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N.B.: When citing this work, cite the original article.

Original Publication:

Onur Parlak, Anthony Turner and Ashutosh Tiwari, On/off-switchable zipper-like bioelectronics on a graphene interface., 2014, Advanced Materials, (26), 3, 482-486.

<http://dx.doi.org/10.1002/adma.201303075>

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<http://www.wiley-vch.de/publish/en/>

Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-102942>

On/off-switchable zipper-like bioelectronics on a graphene interface

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Keywords: Smart interface, bioelectrocatalysis, switchable bioelectronics, graphene

Smart and flexible bioelectronics on graphene have emerged as a new frontier in the field of biosensors.^[1] Graphene has begun to be seen as an ideal signal transducer and a promising alternative for the production of low-cost bioelectronic devices.^[2] However, biological systems used in these devices suffer from a lack of control and regulation. There is an obvious need to develop “switchable” and “smart” interfaces for both fundamental and applied studies.^[3] Here, we report for the first time the fabrication of an on/off-switchable zipper-like graphene interface, which is used to manipulate biomolecular interactions. Using electrochemical measurements, we demonstrate that interfacial bio-electrochemical properties can be tuned by modest changes in temperature. Such an ability to independently regulate the behaviour of the interface has important implications for the design of novel bioreactors, biofuel cells and biosensors with inbuilt self-control features.

Bioelectronics is a rapidly growing interdisciplinary research area which encompasses bio-devices such as biosensors, biofuel cells, and bioreactors.^[4] Recently, pH, light, magnetic fields and potential-triggered on/off-switchable or tunable electron-carrier functional interfaces, have been widely reported as a means to modulate electrobiocatalysis on an adjustable platform.^[5] In order to control the operation of such bioelectronic devices using

these external stimuli, signal-responsive smart functional interfaces are required.^[6] Incorporation of stimuli-responsive materials as an interface element provides a way to switch from the ‘on state’ to an ‘off state’ or to tune the rate of catalytic reactions on the electrode surface. In the field of switchable bioelectronics, most of the stimuli have been restricted to light, magnetic field, and pH.^[7] However, temperature-controlled bioelectronics could provide a promising direct approach to investigate the effect of external temperature on electrocatalytic and bioelectrocatalytic processes on functionalised nanostructured electrodes.^[8] Although a number of related studies have been reported for temperature controlled bioelectronics and biocatalytic applications, most of them have focused on biocatalysis using a range of bulk materials such as hydrogels, polymer brushes and polymeric interfaces.^[9]

Recent advances in graphene research provide an opportunity to enhance the performance and applicability of current approaches to the design of bio-devices.^[10] In spite of intense activity in graphene research, there has been no report of a switchable graphene interface to manipulate bioelectrochemical interactions, despite the fact that this provides a fundamental tool if graphene is to be used in conformal modification. Motivated by this, we functionalised graphene to create a switchable interface for electrochemical bioreactions. To our knowledge this is the first such report and could pave the way for a wide variety of important applications. In particular, switchable graphene surfaces have significant potential in the design on/off-switchable bioelectronics as well as controllable bio-catalysis.

The present manuscript aims to address the design and development of a novel auto-switchable graphene bio-interface that is capable of positively responding, by creating unique “zipper” nanoarchitectures. The zipper consists of a two-dimensional graphene donor and a polymeric receptor, which are rationally assembled together based in a stoichiometric donor-receptor interaction. Preferably, at a relatively low temperature (i.e., at 20 °C) the active donor-receptor interaction (hydrogen bonding) creates a coalescence of the graphene interface,

thereby causing considerable shrinkage in the donor-to-receptor interface. Thus access of an associated enzyme to its substrate is largely restricted, resulting in a decrease in the diffusion of reactants and the consequent activity of the system. In contrast, at a comparatively high temperature (i.e., at 40 °C) the donor-receptor interaction was subverted. As a result, the biosubstrate could freely access the enzyme facilitating bioelectrocatalysis. More importantly, this provides the first example of responsive bioelectronics being achieved on a two-dimensional graphene interface by controlling the external temperature as an on/off-switchable model.

