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# An electrochemical chiral sensing platform for propranolol enantiomers based on size-controlled gold nanocomposite

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ABSTRACT

The size-controlled gold nanoparticle–methylene blue–multiwalled carbon nanotubes nanocomposite (nanoAu–MB–MWNTs) was successfully synthesized as electrochemical redox-probe indicator and immobilization matrix, which calf thymus double-stranded DNA (ctDNA) could be adsorbed for chiral sensing propranolol (PRO) enantiomers. Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and ultraviolet–visible spectroscopy (UV–vis) were used to characterize the functionalized nanocomposite. The DNA-immobilized sensing platform (ctDNA/nanoAu–MB–MWNTs/GCE) showed a larger electrochemical response for *S*-PRO than *R*-PRO, in which the association constant ($\beta$) was calculated to be $1.154 \times 10^4$ L mol$^{-1}$ for *S*-PRO and $4.638 \times 10^3$ L mol$^{-1}$ for *R*-PRO. Quartz crystal microbalance (QCM) and electrochemical impedance spectroscopy (EIS) had confirmed the higher affinity for *S*-PRO with ctDNA. The experimental parameters such as acidity, incubation time, size of nanoAu and the amount of ctDNA were assessed to obtain the optimized conditions. Moreover, the proposed sensor was applied to determine the enantiomeric ratios of *R*-PRO in mixture solutions and high-performance liquid chromatography (HPLC) was used to validate the results by calculating *F*- and *t*-test values at 95% confidence level.

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## 1. Introduction

Chirality is one of the significant biochemical signatures in life since many macromolecular substances in biological systems have intrinsic chiral preference [1]. Drug enantiomers often show different pharmacological and metabolic behaviors in biological systems, for example, propranolol (PRO) enantiomers have obvious difference in pharmacodynamic profiles, in which the metabolism and elimination of *S*-PRO is faster than *R*-PRO and the activity of *S*-PRO is 100 times better than its isomer [2]. Presently, chiral analysis of PRO enantiomers is most commonly ascertained using chromatographies [3,4] and capillary electrophoresis [5,6], but they are laborious as well as unable to meet the needs of rapid determination. In addition, other methods such as surface enhanced Raman scattering [7], room temperature phosphorescene [8], fluorescence spectrophotometry [9] and nuclear magnetic resonance [10], suffer from high cost and complicated operation. Electrochemical methods based on various chemically modified electrodes have gained significant interest for the determination of organic molecues [11,12], attributing to simplicity of operation, short analysis time, excellent sensitivity and low cost [13,14].

Gold nanoparticle (nanoAu) have become one of the most popular materials in the construction of electrochemical sensor since it can provide a favorable microenvironment for biomolecules [15], facilitate the electron transfer between the immobilized redox proteins and electrode surfaces [16], and be controlled the experimentally size and surface morphology [17]. One way to incorporate nanoAu onto the electrode surfaces is to mix it with other material as immobilization matrix. Functionalized carbon nanotubes with desirable metal materials [18], biomolecules [19] or redox dye [20] have widely used to fabricate sensitive electrochemical sensors. For example, methylene blue–multiwalled carbon nanotubes nanohybird (MB–MWNTs) had been synthesized via $\pi$–$\pi$ stacking interaction to perform excellent redox electrochemical activities [21]. Moreover, horseradish peroxidase was incorporated into the multiwalled carbon nanotube/thionine/gold nanoparticle film to retain its bioactivity and electrocatalytical activity [15].

DNA, a polymer of nucleotide units consisting of a ribose sugar, a phosphate and a heterocyclic aromatic base, plays a crucial role in life science. The right handed double helical structure makes DNA as stereselective recognition sites for chiral enantiomers [22,23]. Calf thymus DNA (ctDNA), with a relatively low protein content but a

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highly polymerized skeleton, has been widely used as chiral selector to understand the specifity of chiral molecules such as amino acids, metallo-supramolecular complexes and antimicrobial drugs [24–26]. The previous study found that racemic PRO could inhibit DNA synthesis [27], while there were a few reports on discussing the interaction between ctDNA and PRO enantiomers. In this study, the size-controlled nanoAu were noncovalently attached to multiwalled carbon nanotubes (MWNTs) in the presence of methylene blue (MB) to prepare nanoAu–methylene blue–multiwalled carbon nanotubes nanocomposite (nanoAu–MB–MWNTs) as the biocompatible matrix for ctDNA to chiral sensing PRO enantiomers. Through sensing redox current signals of MB, the strategy based on functionalized nanocomposite would be competent for the development of electrochemical chiral sensor.

## 2. Experimental

### 2.1. Reagents and solutions

Calf thymus DNA (ctDNA), *R*-propranolol hydrochloride (*R*-PRO), *S*-propranolol hydrochloride (*S*-PRO), pristine multiwalled carbon nanotubes (MWNTs), gold chloride tetrahydrate ($HAuCl_4{\cdot}4H_2O$, 99.99%) and methylene blue (MB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trisodium citrate (99.5%) and other analytical grade chemicals were obtained from Chemical Reagent Co. (Chongqing, China). ctDNA were used as received since the purity was sufficiently high as determined from optical measurements ($OD_{260}/OD_{280}$ was higher than 1.8, where OD represents the optical density). Double distilled water was used throughout the experiments.

