activated by phorbol 12-myristate 13-acetate (PMA), which also affects shape and adhesion. Different cell types express different subsets of isoforms. In tracheal epithelial cells, the activation of beta and epsilon isoforms by PMA was followed by their degradation. We sought to determine whether such activation-degradation patterns affected protrusion formation or turnover. Cells were treated by transcriptional knockdown (KD) and the prevalence of each protrusion class was analyzed by computerized morphometry. Latent factors for filopodia (#4) and nascent neurites (#7) were calculated based on geometric variables determined for known populations. At zero time, factor #7 was elevated by alpha KD. Control samples, into which a random KD sequence had been introduced, showed an increase in neurites throughout the PMA exposure. During the time course, all KD samples differed from controls at one or more times. Epsilon KD followed by PMA drastically downregulated the isoform by 15 h and decreased #7 values relative to control. Alpha KD was similar suggesting that the early KD effect (increasing #7) was reversed later on. Beta KD cells resembled control at 15 h but alpha KD at the 5-h time. Filopodia were eliminated by a 2-h PMA exposure regardless of the KD agent introduced. Control samples remained depressed, but epsilon and alpha KD samples partially recovered by 5 h. Alpha KD declined again dramatically by 15 h. The time courses suggest that the main effects were exerted by the actin-binding PKC epsilon, but alpha, which is not actin-binding, could have similar effects. The data suggest that competitive binding on RACKs may be occurring, complicating the picture of kinase regulation of adhesion and protrusive activities.

**859-Pos Board B614**

**In Vitro Reconstitution of Remodeling Actin Asters - Steps towards a Minimal Active Actomyosin Cortex**

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A major aim of our work is to understand the mechanisms behind dynamic organization of the cellular plasma membrane, especially local heterogeneities such as nanometer sized lipid domains (Mayor and Rao, 2004). As reported previously, glycosyl-phosphatidylinositol-anchored protein (GPI-AP) organization in nano-clusters in the plasma membrane is driven by the activity of cortical actin (Goswami et al., 2008).A recent theoretical framework and its experimental verification suggests that the engagement of short actin filaments together with myosin-motor like activity at the inner leaflet is sufficient to explain all the unusual features of GPI-AP organization at the outer leaflet (Gowrishankar et al., 2012).

Here, we present a strategy to reconstitute cortical actin dynamics *in vitro* on supported lipid bi-layers. This allows us to explore the role of proteins thought to be involved in actin cluster formation and to test predictions of the theoretical model. In a first step, we investigate how the diffusion of membrane bound actin binding proteins is affected by actin filaments of varying lengths. Then, we increase the complexity of the system including myosin motors, and actin modifying proteins, and identify conditions under which actin remodeling, i.e. transient formation of actin asters, occurs. As suggested by observation in cells and by the theoretical framework, short actin filaments ($< 1$ μm) are the main source of fast remodeling events whereas longer filaments create a more static meshwork, which can confine membrane bound particles. In summary, we introduce a new kind of minimal dynamic actin cortex, and show how dynamic short actin filaments can drive the organization of membrane components.

**860-Pos Board B615**

**Role of CARP as a Bio-Mechanosensor**

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Cardiomyocyte mechanical stretch induces hypertrophic gene expression, however, the mechanisms for this are poorly understood. Cardiac ankyrin repeat protein (CARP) is highly expressed in cardiomyocytes and interacts with the spring domain of sarcomeric titin and is also localized in the nucleus. This dual localization suggests that CARP may couple titin spring mechanics to muscle gene expression. CARP is also expressed in cardiac fibroblasts (CF) and that CARP interacts with the transcription factor GATA4. We hypothesize that CARP is a bio-mechanosensor that upon cell stretch, translocates to the nucleus and interacts with GATA4 to induce gene expression. Methods: Neonatal Rat Ventricular Myocytes (NRVMs) and CF were isolated from 1-2 day old rats and cultured on BioFlex plates. After 2 days, NRVMs were transfected with CARP siRNA (50nM) and/or a GATA4-luciferase vector. NRVMs and CF were stretched 5-10% for 60 min (S60) to 48hr at 1Hz using the Flexcell system. Cells were fixed or lysed for microscopy, western blotting, or luciferase assay. Results: Unstretched NRVMs have predominantly sarcomeric CARP immunostaining with low nuclear CARP. CARP translocates to the nucleus with S60 and remains nuclear up to 48hr stretch. ERK inhibition with U0126 prevented S-60-induced CARP nuclear translocation. NRVM S60 induced GATA4 phosphorylation and increased GATA4-luciferase expression, which are both inhibited with CARP siRNA. NRVM S60 followed by cessation of stretch for 60 min (SC60) resulted in depletion of nuclear CARP. CF show nuclear CARP, which becomes cytoplasmic with S60. CF subjected to S60 followed by SC60 showed re-localization of CARP to the nucleus. Conclusion: We conclude that CARP is involved in stretch-mediated signaling in cardiac myocytes and fibroblasts. Our data further suggest disparate mechano-sensing roles for CARP in these cells types.

