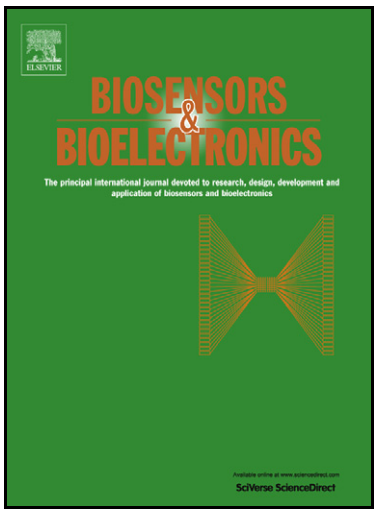

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Combination of cascade chemical reactions with graphene-DNA interaction to develop new strategy for biosensor fabrication

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Abstract

Graphene, a single atom thick and two dimensional carbon nano-material, has been proven to possess many unique properties, one of which is the recent discovery that it can interact with single-stranded DNA through noncovalent π-π stacking. In this work, we demonstrate that a new strategy to fabricate many kinds of biosensors can be developed by combining this property with cascade chemical reactions. Taking the fabrication of glucose sensor as an example, while the detection target, glucose, may regulate the graphene-DNA interaction through three cascade chemical reactions, electrochemical techniques are employed to detect the target-regulated graphene-DNA interaction. Experimental results show that in a range from 5 μM to 20 mM, the glucose concentration is in a natural logarithm with the logarithm of the amperometric response, suggesting a best detection limit and detection range. The proposed biosensor also shows

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favorable selectivity, and it has the advantage of no need for labeling. What is more, by controlling the cascade chemical reactions, detection of a variety of other targets may be achieved, thus the strategy proposed in this work may have a wide application potential in the future.

Keywords: graphene; DNA; cascade; biosensor; glucose; electrochemistry

1. Introduction

Since the first description of graphene in 2004 (Novoselov et al., 2004), this single atom thick and two dimensional carbon nano-material has attracted a great research interest because of its unique properties, such as superior electric conductivity (Zhou et al., 2009; Tang et al., 2009; Service, 2009), large surface area (Geim and Novoselov, 2007; Park and Ruoff, 2009), and excellent mechanical strength (Lee et al., 2008; Alwarappan et al., 2009). It also shows a huge potential for biological applications, eg. Antibacterial (Hu et al., 2010; Akhavan and Ghaderi, 2010; Liu et al., 2011), therapy (Yang et al., 2010; Markovic et al., 2010; Tian et al., 2011), and biosensing (Schedin et al., 2007; Shan et al., 2009; Song et al., 2010; Tang et al., 2010), etc. In biosensing area, graphene is playing an increasingly important role, so a number of biosensors have been developed by using different properties of graphene for the detection of proteins (Mohanty and Berry, 2008; Ohno et al., 2009), DNA (Nelson et al., 2010; Dong et al., 2010; Stine et al., 2010; Lim et al., 2010), metal ions (Li et al., 2009a, 2009b), and small molecules (Lin et al., 2009; Liu et al., 2009; Shang et al., 2008).

Recently, it is reported that single-stranded DNA (ssDNA) may bind to graphene with a high affinity through π-π stacking interaction (Ortmann et al., 2005; Gowtham et al., 2007; Antony and Grimme, 2008), while the affinity for double-stranded DNA (dsDNA) or well-folded ssDNA is much lower. This discovery may provide a great opportunity for the fabrication of graphene-

based DNA sensors and aptasensors (Jang et al., 2010; He et al., 2010; Xing et al., 2012; Lu et al., 2010; Balapanuru et al., 2010; Zhao et al., 2011). For example, Jang et al. built a platform for the assay of helicase unwinding activity that relied on the preferential binding of graphene oxide to ssDNA over dsDNA (Jang et al., 2010). Xing et al. developed a novel aptasensor for the detection of adenosine deaminase activity on the basis of graphene oxide (Xing et al., 2012). In this lab, we proposed a graphene quantum dots-based platform for the detection of DNA and thrombin (Zhao et al., 2011). Despite the success in the fabrication of different kinds of graphene-based DNA sensors and aptasensors, it is still expected that the graphene-DNA interaction may be extended to wider applications.

In this paper, we report a novel graphene-based biosensing strategy, which can be used to fabricate many kinds of biosensors. In principle, cascade chemical reactions triggered by the detection target (here glucose is adopted as a model) are skillfully combined with the changes of the graphene-DNA interaction, which can be subsequently detected with electrochemical techniques. Consequently, the quantitative information of the detection target can be correlated with the changes of the graphene-DNA interaction, thus the correlation can be employed to develop electrochemical biosensors for the detection of various targets. The proposed strategy may also present a novel concept to integrate chemical reactions with the graphene-DNA interaction, which is helpful for further development of a diversity of biosensors.

2. Experimental

2.1. Reagents

Graphene dispersion was purchased from Nanjing XF-Nano Materials Tech. Co., Ltd. Glucose oxidase (GOx) and glucose were from Sigma-Aldrich. DNA oligonucleotide (HPLC purified, palindrome sequence: 5′-GCT AGA GAT TTT AAA TCT CTA GC-3′) was obtained from

Shanghai Sangon Biotech. CO., Ltd. Other chemicals were all of analytical grade and used as received. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system to a specific resistance of >18 MΩ cm. All the experiments were performed under room temperature (25 ^{o}C) unless otherwise specified.

