

## ORIGINAL ARTICLE

# Supramolecular nanofiber formation from commercially available arginine and a bola-type diacetylenic diacid *via* hydrogelation

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**A diacetylenic-acid arginine salt gelled water. The supramolecular structure and optical properties of the resultant hydrogel were examined by optical microscopy, electron microscopy, X-ray diffraction and ultraviolet (UV)-absorption and circular dichroism (CD) spectroscopies. Microscopic images showed that 10, 12-docosadienedioic acid arginine salt formed a fibrous structure. X-ray diffraction and nuclear magnetic resonance measurements indicated guanidinium–carboxylate interaction. Upon UV irradiation, the gel polymerized to diacetylene oligomers, with a concomitant color change from white to orange. After the removal of arginine, CD spectra indicated that the diacetylene oligomers maintained chirality.**

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**Keywords:** arginine; diacetylene; hydrogel; polymerization; self-assembly

## INTRODUCTION

Low-molecular-weight gelators (LMWGs) have attracted much interest because of their unique features and potential applications as new soft materials.<sup>1,2</sup> The LMWGs construct supramolecular 3D networks consisting of nanofibrous assemblies of amphiphilic molecules.<sup>3–9</sup> Noncovalent interactions such as hydrogen-bonding, van der Waals,  $\pi$ – $\pi$  and electrostatic interactions function as driving forces to stabilize these supramolecular nanostructures.<sup>10,11</sup>

Polymerization of constituent molecules in supramolecular self-assemblies<sup>12</sup> will provide stable covalent assemblies. In particular, polymerization of the LMWGs with diacetylene groups has been actively investigated,<sup>13–16</sup> as simple photoirradiation induces polymerization or oligomerization if they arrange and align in a good order. The connection of amide, aromatic or ionic groups to diene motives through synthetic derivation enables one to attain appropriate molecular arrangement to the polymerization. In most cases, however, the derivation requires several tedious steps. Moreover, a careful handling is necessary at every step to avoid photopolymerization of the synthetic intermediates under light.

We have widely investigated the supramolecular nanostructures self-assembled from bola-amphiphiles.<sup>16–23</sup> For example, we previously reported that butadiyne 1-glucosamide connected bola-amphiphiles self-assemble to form nanofibers, and polymerization by photoirradiation causes the gelation of supramolecular networks in organic solvents.<sup>16</sup> In this synthesis, we always needed multistep derivation to attain the gelation.

In the present work, we propose a new simple bola-type diacetylenic gelator. Inspired by the reports on gel-like systems of fatty acids and arginine,<sup>24,25</sup> we examined a salt of arginine and bola-type diacetylene diacid 10, 12-docosadienedioic acid (DDA) for hydrogelation (Figure 1). The salt gelator requires no multistep synthetic derivation. The hydrogel system showed formation of nanofibers and their stabilization by photo-oligomerization as well as chirality transfer to the nanofibers after the removal of the chiral amino acid.

## EXPERIMENTAL PROCEDURE

### Materials and general methods

DDA, 5, 7-dodecadienedioic acid and 10, 12-octadecadienoic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). L-arginine and D-arginine were purchased from Tokyo Chemical Industry (Tokyo, Japan). The other chemicals were commercially available in high-purity grades and were used without further purification. The structures of intermediates and the final products were confirmed by nuclear magnetic resonance (NMR) spectroscopy. <sup>1</sup>H-NMR spectra were recorded on a JEOL 400 spectrometer (JEOL, Tokyo, Japan) using 3-(trimethylsilyl) propionic-2, 2, 3, 3-d<sub>4</sub> acid sodium salt as an internal standard.

### Measurements

Electronic absorption spectra were recorded on a Hitachi U-3300 spectrophotometer (Hitachi, Tokyo, Japan). Circular dichroism spectra were recorded on a JASCO J-820 spectrometer (JASCO, Tokyo, Japan). Scanning electron micrographs were obtained using an S-4800 electron microscope (Hitachi

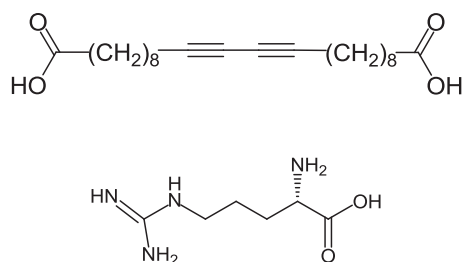
High-tech Inc., Tokyo, Japan). Bright-field microscopic images were obtained using an Olympus IX71 microscope (Olympus, Tokyo, Japan). Powder X-ray diffraction (XRD) patterns of DDA powders and freeze-dried hydrogels (xerogels) were taken by a flat camera method on a Rigaku diffractometer (Type 4037, Rigaku, Tokyo, Japan) using a graded elliptical side-by-side multilayer optics monochromated at  $\text{CuK}\alpha$  radiation ( $\lambda = 0.1542 \text{ nm}$ , 40 kV, 30 mA) and an image plate (Rigaku R-Axis IV system, Rigaku).

