Electronic Supporting Information

Novel electrochemiluminescence solid−state pH sensor based on i-motif forming sequence and rolling circle amplification

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1. Experimental Section

Reagents. Luminol was obtained from TCI (Tokyo, Japan). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) were bought from Shiwu Chemical Reagent Co., Ltd. (Shanghai, China). Tris(2-carboxyethyl)-phosphine hydrochloride (TCEP) was gained from BBI Life Sciences (Shanghai, China). 1-Hexanethiol (HT) was purchased from ACROS Organics (Belgium, USA). Dichlorotris(1,10-phenanthroline) ruthenium (II) hydrate ($Ru(phen)_3Cl_2$) was obtained from Suna Tech. Inc. (Suzhou, China). Glucose and glucose oxidase (GOx) were bought from Sigma-Aldrich. H_2O_2, NaH_2PO_4, Na_2HPO_4, $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, NaOH, HCl and KCl were bought from Beijing Chemical Works (Beijing, China). Hemin was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Hemin solution (10 mM) was prepared by dissolving in DMSO, and then diluted to 0.5 mM by phosphate buffer solution (PBS, pH 7.6) containing 50 mM KCl and 0.05% Triton X-100. Deoxyribonucleoside 5′-triphosphates mixtures (dNTPs), T4 DNA ligase, phi29 DNA polymerase, and corresponding enzyme working buffer solutions were obtained from Thermo Fisher Scientific. The buffers with different pH were obtained by adjusting 0.1 M PBS buffer to pH values from pH 4.00 to 8.00 with NaOH or H_3PO_4 at room temperature. Ultrapure water (18.25 MΩ·cm, Milli-Q, Millipore) was used in all experiments.

All DNA oligonucleotides were custom-synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China) and were dissolved in 10 mM PBS (pH 7.6, 1 mM EDTA). In order to make sure efficient hybridization between DNAs, the dissolved DNA solutions were firstly heated at 95 ℃ for 5 min, then slowly cooled to room temperature and stored at 4℃ for further use.

Table S1 The detailed nucleic acid sequences used in experiments.

capture	5’-TAAGTGTTAGTGATTGTGTTAG TTTTTTTT-C6-SH-3’
primer	5’-CCCTAACCCAATCCCTAACCCTTATCACACGAATTCATCTGAC-3’
padlock	5’-p-ATTCGTGTGAAACCCAACCCGCCCTACCCAAAAGTCAGATGA-3’

Note: The same color marked in the sequences represents they are the complementary sequences. The three mismatched bases are highlighted with underline. The “p” at the 5’−end of padlock indicates phosphate.

Apparatus. The ECL experiments were carried out with MPI-E ECL instrument (Xi’an, China) with photomultiplier tube (PMT) voltage of 600 V. The potential was swept from 0 to 0.6 V with the scan rate of 0.1 V/s. All the electrochemical properties were characterized on a CHI 660A electrochemical workstation (Shanghai CH Instruments, China). The differential pulse

voltammograms (DPV) signal was recorded in 0.1 M PBS (pH 7.4, 0.1 M KCl) at a potential range from −0.6 to 0.1 V and an amplitude of 0.05 V. The electrochemical impedance spectroscopy (EIS) was carried out in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$ with a frequency from 0.1 to 10^5 Hz. All ECL and electrochemical experiments were done with a three-electrode system, composed of glassy carbon electrode (GCE, Φ=3 mm) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl as the reference electrode. Circular dichroism (CD) spectral measurements were performed on a Jasco J-820 Circular Dichroism Spectra polarimeter (Tokyo, Japan) with the wavelength range of 220–350 nm and the scan rate of 100 nm/min. The obtained CD spectra were presented as the average of three successive scans which has been subtracted the background response of corresponding buffer.

