

Support Information

Enhanced Light-Driven Catalytic Performance of Cytochrome P450 Confined in Macroporous Silica

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Experimental

Materials and reagents

Tetramethyl orthosilicate (TMOS), poly-(ethylene oxide)-poly(propylene oxide) - poly(ethylene oxide) (EO₂₀PO₇₀EO₂₀, M_n=5800, denoted as P123), ascorbic acid (AA), 7-ethoxytrifluoromethyl coumarin (7-EFC), 4-nitrophthalic anhydride and CYP3A4 isozyme were obtained from Sigma-Aldrich Co. Ltd. (St. Louis, MO, USA). Graphite was purchased from XFNANO Materials Tech Co. (Nanjing, China). Phosphate buffer solution (PBS, 0.1 M, pH 7.4) was prepared by mixing the stock solution of K₂HPO₄ and KH₂PO₄. MOSF and 2, 9, 16, 23-tetraaminophthalocyanine cobalt (CoTAPc) were synthesized according to the reported methods with a slight modification (See details in SI).^{1,2} The as-obtained CoTAPc was confirmed by FT-IR and UV-vis absorbance spectra (Fig. S1). The graphene oxide (GO) was prepared from natural graphite by a modified Hummers' method.^{3,4} 10 mL 1.0 mg mL⁻¹ of the as-prepared GO suspension was mixed with 0.5 mL 1.0 mM of ascorbic acid, and the pH was adjusted to 8.5-9.0 with 25 wt% ammonia solution. The obtained solution was continuously stirred for 15 min at 95 °C. The mixture was then centrifuged, washed with water, ultrasonicated for 5 h (400 W, 40 kHz) to obtain reduced graphene oxide (RGO) aqueous suspension (1.0 mg mL⁻¹). All other chemicals were of analytical grade and used as received. All aqueous solutions were prepared with deionized water (DI water, 18.2 MΩ cm) obtained from a Milli-Q water purification system.

Apparatus

A 500 W Xe lamp fitted with a 420 nm UV filter (Zolix, China) was used as the irradiation source. Scanning electron microscope (SEM) images were recorded with a field emission scanning electron microscope (FESEM, Hitachi S-4800, Japan). The transmission electron microscope (TEM) images were performed by using transmission electron microscope (JEOL Model JEM 2100, Japan). Photocurrent measurements were performed on a CHI 750C electrochemical workstation (Shanghai Chenhua, China) with a conventional three-electrode system including a modified indium tin oxide electrode (ITO, ca. 0.5 cm²), a Pt wire and a saturated calomel electrode (SCE) as the working, auxiliary and reference electrodes, respectively. Fluorescence spectra were obtained from an F-4600 FL spectrophotometer (Hitachi, Japan). The zeta potential analysis and the dynamic light scattering (DLS) experiments were performed with a Malven zetasizer nano ZS (England). Mass spectra analyses were performed with Agilent triple quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) in positive ion mode with ion spray voltage 5.5 kV, turbo heater temperature of 500 °C, curtain, nebulizer, turbo, and collisionally activated dissociation gases at 40, 50, 60, and 6 psi, respectively.

Preparation and characterization of CoTAPc

2, 9, 16, 23-tetraaminophthalocyanine cobalt (CoTAPc) was synthesized by two-step approach. Firstly, 2, 9, 16, 23-tetranitrophthalocyanine cobalt was prepared by cyclotetramerization from 4-nitrophthalic anhydride in the presence of CoCl₂. Then, 2, 9, 16, 23-tetranitrophthalocyanine cobalt was reduced by Na₂S to form CoTAPc.² The

FT-IR spectra showed that (Figure S1A), the peaks of Pc skeleton vibration at 735, 750, 1095, 1135 and 1346 cm^{-1} , the peak of Co-N coordinate bond at 821 cm^{-1} , the peak of arylamine at 1608 cm^{-1} and the double peaks of $-\text{NH}_2$ at 3331/3207 cm^{-1} illustrated the successful synthesis of CoTAPc. The UV/vis absorbance spectra showed that (Figure S1B), CoTAPc had a typical B band at 320 nm and a Q band at 706 nm in the near-infrared region, which was attributed to the $\pi-\pi^*$ transition from the HOMO to the LUMO of the CoTAPc ring.⁵ Furthermore, when CoTAPc combined with RGO and then assembled CYP3A4, the Q band peak position of CoTAPc in the UV/vis absorbance spectra has no significant change (Figure S2), indicating the stable photoactivity of CoTAPc during the preparation process of CYP3A4/CoTAPc/RGO.

