

Hydrophobic Chromophores in Aqueous Micellar Solution Showing Large Two-Photon Absorption Cross Sections**

By Yanqing Tian, Ching-Yi Chen, Yen-Ju Cheng, A. Cody Young, Neil M. Tucker, and Alex K.-Y. Jen*

A series of new hydrophobic two-photon absorbing (2PA) chromophores with varied electron-donating groups in quasi-linear and multibranched structures are synthesized to correlate their structure/photophysical property relationships. The feasibility of using these large two-photon absorption cross-sectional (δ , expressed in $\text{GM} = 1 \times 10^{-50} \text{ cm}^4 \text{ s photon}^{-1} \text{ molecule}^{-1}$) materials in aqueous solution is also explored. All four hydrophobic 2PA materials can be encapsulated into micelles generated by dispersing an amphiphilic block copolymer, poly(methacrylic acid)-*block*-polystyrene (PMAA-*b*-PS), into water. The micellar nanostructures are characterized using dynamic light scattering, atomic force microscopy, and transmission electron microscopy. After these dyes are incorporated into micelles, they exhibit strong fluorescence in water. It is found that the quantum yield and δ values of these chromophores are strongly dependent on the diameters of the micelles, concentrations of the PMAA-*b*-PS, and molecular structures of the 2PA chromophores. One of the compounds that has a strong triaryl amino donor and a multibranched structure exhibits a large δ value of 2790 GM and high quantum yield (0.56) in micelle-containing water. Although this value is smaller than the original value of 5300 GM in toluene, it is still substantially larger than the values of most water-soluble 2PA materials, which have δ values of less than 100 GM.

1. Introduction

Over the past few years, intensive studies have been focused on developing two-photon absorbing (2PA) materials that can be excited in the near IR region (700–1000 nm) because of their potential applications in frequency upconversion lasing, high-density optical storage, two-photon fluorescence microscopy (TPM), and photodynamic therapy.^[1] Until now, most of the 2PA materials with large 2PA cross sections (δ , expressed in $\text{GM} = 1 \times 10^{-50} \text{ cm}^4 \text{ s photon}^{-1} \text{ molecule}^{-1}$) that have been developed so far are based on the molecular motifs of either linear or quasi-linear D- π -D, D- π -A- π -D, A- π -D- π -A conjugates (D: electron-donating group, A: electron-accepting group, and π : conjugation pathway). Upon excitation, a substantial sym-

metric intramolecular charge redistribution takes place in these molecules, resulting in high 2PA cross sections.^[2] Although many of these 2PA materials have δ value greater than 2000 GM,^[3] their poor solubility in water prevents them from being used in biological applications.

For biological imaging, TPM represents a noninvasive method that possesses the advantages of reduced photobleaching, minimal phototoxicity, and excitation confinement exclusively to the focal plane.^[4,5] In order to obtain a good signal-to-noise ratio, the 2PA materials used in TPM should have large two-photon action cross sections ($\eta\delta$; η is quantum yield) in polar aqueous media. However, most of the commonly used water-soluble 2PA materials only exhibit δ values that are less than 100 GM, which gives a $\eta\delta$ value in the range of 1–50 GM.^[6] Recently, Bazan et al. have successfully developed a series of water-soluble 2PA materials^[7] based on 3D [2.2]paracyclophane as the core. These materials exhibit quite large δ values, up to 700 GM ($\eta\delta$ is about 300 GM) in water.^[7b] They have also demonstrated that hydrophilic 2PA materials can be encapsulated into micelles of sodium dodecyl sulfate in water.^[7c]

Because most 2PA chromophores are hydrophobic, it would be ideal if a simple method could be developed to ensure 2PA materials are biologically compatible while maintaining high $\eta\delta$ values in polar aqueous media. In the literature, there are several reports concerning the use of amphiphilic block copolymers to form micelles in water to incorporate hydrophobic drugs.^[8a] However, little attention has been paid to incorporating 2PA materials into micelles. Only one earlier report from Prasad et al. has demonstrated the feasibility of encapsulating hydrophobic 2PA chromophores into poly(D,L-lactide-co-glycolide) to get aqueous dispersion of 2PA nanoparticles.^[8b] Here, we report a simple method to bring hydrophobic 2PA chromo-

[*] Prof. A. K.-Y. Jen, Dr. Y. Q. Tian, C.-Y. Chen, Dr. Y.-J. Cheng, A. C. Young, N. M. Tucker
Department of Materials Science & Engineering,
University of Washington
Box 352120, Seattle, WA 98195 (USA)
E-mail: ajen@u.washington.edu

Prof. A. K.-Y. Jen, N. M. Tucker
Department of Chemistry, University of Washington
Box 351700, Seattle, WA 98195 (USA)

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phores into an aqueous solution by encapsulating them into micelles formed from an amphiphilic block copolymer poly(methacrylic acid)-*block*-polystyrene (PMAA-*b*-PS). A large $\eta\delta$ value of 1560 GM can be achieved in aqueous media by using this method.

