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BODIPY-based metal-organic frameworks as efficient electrochemiluminescence emitters for telomerase detection

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Table of Contents

Experimental

Experimental

Materials and reagents. The dipyridine-functionalized BODIPY ligand (BDP) was prepared according to the literature.[S1] Zinc nitrate tetrahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), terephthalic acid (BDC), tri-n-propylamine (TPA, >99%) and triethylamine (TEA) were obtained from Sigma Aldrich Co., Ltd. (Shanghai, China). 1-(3-(dimethylamino) propyl)-3-ethylcarbodiimide hydrochloride (EDC, purity ≥98%), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS), N-hydroxysuccinimide (NHS), and hydroquinone were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Potassium persulfate ($K_2S_2O_8$), Tetrachloroauric acid ($HAuCl_4$), N, N-dimethylformamide (DMF), Concentrated nitric acid (HNO_3), trisodium citrate ($C_6H_5Na_3O_7 \cdot 3H_2O$) and streptavidin (SA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phosphate buffer solution (PBS, 0.1 M) was prepared from sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), which were acquired from J&K Scientific Co., Ltd. (Beijing, China). MCF-7 cells were from KeyGen Biotech. Co. Ltd. (Nanjing. China). A human telomerase (TE) ELISA kit was purchased from Yuanju Biotechnology Co. Ltd (Shanghai, China). Ultrapure water was obtained from a Millipore water purification system (Milli-Q, ≥18.2 MΩ cm^{-1}), applied throughout for all the solutions. The 1×TRAP buffer contained 20 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween-20, 1 mM egtazic acid (EGTA) and 0.1 mg mL^{-1} bovine serum albumin (BSA). The deoxynucleotide solution mixture (dNTPs) and dithiothreitol (DTT) along with all the DNA oligonucleotides were supplied by Sangon Inc. (Shanghai, China). Other reagents were analytical grade without purification.

The sequences of DNA oligonucleotides are as follows:

B-T: 5′-Biotin-AGGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAACCCTAA CTCTGCTCGACGGATTTTTTTTTTTTTT–SH–3′

Primer (**P**): AATCCGTCGAGCAGAGTT

Apparatus. Powder X-ray diffraction (PXRD) data were acquired from a Bruker D8-Advance diffractometer with a Cu-sealed tube (λ = 1.54178 Å) at 40 kV and 40 mA with 0.2 s per step. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were taken on an S-4800 scanning electron microscope (Hitachi, Japan) and JEM-2010 transmission electron microscope (JEOL, Japan), respectively. Photoluminescence (PL) spectra were collected on a FLS-980 fluorescence spectrophotometer (Edinburgh Instrument., U.K.). Infrared spectra were recorded on a Vector 22 Fourier transform infrared (FT-IR) spectrometer (Bruker Optics, Germany). Ultraviolet and visible diffuse-reflectance spectra (UV-Vis DRS) were obtained on a UV-3600 UV-Vis-NIR spectrophotometer (Shimadzu Co., Japan). X-ray photoelectron spectroscopy (XPS) was carried out using an ESCALAB 250 spectrometer (Thermo-VG Scientific Co., U.S.A.) with ultra-high vacuum generators. The analysis of thermal stability was performed on a thermogravimetric (TG) instruments (Netzchen, STA449F3) in the range 25-800 ℃ under a nitrogen atmosphere with a flow rate of 10 mL/min and a heating rate of 10 ℃ min^{-1}, and the specimen mass was about 10 mg. The data of dynamic light scattering (DLS) was performed on a Brookhaven 90Plus in dispersion liquid. Cyclic voltammetry (CV) was measured on CHI 630D electrochemical work station (Shanghai CH Instruments, China), while step pulse (SP) and ECL experiments were carried out in a self-made cell on MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Co., Ltd. China) using a three-electrode system including a glassy carbon electrode (GCE) working electrode, a Pt counter electrode, and an Ag/AgCl (saturated) reference electrode. The

ECL spectrum was obtained through measuring the ECL signals at different wavelengths through a number of filters and the Gaussian distribution fitting. Modified electrodes were prepared by decorating 20 μL of dispersion of either ligands or MOFs containing 1.0 mg mL^{-1} BDP. Electrochemical impedance spectroscopic (EIS) measurements were performed on a PGSTAT30/FRA2 system (Autolab, the Netherlands) in 0.1 M KCl aqueous solution containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1). Polyacrylamide gel electrophoresis (PAGE) was carried out using 15% polyacrylamide gel and scanned with a Molecular Imager Gel Doc XRS (BIO-RAD, USA).

Synthesis of MOF. Zn $(NO_3)_2 \cdot 6H_2O$ (29.6 mg), BDP (24.0 mg) and BDC (12.4 mg) were ultrasonically dissolved in a mixture of 8 mL of N-N′-dimethylformamide (DMF) and 2 mL of ethanol. After 3.75 μL of 68% nitric acid was added, the resulted mixture was sealed in the autoclave and placed in a preheated oven at 85 °C for 48 h. The BDP-based MOF was harvested by centrifuging and washed with DMF and CH_3OH several times.

Synthesis of MOF-SA. SA was functionalized onto the surface of MOF through the formation of amide bonds between the exposed carboxyl part of MOF and the amino group of SA to obtain MOF-SA as a signal tag. First, 1.0 mL of MOF dispersion in water (1.0 mg mL^{-1}) was incubated with 400 μL solution of 1- (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (400 mM) and N-hydroxy succinimide (NHS) (100 mM) for 1 h. After being centrifuged at 10000 rpm for 20 min and redispersed in PBS, the dispersion was incubated at room temperature with 200 μL of SA (0.5 mg mL^{-1}) for 4 h, and then washed with phosphate buffer for 3 times to obtain MOF-SA. MOF-SA was stored at 4 ℃ for further use.