Preparation of enzymatic reactor

CoTAPc/RGO assembly was obtained as follows: 1 mL N,N' -dimethylformamide (DMF) solution containing 10 mg of CoTAPc was added into 10 mL of 1.0 mg mL^{-1} RGO suspension, mixed for 30 min, followed by ultrasonication for 15 min. Then the mixture solution was centrifuged, washed with water and dried in air for 24 h. The assembly of CYP3A4 and CoTAPc/RGO was prepared as follows: 2.0 mg of CoTAPc/RGO was redispersed in 0.5 mL 0.1 M PBS (pH 7.4), and reacted with 1.0 mL glutaraldehyde (GA, 2.5 wt%) under constant stirring for 0.5 h. After centrifugation, the GA/CoTAPc/RGO precipitate was washed with water and dispersed in 0.5 mL deionized water. Then, 50 μL of CYP3A4 (1.0 μM) was added, and incubated for 24 h at 4 $^\circ\text{C}$ with mild stirring, followed by centrifugation and

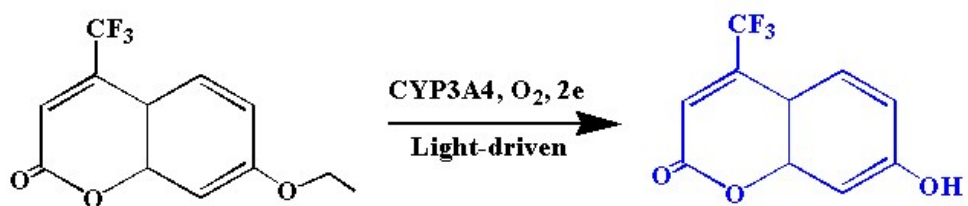
washing. Subsequently, the as obtained CYP3A4/CoTAPc/RGO was re-dispersed in 0.5 mL 0.1 M pH 7.4 PBS, followed by adding 5 mg of as-synthesized MOSF, and mild stirring at 4 °C for 48 h. Finally, the as-prepared CYP3A4/CoTAPc/RGO/MOSF were washed and dispersed in 5 mL of 0.1 M PBS (pH 7.4), and stored at 4 °C for the following use.

Preparation of CYP3A4/CoTAPc/RGO/ITO electrode

Before preparation, ITO conductive glasses were sonicated in acetone, ethanol, water and 4% NaOH solution for 15 min, respectively, followed by drying them at 120 °C for 2 h. Then 50 μ L of as-prepared CYP3A4/CoTAPc/RGO dispersion in 0.1 M pH 7.4 PBS was coated on the surface of ITO electrode and dried in the ambient environment. So, the CYP3A4/CoTAPc/RGO/ITO electrode was constructed for investigation of the mechanism of light-driven enzymatic reaction and the electron transfer between photosensitizer and enzyme under the light irradiation. For comparison, 50 μ L DMF solution containing 2 g L⁻¹ CoTAPc was coated on the ITO to prepared a CoTAPc/ITO electrode, and 50 μ L of 2 g L⁻¹ CoTAPc solution in 0.1 M pH 7.4 PBS was coated on the ITO to prepared a CoTAPc/RGO/ITO electrode.

Light-driven bioconversion of 7-EFC by CYP3A4/CoTAPc/RGO/MOSF

50 μ L of 2 mM 7-EFC solution was added to 1.0 mL 0.1 M pH 7.4 PBS containing 1.0 mg mL⁻¹ CYP3A4/CoTAPc/RGO/MOSF and 0.1 M AA. The mixture solution was stirred mildly and irradiated under the visible light for different time, the conversion of 7-EFC was monitored by fluorescence spectroscopy and mass spectrometry.



Scheme S1. Bio-conversion process of 7-ethoxytrifluoromethyl coumarin catalysed by CYP3A4 with light-driven way.

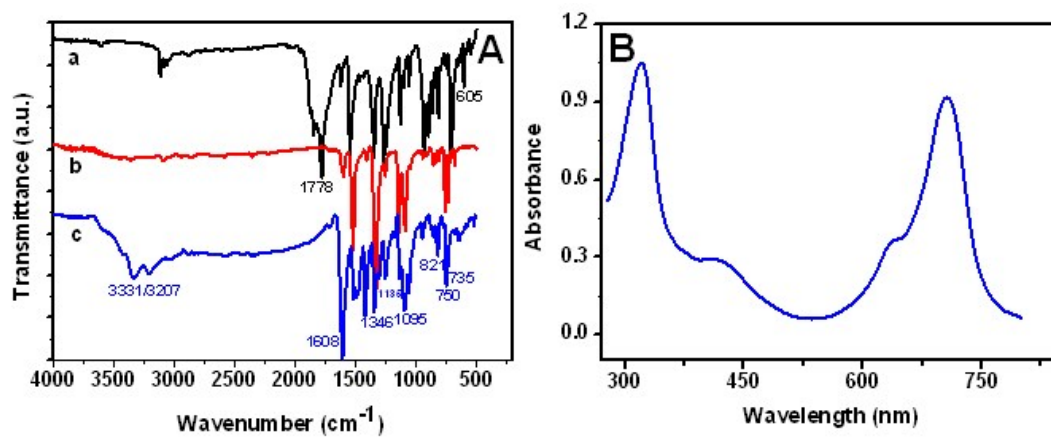


Figure S1. (A) FT-IR spectra of 4-nitrophthalic anhydride (a), intermediate (b) and product CoTAPc (c). (B) UV-vis spectra of CoTAPc.

