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From self-assembled toroids to dynamic nanotubules

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Supramolecular nanostructures based on self-assembly processes have received considerable attention because these structures can lead to dynamic nanomaterials. Among the diverse self-assembled nanostructures, toroids are interesting nanostructures with the potential for many applications such as channel formation because of their unique symmetrical and annular shape. Notably, a further stacking of the toroids can produce well-defined one dimensional nanotubules with responsive properties triggered by external stimuli such as temperature, ionic strength, and guest molecules. This review introduces recent research in the area of self-assembled nanostructures from toroids to dynamic nanotubules.

Introduction

Extensive investigations on the self-assembly behavior of synthetic amphiphiles have created various supramolecular architectures that have a well-defined shape and size including vesicles, spherical micelles, cylindrical micelles, tubes, ribbons, bilayers, helices, networks, and toroids. These self-assembled nanostructures constructed *via* secondary forces such as hydrogen bonding, electrostatic, van der Waals interaction, and hydrophobic interaction can be a powerful tool to develop specific functional supramolecular materials.[1–4] Among the many different morphologies, toroid or nanoring structures have a unique symmetrical and annular shape. Despite the relatively small number of studies for toroidal nanostructures, the developments and applications of self-assembled toroids have been a great challenge in the materials science, nanochemistry, and biomimetic or bioinspired chemistry.[5–9] Toroidal nanostructures can be prepared *via* molecular design in a broad sense. When the bulky and hydrophilic dendrimers are placed in the middle part of hydrophobic segments, curvature at the interface between each building block can be induced due to their steric repulsion. As a result of curvature, specific amphiphiles self-assemble into highly curved toroidal nanostructures. Interestingly, some toroidal nanostructures show an attractive further transformation into well-defined nanotubules through stacks on top of one another.[10–12] In contrast with previous nanotubules which have a limitation on dynamic response characteristics, the nanotubules based on the stacking of toroidal nanostructures show stimuli-responsive properties. In this mini-review, we will focus on recent advances in the construction of toroidal nanostructures and dynamic nanotubules based on self-assembly in aqueous solution.

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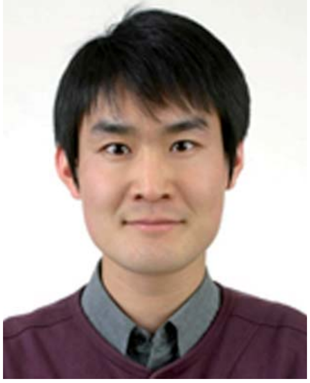

Yongju Kim graduated from Seoul National University, Korea, with B.S. and Ph.D. degrees in chemistry in 2005 and 2012, respectively. His thesis focused on the development and construction of a chemical library for control of biological functions. He then joined Prof. Myongsoo Lee's National Creative Research Initiative Center for Supramolecular Nano-Assembly in a postdoctoral position (2012). His research interests are synthesis and development of self-assembling molecules including synthetic amphiphiles and peptide molecules for the biological applications.

Taehoon Kim received a bachelor degree (2007) in chemistry from Yonsei University, Korea, and now is a last semester graduate student pursuing his M.D. degree at Seoul National University under the supervision of Prof. Myongsoo Lee. His current research focuses on the development of multivalent ligands through linearly self-assembling behavior of carbohydrate-tethered aromatic amphiphiles.

Toroidal nanostructures from self-assembly

Self-assembly of an amphiphilic rigid–flexible macrocycle (**1**) consisting of a hexa-*p*-phenyl rod and a poly(ethylene oxide) (PEO) coil was reported as the formation of toroidal nanostructures with a hydrophilic exterior and interior in bulk and solution (Fig. 1).[13] The well-defined cyclic molecule was prepared through ring-close metathesis reactions between two allyl groups of rod–coil precursors. In the aqueous solution, the rods are aligned axially with their preferred direction, and the interior and exterior are filled by the coil segments, generating the barrel-like supramolecular toroids illustrated in Fig. 1c. The average diameter of the aggregates was observed to be about 7 nm uniformly on the basis of dynamic light scattering (DLS) studies. The transmission electron microscopy (TEM) images showed that there is an obvious contrast between the periphery and centre in the aggregates, characteristic of the projection images of hollow cylinders, and the diameter of the internal pore was about 2 nm (Fig. 1b).