The bioelectrode was prepared by attaching hierarchically self-assembled graphene–gold nanoparticle-cholesterol oxidase (ChOx, from *Streptomyces* sp.) bioconjugate and poly(*N*-isopropylacrylamide) (PNIPAAM). Construction of this zipper-like graphene-polymer donor-acceptor interface, generated a temperature-gated switchable surface. When the temperature is below the lower critical solution temperature (LCST), the graphene/PNIPAAM is expanded, mainly due to the hydrogen bonding between – sulfonate (graphene), amide (PNIPAAM) groups and water molecules, whereas the graphene interface is collapsed above the LCST, since the hydrogen bonding is broken. The temperature variation alters the surface affinity of graphene/PNIPAAM from hydrophilic to hydrophobic. This unique property of graphene/PNIPAAM was exploited in a bioconjugate containing gold nanoparticles (Au NPs) and cholesterol oxidase in a template-directed self-assembly system. Hierarchical interactions were achieved post functionalisation of graphene, by designing a stimuli-responsive interface using the negatively charged surfactant, sodium dodecyl benzene sulfonate (SDBS). Surfactant functionalisation not only stabilised the surface (Figure S9), but also directed the self-assembly of both the enzyme and the Au NPs. Here, incorporation of Au NPs with graphene for biosensing applications has also delivered some important outcomes. The Au NPs had a catalytic effect on the electrochemical reactions and prevented the thermal aggregation of enzyme on the electrode surface thus enhancing biocatalytic efficiency.^[11] The

on/off-switching behaviour of the graphene/PNIPAAm modified electrode surface was demonstrated towards the electroactive ferro/ferri cyanide ($\text{Fe}[(\text{CN})_6]^{3-/4-}$) couple using cyclic voltammetry, and towards cholesterol by chronoamperometry in PBS at pH 7.4.

Scheme 1 illustrates the reversible conformational change of the zipper-like graphene interface and on/off- switchable diffusion of electroactive species and substrate on the modified electrode surface. To prove the temperature sensitive character of bio-electrodes, cyclic voltammetry, impedance spectra and chronoamperometric measurements were carried out at two different temperatures, “ON” state (40 °C) and “OFF” state (20 °C). In order to demonstrate the “ON performance” of the modified electrode, 40 °C was chosen as a representative temperature. The most common optimum temperature for enzymes is typically around 40 °C, although the optimum temperature of cholesterol oxidase is actually 60 °C.^[12] The choice of 40 °C has the added advantage of being representative of mammalian physiological temperatures. A temperature of 20 °C was chosen for the “OFF” state as representative of average room temperature.

Figure 1 shows a cyclic voltammogram (CV) of a modified electrode containing graphene-gold NPs-enzyme conjugated structures in 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ aqueous solution (0.1 M KCl as the supporting electrolyte). It can easily be seen from Figure 1 that there is an apparent peak current difference between two modified electrodes at the two different temperatures. This difference reflects to the temperature-sensitive on/off switchable property of the graphene interface toward $\text{Fe}(\text{CN})_6^{3-/4-}$ and is defined by the change of interaction on a two-dimensional surface. When the temperature is 20 °C, the bond interaction starts to change between graphene, polymer and water molecules. This cause considerable shrinkage at the interface and suppress the diffusion of electroactive species through to the electrode surface. However, if the surrounding temperature is 40 °C, the donor-acceptor interactions change and make the surface more accessible. This would help to increase the permeability and provide easy diffusion of electro-active species through the electrode surface, resulting in large peak

currents. In addition, the electrochemical properties of the modified electrodes were characterised by measuring voltammetric response in 0.1 M PBS solution containing 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$. The results are shown in Figure S4. The CV responses of all modified electrodes displayed a classical sigmoidal shape with different peak-to-peak potential separations. The larger peak separations indicate slow electron transfer kinetics. After incorporation of conductive elements such as graphene and Au NPs in the polymer medium, the peak currents of $\text{Fe}(\text{CN})_6^{3-/4-}$ increased relative to the electrodes modified by only with polymer or graphene-polymer assembly, indicating that the incorporation of nanoparticles played an important role in creating a higher electroactive surface area and providing the conductive interlayer for the electron transfer of $\text{Fe}(\text{CN})_6^{3-/4-}$.

