Nanostructure Assembly

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Mechanical-Force-Induced Nucleation and Growth of Peptide Nanofibers at Liquid/Solid Interfaces**

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Self-assembling peptides have emerged as new functional nanobiomaterials and received considerable attention in the areas of nanochemistry and biomedical engineering.[1] In this category are ionic-complementary peptides, which contain a repeating charge distribution and alternating hydrophobic and hydrophilic residues in the amino acid sequence; this leads to an unusual combination of amphiphilicity and chemical complementarity. Such peptides can self-assemble into stable nanostructures through electrostatic interactions, hydrogen bonds, and hydrophobic interactions.[1a,2] Their nanostructures have a large number of potential applications, such as templates for nanofabrication,[3] scaffoldings for tissue repair and engineering,[4] nanocarriers for drug and gene/small interfering RNA delivery,[5] and surface modifiers for enzyme immobilization in biosensors.[6]

Many of these processes involve interface and surface patterning with peptide micro-/nanostructures. Therefore, it is critical to understand and control the peptide assembly on a surface for the development of these applications. Several studies have shown that the surface can facilitate and/or direct the assembly of peptides into patterned nanostructures.[7] Such patterns are usually generated according to the natural propensity of the peptides and the surface crystallography. Although the solution conditions can help to control the overall density of the peptide nanostructure over an entire surface,[7a] it would be very useful to develop techniques that produce patterns over local regions of a surface for the fabrication of nanoscale devices.

To precisely control the peptide assembly and pattern the assembled nanostructures at desired locations on a surface, we adopt a mechanochemical approach by using atomic force microscopy (AFM). Mechanical force/stress has recently been applied to induce chemical reactions at the atomic level[8] and to deliver single molecules to specific locations.[9] Moreover, the indentation of an AFM tip can actually break a self-assembled protein nanotube.[10] Using such an approach, through the application of a relatively gentle mechanical force from a tapping AFM tip, we expect that peptide-assembled nanostructures can be broken into fragments, which in turn can serve as nuclei for further assembly through a nucleation and growth mechanism. In addition, the mechanochemical control over the nucleation process may aid the understanding of protein aggregation and amyloid-fiber formation. The use of tapping-mode AFM for such a purpose has several advantages. First, it provides the high quality of AFM imaging for biological samples. Second, the application of mechanical force and the AFM imaging can be achieved simultaneously, whereas the two are often operated separately in contact-mode AFM.[11] The applied force by tapping mode is small enough for imaging, but large enough to disrupt the non-covalent interactions that hold the peptide assemblies. To our knowledge, this is the first study where a mechanochemical approach has been used for peptide nanoassembly and surface patterning. The studied peptide, EAK16-II, has the molecular structure shown in Figure 1a. This peptide predominately adopts a β-sheet secondary structure, which leads to an amphiphilic structure with hydrophilic residues on one side and hydrophobic residues on the other side. Such a

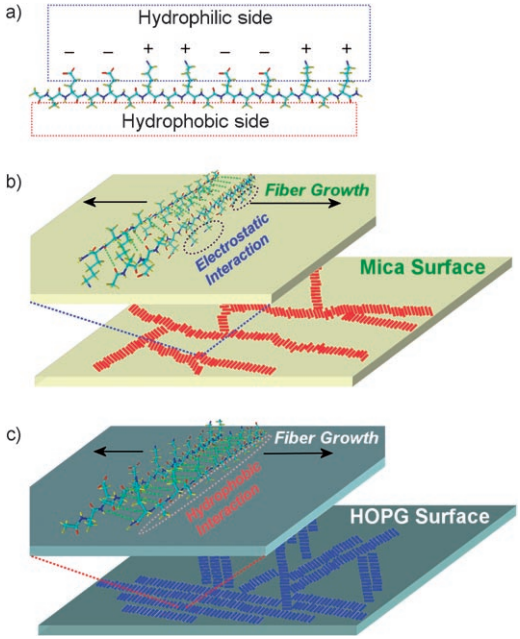

Figure 1. a) The molecular structure of EAK16-II. Interfacial orientation of EAK16-II assemblies on b) mica and c) highly ordered pyrolytic graphite (HOPG) surfaces. These two model surfaces serve as examples of liquid/hydrophilic and liquid/hydrophobic interfaces, respectively. The peptide can interact with mica through electrostatic interactions and with HOPG through hydrophobic interactions. These may result in different patterns of peptide assemblies. The green dotted lines represent hydrogen bonds between two peptide backbones.