## RESULTS AND DISCUSSION

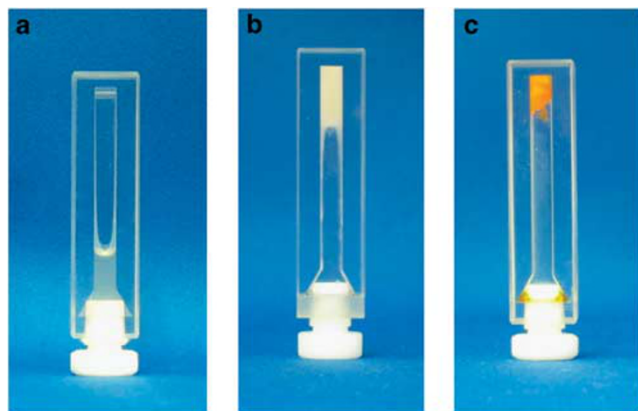
### Hydrogelation behavior of the arginine–DDA salt

In a capped glass bottle or quartz cell, a gelator in water was dissolved by heating, and the mixture was allowed to stand at room temperature. After 24 h, the gelation was tested by turning the glass vial upside down. When the solid aggregate mass was stable to the inversion of the container at room temperature, the sample was recognized to form a gel.

The results of the gelation tests are shown in Figure 2. As DDA scarcely dissolves in pure water, we dissolved DDA in aqueous sodium hydroxide by heating. After 24 h at room temperature, the sample showed a slightly turbid suspension. Without arginine, DDA forms no hydrogel. In contrast, DDA dissolved in water by heating in an aqueous arginine solution. After 24 h at room temperature, a white hydrogel was obtained (Figure 2b, the DDA–arginine hydrogel). The result indicates that the salt formation of DDA with L-arginine led to supramolecular hydrogelation. When the hydrogel was irradiated with ultraviolet (UV) (the wavelength = 254 nm, 20 W UV lamp) for 3 h, the color changed from white to orange with maintaining the gel state (Figure 2c). The change in color suggests photopolymerization or oligomerization of the diacetylene group.



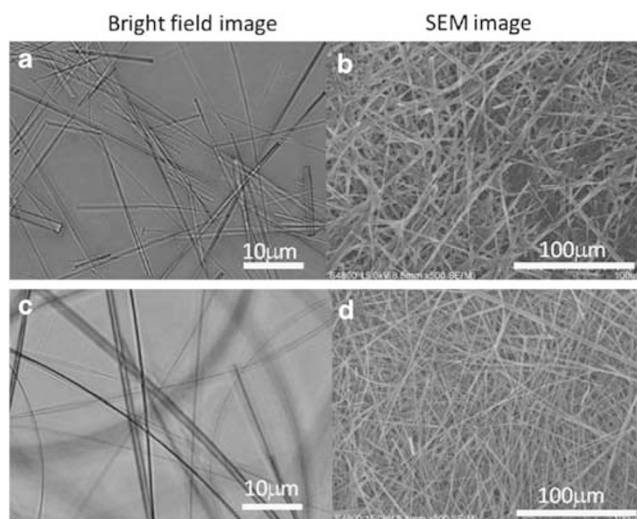
**Figure 1** 10, 12-Docosadiynoic acid (DDA) and L-arginine.



