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Lattice modulation effect of liquid–solid interface on peptide assemblies

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ABSTRACT

We illustrate the single molecule level analysis of the commensurability of the peptide assemblies with the graphite lattice at liquid–solid interface. The pristine peptide assembly was observed to display commensurate registration to graphite lattice, while the introduction of chaperone molecules induces a slight mismatch of the peptide and graphite lattices leading to Moiré patterns. The detailed analysis of the Moiré pattern could provide information of the structural changes of the peptide assembly and the involved peptide–peptide interactions.

1. Introduction

Surface-assisted self-assembly of peptides at liquid–solid interfaces has attracted considerable interest during the last decade due to its wide applicability in many areas of chemistry, biology, medical sciences and nanotechnology [1–6]. It is also of vital importance in the research of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Type II diabetes [7–10].

Liquid–solid interfaces are often introduced as a model system and simulated physiological environment to investigate a variety of disease-related pathogenesis [11–15]. Recent reports have shown that substrates or interfaces are capable of providing templates for denaturation and aggregation of amyloid peptides into fibrils [5,12,16,17]. In vitro studies further clarified the surface modulating effect. It has been found that the self-assembly of peptides into amyloid fibrils was greatly accelerated on the solid surface, and the resulting fibril structures were more uniform than those formed by conventional solution-based method [18]. Among those studies, the most important and challenging thing is the substrate effect. That is, the assembled structures are all oriented in a well-defined manner, indicating an epitaxial growth mechanism and the hexagonal symmetry of the underneath substrate [19–21].

While extensive studies have endeavored to unveil the molecular mechanism, the detailed atomic structure of the surface assembly still remains elusive, largely due to the current limited experimental resolutions and the complex nature of the epitaxial growth [7,10,22]. To elucidate the substrate effects on the peptide assembly structure, high resolution experimental data including the peptide assembly structure and the atomic structure of the substrate surface should be collected.

Scanning tunneling microscopy (STM) has become a useful tool for direct observation of the assembly structures at the single-molecule level [23–30]. In recent years, it has been used to investigate peptide assemblies on graphite substrate and has gained a set of useful information, such as the binding sites of amyloid probes, core regions and folding sites of amyloid peptides, etc. [31–33].

Moiré patterns often exist in a mismatch of two patterns such as the substrate lattice and assembled molecules [34,35]. They can be used to detect the tiny displacement of the assembly on substrate lattice for its sensitivity to tiny shifts and rotations. A common way to analyze the Moiré pattern is 2D-fast Fourier transformation (2D-FFT), which can provide detailed structural information of the STM images and the geometrical relationship between signals in reciprocal space. If high resolution STM images of peptide assembly structure and the resulting Moiré pattern could be obtained, then more rigorous analysis of the peptide assembly mechanism could be possible. This kind of studies is readily feasible for π-conjugated organic molecules, and many results have been reported [36,37]. As for peptide molecules, the non-planar structure, lacking of π-conjugated moieties, and the complex conformations make this study a challenging task.

In this work, a co-assembly strategy is applied for possible high resolution imaging and also the generation of Moiré patterns with introduction of small molecules. The peptide assembly structures at liquid–solid interface with and without chaperone molecules are studied by STM. The peptide hIAPP$_{20-29}$(SNNFGAILSS) (Scheme 1a) is a key sequence of human islet amyloid peptide (hIAPP) [38]. Highly oriented

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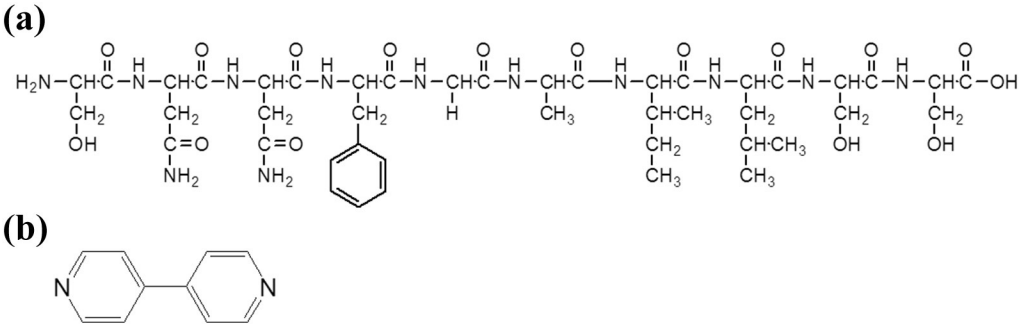

Scheme 1. Molecular structures of $hIAPP_{20-29}$ (a), and the chaperone molecules 4Bpy (b).

pyrolytic graphite (HOPG) is taken as a model substrate. The pristine peptides assemble with a perfect lattice matching. 2D-FFT analysis indicates that the introduction of chaperone molecules, 4,4′-bipyridyl (4Bpy) (Scheme 1b), induces a slight mismatch of the peptide lattice with the underneath graphite. The lattice mismatch further results in a Moiré pattern.

