Supporting information

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

Mostafa Salehirozveh, [a] Robin Bonné, [b] Peeyush Kumar, [c] Farbod Abazar, [d] Parisa Dehghani, *[e] Ivan Mijakovic,*[ag] Vellaisamy A. L. Roy [ef]

[a.] *Division of Systems and Synthetic Biology, Department of Life Sciences, Chalmers University of Technology, SE-41296, Gothenburg, Sweden*
[b.] *Center for Electromicrobiology CEM, Aarhus University, Ny Munkegade 114, 8000 Aarhus, Denmark*
[c.] *Department of Engineering, Johannes Kepler University Linz – JKU, Austria*
[d.] *Department of Information Engineering, University of Pisa, UNIPI, Pisa, Italy*
[e.] *James Watt School of Engineering, University of Glasgow, Glasgow G12 8QQ, UK E-mail: Parisa.Dehghani@glasgow.ac.uk*
[f.] *School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Hong Kong*
[g.] *The Novo Nordisk Foundation Center for Biosustainability Technical University of Denmark DK-2800 Kongens Lyngby, Denmark*
E-mail: ivan.mijakovic@chalmers.se
E-mail: ivmi@biosustain.dtu.dk

Method:

Synthesis of GO

Natural graphite and $NaNO_3$ are added to concentrated H_2SO_4 and stirred for minutes. Then $KMnO_4$ was added gently while storing and maintaining the mixture below 20 °C by keeping it in the ice bath. Next, the mixture was stirred for some minutes at 35 °C. After that, the temperature increased to nearly 100 °C by adding the distilled water slowly to the mixture. Then, by maintaining the mixture at this temperature, it gradually became brown. Then, to eliminate the residual permanganate, manganese dioxide and manganese sulfate, the H_2O_2 (30%) was added slowly. As a result, the dark brown mixture turned gradually to bright yellow. Finally, the product was rinsed with DI water, ethanol, acetone, and a filter to remove impurities, large particles, and residual acid (1). This step is repeated several times until it approaches a *neutral pH* (1,2). To ensure complete removal of ionic impurities, the graphite oxide was dialyzed for a few days (3). To obtain the GO, the graphite oxide product was exfoliated in an ultrasound bath for 2 hours (1–4). It's worth highlighting that utilizing exfoliated graphene for the synthesis of GO results in higher-quality GO. After that, using sonication decreased the GO sheets' size to improve the connection between single-layer of GO. Herein, the 1 mg/mL suspension of GO in DI water was prepared and sonicated for 12 h at a power of 100 W. In the next step, in order to remove the large sheets and have a uniform size of nanosheets, the GO dispersion was centrifuged twice with 1000 and 4000 rpm for 1 h. At last, the average size and thickness of GO sheets studied by AFM were 2-4 µm and ~1.1 nm, respectively.

Pseudo-reference electrode fabrication

To construct the pseudo-reference electrode, 200 nm silver was deposited by an electron beam in conjunction with a shadow mask for precise fabrication. After drying the deposited silver electrodes, they were subjected to a chlorination process using $FeCl_3$. Chlorination was conducted for 60 seconds with varying concentrations of $FeCl_3$, where the average estimated thickness of the AgCl layer was around 123 nm (5). The morphological characterisation of the fabricated pseudo-reference electrode was studied using an SEM coupled with an EDX analysis system. The electrochemical performance of the electrode was assessed using a potentiostat/galvanostat Autolab.

Fabrication of Flexible Aptasensor

Flexible electrochemical kits were fabricated on polyimide substrates by using an electron beam to deposit the Ti/Au (20/100 nm). The reduced graphene oxide (r-GO) was drop-cast on the electrode surface and dried at room temperature. For the immobilization of DNA aptamers, the electrodes were incubated with the 1-Pyrenebutyric acid N-

hydroxysuccinimide ester (PBASE) and consequently with aptamer solution and then rinsed with PBS and distilled water. Each step of electrode modification was characterized by Electrochemical Impedance Spectroscopy (EIS) and atomic force microscopy (AFM).