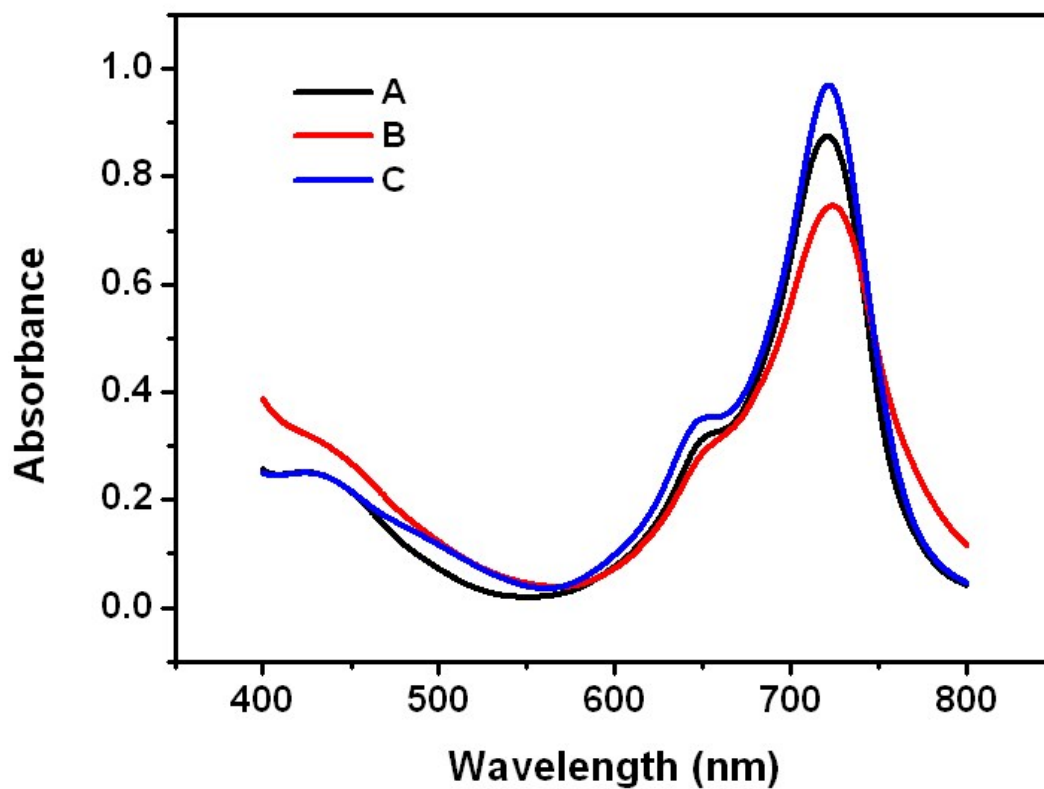


Figure S2. UV-vis spectra of CoTAPc (A), CoTAPc/RGO (B) and CYP3A4/CoTAPc/RGO.

