Platform AL: Atomic Force Microscopy

1776-Plat An Ultrastable Atomic Force Microscope for Structural Biology

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Historically, the atomic force microscope (AFM) community has focused on developing sharper tips and higher sensitivity force detection schemes for increased resolution. Yet, lateral drift remains a critical, largely unaddressed issue that limits tip-sample registration, signal-to-noise ratio, and image resolution. A handful of methods actively minimize drift, but these techniques restrict the imaging mode of the microscope or do not yield atomic-scale (≈100 pm) stability. Here, we demonstrate that by scattering a laser off the apex - not the back side - of a commercial AFM tip, we can locally measure and thereby actively control a tip's lateral position to 12 pm (rms @ 10 Hz) in ambient conditions for 100 s. Furthermore, in conjunction with advances in sample stabilization, we achieved atomic-scale tip-sample lateral stabilization and registration during imaging. This work extends atomic-scale stability and registration previously restricted to cryogenic temperatures and ultra high vacuum, to a wide range of operating environments including aqueous conditions. With this level of control, this ultrastable AFM enables returning to a particular feature in an image, such as a domain of a protein, and hovering the tip over this feature for extended periods - allowing detailed kinetic study of the protein's structural dynamics.

1777-Plat Topographic and Phase Images of DNA and Amyloid-Like Insulin Fibrils in Fluid Using Direct Magnetic Actuation

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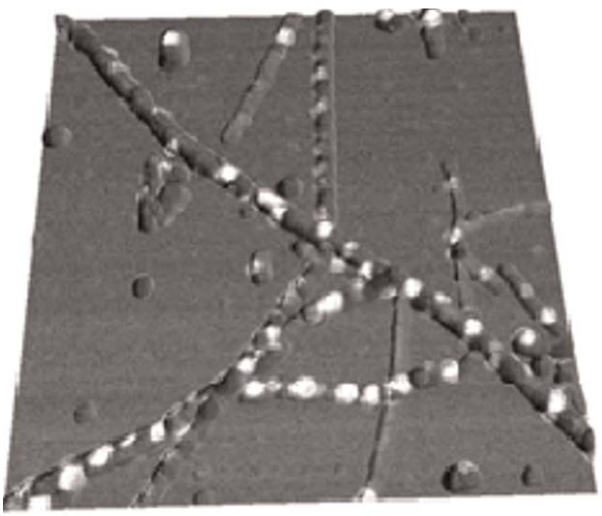
Direct magnetic actuation of an AFM cantilever allows simplified tuning, operation and phase response interpretation in fluid. In contrast to other methods, the phase response is similar to that of cantilevers driven in air. The iDrive™ method uses Lorentz Force to actuate the cantilever as an oscillating current is driven across the cantilever. Using a 100μm-long cantilever (k=0.09 N/m), we observed cantilever tunes in fluid that had near-perfect overlap with the thermal noise power spectrum and very smooth phase response. These characteristics enable advanced techniques like Q-control and DualAC imaging. We demonstrate the utility of this technique by acquiring high-resolution images of lambda-digest DNA and insulin fibrils. An example of combined topographic and phase imaging is shown in the insulin fibril image where the phase contrast is "painted" onto the rendered topography. Insulin fibrils belong to the family of amyloid fibrils and their structure consists of parallel beta sheets that are stacked perpendicular to the fibril axis. Structure of amyloid fibrils at the mesoscopic level is poorly understood and this recent data may provide some insight into their assembly.



1778-Plat Electrophoretic Deposition Of Proteins Using Atomic Force Control

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Capillary electrophoresis (CE) is a rapid and efficient technique for separation of a variety of compounds including proteins and other bio-molecules. Research in our laboratory has shown that cantilevered quartz nanopipettes can be used for Fountain Pen NanoLithography (FPN) with atomic force controlled delivery of liquid phase chemicals such as etchants, proteins etc. By combining these two techniques we can achieve the correlation of the separation of chemicals in time with spatially controlled nanodeposition to obtain Atomic Force Controlled Capillary Electrophoresis or ACCE. Here we demonstrate electrophoretic delivery of Bovine Serum Albumin (BSA) upon mirrorepoxy substrate, using atomic force microscopic (AFM) techniques. The electric field is applied between two electrodes that are positioned on the nanopipette itself. This allows the use of variety of substrates for different applications. A cantilevered nanopipette with 0.1–0.5 micron aperture sizes is filled with a solution of BSA in pH 8 buffer where the protein is negatively charged. Positive polarity of the applied voltage causes the protein to migrate to the substrate while negative polarity reverses the flow direction of the protein and moves it away from the substrate. Additional mechanisms of the voltage control of deposition are also described including electroosmotic flow (EOF). The detection of the protein on the substrate is achieved by reflection fluorescence near field imaging (NSOM) techniques.

