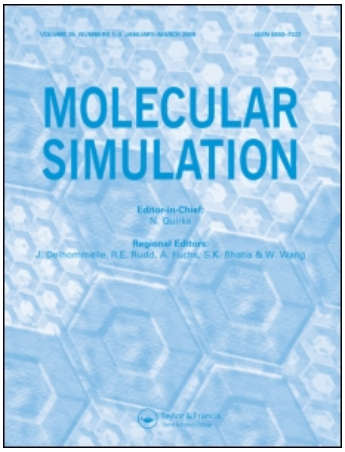

Molecular Simulation

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

Structure of a self-assembled single nanotube of cyclo[(-d-Ala-l-Ala)$_4$-]

Jie Cheng[a]; Jingchuan Zhu[a]; Bo Liu[b]; Zhouxiong Liao[a]; Zhonghong Lai[a]
[a] School of Materials Science and Engineering, Harbin Institute of Technology, Harbin, People's Republic of China [b] School of Chemical and Environmental Engineering, Harbin University of Science and Technology, Harbin, People's Republic of China

To cite this Article Cheng, Jie , Zhu, Jingchuan , Liu, Bo , Liao, Zhouxiong and Lai, Zhonghong(2009) 'Structure of a self-assembled single nanotube of cyclo[(-d-Ala-l-Ala)$_4$-]', Molecular Simulation, 35: 8, 625 — 630
To link to this Article: DOI: 10.1080/08927020902787788
URL: http://dx.doi.org/10.1080/08927020902787788

Molecular Simulation
Vol. 35, No. 8, July 2009, 625–630

Taylor & Francis
Taylor & Francis Group

Structure of a self-assembled single nanotube of cyclo[(-D-Ala-L-Ala)$_4$-]

Jie Cheng[a]*, Jingchuan Zhu[a], Bo Liu[b], Zhouxiong Liao[a] and Zhonghong Lai[a]

[a]*School of Materials Science and Engineering, Harbin Institute of Technology, Box 433, No.92 West Dazhi Street, Harbin 150001, People's Republic of China;* [b]*School of Chemical and Environmental Engineering, Harbin University of Science and Technology, Harbin, People's Republic of China*

(*Received 29 July 2008; final version received 27 January 2009*)

Diameter and wall thickness of self-assembled peptide nanotube of cyclo[(-D-Ala-L-Ala)$_4$-] were characterised by molecular simulation. In order to verify the existence of peptide nanotube of cyclo[(-D-Ala-L-Ala)$_4$-], cyclo[(-D-Ala-L-Ala)$_4$-] was firstly synthesised through Fmoc solid-phase synthesis method and then self-assembled in trifluoroacetic acid. Based on the results of experiment, the single nanotube structure was further characterised by molecular dynamics (MD) employing the COMPASS force field. The results indicate that cyclo[(-D-Ala-L-Ala)$_4$-] is self-assembled into nanotube bundles of about 0.5 μm in diameter and 10 μm in length; the inner and outer diameter of the single nanotube is 8.5 and 15.9 Å, respectively, and the nanotube wall thickness is 3.3 Å.

Keywords: cyclic peptide; self-assembled nanotube; molecular structure; molecular dynamics

1. Introduction

In recent years, extensive work has been done on cyclic peptide nanotubes (SPNs) due to their specific structure and function, which are suitable for applications in biology, chemistry and materials science [1–5].

In 1974, on the basis of investigations of the structural characteristics of linear polypeptides, De Santis [6] predicted the possibility of forming cylindrical structures from D,L-cyclic peptides. In 1993, Ghadiri and his group [7,8], for the first time, synthesised the hollow tubular structures. Subsequently, a number of investigations into cyclic peptide nanotubes have reported their synthesis, characterisation and specific functional applications [9–12]. These studies highlight the usage of cyclopeptide nanotubes as models for mimicking biological channels, as molecular adapters for a pore-forming protein, and as transport vehicles in drug delivery systems and other nanostructural materials [13–15].

