



Supporting Information

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## Quantum Dot-Cytochrome P450 Nanohybrids as Photocatalysts \*\*

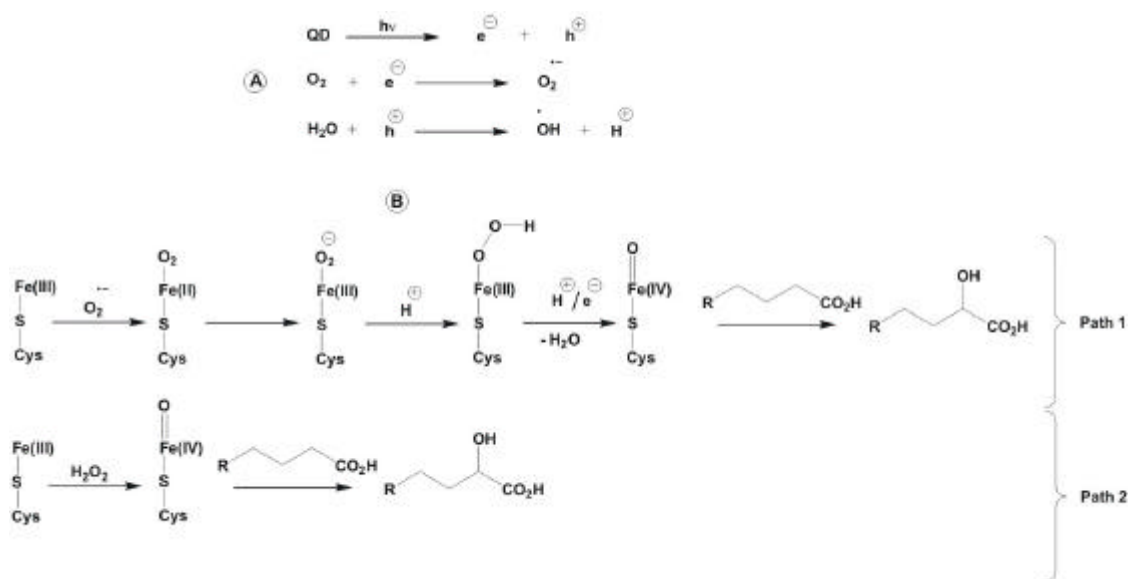
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### Potential catalytic mechanism of P450-Cds photocatalysis

In general the catalytic cycle of cytochrome P450 enzymes, involves substrate binding, reduction of the ferric heme ( $\text{Fe}^{\text{III}}$ ) to the ferrous state ( $\text{Fe}^{\text{II}}$ ) by electron transfer from the heme iron-center, binding of molecular oxygen to the ferrous iron, and transfer of a second electron to the resulting ferrous dioxy complex to form a ferric peroxy anion. The ferric peroxy anion is then protonated, giving rise to a ferric hydroperoxy complex. Heterolysis of the oxygen-oxygen bond in this intermediate, results in elimination of a molecule of water and formation of a ferryl species ( $\text{Fe}^{\text{IV}}=\text{O}$ ), which is thought to be responsible for most of the oxidations catalyzed by the enzyme. A possible alternative mechanism to circumvent the stepwise activation of molecular oxygen requires  $\text{H}_2\text{O}_2$  or organic peroxides as the co-substrate, which can "shunt" the oxidation mechanism.<sup>[1, 2]</sup> Hence, activated oxygen is crucial for the generation of ferryl species, which is responsible for the various oxidation reactions initiated by P450.