Polyacrylamide gel electrophoresis (PAGE) analysis. PAGE was employed to verify the RCA reaction, which was performed in 15% native polyacrylamide gel and Tris−borate−EDTA buffer (TBE, 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) with a constant potential of 100 V for about 2 h at ice-bath. The samples in every lane were in stoichiometric equivalents in a buffer solution. Lane 1−3 samples were capture, primer and padlock DNA solution, respectively. Lane 4 sample was the mixture of capture and primer which have reacted at 25 °C for 1 h. Lane 5 sample was obtained via adding padlock DNA into lane 4 products at 25 ℃ for 1 h. The acquisition of lane 6 sample was divided into two steps. First, 0.2 U/μL T4 DNA ligase in 1×T4 DNA ligase buffer were reacted with reaction production of lane 5 at 25 ℃ for 1 h to obtain RCA template. Second, the linked padlock DNA would start the RCA process after adding 1 mM dNTPs, 0.5 U/μL phi29 DNA polymerase and 1×phi29 DNA polymerase buffer at 37 ℃ for 1 h. Subsequently, we added the reaction products into corresponding lanes with commercial loading solution. The completed gel was stained by silver ions and obtain the image by scanner.

Fabrication of the sensor and ECL detection of pH. A bare GCE (Φ = 3 mm) was polished using our previous method.[1] The well-polished GCE was immersed in 1% $HAuCl_4$ solution to obtain an electrodeposited AuNPs under constant potential of −0.2 V for 30 s. The AuNP/GCE was cleaned with ultrapure water and dried using nitrogen. 2 μM capture DNA was incubated with isometric 5 mM TCEP for 40 min at room temperature to break the disulfide bond. Subsequently, the dried electrode was modified with TCEP-pretreated capture DNA for 16 h at 4 °C to obtain capture/AuNP/GCE. Next, the capture/AuNP/GCE was incubated with 5 μL of HT (1 mM) at 25

°C for 1 h to block nonspecific sites (HT/capture/AuNP/GCE). Then, 10 μL of primer DNA (1 μM) was casted on the surface of the modified electrode at 25 °C for 1 h to obtain primer/HT/capture /Au/GCE.

Afterwards, 10 μL of a mixed solution containing padlock DNA (0.8 μM), T4 DNA ligases (0.2 U) and 1× T4 ligase buffer was dropped on the primer/HT/capture/AuNP/GCE at 25 ℃ for 1 h to obtain padlock/primer/HT/capture/AuNP/GCE. The circulated padlock DNA would act as RCA template to execute the RCA process under the assistance of 1 mM dNTPs, 1 U phi29 DNA polymerase in 1×phi29 DNA polymerase buffer at 37 ℃ for 1 h. The finally formed RCA/padlock/primer/HT/capture/AuNP/GCE was used for pH determination

The pH sensor of RCA/padlock/primer/HT/capture/AuNP/GCE was immersed into buffer with different pH and reacted for 10 min at room temperature and then rinsed with the corresponding pH buffer.[2] Continuously, the sensor was incubated with 10 μL hemin solution (0.5 mM) for 2 h in dark at room temperature to form H/G4 complexes. The ECL signal was measured in the solution of 0.1 M PBS (5 mL, pH 7.6) containing 0.5 μM luminol and 1 mM H_2O_2 at room temperature with a three-electrode system. The ECL measurement was operated at a voltage of 0 to 0.6 V with the scan rate of 100 mV/s. The voltage of the photomultiplier tube (PMT) was set at 600 V.

The detection of glucose. GOx (4 μL, 4 mg/mL) was added into 200 μL of the different concentrations of glucose (1 μM, 5 μM, 15 μM, 30 μM, 50 μM, 0.1 mM, 0.5 mM, 1 mM and 5 mM) in the PBS solution (0.1 mM, pH 7.0) and the mixture was kept at 37 ℃ for 3 h. After that, the pH sensor was immersed into the mixture and incubated for 10 min. The remaining steps are the same as pH detection.

