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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].

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16-Plat Collective Dynamics of Phospholipid Bilayers: A Combined Neutron Scattering and Molecular Dynamics Study

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Biological membranes, which function at physiological temperature, are subject to a variety of thermal and non-equilibrium fluctuations that span a wide range of time and length scales. Describing and characterizing these membrane fluctuations at molecular level may lead to a better understanding of the structure-function relationship of biomembranes. Recently, to investigate the collective dynamics of phospholipid bilayers we have employed inelastic neutron scattering techniques based on last generation neutron sources. However, even using the most intense neutron scattering sources, the scattering intensity from single bilayer systems is very weak, thus requiring samples of highly ordered stacked lipid membranes. In order to investigate the effect of the coupling between the stacked bilayers on their collective dynamics we have employed large scale, all atom molecular dynamics (MD) simulations. We have used a model system of water solvated stacked 1,2-dimyristoylphosphatidylcholine (DMPC) bilayers in the liquid crystal phase at room temperature. The static and dynamic structure factors computed from the MD trajectories were compared to the ones obtained from neutron scattering experiments. Also, the dynamic structure factor was used to determine the dispersion relation of the lipid acyl chains. Furthermore, we have also studied the dynamics of the water layers separating the stacked lipid bilayers by focusing on the relationship between the width of the water layer and the strength of the coupling between the bilayers. Thus, we have been able to investigate the dynamics of the membrane-water system separately but simultaneously.

17-Plat Di-4-aneppdhq As A New Probe For Lipid-rafts Imaging In Living Cardiac Cells

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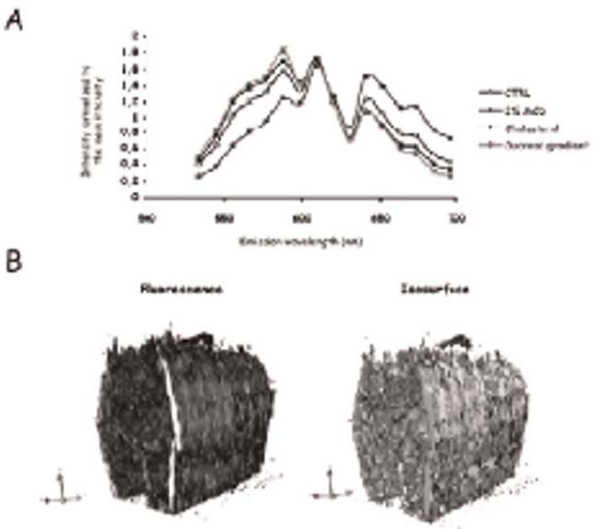
Lipid-rafts are heterogeneous liquid-ordered membrane microdomains playing a critical role in cell signaling. Limited tools are available to image the lipid-raft population in cell membranes. To develop a lipid-raft visualization tool, HL-1 cells were incubated with Di-4-ANEPPDHQ. Emission spectra from a 488 nm excitation wavelength were analyzed. Z-stack acquisitions were obtained and deconvolved. Five discrete emission peaks were identified (wavelengths of [539–571]; [571–593]; [593–625]; [625–657]; [657–689] nm; panel A). Each peak corresponded to distinct 3-D membrane structures (Panel B). Cholesterol depletion with methyl-β-cyclodextrin decreased both [539–571] and [571–593] peaks (34.5% and 28.2% respectively; n=13) and increased [625–657] and [657–689] peaks (26.9% and 52.2% respectively; n=13), panel A. Cholesterol enrichment augmented [539–571] and [571–593] peaks (12.7% and 6.24%; n=6) with a reduction of [625–657] and [657–689] peaks (14.4% and 21.4%; n=6). The emission spectra of lipid-raft enriched fractions (sucrose gradient) exhibited more pronounced [539–571] and [571–593] peaks (18.1% and 12.1% increase respectively; n=3).

Interestingly, these were found to be more prominent in freshly isolated cardiomyocytes 16.8% and 24.2% respectively; n=10).

CONCLUSION: Di-4-ANEPPDHQ differentially stains membrane microdomains in living cardiac cells and may be a useful tool for lipid-microdomain visualization.



18-Plat Lipid Overload with Saturated and Monounsaturated Fatty Acids Has Differential Effects on Antigen Presentation

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Lipid overload, associated with obesity, occurs when saturated fatty acids (SFAs) accumulate in non-adipose tissues. Cells of these tissues use MHC class I molecules to present antigen to T cells in order to eliminate pathogens. Since obesity is associated with impaired immune responses, we tested a new hypothesis: lipid overload with SFAs modifies MHC class I antigen presentation. Antigen presenting cells (APCs) were fed either the saturated palmitic acid (PA) or the monounsaturated oleic acid (OA), to respectively model the high fat Western or Mediterranean diets. PA-treatment lowered APC lysis by activated cytotoxic T lymphocytes and inhibited the ability of APC to stimulate naïve T cells. Inhibition of immune responses with PA was due to lowered MHC class I surface expression and APC-T cell conjugation.

