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Simultaneous, Segregated Storage of Two Agents in a Multicompartment Micelle

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The ability of micelles—whether formed of block copolymers, lipids, or small-molecule surfactants—to package, transport, or otherwise manipulate small molecules in an inhospitable environment is well known.[1] In recent years, interest in micelles as potential delivery vehicles for pharmaceuticals, gene therapy agents, pesticides, personal care products, and even food formulations has grown enormously. In many cases one might envision wishing to deliver two or more active agents to the same place at the same time, but where the two agents are in some sense incompatible, e.g., they might undergo chemical reaction before reaching the site of interest.[2] Although it is possible that one could prepare micellar aggregates of two distinct types, and thereby sequester the two agents separately, this does not solve the problem of guaranteeing that both agents arrive at the same place, at the same time, and in a prescribed stoichiometric ratio. Multicompartment micelles, however, offer just this possibility: by dividing the core of a micelle into two or more distinct nanodomains, different agents might be transported simultaneously within one micelle while being kept separate within the various core compartments.[3−8] In this report we demonstrate that a prototypical multicompartment micelle, made by the self-assembly of a mikto-arm (coming from the Greek word, meaning "mixed")[9] star terpolymer, can sequester two different small-molecule agents in separate domains.

The concept of a multicompartment micelle was proposed by Ringsdorff approximately 9 years ago, and steps toward the realization of this vision have been reviewed.[10] We recently demonstrated unambiguously the formation of such micelles by the self-assembly of mikto-arm star block copolymers, in which one block of polyethylene oxide (PEO) confers water dispersibility, while the two other blocks of polyethylethylene (PEE) and polyperfluoropropylene oxide (PFPO) segregate within the micelle cores.[11] The synthesis and characterization of such polymers have been described.[12] In this study a particular polymer, designated μ-EOF(2-13-2), has been used. The numbers 2, 13, and 2 refer to the mean molar masses (kDa) of the PEE, PEO, and PFPO blocks, respectively. Cryogenic transmission electron microscopy (cryoTEM) has been used to image the micellar structures that result after direct dissolution of copolymer in water. An example for this particular polymer is shown in Figure 1; the gray patches are micellar cores, within which the darker portions are the fluorocarbon domains and the lighter portions are the hydrocarbon domains. As documented elsewhere, these domains are typically about 5 nm thick and up to 10 nm long.[11] The well-solvated PEO coronas are not directly visible here, although they can be discerned by careful cryoTEM measurements. The effect of relative block lengths on the resulting micellar structures has been explored.[11]

To demonstrate simultaneous selective uptake of two small molecules by the micelles illustrated in Figure 1, we chose two negligibly water-soluble chromophores: pyrene (Py), which is selectively dissolved in the PEE domains, and a fluoronaphthalene derivative, 1-naphthyl perfluoroheptanyl ketone (NFH), which is

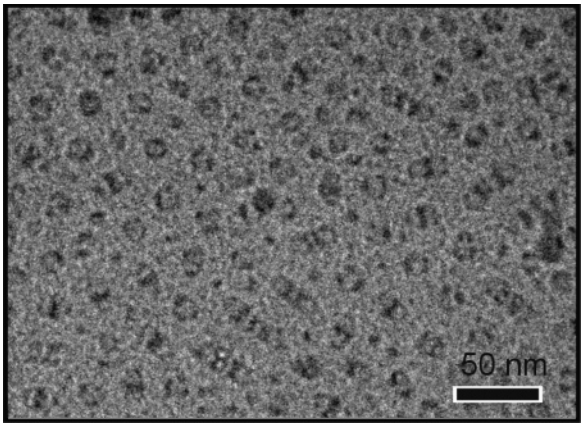

Figure 1. CryoTEM image obtained from a 1 wt % aqueous solution of μ-EOF(2-13-2).

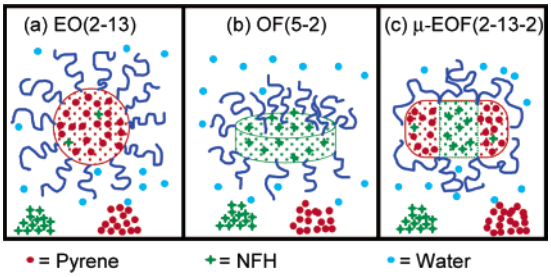

Figure 2. Schematic illustration of selective storage of dye molecules in different micelle solutions.

preferentially dissolved in the fluorinated PFPO domains. The latter dye was synthesized by Friedel−Crafts addition of perfluorooctanoyl chloride to naphthalene with anhydrous $AlCl_3$ as catalyst.[13] The overall experimental scheme is illustrated in cartoon form in Figure 2. Micelles of a diblock copolymer EO(2-13) with a purely hydrocarbon core solubilize Py but only a trace of NFH (Figure 2a). Micelles of a diblock copolymer OF(5-2) with a purely fluorocarbon core solubilize only NFH (Figure 2b). The multicompartment micelles, in contrast, solubilize both dyes, and to nearly the same extent as their respective diblocks. These processes are quantified by UV−vis absorption spectrophotometry.

