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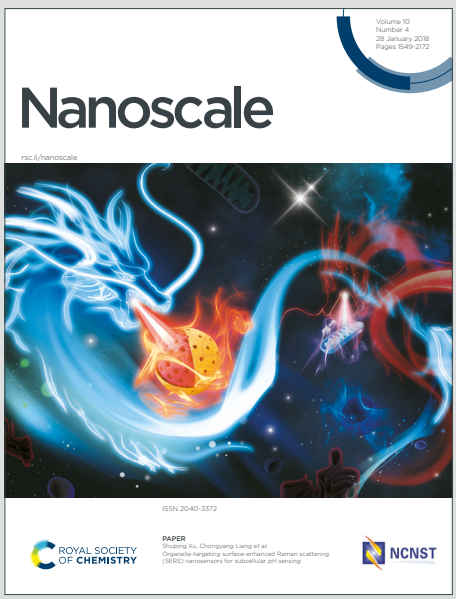

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ARTICLE

Enhanced Detection of Brain-Derived Neurotrophic Factor (BDNF) Using a Reduced Graphene Oxide Field-Effect Transistor Aptasensor

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Neurodegenerative diseases, characterized by the progressive deterioration of neuronal function and structure, pose significant global public health and economic challenges. Brain-Derived Neurotrophic Factor (BDNF), a key regulator of neuroplasticity and neuronal survival, has emerged as a critical biomarker for various neurodegenerative and psychiatric disorders, including Alzheimer's disease. Traditional diagnostic methods, such as Enzyme-Linked Immunosorbent Assay (ELISA) and Electrochemiluminescence (ECL) assays, face limitations in terms of sensitivity, stability, reproducibility, and cost-effectiveness. In this research, we developed the first electrical aptasensor for BDNF detection, constructed on a flexible polyimide (PI) membrane coated with reduced graphene oxide (r-GO) and utilizing an extended-gate field-effect transistor (EGFET) as the transducer. Comprehansive characterization of the sensor, coupled with the fine-tuning of aptamer concentration and the binding time of DNA aptamers to the chemical linker, was achieved through Electrochemical Impedance Spectroscopy (EIS) to boost sensitivity. Consequently, by utilizing the unique properties of r-GO and DNA aptamers, the aptasensor exhibited exceptional detection abilities, with a detection limit as low as 0.4 nM and an extensive response range spanning from 0.025-1000 nM. The flexible PI-based electrode offers exceptional stability, affordability, and durability for home diagnostics, enriched by the reusability of its electronic transducer, making the device highly portable and suitable for prolonged monitoring. Our aptasensor surpasses traditional methods, showcasing superior real-time performance and reliability. The high sensitivity and specificity of our aptasensor highlight its potential to significantly improve early diagnosis and therapeutic monitoring of neurodegenerative diseases such as Alzheimer's, representing a considerable advancement in the diagnosis and management of such conditions.

Keywords: Brain-Derived Neurotrophic Factor, Biosensor, Aptamer, reduced Graphene Oxide, Field Effect Transistor.

Introduction

Neurodegenerative diseases are marked by the progressive deterioration of neuronal function and structure, often culminating in the disorder and death of neuronal cell.(1) Millions of individuals are afflicted by these conditions, which manifest through clinical symptoms including disrupted selective functions, neuroinflammation, neuronal and synaptic damage, and protein aggregation. The impact of neurodegenerative diseases on public health and the economy is profound. However, the incomplete understanding of these diseases in all their facets has hindered the development of effective clinical treatments.(2, 3) Neurodegenerative disorders have reached pandemic proportions globally, with most remaining incurable due to the unclear mechanisms underlying these diseases and the lack of effective clinical therapies. The limited number of clinical treatments available have largely failed in therapeutic application, primarily because there are no commercially available methods for early diagnosis based on specific biomarkers at initial stages of the neurodegenerative diseases.(2, 4)

Brain-derived neurotrophic factor (BDNF) is a regulatory protein essential for modulating neuroplasticity and neuronal survival in the nervous system, playing a critical role in memory and learning. It is critically involved in promoting neurons' growth, repair, differentiation, and survival.(5) BDNF is a significant biomarker for a range of neurological and psychiatric disorders. Its levels in blood serum can vary substantially depending on factors such as age, gender, and overall health. Diminished

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BDNF levels are correlated with neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and multiple sclerosis.(6-9) While some studies provide vague evidence regarding the use of BDNF as a biomarker for neurodegenerative diseases,(10, 11) numerous meta-analyses confirm the correlation between Brain disorders and serum BDNF.(12, 13)

Enzyme-linked immunosorbent assay (ELISA) is considered the gold standard technique and can routinely be used for BDNF diagnosis in clinical trials. Nevertheless, the accuracy of the measurements is called into question due to variations observed among different commercially available ELISA kits used on the same human serum samples.(14) On the other hand, the low sensitivity, stability, and high cost of current biosensors and sensing platforms for BDNF detection underscore the urgent demand for the development of novel analytical tools based on sensitive recognition elements with high specificity.(15) These tools should enable the rapid detection of BDNF to improve the early diagnosis of neurodegenerative diseases and expedite therapeutic interventions. To address the limitations of the most used analytical technologies, the development of flexible graphene-based biosensors has gained significant attention. These biosensors offer unique characteristics, such as high sensitivity, excellent mechanical, optical, and electrical properties, and broad applications in the field of biosensing. In this context, traditional diagnostic strategies based on antibody-antigen interactions encounter issues such as reduced stability and limited shelf life. Conversely, the aptamer-based approach offers several advantages. Aptamers, with their streamlined and cost-effective production processes, ensure reduced batch-to-batch variation and eliminate the need for an immune response or the use of animals in their production. These characteristics expedite manufacturing and enhance the stability and longevity of biosensors, making aptamers an ideal candidate for biosensor applications.(16, 17)

Graphene-based nanomaterials demonstrate ambipolar transfer characteristics and exceptionally high electrical conductivity (200,000 $cm^2V^{-1}s^{-1}$), rendering them highly suitable for a broad spectrum of applications. These encompass nanoenergy,(18) batteries,(19, 20) energy storage, surface engineering,(21) and biosensing.(22-24) Graphene oxide (GO) is recognised as a highly promising material with significant potential for use in various ultra-sensitive biosensors, particularly graphene-based field-effect transistor (G-FET) biosensors. The exceptional sensitivity of graphene oxide FET biosensors is attributed to the adsorption of molecules, which induces charge transfer on the extended gate surface. This process alters the conductivity of graphene oxide and results in a shift in the Fermi energy.(25) Additionally, the conductivity of graphene oxide can be modified by molecule adsorption on its surface, even without shifts in the Fermi energy. Accordingly, the non-covalent binding of target biomolecules to graphene oxide can significantly modify the carrier density and electrical conductivity of graphene-based FETs, even at low biomolecule concentrations.

