CHARACTERIZATION OF BIOMOLECULES USING AN APTAMER-BASED GRAPHENE NANOSENSOR

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ABSTRACT

We present binding properties of biomolecules and their dependence on ionic strength and temperature using a microfluidic aptameric graphene field-effect transistor (FET) nanosensor. Aptamer-immobilized graphene is used to recognize target molecules, and the resulting changes in graphene conductance is measured to study the binding kinetics of biomolecules. Binding properties of aptamer-protein interactions at different ionic strength and temperature are investigated, yielding insight into the pharmacologic basis of biomolecular recognition.

INTRODUCTION

Understanding binding properties of biomolecules and their dependence on environmental conditions such as temperature and ionic strength is of great interest to basic science studies and applied pharmacology. While successful characterization of biomolecular binding properties with multiple methods (e.g., optical, electrochemical and electromechanical) [1-3], the existing approaches for binding studies commonly either require molecular labeling groups or involve complex sensor structure and instrumentation. In contrast, graphene nanosensors, configured as graphene field-effect transistors (FET), are attractive for biomolecular binding studies because its high carrier mobility [4], resulting in high sensitivity to charged molecules. However, existing graphene nanosensors are typically limited to equilibrium binding measurements [5]. Here we present an approach for studying biomolecular interactions under different levels of ionic strength and temperature using a microfluidic graphene FET nanosensor, which is activated by analyte-specific aptamer for target molecule recognition. This approach allows label-free, direct characterization of biomolecular binding properties with one-step electrical readout so that the potential inaccurate transductional conversions commonly found in existing platforms, where the binding kinetics are characterized by fluorescent intensity, resonate frequency or refractive index, can be eliminated. In addition, graphene surface is much easier to be functionalized with molecules compared with gold surface-based approaches (e.g., surface plasmon resonance and quartz crystal microbalance), which require more steps and controlled environmental condition such as pH. In this work, the embedded micro temperature sensor that is exactly close to the graphene sensing region enables accurate temperature control for temperature-dependent biomolecular interaction. Also, the approach features on-chip gate electrode to eliminate the use of cumbersome external wire electrodes, allowing transition-state thermodynamic parameters to be determined simultaneously in an integrated, miniaturized manner. At the same time, the approach allows binding study at different ionic strength and temperatures by simply normalizing the yielded signal to fraction of aptamer bound, even though the graphene is sensitive to environment conditions. As such, the approach is more robust for binding studies under different circumstances. We demonstrate the utility of our device by characterizing the kinetics of the binding between the Immunoglobulin E (IgE) and its specific aptamer (D17.4) as a representative at different Na^+ and Mg^{2+} ionic strength, and energetics at varying temperatures, yielding insight into the pharmacologic basis of biomolecular recognition.

PRINCIPLE AND DESIGN

The nanosensor is configured as an electrolyte-gated graphene FET (Figure 1a) where graphene is the conducting channel, formed between drain and source electrodes on the SiO_2 substrate, with an embedded gate electrode (Figure 1b). Pyrene group terminated 1-pyrenebutanoic acid succinimidyl ester (PASE) is coupled to graphene via - stacking, and D17.4 aptamer then attached to the free end of PASE by forming amide bond (Figure 1c). When exposed to the target analyte, captured IgE by the aptamer varies carrier concentration in the graphene, yielding detectable signal. A polydimethylsiloxane (PDMS) microfluidic channel is bound to the substrate for analyte and buffer introduction to initiate the association and dissociation. On-chip temperature sensor and peltier module are used for the closed-looped temperature control.

