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# Signal amplification architecture for electrochemical aptasensor based on network-like thiocyanuric acid/gold nanoparticle/ssDNA

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ABSTRACT

In this work, we described signal amplification architecture for electronic aptamer-based sensor (E-AB), which is applicable to a wide range of aptamers. Herein, we only take lysozyme as the representative sensing target. The amplification method was based on the network of thiocyanuric acid (TCA)/gold nanoparticles (AuNPs) modified with ssDNA. The binding event can be detected by a decrease in the integrated charge of the surface-bound $[Ru(NH_3)_6]^{3+}$ which electrostatically absorbed onto the negatively charged phosphate backbones of DNA. In the presence of target molecules, a large amount of TCA/AuNP/ssDNA network associated with $[Ru(NH_3)_6]^{3+}$ would be removed from the electrode surface, leading to a significant decrease of redox current. Cyclic voltammetry (CV) signals of $[Ru(NH_3)_6]^{3+}$ provides quantitative measures of the concentrations of lysozyme, with a linear calibration ranging from 5 pM to 1 nM and a detection limit is 0.1 pM. The detection limit of the proposed sensor is one order of magnitude and three orders of magnitude more sensitive than the detection limits in the absence of TCA (5 pM) and in the absence of TCA/AuNP/ssDNA network (0.5 nM). This amplification method is promising for broad potential application in clinic assay and various protein analysis.



## 1. Introduction

Aptamers (short nucleic acid-binding species) can bind to a broad range of specific targets (Famulok et al., 2007; Tombelli and Mascini, 2009; Liu et al., 2009; Sefah et al., 2009) with an affinity and a specificity comparable to that of antibodies, making them promising candidates for bio-detection applications (Tombelli et al., 2005). A variety of aptamer-based biomolecules recognition and detection have been developed in connection to fluorescence (Chang et al., 2010), surface-enhanced Raman spectroscopy (Cho et al., 2008), microgravimetric (Minunni et al., 2004), and electrochemistry (Baker et al., 2006; Zuo et al., 2007; Jin et al., 2007; Zayats et al., 2006; Willner and Zayats, 2007), and so on. Among these methods, electrochemical methods have attracted most attention in the development of aptasensors because of their high sensitivity, simple instrumentation, low production cost, fast response, and portability. Recently, electrochemical aptamer-based (E-AB) sensors have emerged as a promising and versatile new biosensor platform. Heeger, Plaxco, and others developed a series of novel E-AB sensors for various target molecules (Xiao et al., 2005; Baker et al., 2006; Radi et al., 2006). Xiao et al. (2005) developed a potentially universal E-AB sensor for thrombin by using target-induced strand displacement ; Fan et al. report a target-responsive electrochemical aptamer switch (TREAS), which is a signal-on sensor featuring both generalizability and simplicity in design, toward reagentless detection of adenosine triphosphate (ATP) (Zuo et al., 2007). All these E-AB sensors are based on binding-induced conformational changes of redox-tagged and surface-confined aptamers, which have proven to be highly sensitive and selective.

To further improve the sensitivity of E-AB sensors, signal amplification method is very useful or even necessary for the protein detection at low levels (Laios et al., 2001; Zhang et al., 2003). During the past decade, oligonucleotide-functionalized gold nanoparticles (AuNPs) have been employed as amplification tags for novel biosensing protocols due to their ease of bioconjugation with biomolecules and their excellent electrochemical properties (Brakmann, 2004; Niemeyer, 2001). It has been reported that each Au nanoparticle can be loaded with approximately a hundred or so DNA strands (Thaxton et al., 2005; Zhang et al., 2006). Shen et al. (2008) developed an electrochemical DNAzyme sensor for sensitive detection of $Pb^{2+}$ detection, taking advantage of catalytic reactions of a DNAzyme upon its binding to $Pb^{2+}$ and the amplification effect of DNA-Au bio-bar codes, where $[Ru(NH_3)_6]^{3+}$ was selected as electrical signal readout ). Recently, we reported aptamer-based electrochemical approach to the detection of thrombin, lysozyme, and L-histidine based on gold nanoparticles (Li et al.,

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2010a, b; Li et al., 2011). In order to enhance the sensitivity of the aptasensor, gold nanoparticles were used to immobilize more anti-thrombin DNA aptamers on the electrode surface via self-assembly chemistry. However, these methods suffer the disadvantages of time-consuming assay procedures and relatively low sensitivity.

