Electronic Supporting Information

Chronopotentiometric Aptasensing with Signal Amplification Based on Enzyme-Catalyzed Surface Polymerization

Shuwen Liu, [ad] Jiawang Ding *[abcd] and Wei Qin *[abcd]

[a] Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS); Shandong Key Laboratory of Coastal Environmental Processes, YICCAS, Yantai, Shandong 264003, P. R. China

[b] Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qing-dao), Shandong 266237, P. R. China

[c] Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, Shandong 266071, P. R. China

[d] University of the Chinese Academy of Sciences, Beijing 100049, P. R. China

*Telephone: +86 535 2109156. Fax: +86 535 2109000.

*E-mail: jwding@yic.ac.cn (J. W. Ding), wqin@yic.ac.cn (W. Qin).

S1. Experimental section

Reagents and materials

All the oligonucleotides were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of synthetic oligonucleotides are listed in Table S1. Antibiotics including kanamycine, oxytetracycline, chloramphenicol, streptomycin, ampicillin, gentamicin, and spectinomycin, calcium ionophore N,N-dicyclohexyl-2-[2-(dicyclohexylamino)-2-oxoethoxy] acetamide (ETH 129), high-molecular-weight poly(vinyl chloride) (PVC), tetradodecylammonium tetrakis(4-chlorophenyl)-borate (ETH 500), sodium tetrakis- [3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), 2-nitropheny octyl ether (*o*-NPOE), poly(sodium 4-styrenesulfonate) (NaPSS), 3,4-ethylenedioxythiophene (EDOT), horseradish peroxidase (HRP, ≥ 263 units/mg solid) and dopamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) were purchased from Xianfeng Nanotech Port Co., Ltd. (Nanjing, China), and their characteristics are as follows: outer diameter, 10-20 nm; length, 5-15 μm; purity, > 95%. 1-Ethyl-3-(3-(dimethylamino) propyl) carbodiimide (EDC, hydrochloride form, > 98.5%) and N-hydroxysuccinimide (NHS, > 98%) were purchased from Aladdin Reagent Database Inc. MES (2-morpholino-ethanesulfonic acid) buffer (0.05 M pH = 6.0) were purchased from Leagene Biotechnology. Streptavidin labelled magnetic beads (MBs, 10 mg mL^{-1}) with an average particle size of 1.0 μm were purchased from BioCanal Scientific Inc. (Wuxi, China). The phosphate buffered (PBS, 0.01 M, pH 8.0) solution was used in the experiment. All other chemical reagents were purchased from

Sinopharm Chemical Reagent (Shanghai, China). All chemicals were analytical grade and used without further purification. Aqueous solutions were prepared with freshly deionized water (18.2 MΩ specific resistance) obtained with a Pall Cascada laboratory water system.

Membrane preparation and EMF measurements

PEDOT/PSS films were polymerized on glassy carbon (GC, 3 mm in diameter) electrodes by applying a 0.014 mA current for 714 s in aqueous solution containing 0.1 M NaPSS and 0.01 M EDOT as described in the literature.[1] After polymerization, 80 μL of the membrane cocktail solution was drop-cast on the GC/PEDOT. The pulsed current-controlled calcium-selective membrane contained 20 mmol/kg ETH 129**,** 10 wt% ETH 500, 29.7 wt% PVC and 59.4 wt% *o*-NPOE. And the conventional calcium-selective membrane contained 0.46 wt% ETH 129, 0.48 wt% NaTFPB, 33.02 wt% PVC and 66.04 wt% *o*-NPOE. 200 mg of the membrane components was dissolved in 2 mL tetrahydrofuran. After dried at room temperature, the pulsed - current-controlled and the conventional calcium-selective electrodes were conditioned in 10 mM NaCl and 10^{-3} M $CaCl_2$ overnight, respectively. All measurements were carried out at room temperature using a CHI 660E electrochemical work station (Shanghai Chenhua Apparatus Corporation, Shanghai, China). A conventional three-electrode cell with an ISE as the working electrode, a platinum wire as the auxiliary electrode, and Ag/AgCl (3.0 M KCl) as the reference electrode was used.

Preparation of the MWCNT-HRP-linker DNA complexes

The preparation of MWCNT-HRP-linker DNA was according to the reported method.[2] The carboxyl groups on the MWCNTs were activated in 1.0 mL of MES buffer solution containing 110.0 mg EDC and 13.0 mg NHS for 30 min, and then the activated MWCNTs-COOH were purified by centrifugation (12000 rpm, 15 min) to discard the supernatant. The activated MWCNTs-COOH were rinsed with water three times and dispersed into 1.0 mL of the PBS (0.01 M pH = 7.4) solution containing 1.0 μM of the linker DNA and 0.5 mg mL^{-1} of HRP, followed by a 4 h reaction at room temperature under magnetic stirring. After centrifugation and being washed with the PBS solution, the obtained MWCNT-HRP-linker DNA complexes were dispersed in 1.0 mL of the PBS solution (0.01 M pH=7.4) for future use.

