

Molecularly Imprinted Polymer Coating-Assisted CsPbBr₃ Perovskite Quantum Dots/TiO₂ Inverse Opal Heterojunctions for the Photoelectrochemical Detection of Cholesterol

Xuan Wang^{1†}, Fankai Lin^{1†}, Xiaoyu Zhou^{2†}, Yunfei Miao¹, Dongwei Feng¹, Peng Huang³, Axin Liang¹, Bo Tang⁴, Huipeng Hou¹, Mingxing Ren¹, Lina Geng^{1*}, Aiqin Luo^{1*}, Yulin Deng^{1*}

¹ School of Life Science, Beijing Institute of Technology, 5 South Zhongguancun Street, Haidian District, Beijing 100081, P.R. China.

² Cellomics (Shenzhen) Limited, Shenzhen, Guangdong, China;

Department of Biomedical Sciences and Tung Biomedical Sciences Centre, City University of Hong Kong, Hong Kong, China

³ MIIT Key Laboratory for Low Dimensional Quantum Structure and Devices, School of Materials Sciences and Engineering, Beijing Institute of Technology, 5 South Zhongguancun Street, Haidian District, Beijing 100081, P.R. China.

⁴ College of Food and Bioengineering, Bengbu University, Bengbu 233030, P.R. China.

1. Experimental

1.1 Reagent

Triglyceride (TG), Cesium Bromide (CsBr), Ethylene glycol dimethacrylate (EGDMA), Azodiisobutyronitrile (AIBN), methacrylic acid (MAA), and Triton X-100 were bought from Shanghai Aladdin Biochemical Technology Co., Ltd.. N, N-dimethylformamide (DMF), potassium ferricyanide, ethyl acetate (EA), N-hexane, toluene, acetonitrile, dichloromethane, chloroform, and Na₂SO₄ were supplied by Beijing Tongguang Fine Chemical Company. Cholesterol, Methyl methacrylate, oleylamine and tetrabutyl titanate were bought from Meryer Chemical Technology CO., Ltd. (Shanghai, China). Isopropyl alcohol was bought from Beijing Chemical

Works. The indium tin oxide-coated glasses (ITO, 10 Ω /square) used in this study were purchased from Luoyang Guluo glass Co. Ltd (Luoyang, China).

1.2 The preparation process of TiO₂ IOPCs

TiO₂ IOPCs on ITO glass was prepared by the normal colloidal crystal template method. First, The PMMA nanospheres were prepared in the reference to the literature [51]. A solution containing deionized water (48 mL) and 5 mL methyl methacrylate (MMA) was heated at 90 °C under magnetic stirring at 150 rpm. After that, potassium persulfate solution (19 mg/mL) was slowly added. The solution was further heated at 90 °C for 1.5 hours. After 5 mL prepared PMMA colloidal solution was mixed with 30 mL of ultrapure water and treated by 15 minutes of ultrasound, the obtained PMMA colloidal suspension was ready to be used.

Second, the indium tin oxide conductive glass (ITO) substrate was pretreated by ultrasonic washing in sequence with glass cleaning solution (15min), ultrapure water (10min), alcohol (10min), acetone (10min), isopropanol (10min), ultrapure water (10min) and alcohol (10min). Finally, ITOs were dried with N₂.

Third, the PMMA photonic crystal structure was prepared by the self-assembly method of vertical deposition. The clean ITO glass slide was vertically immersed into the prepared PMMA microsphere colloidal suspension and placed in an oven at 32 °C for 48 hours. The container was left open to let the water slowly evaporate. PMMA microspheres were then slowly self-assembled into a highly ordered array on the ITO substrate due to the surface tension produced in the process of liquid evaporation.

