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Electronic Supplementary Information

Electrophoretic Separation in Microfluidic Paper-based Analytical Device with On-Column Wireless Electrogenerated Chemiluminescence Detector

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Reagents

All reagents were of analytical grade and directly used for the following experiments as supplied. Ultrapure water obtained from a Millipore water purification system (resistivity ≥ 18.2 MΩcm) was used in all assays and solutions. Amino acids were obtained from Shanghai Biochemical Co. Whatman chromatography paper #3MM, a medium thickness paper (0.34 mm) used extensively for general chromatography and electrophoresis, was purchased from GE Healthcare Worldwide and used with further adjustment of size (A4 size). Poly(dimethyldiallylammonium chloride) (PDDA) (20%, w/w in water, molecular weight = 200 000-350 000) was purchased from Alfa Aesar. Multi-walled carbon nanotubes (CNTs, diameter, 30-50 nm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). PDDA-CNTs were prepared according to the reported method [1]. CdS NPs (5.3 nm) were prepared in water [2].

Preparation of μ-PED

Wax was used as the paper hydrophobization and insulation agent to construct hydrophobic barrier on paper in this work. Wax-printing in bulk on A4 paper sheets was performed using a Xerox Phaser 8560N color printer set to the default parameters in a high-resolution printing mode for photoquality printing (Figure S1). Then the wax-printed paper sheet was baked at 130 °C for 150 s to let the printed wax melt and penetrate through the paper to form the hydrophobic patterns (Figure S2). The

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unprinted area (paper channel, paper reservoirs, and the ECL reporting zone) still maintained good hydrophilicity, flexibility, and porous structure and will not affect the further applications [3].

The ECL reporting zone was then modified through sequentially assembling positively charged PDDA-functionalized CNTs (PDDA-CNTs) and negative charged CdS NPs onto the surfaces of interwoven cellulose fibers in the ECL reporting zone (Scheme 1B4 and Scheme 2e) according to the procedure reported previously [1]. For the modification of ECL reporting zone, briefly, 20 μL solution of PDDA-CNTs and 20 μL solution of CdS NPs was alternately dropped into the ECL reporting zone and kept it for 10 min. After each dropping step, the ECL reporting zone was rinsed thoroughly according to the method demonstrated in our previous work [4]. Finally, the resulted paper sheet was dried and cut into individual μ-PED by scissors.