Preparation of recognition probe. First, 100 μM biotin-modified hairpin DNA (BT, 6 μL) was mixed with 100 mM dithiothreitol buffer (8 μL) and left for 2 h, then 3 μM of the primer (**P**) was added in equal amounts. The recognition probe (**BT-P**) was then obtained after being stored in a 90 °C water bath for 5 min and gradually cooling to room temperature.

Polyacrylamide hydrogel electrophoresis (PAGE). To avoid the influence of substituents, unsubstituted single-stranded DNA **T** was applied for analysis. The 15% native PAGE was prepared with 1×Tris-Borate-EDTA (TBE) buffer. 7 μL DNA sample was mixed with 1.5 μL×3 loading buffer to prepare the loading sample. The gel was injected into 1×TBE buffer and carried out at 110 V for 100 min. After 30 min of drying with 4S Green Plus Nucleic Acid Stain, scanning was performed with Molecular Imager gel Doc XR (Bio-RAD, USA).

Synthesis of BT-P-gold nanoparticles (AuNPs). Au-NPs were synthesized through sodium citrate reduction method.[S2] 50 mL $HAuCl_4$ solution (0.02%, W/V) was continuously heated under vigorous agitation until boiling. Afterwards, 2.5 mL trisodium citrate aqueous solution (1%, W/V) was quickly added and the mixture was stirred for another 30 minutes till the solution turned wine red. After naturally cooled to room temperature, the AuNPs were filtered by 0.22 μm sterile syringe filter and stored at 4 ℃. The BT-P-AuNPs were assembled via Au-S bonding. Briefly, 150 μL of **BT-P** (10 μM) was added into 350 μL of AuNPs solution, and then kept at -20 °C for 2 h.

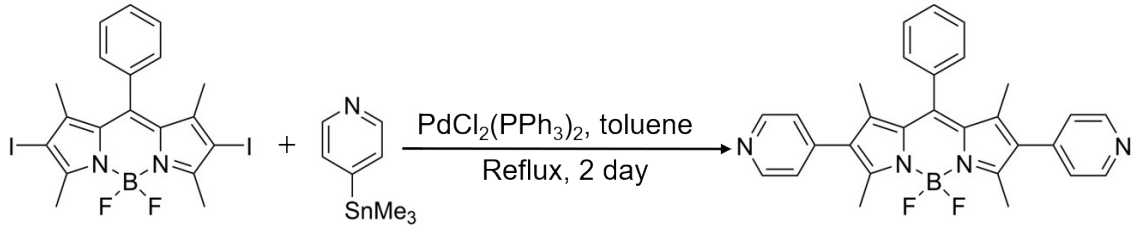
Cell extraction of telomerase. The extraction of telomerase was achieved through the general CHAPS method.[S3] The cells were placed in a 1.5 mL tube and washed three times with PBS (0.1M pH 7.4), along with centrifugation at 2000 rpm at 4 °C for 5 min. The centrifuged cells were added to 500 μL CHAPS lysate and incubated in an ice bath for 30 min, then centrifuged at

12000 rpm at 4 °C for 20 min. The supernatant from the centrifuge tube was collected in a new centrifuge tube for immediate use.

Detection of telomerase activity. The glassy carbon electrode (GCE) was prepared by polishing with 1.0 and 0.05 μm alumina powder and continuous ultrasonic treatment with pure water and ethanol. Subsequently, 5 μL of **BT-P**-AuNPs was dropped onto the clean electrode for 2 h at 37 °C.

For telomerase extension reaction, the pretreated GCE was incubated with 20 μL of extension solution containing the mixture of 10 μL of telomerase solution and 2 mM of dNTPs mixture in 1×TRAP buffer. After incubation of 90 min at 37 ℃, 10 μL MOF-SA was added on the pretreated GCE and stored at 37 ℃ for another 90 min. Lastly, the modified electrode was dipped in PBS (0.1 M, pH 7.4) for ECL detection at a 700 V photomultiplier tube (PMT).

Supporting Figures



Scheme. S1 Synthetic route of BDP ligand.

