Supplementary Material (ESI) for Soft Matter

Supplementary Information

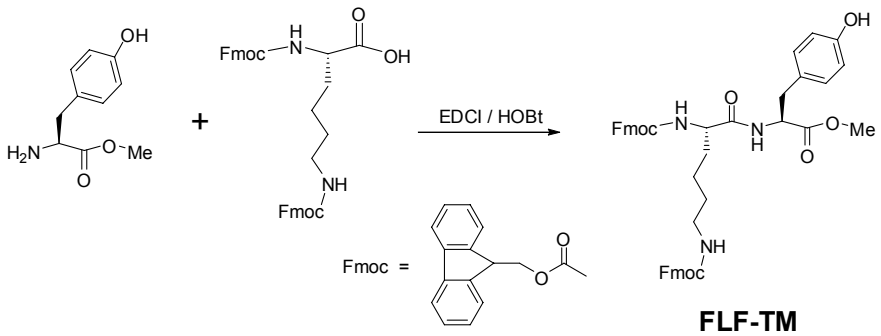
Organo- and hydrogels derived from cyclo(L-Tyr-L-Lys) and its ε-amino derivatives

Zhiguo Xie, Aiying Zhang, Lin Ye and Zeng-guo Feng*

School of Materials Science & Engineering, Beijing Institute of Technology
Beijing 100081, China

1. Experimental

1.1 Synthesis and characterization of Fmoc-Lys(Fmoc)-Tyr-OMe (1, FLF-TM)



Scheme S1 Synthetic description of **FLF-TM**

5.91 g Fmoc-Lys(Fmoc)-OH (10 mmol) was dissolved in 60 ml THF/DMF (1:1), and stirred under a nitrogen atmosphere in the ice bath for 0.5 h. Afterwards 2.30 g EDCI (12 mmol) and 1.62 g HOBt (12 mmol) were added to the solution and stirred for another 1 h. Then 2.34 g tyrosine methyl ester (Tyr-OMe) was added. The mixture was stirred for 1 h at 0 ^{o}C and another 24 h at 40 ^{o}C. The crude product was precipitated in 1000 ml 0.1 M HCl solution, filtrated and dried under vacuum. It was finally obtained as a white solid (6.52 g, yield: 85%) after flash chromatography (eluent: CH_2Cl_2/THF=20/1 v/v gradient to THF). m.p. 127~129 ^{o}C, $[\alpha]_D^{20}$ = -10.2 (C=1.0 in THF), ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C) δ: 1.24 (m, 2H), 1.38 (m, 2H), 1.50 (m, 2H), 2.82 (m, 2H), 2.95 (m, 2H), 3.55 (s, 3H), 4.00 (m, 1H), 4.18 (m, 2H), 4.27 (m, 4H), 4.38 (q, *J*=7.3 Hz, 1H), 6.63 (d, *J*=8.4 Hz, 2H), 6.97 (d, *J*=8.4 Hz, 2H), 6.63 (d, *J*=8.4 Hz, 2H), 7.28 (t, *J*=7.4 Hz, 4H), 7.29 (m, 1H), 7.37 (t, *J*=7.4 Hz, 4H), 7.42 (m, 1H), 7.55 (d, *J*=7.3 Hz, 4H), 7.55 (d, *J*=7.5 Hz, 4H), 8.21 (d, *J*=7.3 Hz, 1H), 9.22 (s, 1H). MALDI-TOF-MS: calcd for $C_{46}H_{45}N_3O_8$, 767.3, found, 790.6 ($M+Na^+$), 806.5 ($M+K^+$); Anal. calcd for $C_{46}H_{45}N_3O_8$: C 72.00, H 5.87, N 5.48; found C

* Corresponding author. E-mail: sainfeng@bit.edu.cn

71.79, H 6.00, N 5.42.

1.2 Computational Methods

The software module Forcite implemented in Materials Studio (version 4.0) was used to acquire the computational molecular conformation of **1** and **2g**. The molecular model of **1** was geometry optimized *via* molecular mechanics by universal force field according to the correlation of protons found in 2-D NOESY result (SI 2.3.2). The molecular model of **2g** was constructed on the base of model of **1**, and further geometry optimized *via* molecular mechanics by universal force field.

