EFRC: CENTER FOR LIGNOCELLULOSE STRUCTURE AND FORMATION (CLSF)

UPDATED: AUGUST 2016

AWARDS: $21.0M (August 2009 – July 2014); $12.5M (August 2014 – July 2018)	
WEBSITES: http://science.energy.gov/bes/efrc/centers/clsf/; http://www.lignocellulose.org/	
TEAM: **Penn State University (Lead)**: Daniel J. Cosgrove (Director), Charles Anderson, Ying Gu, Seong H. Kim, Manish Kumar, Janna Maranas, Karl Mueller, B. Tracy Nixon, Ming Tien; **Massachusetts Institute of Technology**: Mei Hong; **North Carolina State Univ.**: Candace Haigler, Yaroslava Yingling; **Oak Ridge National Laboratory**: Hugh O'Neill; **Stony Brook Univ.:** Chang-Jun Liu; **Univ. of Rhode Island**: Alison Roberts; **Univ. of Texas at El Paso:** James Kubicki; **Univ. of Virginia**: Jochen Zimmer	

SCIENTIFIC MISSION AND APPROACH

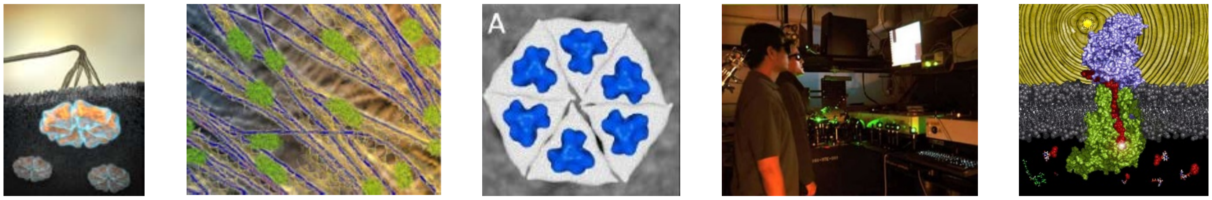
The goals of CLSF are to develop a detailed nano- to meso-scale understanding of plant cell walls, from cellulose microfibril (CMF) formation to the assembly of CMFs with other wall components to form the versatile plant cell wall. Cellulosic biomass (lignocellulose) holds great promise as a large-scale, renewable and sustainable source of liquid biofuels for transportation, if technical obstacles stemming from its complex, hierarchical structure could be overcome. CLSF's research will form the scientific foundation for designing methods to deconstruct cell walls and for coaxing plants into making modified walls for significant advances in sustainable energy and materials. The research is organized around two interconnected themes that lie at the interface of biology and physics:

1) **Cellulose Synthesis and Crystallization:** Elucidate how plants synthesize CMFs, including how cellulose synthases (CESAs) polymerize and secrete individual glucan chains and how multimeric cellulose synthase complexes (CSCs) assemble multiple chains into 3-nm-wide CMFs. CMFs are long, crystalline arrays of glucans that form the major load-bearing component of the plant cell wall.

2) **Structure and Assembly of Cell Walls:** Elucidate the rules of assembly by which CMFs interact with diverse matrix polymers to form complex, multilamellate structures, ranging from highly extensible primary cell walls in growing tissues to rigid lignified secondary cell walls in xylem and other cells.

SELECTED SCIENTIFIC ACCOMPLISHMENTS

- Determined the 3D molecular structure for the catalytic domain of a plant CESA. The structure was first proposed based on computational modeling, then confirmed experimentally in a bacterial CESA.
- For the first time, characterized *in-vitro* cellulose biosynthesis in a bacterial cellulose synthesis complex reconstituted from purified components.
- Computationally predicted a deleterious CESA mutation that was then verified through genetic testing and live cell imaging of cellulose synthesis complexes, opening up the possibility of rationally designing CESAs to engineer the properties of cellulose, biomass and biomaterials.
- Discovered that CSC is made from three types of CESAs in equal proportion. Additional experiments and modeling indicate that CSC contains 18 CESAs (3 in each lobe of 6), contrary to the long held assumption that CSC contains 36 CESAs.
- Elucidated the connection between CMF orientation and the cytoskeleton: a protein, CSI1, binds to CESA and microtubules, providing the long-hypothesized link between these two components.
- Developed Sum Frequency Generation Spectroscopy to quantify the amount, orientation, and mesoscale packing of crystalline CMFs in intact plant cell walls. This tool revealed CMFs in secondary cell walls are often aligned in anti-parallel directions.
- Used atomic force microscopy and solid-state NMR to discern direct cellulose-cellulose junctions and cellulose-pectin interactions that dominate primary cell wall structure and its mechanical properties.