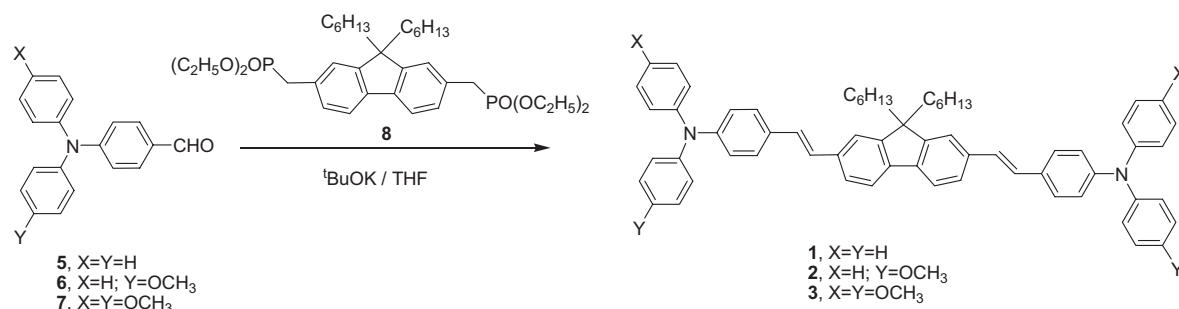
2. Results and Discussion

2.1. Design, Synthesis, and Nanostructure

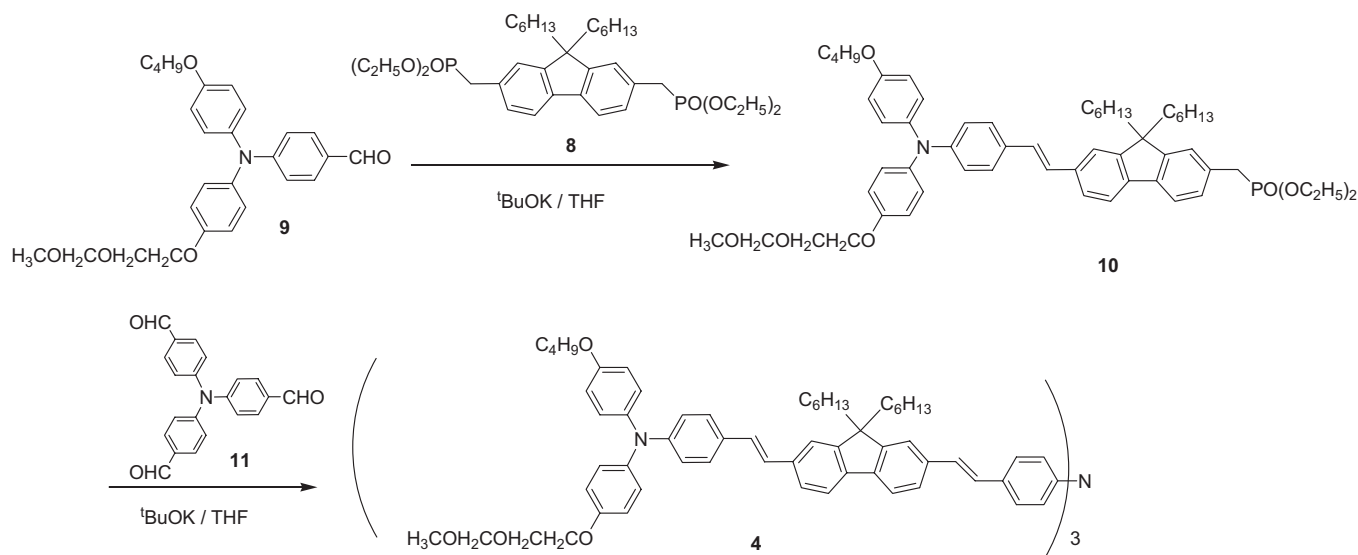
The detailed synthesis of the 2PA materials **1–4** containing a D- π -D motif is shown in Schemes 1 and 2. Compounds **1**, **2**, and **3** were designed to study the influence of donor strength on 2PA cross sections. Compound **4** is a multibranched 2PA chromophore that was designed to explore the cooperative effect for enhancing the δ value.^[3b,9]

The structure and synthetic procedure for the synthesis of the amphiphilic block copolymer PMAA-*b*-PS are given in Figure 1 and Scheme 3, respectively. The block copolymer PMAA-*b*-PS was synthesized using the atom transfer radical

polymerization (ATRP) process.^[10] A PtBMA macroinitiator was prepared according to the procedure published by Karanam et al.^[11] and used to prepare the PtBMA-*b*-PS block copolymer. The *tert*-butyl group was removed at the last stage using acidic condition similar to those reported by Wang et al.^[12] The obtained PS-*b*-PMAA possesses good solubility and can be dissolved in high concentrations (32 mg mL⁻¹ in tetrahydrofuran (THF)). PMAA-*b*-PS forms micelles^[13] in water with the hydrophobic PS as the core and the hydrophilic PMAA as the shell. The core of the micelle (PS) can act as a reservoir for accommodating the hydrophobic 2PA chromophores. The shell (PMAA) has a brushlike protective corona that ensures the dispersion of the micelles in water. This micelles characteristic enables a stable dispersion of water-insoluble 2PA chromophores into the aqueous system. Depending on the approach used (see Experimental section for details) to prepare the micelles, the average diameter of the micelles ranged from 66 to 138 nm (Table 1), as determined by dynamic light scattering (DLS) in solution (Fig. 2a and b). Further investigations on these nanostructures were carried out using atomic force microscopy (AFM) and transmission electron



Scheme 1. Preparation of compounds **1**, **2**, and **3**.



Scheme 2. Preparation of compound **4**.

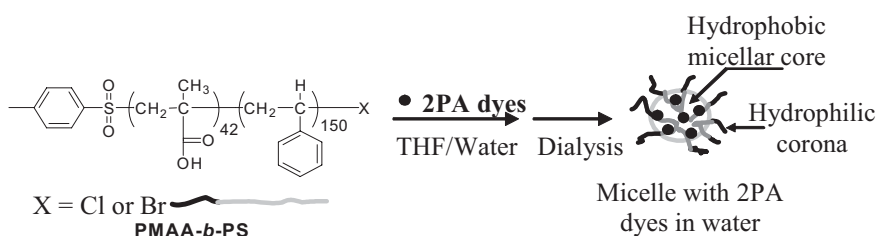
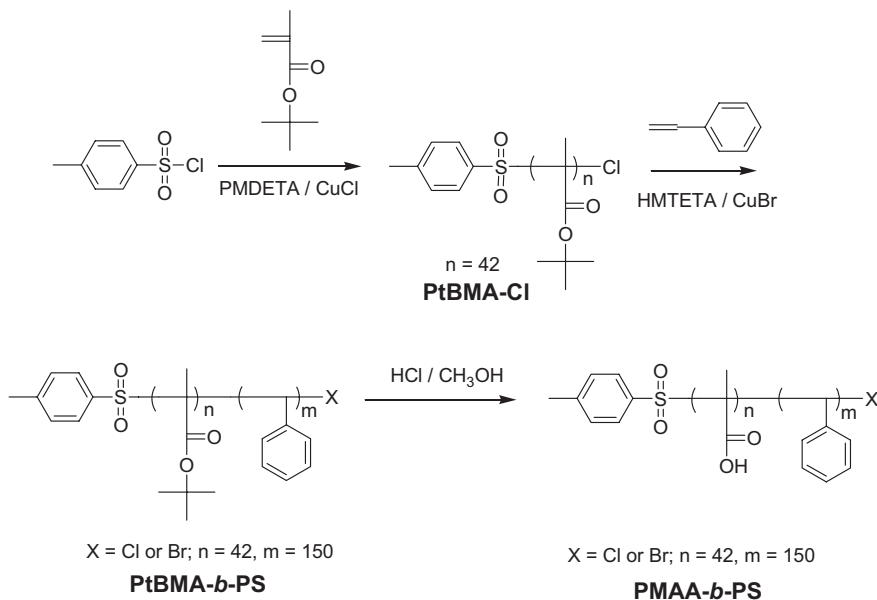


