

Cite this: *Chem. Sci.*, 2018, 9, 8228

All publication charges for this article have been paid for by the Royal Society of Chemistry

Received 23rd May 2018
Accepted 26th August 2018

DOI: 10.1039/c8sc02276c

rsc.li/chemical-science

New self-assembling peptide nanotubes of large diameter using δ -amino acids†

Alejandro Lamas, Arcadio Guerra, Manuel Amorín * and Juan R. Granja *

Here we show that 4-aminocyclohexanecarboxylic acid is a rigid stretcher building block for the preparation of cyclic peptides that self-assemble to form peptide nanotubes with large diameter and hydrophobic pores. The hydrophobic properties of the resulting nanotubes provided by the two methylene groups per δ -residue allow the encapsulation of C_{60} moieties forming a new type of bionanopeapod structure.

1 Introduction

Self-assembling cyclic peptide nanotubes (SCPNs) are hollow cylindrical-shaped supramolecular structures formed by the stacking of cyclic peptides (CPs) in a flat conformation.¹ Their simple synthesis, precise control of the internal pore dimensions, the biocompatibility and easy modification make them especially suitable for the development of new nanobiomaterials.² Recent studies have shown that these supramolecular polymers are one of the known most robust nanofibers derived from proteinaceous materials.³ In fact, the same authors have made composites with polymers as a structural filler to reinforce their mechanical strength.⁴ All these properties have given rise to search for applications in fields ranging from biology (antimicrobials, drug delivery...) to materials science (biomaterials, molecular electronics, sensing and so on).^{5,6} Among the pioneering SCPNs in which CPs made of α -amino acids of alternating opposite chirality (D,L - α -CP)⁷ were used, other amino acids (β -, γ -, δ - or ϵ -residues) have been also reported for the preparation of nanotubes.⁸ Especially attractive are the CPs that contain γ -amino acids (α,γ -CPs) because they form nanotubes with partially hydrophobic or functionalized cavities.^{9,10}

In this sense, nanotubes with hydrophobic cavities are especially demanded for their potential implementation in the design of new drug or diagnostic agent carrier systems.¹¹ We envisioned that 4-aminocyclohexanecarboxylic acid (δ -Ach) would be a promising residue in the preparation of nanotubes with hydrophobic properties because of the two methylene groups of each residue that would be projected towards the

inner cavity of the nanotube. In this article, new hybrid α,δ -cyclic peptides (D,L - α,δ -CPs) with self-assembling properties are described. These peptides allow the formation of nanotubes with large internal diameters and hydrophobic properties. The resulting ensemble is able to dissolve and encapsulate fullerene in aqueous media inside of the tubular pore.

2 Results and discussion

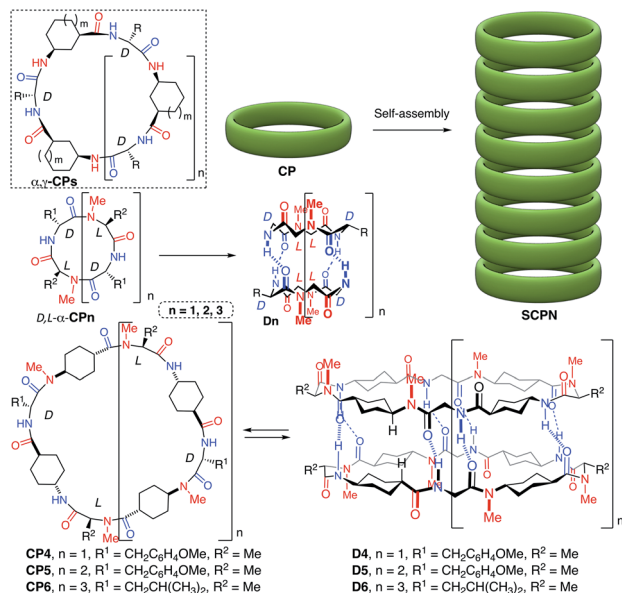
2.1 Synthetic studies and dimer characterization

For the design of these new nanotube forming cyclic peptides we used D,L - α -CPs as the basic component and incorporated one δ -Ach residue after each α -amino acid (Scheme 1) to ensure the adoption of a flat conformation. In addition, this design would also assure that the hydrogen-bond donors and acceptors were complementary in each face of the CP, facilitating the nanotube formation. To carry out these studies we started with dimers forming D,L - α -CPs of different lengths,¹² tetramers ($n = 1$), hexamers ($n = 2$) and octamers ($n = 3$). In these models (CP1, CP2 and CP3) the amide protons in one face of the β -strand are substituted by methyl groups through the alkylation of all the amino acids of the same chirality to prevent hydrogen bond formation through this face. Therefore, the assembling process is restricted to dimers that are soluble in organic solvents. To ensure that all the N -alkyl groups are pointing in the same direction, in these new hybrids the N -methylated δ -Ach residues should be coupled to the N -methylated α -residue (Scheme 1). Consequently, the non-methylated δ -amino acids should be attached to the α -residues of opposite chirality (D -residue) through a secondary amide connection. With these design principles, cyclic peptides CP4, CP5 and CP6 were synthesized following the general scheme illustrated in Scheme 1S in the ESI.† The resulting dimers D4, D5 and D6 (Scheme 1) should be formed by the hydrogen-bonding interactions between the amide groups of D -amino acids and the NH group of δ -Ach with the carbonyl group of ^{Me}N - δ -Ach.