2.2. Fabrication of graphene coated electrode

The surface of a substrate glassy carbon electrode (GCE) was firstly polished to mirror smoothness with fine sand papers and 1.0, 0.3, 0.05 μm alumina slurry on silk in sequence. Then, it was separately ultrasonicated in ethanol and doubly distilled water for about 5 min to remove any adhesive particles. After thoroughly rinsed with pure water and dried with nitrogen, a clean bare electrode was prepared and could be ready for further modification.

Graphene dispersion (0.05 mg/ml) was ultrasonicated for about 10 minutes to make the graphene disperse evenly. Then, 15 μL of the graphene dispersion was cast on the GCE surface, followed by the complete evaporation of water at room temperature for about 16 hours. A uniform thin layer of graphene was consequently prepared on the GCE surface.

2.3. Glucose-regulated graphene-DNA interaction

Glucose with different concentrations was added to a mixture of GOx, $FeCl_2$, and DNA (20 μg/ml GOx, 2 mM $FeCl_2$, 0.1 μM DNA, in 10 mM TE buffer, pH 7.4). The solution was firstly incubated in 37 ºC for 5 hours to allow chemical reactions to proceed sufficiently. Then, 15 μL of the solution was cast on the surface of the above prepared graphene modified GCE for 30 minutes, followed by rinsing the electrode gently with double-distilled water. After dried with nitrogen, the electrode was ready for electrochemical measurements.

2.4. Electrochemical measurements

Electrochemical measurements were performed on a model 660C Electrochemical Analyzer (CHI Instruments) with a TE buffer (10 mM, pH 6.0, containing 5 mM $[Fe(CN)_6]^{4-/3-}$ as electrochemical probe and 1 M KNO_3 to improve the conductivity). A three-electrode system was used for the measurements, in which the graphene modified GCE was adopted as the working electrode. A saturated calomel reference electrode (SCE) and a platinum electrode served as the reference and counter electrode, respectively. All the electrochemical measurements were repeated at least four times. The parameters for electrochemical measurements have been described in the captions of figures without repetitious description here.

2.5. Polyacrylamide gel electrophoretic analysis

Besides the electrochemical measurements, the samples of oligonucleotides were also monitored by polyacrylamide gel electrophoresis. DNA oligonucleotides were dissolved into 100 μM mother liquor with TE buffer (10 mM, pH 7.4), and stored at 4 ºC. Before the electrophoresis, they were incubated at 90 ºC for 15 min first, followed by natural cooling to room temperature to ensure that the oligonucleotides had self-folded or hybridized each other to form dsDNA. $FeCl_2$ was prepared freshly before use, since it could be easily oxidized. The DNA samples were loaded onto a 30% non-denaturing polyacrylamide gel. The electrophoresis experiments were performed at 100 V for 1 h. 1×TBE buffer were used to prepare the gels, also served as the running buffer. The gel was stained with SYBR Green II.

2.6. Detection of glucose in blood serum samples

Blood serum was collected from three independent blood samples by centrifugation. Macromolecules in the blood serum samples were removed by centrifuge filtration (using an Amicon filtration device 30,000 cut-off from Millipore) to avoid any interference. The filtrate was then diluted 10 times by TE buffer (10 mM, pH 7.4), and was ready for electrochemical measurements. Glucose concentration of the blood serum samples was also determined using an automatic biochemical analyzer (Unicel DxC 800 Synchron Clinical Systems from Beckman).

3. Results and discussion

3.1. Mechanism of the sensing strategy

Glucose may trigger three cascade chemical reactions in the presence of GOx, $FeCl_2$, and DNA. The reaction equations are as follows:

$$\text{Glucose} + O_2 \rightarrow \text{Gluconic acid} + H_2O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- \quad (2)$$

$$\cdot OH + \text{DNA} \rightarrow \text{DNA segments} \quad (3)$$

Briefly, the GOx-catalyzed oxidation of glucose leads to the generation of hydrogen peroxide (H_2O_2) (Equation (1)), which subsequently triggers the launching of Fenton reaction in the presence of ferrous iron (Fe^{2+}) (Equation (2)). The highly reactive hydroxyl radical ($\cdot OH$) generated by the Fenton reaction can result in DNA damage that causes DNA breakage (Equation (3)). These three cascade reactions may proceed automatically and rapidly under room temperature in one pot without any auxiliary measures.

It is known that only bases-exposed ssDNA can interact with graphene. So, if a well designed DNA sequence is introduced, the graphene-DNA interaction may be regulated by glucose. Here, we have elaborately designed a palindrome DNA sequence which can self-fold or hybridize each other to form dsDNA with a melting temperature to be ~56 ^{o}C (Figure S1). After glucose is

introduced to trigger the cascade reactions, the palindrome DNA can be cleaved into pieces, whose bases may be exposed to interact with graphene. Since the changes of the interaction can be easily and sensitively detected by using electrochemical technique, electrochemical biosensor for the detection of glucose can thereby be developed. The overall scheme of the sensing strategy has been shown in Scheme 1.

