

Formation of Polymeric Nanocubes by Self-Assembly and Crystallization of Dithiolane-Containing Triblock Copolymers

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Abstract: Template-free fabrication of non-spherical polymeric nanoparticles is desirable for various applications, but has had limited success owing to thermodynamic favorability of sphere formation. Herein we present a simple way to prepare cubic nanoparticles of block copolymers by self-assembly from aqueous solutions at room temperature. Nanocubes with edges of 40–200 nm are formed spontaneously on different surfaces upon water evaporation from micellar solutions of triblock copolymers containing a central poly(ethylene oxide) block and terminal trimethylene carbonate/dithiolane blocks. These polymers self-assemble into 28 ± 5 nm micelles in water. Upon drying, micelle aggregation and a kinetically controlled crystallization of central blocks evidently induce solid cubic particle formation. An approach for preserving the structures of these cubes in water by thiol- or photo-induced crosslinking was developed. The ability to solubilize a model hydrophobic drug, curcumin, was also explored.

Among the key structural parameters of nanomaterials, morphology or shape is known to influence a wide spectrum of their physical and chemical properties.^[1] Particles of various shapes also differ in their potential biological impacts in vivo.^[2] In particular, cubic polymeric microparticles show enhanced uptake by breast cancer cells because they offer a larger contact area with a cell compared to a sphere.^[3]

Anisotropic polymeric nanostructures can be generated via top-down routes, for example, particle replication in nonwetting template (PRINT), lithography, and template-induced printing.^[2f,4] These routes produce controlled particle shapes, but are limited in a minimum achievable size and require special equipment. Bottom-up self-assembly presents

an alternative route for anisotropic polymeric particles formation. In particular, block copolymers can self-assemble into micelles with a range of morphologies, such as spheres, cylinders, lamellae, vesicles, toroids, rods, lenticular and rectangular platelets,^[5] which can be employed as templates for particle formation. Two different strategies were reported for cubic polymeric nanoparticles formation by self-assembly. Pioneering work by Zhang et al. showed that cubes with edge sizes between 200 and 600 nm can be formed by evaporation-induced aggregation of polystyrene-block-poly(acrylic acid) micelles in the presence of organic solvent at 70–120 °C.^[6] Another study by Tu et al. reported that poly(ϵ -caprolactone) can form nanocubes upon evaporation from dichloromethane solution at 100 °C.^[7]

Herein, we report an operationally simple method to form polymeric cubic nanoparticles by the aqueous self-assembly of amphiphilic dithiolane-containing ABA triblock copolymers^[8] followed by evaporation-induced crystallization of the poly(ethyleneglycol) (PEG) central blocks. The versatile chemistry of the hydrophobic dithiolane cores of these micelles provides a facile mechanism for chemical or photo-chemical crosslinking to fix the hierarchical structure following self-assembly.

We recently described the synthesis and properties of water-soluble triblock copolymers containing a central PEG block and terminal poly(trimethylene carbonate/dithiolane) (PTMCDT) blocks (Figure 1).^[8] Some of these triblock copolymers spontaneously self-assemble in water to form flower micelles at low concentrations.^[9] We selected the triblock copolymer **1** synthesized from PEG diols of $M_n = 14$ KDa with ten units of TMCDT (five in each terminal block; PEG14K/DP10) as a model polymer for this study. This polymer was soluble in water up to approximately 160 mg mL⁻¹ (at higher concentration it formed physical hydrogels), and in dilute solution it readily formed flower micelles at room temperature with critical micelle concentration (CMC) of 0.35 mg mL⁻¹.^[8b] The micelles had a mean size of 28 ± 5 nm by dynamic light scattering (DLS) and a mean ζ -potential of -10 ± 2.3 mV. The mean aggregation number for this polymer by static light scattering was 7.5 ± 1.4 .

