Supporting Information

Dual-wavelength responsive photoelectrochemical aptasensor based on ionic liquid functionalized MOFs and noble metal nanoparticles for the simultaneous detection of multiple tumor markers

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1.Experimental section

1.1Materials and apparatus

1,4-Dibromomethylbenzene，Methyl 4-(bromomethyl)benzoate， polyvinyl pyrrolidone,Mw=30000)，Triethylamine，N-imidazole,1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride （EDC），N-Hydroxy succinimide （NHS），Tris (2-carboxyethyl)phosphine （TCEP） purchased from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China)，Zinc nitrate hexahydrate， Sodium hydroxide， L(+)-Ascorbic acid， Chloroauric acid， Disodium hydrogen phosphate，Potassium Phosphate Monobasic， Sodium chloride，Potassium carbonate， Silver nitrate, Sodium borohydride buy from China Pharmaceutical Holding Chemical Testing Co., Ltd. (Shanghai, China).Carcinoembryonic antigen (CEA) and Carbohydrate antigen 15 - 3 (CA153) were purchased from Linc-Bio Science Co. (Shanghai, China).Bovine serum albumin (BSA), glutamate (Glu) and glycine (Gly) are purchased from Shanghai Ruji Biotechnology Co., Ltd. (Shanghai, China).Neuron-specific enolase (NSE) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Immunoglobulin G (IgG) is purchased from Beijing Sunshine Biotechnology Co., Ltd. (Beijing, China). α-fetoprotein (AFP) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Human serum albumin (HSA) is provided by Shanghai Pufei Biotechnology Co., Ltd. (Shanghai, China). All synthesized DNA sequences are provided by Sangon Inc. (Shanghai, China). All other reagents are analytically pure and used as original. All solutions were prepared with ultrapure water. Human serum samples are provided by Wuhan University People ' s Hospital (Wuhan) friendship.

capture aptamer：

CEA:3'-TTAACTTATTCGACCATA-C6-NH_2-5'

CA153:3'-TTTTGCTCCCTGGCAGAT-C6-NH_2-5'

Recognition aptamer modified by noble metal nanoparticles:

CEA:3'-CCCATAGGGAAGTGGGGGA-C6-SH-5'

CA153:3'-AAGCAGTTGATCCTTTGGATACCCTGG-C6-SH-5'

Scanning electron microscopy (SEM) was carried out with SU 8010 scanning electron microscope (Hitachi, Japan). Transmission electron microscopic (TEM) images were obtained on FEI Tecnai G220S-TWIN instrument (FEI Company, Netherlands). X-ray diffraction (XRD) patterns were acquired by D8 ADVANCE (BRUKER, Germany). UV-Vis-NIR spectrum is measured on Cary 5000 spectrophotometer (Agilent Technologies, USA). 400nm and 520 nm LED lamp (150 mW), 808 nm laser lamp (200 mW) used as excitation source. Electrochemical

measurements were performed with CHI 660E electrochemical workstation (Chenhua Corp., Shanghai, China). A conventional three-electrode system was employed with a glassy carbon electrode with a diameter of 3mm (GCE, Wuhan Gaossunion Technology Co., Ltd., China), a Pt wire auxiliary electrode and a saturated calomel reference electrode (SCE).

1.2. PMBCBImBr$_2$ ionic liquid synthesis

4-(Bromomethyl)benzoate (0.44 g, 15 mmol) was dissolved in 150 mL acetonitrile. Under nitrogen atmosphere, N-imidazole (1.02 g, 15 mmol) and anhydrous potassium carbonate (3.36 g, 24 mmol) were loaded into a flask, and kept reacting at 70 ^{o}C for 24 h. As the reaction has been accomplished, the solvent was removed using distillation under a vacuum. The crude product was extracted with dichloromethane and saturated saltwater. After removing the solvent with vacuum distillation, methyl 4-imidazolylmethylbenzoate (1.98 g, 9.2 mmol) was obtained. Methyl 4-imidazoliummethylbenzoate (0.63 g, 2.8 mmol) was dissolved in 30 mL acetonitrile, and heated to 70 ^{o}C with the protection of nitrogen. Subsequently, 1,4-dibromomethylbenzene (0.37 g, 1.4 mmol) and acetonitrile (30 mL) were added to the reaction system. After reacting for 8 h, the product was separated by vacuum filtration, and successively washed with dichloromethane and ultrapure water. After that, six times of potassium hydroxide was used to hydrolyse the product in a mixed solvent containing ethanol, methanol and ultrapure water and kept stirring at 80 ^{o}C for 4 h. After being cooled to room temperature, hydrochloric acid solution was used to adjust the pH value to 2.0. As being separated with vacuum filtration and washed with dichloromethane and ultrapure water, PMBCBImBr$_2$ ionic liquid was prepared successfully and was characterized by ^{1}H-NMR, FT-IR and HPLC-MS. The schematics for PMBCBImBr$_2$ ionic liquid synthesis and all results for characterizations are shown in Figure S1-S4.

