Preparation and Characterization of Assembled Nanostructures by Peptide Amphiphiles

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Abstract
The aim of this research is to investigate the pH effect on the assembled nanostructures of peptide amphiphiles (PAs). Controlling the sizes and shapes of these assembled nanostructures is of great importance for their ultimate applications in drug delivery, tissue engineering, and regenerative medicine. A complete characterization of these nanostructures can lead to further knowledge of how to control their structural features by manipulating their assembly environment. In this work the nanostructures were imaged and analyzed by atomic force microscopy (AFM) operating at tapping mode. AFM imaging results show a consistent effect of pH on the samples' height and width. A consistent change of assembled nanostructures was observed with variation in NaOH concentration.

Introduction
Self-assembly is often used in biology to build scaffolds for complex materials and composites.[1] Self-assembling scaffolds can be used to grow composites such as bone as well as complex cell networks like neural progenitor cells.[1,2] One example of these self-assembling scaffolds is produced through the self-assembly of peptide amphiphiles (PAs). These amphiphilic molecules exhibit both surfactant and peptide properties and can assemble into a large array of nanostructures under different solution conditions.[1,2,3] There is a close relationship between the chemistry of the peptides, the assembly behavior of the molecules, and the properties of the material.[1,2,3] Deciphering the assembly mechanisms of these peptide amphiphiles is critical to the rational design of peptide chemistry and consequent assembly of these molecules into useful materials.[3] A complete characterization of the self-assembled system under different assembly conditions is required. AFM techniques can provide useful size and shape information.

Background

Peptide Amphiphiles
The ability of molecules to create nano-sized particles by themselves with little or no human effort is tremendous and is a significant improvement and simplification for the nano-scale fabrication of structures.[3] Amphiphiles made of a small chain of amino acids and a short alky tail can self-assemble into a variety of structures when dissolved in water.[3] Figure 1 shows the molecular structure of the C_{16}VEVE peptide amphiphile (PA) used in this work.

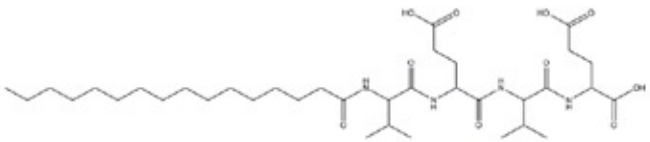

Figure 1. Molecular structure of $C_{16}H_{31}$OVEVE molecule used in this work.

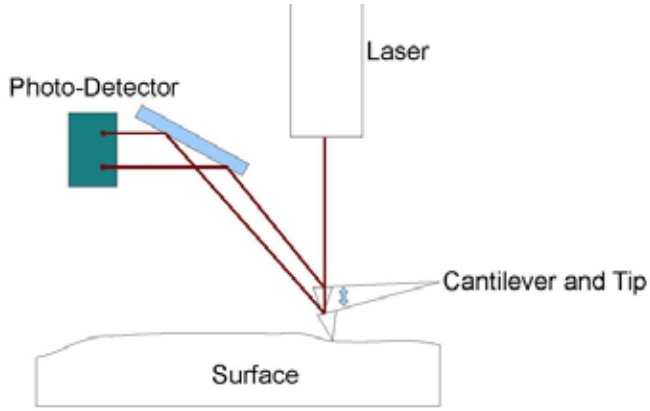

Figure 2. Diagram showing the basic concept on how tapping-mode AFM works.

These molecules can assemble into belts, fibers, ribbons, spheres, or micelles, depending on their molecular structure and aqueous environment.[3] Assembly occurs because of the combined hydrophobic and hydrophilic properties of the PA. The hydrophilic heads favor the water, while the hydrophobic tails hide away from water.[1] Variation of solution pH and peptide concentration can change the assembled structures in a variety of ways. This work demonstrates how solution pH and peptide concentration can influence the morphological transition between nanobelts, nanoribbons, and other nanostructures.

Atomic Force Microscopy
The nanostructures were characterized using the tapping-mode AFM technique.[3] The tapping mode oscillates the cantilever and tip; as the tip gets closer to the surface, the oscillation is distorted.[4] This distorted oscillation is detected by a laser beam reflecting off the cantilever onto a photo-detector and addressed by the feedback loop. The AFM reads a change in height, giving a 3D image of the surface without ever being in contact with it.[4] Figure 2 shows the basic concept of the oscillating tip and how the photo-detector identifies the change in the laser position.

