Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Materials

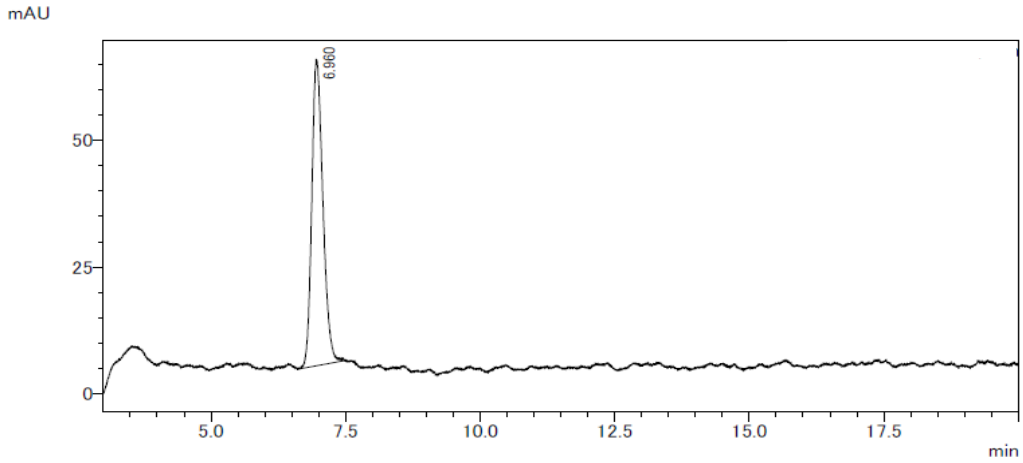
Packing Arrangement of ^{13}C Selectively Labeled Sequence Model Peptides of *Samia cynthia ricini* Silk Fibroin Fiber studied by Solid-state NMR

Tetsuo Asakura, Kenta Miyazawa, Yugo Tasei, Shunsuke Kametani, Yasumoto Nakazawa, Akihiro Aoki and Akira Naito

Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, 184-8588, Japan.

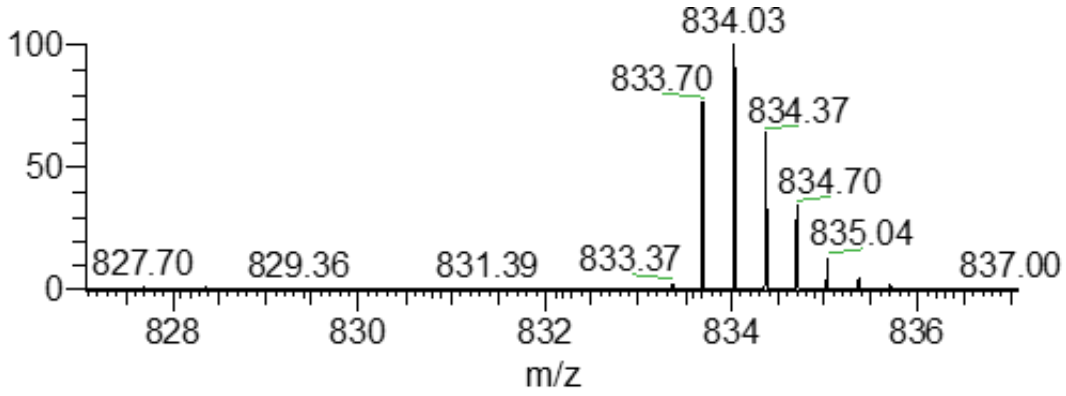
Supporting Materials 1: Liquid chromatography (LC)

The purities of the peptides were determined as more than 95% as follows.
Synthesized peptide (34 mer) was analyzed using a LC 30AD (Shimadzu, Japan) liquid chromatography system with an Inertsil ® ODS-3 column. The eluent composition was 0.1 % acetonitrile and 0.1 % formic acid aq. The flow rate is 0.20 ml/min.
An impurity peak could not be detected in the following LC spectrum of the peptide using UV chromatogram.



Supporting Materials 2: High-resolution FT-MASS (ESI, Posi mode)

Synthesized peptide was dissolved in solvent mixed with 0.1 % formic acid aq. and 0.1 % acetonitrile. Then 1 μl of this solution (100 ppm) was injected at 40 ºC.
The $[M+3H]^{3+}$ was 833.7 and therefore the molecular weight was 833.7×3-3=2498.