1.3 Synthesis of PMBCBImBr$_2$ ionic liquid functionalized Zn-MOF

PMBCBImBr$_2$ (0.375 g, 0.56 mmol) and $Zn(NO_3)_2 \cdot 6H_2O$ (0.372 g, 1.25 mmol) were dissolved in 5 mL dimethylformamide with ultrasonication. 1.66 g PVP was dissolved into a mixed solvent containing 30 mL ethanol and 65 mL DMF. Then, 2.5 mL of PMBCBImBr$_2$ ionic liquid was injected into the mixture. Subsequently, a small amount of triethylamine solution (4 μl, 0.1 mmol) and 2.5 mL of $Zn(NO_3)_2$ solution were added successively. After reacting for 1 h, a white solid product was precipitated from the mixture. The mixture was immersed into an ice bath to stop the reaction. The solid product was centrifuged and thoroughly washed. After being dispersed in anhydrous ethanol, the mixture was heated and kept to reflux for 5 h to exchange DMF in the pores of Zn-MOFs. After filtration, the solid product was dried overnight in a vacuum oven at 40 °C to obtain white crystals, namely, ionic liquid functionalized Zn-MOFs. As-synthesized Zn-MOFs were characterized by scanning electron

microscopy, nitrogen adsorption/desorption isotherm analysis, and X-ray photoelectron spectroscopy (XPS). All results were shown in Supporting Information.

1.4 Gold nanoparticles synthesis and integration with CEA aptamer

AuNPs were synthesized according to the previous report. Four milliliters of 1% chloroauric acid and 96 mL ultrapure water were added to a flask, and were heated to 100 ^{o}C under stirring. Then, 10 mL of trisodium citrate solution (38.8 mmol L^{-1}) was quickly added, and kept reacting for 15 min. After the solution turns from grayish-blue to red, the reaction was stopped, and cooled to room temperature. According to the UV-Vis absorption spectra, the concentration of gold nanoparticles can be calculated to be 106 nmol L^{-1} . 100 μL of CEA aptamer solution (100 μmol L^{-1}) and 2 μL of TCEP reductant at a concentration of 50 mmol L^{-1} (DNA: TCEP = 1: 10) were mixed with Tris-HCl solution (10 mmol L^{-1}, pH = 7.4). One hour later, the disulfide bond existed in the DNA chain was broken. 10 times diluted AuNPs solution (2 mL) was reacted with CEA aptamer (17.3 μL, 98 mmol L^{-1}) with stirring at room temperature. After 16 h, 10 μL of 2 mol L^{-1} NaCl solution was added to the mixture with a speed of 120 μL/h. After NaCl concentration reached to 60 mmol L^{-1}, the mixture was incubated for 24 h. Then, the precipitate was separated with centrifugation. After being thoroughly washed with ultrapure water, it was dispersed in 16.9 mL Tris-HCl (10 mmol L^{-1}, pH = 7.4, 50 mmol L^{-1} NaCl), and stored in 4 ^{o}C for further use.

1.5 Silver nanosheets synthesis and integration with CA153 aptamer

According to the previous report, the method for synthesizing AgNS is slightly modified. 70 μL of silver nitrate solution (70 mmol L^{-1}), 300 μL of sodium citrate solution (80 mmol L^{-1}) and 80 μL hydrogen peroxide solution (80 mmol L^{-1}) were successively added to 24.25 mL ultrapure water with violent stirring at room temperature. 5 min later, 250 μL of sodium borohydride solution (0.1 mol L^{-1}) was added to the mixture. The solution turns yellowish immediately. Kept stirring for 5 min and standing for about half an hour, the solution turns dark blue, meaning the formation of triangle silver nanoparticles. A combination of transmission electron microscopy (TEM) imaging (for size measurement) and inductively coupled plasma mass spectrometry (ICP-MS, for Ag concentration) was used to determine the concentration of silver nanosheets, the concentration of AgNSs can be calculated to be 140 nmol L^{-1}. 100 μL of CA 153 aptamer solution (100 μmol L^{-1}) and 2 μL of TCEP reductant at a concentration of 50 mmol L^{-1} (DNA: TCEP = 1: 10) were mixed with Tris (10 mmol L^{-1}, pH = 7.4). One hour later, the disulfide bond existed in the DNA chain was broken. 10 times diluted AgNSs solution (2 mL) was used for reacting with CA153 aptamer (28.6 μL, 98 mmol L^{-1}) under stirring at room temperature. After 16 h, 10 μL of 2 M NaCl solution was added to the mixture with a speed of 120 μL/h. After NaCl concentration reached to 60

mM, the mixture was incubated for 24 h. Then, the precipitate was separated with centrifugation. After being thoroughly washed with ultrawater, it was dispersed in 28 mL Tris-HCl (10 mmol L^{-1}, pH = 7.4, 50 mmol L^{-1} NaCl), and stored in 4 ^{o}C for further use.