Figure 1. The route used to incorporate hydrophobic 2PA material in water using PMAA-*b*-PS.



Scheme 3. Synthesis of PMAA-*b*-PS.

microscopy (TEM) in the dried state (Fig. 2c–f).

2.2. Linear Photophysical Properties

With an increasing numbers of electron-donating methoxy groups at the para position of the aromatic amino moieties, a clear red-shift of the absorption and emission maxima was observed in **1**, **2**, and **3** (Fig. 3), indicating better charge transfer properties in these compounds. The absorption and emission maxima of **4** are also red-shifted compared to those of **3** because of better electron delocalization of the central amino group in **4**, showing the influence of the multibranched structure on the physical properties.

All of the 2PA materials **1–4** were completely insoluble in water and no fluorescence could be observed. In contrast, after the hydrophobic 2PA materials were encapsulated in the hydrophobic core of micelles (Fig. 1), fluorescence was observed. Optical images of **3** in toluene (a), micelle-containing water (b), and pure water (c) are shown in Figure 4. Because the 2PA materials were loaded in the core of the micelles, they were protected from exposure to the aqueous environment. As a result, their photophysical properties (UV-vis spectra, fluorescence emission spectra, and quantum yields) were not affected by the change in pH from 1 to 14.

Table 1. Properties of Compounds 1–4.

Run	Samples and solvents	$\lambda_{\max}^{\text{abs}} [b]$ [nm]	$\lambda_{\max}^{\text{em}} [c]$ [nm]	$\eta [d]$	$\Delta\lambda_{\text{St}} [e]$ [nm]	$\eta\delta [f]$ [GM]	$\delta [g]$ [GM]	$\lambda_{\max}^{2\text{PA}} [h]$ [nm]	$D_h [i]$ [nm]
1	1 in toluene	411	454	0.98	43	560	570	745	–
2	2 in toluene	416	460	0.95	44	640	670	745	–
3	3 in toluene	421	467	0.98	46	770	790	745	–
4	4 in toluene	428	472	0.86	44	4600	5300	750	–
5	1 in water (0.1) A [a]	414	458	0.77	44	160	210	740	119 (0.08)
6	2 in water (0.1) A [a]	418	468	0.70	50	210	300	740	124 (0.07)
7	3 in water (0.1) A [a]	424	473	0.78	49	300	380	740	125 (0.10)
8	4 in water (0.1) A [a]	432	483	0.23	51	560	2470	750	116 (0.08)
9	4 in water (0.3) A [a]	432	479	0.41	47	1030	2520	750	138 (0.07)
10	4 in water (0.5) A [a]	432	479	0.56	47	1560	2790	750	131 (0.08)
11	2 in water (0.1) B [a]	418	469	0.40	51	90	230	740	66 (0.09)
12	4 in water (0.1) B [a]	432	485	0.06	53	110	1830	750	73 (0.11)

[a] The numbers in parenthesis are the concentration of the block copolymer of PMAA-*b*-PS in water with a unit of mg mL^{−1}. A and B are the preparation methods used for generation of micelles; see the Experimental section for details. [b] Maximum absorbance. [c] One-photon emission maximum. [d] Quantum yield. [e] Stokes shift calculated from $\lambda_{\max}^{\text{em}} - \lambda_{\max}^{\text{abs}}$. [f] Maximum two-photon action cross section. [g] Maximum two-photon cross section. [h] λ_{\max} of two-photon absorption spectra. [i] The average diameters of the micelles in nanometers determined by dynamic light scattering. The values in parenthesis are the polydispersity of the micelles.

