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Reversible Photochemical Control of Singlet Oxygen Generation Using Diarylethene Photochromic Switches

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Supporting Information

ABSTRACT: Reversible noninvasive control over the generation of singlet oxygen is demonstrated in a bicomponent system comprising a diarylethene photochromic switch and a porphyrin photosensitizer by selective irradiation at distinct wavelengths. The efficient generation of singlet oxygen by the photosensitizer is observed when the diarylethene unit is in the colorless open form. Singlet oxygen generation is not observed when the diarylethene is converted to the closed form. Irradiation of the closed form with visible light (>470 nm) leads to full recovery of the singlet oxygen generating ability of the porphyrin sensitizer.

Singlet oxygen ($^1O_2$), the first excited state of molecular oxygen, is highly reactive and can damage organic materials and biological tissues.[1] $^1O_2$ has been studied intensely over several decades to understand a variety of processes, including photodegradation, photobleaching, photochemical synthesis, etc.[2] One of the most important applications of singlet oxygen generation is in photodynamic therapy (PDT), which is used clinically to treat diseases through exposure of tissue to light.[3] Upon irradiation, photosensitizers transfer energy to triplet oxygen ($^3O_2$) and generate $^1O_2$, which can react with bacteria, tumors, or diseased cells and destroy them by chemical oxidation.

The development of $^3O_2$ sensitizers in which the amount of $^1O_2$ produced is controllable and can be regulated has received increasing attention recently, since it could provide a way for efficient and selective control in PDT and limit nonspecific photodamage in the body.[4] Several approaches for controlling generation and deactivation of $^1O_2$ have been reported, e.g., through environmental changes (including solvent or pH),[5] programming with enzymes or DNA,[6] or applying nanomaterials, e.g., carbon nanotubes and quantum dots.[7] These systems provide for either On/Off or high/low functionality to the sensitizers toward generation of $^1O_2$, which can minimize side effects under prolonged exposure to light. However, the change in chemical environments generally needed in these systems is invasive in the context of PDT and the precise control of reversibility is often complex. Furthermore, several of the proposed methods for control over the activation of $^1O_2$ are not tolerable and/or are toxic in the body, which limits their application in PDT. In the case of activation of photosensitizers, by cleavage from a polymer or a short peptide sequence upon enzymatic digestion, or dissociation from nanomaterials upon binding to the target, they cannot be reverted to their original state, i.e., to the off state for $^1O_2$ generation. Therefore, the challenge is to design a system that can switch on and off $^1O_2$ generation efficiently, reversibly, and noninvasively with the potential for high tempo-spatial control.

Here, a noncovalent strategy to regulate $^1O_2$ generation by photosensitizers is described where use is made of the two states of diarylethene photochromic switches, in which the on and off switching of $^1O_2$ generation is fully reversible and can be achieved upon irradiation with light at distinct wavelengths (Figure 1).

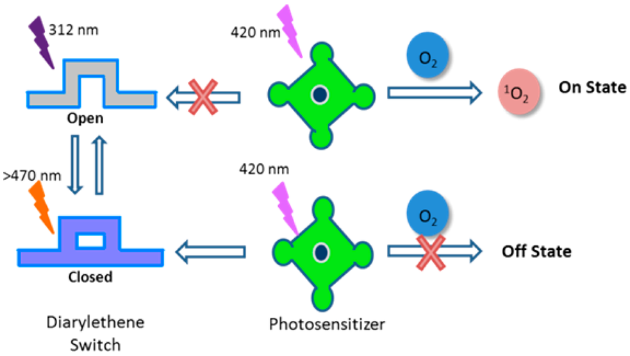

Figure 1. Photochemical control of the generation of $^1O_2$ by a photosensitizer using diarylethene switches.

Diarylethene molecular switches can be interconverted between their colorless open and colored closed forms with UV and visible light, respectively.[8] The structural differences between the states result in a large difference in properties, e.g., electronic energy levels, color, polarizability, and conformational flexibility. Due to their thermal stability, fatigue resistance, and high efficiency in photoisomerization, diarylethene switches have been applied in areas such as information storage in nanotechnology, photonic devices, biochemical reactivity, etc.[9]

Zinc−tetraphenylporphyrin (ZnTPP), a widely used photosensitizer, and its derivatives have been applied clinically in PDT.[3a,10] It was chosen as the photosensitizer in the present study because of its high quantum yield ($\Phi = 0.84$) toward $^1O_2$ generation and well-studied photochemistry.[11] In the present system a noncovalent approach is taken over a covalent

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approach to circumvent the loss in functionality that follows the reaction of $^1O_2$ generated with the switching unit.

