



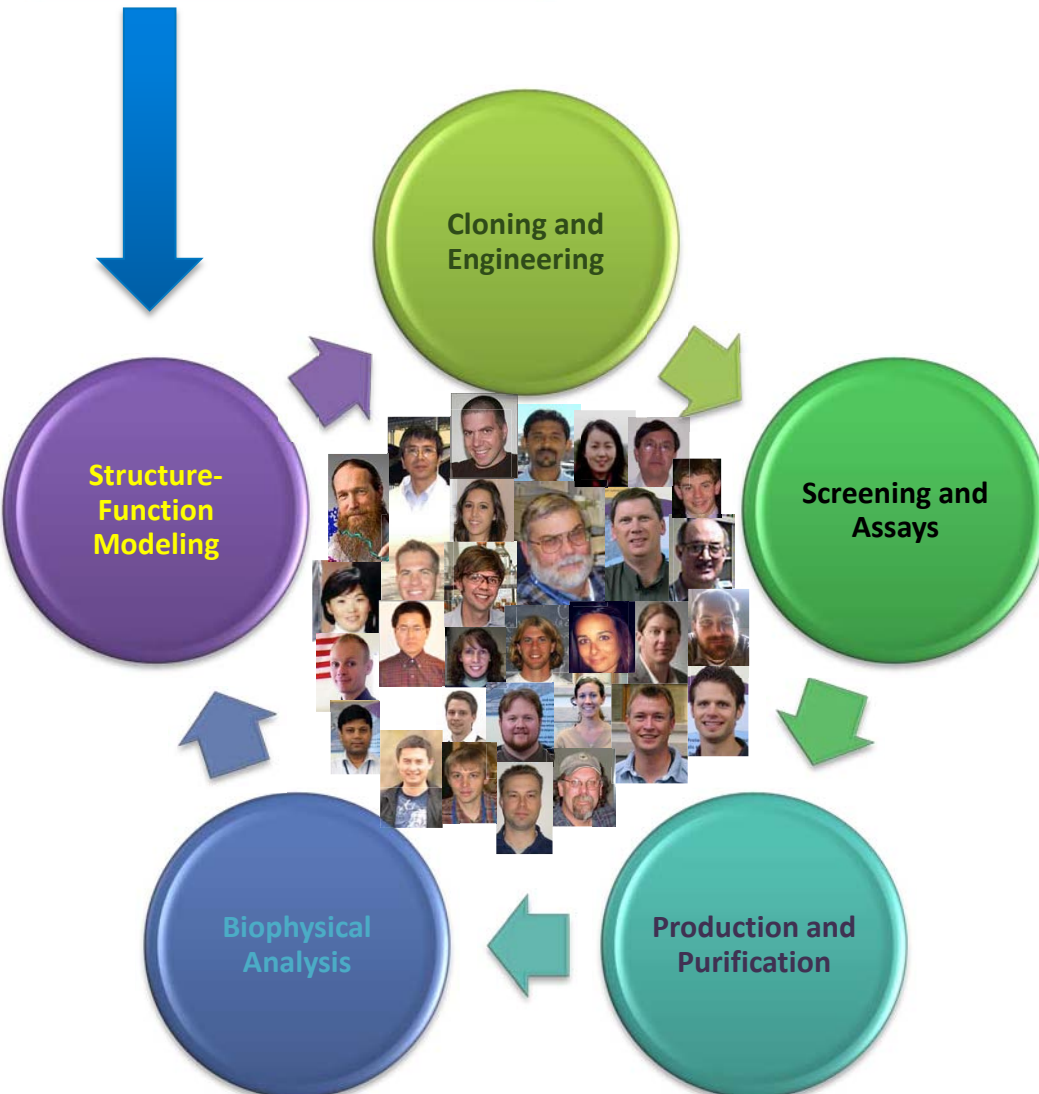
NREL Cell free enzyme capabilities


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July 30, 2017

Enzyme Engineering at NREL

Identify Needed Activity

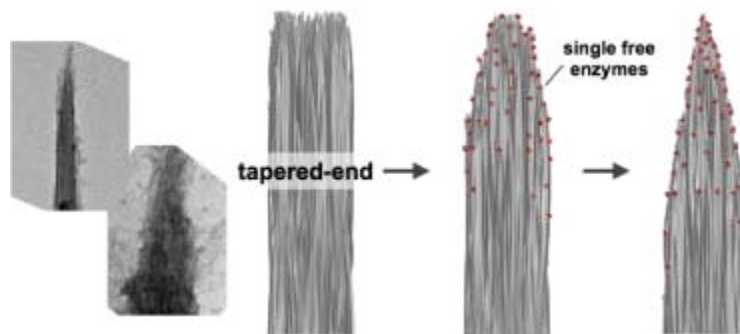
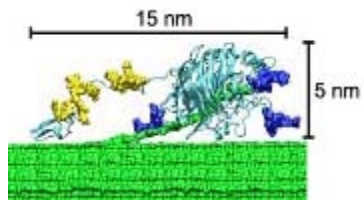