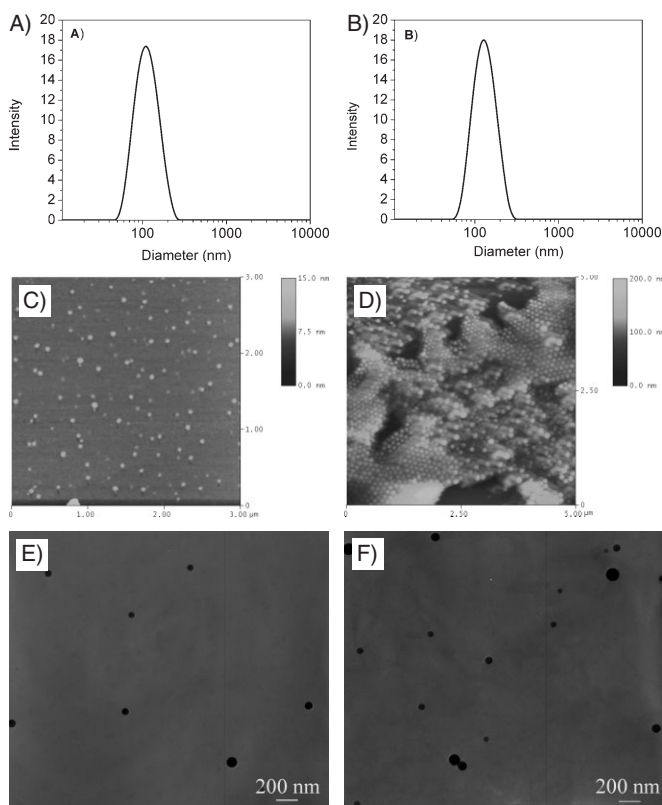


Figure 2. A,B) DLS, C,D) AFM images, and E,F) TEM images of two typical micellar solutions prepared using Method A, as described in the Experimental section. A, C, and E relate to PMAA-*b*-PS without 2PA chromophore. B, D, and F relate to PMAA-*b*-PS with **4** (run 10 in Table 1). AFM and TEM images are from the dry states of the micellar solutions. Average diameters obtained from A, B, C, D, E, and F are 110, 131, 79, 110, 64, and 74 nm, respectively. Note that the observed sizes in the dry state are much smaller than those in solution (110 and 131 nm for PMAA-*b*-PS and PMAA-*b*-PS with **4**). DLS indicates that the micelles are swollen in water due to the soluble PMAA block, whereas TEM and AFM observations show the dried aggregates. The size difference between AFM and TEM is possibly due to the different substrates (for AFM, mica is the substrate; for TEM, a carbon-coated copper grid is the substrate).

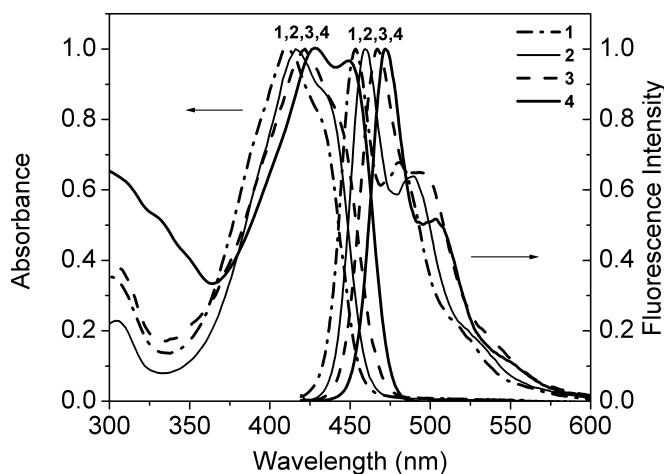


Figure 3. The normalized absorption and emission spectra of **1–4** in toluene.

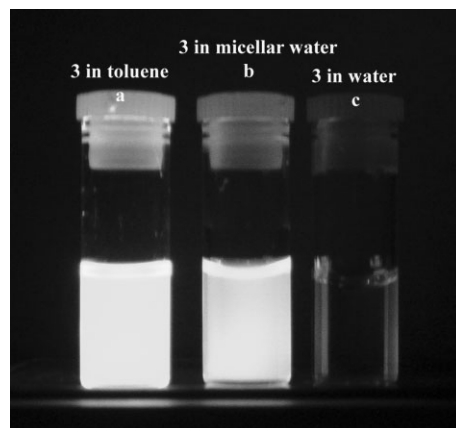


Figure 4. The single-photon fluorescence image irradiated at 365 nm. Samples of b and c are prepared using Method A.

In addition, there is no obvious change of their photophysical properties after these aqueous solutions were stored at room temperature for more than two weeks.

Compared to their photophysical behavior in toluene, 2PA dyes **1–4** in micelles exhibited decreased quantum yields and a slight bathochromic shift of their absorbance and emission maxima. This suggests that aggregates^[14] formed within the micelles. However, because the 2PA molecules were dispersed in and diluted by the hydrophobic PS segments, the aggregation-induced self-quenching is much less serious compared to that of the same molecules dispersed in water. Indeed, a very high quantum yield (0.78) could be obtained for **3** dispersed in micelle-containing water. Interestingly, we found that the decrease of quantum yield for **1–3** was less severe than that of **4**. When the molar concentrations of the 2PA material and the block copolymer were kept constant, compound **4**, which has a higher molecular weight and a larger size, has greater tendency to form aggregates. This results in the decrease of its quantum yield. Nevertheless, the quantum yield of **4** was found to increase with the increase of polymer concentration. This can be explained by the diluting effect from the increased number of micelles. The size of the micelles also affects quantum yields. Much lower quantum yields of dyes were found in smaller micelles (run 6 vs. run 11 or run 8 vs. run 12) because of more severe aggregation.

2.3. Two-Photon Absorption Spectroscopy

2PA cross sections of the compounds were measured by using the two-photon-induced fluorescence method^[6b] with the standard fluorescein in 1 M NaOH aqueous solution as the reference. An increase in the electron-donating strength of **1–3** clearly results in an increase of their δ values (Table 1). It is well known that the 2PA process is a third-order nonlinear optical property which is strongly dependent on intramolecular charge transfer (ICT).^[2] For the symmetrical D- π -D 2PA dye, a stronger donor induces an increase of symmetrical charge transfer from the ends of molecules to the middle due to the resonance enhancement, resulting in an enhanced δ value.^[2]

Compound **4** exhibits a large δ (5300 GM) in toluene (Fig. 5) as a result of the cooperative enhancement^[3b,9] through its multibranched structure.

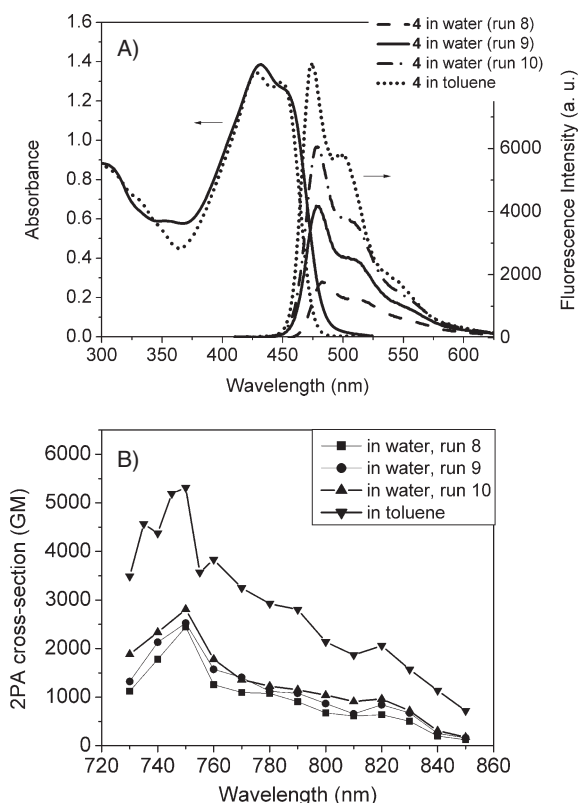


Figure 5. A) Absorption and fluorescence spectra of **4** and B) two-photon absorption spectra of **4** in water and toluene. The fluorescence intensity shown in A reflects the trends of their quantum yields.

