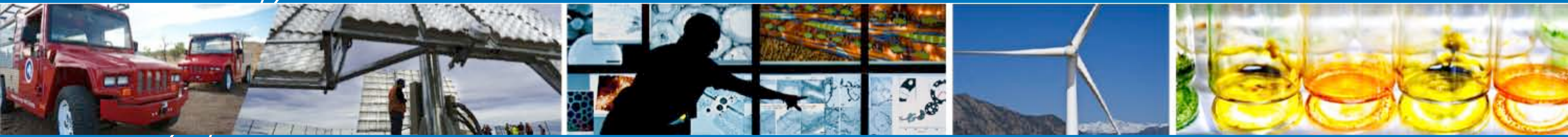
DOE Bioenergy Technologies Office (BETO)
2015 Project Peer Review

Biochemical Process Modeling and Simulation



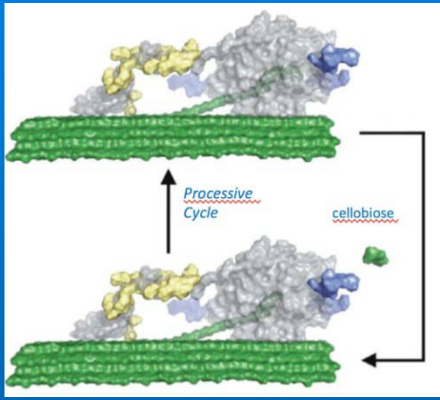
25 March 2015
Biochemical Platform

Michael Crowley
NREL

This presentation does not contain any proprietary, confidential, or otherwise restricted information

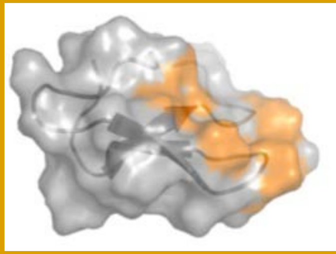
Goal Statement

Understand relevant processes at Molecular Level



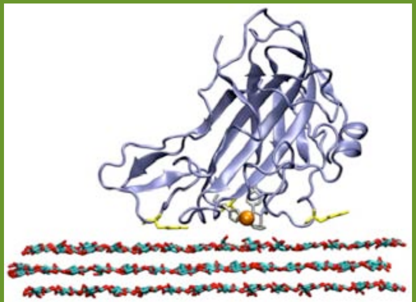
Cellulase Action

Predict improved enzymes, pathways, and process parameters.



