nanofibrillar matrix that has demonstrated promise in tissue engineering approaches for the repair of the injured spinal cord and is architecturally mimetic for the capillary basement membrane at the blood brain barrier. We will present quantitative investigations of the nanofibrillar matrix, which are achieved through use of a dynamic new mode of atomic force microscopy, Scanning Probe Recognition Microscopy (SPRM). SPRM uniquely allows auto-tracking along individual nanofibers, which are then compiled into a statistical representation of the nanofibrillar matrix as a whole. Complementary transmission electron microscopy (TEM) and nuclear magnetic resonance (NMR) investigations are performed to assess nanofiber internal structures that contribute to elasticity, and growth factor binding site information, respectively. Results of investigations of unmodified nanofibers and nanofibers covalently modified with fibroblast growth factor-2 (FGF-2), a prevailing cytokine involved in regulation of the growth of astrocytes, neurons, and other neural cells, will be presented. Astrocytes are neural cells that can be considered to be the cellular bridge between the capillary basement membrane and neurons. They are therefore directly responsive to the biochemical cues and physical properties of the native or prosthetic basement membrane. We will present results achieved through SPRM, immunocytochemistry, and Western blot techniques that indicate that there are significant differences in the astrocyte response to 2D planar substrates versus 3D nanofibrillar substrates versus 3D nanofibrillar substrates that are covalently modified with FGF-2, mimicking the sequestration of growth factors on the basement membrane. Differences in astrocyte physiology, substrate probing through lamellipodia and filopodia extension and FGF-2 up-regulation will be discussed.

984-Pos

Misfolding and Aggregation of Amyloid Beta Peptide: Single Molecule AFM Force Spectroscopy

Bo-Hyun Kim, Yuri L. Lyubchenko.
University of Nebraska Medical Center, Omaha, NE, USA.

Misfolding and aggregation of amyloid beta (Aβ) peptide result in development of Alzheimer's disease, and Aβ dimers are considered as the smallest neurotoxic species. The aggregates formed by Aβ-peptides have been characterized by various techniques, but our knowledge on the molecular mechanism underlying the processes of misfolding and the early stages of aggregation of the peptides is limited. We have shown earlier that AFM force spectroscopy is capable of detecting protein misfolded states and characterizing the initial stages of the protein aggregation. Importantly, we showed that α-synuclein dimers are stable transient states playing role of triggers in the process of the protein self-assembly in nanopartiles and fibrils. Here we applied the sample methodology to probing and characterizing of misfolding of Aβ40 peptide. The protein was immobilized on the AFM tip and the surface and the interaction between the proteins was measured in multiple approach-retraction cycles. Using this approach was able to analyze interprotein interactions at single molecule level. The force spectroscopy analysis provided us with the following important information. First, using Dynamic Force Spectroscopy (DFS) approach we characterized pathways of Aβ40 misfolding. The lifetimes of transient Aβ40 dimers can be as long as several seconds suggesting that formation of the states with such a lifetimes can trigger the aggregation. Second, the pathways for the misfolding and aggregation depend on pH leading to a rather complex energy landscape reconstructed from the DFS data. Third, the analysis of the contour lengths supported the conclusions on various aggregation pathways and led to the models for such conformations of Aβ40.

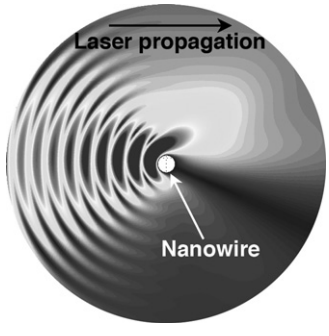
This work is supported by DE-FG02-08ER64579 grant from DOE.

985-Pos

Nanowires As AFM Cantilevers: A Detection Scheme to Gently Image Soft Biological Materials in Fluids

Babak Sanii, Paul D. Ashby.
Lawrence Berkeley National Lab - Molecular Foundry, Berkeley, CA, USA.

Performing AFM on soft materials in fluids (e.g., living cells) is challenging due to their ready deformation by the tip. The thermal force-noise of the cantilever is the principal limitation to reducing sample deformation and minimizing a cantilever's cross- section reduces its noise significantly. However, the minimum size of the cantilever is currently limited by a conventional deflection detection scheme, which requires a large surface area for laser specular reflection. Here we develop an optical technique to use nanowires as cantilevers, and show that we achieve a force noise in water that is orders of magnitude gentler than conventional AFM. This is a significant milestone towards non-invasive scanning probe imaging of biological processes on the surfaces of vesicles and cell membranes.



