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# Hierarchical Self-Assembly of Bolaamphiphiles with a Hybrid Spacer and L-Glutamic Acid Headgroup: pH- and Surface-Triggered Hydrogels, Vesicles, Nanofibers, and Nanotubes

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Bolaamphiphiles with L-glutamic acid headgroups and hybrid linkers, each composed of two rigid benzene rings and different polymethylene units, were designed, and morphological controls of the hierarchical self-assemblies were realized via changing solution pH and application to solid surfaces. At a low pH of 3, bolaamphiphiles formed hydrogels with water and molecules with short and long spacers formed nanofibers and helical nanoribbon–nanotubes, respectively. In a pH 12 aqueous solution, vesicles were observed from cryo-TEM measurements for amphiphiles with short spacers that could transfer to huge vesicles when cast onto a mica surface. Amphiphiles with longer spacers self-assembled into nanoparticles in a pH 12 aqueous solution while micellar fibers were formed on a mica surface. Those assemblies were characterized with UV–vis, CD, and FT-IR spectroscopy and AFM and TEM observations. With molecular structure modification and the fine tuning of conditions, morphology transitions between various nanostructures were obtained from the self-assembled bolaamphiphiles. The environmental pH can induce different interaction modes between the headgroups, and at high pH, there are strong interactions between molecular assemblies and the mica surface. It is suggested that the active headgroups, rigid necks, and flexible linkers with different lengths render molecules with diverse aggregation morphologies.

### Introduction

The self-assembly of amphiphiles into diverse nano/micro nanostructures as well as their morphological changes is one of the central issues in colloid and interface science.<sup>1</sup> The interactions between the hydrophilic headgroups and hydrophobic alkyl chains played important roles. Therefore, subtle molecular design will affect the self-assembly of the amphiphiles. Different from the conventional amphiphilic molecules simultaneously containing one headgroup and one or more alkyl chains, bolaamphiphiles, in which the two hydrophilic ends are covalently connected by a spacer, have attracted much attention recently.<sup>2–4</sup> Because of the covalent connection of the two headgroups, this kind of molecule could behave cooperatively in the self-assembly process, and various morphologies such as monolayers, micelles, vesicle, nanofibers, nanoribbons, and nanotubes could be formed.<sup>2,5–8</sup>

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Except for modifying the molecular structure,<sup>9–11</sup> a morphological change can also be realized via changing temperature,<sup>12</sup> pH value,<sup>13,14</sup> photoirradiation,<sup>8</sup> or doping with other components.<sup>14–17</sup> For example, Shimizu and co-workers synthesized bolaamphiphiles with a 1-glucosamide headgroup and alkyl spacers and found that the self-assembled supramolecular structures of the molecules in water strongly depended on the even or odd number of methylene units.<sup>9</sup> They also reported that bolaamphiphiles with thymidylic acid headgroups could self-assemble into nanofibers and the helicity of the nanofibers could convert reversibly upon UV light irradiation.<sup>8</sup> Huang et al. have reported the transition of different aggregates in a mixed cationic bolaamphiphile/SDS system, which depends on the molar ratio and concentrations of two components.<sup>18</sup>

To control the assembled morphologies of the bolaamphiphiles, good molecular building blocks are usually related to appropriate functional groups, which can regulate the interaction between the spacers and the headgroups. Previously, we designed the bolaamphiphiles with L-glutamic acid as the terminal groups and constructed the helical nanotubes through gel formation.<sup>19</sup> We have also introduced a hybrid spacer into bolaamphiphiles to regulate the corresponding gelating properties in organic solvents.<sup>20</sup> In the

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hybrid spacer, the rigid aromatic rings were placed close to both of the hydrophilic headgroups with a flexible hydrocarbon chain between them and the terminal groups were blocked by using ester derivatives. It has been found that with the introduction of the hybrid spacer the bolaamphiphiles showed interesting self-assembly properties in organic solvents.<sup>10,14,21,22</sup>