We observed that upon deposition of micellar solution of copolymer **1** on various surfaces and subsequent drying for at least 4 h in a vacuum desiccator (ca. 10 kPa) at room temperature, it spontaneously formed nanoparticles of well-defined cubic or cuboidal shape (Figure 2a). This cubic morphology was consistently observed on various substrates such as aluminum, silicon, glass, or formvar, and therefore was not attributed to a specific surface effect (Figure 2). Over

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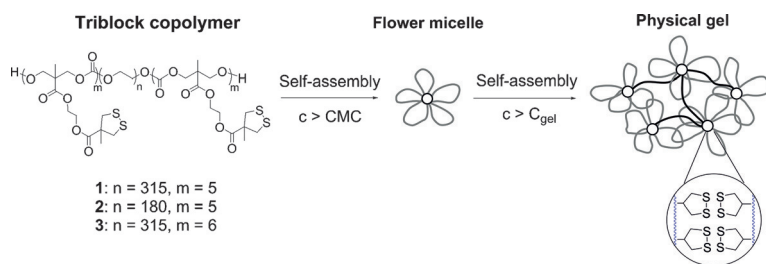


Figure 1. Self-assembly of dithiolane-containing triblock copolymers in water.

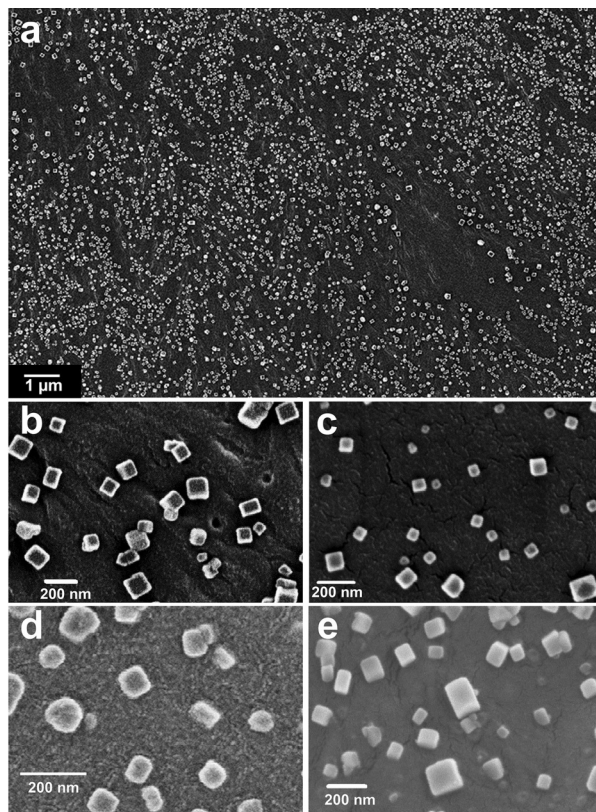


Figure 2. Cubic nanoparticles formed on various surfaces: a) aluminum SEM stub; b) aluminum stub, high magnification; c) silicon wafer; d) glass slide; e) Cu/formvar grid.

50 samples were prepared and tested by a high-resolution field emission scanning electron microscope (FESEM) to verify the particle morphology. Notably, these cubic morphologies were formed spontaneously at room temperature without any pre-treatment or additives. There are about 5450 nanocubes in the area of $20 \times 13.5 \mu\text{m}^2$ formed on aluminum surface (Figure 2a,b), while the mean edge size of these cubes is 107 ± 36 nm. The size of the cubes was not significantly affected by the initial polymer concentration (Figure S1 in the Supporting Information).

To verify that these cubic nanoparticles are formed by the triblock polymer rather than inorganic or metal impurities, energy dispersive X-ray (EDX) characterization was performed. The results confirmed the absence of any salts or inorganic elements other than those originating from the SEM stub or sputter coating (Figure S2(a)). This result was

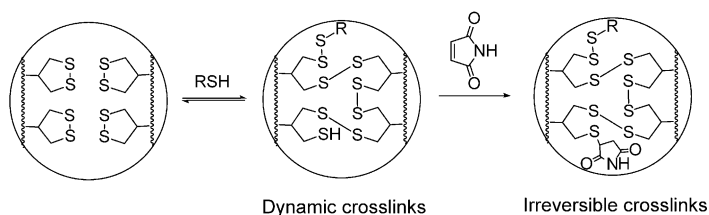
corroborated by transmission electron microscope (TEM)-coupled EDX, which enabled the elemental analysis of spatially resolved individual cubes (Figure S2(b)). Only carbon, oxygen, and sulfur were detected in addition to Cu originating from the copper grid.

