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Carbon dots-based nanoscale covalent organic framework as a new emitter combined with CRISPR/Cas12a mediated electrochemiluminescence biosensor for ultrasensitive detection of bisphenol A

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Table S1. Sequence information of oligonucleotides used in this work.

Name	Sequences (5′ to 3′)
crRNA	UAAUUUCUACUAAGUGUAGAUUCCUGCGGAUUUACUGCCUG
activator DNA	CCCAGGCAGTAAATCCGCAGGA
BPA aptamer*	NH_2-C_6-$(T)_{10}$-GGATAGCGTTCCTGCGGATTTAC
Fc-ssDNA	SH-C_6-TTATTTTATTTTATT-Fc
ss-DNA for gel electrophoresis	AGTTTCTGAAGTAGATATGGCAGCACATAATGACATATT

*Indicates the selection of a truncated BPA aptamer.[1]

Materials and reagents

1, 3, 5-Tris (4-aminophenyl) benzene (TPB), chloroplatinic acid ($H_2PtCl_6·6H_2O$), estradiol (E2), tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Innochem technology Co., Ltd (China, Beijing). Ethanol (C_2H_5OH) was purchased from Sigma Aldrich Co., Ltd (Sigma, USA). Glutaraldehyde, 1,4-dioxane ($C_4H_8O_2$), bisphenol A (BPA), bisphenol S (BPS), diethylstilbestrol (DES), potassium chloride (KCl), 6-mercapto-1-hexanol (MCH), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) were obtained from Aladdin Reagent Co. Ltd (China, Shanghai). Potassium persulfate ($K_2S_2O_8$), potassium ferricyanide ($K_3[Fe(CN)_6]$), ferrocyanide potassium ($K_4Fe(CN)_6·3H_2O$), N-

hydroxysuccinimide (NHS), Tris(2,2-bipyridyl) dichlororuthenium (II) hexahydrate，1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) were purchased from Macklin Reagent Co. Ltd (China, Shanghai). Estrone (E1) and estriol (E3) were purchased from Ark Pharm Reagent Co. Ltd (USA). Cas12a and 10×Cas12a reaction solutions were purchased from Guangzhou Meige Biotechnology Co., Ltd. (China, Guangzhou). Carboxy magnetic beads (MB) were purchased from Tianjin Besile Chromatography Technology Development Center (China, Tianjin). Ultrapure water (resistivity >18.2 MΩ cm^{-1} at 25 °C) was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). All reagents were of analytical grade and used without further purification unless otherwise indicated. All other HPLC-purified oligonucleotides and RNA were acquired from Shanghai Sangon Bioengineering Co., Ltd and listed in the **TableS1**.

Apparatus and instrumentation

MPI-B electrochemiluminescence detection system (Xi'an Remax Electronic Science & Technology Co., Ltd.). CHI760E Electrochemical Workstation (Shanghai Chenhua Instrument Co., Ltd.). PGSTAT 128N Electrochemical Workstation (Swiss Metrohm China Co., Ltd.). SUPRA 55 Sapphire field emission scanning electron microscope (SEM) (Carl Zeiss, Germany). D8 ADVANCE X-ray powder diffractometer (XRD) (Broke AXS, Germany). Nicolet Is10 Fourier transform infrared spectrometer (IR) (Thermo Scientific, USA). Hitachi F-4600 fluorescence spectrophotometer (Hitachi, Japan). JEOL JEM 2100Plus transmission electron

microscope (TEM) (JEOL, Japan). Thermo Scientific K-Alpha X-ray photoelectron spectrometer (XPS) (Thermo Fisher, USA). The electrochemical test including cyclic voltammetric (CV) and electrochemical impedance spectroscopy (EIS). CV measurement was performed at a scan rate of 100mV/s, and the scan range is -0.2~0.6 V，EIS was tested in a 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ solution (containing 0.1 mM KCl) within a frequency range of 0.1 Hz to 100 kHz.