While cyclopeptide nanotubes are prone to congregate, it is difficult to characterise their structures and properties. Therefore, some investigators adopted *N*-alkylation substituents on alternating amino acid residues to stop the self-assembly at the cyclopeptide dimer stage to increase the solubility [16,17]. Although the structure and properties of the dimers, which represent some structural features and properties of the cyclopeptide nanotubes, can be quickly obtained by this method, it has not been confirmed in experiment whether the cyclic peptides can stretch to a longer nanotube, and the character of a single nanotube is also unreported.

Since D,L-Ala cyclopeptide nanotube has the simplest structure, it is important to investigate its structure. In this study, we synthesised peptide of cyclo[(-D-Ala-L-Ala)$_4$-] through Fmoc solid-phase synthesis method and the cyclopeptide nanotube via self-assembling. The morphology and structure of the self-assembled cyclopeptide nanotubes were explored by mass spectrometry (MS), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Furthermore, the molecular structure of the single cyclo[(-D-Ala-L-Ala)$_4$-] nanotube was characterised by molecular dynamics (MD) modelling based on the COMPASS force field. It is hoped these results may provide evidence for the design and application of the cyclic peptide nanotubes.

2. Experiment and simulation

As shown in Figure 1, D,L-Ala linear multipeptide was synthesised by Fmoc solid-phase peptide synthesis method [18], and then cyclised in a dilute solution to reduce the subsidiary [12,19]. The dried cyclic peptides were redissolved in neat trifluoroacetic acid and self-assembled into nanotubes at room temperature for 48–72 h [20]. All reagents are commercially available and used without further purification.

The characterisation of MS is recorded on an Agilent 1100 LC-MS. Positive and negative scan patterns were adopted with ionisation source ESI. FT-IR is recorded at 298 K on Nicolet Nexus 470 by using solid tablet with