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molecular structure allows it not only to self-assemble but also to interact with both hydrophilic and hydrophobic surfaces through electrostatic and hydrophobic interactions, respectively (Figure 1b and c).

The experiments were carried out by first depositing EAK16-II nanofibers on two model surfaces: negatively charged mica and hydrophobic HOPG. In situ AFM was then used both to apply a mechanical force to the nanofibers on the surfaces and to monitor the force-induced/directed peptide assembly in real time. The work done by the tapping AFM tip on the biomolecules could be manipulated by adjusting the amplitude of the tip oscillation, scan area, and scan speed.

Figure 2a–c shows that EAK16-II nanofibers on a mica surface can be broken up by an AFM tip in tapping-mode operation in water. After scanning over a large area of $2\times 2\ \mu m^2$, the nanofibers appear continuous (Figure 2a). However, when a subsequent scan is performed over the $500\times 500\ nm^2$ dotted area, the nanofibers in this region appear to be discontinuous and broken (Figure 2b). Repeated scans over the same location lead to further truncation and displacement of nanofiber segments (Figure 2c). Thus, in the absence of additional peptide dissolved in the solution, the applied mechanical force from the AFM tip can "cut" the nanofibers into shorter segments. This indicates that the force or work applied by the AFM tip has exceeded the bonding strength or cohesive energy in the peptide nanofibers, which is mainly derived from peptide-backbone hydrogen bonding and electrostatic/hydrophobic interactions between the peptide and the substrate.

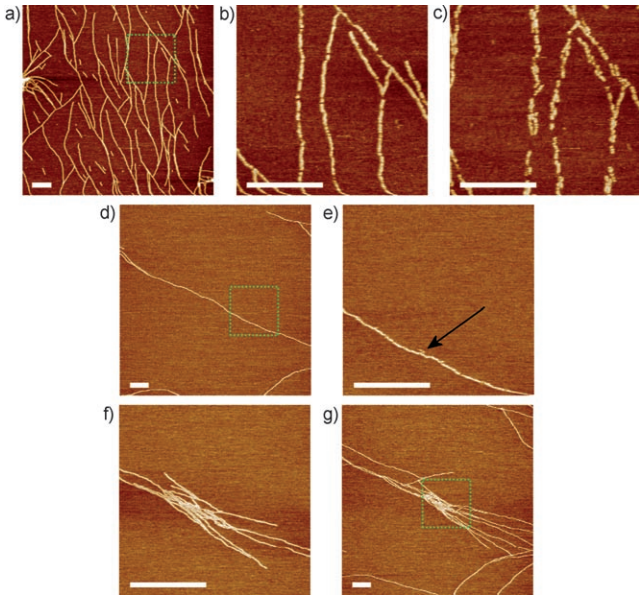

Figure 2. AFM images showing the effect of the mechanical force applied by an AFM tip on EAK16-II nanofibers formed on mica in water (a–c) and in 2 μM EAK16-II solution (d–g): a) $2\times 2\ \mu m^2$ scan area; b) zoom-in scan of area indicated in (a); c) fourth scan at the same location as (b); d) $2\times 2\ \mu m^2$ scan area; e) zoom-in scan of area indicated in (d); the arrow points to broken nanofibers serving as nuclei for subsequent nanofiber growth; f) third repetitive scan at the same location as (e) showing many new growing segments; g) large-scale scan after (f) showing that the mechanical force has induced local nanofiber growth in the indicated area. The scale bars correspond to 200 nm.

