Electronic Supplementary Information

Zirconium-Based Metal-Organic Framework Sensitized by Thioflavine-T for Sensitive Photoelectrochemical Detection of C-Reactive Protein

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Materials and reagents

N,N'-diethylformamide (DEF), 4,4′,4″-s-triazine-2,4,6-triyl-tribenzoic acid (H_3TATB), acetone, $ZrOCl_2 \cdot 8H_2O$, N,N'-dimethylformamide (DMF), gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), hexanethiol (HT), human immunoglobulin G (IgG), bovine serum albumin (BSA), human serum albumin (HSA) and thrombin were bought from Sigma Chemical Co. (St. Louis. MO, USA). Thioflavin-T (Th-T) was purchased from Tokyo Chemical Industry Co., Ltd. C-Reactive Protein (CRP) was obtained from Sino Biological Inc. Carboxyl modified magnetic bead (MB) was purchased from Tianjin BaseLine Chrom Tech Research Centre (Tianjin, China). Hydrogen peroxide (H_2O_2) was obtained from Chemical Reagent Co., Ltd. (Chongqing, China). $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$ were obtained from Beijing Chemical Reagent Co. (Beijing, China). T4 ligase, 10 × T4 ligase reaction buffer, Phi29 polymerase, 10 × phi29 polymerase reaction buffer and deoxyribonucleoside triphosphate (dNTPs) were purchased from Nanjing Vazyme Biotech Co., Ltd. (Nanjing, China). Carcino-embryonic antigen (CEA) and alpha fetal protein (AFP) were supplied by Biocell (Zhengzhou, China). N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) were purchased from Shanghai Medpep Co., Ltd. (Shanghai, China). 0.1 M phosphate buffered solution (PBS, pH 7.4) was prepared with 0.1 M KH_2PO_4, 0.1 M KCl and 0.1 M Na_2HPO_4. Other chemicals were of analytical grade and utilized in this work without further purification. Ultrapure water was employed for preparing solution in this work. The oligonucleotides sequences listed in Table S1 were ordered from

Sangon Inc. (Shanghai, China).

Table S1. The specific oligonucleotides sequences.

Oligonucleotide	Sequences (from 5' to 3')
aptamer	CGA AGG GGA TTC GAG GGG TGA TTG CGT GCT CCA TTT GGT GTT TTT TTT TTT T-NH_2
primer	CCA AAT GGA GCA CGC AAT CAC CCC TCG AAA AAG TTA GG
capture	GTG CTC CAT TTG GAA AAA A-SH
padlock probe	p-CGA GGG GTG ATC CCT AAC CCT AAC CCT AAC CCT AAC TTT TT

Apparatus

The photoelectrochemical (PEC) test was performed on a PEC workstation (Ivium, Netherlands), which was conducted at room temperature in a three-electrode system by using a platinum wire as the counter electrode, a glassy carbon electrode (GCE, Φ = 4 mm) as the working electrode and a calomel (saturated KCl) electrode as the reference electrode. Electrochemical characterization was carried out on a CHI 760E electrochemistry workstation (Shanghai Chenhua Instrumission, China). Scanning electron microscopy (SEM, S-4800, Hitachi, Japan) was used for nanomaterial characterization. The powder X-ray diffraction (XRD) pattern characterization was performed on a XD-3 X-ray diffractometer with Cu Kα radiation (Purkinje, China).

PEC measurement

PBS (5 mL, 0.1 M, pH 7.4) containing 0.06 M H_2O_2 was utilized for PEC

measurements. An UV light with a wavelength of 365 nm produced by LED lamp was served as the excitation light source and the applied voltage was set to 0.0 V.

Experimental section

Preparation of PCN-777 and Th-T solution

The PCN-777 was prepared according to the previous reports.[1] In brief, 60 mg H_3TATB, 200 mg $ZrOCl_2 \cdot 8H_2O$ and 0.6 mL trifluoroacetic acid were added into 12 mL DEF with continuous ultrasonication, followed by heating at 120 ºC for 12 h. Ultimately, the acquired white precipitate was centrifugally washed several times with DMF and acetone, respectively. For preparation of Th-T solution, 2 mg Th-T power was weighed and then added into 2 mL ultrapure water. After 5 mins of ultrasonication, bright yellow homogeneous solution was obtained, which was stored at 4 ºC before use.