Based on the plot of band gap energy, valence band positions and conduction band position, CdS QDs have VB positions deep enough to oxidize water or hydroxide ions to generate hydroxyl radicals<sup>[3]</sup> and has CB positions shallow enough to reduce O<sub>2</sub> to form superoxide radicals. The hydroxyl radicals formed in this photoirradiation process can form hydrogen peroxide<sup>[4, 5]</sup> with rate constants in the order of 10<sup>9</sup> L mol<sup>-1</sup> s<sup>-1</sup>.<sup>[6]</sup>

As depicted in Scheme S1A, CdS QDs can, therefore, produce two initiators (superoxide radical anion and/or hydrogen peroxide) which can activate the P450 enzyme. Since the formation of the superoxide radical anion and/or hydrogen peroxide initiators occurs at the surface of the QD, and thus, in immediate proximity of the electrostatically bound P450 enzyme, this process, indeed, activates the enzyme, as schematically shown in Scheme S1B (Path 1 and 2). In path 1 of Scheme S1, the e<sup>-</sup> is likely provided by a sacrificial electron donor (e.g., organic compounds of the ligand shell or the buffer)



**Scheme S1.** A. Generation of electron and hole from QDs and subsequent generation of activated species; B. Superoxide and hydroxyl radical initiated P450 reactions.

**Experimental Section**

All compounds were obtained from Sigma Aldrich. UV-Vis spectra were recorded on a CaryBio300 UV-Vis spectrometer using quartz glass cuvettes. Fluorescence spectra were recorded on a Cary Eclipse Fluorescence Spectrophotometer by exciting at 323 nm with both excitation and emission slit widths of 5 nm. The extinction coefficient of  $2 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$  of the CdS nanoparticles was calculated from the concentration of Cd, determined by ICP, and the size of the QDs, determined by TEM analysis. ESI-LCMS were carried out using a Thermo Quest Finnigan and HPLC was done on an Agilent 1100 series HPLC apparatus.

Mass spectrometry studies:

After irradiating an aqueous solution (2 mL) containing CdS (20  $\mu\text{L}$  of 20  $\mu\text{M}$ ), P450 (40  $\mu\text{L}$  of 10  $\mu\text{M}$ ), and myristic acid (5  $\mu\text{L}$  of 8 mM) with UV light (365 nm) for 20 min, the reaction mixture was extracted with chloroform (5 ml) and dried over magnesium sulfate. The solvent was evaporated and the solid residue was redispersed in methanol (1 ml). A 10  $\mu\text{L}$  portion of this solution was injected into the ESI LCMS apparatus equipped with a reverse-phase column (Ultra Aqueous (Restek)  $\text{C}_{18}$  5  $\mu\text{m}$  250 x 4.6 mm) kept at room temperature and eluted with  $\text{MeOH-NH}_4\text{COOCH}_3$  (10 mM), (98:8), v/v) at a flow rate of 0.8 mL/min.

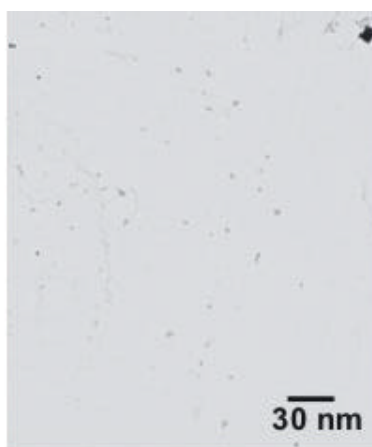
HPLC analysis of hydroxylation reactions

Separate solutions containing different ratios of CdS:P450 (1:1, 1:2, 1:3, 1:4, 1:5) were prepared. To this end, 20  $\mu\text{L}$  each of CdS from a 20  $\mu\text{M}$  stock solution and a 10  $\mu\text{M}$  stock solution of P450 were used. Each of the solutions was mixed with 5  $\mu\text{L}$  of 8 mM Myristic acid in ethanol and

then diluted to a final volume of 1 mL with PBS (20 mM sodium phosphate, 50 mM NaCl, pH 7.3). The 1:1, CdS:P450 solution was used as the standard for investigating the reaction as a function of irradiation time. The turnover reported was obtained from the equimolar mixture of CdS and P450 with an irradiation time of 2 min.

