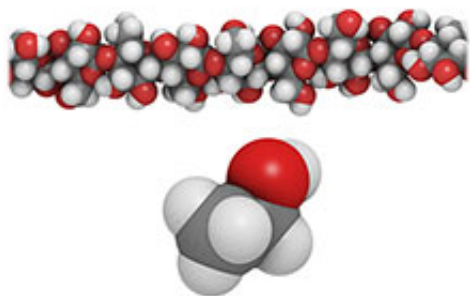


New protein probes find enzymes for biofuel production

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New protein probes are helping find the best microbial enzymes to break down cellulose (top) as part of the process to convert biomass into biofuels, such as ethanol (bottom).

New protein probes are now helping scientists find the best biomass-to-biofuel production enzymes that nature has to offer. Turning biomass into biofuel hinges on the breakdown of the energy-rich primary component of plant matter, cellulose. Cellulose is a polysaccharide, or 'many sugars' bonded together. For biofuel production, the bonds between the many sugars must be broken so that those sugars can then be further processed, for example, fermented to make ethanol. But breaking these bonds is no small feat because they are strong. The best known candidate for this job? A group of microbe-made enzymes called glycoside hydrolases, or GHs.

To help find the most efficient GHs in nature, a team of EMSL users built a suite of probes purposefully designed to bind to known GH active sites and each containing a handle to which a reporter can be attached, enabling further analysis such as the visualization of probe-bound proteins using fluorophores or the isolation of probe-bound proteins for mass spectrometry characterization. Importantly, because GHs share close catalytic similarities, the probes bind not just to known GHs but to previously undiscovered GHs as well. The

team demonstrated the effectiveness of the probes by incubating them with the secretome of—or all of the proteins secreted by—*Clostridium thermocellum*, a biofuel-relevant [bacterium](#) that has remarkably effective cellulose degradation machinery and can even convert cellulose into ethanol directly. [Mass spectrometry](#) tools at EMSL were used to identify the proteins of the secretome that were bound by the probes and proved the probes to be GH selective and specific.

This novel approach is a high-throughput way to find biofuel-relevant enzymes in complex mixtures and can be used to study any microbe. Future efforts are focused on further optimizing the selectivity of the probe suite and expanding its application to fungi.

More information: Chauvigné-Hines L., 2012. Suite of Activity-Based Probes for Cellulose-Degrading Enzymes. *Journal of the American Chemical Society* 134(50):20521–20532. [DOI 10.1021/ja309790w](#)

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