Diblock-Type Supramacromolecule via Biocomplementary Hydrogen Bonding

Atsushi Noro,* Yutaka Nagata, Atsushi Takano, and Yushu Matsushita*

Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

Received March 11, 2006; Revised Manuscript Received April 10, 2006

Control of nanostructure formation by a diblock-type supramacromolecule via biocomplementary hydrogen bonding has been achieved. Two different homopolymers, poly(4-trimethylsilylstyrene) and poly(styrene-d_8), that are end-decorated with complementary oligonucleotides, i.e., thymidine phosphates and deoxyadenosine phosphates, were prepared by using the phosphoramidite method and blended successively. Association behavior in a blend solution was examined with NMR, and a cast bulk film obtained from the solution has been confirmed to show a nanophase-separated structure by transmission electron microscopy and X-ray scattering. Suppression of this nanostructure formation of a block-type supramacromolecule was also attained by adding a smaller agent as an inhibitor.

Introduction

Structures of block copolymers have been studied for four decades focused on their nanophase-separated structures since they could be utilized as highly functional materials, and many research achievements have been attained in a variety of basic[1−5] and applied[6−11] studies. Recently, in macromolecular science, many researchers are interested in introducing noncovalent bonding to connect the same polymer species or different species.[12−18] There are excellent works such as supramolecular polymer networks from polymers terminated with 2-ureido-4-pyrimidone including quadruple hydrogen bonding[19−20] and supramolecular conjugates as the result of self-assembling of multicomponent materials with metal-to-ligand coordination[21,22] or multiple complementary hydrogen bonding.[23−25] Particularly, studies on intermolecular association by using complementary hydrogen bonding interaction from DNA or nucleobase units have attracted a lot of attention.[25−33]

A diblock-type supramacromolecule can be formulated by combining end-decorated chemically different macromolecules as schematically shown in Figure 1a, and if nucleotide units are used for the proposed end-functional groups, the diblock-type supramacromolecule can form a nanophase-separated structure and be decomposed into parent polymers by exposure under a certain external field such as shear flow or temperature. Moreover the associated molecule can be dissociated by inserting inhibitors for hydrogen bonding, which is also illustrated in Figure 1a. Realization of association−dissociation control can lead to very useful functions where advanced polymeric complex materials with nanoscale periodicity could be produced.

We have developed the efficient synthetic method of oligonucleotides-terminated polymers applying the DNA synthesis called the phosphoramidite method.[34] We should obtain a diblock-type supramacromolecule if two different polymers with complementary oligonucleotides are successfully prepared. In this communication, we report on preparation of two different homopolymers end-decorated with complementary oligonucleotides and the successful formation of a diblock-type supramacromolecule from blend solution via hydrogen bonding and a nanophase-separated structure in bulk. The dissociation behavior of a block-type supramacromolecule into component polymers

