domains of viral packaging motors govern their force generation and determine the velocities of packaging. A Q motif mutant of the phage λ DNA packaging motor, Y46F, was shown to have a decreased velocity (~40% less than WT), increased slipping (~10X WT), and steeper force-velocity dependence (~6X WT), showing that the Q motif governs the force generation in translocation and DNA-motor interactions. In addition, we show that mutants with residue changes located in a previously undetermined domain of the motor, T194M and G212S, package dsDNA into viral capsids at ~8X and ~ 3X slower velocities than wild type (WT), respectively. Meanwhile a T194M pseudo-revertant (T194V) showed a near restoration of the WT velocity. The single molecule measurements of motor mutant translocation dynamics, genetic screening experiments, and structural modeling of ring ATPase dsDNA translocases suggest the location of a ''velocity controller'' domain within the phage λ packaging motor downstream the putative Walker B motif, which might be generalizable to other ring ATPase nucleic acid translocases. Importantly, this evidence may aid in explaining the different packaging rates of various dsDNA phages.

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Counting RAD51 Proteins Disassembling from Nucleoprotein Filaments Under Tension

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The central catalyst in eukaryotic ATP-dependent homologous recombination consists of RAD51 proteins, polymerized around single-stranded DNA. This nucleoprotein filament recognizes a homologous duplex DNA segment and invades it. After strand exchange, the nucleoprotein filament should disassemble in order for the recombination process to complete. The molecular mechanism of RAD51 filament disassembly is poorly understood. Here, we have combined optical tweezers with single-molecule fluorescence microscopy and microfluidics to reveal that disassembly results from the interplay between ATP hydrolysis and release of the tension stored in the nucleoprotein filament [1]. Applying external tension to the DNA, we found that disassembly slows down and can even be stalled. We quantified the fluorescence of RAD51 patches and found that disassembly occurs in bursts interspersed by long pauses. Upon relaxation of a stalled complex, pauses were suppressed resulting in a large burst. These results imply that tension-dependent disassembly takes place only from filament ends, after tension-independent ATP hydrolysis. This integrative single-molecule approach allowed us to dissect the mechanism of this key homologous recombination reaction step, which in turn clarifies how disassembly can be influenced by accessory proteins.

[1] van Mameren et al., Nature, 457, 745 - 748 (2009)

Membrane Dynamics & Bilayer Probes

3454-Pos

Revealing the Microstructural Changes in Tissues In-Situ with Positron Annihilation Spectroscopy

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In this work we present a novel and promising tool for characterizing the microstructural changes in biomaterials, namely mammalian lens. Positron annihilation lifetime spectroscopy (PALS) is a widely used tool to study atomic scale defects in semiconductors and routinely used to study the voids in polymer materials [1]. Through the increased understanding of the biomolecular materials, results from PALS experiments can now be compared with simulations and further analysis of the results is possible. Recently we have showed that PALS can be applied to study and characterize free volume changes in lipid bilayers and the results are in full agreement with MD simulations [2]. In biomolecular material, a thermalized positron forms a meta-stable bound state, Positronium (Ps), with an electron from the material. This o-Ps-atom can be used as a probe, due to the Ps lifetime in the material being strongly affected by the free volume characteristics of the probed material.

Here we present results obtained by studying the temperature dependent changes in free volume parameters in mammalian lenses, lipids separated from the lenses and controlled Spingomyelin-cholesterol mixtures [3]. All the measurements provide strong evidence towards a minor structural reorganization near 35°C, far below strict phase transition temperatures. This change indicates a transition to liquid order phase and is not visible with conventional experimental methods used in the study, probably due to the microstructural scope of the change which does not provide strong enough signal for e.g. DSC.

[1] O. E. Mogensen, *Positron annihilation in Chemistry* (Springer-Verlag, Heidelberg, 1995)
[2] P. Sane et al, J. Phys. Chem. B: **113**:1810-12 (2009)
[3] P.Sane et al, ''*Temperature Induced Structural Transition in-situ in Porcine Lens - Changes Observed in Void Size Distribution",* submitted to BioPhys. J. on 7.9.2009

3455-Pos

The Use of Time-Resolved Fluorescence Anisotropy to Reveal Domain Structures in Model Membrane Vesicles: Prospects for Applications to Cell Membranes

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Cell membranes are thought to consist of structurally distinct regions within which reside functionally-interacting cell membrane components. Although much research has been directed toward the detection of these regions, variously known as domains or rafts, they still remain elusive entities. A number of techniques have used probes that tend to reside in these regions and using this approach structures have been identified using florescence spectroscopic approaches. Here we have investigated the motion of the probes rather that their presence to identify the presence of domain regions using giant unilamellar vesicles (GUV) to model cell membranes. Using the electroformation method, GUV were made using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, sphingomyelin and cholesterol in various proportions. The fluorescent probes, diphenyhexatriene (DPH), 1-acyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl) amino]hexanoyl}-sn-glycero-3-phosphocholine (C6-NBD-PC), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) were pre-incorporated in the GUV. The time-resolved anisotropy of the probes was determined using 2-photon excitation from a Ti-sapphire laser (180 fs pulses at 75 MHz and 750 nm) coupled to a microscope. Excitation was performed with an incident vertically polarized light and a Y-output acquisition, generating simultaneous vertical and horizontal detection, using a multichannel photon multiplier tube. The output signal was fed through a Becker and Hickl SPC-830 module and anisotropy analysis software. The set up allows for picosecond time resolution as well as diffraction limited image resolution needed for the domain identification. It was found that each probe had two distinct motions corresponding to different orientations and locations in single-component vesicles, as known for DPH, but not previously observed for the NBD probes. Using polarization direction selectivity these two locations could be separately examined using the current development. The data shows that NBD selectively locates into domain regions in three-component vesicle systems.

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Protein-Induced Shape Changes in Phase-Separated Vesicles

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We explore changes in curvature in vesicles composed of a mixture of phospholipids and cholesterol upon the binding of protein to the membrane. Experiments were done with model peptides and with members of the BAR family of proteins. Vesicles were prepared using electroformation with a mix of phosphatidylcholine, cholesterol, and sphingomyelin. As a sample is lowered through temperature Tmix, a homogeneous vesicle phase separates into two fluid phases with distinct compositions. A line tension at the boundary between the phases deforms the membrane, producing buds with a shape determined by a balance of membrane and line tension. Green fluorescent protein (GFP) with a 6X histidine tag was added to the solution, where it bound to Ni-chelating phospholipids present in the membrane at concentrations ranging from 0.1 to 5 mol%. Domain budding was recorded before and after GFP addition using both differential interference and fluorescence microscopy. Analysis of the shape of the vesicle before and after addition of GFP allows for the determination of the change in line tension owing to the GFP binding. Similar results were obtained with vesicles introduced to poly-L-histidine. A possible mechanism for the change in shape seen with both GFP and poly-L-histidine binding is formation of protein-Ni-chelating lipid complexes that sit at the boundary between phases and lower the line tension. Preliminary investigations into shape changes caused by BAR domains of Drosophila amphiphysin revealed fine vesicle tubulation that initiated at the boundary between the two lipid phases. The addition of both model proteins (GFP and poly-L-histidine) and biologically relevant protein (BAR domains) into our system allows for a broader understanding of the effect of protein, which are ubiquitous in cell membranes, on phase separation and budding.

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