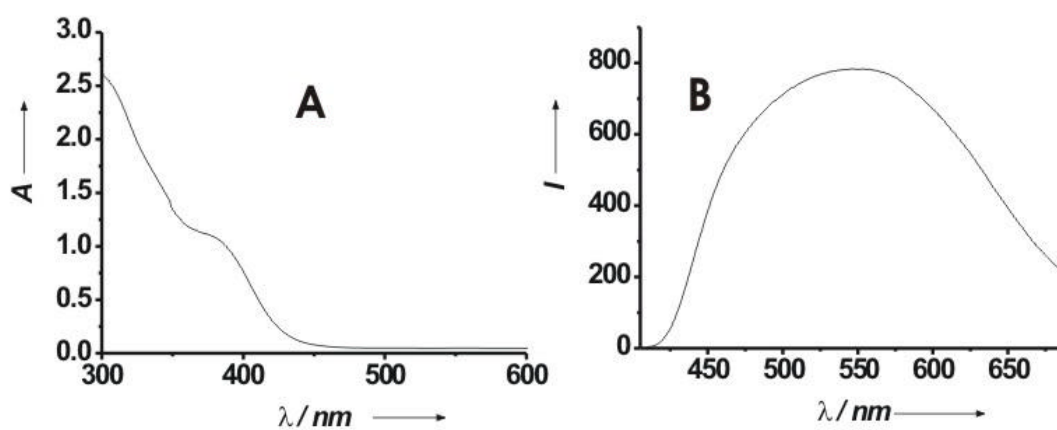
Subsequent to irradiation, each of the reaction mixtures was extracted with 3 mL of EtoAc. The solvent was removed under reduced pressure. The oily residue was redispersed in 150  $\mu$ L EtoAc and derivatized with 9-anthryldiazomethane (60  $\mu$ L from a stock solution of 1 mg/mL) by incubation at room temperature for 2h. 5  $\mu$ L of 1 mM hydroxylauric acid were also added as the internal standard to each of the reaction solutions prior to derivatization. A 10  $\mu$ L portion of the derivatized solution was injected into the HPLC apparatus equipped with a reverse-phase column (Nucleodur Gravity C 18, Macherey-Nagel) and eluted with CH<sub>3</sub>CN-H<sub>2</sub>O. The elution gradient was 40:60 (CH<sub>3</sub>CN:H<sub>2</sub>O) upto 5 min with a flow rate of 1.2 mL/min, 80:20 (CH<sub>3</sub>CN:H<sub>2</sub>O) from 5.1 to 10 min with a flow rate of 1.2 mL/min, 100 % CH<sub>3</sub>CN from 10.1 to 20 min with a flow rate of 1.5 mL/min. The fluorescence intensity of the hydroxylated acid ester derivatives was monitored at 412 nm with excitation at 365 nm using a fluorescence detector of the HPLC. The retention times of hydroxylauric acid,  $\beta$ -hydroxymyristic acid,  $\alpha$ -hydroxymyristic acid and myristic acid were 5.9, 11.5, 11.9, and 13.6 min, respectively. The nature of the products was confirmed by HPLC analysis of authentic samples and ESI-LCMS analysis.