In this article, we changed the terminal groups of the bolaamphiphiles into active carboxylic acids while keeping the hybrid spacer. The –COOH group can be either protonated or deprotonated at certain pH values, which will largely change the hydrogen bonding and ionic interactions.<sup>23</sup> With such a simple molecular design, we have easily regulated the interactions between the bolaamphiphiles and realized diverse morphologies from vesicles to nanofibers and nanotubes in water. Moreover, these morphological changes are controlled or modulated by the integrated effect of several influencing factors. These influencing factors include the length of alkyl linkers, the pH value, and the solid substrate used for the measurements. In the case of bolaamphiphiles, management of multiple influencing factors rather than one to modulate the self-assembled nanostructures is very important but is still rarely studied.

However, because the solid/liquid interface is different from the bulk solution, solid surfaces can also be used as an important means to control supramolecular assemblies.<sup>24–26</sup> From different kinds of solid substrates, mica is relatively special because freshly cleaved mica surfaces covered with aqueous solution are negatively charged.<sup>27</sup> At higher pH values, because the headgroups of the bolaamphiphiles are also charged, they interacted strongly with solid surfaces, especially with mica. Therefore, with these bolaamphiphiles, higher-order nano/micro structures were observed through further hierarchical self-assembly on solid surfaces. The present work shows that by using cooperative interactions between the headgroups and the surface and by using rigid necks as well as different lengths of flexible linkers, various morphologies can be obtained and their changes can be regulated in a controlled manner.

#### **Experimental Section**

**Synthesis.** Solid reagents were used as obtained from commercial suppliers without further purification, and solvents were freshly distilled before use. Milli-Q water (18.2 M $\Omega$  cm) was used in all cases. Bolaamphiphiles with esterified L-glutamic acid head-groups (**BEC4** and **BEC12**) were prepared according to the methods described previously,<sup>20</sup> and the target molecules (**BECA4** and **BECA12**) were obtained from the hydrolysis of corresponding esterified derivatives.

1,12-Dodecyloxy-bis(4-benzoyl-L-glutamic acid) (BECA12). 1,12-Dodecyloxy-bis(4-diethyl benzoyl-L-glutamate) (BEC12, 500 mg) was suspended in 30 mL of ethanol, and 15 mL of an aqueous solution of sodium hydroxide (2 M) was slowly added to the mixture at 0 °C, which was again stirred overnight at room temperature. The reaction mixture was acidified to pH 3 with dilute HCl, and the precipitate was filtered off. BECA12 (400 mg)

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was obtained as a white solid (93% yield). HRMS (FAB): m/z 699.3134 (m/z calcd for  $[C_{36}H_{48}N_2O_{12}-H]^+$ , 699.3129). <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  1.23 (m, 12H), 1.37 (t, 4H), 1.69 (t, 4H), 1.92 (m, 2H), 2.04 (m, 2H), 2.32 (t, 4H), 3.99 (t, 4H), 4.35 (m, 2H), 6.96 (d, 4H), 7.82 (d, 4H), 8.4 (d, 2H), 12.33 (s, 4H). Elemental analysis calcd for  $C_{36}H_{48}N_2O_{12}$ : C 61.70%, H 6.90%, N 4.00%. Found: C 61.79%, H 7.08%, N 3.87%.

Compounds **BECA4** and **BECA12** were prepared in similar manners.

1,4-Butyloxy-bis(4-benzoyl-L-glutamic acid) (BECA4). Yield 60%. HRMS (FAB): m/z 587.1882 (m/z calcd for [C<sub>28</sub>H<sub>32</sub>N<sub>2</sub>O<sub>12</sub>-H]<sup>+</sup>, 587.1877). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  1.88–1.96 (m, 6H), 2.05 (m, 2H), 2.32 (t, 4H), 4.09 (t, 4H), 4.36 (m, 2H), 6.98 (d, 4H), 7.83 (d, 4H), 8.41 (d, 2H), 12.34 (s, 4H). Elemental analysis calcd for C<sub>28</sub>H<sub>32</sub>N<sub>2</sub>O<sub>12</sub>: C 57.14%, H 5.48%, N 4.76%. Found: C 56.84%, H 5.46%, N 4.80%.

