were observed by negative staining EM, and their 3D reconstructions (computed by single particle methods) compared. The wild type reconstruction showed, at 4 nm resolution, the near-helical organization of the myosin heads, intramolecular head-head interactions, and immunoglobulin/fibronectin domains of titin and MyBP-C (Zoghbi et al., 2007, Biophys. J. *92*. 373a.). MyBP-C knockout filaments looked similar to the wild type in negative stain (Kensler 2007, Biophys. J. *92*. 297a.). However, the MyBP-C knockout reconstruction had a lower resolution (7 nm), suggesting that MyBP-C helps to stabilize the relaxed array of myosin heads in wild-type filaments. Titin domains were not distinguished at this resolution. Some of the intramolecular head-head interactions observed in wild-type filaments were absent in the knockout. Since such head interactions are important for the relaxed conformation of the filament, our results suggest that MyBP-C knockout filaments might not relax as fully as the wild-type. Poorer relaxation in the absence of MyBP-C may help explain compromised cardiac relaxation in MyBP-C knockout mice, and may also be related to the abnormal diastolic function in humans with HCM.

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7-Subg Computational Dissection of Intramolecular Interface Binding Energies in Various Myosin States

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Several crystal structures of the head domain of myosin are thought to represent various states in the crossbridge cycle. Here we offer an objective way to examine the various aspects of the energies involved in the myosin molecule and test hypotheses of force production and regulation. For instance, the 50k/25k cleft, the HW helix[1] and the 7 strand beta sheet are currently implicated as substructures capable of storing stress (tension) and productively releasing it as work at later points in the crossbridge cycle. We examined these energies and the implication of intramolecular interface (intraface) formation, dissolution and motion by computing ΔΔG of specific amino acids and their binding to neighboring substructures by virtual alanine scanning[2] intrafacial energies. We then compared these values in identical amino acids at various states in the crossbridge cycle. This produced a ΔΔΔG value describing how energy shifts inside the head at various states in the crossbridge cycle. These values represent the shifts in stress within myosin and within the crossbridge cycle. These intrafacial energy values and stress and strain estimates, combined with the empirical values of free energy of ATP hydrolysis, enthalpy and work output set limits for values of the energy necessary (and perhaps mechanism) to produce regulation as well as the interfacial energies associated with the actin myosin interface.

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Biological Fluorescence Subgroup

8-Subg In Vivo and In Vitro Studies of Endophilin Oligomerization

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Endophilin, which is involved in membrane vesiculation in receptor mediated endocytosis and vesicle trafficking, is a 40-kDa SH3 domain-containing protein that binds to the PRD domain of dynamin and to synaptojanin, a phosphoinositide phosphatase implicated in endocytosis. The N-terminus of endophilin contains a so-called BAR domain and the recent solution of its structure has suggested a mechanism for the ability of endophilin to induce membrane curvature. It has been suggested that dimerization of BAR domains results in a concave, positively-charged surface that can interact with, and thereby deform, membranes containing negatively charged lipids. It has been also been suggested that endophilin may be a monomer in the cytoplasm which can then dimerize upon binding to membranes or perhaps upon binding, via its SH3 domain, to dynamin's PRD domain. To clarify these issues we have studied the oligomeric state of endophilin, both in vitro (using AUC and fluorescence polarization) and in vivo. EGFP-endophilin, expressed in CV-1 cells, were studied using two-photon fluorescence correlation spectroscopy (FCS). The FCS data were analyzed using the Q-analysis method which allowed for determination of the intrinsic "brightness" of the labeled protein complexes and hence its aggregation state in the cytoplasmic regions of the cell. Despite a relatively high Kd (~5 micromolar) observed in vitro, the in vivo measurements indicate that endophilin is dimeric in the cytoplasm even at submicromolar concentrations.

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Membrane Structure and Assembly Subgroup

9-Subg Hydration of POPC Bilayers Studied by ^{1}H-PFG-MAS-NOESY and Neutron Diffraction

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Location of water molecules in POPC bilayers and the lifetime of water-lipid associations was studied by nuclear Overhauser en-

hancement spectroscopy (NOESY) under conditions of magic angle spinning as well as by neutron scattering. NOESY with application of pulsed magnetic field gradients (PFG) improved detection of very weak crosspeaks like those between water and lipids. NOESY spectra were recorded at mixing times from 5 to 800 ms and all water-lipid and lipid-lipid cross-relaxation rates determined by a matrix approach. The analysis shows that water molecules interact almost exclusively with sites of the lipid-water interface including choline-, phosphate-, glycerol-, and carbonyl groups, in perfect agreement with measurements of water density distribution by neutron diffraction at identical conditions. Water does not penetrate beyond lipid carbonyl groups to a significant extent. The lifetime of water associations with any segment of lipids is rather short, on the order of 100 ps. The low cross-relaxation rates between water and hydrophobic methylene protons could be as much the result of infrequent chain upturns towards the lipid-water interface as of an occasional deep penetration of water molecules into the hydrophobic core. The very low water content in the bilayer center indicates that water permeation through bilayers must be a very rapid event.

