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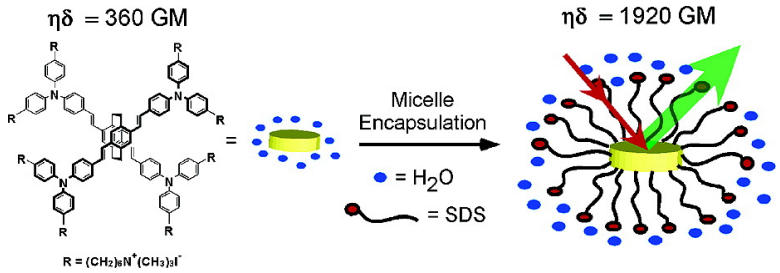

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Two-Photon Absorption in Aqueous Micellar Solutions

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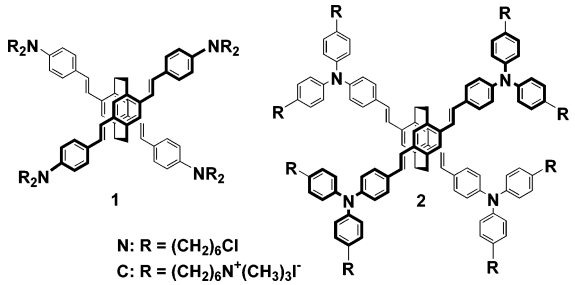
Fluorescent tags for biological imaging by two-photon fluorescence microscopy (TPM) are under substantial examination.[1,2] Best signal-to-noise ratio is obtained with chromophores with a large two-photon action cross-section, which is defined by $\eta\delta$, where η is the fluorescence quantum yield and δ is the two-photon absorption (TPA) cross-section. Molecular guidelines have been developed for obtaining organic chromophores with large δ values. One widely cited example involves quasi linear D-π-D structures, where D is a donor group and π corresponds to a π-conjugated bridge.[3,4] The effect of the medium, and particularly water, on the magnitude of δ remains less precisely understood, and there are conflicting expectations based on theoretical analysis.[5] For example, we recently reported significant decreases in δ and η when modified D-π-D structures are measured in water, relative to the values obtained in organic solvents, such as toluene.[6,7] Even more poorly understood are the effects of different local environments, for example, membranes, vesicles, or intracellular fluid, on $\eta\delta$.

Micelles are dynamic microheterogeneous structures containing surfactant molecules and constitute an important research subject.[8,9] It is possible within their internal environment to include some compounds that are insoluble in water, to perturb the kinetics of many photophysical processes, and to provide structural mimics of biological membranes.[10,11] Sodium dodecyl sulfate (SDS) is a typical anionic surfactant that displays a critical micelle concentration (cmc) of 8.1 mM at 25 °C and forms spherical micelles of low polydispersity in aqueous solution.[12]



In this contribution, we report the linear and TPA spectroscopies of the water-soluble [2.2]paracyclophane-based fluorophores,[6] 4,7,12,15-tetra[*N*,*N*-bis(6''-(*N*,*N*,*N*-trimethylammonium)hexyl)-4′-aminostyryl]-[2.2]paracyclophane octaiodide (**1C**) and 4,7,12,15-tetra[*N*,*N*-bis(4′′-(6′′′-(*N*,*N*,*N*-trimethylammonium)hexyl)phenyl)-4′-aminostyryl]-[2.2]paracyclophane octaiodide (**2C**) in the presence of SDS micelles. Where appropriate, a comparison is made against the neutral counterparts, 4,7,12,15-tetra[*N*,*N*-bis(6′′-chlorohexyl)-4′-aminostyryl]-[2.2]paracyclophane (**1N**) and 4,7,12,15-tetra[*N*,*N*-bis(4′′-(6′′′-chlorohexyl)phenyl)-4′-aminostyryl]-[2.2]paracyclophane (**2N**), which are soluble in nonpolar organic solvents. These data show large enhancements of η and δ in aqueous micellar solution by incorporation of the optically active units within hydrophobic microenvironments.

Previous studies showed that differences in absorption and photoluminescence (PL) between **N** and **C** compounds stem from interactions between the solvent and the optically active fragment and are not influenced by the neutral or ionic groups attached to the conjugated framework.[6] The spectra of the **N** and **C** compounds in the current study are reported in toluene and aqueous media, respectively. The PL maxima (λ_{PL}) display a solvatochromic effect (λ_{PL} in nm: **1N** = 486, **1C** = 553, **2N** = 492, and **2C** = 537; see Table 1) due to the strong intramolecular charge transfer (ICT) character of the excited state. In water, the PL spectra are broad, and one observes considerably lower η values (0.04 for **1C**, 0.52 for **2C**).

Table 1. Spectroscopy Summary

	[SDS]	λ_{abs} (nm)	λ_{PL} (nm)	η^a	$\lambda_{TPA}{}^b$ (nm)	δ (GM)	$\eta\delta$ (GM)
1C	0 M	435	553	0.04	725	370	15
1C	0.05 M	434	484	0.84	730	1550	1300
1N[c]		434	486	0.92	725	1290	1180
2C	0 M	430	537	0.52	750	690	360
2C	0.2 mM	430	497	0.40	770	820	330
2C	0.05 M	440	488	0.95	770	2020	1920
2C	0.1 M	440	488	0.94	770	2050	1930
2N[c]		441	492	0.92	770	2080	1910

[a] Fluorescein (pH=11) as a standard. [b] TPA maximum. [c] In toluene.

