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Singlet Oxygen Generation via Two-Photon Excited FRET

William R. Dichtel,[†] Jason M. Serin,[†] Carine Edder,[†] Jean M. J. Fréchet,*[,†] Michael Matuszewski,[§] Loon-Seng Tan,[§] Tymish Y. Ohulchanskyy,[‡] and Paras N. Prasad[‡]

Department of Chemistry, University of California, Berkeley, California 94720-1460, Institute for Lasers, Photonics and Biophotonics and Department of Chemistry, State University of New York, Buffalo, New York 14260-3000, and Polymer Branch, Air Force Research Laboratory/MLBP, 2941 P Street, Suite 136, Area B, Building 654, Wright-Patterson AFB, Ohio 45433-7750

Received December 11, 2003; E-mail: frechet@cchem.berkeley.edu

Uphill energy conversion through the use of two-photon absorbing chromophores and subsequent energy transfer is an attractive scientific frontier. Among many possible targets, compounds capable of generating singlet oxygen following efficient two-photon absorption (TPA)[1−5] could broaden the applicability of singlet oxygen generation. Porphyrin sensitizers have low TPA cross sections,[6] limiting their usefulness in two-photon absorption applications. This limitation has stimulated interest in the discovery of porphyrins with enhanced TPA cross sections through chemical modification of the porphyrin chromophore.[7,8]

Presented here is a new approach, in which donor chromophores capable of efficient TPA are covalently attached to a central porphyrin acceptor. After TPA, the donors transfer their excited-state energy to the porphyrin via fluorescence resonance energy transfer (FRET),[9−11] where intersystem crossing (ISC) and singlet oxygen generation occur. Because the donor chromophores are chosen for their high TPA cross section (8100 GM, 1 GM = 10^{-50} cm^4 s),[12] this approach has the potential to enhance the effective TPA cross section of the porphyrin well beyond what is available by direct modification. Another advantage of this approach is its versatility; other donor chromophores or functional groups (such as peripheral solubilizing hydrophilic moieties) can be incorporated into future designs without interfering with the desirable properties of the central porphyrin acceptor.

The target porphyrin (**1**, Figure 1), containing eight AF-343 two-photon absorbing donor chromophores[12] in a multivalent dendritic configuration,[13] was synthesized from tetrakis(3′,5′-dihydroxyphenyl)porphyrin[14] and AF-343 derivative (**2**)[12] in the presence of K_2CO_3 in DMF. The presence of all eight donor chromophores in compound **1** was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as well as by NMR. Compound **1** is soluble in most organic solvents but can be purified by precipitation from an ethyl acetate/hexanes mixture and is well-behaved in silica gel chromatography.

Prior to performing photophysical measurements, preparative TLC purifications were performed on **1**, **2**, and porphyrin model compound tetrakis(3′,5′-dimethoxyphenyl)porphyrin[15] (**3**) to ensure their purity. Figure 2 shows the steady-state absorption spectra of **1**, **2**, and **3**. The absorption spectrum of **1** shows the characteristic peaks at 308 and 390 nm of compound **2** in addition to the porphyrin Soret band at 421 nm and less intense Q-bands, the first of which appear at 513 nm.

The emission spectra of **1**, **2**, and **3** shown in Figure 3 confirm that the AF-343 donor transfers energy with high efficiency to the porphyrin acceptor. Excitation of **2** at 385 nm results in the expected emission from the AF-343 chromophore with a λ_{max} at 492 nm.

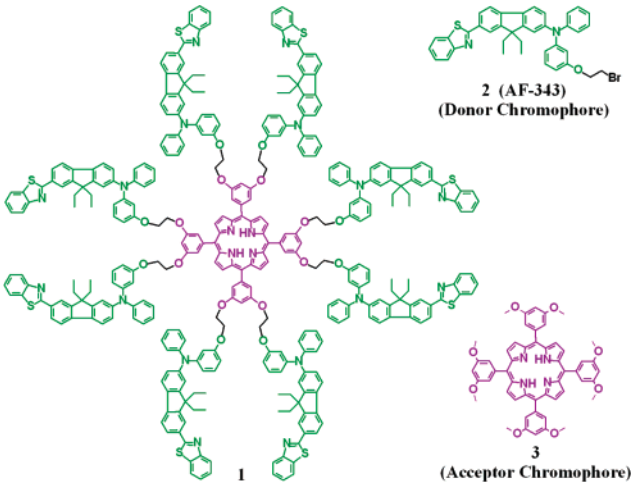

Figure 1. Molecular structure of the target system (**1**), the component AF-343 donor chromophore (**2**), and a porphyrin model compound (**3**).

