elastic response of cells. In the cell, these networks are far from equilibrium: their mechanical properties can reflect internal active force generation by molecular motors. We develop a simple three-component *in vitro* model system consisting of myosin II, actin filaments, and cross-linkers [1]. By measuring the dynamics and mechanical properties, we quantify the effects of non-equilibrium stresses arising from motor activity. We show theoretically and experimentally how this motor activity can result in a 100-fold stiffening of the cytoskeleton. We present a quantitative theoretical model connecting the large-scale mechanical properties of this active gel to molecular force generation [2]. Based in part on this theoretical model, we investigate and explain the large shape fluctuations observed for microtubules *in vivo* [3]. Although microtubule bending is suppressed by the surrounding elastic cytoskeleton, large motor-induced forces cause significant bending fluctuations on short length scales, which are then frozen-in by the surrounding matrix. These lateral bending fluctuations naturally result in wandering of the orientation of the microtubule tip, and an apparent persistent random walk of the microtubule, with a small non-equilibrium persistence length approximately 100 times smaller than that resulting from thermal fluctuations alone. Thus, large non-thermal forces govern the growth of microtubules and can explain the highly curved shapes observed in the microtubule cytoskeleton of living cells.

References

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972-Symp Architectural Dynamics of the Meiotic Spindle Revealed by Single-Fluorophore Imaging

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Bipolarity of the meiotic spindle, required for proper chromosome segregation, is maintained throughout cell division despite rapid microtubule turnover. How this is achieved has remained mysterious as determining the organization of individual spindle microtubules has been difficult. Here, we develop single fluorophore speckle imaging to examine microtubule organization in the vertebrate meiotic spindle. We find that the mean length of microtubules is ~40% of spindle length. Long and short filaments distribute randomly throughout the spindle and those in close proximity can move in the same direction with highly heterogeneous velocities. The ratio between microtubule and spindle lengths remains unchanged as spindles elongate upon dynein/dynactin inhibition. However, maintaining this ratio depends on proper kinesin-5 function. Our data suggest that force transmission within the spindle must be understood in terms of crosslinking dynamics of a tiled array of individual filaments, most of which do not span the distance from the pole to the metaphase plate.

Symposium 12: Non-Conducting Functions of Ion Channels

973-Symp Mutations in ion channels and their auxiliary subunits can lead to neurological or cardiovascular diseases

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Mutations in ion channels and their auxiliary subunits can lead to neurological or cardiovascular diseases. In recent years it has become apparent that ion channels are part of large, multi-protein complexes, comprising not only the channel pore and its auxiliary subunits, but also components of the cytoskeleton, regulatory kinases and phosphatases, trafficking proteins, extracellular matrix proteins, and possibly even other ion channels. Sodium channel beta subunits do not form the ion-conducting pore, but are multifunctional proteins that play critical roles in modulation of channel function, regulation of channel expression levels at the plasma membrane, cell adhesion, neurite outgrowth, and transcription. Beta subunits signal through multiple pathways on multiple time scales in a tissue-specific, and possibly even subcellular domain-specific, manner . This feature makes sodium channels unique among the superfamily of voltage- and ligand-gated ion channels. In vitro evidence suggests that sodium channel beta subunits serve as critical communication links between adjacent cells, the extracellular environment, and intracellular signaling mechanisms, possibly including other ion channels. We propose that disruption of any member of a sodium channel signaling complex in vivo has the potential to disrupt channel function, resulting in paroxysmal disease, such as epilepsy or cardiac arrhythmia. In addition, because beta subunits can function as cell adhesion molecules in the absence of the ion conducting pore, mutations in beta subunit genes may result in defects in axon guidance or cell-cell communication. Understanding the molecular composition of individual sodium channel signaling complexes in excitable cells, as well as the conducting and non-conducting functions of the beta subunits may yield important insights into the molecular basis of inherited disease.

974-Symp Role of an Ion Channel Regulatory Protein Complex in Neuronal Physiology

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Many ion channels are intimately associated with one or more auxiliary proteins that participate in the regulation of channel activity. While the molecular details of ion channel regulatory protein complexes have been widely studied, their physiological roles remain poorly understood. We have taken advantage of Drosophila genetics to explore the role of the Slowpoke channel binding protein Slob in the modulation of large conductance calcium-dependent potassium channel activity in vivo. Slob has been shown previously to exhibit protein kinase activity in vitro, although its physiological substrates have not yet been identified. Patch

recordings from neurons in the brains of living flies reveal changes in macroscopic outward current in Slob null and Slob over-expression flies. These changes are consistent with the effects of Slob described previously in a heterologous expression system. Furthermore, in vivo single channel recordings demonstrate large changes in Slowpoke channel activity in Slob null and Slob over-expression flies. Our results provide evidence that an ion channel regulatory protein complex can modulate neuronal physiology, and ultimately behavior, in an intact organism.