*Corresponding author. Email: t.j.cheng@163.com

ISSN 0892-7022 print/ISSN 1029-0435 online

DOI: 10.1080/08927020902787788
http://www.informaworld.com

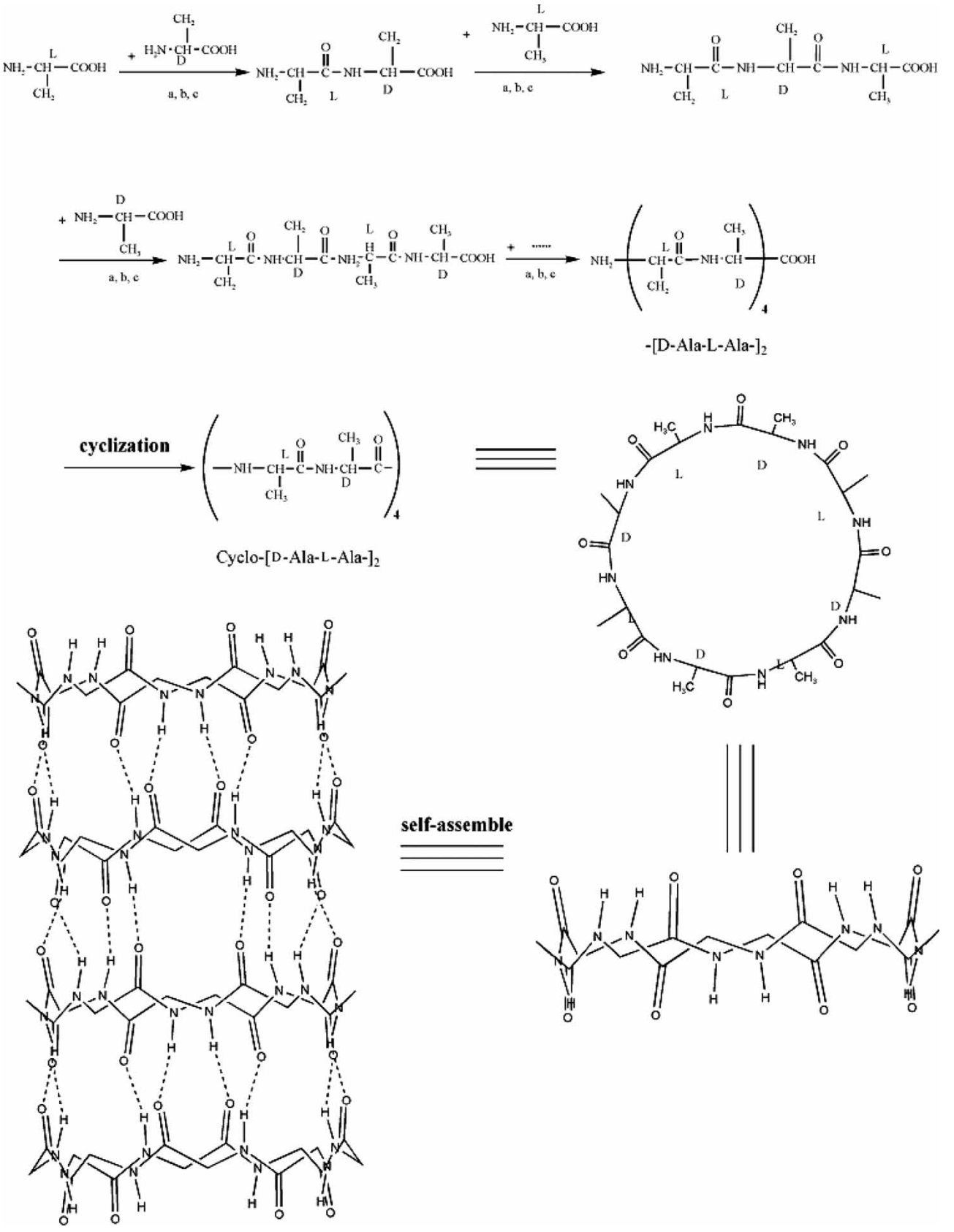

Figure 1. Synthesis of cyclo-[L-Ala-D-Ala-]$_4$ and self-assembly of nanotubes.

KBr. SEM is operated on a S-4700 after the sample is sprayed with gold for 8–10 min.

An infinite single nanotube model with three-dimensional periodical boundary is built to investigate the detailed structure of single nanotube.

Firstly, a single nanotube composed of 10 peptide rings was built with antiparallel rings arrangement; between neighbouring rings there are eight intermolecular H-bonds. Previous investigations have shown that cyclic peptides adopt a flat ring-shaped conformation with groups of C═O and N—H roughly perpendicular to the ring plane. Such conformation facilitates the rings stacking together to form a hollow tubular structure by means of inter-ring H-bonds. There are two stacking arrangements in the nanotube: one is parallel and the other is antiparallel. Since almost all studies indicate that the antiparallel arrangement is dominant [7,21], we stack the rings together antiparallel. The model was optimised with molecular mechanics (MM). The maximum deviation of root mean square gradient was 0.001 kcal/mol. Atomic-based summation method was used to calculate the long-range van der Waals interactions and the electrostatic interactions. Spline width, cut-off distance and buffer width are 1, 9.5 and 0.5 Å, respectively.

Then, the single nanotube was put in a three-dimensional periodical boundary box, with the axis of the nanotube parallel to axis-*a*. Lattice-*a* is equal to the length of nanotube so that the terminal rings can be hydrogen bonded with the other rings in their neighbouring cells. Thus, the nanotube will stretch to a single infinite nanochannel when they repeat along axis-*a*. Lattices *b* and *c* are set to 30 Å, and angles α, β and γ are set to 90°, respectively. The infinite single nanotube model is also optimised by MM, and the optimised model is shown in Figure 2.