986-Pos

Nucleosome Dynamics : Atomic Force Microscopy Reveals its Intimity

Pascale Milani[1], Zofia Haftek-Terreau[2], Guillaume Chevereau[1], Philippe Bouvet[3], Françoise Argoul[1], Alain Arneodo[1].
[1]CNRS-Laboratoire Joliot-Curie USR 3010-Laboratoire de physique UMR 5672, Lyon cedex 7, France, [2]CNRS-Laboratoire Joliot-Curie USR 3010, Lyon cedex 7, France, [3]CNRS-Laboratoire Joliot-Curie USR 3010-Laboratoire de Biologie Moléculaire de la Cellule, Lyon cedex 7, France.

Recent genome-wide nucleosome mappings along with bioinformatics studies have confirmed that the DNA sequence plays a more important role in the collective organization of nucleosomes in vivo than previously thought. Yet, in living cells, this organization of nucleosomes also results from the action of various external factors like DNA binding proteins and chromatin remodelers. To decipher the code for intrinsic chromatin organization and dynamics, there is thus a need for in vitro experiments to bridge the gap between computational models of nucleosome sequence preferences and in vivo nucleosome occupancy data. Here we first combine atomic force microscopy (AFM) in liquid and theoretical modeling to demonstrate that the main sequence signaling in vivo are high energy barriers that locally inhibit nucleosome formation rather than favourable positioning motifs. We show that these excluding genomic energy barriers condition the collective assembly of neighboring nucleosomes consistently with equilibrium statistical ordering principles. The analysis of two gene promoter regions in S.cerevisiae and the human genome indicates that these genomic barriers direct the intrinsic nucleosome occupancy of regulatory sites, thereby contributing to gene regulation. We further apply time-lapse AFM imaging to directly visualize the dynamics of a single nucleosome nearby a genomic excluding energy barrier. The observation, in the absence of remodelers, of the unwrapping and/or ejection of this nucleosome suggests that the sequence-dependent intrinsic nucleosome dynamics can contribute to chromatin remodeling. These results provide novel hypotheses about chromatin dynamics and its contribution to gene regulation.

987-Pos

Mechanically Induced Cell Signaling Stimulates Real-Time Cytoskeleton Remodeling

Soon-Mi Lim, Jerome Trzeciakowski, **Andreea Trache.**
Texas A&M Health Science Center, College Station, TX, USA.

External mechanical stresses alter the structural and functional properties of the cells, leading to rapid responses that induce adaptive changes to the external environment. The extracellular matrix is responsible for a complex cross-talk needed for transmitting environmental signals to the cell through the focal adhesions as mediators of the process. An Atomic Force Microscope (AFM) probe functionalized with fibronectin was able to mechanically stimulate the apical surface of a live smooth muscle cell inducing significant changes in cell shape that can be recorded in real time by optical imaging. Due to the strong focal adhesion formed around the AFM tip, the cytoskeletal elements are directly manipulated through a matrix-integrin-actin linkage between the cell and the fibronectin coated tip. Following each controlled upward movement of the cantilever, the cell responds by presenting a biphasic change in height dependent of the treatment applied, and independent of time. In the same time, the cell reinforces its attachment to the substrate to better resist the mechanical stimulation by increasing focal adhesion and actin area at the basal cell level. Our measurements showed significant differences between control cells and cells where the intracellular tension was modulated by RhoA. Thus, in cells transfected with RhoA constitutively active the cell reactive-response presents higher amplitude than control because the cell is stronger due to the presence of more actin fibers. A different response was found when cells were transfected with RhoA dominant negative, which decreases intracellular tension, such that actin filaments are present only at the cell boundaries. Under these conditions, at the same force level, the AFM tip detaches from the apical cell surface. These innovative approaches offer new information for understanding live cell remodeling and dynamics in response to mechanical force.