Synthesis of Pt NPs

Pt NPs were prepared according to the reported method.[2] In brief, 2 mL of 0.038 M H_2PtCl_6 solution was added into 100 mL ultrapure water and heated to boiling. Then 1 mL of 0.3 M sodium citrate was injected into the above solution under vigorous stirring to reduce H_2PtCl_6 to Pt NPs. After 40 min, the solution turned black. The solution was cooled down to room temperature under stirring, then concentrated and purified with an ultrafiltration tube (3 KD, 7500 rpm, 30 min) and stored at 4 °C in the dark for further use.

Preparation of water samples

We collected real water samples including the Yong jiang river water, bottled water and tap water. Water sample was purified with a 0.45 μM filter membrane before the measurement to eliminate the solid impurity, and then kept them in a clean glass bottle for further use. Finally, 1×10^{-9}, 1×10^{-8}, 1×10^{-7} mol/L BPA solutions were added to the water samples respectively.

Experiment and calculation of ECL efficiency

We prepared 0.1 M $K_2S_2O_8$ solution containing 1 mM $[Ru(bpy)_3]^{2+}$ and 0.1 M Bu_4NPF_6 as the standard (st) of the calculation of the overall ECL efficiency. Then 0.075 mg/ml of CDs and CD-COF were prepared respectively, 5 μL of each were dropped on the electrode surface, dried naturally. In order to compare the ECL efficiency of CDs/ $S_2O_8^{2-}$, CD-COF/$S_2O_8^{2-}$, CD-COF/$S_2O_8^{2-}$/Bu_4N^+ systems, the modified electrode was tested in 10 mL of 0.1M PBS (pH 7.4) containing 0.1M $K_2S_2O_8$ solution. For CD-COF/$S_2O_8^{2-}$/Bu_4N^+ system, 1 mM Bu_4NPF_6 needed to be added to the above solution. The voltage of the photomultiplier tube was set to 600 V, and the scanning potential ranged from -2 to 0 V.

The quantum efficiency for ECL is defined as the number of photons per electron transferred. The relative ECL efficiency was calculated using the relation below:[3]

$$\emptyset_x = \emptyset_{st}\left(\frac{\int_0^t Idt}{\int_o^t idt}\right)_x \Big/ \left(\frac{\int_0^t Idt}{\int_o^t idt}\right)_{st}$$

Here, $Ø_x$ represents the ECL efficiency of sample, $Ø_{st}$ is the ECL quantum efficiency of $[Ru(bpy)_3]^{2+}$ (1 mM and 0.1 M Bu_4NPF_6/acetonitrile) via annihilation, taken as 5.0%, I is ECL intensity, i is current value, and x is the sample. The ECL efficiencies of different ECL systems were calculated based on eq1 and displayed in **Table 1.**