Fig. S1



**Fig. S1.** TEM micrographs of mercaptoacetic acid functionalized QDs of CdS.

Fig. S2

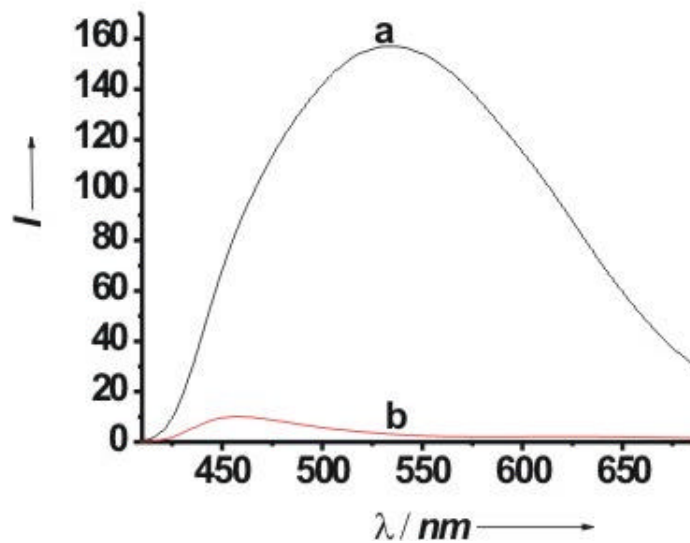


**Fig. S2.** Absorption (A) and Emission (B) spectra of mercaptoacetic acid functionalized CdS QDs.

### Purification of QD-P450<sub>BSβ</sub> nanohybrids

The QD-P450<sub>BSβ</sub> nanohybrids were purified by ultrafiltration using a 100 KD molecular MWCO and the negligible fluorescence of QDs in the dialysate clearly suggested that all QDs are retained (Figure S3) The molecular weight of P450<sub>BSβ</sub> is ~ 48 Kda and that of the conjugates with 6 molecules of P450 per CdS is estimated to be approximately 500 Kda.

**Fig. S3**



**Figure S3.** Photoluminescence a) of the QDs-P450 before and b) that of the dialysate after passing through the membrane.

### Theoretical consideration of surface coverage

Theoretical number of enzymes per QD derived from steric considerations is given by the equation<sup>[7]</sup>

