the country. We utilized this assay to identify 20 different amyloid subtypes distributed among 4167 patients, allowing for confident individualized treatment. Data mining these clinical amyloid proteomes revealed surprising new insights in to the clinical biology of amyloidosis. We identified a universal proteomic signature (APOE, SAP and APOA4) that can more sensitively detect amyloid laden tissues when compared to traditional Congo red staining of fat aspirate specimens. We also detected amino acid sequence abnormalities that are associated with hereditary amyloidosis syndromes. In summary, amyloidosis patient care was optimized based on accurate determination of the amyloid type using tissue shotgun proteomics technology.

Subgroup: Membrane Structure & Assembly

7-Subg

Protein Gymnastics in Thelipid Bilayer: Lipides as Determinants of Protein Structure

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The orientation of transmembrane domains of polytopic membrane proteins with respect to the plane of the lipid bilayer is determined by a complex interplay between topogenic signals residing within the protein sequence, interaction of the protein with the translocon membrane insertion machinery, short-range and long-range interactions within the protein and the final environment of the protein largely determined by membrane lipid composition. Systematic alteration of lipid composition at steady state or dynamically after membrane protein assembly uncovered a role for lipid-protein interactions in determining initial membrane protein topogenesis as well as in dynamic topological re-organization after initial protein folding. Alteration of the charges within protein extramembrane domains coupled with changes in lipid composition demonstrated a synergistic and reciprocal relationship between protein and lipid charges in determining orientation of transmembrane domains. These results led to the Charge Balance Hypothesis, which posits that protein topogenic signals are decoded in accordance with positive-inside rule initially by the translocon but finally interpreted by electrostatic interactions between protein extramembrane domains and the membrane surface charge as determined by the collective membrane lipid head group composition. The steady state and dynamic nature of membrane protein organization observed in whole cells, as a function of the Charge Balance Hypothesis, has been faithfully reproduced with membrane proteins reconstituted in proteoliposomes thus establishing that such initial and dynamic protein organization is dependent solely on direct lipid-protein interactions independent of other cellular factors. Therefore, membrane protein topological organization can be viewed as highly dynamic rather than stable and static. The dynamic view of protein topological organization as influenced by the membrane lipid environment reveals previously unrecognized possibilities for cellular regulation and understanding of disease states resulting from misfolded membrane proteins. Supported in part by NIH grant R37-GM20478.

8-Subg

How Lipids Mediate Pten Tumor Suppressor Function

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Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an important regulator of the PI 3-kinase signaling pathway. Mutation or deletion of one copy of this protein results in a tumorigenic state and PTEN has been identified as the second most important human tumor suppressor, rivaled only by p53. Not surprisingly, PTEN function is tightly regulated at the post-translational level as well as through interactions with lipids and other proteins. We have shown that PTEN binds synergistically to phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) and phosphatidylserine (PS), which leads to membrane association and allosteric activation. In this talk we will present molecular details about the interaction of $PI(4,5)P_2$ with PTEN's N-terminal end as well as the interaction of PS with PTEN's C2 domain. In addition, we will discuss how PTEN function is affected by the lateral distribution of phosphoinositide lipids.

9-Subg

New Insights into Mitochondrial Permeabilization in Apoptosis

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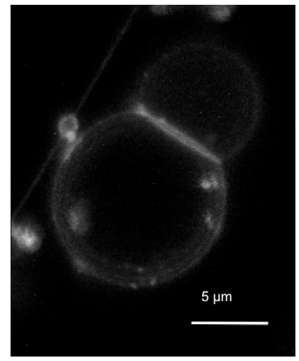
Mitochondria undergo dramatic changes during apoptosis, which include extensive fragmentation and the permeabilization of the outer mitochondrial membrane, which is considered a point of no return in the cell's commitment to die. These alterations are controlled by the proteins of the Bcl-2 family in collaboration with the mitochondrial machinery for fission and fusion. Despite their central role, the molecular mechanisms involved remain poorly understood. To tackle these problems, we have used a combination of advanced microscopy methods in cells and in reconstituted systems. Here we present our results with Bax, a proapoptotic protein of the Bcl-2 family involved in mitochondrial permeabilization, and Drp1, a dynamin-like protein responsible for mitochondrial division. Studies with Giant Unilamellar Vesicles show that Bax forms stable, large membrane openings, which are affected by other Bcl-2 proteins and by Dpr1. Interestingly, Drp1 has a strong effect on the organization of the membrane. Using single molecule microscopy, we have analyzed the stoichiometry of Bax oligomers in the lipid bilayer. Finally, the analysis of Bax organization at the nanoscale by superresolution microscopy in cells undergoing apoptosis reveals striking structures that seem to play a role in the mitochondrial alterations in apoptosis.



