Low-cost platform for determination of NTproBNP in saliva using graphene-based aptasensor

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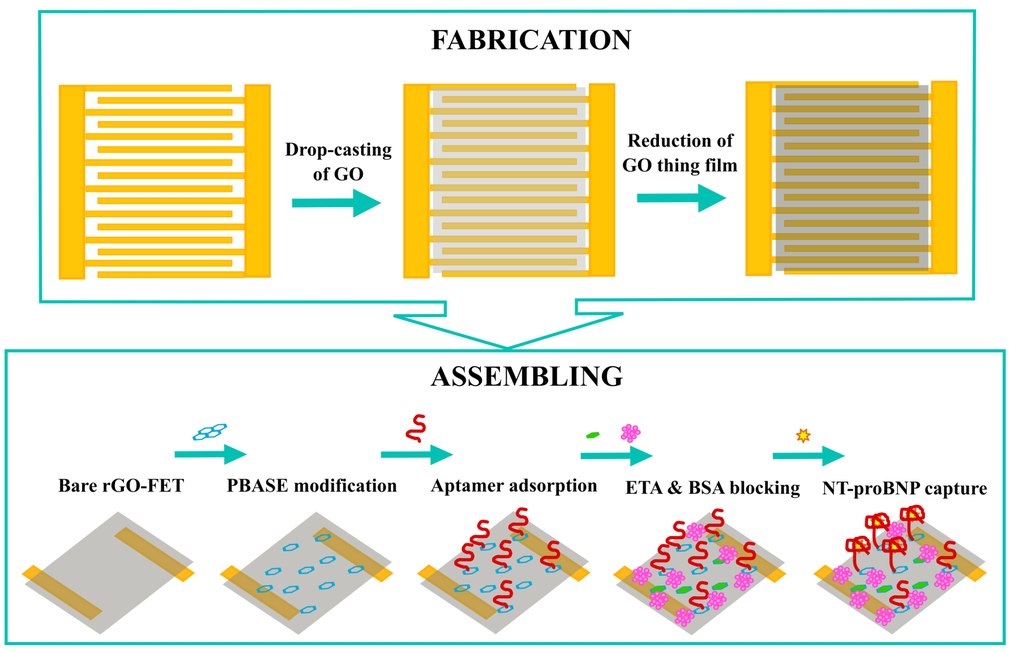
ABSTRACT Detection of trace concentrations of biomarkers can be essential if non-invasive analysis is employed, such as sampling of saliva, urine, or sweat. A significant early-stage heart failure biomarker N-terminal pro-B-type natriuretic peptide (NT-proBNP) can be found in saliva in concentrations of thousand times less than in blood plasma suggesting that non-invasive analysis require outstanding low detection limit. In this research, we propose a reduced graphene oxide-based FET device to develop a biosensor using specific anti-NT-proBNP aptamers for the detection of NT-proBNP in saliva. To address the need for low concentration detection in saliva, we included a correlation analysis of two FET parameters, Dirac point shift and transconductance variation to provide reliable biosensor performance. Namely, depending on the ionic strength of the solution, we found that correlation is significant (>0.8) for 0.01X PBS, while it is zero for 0.1X PBS. This can be associated to the long aptamer chain, consisted of 72 bases, that has significant effect on doping of rGO channel and such effect is screened by the ions of higher concentrations (> 16 mM). The analysis shows that chemical interaction between target and receptor is transformed into electrical signal, which is a result of either direct doping effect or charge mobility change. With such an approach, we observed a broad dynamic range of NT-proBNP concentrations in 0.01X PBS of 10^0 – 10^5 fg mL^{-1}. Additionally, femtomolar detection of NT-proBNP is achieved in artificial saliva with limit of detection of 41 fg mL^{-1}.

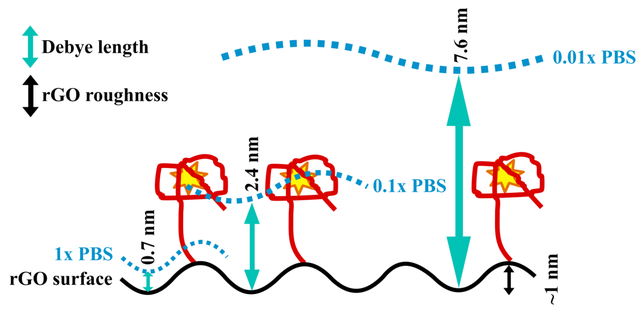
BIOSENSOR DEVELOPMENT



Modification of gold interdigitated electrodes (G-IDE222, Drop Sens) by 0.2 mg mL^{-1} GO suspension in water/NMP drop-casting for 2 hours. GO thin film was reduced by hydrazine vapor for 2 hours and thermall annealing for 1 hour at 200 °C.