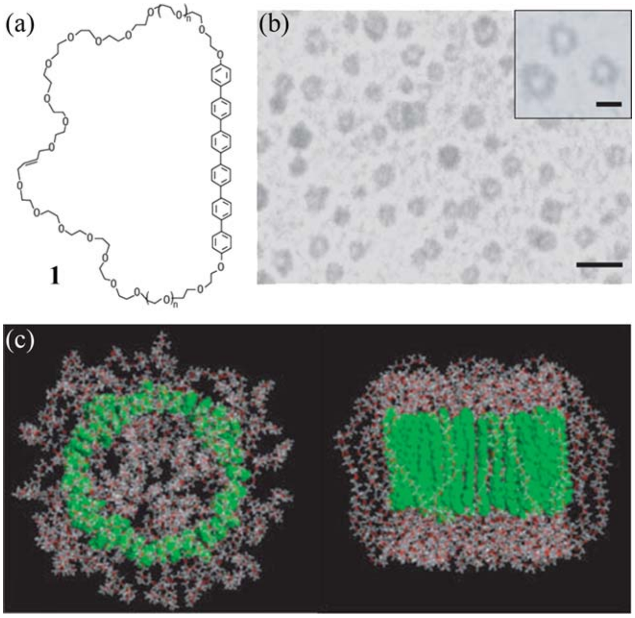

Fig. 1 (a) Schematic molecular structure of an amphiphilic rigid–flexible macrocycle (**1**). (b) TEM image in an aqueous solution. The scale bar represents 20 nm. Inset: higher-magnification image showing the contrast between the periphery and center. The dark areas arise because the rod segments are stained more than the coils. The scale bar represents 5 nm. (c) Top view (left) and side view (right) of a toroidal nanostructure by molecular modeling of **1** (rod segments are colored green, oxygen and hydrocarbons are colored red and grey, respectively). Reprinted with permission from ref. 13. Copyright (2005) Nature Publishing Group.

Amphiphilic dumbbell-shaped molecules consisting of a certain length of hydrophobic alkyl chains and hydrophilic oligoether dendrons at each end of the rod segment also form a self-assembled toroidal structure (Fig. 2a).[14] From TEM studies with cast samples stained with uranyl acetate, aggregates of molecule **2** with the shortest hexyl hydrophobic chains showed the coexistence of spherical and short undulated cylindrical micelles with diameters of about 15 nm, indicative of an interdigitated bilayer packing of molecular dumbbells (Fig. 2b). Molecule **4**, with the longest tetradecyl chains, exhibited elongated and entangled cylindrical micelles with a uniform diameter of 18 nm and lengths of at least several micrometers (Fig. 2d). More remarkable phenomena were found in molecule **3** with intermediate decyl chains. The molecular dumbbell **3** self-assembled into toroidal nanostructures which have a cross-sectional diameter of 16 nm, and ring diameters in the 70 to 300 nm range (Fig. 2c). The authors performed a time-dependent TEM study in order to gain insight into the formation mechanism of these unusual toroidal aggregates. The results showed that kinetically driven spherical micelles at the initial stage spontaneously transform into thermodynamically stable toroids in a week. The authors suggested that the hydrophobic effect associated with hydrophobic alkyl chain length dominates the overall interfacial area, causing the discrete spherical, toroidal nanostructure to transform into elongated cylindrical aggregates.

Myongsoo Lee received a bachelor degree in Chemistry from Chungnam National University, Korea, in 1982 and his Ph.D. degree in Macromolecular Science from Case Western Reserve University in 1993. After a short postdoctoral appointment at the University of Illinois at Urbana-Champaign, he joined the Faculty of Chemistry at Yonsei University (1994) and then moved to Seoul National University in 2009, where he is presently a Professor of Chemistry. In 2002, he became a director of the National Creative Research Initiative Center for Supramolecular Nano-Assembly. He received the PSK–Wiley Polymer Science Award for Young Scientist (2001), Yonsei Academy Award (2003), Scientist Award of This Month, Korea Ministry of Science & Technology (2006), Samsung Polymer Science Award (2008), and KCS Academy Award (2009). His main research interests include self-assembling molecules, controlled supramolecular structures, and peptide assembly.

In order to develop self-assembled peptide nanostructures similar to naturally occurring toroidal proteins, an organic–peptide hybrid T-shape building block that consisted of a β-sheet forming peptide and an oligo(ethylene oxide) dendrimer has been synthesized (Fig. 3a).[15] Because the steric repulsion of the bulky and hydrophilic dendrimers placed in the middle part of the peptides induces curvature at the interface between each building block, the T-shape molecules can self-assemble into highly curved toroidal nanostructures (Fig. 3b). The toroids consist of highly uniform diameters (~11 nm), indicating a single layer of T-shape building blocks based on an anti-parallel β-sheet interior and a hydrophilic oligo(ethylene oxide) dendrimer exterior. In contrast with natural β-barrel proteins including very long polypeptide chains, it is noteworthy that relatively short peptide building blocks can mimic the natural β-barrel protein through a