1.6 Fabrication of PEC aptasensors

The glassy carbon electrode (GCE, d = 3 mm) was polished with alumina suspension, followed by ultrasonic cleaning in nitric acid solution, ethanol and ultrapure water. Then, PMBCBImBr_2-MOF nanoparticles (6.0 μL, 10 mg mL^{-1}) were coated onto the GCE surface. As being dried under 37 °C, a PMBCBImBr_2-MOF/GCE was fabricated. Immersed PMBCBImBr_2-MOF/GCE in an EDC/NHS (molar ratio: 1: 2) solution to activate the carboxyl group on PMBCBImBr_2-MOF/GCE. Subsequently, 8 μL mixed solution containing 10 nmol L^{-1} NH_2-CEA aptamer and 10 nmol L^{-1} NH_2-CA153 aptamer was drop coated onto the PMBCBImBr_2-MOF/GCE surface. After incubation at 4 ^{o}C for 12 hours, the unfixed aptamer was removed by washing with phosphate buffer (0.01 mol L^{-1}, pH 7.4) to obtain a CEA-Apt1&CA153-Apt1/PMBCBImBr_2-MO/GCE. Then, it was incubated into a BSA solution (0.25%) for 30 min to block the nonspecific binding site under stirring. After being washed with phosphate buffer (0.01 M, pH 7.4) and dried at room temperature, the desired photoelectrochemical immunosensing platform based on BSA/CEA-apt1&CA153-apt1/PMBCBImBr_2-MOF/GCE was obtained. The BSA/CEA-Apt1&CA153-Apt2/PMBCBIMBr_2-MOF/GCE was soaked in CA153 and CEA antigen solution with different concentrations, and the specific binding was performed at 37 ^{o}C for 20 min. Washed CA153&CEA/CA153-Apt1&CEA-Apt1 (BSA) with buffer solution, then 6 μL AgNSS-CA153-Apt2 and 6 μL AuNPS-CEA-Apt2 were added successively and incubated at 37 ^{o}C for 25 min. As being rinsed with phosphate buffer (0.01 mol L^{-1}, pH 7.4) and dried under room temperature, the electrode can be used for immunoassay.

Table 1. Dual-channel sandwich type PEC aptasensor compared with previous reports

Detection targets	Methods	Linear range	Detection	reference
CEA，CA153	PEC immunosensor	0.005~5 ng mL^{-1} 0.05~25 U mL^{-1}	2.85 pg mL^{-1} 0.028 U mL^{-1}	This work
CEA, AFP	PEC immunosensor	0.05 ~ 45 ng mL^{-1} 1~90 ng mL^{-1}	0.2 ng mL^{-1} 0.5 ng mL^{-1}	1
CEA,NSE, CYFRA21-1	Electrochemical immunosensor	0.01~100 ng mL^{-1}	6.3 pg mL^{-1} 7.9 pg mL^{-1} 8.5 pg mL^{-1}	2
CEA, AFP, CA199, CA724	Electrochemical immunosensor	0.01~100 ng mL^{-1} 0.01~100 ng mL^{-1} 0.01~100 U mL^{-1} 0.01~100 U mL^{-1}	8.1 pg mL^{-1} 6.3 pg mL^{-1} 0.008 U mL^{-1} 0.007 U mL^{-1}	3
CA15-3, CA19-9	Electrochemical immunosensor	1~100 U mL^{-1} 5~100 U mL^{-1}	0.3 U mL^{-1} 1.6 U mL^{-1}	4
CEA,CA153	Immunochromatography test strips	0.01~10 ng mL^{-1} 0.05~10 U mL^{-1}	0.06 ng mL^{-1} 0.09 ng mL^{-1}	5
CA153,CEA	Electrochemiluminescence immunosensor	2.0~100 U mL^{-1} 1.0~70 ng mL^{-1}	0.8 ng mL^{-1} 0.65 ng mL^{-1}	6
CA125, CA15-3	SERS immunosensor	0.1 U mL^{-1}~1 kU mL^{-1}	0.01 U mL^{-1} 0.01 U mL^{-1}	7

Table 2 Determination of CA153 and CEA in serum samples

Sample		ELISA	PEC aptasensor	RSD (%)	Relative error (%)
CA153	1	0.528	0.530	3.55%	0.38%
(U mL^{-1})	2	2.873	2.85	1.74%	-0.84%
	3	3.413	3.38	2.04%	-0.97%
CEA (ng mL^{-1})	1	1.786	1.765	1.91%	-1.18%
	2	5.421	5.32	1.07%	1.86%
	3	4.473	4.558	2.89%	-1.88%

Table 3 Recoveries for determining CA153 and CEA in serum samples.