988-Pos

AFM and SMFS of Clathrin Triskelia under Fluid

Svetlana Kotova[1], Eileen M. Lafer[2], Paul D. Smith[1], Ralph Nossal[3], Albert J. Jin[1].
[1]NIBIB/National Institutes of Health, Bethesda, MD, USA, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]NICHD/National Institutes of Health, Bethesda, MD, USA.

Atomic force microscopy (AFM) and single molecule force spectroscopy (SMFS) have been used to characterize the structure and interactions of clathrin triskelia, which are principal components of the protein coats surrounding certain plasma-membrane-derived vesicles involved in receptor-mediated endocytosis. Time sequence AFM images of wet triskelia resting on mica surfaces clearly demonstrate conformational fluctuations within individual triskelia, further strengthening indirect inferences from earlier AFM and electron microscopy of dried protein samples. Related studies using SMFS reveal a series of internal energetic barriers that characterize triskelion heavy chain domain unfolding. Protein sequence and force spectrum alignment analyses suggest that these features correspond to the unfolding of numerous alpha-helix hairpins of ca. 30 amino acid residues and cooperative unraveling of several hairpin domains up to the size of the known repeating motif of ca.145 amino acid residues. The dynamic domain rupture forces range from 10s of pN to over 500 pN, increasing continuously as the stretching loading rate increases, in accordance with the Bell model. To further understand the molecular functionality of clathrin, specific clathrin-substrate and clathrin-tip attachments via antibodies are being explored in ongoing investigations.

989-Pos

Elasticity Mapping of Pore Suspending Cell Membranes

Andreas Janshoff.
Institute of Physical Chemistry, Goettingen, Germany.

The mechanics of cellular membranes is governed by a non-equilibrium composite framework consisting of the semiflexible filamentous cytoskeleton and extracellular matrix proteins linked to the lipid bilayer. While elasticity information of plasma membranes has mainly been obtained from whole cell analysis, techniques that allow addressing local mechanical properties of cell membranes are desirable to learn how their lipid and protein composition is reflected in the elastic behavior on local length scales. Here, we introduce a novel approach based on a highly ordered porous matrix that allows elastic mapping on biomembranes on a submicrometer length scale. Spatially resolved indentation experiments carried out with a combined atomic force and fluorescence microscope permit to relate the supramolecular structure to the elasticity of cellular membranes. We found that pre-stress governs the response of black lipids membranes while a strong correlation between the density of the actin cytoskeleton and the measured membrane elasticity exists for native membrane fragments.

990-Pos

Development of Affine Surfaces for Specific Binding of Bacterial Fragments from Solutions Using AFM

Evgeniy V. Dubrovin[1], Galina N. Fedyukina[2], Sergey V. Kraevsky[3], Tatyana E. Ignatyuk[3], Igor V. Yaminsky[1], Sergei G. Ignatov[2].
[1]Faculty of Physics of Moscow State University, Moscow, Russian Federation, [2]State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russian Federation, [3]Alikhanov Institute for Theoretical and Experimental Physics, Moscow, Russian Federation.

In this work we have investigated biofunctional surfaces on glow discharged mica and their specific binding of bacterial cells and their fragments from aqueous solutions. Atomic force microscopy (AFM) was used to obtain the topography and to probe the mechanical properties of the biological surfaces. The method of quantitative estimation of the level of bound material was introduced using special processing software. We have found the conditions under which there was the pronounced effect of binding of bacterial fragments to their affine surface while the level of non-specific binding was very low. Our results demonstrate promising system for detection of bacterial fragments from solutions using AFM.

Acknowledgements. Support from ISTC (project 3245) and federal target program ''Scientific and educational research personnel to innovative Russia'' (projects P717, P973, P255) is acknowledged.

991-Pos

Force Measurement and Intracellular Operation Using Customized AFM Cantilever

Shinichi Machida[1,2], Takahiro W. Nakayama[1], Ichiro Harada[1], Rehana Afrin[1], Tomonobu Nakayama[2,3], Atsushi Ikai[1].
[1]Tokyo Institute of Technology, Yokohama, Kanagawa, Japan, [2]National Institute for Materials Science, Tsukuba, Ibaraki, Japan, [3]Univ. of Tsukuba, Tsukuba, Ibaraki, Japan.

