

α,γ -Peptide Nanotube Templating of One-Dimensional Parallel Fullerene Arrangements

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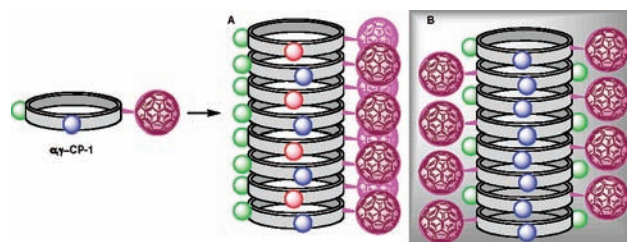
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The potential of functional 1D, 2D, and 3D nanostructured materials has intensified the quest for their fabrication because of their technological applications in biosensing, pharmacology, and catalysis.¹ Supramolecular chemistry provides an important method (a bottom-up approach) for the development and preparation of nanoscale objects and devices in which small molecules are combined in a predetermined way and linked by means of noncovalent interactions.² In this regard, among the most significant examples are the self-assembling peptide nanotubes (SPNs), in which cyclic peptides (CPs) in a flat conformation stack on top of one another to form cylindrical structures.³ The amino acid side chains are oriented outward in a very predictable and ordered fashion at the nanotube surface, thus providing the opportunity to design nanotubes with function-based properties.⁴

In the past few years, we have been working on the design of α,γ -SPNs. Although our initial aims were particularly focused on dimeric models (the most basic SPN structure) because of their relative ease of characterization, we were able to show their improved self-assembly properties and control of the ensemble diameter.^{5,6} We present here for the first time the full characterization of a single α,γ -SPN bearing fullerene side chains. The nanotube-directed self-assembly process allows the C₆₀ moieties to be organized into 1D structures that may have applications in the preparation of nanosized wires (Scheme 1).⁷ The fullerene moiety was selected to aid in the characterization of the α,γ -SPN as well as for its important chemical, supramolecular, electrochemical, and photophysical properties that may have a role in future applications in the preparation of nanosized wires.⁸ Numerous different types of supramolecular arrangements have been described, depending on the conditions and surface properties under which the fullerenes were deposited on the surface.⁹ However, from a technological and scientific point of view, new methods (such as that described here) in which the fabrication of ordered arrays of fullerenes on solid surfaces is directed by nanotube formation are needed.¹⁰

For the nanotube preparation, an α,γ -cyclic octapeptide with a nonsymmetrical structure was designed to disfavor highly ordered nanotube organization. The introduction of hydrophilic residues increases the water solubility of the CP and at the same time helps to induce the self-assembly process under appropriate and controlled conditions (Scheme 1).¹¹ In order to reduce the register freedom on the nanotube (Scheme 2A and SI-1 in the Supporting Information),¹² hydrophilic amino acids were selected to form complementary interstrand salt-bridge interactions once the self-assembly

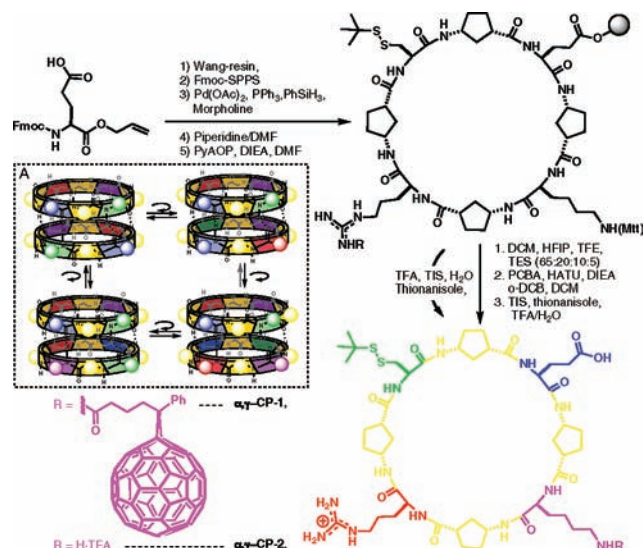
Scheme 1. Model for Peptide Nanotube Formation from α,γ -CP-1 in Which the Self-Assembly Process Can Be Directed by (A) Salt-Bridge Interactions (Red and Blue Balls) or (B) Induced by the Surface Properties



process had taken place. These salt-bridge interactions must induce the predominant formation of only one β -sheet register in the nanotube and also reinforce the SPN formation.

