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Probing cell membrane mechanics by magnetic particle actuation and 3D rotational particle tracking

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to study the mechanics of single cells and subcellular components such as the nucleus, we developed a unique system that couples an Atomic Force Microscope (AFM) with a new imaging technique called PRISM - Pathway Rotated Imaging for Sideways Microscopy. PRISM allows for simultaneous horizontal and vertical fluorescence imaging of a single cell. The combined AFM and PRISM system enables acquisition of 3D images of cell structure with accompanying piconewton resolution force measurements. We use this new technique to observe nuclear deformation from the side in real time. From these experiments we can measure a cell's mechanical response (AFM) and strain maps of punctate labeled nuclei (PRISM). Additionally, we will show how external forces induce a strain in chromosome territories. Our larger goals are to investigate how external mechanical stimuli structurally affects the genome and thereby alters gene expression, motility and differentiation.

234-Plat
Imaging Mechanical Force Transmission at Single Integrins in Living Cells
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Mechanical interactions between cells and the extracellular matrix (ECM) exert a profound influence on cell migration, proliferation, and stem-cell differentiation. However, how cells generate and detect mechanical force remains poorly understood, in part due to a lack of methods that visualize molecular-scale forces in living cells. Here we describe a Förster resonance energy transfer (FRET)-based molecular tension sensor (MTS) that allows us to directly visualize cellular forces at the single molecule level. We designed a MTS that binds to a glass coverslip via avidin or covalent bond at one end and presents an integrin binding site at the other. Cellular integrins bind surface immobilized MTSs and transmit force to the FRET module, resulting in decreased FRET with increasing load. In agreement with previous work, we find that force generation is largely confined to dense integrin-containing assemblies termed focal adhesions (FAs). The enhanced spatial resolution of our measurement in comparison to previous techniques allows us to directly visualize the distribution of forces within FAs. We observe localized force generation that is only weakly correlated with paxillin recruitment, a standard marker for FA maturity, consistent with the proposal that FAs are structurally heterogeneous on the submicron length scale. FRET values measured for single MTS molecules are consistent with tensions ranging from 1 to 5 pN, substantially less than those required to rupture integrin-ligand bonds. These relatively modest tensions suggest that the collective contribution of numerous weak integrin-binding interactions can be sufficient to drive robust cell adhesion, and by extension mechanotransduction.

235-Plat
The Role of Arp2/3 in DRG Growth Cones Motility
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The Arp2/3 complex simultaneously controls nucleation of actin polymerization and filament branching. We used optical tweezers and video imaging to analyse the role of Arp2/3 in the motility of lamellipodia from developing growth cones (GCs) of isolated Dorsal Root Ganglia (DRG) neurons. Kymograph analysis shows that inhibition of Arp 2/3 complex with 100 μM CK548 completely abolished the lamellipodia motion. Surprisingly when the activity of Arp2/3 is inhibited by 25 μM CK548, lamellipodia shrank for 6-8 min but then reappears again and show an almost normal motility. The average period to complete protrusion and retraction cycles of lamellipodia 76 s in control, increased to 110 s after the recovery from the exposure to this drug. The drug CK548 decreased, initially, the ability of the lamellipodia to lift up in the axial direction, but after the recovery lamellipodia were able to lift 4 μm up above the coverslip as in control conditions. The force generated by lamellipodia and their maximal protrusion velocity decreased when the concentration of drug CK548 is increased from 25 to 100 μM. After the recovery of an almost normal motility, ruffles were present but had a different shape and were more fragmented and invaginated, suggesting an altered cytoskeleton. These results show that Arp 2/3 is important in maintaining the morphology and the motility of the neuronal growth cones but in neuronal lamellipodia several distinct pathways contribute to actin filament branching and to their cross-linkage.

236-Plat
Probing Cell Membrane Mechanics by Magnetic Particle Actuation and 3D Rotational Particle Tracking
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Menno W.J Prins[1,3].
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The mechanical properties of the cell membrane and the actin cortex determine a variety of cellular processes. An accurate description of their mechanics and dynamics necessitates a measurement technique that can capture the inherent anisotropy of the system. We combine magnetic particle actuation with rotational and translational particle tracking to simultaneously measure the mechanical stiffness of the membrane and the actin cortex in living cells in three rotational and two translational directions.
We demonstrate the technique by targeting various types of membrane receptors. When using particles that bind via integrins, we measured an isotropic stiffness and a characteristic power-law dependence of the shear modulus on the applied frequency. When using particles functionalized with immunoglobulin G, we measured an anisotropic stiffness with a strongly reduced value in one dimension. We suggest that the observed reduced stiffness is caused by a local detachment of the membrane from the subjacent cytoskeletal cortex.
Furthermore, we use functionalized particles as phagocytic targets for macrophages. Although phagocytosis is an inherently mechanical process, little is known about the forces and energies that a cell requires for internalization. We use our technique to measure the stiffnesses of the phagocytic cup as a function of time. The measured values and their time-dependence can be interpreted with a model of a pre-stressed membrane connected to an elastically deformable actin cortex. A comparison of model and data allows a determination of the speed at which the membrane advances around the particle. This approach is a novel way of measuring the progression of phagocytic cups and their mechanical properties in real-time.
We expect that our technique will enable new insights into the mechanical properties of cells and will help to better understand numerous cellular processes.

237-Plat
Tight Coupling Between Nucleus and Cell Migration through the Perinuclear Actin Cap
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Eukaryotic cells alternate between episodes of fast and persistent movements and ''hesitation'' episodes of low speed and low persistence. Unlike the well-understood tumbling dynamics in E. Coli, the molecular mechanism that controls the dynamic changes in morphology, speed, and persistence of eukaryotic migratory cells remains unclear. Here, we show that the movement of the interphase nucleus during random cell migration switches intermittently between two distinct modes - rotation and translocation - that follows with high fidelity the sequential rounded and elongated morphologies of the nucleus and the cell, respectively. Nuclear rotation and translocation respectively mediate the stop-and-go motion of the cell through the dynamic formation and dissolution of the contractile perinuclear actin cap, which is dynamically coupled to the nuclear lamina and the nuclear envelope through LINC protein complexes. A persistent cell movement driven by the actin cap and accompanied by actin cap-mediated nuclear translocation, is stopped following actin cap disruption, which in turn allows for the cell to repolarize thanks to dynein for its next persistent move thanks to dynein-mediated nuclear rotation.

Workshop: Polarizable Force Fields from Biomolecular Simulations

238-Wkshp
Development of a Polarizable Force Field for Macromolecules Based on the Classical Drude Oscillator
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Accurate potential functions based on simple and computationally tractable functional forms are essential for meaningful molecular dynamics simulation studies of biomolecular systems. Towards this need we have developed a polarizable force field based on the classical Drude oscillator model. Built on a foundation of parameters optimized targeting a range of experimental and quantum mechanical (QM) data for small molecules representative of macromolecules, force fields for proteins, lipids, nucleic acids and carbohydrates have been developed. Extension of the polarizable model from small molecules to macromolecules required additional optimization of the electrostatic parameters in the context of the biopolymers, iterative optimization of the torsion potentials targeting both QM and experimental data and the inclusion of pair specific Lennard-Jones parameters. Examples from optimization of the polarizable model for proteins and nucleic acids will be presented, including results from explicit solvent simulations of 100 ns and longer.