Supporting Information

Sensitive and noninvasive cyclic peptide-based electrogenerated chemiluminescence biosensing method for the determination of sweat glucose

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1. Experiment

1.1 Reagents and apparatus

The cyclic peptides, including cyclo-[-CNDNHCRDNDC-] (MW = 1306.33 g/mol, CP1), cyclo-[-CHNDNHCHDNDHC-] (MW = 1561.58 g/mol, CP2) were chemically synthesized by Shanghai Apeptide Co., Ltd. (China). 6-Mercapto-1-hexanol (MCH), Nafion (5 wt%) and CH_3CN were obtained from Sigma-Aldrich (USA). Ascorbic acid (AA), uric acid (UA) and lactic acid (LA) were obtained from Wokai Chemical Reagent Co., Ltd (China). Fructose, sucrose, maltose, lactose and tri-n-propylamine (TPA, 98%) were supplied by Sinopharm Chemical Reagent Co., Ltd. (China). Millipore Milli-Q water (18.2 MΩ•cm) was used in all experiments.

CHI 660 electrochemical workstation (Chenhua Instruments Co., China), MPI-E ECL detector (Xi'an Remax Analysis Instruments Co., Ltd, China), UV-Vis spectrophotometer (UV-2450, Shimadzu Corporation, Japan), nuclear magnetic resonance spectrometer (Bruker Advance III 400 spectrometry, Germany), electrospray ionization mass spectrometer (ESI-MS, Bruker Maxis UHR-TOF, Germany), and fluorolog-3 fluorescence spectrophotometer (Horiba JY, USA) were used in this work.

1.2 Synthesis of $[Ir(ppy)_2(CH_3CN)Cl]$ and ECL probe

An Ir(III) solvent complex ($[Ir(ppy)_2(CH_3CN)Cl]$, Ir1-CH_3CN) was synthesized and used as ECL signal precursor according to references[1] and our previous work[2]except CH_3CN used as solvent. Firstly, chloro-bridged Ir(III) dimer $[Ir(ppy)_2(\mu\text{-}Cl)]_2$ was synthesized according to the reference.[3] In brief, $IrCl_3•3H_2O$ (353 mg, 1.0 mmol) and 2-phenylpyridine (ppy) (341 mg, 2.2 mmol) were carefully added into 40 mL of the mixture of 2-ethoxyethanol/H_2O (v:v = 3:1) solvent. The mixture was refluxed at 120 °C for 24 h under N_2 atmosphere. On cooling, the precipitate was deposited, which was collected by filtration, and washed three times with water, ethanol and n-hexane. After that, the crude product was further purified by recrystallizing with CH_3OH and CH_2Cl_2. Yellow product was obtained and dried in an oven at 40 °C overnight. Secondly, $[Ir(ppy)_2(\mu\text{-}Cl)]_2$ (5.0 mg) was dissolved in 20 mL of CH_3CN and the mixture was stirred for 30 min at room temperature. After that, a yellow solid $[Ir(ppy)_2(CH_3CN)Cl]$ was obtained.

$[Ir(ppy)_2(CH_3CN)Cl]$ ^1H NMR (400 MHz, CD_2Cl_2-d_2) δ 9.25 (2H, d, J = 5.4 Hz), 7.94 (2H, d, J = 8.1 Hz), 7.80 (2H, t, J = 7.9 Hz), 7.56 (2H, d, J = 7.9 Hz), 6.82 (4H, q,

J = 6.8 Hz), 6.60 (2H, t, J = 7.5 Hz), 5.87 (2H, d, J = 7.8 Hz), 1.97 (3H, s). ESI-MS (m/z): calculated for $[C_{24}H_{19}IrN_3]^+$ $[M]^+$: 542.1204; found: 542.1210.

The ECL probe in this work was prepared by chemically conjugating the Ir1-CH_3CN onto the cyclic peptide by coordination interaction via His within the cyclic peptide. In detail, 200 μL of 1 mM $[Ir(ppy)_2(CH_3CN)Cl]$ in CH_3CN was mixed with 800 μL of 12.5 μM cyclic peptide in 10 mM PB (phosphate buffer, pH 7.4, 0.0018 M NaH_2PO_4 and 0.0082 M Na_2HPO_4) for 2 h at room temperature.

