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Self-assembling triazolophanes: From croissants through donuts to spherical vesicles

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Macrocyclic compounds M1-M3 with different ring sizes containing amide and triazole units were synthesized. These triazolophanes displayed a variety of self-assembled structures such as hemi-toroids, toroids, and vesicles in a concentration dependent manner. Detailed ultramicroscopic and crystallographic investigations delineated a hierarchical mechanism of self-assembly.

The physico-chemical basis of morphogenesis continues to intrigue the scientists for over half a century. The formations and transformations of biomolecular assemblies are intricately linked with the molecular structure. The link between functional biomolecular assemblies and molecular structure is an abiding theme of biochemical research. The fabrication of specific 2D and 3D assemblies from small molecules by the process of self-assembly is a powerful strategy for the generation of functional materials and is also useful to unravel the mechanism of chemical basis of morphogenesis. Design and synthesis of molecules with the ability to self-assemble to specific 3D shapes such as toroids and vesicles have attracted attention recently. Despite the diverse applications of vesicles, the mechanism of their formation is not well understood. Toroidal structures are observed in the self-assembly of proteins, block copolymers, and surfactants and other organic molecules. Fabrication of toroidal nanostructures with uniform size and shape is a challenging endeavor, strategies such as flow-induced synthesis and solidification of polymer droplets in a microfluidic device are used for this purpose.

Here we present a class of triazolophanes with a hierarchical mechanism of assembly to toroids and vesicles. We envisaged that macrocyclic compounds incorporating aromatic and amide groups may impart rigidity and self-assemble to form a variety of 3D architectures based on conventional and non-conventional hydrogen bonds. The triazole unit is considered as a mimic of an amide bond, apart from that it adds rigidity to the structure and provides a non-classical H-bond donor site. We set out to synthesize a variety of triazolophanes making use of a Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The first series of macrocycles based on an isophthaloyl unit resulted in insoluble compounds. The high insolubility of amide-linked isophthaloyl-based triazolophanes is believed to be as a result of intermolecular interactions due to its flat structure leading to strong π-π stacking and hydrogen bonding interactions. This prompted us to place a t-butyl substituent on the phenyl ring (Fig. 1) to reduce the intermolecular stacking interactions in order to improve the solubility. The cyclophanes M1-M3 with amide rigidity and hydrogen bond donor and acceptor moieties are designed for investigating the self-assembly behavior.

Macrocycles M1-M3 were synthesized by a CuAAC reaction between dialkynes and the corresponding diazides with ~40% yield (ESI†). The relatively rigid molecular framework of M1-M3 and the amide linkages confer them with self-assembling properties. The acidic triazole CHs are additional structural attributes of these molecules that can facilitate self-assembly by non-classical CH...X (X = O, N) interactions.

Figure 1: Structures of triazolophanes M1, M2 and M3.

The self-assembly of M1-M3 were studied by electron microscopy, atomic force microscopy (AFM) and X-ray crystal structure analysis. In order to probe the self-assembly of macrocycles, isomeric triazolophanes M1 and M2 with slight difference in the linker regions were synthesized. Macrocycle M3 with leucine in the framework was synthesized in order to see the effect of chirality and macrocyclic size on the self-assembly. Solutions of these compounds (M1-M3) with different concentrations in 1:1 chloroform:methanol were placed on freshly cleaned mica and allowed to evaporate in the air and analyzed by AFM. Mica provides a macroscale atomically flat surface by simply removing the top layers. This removes the influence of surface roughness on the self-assembly process. Careful analysis of AFM data revealed a variety of structures, such as hemi-toroids, toroids and vesicles. At 0.1 mM, the macrocycles show mostly hemi-toroids as...
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Figure 2: Ultramicroscopic analysis of the self-assembly of triazolophanes. Only selected images are presented (I) (a) AFM image of M2 at 0.1 mM showing hemi-toroids. Inset shows a single magnified hemi-toroid (b) AFM image of M2 at 0.25 mM showing the toroids (c) AFM image of M2 at 1 mM showing the vesicles (d) SEM image of M2 at 0.25 mM showing mostly toroids (e) FE-SEM image of M2 at 0.5 mM showing toroids with decreasing internal cavity (f) FE-SEM and AFM image of M2 at 1 mM showing vesicles (II) Schematic representation showing the evolution of vesicles. The increase in concentration results in transformation of hemi-toroids (left) to vesicles (right).