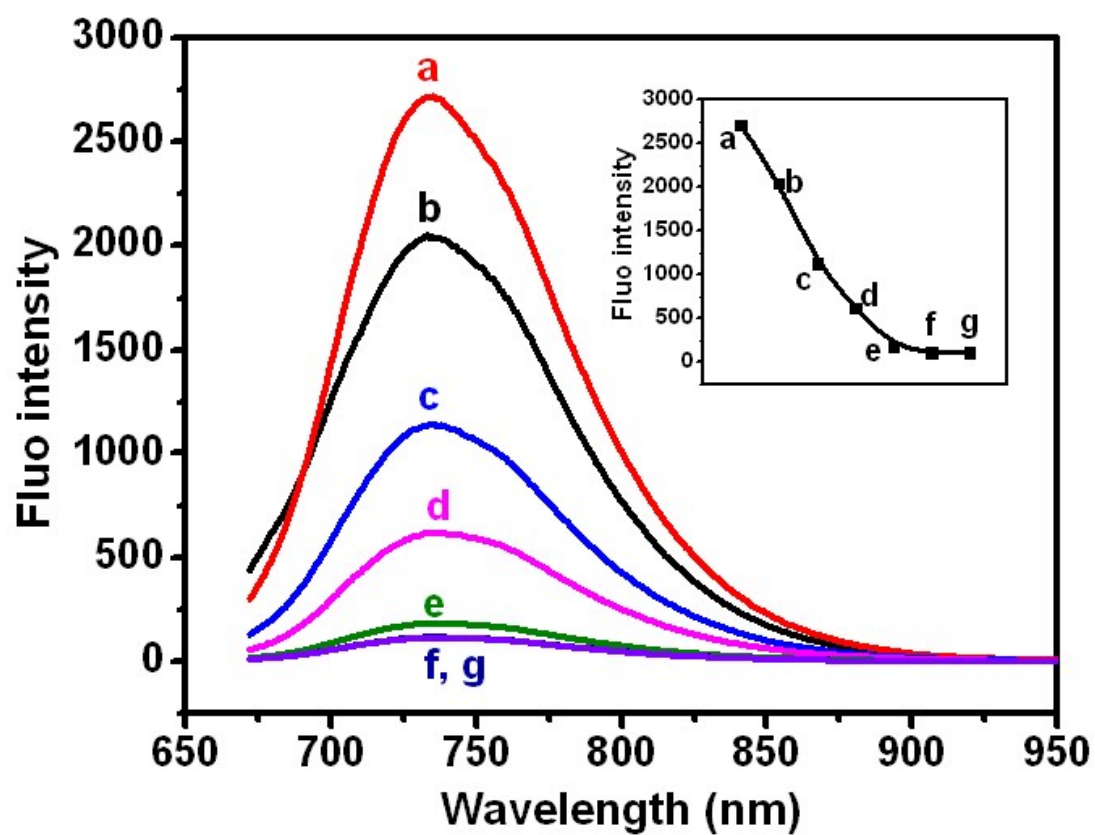


Figure S3. Fluorescence spectra of CoTAPc with different mass ratio of CoTAPc to RGO, (a) 1:0, (b) 10:1, (c) 8:1, (d) 5:1, (e) 2:1, (f) 1:1, (g) 1:5.

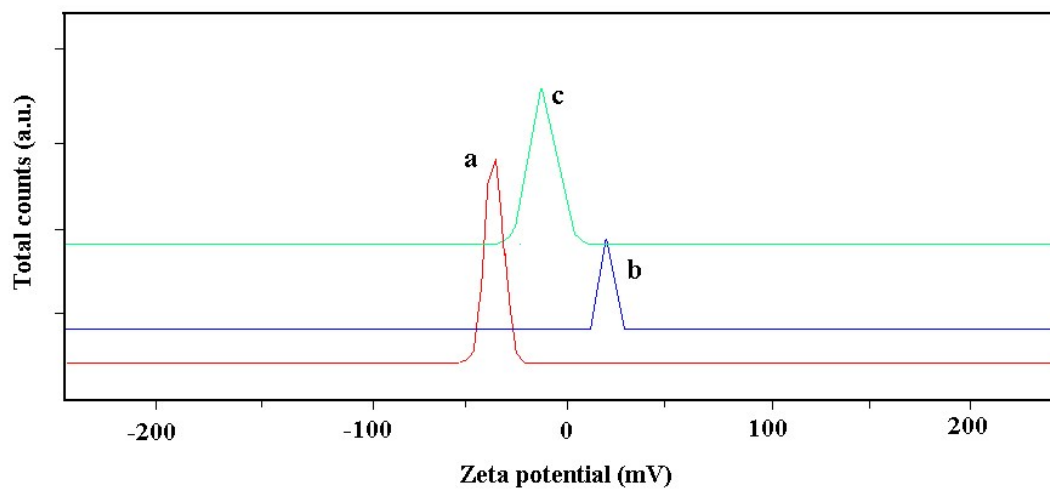


Figure S4. Zeta potential distributions of MOSF (a), CYP3A4/CoTAPc/RGO (b) and CYP3A4/CoTAPc/RGO/MOSF (c).

The zeta potential of MOSF was measured to be -38.5 mV in 0.1 M of pH 7.4 PBS solution (Curve a, Fig. S3), showing that MOSF was negatively charged.