$$N_{p450} = 0,65((R_2^3 - R_1^3))/Rp^3$$

Where  $R_1$  is the QD radius

$R_2 \sim R_1 + 2Rp$  where  $Rp$  is the radius of enzyme

In our case

$$R_1 = 1.5 \text{ nm}$$

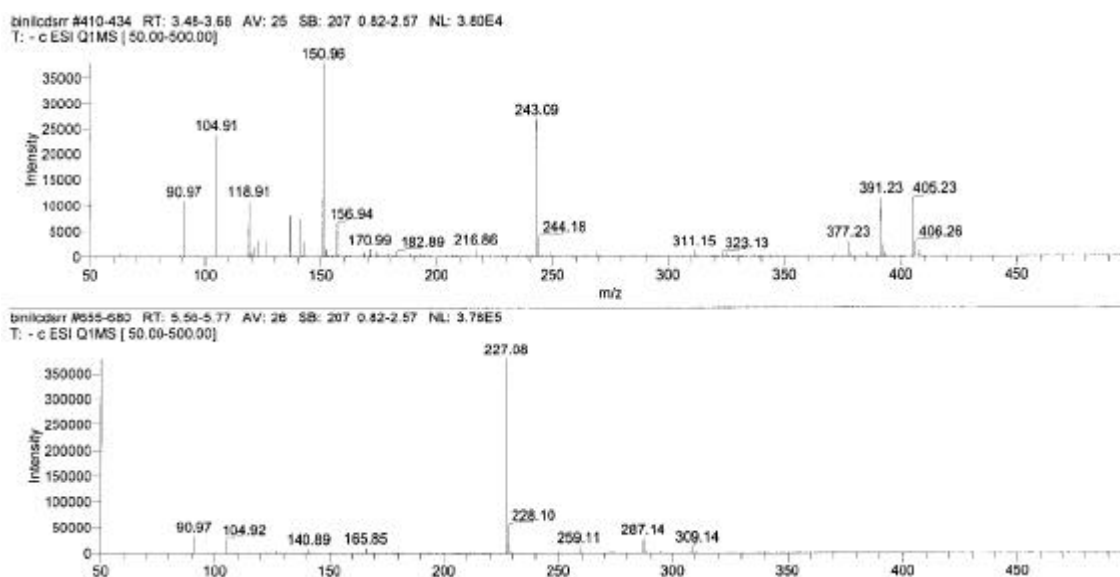
$$Rp = 3 \text{ nm}$$

$$R_2 = 7.5 \text{ nm}$$

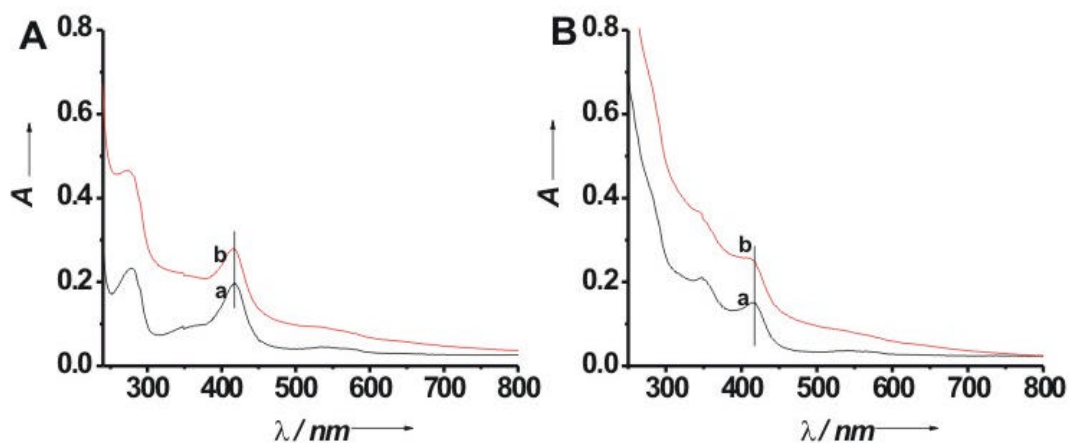
Therefore, the theoretical number of enzyme that can attach per QD =  $0.65((7.5)^3 - (1.5)^3)/3^3$

$$= 10.$$

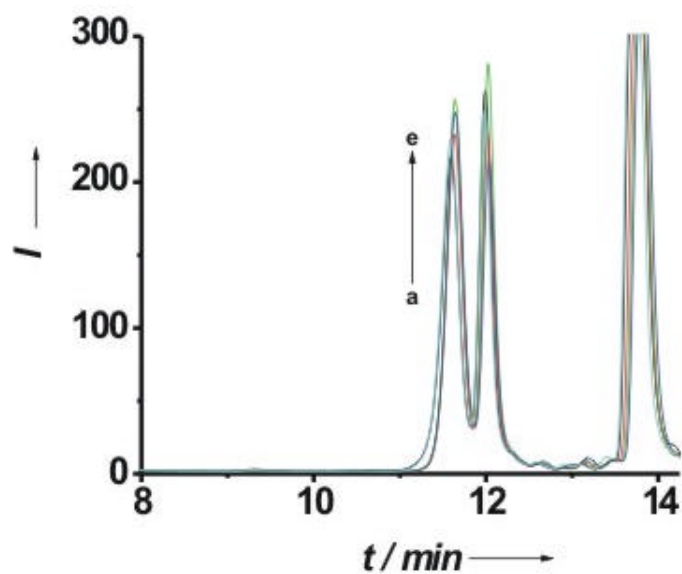
Fig. S4



**Figure S4:** Mass spectrometry analysis of CdS-P450 nanohybrid system irradiated in the presence of myristic acid. The 243.09 peak between a retention time 3.48-3.68 correspond to the  $[M-H]^+$  of hydroxymyristic acid and the 227.08 peak between a retention time 5.56-5.77 correspond to the  $[M-H]^+$  of myristic acid.