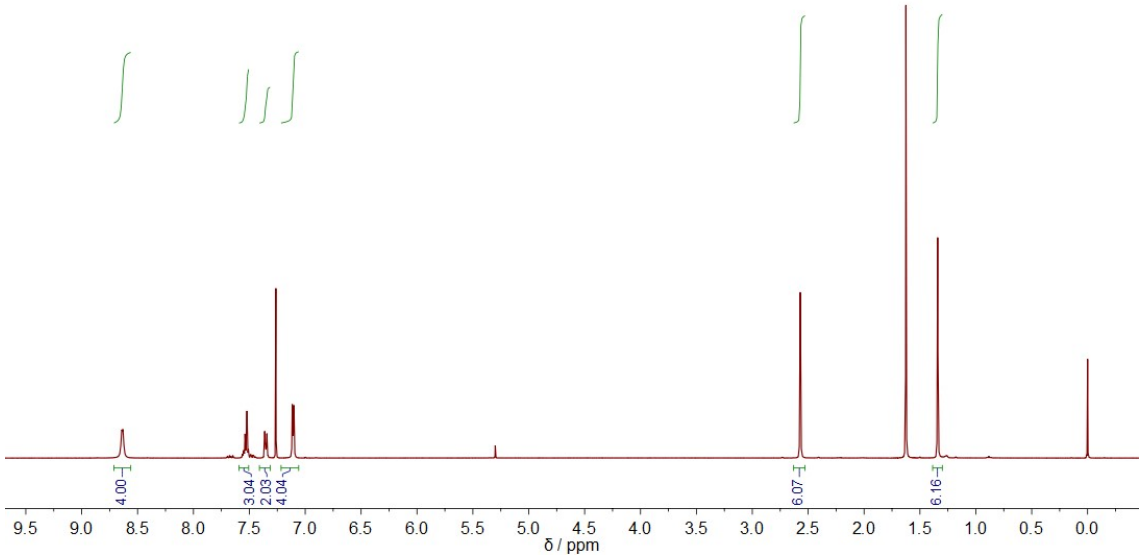


Fig. S1 ^{1}H NMR spectrum of BDP in $CDCl_3$.

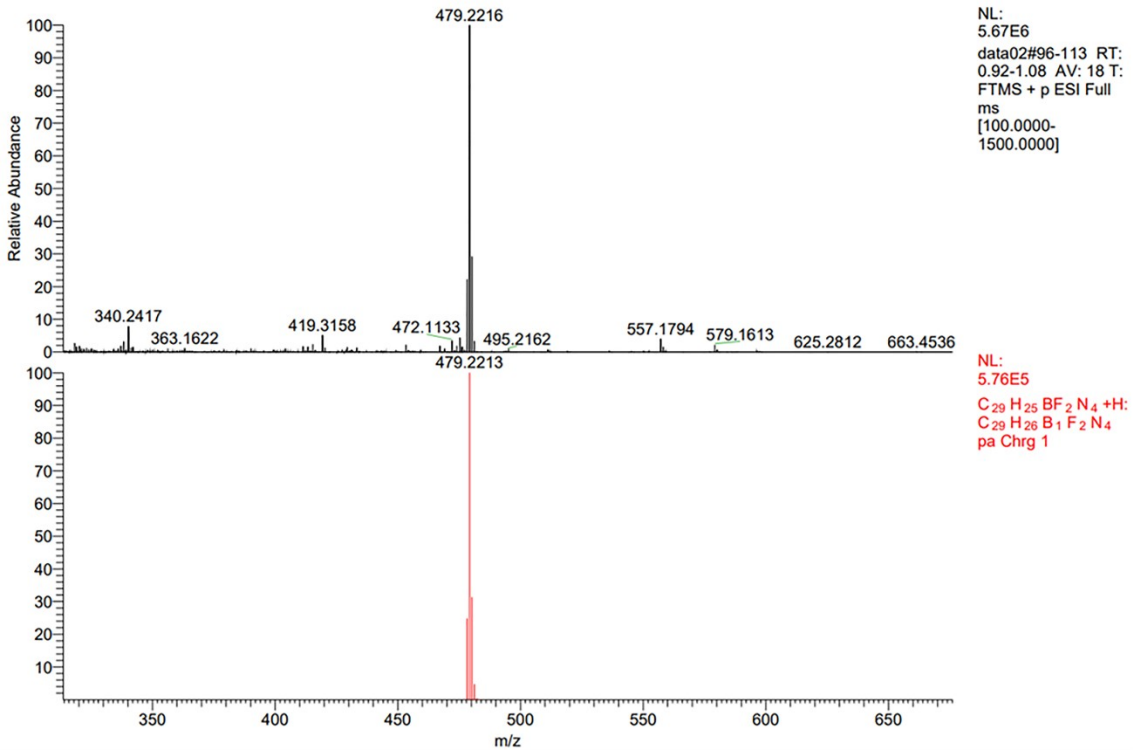


Fig. S2 Experimental (black) and theoretical (red) mass spectrum of BDP.

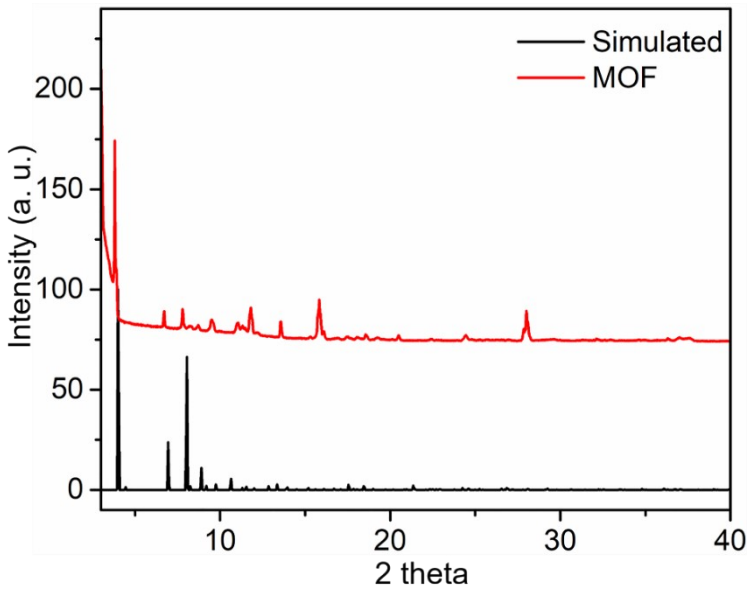


Fig. S3 Simulated (black) and experimental (red) PXRD patterns.

