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A Torsional Strain Mechanism To Tune Pitch in Supramolecular Helices**

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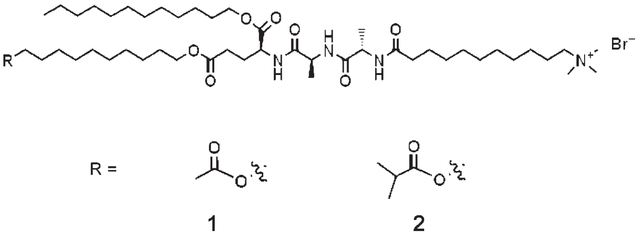
Inspired by biology, contemporary chemistry is challenged with the synthesis of architecturally defined functional structures that are much larger than ordinary molecules.[1] One emerging strategy is the use of noncovalent interactions in polymolecular assemblies to craft the shapes, dimensions, and functions of nanostructures. The scientific goal is to gain access to unknown static and dynamic functions with supramolecular systems which could be designed with the same ease that small molecules are now synthesized. Supramolecular pores,[2] tubes,[3] cylinders,[4] helices,[5] and mushroomlike noncentrosymmetric clusters[6] have been among the targets of recent work in this area. We report here a torsional strain mechanism to tune the pitch of micrometer-long helical assemblies in the range of tens to hundreds of nanometers.

Helices in biology, such as double-helical DNA, protein coiled coils, and twisted β sheets,[7] have inspired an extensive amount of work on synthetic helical nanostructures.[5] Artificial structures containing peptide β sheets have been of particular interest[8,9] since they only require short amino acid sequences to form and also because of their relevance in diseases linked to amyloid fibrils. During our recent work on the functionalization of cylindrical nanofibers formed by tripeptide amphiphiles in organic solvents (such as cyclohexyl chloride),[9,10] we found that simple modification of the compounds led to nanostructures with dramatically different morphologies.



Compounds **1** and **2** were synthesized with an acetate and a dimethyl acetate end group, respectively, and we used atomic force microscopy (AFM) to examine the morphology of the supramolecular aggregates they form. At a concentration of 1% (by weight) both compounds dissolve in cyclohexyl chloride at about 80 °C and form translucent self-supporting gels upon cooling to room temperature. AFM studies of the diluted gels dried on silicon substrates revealed straight cylindrical fibers for compound **1** (Figure 1 a). In contrast, AFM images of the aggregates formed by molecules of compound **2** show helices with a regular pitch (Figure 1 b) of 22(±2) nm (Figure 1 c), and the orientation of the height contour clearly indicates these helices have a left-handed sense.

The significant change in the morphology of the assembly when the acetate end group in **1** was replaced by a dimethyl acetate substituent in **2** suggested that a bulkier substituent at the terminus of the alkyl segment causes twisting of the initially cylindrical assemblies. To gain a mechanistic view of this process, we considered first the driving force for aggregation in these systems. In a low polarity solvent, the amphiphilic molecules studied here assemble as a result of solvophobic interactions as well as the formation of an intermolecular β sheet between the peptide segments.[9] The charged tetraalkylammonium head groups are buried inside the nanofibers as a result of their low affinity for the organic solvent, and the less polar tails are present on the surfaces of the nanofibers. Infrared absorbance spectroscopy reveals the formation of parallel β sheets[11] in these aggregates. To establish the twisted nature of the β sheets[7] in these systems, we synthesized a diacetylene-containing tripeptide amphiphile compound without any bulky substituent in the alkyl segment and subsequently polymerized the diacetylene moieties to introduce chromophores into the aggregates. Circular dichroism spectroscopy on the polymerized aggregates confirmed the helical nature of the β sheets in the cylindrical nanostructures, which do not reveal any helicity under AFM.[11] This finding is consistent with the twisted nature (with left-sense handedness) of the β sheets of almost all natural proteins.[7]

