



# A novel amperometric glucose biosensor based on ternary gold nanoparticles/polypyrrole/reduced graphene oxide nanocomposite



Kaiwen Xue<sup>a</sup>, Shenghai Zhou<sup>a</sup>, Hongyan Shi<sup>a</sup>, Xun Feng<sup>a</sup>, Hua Xin<sup>b,\*</sup>, Wenbo Song<sup>a,\*</sup>

<sup>a</sup> College of Chemistry, Jilin University, Changchun 130012, China

<sup>b</sup> China–Japan Union Hospital, Jilin University, Changchun 130033, China

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## ABSTRACT

A ternary nanocomposite comprised of dispersed small gold nanoparticles (AuNPs) on the surface of reduced graphene oxide (RGO) was achieved via facile wet-chemical routes by using polypyrrole (PPy) as a linker. Highly dispersed small AuNPs supported on PPy/RGO surface have been evidenced by the TEM and SEM characterization. The composite exhibited excellently electrocatalytic property toward O<sub>2</sub> reduction compared with RGO/PPy, RGO and PPy. Based on the oxygen reduction activity and compatibility of the composite, its prospective in biosensing was further exemplified. Upon encapsulation of glucose oxidase via chitosan cross linking, a glucose electrochemical biosensor was constructed. The biosensor manifested a reproducible amperometric response to glucose in a linear range of 0.2–1.2 mM ( $R=0.986$ ) with a sensitivity of 123.8  $\mu\text{A mM}^{-1} \text{cm}^{-2}$ . The results indicate that the ternary nanocomposite is promising in biosensor fabrication.

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## 1. Introduction

Electrochemical biosensors, which possess attractive features with high sensitivity, fast in situ detection, excellent selectivity and low cost, have attracted extensive attention for a long time [1–4]. The electrochemical biosensors based on nanomaterials including metal nanoparticles, carbon nanotubes, graphene and metal oxides have recently been investigated intensively, due to their unique chemical and physical properties [5–8]. The nanocomposites combining two or several different components are expected to further improve the deficient characteristics of each component [9], leading to promising application in biosensing.

Graphene, as the thinnest material in universe, has attracted extensively attention for both experimental and theoretical science [10]. Because of the unique properties including fast electron transportation, excellent thermal conductivity, high specific surface area and good biocompatibility, graphene and its nanocomposites have demonstrated attractive application in electrochemical bio-sensing [11–15]. For example, Shan et al. prepared AuNPs/graphene nanocomposites protected by polyvinylpyrrolidone, realizing the direct electron transfer of GOD and exhibiting

good amperometric responses to glucose [14]. Wu et al. constructed PtNPs/graphene/GOD/chitosan nanocomposite film, showing satisfied sensitivity and good reproducibility for glucose determination. The above improved biosensing performances were ascribed to the excellent electrical conductivity and large surface area of graphene, as well as the superiority by combining graphene and metal nanoparticles [16].

Due to the high surface area, excellent conductivity, catalytic and biocompatible properties, AuNPs have been extensively used to construct various sensors, aiming at improving the performances in electrochemical detection of some small molecules (such as glucose, ascorbic acid, dopamine, uric acid, and nitrite ion) [6,17–19]. The AuNPs-based bio-electrocatalytic system with glucose oxidase was designed by Willner et al. [20], and the AuNPs-reconstituted enzyme electrodes demonstrated a high electron-transfer turnover rate of 5000  $\text{s}^{-1}$  (7-fold higher than that of native GOD). In addition, nanocomposites of AuNPs-polypyrrole [21–24] and AuNP-(3-mercaptopropyl)-trimethoxysilane (MPS) [25] have also been used for fabricating sensors and biosensors with excellent performances.

Because of the excellent mechanical strength and electrical conductivity [26,27], conducting polymers have showed promising application in supercapacitors, fuel cells and sensors [28,29]. As one of the most popular conducting polymers, the PPy possesses good electrical conductivity, environmental stability and good biocompatibility. The composite of PPy and graphene oxide (GO) revealed an enhanced electrical conductivity and demonstrating excellent sensing performance for H<sub>2</sub>O<sub>2</sub> [29–31].

\* Corresponding authors. Tel.: +86 0431 85168352; fax: +86 0431 85167420.  
E-mail addresses: [xinhua7254@aliyun.com](mailto:xinhua7254@aliyun.com) (H. Xin), [wbsong@jlu.edu.cn](mailto:wbsong@jlu.edu.cn) (W. Song).

Although small AuNPs nanoparticles are of particular importance in efficient catalysis and sensitive detection, the ease of aggregation often leads to deterioration of the performance. For obtaining highly dispersed small AuNPs, one of the effective routes is to precisely control AuNPs growth on a high specific nanomaterial surface that is pre-anchored by a linker. In previous works, two dimensional RGO nanosheets have been extensively investigated as a support, due to their large surface area and fast electron transfer activity. However, these nanosheets are prone to aggregate, leading to poorly dispersed catalytic particles with deteriorated performance. PPy possesses positive charges and good conductivity, which is prospective for functionalization of RGO and acts as effective linkers for precise confining small catalytic particles. Particularly, the well-established PPy/RGO synthetic routes as well as the biocompatibility are attractive to sensor fabrication. Based on above considerations, we designed a ternary nanocomposite by dispersed small AuNPs on RGO surface by using PPy as a linker. The small AuNPs well dispersed in AuNPs/PPy/RGO was expected to promote electron transfer among the composite and possess good biocompatibility upon GOD immobilization. Mean-time, the composite biocompatibility upon GOD immobilization and its prospective in biosensing was further exemplified, based on the decrease of  $O_2$  reduction current resulting from consumption of  $O_2$  during GOD-catalyzed glucose oxidation.