1.3 Fabrication of ECL biosensor

Prior to its use, the glassy carbon electrode (GCE, 2.0 mm diameter) was carefully polished with 0.3 and 0.05 μm Al_2O_3 powder on fine polishing paper and then ultrasonically cleaned in water and ethanol.

Gold nanoparticles (AuNPs) with a diameter of ~12 nm were prepared by citrate reduction of $HAuCl_4$ in aqueous solution. In brief, 100 mL of 0.01% $HAuCl_4$ was brought to reflux, and then, 4 mL of 1% sodium citrate was introduced while stirring. The gold nanoparticles suspension was then kept boiling for another 30 min and left to cool to room temperature.

The mixture of AuNPs/Nafion was prepared by mixing 0.5% Nafion and AuNPs (v:v = 1:2) and sonicating for 30 min. Then, 10 μL of this mixture was dropped onto the surface of the cleaned GCE and allowed to dry to obtain AuNPs/Nafion/GCE. The AuNPs/Nafion/GCE was immersed in 100 μL Ir1-CP1 for 4 h and then into 100 μL of 1.0 mM MCH for 30 min. In each step, the electrodes were washed with 10 mM PB (pH 7.4). The obtained form Ir1-CP1/AuNPs/Nafion/GCE was used as ECL biosensor.

1.4 ECL measurement

A three-electrode system with modified GCE, a platinum counter electrode, an Ag/AgCl reference electrode (saturated KCl) was used. The prepared Ir1-CP/AuNPs/Nafion/GCE was dipped into glucose solution with different concentrations for 30 min. ECL measurement was performed in 1.0 mL of 0.1 M phosphate buffer solution (PBS, 0.018 M NaH_2PO_4, 0.082 M Na_2HPO_4, and 0.1 M KCl, pH 7.4) containing 50 mM TPA under triangular potential scan at 0.1 V/s unless otherwise stated. The voltage of the photomultiplier tube (PMT) was set to -900 V. The decrease peak of ECL intensity at 1.15 V ($\Delta I = I_0 - I_s$) was used as analytical signal for the determination of glucose, in which I_0 and I_s was the ECL intensity in the absence and presence of glucose, respectively.

2. Results

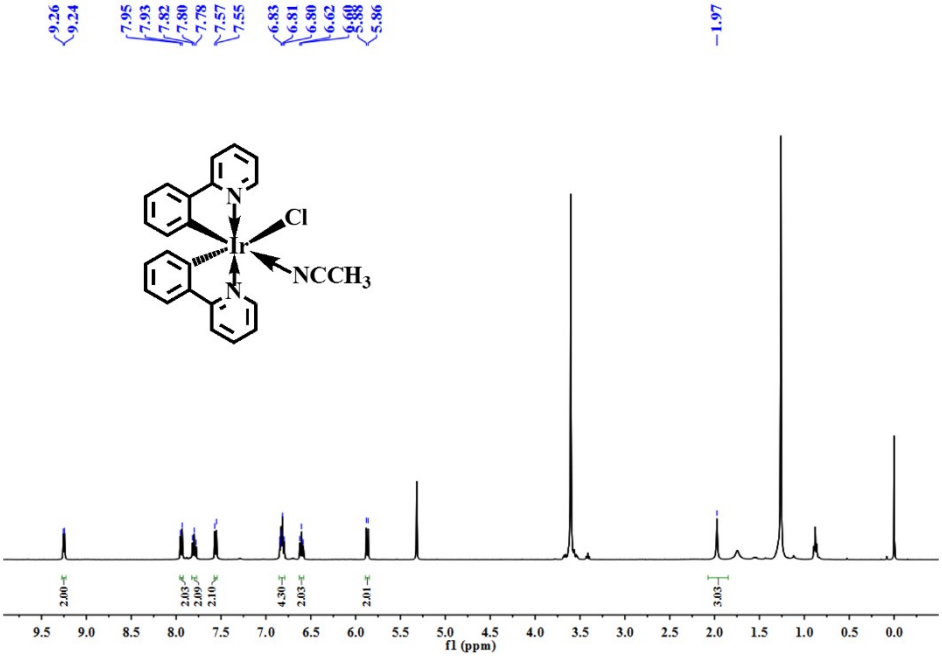

Fig. S1 ^{1}H NMR spectrum of $[Ir(ppy)_2(CH_3CN)Cl]$ in CD_2Cl_2-d_2. (400 MHz)

