

Selected Paper

Immobilization of Polythiophene Chirality Induced by a Helix-Forming β-1,3-Glucan Polysaccharide (Schizophyllan) through Sol–Gel Reaction

Shuichi Haraguchi,[1] Munenori Numata,[2] Kenji Kaneko,[3] and Seiji Shinkai*[1]

[1]Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395

[2]Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, 1-1-1 Noji Higashi, Kusatsu 525-8577

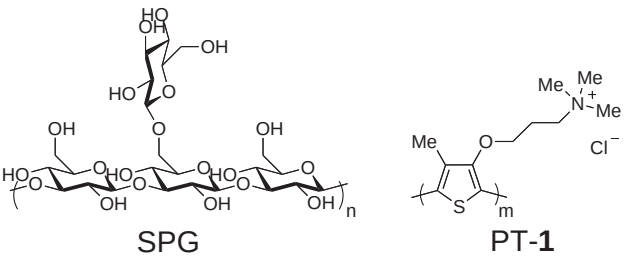
[3]Department of Material Science and Engineering, Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395

Received January 16, 2008; E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp

It is known that an achiral water-soluble polythiophene (PT-**1**) is chirally twisted by complexation with a natural helix-forming polysaccharide, shizophyllan (SPG). We found that the chirality can be immobilized by sol–gel reaction of tetraethoxysilane (TEOS) with benzylamine as a catalyst ("solution mechanism"), where SPG acts not only as a chirality inducer for PT-**1** but also as a chemical glue to adsorb silica particles onto the composite surface. When a complex with amine-appended SPG (SPG-NH) was used as a template, the sol–gel reaction proceeded according to the "surface mechanism," where silica particles were formed mainly on the composite surface due to the catalytic effect of the covalently bound amino groups and thus the reaction conditions became milder. It was confirmed that under the "surface mechanism" the chemical and physical properties of the original PT-**1**/SPG-NH composite are maintained more efficiently.

Recently, there has been a growing interest in the development of conjugated-polymer (CP)-based molecular wires in view of their theoretical importance for the exploration of new physical properties arising from the π-electron delocalization and for the potential applications to molecular electronics[1] and sensory signal amplifications.[2] CPs are often described as one-dimensional molecular wires in current nanotechnology programs, but they are usually obtained as two- or three-dimensional figures due to their strong aggregation tendency both in solution and solid phases. This makes it a challenging subject to fabricate CP molecular wires on a single-molecular scale. Hitherto, several intriguing strategies have been developed to solve this problem by designing insulated molecular wires through covalent or noncovalent approaches, in which the CP backbones are encapsulated by a protective sheath, such as threading CPs through cyclophanes[3] or cyclodextrins,[4] wrapping them within dendrimeric wedges,[5] limiting interchain interactions, etc. More recently, we reported our novel findings on the preparation of a novel supramolecular chiral insulated molecular wire by self-assembling between an achiral water-soluble polythiophene (PT-**1**) and a natural polysaccharide, schizophyllan (SPG: Scheme 1).[6] To the best of our knowledge, this has become the first observation of supramolecularly insulated molecular wires with a helical structure in a chiral sense.

SPG is a β-1,3-glucan polysaccharide and is known to exist as a right-handed triple helix (t-SPG) in water but as a single random coil (s-SPG) in dimethyl sulfoxide (DMSO).[7] When water is added to its DMSO solution, s-SPG collapses owning to hydrophobic interaction and retrieves its original triple helix structure (renaturation).[7] These specific structural characteristics make s-SPG form stable water-soluble complexes with certain polynucleotides, single-walled carbon nanotubes, and hydrophobic polymers during its renaturation process.[8] We thus confirmed that SPG can be used as a one-dimensional host to trap single-chain PT-**1** to form insulated molecular wire and the main chain of PT-**1** is chirally twisted owing to the right-handed helical structure of SPG.[6] Here, it occurred to us that if this chirality can be fixed and the composite obtained in solution can be "solidified," it would act as an interesting functional material. It is already known that certain polysaccharides and saccharide-appended molecular assemblies can act as templates to adsorb silica particles onto their surfaces.[9,10] This suggests a possibility that the PT-**1**/SPG composite would be immobilized by sol–gel reaction occurring on the surface of SPG. We have found that (1) sol–gel reaction of tetraethoxysilane (TEOS) in the presence of the PT-**1**/SPG composite yields fibrous silica and (2) the red-shifted absorption maximum and the CD activity of PT-**1** are maintained even after sol–gel reaction: that is, the unique characters of PT-**1** induced



Scheme 1. Chemical structures of schizophyllan (SPG) and cationic polythiophene (PT-**1**).

