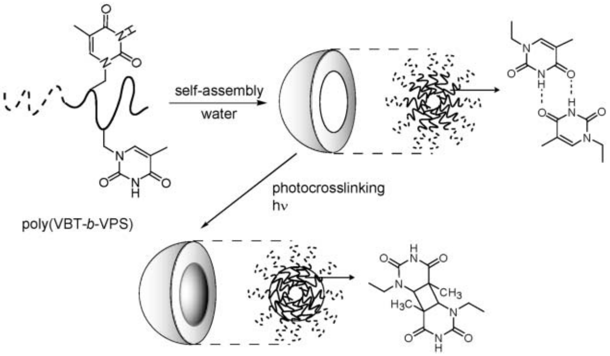
Core-bound polymeric micellar system based on photocrosslinking of thymine†

Kei Saito,[a] Laura R. Ingalls,[a] Jun Lee[b] and John C. Warner*[a]

Received (in Austin, TX, USA) 17th January 2007, Accepted 22nd February 2007
First published as an Advance Article on the web 21st March 2007
DOI: 10.1039/b700686a

Bioinspired core-bound polymer micellar aggregates were synthesized by photocrosslinking thymine-functionalized cores using short UV irradiation; H-bonding between thymines in the core is also believed to increase micellar aggregate stability.

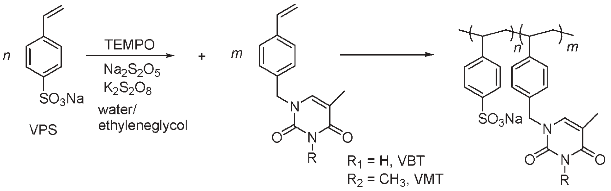
In our efforts to develop environmentally benign materials using the principles of green chemistry,[1] we have explored a number of bioinspired mechanisms.[2] Nature provides abundant and elegant examples of the synthesis of materials in terms of both atom economy and energy utilization. By identifying naturally occurring mechanisms that can be extrapolated to synthetic systems, we hope to develop novel materials based on the principle of green chemistry. One such natural mechanism involves the H-bonding and photodimerization of thymine within DNA.[3,4] Thymine, one of the nucleic bases in DNA, features the ability to form relatively strong hydrogen bonds as well as the ability to photocrosslink. The hydrogen bonding of thymine is well known to stabilize the double helix structure of DNA.[3] The photodimerization of thymine, a UV induced $2\pi + 2\pi$ photocyclization, results in the covalent dimerization of adjacent thymines and disrupts the helical structure of DNA. This process has been linked with the development of certain forms of skin cancer.[4] Deriving inspiration from this biological mechanism, we have sought to create novel materials.[5] Self-assembling amphiphilic block copolymer nanoparticles obtained in aqueous media through a micellization process are of interest because of their potential for applications in nanofabricated materials.[6] Unfortunately, these amphiphilic block copolymer micelles often disassociate into unimers when diluted or subjected to elevated temperatures. It is critical that the amphiphilic block copolymer micelles be stable if they are to be of utility in any practical application. We report here the preparation and properties of a core-bound micellar system obtained from poly(vinylbenzylthymine)-*b*-poly(styrene sulfonic acid sodium salt) by leveraging H-bonding and photocrosslinking properties of thymines (Scheme 1).



Scheme 1

The hydrophobic block was composed of thymine-functionalized monomer vinylbenzylthymine (VBT), synthesized from *para*-vinylbenzyl chloride and thymine in aqueous ethanol, and the hydrophilic block was composed of vinylphenylsulfonate (VPS). We chose VPS as the hydrophilic block monomer because sulfonic acid functionalized polymers have found use in various applications such as polymer electrolyte fuel cell membranes and other high proton conductive materials.[7] The block copolymer synthesis was carried out by a TEMPO-mediated living radical polymerization system in a water–ethylene glycol mixture (Scheme 2).[8] It is worth noting that this living radical polymerization can be carried out in an aqueous mixture, a principle of green chemistry. The use of water also allows the polymerization of VPS without the protection and the subsequent deprotection of sulfonic acid, another principle of green chemistry. Both low molecular weight (MW) poly(vinylbenzylthymine)-*b*-poly(styrene sulfonic acid sodium salt), poly(VBT-*b*-VPS)-L ($M_n = 2.2 \times 10^4$, $M_w/M_n = 1.58$), and high MW poly(VBT-*b*-VPS)-H ($M_n = 8.1 \times 10^4$, $M_w/M_n = 1.59$) were synthesized. 3-Methyl-1-(4-vinylbenzyl)thymine (VMT) was obtained by methylation of VBT and the amphiphilic block copolymer of VMT and VPS, poly(VMT-*b*-VPS) ($M_n = 3.5 \times 10^4$, $M_w/M_n = 1.72$), was synthesized to examine the effect of hydrogen bonding on the stability of micelles.

