collagen and its associated proteoglycan decorin (Magzoub et al., Faseb J. 2008). Our studies also revealed an unexpected dependence of diffusion in deep brain on the size of the diffusing macromolecule (Zador et al., Faseb J. 2008). Here, we report a new method to measure diffusion in the ECS using a dual-lumen device consisting of a micropipette for fluorescent dye delivery in close proximity to a guide barrel for introduction of the optical microfiber. Small quantities of extracellular fluorescent dyes are delivered by iontophoresis or pulsed pressure injection, and the kinetics of dye diffusion measured with the optical microfiber. With this method we found slowed diffusion of a small dye, calcein, in tissue vs. saline, D_o/D, of 3.9 ± 0.5 in brain cortex, in agreement with prior data, with substantial slowing of ECS diffusion in skeletal muscle (D_o/D = 229 ± 21) and kidney (D_o/D =134 ± 10). Relative diffusion of a larger dye, 70 kDa FITC-dextran, was similar in brain cortex to that of calcein ($D_o/D = 4.4 \pm 0.5$), though different in kidney ECS (D_o/D = 357 ± 16). This new microfiberoptic method has utility for measurement of diffusion of any fluorescent molecule deep in living tissue as well as for measurements of anisotropic diffusion.

92-Plat Limiting Calmodulin Revealed by Image Correlation Spectroscopy

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Protein-protein interactions depend on the ability of proteins to move from one place to another in search of their respective targets. The passive transport of signaling molecules is potentially hindered by obstacles or by its amount of available (mobile) pool. Calmodulin (CaM) a Ca^{2+} sensor protein searches among a wide number of targets, posing the question on whether there is enough non-bound CaM that can move freely through the cytoplasm to activate all of its targets. To address this question, we have used Raster Image Correlation Spectroscopy (RICS) to study the spatial distribution and mobility of CaM inside cells at rest and following elevation of intracellular Ca^{2+}. RICS can be performed on data collected with a standard confocal microscope and allows one to quantify the availability and diffusivity of a tagged protein from fluorescent fluctuations over space and time. Diffusion coefficients from a few hundred $\mu m^2/s$ to less that 1 $\mu m^2/s$ can be resolved using RICS. At the whole cell level on cells expressing GFP tagged CaM, only ~ 15% of the fluctuations generate a diffusion rate of > 2 $\mu m^2/s$, while the rest come from the immobile pool or the background motion of the cell. This rate is comparable to the diffusion rate of a target protein CaM Kinase II tagged with GFP (GFP-CaMKII). A non-interactive molecule like GFP, which diffuses at a similar rate as GFP-CaM in solution, has a diffusion coefficient of ~ 20 $\mu m^2/s$ when measured inside the cells. We conclude that the intracellular diffusion of GFP-CaM is consistent with the hypothesis that CaM is sequestered by the wide number of targets and its availability for signal transduction is limited.

Platform I: Cell Mechanics & Motility

93-Plat Uniaxial Loading Of Neurons Results In Cyoskeletal Reorganization

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Understanding how mechanical forces affect growth and signaling of neurons is vital to regenerative therapies used for the treatment of neurodegenerative disease or trauma. Research on the mechanical properties of neurons has largely been restricted to studies on the effect of surgical procedures, such as limb lengthening, at the tissue level. Consequently, there is a dearth of information on the response of individual neurons to mechanical loading. Additionally, while there are several commercially available cell-stretchers, many are not intended for high magnification fluorescence microscopy, and are extremely costly. In order to address these issues, we have designed and fabricated a cell stretching device capable of uniaxially loading cells. Design criteria include the ability to image both live cell morphology and the movement of fluorescently labeled subcellular organelles during loading Cells are cultured on a flexible lysine-coated silicone membrane and inverted onto a glass-bottomed aluminum culture chamber. The membrane is attached to multidirectional actuators, which allow its positioning within the culture chamber and also control membrane strain. In this study, we probed the response of mouse neuroblastoma cells to static mechanical loading (n=42). Immediately upon loading, cells stretch with the substrate, thereby exhibiting viscoelastic behavior. This is followed within 10–15 minutes by an active response phase during which the cell body shrinks and neurites retract and/or elongate both parallel and perpendicular to the axis of strain. These results strongly indicate dramatic cytoskeletal reorganization in response to mechanical loading. The effect of chronic loading on the directionality and dynamics of neurite growth is currently being investigated with a focus on this reorganization.

