for live-cell imaging. However, performance of the technique is context-dependent: e.g., weak fluorescence signals and clustered sub-resolution structures typically yield poor deconvolution results. We have evaluated such difficulty using realistically simulated TIRF images of GLUT4 glucose transporters in cultured adipocytes, whose average diameter of 75nm is far below the optical resolution. An essential image-processing step isolating regions of high information content from a TIRF image was discovered, which enables subsequent deconvolution resolution approaching 100nm. Detailed analysis of deconvolution results as a function of signal-to-noise qualities of the original images suggests that super-resolution details can be resolved with TIRF images of live cells acquired at speed up to 10 fps.

3299-Pos Board B346

Trimming the resolution gap in the study of molecular and cellular events by means of High Data Output and automated three-dimensional Correlative Light-Electron Microscopy approach

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Correlative light/electron microscopy (CLEM) allows the simultaneous observation of a given subcellular structure by fluorescence light microscopy (FLM) and electron microscopy. The use of this approach is becoming increasingly frequent in molecular and cellular biophysics. Here we report on a new high data output three-dimensional (3D) CLEM method based on the use of cryosections (Vicidomini et al.,Traffic, 2008). We successfully applied the method to analyze the structure of rough and smooth Russell bodies used as model systems. The major advantages of this approach are the following: (i) the ability to correlate several hundreds of events at the same time, (ii) the possibility to perform 3D correlation, (iii) the potential to immunolabel both endogenous and recombinantly expressed proteins at the same time and (iv) the effective combination of the high data analysis capability of FLM with the high precision-accuracy of transmission electron microscopy in a CLEM hybrid morphometry analysis. We have identified and optimized critical steps in sample preparation, defined routines for sample analysis and retracing of regions of interest, developed software for semi/fully automatic 3D reconstruction and defined preliminary conditions for an hybrid light/electron microscopy morphometry approach. The relevance of the presented approach is further enhanced by two important key elements, namely: the development of optical nanoscopy methods and the potentiality for exploring different correlative frameworks like optical nanoscopy vs. optical microscopy adding scanning force microscopy techniques.

3300-Pos Board B347

Seeing Multifunctional Nano- and Micro-particles Suitable for Imaging & Therapy Using Freeze-fracture Electron Microscopy

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The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics, is frequently depending upon their morphology adopted in a biological relevant environment. Freeze-fracture electron microscopy (ff-em) as a cryofixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, and imaging molecules[1-3]. At a resolution limit of 2 nm we are able to study the fate of such carriers related to their pay load, application milieu [4], and during their interaction with cells.
Using ff-em we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (free and coupled to drug-loaded immunoliposomes), micelles (spherical-, disc-, and worm-type micelles) [5], small unilamellar liposome [6], multilamellar liposome, niosomes [7], cationic liposome/DNA complexes [8,9], polymer- or lipid-stabilized gas bubbles [10], cochleate cylinder, depofoam particles, and drug crystals. Recently we explored liposome-, virosome-, and virus-based vaccines, including measles vaccine powders, by ff-em.
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3301-Pos Board B348

New Approach To Quantitative Subcellular Imaging Of Phosphorus And Calcium Using Energy-filtered Transmission Electron Microscopy And Tomography

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Analytical electron microscopy provides high-resolution distributions of chemical elements by measuring characteristic core-edge signals that originate from interaction of the incident electrons with a thin section of a cell. Such elemental images give information about the organization of specific biomolecules within cellular organelles, as well as the distribution of ions involved in regulation of cellular processes. For example, mapping phosphorus enables visualization of nucleic acid, and calcium provides distributions of a major intracellular second messenger. Elemental mapping at ~10 nm spatial resolution is typically achieved using energy-dispersive x-ray spectroscopy (EDXS) or electron energy loss spectroscopy (EELS) in a scanning transmission electron microscope (STEM). We have developed a complementary approach based on energy-filtered transmission electron microscopy (EFTEM). It is demonstrated that quantitative 2D elemental distributions containing ~10^6 pixels can be obtained from large regions of cells, and that 3D elemental distributions can be obtained when EFTEM is combined with electron tomography. It is found that an accurate elemental distribution can be derived from just two energy-selected images, above and below a core-edge. However, since the core-edge signals for elements like calcium and phosphorus are relatively weak, it is important to model the spectral background carefully by correcting for plural scattering. We have applied quantitative ETFEM imaging and tomography to determine the 3D distributions of DNA in the cell nucleus, and to measure calcium in mitochondria of neurons.

