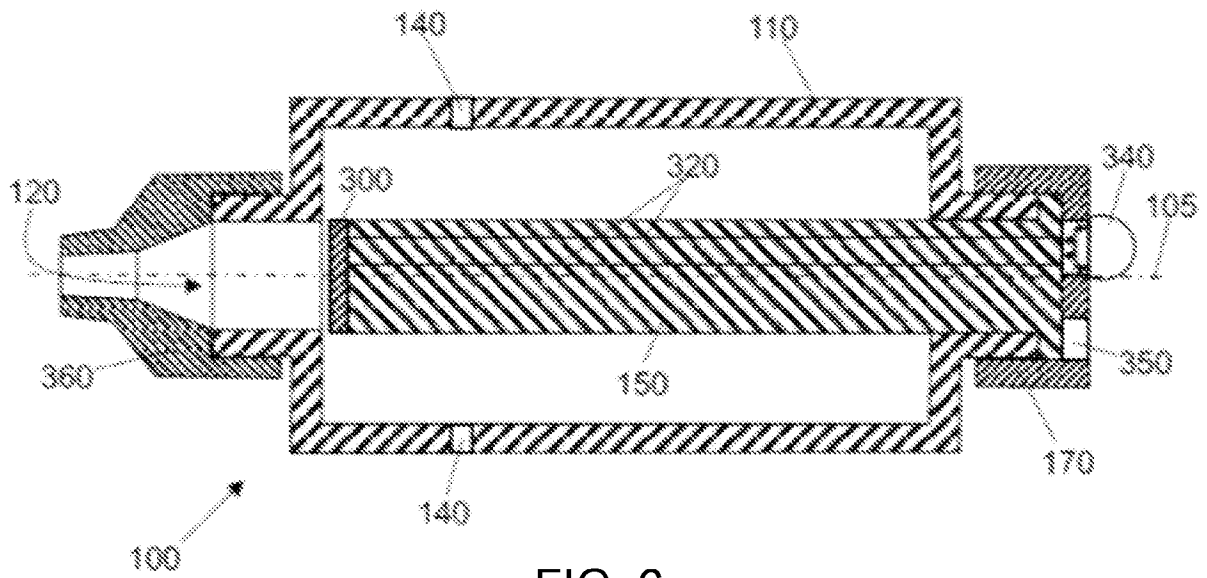


FIG. 6

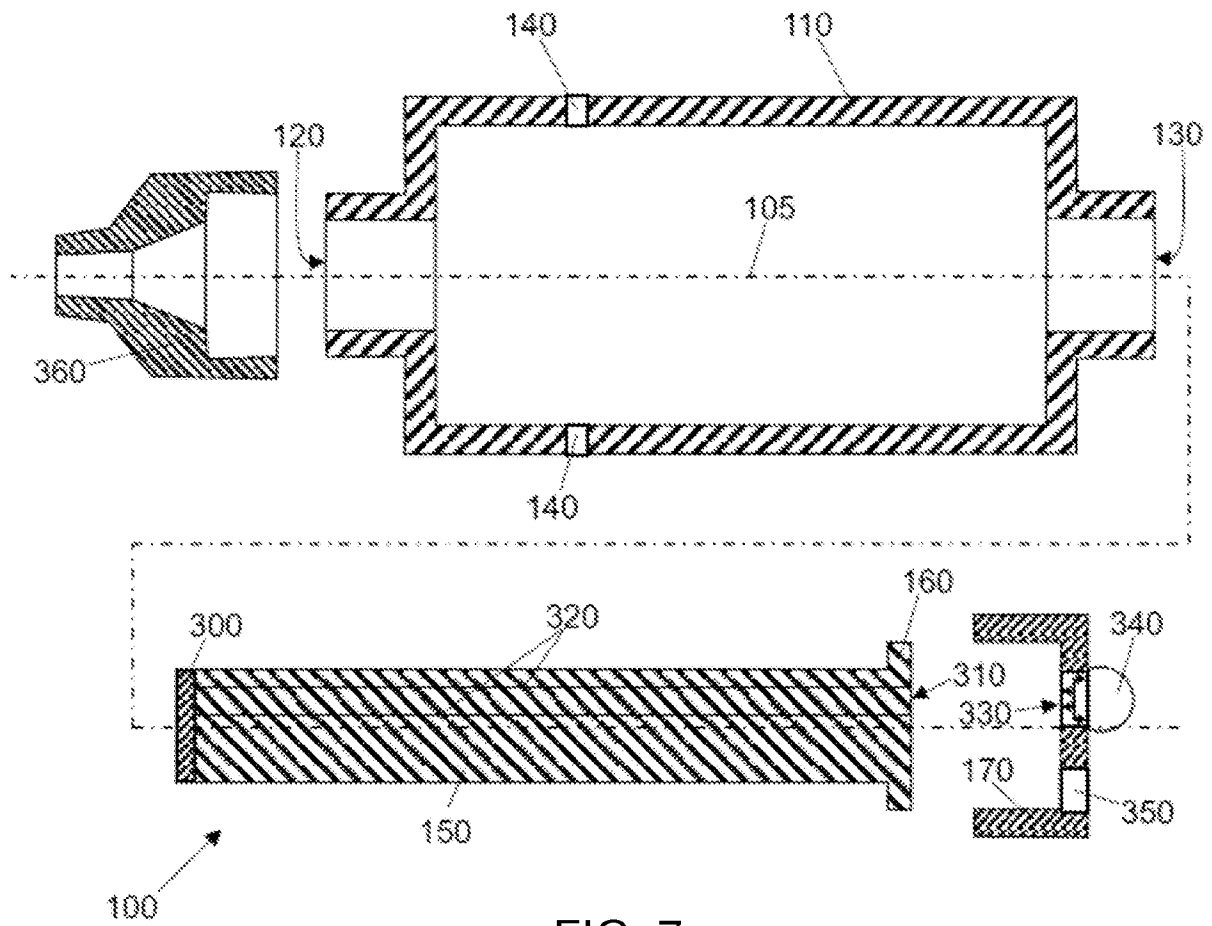


FIG. 7

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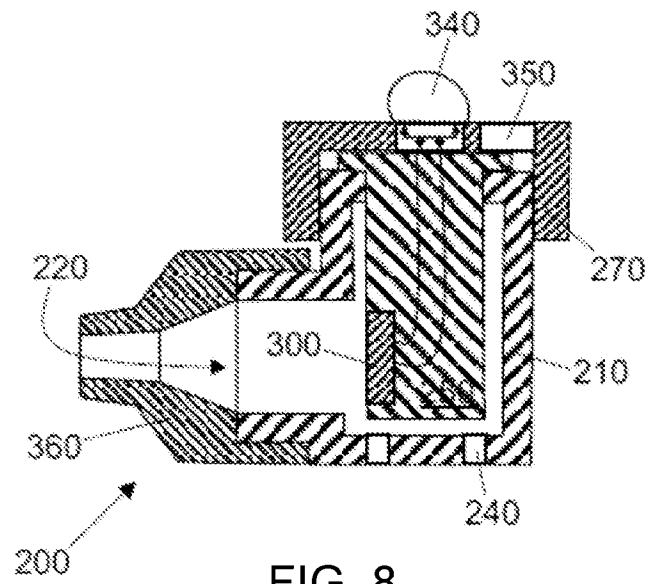