**Instruments for Measurement.** High-resolution mass spectra (HRMS) were recorded on a Bruker Daltonics Apex-II spectrometer. Elemental analysis was carried out with a Flash EA Carlo-Erba-1106 Thermo-Quest. Jasco UV-550 and J-810 CD spectrometers were used for the UV-vis and CD spectral measurements, respectively. FTIR spectra were obtained with a Jasco FT/IR-660 plus spectrophotometer with a wavenumber resolution of 4 cm<sup>-1</sup>. Atomic Force Microscopy (AFM) was performed in tapping mode (Nanoscope IIIa, Digital Instruments, Inc.) with silicon cantilever probes. The scanning rate was usually 1 Hz. Cryo-TEM was performed on a JEOL JEM-2200FS TEM operating at 200 kV and equipped with a Gatan cryoholder. For standard TEM measurements, a JEOL 1011 system operating at 200 kV was used.

**Procedures.** The bolas (**BECA4** and **BECA12**,  $1 \times 10^{-3}$  mmol) were dispersed in 10 mL of 0.02 M NaOH, and the corresponding mixture was treated with ultrasound and stirred for 1 h at 80 °C. The resulting solution was titrated very slowly with 1 M HCl under stirring to obtain an aqueous solution with a certain pH; the sample was again heated to 80 °C for 1 h and slowly cooled to room temperature. The hydrophilic silicon wafers were prepared as follows: (1) The wafers were kept in acetone under ultrasound for 15 min and rinsed with distilled water. (2) The wafers were then cleaned by immersing them in piranha solution (a mixture of 7:3 (v/v) 98% $H_2SO_4$  /30%  $H_2O_2$ ) at 90 °C for 30 min and washing them with distilled water. (3) The obtained clean wafers were again immersed in a mixture of 1:1:5 (v/v/v) 25% ammonia/30% H<sub>2</sub>O<sub>2</sub>/distilled water at 70 °C for 20 min and washed with distilled water. (4) Finally, the silicon wafers were immersed in 1:1:5 (v/v/v) 37% hydrochloric acid/30% H<sub>2</sub>O<sub>2</sub>/distilled water at room temperature for 30 min, washed thoroughly with distilled water, and dried in a stream of clean nitrogen. For the AFM measurement, freshly cleaved mica or hydrophilic silicon wafers were first deposited with 20  $\mu$ L of the corresponding samples with certain pH values. This piece of substrate was kept at room temperature for 30 min and then stored under vacuum for 3 days to remove additional solvent. For the cryo-TEM measurements, a small droplet of solution was placed on a holey carbon film, which was further supported on a TEM copper grid. The sample-loaded grid was then plunged into liquid nitrogen, transported under liquid nitrogen, and mounted onto the cryoholder of the TEM. The temperature of the sample was maintained at -178 °C. During the entire measurement, the sample was under vacuum. For the standard TEM measurements, a drop of samples was cast on a copper grid, which was again dried under vacuum. The samples for standard TEM measurements at room temperature were stained with 2% uranyl acetate solution. In the case of FT-IR measurement, the bola samples with different pH value were cast onto a freshly cleaned silicon wafer and dried under vacuum. In the process of obtaining the UV and CD spectra, a quartz cuvette with a 10 mm width was used.