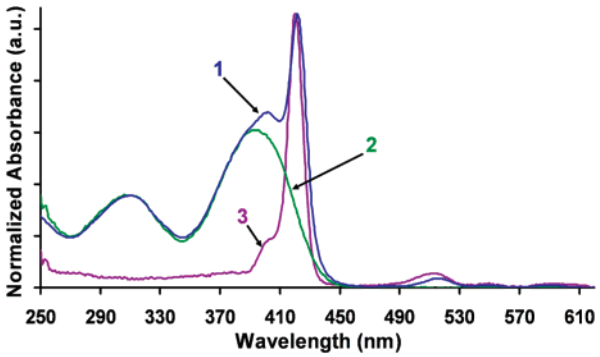

Figure 2. Absorption spectra of **1**, **2**, and **3** in chloroform at room temperature. The spectrum of **2** is normalized to the spectrum of **1** at 385 nm, while the height of the Soret band at 420 nm in the spectrum of **3** is normalized to the height of the Soret band of **1**.

The emission of **2** shows spectral overlap with the absorption of **3** (Figure 2), which is necessary for efficient FRET. When **1** is excited at the same wavelength, the AF-343 emission is almost completely quenched, and emission is seen predominantly from the porphyrin, corresponding to a calculated energy transfer efficiency of 97%.[16] Compound **1** shows an enhanced emission when the porphyrin is excited directly at 424 nm, but this is due to the higher molar absorptivity of **1** at 424 nm than at 385 nm and is not evidence of inefficient energy transfer.

With the knowledge that the AF-343 chromophore is capable of efficient energy transfer to the porphyrin, the emission spectra of compounds **1** and **3** were measured using a 780 nm fs mode locked Ti:Sapphire laser (Spectra-Physics Tsunami) as the excitation source

[†] University of California.
[‡] State University of New York.
[§] Wright-Patterson AFB.

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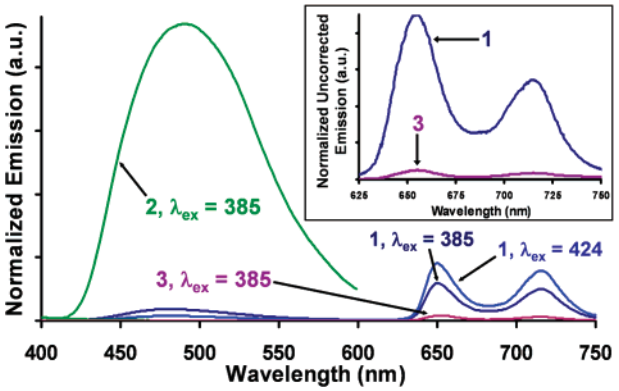

Figure 3. Fluorescence spectra of **1**, **2**, and **3** in chloroform at room temperature under single photon excitation conditions normalized to the absorption spectra (Figure 2). A near complete quenching of the donor emission is observed upon excitation of **1** at 385 nm, resulting in a significant increase in porphyrin emission from **1** relative to the porphyrin chromophore alone (**3**) excited at the same wavelength. Inset: Normalized emission of **1** and **3** upon laser excitation (780 nm) of both compounds in benzene-d_6 at room temperature.

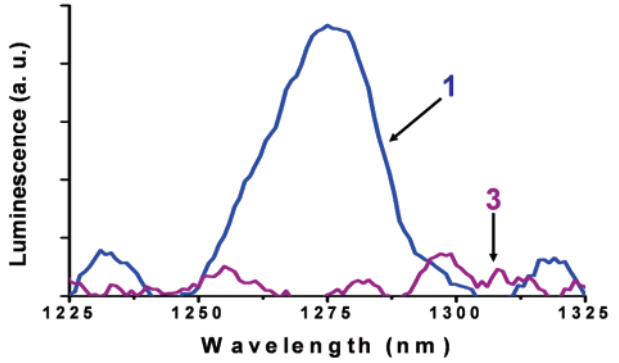

Figure 4. Oxygen luminescence spectra obtained from oxygen-saturated solutions of **1** and **3** in benzene-d_6 irradiated with a 780 nm laser. Oxygen luminescence is observed from the solution containing **1**, but the detection conditions were not sensitive enough to observe a luminescence signal from solutions of **3**. Baseline correction and Savitsky−Golay smoothing have been applied to each spectrum.

(Figure 3, inset). Under these conditions, emission from **1** is 17 times more intense than the emission from **3**, as measured by integrating each signal from 600 to 755 nm. This result affirms the design of compound **1**; the TPA of the donor chromophores can be utilized to efficiently generate porphyrin excited states.