— Please insert Scheme 1 here —

3.2. Glucose-triggered cascade chemical reactions

In order to achieve the goal depicted above, the glucose-triggered cascade chemical reactions should firstly be confirmed. Since the end product is the DNA segments, polyacrylamide gel electrophoresis is an appropriate method in the characterization of the changes of DNA. As is shown in Figure 1, the palindrome DNA alone (lane 2 and 6) shows a clear band around 20 bp, suggesting the well formation of dsDNA (23 bp). While the DNA is incubated with H_2O_2 and Fe^{2+} to allow the occurrence of Fenton-induced DNA damage reactions (Equation (2) and (3)), it can be observed that the DNA bands disappear (lane 3-5, reaction time is 30 min, 1 h, and 2 h, respectively), suggesting the breakage of the DNA chains. On the other hand, if glucose and GOx instead of H_2O_2 are introduced to trigger the cascade chemical reactions (Equation (1) to (3)), the DNA bands also disappear gradually (lane 7-9, reaction time is 30 min, 1 h, and 2 h, respectively). The electrophoresis results have well confirmed the occurrence of glucose-triggered cascade chemical reactions, which may lay the foundation for the further electrochemical sensing.

— Please insert Figure 1 here —

3.3. Interaction between graphene and DNA

The graphene-DNA interaction can be well revealed by monitoring the electron transfer behavior of the $[Fe(CN)_6]^{3-/4-}$ probes at the graphene modified electrode.[38] After DNA adsorbs onto the surface of the graphene coated electrode through π-π stacking interaction, electron transfer between the electrode and the probes may be inhibited due to both the steric and electrostatic exclusion of DNA to the probes. The more the DNA molecules are adsorbed on the surface of the electrode, the more drastic the inhibition will be. So the changes of the electrochemical signals obtained from the probes may reveal the state of the graphene-DNA interaction. Based on this fact, we have firstly examined the electron transfer behavior of graphene alone coated electrode by using the techniques of cyclic voltammetry (CV) and differential pulse voltammetry (DPV). As is shown in Figure 2A, a well-defined couple of peaks representing the redox reaction of $[Fe(CN)_6]^{3-/4-}$ at the surface of the graphene modified electrode can be observed, suggesting a good conductivity of graphene. In fact, the peaks currents obtained at the graphene coated electrode are even a little higher than the bare electrode, so the surface of the graphene modified electrode is somewhat more suitable for the redox reaction of the probes. The results have also been confirmed by using the technique DPV, which is more sensitive for electrochemical measurement (Figure 2B). Also because of this reason, DPV has been employed for the following experiments.

— Please insert Figure 2 here —

Figure 3 shows that the peak current will decrease gradually if the intact palindrome DNA with different concentrations is introduced in the sensing system, suggesting that more and more DNA molecules have been adsorbed onto the surface of the graphene coated electrode. As we have described in section 3.1, the palindrome DNA is designed to be able to self-fold or hybridize each other to form dsDNA, which is unfavorable for graphene-DNA interaction. Nevertheless, the actual experimental results show that the interaction between graphene and

intact palindrome DNA molecules still occurs. Actually, there is no contradiction here. Due to the competitive action of graphene with the bases in the DNA, possible folding or hybridization might be inhibited, thus the palindrome structure may retard the interaction but not block it totally. Therefore, with the increased concentration of the employed DNA, decrease of the peak currents can still be observed. If an unfolded reference DNA with no palindrome sequence is employed to interact with the immobilized graphene, experimental results show that the peak current decreases much more rapidly in comparison with the palindrome DNA (Figure S2). So, the results are expected, and also confirm the above speculation.

— Please insert Figure 3 here —

In order to obtain a suitable concentration of the palindrome DNA for the following biosensing experiments, we have studied the influence of the DNA concentration on the current changes in the presence and absence of glucose-triggered cascade reactions (Figure S3). Results show that, if 0.1 μM DNA is adopted, a maximum extent of the current changes can be obtained. The result suggests that at such a concentration, the palindrome DNA has week interaction with graphene due to the imbedded bases. And, the interaction has the potential to become much stronger while the bases are exposed after breakage caused by glucose-triggered cascade chemical reactions. So, we have fixed the concentration of the palindrome DNA at 0.1 μM for the following experiments.

3.4. Detection of glucose

On the basis of the above work, we have then connected the glucose-triggered cascade chemical reactions with the electrochemical detection of glucose. So, glucose with various concentrations is employed to trigger the cascade reactions, the end product of which is allowed to interact with graphene. As is shown in Figure 4A, the peak currents of the differential pulse

voltammograms decrease apparently along with the increase of glucose concentration. Further studies reveal that the glucose concentrations are in a natural logarithm with the logarithm of the currents values of the voltammograms in a wide range from 5 μM to 20 mM, as is shown in Figure 4B. The fitting equation goes to $Y = 1.95 + 0.13 \times Ln(X + 0.043)$, $R^2 = 0.98$. Therefore, the detection range can be as low as 5 μM and as high as 20 mM, both of which are better than the previous reports (Shan et al., 2009; Wu et al., 2007; Tsai et al., 2005; Zhu et al., 2007). The relative standard deviations (RSD) of the data points from four independent experimental results are calculated to be 1.5%~3.5%, suggesting a good reproducibility.

— Please insert Figure 4 here —

Some biomolecules that usually interfere in the electrochemical detection of glucose have been introduced to investigate the selectivity of the biosensor. So, ascorbic acid (AA), dopamine (DA) and uric acid (UA) instead of glucose are adopted as controls. As is shown in Figure 5, the addition of AA, DA or UA can only produce a very small decrease of the amperometric response. The variation of the currents (ΔI) for the controls can be about 16~24 times lower than that for glucose, suggesting a good selectivity of the proposed biosensor. In fact, since the first step in the cascade reactions is the GOx-catalyzed oxidation of glucose, the specificity can be well guarantied due to the specific enzymatic catalysis. We have also adopted blood serum as a real sample to study the anti-interference ability of the biosensor. Results show that glucose in blood serum can be well detected with an acceptable relative deviation of maximum 10.5% (Table S1). It should also be noted here, because the hydroxyl free radical is a non-selective oxidant, the presence of organic molecules in the samples may interfere with the detection. So, if untreated blood serum or spiked urine samples are directly used, results show that the cascade reactions are affected (lane 3 and 5 in Figure S4). Nevertheless, if the concentration of the organic molecules is confined in a reasonable limit through diluting, centrifuge filtration, or

some other methods, the cascade reactions still proceed (lane 6 in Figure S4), and the biosensor still works.

