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Identify Molecular Structural Features of Biomass Recalcitrance Using Non-destructive Microscopy and Spectroscopy

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Lignocellulosic biomass has long been recognized as a potential sustainable source of mixed sugars for fermentation to fuels and other bio-based products. However, the chemical and enzymatic conversion processes developed during the past 80 years are inefficient and expensive. The inefficiency of these processes is in part due to the lack of knowledge about the structure of biomass itself; the plant cell wall is indeed a complex nano-composite material at the molecular and nanoscales. Current processing strategies have been derived empirically, with little knowledge of the nanostructure of the feedstocks, and even less information about the molecular processes involved in biomass conversion. Substantial progress towards the cost effective conversion of biomass to fuels is contingent upon fundamental breakthroughs in our current understanding of the chemical and structural properties that have evolved in the plant cell walls which prevent its disassembly, collectively known as "biomass recalcitrance."

In nature, biomass degradation is a process of molecular interaction and reaction between plant cell wall polymers (i.e., cellulose and matrix polymers) and cellulolytic microbes and their secreted enzymes (Figure **A**). An integrated system (Figure **B**) has been set up to combine microscopic and spectroscopic modules that allow us to characterize biomass conversion processes at high spatial and chemical resolution. For example, atomic force microscopy (AFM) is used to map the surface topography of the plant cell wall and the binding of microbial cells and enzymes to the walls; total internal reflection fluorescence (TIRF) microscopy and single molecule spectroscopy is used to track the distributions and movements of labeled microbial cells and enzymes; and spectroscopy (e.g., coherent anti-stokes Raman scattering, CARS, see also Poster by *Friedrich et al.*) is used to monitor the resultant chemical changes in cell wall polymeric component during biochemical, as well as chemical, conversions of biomass.

Preliminary results have demonstrated that integrated analysis of the same cell wall samples by diverse microscopic and spectroscopic approaches is critical for characterizing the degradation processes. In the examples illustrated, correlative imaging of AFM (Figure **C**-**E**) and TIRF-M (Figure **F** and **G**) provides molecular resolution of surface structure and chemistry of cell walls. AFM has been shown to be a powerful tool for imaging biomolecules because of its potential atomic level resolution and its ability to image surfaces under appropriately-buffered liquids [1]. Precise measurement of cellulose microfibrils (Figure **C**) has been reported [1, 2] and individual cellulose chains (Figure **D**, Ding, unpublished) can be visualized. Using a flow-cell, the same cell wall sample can be thermo-chemically treated and imaged to monitor the structural changes that occur during pretreatment. Figure **E** shows particles precipitated on the wall surface after dilute acid pretreatment at 140ºC. These particles are probably lignin-carbohydrate-complexes (LCC) generated by partial hydrolysis with acid (Ding, unpublished). The cell wall can also be specifically labeled by a fluorescently-tagged carbohydrate-binding module (CBM) and imaged by TIRF-M. Figure **F** shows a CBM3-GFP (green fluorescence protein) labeled cell wall, in this

case, the family 3 CBM specifically recognizes the planar face of cellulose [3]. This image therefore reveals cellulose distribution in the cell wall. Using similar technique, individual cellulase species can be labeled with fluorescent protein and imaged using TIRF-M at the single molecule level. Figure **G** shows GFP-labeled *Trichoderma* cellobiohydrolase-I (CBH I) selectively bound on areas of an individual cellulose microfibril also labeled with CBM3-RFP (red fluorescence protein) [4].

In summary, we have demonstrated non-destructive approaches to characterize the biomolecules involved in biomass conversion processes using integrated microscopy and spectroscopy. The methods are initially developed using corn stover biomass and maize plant. As a next step, we intend to employ this imaging system to characterize more energy plants, such as switchgrass and poplar wood, and their chemical and biological conversion processes to biofuels.

References

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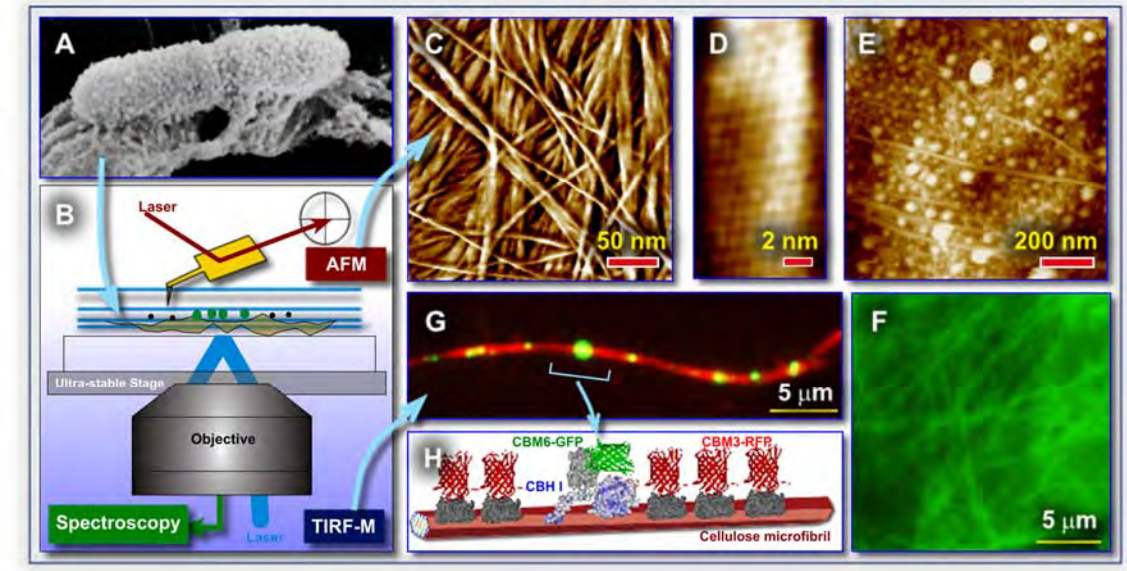

Figure. Advanced imaging techniques used for characterizing biomass conversion. **A**. TEM of *Acetivibrio cellulolyticus* cell binds to lignocelluloses material. **B.** An integrated imaging system combining AFM, TIRF-M and spectroscopic modules. **C.** AFM of maize primary cell walls. **D.** individual microfibril showing cellulose chains. **E.** Thermochemically pretreated cell wall showing lignin-cabohydrate-complexes. **F.** TIRF-M of CBM-GFP labeled cell wall showing cellulose microfibril network. **G.** TIRF-M of CBM-RFP labeled single cellulose microfibril and CBM-GFR labeled cellulase (CBHI).

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