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# One-pot green synthesis of mussel-inspired myoglobin–gold nanoparticles–polydopamine–graphene polymeric bionanocomposite for biosensor application

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ABSTRACT

A novel myoglobin–gold nanoparticles–polydopamine–graphene (MGPG) polymeric bionanocomposite was applied for studying the immobilization and direct electrochemistry of redox protein. The MGPG bionanocomposite was synthesized by an efficient one-pot green polymerization approach. The direct electrochemistry studies of the MGPG bionanocomposite modified electrode demonstrated that the immobilized myoglobin (Mb) retained its bioactivity and exhibited strongly enhanced direct electrochemical redox activity. This is mainly attributed to the proper orientation of the immobilized Mb due to in-situ oxidative polymerization process, the large specific surface area of the nanocomposite, and the good conductivity of the graphene-Au nanoparticles complex. Hence, the present polymeric bionanocomposite displayed good electrocatalytic activity to the reduction of $H_2O_2$. The proposed method is highly recommended as a novel biosensing platform for biomolecules immobilization, which can further expand the applications of biosensing and biocatalysis, such as construction of third-generation biosensors and the detection of $H_2O_2$ released by cells.



## 1. Introduction

In the last few decades, the immobilization and the direct electrochemistry of native redox proteins attracts continuously renovated attention, which can not only provide a desirable model for fundamental studies on the redox mechanism of the proteins in biological systems, but also enable the establishment of the third-generation biosensors, biomedical devices, and enzymatic bioreactors [1,2]. However, due to the electroactive centers of redox proteins are often deeply buried within the structure of biomacromolecules and the proteins are easy to be irreversibly denatured, the direct electrochemistry between the redox proteins and the substrate electrode is difficult to occur [3,4]. Therefore, great efforts have been made to retain high bioactivity of the redox proteins and facilitate their direct electrochemistry by using promoters, mediators, or other special materials to modify the electrodes [5–9]. Among them, nano-materials have become the preferred choice for immobilizing proteins and studying their direct electrochemistry because of their high surface areas, good biocompatibility, excellent adsorption and penetrability, and unique catalytic properties [8,10–15].

Graphene, a two-dimensional sheet of sp2 conjugated atomic carbon, has attracted increasing attentions in different fields including capacitors, cell images, biosensors, drug delivery, and solar cell in recent years because of its unique electrical, optical, and mechanical properties [16–18]. Due to its good electrical conductivity and excellent electrocatalytic activity, graphene could efficiently enhance the detection sensitivity in electrochemistry biosensor field. In the meantime, benefiting from the high surface area, good chemical stability and flexibility, graphene sheets have been an attractive choice as the immobilization platform for new functional graphene sheets composites [19–21]. These graphene-based functional materials have been extensively used to immobilize proteins and applied in the study of direct electrochemistry of proteins [9,22,23]. Furthermore, entrapment or encapsulation of biomolecules within biocompatible materials by using facile procedures is certainly desirable. Recently, inspired by the mussel adhesive proteins, polydopamine (PDA) can be deposited on virtually any surface on the basis of dopamine self-polymerization at alkaline pH [24,25]. Moreover, due to the good stability, excellent biocompatibility in vivo, the multifunctional PDA-based nano-composites with interesting properties have been proven to be the excellent materials for biomedical applications [26]. This approach is simple, inexpensive, quick, and "green". Considering these features, over the past few years,

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PDA-based electrochemical biosensors have been widely investigated, and variety high-performance biosensors have been developed [27–31].

Herein, we report on the preparation of multi-functional myoglobin–gold nanoparticles–PDA–graphene polymeric (MGPG) bio-nanocomposite by chemical polymerization for the immobilization and direct electron transfer studies of myoglobin (Mb). It was found that the MGPG bionanocomposite film modified electrode could provide a favorable microenvironment for Mb to realize the direct electron transfer between the immobilized Mb and electrode. We presume that this bionanocomposite represents a new matrix immobilization of biomolecules for electrochemical bioassays.

## 2. Experimental

### 2.1. Materials

Graphite flakes (99.99%, 325 mesh) and dopamine were purchased from Alfa Aesar. Horse heart Mb (MW 17,800) and ascorbic acid were acquired from Sigma. Hydrogen peroxide (30%), potassium ferricyanide, and $HAuCl_4 \cdot 4H_2O$ were obtained from Sinopharm Chemical Reagent Co Ltd. (Shanghai). 0.1 M phosphate buffer solution (PBS, pH 6.98) was used as supporting electrolyte. All solutions were prepared with ultrapure water (18.2 MΩ cm resistivity) from a Millipore system.

### 2.2. Characterization

Electrochemical experiments were performed on a CHI 760D Electrochemical Workstation (CH Instrument, USA). A three-electrode system was used with an Ag/AgCl as the reference electrode, a platinum wire as the auxiliary electrode, and the modified GCE as the working electrode. The electrolyte solutions were purged with $N_2$ for at least 20 min to remove $O_2$ and kept under $N_2$ atmosphere during measurements. UV–vis absorption spectra were acquired with a UV-2450 spectrophotometer (Shimadzu). Scanning electron microscopy (SEM) images were collected on a Quanta 200F scanning electron microscope (FEI, USA). To analyze the elements of MGPG polymeric bionanocomposite, energy dispersive X–ray (EDS) analysis was also carried out.

