Support information

Cytochrome P450 Enzyme Functionalized-Quantum Dots as Photocatalysts for Drug Metabolism

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Experimental Section

Reagents:

Indium tin oxide (ITO) electrode (sheet resistance ≤10 Ω/square) was purchased from Wuhan Lattice Solar Technology Co., Ltd. (Wuhan, China). Superoxide dismutase (SOD, from bovine erythrocytes, EC 1.15.1.1, ≥3000 units/mg protein, powder), 3-aminopropyltriethoxysilane (APTES, 99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), tolbutamide (≥99%, powder), sulfaphenazole (≥99%, powder) were obtained from Sigma-Aldrich Chem. Co. Thioglycolic acid (TGA) stabilized CdTe quantum dots (QDs) were purchased from Nanjing Janus New-Material Co., Ltd. (Nanjing, China). Recombinant human cytochrome P450 2C9*1 (CYP2C9*1, 1 μM, the most common human CYP2C9 allele) with cyt P450-reductase and cyt b5 was purchased from BD Biosciences Medical Devices Co., Ltd. (Shanghai, China). The activity of CYP2C9 was tested by using P450-Glo™ CYP2C9 assay kit from Promega Biotechnology Co., Ltd. (Beijing, China). The stock solutions of 50 mM tolbutamide were prepared in ethanol. 0.1 M pH 7.4 phosphate buffer solutions (PBS) were prepared by mixing the stock solution of Na_2HPO_4 and NaH_2PO_4. Distilled water was used throughout the study. Other reagents were of analytical grade unless otherwise specified and were used as received.

Instrument:

A 150 W Xe lamp (Zolix, China) with a spectral range of 200-1800 nm was used as the irradiation source. The illuminating light intensity was 16 mW cm^{-2}. Light pulses were generated by manually opening and closing of an aperture. Photocurrent was measured on a computer-controlled electrochemical analyzer (CHI832B, CHI Instrument) with the conventional three-electrode system, where the modified ITO electrode with an area of 0.49 cm^2 was employed as the working electrode, a platinum wire served as counter electrode, and a saturated calomel electrode (SCE) was used as reference electrode. The electrochemical impedance spectroscopy (EIS) was measured on VersaSTAT 3 (Riceton Applied Research, UK). Scanning electron microscopic (SEM) images were obtained using scanning electron microscopy (SEM, JEOL JSM-5610LV, Japan) at an acceleration voltage of 5 kV. The size and morphology of QDs were analyzed with transmission electron microscope (TEM, S-2400N, HITACHI, Japan) at an accelerate voltage of 200 kV. The UV–vis spectra were performed on a UV-2450 UV–vis spectrometer (Shimadzu, Japan) and the photoluminescence (PL) spectra were recorded on Fluoromax-4 spectrofluorometer (Horiba, Japan). For P450-Glo™ CYP2C9 assay kit, the substrate can be converted by CYP enzymes to luciferin products, whose luminescence spectra were detected on Fluoromax-4 spectrofluorometer by blocking the exiting light.

The reaction mixture from light-triggered biocatalytic conversion of tolbutamide to 4-hydroxytolbutamide was analyzed by high performance liquid chromatography-mass spectrometry (HPLC-MS). An Agilent series 1290 HPLC system (Agilent, Palo Alto, CA, USA) was used in this study. A solution of 5 μL sample was injected into a ZORBAX Eclipse XDB-C18 column (150×2.1 mm I.D., 3.5 μm; Agilent Technology), and the separation was performed using a mobile phase of 40% methanol and 60% water at a flow rate of 0.2 mL min^{-1} with UV detection wavelength

at 230 nm. For ESI–MS, an Agilent 6460 QQQ mass spectrometer (Agilent, Palo Alto, CA, USA) was operated in the positive ion mode under these conditions: gas temperature 350 °C, gas flow 8 L min-1, nebulizer gas pressure 45 psi, sheath gas temperature 250 °C, sheath gas flow 10 L min^{-1}, and capillary voltage 4 kV. The total run time was 30 min. The autosampler needle was rinsed with methanol and water before and after each injection.

Preparation of CYP2C9 modified electrodes:

Before modification, ITO electrodes were sonicated in acetone, ethanol, water and 4% NaOH, respectively, for 15 min each, followed by drying them at 120 ^{o}C for 2 h. Then, the ITO slice with a fixed area of 0.49 cm^2 was dipped into 1% APTES dissolved in toluene for 16 h, which resulted in a self-assembled monolayer of APTES on the electrode surface. After exhaustive rinsing with ethanol and dried with a stream of nitrogen, APTES modified ITO electrodes (APTES/ITO) were ready for further modification.

