

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

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**Project Goals:** Determine mechanical cell wall properties and thylakoid membrane organization in chloroplasts for improving lignocellulosic biomass production.

Plant cell walls are the storehouse of complex sugars that if separated out efficiently from the wall lignins, can be fermented to produce commercially viable biofuel. There have been several approaches to genetically re-engineer the cell walls in various feedstocks with the ultimate goal of making the walls more accessible to deconstruction processes. At the same time, the modified cell walls need to maintain enough strength for the plants to grow and be functional. We pioneered a structural approach to determine the mechanical properties of plant cell walls. We are using qualitative and quantitative analysis of cryo electron tomography images and 3D computer-aided design (CAD) programs to develop realistic 3D macromolecular resolution models of various cell walls. Results of static stress-strain simulations of cell wall models allow an assessment of cell wall properties, which can be used for wall design improvement and optimization.

In addition to the cell wall project, we are studying structure and dynamics of thylakoid networks in chloroplasts of land plants. These organelles define plants, and they are responsible for photosynthesis as well as numerous other functions. Optimization of photosynthesis and carbon-fixation are key processes for biomass generation. Chloroplasts harvest the light and produce the biochemical energy that allows cell wall biomass production. 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. These sheet-like membrane-bound compartments are arranged into regularly spaced stacks, called grana and non-stacked stroma thylakoids. To understand the interplay between the molecular processes of light harvesting and carbon-fixation and the morphology of thylakoid network, it is necessary to know how these networks develop, what the three-dimensional (3D) organization of thylakoid membranes at different time points of differentiation looks like and what kind of the structural changes take place under changing light conditions. Our approach aims at combining cryo immobilization techniques with focused ion beam/scanning electron microscopy (FIB/SEM). Cryo-immobilization can be achieved by high-pressure freezing (HPF), which allows for the vitrification of cells or whole tissues. Combined with a recently developed quick protocol for freeze-substitution (FS) and resin embedding, this method leads to sample preservation that is very close to the native state without imposing size limitations. With the exception of a few very small cells, more complex eukaryotic specimens must be thinned to make them transparent to the electron beam. In contrast, using FIB/SEM, there is no need for thin samples. This technology can be used to directly cross-section and image biological samples, such as cells or tissues for 3D reconstructions. By combining these two methods, we plan to obtain 3D volumes of chloroplasts in a close to native condition and in their native cellular context.

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