FIG. 8

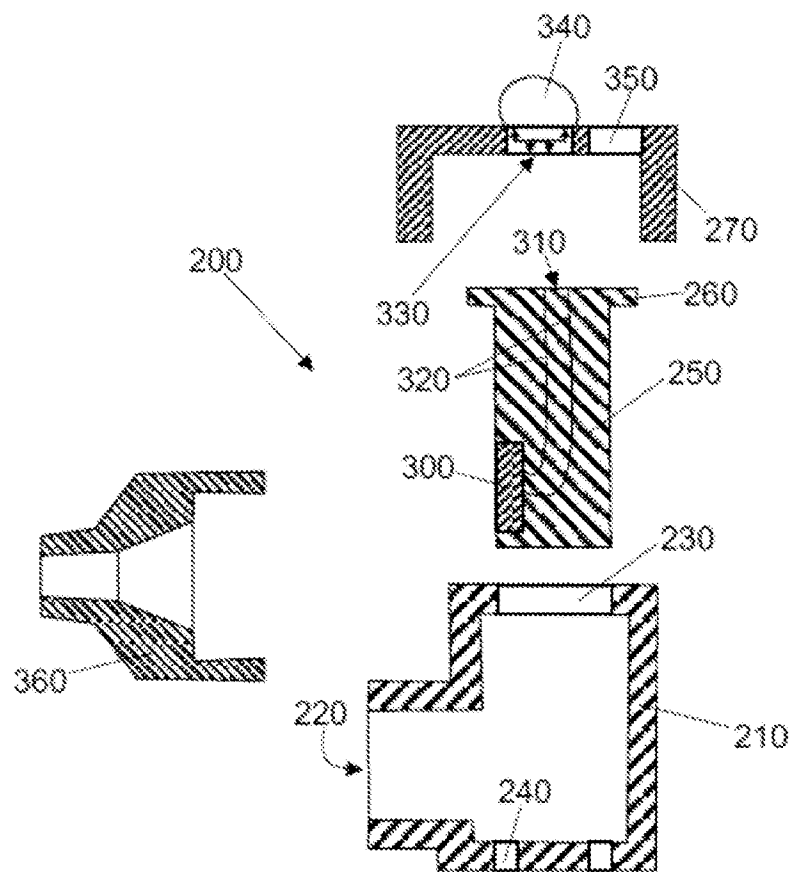


FIG. 9

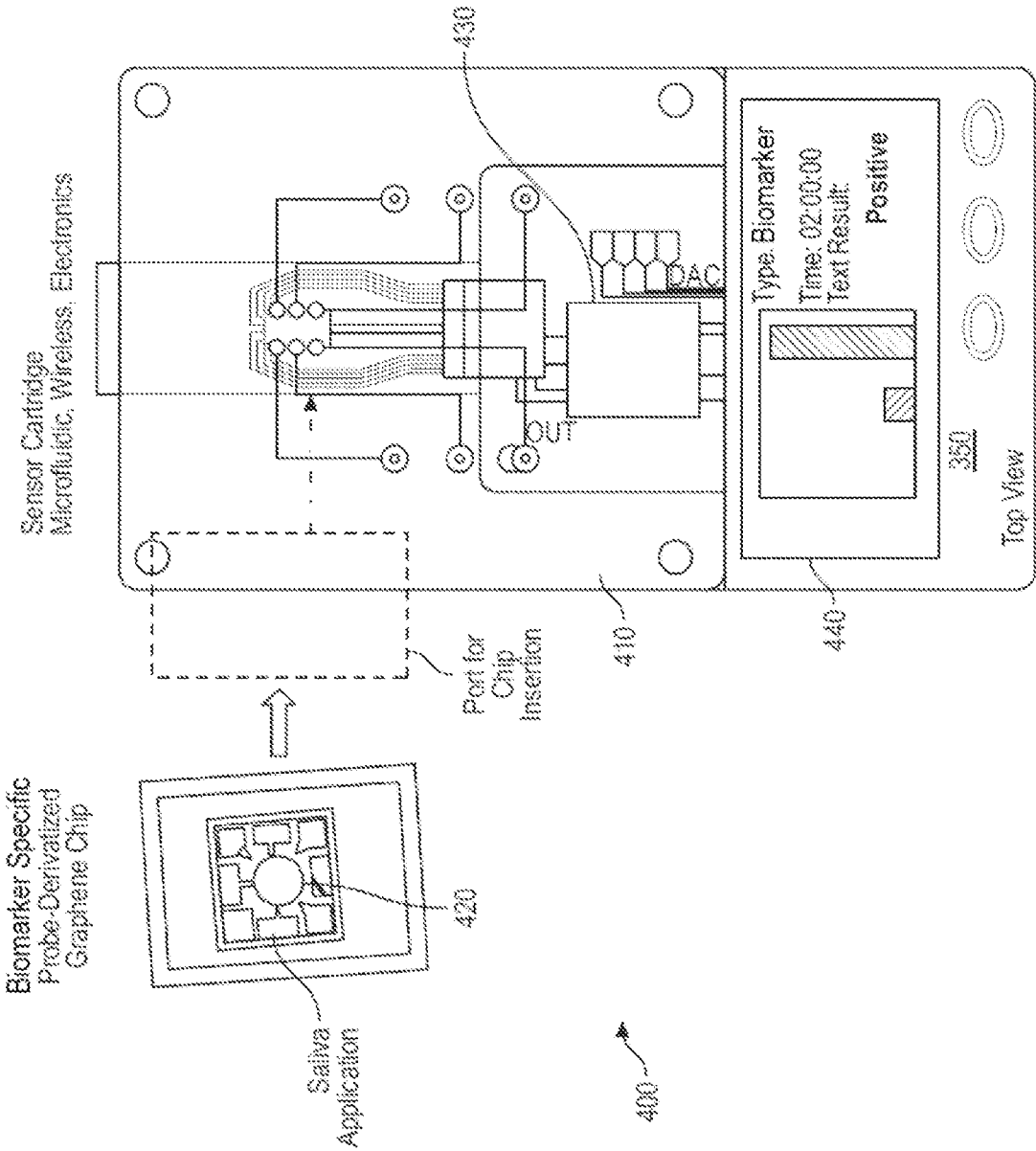


FIG. 10

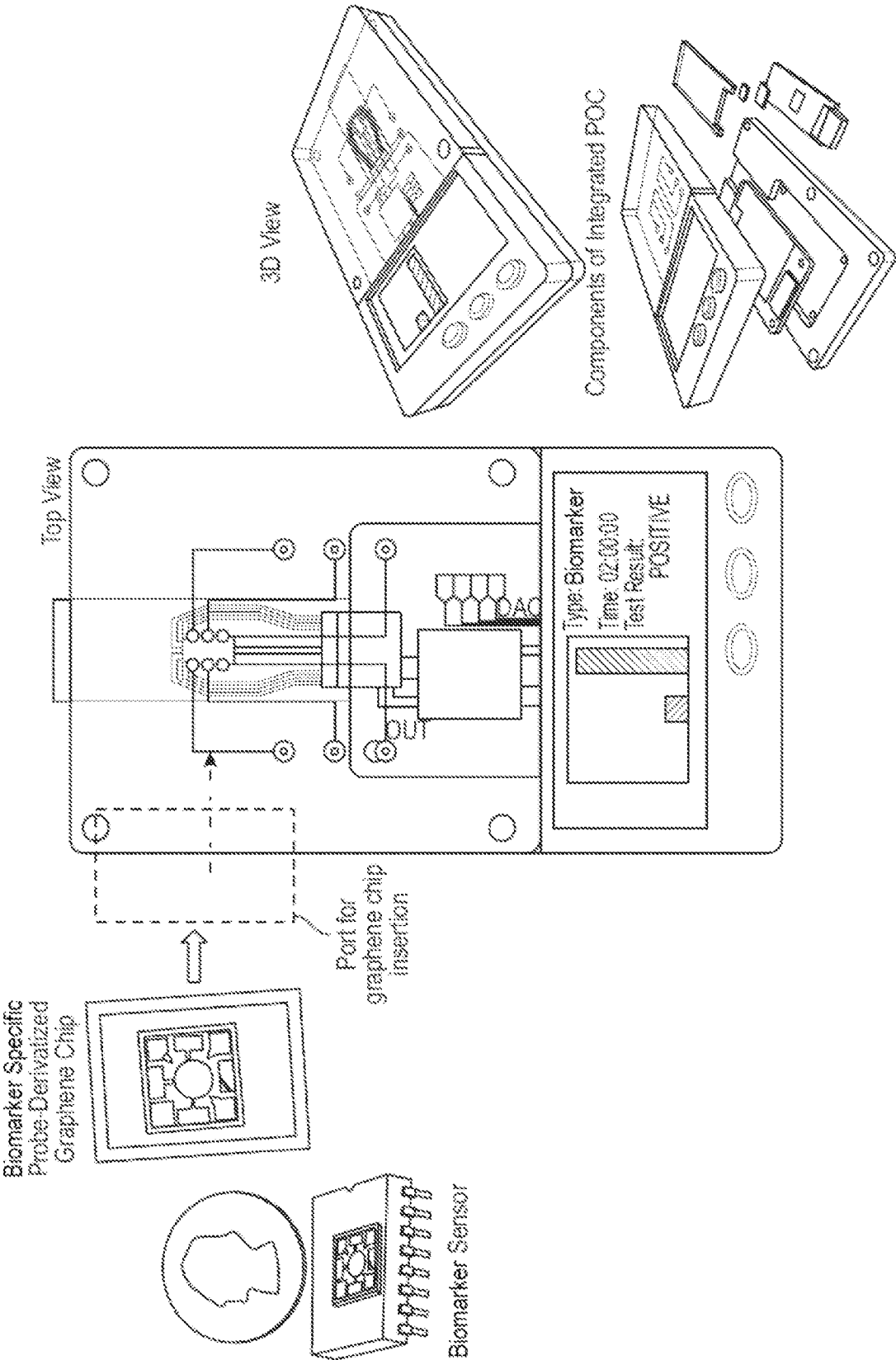


FIG. 11A

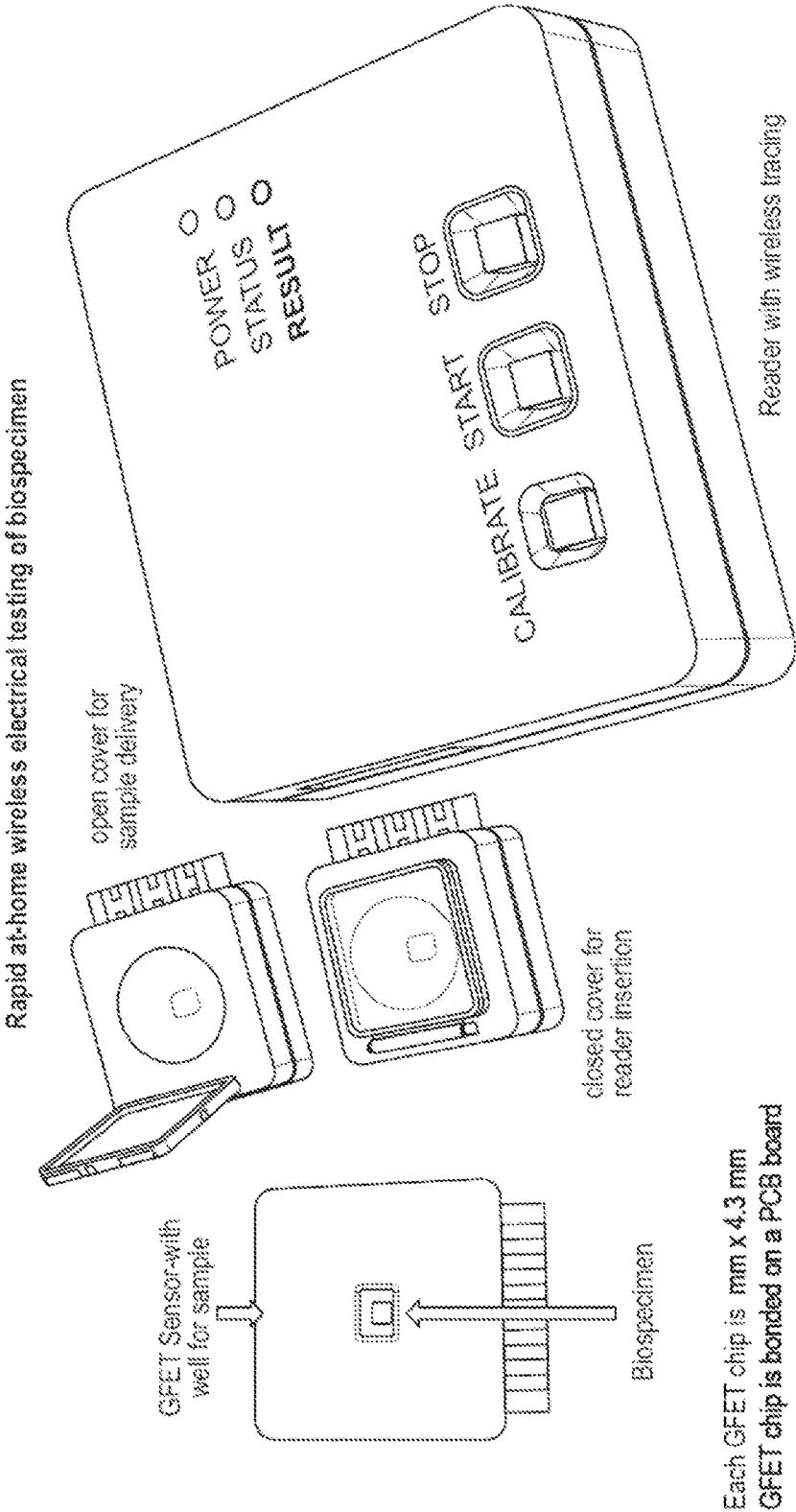