To prepare the CdTe QDs modified ITO electrodes (QDs/ITO), the TGA-capped QDs solution (10 μM) was firstly activated by adding 1 mg EDC into 30 μL of CdTe QDs solution at room temperature for 15 min. Subsequently, 15 μL of the above solution was dropped onto the surface of ITO/APTES and stored at 4 °C overnight to obtain the QD/ITO electrode.

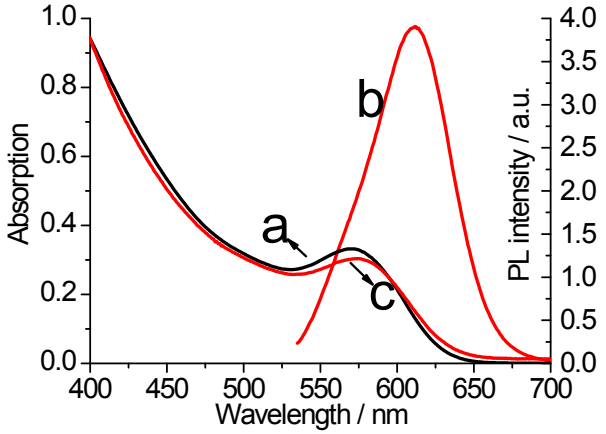
For the synthesis of CYP2C9 conjugated CdTe QDs (CYP2C9/QDs), 10 μL of CYP2C9 solution was added into 15 μL of the above EDC activated QDs solution and mixed gently at room temperature for 1 h, followed by stored at 4 °C overnight. For comparison, CYP2C9 solutions with different concentrations were added into the same amount of QDs solution. It should be noted that QDs were greatly in excess of CYP2C9 to ensure remaining adequate carboxyl groups for further immobilization onto the ITO electrode.

CYP2C9/QDs nanohybrids were immobilized on the ITO electrode by the covalent binding of the carboxyl groups on QDs with the amino groups in APTES. To prepare the CYP2C9/QDs modified ITO electrodes, the obtained 25 μL of CYP2C9/QDs solution was activated again by adding 0.5 mg EDC and mixed gently at room temperature for 15 min, and dropped onto the surface of the APTES/ITO electrode. Then, the electrodes were stored at 4°C overnight to obtain the CYP2C9/QDs/ITO electrode.

Fig. S1 (A) UV-vis spectra of (a) CdTe QDs and (c) CYP2C9/QDs solutions. (b) PL spectra of CdTe QDs solution. (B) TEM image of CdTe QDs.

CdTe QDs (λ_{em} = 615 nm, λ_{ex}= 530 nm) used here exhibited a characteristic UV-*vis* absorption at 573 nm (curve a). Then, the particle size of CdTe QDs was calculated to be ~3.4 nm, which was in a good agreement with the result from the TEM image (Fig. S1B). By using the extinction coefficient of 1.2×10^5 M^{-1} cm^{-1}, the concentration of stock CdTe QDs solution was estimated to be 10.89 μM.[1]

Compared with the UV-vis absorbance spectra of CdTe QDs (curve a), the conjugation of CYP2C9 molecules did not affect the UV-vis spectra of CdTe QDs obviously, only with a small red-shift from 573 nm to 578 nm (curve c).



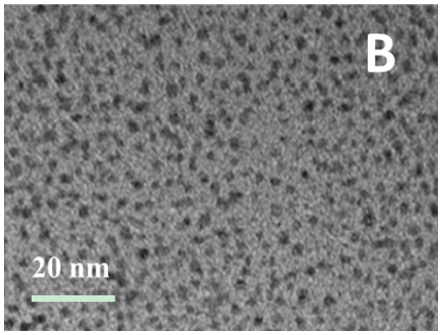


Fig. S2 Dependence of photocurrents on the applied potentials at the QDs/ITO electrode in 0.1 M pH 7.4 PBS. Insert: photograph of the QDs/ITO electrode under a UV lamp (λ_{ex}=365 nm).

