Polymeric Nanostructures

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Ultrathin Alternating Copolymer Nanotubes with Readily Tunable Surface Functionalities**

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Abstract: *Well-defined ultrathin nanotubes (30 nm in diameter and of micrometer-scale length) were generated through the self-assembly of a novel alternative copolymer synthesized using an epoxy–thiol click-chemistry reaction. The self-assembly mechanism was investigated both by experiments and using dissipative particle dynamics (DPD) simulations. The obtained nanotubes can be readily functionalized with carboxy groups, amino groups, peptides, or other groups by simple modular click copolymerization.*

The self-assembly of amphiphilic molecules into nanotubes has drawn tremendous attention for their diverse range of applications and various small-molecule surfactants, such as phospholipids, glycolipids, and peptides, have been reported to form nanotubes.[1] Compared to nanotubes made from small amphiphiles, nanotubes from amphiphilic polymers exhibit a higher stability and are more flexible in adjusting compositions and functionalities. In addition, polymeric nanotubes are very important and are ubiquitous in nature being present, for example, in the tobacco mosaic virus, ionic channels, and in chromosomes. To date, polymeric nanotubes have been prepared through the self-assembly of biomacromolecules such as DNA and proteins.[2] They can also be constructed from the self-assembly of synthetic polymers with different molecular architectures,[3] such as block copolymers (coil–coil diblock copolymers,[4] rod–coil diblock copolymers,[5] and triblock copolymers[6]), macrocyclic polymers,[7] and branched or dendritic polymers.[8] However, most reports on synthetic polymeric nanotubes employ linear di- or triblock copolymers.

As an important type of linear copolymers, alternating copolymers are regular polymers with alternating repeating units. Alternating polymers have demonstrated great potential in photoelectric applications.[9] However, research on the self-assembly of alternating copolymers has been scarce, likely as a result of at least three limitations.[10] The limited range of polymer compositions may be considered as the first limitation as most of the reported alternating copolymers in self-assembly studies are prepared through the radical copolymerization of a few vinyl monomers with maleic anhydride or its derivatives. The second limitation is the self-assembled morphology, as relatively few supramolecular structures have been obtained through the self-assembly of alternating copolymers. Finally, functionalization of these structures is also particularly difficult. Thus, it is necessary to extend the range of polymer compositions, the self-assembly structures, and the functionalization methods employed to advance the field of alternating copolymer self-assembly.

Herein, we report a novel alternating copolymer synthesized through an epoxy–thiol click reaction (Scheme 1) between 1,4-butanedithiol and butadiene diepoxide. This amphiphilic alternating copolymer could self-assemble into well-defined nanotubes (NTs) (Scheme 1) around 30 nm in diameter and with micrometer-scale length. The polymeric nanotubes are ultrathin and have a thickness of 1–2 nm, which is as thin as that of small-molecule nanotubes. Most importantly, the modular click-chemistry approach provides a facile method to functionalize the obtained nanotubes by simple click copolymerization with functional dithiols or amines. As a demonstration, the positively charged nanotubes with tertiary amino groups, negatively charged nanotubes with carboxy groups, and peptide-immobilized nanotubes were readily prepared. The facile synthesis and one-step functionalization process make this type of alternating copolymer very promising in the field of self-assembly and in subsequent applications.

The alternating copolymer, named as poly(2,3-dihydroxy butylene-*alt*-butylene di-thioether (P(DHB-*a*-BDT)), has a number-average molecular weight of 20700 Da, and a polydispersity index (PDI) of 2.63. Polymer nanotubes were fabricated by the dropwise addition of water (about $0.1\ mL\,h^{-1}$) into solution of the copolymers in dimethyl sulfoxide (DMSO) with stirring to yield 0.05–1.00 $mg\,mL^{-1}$ polymer suspensions. The detailed molecular characterization