#### Results

**Self-Assembly in Aqueous Solutions.** The two designed bolaamphiphiles are shown in Scheme 1. These bolaamphiphiles

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have L-glutamic acid as the terminal groups and hybrid spacers. We designed the hybrid spacer containing the aromatic benzene ring as the rigid part and the hydrocarbon as the flexible part. Because chain lengths of 4 and 12 methylene units showed obviously different assembly properties, we selected compounds with an alkyl chain length of 2. The two bolaamphiphiles, abbreviated as BECA4 and BECA12, were easily obtained from the hydrolysis of corresponding ester derivatives<sup>20</sup> in an ethanol/water mixture with sodium hydroxide and subsequent acidification with dilute HCl. Both compounds can form hydrogels<sup>28</sup> with water at lower pH values. For BECA4, a hydrogel was obtained within the pH range of 5.5 to 3 and a precipitate appeared at pH values lower than 2. At pH 3 and room temperature, the critical gelation concentration<sup>29</sup> (CGC) (wt %) is as low as 0.5 wt %. Moreover, the obtained hydrogels from BECA4 at pH 3 are stable, the sol-gel transition temperature<sup>29</sup> ( $T_{gel}$ ) of the 2 wt % hydrogel is about 81 °C, and the corresponding hydrogel can be kept at room temperature for more than 3 weeks. Hydrogels of BECA12, which are also obtained at pH 3, can remain for about half an hour at room temperature and then start to shrink via precipitation.

We studied the aggregation behavior of the bolaamphiphiles in aqueous solution at a concentration about  $10^{-4}$  M, which is far below the critical gelation concentration, and the molecules do not precipitate at pH 3. At higher pH values, disperse solutions are formed. The p $K_a$  values for the two carboxylic acid groups in glutamic acid are p $K_1 = 2.13$  and p $K_2 = 4.32$ . At low pH, strong hydrogen bonding between the carboxylic acid groups seems to be the triggering force for the assembly, and at higher pH, the ionic interaction between headgroups is suggested to be important. In this context, we studied the self-assembly of bolas in aqueous solution as well as on solid surfaces, mainly at pH 3 and 12.

UV-Vis and CD Spectra. The aggregation behavior of bolas in aqueous solution at different pH values was studied with UV– vis spectroscopy, as shown in Figure 1. At pH 12, the  $\lambda_{max}$  values of both **BECA12** and **BECA4** appear at 255 nm, which can be ascribable to the phenyl group. However, when the solution was titrated to a lower pH value, a red shift was observed in the case of **BECA4** (Figure 1a) and a strong blue shift was detected for **BECA12** aggregation (Figure 1b). At pH 3, the  $\lambda_{max}$  from **BECA4** shows about a 2 nm red shifts compared to that of its pH 12 solution. In contrast, when the pH value decreased from 12 to 7 and further to 3, 2 nm and 7 nm blue shifts could be detected, respectively, from the UV–vis spectra of **BECA12** assemblies. These results indicated that **BECA4** may form weak J aggregates whereas **BECA12** may form relatively strong H aggregates when the systems were converted to lower pH values.



Figure 1. UV-vis and CD spectra of a bolas aqueous solution  $(1 \times 10^{-4} \text{ M})$  at pH 12 (-), pH 7 (-·-), and pH 3 (···): (a) UV-vis spectra of **BECA4**; (b) UV-vis spectra of **BECA12**; (c) CD spectra of **BECA4**; and (d) CD spectra of **BECA12**.

Circular dichroism (CD) spectroscopy is a powerful technique for studying molecular self-assembly with chirality. Figure 1c,d show the CD spectra of the assemblies under various conditions. Although the CD signals at pH 12 are relatively weak, it still suggests molecular aggregation at a very high pH value. However, for BECA12 in a pH 3 aqueous solution, a strong negative band at 262 nm with the crossover blue shifted to 241 nm was detected (Figure 1d). The strong CD signal indicated a highly ordered aggregation within the molecular assemblies.<sup>30</sup> Compared with BECA12, BECA4 did not give a good CD signal with a regular exciton-type Cotton effect (CE), even though it formed a stable hydrogel at pH 3 with a critical gelation concentration (CGC) (wt %) of as low as 0.5 wt %. The poor CD signals suggest a weak interaction between the phenyl groups in the case of BECA4 assemblies. More interestingly, although the bola molecules with carboxylic acid headgroups exhibit stronger H-bonding interactions with each other at lower pH values, the intensity of the BECA4 CD signal at pH 7 is still even weaker than at pH 12 (Figure 1c), indicating that BECA4 self-organizes into a very ordered structure at pH 12. Moreover, for the CD spectra of bolas at different pH values, only the BECA12 in pH 3 aqueous solution shows a negative band whereas all of the other cases give a positive

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**Figure 2.** Cryo-TEM images of **BECA 4** (a, b) and **BECA 12** (c, d) self-assembled in a pH 3 aqueous solution.

