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Cytochrome P450 enzyme functionalized-quantum dots as photocatalysts for drug metabolism

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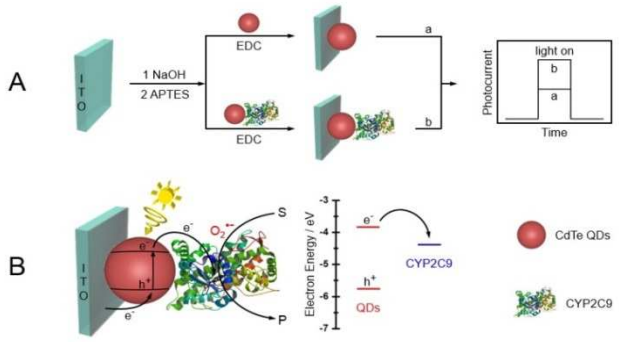
On the basis of the photo-induced electron transfer (PET) from CdTe quantum dots (QDs) to cytochrome P450 2C9 (CYP2C9), a light-controlled drug metabolism system was successfully designed by using CYP2C9 functionalized-CdTe QDs as photocatalysts.

Recently, quantum dots (QDs) have received considerable attention due to their unique properties, such as high fluorescence quantum yields, stability against photobleaching, and size-controlled luminescence properties.[1] QDs can serve as photoelectrochemically active species. When QDs are excited with light energy exceeding their band gap, electrons are promoted from the valence band (VB) to the conduction band (CB) with the generation of electron-hole pairs. The ejection of the CB electrons to the electrode, with the concomitant transfer of electrons from an electron donor, yields an anodic photocurrent. In contrast, transfer of the CB electrons to an electron acceptor, followed by the supply of electrons from the electrode to neutralize the VB holes, yields a cathodic photocurrent.[2] Photocurrents can be regulated by the participation of charge-transfer mediators, redox-active intercalators and redox-active proteins, which would be used for the operation of a DNA machine, the detection of small molecules and the assay of enzyme activities.[3]

The activity of enzymes is usually evaluated by two general pathways. On one hand, the products generated or the substrates consumed in the catalytic reactions may act as electron acceptor or electron donor units, which can control the intensity of photocurrents.[4,5] On the other hand, the active center of redox proteins may mediate the charge transfer of QDs, influencing the direction or intensity of photocurrents in the hybrid system. Recently, the photo-induced reduction of heme moiety within myoglobin by the photosensitized CdTe QDs covalently attached to myoglobin has been demonstrated.[6b] Besides, the photo-induced electron transfer (PET) from CdSe/ZnS QDs to the fully oxidized cytochrome c (cyt c) has been achieved, which leads to an enhancement of the cathodic photocurrent at the QDs modified gold electrode.[7]

Inspired by the PET from QDs to hemoproteins, a light-controlled drug metabolism system was designed by using cytochrome P450s (cyt P450s) functionalized-QDs as photocatalysts. Cyt P450s are a group of heme-containing proteins that participate in the metabolism of more than 60% of all clinically used drugs.[8] The proposed catalytic cycle of cyt P450s features delivery of electrons from NADPH to cyt P450 reductase (CPR) for subsequent delivery to cyt P450 heme.[8c,d] Herein, CdTe QDs are introduced as the electron supplier by replacing the electron donation from expensive NADPH, while the electrode at appropriate bias potentials supply electrons for neutralizing the VB holes of QDs and monitoring the occurrence of the light-induced catalytic process (Scheme 1).



Scheme 1 (A) Fabrication of the photocatalysts and photoelectrodes. (B) Illustration of the electron transfer steps of the CYP2C9/QDs/ITO electrode upon irradiation.