The effect of the scan area on the breakage of peptide nanofibers on the mica surface may be related to differences in the total work done by the AFM tip. The total work, W_{total}, that an AFM tip does on a nanofiber can be approximated by using Equation (1), in which F_{spring} represents the tension in the spring that the AFM tip applies to the nanofiber during each tap, S_n represents the displacement of the AFM tip as it interacts with the nanofiber during the n^{th} tap, and m is the total number of taps by the tip on a nanofiber during a scan.

$$W_{total} = \sum_{n=1}^{m} F_{spring}\, S_n \qquad (1)$$

The damping force due to friction and the driving force that oscillates the cantilever are ignored as they are negligible compared to the F_{spring} value. When the AFM tip scans the surface, it applies an intermittent force to the surface during each tap. This intermittent force is determined by the mechanical properties (for example, spring constant k) and amplitude, x, of the oscillating tip and can be described by Hooke's law, that is, $F_{spring} = kx$. The amplitude of the tip oscillation/displacement is about 3–4 nm for tapping-mode AFM imaging (see the Supporting Information). For a given tip with a fixed k value, the F_{spring} value is nearly constant (≈1–2 nN) during each scan. Thus, the total work done on the nanofiber is primarily determined by the number of taps (m), which is inversely proportional to the scan area at a given scan speed and oscillation frequency. For example, the number of taps on a nanofiber is estimated to be ≈69–77 for a scan area of $2000\times 2000\ nm^2$; it increases to ≈275–310 when scanning over a smaller area of $500\times 500\ nm^2$ (see the Supporting Information). The number of taps will determine the total work applied to a nanofiber by the AFM tip. If the total work, or energy, applied overcomes the cohesive energy of the nanofiber, the nanofiber will break. Such control by the number of taps in tapping-mode AFM provides simultaneous imaging and fiber "cutting". This is different from the operation of contact-mode AFM, for which the concept of force threshold is used in determining whether to "cut" a sample in AFM nanolithography and nanografting.[11]

One application of this discovery is to locally induce/control the growth/morphology of peptide nanofibers on surfaces. Previous studies have demonstrated that surface-assisted nanofiber growth follows a nucleation and growth mechanism.[7a] In the presence of EAK16-II molecules in solution, a nanofiber "seed" can grow by addition of EAK16-II molecules at its active ends. This suggests that the truncation of the peptide nanofibers by the AFM tip can lead to the growth of new nanofibers due to an increase in the number of active ends (Figure 2d–g). After three zoom-in scans ($500\times 500\ nm^2$) of an original single nanofiber, many new segments appear (Figure 2f). This new growth may either repair the breakage or form a new branch depending on the orientation of the truncated nanofibers and the growth direction. As shown in the larger scale scan in Figure 2g, the nanofiber density is higher in the scanned area than elsewhere. This shows that nanofiber formation can be promoted at a desired location on the mica surface.

In addition to the mica surface, mechanical-force-induced peptide assembly and nanofiber growth are also observed on a hydrophobic HOPG surface. In the absence of peptide molecules in solution, the continuous long nanofibers observed initially (Figure 3a) are truncated after four zoom-in scans ($500 \times 500\ nm^2$; Figure 3b). Evidently, some nanofibers have detached from the HOPG surface. On the other hand, when peptide molecules are present in solution, repeated zoom-in scanning results in many short nanofibers on the surface (Figure 3c and d); the number of short nanofibers increases dramatically and covers the whole scan area ($1\ \mu m^2$) after several scans. Another feature with the nanofibers is that the adsorbed fibers tend to be packed in hexagonal patterns that resemble the crystallographic symmetry of the HOPG surface (Figure 3e).[12] A comparison of the increase of surface coverage by the nanofibers over time with and without repetitive scanning (Figure 3f) indicates that the mechanical force can induce nanofiber formation. Not only does the surface coverage increase but also the coverage reaches as high as 80%, more than twice that achieved without the mechanical force.