2. Supplementary Figures and Discussions

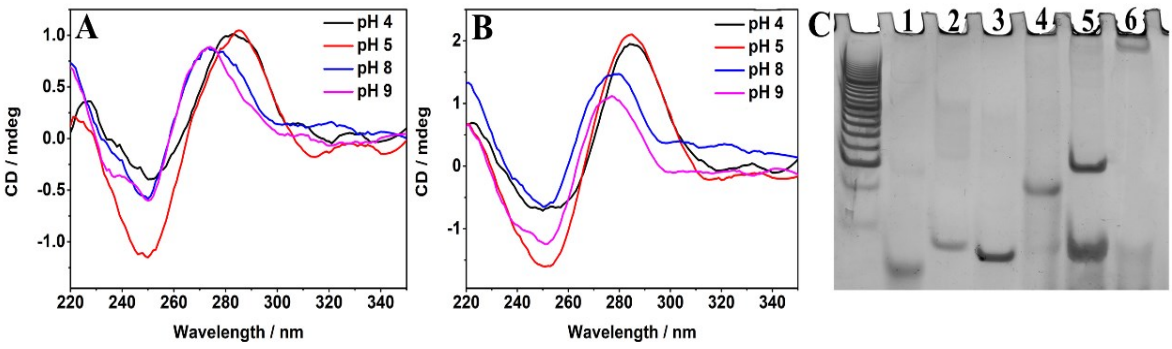

Fig. S1 CD spectra of (A) primer DNA and (B) the mixture of capture and primer DNA at different pH conditions. The CD spectra were recorded with 2 μM DNA samples. (C) PAGE image of

different samples: (1) capture, (2) primer, (3) padlock, (4) capture+primer, (5) capture+primer+padlock+T4 ligase and (6) capture+primer+padlock+T4 ligase+phi29 polymerase+dNTPs. The PAGE experiment was carried out with 1 μM DNA samples.

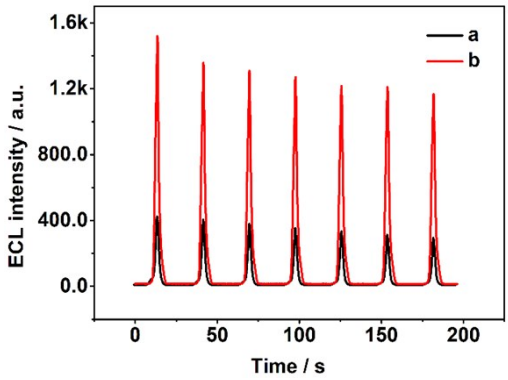

Fig. S2 ECL responses of (a) capture/AuNP/GCE and (b) primer/HT/capture/AuNP/GCE after incubating with 50 mM $Ru(phen)_3Cl_2$ at 37 ℃. Potential range: 0−1.4 V; PMT: 400V. Electrolyte: 0.1 M PBS containing 0.1 M triethylamine.

Optimization of experimental conditions. To optimize the analytical performance, we explored two factors which would affect the sensitivity of the sensor hereby, including the concentration of capture and the incubation time of sensor in hemin solution. As shown in Fig. S3A, the ECL intensity increased with increasing concentration of capture DNA and then slightly decreased after the concentration reaching to 1 μM, which might be due to the fact that higher packing density of capture DNA which brought stereospecific blockade wasn't beneficial for the formation of H/G4 catalytic structure. Thus 1 μM was chosen as the optimum concentration of capture DNA. The incubation time of in hemin solution also significantly influenced the finally signal., The ECL intensity increased with increasing incubation time of hemin incubation and tended to be steady after 2 h in Fig. S3B, which might own to the fact that 2 h was enough to reach a saturated binding amount between G4 and hemin. Therefore 2 h was regarded as the most optimum incubation time.