94-Plat The Limits of Filopodium Stability

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Filopodia are long, finger-like membrane tubes supported by cytoskeletal filaments, that are thought to play a mechanosensing role in cell motility and axon growth. Their physical shape is determined by the properties of the stiff actin filament bundles found inside them and by the interplay between membrane surface tension and rigidity.

Although one might expect the Euler buckling instability to limit the length of filopodia, we show, through simple energetic considerations, that this may not be the case. By further analyzing the statics of filaments inside membrane tubes, and through computer simulations that capture membrane and filament fluctuations, we show under which conditions filopodia of arbitrary lengths are stable, and that in these filopodia the filaments adapt a helical shape (the attached figure an unstable filopodium in the process of

collapsing). We will also discuss several in vitro experiments where this kind of stability has already been observed.

95-Plat Physical Plasticity of the Nucleus in Stem Cell Differentiation

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Cell differentiation in embryogenesis involves extensive changes in gene expression and structural organization within the nucleus, including chromatin condensation and nucleoprotein immobilization. Nuclei within naive stem cells were therefore hypothesized to be *physically* plastic and also more deformable than nuclei in differentiated cells. Micromanipulation methods indeed show that nuclei in human embryonic stem cells are highly deformable and stiffen 6-fold through terminal differentiation, and that nuclei in human adult stem cells possess an intermediate stiffness and deform irreversibly. Since the nucleo-skeletal component Lamin A/C is not expressed in either type of stem cell, this protein was knocked down in human epithelial cells - imparting a deformability similar to that of adult hematopoietic stem cells. Rheologically, lamin-deficient states prove to be the most fluid-like, especially within the first ~10 sec of deformation. Nuclear distortions that persist longer than this are irreversible, and fluorescence imaged microdeformation with photobleaching confirms that chromatin indeed flows, distends, and reorganizes while the lamina stretches. The rheological character of the nucleus is thus set largely by nucleoplasm/chromatin, while the extent of deformation is modulated by the lamina.

96-Plat Assembly Of The Cytokinetic Contractile Ring In Fission Yeast By A Search, Capture, Pull, And Release Mechanism

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We used high time resolution quantitative confocal microscopy and numerical simulations to study the assembly of the actomyosin contractile ring in fission yeast. In fission yeast the contractile ring is formed from the condensation of a broad band of ~63 membrane-bound "nodes" containing myosin-II, formin Cdc12p, and other proteins (Wu et al. J. Cell Biol. 2006). In cells expressing myosin regulatory chain Rlc1p labeled with GFP, we discovered that nodes make many starts, stops, and changes of direction as they condense into a ring. In a typical movement episode which lasts ~ 20 s, nodes move with speed ~ 30 nm/s. Estimates of the node friction coefficient from the mean square displacement of stationary nodes imply that node movement requires a force $F > 4$ pN, in the same range as the force exerted by a few molecular motors. The highly stochastic pattern of node motions indicates that actin filaments nucleated by formin Cdc12p establish transient actomyosin connections between nodes. Time lapse imaging of cells expressing calponin homology domain tagged with GFP to mark actin filaments showed transient linear elements extending in all directions (at ~200 nm/s) from Rlc1p-tdTomato nodes and establishing connections among them. Some nodes connected by actin filaments moved along the direction of the linear element. We propose a model with traction between nodes depending on transient connections established by stochastic search and capture ("search, capture, pull and release"). Numerical simulations of the model using parameter values obtained from experiment condense nodes into a continuous ring through life-like stop-go movements.

97-Plat Mechanical Architecture of the Mitotic Spindle in *Xenopus* Egg Extracts

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The accurate segregation of chromosomes is required for genome stability. The mitotic spindle is a multi-component macromolecular machine composed of cytoskeletal networks, molecular motors, and other regulatory molecules, of which role is to partition the duplicated genome to the daughter cell in each cell division. Microtubules, dynamic polymers of the cytoskeletal protein tubulin, provide the mechanical framework for mitotic spindle function. In