Singular Research Centre in Chemical Biology and Molecular Materials, (CIQUS), Organic Chemistry Department, University of Santiago de Compostela (USC), 15782 Santiago de Compostela, Spain. E-mail: juanr.granja@usc.es; manuel.amorin@usc.es

† Electronic supplementary information (ESI) available: Including synthetic experimental details, DFT calculation details and additional figures (relevant NMR data) and schemes (synthetic schemes for peptide and amino acid preparation). See DOI: 10.1039/c8sc02276c

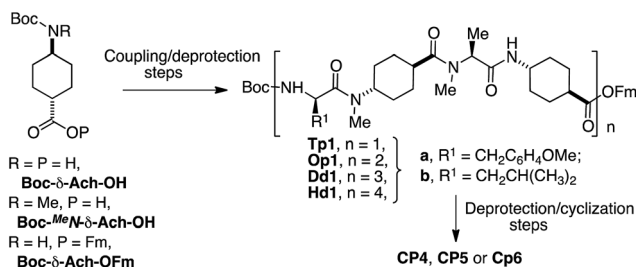




Scheme 1 Top: Peptide nanotube models formed by stacking of cyclic peptides (SCP) and structure of α,γ -cyclic peptide hybrids. Bottom: Structure of the cyclic peptides and dimers of D,L - α -CP (centre) and D,L - α,δ -CP (bottom) in which CP4 corresponds to $n = 1$ (octamer), CP5 to $n = 2$ (dodecamer) and CP6 to $n = 3$ (hexadecamer).

The synthesis (Scheme 2, see the ESI and Scheme 1S† for more details) started from commercially available *trans*-*N*-Boc-4-aminocyclohexanecarboxylic acid (**Boc- δ -Ach-OH**) that was transformed into **Boc-^{Me}N- δ -Ach-OH** by treatment with sodium hydride and iodomethane in DMF followed by hydrolysis with lithium hydroxide of the resulting methyl ester. Finally, **Boc- δ -Ach-OH** was also transformed into **Boc- δ -Ach-OFm** under simple esterification conditions. This compound was converted into the corresponding tetrapeptides (**Tp1a** or **Tp1b**, Scheme 1S†) through the sequential coupling/deprotection steps of the corresponding Boc-protected amino acids (^{Me}*N*-Ala, ^{Me}*N*- δ -Ach and *D*-Tyr for **Tp1a**, and ^{Me}*N*-Ala, ^{Me}*N*- δ -Ach and *D*-Leu for **Tp1b**). The resulting tetrapeptides, through successive deprotection and coupling processes, were transformed into the corresponding linear octa-, dodeca- and hexadecapeptides (**Op1**, **Dd1** and **Hd1a** and/or **b**) that were finally cyclized to provide **CP4**, **CP5** and **CP6**, respectively, in yields ranging from 34 to 64%.

The CPs were soluble in nonpolar solvents, *i.e.* deuteriochloroform, allowing the analysis of the self-assembling



Scheme 2 Synthetic strategy used in the synthesis of cyclic peptides CP4, CP5 and CP6. For more details, see Scheme 1S in the ESI.†

properties by NMR experiments. The ^1H NMR spectra in deuteriochloroform of the three compounds are well defined and highly symmetrical with $J_{\text{NH},\gamma\text{H}}$ coupling constants larger than 7.0 Hz. This indicates that the peptides must exist in an all-*trans* conformation characteristic of the flat-ring-shaped structure (Fig. 1). The main difference is the chemical shift of the amide protons. While in this solvent the signals of these protons of **CP4** appear at δ 6.48 (NH_{Tyr}) and 6.23 ($\text{NH}_{\delta\text{-Ach}}$) ppm, independently of peptide concentration, the signals of amide protons are shifted downfield, δ 8.68 (NH_{Tyr}) and 7.78 ($\text{NH}_{\delta\text{-Ach}}$) ppm for **CP5** and 8.32 (NH_{Leu}) and 7.91 ($\text{NH}_{\delta\text{-Ach}}$) ppm for **CP6**. These results suggest that the small CPs remain as monomers in solution with the amide protons not involved in a hydrogen bonding interaction. This was also confirmed by solution and solid state FTIR (Fig. 1aS†) that show an amide A band at 3400 cm^{-1} that corresponds to the stretching of amide protons not involved in hydrogen bonding.¹³