Two critical factors were identified for the formation of cubic nanoparticles: the pre-assembly of triblock copolymers in water, and the evaporation rate. In a control experiment, homopolymers derived from the two constituent blocks, PEG and PTMCDT, were dissolved in water (or acetone) and dried under the same conditions. Neither of these homopolymers formed cubic nanoparticles; while PTMCDT formed an amorphous film without any nanostructures, PEG formed particulate irregular clusters (Figure S3(a)). A diblock copolymer of PEG and PTMCDT (PEG5k/DP6) did not form cubes either (Figure S3(b)). In addition, when copolymer **1** was dissolved in acetone, a good solvent for both blocks, no cubic nanostructures were observed upon drying (Figure S3(c)). Taken together, these results clearly show that the self-assembly of triblock copolymer **1** in water is essential for the formation of cubes. We examined two other triblock copolymers: copolymer **2** (PEG8k/DP10) and **3** (PEG14k/DP12) with different block lengths from that of copolymer **1**. Micellar solutions of copolymers **2** and **3** were subjected to the same drying procedure and both formed morphologically similar cubic nanoparticles with size ranging from 70 nm to 220 nm (Figure S3(d,e)). Therefore, the formation of cubes is not unique to a specific PTMCDT-PEG-PTMCDT but is a general phenomenon for this class of triblock copolymers as they undergo similar self-assembly in water.

Water evaporation rate plays an important role in the cube formation process. Aqueous micellar solutions of the triblock copolymers deposited on a surface and dried for at least 4 h at about 10 kPa at room temperature consistently resulted in the formation of cubic nanostructures. However, slower air drying produced irregular structures (Figure S4(a)), while rapid evaporation by lyophilization resulted in an interconnected macroporous network (Figure S4(b)). When the drying at about 10 kPa is stopped after 1 h, irregularly shaped particles with softer edges are formed, which is suggestive of a transition phase between micellar aggregates and the cubes (Figure S4(c)).

Having established that the pre-assembly of triblock copolymers in water is a pre-requisite for the cube formation, we hypothesized that the polymeric flower micelles serve as building blocks for the next-stage assembly during water evaporation. To test this hypothesis, we cross-linked the core of these micelles to prevent them from dissociation and rearrangement during the drying process. The crosslinking was achieved by either photo-induced disulfide exchange^[10] or thiol-initiated ring-opening polymerization of dithiolanes^[11] followed by maleimide capping^[8b] (Figure 3). Both methods stabilize the micelles, as evidenced by the observation that the crosslinked micelles did not dissemble but swelled when suspended in acetone. When the crosslinked micellar solutions of copolymer **1** were deposited on an

a. Chemical crosslinking



b. Photo-crosslinking

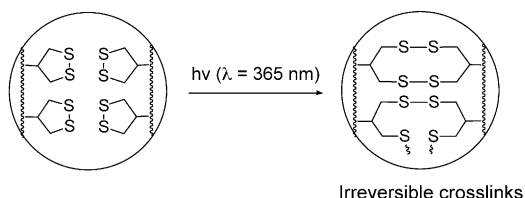


Figure 3. a) Chemical crosslinking of micelles through a thiol-initiated ring-opening cascade of dithiolanes, and subsequent capping the free thiols irreversibly by maleimide. b) Photo-crosslinking of dithiolanes.

aluminum substrate and dried at about 10 kPa, they formed very similar cubic nanoparticles (Figure 4a,b), which implies that the micelles do not dissociate during the cube formation.