— Please insert Figure 5 here —

3.5. Discussion

We have succeeded in combining cascade chemical reactions with graphene-DNA interaction for biosensing applications by fabricating a novel glucose biosensor. To extend the application of the strategy for the detection of other targets, diverse cascade chemical reactions can be employed. For example, hypoxanthine, cholesterol, choline, and etc. can be detected using the same cascade chemical reactions by replacing GOx with the corresponding oxidase (xanthine oxidase, cholesterol oxidase, choline oxidase, etc.). Moreover, the hydroxyl radical produced in the cascade reactions can also be produced through lipid peroxidation and some other reactions. In addition to the hydroxyl radical, some other free radicals (eg. peroxynitrite, alkoxyl radical, sulfur-centered radical, and nitrogen-centered radical) and chemical agents (eg. alkylating agents) can also induce or accelerate the DNA damage. On the other hand, a large number of species can interrupt or repair the DNA damage. Therefore, a variety of molecules, enzymes, and even reactions can be used for the extension of the cascade chemical reactions, which may actually be employed to develop more kinds of biosensors.

4. Conclusions

In summary, we have proposed a novel strategy for the development of many kinds of biosensors by combining cascade chemical reactions with graphene-DNA interaction. In our strategy, a palindrome DNA sequence is designed to be cleaved into DNA pieces under target-triggered cascade chemical reactions, and subsequently the exposed DNA bases may interact

with graphene through noncovalent π-π stacking. Therefore, we are able to detect the target (glucose) by monitoring the change of the interaction between graphene and the employed DNA molecules. The biosensor may present a detection range as wide as 5 μM ~ 20 mM, and show a favorable selectivity against some potential interference. Since this is the first time for the property of interaction of graphene with DNA to be connected with cascade chemical reactions, more studies can be followed for fabrications of more biosensors. This novel concept to connect chemical reactions with the graphene-DNA interaction may also provide a broader scope for the application of graphene in the future.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version.

Acknowledgements

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Figure Captions

Scheme 1 Schematic illustration of the electrochemical glucose biosensor based on the interaction between graphene and DNA.

Figure 1 Electrophoretogram: DNA ladder (lane 1); 1 μM palindrome DNA alone (lane 2 and 6); palindrome DNA incubated with H_2O_2 and $FeCl_2$ (2 mM for both) for 30 min (lane 3), 1 h (lane 4), and 2 h (lane 5); palindrome DNA incubated with glucose, GOx, and $FeCl_2$ (2 mM, 20 μg/ml, and 2 mM, respectively) for 30 min (lane 7), 1 h (lane 8), and 2 h (lane 9). The numbers in the left indicate the base pairs.

Figure 2 (A) Cyclic voltammograms and (B) Differential pulse voltammograms obtained at a bare GCE (solid line) and a graphene coated GCE (dash line) in an electrolyte containing 5 mM electrochemical probe $[Fe(CN)_6]^{3-/4-}$. CV was carried out with a scan rate of 100 mV s^{-1}. DPV was carried out with pulse amplitude of 50 mV and pulse width of 50 ms.

Figure 3 Differential pulse voltammograms obtained at a graphene coated GCE, which has been incubated with DNA for 30 minutes to allow graphene-DNA interaction occur. DNA concentrations from a to h: 0 μM, 0.1 μM, 0.2 μM, 0.5 μM, 1 μM, 5 μM, 10 μM, 20 μM. Others same as in Figure 2B.

Figure 4 (A) Differential pulse voltammograms obtained at a graphene coated GCE, which has been incubated with the product of glucose-triggered cascade reactions for 30 minutes to allow graphene-DNA interaction to occur. DNA concentration: 0.1 μM. Glucose concentration from a