3302-Pos Board B349

Investigating the Protective Effects of Milk Phospholipids Against Ultraviolet Exposure Using Confocal Reflectance Microscopy

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Current research on bioactive molecules in milk have documented health advantages of bovine milk and its components. Milk phospholipids, selected for this study, represent molecules with great potential benefit in human health and nutrition. In this study we used confocal reflectance microscopy to monitor changes in skin morphology upon exposure to ultraviolet light and evaluate the potential of milk phospholipids in preventing photodamage to skin. We imaged skin equivalent models based on human keratinocytes and dermal fibroblasts cultured in a collagen matrix. We compared images from skin equivalent models with (a) no exposure to UV light, (b) exposure to a dose of 60 mJ/cm^2 of UVB exposure, triple the minimal erythema dose, (c) exposure to milk phospholipids in the media, and (d) exposure to milk phospholipids in the media followed by exposure to UV light. Specimens were imaged directly after exposure, 24 hours after exposure, and 48 hours after exposure. The results suggest that milk phospholipids act upon skin cells in a protective manner against the effect of ultraviolet radiation. Preliminary experiments determining the mechanisms by which the benefits occur are underway.

3303-Pos Board B350

Biomineralization By The Marine Tubeworm Hydroides Dianthus: Structure And Composition Of The Adhesive Cement

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The structure and composition of the adhesive cement of Hydroides Dianthus was studied using a variety of characterization techniques, including XRD, FTIR, SEM, EDX, and AFM. The cement was determined to be a composite of inorganic CaCO3 crystals in an organic matrix, with the organic component making up only a small fraction of the material. Two polymorphs of CaCO3, in roughly equal proportions, were identified in both the tube shell and the cement via XRD and FTIR: aragonite (CaCO3), and magnesium calcite ((Ca,Mg)CO3). Electron microprobe and EDX measurements also confirmed the presence of magnesium. SEM imaging revealed two distinct crystal habits, and EDX measurements allowed for the identification of crystals with an acicular habit as aragonite, and crystals exhibiting a triangular layered structure as magnesium calcite. AFM measurements in sea water and in air were performed in order to determine the elastic moduli of the various components of the composite cement. For the inorganic component, moduli in the range of ~3 GPa were observed in the wet state, and values in the range of ~11 GPa were

observed in the dry state, both of which are consistent with literature values for CaCO3.

Molecular Motors & Force Spectroscopy II

3304-Pos Board B351

Single Molecule Force Spectroscopy and Steered Molecular Dynamics Simulations Reveal the Mechanical Design of the Third FnIII Domain of Tenascin-C

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By combining single molecule atomic force microscopy, proline mutagenesis and steered molecular dynamics simulations, we investigate the mechanical unfolding dynamics and mechanical design of the third FnIII domain of tenascin-C (TNfn3) in detail. The mechanical stability of TNfn3 is found to be similar to that of other constituting FnIII domains of tenascin-C, and the unfolding process of TNfn3 is an apparent two-state process. The hydrophobic core packing of TNfn3 was previously reported as the key element of its mechanical stability. Here, employing proline mutagenesis to block the formation of backbone hydrogen bonds and introduce structural disruption in β sheet, we showed that not only hydrophobic core packing plays important roles in determining the mechanical stability of TNfn3, backbone hydrogen bonds in β hairpins are also responsible for the overall mechanical stability of TNfn3. Furthermore, proline mutagenesis revealed that the mechanical design of TNfn3 is very robust and proline substitution in β sheets only leads to mild reduction in mechanical stability. We also compare the AFM results with those of SMD simulations to understand the molecular details underlying the mechanical unfolding of TNfn3. We found that the mechanical unfolding and design of TNfn3 is significantly different from its structural homologue the tenth FnIII domain from fibronectin. These results serve as a starting point for systematically analyzing the mechanical architecture of other FnIII domains in tenascins-C and will help to gain a better understanding of some of the complex features observed for the stretching of native tenascin-C.

3305-Pos Board B352

Single-molecule Force Spectroscopy Reveals Engineered Metal Chelation Is A General Approach To Enhance Mechanical Stability Of Proteins

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Significant mechanical stability is an essential feature shared by many elastomeric proteins, which function as molecular springs in a wide variety of biological machinery and biomaterials of superb mechanical properties. Despite the progress in understanding molecular determinants of mechanical stability, it remains challenging to rationally enhance the mechanical stability of proteins. Using single molecule force spectroscopy and protein engineering techniques, we demonstrate that engineered bi-histidine metal chelation can enhance the mechanical stability of proteins significantly and reversibly. Based on simple thermodynamic cycle analysis, we engineered a bi-histidine metal chelation site into various locations of the small protein, GB1, to achieve preferential stabilization of the native state over the mechanical unfolding transition state of GB1 through the binding of metal ions. Our results demonstrate that the metal chelation can enhance the mechanical stability of GB1 by as much as 100 pN. Since bi-histidine metal chelation sites can be easily implemented, engineered metal chelation provides a general methodology to enhance the mechanical stability of a wide variety of proteins. This general approach in protein mechanics will enable the rational tuning of the mechanical stability of proteins. It will not only open new avenues toward engineering proteins of tailored nanomechanical properties, but also provide new approaches to systematically map the mechanical unfolding pathway of proteins.

