Sensing and Plotting Single Molecule Binding Events with the Atomic Force Microscope

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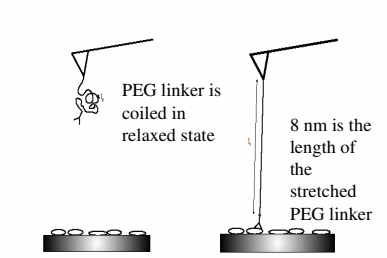
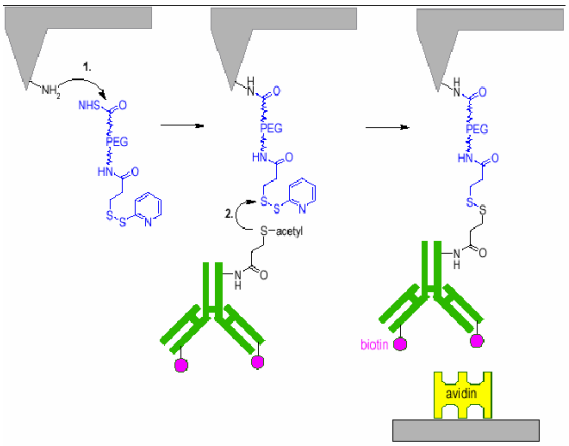
SPM/AFM (scanning probe microscopy/atomic force microscopy) offers many unique advantages to the study of biological process at the nanometer scale. ASM/AFM allows scientists to visualize, probe, and analyze the structure of biological molecules in their native environments with unprecedented resolution and without the need for rigorous sample preparation. Nanoscale binding events are known to be the primary events in a variety of biological phenomenon, from DNA replication and RNA transcription, to immune response, tissue growth, cellular differentiation, and the action of drugs, hormones, and toxic substances, to name just a few. SPM/AFM is also unique in its ability to detect nanoscale molecular forces such as those that occur on an SPM/AFM tip as it approaches and then retracts from a surface. This property has exposed the possibility of measuring inter- and intra-molecular forces at the single molecule level.

Force-spectroscopy is often used to study and quantify binding interactions between purified ligands immobilized on an SPM/AFM tip and discrete target molecules on a surface. In this technique, an SPM/AFM tip and ligands come into contact with a surface that contains complimentary targets or receptors. As the ligands and the targets contact, a ligand-receptor bond is formed. Upon retraction of the SPM/AFM tip, the ligand-receptor binding complex deforms until the attractive interactions which hold the complex together unbind. The forces of unbinding are detected and recorded by the SPM/AFM. By performing many unbinding experiments, rate constants and the affinities of binding can be calculated. 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 structural data is often lacking from these experiments. These experiments also tend to be lengthy, taking hours to complete, which is a problem when dealing with samples that easily degrade.

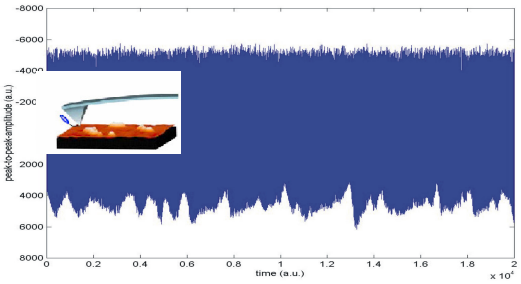
Fast structural and recognition results are useful in recognition studies of biological mixtures. For mixed samples, like protein-protein or DNA-protein complexes, and biological surfaces such as cells, or membranes, high-resolution topography imaging can be combined with force detection. TREC (*T*opography and *REC*ognition imaging) is a powerful, new SPM/AFM imaging mode that combines molecular-scale imaging with the sensitivity of a picoNewton-scale single molecule biosensor. With TREC, 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 an SPM/AFM tip. The PEG tether gives a ligand the freedom to move freely in a defined space and to reorient in order bind properly to an immobilized target. When a recognition event between the ligand and the target occurs, the SPM/AFM detects the minute forces that are required to break the interactions which are involved in molecular binding. The technique is being used to measure binding forces between antibodies and antigens, drugs/hormones and receptors and between protein-DNA complexes. When combined with MAC Mode™ (magnetic AC mode) SPM/AFM, entire maps of specific binding events between ligands and targets can be obtained in real time. The TREC maps are generated by scanning the PEG-tethered ligand across the surface to detect single molecule binding events. Optimized SPM/AFM cantilevers and MAC Mode provide the prerequisite extremely clean, sensitive and precise SPM/AFM signals, and permit the ligands to be kept in close proximity to their receptors. This allows for efficient biomolecular recognition and for gentle interaction between the tip and sample during scanning. PicoTREC™ is the hardware component of the system that records two very important, but separate, images simultaneously. One image provides the topography of the target molecules that are immobilized on the surface. The second image displays a map of binding interactions between the ligands on the SPM/AFM tip and the target molecules that are immobilized on the surface. 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 comparison to the corresponding topography image.



