

Controlled Fabrication of Uniform Digital Nanorods from Precise Sequence-Defined Amphiphilic Polymers in Aqueous Media

Qiang-Qiang Shi^a, Xin Zhou^a, Jie Xu^a, Ning Wang^a, Jia-Lin Zhang^a, Xiang-Long Hu^{a,b*}, and Shi-Yong Liu^{a*}

^a CAS Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei 230026, China

^b School of Biomedical Engineering and Suzhou Institute for Advanced Research, University of Science and Technology of China, Suzhou 215123, China

Electronic Supplementary Information

Abstract Compared with spherical micelles, rod/worm-like micelles not only have extended blood circulation duration, but also exhibit favorable cellular uptake behavior, which is promising for next-generation nanomedicine and biomaterials. However, the controllable fabrication of narrowly dispersed nanorods in aqueous media is still challenging. Herein, the methodology of thermal annealing was developed for the fabrication of helical nanorods as well as a series of nanorods with different lengths. The thermal annealing process generally consisted of adding a percentage of organic solvent (10%(V/V) or 20%(V/V)) to the digital micellar aqueous dispersion, followed by heating at 90 °C for 1 h, then cooling naturally to room temperature, and dialyzing against water to remove the organic solvent. Right-handed helical nanorods were afforded by the treatment of 45 nm digital micelles in the presence of 10%(V/V) dioxane, while left-handed helical nanorods were obtained in the presence of 20%(V/V) dioxane. Meanwhile, the controlled growth of rod-like digital micelles was achieved after thermal annealing in the presence of different types of organic solvents, and the length of the annealed nanorods was correlated with the types of organic solvent. Furthermore, no matter the size of initial digital micelles, they all exhibited similar trend of rod growth in the presence of a certain amount of organic solvent, allowing for controllable formulation of narrowly dispersed nanorods. In addition, supramolecular self-assembly by amphiphilic dendritic oligourethane readily fabricated diverse uniform nanorods in aqueous media. Overall, this work provided an attractive methodology to fabricate uniform digital nanorods.

Keywords Sequence-defined amphiphilic polymers; Digital nanorods; Thermal annealing; Dendritic oligourethane; Self-assembly

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INTRODUCTION

Rational supramolecular self-assembly to afford functional nanomaterials has attracted considerable interest for both applied and fundamental researchers.^[1–12] In particular, supramolecular assembly of block copolymers (BCPs) has provided a powerful and versatile bottom-up strategy to construct aggregates with well-defined nanostructures, attracting extensive attention.^[13–20] Among them, the aqueous self-assembly of amphiphilic BCPs has been widely employed to fabricate various morphological nanostructures, such as spherical micelles, cylinders/worm-like micelles, vesicles, and disk-like nanoparticles, revealing distinct applications in diagnosis, biomedicine, and other fields.^[21–29] Specifically, for the application in biology and medicine,^[30] some literatures

reported that rod or worm-like micelles exhibited longer *in vivo* blood circulation time and distinct cellular uptake behavior compared to spherical counterparts, which was significantly favorable for the development of next-generation nanomedicines.^[31–34]

However, it was extremely challenging to fabricate length-controlled and narrowly dispersed nanorods by the self-assembly of conventional BCPs.^[35–42] Manners and coworkers developed a seed growth strategy to easily prepare fibrous micelles with controlled length and low distribution, which was efficient and facile to achieve *via* crystallization-driven self-assembly in organic solvents.^[43–46] Nevertheless, the formulation of rod or fibrous micelles in aqueous media has received much attention, yet has been rarely concerned.^[47–50] Although the nanostructures fabricated from the assembly in aqueous media are not as abundant as those obtained in organic solvents, the nanoparticle aqueous dispersion is attractive for extensive biomedical applications.^[51–53] In addition, the inherent polydispersity of chain length for conventional BCPs and the uncertainty of the main chain sequence both have a great influence on the structure of self-assembled

* Corresponding authors, E-mail: huxlong@ustc.edu.cn (X.L.H.)

E-mail: sliu@ustc.edu.cn (S.Y.L.)

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nanoparticles.^[54,55]

The situation has been improved in the last decade with the rapid development of precision polymers.^[56–61] Benefiting from the design and successful synthesis of sequence-defined amphiphilic polymers, some smart systems have been increasingly reported to fabricate various nanostructures with controlled properties in aqueous media, including nanorods or nanofibers.^[52,62–65] Typically, Sleiman *et al.* reported a class of precision amphiphiles, DNA-*b*-oligomer, and prepared length-controlled nanorods in aqueous media.^[62,65] Meanwhile, the adjustment of length and sequence of hydrophobic segment or the length of DNA not only achieved the transition from nanorods to nanospheres, but also could control the nanorods length.^[62,65] Very recently, Liu *et al.* reported the preparation of digital micelles with lengths strictly controlled by the sequences of encoded amphiphiles in aqueous media and used for *ex vivo* label-free quantification.^[52]

Herein, based on our previous work on digital micelles, we further investigated the effect of thermal annealing process on the physicochemical properties of digital micelles.^[52] Specifically, the morphological transformation of the digital micelles upon annealing was investigated concerning different annealing conditions and different digital micelles, separately. And accordingly, a promising method to fabricate nanorods with controllable length was proposed by the thermal annealing process. A precision amphiphilic dendritic oligourethane was further prepared to examine the self-assembly behavior, affording rod-like nanostructures in aqueous media in a controlled manner. This work provided a charming methodology for the fabrication of nanorods in water.