MD simulation of 2 ns was performed to the model using the NVT ensemble with a time step of 1 fs at 298 K, and a Nose thermostat was employed to keep the temperature of the system constant during MD [22]. Ewald method was used to calculate the long-range interactions with an accuracy of 0.001 kcal/mol and update width of 3 Å. Every 1000 fs, the molecular structure was stored in the trajectory file for analysis.

All the simulation work is conducted with Materials Studio® software (Accelrys Inc., San Diego, CA, USA) on a sgi3800 origin sever using a COMPASS force field [23–25].

3. Results and discussion

3.1 Structure and morphology of nanotubes

The results of MS, FT-IR and SEM are shown in Figure 3. The mass of cyclo[(-D-Ala-L-Ala)$_4$-] is 586.8. MS spectrum shows a peak at 585.3 (Figure 3(a)), corresponding to the mass of cyclo[(-D-Ala-L-Ala)$_4$-], indicating the existence of cyclo[(-D-Ala-L-Ala)$_4$-]. In the nanotube, the peptide ring stacks together by means of H-bonds, which pertain to the protein β-sheet arrangement. The β-sheet nature of the hydrogen bonding is supported by FT-IR spectra recorded in nanotube (Figure 3(b)), which displays amide I and II bands at 1631.68 and 1535.98 cm^{-1}, respectively (positions for typical β-sheets [26]). Hydrogen bonding by N—H is further supported by amide A bands near 3276.73 cm^{-1} [27–29], while a band that appears at 3448.5 cm^{-1} may correspond to the N—H bond of the cyclopeptide monomer [30]. These characters are similar to those found in nanotubes and dimers composed of D,L-cyclic peptides [7,8,22].

SEM shows the morphology of self-assembled cyclo[(-D-Phe-L-Ala)$_4$-] nanotube (Figure 3(c)). It can be found that the nanotubes formed predominantly bundles with 0.5 μm in diameter and 10 μm in length, which is composed of parallel nanotubes.

As discussed above, data from MS and FT-IR confirmed that the [(-D-Ala-L-Ala)$_4$-] cyclopeptide has been synthesised and self-assembled into nanotubes.

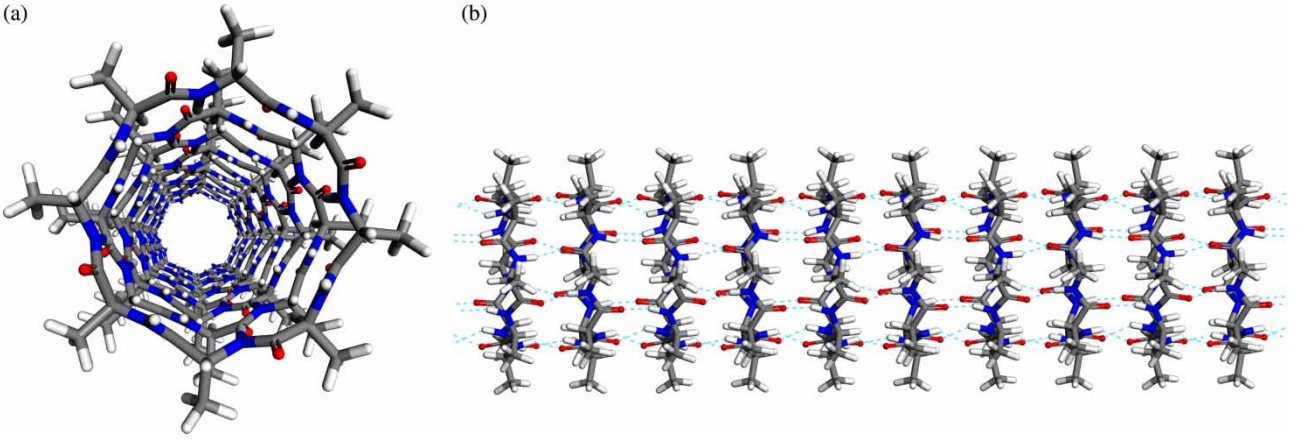

Figure 2. The optimised infinite single nanotube model. (a) Top view and (b) side view.