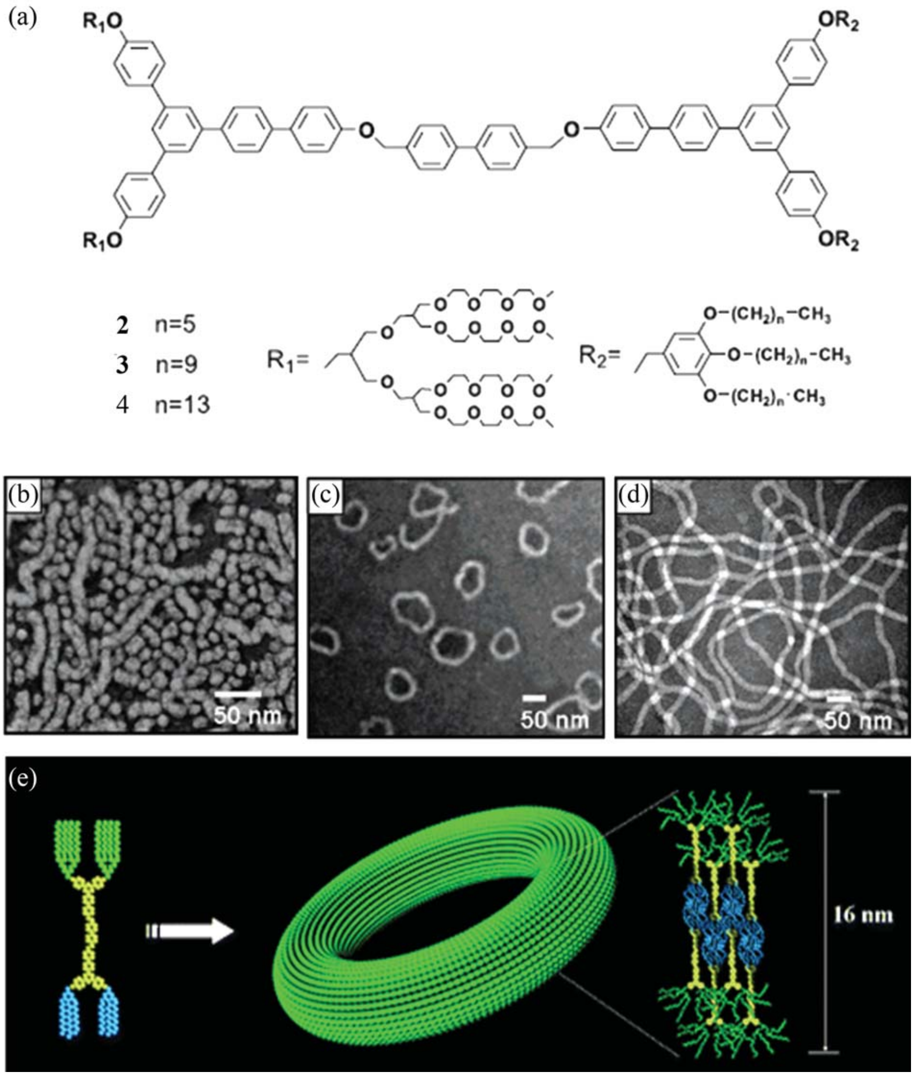

Fig. 2 (a) Schematic structure of amphiphilic dumbbell molecules (**2–4**). TEM images of (b) spherical, short cylindrical morphology of **2**, (c) toroidal morphology of **3**, and (d) long cylindrical morphology of **4** in an aqueous solution (0.01 wt%). (e) Schematic representation of a nanoring from amphiphilic molecular dumbbells. Reprinted with permission from ref. 14. Copyright (2006) American Chemical Society.

non-covalent self-assembly process. As the height of the toroid (4 nm) corresponds to that of common lipid bilayer membranes (2–4 nm), the self-assembled toroids may act as pores or channels across the membranes. In this context, an additional T-shaped building block (**6**) based on a hydrophobic alkyl chain dendrimer was prepared to enable the outer surface of the toroid nanostructure to interact with the hydrophobic space of lipid bilayer membranes. Indeed, the unique toroidal nanostructures showed an ability to selectively transport molecules across lipid membranes depending on their charge. A facile and versatile approach to biocompatible toroid nanostructures formed from the self-assembly of small synthetic peptides suggests that the function of the β-barrel mimics can be easily tuned by a simple change in dendrimer polarity and/or β-sheet peptide configuration.

It has been found that toroidal structures can be formed as discrete intermediates during the fibrillization of β-amyloid and α-synuclein.[16] A β-sheet peptide-based block molecule conjugated with a highly charged and flexible hydrophilic block was envisioned to self-assemble into a toroidal nanostructure by adopting molecular structural requirements for toroid formation from synthetic amphiphiles (Fig. 4).[17] The block copolypeptides (**7**) have a charged hydrophilic poly (L-arginine) block which is attached to a β-sheet forming block. The multiple positive charges of the arginine residues are placed between flexible oligo(ethylene glycol) linkers to inhibit the formation of higher aggregates and enhance the solubility of peptide nanostructures in an aqueous solution. As shown in Fig. 4b, discrete toroidal nanostructures were found to coexist with β-ribbon nanostructures under optimal conditions and the diameter of the toroid showed a highly uniform size (9.8 ± 0.8 nm). The coexistence of toroids and β-ribbons, together with the uniform toroid sizes, suggests that toroids are likely to form *via* end-to-end connections of β-ribbons (Fig. 4c). The N-terminal part of the block copolypeptide could be modified to contain carbohydrates such as mannose and glucose, still exhibiting the

