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# An electrochemical molecular recognition-based aptasensor for multiple protein detection

Lin Cheng, Jie Zhang, Yan Lin, Qiong Wang, XiuXiu Zhang, YanHua Ding, Hanfeng Cui*, Hao Fan*

*Department of Pharmacy, JiangXi University of Traditional Chinese Medicine, JiangXi 330004, People's Republic of China*

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ABSTRACT

This article reports a simple electrochemical approach for the detection of multiple proteins (thrombin and lysozyme) using Dabcyl-labeled aptamer modified metal nanoparticles (DLAPs). DLAPs were immobilized on β-cyclodextrins (β-CDs) modified electrode by means of host–guest self-assembly. During the time of detection, the aptamers' structure will change due to the specific binding with corresponding proteins that forced DLAPs far away from the electrode that had been modified by β-CDs. Thus, the capture of target proteins onto DLAPs was translated via the electrochemical current signal offered by metal nanoparticles. Linearity of the aptasensor for quantitative measurements was demonstrated. Determinations of proteins in human real serum samples were also performed to demonstrate detection in real clinical samples.



In cells, many biological complexes are composed of more than two proteins. Among these are DNA replication forks, RNA transcription complexes, ribosomes, proteosomes, histones, T cells, B cells, NKT cell antigen receptor complexes, cytoskeletal focal adhesion complexes, nucleotide excision repair complexes, RNA splicing complexes, and ASTRC that modifies RNA stability during the activation of immunocytes. In clinical diagnosis, simultaneous multi-analyte assays are promising analytical method for their intrinsic advantages such as short analytical time, small sample volume, high test efficiency, and low cost compared with parallel single-analyte assays [1,2].

Recently, many researchers have focused on simultaneous detection multi-analyte methods based on the identical sensing interface using multiple labels [3–5]. For example, Wang and coworkers [6] reported highly sensitive and selective simultaneous bioelectronics detection of thrombin and lysozyme by the coupling of CdS and PbS quantum dot immobilization of the corresponding aptamers. Tang and coworkers [7] used a magnetic graphene-based immunosensing probe and distinguishable signal tags (HRP–thionine and HRP–ferrocene conjugates) as tracers for simultaneous detection of α-fetoprotein and carcinoembryonic antigen. Most of the multiple electrochemical protein sensors reported until now also require multiple probe DNA pre-immobilization on the solid substrates surface or the subsequent addition of a label. These methods need to have multi-step operation and specific binds for many times, and all of these will lead to time-consuming problems in building complexes. So, it is necessary to develop multiple electrochemical protein sensors with simple structure and easy operation.

In our previous work [8,9], we investigated the DNA and protein detection signal analyte methods using molecular recognition technology. Inspired by this, here we attempted to construct multiple electrochemical protein sensors with simple structure and easy operation on the basis of Dabcyl-labeled aptamer modified metal nanoparticles (DLAPs).

Aptamers are factitious DNA or RNA ligands evolved by an in vitro selection technique called SELEX (systematic evolution of ligands by exponential enrichment) [10] that can specifically bind to a variety of targets [11]. Aptamers as a kind of novel molecules for the use of recognition were first suggested by Gold and Szostak [12,13]. Compared with antibodies, aptamers have more advantages such as chemical synthesis, easy modification, high stability,

*Abbreviations:* DLAP, Dabcyl-labeled aptamer modified metal nanoparticle; CD, cyclodextrin; TEM, transmission electron microscope; ESI, electrospray ionization; MS, mass spectrometry; BSA, bovine serum albumin; IgG, immunoglobulin G; DMF, dimethylformamide; PBS, phosphate-buffered saline; DPV, differential pulse voltammetry; RSD, relative standard deviation.

* Corresponding authors.
*E-mail addresses:* cuihanfeng@126.com (H. Cui), fanhao11@aliyun.com (H. Fan).

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target versatility, easy to stock, and resistant to denaturation and degradation [14]. Due to these advantages, aptamers have attracted more and more attention. To date, more than 200 aptamers have been generated against various targets ranging from small molecules to live cells such as metal ions [15–17], organic dyes [18], amino acids [19], nucleotides [20], and cells [21]. With the development of research, aptamers have become applied more broadly in the fields of diagnostics, therapeutics, research, separation, and bioanalysis [22,23]. Aptamers for detection have been developed on the basis of different technologies such as fluorescence [24,25], surface plasmon resonance spectroscopy [26], microgravimetry [27], quartz crystal microbalance [28], and electrochemistry [29].

Molecular recognition technology, defined as the supramolecular noncovalent interaction between the "host" and "guest" molecules, has played an important role in the chemical sensing field such as the supramolecular sensor chip for metal ion detection [30] and fluorescent DNA detection [31]. Cyclodextrins (CDs) are one kind of special oligosaccharides that consist of six, seven, or even eight glucose units (named a-, β-, or g-CD, respectively) and are characterized by a toroidal form with a hydrophobic inner cavity and a hydrophilic outer side. Their unique "cage" structure endows CDs and their derivatives with outstanding recognition and encapsulation abilities to their guest molecules, and consequently they have been employed in organic chemistry, electrochemistry, and the pharmaceutical field as the host molecule. Recently, CDs modified electrodes as selective electrodes were also applied to recognize organic molecules such as thioridazine and aminobiphenyl [32–35].

