Photocatalysis

DOI: 10.1002/anie.200503084

Nanohybrids Composed of Quantum Dots and Cytochrome P450 as Photocatalysts**

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Semiconductor nanomaterials, such as quantum dots (QDs), quantum rods (QRs), and nanotubes, have potentials in a wide variety of fields ranging from nanoelectronics[1] and nanophotonics to the broad area of nanosensing[2–7] and bioimaging.[8,9] Moreover, the unusual physical and chemical properties of nanomaterials resulting from the spatial confinement of electrons have made them attractive candidates for the fabrication of new devices in energy technology.[10] For example, the absorption of light and the occurrence of electron-transfer reactions at the semiconductor/liquid interface have been investigated in detail for the design of solar cells.[11] In addition, the photocatalytic activity of semiconductor QDs has been a subject of intense research ever since their initial description,[12] and organic reactions carried out with QDs as photosensitizers have also been reported.[13–17]

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[**] This work was supported by the Deutsche Forschungsgemeinschaft and the Alexander von Humboldt Foundation (research fellowship for B.I.I.). We thank Dr. Isamu Matsunaga for the generous donation of the plasmid pQE-30tBSb for overexpression of recombinant P450, Andreas Arndt for help with the overexpression, and Dr. Ljiljana Fruk for help setting up the HPLC assay.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Currently, nanotechnology and biotechnology are being joined to develop novel materials and devices, leading to the establishment of the new field of nanobiotechnology.[18] In this context, we report here on the photoactivated organic transformation catalyzed by hybrid devices composed of semiconductor nanoparticles and the enzyme cytochrome $P450_{BS\beta}$. $P450_{BS\beta}$ belongs to the broad class of monooxygenase enzymes which are well known to catalyze a range of stereospecific and regioselective oxygen-insertion reactions into organic compounds.[19] In particular, $P450_{BS\beta}$ catalyzes the hydroxylation of long-chain fatty acids at the α and β positions using hydrogen peroxide as an oxidant.[20]

The choice of this hybrid system was based, on the one hand, on the knowledge that radical species are involved in the catalytic cycle of P450-mediated oxygenation reactions[21–23] and, on the other hand, on the results of previous studies showing that the irradiation of CdS QDs leads to the formation of excitons (e^- and h^+), which generate superoxide ($O_2^{\bullet-}$) and hydroxyl ($OH^\bullet$) radicals in aqueous solutions.[24–26] We speculated that the superoxide and hydroxyl radicals could activate the enzymes adsorbed at the QD surface, thereby inducing specific monooxygenation of a suitable substrate. Such a photoactivation of enzymes could have significant advantages over conventional activation of $P450_{BS\beta}$ enzymes using H_2O_2, because the light-induced reaction would offer a much higher degree of control for the on/off switching of chemical reactivity than that possible in chemically initiated reactions.

To prepare the QD/$P450_{BS\beta}$ nanohybrids, recombinant $P450_{BS\beta}$ enzyme containing a 6×His tag at its C-terminus,[21] was overexpressed in *E. coli* and purified by nickel nitriloacetate (Ni-NTA) chromatography. CdS nanoparticles approximately 3 nm in diameter capped with a layer of mercaptoacetic acid were prepared in reverse-micellar medium, as previously described.[24] The $P450_{BS\beta}$ enzyme was attached to the CdS QDs by means of the electrostatic interaction between the positively charged hexahistidine tail of the enzyme and the negatively charged mercaptoacetic acid ligand shell of the CdS QDs (Figure 1).

For the preparation of the QD/$P450_{BS\beta}$ hybrid materials, the number of $P450_{BS\beta}$ molecules attached per QD was measured by means of FRET-quenching studies (FRET = fluorescence resonance energy transfer) between CdS (donor) and the heme enzyme (acceptor). Defined volumes of 6×-His-tagged $P450_{BS\beta}$ were titrated against a stock solution of CdS QDs, and the change in fluorescence intensity at 550 nm was monitored. A similar method was recently used to establish the interactions between CdSe QDs and cytochrome c.[27] The concentration of $P450_{BS\beta}$ was determined by means of CO differential absorption studies,[28] whereas the concentration of CdS QDs was calculated from inductively coupled plasma mass spectrometry.[24] Figure 2A shows the fluorescence spectra of QD/$P450_{BS\beta}$ nanohybrids. Each spectrum represents QDs mixed with variable molar equivalents of $P450_{BS\beta}$. The decrease in fluorescence emission of CdS QDs at 550 nm upon titration with $P450_{BS\beta}$ is shown in Figure 2B. It is evident that the continuous quenching of QD fluorescence ceases at approximately six molar equivalents of the $P450_{BS\beta}$ per QD. This surface coverage correlates well with theoretical considerations based on the size of the QDS and the enzyme (see the Supporting Information). The slight decrease in fluorescence observed on further increase of $P450_{BS\beta}$ concentration might be attributed to solution-based quenching effects.[29]