Published on the web August 11, 2008; doi:10.1246/bcsj.81.1002

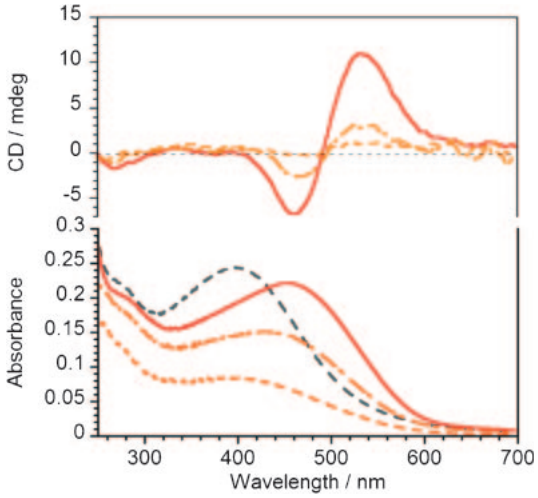


Figure 1. UV–vis absorption spectra (lower) and CD spectra (upper); (black dashed line) PT-**1** itself, (red solid line) PT-**1**/SPG, (orange dashed line) PT-**1**/SPG after 2 and 10 days sol–gel reaction; for experimental conditions see the text. The ordinate unit for PT-**1**/SPG after sol–gel reaction is arbitrary.

by SPG complexation can be immobilized and converted to "solid" by sol–gel reaction.

Results and Discussion

The PT-**1**/SPG composite was prepared, according to a reported procedure,[6] by mixing 0.5 mL of 6.0 mmol dm^{-3} (in monomer unit) PT-**1** DMSO solution and 0.5 mL of 12 mmol dm^{-3} (in repeating unit) SPG DMSO solution with 1.9 mL of water. The solution thus prepared consisted of water:DMSO = 95:5 v/v, [PT-**1**] = 0.15 mmol dm^{-3} (in monomer unit), and [SPG] = 0.30 mmol dm^{-3} (in repeating unit). As shown in Figure 1, the absorption maximum (λ_{max}) of PT-**1** itself appears at 400 nm, whereas the PT-**1**/SPG composite gives the λ_{max} at 458 nm. The longer wavelength shift implies that conjugation of the PT-**1** main chain is elongated by complexation with SPG. In accord with the previous report,[6] a CD band having a positive exciton coupling with $\lambda_{\theta=0}$ = 491 nm is observed, indicating that the dipoles in the PT-**1** main chain possess a right-handed helical motif.

This composite solution was mixed with benzylamine (as catalyst) and ethanol solution containing TEOS: the final solution consisted of ethanol:water:DMSO = 7.00:2.95:0.05 v/v, [PT-**1**] = 0.045 mmol dm^{-3} (in monomer unit), [SPG] = 0.09 mmol dm^{-3} (in repeating unit), [benzylamine] = 0.47 mmol dm^{-3}, and [TEOS] = 0.34 mmol dm^{-3}. In a separate experiment, we confirmed that the addition of the same amount of ethanol scarcely changes the absorption and CD spectra of the PT-**1**/SPG composite, indicating that the composite is not decomposed by added ethanol. The sol–gel reaction was continued at 25 °C for 10 days, taking an aliquot every day for TEM observations. The samples were well washed with ethanol, deposited on a carbon-coated grid, and dried well before TEM observations. As shown in Figure 2, arrays of dots appeared after 2 days (Figure 2A), which gradually grew up to silica fibers (Figures 2B and 2C). The average diameter is ca. 15 nm. As large silica fiber cannot be found in these