Fourth, the PMMA template photonic crystal structure was sintered at 120 °C for 40

min. Next, after 5mL tetra butyl titanate was completely dissolved in 5mL ethanol, 0.5mL deionized water and 0.5 mL nitric acid was added. This solution was stirred for 12 hours to form a colorless transparent titanium ion precursor solution. This TiO₂ sol-gel precursor solution was introduced and infiltrated into the voids of PMMA photonic crystal templates with capillary force. After fully drying at 25 °C for 12 hours, the resulting products were put into the tube furnace for calcining at 500 °C for 6 h to remove the PMMA template. The TiO₂ IOPCs structure was then obtained, displaying strong opalescence under incident light.

1.3 The synthesis of CsPbBr₃ nanocrystals

Colloidal CsPbBr₃ nanocrystals was synthesized fabricated following the reported ligand assisted reprecipitation (LARP) synthesis method including: a mixture of 0.2mmol CsBr and 0.2mmol PbBr₂ was dissolved in 1 mL of DMSO with 120μL of oleylamine and 0.5 mL of oleic acid to form a precursor solution. Under vigorous stirring, one milliliter of precursor solution was dropped into ten milliliter toluene or tertiary butanol. Bright CsPbBr₃ colloidal nanocrystals was then obtained after centrifugation at 5000 rpm for 3 mins.

1.4 The preparation of MIP@TiO₂ IOPCs/ CsPbBr₃ composite electrode

The colloidal CsPbBr₃ solution was obtained by the dispersing of CsPbBr₃ ODs in toluene. Precipitation was carried out in order to remove the large size particles. The remaining small particles was dispersed in toluene to form colloidal solution. After that, a certain amount of CsPbBr₃ solution was dropped onto TiO₂ IOPCs. With a 6-hour volatilization of toluene in an air-dry oven, the CsPbBr₃ were then self-assembled

slowly in the void space of TiO₂ IOPCs.

To prepare MIP_S prepolymerization solution, 1 mmol cholesterol and 6 mmol MAA, and 35 mmol EGDMA were dissolved in a 4mL mixture of toluene and chloroform (7:1, V/V). After the ultrasonic treatment for 10 minutes, the mixture was put in 4 °C for 12 hours. Finally, 20.7 mg of AIBN was added. After 10 minutes of ultrasonic treatment, the solution was purged with nitrogen to remove the air for 10 minutes.

Next, 20 μL pre-polymerization solution was dropped onto and slowly coated the TiO₂ IOPCs/CSPbBr₃ on the ITO substrate. The photopolymerization was carried out under irradiation by an ultraviolet lamp (365 nm) for 3 hours. MIP film capped TiO₂ IOPCs/CsPbBr₃ composite electrode was obtained. Finally, the electrode was vertically inserted into n-hexane-ethyl acetate solution (3:1, V/V) and soaked for 15 minutes in order to remove the cholesterol template molecule. After the elution of template, the electrode was put into a vacuum drying oven and dried for 3 hours before standby. To prepare a control, the preparation process of non-molecularly imprinted polymers (NIPS) was the same as that of MIPs, except that the template molecule cholesterol was not added in the pre-polymerization solution.

1.5 Characterization

Scanning Electron Microscopes Phenom Pro Desktop (SEM, Thermo) was used to observe the surface morphology of different electrodes during preparation. The phase structure and purity of different electrodes were analyzed using multifunctional powder X-ray diffraction analysis (Xpert powder, Panaco, Netherlands). Fourier transform infrared spectra (FTIR) was performed on IRAffinity-1s (Shimadzu, Japan). The

analysis of fluorescence intensity of CsPbBr₃ QDs in different organic solvents was carried out by a fluorescence spectrometer (rf-5301, Shimadzu, Japan). The absolute photoluminescence quantum yield measurement of solutions were determined using a fluorescence spectrometer (calibrated multichannel spectrometer, PMA12) with an integrated sphere (C9920-02, Hamamatsu Photonics, Japan). Time-resolved PL spectra were recorded on a Horiba Scientific DeltaFlex TCSPC lifetime fluorometer (excitation: 365 nm). Ultraviolet-visible (UV-Vis) extinction spectra were measured with a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan).