Dialysis was used to form micellar aggregate and the micellar aggregate size was measured using dynamic light scattering (DLS). Low MW poly(VBT-*b*-VPS)-L ($M_n = 2.2 \times 10^4$) formed a small average diameter micelle aggregate (87 nm) and the high MW poly(VBT-*b*-VPS)-H ($M_n = 3.5 \times 10^4$) formed a large micelle aggregate (164 nm), suggesting a relationship between the polymer MW and size of micellar aggregates and thus the size of micellar



Scheme 2

---

[a] *Center for Green Chemistry, University of Massachusetts Lowell, One University Avenue, Lowell, MA 01854, USA. E-mail: John_Warner@uml.edu; Fax: 978-934-2074; Tel: 978-934-4543*
[b] *Center for High-rate Nanomanufacturing, University of Massachusetts Lowell, One University Avenue, Lowell, MA 01854, USA*

† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/b700686a

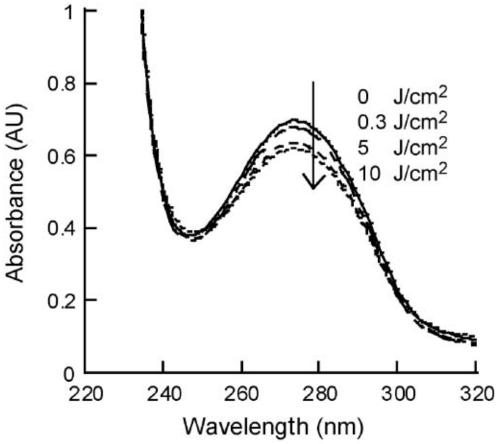


Fig. 1 The UV absorption spectra of poly(VBT-*b*-VPS)-H micellar aggregates solution upon irradiation with 254 nm UV light at 0, 0.3, 5, 10 J cm^{-2}.

aggregates can be somewhat controlled. Photocrosslinked micellar aggregates were prepared by pouring a micelle solution into a quartz tube and irradiating at short wave UV at 0.3, 5, and 10 J cm^{-2}. It has been shown previously that thymine has an absorption at 270 nm which decreases as a result of 2π + 2π photodimerization.[9] After each irradiation, the UV spectrum of the micellar aggregate solution was measured. As the irradiation dose increases, the intensity of the absorption at 270 nm decreases (Fig. 1), consistent with photocrosslinking of the thymine units in the micellar aggregates core. The decrease in intensity of the peak at 270 nm corresponds to ~15% crosslinking of thymine units which is the maximum degree of crosslinking obtained using 254 nm wavelength UV light. It is to be noted that this only ~15% crosslinking of the thymine units leaves the remainder of the thymine units to dissociate freely (discussed in the CMC section below). As expected, DLS measurements showed no significant change in average aggregate size upon irradiation.

TEM images of poly(VBT-*b*-VPS)-H micellar aggregates were obtained from aqueous solution containing uranyl acetate as a negative stain on carbon-coated copper grids. TEM images before and after photocrosslinking show approximately spherical shaped micellar aggregates having diameters in the 100–140 nm range. The AFM image of dried poly(VBT-*b*-VPS)-H core-photocrosslinked micellar aggregates on freshly cleaved mica was obtained in the tapping mode (Fig. 2). The image shows an elliptical object of larger weight and lower height than is consistent with the flattening of the labile and fluid micellar aggregates upon drying on a flat mica surface during the sample preparation.

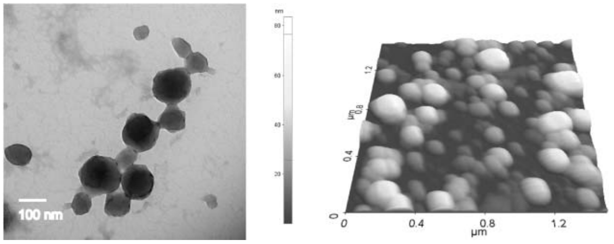


Fig. 2 TEM and AFM of poly(VBT-*b*-VPS)-H photocrosslinked micellar aggregates (5 J cm^{-2} UV irradiation).

