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Supporting information

Anodic electrochemiluminescence from $CsPbBr_3$ perovskite quantum dots for alkaline phosphatase assay

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Chemicals and Materials

Lead bromide ($PbBr_2$, 99.0%), oleic acid (OA, 85%), oleylamine (OAm, 80-90%) were purchased from Aladdin. Caesium bromide (CsBr, 99.5%) was obtained from Macklin Biochemical Co., Ltd. N, N-Dimethylformamide (DMF, 99.5%) and toluene (99.5%) were custom-made from Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). L-ascorbic acid (H_2A), alkaline phosphate (ALP), ascorbic acid 2–phosphate (AAP), horseradish peroxidase (HRP), acetylcholinesterase (AChE), glucose oxidase (GOx), L-Proline (L-Pro) and L-Arginine (L-Arg) were achieved form Yuanye Biotechnology Co., Ltd. (Shanghai, China). All aqueous solutions in the experiment were prepared with doubly distilled water.

Apparatus and Measurements

Ultraviolet-visible (UV−vis) absorption spectra were performed using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Fluorescence (PL) spectra were recorded on a Fluoromax-4 fluorescence spectrofluorometer (Horiba, USA). Transmission electron microscopy (TEM) measurements were taken on a JEM-2100 transmission electron microscope (JEOL Ltd.). Scanning electron microscopy (SEM) measurements were conducted on a Phenom ProX and Zeiss Ultra Plus scanning electron microscope. X-ray diffraction (XRD) patterns were recorded using a Rigaku Americas Miniflex Plus powder diffractometer. Electrochemical (EC) experiments were recorded with a CHI660E electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd. China). The ECL emission measurements were conducted on an MPI-M electrochemiluminescence analyzer (Xi'an Remex Analytical Instrument Co., Ltd., China).

Preparation of $CsPbBr_3$ QDs and $CsPbBr_3$ QDs|GCE

$CsPbBr_3$ QDs were prepared according to previous reported by Zeng et al[1] with trifling revise. Typically, equimolar amounts of CsBr (42.6 mg) and $PbBr_2$ (73.4 mg) were dissolved in DMF (5 mL) and stirred vigorously until the solids were completely dissolved. Then, a mixture of OA (0.5 mL) and OAm (0.2 mL) was rapidly added to stabilize the precursor solution. After 1 minutes, 1 mL of the precursor solution was quickly added into stirred toluene (10 mL). Strong green emission was appeared gradually after the injection. All operations were done at room temperature and did not require inert gas protection. The final yellow solution was centrifuged at 8000 rpm for 5 minutes, the supernatant was discarded and the precipitate was re-dispersed in 1 mL n-hexane.

Prior to use, the surface of glassy carbon working electrode (GCE, diameter 3 mm) was polished sequentially using 0.3 μm and 0.05 μm alumina slurry. Subsequently, GCE was carefully cleaned by ultrasonic with doubly distilled water and ethanol, and then dried under a nitrogen atmosphere. After that, 5 μL of the prepared $CsPbBr_3$ QDs hexane dispersion was cast onto the freshly cleaned GCE surface and evaporated in air to form a homogeneous film.

EC and ECL Measurement

The ECL-potential profiles and EC curves were measured with a three-electrode system including a GCE working electrode, a platinum (Pt)-wire counter electrode, and a saturated calomel electrode (SCE) reference

electrode. The electrolyte in EC and ECL analysis were conducted in 0.1 M PBS solution. The ECL and EC signals were performed via scaning between different potential ranges at the photomultiplier tube (PMT) voltage of 600V.

ALP determination

300 μL different concentration of ALP (0 to 4 U/L) and AAP (200 μL, 500 nM) were incubated for 30 min at 37 °C. Then, 400 μL mentioned reactants were served as electrolyte in EC and ECL analysis. The ECL and EC spectra were recorded for the detection of ALP.

