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Self-Assembly of 1-D *n*-Type Nanostructures Based on Naphthalene Diimide-Appended Dipeptides

Hui Shao,[†] Tuan Nguyen,[‡] Natalie C. Romano,[‡] David A. Modarelli,[‡] and Jon R. Parquette*[,†]

Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio 43210, and Department of Chemistry and The Center for Laser and Optical Spectroscopy, Knight Chemical Laboratory, The University of Akron, Akron, Ohio 44325-3601

Received July 29, 2009; E-mail: parquett@chemistry.ohio-state.edu

The self-assembly of one-dimensional (1-D) nanostructures composed of electronically active constituents has tremendous potential to enhance the performance of optoelectronic devices.[1] Although most nanostructured electronic materials are derived from inorganic components, the self-assembly of organic π-systems has recently emerged as a method to tune the properties of thin film materials and devices.[2] The majority of these systems are *p*-type organic semiconductors that organize in organic media.[3] Consequently, the development of strategies to produce π-electronic nanostructures from small, *n*-type organic building blocks in water remains as a significant challenge. Herein, we demonstrate a simple method for fabricating *n*-type 1-D nanostructures via the β-sheet assembly of dipeptides bearing a 1,4,5,8-naphthalenetetracarboxylic acid diimide (NDI) side chain into either helical nanofibers or twisted nanoribbons.

Aromatic diimide derivatives serve as electron acceptors for solar energy conversion,[4] molecular electronics,[5] and *n*-type organic semiconductors.[6] Intermolecular $\pi-\pi$ interactions in such planar aromatic molecules can be modulated with solubilizing side chains to facilitate self-assembly.[7] The poor directionality of $\pi-\pi$ overlap makes the design of 1-D nanostructures with well-defined morphologies quite difficult using this strategy.[2] The β-sheet self-assembly of short peptides[8,9] provides a potential strategy to control the long-range spatial organization of functional chromophores.[10,11] The construction of π-electronic nanostructures from suitably functionalized dipeptide building blocks has not yet been achieved.

Recently, we observed that a 16-mer peptide−dendron hybrid could be induced to form either soluble nanotubes or amyloid-like nanofibrils.[12] The two structures could be interconverted by modulating the extent of charge repulsion with changes in pH or in salt concentration. Most amyloid-type fibrillar aggregates form insoluble infinite networks.[13] We found that attenuating the self-assembly process, which was driven by hydrophobic $\pi-\pi$ association in water, with the electrostatic repulsion of protonated lysine groups[14] produced finite, soluble, nanoscale assemblies.[12] We demonstrate in this work that this strategy also permits the controlled self-assembly of simple dipeptide−NDI conjugates into well-defined 1-D nanostructures.

Our design strategy focused on a series of dilysine peptides functionalized at the ϵ-amino position with an NDI chromophore (Figure 1). We reasoned that the hydrogen-bonding along the peptide backbone and $\pi-\pi$ association of NDI chromophores, which strongly drives β-sheet assembly in water,[15] would be mitigated by the electrostatic repulsions of adjacent protonated lysines, resulting in soluble, well-ordered nanostructures (Figure 1.).[14] Generally, designed β-sheet-forming peptides display sequences that alternate hydrophobic (H) and polar (P) residues.[16] Notably, this design exemplifies a minimal alternating H/P sequence.