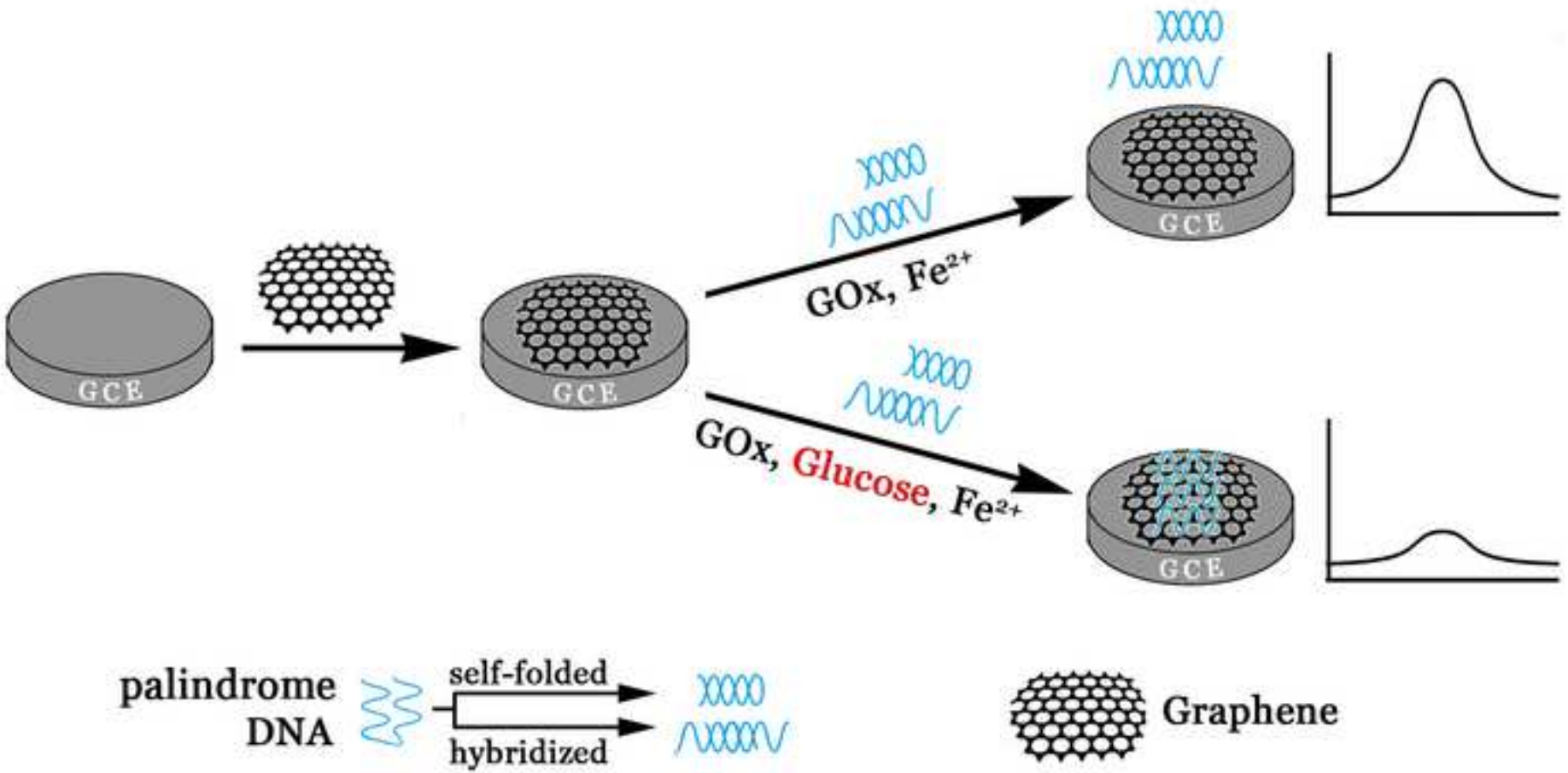
to i: 0 μM, 5 μM, 25 μM, 100 μM, 500 μM, 1 mM, 5 mM, 10 mM, 20 mM. Others same as in Figure 2B. (B) Plots of the glucose concentration against the logarithm of the current value.

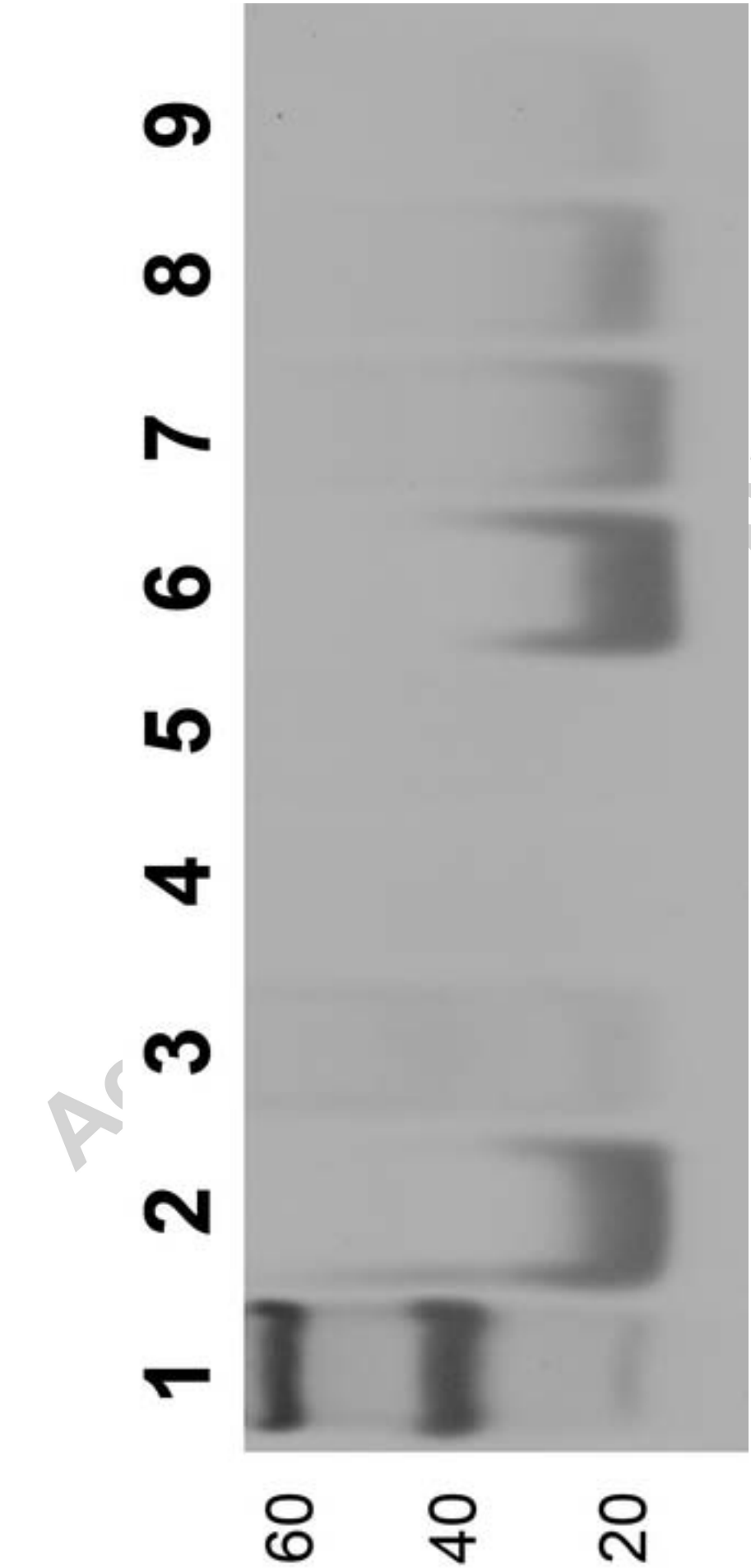
Figure 5 (A) Differential pulse voltammograms obtained by adopting 20 mM AA, DA, and UA as controls. Others same as in Figure 4A. (B) Histograms of the current values in the cases of AA, DA, UA and glucose.