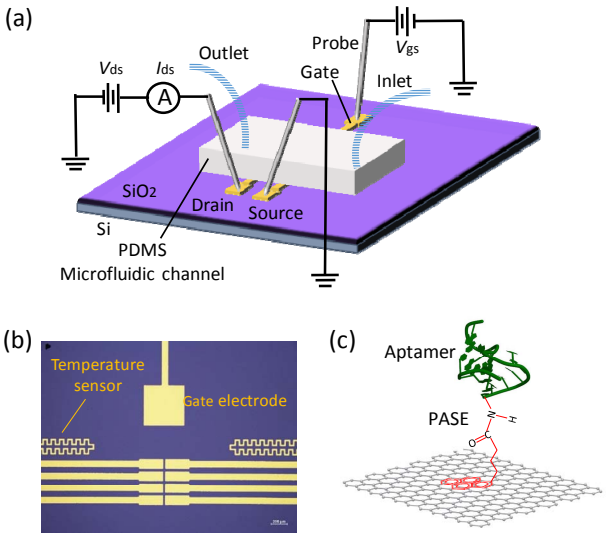


Figure 1: (a) Schematic of microfluidic graphene aptasensor configured as solution-gated field-effect transistor. Gate electrode is embedded on the chip. A microfluidic channel was bound to the chip to introduce the analyte and buffer. (b) Micrograph of sensing region. Gate electrode and temperature sensor are embedded on chip (c). Graphene surface functionalization. Aptamer is coupled to graphene surface via PASE that is terminated to graphene via - interaction.

EXPERIMENTAL

The device was fabricated using micro and nanofabrication techniques on an oxidized silicon wafer. Thermal assisted bilayer lift-off process [6] was used to fabricate the electrodes. After cleaning by piranha, two layers of photoresist with different reflow temperature were spin-coated on top of substrate, and the source, drain and gate electrode then were patterned through photolithography. After developing, a layer of 5/45 nm Cr/Au was deposited using thermal evaporation, and the remaining resist were completely removed by immersing the whole wafer in the remover PG. Graphene synthesized via chemical vapor deposition (CVD) was transferred onto the substrate by following a previously reported protocol [7] to connect the source and drain electrodes (Figure 1a). To achieve noncovalent functionalization of graphene surface, the graphene FET was immersed for 2 hours at room temperature in a dimethylformamide (DMF) solution of 5 mM 1-pyrenebutanoic acid succinimidyl ester (PASE), which served as a linker. After rinsing thoroughly with ethanol, phosphate buffered saline (PBS) buffer and deionized water, the graphene sensor was immersed in a solution of D17.4 aptamer in PBS (concentration: 1 μM) for 12 hours at room temperature. During the operation, the association was initiated by introducing the analyte, and dissociation was induced by switching to buffer when the association reached equilibrium state. Binding buffer containing 2.9 mM Na_2HPO_4, 1 mM KH_2PO_4 with different salts ionic strength were prepared to study the ion-dependent binding kinetics, and temperature-dependent biomolecular interactions were investigated by measuring the response of graphene nanosensor to the interaction of IgE-D17.4 at different temperature. Temperature control enabled by the on-chip temperature sensor and a peltier module was achieved by a PID control system. IgE at 5 nM concentration in the buffer was prepared. The microfluidic chamber introduced both the IgE and buffer at 5 μL/min throughout the measurement. Source-drain current I_{ds} at an appropriate fixed gate voltage V_{gs} was measured throughout the experiments to monitor the association and dissociation events.

RESUSLTS AND DISSCUSSION

The graphene was verified to be a single layer via Raman spectroscope (Figure 2), where a G band at ~1580 cm^{-1} and 2D band at 2685 cm^{-1} in Raman spectrum were characteristics of single-layer graphene [8]. The surface functionalization was further validated by measuring the transport characteristics of graphene channel at completion of PASE and aptamer functionalization respectively (Figure 3), the obtained results showed good agreement with the reported results [9].

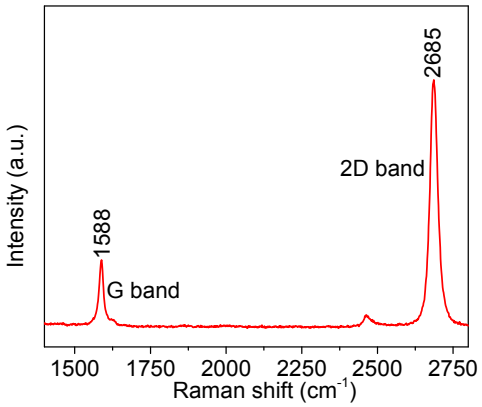


Figure 2: Raman spectrum of a single-layer graphene flake used in FET's channel. Signature peaks of G- and 2D bands were observed indicating the characteristics of single-layer graphene.