Metal–oxide–semiconductor field-effect transistors (MOSFETs), functioning as transducers, generate electronic signals with high sensitivity in response to specific biochemical reactions, making them widely utilized in the development of biosensors. The extended gate FET (EG-FET) variant effectively addresses application drawbacks by extending the recognition sites.(26-29) The configuration of EGFETs makes them highly suitable for biosensing applications due to the adjustable and replaceable recognition sites on the sensing platform, tailored to specific targets. To the best of our knowledge, there are no reports on the design and fabrication of graphene oxide EGFETs for BDNF detection. In this study, a comprehensive characterization of the sensor, in conjunction with the meticulous optimization of aptamer concentration and the binding duration of DNA aptamers to the chemical linker, was accomplished through Electrochemical Impedance Spectroscopy (EIS) to enhance sensitivity. This research pioneers the use of DNA aptamers for BDNF detection via EIS and reduced graphene oxide field-effect transistors (r-GOFET), representing a significant advancement in biosensing technology.(7, 8) As a result, by leveraging the distinctive properties of reduced Graphene Oxide (r-GO) and DNA aptamers, the aptasensor demonstrated remarkable detection capabilities, achieving a detection threshold as low as 0.4 nM and maintaining an extensive linear response range extending from 0.025-1000 nM. The adaptable polyimide (PI)-based electrode provides outstanding stability, cost-effectiveness, and longevity for at-home diagnostics, further augmented by the reusability of its electronic transducer, rendering the device highly portable and appropriate for extended monitoring. This advancement provides a powerful tool for early neurodegenerative disease diagnosis. In addition, we have developed gold (Au) and silver chloride (Ag/AgCl) electrodes on flexible polymer (Polyimide) substrates, which offer high stability and make long-term monitoring possible.(7) These flexible electrodes are compared with conventional methods, demonstrating superior performance and reliability. Flexible biosensors are at the forefront of a rapidly expanding research field, utilizing multidisciplinary approaches in materials science, fabrication techniques, and design innovations.

Results and discussion

Pseudo-reference electrode characterization. An alternative approach to depositing the silver layer involves using undiluted aqueous $FeCl_3$ solutions for chemical oxidation. The primary objective of the proposed pseudo-reference electrode fabrication method is to develop a fully compatible large-scale production process. In this study, a homogeneous AgCl-coated layer depicts in Figure S1a. Energy-dispersive X-ray (EDX) analysis further confirms the formation of the AgCl layer, evidenced by the presence of a chlorine peak in addition to the silver peak (Figure S1b and S1c). The performance of the electrode evaluates through electrochemical characterization using cyclic voltammetry (CV) in a buffer solution. The initial assessment involved varying the scan rate and comparing the results with those obtained by commercial Ag/AgCl electrode.

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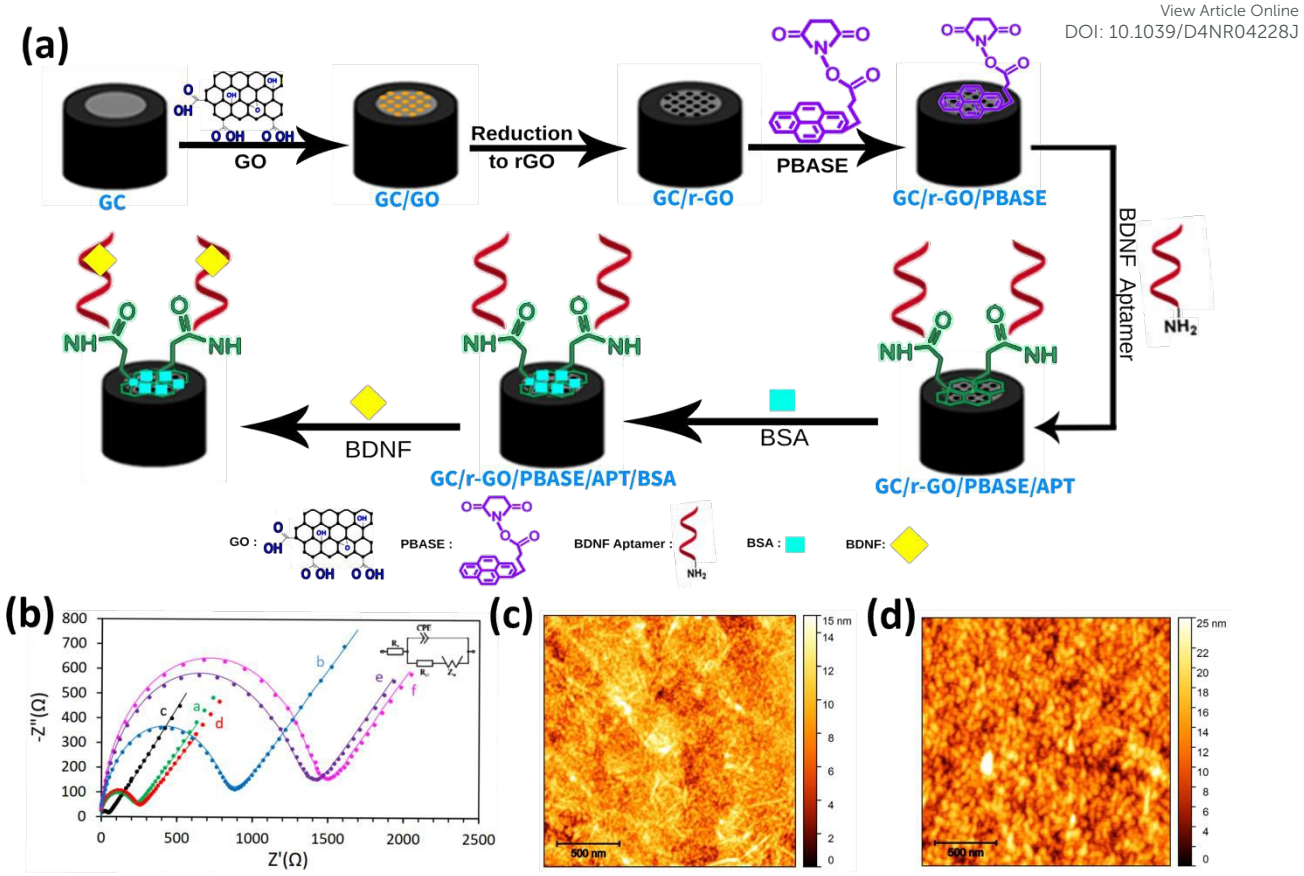

Figure 1 (a) Schematic diagram of the fabrication and operation of the aptasensor. (b) EIS complex plane plots obtained on (a) GC, (b) GC/GO, (c) GC/rGO, (d) GC/rGO/PBASE, (e) GC/rGO/PBASE/APT and (f) GC/rGO/PBASE/APT/BSA electrodes in 0.1 M KCl electrolyte solution containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Here, dots and dash lines represent the experimental and the fitted data respectively, based on modified Randle's model (Inset of the Figure).AFM images of GC/rGO/PBASE/APT electrode (c) after binding with BDNF(d).