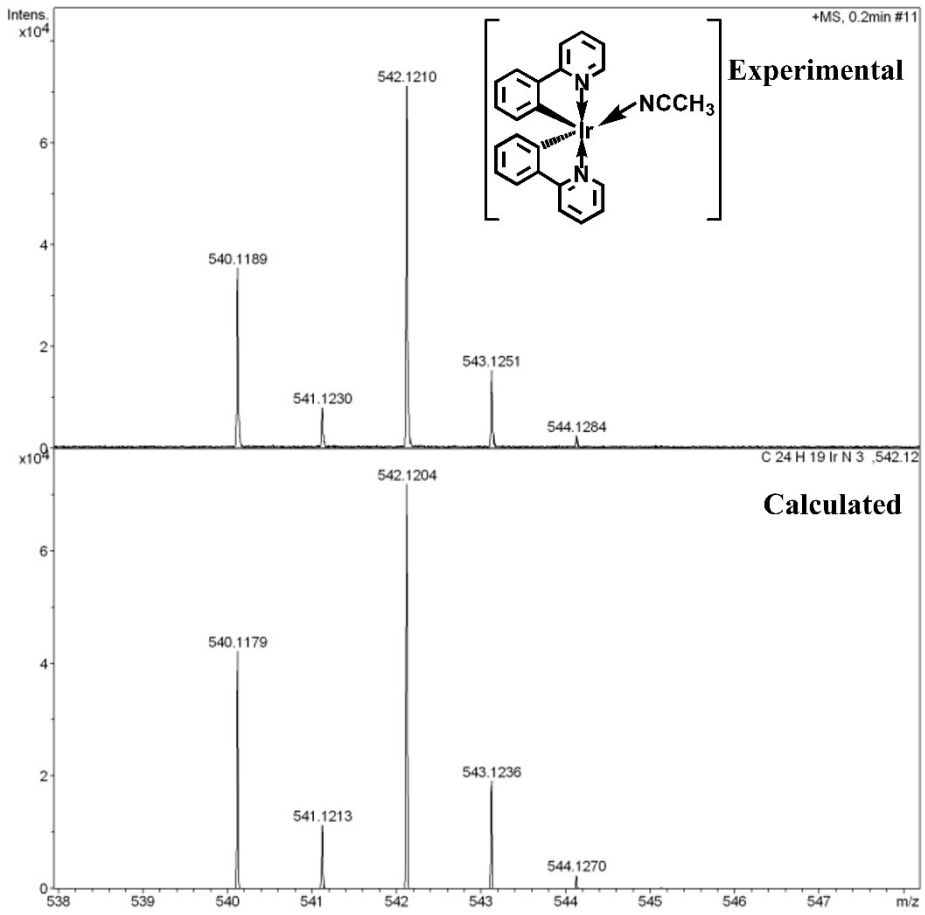

Fig. S2 Mass spectrum of $[Ir(ppy)_2(CH_3CN)]^+$ in methanol. $[M]^+$ 542.1210 (calculated, 542.1204)

