Supporting Information

for

Light-Induced Triggering of Peroxidase Activity Using Quantum Dots **

Ljiljana Fruk, Vidya Lakshmi Rajendran, Mark Sprengler, and Christof M. Niemeyer*

A.) Physical adsorption of HRP to CdS quantum dots

To investigate whether adsorption of the HRP to the QDs occurs, we used fluorescence titration method, in which the fluorescence of the QDs is quenched by the heme-containing HRP. To this end, defined volumes of HRP were added to a stock solution of CdS QDs and the change in the fluorescence intensity at 550 nm was monitored. As shown in Fig. S1, the QDs fluorescence continuously decreased until about three molar equivalents of the HRP were added. At higher ratios, the decrease in fluorescence ceased upon addition of more aliquots, thus suggesting that each nanoparticle is covered with about 3-4 HRP molecules. These results were supported by DLS measurements which indicated that average particle diameters increased from 5.55 nm (QDs only) to 19.85 nm upon addition of three molar equivalents of HRP and further
additions did not significantly increase the average particle size (Table S1). DLS measurements of HRP QD solutions were carried using Zetasize Nano Instrument (Malvern Instruments, UK). The solutions of CdS-HPR nanohybrid are filtered before DLS measurements using Whatmann 0.1 µm membrane filter.

![Graph](image)

**Figure S1:** The luminescence quenching at 550 nm upon the addition of a) HRP b) CCP and c) Mb. To eliminate effects from the dilution, the data were normalized against unconjugated QDs.
Table S1: Particle diameter obtained by DLS measurements of CdS QD’s in presence of different amount of HRP enzyme

<table>
<thead>
<tr>
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<th>Average particle diameter (nm)</th>
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<tr>
<td>CdS QDs</td>
<td>5.56 ± 0.45</td>
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<tr>
<td>HRP</td>
<td>8.04 ± 0.53</td>
</tr>
<tr>
<td>CdS:HRP 1:1</td>
<td>13.35 ± 0.35</td>
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<tr>
<td>CdS:HRP 1:2</td>
<td>19.50 ± 0.42</td>
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<tr>
<td>CdS:HRP 1:3</td>
<td>19.85 ± 0.35</td>
</tr>
<tr>
<td>CdS:HRP 1:4</td>
<td>15.85 ± 0.07</td>
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</table>

Note that average particle diameters increase until about 3 molecules of HRP are adsorbed at the QD’s surface. The addition of fourth molecule is not favorable due to electrostatic and stercical reasons. Therefore, an increasing amount of free HRP molecules (average diameter of about 8 nm) in the solution leads to a reduction of the average particle diameter measured.

B.) Amplex Red oxidation by HRP

Figure S2: Oxidation of Amplex Red substrate by HRP in the presence of various amounts of QDs. The 1_1 ratio corresponds to 5 pmol HRP and 5 pmol QDs in a total volume of 150 uL reaction mixture (i.e., 33.3 nM concentration of each compound). 1_0, 0_1 and 0_0 are the controls, containing QD’s only, HRP only and Amplex Red only, respectively.
Figure S3: Effect of the continuous irradiation on the peroxidase activity of 5 pmol HRP in the presence of QD’s. Standard H$_2$O$_2$ and Amplex Red protocol was used to determine the residual peroxidase activity of the enzyme after the irradiation.

C.) Formation of hydroxyl radicals by irradiation of QDs

Fig S4: Disodium terephthalate assay to monitor the formation of hydroxyl radicals upon irradiation. The assay was carried out as described previously.[2]
D.) ABTS, HPA, guaiacol, and KI as peroxidase substrates

To investigate whether the QD/HRP system can be used to oxidize other substrates besides Amplex Red, experiments with common HRP substrates, i.e., 2,2'-azino-bis (3-ethylbenzthiazolin-6-sulfonat (ABTS), 3-(4-hydroxyphenyl) propionic acid (HPPA), guaiacol and potassium iodide (KI) were carried out.

In the case of ABTS and KI as peroxidase substrates, no significant product formation was observed during 15 min of in situ QD-HRP irradiation. However, significant amounts of oxidation product were obtained when a pre-irradiated QD solution was used (Fig S5). The experiments revealed that significant amounts of the ABTS oxidation product were formed after 5 min of in situ irradiation, however, the amount of product decreased with increasing times of irradiation.

![Fig S5: Peroxidase activity of 20 pmol HRP in presence of 200 pmol QD (preirradiated and in situ irradiated) using a) ABTS and b) KI as a substrate.](image)
This observation was confirmed by measuring the amount of ABTS$^+$ radical cations formed by using QD solutions which were pre-irradiated for variable times, and thus, contained increasing concentrations of oxygen radicals (Fig. S6).

**Fig. S6:** Concentration of ABTS radical cations formed by 20 pmol HRP and 200 pmol QDs pre-irradiated for variable times. Note that the product concentration decreases with increasing amounts of oxygen radicals formed by QD irradiation (for comparison, see Fig. S4).

It is well known that ABTS$^{[3]}$ and also guaiacol$^{[4, 5]}$ generate coloured radical intermediates and products in the presence of peroxidases. For instance, the oxidation of ABTS in the presence of peroxidases leads to the formation of the green coloured radical cation ABTS$^+$ (Scheme S1).
It is evident from Fig. S4, that increased irradiation times lead to the formation of greater amounts of oxygen radicals. Fig. S6 indicates that about 17 µM of ABTS radical cations were formed by using a QD solution pre-irradiated for 5 min, and extended irradiation times efficiently reduce the amounts of ABTS radical cations formed. We therefore hypothesized that radical quenching may occur when sufficient amounts of radicals have been generated by the irradiation of the QDs. This results of Fig. S4 and Fig. S6 support the hypothesis, that the oxygen radicals produced by QD irradiation react with and decrease the amount of ABTS radical cations produced by HRP. A likely explanation includes radical recombination reactions between oxygen radicals and the ABTS radical cations.