Cotton effect. These results also suggest the formation of higherorder hierarchical nanostructures of **BECA 12** in solution.

*Cryo-TEM Measurements in a pH 3 Aqueous Solution.* The original morphologies of these molecular assemblies in aqueous solution were studied via cryo-TEM measurement. For BECA4 in pH 3 water, two different kind of nanofibers were observed in the same sample, including mass amounts of very thin fibers (Figure 2a) and small amounts of broad fibers (Figure 2b). The thin fibers have lengths greater than micrometers, and the widths of the thin fibers are quite uniform at about 2 to 3 nm, which is on the singlemolecule scale. In contrast, the widths of the broad fibers are different from each other; fibers with widths ranging from 10 nm to more than 50 nm can be found. Moreover, some striations can also be detected in the broad fibers, indicating that these broad fibers may come from the assembly of thin fibers. Except for cryo-TEM measurements, standard TEM measurements were also performed on dry samples at room temperature. For BECA4 assemblies at pH 3, only interlaced broad fibers were found in the standard TEM measurements (Figure S1a) and the striations on the fibers are very clear, further demonstrating the hierarchical assembly of BECA4 molecules from thin fibers to broad fibers.

Different from the case of **BECA4**, the cryo-TEM images from the assembly of long bolaamphiphiles (**BECA12**) at pH 3 show flexible nanoribbons (Figure 2c) with a width of several tens of nanometers to two hundred nanometers. Some of the flexible nanoribbons can also be distorted and turned into helical forms (Figure 2d). However, the helical morphologies can hardly be recognized from standard TEM measurements, in which the dry samples were stained with 2% uranyl acetate and the nanoribbons from **BECA12** assemblies at pH 3 form only tangled aggregates upon drying (Figure S1b).

*Cryo-TEM Measurements in a pH 12 Aqueous Solution.* Figure 3 shows the cryo-TEM images of **BECA 4** and **BECA 12** self-organization in a pH 12 aqueous solution. Compared with the results at lower pH values, the cryo-TEM images of both of the bolas reveal completely different assembly morphologies. For **BECA4** in pH 12 water, vesicles with a diameter of about 40–60 nm were identified. The thickness of the vesicle walls can be established as



Figure 3. Cryo-TEM images of BECA 4 (a) and BECA 12 (b) selfassembly in a pH 12 aqueous solution.

about 3-3.2 nm, which is in the range of molecular monolayer. Besides the vesicles, flexible lamellae can also be detected from the cryo-TEM study (Figure 3a), and some of the lamellae may come from incomplete vesicles. Smaller vesicles can also be detected from normal TEM measurements (Figure S1c). Interestingly, some larger vesicles with a diameter of about 100 nm formed upon drying. The larger vesicles look like multilamellar vesicles, which may come from the aggregation of smaller vesicles (Figure S1d). **BECA 12** with a C12 alkyl chain spacer forms nanoparticles with a diameter of about 100–20 nm in a pH 12 aqueous solution (Figure 2b), and from their appearance, all of the nanoparticles are solid. However, neither nanoparticles nor any other regular morphology can be observed from normal TEM measurements on dry samples on a copper grid.

**Self-Assembly on a Solid Surface.** Because the solid surface may endow the supramolecular assemblies with many novel structures, we transferred the samples from aqueous solutions to a mica surface and studied them with AFM. The mica sheets that we used for the investigation are newly cleaved Muscovite mica, which is the standard substrate for AFM measurements.