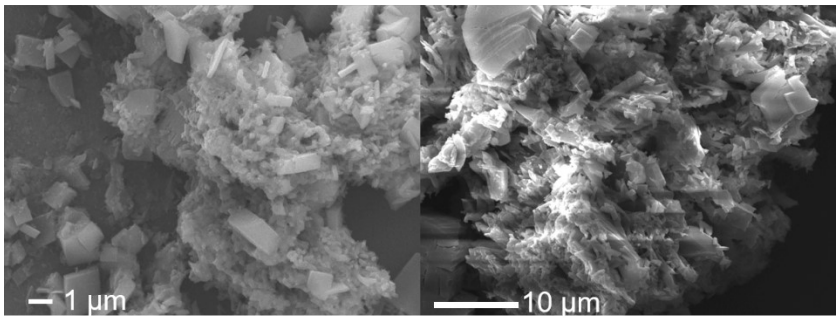


Fig. S4 SEM images of MOFs.

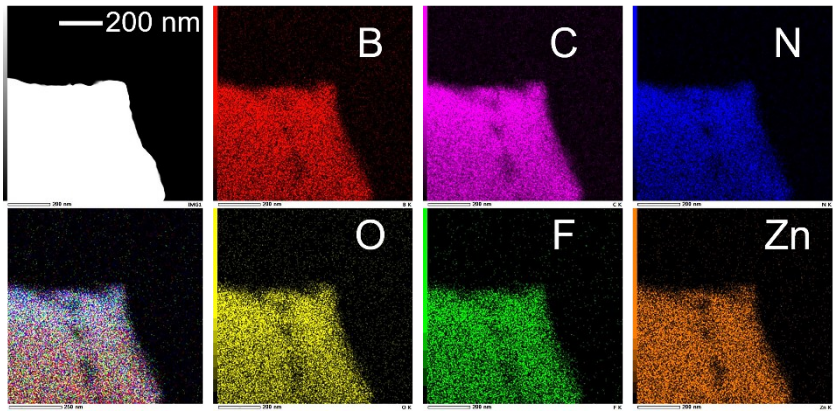


Fig. S5 TEM image and EDS mapping of MOFs.

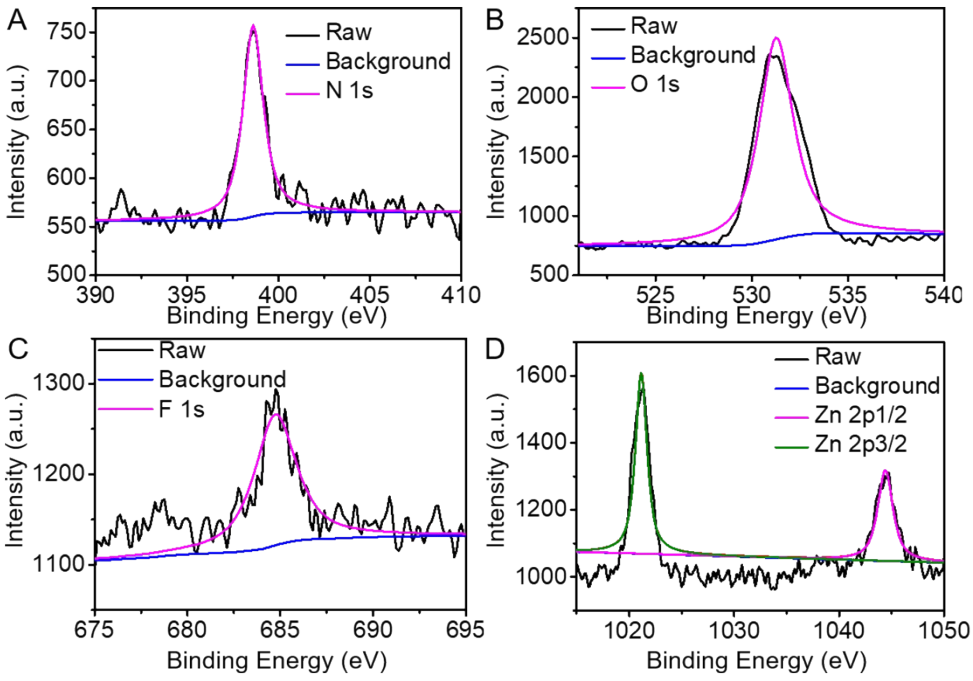


Fig. S6 High-resolution XPS spectra of (A) N, (B) O, (C) F and (D) Zn in MOFs.

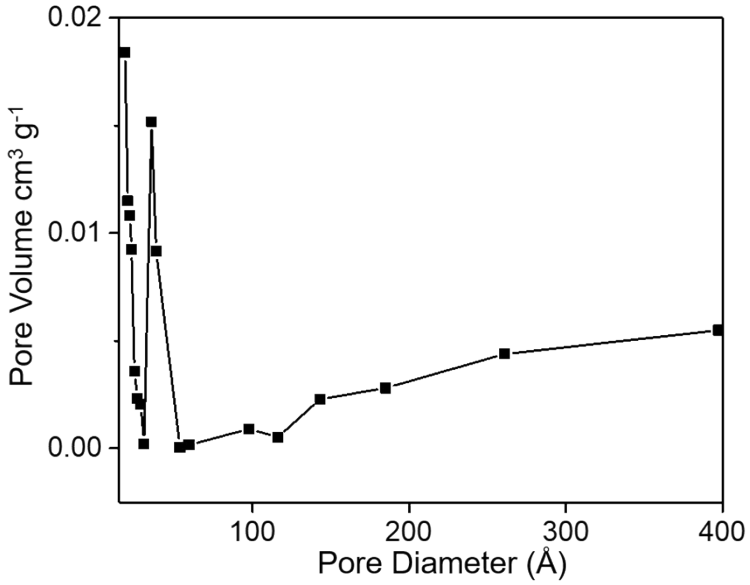


Fig. S7 Pore distribution of MOFs.

