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Compartmentalized host spaces accommodating guest aromatic molecules in a chiral way in a helix-peptide-aromatic framework[†]

Hirotaka Uji, ២ a Junya Ogawa, a Kenji Itabashi, b Tomoya Imai ២ c and Shunsaku Kimura 🕩 *a

A novel host molecular assembly of a free-standing flat nanosheet with compartmentalized spaces was prepared using a bolaamphiphilic peptide composed of two amphiphilic helical peptides and an oligo(naphthaleneethynylene) (ONE) unit at the center of the molecule. The nanosheet possesses void host spaces that can accommodate two mol-equivalent ONE groups to form columns of ONE groups in a right-handed helical way and ONE channels over a long distance. The present molecular system therefore can provide a chiral pore channel for relatively large molecules.

Producing synthetic 2D materials on a length scale of 10 nm-10 µm has been an attractive challenge,1 and free-standing nanosheets and free-floating sheets can be prepared using self-assembly methods.^{2,3} Functionalized ultrathin materials comprising aromatic groups at the core region have also been reported.^{4,5} To the best of our knowledge, however, free-standing nanosheets with void spaces that have an ability to accommodate guest molecules have not been reported so far, which makes a vivid contrast to metal-organic frameworks (MOFs). MOFs have been attracting increasing attention as functional porous materials applicable for various purposes.⁶ MOFs are advantageous for their simple preparation and system designs with high versatility and tunability for relevant functions. One of the current challenges for porous materials is achieving significant porosity characterized by large intermetallic separation and wide accessibility.⁷ However, they are generally poor in terms of thermal and mechanical stability. When the requirement of crystallinity of the porous material is removed, large porosity is attainable using metal-organic polyhedra (MOP),

which can be dispersed in a polymer membrane⁸ and selfassembled into supramolecular polymers.9 MOP can provide cavities of ca. 1.5 nm and more,^{8,10} but channels composed of continuous and relatively large cavities are still difficult to obtain. One way to solve the instability problem of the large pore and channel structure generated by the self-organization of organic linkers by adding a space-filled component to the organic linkerframe structure is considered and proposed here. As an analogy, our idea can use a house construction method of frame wall construction using walls and floors or ceilings as flat space-filled components to support the house, which is in contrast to the axis construction method using only pillars and beams as vertical and horizontal axes, respectively. With the aim of obtaining a large pore and long channel structure, we designed here the molecular assembly of a free-standing sub-µm sheet with ca. 10 nm thickness including the void host space at the center.

Another challenge here with regard to the pore and channel structure is to accommodate guest molecules in a chiral arrangement. Chiral environments have been provided in MOFs using chiral organic linkers to achieve an enantioselective reaction¹¹ and enantioselective recognition.¹² Apart from these instances, we report here the arrangement of guest aromatic molecules in a spiral way in the void spaces. Thereby, achiral aromatic molecules self-assemble in a one-handed helical array without any help from covalent joints. Further, the continuous pores make channels at a long-length scale of µm, where the aromatic molecules are filled in with a retention of π -stacking. A homochiral form of the porous organic cage was reported,¹³ however, which provided an isolated space. Chirality control at a long-length scale, which is still a challenging task,¹⁴⁻¹⁶ is targeted here. Homochiral porous molecular networks were also reported, however, using substrate surfaces to organize molecules into two-dimensional arrays.¹⁷ On the other hand, in the present paper, a free-standing twodimensional sheet was prepared for chiral channel environments without the help of an interface.