As demonstrated in Figure S1d, there is no significant difference between the CV curves of the fabricated pseudo-reference electrode and the commercial Ag/AgCl electrode, indicating comparable performance and validating the efficacy of the fabrication method.(30)

Characterization of the aptasensor. A schematic representation of the aptasensor's structure is presented in Figure 1a. Each step involved in electrode modification characterized using Electrochemical Impedance Spectroscopy (EIS) methodology. The Nyquist plots, depicting the impedance spectra of various electrode configurations after each step of modification, are shown in Figure 1b. Here, the results demonstrate that modification of the bare glassy carbon (GC) electrode (plot a) with a GO film (plot b) leads to a significant increase in the R_{ct}. Specifically, the introduction of GO onto the GC electrode surface results in a marked elevation in the R_{ct} value compared to the unmodified GC electrode, due to the interaction between the electrolyte species in the solution and the oxygen-containing functional groups on the GO sheet-based electrode.(31-34) However, this trend is somewhat reversed when considering the GC/r-GO electrode. After the reduction of GO by hydrazine, the R_{ct} decreases (plot d). This decline in R_{ct} can be attributed to the high conductivity and substantial surface area of r-GO, which promotes and facilitates the charge transfer process at the electrode interface.(35-37) When the graphene sensor surfaces modified with 1-pyrenebutanoic acid succinimidyl ester (GC/r-GO/PBASE), a molecular linker, the R_{ct} exhibited only a slight shift, and the electron transfer resistance showed a modest increase (plot e).(38, 39) Subsequently, after incubation with aptamer molecules (GC/r-GO/PBASE/APT), the R_{ct} values increases significantly (Plot e). This heightened signal can be attributed to two factors: the blocking effect of the aptamer molecules on the electrode surface, and the repulsive force between the negatively charged phosphate groups of DNA and the redox probe.(40) Additionally, the adsorption of BSA onto the surface of the GC/r-GO/PBASE/APT electrode results in a further increase in the R_{ct} value (plot f). These results are consistent with findings from recent studies, confirming the reliability and validity of our observations. Consequently, the presence of BDNF leads to a substantial increase in the R_{ct} value. The Randles model parameters are presented in Table S1.

To gain deeper insights into the immobilization of aptamer molecules on the electrode surface, Atomic Force Microscopy (AFM) analysis was performed on GC/r-GO/PBASE and GC/r-GO/PBASE/APT-modified electrodes. The root mean square (RMS) surface roughness values were determined to be approximately 2 nm and 4 nm, respectively, as shown in Figures 1c and 1d. These findings confirm the successful binding between BDNF and DNA aptamer, as evidenced by the increased surface roughness.

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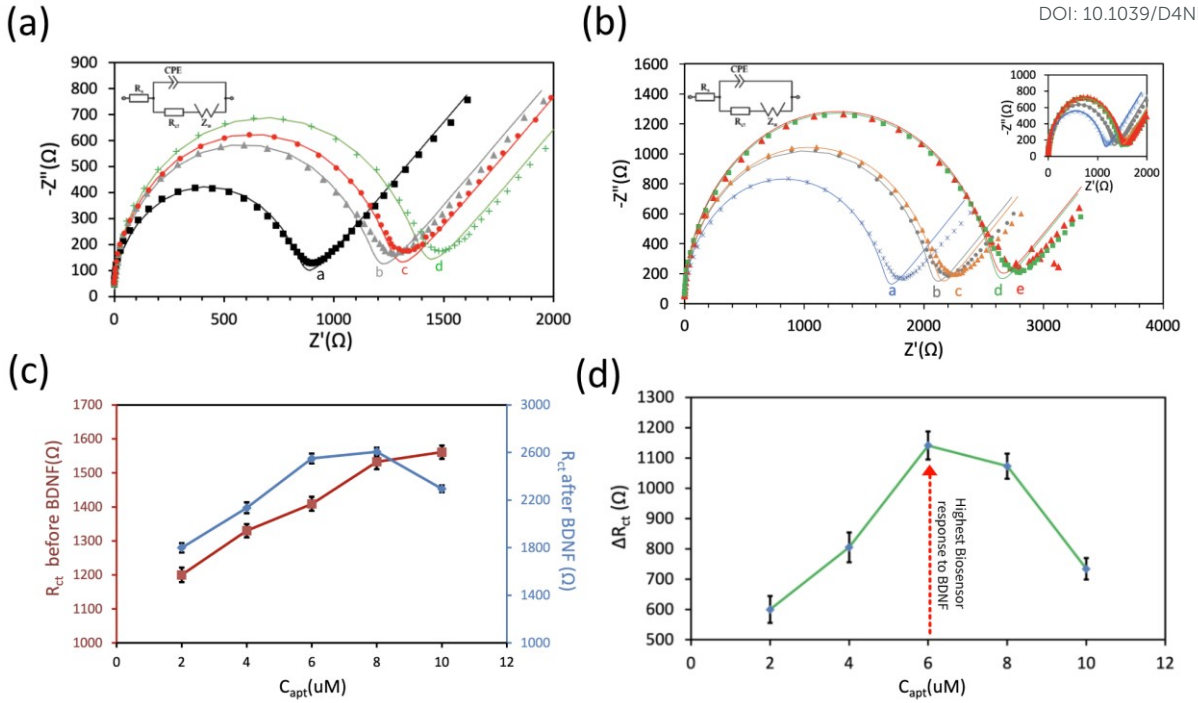

Figure 2 (a) EIS complex plane plots were obtained for GC/r-GO/PBASE/APT at different aptamer incubation time in 0.1 M KCl electrolyte solution containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. (b) EIS complex plane plots obtained for GC/r-GO/PBASE/APT at different aptamer concentrations and its response to 12.5 nM BDNF in 0.1 M KCl electrolyte solution containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Here, dots and dash lines represent the experimental and the fitted data respectively, based on modified Randle's model (Inset of the Figure). (c) Extracted Rct from plot (B). (d) the ΔR_{ct} of GC/r-GO/PBASE/APT electrode with different aptamer concentrations (2 to 10 µM) and 12.5 nM BDNF protein.