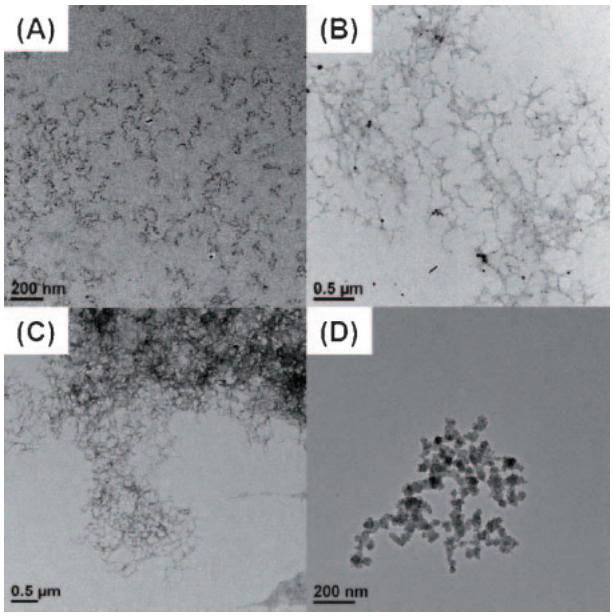


Figure 2. TEM images for the samples obtained by sol–gel reaction in the presence of PT-**1**/SPG complex; (A) after 2 days, (B) after 3 days, (C) after 10 days, and (D) the same reaction in the presence of only PT-**1** after 10 days.

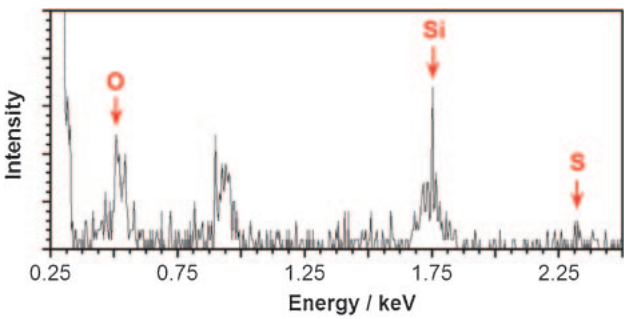
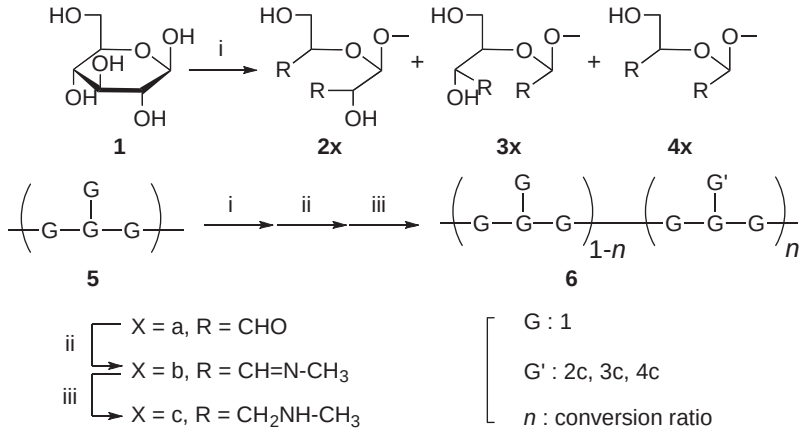


Figure 3. EDX spectrum for the nano-fiber after 10 days sol–gel reaction.

TEM images, one can presume that SPG wrapping PT-**1** efficiently suppresses aggregation or bundling of the PT-**1**/SPG composites. On the other hand, when the sol–gel reaction was carried out in the presence of only PT-**1**, the resultant image was a connection of large dots (Figure 2D), clearly different from the nano-fibers observed for the PT-**1**/SPG composite.