The electron transfer properties of the bare glassy-carbon electrode (GCE) and PNIPAAm/graphene/Au NPs/ChOx conjugate-immobilised electrodes at 20 °C and 40 °C were characterised by electrochemical impedance spectroscopy. Randles circuit was chosen to fit the impedance outputs (inset of Figure 1b). The results are shown as Nyquist plots of spectra which contain semi-circular and linear portions. The plots indicate both electron transfer limited (semi-circular part) and diffusion processes (linear part) occurring at the same time. The charge transport resistance (R_{CT}) at the electrode surface can be quantified based on the diameter of the semi-circular part of the plot and calculated using a frequency response analyser (FRA-version 4.9.007). Calculations showed that the modified electrodes had a much higher R_{CT} value than the bare GC electrode (0.059 k Ω). Thus, the presence of a polymer layer on the surface of the electrode hinders the electron transfer. Moreover, a significant difference between two R_{CT} values of graphene-modified electrodes was observed. The R_{CT} value for the bio-electrode at “ON” state was calculated to be 0.560 k Ω , but at “OFF” state, the electron transfer resistance increased markedly to 4.25 k Ω , i.e. a ~10 times higher resistance than at the higher temperature. This shows that hydrophilicity/hydrophobicity

changes of the electrode interface affect not only diffusion of electroactive species and/or substrate, but also influence charge transfer properties.

Figure 2 shows the effect of temperature change on the hydrophilicity/hydrophobicity of a PNIPAAm/graphene/Au NPs/ChOx modified glassy-carbon surface. The surface showed more hydrophilic character at 20 °C, due to the hydrogen bonding between sulfonate groups of graphene, amide groups of polymer and water molecules. The water contact angle was measured as $48.19^\circ \pm 2.15$ for 20 °C. However, after heating the sample to around 40 °C, the surface character changed to a hydrophobic state, due to the collapsing of hydrogen bonding between polymer and graphene surface. The water contact angle was measured as $97.49^\circ \pm 4.08$ for 40 °C. The change of contact angles and the change of hydrophilicity/hydrophobicity character on the surface of glassy carbon are considered to be the controlling mechanism behind diffusion of electroactive species and the substrate for the bioelectrocatalytic process.

The structure change of graphene-based bionanocomposite on the electrode surface was investigated by *in-situ* temperature-controlled scanning electron microscopy (SEM) (Figure S1). The temperature of the surrounding was controlled by using additional electrical heating / water cooling equipment, as described and demonstrated in Figure S1. The sample was prepared by simply dropping of 20 μ L polymer bionanocomposite solution on a gold disk and leaving overnight at 4 °C before SEM examination. As can be seen in **Figure 3** and Figure S2, the surface morphologies were obviously different. At 20 °C (a), the structure has contained any narrow channels and pores, while at 40 °C (b), the surface displayed more compact and rough characteristics.

The further electrochemical and stability/ reliability characteristics of the modified electrodes were investigated by chronoamperometry. **Figure 4a** shows typical amperometric responses of the PNIPAAm/graphene/Au NPs/ChOx immobilised electrodes for successive additions of cholesterol at an applied potential of +0.6 V under two different temperature conditions. At 40 °C, the electrode exhibited a fast response time of just under 5s to steady-