The absorption spectra of the two chromophores solubilized in the various micelle solutions are shown in Figure 3. The Py absorption at 335 nm and the NFH absorption at 298 nm were used for quantitative analysis. Extinction coefficients were determined to be 34 600 $L \cdot mol^{-1} \cdot cm^{-1}$ for Py in squalane (a chemical analogue to PEE) and 9700 $L \cdot mol^{-1} \cdot cm^{-1}$ for NFH in PFPO homopolymer, respectively, as shown in Figure S7. Neither dye dissolves in water enough to be quantified by absorption spectrophotometry, nor does Py in PFPO (Figure S8).

Each dye was exposed separately to a 0.5 wt % aqueous solution of a PEE−PEO diblock copolymer, EO(2-13), which forms

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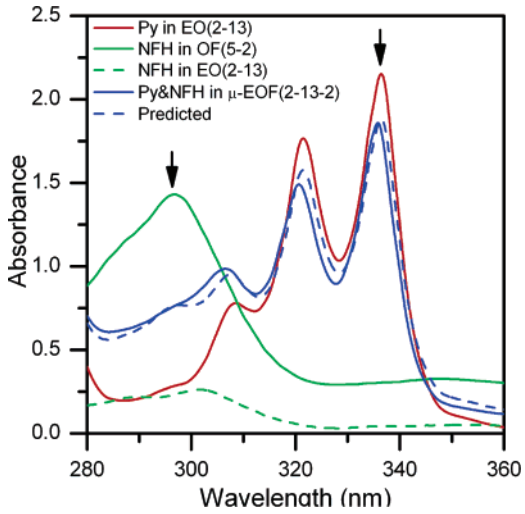

Figure 3. Absorption spectra of Py, NFH, and Py/NFH in 0.5 wt % EO(2-13), OF(5-2), and μ-EOF(2-13-2) micelle solutions.

spherical micelles (Figure S3) with a mean radius of 30 nm (by dynamic light scattering, DLS), to determine the storage capacity. This diblock was actually an intermediate product along the synthetic path to μ-EOF(2-13-2).[12] The resulting spectra in Figure 3 (solid red and dashed green curves) indicate that the EO(2-13) micelles took up 0.16 Py molecule per polymer chain but only 0.035 NFH molecule per polymer chain, indicating a preference for Py by a factor of about 5. NFH was exposed separately to a 0.5 wt % aqueous solution of a PEO−PFPO diblock copolymer, OF(5-2), which forms micelles (Figure S5) with a mean radius of 48 nm (by DLS, Figure S4). In this case, the diblock was prepared by direct coupling of end-functionalized PEO and PFPO homopolymers (Supporting Information). The fluoropolymer core took up 0.20 NFH molecule per polymer chain but only a negligible amount of Py (the solubility of Py in PFPO is about 8×10^{-8} mol/L). This result indicates an overwhelming preference of fluorinated compounds for fluorinated micelle cores.[14] Furthermore, we exposed Py and NFH simultaneously to 0.5 wt % aqueous solutions of OF(5-2) and EO(2-13), respectively. As expected, only NFH was absorbed in OF(5-2), with up to 0.22 NFH molecule per OF(5-2) chain. On the other hand, EO(2-13) takes up only Py, with up to 0.18 Py molecule per EO(2-13) chain, from the mixture containing both Py and NFH (Figure S9). This interesting result reflects the higher affinity of the PEE domain to Py over NFH, which leads to almost exclusive preferential absorption of Py into PEE domains. These results demonstrate that both the PEE and PFPO domains will preferentially absorb only one of the two dye molecules. Subsequently, multicompartment micelles which contain both fluorocarbon and hydrocarbon domains formed by μ-EOF(2-13-2) were tested for simultaneous uptake of Py and NFH.

