

Semiconductor Quantum Dots for Photodynamic Therapy

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Photodynamic therapy (PDT) is an emerging cancer treatment that takes advantage of the interaction between light and a photosensitizing agent to initiate apoptosis of cancer cells.¹ In PDT, the photosensitizing agent becomes activated by light but does not react directly with cells and tissues. Instead, it transfers its triplet state energy to nearby oxygen molecules to form reactive singlet oxygen (¹O₂) species, which cause cytotoxic reactions in the cells.² The increasing popularity of this treatment method is largely due to its selectivity: only tissues that are simultaneously exposed to the photosensitizer and light, in the presence of oxygen, are the ones subjected to the cytotoxic reactions during PDT.^{1,2} Thus, under ideal circumstances only diseased tissues are eradicated, leaving the surrounding healthy cells undamaged.

Since the first demonstration of the photodynamic action in 1900,³ great effort has been devoted towards the development of photodynamic therapy agents, which have specific light absorption and tissue distribution properties. A first-generation photosensitizer that has been accepted for clinical use is the hematoporphyrin derivative, Photofrin.⁴ The clinical success of Photofrin has inspired the development of new PDT photosensitizers, which could offer improvement in optical and chemical properties.⁵ Among the more promising second-generation photosensitizers that are currently being evaluated for PDT applications are the phthalocyanines (Pc's).⁶ Pc derivatives have favorable photophysical and chemical properties, which include strong absorbance at long wavelengths and chemical tunability through substituent addition on the periphery of the macrocycle or on the axial ligands.^{2,6} However, as with most photosensitizing agents, Pc's have poor solubility in water and tend to aggregate in aqueous solutions, which can result in loss of photochemical activity and affect their cell-penetrating properties.^{2,7} To resolve this issue, nanoparticles are currently being explored as potential delivery systems for PDT photosensitizers.⁷ Gold nanoparticles coated with a zinc-Pc photosensitizer were prepared to facilitate the development of a hydrophilic three-component nanoparticle system (photosensitizer/gold/transfer agent), which was shown to generate ¹O₂ with enhanced efficiency as compared to the free Pc.^{7a} Along this direction, silica-based nanoparticles were recently developed to entrap water-insoluble photosensitizing agents and were shown to be an effective PDT drug carrier in aqueous media.^{7b} These developments illustrate the potential of nanoparticle-based PDT for cancer therapy applications.

Nanomaterials that offer great promise in PDT applications are semiconductor quantum dots. Quantum dots (QDs) in the size range of 1–6 nm are not quite small molecules nor bulk crystals. The size of QDs gives them unique optical properties that can be precisely tuned from the UV to the infrared region by changing their size and composition.⁸ Their emission properties result from quantum-confinement effects and can be tuned to emit into the near-IR region as opposed to the visible emission of most conventional photosensitizers. Since there is minimal light scattering and absorption in the near-IR region of the spectrum, light of low

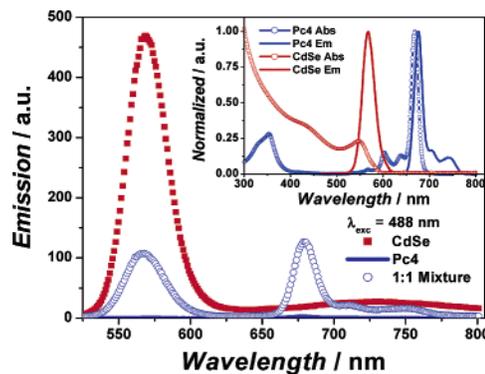


Figure 1. Indirect activation of a phthalocyanine-based PDT photosensitizer using 488-nm excitation, through CdSe quantum dot-assisted FRET. The inset shows the spectral features of the free silicon phthalocyanine (Pc4) and CdSe quantum dot, respectively.

intensity can be used to penetrate tissue to depths of several centimeters, thereby allowing access to deep-seated tumors. Furthermore, due to their large transition dipole moment, QDs are strong absorbers,⁸ making them ideal agents for PDT applications. More importantly, the surface coating of QDs can be functionalized to make them both water soluble and biocompatible,⁹ which facilitates systemic delivery.

So far, most of the work done on semiconductor QDs in the area of biomedicine has focused on fluorescence imaging applications.⁹ Here, we report on the applicability of semiconductor QDs for PDT. CdSe QDs with an average diameter of 5 nm were synthesized using previously reported methods¹⁰ and linked to a silicon Pc photosensitizer (Pc4) through an alkyl amino group on the photosensitizer's axial substituent.^{6a} Pc4 is a known PDT agent currently undergoing clinical trials.^{1a} As a first step, the spectral interaction between the QD and Pc4 was investigated. For excitation wavelengths between 550 and 630 nm, Pc4 can be directly activated. However, as Figure 1 illustrates, the QD is needed as primary energy donor if wavelengths between 400 and 500 nm are to be used for excitation. Conjugation of the QDs afforded the indirect excitation of Pc4 at 488 nm through a fluorescence resonance energy transfer (FRET)^{1a} mechanism from the QD to Pc4, and the Pc4 emission could be observed at 680 nm (Figure 1). By monitoring the decrease in emission intensity of the energy donor (QD), a 77% FRET efficiency can be estimated. The CdSe QD emission at 568 nm was used to activate the Pc4 photosensitizer. The combination of semiconductor QDs and PDT photosensitizers enabled the use of an excitation wavelength where the photosensitizer alone does not absorb (inset of Figure 1). To our knowledge, this is the first demonstration of utilizing QD-based FRET to facilitate excitation of a PDT photosensitizer, which is known to generate reactive ¹O₂ species available for photodynamic cancer therapy. Since the QD exhibits a broad absorption spectrum (inset of Figure 1), its conjugation to the Pc4 photosensitizer provides the flexibility of

