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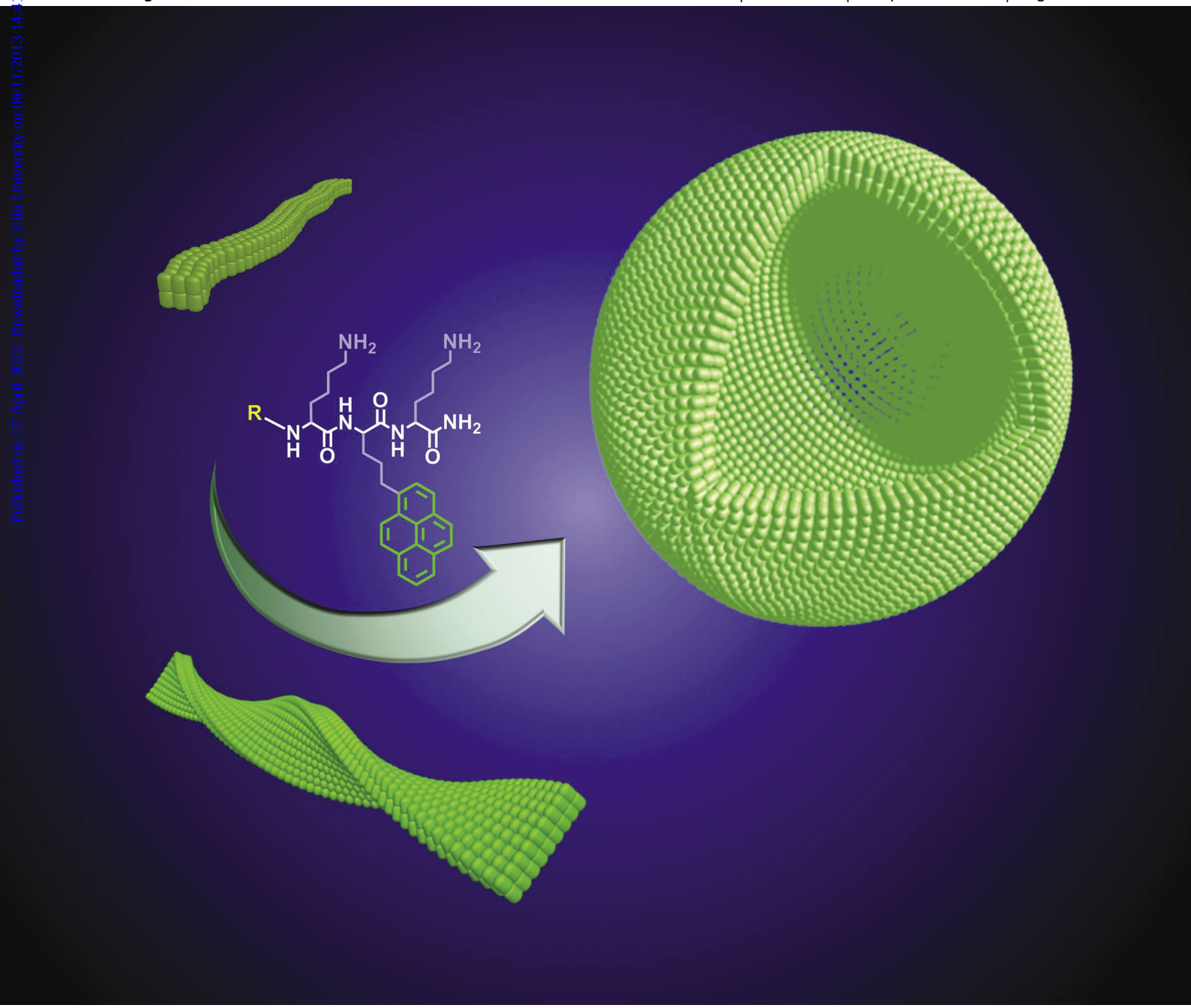

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Control of peptide assembly through directional interactions†

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We demonstrate the self-assembly of tripeptide amphiphiles into spherical hollow capsules from linear nanoribbons *via* control of the molecular packing. We achieved a transition of arrangement from anisotropic to isotropic by an elaborate design of the molecular architecture.