Fluorescence anisotropy measurements showed an associated change in acyl chain structure of the plasma membrane, which could modify the immunological synapse. OA-treatment had no effect on antigen presentation and when fed together with PA, reversed the phenotypic effects of PA. Protection from functional changes in APCs by OA correlated with its distinct cellular metabolic fate. OA-treatment conferred protection by accumulating fatty acids into triglyceride-rich lipid droplets of APCs. Our findings establish for the first time a link between lipid overload and antigen presentation and suggest that dietary SFAs in non-adipose cells of obese individuals could impair immunity by affecting MHC I-mediated antigen presentation; this could be prevented by accumulation of triglycerides rich in monounsaturated fatty acids.

19-Plat Single Quantum Dots Tracking Reveals the Dynamic Repartitioning and Bimodal Diffusion of GPI-anchored Probes in the Plasma Membrane

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The heterogeneous diffusion of biomolecules in the plasma membrane of live cells is now widely accepted. However, whether these lateral heterogeneities are induced by the dynamic scaffolding of the membrane or originate from lipid and/or protein microdomains remains contentious. Here we have studied the diffusional dynamic of a raft-associated glycosylphosphatidylinositol-anchored avidin test probe (av-GPI) which reports on plasma membrane heterogeneities in HeLa cells when targeted with biotinylated peptide-coated quantum dots. Quantum dots allowed high resolution and long-term tracking of single av-GPI. The diffusion trajectories of the latter were analyzed using the probability distribution of the squared displacements to allow an unbiased detection and classification of their diffusive and sub-diffusive behaviors. Using dual-color total internal fluorescence microscopy imaging, changes in the lateral diffusion coefficients of av-GPI were correlated with the position of glycosphingolipid GM1-rich domains and with caveolae, marked respectively with fluorescent cholera toxin B and caveolin-1-EGFP. We show that av-GPI experience two distinct diffusion regimes (fast and slow diffusion) in the plane of the plasma membrane and confirm this heterogeneous mobility by FRAP and single dye tracking. We demonstrate that the slow diffusion regime is induced by interaction of av-GPI with membrane glycosphingolipid GM1-rich microdomains located in close proximity to caveolae. While the GPI-test probes dynamically partition in and out of GM1-rich microdomains they rarely enter caveolae. We further show that the diffusion inside GM1-rich domains, but also in the rest of the plasma membrane is cholesterol-dependent and that the sub-cortical actin cytoskeleton also influences the diffusion of fast av-GPI. We thus provide direct evidences that cholesterol/sphingolipid-rich microdomains can impede the free diffusion of GPI-anchored proteins in the plasma membrane of live cells.

20-Plat Vibrational Lifetime of the Anesthetic Gas Nitrous Oxide as a Probe of Interfacial Water in Biological Systems

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Ultrafast infrared spectroscopy was used to study the vibrational energy relaxation of the anesthetic gas nitrous oxide (N_2O) solvated in model biological systems. The sensitivity of the antisymmetric stretching mode of N_2O to its local environment is demonstrated by measuring the vibrational lifetime of N_2O in lipid bilayers as a function of hydration. As the number of water molecules per lipid headgroup is decreased, the lifetime of the antisymmetric stretching mode in the aqueous region of the lipid bilayer increases significantly from the value obtained in bulk water. Our results suggest that vibrational energy relaxation of N_2O in the aqueous region is controlled by resonant energy transfer to water modes. Molecular Dynamics simulations show that there is orientational ordering of water molecules near the lipid headgroups. Ordering of water molecules alter the energy transfer rate, making N_2O an excellent reporter/marker of the local ordering of water structure and dynamics. The ability for N_2O to probe oriented water molecules is further demonstrated with preliminary measurements on N_2O dissolved in hydrated nucleic acids.

21-Plat One Antimicrobial Peptide (Gramicidin S) Can Affect The Function Of Another (Gramicidin A Or Alamethicin) Via Effects On The Phospholipid Bilayer

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Perturbations in phospholipid bilayer material properties (thickness, lipid intrinsic curvature and elastic moduli) modulate the free energy differences between different membrane protein conformations. When both curvature and elastic moduli are altered by the adsorption of amphiphiles at the bilayer/solution interface, changes in elastic moduli (and channel-bilayer hydrophobic mismatch) often are more important than changes in intrinsic curvature in determining the bilayer deformation energy: for both positive and negative changes in curvature, a concomitant increase in bilayer elasticity stabilizes gramicidin A (gA) channels and the higher conductance states in alamethicin (Alm) channels (Ashrafuzzaman and Andersen, Biophys J. *421 A*, 2007). We now explore whether one membrane protein can modulate the activity of another via changes in the physical properties of the host bilayer using subthreshold concentrations of gramicidin S (GS) to perturb the stability and

function of ion channels formed by two antimicrobial peptides, gA and Alm. We find that GS stabilizes both gA channels and the higher conductance states in Alm channels, indicating that GS increases bilayer elasticity at concentrations below those at which it by itself causes breakdown of the bilayer barrier properties. The presence of one membrane protein may affect the stability and function of another membrane protein through changes in the physical properties of the host bilayer, a finding likely to be relevant for the function of biological membranes and possibly for the mode of action of antimicrobial peptides.

