



# Potentiometric aptasensing of small molecules based on surface charge change

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## ABSTRACT

Solid-state potentiometric sensors based on surface charge change are well-established tools for measuring ions and biological species. However, their use for detection of small molecules with high sensitivity and good selectivity is still elusive. In this work, a novel potentiometric aptasensing platform for small molecules is presented, using bisphenol A (BPA) as a model. The proposed sensor can be prepared by layer-by-layer assembling of carboxylated multiwall carbon nanotubes, poly(diallyldimethylammonium chloride) (polycation), and aptamer (polyanion) on the electrode surface. The presence of BPA induces the conformational change and detachment of the aptamer at the surface of the modified electrode, which leads to a variation of the surface charge (negative to positive) and therefore a potential change. The introduction of polyions can cause substantial charge change on the electrode surface, thus improving the sensitivity of the sensor. The morphology and electron-transfer properties of the electrode have been characterized. Under optimum conditions, the present sensor shows a stable response to BPA in the concentration range from  $3.2 \times 10^{-8}$  to  $1.0 \times 10^{-6}$  M with a detection limit of  $1.0 \times 10^{-8}$  M. The proposed methodology can be used for sensitive potentiometric sensing of other small molecules involved in aptamer/target binding events.

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## 1. Introduction

Potentiometric sensors based on solvent polymeric membrane ion-selective electrodes (ISEs) were developed in the 1970s [1]. In recent years, they have been improved in terms of sensitivity, selectivity, reversibility, and reliability [2–4]. With the introduction of various synthetic receptors, new materials, and deeper theoretical models [5–8], potentiometry based on solvent polymeric membrane ISEs has become a well-established tool for ion analysis. In parallel, researchers have designed solid-state potentiometric sensors, which are tailored with recognition elements on the surfaces of the electrodes. In these solid-state sensors, the target-induced adsorption, desorption, or uptake of ions or charged molecules on the surfaces of electrodes can change the surface potentials of the electrodes [9–12].

Early researches on solid-state sensors focused mainly on the detection of ions such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  by using the precipitate-based electrodes [10,13]. The introduction of nanomaterials and

novel recognition molecules was a key turning point in this field. In 2012, Michalska et al. [14] developed a potentiometric sensor based on the dithizone modified gold nanoparticle film for the determination of copper ions. In this method, the cations diffuse into the gold nanoparticle layer and interact with dithizone to produce the surface charge change. Similarly, a solid-state potentiometric sensor with improved selectivity for copper ions was designed using an ion-selective gold–thiol film [15].

Solid-state potentiometric sensors for macromolecules and whole cells have also been developed. In 1975, Janata developed an immunoelectrode based on surface recognition [16]. The target antigen with net electrical charges could induce a charge change on the surface, along with a subsequent potential change. However, the low efficiency of the immobilization procedures restrict its wide applications. Subsequently, research in this area encountered a bottleneck until the introduction of novel materials and the optimization of the modification processes. In 2009, Rius et al. [17] developed a potentiometric aptasensor using aptamer functionalized single-walled carbon nanotubes as a transducing material for immediate detection of living bacteria. Although the sensor could detect bacteria rapidly, the potential changes are small, even with the introduction of dual aptamers [18]. So far, none solid-state

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potentiometric sensors have been developed for sensitive detection of small molecules.

In this paper, a novel potentiometric aptasensing platform for small molecules based on surface charge change is proposed. Bisphenol A (BPA), which is an endocrine disrupter, was selected as a model. An aptamer with excellent selectivity and high binding affinity to BPA is used as a recognition element [19]. In recent years, a number of aptasensors for BPA based on optical, electrochemical, and mass-sensitive transductions have been developed [20,21]. Among these methods, electrochemical aptasensors have been received considerable attention due to their advantages of rapid response, ease of use, and low cost. As one kind of simple, field-portable, and widespread electrochemical methodology, potentiometric sensors have been adopted as promising transducers for aptasensing. In our previous research, a potentiometric aptasensing strategy based on DNA nanostructures has been designed for the detection of BPA with high sensitivity [22]. However, our previous system for BPA needs additional signal reporter of protamine, thus adding more complexity. Herein, a simple and direct potentiometric aptasensing platform is designed via a layer-by-layer technique. For the present work, the charged materials, carboxylated multiwall carbon nanotubes (CNTs), poly(diallyldimethylammonium chloride) (PDDA, polycation), and the aptamer (polyanion), are immobilized layer-by-layer on the electrode surface. The target-binding induced release of the aptamer from the surface of the electrodes causes a charge distribution change and subsequently a potential change. It is anticipated that the introduction of an aptamer for the specific target allows the sensing methodology with high generality and good selectivity.