In this system (Figure 1), the on and off switching of $^1O_2$ generation by ZnTPP relies on differences between the triplet energies of the open and closed diarylethene switches. Upon excitation into the Soret band (ca. 420 nm) of ZnTPP, energy transfer occurs from the ^{3}ZnTPP* to $^3O_2$, generating $^1O_2$ (on state), while energy transfer does not occur between ^{3}ZnTPP* and the open form of the diarylethene switches. The off state for $^1O_2$ generation can be achieved by brief irradiation with UV light, which converts the diarylethene to the closed form. Energy transfer from ^{3}ZnTPP* to the closed form of the diarylethene switches competes effectively with energy transfer to $^3O_2$, with the result that $^1O_2$ generation is shut down. The on and off states for $^1O_2$ generation can be accessed by alternate brief irradiation with UV (λ = 312 nm) and visible light (>470 nm).

Two diarylethene switches were used (Figure 2) to turn on and off the generation of $^1O_2$. The pyridine unit of *N,N*-dimethyl-4-(5-methyl-4-(2-(2-methyl-5-(pyridin-4-yl)thiophen-3-yl)cyclopent-1-en-1-yl)thiophen-2-yl)aniline (**1**) (Figure 2) can coordinate to ZnTPP. In addition to increasing the effective local concentration[12a] and thereby facilitating energy transfer quenching, coordination can potentially enhance the photochromic response of the dithienylethene unit also as shown previously by Tian and co-workers.[12b] The dimethylamine substituent is incorporated to increase the binding affinity of **1** in the closed form. At the photostationary state ($PSS_{312\ nm}$), 88% of **1** is in the closed form, as determined by ^1H NMR spectroscopy. A second diarylethene switch, 1,2-bis(2′-methyl-5′-phenylthien-3′-yl)cyclopentene (**2**),[12c] was used to evaluate the necessity of coordination to achieve regulation of $^1O_2$ generation.

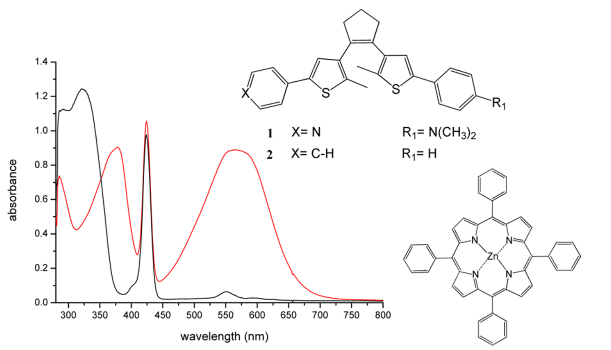

Figure 2. UV/vis absorption spectra of **1** (40 μM) and ZnTPP (2 μM) in air equilibrated toluene at room temperature. The open form of **1** with ZnTPP (black line) and at the $PSS_{312\ nm}$ (red line). For individual spectra see Figure S1 (SOI).

The UV/vis absorption spectrum of ZnTPP undergoes a red shift and decrease in absorption at the Soret band upon addition of the open form of **1** (Figure S1) in air equilibrated toluene indicating coordination. Titration of ZnTPP with the open or closed forms of **1** monitored by UV/vis absorption spectroscopy indicates the formation of 1:1 axially coordinated complexes (Figures S2, S3).[13] The association constants for the open and closed forms are 1.2(±0.3) 10^4 M^{-1} and 1.5(±0.3) 10^4 M^{-1}, respectively.

Irradiation of the open form of **1** mixed with ZnTPP at 312 nm leads to a decrease in absorption between 280 to 350 nm and a concomitant increase in two new bands at 375 and 576 nm (Figure 2, red), which are characteristic of the closed form. The initial spectrum recovered fully upon irradiation with visible light (>470 nm). The presence of ZnTPP does not affect the photoswitching of **1**. Importantly, the Soret band of ZnTPP corresponds to a minimum in absorption for both the open and closed forms of **1** and **2**, minimizing photoinduced changes to the switches upon excitation into the Soret band.

Photosensitized $^1O_2$ generation was monitored directly by near-infrared emission spectroscopy, through the phosphorescence of $^1O_2$ at ca. 1270 nm.[1] The emission spectrum of $^1O_2$ in ZnTPP containing solution is identical in the absence and presence of the open form of **1** (Figure 3, black and Figure S4).