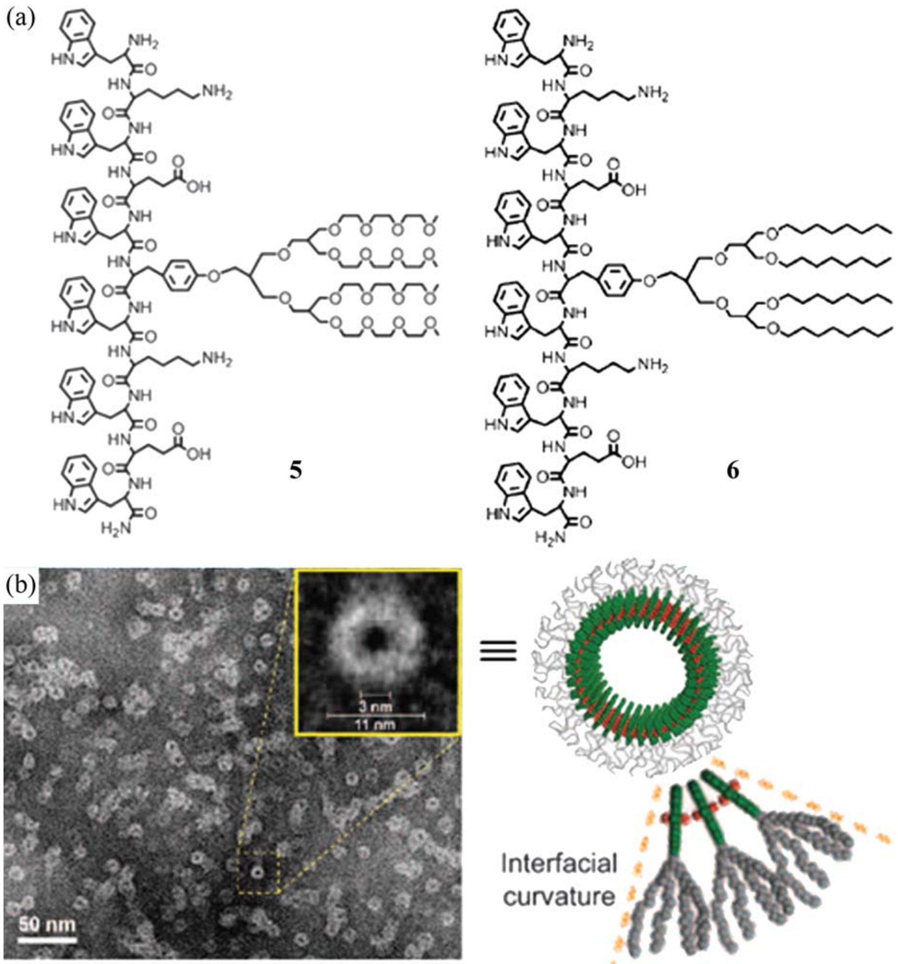

Fig. 3 (a) Schematic structure of amphiphilic dumbbell molecules (**5–6**). (b) Negative-stain TEM image of peptide **5** in 20 mM KF and model of self-assembled β-barrels from peptide **5**. Reprinted with permission from ref. 15. Copyright (2011) John Wiley and Sons.

ability to form toroidal nanostructures. The multivalent effect of carbohydrates in the toroidal nanostructures could enhance the affinity of carbohydrate–protein interactions based on lectin agglutination assays.[18,19] These results imply that the carbohydrate-decorated toroids can be used as multivalent ligands with unique circular nanogeometry.

Dynamic nanotubules from stacking of toroidal nanostructures

Nanotubules through self-assembly of small molecules are a major structural feature of biological systems, as exemplified by tobacco mosaic virus and cytoplasmic microtubules.[20,21] Many studies have focused on the tubular nanostructures through self-assembly of lipid molecules,[22] aromatic amphiphiles[23–27] and oligopeptides[28,29] using various non-covalent interactions.[30] However, despite recent advances in synthetic tubular nanostructures based on self-assembly, the incorporation of dynamic characteristics has remained a challenge. The stacking of ring-shaped nanostructures can provide one of the best candidates to generate dynamic nanotubules.[31,32]

A nanotubule could be induced by guest (C_{60}) encapsulation in the hydrophobic interior of the toroidal nanostructure which was constructed from coassembly of laterally grafted rod amphiphiles (Fig. 5).[10] Amphiphile **8** with a laterally grafted oligoether dendrimer and a branched alkyl chain at an opposite face aggregates into planar 2D sheets in water. Interestingly, the two-dimensional (2D) structures were broken up into a ribbonlike 1D structure through the addition of amphiphile **9** containing only a hydrophilic dendrimer. Eventually, the 1D structures were transformed into discrete toroidal nanostructures at a **9** content of 80 mol%, exhibiting that the diameters of the exterior and internal dimensions were measured to be ~10 and ~2 nm in the TEM image, respectively. The formation of toroids can be easily explained by induction of high curvature through co-assembly of amphiphiles **8** and **9**, consisting of a hydrophobic cavity and a hydrophilic exterior. Subsequently, the toroids stack to form nanotubules triggered by encapsulation of fullerenes (C_{60}) in their hydrophobic interior, showing a diameter of 10 nm and a regular spacing of 3.3 nm along the cylinder axis. The aggregation of the tubular structure based on stacking of the individual toroids was

