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# Enzymatically catalytic deposition of gold nanoparticles by glucose oxidase-functionalized gold nanoprobe for ultrasensitive electrochemical immunoassay

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ABSTRACT

A novel ultrasensitive immunoassay method was developed by combination of the enzymatically catalytic gold deposition with the prepared gold nanoprobe and the gold stripping analysis at an electrochemical chip based immunosensor. The immunosensor was constructed through covalently immobilizing capture antibody at a carbon nanotube (CNT) modified screen-printed carbon electrode. The gold nanoprobe was prepared by loading signal antibody and high-content glucose oxidase (GOD) on the nanocarrier of gold nanorod (Au NR). After sandwich immunoreaction, the GOD-Au NR nanoprobe could be quantitatively captured onto the immunosensor surface and then induce the deposition of gold nanoparticles (Au NPs) via the enzymatically catalytic reaction. Based on the electrochemical stripping analysis of the Au NR nanocarriers and the enzymatically produced Au NPs, sensitive electrochemical signal was obtained for the immunoassay. Both the GOD-induced deposition of Au NPs by the nanoprobe and the sensitive electrochemical stripping analysis on the CNTs based sensing surface greatly amplified the signal response, leading to the ultrahigh sensitivity of this method. Using carcinoembryonic antigen as a model analyte, excellent analytical performance including a wide linear range from 0.01 to 100 ng/mL and a detection limit down to 4.2 pg/mL was obtained. In addition, this immunosensor showed high specificity and satisfactory reproducibility, stability and reliability. The relatively positive detection potential excluded the conventional interference from dissolved oxygen. Thus this electrochemical chip based immunosensing method provided great potentials for practical applications.



## 1. Introduction

Recently, electrochemical immunosensors which combine the unique advantages of the high specificity of immuno-recognition and the cost, small sample consumption and convenient operability of electrochemical sensors have shown great potentials for clinical diagnosis (Fan et al., 2015; Lai et al., 2014; Wan et al., 2013). In order to achieve accurate measurement of low-abundant protein biomarkers for the early disease diagnosis, nanomaterials which possess excellent electroconductivity, good biocompatibility and highly specific area have been widely used in this field for improving the sensitivity of the immunoassay methods (Zhang et al., 2010; Haque et al., 2012; Lei and Ju, 2012; Chikkaveeraiah et al., 2012; Pei et al., 2013). Compared with their conventional applications in the electrode modification for fabricating useful nanosensing surfaces with good bioaffinity and fast electron transfer ability (Zhang et al., 2010; Haque et al., 2012), more attentions are focused on their versatile roles as novel nanocarriers for loading high-content signal labels and designing various useful nanoprobes (Lei and Ju, 2012; Chikkaveeraiah et al., 2012; Pei et al., 2013). When these nanoprobes were used for the signal tracing of immunoassays, the signal response corresponding to each biorecognition event could be greatly amplified, thus leading to the dramatic enhancement of the analytical sensitivity comparing to the conventional single-label methods.

Up to now a great variety of nanomaterials such as carbon nanotubes (CNTs) (Yu et al., 2006; Feng et al., 2014a), gold nanoparticle (Au NPs) (Cui et al., 2008; Gao et al., 2013) and graphene (Du et al., 2011; Jia et al., 2014) have been successfully used to load various electrochemical labels for preparing many useful nanoprobes in immunoassays. Among these nanoprobes, horseradish peroxidase (HRP), which can produce sensitive electrochemical

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signal through enzymatically catalytic reaction, was the most commonly used signal label due to its cheap cost and commercial availability. However, the interference from the dissolved oxygen reduction greatly limited the practical applications of the HRP based electrochemical immunoassay methods (Lai et al., 2012). Compared with HRP biomolecule, the noble-metal nonenzymatic labels such as Ag NPs and Au NPs can be conveniently measured by electrochemical stripping analysis at relatively positive potential range (Leng et al., 2010; Ho et al., 2010; Li et al., 2010), thus completely excluding the conventional dissolved oxygen interference. For example, we previously designed an Ag NPs-functionalized CNTs nanoprobe (Lai et al., 2011a) and an Au NPs-functionalized polydopamine nanoprobe (Lai et al., 2013) and successfully developed two electrochemical immunoassay methods without the dissolved oxygen interference problem, respectively. Additionally, recent researches have shown that some enzyme labels can be also used to induce the deposition of noble-metal nanoparticles via the enzymatically catalytic reaction (Lai et al., 2011b; Zhang et al., 2011). This phenomenon provides the possibility of the development of a novel ultrasensitive signal tracing strategy by combination of the enzymatically catalytic deposition of nanoparticles and their electrochemical stripping analysis.