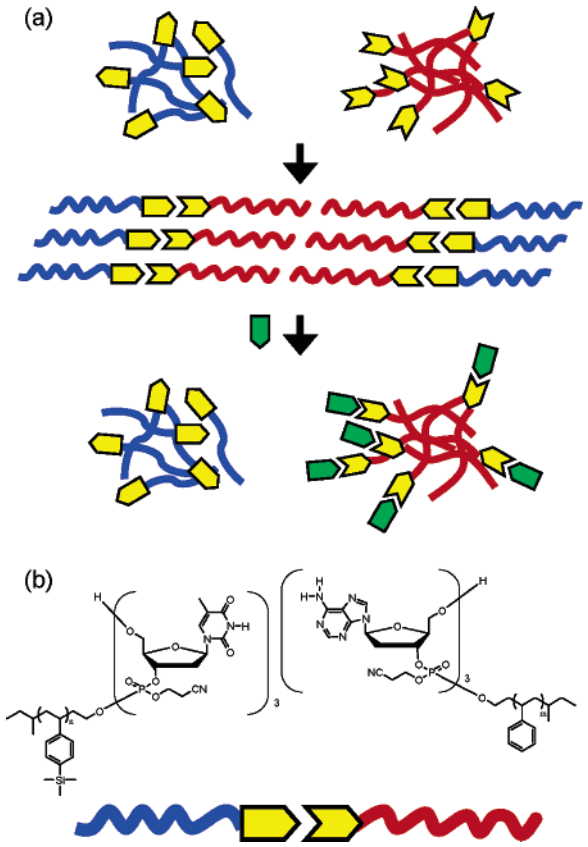

Figure 1. (a) Schematic illustrations for formation of a diblock-type supramacromolecule via hydrogen bonding (nanophase separation) and dissociation of the supramacromolecule by adding an inhibitory agent (macrophase separation). (b) Chemical structures of polymers end-decorated with oligonucleotides used in this study and schematic illustrations of complex formation. The left polymer is PTMSS-T_3, whereas the right one is PS_{d8}-A_3, where the styrene unit is perdeuterated.

* Corresponding authors. Phone: +81-52-789-4604. Fax: +81-52-789-3210. E-mail: yushu@apchem.nagoya-u.ac.jp (Y.M.); noro-a@nagoya-u.jp (A.N.).

10.1021/bm0602280 CCC: $33.50
Published on Web 04/29/2006

was examined in solution and in bulk by adding a small molecule as an inhibitor.

Experimental Section

The two types of polymers used in this study are poly(4-trimethylsilylstyrene) abbreviated to PTMSS ($M_n = 11\,000$, $M_w/M_n = 1.03$) and poly(styrene-d_8) called PS_{d8} ($M_n = 10\,000$, $M_w/M_n = 1.03$). Both polymers were synthesized via living anionic polymerizations, and a hydroxyl group was introduced on one end of each polymer. ^{1}H NMR measurements were performed on each step of synthesis by using Varian Unity Inova 500 MHz to confirm the molecular structures. Deuterated polystyrene was used instead of the hydrogenated one on purpose to analyze the ^{1}H NMR spectrum effectively.[34] The selected three nucleotide units were each introduced sequentially on the ends of these polymers, that is, three thymidine phosphates for PTMSS and three 2′-deoxyadenosine phosphates for PS_{d8}, using the previously developed phosphoramidite method. The principle reactions of this method are known to be mild but efficient and capable of incorporating multiple nucleotides (see the Supporting Information). Figure 1b shows chemical structures of polymers we aimed to prepare, and the obtained polymers were coded as PTMSS-T_3 and PS_{d8}-A_3.

To observe the structures in bulk, polymer thin films were obtained from chloroform solutions on Teflon Petri dishes by casting them at room temperature for 1 day under the similar initial preparation condition for NMR studies. Bulk structures of samples were observed by transmission electron microscopy (TEM) by using H-800 of Hitachi for an ultrathin section without staining and also by a small-angle X-ray scattering (SAXS) apparatus, Rigaku Nano Viewer.

Results and Discussion

Molecular structural analysis was performed by ^{1}H NMR as shown in Figure 2, panels a and b. The multiple peaks for imide protons (−CON**H**CO−) in PTMSS-T_3 are found at 8.9−9.8 ppm as shown with a broken box in Figure 2a (details of analysis and more data are in the Supporting Information). The blend solution of PTMSS-T_3 and PS_{d8}-A_3 in the molar ratio of 1:1 in chloroform-d_1 (32 mg/30 mg in $CDCl_3$ of 1.3 g) was prepared by simply weighting each polymer powder and then dissolving into the solvent. Association behavior of polymers in solution was also observed by using ^{1}H NMR. In Figure 2c, the broader peak of imide protons in PTMSS-T_3 shifted and located at 10.2−11.0 ppm as the result of conjugation, referring to the original location of the peaks at 8.9−9.8 ppm in Figure 2a. From these experiments, it has been clearly found that hydrogen bonding generated between PTMSS-T_3 and PS_{d8}-A_3.

Figure 3a is a TEM image for the blend film showing the lamellar-like two-phase structure whose domain size is roughly estimated to be about 20 nm. The darker phase represents PTMSS due to comparatively high electron density from silicon atoms, whereas the brighter phase represents PS_{d8} and oligonucleotides. Figure 3b is fast Fourier transform data obtained from the TEM image using an application software, ImageJ. It displays a diffused circle at around 0.3 nm^{-1} in terms of q ($=4\pi \sin\theta/\lambda$), suggesting poor orientation of microdomains in the structure.

