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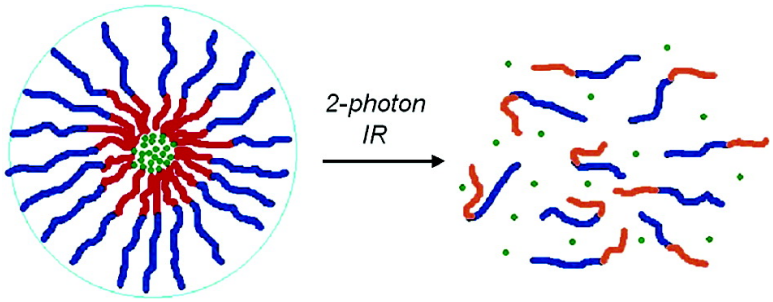

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Synthetic Micelle Sensitive to IR Light via a Two-Photon Process

Andrew P. Goodwin, Justin L. Mynar, Yingzhong Ma, Graham R. Fleming, and Jean M. J. Fréchet*

Department of Chemistry, University of California, MS 1460, Berkeley, California 94720-1460

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Much exciting work has been done to encapsulate drug molecules in macromolecular structures, such as micelles[1] and liposomes,[2] that can accumulate in a tumor and release their payload. Despite significant advances in tumor targeting,[3] however, these therapeutics will spread throughout the body, as well.[4] If the stability of the drug complex could be controlled by a spatially directed external agent, such as light, the drug might be released at the tumor site exclusively. While skin absorbs UV and visible light quite readily, light between 750 and 1000 nm has been shown to penetrate the skin with fewer risks of damage to the irradiated area.[5a] Infrared light has been used for local generation of singlet oxidation for photodynamic therapy[5] and liposome degradation,[2f] but to our knowledge, no one has synthesized a micellar system capable of encapsulating a hydrophobic molecule and releasing it under infrared light.

Functional micelles have found extensive use in drug delivery for their ability to encapsulate and retain hydrophobic drugs in their self-assembled interior. When exposed to a specific stimulus, such as pH change,[6] the hydrophobic tail of molecules constituting the micelle becomes hydrophilic and the micelle is destroyed. 2-Diazo-1,2-naphthoquinones (DNQ) constitute an attractive trigger group because their UV-induced Wolff rearrangement[7] can result in a drastic change in water solubility, a property that has led to the widespread incorporation of DNQ in industrial UV photoresists. The photoproduct, a 3-indenecarboxylic acid of pK_a = 4.5,[8] should be ionized in aqueous buffer and would, therefore, be strongly hydrophilic (Figure 1). Moreover, Urdabayev and Popik recently discovered that DNQ undergoes this same reaction via a two-photon process under 800 nm laser light,[9] a useful wavelength for external targeting.

Functional amphiphile **4** was designed as a PEG-lipid with DNQ on the end of a hydrophobic tail. Its synthesis (Scheme 1) involved treatment of 12-aminododecanoic acid with di-*tert*-butyldicarbonate to protect the amine as the *tert*-butyl carbamate followed by esterification of the free acid with poly(ethylene glycol) monomethyl ether (Aldrich, $M_n \sim 750$) using DCC coupling to install the hydrophilic segment of the amphiphile. The amine was then deprotected with trifluoroacetic acid and coupled to 2-diazo-1,2-naphthoquinone-5-sulfonyl chloride to afford the final product in 86% yield over four steps.

To determine if this molecule indeed formed micelles in water, its ability to encapsulate Nile Red, a fluorescence probe, was examined.[10] Excitation of the hydrophobic Nile Red with 550 nm light in an aqueous medium results in a relatively low fluorescence with a λ_{max} of 660 nm. However, if the dye resides in a hydrophobic environment, such as the interior of a micelle, its fluorescence emission intensity increases dramatically and experiences a blue shift. Solutions with varying concentrations of **4** in 10 mM phosphate buffer at pH 7.4 were treated with Nile Red and equilibrated 2 h to allow for encapsulation. The fluorescence emission spectra for these solutions excited at 550 nm show a pronounced increase in emission intensity with increasing concentration of **4** (Figure 2a). A plot of the relative fluorescence intensity at 642 nm (λ_{max}) versus the log of concentration shows a nonlinear relationship, suggesting formation of multimolecular micelle (Figure 2b). At low concentrations (e.g., 0.05 mg/mL), the weak emission indicates that the Nile Red is in water and, thus, few micelles are present. The steady increase in emission with increasing concentration shows the formation of micelles. The observed inflection point corresponds to a critical micelle concentration (CMC) of 0.15 mg/mL, or 0.13 mM. Further evidence of micelle formation was provided by dynamic light scattering (DLS), which at 1.0 mg/mL, showed particles of approximately 7 nm in diameter (see Supporting Information). Below the CMC, the scattering intensity fell below the detection limit of the instrument.

To demonstrate the sensitivity of the micelle to UV light, the change in Nile Red emission was examined as a function of the photoreaction of **4** at concentrations above and below the CMC. Solutions of **4** at concentrations of 0.05 and 1.0 mg/mL in 10 mM phosphate buffer at pH 7.4 were equilibrated with Nile Red as

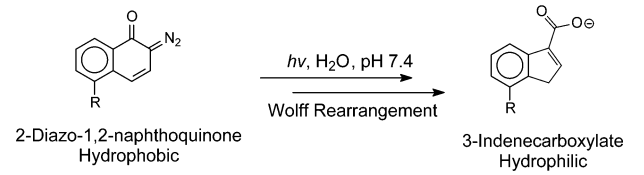
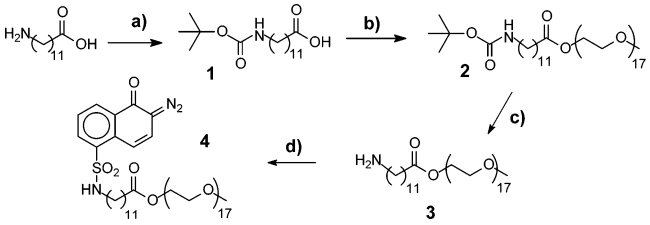


Figure 1. Solubility change in 2-diazo-1,2-naphthoquinone derivatives after Wolff rearrangement in buffered water.