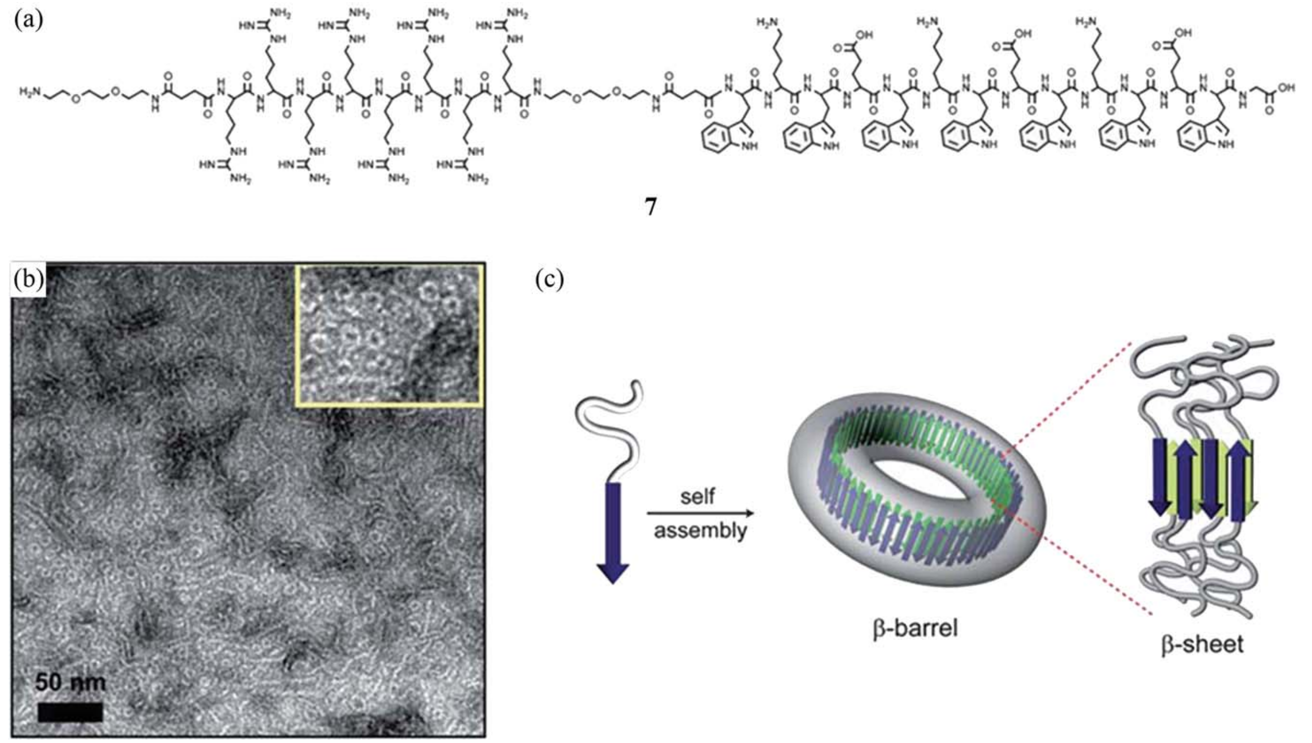

Fig. 4 (a) The representative chemical structure of a block copolypeptide (**7**) based on poly(L-arginine) and β-sheet peptide. (b) Negative-stain TEM image of the block copolypeptide in PBS. (c) Schematic representation of a toroidal nanostructure from the block copolypeptide (**7**). Reprinted with permission from ref. 17. Copyright (2011) John Wiley and Sons.

confirmed by the TEM image (Fig. 5b) and this aggregation could be explained by hydrophobic interaction on the top and bottom of the toroids caused by encapsulation of hydrophobic C_{60} molecules in aqueous solution. The results demonstrate that the coassembly of laterally grafted amphiphilic analogues leads to the formation of water-soluble toroids with a hydrophobic interior. Notably, the toroids can efficiently encapsulate fullerene within their internal cavities and transform into stimuli responsive nanotubules. This unique assembly could be successfully utilized to spatially order the fullerene, which might further broaden the application scope of fullerenes.

The laterally grafted bent-rod amphiphiles **10**, which consist of *meta*-linked aromatic segments with an internal angle of 120° and an oligoether dendrimer, can self-associate to form toroidal macrocycle structures through a combination of shape complementarity and phase separation of dissimilar blocks (Fig. 6).[11] The resulting hexameric toroidal macrocycles stack together with mutual rotation in the same direction to form helical nanotubules. The internal diameter of the nanotubules is 3 nm and the external diameter is 6.5 nm from TEM and small angle X-ray scattering (SAXS), which is in good agreement with the molecular dynamics simulations. More importantly, these helical nanotubules are segmented into sliced nanotubules with a highly regular diameter of 8 nm while maintaining helical order in these discrete nanostructures upon addition of a silver salt. This dissociation into toroids upon addition of silver salt might be understood by considering the space-filling requirements of the tubular cavity. Because the internal cavity of nanotubules requires more space to efficiently encapsulate the guest silver salts through metal–nitrile coordination, the elongated nanotubules break up into shorter objects with helical order without sacrificing π–π stacking interactions. The preservation of the shape-persistent hexameric macrocycles during this transition is responsible for the retention of supramolecular chirality. The results represent a significant example of dynamic helical nanotubules that are able to respond to external triggers by segmentation into discrete nanostructures with preservation of their supramolecular chirality. Furthermore, this transition should provide an insight into the dynamic control of the regular dissociation of 1D chiral structures.

