Real-Time Observation of Superstructure-Dependent DNA Origami Digestion by DNase I using High-Speed AFM

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DNA origami nanostructures are promising tools for numerous biomedical applications, ranging from diagnostics to drug delivery and targeted therapy [1]. They are not only intrinsically biocompatible, biodegradable, and noncytotoxic, but can be synthesized in a wide variety of different shapes and sizes, and further functionalized in a precisely controlled manner with various organic and inorganic species. This enables their defined loading with therapeutic cargos and may be further exploited to facilitate cell targeting, cellular uptake, and cargo release. The performance of such DNA origami vehicles strongly depends on their structural and shape integrity. Unfortunately, previous studies have observed that DNA origami nanostructures are rapidly degraded in biological media, which poses serious limitations for their application in such environments [2]. Two major factors have been identified to contribute to the limited stability of DNA origami nanostructures in biological media: low Mg^{2+} concentrations and the presence of nucleases. While the former issue may be at least partially circumvented by rational design choices that ensure sufficient stability in selected media [3], DNase attack so far appears to be more difficult to control.

In this work [4], we study the degradation of four well established and structurally distinct 2D DNA origami designs (different lattice types, different edge types, and different flexibility) under the attack of DNase I using high-speed atomic force microscopy (HS-AFM). The temporal resolution in our experiments ranged from 5 to 10 seconds per frame, thus allowing a real-time observation of the digestion process. Our results reveal that digestion of the different DNA origami exhibits a superstructure dependence (see Fig. 1). Furthermore, we could identify structural features of each DNA origami design that are most susceptible and most resistant to DNase I digestion, respectively. The results acquired for DNA origami nanostructures immobilized at a solid surface are compared to digestion profiles obtained under identical conditions in bulk solution. It is found that DNA origami designed on the square lattice without twist-correction show remarkably different digestion profiles in bulk solution and at the solid-liquid interface, which is attributed to adsorption-induced shape distortions and strain build-up. Our findings may thus not only help in creating more resilient DNA origami nanostructures, but could also be applied in designing structures with building blocks possessing distinct susceptibilities to nucleases.

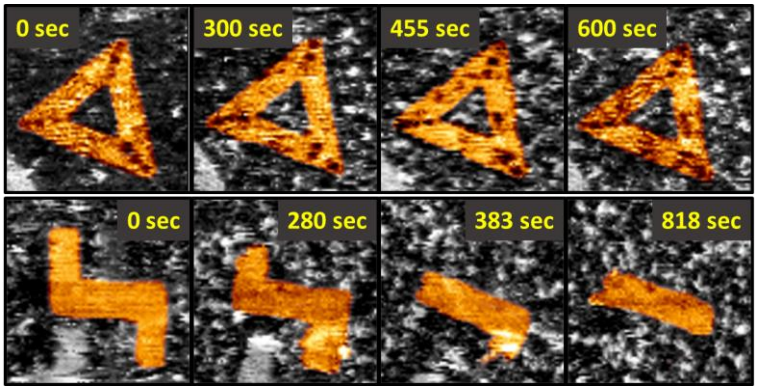

Figure 1: HS-AFM images of the digestion of two different DNA origami nanostructures by DNase I.

References

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