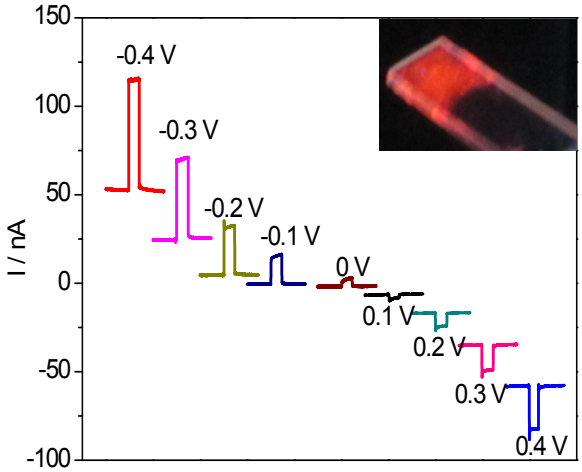


Fig. S3 Agarose gel electrophoretic analyses of the CYP2C9/QDs solution. The molar ratios of CYP2C9 to QDs were 0:60, 3:60 and 6:60 from left to right lanes. A 0.5% agarose gel was used with 1×TBE buffer (40 mM Tris-acetate, 1 mM EDTA) and the bands were separated at the applied voltage of 120 V for 15 min. The negatively charged CdTe QDs were found to run smoothly toward the anode. As the CYP2C9 to QDs molar ratios increased from 0:60 to 6:60, less free QDs appeared in the lanes because of the increased size and decreased negative charge.

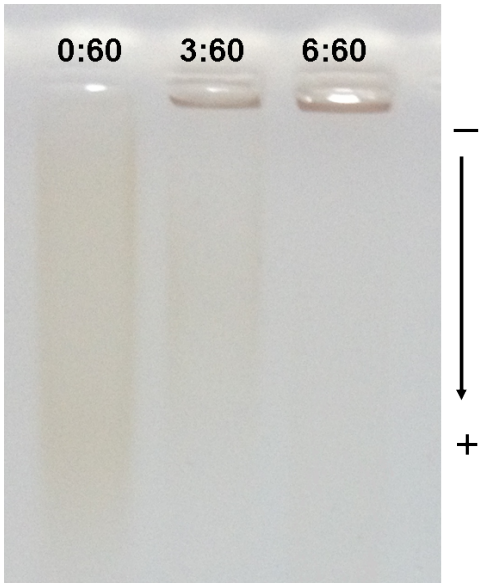


Fig. S4 The luminance produced by (a) free CYP2C9, (b) CYP2C9/QDs and (c) QDs in the P450-Glo™ CYP2C9 assay. The enzymatic activity of CYP2C9 in CYP2C9/QD nanohybrids was confirmed by P450-Glo™ CYP2C9 assay kit. The luminance produced by the CYP2C9/QDs nanohybrid was only ~47.15% of that by the free CYP2C9. The decreased bioactivity of CYP2C9 is likely due to the interaction between the immobilized CYP2C9 proteins and QDs, which may hinder the substrate diffusion from/to the enzyme.[2]

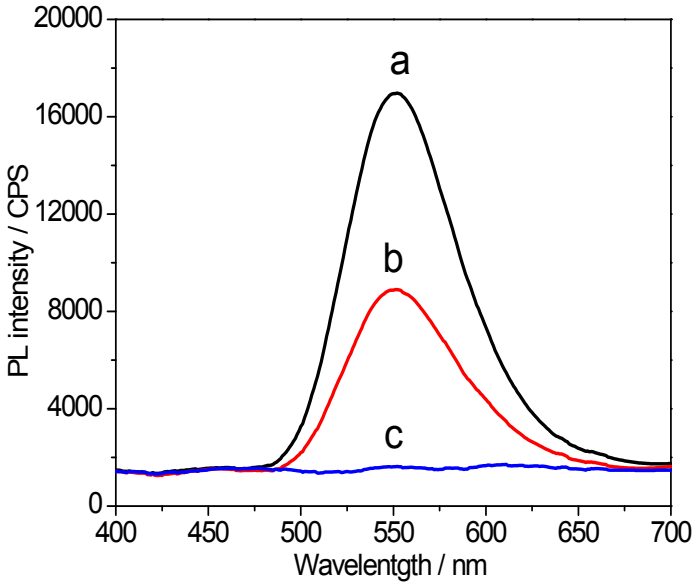
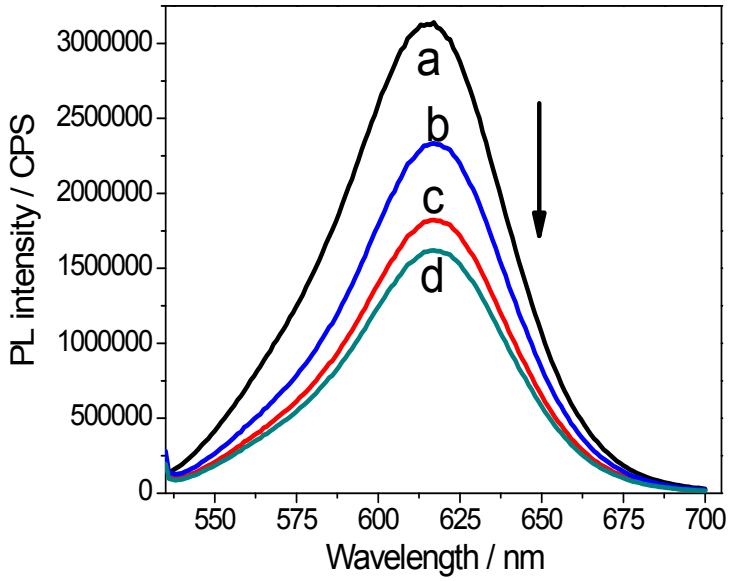