## 2. Experimental section

### 2.1. Materials

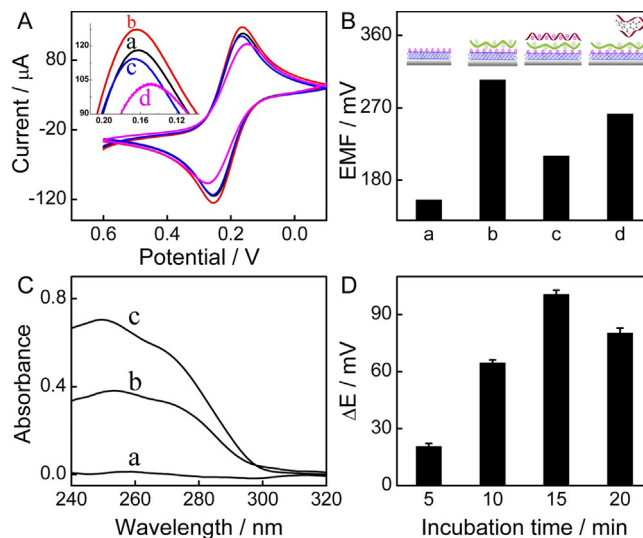
An aptamer with a dissociation constant of 8.3 nM toward BPA was used [19]. The sequence was: 5'-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA-3'. More information of the materials is available in the Supporting Information.

### 2.2. Electrode preparation

As illustrated in Scheme 1, chemically modified glass carbon electrodes (GC/CNTs/PDDA/aptamer) were fabricated using the layer-by-layer technique. The detailed procedures are shown in the Supporting Information.

### 2.3. Electrochemical measurements

Potentiometric measurements were performed using a double-junction Ag/AgCl (3.0 M KCl) reference electrode with 1.0 M LiOAc as a salt bridge electrolyte in the galvanic cell as follows: Ag/AgCl/3.0 M KCl/1.0 M LiOAc/binding buffer/modified electrode. The binding buffer was Tris-buffered saline solution (50 mM Tris base, 100 mM NaCl, 25 mM KCl, 10 mM MgCl<sub>2</sub>, pH 7.4). The electromotive force (EMF) measurements were carried out using a CHI 760D electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China). In this work, the open circuit potential (OCP) of the proposed sensor recorded in the binding buffer was used as the baseline. Then, 20  $\mu$ L of BPA at different concentrations was added on the electrode surface for incubation. After incubation for 15 min and washing with binding buffer, the potential response was recorded in the binding buffer. The potential change between the baseline and the potential recorded at 100 s was used for quantification of BPA.

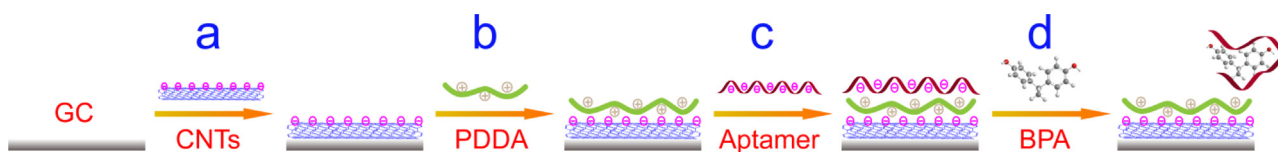


**Fig. 1.** (A) Cyclic voltammograms recorded in 0.1 M KCl containing 5 mM Fe(CN)<sub>6</sub><sup>4-3-</sup>, for the electrodes: (a) GC, (b) GC/CNTs, (c) GC/CNTs/PDDA, and (d) GC/CNTs/PDDA/aptamer. (B) Open circuit potentials of (a) GC/CNTs, (b) GC/CNTs/PDDA, (c) GC/CNTs/PDDA/aptamer, and (d) GC/CNTs/PDDA/aptamer after incubation with 10<sup>-7</sup> M BPA for 15 min. Potential responses of the modified electrodes were recorded for 100 s. (C) UV spectra of (a) the incubation solution alone after incubation for 15 min, and (b) the incubation solution in the presence of 10<sup>-5</sup> M BPA after incubations for 10, and (c) 15 min with the GC/CNTs/PDDA/aptamer electrode. 5  $\mu$ L of 10<sup>-5</sup> M aptamer was used to prepare the electrode. (D) Potentiometric responses of the electrodes to 10<sup>-6</sup> M BPA with different incubation times. Error bars represent one standard deviation for three measurements.