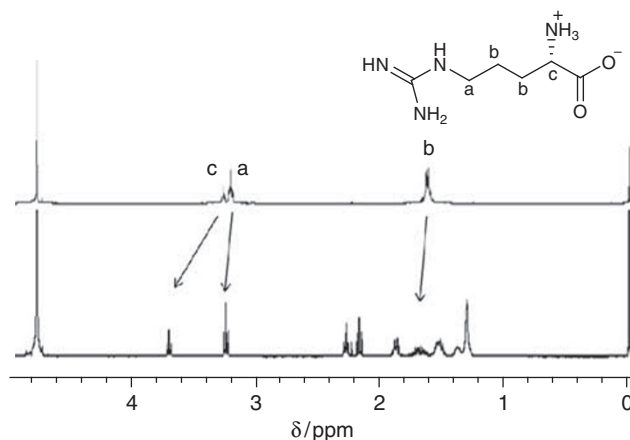
**Figure 2** Photographs of the (a) DDA dispersion, (b) DDA–arginine hydrogel and (c) hydrogel after photoirradiation. DDA: L-arginine = 1: 2.5 (mol/mol), 2.5 wt% DDA.

Figure 3 shows microscopic images of the DDA–arginine hydrogel before and after photoirradiation. Both the hydrogels clearly showed a fibrous structure. This result suggests that the resultant fibrous structures provide a three-dimensional network structure, which entraps water molecules in the interstitial spaces of the network. The scanning electron micrographs image of the hydrogel revealed a tape-like morphology (Figure 3b). The fibers were 0.1–1  $\mu\text{m}$  wide and tens of  $\mu\text{m}$  long (Figures 3b and d). No morphological change was observed between the samples before and after photoirradiation, while the bright-field microscopic images showed the fiber color changed to pink (Figures 3a and c). This result suggests the occurrence of photopolymerization in the fibrous structures without any drastic change of molecular arrangement.

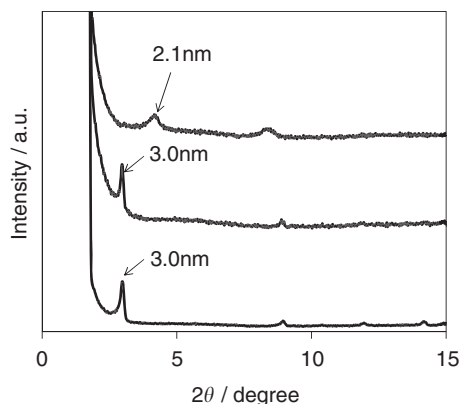
We examined the interaction between L-arginine and DDA using 400-MHz  $^1\text{H}$ -NMR spectroscopy (Figure 4). L-arginine gave three peaks at 3.27, 3.21 and 1.63 p.p.m. in deuterium oxide. Upon addition of DDA, the L-arginine peaks shifted to 3.73, 3.27 and 1.64–1.80 p.p.m. (a multiplet peak), respectively. This observation is



**Figure 3** Microscopic images of the (a, b) DDA hydrogel and (c, d) gels after photoirradiation. (a) and (c): bright-field light microscopic images. (b) and (d): scanning electron microscopic images. A full color version of this figure is available at *Polymer Journal* online.



**Figure 4** NMR spectra of L-arginine (upper) and DDA–arginine solution diluted from the hydrogel (lower).



**Figure 5** XRD diffractograms of the DDA powders (upper), the xerogel from the DDA–arginine hydrogel (middle), and the xerogel from the hydrogel after photoirradiation (bottom). The  $d$ -spacing values of (001) peaks are shown with arrows.

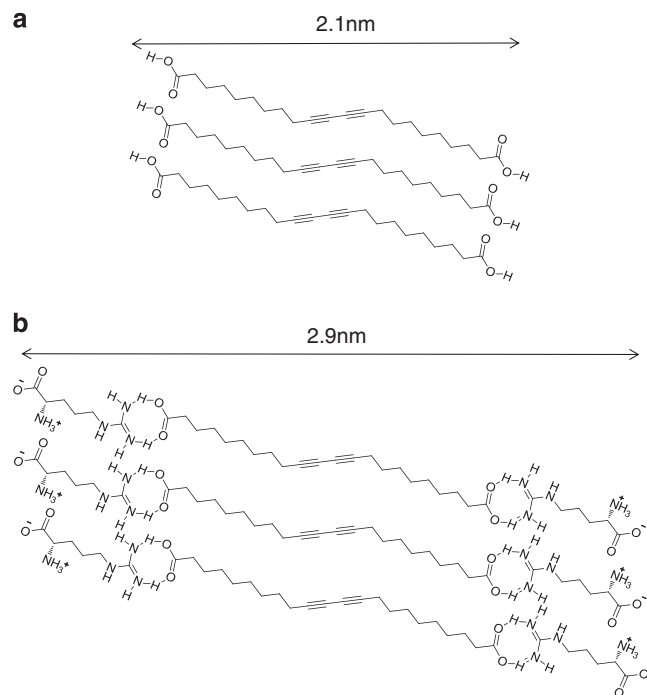
concomitant with the fact that L-arginine and DDA interact with each other owing to salt formation.