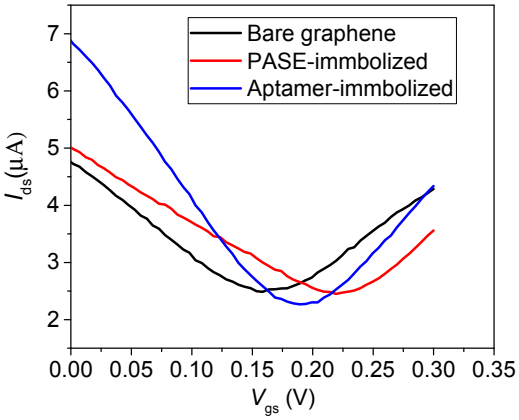


Figure 3: Characterization of graphene surface functionalization. Transport characteristics are measured at completion of every functionalization step. Functionalization of PASE and aptamer induced p-doping and n-doping respectively to the graphene.

The binding kinetics at different ionic strength of Na^+ and Mg^{2+} then were investigated (Figure 4, 5); the results suggested that both association and dissociation was enhanced by the increasing level of salt ions, which can be explained as that, the presence of salt ions pre-fold the conformational structure of aptamer through electrostatic interaction with backbone of DNA strand, favoring the target recognition during association. On the other hand, salt ions may also decrease the conformational flexibility of aptamer in the dissociation period, resulting in less capable of target recognition [3].

Dissociation of IgE-aptamer complex was studied at various temperatures 10℃, 20℃ and 25℃ to understand the effect of temperature on IgE-D17.4 interactions. The temperature-dependent dissociation profiles (Figure 6) showed the decrease in affinity with increasing temperature, which can be attributed to the progressive destabilization of aptamer structure [4]. Simulation was carried out to demonstrate the thermal distribution inside the chamber at 10℃. The simulation showed good uniformity for IgE-D17.4 interactions (Figure 7).

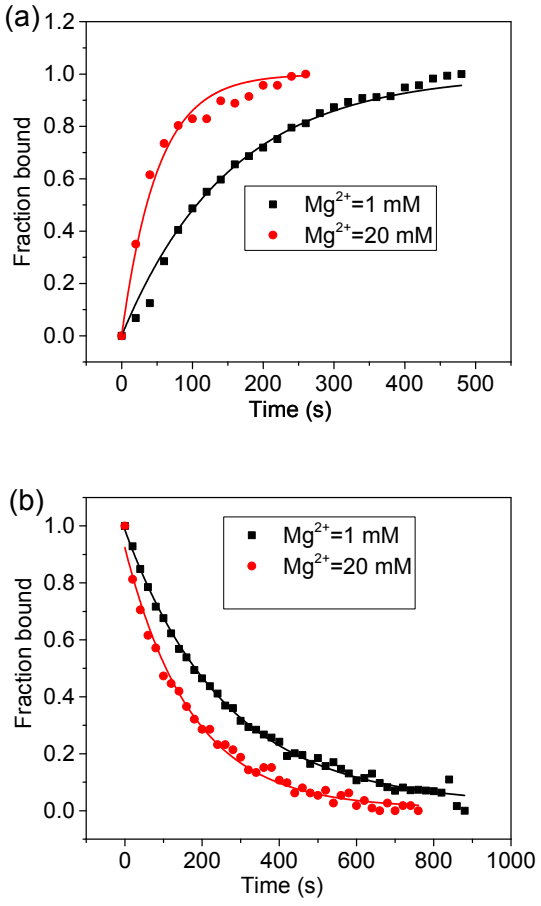
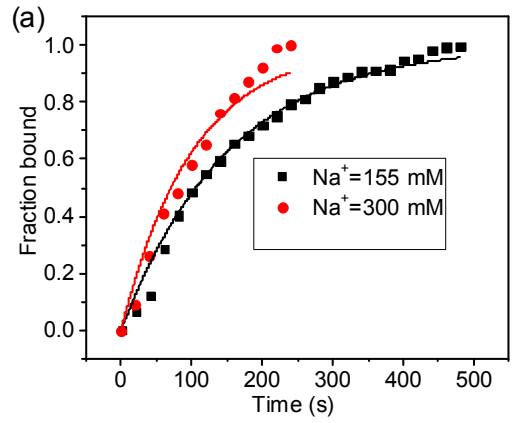


Figure 4: Effect of Mg^{2+} on IgE-D17.4 association and dissociation. (a) Association and (b) Dissociation profiles of IgE-aptamer interaction at various Mg^{2+} concentrations 1 mM and 20 mM in the buffer containing 2.9 mM Na_2HPO_4, 1 mM KH_2PO_4, 155mM NaCl.