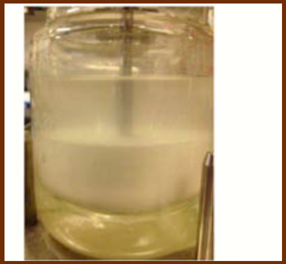
Lignin Binding

Test and select best hypotheses from experiment



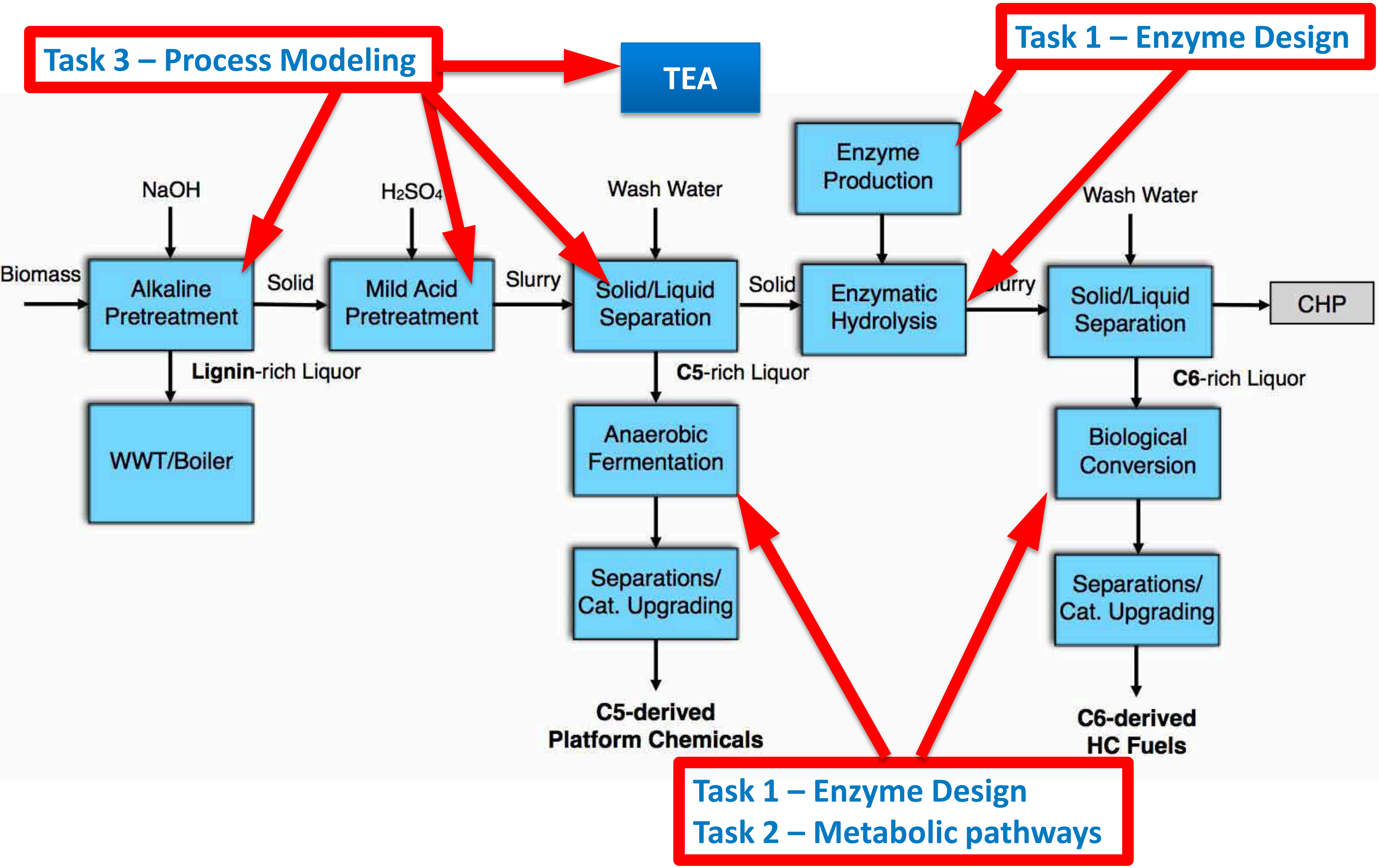
LPMO mechanism

Streamline path to improved biofuel processes

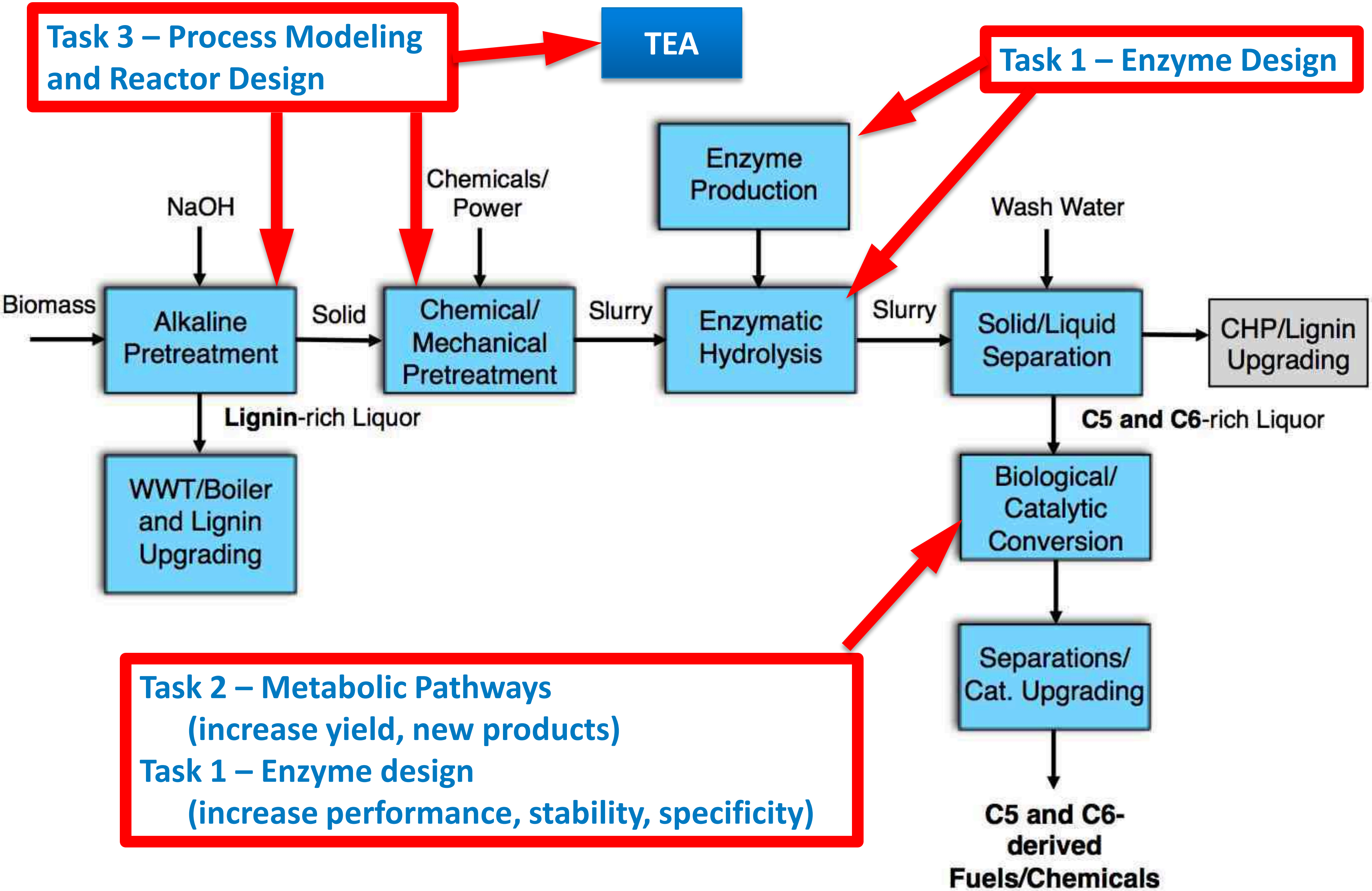


Reactor Design

Project Context in 2017 Process Target



Project Context in 2022 Process Target



Quad Chart Overview: Project 2.5.1.100

Timeline

Project start	FY14-Q1
Project end	FY17-Q4
Percent complete	31%

Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date)
DOE Funded	NA	NA	1,593K	5,362K

Barriers

(associated task)

Bt-B. Biomass and Feedstock Recalcitrance (1,3)

Bt-C. Reactor Feed Introduction (3)

Bt-D. Pretreatment Processing and Selectivity (3)

Bt-E. Reactor Design and Optimization (3)

Bt-F. Hydrolytic Enzyme Production (1,2)