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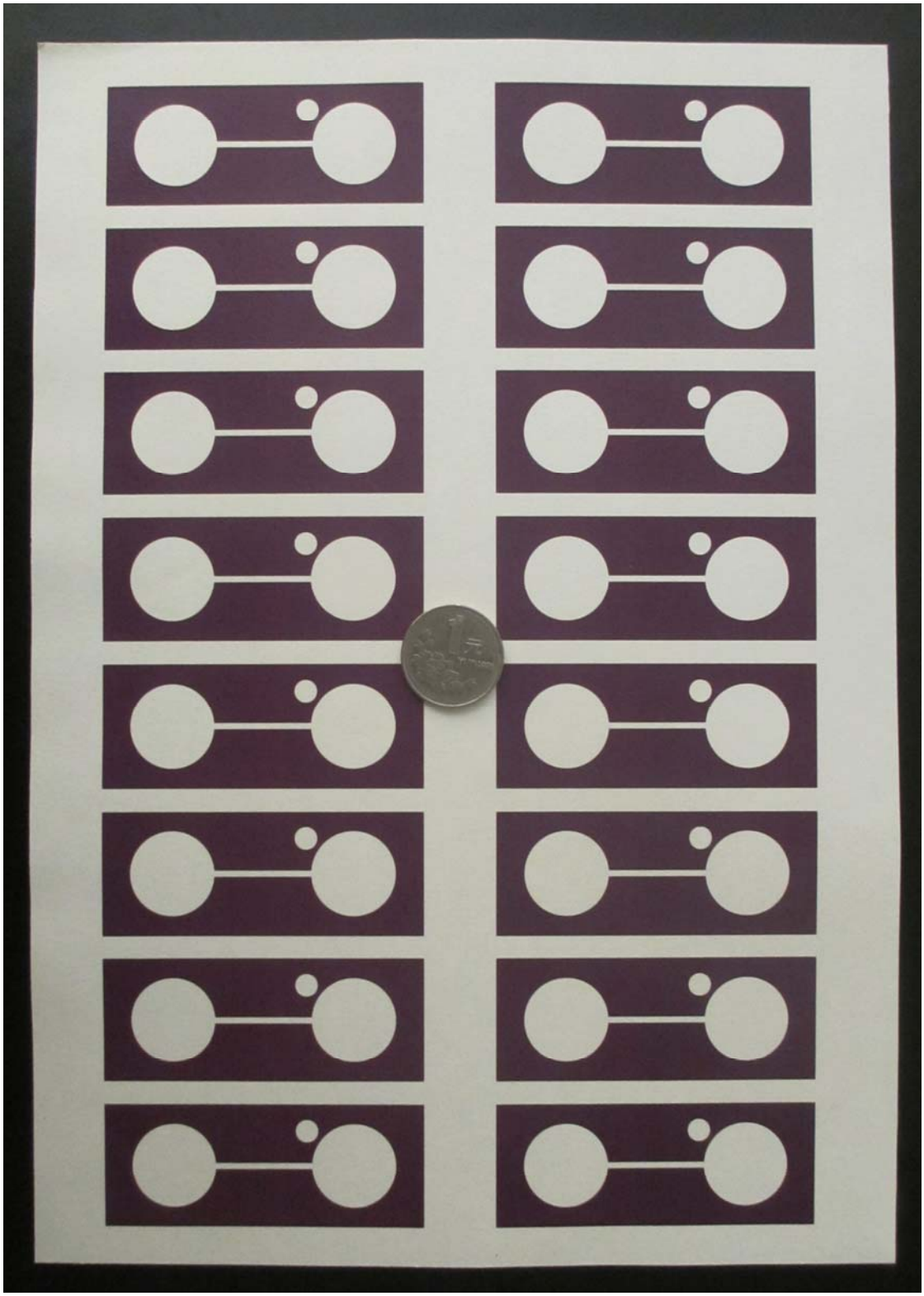