Preparation of the functionalized magnetic beads

Before use, all the oligonucleotides in PBS buffer (0.01 M pH = 7.4) were heated at 95 ^{o}C for 5 min to dissociate any intermolecular interaction and cooled quickly on 0 ^{o}C ice-water mixture.[3] The OTC aptamer and the linker DNA assembled on the MWCNTs were immobilized on the magnetic beads via DNA hybridizations. Firstly, 100 μL of the biotin-modified capture DNA (50 μM) was first incubated with 100 μL of the streptavidin modified magnetic beads (10 mg mL^{-1}) with 30 min shaking at room temperature via the biotin-streptavidin interaction. The immobilization efficiency was measured by UV-vis spectroscopy and the concentration of the capture DNA on the magnetic fluid was ca 10 μM (Fig. S1). It should be noted that the added volumes of the PBS buffer were equal to the volumes of the original magnetic fluid after each magnetic separation.

10 μL of the capture DNA modified magnetic beads were then incubated with 1.0 μL of 100 μM aptamer and 100 μL of the MWCNT-assisted multiple HRP label with 1 h shaking at room temperature. After magnetic separation and being washed with 100 μL of PBS, the sandwich functionalized magnetic beads (FMBs) were obtained, which were resuspended to the 100 μL of PBS buffer.

OTC detection

1.0 μL of OTC at different concentrations was first incubated with 100 μL of the FMBs in the PBS buffer (0.01 M pH = 7.5) with 1 h shaking at room temperature. After magnetic separation and being washed with the PBS buffer (0.01 M pH = 7.5), the resulting MBs were dispersed in the PBS blocking buffer (0.01 M pH = 8.0). Then, 7.5 mg mL^{-1} dopamine and 2 μL 1 M H_2O_2 were added into the final volume of 100 μL of the PBS buffer blocking solution which was drop-coated on the Ca^{2+}-ISM and allowed for a 45 min HRP-catalyzed polymerization to form polydopamine (PDA). It should be noted that a relatively long incubation time (1 h) and polymerization time (45 min) were chosen in order to achieve highly sensitive measurements. However, the incubation and polymerization times can be shortened for measuring the analyte at concentrations higher than 1.0 nM. The resultant electrode was rinsed with the PBS buffer (0.01 M pH = 8.0) slightly and used for chronopotentiometric measurements. Control experiments were done following the same procedures. For the chronopotentiometric measurements, the procedures switching between the galvanostatic and potentiostatic steps were designed according to our previous research.[4] The open-circuit potential of the electrode in 10 mM NaCl was first

recorded for 1 s. Then, a cathodic pulsed current of 2 μA with a duration of 2 s was applied, which was followed by using a constant potential for 100 s to refresh the membrane. The high salinity background in seawater may have an effect on the interactions between the aptamer and OTC in seawater. Therefore, seawater samples were analyzed by the proposed potentiometric aptasensing platform with the standard addition method.

S2. Results and discussions

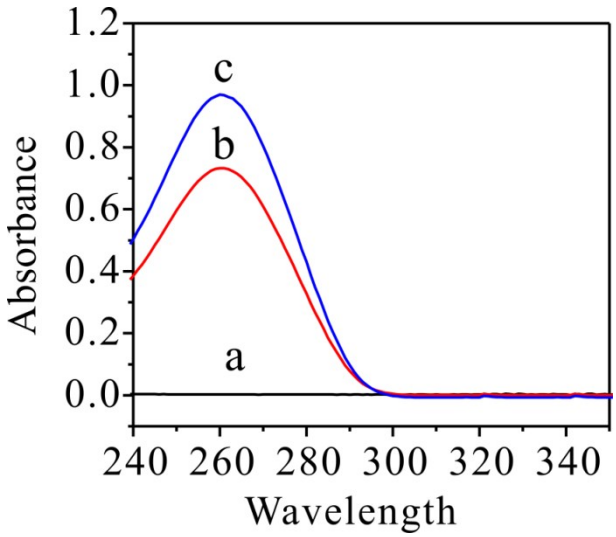

Fig. S1 UV-vis spectra of the 0.01 M pH 7.4 PBS buffer (a), the capture DNA solution (50 μM) after incubation with 100 μL of the magnetic beads for 0.5 h (b), and the capture DNA solution alone (c).