Figure 3: (a) AFM image of M2 (b) Cross section along the line in (a). (c) Cartoon representation of toroid at 0.25 mM with dimensions estimated by SEM, TEM and AFM. Structure formed en route to becoming vesicles. Increasing concentration resulted in gradual changes from hemi-toroid to vesicles (Fig. 2). Similarly, dilution also resulted in vesicles to hemi-toroids, supporting that the transformations are bidirectional in nature. The definite concentration dependence on the topology indicates that the desired shape could be attained by change of concentration.

The macrocycle design can incorporate chiral units like amino acids in its framework. This has been demonstrated by synthesizing macrocycle M3 containing leucine in its framework. Macrocycle M3 shows self-assembly behavior similar to that of M1-M2 (Fig. 2, and Figs. S5-S7, ESI†). This opens a wider opportunity for the design and synthesis of self-assembling amino acid-based triazolophanes. Interestingly, the CD spectrum of M3 showed no characteristic CD signal indicating that the macrocycle has no specific folded conformation in solution. The
formation of vesicles for all macrocycles (M1-M3) follows the same path as revealed from their detailed ultra-microscopic studies (Fig. 2, Figs S1-S7, ESI†).

Dynamic light scattering (DLS) of M2 and M3 at different concentrations in 1:1 MeOH/CHCl₃, revealed the presence of particles with size in the range of ~198-478 nm, matching well with microscopic studies (Fig. S8, ESI†) demonstrating the presence of aggregate structures in solution.

SEM equipped with focused ion beam (FIB) was used to get finer details of toroids and vesicles (Fig. 4 and Fig. S9, ESI†). The well-formed toroid from triazolophane M2 was subjected to FIB milling using a gallium ion beam. The selected portion of the toroid was excised out (Figs. 4b, 4d) which revealed that the internal cavity of the toroid is empty. Similarly, a portion of the vesicles from M1 and M3 were sliced off, which revealed the hollow interior (Fig. S9, ESI†). Interestingly, in the TEM images, the thickness of membrane of the vesicles could not be seen, and hence appeared as dark spheres (Figs. S1c, S1f and S5c-5e, ESI†). This is also observed by other researchers and is attributed to the soft and rubbery-like nature of the vesicles.

Figure 4: FE-SEM images of M2 before (a and c) and after FIB milling (b and d). The red circles indicate the portions where FIB milling was performed. The observed surface softening of toroids after milling is due to high FIB energy employed in the experiment.¹⁴