The CdTe QDs are functionalized with thioglycolic acid (TGA) offering plenty of carboxylic acid end groups, thus can be easily linked to the amino groups of 3-Aminopropyltriethoxysilane (APTES) immobilized on the indium tin oxide (ITO) electrodes (Scheme 1A). CdTe QDs adhere well to the ITO surface, as evidenced by the fact that the film still emitted orange-red light under a UV lamp (λ_{ex}=365 nm) even after several washing cycles (insert in Fig. S2). To character the photoelectric property of the QDs film, photocurrents were recorded by illumination of the QDs/ITO electrode with a white light source (Xe lamp).[5] It was observed that not only intensity but also direction of photocurrents was strongly influenced by the applied potentials (Fig. S2). For potentials more negative than +0.05 V, cathodic photocurrents can be observed, whereas anodic photocurrents are enforced at positive potentials.[9b] It should be mentioned that the APTES/ITO electrode showed no photocurrent. Therefore, the obtained photocurrents on the QDs/ITO electrode resulted from the generation of the electron-hole pair and the electron transfer between CdTe QDs and the electrode.[7a,9] At the applied potentials of -0.3, -0.2 and -0.1 V, no more than 5.0% of the initial photocurrents decreased after 20 cycles of exposure, indicating

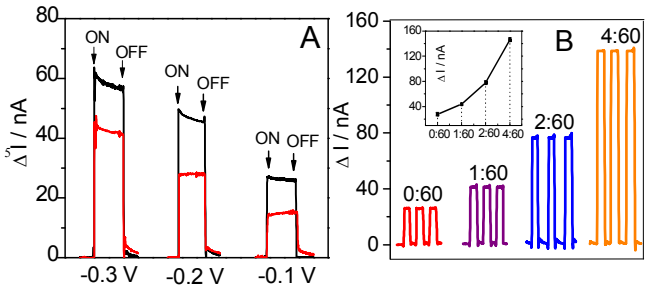


Fig. 1 (A) Photocurrent responses at potentials of -0.1, -0.2 and -0.3 V on the QDs/ITO (in red) and CYP2C9/QDs/ITO (in black) (CYP2C9:QDs ratio of 1:60) electrodes upon the illumination of white light. (B) Photocurrent responses on the CYP2C9/QDs/ITO electrode at -0.2 V with different molar ratios of CYP2C9 to QDs.

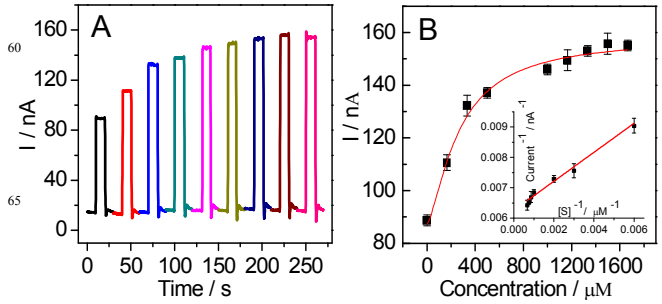


Fig. 2 (A) The cathodic photocurrents on the CYP2C9/QDs/ITO electrode (CYP2C9:QDs ratio of 2:60) in air-saturated 0.1 M pH 7.4 PBS with successive addition of tolbutamide at the applied potential of -0.2 V. (B) Calibration plots illustrating the electrode response to tolbutamide addition. Insert: double-reciprocal plot of the photocurrents and the concentrations of tolbutamide.

the good stability of the QDs/ITO electrode, which was important for the construction of a photoelectrochemical biosensor.

For the preparation of the CYP2C9/QDs photocatalysts, CYP2C9 was tethered to QDs by the covalent binding of the protein's amino groups to the carboxyl end of TGA ligand shell of CdTe QDs, offering a favourable orientation for ET.[6a] Actually, the surface of CYP2C9 (Ip=8.13) was positively charged in pH 7.4 PBS. It was inevitable that a certain amount of CYP2C9 was bound to the negatively charged CdTe QDs by weak electrostatic interaction.[6b] The successful association of CYP2C9 to CdTe QDs was confirmed by the agarose gel electrophoretic experiments (Fig. S3). Based on an assumption that CYP2C9 molecules were close-packed as spheres around a central QD, and using the sizes of the QD particle (R = 17 Å) compared with the protein (Rp = 55 Å), the theoretical number of CYP2C9 that can be packed around per QD particle was estimated to be 8.[10]