Figure S1. Wax-patterns of μ-PEDs on a paper sheet (A4) before baking

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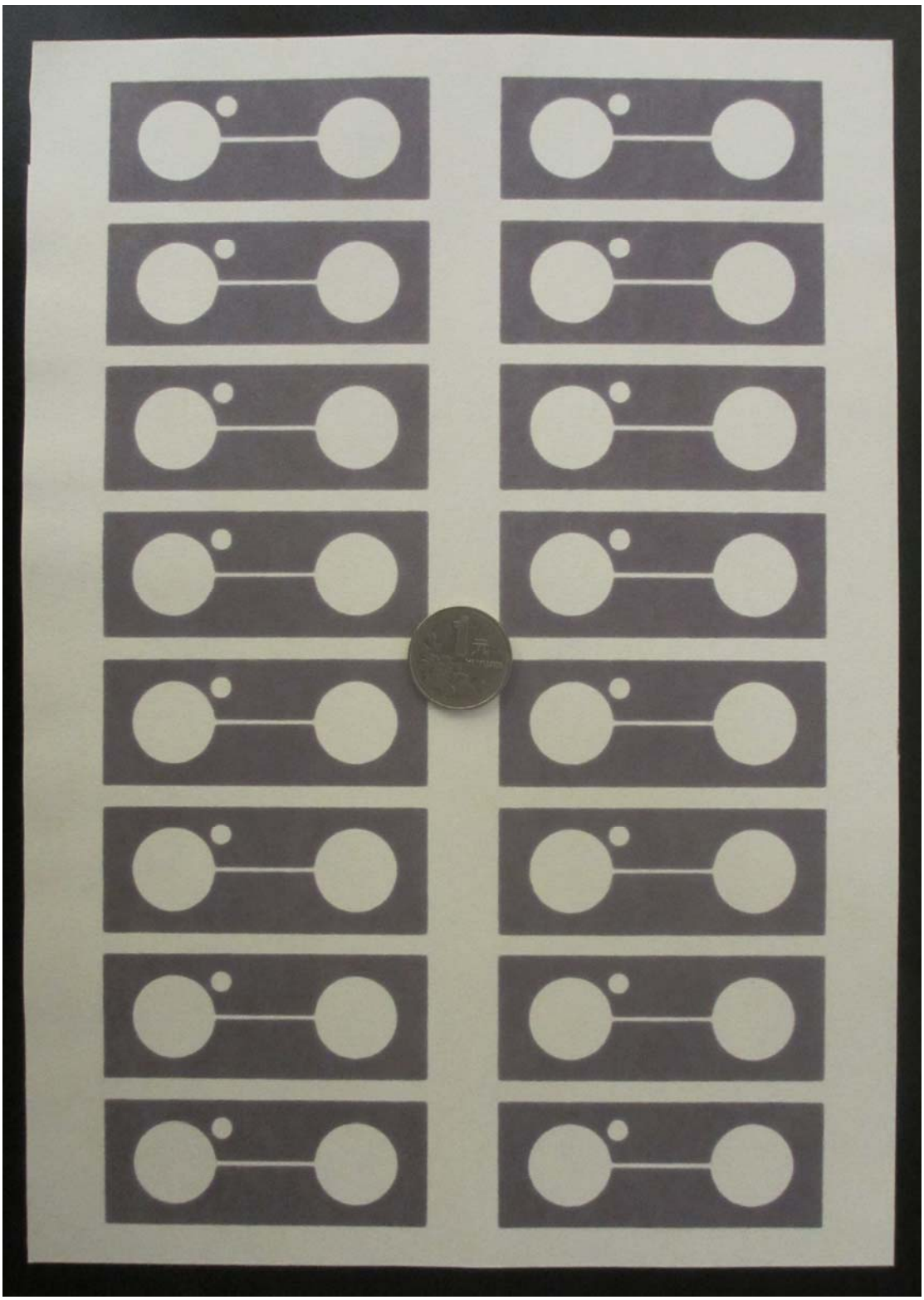

Figure S2. Wax-patterns of μ-PEDs on a paper sheet (A4) after baking.

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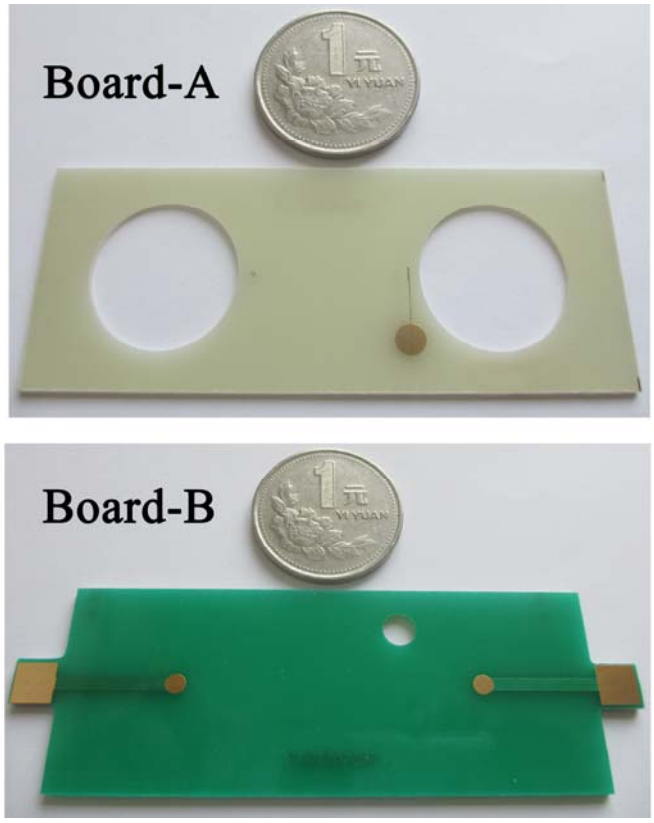

Figure S3. The picture of Board-A and Board-B.

Figure S4. The clamped μ-PED@ECL.