The self-assembly of peptide amphiphiles into well-organized nanostructures has been the subject of intense study due to the fundamental importance of understanding the natural peptide assembly and their potential applications.[1] Synthetic peptide amphiphiles have adopted secondary structures like α-helices and β-sheets, and further organized into more complicated nanostructures.[2,3]

Recently, the construction of one-dimensional (1D) structures from short peptides such as di- or tripeptide molecules has attracted considerable attention because of their simple synthetic procedure and well-defined nanostructures with potential applications and practical implications.[4] Such nanostructures include nanoribbons, twisted ribbons, and nanotubes obtained from the self-assembly of simple peptides.[5] Such peptides usually consist of three main segments: (i) a hydrophobic group, commonly a side chain of an amino acid, such as phenylalanine and tryptophan, or aromatic segments that drive aggregation through hydrophobic and π–π interaction; (ii) a β-sheet-forming peptide backbone that promotes 1-D nanofiber structures; (iii) a hydrophilic group either at the *N* or *C* terminal of the peptide amphiphiles or in the side chain of an amino acid (*i.e.* lysine or glutamic acid) that provides charge repulsion to prevent undefined agglomeration but instead promotes well-defined nanostructures.[6]

In small peptide systems, the formation of β-sheet structures is the main driving force for assembly, and 1-D nanostructures are commonly observed due to the directional ordering derived from β-sheet hydrogen bonding. Thus, tuning of β-sheet characteristics by modifying the molecular architecture will be a rational strategy for the control of aqueous nanostructures. For this reason, we designed three tripeptides based on lysine, where the middle lysine is functionalized with pyrene at its ε-position acting as a hydrophobic unit. The *N*-termini differ and are hydrogen, acetyl and Fmoc (fluorenyl-9-methoxy-carbonyl), respectively (Fig. 1). Our key strategy to tune β-sheet characteristics is this variation of the *N*-terminal, as self-assembly of these tripeptides requires a delicate balance between electrostatic repulsion (here: lysine ammonium side-chains), hydrogen bonding (stabilized by hydrophobic *N*-termini), and hydrophobic π–π interactions induced by the pyrene units. Herein, we present the self-assembly of these tripeptides into 0-D vesicle, 1-D twisted ribbon and 1-D flat ribbon structures by changing the molecular packing from an isotropic to an anisotropic arrangement induced by β-sheet formation (Fig. 1).

Transmission electron microscopy (TEM) image of tripeptide **1** functionalized with Fmoc at the *N*-terminal revealed the formation of fibers with uniform diameters of 6.7 nm and lengths reaching the

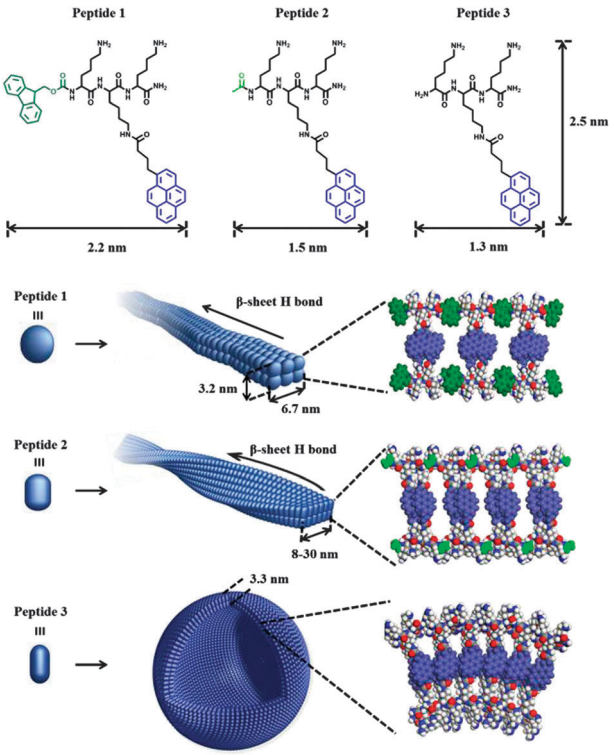

Fig. 1 Molecular structures of the tripeptides and schematic presentation of their self-assembled nanostructures. Peptide **1** which has similar height and width aggregates into a flat ribbon. Peptide **2** which can be described by rectangular shape forms a twisted ribbon. Peptide **3** which has a more narrow width assembles into a vesicle structure.