Recently, thermoresponsive nanotubules that undergo a reversible swelling–unswelling action together with helicity inversion were reported. The nanotubule was based on a modified bent-shaped aromatic amphiphile **11** in which the central benzene core of amphiphile **10** was replaced with pyridine known to form a water cluster through hydrogen bonding (Fig. 7a).[12] Similar to amphiphile **10**, bent-shaped aromatic amphiphiles **11** self-assembled into hexameric toroidal nanostructures in an aqueous solution, containing 11 nm external diameter and 4 nm internal diameter (Fig. 7b). As the concentration of the solution was increased, the signal intensity of circular dichroism (CD) spectra began to increase, indicating chiral nanotubules were formed through spontaneous one dimensional stacking of hexameric toroidal macrocycles with a mutual rotation in one direction (Fig. 7c and d).

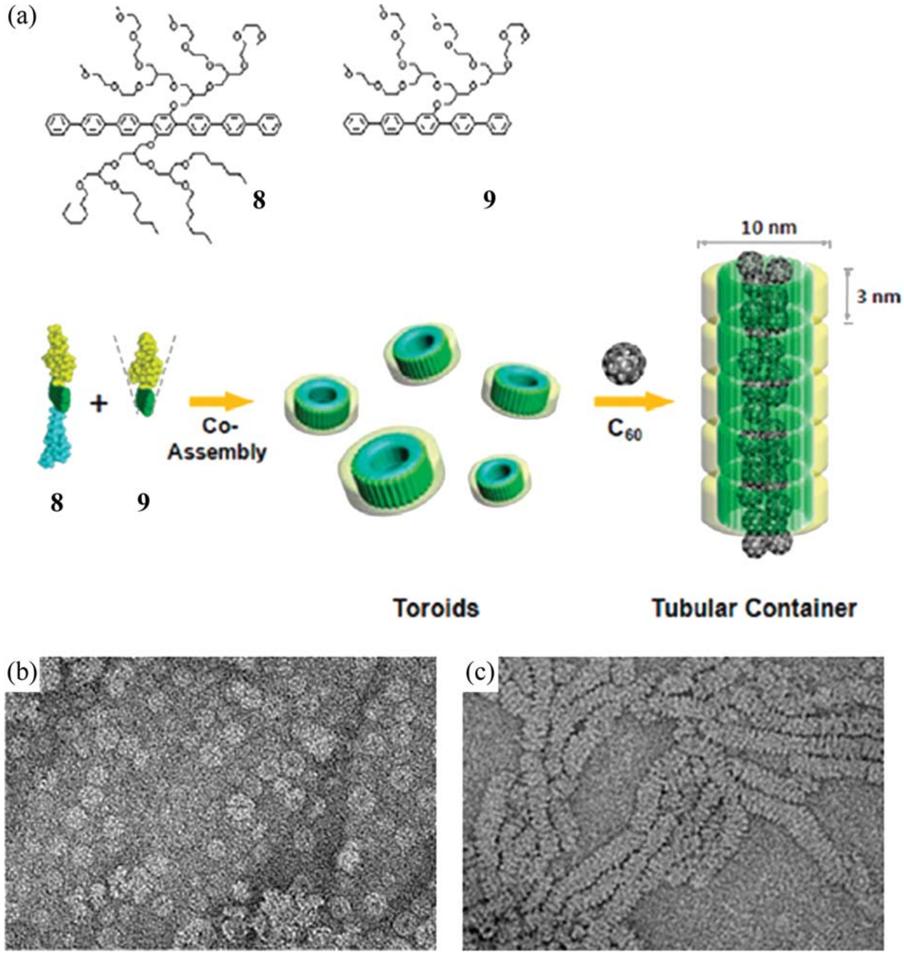

Fig. 5 (a) Chemical structures of **8** and **9**, and schematic illustration of the stacking of toroids in a 1D manner upon addition of C_{60} guest molecules. TEM images of (b) toroidal nanostructures and (c) the nanotubules of **8** and **9** after mixing with 30 mol% C_{60}. Reprinted with permission from ref. 10. Copyright (2009) American Chemical Society.

