

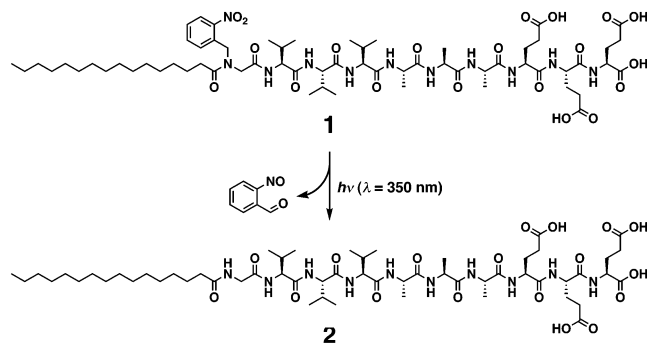
Quadruple Helix Formation of a Photoresponsive Peptide Amphiphile and Its Light-Triggered Dissociation into Single Fibers

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Building well-defined higher-order architectures through supramolecular chemistry is one of the challenges in nanotechnology and is critical for our understanding of biological self-assembly. Secondary structures, such as α -helices and β -sheets in proteins and the double-helix of nucleic acids, generate stable tertiary and quaternary structures with critical roles in the three-dimensional architectures in biology. Furthermore, conformational changes of proteins can switch functional states such as enzymatic activities.¹ Inspired by these switchable structures seen in biomolecules, we have investigated here the synthesis of photoresponsive peptide amphiphiles (PAs), well-known to self-assemble into supramolecular nanofibers.² The single-handed helical structure has always been an attractive target, and more recently, there is also interest in multiple helices.^{3–5} Here we report the discovery of a quadruple helical fiber formed by photoresponsive PA **1** and its conversion into single fibers upon photochemical cleavage of the 2-nitrobenzyl group in **1**.



Compound **1** contains a palmitoyl tail, the 2-nitrobenzyl group, and an oligopeptide segment GV₃A₃E₃. Based on previous work in our group,² the amphiphilic structure of **1** is expected to drive self-assembly into cylindrical nanofibers. The nitrogen of the *N*-terminal amide of **1** has a 2-nitrobenzyl group that can be cleaved by irradiation at 350 nm to afford **2**.⁶ Hartgerink et al. have reported that hydrogen bonds on the amino acids close to the core play a major role in directing self-assembly into cylindrical structures.⁷ In their research, even a methyl substitution at the amide groups affects considerably the secondary structure formation of PAs. Because of the lack of hydrogen bonding on the amide closest to the alkyl segment and the bulkiness of the 2-nitrobenzyl group, we expected that **1** and **2** would differ in their supramolecular architecture after self-assembly. In a recent paper on different amphiphilic molecules, termed peptide lipids, we reported the twisting of a cylindrical supramolecular assembly with regular pitch as a result of bulky substituents near a β -sheet forming tripeptide.³

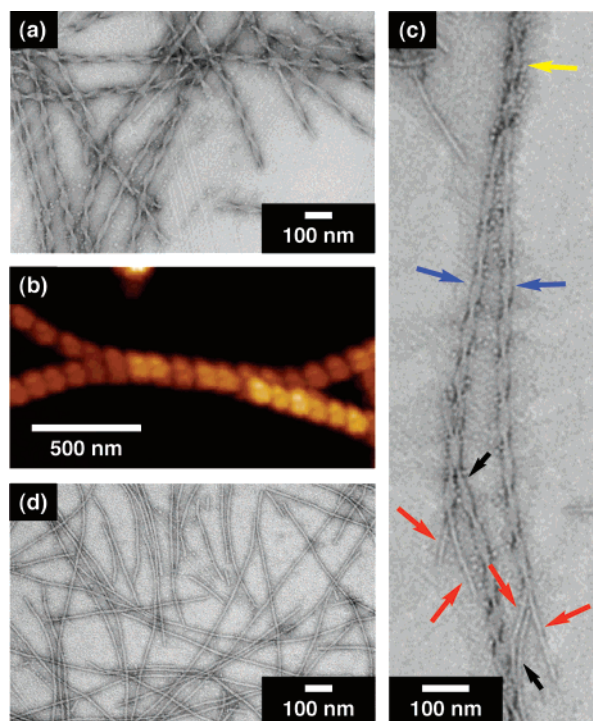


Figure 1. (a) TEM and (b) AFM images of **1** annealed at 80 °C at a concentration of 7.4×10^{-4} M in pH 11 water containing NH₄OH for 30 min and slowly cooled to 25 °C over 90 min followed by air-drying on the substrate. (c) TEM image of a quadruple fiber; the yellow arrow points to the quadruple strand which uncoils into double helices (blue arrows) and further into single fibers (red arrows). Additional fibers are seen in the micrograph (black arrows). (d) TEM image of an air-dried pH 11 water solution of photoirradiated (350 nm, 250 W, 5 min) **1** after annealing.

Our interpretation was that torsional strain by the bulky groups on twisted β -sheets resulted in relaxation of the assembly into a superhelix. Compound **1** was synthesized employing a standard Fmoc solid-phase peptide synthesis method (see Supporting Information). The successful synthesis and the purification of **1** have been confirmed by IR spectroscopy, MALDI-TOF MS spectrometry, and HPLC (Figures S1–S3). Compound **2** has also been synthesized separately for comparison.