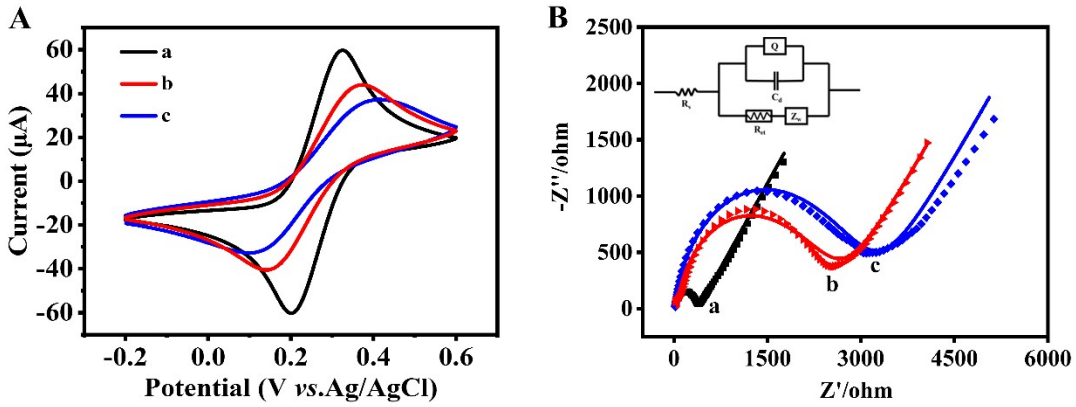

Fig. S3 (A) Cyclic voltammograms and (B) experimental (line) and simulating (dot) Nyquist plots of electrochemical impedance spectra obtained at (a) bare GCE, (b) AuNPs/Nafion/GCE and (c) Ir1-CP1/AuNPs/Nafion/GCE. The measurement conditions: 0.1 M PBS (pH 7.4) containing 5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4[Fe(CN)_6]$ (A) Scan rate, 100 mV/s. (B) The biased potential of 0.22 V, the frequency from 100 kHz to 0.1 Hz, and the amplitude of 5.0 mV.

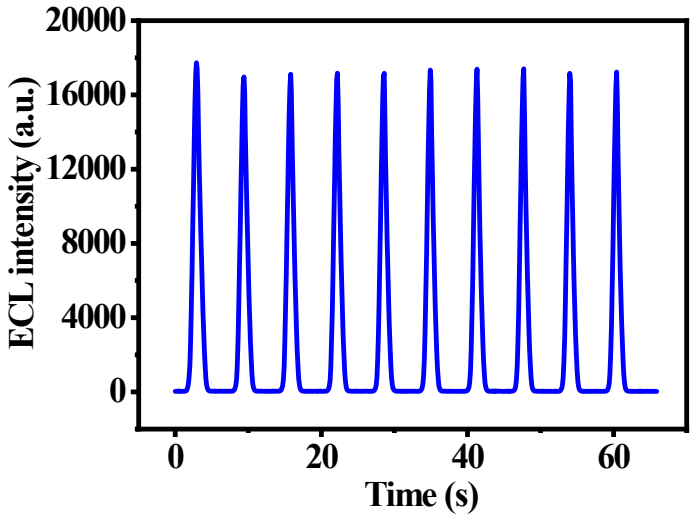

Fig. S4 Representative ECL signals of Ir1-CP1/AuNPs/Nafion/GCE obtained in 0.1 M PBS (pH 7.4) containing 50 mM TPA from continuous potential scanning over ten cycles between 0 and +1.6 V *vs.*Ag/AgCl with scan rate of 0.5 V/s.

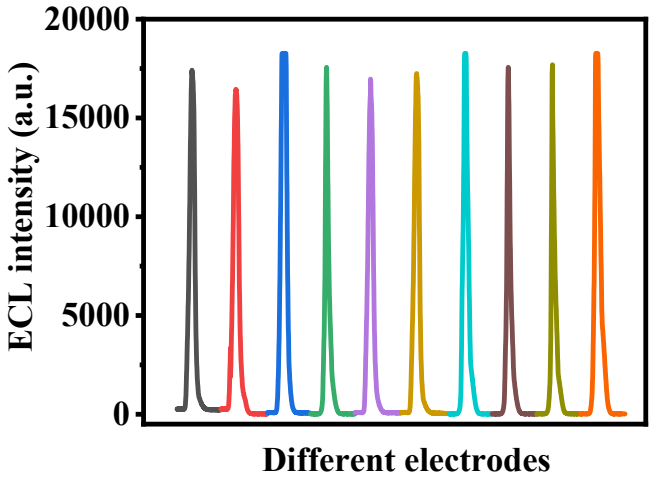

Fig. S5 ECL signals obtained in 0.1 M PBS (pH 7.4) containing 50 mM TPA from ten modified electrodes (Ir1-CP1/AuNPs/Nafion/GCE) by potential scanning over one cycle between 0 and + 1.6 V *vs.* Ag/AgCl with a scan rate of 0.1 V/s.