Because of the aggregation of the 2PA molecules in the micelles, their δ values decrease (Table 1) when compared to those measured in toluene. However, the trend of their 2PA behavior is consistent with that observed in toluene, δ increases with the increasing donor strength of the dye, and the multibranched compound **4** exhibits the highest δ value in water. Although the $\eta\delta$ value of **4** increases significantly as a result of the diluting effect from the increase of polymer concentrations (runs 8, 9, and 10 in Table 1), the maximum δ value only increases slightly. This is because the size of the micelles also affects the δ values. Smaller δ values were found in smaller micelles (run 6 vs. run 11 or run 8 vs. run 12).

In our experiments, a $\eta\delta$ value as high as 1560 GM (corresponding to a δ value as high as 2790 GM) could be realized for compound **4** by controlling the experimental conditions, as given in Table 1 and Figure 5. To the best of our knowledge, this is the first example of using hydrophobic 2PA materials in aqueous media to achieve such high δ values (2470 to 2790 GM) in water. These numbers are almost one order higher than those reported for the commonly used water-soluble 2PA dyes^[6] and they are among the highest ones reported for 2PA materials in aqueous media.

3. Conclusions

A simple method has been employed to encapsulate hydrophobic 2PA materials into amphiphilic block copolymer to bring them into water. Because the 2PA materials could be well-dispersed in the hydrophobic environment of the micelles, compound **4** exhibits a large two-photon action cross section of 1560 GM (corresponding to a δ value of 2790 GM) in water. Further investigation is in progress for the use of these materials in biological imaging and sensing by controlling the size of the micelles and modifying the carboxylic functional groups.

4. Experimental

General Information: ^1H NMR spectra were measured using a Bruker 300 spectrometer operating at 300 MHz with trimethylsilane (TMS) as an internal standard and reference for chemical shifts. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and high-resolution mass spectrometry (HRMS) were performed by the University of Washington Bio Mass Spectrometry Lab. Thermal transitions were measured on a TA Instruments differential scanning calorimeter 2010 with heating and cooling rates of 10°Cmin^{-1} . UV-vis spectra were recorded with a Hewlett Packard 8452 A Diode Array UV-vis spectrophotometer. One-photon fluorescence spectra were recorded with a Perkin Elmer Luminescence Spectrometer LS 50B using a Xenon lamp as a light source with the emission and excitation slit of 5 nm. Molecular weights of polymers were determined using a Waters 1515 GPC coupled with UV and RI detectors, in reference with a series of polystyrene standards with THF as the eluent.

Atomic force microscopy (AFM, NanoScope III, Digital Instruments) equipped with an integrated silicon tip/cantilever with a resonance frequency ca. 240 kHz in height and phase image models were utilized for the observation of morphologies. Polymer solutions (4 μL) were dropped on a mica substrate and dried at room temperature. The AFM topographies showed no evidence of tip-induced modification during successive scans.

Transmission electron microscopy (TEM) images were obtained using a Philips EM410 microscope operated at 100 kV. TEM samples were prepared by dropping a liquid sample (4 μL) onto a carbon-coated TEM copper grid and then dried in air overnight. In order to enhance the contrast of images, the specimen on the TEM grids were stained by exposing them to a vapor of a 0.5 wt % RuO_4 aqueous solution for 1 min.

Dynamic light scattering (DLS) measurements were performed using a Malvern Nano-ZS instrument equipped with a 4 mW He-Ne laser (633 nm) with an output at a scattering angle of 90° . The solution was passed through a 0.45 μm Millipore Millex-HN filter to remove dust before DLS measurements.

Preparation of Micelles and Incorporation of the 2PA Chromophores: Micelles were prepared using two methods by modifying a reported procedure [15a] for preparing PMAA-*b*-PS micelles. Method A: A mixture of 100–200 μL of the block polymer solution (8–32 mg mL^{-1} in THF) and 100 μL of the 2PA material (ca. $5 \times 10^{-4} \text{ mol L}^{-1}$ in THF) was added slowly to 8 mL of deionized (DI) water, resulting in a concentration of PMAA-*b*-PS of 0.1–0.5 mg mL^{-1} and 2PA dyes of ca. $6 \times 10^{-6} \text{ mol L}^{-1}$ in aqueous solution. The mixture was first stabilized at room temperature for one day and then dialyzed against DI water using dialysis membrane (weight-average molecular weight M_w cut off ca. 1000 Da) for one day to remove THF. Finally, the solutions were filtered through a 0.20 μm microfilter to eliminate excess 2PA dye. The exact amount of each 2PA dye loaded into the micelles was then determined using UV-vis spectroscopy. In general, more than 85 % of the 2PA materials could be incorporated into micelles. Method B: Similar to Method A, however, the concentration of PMAA-*b*-PS used for micelle preparation was 1.0 mg mL^{-1} in THF and the volume of THF was

1.0 mL. The water volume used in Method B is 10 mL. The final polymer concentration in the aqueous solution after dialysis was controlled to be 0.1 mg mL⁻¹. According to DLS, the average diameter of the micelles prepared using Method B (66–73 nm) is much smaller than those prepared using Method A (110–138 nm). It is known that the morphology and dimensions of the resulting aggregates (in this case the micelles) are determined by the balance of three force contributions to the free energy: core-chain stretching, corona-chain repulsion, and interfacial tension between the core and outside solution. Consequently, factors that alter the above balance can be employed to control the aggregate shape and size [15b,c].