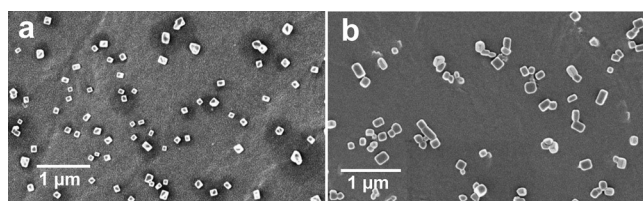


Figure 4. SEM images of nanocubes formed from crosslinked micellar solution of copolymer **1**. a) chemically crosslinked micelles; b) photo-crosslinked micelles.

To assess whether the formation of these cubic nanostructures was influenced by crystallization of the PEG sequences upon drying, nanocubes derived from copolymer **1** were collected from a glass vial and analyzed by differential scanning calorimetry (DSC) (Figure S5(a)). The first heating and cooling scans revealed an endothermic melting peak at 58.5°C and an exothermic crystallization peak at 36.4°C respectively, which is consistent with the thermal behavior of semi-crystalline PEG.^[12] The degree of crystallinity calculated by comparing the melting enthalpy of the sample to that of a 100% crystalline PEG was 70%, indicative of very efficient crystallization upon formation of the cubes. The crystallinity was further confirmed by the wide angle X-ray diffraction (WAXD) studies on the cubes pre-formed on silicon wafers (Figure 5). Two peaks at $q = 1.36 \text{ 1/Å}$ ($2\theta \text{ (CuK}\alpha\text{)} = 19.4^\circ$) and at $q = 1.65 \text{ 1/Å}$ ($2\theta = 23.6^\circ$) were observed, similar to diffraction pattern of crystalline PEG.^[13] When the pre-formed cubes were heated to 70°C on an aluminum substrate for 1 h, their cubic morphology was destroyed (Figure S5(b)). These results imply that the evaporation-induced crystallization of PEG block of micelles is one important factor for the formation of cubes. This crystallization is likely kinetically

controlled and is constrained by the pre-assembly of the micellar aggregates under particular drying conditions.

On the basis of data collected and literature precedent for the solution self-assembly of ABA triblock copolymers,^[14] one possible mechanism for the formation of these nanocubes is proposed in Figure 6. In the first step, the amphiphilic triblock copolymers **1–3** self-assemble in water to form spherical flower micelles.^[8b] As the concentration of these micelles rises upon evaporation, aggregation of the micellar structures into a transient ordered mesophase^[14b,d] is proposed to position the PEG coronas into an optimal morphology for crystallization. The existence of ordered mesophases in concentrated micellar solution has been reported for a variety of ABA triblock copolymers.^[14] In particular, a face-centered cubic (FCC) liquid crystalline phase was

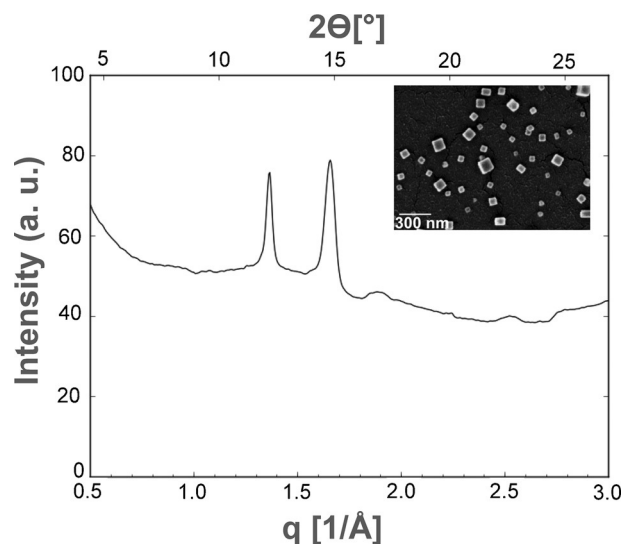


Figure 5. WAXD curve of cubes formed by copolymer **1** on a silicon wafer. Inset: SEM image of copolymer **1** cubes on a silicon wafer.