state. The calibration curve for the modified electrode is shown in Figure S6. The linear range was 0.05 to 1.8 mM with a sensitivity of $0.33 \pm 0.67 \mu\text{A}/\mu\text{M}/\text{cm}^2$. The sensitivity was calculated by dividing the slope of the calibration curve by the surface area of the bare electrode (0.07 cm^2). The detection limit for this modified electrode was $0.2 \mu\text{M}$ ($S/N = 3$). However, when the surrounding temperature is 20°C , the catalytic behaviour of the enzymatic reaction was almost completely suppressed and no obvious signal increment was observed for the successive addition of cholesterol. In addition to the electrochemical responses at 20°C and 40°C , electrochemical performance of the PNIPAAm/graphene/Au NPs/ChOx reactor were tested at intermediate temperatures. The results are shown in Figure S10. At moderately low temperatures, the reactor displayed almost no electrochemical signal, which shows that this temperature range had insufficient energy to break the hydrogen bonding of donor-acceptor interactions. However, above 32°C , which is the LCST of PNIPAAm, the reactor began to generate an electrochemical signal. This shows that the interaction between donor-acceptor branches had begun to collapse and bioelectrocatalytic reactions were thus permitted. In order to define the reproducibility of this reversible enzymatic reaction for both temperature ranges, the same electrodes were cycled successively 7 times (Figure 4b). The results in Figure 4b were obtained based on each chronoamperometric measurement, and each data point was recorded after sequential 1.0 mM cholesterol additions at 20°C and 40°C . The electrodes which had been used for electrochemical measurements at 40°C , were placed in a solution at 20°C and the same procedure was performed. The switching time between the two different temperatures was no longer than 1 min for successive cycles. It can be seen that after 7 runs, there was no significant current difference between each repetitive cycle for both temperatures. So as to determine specificity of the enzymatic reaction, the interference effect of compounds having similar chemical structures, such as progesterone and cholate (Figure S12b), was studied under “ON” state conditions (40°C). The chronoamperometric results are shown in Figure

S11. The results were typical amperometric responses for PNIPAAm/graphene/Au NPs/ChOx immobilised electrodes with successive additions of cholesterol at an applied voltage of +0.6V. After reaching 1.0 mM cholesterol concentration, 1.0 μ M progesterone, which is ~50 times higher concentration than progesterone level in human blood, and cholate (1.0 μ M) solutions were added, successively.^[13] No current difference was observed following both additions. Thereafter, more cholesterol solution was added and an amperometric response was observed. This shows that the electrocatalytic reaction was unaffected by the presence of progesterone and cholate at any level likely to be encountered physiologically. Other potential interferents, 5-cholesten-3-one and 4-cholesten-3-one, which have similar chemical structure to cholesterol (Figure S12a), are produced in equimolar amounts after the oxidation of cholesterol in each cycle. It can be seen that, there was also no significant current difference between successive additions of cholesterol. This shows that the modified electrode was cholesterol specific in its on/off-switchable ability. In addition, further chronoamperometric measurements were performed to show the synergistic effect of the graphene-Au NPs assembly in the polymeric medium. For this purpose, PNIPAAm/ChOx-modified (without graphene-Au NPs) electrodes were prepared and measured at two different temperatures (Figure S5). The results showed that presence of both graphene and Au NPs enhances the amperometric response and stabilises the electron transfer.

The catalytic properties of the modified electrodes were also investigated at low and high temperatures and the results are shown in Figure S7. The apparent Michaelis-Menten constant (K_m^{app}) was calculated using the Lineweaver-Burk plot according to the following equation:^[14]

$$1/I = (K_m^{app}/I_{max})(1/C) + 1/I_{max} \quad (1)$$

where I represents the steady-state current after addition of cholesterol. The maximum current ($I_{\max}$) was determined at saturated level of cholesterol, and C is the concentration of cholesterol. The values of $I_{\max}$ and K_m^{app} were calculated for 40 °C by the analysis of the intercept and the slope of the plot and found to be 0.04 mA and 1.56 mM, respectively. The K_m^{app} value reflects the strong affinity between the enzyme and its substrate, or in this case, the ease of diffusion. However, the catalytic behaviour of the enzymatic reaction was completely suppressed at 20 °C due to changes in hydrogen bonding between the polymer acceptor and graphene donor interface, which helps to modulate diffusion of the substrate for the catalytic enzyme reaction.