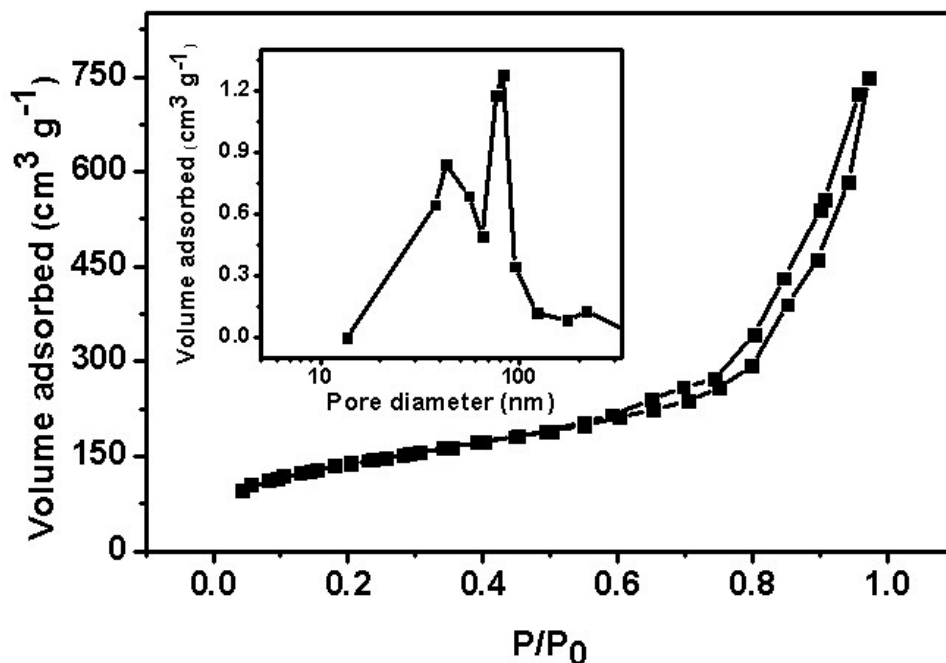


Figure S5. Nitrogen sorption isotherms and pore size distribution curve of MOSF (Inset).

The nitrogen adsorption and desorption analysis showed that the MOSF material had a large surface area and pore volume, which were $402 \text{ m}^2 \cdot \text{g}^{-1}$ and $1.31 \text{ cm}^3 \cdot \text{g}^{-1}$, respectively (Figure S5), and the pore diameter calculated from the adsorption branch by the Barrett–Joyner–Halenda (BJH) method was centered at ca. 80 nm with a relatively narrow size distribution (Inset of Fig. S4), in accordance with the TEM result.

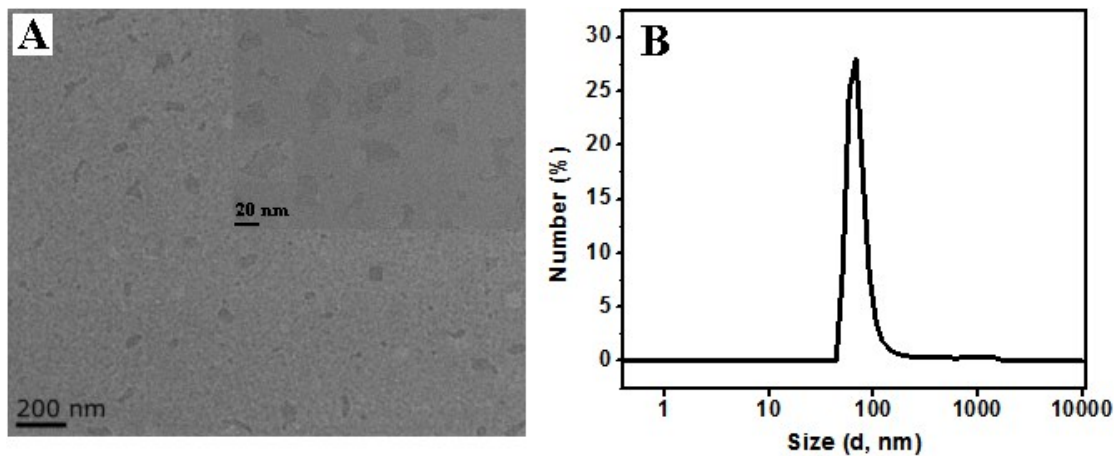


Figure S6. (A) TEM image of RGO, the inset was a enlarge TEM image of RGO; (B) Size distribution of CYP3A4/CoTAPc/RGO measured by the dynamic light scattering technique.

The TEM image showed that the as-prepared RGO was a translucent thin nanosheet with the size distribution in 25-40 nm (Fig. S5A).

