**1013-Plat**
**Stem Cell Biophysics: Pre-differentiation Dynamics of Stress Fiber Polarization on Elastic Matrices**
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Human mesenchymal stem cells (hMSCs) are mechanosensitive and specify their lineage based on the stiffness of their environment. That is, a purely mechanical cue is sufficient to cause cells on gels with an intermediate stiffness (Young's modulus E = 11 kPa) to adopt a spindle-like morphology characteristic of muscle cells and to up-regulate muscle markers such as MyoD. Inhibition of myosin II by blebistatin blocks this lineage specification. We analyzed the shape changes that occur at early times in cells cultured on soft to stiff (1, 11, and 34 kPa) gels using an automated image analysis algorithm to correlate morphological changes with stress fiber formation and orientation. While the total production of stress fibers increases monotonically with substrate stiffness similar to the trend in projected cell area, the orientation shows a maximum at intermediate stiffness, similar to the polarization. These early time changes are not due to changes in gene expression, which occur only after several days, but must instead have a more rapid biophysical basis. Both the myosin inhibition and correlation between stress fiber orientation and cell morphology suggest a critical role for these contractile structures in mechanosensing. To help dissect their function we used a multi-color hybrid fluorescence and atomic force microscope to locate specific stress fiber proteins such as actin and myosin and to correlate their presence with specific features in the high resolution AFM images. AFM also offers the possibility of determining the mechanics of the stress fibers themselves, and thus of integrating their properties into a more complete picture of the cell's mechanics and ultimately mechanosensitivity.

**1014-Plat**
**Contractile Force Generation Enhanced Tumor Cell Invasion, But Decreased Tumor Growth**
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The process of metastasis formation that includes cell invasion has been shown to cause malignant progression of tumors. The impact of cell mechanical properties on the malignancy of tumor cells has not been investigated systematically. Highly-invasive tumor cells expressed significantly higher amounts of the α5β1 integrin compared to weakly-invasive tumor cells. We hypothesized that the ability to generate contractile forces is a prerequisite for cell invasion and that α5β1-expression increased cell invasion through enhanced generation of these contractile forces. We analyzed whether α5β1-high or α5β1-low expressing breast carcinoma cells differ in their ability to invade into a 3-D collagen fiber matrix. Our results show that higher α5β1 expression increased cell invasiveness. We found that the increased 3-D motility of α5β1-high expressing cells depends on the integrin α5β1 activating traction generation. The increased invasiveness was inhibited by addition of myosin light chain kinase inhibitor ML-7 or by addition of the ROCK kinase inhibitor Y27632. Furthermore, we analyzed whether α5β1-high and α5β1-low cells formed tumors in mice. The tumor formation and growth is impaired in α5β1-high compared to α5β1-low cells. The integrin α5β1 acts as an enhancer of cell invasiveness where contractile forces are necessary to overcome the viscous drag, but as a suppressor of primary tumor formation and growth where increased motility is rather a hindrance for cell clustering to form tumors.

**1015-Plat**
**Pushing Off The Walls: A Mechanism Of Cell Motility In Confinement**
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Cell migration is involved in crucial processes in biology and physiopathology such as immune response and carcinogenesis. In these cases cells migrate through tissues composed of densely packed cells and/or extracellular matrix with a small mesh size. Despite extended studies of cell motility on 2D substrates it is not clear whether these results can be generalised to migration in confined environments such as tissues. We propose a novel, simple mechanism of cell motility, which relies mainly on the coupling of actin polymerization at the cell membrane to geometric confinement. We consider a polymerizing viscoelastic cytoskeletal gel confined in a narrow channel, and show analytically that spontaneous motion occurs. Interestingly, this mechanism does not require specific adhesion with the channel walls, and yields velocities potentially larger than the polymerization velocity of the gel. The contractile activity of myosin motors is not necessary to trigger motility in this mechanism, but is shown quantitatively to increase the velocity of motion. Our model qualitatively accounts for recent experiments that show that cells without specific adhesion proteins are motile only in confined environments while they are unable to move on a flat surface, and could help in understanding the mechanisms of cell migration in more complex confined geometries such as living tissues.

## Platform N: Membrane Dynamics & Bilayer Probes

**1016-Plat**
**High-Resolution Far-Field Fluorescence STED Microscopy Reveals Nanoscale Details of Molecular Membrane Dynamics**
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Prominent problems in biology cannot be solved due to the limited resolution of conventional optical microscopy. For example, a whole range of membrane-associated processes are considered to be mediated through cholesterol-assisted interactions such as the formation of lipid nanodomains or 'rafts'. The direct and non-invasive observation of lipid or membrane protein dynamics in living cells, which are believed to occur on small spatial scales, is impeded by the resolution limit of >200nm of a conventional far-field fluorescence microscope or by the limited time resolution of single-particle tracking. We combine single-molecule based techniques such as fluorescence correlation spectroscopy (FCS) with stimulated emission depletion (STED) far-field microscopy to accesses a superior spatial and temporal resolution for observing the diffusion characteristics of molecules in the plasma membrane of living cells. Tuning the detection area between 250 nm and 30 nm in diameter, we directly reveal marked differences between different lipid or molecular classes. For example, sphingolipids or 'raft'-associated proteins are transiently trapped on the nanoscale in cholesterol-mediated molecular complexes. The presented direct detection of molecular dynamics in nanoscale areas of tunable size constitutes a powerful approach to study the dynamics of biomolecules in living cells.