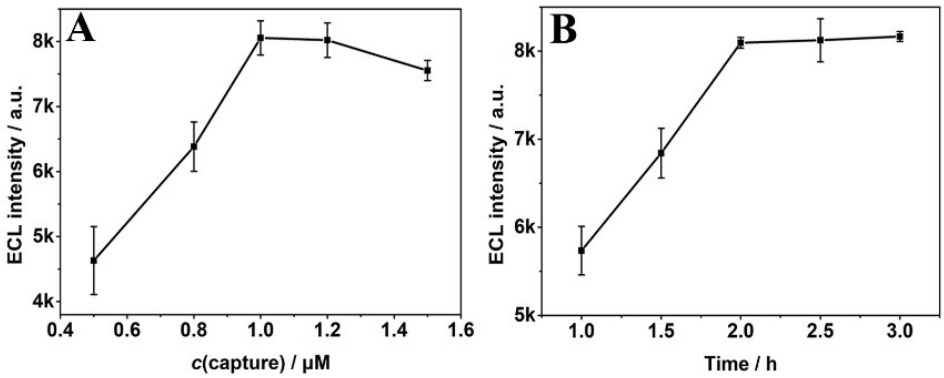

Fig. S3 Optimization of the detection conditions. The effects of (A) the concentration of capture DNA and (B) the incubation time of hemin.

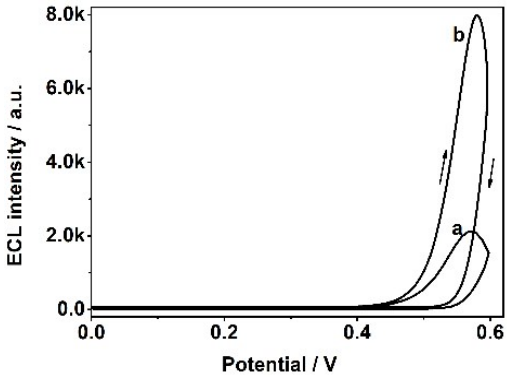

Fig. S4 ECL signals before (a) and after (b) the formation of H/G4 complexes.

Transitional pH for i-motif forming sequence. The transitional pH (pH_T) where the sequence is 50% folded could be used to character i-motif forming sequence.[3,4] The primer DNA solid powder was dissolved in water to 100 μM and then diluted to 10 μM (total volume of 200 μL) with buffer at different pH (4.00, 4.83, 5.00, 5.17, 5.55, 6.00, 6.21, 6.49, 6.75, 6.89, 7.17, 7.40, 7.48, 7.73 and 8.00). Final analysis and manipulation of the CD data was carried out using Origin. As it was shown in the Fig. S5A, CD spectra showed a significant change as a function of pH. At pH 4.00, the CD spectrum showed a positive band at 286 nm and a negative band at 256 nm, which is consistent with the characteristic bands of i-motif reported previously.[5,6] When the pH increased 8.00, the positive peak decreased and shifted to 272 nm, the negative peak shifted to 247 nm, which was the characteristic peaks of a random-coiled, primer DNA singled strand. The pH_T of i-motif was identified from the inflection point of the titration curve by plotting CD intensity at 286 nm vs pH value. The pH_T of primer DNA was 6.2 according to Fig. S5B.

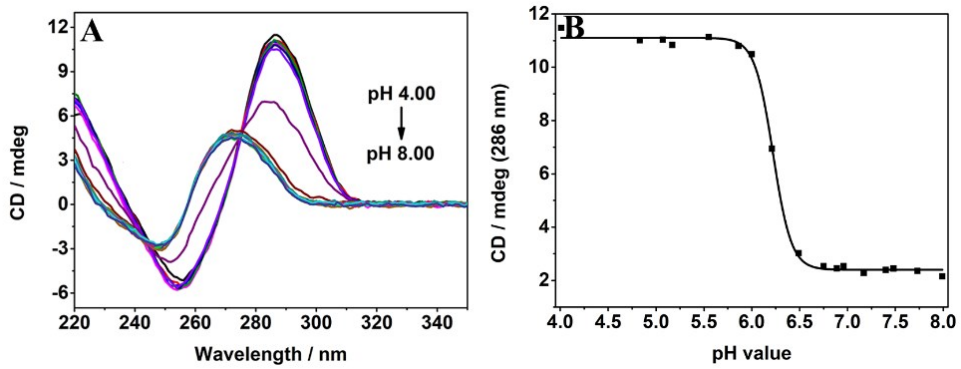

Fig. S5 (A) The CD spectra for primer DNA in different pH solution. (B) Plot of CD intensity at 286 nm versus pH value.