Figure 5 shows the XRD patterns of the DDA powders and xerogels from the DDA–arginine hydrogels before and after photoirradiation. The XRD pattern of the DDA powders (the upper diffractogram in Figure 5) gave Bragg peaks at  $d$ -spacing of 2.1 and 1.0 nm, whose ratio is practically 1:1/2. The dried DDA–arginine hydrogel before photoirradiation (the middle diffractogram) showed the peaks at 3.0 and 1.0 nm in the small-angle region (the ratio of 1:1/3), and the hydrogel after photoirradiation (the bottom diffractogram) gave  $d$ -spacing values of 3.0, 1.0, 0.7 and 0.5 nm (practically 1:1/3:1/4:1/5). The relatively sharp diffraction peaks indicate an ordered arrangement of the molecules in a layered structure of the gel, where each layer is composed of a bola-type diacid with or without arginine. The layers were 2.1, 3.0 and 3.0 nm thick for the DDA powders, the xerogels from the hydrogels before and after photoirradiation, respectively. The relatively greater layer thickness of the gels suggests that arginine molecules were inserted between the DDA layers *via* salt formation. This structural feature is in good agreement with the NMR result. As the diffraction patterns were almost similar to each other before and after photoirradiation, the microscopic observations can also support that the molecular arrangement in gels was not drastically affected by polymerization of DDA.

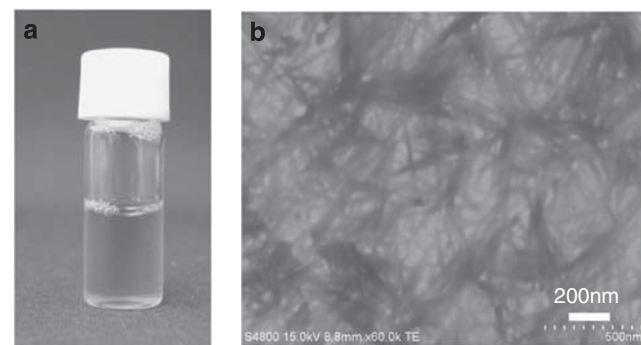
Figure 6 schematically illustrates the molecular arrangement of DDA in a solid state and the DDA–arginine hydrogel. As the obtained layer thickness of DDA (2.1 nm) is shorter than the fully extended molecular length of DDA (2.9 nm), the DDA molecules tilt in the solid layer structure. In the layer structure of the DDA–arginine hydrogels, the guanidinium group of L-arginine interacts with the carboxylate moieties of the DDA molecule. Generally guanidinium–carboxylate interaction proves to be a strong noncovalent bond, as reported for fatty acids and arginine.<sup>24,25</sup> The present NMR and XRD results strongly support this DDA–arginine interaction. At the pH, the amino acid forms a zwitterion of  $\text{-NH}_3^+$  cation and  $\text{-COO}^-$  anion at the amino acid  $\alpha$  position.<sup>24,25</sup> It is very likely that the zwitterion forms salt bridges between the neighboring layers and stabilizes the layered structure in the nanofibers.

#### Characterization of the hydrogel after arginine removal

We can expect that, under low pH conditions, the guanidinium–carboxylate interaction decreases. Therefore, arginine becomes



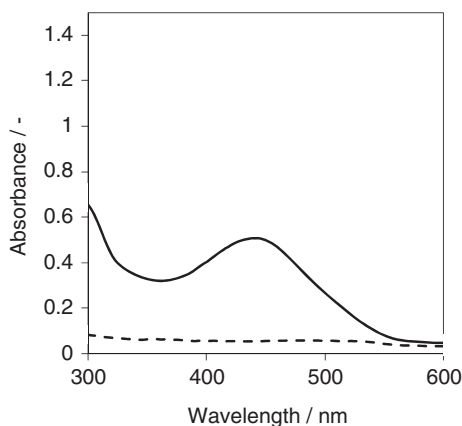
**Figure 6** Schematic illustrations of the (a) bilayer structures of the DDA powders and (b) DDA–arginine hydrogel.