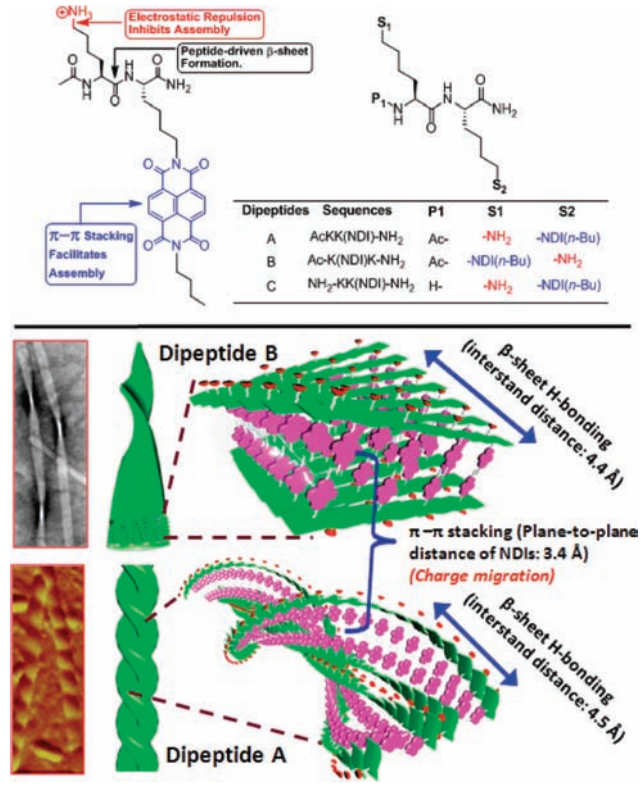

Figure 1. Design of minimal H/P-type dipeptide-NDI sequences (Top). Assembly of dipeptide-NDI conjugates **A** and **B** into left-handed fibers and ribbons (Bottom). Interstrand distances of β-sheets and plane-to-plane distance of NDIs shown determined by XRD.

Transmission electron microscopy (TEM) imaging of dipeptide **A** revealed the formation of helical, micrometer-long nanofibers with uniform diameters of 10 ± 1 nm (Figure 2a). Atomic force microscopy (AFM) images of the fibrils of dipeptide **A** confirmed a left-handed superhelical twist with a pitch length of 50 ± 4 nm and average heights of 7 ± 1 nm (Figure 2b inset and Supporting Information), resembling several disease-related amyloids such as Aβ1-40, hen lysozyme.[17] In contrast, dipeptide **B**, which reverses the positions of the NDI and free amine groups, appeared as flattened, twisted nanoribbons with sizes of 20−70 nm in width and 6 (flat)−16 nm in height (Figure 2c and d, Supporting Information). These nanoribbons also exhibit a left-handed twist (Figure 2c, inset) and a constant height distribution along the long axis of the ribbons, indicating a relatively smooth surface (Figure 2d, inset). In contrast to **A** and **B**, the self-assembly of dipeptide **C** was significantly attenuated by the increased electrostatic repulsion contributed by the additional protonated amine, resulting in no observable nanostructures at 250 μM by TEM (Supporting Informa-

[†] The Ohio State University.
[‡] The University of Akron.

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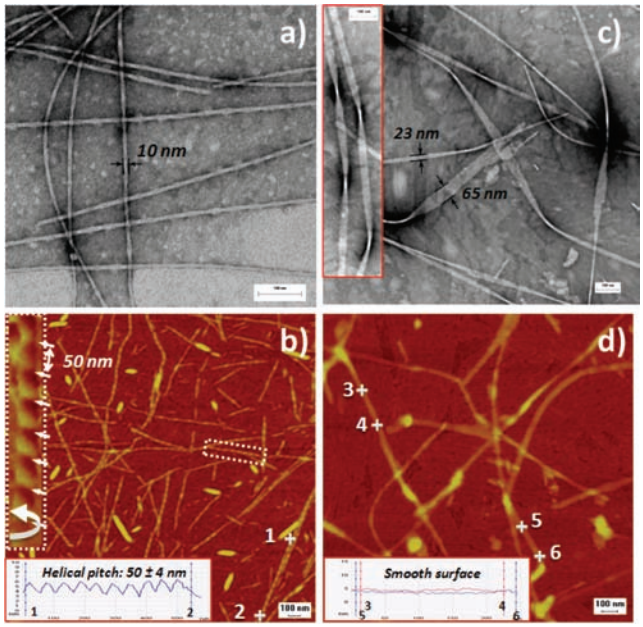

Figure 2. Self-assembly of dipeptide **A** and **B** in water (250 μM). Dipeptide **A**: (a) TEM image (carbon-coated copper grid); uranyl acetate as negative stain. (b) Tapping-mode AFM image on freshly cleaved mica. Inset: a single fiber showing left-handed helicity along the long axis of nanofiber; section analysis between two cross in AFM image showing regular helical pitch. Dipeptide **B**: (c) TEM image. Inset: two ribbons showing left-handed twists. (d) Tapping mode AFM image. Inset: section analysis between two cross in AFM image showing smooth surface of ribbons. Scale bar: 100 nm.

tion). However, at very high concentrations (25 mM), nanofibers could be observed by TEM (Supporting Information). Cryo-TEM analysis further confirms that the observed nanostructures from dipeptide **A** and **B** are formed in an aqueous solution rather than during the evaporation of water (Supporting Information).