The software module Reflex Plus implemented in Materials Studio (version 4.0) was employed to solve the crystal structure of cyclo(L-Tyr-L-Lys) in xerogel state. The powder pattern was indexed by TREOR90 program using the first 22 reflections ($2\theta<27^{o}$), and a monoclinic cell with highest figures of merit (FOM=15) was obtained. The cell and profile parameters (function: Pseudo-Voigt) was refined by Pawley method ($2\theta<40^{o}$) to reach the R_{wp} at 6.4 %. The space groups were then determined to be A2 (No. 1, FOM=115) (Z=4) by means of a trial and error method using the Pawley method among the space-group candidates consistent with systematic absences. The other candidates of space group among the top 11 with positive FOM value were given up for the false density or it may cause chiral inversion, which are unsuitable for chiral molecular. The cell parameters after Pawley refinement were as follows: a (19.31), b (6.11), c (14.56), β (94.68). A careful comparison to the crystal cell of cyclo(L-Gly-L-Tyr) and cyclo(L-Ser-L-Tyr)[1] showed they have the very close short crystallographic repeat distance (6.17 *vs* 6.19), which are corresponded to arrangement of diketopiperazine and aromatic ring in rows. Herein, we assumed the cyclo(L-Tyr-L-Lys) has similar packing mode along the short axes, therefore we constrained the torsion of tyrosine residue to the value similar to that in cyclo(L-Gly-L-Tyr) and cyclo(L-Ser-L-Tyr) crystal so as to reduce the total degree of freedom in the subsequent process. Before the structure solution, the molecular model deduced from 2-D NOESY with constrained Tyr residue was putted into the unit cell, as well as one crystal water molecular as initial conformation model. And a number of degrees of freedom describing position and orientation of cyclo(L-Tyr-L-Lys) and water molecular as well as torsions of the Lys residue were defined. Real structure solution was then carried out by using Simulated Annealing method with preferred orientation correction *via* March–Dollase method. After several cycles of trial, a reasonable crystal structure with sufficiently low R_{wp} value was selected to perform further Rietveld refinement. For the subsequent Rietveld refinement, the following conditions were applied. 1) The pseudo-Voight function was used for simulating the peak shape. 2) Line shift was refined with instrument geometry: Bragg-Brentano. 3) The background was determined by linear interpolation using 20 terms. 4) The Berar-Baldinozzi method was used for asymmetric refinement. 5) The March–Dollase method was applied to correct the effects of preferred orientation. 6) Global isotropic factors were used to refine the temperature factor. All the parameters of unit cell were checked on to be refined. The translation and rotation of cyclo(L-Tyr-L-Lys) and water molecular as well as all the torsions inside of Tyr and Lys residue were defined as freedom as structure parameters. The refinement was repeated for several cycles until to reach a relative

[1] C. F. Lin, L. E. Webb, *J. Am. Chem. Soc.* **1973**, *95,* 6803-6811.

constant value of R_{wp}. The further Rietveld with energy refinement was carried out to refine both of the energy of structure and the R_{wp} value. The aim is to find solutions that optimally meet two different objectives. The first objective is that the simulated pattern must match the experimental diffraction data. The second objective is that the potential energy of the structure has to be close to the minimum. Herein, Energy Weight was set 50 %, and COMPASS was selected as force field, and the other parameters mentioned in the Rietveld refinement were all checked on. After several cycles of refinement, a well-solved crystal structure with three-dimensional network of hydrogen bond and no close contacts was obtained, with the final R_{wp}: 10.4 %, R_{wp} (without background): 25.82 %, and R_p: 7.68 %. (March-Dollase parameters: a*= 0.39566; b*= 0.82437; c*= -0.40480; R_0= 1.35671)