10-Subg

Effects of Phosphoinositides and their Derivatives on Endomembrane Morphology and Function

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No abstract.

11-Subg

Molecular Basis of the Assembly and Budding of the Ebola Virus from the Plasma Membrane of Human Cells

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Lipid enveloped viruses replicate and bud from the host cell where they acquire their lipid coat. The Ebola virus, which buds from the plasma membrane of the host cell causes viral hemorrhagic fever and has a high fatality rate. To date little is known about how the plasma membrane mediates budding and egress of the Ebola virus. My lab is investigating the molecular basis of the plasma membrane assembly, budding and egress of this virus, which is regulated by the matrix protein, VP40. We use biochemical and biophysical tools along with cellular imaging and viral replication assays to investigate how VP40 interacts with the plasma membrane of human cells to regulate viral replication. This presentation will outline the molecular basis of VP40 association with plasma membrane lipids and how lipid-protein interactions regulate VP40 oligomerization and plasma membrane bending. Furthermore, VP40 plasma membrane binding displays sensitivity to the lipid composition in the plasma membrane, which can be altered to inhibit Ebola assembly and egress.

12-Subg

Vesicles in Electric Fields

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Giant vesicles provide exceptional biomembrane models for systematic studies on the effect of electric fields because the membrane response can be directly visualized under the microscope (1-3). In AC fields, the dependence of the vesicle morphology on both field frequency and media conductivity has been characterized recently (3, 4). The theoretical models for the observed morphological transitions (5, 6) predict the dependence of the frequency of the prolate-oblate transition on the vesicle size. We used this prediction to develop a method for measuring the membrane capacitance (7). At a fixed field frequency and increasing field strength, the degree of vesicle deformation increases and can be used to deduce the membrane bending rigidity (8). Inhomogeneous AC fields trigger flows on the membrane surface visualized by domain movement (9). When exposed to strong DC pulses, giant vesicles porate. Using the dynamics of the pore closure, we developed an approach for measuring the edge tension and evaluate the membrane stability (10). The response of both fluid- and gel-phase membranes will be discussed. We also established an electrofusion protocol for creating multicomponent giant vesicles with precisely known composition and used it to locate tie lines in the region of coexistence of liquid-ordered and liquid-disordered phases (11).

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13-Subg
Lipid Nanotubes as a Tool for Studying Nanoscale Proteo-Lipid Domains
Anna Shnyrova, PhD.
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Membrane curvature can play a decisive role in demixing of membrane bound proteins, allowing for formation of fluid-like or gel-like proteo-lipid domains responsible of distinct shape (shape creators) and/or function (e.g. topological remodeling). We take advantage of the nanoconfinement offered by the lipid membrane tethers or lipid nanotubes to access such domains and reveal the fine details of their dynamic life. By combining conductance and fluorescence measurements on a lipid nanotube we are able to monitor the nucleation, stepwise growth and disassembly of individual dynamin1 nanodomains and observe reversible changes in membrane shape and topology produced by them. The sensitivity of this method relies on the nanoconfinement of the tube and the correlative analysis of the data, and gives the spatial and temporal resolution needed for the study of dynamic elasticity of non-homogeneous membranes at nanoscales.

14-Subg
How Cells Exploit Forces to Sense and Respond to their Environments
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Cells recognize physical features in their environments by exploiting mechanical forces generated by their motors which pull on distal extracellular anchoring points. Filopodia have been described previously as the ''sticky fingers'' that help cells to explore their environments and help immune cells to clear pathogens. Here we will ask how cells exploit the interplay between filopodia and lamellipodia to explore their environments, recognize surface properties and find their pray. Myosin-generated tensile forces acting on filopodia are utilized by cells to pull on external adhesive objects. If the external objects can be deflected, the filopodia adhesion will grow as filopodia and object align, but filopodia often peel off from flat surfaces. Tensile forces acting on filopodia are thus used by cells to distinguish between deflectable nanofibrillar environments and flat surfaces. Only if lamelliopdia are in contact with flat surfaces, the tensile forces acting on filopodia can steer the protrusion of lamellipodia. This synchronized movement is needed for the physical removal of surface adhering pathogens for example by macrophages. Membrane tension might play a still poorly understood role in the local coordination of events.

