Bruker BioAFM NanoRacer

Investigating Molecular Dynamics with High Spatiotemporal Resolution Atomic Force Microscopy

Atomic force microscopy (AFM) is a powerful tool, which allows the comprehensive study of mechanical properties and interactions with nanometer resolution. The ability of AFM to obtain three-dimensional topography images of biological molecules and complexes under near-physiological conditions together with the recently achieved high-temporal resolution makes high-speed AFM (HS-AFM) a perfect tool for investigating dynamic biological processes.

The newly developed High-Speed AFM (NanoRacer®) enables scanning speeds of over 50 frames per second. The high-speed study of the time-resolved dynamics associated with cellular processes and the binding mechanisms of individual biomolecules is, therefore, possible. For example, the dynamics of individual protein binding behavior, two-dimensional protein assemblies, motor proteins, membrane trafficking, structural transitions of nucleic acids, can now be observed. Deoxyribonucleic acid (DNA) has an inherent structure of two alpha helical strands, held together by hydrogen bonds. Due to their hydrophobic organic base backbone, the strands carry a lot of torsional stress and twist around the axis to minimize contact with water. This enables, a myriad of structural DNA transitions on its own which can be monitored with HS-AFM on the millisecond scale (**Fig.1**).

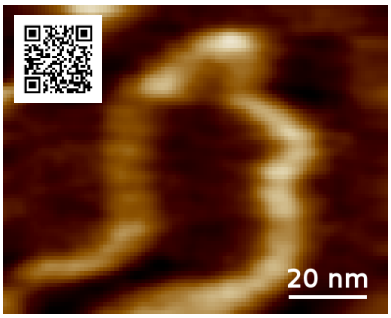

Fig.1 *Individual DNA molecules imaged in liquid on a polycationic substrate at 50 frames/sec. Follow the QR code to watch the entire 1000 frame sequence.*

DNA molecules *in vivo* and *in vitro* can vary in length and form, which can give rise to topological differences, which in turn affects the classical inter-strand base-pairing and is referred to as supercoiling [1]. Various factors can affect the degree of DNA supercoiling, among these being the interactions with molecules, such as other nucleic acids, DNA-specific enzymes, etc. With certain protocol optimizations [2], DNA can be imaged at a state in which it carries mostly torsional energy, and starts to exhibit partial dehybridization (unwinding of the double-strand region), as an attempt to minimize the torsional stress on the entire super-coiled construct (**Fig. 2**). This carries the potential to

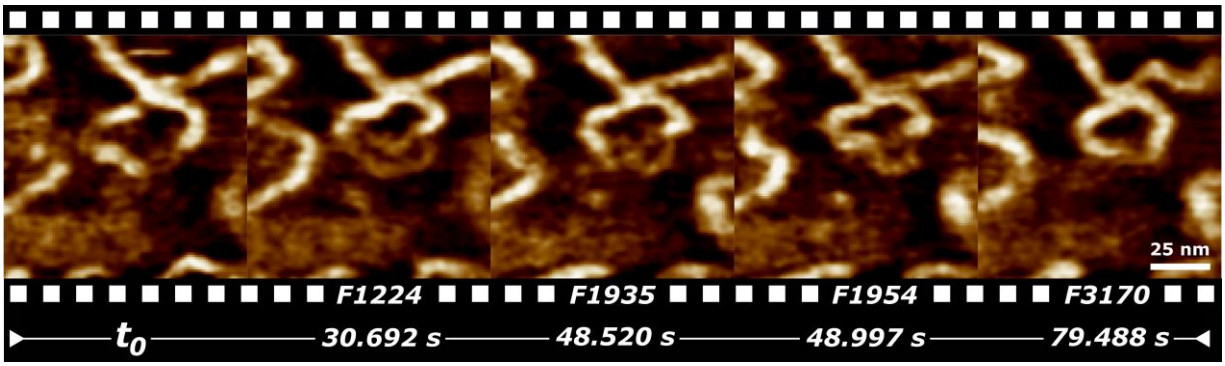

Fig.2 *Selected frames from a high-speed AFM video describing the thermodynamic single strand fluctuations of a dehybridized DNA helix in liquid. The video recorded at 40 frames/sec features three different kinetic regimes – dehybridization, metastable phase and closing of the DNA bubble (strand loop).*

study the kinetics of DNA double-strand de-/rehybridization in fundamental processes in molecular and cellular biology, such as transcription, replication, recombination, repair, and even PCR (polymerase chain reaction) [3].

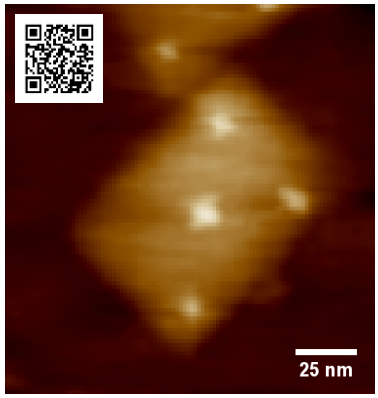

Fig.3 *DNA origami nanostructures containing 5 biotin binding sites on mica imaged in buffer in the presence of streptavidin. Streptavidin binding/unbinding can be observed on top of the DNA origami nanostructures as bright dots appearing/disappearing. The video is taken at 50 frames/sec and consists of over 1400 frames. In collaboration with C.M. Domínguez, C.M. Niemeyer, Institute for Biological Interfaces (IBG-1), KIT (Germany). Follow the QR code to watch the entire video*

DNA origami nanostructures (DONs) have emerged as excellent molecular pegboards for the immobilization of ligands on surfaces to study early signaling events in adherent cells. The bottom-up self-assembly of such supramolecular architectures can be harnessed to create bioinstructive materials, such as, nanocomposites for cell receptor stimulation [4] or biosensor surfaces for investigation of nanoscale effects on early cell signaling [5]. These applications take advantage of the effective linkage between receptor ligands and the DONs through high-affinity biotin-streptavidin bridges. High-speed AFM (HS-AFM) data has been obtained from DONs containing biotin binding sites, imaged in fluid in the presence of streptavidin at 20 ms per frame (**Fig. 3**). The occupation of each binding site can be analyzed, thus potentially revealing details on the binding properties and dynamics, which could be tailored by changing the chemical nature of the nanoscale binding sites [6].

The NanoRacer® High-Speed AFM marks a quantum leap in quantitative imaging capabilities. The real-time visualization of dynamic biological processes with nanometer resolution has been designed with the concept of ease-of-use to enable researchers to focus on discovering new and exciting possibilities for Life Science applications, and gain an in-depth understanding of complex biological systems and molecular mechanisms, in a way not possible until now.

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[2] Bruker Nano Surfaces, 'High-speed imaging of DNA submolecular structure and dynamics', *Microscopy and Analysis*, no. SPM Supplement, 2019.

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[5] P. Lanzerstorfer, U. Müller, K. Gordiyenko, J. Weghuber, and C. M. Niemeyer, 'Highly Modular Protein Micropatterning Sheds Light on the Role of Clathrin-Mediated Endocytosis for the Quantitative Analysis of Protein-Protein Interactions in Live Cells', *Biomolecules*, vol. 10, no. 4, p. 540, 2020.

[6] C. M. Domínguez, A. Kraus, H. Haschke, and C. M. Niemeyer *(in preparation)*.