Determination of Quantum Yields: Fluorescence quantum yield measurements were carried out using a Perkin-Elmer LS 50B luminescence spectrometer. The fluorescence quantum yields of samples in solutions were recorded by using 9,10-diphenylanthracene ($\eta = 0.90$) [16] excited at 390 nm. η was calculated according to the following equation [17]

$$\eta_s = \eta_r \left(\frac{A_r}{A_s} \right) \left(\frac{I_s}{I_r} \right) \left(\frac{n_r^2}{n_s^2} \right) \quad (1)$$

where (η_r) and (η_s) are the fluorescence quantum yields of the standard and the sample, respectively, A_r and A_s are the absorbance of the standard and the measured sample at the excitation wavelength, respectively, I_r and I_s are the integrated emission intensities of standard and the sample, respectively, and n_r and n_s are the refractive indices of the corresponding solvents of the solutions, respectively.

Determination of Two-Photon Cross Sections: Two-photon excitation spectra were measured using the two-photon induced fluorescence technique [6b] using a mode-locked Ti:Sapphire laser excitation source (Coherent, Mira 900). The laser provides a pulse of approximately 120 fs pulse width at a pulse repetition frequency of 76 MHz in the wavelength range of 710–1000 nm. The pumping wavelengths were determined by a monochromator–charge coupled device (CCD) system. Fluorescein in pH 11 water, which has been well characterized in the literature [6b], was used as a reference (r). The two-photon absorption cross section of a sample compound (s) can be calculated at each wavelength according to

$$\delta_s = \frac{S_s \eta_r \phi_r C_r}{S_r \eta_s \phi_s C_s} \delta_r \quad (2)$$

where S is the detected two-photon induced fluorescence signal, η is the fluorescence quantum yield, and C is the concentration of the chromophore, ϕ is the overall fluorescence collection efficiency of the experimental apparatus. The concentration of the solutions were in the range of 4×10^{-6} to 9×10^{-6} M. The measurements were conducted in an intensity regime where the fluorescence signal showed a quadratic dependence on the intensity of the excitation beam. The uncertainty in the measured cross sections is about 15%.

Materials: *N,N,N',N',N''*-pentamethyldiethylenetriamine (PMDETA), 1,1,4,7,10,10-hexamethyl triethylenetetraamine (HMTETA), anisole, *p*-toluenesulfonyl chloride (*p*-TsCl), potassium *tert*-butoxide, and other chemicals were purchased from Aldrich and used without purification. CuCl and CuBr (Aldrich) were washed with acetic acid, ether, and then dried under vacuum. THF was distilled from sodium/benzophenone under nitrogen. Styrene and *tert*-butyl methacrylate (Aldrich) were distilled over CaH₂ under reduced pressure. 2,7-Bis(diethylphosphono)-9,9-dihexylfluorene (**8**) [18], compounds **5** [3b], **6** [3b], **7** [3b], **9** [19], and **11** [20] were prepared according to known procedures.

Synthesis of 1: Compound **5** (150 mg, 0.55 mmol) and compound **8** (151 mg, 0.25 mmol) were dissolved in 10 mL dry THF. 0.8 mL of potassium *tert*-butoxide (1 M in 2-methyl-2-propanol) was added to the solution. The mixture was stirred at room temperature overnight. After the solvent was removed under reduced pressure, the residue was purified by column chromatography with hexane/ethyl acetate (20:1 by volume) to yield 160 mg of yellow powder with a yield of 74%. ¹H NMR (300 MHz, CDCl₃): δ = 7.69 (m, 2H), 7.49 (m, 8H), 7.30 (m, 8H), 7.17 (m, 12H), 7.10 (m, 8H), 2.00 (m, 4H), 1.06 (m, 12H), 0.73 (t, 6H), 0.67

(m, 4H). ¹³C NMR (125 MHz, CDCl₃): δ = 151.5, 147.6, 147.2, 140.4, 136.5, 131.8, 129.3, 127.8, 127.4, 125.4, 124.6, 124.5, 123.6, 123.0, 120.5, 119.8, 54.9, 40.5, 31.5, 29.7, 23.8, 22.6, 14.0. EI-HRMS: m/z calculated for C₆₅H₆₄N₂ (M^+) 872.5070, found 872.5065.

Synthesis of 2: Prepared following a procedure similar to **1**. Yield: 68%. ¹H NMR (300 MHz, CDCl₃): δ = 7.68 (m, 2H), 7.50 (m, 8H), 7.44 (m, 4H), 7.29 (m, 4H), 7.14 (m, 12H), 7.04 (m, 6H), 6.91 (m, 4H), 3.86 (s, 6H), 2.00 (m, 4H), 1.06 (m, 12H), 0.73 (t, 6H), 0.67 (m, 4H). ¹³C NMR (125 MHz, CDCl₃): δ = 156.3, 151.5, 147.8, 140.3, 136.5, 129.1, 127.4, 127.2, 125.4, 123.4, 122.3, 122.2, 120.5, 119.8, 114.8, 55.5, 54.9, 40.5, 31.5, 29.7, 23.7, 22.6, 14.0. EI-HRMS: m/z calculated for C₆₇H₆₈N₂O₂ (M^+) 932.5281, found 932.5281.

Synthesis of 3: Prepared following a procedure similar to **1**. Yield: 65%. ¹H NMR (300 MHz, CDCl₃): δ = 7.63 (m, 2H), 7.43 (m, 4H), 7.37 (m, 4H), 7.09 (m, 12H), 6.86 (m, 12H), 3.81 (s, 12H), 2.02 (m, 4H), 1.09 (m, 12H), 0.73 (t, 6H), 0.67 (m, 4H). ¹³C NMR (125 MHz, CDCl₃): δ = 155.9, 151.4, 140.8, 140.2, 136.6, 127.5, 127.1, 126.6, 125.3, 120.5, 120.4, 119.7, 114.7, 55.5, 54.9, 40.5, 31.5, 29.7, 23.7, 22.5, 14.0. EI-HRMS: m/z calculated for C₆₉H₇₂N₂O₄ (M^+) 992.5492, found 992.5494.