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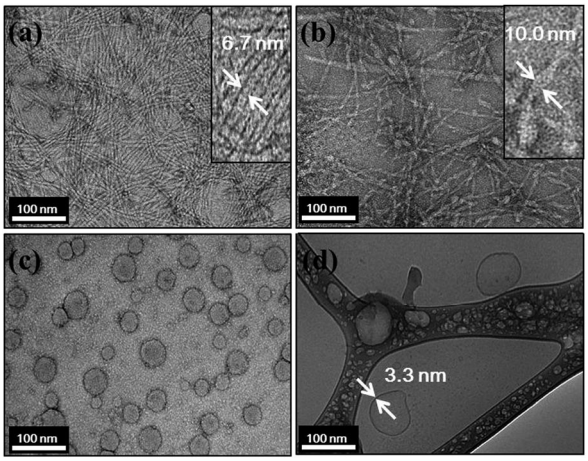

Fig. 2 Negatively stained TEM images in water at 200 μM. (a) Tripeptide **1**, (b) **2**, (c) **3** and insets are magnified images of (a) and (b). (d) Cryo-TEM images of tripeptide **3** (5 mM aqueous solution).

micrometer-scale (Fig. 2a). Atomic force microscopy (AFM) images of tripeptide **1** confirmed that this fibril is of a flat ribbon like structure with an average height of 3.2 nm (Fig. S3, ESI†). In contrast, tripeptide **2** functionalized with an acetyl group at the *N*-terminal formed twisted nanoribbons with sizes of 8–30 nm in width and several hundred nanometers in length (Fig. 2b). Interestingly, tripeptide **3** showed a spherical shaped aggregation in TEM with a radius of about 40 nm (Fig. 2c). Considering the distance between the hydrophilic amine at the ε-position of lysine and the pyrene units in **3** to be 2.5 nm, the dimension of the spheres exceeds the extended molecular length by a factor of about 16, which strongly suggests that the objects might be vesicles rather than simple micelles. In addition, the concaveness which is observed in the negatively stained TEM image indicates the shape of shrunk spheres formed during the drying process, providing further evidence of the hollow nature of the vesicular structures (Fig. 2c). To further corroborate the formation of vesicles, we performed a cryo-TEM investigation. As shown in Fig. 2d and Fig. S8 (ESI†), the micrograph shows hollow spherical objects with a uniform wall thickness of 3.3 nm, which is slightly less than twice the extended molecular length, suggesting an interdigitated bilayer molecular packing of tripeptide **3** (Fig. 1).

The self-assembling behavior of the tripeptides was further scrutinized by fluorescence spectroscopy and dynamic light scattering (DLS) experiments. The absorption maxima of tripeptides **1** and **2** in aqueous solution are red-shifted and the intensities decreased with respect to the monomers in acetonitrile. This absorption behavior and the observed quenching of fluorescence indicate J-type aggregation of the pyrene segments within the ribbon structure (Fig. S4 and S5, ESI†).[7] Remarkably, the fluorescence of tripeptide **3** only showed the excimer peak at longer wavelength regions in water, which is an indication of inter-pyrene electronic communication between closely packed pyrene units in the excited state (Fig. 3a).[8] DLS experiments of **3** showed a monomodal size distribution with an average hydrodynamic radius (R_H) of 40 nm (Fig. 3b). In comparison, tripeptides **1** and **2** showed bimodal distributions and hydrodynamic radii much larger than that of **3**. These results correlate with the TEM data.

Fourier-transform infrared (FTIR) spectroscopy and circular dichroism (CD) studies were applied to determine the secondary

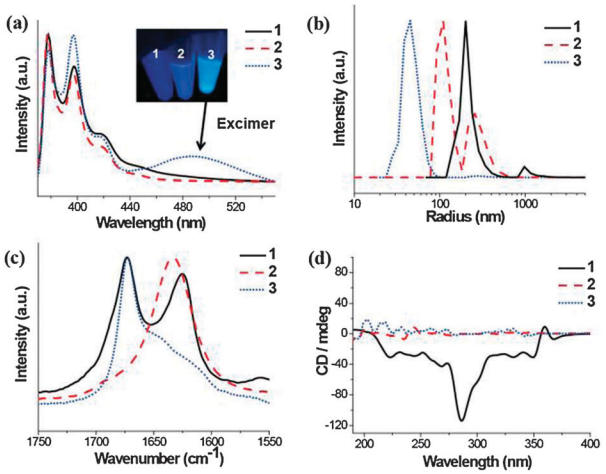

Fig. 3 (a) Fluorescence spectrum (λ_{ex} = 340 nm) of tripeptides in aqueous solution at 1 mM. Only **3** shows the excimer peak at 490 nm. Inset: 1 mM aqueous solutions of **1** (left), **2** (middle), **3** (right) illuminated by a UV (365 nm) lamp. (b) R_H distribution of peptides obtained by DLS (200 μM aqueous solutions). (c) FTIR spectra of peptides in D_2O at 1 mM. (d) CD analysis of peptide secondary structures (1 mM aqueous solution) **1** (solid), **2** (dashed) and **3** (dotted).