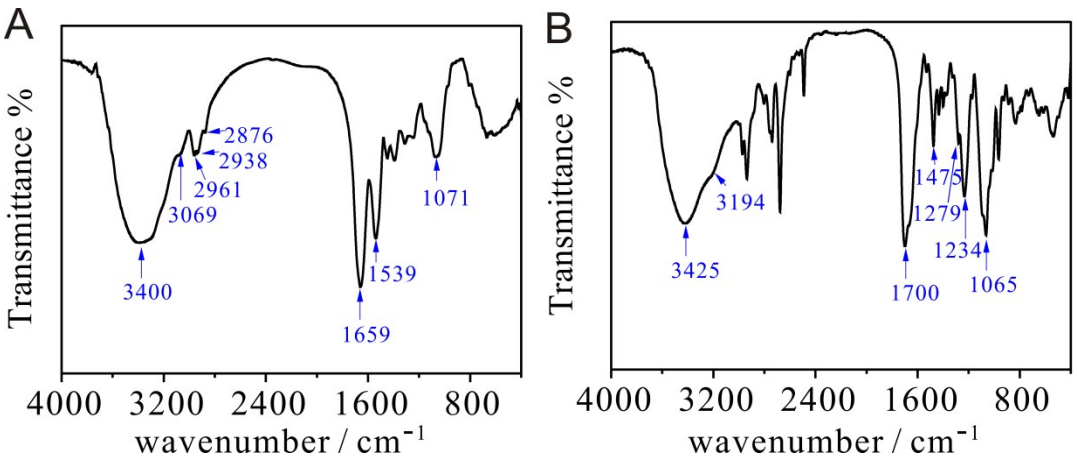

Fig. S2 FTIR spectra of HRP (A) and the linker DNA (B).

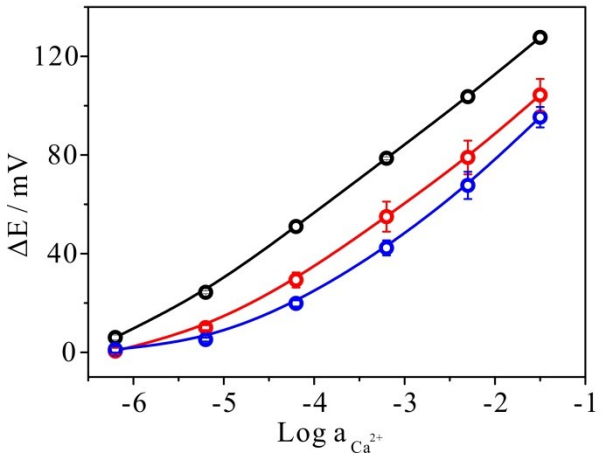

Fig. S3 Conventional potentiometric responses of Ca^{2+}-ISEs to Ca^{2+} in 0.01 M NaCl without (in black) and with dopamine autopolymerization (in red), or HRP-induced dopamine polymerization for 1 h (in blue). For HRP-induced dopamine polymerization, a 100 μL of the blocking buffer solution containg 7.5 mg mL^{-1} dopamine, 3.2×10^{-3} U mL^{-1} HRP, and 20 mM H_2O_2 was drop-coated on the Ca^{2+}-ISM. Error bars represent one standard deviation for three measurements.

Characterization by UV and EIS

As proven by the UV-Vis spectroscopic measurement, the presence of HRP can rapidly trigger the formation of PDA with an increase in absorbance at 420 nm, and the PDA aggregates begin to precipitate after 30 min (Fig. S4). The electrochemical impedance measurements reveal that the phenolic hydroxyl groups of catechol with strong adhesion allow a large amount of PDA deposited on the PVC membrane surface, which leads to an increase in the charge transfer resistance (Rc) (Fig. S5). Compared with the Rc value (9.86 KΩ) of the PVC membrane treated with dopamine autopolymerization, an increased value of Rc (11.83 KΩ) was obtained, indicating a larger blocking effect in the presence of HRP.

