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Shell-encoded Au nanoparticles with tunable electroactivity for specific dual disease biomarkers detection

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ABSTRACT

The exploration of electroactive labelling with tailorable and strong differential pulse voltammetry (DPV) responses is of great importance in accurate and sensitive screening of a panel of biomarkers related to cancer. Herein, shell-encoded gold nanoparticles (Au NPs) are fabricated and give rise to shell species-dominated DPV peak potentials. Two independent DPV peaks appear at −0.08 V for Au@Cu_2O core-shell NPs and 0.26 V for Au@Ag core-shell NPs. Shell-encoded Au NPs drastically exhibit shell thickness-tunable amplified peak currents. The non-interfering and amplified DPV responses enable shell-encoded Au NPs to be an alternative electrochemical signal amplifier for dual screening of carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP). The limits of detection (LODs) are calculated to be 1.8 pg/mL for CEA and 0.3 pg/mL for AFP. In comparison to the parallel single-analyte assays, shell-encoded Au NPs engineered electrochemical aptasensors offer multiplexing capability and show significant prospects in biomedical research and early diagnosis of diseases.

1. Introduction

Multiplex and strong electroactive labelling is significant for the fabrication of a portable fast and sensitive electrochemical sensor for the point-of-care testing of multiple targets. Currently, most electroactive labelling-driven electrochemical sensors are established depending on the electroactive organic compounds, metal salts and enzymes (Chen et al., 2013; Hu et al., 2014; Li et al., 2016; Lin et al., 2011; Wang et al., 2014). However, it is very difficult to enhance the electroactivity for a single compound without loading them on the extra supporters (e.g. graphene nanocomposites, mesoporous silica and platinum porous NPs. *etc*) (Chen et al., 2013; Zhou et al., 2016). The loading effects and the properties of supporters, as well as the cross-talk interference would further influence the electrochemical performances of electroactive labelling (Song et al., 2014). Alternatively, a family of semiconductor nanocrystals and metal nanomaterials featured with large surface area-to-volume ratios exhibit instinctive and efficient electrochemical responses (Fang et al., 2015; Kokkinos et al., 2016, 2013; Liu et al., 2004; Song et al., 2014; Wan et al., 2014; Wang et al., 2015; Zhao et al., 2015; Zhu et al., 2016). However, their application still faces inevitable challenges: 1) The fabrication of semiconductor nanocrystals faces complication in the preparation process and the misgivings of collection in its practical applications (Song et al., 2014); 2) The electrochemical signals of semiconductor nanocrystals are generated replying on harsh strong acidic conditions (Kokkinos et al., 2016, 2013); 3) The poor regulation for electrochemical peak potentials and electroactivity limits the accurate and sensitive multi-analyte detection. Tremendous efforts have been devoted to the rational synthesizing of various NPs with distinct sizes in order to adjust the electrochemical performances, but this requires different harsh synthesis methods. And the size-dependent electrochemical responses of the aforementioned NPs restrict the production of strong DPVs. Given these limitations, the exploration of tailored electroactive metal NPs with reinforced electrochemical reactivity and convenient storage and treatment, enables the sensitive and multiple identification of targets.

Excellent electroactive NPs should be easily oxidized for the production of narrow and obvious DPV peaks. Contradictorily, the easy oxidization would reduce the stability of NPs. One of the most popular strategies is to develop shell-driven core-shell NPs as electro-

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active labels, in order to amplify the electrochemical responses and enhance the stability of NPs. It was reported that shell-deposited core-shell NPs exhibited intriguing local electromagnetic field, and thus synergistically produced amplified chirality, surface enhanced raman spectroscopy (SERS) signal and catalytic performances (Huang et al., 2015; Lu et al., 2016; Pang et al., 2015; Sun et al., 2015; Zhao et al., 2014b, 2014c, 2016, 2014d). Nevertheless, little attention is paid to exploit the shell-engineered electrochemical responses of metal NPs for the multiple detection application. Detailed and quantitative understanding of shell species-dominated peak potentials and shell thickness-driven peak intensity is urgently needed to make use of the advantages of both components, and holds promise for exploration the fascinating multifunctionalities of heterostructures.

The selection of narrow-band gap nanoshells can effectively improve electrical properties of composites. Attractively, Cu_2O exhibits a small band gap of 2.17 eV, and Ag has even stronger plasmon resonances, and higher electrical and thermal conductivity (Lai et al., 2011; Liu et al., 2012; Xiong et al., 2014). Au NP core acting as electron traps accelerates the electron transfer and introduces efficient interfacial charge separation in the composites. Rationally understanding the electrochemical performances of shell-encoded Au NPs, involving Au@Cu_2O core-shell NPs and Au@Ag core-shell NPs, provides the keystone for the fundamental investigation of the electron transfer process and the rational design of hybrid electroactive labels for the fabrication of multiple electrochemical sensors.