These results are consistent with prior literature findings,(23, 41, 42) and affirm the high density of BDNF-aptamer and BDNF binding affinity to the electrode surface, in excellent agreement with the EIS results. Also, the elemental structure of synthesized GO characterized with FTIR and XRD (Figure S2). Here, in the FTIR image of GO, the vibration peaks observed include ~1058 cm^{-1} (C-O-C), ~1228 cm^{-1} (C-O), and ~1622 cm^{-1} (C=C). Additionally, there are bending vibration peaks at ~1374 cm^{-1} (O-H) and stretching vibrations at ~1740 cm^{-1} (C=O). A broad peak in the range of 3200-3500 cm^{-1} is attributed to O-H stretching from alcohol groups and water molecules, superimposed on the O-H bending vibrations of COOH groups. Moreover, the structural properties of the synthesized GO studied by XRD analysis, illustrating the distinct XRD peak of GO at ~10°. These results confirm the success of the oxidation process during GO synthesis and indicate the presence of numerous oxygen-containing functional groups on the GO surface.

Optimization of analytical parameters. To achieve the highest sensitivity of the proposed aptasensor, several key experimental parameters, such as aptamer incubation time and aptamer concentration, thoroughly investigated and optimized using the EIS technique. The experimental results reveals that a 1-hour incubation period was sufficient for DNA aptamers to interact effectively with PBASE, leading to the maximum ΔR_{ct} (Figure 2a). Various aptasensors developed using different aptamer concentrations, and 12.5 nM BDNF employed to compare the response of the aptasensors in terms of their ΔR_{ct}. The concentration of the aptamers significantly influences the detection capability of the aptasensor, as illustrated in Figure 2b. Figures 2c and 2d demonstrate that as the aptamer concentration increases from 2 to 10 µM, the R_{ct} intensity gradually enhances. The maximum ΔR_{ct} for 12.5 nM BDNF protein achieved at an aptamer concentration of 6.0 µM. Beyond this concentration, ΔR_{ct} slightly decreases. This decrease may be attributed to an excess of aptamers on the electrode surface, which can hinder the necessary conformational change when the aptamer binds to the BDNF protein, making the redox moiety less accessible to the electrode surface.(43-45) Consequently, an incubation time of 1 hour and an aptamer concentration of 6 µM determined to be the optimal conditions for aptamer binding to the surface of the GC/r-GO/PBASE electrode.

Analytical performance of the aptasensor. To evaluate the analytical performance of the proposed aptasensor, a range of BDNF concentrations from 0.025-1000 nM employed, and the corresponding Nyquist plots and aptasensor responses recorded (as illustrated in Figure 3a). As the BDNF concentration increased, a noticeable change in the Nyquist plots observed, and characterized by a significant increase in the R_{ct} value. The obtained data are illustrated in Figure 3b, which demonstrates the aptasensor response as a function of BDNF concentration. A linear increase in the aptasensor response is evident as the BDNF concentration rises, as described by Equation (1).

$$\Delta R_{ct}\,(\Omega) = 95.51\,[BDNF](nM) + 41.63 \qquad R^2=0.97 \qquad (1)$$

At high BDNF concentrations, the calibration curve deviates from linearity due to the saturation of the electrode surface.

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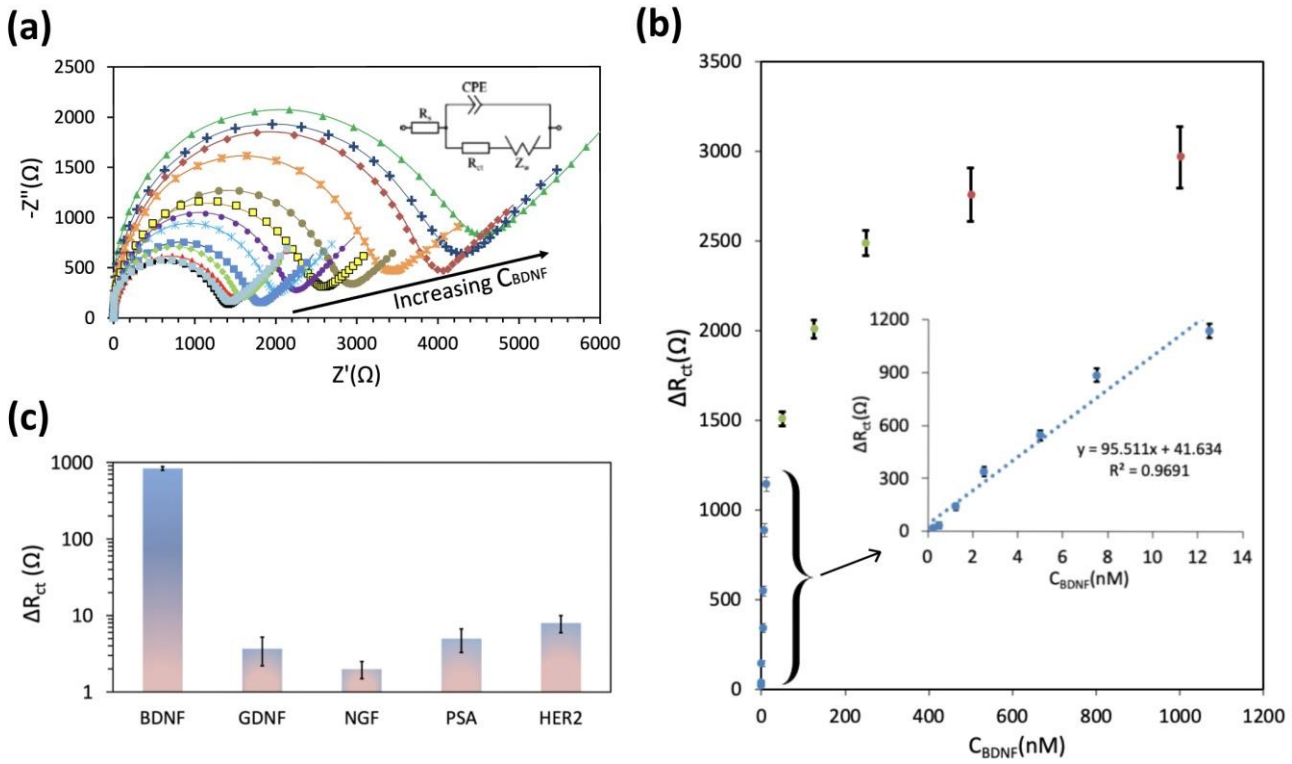

Figure 3 (a) Nyquist plots of the aptasensor at various concentrations of BDNF. Here, dots and dash lines represent the experimental and the fitted data respectively, based on modified Randle's model (Inset of the Figure) (b) Calibration curve of the aptasensor in the probe solution with different BDNF concentrations from 0.025-1000 nM. The inset displays the resulting linear range. (c) Selectivity of the aptasensor for BDNF detection compared to other biological interferents. BDNF was used at 50 nM, while the other tested molecules were each used at 10 µM.

