Micro-Spectroscopic Imaging of Lignin-Carbohydrate Complexes in Plant Cell Walls and Their Migration During Biomass Pretreatment

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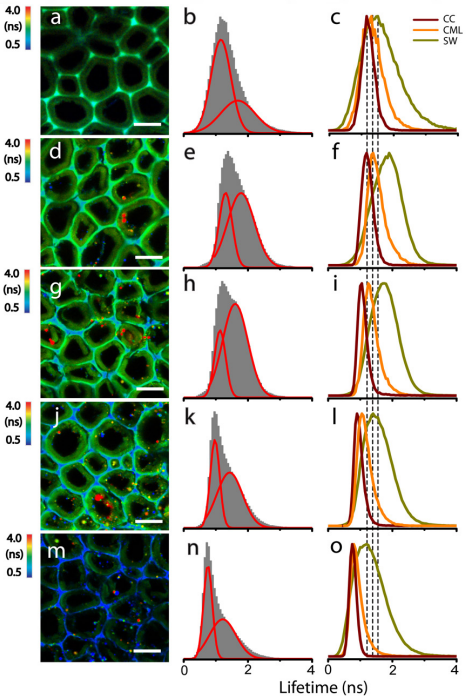
ABSTRACT Lignin is the second abundant biopolymer in lignocellulosic biomass. In plant, lignin provides necessary physical support for plant growth and to resist pathogen attack. Lignin content is considered to be a major factor that negatively affects the process of deconstructing biomass to simple sugars by cellulosic enzymes. Challenges of characterizing lignin have always been to gather complementary and unaltered information from in situ. Here we report a combined microspectroscopic approach to probe in situ lignin content and structural changes with regards to local environmental conditions. Using fluorescence lifetime imaging microscopy (FLIM) and Stimulated Raman Scattering (SRS) microscopy, we observe two types of lignin, the dense and the loose lignin, on natural poplar cell wall. These two types of lignin are released from cell wall during maleic acid pretreatment via two hypothetic pathways. We believe that the loosely formed lignin in secondary wall could be the key barrier for enzyme digestion. This study provides new insights into the rational design of biomass pretreatment process.

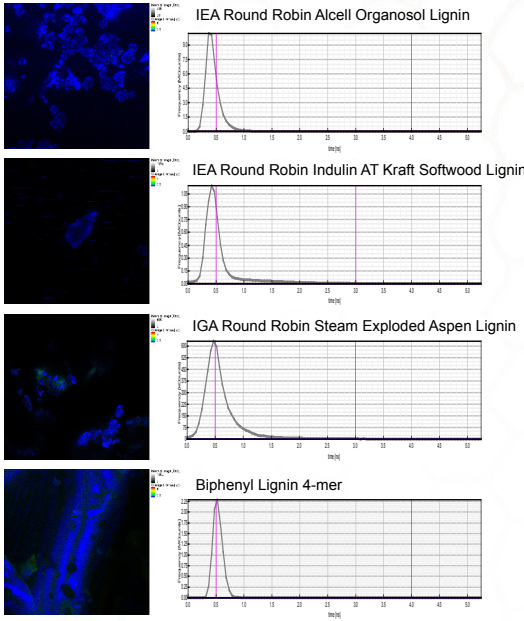
Lignin Autofluorescence Lifetime Distribution in Cell Wall Layers



↑ FLIM analysis results of lignin fluorescence lifetime are displayed. (*left to right*) 1st column (Cell Wall FLIM, panel a, d, g, j and m) shows the representative FLIM images of cell wall lignin autofluorescence, scale bar = 10 mm. 2nd column (Overall Cell Wall Lifetime Distribution, panel b, e, h, k and n) shows the ensemble cell wall lignin fluorescence lifetime distributions from all the cell wall layers. The two red curves are the two fitted Gaussian peaks by fitting the overall histogram. They represent the fluorescence lifetime distributions of dense and loose lignin in cell walls. 3rd column (Cell Wall Layer Lifetime Distribution, panel c, f, i, l and o) shows the individual cell wall layer (CC: cell corner; CML: compound middle lamella; and SW: secondary cell wall) lignin fluorescence lifetime distributions. These results are also compared for different pretreatment concentrations: (*top to bottom*) the 1st row shows the results from untreated cell walls, and the 2nd to 5th row are for the 0.025 to 0.25 M maleic acid pretreated poplar cell walls. Scale bar = 10 um.