The cell is the smallest unit of living matters and a great deal of interest centers upon engineering of this smallest unit using recently developed nanotechnological methods, not only for basic biological research, but also for medical applications. In this presentation, we show demonstration of direct intracellular manipulation and force measurement using specially customized cantilevers for atomic force microscopy (AFM).

Our focus of intracellular manipulation is the cytoskeleton of a fibroblast cell. In order to perform the manipulation and mechanical force measurement of the cytoskeleton, we modified the shape of an AFM probe into a hook using focused ion beam (FIB) technique. The refined-hook on top of the cantilever tip was designed to allow us to pick up, pull and finally cut a filamentous structure in the cell.

Fibroblast cells expressing fused green fluorescence protein (GFP) or red fluorescence protein (RFP) to actin were used to visualize actin stress fibers (SF) under the fluorescence microscope. We successfully performed force measurement and manipulation of stress fibers of semi-intact cells and in living cells. The measured force to cut SF varies from 20 nN to 40 nN depending on their thickness and situations. This is the first attempt to directly manipulate intracellular structures by mechanical means to obtain quantitative strength data on the cytoskeletal structures. We will discuss the details of analysis of force measurements and the future possibility of ''Single Cell Operation'' using customized AFM cantilevers.

992-Pos

Binding Kinetics and Binding Site Locations of Cytoadherent Molecules on the Surface of Malaria Infected Cells

Ang Li[1], Shyong Wei Tan[2], Chwee Teck Lim[3].
[1]Singapore-MIT Alliance, National University of Singapore, Singapore, Singapore, [2]Dept of Microbiology, National University of Singapore, Singapore, Singapore, [3]Dept of Mech Eng and Div of Bioeng, National University of Singapore, Singapore, Singapore.

It has been known for over a century that *Plasmodium falciparum* infected cells develop specific knob like structures on the surface that mediate cytoadherence to endothelial cells lining the blood vessel wall. This helps the malaria parasites to survive from splenic clearance. Although an array of ligand-receptor complexes have been demonstrated to be involved in cytoadherence and thus been proposed for drug targets, detailed binding kinetic properties and binding site locations of these complexes are largely unknown. We utilized atomic force microscopy based single-molecule force spectroscopy technique to investigate the binding kinetics of selected endothelial receptors with living malaria infected cells at different temperatures and also to map the binding sites on the surface of fixed cells to identify whether the corresponding ligand is knob associated protein or other surface bearing protein. Our results showed significantly different binding kinetics of the two selected endothelial receptors at different temperatures which explained the different rheological behaviors observed under flow conditions. Furthermore, the force mapping results on the binding locations helped us better understand how the surface structural changes facilitate cytoadherence and contribute to the disease.

993-Pos

The Mechanical Properties of Dry, Electrospun Fibrinogen Fibers

Justin L. Sigley, Stephen Baker, Christine Carlisle, Joel Stitzel, Joel Berry, Keith Bonin, Martin Guthold.
Wake Forest University, Winston-Salem, NC, USA.

Due to their low immunogenicity, fibrinogen fibers may be ideal candidates for tissue engineering scaffolds, drug delivery vehicles and other medical devices. However, their mechanical properties are incompletely understood. We have electrospun nanoscopic fibrinogen fibers from a solution of 6% by wt. bovine fibrinogen, 6% minimum essential medium (MEM), and 88% 1,1,1,3,3,3-hexaflouro-2-propanol (HFP). We used a combined atomic force microscopic (AFM)/optical microscopic technique to study the mechanical properties of individual fibers in dry, ambient conditions. Mechanical testing of fibers was done using the AFM to laterally stretch individual fibers suspended over 12 μm wide grooves in a transparent substrate. The optical microscope, located below the sample, was used to monitor the stretching process. Fibers ranging in diameter from 30-200 nm can stretch to 2.34 times their original length before rupturing at a stress of 5.9 GPa. These fibers behave elastically for stretches up to 1.19 times the original length of the fiber. Incremental stress-strain curves were collected to measure the viscoelastic behavior of the individual fibers. The total stretch modulus was 6.0 GPa while the relaxed elastic modulus was 4.1 GPa. While held at constant strain, fibrinogen fibers display a fast and slow relaxation time of 1.5 s and 16 s respectively. Dry electrospun fibrinogen fibers are about 1000 times stiffer than wet electrospun fibrinogen fibers, and nearly as extensible.