From Equation (1) the estimated limit of detection (LOD) and sensitivity for GC/r-GO/PBASE/APT/BSA electrode were 31.3 nM (S/N = 3) and 95.5 $\Omega.nM^{-1}$, respectively. The selectivity of the proposed aptasensor investigated in the presence of some interfering species such as Glial cell line-derived neurotrophic factor (GDNF), Nerve growth factor (NGF), Prostate-Specific Antigen (PSA), and human epidermal growth factor receptor 2 (HER2). These proteins were chosen because of their structural resemblance to BDNF.(46) As illustrated in Figure 3c, a significant response is noted only with the addition of BDNF, while the response to 10 µM of any other species is less than 1% of the response elicited by 50 nM BDNF. These results demonstrate the high accuracy and selectivity of the proposed aptasensor in the complex matrix of human blood serum. The high selectivity of the aptasensor attributes to the effective immobilization of BDNF aptamer molecules and the efficient blocking effect of BSA on the GC/r-GO/PBASE/APT/BSA electrode. To confirm that the observed signal originated from the specific interaction between the aptamer and BDNF, a synthesized mutated non-binding aptamer was immobilized on the sensor surface. As anticipated, no significant response was observed upon exposure to BDNF or other biomolecules, as shown in Figure S3a. Furthermore, the long-term stability and performance of the sensors were evaluated over a 30-day period using three independent sensors. The relative response exhibited a decline of approximately 7% after 5 days and 21% after 30 days, as illustrated in Figure S3b, indicating acceptable stability over the investigated timeframe.

BDNF detection with EGFET. As illustrated in schematic Figure 4, the flexible polyimide (PI) electrode serves as an extended sensing element and modified with r-GO/PBASE/APT/BSA within an extended-gate field-effect transistor configuration (aptaEGFET). This design capitalizes on the selective properties of the r-GO/PBASE/APT/BSA electrode, which specifically engineered to recognize and bind to BDNF. When incorporated into the EGFET system, the binding of BDNF induces changes in the electrical properties of the r-GO/PBASE/APT/BSA electrode, which then transduced into measurable signals. This configuration optimized to provide high sensitivity and selectivity for BDNF, making it an effective tool for detecting and quantifying BDNF in complex sample matrices. In this EGFET setup, the gate terminal of the conventional n-MOSFET functions as the transducer, interfacing with the molecular recognition component of the sensor.

The negatively charged aptamers, which serve as recognition sites on the surface of the modified electrode, enable the specific binding of BDNF within the r-GO/PBASE/APT/BSA modified electrode matrix. Applying a positive bias to the floating gate electrode (reference electrode) attracts positively charged BDNF molecules (isoelectric point 9.43) at pH 7 to the r-GO/PBASE/APT/BSA modified electrode surface, where they bind to the aptamers. This binding interaction induces changes in the surface potential due to charge accumulation in the

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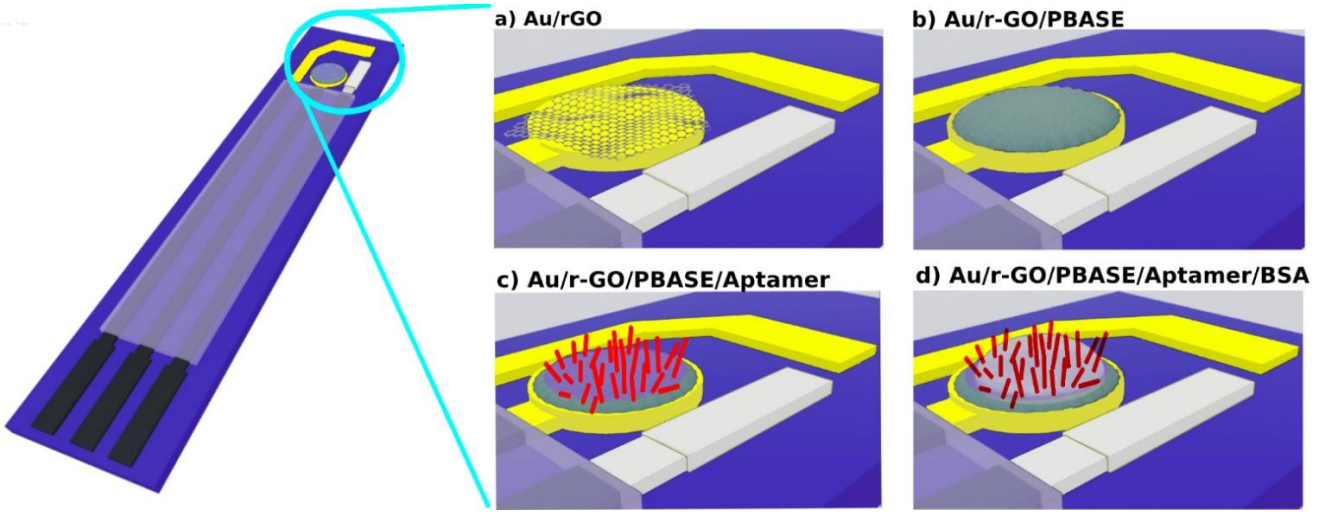

Figure 4 (a) Schematic image of the Au electrode deposited on the surface of polyimide (PI). The functionalization of modified r-GO/PBASE electrode (b) before and (c) after aptamer immobilization, and (d) after blocking the surface with BSA is illustrated. Here, the aptamers are presented by red rods.

recognition layer.(47) For optimal detection, the Debye length of the modified aptaEGFET must be comparable to or exceed the size of the recognition element when interacting with BDNF. If the Debye length does not sufficiently overlap with the size of the recognition element, the charge field from the BDNF molecules will be screened, resulting in a significant decrease in detection sensitivity.(48)

$$\lambda_D = \frac{1}{\sqrt{4\pi l_B \sum_i \rho_i z_i^2}} \quad (2)$$

Here, l_B denotes the Bjerrum length (0.7 nm), while ρ_i, and z_i^2 represent the ion density and valence, respectively. As indicated by Equation (2), the Debye length λ_D diminishes with increasing PBS concentration. Previous studies have shown that λ_D significantly impacts target detection. Due to the charge screening effect, BioFETs achieve optimal performance in environments with relatively low ionic strength.

