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# Design of Self-Assembling Peptide Nanotubes with Delocalized Electronic States[**]

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## Electronically active biomaterials via directed peptide self-assembly

Redox-promoted self-assembly of an eight-residue cyclic D,L-α-peptide bearing four 1,4,5,8-naphthalenetetracarboxylic diimide (NDI) side chains results in the formation of electronically delocalized peptide nanotubes hundreds of nm in length. The supramolecular approach described provides a rational basis for the design and fabrication of 1-D materials with potential utility in optical and electronic devices.

### Keywords

supramolecular chemistry; self-assembly; nanotubes; cyclic peptides; charge delocalization

A central consideration in bottom-up approaches aimed at the design and fabrication of nanoscale functional materials is the ability to predictably associate the properties of building blocks at the molecular level to the materials' large scale order and function. In this regard, the principles of supramolecular chemistry have become increasingly useful in materials design including the fabrication of electronic systems.[1,2] Here we describe an eight residue cyclic D,L-α-peptide bearing four cationic 1,4,5,8-naphthalenetetracarboxylic diimide (NDI) side chains that undergoes redox-triggered self-assembly in aqueous solution into peptide nanotubes[3] possessing highly delocalized electronic states. Using this approach, isolated peptide nanotubes hundreds of nm in length can be obtained and adsorbed onto solid surfaces. The studies described here provide a rational approach for the design of electronically active peptide nanotubes with potential utility in optical and electronic devices.[1h,j,2b,f,4,5]

Self-assembling cyclic D, L-α-peptide nanotubes are electronically insulating[6] as are most biomaterials derived from natural amino acids. Theoretical studies have demonstrated that the bandgap of peptide nanotubes can be altered by modifying cyclic peptide sequence, but natural amino acid side chains are not sufficient to create electronically conductive assemblies.[6e] Previous studies have shown that NDI derivatives in extended ring stacked configurations can be used for fabrication of organic electronic materials.[4,5,7] We hypothesized that hydrogen-bond-directed self-assembly of NDI-bearing cyclic D,L-α-peptides (Figure 1) could be used to juxtapose and thus promote intermolecular stacking of NDI side chains to provide ordered electronically active biomaterials (Figure 2a). In a recent study, we explored the basic design concept in the context of dimeric cyclic D,L-α-peptide cylindrical ensembles and showed that intermolecular peptide self-assembly is an effective template for promoting ring stacking and

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**Supporting Information Available:** Supplementary figures, synthetic methods, and experimental details.

charge transfer between NDI side chains.[8] In the present study, we report the utility of this approach in the fabrication of peptide nanotubes with extended charge delocalized states.

The $C_4$ symmetric sequence structure of the eight-residue cyclic peptide **1** (Figure 1) having four homochiral NDI residues was designed to enforce inter-subunit juxtaposition of NDI side chains in the self-assembled nanotubular configuration.[3b] Force field minimization modeling suggested that the peptide nanotube scaffold formed by **1** could template extended stacking of lysine-modified NDI side chains (Figure 2a). In the model, the NDI side chains tilt with respect to the peptide rings (4.7 Å intersubunit distance) minimizing the distance between the aromatic moieties (3.6 Å). Such tight packing is required for efficient charge delocalization and has been observed in many conductive materials based on aromatic ring stacking.[2,9] In addition, introduction of the eight primary amine side chain substituents were expected to afford aqueous solubility, inhibit peptide self-aggregation, and disfavor non-specific hydrophobic NDI-NDI interactions through intermolecular electrostatic repulsions.[10] Dipeptide **2** (Figure 1), which manifests the smallest repeating unit of **1** was prepared as a model compound to probe the unique effects on the structural and functional properties of **1** deriving from the cyclic D,L-α-peptide architecture.