Fourier-transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) was used to determine the secondary structures of dipeptides within the self-assembled nanostructures (Supporting Information). In addition to the absorptions at *ca.* 1660 and 1704 cm^{-1} due to the symmetric and asymmetric stretching of the imide carbonyl of the NDI group, dipeptides **A** and **B** displayed a strong amide I ($\nu_{C=O}$) band at 1612 and 1608 cm^{-1}, respectively, characteristic of aggregated intermolecular β-sheets.[18] No such β-sheet absorption bands, however, were shown in the FTIR spectrum of dipeptide **C**. For comparison, in a 2,2,2-trifluoroethanol (TFE) solution, FTIR spectra of all three dipeptides showed a strong absorption at 1640 cm^{-1}, indicating that a random coil structure was adopted. Similarly, no discernible assemblies could be observed by TEM for all three peptides in TFE.

XRD patterns taken from dipeptides **A** and **B** likewise indicated the presence of ordered β-sheet assemblies within the nanostructures (Figure 3b). Reflections were observed with *d*-spacings of 4.4 Å (**A**) and 4.5 Å (**B**), which are characteristic of the interstrand spacings in "cross-β" patterns.[19] Likewise, intersheet spacings of 9.9 Å (**A**) and 9.1 Å (**B**), respectively, also support a β-sheet structure. No apparent reflections in XRD pattern were observed for dipeptide **C**, consistent with the lack of any long-range order observed by TEM and AFM.

As shown in Figure 3a for **A**, all three dipeptides exhibited UV−vis absorptions at 240 nm (band II) and in the range of 300−400 nm (band I), corresponding to $\pi-\pi^*$ transitions polarized along the short and long axis of the NDI chromophore, respectively.[20] Band I absorptions for **A** and **B** were red-shifted by 8 and 6 nm and exhibited 50% and 38% decreases in intensity, respectively, going from TFE to water. The decreased intensity and red-shifting of the band I absorptions indicate that *J*-type $\pi-\pi$ interactions contribute to the formation of the nanostructures of **A** and **B**.[21] The XRD patterns shown in Figure 3b reveal the $\pi-\pi$ stacking distances within the nanostructures to be 3.42 Å for **A** and 3.35 Å for **B**, similar to the interplanar distances present in the crystal structure of NDIs.[22]

To investigate the relative orientation of the NDI chromophores within the assemblies, circular dichroism (CD) studies were carried out for **A**−**C** in both water and TFE (Figure 3c). All three peptides displayed flat signals in TFE, in which the peptides did not assemble. Similarly, only weak transitions could be discerned between the 300−400 nm (band I) region of the CD spectrum of **C** in water. In contrast, both **A** and **B** showed strongly negative excitonic Cotton effects in water corresponding to both $\pi-\pi^*$ absorption bands I and II (between 260 and 220 nm). In particular, band II, which is polarized along the short axis of NDI, shows a negative bisignet couplet centered at 242 nm indicating a left-handed, *M*-type helical arrangement of adjacent NDI transition dipoles within the nanofiber and nanoribbon assemblies of **A** and **B**.[20] Both assemblies displayed exceptional thermal stability, indicated by the Cotton effects remaining in the CD spectra of **A** and **B** at temperatures as high as 90 °C (Supporting Information).