Result and Discussion

Pseudo-reference electrode characterization

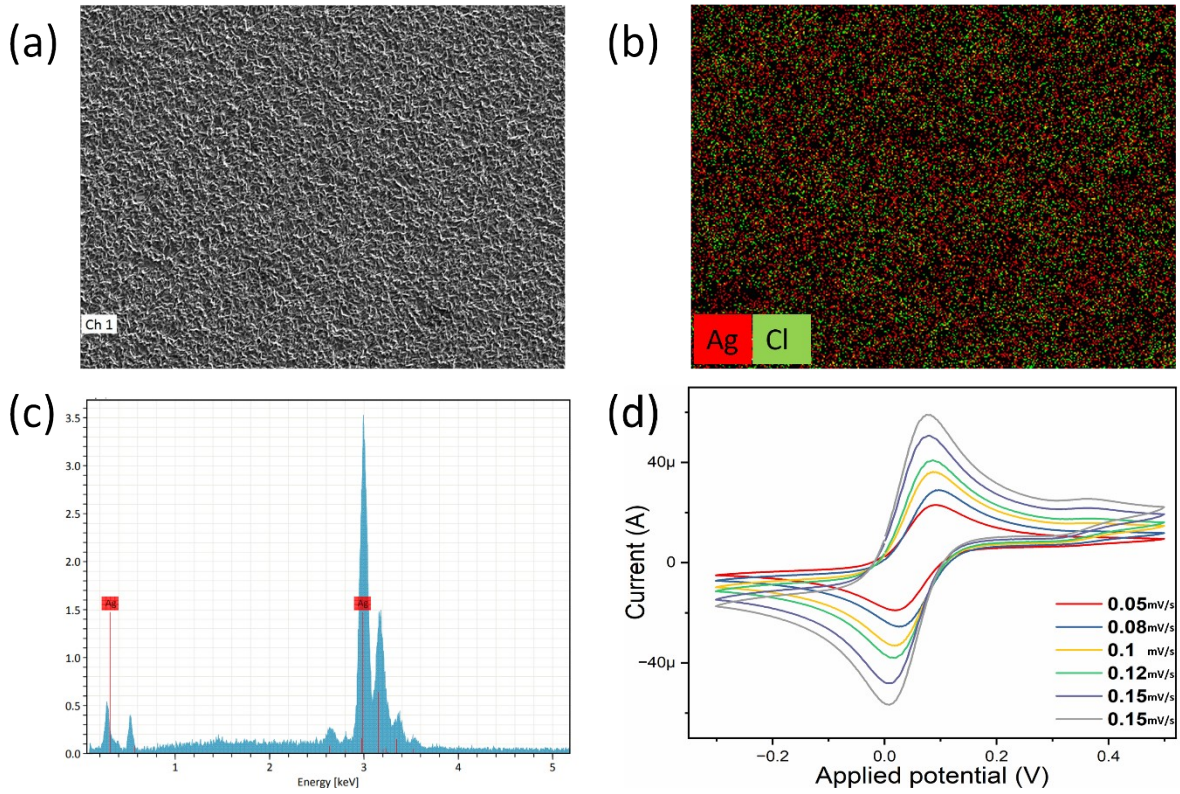

Figure S1 (a) SEM image of the silver electrode after chlorination with $FeCl_3$. (b) The EDX mapping (b) and spectra (c) of the Ag/Cl electrode. Cyclic voltammetry (CV) to investigate the performance of pseudo-reference electrodes in buffer solution at different scan rates (d).

Chemical analysis

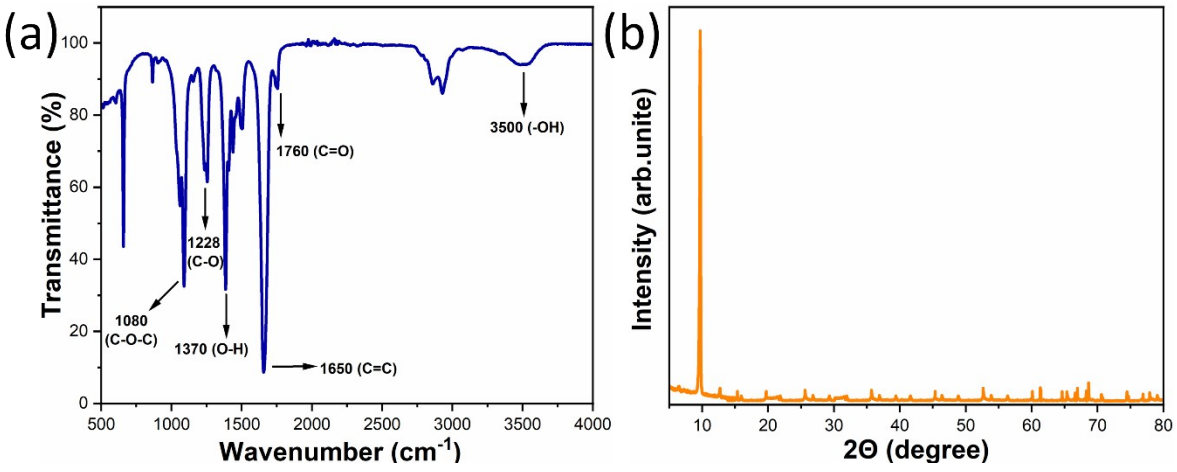

Figure S2 (a) XRD image of GO with a distinct peak at approximately 10°, (b) FTIR spectra of synthesized GO.

