

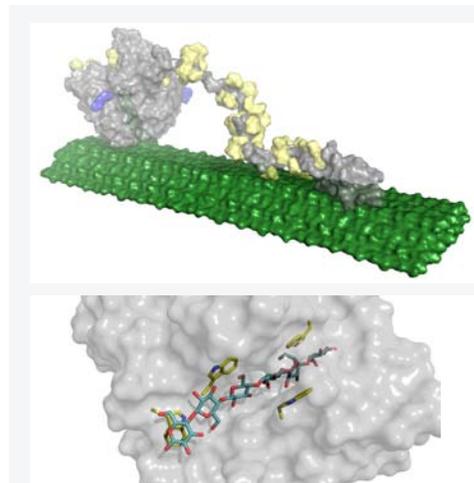
## Computer Simulations Reveal Multiple Functions for Aromatic Residues in Cellulase Enzymes

NREL researchers use high-performance computing to demonstrate fundamental roles of aromatic residues in cellulase enzyme tunnels.

National Renewable Energy Laboratory (NREL) computer simulations of a key industrial enzyme, the *Trichoderma reesei* Family 6 cellulase (Cel6A), predict that aromatic residues near the enzyme's active site and at the entrance and exit tunnel perform different functions in substrate binding and catalysis, depending on their location in the enzyme. These results suggest that nature employs aromatic-carbohydrate interactions with a wide variety of binding affinities for diverse functions. Outcomes also suggest that protein engineering strategies in which mutations are made around the binding sites may require tailoring specific to the enzyme family. Cellulase enzymes ubiquitously exhibit tunnels or clefts lined with aromatic residues for processing carbohydrate polymers to monomers, but the molecular-level role of these aromatic residues remains unknown. *In silico* mutation of the aromatic residues near the catalytic site of Cel6A has little impact on the binding affinity, but simulation suggests that these residues play a major role in the glucopyranose ring distortion necessary for cleaving glycosidic bonds to produce fermentable sugars. Removal of aromatic residues at the entrance and exit of the cellulase tunnel, however, dramatically impacts the binding affinity. This suggests that these residues play a role in acquiring cellulose chains from the cellulose crystal and stabilizing the reaction product, respectively. These results illustrate that the role of aromatic-carbohydrate interactions varies dramatically depending on the position in the enzyme tunnel. As aromatic-carbohydrate interactions are present in all carbohydrate-active enzymes, the results have implications for understanding protein structure-function relationships in carbohydrate metabolism and recognition, carbon turnover in nature, and protein engineering strategies for biofuels production.

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**Reference:** Payne, C.M.; Bomble, Y.J.; Taylor, C.B.; McCabe, C.; Himmel, M.E.; Crowley, M.F.; Beckham, G.T. (2011). "Multiple Functions of Aromatic-Carbohydrate Interactions in a Processive Cellulase Examined by Molecular Simulation." *J. Biol. Chem.* 286, 47, pp. 41028-41035.



*Top: The T. reesei Cel6A enzyme catalytically engaged on a cellulose crystal (green). Bottom: A closer view of the catalytic domain showing the cellulose chain in blue and red, and the large aromatic residues (tryptophans) in yellow and blue. The tunnel entrance where the chain is threaded into the enzyme is on the left, and the bond cleavage occurs between the fourth and fifth glucose monomers counting from left.*

### Key Research Results

#### Achievement

NREL biomass research suggests that natural aromatic-carbohydrate interactions occur over a very large range of binding affinities for multiple functions.

#### Key Result

The varying roles of aromatic-carbohydrate interactions depend on position in the enzyme tunnel.

#### Potential Impact

Understanding the functional role of aromatic residues in the enzyme active site is necessary for the rational design of enhanced carbohydrate-active enzymes.