2. Results and discussions

2.1 Optical photos of gel formation during preparation of cyclo(L-Tyr-L-Lys).

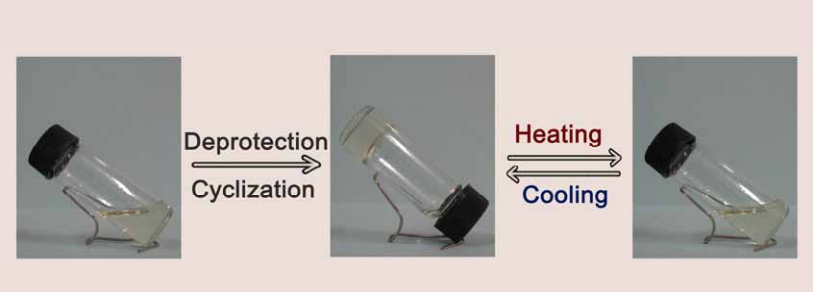


Figure S1 Solution of 25 mg **FLF-TM** in 1 ml 20 wt% piperidine/DMF (left) and organogel formed in situ upon standing after deprotection and cyclization (middle), which turn to solution when heating (right) and back to gel when cooling.

2.2 ^{1}H NMR analysis of gel systems.

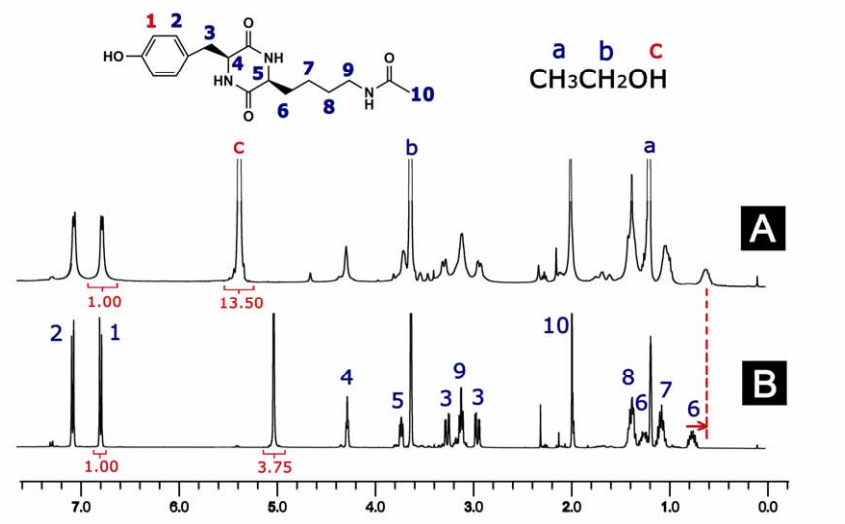


Figure S2 ^{1}H NMR spectra of **2a** in ethanol-d_6 (1.5 wt%) at 25 °C (A) and 50 °C (B).

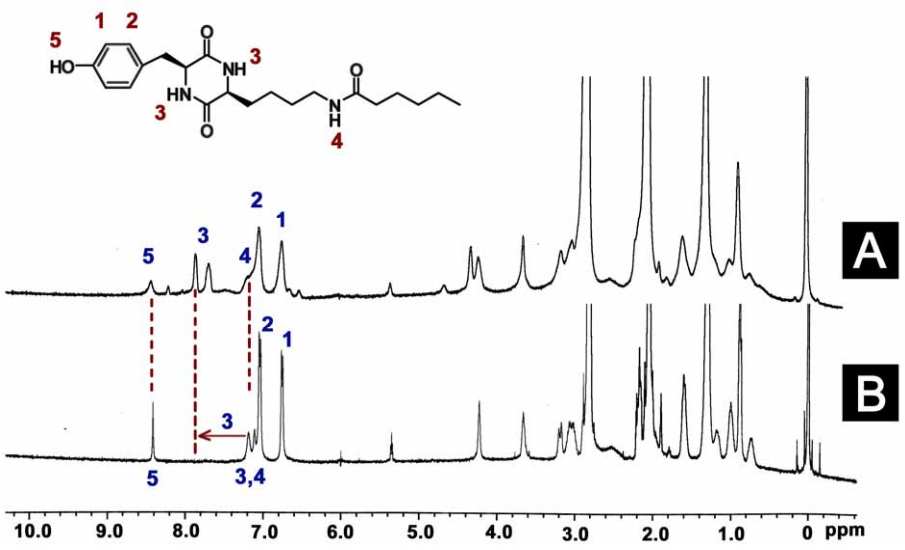


Figure S3 ^{1}H NMR spectra of **2e** in acetone-d_6 at 0.4 wt% (A, gel) and 0.1 wt% (B, solution) at 25 °C.