In addition to covalent photocrosslinking, H-bonding in the core might be expected to confer additional stability on the micellar aggregates. For example, in spite of its higher molecular weight, micelle aggregates of poly(VMT-*b*-VPS) ($M_n = 3.5 \times 10^4$) are smaller in size (48 nm) than the corresponding low MW poly(VBT-*b*-VPS)-L ($M_n = 2.2 \times 10^4$), suggesting that factors other than polymer MW are involved. We believe that H-bonding between thymine units in the micellar core could be playing an important role, *e.g.*, the smaller aggregate size in poly(VMT-*b*-VPS) could be due to the 3-methyl substituent that would disrupt H-bonding between thymine units in the micellar core. Examination of the micellar aggregate solution by IR was carried out in an attempt to demonstrate the H-bonding of thymine units but this was inconclusive because of the multiple broad peaks around 3000–3500 cm^{-1}. However, secondary evidence consistent with H-bonding is provided by critical micelle concentration (CMC) measurements on these systems.

The CMC of a given system is a measurement of the ease of formation of micelles and therefore an indirect measurement of the stability of the micelle. A lower CMC value indicates higher stability. The CMC value of the block copolymer system was determined by fluorescence measurements in aqueous solution using pyrene as a probe.[10] Pyrene has a strong fluorescence in a nonpolar environment, but in a polar environment such as water, fluorescence quenching is greatly enhanced. Thus the CMC can be determined by measuring the micelle concentration point at which the fluorescence emission intensity of pyrene becomes constant. The CMC of the poly(VMT-*b*-VPS) system was compared to poly(VBT-*b*-VPS)-H to examine the role of H-bonding in the stability of the micelle (Table 1). Our hypothesis is that the poly(VMT-*b*-VPS) system is incapable of forming self-assembled H-bond dimers in the core because of methylation, and consequently, has a much higher CMC (140 ppm) than the poly(VBT-*b*-VPS)-H system (31 ppm). It must be pointed out that the higher CMC value of the poly(VMT-*b*-VPS) system could also be due to other factors, such as its low molecular weight compared to the poly(VMT-*b*-VPS) system or steric inhibition to micellar packing due to the 3-methyl substitution, *etc.*, although the inability to H-bond in the micellar core could still be dominant. It is unclear at the present time how quantitative the cumulative effects of H-bonding and the size of the H-bonded core would be in increasing micellar stability, and whether they would be enough to tip the scale against classical aggregation forces such as hydrophobic interaction. In any case, when comparing the two forces, *i.e.*, H-bonding and photocrosslinking, the latter is more straightforward.

For example, the CMC values of the poly(VBT-*b*-VPS)-H system that had been irradiated at the different irradiation doses (0, 0.3, 5 J cm^{-2}) were measured to determine the effect of

Table 1 Critical micelle concentration (CMC) values of block copolymer systems

Entry	Polymer code	Irradiation/ J cm^{-2}	CMC ppm/µg mL^{-1}
1	poly(VMT-*b*-VPS)	0	140
2	poly(VBT-*b*-VPS)-H	0	31
3	poly(VBT-*b*-VPS)-H	0.3	27
4	poly(VBT-*b*-VPS)-H	5	9.8

photocrosslinking on the stability of the micellar system (Table 1). The CMC of the poly(VBT-*b*-VPS)-H system decreases with increased irradiation dosage, *e.g.*, with 5 J cm^{-2} UV irradiation, the CMC becomes 3 times smaller (9.8 ppm) compared to the non-irradiated control (31 ppm). Micelles having size >100 nm have been observed previously.[10,11] Our CMC studies are meant merely to highlight the effect of photocrosslinking and potential H-bonding in influencing the aggregation behavior of this class of polymers.

It is interesting to note that our ~15% crosslinked system is different from conventional core-crosslinked micellar systems which are much more crosslinked and cannot, therefore, dissociate to unimers (below their uncrosslinked CMC). Our lightly cross-linked system can reversibly dissociate into micellar aggregates and unimers (albeit, ~15% crosslinked), opening up new opportunities to possibly tune the aggregate size, shape, *etc*, by UV irradiation.

