



# Conductive catalytic redox hydrogel composed of aniline and vinyl-ferrocene for ultrasensitive detection of prostate specific antigen

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## ABSTRACT

In this work, a conductive catalytic redox hydrogel composed of aniline and vinyl-ferrocene was prepared which showed good conductivity and strong electrochemical redox signal at 0.04 V (vs. Ag/AgCl). The hydrogel was simply generated in situ on the glassy carbon electrode to fabricate an amperometric immunosensor. The redox hydrogel provided a three dimensional porous structure, which improved the specific surface area of modified electrode. Based on the catalytic activity of ferrocene toward the oxidation of ascorbic acid, the electrochemical signal can be amplified. Gold nanoparticles were modified on the redox hydrogel by electrochemical deposition to promote electron transfer, which also can immobilize antibody. Prostate-specific antigen (PSA), a significant indicator of prostate cancer, had been measured as a model tumor marker. Under the optimized conditions, a linear range of the prostate specific antigen concentrations from 0.001 to 200 ng mL<sup>-1</sup> with a detection limit of 0.54 pg mL<sup>-1</sup> ((S/N)=3) was obtained. Importantly, the detection of PSA in the human blood serum samples showed satisfactory accuracy with the enzyme linked immunosorbent assay results, which showed the redox hydrogel possessed huge potential for the detection of other tumor markers.

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## 1. Introduction

The precise and sensitive detection of tumor markers could extremely increase the treatment efficiency of cancers, which is vital in the clinical diagnosis and biochemistry research [1–3]. A great variety of interfacial strategies have been used to measure tumor markers, such as the traditional enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance, fluorescent immunoassay, and electroanalysis [4–8]. Because of the innate high sensitivity, low detection limit, extreme simplicity and miniaturization, electrochemical measurement has drawn more and more attention [8]. Since no additional labeling step is needed, label-free amperometric immunosensor can directly detect the change of electrochemical signal resulted by the specific recognition between antibodies and antigens, it has been commonly employed to quantitative determination of tumor markers [9,10]. The substrate with good conductivity, strong current signal, high catalysis and large special surface area are crucial factors for high performance label-free amperometric biosensor [11,12].

Recently, a number of composites substrates have been designed to prepare label-free amperometric immunosensor, such as metal nanocomposites, carbon nanocomposites and conductive polymers [13–18]. Although metal nanocomposites with good conductivity and catalysis can promote electron transfer, amplify current signal and immobilize proteins, the signal species was need to be introduced. Carbon materials also can increase the conductivity and special surface area of electrode, but complex operations often are required for the activation process. Conductive polymers were easy to synthesize on the surface of electrode, however, they commonly do not have an electrochemical signal. Potassium ferricyanide is often introduced in the electrolyte solution with high concentration as redox species of these immunosensors [19,20]. For this process, the inactivation of proteins can be easily caused [21,22]. In addition, some conductive hydrogels with heavy metal ion as redox species have been prepared to develop amperometric immunosensors [23,24]. However, heavy metal ions are easy to pollute the environment. So it is of great significance to find a new conductive substrate with redox species, good conductivity and catalysis for a better performance of label-free electrochemical biosensor, which was fabricated by one-pot method.

In this work, a conductive catalytic redox hydrogel was prepared by a simple and convenient method as substrate for label-free elec-

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trochemical immunosensor. A novel porous hydrogel was achieved by a feasible strategy using aniline and vinyl ferrocene (Fc) as monomers and phytic acid as gelator. It was simply generated in situ on the glass carbon electrode (GCE). The advantages of the as-prepared redox hydrogel are as follows: (1) the remarkable electronic conducting property [25]; (2) the redox hydrogel with abundant water is helpful for ionic conduction [26]; (3) strong electrochemical signal at 0.04 V (vs Ag/AgCl); (4) excellent catalytic activity toward the oxidation of ascorbic acid for the amplification of peak current [27,28]. (5) the convenient and simple manufacturing process. Then the redox substrate was obtained through electrochemical deposition a gold nanoparticles layer onto the surface of redox hydrogel modified GCE. Gold nanoparticles were used to promote electron transfer, enhance electrical conductivity, and immobilize antibody [29,30]. The immunosensor showed excellent performance, which is expected to be a worthy strategy for detection of PSA.

## 2. Experimental

### 2.1. Materials and reagents

PSA, mouse anti human monoclonal antibody (anti-PSA), neuron specific enolase (NSE), alpha fetoprotein (AFP), carcinoembryonic antigen (CEA) and human immunoglobulin G (IgG) were bought from Linc-Bio Company (Shanghai, China). Disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, albumin from bovine serum (BSA) and ethanol were purchased from Beijing Chemical Reagents Company (Beijing, China). Chloroauric acid, vinyl Fc, ascorbic acid (AA) and D-(+)-glucose were achieved from Alfa Aesar (Tianjin, China). Aniline was bought from Aladdin Industrial Corporation (Shanghai, China). Phytic acid and ammonium persulfate were got from Sigma-Aldrich.