Upon heating, the dehydration of pyridine and oligoether dendrimer through the loss of hydrogen bonding interaction with water induces a contraction of the helical nanotubules to accomplish the fully overlapped arrangement of aromatic units for π–π stacking interactions. The dehydrated nanotubules show a considerable decrease in external (7 nm) and internal diameters (3 nm), providing a 47% reduction in its cross-sectional area (Fig. 8b). Interestingly, the chirality inversion between the expanded nanotubule and the contracted nanotubule was observed with temperature variation (Fig. 8d). The circular dichroism (CD) spectra of **11** showed a signal change from a negative Cotton effect at 369 nm into a positive state upon gradual heating (Fig. 8a), demonstrating that the helicities of the tubular nanostructures were highly dynamic and reversible in response to temperature. The exact handedness of the helical nanotubules and the inversion of handedness were confirmed using atomic force microscopy (AFM) experiments, which successfully visualize mirror-image helices of expanded and contracted states (Fig. 8c). This amazing thermal responsive chiral nanotubule can have a broad range of applications because the chirality and the size are variable depending on the temperature change.

Conclusion

Nanostructures and nanomaterials recently have attracted attention due to their unique and valuable properties compared to those of conventional bulk materials. Nanostructures can display significantly different properties depending on their physicochemical parameters such as size, morphology, and stability. Among the many different nanostructures, toroidal nanostructures have a unique symmetrical and annular shape and can be prepared successfully through self-assembly of the rigid–flexible block molecules and peptide-based amphilphiles. Notably, a further stacking of the toroidal nanostructures can produce well-defined one dimensional nanotubules which have enabled the construction of highly versatile and dynamic nanostructures. The nanotubules based on the stacking of toroidal nanostructures show responsive properties triggered by external stimuli such as temperature, ionic strength, and guest molecules. Although only a few studies related to toroids and nanotubules have been reported, it is obvious that more intensive research efforts will be achieved because of the fascinating functionalities and dynamic properties. Therefore, the development of new toroidal nanostructures and dynamic

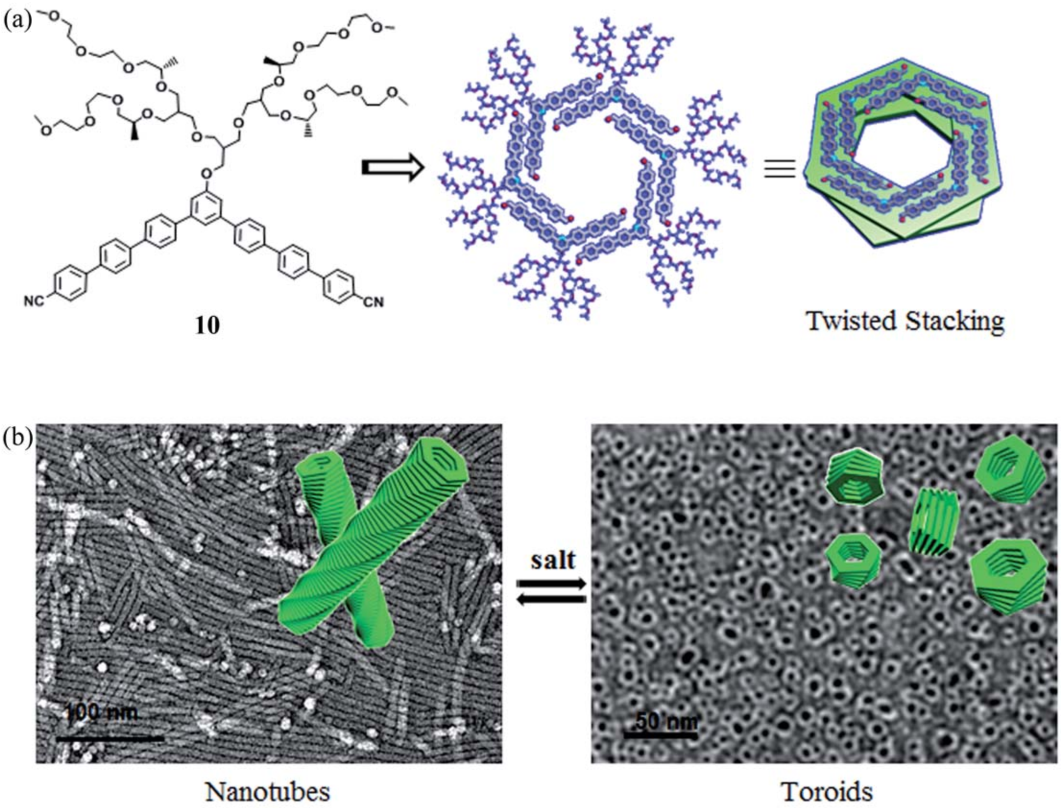

Fig. 6 (a) Schematic representation of helical stacking of hexameric toroidal macrocycles. (b) Dissociation of nanotubules into toroidal stacks and TEM images. Reprinted with permission from ref. 11. Copyright (2008) John Wiley and Sons.