**1017-Plat**
**3D Tracking of Antibody-Receptor Dynamics on RBL Cells.**
**Nathan P. Wells**, Guillaume A. Lessard, Peter M. Goodwin, James H. Werner.
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We have previously demonstrated the ability to track single quantum dots freely diffusing in three dimensions using a custom confocal microscope. The microscope is capable of both following the trajectory of a single (fluorescent) molecule and recording the time resolved (fluorescent) photon stream. With both positional and lifetime information, the microscope can provide fluorescent lifetime trajectories (FLT) analogous to fluorescent lifetime imaging (FLIM). Using this microscope we have recently begun to study the signal cascade process in the IgE-FcεRI antibody-receptor system in live RBL cells. We have demonstrated the ability to track fluorescently labeled IgE docked to the FcεRI receptors and observe 3D motion on the membrane. We are currently working to observe and track the endocytosis of the antibody receptor complex on a single molecule basis.

**1018-Plat**
**Physiological Membrane Tension Causes An Increase In Lipid Diffusion: A Single Molecule Fluorescence Study**
**Hari S. Muddana**, Ramachandra Rao Gullapalli, Tristan Tabouillot, Peter J. Butler.
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The quantitative relationship between lipid bilayer tension and lipid dynamics is currently unknown. We used time-correlated single photon counting (TCSPC) and fluorescence correlation spectroscopy (FCS) to determine diffusion of DiI, a lipoid dye, in micropipette-aspirated model membranes. Fluid-phase giant unilamellar vesicles (GUVs) were prepared from DOPC lipid using electroformation and stained with nanomolar concentrations of DiIC12. When GUVs were stressed from 0.02 to 0.1 mN/m, the diffusion coefficient increased monotonically from $9 \times 10^{-8}$ to $13 \times 10^{-8}$ cm2/sec. Thus, for the first time, we show that physiological tensions on the order of those experienced when cells

are subjected to fluid shear stress or osmotic swelling induce substantial changes in lipid diffusion. These results suggest that tension directly causes changes in lipid diffusion, which may play a role in activation of integral membrane proteins. Conversely, this direct relationship may allow one to determine membrane stresses in cells from measured diffusion coefficients.

**1019-Plat**
**Hydrophobic Mismatch: A universal Tool for Clustering, Demixing, and Sorting of Transmembrane Proteins**
**Ulrich Schmidt**, Gernot Guigas, Matthias Weiss.
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Sorting of transmembrane proteins is a central task of eucaryotic cells, in particular in the secretory pathway.
Due to a lack of an organizing mastermind the decision whether a membrane protein participates in secretory transport or not has to be made by a self-organization process on the molecular scale, e.g. via cluster formation. We show by means of coarse-grained membrane simulations that hydrophobic mismatching can drive cluster formation of transmembrane proteins [1]. Also, proteins with different degrees of hydrophobic mismatching can segregate and form homo-oligomers. In addition, we show that proteins partition into the lipid phase with the smallest hydrophobic mismatch if the membrane has a heterogeneous composition. Our data thus indicate that hydrophobic mismatching may help to organize trafficking along the secretory pathway in living cells.
[1] U. Schmidt, G. Guigas & M. Weiss, Phys. Rev. Lett. 101, 128104 (2008).

**1020-Plat**
**Backbone Conformation and Dynamics of the Lipid-Modified Membrane Anchor of Human N-Ras Investigated by Solid-State NMR and Molecular Dynamics Simulations**
**Alexander Vogel**[1], Guido Reuther[1], Kui-Thong Tan[2], Herbert Waldmann[2], Michael F. Brown[3], Scott E. Feller[4], Daniel Huster[5].
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Many proteins involved in signal transduction are anchored to membranes by covalently attached lipid modifications. In this study we investigated the conformation and dynamics of the backbone and side chains of the N-Ras membrane anchoring domain. Experimental solid-state NMR studies involved doubly lipid-modified uniformly $^{13}C$ and $^{15}N$ labeled heptapeptides representing the C-terminus of N-Ras, which were incorporated into DMPC bilayers. A structural model of the peptide was calculated on the basis of isotropic chemical shifts, explicit torsion angle measurements, and nuclear Overhauser effects determined by solid-state NMR. The amplitude of molecular motions was assessed by $^{1}H$-$^{13}C$ order parameter measurements using separated local field NMR. For determination of the correlation times of the motions, $T_1$ and $T_2$ relaxation times were measured and analyzed using a generalized relaxation approach. To further understand the dynamics of Ras, molecular dynamics simulations of the molecule in lipid bilayers were conducted. In generating starting conditions for the simulation, special attention was paid to the backbone conformation since transitions between conformations were found to be rare events in a previous simulation of 100 ns length on this system [1]. Therefore, the experimentally determined conformation of the peptide backbone was equilibrated using a replica exchange technique in an explicit membrane environment. This enabled us to identify different conformers and to assess their relative probability. The resulting distribution of conformations was used subsequently for a long conventional MD simulation that was analyzed with regard to the experimental data. The combined simulations and experimental approach enabled a detailed model of the dynamics of the peptide to be obtained.
[1] Vogel, A. Tan, K.-T. Waldmann, H. Feller, S.E. Brown, M.F. Huster, D. *Biophys. J.* **2007**, *93*, 2697-2712.