ECL spectra of $CsPbBr_3$ QDs

Fluorescence (FL) is mainly caused by the excitation and emission of the core of quantum dots, while the ECL process mainly come from the annihilation of the surface electrons and holes of the quantum dots. Comparing the ECL spectrum with the FL spectrum is conductive to identify the excited states in the ECL process. Fig. S2 shown the ECL scattered points obtained from the filter diaphragm simulation in the potential range of 0-1.0 V. By performing curve simulation on these scattered points, the spectral wavelength corresponding to the maximum ECL intensity was 506 nm, which was in good agreement with the FL peak position, indicating the emission of ECL and FL has a similar mechanism, i.e. the radiative electron relaxation of the $CsPbBr_3$ QDs* excited state in the eigen band gap.

optimizing conditions of ALP assay

ALP catalyzes the hydrolysis of a variety of orthophosphoric monoesters to yield phosphate and the corresponding alcohols/phenols. In this detection platform, AAP is chosen as the substrates of ALP, which can be converted to H_2A and phosphate. The concentration of AAP is the key to the success of the experiment, because the concentration of AAP directly affects the production of H_2A. As shown in Fig. S3, the ECL increasing rate increased gradually with the increasing of AAP and reached a plateau when AAP concentration was 500 nM. Therefore, 500 nM was chosen in the further experiments. The enzyme activity of ALP depends on the pH value of the buffer solution and reaction time. A detection PBS pH of 8.0 was found for the best ECL increasing rate (Fig. S4). The effect of reaction time on production system of H_2A was chosen as 30 min (Fig. S5). Therefore, pH 8.0 of PBS and 30 min of reaction time was chosen in the further experiments.

Limit of Detection Calculation

The limit of detection was calculated based on corresponding literatures[2], an ECL measurement for blank samples was executed with three parallel tests first, which exhibited average ECL intensity (I_B) of 622 with standard deviation (S_B) of 21.378. With signal-to-noise ratio value (k) of 3, the smallest detectable signal (I_L) could be calculated as:

$I_L = I_B + k\,S_B = 686.1327$

I_L refers to the I value, I_L=I=686.1327

I_0 was the ECL intensity of $CsPbBr_3$ QDs in the absence of ALP, I_0=639

$(686.1327-639)/\ 686.1327 = 0.35 \lg C_{ALP} + 1.17$

$\lg C_{ALP}$ =-3.15, then C_{ALP} =0.000714 U/L.

Thus, the limit of detection was calculated to be 0.714 mU/L by the linear equation.

Evaluation of stability, specificity and reproducibility for ECL sensor

Stability, reproducibility and selectivity are the three important indicators to evaluate an ECL sensor. In this study, the ECL emission of different concentration of ALP in the range of 0.015 U/L to 15.625 U/L under consecutive potential scanning for three cycles was recorded to attest its stability. As demonstrated in Fig. S6, the proposed ECL biosensor possessed relatively stable at diverse concentration of ALP with the relative standard deviation (RSD) of 0.78-1.34%.

Simultaneously, the reproducibility of the ECL biosensor was monitored by five parallel measurements with the same concentration of ALP. In order to reduce the absolute deviation due to the difference between the electrodes, the ECL sensor used the same working electrode in this work. As shown in Fig. S7, the RSD was calculated to be approximately 1.15%, indicating benign stability of the designed ECL system.

The selectivity of the proposed ECL sensor was evaluated by ECL intensity toward blank and several interferents including GOx, HRP, AChE, L-Pro, L-Arg, K^+ and Na^+. As can be seen form Fig. S8, the ECL signal of the interfering substances were nearly identical to the blank. In addition, when these interfering substances were present in ALP, the ECL intensity induced by ALP had no prominent disparity, implying the outstanding selectivity of the propounded sensor.

Real sample analysis

To evaluate the possibility of the as-proposed ALP sensor for clinical application, a series of samples were prepared by adding different concentrations of ALP to human serum obtained from Lianshui People's Hospital (standard addition method). The results (Tables S2) exhibited that satisfactory recoveries (98.5–105%) were obtained, indicating that the proposed ECL-sensor has good analytical performance for specific ALP in real serum.

