

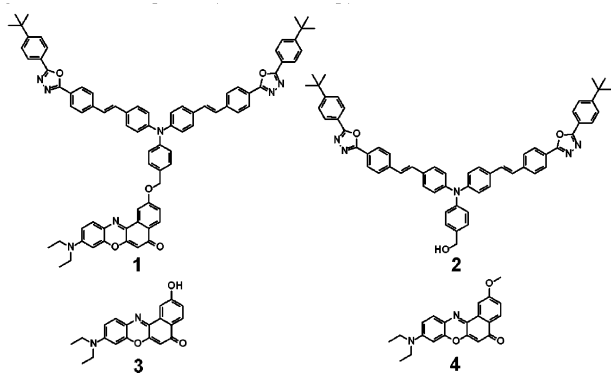
## Fluorescence Resonance Energy Transfer in a Novel Two-Photon Absorbing System

Darryl W. Brousmiche,<sup>†</sup> Jason M. Serin,<sup>†</sup> Jean M. J. Fréchet,<sup>\*,†</sup> Guang S. He,<sup>‡</sup> Tzu-Chau Lin,<sup>‡</sup> Sung Jae Chung,<sup>‡</sup> and Paras N. Prasad<sup>\*,‡</sup>

Department of Chemistry, University of California, Berkeley, California 94720-1460, and Institute for Lasers, Photonics and Biophotonics, State University of New York at Buffalo, Buffalo, New York 14260-3000

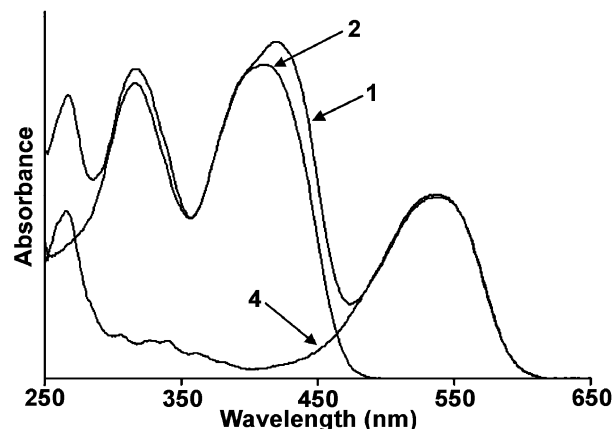
Received October 8, 2002; E-mail: frechet@cchem.berkeley.edu and pnprasad@acsu.buffalo.edu

A novel bichromophoric system (**1**) has been synthesized, which undergoes near quantitative, nonradiative fluorescence resonance energy transfer (FRET) from a two-photon absorbing chromophore to a Nile red derivative when excited by either high-intensity IR (two-photon) or UV–visible (single-photon) radiation. Through FRET, the two-photon absorbing moiety in **1** effectively imparts a large two-photon absorbing (TPA) cross-section to the Nile red acceptor and permits its excitation upon irradiation of **1** with high-intensity IR light. Unlike previous systems,<sup>1</sup> where both donor and acceptor have large TPA cross-sections, this value is small for the Nile red chromophore alone. Utilizing two-photon excitation in combination with efficient FRET in this manner provides an effective method by which IR radiation can be used to activate molecules that absorb in the UV–visible and have little or no two-photon absorptivity in the IR. This novel approach could find applications in biomedical imaging,<sup>2</sup> two-photon pumped lasing<sup>3</sup> and optical power-limiting.<sup>4</sup> Two-photon absorbing systems have also been used in such diverse areas as 3-D optical data storage,<sup>5</sup> photoacid generation,<sup>6</sup> and photodynamic therapy.<sup>7</sup>