On the other hand, the other two CPs must form in this solvent (CDCl_3) the corresponding dimers, **D5** and **D6**, with a very large association constant ($K_a > 10^5\text{ M}^{-1}$), as deduced from the location of the amide signals (8.68 and 7.78 ppm for **CP5** and 8.32 and 7.91 ppm for **CP6**) that remains constant at concentrations as low as $1 \times 10^{-4}\text{ M}$ (Fig. 2S†). NOE cross peaks (Fig. 1) between Tyr amide protons (8.68 ppm) and the H_δ of $\delta\text{-}^{\text{Me}}\text{N-Ach}$ (4.45 ppm) and between the latter and H_α of $\delta\text{-}^{\text{Me}}\text{N-Ach}$ (2.54 ppm) for **CP5** are consistent with the proposed anti-parallel dimeric structure. Similar cross peaks were also observed for **CP6** (see ESI, Fig. 3S†). Dimer formation was also verified by electrospray ionisation-time-of-flight (ESI-TOF) mass

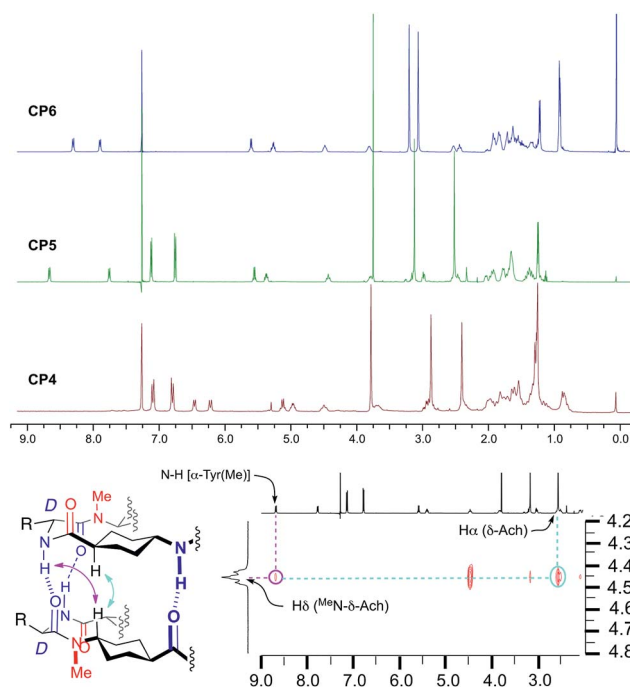


Fig. 1 Top: ^1H NMR spectra of CP4 (bottom, red), CP5 (media, green) and CP6 (top, blue). Bottom: Selected NOESY spectra of **D5** (5 mM in CDCl_3); on the left the coloured arrows denote the main characteristic cross peaks, magenta for NH (Tyr) and cyan for $\text{H}_\delta(\delta\text{-}^{\text{Me}}\text{N-Ach})$, $\text{H}_\delta(\delta\text{-}^{\text{Me}}\text{N-Ach})$ and $\text{H}_\alpha(\delta\text{-Ach})$.



spectroscopy that showed peaks corresponding to the dimeric forms with two (1580.9 for CP5 and 1851.3 for CP6) and three charges (1054.3 for CP5 and 1234.5 for CP6), as deduced from the isotopic distribution, see Fig. 4S in the ESI† The FTIR spectra (Fig. 1bS and cS†) also confirm the formation of the dimer assemblies with an amide A band near 3315 cm^{-1} that suggests an inter-monomer distance of around $4.85\text{--}4.90\text{ \AA}$.¹³ These experiments confirm that the intercalation of the rigid δ -Ach residues into dimers forming self-assembling D,L - α -CPs provides a new class of CPs that adopt a flat conformation and stack to form torus-shaped supramolecular ensembles; see Fig. 5S in the ESI† for a computer generated model of CP5. The rigidity of this residue allows the formation of a large pore structure with diameters as big as 30 \AA , the largest published for this type of tubular assembly.

2.2 Nanotube characterization

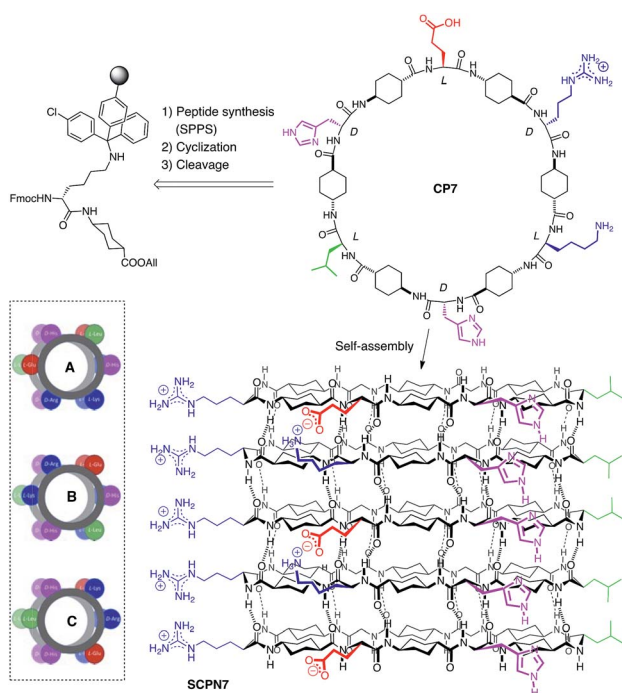
After confirming the assembling properties of the D,L - α , δ -CPs with dimeric models, we extend the studies to the formation of SCPNs. For that purpose, cyclic dodecapeptide CP7 (Scheme 3) was designed. The incorporation of Glu and Lys residues should provide CPs with improved solubility in aqueous media and also provide some control over cyclic peptide stacking by salt bridge formation. His, in addition to the participation in hydrogen bonding interactions, should give some chemical control (pH modification) over the assembling process under neutral conditions.¹⁴ In the resulting nanotube, the more