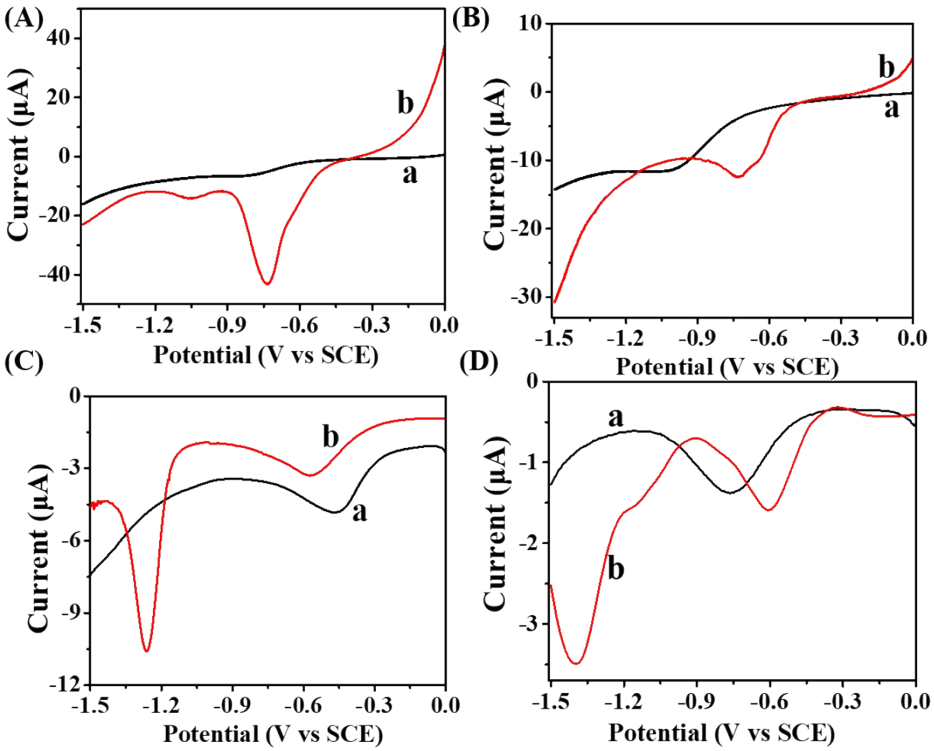

Fig. S1 LSV profiles of bare GCE (a, black line) and $CsPbBr_3$ QDs|GCE (b, red line) in PBS (A) and 10 mM H_2A (B). DPV profiles of bare GCE (a, black line) and $CsPbBr_3$ QDs|GCE (b, red line) in PBS (C) and 10 mM H_2A (D).

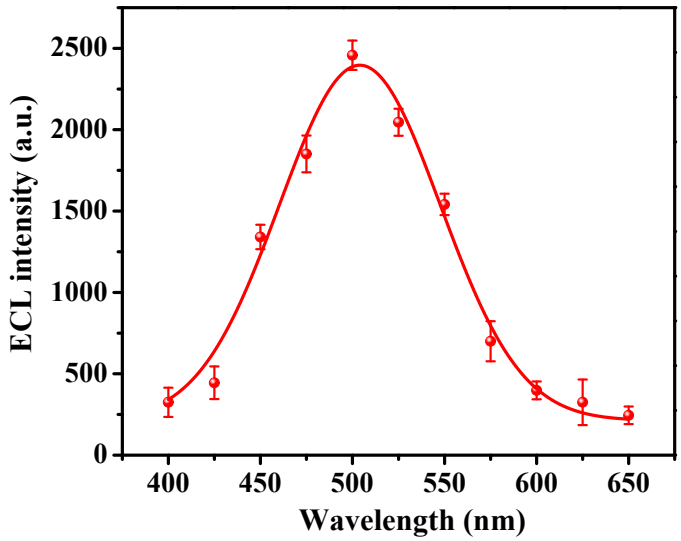

Fig. S2 ECL spectra of $CsPbBr_3$ QDs.

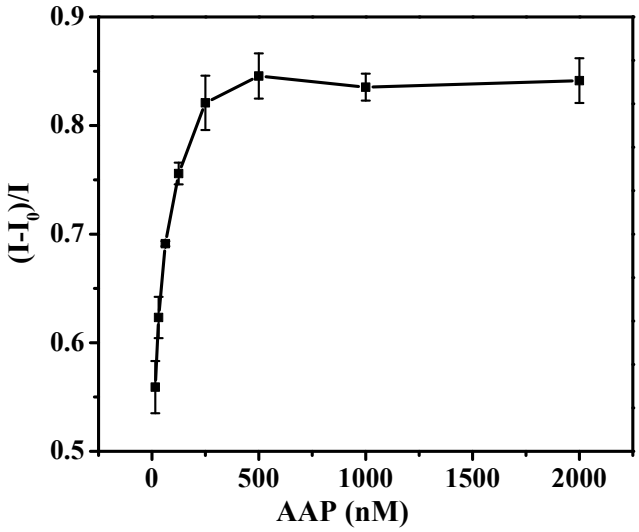

Fig. S3 The effect of the concentration of AAP on ECL sensor in the presence of ALP.

