

Genome-Enabled Assembly of Carbohydrate-Active Enzymes for the Deconstruction of Biomass

Evan Glasgow^{1,2*} (eglasgow@wisc.edu), Emily Beebe,^{1,2} Kirk Vander Meulen,^{1,2} Lai Bergeman,^{1,2} Johnnie Walker,^{1,2} Taichi Takasuka,^{1,2} Christopher Bianchetti,^{1,2} Savannah Mills,^{1,2} Bob Smith,^{1,2} Craig Bingman,^{1,2} Adam Book,^{1,2} Robb Stankey,^{1,2} Gina Lewin,^{1,2} Evelyn Wendt-Pienkowski,^{1,2} Cameron Currie,^{1,2} Steven Karlen,^{1,2} Hoon Kim,^{1,2} John Ralph,^{1,2} Leonardo Sousa,^{1,3} Bruce Dale,^{1,3} Sam Deutsch,⁴ Kai Deng,⁵ Trent Northen,⁵ Seema Singh,⁵ Ken Sale,⁵ Blake Simmons,⁵ Paul Adams,⁵ Michael G. Hahn,⁶ Sivakumar Pattathil,⁶ and **Brian Fox**^{1,2}

¹Great Lakes Bioenergy Research Center (GLBRC), Madison, WI ²University of Wisconsin-Madison, Madison, WI; ³Michigan State University, Lansing, MI; ⁴Joint Genome Institute, Walnut Creek, CA, ⁵Joint Bioenergy Institute, Emeryville, CA, ⁶Bioenergy Science Center, Oak Ridge, TN

<https://www.glbrc.org/research/deconstruction>

Project Goals: GLBRC deconstruction research focuses on basic physical, chemical, and enzymatic strategies to overcome biomass recalcitrance. Carbohydrate-active enzymes are especially promising as powerful, specific, and green catalysts for digestion of plant polysaccharides into valuable sugars for downstream conversion into fuels. With the tools for high-throughput gene and protein synthesis in hand, GLBRC aims to 1) functionally annotate large families of putative carbohydrate-active enzymes, 2) produce high-resolution structural models of the best-performing enzymes, and 3) engineer recombinant variations with improved activity, as well as formulate synergistic combinations of enzymes to accelerate biomass hydrolysis in industrial settings. This work is part of a larger effort shared between GLBRC-supported laboratories and all DOE Bioenergy Research Centers, with the grand challenge of unlocking the renewable carbon reservoir in lignocellulosic biomass.

Overcoming the recalcitrance of lignocellulosic biomass is the primary deconstruction aim of GLBRC. The impetus for this aim is the need for increased yields from plant materials used in the production of bioenergy and renewable carbon fuel. GLBRC approaches this problem from multiple angles simultaneously: genetic engineering of plants with more accessible cell walls, development of chemical pretreatment methods to enable enzymatic digestion of lignocellulosic materials, discovery and development of enzymes with enhanced activity against plant polysaccharides, and design of microbial fermentation processes to convert biomass into valuable chemical commodities.

Within the enzymology sector of deconstruction research, the overall strategy follows a progression of phylogeny to function to structure. By sourcing genomic sequences from databases (such as CAZy) or from metagenomic analyses of diverse microbial communities, high-throughput gene synthesis of interesting carbohydrate-active targets is achieved via

collaboration with the DOE Joint Genome Institute. Cell-free translation reactions allow preparation of microgram amounts of enzyme for initial screening against polysaccharides of different monomeric compositions and chemical linkages. Enzymes with interesting reaction profiles can easily be scaled up to milligram quantity in a cellular expression system, permitting detailed biochemical characterization and crystal structure determination. This approach has been successfully applied to glycoside hydrolase family 55 (GH55) enzymes, of which the laminarinase SactELam55A (whose structure was recently solved) is a member.

A current focus is on subfamily 4 of glycoside hydrolase family 5 (GH5_4), a clade of about two hundred enzymes with broad specificity. Total functional coverage of this group is desired, starting with assignment of cellulase, mannanase, and xylanase activities to each member. In particular, multispecific enzymes with temperature and pH optima that coincide with industrial process conditions are sought, and a number of new thermophilic and acidophilic enzymes have been identified. Fusions of enzymes with carbohydrate-binding modules (CBMs) has been investigated with the well-studied cellulase CelE, and this strategy may also prove useful on newly discovered catalytic domains. Ultimately, novel combinatorial mixtures of enzymes with synergistic activities are tested on pure substrates and on pre-treated biomass samples including genetically-engineered poplar strains with altered lignocellulose structure, which were developed at GLBRC. Glycome profiling and nanostructure-initiator mass spectrometry (NIMS) are used in collaboration with DOE Bioenergy Science Center (BESC) and Joint Bioenergy Institute (JBEI) to identify specific reactions of glycoside hydrolases with polysaccharide epitopes in plant cell walls and to quantify the product distributions and kinetics of these reactions.

Thorough, rational sampling of uncharacterized enzyme space and detailed mechanistic experiments ensure a high probability of adding versatile catalysts to the existing armory of biomass deconstruction tools.

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