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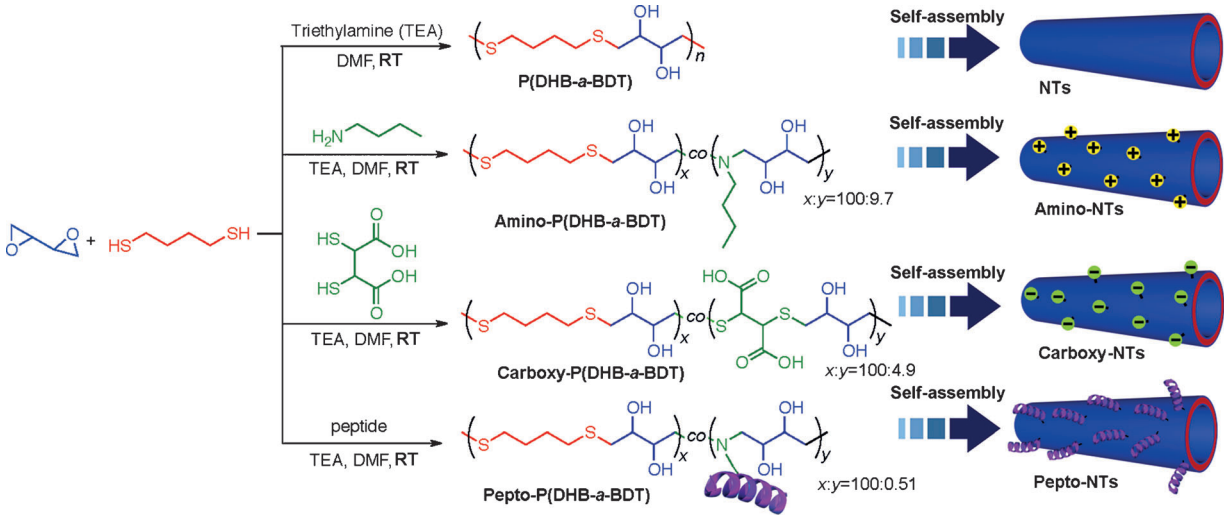

Scheme 1. Synthesis, self-assembly, and functionalization of the alternating copolymers of P(DHB-*a*-BDT)s. DMF = dimethylformamide.

of P(DHB-*a*-BDT) polymers and their functionalized derivatives are shown in Figures S1–S5 in the Supporting Information.

Formation of nanotubes from P(DHB-*a*-BDT)s in water/DMSO mixtures was confirmed by transmission electron microscopy (TEM) and atomic force microscope (AFM) as shown in Figure 1. The TEM images (Figure 1 a, b; Figure S6) depict typical nanotube morphology with a significant contrast of electron density between bright central channel and dark walls. Most of the nanotubes are straight, although some branched nanotubes with Y-shaped junctions have also been observed (Figure S6b). The diameter of the tube is approximately 25–35 nm. Additional evidence for the nanotube structure is provided by the TEM images of the ultrathin sections of the nanotubes embedded in epoxy resin, showing a hollow tubular pattern in longitudinal section (Figure 1 c; Figure S7) and a donut-shaped pattern in horizontal cross section. The nanotubes in ultrathin sections are somewhat irregular, likely because of the sampling process. The AFM image (Figure 1 e) shows the self-assemblies are nanofibers or nanotubes with width 73.8 nm, height 2.4 nm, and are up to several micrometers in length. The height-to-width ratio is up to 1:30.8, which further supports they are collapsed nanotubes rather than nanofibers. A height of 2.4 nm is very small, suggesting that the collapsed nanotube might have a unilamellar structure as shown in the representation in Figure 1 e. Thus, the height of the tube should represent the thickness of two collapsed nanotube membranes, that is, a single nanotube membrane should have a thickness of 1.2 nm. In the XRD pattern the nanotubes have a 1.2 nm periodic layer (Figure S8), in good agreement with the calculation of a 1.2 nm-thick membrane from the AFM images and confirming the unilamellar nanotube structure depicted in Figure 1 e. Such an ultrathin membrane thickness is quite rare for polymeric nanotubes which are generally more than 10 nm in thickness as a result of the block length.[4–10] It is comparable to the membrane thickness of small molecular nanotubes, such as lipid nanotubes. The diameter of the nanotubes is quite uniform according to the sectional height profile, but it is significantly bigger than that measured from TEM (25–35 nm), which might be attributed to the tip-induced image

