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Two-Photon Absorbing Block Copolymer as a Nanocarrier for Porphyrin: Energy Transfer and Singlet Oxygen Generation in Micellar Aqueous Solution

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Porphyrin-based derivatives are widely used as photosensitizers for singlet oxygen ($^1O_2$) generation, which is a key step for photodynamic therapy.[1] However, most porphyrins absorb light in the visible region and have relatively low two-photon absorption (2PA) cross-sections (δ: 1−10 GM; 1 GM = 10^{-50} cm^4 s photon^{-1}).[2] These characteristics limit their effectiveness when excited with a near-infrared (NIR) light source (ranging from 700 to 1000 nm), which would allow deeper penetration and less damage to biological tissues.[3] Direct chemical modification of porphyrins by preparing porphyrin dimers and π-conjugated porphyrins[4] is a commonly used approach to solve these problems. Alternatively, the utilization of fluorescence resonance energy transfer (FRET)[5] in light harvesting dendrimers[6,7] and silica nanoparticles[8] is also quite effective. In these dendrimers, the peripheral donors possess a relatively large two-photon cross-section that can efficiently absorb NIR light and transfer the energy to the porphyrin core. As a result, these porphyrin-containing dendrimers could be used to efficiently generate $^1O_2$.[6]

Herein, we report the use of a new amphiphilic 2PA-chromophore-containing block copolymer to enhance the efficiency of $^1O_2$ generation. This is realized by using the block copolymer (**P1**, Figure 1) as a nanocarrier to encapsulate a hydrophobic porphyrin photosensitizer (**1**, Figure 1) inside its micelles. In aqueous solution, **P1** forms micelles with the hydrophilic poly(ethylene glycol) (PEG) as the corona to ensure good water solubility. The 2PA-chromophore-containing hydrophobic core provides a good environment to accommodate the hydrophobic **1**. Due to the size confinement in these micelles, efficient FRET from the 2PA chromophore to porphyrin occurs, which enhances the effective 2PA cross-sections of the porphyrin.

This approach combines several advantages: (1) numerous photosensitizers can be easily incorporated into an aqueous system without encountering tedious chemical modifications; (2) biocompatible 2PA block copolymers can be used as nanocarriers for bioapplications; and (3) the FRET through noncovalently bonded donors and acceptors within micelles enhances the efficiency of $^1O_2$ generation under NIR light irradiation.

The structures of two block copolymers synthesized (**P1** and **P2**) are shown in Figure 1. In order to reduce chromophore aggregation within the micelles, the 2PA monomer (**M**) was copolymerized with styrene to produce **P1**.[9] The aryl amino-containing donors were connected with a fluorene bridge through triple bonds to enhance its photostability.[10] To differentiate the contribution of FRET to $^1O_2$ generation, **P2** without the 2PA chromophore was also synthesized for comparison.

Micelles were prepared in aqueous solution using the typical dialysis method and were characterized by dynamic light scattering (DLS) and atomic force microscopy (AFM). The average diameters of the micelles are listed in Table 1.

The fluorescence spectrum of the 2PA chromophore overlaps with both the Soret and Q-band absorptions of **1** (see Supporting
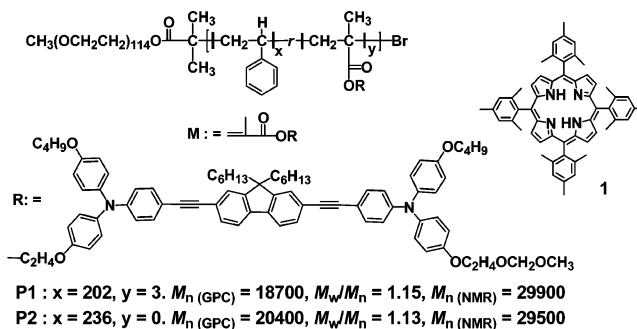

Figure 1. The chemical structures of **P1**, **P2**, and **1**.