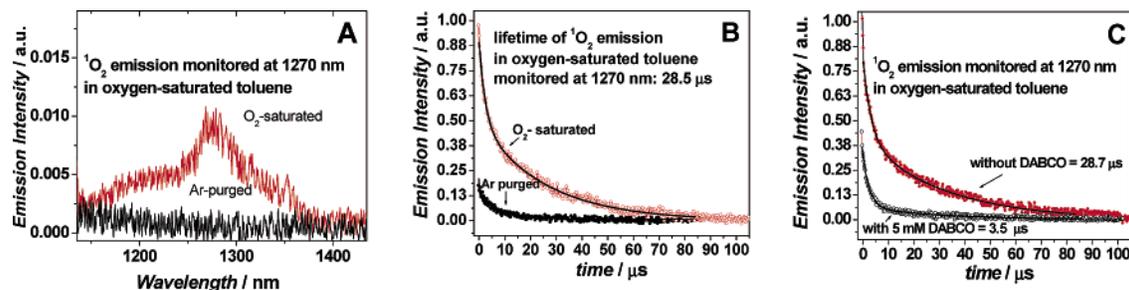


Figure 2. $^1\text{O}_2$ dynamics through semiconductor QDs in toluene solution following 488-nm excitation. (A) Emission spectra of the $^1\text{O}_2$ generated from CdSe QDs. (B) Decay kinetics of $^1\text{O}_2$ emission observed at 1270 nm. (C) Quenching of $^1\text{O}_2$ emission in the presence of DABCO.

utilizing variable excitation wavelengths to activate the sensitizer molecule. Moreover, the spectral properties of the QDs can be adjusted to match those of any PDT photosensitizer by simply adjusting the size of the QD.

In the process of investigating the two-step energy-transfer mechanism in the QD/Pc4/oxygen system, we found that semiconductor QDs alone can actually generate $^1\text{O}_2$ without a mediating Pc4 molecule (Figure 2A). The radiative relaxation of $^1\text{O}_2$ causes emission at 1270 nm,¹¹ which is monitored using laser-flash photolysis (Spectra Physics Pro 230-MOPO 730) and a liquid nitrogen-cooled Ge detector (North Coast EO-817P) positioned behind a 1200-nm long-pass filter and coupled to a digital oscilloscope (LeCroy LT 342). Shown in Figure 2B is the decay kinetics of the $^1\text{O}_2$ emission measured at 1270 nm after 488-nm excitation in toluene, in argon-purged solution (solid circles), and in oxygen-saturated solution (open circles). To evaluate the $^1\text{O}_2$ quantum yield in toluene, perinaphthenone (Aldrich, $\Phi_{\Delta} = 1.0$) was used as reference compound. Using CdSe QD with 65% emission quantum yield, we found the $^1\text{O}_2$ quantum yield to be $\sim 5\%$. In comparison, the Pc4 photosensitizer was reported to have a $^1\text{O}_2$ efficiency of 43%.¹² A test experiment was conducted to demonstrate the quenching of the $^1\text{O}_2$ emission after addition of 1,4-diazabicyclo[2.2.2]octane (DABCO, Acros Organics), in oxygen-saturated solution (Figure 2C).

The O_2 molecules are likely to intercalate in the nonpolar¹⁻⁴ TOPO layer at the QD surface. One can expect a similar interaction in water-soluble phospholipid-capped QDs, with one of the limitations in efficiency being the solubility of O_2 in water (1.27×10^{-3} M) as compared to toluene (9.88×10^{-3} M).¹³

Theory predicts that the lowest excited state of CdSe QDs is a triplet state,¹⁴ often referred to as “dark state” since it is not observable in the steady-state absorption spectrum. The results above show that the triplet character of QDs can be used to generate $^1\text{O}_2$. Our femtosecond time-resolved studies (employing a pump-probe system driven by a Clark MXR CPA 2001 laser) suggest that the low $^1\text{O}_2$ quantum yield can be due to carrier trapping and nonradiative carrier recombinations occurring on the early picosecond time scale.^{10,15} In addition, the photoexcited QDs and ground-state triplet oxygen ($^3\text{O}_2$) have to be in contact to undergo triplet energy transfer (TET), and spin statistics allows only a small fraction¹⁶ of the QD– $^3\text{O}_2$ contact pairs to form $^1\text{O}_2$. On the positive side, semiconductor QDs display tunable absorption properties and have excellent photostability,⁹ which allows reliable studies in clinical trials. Moreover, the amount of defect sites on the QD surface can be controlled to some extent by using additional surface passivation layers,¹⁰ which may systematically improve $^1\text{O}_2$ generation.

In summary, we have demonstrated that CdSe QDs can be used to sensitize either a PDT agent (e.g., Pc4) via a FRET mechanism or molecular oxygen through a TET mechanism, respectively (Figure 3). Both processes are useful for PDT applications and could

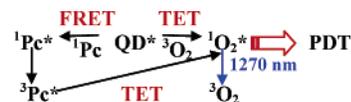


Figure 3. Schematics of the $^1\text{O}_2$ generation in QD-based PDT systems. pave the way for the development of a novel class of PDT sensitizers, with optical properties that can be tuned to treat both shallow and deep-seated tumors. We are currently investigating singlet oxygen generation on less toxic QDs¹⁵ and are evaluating different surface-coating techniques to increase the $^1\text{O}_2$ quantum yield.

Acknowledgment. We thank Professor M. E. Kenney for providing the Pc4 sample, and we dedicate this paper for his 75th birthday.

Supporting Information Available: Figure of QD excited-state quenching by oxygen (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0386905