### 2.3. Preparation of graphene

Graphene oxide (GO) was synthesized from natural graphite powder using a modified Hummers and Offeman's method [32,33]. Graphene was prepared by the chemical reduction of GO according to the literature [34]. In brief, 100 mL of GO dispersion was mixed with certain amount of ascorbic acid solution. After ultrasonication for 1 h at 60 °C, the graphene sheets were obtained by centrifugation and washed with water for several times and dried in vacuum at 60 °C overnight.

### 2.4. Preparation of MGPG polymeric bionanocomposite

In a typical procedure, 300 μL of 2% $HAuCl_4$ and 10.0 mg Mb were added to 0.5 mg $mL^{-1}$ as-prepared graphene solution (1.0 mL PBS, pH 6.98) under vigorous stirring at 4 °C. Then, 60 mM dopamine (500 μL) was added dropwise to the mixture under continuous magnetic stir. After reaction for 30 min, the product was obtained by centrifugation and washed with water for several times, finally redispersed in pH 6.98 PBS (2 mL) and stored at 4 °C when not used.

### 2.5. Electrode preparation

Prior to the modification, glassy carbon electrode (GCE, 3 mm diameter) was polished with 1.0, 0.3 and 0.05 μm alumina slurry, respectively. The electrode was then successively sonicated in 1:1 nitric acid, acetone and doubly distilled water, and rinsed with distilled water. Then, 6 μL of the MGPG polymeric bionanocomposite solution was dripped onto the surface of the well-polished GCE.

## 3. Results and discussion

### 3.1. Characterization of MGPG polymeric bionanocomposite

Herein, MGPG polymeric bionanocomposite was prepared by the one-pot in situ polymerization of dopamine triggered by oxidizing reagent $HAuCl_4$ in the mixture. As can be seen from the SEM images, the shape of graphene was planar sheet-like (Fig. 1A). After the in situ

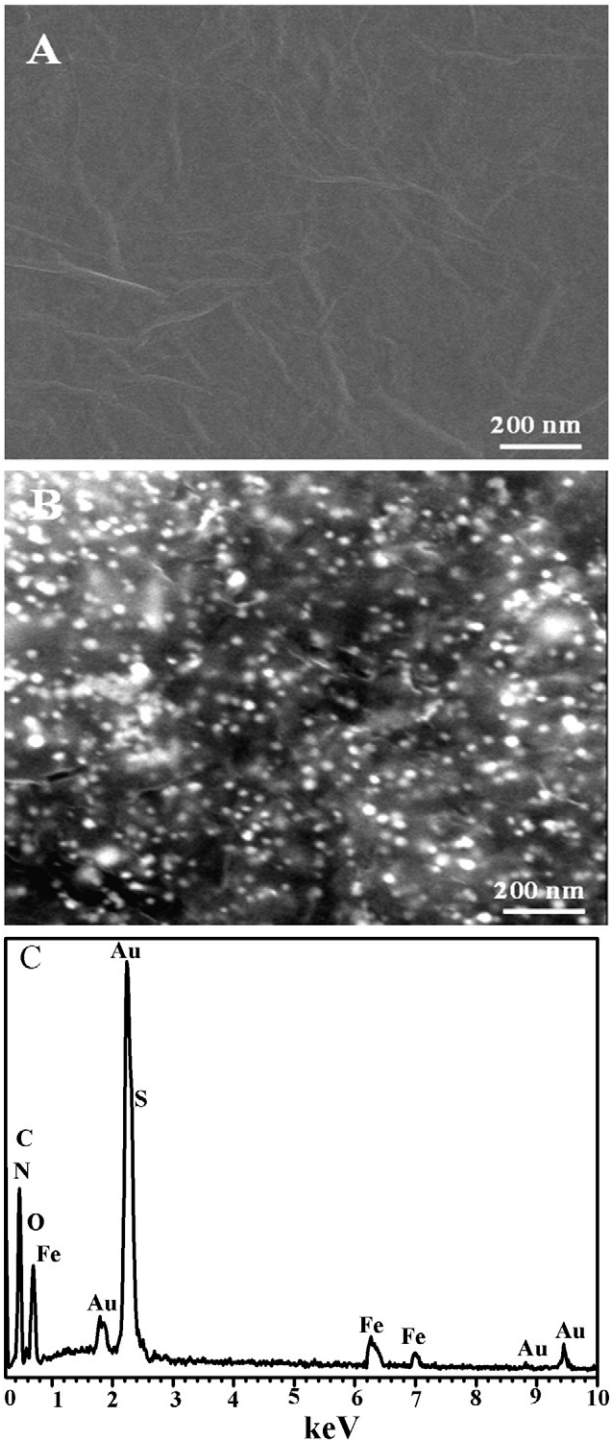


**Fig. 1.** SEM images of graphene (A) and MGPG polymeric bionanocomposite (B).