Structural Studies of Biomass Degrading Enzyme Systems

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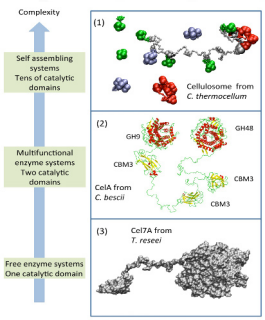
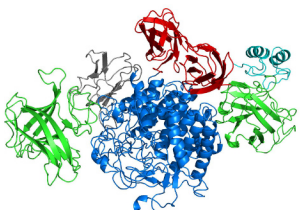
Overview:

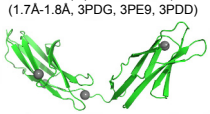
In Nature, bacteria and fungi have evolved multiple strategies to disrupt and deconstruct plant cell walls for their own utilization. These methods employ one of the following broad strategies for biomass deconstruction: mono-functional enzymes, multi-functional enzymes, and highly aggregated (cellulosomal) enzymes; all of which include enzymes such as: cellulases, hemicellulases, and other polysaccharide degrading enzymes. Each of these enzyme systems relies on the synergistic interaction between the enzymes or functional domains of the enzymes to enhance the rate of biomass conversion into monomeric sugar. In 1950, Elwyn T. Reese was the first researcher to report synergistic effects by mixing cellulase fractions. In this keystone report, Reese et al. described this behavior which is now known as endo- and exo-cellulase synergism. Today, inter-molecular synergy has been reported for almost every known cellulolytic fungal and bacterial system studied in detail. In fungal, and some bacterial systems, cellulases are secreted as independent, mono-functional molecules with functionalities that complement each other to create an inter-molecular synergistic enzyme cocktail. In contrast to the mono-functional cellulase system, some cellulolytic anaerobic bacteria and fungi utilize cellulases that self-assemble onto to a protein scaffold attached to the cell surface. In this case inter-molecular synergy relies on the proximity of enzymes with complementary functionalities. Finally, with the recent discovery of multi-functional enzymes, scientists have also described intra-molecular synergy wherein multiple catalytic domains of different functions are combined into a single molecule. In this poster, we summarize the three distinct paradigms of cellulase synergy, provide examples of their performance, and present some protein structures determined for the key members of the enzymatic families involved.

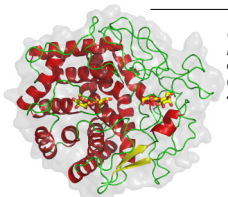


Complexity of the three cellulase systems used in Nature by microorganisms to degrade lignocellulose (Brunecky et al. 2013).

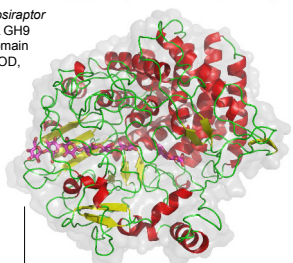


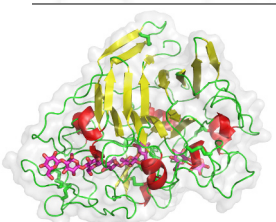
Clostridium thermocellum CbhA cellulase.
CBM4-IG-like domains (2.1Å, 3K4Z)
Fibronectin(III)-like domains (X-domains) (1.7Å-1.8Å, 3PDG, 3PE9, 3PDD)



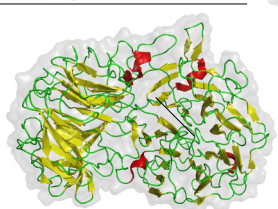


Caldicellulosiraptor bescii CelA GH9 catalytic domain (1.56Å, 4DOD, 4DOE)



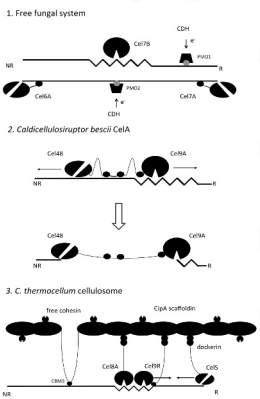


Fungal Family 7 cellulase (1.75Å)