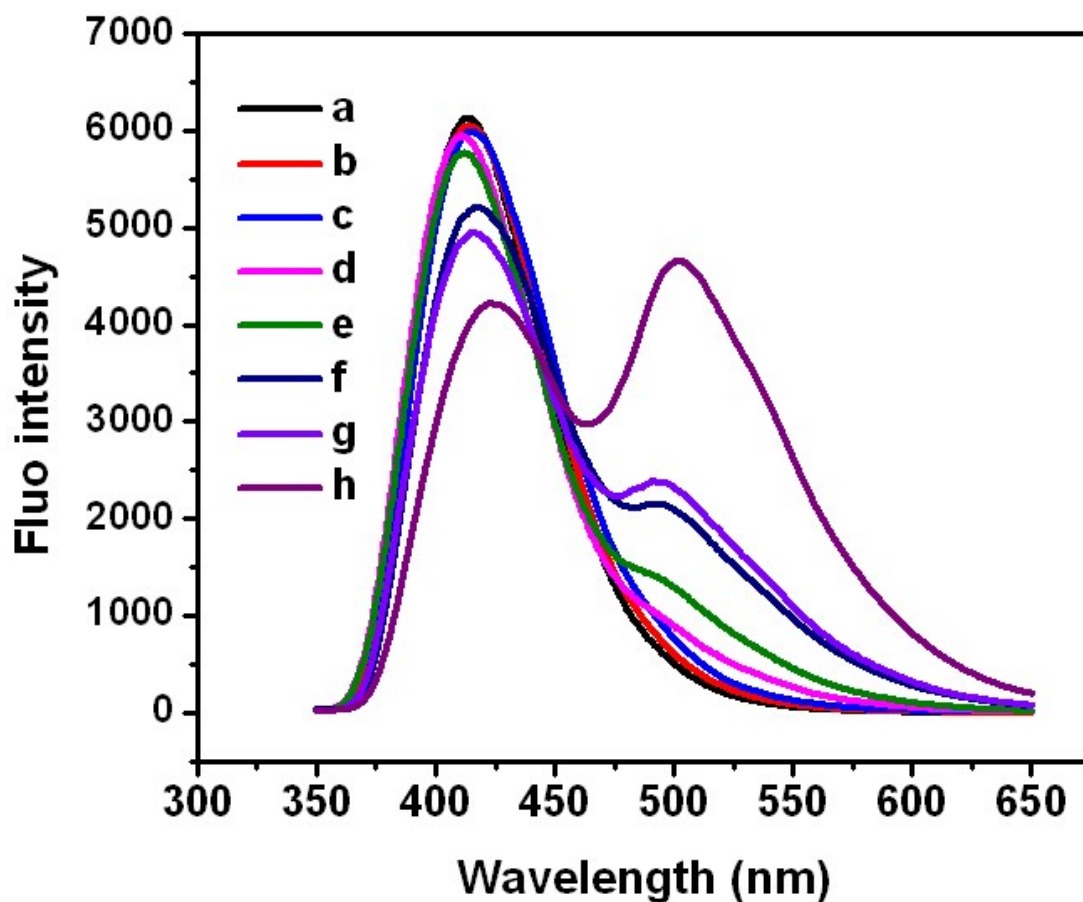


Figure S7. Fluorescence spectra of 7-EFC (a) and reaction mixtures of 7-EFC (b-h) in the 0.1 M pH 7.4 PBS containing different systems after visible light irradiation for 4 h. (b) CYP3A4/RGO, (c) CYP3A4/RGO/MOSF, (d) CYP3A4/CoTAPc, (e) CYP3A4/CoTAPc/RGO, (f) CYP3A4/CoTAPc/MOSF, (g) CYP3A4/CoTAPc/RGO/SiO₂, (h) CYP3A4/CoTAPc/RGO/MOSF. Concentration of 7-EFC: 100 μ M; Pore diameter of MOSF: \sim 80 nm; Excitation wavelength: 333 nm.