The interaction between CdTe QDs and the immobilized CYP2C9 were analyzed by means of PL quenching studies. The progressive quenching of the fluorescence emission was detected when the molar ratios of CYP2C9 to CdTe QDs changed from 0:60 to 6:60, with an estimated loss of ~48.35% at the ratio of 6:60 (Fig. S5), suggesting the successful PET process from CdTe QDs to CYP2C9.[1d,10b,c] This PET process can be explained by their energy levels. The reduction peak of CdTe QDs on the ITO electrode was observed at -0.87 V (vs. SCE). When this information was coupled with the band gap of 1.93 eV obtained from the UV-vis absorption spectrum, the CB and VB positions of CdTe QDs can be quantified as -3.87 eV and -5.80 eV, respectively.[11] The energy level of CYP2C9 was calculated to be -4.36 eV, according to its formal redox potential at -0.38 V (vs. SCE).[8d] Therefore, the redox energy of CYP2C9 lies between the CB and VB of CdTe QDs, providing the possibility of PET from CdTe QDs to the surface-bonding hemoproteins.[12] Furthermore, the feasibility of the electron transfer from the photo-exited CdTe QDs to CYP2C9 was estimated by the application of the Rehm-Weller model (ESI†). Based on this, the Gibbs energy change (ΔG_{ET}) for the electron transfer process was calculated to be -1.60 eV, indicating the thermodynamic feasibility of the PET process from CdTe QDs to CYP2C9.

When CYP2C9/QDs were immobilized onto the ITO slice, the oxidized hemoprotein in CYP2C9 acted as an electron acceptor. To maintain CYP2C9 proteins in the oxidation state, potentials more positive than its formal potential should be applied.

At -0.3 and -0.2 V, the dark current of the CYP2C9/QDs/ITO electrode was slightly higher than that of the QDs/ITO electrode (an increase of 6.35 nA at -0.3 V and 3.88 nA at -0.2 V, respectively). Upon illumination, there was an obvious enhancement of cathodic photocurrents on the CYP2C9/QDs/ITO electrode, and the cathodic photocurrent increased with decreasing potentials (Fig. 1A). However, the difference between the photocurrents for the CYP2C9/QDs/ITO and QDs/ITO electrodes had the largest value of 18.51 nA at -0.2 V. Fig. 1B shows the dependence of photocurrents at -0.2 V on the molar ratios of CYP2C9 to QDs. The photocurrents continued to increase when the ratios varied from 0:60 to 4:60, implying that more CYP2C9 proteins immobilized in the film would accelerate the electron transfer from QDs to CYP2C9 and make the cathodic photocurrents larger. All these results are consistent with the mechanism outlined in scheme 1B. The fixed CdTe QDs on the ITO electrode have worked as a light-controlled switch for the heterogeneous electron transfer to CYP2C9 in the oxidized state, which can trap the CB electrons in the surface traps of QDs and give rise to the cathodic photocurrents at the CYP2C9/QDs/ITO electrode.[2,7a] Meanwhile, the VB holes generated in QDs are filled through the electron transfer from the electrode.

To evaluate the concept of light-driven drug metabolism on the CYP2C9/QDs/ITO electrode, tolbutamide was chosen as the model which can be metabolized by CYP2C9 in the presence of oxygen to yield 4-hydroxytolbutamide. Fig. 2A displays the cathodic photocurrents at -0.2 V after successive additions of tolbutamide. There was an obvious enhancement of photocurrents with increase of tolbutamide. Plotting the values of photocurrents at -0.2 V as a function of the concentrations of substrate showed a hyperbolic behavior that can be fitted with a Michaelis-Menten model (Fig. 2B). According to the meaning of Michaelis-Menten equation,[13a] the apparent Michaelis-Menten constant K_m^{app} was calculated to be 76.28±3.59 μM. This was in a good agreement with other reports, where tolbutamide hydroxylation exhibited simple Michaelis-Menten kinetics with K_m^{app} values ranging from 60 to 400 μM.[8d,13b]

In contrast, no increased photocurrent was observed in control experiments: (1) with addition of equivalent aliquot (10 μL) of ethanol or PBS; (2) on the QDs/ITO electrode without CYP2C9; (3) without irradiation; (4) without dissolved oxygen in solution.