In the case of guaiacol as peroxidase substrate, no oxidized products were observed in the reaction mixture comprising HRP, pre-irradiated QDs and guaiacol. This result provided additional support for our hypothesis of radical quenching, because the peroxidase-mediated oxidation of this substrate also includes radical intermediates (Scheme S2).
Scheme S2: Mechanism of guaiacol product formation in presence of peroxidases and $\text{H}_2\text{O}_2$

The mechanism of KI oxidation by HRP is still not fully understood.\[6\] However, the lack of product formation upon in situ irradiation suggests that in this case radical species might be involved.

The oxidation of HPPA was readily achieved by in situ irradiation of QDs in the presence of HRP (Fig. S7).

Figure S7: 3-(4-hydroxyphenyl) propionic acid (HPPA) assay in the presence of 250 pmol of peroxidases and 10 molar equivalents of pre-irradiated quantum dots. The concentration of HPPA was 12.5 mM. The fluorescence of the product formed was measured at 410 nm emission wavelength using 310 nm excitation.
E.) Experimental Details

Chemicals. All chemicals used for the synthesis of CdS quantum dots, guiacole, 2,2’-azino-bis (3-ethylbenzthiazolin-6-sulfonat (ABTS), potassium iodide and 3-(4-hydroxyphenyl) propionic acid (HPPA) were obtained from Sigma Aldrich and used without further purification. N-acetyl-3,7-dihydroxyphenoxazin (Amplex Red) was obtained from Molecular Probes and H₂O₂ from Fluka (fresh solution was prepared before each experiment).

Enzymes. Myoglobin (horse heart) and horseradish peroxidase were purchased from Sigma. Cyp152A1 was overexpressed in E.coli using plasmid pQE-30tBSb obtained from Dr. Isamu Matsunaga and purified by nickel nitorilo-acetate (Ni-NTA) chromatography as previously described.[7] CCP was cloned from S.cerevisiae chromosomal DNA, overexpressed in E. coli using pQE-30CCPwt plasmid and purified using nickel Ni-NTA chromatography yielding the C-terminal His₆-tagged CCP. The enzyme was purified to homogeneity and characterized by SDS-PAGE, MALDI-MS and enzyme kinetic measurements (M. Spengler, J. Müller, C. M. Niemeyer, unpublished results).

Synthesis of Quantum Dots. An AOT/heptane microemulsion was prepared by dissolving AOT (7 g) in a mixture of water (2 ml) and heptane (100 ml). This was divided into two portions of 61 ml and 41 ml. To the former was added 240 µl of Cd(ClO₄)₂ (1 M) and to the latter was added 160 µl of Na₂S (1M). The two solutions were stirred separately for 1 h. These two solutions were mixed and stirred under argon for 1 h. To this, 160 µl of mercaptoacetic acid (1 M) was added and the solution was stirred under argon for 8 h. The QDs were precipitated by the addition of pyridine. They
were filtered and washed separately with heptane, butanol, ethanol and methanol. The precipitated nanoparticles after vacuum drying were redispersed in PBS buffer (pH 7). The nanoparticle concentration was determined from the extinction coefficient of CdS $2 \times 10^4 \text{M}^{-1}\text{cm}^{-1}$.[8] Quantum Dots for peroxidase activity studies were irradiated with 366 nm light.

**Peroxidase measurements.** Amplex Red (AR) solution was prepared according to the manufacturer’s instructions as a 10 mM stock in DMSO. End concentration of Amplex Red in peroxidase assays was 60uM. In a typical microtitre plate format assay, desired amounts of the enzyme were mixed with AR reagent, followed by addition of pre-irradiated quantum dots, and the fluorescence measurements were carried out using 540 nm excitation and 590 nm emission wavelengths. Every measurement was done in triplicate. Other peroxidase substrates were prepared according to the manufacturer’s instructions with following end concentrations: ABTS 15 mM in phosphate citrate buffer pH 5.5; guaiacol: 15 mM in pH 7 phosphate buffer; HPPA: 12.5 mM in pH 7 phosphate buffer; KI: 2 mM in sodium acetate buffer pH 5.2.

**Stability studies** (Fig S3). 5.5 pmol of HRP and 55 pmol QDs were placed in quartz cuvette and continuously irradiated using 366 nm light. Aliquots were taken such that the final concentration of the enzyme was 5 pmol. 1 µL of AR (10mM) and 7 µl H$_2$O$_2$ (20mM) solution (both in KPi pH 7.4) were added, the mixture was stirred for 1 minute after which the fluorescence of the samples was measured.

**Switchability.** 50 pmol HRP in phosphate buffer pH 7.4 were mixed in a cuvette with a two- or ten-fold molar excess of QDs. To this, AR stock solution was added such that the end
concentration in 1 ml volume was 60µM. The cuvette was placed in fluorimeter and the sample was irradiated with 366 nm lamp placed on the top of the cuvette. The irradiation was started and stopped in regular time intervals. The same procedure was repeated using 50 pmol CCP and Mb and 250 pmol Cyp154A1.

References