recent years, most of the protein components of the mitotic spindle have been identified. The roles of key molecules (kinesins, cytoplasmic dyneins, microtubule-associated proteins, etc.) in spindle dynamics are being characterized in vitro and in vivo using chemical and protein-based inhibition. Various studies suggested that the forces generated in the mitotic spindle are finely balanced through the action of the key molecules, such that the shape of mitotic spindle is maintained nearly constant during metaphase. This maintenance of mitotic spindle morphology is one of the most critical steps during mitosis. However, the mechanical architecture of the mitotic spindle is still unclear. Here, we report a new approach based on force measurements for studying the mechanical framework of in vitro assembled mitotic spindle. *Xenopus* egg extract is an excellent system for manipulating the mitotic spindle directly using biophysical methods such as force-calibrated micro-needles, which have been applied for the measurements of force generation in skeletal myofibrils. The approach is based on a unique combination of measurements of both force-dependent deformability and rigidity of whole mitotic spindle. By using specially designed force-measurement system, the force-compression curve of the mitotic spindle was determined for the first time. We have examined reorganization processes of the mitotic spindle after large deformation by applying external forces. We discuss the current progress in the research on the mechanical architecture of the mitotic spindle.

98-Plat Reorientation Of The Mechanical Properties Of The Cytoskeleton Of Endothelial Cells Resulting From Flow Shear Stresses

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Adherent cells undergo remodeling in response to external mechanical stimuli, with a consequent redistribution of intracellular forces. This redistribution depends on the composition and structural directionality of the cytoskeleton, and has been shown to play an important role in cell function. We have analyzed the temporal changes in the magnitude and directionality of the cytoplasm shear modulus (G) in cultures of confluent vascular endothelial cells subject to continuous shear flow. For this purpose, we have extended the Multiple Particle Tracking Microrheology technique, which is based on the analysis of the Brownian dynamics of intracellular markers. Our approach considers the complete second-order tensor of marker displacements, allowing us to determine at each position the principal directions of highest and lowest G. Every half-hour after the application of shear, the random motion of the mitochondria is tracked using phase-contrast microscopy. This non-invasive method has been validated by tracking micro-beads injected into static cells. Prior to the application of flow, the shape of the cells is polygonal without orientation, and G is isotropic in average for the cell population. The shear modulus of individual cells, however, exhibits directionality with varying orientations. After the application of flow, the cells elongate and the stress fibers gradually reorient parallel to the flow. Our measurements show that, as the stress fibers become aligned with the flow, the local regions with the lowest G are oriented parallel to the flow, while those with the highest G are oriented perpendicular to it. This reorientation of the principal directions of the shear modulus is found to occur with little changes in its overall magnitude in the whole cell.

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99-Plat Rescue Of Mechanical Function, Morphology And Signaling By Desmin Plasmid Transfection Into Muscles From Desmin Knockout Mice

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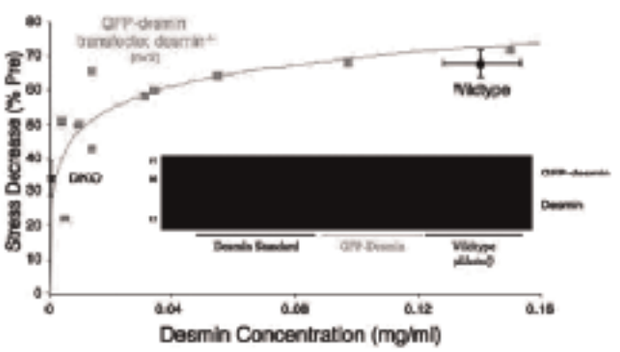
The muscle-specific intermediate filament desmin has been described as the mechanical integrator of the muscle cell. We probed this function by transfecting adult desmin knockout (DKO) muscle with plasmid DNA encoding desmin and measuring the resulting structural and functional changes. Mice were transfected by electroporation and, after 7 days, when protein expression peaked, we measured the regularity of the myofibrillar lattice and several responses to mechanical stretch including the deformability of nuclei, stress production, injury and c-Jun-kinase (JNK) phosphorylation. Desmin transfection reversed essentially all of the knockout phenotype in a quantitative manner: Z-disks were re-aligned, myonuclear deformability was restored, and stress production and the resulting injury response was also returned to near-wildtype levels in a dose-dependent, although in a nonlinear manner (Figure). Furthermore, the stretch-induced activation of JNK phosphorylation, which was not detectable in the DKO muscles, was restored. These results demonstrate that the desmin intermediate filament is primarily respon-sible for the spatial alignment of Z-disks and plays a central role in transducing mechanical stress:

1. from the force-generating cytoskeletal network to the surrounding tissue and
2. to the intracellular signaling apparatus.