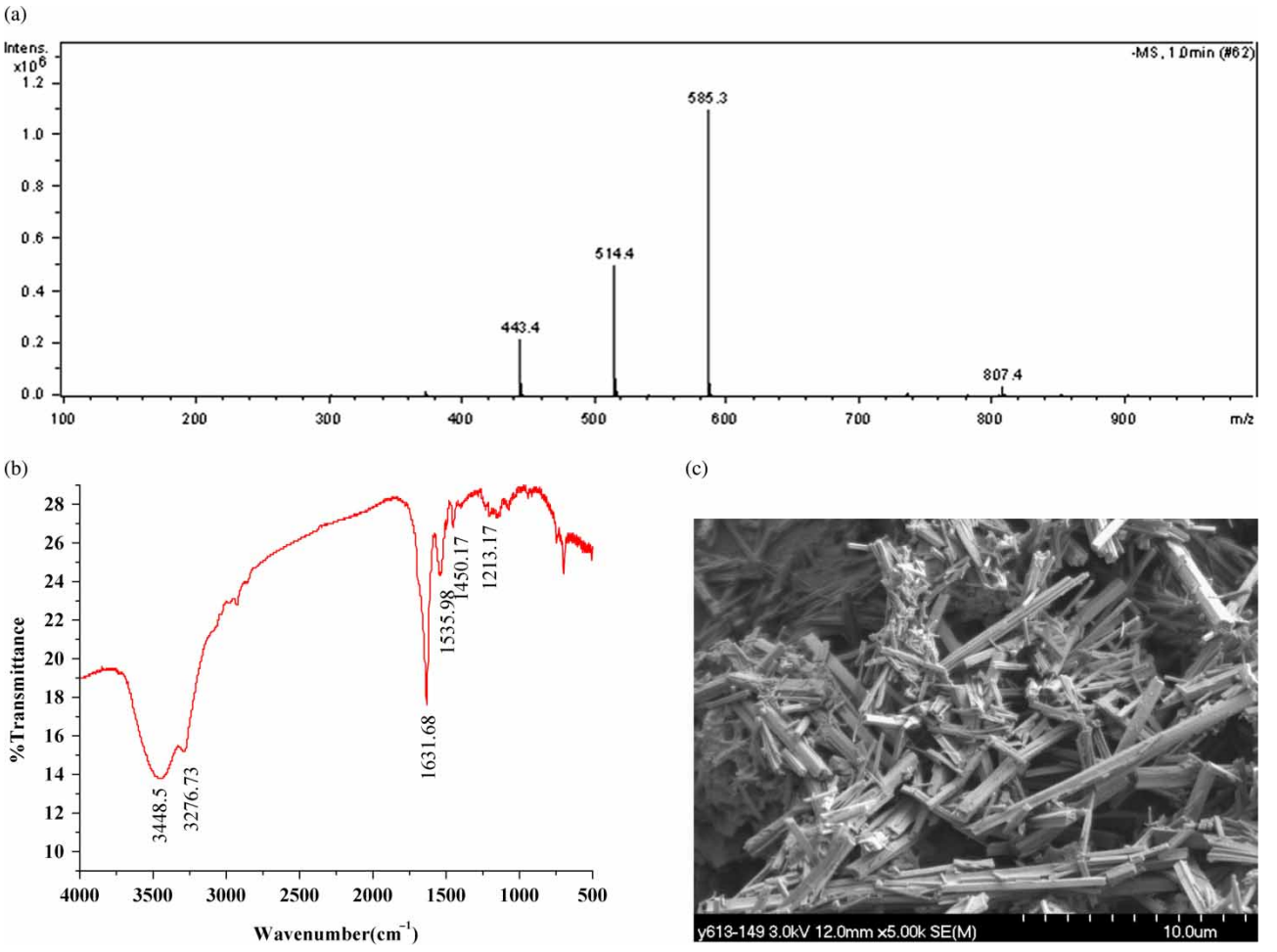

Figure 3. Structure and morphology of self-assembled nanotubes of cyclo[(-D-Ala-L-Ala)$_4$-]. (a) MS spectrum, (b) FT-IR spectrum and (c) SEM image.