2.3 Crystal solution of xerogel of 1.

To solve the crystal structure of the xerogel of **1**, firstly its solution conformation was investigated by ^{1}H NMR analysis. Coupling constant of tyrosine residue protons implied the folded form between phenol and diketopiperazine ring in DMF-d_7, DMSO-d_6 and D_2O (Table S1 in SI 2.3.1). 2D-NOESY spectrum further confirmed this folded form in D_2O by observation of cross-peaks between lysyl residue and aromatic protons (Figure S2 in SI 2.3.2). Secondly, by using Reflex plus module in Materials Studio,[2] a monoclinic unit cell was created after Powder Indexing of XRD data of the xerogel by TREOR90 program and Pawley refinement. The molecular model of **1** deduced from 2D-NOESY (Figure S3 in SI 2.3.2) was then added into the unit cell as well as one crystal water (calculated from its TG results as shown in figure S5 in SI 2.3.3). Thirdly, a real structure solution was performed by using Simulated Annealing method with preferred orientation correction *via* March-Dollase method. The position, orientation and conformation (torsion of single bond) were set as degrees of freedom to reach possible crystal packing. At last, the generated best structure was refined *via* Rietveld method and further Reitveld refinement with energy by COMPASS force field, and a high quality fit was acquired between the simulated and experimental powder patterns as show in up part of figure S6 in SI 2.3.4. The final energy optimized crystal structure was obtained as shown in figure S7 in SI 2.3.4, with three-dimensional network of hydrogen bond and no close contact. Hydrogen bonds of the N-H···O type bind the diketopiperazine rings in rows parallel to the *b*-axis. The two N-H···O distances for each diketopiperazine ring are close to those found in other crystal examples of cyclic dipeptide [1] (a detailed comparison listed in Table S2). The refined March-Dollase parameters suggested a kind of needle-like crystals with preferred orientation direction paralleled the *b*-axis as depicted by the arrows. The preferred orientation results were consistent with the TEM and IR findings and other self-assembly forms of cyclic dipeptide derivatives, like tapes and layers.[3] However, this xerogel

[2] R. Tamura, M. Mizuta, S. Yabunaka, D. Fujimoto, T. Ariga, S. Okuhara, N. Ikuma, H. Takahashi, H. Tsue, Chem. Eur. J. 2006, 12, 3515.

[3] G. T. R. Palmore, M. T. McBride, Chem. Commun. 1998, 145–146.; (b) J. C. MacDonald, G. M. Whitesides, Chem. Rev. 1994, 94, 2383.

crystal structure should be taken with some caution as molecular packing may change to a certain extent during sample preparation.

2.3.1 Estimation of conformer fractions f (χ) by ^{1}H-^{1}H coupling constants.

Conformation between phenol and diketopiperazine ring was evaluated according to the method previously applied in cyclic dipeptides with two aromatic side chains.[4] Fraction of three potential conformers was calculated *via* the following equation (1):

$$\begin{aligned} f(u_1) &= (J_{\alpha-\beta l} - J_G)/(J_T - J_G) \\ f(u_2) &= (J_{\alpha-\beta h} - J_G)/(J_T - J_G) \\ f(f) &= 1 - f(u_1) - f(u_2) \end{aligned} \quad (1)$$

where the values of J_T and J_{TG} are set 13.85 and 3.55 Hz respectively.

As summarized in Table S1, the folded form of phenol and diketopiperazine ring is the dominant conformation in d_7-DMF, d_6-DMSO and D_2O solution.

