Electronic Supporting Materials (ESM):

Electrochemiluminescence dual "turn-on" strategy for alkaline phosphatase detection using dual quenching Ru(bpy)$_3$$^{2+}$ encapsulated zeolite imidazole metal organic framework

Experimental section

Materials and reagents.

Tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate, alkaline phosphatase (bovine intestinal mucosa) and goat serum samples, tripropylamine (TPA) were purchased from Sigma-Aldrich (Beijing, China). Imidazole-2-carboxaldehyde (2-ICA), zinc acetate dihydrate (Zn(CH$_3$COOH)$_2$·2H$_2$O), 2,6-dichloroindophenol sodium salt hydrate (DCIP) were purchased from Macklin (Shanghai, China). 2-Phospho-L-ascorbic acid trisodium salt (AA2P) was purchased from Aladdin (Shanghai, China). Tris-HCl buffer solutions (0.05 M) were used throughout all the experiments. All these chemicals were analytical-reagent grade and used without any further purification.

Instrumentation.

All electrochemiluminescence measurements were performed by the MPI-A capillary electrophoresis electrochemiluminescence detector produced by Changchun Institute of Applied Chemistry (Changchun, China) and Xi'an Remex Electronics Company (Xi'an, China). Glass carbon electrode (GCE) (φ = 3 mm) was used as working electrode, platinum wire as counter electrode and Ag/AgCl as reference electrode (saturated KCl). GCE working electrode was polished with 0.05 μm alumina before each use and then cleaned with ultrapure water in an ultrasonic bath. ECL signals were amplified by photomultiplier tubes and transmitted to the computer. Scanning electron microscopy (SEM) images were taken by a SU8020 scanning electron microscope (Hitachi, Japan). Transmission electron microscopy (TEM) and electron energy-loss spectroscopy (EELS) images were respectively recorded on a FEI Tecnai G2 F30 and OXFORD microscope (Hitachi, Japan). UV-visible absorption spectra were performed on UV2550 spectrophotometer (Shimadzu, Japan).

Synthesis of Ru(bpy)$_3$$^{2+}$@ZIF-90

According to the reported work with some modifications, Ru(bpy)$_3$$^{2+}$@ZIF-90 were synthesized via a one-step self-assembly of Zn^{2+} and imidazole-2-carboxyaldehyde (2-ICA).[1] Firstly, Ru(bpy)$_3$$^{2+}$ (2 mL, 10 mM), Zn(CH$_3$COOH)$_2$·2H$_2$O (2 mL, 0.1 M) and 2-ICA (2 mL, 0.2 M) were added to the glass flask and kept stirred vigorously for 15 min. Then, 6 mL of DMF was added and stirred at room temperature (25°C) for 14 h. The resulting precipitates were washed three times with DMF, methanol and ultrapure water by centrifugation in order to remove the supernatant. Finally, the precipitates were resuspended with 5 mL water to obtain Ru(bpy)$_3$$^{2+}$@ZIF-90

solutions and were stored in 4 °C refrigerator. Before each use, Ru(bpy)$_3^{2+}$@ZIF-90 solutions were ultrasonically dispersed. 20 μL of Ru(bpy)$_3^{2+}$@ZIF-90 was used in each measurement.