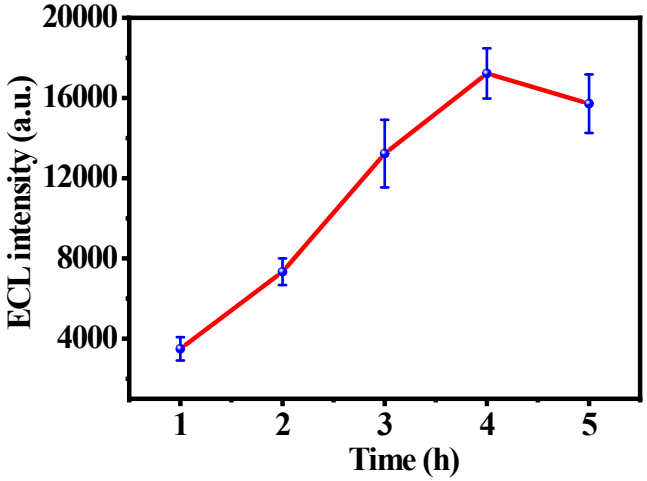

Fig. S6 Effect of self-assembly time of Ir1-CP1 on the ECL intensity of the obtained Ir1-CP1/AuNPs/Nafion/GCE.

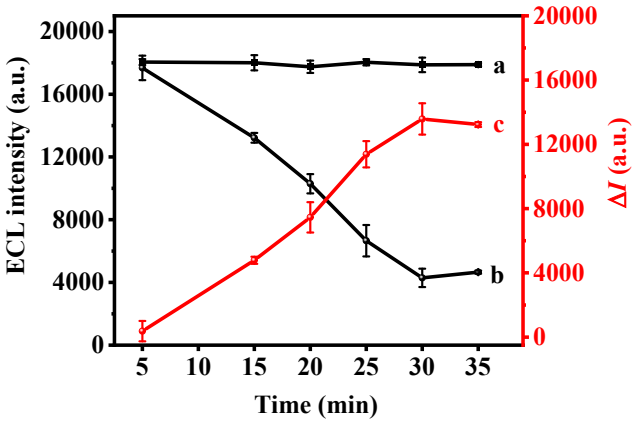

Fig. S7 Optimization of incubation time for 10 mM glucose. ECL intensity of Ir1-CP1/AuNPs/Nafion/GCE in the absence of glucose (I_0, a) and in the presence of glucose (I_s, b), $\Delta I = I_0 - I_s$ (c).

