$Ca^{2+}$. Because major components of the spasmoneme, the contractile organelle inside the stalk, are EF-hand $Ca^{2+}$-binding proteins including spasmin and centrin, the spasmonemal contraction is thought to be related to other centrin-based motility mechanisms. This study describes how stall force affects contractions of live *Vorticella*. To impede contractions, we applied hydrodynamic drag force to *Vorticella* in a microfluidic channel with Poiseuille flow of viscous PVP solution. This method enables controlling the stall force by changing flow rate and the viscosity of the solution. Cell dimension measurements show that the zooid is elongated by the flow in relaxed and contracted states keeping roughly constant volume. As the stall force increases, the end-to-end length of the contracted stalk increases while that of the relaxed stalk is almost constant, and maximum contraction speed decreases while contractions take longer time. Furthermore, the time lag in contraction commencement between the zooid and the stalk also increases. We measured time differences in movement start among polystyrene beads attached to the stalk, and they increase with increasing stall force. These increasing time lags imply that the stalk cannot contract until it develops force great enough to overcome the stall force. The stall force affects the relaxation of *Vorticella* because relaxations take longer time as the stall force increase and the extending stalk resumes its contraction after the stall force is removed. It seems that although the spasmoneme retains contractile force, the stall force extends the stalk.

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**Tuning Cellular Mechano-Response Using Biomembrane-Mimicking Substrates of Adjustable Fluidity**

**Daniel E. Minner**[1], Philipp Rauch[2], Amanda P. Siegel[1], Johannes Stelzer[2], Joseph Kás[2], Guilherme Sprowl[3], Kevin Harvey[4], Rafat Siddiqui[4], Simon Atkinson[1], Christoph A. Naumann[1].
[1]Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]University of Leipzig, Leipzig, Germany, [3]George Washington Community High School, Indianapolis, IN, USA, [4]Methodist Research Institute, Indianapolis, IN, USA.

An important aspect of mechanobiology is that tissue cells are anchorage-dependent and respond to viscoelastic changes in their environments. The mechanosensitivity of cells is believed to play an important role in processes such as cancer cell migration and stem cell differentiation. Previously, cellular mechano-response has been mainly studied using μm-thick polymeric films of adjustable viscoelasticity. Here we report on the design and characterization of alternative cell substrates based on 8-40nm thick polymer-tethered phospholipid membranes where cellular mechano-response can be regulated by tuning bilayer fluidity. Two complementary membrane systems are employed to span a wide range from low to high bilayer fluidity. Low to medium bilayer fluidity is achieved by using a single polymer-tethered lipid bilayer of adjustable tethering concentration. Medium to high bilayer fluidity is obtained through the regulation of the number of bilayers in a stack of polymer-tethered lipid bilayers. Changes in bilayer fluidity in these substrates have been confirmed through wide-field single molecule tracking of fluorescently labeled lipids. To facilitate the adsorption and migration of cells, these biomembrane-mimicking substrates contain bilayer-cell linkages of well-defined concentrations. Phase contrast microscopy experiments on PC12 neurons show that dendritic growth can be tuned by modifying the tethering concentration in a single polymer-tethered lipid bilayer. Comparing phase contrast and epifluorescence microscopy experiments on 3T3 fibroblasts containing GFP-actin, which were plated on multi-bilayer stacks, revealed profound changes in cellular phenotype, projected cell area, cell migration, and cytoskeletal organization with the number of bilayers in the stack. For example, on very fluid substrates, neuron-like, dendritic fibroblasts were observed. The described substrates are particularly significant because, unlike in the case of polymeric films, substrate-cell linkages are free to move and matrix remodulation caused by adsorbed cells is largely suppressed.

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**Cell Contact, Substrate Mechanics And Boundary Conditions In The Movement Of Epithelial Sheets**

**Michael P. Murrell**[1], Paul Matsudaira[2], Roger Kamm[1].
[1]MIT, Cambridge, MA, USA, [2]National University of Singapore, Singapore, Singapore.

The directed and highly coordinated movement of epithelial cells can be found in various vertebrate systems, from the separation of tissues in early development through the renewal of tissues in the adult. How the cells coordinate their movement in a sheet remains unexplained, especially in physical terms, as the movement involves a complex balance of forces generated at multiple length-scales. The forces that stem from the cellular level, and their mediation by the physical environment that allow them to manifest a highly correlated, multicellular pattern of movement is dependent upon the relationship between local influences such as cell-cell contact and the ability for cells to deform the substrate, with longer-ranged influences, such as physical and geometric constraints placed on the population of the sheet. Therefore, in this study, we quantitatively explore the dependence between cadherin-mediated contact, substrate mechanics, and boundary conditions on the motility of two-dimensional epithelial sheets.