structure within the self-assembled nanostructures.[9] As shown in Fig. 3c, the FTIR spectrum of tripeptide **1** shows a strong band at 1625 cm^{-1} and a weak signal at 1675 cm^{-1}, which are typical signals of antiparallel β-strands.[10] In contrast, tripeptide **2** showed a broad peak at 1633 cm^{-1}, indicative of the weak β-sheet hydrogen boding within the twisted ribbon. Tripeptide **3** showed a strong peak at 1672 cm^{-1} which is assigned to the trifluoroacetate counterions of the ammonium group in lysine units.[11] This indicates that tripeptide **3** adopts a random coil structure between the peptide backbone, as revealed by the lack of the characteristic peaks of hydrogen bonding in the FTIR spectrum. The CD spectrum of tripeptide **1** showed pronounced negative Cotton effects in water, while **2** and **3** displayed flat signals. This indicates that Fmoc-containing tripeptide **1** assembles into highly oriented chiral structures in water *via* strong β-sheet formation (Fig. 3d).

We propose a packing model for the self-assembly of the tripeptides as shown in Fig. 1. In the case of tripeptide **1**, the 6.7 nm width of the ribbon structure fits well with the length of three pairs of stacked β-sheets in an antiparallel packing fashion (the length of one fully extended monomeric unit was determined to be 2.2 nm from molecular modeling). Furthermore, the 3.2 nm height of the nanoribbon determined by AFM is comparable to the calculated height of an interdigitated β-sheet bilayer stack, presumably driven by hydrophobic and π–π stacking interactions of overlapped pyrene units (Fig. 1). The nanoribbons are composed of a hydrophobic pyrene interior in the bilayer β-sheet peptide domains, and a hydrophilic exterior consisting of the side chain of two lysines facing the aqueous interface. The β-sheet structure might be further stabilized by intermolecular π–π interaction between the fluorenyl groups of neighboring antiparallel β-sheets.

The decreasing hydrophobicity of the *N*-terminal group from Fmoc to acetyl induced a morphological transition from a long flat ribbon to a short twisted ribbon. This is because of a loss of π–π interactions between neighboring antiparallel

β-sheets, which results in a destabilization of the β-sheet and a premature termination of ribbon growth. The resulting decreased interaction between β-sheets causes weak directional ordering along the peptide backbone, resulting in the increase in width and formation of a twisted structure rather than a laterally finite flattened ribbon.

The removal of the hydrophobic group from the *N*-terminal changes the molecular packing from an anisotropic conformation to an isotropic one. Tripeptides **1** and **2** show directional order forming β-sheets induced by the hydrophobic interactions at *N*-termini, resulting in a 1-D nanoribbon structure. In the case of peptide **3**, the absence of the hydrophobic interactions at *N*-termini increases the flexibility of the molecular arrangement, which provides the possibility of forming curvature. Also, by the flexibility of the molecular arrangement, peptide **3** can make a denser packing structure with a face-to-face overlapping of inter-pyrene units resulting in excimer formation. These results suggest that tripeptide **3** adopts an isotropic planar conformation rather than the directional ordered anisotropic conformation like **1** and **2**, leading to hollow vesicular structures.

In conclusion, we reported the rational design of tripeptide amphiphiles for the formation of 0-D and 1-D nanostructures and explained the self-assembling behavior based on their molecular architecture. Tripeptide **1** forms 1-D flat ribbons through an anisotropic arrangement of peptides by strong β-sheet formation stabilized with intermolecular fluorenyl π–π interaction at the *N*-terminal Fmoc. Peptide **2** with acetyl as a smaller hydrophobic *N*-terminal, aggregates into a twisted ribbon structure due to weaker β-sheet formation. The removal of the hydrophobic group from the *N*-terminal induces an isotropic molecular packing, resulting in 0-D spherical nanocapsules. The results here imply that a delicate balance between β-sheet formation and hydrophobic π–π interaction plays a critical role in the self-assembly of short peptide amphiphiles. We believe that this fundamental understanding of peptide self-assembly will provide an important strategy to design well-defined nanostructures using small peptide building blocks for advanced nano- and biomaterials.

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