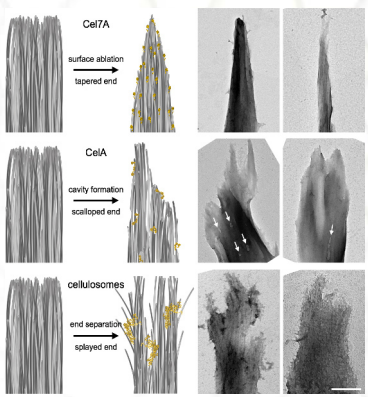


Acidothermus cellulolyticus GH74 (1.8Å, 4LGN)

Family 48 glycoside hydrolase:
Caldicellulosiraptor bescii CelA (2.4Å, 4EL8)
Hahella chejuensis (1.75Å, 4FUS)
Termobifida fusca (1.6Å, 4JJJ)
Few more in progress

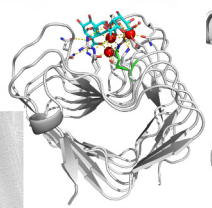


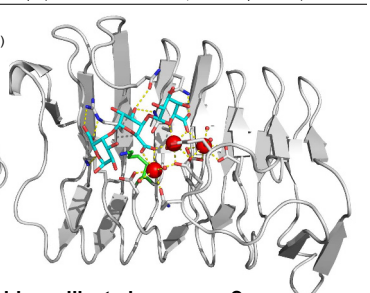
Depiction of the mechanism of action of the three natural cellulose degrading systems known today. The 1) mono-functional system (free enzymes) deployed by most filamentous fungi (*T. reesei*) and bacteria, 2) the multi-functional system used by some bacteria (*C. bescii*), and the 3) highly aggregated system used by very few bacteria (*C. thermocellum*) and fungi.



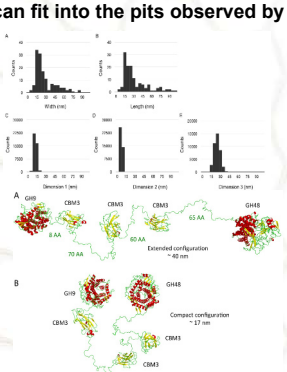
Illustrations and transmission electron micrographs of digested Avicel particles. Imaging of these substrates has revealed new insights into different strategies employed by cellulase systems to deconstruct biomass. In the mono-functional enzyme system, where individual cellulases are limited to ablating the surface, the particle obtains a smooth, tapered end. The tapering of one end of the particle can be understood by the abundance of the reducing-end oriented exoglucanase, Cel7A, in CTec2 and that the microfibrils that make up these small Avicel particles must be oriented in parallel. In the CelA digested particles the same end displays a more irregular, scalloped morphology. In addition, a unique feature of CelA digested particles is the appearance of excavated cavities (white arrows). Cellulosome digested Avicel particles display separation of individual cellulose microfibrils creating more splayed particle end morphology. Scale bar = 500 µm (modified from Resch et al. 2013, and Brunecky et al. 2013)

Caldicellulosiraptor bescii pectate lyase
PL3 (1.2Å-1.8Å, 3T9G, 4EW9, in progress)



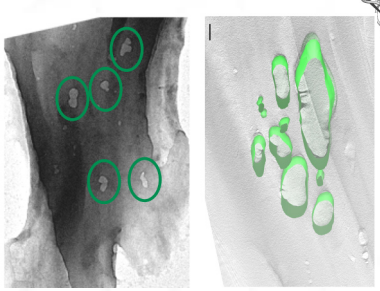


CelA can fit into the pits observed by TEM



Histogram of cavities dimensions created by CelA (A,B) extracted from TEM micrographs shown in Figure 2E,F. Dimensions of CelA over a 40 ns molecular dynamics simulation when aligned on the principal axis at each step (C-E).

Crystal structure of CelA solved at NREL:
Typical extended (A) and compact (B) configurations of CelA found over a 40 ns molecular dynamics simulation.



TEM micrographs of small Avicel PH101 particles from digestions at ~65% conversion. CelA digested particles display cavities of various sizes on the surface (green). Plate on the right is one tilt image from a tomographic study of a cavity field. The length insert bar indicates 50 nm.

Would you like to know more?

Revealing Nature's Cellulase Diversity: The Digestion Mechanism of *Caldicellulosiruptor bescii* CelA
Roman Brunecky, Markus Alahuhta, Qi Xu, Bryon S. Donohoe, Michael F. Crowley, Irina A. Kataeva, Sung-Jae Yang, Michael G. Resch, Michael W. W. Adams, Vladimir V. Lunin, Michael E. Himmel, and Yannick J. Bomble
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