## 3. Results and discussion

As illustrated in Scheme 1, CNTs, PDDA, and the aptamer were successively modified on the electrode via electrostatic and  $\pi$ - $\pi$  interactions, thus forming an electric double layer at the electrode/solution interface. The presence of BPA could induce the conformational change and further detachment of the aptamer from the surface of the modified electrode. This causes the charge distribution change of the electric double layer and therefore induces a potential change. Indeed, previous reports demonstrated that an increase of any excess negative or positive charges on the surface of the electrode can lead to a potential response [16,23]. More importantly, polyions with large amounts of charges assembled on the surface could induce much larger surface charge changes for improving the sensitivity of the potentiometric sensors.

To characterize the fabrication process of the layer-by-layer assembly, cyclic voltammetry (CV) was performed (Fig. 1A). Compared with the bare glass carbon electrode (GC) (Fig. 1A, curve a), the electrode modified with CNTs exhibits better conductivity (Fig. 1A, curve b). However, PDDA and the aptamer could block the electron transfer and induce lower peak currents (Fig. 1A, curve c and d). Field-emission scanning electron microscopy pictures also indicate the layer-by-layer modification process (Fig. S1). Moreover, the charge distribution or density on the electrode surface will change during the process of the layer-by-layer assembly. Therefore, the open circuit potentials of the modified electrode were measured (Fig. 1B). Compared with GC/CNTs (Fig. 1B, column a), the modification of PDDA with a large positive charge changes the charge distribution on the electrode surface from negative to positive, which induces a large increase of the potential change (Fig. 1B, column b). Furthermore, the adsorption of aptamer leads to a decrease of the OCP (Fig. 1B, column c). These results demonstrate that the electrode was successfully prepared by the layer-by-layer assembly.



**Scheme 1.** Schematic illustrations of the preparation of the GC/CNTs/PDDA/aptamer film by successively assembling (a) negatively charged CNTs on GC, (b) positively charged PDDA, and (c) negatively charged aptamer, and (d) the interaction with the target.

According to Fig. 1B (column d), the incubation of the modified electrode with the target molecules induces the recovery of the OCP, which is probably due to the detachment or release of the aptamer into the sample solution. The release of the aptamer from the electrode was confirmed by using UV spectroscopy. In contrast to the incubation solution without BPA (Fig. 1C, curve a), the incubation solution with BPA causes an obvious UV absorption (Fig. 1C, curve b). Moreover, the amount of released aptamer can be increased by increasing the incubation time (Fig. 1C, curve c).

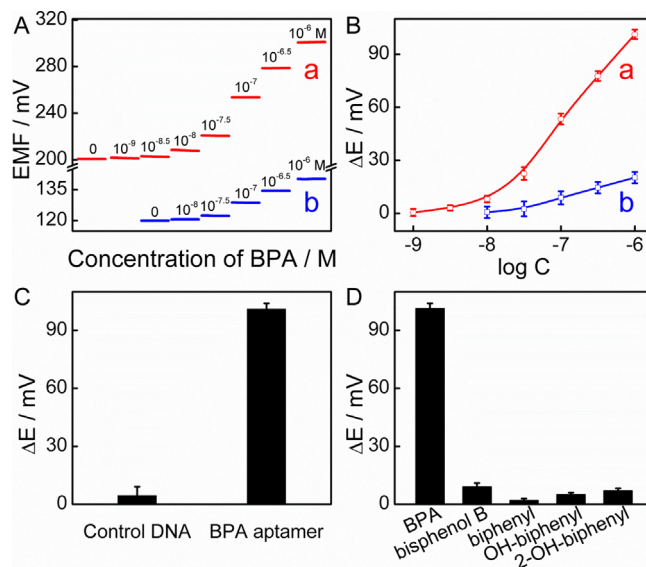
A similar phenomenon can also be observed when potentiometric measurements with different incubation times were carried out. During the incubation, a conformational change of the aptamer occurred that weakened the interactions between PDDA and the aptamer. The conjugate of the aptamer and BPA dropped away when the electrodes were rinsed or placed in the stirring solution. The decrease of the aptamer on the electrode surface induced the surface charge change of the working electrode, thus changing the potential of the electrode. Furthermore, the higher binding constant of BPA and the aptamer made the separation between the aptamer and PDDA easier [24]. As shown in Fig. 1D, the response of the modified electrode increases with the incubation time for up to 15 min, and then decreases gradually, which might be due to the dissolution of PDDA. The incubation time of 15 min was selected for further experiments.