Herein, based on E-AB sensing mechanisms, we proposed a label-free, electrochemical approach for the detection of lysozyme. Since altered blood serum lysozyme levels have been detected in patients with leukemia (Pascual et al., 1973), kidney problems (Porstmann et al., 1989) and breast cancer (Serra et al., 2002), lysozyme serves as an ideal and representative target due to pressing needs for its rapid detection in clinical settings. In this paper, the most important consideration is signal enhancement. This was accomplished firstly by using 15 nm AuNP as carriers to load large amounts of ssDNA (average loading ca. 80 aptamer units per particle), and then by the addition of thiocyanuric acid (TCA), a star-shaped trithiol molecule, which readily captures more AuNPs and forms a TCA/AuNP network through strong interactions between AuNPs and the three thiol groups of each TCA molecule. The fabrication steps of the sensor are shown in Fig. 1. Firstly, AuNP modified with 5′–SH 15-mer ssDNA (ssDNAI) linked up together by TCA to form a TCA/AuNP/ssDNA network (TCA/AuNP/ssDNAI), then, a 5′-thiol-modified 42-base anti-lysozyme aptamer (ssDNAII) was immobilized onto the electrode surface via gold-sulfur affinity. It is sufficiently long to extend above the 6-mercaptohexanol (MCH) layer for complete hybridization to the TCA/AuNP/ssDNAI through complementary bonds. As usual, $[Ru(NH_3)_6]^{3+}$ could be bound electrostatically to the anionic DNA backbones. The binding lysozyme can be detected by a significant decrease in the integrated charge of the surface-bound $[Ru(NH_3)_6]^{3+}$ due to the removing of whole TCA/AuNP/ssDNAI network, which associated with a large number of $[Ru(NH_3)_6]^{3+}$. Cyclic voltammetry (CV) signals of $[Ru(NH_3)_6]^{3+}$ provide quantitative measures of the concentrations of lysozyme. This novel amplification protocol by TCA/AuNP/ssDNA network provides a very efficient way to improve the sensitivity, and may offer a promising approach for simple, rapid and sensitive detection of other proteins and small molecules.