Table S1. Experimental values of vicinal ^{1}H-^{1}H coupling constants and Tyr side-chain conformer fractions f (χ) estimated from the $J_{\alpha-\beta}$ values.

Solvent	Tyr residue						Lys residue
	Coupling constants[b] (Hz)			Fraction of conformers f(x)			
	$J_{\alpha-\beta l}$	$J_{\alpha-\beta h}$	$J_{\beta l-\beta h}$	u_1	u_2	f[c]	$\Delta\delta_{max}$ of δ_β[d]
d_7-DMF[a]	4.8	4.52	13.7	0.12136	0.09417	**0.78447**	0.45(sol) 0.6(gel)
D_2O	3.02	4.8	14.34	-0.0515	0.12136	**0.9301**	1.28
d_6-DMSO	2.54	4.34	13.46	-0.0981	0.0767	**1.02136**	0.98

a: Solution sample of 0.4 wt% at 60 °C.

b: the two β-protons are distinguished by appending an h for that in higher field and l for that in lower field.

c: Fraction of folded comformer.

d: Calculated maximum upfield shift of lysyl β-protons relative to that of cyclo(L-Lys-L-Lys), (δ=1.65 ppm, found in ref.[5])

2.3.2 NOESY spectrum and molecular model evaluated by Materials Studio.

[4] T. Yamazaki, K. I. Nunami, M. Goodman, *Biopolymer* 1991, **31**, 1513.

[5] M. A. Majó, J. J. Bou, C. Herranz, S. Muñoz-Guerra, *Macromol. Chem. Phys.* **2006**, *207,* 615-620.

The folded conformation in D_2O solution is further confirmed by NOESY study of intramolecular interaction. Nuclear Overhauser effect (NOE) is caused by dipole-dipole interaction or cross-relaxation and can provide unique spatial relationship between two nuclei of less than 5 Å in distance. From the NOESY spectrum as shown in Figure S4, the cross-peak (in blue frame) could be distinctly observed between lysyl residue proton 6-8 and phenoxy proton 1-2. A reasonable description is the folded conformation (as shown in Figure S5, left) makes the two residues keeping in close distance, in good agreement with ^1H-^1H coupling constants as described above.

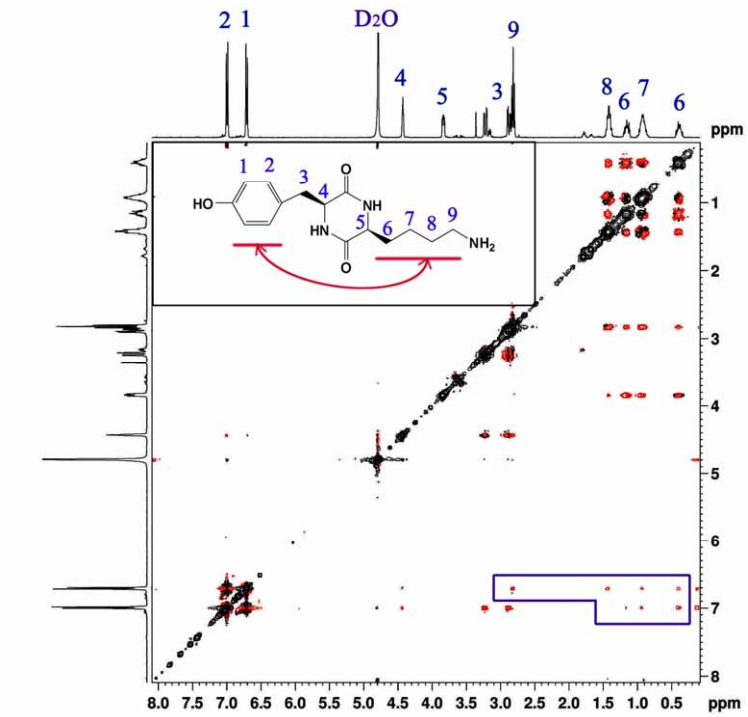


Figure S4. 2D-NOESY spectrum of cyclo(L-Tyr-L-Lys) in D_2O (inset: molecular structure and correlation of protons)

