## Symposium 7: Protein and Cellular Mechanics

**1097-Symp**
**How Mechanical Forces Can Switch On and Off Protein and Cell Binding Sites**
**Viola Vogel**.
Inst Bio Oriented Mat, Zurich, Switzerland.
How do cells sense whether proteins are stretched or relaxed? While mounting evidence exists that cells and tissues sense mechanical stimuli and convert them into biochemical signals, knowledge about the underpinning mechanisms is sparse. A multitude of structural mechanisms have evolved among extracellular and cytoplasmic proteins that are part of force-bearing protein networks, each enabling distinct modes of mechano-chemical signal conversion. The structural motives include designs by which force can destroy recognitions sites, or alternatively open up cryptic sites that can then recruit other proteins in a force-up-regulated manner. Here we will discuss how the stretching of fibronectin fibers, which form the most extensible protein fibers known so far, can activate or destroy protein and cell binding sites over a wide range of mechanical strains. Stretching of fibronectin fibers thus not only increases their Young's moduli, over orders of magnitude until they rupture when stretched to a few MPa, but their biochemical display is altered in intricate ways as well. Deciphering the underlying engineering design principles by which extracellular matrix proteins can serve as mechano-chemical signalling switches is not only essential to learn how cells sense and respond to mechanical forces, and probe the physical properties of their environments. It has far reaching implications in tissue engineering, systems biology and medicine.

**1098-Symp**
**Nanoscale Protein Architecture of Focal Adhesions**
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Focal adhesions (FAs) mediate cell interactions with their extracellular matrices (ECMs) and consist of integrin ECM receptors linked to the actin cytoskeleton via plasma-membrane-associated protein plaques. Despite their fundamental importance in multicellular organisms, the three-dimensional organization of proteins within FAs is unknown. Here we determine FA molecular architecture by using 3D superresolution microscopy (interferometric Photo-Activated Localization Microscopy) to map nanoscale protein organization. We find that the FAs consist of partially overlapping proteinspecific vertical layers of 15-50 nm thickness, with integrins and actin separated by a 30-50 nm FA core which is spanned by talin tethers. This reveals a structural basis for FA function whereby a multilaminar core architecture mediates the interdependent cell processes of adhesion, signaling, force transduction, and actin cytoskeletal regulation.

**1099-Symp**
**Force Probing the Molecular Mechanics of Cell Rounding**
**Daniel J. Mueller[1]**, Martin P. Stewart[2], Jonne Helenius[2], Yusuke Toyoda[3], Subramaniam P. Rmanathan[2], Anthony A. Hyman[3].
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During mitosis tissue culture cells undergo a dramatic shape change, from essentially flat to nearly spherical. The forces and mechanisms that drive this shape change remain unexplained. Here we use assays based on atomic force microscopy to measure the height and rounding force of single mitotic cells. We show that under our conditions, human cells exert forces approaching 100 nN when they round up. The force depends not only on the actomyosin cortex but also on trans-membrane ion gradients. In further experiments we demonstrate which membrane proteins are coupled to and regulated by the actomyosin cortex to establish a hydrostatic pressure that rounds up the cell. By using single-molecule force spectroscopy we look inside these individual membrane proteins to quantify by which interactions and mechanisms they are functionally regulated. Based on these results we introduce an advanced model of cell rounding in which a hydrostatic outward pressure, and contractile actomyosin cortex forces govern shape.

**1100-Symp**
**Regulation of Mechanical Equilibrium in Multicellular Arrangements**
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We investigated the physical laws governing the mechanical equilibrium of multi-cellular arrangements. Breaking and maintaining this equilibrium are the fundamental basis for embryonic morphogenesis and tissue homeostasis. It notably plays a key role in epithelium-mesenchymal transition (EMT) during normal development and tumor transformation. Since multi-cellular equilibrium relies on a spatial regulation of the force balance between cell-cell and cell-extra cellular matrix (ECM) adhesions, we studied human epithelial cell pairs confined on defined ECM micro-patterns geometries. We developed an automated tracking method to quantify the cell movements in high throughput time-lapse acquisitions. We found that cell pairs could adopt different behaviors depending on pattern geometries. A complete survey over many different geometries showed that cells adopted all graded phenotypes from continuous cell migration to static mechanical equilibrium. After induced EMT the stability of cell pair configuration was affected.
We used cytoskeleton observations and physical modeling to identify the physical parameters implicated in the establishment of mechanical equilibrium. Current physical models of multi-cellular equilibrium, which consider a constant line tension along the perimeter, surface tension of the membrane and adhesion energies could not account for spatial arrangements we observed. Immuno-fluorescent labellings and in vivo expression of actin marker revealed three types of actin cables: cables above adhesive regions connecting two ECM adhesion sites and cables above non adhesive regions connecting either two ECM adhesion sites or one ECM and one cell-cell adhesion site. Preliminary nano-ablation experiments to severe actin cables suggested that tension could vary in each type of cable. It seems that cells develop an anisotropic distribution of line tension in response to local adhesiveness. We currently investigate with experimental and numerical approaches whether anisotropic distribution of tension could be the regulator of the spatial arrangement we observed in various microenvironment geometries.

## Symposium 8: Structure and Dynamics of Membrane Transporters

**1101-Symp**
**A Dynamical View of Membrane Transporter Function at Sub-Angstrom Resolution**
**Emad Tajkhorshid**.
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Membrane transporters provide the main mechanism for active exchange of materials between the cytoplasm and a cell's environment in a highly selective manner. These complex molecular machines present a structurally diverse group of pumps evolved to efficiently couple various sources of cellular energy to the selective transport of different molecules. Depending on the source of energy used and the type of the substrate transported, different protein architectures and, thus, different mechanisms are employed by membrane transporters. Active transporters undergo various degrees of protein conformational changes (induced, e.g., by ATP hydrolysis or by binding of the substrate and the co-transported ions) during their transport cycle. In other words, they adopt distinct conformational states during their function. Due to the difficulties associated with structure determination of membrane proteins, however, for the majority of structurally-known transporters only one of the major functional states has been structurally characterized. Substrate binding and translocation along the transport pathway in membrane transporters are closely coupled to numerous stepwise protein conformational changes of various magnitudes and forms that are induced by and/or coordinated with the energy-providing mechanisms. A detailed description of the mechanism of membrane transporters, therefore, relies on high-resolution methodologies that can describe the dynamics of the process at an atomic level. In this talk, latest results of molecular dynamics simulations performed on a number of membrane transporters with diverse mechanisms, and the molecular events involved in their function revealed by these simulations will be presented.

**1102-Symp**
**Ion Transport by the Sodium Pump**
**Hanne Poulsen**.
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The first crystal structure of the Na+,K+-ATPase revealed the potassium-bound form of the pig kidney enzyme at 3.5 Å resolution. This large membrane protein complex consists of an alpha subunit similar to the Ca2+-ATPase, a heavily glycosylated beta subunit and a small regulatory gamma subunit (also known as FXYD2). The electrogenic transport performed by the Na+,K+-ATPase causes extrusion of three sodium ions and uptake of two potassium ions per ATP split. The gradients thus formed are of fundamental importance in physiology as they