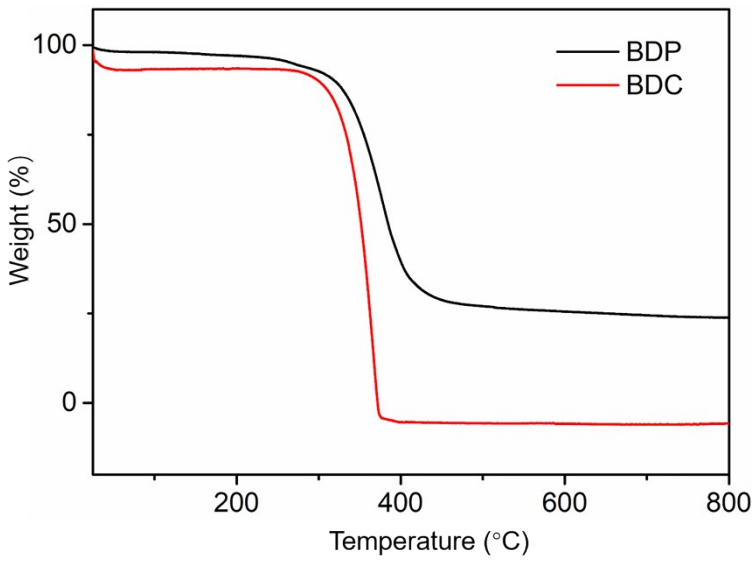


Fig. S8 Thermogravimetric analysis of BDC and BDP ligands.

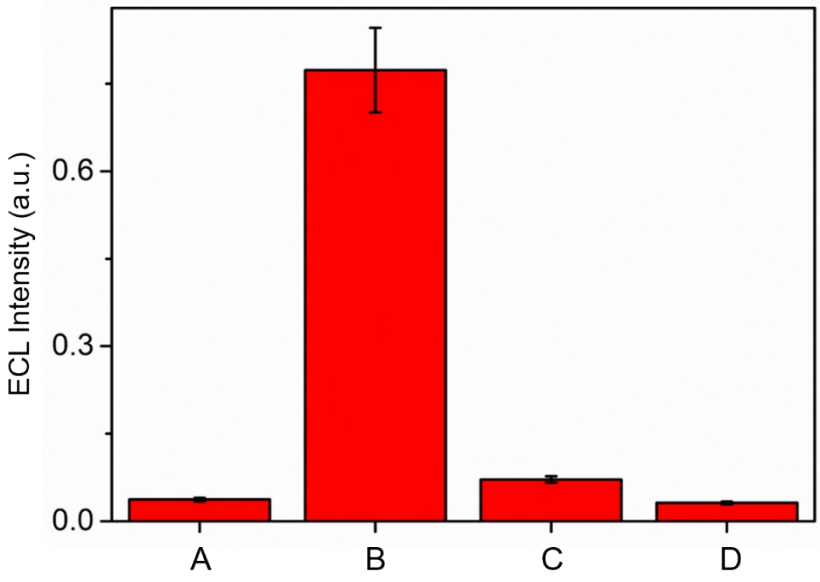


Fig. S9 ECL intensity of MOFs modified GCEs in 0.1 M PBS containing (A) 100 mM H_2O_2, (B) 100 mM $K_2S_2O_8$, (C) 10 mM TPA and (D) 10 mM TEA.

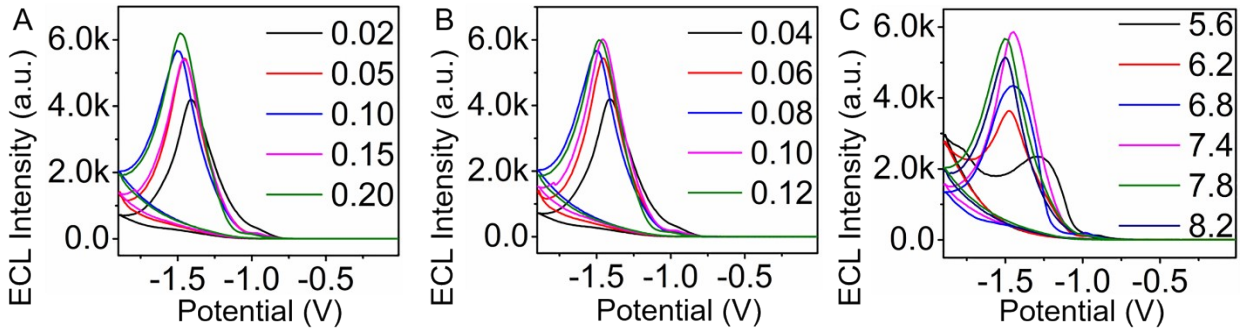


Fig. S10 Dependence of ECL intensity of MOFs modified GCEs on (A) scanning rate (V s^{-1}), (B) concentration of $K_2S_2O_8$ (mol L^{-1}) and (C) pHs in 0.1 M PBS.