The analysis of Electrochemical Impedance Spectroscopy (EIS) measurements was performed using Nyquist plots and the parameters obtained from equivalent circuit modeling of the electrochemical system, as summarized in Table S1. A Randles equivalent circuit was employed to model the Nyquist diagram, comprising solution resistance (R_s), a constant phase element (CPE) representing the capacitance of the electrochemical double layer, charge transfer resistance (R_{ct}) associated with the interfacial electron transfer process. The solution resistance (Rs ≈ 100 Ω) was considered negligible and excluded from further discussion due to its relatively constant and minimal contribution compared to other resistance components.

Table S1 Fitting EIS parameters of GC electrodes at each functionalization steps.

Samples	CPE (F)	R_{ct} (Ω)
GC	1.2×10^{-6}	198±14
GC/GO	4.2×10^{-7}	779±36.2
GC/rGO	3×10^{-7}	52±9.4
GC/rGO/PBASE	6×10^{-7}	225±19.3
GC/rGO/PBASE/APT	3.9×10^{-7}	1260±99.6
GC/rGO/PBASE/APT/BSA	4.1×10^{-7}	1409±128.7

Figure S3 illustrates the long-term monitoring of biosensor performance that the response was lost 7% after 5 days and 21% after 30 days. Therefore, it can be concluded that our BDNF biosensor, developed with an aptamer, is well-suited for long-term BDNF measurements and offers a broad linear working range.

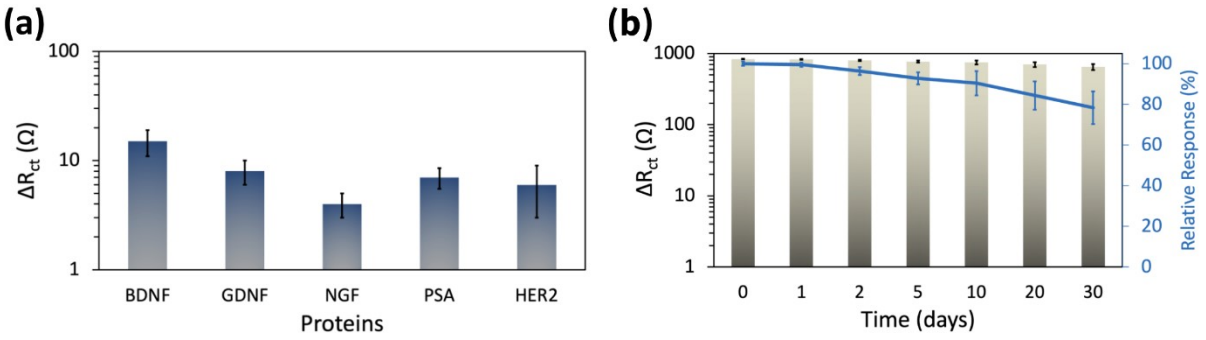

Figure S3 (a) The control experiments involve using a mis-matched (non -binding) aptamer functionalized sensor to prove that the aptasensor response is due to the aptamer-BDNF binding, (b) The lifetime of BDNF aptasensor for a period of 30 days. The study is carried out with 50 nM BDNF, while the other tested molecules were each used at 10 µM.

References

1. Chhowalla M, Shin HS, Eda G, Li LJ, Loh KP, Zhang H. The chemistry of two-dimensional layered transition metal dichalcogenide nanosheets. Nature Chemistry 2013 5:4 [Internet]. 2013 May;5(4):263–75. Available from: https://www.nature.com/articles/nchem.1589

2. Kurapati R, Kostarelos K, Prato M, Bianco A, Kurapati R, Bianco A, et al. Biomedical Uses for 2D Materials Beyond Graphene: Current Advances and Challenges Ahead. Advanced Materials [Internet]. 2016 May;28(29):6052–74. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/adma.201506306

3. Morales-Narváez E, Merkoçi A, Merkoçi A, Morales-Narváez E. Graphene Oxide as an Optical Biosensing Platform. Advanced Materials [Internet]. 2012 May;24(25):3298–308. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/adma.201200373

4. Balendhran S, Walia S, Alsaif M, Nguyen EP, Ou JZ, Zhuiykov S, et al. Field effect biosensing platform based on 2D α-MoO3. ACS Nano [Internet]. 2013 May;7(11):9753–60. Available from: https://pubs.acs.org/doi/full/10.1021/nn403241f

5. Dunare C, Marland JRK, Blair EO, Tsiamis A, Moorel F, Terry JG, et al. Test structures for characterising the silver chlorination process during integrated Ag/AgCl reference electrode fabrication. IEEE International Conference on Microelectronic Test Structures. 2019 Mar 1;2019-March:58–63.