Peptide Self-Assembly

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# Bioinspired Design of Nanocages by Self-Assembling Triskelion Peptide Elements**

*Surajit Ghosh, Meital Reches, Ehud Gazit,* and Sandeep Verma**

One of the key characteristics of self-assembled biological building blocks is their ability to form well-ordered structures without the need for prepatterning.[1–9] The ability to form such structures is based on a combination of specific molecular recognition as well as specific architectures. Over the years, various natural self-assembling systems have served as inspiration for the design of novel building blocks on the nanoscale.[1–9]

The triskelion structure of the clathrin protein represents a unique example in which particular architecture of the building blocks facilitates the formation of well-ordered cagelike nanostructures.[4,10] Here, we have explored the use of the triskelial architecture, as is employed by the clathrin units, by combining them with remarkably smaller and simpler building blocks. This concept led us to explore tris(2-aminoethyl)amine (tren) as a scaffold for the conjugation of three aromatic homodipeptide units. The design of the molecular building block is based on the premise of deriving an ordered architecture from specific molecular recognition modules. For a recognition module, we employed a homoaromatic dipeptide motif—a concept shown to be very useful in the design of peptide structures on the nanoscale.[11–16]

In the present work, we envisioned that tripodal conjugation of aromatic dipeptides to tren (Figure 1) would afford a bioinspired construct, eventually resulting in interesting bioinspired self-assembled structures. The designed tripodal triskelion ditryptophan conjugate exhibits an overlap

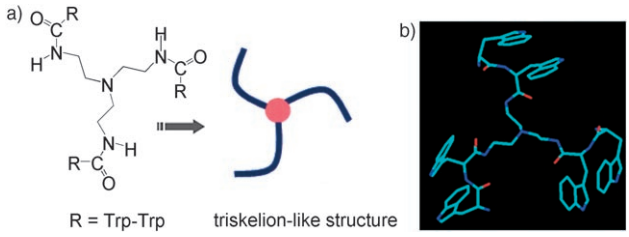


***Figure 1.*** a) Molecular structure of a tripodal dipeptide derivative and rendering of a triskelion-like structure with three dipeptide legs radiating from a central nitrogen hub of tren (triskelion ditryptophan conjugate). b) Molecular mechanics (MM+)-optimized structure of the synthetic triskelion ditryptophan conjugate (O red, N dark blue, C pale blue).

of indole aromatic rings in the MM+-optimized structure, suggesting that π stacking may play a crucial role in the self-assembly of synthetic triskelions. Our design strategy was validated as the triskelion ditryptophan conjugate exhibited rapid self-organization into spherical nanosized structures when incubated in a methanol/water mixture at 37 °C for 7 days. Spherical structures were observed using transmission electron microscopy (TEM) with negative staining (Figure 2a). High-resolution field-emission gun TEM (HR-TEM) verified the occurrence of circular patterns with a clear view of a double-layer outer boundary (Figure 2b, c and

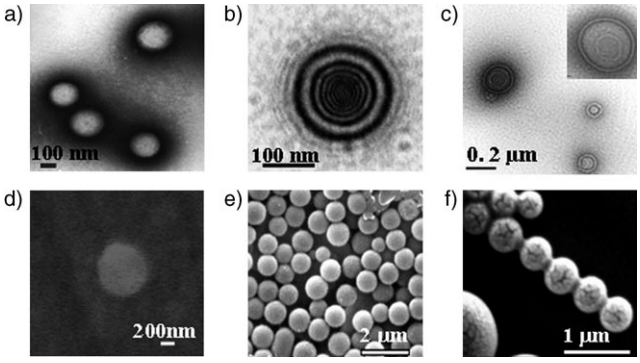


***Figure 2.*** Electron microscopy analysis of the self-assembled structures formed by the triskelion ditryptophan conjugate. a) TEM image. b, c) HR-TEM images of the ditryptophan conjugate in 60% and 90% methanol/water mixtures, respectively. The insets show higher-magnification images. A bilayer-like boundary is distinctively visible for self-assembled structures in the different methanol/water combinations. d) E-SEM image of self-associated ditryptophan conjugate under moist conditions. e) SEM images of the ditryptophan conjugate incubated for 7 days in 90% methanol/water. The appearance of spherical vesicular structures is uniformly observed. f) Beads-on-a-string attachment of the vesicular structures.

