# Intralayer hydrogen-bond-directed self-assembly of nano-fibers from dicarboxylic valylvaline bolaamphiphiles

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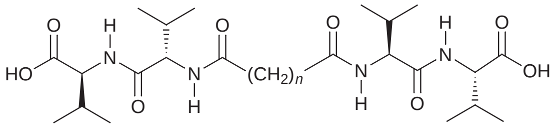
**Dicarboxylic L-valyl-L-valine bolaamphiphiles produced nanoscale fibers with widths of 10–30 nm, *via* proton-triggered self-assembly in water, which are dominated by both intralayer, lateral hydrogen-bond networks between end carboxylic acid groups and parallel β-sheet networks between amide groups.**

Carboxylic amphiphiles form a variety of self-assemblies such as micelles, vesicles, fibers, and crystals in water. The formation behavior strongly depends upon hydration states, salt concentrations, and pH conditions in aqueous dispersions.[1,2] In particular, the end carboxy groups exist as carboxylate anions (soap), acid–soaps, acids, or a mixture of these. To date it is still uncertain whether inter- or intra-layer carboxy interactions enforce the crystalline order and whether they dominate supramolecular structures or not.[3] A large number of crystal structures have been reported for fatty acids that form an acid-acid cyclic dimer,[3,4] whereas only one crystallization was achieved for potassium palmitate soap.[3,5] This indicates that no specific interlayer interactions are dominant in the soaps. We have recently demonstrated the formation of rod-like micelles, supramolecular microtubes,[6] and needle-shaped crystals[7] in water from dicarboxylic glycylglycine bolaamphiphiles. In addition to hydrophobic interactions between the alkylene spacers, the predominant forces driving these self-assemblies involve anion-anion, intralayer acid-anion, and interlayer acid-acid interactions, respectively.[8] Here we describe proton-triggered nano-fiber formation from valylvaline bolaamphiphiles **1–3**, which is dominated by intralayer acid-acid and amide hydrogen-bond networks. Furthermore, the separate contributions of the inter- and intra-layer interactions on the supramolecular structures was evaluated using FT-IR analysis.



**1** $n = 6$
**2** $n = 8$
**3** $n = 10$

The dicarboxylic bolaamphiphiles **1–3** with an L-valyl-L-valine moiety at each end were synthesized by condensation of alkane-1,*n*-dicarboxylic acid ($n$ = 6, 8 and 10) with 2 equiv. of *C*-protected L-valyl-L-valine benzyl ester, followed by deprotection of the *C*-terminal.‡

The obtained white powders of **1–3** are sparingly soluble in water ($< 0.1$ mg ml$^{-1}$ at 23 °C), but become highly soluble ($> 50$ mg ml$^{-1}$ at 23 °C) after neutralization with alkali hydroxides. When alkaline aqueous solutions of **1–3** (10 mM, 2 equiv. NaOH, pH $>$ 7) were slowly acidified by vapor diffusion of 1–5% AcOH into the solution,[7,8] the bolaamphiphiles **2** ($n$ = 8) and **3** ($n$ = 10) produced a hydrogel in 1–2 weeks. In contrast, the bolaamphiphile **1** ($n$ = 6) with a shorter oligomethylene bridge formed a crystalline solid as a precipitate. We obtained these self-assemblies at pH 3.4, 4.3 and 4.9 for **1**, **2** and **3**, respectively. From pH titration results,[8] the original carboxylate anions of **1–3** proved to be fully protonated at these pH values. Elemental analyses of the crystal from **1** and the dehydrated gels from **2** and **3** are also consistent with the protonated state.§

Energy-filtering transmission electron microscopy (EF−TEM) is a useful tool for observing low-contrast organic and biological samples without staining.[9] Furthermore, unstained TEM observations have the advantage of avoiding awkward microscopic artifacts. EF−TEM of the hydrogel revealed that the gels from **2** and **3** are comprised of a number of fibrous assemblies with micrometers lengths [Fig. 1(a)]. High-resolution EF−TEM images clearly show the presence of nanoscale fibers with widths of 10–30 nm [Fig. 1(b)]. No significant intertwisted fibers, observed for organogels made of N-protected bolaform amides,[10] can be seen. Furthermore, small angle X-ray diffraction analysis of the fibers from **2** and **3** displayed no crystalline Bragg reflections. These findings indicate that the proton-triggered self-assembly provides supramolecular fibers made of the fully protonated bolaamphiphiles **2** or **3**.