For example, the control of the CMC of the block copolymer micellar system by photocrosslinking should prove of use in the controlled release of materials encapsulated in the micelles. Furthermore, micellar aggregates from VBT and VPS block copolymers have the potential to encapsulate guest materials by H-bonding with the attached thymine in the core. It is known that photocrosslinking of thymine can be reversed either by exposure to lower wavelength UV irradiation or enzymatically.[12] Either of these mechanisms should allow one to reversibly control the photocrosslinking of these thymine functionalized micellar aggregates. Investigation of the controlled release of encapsulated materials and the reversible core-photocrosslinked micelle is in progress.

The authors thank the Pfizer Corporation for financial support. We also thank Dr Timothy Kotyla and Dr Robert J. Nicolosi for useful discussions and providing the DLS instrument, Dr Jayant Kumar for providing the fluorometer and Dr Roger Boggs and Dr Sanjeev K. Manohar for fruitful discussions.

Notes and references

1 P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, London, 1998.

2 J. C. Warner, *Pure Appl. Chem.*, 2006, **78**, 2035–2041.

3 J. D. Watson and F. H. C. Crick, *Nature*, 1953, **171**, 737–738; A. K. Boal, F. Ilhan, J. E. DeRouchey, T. Thurn-Albrecht, T. P. Russell and V. M. Rotello, *Nature*, 2000, **404**, 746–748; K. Viswanathan, H. Ozhalici, C. L. Elkins, C. Heisey, T. C. Ward and T. E. Long, *Langmuir*, 2006, **22**, 1099–1105.

4 A. A. Lamola and J. P. Mittal, *Science*, 1966, **154**, 1560–1561; W. Harm, *Biological Effects of Ultraviolet Radiation*, Cambridge University Press, Cambridge, 1980.

5 C. M. Cheng, M. J. Egbe, M. J. Grasshoff, D. J. Guarrera, R. P. Pai, L. D. Taylor and J. C. Warner, *J. Polym. Sci., Part A: Polym. Chem.*, 1995, **33**, 2515–2519; C. Kiarie, J. Bianchini, S. Trakhtenberg and J. C. Warner, *J. Macromol. Sci., Pure Appl. Chem.*, 2005, **42**, 1489–1496; S. Trakhtenberg, Y. Hangun-Balkir, J. C. Warner, F. Bruno, J. Kumar, R. Nagarajan and L. A. Samuelson, *J. Am. Chem. Soc.*, 2005, **127**, 9100–9104.

6 I. W. Hamley, *Encyclopedia of Polymer Science and Technology*, ed. H. F. Mark, Wiley, New Jersey, 3rd edn, 2003, Vol. 1, pp. 457–482; N. Hadjichristidis, S. Pispas and G. Floudas, *Block Copolymers*, Wiley, New Jersey, 2003, pp. 203–231; X. Zhang, M. Wang, T. Wu, S. Jiang and Z. Wang, *J. Am. Chem. Soc.*, 2004, **126**, 6572–6573; F. Liu, M. Wang, Z. Wang and X. Zhang, *Chem. Commun.*, 2006, 1610–1612.

7 K. Matsumoto, T. Hirabayashi, T. Harada and H. Matsuoka, *Macromolecules*, 2005, **38**, 9957–9962; K. Saito, T. Masuyama and H. Nishide, *Bull. Chem. Soc. Jpn.*, 2005, **78**, 1699–1701.

8 B. Keoshkerian, M. K. Georges and D. Boils-Boissier, *Macromolecules*, 1995, **28**, 6381–6382; M. Bouix, J. Gouzi, B. Charleux, J. Vairon and P. Guinot, *Macromol. Rapid Commun.*, 1998, **19**, 209–213.

9 R. B. Setlow, *Biochim. Biophys. Acta*, 1961, **49**, 237–238.

10 A. V. Kabanov, I. R. Nazarova, I. V. Astafieva, E. V. Batrakova, V. Y. Alaknov, A. A. Yaroslavov and V. A. Kabanov, *Macromolecules*, 1995, **28**, 2303–2314; H. Liang, T. Yang, C. Huang, M. Chen and H. Sung, *J. Controlled Release*, 2005, **105**, 213–225.

11 T. Bronich, P. A. Keifer, L. S. Shlyakhtenko and A. V. Kabanov, *J. Am. Chem. Soc.*, 2005, **127**, 8236–8237.

12 J. Whitfield, A. Morelli and J. C. Warner, *J. Macromol. Sci., Pure Appl. Chem.*, 2005, **42**, 1541–1546.