Fig. S5 PL quenching of the CdTe QDs dependent on the molar ratio of CYP2C9 to QDs. (a) 0:60, (b) 2:60, (c) 4:60, (d) 6:60. Defined volumes of CdTe QDs solution were titrated against CYP2C9, and the change in the fluorescence intensity at 615 nm was monitored (λ_{ex}= 530 nm).



The feasibility of the electron transfer from the photoexited CdTe QDs to CYP2C9 is estimated by the application of the Rehm-Weller model. According to this model, the Gibbs energy change (ΔG_{ET}) for the electron transfer process may be derived by employing the equation:[3]

$$\Delta G_{ET}=E^{1/2}_{(ox)}-E^{1/2}_{(red)}-E_S+C \quad (1)$$

where $E^{1/2}_{(ox)}$ is the one-electron oxidation potential of CdTe QDs (0.6 V vs. NHE),[3c] $E^{1/2}_{(red)}$ is the one-electron reduction potential of CYP2C9 (-0.14 V vs. NHE),[4] E_S is the excited state energy of CdTe QDs (2.34 eV from the PL wavelength) and C is the coulombic term. Since the electron transfer reaction was in water, the coulombic term in the above expression can be neglected. Based on this, the Gibbs energy change (ΔG_{ET}) for the electron transfer process was calculated to be -1.60 eV.

Fig. S6 Photocurrent response of the QDs/ITO electrode in nitrogen-purged (red) and air-saturated (black) 0.1 M pH 7.4 PBS at applied potentials of -0.2 and -0.3 V (vs. SCE).

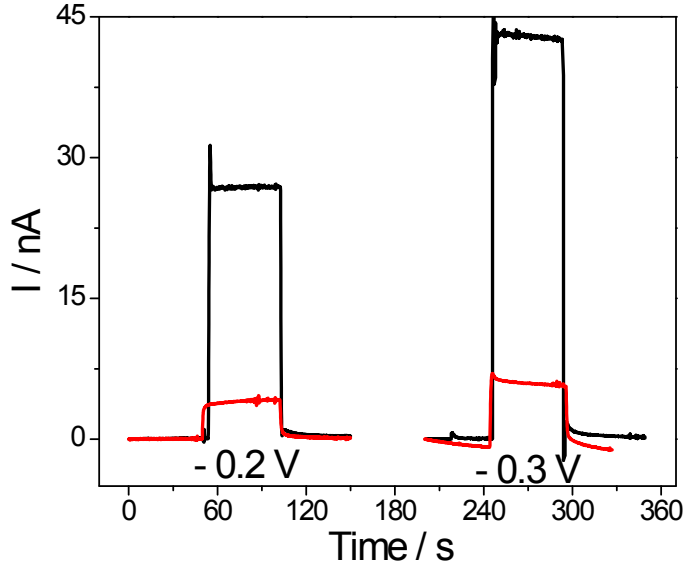


Fig. S7 Photocurrent response of the QDs/ITO electrode in air-saturated 0.1 M pH 7.4 PBS with (a) 0, (b) 30 units mL^{-1}, (c) 60 units mL^{-1}, (d) 90 units mL^{-1}, and (e) 120 units mL^{-1} SOD at an applied potential of -0.2 V (vs. SCE).