Highlights

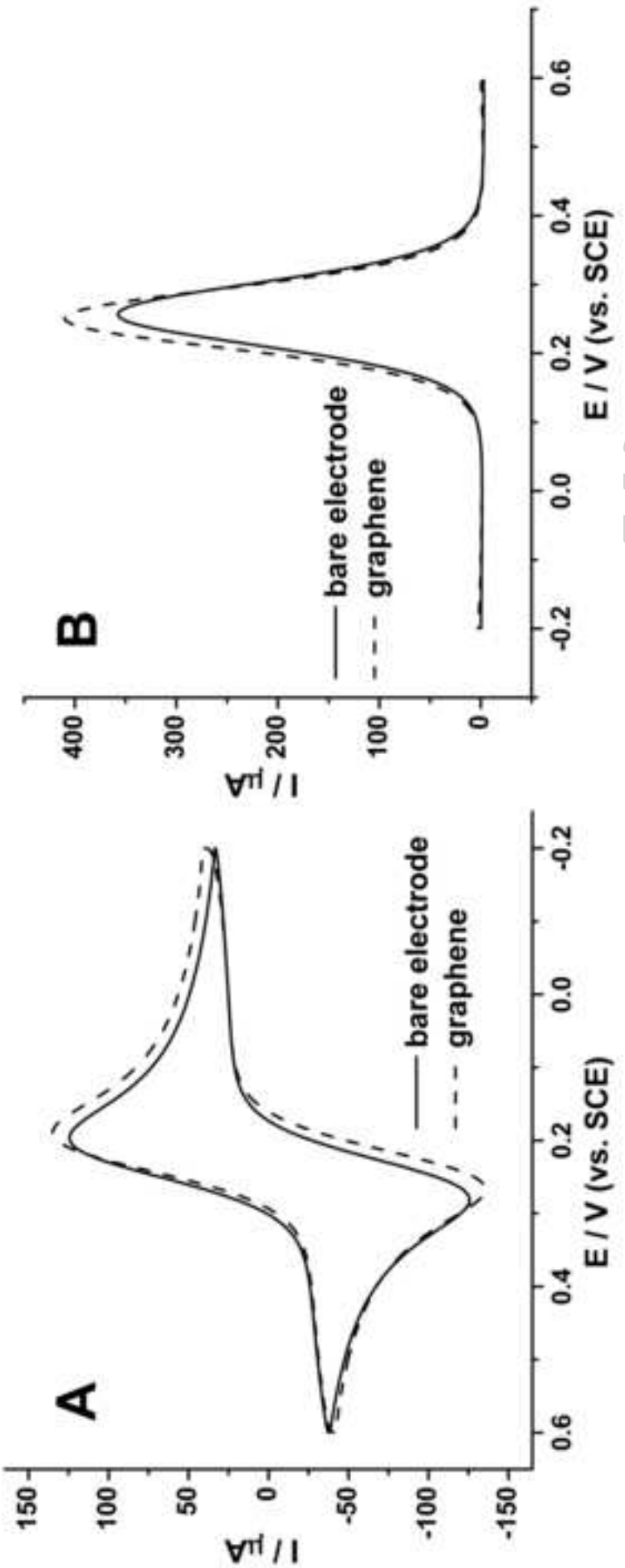
- Combination of cascade chemical reactions with graphene-DNA interaction.
- A broader scope for the application of graphene in biosensing can be achieved.
- Development of electrochemical study on target-regulated graphene-DNA interaction.
- Glucose can be well detected with a superior detection limit and detection range.