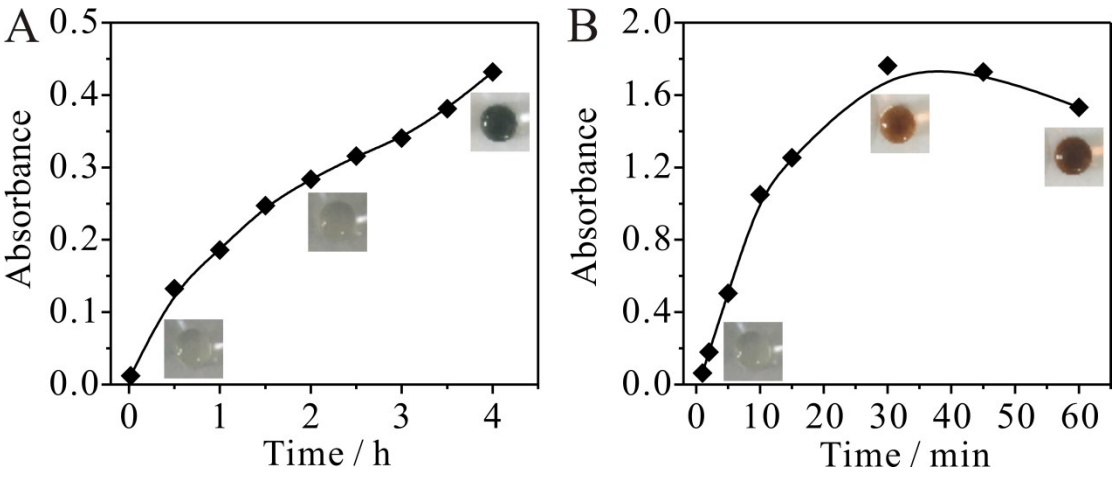

Fig. S4 Time dependences of the UV-Vis absorbance measured at 420 nm for the dopamine solutions with 20 mM H_2O_2 in the absence (A) and presence (B) of HRP (3.2×10^{-3} U mL^{-1}). The inset shows the camera images of the PDA layers coated on the PVC membrane surfaces without (A) and with (B) HRP at different polymerization times.