To effectively functionalize the electrode, a 0.75 nm thick PBASE layer employed as a linker between the aptamers and the r-GO sheet. PBASE binds to the r-GO surface via π–π interactions involving its aromatic carbon rings. Additionally, the amine group at the terminal end of the aptamer, which participates in the EDC (1-ethyl-3-(dimethylaminopropyl) carbodiimide)-NHS (N-hydroxysuccinimide) coupling reaction with PBASE, measures approximately 0.91 nm. Considering that BDNF, with an estimated size based on its molecular weight (27,000 g/mol) and average protein density (1.37 g/cm^3), is approximately 4 nm, the combined size of the BDNF-aptamer-PBASE complex is approximately 8.91 nm, which has a Debye length of approximately 10 nm. This ensures that the given the Debye lengths in various PBS concentrations (0.7 nm in 1× PBS, 2.3 nm in 0.1× PBS, 7.3 nm in 0.01× PBS, 10 nm in 0.005× PBS, and 24 nm in 0.001× PBS) the PBS solution diluted to 0.005× PBS, charged BDNF-aptamer conjugates, with a combined length of approximately 8.91 nm, fall within the Debye screening length of the solution.(49-51)

To assess the efficacy of the modified aptaEGFET for BDNF detection, a systematic experimental investigation performed. This evaluation involved measuring the responses of the modified aptaEGFET to various BDNF concentrations in a PBS buffer solution. Semiconductor parameter analysis (K2450) employed for characterizing the modified aptaEGFET. The operational mechanism and sensitivity of the modified aptaEGFET further elucidated by analysing the V_{gs}–I_{ds} (Gate Voltage vs. Drain Current) electrical characteristics, which provided insight into the performance of the sensing platform. Our hypothesis posits that the positively charged BDNF molecules approach and bind to the surface of the modified aptaEGFET. This interaction induces a change in the surface potential (ψ) of the electrode. Notably, this change in surface potential directly affects the threshold voltage (V_T) at the interface between the electrolyte and the modified aptaEGFET. Consequently, variations in BDNF concentration (C) lead to corresponding changes in V_T, which can be mathematically represented by the following relationship (Equation 3)(52):

$$V_{T(EGFET)} = V_{T(FET)} - \frac{\psi_M}{q} + E_{REF} + \chi^{Sol} - \psi(C) \quad (3)$$

In this context, the V_T of the MOSFET is denoted as $V_{T(FET)}$, representing the gate threshold voltage for each individual MOSFET. The surface potential of the metal gate is expressed as ψ_M, while the potential of the reference electrode is defined by E_{REF}. Additionally, the surface dipole potential of the buffer solution is represented by χ^{Sol}. This equation highlights that the sensing mechanism relies on detecting changes in electrical properties, specifically the V_T, at the interface between the electrolyte and the modified aptaEGFET. These changes apply by the specific binding of BDNF molecules to the aptamers on the electrode surface. This approach facilitates the quantification of BDNF by measuring the alterations in V_T (ΔV_T). When a voltage is applied to the extended gate, the ψ changes due to the accumulation of positive charges from BDNF binding, resulting in a leftward shift in the V_{gs}–I_{ds} curves of the n-channel

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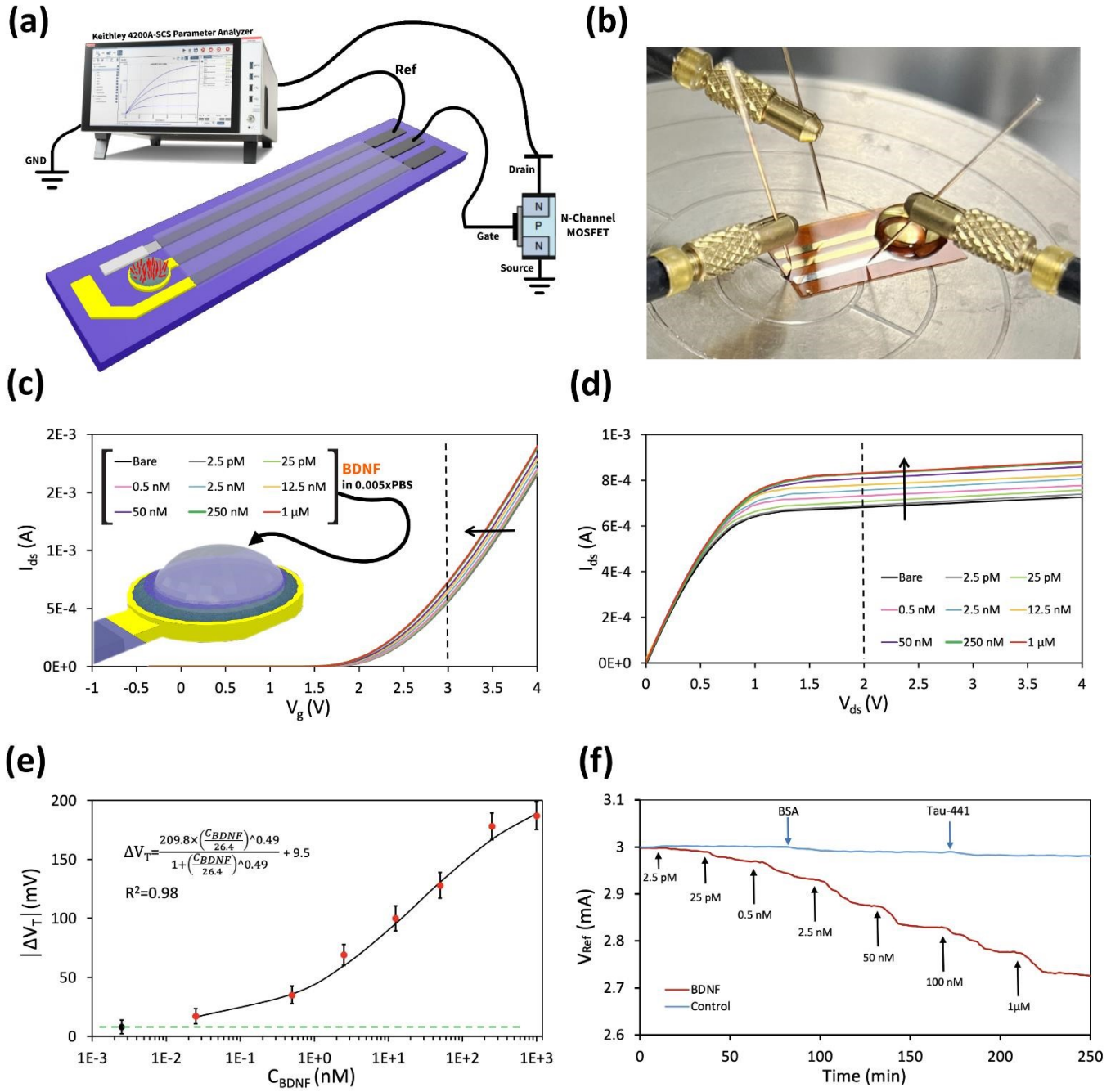

Figure 5 (a) The schematic and (b) real image of the aptaEGFET. (c) Gate voltage-drain Current– (I_D– V_{gs}) at V_D=2 V and (d) drain voltage-drain current (V_{ds}-I_{ds}) at V_g=3 V characteristics after applying different concentrations of BDNF to r-GO/PBASE/APT/BSA modified electrode. (e) The calibration curve and LOD after adding different levels of BDNF. The baseline of BDNF response, indicated by the green line, is characterized as the mean response augmented by a single standard deviation of three to r-GO/PBASE/APT/BSA modified electrode of aptaEGFETs when subjected to 0.005x PBS buffer solution in the absence of BDNF. The fitted data to a Hill–Langmuir model for BDNF on r-GO/PBASE/APT/BSA modified electrode of aptaEGFET is presented. (e) Real-time detection using an aptaEGFET for BDNF detection was investigated. BDNF was added sequentially at different levels. Control samples like BSA and Tau-441, were added at a concentration of 500 nM.

