

## Precision Labeling of Membrane Fatty Acids in *Bacillus subtilis* and the Impacts on the Cellular Proteome and Lipidome

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<http://cmb.ornl.gov/index.php/research/bioenergy/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.**

In order to perform structural studies on the disruption of biomembranes additional tools to selectively manipulate biomembranes *in vivo* are needed. We previously developed a system to control both the chemical and isotopic composition of the hydrophobic region within the cellular membrane of the model bacterium, *Bacillus subtilis*.<sup>1</sup> Briefly, blocking *de novo* fatty acid (FA) biosynthesis using cerulenin as an irreversible inhibitor of beta-ketoacyl-ACP synthase II (FabF), in a strain that is unable to catabolize FAs ( $\Delta$ *fadN*), forces growing cells to take-up exogenous FAs from the growth medium to construct their membrane. This feeding strategy has enabled the biophysical properties of biomembranes in viable bacterial cells to be studied using neutrons as a

non-destructive probe. If cells are grown in a high D<sub>2</sub>O background while being fed protiated FAs, the membrane can be “visualized” using small-angle neutron scattering (SANS) techniques due to the differences in scattering length density between hydrogen and deuterium. By tuning isotopic contrast within the plane of the membrane, we have also shown that lateral structure exists in viable bacterial cells consistent with the presence of lipid domains (aka lipid rafts). Having demonstrated the potential of our approach to study bacterial membranes using SANS techniques *in vivo*, we now aim to expand the utility of our methods by determining how microbial membranes respond to second-generation biofuels and plant biomass pretreatment solvents. Amphiphilic solvents partition into biomembranes affecting their nanoscale structure and ability to form an effective barrier to the extracellular environment. A better understanding of how membranes respond to different solvents at the molecular scale will improve molecular dynamics simulations and potentially, the ability to engineer more resilient biomembranes.

While our strategy to introduce neutron contrast in the bilayer by feeding exogenous FAs has been effective, it is necessary to understand the systemic changes in *B. subtilis* cells induced by the labeling procedure itself. In this work, analysis of cellular membrane compositions was paired with shotgun proteomics to assess how the proteome changes in response to the directed incorporation of exogenous FAs into the membrane of *Bacillus subtilis*. Key findings from this analysis include an alteration in lipid headgroup distribution, with an increase in phosphatidylglycerol lipids and decrease in phosphatidylethanolamine lipids, possibly providing a fluidizing effect on the cell membrane in response to the induced change in membrane composition. Changes in the abundance of enzymes involved in FA biosynthesis and degradation are observed; along with changes in abundance of cell wall enzymes and isoprenoid lipid production. The observed changes may influence membrane organization, and indeed the well-known lipid raft-associated protein flotillin was found to be substantially down-regulated in the labeled cells – as was the actin-like protein MreB. Taken as a whole, this study provides a greater depth of understanding for this important cell membrane experimental platform and presents a number of new connections to be explored in regard to modulating cell membrane FA composition and its effects on lipid headgroup and raft/cytoskeletal associated proteins.

## References

1. Nickels JD, Chatterjee S, Stanley CB, Qian S, Cheng X, Myles DAA, Standaert RF, Elkins JG, Katsaras J (2017) The *in vivo* structure of biological membranes and evidence for lipid domains. *PLoS Biol* 15(5): e2002214. <https://doi.org/10.1371/journal.pbio.2002214>

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