Applying new models to describe biomembrane structure and solvent partitioning in living cell membranes and membrane mimics.

Jonathan D. Nickels[1]* (nickeljo@ucmail.uc.edu), Micholas Dean Smith[2,3], Luoxi Tan[1], Haden L. Scott[3], John Katsaras[3], Hugh M. O'Neill[3], Sai Venkatesh Pingali[3], Jeremy C. Smith[2,3], James G. Elkins[3], Brian H. Davison[3]

[1]University of Cincinnati, Cincinnati, Ohio; [2]University of Tennessee, Knoxville, Tennessee; [3]Oak Ridge National Laboratory, Oak Ridge, Tennessee

https://cmb.ornl.gov/dynamic-visualization-of-lignocellulose/

Project Goals: The development of renewable biofuels is a key mission of the DOE Genomic Science program. Lignocellulosic biomass has the potential to be an abundant, renewable source material for production of biofuels and other bioproducts. The use of organic solvents to optimize biomass pretreatment has shown considerable promise, but their disruption of microbial membranes is key to toxic effects limiting fermentation titers. The Oak Ridge National Laboratory (ORNL) Scientific Focus Area (SFA) Biofuels Program utilizes multi-length scale imaging with neutron scattering complemented by high performance computer simulations, NMR, biochemistry and targeted deuteration to provide fundamental knowledge about the molecular forces that drive solvent disruption of the critical assemblies of that comprise plant cell walls and microbial biomembranes.

Amphiphilic co-solvents have a significant impact on the structure, organization, and physical properties of lipid bilayers. The cell membrane is defined by its transverse structure, an approximately five nanometer thick selectively semi-permeable lipid membrane; but it is so much more. Compositionally complex, dynamic, and organized in both the transverse and lateral dimensions, understanding the cell membrane structure – and the role that structure plays in cellular function, communication, and environmental sensing is an active scientific effort. Describing the mutual impact of partitioning and induced structure changes is therefore a crucial consideration in bioenergy research for microbial solvent tolerance in the production of biofuels and other fermentation products where molecules such as ethanol, butanol, or acetic acid might be generated by fermenting microbes; or when residual solvents such tetrahydrofuran (THF) are present from cellulose extraction procedures. Small angle neutron scattering (SANS) is a key method for studying lipid and polymer bilayer structures, with many models for extracting bilayer structure (thickness, area per lipid, etc.) from scattering data in use. However, the molecular details of co-solvent partitioning are conflated with induced changes to bilayer structure, making interpretation and modeling of the scattering curves a challenge. To address this issue, we present a model of bilayer structure which includes a two-term partition constant accounting for the localization of the co-solvent within the bilayer. We validate this model using

a series of SANS measurements of lipid vesicles in the presence of the co-solvent THF, showing several strategies of how to deploy the two-parameter partition coefficient model to describe scattering data and extract both structure and partitioning information from the data. The associated code will be publicly deposited pending publication. Molecular dynamics (MD) simulations are used to both evaluate underlying assumptions of the new data fitting model and illustrate its complementary approach to the data fitting procedure for our model membrane standard (phosphatidylcholine lipids) and a lipid mixture mimicking the *B. subtilis* cell membrane extract. We subsequently demonstrate the use of MD derived estimates of solvent partitioning to refine our SANS modeling. The new structure/partitioning model has been applied to solvent partitioning in the cell membrane of *Bacillus subtilis.* An updated and improved method of isotopic labelling has been developed. Previously, we have devised a novel isotopic labelling approach to enable direct *in vivo* structural study of the cell membrane of the gram-positive organism, *B. subtilis*, using neutron scattering. This was accomplished through a genetic inhibition of fatty acid degradation (*ΔyusL*) and a chemical inhibition of fatty acid biosynthesis through cerulenin. Here, we improve upon the previous system by introducing a dCas9/sgRNA-*fabF* complex that blocks transcription of the essential *fabF* gene when under xylose induction. This leads to greater sensitivity to cerulenin and more robust cell growth when supplementary fatty acids are introduced. A subtle change in fatty acid uptake is noted which manifests as an increase in the membrane thickness determined via neutron scattering. This enables improved investigations of cellular uptake and utilization of fatty acids, cell membrane structure and organization as a phenotypic response to metabolic and environmental changes. SANS observations of live cells and lipid extracts in the presence of co-solvents reveal bilayer thinning and estimates of partitioning of the co-solvent. These are currently being analysed using the new model. Initial analysis of complementary MD simulations compares favorably to both structure (thinning) and solvent partitioning from SANS.

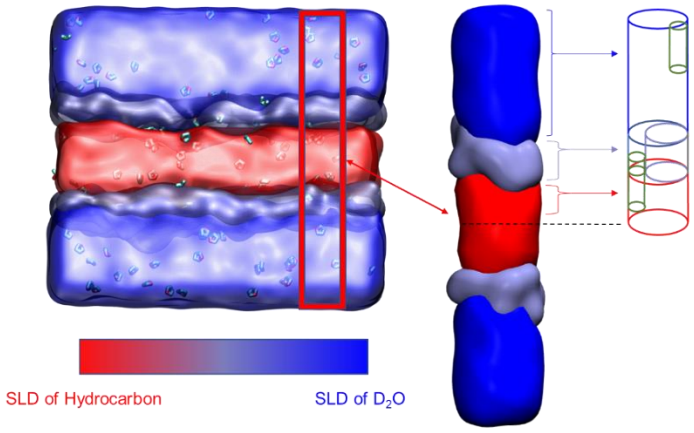

Figure 1. The two-parameter partition constant model presented for the case of a lipid bilayer (DMPC) in water with THF as a co-solvent. The bilayer structure is approximated as a three-slab model: the hydrophobic core containing a portion of the lipid and co-solvent – central symmetric outer layers containing a portion of the lipid, water, and co-solvent – and the bulk solvent containing water and co-solvent. Given a knowledge of the bilayer chemistry, atomic scattering length and molecular volumes, these relationships can define the partitioning of the co-solvent and bilayer structure using the area per lipid (APL), number of water molecules per lipid headgroup (N_w), the partition constant (K_p) and the *co-solvent localization constant* (P_s).

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