3.2 Structure of single nanotube

Although single nanotubes in the bundle are parallel to each other, it is still difficult to measure the diameter of a single cyclopeptide nanotube in experiment. MD simulation provides a microscopic picture at atomistic level; some information of single nanotube can be obtained from MD trajectories.

Information derived from MD trajectories is meaningful only at equilibrium. There are two criteria to determine whether the system reached equilibrium: one is energy and the other is temperature. In our simulation, the energy and temperature have rapidly reached equilibrium at about 1000 fs. The energy vibrates in range of 800–900 kcal/mol, and the temperature vibrates in range of 260–300 K, respectively.

In MD simulation, lengths of both lattice-*b* and -*c* ($b = c = 30$ Å) in the initial model are much longer than the nanotube diameter; this ensures the nanotube is not affected by the periodic image. In the initial model, the resulting length of the nanotube after MM is 48 Å, and the average distance between centroids of neighbouring rings is 4.8 Å, which agrees with that of other cyclic peptide nanotube [9]. In addition, the hydrogen-bonding distance (N—O) between neighbouring peptide rings is 2.9–3.1 Å, which is in accord with the experiment results as well [31]. These mean that the initial model used in MD simulation is reasonable.

During MD simulation, the cyclic peptides are hydrogen bonded by eight H-bonds in each neighbouring rings, and the distances between centroids of neighbouring rings vibrate in the range of 4.5–5.2 Å. This shows that the nanotube is very stable. Moreover, the distances between centroids of neighbouring rings are also in good agreement with the results of the experiment [9].

Diameter of the nanotube is measured from the profiles of concentration distribution of lateral nanotube (Figure 4). The two main peaks in each profile attribute to atoms of backbone. The inner diameter is the distance between the

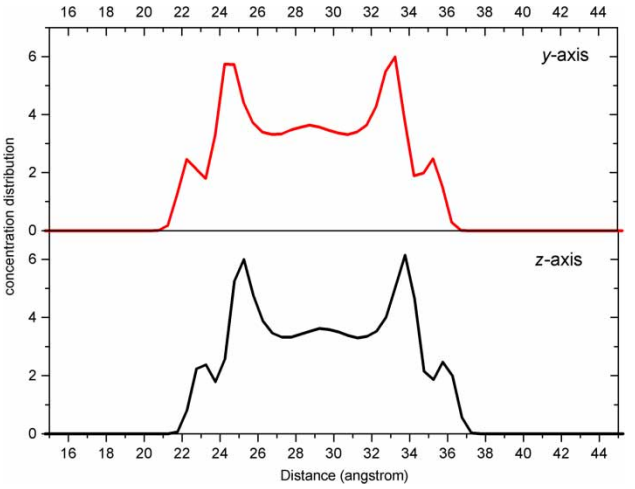

Figure 4. Concentration distribution of nanotube along *y*- and *z*-axis. The distance between two main peaks represents the internal diameter and the width of the profile is outer diameter. The internal diameter is about 8.5 Å and the outer diameter is about 15.9 Å.

two main peaks, the outer diameter is the width of the profile and the nanotube wall thickness is obtained from the difference of inner and outer diameters. It can be seen from Figure 4 that the inner diameter is 8.5 Å, which corresponds to the values of other cyclic peptide nanotubes with the same number of amino acid residues [32], and the outer diameter is 15.9 Å, which is less than that of other cyclic peptides with larger side-chain groups [7], and the thickness of nanotube wall is 3.3 Å.