CRP converting procedure

Scheme 1A presented the target (CRP) converting procedure. Firstly, 50 μL carboxyl modified magnetic bead (MB) and 50 μL coupling reagent (20 mM EDC and 10 mM NHS) were mixed for 1 h to activate carboxyl of MB. The supernatant was removed via magnetic separation and then the activated MB was redispersed in 50 μL ultrapure water. Afterwards, 50 μL 8 μM amino modified aptamer was added into the above solution and kept for 1 h, which could connect aptamer to MB surface via acylation reaction. Subsequently, 50 μL 8 μM primer was added and incubated at 37 ºC for 2 h to achieve hybridization between aptamer and primer, leading to the generation of DNA duplex (aptamer/primer). Then, 50 μL CRP with different

concentrations was dropped into the mixture for reaction at 25 ºC. After 30 mins, the aptamer-CRP complex structure would be generated by specific binding and primer could be released from DNA duplex. Finally, primer converted from CRP was separated though magnetic force and then utilized for construction of PEC sensing.

Fabrication of the PEC Biosensor

After polishing with α-Al_2O_3 powder and washing with ultrapure water, the GCE was coated by 20 μL PCN-777 solution (1 mg/mL), and then dried at 37 ºC for generating an uniform film. Next, the PCN-777 modified GCE was submerged in 1% $HAuCl_4$ solution for electrodeposition of gold nanoparticles (DepAu), which was run at a constant potential of −0.2 V for 20 s. Afterwards, 20 μL 2 μM capture was attached on DepAu/PCN-777/GCE surface at 4 °C overnight, followed by blocking nonspecific sites with 15 μL 0.1 mM HT. Then, 20 μL primer converted from CRP was dropped on the modified GCE surface, and incubated for 2 h at 37 °C. After incubating of 20 μL 2 μM padlock for 1 h, 5 μL 10 U T4 ligase and 5 μL 10 × T4 ligase reaction buffer were added and kept for 2 h. Subsequently, 2 μL 10 U phi29 polymerase, 5 μL 10 × phi29 polymerase reaction buffer and 5 μL 10 mM dNTPs were simultaneously dropped on the acquired GCE surface for 2 h at 30 °C to achieve RCA, which could generate a large number of periodic G-rich single-stranded DNA (ssDNA). Ultimately, the obtained GCE was coated with 15 μL 1 mM Th-T solution at room temperature for 0.5 h, and Th-T could specifically bind with periodic G-rich ssDNA to form numerous G-quadruplex structures. The fabrication of PEC biosensor was shown in Scheme 1.

Condition optimization

The concentration of H_2O_2 in supporting electrolyte is an important consideration that influences the PEC signal. As illustrated in Figure S1A, with the increase of the concentration of H_2O_2 from 0.02 M to 0.06 M, the PEC signal was observed to be gradually increased. And then the PEC response reached a plateau when the H_2O_2 concentration surpassed 0.06 M. The result hinted that the optimized H_2O_2 concentration was 0.06 M. In addition, the selection of excitation wavelength has a tremendous impact on the generation of PEC signal. The PEC signal that generated by excitation with different wavelength light sources (660, 523, 460 and 365 nm) was recorded in Figure S1B. The PEC signal excited by the light with the wavelength of 365 nm (column d) was larger than that of other columns. Thus, the optimal excitation wavelength was chosen to be 365 nm.

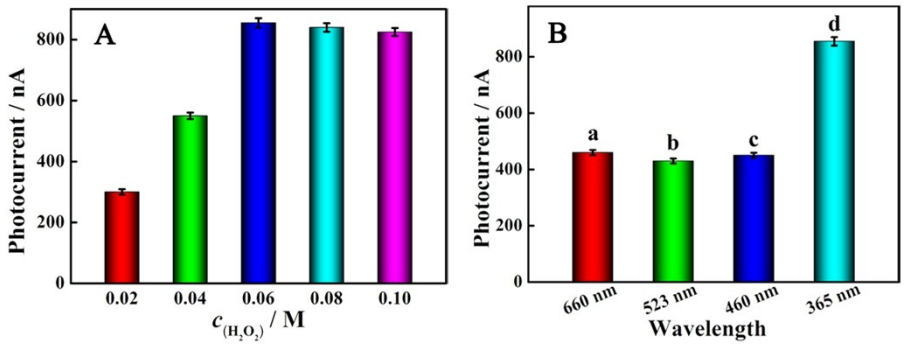

Figure S1. Effect of the H_2O_2 concentration (A) and the irradiation wavelength (B) on the PEC signal.

Electrochemical characterization of the PEC biosensor

Electrochemical impedance spectroscopy (EIS) is an effective and facile technique for characterizing the stepwise assembly procedures of the PEC biosensor.