Scheme(1)

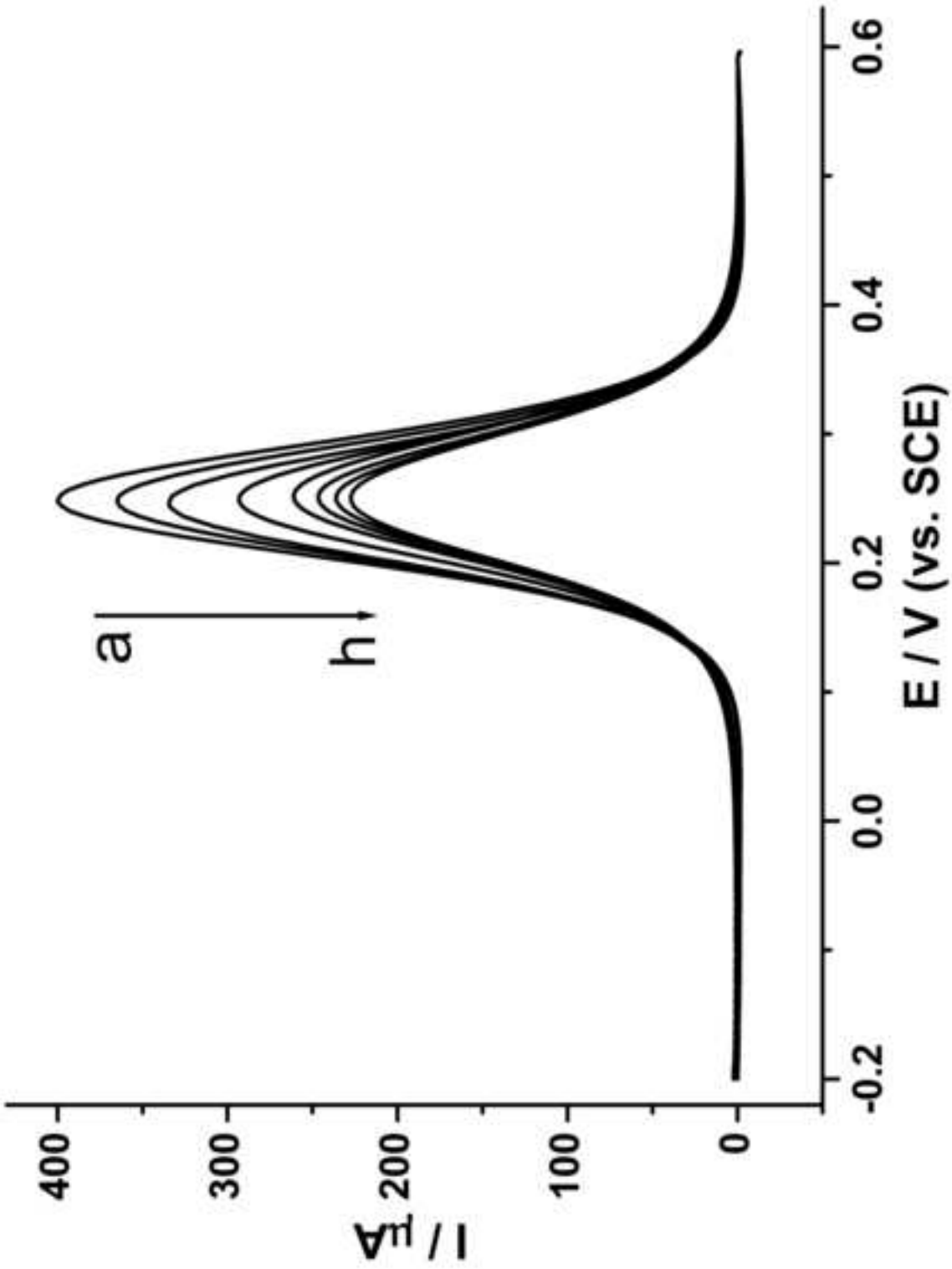




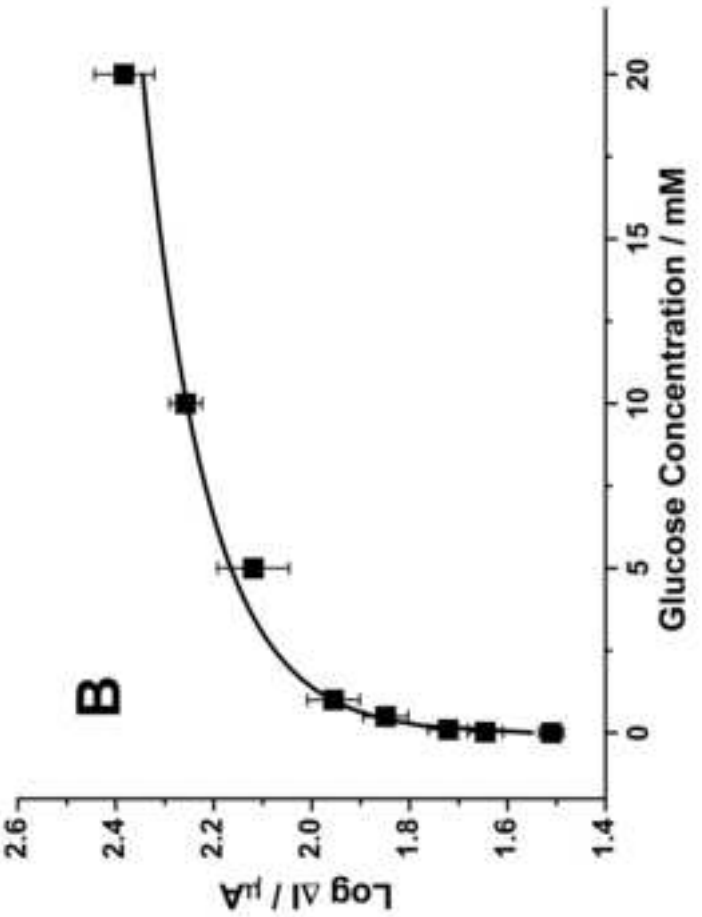
Figure(1)