MOSFET (Figure 5b) at a constant V_{ds} = 2V. As illustrated in Figure 5b, V_T decreases with rising BDNF concentration, reflecting the enhanced carrier density leading to a higher current through the channel. Additionally, Figure 5d demonstrates that the relationship between the drain current (I_{ds}) and drain voltage (V_{ds}) can be utilized to evaluate the responses of the r-GO/PBASE/APT/BSA in the presence of BDNF. The I_{ds} in the saturation region can be calculated as follows (47):

$$I_{ds,max} = \frac{\mu_0 C_{OX}}{2} \times \frac{W}{L} \times \left(V_{ref} - V_T\right)^2 (1 + \lambda V_{ds}) \quad (4)$$

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where electron mobility is depicted by μ_0 in the channel with the width-to-length ratio of W/L, and length modulation of λ. The capacitance of the unit era is shown as C_{OX}, and the voltages apply to the reference and drain electrodes are denoted by V_{ref} and V_T, respectively.

As depicted in Figure 5d, the application of a constant gate voltage (V_g = 3 V) correlates with an increase in $I_{ds,max}$ as the BDNF level increases. This enhancement results from the binding interaction between BDNF and the modified aptaEGFET, leading to an increase in the effective voltage applied to the gate and a subsequent rise in I_d.

The modified aptaEGFET evaluated against various BDNF concentrations to validate its detection effectiveness using BDNF-aptamers. Figure 5e illustrates the resulting I_{ds}-V_{gs} characteristics fitted using the Hill-Langmuir model, which represents the affinity binding of a BDNF by an aptamer (Equation 5) (53, 54):

$$\Delta V_T = A\frac{\left({}^{C}/_{K_d}\right)^n}{1+\left({}^{C}/_{K_d}\right)^n} + Z \quad (5)$$

In this model, the maximum signal resulting from all aptamers binding to BDNF is denoted as A, the BDNF concentration is C, the effective dissociation constant at which half of the aptamers are occupied by BDNF is K_a, the Hill coefficient is n, and Z is an offset parameter accounting for ΔV_T in the absence of BDNF.

The best fit to the data yielded the following values: A=209.8 mV, K_d=26.4 nM, n=0.49, and Z=9.5 mV. The best-fit value for the offset parameter Z=9.5 mV aligns with the measured responses of devices exposed to pure 0.005× PBS buffer. The best-fit value for K_d=26.4 nM for soluble BDNF falls within the expected range of 0.025−1000 nM. The best-fit value for n=0.49 suggests negative cooperativity in the binding of BDNF to the modified aptaEGFET, which may be attributed to protein−aptamer interactions upon binding or increased charge carrier scattering with increased ligand binding. The LOD of 0.4 nM achieved based on the "3σ" rule.(55, 56) The aforementioned LOD is notably significant, given that the normative serum concentrations of BDNF have been documented to range from 8-40 ng/mL. Furthermore, the achieved LOD is comparable to that of graphene-based FET sensors reported for the detection of other biomolecules, underscoring the competitive sensitivity of the proposed sensing platform.(57, 58)

Real-time monitoring and specificity checking of aptaEGFET for detection of BDNF performed by recording the sensor reference voltage (V_{ref}) at constant I_{gs} = 0.6 µA and voltage of V_{ds} = 2V (Figure 5f). No washing steps or reaction buffer changes were used during the measurements. Initially, the electrodes placed in a 0.005× PBS buffer solution for approximately 30 minutes to stabilize their potential. BDNF targets then introduced to the sensing surface of the aptaEGFET-modified electrodes functionalized with aptamer probes. Seven different target concentrations (2.5 pM, 25 pM, 0.5 nM, 2.5 nM, 50 nM, 100 nM, and 1 µM) added consecutively at 30-minute intervals. Each addition of BDNF concentration resultes in clearly distinguishable drops in V_{ref}.

The performance of the aptaEGFET in detecting BDNF benchmarked to previous studies. Table 1 provides a comparative analysis of various biosensors for BDNF detection, showcasing the advantages of our aptaEGFET. This study exhibits superior sensitivity with a low LOD and a broad linear detection range, utilizing an aptaEGFET. This performance aligns it with advanced FET biosensors and makes it comparable to electrochemical, and optical sensors. The aptaEGFET presents a reliable candidate for early neurodegenerative disease diagnosis.

Table 1 Comparison study of different sensor for BDNF detection.

Method	Recognition element	Clinical samples	Limit of detection	Range of Detection	Ref.
Optical	Aptamer	Human Serum	0.2 ng/mL	0.41 to 250 ng/mL	(59)
DPV	MIP	-	9 pg/mL	10–40 pg/mL	(15)
EIS	Antibody	Mice cerebrospinal fluid	-	10 fg/mL-10 ng/mL	(46)
DPV	Antibody	Human serum	0.2 ng/mL	0.1-2.0 ng/mL	(60)
Colourimetric	Antibody-AuNPs	Artificial tear	14.12 pg/mL	25 -300 pg/mL	(61)
EIS	Antibody-AuNPs	Human serum	1.5 ± 0.012 pg/mL	4-600 pg/mL	(62)
CV, DPV	Antibody-MWCNTs	Human serum	5 pg/mL	0.01-100 ng/mL	(63)
CV, DPV	MIP	Human serum	6 pg/mL	0.01-0.06 ng/mL	(64)
EGFET	Aptamer	-	10.8 ng/mL (0.4 nM)	6.7 ng/mL-27 µg/mL (0.025-1,000 nM)	**This work**

Experimental

Material. PBASE, buffer phosphate saline (PBS), ethanolamine, hydrazine monohydrate (85%), DNA aptamer specific to BDNF (5'-NH_2-(T20)-GGATTTGAGCTTATGTGGCATAGGTTGCCTGGGTG GGTGGGGTCGGGGAA-3') and mutated non-binding aptamer to BDNF (5'-NH_2-(T20)-GGATTTGAGCTTATGTGGCATAGGTTGCCTCCGTGGCTGGCGTCG GGGAA-3') (65), and $KMnO_4$ (99%), $NaNO_3$ (99%), H_2O_2 (30%), H_2SO_4 (98%), Graphite (cat #332461), purchased from Sigma-Aldrich. Disodium hydrogen phosphate (Na_2HPO_4), potassium ferrocyanide ($K_4Fe(CN)_6$), and potassium ferricyanide ($K_3Fe(CN)_6$) were purchased from Merck. The 3.0 µm Pore Size Whatman membrane filters were purchased from Tisch Scientific. BDNF Protein was purchased from Bio-Techne.

