13-14. This region undergoes significant conformational changes during sugar translocation. Moreover, the results from the thio-D-glucose coupled via a long heterobifunctional crosslinker with a small end group showed the possibility to observe the D-glucose transport pathway of the SGLT1. These studies demonstrate that AFM is a powerful method to explore the structural and functional dynamics of plasma membrane transport proteins in live cells on a single molecule level.

979-Pos

Simultaneous Topography and Recognition (TREC) of Proteins in the Pathological Deposits in Pseudoexfoliation Syndrome using AFM

Rhiannon Creasey[1], Chris Gibson[1], Shiwani Sharma[1], Jamie Craig[1], Tom Becker[2], Peter Hinterdorfer[3], Nicolas Voelcker[1].
[1]Flinders University of South Australia, Adelaide, Australia, [2]Curtin University of Technology, Perth, Australia, [3]Johannes Kepler Universität Linz, Linz, Austria.

Protein aggregation is of significant interest to various disciplines; it can be the cause of debilitating diseases, or the foundation of advanced nanomaterials. One ocular disease hallmarked by protein aggregation is known as Pseudoexfoliation Syndrome (PEX). This condition is caused by the formation of insoluble aggregates, and is characterised by deposition of fibrillar proteinaceous material on the anterior lens capsule. PEX deposits in the eye block the aqueous outflow mechanisms, which can lead to an elevation in intraocular pressure and subsequent glaucoma. Glaucoma is the second leading cause of irreversible blindness worldwide, and PEX is the most common known risk factor for glaucoma.

Proteomic analyses have revealed an association of various genetic markers and protein expression with PEX; however a complete explanation for disease susceptibility is not known. As the aggregates are a complex arrangement of proteins, the ultrastructure is poorly characterised and many protein constituents of the aggregates remain unknown. This study addresses the critical issue of determining the molecular nature of PEX on lens capsules in their native state by atomic force microscopy (AFM) based antibody recognition imaging. This AFM methodology is referred to as Topography and RECognition imaging (TREC). Proteins identified as being implicated in the PEX pathophysiology are detected by an AFM probe modified with the appropriate antibody. Topographical AFM images and antibody recognition images are obtained simultaneously to determine the specific location of proteins in and around PEX aggregates. This data, combined with data from proteomic and genetic analyses, is leading to an improved understanding of the pathophysiological basis of PEX. A more complete understanding of the pathophysiological basis for the disease will lead to earlier detection methods and treatments that target the disease instead of the subsequent glaucoma.

980-Pos

Deciphering Podosome Physical Properties in Human Macrophage by Atomic Force Microscopy

Anna Labernadie.
IPBS-CNRS UMR 5089, Toulouse, France.

Macrophages belong to phagocytes which constitute the first line of host defense. Directed migration and adhesion of these cells through anatomic boundaries is crucial for their functions. Moreover, tissue infiltration of macrophages has been shown to worsen many pathologies such as atherosclerosis, chronic inflammation and cancer. Our research focuses on understanding the adhesive and motile behaviors of macrophages with particular emphasis on podosomes, dynamic actin-rich structures only found in macrophage and macrophage-derived cells required for normal adhesion and migration. Although the minimal structural feature of podosome can be defined as an F-Actin-rich core surrounded by a ring containing proteins such as Vinculin and Integrins, the mechanisms involved in podosome biogenesis and architecture are poorly understood. Thanks to the atomic force microscope operated in liquid, it is now possible to explore cells at the nanoscale in terms of topographic and force measurement mode. Here we choose to use this powerful technique to explore the mechanisms involved in biogenesis, bio-physical properties and architecture of podosome in human monocyte-derived macrophage. Micro-contact printing technique was used to generate patterns of different physiological extra-cellular matrix (ECM) proteins in order to delineate podosome formation in vitro and investigate the influence of the nature of the substrate on their bio-physical properties knowing that the molecular recognition of ECM protein inducing podosome formation involved an integrin-dependant signaling. Our preliminary experiments using AFM allowed us to measure the height, the dynamic and the Young's modulus of podosomes in different situations. We will present our last results of this ongoing work.

981-Pos

Geometric Influences on Radial Indentation of Microtubules

Zachary J. Donhauser, William B. Jobs, Edem C. Binka.
Vassar College, Poughkeepsie, NY, USA.