The structures of the cast film of the blend, together with two homopolymers, PTMSS-T_3 and PS_{d8}-A_3, were also observed by SAXS. Figure 4a compares circularly averaged one-dimensional SAXS images from film edges for PTMSS-T_3, PS_{d8}-A_3, and the blend. The top two scattering patterns for end-decorated homopolymers in Figure 4a show only one scattering peak, their positions are 0.73 nm^{-1} for PTMSS-T_3 and 0.60 nm^{-1} for PS_{d8}-A_3. These peaks are associated with correlation hole peaks for end-decorated polymers,[35] and their two-dimensional patterns are shown in Figure 4, panles b and c. On the other hand, the blend clearly shows two scattering peaks, which are located at 0.27 and 0.66 nm^{-1}, and the two-dimensional pattern is shown in Figure 4d. The appearance of the scattering peak at lower q indicates the formation of a large structure, whose repeating distance is determined to be 23 nm from the q value at the peak maximum, i.e., 0.27 nm^{-1}. This size is quite consistent with the result from TEM observation and is much larger than the size of a phase-separated structure, 11 nm, for a bulk polystyrene with the molecular weight of 1.3×10^4 having five nucleotides on one chain end in bulk.[34] Therefore, a diblock-type supramacromolecule has been formed

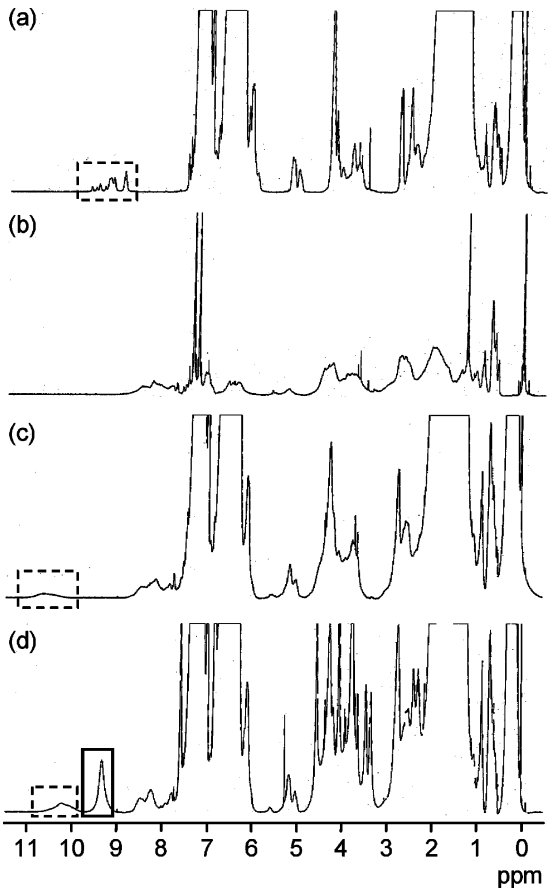

Figure 2. ^{1}H NMR charts: (a) PTMSS-T_3, (b) PS_{d8}-A_3, (c) the blend of two end-decorated homopolymers, and (d) the blend of PTMSS-T_3/PSd8-A_3/DMTr-T with mole ratio of 1/1/5. Three boxes drawn with broken lines at (a), (c), and (d) are showing the peaks of imide protons (−CON**H**CO−) for thymine in PTMSS-T_3, whereas a box drawn with a solid line in (d) is for imide proton in DMTr-T.

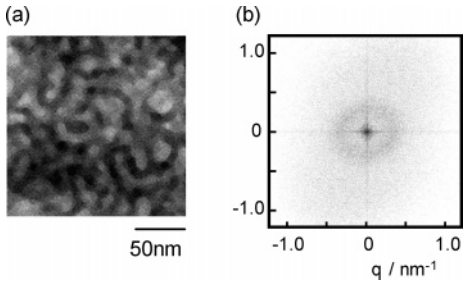

Figure 3. (a) TEM image for the blend of two end-decorated homopolymers. The darker phase is for PTMSS polymer, while the brighter phase is for PS_{d8} and oligonucleotides. (b) A FFT image from a TEM image by using ImageJ. Horizontal and vertical axes are scattering vectors, q ($=4\pi \sin\theta/\lambda$).