An important criterion in the design of materials based on the cyclic D,L-α-peptide nanotube architecture is the installation of a mechanism for promoting the process of self-assembly. We have shown previously that modulation of electrostatic interactions can be used to trigger cyclic D,L-α-peptide nanotube formation.[3a,b,11] A salient feature of the present design is that the NDI moieties can be reduced, through mild chemical or electrochemical methods, resulting in anionic radicals that are stable in the absence of oxygen[4,7] (Figure 2b). Furthermore, NDI anion radicals have been shown to self-associate in aqueous solution[12] and can be deposited as films with ohmic conductivity.[4] Therefore, we envisioned that cyclic D,L-α-peptide self-assembly in aqueous solutions could be promoted via decrease of the net charge on cyclic D,L-α-peptide **1** as a result of radical anion formation in the presence of sodium dithionite and the potential contributions arising from attractive interactions between NDI-NDI radical anion side chains (Figure 2c).[4,7] The redox-mediated cyclic peptide self-assembly thus should serve a dual role in our design in which NDI radicals act as both shepherds for peptide self-association and the foundation for electronic properties of the nanotubular assembly.

Peptides **1** and **2** were reduced under anaerobic conditions by treatment with an excess of sodium dithionite in $D_2O$. Formation of NDI radical anions were confirmed by the appearance of a new peak at 444 nm in the visible region of the absorption spectra (Figure 3a). The process can be reversed by quenching radical anions upon introduction of air/oxygen into the sample. Previous studies have suggested that NDI radical anions can π-stack to form aggregates in aqueous solution that give rise to characteristic near-infrared (NIR) absorption bands as a result of electron delocalization.[4,7] The wavelength of the NIR absorption band has been suggested to correlate with the aggregate size and the extent of charge delocalization within NDI stacks. The NIR spectrum obtained for **1** (Figure 3b) shows a broad multicomponent absorption with a maximum centered around 2200 nm and a weaker absorption band at 1200 nm. The observed peak at 2200 nm suggests extended charge delocalization, while the smaller peak at 1200 nm can be attributed to NDI dimers[4,7] formed most likely through intramolecular NDI-NDI radical anion interactions within monomeric cyclic peptides. Comparison of peptide **1** and **2** NIR spectra at equal concentration of NDI groups (1.5 mM) reveals an increase of ~900 nm in $\lambda_{max}$ for cyclic peptide **1** relative to control compound **2** (Figure 3c). The presence of a NIR band at 2200 nm in peptide **1** as well as its significant red shift with respect to that observed for control dipeptide **2** highlight the role of the cyclic D,L-α-peptide nanotube architecture and self-assembly in promoting charge delocalized NDI anion radical stacks.

Atomic force microscopy (AFM) images of **1** adsorbed on mica from a reduced solution revealed a fibrous material consistent with formation of mainly isolated peptide nanotubes hundreds of nanometers in length and 2–3 nm in height (Figure 4). Self-assembled peptide nanotubes can also be adsorbed onto other surfaces including hydroxylated hydrophobic pyrolytic graphite (HOPG) and silicon oxide (Figure S1a,b). The presence of adsorbed peptide nanotubes on distinct surfaces as imaged by AFM strongly suggest that nanotubes are formed in solution in the presence of sodium dithionite and then physisorbed onto the solid surfaces. Furthermore, AFM imaging of cyclic peptide **1** in the absence of reducing agent and the control dipeptide **2** in the reduced state gave only amorphous structures (Figure S1d,f). Moreover, exposing the reduced solution of cyclic peptide **1** to air prior to adsorption on the solid support resulted in small amorphous aggregates (Figure S1e). The peptide nanotubes demonstrate high structural stability when supported by a substrate and persist even after two months exposure to ambient temperature and atmosphere.

In summary, we have shown that the long range supramolecular order afforded by the directed backbone hydrogen bonding interactions in self-assembling cyclic D,L-α-peptide nanotubes can provide a facile method for the preparation of a new class of synthetic biomaterials that exhibit extended charge delocalized states. Furthermore, the potential utility of this and other appropriately functionalized self-assembling peptide nanotubes in the design and fabrication of optical and electronic devices would likely benefit from available methods for the preparation of peptide nanotube films using Langmuir deposition techniques[13] and high-resolution patterning of cyclic D,L-α-peptides using dip-pen nanolithography[14] (see Supporting Information).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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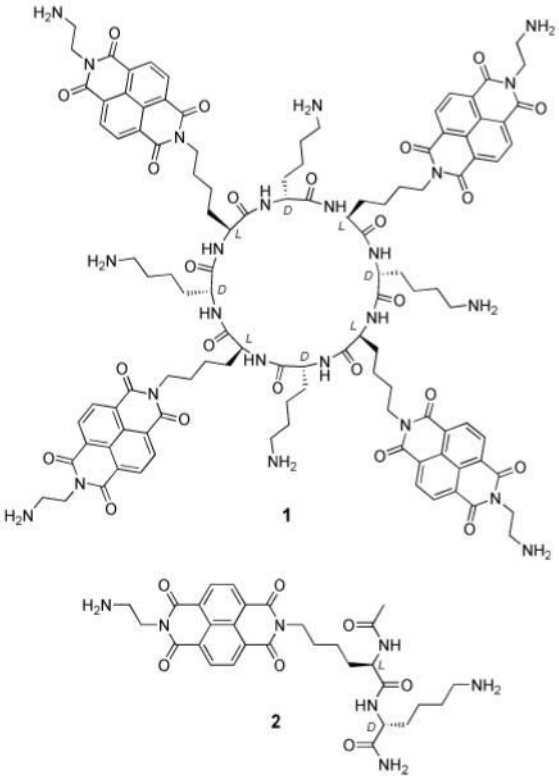