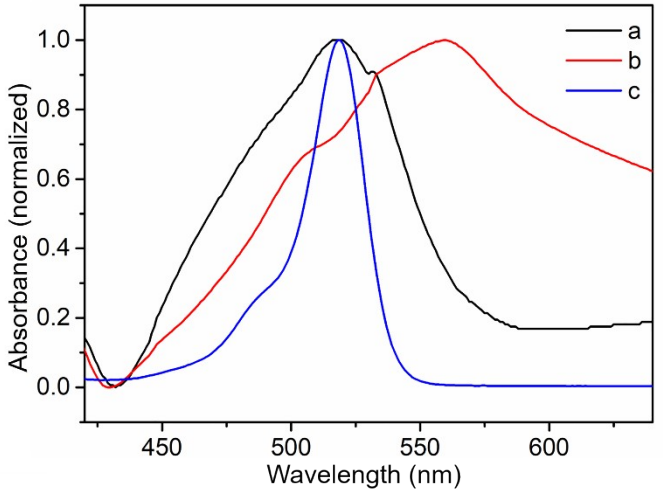


Fig. S11 Normalized UV-vis spectra of 1.0 mg mL^{-1} MOF (a), 1.0 mg mL^{-1} BDP in water (b) and 1.0 mg mL^{-1} BDP (c) in ethanol.

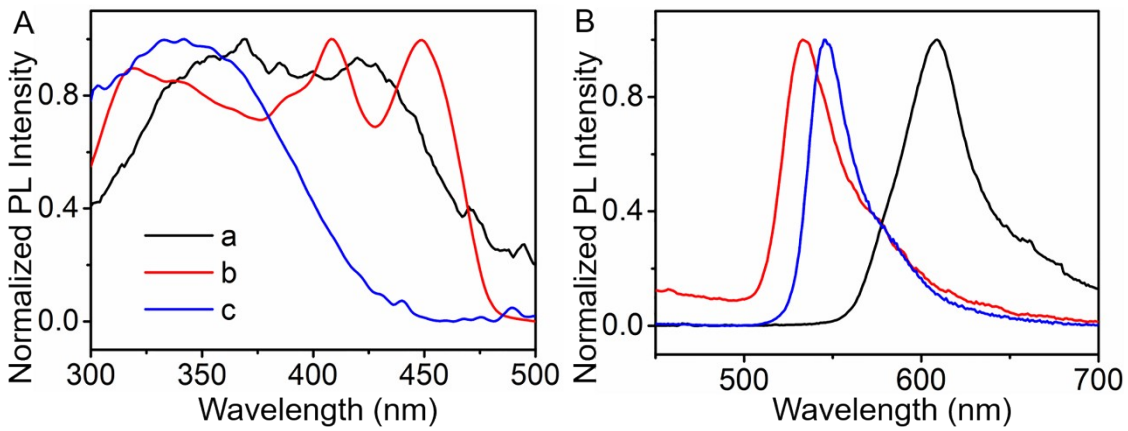


Fig. S12 Normalized fluorescence (A) excitation and (B) emission spectra of 1.0 mg mL^{-1} BDP (a), 1mg mL^{-1} MOF (b) in water and 1.0 mg mL^{-1} BDP (c) in ethanol.

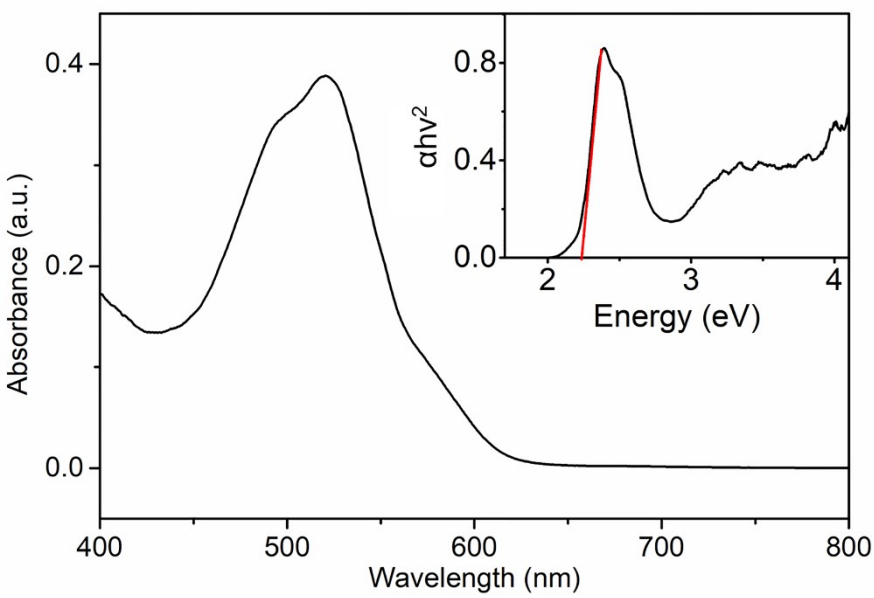


Fig. S13 UV-vis DRS and band gap energy (inset) of MOFs.