[*] M. Reches, Prof. E. Gazit
Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences
Tel Aviv University, Tel Aviv 69978 (Israel)
Fax: (+972) 3-640-5448
E-mail: ehudg@post.tau.ac.il
S. Ghosh, Dr. S. Verma
Department of Chemistry
Indian Institute of Technology-Kanpur
Kanpur-208016 (UP) (India)
Fax: (+91) 512-259-7436
E-mail: sverma@iitk.ac.in

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inset). These spherical structures could also be detected using environmental scanning electron microscopy (E-SEM) under moist conditions (Figure 2d). In addition, SEM afforded three-dimensional images of uniformly distributed spherical structures and also certain segments where more than one spherical structure was arranged in a bead-on-a-string orientation (Figure 2e,f).

Ultrastructural details of triskelion self-assembly were studied by atomic force microscopy (AFM). Highly regular multilamellar nanosized ring structures were observed upon 7 days incubation in 60% methanol/water solution when probed by AFM on a freshly cleaved mica surface (Figure 3a). A densely organized central core is surrounded by multilayered concentric nanorings of varying sizes, with interring separation distances ranging from 50 to 200 nm. This organization was independent of the surface used, as similar structures were also observed on silicon as well as highly organized pyrolytic graphite (HOPG) surfaces (see the Supporting Information), thus suggesting that self-organization is inherently engendered in the triskelion building block. The ringlike morphologies result from the dried samples in AFM and HR-TEM, while a vesicular morphology is evident in SEM and E-SEM, techniques which afford three-dimensional structures.

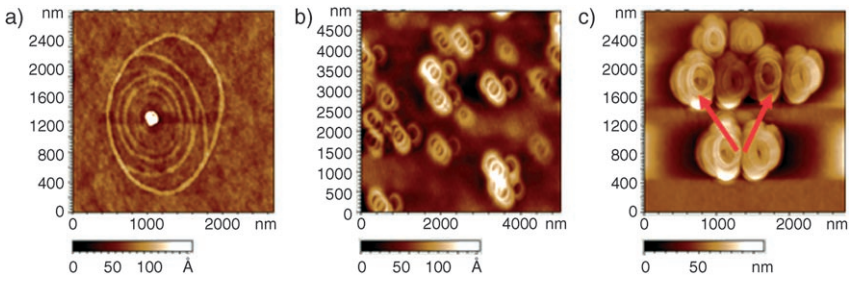


***Figure 3.*** Multilamellar structures from the ditryptophan conjugate. a, b) Self-assembled nanorings formed by the triskelion ditryptophan conjugate on a freshly cleaved mica surface after incubation during 7 days (1 mM) in 60% and 90% methanol in water, respectively. The occurrence of concentric nanorings suggests hierarchical organization of the synthetic triskelion. c) Vertically stacked clustered nanorings in pure methanol. The red arrows point towards voids formed as a result of ordered stacking.

Encouraged by the preliminary observations, the possible effects of solvent composition were studied on the nanostructures formed. Self-association of the triskelion ditryptophan conjugate in 70% and 80% methanol resulted in morphologies similar to those observed for 60% methanol, but the pattern was slightly different for 90% methanol solution whereby thicker nanorings were observed possibly as a result of the coalescence of finer nanoring structures (Figure 3b). Ageing in 100% methanol resulted in extensive aggregation through a vertical stacking of the nanorings (Figure 3c). Unlike concentration effects reported for the D-Phe-D-Phe dipeptide, in which tubular structures morphed into vesicles upon dilution, the morphology of the triskelion nanocage was not affected over a concentration range of 0.02–1 mM.[17]

Interestingly, the appearance of ordered structures was almost instantaneous when investigated by AFM. The formation of nanorings was observed within 5 s of incubation (see the Supporting Information). Prolonged incubation just refines the structure, but the gross morphology remains similar to what is seen after a few seconds of incubation. Detailed analysis of samples from various time points indicated shape-persistent nanorings from a sample incubated for 5 s to one incubated for 6 days (see the Supporting Information). Interestingly, incubation of the triskelion conjugate for 20 days revealed the formation of thicker rings, thus indicating occurrence of a nanoscale structure that displays remarkable structural integrity (see the Supporting Information). Importantly, nanocage patterns were exclusively formed without any indication of other self-assembled structures such as fibrils, filaments, and tapes.