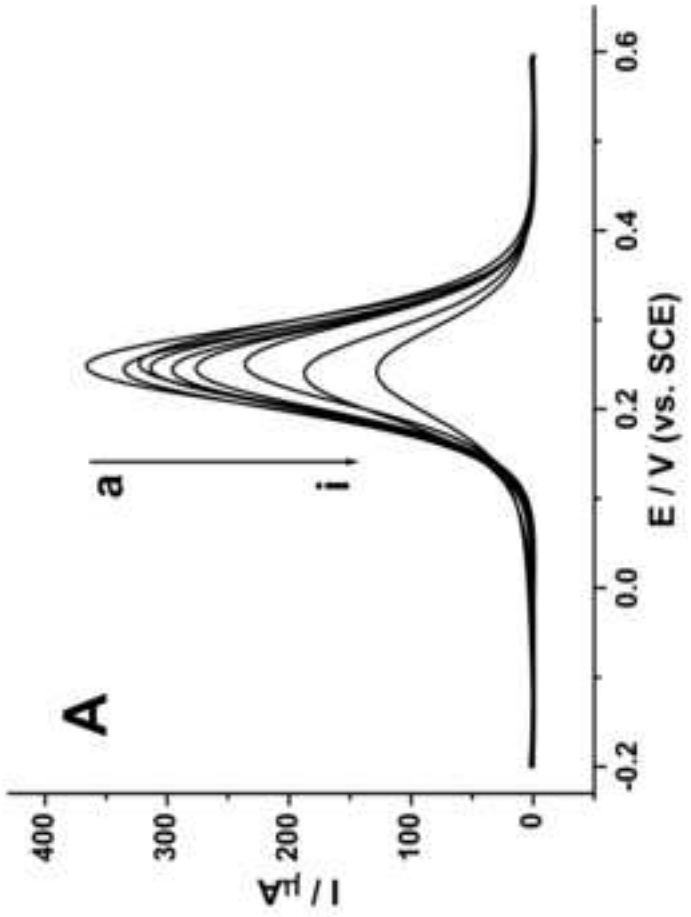


Figure(2)

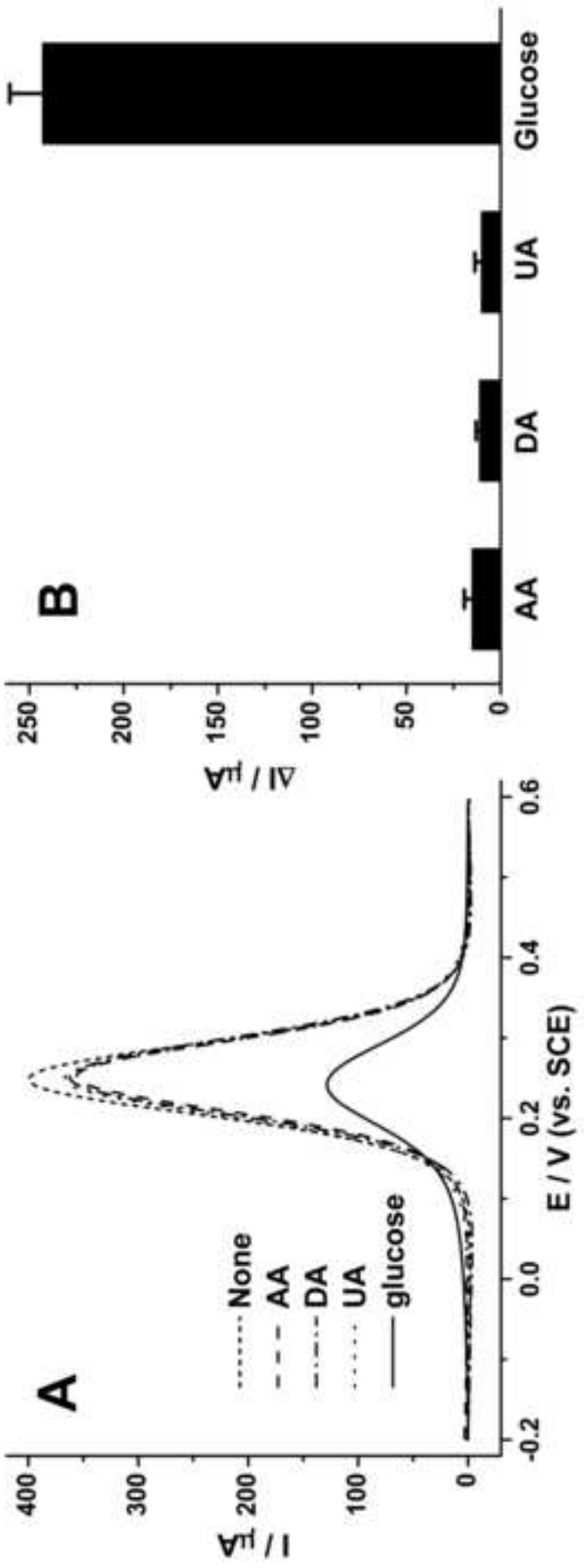


Figure(3)


B
Log Δi / μA
Glucose Concentration / mM
2.6
2.4
2.2
2.0
1.8
1.6
1.4
0
5
10
15
20


A
a
i
I / μA
E / V (vs. SCE)
400
300
200
100
0
-0.2
0.0
0.2
0.4
0.6

Figure(4)



Figure(5)