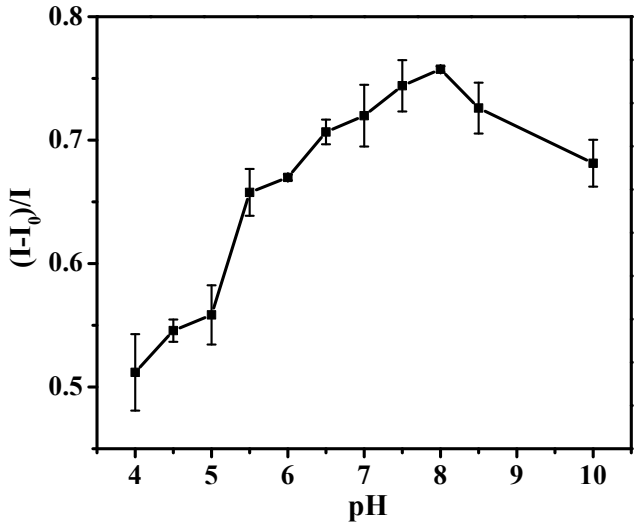

Fig. S4 The effect of the pH of PBS on ECL sensor in the presence of ALP.

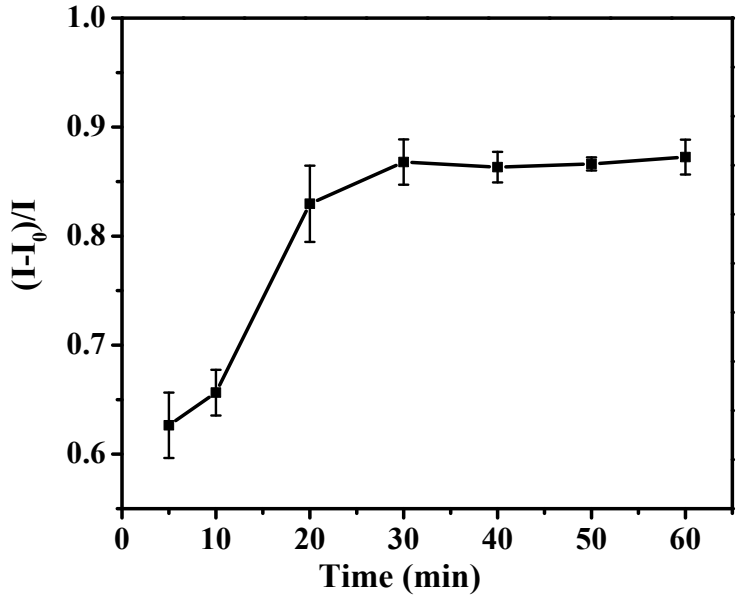

Fig. S5 The effect of reaction time on ECL sensor in the presence of ALP.

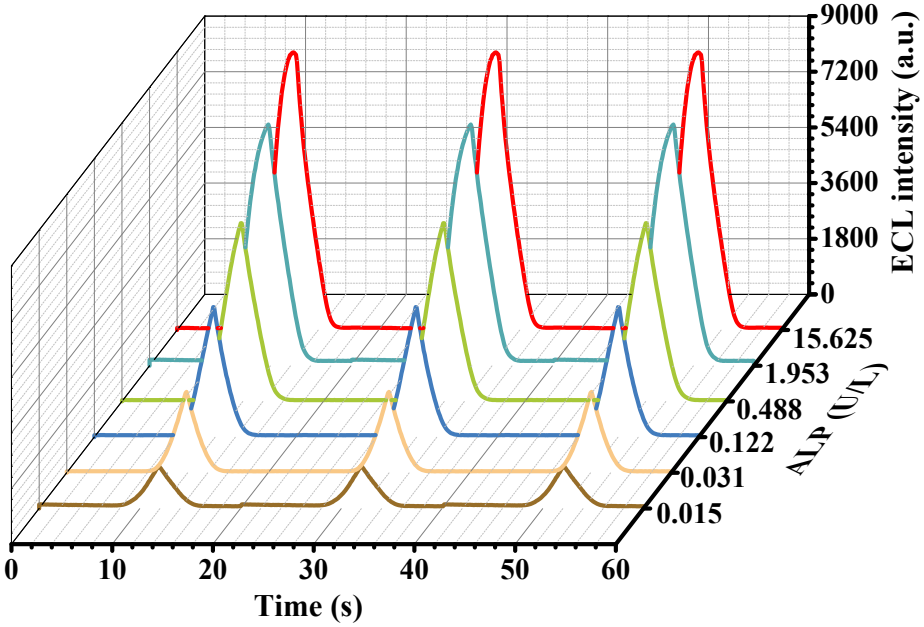

Fig. S6 ECL signals under continuous scan at different concentration of ALP.

