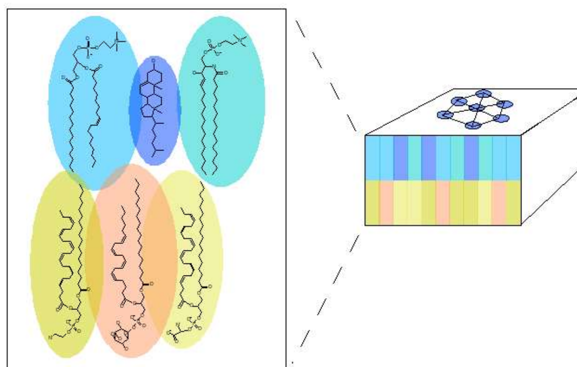
Nanoscale Organization of Biological Membranes

My primary interest is discerning the molecular organization of biological membranes. These cell "walls" are composed from a diverse blend of lipid and protein molecules which regulate membrane function. The modern view of membrane structure dictates that a highly organized lateral packing of lipid and protein molecules within the plane of the membrane is essential for proper cell functioning [1]. Additionally, the inner and outer leaflets of cell membranes are compositionally and structurally unique, further complicating the picture. I ask the question, "What are the molecular details that result in such complex yet highly organized structures?"

Membrane Lateral Organization

Membrane lipids and associated proteins are proposed to be heterogeneously distributed within the bilayer and specific structure related interactions are thought to drive this. The sizes and lifetimes of these "lipid micro-domains" (a.k.a "lipid rafts") are controversial [2]. In earlier work, I addressed this issue and identified a cholesterol-induced clustering mechanism in model neural membranes comprising omega-3 polyunsaturated phospholipids and cholesterol. The combined solid state nuclear magnetic resonance (NMR) and X-ray diffraction (XRD) work defined a molecular mechanism driving cholesterol-rich and cholesterol-poor regions of the bilayer, estimated to be smaller than 160 Å in radius and existing for less than 10^{-5} s [3]. I also illustrated how this cholesterol-clustering effect could affect the function of membrane proteins [4] since it is known that inhibition/activation of certain membrane protein families is dependent on cholesterol [5]. Yet a more accurate description of micro-domains remains elusive. Visualizing lipid and protein lateral distribution at 1-10 Å correlation lengths is necessary to define the structure of a lipid/protein domain. These measurements are challenging, however, since it is difficult to observe such short length scales under the highly dynamic "fluid" condition analogous to that *in vivo*. One technique capable of revealing atomic scale structure in fluid samples is X-ray scattering.



Trans-Membrane Asymmetry

Biological membranes are asymmetric: the inner and outer leaflets each posses different varieties of lipids and proteins. While this is a generally accepted fact, only a handful of works have attempted to elucidate the complete asymmetric structure of a membrane at atomic scale. I set about to design an experimental method which would enable asymmetric structure determination under physiological conditions. I developed a theory and experimental protocol utilizing X-ray scattering of vesicles in solution [6]. With this new method I was able to show a difference in structure between inner and outer leaflets of a unilamellar vesicle. In support, a similar lipid bilayer profile was reported in a recent crystallographic study [7]. I am currently investigating the vesicle/protein interaction with the intent of establishing an atomic scale binding assay. The lipid mixture is modified to reveal protein affinity for specific lipids and additionally cofactors (e.g. Ca^{2+} ions) are easily titrated into these "solution" samples during X-ray measurements. The use of unilamellar vesicles ensures that all outer leaflets are available for interactions.

Planned Research

Biophysics Laboratory

I plan for a laboratory capable of constructing and characterizing vesicle/protein assemblies. Considering the hundreds of lipid and thousands of protein species present in a cellular membrane, I will narrowly focus my work on specific systems. I am particularly interested in neuronal membranes which predominantly contain cholesterol, polyunsaturated phospholipids and membrane-anchored soluble protein assemblies. Lipids and soluble proteins are available commercially and their assembly into complexes requires only modest equipment. Novel proteins are available either through core facilities (e.g. peptide synthesizer) or through collaborations, typically with biochemists or structural biologists. Building an in-house X-ray scattering facility is desirable, but not necessary, and can be added later as major funding is acquired. A small computer cluster using off-the-shelf PC's running LINUX can be used for computational modeling of membranes in parallel with X-ray experiments. Recent advances in coarse grained molecular dynamics methods enable tractable simulations of membranes [8].

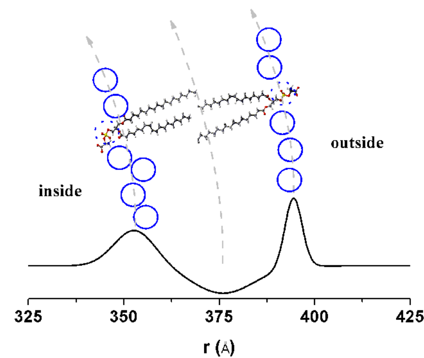
Synchrotron X-ray Scattering from Vesicle/Protein Assemblies

I have designed experimental protocols for observing vesicle structure in solution at both small angle X-ray scattering (SAXS) beamlines (SSRL BL4-2 and ALS 12.3.1.) and protein crystallography (PX) beamlines (SSRL 11-1 and ALS 8.2.1). The SAXS lines have the advantage of providing *in situ* experimentation (e.g. stopped flow, titration, laser catalyzed reactions) at unparalleled signal-to-noise while the PX lines offer the advantages of ease in tunability and automated data collection for large (~100) sample sets. I plan on continued utilization of these facilities for my future studies.