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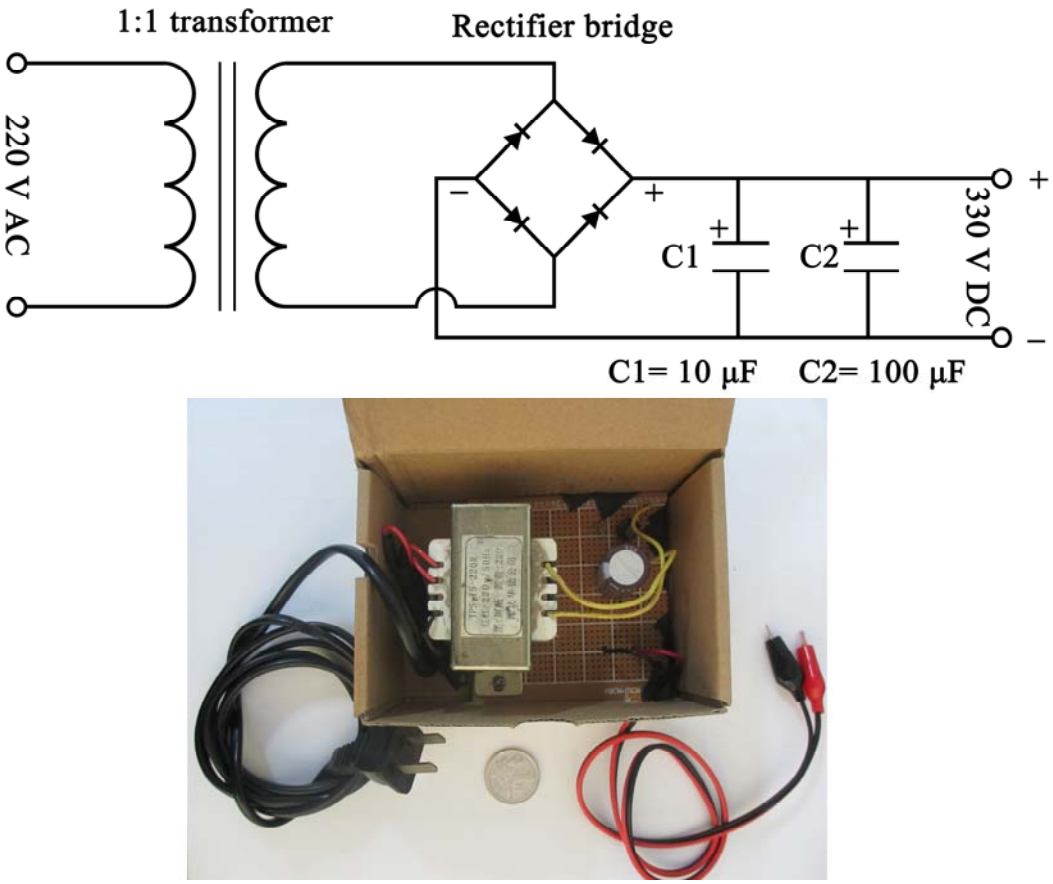
Design of the portable rectifier

In the conventional ECL electrophoretic methods, to drive the electrophoretic separation, an external electrophoretic power supply is required [5]. However, the expensive and sophisticated power supply makes the instrument complicated and departs from the portable and low-cost trend for μ-PADs. Hence, a strategy for substitution of these expensive and sophisticated electrophoretic power supply are highly deserved. In this work, a novel portable and low-cost rectifier was designed as a proof-of-concept electrophoretic power-supply, which could directly utilize the most commonly used chinese (as a model) alternating power supply (220 V), to drive the electrophoretic separation and triggering the ECL reaction in this μ-PED. The electronic circuit diagram for this rectifier is given in Scheme S1. All electronic components used in this rectifier were purchased from a local electronics store. In this rectifier, the 1:1 transformer was employed as a isolator to keep the rectifier safe for end users. Through the rectifer bridge and the two capacitors (C1 and C2), the alternating current (AC, 220 V) could be onverted into direct current (DC, 330 V), which was employed as the separation potential in this work. The component cost estimation of this home-made rectifer was shown in Table S1.



Scheme S1. Detail circuit diagram and picture of this rectifier.