Supporting Materials 3. Model building of the sequence model peptide with β-sheet forms.

In order to obtain the co-ordinate of the inter-molecular structure of the main part (20 mer), GAGG$(A)_{12}$GGAG, of the model peptide (34 mer), GGAGGGYGGDGG$(A)_{12}$GGAGDGYGAG in the crystalline state, the MD simulation and MM minimization was performed to optimize the peptide structure according to the following manner.

1) The starting structure is the co-ordinates of $(Ala)_4$ with anti-parallel β-sheet alignment in the crystal, as determined by X-ray analysis by us (Space group $P2_12_12$; Fig. S1).[32] The structure of the central $(Ala)_2$ unit of the $(Ala)_4$ chain was inserted into the center of the $(Ala)_4$ chain to create $(Ala)_6$ chain in the crystal with anti-parallel β-sheet structure. Then the unit cell length of $(Ala)_6$ crystal along molecular chain was elongated by the length of $(Ala)_2$ unit to perform optimization and create new co-ordinate of $(Ala)_6$ crystal with space group $P2_12_12$ (Fig. S2). This process was repeated to create new co-ordinate of $(Ala)_8$ crystal with space group $P2_12_12$ as shown in Fig. S3.
2) In the next step, N-terminal and C-terminal Ala residues were cut from the $(Ala)_8$ chain. The structure of $(Ala)_6$ without N- and C-termini in the crystal was obtained and then optimized by MD simulation and energy minimization by MM calculation under fixed cell parameter (now space group is changed to P_1) as shown in Fig. S4B. In this structure, β-sheet chain is connected between the cell to create poly-Ala chains.
3) In the next step, (the co-ordinate of GAGG$(A)_{12}$GGAG molecules with anti-parallel β-sheet structure in the crystal with space group P1 was prepared.) The structure of $(Ala)_6$ molecules were expanded to $(Ala)_{20}$ which was the same number of amino acid residues of the main part of the model peptide (34 mer). Four $(Ala)_{20}$ molecules with anti-parallel β-sheet structure were combined to produce one layer and then four layers with anti-parallel pattern along the layer direction were stacked. Consequently, the co-ordinates of 4x4 (=16) $(Ala)_{20}$ molecules with anti-parallel β-sheet structure in the crystal were obtained (Fig. S5).
4) In order to get more stable packing structure, one layer which consists of four $(Ala)_{20}$ molecules with anti-parallel β sheet structure is moved to the position between two layers by shifting half inter chain distance to avoid the overlapping of the β-sheet chain. In this crystal, cell length of perpendicular to the β-sheet layer became short and thus, energy optimization was performed by changing 0.1 Å step.

The optimized packing structure in the crystal is shown in Fig. S6. Notably, there are two types of molecules in which inter molecular distances are slightly different each other as shown in Fig. S6C.

5) The crystal structure of 4x4(=16) $(Ala)_{20}$ molecules with minimum energy was obtained as follows. Space group: P_1, A: 75.00 Å (molecular direction), B: 18.41 Å (β-sheet direction), C: 19.50 Å (cross β-sheet direction), α: 90°, β: 90°, γ: 90°.
6) To get packing structure of $GAGG(A)_{12}GGAG$ peptide, N-terminal $(A)_4$ structure changed to GAGG and C-terminal $(A)_4$ structure changed to GGAG and further optimized using same cell dimension. The optimized structure is defined as no shifted packing model and the packing diagram is shown in Fig. 6 A.
7) In a second packing model, one layer is shifted by two residues to the chain direction and optimized the structure using the same cell parameter. The optimized structure is defined as two shifted packing model and the packing diagram is shown in Fig. 6 B.
8) All optimization were performed using Material Studios Discover (Accelrys Inc.) with force field pcff. The condition in MD simulation is MD NVT 373K 50,000step and Minimization cell fix 5,000step.