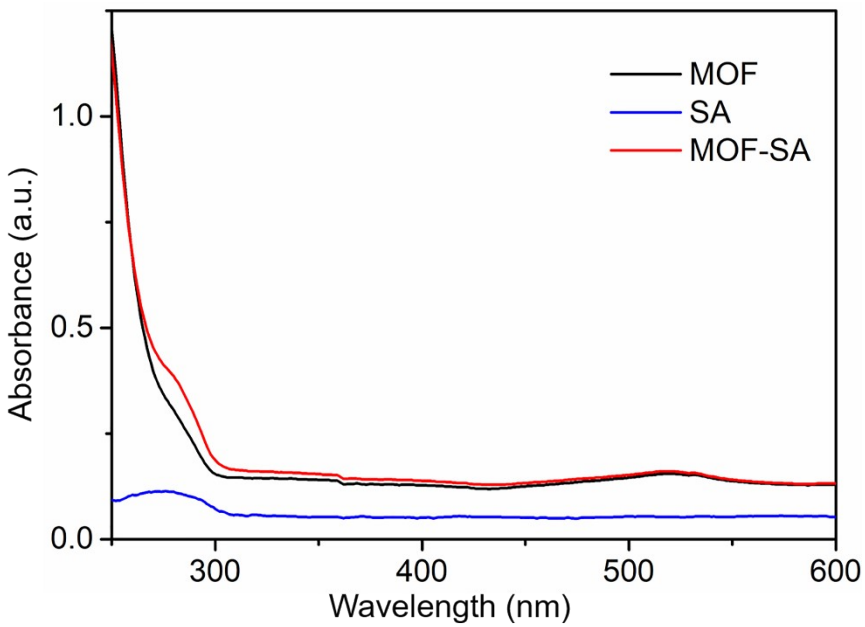


Fig. S14 UV-vis spectra of 1.0 mg ml^{-1} MOF (black), 1.0 mg ml^{-1} SA (blue) and 1.0 mg ml^{-1} MOF-SA (red) in water.

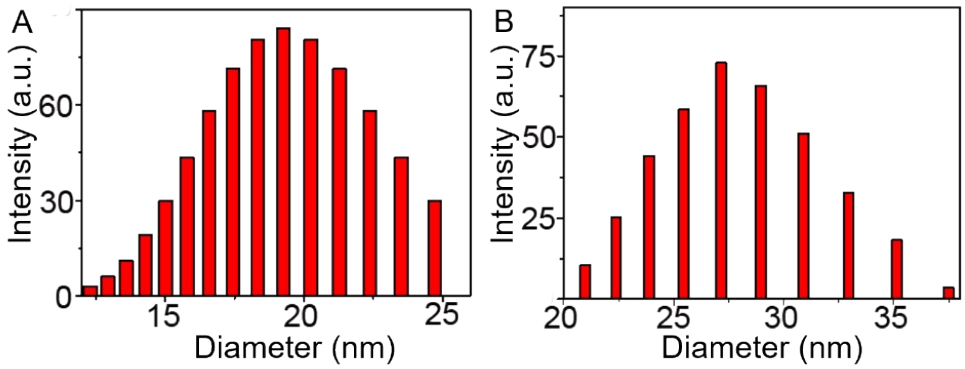


Fig. S15 DLS analysis of (A) AuNPs and (B) **BT-P**-AuNPs.

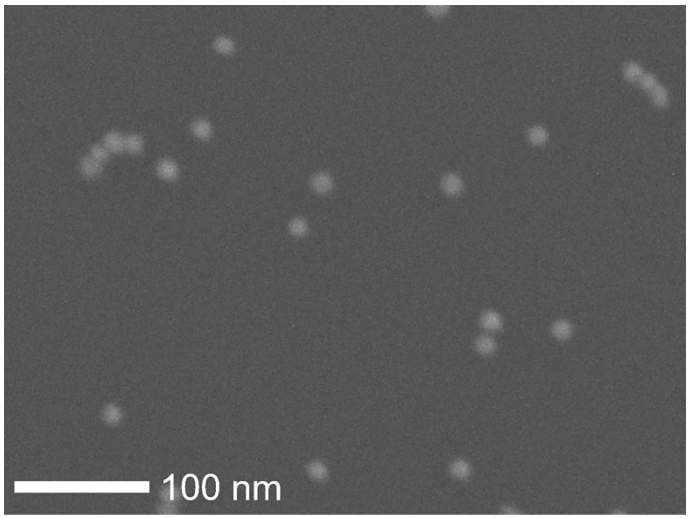


Fig. S16 SEM image of AuNPs.

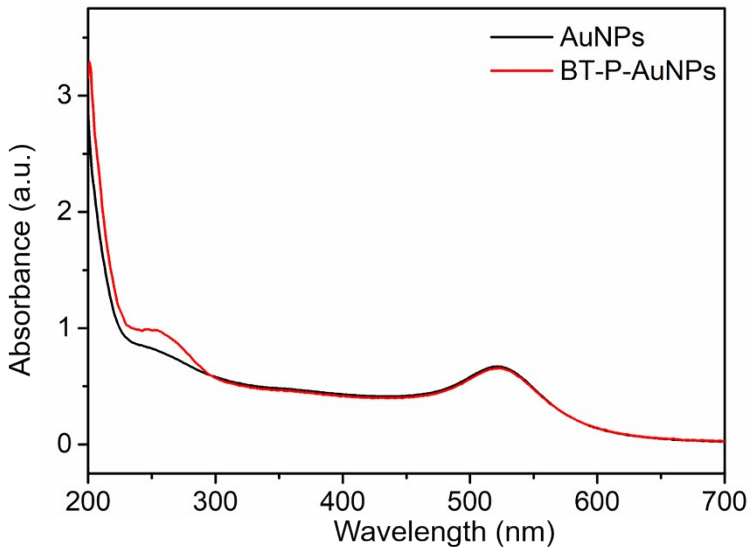


Fig. S17 UV-vis spectra of AuNPs and **BT-P**-AuNPs.