Table S1. Component cost estimation of this home-made rectifer

Component	Cost
Transformer	$3.0
Rectifer bridge	$0.3
Capacitors (C1 and C2)	$1.2
Circuit board	$1.5
Total	$6.0
Conventional power supply for electrophoresis	$650-$5000

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Electrophoretic operation and assay procedures of this μ-PED

This μ-PED has separated the operational procedures into three main steps, included clamping the device, adding the solutions, and connecting the rectifer under a specific sequence. First, as shown in Scheme 1D and Figure S4, the μ-PED was clamped between the two compatibly designed circuit boards. The next stage of the μ-PED operation was the pre-wetting procedure of the separation channel, which was performed by dropping 0.1 mL electrophoresis medium into the two paper reservoirs (Scheme 1B5), respectively, through the pierced reservoir zones on Board-A (Scheme 1A1). The paper channel (Scheme 1B6) could be completely wetted after 2 min through the normal wicking action. After the pre-wetting procedure, 0.1 M PBS (pH 7.4) containing 0.2 M $K_2S_2O_8$ and 0.1 M KCl (10 μL) were added into the modified ECL reporting zone through the hole on Board-B (Scheme 1C8, 5.0 mm in diameter).

Then, the clamped μ-PED was put into a model cassette (demonstrated in our previous work [6, 7]) and connected with the rectifier, followed by the addition of sample solution (1 μL) into the separation channel through the sample zone on Board-A (Scheme 1A2, 1.0 mm in diameter). Immediately after that, the μ-PED in the cassette was left free of external light through closing the black cover [6, 7] to perforem electrophoretic separation. Electrophoresis in the μ-PED@ECL was driven by the rectifier (330 V), which was applied at the injection side with the detection side held at ground potential.

Table S2. Migration and detection of each amino acid under different ΔE_{BPE}

ΔE_{BPE} /V	54 pM Serine				0.12 nM Aspartic acid				0.67 nM Lysine			
	T_m[a] /s	RSD	I_{ECL}[b]	RSD	T_m[a] /s	RSD	I_{ECL}[b]	RSD	T_m[a] /s	RSD	I_{ECL}[b]	RSD
0.55	-	-	-	-	-	-	-	-	-	-	-	-
0.78	-	-	-	-	-	-	-	-	-	-	-	-
1.0	108	1.1%	60	3.4%	137	1.0%	71	4.4%	297	1.2%	65	4.0%
1.23	109	1.3%	151	4.6%	138	1.4%	90	4.3%	298	1.4%	138	4.8%
1.45	110	1.4%	509	3.6%	135	1.1%	141	3.5%	303	1.2%	327	3.9%
1.68	107	1.2%	1142	4.0%	136	1.1%	460	3.3%	301	1.1%	706	4.3%
1.9	108	1.0%	1710	3.8%	134	1.3%	849	4.5%	302	1.2%	1124	3.7%
2.13	106	1.2%	2102	3.6%	137	1.2%	1208	3.3%	297	1.4%	1546	4.2%
2.35	109	1.5%	2301	3.5%	135	1.1%	1445	3.7%	299	1.2%	1782	3.8%
2.58	107	1.4%	2028	4.3%	137	1.4%	1423	3.9%	300	1.1%	1751	3.9%
2.8	108	1.1%	1898	3.7%	138	1.5%	1378	4.2%	298	1.2%	1608	4.4%

[a] Average migration time of eleven repeats. [b] Average ECL intensity of eleven repeats.