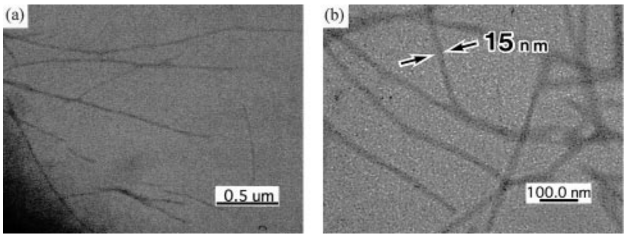


**Fig. 1** (a) Energy-filtering transmission electron micrograph of the nanofibers made of 3. (b) High-resolution image of the same specimen.

FT-IR spectra of the dried self assemblies from **1–3** were measured on a CaF$_2$ plate. Fig. 2 shows a partial FT-IR spectrum of the dehydrated fibers from **3**. The CH$_2$ antisymmetric and symmetric stretching bands of **3** appear at 2930 and 2856 cm$^{-1}$, respectively. The CH$_2$ scissoring band gives a single sharp peak at 1467 cm$^{-1}$. These band characteristics

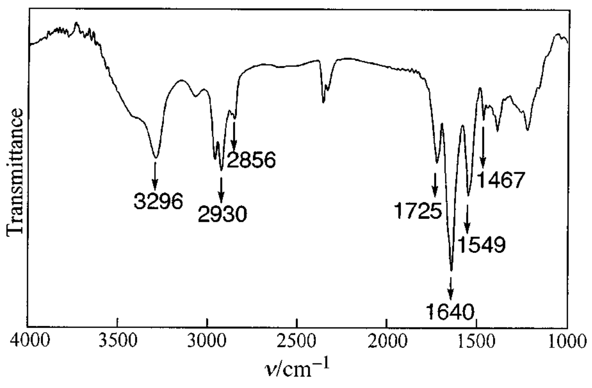


**Fig. 2** FT-IR spectrum of the dehydrated fibers from **3** in the region of 1000–4000 cm$^{-1}$

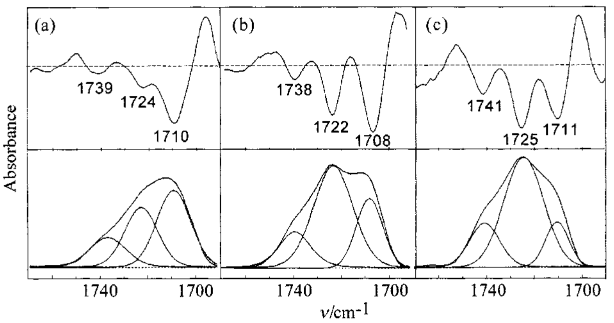


**Fig. 3** FT-IR spectra in the $CO_2H$ band region of the dried self-assemblies from (a) **1**, (b) **2** and (c) **3**. Top: second-derivative plots. Bottom: curve-fitting results.

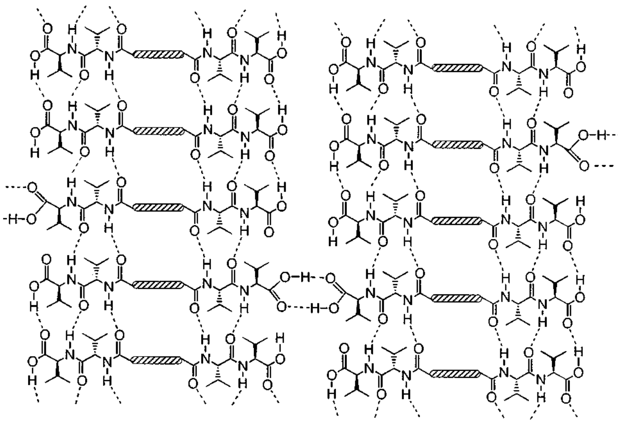


**Fig. 4** A possible model for the predominant intralayer hydrogen-bond network within the nano-fibers from **2** or **3**. Interlayer acid-acid hydrogen bonds are partly depicted.

suggest that the alkylene bridge of **3** has a high *trans* conformational population[11] and that its packing mode is triclinic.[12] On the other hand, the presence of a parallel β-sheet network between amide groups can be confirmed by the N–H stretching, with the amide I and II bands at 3296, 1640 and 1549 $cm^{-1}$, respectively.[13] This is in contrast with the three-dimensional networks of hydrogen bonds found within micro-tube membranes and crystals of oligoglycine bolaamphiphiles.[7,8] The isopropyl groups of the valine residue enforce two-dimensional hydrogen-bond networks due to steric hindrance.