The design outlined above was used to prepare α,γ -CP-2 by Fmoc solid-phase synthesis on a Wang resin, and this compound was cyclized prior to cleavage with trifluoroacetic acid (TFA) (Scheme 2). The use of the methyltrityl group for protection of the Lys side chain allowed its selective removal by treatment with trifluoroethanol (TFE) in dichloromethane (DCM) before peptide cleavage. The resulting lysine-deprotected resin-linked CP was then coupled with PCBA¹³ in *o*-dichlorobenzene/CH₂Cl₂ in the presence

Scheme 2. Structures of α,γ -CP-1 and α,γ -CP-2 and the Solid-Supported Synthetic Strategy Followed for Their Preparation (A Model of the Four Possible β -Sheet Registers Corresponding to the α,α -Face Hydrogen-Bonding Interaction Is Shown in Inset A)



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of HATU and DIEA to generate, after cleavage with the TFA cocktail, α,γ -CP-1. While α,γ -CP-2 was purified by reversed-phase HPLC before nanotube formation studies, α,γ -CP-1 was simply resuspended in Et₂O, centrifuged, decanted, and used without further purification. As a consequence of the 180° orientation between the Arg and Glu residues and the characteristic antiparallel β -sheet-type structure of the nanotube interactions, SPN formation directed by the peptide self-assembly process would give rise to structures in which all of the fullerene moieties are oriented toward the same nanotube side (model A, Scheme 1). On the other hand, anionic surface-induced self-assembling systems, such as mica,¹⁴ should direct all of the Arg residues toward the same nanotube face to interact with the surface, while the Glu side chain, if protonated, could also participate in SPN stabilization through hydrogen-bonding interactions (model B, Scheme 1).¹⁵ In this second nanotube arrangement, the fullerene moieties would be alternately oriented toward the two sides of the nanotube.

With this aim in mind, α,γ -CP-2 was dissolved under acidic conditions (1% TFA), and the resulting solution was placed on a mica surface and removed after 5–10 min. Atomic force microscopy (AFM)¹⁶ images of this adsorbed CP revealed the formation of long fibrous structures that were several micrometers long, 5 nm in height, and more than 100 nm wide (Figure 1A,B). These structures may consist of several SPNs packed in a parallel manner on the anionic surface through the interaction of the Lys side chains with the mica, while the Arg and Glu side chains participate in inter- and intratubular salt-bridge formation. In this type of arrangement, the Cys *tert*-butyl groups are exposed to the solvent medium. In order to reduce the exposure of these groups, a second nanotube sheet must be placed on top of the already-deposited SPN layer (Figure 1C). Additionally, shorter agglomerated structures were also observed. In this case, these arrangements have needle-shaped structures that are 2.5–3.0 nm in height, which corresponds to the expected single-nanotube diameter. The SPNs are probably deposited together as a result of nonspecific hydrophobic interactions or salt-bridge formation (see Figures SI-1–SI-4 in the Supporting Information).

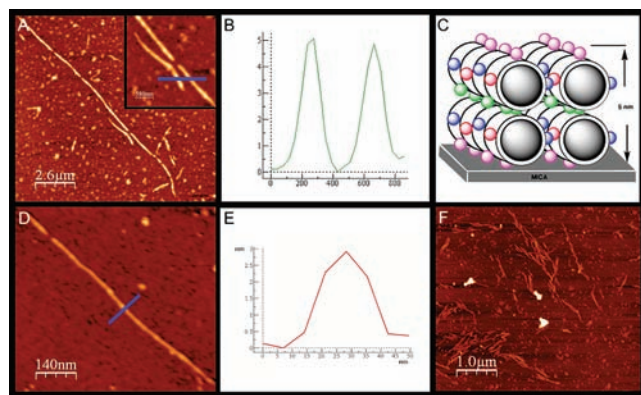


Figure 1. (A) AFM image of α,γ -CP-2 adsorbed on mica, showing long linear structures. Inset: magnified image of some of the long linear structures. (B) Profile obtained along the blue line shown in the inset of (A). (C) Schematic illustration of the bilayer arrangement proposed for α,γ -CP-2 deposition on mica. (D) AFM image of an α,γ -CP-1 sample adsorbed on mica, showing a single nanotube. (E) Height profile measured along the blue line shown in (D). (F) AFM image of α,γ -CP-1 adsorbed on mica, showing bundles of nanotubes.

On the basis of the above results, we decided to characterize α,γ -CP-1, in which the introduction of the fullerene moiety not only would help to characterize the nanotube structure but, as mentioned earlier, also would have possible applications in mo-

lecular electronic components such as nanowires because the SPN formation represents a new way to template the 1D supramolecular organization of C₆₀. AFM images of α,γ -CP-1, which contains only one basic group (Arg) to interact with the surface and was deposited under similar conditions on mica, revealed the formation of small, needle-shaped structures ranging from 3.0 to 3.5 nm in height and packed together via fullerene-directed nanotube aggregation (Figure 1D,E), although large and isolated linear organizations were also observed (Figure 1F).