Sample		Original Concentration	Diluted Concentration	Added	Detected	Recovery (%)	RSD (%)
CA153 (U mL^{-1})	1	84.24	4.212	1.0	5.31	101.88%	3.40%
	2	8.185	1.637	1.0	2.638	100.04%	0.97%
	3	71.04	3.552	1.0	4.457	97.91%	2.89%
CEA (ng mL^{-1})	1	17.06	3.412	1.0	4.227	96.94%	4.19%
	2	4.82	0.964	1.0	1.968	100.20%	0.99%
	3	44.76	1.492	1.0	2.577	103.41%	0.89%

Under normal circumstances, a person's CEA level is higher than 5.0 ng mL^{-1}, there is a risk of illness.[8-9] Based on serum CA153 levels, breast cancer CA153 levels are usually divided into three levels: 5-25 U mL^{-1}, early, 25-100 U mL^{-1}, medium and > 100 U mL^{-1}, late.[10]

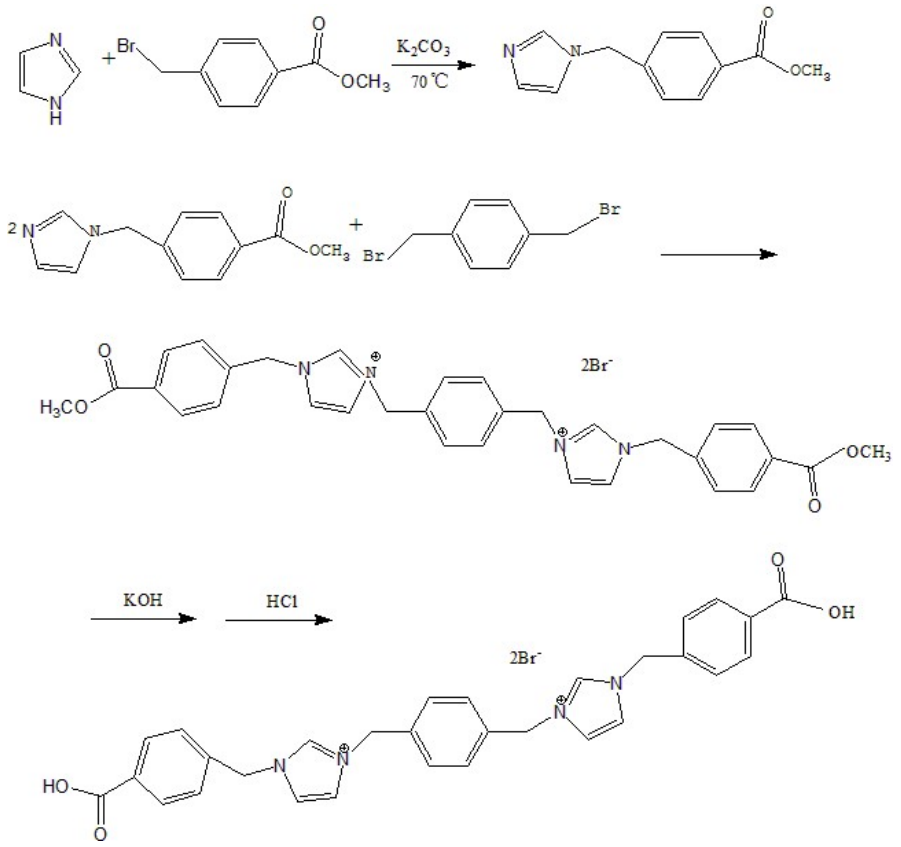

Figure S1 Scheme for the synthesis of $PMBCBImBr_2$ ionic liquid.

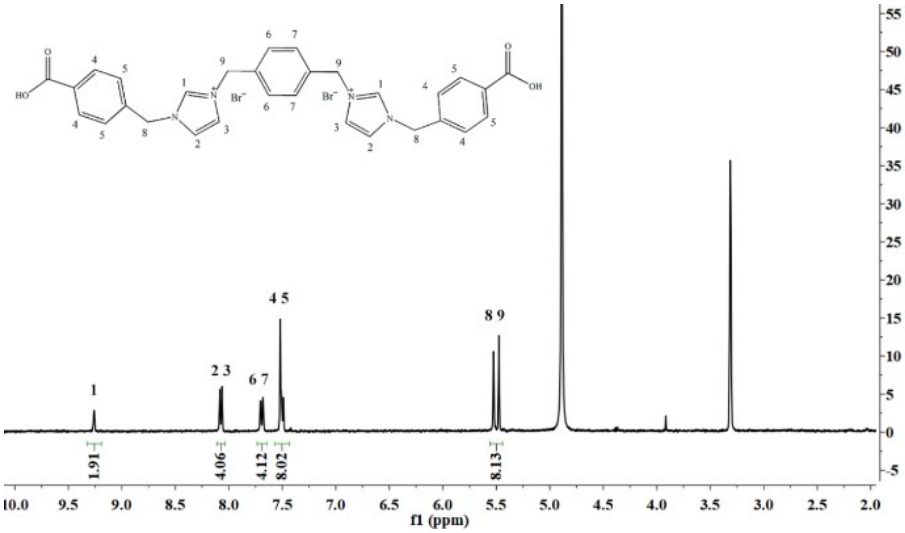

Figure S2 ^{1}H NMR spectrum of PMBCBImBr$_2$ ionic liquid.

