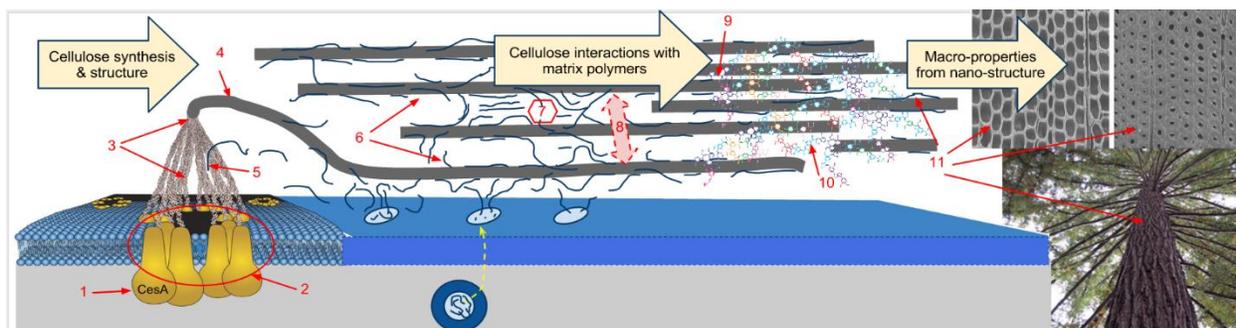


Center for Lignocellulose Structure and Formation (CLSF)
EFRC Director: Daniel J. Cosgrove
Lead Institution: Pennsylvania State University
Start Date: August 2009

Mission Statement: *To develop a nano- to meso-scale understanding of plant cell walls, the main structural material in plants, and the mechanisms of their assembly, forming the foundation for significant advances in sustainable energy and novel biomaterials.*

Cellulosic biomass (lignocellulose) holds great promise as a large-scale, renewable and sustainable source of liquid biofuels for transportation, if we could overcome technical obstacles stemming from its complex, hierarchical structure. Despite its huge economic importance, many aspects of lignocellulose structure and formation remain shrouded in mystery. For instance, little is known of the details of how the cellulose-synthesizing nano-machine at the cell surface links simple sugar molecules into long strands and extrudes them at the cell surface in such a way that they make a strong, insoluble and highly inert crystalline fibril. Likewise, the processes by which simple polymers are transformed into a strong and recalcitrant biomaterial are not well understood.

CLSF goals are to develop a detailed nano- to meso-scale understanding of plant cell wall structure and its mechanism of assembly, from the molecular mysteries of how glucose is assembled by cellulose synthase complexes to form cellulose microfibrils to the orderly, hierarchical interaction of cellulose with other components to form cell walls with diverse properties. The diagram below sketches some of the key points for lignocellulose formation, starting with cellulose synthesis by cellulose synthases (CESA) organized into a protein complex embedded in the plasma membrane (left, points 1-2), followed by cellulose crystallization and interactions with hemicelluloses to form a cohesive wall (points 3-8) and then changes that accompany lignin polymerization within the wall (points 9-11).



New understanding of these processes will form the scientific foundation for designing rational, science-based pretreatments to deconstruct cell walls and for using genetic techniques to coax plants into making modified walls for significant advances in sustainable energy and novel materials. It will also yield insights into biomimetic ways to transform simple molecules into complex polymeric materials with diverse physical and chemical properties.

CLSF research is organized around two themes concerned with the process of cellulose synthesis and the rules of assembly of cell wall components to make a cell wall with specific physical properties.

CLSF Theme 1 probes the mechanism of cellulose microfibril (CMF) formation. Specific objectives include: (1.1) Determination of the structure and in-vitro activity of plant cellulose synthase (CESA) by biochemical means, X-ray crystallography and electron microscopy, as well as by computational modeling of the dynamics of glucan synthesis by a CESA protein. (1.2) Assessing the roles of parts of the CESA protein for cellulose synthesis by genetic modification and analysis of CESA activity and cell wall phenotypes. (1.3) Analysis of the contributions of CESA isoform to the activity of the Cellulose Synthesizing Complex (CSC) and properties of the microfibril and macrofibril through microscopic and genetic experiments. (1.4) In-depth characterization of the plant CSC by isolating an active CSC, modeling CMF formation in silico, and identifying proteins that interact with CSCs that synthesize cellulose in secondary cell walls. (1.5) Reconstitution of a functional CSC from purified plant components by assembling the essential components of the plant CSC.

CLSF Theme 2 investigates the nano- and meso-scale structure and assembly of cell walls and the basis for their important physical and biological properties. Research objectives include: (2.1-2.2) Analysis of mesoscale wall architecture and dynamics by Atomic Force Microscopy (AFM), small-angle neutron scattering, and solid-state Nuclear Magnetic Resonance (ssNMR) in combination with enzymatic and genetic modifications of the wall. (2.3) Testing of the current model of the grass cell wall using enzymatic, biomechanical and physical approaches. (2.4) Testing how matrix polymer delivery relates to cellulose biosynthesis and order using click-labeling of matrix polysaccharides combined with analysis by AFM and transmission electron microscopy. (2.5) Analysis of the mobility of water, polysaccharides and proteins by neutron scattering and ssNMR to assess influence of wall components and wall charge on wall dynamics. (2.6-2.7) Incorporation of new as well as existing data into computational models of primary and secondary cell wall architecture and material properties. (2.8) Analysis of the physical effects of lignin polymerization in cell wall explants and analogs, including mobility of water and polysaccharides. (2.9) Development and use of sum frequency generation (SFG) spectroscopy (a) to analyze meso-scale organization of cellulose in single cell walls and (b) to refine the interpretation of SFG spectra.

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