EXPERIMENTAL

General Considerations

All the reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., and used as received unless specific need. (S)-1-bromo-3,7-dimethyloctane was obtained according to a previous literature.^[66] All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III NMR 400 MHz spectrometer operating in the Fourier transform mode. Molecular weights and molecular weight distributions were determined by size exclusion chromatography (SEC) equipped with Waters 1515 pump and Waters 2414 differential refractive index detector (set at 35 °C). It used a series of two linear Styragel columns (HR2 and HR4) at an oven temperature of 35 °C. The eluent was tetrahydrofuran (THF) at a flow rate of 1.0 mL/min. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were acquired on an Autoflex Speed MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) equipped with a Smart beam-II laser (355 nm, 1 kHz, Bruker Daltonics). The instrument was calibrated prior to each measurement with external PMMA standard and peptide calibration standard II (Bruker Daltonik GmbH, Germany) around the investigated MW range. All MALDI-TOF mass spectra were processed with 'Compass for flexSeries 1.4' software package (Bruker Daltonik GmbH, Germany).

Thermal Annealing Procedures for Digital Micelles

Typically, a general thermal annealing process was performed in the presence of 10%(V/V) organic solvent. Take the thermal annealing of 45 nm nanorods (self-assembled from T-**00000000**-dPEG) as an example, the aqueous dispersion of 45 nm nanorods (0.1 mg/mL, 450 μ L) was added into the capped vial, and then organic solvent (50 μ L) was added, and the sample vial was sealed well. Next, the sample vial was heated at 90 °C for 1 h, then the heating was stopped and the mixture cooled down to room temperature. When the thermal annealing was completed, the aqueous dispersion was dialyzed against deionized water to remove organic solvent using a dialysis bag.

Similarly, a typical thermal annealing process in the presence of 20% organic solvent was as followed. The aqueous dispersion of 45 nm nanorods (0.1 mg/mL, 400 μ L) was added into the capped vial, and then 100 μ L of organic solvent was added, and the sample vial was sealed well. The next thermal annealing and dialysis operations were as described above.

Small-angle X-ray Scattering (SAXS) Characterization

SAXS was performed at the beamline BL19U2 of Shanghai Synchrotron Radiation Facility (SSRF). A Pilatus 1M detector, mounted on railings for position adjustment, was used for the SAXS data acquisition. Data was collected with an X-ray energy of 13.5 keV (wavelength $\lambda=0.9184$ Å⁻¹). A typical sample volume of 60 μ L was tested using an automated sample changer at a sample concentration of *ca.* 2.5 mg/mL. Samples were subjected an X-ray exposure time of 1.0 s for each measurement, and each repeated for ten times (q ranges from 0.008 Å⁻¹ to 0.45 Å⁻¹, $q = (4\pi/\lambda)/\sin(\theta/2)$, where θ is the scattering angle). The acquired 2D scattering data were then reduced to plots of 1D intensity versus wave vector *via* azimuthal integration around the beam center using the BioXTAS RAW software. Background scattering patterns were recorded from pure water. The background data was then subtracted from the experimental data. The data fitting was conducted using the Igor software.

Synthesis of D-(01')₃-(OEG₈-Trt)₄

Synthetic routes employed for the preparation of D-(01')₃-(OEG₈-Trt)₄ are shown in Figs. S3–S5 (in the electronic supplementary information, ESI).

Synthesis of methyl 3,4-bis(((S)-3,7-dimethyloctyl)oxy)benzaldehyde

(S)-1-bromo-3,7-dimethyloctane (8.0 g, 36.2 mmol, 2.2 equiv.), 3,4-dihydroxybenzaldehyde (2.3 g, 16.4 mmol, 1.0 equiv.), K₂CO₃ (6.8 g, 49.2 mmol, 3.0 equiv.), and 200 mL of DMF were mixed and stirred at room temperature under nitrogen protection for 24 h. Upon completion, the solvents were removed under reduced pressure. The residues were mixed with 300 mL of ethyl acetate (EA) and washed with 200 mL of saturated NaCl solution for three times. Then the organic phase was collected and dried over anhydrous Na₂SO₄, filtered, and the solvent was removed under reduced pressure. The residues were further purified by silica gel column chromatography using PE/EA (V/V=9/1) as eluent to afford 3,4-bis(((S)-3,7-dimethyloctyl)oxy)benzaldehyde (5.6 g, yield: 81.2%). ¹H-NMR (400 MHz, CDCl₃, δ , ppm, Fig. S6 in ESI): 9.83 (s, 1H), 7.45–7.37 (m, 2H), 6.95 (s, 1H), 4.10 (dtq,

$J=13.7, 6.9, 3.6, 2.7$ Hz, 4H), 2.16–0.77 (m, 42H).

Synthesis of (3,4-bis(((S)-3,7-dimethyloctyl)oxy)phenyl)methanol

NaBH_4 (899.6 mg, 23.8 mmol, 2.0 equiv.) was slowly added into a solution of 3,4-bis(((S)-3,7-dimethyloctyl)oxy)benzaldehyde (5.0 g, 11.9 mmol, 1.0 equiv.) in 100 mL anhydrous THF, and stirred at room temperature for 4 h. Upon completion, the reaction mixture was quenched by the addition of 20 mL of methanol and all the solvents were removed under reduced pressure. Then the obtained residues were dissolved in 300 mL of EA and washed with 200 mL of saturated NaCl solution for three times. The organic phase was collected and dried over anhydrous Na_2SO_4 , filtered, and the solvent was removed under reduced pressure to afford the resultant (3,4-bis(((S)-3,7-dimethyloctyl)oxy)phenyl)methanol (4.8 g, yield: 95%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S7 in ESI): 6.94–6.73 (m, 3H), 5.04 (t, $J=5.7$ Hz, 1H), 4.39 (d, $J=5.7$ Hz, 2H), 3.94 (qd, $J=9.6, 5.4$ Hz, 4H), 2.06–0.75 (m, 40H).