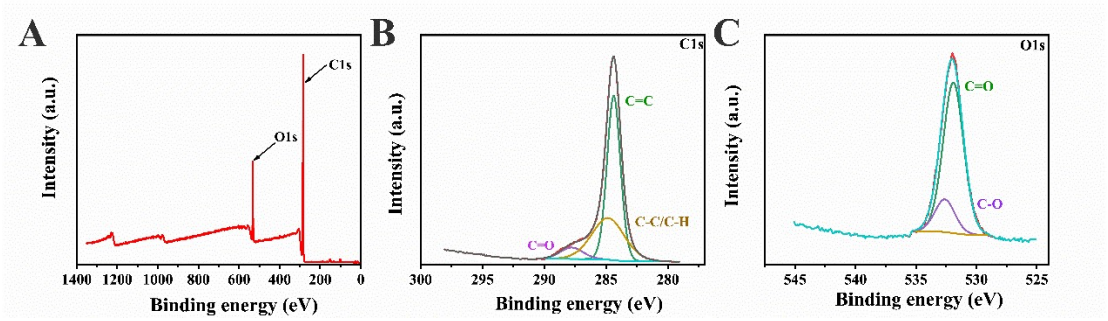

Fig. S1. XPS survey (A), C1 s (B), O1 s (C) spectra of CDs

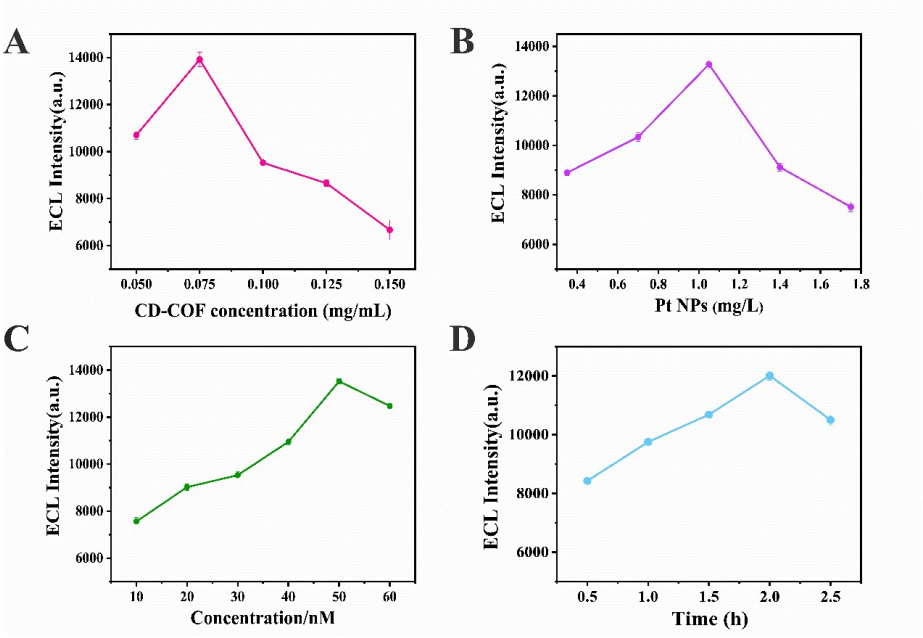

Fig. S2. Optimizations of CD-COF concentration (A). Pt NPs concentration（B）. Cas12a-crRNA concentration (C) and cleavage time on Fc-ssDNA by the CRISPR-Cas12a system (D).

Table S2. Comparison of other methods for BPA detection

Methods	Linear range (M)	Detection limit M)	Ref.
Electrochemical	1.0×10^{-8} - 5.0×10^{-5}	3.2×10^{-9}	4
Photoelectrochemistry	5.0×10^{-10} -5.0×10^{-5}	1.8×10^{-10}	5
ECL	1.0×10^{-9} - 5.0×10^{-4}	3.4×10^{-10}	6
ECL	9.0×10^{-16} - 4.0×10^{-10}	3.0×10^{-16}	7
ECL	1.0×10^{-10} - 1.0×10^{-4}	3.3×10^{-11}	8
ECL	1.0×10^{-14} - 1.0×10^{-5}	2.2×10^{-15}	This work

Calculation of the limit of detection

According to related references and the IUPAC recommendation[9] the limit of detection (LOD) in our work was determined as LOD = k S_B/m, where S_B represents the standard deviation of the blank signals (n_B=3), m represents the analytical sensitivity which can be estimated as the slope of calibration curve at lower concentration ranges and k represents the number of blank samples setting as 3. Fig. 6C presented that ECL Intensity values (I/a.u.) was linearly related to the concentration of BPA (C/M) at a low concentration range. The corresponding linear equation was ΔI = 1089.48 lgC + 16056.81 and the S_B of three times blank sample signal was about 80.31. Therefore, the LOD of the ECL biosensor was determined as 2.21 fM (LOD = 3 × 80.31 ÷ 1089.48 = 2.21 fM).

Table S3. Analysis of BPA in water samples.

Sample	Added（nmol/L）	Found（nmol/L）	Recovery（%）	RSD（%，n=3）
Yongjiang river water	1	0.98	98	1.32
	10	10.95	110	1.29
	100	96.45	96	2.05
Barreled water	1	1.04	104	2.71
	10	10.82	108	1.23
	100	95.70	96	2.36
Tap water	1	0.91	91	0.61
	10	10.33	103	1.96
	100	107.96	108	2.12

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