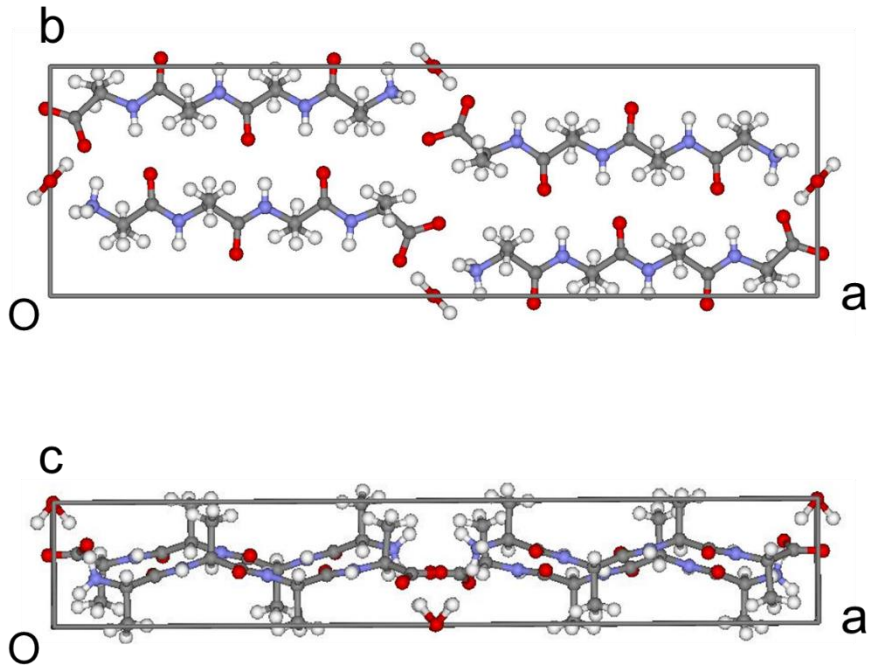


Fig. S1. Packing diagram of $(Ala)_4$ molecules in the unit cell of the crystal with space group $P2_12_12$ in view of ab (top) and ac (bottom) planes. Four $(Ala)_4$ molecules form anti-parallel β-sheet structure. The cell dimension is as follows.

a: 31.44 Å (direction of molecular chain axis).

b: 9.38 Å (direction in the β-sheet perpendicular to a axis).

c: 5.11 Å (perpendicular to the β-sheet layer).

$\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$

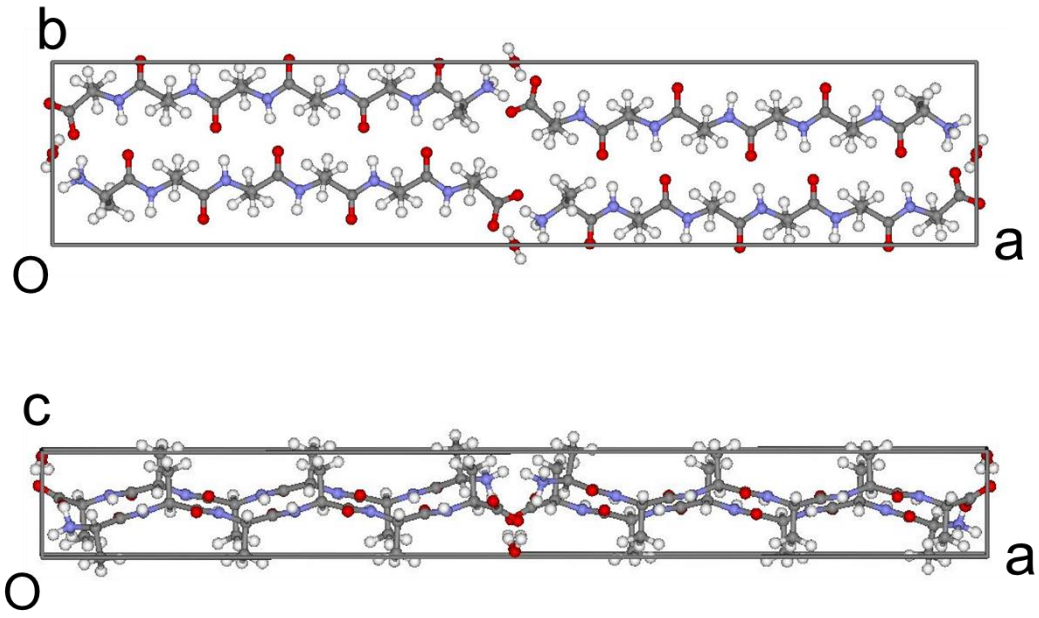


Fig. S2. Packing diagram of $(Ala)_6$ molecules in the unit cell of the crystal with space group $P2_12_12$ in view of ab (top) and ac (bottom) planes. Four $(Ala)_6$ molecules form anti-parallel β-sheet structure. The cell dimension is as follows.

a: 46.47 Å (direction of molecular chain axis).

b: 9.38 Å (direction in the β-sheet perpendicular to a axis).

c: 5.11 Å (perpendicular to the β-sheet layer).