favourable arrangement (pairwise C, inset Scheme 3) should align all the Arg and Leu residues on top of each other but in opposite sides. This linear arrangement of guanidinium groups in the nanotube should facilitate the deposition of the nanotube on an anionic mica surface. The peptide was synthesized by solid phase synthesis on a trityl resin and cyclized prior to its cleavage by attaching the first dipeptide Fmoc-Lys- δ -Ach-Oallyl through the Lys side-chain (Scheme 2S†).^{15,16} After cleavage with the TFA cocktail,¹⁷ the peptide was precipitated, washed and purified. NMR and MS confirmed the formation of CP7 (Fig. 6S†). IR spectra showed intense bands at 1537 and 1627 cm^{-1} identified as amide I and amide II, respectively, corresponding to amide bonds involved in hydrogen bonded anti-parallel β -sheet structures (Fig. 7S†).¹³ The amide A stretching band at 3270 cm^{-1} confirmed the tight ring-to-ring stacked tubular structure with an average inter-subunit distance of $4.75\text{--}4.80\text{ \AA}$.¹⁸ Nanotube formation in solution was confirmed by using thioflavin T (ThT), a probe used to study amyloid diseases such as Alzheimer, diabetes or prion diseases, that is known to fluoresce upon binding to β -sheet structures.^{19,20} Addition of increasing amounts of CP7 to an aqueous acidic solution containing ThT ($20\text{ }\mu\text{M}$) did not produce any change in the emission band (excitation 420 nm) at 485 nm (Fig. 8aS†). On the other hand, similar additions of CP7 to a solution at pH 8.1 (in water or TRIS buffer) gave rise to a clear increase in dye emission that confirms the interaction of ThT with cyclic peptide assemblies (Fig. 8bS†). This was observed at CP7 concentrations higher than $50\text{ }\mu\text{M}$. In any case nanotube formation was confirmed above this concentration under slightly basic conditions in which His residues are mainly deprotonated.

Drop casting of an aqueous solution of CP7 in TRIS buffer (2.0 mM , pH ~ 8.1) on mica afforded fibrous structures with lengths of a few μm and average heights of $3.3\text{--}3.9 \pm 0.30\text{ nm}$ (Fig. 2a, b and 9S†). Similar results were obtained from aqueous solutions of CP7 at the same pH. These fibrous structures are in keeping with those expected for a nanotube composed of the corresponding dodecapeptide rings.

2.3 Fullerene encapsulation and peptide molecular peapods

Computational models suggest that the hydrophobic properties and diameter size of the α , δ -SCPn made of twelve residues, such as CP7, would be suitable for the encapsulation of fullerene to



Scheme 3 Structures of cyclic peptide CP7, synthesized on a solid support (trityl resin), and the corresponding SCPN model, in which the CPs are stacked in an antiparallel-like β -sheet structure. The inset shows the top view of the three inter-cyclic peptides pairwise that can be formed between each pair of CPs considering the antiparallel β -sheet structure.

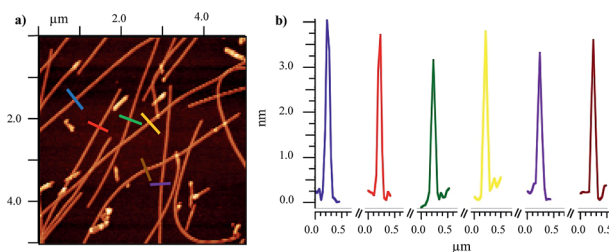


Fig. 2 (a) AFM topography micrographs of SCPN7 deposited over mica (grade V-I muscovite) from aqueous solutions ($250\text{ }\mu\text{M}$, pH ~ 8.1). (b) AFM height profiles along the transects in different colors shown in (a).



form peptide nanopeapod-type structures (Fig. 3c).^{21–23} Therefore, 1.5 mL of a water solution of CP7 (1.0 mM) at pH 8.1 was added to an Eppendorf tube containing C₆₀ (0.5 mg) and then sonicated for 40 min. The resulting solution was centrifuged (7500 rpm) for 10 min to afford a brownish aqueous solution that was stable for few weeks. The UV/vis spectrum shows the absorption band at 345 nm characteristic of buckminsterfullerene dispersed in water as almost individual molecules (Fig. 10S†).²⁴ Considering the proposed UV-vis molar extinction coefficients in aqueous media [30.000–60.000 M⁻¹ cm⁻¹ for the λ_{max} (360)], we estimated a C₆₀ concentration of 70 μM.²⁵ In addition to this band, long wavelength absorption bands between 400 and 480 nm were also observed. They have been assigned to the interaction between different C₆₀. This suggests that the entrapped fullerenes must be aligned inside the SCPN7. The UV absorption spectrum characteristic of the C₆₀ moiety was essentially the same with time. It is worth mentioning that acidic solutions (pH 3.5) containing similar CP concentration (1.5 mM) or acidic (pH 3.5) or basic aqueous (pH 8.1) solutions did not dissolve C₆₀ under similar conditions. This confirms the requirement of SCPN7 formation for dissolving the fullerene. In addition, the disassembly of peptide nanotubes by acidification of the basic solution containing CP7 and C₆₀ produces the precipitation of the encapsulated fullerene. This suggests, considering the CP concentration used in the solubilization (1.0 mM), that one molecule of C₆₀ was dissolved per each twenty molecules of CP7.