The obtained silica nano-fibers were characterized using several spectroscopic methods. Figure 3 is an energy dispersive X-ray spectroscopy (EDX) spectrum of the silica nano-fiber after 10 days. One can identify large O and Si peaks as well as a weak S peak. The S peak supports the presence of PT-**1** in this nano-fiber. Assuming that the O and Si peaks originated from SiO_2, then the intensity of the O peak is stronger than the theoretical intensity by 30%. One can consider, therefore, that this 30% excess is attributed to the presence of SPG. As a conclusion, this EDX spectrum evidences that the nano-fiber is a ternary composite of SiO_2, SPG, and PT-**1** (in the order from outside to inside). We also confirmed in the ATR FTIR spectra of each compound that silica has the characteris-



Scheme 2. Preparation and chemical structure of SPG-NH. Reagent and conditions: (i) $NaIO_4$, H_2O, 4 °C, 2 days, (ii) methylamine, DMSO, rt, 2 days, (iii) $NaBH_4$, DMSO, rt, 1 day.

tic Si–O–Si band at 1062 cm^{-1} and SPG has the C–O stretching band at 1049 cm^{-1}. The PT-**1**/SPG/silica ternary composite obtained here gave a broad band centered at 1049 cm^{-1}, suggesting that it involves both SPG and silica. The UV–vis absorption spectra were measured several times as the sol–gel reaction proceeded. After 2 days, the λ_{max} appeared at 431 nm, which slightly shifted to shorter wavelength from that of the aqueous PT-**1**/SPG composite (458 nm). The λ_{max} gradually shifted to shorter wavelength and after 10 days it reached a fixed wavelength of 419 nm. In the CD spectrum, a positive exciton coupling band, similar to that of the aqueous PT-**1**/SPG composite, was observed with $\lambda_{\theta=0} = 491$ nm and the CD pattern scarcely changed with reaction time. These spectroscopic results consistently support the view that the silica nano-fiber shown in Figure 2 is composed of silica, SPG and PT-**1** (from outside to inside) and the right-handed motif of PT-**1** induced by inherent right-handed helicity of SPG is immobilized in the silica gel by sol–sol reaction. On the other hand, the blue shift of the absorption maximum is related to shortage of the conjugatation length induced by sol–gel reaction.

Transcription of organic templates into shape-defined inorganic materials is generally believed to occur because of charge or hydrogen-bonding interactions between the template and the inorganic precursor; in this case, the formation of the inorganic material is always promoted by a catalyst present in solution ("solution mechanism").[11] In fact, we confirmed that sol–gel reaction of TEOS with the PT-**1**/SPG composite as a template does not proceed at all in the absence of benzylamine. On the other hand, if the catalyst is covalently bound to the organic template, the transcription process can take place via initial formation of the inorganic material (here silica) exclusively on the template surface ("surface mechanism").[11] As mentioned above, the "solution mechanism" using benzylamine as a catalyst can result in fibrous silica composite, but the λ_{max} of PT-**1** in the composite shifts to shorter wavelength by 39 nm. This implies that micro silica particles formed in solution are deposited onto the PT-**1**/SPG composite surface and reduced the main-chain conjugation length of PT-**1**. We expected, therefore, that the "surface mechanism," which can occur under milder reaction conditions, would much less influence the helical structure and the conjugation length of PT-**1** included

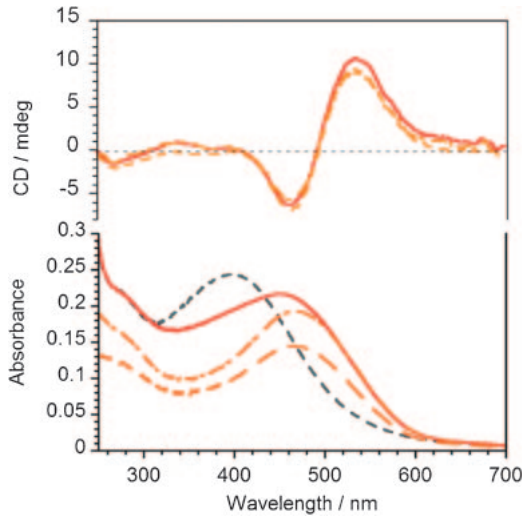


Figure 4. UV–vis absorption spectra (lower) and CD spectra (upper); (black dashed line) PT-**1** itself, (red solid line) PT-**1**/SPG-NH, (orange dashed line) PT-**1**/SPG-NH after 2 and 10 days sol–gel reaction; for experimental conditions see the text. The ordinate unit for PT-**1**/SPG-NH after sol–gel reaction is arbitrary.

in SPG. We thus introduced amino groups, which are expected to work as a catalyst for the surface mechanism, into SPG according to a reported procedure (periodate oxidation of the glucose side-chain followed by reductive amination with methylamine: Scheme 2).[12] The content of the amine-pendent glucose unit in thus obtained SPG-NH was estimated to be 14.2 mol % from elemental analysis.

