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**

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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.

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**985-Pos**

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

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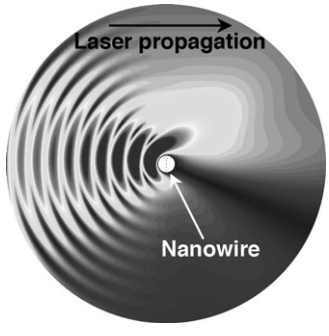
Performing AFM on soft materials in fluids (e.g., living cells) is challenging due to their ready deformation by the tip. The thermal force-noise of the cantilever is the principal limitation to reducing sample deformation and minimizing a cantilever's cross- section reduces its noise significantly. 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**

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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**

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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**

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