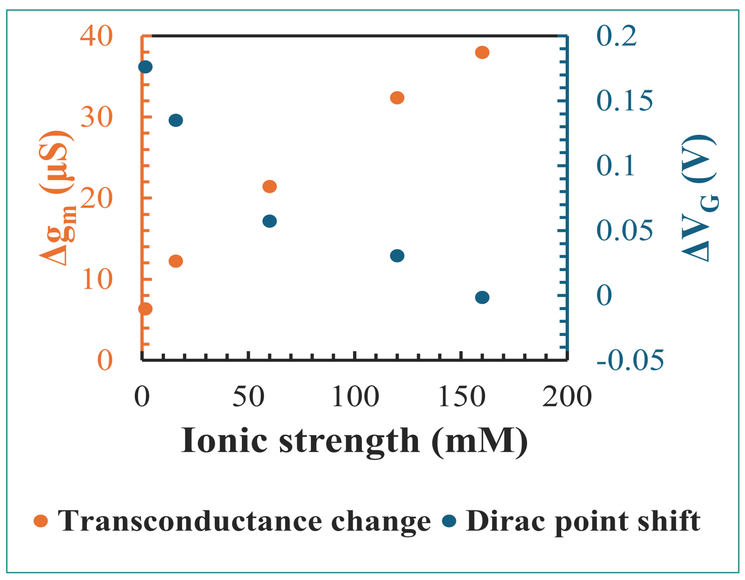
rGO-FET channel was functionalized as follows: rGO was modified with PBASE linker to enable N20 (anti-NTproBNP) aptamer to covalently attach during overnight incubation in 1x PBS. The unreacted PBASE were blocked by ethanolamine and remaining surface by BSA [1,2].

SENSOR CALIBRATION DUE TO BUFFER IONIC STRENGTH



EDL formation due to different buffers

The electric double layer (EDL) formed in the vicinity of the graphene surface is described by Debye length (DL) [3]. Increasing the DL, the ion screening is weaker, lifting the signal damping.



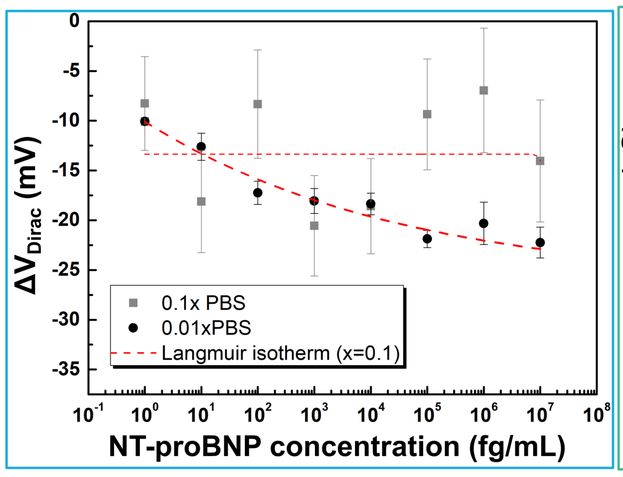
Ionic strength variation influence on rGO-FET key parameters

Ionic strength change induces changes of the electrical properties of rGO-FET since gating is realized by buffer ions. Two important characteristics are observed:

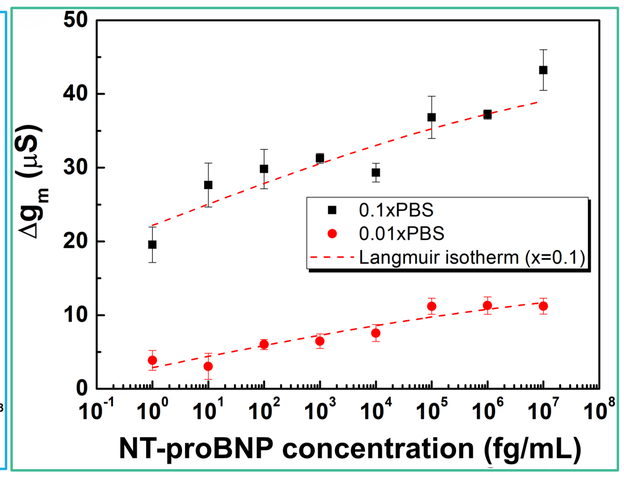
1. Dirac point: left-shift observed for ion concentration increase;
2. Transconductance: increase for ion concentration increase.