AFM Measurements of pH 3 Assemblies. Figure S2 shows AFM images of BECA4 and BECA12 assemblies cast on a mica surface at pH 3. Very long, twisted helical nanofibers were identified from the aggregation of **BECA4** at pH 3 on the mica surface; the fibers could be more than  $10 \mu m \log$  with an average height of about 40 nm (Figure S2a). The magnified image clearly shows that many small fibers twist with each other in a helical manner and that the huge nanofibers come from smaller analogues (Figure S2b). The hierarchical assembly from thin fibers to huge fibers is identical to the results from cryo-TEM measurements, but the twisting in a helical manner is observed only on a solid surface. However, a thorough drying process is also important to obtaining the helical manner. For the AFM measurement, the samples on the solid substrates were kept at room temperature for 30 min and then stored under vacuum for 3 days to remove additional solvent. Without 3 days of drying, the huge nanofibers coming from small twisted fibers can hardly be recognized (figure S3).

At pH 3, helical nanotubes can be detected from the **BECA12** assembly on the mica surface (Figure S2c,d) with a height of 7.5 nm and an apparent helical pitch of 50 nm. Compared to the results from the cryo-TEM study, the helical nanotubes on the mica surface may come from the assembly of nanoribbons upon drying. Some straight ribbons, which did not roll up into nanotubes, can also be detected on the mica surface (Figure S2c).

Except for the negatively charged mica surface, we also studied assemblies on the hydrophilic silicon wafer for comparison. **BECA4** also assembles into intertwined fibers (Figure S4a), and **BECA12** forms nanotubes (Figure S4b) on a hydrophilic silicon surface at pH 3, which is similar to the case on the mica surface. At low pH,



**Figure 4.** AFM images of **BECA4** (a, b) and **BECA12** (c, d) at pH 12 on the mica surface.

the two carboxylic acid groups in glutamic acid could be mostly protonated and there is no strong ionic interaction between the headgroups. In this context, the negative charges on the mica surface presumably do not influence on the assembly greatly at pH 3.

*AFM Measurements of pH 12 Assemblies.* The negatively charged mica surface changed the self-assembled morphologies dramatically at higher pH. For **BECA4** at pH 12 on the mica surface, huge compressed vesicles with a diameter of more than 1  $\mu$ m were observed (Figure 4a). The heights of corresponding assemblies are only 40 nm (Figure 4a,b), which is much smaller than the apparent diameter, indicating a depressed hollow structure. Although the horizontal distance obtained from AFM measurements is not precise because of the size and movement of the tips, the **BECA4** vesicles on the mica surface are still many times larger than those determined from the cryo-TEM study. However, for **BECA4** assemblies on hydrophilic silicon surface at pH 12, only nanospheres with a diameter of around 100 nm and heights of several nanometers can be detected from AFM measurements (Figure S4c).

**BECA12** with a longer spacer exhibited different aggregation behavior. It is very unusual for **BECA12** to self-organize into nanofibers at pH 12 on a mica surface (Figure 4c). In general, these fibers distribute on the mica surface with dendritic style, and many of the branchs are aligned parallel to each other. The magnified image shows that the nanofibers are rigid and nonhelical. The average heights of the fibers are from 3.5 to 5 nm (Figure 4d). However, on the hydrophilic silicon surface at pH 12, the nanofibers from the **BECA12** assembly are much thinner and shorter and many of them break into smaller parts (Figure S4d).

Interestingly, although **BECA4** and **BECA12** show totally different aggregation behaviors at pH 3 and 12 on the mica surface, they present similar assembled morphologies at pH 7. The AFM images show that both **BECA4** and **BECA12** form platelets on the mica surface at pH 7. In the case of **BECA4**, the thickness



**Figure 5.** FTIR spectra of bolaamphiphiles at different pH values: (a) **BECA4** at pH 3, (b) **BECA4** at pH 12, (c) **BECA12** at pH 3, and (d) **BECA12** at pH 12.

of the platelets is uniformly 2.5 nm, corresponding to a monolayer of **BECA4** (Figure S5a). However, the thickness of **BECA12** platelets at pH 7 on the mica surface was evenly determined to be 3.6 nm, which is also the height of the **BECA12** monolayer (Figure S5b). At pH 7 on solid surfaces, because of relatively weak interactions between the headgroups, the long spacer lengths of the building blocks do not lead to different aggregation behavior.