Synthesis of tert-butyl 4-((2,4-diformylphenoxy)methyl)benzoate

4-Hydroxyisophthalaldehyde (8.0 g, 53.3 mmol, 1.0 equiv.), K_2CO_3 (9.6 g, 69.3 mmol, 1.3 equiv.) and 1-(bromomethyl)-4-(tert-butoxymethyl)benzene (12.3 g, 48.0 mmol, 0.9 equiv.) were dissolved in 100 mL anhydrous DMF and kept stirring at 80 °C for 12 h. Upon completion, the reaction mixture was cooled to room temperature and all solvents were removed under reduced pressure. The residue was dissolved in 300 mL of EA and washed with 100 mL of saturated NaCl solution for three times, then the organic layer was dried over anhydrous Na_2SO_4 , filtered, and evaporated to dryness under reduced pressure. The crude product was further purified by silica gel column chromatography to afford 4-((2,4-diformylphenoxy)methyl)benzoate (13.4 g, yield: 82.0%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S8 in ESI): 10.46 (s, 1H), 9.97 (s, 1H), 8.31–7.47 (m, 7H), 5.51 (s, 2H), 1.55 (s, 10H).

Synthesis of 4-((2,4-diformylphenoxy)methyl)benzoyl azide

4-((2,4-Diformylphenoxy)methyl)benzoate (10.0 g, 29.4 mmol) was dissolved in 200 mL of formic acid and stirred for 2 h at room temperature. Upon completion, all the solvents were removed under reduced pressure to afford 4-((2,4-diformylphenoxy)methyl)benzoic acid as a solid product, which was directly used for the next process. Then, 4-((2,4-diformylphenoxy)methyl)benzoic acid (7.0 g, 24.6 mmol, 1.0 equiv.) was dissolved in 200 mL of THF and TEA (3.0 g, 29.5 mmol, 1.2 equiv.) was added into the mixture. Subsequently, DPPA (8.1 g, 29.5 mmol, 1.2 equiv.) was added dropwise into mixture. After stirring for 5 h at room temperature, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (PE/EA=6/1, V/V) to afford 4-((2,4-diformylphenoxy)methyl)benzoyl azide (6.5 g, yield: 86.0%). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm, Fig. S9 in ESI): 10.57 (s, 1H), 9.97 (s, 1H), 8.38 (s, 1H), 8.10 (d, $J=7.4$ Hz, 3H), 7.56 (d, $J=7.9$ Hz, 2H), 7.17 (d, $J=8.6$ Hz, 1H), 5.38 (s, 2H).

Synthesis of D-01'-Ald₂

2Cit-OH (4.0 g, 9.5 mmol, 1.0 equiv.), 4-((2,4-diformylphenoxy)methyl)benzoyl azide (3.5 g, 11.4 mmol, 1.2 equiv.), DBTL (60.0 mg, 0.095 mmol, 0.01 equiv.) and anhydrous toluene (10 mL) were dissolved in a reaction flask. After azeotropically removing

toluene under reduced pressure at room temperature, another portion of 10 mL of anhydrous toluene was added, and the azeotropic distillation cycle was repeated for three times in total. Next, 50 mL of anhydrous 2-methyltetrahydrofuran (MeTHF) was added to the reaction flask and stirred at 80 °C for 4 h. Upon completion, the reaction mixture was cooled to room temperature and all the solvents were removed under reduced pressure. The residue was further purified by silica gel column chromatography to afford **D-01'-Ald₂** (6.1 g, yield: 92.0%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S10 in ESI): 10.39 (s, 1H), 9.96 (s, 1H), 9.81 (s, 1H), 8.28–8.13 (m, 2H), 7.58–7.41 (m, 5H), 7.04 (s, 1H), 6.95 (s, 2H), 5.33 (s, 2H), 5.05 (s, 2H), 3.98 (dq, $J=12.8, 6.3$ Hz, 4H), 1.88–0.73 (m, 40H).

Synthesis of D-01'-OH₂

D-01'-Ald₂ (6.0 g, 8.5 mmol, 1.0 equiv.) was dissolved in 100 mL THF, and NaBH_4 (642.6 mg, 17.0 mmol, 2.0 equiv.) was then slowly added to the mixture at 0 °C. After stirring for 1 h, the solvent was removed under reduced pressure. The residue was dissolved in 300 mL EA and washed with 200 mL saturated NaCl solution for three times, then the organic layer was dried over anhydrous Na_2SO_4 , filtered, and evaporated to dryness under reduced pressure to afford **D-01'-OH₂** (5.4 g, yield: 90.0%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S11 in ESI): 9.75 (s, 1H), 7.62–7.30 (m, 6H), 7.16–6.91 (m, 6H), 5.09–4.94 (m, 6H), 4.51 (d, $J=5.4$ Hz, 2H), 4.41 (d, $J=5.5$ Hz, 2H), 3.97 (q, $J=6.2$ Hz, 4H), 1.81–0.79 (m, 40H).

Synthesis of D-(01')₃-Ald₄

Following similar procedures described above, **D-(01')₃-Ald₄** was prepared using **D-01'-OH₂** and 4-((2,4-diformylphenoxy)methyl)benzoyl azide as the starting materials. $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S12 in ESI): 10.38 (d, $J=1.9$ Hz, 2H), 9.94 (s, 2H), 9.89–9.71 (m, 3H), 8.30–8.09 (m, 4H), 7.57–7.33 (m, 16H), 7.18–6.89 (m, 5H), 5.37–4.98 (m, 12H), 3.96 (dq, $J=15.8, 9.5, 6.7$ Hz, 4H), 1.78–0.72 (m, 43H).

Synthesis of D-(01')₃-OH₄

Following similar procedures described above, **D-(01')₃-OH₄** was prepared from the reduction of **D-(01')₃-Ald₄** precursor by NaBH_4 . $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S13 in ESI): 9.85–9.70 (m, 3H), 7.51–7.30 (m, 15H), 7.18–6.90 (m, 9H), 5.22–4.95 (m, 16H), 4.51 (dd, $J=5.4, 2.0$ Hz, 4H), 4.40 (dd, $J=9.5, 5.4$ Hz, 4H), 4.02–3.91 (m, 4H), 1.76–0.80 (m, 40H).