FIG. 11B

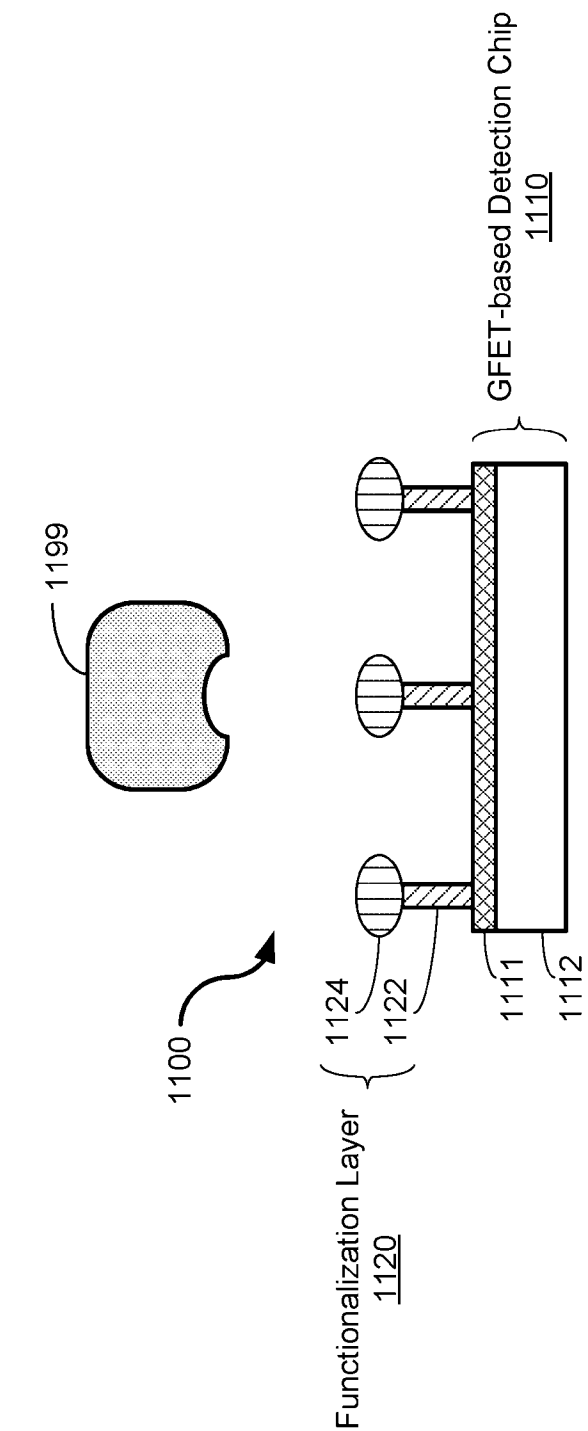


FIG. 11C

FIG. 12A

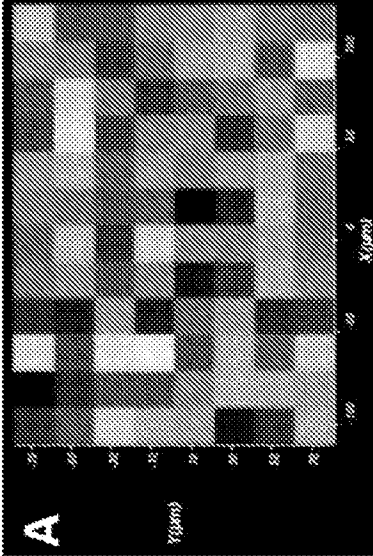
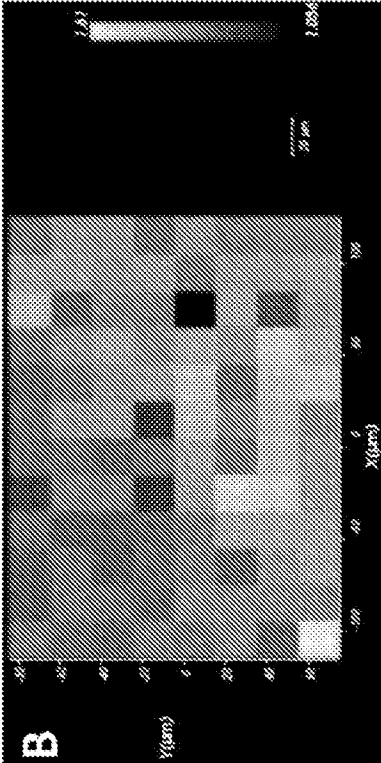
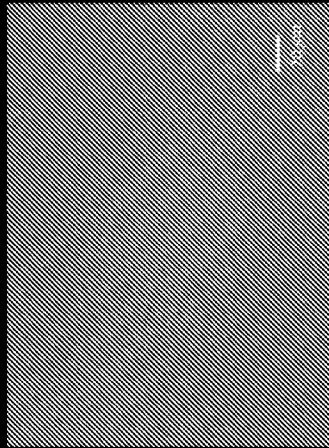