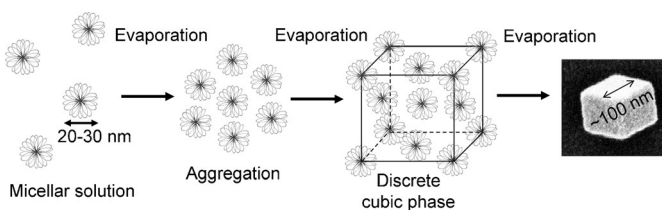


Figure 6. Possible mechanism of cube formation through micelle aggregation into nanometric cubic phase.

reported for PPO-PEO-PPO (PPO = poly(propylene oxide)) architectures (hydrophobic A and hydrophilic B block), analogous to those described herein.^[14b,c] Upon further drying, the self-assembly constrained crystallization of PEG leads to the cubic shape of resultant solid nanoparticles. This proposed mechanism is supported by several of our observations:

1) self-assembly into micelles is a pre-requisite of cube formation; the triblock copolymers crystallized from acetone solution do not form cubic structures, even under analogous drying conditions; 2) when the evaporating process is interrupted, a transition phase, which presents the features of both spherical aggregates and the cubes, is formed; 3) when the core of micelle is crosslinked prior to evaporation to prevent micelle rearrangement, it results in similar cubic morphology to those of free micelles, indicating that micelles act as building blocks in cube formation; and 4) a kinetically controlled crystallization of triblock copolymer is required for cube formation; when the cubes are heated above the polymer melting point, the cubic particles disappear and do not re-form upon cooling to room temperature. This proposed mechanism is in partial agreement with the mechanism proposed by Zhang et al. for cubic particles of block copolymers formed through self-assembly in the presence of organic additives.^[6] While more detailed investigations on the domain structure and ordering of micelles within the cube particles is underway to provide further evidence for the proposed mechanism, the results reported here represent a novel strategy for generating novel nanostructures by solution self-assembly and crystallization.

The cubic nanoparticles assembled from the triblock copolymer micelles readily dissociate to micelles once re-dispersed in water, as evidenced by DLS (Figure 7, dashed

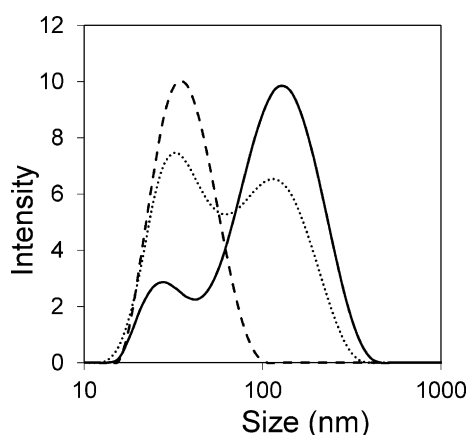


Figure 7. Average particles size (DLS) of aqueously dispersed nanocubes. Dashed curve: non-crosslinked nanocubes dissolved in water. Solid curve: nanocubes that were crosslinked (by treatment with thiol and maleimide) and then dispersed in water. Dotted curve: photo-crosslinked micelles that were assembled into cubes and subsequently crosslinked with thiol/maleimide prior to re-dispersing in water.

curve). While this is useful for some potential applications, such as support for nanocatalysts or cubic shell formation, for a variety of others this presents a disadvantage. To preserve the high-order structure resulting from micelle assembly and crystallization, we developed a facile method for crosslinking the nanocubes after their formation. In the past we observed that these triblock copolymers form physical gels derived from interconnected flower micelles at aqueous concentrations above 15 wt %.^[8b] As the hydrophobic dithiolane-enriched micellar cores of these gels can be crosslinked