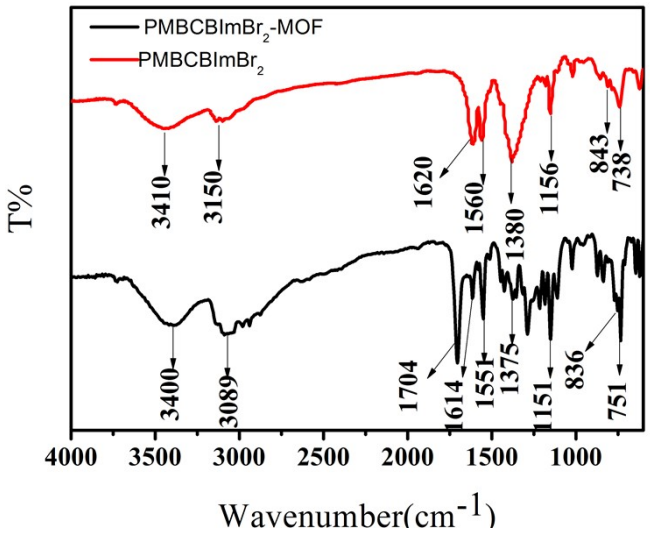

Figure S3 FTIR spectra of $PMBCBImBr_2$ ionic liquid and $PMBCBImBr_2$-MOF.

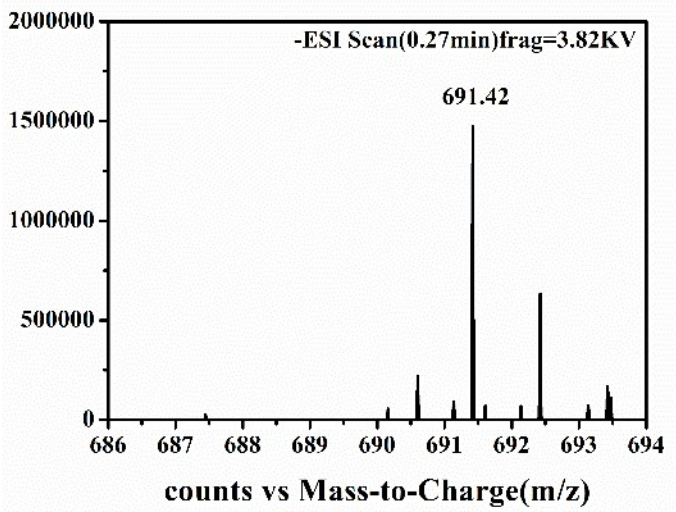

Figure S4 HPLC-MS spectrum of $PMBCBImBr_2$ ionic liquid.

The HPLC-MS spectrum (Figure S4) indicates that the m/z of $PMBCBImBr_2$ is 691.42 ($[M+Na]^+$). The results show that $PMBCBImBr_2$ ionic liquid has been successfully synthesized.

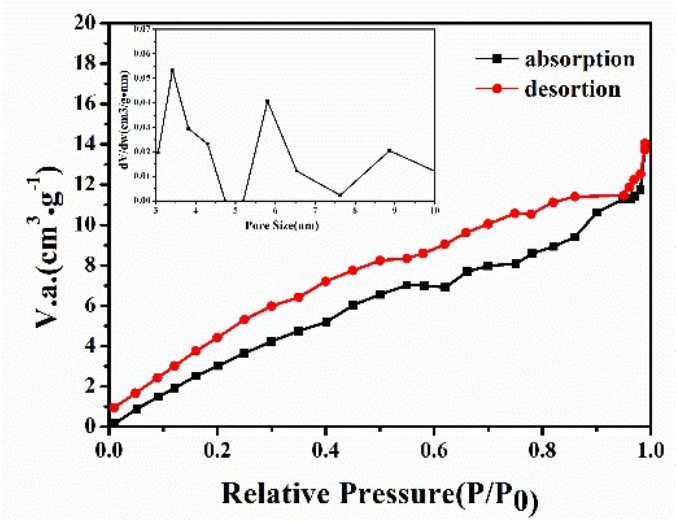

Figure S5 Nitrogen adsorption/desorption isotherm of PMBCBImBr$_2$-MOF.

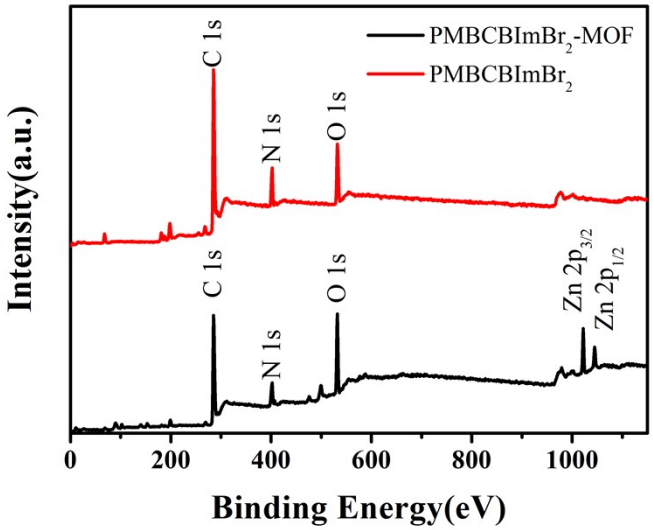

Figure S6 XPS spectra of $PMBCBImBr_2$ ionic liquid and $PMBCBImBr_2$-MOF.