Additions of the basic solution containing CP7 and C₆₀ to a TRIS solution containing ThT (20 μM) showed the characteristic emission at 485 nm that confirms the β-sheet structure characteristic of peptide nanotubes (Fig. 11S†). Finally, FTIR spectra showed intense bands at 1542, 1620 and 3277 cm⁻¹ that, once again, confirmed the SCPN formation (Fig. 12S†).¹³

The AFM images of the basic solution of CP7 and C₆₀ also showed a few μm long horizontal fibrous structures with heights of 3.9–4.5 ± 0.41 nm slightly higher to those observed for the solutions of the CP alone (Fig. 3a and 13S†), perhaps as a consequence of the higher rigidity of the cyclic peptide peapod structure.²⁶ No spherical particles of 1 nm in height deposited on the nanotube were observed in any case, confirming that C₆₀ must be encapsulated on the nanotube cavity. These results confirm the ability of the cyclic peptide to assemble into a tubular structure after fullerene encapsulation. Interestingly, the SCPN7 solution was not capable of dissolving C₇₀, perhaps due to its larger size that must not fit in the nanotube cavity following the best Rebek's packing coefficient.²⁷ The solution containing CP7 and C₆₀ was also deposited on a carbon holey grid that appeared in scanning transmission electron microscopy (STEM) images as long fibers of a few μm long that correspond to individual nanotubes (Fig. 3b). Interestingly the deposition of more than one-week aged solutions showed the formation of small nanotube bundles (Fig. 16S†). Similar results were also observed by AFM (Fig. 14S†).

3 Conclusions

We have showed that 4-aminocyclohexanecarboxylic acid (δ-Ach) is an useful building block for the preparation of self-assembling cyclic peptides with large diameter and hydrophobic internal cavities. δ-Ach is a rigid amino acid in which *trans*-1,4 disposition makes it a suitable component of cyclic peptides that must adopt a flat conformation. Especially suited for this purpose *δ,ε*-α-CPs were transformed into α,δ-hybrids by incorporating δ-Ach residues in tetra-, hexa- and octa-peptides. The resulting peptides can stack through an antiparallel-like interaction. The disposition of two methylene groups of the cyclohexyl moiety towards the lumen of the nanotube cavity provided hydrophobic properties. These hydrophobic properties and the appropriate nanotube internal diameter were efficiently implemented for the encapsulation of fullerene C₆₀. These results provide new supramolecular polymers with tuned assembling properties that might make them suitable for the development of efficient drug delivery platforms. The novel peptide nanopeapod structures described here might find applications in field-effect transistors, magnetic or data storage nanodevices, nanoscale lasers and so on.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Spanish Agencia Estatal de Investigación (AEI) and the ERDF (CTQ2016-78423-R), and by the Xunta de Galicia and the ERDF (EM2014/011, ED431C 2017/25 and Centro singular de investigación de Galicia accreditation 2016-2019, ED431G/09). We also thank the ORFEO-CINCA network and MINECO (CTQ2016-81797-REDC). We thank Dr

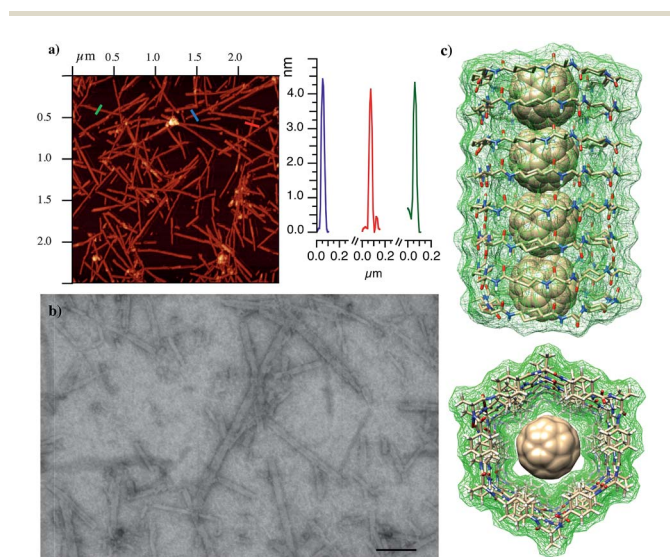


Fig. 3 (a) AFM topography micrographs from aqueous solutions of CP7 and C₆₀ deposited over mica (100 μm, pH ~ 8.1) and AFM height profiles along the transects in different colors. (b) STEM image of an aqueous solution of CP7 and C₆₀ after deposition on a carbon holey grid (scale bar 100 nm). (c) Side and top views of a computer assisted model of C₆₀ encapsulated in the inner cavity of SCPN7.