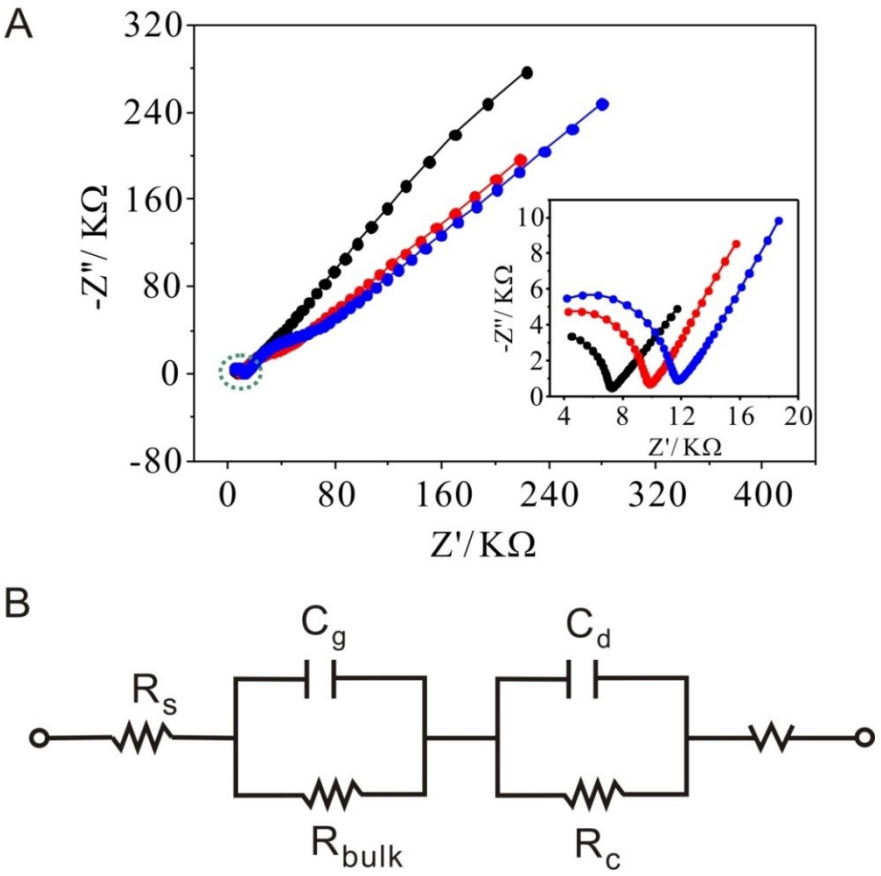

Fig. S5 (A) Impedance spectra of pulsed chronopotentiomertric GC/PEDOT-PSS/Ca^{2+}-ISE recorded in 10^{-4} M $CaCl_2$ with a 0.01 M NaCl background solution. The impedances of the electrode without (in black) and with the DA polymerization in the absence (in red) and presence (in blue) of HRP were recorded. The inset shows the magnification of the impedance spectra in the high frequency region (green circle). For the HRP-induced dopamine polymerization, a 100 μL of the blocking buffer solution containg 7.5 mg mL^{-1} dopamine, 3.2×10^{-3} U mL^{-1} HRP, and 20 mM H_2O_2 was drop-coated on the Ca^{2+}-ISM. Other conditions are shown as follows: initial potential, open circuit potential; excitation amplitude, 100 mV; frequency range, 0.01-100,000 Hz. (B) Equivalent circuit to fit the impedance spectra. The circuit components are the bulk resistance R_{bulk}, the geometric capacitance of the membrane C_g, the capacitance of the double electric layer C_d, the charge-transfer resistance R_c, and the solution resistance R_s, respectively. The Warburg impedance is attributed to the diffusion species in the membrane.

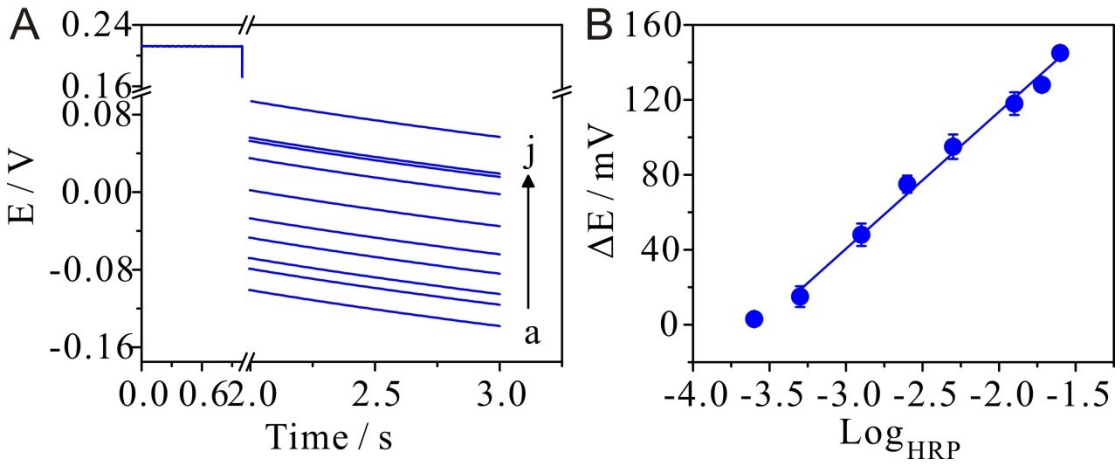

Fig. S6 (A) Pulsed chronopotentiomertric responses of the calcium-selective membrane blocked by the HRP-catalyzed DA polymerization for measuring 10^{-4} M Ca^{2+} (a-i) in 0.01 M NaCl in the presence of 2.5×10^{-2} (a), 2.0×10^{-2} (b), 1.3×10^{-2} (c), 5.0×10^{-3} (d), 2.5×10^{-3} (e), 1.3×10^{-3} (f), 5.0×10^{-4} (g), 2.5×10^{-4} (h) and 0 U mL^{-1} (i) of HRP. The chronopotentiomertric responses of 10^{-4} M Ca^{2+} (j) in 0.01 M NaCl without blocking. (B) Corresponding calibration plot of the potential change versus logarithm of the concentration of HRP. The chronopotential difference (ΔE) between the responses in the absence (i) and presence of different concentrations of HRP (a-h) was used for quantification. Error bars represent one standard deviation for three measurements. The polymerization conditions are shown as follows: 0.01 M pH 8.0 PBS; 7.5 mg mL^{-1} dopamine; 20 mM H_2O_2; 45 min polymerization time.

Selection of substrates for HRP

Previous reports have shown that HRP can effectively catalyze a broad range of substrates to form polymeric layers on the electrode surfaces,[2,5,6] which can decrease the electroactive areas of the electrodes and block the ion and/or charge transfer processes. In this work, epinephrine, aniline and dopamine were selected as substrates to investigate the blocking effects on the ion transfer across the polymeric membrane

(Fig. S7). The blocking ability of each substrate was compared by measuring 10^{-4} M Ca^{2+} in 0.01 M NaCl background in the absence and presence of HRP. Although all the three substrates can be polymerized and deposited on the membrane in the presence of HRP, neither polyepinephrine nor polyaniline could firmly be fixed on the PVC membrane. In contrast, PDA can strongly adhere to the electrode and show the best sensitivity.