- **Glycosidase Hydrolase Systems**
 - Fungal- cellulases, hemicellulases, accessory enzymes
 - Bacterial- thermo/caldiphilic cellulases, multi-functionals, cellulosomes
 - Niche-enzyme- rumen, aquatic crustaceans, social amoebae
 - **Natural diversity cellulase screening**
 - Enabled through JGI sequencing
 - Learn and Apply approach
 - International collaborations
- 
- Fungi, yeast, crustaceans, protozoa, social amoebae, bacteria.....
 - **Other Biomass-Active Enzymes**
 - LPMOs, peroxidases, laccases, lyases, CDH
 - **Metabolic Enzymes**
 - Carboxylic acid reductases, transporters, synthases, dehydrogenases



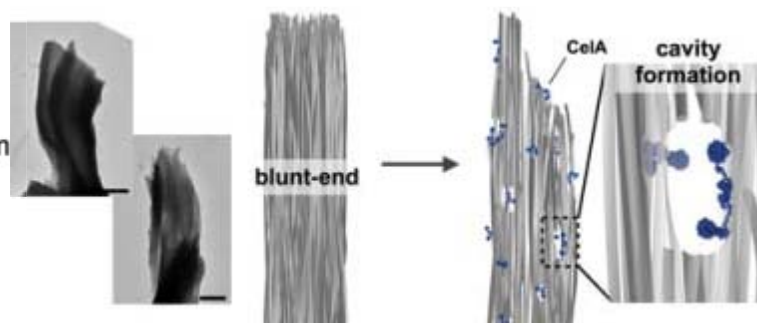
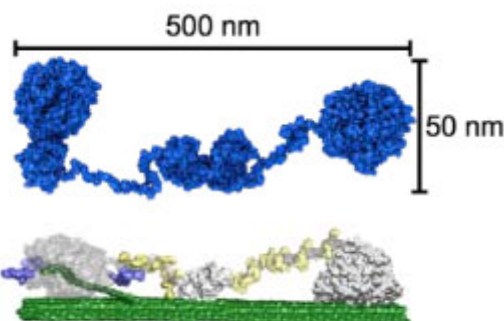
Tunable biocatalysts for biomass conversion

Free Enzymes



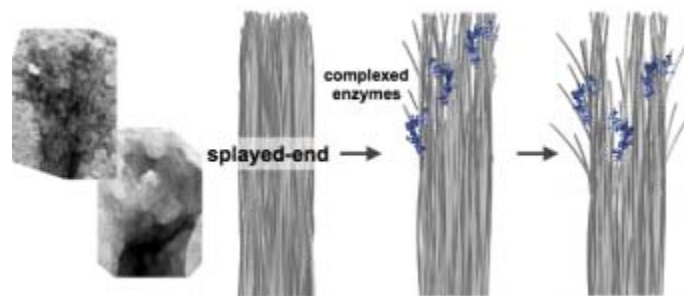
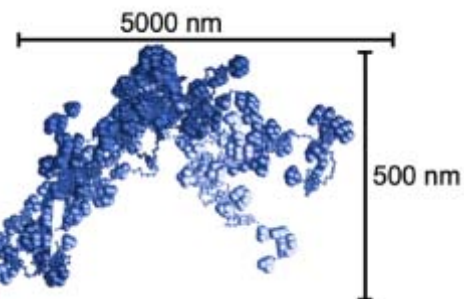
Free fungal enzymes ablate only surfaces of cellulose bundles

Multifunctional Enzymes



Bacterial CelA and synthetic fungal multifunctional enzymes excavates cavities into particle surfaces, fragment and fibrillate avicel and create nano-crystals - > materials

Cellulosomal Enzymes



Bacterial cellulosomes divide cellulose bundles, increasing accessible surface area