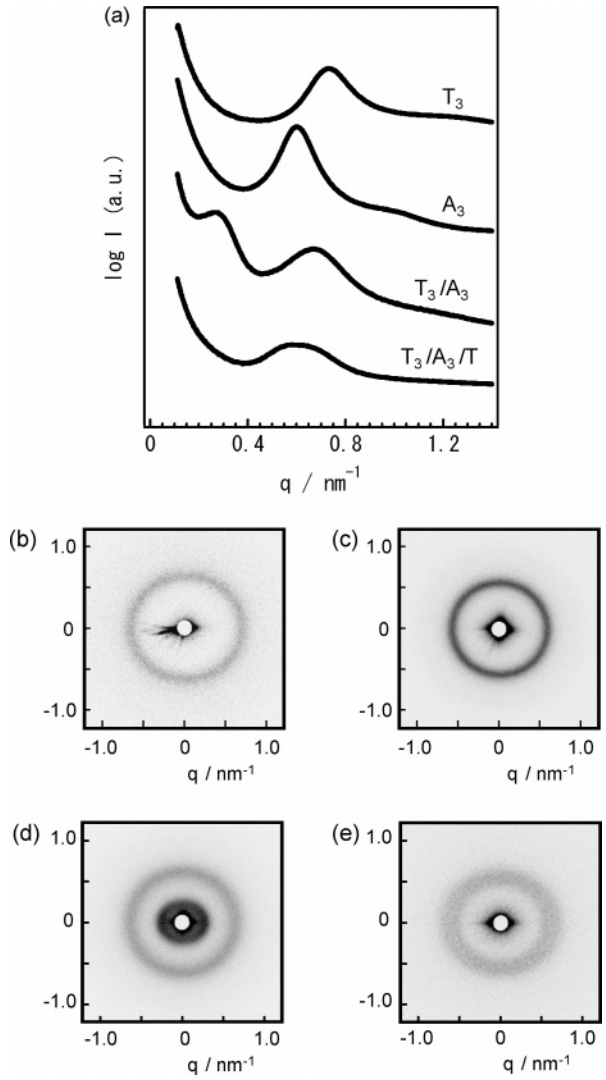

Figure 4. SAXS results for homopolymers and blends. (a) One-dimensional SAXS profiles. The horizontal axis expresses scattering vector, q ($=4\pi$ sin θ/λ), whereas the vertical one shows diffracted intensities in logarithmic scale. The top curve is for PTMSS-T_3 (T_3), the second one for PS_{d8}-A_3 (A_3), the third one for the blend of two polymers (T_3/A_3), and the bottom one for the blend of three components (T_3/A_3/DMTr-T). Panels b−e are two-dimensional SAXS patterns: (b) PTMSS-T_3, (c) PS_{d8}-A_3, (d) the blend of two end-decorated homopolymers, (e) PTMSS-T_3/PS_{d8}-A_3/DMTr-T blend with mole ratio of 1/1/5. Horizontal and vertical axes are scattering vectors, q.

from two parent end-decorated homopolymers via biocomplementary hydrogen bonding and this leads to forming a nanophase-separated structure in bulk. The scattering peak at higher q is broad compared with the one at lower q, and its location is in the middle of the peaks for PTMSS-T_3 and PS_{d8}-A_3. This may due to the coexistence of two different phases from two free homopolymers in the blend.

Comparing the results from TEM and SAXS observations, the peak at 0.27 nm^{-1} must be the first-order diffraction for the nanophase-separated domain structure. In general, the lamellar structure gives integer-number order peaks in a SAXS profile. They should appear at 0.54 nm^{-1} for the second and 0.81 nm^{-1} for the third but located at the shoulder range of the large peak above-mentioned. In addition, the orientation of lamellae is poor so that the intensity of the higher order peaks was weakened. Thus, the higher order peaks were considered to be buried by the correlation hole peaks.