→ Artificial lignin-carbohydrate composites are prepared by co-precipitation of biphenyl lignin 4-mer and carboxymethyl cellulose, CMC (as the carbohydrate) from solution. The moisture content was removed by vacuum and incubated at 90°C for overnight. The lignin-carbohydrate complex thin film is then imaged under FLIM to obtain the fluorescence lifetime distribution across the film. We find that increasing carbohydrate content in the complex leads to the increase of lignin fluorescence lifetime. The 50% lignin and 50% carbohydrate composite shows very broad fluorescence lifetime distribution from 1 ns to 3 ns. The very dilute lignin content composite (5% lignin and 95% carbohydrate) shows a sharp distribution fluorescence lifetime centered at around 3.5 ns. Those observations are consistently showing that the lower the lignin concentration, the longer its fluorescence lifetime

Lignin Model Compound Autofluorescence Lifetime Distribution



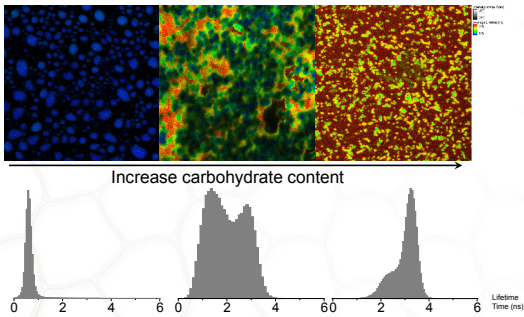
↑ Fluorescence lifetime distribution of lignin model compounds as model of dense lignin unanimously show narrow fluorescence lifetime distribution at 0.5 ns. This short fluorescence lifetime is also observed for the dense lignin present in cell wall.

Varying CMC: biphenyl Lignin 4-mer Ratio

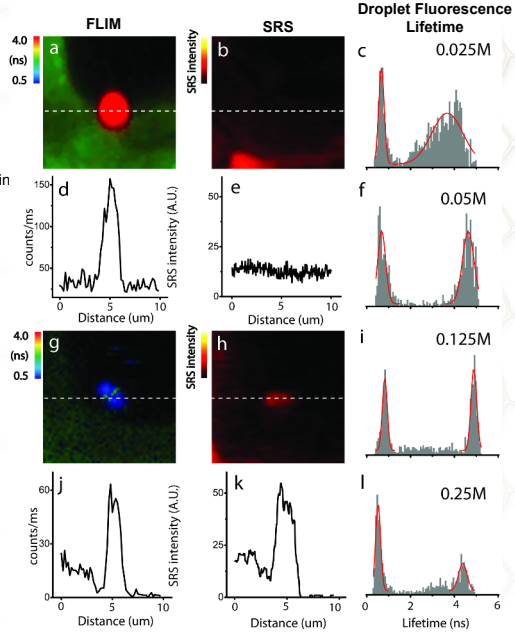
100% Biphenyl lignin + 0% CMC Overnight, 90-95 °C

50% Biphenyl lignin + 50% CMC Overnight, 90-95 °C

5% Biphenyl lignin + 95% CMC Overnight, 90-95 °C

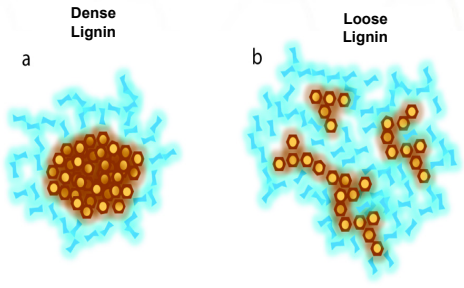


Two types of LCC Droplets after Pretreatment



↑ FLIM (left column) and SRS (middle column) dual-view images for the same droplet and linescan profile for comparison (scale bar = 10 um). (a,b) a long lifetime LCCs droplets shows strong fluorescence intensity with very low SRS signal at lignin resonance frequency. (d, e) Linescan profiles for a and b respectively. (g,h) a short lifetime LCCs droplets shows both strong fluorescence intensity and strong SRS signal at lignin resonance frequency. (j, k) Linescan profiles for g and h respectively. The ensemble fluorescence lifetime distributions of ~ 50 droplets at various of concentrations of maleic acid treatment, 0.025M (c), 0.05M (f), 0.125M (i) and 0.25M (l).

Dense and Loose Lignin



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