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U.S. Department of Transportation

SunGrant INITIATIVE South Central Region

Project Title: *Structure Dynamics-Guided Biocatalyst Improvement*

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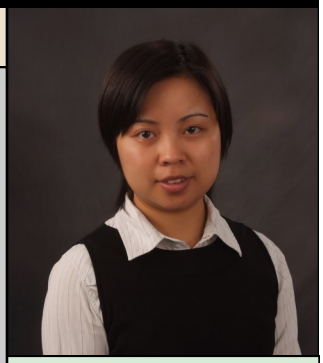

Project Goals

The project aimed to develop a novel structure dynamics-guided platform for biocatalyst improvement and employ the platform to improve the biocatalysts like cellulases for higher efficiency and better inhibitor resistance. The ultimate goal for this research is to produce the cost-effective biocatalysts with higher efficiency, more inhibitor resistance, improved thermostability, better substrate specificity, enhanced acidic tolerance, and superior compatibility for expression in different systems. The biocatalyst improvement will help to improve the biomass conversion efficiency and revolutionize the biorefinery procedures. The two major objectives are: I) Comparative structure dynamics profiling for three cellulases upon binding with different substrates and inhibitors and II) Developing improved cellulases based on the structure dynamics analysis.

Project Outcomes

- Performed the HDX analysis of both CBHI and EGI enzymes and applied the HDX analysis to xylanase enzymes. The HDX mass spectrometry data was combined with comparative genome analysis and identified important regions for enzyme functions. The novel approach will enable the finding of new important regions for enzyme improvement and proprietary enzyme improvement strategies.
- Successfully engineered a *T. reesei* xylanase to allow the expression in *E. coli* to reach similar activity as in *T. reesei.* Traditionally, the expression of *T. reesei* enzymes in *E. coli* and yeast resulted in extremely low activity (1/30th). The xylanase expressed in *E .coli* showed much lower activity than the wild type xylanase expressed in *T. reesei*. However, with the mutant that was engineered, SprF xylanase expressed in *E .coli* showed comparable activity to that of wild type enzyme expressed in *T. reesei* at 50 degree. The activity is also retained at higher temperature of 70 degree. These results have overcome the problem and enabled the T. reesei and other fungal enzymes to be used for en-

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