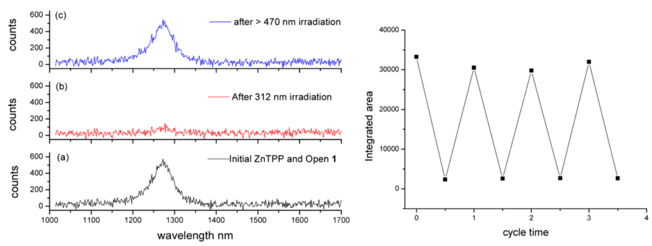

Figure 3. NIR emission spectra of $^1O_2$ generated by ZnTPP (6 μM) in the presence of **1** (30 μM) in air equilibrated toluene; excitation at 405 nm (4 mW). $^1O_2$ generation was observed with the open form of **1** present (left, black) and was switched off (left, red) by brief irradiation at 312 nm. $^1O_2$ generation was recovered by brief irradiation at >470 nm (left, blue). The reversibility of $^1O_2$ generation was monitored through the integrated area of the emission band at 1270 nm over four cycles (right).

Brief irradiation of a solution of ZnTPP and **1** at 312 nm, to generate the closed form of **1**, results in a remarkable decrease (>93%) in the intensity of the $^1O_2$ emission at 1270 nm (Figure 3, red). Further irradiation of the solution with visible light (>470 nm), to convert **1** back to the open form, resulted in the full recovery of $^1O_2$ emission intensity at 1270 nm (Figure 3, blue). The change in emission intensity at 1270 nm (i.e., the integrated area of the emission) was monitored over four cycles of switching between the open and closed form of **1** (Figure 3, right). These changes indicate that the on and off switching of $^1O_2$ generation is reversible and the switching system is stable. Compound **2**, which cannot bind to ZnTPP, shows essentially the same on and off switching behavior of $^1O_2$ generation by ZnTPP (Figure S5).

The change in the intensity of the phosphorescence of $^1O_2$ generated by sensitization by ZnTPP in the presence of the closed form of **1** and of **2** was studied using Stern–Volmer plots (Figure S6). The quenching constant for the closed form of **2** is $(8.2 \pm 0.3) \times 10^9\ M^{-1}\ s^{-1}$, which indicates that the energy transfer is a diffusion controlled process.[14] The nonlinear Stern–Volmer plot for the closed form of **1** yields a dynamic quenching constant under diffusion control of $(8.2 \pm 0.3) \times 10^9\ M^{-1}\ s^{-1}$ and a static quenching constant from the formation of the complex of $(3 \pm 0.3) \times 10^8\ M^{-1}\ s^{-1}$, respectively. The switching on and off of $^1O_2$ generation by ZnTPP in the presence of **1** is also achieved in solvents (Figure S7), such as cyclohexane, tetrahydrofuran, ethanol, and a mixture of ethanol and D_2O (4:1). Furthermore, the fact that compound **1** can control $^1O_2$ generation by free-base tetraphenylporphyrin (H_2TPP) confirms that coordination is not essential to its function (Figure S8).

The effect of the switches in the open and closed states on the fluorescence of ZnTPP was also examined. The emission spectrum of ZnTPP (2 μM) with **1** (40 μM) in toluene was measured with excitation at 405 nm (Figure 4, black). ZnTPP

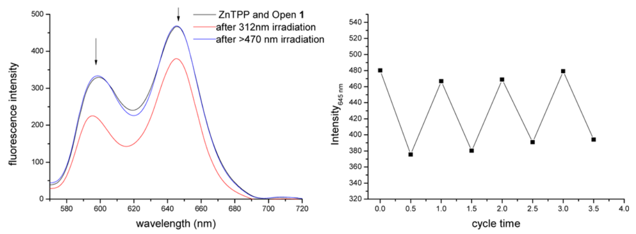

Figure 4. Emission spectra (left) of ZnTPP (2 μM) in the presence of the open form of **1** (40 μM) (black line), after brief irradiation at 312 nm to the PSS (red line), and recovery upon brief irradiation with visible light (>470 nm) (blue line). The reversibility of fluorescence quenching was monitored by the change in emission intensity at 645 nm over four cycles (right).

shows two emission bands with maxima at 596 and 645 nm in toluene, respectively, where neither the open nor closed forms of **1** emit. In the presence of the open form of **1**, the emission maxima of ZnTPP shifts to 598 and 646 nm, respectively, due to the coordination of **1** to the Zn(II) ion. A decrease in fluorescence intensity of ZnTPP was observed when the solution was irradiated briefly at 312 nm (Figure 4, red). The change in intensity at 645 nm was monitored by alternate irradiation at 312 nm and >470 nm over four cycles (Figure 4, right) with good reversibility observed. The ratio of fluorescence intensity between the open form and $PSS_{312\ nm}$ is ca. 1/0.8, which is comparable to that of related systems used to control the fluorescence of zinc−porphyrins.[15] Compound **2** in the open and closed form does not affect the fluorescence of ZnTPP as expected, due to the intermolecular nature of the quenching and the short fluorescence lifetime of ZnTPP (2.0 ns).[16]