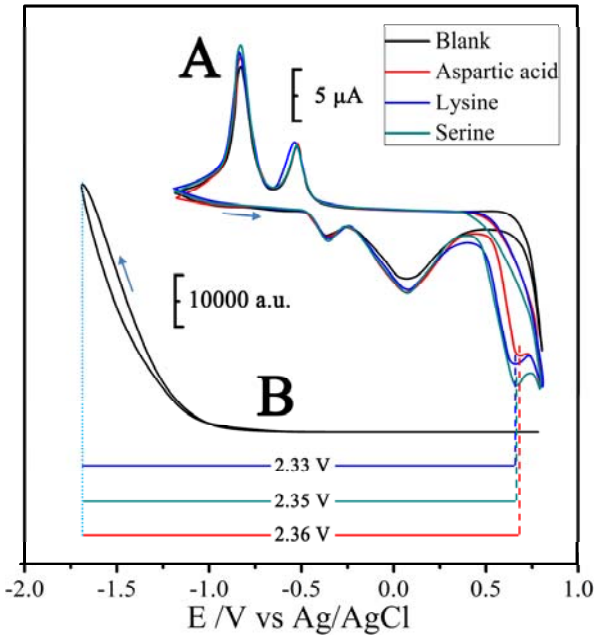

Figure S5. (A) Cyclic voltammograms of Cu electrode in 50 mM NaOH containing 0 mM and 0.01 mM amino acids; (B) ECL intensity-potential curve of CdS NPs modified gold electrode in 0.1 M PBS (pH 7.4) containing 0.2 M $K_2S_2O_8$ and 0.1 M KCl. Scan rate, 50 $mV \cdot s^{-1}$.

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Electrical coupling of this η-cBPE

In order to verify the coupling between electrochemical sensing of the three amino acids on the anodic pole and reporting of their existence by the cathodic ECL emission from the CdS NPs-$K_2S_2O_8$ ECL system on the gold cathodic end, the cyclic voltammograms (CVs) of the three amino acids and the ECL intensity-potential curve of the CdS NPs-$K_2S_2O_8$ ECL system were obtained in conventional three-electrode cell (Figure S5). For CV, the amino acids were present at a concentration of 0.01 mM respectively, the electrolyte was aqueous 50 mM NaOH, and the working electrode was copper electrode. In the case of ECL intensity-potential curve, gold working electrode was modified with CdS/PDDA-CNT according to the procedures reported previously [2, 8] and the electrolyte was 0.1 M PBS (pH 7.4) containing 0.2 M $K_2S_2O_8$ and 0.1 M KCl. In addition, Ag/AgCl reference electrode and platinum wire counter electrode were employed in the above investigations.

As shown in Figure S5A, the CV in blank electrolyte solution exhibited two anodic waves at ~-0.35 and ~+0.08 V, corresponding to the formation of Cu(I) and Cu(II) oxides, respectively, and the cathodic waves at -0.5 and -0.8 V represented the reduction back to Cu(1) and Cu(0). Upon the addition of amino acids, the CVs showed an obviously increased anodic currents in the +0.4 V - +0.8 V range due to the electrocatalytic oxidation of amino acids. The anodic peak potential for the electrocatalytic oxidation of each amino acid (indicated by the dashed lines in Figure S5) was: serine, 0.67 V; aspartic acid, 0.68 V; lysine, 0.65 V. ECL intensity-potential curve (Figure S5B) on the CdS/PDDA-CNTs modified Au electrode in 0.1 M PBS

(pH 7.4) containing 0.2 M $K_2S_2O_8$ and 0.1 M KCl showed one cathodic ECL peak at -1.68 V (indicated by the dotted line in Figure S5), resulting from the reaction between CdS NCs and $S_2O_8^{2-}$. The potential differences between the cathodic peak potential of the ECL process and the anodic peak potential of each amino acid were shown at the bottom of Figure S5.

Table S3. Separation and detection of these amino acids under different L_{SC}

L_{SC} /mm	54 pM Serine				R	0.12 nM Aspartic acid				R	0.67 nM Lysine			
	T_m[a] /s	RSD	I_{ECL}[a]	RSD		T_m[a] /s	RSD	I_{ECL}[a]	RSD		T_m[a] /s	RSD	I_{ECL}[a]	RSD
30	194	1.3%	1758	3.5%	1.67	238	1.5%	716	3.8%	4.54	535	1.4%	879	4.1%
25	148	1.4%	1993	3.3%	1.61	186	1.4%	1085	4.7%	3.78	410	1.3%	1237	3.7%
20	108	1.5%	2314	4.1%	1.52	136	1.5%	1453	3.4%	3.52	300	1.5%	1788	3.6%
15	72	1.3%	2657	3.6%	0.85	95	1.3%	1851	4.6%	2.68	203	1.4%	2124	4.4%
10	43	1.1%	2952	4.2%	0.33	54	1.2%	2226	3.4%	1.73	120	1.5%	2704	3.8%

[a] Average value of eleven repeats.