I have developed a flexible model for observing structure, interactions and fluctuations of unilamellar vesicles under physiological conditions. I plan to further develop this method as applied to the observation of 1) lateral and asymmetric structure of macromolecular vesicle/protein assemblies 2) binding and fusogenic interactions between macromolecular assemblies and 3) volume and pressure fluctuations in vesicles as related to protein binding. I will continue experiments already in progress at Stanford Synchrotron Radiation Laboratory (SSRL) and the Advanced Light Source (ALS).

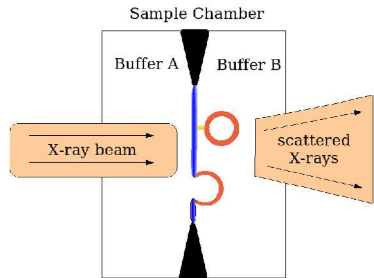
Structure

The nearly universal approaches of deriving membrane structure from X-ray scattering are either to perform integral transforms on the observed scattering curve or to perform a Fourier synthesis of factors derived from the Bragg diffraction. **I take a different and novel approach.** I utilize unilamellar vesicles which possess a distinct "inside" and "outside" analogous to the situation *in vivo*. Next, instead of transforming the intensity, I assume a general, parameterized form for the electron density of the membrane and then a continuous, well behaved analytic expression for the scattered intensity is derived. This method is explained in detail in our forthcoming publication [6]. The result is shown at right where the asymmetric structure of a unilamellar lipid vesicle is revealed. It is straightforward to add additional peaks to the model to facilitate more features e.g. protein binding to the outer surface. Solvent contrast variation techniques and labeling with heavy atoms or halogens can be used to aid in discriminating between molecular species.



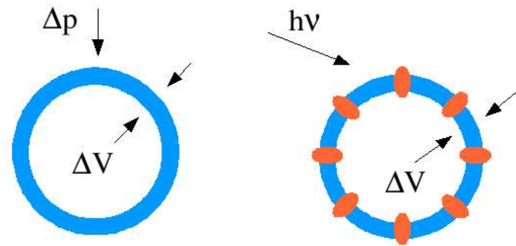
Interaction

I am developing an experimental protocol to observe the in-plane docking of vesicles to a single flat membrane. Typical SAXS experiments are performed at a dilution where particle-particle interactions can be ignored. Confining them to a 2-D space however will enable a study of their clustering as a function of specific molecular constituents, e.g. cholesterol, added to the membrane. In neural tissues, vesicles are docked *via* the membrane anchored SNARE complex (shown in yellow). Several works have suggested that the fusion of lipid vesicles is dependent on cholesterol concentration in the membrane. It is possible that vesicle fusion is directly related to the lipid mediated organization of SNAREs. The experiments I propose here will be able to answer the general question of how lipids, proteins and assemblies aggregate at the 2-D surface of a membrane. The use of a solution cell (as shown above) allows *in situ* observations since critical components can be injected during the experiment.



Fluctuation

X-ray scattering measurements of volume fluctuations within the core of the vesicle are possible utilizing the methods I've developed [9]. One question that has eluded biomembranes researchers for years is the description of compressibility within a membrane bilayer [10]. Typical studies measure pressure *vs.* area on monolayer films using the Langmuir-Blodgett method. An ideal experiment would allow the measurement of volume fluctuations in a unilamellar vesicle. To study the statistical thermodynamic properties of vesicles, volume fluctuations will be observed as the hydrostatic or osmotic pressure is varied. In this way, compressibility as a function of depth, and therefore membrane asymmetry, may be observed. This type of study is unprecedented. In another example, volume fluctuations may be observed during light catalyzed protein (shown in orange above) conformational changes.



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Teaching Philosophy, Experience and Interests

My undergraduate teaching philosophy is simple:

1. Be prepared: know how to do **every** problem in the book
2. Be humble: teach **to** the student (not **at** the student)
3. Be real: provide plenty of examples of actual research

As a graduate student, I had approximately 4 yrs (including summers) experience teaching undergraduate physics recitation and lab. I understood the important difference between teaching engineering *vs.* biomedical students and had great success as measured by formal evaluation as well as personal interactions. I look forward to teaching undergraduate physics again, with a special interest in encouraging talented engineering, biology and chemistry majors to pursue a deeper physics education. My formal training in physics, as well as chemistry/biochemistry and the medical sciences enables me to uniquely relate to students in a cross-disciplinary environment.

At the graduate (and advanced undergraduate) level, I'm most interested in teaching the traditional classes of E+M, Statistical Mechanics and Fluid Dynamics with a specialty in Biophysics/Soft Condensed Matter emphasizing the topics of scattering and magnetic resonance. I plan to incorporate "learning-by-doing" into the class time by utilizing Mathematica notebooks I've written, so far, on the topics of scattering theory and molecular order. With this tool, students have a comprehensive, yet compact overview of the field and then can simultaneously explore the foundations of theory as well as simulate experimental data in an extremely flexible programming and publishing environment. This type of tool is invaluable to their future as physicists and I would certainly encourage students to develop similar ideas in their own research.