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COMMUNICATION

Branched peptide fibers self-assembled from gemini-like amphiphilic peptides†

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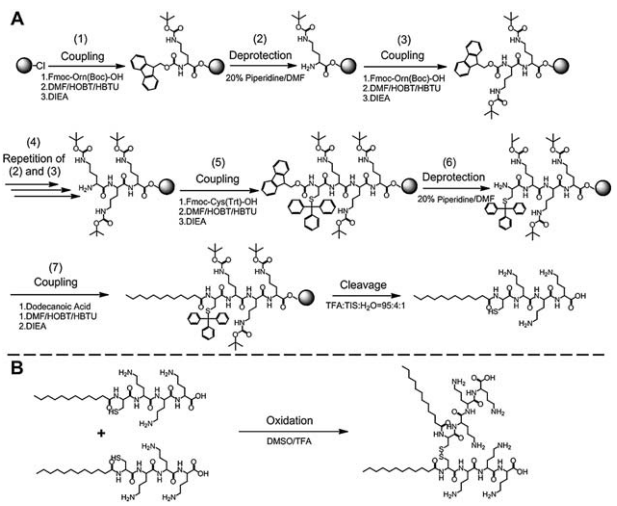
The development of gemini-like amphiphilic peptides to introduce branching into fibers is reported. The proper balance of hydrophobic interactions and hydrogen bonding leads to the formation of long branched fibers. However, due to the damage of the balance, the long fibers turn to short nanofibers with nearly no observable branches.

Branched protein fibers formed by actin, collagen, and fibrin *etc.*, can be found widely in nature and some branched fibers are identified as being related to some diseases, including Alzheimer's disease[1] and Duchenne muscular dystrophy.[2] On the other hand, natural branched fibrous structures, such as capillaries in the blood vessel system and bird feathers, play a particularly significant role in biological bodies.[3] Building branched nanostructures through supramolecular chemistry is critically important for the better understanding of biological self-assembly and it provides a new direction for controlling the fabrication of advanced nano-sized materials and devices.

Due to good biocompatibility, straight forward chemical modification, inherent molecular recognition *etc.*, peptide assemblies have attracted great research interest. It was found that linear surfactant-like hepta- and octapeptides are able to self-assemble into a network of open-ended nanotubes with junctions or branches connecting the nanotubes.[4] Co-assembly of T-shaped peptides and their corresponding linear building blocks can also introduce branches into the straight self-assembled fibers.[5]

Gemini structure is of special interest and studies have revealed the ability of gemini surfactants to form branched assemblies although these assemblies are not fibers.[6–8] In this paper, a series of gemini-like amphiphilic peptides (GAPs) with different alkyl tail lengths, *i.e.* $(C_{10}–C–O_3)_2$, $(C_{12}–C–O_3)_2$, $(C_{14}–C–O_3)_2$ and $(C_{16}–C–O_3)_2$, were designed as represented in Scheme 1. First, corresponding peptide amphiphiles were synthesized by the solid phase peptide synthesis (SPPS) technique. Then, the peptide amphiphiles were directly connected into GAPs through the formation of disulfide bonds by using the method of DMSO–TFA oxidation.[9,10] The molecular structure of the GAPs thus obtained is illustrated in Scheme S1.†

These lyophilized GAPs were dissolved in an acetic acid–sodium acetate buffer solution at pH 4 at 10 mg ml^{-1} for 12 hours to form stable assemblies. As revealed by transmission electron microscopy (TEM) analysis, long fibers that extend for many microns with branches were observed in the self-assembled $(C_{10}–C–O_3)_2$ (Fig. 1A) and the fiber bundles were visible (as labeled by arrow i in Fig. 1A). A much higher density of the long branched fibers can be formed by the self-assembly of $(C_{12}–C–O_3)_2$, in which almost all the fibers had branches and highly ordered branched fibers were also present. A representative assembly of elongated, highly ordered and multiple-level branched fibers is shown in Fig. 1B. The high magnification image in Fig. 1C provides further indication of the distinct structure of the branched fibers. The "trunks" are fiber bundles which are made up of multiple fibers aligned with one another (as labeled by arrow ii). The branches originate from the individual fibers (as labeled by arrow iii). These branched structures assembled from $(C_{10}–C–O_3)_2$ and $(C_{12}–C–O_3)_2$ show some morphological similarities to the reported branched peptide fibers formed by the co-assembly of T-shape peptides and linear peptides.[5] However, they are different to most of the reported branched thread-like micelles formed by linear or gemini surfactants, which are flexible and irregularly branched.[7,11,12]