A broad band due to the carboxylic acids ($CO_2H$) appears around 1725 $cm^{-1}$, whereas no carboxylate anion ($COO^-$) bands can be seen around 1600 $cm^{-1}$. Fig. 3 shows FT-IR spectra in the $CO_2H$ band region for the dried self-assemblies of **1–3** and their second derivative plots. Curve-fitting analysis also supports the validity of this deconvolution for the $CO_2H$ band. As a result, we found three independent absorption bands at 1741, 1725, and 1711 $cm^{-1}$ for **3**. The absorption bands near 1740 and 1725 $cm^{-1}$ can be assigned to the C=O stretching vibration of the free, non-hydrogen bonded and laterally hydrogen-bonded $CO_2H$ groups, respectively.[14] On the other hand, the bifurcated hydrogen bond of the $CO_2H$ groups, which is commonly observed in the solid state, displays a band around 1710 $cm^{-1}$. The area ratio of each band for **3** is calculated to be 20:65:15 for the non-hydrogen-bonded:laterally hydrogen-bonded:bifurcated $CO_2H$ groups. The nano-fibers made of **2** also display three FT-IR bands at 1738, 1722, and 1708 $cm^{-1}$ with an area ratio of 15:60:25, respectively. However, the $CO_2H$ band area ratio of **1** is 20:35:45. These results indicate that intralayer, lateral hydrogen-bond networks are of great importance in the nano-fiber formation from **2** and **3**. On the basis of the FT-IR analyses, a possible hydrogen-bond network within the nano-fibers is shown in Fig. 4. Intralayer interactions between the carboxylic peptide head groups enforce an extended molecular sheet. Similar structures were also depicted for the organogel-forming bolaform amides with no interacting end functionalities.[10] On the other hand, interlayer cyclic dimer formation by the $CO_2H$ groups determines the crystalline structure of **1**. The bolaamphiphile **1** probably requires stronger interlayer interactions to compensate for a decrease in hydrophobic interactions.

In conclusion, the FT-IR deconvolution analysis of the $CO_2H$ bands clarified that intra- and inter-layer interactions between the head groups of **1–3** control the formation of supramolecular nano-fibers and the crystalline solid, respectively.

## Notes and References

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‡ *Selected data* for **1**: yield 69 %, mp 193 °C; $[\alpha]_D$ −46.9. For **2**: yield 79%, mp 184 °C; $[\alpha]_D$ −46.1. For **3**: yield 61%, mp 134 °C; $[\alpha]_D$ −39.9; $\delta_H$(270 MHz, $[^2H_6]$DMSO) 0.83 [d, *J* 5.4, 24H, $(CH_3)_2CH \times 4$], 1.20 [m, 12H, $CH_2(CH_2)_6CH_2$], 1.45 [m, 4H, $CH_2(CH_2)_6CH_2$], 1.99 [m, *J* 5.4, 4H, $(CH_3)_2CH \times 4$], 2.08 (t, *J* 5.4, 4H, $COCH_2CH_2 \times 2$), 4.08 (t, *J* 5.4, 24H, $NHCHCO \times 2$), 4.24 (t, *J* 5.4, 2H, $NHCHCOOH \times 2$); 7.79 (d, *J* 8.1, 2H, $NHCHCO \times 2$); 7.88 (d, *J* 8.1, 2H, $NHCHCO \times 2$). These *C*-terminated bolaamphiphiles **1–3** are analogues of the *N*-protected bolaform amides reported by Hanabusa and co-workers (ref. 10).

§ Calc. for the fully protonated state of **3** ($C_{32}H_{58}O_8N_4 0.5H_2O$): C, 60.44; H, 9.35; N, 8.82. Found: C, 60.24; H, 9.27; N, 8.90%.

¶ EF−TEM of the specimens was performed at 80 keV using an analytical electron microscope (Carl Zeiss EM 902) with a Castain-Henry type electron energy filter at room temperature.

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