CONFORMATION-SELECTIVE ENRICHMENT OF APTAMER-BOUND NEUROPEPTIDES BY DIELECTROPHORESIS

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ABSTRACT

Neuropeptide Y (NPY) offers diagnostic information on stress, depression and neurotrauma and can be non-invasively collected from peripheral bio-fluids. However, detection at high spatial and temporal resolution has been limited by poor sensitivity, long assay times and the inability to scale-down sample volumes. Herein, sensitive and rapid electrochemical detection of NPY at picomolar levels from sub-nanoliter droplets is accomplished by utilizing negative dielectrophoresis (nDEP) for selective preconcentration of aptamer-bound NPY away from constricted regions of a nanochannel. We envision the utility of this methodology towards off-line detection of neuropeptides from microdialysis samples in bio-fluid matrices that include interfering electroactive peptides.

KEYWORDS: Neuropeptides, graphene, dielectrophoresis, nanochannel, electrochemistry

INTRODUCTION

Neuropeptides are a class of peptides that are vital to the transmission and modulation of neurological signals. Their sensitive measurement at high spatial and temporal resolution, usually in the form of analyte droplets collected by microdialysis at varying locations or time points from tissues, offers valuable information on the chemistry, biology and pharmacology of the nervous system. However, the sensitivities offered by current methods, such as immunoassays and HPLC-MS are not sufficient for diagnostic information, given the presence of neuropeptides at picomolar levels within subnanoliter droplets collected by microdialysis. In our recent work [1], Neuropeptide Y (NPY) was detected based on signal from the electrochemical oxidation of its tyrosine moiety. The detection sensitivity was enhanced by electrokinetic preconcentration of NPY on graphene-modified electrodes in a nanochannel that were optimized for high conductivity and fast adsorption kinetics. However, selectivity in the presence of other tyrosine containing peptides, such as Orexin A, was limited. Herein, we explore the enhancement of the selectivity by utilizing aptamer-bound NPY to enable selective preconcentration of NPY by negative dielectrophoresis (nDEP) away from constricted regions of a nanochannel. Aptamers are nucleic acid molecules that fold into three-dimensional conformations that cause a characteristic dielectric property, with clefts to enable specific binding to a selected target molecule. Hence, through coupling frequency-selective dielectrophoretic trapping of aptamer bound NPY to its detection by electrochemistry within sub-nanoliter samples, we envision its utility towards off-line detection from microdialysis samples within bio-fluid matrices that include interfering electroactive peptides.

EXPERIMENTAL

The microfluidic chip utilizing ~0.1 nL sample volume, consists of a quartz substrate nanofabricated with the channel structures and bonded to a glass cover-slip that is microfabricated with electrochemical working, counter and reference electrodes (Figure 1). Electrochemical detection electrodes (30 μm width for coupling to nDEP) are fabricated on cover-slip glass by converting the patterned resist to glassy carbon (GCE) by standard pyrolysis methods, followed by electro-reduction of graphene oxide (ERGO) and electrodeposition of AuNPs at -0.8 V for 60 s in 1 mM $HAuCl_4$. The chip includes a Pt counter electrode and a Ag/AgCl reference electrode, which is prepared by electrodeposition with Ag and treatment with $FeCl_3$ for chloridizing the surface layer. This cover-slip is bonded to the nano-slit quartz substrate after ensuring that the length-edge of graphene-modified working electrode is orthogonally

aligned to be within ~1 µm from the constriction tip, to enable preconcentration in the proximity of the electrodes by negative DEP (nDEP). The nDEP behavior of NPY under an AC field (300 V_{pp}/cm at 3 MHz frequency) is driven by external Pt electrodes (at inlet and outlet) using a custom designed voltage amplifier, with an additional DC field of 1.5 V/cm to enhance electrokinetic transport of NPY towards the constriction. The preconcentration profile is optimized by observation of fluorescently labeled NPY (Phoenix) under inverted microscopy (Zeiss, Z1) using an EMCCD (Hamamatsu). Following optimization, electrochemical analysis by Differential Pulse Voltammetry (DPV) is carried out (Solartron) using the patterned graphene-modified electrodes.