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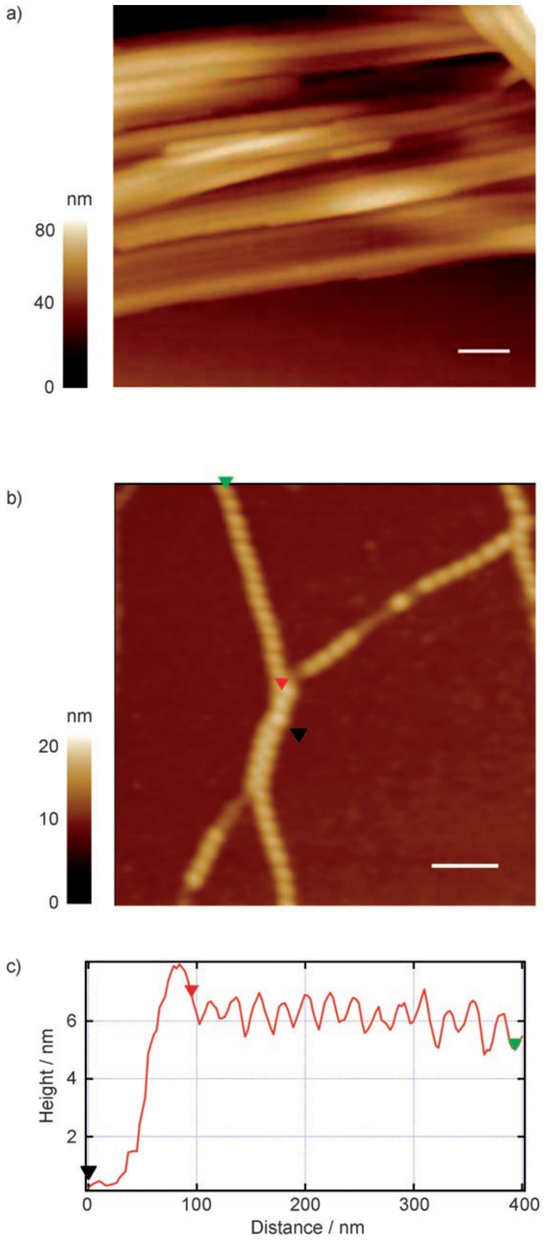


Figure 1. AFM images of the aggregates formed by compounds: a) **1** and b) **2** in cyclohexyl chloride. c) The height profile along the line connecting the reverse triangles in (b), with the corresponding positions labeled. Statistical analysis show that these helices have a pitch of 22(±2) nm. Scale bars: 100 nm.

The twisted β sheets in cylindrical assemblies indicate the helical ones formed by compound **2** are actually superhelices (Figure 2a). The β sheets in these aggregates define the primary helices (Figure 2b), and the presence of the bulky end groups could be generating torsional strains that drive the formation of the superhelix (Figure 2c). Torsional strain caused by steric repulsion between the bulky substituents may overwind the β sheets. We propose that the axis of the β sheets forms a superhelix to accommodate the bulky groups on the periphery of the β sheets without disrupting the hydrogen-bonded network (Figure 2c). The observed left-sense in the assemblies formed by compound **2** is consistent with superhelices made by overwinding left-handed primary helices (Figure 2a).[12] The primary helical structures cannot be resolved with AFM, presumably because of the presence of multiple β sheets inside the fibers and the flexible alkyl tails on the surface.

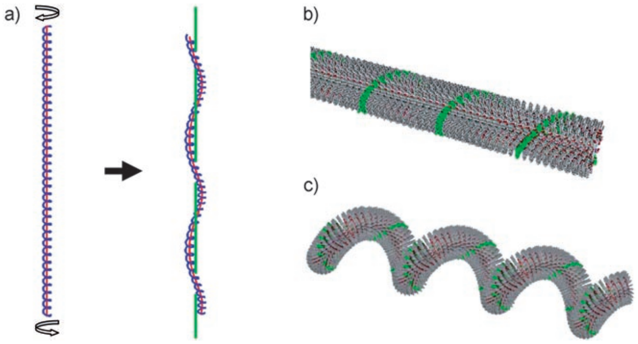
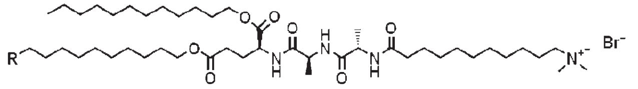