*FT-1R Measurements*. The FT-IR spectra were also measured in order to clarify further the self-assembly properties of the bolas at different pH values. For the molecules at pH 12, a strong vibrational band was observed at 1606 cm<sup>-1</sup> (**BECA4**) and 1591 cm<sup>-1</sup> (**BECA12**) respectively, which can be assigned to the antisymmetric vibration of the carboxylate (Figure 5b,d). However, the spectra change dramatically when the pH values were decreased to 3. For **BECA4**, a peak emerges at 1715 cm<sup>-1</sup> that is the carbonyl stretching band with hydrogen bonding (Figure 5a). In the case of **BECA12**, the new carbonyl stretching bands appear at 1715 and 1693 cm<sup>-1</sup> respectively, indicating the coexistence of different hydrogen bonds (Figure 5c). For **BECA12** at pH 3, the amide I bands present at about 1629 cm<sup>-1</sup> with the amide II bands at around 1572 cm<sup>-1</sup> clearly signify the hydrogen bonding from the amide group (Figure 5c).

The CH<sub>2</sub> vibrational band is very sensitive to the packing of the alkyl chain, and a significant change was found for the assemblies of bolaamphiphiles under different conditions in the case of **BECA12**. The CH<sub>2</sub> antisymmetric and symmetric stretching bands of **BECA12** appear at 2923 and 2853 cm<sup>-1</sup> at pH 12 (Figure 5d), and the corresponding peaks shift to 2916 and 2852 cm<sup>-1</sup>, respectively, when the pH value was changed to pH 3 (Figure 5c). It is well known that the lower wavenumbers (ca. 2916–2918 and 2848 cm<sup>-1</sup>, respectively) indicate a highly ordered, well-organized arrangement of the CH<sub>2</sub> hydrocarbon chains and the higher wavenumbers indicate the disordering of the hydrocarbon chains.<sup>31,32</sup> Thus, alkyl linkers in **BECA12** adopt all-trans and highly ordered assemblies at lower pH values but a more gauche

conformation at higher pH values. In the case of **BECA4**, there are no significant changes from  $CH_2$  antisymmetric and symmetric stretching bands when the pH value is transformed from 12 to 3.

## Discussion

For the bolaamphiphiles, the self-assembly properties are strongly dependent on many noncovalent interactions, such as ionic interactions, hydrophobic interactions,  $\pi - \pi$  interactions, and hydrogen bonding. In the present case, besides the strong interactions between the spacers, the interaction between headgroups plays an important role, and the rigid aromatic rings close to the hydrophilic headgroups could also magnify the effect from interactions. Furthermore, such interactions between the headgroups can be modulated by the environment, such as different pH values or solid surfaces. Hybrid spacers with different alkyl chain lengths could affect the packing differently according to subtle changes in the environment. Because of all of these cooperative interactions, various nanostructures are suggested to be formed.

At pH 3, more protonated headgroups with stronger hydrogen bonds could be achieved. The hydrogen bonding have been proved by FT-IR spectra. For **BECA4**, because of strong hydrogen bonding from several functional groups and the rigidity of the phenyl neck, short chains with four methylene units cannot be managed well to maintain a monolayer at pH 3. Instead, the molecules have to be twisted with each other to form very thin nanofibers with the diameter of each section on the single-molecule scale (Figure 6), as proven with cryo-TEM measurements. The rigid phenyls in **BECA4** cannot be arranged into a more ordered way, so the assemblies give a weak, irregular CD signal.