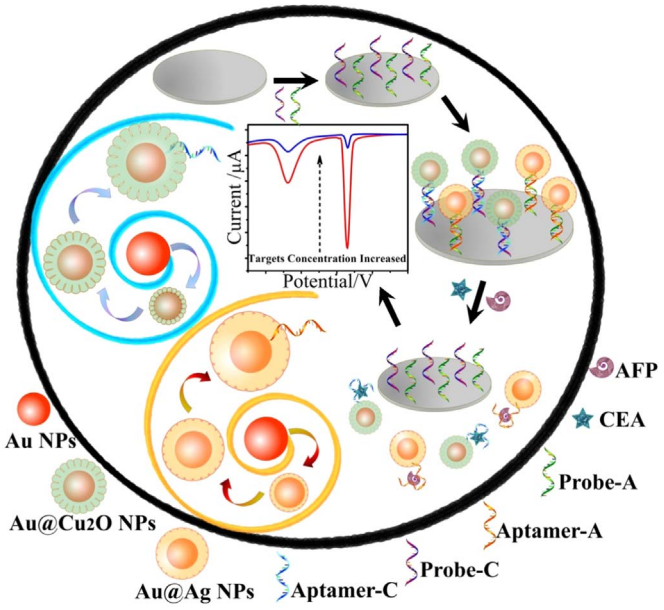
The explosive development of electrochemical aptasensors employing tailored electroactive labelling opens new horizons for the fabrication of reliable sensors for the detection of multiplex disease biomarkers. Since most cancer diseases are associated with the presence of more than one biomarker, the development of simultaneous measurement of co-existing biomarkers is beneficial to improving the detection accuracy and provides more precise information on prognostic and treatment (Chikkaveeraiah et al., 2012; Guo et al., 2016; Wu and Qu, 2015; Xu et al., 2015). In particular, CEA is one of the most extensively used clinical tumor markers, and AFP is an important tumor marker employed for the early diagnosis of the patients with liver cancer (Hu et al., 2015; Pang et al., 2015). Simultaneous detection of the trace amounts of CEA and AFP in biological samples could accurately predict liver cancer.

In this manuscript, shell-encoded Au NPs, involving Au@Cu_2O core-shell NPs and Au@Ag core-shell NPs, were fabricated by the deposition of different amounts of Cu and Ag precursors. Cu_2O shell and Ag shell encoded Au NPs exhibited non-interfering DPV responses at −0.08 V and 0.26 V, respectively. The currents were largely amplified with the increasing thickness of shells. The adoption of shell-encoded Au@Cu_2O core-shell NPs and Au@Ag core-shell NPs as two novel electrochemical signal amplifiers could dramatically improve the performance of electrochemical aptasensors (Scheme 1). Shell-encoded Au NPs engineered electrochemical aptasensors offer multiplexing capability and demonstrate exquisite specificity owing to high affinity and specificity of aptamers against the targets. We envision that clinical translation of this assay may further enable asymptomatic surveillance of cancer survivors and speedy assessment of patient condition through a simple blood test.

2. Experimental section

2.1. Chemical reagents and materials

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$), sodium citrate, PVP, cupric nitrate ($Cu(NO_3)_2$), Hydrazine hydrate ($N_2H_4{\cdot}H_2O$, 85% w/w), ascorbic acid, silver nitrate ($AgNO_3$), 4-aminobenzenesulfonic acid, L-cysteine, glutathione, arginine, histidine, valine, tryptophan and lysine were all obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. CEA was purchased from Elabscience Biotechnology Co., Ltd. AFP, PSA, thrombin and human Ig G were purchased from Sigma-Aldrich. Aptamers and complementary DNA fragments purified by HPLC were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Table S1) (Huang et al., 2012; Zhou et al., 2015b, 2014). All reagents were of analytical grade and were used without further purification. All solutions were prepared with deionized water (18.2 MΩ) obtained from a Milli-Q water purifying system.



Scheme 1. Schematic illustration of shell-encoded Au NPs with tunable electroactivity for specific dual disease biomarkers detection.

2.2. Synthesis of shell-encoded Au@Cu_2O core-shell NPs

Au NPs with average sizes of 18.2 ± 1.6 nm were synthesized according to our previous reported methods (Fig. S1a) (Zhao et al., 2013). Shell-encoded Au@Cu_2O core-shell NPs were obtained by depositing $Cu(NO_3)_2$ solution on the surface of Au NPs (Zhang et al., 2011). First, an amount of 7.75 g PVP was dispersed in 96.875 mL 40 mM $Cu(NO_3)_2$ solution. An aliquot of 2 mL 2 nM Au NPs were added into the as-prepared $Cu(NO_3)_2$-PVP solution under vigorous stirring, followed by immediate introduction of $N_2H_4{\cdot}H_2O$ solutions (35%, w/w). The reaction solution was stirred at room temperature. After 2 min, Au@Cu_2O core-shell NPs were obtained through centrifugation and washed by ultrapure water for three times. The preparation of shell-encoded Au@Cu_2O core-shell NPs required the precise controllable of the amount of $Cu(NO_3)_2$-PVP solution and $N_2H_4{\cdot}H_2O$ solution. When 3.125 mL, 6.25 mL, 12.5 mL, 25 mL, 50 mL, 100 mL $Cu(NO_3)_2$-PVP solution and 4.25 μL, 8.5 μL, 17 μL, 34 μL, 68 μL, 136 μL $N_2H_4{\cdot}H_2O$ solutions were respectively used, shell-encoded Au@Cu_2O core-shell NPs named as Au@$Cu_2O_{(1)}$ NPs, Au@$Cu_2O_{(2)}$ NPs, Au@$Cu_2O_{(3)}$ NPs, Au@$Cu_2O_{(4)}$ NPs, Au@$Cu_2O_{(5)}$ NPs and Au@$Cu_2O_{(6)}$ NPs were obtained.

2.3. Synthesis of shell-encoded Au@Ag core-shell NPs

Au@Ag core-shell NPs were obtained by depositing $AgNO_3$ solution on the surface of Au NPs (Zhao et al., 2014b, 2014c). First, an aliquot of 4 mL 0.1 M PBS solution and 2 mL PVP solution (1%, w/w) were mixed together. An amount of 2 mL 2 nM Au NPs and 1 mL 0.1 M ascorbic acid were added and mixed evenly. And then, 3 mM $AgNO_3$ solution was added into the above solution, and the mixture solution was shaken for 3 h under dark conditions at room temperature. Au@Ag core-shell NPs were obtained through centrifugation and washed by ultrapure water for three times. The preparation of shell-encoded Au@Ag core-shell NPs required the precise controllable of the amount of