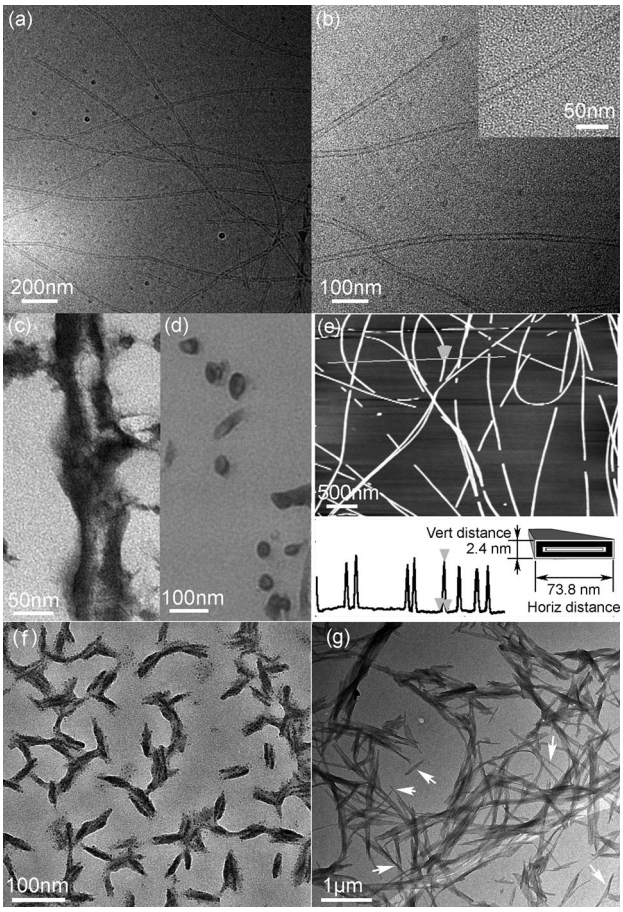

Figure 1. Morphology characterization of P(DHB-*a*-BDT) self-assemblies. a, b) TEM images of air-dried nanotubes. c, d) TEM images of ultrathin sections of nanotubes (embedded in epoxy resin). e) AFM image of collapsed nanotubes and a representation of the unilamellar structure. f) TEM image of nanosheet-like intermediates. g) TEM image of microsized nanoribbon-like intermediates.

broadening effect during the AFM measurements.[11] As the nanotubes are uniform in diameter, they tend to aggregate into bundles (Figure S9) driven by solvent evaporation.[12]

To address the tubular self-assembly mechanism, the intermediates formed during the self-assembly of P(DHB-*a*-BDT)s in water/DMSO were collected for TEM analyses. At the beginning of the process, nanosheets shorter than 100 nm were formed (Figure 1 f). Subsequently, micrometer-long nanoribbons were obtained, and some ribbons were observed to roll up into tubular structures (white arrows, Figure 1 g). These intermediates suggest the formation of P(DHB-*a*-BDT) nanotubes probably proceeds with the growth of nanosheets and nanoribbons. Additionally, differential scanning calorimetry (DSC) data suggest a crystalline structure exists in the as-prepared nanotubes with a melting point of 52.3 °C (Figure S5), which can be attributed to the highly ordered packing of alkyl chains in the nanotubes. It indicates that the nanotubes are possibly formed under a crystallization-driven self-assembly process.[13]

To further verify the experimental results and unveil the detailed mechanism for nanotube formation, dissipative particle dynamics (DPD) simulations were performed to explore the self-assembly process of P(DHB-*a*-BDT)s. Unlike for the self-assembly of block copolymers, theoretical studies on the self-assembly of alternating copolymers are rare[10d,14] and no DPD study has been performed to date. To capture the essential features of P(DHB-a-BDT) copolymers, a model molecule $(A_2B_1)_{16}$ was constructed in DPD simulations (Figure 2 a). In this system, one "A" bead represents a hydrophobic CH_2–CH_2–S–CH_2 unit, while one "B" bead denotes a hydrophilic OH–CH_2–CH_2–OH unit. Thus, A_2B_1 consists of one repeat unit in the synthesized alternating copolymer of P(DHB-*a*-BDT). For simplicity, the degree of polymerization is set as 16 in this DPD model. The details of the simulation model and method are described in the Supporting Information (Figures S10 and S11, Table S1).

Figure 2 displays snapshots of the self-assembly process at different time intervals of the alternating copolymer calculated through DPD simulations. Starting from a random state (Figure 2 b), the amphiphilic P(DHB-*a*-BDT)s aggregate into many small sheets at 11.40 ns (Figure 2 c). These small sheets gradually fuse to form a large ribbon-like aggregate at 22.04 ns (Figure 2 d, Figure S12). Thereafter, the ribbons roll up and finally close up to form a complete nanotube from 30.40 ns to 60.80 ns (Figures 2 e–h). Thus, as shown in the representation in Figure 2 i, the self-assembly of P(DHB-*a*-BDT) nanotubes involves a pathway from unimers through small nanosheets and then large nanoribbons to form nanotubes through sequential sheet fusion and ribbon rolling and closing processes. The results of these simulations are in good agreement with the experimental data as shown in Figure 1.