3306-Pos Board B353

Mechanical function and Biophysical Properties of the REJ region of Polycystin-1

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Mutations of Polycystin-1 (PC1) account for 85% Autosomal dominant polycystic kidney disease (ADPKD), which is the most common life-threatening inherited disease worldwide. PC1 has been implicated to be involved in renal tubule and kidney morphogenesis as a mechanosensor and transduce the signals into cellular response. Most domains of the long PC1's ectodomain of are mechanical stable Ig-like motifs and may function as effective force transmitters to regulate the multi-function properties of PC1. The REJ region is a major component of PC1s ectodomain (30% or ~1000 aa); however its structure and function remains unknown. Here we used protein engineering in combination with single-molecule AFM and circular dichroism (CD) techniques to elucidate the structure and mechanical properties of this region. Our studies indicates that the REJ region has complex mechanical properties. Stretching a protein construct which includes four PKD Ig-like domains and the complete REJ region, resulted in saw-tooth patterns with 3-10 force peaks with a wide range of unfolding forces of 50-250 pN, suggesting that the extra force peaks must originate from the REJ region. We also made several REJ constructs (I27)3-REJ FN4-(I27)2 and (I27)3-REJ FN3,4-(I27)2 and expressed them in bacteria and insect cells. Stretching these constructs generated peaks characteristic of the unfolding of titin I27 as well as other more complex unfolding events which we attribute to the unfolding of REJ domains. The complexity of the REJ domain unfolding force patterns suggests that these domains may have unfolding intermediates. These results support the hypothesis that PC1 is a mechano-transducer with a novel molecular architecture and elastic properties well-suited for sensing and transmitting distinct mechanical signals with a wide range of strengths.

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3307-Pos Board B354

Effect Of Temperature On The Mechanical Properties Of Fibronectin

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Fibronectin (Fn) is a multi-domain protein in the extracellular matrix whose primary function is to provide mechanical strength for cell adhesion. In particular the fibronectin cell binding fragment containing exclusively fibronectin type III repeats (FnIII) are studied due to their similarity in structure, their mechanical strength and their direct involvement in cell binding. Previous experimental studies on the mechanical properties of FnIII using single molecule force spectroscopy have focused on the mechanical strength hierarchy of FnIIIs and the folding intermediate of FnIIIs under physiological conditions. Here, we want to explore the mechanical unfolding of FnIII under conditions that disrupt the folding of the protein. In particular, we report studies of the effect of temperature on the mechanical strengths of FnIII.

3308-Pos Board B355

Nanomechanical Manipulation Of Skeletal-muscle Titin With Force-ramp Optical Tweezers

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Titin is a filamentous protein that spans the half sarcomere and functions as a molecular spring, a sarcomeric template, and possibly as a mechanosensor. The molecule has become a popular experimental model for exploring mechanically-driven protein folding because of its structure as a tandem array of similar beta-barrel domains. The force versus extension curve of titin, recorded in constant-displacement-rate experiments, is characterized by entropic-chain behavior onto which sudden, stepwise contour-length fluctuations caused by domain unfolding are superimposed. Recent force-clamp experiments revealed a complex, multi-stage force response during folding, suggesting that the unfolded chain collapses not solely by entropic mechanisms.

To explore the nanomechanical detail in titin's folding and unfolding, here we stretched single molecules of purified skeletal-muscle titin with force-ramp optical tweezers. Titin was extended in cycles of stretch and relaxation, during which the loading rate was kept constant by using a fast (500 Hz) feedback. Loading rates ranged between 1-10 pN/s, and minimal relaxation loads were 2-10 pN. We found that above 50 pN partially unfolded titin molecules often deviated significantly from the pure wormlike-chain behavior and displayed a variable stretch modulus of tens to hundreds of pN. When partially unfolded titin was relaxed to 2 pN and restretched immediately, we did not observe significant domain refolding. Thus, while enthalpic elasticity mechanisms may assist the collapse of the unfolded and extended protein chain, complete domain refolding requires longer periods of time spent in a highly contracted state at low (< 2 pN) forces.

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Mechanical Properties of Type IV Pili in Pseudomonas Aeruginosa

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Type IV pili (Tfp) are thin flexible protein filaments that extend from the cell membrane of bacteria such as *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*. The mechanical properties of Tfp are of great importance since they