Synthesis of D-(01')₃-(OEG₈-Trt)₄

Trt-OEG₈-AA (379.0 mg, 0.50 mmol, 2.0 equiv.), **D-(01')₃-OH₄** (320.0 mg, 0.25 mmol, 1.0 equiv.) and DBTL (15.8 mg, 0.025 mmol, 0.01 equiv.) were charged into 10 mL of anhydrous toluene. After azeotropically removing toluene under reduced pressure at room temperature, another portion of 10 mL of anhydrous toluene was added, and the azeotropic distillation cycle was repeated for three times in total. Then 10 mL of MeTHF was added and the mixture was stirred at 80 °C under nitrogen protection. Upon completion, the solvent was removed under reduced pressure. The residue was further purified by silica gel column chromatography to afford the resultant **D-(01')₃-(OEG₈-Trt)₄** (587.0 mg, yield: 56.0%). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ , ppm, Fig. S14 in ESI): 9.77 (d, $J=20.1$ Hz, 3H), 9.53 (d, $J=17.7$ Hz, 4H), 7.80–6.48 (m, 96H), 5.20–4.97 (m, 21H), 4.08–3.91 (m, 12H), 3.87–3.38 (m, 115H), 1.53–0.79 (m, 41H).

Self-assembly of $D-(01')_3-(OEG_8-Trt)_4$

A typical self-assembly procedure was as following. The solution of $D-(01')_3-(OEG_8-Trt)_4$ in dioxane (1 mg/mL, 1 mL) was placed in a 20 mL capped vial with a magnetic stirring bar, then 9 mL of deionized water was added slowly through a syringe pump at a rate of 1.0 mL/h at 25 °C under magnetic stirring. After completion of water addition, the aqueous dispersion was further stirred for 2 h and dialyzed against deionized water for 12 h and replaced with fresh deionized water approximately every 3 h, thus affording the resulting micellar dispersion.

RESULTS AND DISCUSSION

Construction and Characterization of Helical Nanorods

Typically, thermal annealing allowed further alignment of the nanoparticles into more advanced structures.^[65,67] Based on previous explorations on digital micelles, we further investigated the effect of thermal annealing process on the structure and transformation behavior of digital micelles. In this work, typical process of thermal annealing was performed, a certain amount of organic solvent (10% or 20% of the total solvent of the system) was added to the aqueous dispersion of

digital micelles at first, and the mixture was incubated in oil bath at 90 °C for 1 h. After that, the heating was stopped, and the whole system was allowed to cool naturally to room temperature, followed by removal of all the organic solvents by dialysis against water.

Initially, we investigated the thermal annealing of 45 nm nanorods assembled from encoded amphiphile T-**00000000**-dPEG. When the 45 nm nanorods were thermally annealed in the presence of 10% dioxane, the resulting nanostructures were characterized by cryogenic-transmission electron microscopy (cryo-TEM). Surprisingly, the cryo-TEM image clearly showed that the initial 45 nm nanorods transformed into right-handed helical nanorods. Measurement of right-handed helical nanorods (over 100 nanorods) using FiberApp,^[68] indicated helical nanorods of average length $L_n=142.4$ nm, $L_w=162.9$ nm, $L_w/L_n=1.14$, in which L_n and L_w were number-average and weight-average contour lengths, respectively,^[44] exhibiting a diameter of ~ 16.6 nm (Fig. 1a, Fig. S1 in ESI). The nanostructures fabricated from thermal annealing were further characterized by SAXS, affording a core radius to be ~ 7.0 nm, a thickness of ~ 1.8 nm, when fitting of the SAXS data with a core-shell cylinder model (Fig. 1b). The fitting result was in consistent with the observation from cryo-TEM analy-

