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.

References

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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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13-Symp Kinesin Head-Head Communication to Modulate Microtubule-Motor Interactions

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Eg5/KSP is the kinesin-related motor protein that generates the major plus-end directed force for mitotic spindle assembly and dynamics. Recent work using a dimeric form of Eg5 has found it to be a processive motor; however, its mechanochemical cycle is different from that of conventional Kinesin-1. Dimeric Eg5 appears to undergo a conformational change shortly after collision with the microtubule that primes the motor for its characteristically short processive runs. To better understand this conformational change as well as head-head communication during processive stepping, equilibrium and transient kinetic approaches were used. By contrast to the mechanism of Kinesin-1, microtubule association triggers ADP release from both motor domains of Eg5. One motor domain releases ADP rapidly while ADP release from the other occurs after a slow conformational change at ~1 s-1. Therefore, dimeric Eg5 begins its processive run with both motor domains associated with the microtubule and in the nucleotide-free state. During processive stepping however, ATP binding with ATP hydrolysis signals rearward head advancement 16 nm forward to the next microtubule binding site. This alternating cycle of processive stepping is proposed to terminate after a few steps because the head-head communication does not sufficiently control the timing to prevent both motor domains from entering the ADP-bound state simultaneously.

14-Symp Lever mechanisms in protein motors

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The powerstroke forward movement of myosin's converter subdomain is amplified by a lever that extends from it. The lever is conventionally identified as the series of alpha-helical IQ motifs that follows the converter, each stabilised by a calmodulin-family light chain. Myosin 5 has six IQ motifs, and the long lever this produces enables the two-headed molecule to take many long steps along actin filaments without following the actin helical tracks. An additional feature of the long strides is to allow an intramolecular gating between the heads: the trailing head inhibits product release from the leading head. Our electron microscopy indicated it does this by preventing the converter of the leading head moving forward to the end of its powerstroke. Truncation of the lever to just two IQ motifs abolishes inhibition of product release and reduces the number of steps taken in a run. We find that the two heads of this construct bind on adjacent actin subunits and that the trailing head does not inhibit converter movement in the leading head, accounting for the changed behaviour. We previously showed that in myosin 10, the sequence adjacent to the IQ motifs that had been predicted to form coiled coil (and therefore to create a two-headed molecule) is instead stable as a single alpha-helix (a SAH domain), and does not dimerise the molecule. This raised the possibility that the SAH domain could act as a further extension of the lever. We have tested this by inserting the predicted SAH domain of Dictyostelium myosin M into the myosin 5 2IQ construct. We find this chimera takes longer steps and makes longer runs along actin, and electron microscopy shows the narrow SAH domain between the calmodulins and the tail. Thus the SAH domain can function as a lever.

Platform A: Membrane Dynamics & Bilayer Probes

15-Plat Lipid Bilayer Dynamics Investigated by Combined Solid-State NMR Relaxation and Molecular Dynamics Simulations

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NMR relaxation is a powerful tool for the investigation of membrane dynamics since it is intimately related to the noise spectrum due to random fluctuations of the lipids [1]. Yet several problems center about the question of how NMR relaxation data can be related to molecular and collective motions of lipid bilayers. The main reason for the controversy is the lack of comprehensive experimental data over a sufficiently broad range of frequency. It is not currently possible to decide unequivocally among the various possible motional mechanisms for the relaxation and their correspondence to emergent membrane properties [2]. Therefore, in this work we extended the range of ^{2}H NMR spin-lattice (R_{1Z}) and quadrupolar order (R_{1Q}) relaxation rates for DMPC-d_{54} in the L_α phase by acquiring additional R_{1Z} relaxation rates at a high magnetic field strength of 17.6 T. The data indicate that a continuous distribution of relaxation rates is evident in membrane lipid bilayers. A composite membrane deformation model describing molecular and collective motions can account for the combined data [2]. We also conducted MD simulations of DMPC bilayers to establish the atomistic fluctuations that underlie the lipid dynamics. The simulations featured newly derived force field parameters for head group torsions and the equilibration was done using a replica exchange MD simulation technique. Our MD simulations are able to reproduce the experimental data and yield further insights into the motions that govern the NMR relaxation in bilayers. Extension to bilayer systems containing peptides provides additional insight into dynamical coupling in proteolipid systems [3].

References

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