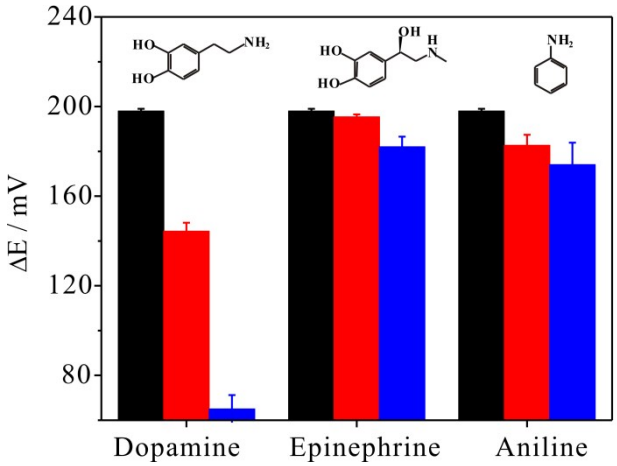

Fig. S7 Potential differences for measuring 10^{-4} M Ca^{2+} in 0.01 M NaCl in the absence (in black), and presence of the substrate with (in blue) and without (in red) the HRP-catalyzed polymerization. 100 μL of 10 mM PBS (pH 8.0) containing 7.5 mg mL^{-1} substrate (dopamine or epinephrine) or 100 μL of 0.1 M acetate buffer solution (pH 4.0) containing 30 mM aniline, 3.2×10^{-3} U mL^{-1} HRP, and 20 mM H_2O_2 were drop-coated on the Ca^{2+}-ISE and allowed to react for 45 min for dopamine, and 2 h for epinephrine and aniline, respectively. Error bars represent one standard deviation for three measurements.

Optimization of the experimental parameters

The experimental parameters such as the dopamine concentration, dopamine polymerization time, and pH of the buffer solution were optimized (Fig. S8). The effect of the dopamine concentration was examined between 2 and 20 mg mL^{-1}. As shown in Fig. S8A, the potential change (purple curve) gradually increases from 2 to 7.5 mg mL^{-1} because the amount of dopamine dominates the blocking effect. However, the potential change decreases at concentrations higher than 7.5 mg mL^{-1}, probably due to the increased background potential caused by the dopamine autopolymerization. Thus, the optimum dopamine concentration was chosen as 7.5 mg mL^{-1}. With increasing the polymerization time (Fig. S8B), the potential change gradually increases from 0 to 45 min and then decreases due to the autopolymerization effect. In order to obtain a large potential change, the polymerization time was controlled at 45 min. The buffer pH plays a key role in dopamine polymerization. Previous reports have shown that the autopolymerization of dopamine can occur at pH 8.5. In this work, in order to reduce autopolymerization of dopamine and obtain higher enzyme activities, pH 8.0 was selected for further experiments (Fig. S8C).

One unique feature of the current-pulse based ISEs is that the super-Nernstian region of the electrode can be modulated by the magnitude and duration time of the applied current in a single pulse.[4] As shown in Fig. S9A, when the electrode coated with the dopamine in the absence of HRP (in red), the potential difference of Ca^{2+}-ISM increases with the tested cathodic current amplitude from 1 to 2 μA and does not

change obviously between 2 to 4 μA. When a larger cathodic pulsed current 6 μA is applied (in black and red), the background ions or oppositely charged ions could be extracted along with the calcium into the membrane and resulted in the decrease of the potential differences. For the electrode modified with dopamine in the presence of the HRP (in blue), the change of the potential difference at various current amplitude is not obvious which can be attributed to the prominent blocking effect of PDA in the presence of HRP. Therefore, the cathodic current of 2 μA was applied. As depicted in Fig. S9B, the potential difference for measuring 10^{-4} M Ca^{2+} gradually increased from 0.5 to 2 s and then reaches a plateau to 4 s. Further increasing the duration may deteriorate the selectivity and result in a decreased membrane potential. Therefore, the cathodic current amplitude of 2 μA with a pulse duration of 2 s was selected for subsequent experiments.