Bt-G. Enzyme Efficiency (1,2)

Bt-I. Catalyst Efficiency (1,2)

Partners

- **Internal Partners**
  - 2.5.4.100 Enzyme Engineering and Optimization
  - 2.3.2.105 Biological Upgrading of Sugars
  - 2.4.3.102 Targeted Microbial Development
  - 2.2.3.100 Pretreatment and Process Hydrolysis
  - 2.1.0.100 Biochemical Platform Analysis

Other Collaborations

UCSD/SD Supercomputer Center
U. Kentucky
ORNL
Swedish Univ. Agricult. Sciences
University of Portsmouth, UK
University of York, UK
Purdue
UC Berkeley
Norwegian U. Life Sciences
University of Tokyo
University of Oxford, UK

NSF Xsede Computer Resource
U. Colorado, Boulder
U. Colorado,Denver
U. Virginia
Vanderbilt University
Univ. South Florida
Penn State University
University of Michigan

1 - Project Overview

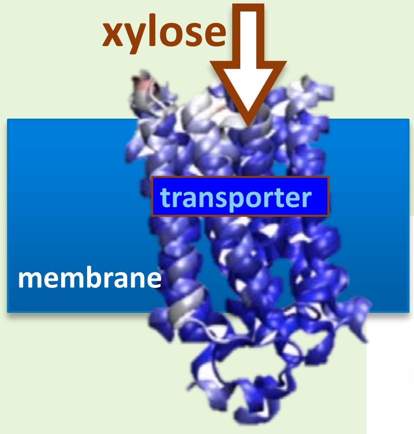
Objectives:

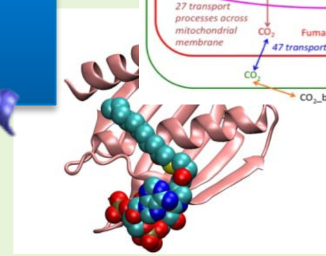
Improve enzymes,

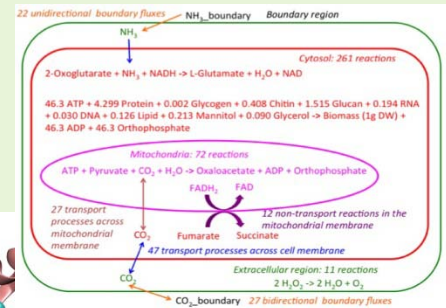
metabolic pathways,

and industrial processes for biofuel production.

Contribute to achieving 2017 and 2022 targets







History:

FY13 Subtasks to Targeted Conversion Research Task (Himmel) and Chemical Conversion (Johnson).

FY14 split off into independent Project with two original tasks and an additional metabolic modeling task.

Context:

Aligned with multiple projects

- 2.5.4.100 Enzyme Engineering and Optimization
- 2.3.2.105 Biological Upgrading of Sugars
- 2.4.3.102 Targeted Microbial Development
- 2.2.3.100 Pretreatment and Process Hydrolysis
- 2.1.0.100 Biochemical Platform Analysis

2 – Approach (Technical)

- *Approach:*
  - ***Complement Experiment and Measurement** with **Theory, Simulation and Modeling.***
  - *Strong communication between experimental and modeling efforts*
  - *Target worst bottlenecks in processes.*
- *Objective:*
  - *Gain insight*
  - *Guide experiment and design*
  - *Increase efficiency*
- *Success Factors:*
  - *Insights*
  - *Reduced time to solution: increasing titer, efficiency, speed*
  - *Reduced cost of biofuels*
  - *New routes to advanced fuels*
- *Go/No-Go Decisions*
  - *Deadlines: If predictions for improvement cannot be delivered in time for Targets, research efforts are terminated and redirected to achievable goals.*
  - *Success of predictions: If predictions do not result in improvement, approach is abandoned for better approaches.*

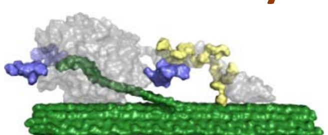
2 – Approach (Management)

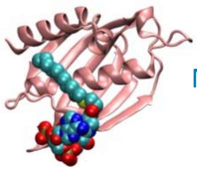
Project is split into tasks according to type of modeling and managed by person with appropriate expertise.

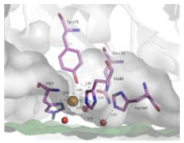
Project

Biochemical Process Modeling and Simulation
M. Crowley

[Task 1] Mechanisms and Enhancement of enzymes. M. Crowley


Structure/function

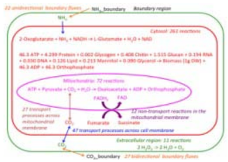

Specificity

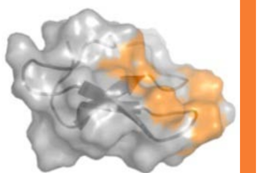

Mechanism/barriers

Molecular Dynamics
Quantum Mechanics

[Task 2] Metabolic Modeling and Pathway Engineering Y. Bomble

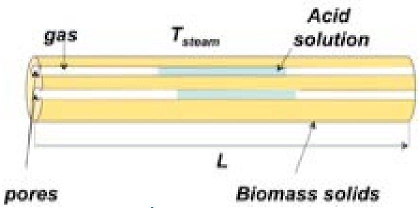
Metabolic Pathway
Flux analysis
Kinetic modeling
Sequence Analysis
Rosetta Design