CLSF research, from left: Model of cellulose synthesis complex; Model of a primary cell wall: CMF (blue), xyloglucan (green), and pectins (yellow); Experimental evidence for 18 CESAs within the CSC rosette; Yong Bum Park and Chris Lee, CLSF members, utilize sum frequency generation vibration spectroscopy to detect crystalline cellulose in lignocellulosic materials; Afterlife of a Photon – Winner of the 2015 EFRC Poetry of Science Contest (http://www.energyfrontier.us/content/afterlife-photon)

IMPACT

- When the CLSF was initiated, a vast communication gap existed between the physical chemists who study cellulose structure and the biologists who are discovering how this material is synthesized by plants. CLSF helped to bridge this gap by organizing and sponsoring two symposia at the American Chemical Society meeting in 2011 and 2012 and a 3-day international symposium at Penn State in 2013 with about 150 attendees.
- Overthrew the “tethered network” model of primary cell walls that dominated the field for the last 25 years. Xyloglucans were postulated to form load-bearing tethers linking dispersed CMFs into a cohesive network, but this was refuted when enzymatic cutting of xyloglucans did not loosen the cell wall. Computational modeling, NMR experiments and atomic force microscopy indicate a cell wall model where strength is controlled at limited, direct junctions formed between CMFs.
- CLSF researchers are recognized as leaders in the cell wall community: they garnered more invitations to speak at the last two international cell wall conferences than any other group.

PUBLICATIONS AND INTELLECTUAL PROPERTY

As of May 2016, CLSF had published 132 peer-reviewed publications cited over 1,600 times and filed one US patent application. A selection of highly cited papers are:

- Park, Y. & Cosgrove, D. A Revised Architecture of Primary Cell Walls Based on Biomechanical Changes Induced by Substrate-Specific Endoglucanases. *Plant Physiology* **158**, 1933-1943, doi:10.1104/pp.111.192880 (2012). [**105 citations**]
- Li, S., Lei, L., Somerville, C. & Gu, Y. Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. *PNAS* **109**, 185-190, doi:10.1073/pnas.1118560109 (2012). [**97 citations**]
- Wang, T. *et al.* Sensitivity-enhanced solid-state NMR detection of expansin's target in plant cell walls. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 16444-16449, doi:10.1073/pnas.1316290110 (2013). [**65 citations**]
- Park, Y. & Cosgrove, D. Changes in Cell Wall Biomechanical Properties in the Xyloglucan-Deficient xxt1/xxt2 Mutant of Arabidopsis. *Plant Physiology* **158**, 465-475, doi:10.1104/pp.111.189779 (2012). [**64 citations**]
- Cosgrove, D. J. & Jarvis, M. C. Comparative structure and biomechanics of plant primary and secondary cell walls. *Frontiers in Plant Science* **3**, doi:10.3389/fpls.2012.00204 (2012). [**57 citations**]
- Sethaphong, L. *et al.* Tertiary model of a plant cellulose synthase. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 7512-7517, doi:10.1073/pnas.1301027110 (2013). [**55 citations**]
- Barnette, A. *et al.* Selective Detection of Crystalline Cellulose in Plant Cell Walls with Sum-Frequency-Generation (SFG) Vibration Spectroscopy. *Biomacromolecules* **12**, 2434-2439, doi:10.1021/bm200518n (2011). [**43 citations**]
- Omadjela, O. *et al.* BcsA and BcsB form the catalytically active core of bacterial cellulose synthase sufficient for in vitro cellulose synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 17856-17861, doi:10.1073/pnas.1314063110 (2013). [**43 citations**]
- Bashline, L. *et al*. The Endocytosis of Cellulose Synthase in Arabidopsis Is Dependent on μ2, a Clathrin-Mediated Endocytosis Adaptin. *Plant Physiology* **163**, 150-160, doi:10.1104/pp.113.221234 (2013). [**40 citations**]