Developing next-generation modeling techniques to analyze biomembrane neutron scattering data

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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 biomolecules that comprise plant cell walls and microbial biomembranes.

The cellular membranes of fermentative microorganisms are a major target for the toxic effects of advanced biofuels, including *n*- and *iso*-butanol as well as solvents used in the pretreatment of lignocellulosic biomass such as tetrahydrofuran (THF). These amphiphilic molecules partition into the lamellar structure of the membrane bilayer affecting its viscosity, stability, and structure, both in the transverse and lateral directions. Small-angle neutron scattering (SANS) is ideally suited to measure structural properties of membranes, due to its probe-free nature that enables measurements with minimal perturbation to the membrane and its broad spatial resolution (i.e., ~1-100 nm). However, improved models are needed to analyze and extract the maximum information from the SANS data measured from the heterogeneous lamellar structures of microbial membranes.

Our team has studied model microbial membranes in the presence of co-solvents, Figure 1 (Smith et al. 2020), and is currently looking at studies of bacterial lipid extracts and the membranes of living bacteria. These studies have already revealed differences in the partitioning and localization of co-solvents, along with clear effects on membrane structure. As the membrane compositions become more complex and more biologically relevant, there is a clear need for reliable and robust structural models that can credibly extract structural information, despite the complex composition of biological membranes and the variable and broad spatial distribution of the co-solvent molecules partitioned within the membrane.

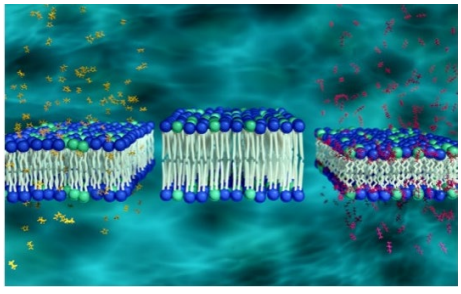

Figure 1. SANS and MD simulations reveal different partitioning and structural effects of THF (left, yellow) and *n*-butanol (right, purple) in model microbial membranes (bilayer of white fatty acids and blee or green headgroups).

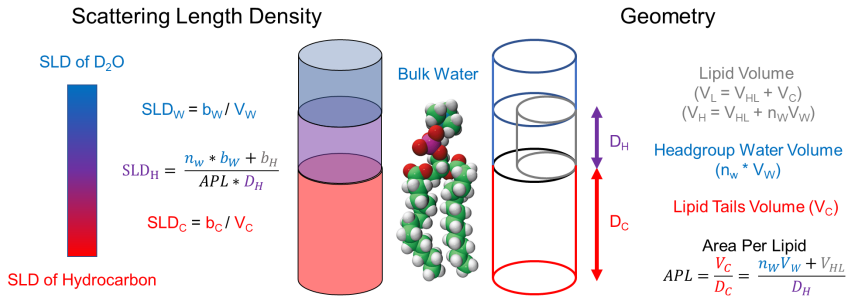

Figure 2. Implementation of the slab model for symmetric lamellar structures, shown here on a per lipid basis in one leaflet. Our implementation in Tan et al. (2021) provides a self-consistent fitting algorithm to extract the key parameters of area per lipid (APL) and water content in the head group region (n_H).

A robust solution to this question is a slab model, a relatively simple structural description that can be used to describe lamellar phases including lipid bilayers. To a first approximation, membrane structure can be described by three slabs (or sheets) namely, a central solvent-free core and two symmetric outer layers composing the solvated shell. This model well-describes the distribution of neutron scattering length density within a membrane sample and therefore is applied to interpreting SANS spectra derived from membrane lipid bilayers. Prior implementation of this model in common scattering software packages was prone to generating unreliable results due to the covariance of scattering length density and bilayer thickness. Here, we report on an improvement to the existing models within the publicly available software suite, *SasView*, which enforces physical consistency through the area per amphiphile molecule and number of solvent molecules included within the solvent-exposed outer layer. The model was applied to fit standard lipid bilayer scattering data sets, determine structural parameters consistent with prior literature values, and illustrate the typical and ideal cases of fitting for neutron scattering data obtained using single or multiple contrast matching conditions. The model has been submitted for inclusion in subsequent releases of *SasView* and will aid the broader scattering community studying lamellar structures.

Adding self-consistency to this model is an important first step to the development of lamellar models capable of describing the partitioning of co-solvents between the membrane and the bulk solvent, and the resulting changes in membrane structure. The improved slab model provides the opportunity to compare experimental membrane thickness and solvent positioning data to those quantities predicted by molecular dynamics simulations of biomembranes under solvent stress. Further, this comparison will permit future, experimentally guided, improvements to molecular mechanics membrane force-field accuracy, ultimately leading to more predictive computational tools for the study of membrane behavior under environmental stress.

References

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2. Smith, M. D., et al. “Solvent-induced membrane stress in biofuel production: molecular insights from small-angle scattering and all-atom molecular dynamics simulations.” 2020, *Green Chem* 22(23), 8278-8288. DOI: 10.1039/d0gc01865a

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