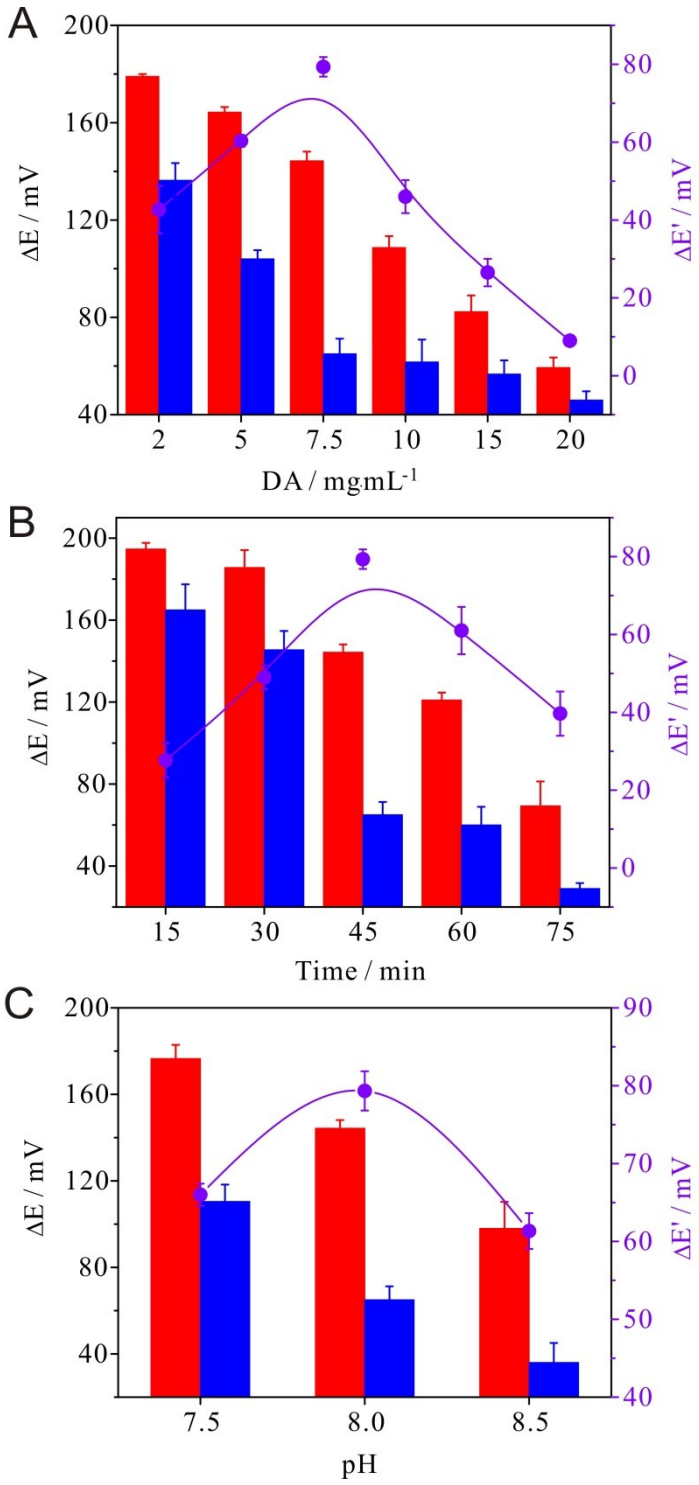

Fig. S8 Effects of the amount (A), polymerization time (B), and pH (C) of the DA solution on the blocking effect. The potential differences of the Ca^{2+}-ISM blocked by the PDA in the absence (red) and presence (blue) of HRP were measured. Then, the potential differences between the red and blue curves were calculated (purple curves).

The potential differences were obtained by measuring 10^{-4} M Ca^{2+} in 0.01 M NaCl. The polymerization conditions are shown as follows: 0.01 M pH 8.0 PBS, 7.5 mg mL^{-1} dopamine, 3.2 × 10^{-3} U mL^{-1} HRP, 20 mM H_2O_2 and 45 min polymerization time. Error bars represent one standard deviation for three measurements.