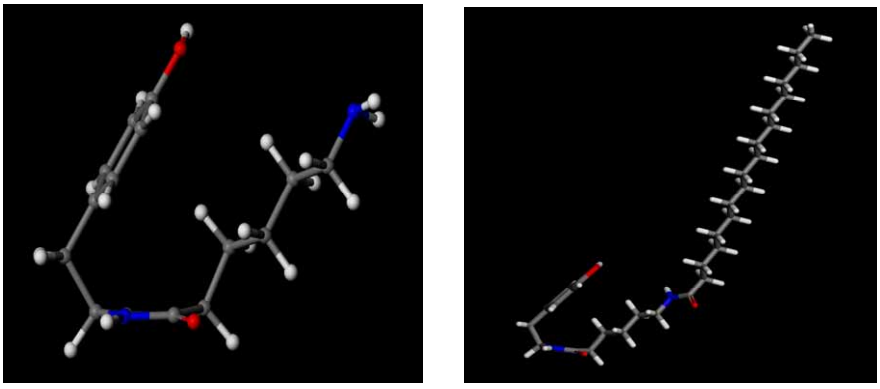


Figure S5. 3D models of **2g** (Right) and **1** (Left) illustrated by Materials Studio, in the latter the spatial distances between atoms are in accordance with the 2D-NOESY finding.

2.3.3 TG curves and the calculation of number of crystal water in the xerogel of 1.

TG curve of xerogel obtained from hydrogel of **1** is shown in Figure S6. The number of crystal water was evaluated by the following equation:

Molecular formula: $C_{15}H_{21}N_3O_3 \cdot n\ H_2O$ (M=291+18·n)

$$n = \frac{(90.34 - 85.05) \times 291 / 85.05}{18} = \frac{18.1}{18} \approx 1$$

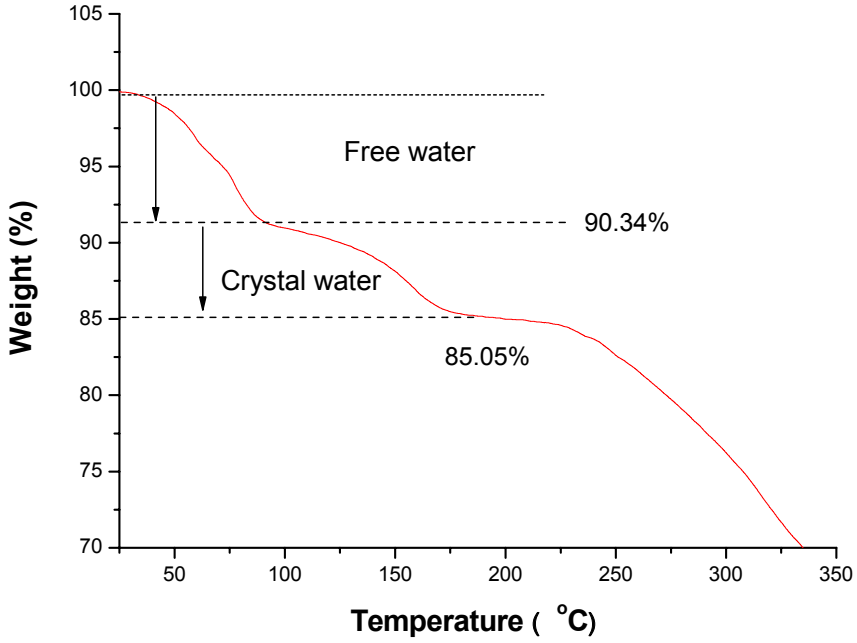


Figure S6. TG curve of the xerogel from hydrogel of **1** (1.5 wt%)

2.3.4 Illustration of crystal packing generated by Materials Studio.

Experimental and simulated XRPD patterns of xerogel prepared from the hydrogel of **1** are shown in Figure S7.