The DPD simulations can provide further details on the nanotube structure. First, as shown in Figure 2 j, the alternating polymer adopts a monolayer core–shell structure with a hydrophilic hydroxy-rich shell and a well-ordered hydrophobic alkyl chain core, which supports the crystallization-driven self-assembly process. Second, the membrane thickness of the nanotube can also be obtained according to the density distribution profile (Figure 2 k) along the radial direction *r*, that is, from the center to the outside wall of the nanotube. Specifically, the membrane thickness is calculated

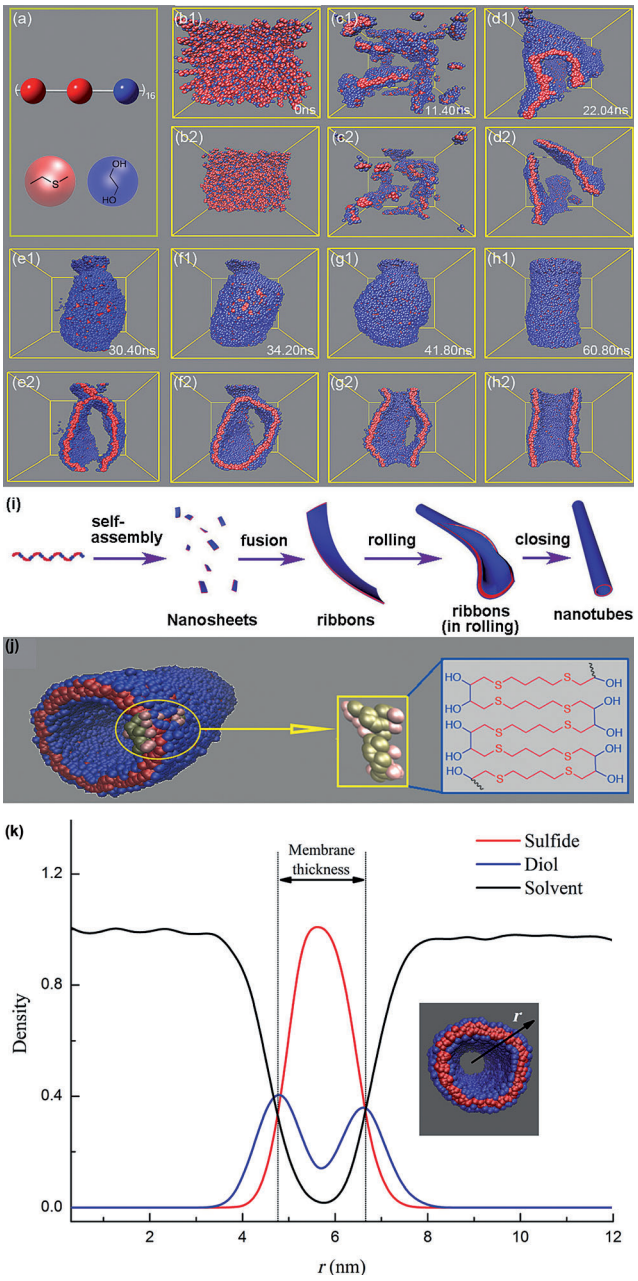

Figure 2. DPD simulations showing the self-assembly of the P(DHB-*a*-BDT) alternating copolymers captured at different time intervals. a) The model $(A_2B)_{16}$ for one P(DHB-*a*-BDT) molecule. Red bead = sulfide segment; blue bead = diol segment. b) Randomly distributed P(DHB-*a*-BDT) unimers in solution (t = 0 ns). c) Formation of small sheets (t = 11.40 ns). d) Ribbon formation (t = 22.04 ns). e) Formation of a rolling ribbon (t = 30.40 ns). f, g) Molecule rearrangement and nanotube closing (t = 34.20 ns (f); 41.80 ns (g)). h) Formation of the final nanotube structure (t = 60.80 ns). b1–h1) 3D views; b2–h2) Cross-section views. Solvent beads are removed for clarity. i) Scheme of the proposed self-assembly pathway for nanotubes. j) A cross-section view of the nanotube showing the membrane structure. The expanded portion shows the molecular packing model in the nanotube membrane using one labelled molecule. k) Radial density distributions of the sulfide segment (red) and diol segment (blue) in the nanotube.

from the distance between the peaks of density distribution of the diol segments located at the internal and external leaflets of the nanotube. The calculated membrane thickness of the nanotube is 1.8 nm, which is slightly larger than that obtained from the AFM results. Such a difference might result from the dried and collapsed state of the polymer chains in the AFM measurements.