Density functional theory calculations of the energies of the lowest singlet and triplet state were performed for compounds **1** and **2** (Table 1). The energy differences between the open

Table 1. Calculated Energies of the Switches and ZnTPP

	ZnTPP[17a]	open **1**	closed **1**	open **2**	closed **2**
S_1 (eV)	2.30	3.64	2.30	3.74	2.41
T_1 (eV)	1.61	2.89	1.23	2.98	1.30

and closed forms of the switches at the lowest singlet and triplet excited states are relatively large, which provides the possibility to switch on and off energy transfer with a photosensitizer that has a singlet and/or triplet energy lying within the gap. Considering the first triplet energy of ZnTPP (1.61 eV)[17a] and H_2TPP (1.43 eV),[17b] respectively, energy transfer can only occur from ZnTPP or H_2TPP to the closed form of **1** (1.23 eV), but not to the open form (2.89 eV). The lowest triplet excited state of the closed switches (1.23 eV for closed **1** and 1.30 eV for closed **2**, respectively) was calculated to be higher than the lowest excited state of $^3O_2$ (0.97 eV),[1] which makes the pathway competitive with energy transfer to $^3O_2$ and results in effectively switching off the generation of $^1O_2$. The first singlet excited state of ZnTPP (2.30 eV) is also close to the closed form of **1** (calculated as 2.30 eV), but not to the open form (calculated as 3.64 eV), and hence Förster resonance energy transfer (FRET) is allowed if the ZnTPP and the switches are in sufficient proximity.

Energy transfer between the triplet state of ZnTPP and the closed form of **1**, but not the open form, was further confirmed by transient absorption spectroscopy by monitoring the change in absorbance at 460 nm upon ns-pulsed laser excitation. ZnTPP has a triplet life of ca. 30 μs (Figure S9),[18] even in the presence of the open form of **1** (Figure 5, left). When mixed

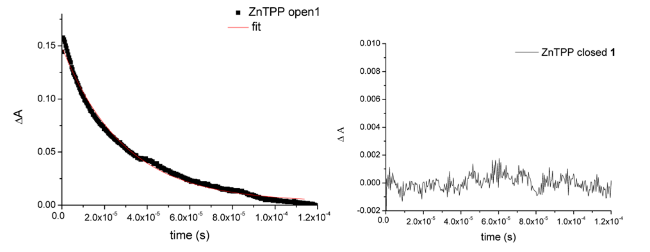

Figure 5. Transient absorption at 460 nm of ZnTPP (100 μM) mixed with the open form of **1** (100 μM) (left), and ZnTPP (40 μM) mixed with the closed form of **1** (80 μM) (right) in toluene. All samples were prepared in an argon atmosphere by at least three freeze−pump−thaw cycles.

with the closed form of **1**, the lifetime of ^{3}ZnTPP* is reduced to <0.5 μs (Figure 5, right), which indicates efficient triplet energy transfer between ZnTPP and the closed form of **1**.

In summary, the present system shows the potential for efficiently and reversibly switching on and off $^1O_2$ generation by irradiation at distinct wavelengths without affecting the photochemical performance of the diarylethenes. Both coordinating and noncoordinating systems show similar efficiency in controlling $^1O_2$ generation; however, in the case of the coordinating system, control over the fluorescence of ZnTPP can be achieved simultaneously. The large difference in excited state energies between the open and closed forms of the diarylethenes is central to control over $^1O_2$ generation, and fine-tuning of the efficiency can therefore be achieved by variation of the substituents on the dithienylethene unit to optimize the selectivity that can be achieved in addressing each component. As a final remark it should be noted that the concentrations employed in the present study are within range of normal therapeutic doses, in particular in topical application, and hence future efforts will be directed toward developing water-soluble diarylethene switches that will enable noninvasive control over $^1O_2$ generation for application in PDT. Ultimately, the use of one- and two-photon excitation with NIR light[19] for both photochemical switching of the dithienylethene unit and the generation of singlet oxygen will enable the application of this approach in highly localized deep tissue treatments.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, synthetic procedures and characterization data, spectroscopic data, and computational details are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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