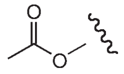
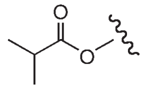
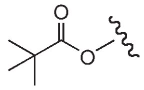
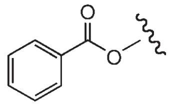
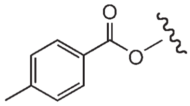
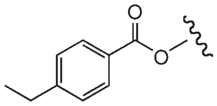
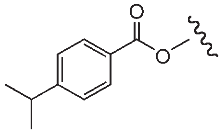
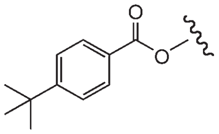
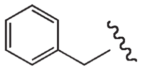
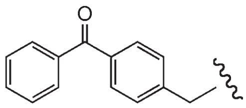
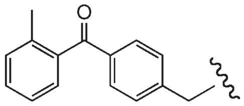
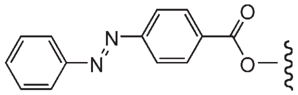
Figure 2. a) Schematic representation of a twisted helical telephone cord: when a left-handed cord (primary helix) is overwound, its axis (red) relaxes into a secondary helix (superhelix), and the superhelix is also left-handed (the green line represents the axis of the superhelix). The pitch of the superhelix is always larger than that of the primary helix, and can be varied by changing the magnitude of the torsional strain applied. A larger torsional strain leads to a smaller pitch and vice versa. The superhelix has handedness opposite to the primary helix if the primary helix is unwound (not shown). b) Schematic representation of an aggregate of tripeptide amphiphile molecules shaped as a cylindrical nanofiber. One of the internal twisted β sheets of the aggregate is highlighted in green, and defines a left-handed primary helix. c) Schematic representation of a superhelix formed by the supramolecular aggregate as a result of sterically driven internal strain. One of the β sheets in the superhelix is highlighted in green, with its axis now making a secondary helix with a much larger pitch.

To provide support for the torsional strain mechanism in the formation of the superhelices, we synthesized three series of additional compounds **3–11** (Table 1) with end groups of widely different bulkiness. Compounds **3–11** all formed self-supporting gels in cyclohexyl chloride. AFM measurements indicate the formation of helices with the pitch values listed in Table 1; the estimated van der Waals volume[13] of the end groups are also given. Straight cylindrical fibers are described as assemblies having infinite pitch, and as expected the helices are all left-handed when L-amino acids are used in the synthesis. Within each of the series (**1–3, 4–8**, and **9–11**), the pitch of the helices decreases as the van der Waals volume of the end groups increases. This trend is qualitatively consistent with the van der Waals picture of liquids and solids,[14] in which the steric effects produced by the short-range repulsive forces primarily determine the arrangement of the molecules. Interestingly, there appears to be a lower limit in the pitch values for the first two series and the value is the same (ca. 22 nm) in both series, despite the structural differences in their end groups. This common limiting value may result from the requirement to maintain the hydrogen bonds in the β sheets and to change it further may require variation in the peptide sequence.

The pitch of the helices appears to also depend on the shape and packing of the end groups within the assemblies. This effect can account for the apparent discrepancy when comparing compounds across the series listed in Table 1. For

Table 1: Chemical structure of **1**–**12**[a], pitch of the supramolecular helices they form[b], and estimated van der Waals volumes[c] of their R substituents.



Compound[a]	R	Pitch [nm][b]	van der Waals volume [$\AA^3$][c]
1		∞	58.1
2		22(±2)	92.7
3		22(±2)	110.0
4		∞	113.4
5		160(±30)	130.7
6		40(±3)	148.0
7		22(±2)	165.3
8		22(±2)	182.6
9		∞	104.4
10		90(±10)	218.2
11		60(±10)	235.5
12		74(±6)	292.3

[a] All compounds were synthesized using L-amino acids. [b] The pitch values are averages obtained from AFM height images of tens of nanostructures.[c] van der Waals volumes are calculated for compounds with the formula R-H by using the empirical equations in Ref. [13].

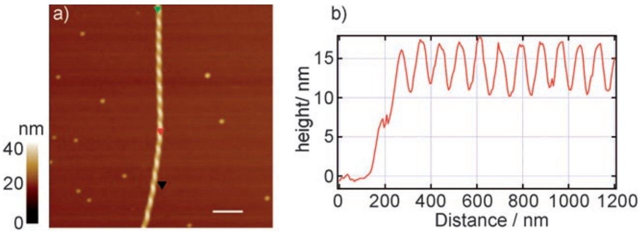


Figure 3. a) AFM image of the aggregates formed by **10** and b) height profile along the line connecting the reverse triangles in (a). The pitch of the helices is 90(±10) nm from statistical analysis. Scale bar: 200 nm.

example, we expect that the phenyl groups in compound **4** would tend to stack, thereby reducing the torsional strain in the assembly. Similarly, the nearly disclike shape of benzophenone[15] in **10** would explain the large pitch in the supramolecular aggregate (Figure 3), while deviation from planarity in **6** and **7** can lead to greater strain and thus a smaller pitch.