Double distilled water (DI) was used throughout the work. GDNF, NGF, PSA, and HER2 were purchased from fisher scientific. All chemicals were of analytical grade and used without further purification. All solutions were prepared with high-purity nitrogen (99.99 % purity) before the experiments. CD4007 (MOSFET), and PI film were purchased from RS components.

Fabrication of aptasensor. The glassy carbon electrode (GCE) cleaned by polishing with 0.05 µm alumina slurry. 5 µL of GO concentrations between 0.1 µg/mL diluted in ultra-pure water (UPW) dropped cast on the freshly GCE surface. A sealed vessel at 70 degrees Celsius for 3 hours used to reduce GO to r-GO using hydrazine vapour ($NH_2NH_2 \cdot xH_2O$, reagent grade, N_2H4: 50–60%). For the immobilization of the DNA aptamers on the electrode surface, 10 mM PBASE in DMF used to functionalize graphene devices for 120 minutes at room temperature to obtain GC/r-GO/PBASE. By the two different moieties of PBASE, it serves two different functions as a heterobifunctional linker (66, 67). PBASE pyrenes stack on the graphene surface by π–π stacking interaction and the succinimide moiety reacts with the amino group at the 5′ end of the probe DNA (68). To obtain GC/r-GO/PBASE/APT, a total of two microliters of aptamer solution placed on the GC/r-GO/PBASE electrode surfaces at room temperature for different incubation times and aptamer concentrations in PBS. GC/r-GO/PBASE/APT electrodes washed with PBS and distilled water for 10 minutes to remove non-attached aptamer molecules. To block unreacted surface sites, this modified electrode immersed in 1 mg/mL bovine serum albumin (BSA) solution for 60 minutes. PBS and distilled water used to thoroughly rinse the electrode after this step. It is named the GC/r-GO/PBASE/APT/BSA electrode. Electrochemical impedance spectroscopy (EIS) used to investigate each step of electrode modification. To detect BDNF protein, a 2.0 microliters aliquot of the protein dropped-cast on the GC/r-GO/PBASE/APT/BSA electrode for 1 hour. The electrode surface washed in ultra-pure water and PBS for 10 minutes to remove BDNF that may have remained unabsorbed or weakly absorbed on it. The change in charge transfer resistance (R_{ct}) used as a biosensor response for measuring the concentration of BDNF.

Apparatus and Measurements. An infrared spectrometer equipped with a Jasco 6300 FTIR spectrometer used to record FTIR spectra of solid-state samples (KBr matrix). The thickness of GO investigated using an atomic force microscope (AFM) from Park System XE-100E. Autolab PGSTAT 302N potentiostat/galvanostat (Eco Chemie, Utrecht, Netherlands) used for all electrochemical measurements. In all electrochemical experiments, a three-electrode system used with an Ag/AgCl electrode (saturated) in 3 M KCl, a platinum counter electrode, and modified glassy carbon electrodes. EIS performed in a 0.1M $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ at a potential of 0.22 V, with an oscillation potential of 5 mV, at 10 kHz to 0.1 Hz. All electrochemical experiments conducted at room temperature and pH 7. The modified Randles circuit model employed to fit the EIS data.

Various concentrations of BDNF ranging from 0.025-1,000 nM prepared in 0.005× PBS at pH 7. To examine the EFGET response to different BDNF concentrations, 100 µL of the sample applied to the surface of a modified electrode for 30 min followed by a rinsed with DI water and gentle drying with N_2 gas. This electrode pre-incubated in a 0.005× PBS solution for 30 minutes to stabilise. Subsequently, the measurement conducted in a 0.005× PBS solution. The gate voltage (V_g) varied from 0 to 5 V, while a constant voltage of 2 V maintained between the source and drain contacts to ensure the MOSFET operates in the linear regime. The I_{ds}–V_{gs} characteristics of the n-channel MOSFET documented as the response of the EGFET sensor to various BDNF concentrations. Additionally, V_g held at a steady 3 V, and the drain voltage (V_{ds}) varied from 0 to 5 V to examine the I_{ds}–V_{ds} characteristics of the sensor.

Conclusions

This study presents a novel electrochemical aptasensor based on reduced-graphene oxide field-effect transistors (r-GO-FETs) for the detection of Brain-Derived Neurotrophic Factor (BDNF). The use of DNA aptamers as recognition elements, combined with the exceptional electrical properties of reduced graphene oxide (r-GO), resulted in a highly sensitive and selective biosensor. By employing Electrochemical Impedance Spectroscopy (EIS) to optimize the sensor, we significantly enhanced sensitivity and achieved a lower limit of detection (LOD), with a detection limit of 0.4 nM and a wide linear response range from 0.025-1,000 nM. This optimization offers a considerable improvement in sensor performance across a broad dynamic range of BDNF detection. Additionally, the aptaEGFET exhibited high specificity in complex biological matrices, making it a promising tool for early diagnosis and monitoring of neurodegenerative diseases. The flexible polymer substrate, along with the integration of gold and silver chloride electrodes, further enhanced the stability and performance of the biosensor. This research underscores the potential of aptamer-based biosensors in clinical diagnostics, offering a cost-effective and reliable alternative to conventional methods. Future work will focus on further optimization and clinical validation to establish this technology as a standard diagnostic tool for neurodegenerative disorders.

Author contributions

Mostafa Salehirozveh: Conceptualization, Visualization, Writing – review & editing, Writing – original draft, Formal analysis, Validation, Methodology, Investigation, Formal analysis, Data curation. **Robin Bonne**: Writing – review & editing, Methodology. **Peeyush Kumar**: Investigation, Resources. **Farbod Abazar**: Writing – review & editing, Software. **Parisa Dehghani**: Writing – review & editing, Methodology, Formal analysis. **Vellaisamy A. L. Roy**: Writing – review & editing, Resources, Project administration, Supervision. **Ivan Mijakovic**: Writing – review & editing, Resources, Project administration, Supervision.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

The following Supporting Information is available free of charge at the RSC website **https://pubs.rsc.org.** Additional details on material, Substrate preparation, Pseudo- reference electrode fabrication, Electrochemical measurement, Electrical setup and analysis, Readout solution preparation, CV analysis of pseudo-reference electrode, XRD, FTIR characterizations.

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Data availability

The following Supporting Information is available free of charge at the RSC website https://pubs.rsc.org Additional details on material, Substrate preparation, Pseudo- reference electrode fabrication, Electrochemical measurement, Electrical setup and analysis, Readout solution preparation, CV analysis of pseudo-reference electrode, XRD, FTIR characterizations.