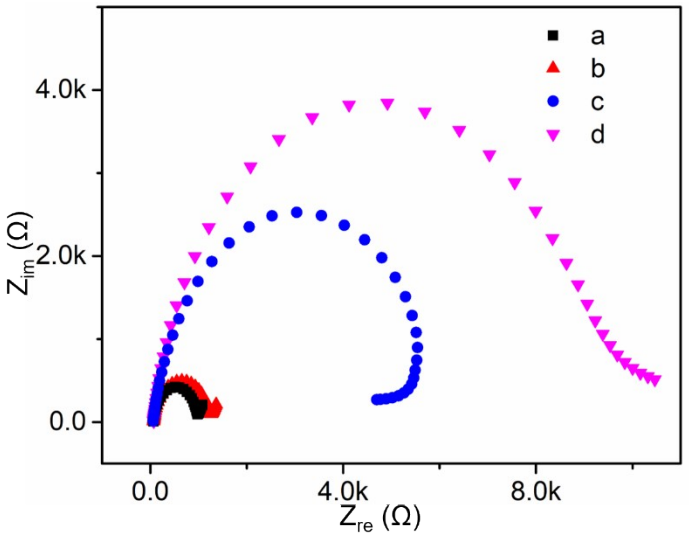


Fig. S18 EIS response of bare GCE (a) and **BT-P**-Au modified GCEs (b), and sequential incubation of telomerase and dNTPs (c) and MOF-SA(d) in the aqueous solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ as redox probe.

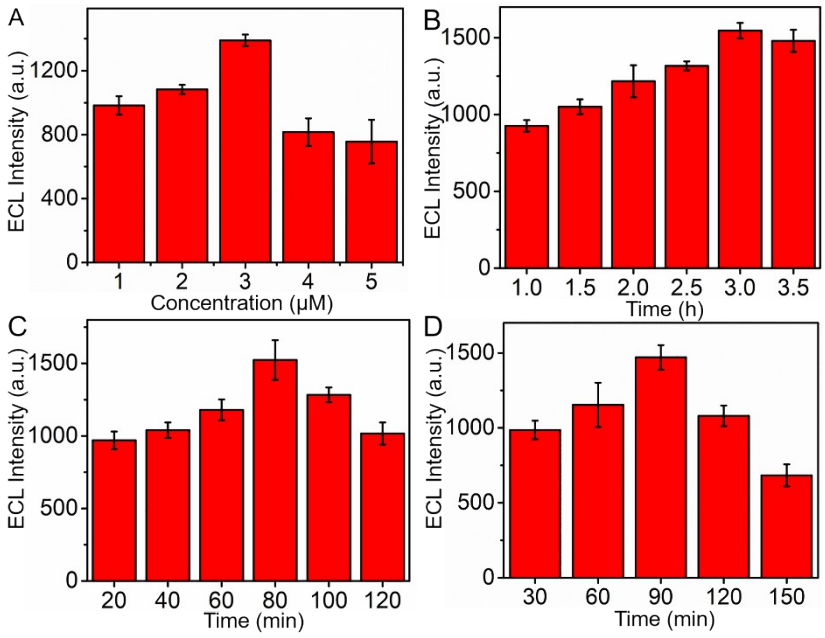


Fig. S19 Effects of (A) concentration of **BT-P**, (B) incubation time of **BT-P**-AuNPs, (C) incubation time for telomerase extension reaction, and (D) hybridization time of MOF-SA on GCE on ECL intensity in the electrochemical biosensing system.

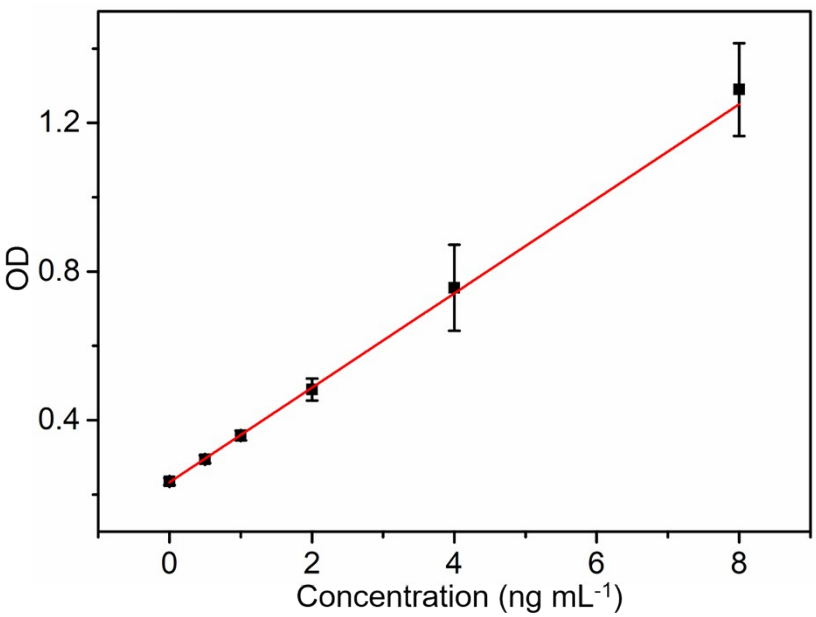


Fig. S20 Standard telomerase concentration curve obtained with a human telomerase ELISA Kit.

Supplemental Table

Table S1. Fluorescence decay time of BDP, MOF in water, and BDP in ethanol.

Samples	**τ_1 (ns) (Rel%)**	**τ_2 (ns) (Rel%)**	**τ_{av} (ns)**
BDP	0.7367 (71.08)	3.1096 (28.92)	1.0507
BDP-MOF	0.8177 (14.64)	3.7327 (85.36)	3.2884
BDP in ethanol	0.6476 (7.55)	4.8990 (92.45)	4.5780

Supporting references

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S2 J. R. Yang, P. F. Dong, Y. Q. Wang, T. R. Liu, Y. Y. Huang and J. P. Lei, *Analyst,* 2021, **146**, 1859-1864.

S3 P. F. Dong, L. Y. Zhu, J. Huang, J. J. Ren and J. P. Lei, *Biosens. Bioelectron.,* 2019, **138**, 111313.