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

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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

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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.

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991-Pos

Force Measurement and Intracellular Operation Using Customized AFM Cantilever

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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

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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

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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.