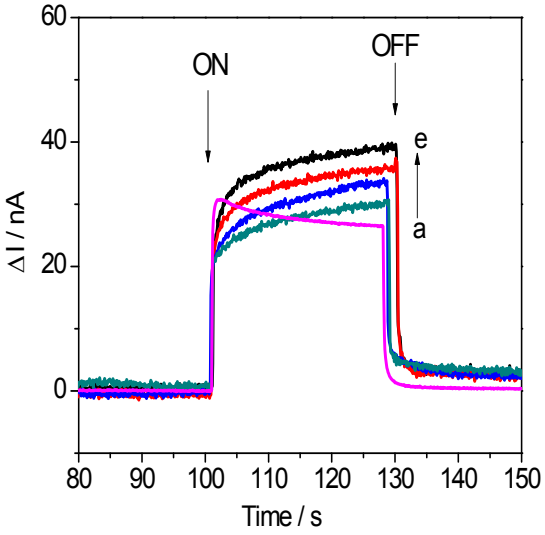
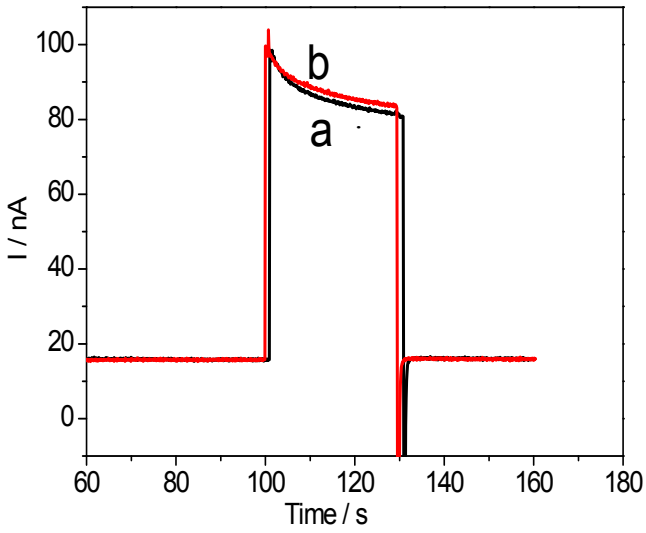


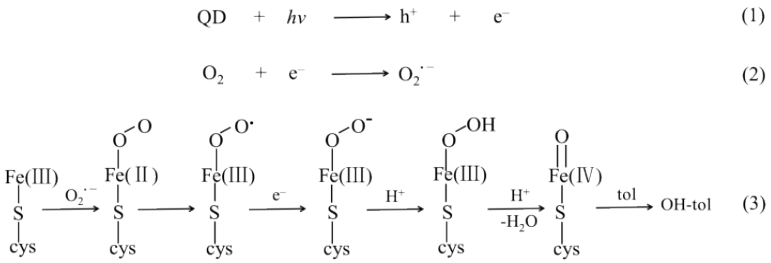
Fig. S8 Photocurrent responses of the CYP2C9/QDs/ITO electrode (CYP2C9:QDs ratio of 2:60) in air-saturated 0.1 M pH 7.4 PBS with 100 units mL^{-1} SOD (a) before and (b) after addition of 166 μM tolbutamide at an applied potentials of -0.2 V (vs. SCE).



It is known that oxygen can easily trap electrons from the CB of some QDs. In this study, the CB position of CdTe QDs is -3.87 eV, shallow enough to reduce oxygen into superoxide radicals ($O_2^{\cdot-}$).[5,6a] In the oxygen-free solution, the cathodic photocurrent of the QDs/ITO electrode can be observed. However, its value was much smaller than that in air-saturated buffer at both -0.2 and -0.3 V (Fig. S6). This suggested that oxygen can be reduced into $O_2^{\cdot-}$ by the generated electrons during illumination of the QDs/ITO electrode.[5] Further evidence was provided by the measurement of the cathodic photocurrents with addition of superoxide dismutase (SOD), a kind of effective $O_2^{\cdot-}$ scavenger.[6b] The presence of SOD in the air-saturated buffer can accelerate the oxygen reduction process on the QD/ITO electrode and make the photocurrents at -0.2 V gradually increase (Fig. S7), which should be the result of the elimination of $O_2^{\cdot-}$ catalyzed by SOD.