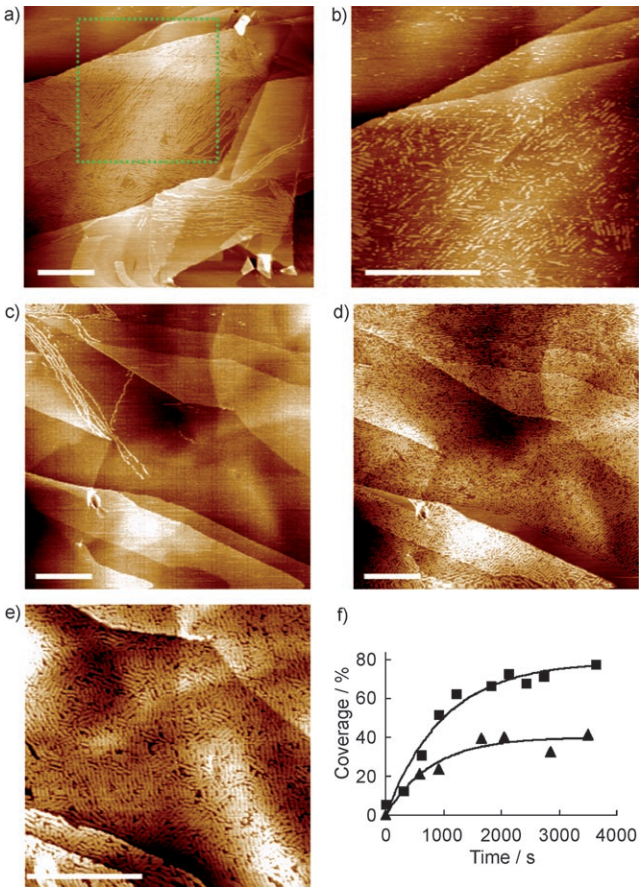

Figure 3. AFM images of EAK16-II nanofibers formed on HOPG in water (a and b) and 4 μM EAK16-II solution (c and d) under a mechanical force applied by a tapping AFM tip: a) large-scale $1 \times 1\ \mu m^2$ scan; b) fourth zoom-in scan of dotted area in (a); long peptide nanofibers have been broken into short nanofiber segments under the mechanical force; c) first and d) tenth $1 \times 1\ \mu m^2$ scan at the same location; the amount of peptide nanofibers on the HOPG surface increases with the number of repeated scans; e) zoomed-in scan image after the tenth scan reveals many truncated nanofibers formed on the HOPG surface. f) Surface coverage of peptide nanofibers as a function of time; coverage at one location with repeated scans: ■; different locations scanned only once: ▲. The scale bars correspond to 200 nm.

The mechanical-force-induced nucleation and growth of peptide nanofibers may provide insights into a number of biological phenomena and developing new biomedical applications. It has been found that applying sonication to proteins can cause their aggregation into structures resembling amyloid fibrils.[13] Although sonication is a complex process involving mechanical/shear stress, local heating, the formation of liquid/gas interfaces, and free radical reactions,[14] it causes the formation of active seeds that facilitate protein aggregation into amyloid fibrils. Recently studies have shown that sonication can break peptide nanofibers into short fragments.[15] Thus, the results of our study suggest that this may be related to the mechanical force applied by sonication, rather than to other factors.

Thus far, we have demonstrated one approach for using an AFM tip to apply mechanical force and induce the local growth of peptide assemblies/nanofibers on both mica and HOPG surfaces. Such mechanochemical control over peptide assembly on surfaces could potentially be used to fabricate patterns with specific shapes at desired locations. This can be achieved by first modifying the surface with peptide nanofibers and then removing the nanofibers locally with the AFM tip in an acidic environment (for example, 1 mM HCl). This is possible due to the effect of the pH value on the adsorption of peptide molecules/nanofibers on the surface. Using such an approach, we were able to fabricate peptide-nanofiber patterns on HOPG (see the Supporting Information), and alternating hydrophilic and hydrophobic stripes could be created.[6] Such a structure could serve as a template to pattern proteins or enzymes by selective adsorption due to variations in protein affinity based on differences in the hydrophobicity of the surface. Many proteins/enzymes can also be immobilized on the peptide nanofibers to construct biosensors.[16]

In summary, mechanical force applied by the tapping of an AFM tip can break/overpower the weak interaction or cohesive energy in EAK16-II nanofibers on both mica and HOPG surfaces. Such a process can be controlled by adjusting the AFM-tip scan area and scan speed. The broken nanofibers can serve as seeds for new nanofiber growth. The mechanical-force-induced nucleation and growth of the peptide nanofibers allow one to locally grow nanofibers that densely cover specific regions of the surface. A new AFM-lithography method based on the application of a mechanical force to peptide nanofibers in an acidic solution is proposed for the fabrication of an alternating hydrophilic and hydrophobic patterned surface on HOPG. These patterned peptide nanofibers could serve as templates to immobilize proteins/enzymes, DNA, and cells for biomolecular sensing and other biomedical applications.

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