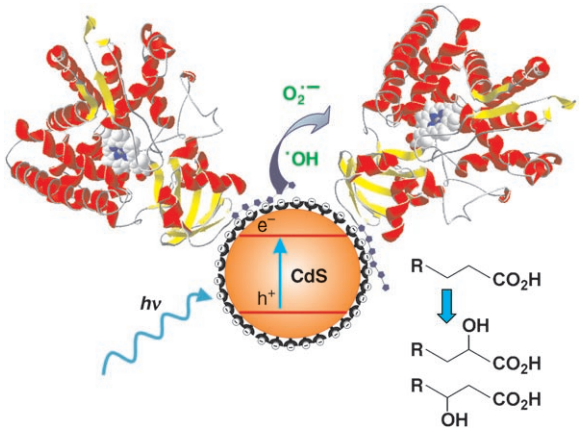

Figure 1. Monooxygenation of myristic acid (R = $(CH_2)_{10}CH_3$) using $P450_{BS\beta}$/QD nanohybrids.

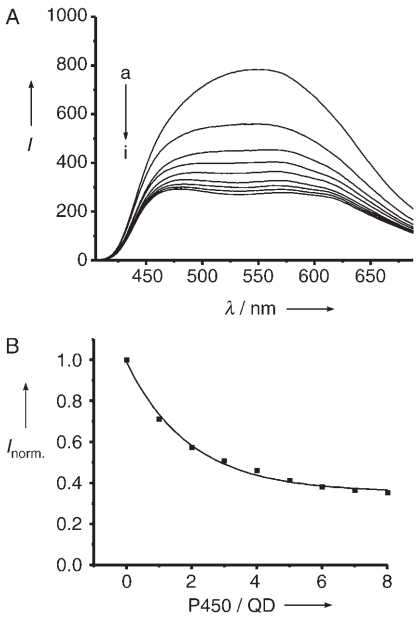

Figure 2. A) Photoluminescence quenching of CdS in the presence of different concentrations of the 6×His-$P450_{BS\beta}$ enzyme. [CdS]/[$P450_{BS\beta}$] ratio: a) 1:0, b) 1:1, c) 1:2, d) 1:3, e) 1:4, f) 1:5, g) 1:6, h) 1:7, i) 1:8. B) Luminescence quenching at 540 nm upon bioconjugate formation at increasing $P450_{BS\beta}$/CdS ratios (data normalized against unconjugated QDs).

In an initial demonstration of the QD/$P450_{BS\beta}$ nanohybrid-mediated photocatalysis we chose the well-established hydroxylation of myristic acid as a model reaction,[21] and we investigated whether hydroxymyristic acid products are formed by using HPLC and ESI-LCMS analysis. To this end, myristic acid (8 mM) in EtOH was added to the $P450_{BS\beta}$/QD hybrid solution (20 μM), and the mixture was irradiated

for 20 minutes at room temperature. The reaction mixture was extracted with chloroform, and the products were analyzed by LC/ESI-MS. This analysis revealed the characteristic mass peak of hydroxymyristic acid ($[M-H^+]=243.09$, calculated $M=244.37$, see the Supporting Information). In contrast, no product peaks were observed in control experiments with: 1) pure QDs in the absence of $P450_{BS\beta}$; 2) with pure $P450_{BS\beta}$ in the absence of QDs; and 3) without irradiation.