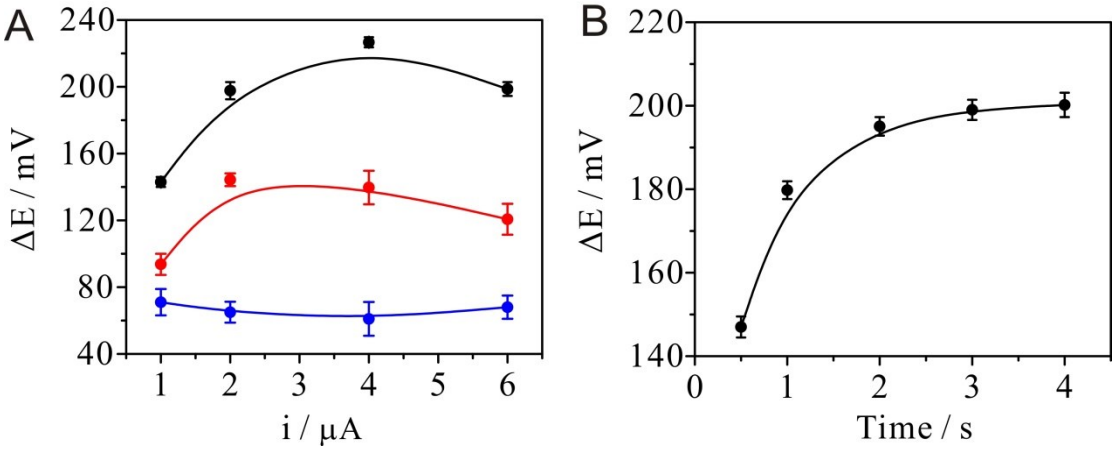

Fig. S9 (A) Effects of the current amplitude on the potential difference for measuring 10^{-4} M Ca^{2+} in 0.01 M NaCl without the DA polymerization (in black), with the DA autopolymerization (in red) and with the HRP-induced DA polymerization (in blue) for 45 min. The polymerization conditions are shown as follows: 0.01 M pH 8.0 PBS, 7.5 mg mL^{-1} dopamine, 3.2 × 10^{-3} U mL^{-1} HRP, 20 mM H_2O_2 and 45 min polymerization time. (B) Influence of the current pulse duration time on the potential difference for measuring 10^{-4} M Ca^{2+} with 0.01 M NaCl. Error bars represent one standard deviation for three measurements.

Reversibility

The reversibility of the electrode is determined by the detachment of the PDA from the ion-sensitive membrane. In this work, the decomposition/destruction of the PDA layer formed by noncovalent interactions under an alkaline solution would lead to detachment of PDA.[8,9] The electrode can be used at least for 3 cycles. Moreover, the reversibility of the electrode could be improved by using strongly alkaline solutions (e.g., pH 13).

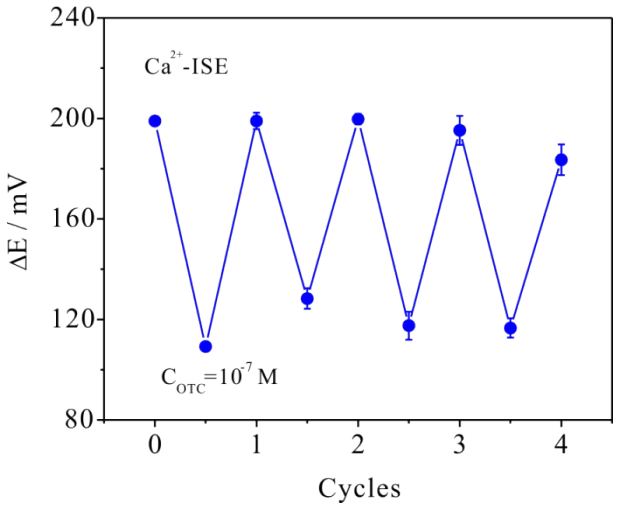

Fig. S10 Reversibility of the Ca^{2+}-ISE for measuring 10^{-4} M Ca^{2+} in 0.01 M NaCl in the presence of 10^{-7} M OTC. After each measurement, the Ca^{2+}-ISE was immersed in 0.01 M NaOH for 0.5 h and then conditioned in 0.01 M NaCl for 4 h. The polymerization conditions are shown as follows: 0.01 M pH 8.0 PBS, 7.5 mg mL^{-1} dopamine, 20 mM H_2O_2 and 45 min polymerization time. Error bars represent one standard deviation for three measurements.

Table S1. DNA sequences used in this work

Oligonucleotide	Sequence (5'to 3')
Capture DNA	biotin-TTTTTTACACAACAC
OTC aptamer	ACGTTGACGCTGGTGCCCGGTTGTGGTGCGAGTGTTGTGT
Scrambled sequence	ACGTTGACGAAACCATTATACGCGAGGATTGGTGTTGTGT
Linker DNA	CGTCAACGTTTTTTTTTT-NH_2

Table S2. Comparison of the potentiometric aptasensor with reported methods for OTC detection

Method	Linear range (nM)	Detection limit (nM)	Real Samples	Reference
Electrochemical aptasensor	22 - 1300	21	blood serum urine	10
Aptamer-based fluorescence assay	2.2 - 109	1.8	pork, milk, honey	11
Fluorescence sensor	100 -1800	30	honey	12
Electrochemical sensor	800 - 40000	120	chicken, fish, shrimp	13
Sandwich immunoassay	4 - 500	4	fish	14
Fluorescence biosensor	25 - 1750	9.5	milk	15
Potentiometric aptasensor	10 - 100	9.8	sea water	16
Potentiometric aptasensor	0.1 - 1000	0.028	sea water	this work

Table S3. Results for OTC detection with the proposed method in seawater (n=3)

Sample	Added (nM)	Proposed method (nM)	Recovery (%)
1	1	1.0 ± 0.1	100
2	10	9.5 ± 0.8	95
3	50	47.2 ± 2.4	94

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