Sunday, February 3, 2008

Symposium 1: The Biophysics of the Immune Response

10-Symp Cellular and Molecular Choreography of Lymphocyte Activation

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We are investigating cellular and molecular mechanisms that allow T cells to respond to presentation of a specific antigen while avoiding inappropriate triggering. Observed within lymphoid organs in vivo by two-photon microscopy, T cells migrate in a three-dimensional random walk at 10 - 12 um/min and respond to specific antigen presented by dendritic cells. Ca2+ signaling is triggered by contact with the antigen presenting cell, is required for gene expression via the NF-AT transcription factor pathway, and is sustained by Ca2+ release-activated Ca2+ (CRAC) channels, a type of store-operated Ca2+ channel. Having shown that Drosophila S2 cells express CRAC current with the same biophysical properties as in human T cells, we used an unbiased genome-wide RNA interference screening approach to identify molecules that underlie store-operated Ca2+ influx. We identified an ER-resident single-span transmembrane protein, Stim with a critical EF-hand domain, as the Ca2+ sensor and the messenger to the plasma membrane; and a four-span transmembrane protein, olf186-F (now renamed Orai), as the channel. Overexpression of Stim and Orai together markedly increased CRAC current. A conservative point mutation from glutamate to aspartate at position 180 in the conserved S1-S2 loop of Orai transformed the ion selectivity properties of CRAC current from Ca2+-selective and inwardly rectifying to Na+-selective and outwardly rectifying. Our results indicate that Stim (STIM1 in human T cells) senses the depletion of ER luminal Ca2+, forms puncta and translocates 'empty handed' to the plasma membrane, where it interacts with Orai (ORAI1) subunits that embody the pore of the CRAC channel.

11-Symp Structural Immunology - Measuring Where To Stick

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Recent results [1] on the structure and function of a receptor protein tyrosine phosphatase, RTPTμ, will be presented. In addition to their intercellular catalytic domains which bear the phosphatase activity, the RPTPs are cell surface receptor type molecules and in many cases have large extracellular regions. CD45 is one such example. What role can these extracellular regions play in function? For RTPTμ the extracellular region is known to mediate homophilic adhesion. Sequence analysis indicates that it comprises of six domains: an N terminal MAM (meprin/A5/μ), one immunoglobulin-like domain and four fibronectin type III (FN) repeats. We have determined the crystal structure of the entire extracellular region for RTPTμ in the form of a functional adhesion dimer. The physical characteristics and dimensions of the adhesion dimer suggest a mechanism by which the location of this phosphatase can be influenced by cell-cell spacings.

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Symposium 2: Mechanoenzymes

12-Symp F1Fo-ATPase

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Synthesis of ATP from ADP and phosphate is the major reaction that provides the 'chemical energy currency' for living organisms. This reaction is performed by a stepwise internal rotation of subunits of the enzyme F_oF_1-ATP synthase. The bacterial enzyme also catalyzes the reversed chemical reaction, i.e. ATP hydrolysis. The opposite direction of rotation during ATP synthesis and hydrolysis was confirmed by single-molecule fluorescence resonance energy transfer, FRET, using specific labeling of the rotary subunits γ (or ε) in the F_1 motor and the stator subunits [1,2]. Subsequently we investigated the proton-driven rotation of the c-subunits in the F_o motor. FRET artifacts could be minimized by 'duty cycle optimized alternating laser excitation', DCO-ALEX. Rotary movements with stochastic single stepsizes between 36° and 144° were determined by Hidden Markov Models [3]. As the two coupled motors of F_oF_1-ATP synthase showed apparently different step sizes, this mismatch has to be unraveled by mapping the contributions of rotor and stator subunits for transient energy storage, i.e. by locating elastic deformations. Actually we aim at identifying the action mode of the allosteric inhibitor aurovertin B, which modulates single F_oF_1 activity either by intermittent blocking or by slowing down rotation.

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