4. Conclusions

In conclusion, cyclo[(-D-Ala-L-Ala)$_4$-] peptide is synthesised through Fmoc solid-phase synthesis and self-assembled into nanotube bundles with 0.5 μm in diameter and 10 μm in length. Molecular modelling results show that the internal diameters of the single cyclo[(-D-Ala-L-Ala)$_4$-] nanotube is 8.5 Å and the outer diameter is 15.9 Å. The thickness of the nanotube wall is 3.3 Å.

References

[1] J.D. Hartgerink, T.D. Clark, and M.R. Ghadiri, *Peptide nanotubes and beyond*, Chem. Eur. J. 4 (1998), pp. 1367–1372.

[2] R.J. Brea, M.E. Vazquez, M. Mosquera, L. Castedo, and J.R. Granja, *Controlling multiple fluorescent signal output in cyclic peptide-based supramolecular systems*, J. Am. Chem. Soc. 129 (2007), pp. 1653–1657.

[3] A. Janshoff, K.P.S. Dancil, C. Steinem, D.P. Greiner, V.S.Y. Lin, C. Gurtner, K. Motesharei, M.J. Sailor, and M.R. Ghadiri, *Macroporous p-type silicon Fabry-Perot layers. Fabrication, characterization, and applications in biosensing*, J. Am. Chem. Soc. 120 (1998), pp. 12108–12116.

[4] G. David and B.M. Pierre, *Self-assembly of cyclic peptides into nanotubes and then into highly anisotropic crystalline materials*, J Angew. Chem. Int. Ed. 40 (2001), pp. 4635–4638.

[5] M.R. Ghadiri, *Self-assembled nanoscale tubular ensembles*, Adv. Mater. 7 (1995), pp. 675–677.

[6] P.D. Santis, S. Morosetti, and R. Rizzo, *Conformational analysis of regular enantiomeric sequences*, Macromolecules 7 (1974), pp. 52–58.

[7] M.R. Ghadiri, J.R. Granja, R.A. Milligan, D.E. McRee, and N. Khazanovich, *Self-assembling organic nanotubes based on a cyclic peptide architecture*, Nature 366 (1993), pp. 324–327.

[8] M.R. Ghadiri, J.R. Granja, and L.K. Buehler, *Artificial transmembrane channels from self-assembling peptide nanotubes*, Nature 369 (1994), pp. 301–304.

[9] H.S. Kim, J.D. Hartgerink, and M.R. Ghadiri, *Oriented self-assembly of cyclic peptide nanotubes in lipid membranes*, J. Am. Chem. Soc. (1998), pp. 4417–4424.

[10] T.D. Clark, J.M. Buriak, K. Kobayashi, M.P. Isler, D.E. McRee, and M.R. Ghadiri, *Cylindrical beta-sheet peptide assemblies*, J. Am. Chem. Soc. 120 (1998), pp. 8949–8962.

[11] S. Fernandez-Lopez, H.S. Kim, E.C. Choi, M. Delgado, and J.R. Granja, *Antibacterial agents based on the cyclic D,L-alpha-peptide architecture*, Nature 412 (2001), pp. 452–455.

[12] R.J. Brea, M.P. Lopez-Deber, L. Castedo, and J.R. Granja, *Synthesis of omega-(hetero)arylalkynylated alpha-amino acid by sonogashira-type reactions in aqueous media*, J. Org. Chem. 71 (2006), pp. 7870–7873.

[13] J. Sanchez-Quesada, M.R. Ghadiri, H. Bayley, and O. Braha, *Cyclic peptides as molecular adapters for a pore-forming protein*, J. Am. Chem. Soc. 122 (2000), pp. 11757–11766.

[14] W.S. Horne, N. Ashkenasy, and M.R. Ghadiri, *Modulating charge transfer through cyclic D,L-alpha-peptide self-assembly*, Chem. Eur. J. 11 (2005), pp. 1137–1144.