**Figure 1.**
Chemical structures of the NDI-modified peptides used in this study. For details regarding the synthesis of **1** and **2**, see Supporting Information.

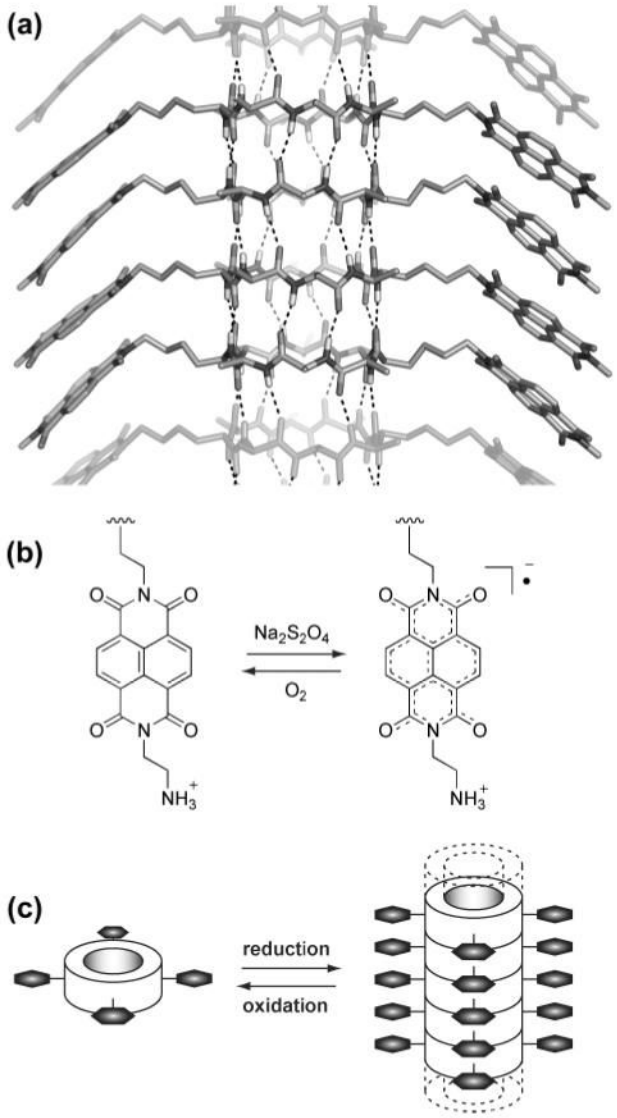


**Figure 2.**

(a) Calculated model of cyclic D,L-α-peptide **1** in a self-assembled tubular configuration illustrating the role of intermolecular β-sheet-like backbone hydrogen bonding in juxtaposing and stacking of aromatic NDI side chains (most side chains are omitted for clarity). (b) Reversible reduction of the NDI side chains in **1** and **2** to the corresponding NDI anion radicals. (c) Schematic illustration of redox-promoted peptide nanotube self-assembly.