We could also observe subtle changes in the optical absorption of P450 in the presence of the myristic acid substrate. An approximately 5-nm blue shift of the 417-nm absorption of the $P450_{BS\beta}$ attached to the CdS QD was observed (Figure S5B in the Supporting Information), and $P450_{BS\beta}$ alone also showed an $\approx$3-nm blue shift in the presence of myristic acid (Figure S5A). As reported earlier, this blue shift is indicative of the binding of the substrate to the active site of P450.[22,30] Although substrate binding is not a prerequisite for electron transfer,[31] the spectroscopic results together with the nonformation of hydroxymyristic acid products in the absence of $P450_{BS\beta}$ provides evidence that the myristic acid is bound to the active site of the $P450_{BS\beta}$ in the CdS/$P450_{BS\beta}$ nanohybrids.

To further elucidate this system, the product yields of similar reactions were determined systematically using a standardized assay which included esterification of the fatty acids and their subsequent HPLC analysis using hydroxylauric acid as an internal standard for quantification[21] (For a detailed protocol, see the Supporting Information.) Various experiments were carried out under different irradiation times, as well as different surface coverages of the CdS QDs with $P450_{BS\beta}$. The results indicated that both α- and β-hydroxymyristic acid isomers are formed in approximately equal amounts in the $P450_{BS\beta}$/CdS photocatalysis, in analogy to the hydrogen peroxide initiated reaction.[20] As expected, the amount of hydroxylation products increased with increasing amounts of P450 per CdS (Figure 3A) as well as increasing irradiation time (Figure 3B).

To further investigate the P450 enzyme activity, controls were carried out with a binary system composed of CdS QDs and $P450_{BS\beta}$ without the 6×-His tag. Under standardized conditions, the system produced similar amounts of the hydroxylation products, as determined by the HPLC assay (see Figure S7). Hence, it appeared questionable whether the close proximity of the two components in the $P450_{BS\beta}$/CdS nanohybrids is, indeed, beneficial for the enzymatic activity of this system. To estimate proximity effects, we compared the turnover of the $P450_{BS\beta}$/CdS nanohybrids with that of the native enzyme activated with H_2O_2. The turnover of the $P450_{BS\beta}$/CdS system was found to be 61 min^{-1}, while that of P450 with H_2O_2 was 122 min^{-1}. The value is even about six times less than that reported for a similar $P450_{BS\beta}$ catalysis (363 min^{-1}), and therefore our results indicate that the activity of the $P450_{BS\beta}$ in the nanohybrids is significantly less than that of the native enzyme. This might be a consequence of the negative effects of the attached nanoparticles on the enzyme's catalytic performance, such as the deceleration of diffusion processes or the restriction of conformational degrees of freedom. Alternatively, (partial) denaturation of the enzyme molecules might occur during the adsorption and purification steps. Nonetheless, despite this reduction in turnover, the $P450_{BS\beta}$/CdS nanohybrids were capable of producing similar amounts of hydroxylation products as the native enzyme. Therefore, these results suggest that the close proximity between the enzymes and the QDs in the $P450_{BS\beta}$/CdS nanohybrids improves the overall catalytic power over that of the system containing the two components in separated form.