Synthesis of 10: Compound **9** (2.09 g, 4.44 mmol) and compound **8** (3.12 g, 4.92 mmol) were dissolved in 20 mL dry THF. 10 mL of potassium *tert*-butoxide (1 M in 2-methyl-2-propanol) was added into the solution at 0 °C. Then the mixture was stirred at room temperature for 2 h. After the solvent was removed under reduced pressure, the residue was purified by column chromatography with hexane/ethyl acetate (4:1 v/v) to yield 1.6 g of oil with a yield of 39%. ¹H NMR (300 MHz, CDCl₃): δ = 7.63 (m, 4H), 7.48 (m, 4H), 7.08 (m, 6H), 6.89 (m, 6H), 4.75 (s, 2H), 4.14 (m, 4H), 4.02 (m, 6H), 3.43 (s, 3H), 3.30 (d, 2H), 1.99 (m, 4H), 1.80 (m, 2H), 1.58 (m, 2H), 1.25 (m, 6H), 1.05 (m, 15H), 0.79 (t, 6H), 0.65 (m, 4H).

Synthesis of 4: Compound **11** (118 mg, 4.44 mmol) and compound **10** (1.2 g, 1.29 mmol) were dissolved in 50 mL dry THF. 2 mL of potassium *tert*-butoxide (1 M in 2-methyl-2-propanol) was added to the solution at room temperature. The mixture was stirred at room temperature overnight. After the solvent was removed under reduced pressure, the residue was purified by column chromatography with hexane/ethyl acetate (4:1 by volume) to yield 0.5 g of yellow powder with a yield of 52%. ¹H NMR (300 MHz, CDCl₃): δ = 7.68 (m, 6H), 7.48 (m, 18H), 7.40 (m, 6H), 7.07 (m, 30H), 6.87 (m, 18H), 4.75 (s, 6H), 4.16 (m, 6H), 3.94 (m, 12H), 3.44 (s, 9H), 2.02 (m, 12H), 1.81 (m, 6H), 1.56 (m, 6H), 1.03 (m, 45H), 0.80 (m, 30H). ¹³C NMR (125 MHz, CDCl₃): δ = 156.1, 155.5, 152.0, 148.6, 147.0, 141.5, 141.0, 140.6, 137.2, 136.8, 132.9, 130.2, 128.6, 128.1, 127.9, 127.6, 127.2, 126.9, 125.9, 125.8, 124.8, 121.0, 120.3, 115.9, 115.7, 97.0, 68.3, 68.0, 66.5, 55.7, 55.4, 41.0, 31.9, 31.8, 30.2, 24.2, 23.1, 19.7, 14.5, 14.3. EI-HRMS: m/z calculated for C₁₈₃H₂₁₀N₄O₁₂ (M^+) 2655.5945, found 2655.5943 (calculated from the +3 charge state).

Preparation of PtBMA Macroinitiator: CuCl (49.2 mg, 0.5 mmol), PMDETA (100 μ L, 0.5 mmol), and *tert*-butyl methacrylate (3.0 g, 0.02 mol) were charged in a dry 50 mL three-neck round-bottomed flask, and the flask was sealed with a rubber septum. The reaction mixture was bubbled with argon for 30 min to remove traces of oxygen. *p*-TsCl (94.2 mg, 0.5 mmol) and anisole (8 mL) were added to a second, dry, 25 mL three-neck round-bottomed flask that was then sealed with a rubber septum. This initiator solution was also bubbled with argon for 30 min to remove traces of oxygen. Finally, the initiator solution was added via a degassed syringe to the monomer solution and the flask was immersed in an oil bath at 90 °C overnight. After completion of the reaction, THF (100 mL) was added to the flask, and a magnetic stirrer was used to dissolve the polymer. The resulting green (Cu^{II} complex) polymer solution was passed through an Al₂O₃ column to remove the copper complex. The resulting colorless polymer solution was concentrated under vacuum, after which the polymer was collected and dried under vacuum for three days at 50 °C. 1.0 g white powder was obtained. Yield: 33%. Number-average molecular weight $M_n(\text{GPC}) = 7500 \text{ g mol}^{-1}$, $M_w/M_n = 1.22$; degree of polymerization (n) (determined by NMR) = 42; $M_n(\text{NMR}) = 5800 \text{ g mol}^{-1}$.

Preparation of PtBMA-*b*-PS: A mixture of styrene (3 mL, 0.02 mol), PtBMA initiator (150 mg, ~0.026 mmol), Cu(I)Br (11.5 mg, 0.08 mmol), and HMTETA (23 μ L, 0.08 mmol) in 1 mL of anisole was degassed three times by using a freeze–pump–thaw process and then

polymerized at 80 °C under nitrogen for 14 h. After cooling to room temperature, the mixture was passed through an Al₂O₃ column to remove the copper complex. The obtained polymer was precipitated into methanol twice to get 630 mg of pure block copolymer with a yield of 21 %. $M_{n(\text{GPC})} = 39\,700 \text{ g mol}^{-1}$, $M_w/M_n = 1.44$; m (determined by NMR) = 150; $M_{n(\text{NMR})} = 21\,400 \text{ g mol}^{-1}$. FTIR: 1719 cm⁻¹ (–COOC₄H₉).

Preparation of PMAA-*b*-PS: PtBMA-*b*-PS (300 mg) was suspended in a solution of 3 mL HCl (36 %) in 30 mL methanol, and heated under reflux for 24 h. The resulting solution was filtered to remove the insoluble impurities. The light-yellow solution was concentrated and precipitated in petroleum ether. The resulting white powder was dried in vacuum at 40 °C for about 24 h. $M_{n(\text{GPC})} = 61\,200 \text{ g mol}^{-1}$, $M_w/M_n = 1.56$. $M_{n(\text{NMR})} = 19\,100 \text{ g mol}^{-1}$ (calculated from its precursor PtBMA-*b*-PS). FTIR: 1702 cm⁻¹ (–COOH). Presumably, the quite different polarities between PMAA-*b*-PS and PtBMA-*b*-PS result in their different relative molecular weights and polydispersities.