P1 : x = 202, y = 3. $M_{n\,(GPC)}$ = 18700, M_w/M_n = 1.15, $M_{n\,(NMR)}$ = 29900
P2 : x = 236, y = 0. $M_{n\,(GPC)}$ = 20400, M_w/M_n = 1.13, $M_{n\,(NMR)}$ = 29500

Table 1. Typical Physical Properties of **P1** and **P2**

systems	ratio[a]	ET (%)		AM[d]	Average sizes of the micelles	
		1PF[b]	2PF[c]		DLS[f]	AFM[g]
P1	0			0	59 (0.14)	53 ± 11
1/P1	0.1	47	50	e	66 (0.14)	50 ± 12
1/P1	0.2	65	71	2.7	67 (0.13)	48 ± 10
1/P1	0.6	86	89	6.5	70 (0.14)	52 ± 11
1/P1	1.0	91	96	4.4	73 (0.14)	52 ± 15
1/P2	1.0				77 (0.18)	54 ± 14

[a] Molar ratio of **1** to **P1** or **P2** determined by UV−vis spectra. *[b]* Energy transfer efficiency irradiated at 380 nm. *[c]* Energy transfer efficiency excited at 800 nm. *[d]* Amplification using **P2** as counterpart excited at 800 nm. Integrated emission intensity of **1** was collected from 625 to 750 nm. *[e]* The signal is too weak to detect. *[f]* Determined by dynamic light scattering in solution at 25 °C. The data in the parentheses are the polydispersities. *[g]* Observed by AFM using dried sample on mica surface.

Information for details), indicating that efficient FRET can occur from the 2PA chromophore to **1**. Sensitizer **1** is insoluble and does not fluoresce in an aqueous solution. However, once it was encapsulated within a micelle, the aqueous solution began to fluoresce. For this study, the concentration of **P1** was kept constant (0.075 mg/mL, which corresponds to a 2PA chromophore concentration of 7.5 μM). When the concentration of **1** in **P1** (simplified as **1/P1**) was increased, the fluorescence intensity of the 2PA chromophore decreases (Figure 2A). The energy transfer efficiency can be calculated to be as high as 91%.[11] This indicates that effective FRET indeed occurs within these micelles.[12] It also proves that the nanoconfinement provided by the 2PA block copolymer helps efficiently generate the porphyrin excited state through energy transfer.

P1 in aqueous solution exhibits 2PA cross-sections of 288 GM at 750 nm and 120 GM at 800 nm when excited with a femtosecond mode lock Ti-sapphire laser. Although these values are moderate compared to other values reported in the literature,[13] they are still much higher than those from the porphyrins alone. The energy transfer efficiencies of **1/P1** excited at 800 nm are similar to those excited at 380 nm (Table 1). Furthermore, the emissions of **1** in **1/P1** through FRET are 2.7−6.5[14] times higher than the emissions
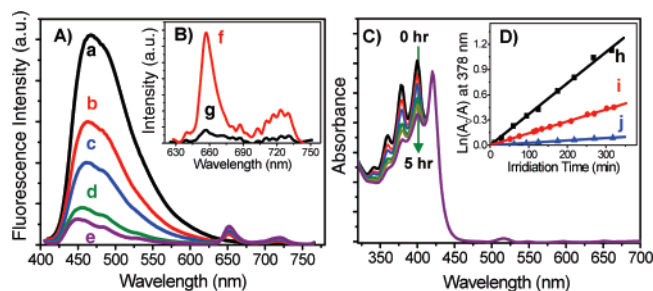
Figure 2. (A) The fluorescence spectra of **1** in **P1** with various loading ratio (**1**/**P1** in mole: 0, 0.1, 0.2, 0.6, and 1 for curves of a, b, c, d, and e) under the illumination of 380 nm in water. (B) The two-photon-induced emission of **1** in **P1** (f) and in **P2** (g) with the ratio of 1/1 irradiated at 800 nm. (C) The typical photochemical process of **1**/**P1**/ADPA under the excitation of 800 nm in D$_2$O. The absorbance of **1** at 420 and 515 nm has no change, indicating the stability of **1** under the experimental condition. (D) The first-order plots for the photooxidation of ADPA in **1**/**P1**/ADPA (curve h), **1**/**P2**/ADPA (curve i), and **P1**/ADPA (curve j) against irradiation time. A_0 is the absorbance at 378 nm before irradiation. A is the absorbance after irradiation. The ratio of **1** to **P1** or **P2** in mole is 0.6/1

of **1** in the **1**/**P2** systems because no 2PA-chromophore-enhanced FRET was involved in **1**/**P2** (Table 1 and Figure 2B). This confirms that efficient FRET only occurs with the aid of 2PA chromophores.