Carlos Vázquez-Vázquez and Miguel Cuerva (IIT, USC) for their help in the AFM characterization.

Notes and references

- 1 D. Bong, T. Clark, J. R. Granja and M. Ghadiri, *Angew. Chem., Int. Ed.*, 2001, **40**, 988–1011; R. J. Brea, C. Reiriz and J. R. Granja, *Chem. Soc. Rev.*, 2010, **39**, 1448–1456; R. Chapman, M. Danial, M. L. Koh, K. A. Jolliffe and S. Perrier, *Chem. Soc. Rev.*, 2012, **41**, 6023–6041; N. Rodríguez-Vázquez, M. Amorín and J. R. Granja, *Org. Biomol. Chem.*, 2017, **15**, 4490–4505.
- 2 M. J. Webber, E. A. Appel, E. W. Meijer and R. Langer, *Nat. Mater.*, 2016, **15**, 13–26; R. Dong, Y. Zhou, X. Huang, X. Zhu, Y. Lu and J. Shen, *Adv. Mater.*, 2014, **27**, 498–526; S. Koutsopoulos, *J. Biomed. Mater. Res.*, 2016, **104**, 1002–1016; A. Fuertes, M. Juanes, J. R. Granja and J. Montenegro, *Chem. Commun.*, 2017, **53**, 7861–7871.
- 3 D. J. Rubin, S. Amini, F. Zhou, H. Su, A. Miserez and N. S. Joshi, *ACS Nano*, 2015, **9**, 3360–3368; L. Ruiz, P. VonAchen, T. D. Lazzara, T. Xu and S. Ketten, *Nanotechnology*, 2013, **24**, 195103.
- 4 D. J. Rubin, H. T. Nia, T. Desire, P. Q. Nguyen, M. Gevelber, C. Ortiz and N. S. Joshi, *Biomacromolecules*, 2013, **14**, 3370–3375.
- 5 M. R. Ghadiri, J. R. Granja and L. K. Buehler, *Nature*, 1994, **369**, 301–304; J. R. Granja and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1994, **116**, 10785–10786; S. Fernandez-Lopez, H. S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxon and M. R. Ghadiri, *Nature*, 2001, **412**, 452–456; M. Richman, S. Wilk, M. Chemerovski, S. K. T. S. Wärmländer, A. Wahlström, A. Gräslund and S. Rahimpour, *J. Am. Chem. Soc.*, 2013, **135**, 3474–3484; Y. Wang, S. Yi, L. Sun, Y. Huang, S. C. Lenaghan and M. Zhang, *J. Biomed. Nanotech.*, 2014, **10**, 445–454; M. Danial, C. M. N. Tran, K. A. Jolliffe and S. Perrier, *J. Am. Chem. Soc.*, 2014, **136**, 8018–8026; M. Li, M. Ehlers, S. Schlesiger, E. Zellermann, S. K. Knauer and C. Schmuck, *Angew. Chem., Int. Ed.*, 2016, **55**, 598–601; J. Chen, B. Zhang, F. Xia, Y. Xie, S. Jiang, R. Su, Y. Lu and W. Wu, *Nanoscale*, 2016, **8**, 7127–7136; Y. Zhao, L. J. Leman, D. J. Search, R. A. Garcia, D. A. Gordon, B. E. Maryanoff and M. R. Ghadiri, *ACS Cent. Sci.*, 2017, **3**, 639–646; S. C. Larnaudie, J. C. Brendel, I. Romero-Canelón, C. Sanchez-Cano, S. Catrouillet, J. Sanchis, J. P. C. Coverdale, J.-I. Song, A. Habtemariam, P. J. Sadler, K. A. Jolliffe and S. Perrier, *Biomacromolecules*, 2017, **19**, 239–247.
- 6 C. Steinem, A. Janshoff, M. S. Vollmer and M. R. Ghadiri, *Langmuir*, 1999, **15**, 3956–3964; M. S. Vollmer, T. D. Clark, C. Steinem and M. R. Ghadiri, *Angew. Chem., Int. Ed.*, 1999, **38**, 1598–1601; W. S. Horne, N. Ashkenasy and M. R. Ghadiri, *Chem.–Eur. J.*, 2005, **11**, 1137–1144; N. Ashkenasy, W. S. Horne and M. R. Ghadiri, *Small*, 2006, **2**, 99–102; J. Couet, J. D. J. S. Samuel, A. Kopyshv, S. Santer and M. Biesalski, *Angew. Chem., Int. Ed.*, 2005, **44**, 3297–3301; R. de la Rica, C. Pejoux and H. Matsui, *Adv. Funct. Mater.*, 2011, **21**, 1018–1026; T. Xu, N. Zhao, F. Ren, R. Hourani, M. Tsang Lee, J. Y. Shu, S. Mao and B. A. Helms, *ACS Nano*, 2011, **2**, 1376–1384; M. Mizrahi, A. Zakrassov, J. Lerner-Yardeni and N. Ashkenasy, *Nanoscale*, 2012, **4**, 518–524; M. Danial, C. M.-N. Tran, P. G. Young, S. E. B. Perrier and K. A. Jolliffe, *Nat. Commun.*, 2013, **4**, 2780; M. Potnuru and N. Madhavan, *Polym. Chem.*, 2016, **7**, 31–35.