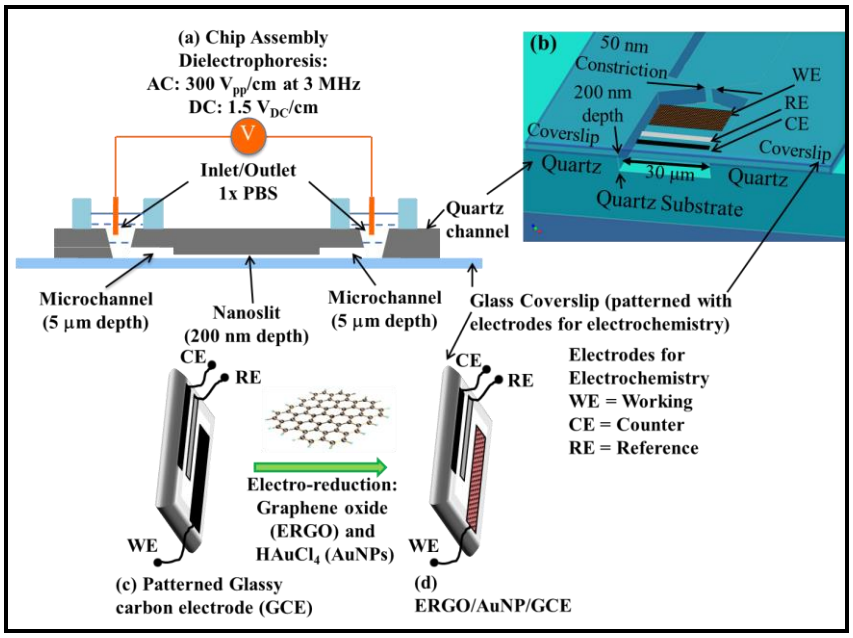

Figure 1: Schematic of nano-slit device for DEP enrichment on patterned graphene in the nanochannel.

RESULTS AND DISCUSSION

Dielectrophoresis (DEP) is capable of frequency-selective preconcentration of polarized biomolecules by their selective translation in a spatially non-uniform electric field, either towards (by positive DEP) or away (by negative DEP) from localized regions of high field. DEP behavior is determined by the polarizability of the biomolecule, which is highly influenced by its conformation that causes a characteristic dielectric frequency response. For instance, as per Figure 2, while nDEP preconcentration of free NPY requires 300 V_{pp}/cm and 3 MHz, a power bandwidth that is achievable only with custom amplifier circuits, aptamer-bound NPY can be selectively preconcentrated at a lower power bandwidth of 100 V_{pp}/cm and 500 kHz, which is easily accomplished with a portable commercial amplifier. Furthermore, the aptamer-bound NPY molecules preconcentrated on graphene-modified electrodes in the nanochannel retain their electrochemical activity due to tyrosine, in spite of conjugation to the aptamer, similar to prior work on aptamer-bound dopamine molecules. However, this methodology has not been previously applied to selectively preconcentrate analytes, as demonstrated here. As a result, we demonstrate in Figure 3 that the non-specific signal from thousand-fold higher Orexin-A on NPY detection can be eliminated. While DEP has been extensively applied previously to sort micron-sized biological cells based on their electrophysiology, its application to separate nanoscale biomolecules, such as aptamers, in physiological media is limited by the cube-fold drop in trapping force with size of the polarized particle and an increase in disruptive electrothermal flow due to non-uniform Joule heating. We have overcome this limitation by using a nano-slit device design with sharp constrictions to enhance trapping forces, while reducing Joule heating due to enhanced heat dissipation at nanochannel interfaces [2, 3].

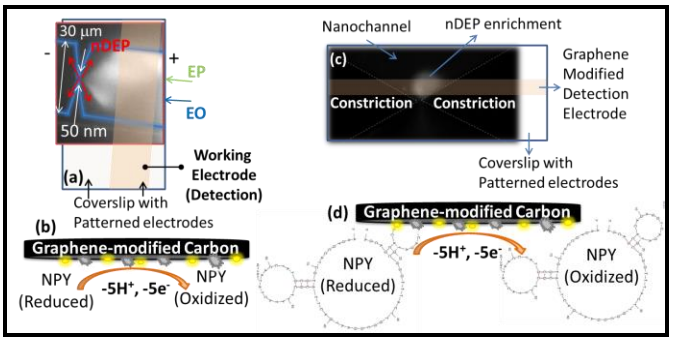

Figure 2: (a) DEP enrichment of free NPY (3 MHz); (b) detection by voltammetry, as in [1] versus: (c) DEP enrichment of NPY-bound aptamer (0.5 MHz) for detection (d), as per Fig. 3 of this report.

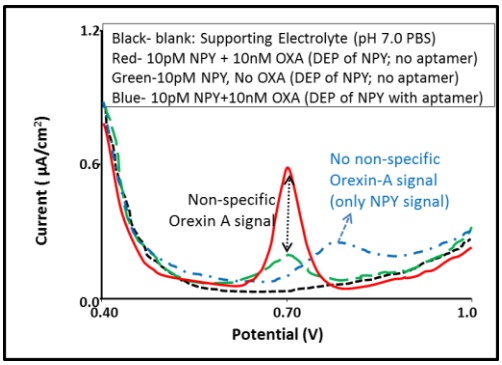

Figure 3: DEP accumulation as per Figure 2a & b, using 10 pM NPY with 10 nM Orexin-A shows significant non-specific signal from Orexin (red versus green), which can be eliminated (blue) by using DEP on aptamers (as per Figure 2c and d).

CONCLUSION

In summary, DEP preconcentration of aptamer-bound NPY enhances electrochemical detection sensitivity and selectivity, enabling far more rapid assay times than immunoassays and with far less sample volumes. We envision application within off-line detection from dialysates for measurement of neuropeptides at high spatial and temporal resolutions.

ACKNOWLEDGEMENTS

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