To form closed 3D surfaces such as toroids and vesicles, a significant curvature in the self-assembly is required, suggesting a possible non-planar assembly adopted by triazolophane. In order to get insight into their self-assembly, we attempted crystallization of triazolophanes. Macrocycle M2 was crystallized from a mixture of methanol, chloroform and acetonitrile. The X-ray crystal structure of macrocycle M2 revealed an asymmetric unit comprised of two molecules of M2 (Fig. 5a, Fig. S10 and Table S1, ESI†) which differ in the arrangement of triazole units. In one conformer, both the triazole CHs are pointing to same side (syn), while in the other, CHs of triazoles are pointing in opposite directions (anti). The amide carbonyls adopt an anti-conformation in both forms. The phenyl ring carrying i-butyl group is almost perpendicular to the plane of the 1,4-phenyl unit. The macrocycle M2 has both a concave and convex surface. In the syn conformer, the nitrogens are on the concave face. The packing diagram reveals a very unique self-assembly pattern, in which different conformers of M2 (syn and anti) arrange alternately to form a cyclic tetrad resembling a toroid (Fig. 5b, Figs. S11-S12, ESI†). Symmetry expansion reveals a circular tetrameric framework in the unit cell (maximum width 27 Å), in which each of the syn and anti molecules of M2 are arranged in an alternate “up(syn)–down(anti)–down(syn)–up(anti)” fashion (Fig. S12, ESI†). The alternate “syn–anti” motif coupled with an up-down packing arrangement of the macrocycles results in the formation of a circular assembly with a hole positioned at the centre. The tetrameric supramolecular assembly in the unit cell is held together by four CH-...N and two CH-...O hydrogen bonds (Table S2, ESI†). The methylene attached to the triazole of anti conformer bonds to the triazole nitrogen of the syn conformer (2.69 Å), and one of the benzylic hydrogens of the syn conformer makes a non-covalent contact with the amide carbonyl of the anti-conformer (2.56 Å). Each molecule in the tetrad makes three hydrogen bond contacts with its two neighbors (C25B-H25C-...N6A; C15A-H15A-...O2B and C25B-H25D-...N3A). Both triazole moieties of the syn conformer take part in the hydrogen bonding. The anti conformer provides both the methylene protons attached to triazole and the amide carbonyl. A noteworthy point is that the molecules in the tetrad are held only by weak intermolecular non-classical hydrogen bonds. The tetrad assembly generates a hole at the centre that has a diameter of approximately ~10 Å.

Careful analysis of the packing revealed that the syn conformers are assembled by two N-H-...O (N1A-H1A-...O2A; N6A-H6A-...O1A) and two C-H-...O interactions (C1A-H1AA-...O1A; C23A-H23AA-...O1A) to form stacks.
In a similar way, anti conformers form stacks, wherein two intermolecular N-H…O (N1B-H1B–O2B; N8B-H8B…O1B) H-bonds are present. The separate syn and anti stacks indicate a clear case of self-sorting. The syn conformers in the tetrad make interactions with upper layer of anti conformers by (C15A-H15B–N6B; C15A-H15B–N7B) hydrogen bonds (Fig. S13, ESIF). Taken together the results from microscopic analysis and X-ray structure analysis imply that array of M2, with self-sorted stacks of syn and anti molecules and their inter-stack interactions lead to a continuous surface (Fig. 5c), which may eventually fold into hemi-toroids, toroids and vesicles (Fig. S14, ESIF).

Bensimon and coworkers reported the self-assembly of phospholipids to various topological genuses.19 Their experimental and theoretical studies indicated that the shape of vesicle is a result of minimization of its elastic curvature under various physical constraints. Our experimental results suggest that the intrinsic tendency of triazolophanes to form toroids may be due to its bent molecular architecture. The bent molecular shape causes spontaneous curvature to the assembly leading to minimum energy toroidal architecture. Increase in concentration leads to assembly of more molecules in the poloidal direction leading to vesicles (Fig. S14, ESIF).

In conclusion, we have designed, synthesized and studied the self-assembling behaviors of a series of triazolophanes by ultramicroscopy and X-ray crystallography. The studies presented here serve to support new hypothesis regarding the mechanism of vesicle formation. Furthermore, the 3D assemblies have many applications in chemistry and biology. The physiochemical basis of vesicle morphogenesis from simple molecular building blocks has profound significance in evolutionary point of view. The concentration dependent self-assembly from hemi-toroid to vesicle through the intermediacy of a toroid is a new finding and we believe this study provide deep insight into the mechanism of vesicle formation. The finer details of the internal structure obtained from FIB milling experiments, support the hollow and robust nature of self-assembled structures. We acknowledge DST, CSIR for financial support and IITD for instrumental facilities. We also acknowledge DST-FIST for HRMS and single crystal XRD facilities at IITD. We thank Prof. Narayanan Kurur and Prof. Nalin Pant, Department of Chemistry, IITD for discussions. We thank Prof. S. Aravindan and Mr. Amit Gupta, Department of Mechanical engineering, IITD for help in FIB experiments. JPP acknowledges the NSF-MRI program (grant No. CHE-1039027) for funds to purchase the X-ray diffractometer.

Notes and references