**1021-Plat**
**Subdiffusion And Diffusion Of Lipid Atoms And Molecules: Relating The Dynamics Of Lipids To Neutron Scattering Experiments**
**Elijah Flenner**[1,2], Jhuma Das[1], Maikel Rheinstädter[1], Ioan Kosztin[1].
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Inelastic neutron scattering (INS) experiments, based on latest generation of neutron sources, allow us to gain insight into the complex dynamics of lipid molecules in biologically relevant phospholipid bilayers. However, the proper interpretation of the INS scattering experiments requires theoretical and computational models that correctly capture the main features of lipid dynamics at atomic and molecular levels. To this end, here we use a 0.1 microsecond all-atom molecular dynamics simulation to investigate the dynamics of lipid atoms and molecules in a hydrated diyristoyl-phosphatidycholine (DMPC) lipid bilayer. First, as predicted by theories of polymer dynamics, we identify three well separated dynamic regimes in the mean square displacement of the lipid atoms and molecules: (1) a ballistic regime where the mean square displacement increases as the square of time for $t < 10$ femtoseconds; (2) a subdiffusive regime where the mean square displacement increases with a sub-linear power law for times between 10 picoseconds and 10 nanoseconds; and (3) a Fickian diffusion where the mean square displacement increases linearly in time for $t > 30$ nanoseconds. Next, we show that the cumulant approximation of the self-intermediate scattering function (which is the inverse Fourier transform of the dynamic structure factor measured in INS experiments) is in very good agreement with the simulation results, and allows us to connect the three time scales in the mean square displacement to the interpretation of neutron scattering results. Finally, we focus on the hydrogen atoms (which represent the main source of the incoherent INS signal) in the lipids and draw conclusions about the lipid dynamics by examining the wave-vector dependence of the intermediate scattering function.
Computer time was generously provided by the University of Missouri Bioinformatics Consortium.

**1022-Plat**
**Towards Subcellular Tissue Sampling by Near-Field Laser Ablation: A 'Protein Microscope' to Map Peptide Distributions in Cells**
**Mark E. Reeves**[1], Jasper Nijdam[1], Benjamin Gamari[1], Deepa Raghu[1], Andrew Gomella[1], Joan A. Hoffmann[2].
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We report on the development of a new instrument, dubbed a 'Protein Microscope,' that uses near-field optical techniques to increase the spatial resolution of atmospheric pressure matrix-assisted laser desorption and ionization (AP-MALDI). This functions as a novel front-end for time-of-flight mass spectrometry. Standard protein identification techniques involve homogenization of a tissue sample, which destroys all spatial and temporal information about the expressed proteins. Our new NSOM-based instrument will allow the identification and mapping of proteins expressed in intact cells and tissues, which is of great interest as protein expression connects genomic information with the functioning of an organism. This poster will focus on the development of near-field-based ablation of sub-cellular-sized regions of tissue and plant samples.

**1023-Plat**
**A Biomolecular Photodiode For Imaging Of Cell Membrane Potential**
**Daniel R. Cooper**.
McGill University, Montreal, QC, Canada.
Despite the recognized importance of electrical signals in many biological systems, there has been very limited success in the creation of a robust fluorescent voltage sensor. Using standard molecular biology techniques, we have created a biomolecular photodiode consisting of a membrane-bound cytochrome c protein fused with a GFP (green fluorescent protein) variant. A similar photodiode assembly has been shown to produce unidirectional photocurrent in vitro with the cytochrome acting as an acceptor of excited electrons from the FP donor upon excitation with visible light. Electron transfer between the cytochrome and the FP is a highly voltage dependent process. By embedding this assembly in the plasma membrane of living cells, it is subjected to the same electric potential as the membrane. As the membrane potential of the cell changes over ~100 mV, as in an action potential, the extent of electron transfer should vary significantly, manifesting as a change in fluorescence intensity of the FP donor. As this is a very fast process with a high sensitivity to changes in electric potential, this biophotodiode is expected to form a robust sensor of electrical activity in cells. The feasibility of the sensor is investigated in several ways, including modeling, electrophysiology, and direct application of current to purified membrane fragments.

## Platform O: Phototransduction: Signaling Events Downstream of Photon Absorption

**1024-Plat**
**Structure and Dynamics of Signal Transducing Membrane Complexes**
**Theodore G. Wensel**, Feng He, Qiong Wang, Zhixian Zhang.
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We have used electron cryo-microscopy (cryo-EM) of single particles (individual protein complexes) and two-dimensional crystals along with fluorescence resonance energy transfer (FRET) and fluorescence recovery after