FIG. 12B



C



D



FIG. 12C

FIG. 12D

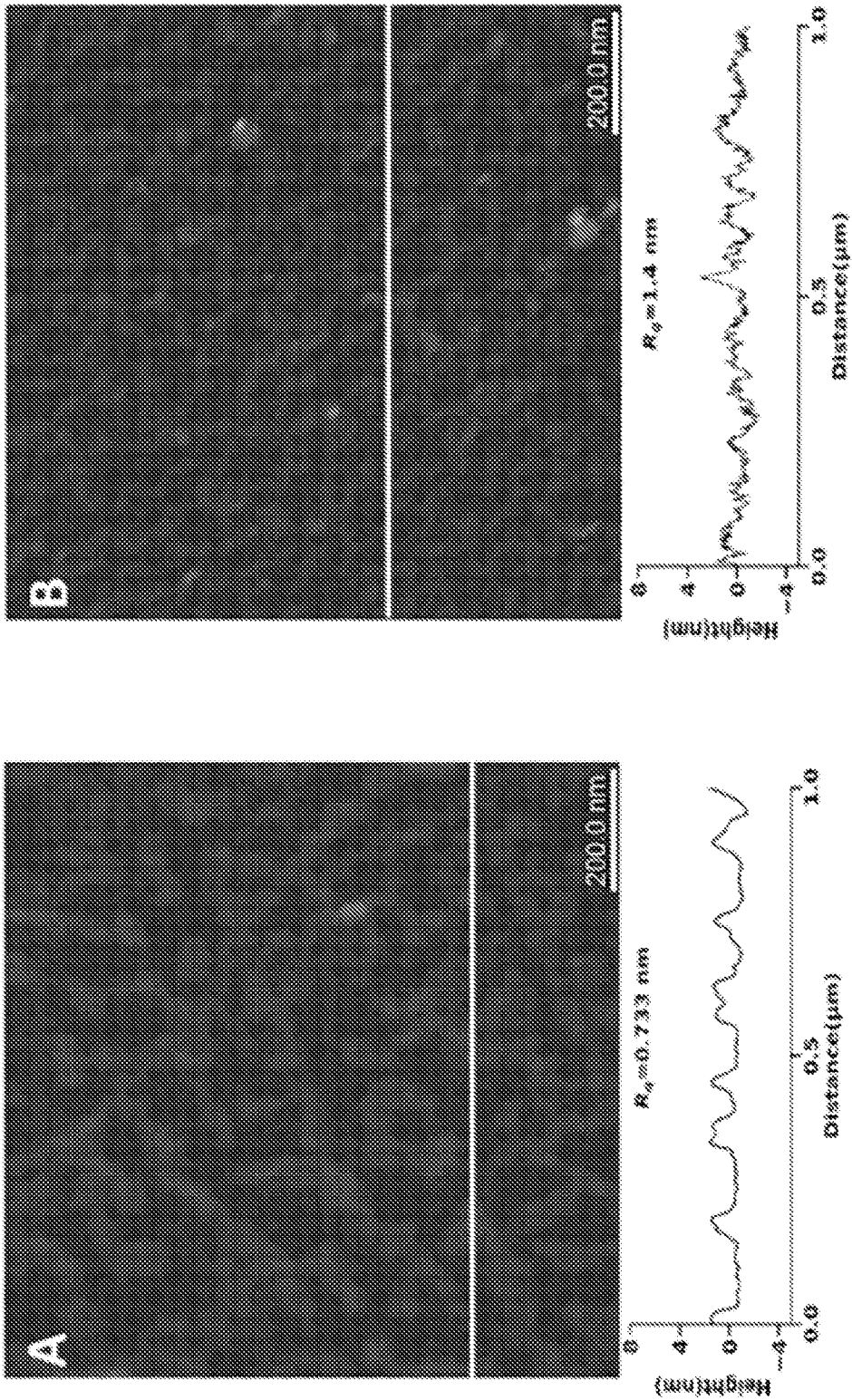


FIG. 13A

FIG. 13B

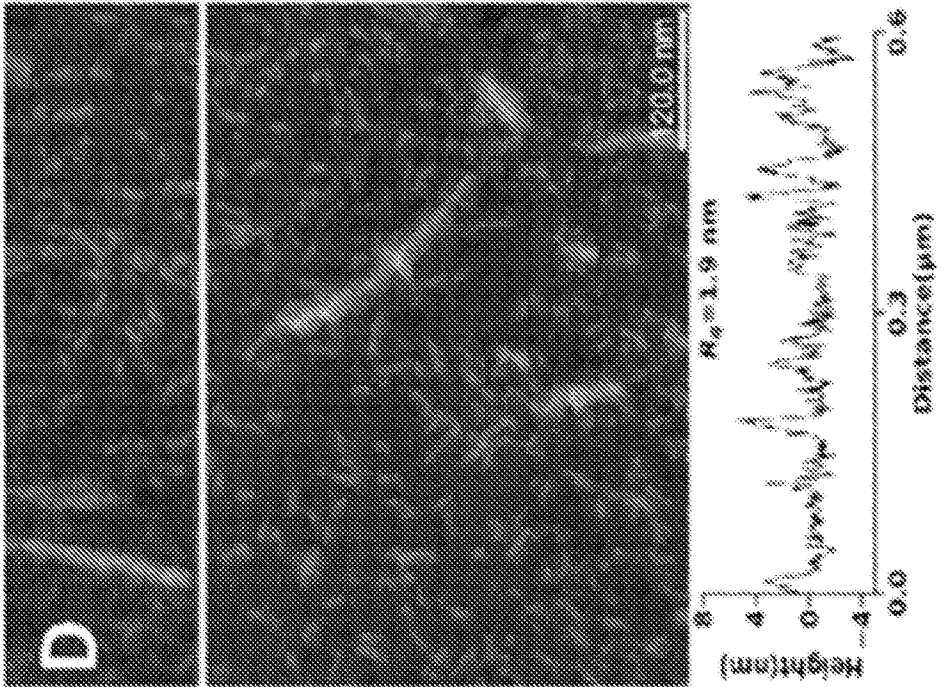


FIG. 13D

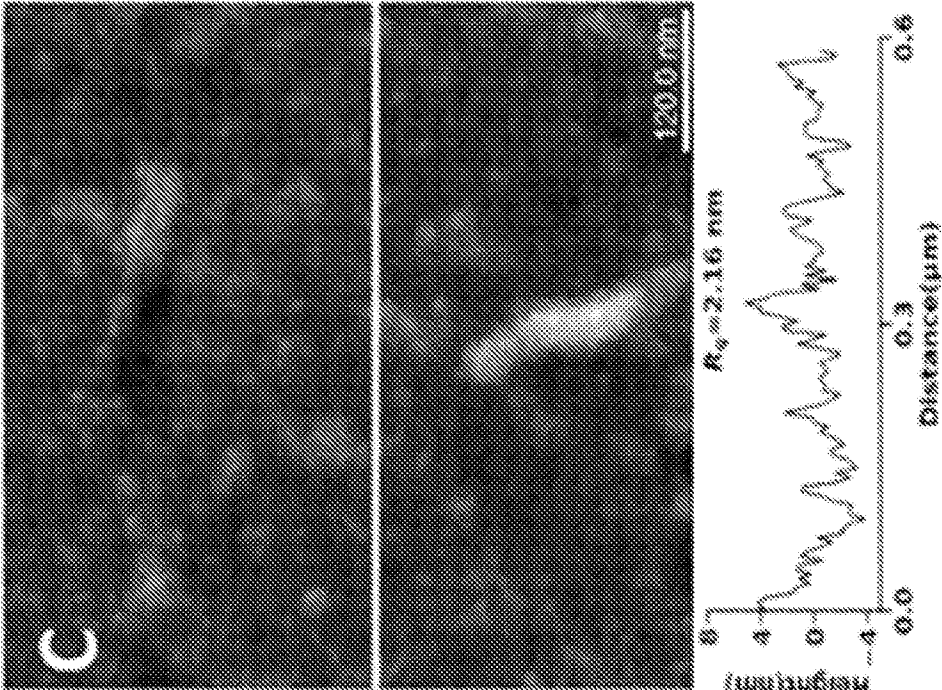


FIG. 13C

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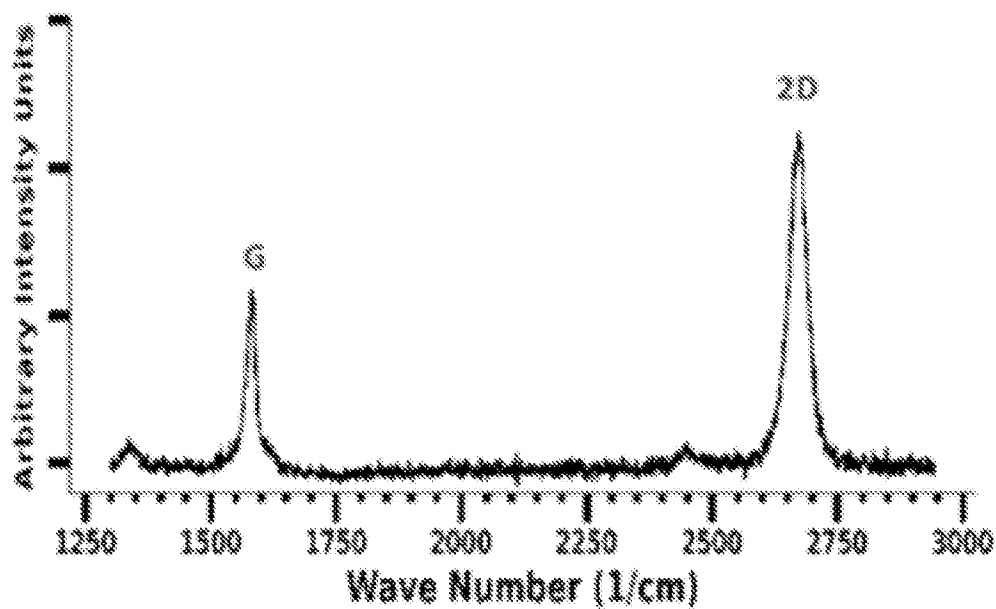