As shown in Figure S2, compared with that of bare GCE (curve a), the charge-transfer resistance (R_{et}) of the PCN-777 modified electrode presented obvious enhancement (curve b), which might be derived from the poor conductivity of PCN-777. After the electrodeposition of Au nanoparticals (DepAu), the R_{et} decreased dramatically (curve c) because of the excellent conductivity of Au nanoparticals. The R_{et} increased successively when capture, nonconductive HT and primer were continuously incubated on the DepAu/PCN-777/GCE surface, which were presented in curve d, curve e and curve f, respectively. With the immobilization of padlock and T4 ligase, the R_{et} increased evidently (curve g), which illustrated that the padlock was successfully bound to the modified electrode surface. Once the above electrode was incubated with phi29 polymerase and dNTPs, the R_{et} increased significantly (curve h), suggesting that the rolling circle amplification (RCA) was on schedule. Ultimately, an evidently decreased R_{et} was found when the introduction of Th-T occurred (curve i), because Th-T might promote the negatively charged probe to access the electrode surface. These results confirmed the successful assembly of the sensing interface.

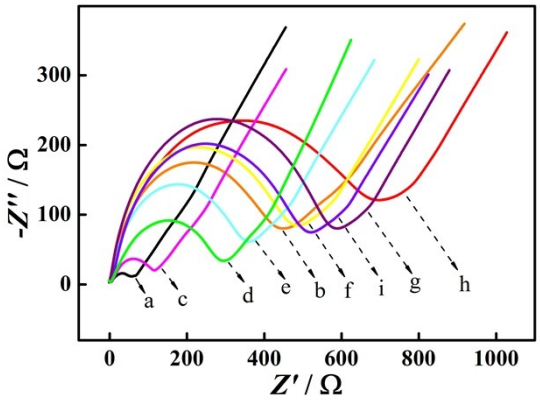

Figure S2. EIS responses of (a) bare GCE, (b) PCN-777/GCE, (c) DepAu/PCN-777/GCE, (d) capture/DepAu/PCN-777/GCE, (e) HT/capture/DepAu/PCN-777/GCE, (f)

primer/HT/capture/DepAu/PCN-777/GCE, (g) T4 ligase + padlock/primer/HT/capture/DepAu /PCN-777/GCE, (h) phi29 polymerase + dNTPs/T4 ligase + padlock/primer/HT/capture/DepAu /PCN-777/GCE and (i) Th-T/phi29 polymerase + dNTPs/T4 ligase + padlock/primer/HT /capture/DepAu/PCN-777/GCE in PBS containing KCl (0.1 M) and $[Fe(CN)_6]^{3-/4-}$ (5 mM).

Table S2. Comparison of different methods for protein analysis.

Analytical method	LOD	Linear range	Ref.
fluorescence	10 pM	0-1 nM	2
electrochemistry	0.76 pM	2 pM-20 nM	3
surface plasmon resonance	10 pM	10 pM-100 nM	4
PEC	30 fM	100 fM-10 nM	5
PEC	0.5 pM	1 pM-10 nM	6
PEC	16 fM	50 fM-50 nM	**Our work**

SEM and EDX spectrum characterization

Nanomaterials were gradually modified on the surface of conductive glass (CG) and then they were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) spectrum. Figure S3A was a SEM image of PCN-777/CG, which presented homogeneous octahedrons with average diameter of 2.5 μm. The SEM image of gold nanoparticles (Au NPs) electrodeposited on bare CG surface was shown in Figure S3B and a large number of uniform flower-like shape could be obviously observed. After electrodepositing a layer of Au NPs (DepAu) for 15 s on the PCN-777 modified CG surface, homogeneous octahedrons and flower-like structures could be simultaneously found in Figure S3C. As illustrated in Figure S3D, a flat film covered the modified CG surface when capture was incubated on the

surface of DepAu/PCN-777/CG. Moreover, its EDX spectrum was recorded for further proving the successful immobilization of capture, which was shown in Figure S3G. The existence of C, N, O and P elements indicated that the oligonucleotides sequence (capture) was successfully coated on the modified CG surface. Phosphate buffered solution was used for diluting oligonucleotides sequences, thus Na, Cl and K elements could also be found in Figure S3G. With the immobilization of nonconductive HT, some bright areas were presented in Figure S3E, which originated from electrons gathering around the nonconductive HT. Finally, the introduction of primer led to a uniform film covering the modified CG surface (Figure S3F). The SEM and EDX results of these states demonstrated that the immobilization of nanomaterials was successful during the construction processes of the PEC biosensor.