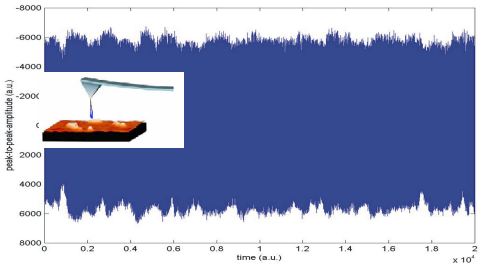
Flexible, polyethylene glycol linker



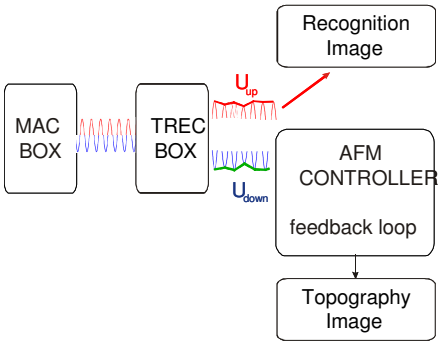
Immobilizing a specific ligand (biotinylated IgG) to an SPM/AFM tip and a target (avidin) to mica.



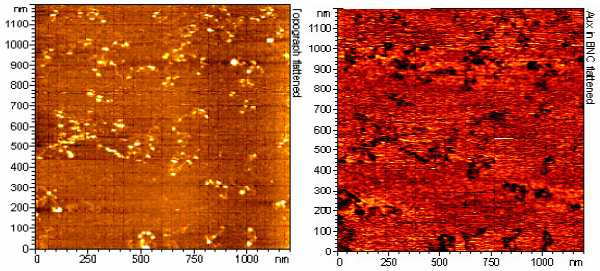
MAC Mode signal in the absence of a recognition event



MAC Mode signal in the presence of a recognition event



PicoTREC simultaneously records topography and recognition images using the maxima and minima of each sinusoidal cantilever oscillation



Simlutaneous Topography (left) and RECognition (right) images of chromatin (histone-DNA complexes) using antihistone H3 modified SPM/AFM tips. Recognition sites are indicated by dark areas

REFERENCES

1. Ebner A., *et al*: ChemPhysChem. 6, 897-900 (2005)
2. Kienberger, F., *et al*: BIOforum Europe 06/2004, 66-68 (2004)
3. Liu, Y. Z., *et al*: Langmuir. 1r, 8547-8548 (1999)
4. Hinterdorfer, P.: Handbook of Nanotechnology (Ed.: B. Bushan), Springer Verlag, Heidelberg, 475–494 (2004)
5. Stroh C. M., *et al*: PNAS 101, (2004)
6. Stroh C. M., *et al*: Biophys. J. (2004)
7. Noy, A., *et al*: Annu. Rev. Mater. Sci. **27,** 381–421 (1997).
8. Allison D. P., *et al*: Curr. Opinion Biotechn. 13, 47–51 (2002)
9. Riener C., *et al*: Anal. Chim. Acta 497, 101–114 (2003)
10. Kienberger F., *et al*: Single Mol. 1, 123–128 (2000)
11. Han, W., *et al*: Nature. 563 (1997)
12. Wang, H., *et al*: *Biophys. J.* **83,** 3619–3625 (2002)
13. Kienberger F., *et al*: Biol. Proc. Online 6, 120–128 (2004)
14. Raab A., *et al*: Nat. Biotechnol. 17, 901–905