The molecular structure of the free-standing nanosheet with pore and channel structures is shown as S29L8ONE in Fig. 1. Two chains of the AB-type amphiphilic polypeptide,



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^a Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan. E-mail: shun@scl.kyoto-u.ac.jp

^b Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

^c Research Institute for Sustainable Humanosphere, Kyoto University, Uji,

Kyoto 611-0011, Japan

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Fig. 1 (A) Chemical structures of S29L8ONE and BocONE. (B) Schematic illustration of S29L8ONE. (C) Molecular model of S29L8ONE without poly(sarcosine) moieties. (D) Schematic illustration of the arrangement of two adjacent Leu-Aib helices (left) and the molecular assembly structure prepared from S29L8ONE (right). Black and grey balls of isobutyl moieties are protruding over the front side and back side of the helices, respectively. (E) Schematic illustration of the column structure of the ONE groups. (F) Schematic illustration of the channel structure of the ONE groups.

poly(sarcosine)-*b*-(ι -Leu-Aib)₄, were symmetrically connected to the oligo(naphthalene-ethynylene) (ONE) core. Previously, poly-(sarcosine)-*b*-(ι -Leu-Aib)₆ was reported to self-assemble into a nanotube morphology as a result of the association of hydrophobic helices in water.¹⁸ The favourable space filling of Leu-side chains between neighboring helices in a knob-and-hole arrangement is the origin of the robust self-assembled monolayer.¹⁹ The blocks of poly(sarcosine)-*b*-(ι -Leu-Aib)₄ in S29L8ONE are therefore expected to self-assemble in water into two monolayers acting as walls and sandwiching the ONE cores as pillars (Fig. 1D and E).

Since the diameter of the helices is around 1.5 nm, which is thicker than the flat ONE group, there will be a void space in the immediate surroundings of the ONE cores where two additional aromatic groups can be accommodated in a face-to-face arrangement with *ca.* 5 Å intervals (Fig. 1E). In addition, the naphthyl groups of the ONE moiety are expected to contribute to the channel formation due to the kink structure *via* linkages at the β position leading to successive connections of the ONE cores through π -stacking of the naphthyl groups (Fig. 1F).^{20–22}

The peptide and ONE components of S29L8ONE were synthesized separately, and subsequently combined with one another (synthetic details are available in the ESI,† Fig. S1–S3). The Leu-Aib alternating sequence was synthesized using the conventional liquid-phase method. The ONE component was synthesized using Sonogashira coupling reactions starting from 2,5-dibromohydroquinone. After these components were combined, poly(sarcosine) chains were grown by Sar-NCA polymerization from the Leu N-terminus.

Molecular assemblies were prepared using the injection method. A 10 μ L DMF solution of S29L8ONE was injected into 1 mL MilliQ water (1% DMF) at 4 °C followed by heat treatment at 50 °C for 1 h. Molecular assemblies as small as *ca.* 10 nm were observed using TEM (Fig. 2A). Liquid AFM was used to obtain the topological image, which clearly shows a flat nanosheet morphology with a height of 10.0 \pm 2.0 nm (Fig. 2B and C, and the height histogram is shown in Fig. S4, ESI†). The side-by-side association of helices is considered to be the driving force of the formation of the self-assemblies (Fig. 1D). Previously we have



Fig. 2 TEM and AFM measurements of molecular assemblies. (A–C) Negatively stained TEM image with 2% uranyl acetate (A), liquid AFM image (B), and height profile (C) of molecular assemblies prepared from S29L8ONE (9.5 × 10^{-2} mM) with heating treatment at 50 °C for 1 h. (D) TEM image of molecular assemblies prepared from S29L8ONE (9.5 × 10^{-2} mM) + BocONE (1/2 mol/mol) with heating treatment at 50 °C for 1 h. (E–G) TEM image (E), liquid AFM image (F), and height profiles (G) of molecular assemblies prepared from S29L8ONE (9.5 × 10^{-2} mM) + BocONE (1/2 mol/mol) with heating treatment at 50 °C for 1 h. (E–G) TEM image (E), liquid AFM image (F), and height profiles (G) of molecular assemblies prepared from S29L8ONE (9.5 × 10^{-2} mM) + BocONE (1/2 mol/mol) with heating treatment at 90 °C for 24 h.

analysed a nanosheet morphology prepared from a mixture of poly(sarcosine)-*b*-(L-Leu-Aib)₆ and poly(sarcosine)-*b*-(D-Leu-Aib)₆ to possess a 7.4 nm thickness,²³ where the thickness of the poly(sarcosine) layer was estimated to be *ca.* 2.8 nm. Accordingly, the total thickness of two poly(sarcosine) layers of 5.6 nm, two helix-block layers of 2.4 nm, and the ONE layer of 2 nm is estimated to be 10 nm, which is consistent with the thickness observed with AFM. S29L8ONE is therefore suggested to self-assemble into a monolayer with two hydrophobic helix layers flanking the ONE layer with void spaces. Further heating at 90 °C for 24 h, however, made the molecular assemblies transform into irregular aggregates, suggesting that the tiny nanosheets are not thermodynamically stable (Fig. S5, ESI†).