**861-Pos Board B616**

**Primary Cilia Respond to Uniaxial Strain by Reorienting and Elongating along the Axis of Stretch**

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The primary cilium is a ubiquitous, microtubule-based organelle that protrudes from the cell surface and acts as the cell's antenna to sense extracellular signals, such as growth factors, fluid flow and developmental morphogens. Ciliary defects have been implicated in a diverse spectrum of diseases including retinal degeneration, polycystic kidney disease, mental retardation, obesity and situs inversus. In order to sense extracellular signals appropriately, cells must have primary cilia of certain lengths in each organ or tissue. However, it is unclear how cells control ciliary length. We hypothesize that assembly and disassembly of primary cilia are affected by the cellular environment, including mechanical stress from the substrate and cell shape. Recent studies indicate a relationship between the dynamics of actin filaments and the length of cilia. To test whether cell environment is involved in cilia length and orientation, we investigated the effect of uniaxial strain on cilia in retinal pigment epithelial (RPE) cells using a polydimethylsiloxane (PDMS) stretching device. After uniaxial strain, cilia elongated and rotated along the axis of stretch. In addition, we found that this behavior is dependent on actin network integrity and myosin activity. These novel findings propose a potential link between primary cilia behavior, cellular mechanosensation and actomyosin contractility. This stretching system can be used for understanding other biological problems related with mechanical stress, such as organelle size and orientation of cell division.

**862-Pos Board B617**

**Measuring Sub-Cellular Rheology in Zebrafish Embryos**

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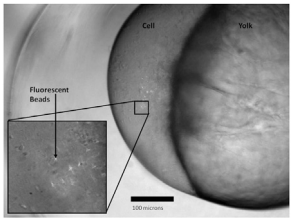
Cells are more than just bags of chemicals. The cell's mechanical properties, in addition to its chemical properties, play a large role in determining its motility, shape, differentiation, adhesion, and growth. Our goal is to measure these mechanical properties of the cell, specifically the frequency-dependent viscoelasticity of the cytoplasm at the sub-cellular level. Measurements on this scale allow us to link cytoskeletal rearrangements that occur during development with cellular viscoelastic evolution. Accordingly, we used particle tracking microrheology in the large (~500-micron-diameter) one-cell zebrafish embryo to accurately measure these parameters on a sub-cellular scale. In particle tracking microrheology, the Brownian motion of injected beads is tracked to determine both the viscous and elastic moduli of the cytoplasm. We injected fluorescent beads into the one-cell stage of the zebrafish embryo (see Figure) and assigned the beads to separate domains based on their radial, axial, and temporal positions within the cell. Viscoelastic values measured in each domain were compared with fluorescent data of the embryo's cytoskeleton to assess the current models of how cytoskeletal variations affect cellular rheology.




**863-Pos Board B618**

**Mapping Internal Stress of in Vitro Cytoskeletal Networks with UV-Laser Ablation**

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In vitro networks open up the possibility of investigating cytoskeletal components individually and in a very controlled environment. The process of

assembly may lead to kinetically trapped networks [1], thus giving rise to internal stress. To investigate this phenomenon local probing of the stress field is necessary, which is achieved by means of diffraction limited UV-laser ablation: We produce small cuts inside in vitro networks of fluorescently labeled actin and its crosslinkers, or keratin. Analysis of simultaneously acquired confocal and bright field images of the system's reaction reveal relaxation properties and show anisotropies in the viscoelastic behavior of these networks.

[1] Schmoller et al.: Internal stress in kinetically trapped actin bundle networks. Soft Matter, 2008, 4, 2365-2367.

**864-Pos Board B619**

**Investigating the Relationship between Cellular Mechanics and Beta Amyloid in Alzheimer's Disease**

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The beta amyloid (Aβ) peptide was first implicated in the formation of amyloid plaques, a characteristic of Alzheimer's disease (AD), nearly 30 years ago. Misfolding of the Aβ peptide into nonnative conformations promotes the formation of aggregates such as oligomers, annular aggregates and fibrils that can impair cellular function. Although these aggregates are known to be toxic, the exact mechanism of their cytotoxicity remains unclear. As aging is associated with AD, we explored how cytoskeletal degradation, associated with the aging process, modulates a cell's ability to cope with exposure to exogenous Aβ. In this study, hypothalamic neurons, of the GT1-7 cell line, were treated using a variety of cytoskeletal altering drugs. The mechanics of the altered neurons were examined by atomic force microscopy-based techniques (force-distance curve and force volume analysis) and the altered neurons were then exposed to the Aβ peptide to determine how the cytoskeletal network affects peptide binding and toxicity. Binding studies were performed using fluorescence activated cell sorting and toxicity was examined using a variety of biochemical assays. Finally, the topography and mechanics of the the aged neurons exposed to Aβ were examined to determine the impact of Aβ binding. This research gives a mechanistic understanding of AD pathology in relation to the cytoskeleton and examines the potential for cytoskeletal stabilization as a therapeutic intervention for combating AD.