In conclusion, we have demonstrated for the first time an on/off-switchable zipper-like graphene interface with a simple-to-use, inexpensive and high-order system in a model biosensor, which is highly selective, sensitive and stable. The electrochemical measurements showed that the interfacial electrochemical properties can be tuned by varying the surrounding temperature. We believe that the fundamental design behind the present work is both attractive and novel. Ideally, it could make significant contributions to advanced biocatalysis and material sciences, leading to self-switching bio-catalysis by utilising reusable, cost-effective and simply-made nanomaterials. In nature, reactions are compartmentalised, and attempts to construct artificial systems have generally resulted in vastly inferior performance. This biotechnological problem could be solved by the use of zipper-like graphene interfaces to segregate reaction conditions at a molecular level. Conditions could then either be switched in a single compartment, by the use of graphene zipper, or the products of one reaction in a sequence passed to the second reaction in the biological chain by intelligent switching of the nanomaterial.

Experimental Section

Temperature controlled scanning electron microscopy (SEM) was performed using an LEO 155 Gemini (Zeiss, OR, USA) by attaching a H1002 hot-stage module for heating and water cooling apparatus, as shown in the supporting information. Water contact angle measurement was performed using a CAM 200 optical contact angle meter (KSV Instrument, Helsinki, Finland). All voltammetric and amperometric measurements were carried out with an Ivium Stat.XR electrochemical analyser (Eindhoven, Netherlands). Impedance measurements were carried out with Autolab potentiostat-galvanostat. A three-electrode cell with glassy carbon (GC) working electrode, having 0.07 cm^2 surface area, and platinum wire auxiliary and Ag/AgCl (3 M KCl) reference electrodes were used in the voltammetric and amperometric measurements.

Synthesis of zipper-like graphene: Graphene nanosheets were produced using the Hummers method^[15] (Graphene Supermarket, USA) and graphene based bioconjugate containing cholesterol oxidase and gold nanoparticles was prepared according to our previous report with minor modifications.^[16] Then, graphene/ChOx/Au NPs conjugate was incorporated in amine-terminated PNIPAM polymer matrix to obtain temperature responsive graphene-polymer hybrid structure. In a typical preparation, 1 mg of graphene-ChOx-Au NPs conjugate was added to 1 mL of 1mg/mL PNIPAM aqueous solution to form homogenous solution with ultrasonication.

Fabrication of on/off-switchable electrode interface: Prior to immobilisation of self-assembled graphene based bioconjugate, the glassy carbon electrode (GCE) surface was carefully polished with 1, 0.3, and $0.5\text{ }\mu\text{m}$ of α -alumina slurry successively and washed with deionised water. In order to obtain the functionalised electrode surface for further immobilisations, the electrode surface was first oxidised in PBS (pH 7.4) by applying a 0.7 V DC potential for 2 min. Then the surface was activated by EDC/NHS solution ([EDC] = [NHS] = 5 mM) which was prepared with cold water and then equilibrated for 2h at room

temperature. The resulting succinimidyl ester-terminated surface was rinsed with water and dried under a stream of nitrogen. Then graphene-based bioconjugate solution (15 μ L) was dropped onto activated GCE and dried for 8 h at 4 $^{\circ}$ C. For chronoamperometric measurements, fresh cholesterol solution was prepared in 10% t-octylphenoxy polyethoxy ethanol (Triton X-100) for each experiment.

Supporting Information

Supporting Information is available online from the Wiley Online Library or from the author.

Acknowledgements

The authors wish to acknowledge the Swedish Research Council (VR- 2011-6058357), European Commission (PIIF-GA-2009-254955), IGEN and LIST (Linköping University) for generous financial support to carry out this research.

Received: ((will be filled in by the editorial staff))

Revised: ((will be filled in by the editorial staff))

Published online: ((will be filled in by the editorial staff))

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Scheme 1. Schematic representations of on/off switchable bioelectrocatalytic graphene interface.