22-Plat New Insights into Red Blood Cell Membrane Dynamics

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The dynamical behavior of the red blood cell (RBC) and the relationship of membrane fluctuations with viscoelasticity are still open questions. We report both forced and spontaneous motions of microbeads tightly bound to the RBC membrane. To assess the nature of forces driving membrane dynamics we studied the effects of temperature and ATP depletion. We found that RBC stiffness was nearly frequency independent and that spontaneous bead motions were sub-diffusive or caged for almost all time lags experimentally accessible (~ 0.05–50 sec). We found that from room temperature to febrile 41°C cell stiffness approximately doubled. Consistent with this finding, increased temperature slowed down the spontaneous motion of the beads. ATP depletion led to a much larger stiffening response, approximately an order of magnitude. These measurements indicate that ATP associated effects dominate thermal ones. Moreover, our data relate spontaneous fluctuations directly to dissipation seen in driven motion and allows us to investigate the extent to which the fluctuation-dissipation theorem holds in RBCs.

Platform B: Voltage-gated Ca Channels

23-Plat Calmodulin-dependent Gating of Calcium Channels in the Absence of β Subunits

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It is generally accepted that, to generate calcium currents in response to depolarization, $Ca_v1.2$ calcium channels require association of the poreforming α1C subunit with the β and α2δ accessory subunits. At least one calmodulin molecule (CaM) is tethered to the C-terminal α1C-LA/IQ region and mediates Ca^{2+}-dependent inactivation (CDI) of the channel. β subunits are stably associated with the α1C-AID site of the cytoplasmic linker between internal repeats I and II and also interact dynamically, in a Ca^{2+}-dependent manner, with the α1C-IQ motif. Here we describe a surprising discovery that CaM supports calcium channel voltage gating on transient coexpression with α1C/α2δ in COS1 cells in the absence of β subunits. The β-free CaM-activated α1C/α2δ channels exhibited CDI. Real time PCR with primers complementary to monkey β subunits did not reveal an induction of endogenous β subunits in response to overexpression of CaM indicating that calcium channel-free environment of the COS1 cell expression system was not compromised by CaM. Similar to CaM, transient expression of the calcium-insensitive CaM_{1234} mutant activated β-free $Ca_v1.2$ channels and stimulated the plasma membrane targeting of α1C/α2δ complexes, but did not support CDI. Our results suggest that there is a calcium-independent crosstalk between CaM and β subunits that, in the absence of β, allows for facilitation of the channel voltage gating via interaction with CaMs other than the regularly tethered one. This effect is altered or abolished by the α1C-β association. Thus, transient expression of CaM creates conditions when the channel gating, inactivation, CDI and plasma-membrane targeting occur in the absence of β.

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24-Plat

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25-Plat Origin Of The Voltage Dependence Of G Protein Regulation Of P/Q-type Ca^{2+} Channels

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G protein (Gβγ)-mediated voltage-dependent inhibition of N- and P/Q-type Ca^{2+} channels contributes to presynaptic inhibition and short-term synaptic plasticity. The voltage dependence arises from the dissociation of Gβγ from the inhibited channels, and it is this property that permits high frequency action potential firing to relieve the inhibition of synaptic N- and P/Q-type Ca^{2+} channels by G-protein coupled receptors. The molecular and biophysical mechanisms underlying Gβγ unbinding remain largely unclear. We have investigated the structural elements and conformational changes that produce the voltage dependence. We found that voltage-dependent Gβγ inhibition required the calcium channel β subunit ($Ca_v\beta$) and a rigid α-helical structure between the AID, the primary $Ca_v\beta$ docking site on the channel α_1 subunit, and the pore-lining IS6 segment. Gβγ inhibition of P/Q-type channels was reconstituted in inside-out membrane patches from *Xenopus* oocytes by applying purified Gβγ directly to the cytoplasmic side of the channels. Channels devoid of Caβ (produced by removing a mutant $Ca_v\beta$ with a reduced affinity for the AID) or containing a WT $Ca_v\beta$ but bearing a helix-disrupting insertion between the AID and IS6 were still inhibited by Gβγ, but without any voltage dependence. Furthermore, a truncated $Ca_v\beta$ containing only the AID-binding guanylate kinase (GK) domain could fully confer the voltage dependence. These results suggest that depolarization-triggered movement of IS6, coupled to the subsequent conformational change of the Gβγ-binding pocket through a rigid a-helix induced partly by