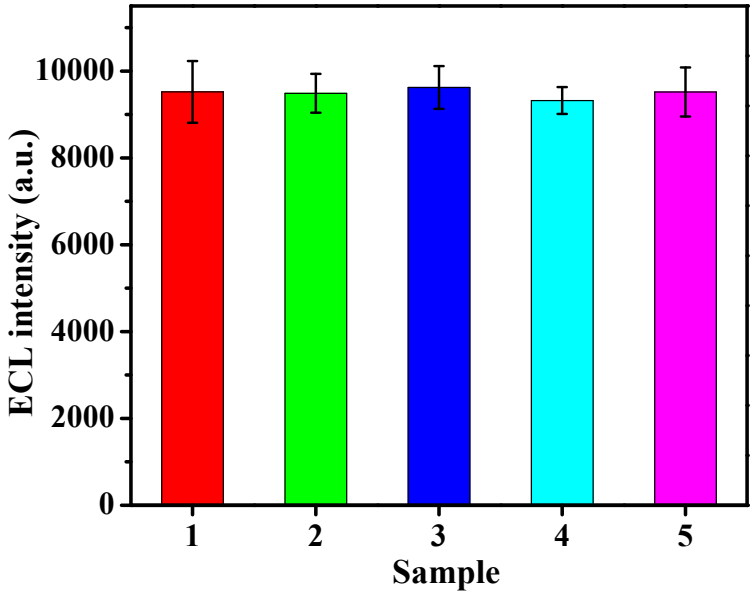

Fig. S7 Reproducibility studies of the ECL sensor.

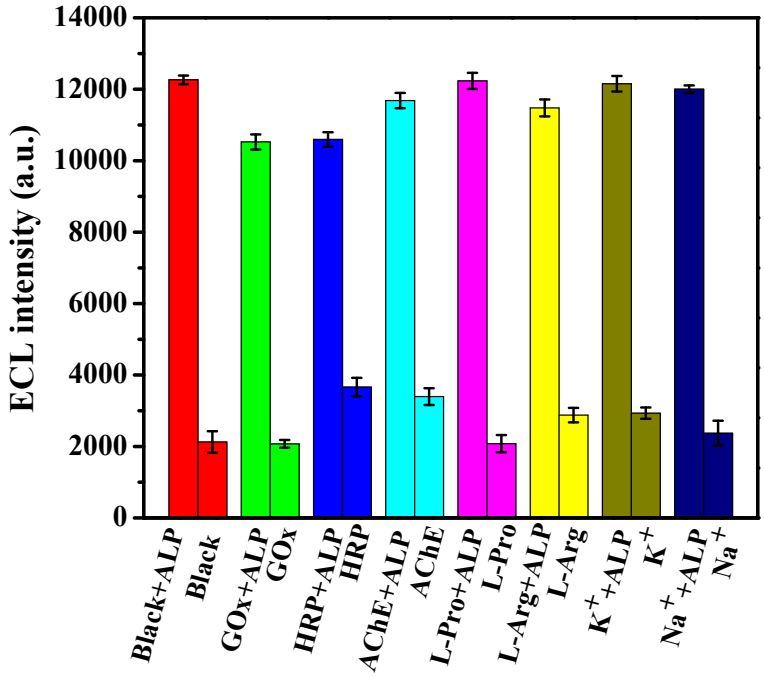

Fig. S8 The selectivity of adding the interfering substances to the ALP sensor.

Table S1. Various sensing strategies for detection of ALP.

Method	**Materials**	**LOD (U/L)**	**Ref.**
Colorimetry	Au NPs	27	[3]
Fluorescence	Coumarin@Tb-GMP	10	[4]
Fluorescence	$CuInS_2$ QDs	3.6	[5]
Electrochemiluminescence	CdSe NPs	2.0	[6]
Fluorescence	CQDs	1.4	[7]
Electrochemical	Fc	0.4	[8]
Fluorescence	Au/Ag NCs	0.00076	[9]
Electrochemiluminescence	$CsPbBr_3$ QDs	0.00071	This work

Table S2. ALP detection in serum samples by this ECL sensor.

Number	Added (U/L)	Found (U/L)	Recovery (%, n = 3)	RSD (%, n = 3)
Serum 1	0.02	0.0204	102.0	2.08
	0.05	0.0504	100.8	0.57
	0.10	0.0985	98.5	1.09
Serum 2	0.02	0.0205	102.5	0.32
	0.05	0.0517	103.4	0.50
	0.10	0.0995	99.5	2.42
Serum 3	0.02	0.021	105.0	0.53
	0.05	0.0503	100.6	0.26
	0.10	0.103	103.0	1.32

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