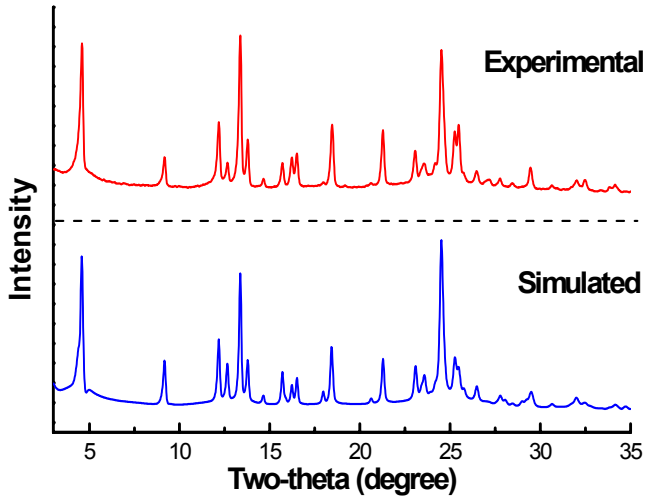


Figure S7 Experimental and simulated XRPD patterns of xerogel prepared from the hydrogel of **1** (1.5 wt% in water)

As shown in Figure S8, hydrogen bonds of the N-H···O type bind the diketopiperazine (DKP) rings in rows parallel to the *b*-axis. As shown in Table S2, the two N-H···O distances for each DKP ring are close to those found in other crystal examples of cyclic dipeptides. Each phenolic oxygen is hydrogen bonded to two nitrogen atoms of amine group directly and through a water molecule.

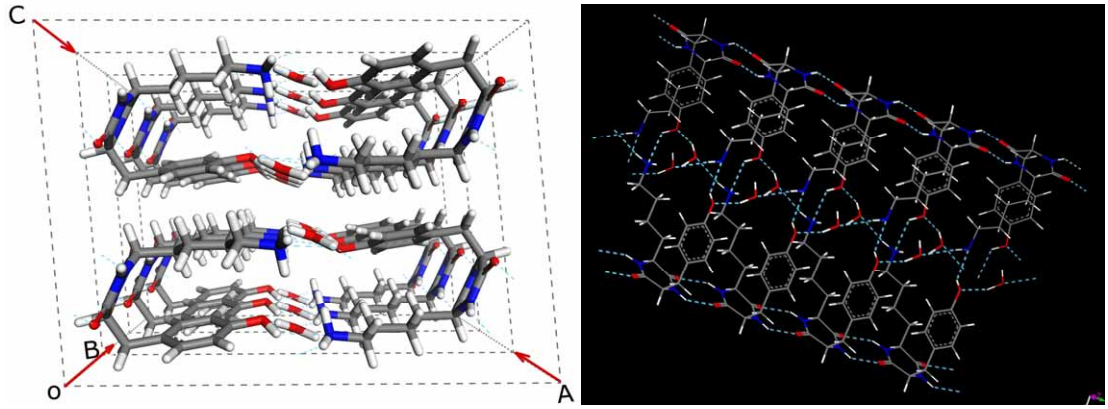


Figure S8. (Left) Perspective projection of molecular packing of and hydrogen bonding in the xerogel phase of **1**, viewed perpendicular to the *ac* plane, cell parameters: *a* (19.61), *b* (6.21), *c* (14.79), *β*(94.74), space group: A2, Z=4. (Right) Supplementary images of crystal packing of xerogel generated by Materials Studio

Table S2 Hydrogen bonds between diketopiperazine (DKP) rings

Crystal	$N_{Tyr}H\cdots O$ (Å)[a]		$N_{Lys}H\cdots O$ (Å)[b]	
	N···O	H···O	N···O	H···O
cyclo(L-Tyr-L-Gly)[c]	2.89	1.82	2.88	1.81
cyclo(L-Tyr-L-Ser)[d]	2.90	1.80	2.93	1.87
cyclo(L-Tyr-L-Lys)[e]	**2.83**	**1.88**	**2.91**	**1.90**

a: N_{Tyr} means the nitrogen atom of tyrosine side.

b: N_{Lys} means the nitrogen atom of lysine side.

c, d: values obtained from ref. 3 in SI.

e: values obtained from the model.