### 2.2. Apparatus

All the electrochemical detections were done on the CHI1140 electrochemical workstation which was obtained from Chenhua Instruments Co. (Shanghai, China). Scanning electron microscopy (SEM) images were got from a Hitachi S-4800 SEM with an energy dispersive X-ray spectrometer (EDS). The vortex instrument was obtained from Shanghai Huxi Analysis Instrument Factory Co. An ordinary three-electrode system with a glassy carbon electrode (working electrode) with 4 mm in diameter, an Ag/AgCl electrode (saturated KCl) (reference electrode) and a platinum wire (auxiliary electrode) was used in this experiment. In the all experiment procedures, the water used was obtained by the Olst ultrapure K8 apparatus with a 18 M $\Omega$ -cm resistivity. X-ray photoelectron spectroscopy (XPS) analysis was finished on an Escalab 250 X-ray Photoelectron Spectroscopy with an Al (mono) K $\alpha$  radiation (ThermoFisher, American).

### 2.3. Preparation of the redox hydrogel

The hydrogel was prepared based on the literature with some modification [31]. Briefly, Solution A: 0.46 mL phytic acid and 1 mL H<sub>2</sub>O were mixed together by ultrasonic for 5 min, then 229  $\mu$ L aniline and 0.015 g vinyl Fc were sequentially injected and sonicated 15 min. Solution B: 0.5 mL H<sub>2</sub>O was used to dissolve 0.143 g ammonium persulfate by ultrasonic for 1 min. After that, solution B was mixed with solution A quickly by vortex for 10 s and the mixture was stored at 4 °C. Finally, the obtained redox hydrogel was purified by dialysis (8000–14,000 molecular weight cut off) for three days to remove oligomers from polymerization and excess crude materials. The prepared hydrogel was dried under vacuum at 65 °C.

### 2.4. Fabrication of the label-free electrochemical immunosensor

The GCE was prepared according to the previous reported work [32,33]. First, 3  $\mu$ L solution B was dropped on the surface of neat GCE, 7  $\mu$ L solution A was also dropped on the surface. Then the electrode was placed in the freezer to polymerize for 10 min for forming a thin homogeneous redox hydrogel film at 4 °C. Subsequently, the modified electrode was immersed in the double-distilled water at 37 °C for 30 min. Finally, a shiny film was obtained on the surface of GCE. The redox hydrogel/Au composite was acquired by electrochemically deposited the gold nano-particles (AuNPs) on the modified GCE by exerting a constant potential of –0.2 V (vs Ag/AgCl) for 20 s in the 0.5 mM HAuCl<sub>4</sub> solution containing 0.1 M KCl. After washing with purified water for three times, 80  $\mu$ L of 200  $\mu$ g mL<sup>–1</sup> anti-PSA solution was spread on the GCE at 4 °C for 12 h. Then 50  $\mu$ L 1% BSA solution was used to block the nonspecific sites. After reacting for 1 h at room temperature, the electrode was washed with purified water for three times and incubated with 80  $\mu$ L of different concentrations of PSA for 1 h at room temperature.

### 2.5. Measurement procedure

The electrochemical measurements were performed in the phosphate buffer saline (PBS) (pH 7.0). A square wave voltammetry (SWV) scan from –0.6 to 0.8 V (vs Ag/AgCl) with pulse amplitude of 25 mV, a frequency of 15 Hz, and quiet time of 2 s was implemented to keep track of the electrochemical responses at 0.04 V (vs Ag/AgCl) for quantitative measurement of PSA.

## 3. Results and discussion

### 3.1. Characterization of the redox hydrogel/Au composites

A reddish-brown hydrogel was synthesized by using aniline and vinyl ferrocene (Fc) as monomers and phytic acid as gelator (Fig. S1). The porous hydrogel (Fig. 1A) can effectively improve the surface area of GCE and highly increase the conductivity. Compared with the redox hydrogel, the redox hydrogel/Au heterostructure was obviously observed (Fig. 1B) after the electrochemical deposition. Energy dispersive X-ray spectrum (EDX) showed the constitutions of the redox hydrogel before and after modified with AuNPs (Figs. 1C and S2). Six major elements were found in the redox hydrogel/Au heterostructure: Fe, Au, C, N, O, and P. Obviously, Au element came from the AuNPs, and Fe, P, C, N and O originated from the redox hydrogel. Besides, the components of redox hydrogel were characterized by XPS. The spectrum showed the major constitutions of the redox hydrogel were Fe, C, N, O, and P (Fig. S3).

### 3.2. Immunoassay using the redox hydrogel/Au composites

The label-free amperometric immunosensor based on the redox hydrogel/AuNPs was developed as clarified in Scheme 1. Firstly, the redox hydrogel was obtained directly in situ by mixing the reactants on the surface of GCE, which can facilitate the electron and ion transferring and enlarge the specific surface area [25,30]. In the process, the redox hydrogel was strongly immobilized on the GCE. Following that, gold nanoparticles were acquired by electrochemically deposited on the modified GCE to immobilize anti-PSA. In addition, gold nanoparticles can enhance electrical conductivity. Then, anti-PSA was directly attached to the Au/redox hydrogel modified electrode. After blocked with BSA, a label-free electrochemical immunosensor for PSA was obtained. Following that, the electrode was incubated in PSA solution and PSA were fixed on the electrode through their specific recognition with anti-PSA. Because PSA as a nonconductor obstructed the electron transfer, the SWV peak currents decreased with the increasing of PSA concentration.

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