Inhibition for the formation of a block-type supramacromolecule by adding an inhibitor was also examined. In Figure 2d, a ^1H NMR chart for a solution of the blend composed of three components, that is, PTMSS-T_3, PS_{d8}-A_3, and DMTr-T (5′-O-(4,4′-dimethoxytrityl)thymidine, transgenomic) are shown. The mole ratio of PTMSS-T_3/PS_{d8}-A_3/DMTr-T in the blend is 1/1/5 (32/30/7.5 mg in $CDCl_3$ of 1.3 g). From this figure, we have found that imide protons in PTMSS-T_3 were shifted upfield from 10.2 to 11.0 ppm in Figure 2c to 9.8−10.6 ppm by adding 5 equiv of DMTr-T (more data in the Supporting Information). This result suggests that formation of hydrogen bonding between PTMSS-T_3 and PS_{d8}-A_3 was partially suppressed in solution by adding DMTr-T. To observe the structure of the blend from three components in bulk, a thin film of the blend in the mole ratio of 1/1/5 was also prepared from a chloroform solution by casting. The bottom scattering profile in Figure 4a is a one-dimensional SAXS pattern from the film edge of the three-component blend, whose two-dimensional SAXS pattern is also shown in Figure 4e. The scattering peak at lower q disappeared in Figure 4a, and only one scattering peak at higher q exists similar to the case of two homopolymers on the top and the second, though the peak broadened considerably. This result suggests that PTMSS-T_3 and PS_{d8}-A_3 were prevented from forming a diblock-type supramacromolecule by adding a large excess amount of DMTr-T as an inhibitor since this small molecule has an ability to form the association structure with PS_{d8}-A_3 preferencially owing to higher mobility (association behavior between PS_{d8}-A_3 and DMTr-T in the Supporting Information). This must be the reason for the disappearance of the peak at lower q. The peak broadening is quite natural because the most intensified position of the scattering peak for PTMSS-T_3 and that for an aggregate of PS_{d8}-A_3 and DMTr-T are comparatively different, and these two peaks form a new broad peak.

In conclusion, we have demonstrated preparation of a block-type supramacromolecule via biocomplementary hydrogen bonding by blending two different homopolymers end-decorated with oligonucleotides. The association behavior in solution was examined and confirmed by NMR, and a nanophase-separated structure in bulk was observed by SAXS and TEM. Suppression of the formation of a block-type supramacromolecule in solution and in bulk was also investigated by using a low molecular weight inhibitor, where a nanophase-separated structure was not formed preferentially. Association−dissociation behavior of the present blend system caused by biocomplementary hydrogen bonding and the control of nanostructure formation is very interesting for basic and also applied fields and this research field will extend widely soon.

Acknowledgment. We greatly thank Dr. S. Arai at the Ecotopia Science Institute in Nagoya University for his help in taking transmission electron micrographs and also Mr. T. Hikage at High Intensity X-ray Diffraction Laboratory in Nagoya University for his help in measuring X-ray scattering. This work was supported by Grants-in-Aid for Scientific Research (Exploratory Research #17651066) from the Ministry of Education, Science, Sports, and Culture, and was also supported by the 21st century COE Program entitled "The Creation of Nature-Guided Materials Processing". We, especially A.N., thank them for their financial assistance.

Supporting Information Available. Details of syntheses, characterizations, and morphological observations for oligonucleotides-terminated polymers themselves and the blend.