A 0.5 wt % aqueous solution of μ-EOF(2-13-2) was exposed to excess Py and NFH, and the resulting spectrum was analyzed by the standard application of Beer's law at the two wavelengths indicated in Figure 3. The absorption spectrum (solid blue curve) of the μ-EOF(2-13-2) micelle solution exhibited the strong characteristic absorbance bands of both Py and NFH. Quantitative analysis indicated that the multicompartment micelle took up 0.17 Py and 0.14 NFH molecule per polymer chain. These results compare favorably with the capacities determined from the diblock copolymer micelles. The reduced value for NFH presumably reflects its exclusion from the PEE domains, due to the presence of Py. Considering the large partition coefficients of dye molecules between PEE and PFPO domains, it is reasonable to infer that each dye has been selectively confined in its preferred compartment. To test the effect of micelle environments on load capacity, both Py and NFH dyes were also separately exposed to μ-EOF(2-13-2) micelle solutions. The results indicated that μ-EOF(2-13-2) took up 0.16 Py molecule per polymer chain or 0.22 NFH dye molecule per polymer chain, similar to the previous cases. In this instance more NFH is taken up, as the PEE domain is unoccupied by Py. The comparable storage capacities of PEE and PFPO domains in both diblock and triblock micelles indicate that the solubilization of small molecules is not strongly coupled to the micelle structure, as one might expect.

The multicompartment micelles formed from μ-EOF(2-13-2) have the same core-forming blocks as the combination of EO(2-13) and OF(5-2). Therefore, the maximum loading of Py and NFH in μ-EOF(2-13-2) micelles could be estimated from the storage capacities of EO(2-13) and OF(5-2). This expectation is demonstrated in Figure 3, where the dashed blue spectrum is the appropriately weighted sum of the spectra of Py in EO(2-13) micelles (solid red curve) and of NFH in OF(5-2) (solid green curve); the experimental result is the solid blue curve. The predicted spectrum matches very well with experimental data.

These results demonstrate conclusively that it is possible to create multicompartment micelles that can solubilize two distinct molecules within separate nanoscopic compartments. Furthermore, there is relatively little interaction between the two solubilization efficiencies, in the sense that the ability of the multicompartment micelle to take up a certain "package" can be anticipated on the basis of measurements using the appropriate diblock copolymer micelle, independent of the presence or absence of the other "package".

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Supporting Information Available: Experimental details and Figures S1−S9. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Alexandridis, P., Lindman, B., Eds. *Amphiphilic Block Copolymers: Self-Assembly and Applications*; Elsevier: Amsterdam, 2000.
(2) Savic, R.; Luo, L.; Eisenberg, A.; Maysinger, D. *Science* **2003**, *300*, 615−618.
(3) Kotzev, A.; Laschewsky, A.; Adriaensens, P.; Gelan, J. *Macromolecules* **2002**, *35*, 1091−1101.
(4) Stähler, K.; Selb, J.; Candau, F. *Langmuir* **1999**, *15*, 7565−7576.
(5) Kujawa, P.; Goh, C. C. E.; Calvet, D.; Winnik, F. M. *Macromolecules* **2001**, *34*, 6387−6395.
(6) Erhardt, R.; Zhang, M.; Boeker, A.; Zettl, H.; Abetz, C.; Frederik, P.; Krausch, G.; Abetz, V.; Müeller, A. H. E. *J. Am. Chem. Soc.* **2003**, *125*, 3260−3267.
(7) Weberskirch, R.; Preuschen, J.; Spiess, H. W.; Nuyken, O. *Macromol. Chem. Phys.* **2000**, *201*, 995−1007.
(8) Brannan, A. K.; Bates, F. S. *Macromolecules* **2004**, *37*, 8816−8819.
(9) Hadjichristidis, N. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 857−871.
(10) Laschewsky, A. *Curr. Opin. Colloid Interface Sci.* **2003**, *8*, 274−281.
(11) Li, Z.; Kesselman, E.; Talmon, Y.; Hillmyer, M. A.; Lodge, T. P. *Science* **2004**, *306*, 98−101.
(12) Li, Z.; Hillmyer, M. A.; Lodge, T. P. *Macromolecules* **2004**, *37*, 8933−8940.
(13) Olah, G. A., Ed. *Friedel−Crafts and related reactions*; Interscience Publishers: New York, 1963; Vol. 3.
(14) Matsumoto, K.; Mazaki, H.; Nishimura, R.; Matsuoka, H.; Yamaoka, H. *Macromolecules* **2000**, *33*, 8295−8300.

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