As described above, the molecular assemblies of S29L8ONE will have void spaces to accommodate two flat guest molecules on the basis of 5 Å intervals available for π -stacking. Accordingly, a mixture of S29L8ONE and BocONE at a molar ratio of 1/2 was selfassembled in water. After heat treatment at 50 °C for 1 h, some aggregates of a few tens of nanometers, in addition to the tiny molecular assemblies, were observed using TEM (Fig. 2D). The hydrophobic guest molecules of BocONE may be difficult to incorporate into the molecular assemblies. However, further heating at 90 °C for 24 h afforded molecular assemblies of a squaresheet morphology with a length of ca. 1 µm, as indicated by the TEM (Fig. 2E) and liquid AFM (Fig. 2F and Fig. S6, ESI[†]) observations, which were in contrast to the irregular aggregates observed in the absence of BocONE (Fig. S5, ESI⁺). The AFM height profiles clearly show the formation of nanosheets with a height of ca. 10 nm (Fig. 2G), which is consistent with the dimension of the S29L8ONE monolayer. The surface roughness decreased down to 0.5 nm, supporting the formation of the homogeneous nanosheet structure. Structural evolution with different heating treatments should follow a path which can be controlled by a combination of thermodynamics and kinetics.²⁴⁻²⁶ It is suggested that the induced type of host-guest interaction stabilizes the molecular assemblies thermodynamically, and the driving force is considered to be the combination of hydrophobic interactions and π -stacking between the ONE groups.

Molecular assemblies with varying amounts of the guest molecule BocONE were analysed using UV-vis, fluorescence, and CD spectroscopy. BocONE alone shows an absorption tail over 450 nm, which should be due to self-aggregation. The tailing disappears in the presence of S29L8ONE, meaning that BocONE should disassemble upon incorporation into the S29L8ONE assemblies (Fig. 3A). Fluorescence spectra of the mixtures of S29L8ONE and BocONE, however, show the additive spectra of S29L8ONE and BocONE in water, suggesting that any specific emissive species are not formed with heat treatment at 50 °C for 1 h (Fig. 3B).

With heat treatment at 90 °C for 24 h, drastic changes were observed in the UV-vis and fluorescence spectra. A new absorption signal at 425 nm and a long tailing appeared in the UV-vis spectra (Fig. 3C). The former absorption is ascribable to a partial overlap of the ONE groups, and the latter may correspond to a successive overlap of the ONE groups over a long-distance. The time-course of the UV-vis spectral change revealed that the molecular assemblies were converted into a new structure with time (Fig. 3E).



Fig. 3 Spectra of molecular assemblies prepared from S29L8ONE (9.5 × 10⁻² mM) + BocONE. (A–D) Molar ratio dependent UV-vis spectra (A and C) and fluorescence spectra (B and D) with heat treatment at 50 °C for 1 h (A and B) and at 90 °C for 24 h (C and D) in MilliQ. (E and F) Heat treatment time dependent UV-vis spectra (E) and CD spectra (F) of molecular assemblies prepared from S29L8ONE (9.5 × 10⁻² mM) and BocONE at a molar ratio of 1/2 in MilliQ.

It is notable that the absorption in the range from 250 nm to 400 nm decreases in intensity with the emergence of a new peak at 425 nm, indicating that the long tailing over 600 nm is due to elongated π -stacking with less contribution from the light scattering of the large molecular assemblies. The hypochromism around 250 nm to 400 nm also supports the idea that the ONE groups begin to take regular positions in close proximity to the H type in the molecular assembly with heat treatment at 90 °C for 24 h.