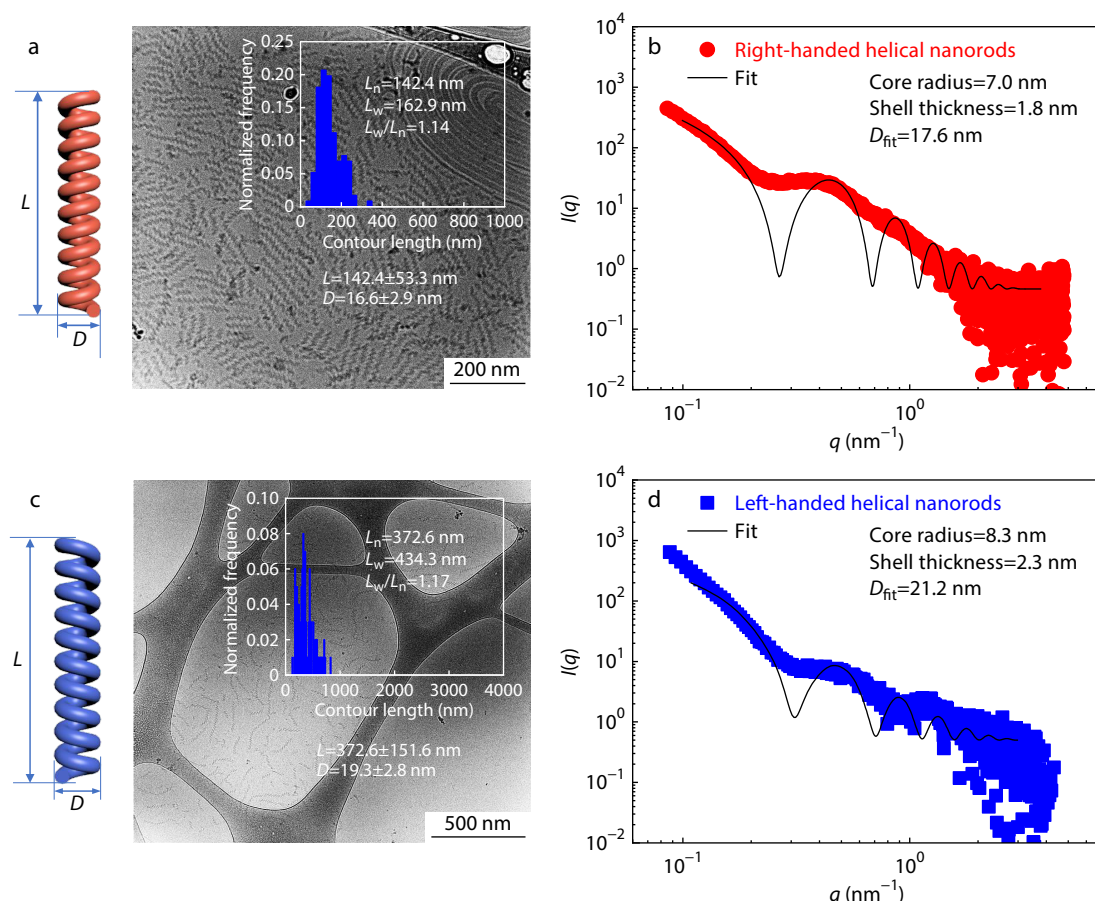


Fig. 1 (a) Cryo-TEM image of right-handed helical nanorods formed from thermal annealing of 45 nm nanorods aqueous dispersions in the presence of 10% dioxane for 1 h; (b) SAXS data recorded for the aqueous dispersion of corresponding right-handed helical nanorods; (c) Cryo-TEM image of left-handed helical nanorods formed from thermal annealing of 45 nm nanorods aqueous dispersions in the presence of 20% dioxane for 1 h; (d) SAXS data recorded for the aqueous dispersion of corresponding left-handed helical nanorods. The dark lines in (b) and (d) indicated fitting results according to the core-shell cylinder model.

sis. It was worth noting that the length of the nanorods could not be measured due to the limitation of angle range in the SAXS test. Thus, the transformation of non-helical nanorods to helical nanorods was achieved by the distinct thermal annealing process. We conjectured that it probably resulted from the chirality of the chiral carbon in T-**00000000**-dPEG being amplified during the annealing process to form helical nanorods.^[69]

Furthermore, organic solvent played an important role in the annealing process.^[70] Upon heating for the nanorod dispersion, the polymer chains became more active and flexible, which could adopt to a low energy state due to the input of external energy. Subsequently, when the nanorods dispersion was cooled to room temperature, the polymer chains were frozen and the resultant nanostructures were fixed.

Next, we attempted to increase the organic solvent (dioxane) volume ratio to 20% and perform the same thermal annealing steps as described above. Similarly, the nanostructures after thermal annealing were first characterized by cryo-TEM. Interestingly, cryo-TEM images clearly showed the transformation of the initial 45 nm nanorods into left-handed helical nanorods with an average length of $L_n=372.6$ nm, $L_w=434.3$ nm, $L_w/L_n=1.17$, and a diameter of ~ 19 nm (Fig. 1c, Fig. S2 in ESI). The SAXS data was fitted using the core-shell cylinder model, revealing a core radius of 8.3 nm and a shell thickness of ~ 2.3 nm, which agreed well with the cryo-TEM result (Fig. 1d). Comparing the results of thermal annealing of 45 nm nanorods in the presence of 10%(V/V) and 20%(V/V) dioxane respectively, it was observed that a larger percentage of organic solvent allowed the nanorods to become longer. Meanwhile, the presence of 20%(V/V) dioxane in the thermal

annealing system also led to typical change in helix orientation of the nanorods. Notably, the increase of organic solvent volume during the thermal annealing process was postulated to further adjust the polymer chains in the digital micelles. The unique annealing conditions in the presence of 10% and 20% dioxane could induce probable arrangement and distortion of polymer chains within the nanorods.^[70,71] Furthermore, the presence of plentiful chiral carbons within polymer chains could potentially amplify the resultant chirality of self-assembled nanorods, thus promoting the chiral transformation during thermal annealing.^[72,73]

Thermal Annealing of 45 nm Digital Micelles in the Presence of Different Types of Organic Solvents

In addition, diverse organic solvents were investigated for the thermal annealing of the 45 nm nanorods self-assembled from T-**00000000**-dPEG. First, we explored the morphological transformation of 45 nm digital micelles after thermal annealing in the presence of 20%(V/V) DMSO. Detailed characterization of the thermally annealed nanostructures using cryo-TEM revealed an increase in nanorods length, but no helical nanorods were found. The average lengths of nanorods ($L_n=337.9$ nm, $L_w=373.1$ nm, $L_w/L_n=1.10$) were obtained by measuring nanorods with FiberApp (Figs. 2a and 2b). Subsequently, DMSO was replaced with THF and the same thermal annealing operation was performed. Cryo-TEM images showed nanorods exhibiting further growth with average length ($L_n=497.0$ nm, $L_w=559.3$ nm, $L_w/L_n=1.13$, Figs. 2c and 2d). Notably, when the organic solvent was further changed to acetonitrile in the same volume ratio, the original 45 nm nanorods were transformed into ultra-long advanced fibers after thermal annealing. Notably, when the organic solvent was further changed to acetonitrile, upon