We first prepared the PT-**1**/SPG-NH complex by the same method mentioned above. As both the UV–vis and CD spectra (Figure 4) were very similar to those for the PT-**1**/SPG complex, one may regard that a similar complex is also formed from PT-**1** and SPG-NH. Sol–gel reaction was carried out without catalyst, expecting that the covalently bound amino groups would initiate the reaction. As a reference experiment, we confirmed that in the presence of the PT-**1**/SPG complex sol–gel reaction does not proceed at all without catalyst (vide supra). Very interestingly, the λ_{max} of the PT-**1**/SPG-NH complex in the UV–vis spectrum (461 nm) is scarcely changed (or

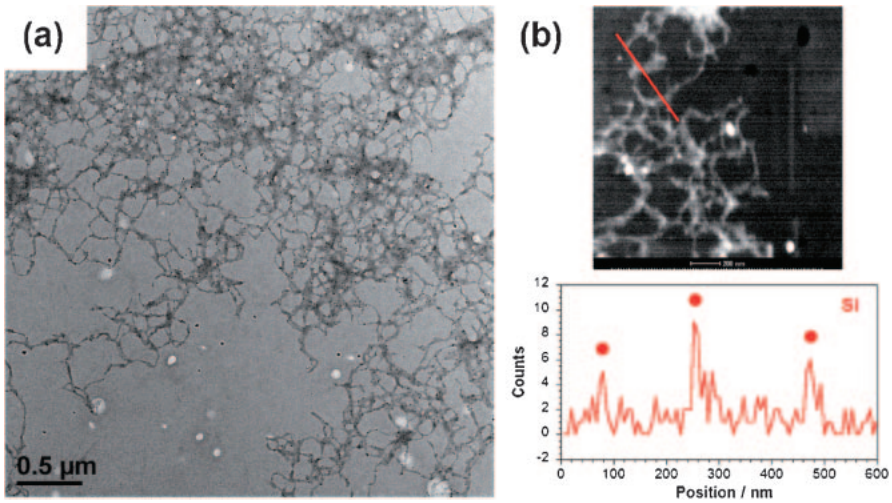


Figure 5. (a) TEM image for the sample obtained by 2 days sol–gel reaction in the presence of PT-**1**/SPG-NH complex, (b) EDX pattern for Si atom; ● indicates the position where EDX crosses over the white fiber.

slightly shifts to longer wavelength) even after sol–gel reaction according to the "surface mechanism." This implies that the main-chain conjugation length was scarcely affected by sol–gel reaction: that is, in order to immobilize the higher-order structure as it is in the silica matrix, the "surface mechanism" is superior to the "solution mechanism."

Figure 5 shows a TEM image and an EDX pattern for Si atom. It is seen from the TEM image that clean fibrous composites are formed according to the "surface mechanism." Comparison of the 2 days TEM images (Figure 2 and Figure 4) indicates that the reaction speed according to the "surface mechanism" is faster than the "solution mechanism" under the present sol–gel reaction conditions, although the latter can be accelerated by the increase in the catalyst concentration.

Conclusion

In summary, we found that a few characteristic properties of PT-**1** induced by inclusion in SPG can be immobilized by sol–gel reaction of TEOS. Therein, SPG plays bimodal roles, i.e., as a chirality inducer for PT-**1** and as a chemical glue for silica particles. The right-handed helical motif of PT-**1** can be maintained by both the "solution mechanism" and the "surface mechanism," but only the "surface mechanism" can maintain longer conjugation length. We believe, therefore, that the present concept is useful for material sciences by solidifying functional polymers and aggregates, maintaining their original chemical and physical properties.

