the adhesion and migration of prostate cells in various stages of metastasis. The effect of external forces on a synthetic gene network in E.coli is being studied to determine if they impact intrinsic or extrinsic stochasticity. The stochasticity is monitored through the expression of three different fluorescent proteins CFP, YFP, and RFP. The emission intensities as a function of applied force are monitored to discern the effect of applied force on gene stochasticity. The influence of mechanical stress on cancer metastasis is being investigated by determining the expression levels of membrane and cytoplasmic proteins as a function of applied force. Additionally the cell-cell adhesion, cell-matrix adhesion, cell stiffness and elasticity, and expression levels of membrane proteins are determined by AFM. The AFM cantilever is employed to exert a local force and measure the response of the force in terms of the expression of adhesion proteins, and cell-cell and cell-matrix adhesion. Significant results of these studies will be presented.

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Strain Stiffening And Soft Glassy Rheology In A Generalized Sliding Filament Model

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Despite their enormous complexity and structural diversity, most biological materials show a remarkably similar viscoelastic phenomenology: nonlinear elasticity, power-law or logarithmic stress relaxation, and plastic length adaptation. Here we present a simple model based on Huxley's sliding filament model to demonstrate that such behavior can arise from generic structural properties, independent of the actual molecular constituents of the system. We compare the model predictions to data from active and passive microrheological experiments on epithelial cells and fibroblasts, smooth muscle tissue, and extracellular matrix protein networks.

The material is represented by an uniaxial arrangement of infinitely stiff filaments crosslinked with parallel elastic elements that have a distribution of attachment angles. When the system is sheared or stretched, elements start to align, leading to strain stiffening due to a geometric recruitment of springs. The elastic elements have force-dependent average lifetimes described by energy traps with a broad distribution of energy trap depths. Broken links can reattach at random positions and attachment angles after unbinding. Such nanoscale structural rearrangements lead to viscous flow and plastic length adaptation on a macroscopic scale. Due to a broad distribution of energy trap depths, the system displays power law stress relaxation and soft glassy rheology.

The model is capable of qualitatively reproducing experiments, and gives quantitative agreement for creep compliance, stress stiffening and plasticity in the case of cell microrheology. These results suggest that recruitment and dynamic unbinding of elastic elements are the common mechanism underlying the mechanical behavior of many complex biological materials from single cells to whole tissues.

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Mechanical perturbation of T cell actin retrograde flow

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The interplay between the plasma membane morphology and the actin network at cell-cell interfaces is believed to play an important role in various signaling pathways. Here, we manipulate the curvature of the membrane and the conforming actin at hybrid cell-supported membrane junctions. We demonstrate that the micron scale protein patterns in the T cell immunological synapse are altered merely by the curvature imposed by the supporting substrate. The radial symmetry of actin and other signaling proteins breaks, and the shape of the cell junction elongates up to three fold across one-dimensional (1-D) grooves. Cell aspect ratio is dependent on groove frequency and curvature. Our observations show that geometrical perturbations at membrane junctions can remodel actin retrograde flow.

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Non-linear Rheology Of Collagen Type I Gels Probed On The Length Scale Of A Migrating Cell

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Recent investigations showed that the dimensionality of the environment in which living cells are cultured - flat 2D culture wells versus 3D biopolymer networks - has a strong effect on cell morphology, metabolism and migration. The reason for these differences are unclear. What is lacking is a fundamental understanding of the mechanical and morphological properties of 3D matrices at varying length scales.

We probe the local microrheology of a series of reconstituted collagen gels with different concentrations (1.2 - 2.4 mg/ml) by applying a calibrated force on embedded magnetic particles (Ø4.5μm) using magnetic tweezers. The resulting strain field within the matrix is visualized by tracking the positions of polystyrene spheres (Ø1μm) embedded in the collagen gels. This strain field is compared to expectations from continuum theory. In addition, the local microrheology is compared to bulk rheological properties measured in a cone-plate rheometer. At low forces and strains below 3%, local and bulk rheological properties agree closely, and the strain field follows that of a continuum linear elastic material. At higher strains, marked non-linear strain stiffening occurs, showing an increase in modulus of nearly 20-fold until the material eventually yields. Because of the non-uniform shear conditions around magnetic beads in the local microrheology experiments, the non-linear stiffening appeared to be less pronounced, but the strain field spread much farther out than expected from continuum theory. These data suggest that the strain stiffening behavior of collagen gels, together with the well-documented ability of cells to sense the stiffness of their surroundings, could account for the differences in cell behavior seen in 2D versus 3D culture conditions.

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The Role of Quaternary Structure in the Signaling Mechanisms of PAS Sensor Domains

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The modular nature of proteins containing PAS (Per-ARNT-Sim) or other sensor domains enables signaling networks to be diverse and poses an interesting question: how can sensor domains with largely conserved tertiary structures regulate effector domains with very diverse structures and functions? We address this question by examining signal processing by the PAS sensor domain, which can regulate the activity of covalently linked effector domains such as a kinase, phosphodiesterase or DNA binding domains. In many cases oligomerization of sensor proteins is essential for signal transduction. We present the structure of a heme-PAS domain dimer from *Bradyrhizobium japonicum* (*bj*FixLH) in a new space group (P1) and at higher resolutions (1.5-1.8 Å) than those previously obtained. Interestingly, *bj*FixLH can form two different dimers in the same crystallization solution, where the monomers in one dimer are rotated ~175° relative to the second. This suggests that PAS monomers are plastic and that two quite distinct quaternary structures are closely similar in free energy. Comparison of PAS domain dimers using screw rotation analysis reveals that PAS monomers adopt a discrete range of monomer orientations. Similar to the light-sensitive PAS domain YtvA-LOV from *Bacillus subtilis*, *bj*FixLH undergoes signal-induced quaternary structural changes where monomers rotate ~2° relative to each other. Signal-induced quaternary structural changes accommodate the ability of PAS sensor domains to regulate a wide variety of effector domains since PAS and effector domains would not be required to interact with each other in a structure-specific manner. Our results will guide the rational design of novel PAS signaling proteins.

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A 3D Cell Traction Force Measurement Technique Based on Collagen Fiber Tracking

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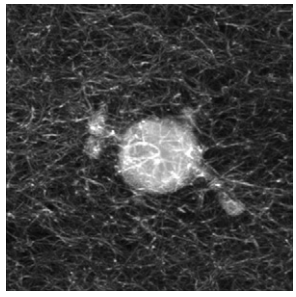
Mechanical interactions between cells and the extracellular matrix play an important role in determining essential cell behaviors such as cell migration, proliferation, wound healing and metastasis. While creative techniques have been recently devised and successfully implemented to measure the forces a cell can generate on a two-dimensional substrate, three-dimensional measurements have yet to be validated. Because many cells, in their physiological environment, live in a 3D matrix rather than on a 2D surface, a true understanding of cell-matrix interactions requires robust 3D force measurements.



We describe a new experimental technique and image analysis tools to measure forces generated by cells in a 3D reconstituted collagen matrix. This technique is based on confocal imaging of fluorescently-labeled collagen fiber networks around