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Two Self-Assembly Pathways of a Peptide Hydrogel Studied by Atomic Force Microscopy

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Introduction

Peptide-based hydrogels are being investigated over almost 30 years for, amongst others, drug delivery, tissue engineering and wound healing [1,2]. The hierarchical self-assembly of the peptides is steered by the amino acid composition and external physicochemical parameters. The macroscopic properties of the resulting soft materials will depend on the fibril and network morphology. One class of peptide-hydrogels comprise β-sheet based materials. Here, the formed β-sheets will elongate and form a fibril. These fibrils will align and interact with each other forming the cross-links needed for hydrogel network formation. Unfortunately, the full assembly mechanisms of these networks are not revealed yet. Common techniques to investigate the network morphology, such as atomic force microscopy (AFM) in air and cryogenic electron microscopy (cryo-EM), lack dynamic features. Additionally, the sample preparation might influence the self-assembly process. In this work, peptide self-assembly dynamics were investigated under native conditions by means of high-speed atomic force microscopy (HS-AFM) experiments in liquid [3]. The general AFM set-up is depicted in Figure 1. We optioned for a model peptide, KFE8 (Ac-FKFEFKFE-NH_2) [4], which is known to form β-sheet containing hydrogels due to its alternating hydrophobic and hydrophilic amino acids. After the formation of a β-sheet double layer to create a hydrophobic core, it was described that this peptide will form helical ribbons as intermediate structures before evolving into larger fibrils.

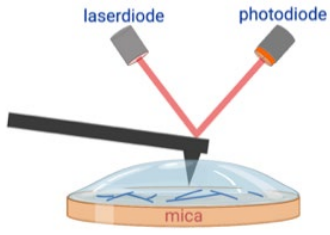

Fig. 1. Illustration of the AFM set-up.

Results and Discussion

In the first experiments, the self-assembly was followed *in-situ,* meaning the fibril formation took place at the interface between the mica surface and the water droplet. The scans, recorded with a speed up to 0.3 frames per second, were combined in videos and selected scans are depicted in Figure 2. It was observed that the formation of the fibrils occurred mostly via secondary nucleation. Besides, a bi- directional growth was visualized, the fibril elongated until the growing end reached the surface of another fibril. To form a network structure, the fibrils further branched and aligned. A stabilized 2D network was formed within a few minutes. Remarkably, polymorphism and the existence of different intermediate morphologies was not observed.

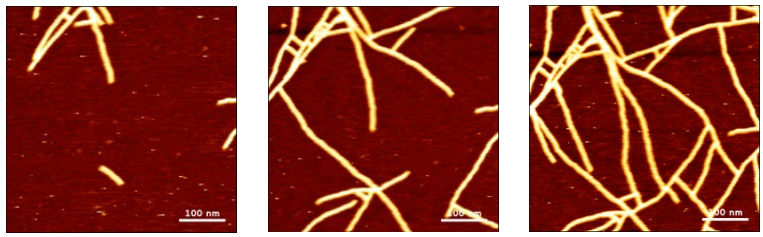

Fig. 2. AFM scans of the in-situ self-assembly at 85 s, 127 s and 235 s.

In a subsequent experiment, the self-assembly occurred in solution without being in contact with the mica surface (*ex-situ)*. Aliquots of this solution were dropped on mica and scanned in liquid. After the fast formation of large spherical aggregates during the first seconds, helical ribbons were observed on the AFM micrographs (Figure 3). Over time, these ribbons elongated prior to condensation towards nanotubes, being the most stable morphology. Both the nanotubes and the intermediate ribbons aligned sideways and formed cross-links. Under the applied conditions, slow kinetics were observed, the transition to a mature network required over 3 months. The material visible in the background of the images, consists of the 2D network formed on the mica surface, indicating the existence of two self-assembly pathways.

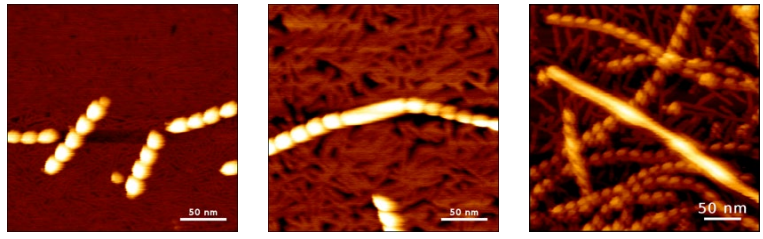

Fig. 3. AFM scans of the ex-situ self-assembly, fltr: the formation of helical ribbons, the structural change from ribbons to nanotubes and the presence of a nanotube.

In conclusion, two distinct self-assembly mechanisms were revealed, being at the surface-liquid interface and in the bulk solution. For the former, a fast-growing network composed of small uniform fibrils is formed, while for the latter, a more extended network resulted after the formation of an intermediate morphology. Both processes could be captured at high resolution and dimensions of these structures could be determined. Moreover, our findings provided novel dynamic insights into the understanding of a β-sheet forming peptide-based material (KFE8). This technique leads the way to further studies on the influence of physicochemical parameters and peptide composition on the assembly kinetics and morphological transitions of self-assembling systems.

Acknowledgments

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