Fluorescence spectra show monomer emission at 425 nm and 445 nm and emission over 490 nm with vibrational structures (Fig. 3D). The latter is considered to be emitted from the partial overlap species because the excitation spectrum indicates a contribution from the partial overlap species at 425 nm (Fig. S7H, ESI†). These drastic changes in the UV-vis and fluorescence spectra indicate that the assembly evolution could be controlled primarily by π -stacking interactions between the ONE groups.

The spatial arrangement of the ONE groups in the molecular assemblies of a mixture of S29L8ONE and BocONE (1/2 mol/mol) was analysed using CD measurements (Fig. 3F). An induced Cotton effect with a large exciton coupling centered at 425 nm appeared with heat treatment at 90 °C in a similar time scale to the appearance of the absorption signal at 425 nm (Fig. 3E). Since the intensity of the Cotton effect due to the exciton coupling changed the sign from positive to negative with a shift to shorter wavelengths, four ONE groups, two from the cores and two from the guests, take a right-handed helical arrangement to form columns (Fig. 1F). It is speculated that these columns are successively aligned





Fig. 4 Electron diffraction pattern with an electron beam perpendicular to the axis of the molecular assembly prepared from S29L8ONE (9.5 \times 10⁻² mM) + BocONE at a molar ratio of 1/2 in MilliQ with heat treatment at 90 °C for 24 h. Inset axes *a** and *c** correspond to the axes *a* and *c* in Fig. 2D.

to form channels of the ONE groups retaining partial π -stacking and are assignable to the absorption tailing over 600 nm without chirality because any induced CD observed over 600 nm was insignificant.

The square-sheet morphology with heat treatment at 90 °C should be a thermodynamically stable structure considering the combination of π -stacking interactions between the ONE groups and hydrophobic interactions among the helical peptides. Therefore, electron diffraction from the molecular assembly of a mixture of S29L8ONE and BocONE (1/2 mol/mol) was measured (Fig. 4). The diffraction spots can be indexed using a = 14.2 Å and c = 7.85 Å, where the *a*- and *c*-axes correspond to the shorter and longer axes of the rectangular nanosheet molecular assemblies (Fig. 2D). The spacing of 14.2 Å corresponds to the helix diameter, which supports the tight association of helices in a side-by-side way. Further, there are systematic absences in the diffraction pattern at the intersections of odd-numbered layer lines and a central meridian, suggesting that a twofold symmetry exists along the c axis. In addition, a unit cell has an axial spacing of 3.93 Å along the *c* axis (Fig. 4, l = 2), indicating that the ONE groups are regularly arranged in line and keep this spacing (Fig. 1E) to form columns along the c axis. These columns successively connect to form channels along the a axis with intervals of the helix diameter of 14.2 Å. The ONE core of S29L8ONE aligns along the *a* axis with a tilting of the long axis toward the *b* axis (Fig. 1D). The tilting angles of the neighbour ONE cores change the sign from negative for the back row to positive for the front row (Fig. 1E). The two accommodated guest molecules of BocONE should rotate the long axis between these two ONE cores, resulting in a right-handed helical arrangement. Furthermore, these ONE channels are expected to show twofold symmetry around the *c* axis, because both S29L8ONE and BocONE have point symmetry in the molecular structure.

Considering the results of the CD spectra and the helix diameter, two neighboring ONE cores of two 29SL8ONE units clamp two BocONE molecules in a right-handed helical way along the c axis (Fig. 1F). In the ONE column, the long molecular axes of the ONE groups should rotate in a right-handed way. However, the reason for the induction of the right-handed rotation remains unknown. At the moment, we speculate that two adjacent helices associate together with a tilting of the helix axis to accommodate isobutyl groups in a knobs-into-holes arrangement, which generates a chiral environment to induce the right-handed helical arrangement of the ONE cores (Fig. 1D).

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Conflicts of interest

There are no conflicts to declare.

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