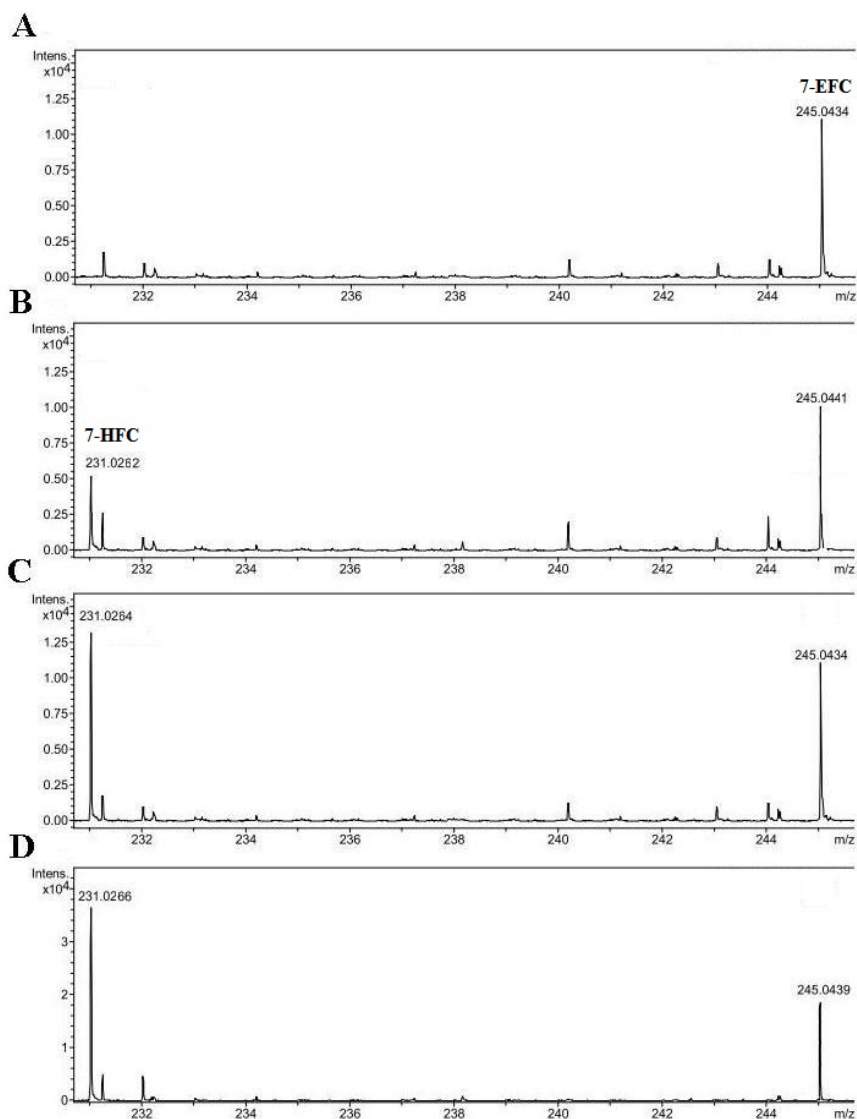


Figure S8. Mass spectra of the reaction mixture of 7-EFC after light-driven biocatalysis by CYP3A4/ CoTAPc/RGO/MOSF for different time. (A) 0 h; (B) 1.0 h; (C) 2.0 h and (D) 4 h.

The light-driven bioconversion of 7-EFC by CYP3A4/CoTAPc/RGO confined in the MOSF could be analyzed by electrospray ionization-mass spectrometry (ESI-MS). As shown in Fig. S7, the initial pure 7-EFC showed a MS peak at 245.04 m/z corresponded to the molecular ion of 7-EFC (Fig. S7A). Under visible light irradiation, 7-HFC converted from 7-EFC by O-deethylation was observed, whose MS peak was at 231.02 m/z (Fig. S7B). Furthermore, with increasing the light irradiation time, the ratio of peak intensity at 231.02 m/z and peak intensity increased (Fig. S7B-S7D), which demonstrated the successful light-driven conversion process that generated 7-HFC from 7-EFC.

Table S1. Light-driven bioconversion of 7-EFC catalysed by different systems.

Systems	Bioconversion of 7-EFC	Conversion rate of 7-EFC
CYP3A4/RGO	-	
CYP3A4/RGO/MOSF	-	
CYP3A4/CoTAPc	+	2.8%
CYP3A4/CoTAPc/MOSF	+	8.9%
CYP3A4/CoTAPc/RGO	+	4.4%
CYP3A4/CoTAPc/RGO/SiO ₂	+	12.1%
CYP3A4&CoTAPc/RGO/MOSF	+	8.2%
CYP3A4/CoTAPc/RGO/MOSF	+	56.5%

The conversion rate of 7-EFC in the solution containing CYP3A4/CoTAPc/RGO/MOSF was about 56.5% after visible light irradiation for 4 h. Control experiments showed that, in the case when the CYP3A4 was directly immobilized on RGO sheets (CYP3A4/RGO), regardless of being loaded into the pores of MOSF or dispersed in the bulk aqueous solution, no obvious product was obtained (curve b and c, Fig. S6), indicating no electron could be supplied to CYP3A4 for substrate conversion without CoTAPc. Moreover, when CYP3A4 was covalently combined with CoTAPc (CYP3A4/CoTAPc) or further immobilized on the RGO (CYP3A4/CoTAPc/RGO), only very small amount of 7-HFC could be obtained (curve d and e, Fig. S6). After confining CYP3A4/CoTAPc in the MOSF (CYP3A4/CoTAPc/MOSF), the conversion rate was about 8.9% (curve f, Fig. S6). However, the conversion rate was still much smaller than that with CYP3A4/CoTAPc/RGO/MOSF (~56.5%) due to the lack of RGO as electron mediator. In addition, when the CoTAPc was assembled with CoTAPc/RGO via electrostatic interaction, the conversion rate was only 8.2% after 4 h light irradiation probably because of the instability of the assembly.

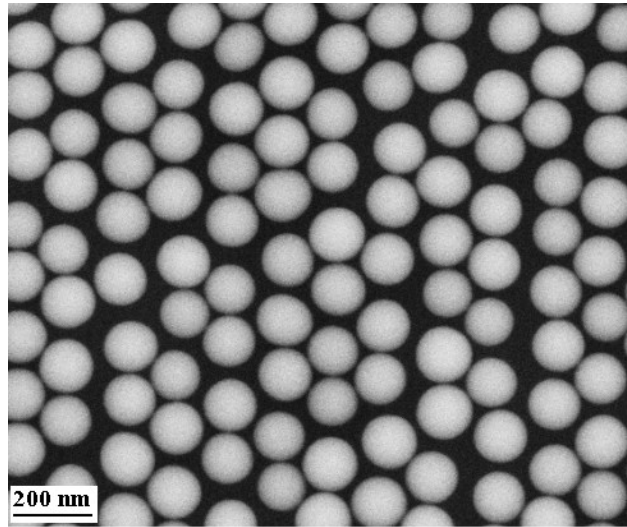


Figure S9. SEM image of silica nanoparticles.