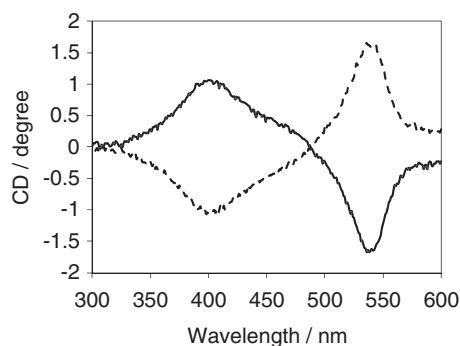
**Figure 7** A photograph of the DDA derivative dispersed in sodium hydroxide (a) and a scanning electron micrograph image of the dried sample (b) after the removal of L-arginine from the photoirradiated DDA–arginine hydrogel. A full color version of this figure is available at *Polymer Journal* online.

soluble, but DDA derivatives are insoluble.<sup>24,25</sup> Washing the hydrogel sample with hydrochloric acid (1 M) we successfully removed L-arginine from the photoirradiated DDA–arginine hydrogel. After filtration and evaporation of the solvent, the residue was dried *in vacuo*. The complete removal of L-arginine was confirmed in the NMR spectrum. Figure 7 shows the sample dispersion in aqueous sodium hydroxide and the scanning electron micrograph image of the dried sample. In contrast to the dispersion of the unirradiated DDA (Figure 2a), the photoirradiated DDA derivative gave an orange suspension (Figure 7a), which indicates the polymerization or oligomerization in the fibers. The scanning electron micrograph image showed fibrous structures 10–40 nm wide (Figure 7b).

Figure 8 shows UV spectra of the DDA derivative and DDA in aqueous sodium hydroxide. The DDA solution showed no distinct peak in the wavelength region from 300 to 600 nm. In contrast, the photoirradiated DDA derivative showed a broad peak at around 438 nm with an overlapped weak peak at around 540 nm. The



**Figure 8** UV spectra of DDA derivative (solid line) and DDA (dashed line) in aqueous sodium hydroxide (0.06 wt% DDA or DDA derivative in 0.5 M sodium hydroxide).



**Figure 9** CD spectra of the DDA derivatives after removal of L-arginine (solid line) and D-arginine (dashed line) (0.06 wt% DDA derivative in 0.5 M sodium hydroxide).

spectrum is typical of diacetylene oligomers. Evidently, the DDA sample contained the oligomers formed by the photopolymerization. Polhuis *et al.*<sup>26</sup> have reported that a synthetic tetra-diacetylene shows an UV absorption maximum around 360 nm. It implies the present sample contained tetramers or longer oligomers, though the UV absorption maximum can change sensitively to the molecular structure. The precise polymerization degree of the oligomers was not successfully measured by mass spectroscopy or gel permeation chromatography.

In the system we found that the chirality of arginine in the hydrogels was transferred to the DDA oligomers in the fibrous structure. Two hydrogels were separately prepared with DDA and L-arginine or D-arginine. After photoirradiation, the arginine was completely removed from the hydrogel samples. Figure 9 shows circular dichroism (CD) spectra of the DDA derivatives after removal of L-arginine and D-arginine. Both DDA derivative solutions exhibit prominent absorption bands at 400 and 540 nm, which are assignable to diacetylene oligomers. These spectra showed a mirror image of each other. These CD spectra are similar to the reported CD spectra of the chiral poly-diacetylene.<sup>27,28</sup> Although the arginine as a chirality source was removed, the DDA derivative was found to maintain the chirality. These results clearly indicate that the oligomerized DDA nanofibers memorize the chirality of the arginine.

It should be noted that the chemical structure of diacetylenic acid is critical to the nanofiber formation in the hydrogel. The arginine salts of 5,7-dodecadienedioic acid (a short-chain analog of DDA) and 10,

12-octadecadiynoic acid (a monoacid with a diacetylene group) form no hydrogel. The observations suggest that the bola-form diacid structure with a sufficient hydrophobic moiety is required to facilitate the appropriate molecular stacking in the layered structure. In this manner, the balance of a hydrophilic group and a hydrophobic group is important to form a supramolecular aggregate like other LWMGs.<sup>11</sup>

It has been reported that guanidinium chloride forms a fibrous nanostructure with 12-hydroxystearic acid in water.<sup>29</sup> In place of arginine, guanidinium chloride was mixed with DDA. As a result, no fibrous structure was observed in the mixture, though the guanidinium cation interacted with the carboxylic acid of DDA.