Scheme 1. Synthesis of Functional Amphiphile[a]



[a] Reagents and conditions: (a) Boc_2O, Et_3N, CH_2Cl_2, 99%; (b) poly(ethylene glycol) monomethyl ether, DCC, DMAP, DPTS, CH_2Cl_2, 90%; (c) TFA, CH_2Cl_2; (d) 2-diazo-1,2-naphthoquinone-5-sulfonyl chloride, Et_3N, CH_2Cl_2, 97% from **2**.

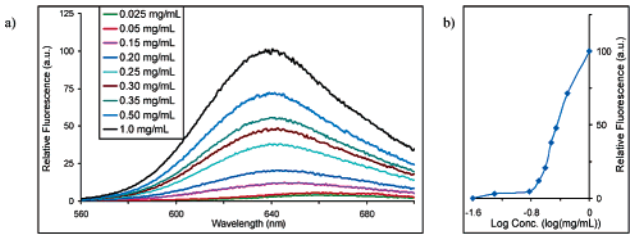


Figure 2. (a) Emission spectra of Nile Red (λ_{exc} = 550 nm) in solutions of varying concentrations of **4** in 10 mM phosphate buffer at pH 7.4. (b) Emission intensity at 642 nm versus the log of concentration (mg/mL) of **4**.

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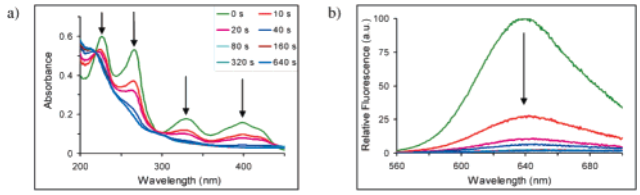

Figure 3. (a) UV−vis spectra of **4** at 0.05 mg/mL in 10 mM phosphate buffer at pH 7.4 at various timepoints during irradiation at 350 nm. (b) Fluorescence measurements of Nile Red encapsulated in 1.0 mg/mL of **4** in 10 mM phosphate buffer at pH 7.4 at same timepoints.

before. The solutions were irradiated at 350 nm, and both the UV absorbance and fluorescence emission spectra were measured at various times. With increasing irradiation time, the UV absorption maxima at 330 and 399 nm decreased steadily, showing conversion of DNQ to the 3-indenecarboxylate (Figure 3a). Also observed was a slight shoulder at approximately 255 nm, which supports formation of the deprotonated carboxylate.[9a] This is expected to change the amphiphilic properties of the molecule and destroy any micellar formation.

During this same experiment, the emission from the Nile Red decreased sharply and shifted from λ_{max} 642 to 660 nm, indicating that the Nile Red was being released into water (Figure 3b). Also observed were a sharp decrease in light scattering intensity and an increase in surface tension consistent with that seen in other photosensitive micelles[11] (Supporting Information). When the same experiment was performed on the sample with a concentration of **4** below the CMC, the UV absorbance changed as before, but the fluorescence of the Nile Red was low and changed very little. Thus, the change in fluorescence is caused by the destruction of a micelle, a result of the photoreaction of **4**.

Analogous studies were performed to show the sensitivity of **4** to infrared light. Two solutions of **4** with Nile Red were prepared as in the above experiment but instead irradiated with a Ti-sapphire pulse laser at 795 nm. The fluorescence emission of Nile Red was monitored again through fluorescence at various times (Figure 4a). After only 15 min of irradiation, the fluorescence emission decreased by about 60%, while after 30 min, the emission fell to 25% of its original intensity. Again, a system with **4** at a concentration of 0.05 mg/mL with Nile Red showed little to no fluorescence change, while a solution of Nile Red in 20 mM sodium dodecyl sulfate showed no decrease in emission following irradiation (Figure 4b). Thus, it appears that the Nile Red has been released due to the destruction of an IR-sensitive micelle.

This constitutes the first demonstration of a micellar system sensitive to infrared light via a two-photon photoreaction at wavelengths that are potentially useful for the photoactivation of drug carriers within a living system. We plan to adapt this technology for use in novel drug delivery platforms and pursue appropriate biological studies.

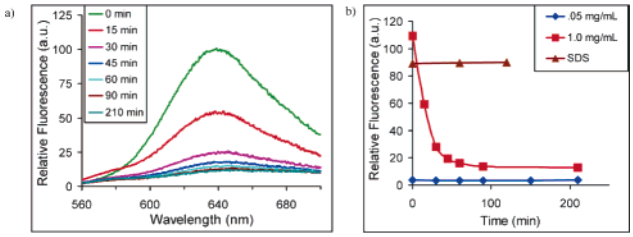

Figure 4. (a) Emission spectra of Nile Red (λ_{exc} = 550 nm) encapsulated in 1.0 mg/mL of **4** in 10 mM phosphate buffer at pH 7.4 after irradiation at 795 nm. (b) Fluorescence emission intensity of Nile Red at 640 nm versus time of irradiation. The relative intensities of Nile Red in solutions containing **4** are comparable, but these are not comparable with the data obtained from Nile Red in SDS.

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Supporting Information Available: Experimental details, light scattering experiments, and additional photophysical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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