The aforementioned DPD simulation results are based on a monodisperse polymer system. We also systematically studied the effect of molecular weight distribution on the self-assembly behavior by changing the PDI (from 1.64, to 2.07, 2.23, and 2.61). As shown in the Supporting Information (Figures S13–S16, Table S2), all simulation systems have a similar trajectory to form a nanotube structure, and the molecular packing model for each system is the same as that of the monodisperse system. Thus, it can be concluded that the self-assembly process in the P(DHB-*a*-BDT) system is not related to the molecular weight distribution.

Furthermore, the modular nature of click chemistry has also been exploited to functionalize the nanotubes. Carboxy groups, amino groups, or peptides were introduced onto the copolymers by click copolymerization of 1,4-butanedithiol and butadiene diepoxide with functional monomers such as 1-amino-butane, dimercaptosuccinic acid, and peptides (Scheme 1). The self-assembly of these functionalized copolymers was carefully studied by AFM, TEM, confocal laser scanning microscopy (CLSM), and zeta-potential measurements. All AFM images show highly collapsed fibrous structures with height-to-diameter ratios above 17 (Figures 3 a–c), indicating the formation of nanotubes. Additionally, the TEM images (Figures 3 d–f; Figures S17–S19) also confirm that polymers self-assemble to form nanotubes. All of these nanotubes showed the expected properties. For example, the zeta potential of nanotubes functionalized with dimercaptosuccinic acid (carboxy-NTs) is −36.6 mV, while the nanotubes functionalized with 1-aminobutane (amino-NTs) is +49.5 mV (Figure 3 g). Additionally, the CLSM image of the nanotubes functionalized with FITC-labelled peptides (pepto-NTs; FITC = fluorescein isothiocyanate) show highly green-fluorescent fibrous structures (Figure 3 h; Figure S20). The functionalized nanotubes can also be prepared through the co-assembly of P(DHB-*a*-BDT)s and the functionalized derivatives with a weight ratio of 1:1 (Figure S21). This co-assembly method is very useful to endow the nanotubes with adjustable compositions and functionalities.

In conclusion, herein we have demonstrated the formation of ultrathin nanotubes by the self-assembly of a novel alternating copolymer obtained through click-chemistry reactions between dithiol and diepoxide. Experimental data together with DPD simulations suggest a sequential self-assembly pathway including formation of small sheets, fusion of small sheets into large ribbons, rolling of ribbons, and the closing of the rolled ribbons to form nanotubes. In addition, several types of functional groups, such as carboxy groups, amino groups, or peptides, have been introduced into the nanotubes by simple modular click copolymerization with functionalized dithiols or amines. This work indicates that alternating copolymers are very promising precursors in self-assembly to construct well-defined nanostructures with tunable surface functionalities. In addition, we expect that the as-prepared nanotubes might have application in biomedical research.

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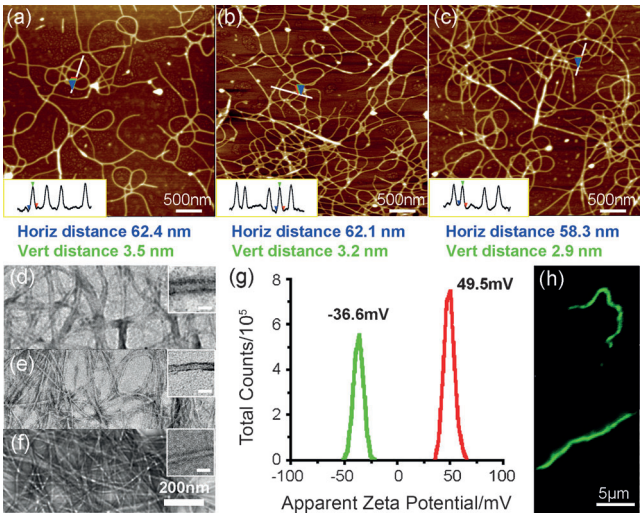

Figure 3. Characterization of the functionalized nanotubes. a–c) AFM images and d–f) TEM images of nanotubes functionalized with a, d) carboxy groups (carboxy-NTs), b, e) amino groups (amino-NTs), and c, f) peptides (pepto-NTs). g) Zeta potential of carboxy-NTs (green) and amino-NTs (red). h) CLSM fluorescence micrograph of nanotubes functionalized with FITC-peptides (1 mg mL^{-1} in water). Inset in (d–f): scale bars = 30 nm.

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