Those materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Matsuo, M.; Sagae, S.; Asai, H. *Polymer* **1969**, *10*, 79−87.
(2) Helfand, E. *Macromolecules* **1975**, *8*, 552−556.
(3) Matsushita, Y.; Mori, K.; Saguchi, R.; Nakao, Y.; Noda, I.; Nagasawa, M. *Macromolecules* **1990**, *23*, 4313−4316.
(4) Mogi, Y.; Kotsuji, H.; Kaneko, Y.; Mori, K.; Matsushita, Y.; Noda, I. *Macromolecules* **1992**, *25*, 5408−5411.
(5) Bates, F. S.; Fredrickson, G. H. *Annu. Rev. Phys. Chem.* **1990**, *41*, 525−557.
(6) Jenekhe, S. A.; Chen, X. L. *Science* **1998**, *279*, 1903−1907.
(7) Zhao, C.; Huo, Q.; Feng, J.; Chmelka, B. F.; Stucky, G. D. *J. Am. Chem. Soc.* **1998**, *120*, 6024−6036.
(8) Discher, B. M.; Won, Y.-Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143−1146.
(9) Cheng, J. Y.; Ross, C. A.; Chan, V. Z.-H.; Thomas, E. L.; Lammertink, R. G. H.; Vancso, G. J. *Adv. Mater.* **2001**, *13*, 1174−1178.
(10) Dubertret, B.; Skourides, P.; Norris, D. J.; Noireaux, V.; Brivanlou, A. H.; Libchaber, A. *Science* **2002**, *298*, 1759−1762.
(11) Sundrani, D.; Darling, S. B.; Sibener, S. J. *Nano Lett.* **2004**, *4*, 273−276.
(12) Ruokolainen, J.; Makinen, R.; Torkkeli, M.; Makela, T.; Serimaa, R.; ten Brinke, G.; Ikkala, O. *Science* **1998**, *280*, 557−560.
(13) Harada, A.; Kataoka, K. *Science* **1999**, *283*, 65−67.
(14) Russell, T. P. *Science* **2002**, *297*, 964−967.
(15) Pispas, S.; Floudas, G.; Pakula, T.; Lieser, G.; Sakellariou, S.; Hadjichristidis, N. *Macromolecules* **2003**, *36*, 759−763.
(16) Jiang, S. M.; Gopfert, A.; Abetz, V. *Macromolecules* **2003**, *36*, 6171−6177.
(17) Akiba, I.; Masunaga, H.; Sasaki, K.; Jeong, Y.; Sakurai, K.; Hara, S.; Yamamoto, K. *Macromolecules* **2004**, *37*, 1152−1155.
(18) Asari, T.; Matsuo, S.; Takano, A.; Matsushita, Y. *Macromolecules* **2005**, *38*, 8811−8815.
(19) Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Hirschberg, J. H. K. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601−1604.
(20) Yamauchi, K.; Lizotte, J. R.; Hercules, D. M.; Vergne, M. J.; Long, T. E. *J. Am. Chem. Soc.* **2002**, *124*, 8599−8604.
(21) Lohmeijer, B. G. B.; Schubert, U. S. *Angew. Chem., Int. Ed.* **2002**, *41*, 3825−3829.
(22) Beck, J. B.; Rowan, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 13922−13923.
(23) Yang, X.; Hua, F.; Yamato, K.; Ruckenstein, E.; Gong, B.; Kim, W.; Ryu, C. Y. *Angew. Chem., Int. Ed.* **2004**, *43*, 6471−6474.
(24) Binder, W. H.; Bernstoff, S.; Kluger, C.; Petraru, L.; Kunz, M. J. *Adv. Mater* **2005**, *17*, 2824−2828.
(25) Li Z.; Zhang Y.; Fullhart, P.; Mirkin, C. A. *Nano Lett.* **2004**, *4*, 1055−1058.
(26) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607−609.
(27) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. *Nature* **1996**, *382*, 609−611.
(28) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849−1862.
(29) Boal, A. K.; Ilhan, F.; DeRouchey, J. E.; Thurn-Albrecht, T.; Russell, T. P.; Rotello, V. M. *Nature* **2000**, *404*, 746−748.
(30) Fogleman, E. A.; Yount, W. C.; Xu, J.; Craig, S. L. *Angew. Chem., Int. Ed.* **2002**, *41*, 4026−4028.
(31) Yamauchi, K.; Lizotte, J. R.; Long, T. E. *Macromolecules* **2002**, *35*, 8745−8750.
(32) Sivkova, S.; Rowan, S. J. *Chem. Comm.* **2003**, *19*, 2428−2429.
(33) Immoos, C. E.; Lee, S. J.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2004**, *126*, 10814−10815.
(34) Noro, A.; Nagata, Y.; Tsukamoto, M.; Hayakawa, Y.; Takano, A. Matsushita, Y. *Biomacromolecules* **2005**, *6*, 2328−2333.
(35) de Gennes, P.-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.

BM0602280