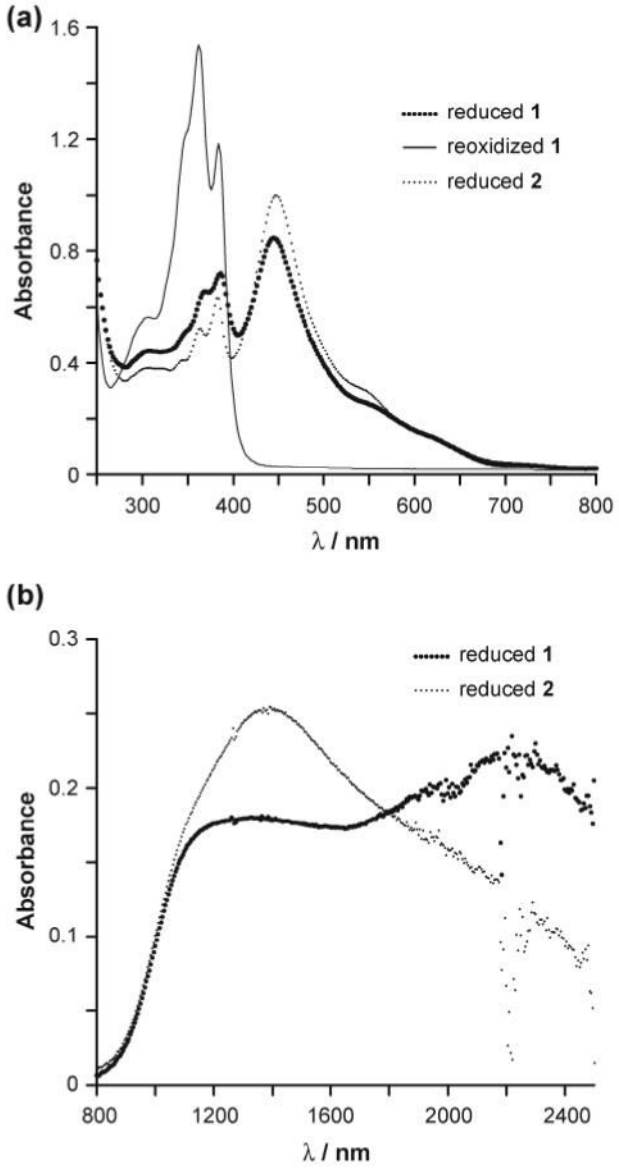


**Figure 3.**

(a) UV-VIS spectra of peptides **1** and **2** in the reduced state and peptide **1** in its native state. (b) Near-infrared spectra of **1** and **2** in their reduced states. Each peptide sample is 1.5 mM in NDI groups, and reduction is accomplished by exposure to 3.3 mM $Na_2S_2O_4$ in $D_2O$ under inert atmosphere. Spectra in (b) were background corrected against the same sample reoxidized by exposure to air in order to minimize background absorption and distortions in the NIR resulting from small differences arising from residual $H_2O$ in peptide and dithionite stock solutions. The noise around 2200 nm is an instrument artifact.

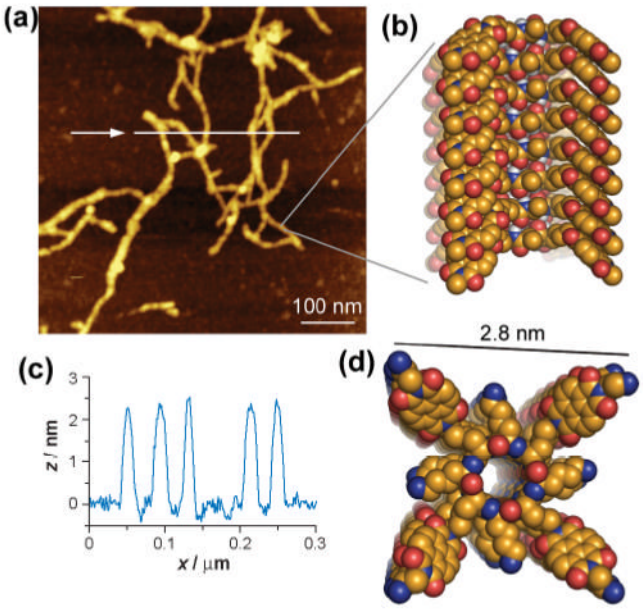


**Figure 4.**

(a) Atomic force microscopy (AFM) image of reduced cyclic peptide **1** adsorbed on mica with (b) a suggested model for the organization of the self-assembled cyclic peptide within the fibrous material. (c) The lateral cross-section is consistent with the calculated diameter of the self-assembled peptide nanotube as shown in (d).