1.6 The electrochemical and PEC characterization

Electrochemical impedance spectroscopy (EIS) was taken through a model CHI660D electrochemical analyzer (Chenhua, Shanghai, China). A three-electrode system comprised a platinum wire as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode, and the modified electrode with a geometrical area of 1.0 cm² as the working electrode, was used in this work during photochemical and electrochemical analysis. The electrolyte contained 5 mmol/L [Fe(CN)₆]^{3-/4-}, 0.5 mol/L Na₂SO₄ and 0.1 mol/L CsBr in pH=7.0 phosphate buffer. The frequency range was set as 1 Hz to 100 kHz, DC voltage was 0.276 V and AC amplitude was 5 mV.

The PEC experiments were recorded on a PEC1000 instrument (Beijing Perfectlight Technology Co, LTD). The PEC measurements with the same three-electrode system as EIS were carried out in pH=7.0 phosphate buffer containing 0.5 mol/L Na₂SO₄ solution and 0.1 mol/L CsBr at the bias potential of 0.6 V under the irradiation of visible light (500 mW·cm⁻²).

Results and discussions

2.1 Experimental optimization

2.1.1 Optimization of inverse opal structure assembly

TiO₂ IOPCs were prepared by employing the colloidal crystal template method in which the PMMA photonic crystals were self-assembled. The experimental conditions influencing the size of PMMA microspheres and the size distribution of PMMA microspheres are critical for the preparation of monodispersed and high-quality PMMA colloidal spheres. The proper rotating speed was 150 rpm, and the proper dipping speed of the initiator was 1.5 mg/min. The ITO glass substrate was then vertically immersed into the prepared PMMA colloidal suspension to allow the formation of the PMMA opal structure. After being placed in an oven at 32 °C for 3 days, PMMA colloidal spheres were slowly self-assembled into a highly 3D ordered array on the ITO substrate driven by the surface tension produced due to liquid evaporation. Figure S1A shows the SEM data of inverse opal. PMMA microspheres are approximately 200 nm. Furthermore, PMMA colloidal microspheres self-assembled into a periodic face-centred cubic (FCC) structure in three-dimensional space. The assembly was regular over a relatively large area. Figure S1B displays the assembled PMMA inverse opal with bright-green structure colour.

2.1.2 Optimization of the preparation of MIPs

The suitable porogen for molecularly MIPs was investigated to avoid the decomposition of CsPbBr₃ QDs during the preparation of MIPs. Based on the study of organic solvents including N-hexane, toluene, ethyl acetate, dichloromethane, chloroform, acetonitrile, acetic acid, ethanol and DMF were compared. As shown in Figure S1C, in nonpolar solvents (i.e. n-hexane, toluene and dichloromethane), the

CsPbBr₃ QD solution with a bright green colour maintained characteristic peaks in the fluorescent spectra. In solvents with high polarity (i.e. N, N-dimethylformamide and ethanol), CsPbBr₃ QDs decomposed with the disappearance of green fluorescence. Since toluene was also adopted as the dispersion solvent for the prepared perovskite QDs, a mixed solution of toluene and chloroform was chosen as the solvent for preparing the MIPs.