Scheme 1 The synthesis of gemini-like amphiphilic peptides (GAPs), for example $(C_{12}–C–O_3)_2$. (A): $C_{12}–C–O_3$ was synthesized by the solid phase peptide synthesis (SPPS) technique; (B): GAP $(C_{12}–C–O_3)_2$ was synthesized through the formation of disulfide bonds between $C_{12}–C–O_3S$ in the cleavage mixture by using the method of DMSO–TFA oxidation.[9,10]

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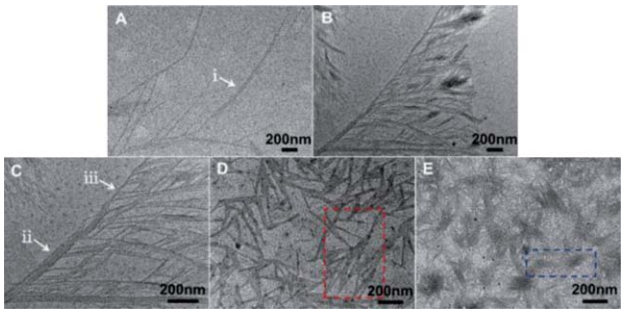


Fig. 1 TEM images of the GAPs in buffer solution at pH 4 at 10 mg ml^{-1} ((A): $(C_{10}–C–O_3)_2$, (B): $(C_{12}–C–O_3)_2$, (C): $(C_{12}–C–O_3)_2$ with high magnification, (D): $(C_{14}–C–O_3)_2$, (E): $(C_{16}–C–O_3)_2$).

Interestingly, $(C_{14}–C–O_3)_2$ and $(C_{16}–C–O_3)_2$ only form short fibers with nearly no observable branches as shown in Fig. 1C and D, respectively, and the persistence lengths of the nanofibers appear to be a few nanometres. As indicated by the broken-line panes (red in Fig. 1C and blue in Fig. 1D), many of the short fibers entangle with one another and pack into nanofiber bundles.

Circular dichroism (CD) spectra and Fourier transform infrared spectroscopy (FT-IR) were used to study the molecular configurations of the assembled GAPs. For $(C_{10}–C–O_3)_2$, as shown in Fig. 2A, a negative band near 215 nm and a positive band near 194 nm were found. These characteristic peaks are consistent with a β sheet-like conformation of the amide bond.[13,14] A negative band near 216 nm and a positive band near 195 nm were observed in Fig. 2B, also suggesting the presence of the β sheet-like conformation in the $(C_{12}–C–O_3)_2$ assemblies. As indicated in Fig. 2C a β sheet-like conformation can also be found in the assembled structures of $(C_{14}–C–O_3)_2$ with the negative band near 215 nm and positive band near 195 nm. In addition, the assemblies of $(C_{16}–C–O_3)_2$ also adopt the same β sheet-like conformation with the negative band near 214 nm and positive band near 196 nm (Fig. 2D). FT-IR analysis of the assembled structures formed by $(C_{10}–C–O_3)_2$ (Fig. 2E) showed a 1632 cm^{-1} peak at the amide I region, which is further evidence of the β sheet-like conformation. Meanwhile, the other peak around 1680 cm^{-1} at the amide I region also suggested the formation of the β sheet-like conformation *via* the antiparallel hydrogen bonding interactions of the GAPs.[13,15,16] A similar antiparallel β sheet-like conformation was also confirmed by the FT-IR spectra for $(C_{12}–C–O_3)_2$ (Fig. 2F), $(C_{14}–C–O_3)_2$ (Fig. 2G) and $(C_{16}–C–O_3)_2$ (Fig. 2H).