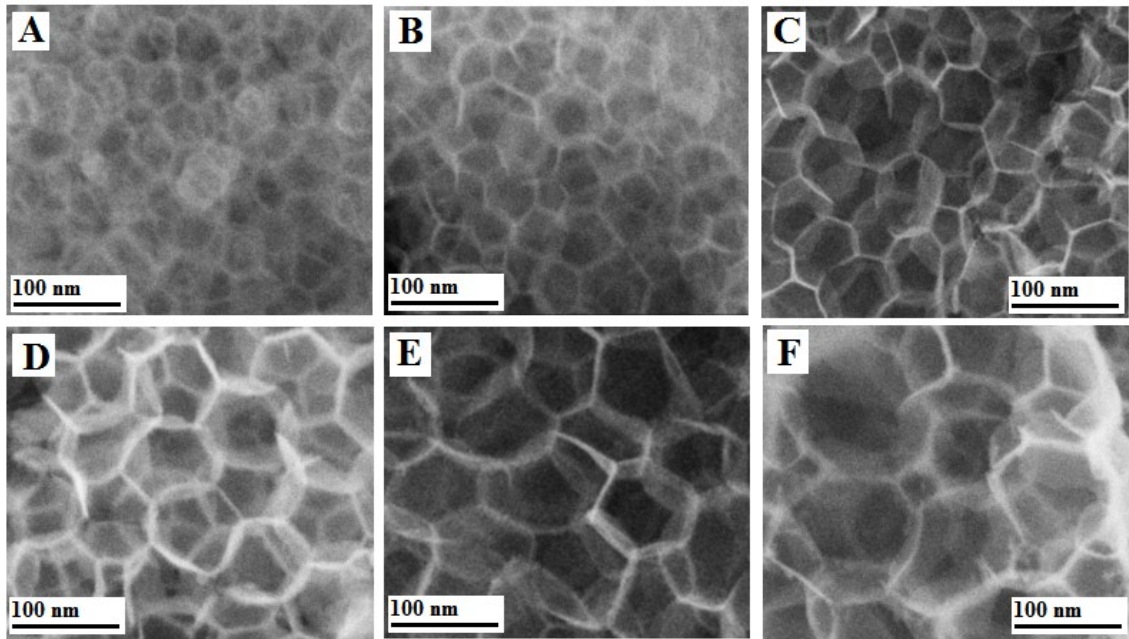


Figure S10. SEM images of MOSFs with different pore diameter. A: 30 nm; B: 40 nm; C: 60 nm; D: 80 nm; E: 100 nm; F: 120 nm.

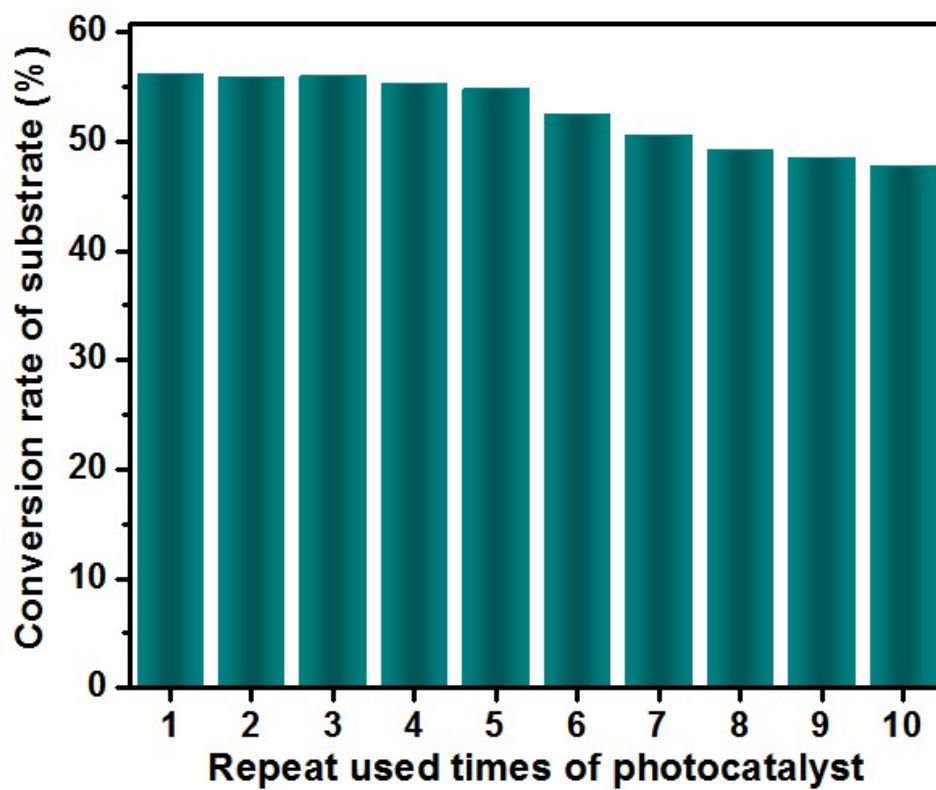


Figure S11. Change of substrate conversion rate when CYP3A4/CoTAPc/RGO/MOSF photocatalyst was repeat used.

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