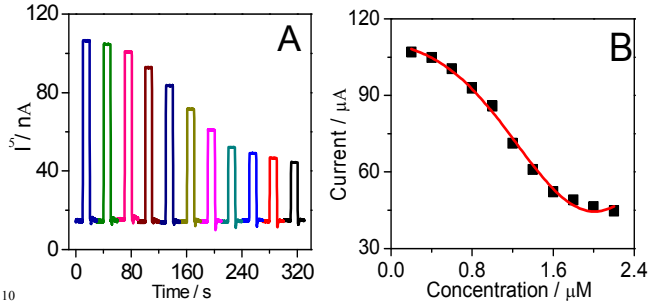


Fig. 3 (A) Analysis of the inhibition effect on 4-hydroxylation of tolbutamide by sulfaphenazole. The cathodic photocurrents at -0.2 V on the CYP2C9/QDs/ITO electrode (CYP2C9:QDs ratio of 2:60) by varying sulfaphenazole concentrations in 0.1 M pH 7.4 PBS containing 75 μM tolbutamide. (B) Changes in bioelectrocatalytic currents at -0.2 V upon addition of sulfaphenazole.

Therefore, the increased photocurrents on the CYP2C9/QDs/ITO electrode resulted from the catalytic reaction of CYP2C9 to tolbutamide, which can be light-triggered in the help of dissolved oxygen. Five parallel measurements were carried out with the same CYP2C9/QDs/ITO electrode and the relative standard deviation (RSD) of the calculated apparent Michaelis-Menten constant K_m^{app} was ~4.7%, indicating the acceptable stability of the photocatalyst. Furthermore, the CYP2C9/QDs/ITO electrode was kept under 4°C for 10 days, and 95% of its initial photocurrent response was remained. Furthermore, the significant role played by oxygen in the light-triggered catalytic process was also investigated by using superoxide dismutase (SOD) (ESI†).

To confirm this light-driven drug metabolism, the system was switched on irradiation by the CYP2C9/QDs/ITO electrode in the presence of 600 μM tolbutamide at an applied potential of -0.2 V (vs. SCE). After one hour, a new peak at 287.3 m/z corresponding to the molecular ions of 4-hydroxytolbutamide emerged in electrospray ionization-mass spectrometry (ESI-MS) (Fig. S9), which further demonstrated the successful light-driven hydroxylation of tolbutamide by the CYP2C9/QDs/ITO electrode.

Sulfaphenazole was identified as a strong, competitive and very selective inhibitor of CYP2C9 for its high affinity toward CYP2C9.[14] The inhibition effects of sulfaphenazole on tolbutamide hydroxylation by the CYP2C9/QD photocatalysts was then investigated. As shown in Fig. 3A, the photocurrent at -0.2 V continued to decrease to a stable value by varying the concentrations of sulfaphenazole from 0.2 to 2.2 μM in the aerobic solution with tolbutamide. When the current versus the sulfaphenazole concentration was plotted (Fig. 3B), the mean IC_{50} value (concentration of inhibitor producing 50% inhibition) of sulfaphenazole for the inhibition of CYP2C9-dependent hydroxylation of tolbutamide was determined to be 1.2 μM. This inhibition experiment again suggests that the CYP2C9/QDs film provides a favorable bioenvironment for maintaining the bioactivity of immobilized enzymes.

Conclusions

As a summary, on the basis of the direct electron transfer from photo-exited QDs to CYP2C9, a light-controlled drug metabolism system is successfully designed by using CYP2C9 functionalized-CdTe QDs as photocatalysts. The constructed system promises the photo-induced catalytic reaction of the CYP2C9/QDs nanohybrids with specific substrates in the presence of oxygen, and the direct detection of catalytic signals with the electrode. This novel light-driven drug metabolism system can be expanded to other cyt P450s for applications in drug discovery and development by monitoring substrate metabolism and enzyme inhibition. Other applications may include as photocatalysts in synthetic organic chemistry and as photosensitizers for intracellular reactions.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, characterization data, the calculation of ΔG_{ET}, the catalytic mechanism. See DOI: 10.1039/b000000x/

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