In addition to two strong single-photon absorptions at 320 and 410 nm, the donor chromophore **2** has a large TPA cross-section in the 800-nm region (6700 GM ( $\pm 15\%$ ); 1 GM =  $10^{-50}$  cm<sup>4</sup>·s), thereby enabling absorption of high-intensity light in this spectral range.<sup>8</sup> Our labs have previously incorporated **2** into the periphery of a series of poly(aryl ether) dendrimers in an effort to design molecules with large TPA cross-sections by increasing the chromophore density.<sup>9</sup> The Nile red derivative **3**<sup>10</sup> was chosen as the acceptor chromophore due to the favorable overlap of its absorbance spectrum with the emission spectrum of **2**. It is readily functionalized, due to the presence of the phenol group and has been used previously as a label for biomolecules.<sup>11</sup>

Synthesis of the target compound **1** was accomplished through a Mitsunobu coupling of **2** and **3** in THF. Compound **4** was also



**Figure 1.** Linear steady-state absorption spectra of **1**, **2**, and **4** at rt in CHCl<sub>3</sub> normalized to the  $\lambda_{\text{ex}}$  used in emission studies (405 and 540 nm).

synthesized, via reaction of **3** with CH<sub>3</sub>I in basic DMSO (NaOH), to model the behavior of the Nile red moiety of **1** in the two-photon excitation experiments. Unlike **3**, both **1** and **4** are highly soluble in common organic solvents. All compounds were purified extensively using preparatory TLC immediately prior to photophysical analysis. The linear absorption spectra of **1**, **2**, and **4** in CHCl<sub>3</sub> are shown in Figure 1. The spectrum of **1** is a composite of the model compounds **2** and **4**, with the bands at 320 and 405 arising from the two-photon absorbing chromophore, and the bands at 270 and 540 arising from the Nile red moiety.

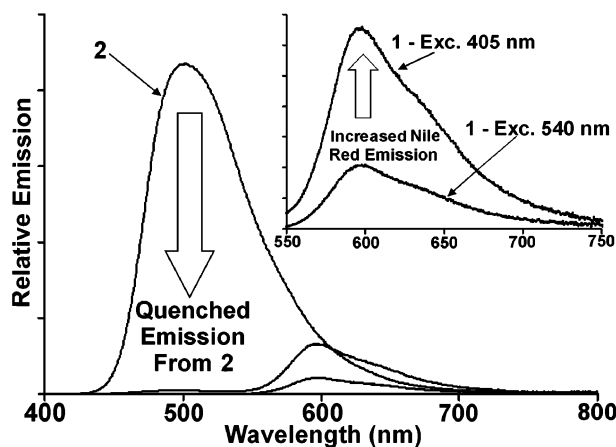
The single-photon induced emission spectra of **1** and **2** in chloroform are illustrated in Figure 2 (normalized for excitation at 405 nm,  $\sim 1.7 \times 10^{-6}$  M). Irradiation of **1** at 405 nm resulted in emission mainly from the Nile red chromophore (595 nm), with <1% of the emission at 510 nm compared to irradiation of **2** at the same wavelength, indicating very efficient energy transfer<sup>12</sup> (>99%). Excitation of **1** at 405 nm results in a  $\sim 3.4$ -fold increase in the area of emission from the Nile red moiety, compared to its direct excitation (540 nm). This “antenna effect” is the result of the larger extinction coefficient of **1** at 405 nm compared to 540 nm. Similar results are obtained in CH<sub>3</sub>CN and THF.

Studies of **1** at  $1.7 \times 10^{-7}$  M show the same energy-transfer efficiency and antenna effect as at higher concentrations, eliminating the possibility of radiative (trivial) energy transfer. Dexter-type (through-bond) energy transfer has been discounted in similar systems<sup>13</sup> and is ruled out in **1**, due to the methylene spacer between the two chromophores. Thus, the attenuation of fluorescence at 510 nm from **1** compared to **2** is believed to result from a nonradiative, Förster-type FRET from the two-photon absorbing chromophore to the Nile red moiety.