- 7 M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee and N. Khazanovich, *Nature*, 1993, **366**, 324–327; J. D. Hartgerink, J. R. Granja, R. A. Milligan and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1996, **118**, 43–50; N. Khazanovich, J. R. Granja, D. E. McRee, R. A. Milligan and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1994, **116**, 6011–6012; M. R. Silk, J. Newman, J. C. Ratcliffe, J. F. White, T. Caradoc-Davies, J. R. Price, S. X. B. Perrier, P. E. Thompson and D. K. Chalmers, *Chem. Commun.*, 2017, **53**, 6613–6616.
- 8 D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher and L. B. McCusker, *Helv. Chim. Acta*, 1997, **80**, 173–182; S. Leclair, P. Baillargeon, R. Skouta, D. Gauthier, Y. Zhao and Y. L. Dory, *Angew. Chem., Int. Ed.*, 2004, **43**, 349–353; W. S. Horne, C. D. Stout and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2003, **125**, 9372–9376; A. Ghorai, S. K. Reddy, B. Achari and P. Chattopadhyay, *Org. Lett.*, 2014, **16**, 3196–3199.
- 9 M. Amorin, L. Castedo and J. R. Granja, *J. Am. Chem. Soc.*, 2003, **125**, 2844–2845; J. Montenegro, C. Vázquez-Vázquez, A. Kalinin, K. E. Geckeler and J. R. Granja, *J. Am. Chem. Soc.*, 2014, **136**, 2484–2491; M. Cuerva, R. García-Fandiño, C. Vázquez-Vázquez, M. A. López-Quintela, J. Montenegro and J. R. Granja, *ACS Nano*, 2015, **9**, 10834–10843; A. Fuertes, H. L. Ozores, M. Amorín and J. R. Granja, *Nanoscale*, 2017, **9**, 748–753.
- 10 C. Reiriz, C. Amorín, R. García-Fandiño, L. Castedo and J. R. Granja, *Org. Biomol. Chem.*, 2009, **7**, 4358–4361; R. Hourani, C. Zhang, R. van der Weegen, L. Ruiz, C. Li, S. Ketten, B. A. Helms and T. Xu, *J. Am. Chem. Soc.*, 2011, **133**, 15296–15299; N. Rodríguez-Vázquez, R. García-Fandiño, M. Amorín and J. R. Granja, *Chem. Sci.*, 2016, **7**, 183–187; N. Rodríguez-Vázquez, M. Amorín, I. Alfonso and J. R. Granja, *Angew. Chem., Int. Ed.*, 2016, **55**, 4504–4508; N. Rodríguez-Vázquez, R. García-Fandiño, M. J. Aldegunde, J. Brea, M. I. Loza, M. Amorin and J. R. Granja, *Org. Lett.*, 2017, **19**, 2560–2563.
- 11 W. B. Liechty, D. R. Kryscio, B. V. Slaughter and N. A. Peppas, *Annu. Rev. Chem. Biomol. Eng.*, 2010, **1**, 149–173; M. McKenzie, D. Betts, A. Suh, K. Bui, L. D. Kim and H. Cho, *Molecules*, 2015, **20**, 20397–20408; D. Douroumis and A. Fahr, *Drug Delivery Strategies for Poorly Water-Soluble Drugs*, John Wiley & Sons, Ltd., Chichester, West Sussex, 2012.
- 12 M. R. Ghadiri, K. Kobayashi, J. R. Granja, R. K. Chadha and D. E. McRee, *Angew. Chem., Int. Ed.*, 1995, **34**, 93–95; T. D. Clark, J. M. Buriak, K. Kobayashi, M. P. Isler, D. E. McRee and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1998,