[15] G. Ashkenasy and M.R. Ghadiri, *Boolean logic functions of a synthetic peptide network*, J. Am. Chem. Soc. 126 (2004), pp. 11140–11141.

[16] W.S. Horne, C.D. Stout, and M.R. Ghadiri, *A heterocyclic peptide nanotube*, J. Am. Chem. Soc. 125 (2003), pp. 9372–9376.

[17] W.S. Horne, M.K. Yadav, C.D. Stout, and M.R. Ghadiri, *Heterocyclic peptide backbone modifications in an α-helical coiled coil*, J. Am. Chem. Soc. 126 (2004), pp. 15366–15367.

[18] S.S. Wang, R. Makofske, and A. Bach, *Automated solid phase synthesis of thymosinal*, Int. J. Peptide Protein Res. 15 (1980), pp. 1–4.

[19] M. Amorin, L. Castedo, and J.R. Granja, *New cyclic peptide assemblies with hydrophobic cavities: the structural and thermodynamic basis of a new class of peptide nanotubes*, J. Am. Chem. Soc. 125 (2003), pp. 2844–2845.

[20] J.D. Hartgerink, J.R. Granja, R.A. Milligan, and M.R. Ghadiri, *Self-assembling peptide nanotubes*, J. Am. Chem. Soc. 118 (1996), pp. 43–50.

[21] G. Chen, S. Su, and R. Liu, *Theoretical studies of monomer and dimer of cyclo[(L-Phe^1-D-Ala^2)$_n$] and cyclo[(-L-Phe^1-D-^{Me}N-Ala^2)$_n$](n=3–6)*, J. Phys. Chem. B 106 (2002), pp. 1570–1575.

[22] W.G. Hoover, *Canonical dynamics: equilibrium phase-space distributions*, Phys. Rev. A 31 (1985), pp. 1695–1697.

[23] H. Sun, Ab initio *calculations and force field development for computer simulation of polysilanes*, Macromolecules 28 (1995), pp. 701–712.

[24] H. Sun, *COMPASS: an* ab initio *force-field optimized for condensed-phase applications-overview with details on alkane and benzene compounds*, J. Phys. Chem. B 102 (1998), pp. 7338–7364.

[25] H. Sun, P. Ren, and J.R. Fried, *The COMPASS force field: parameterization and validation for polyphosphazenes*, Comput. Theor. Polym. Sci. 8 (1998), pp. 229–246.

[26] S. Krimm and J. Bandekar, *Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins*, Adv. Protein Chem. 38 (1986), pp. 181–364.

[27] V. Pavone, E. Benedetti, B.D. Blasio, A. Lombardi, C. Pedone, L. Tomasich, and G.P. Lorenzi, *Regularly alternating L,D-peptides. III. Hexacyclic peptides from valine or phenylalanine*, Biopolymers 28 (1989), pp. 215–223.

[28] X. Sun and G.P. Lorenzi, *On the stacking of β-rings: the solution self-association behavior of two partially* N*-methylated cyclo(hexaleucines)*, Helv. Chim. Acta 77 (1994), pp. 1520–1526.
[29] J. Bandekar, *Amide modes and protein conformation*, Biochim. Biophys. Acta 1120 (1992), pp. 123–143.
[30] M. Saviano, A. Lombardi, C. Pedone, B.D. Blasio, X.C. Sun, and G.P. Lorenzi, *A structural two-ring version of a tubular stack of β-rings in crystals of a cyclic D,L-hexapeptide*, J. Inclusion Phenom. 18 (1994), pp. 27–36.
[31] M. Amorin, L. Castedo, and J.R. Granja, *New cyclic peptide assemblies with hydrophobic cavities: the structural and thermodynamic basis of a new class of peptide nanotubes*, J. Am. Chem. Soc. 125 (2003), pp. 2844–2845.
[32] J.R. Granja and M.R. Ghadiri, *Channel-mediated transport of glucose across lipid bilayers*, J. Am. Chem. Soc. 116 (1994), pp. 10785–10786.