15-Subg
Determining the In-Plane and Out-of-Plane Structure of Model Membranes; Two Recent Examples
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With the exception of hydrogen, neutrons are found in all atomic nuclei. Importantly, unlike X-rays, neutrons are able to differentiate between the different isotopes of the same element. In biology, the classic example is the isotopic substitution of hydrogen for deuterium, allowing one to selectively tune the sample's contrast *in situ* with minimal or no change to its native structure. Biological membranes are believed to exist in a disordered state, a fact that presents unique challenges to elucidating their fine structure. In the case of model membranes, to overcome this difficulty we have developed the Scattering Density Profile (SDP) model, which combines neutron and x-ray scattering data, with molecular dynamics simulations to yield robust structural data, including the much sought after area per lipid needed by simulators to refine their force fields. In addition to one-dimensional structural data along the membrane, we have recently exploited the contrast variation offered by neutron scattering (exchange of hydrogen for deuterium), to study - with unprecedented accuracy - the lateral phase separation (in-plane structure) of so-called ''raft'' forming mixtures. We hope that in the near future we will apply this knowledge to address the question that has vexed biologists and confounded experimentalists for over 40 years: do membrane domains exist *in vivo*?

16-Subg
Membrane Fusion by X-Rays: From Model Membranes to Organelles
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Understanding the physical mechanisms underlying membrane fusion requires a multi winged approach, involving model systems as well as biological membranes. We study fusion intermediates occurring in form of ordered passages or stalks connecting neighbouring bilayers in multilamellar model membrane stacks. The stalks exhibit long range crystalline order with rhombohedral symmetry in a fluid 'host' membrane stack, which is studied by high resolution x-ray diffraction under grazing incidence angles. Information on membrane curvature, and hydration interaction can be revealed by analyzing the quantitative electron density maps, collected for controlled environmental parameters and membrane composition [1]. Phase diagrams can be analyzed in view of stabilizing or destabilizing agents for stalk formation.
While in these equilibrium phase, dehydration forces bring bilayers together favoring at some point the formation of stalks, it is specific membrane proteins and their interaction which set the local boundary conditions for membrane apposition in biological membrane fusion. In view of studying fusion in the presence of SNARE proteins, we have started a x-ray structural characterization of synaptic vesicles (SV) by small-angle x-ray scattering, and currently extent this work towards studies of SV dockled to and interaction with model bilayers [2].
Finally we present a novel high resolution x-ray imaging scheme capable of yielding a magnified hologram of a freely suspended lipid membrane illuminated by highly divergent and coherent x-ray beams. We propose this setup to image fusion trajectories at high resolution in future experiments [3].
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17-Subg
Some of my Greatest Mistakes
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2014 Thomas E. Thompson Award

Subgroup: Bioenergetics

18-Subg
FOF1-ATP Synthase Dimers and The Mitochondrial Permeability Transition Pore from Yeast to Mammals
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The mitochondrial permeability transition pore (PTP) is a voltage-dependent channel that allows solutes of molecular mass $\leq$ 1.5 kDa to equilibrate across the inner membrane. Matrix Ca^{2+} accumulation, together with Pi and a set of compounds collectively called ''inducers'', is necessary to induce PTP opening. In mammals cyclosporin (Cs) A desensitizes the PTP through its binding to cyclophilin D, a matrix protein that facilitates PTP opening. Yeast and Drosophila mitochondria also possess Ca^{2+}-activated channels which, at variance from the mammalian PTP, are insensitive to CsA and inhibited rather than activated by Pi. We show (i) that the permeability properties of the Drosophila channel, which displays selectivity toward Ca^{2+} and H^{+}, are not modified by expression of human cyclophilin D; and (ii) that, in keeping with our recent demonstration that the mammalian PTP forms from dimers of the FOF1-ATP synthase, Ca^{2+}-dependent currents can be elicited in reconstitution experiments with purified dimers of the yeast enzyme. We are currently investigating the effect of genetic ablation of FOF1-ATP synthase subunits that mediate dimerization on PTP opening in yeast, Drosophila and mammalian mitochondria. Our findings suggest that the PTP-forming ability of FOF1-ATP synthase has been conserved in evolution, and that the channels display species-specific features.

19-Subg
The C-Subunit of the ATP Synthase Forms the Pore of the PTP
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Mitochondria maintain tight regulation of inner mitochondrial membrane (IMM) permeability to sustain ATP production. Stressful events cause cell