Experimental

Materials and Methods. Native schizophyllan ($M_w = 1.5 \times 10^5$) was kindly supplied by Mitsui Seito, Co., Ltd., (Japan). The other chemicals were purchased from Aldrich or Tokyo Kasei Chemicals. UV–vis and circular dichroism (CD) spectra were measured on a Shimadzu UV-2500PC spectrometer and JASCO 720WI Circular Dichroism Spectrometer. IR spectra were measured on a Perkin-Elmer Spectrumone Fourier transform infrared spectrometer attached to a Universal ATR Sampling Accessory. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were acquired using a JEOL TEM-2010 (accelerate voltage 120 kV) and a TECNAI-20, FEI (accelerate voltage 200 kV), respectively. Energy dispersive X-ray spectroscopy (EDX) spectra and EDX line scan profiles were obtained using a TECNAI-20, FEI.

Systhesis of SPG-NH. SPG (100 mg) was dissolved in distilled water. $NaIO_4$ solution (0.04 equivalent against the side chain of SPG) was poured into the SPG solution. The mixture was stirred for 2 days at 4 °C. After dialysis with water, it was lyophilized. The white solid thus obtained by lyophilization was dissolved in DMSO and 2 mL of methylamine solution was added to this DMSO solution. The solution was stirred for 2 days at room temperature. Then, $NaBH_4$ (excess) was added into the solution and stirred for 1 day. Excess $NaBH_4$ was quenched by AcOH and dialyzed in acidic water, basic water, and then distilled water. After the solution was lyophilized, the resultant SPG was modified with the amino groups. The content of the introduced amino groups was identified by nitrogen elemental analysis, which was estimated to be 14.2%.

Sol–Gel Process. The polythiophene derivative PT-**1** and the complex with SPG were prepared with the method described previously. The PT-**1**/SPG complex was prepared by adding s-SPG DMSO solution to aqueous PT-**1** solution, and the mixed solution was incubated for 12 h at room temperature. The ratios of the s-SPG and PT-**1** solutions were chosen so that, after mixing, the concentrations of PT-**1** and SPG were 1.5×10^{-4} and 3.0×10^{-4} M, respectively, and the volume fraction of water in the mixture (Vw) was 0.95. The PT-**1**/SPG complex was utilized as an organic template in ethanol/water mixtures ([SPG] = 9.0×10^{-5} M, [PT-**1**] = 4.5×10^{-5} M, 6.6 mL), to which 70 μL of tetraethoxysilane (TEOS) and 50 μL of benzylamine were added at room temperature. The final solution consisted of ethanol: water:DMSO = 7.00:2.95:0.05 v/v, [PT-**1**] = 0.045 mmol dm^{-3} (in monomer unit), [SPG] = 0.09 mmol dm^{-3} (in repeating unit), [benzylamine] = 0.47 mmol dm^{-3}, and [TEOS] = 0.34 mmol dm^{-3}. The obtained fluid reaction mixture was allowed to stand for 2–10 days, leading gradually to precipitate of the pale yellow silica composites. After the reaction, the mixture was treated with a centrifuge (8500 rpm) for 30 min and the supernatant that contained uncomplexed PT-**1**/SPG was pipetted off. The precipitated PT-**1**/SPG/silica composite was then dispersed into wa-

ter and EtOH (500 mL and 500 mL). After repeating this centrifuge process three times, the objective suspension was obtained. The composite silica powder was obtained by lyophilization. Sol–gel transcription of PT-**1**/SPG-NH complex was also carried out according to the same method as PT-**1**/SPG. In this system no catalyst was added. Supramolecular complex from SPG-NH and PT-**1** was utilized as an organic template in ethanol/water mixtures ([SPG-NH] $= 9.0 \times 10^{-5}$ M, [PT-**1**] $= 4.5 \times 10^{-5}$ M, 6.6 mL), to which 70 μL tetraethoxysilane (TEOS) were added at room temperature. After 2–10 days sol–gel reaction, the precipitated PT-**1**/SPG-NH/silica composite was purified as described above.

This work was supported by a grant in aids: No. 18655048, and a grant in aid for Global COE Program, "Science for Future Molecular Systems" from MEXT of Japan.