either by light or by the addition of thiols and maleimide,^[8b] we exploited this versatile chemistry to crosslink the nanocubes. First, the pre-formed cubes were dispersed in a diethyl ether solution (a poor solvent for copolymer **1**) of a thiol crosslinker such as 3,6-dioxo-1,8-octanedithiol, which promotes the ring-opening and crosslinking of dithiolane cores in the cubes. Next, the crosslinked cubes were collected, washed with diethyl ether, and then re-dispersed in a diethyl ether solution of maleimide, which reacts with the free thiols to prevent de-crosslinking.^[8b] After these treatments, cubes were dispersed in water and their sizes showed bimodal distribution by DLS (Figure 7, solid curve). One set of particles exhibited an average hydrodynamic diameter of 122 nm, roughly corresponding to the size of nanocubes, and the other of approximately 28 nm, corresponding to the size of copolymer **1** micelles in water.^[8b] These data indicate that a significant fraction of copolymer **1** in the nanocubes can be crosslinked, preserving the hierarchical structure of the nanocube. As this thiol/maleimide crosslinking strategy is not 100 % efficient in crosslinking all the chains,^[8b] the remaining free chains can re-assemble into micelles in water. When the same post-formation crosslinking was performed on cubes assembled from core-crosslinked micelles (through photo-crosslinking), the fraction of free micelles upon re-dispersion in water was significantly larger (Figure 7, dotted curve). This is likely due to the fact that crosslinking of the micellar cores reduces the availability of polymer chains for rearranging and bridging, and thus disfavors the intermicellar bridge formation required for the preservation of high-order assembly of micelles. These studies provide further support for our proposed mechanism where micelles constitute the basis of the high-order assembly. Post-formation crosslinking of the cubes provides a means to preserve the hierarchical structure of these triblock copolymers.

To assess the potential of these triblock copolymers to sequester hydrophobic molecules in water, we investigated the encapsulation of curcumin as a representative hydrophobic therapeutic.^[15] Curcumin was loaded into the triblock polymer micelles by nanoprecipitation. While copolymer **1** had rather low loading capacity due to the low content of hydrophobic blocks, the drug loading content (DLC) and efficiency (DLE) could be increased by simply decreasing the hydrophilic PEG block length (Table S1). The most hydrophobic triblock copolymer PEG4.6K/PTMCDT DP 10 had DLC = 10.5 wt % and DLE = 81.5 wt %. The effective concentration of curcumin dispersed in the nanoparticles ($187 \mu\text{g mL}^{-1}$) is more than 300 times higher than its solubility in pure water ($0.6 \mu\text{g mL}^{-1}$). In addition to solubilizing curcumin, the hydrophobic micellar cores also effectively prevent curcumin hydrolysis (Figure S6).^[16] Moreover, no precipitation was observed from these curcumin-loaded polymer solutions for at least 48 h. When the cubes were formed from curcumin-loaded micelles of copolymer **1**, they had slightly irregular morphologies (Figure S7).

In summary, we report a template-free method for preparing nanoparticles (40–200 nm) with unusual cubic morphology based on solution self-assembly and evaporation-induced crystallization of a class of dithiolane-containing triblock copolymers (PTMCDT-PEG-PTMCDT). Upon

vacuum drying at room temperature, micellar solutions of copolymers with various block lengths can lead to the formation of nanocubes on a variety of surfaces. We propose that the micelles serve as the building blocks for nanocubes formed via higher-order assembly, and that micelle aggregation is controlled by the crystallization of PEG. We also develop an approach for preserving the supramolecular structure of cubes in aqueous media through dithiolane crosslinking after the cube formation. Non-crosslinked cubes that are soluble in water can be utilized as supports for synthesizing of hollow cubic nanocatalysts or cubic drug-delivery shells in non-aqueous media, and be later removed with water. Cubes with intermicellar crosslinking can serve as nanocarriers for therapeutics and might potentially affect their in vivo impact.^[2,3] While the mechanism and structural factors that govern the cubic shape merit further investigation, this study provides a simple way of creating non-spherical polymeric nanoparticles through multi-step self-assembly.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: dithiolane crosslinking · micelle aggregation · nanocubes · self-assembly · triblock copolymers

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