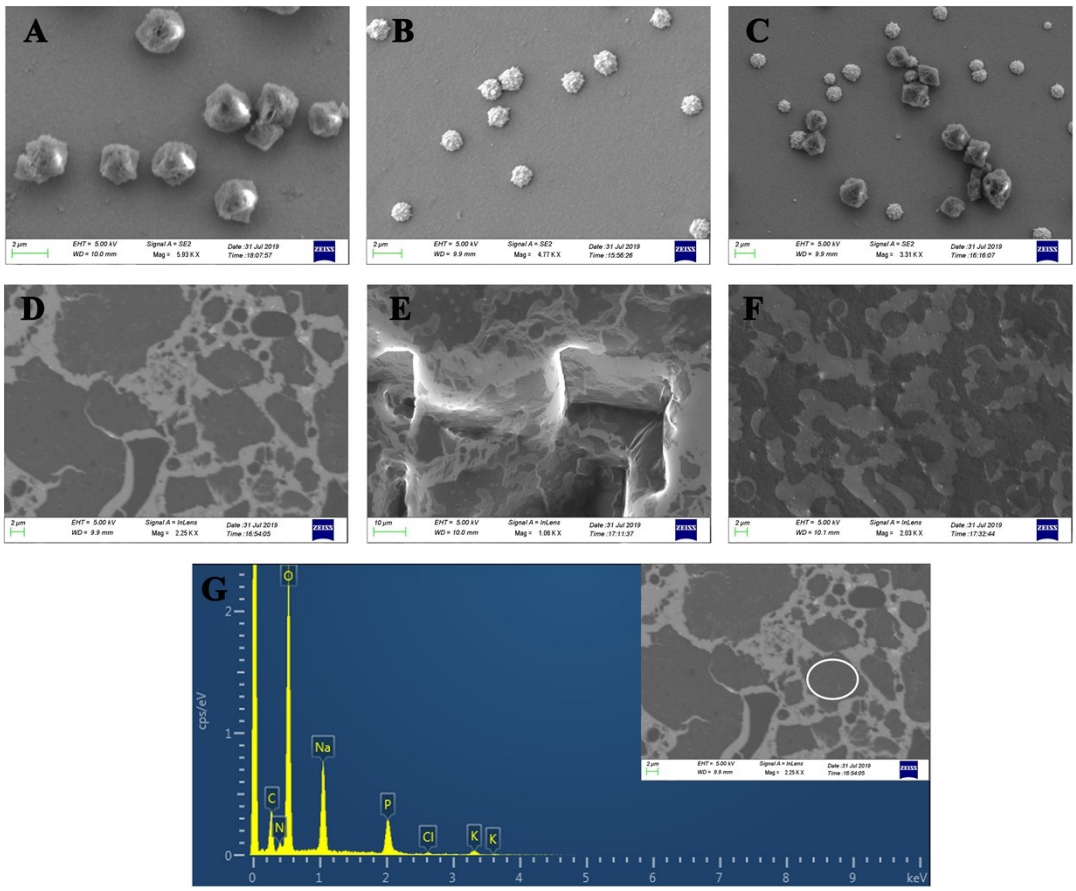

Figure S3. SEM of PCN-777/CG (A); DepAu/CG (B); DepAu/PCN-777/CG (C); capture/DepAu/PCN-777/CG (D); HT/capture/DepAu/PCN-777/CG (E); primer/HT/capture /DepAu/PCN-777/CG (F). EDX spectrum of capture/DepAu/PCN-777/CG (G).

PEC analysis of CRP

According to related references[7, 8], the limit of detection (LOD) in our work was defined as LOD = $3S_B/m$, where S_B was the standard deviation of the blank and m was the analytical sensitivity, in which m could be estimated as the slope of calibration plot at low concentration ranges. The changed trends of photocurrent values with the increasing CRP concentration was presented in Figure S4. The insert demonstrated that the photocurrent values (I / nA) was linearly related to the concentration of CRP (c / pM) at low concentration range. The corresponding linear equation was $I_{(nA)}$ =

155.4$c_{(pM)}$ + 424.1 and the S_B of twenty times zero-dose was about 0.83. Therefore, the LOD of the proposed PEC biosensor was 0.016 pM (16 fM), demonstrating a high sensitivity of the designed strategy for CRP detection.

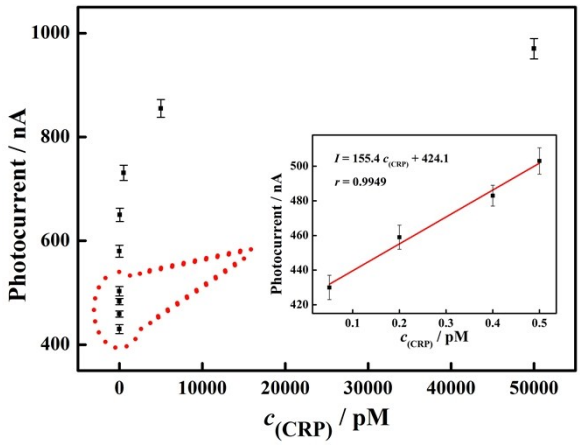

Figure S4. The changed trends of PEC response with the increasing of CRP concentration. The insert showed the calibration curve of PEC response *vs* the CRP concentration at low concentration range.

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