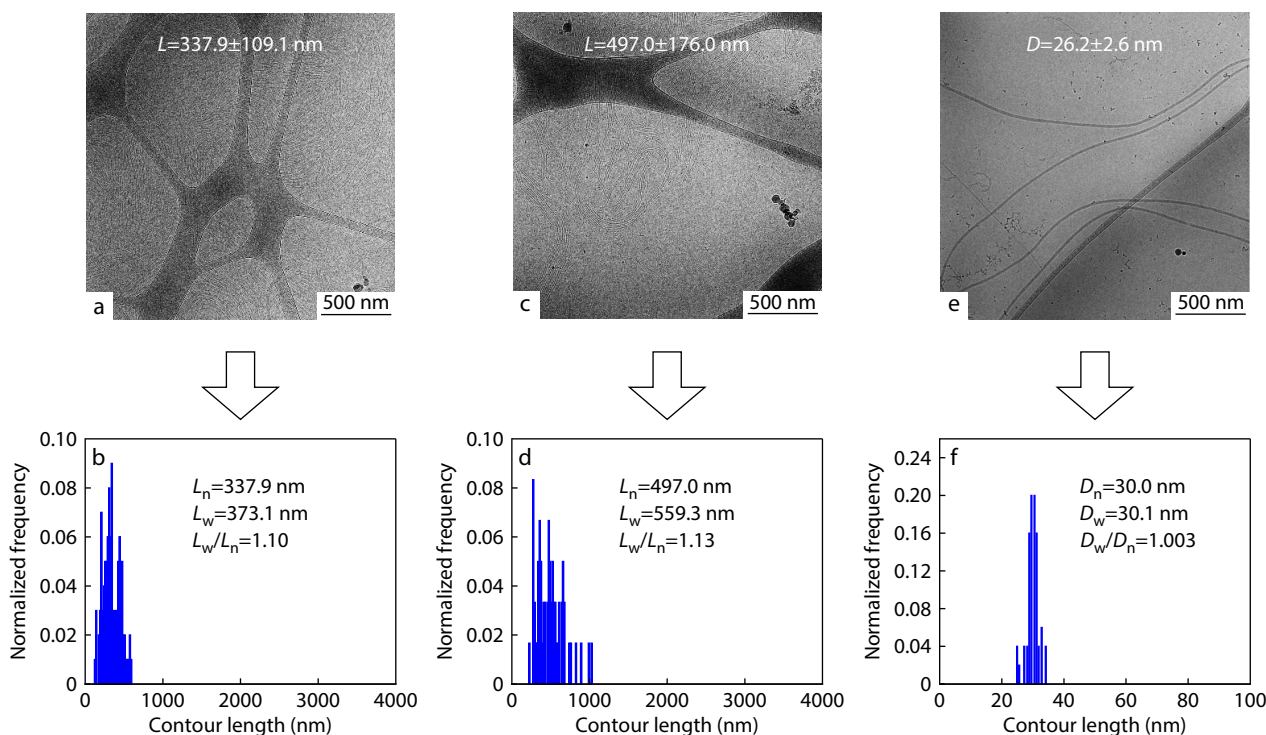


Fig. 2 Cryo-TEM images and histograms of the nanostructures of 45 nm nanorods after thermal annealing in the presence of diverse organic solvents at 20%(V/V): (a, b) DMSO, (c, d) THF, (e, f) acetonitrile.

undergoing the same thermal annealing, the original 45 nm nanorods were transformed into ultra-long advanced fibers with lengths beyond the tested range and average diameter of ~30 nm (Figs. 2e and 2f). Overall, for the 45 nm nanorods, the type and content of organic solvents in the nanoparticle dispersions during the thermal annealing process had significant effect on the morphological transformation of the nanorods, and the presence of organic solvents all led to further growth of the nanorods (Fig. 3).

Thermal Annealing of Different Types of Digital Micelles

Next, the generalization of thermal annealing with 20%(V/V) dioxane was investigated for the nanorod growth of digital micelles. Different types of digital micellar dispersions were performed separately, and the obtained nanostructures were characterized in detail by cryo-TEM and SAXS, respectively. First, the 28 nm nanorods self-assembled from T-01010101-dPEG were thermally annealed, and similarly, cryo-TEM results revealed longer nanorods with an $L_n=52.7$ nm, $L_w/L_n=1.10$ (Fig. 4a). The fabricated longer nanorods from thermal annealing were further verified by SAXS analysis, the fitting of SAXS data with a core-shell cylinder model indicated a core radius of ~6.1 nm, a thickness of ~2.3 nm and a rod length of ~62.8 nm (Fig. 4b).

Similarly, the length of 78 nm nanorods self-assembled

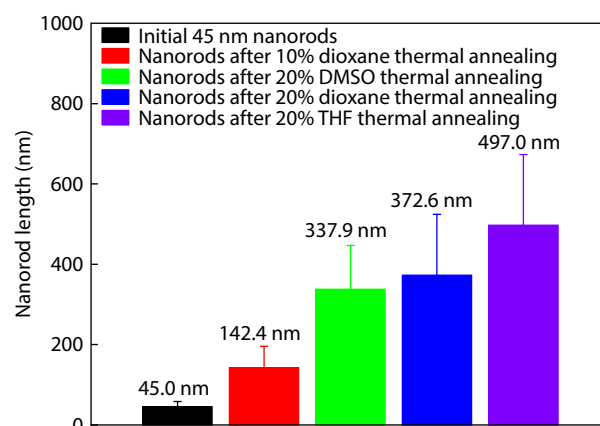


Fig. 3 Solvent-dependent length change for the 45 nm nanorods upon treating with diverse organic solvents in the thermal annealing.

from T-11111111-dPEG was further increased upon thermal annealing under the same condition, affording the value of L_n to be 228.3 nm, and L_w/L_n to be 1.11 (Fig. 4c). The nanostructures obtained by thermal annealing were characterized by SAXS, revealing a core radius of ~5.2 nm, a thickness of ~1.7 nm (Fig. 4d). Finally, to further confirm the contribution of thermal annealing to the increase of nanorod length, we also investigated the changes of 245 nm nanorods self-assembled