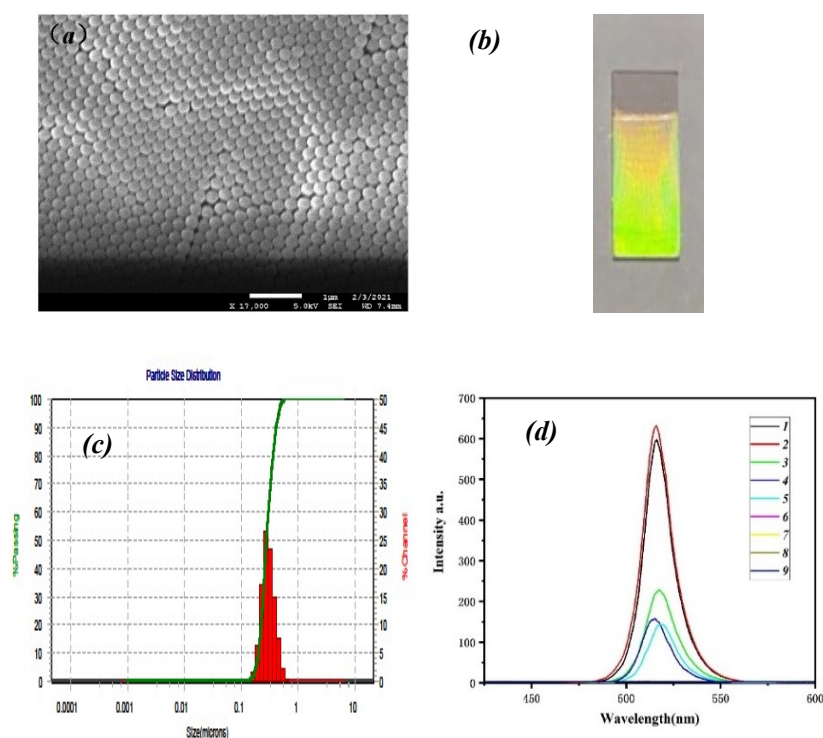


Figure S1 (a) The SEM image of PMMA opal structure; (b) the opal assembled PMMA with bright green structure color; (c) The size distribution analysis of PMMA microspheres; (d) The fluorescent emission spectra of QDs in different solvents, including organic solvent, including 1. N-hexane, 2. toluene, 3. ethyl acetate, 4. dichloromethane, 5. chloroform, 6. acetonitrile, 7. acetic acid, 8. ethanol and 9. DMF were compared.

2.1.3 The PL analysis of CsPbBr₃

The absolute photoluminescence quantum yield measurement of CsPbBr₃ was carried

out. As shown in Table S1, the average photoluminescence quantum yield (PLDY) turned out to be about 89.64% under 369.54nm.

Table S1 PL analysis of CsPbBr₃

ABS	0.118	0.160	0.113	0.161	0.077
PLQY	0.737	1.077	0.977	0.841	0.850

2.1.4 The optimization of bias potential

During the PEC analysis, since the bias voltage influenced the recombination of photogenerated electron/hole pairs, different bias voltages on the photocurrent of MIP@TiO₂ IOPCs/CsPbBr₃ were compared. It was found that the photocurrent response initially increased with increasing applied voltage. This indicated that the positive voltage contributed to the separation of electrons and holes. When the applied voltage exceeded 0.6 V, the photocurrent response decreased rapidly. Moreover, the photocurrent was unstable. These phenomena might be attributed to the destruction of the heterostructure structure of TiO₂ and CsPbBr₃ under excessive bias. Therefore, 0.6 V was found to be the proper bias potential in this study.

2.1.5 Proper elution time during MIP preparation

Additionally, the proper duration time for template elution was investigated. N-Hexane and ethyl acetate (V:V=3:1) were used as the solvents for template elution. As shown in Figure S2, with increasing washing time, the photocurrent of MIP@TiO₂ IOPCs/CsPbBr₃ slowly peaked after 15 minutes, indicating that the template molecules had been fundamentally removed. Therefore, in the subsequent experiments, the elution time of the template from the MIP membrane was set to 15 minutes.

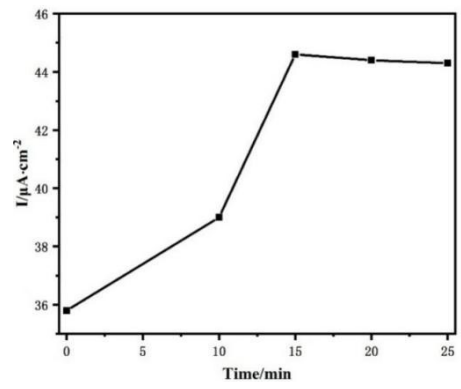


Figure S2 I-t curves of TiO₂ IOPCs/ CsPbBr₃ QDs/ MIPs prepared with different elution time.