The ability of the system to generate singlet oxygen from porphyrin was evaluated using the disodium salt of 9,10-anthracenedipropionic acid (ADPA) as a water-soluble $^1O_2$ sensor.[15] The generated $^1O_2$ can react with ADPA which results in bleaching ADPA to its corresponding endoperoxide. The progress of the reaction could be monitored by observing the decrease of the ADPA absorption intensity at 378 nm when pumped with an 800 nm light. For comparison, three different oxygen-saturated aqueous solutions[16] (**1**/**P1**/ADPA, **1**/**P2**/ADPA, **P1**/ADPA) with the same concentration of ADPA and/or **1** were studied. Without **1**, almost no ADPA absorption intensity change was observed throughout the time (curve j), indicating that bleaching of ADPA by the irradiating light itself was negligible. A noticeable time-dependent decrease in the ADPA absorbance in mixtures containing **1** was observed (Figure 2C). This observation shows both the generation of $^1O_2$ and its subsequent diffusion out of micelles. The values of the observed rate constants of **1**/**P2**/ADPA and **1**/**P1**/ADPA are $(1.41 \pm 0.2) \times 10^{-3}$ and $(3.69 \pm 0.3) \times 10^{-3}$ min^{-1}, respectively. Thus, the bleaching rate of the system with the 2PA chromophore is 2.6-fold faster than that without the 2PA chromophore. This confirms that the FRET through the 2PA chromophore contributes significantly to the $^1O_2$ generation.

In conclusion, a novel water-soluble amphiphilic 2PA-chromophore-containing block copolymer was prepared. Hydrophobic porphyrin was incorporated into an aqueous solution with the assistance of micelles. Efficient energy transfer (as high as 96%) from the 2PA chromophore to porphyrin was observed in micellar aqueous solutions under the 2PA conditions. Furthermore, the efficient energy transfer contributes to effective singlet oxygen generation (by a factor of 2.6 at 800 nm) from the porphyrin guest molecules. We have developed a new strategy to use covalently bonded donors to trap acceptors within an ideal nanoenvironment for improving the efficiency of singlet oxygen generation under NIR light irradiation in aqueous solution. This advancement may generate further interest in developing efficient two-photon block copolymers for bioapplications.

Supporting Information Available: Syntheses and experimental details, UV−vis spectra, fluorescence spectra, DLS and AFM images (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The energy transfer efficiency was determined by studying the quenching of the donor fluorescence from 410 to 620 nm in the presence of the acceptor at the same excitation wavelength (380 nm).

(12) It should be noted that the slight quenching effect of **1** to **P1** (3, 5, and 10% for **1**/**P1** with the molar ratios of 0.1/1, 0.6/1, and 1/1, respectively) was observed in their THF mixtures without micellar structures, possibly due to charge or electron transfer. However, these quenching effects were much smaller than those in the micelles. Thus, we believe that the size confinement in micelles may facilitate the requirement of the distance between the donor and acceptor (less than 10 nm) for FRET, though we did not calculate the exact distance between the donor and acceptor in micellar centers.

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(14) The amplification of **1**/**P1** determined by using **1**/**P2** as counterpart is affected by two factors: energy transfer efficiency and concentration of **1** within the micelles. The large amplification is observed in the molar ratio of 0.6/1 due to the fact that it has similar energy transfer efficiency but less aggregation of **1** within the micelles compared to that of a molecular ratio of 1/1.

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(16) These experiments were performed by adding 25 μL of ADPA (2 mg/mL) into 2.5 mL of the micellar solutions of **P1**, **1**/**P1**, and **1**/**P2** with the same concentration of block copolymer and/or **1** at room temperature. Here, D$_2$O was used to replace H$_2$O due to the concern of the much longer lifetime of the $^1O_2$ in D$_2$O. The solution was bubbled with oxygen for 5 min to get an oxygen-saturated aqueous solution.

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