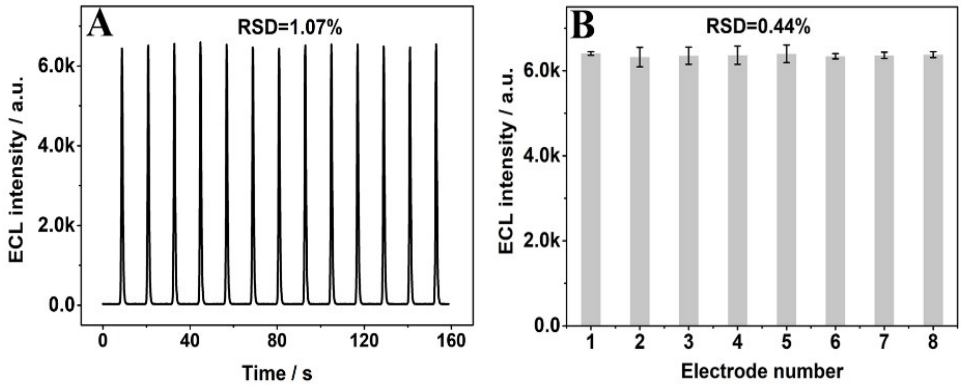

Fig. S6 (A) The ECL response of the constructed sensor at pH 6.0 under 13 continuous potential cycles. (B) The ECL response intensity of eight parallelly prepared sensors to pH 6.0 solutions.

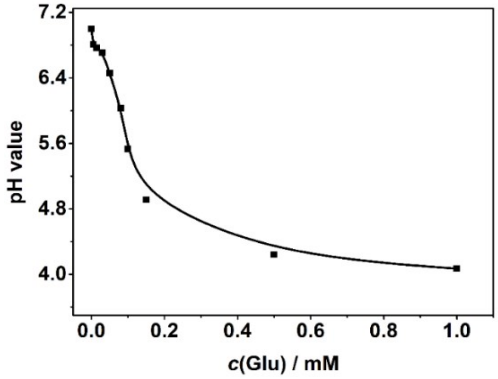

Fig. S7 The pH value of glucose solution with different concentrations after incubating with GOx at 37 ℃ for 3 h.

Table S2 Comparison of the performance of the proposed sensor with other i-motif-based pH sensors.

Method	Application	Dynamic response range	linear range	Reference

Colorimetry	pH sensor	pH 5–8.3	–	7
Fluorescence	pH sensor	pH 5–7.5	–	8
Fluorescence	pH sensor	pH 6.5–7.4	pH 6.5–7.4	9
Fluorescence	pH sensor	pH 5–7.5	pH 5–6.5	5
Fluorescence	pH sensor	pH 5.3–6.6	–	10
Fluorescence	pH sensor	pH 5.2–7	pH 5.8–6.4	11
Fluorescence	pH sensor	–	pH 6.25–7.75	12
Fluorescence	pH sensor	–	pH 5.5–7	13
ECL	pH sensor	pH 4–7.4	pH 6–7.4	this work

Table S3 Glucose detection in human serum sample. The human serum samples were diluted 500−fold for detection by using the ECL−pH sensor.

Sample	Determined (mM)	Added (mM)	Found (mM)	Recovery (%)	RSD (%, n=3)
1	4.83	20	23.12	93.11	4.52
2	5.62	30	33.46	94.94	5.87

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