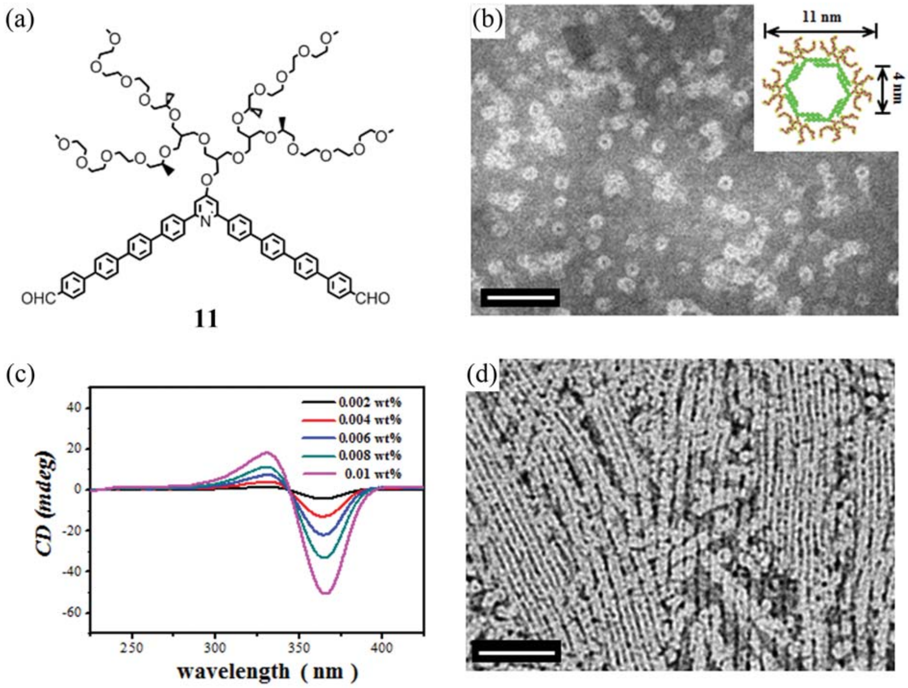

Fig. 7 (a) Molecular structure of bent-shaped rod amphiphiles **11**. (b) TEM image of **11** from 0.002 wt% aqueous solution (scale bar, 50 nm). (c) Circular dichroism (CD) spectra of **11** in aqueous solution at various concentrations. (d) TEM image of **11** from 0.02 wt% aqueous solution (scale bar, 50 nm). Reprinted with permission from ref. 12. Copyright (2012) American Association for the Advancement of Science.

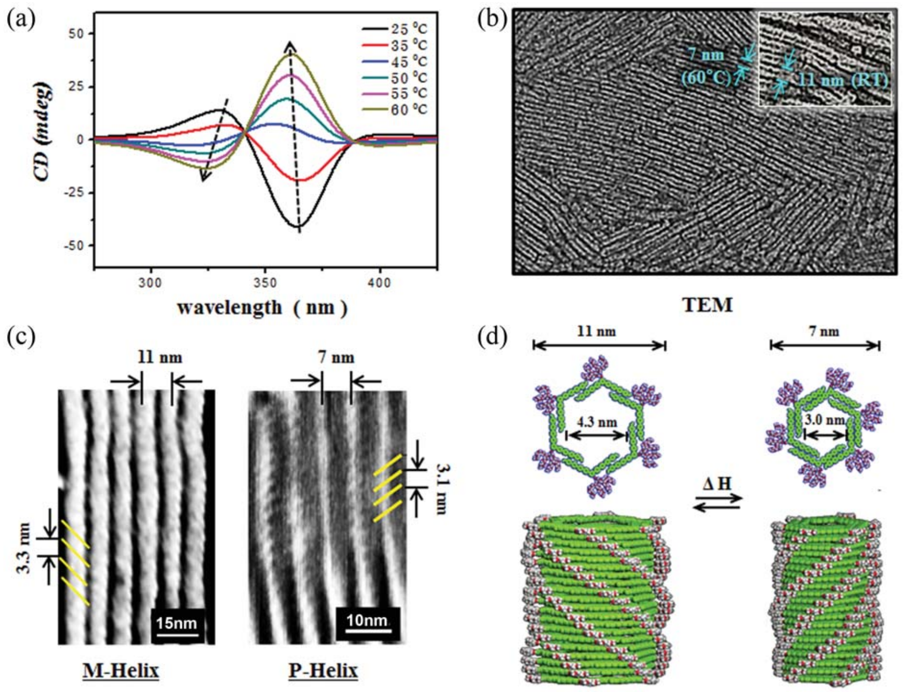

Fig. 8 (a) Temperature dependent CD spectra of **11** (0.01 wt%) in aqueous solution. (b) TEM image of **11** in aqueous solution (0.01 wt%) prepared at 60 °C. (c) AFM phase images of 2D self-assembled **11** at room temperature condition (left) and at heating condition (right) on highly ordered pyrolytic graphite (HOPG). (d) Schematic representation of reversible switching of the tubules between expanded and contracted states with chirality inversion. Reprinted with permission from ref. 12. Copyright (2012) American Association for the Advancement of Science.

nanotubules through self-assembly will be widely applicable to advanced research areas ranging from material chemistry and nanotechnology all the way up to biological science.

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