BPA at different concentrations could lead to the detachments of aptamer to variable extents. The potential responses to BPA in the concentration range of  $1.0 \times 10^{-9}$  to  $1.0 \times 10^{-6}$  M are shown in Fig. 2A. Under the optimal experimental conditions, the sensor exhibits a linear response range from  $3.2 \times 10^{-8}$  to  $1.0 \times 10^{-6}$  M with a slope of 52.7 mV/dec. The detection limit was calculated to be  $1.0 \times 10^{-8}$  M ( $3\sigma$ ). In our previous research, a lower detection limit of 80 pM for BPA could be obtained, which is probably due to the multiple target-binding sites (aptamer) on each of the long concatamer [22]. Compared with our previous aptasensing platform that couples to a signal amplification strategy based on DNA assembly, the present sensor is simple and easy to use. More importantly, the proposed sensor exhibits better sensitivity than that of the existing potentiometric solid-state sensors based on surface charge change, which were designed for proteins and whole cells [17,25–27].

Without the polycation, the aptamer also could be modified on the electrode by utilizing  $\pi$ - $\pi$  stacking interactions between the nucleic acid bases and the carbon nanotubes' walls [28]. The fabricated electrode (GC/CNTs/aptamer) was tested for the detection of BPA. Likewise, the target/aptamer recognition could release the aptamer from the CNTs layer, inducing the change of surface potential. Fig. 2 shows that the sensor indeed responds to BPA at different concentrations (Fig. 2B, a). However, the sensor shows a poor performance in sensitivity (Fig. 2B, b).

Control experiments reveal that no significant potential change was observed for the control DNA (Fig. 2C). More importantly, the presence of other structurally similar molecules (bisphenol B, biphenyl, OH-biphenyl and 2-OH-biphenyl) could not lead to an obvious potential change (Fig. 2D), which confirms the specific interaction between BPA and the aptamer.

To investigate the regeneration of the proposed sensor, 5 regeneration cycles were tested. As shown in Fig. S3, a decrease of ca 20%



**Fig. 2.** (A) Potential responses of the electrodes after incubation in binding buffer with BPA of  $10^{-9}$ ,  $10^{-8.5}$ ,  $10^{-8}$ ,  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ , and  $10^{-6}$  M with (a) the GC/CNTs/PDDA/aptamer electrode and (b) the GC/CNTs/aptamer electrode. Potential responses of the modified electrodes were recorded for 100 s. (B) Calibration curves for BPA with (a) the GC/CNTs/PDDA/aptamer electrode and (b) the GC/CNTs/aptamer electrode, respectively. (C) The potential changes of the control DNA and BPA aptamer modified electrode in the presence of  $10^{-6}$  M BPA. (D) Potential responses of the electrodes to BPA and other structurally similar molecules. The concentrations of BPA and its analogues were  $10^{-6}$  M. Error bars represent one standard deviation for three measurements.

of the potentiometric response was observed after 3 regeneration cycles, which is probably due to the release of PDDA from the membrane into the aqueous sample solution. Experiments revealed that no obvious loss of response was observed after dry storage of the modified electrode at  $4^\circ\text{C}$  for 7 days (see Supporting Information, Fig. S4). The deterioration of the sensor response could be attributed to the dissolution of PDDA.

#### 4. Conclusions

In summary, a potentiometric aptasensing platform for small molecules based on surface charge change has been demonstrated. Polymers with large amounts of charges can be introduced and modified on the electrode for improving sensitivity. The target induced detachment of the aptamer leads to the surface charge change from negative to positive, which can amplify the potential response. Our proposed approach significantly improves the sensitivity and can be extended to determine other analytes that can interact with their aptamers, such as bacteria, proteins, and metal ions. Further applications of the present sensing configuration are currently in progress in our laboratory.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.snb.2017.12.067>.

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