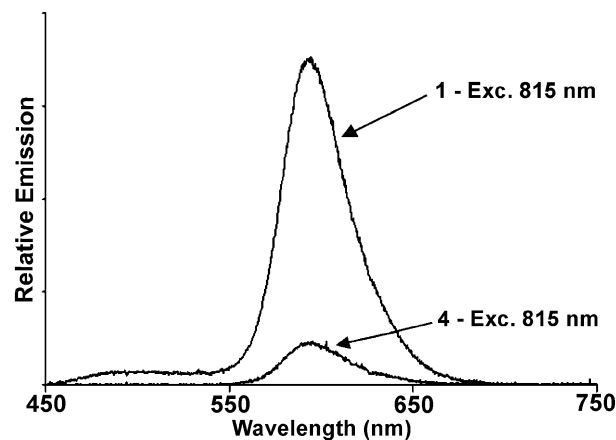
The two-photon induced fluorescence spectra (815 nm laser excitation, 8 ns pulse) of **1** and **4** in THF are shown in Figure 3

<sup>†</sup> University of California, Berkeley.

<sup>‡</sup> State University of New York at Buffalo.



**Figure 2.** Single-photon induced emission spectra of **1** ( $\lambda_{\text{ex}}$  405 and 540 nm) and **2** ( $\lambda_{\text{ex}}$  = 405 nm) at rt in  $\text{CHCl}_3$  (the absorption of **2** is matched to that of **1** at 405 nm to allow comparison). Emission at 510 nm is quenched (>99%) upon excitation of **1** at 405 nm compared to excitation of **2** at 405 nm. (Inset) Expansion of the 550–750 nm region showing the  $\sim 3.4$ -fold increase in Nile red emission arising from excitation of **1** at 405 nm compared to direct excitation at 540 nm.



**Figure 3.** Two-photon induced fluorescence spectra of **1** and **4** at rt in THF (0.001 M,  $\lambda_{\text{ex}}$  = 815 nm, 8-ns laser pulse). Emission at 510 nm is effectively quenched by energy transfer to the Nile red chromophore upon excitation of **1**. This results in an 8-fold increase in Nile red emission compared to excitation of **4** at the same wavelength.

(0.001 M solutions). Irradiation of **1** shows near quantitative FRET from the two photon absorbing chromophore to the Nile red chromophore, as evidenced by the limited emission at 510 nm. Changing the local laser intensity by varying sample position ( $z$ -scan) results in changes in emission intensity (at 595 nm) that follow a quadratic dependence, indicating that the emission from compounds **1** and **4** is due to two-photon excitation. The 8-fold increase in emission at 595 nm from **1**, compared to excitation of **4** at the same wavelength, represents a significant antenna effect. This arises from the much higher two-photon absorbing cross-section of **2** (6700 GM ( $\pm 15\%$ )) at 815 nm in the nanosecond regime, compared to the Nile red chromophore (750 GM ( $\pm 15\%$ )) and indicates that the majority of the emission observed from **1** upon IR laser irradiation can be attributed to absorption and FRET from the two-photon absorbing chromophore. Although reabsorption is not negligible due to the high concentrations used, the possibility of a

radiative energy-transfer mechanism can be excluded by dynamic fluorescence measurements. Emission at 595 nm from **1** displays a fast single-exponential decay with a subnanosecond lifetime that is evidence of a nonradiative energy-transfer mechanism. According to the convolution analysis, a nonexponential temporal behavior with a slow rise followed by a much slower decay process would be observed, if radiative energy transfer were the major mechanism.

This work presents a novel bichromophoric system that is capable of undergoing efficient FRET following either two-photon (IR laser) or single-photon (UV–visible) excitation. The enhanced emission from the Nile red moiety upon excitation of **1** at either 405 or 815 nm (by laser) has potential uses in a number of applications. Through the use of FRET, this system shows that it is possible to utilize high-intensity IR light to activate molecules that, by themselves, have little or no two-photon absorptivity in the IR range.

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**Supporting Information Available:** Experimental details and chemical characterization data for **1** and **4** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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