$\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$

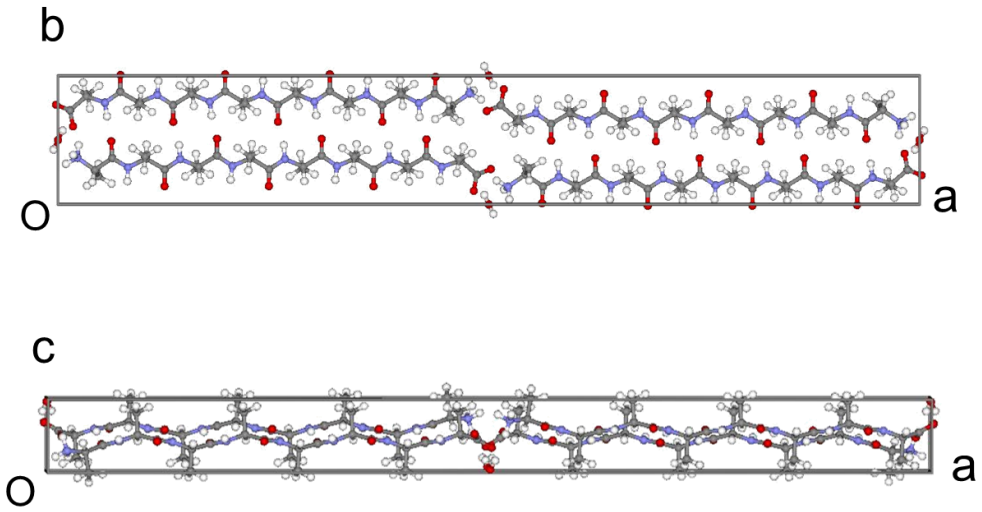


Fig. S3. Packing diagram of $(Ala)_8$ molecules in the unit cell of the crystal with space group $P2_12_12$ in view of ab (top) and ac (bottom) planes. Four $(Ala)_8$ molecules form anti-parallel b-sheet structure. The cell dimension is as follows.

a: 61.50 Å (direction of molecular chain axis).

b: 9.38 Å (direction in the β-sheet perpendicular to a axis).

c: 5.11 Å (perpendicular to the β-sheet layer).