However, molecular monolayer structures are widespread in **BECA12** assemblies at pH 3. Both the nanoribbon and nanotube should be molecular monolayers, as in the case of many other previously reported typical bolaamphiphiles.<sup>2,8,19,21</sup> Although there is also strong hydrogen bonding and a rigid neck, the C12 alkyl spacers in **BECA12** may become long enough to fit the conformation of a monolayer (Figure 6) so that the CD spectra of **BECA12** in a pH 3 aqueous solution show a strong exciton-type Cotton effect, which originates from the strong  $\pi - \pi$  stacking of the benzene ring. This monolayer structure can also be proven by the CH<sub>2</sub> antisymmetric and symmetric stretching bands of FT-IR measurements and the blue shift in the UV spectra.

At pH 12, the heights of **BECA12** fibers on the mica surface are 3.5-5 nm, which is greater than the height of a molecular monolayer but less than the height of a multilayer. Moreover, the FT-IR spectrum shows that the **BECA12** alkyl linkers adopt a more gauche conformation at pH 12, as represented by the CH<sub>2</sub> vibrations appearing at 2923 and 2853 cm<sup>-1</sup>. Thus, a possible structure of the corresponding **BECA12** fibers should be micellar fibers, where the hydrophobic alkyl chain adopted the U-shaped conformation<sup>2,32-36</sup> with the negatively charged headgroup outside (Figure 6). Moreover, a mica surface with a negative charge may also play an important role in the formation of nanofibers at high pH values. The strong ionic interaction could stabilize the fibers.

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Figure 6. Illustration of the self-assembly (BECA4 and BECA12) in pH 3 and 12 solutions as well as on a mica surface.

In the case of **BECA4**, there are no significant changes from  $CH_2$  antisymmetric and symmetric stretching bands when the pH value was transformed from 12 to 3 (Figure 5a,b). Compared with its headgroups and rigid necks, the alkyl chain in **BECA4** maybe too short to be bent enough to form the U shape, so it cannot form micellar fibers at pH 12 on the mica surface. In contrast, vesicles with molecular monolayers working as walls were detected in bulk aqueous solution at pH 12. The negative charges from head-groups are around the vesicles, and the mica surface with charges could help the smaller vesicles to fuse into huge vesicle (Figure 6).

Expect for the noncovalent interactions between molecules, the solid surface also plays an important role during the hierarchical self-assembly of bolaamphiphiles at different pH values. At pH 3, the thin fibers from **BECA4** further aggregate into intertwined nanofibers in a helical manner and the nanoribbons from **BECA12** form helical nanotubes upon drying on hydrophilic silicon or a mica surface. However, for ionic supramolecular assemblies from L-glutamic acid-based bolas at high pH values, the charged mica surface could dramatically change the corresponding nanostructure. Thus, for **BECA4**, smaller vesicles fused into huge vesicles, but for **BECA12**, very long nanofibers appeared on the mica surface.

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## Conclusions

Bolaamphiphiles with glutamic acid headgroups and hybrid linkers were designed, and their hierarchical self-assembly in water as well as on solid surfaces was investigated. The self-assembled morphologies can be controlled cooperatively by using different means and conditions. Concerning the interaction between molecules, the cooperative hydrogen bond and/or the ionic interactions between headgroups and the noncovalent interactions between hybrid linkers render bola assemblies in various nanostructures. Low pH values favor the formation of H bonds between headgroups, which triggers the formation of nanofiber, nanoribbon, and nanotube structures. The higher pH value causes the formation of carboxylates of bolaamphiphiles, and the ionic interaction causes the bolaamphiphiles to prefer to form spherical vesicles or nanoparticles in solution. Because of the presence of rigid necks, the length effect from the flexible spacer can function very unusually during self-assembly. At higher pH values, the negatively charged mica surface also plays an important role in the hierarchical self-assembly; the mica surface can change the smaller vesicles into huge vesicles and transfer the nanoparticles into nanofibers. Through such design of the bolaamphiphiles and modulation of the pH value and with help from the liquid/solid interface, we have realized the controllable morphology transition between diverse self-assembled nanostructures.

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**Supporting Information Available:** Additional AFM and TEM data. This material is available free of charge via the Internet at http://pubs.acs.org.