100-Plat Myosin II Plays a Specific Role in Driving Glioma Invasion of Normal Brain

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The ability of gliomas to invade normal brain enormously limits the efficacy of standard therapies. Targeting this invasive phenotype requires understanding how glioma cells move and what intracellular machinery they use to invade normal brain. In normal brain, glioma migration occurs in a two-step process that resembles how neural and glial progenitor cells migrate *in situ*. These steps consist of extension of a leading process, followed by forward propulsion of the cell body. We show that forward propulsion of the cell body requires the molecular motor myosin II when these cells have to crawl through the mechanically-constrained 3-dimensional matrix that characterizes the brain. By contrast, myosin II activity is unnecessary when these tumor cells migrate on a 2-dimensional surface in the absence of such mechanical constraints. Immunoblots and immunofluorescence reveal that tumors and their associated vasculature markedly up-regulate myosin IIA expression, and RNAi suppression of myosin IIA, but not myosin IIB, blocks tumor cell invasion. Our results thus demonstrate that myosin IIA has a specific and indispensable role in driving glioma invasion of normal brain, presumably by providing internal compressive forces need to propel the cell body and nucleus forward.

Symposium 5: Driving Forces in Macromolecular Binding

101-Symp Complexity in protein-protein interfaces

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Protein-protein interactions are characterized by a striking structural plasticity that allows contact points to adapt to conformational changes and multiple amino acid substitutions. As a result, the biophysics governing the steric and energetic properties of protein-protein interactions is extremely complex. The structure-function data base for human growth hormone (hGH) binding to its receptor (hGHR) is probably the most extensive available for any large protein-protein interface, but attempts to extract universal trends have been hindered by the incompleteness of the data set. We have developed combinatory techniques that have allowed us to access the structural and functional effects of all possible point mutations across the hGH-hGHR interface. This has allowed us to assess the binding effects of introducing all 20 amino acid types in the 35 positions comprising the hGH binding interface. This has produced the most comprehensive picture of adaptability in a large protein-protein interface that has ever been achieved and has led to some new and unexpected insights. We have determined that while there are a few hot-spot residues that are immutable, at least half of the 35 residues can be simultaneously mutated to alanine without affecting binding. Our data challenge the concept of conservative substitutions, especially in the case for hydrophilic residues. Sequence conservation across species is a poor predictor of functional importance. Hydrophobic residues in many instances can replace hydrophilic ones even if that residue is involved in a specific H-bonding or salt-bridge interaction. In a complementary phage display study, we show that high affinity interactions can be achieved using a simple binomial (Tyr, Ser) genetic code. This facilitates building high affinity and specific interactions with a highly restricted chemical diversity and demonstrates that conformational diversity trumps sequence diversity in protein-protein interactions.

102-Symp Biophysics of Interactions in Non-globular Proteins

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Protein-protein interactions mediated by non-globular proteins generally involve more than one folded protein domain in the interaction interface. Experimental results from two different interactions will be discussed; the LRP1/apoE interaction and the NF-kB/IkBa interaction. Isothermal titration calorimetry, SPR, NMR, and amide H/D exchange results taken together help to understand these interactions in more detail. In both of these cases, contributions to the energetics of the interaction come from more than one "hot spot" and are energetically cumulative. In addition, the overall favorable energy of interaction has a contribution from folding upon binding. The functional significance in terms of binding to multiple targets and binding kinetics will also be discussed.

103-Symp Understanding Protein-ligand Interactions: Correlating Structure and Thermodynamics

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Isothermal titration calorimetry (ITC) provides a direct method for determining thermodynamic parameters associated with biomolecular interactions. The understanding of the correlation between the change in thermodynamic parameters for a binding event and the perturbation in structure forms one cornerstone of biophysical science. Clearly, if correlations can be defined then predictive algorithms can be produced which will permit the calculation of compound affinities directly from high resolution structural data (and vice versa). This would have dramatic effects on the temporally and financially expensive processes associated with compound development in the pharmaceutical industry. Although significant effort has been made in this area, particularly in the last decade, the application of these principles to drug development is fraught with inconsistencies and inaccuracies. The SCORPIO database (www.biochem.ucl.ac.uk/scorpio/scorpio.html) provides a repository for thermodynamic data derived solely from ITC and thus is not prone to potential problems in derivation of the enthalpic (and hence entropic) contributions to binding inherent in spectroscopic methods. Data from SCORPIO has been used to assess potential correlations between thermodynamic parameters and structure. These