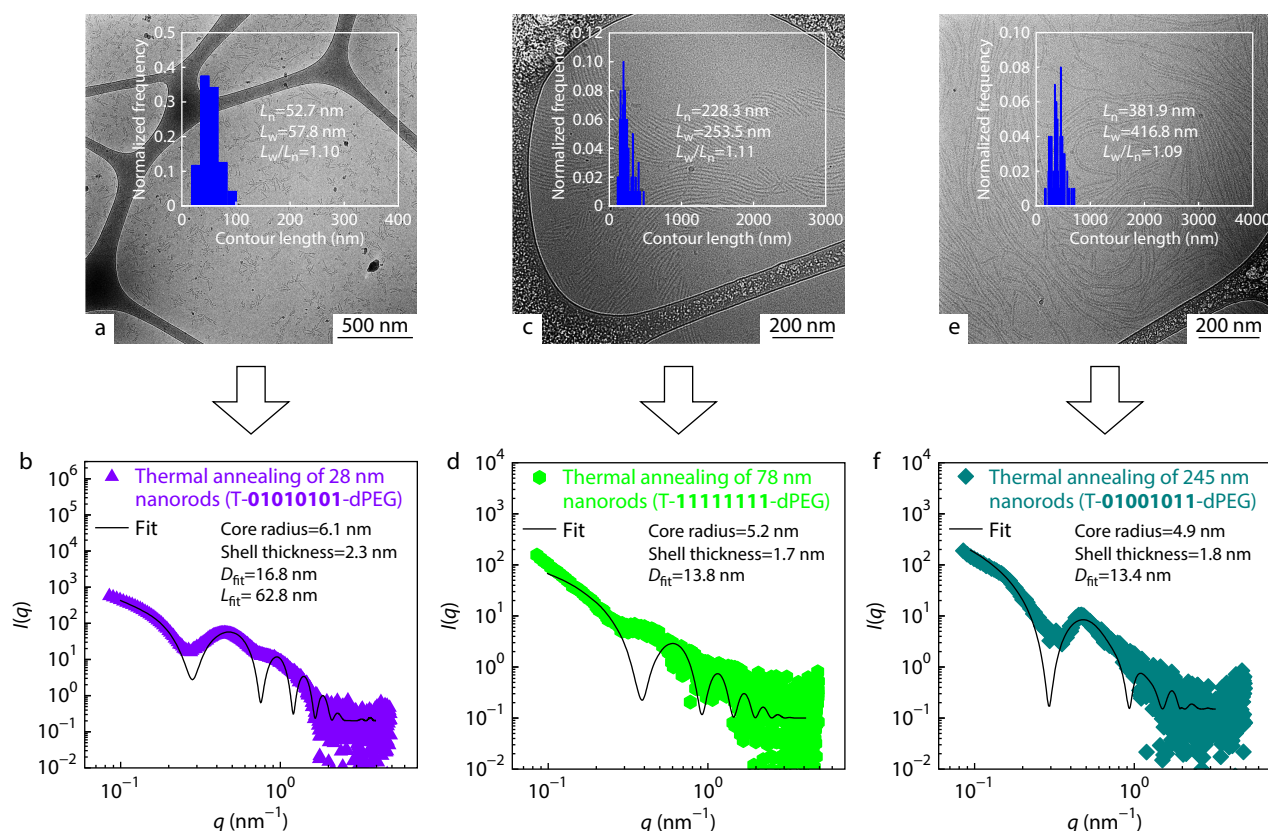


Fig. 4 Cryo-TEM images of the aqueous nanorods formulated from thermal annealing for 1 h in the presence of 20%(V/V) dioxane. (a) 28 nm nanorods self-assembled from T-01010101-dPEG; (c) 78 nm nanorods self-assembled from T-11111111-dPEG; (e) 245 nm nanorods self-assembled from T-01001011-dPEG aqueous dispersions. SAXS data recorded for the corresponding nanorods aqueous dispersions after thermal annealing of (b) 28 nm nanorods; (d) 78 nm nanorods and (f) 245 nm nanorods. The dark lines in (b, d, f) indicated fitting results according to the core-shell cylinder model.

from T-**01001011**-dPEG after thermal annealing. As expected, its cryo-TEM image showed that the length of the thermally annealed nanorods increased further, with the average length of $L_n=381.9$ nm, $L_w=416.8$ nm, $L_w/L_n=1.09$ (Fig. 4e). Complementary SAXS data revealed a core radius of ~ 4.9 nm, a thickness of ~ 1.8 nm (Fig. 4f).

Synthesis and Characterization of **D-(01')₃-(OEG₈-Trt)₄**

The modular synthesis of dendrimer **D-(01')₃-(OEG₈-Trt)₄** started from the preparation of 2Cit-OH (Fig S3 in ESI), which was obtained by a facile two-step process, and the intermediates were characterized by $^1\text{H-NMR}$ analysis (Figs. S6 and S7 in ESI). Subsequently, another building block AA-01'-Ald₂ was further obtained by three-step reactions, which was employed for further dendrimer construction (Fig. S4 in ESI). Both intermediate and final product were characterized in detail by $^1\text{H-NMR}$ analysis (Figs. S8 and S9 in ESI). After obtaining the two building blocks, the resultant dendrimer was readily prepared by a series of iterative reactions (Fig. S5 in ESI). Specifically, 2Cit-OH and AA-01'-Ald₂ were mixed and heated under water-free and inert gas-protected conditions, and the aroyl azide moiety of AA-01'-Ald₂ underwent Curtius rearrangement to *in situ* converted to aroyl isocyanate and coupled with 2Cit-OH catalyzed by DBTL to yield **D-01'-Ald₂**. Subsequently, the aldehyde groups of the newly obtained product were reduced to hydroxyl group in the presence of NaBH_4 . Correspondingly, the synthesized intermediates were

characterized in detail by $^1\text{H-NMR}$, SEC and MALDI-TOF MS analysis (Fig. 5, Figs. S10 and S11 in ESI). The above coupling and reduction reaction processes were further repeated to afford **D-(01')₃-OH₄** (Fig. 5, Figs. S12 and S13 in ESI). To endow the dendrimer with amphiphilicity, Trt-OEG₈-AA was used to terminally cap the peripheral hydroxyl groups (Fig. S5 in ESI). The final product **D-(01')₃-(OEG₈-Trt)₄** was characterized by $^1\text{H-NMR}$ (Fig. S14 in ESI), SEC (Fig. 5a) and MALDI-TOF MS (Fig. 5b) analyses to confirm the successful preparation. The symmetric and narrow SEC elution peak and the single peak of MALDI-TOF MS indicated the uniform distribution and homogeneity of **D-(01')₃-(OEG₈-Trt)₄**.