TWO-PARAMETER ANALYSIS

Experimental setup: spiked samples of NT-proBNP ranging from 1 fg mL^{-1} to 10 ng mL^{-1} in 0.1x and 0.01x PBS solutions. Signal response was obtained from transfer curves measured for each concentration.



DP shift for NT-proBNP conc. change

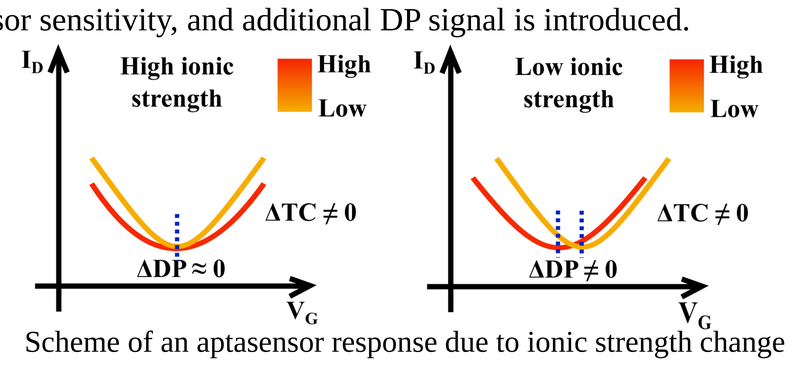


TC change for NT-proBNP conc. change

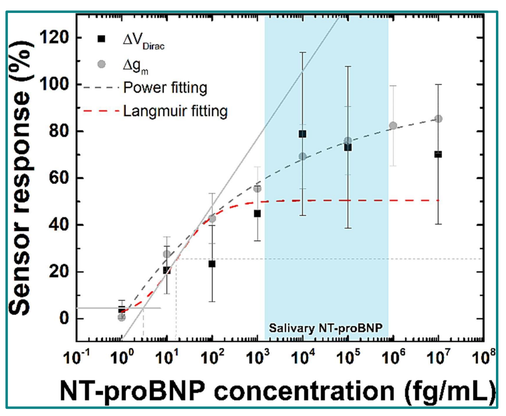
NT-proBNP conc. increase	DP shift (ΔV_G)	TC change (Δg_m)	PCC value
0.1x PBS	No trend	Increases	-0.09
0.01x PBS	Left-shift	Increases	0.86

DISCUSSION & CONCLUSION

DP position is a function of channel doping and the contribution from aptamer conformation upon target binding is significantly influenced by ion screening (EDL capacity) [1]. TC decreases with the increase of adsorbed molecules (charge scattering centers) because carrier mobility weakens as charge scattering is increasing. Therefore, TC itself is not enough to study biosensor sensitivity, and additional DP signal is introduced.



Scheme of an aptasensor response due to ionic strength change



Detection of NT-proBNP in artificial saliva [1]

Detection of NT-proBNP in artificial saliva is observed in the range of 10 fg mL^{-1} to 1 pg mL^{-1}, with LOD of 41 fg mL^{-1}, as extracted from DP shift data. Additionaly, biosensor showed very good selectivity and potential to reuse [1].

ABBREVIATIONS GO: graphene oxide; rGO-FET: reduced graphene oxide field-effect transistor; NMP: 1-methyl-2-pyrolidinone; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PBASE: 1-pyrenebutyric acid N-hydroxysuccinimide ester; PBS: phosphate-buffered saline; BSA: bovine serum albumin

REFERENCES

[1] Jarić et al., **Microchem J.**, 196 (2024), 109611.
[2] Nekrasov et al., **Biosens. Bioelectron.**, 200 (2022), 113890.
[3] Kesler et al., **ACS Nano**, 14 (2020), 16194-16201.

ACKNOWLEDGEMENTS

*This research is supported by **ANTARES**, funded by H2020 Teaming programme under GA No. 664387 (https://doi.org/10.3030/664388) and **NANOFACTS** project, funded by H2020 Twinning programme under GA No. 952259 (https://doi.org/10.3030/952259).*

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