Membrane Dynamics I

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Hydration-Modulated Collective Dynamics of Membrane Lipids are Revealed by Solid-State ^{2}H NMR Relaxation

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Investigations of model membranes aim to understand the atomistic interactions that can explain bulk membrane lipid properties in relation to key biological functions [1]. Solid-state ^{2}H nuclear magnetic resonance (NMR) spectroscopy uniquely provides such information by probing structure and dynamics of membranes [2,3]. Here we examine the effect of water on the liquid-crystalline properties of amphiphilic membrane lipids using NMR relaxometry. We performed NMR longitudinal (R_{1Z}), transverse quadrupolar-echo decay (R_2^{QE}) and quadrupolar Carr-Purcell-Meiboom-Gill (QCPMG) relaxation (R_2^{CP}) experiments on DMPC-d_{54} bilayers, to study membrane-lipid dynamics over time scales ranging from 10^{-9}s to 10^{-3}s. The plots of R_{1Z} rates versus squared segmental order parameters (S_{CD}^2) follow an empirical square-law behavior showing the emergence of collective lipid dynamics [4]. Such a functional behavior characterizes 3-D order-director fluctuations due to the onset of membrane elasticity over atomistic dimensions [4]. The transverse relaxation rates also show similar results at low hydration. Yet at high hydration, a further enhancement versus the functional square-law plot is evident for segments deeper in the bilayers. Additional contributions from slower dynamics involving water-mediated membrane deformation are evident over mesoscopic length scales on the order of bilayer thickness. The slow dynamics at high hydration must be a consequence of modulation of elastic properties of lipid bilayer. Analysis of the QCPMG frequency dispersions as function of hydration and temperature reveals quantitative information on viscoelastic properties of the liquid-crystalline media. Similar studies in the presence of proteins and peptides give insights into optimized lipid hydration for biomembrane function. [1] A. Leftin *et al*. (2014) *BJ*. (in press). [2] K. J. Mallikarjuniah *et al.* (2011) *BJ* 100 98-107. [3] J. J. Kinnun *et al*. (2014) *BBA* (in press). [4] M. F. Brown *et al*. (2002) *JACS* 124, 8471-8484.

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Spot Variation FCS in the 2D Ising Model

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Spot Variation Fluorescence Correlation Spectroscopy (svFCS) was developed to study the movement and organization of single molecules in plasma membranes. This experimental technique varies the size of the confocal beam waist while measuring correlation times using standard FCS methods. For Brownian motion, correlation times should decrease linearly with decreasing excitation area and intersect zero in the limit of vanishing beam waist. When this is not the case, results are frequently interpreted as providing evidence for confined motion, either through corralling or through partitioning into domains. These interpretations rely on the assumption that the FCS measurement provides information on the time-scale of single molecule motions, and not the correlation time of the average composition. In this poster, we use a simple implementation of a conserved order parameter 2D Ising model to explore how temperature and the surface density of tracer molecules impact simulated svFCS measurements within a fluctuating model membrane environment. At low tracer concentration, svFCS largely detects Brownian motion, with some deviation as temperatures approach the critical temperature (TC). When tracer molecules are included at a high enough density, FCS correlation times are dominated by motions of the average composition, which become very slow as temperature is lowered to TC. We explore the cross-over between these two regimes, in addition to the impact of coupling Ising simulations to a rigid cortical cytoskeleton. Possible implications for existing svFCS measurements will also be discussed.

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Universal Approach to FRAP Analysis of Arbitrary Bleaching Patterns

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Fluorescence recovery after photobleaching (FRAP) is a classical biophysical method, that has been used extensively in the study of numerous systems, particularly cell membranes. It consists of irreversibly photobleaching a fluorescently labeled diffusing species, and then following the fluorescence recovery over time. By analyzing the fluorescence recovery dynamics it is possible to extract the diffusion coefficient of the fluorescent species. While the experimental setup is relatively simple, the interpretation of the results is complex, severely limiting the quantitative use of the technique.

With the introduction of digital imaging and the Laser Scanning Confocal Microscope (LSCM), a multitude of variations of the technique became possible. While these modern options offer high flexibility and extend the availability of the method, the initial and boundary conditions are too complex to allow a precise closed, analytical solution of the diffusion problem, and thus quantitative extraction of the diffusion coefficient.

We have developed a fast algorithm that allows the extraction of diffusion coefficients given a stack of images representing a FRAP experiment, and acquired with any method, for a completely arbitrary geometry of the initially bleached area. The algorithm treats the first post-bleach frame as the initial and boundary conditions, and simulates the diffusion of the fluorescent molecules. The lack of closed analytical solutions for complex geometries is not a limiting factor, since the diffusion equation is effectively solved numerically by iterative simulation.

We validated our approach using a well characterized diffusing molecule (DiIC18) and against the well-established analytical procedures for Gaussian (Axelrod, Koppel et al. 1976) and box bleaching geometries (Ellenberg, Siggia et al. 1997). Furthermore, we show that the algorithm can deduce the diffusion coefficient for an arbitrary bleaching geometrys.

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Faster Calculations of Diffusion Constants for Lipids, Water and Proteins

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We have taken two approaches to aid in the calculation of diffusion coefficients: first, we examine the statistical distribution of properties of an ensemble of individual molecules to provide accurate estimates of diffusion coefficients as well as to assess the convergence of those diffusion coefficients. Second, we use non-equilibrium simulations, applying a constant force to selected molecules in near-equilibrium conditions. The mobility and diffusion coefficients are obtained from the terminal drift velocity. The non-equilibrium results are generally found to be within a factor of 1.5 of the experimental constants, converge much more quickly than traditional techniques, and are well-suited for parallelization.

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The Effect of Lipid Bilayers on Membrane-Bound Proteins

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Membrane-induced interactions can play a significant role in the spatial distribution of membrane-bound proteins. We develop a model that combines a continuum description of lipid bilayers with a discrete particle model of proteins to probe the emerging structure of the combined membrane-protein system. Our model takes into account the membrane's elastic behavior, the steric repulsion between proteins, and the quenching of membrane shape fluctuations due to the presence of the proteins. We employ coupled Langevin equations to describe the dynamics of the system. We show that coupling to the membrane induces an attractive interaction among proteins, which may contribute to the clustering of proteins in biological membranes. We further demonstrate that lateral protein diffusion is enhanced by fluctuating forces generated by membrane undulations.

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Structural and Dynamical Properties of POPC Bilayers Supported on Nanoporous Substrates

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The structure, dynamics and organization of lipid bilayers depend on a variety of environmental factors. Here we examine, using molecular dynamics (MD) simulations, the specific effects of nanoporous substrates on palmitoyl-oleyl phosphatidylcholine (POPC) bilayers. We expose POPC bilayers unilaterally and separately to various model Lennard-Jones (LJ) solid substrates differing in surface chemistry. In an earlier study, we had found that substrates with surface hydroxyl densities in the range 10-20% kept POPC bilayers juxtaposed to the substrates. While the bilayers did not interact directly with these hydroxylated substrates, they were separated from substrates by thin (< 0.6 nm) layers of water. Additionally, despite such buffered interactions, these supported bilayers exhibited physical properties notably different from