References

1 a) J. M. Tour, *Chem. Rev.* **1996**, *96*, 537. b) J. Roncali, *Chem. Rev.* **1997**, *97*, 173.

2 a) D. T. McQuade, A. E. Pullen, T. M. Swager, *Chem. Rev.* **2000**, *100*, 2537. b) H.-A. Ho, M. Boissinot, M. G. Bergeron, G. Corbeil, K. Doré, D. Boudreau, M. Leclerc, *Angew. Chem., Int. Ed.* **2002**, *41*, 1548. c) M. R. Pinto, K. S. Schanze, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7505.

3 a) J. Buey, T. M. Swager, *Angew. Chem., Int. Ed.* **2000**, *39*, 608. b) J.-P. Sauvage, J.-M. Kern, G. Bidan, B. Divisia-Blohorn, P.-L. Vidal, *New J. Chem.* **2002**, *26*, 1287.

4 a) F. Cacialli, J. S. Wilson, J. J. Michels, C. Daniel, C. Silva, R. H. Friend, N. Severin, P. Samori, J. P. Rabe, M. J. O'Connell, P. N. Taylor, H. L. Anderson, *Nat. Mater.* **2002**, *1*, 160. b) J. J. Michels, M. J. O'Connell, P. N. Taylor, J. S. Wilson, F. Cacialli, H. L. Anderson, *Chem.—Eur. J.* **2003**, *9*, 6167.

5 a) R. E. Martin, F. Diederich, *Angew. Chem., Int. Ed.* **1999**, *38*, 1350. b) S. Hecht, J. M. J. Fréchet, *Angew. Chem., Int. Ed.* **2001**, *40*, 74. c) T. Sato, D.-L. Jiang, T. Aida, *J. Am. Chem. Soc.* **1999**, *121*, 10658.

6 a) C. Li, M. Numata, A.-H. Bae, K. Sakurai, S. Shinkai, *J. Am. Chem. Soc.* **2005**, *127*, 4548. b) C. Li, M. Numata, T. Hasegawa, K. Sakurai, S. Shinkai, *Chem. Lett.* **2005**, *34*, 1354.

7 a) T. Yanaki, T. Norisuye, H. Fujita, *Macromolecules* **1980**, *13*, 1462. b) T. M. McIntire, D. A. Brant, *J. Am. Chem. Soc.* **1998**, *120*, 6909.

8 a) K. Sakurai, S. Shinkai, *J. Am. Chem. Soc.* **2000**, *122*, 4520. b) M. Numata, M. Asai, K. Kaneko, T. Hasegawa, N. Fujita, Y. Kitada, K. Sakurai, S. Shinkai, *Chem. Lett.* **2004**, *33*, 232. c) S. Haraguchi, T. Hasegawa, M. Numata, M. Fujiki, K. Uezu, K. Sakurai, S. Shinkai, *Org. Lett.* **2005**, *7*, 5605. d) T. Hasegawa, S. Haraguchi, M. Numata, T. Fujisawa, C. Li, K. Kaneko, K. Sakurai, S. Shinkai, *Chem. Lett.* **2005**, *34*, 40. e) M. Numata, T. Fujisawa, C. Li, S. Haraguchi, M. Ikeda, K. Sakurai, S. Shinkai, *Supramol. Chem.* **2007**, *19*, 107.

9 a) I. Ichinose, Y. Hashimoto, T. Kunitake, *Chem. Lett.* **2004**, *33*, 656. b) J. Huang, I. Ichinose, T. Kunitake, *Angew. Chem., Int. Ed.* **2006**, *45*, 2883.

10 J. H. Jung, S. Shinkai, T. Shimizu, *Nano Lett.* **2002**, *2*, 17.

11 K. J. C. van Bommel, S. Shinkai, *Langumuir* **2002**, *18*, 4544.

12 a) K. Koumoto, T. Kimura, M. Mizu, K. Sakurai, S. Shinkai, *Chem. Commun.* **2001**, 1962. b) T. Matsumoto, M. Numata, T. Anada, M. Mizu, K. Koumoto, K. Sakurai, T. Nagasaki, S. Shinkai, *Biochim. Biophys. Acta* **2004**, *1670*, 91.