Transmission electron microscopy (TEM) of **1** revealed interesting assemblies with helical architecture and an aspect ratio exceeding 1000 (Figures 1a and S4), with a nearly uniform width and helical pitch of 33 (± 2) and 92 (± 4) nm, respectively (at least 80% in many micrographs). A TEM image of the terminus of one of the supramolecular structures reveals it to be a quadruple helix (Figures 1c and S5). The examples of quadruple helices are rare, and as far as we know, there has been only one paper reporting a quadruple helix composed of micellar fibers.⁵ The quadruple nature of the helix can be seen in Figure 1c which shows that the helix is actually composed of two smaller helices (blue arrows), and each

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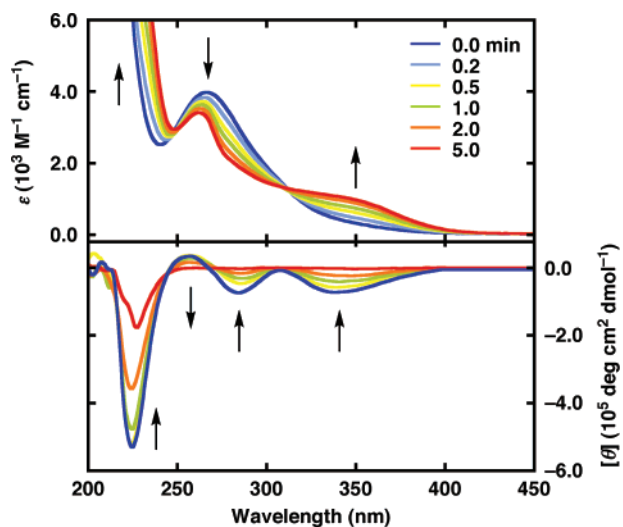


Figure 2. UV-vis (top) and CD (bottom) spectral changes of **1** (7.4×10^{-4} M) in pH 11 water containing NH_4OH upon 350-nm irradiation at 25 °C. Arrows indicate the direction of spectral changes.

of these is composed instead of two individual fibrils (red arrows). The diameter of the nonhelical fibrils is 11 nm, a dimension that corresponds approximately to twice the calculated length of **1** (5.6 nm). Based on TEM images, the width and pitch of the double helices are $24 (\pm 1)$ and $106 (\pm 3)$ nm, respectively. The orientation of the height profile in an AFM image revealed both helices have right-handed sense (Figures 1b and S6). A characteristic negative Cotton effect at 225 nm in the circular dichroism (CD) spectrum as well as an amide I absorption band at 1634 cm^{-1} in the IR spectrum indicate the presence of a β -sheet structure in the aggregates (Figures 2 and S1).^{8,9} Since the amide A band is below 3300 cm^{-1} (3282 cm^{-1}), the peptide segment appears to be stretched to form the intermolecular β -structure.¹⁰ Compound **1** displays an IR absorption peak associated with the vibrational band of C–H bonds in its alkyl chain at 2923 and 2853 cm^{-1} at a frequency which is higher than that of control compound **2** (2920 and 2848 cm^{-1}), suggesting that **1** in its quadruple helix architecture has “liquid-like” packing of alkyl chains and the cylindrical nanofibers of **2** have more ordered nanofiber cores.¹¹ This is consistent with our previous finding that simple nanofibers formed by PAs tend not to have liquid cores.⁸ Considering **2** does not have any absorption longer than 250 nm (Figure S7), the two negative CD bands at 285 and 338 nm and a positive CD band at 257 nm can be attributed to the 2-nitrobenzyl group, suggesting a helical environment for this group in the nanofibers.¹² A bulky 2-nitrobenzyl group close to the core appears to hinder dense packing of the alkyl chains and also leads to the twisting of its supramolecular assemblies.

Interestingly, after a 5-min irradiation ($\lambda = 350 \text{ nm}$) of **1**, the helical structures disappeared completely in the TEM images, and only cylindrical fibrils with a diameter of 11 nm were observed (Figures 1d). UV-vis spectroscopy showed an intensity decrease of the absorption band at 266 nm with an increase of the absorption band around 350 nm having isosbestic points at 249 and 312 nm, and in the CD spectrum, Cotton effects at 225, 257, 285, and 338 nm decreased significantly, thus indicating photocleavage of the 2-nitrobenzyl groups (Figure 2). In MALDI-TOF MS spectrometry, the signals corresponding to **2** were clearly observed after photoirradiation (Figure S2), and HPLC showed nearly complete conversion from **1** to **2** (ca. 97%, Figure S3). A standard sample of **2** (7.4×10^{-4} M) also forms fibers with a diameter of 11 nm in the same solvent conditions as those above (Figure S8). Furthermore, an irradiated sample of **1** has an IR absorption band at 1633 and 3279

cm^{-1} indicating the presence of intermolecular β -sheet structure in the nanofibers.^{8,10} Before and after the photoirradiation, IR absorption bands at 2923 and 2853 cm^{-1} did not show any significant shifts (Figure S1), although the standard sample of **2** has absorption bands at lower wavenumbers (2920 and 2848 cm^{-1}). This result suggests that alkyl tails within the fibers did not rearrange significantly during the conversion from the quadruple helical bundles to the single fibrils.

We conclude that a peptide amphiphile molecule bearing a bulky photocleavable group self-assembles into supramolecular quadruple helical fibers that dissociate into single nonhelical fibrils in response to light. We hypothesize that poorer internal packing of PA molecules caused by sterics creates the opportunity for interactions among molecules in neighboring nanofibers (hydrogen bonds, hydrophobic interactions). This could explain why the well-defined quadruple supramolecular structure becomes thermodynamically favorable. We believe our discovery suggests novel strategies to create dynamic functions in materials through the transformation of higher-order supramolecular architectures using external stimuli such as light.

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Supporting Information Available: Details of synthesis; IR, MALDI-TOF MS, HPLC, UV-vis, TEM, CD data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (12) At 10-fold dilution of a solution of **1** (7.4×10^{-5} M), the CD bands at 257, 285, and 338 nm almost disappeared, indicating they are induced by self-assembly. See Figure S9.

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