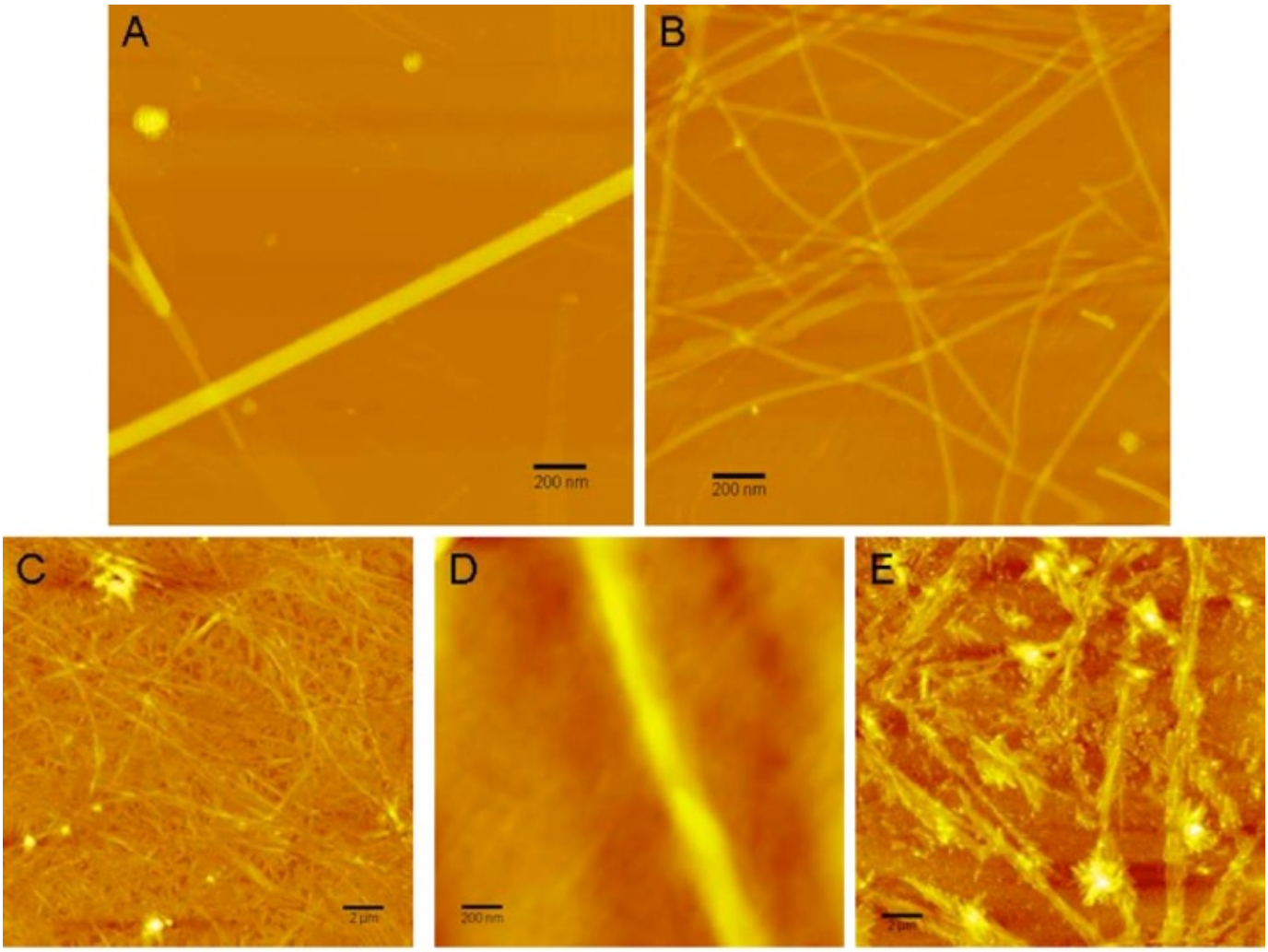

Figure 3. AFM images of $C_{16}H_{31}OVEVE$ with (A) no NaOH, (B) 0.001m NaOH, (C) 0.005m NaOH, (D) 0.010m NaOH, and (E) 0.100m NaOH. Images A and B show individual nanobelt structures, while C, D, and E show bundles of the nanostructures.