Characterization of Nanorods Self-assembled from **D-(01')₃-(OEG₈-Trt)₄**

The nanostructures self-assembled from **D-(01')₃-(OEG₈-Trt)₄** were examined *via* co-solvent assisted self-assembly. Once the nanoparticle dispersion was formulated, transmission electron microscopy (TEM) was utilized to characterize the morphology of the aggregates (Fig. 6a). The diameter of the nanorods was observed to be ~ 30 nm. The amphiphilic dendrimer could self-assemble into relatively uniform nanorods with an average length of $L_n=292.5$ nm, $L_w=321.0$ nm, and $L_w/L_n=1.10$ (Fig. 6b). The rod-like nanostructure fabrication probably rooted in the cooperative hydrogen bonding of carbamate moieties as well as remarkable π - π interactions in the crystalline branched oligourethane.^[52,74]

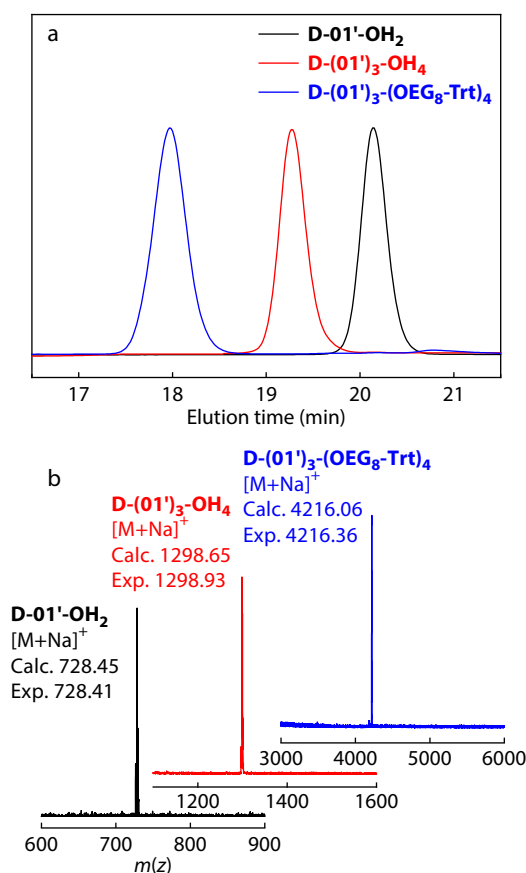


Fig. 5 (a) SEC traces and (b) MALDI-TOF MS spectra recorded for **D-01'-OH₂**, **D-(01')₃-OH₄**, and **D-(01')₃-(OEG₈-Trt)₄**.

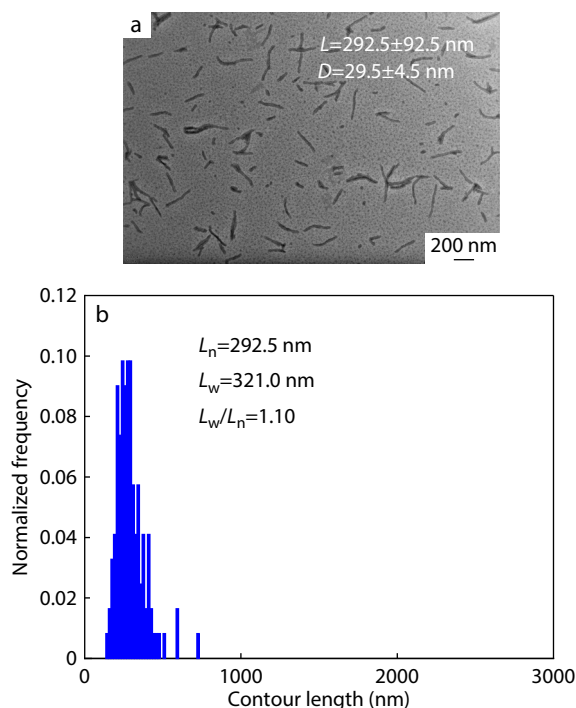


Fig. 6 (a) TEM image of nanorods and (b) histogram of the contour length distribution of nanorods self-assembled from **D-(01')₃-(OEG₈-Trt)₄**.

CONCLUSIONS

In summary, controllable fabrication of uniform digital nanorods was developed from precise sequence-defined amphiphilic polymers in aqueous media by thermal annealing in the

presence of slight organic solvents. Interestingly, the 45 nm nanorods self-assembled from T-00000000-dPEG were thermally annealed in the presence of 10%(V/V) and 20%(V/V) dioxane to obtain right-handed helical and left-handed helical nanorods, respectively. Meanwhile, the length values of the nanorods obtained by thermal annealing were solvent-dependent. Furthermore, the length of different types of nanorods increased significantly after thermal annealing, affording narrowly distributed rod-like micelles in aqueous media. Moreover, the thermal annealing approach was also applicable for extra types of digital micelles, where rod/worm-like micelles were successfully formulated with different lengths. Additionally, relatively uniform nanorods were also obtained in aqueous media by the self-assembly of amphiphilic dendritic oligourethane. Overall, this work provided an attractive methodology for the fabrication size-controllable nanorods in water and was prospective for subsequent biomedical applications.

NOTES

The authors declare no competing financial interest.



Electronic Supplementary Information

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