$\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$

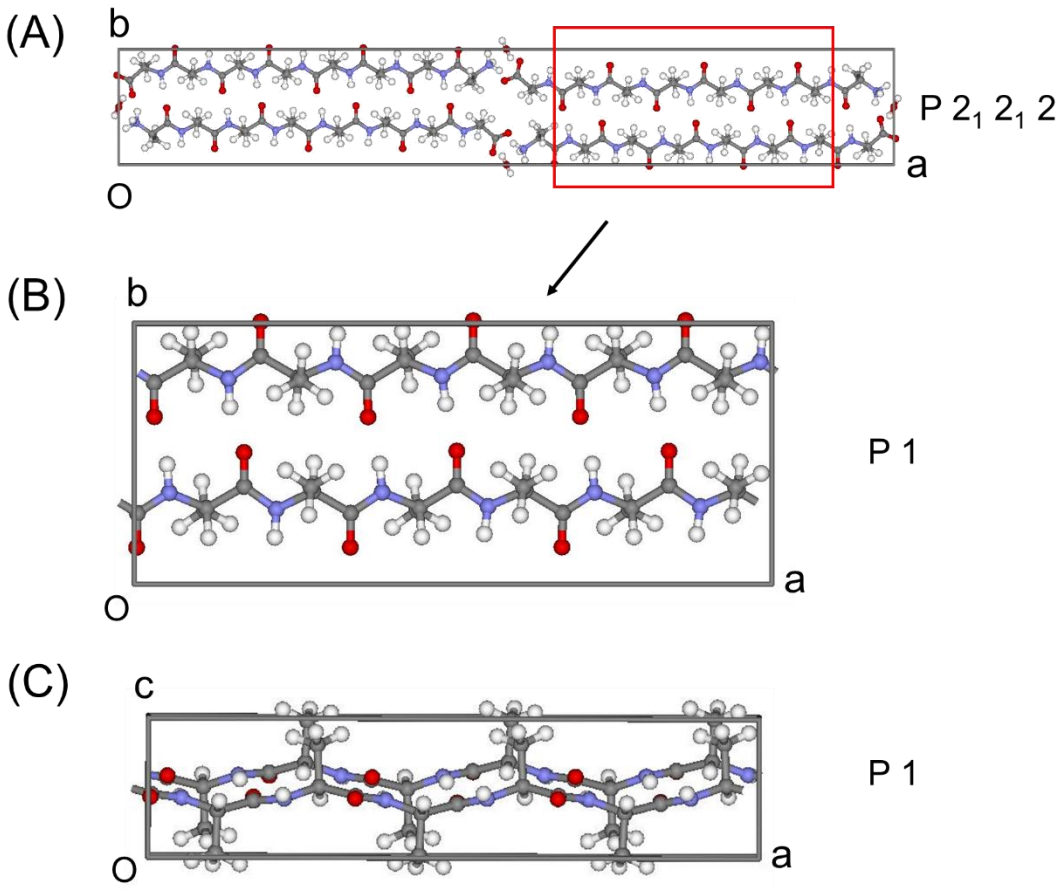


Fig. S4. (A). Packing diagram of $(Ala)_8$ molecule in the unit cell of crystal with space group $P2_12_12$. (B). Red square unit of $(Ala)_8$ crystal with space group P1. Two $(Ala)_6$ molecules without N-Ala and C-Ala residues consist of the unit cell. The cell dimension is as follows.

a: 22.50 Å (direction of molecular chain axis).

b: 9.20 Å (direction in the β-sheet perpendicular to a axis).

c: 5.12 Å (perpendicular to the β-sheet layer).

$\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$

(C). Packing diagram of $(Ala)_6$ crystal with space group P1 in view of ac plane.

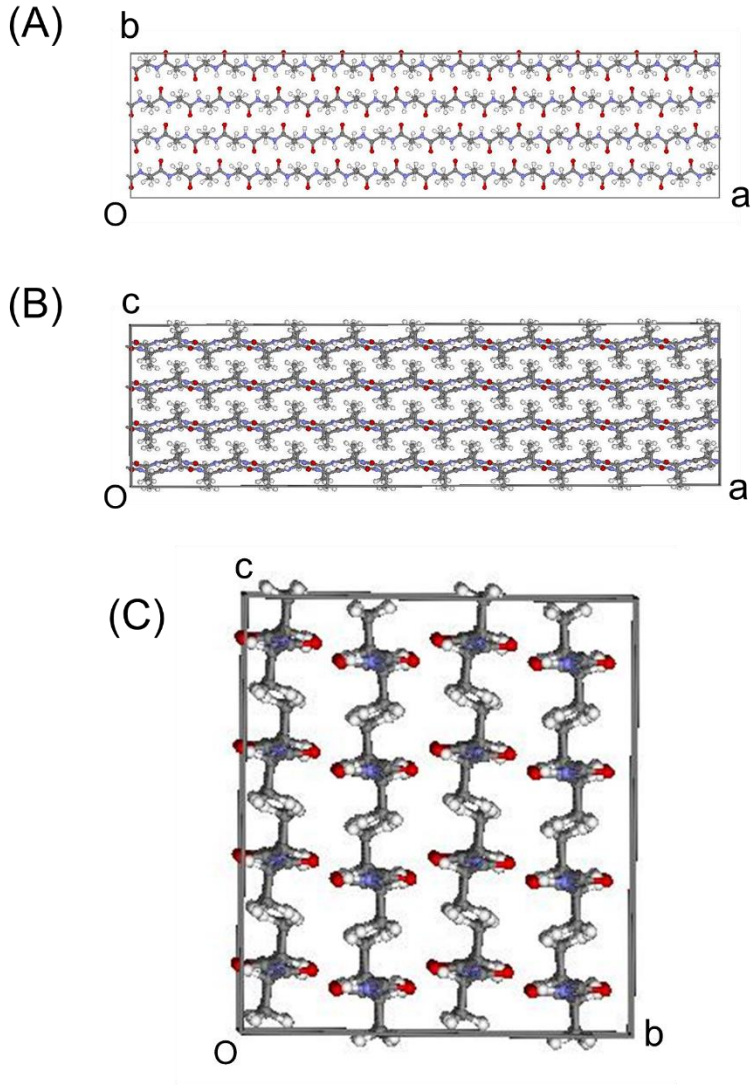


Fig. S5. (A). Packing diagram of $(Ala)_{20}$ molecule in the unit cell of crystal with space group P1. Sixteen molecules consist of one unit cell. The cell dimension is as follows.

a: 75.00 Å (direction of molecular chain axis).

b: 18.41 Å (direction in the β-sheet perpendicular to a axis).

c: 18.41 Å (perpendicular to the β-sheet layer).