As a new kind of gold nanomaterial, gold nanorod (Au NR) has recently attracted considerable attentions in the bioassay field (Huang et al., 2009; Vigderman et al., 2012). Owing to its variable length–diameter ratio and large surface area, Au NR could be served as a new nanocarrier for preparing various useful nanoprobes (Sun et al., 2013; Zang et al., 2013; Zhang et al., 2015). Meanwhile, the unique electrochemical property of Au NR also enabled its direct application as a nonenzymatic label and convenient measurement by electrochemical stripping analysis (Lin et al., 2013).

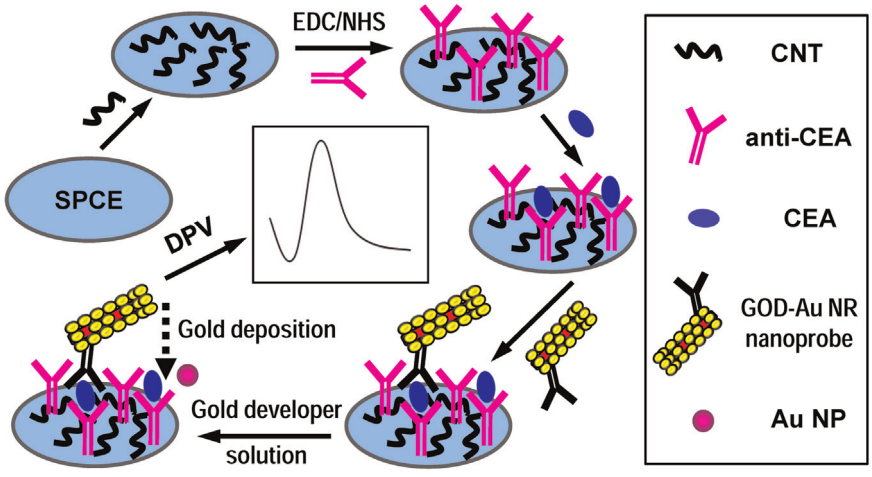
Hence, in this work we prepared a glucose oxidase (GOD)-functionalized Au NR nanoprobe and used it to develop a GOD-catalyzed Au NPs deposition based electrochemical immunoassay method. As shown in Scheme 1, at a CNTs-modified screen-printed carbon electrode (SPCE) based immunosensor, the as-prepared GOD-Au NR nanoprobe could be quantitatively captured onto the immunosensing surface through sandwich immunoreaction. When the gold developer solution containing the glucose substrate, *p*-benzoquinone (PBQ) mediator and gold salt was dropped on the electrode surface in succession, the enzymatically catalytic reaction of GOD on the nanoprobe could make the PBQ mediator to be reduced into hydroquinone, thus leading to the reduction of gold salt into Au NPs deposition on the electrode surface (Zhang et al., 2011). Based on the electrochemical stripping analysis of the captured Au NRs and the enzymatically produced Au NPs, sensitive electrochemical signal could be obtained for the quantitative measurement of the protein analyte of carcinoembryonic antigen (CEA). Because both the enzymatically catalytic reaction of the GOD-Au NR nanoprobe and the sensitive electrochemical stripping analysis at the CNTs based immunosensor greatly improved the signal response of immunoassay, ultrahigh sensitivity of the method was achieved for the accurate measurement of the low-abundant protein analyte. In addition, the relatively positive detection potential of the gold stripping analysis also excluded the conventional dissolved oxygen interference completely.

## 2. Materials and methods

### 2.1. Materials and reagents

CEA and monoclonal rabbit anti-CEA antibodies (clone nos. F13051304 and F14010405) were purchased from Xiamen Boson Biotechnology Ltd. Human IgG (HIgG) and mouse IgG (MIgG) were purchased from Wuhan Boster Biologcial Technology Ltd. Glucose oxidase (GOD, from *Aspergillus niger*), bovine serum albumin (BSA), human serum albumin (HSA), cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$), ascorbic acid, 4-morpholineethanesulfonic acid (MES), poly(styrenesulfonate) (PSS), *p*-benzoquinone (PBQ), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinmide (NHS) were obtained from Sigma-Aldrich Chemical Co. Chloroauric acid ($HAuCl_4 \cdot 3H_2O$), glucose and silver nitrate were purchased from Shanghai Reagent Company. Multi-walled carbon nanotubes (CNTs, diameter 10–20 nm and length 1–2 μm) were purchased from Shenzhen Nanoport Ltd. The clinical serum samples were obtained from The Second Hospital of Huangshi. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments.

Phosphate-buffered saline (PBS) solutions at various pH values were prepared by mixing the stock solutions of 50 mM $NaH_2PO_4$ and $Na_2HPO_4$. A 50 mM pH 7.4 PBS containing 0.05% (w/v) Tween-20 (PBST) was used as washing buffer. A 50 mM pH 7.4 Tris–glycine buffer containing 2% BSA was used as blocking solution. In addition, a 50 mM pH 6.0 MES buffer was prepared for the EDC/NHS activation reaction.




**Scheme 1.** Schematic representation of the immunosensor preparation and the sandwich immunoassay based on the signal tracing of the GOD-functionalized Au NR nanoprobe.