Figure 1 compares the PL and absorption spectra of **1C** and **2C** ([**1C**] or [**2C**] = 10^{-5} M) in water and in the presence of SDS at concentrations above the cmc ([SDS] = 0.05−0.1 M). Under these conditions, the chromophores are incorporated within the micellar cores, and the probability of finding two chromophores within a single micelle is low.[13] As shown in Figure 1, there is virtually no change in the absorption maxima (λ_{abs}); however, a significant increase in the molar absorptivity of **2C** is observed. The PL spectra are blue-shifted, have more pronounced vibronic definition (Table

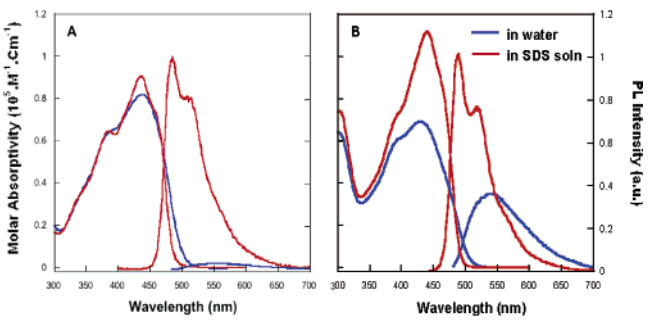


Figure 1. Absorption and PL spectra of **1C** (A) and **2C** (B) ([**1C** or **2C**] = 10^{-5} M and [SDS] = 0.05 M). Areas under the PL spectra are proportional to η.

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1 and Figure 1), and larger η are observed (0.84 for **1C** and 0.95 for **2C**), relative to measurements in the absence of SDS. These PL characteristics are similar to those obtained with **1N** and **2N** in toluene (Table 1). Additionally, we note that the intrinsic PL lifetimes determined by single-photon counting techniques are sensitive to the solvent (in water: 13 ns for **1C**, and 14 ns for **2C**; in toluene: 2.0 ns for **1N**, 1.7 ns for **2N**), and the values obtained in the presence of micelles (2.8 ns for **1C**, 2.1 ns for **2C**) are similar to the values obtained in toluene.[14]

Table 1 also summarizes the effect of [SDS] on the optical properties of **2C**. At [SDS] < cmc (0.2 mM), one observes a substantial blue-shift and appearance of vibronic structure in the PL spectra, with little change in η or in the absorption spectra. At the cmc ([SDS] = 8.1 mM), values for λ_{abs}, λ_{PL}, and η are intermediate between those in water and in toluene (Supporting Information). The spectra at [SDS] = 0.1 M are similar to when [SDS] = 0.05 M. From these observations, we gather that even small quantities of surfactant can modify the emission characteristics of **2C**; however, there is no correlation between λ_{PL} and η. Additionally, there is a saturation of the surfactant effect.

TPA spectra were obtained by using the two-photon-induced fluorescence (TPIF) technique[4] using a femtosecond pulsed laser source (Figure 2). The relative TPIF intensities of the samples ([**1C**] = [**2C**] = ~10^{-5} M) were measured relative to fluorescein in water (pH = 11).[15] Solutions were carefully degassed, and no evidence of photodegradation was observed within the time scale of the TPA measurement. As shown in Figure 2, there is a significant increase in δ values when one compares the results in water (δ_{max} = 370 GM for **1C**, 690 GM for **2C**) with those in the presence of 0.05 M SDS (1550 GM for **1C** and 2020 GM for **2C**). Indeed, the TPA spectra under micellar conditions are similar to the results in toluene (1290 GM for **1C**, 2080 GM for **2C**). The combined maximum $\eta\delta$ values in water are 1300 GM for **1C** and 1920 GM for **2C**, respectively, which are more than 10 times larger than those of the current fluorescent reporters in TPM.[2,6,16] In addition, similar trends are observed in the linear and TPA spectroscopies of the monomeric distyrylbenzene counterparts of **2** in the presence of SDS micelles (Supporting Information).

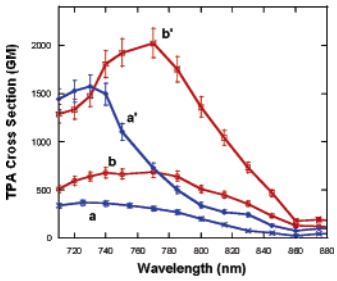

Figure 2. TPA spectra of **1C** (a, a′) and **2C** (b, b′) in water (a, b) and in SDS micellar solution (a′, b′). [**1C**] or [**2C**] = 10^{-5} M, [SDS] = 0.05 M. (GM = 10^{-50} cm^4·s·photon^{-1}).

In summary, we report that the presence of micelles in aqueous media causes a large increase in the δ and $\eta\delta$ of compounds **1C** and **2C**. We attribute this enhancement to the incorporation of the optically active units within the hydrophobic interior of the micelles. There are striking similarities between the optical performance of the charged species within the micellar environment and the neutral analogues in toluene. As a result of the combined increase in δ and η, the measured $\eta\delta$ values of **1C** and **2C** are among the highest reported in an aqueous environment.

We further note that the findings reported herein should be taken into consideration when examining TPM images where fluorescence intensities are used to gauge concentration of labeled substrate in biological samples. Local cell or tissue environments or microstructures may perturb substantially the optical performance of the reporter. These results also suggest that it may be possible to incorporate TPM tags within the interior of hydrophobic structures, such as dendrimers with a charged periphery[17] or polymer nanoparticles, to obtain improved performance. Finally, the dependence of PL lifetime on the polarity of the medium for the [2.2]-paracyclophane structures, due to participation of forbidden states,[14] may be used as a microenvironment probe.[18]

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Supporting Information Available: Complete ref 3, additional UV/vis and PL spectra, and lifetime measurements at different surfactant concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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