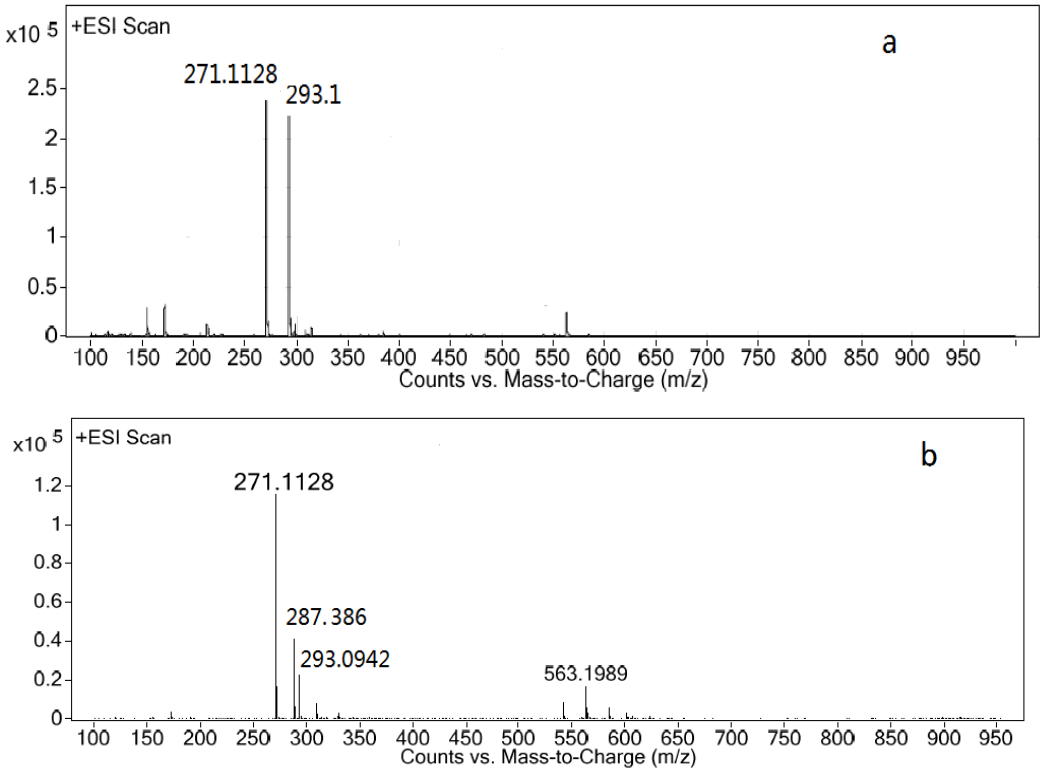
On the knowledge that radical species are involved in the catalytic cycle of P450-mediated oxygenation reactions and reactive oxygen species can active the oxygen-consuming enzymes to catalyze the oxidation of substrates,[2,6] the effect of $O_2^{\cdot-}$ on the hydroxylation process by the CYP2C9/QDs photocatalysts was then studied by using SOD. It was observed that the photocurrent response of the CYP2C9/QDs/ITO electrode to the addition of tolbutamide in the presence of 100 units mL^{-1} SOD was almost negligible (Fig. S8), which demonstrated that $O_2^{\cdot-}$ played a significant role in the light-triggered catalytic process. According to the general catalytic cycle of cyt P450s,[2,7] we speculated that the generated superoxide radicals may bond to CYP2C9 in the immediate proximity and convert the heme center into the ferrous state,

resulting in a fast electron transfer from QDs to CYP2C9.[8] Thus, the hydroxylation process of tolbutamide by the CYP2C9/QDs nanohybrids may be illustrated as follows:



where $O_2^{\cdot -}$ is produced on the surface of CdTe QDs and binds to the hemoprotein for the generation of ferryl species (Fe^{IV}=O), which is thought to be responsible for the cyt P450-based oxidation reaction. Meanwhile, the demanded electrons in the catalytic process can be trapped from the CB electrons in QDs and give rise to the cathodic photocurrent of the CYP2C9/QDs/ITO electrode.

Fig. S9 ESI-MS analysis of the reaction mixture (a) before and (b) after electrolysis for 1 h on the CYP2C9/QDs/ITO electrode with irradiation at an applied potential of -0.2 V (vs. SCE).



Notes and references

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