High-quality protein production for structural studies: From plant cell wall synthesis to microbial production of bioproducts.

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Project Goals: JBEI’s mission is to provide the scientific basis for converting lignocellulosic biomass to renewable, drop-in, liquid transportation fuels and other important bioproducts. In order to achieve this, JBEI researchers use high high-resolution X-ray crystallography and cryo-electron microscopy methods to understand the atomic structures of proteins involved in producing advanced biofuels and bioproducts by providing the foundational understanding of enzyme mechanism and structure/function relationships as well as the ability to engineer function and stability. An important step to obtaining high-resolution structural information is sample preparation, which requires the expression and purification of high-quality protein that typically includes a series of screening steps followed by up scaling and optimization. The workflow will be described for enzymes involved in the synthesis of the plant cell wall, and enzymes used for the microbial production of bioproducts.

Glycosyltransferases (GTs) are enzymes involved in the biosynthesis of plant cell-wall polysaccharides. The cell walls are complex structures that play a key role in plant fitness. Furthermore, these cell walls have been proposed to be a source of renewable energy in the form of biofuels. GTs catalyze the connection of simple monosaccharide sugars into complex polysaccharide sugars, collectively known as hemicelluloses. Due to their essential function in plant cell wall biosynthesis we are interested in their structural characterization and mechanisms of catalysis.

Type I Fatty acid synthases (FAS1) are megadalton-sized enzymes, which contain multiple catalytic domains and function like molecular assembly lines. Corynebacteria utilize the multifunctional type I FAS for the de novo biosynthesis of fatty acids to generate, for example, palmitic acid (C16:0), oleic acid (C18:1) and stearic acid (C16:0 or C18:0), which are incorporated into the cell-membrane phospholipids or used as precursors for mycolic acid biosynthesis. Fatty acids are interesting candidates for future biofuels, because they are highly reduced and have high energy densities. Due to the complex interactions of multiple catalytic domains, the engineering of FAS1 for biofuel production will benefit from detailed structural analysis.

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