ECL dual "turn-off" and "turn-on" detection of ALP

AA2P (20 μL, 5 mM) firstly reacted with different concentrations of ALP in tris-HCl buffer pH 8.6 (200 μL, 50 mM) for 15 minutes. Then 20 μL of Ru(bpy)$_3^{2+}$@ZIF-90 solutions and DCIP (40 μL, 1 mM) were added to the above sample. The whole mixtures were then incubated for 5 minutes. Finally, TPA (20 μL, 50 mM) and appropriate water were added to keep the final volume of the whole solutions at 1 mL. After thoroughly vortex mixed, ECL spectra were recorded. Potential scan range is kept from 0 V to 1.4 V to 0 V. Scan rate is kept at 0.1 V/s. Photomultiplier tube voltage (PMT) is kept at 1000 V.
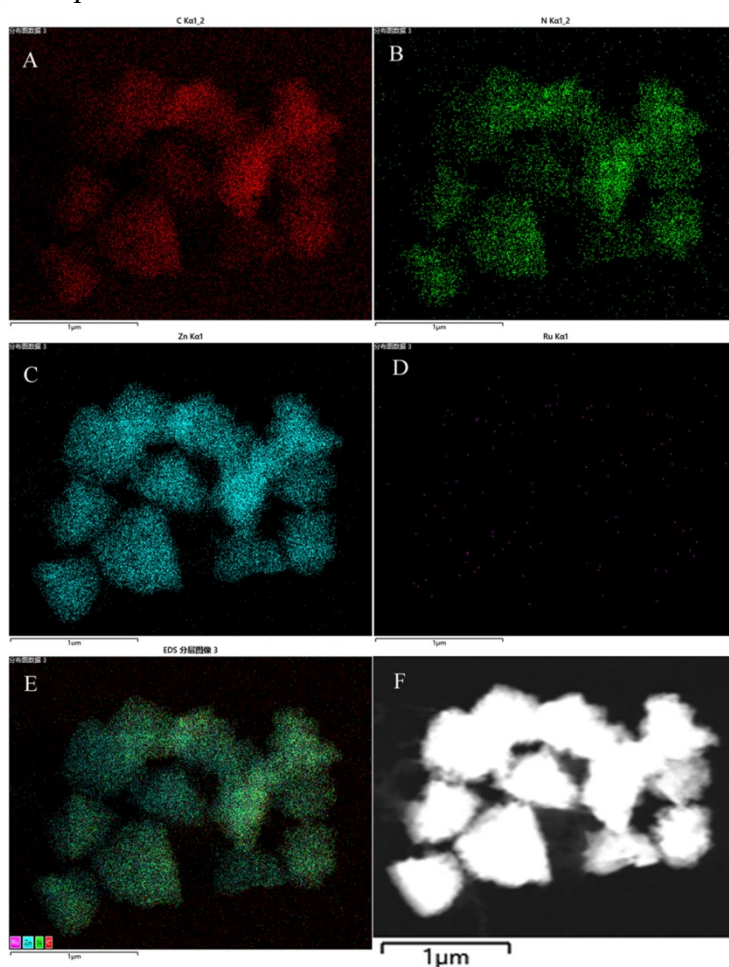

Figure S1. STEM-EELS elemental maps of C (A), N (B), Zn (C), Ru (D) inside Ru(bpy)$_3^{2+}$@ZIF-90, overlay of four micrographs (E) and TEM image of overlay of four micrographs (F). The scale bars are 1 μm.
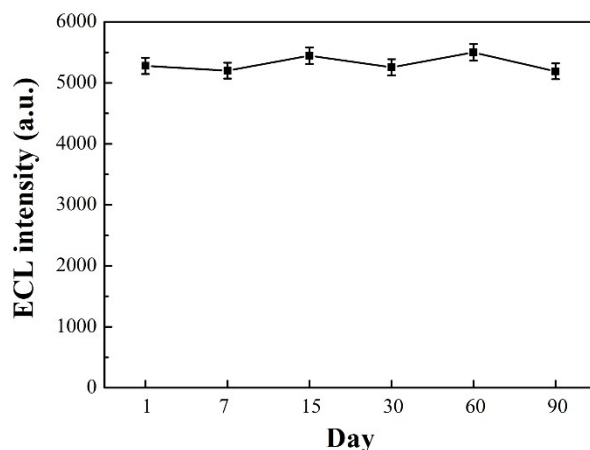
Figure S2. The stability of of the synthesized Ru(bpy)$_3^{2+}$@ZIF-90 solution during three months. All the error bars represent the standard deviation of three measurements.

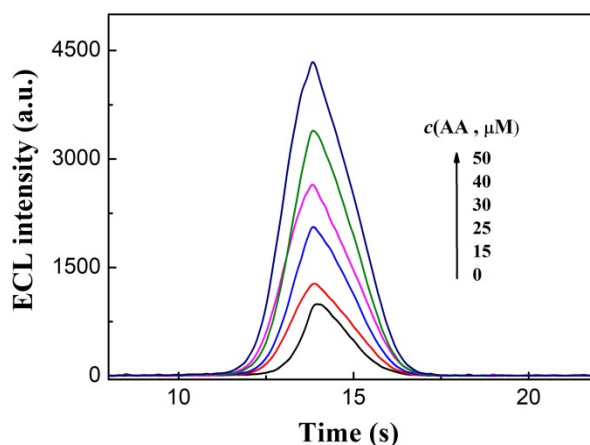**Figure S3.** ECL intensity-time profiles of Ru(bpy)$_3^{2+}$@ZIF-90 applied in ECL "turn-on" detection of AA. c(AA): 0, 15, 20, 30, 40 and 50 µM; c(DCIP): 50 µM; c(TPA): 1 mM; 10 m Tris-HCl buffer solutions: pH 8.6; Ru(bpy)$_3^{2+}$@ZIF-90: 30 µL. Scan range: from 0 to 1.4 to 0 V; Scan rate: 0.1 V/s; Photomultiplier tube voltage: 1000 V.