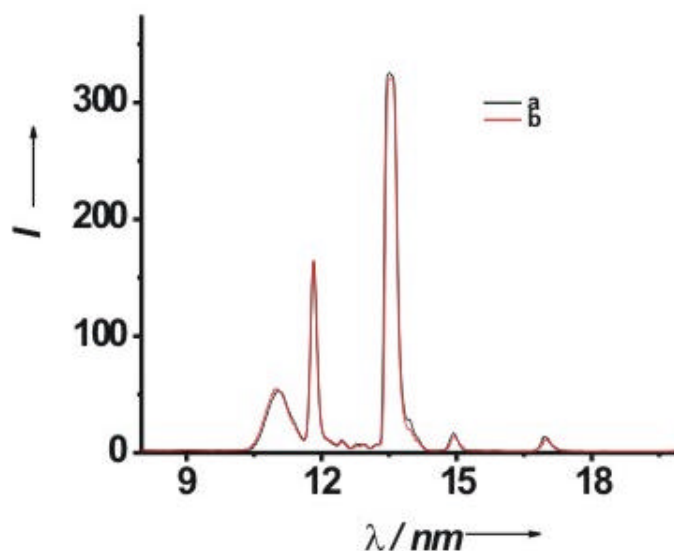


**Figure S5:** Absorption spectrum of **A)** P450 a) in the absence and b) in the presence of Myristic acid; **B)** CdS-P450 a) in the absence and b) in the presence of Myristic acid.



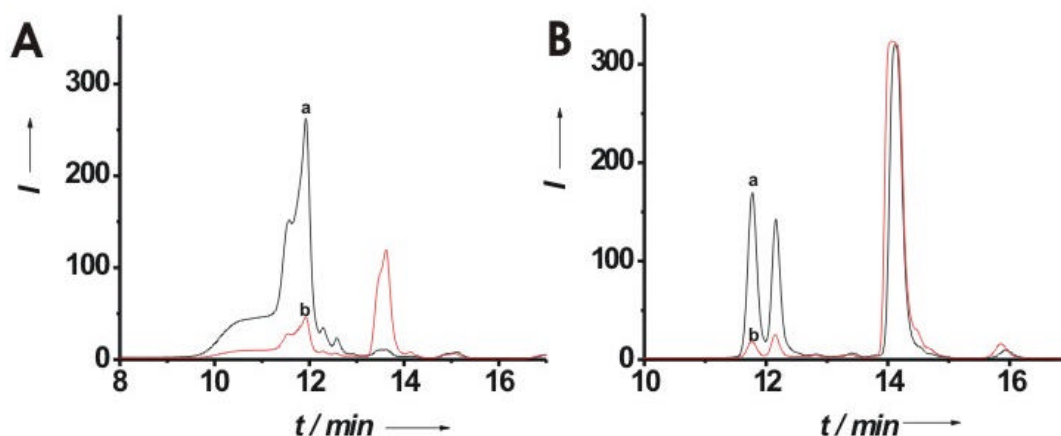
**Figure S6:** Elution profile of myristic acid and its metabolites produced from QD-P450<sub>BS $\beta$</sub>  hybrids with different CdS: P450<sub>BS $\beta$</sub>  ratios: molar ratio CdS: P450<sub>BS $\beta$</sub>  = a) 1:1, b) 1:2, c) 1:3, d) 1:4, e) 1:5. Note that due to the injection volume of 10  $\mu$ L no shoulder appears at retention time  $t=10.8$  min as it does for the injection of 25  $\mu$ L sample volumes.

Fig. S7



**Figure S7:** Elution profile of myristic acid and its metabolites produced from P450; a) with and b) without 6x-His tags in the presence of CdS QDs.

Fig. S8



**Figure S8:** Effect of SOD on yield of hydroxymyristic acid. Elution profile of myristic acid and its metabolites produced from A) QD-P450 hybrids a) irradiation time 10 min, b) same as in 'a' and irradiated in the presence of SOD; B) QD + P450 (w/o His-tag) a) irradiation time 10 min, b) same as in 'a' and irradiated in the presence of SOD.

**References.**

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