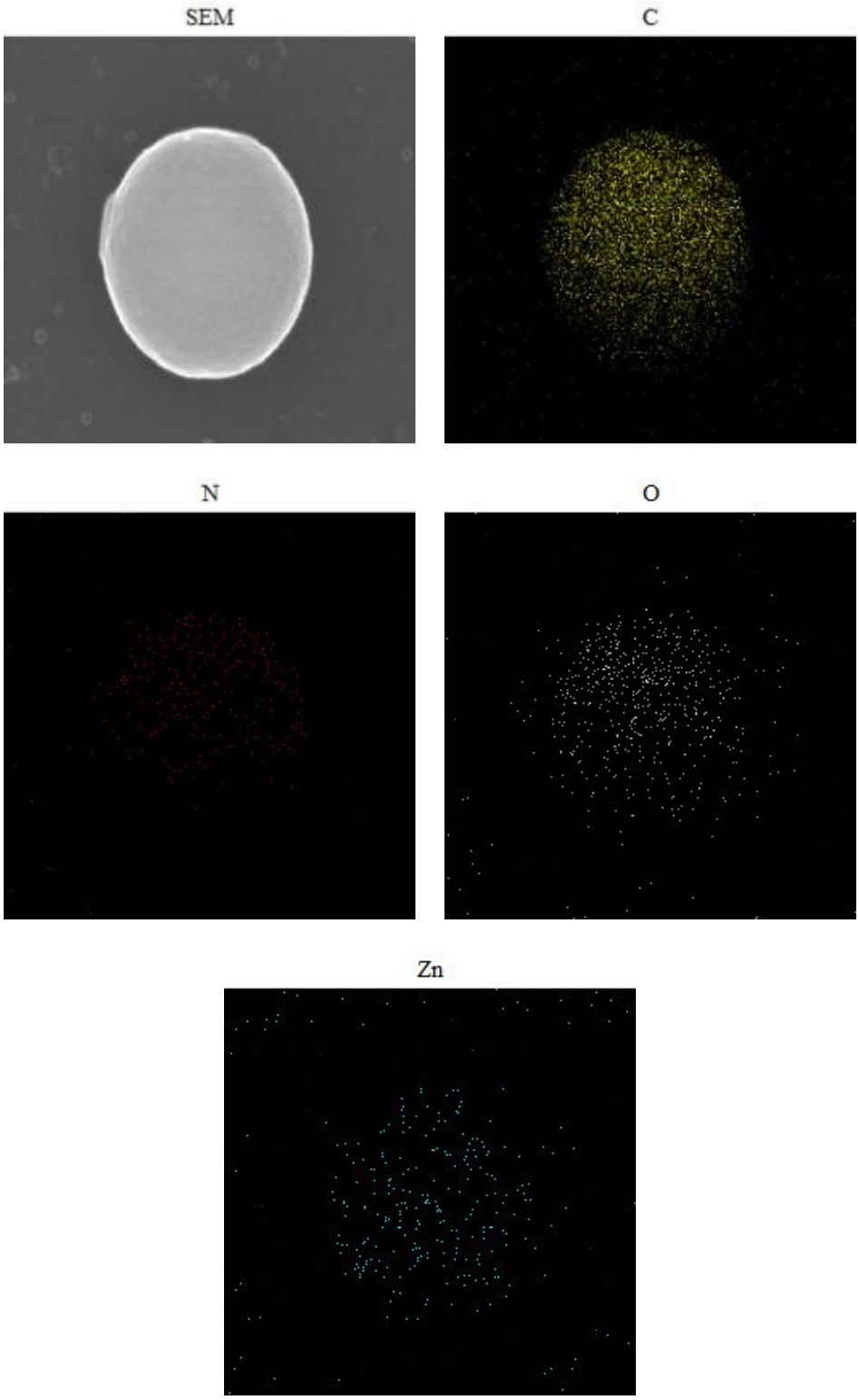

Figure S7 SEM elemental mapping of PMBCBImBr_2-MOF.