Britton–Robinson (BR) buffer solution (0.04 M in each of acetic, phosphoric and boric acids) was adjusted to the desired pH with additions of 0.2 M sodium hydroxide solution were used as supporting electrolytes. Stock solutions of *R*-PRO and *S*-PRO were prepared in 0.04 M BR buffer solution (pH 7.0) and kept in a dark glass stored under refrigeration. For analysis of commercial pharmaceutical product, ten racemic PRO tablets were weighted and powdered. A portion of powder was transferred into a 50 mL calibrated flask and made up to the volume with acetone. The mixture was sonicated for 15 min to provide complete dissolution. After filtered with acetone, the clear supernatant was transferred to a calibrated flask and diluted with 0.04 mM BR buffer (pH 7.0) to yield a final concentration of 5.0 mM PRO.

### 2.2. Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out by CHI604D electrochemistry workstation (Shanghai Chenhua Instruments Co., China). The shift of frequency caused by adsorption of PRO enantiomers on DNA modified quartz plates was investigated by model CHI 440A time-resolved electrochemical quartz crystal microbalance (QCM, Shanghai Chenhua Instruments). Transmission electron microscopy (TEM) was used to estimate the microstructures of the prepared composites (H600, Hitachi, Japan). The morphological characterization was examined by means of a scanning electron microscope (SEM, S-4800, Hitachi, Japan). Ultraviolet–visible absorption (UV–vis) spectra were recorded by an UV-2450 spectrometer (Shimadzu, Japan). The value of pH was measured by pH meter (MP 230, Mettler-Toledo, Switzerland). HPLC used for validating the method was an Agilent model 1100. HPLC experiments were carried out using a chromatographic column Zorbax C18, 250 mm × 4.1 mm, particle size 5.0 μm (Agilent Technologies, Waldbronn, Germany). All these experiments were at a room temperature (25 °C).

### 2.3. Preparation of nanoAu–MB–MWNTs nanocomposite

All glassware used in the following procedure were cleaned in a bath of the prepared chromic acid solution and rinsed thoroughly in double-distilled water. Gold nanoparticle (nanoAu) was prepared by reducing gold chloride solution ($HAuCl_4$) with sodium citrate, and the diameter of nanoAu with $13 \pm 1.3$, $18 \pm 2.1$, $25 \pm 1.5$, $33 \pm 2.6$ and $51 \pm 5.1$ nm were prepared (Fig. S1 in Supporting Information for details). The prepared nanoAu were stored in dark bottles at 4 °C for further use.

Following steps were processed to obtain nanoAu–MB–MWNTs nanocomposite. Firstly, pristine MWNTs were chemically oxidized in a mixture of sulfuric acid and nitric acid (3:1) to introduce carboxylic acid groups [28]. Secondly, MB–MWNTs nanohybrid was synthesized according to the previous reference [19]. Briefly, the acid-treated MWNTs (1.0 mg) and MB (1.5 mg) were dissolved in double-distilled water (10 mL) and sonicated for 2 h at room temperature. The positively charged MB molecules would attach to the MWNTs through an electrostatic interaction to form MB–MWNTs. After the homogeneous solution was centrifuged and rinsed with distilled water to remove excessive MB, the MB–MWNTs solution was prepared in double-distilled water (0.5 mg $mL^{-1}$). Thirdly, the prepared MB–MWNTs (2.0 mL) were added into the solution of nanoAu under vigorous agitation for 8 h. The black suspension was isolated by centrifugation and washing for several times. Finally, nanoAu–MB–MWNTs nanocomposite was dispersed in distilled water (0.5 mg $mL^{-1}$). The schematic processes of nanoAu–MB–MWNTs nanocomposite were illustrated in Scheme 1A.

### 2.4. Fabrication of the chiral sensing platform

The glassy carbon electrodes (GCE, $\Phi = 4$ mm) were carefully polished with 1.0, 0.3 and 0.05 μm alumina slurries to a mirror-like surface, then sonicated in ethanol. The black suspension of nanoAu–MB–MWNTs (8.0 μL) was dropped onto the clean GCE surface (nanoAu–MB–MWNTs/GCE). After dried at room temperature, ctDNA solution (8.0 μL, 0.33 mg $mL^{-1}$) was dropped to get ctDNA/nanoAu–MB–MWNTs/GCE. The preparation processes of the chiral sensing platform for PRO enantiomer was illustrated schematically in Scheme 1B.

### 2.5. Electrochemical measurements

Electrochemical experiments were carried out with three electrode system, in which the bare or the modified glassy was used as working electrode, a platinum wire was applied as the counter electrode and a saturated calomel electrode served as the reference electrode. The CV scan was done from 0.2 to −0.8 V at 100 mV $s^{-1}$ in 0.04 M BR solution (pH 7.0). EIS was measured in 5.0 mM $[Fe(CN)_6]^{4-/3-}$ solutions, in which the frequency range was from 0.1 to $10^5$ Hz in a bias potential of 220 mV vs. SCE, and the amplitude was 10 mV. The peak current of ctDNA/nanoAu–MB–MWNTs/GCE was regard as $I$ and the peak current after ctDNA/nanoAu–MB–MWNTs/GCE immersed in *R*- or *S*-PRO was regard as $I_R$ or $I_S$. The difference of the peak current was given by the following equation: $\Delta I_R = I - I_R$, $\Delta I_S = I - I_S$ and $\Delta I = \Delta I_S - \Delta I_R$.

## 3. Results and discussion

### 3.1. Characterization of the nanocomposite

Fig. 1 showed the UV–vis spectra of nanoAu, MB–MWNTs and nanoAu–MB–MWNTs. The characteristic absorption peak of nanoAu was around 518 nm (Fig. 1a) and MB–MWNTs showed

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