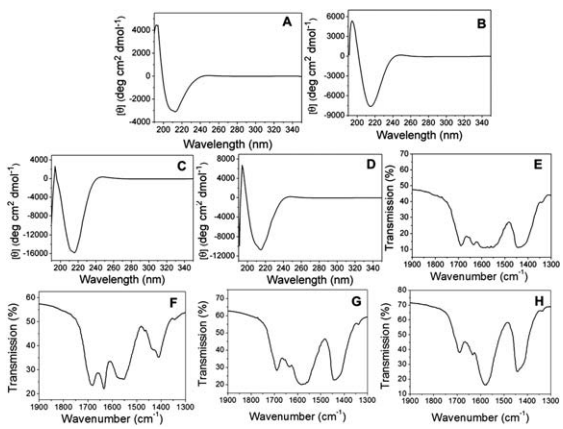


Fig. 2 Analysis of the molecular configurations of the self-assembled GAPs at 10 mg ml^{-1}: (A–D) CD spectra for $(C_{10}–C–O_3)_2$, $(C_{12}–C–O_3)_2$, $(C_{14}–C–O_3)_2$ and $(C_{16}–C–O_3)_2$, respectively; (E–H) FT-IR spectra for $(C_{10}–C–O_3)_2$, $(C_{12}–C–O_3)_2$, $(C_{14}–C–O_3)_2$ and $(C_{16}–C–O_3)_2$, respectively.

Based on the above results, the self-assembled GAPs were formed through the hydrophobic interactions of the alkyl tails and hydrogen bonding of the peptide moieties. Just like with cylindrical micelles,[16] the two alkyl tails of the GAP building blocks pack on the inside of the fibers and the peptide segments are displayed on the surface of the fibers with a β sheet-like secondary structure. The formation of the branched assemblies is attributed to the gemini molecular structure. According to the literature,[6,7] gemini surfactants potentially provide an easy way to form branches because of the presence of the two tails. Branched fibers self-assembled by amphiphilic pseudopeptides with gemini structure were observed for the first time. However, branching may well be inherent in the system, but with shorter fibers formed by GAPs with longer alkyl tails it is likely that detecting the branching is not possible. Here, our experiments indicate that the length of the alkyl tails makes a difference to the self-assembly of the GAPs: the shorter aliphatic chains (C10 and C12) assembled into long fibers while the longer aliphatic chains (C14 and C16) assembled into short fibers. Excepting the influence of slight molecular geometry changes[17] due to the characteristics of the gemini molecular structure, changes in the hydrophobic interactions are more sensitive to the increase of the aliphatic chain length.[18] In general, it was considered that the balance of intermolecular weak non-covalent interactions may play an important role in the precise control of supramolecular architectures in the self-assembly process.[19] Therefore, we proposed that these assemblies formed by GAPs are achieved through synergistic effects of hydrophobic interactions and hydrogen bonding, and the increment of the hydrophobic interactions from C10 to C12, C14 and C16 leads to the damage of the balance of these weak non-covalent interactions, which may impact on their self-assembly behavior.

To further verify this, the thermal behavior of the assembled GAPs was characterized using differential scanning calorimetry (DSC). The DSC traces were obtained using lyophilized assembled GAPs, which allowed the DSC measurements to be performed without interference from solvent evaporation. As shown in Fig. 3A–D, all the assembled GAPs exhibited a fairly broad endothermic trace and the trace peak area is proportional to the enthalpy change (ΔH). It was found that the enthalpy change of the assembled GAPs increased drastically