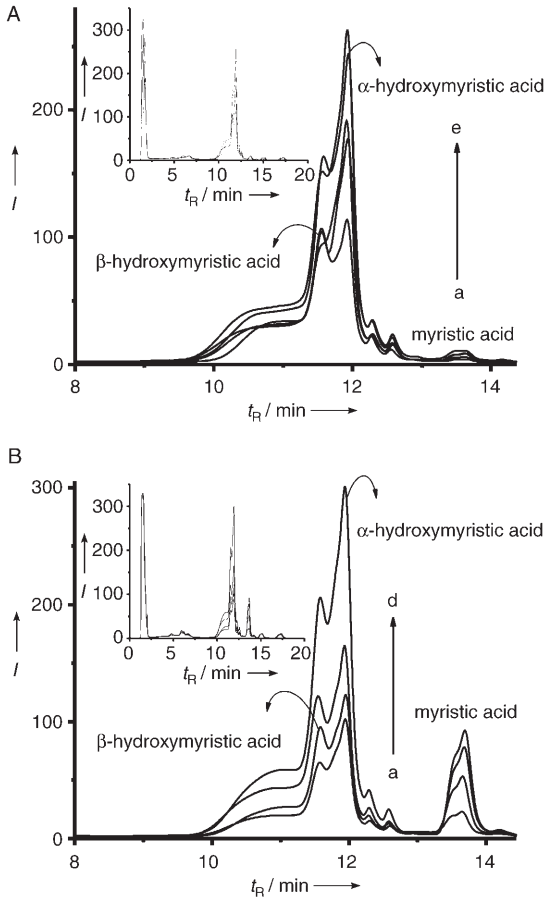

Figure 3. Identification of monooxygenation products by HPLC. Elution profile of myristic acid and its metabolites produced from QD/$P450_{BS\beta}$ hybrids: A) with different CdS/$P450_{BS\beta}$ ratios: a) 1:1, b) 1:2, c) 1:3, d) 1:4. e) 1:5; B) variation of irradiation times: a) 1 min, b) 2 min, c) 5 min, d) 10 min. The insets show complete chromatograms. The left part of the split product peak ($t_R=11.5$ min) corresponds to the β isomer and the right part ($t_R=11.9$ min) to the α isomer of hydroxylmyristic acid. The peak at $t_R=13.6$ min corresponds to myristic acid. The broad shoulder ($t_R=10.8$ min) is an HPLC artifact resulting from large injection volumes (25 μL), see also Figure S6.

To further investigate the $P450_{BS\beta}$/CdS photocatalysis, hydroxylation experiments were carried out in the presence of superoxide dismutase (SOD) enzyme. SOD effectively decomposes superoxide radicals, and we had previously used this activity to prove the identity of $O_2^{\bullet-}$ species produced by CdS QDs.[24] With the $P450_{BS\beta}$/CdS system investigated here, we observed a decrease of the hydroxymyristic acid products formed to approximately 20% chemical yields in the presence of SOD (see Figure S8 in the Supporting Information).

Similar experiments carried out with the two-component system, that is, the mixture composed of CdS QDs and $P450_{BS\beta}$ without the His tag, revealed a decrease of the hydroxymyristic acid products to approximately 10% chemical yields. These results, again, hint at beneficial proximity effects in the $P450_{BS\beta}$/CdS nanohybrids. Apparently, the close distance between the QD and the P450 reduces the SOD-mediated degradation of superoxide radicals. Nonetheless, the results also suggest that the diffusion of radicals and their decomposition by SOD occurs at faster rates than the uptake and conversion of the radicals by the P450 enzyme.

In conclusion, we here report the design of quantum dot/enzyme nanohybrids that are capable of catalyzing an organic transformation through photoactivation of the QDs. The photogenerated excitons (e^- and h^+) produce $O_2^{\bullet-}$ and/or $OH^\bullet$ radicals, which in turn activate the P450 enzymes to catalyze monooxygenation of fatty acid substrates. In our example myristic acid is hydroxylated to give α- and β-hydroxymyristic acid. Although we could clearly prove the chemical composition and the catalytic activity of the novel $P450_{BS\beta}$/CdS nanohybrids, the characterization of their detailed kinetic properties will require more sophisticated studies. Hence, further work will focus on potential proximity effects that might arise owing to the close distance between the enzyme molecules and the surface of the radical-generating nanoparticle. In general, the light-induced triggering of enzyme activity confers explicit advantages over chemical initiation with respect to the control of enzymatic reactions. Moreover, the $P450_{BS\beta}$/CdS nanohybrids should also enable convenient recycling of the catalyst in organic transformations.[32] In addition to the investigation of other semiconductor QDs for improving the catalytic activity of such nanohybrids, which is currently under way, we are also aiming towards applications as photocatalysts in synthetic organic chemistry and as photosensitizers for intracellular reactions.

Received: August 30, 2005
Revised: October 14, 2005
Published online: December 19, 2005