Here, we used the host–guest recognition technique and specific aptamer–protein interaction to construct DLAPs for multiple protein detection. As Fig. 1 shows, $DLAP_1$ was designed based on Dabcyl-labeled anti-thrombin aptamer F1 probe. Its 5′ terminal was labeled with CdS nanoparticles and used as the electrochemical signal-producing marker. Its 3′ terminal had been labeled with one Dabcyl molecule, which is β-CD's typical guest and would be captured by β-CDs modified electrode during the sensor construct procedure. The PdS nanoparticles were conjugated with anti-lysozyme aptamer to construct $DLAP_2$. DLAPs were immobilized on β-CDs modified electrode by means of host–guest self-assembly.

During multiple protein detection, conformational change of the aptamers occurred due to the special bind with corresponding protein and forced DLAPs far away from the electrode surface; thus, the capture of target protein onto DLAPs was translated via the electrochemical current signal offered by released nanoparticles. Compared with previous methods using metal nanoparticles as label, the functional metal nanoparticles play a role of target recognition and signal provider, which makes this current method display simple-step detection and sensitivity. Therefore, this electrochemical aptasensor is expected to have deeply wide applications in multiple protein monitoring and disease diagnosis.

## Materials and methods

### *Apparatus*

A transmission electron microscope (TEM, JEOL, 2000FX, Japan) and a nuclear magnetic resonance spectrometer (Bruker, 400 MHz, Switzerland) were used. High-resolution mass spectral analyses (HRMS) were carried out using electrospray ionization time-of-flight mass spectrometry (ESI–TOF–MS) resources. All voltammetric experiments were performed using a CHI 660 electrochemical analyzer (CHI Instruments, USA). Electrochemical experiments were carried out in a 3-ml electrochemical cell using three-electrode configurations at room temperature (25 °C). A platinum wire served as a counter electrode, and an Ag/AgCl served as reference electrode with saturated KCl solution. An Au electrode with 2 mm diameter was modified with β-CD and used as working electrode. A mercury film electrode was prepared for metal ion detection, which was fabricated by applying a potential of −1.10 V to a polished glassy carbon electrode with 1 mm diameter in a 0.1 M HCl solution containing 100 mg/L $Hg^{2+}$ for 10 min.

### *Chemicals and materials*

The β-cyclodextrins were purchased from Wacker Chemical. Thrombin, lysozyme, bovine serum albumin (BSA), and immunoglobulin G (IgG) were purchased from Dingguo Biotechnology (Shanghai, China). Nitric acid, cadmium chloride, sodium hydroxide, Tris·HCl buffer (20 mM, pH 7.4), sodium acetate buffer (0.1 M, pH 5.3), and other reagents were commercially available and of analytical reagent grade (Dingguo Biotechnology). All oligonucleotides were purchased form Sangon Biotechnology (Shanghai, China), and sequences of all oligonucleotides are listed as follows: anti-lysozyme aptamer, 5′-HS-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACTTAG-Dabcyl-3'; anti-thrombin aptamer, 5′-HS-GTC CGT GGT AGG GCA GGT TGG GGT GAC-Dabcyl-3'.

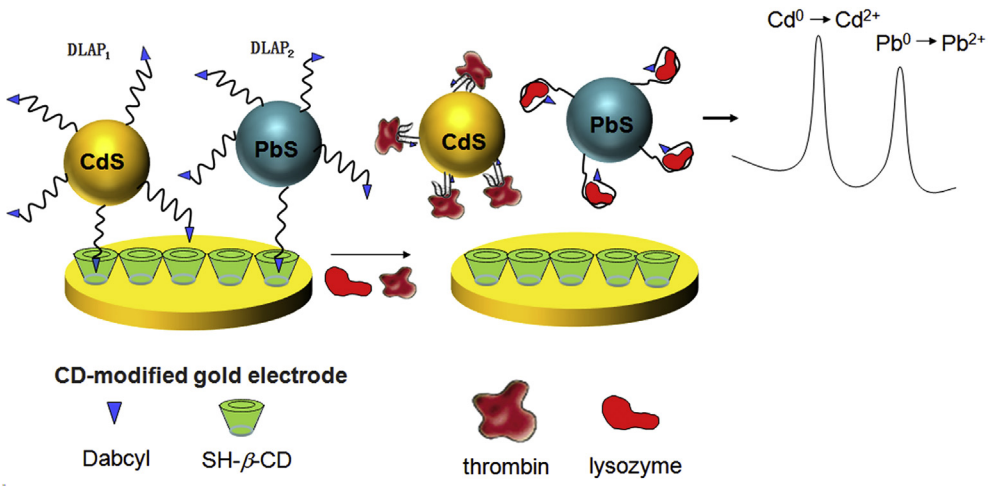


**Fig.1.** Schematic illustration of the electrochemical protein sensing principle.