Approach

Peptide amphiphiles were synthesized using a standard fluorenylmethoxycarbonyl chemistry method reported previously.[5] The peptide was first dissolved in deionized water (0.1%) and put aside for two days. Samples with different pH were then prepared by adding a specified amount of NaOH to the aqueous solutions.

Atomic Force Microscope Preparation

After 24 hours samples were prepared for AFM imaging. Freshly peeled mica was mounted on top of the steel disks specifically made for AFM imaging. A small drop of the PA solutions (2-5μL) was placed onto the mica and left to dry for an hour. Once dry, the samples were imaged using the AFM.

Atomic Force Microscope Imaging and Analysis

Tapping-mode AFM was used to image the delicate PA samples. Silicon tips with an aluminum coating were used to maximize the resolution of the AFM images during tapping mode. After the images were taken, they were processed using the Nanoscope offline AFM program.

Sample	Average Height (nm)	Height Standard Deviation (nm)
BA0: 0.000M NaOH	19.660	4.483
BA1: 0.001M NaOH	4.450	0.440
BA2: 0.005M NaOH	2.804	1.589
BA3: 0.010M NaOH	No Data	No Data
BA4: 0.100M NaOH	No Data	No Data

Table 1. Average heights of the nanostructures with their respective standard deviations.

Results

The AFM images, shown in Figure 3, show different nanostructures formed under different NaOH concentrations. Table 1 shows the results of the average heights of all the samples imaged.

Though images of BA3 and BA4 were taken and analyzed, quantitative data could not be extracted from the images because the size of the individual structures could not be obtained from the bundled multiple nanostructures. During further imaging of those two samples, no individual fibers were found for measurement, probably because of increased entanglement of the nanostructures with high pH.

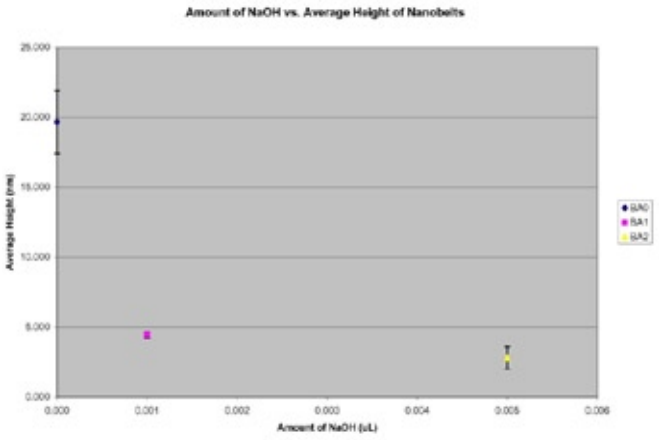

Figure 4. Graph displaying the average height of the nanostructures formed with varying amounts of NaOH.

Figure 4 shows the average heights for the amounts of NaOH in the samples. The graph reveals a dramatic decrease in the height of the nanostructures from BA0 to BA1 but only a slight height difference between BA1 and BA2. It seems that the samples reach a plateau, and an increase of NaOH amount does not lead samples to break into smaller structures. Though BA3 and BA4 could not be analyzed, it is hypothesized that they may lie along that same plateau.

Discussion

Results show that as the amount of NaOH increases, the size of the nanostructures decreases in both height and width. This occurs because the change in pH causes highly charged molecules. The repulsions between molecules lead the stacked nanostructures to separate into individual nanostructures. Eventually, when the structures cannot be broken down further, a plateau is reached at a certain pH. The plateau signifies that the nanostructures formed are at their minimum size, and stacking does not occur at all.

Conclusion

From this work, it has been show that pH could alter the size of the peptide amphiphile assemblies and can be used to tailor nanostructures of specific size in order to create scaffolds for many applications. Further testing must be done to determine how to obtain specific-size nanostructures by altering the pH of the solution. With this knowledge, specific-size nanostructures could be used in many applications, such as drug delivery and scaffolding.

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