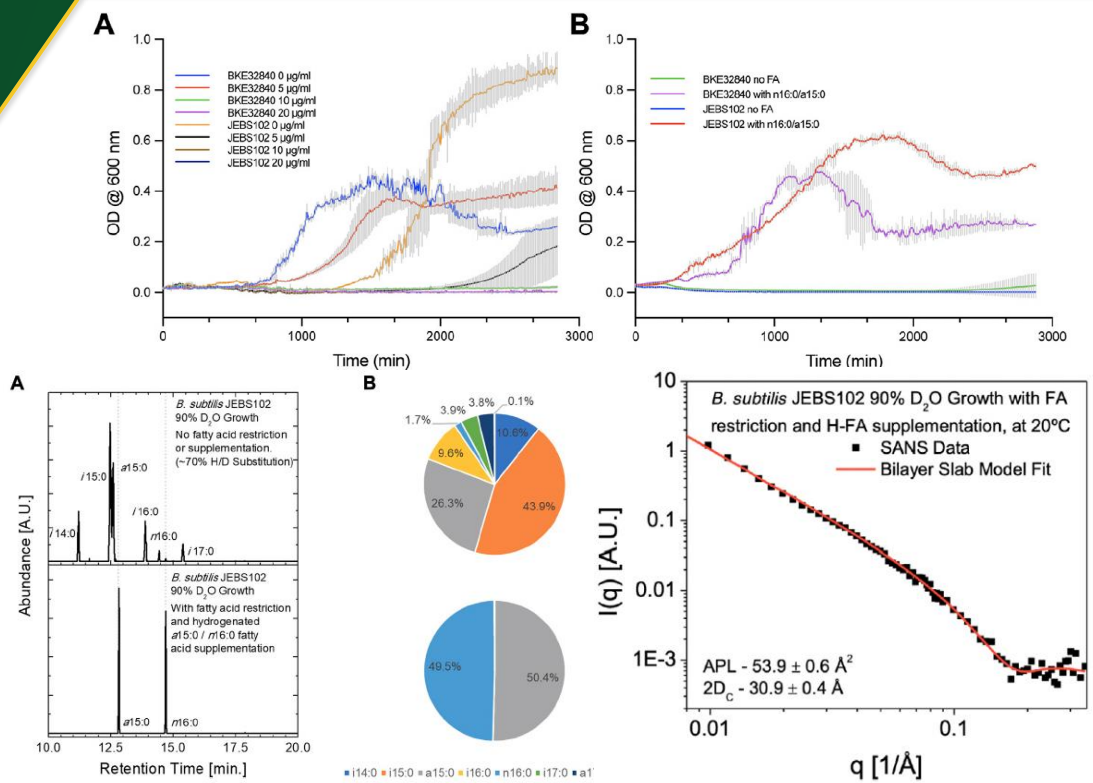
Improved chemical and isotopic labeling of biomembranes in *Bacillus subtilis* by leveraging CRISPRi inhibition of beta-ketoacyl-ACP synthase (*fabF*)



Growth curves showing reduced cerulenin dependency and improved overall cell densities in the new *B. subtilis* strain (top). Fatty acid uptake profiles for the control strain and JEBS102 (left). SANS data applied to determine membrane thickness (right).

Nickels et al., *Front Mol Biosci* (2022). doi: 10.3389/fmolb.2022.1011981

This work is supported by DOE Office of Science, Office of Biological and Environmental Research Genomic Science Program (ERKP752). This work was also supported in part by the DOE Office of Science, Workforce Development for Teachers and Scientists under the Science Undergraduate Laboratory Internships (SULI) program.

Scientific Achievement

- We applied a CRISPR interference system that blocks transcription of the essential fatty acid (FA) synthesis *fabF* gene in *Bacillus subtilis* under xylose induction. With higher inhibition of FA synthesis using CRISPRi, we observed robust cell growth incorporating added labelled FAs.

Significance and Impact

- Differential isotopic labeling of cell membrane systems is an enabling methodology for structural studies in living microbial systems. Our new strain improves upon previous methods to label membranes *in vivo*.

Research Details

- A novel *B. subtilis* strain was constructed that included both a *fadN* deletion and CRISPR dCas9-sgRNA(*fabF*) construct to modulate FA metabolism.
- The strain displayed improvements in FA incorporation into its membrane. This will allow more robust SANS experiments to be conducted on live cells.
- Increased SANS scattering intensities were confirmed and allowed a direct estimate of membrane thickness.