Ultrastructure of plant cells by electron microscopy: towards increased biofuel production

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Project goals: The Physical Analysis group of the Joint BioEnergy Institute focuses on the development and the application of methods for the physical characterization of plants and microbes at the nano- and meso-scale. We support the analysis of JBEI experiments in the Feedstocks, Deconstruction, Fuels Synthesis and Technology divisions by using faithfully preserved samples and a variety of electron microscopy techniques, including traditional SEM and TEM, and wide-field montages TEM, as well as advanced 3D imaging approaches, such as electron tomography, and focused ion beam SEM and serial block face SEM.

The plant biomass suitable for the production of bioenergy and high value chemicals is concentrated in the cell wall. While significant progress has been made in understanding the composition and to some degree the regulation of the cell wall synthesis, little is known about its 3D organization and thus the mechanical properties that result as a consequence from this organization. Any effort aiming to re-engineer cell walls for the purpose of increasing yield or facilitating deconstruction needs to take plant cell wall 3D organization into consideration. Using quantitative 3D analysis of ultrarapid frozen, cryo-sectioned *Arabidopsis thaliana* stems and computer-assisted design (CAD) model building, we have obtained the first accurate 3D model of the primary and secondary cell wall of mature xylem tracheary cells *in situ*. The 3D model shows an unexpected pattern of microfibril orientation with sparse yet stiff hemicellulose cross-linking of microfibrils. Comparing the mechanical simulation results of our experimental model with other proposed models for turgor pressure (compression) and shear forces (bending) reveals how elegantly cell walls are designed by evolution to withstand the mechanical forces imposed on plants, leading to rigid yet flexible mechanical strength.

Plant chloroplasts are the site of photosynthesis and carbon-fixation and, therefore, a foundational understanding of their function and maturation is key for effective biomass generation. To carry out this complex task, in both plants and algae, chloroplasts contain an elaborate architecture of complex lamellar membrane systems, also known as photosynthetic thylakoid membranes. We are focusing on understanding the interplay between the molecular processes of light harvesting, carbon-fixation and ultrastructural thylakoid network organization including changes under changing light conditions. The unicellular green algae *Chromochloris zofingiensis* is known for its

production of biodiesel and a high-value carotenoid and, therefore is an ideal model system for studying chloroplast development. It allows us to trap defined stages of chloroplast development by switching between autotrophy and heterotrophy. Currently, we are working on analyzing the ultrastructural data of chloroplasts at different time points during the photosynthetic breakdown and biogenesis using serial sectioning and electron microscopy. Our recently developed cryo-immobilization sample preparation techniques show superior near-native ultrastructural preservation without imposing any size limitations.

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