$\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$

The diagram is drawn in view of the ab plane.

(B). In view of the ac plane. (C). in view of the bc plane.

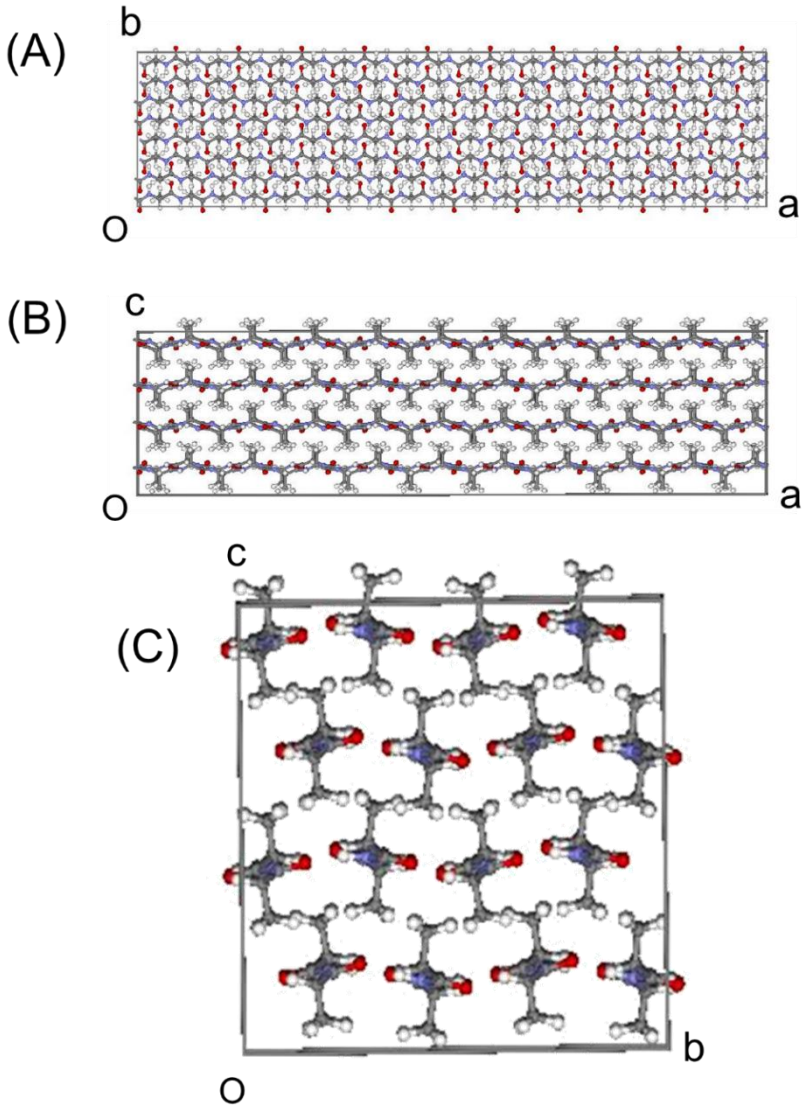


Fig. S6. (A). Packing diagram of $(Ala)_{20}$ molecule in the unit cell of crystal with space group P1. Sixteen molecules consist of one unit cell. In this crystal, one β-sheet layer which consist of four $(Ala)_{20}$ molecules is located between two β-sheet layers by shifting half inter chain distances. The cell dimension is as follows.

a: 75.00 Å (direction of molecular chain axis).

b: 18.41 Å (direction in the β-sheet perpendicular to a axis).

c: 19.50 Å (perpendicular to the β-sheet layer).

$\alpha = 90^o$, $\beta = 90^o$, $\gamma = 90^o$

The diagram is drawn in view of the ab plane.

(B). In view of the ac plane. (C). In view of the bc plane.