## CONCLUSION

We have successfully prepared a hydrogel using a simple salt of a bola-type diacetylenic acid and L- or D-arginine. The hydrogel proved to be made of fibrous structures of DDA-arginine salt. NMR and XRD measurements strongly suggested that guanidinium-carboxylate interaction drives the fibrous structure formation. By photoirradiation, the nanofibers showed a color change from white to orange. This color change indicated that the diyne groups oligomerize in the hydrogel with keeping the fibrous morphology. Even after L- or D-arginine was removed, the oligomerized samples in dispersion maintained the nanofiber structures and the chirality.

The present method may provide some advantages in the preparation of nanofibers. Hydrogelation can occur with a simple salt of the acid component and arginine in water. It requires no multistep, tedious synthetic derivation from a diacetylenic starting material. After photoirradiation, the oligomerized nanofibers are stable in water keeping the fibrous structure. In addition, the arginine can be replaced with another component on the surface of the polymerized fibrous structure. With the salt formation, the nanofibers may show a change in color or dispersibility depending on the amino acid species or its chirality. Further characterization of the relating gel systems are in progress in our laboratory.

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- 1 Wang, C., Chen, Q., Sun, F., Zhang, D., Zhang, G., Huang, Y., Zhao, R. & Zhu, D. Multistimuli responsive organogels based on a new gelator featuring tetrathiafulvalene and azobenzene groups: reversible tuning of the gel-sol transition by redox reactions and light irradiation. *J. Am. Chem. Soc.* **132**, 3092–3096 (2010).
- 2 Dutta, S., Das, D., Dasgupta, A. & Das, P. K. Amino acid-based low-molecular-weight ionogels as efficient dye-adsorbing agents and template for the synthesis of TiO<sub>2</sub> nanoparticles. *Chem. Eur. J.* **16**, 1493–1505 (2010).
- 3 Ojida, A., Mito-oka, Y., Sada, K. & Hamachi, I. Molecular recognition and fluorescence sensing of monophosphorylated peptides in aqueous solution by bis(zinc(II)-dipicolylamine)-based artificial receptors. *J. Am. Chem. Soc.* **126**, 2454–2463 (2006).
- 4 Naota, T. & Koori, H. Molecules that assemble by sound: an application to the instant gelation of stable organic fluids. *J. Am. Chem. Soc.* **127**, 9324–9325 (2005).
- 5 Shiraki, T., Morikawa, M. & Kimizuka, N. Amplification of molecular information through self-assembly: nanofibers formed from amino acids and cyanine dyes by extended molecular pairing. *Angew. Chem.* **120**, 112–114 (2008).
- 6 Terech, P. & Weiss, R. G. Low molecular mass gelators of organic liquids and the properties of their gels. *Chem. Rev.* **97**, 3133–3159 (1997).
- 7 Oda, R., Hue, I. & Candau, S. J. Gemini surfactants as new, low molecular weight gelators of organic solvents and water. *Angew. Chem. Int., Ed. Engl.* **37**, 2689–2691 (1998).
- 8 Abdallah, D. J. & Weiss, R. G. Organogels and low molecular mass organic gelators. *Adv. Mater.* **12**, 1237–1247 (2000).
- 9 van Esch, J. H. & Feringa, B. L. New functional materials based on self-assembling organogels: from serendipity towards design. *Angew. Chem., Int. Ed. Engl.* **39**, 2263–2266 (2000).