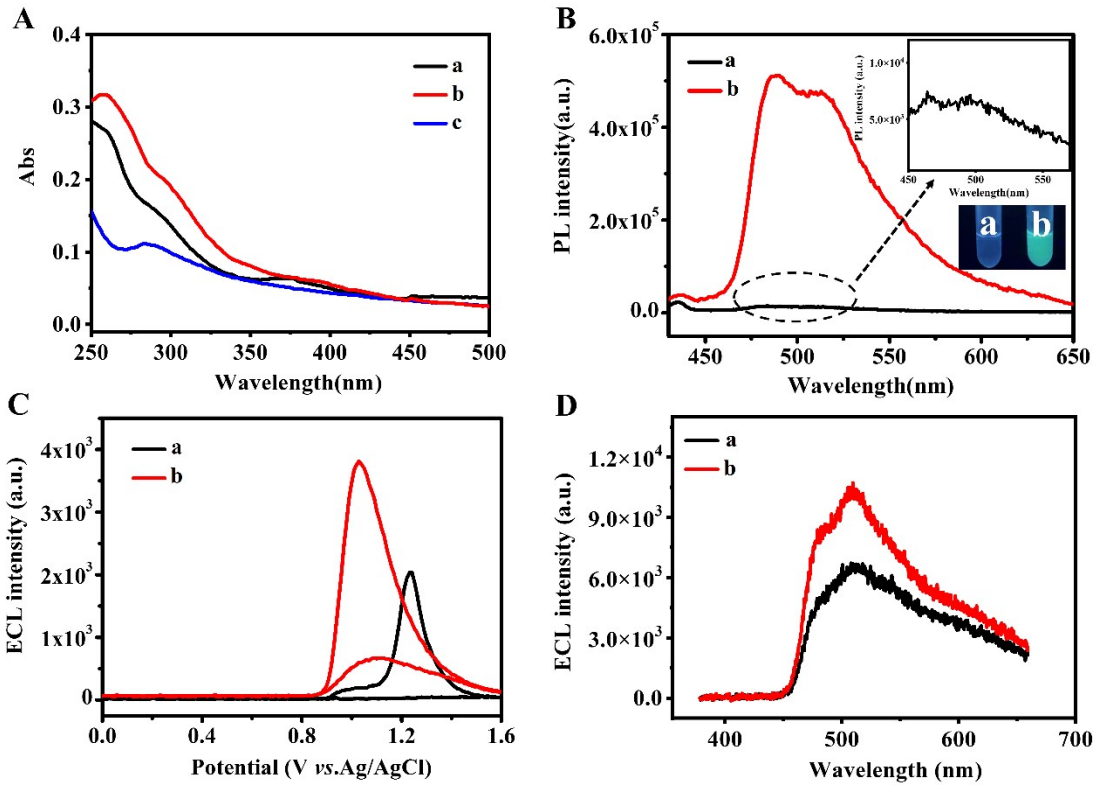

Fig. S8 UV-Vis absorption spectra (A) and PL spectra (B) of 5 μM Ir1-CH_3CN (a), 5 μM Ir1-CH_3CN-50 μM CP2 (b) and 50 μM CP2 (c) in 10 mM PB (pH=7.4). λ_{ex} = 380 nm. Insert, relative photographs under 365 nm irradiation. (C) ECL intensity *vs.* potential profiles of 1 μM Ir1-CH_3CN in the absence (a) and presence (b) of 10 μM CP2 in 0.1 M PBS (pH 7.4) containing 50 mM TPA. PMT: -900 V. Scan rate, 0.1 V/s. (D) ECL spectra of 10 μM Ir1-CH_3CN without (a) or 1 μM CP2 (b). ECL measurement

conditions: 0.1 M PBS (pH 7.4) containing 50 mM TPA by pulsing between 0 V and + 1.28 V (a), or pulsing between 0 V and + 1.18 V (b).

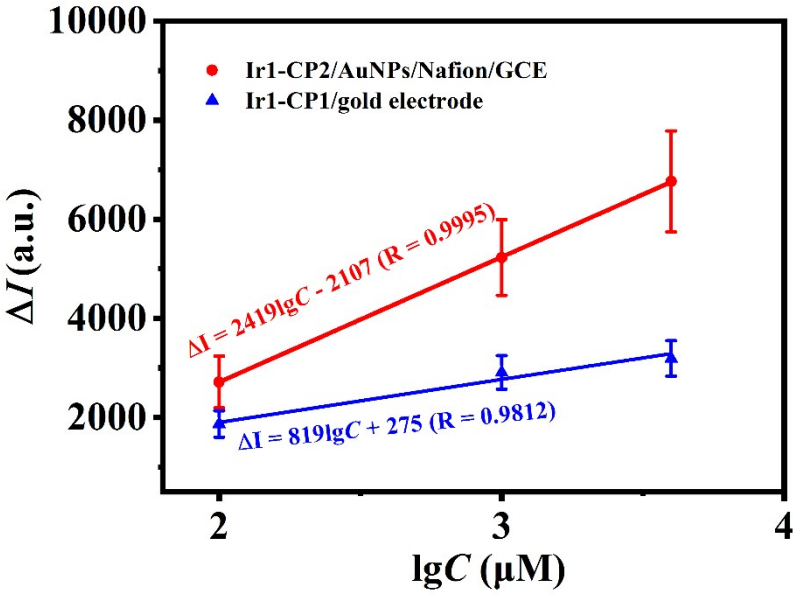

Fig. S9 Comparison of ECL response to glucose at Ir1-CP2/AuNPs/Nafion/GCE and Ir1-CP1/gold electrode.