2. Materials and methods

2.1. Synthetic peptides and molecules

Peptides were obtained from GL Biochem (Shanghai) Corporation Ltd. 4Bpy was obtained from Sigma-Aldrich Co. Ltd. and used without further purification.

2.2. Sample preparation

For pristine peptide assembly, the lyophilized powder of $hIAPP_{20-29}$ (SNNFGAILSS) was dissolved in MilliQ water to a concentration of 0.1 mg/mL. A total of 5 μL of the solution was deposited on a freshly cleaved HOPG surface. STM experiments were performed after the water was evaporated from the HOPG.

For peptide-4Bpy co-assembly, solid powder of 4Bpy was dissolved in MilliQ water to a concentration of 0.2 mg/mL. Then, 100 μL of $hIAPP_{20-29}$ solution and 100 μL of 4Bpy solution were mixed and briefly sonicated. A total of 5 μL of the resulting mixture was deposited on an HOPG surface. STM experiments were performed after the water was evaporated from the HOPG.

It should be noted that the evaporation process can only remove the apparent water from the HOPG surface to reduce the tunneling noise caused by water molecules. A thin layer of water on the HOPG surface has been known by the humidity under ambient conditions, leading to the formation of the liquid–solid interface.

2.3. STM measurements

STM experiments were performed in constant-current mode under ambient conditions (Nanoscope IIIA scanning probe microscope system, Veeco, USA). The tips were newly, mechanically formed Pt/Ir wires (80/20). The STM tunneling conditions are described in the corresponding figure captions. The experiments were repeated more than three times, independently, using different tips for reproducibility.

2.4. Statistical methods

The lengths of the peptide strands in the STM images were measured by using Gwyddion version 2.36 (Czech Metrology Institute, Czech Republic). Step size of 0.325 nm for each residue was assumed for parallel β-sheet structures. The statistical histogram of the length distribution was fitted by using a Gaussian distribution.

3. Results and discussion

3.1. Assemblies of $hIAPP_{20-29}$

The assembly of $hIAPP_{20-29}$ on HOPG surface is shown in Fig. 1a. The peptide molecules assemble side-by-side in lamella structures as shown in the model superimposed at the up-right. Fig. 1b is a zoomed-in image with alternating tunneling conditions to display the structures of the assembled peptides and graphite lattice. The directions of peptides and graphite lattice are shown in Fig. 1b with red and blue lines respectively. The peptides assemble with a perfect lattice matching. As have been shown in the image, the peptide molecules lie in the same direction as graphite [210] and the distance between two peptides is 4.26 ± 0.02 Å which is two times of the distance of graphite lattice (see Figure S1 for the geometrical illustration). Fig. 1c is a 2D-FFT pattern of Fig. 1b. The dashed hexagon indicates the typical hexagonal lattice of graphite. $\overrightarrow{OG1}$ represents the graphite lattice in [210] direction. The points P marked by red circle indicate the peptide main chains' periodicity (see Figure S2 for the inverse FFT image of P and 2P). The overlapping of 2P and G1 is in accordance with the measuring of STM image which further proves the lattice matching.

3.2. Co-assembly of $hIAPP_{20-29}$-4Bpy

Fig. 2a shows the structure of $hIAPP_{20-29}$-4Bpy co-assembly with a molecular model at the up-right. The arrays of bright dots are 4Bpy molecules. The peptides assemble in lamella structures between two arrays of 4Bpy. Different from the structure of peptide self-assemblies, the peptides lie with a small angle deviated from graphite [210] direction, and the distance between two peptides is enlarged to 5.02 ± 0.02 Å. Moreover, peptide molecules form an out-of-register structure with different inter-peptide hydrogen bonds. Fig. 2c is the 2D-FFT of Fig. 2b which is in accordance with the above measurements (see Figure S3 for the inverse FFT image of P and G1). The orientation of $\overrightarrow{OP}$ and $\overrightarrow{OG1}$ directions is separated with an angle of 3.1°. Point 2P is closer to the center which means a larger inter-peptide distance. The introduction of 4Bpy molecules further induces a bright and dim pattern among assembled peptides. The green circles in Fig. 2b indicate the ordered matrix of bright features. It can be proved that the patterns are the Moiré pattern induced by the mismatch of assembled peptides and graphite lattice.

3.3. Causes of the Moiré pattern

Fig. 3a is a large scale image of the $hIAPP_{20-29}$-4Bpy co-assembly. Large scale image is suitable for the observation of Moiré patterns and its FFT image often reveals more detailed structural information. White arrows *a* and *b* indicate the assembly direction of 4Bpy molecules. Fig. 3b is a zoomed-in image of the 2D-FFT pattern of Fig. 3a with the whole 2D-FFT image inserted in top left. Point A in Fig. 3b