The presence of extended aggregates of π-conjugated NDI moieties in self-assembled fibers and ribbons should facilitate long-range charge migration as a result of effective intermolecular π-electron delocalization.[23] The fluorescence spectra of **A**−**C** in TFE displayed maxima at ~408 nm with low energy shoulders at 430 nm that are typical for monomeric *N*,*N*-dialkyl-substituted NDIs (Supporting Information).[24] In water, the fluorescence spectra of all three dipeptides exhibited an additional broad emission band at lower energy (λ_{em} ~498 nm) consistent with inter-NDI electronic communication in the excited state between closely spaced, spatially constrained, NDI groups.[25] Time-correlated single photon counting (TCSPC) experiments with picosecond excitation at 305 nm and monitoring the emission at 420 nm yielded short-lived decays consistent with the NDI singlet excited state lifetime (τ_{Fl} ~16.4 ps, Φ_{Fl} ~0.002).[26] Monitoring the emission at the lower energy band at ~500 nm, however, revealed longer-lived multiexponential decays. The average decay lifetimes (τ_{500nm}) for **A** (57 ps) and **B** (153 ps) were substantially longer-lived (Figure 3d, Supporting Information) than that of **C** (<10 ps). The longer-lived excited states observed for **A** and **B** are not likely to have formed within the lifetime of the excited state but rather arise from preassociated NDIs in the assemblies. Notably, dipeptide **C**, which does not undergo self-assembly, has the shortest lifetime. Time-resolved fluorescence anisotropy experiments were performed (λ_{em} 500 nm) because depolarization of the fluorescence signal within the excited state lifetime is expected to result *only* when energy migration is prominent, since the rotational tumbling of the NDIs within the assemblies, or the assemblies themselves, will be considerably slower than the excited state lifetime. As expected for an amorphous material, the initial anisotropy value (r_0) for **C** was found to be 0.225. Strikingly, zero initial anisotropy was observed for **B**, consistent with rapid depolarization by energy transfer along the twisted nanotape. The intermediate-lived **A** exhibited an initial anisotropy value (r_0 ~0.08) between **B** and **C**. Although both **A** and **B** form β-sheet assemblies, the sharper reflections observed in the diffraction pattern of **B** indicates a higher degree of uniformity within the nanostructure, compared with that of **A**, which shows broader reflections (Figure 3b).

A working model for the self-assembly of **A** and **B** can be envisioned as shown in Figure 1. The extended lengths of **A** and **B** (N−C terminus) are ~11 Å, whereas the distance going from the side chain ammonium to the tip of the NDI alkyl chain is *ca.* 27

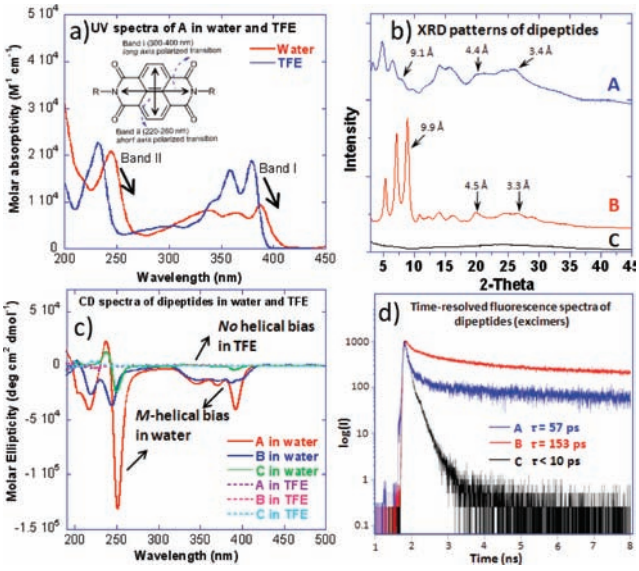

Figure 3. (a) UV spectra of dipeptide **A** (1 mM) in water and TFE. Inset: principal y and z polarized $\pi-\pi^*$ transitions in the NDI chromophore. (b) XRD patterns of dipeptides. (c) CD spectra of dipeptides (250 μM) in water and TFE. (d) Time-resolved fluorescence spectra of **A**−**C** in water (excimer).

Å. Therefore, the ribbons are likely composed of two stacked β-sheet aggregates, in accord with the thickness of flattened ribbons of **B** (*ca.* 6 nm based on AFM measurements, Supporting Information). This arrangement would sequester the hydrophobic NDI-modified side chains within the interior and project the hydrophilic lysine side chains on the surface of the assembly. The helical fibers of **A** may emerge from the intertwining of two small fibrils, resulting in a uniform diameter at ∼10 nm (Supporting Information).[17]

In conclusion, we report the synthesis and self-assembly properties of minimal H/P type β-sheet forming dipeptides bearing an *n*-type organic semiconductor NDI segment. NDI-dipeptides **A** and **B** form amyloid-like 1-D helical nanofibers and twisted nanoribbons in an aqueous solution, depending on the placement of the NDI group. β-Sheet-type hydrogen bonding and $\pi-\pi$ association play important roles in directing the assembly process. A delicate balance between electrostatic repulsion and hydrophobic interactions is critical for the self-assembly. Charge transfer between NDI moieties within 1D assemblies **A** and **B** provides promising applications for optoelectronic nanodevices.

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Supporting Information Available: Experimental, FTIR, AFM, Cryo-TEM, CD, NMR, MS data and time-resolved fluorescence decays for the studied peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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