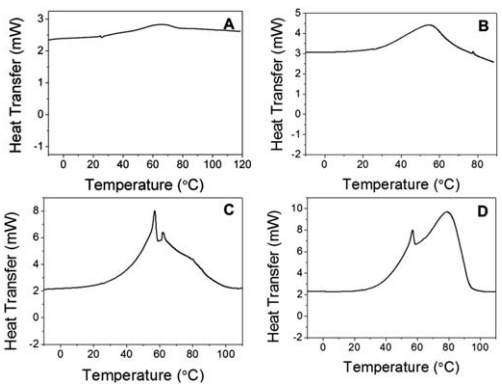
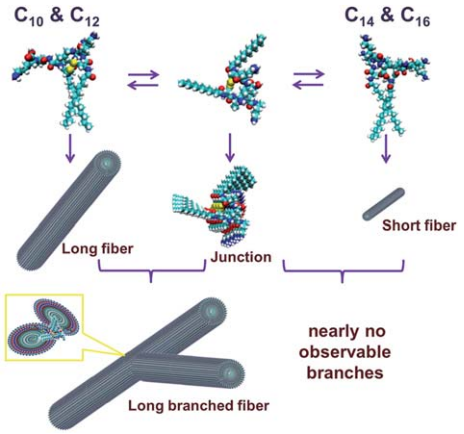


Fig. 3 DSC analysis of the self-assembled GAPs at 10 mg ml^{-1}. (A): Formed by $(C_{10}–C–O_3)_2$; (B): formed by $(C_{12}–C–O_3)_2$; (C): formed by $(C_{14}–C–O_3)_2$; (D): formed by $(C_{12}–C–O_3)_2$.



Scheme 2 A schematic illustration of the self-assembly of GAPs with different alkyl tails into long branched fibers or short fibers.

from 12.3 kJ mol^{-1} ((C_{10}–C–O_3)$_2$) to 67.7 kJ mol^{-1} ((C_{12}–C–O_3)$_2$), 297.0 kJ mol^{-1} ((C_{14}–C–O_3)$_2$) and 390.3 kJ mol^{-1} ((C_{16}–C–O_3)$_2$), respectively. In general, the consuming energy of the endothermic process is used for the destruction of the intermolecular forces of the assembled supramolecular structures. For these GAP analogues with the same peptide segments, the increasing intermolecular force is primarily contributed to by the increment of the hydrophobic interactions from C10 to C16. This analysis suggests that the hydrophobic interactions of the assembled structures increase distinctly with the increase of aliphatic chain lengths.

On the basis of above analysis, branched nanofibers are achieved through the synergistic effects of the hydrophobic interactions of the alkyl tails and the hydrogen bonding of the peptide moieties of the unique gemini molecular structure. For (C_{12}–C–O_3)$_2$, the synergistic effect of the hydrophobic interactions and hydrogen bonding reached the proper balance. This leads to the formation of long fibers and the two tails produce the branch-like conventional gemini surfactants.[6,7] With regards to (C_{10}–C–O_3)$_2$, the relatively weak hydrophobic interactions impacted the balance of the intermolecular interactions, so that its assembly into long branched fibers was not as effective as (C_{12}–C–O_3)$_2$. However, for (C_{14}–C–O_3)$_2$ and (C_{16}–C–O_3)$_2$, the drastically increased hydrophobic interactions resulted in the damage of the balance of the hydrophobic interactions and hydrogen bonding. This change lead to the transformation of the long fibers into short fibers. Moreover, these fibers may be too short to be detected. The self-assembly of GAPs into branched fibers is illustrated in Scheme 2.

In conclusion, we demonstrated the development of GAPs to introduce branching into fibers. Based on the proper synergistic effects of hydrophobic interactions and hydrogen bonding, (C_{10}–C–O_3)$_2$ and (C_{12}–C–O_3)$_2$ can self-assemble into long branched fibers. However, (C_{14}–C–O_3)$_2$ and (C_{16}–C–O_3)$_2$ resulted in short nanofibers with nearly no observable branches, due to the damage of the balance. Such biomimetic branched peptide fibers have significant implications in the understanding of biological self-assembly. Furthermore, branched fibers allow potential applications such as nanoscale templates for microfluidics,[20] artificial blood vessel generation,[21] lung tissue engineering,[22] and fiber-reinforced composite technology.[23]

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