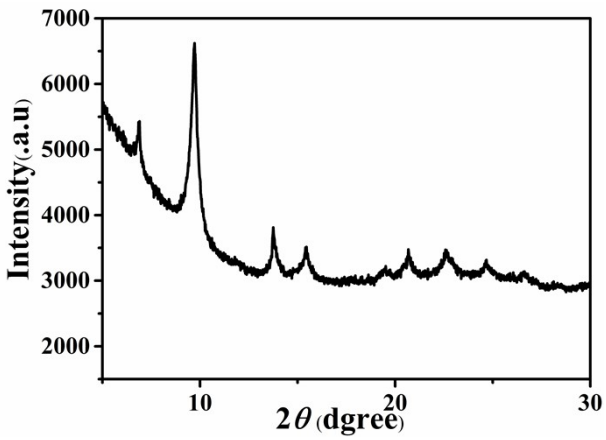

Figure S8. X-ray diffraction pattern of PMBCBImBr$_2$-MOF

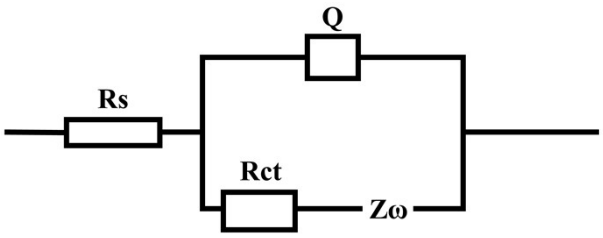

Figure S9. Randles equivalent circuit applied for the fitting of recoded Nyquist plots.

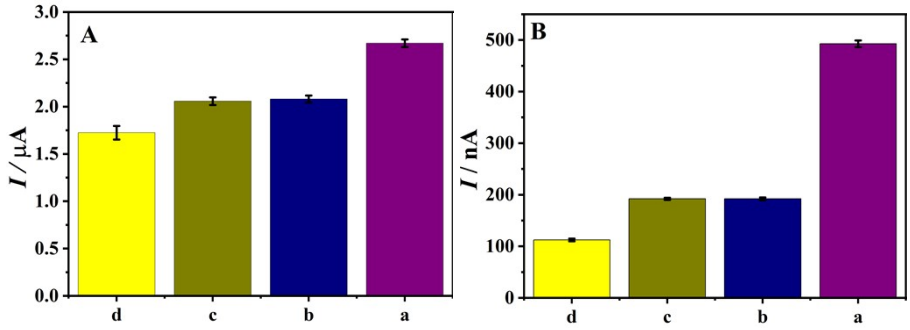

Figure S10. (A) Photocurrent responses of a single-channel sensor marked with AgNSs (a), a dual-channel sensor with only AgNSs-CA153-Apt2 (b), a dual-channel sensor with AgNSs-CA153-Apt2 and AuNPs-CEA-Apt2 (c), and a single-channel sensor marked by AgNSs (d) at 808 nm; (B) Photocurrent responses of a single-channel sensor marked with AuNPs (a), a dual-channel sensor with only AuNPs-CEA-Apt2 (b), a dual-channel sensor with AgNSs-CA153-Apt2 and AuNPs-CEA-Apt2 (c), and a single-channel sensor marked by AgNSs(d) at 520 nm.

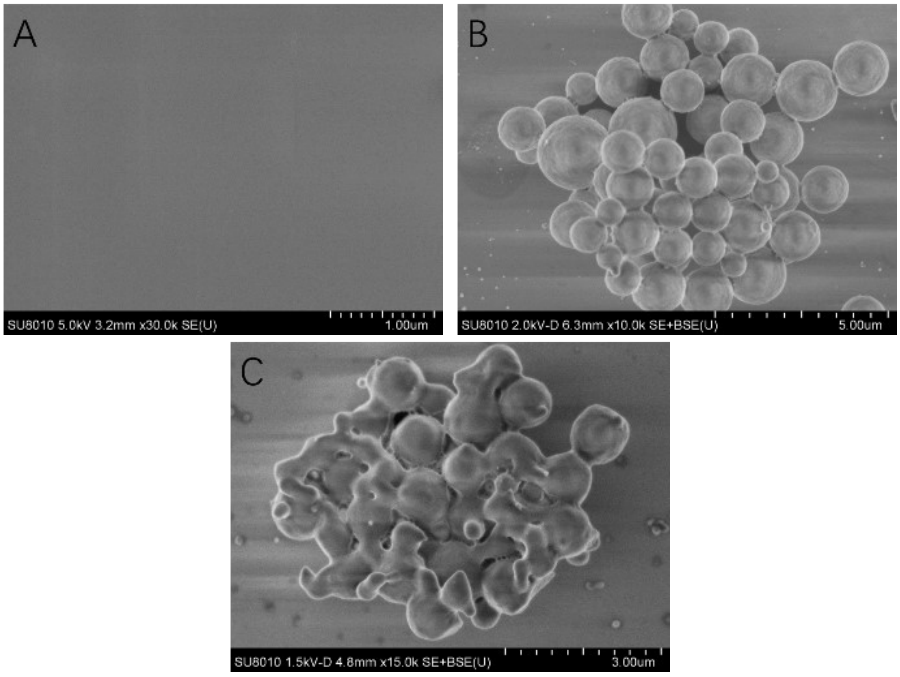

Figure S11 SEM images of a bare GCE (a), a PMBCBImBr_2-MOF/GCE (b) and CA153 Apt1&CEA Apt1/PMBCBImBr_2-MOF/GCE (c).