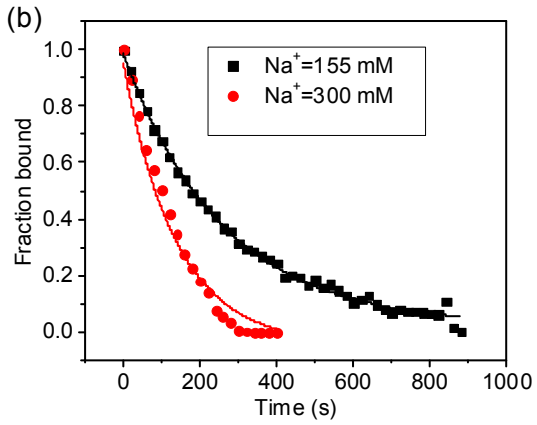


Figure 5: Effect of Mg^{2+} on IgE-D17.4 association and dissociation. (a) Association (b) Dissociation profiles of IgE-aptamer interaction at various Na^+ concentrations 155 mM and 300 mM in the buffer containing 2.9 mM Na_2HPO_4, 1 mM KH_2PO_4, 1 mM $MgCl_2$.

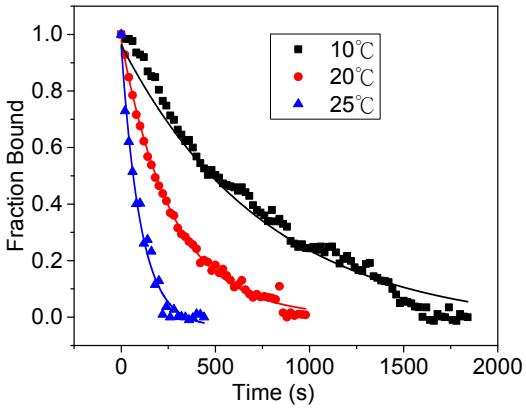


Figure 6: Effect of temperature on IgE-D17.4 interactions. Dissociation profiles of IgE-specific pre-complexed with IgE at various temperatures 10 ℃, 20 ℃ and 25 ℃ in the buffer containing 2.9 mM Na_2HPO_4, 1 mM KH_2PO_4, 1 mM $MgCl_2$.

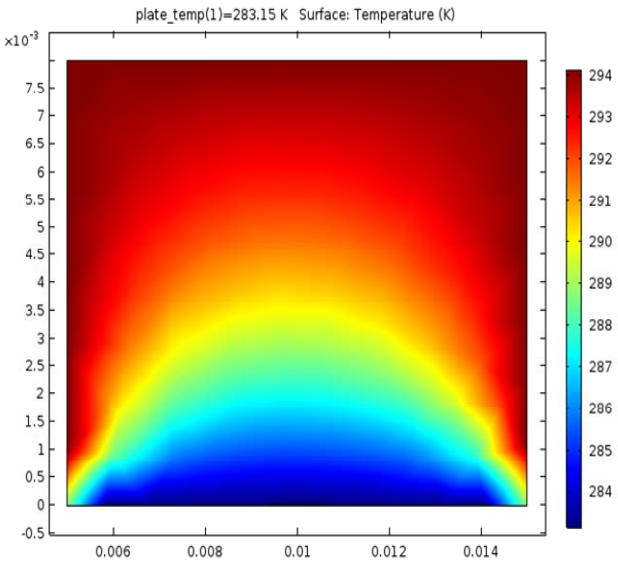


Figure 7: Numerically determined temperature distribution in the channel (20 μm in height) with cross-section view of PDMS at 10℃. Simulation showed good uniformity of temperature distribution.

CONCLUSIONS

We have presented the study of biomolecular interaction using a microfluidic aptameric graphene nanosensor. The nanosensor was configured as a graphene FET with fully integrated structure where on-chip gate electrode and temperature sensor were embedded. The specific recognition of aptamers and target molecules induced changes in the conductance of graphene, which was measured monitor the association and dissociation between aptamers and target molecules. We demonstrated binding kinetics of IgE-D17.4 and their dependence on salt ionic strengthen and temperature, highlighting the great capability of our graphene nanosensor to characterize the biomolecular interaction at different environmental conditions.

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