Characterization of g-FET Biosensors in Action with Liquid-cell TEM

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In order to monitor biological markers of human performance, such as neuropeptide Y (NPY), orexin A, cortisol, interleukin-6 (IL-6), or troponin, biosensors must be able to selectively detect the target peptide with sub-10 pM sensitivity. The use of graphene-based field effect transistor (g-FET) sensors has been used to detect a variety of macromolecules such as RNA, DNA, peptides, and small toxic compounds [1]. The conductive graphene surface is functionalized with biological recognition elements (BREs) specific for the target peptide that consist of short amino acid sequences obtained via the phage display protocol [2]. To fully characterize the BRE, its local deformation and attachment to its target as well as the behavior of target peptide on the graphene surface during sensing (*in operando* bio-sensing) we use a combination of super-resolution microscopy, transmission electron microscopy (TEM), and liquid cell transmission electron microscopy (LC-TEM). Here we present the characterization of a Neuropeptide Y specific g-FET biosensor.

Neuropeptide Y (NPY) is one of the abundant proteins in brain [3], and it is involved in regulation of important biological and pathophysiological functions such as food uptake, energy homeostasis, circadian rhythm and cognition. Neuropeptide Y (NPY) is one of the most abundant proteins in the brain [3], and also serves as a major neurochemical component in stress response and a key element in modulation of emotional-affective behavior [4,5]. NPY levels are therefore a potential biomarker and non-invasive detection of NPY levels in human sweat with wearable g-FET bio-sensors would play an important role in monitoring real-time human response to stressful or threat-related conditions.

To fabricate a sensor specific for NPY, we use a 12 amino acid NPY-specific binding peptide (N3 peptide) as the BRE. Super-resolution microscopy of graphene-BRE-coated spheres demonstrated that the NPY binding was specific. For LC-TEM, Chemical Vapor Deposited few-layer graphene was transferred to a liquid electrochemical TEM cell (Fig 1A) and the NPY-N3 interaction was examined using an image-corrected FEI Titan microscope. When fused with a glutathione S-transferase (GST) tag, the NPY full length protein assembles into a 20nm micelle-like structure in solution containing three copies of GST-NPY dimer, as determined with cryo-electron microscopy and single particle reconstruction (Fig 1C). GST-NPY micelles were able to freely move until binding onto the BRE-graphene surface. Imaging under low-dose conditions minimized electron-water interactions, preventing significant pH change within the liquid cell [6]. The trajectory plot of GST-NPY showed that the micelle appeared to have localized vibration after binding to graphene (Fig 1B) that was on the order of the length of the N3 peptide, demonstrating that the GST-NPY micelle was tethered on graphene through the interaction with N3-peptide and not simply moving by Brownian motion. For *in operando* characterization of NPY binding the current was measured while under flow (Fig1 D) and imaged using HAADF-STEM (Fig 1E). Our characterizations demonstrate that N3-peptide is a robust BRE that is able to detect NPY in real time on functionalized g-FET sensors and is likely to be more robust than traditional NPY antibody-based assays.

References:

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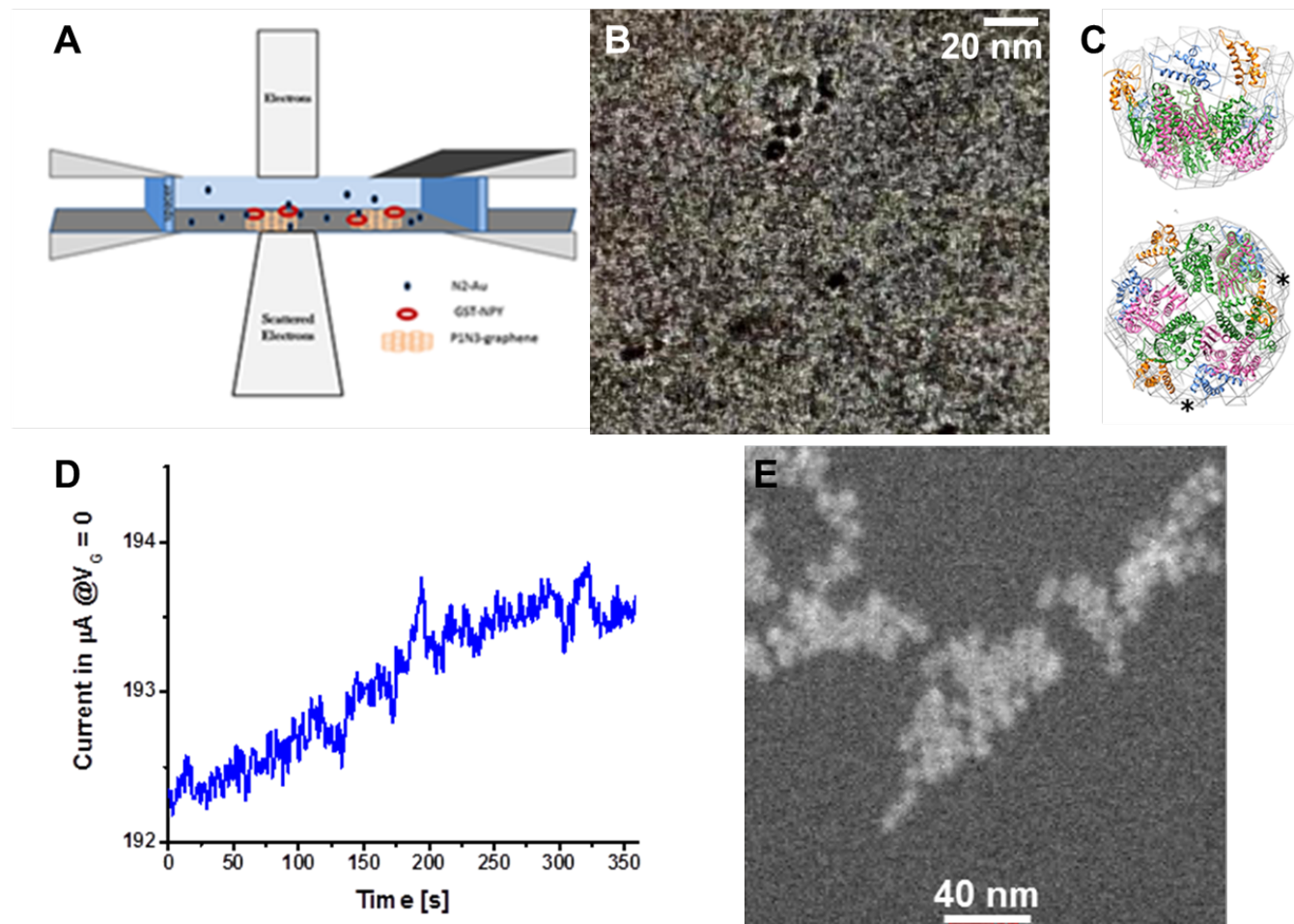
Figure 1. Characterization of g-FET biosensors in action with liquid-cell TEM.
A) Cross-sectional schematic of the 50 nm thick Si3N4 liquid electrochemical TEM cell in the electron beam path showing the BRE P1N3 attached to graphene and its GST-NPY micelle target labeled with N2 Au to aid in visualization. B) CryoTEM image of the 20 nm GST-NPY micelle labeled with N2 Au. C) GST-NPY electron potential map from single particle cryoTEM reconstruction showing the fitting of three copies of GST-NPY dimers in a micelle. Asterisks indicate gold-binding sites. D) Graph of real-time sensing of NPY binding where current increases with time due to binding. $V_{DS} = 0.2$ V, flow rate = 2 µl/min, NPY ~ 2 nM. E) HAADF-STEM image from sensing measurements shown in D.