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NATIONAL RENEWABLE ENERGY LABORATORY

Defining the Interactions of Cellobiohydrolase with Substrate through Structure Function Studies

Cooperative Research and Development Final Report

CRADA Number: CRD-10-409

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In accordance with Requirements set forth in Article XI.A(3) of the CRADA document, this document is the final CRADA report, including a list of Subject Inventions, to be forwarded to the Office of Science and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

CRADA Number: CRD-10-409

CRADA Title: Defining the Interactions of Cellobiohydrolase with Substrate through Structure Function Studies

Parties to the Agreement: Danisco US, Inc.

Joint Work Statement Funding Table showing DOE commitment:

Estimated Costs	NREL Shared Resources
Phase 2	$ 2,361,330.00
Equipment In-Kind	$ 564,864.00
TOTALS	$ 2,926,194.00

Abstract of CRADA work:

NREL researchers will use their expertise and skilled resources in numerical computational modeling to generate structure-function relationships for improved cellulase variant enzymes to support the development of cellulases with improved performance in biomass conversion.

Summary of Research Results:

The CRADA between Danisco (now DuPont Industrial Biosciences) and NREL focused on applications of computational science at NREL to suggest mutations for improving stability and activity of glycoside hydrolase Family 6 (GH6) cellobiohydrolases as well as computational studies aimed at elucidating the mechanisms of this important class of cellulases. We used bioinformatics, Rosetta Design, and multiple molecular dynamics simulation-based methods to suggest various methods to improve GH6 activity. These recommendations for improvements to GH6 activity and stability were provided to our CRADA partner, Danisco, for experimental testing and these experiments are ongoing. In addition, this work encompassed a large set of combined computational and experimental work to examine the Cel6A carbohydrate-binding module and linker binding affinity to cellulose, to survey Cel6A natural diversity, and to examine the viability of domain swapping as a potential means to elucidate the role of the CBM, linker, and CD in catalysis with the aim to design enhanced activity variants of Cel6A. Additionally, we examined in detail the product inhibition mechanisms in the commonly studied *Trichoderma reesei* Cel6A and *Thermobifida fusca* Cel6B enzymes to understand why cellobiose inhibits these enzymes

differently with the aim to more generally understand the important phenomenon in GH Family 6 enzymes. Additionally, we conducted a detailed study on the effects of pH on *T. reesei* Cel6A stability and structure. This study revealed key aspects of pH dependence on GH6 cellulases, which is important for understanding how to engineer these critical enzymes for processes that occur in different pH ranges than the natural enzymes. Overall, this work has resulted in a number of manuscripts published or currently in production that have elucidated multiple features of GH6 activity, structure, and function that resulted in an improved ability to engineer this key cellulase enzyme, which is a primary component of enzyme cocktails for economical production of biofuels in a biochemical conversion process. This learning was passed to Genencor throughout the duration of the CRADA, and experiments based on these computational findings are ongoing.

Subject Inventions Listing: N/A

Report Date: 5/12/2013

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