Figure 1. Cyclic voltammetry (a) and impedance (b) responses of PNIPAAM/Gr/Au NPs/ChOx modified electrodes in the “ON” state (40 °C) and “OFF” state (20 °C) in 5 mM $\text{Fe}[\text{CN}]^{3-/4-}$ and 0.1 M PBS at $50 \text{ mV} \cdot \text{s}^{-1}$ vs. Ag/AgCl reference electrode. The inset in (b) is the equivalent circuit used to fit the data.

Figure 2. Water contact angles of PNIPAAm/Graphene/Au NPs/ChOx modified glassy carbon surfaces at 20 °C and 40 °C. (*Inset:* Photographs show water drop on PNIPAAm/Graphene/Au NPs/ChOx modified glassy carbon surfaces at two different temperatures).

Figure 3. SEM images of PNIPAAm/graphene/Au NPs/ChOx modified surface at 20 °C (a) and 40 °C (b).

Figure 4. Chronoamperometric responses (a) of PNIPAAm/graphene/Au NPs/ChOx modified electrodes at <LCST 20 °C (“OFF” State) and >LCST 40 °C (“ON” State) and (b) dependence of amperometric currents vs. number of cycles for 1.0 mM cholesterol at +0.6 V in PBS solution switched between at two different temperature values.

The Table of Content

An on/off-switchable graphene based zipper-like interface is architected for efficient bioelectrocatalysis. The graphene interface transduces temperature input signal into structural changes of the membrane, resulting in the amplification of electrochemical signals and their transformation into the gated transport of molecules through the membrane.

Keyword: Smart interface, bioelectrocatalysis, switchable bioelectronics, graphene.

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Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma.((please add manuscript number))

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Figure S.1. Set-up for temperature-controlled SEM experiments with lower (a) and higher (b) magnifications, and external temperature control device (c).

Figure S.2. SEM images of PNIPAAm/graphene/Au NPs/ChOx modified surface at 20 °C (a) and 40 °C (b) with higher magnification of Figure 2.

Figure S.3. TEM images of graphene-Au NPs-enzyme assembled structures at different magnifications.

Figure S.4. Cyclic voltammogram of bare and modified electrodes in 5 mM $\text{Fe}[\text{CN}]^{3-/4-}$ and 0.1 M PBS at $50 \text{ mV} \cdot \text{s}^{-1}$ vs. Ag/AgCl reference electrode.

Figure S.5. Chronoamperometric responses of PNIPAAm/ChOx modified (without graphene and Au NPs) electrodes at 20 °C (“OFF” State) and 40 °C (“ON” State) and at +0.6 V in PBS solution.

Figure S.6. Calibration curve for choronoamperometric result (Figure 4a) of PNIPAAM/graphene/Au NPs/ChOx immobilised electrode.

Figure S.7. Lineweaver-Burk plot of PNIPAAm/graphene/Au NPs/ChOx immobilised electrode. The plot was obtained data based on Figure 3a.

Figure S.8. Schematic illustration of quantities in Young's equation.

An appropriate experimental method to identify the properties of surface is the measurement of contact angle of liquids on solid surfaces. The contact angle is used to quantify wettability parameter of surface, which provides a thermodynamic parameter to calculate the solid interfacial tension.

$$\cos \Theta_c = \frac{\gamma_{sg} - \gamma_{sl}}{\gamma_{lg}}$$

Young's equation which gives the measurable quantities, the contact angle Θ_c , the liquid-vapour/gas interfacial tension γ_{lg} , to the non-measurable interfacial tension γ_{sg} and γ_{sl} of the solid-vapour and solid-liquid interfaces, respectively.

Figure S.9. Zeta potential measurement of graphene at various pH values before and after surface modification.

Figure S.10. Reactor performance with varying temperature ranging from 20 to 45 °C.

Figure S.11. Chronoamperometric response of bio-electrode obtained by successive additions of progesterone (1.0 μM), sodium cholate (1.0 μM) in the presence of cholesterol at the potential of +0.6 V vs Ag/AgCl.

Figure S.12. Plausible reaction mechanism of cholesterol with cholesterol oxidase and biproducts (a). Chemical structures of progesterone and cholate (b).