Topography and Recognition Imaging with PicoTREC,

Topography and Recognition Imaging with PicoTREC, a Scanning Probe Microscopy Accessory for Mapping Target Molecules on a Sample Surface

Application Note

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The development of the atomic force microscope (AFM) has allowed scientists and engineers to observe the details of molecular structures with unprecedented resolution and without the need for rigorous sample preparation or labeling. In addition to topographical imaging, AFM can sense nanomechanical and other fundamental properties of sample surfaces, including local adhesion and elasticity. Microscopic adhesion affects a variety of events, from the behavior of ceramics and semiconductors to DNA replication and drug binding in the human body. The AFM offers a tool to study these important parameters on the nanometer scale using a technique that detects forces on the AFM probe tip as it approaches and retracts from a surface.

The Institute for Biophysics at the University of Linz, Austria, has developed a technique in which biological molecules are attached to the AFM tip on the end of a *linker* (Figure 1). The linker is an elastic PEG (polyethylene glycol) tether that gives the sensor molecule the freedom to reorient itself properly to bind to its target on the surface. When a recognition event occurs, the AFM detects the force required to break the bonds that are involved in molecular binding. This technique is being used to detect binding forces between antibody-antigen pairs, drug-receptors, and sense-antisense DNA complexes (1).

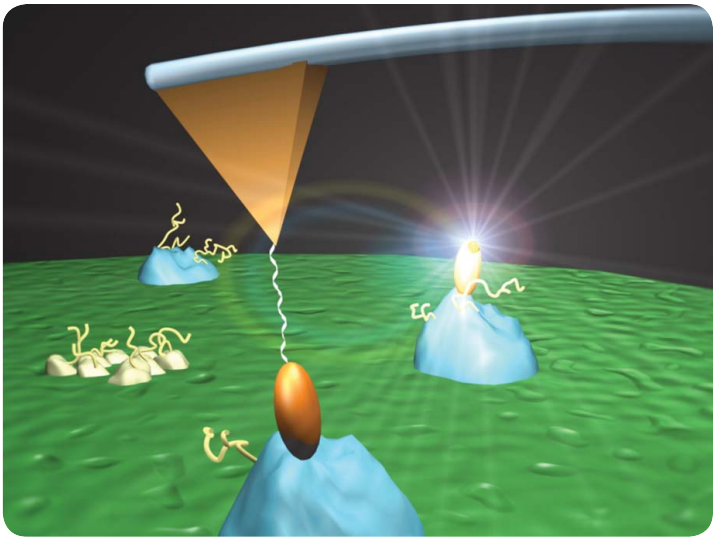

Figure 1. Schematic representation of an AFM tip measuring the nanomechanical properties of a flexible tether bound to a biological molecule (brown) recognizing its target (blue) on a surface. In TREC (topography and recognition) scanning mode, target molecules can be detected on the surface.

When combined with dynamic force microscopy (Agilent's MAC Mode AFM, in particular), entire maps of specific binding sites are obtained by scanning the tethered sensor molecule across the surface to detect binding events (2).

More recently, an Agilent imaging solution known as PicoTREC has been used to simultaneously record two separate images, one that provides the topography of target molecules on a surface and another that displays a map of specifically recognized target molecules on a surface (3, 4). Using MAC Mode, PicoTREC, and optimized AFM cantilevers, a ligand can be kept in close proximity to the surface, allowing efficient recognition and gentle interaction between tip and sample during scanning. PicoTREC resolves molecular recognition during the lateral scan by processing the asymmetric reduction of the oscillation amplitude. In this way, the locations of target molecules are easily determined from their coordinates on the recognition image.

The PicoTREC has been demonstrated with a antihistone antibody tethered to the AFM tip recognizing chromatin molecules bound to a mica surface (Figure 2). The recognition image disappears when avidin-biotin interactions are blocked by free biotin (5). This method eliminates requirements for secondary reporter systems. For example, no fluorescence, radioactivity, or enzyme-linked detection schemes are needed when using PicoTREC. Furthermore, the system is extremely sensitive, since single molecule interactions are readily detected. PicoTREC can be used for nanometer-scale epitope mapping of biomolecules, to localize receptor sites during biological processes, or to expedite force spectroscopy studies on areas of interest.

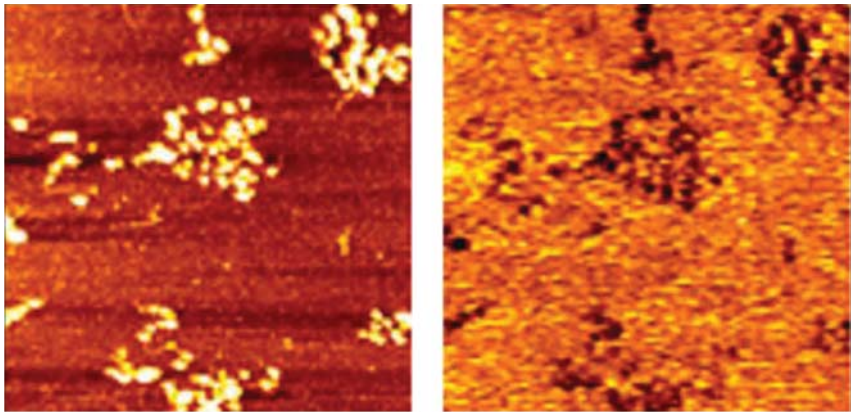

Figure 2. PicoTREC image of MMTV chromatin (DNA and histone protein complex) covalently attached to mica. The Topography image (left) shows individual chromatin molecules. The RECognition image (right) shows single molecule binding events between the antihistone antibody, which has been attached to the AFM tip, and histone molecules on the surface. Scan size approximately 500 nm x 500 nm.

References

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