Identifying Nanoscale Molecular Binding Events With SPM/AFM

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ABSTRACT

PicoTREC™ combines high resolution (nanometer-scale) topographic imaging with *in-situ*, single molecule (picoNewton-scale) detection to map out molecular recognition events on biological samples. When a ligand for a particular target receptor is attached to the tip of an SPM/AFM cantilever, the cantilever becomes a sensitive, chemically selective biosensor for that target. The mechanical force that is required to break the chemical bonds between the ligand on the tip of the cantilever and target molecules immobilized on a substrate can be detected by the SPM/AFM. The locations of these molecular interactions are simultaneously resolved and mapped by PicoTREC. Maps of molecular binding interactions across a variety of surfaces can quickly and easily be obtained with PicoTREC and it is not dependent on fluorescence, radioactivity, or enzyme-linked detection schemes. PicoTREC has been used to image, map and analyze the chemical compositions of a variety of samples; including molecular interactions between nucleic acids and proteins, antibodies and antigens and small ligands and their receptors.

Keywords: PicoTREC, SPM, AFM, scanning probe microscope, atomic force microscope, MAC Mode, molecular mapping, biosensor, antibody-antigen, ligand-receptor, nanobiotechnology

1 INTRODUCTION

Molecular binding events are critical factors in a variety of biological phenomenon. For example, molecular interactions are responsible for the initiation, modulation, and termination of DNA replication, RNA transcription, enzyme activity, infection, immune response, tissue generation, wound healing, cellular differentiation, programmed cell death, and the activities of drugs, hormones, and toxic substances, to name just a few. PicoTREC™ (TREC stands for topography and recognition imaging) is an accessory for the Agilent 5500 SPM/AFM (scanning probe microscope/atomic force microscope) that combines high resolution dynamic mode SPM/AFM imaging with nanometer-scale molecular recognition event detection. PicoTREC is being used to identify and map picoNewton-scale ligand-receptor interactions by utilizing the sensitivity of an SPM/AFM based, single molecule biosensor [1].

Scanning probe microscopy offers many unique advantages over various other methods to the study of biological process at the nanometer scale. It allows scientists to visualize, probe, and analyze the structures of biological molecules in their native environments with unprecedented resolution and without the need for extraneous labels or tags. It does not require rigorous sample preparation; which is generally required for high resolution imaging studies using electron microscopy. Scanning probe microscopy has become an important tool to study the nanomechanical properties of biological samples; including adhesion, hardness, and elasticity [2].

2 SINGLE-MOLECULE RECOGNITION STUDIES WITH THE SPM/AFM

Scanning probe microscopy is unique in its ability to direct and detect single molecule inter- and intra-molecular forces at the picoNewton scale. In force-distance spectroscopy experiments, SPM/AFM can be applied to detect and quantify molecular unbinding interactions between ligands attached to an SPM/AFM tip and discrete receptor molecules immobilized on another surface [3]. When a ligand for a particular receptor target is attached to the tip of an SPM/AFM cantilever, the cantilever becomes a sensitive, chemically selective biosensor [4]. When the molecular entities on the tip of the cantilever are allowed to interact with complimentary receptor molecules immobilized on a substrate, a ligand-receptor bond is formed. Upon retraction of the SPM/AFM tip, the ligand-receptor complex deforms until the attractive interactions which hold the complex together are broken and the complex unbinds. The mechanical force that is required to break the complex between the molecules on the tip and the receptors can be directly detected and recorded by a scanning probe microscope. By performing many unbinding experiments, rate constants and the affinities of unbinding for ligand-receptor complexes can be calculated [5]. Structural data about the binding pocket can also be inferred from this data. Unfortunately, most biological samples are composed of more than one component and important topographic or structural data about the target and the ligand-receptor complex is generally lacking from these force-distance spectroscopy experiments. Also, force-distance spectroscopy experiments often tend to be lengthy, taking hours to complete, which can be a problem with biological samples in unsterile environments or when using samples that denature or decompose easily under experimental conditions [6]. Therefore, the need to generate relatively fast, high resolution results from molecular

recognition experiments is especially evident in studies on biological samples and mixtures.

3 TOPOGRAPHY AND RECOGNITION IMAGING WITH PICOTREC

For mixed biological samples, like protein-protein or DNA-protein complexes and complex biological surfaces such as cells or membranes, high-resolution topographic SPM/AFM imaging can be combined with molecular recognition force detection using PicoTREC. With PicoTREC, biological ligands, such as antibodies or drugs, are attached to the end of relatively short (~8-10 nm), elastic polyethylene glycol (PEG) tether that is in turn attached to the tip of an SPM/AFM cantilever. The PEG tether gives the ligand freedom to diffuse within a defined volume of space [7]. The tether also imparts upon the ligand the ability to reorient as it approaches or comes in contact with the target in order that it may bind properly to the immobilized receptor [8].

PicoTREC is operated using Agilent's patented magnetic AC imaging mode (MAC Mode^{TM}) to permit efficient biomolecular recognition. Optimized SPM/AFM cantilevers and MAC Mode operation provide gentle interactions between the SPM/AFM tip and sample during scanning. The extremely clean, sensitive and precise cantilever oscillation provided by MAC Mode permits the ligands on the tip of the cantilever to be kept in close proximity to the molecular receptors on the sample without damaging the sample [9]. PicoTREC detects the minute forces that are required to break the single molecule interactions involved in molecular binding and generates a map of these single molecule binding events by recording and displaying two very important, but separate, images simultaneously [10, 11]. One of these images is a high resolution, MAC Mode topographic image of the sample. The second image is a map of molecular recognition events between the ligands on the SPM/AFM tip and the receptor molecules on the sample. The images and maps of molecular binding events are obtained in real time.

PicoTREC resolves molecular recognition events during lateral scanning by processing the asymmetric reduction of the MAC Mode oscillation amplitude that occurs when a tip-bound ligand and its immobilized target interact. In this way, the locations of binding interactions can quickly and easily be determined from their coordinates on the recognition image, along with the corresponding MAC Mode topography image of the sample.

4 SUMMARY

Simultaneous nanometer-scale topography and recognition imaging experiments have moved SPM/AFM research beyond basic imaging and molecular recognition studies on mono-component samples. PicoTREC and the Agilent 5500 SPM/AFM have been successfully utilized to identify and map the chemical compositions and surface topographies of various biomolecular recognition systems and biological entities. PicoTREC gives SPM/AFM researchers the ability to image and chemically distinguish between discrete nanometer-scale molecular entities at the single-molecule level, so it is especially useful to analyze the components of heterogeneous samples and to resolve chemical information from samples that can not be distinguished from topographic images alone. PicoTREC has been demonstrated to be applicable to a variety of biological interactions and molecular recognition systems in their native, physiolgical environments; including antibody-antigen [12], nucleic acid-protein [13], and small ligand-receptor interactions [14].

5 REFERENCES

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