1779-Plat Local Viscoelasticity of the Surfaces of Individual Gram-negative Bacterial Cells Measured Using Atomic Force Microscopy

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The cell wall of Gram-negative bacteria is responsible for many important biological functions: it plays a structural role, it accommodates the selective movement of molecules across itself, it undergoes changes made necessary by growth and division, and it transfers information about the environment into the cell. These functions not only suggest that the cell wall is dynamic, but that its mechanical properties are of significant importance. In the present study, we have used a novel, AFM-based approach to probe the mechanical properties of single bacterial cells by applying a constant compressive force to the cell under physiological conditions while measuring the time-dependent displacement (creep) of the AFM tip due to the viscoelastic properties of the cell. For these experiments, we chose a representative Gram-negative bacterium, *Pseudomonas aeruginosa* PAO1, and we used AFM cantilever tips of different size and geometry. We find that the cell response is well described by a three element mechanical model which describes an effective cell spring constant k and an effective time constant τ for the creep motion. Adding glutaraldehyde, an agent that increases the covalent bonding of the cell surface, produced a significant increase in k together with a significant decrease in τ. This work represents a new attempt towards the understanding of the nanomechanical properties of single bacteria while they are under natural fluid conditions which could be of practical value for elucidating, for instance, the biomechanical effects of drugs (such as antibiotics) on pathogens.

1780-Plat Rational Manipulation Of The Mechanical Properties Of A Virus By Protein Engineering

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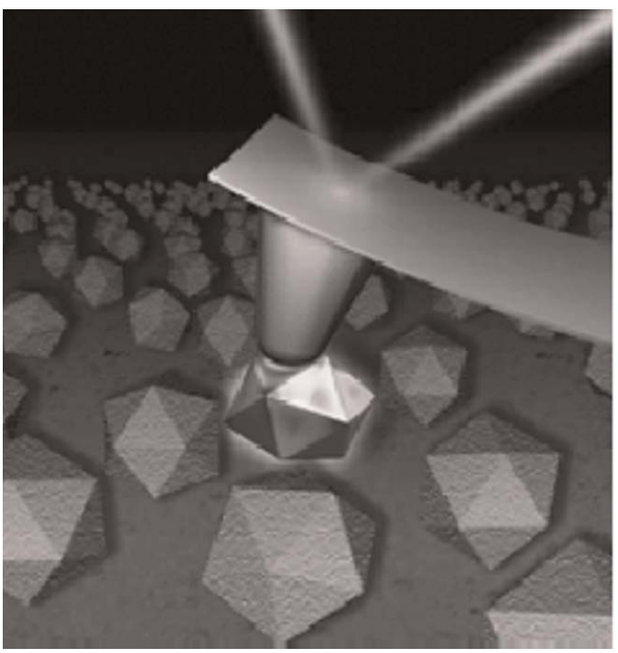
In a previous study we showed that the DNA molecule within a spherical virus, the minute virus of mice, exerts an architectural role by increasing the stiffness of the virus. This mechanical reinforcement is a consequence of the interaction between crystallographically visible DNA patches and the inner capsid wall. We tested this model by using protein engineering. Selected amino acid side chains have been truncated to remove major interactions between the capsid and the visible DNA patches, measuring the effect of the mutations on the stiffness of virus particles by using atomic force microscopy. The mutations do not affect the stiffness of the empty capsid, while they significantly reduce the difference in stiffness between the DNA-filled virion and the empty capsid. The results reveal that intermolecular interactions between individual chemical groups contribute to the mechanical properties of a supramolecular assembly, and identify specific protein-DNA interactions as the origin of the anisotropic increase in the rigidity of a virus. This study demonstrates that it is possible to control the mechanical properties of a protein nanoparticle by the rational application of protein engineering based on a simple mechanical model.



1781-Plat Simultaneous Atomic Force Microscopy and Patch clamp measurments on primary cilia of MDCK cells