- 120, 8949–8962; X. C. Sun and G. P. Lorenzi, *Helv. Chim. Acta*, 1994, **77**, 1520–1526.
- 13 P. I. Haris and D. Chapman, *Biopolymers*, 1995, **37**, 251–263; S. Krimm and J. Bandekar, *Adv. Protein Chem.*, 1986, **38**, 181–364; J. Bandekar, *Biochim. Biophys. Acta*, 1992, **1120**, 123–143.
- 14 Y. Le Duc, M. Michau, A. Gilles, V. Gence, Y. M. Legrand, A. van der Lee, S. Tingry and M. Barboiu, *Angew. Chem., Int. Ed.*, 2011, **50**, 11366–11372; A. Méndez-Ardoy, J. R. Granja and J. Montenegro, *Nanoscale Horiz.*, 2018, **3**, 391–396.
- 15 F. Albericio and F. García-Martín, *Chem. Today*, 2008, **26**, 29–34.
- 16 C. J. White and A. K. Yudin, *Nat. Chem.*, 2011, **3**, 509–524; J. Alsina, F. Rabanal, E. Giralt and F. Albericio, *Tetrahedron Lett.*, 1994, **35**, 9633–9636; P. Romanovskis and A. F. Spatola, *J. Peptide Res.*, 1998, **52**, 356–374.
- 17 C. Reiriz, R. J. Brea, R. Arranz, J. L. Carrascosa, A. Garibotti, B. Manning, J. M. Valpuesta, R. Eritja, L. Castedo and J. R. Granja, *J. Am. Chem. Soc.*, 2009, **131**, 11335–11337.
- 18 H. S. Kim, J. D. Hartgerink and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1998, **120**, 4417–4424.
- 19 M. Krebs, E. Bromley and A. Donald, *J. Struct. Biol.*, 2005, **149**, 30–37; N. Byrne, D. Menzies, N. Goujon and M. Forsyth, *Chem. Commun.*, 2013, **49**, 7729–7732; H. Levine III, *Arch. Biochem. Biophys.*, 1997, **342**, 306–316.
- 20 M. Groenning, *J. Chem. Biol.*, 2009, **3**, 1–18; H. Naiki, K. Higuchi, M. Hosokawa and T. Takeda, *Anal. Biochem.*, 1989, **177**, 244–249.
- 21 G. D. Pantoş, J.-L. Wietor and J. K. M. Sanders, *Angew. Chem., Int. Ed.*, 2007, **46**, 2238–2240; D. Canevet, E. M. Pérez and N. Martín, *Angew. Chem., Int. Ed.*, 2011, **50**, 9248–9259.
- 22 B. W. Smith, M. Monthieux and D. E. Luzzi, *Nature*, 1998, **396**, 323–324; G. Zhang, R. Zhou and X. C. Zeng, *J. Mater. Chem. C*, 2013, **1**, 4518–4526; K. Hirahara, S. Bandow, K. Suenaga, H. Kato, T. Okazaki, H. Shinohara and S. Iijima, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2001, **64**, 115420; J. Lee, H. Kim, S. J. Kahng, G. Kim, Y. W. Son, J. Ihm, H. Kato, Z. W. Wang, T. Okazaki, H. Shinohara and Y. Kuk, *Nature*, 2002, **415**, 1005–1008; D. A. Britz, A. N. Khlobystov, K. Porfyrakis, A. Ardavan and G. A. D. Briggs, *Chem. Commun.*, 2005, 37–39.
- 23 T. Kawase, K. Tanaka, N. Fujiwara, H. R. Darabi and M. Oda, *Angew. Chem., Int. Ed.*, 2003, **42**, 1624–1628; T. Kawase, K. Tanaka, N. Shiono, Y. Seirai and M. Oda, *Angew. Chem., Int. Ed.*, 2004, **43**, 17221–11724; Y. Shoji, K. Tashiro and T. Aida, *J. Am. Chem. Soc.*, 2006, **128**, 10690–10691; E.-X. Zhang, D.-X. Wang, Q.-Y. Zheng and M.-X. Wang, *Org. Lett.*, 2008, **10**, 2565–2568; J. Song, N. Aratani, H. Shinokubo and A. Osuka, *J. Am. Chem. Soc.*, 2010, **132**, 16356–16357; S. Hitosugi, W. Nakanishi, T. Yamasaki and H. Isobe, *Nat. Commun.*, 2011, **2**, 17646; T. Iwamoto, Y. Watanabe, T. Sadahiro, T. Haino and S. Yamago, *Angew. Chem., Int. Ed.*, 2011, **50**, 8342–8344.
- 24 X. Chang and P. J. Vikesland, *Environ. Sci. Technol.*, 2011, **45**, 9967–9974; F. Diederich, J. Effing, U. Jonas, L. Jullien, T. Plesniviy, H. Ringsdor, C. Thilgen and D. Weinstein, *Angew. Chem., Int. Ed.*, 1992, **31**, 1599–1602; H. Ajje, M. M. Alvarez, S. J. Anz, R. D. Beck, F. Diederich, K. Fostiropoulos, D. R. Huffman, W. Kraetschmer, Y. Rubin, K. E. Schriver, D. Sensharma and R. L. Whetten, *J. Phys. Chem.*, 1990, **94**, 8630–8633; W. Kratschmer, L. D. Lamb, K. Fostiropoulos and D. R. Huffman, *Nature*, 1990, **347**, 354–358.
- 25 J. A. Sene, M. V. B. Pinheiro, K. Krambrock and P. J. S. Barbeira, *Talanta*, 2009, **78**, 1503–1507; S. J. Vance, V. Desai, B. O. Smith, M. W. Kennedy and A. Cooper, *Biophys. Chem.*, 2016, **214–215**, 27–32.
- 26 AFM image of a control experiment in which a basic solution of CP7 was sonicated for 40 min and then deposited over mica, showing sorter nanotube structures with similar heights to those observed for the solutions of the CP alone without sonication, see Fig. 15S.†
- 27 S. Mecozzi and J. Rebek Jr, *Chem.–Eur. J.*, 1998, **4**, 1016–1022.