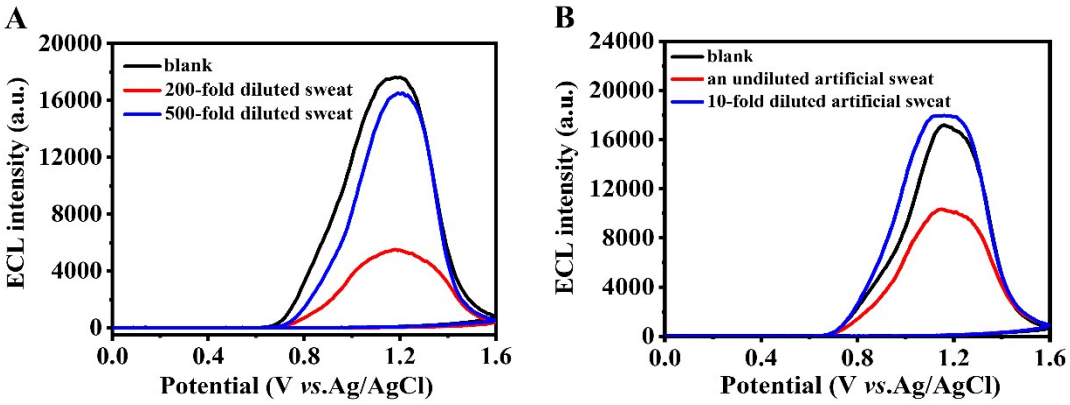

Fig. S10 ECL intensity vs. potential profiles of the ECL biosensor after incubation in different media. (A) (a) blank, (b) 200-fold diluted sweat, (c) 500-fold diluted sweat. (B) (a) blank, (b) undiluted artificial sweat, (c)10-fold diluted artificial sweat.

Table S1 Analytical performance of glucose in references and in this work.

Analytical method	Linear range	Detection limit	Sample	Ref.
Fluorescence	0.05-5 mM	50 μM	intracellular	4
Electrochemistry	20.0-400.0 μM	200 μM	human serum	5
Electrochemistry	0-100 μM	300 nM	sweat	6
Electrochemistry	1-111 mM	32.4 μM	blood	7
Electrochemistry	0.08-30 mM	27 μM	human serum	8
ECL	20 nM-12 mM	1.2 nM	sweat	9
ECL	5-80 μM	0.6 μM	human serum	10
ECL	0.1 mM-10 mM	70 μM	sweat	This work

Table S2 Recovery of glucose in 10-fold diluted artificial sweat sample.

Samples	Added (mM)	Found (mM)	Recovery (%)
1	0.1	0.093	93%
2	0.5	0.48	95%
3	1	1.13	113%
4	5	5.31	106%
5	10	10.96	109%

Table S3 Recovery of glucose in 500-fold diluted human sweat sample.

Samples	Added (mM)	Found (mM)	Recovery (%)
1	0.1	0.093	93%
	1	1.12	112%
2	0.1	0.11	110%
	1	1.07	107%
3	0.1	0.097	97%
	1	0.95	95%

3. References

1 Y. Zhou, K. Xie, L. Kong, F. Chen and D. Sun, *J. Electroanal. Chem.,* 2017, **799**, 122-125.

2 M. Qian, D. Zhang, H. Qi, X. Yang, G. Yin, C. Zhang, J. Guo and H. Qi, *Chin. Chem. Lett.,* 2023, **34**, 107336.

3 S. Lamansky, P. Djurovich, D. Murphy, F.A. Razzaq, H.E. Lee, C. Adachi, P.E.Burrows, S.R. Forrest and M. E. Thompson, *J. Am. Chem. Soc.,* 2001, **123**, 4304-4312.

4 S. Jiang, Y. Zhang, Y. Yang, Y. Huang, G. Ma, Y. Luo, P. Huang and J. Lin, *ACS Appl. Mater. Interfaces*, 2019, **11**, 10554-10558.

5 X. Xu, Y. Ma, S. Ding and Y. Li, *Microchim. Acta*, 2021, **188**, 1-7.

6 Q. Chen, Y. Liu, K. Gu, J. Yao, Z. Shao and X. Chen, *Biomacromolecules*, 2022, **23**, 3928-3935.

7 Z. Liang, J. Zhang, C. Wu, X. Hu, Y. Lu, G. Wang, F. Yu, X. Zhang and Y. Wang, *Biosens. Bioelectron.*, 2020, **155**, 112105.

8 Y. Yang, J. Yang, Y. He and Y. Li, *Sens. Actuators B Chem.,* 2021, **330**, 129302.

9 Y. Sun, P. Li, Y. Zhu, X. Zhu, Y. Zhang, M. Liu and Y. Liu, *Biosens. Bioelectron.*, 2021, **194**, 113600.

10 J. Ye, M. Yan, L. Zhu, J. Huang and X. Yang, *Chem. Commun.*, 2020, **56**, 8786-8789.