Microtubules assembled in vitro exist in a variety of configurations that vary in number of protofilaments, radius, and skew angle of protofilaments relative to the main microtubule axis. Such variations affect microtubule stability, energetics, and assembly/disassembly dynamics. Further, the most abundant microtubule geometries observed in vitro are influenced by assembly conditions and stabilization methods. We have studied the relationship between microtubule geometry and mechanical properties using finite element modeling (FEM). Specifically, we have examined the effects of protofilament number, microtubule radius, and protofilament skew on the radial stiffness (effective radial spring constant of the microtubule wall) of microtubules as measured in atomic force microscopy (AFM) experiments. Our previous AFM work determined that microtubules assembled in the presence of a slowly-hydrolysable GTP analog, GMPCPP, have enhanced radial stiffness relative to those stabilized with paclitaxel. We surmise that in vitro populations of GMPCPP-microtubules and paclitaxel microtubules contain distinct distributions of microtubule geometries, so we have used FEM to examine the relative effect of microtubule geometry on stiffness values we measure. Our modeling results indicate that the changes in stiffness that we have observed experimentally are not simply a result of changes in protofilament number or orientation but instead are likely due to a relative change in material properties (e.g. effective Young's modulus) of the tubulin polymers.

982-Pos

Organization of RAG1/2 and RSS DNA in the Post-Cleavage Complex

Svetlana Kotova[1], Gabriel J. Grundy[2], Santiago Ramon-Maiques[2], Emilios K. Dimitriadis[1], J. Bernard Heymann[3], Alasdair C. Steven[3], Martin Gellert[2], Wei Yang[2].
[1]NIBIB, NIH, Bethesda, MD, USA, [2]NIDDK, NIH, Bethesda, MD, USA, [3]NIAMS, NIH, Bethesda, MD, USA.

V(D)J recombination is central to establishing a functional adaptive immune system. The large repertoire of immunoglobulins and T-cell receptors is generated by combinatorial rearrangement of an extensive array of variable (V), diversity (D), and joining (J) gene segments that are joined to encode the variable domains of the protein chains. The recombination signal sequences (RSS) that flank these gene segments are recognized, paired in a synaptic complex, and cleaved by collaboration of the lymphoid-specific proteins RAG1 and RAG2. After cleavage, the signal ends remain tightly bound to the RAG proteins in a particularly stable Signal-End Complex (SEC).

To obtain 3D structural information about RAG1/2 bound to RSS DNA, isolated and purified SEC were visualized by AFM. To better define the arrangement of the RAG proteins and RSS DNA in the complex, we used RAG1 and RAG2 fused with maltose binding protein (MBP). A wide variety of complex shapes was recorded, however, it was clear that the two DNA chains predominantly exited the SEC complex from adjacent points. The volume of the protein core was consistent with the expected mass of 500 kDa corresponding to (RAG1)2-(RAG2)2 composition. MBP protrusions could be observed on the protein particles marking the N-termini of RAG1 and RAG2. To make their appearance more noticeable, we used selective antibody labeling. Fab-labeled MBPs were clearly identified peripheral to the recombinase core. When only the RAG2 MBPs were labeled, the two DNAs most often exited together from the SEC on the opposite side to the Fabs. Consistent with this observation, when only the RAG1 MBPs were labeled, they were situated closer to the exiting DNAs.

The parallel arrangement of DNA and protein subunits found by AFM is in an excellent agreement with the 3D model based on EM data.

983-Pos

Nanoscale Tissue Scaffold Investigations to Optimize Central Nervous System Prosthetic

Volkan Mujdat Tiryaki[1], Virginia M. Ayres[1], Adeel A. Khan[2], Roberto Delgado-Rivera[3], Ijaz Ahmed[4], Sally Meiners[4].
[1]Michigan State University, East Lansing, MI, USA, [2]Western Michigan University, Kalamazoo, MI, USA, [3]Rutgers University, The State University of New Jersey, Piscataway, NJ, USA, [4]University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA.

The introduction of scaffolding materials with appropriate biochemical cues and physical properties into damaged sites within the central nervous system can encourage endogenous or exogenous cellular re-colonization. The scaffolding material currently under investigation is a synthetic electrospun polyamide