FIG. 13E

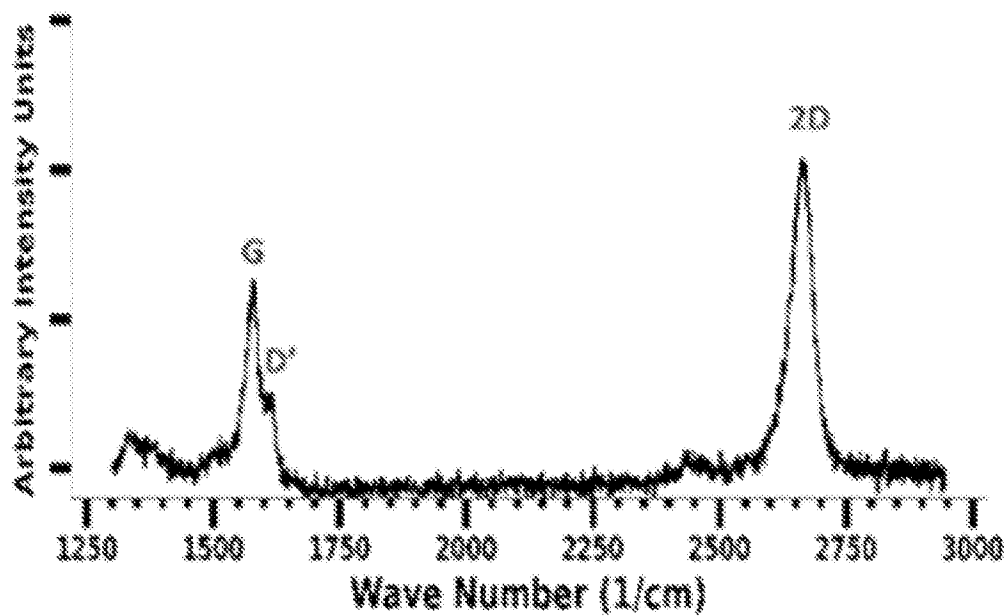


FIG. 13F

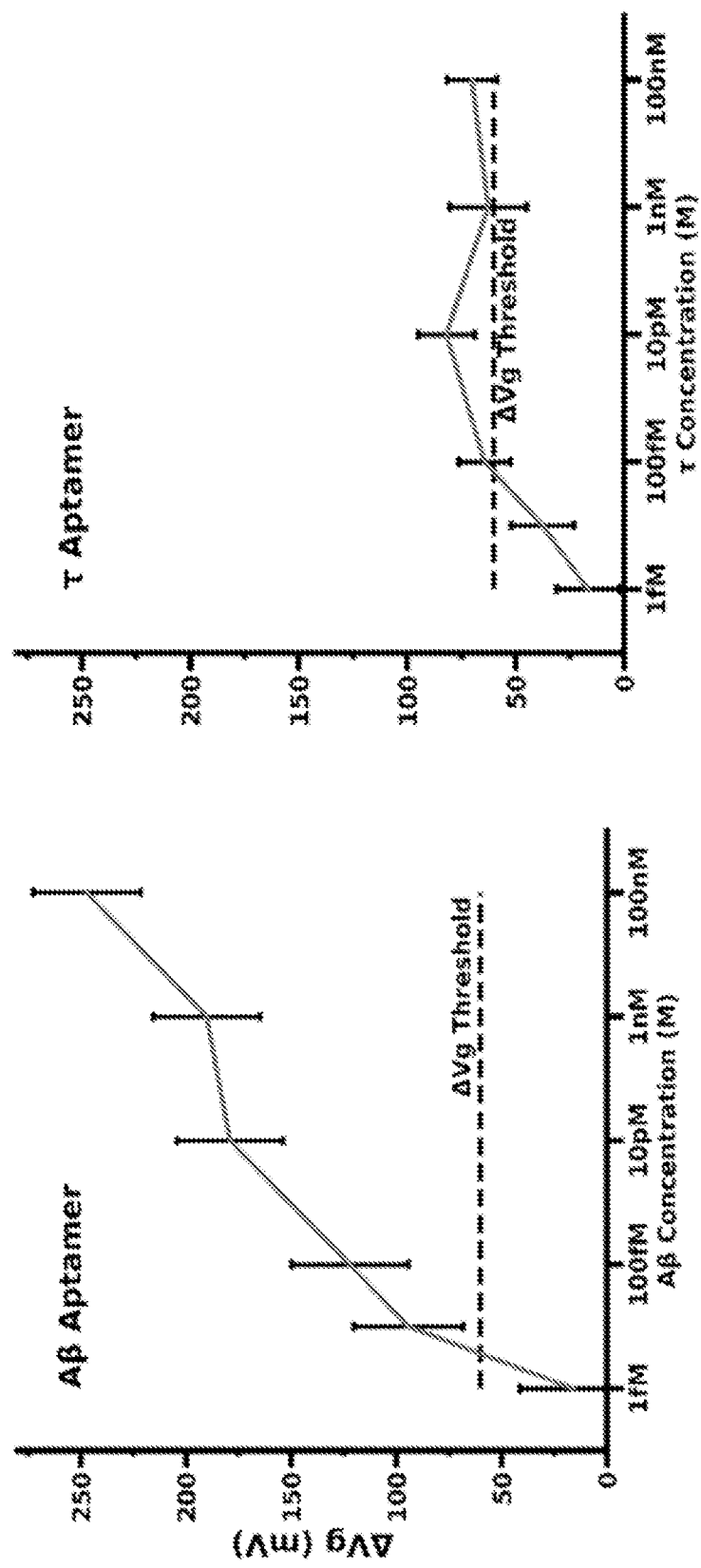


FIG. 14A

FIG. 14B

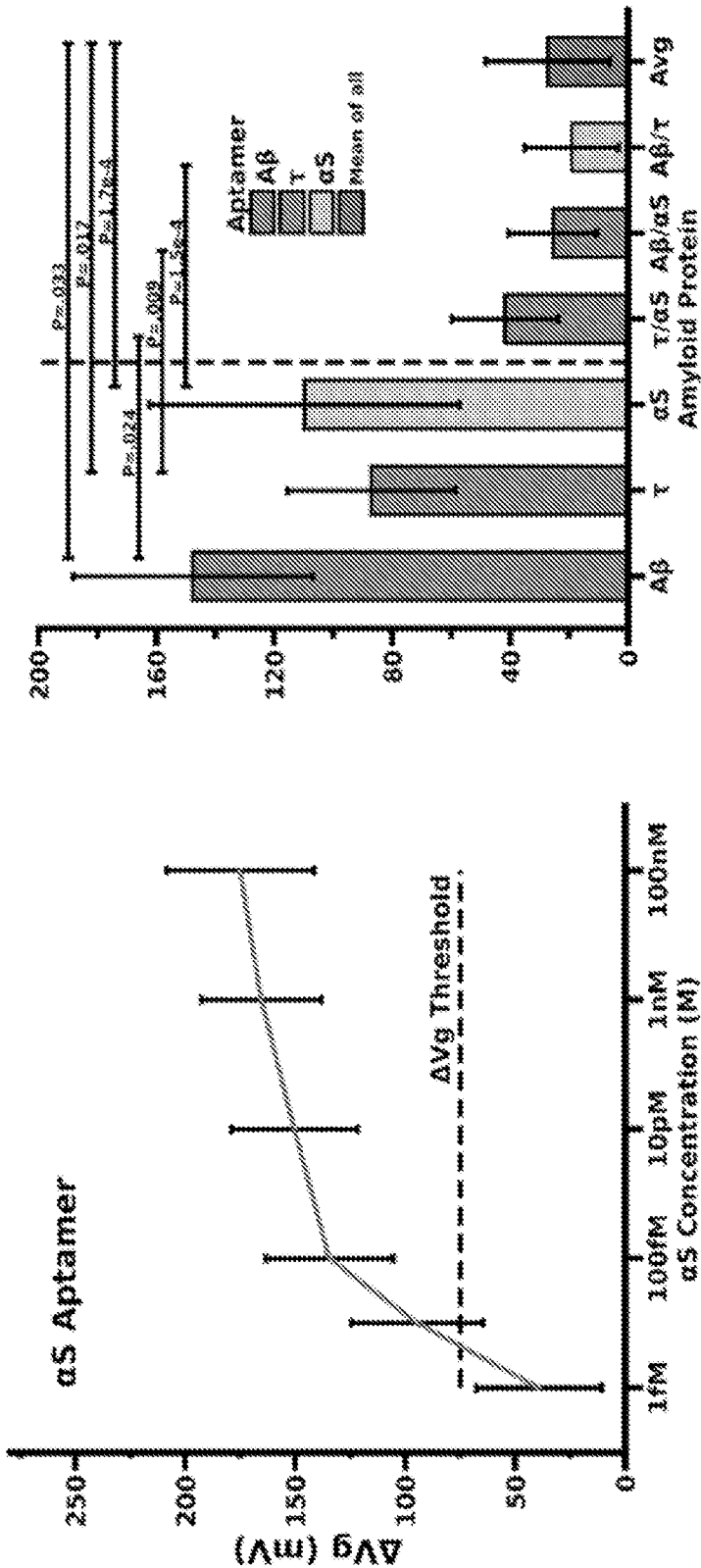


FIG. 14D

FIG. 14C

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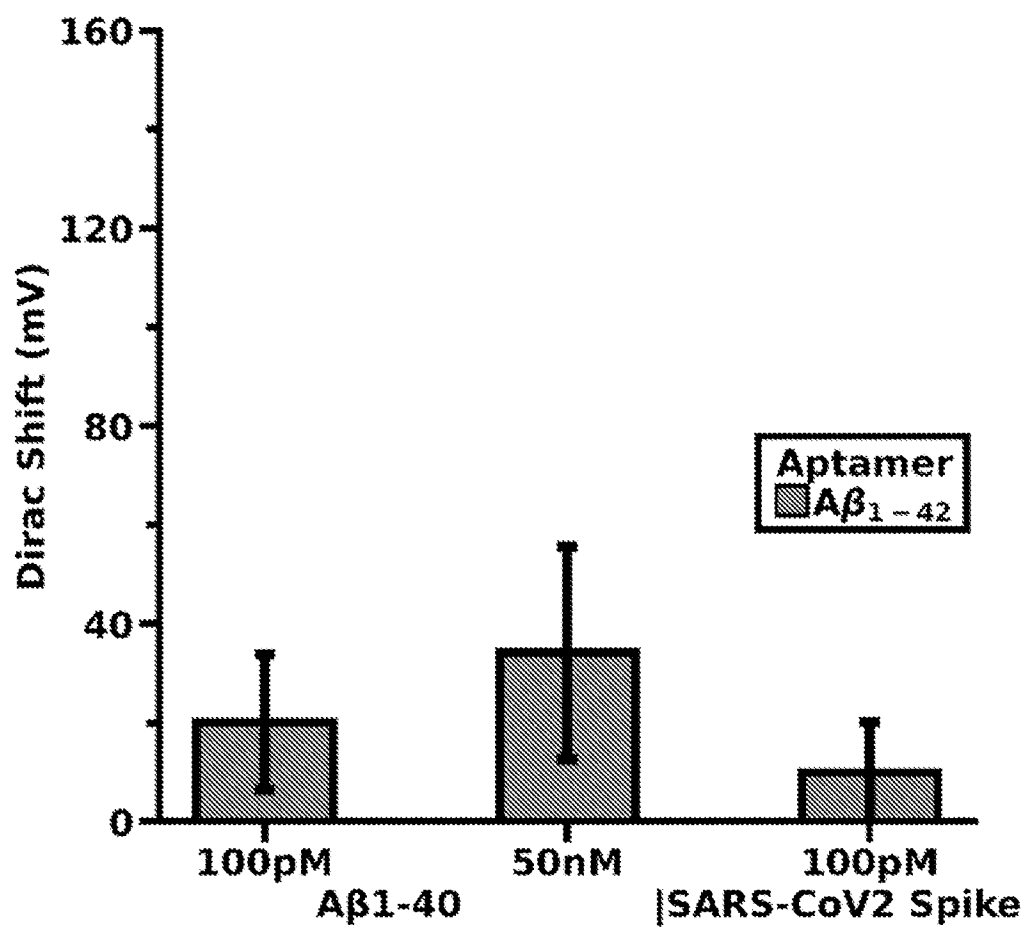