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**TβRIII Restores Normal Cytoskeleton Mechanics In Ovarian Cancer Cells**

**Vinay Swaminathan**[1], Mythreye Karthikeyan[2], E. Timothy O'Brien[1], Gerard Blobe[2], R. Superfine[1].
[1]University of North Carolina- Chapel Hill, Chapel Hill, NC, USA, [2]Duke University, Durham, NC, USA.

Changes in cellular phenotypes in cancer are characterized by alterations of the cytoskeleton and several important signaling pathways in the cell. One of the signaling pathways implicated in controlling proliferation, angiogenesis and apoptosis is the TGF-β signaling pathway. Loss of expression of the TGF- β superfamily co-receptor, TβRIII/betaglycan, occurs in a broad spectrum of cancers, including those of the breast, ovary and prostate. Recent studies have shown that restoration of TβRIII to metastatic populations of ovarian and breast cancer cells suppresses migration via alteration of the cytoskeleton. Using our 3 dimensional force microscope system (3DFM) for passive and active micro-rheology, we compared the response of normal ovarian surface epithelial cells, ovarian cancer cells with reduced TβRIII expression, and ovarian cancer cells with restored expression of TβRIII. We found that cancer cells lacking TβRIII were at least 3× more compliant (less stiff) than either normal epithelial or TβRIII-expressing cancer cells. Our results, are consistent with potential invasiveness being correlated with increased cell compliance, and both being regulated by the TβRIII pathway. Compliance measurements using the 3DFM could be a useful tool to measure invasiveness in the future.

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**Three Dimensional Superresolution Fluorescence Microscopy Reveals Protein Stratification in Focal Adhesions**

**Pakorn Kanchanawong**[1], Gleb Shtengel[2], Michael W. Davidson[3], Harald F. Hess[2], Clare M. Waterman[1].
[1]National Heart Lung and Blood Institute, Bethesda, MD, USA, [2]Howard Hughes Medical Institute Janelia Farm Research Campus, Ashburn, VA, USA, [3]Florida State University, Tallahassee, FL, USA.

Focal Adhesions (FA) are dynamic structures consisting of large numbers (>150) of different proteins that mechanically link the actin cytoskeleton to the extracellular matrix (ECM). Despite the central role of FA in cell migration and the wealth of biochemical and cell biological data on FA proteins, it remains virtually unknown how these proteins are organized within FA. Based on the differential dynamics of distinct FA proteins we previously observed using fluorescent speckle microscopy, we hypothesized that FA proteins may be organized into stratified layers within FA that serve as dissipative elements in a ''molecular clutch'' to form a regulatable, force-transducing link between the actin cytoskeleton and the ECM. To test this hypothesis, we employed a 3-dimensional superresolution fluorescence microscopy technique, interferometric photoactivated localization microscopy (iPALM), to determine sub-20 nm z-axis localizations of several key structural components of FA labeled with photoactivatable fluorescent proteins and expressed in U2OS cells plated on a fibronectin-coated substrate. Within FA, we found that the cytoplasmic face of the plasma membrane, marked by Farnesylated tdEosFP, was localized at ~20-30 nm from the substrate, in agreement with previous electron miocroscopic analyses. Talin and vinculin, putative force transducing elements of FA, were observed within distinct planes parallel to the substrate, with the highest densities at ~35, and ~50 nm above the substrate, respectively. Actin appeared with the highest density at ~90 nm above the substrate plane, and was largely excluded from area adjacent to plasma membrane up to a height of ~50 nm. iPALM reveals for the first time the organization of specific proteins within the nanoscale core of the FA. The protein stratifications in FA provide a structural context for the mechanosensing and mechanotransducing functions of FA.

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**Role of Mechanotransduction in Cellular Processes**

**Narayanan Srividya**, Gayathri Balandaram, Chandima Bandaranayake, Subra Muralidharan.
Washington State University, Pullman, WA, USA.

The role of mechanical properties of cells is gaining increasing attention due to the regulatory role that it plays in cellular processes. In particular the transduction of an applied force on the cell membrane through the cellular components can have significant influence on such phenomena as stochasticity in gene expression and cancer metastasis. We are currently investigating the effect of mechanical forces on the stochasticity of gene expression in E.coli cells and