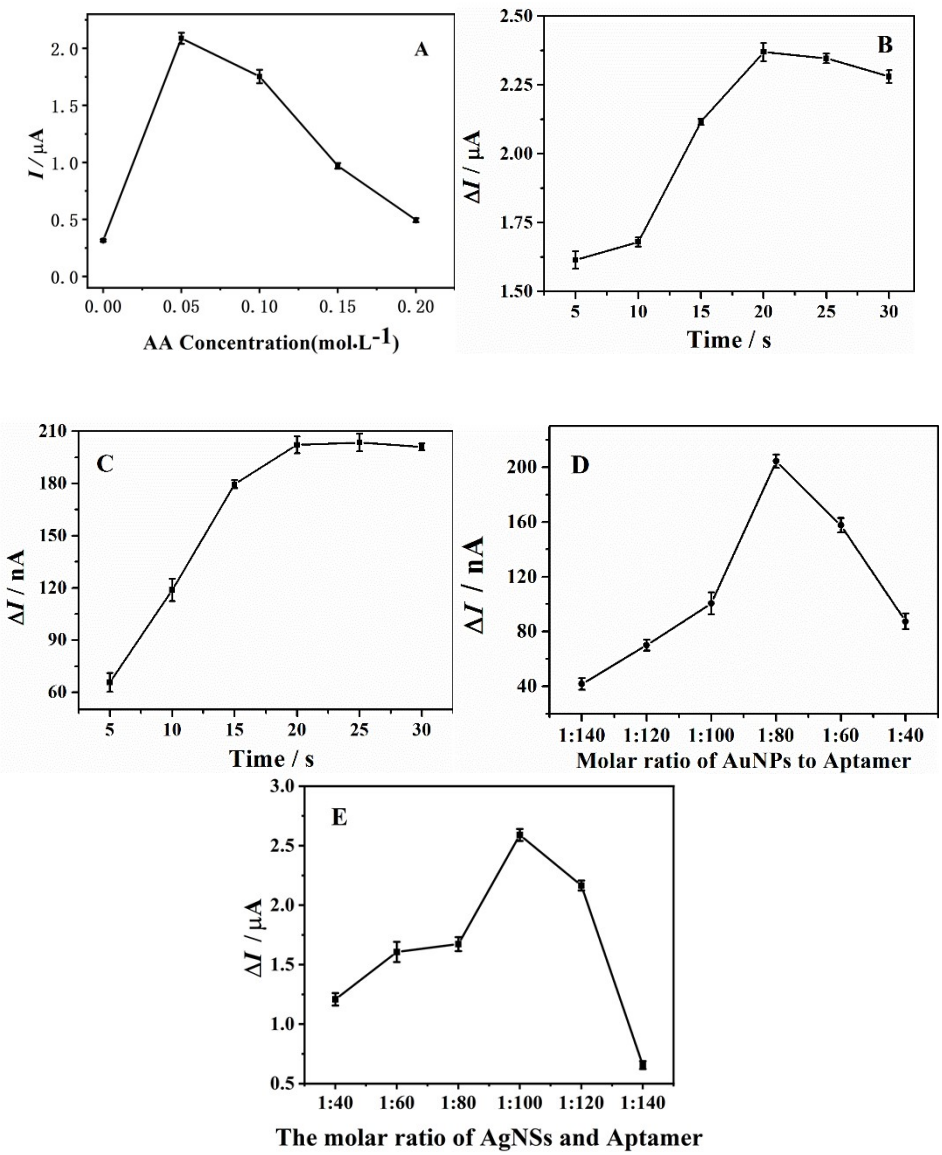

Figure S12 Influence of (A) the concentration of ascorbic acid (AA); (B) the incubation time on the photocurrent response of 1.0 U mL^{-1} CA153; (C) the incubation time on the photocurrent response of 1.0 ng mL^{-1} CEA; (D) the molar ration of AuNPs and CEA-Apt2; (E) the molar ration of AgNSs and CA153-Apt2.

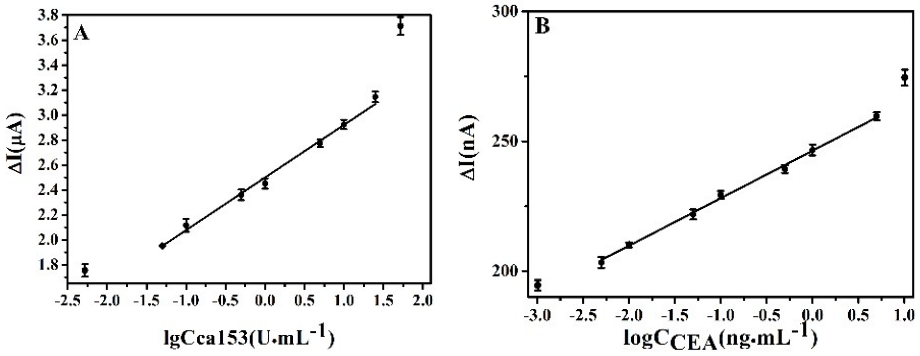

Figure S13 (A) The relationship between the photocurrent variation (Δ*I*) and the logarithm of the CA153 concentration; (B) The relationship between the photocurrent variation (Δ*I*) and the logarithm of the CEA concentration.

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