FIG. 15

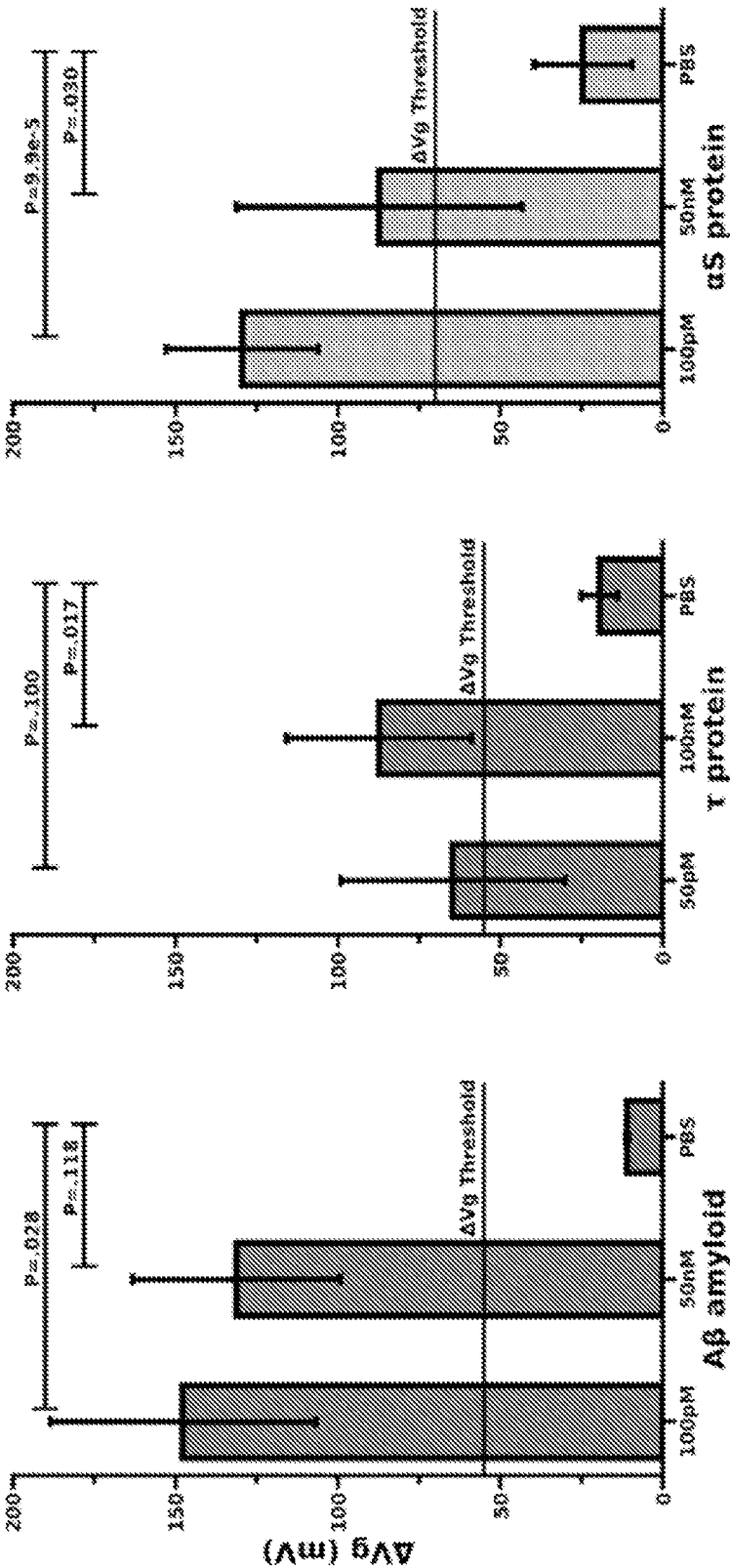


FIG. 16A

FIG. 16B

FIG. 16C

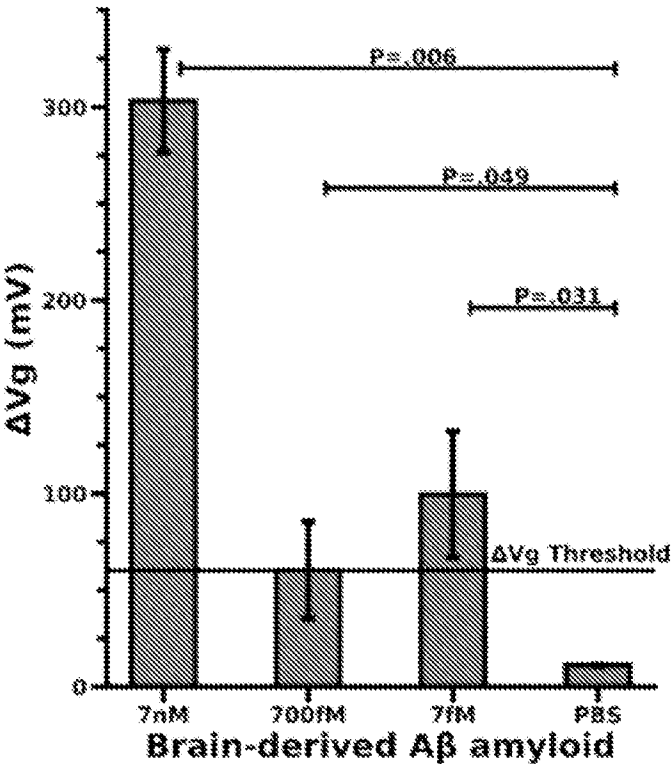


FIG. 17A

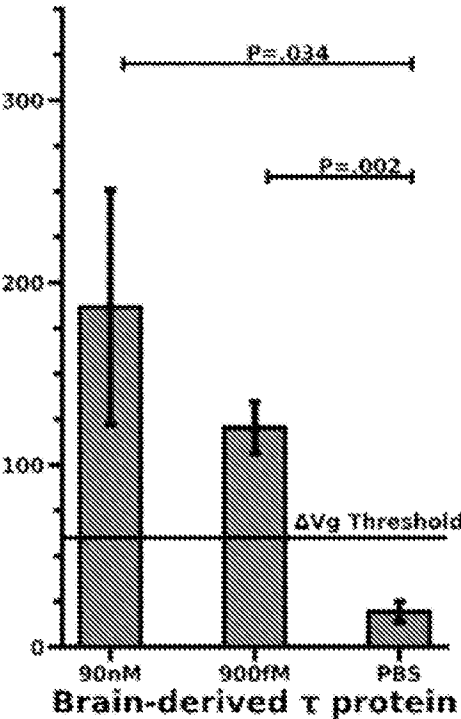


FIG. 17B

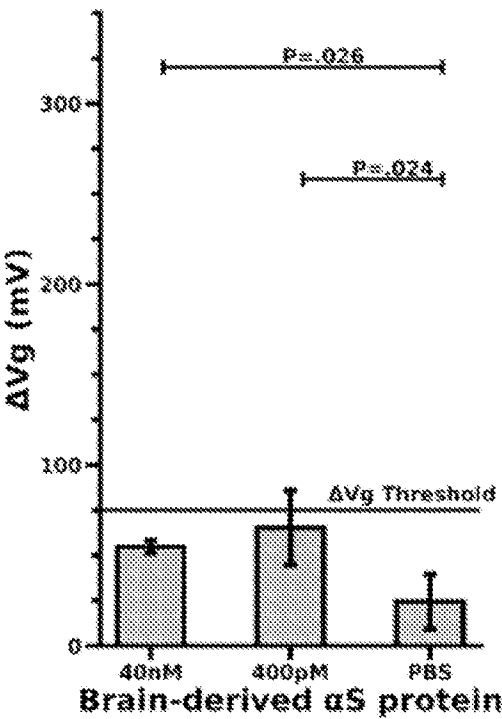


FIG. 17C

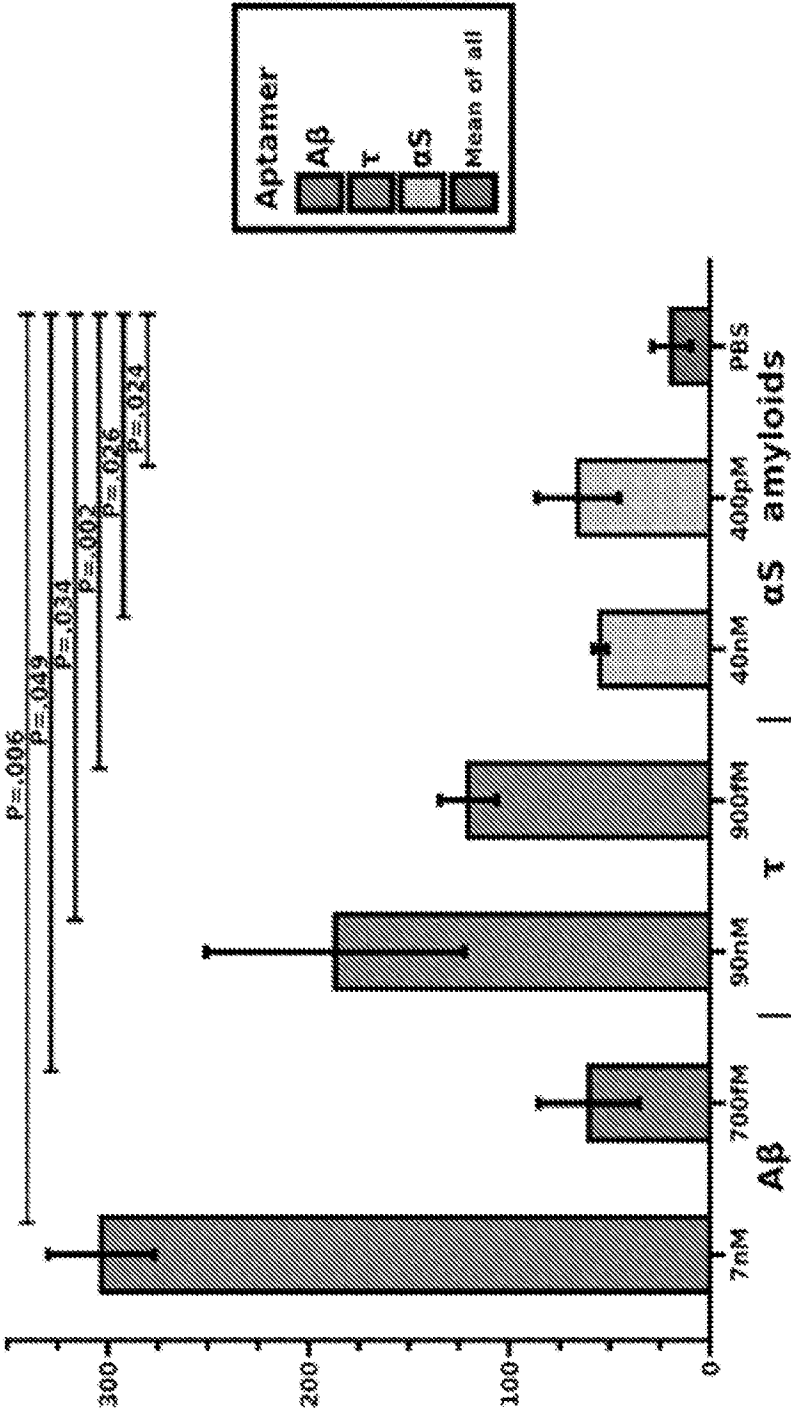


FIG. 17D

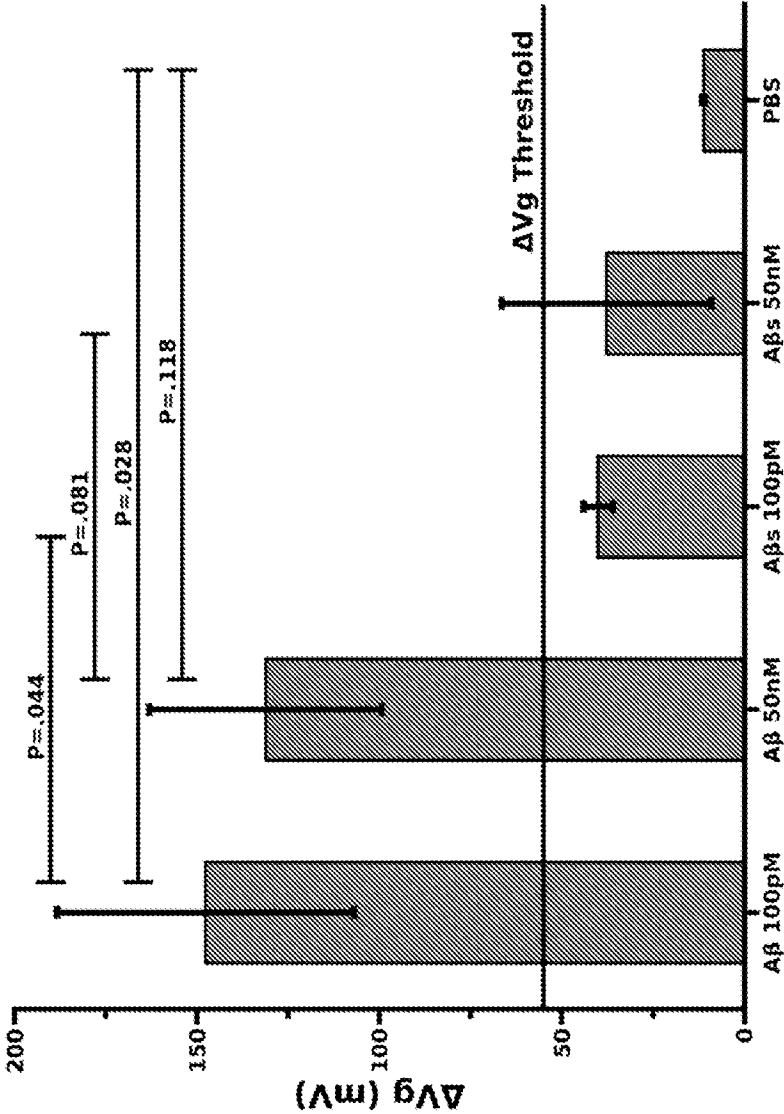


FIG. 18

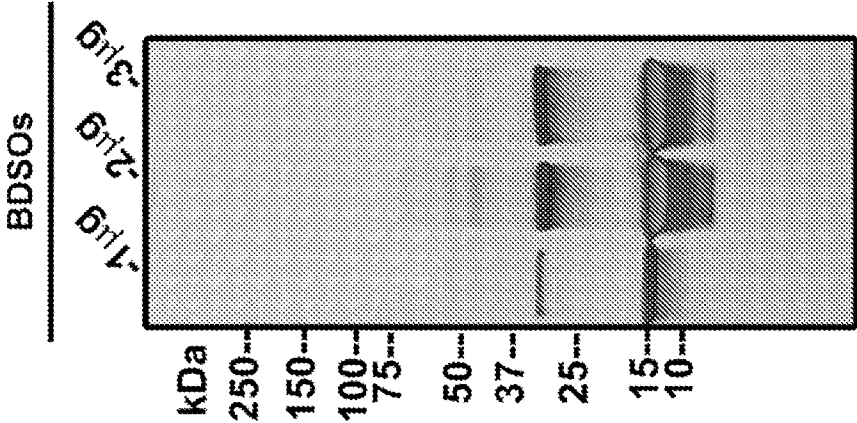


FIG. 19

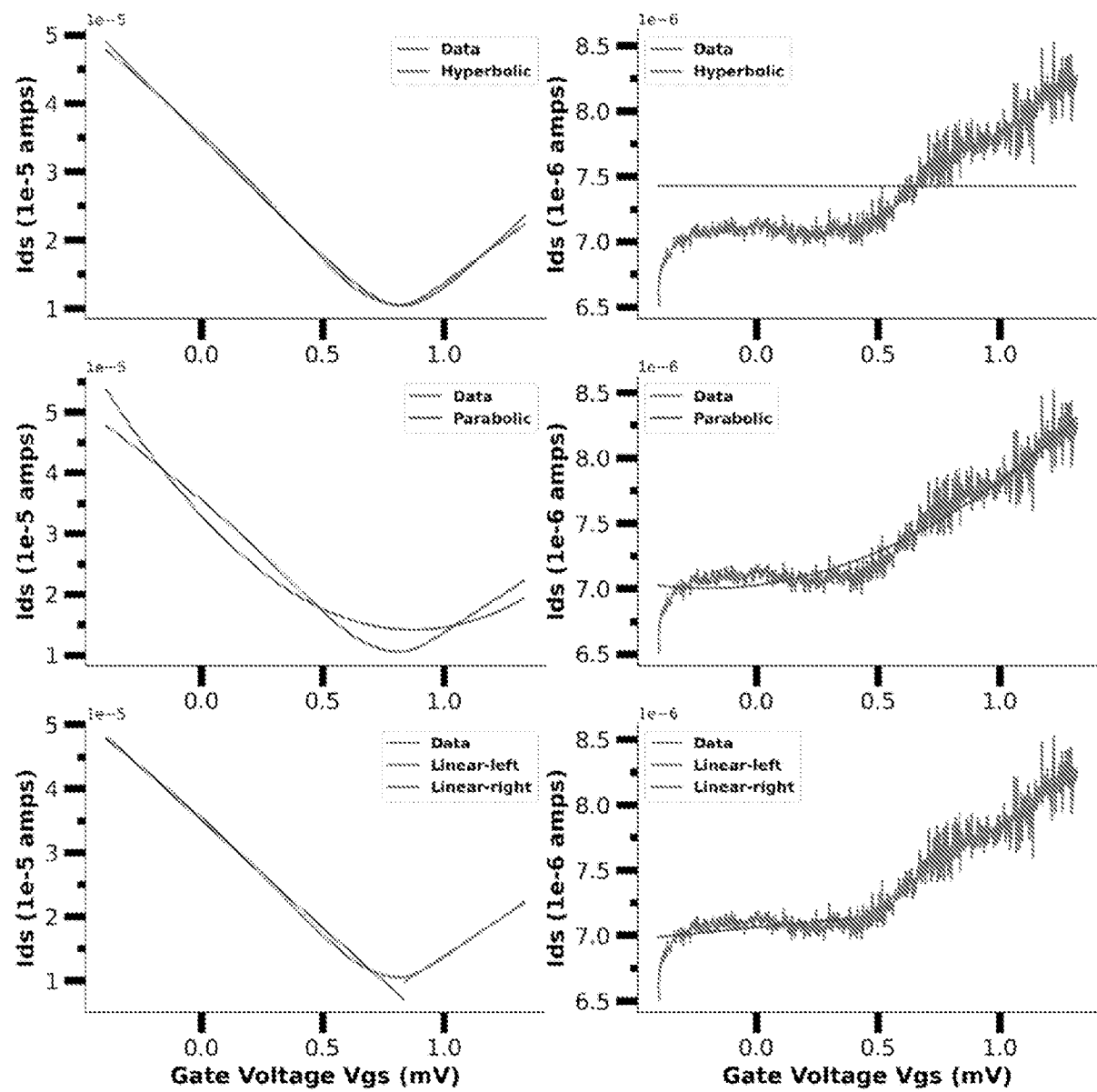


FIG. 20

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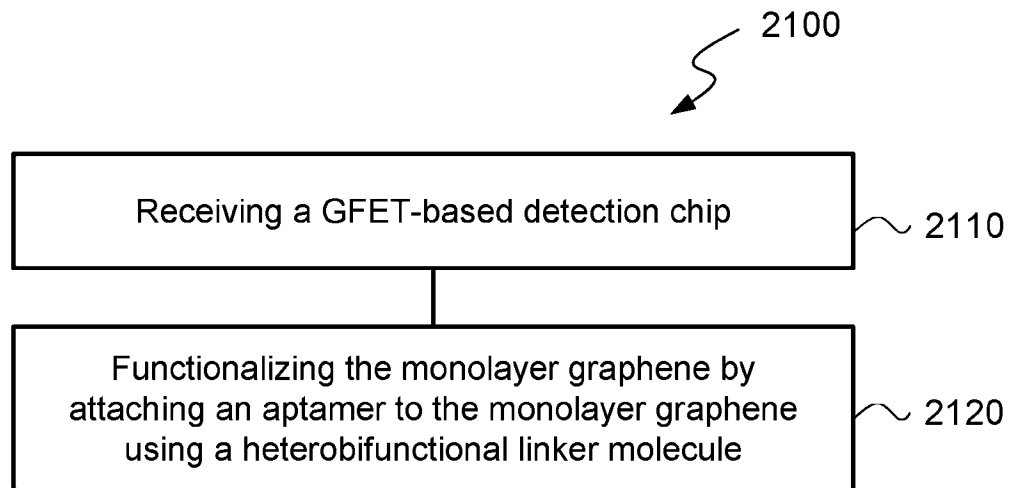


FIG. 21

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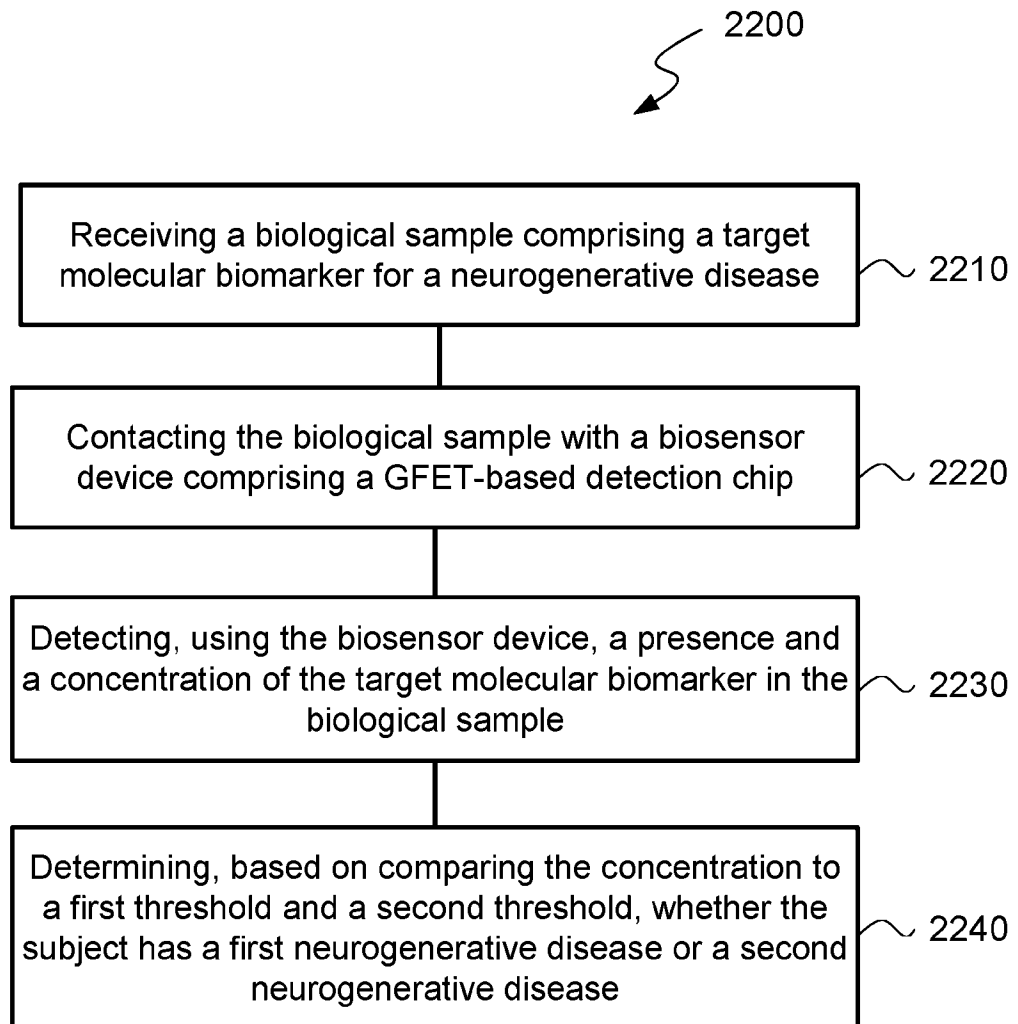


FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/045854

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **G01N 33/483** (2024.01); **H01M 4/02** (2024.01); **A61B 5/145** (2024.01); **G01N 27/41** (2024.01); **G01N 27/44** (2024.01)
CPC: **G01N 33/48721**; **H01M 4/02**; **G01N 27/44791**; **G01N 27/4146**; **B01L 3/502707**; **A61B 5/14532**; **G01N 27/41**

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.
A	US 2023/0236147 A1 (PICCININI et al.) 27 July 2023 (27.07.2023) entire document	1-4, 11-14, 20-27
A	US 2022/0136996 A1 (GRAPHENEA SEMICONDUCTOR SLU) 05 May 2022 (05.05.2022) entire document	1-4, 11-14, 20-27
A	US 2012/0220053 A1 (LEE et al.) 30 August 2012 (30.08.2012) entire document	1-4, 11-14, 20-27
A	WO 2023/149966 A2 (THE TRUSTEES OF BOSTON COLLEGE et al.) 10 August 2023 (10.08.2023) entire document	1-4, 11-14, 20-27
A	US 2021/0239645 A1 (PONTIFICIA UNIVERSIDAD JAVERIANA) 05 August 2021 (05.08.2021) entire document	1-4, 11-14, 20-27



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

21 November 2024 (21.11.2024)

Date of mailing of the international search report

04 December 2024 (04.12.2024)

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/045854

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **5-10, 15-19, 28-32**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).