975-Symp MPS-1 is a serine/threonine kinase

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MPS-1 is an accessory subunit of K^+ channels which belongs to the evolutionarily conserved family of KCNE proteins. MPS-1 is expressed in the metazoan *Caenorhabditis elegans* where it co-assembles with voltage-gated K^+ channel KVS-1 to form a complex that is necessary for the normal function of the sensory apparatus of the worm. Of particular interest is that MPS-1 possesses kinase activity. Thus, MPS-1 can phosphorylate KVS-1 and other substrates. Electrophysiological analysis in CHO cells, shows that MPS-1 activity acts to specifically regulate the magnitude of the macroscopic potassium current by controlling the open probability of the channel. We found that disruption of MPS-1 kinase activity leads to learning-disability in *C. elegans*. Worms bearing a dead kinase variant, obtained by mutating a D to N in the catalytic site retain normal nervous function but cannot habituate to repeated sensory stimulation. Taken together these data indicate that the enzymatic activity of an ancillary subunit can produce plasticity of K^+ channel function to specifically control neuronal excitability and habituation behavior in the nematode.

976-Symp Modulation Of Kv1 Channel Activity By Structural Changes Of An Associated Oxidoreductase

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Beta subunit (Kvβ) of the *Shaker* family voltage-dependent potassium channels (Kv1) assembles with the channel early in biogenesis to form a permanent macromolecule complex. We have shown that Kvβ is a functional aldo-keto reductase that utilizes NADPH as a cofactor. Furthermore, structural changes on Kvβ, for example, oxidation of the Kvβ-bound NADPH, changes Kv1 channel activities. These results suggest that Kvβ could transduce changes in cellular metabolic redox state into altered cell excitabilities, and imply a new way of regulating Kv1 channels. Small-molecule compounds that interact with Kvβ at low-micromolar concentrations were identified using an automated high-throughput screen in addition to a manual screen. Positive hits from the screens were examined for their effects on channel activities and then were co-crystallized with Kvβ for structural analysis. Atomic resolution structures of the complexes revealed different binding sites on Kvβ, likely representing different mechanisms of channel modulation. These small-molecule compounds are used as a tool to probe both the physiological functions of Kvβ, as well as the molecular mechanisms of channel modulation.

Platform AA: Coarse-Grained and Enchanced Sampling Biophysical Simulation Methods

977-Plat Internal Coordinate Molecular Dynamics Using Efficient Multibody Dynamics Algorithms

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In this work a methodology for reduced order modeling of biomolecular systems as sub-structured multi-rigid body articulated systems is presented and the integration of molecular dynamics software with multibody dynamics software to facilitate this modeling effort is discussed. Holonomic constraints are used to selectively curtail the frequency content of the system, thereby allowing for large temporal integration steps. These constraints are used to either freeze out select degrees of freedom or make rigid clusters of sets of interaction sites. An efficient, recursive method based on Kane's method is used for generating and solving the internal coordinate dynamics equations of motion of the coarse-grained bio-molecular and bulk materials. The methodology is verified by simulating several systems including explicit water, alkane chains, Alanine dipeptide and carboxyl terminal fragments of Calmodulin, Ribosomal, Rhodopsin L7/L12 and RuBisCO proteins. The stability and validity of the simulations are studied through thermodynamics properties and conformational analysis of nano-second long simulations of these systems. In these simulations, a speed up of an order of magnitude (or more) is realized as compared to classical molecular dynamics while preserving the essential dynamics of the system (within conservative error bounds). As a part of this work, a freely available, open-source computational tool was developed by combining a classical molecular dynamics software LAMMPS and a multibody dynamics research code called POEMS. This tool gains on the complementary nature of the two codes by coupling the efficient force calculation algorithms in LAMMPS with the efficient algorithm in POEMS for generating and solving the internal coordinate dynamics equations of motion.

978-Plat Accelerating the Convergence of Slow Diffusive Conformational Transitions with Molecular Dynamics

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Slow diffusive conformational transitions play key functional roles in biomolecular systems. Our ability to sample these motions with