Keywords: nanostructures · photochemistry · quantum dots · radicals

[1] T. Mokari, E. Rothenberg, I. Popov, R. Costi, U. Banin, *Science* **2004**, *304*, 1787.
[2] C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128.
[3] E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042.
[4] L. Pavesi, L. D. Negro, C. Mazzoleni, G. Franzco, F. Priolo, *Nature* **2000**, *408*, 440.
[5] A. V. Malko, A. A. Mikhailovsky, M. A. Petrusca, J. A. Hollingsworth, H. Htoon, M. G. Bawendi, V. I. Klimov, *Appl. Phys. Lett.* **2002**, *81*, 1303.
[6] J. M. Bruchez, M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science* **1998**, *281*, 2013.
[7] I. Willner, F. Patolsky, J. Wasserman, *Angew. Chem.* **2001**, *113*, 1913; *Angew. Chem. Int. Ed.* **2001**, *40*, 1861.
[8] W. C. W. Chan, S. Nie, *Science* **1998**, *281*, 2016.
[9] X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, S. Weiss, *Science* **2005**, *307*, 538.
[10] F. Raimondi, G. G. Scherer, R. Kotz, A. Wokaun, *Angew. Chem.* **2005**, *117*, 2228; *Angew. Chem. Int. Ed.* **2005**, *44*, 2190.
[11] M. Gratzel, *Energy Resources through Photochemistry and Catalysis*, Academic Press, New York, **1983**.
[12] M. A. Fox, *Acc. Chem. Res.* **1983**, *16*, 314.
[13] S. Marinkovic, N. Hoffmann, *Chem. Commun.* **2001**, 1576.
[14] S. Marinkovic, N. Hoffmann, *Eur. J. Org. Chem.* **2004**, 3102.
[15] M. Warrier, M. K. F. Lo, H. Monbouquette, M. A. Garcia-Garibay, *Photochem. Photobiol. Sci.* **2004**, *3*, 859.
[16] M. L. Curri, A. Agostiano, G. Leo, A. Mallardi, P. Cosma, M. D. Monica, *Mater. Sci. Eng. C* **2002**, *22*, 449.
[17] I. N. Aleksey, I. A. Shumilin, V. V. Nikandrov, *J. Phys. Chem.* **1996**, *100*, 17544.
[18] C. M. Niemeyer, C. A. Mirkin, *Nanobiotechnology: Concepts, Methods and Application*, Wiley-VCH, Weinheim, **2004**.
[19] H. Waldmann, K. Drauz, *Enzymes Catalysis in Organic Synthesis*, Wiley-VCH, **2002**.
[20] I. Matsunaga, A. Ueda, N. Fujiwara, T. Sumimoto, K. Ichihara, *Lipids* **1999**, *34*, 841.
[21] I. Matsunaga, A. Ueda, T. Sumimoto, K. Ichihara, M. Ayata, H. Ogura, *Arch. Biochem. Biophys.* **2001**, *394*, 45.
[22] D.-S. Lee, A. Yamada, H. Sugimoto, I. Matsunaga, H. Ogura, K. Ichihara, S.-i. Adachi, S.-Y. Park, Y. Shiro, *J. Biol. Chem.* **2003**, *278*, 9761.
[23] A detailed description of potential catalytic pathways is included in the Supporting Information.
[24] B. I. Ipe, M. Lehnig, C. M. Niemeyer, *Small* **2005**, *1*, 706.
[25] J. R. Harbour, M. L. Hair, *J. Phys. Chem.* **1977**, *81*, 1791.
[26] A. Henglein, *Ber. Bunsen-Ges.* **1982**, *86*, 301.
[27] I. Geissbuehler, Dissertation, EPFL (Lausanne, Switzerland), **2005**.
[28] T. Omura, R. Sato, *J. Biol. Chem.* **1964**, *239*, 2370.
[29] E. R. Goldman, I. L. Medintz, J. L. Whitley, A. Hayhurst, A. R. Clapp, T. Uyeda, J. R. Deschamps, M. E. Lassman, H. Mattoussi, *J. Am. Chem. Soc.* **2005**, *127*, 6744.
[30] I. Matsunaga, A. Yamada, D.-S. Lee, E. Obayashi, N. Fujiwara, K. Kobayashi, H. Ogura, Y. Shiro, *Biochemistry* **2002**, *41*, 1886.
[31] F. P. Guengerich, W. W. Johnson, *Biochemistry* **1997**, *36*, 14741.
[32] Initial judgements on the stability of the $P450_{BS\beta}$/CdS nanohybrids can be drawn from the observation that continuous irradiation for 20 minutes led to neither precipitation nor to a decrease in enzymatic turnover. However, our efforts of reusing the nanohybrids for an additional round of catalysis have been unsuccessful so far, probably as a result of enzyme denaturation during the workup and extraction procedures.