The formation of nanorings appears to be dictated by the tryptophan residues and the tripodal scaffold, which results in directed solution-phase self-assembly of the triskelion building block. The tripodal scaffold imparts a slight curvature in synthetic triskelion, as confirmed by its MM+-optimized structure. Note that two indole moieties in a triskelion arm are stacked, thus invoking favorable aromatic interactions. It is likely that the ditryptophan arms may interdigitate to result in the formation of self-assembled polyhedra in a mechanism analogous to the clathrin-pit formation.[10] A similar role of alkylated indole rings was previously described in the formation of stable vesicular structures.[18]

Clathrin-coated vesicles entrap materials for receptor-mediated uptake and cellular trafficking. Thus, it was of interest to investigate guest entrapment in triskelion nanocages. The triskelion was co-incubated with rhodamine B dye for encapsulation to permit fluorescence detection of dye-loaded nanocages. Fluorescence microscopy clearly indicated dye entrapment as revealed by the appearance of fluorescent circular structures (Figure 4a,b). This observation affirms the inspiration behind the synthetic triskelion conjugate and substantiates stepwise growth of vesicular structures around guest molecules. Interestingly, we were able to facilitate the release of dye by simple acidification, which not only led to empty nanocages but also caused deformity in their structure and coalescence (Figure 4c,d). This observation may be attributed to the protonation of the tertiary nitrogen center of the tren scaffold and the dipeptide α-amino group followed by electrostatic repulsion. Thus, these nanocages can serve as potential entrapment agents with a simple release mechanism.

Control experiments with constructs containing a single Trp residue connected to the tren scaffold afforded ill-defined, larger nanoring structures, while Phe-Phe dipeptide conjugation led to the formation of nanotubes with sporadic appearance of vesicular structures (see the Supporting Information). It seems that the Trp-Trp dipeptide is a critical core motif essential for the generation of nanocages. The indole ring of Trp residues enables secondary structure

  

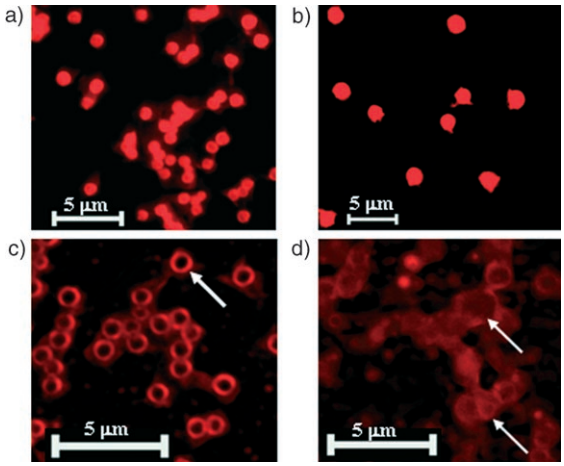


***Figure 4.*** Fluorescent micrographs of entrapment and release of rhodamine dye from nanocages. a,b) Images after 1 and 2 days of incubation, respectively, showing uniform, bright red circular structures indicative of trapped dye. c) Release of dye upon acidification of nanocages at pH 5.5 after 15 h. The arrow indicates an empty cage. The fluorescence remains localized around the periphery after release of the dye without affecting the morphology. d) Release of dye upon acidification of nanocages at pH 2.2 after 15 h. The arrows indicate deformed cages. Incubation at pH 2.2 not only results in the release of fluorescent dye but also causes deformation of the nanocage structure.

stabilization as a result of π-stacking interactions.[11–14,19–20] This property is responsible for events such as biomolecular recognition, protein structure stabilization, and biochemical functions.[21–24] Notably, the MM+-optimized structure of synthetic triskelion displays a parallel displaced type of π–π stacking.

In summary, we have described a bioinspired design paradigm for a self-assembling synthetic peptide triskelion with guest entrapment properties. Other successful forays in bioinspired design of functional self-assembling structures include peptide nanotubes from cyclic peptides,[25] nanotubes and nanovesicles from surfactant-like peptides,[26] conducting nanowires from Sup35p protein,[27] and oligopeptide based β-sheet tapes with useful mechanical properties,[28] among other possible applications.[29–30] We propose that entrapment properties of this synthetic triskelion could be harnessed as delivery vehicles with a possibility of attaching recognition modules through available N termini for the delivery of drugs and cytotoxic agents.

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