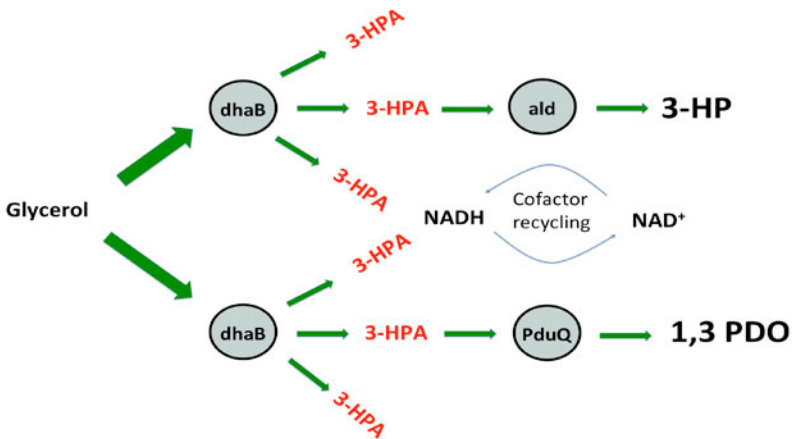
Resch, M. G., B. S. Donohoe, J. O. Baker, S. R. Decker, E. A. Bayer, G. T. Beckham, and M. E. Himmel. (2013). Energy Environ. Sci. 6:1858–1867.

Synthetic Proteomes for the Production of BioProducts

Channeling of metabolites between enzymes and increased stability with cofactor recycling. Plug and play concept that can be used as a robust platform to screen through enzymes from different microorganisms.

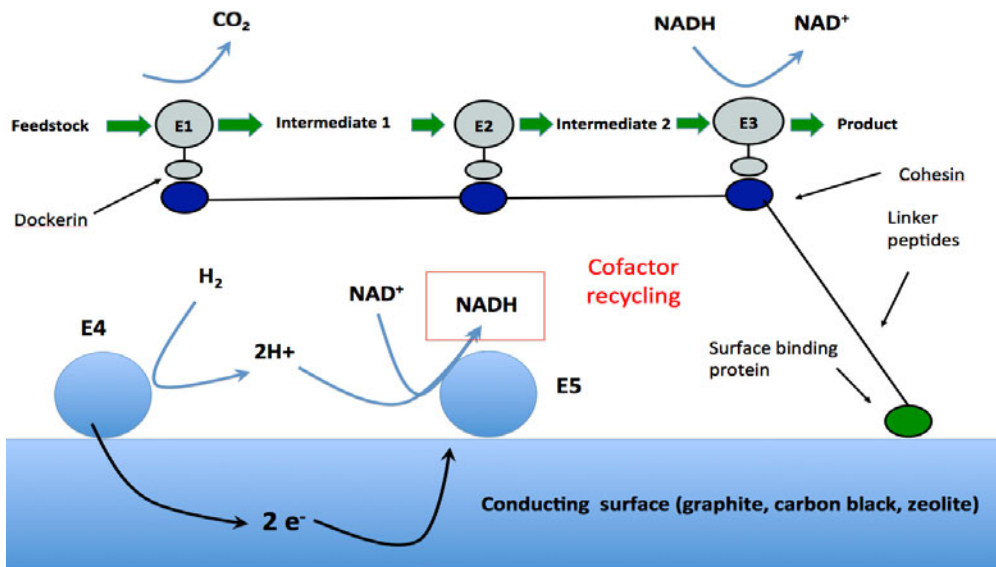
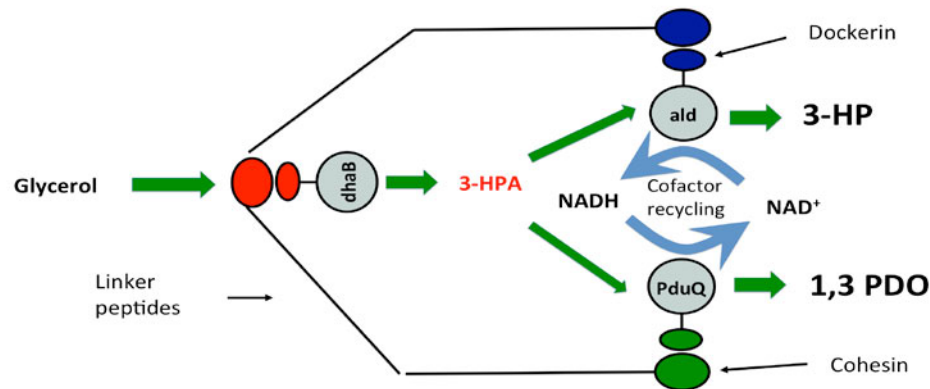
Free Metabolic Pathway

Loss of metabolites due to free diffusion
lack of enzyme stability/ accumulation of toxic intermediates



New Concept: Tethered Metabolic Pathway

Further reduction of toxicity
increased stability / better cofactor recycling



System can be generalized to more complex pathway with adaptor scaffolds.

The concept can be further exploited to allow tethering to surfaces therefore further enhancing stability and recycling.

Surface tethering allows for other types of cofactor recycling strategies.

Thank You!

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