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Primary cilia are solitary, immotile, hair-like structures that protrude from the apical membrane of kidney epithelial cells . Recent work has identified a link between primary cilia malfunction and Autosomal Dominant Polycystic Kidney Disease (ADPKD) suggesting that these organelles might act as flow sensors, signalling changes in luminal flow to control transepithelial ion transport along the nephron. We have imaged Primary Cilia on MDCK cells, using Atomic Force Microscopy (AFM). Cells were grown on glass coverslips and fixed with 2% glutaraldehyde in PBS to assess coverage. Live, unfixed cells were imaged under liquid, and as the cilia remain mobile and not adherent to a substrate, their movement

and orientation may affect the motion of the AFM cantilever. We imaged unfixed cilia using AFM, using a 'peel-off' method whereby a poly-L-lysine-coated coverslip, placed on top of a layer of cells in a Petri dish, is removed in a single movement, and then imaged *in situ*. The nanoscale surface structure of the immobilised cilia can then be imaged with the AFM. From an analysis of AFM data, we have estimated cilium flexibility, and have probed cilium elasticity using AFM in a nanomechanical mode. By correlating AFM images with single-point AFM force curves, we observe a clear difference in stiffness between the cilium and the cell surface. Using a combined AFM/confocal microscope we have correlated the structures imaged by AFM, to fluorescent markers for tubulin and actin. The staining observed by confocal microscopy confirms that the structures protruding from the cells in the AFM images are the primary cilia. In a further development, we will report results from a combination of AFM imaging and patch-clamp electrophysiology to probe how cilium bending is coupled to ion transport.

1782-Plat Atomic Force Microscopy Studies of Leukocyte Transmigration

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Leukocyte trafficking involves the homing of leukocytes to sites of injury during inflammation. It culminates with their migration across the endothelial cell junction, the integrity of which is maintained by adhesion molecules including the junctional adhesion molecule-A (JAM-A). JAM-A is able to form interactions with itself as well as with the integrin LFA-1 (leukocyte function-associated antigen-1). The interaction of LFA-1 expressed on the leukocyte surface with JAM-A as well as the disruption of the homophilic interaction of the JAM-A receptors *in trans* are a critical part of this process. In this study, we used atomic force microscopy (AFM) to characterize the dynamic strength of these interactions in order to determine which (JAM-A/JAM-A v. LFA-1/JAM-A) prevails under pulling forces. In addition we investigated the possible impact that the LFA-1/JAM-A interaction has on the homophilic JAM-A interaction *in trans.* Measurements of unitary LFA-1/JAM-A or JAM-A/JAM-A unbinding forces were obtained between LFA-1 expressing Jurkat cells or JAM-A on the cantilever tip and the JAM-A substrate. All measurements covered three orders of magnitude in force loading rate (50–60000 pN/s). Two loading rate regimes and higher unbinding forces were observed for the LFA-1/JAM-A interaction as compared to one regime and lower unbinding forces for the JAM-A/JAM-A interaction. Our results indicate that the JAM-A/LFA-1 interaction is better able to resist pulling forces than the JAM-A/JAM-A interaction, and imply that LFA-1 may modulate the mechanical strength of the JAM-A homophilic interaction.

1783-Plat Atomic Force Microscopy Exploration of the Extracellular Matrix

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A recent report describes the use of the extracellular matrix (ECM) secreted by HEPG2 cells as a culture surface to promote the *in vitro* establishment of characteristic membrane polarity of hepatocytes (Herrema et. al, Molecular biology of the Cell, vol. *17* (2006)). The re-establishment of the cell membrane polarity is important for global cell function and is thought to be driven by a compromise between cell-cell and cell-surface interactions. It is believed that the cell-surface interactions are influenced by surface properties such as surface energy, charge, topography, modulus and the presentation of ligands at the surface. In the case of the HEPG2 cell-derived ECM surface, the role of these surface properties in achieving the characteristic hepatocyte cell membrane polarity is unknown. We use Atomic Force Microscopy (AFM) and derivative techniques to explore the extracellular matrix on dimensional scales relevant to individual cells. Standard AFM topography imaging yields ECM surface roughness. Nanoindentation measurements provide insight on mechanical properties across the ECM surface. Single molecule force spectroscopy allows examination of the conformational and binding properties of proteins and peptide ligands exposed on the ECM surface. Functionalization of the AFM tip, e.g. via hydrophobic silane chemistry, enables chemical force microscopy probing of specifically targeted domains on the ECM surface. Once a characterization profile is established for the extracellular matrix, we may explore the influence of the underlying substrate on the cell-derived ECM surface. For example, the ECM produced by cells and adsorbed to a hydrophobically-treated glass will be compared to that produced by cells on hydrophilic-treated glass. Correlation of substrate properties to ECM properties to subsequent cell growth will further fundamental understanding of cell-surface interactions and its influence on cell morphology and function.

Symposium 15: Awards Symposium and IUPAB Arne Engstrom Lecture

1784-Symp Breaking the Nanometer Barrier: Biophysics, One Molecule at a Time

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Advances have led to a new field of exploration, dubbed *single molecule biophysics*. Prominent among the enabling technologies is the optical trap, also called 'optical tweezers.' When combined with *in vitro* assays for function, optical traps can perform measurements