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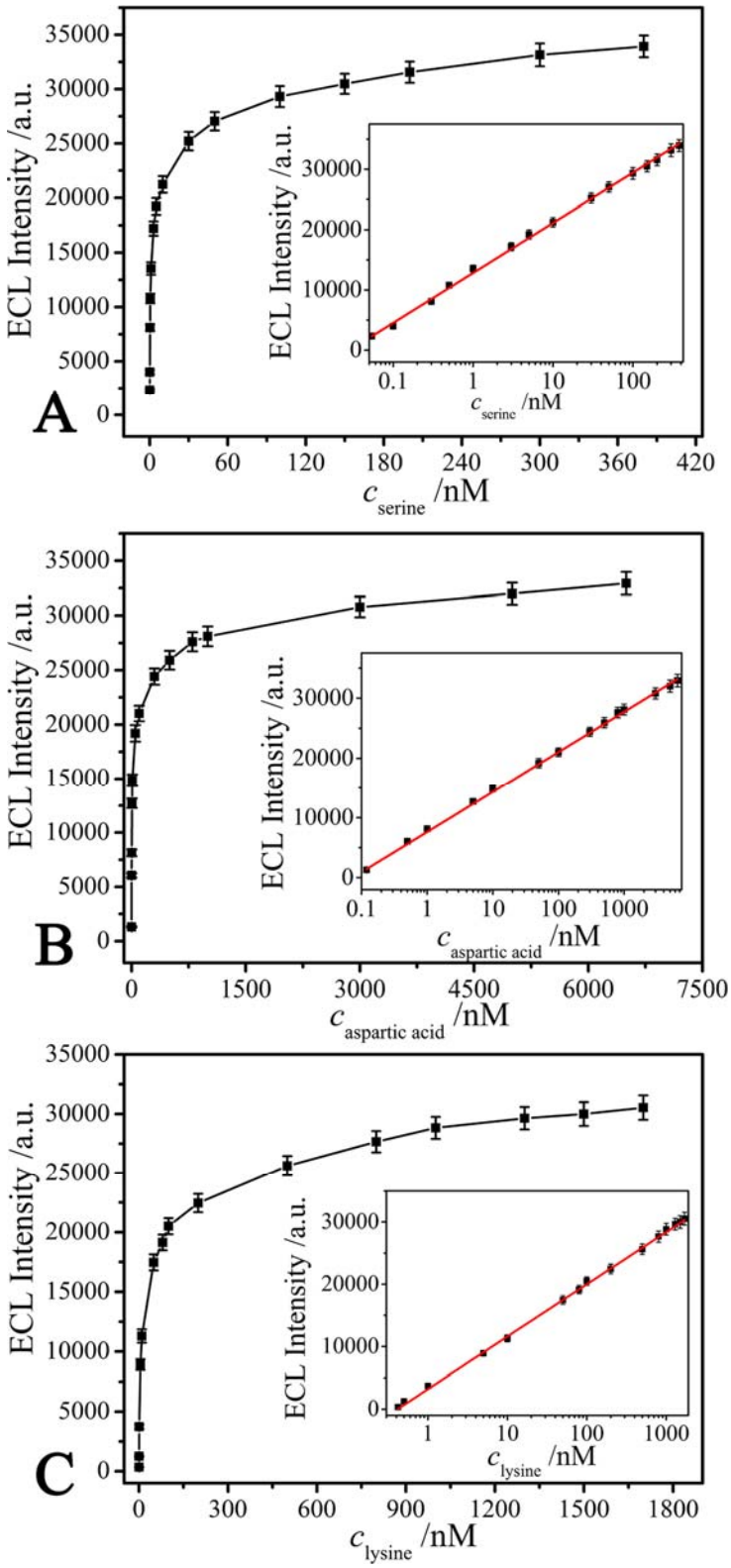

Figure S6. Relationship between ECL intensity and concentration of (A) serine, (B) aspartic acid, and (C) lysine. Insets: logarithmic calibration curve. N =11.

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