It will be some time before we can quantitatively predict the pitch of the helical molecular assemblies, since the collective forces involved are complex and difficult to model in atomistic detail. However, our work clearly shows that steric forces on the twisted β sheets play an important role in the tunability of the pitch in the supramolecular assemblies. We describe this effect here as a torsional strain mechanism to tune the pitch of a superhelix, analogous to the observed macroscopic relaxation of primary helices strained by an applied torque.[12]

The class of compounds studied here share common characteristics with many others that form helical aggregates, such as chirality and the ability to form intermolecular hydrogen bonds or π stacks,[16] and thus we speculate that the superhelix mechanism described here may be quite general. Previously, the formation of helical aggregates by chiral molecules was explained by macroscopic continuum elastic theories.[17] However, a mechanism bridging molecular chirality and helical morphology over larger length scales has not been available.[16] We believe the superhelix formation described here offers a mechanism to fill this gap.

Potential applications for nanostructures with tunable pitch include systems to control stereoselective chemistry[18] and nonlinear optics.[19] In addition, they may also be used for dynamic processes such as sensing or actuation, provided the end groups can be modified in situ to impart greater steric forces through chemical changes. As a proof of principle, we exposed a suspension of nanofibers made of **12** (Table 1) in cyclohexyl chloride to UV radiation with a wavelength of 360 nm,[11] and used AFM to study the actuation of the helices as a result of the *trans*-to-*cis* isomerization of the azobenzene moiety (Figure 4). We observed (Figure 4a and b) that the light-induced molecular isomerization leads to a reduction in the pitch of the supramolecular helices. As the *cis* isomer is less planar than the *trans* isomer,[20] the isomerization should result in an increase of the sterically induced torque, thus leading to a reduction in the superhelical pitch. The decrease in the pitch caused by illumination with UV light appears to vary from nanostructure to nanostructure, thus suggesting an inhomogeneous level of isomerization in each supramolecular assembly. Interestingly, however, each nanostructure appears to have a uniform pitch, which in turn is an indication that the torque-induced actuation is the result of a relaxation process throughout the entire assembly. Many opportunities may thus

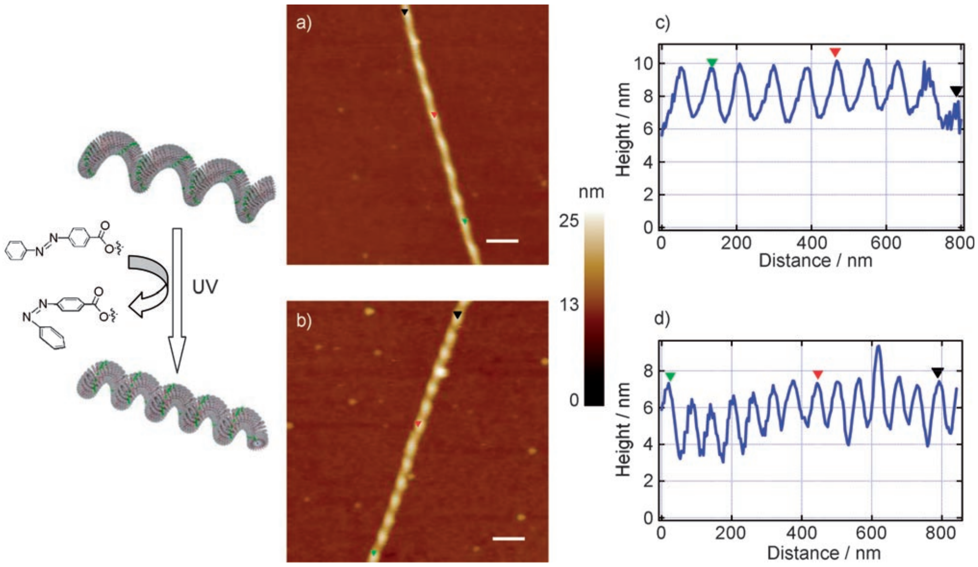


Figure 4. AFM height images of the fibers: a) before and b) after exposure to UV light,[11] as well as the height profiles (c and d) along the straight lines connecting reverse triangles in (a) and (b), respectively. The helices before illumination are made of the *trans* isomer, and have a uniform pitch of 78(±6) nm from statistical analysis. After exposure to UV light the helix pitch is reduced as the light induces formation of the nonplanar *cis* isomer.[20] The pitch value varies from nanostructure to nanostructure, and ranges from 40 nm to 70 nm. As an example, (b) shows one nanostructure with a pitch of 56(±)4 nm. Scale bars: 100 nm.

emerge in the future for artificial dynamic systems since the torque prescribing the pitch can be varied by tuning the interaction between the building blocks within the aggregates.

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