The same sample of α,γ -CP-1 was studied by transmission electron microscopy (TEM). In this case, aliquots of the sample were applied to previously cleaved mica and left to evaporate. The samples were subsequently floated on a water–ethanol mixture and collected over 400 mesh Cu/Rh grids, which were then negatively stained with uranyl acetate solution. Under these conditions, electron microscopy revealed the presence of long filaments (~200 nm) that were ~2.5 nm wide, consistent with the width of the nanotubes. The nanotubes were mainly observed as single entities (arrowheads in Figure 2A) or grouped in pairs (arrowheads in Figure 2B), although other types of aggregations were also observed. The averaging of small areas of these double filaments using maximum-likelihood procedures¹⁷ (Figure 2C) not only confirmed the width of the nanotubes but revealed a periodicity in the structure that we believe is due to the interaction of the stain with the line of fullerenes, which are located on one side in the case of the single nanotubes or both sides in the case of the double ones. The fullerene lines are not always visible but in some cases are contrasted by the stain and detected as a line that is ~1 nm wide (arrow in Figure 2B). These two types of structures correlate quite well with the previously proposed model B (Scheme 1), but the formation of the two parallel nanotubes must be due to fullerene aggregation, in which the C₆₀ moieties are hydrophobically interdigitated to induce their pairing (Figure 2D).

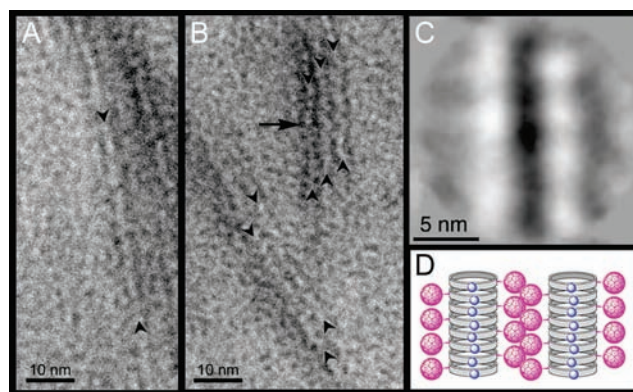


Figure 2. Electron microscopy fields of negatively stained (A) single filaments and (B) two- and three-filament arrangements. Arrowheads indicate the nanotubes, whereas the arrow shows a line of fullerenes. (C) Two-dimensional average image of small areas of the double-filament structure. (D) Model proposed for the nanotube pairs observed by TEM.

Nanotube assemblies were further studied by scanning tunneling microscopy (STM). A 1% TFA solution of α,γ -CP-1 was deposited on a highly ordered pyrolytic graphite surface and allowed to dry. STM images of this surface showed a reduced number of deposited nanotubes, and in most cases, these were found at the edge of graphite plates (Figure 3). Most of the observed structures were characterized by the presence of two parallel lines with heights of 1 nm, and these could correspond to the ordered fullerenes. The peptide backbone was not visible, but the C₆₀ molecules appeared as bright rods because of their high electron density, which resulted

in an increased tunneling probability. The fullerene arrangements were separated in most cases by 6–7 nm, a distance that corresponds to the distance between fullerenes in the previously described model B (Scheme 1), again confirming the peptide nanotube structure and its capability to organize the fullerene deposition.

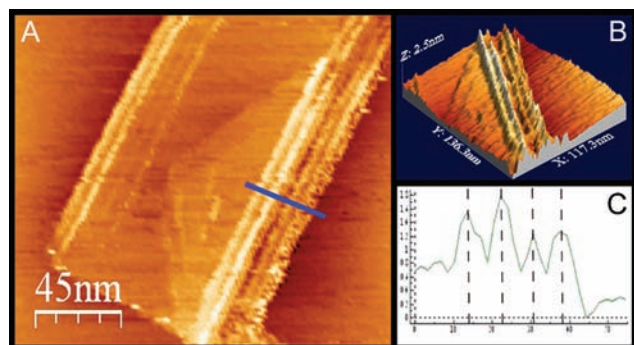


Figure 3. (A) Constant-current STM image of self-assembled α,γ -CP-2 on highly ordered pyrolytic graphite, in which two parallel strands ~ 1 nm in height are observed. (B) 3D topographic STM image representative of the α,γ -CP-1 arrangement to form the observed parallel C_{60} organization. (C) Height profile measured along the blue line shown in (A), confirming the proposed nanotube organization.

In conclusion, we have demonstrated the formation and full characterization by AFM, STM, and TEM of a new class of peptide nanotubes from α,γ -CPs. These CPs are able to align the C_{60} moieties to form 1D fullerene arrangements in which the fullerenes point outward from the nanotube on both sides (180° orientation) of the SPN. It is worth noting that the observed structure relies on the aforementioned design principles in which the formation of the four nonidentical antiparallel β -sheet forms per CP–CP hydrogen-bonding interaction present in the nanotube (D_α, D_γ ; Scheme SI-1 in the Supporting Information)¹² is restricted and controlled by the interstrand salt-bridge interactions. This control of the self-assembly process is able to organize the fullerene deposition to give 1D structures. As a consequence of this special arrangement, the fullerenes form two parallel wires separated by an insulating material, i.e., the peptide nanotube. The precise nanotube register control demonstrated here opens up opportunities not only for 1D alignment of other materials but also for other supramolecular organizations, such as helical. These structure types may have applications as nanowire components and/or in optical and electronic devices.

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Supporting Information Available: Additional AFM, STM, and TEM pictures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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