## 2. Experimental

### 2.1. Materials

Graphite,  $H AuCl_4 \cdot 4H_2O$ , chitosan,  $NaBH_4$ , hydrazine (50 wt%) and ammonia solution (28 wt%) were purchased from Sinopharm Chemical Reagent Co. Ltd. Glucose oxidase (GOD, EC 1.1.3.4, Type X-S, lyophilized powder, 100–250 units/mg) was from *Aspergillus niger*, and D-(+)-glucose ( $\geq 99.5\%$ ) was from Beijing Chemical Factory (Beijing, China). Glucose stock aqueous solutions were stored overnight at room temperature before use. The chitosan aqueous solution (pH 5) was prepared according to previous report [32]. Pyrrole monomer (Aldrich,  $>99.5\%$ ) was distilled under reduced pressure and stored under nitrogen ( $N_2$ ) gas. All other reagents were of analytical grade. All aqueous solutions were prepared with ultra-pure water from a Millipore-Q system ( $18.2 M\Omega cm$ ).

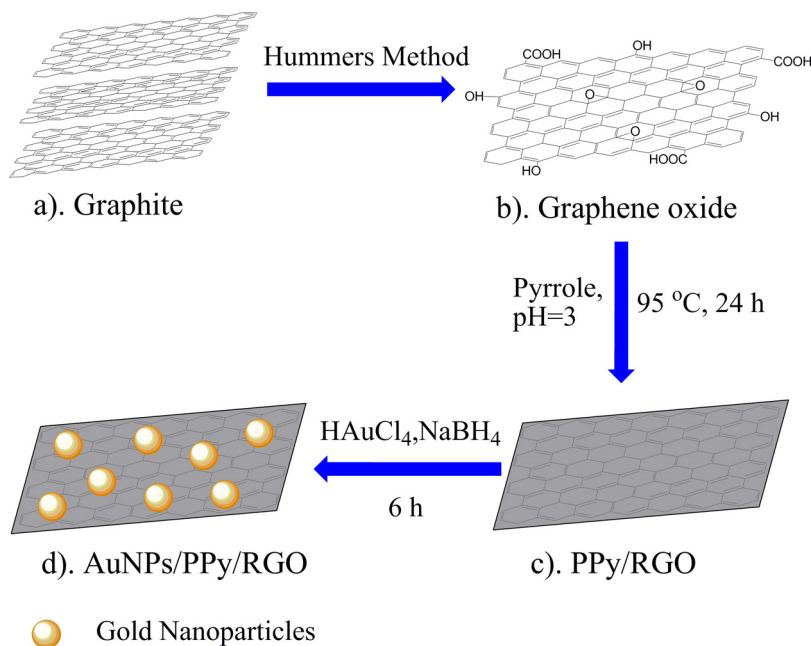
### 2.2. Apparatus and measurements

The morphology of resulting GO, PPy/RGO and AuNPs/PPy/RGO was characterized with a XL 30 ESEM FEG scanning electron microscopy (SEM) at an accelerating voltage of 20 kV and a JEM-2000EXJEOL transmission electron microscopy (TEM) at an accelerating voltage of 200 kV. The samples for SEM and TEM characterization were prepared by dropping ethanol solution of the sample on clean silicon wafer and carbon coating copper grids, respectively. These samples were dried at room temperature before measurement. The transmission spectra of Fourier transform infrared spectroscopy (FTIR) were measured on a Bruker Vertex 70 spectrometer ( $4 cm^{-1}$ ). The samples were prepared by the alkali halide (KBr) pressed pellet method. All electrochemical experiments were performed in a conventional three-electrode cell controlled by CHI 660B Electrochemical Work Station (CH instrument, China). The ternary composite modified glassy carbon (GC) electrode was used as the working electrode. A platinum wire was applied as the counter electrode and a saturated calomel electrode (SCE) served as the reference electrode. All aqueous solutions were freshly prepared with ultra-pure water from a Millipore-Q system ( $18.2 M\Omega cm$ ) before experiment. All electrochemical measurements were carried out at room temperature in 0.05 M phosphate buffer aqueous solution (PBS, pH 7.4), which was prepared by mixing  $Na_2HPO_4$  and  $NaH_2PO_4$  solutions. Prior to experiment,  $N_2$ -saturated PBS was prepared by purging the PBS with high purity nitrogen for at least 15 min, and then kept the cell under nitrogen atmosphere. For  $O_2$  reduction measurements,  $O_2$ -saturated PBS was used, which was similarly obtained by purging the electrolyte with high purity oxygen and kept the cell under oxygen atmosphere. All potential values given below were referred to SCE.

### 2.3. Synthesis of nanomaterials

#### 2.3.1. Synthesis of PPy/RGO

Graphene oxides were synthesized directly from graphite by the modified Hummers method [33]. The synthesis of PPy/RGO is illustrated in Scheme 1. Briefly, 60 mg of GO was homogeneously dispersed in 60 mL ultra-pure water by sonication for 1 h, and the pH was adjusted to 3 by adding hydrochloric acid aqueous solution.



Scheme 1. Schematic diagram of AuNPs/PPy/RGO synthesis.

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