- 10 Yang, Z. & Xu, B. A Simple visual assay based on small molecule hydrogels for detecting inhibitors of enzymes. *Chem. Commun* 2424–2425 (2004).
- 11 John, G. & Vemula, P.K. Design and development of soft nanomaterials from biobased amphiphiles. *Soft Matter* **2**, 909 (2006).
- 12 Fuhrop, J. -H. & Koenig, J. *Membranes and Molecular Assemblies: The Synergistic Approach* (the Royal Society of Chemistry, Cambridge, 1994).
- 13 Jung, J. H., Ono, Y., Hanabusa, K. & Shinkai, S. Creation of both right-handed and left-handed silica structures by sol-gel transcription of organogel fibers comprised of chiral diaminocyclohexane derivatives. *J. Am. Chem. Soc.* **122**, 5008–5009 (2000).
- 14 Song, J., Cheng, Q., Kopta, S. & Stevens, R. C. Modulating artificial membrane morphology: pH-induced chromatic transition and nanostructural transformation of a bolaamphiphilic conjugated polymer from blue helical ribbons to red nanofibers. *J. Am. Chem. Soc.* **123**, 3205–3213 (2001).
- 15 Tamaoki, N., Shimada, S., Okada, Y., Belaisaoui, A., Kruk, G., Yase, K. & Matsuda, H. Polymerization of a diacetylene dicholesteryl ester having two urethanes in organic gel states. *Langmuir* **16**, 7545–7547 (2000).
- 16 Masuda, M., Hanada, T., Yase, K. & Shimizu, T. Polymerization of bolaform butadiyne 1-glucosamide in self-assembled nanoscale-fiber morphology. *Macromolecules* **31**, 9403–9405 (1998).
- 17 Shimizu, T., Kogiso, M. & Masuda, M. Vesicle assembly in microtubes. *Nature* **383**, 487–488 (1996).
- 18 Matsuzawa, Y., Kogiso, M., Matsumoto, M., Shimizu, T., Shimada, K. & Kinugasa, S. Phase behavior and spherical hollow particle formation by dipeptide-based two-headed amphiphiles in a mixed solvent of dimethyl sulfoxide and water. *Colloids Surf* **A297**, 191–197 (2007).
- 19 Shimizu, T., Masuda, M. & Minamikawa, H. Supramolecular nanotube architectures based on amphiphilic molecules. *Chem. Rev.* **105**, 1401–1443 (2005).
- 20 Iwaura, R., Iizawa, T., Minamikawa, H., Ohnishi-Kameyama, M. & Shimizu, T. Diverse morphologies of self-assemblies from homoditopic 1,18-nucleotide-appended bolaamphiphiles: effects of nucleobase and complementary oligonucleotide. *Small* **6**, 1131–1139 (2010).
- 21 Iwaura, R., Kameyama, M. & Shimizu, T. Nanofiber formation from sequence-selective DNA-templated self-assembly of a thymidic acid-appended bolaamphiphile dependence. *Chem. Commun.* 5770–5772 (2008).
- 22 Iwaura, R., Kikkawa, Y., Ohnishi-Kameyama, M. & Shimizu, T. Effects of oligodna template length and sequence on binary self-assembly of a nucleotide bolamphiphile. *Org. Biomol. Chem.* **5**, 3450–3455 (2007).
- 23 Jung, J. H., Rim, J. A., Cho, E. J., Lee, S. J., Jeong, I. Y., Kameta, N., Masuda, M. & Shimizu, T. Stabilization of an asymmetric bolaamphiphilic sugar-based hydrogel by hydrogen bonding interaction and its sol-gel transcription. *Tetrahedron* **63**, 7449–7456 (2007).
- 24 Hirai, A., Kawasaki, H., Tanaka, S., Nemoto, N., Suzuki, M. & Maeda, H. Effects of L-arginine on aggregates of fatty-acid/potassium soap in the aqueous media. *Colloid Polym Sci.* **284**, 520–528 (2006).
- 25 Kaneko, T., Yamaoka, Y., Kaise, C., Orita, M., Sakai, H. & Abe, M. Preparation and characteristics of arginine oleate liquid crystal holding a large amount of water. *J. Oleo Sci.* **54**, 325–333 (2005).
- 26 Polhuis, M., Hendriks, C. C. J., Zuilhof, H. & Sudholter, E. J. R. Synthesis of oligoenynes and oligomeric conjugated diacetylenes. *Tetrahedron Lett.* **44**, 899–901 (2003).
- 27 Manaka, T., Kon, H., Ohshima, Y., Zou, G. & Iwamoto, M. Preparation of chiral polydiacetylene film from achiral monomers using circularly polarized light. *Chem. Lett.* **35**, 1028–1029 (2006).
- 28 Huang, X. & Liu, M. Chirality of photopolymerized organized supramolecular poly-diacetylene films. *Chem. Commun.* 66–67 (2003).
- 29 Fameau, A. -L., Houinsou-Houssou, B., Ventureira, J. L., Navailles, L., Nallet, F., Novales, B. & Douliez, J. -P. Self-Assembly, foaming, and emulsifying properties of sodium alkyl carboxylate/guanidine hydrochloride aqueous mixtures. *Langmuir* **27**, 4505–4513 (2011).