**865-Pos Board B620**

**TIRF and Model Blood Vessels Combined to Elucidate the Role of the Cytoskeleton in Platelet Activation**

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Platelets are cell fragments that play a central role in hemostasis and thrombosis. Typically platelets circulate as thin flat disks; however upon encountering a damaged vessel wall they are recruited to the exposed subendothelial matrix where they adhere and activate. The platelets interact with the exposed matrix proteins via specific adhesive glycoproteins found on the surface of the platelet. Activated platelets have extensive pseudopodia due to a change in the assembly of cytoskeletal proteins such as actin and tubulin. The rearrangement of the cytoskeleton also corresponds with the secretion of granule content leading to the amplification of the platelet stimulation and the formation of a stable platelet-fibrin plug. A popular model assumes that the granules are drawn to the center of the platelet before release occurs, indicating the importance of the cytoskeletal components that drive this centralization leading to the eventual clot formation.

In this study we will examine cytoskeletal protein arrangements in granule release using TIRF to study the activated platelets that aggregate on the protein coatings along a microfluidic channel. These protein coatings will include proteins found in damaged vessel walls such as collagen and fibrinogen. TIRF uses an induced evanescent wave created at the interface of a glass and to selectively excite fluorescent molecules located near the interface. Here we will describe the microfluidic channels we used to model blood vessels. By controlling which proteins are coated on the inside of the channels we are able to visualize platelet activation in a more native setting. We will also discuss methods that were used to stain cytoskeletal proteins to reduce the premature activation of the platelets. A better understanding of these models will lead to a greater understanding clot formation.

**866-Pos Board B621**

**Reconstituted Active Actin Networks in Confinement**

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Giant unilamellar vesicles (GUVs) are a great model system to explore the functional mechanisms of the constantly rearranging cytoskeletal network in a bottom-up approach. Here, we developed a reconstituted active actin network consisting of actin filaments, crosslinking proteins and myosin-II motor filaments in confinement. In this minimal model system, the key parameters are tightly controlled, which leads the way to gain a better understanding of the basic physical principles underlying the complex cytoskeletal dynamics. By means of quantitative fluorescence microscopy and image analysis, the interplay between force generation by molecular motors and the stabilization of the network by crosslinking proteins is identified to be responsible for the highly dynamic structure formation process.

**867-Pos Board B622**

**Force-Dependent Mechanical Properties of Dendritic Actin Networks**

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Branched actin networks generate protrusive forces required for cell motility and movement of sub-cellular structures. While biochemical knowledge of dendritic actin network assembly continues to advance, relatively little is known about how the mechanical properties of networks respond to physical stimuli during growth. By combining surface micropatterning with AFM and TIRF microscopy, we are able to measure mechanical properties of in vitro reconstituted branched networks that grew under different counter forces in a biochemically defined environment and simultaneously measure protein densities at the force-generating surface. Our measurements show that 1) the elasticity of the network increases more strongly than actin density with increasing counter force, 2) the force to plastically deform the network also increases with the increasing counter force, 3) the networks growing under larger counter forces show stronger stress stiffening and softening, while networks growing under small counter forces do not. These results indicate that the structure of actin networks is permanently altered by different counter forces experienced during growth, giving rise to different network mechanical properties. Our AFM-TIRF measurements provide new insight into the role of force in the assembly and mechanical properties of dendritic actin networks.

**868-Pos Board B623**

**Athermal Fluctuations of Probe Particles in Active Cytoskeletal Network**

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A reconstituted active cytoskeletal networks consisting of an actin filament network coupled to myosins (motor proteins) have been shown to display rich in dynamical and mechanical behaviors that is often in contrast to passive, equilibrium system. The motor proteins, which spontaneously generate forces, kept the active cytoskeletal network out of equilibrium. The athermal fluctuations observed in the network are linked to the active force generation by motor proteins which give more relevant information including the interaction with the surrounding materials. In prior studies, only the second moment–also referred to as the mean square displacement or power spectral density–of the athermal fluctuations has been investigated. In equilibrium where the Gaussian statistics are implied, second moment analysis supplies all the necessary information to characterize the fluctuation. There is no reason *a priori* to expect Gaussian statistics in non-equilibrium systems. Indeed, the full displacement distribution of the athermal fluctuations in active cytoskeleton recently probed using video microrheology is found to be far from Gauss. No theoretical model has been found yet that describes the non-Gaussian signature of the distribution. Therefore, in this study, we examine the non-equilibrium statistics and dynamics of the active network by analyzing the athermal fluctuations using a new theoretical model developed by I. Zaid, *et al*. The model, which is based on Lévy statistics, incorporates the thermal and athermal fluctuations and assumes that a single myosin acts as a force dipole. In our results, it was found out the full displacement distribution follows truncated Lévy statistics distribution. Sum action of multiple motor proteins, which drives the probe particle, only slowly converges to Gauss distribution because of the $1/r^2$ spatial decay of the motor impacts.