The ability of **1** to generate singlet oxygen following TPA was evaluated by observing the characteristic luminescence of singlet oxygen at 1270 nm[17] using a SPEX 270M spectrometer (Jobin Yvon) equipped with InGaAs photodetector (Electrooptical Systems) (Figure 4). Oxygen-saturated solutions of **1** showed a measurable luminescence signal upon excitation with the 780 nm laser. No detectable oxygen luminescence was observed from solutions of **3** under the same conditions. The signal observed from compound **1** is direct evidence that the presence of the AF-343 chromophores dramatically enhances the ability of the porphyrin to generate singlet oxygen when irradiated with 780 nm light.

Compound **1** demonstrates that the incorporation of a dendritic array of suitable donor chromophores can enhance the effective TPA cross section of a porphyrin acceptor, allowing more efficient generation of singlet oxygen using 780 nm light. This could increase the generality of photodynamic therapy to tumors below the skin surface. Further enhancement of this effect and the synthesis of more biologically relevant sensitizers are currently underway.

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Supporting Information Available: Experimental details and chemical characterization data of compound **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bhawalkar, J. D.; Kumar, N. D.; Zhao, C. F. a. P. P. N. *J. Clin. Laser Med. Surg.* **1997**, *15*, 201.
(2) Fisher, W. G.; Partridge, W. P., Jr.; Dees, C.; Wachter, E. A. *Photochem. Photobiol.* **1997**, *66*, 141.
(3) Frederiksen, P. K.; Jørgensen, M.; Ogilby, P. R. *J. Am. Chem. Soc.* **2001**, *123*, 1215.
(4) Karotki, A.; Kruk, M.; Drobizhev, M.; Rebane, A.; Nickel, E.; Spangler, C. W. *IEEE J. Sel. Top. Quantum Electron.* **2001**, *7*, 971.
(5) Poulsen, T. D.; Frederiksen, P. K.; Jørgensen, M.; Mikkelsen, K. V.; Ogilby, P. R. *J. Phys. Chem. A* **2001**, *105*, 11488.
(6) Kruk, M.; Karotki, A.; Drobizhev, M.; Kuzmitsky, V.; Gael, V.; Rebane, A. *J. Lumin.* **2003**, *105*, 45.
(7) Drobizhev, M.; Karotki, A.; Kruk, M.; Mamardashvili, N. Z.; Rebane, A. *Chem. Phys. Lett.* **2002**, *361*, 504.
(8) Karotki, A.; Drobizhev, M.; Kruk, M.; Spangler, C.; Nickel, E.; Mamardashvili, N.; Rebane, A. *J. Opt. Soc. Am. B* **2003**, *20*, 321.
(9) Förster, T. *Ann. Phys.* **1948**, *2*, 55.
(10) Förster, T. *Z. Naturforsch., A.* **1949**, *4*, 321.
(11) Weib van der Meer, B.; Coker, G., III.; Simon, S. Y. *Resonance Energy Transfer, Theory and Data*; VCH: Weinheim, 1994.
(12) Tan, L.-S.; Kannan, R.; Matuszewski, M. J.; Khur, I. J.; Feld, W. A.; Dang, T. D.; Dombroskie, A. G.; Vaia, R. A.; Clarson, S. J.; He, G. S.; Lin, T.-C.; Prasad, P. N. *Proc. SPIE* **2003**, *4797*, 171.
(13) (a) Brousmiche, D.; Serin, J. M.; Fréchet, J. M. J.; He, G. S.; Lin, T. C.; Chung, S. J.; Prasad, P. N. *J. Am. Chem. Soc.* **2003**, *125*, 1448. (b) Adronov, A.; Fréchet, J. M. J.; He, G. S.; Kim, K. S.; Chung, S. J.; Swiatkiewicz, J.; Prasad, P. N. *Chem Mater.* **2000**, *12*, 2838.
(14) James, D. A.; Arnold, D. P.; Parsons, P. G. *J. Photochem. Photobiol.* **1994**, *59*, 441.
(15) Rocha Gonsalves, A. M. d.; Varejao, J. M. T. B.; Pereira, M. M. *J. Heterocycl. Chem.* **1991**, *28*, 635.
(16) Gilat, S. L.; Adronov, A.; Fréchet, J. M. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1422.
(17) Roy, I.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Bergey, E. J.; Oseroff, A. R.; Morgan, J.; Dougherty, T. J.; Prasad, P. N. *J. Am. Chem. Soc.* **2003**, *125*, 7860.

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