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- [1] See recent review on 2PA materials and their applications: T. C. Lin, S. J. Chung, K. S. Kim, X. P. Wang, G. S. He, J. Swiatkiewica, H. E. Pudavar, P. N. Prasad, *Adv. Polym. Sci.* **2003**, 161, 157.
- [2] a) M. Albota, D. Beljonne, J.-L. Brédas, J. E. Ehrlich, J.-Y. Fu, A. A. Heikal, S. E. Hess, T. Kogej, M. D. Levin, S. R. Marder, D. McCord-Maughon, J. W. Perry, H. Röckel, M. Rumi, G. Subramaniam, W. W. Webb, X.-L. Wu, C. Xu, *Science* **1998**, 281, 1653. b) M. Rumi, J. E. Ehrlich, A. A. Heikal, J. W. Perry, S. Barlow, Z. Y. Hu, D. McCord-Maughton, T. C. Parker, H. Röckel, S. Thayumanavan, S. R. Marder, D. Beljonne, J.-L. Brédas, *J. Am. Chem. Soc.* **2000**, 122, 9500.
- [3] a) L. Ventelon, S. Charier, L. Moreaux, J. Mertz, M. Blanchard-Desce, *Angew. Chem. Int. Ed.* **2001**, 40, 2098. b) J. Yoo, S. K. Yang, M. Y. Jeong, H. C. Ahn, S. J. Jeon, B. R. Cho, *Org. Lett.* **2003**, 5, 645.
- [4] W. Denk, J. H. Strickler, W. W. Webb, *Science* **1990**, 248, 73.
- [5] a) M. D. Cahalan, I. Parker, S. H. Wei, M. J. Miller, *Nat. Rev. Immunol.* **2002**, 2, 872. b) M. Rubart, *Circ. Res.* **2004**, 95, 1154. c) S. A. Sánchez, E. Gratton, *Acc. Chem. Res.* **2005**, 38, 469.
- [6] a) P. T. C. So, C. Y. Dong, B. R. Masters, K. M. Berland, *Annu. Rev. Biomed. Eng.* **2000**, 2, 399. b) C. Xu, W. W. Webb, *J. Opt. Soc. Am. B* **1996**, 13, 481.
- [7] a) H. Y. Woo, B. Liu, B. Kohler, D. Korystov, A. Mikhailovsky, G. C. Bazan, *J. Am. Chem. Soc.* **2005**, 127, 14721. b) H. Y. Woo, J. W. Hong, B. Liu, A. Mikhailovsky, D. Korystov, G. C. Bazan, *J. Am. Chem. Soc.* **2005**, 127, 820. c) H. Y. Woo, D. Korystov, A. Mikhailovsky, T. Q. Nguyen, G. C. Bazan, *J. Am. Chem. Soc.* **2005**, 127, 13794.
- [8] a) N. Hadjichristidis, S. Pispas, G. A. Floudas, *Block Copolymers-Synthetic Strategies, Physical Properties, and Applications*, Wiley, New York **2002**, p. 397. b) K. Baba, T. Y. Ohulchanskyy, Q. Zheng, T. C. Lin, E. J. Bergey, P. N. Prasad, *Mater. Res. Soc. Symp. Proc.* **2005**, 845, 209.
- [9] a) S.-J. Chung, K.-S. Kim, T.-C. Lin, G. S. He, J. Swiatkiewicz, P. N. Prasad, *J. Phys. Chem. B* **1999**, 103, 10741. b) P. Macak, Y. Luo, H. Norman, H. Ågren, *J. Chem. Phys.* **2000**, 113, 7055.
- [10] a) J. S. Wang, K. Matyjaszewski, *J. Am. Chem. Soc.* **1995**, 117, 5614. b) K. Ohno, Y. Tsujii, T. Fukuda, *J. Polym. Sci. Part A* **1998**, 36, 2473.
- [11] S. Karanam, H. Goossens, B. Klumperman, P. Lemstra, *Macromolecules* **2003**, 36, 3051.
- [12] G. J. Wang, D. Y. Yan, *J. Appl. Polym. Sci.* **2001**, 82, 2381.
- [13] We didn't study the critical micelle concentration (CMC) of the material. However, according to results from DLS, AFM, and TEM, the CMC of this material should be lower than 0.1 mg mL⁻¹ ($5.2 \times 10^{-6} \text{ mol L}^{-1}$).
- [14] We didn't determine exactly how many hydrophobic 2PA molecules were loaded into an individual micelle. However, according to the conditions used here, more than one 2PA molecule was loaded into one micelle.
- [15] a) K. Prochazka, D. Kiserow, C. Ramireddy, Z. Tuzar, P. Munk, S. E. Webber, *Macromolecules* **1992**, 25, 454. b) H. Shen, A. Eisenberg, *J. Phys. Chem. B* **1999**, 103, 9473. c) A. A. Choucair, A. H. Kycia, A. Eisenberg, *Langmuir* **2003**, 19, 1001.
- [16] S. Hamai, F. Hirayama, *J. Phys. Chem.* **1983**, 87, 83.
- [17] H. S. Joshi, R. Jamshidi, Y. Tor, *Angew. Chem. Int. Ed.* **1999**, 38, 2721.
- [18] a) B. Huang, J. Li, P. Shao, J. Qin, G. Yu, Y. Q. Liu, *Synth. Met.* **2005**, 153, 261. b) J. M. Kauffman, G. Moyna, *J. Org. Chem.* **2003**, 68, 839.
- [19] Y.-J. Cheng, J. Luo, S. Hau, D. H. Bale, T. D. Kim, Z. Shi, D. B. Lao, N. M. Tucker, Y. Tian, L. R. Dalton, P. J. Reid, A. K.-Y. Jen, *Chem. Mater.* **2007**, 19, 1154.
- [20] P. Wei, X. D. Bi, Z. Wu, Z. Xu, *Org. Lett.* **2005**, 7, 3199.