Figure S4. The effect of different concentrations of PO$_4^{3-}$ on Ru(bpy)$_3^{2+}$@ZIF-90. c(PO$_4^{3-}$, mM): 0, 0.1, 0.2, 0.3, 0.4 mM; c(TPA): 1 mM; 10 mM Tris-HCl buffer

solutions pH 8.6; Ru(bpy)$_3^{2+}$@ZIF-90: 30 μL. Scan range: from 0 to 1.4 to 0 V; Scan rate: 0.1 V/s; Photomultiplier tube voltage: 1000 V.
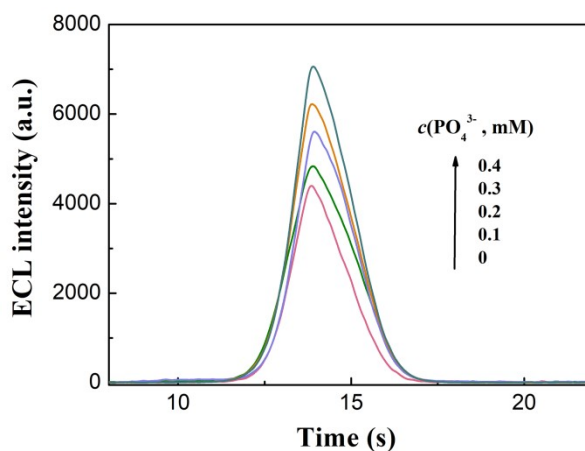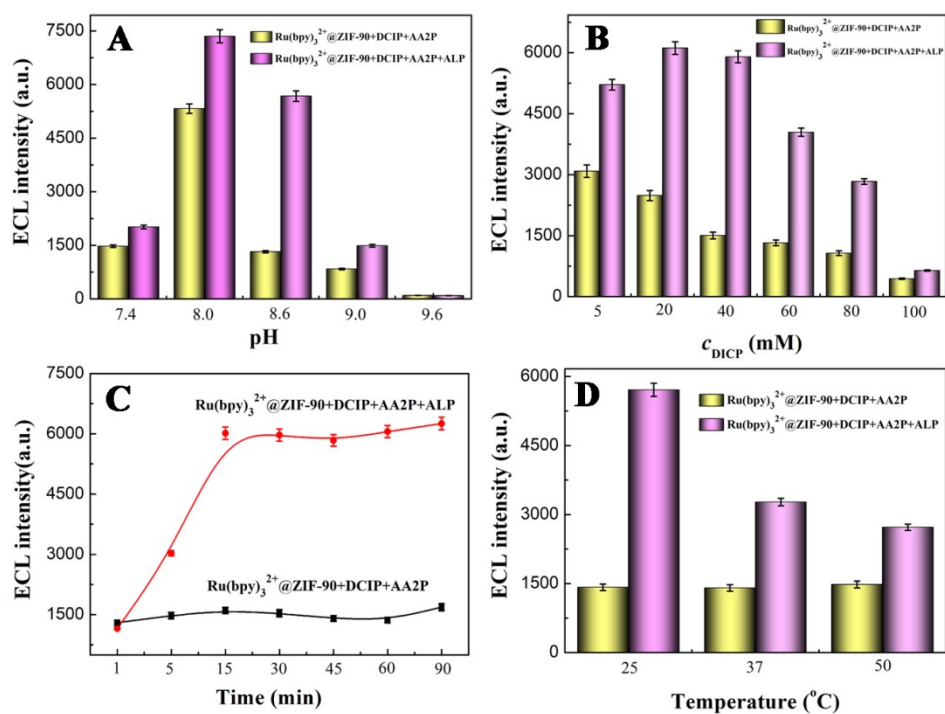

Figure S5. The effect of different pH (A), the concentrations of DCIP (B), the time (C) and temperature (D) for the hydrolysis of AA2P by ALP. All the error bars represent the standard deviation of three measurements.

Table S1. The comparison of different methods for ALP detection.

Method	Detection mechanism	Linear range (U/L)	LOD (mU/L)	Refs
Fluorescence &Colorimetry	off→on	0-24 0-30	50 50	[2]
Fluorescence &Colorimetry	off→on	1-30 0-30	450 300	[3]
Electrochemiluminescence	off→on	0.002-50	7	[4]
Electrochemiluminescence	on→off	0.1-6	37	[5]
Electrochemiluminescence	off→on	2×10^3-60×10^3	2×10^6	[6]
Electrochemiluminescence	off→on	50-10^4	48×10^3	[7]
Electrochemiluminescence	on→off	2-25	2×10^3	[8]
Fluorescence	on→off→on	0.5-100	300	[9]
Fluorescence	off→on→off	0.5-10	193	[10]
Fluorescence	off→on	0.5-20	50	[11]
Electrochemiluminescence resonance energy transfer	off→off→on→on	0.1-100	40	This work

Table S2. Recovery results of ALP detection in serum samples.

Samples	Added ALP (U/L)	Found ALP (U/L, n = 3)	Recoveries (%, n = 3)
1	5	4.90, 5.01, 5.12	100.2±2.2
2	10	9.93, 10.42, 9.77	101.7±2.4
3	40	40.7, 38.9, 39.4	99.2±2.3

Referenecs

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