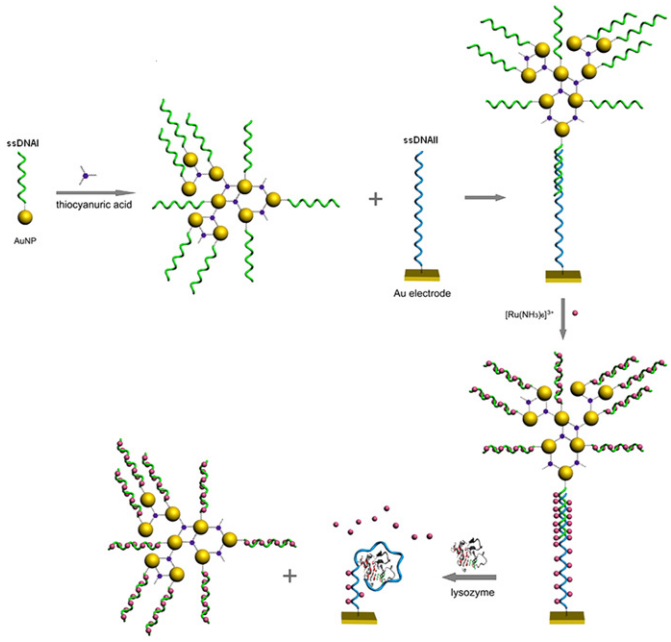


**Fig. 1.** Schematic diagram showing biosensor fabrication and sensing mechanism.

## 2. Experimental

### 2.1. Apparatus and reagents

Electrochemical measurements were performed using CV with an electrochemical analyzer (CHI 660C, CH Instruments). All experiments were performed using a conventional three-electrode system with a fabricated aptasensor or bare gold (diameter, 2 mm) as the working electrode, Ag/AgCl (sat. KCl) as the reference electrode, and a platinum counter electrode. All potentials were referred to the reference electrode. All measurements were carried out in immobilization buffer (I-B: 20 mM Tris-HCl, pH 7.41 with 140 mM NaCl, 20 mM $MgCl_2$ and 20 mM KCl).

Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from the Alfa Aesar (Tianjing) company. Tris-base was purchased from Sigma-Aldrich. DNA oligonucleotides (I) and (II) were synthesized and purified by Takara Inc. (Dalian, China). The sequences of the employed oligomers are as follows:

ssDNAI:5′-SH-$(CH_2)_6$-CTA AGT AAC TCT GCA-3′

ssDNAII:5′-SH-$(CH_2)_6$-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′

### 2.2. Preparation of AuNPs and TCA/AuNP/ssDNAI network

The AuNPs were prepared by citrate reduction of $HAuCl_4$ with sodium citrate according to the literature (Grabar et al., 1995). In brief, 150 mL of 1 mM $HAuCl_4$ was heated to reflux while stirring. Rapid addition of 15 mL of 38.8 mM sodium citrate to the vortex of the solution resulted in a change in solution color from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min, and allowed to cool to room temperature. The prepared Au colloidal nanoparticles were characterized by TEM (transmission electron microscopy) and UV–vis spectrometry (Fig. S1). For AuNP with an average diameter of ca. 15 nm, the UV absorption peak of the AuNP colloid appears at approx. 520 nm, consistent with the absorption of the AuNPs of this size.

The ssDNAI/AuNP conjugates were prepared according to the literature (Ighanian et al., 1997). Briefly, 200 μl of the aqueous AuNP solution (~10 nM) was mixed with 1 μM mercapto-ssDNAI for 16 h, and the solution was centrifuged for 30 min at 12000 rpm to remove the excess oligonucleotides. The red oily ssDNAI/AuNP precipitate was washed with 10 mM Tris buffer (pH 7.41) and then re-dispersed in 20 mM Tris buffer (pH 7.41). The resulting ssDNAI/AuNP conjugates were mixed with 0.1 μM TCA solution for 16 h, and the solution was centrifuged for 30 min at 12000 rpm to remove unbound TCA, forming TCA/AuNP/ssDNAI network.

### 2.3. Sensor preparation

The gold electrode (2 mm in diameter) was polished with 1.0 μm, then 0.3 μm, and finally 0.05 μm alumina powders, and sonicated, first in ethanol, then deionized water (each for 2 min). Afterwards, the electrode was subsequently voltammetrically cycled in 0.5 M $H_2SO_4$ with the potential between −0.2 and +1.5 V at 0.1 V $s^{-1}$ until a representative cyclic voltammogram of a clean gold electrode was obtained.

The cleaned electrode was immersed in a solution composed of 1 μM anti-lysozyme aptamer (ssDNAII), immobilization buffer (I-B: 20 mM Tris-HCl, pH 7.41 with 140 mM NaCl, 20 mM $MgCl_2$ and 20 mM KCl), and 1 mM Tris (2-carboxyethyl) phosphinehydrochloride (TCEP) for 90 min at 37 °C. The surface was then passivated with 1 mM 6-mercaptohexanol (MCH) for 30 min to obtain well-aligned DNA monolayers. The resulting ssDNAII monolayer functionalized surface was treated with 5 μM TCA/AuNP/ssDNAI