Pathway modification

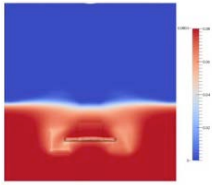

Sequence modification

[Task 3] Mechanistic Process Modeling J. Stickel

Reaction-Diffusion Modeling
Coupled CFD/Rxn-Diffusion
Multi-scale Modeling

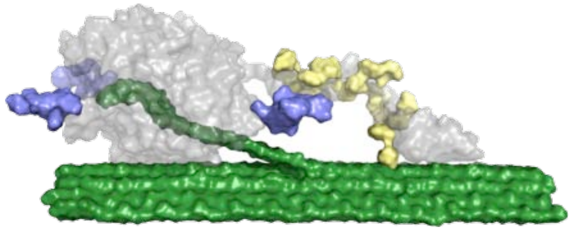


Reaction/diffusion process tuning


Reactor Design

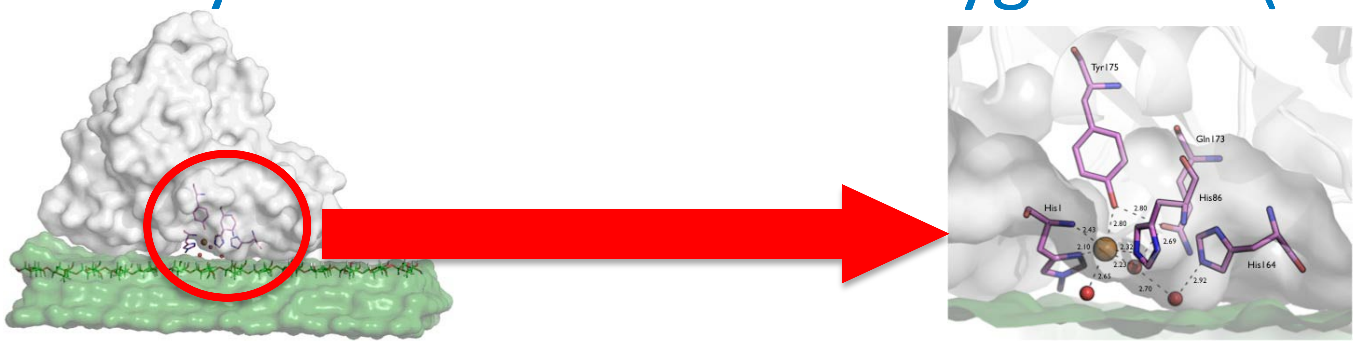
3 – Technical Accomplishments Task 1

- Cel7A Reaction and Processivity Mechanism



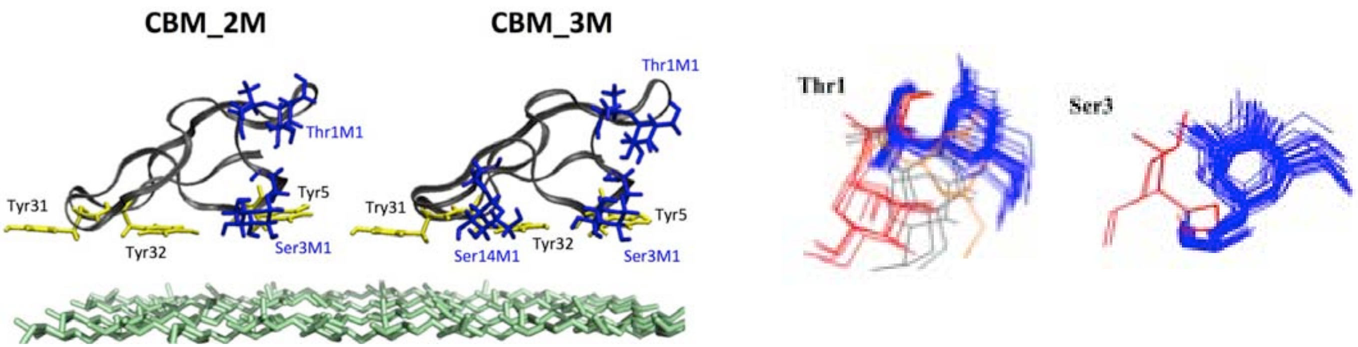
Knott, et al., JACS 2014
Knott, et al., JACS 2013

- Lytic Polysaccharide Mono-Oxygenase (LPMO)



Kim, et al., PNAS 2014

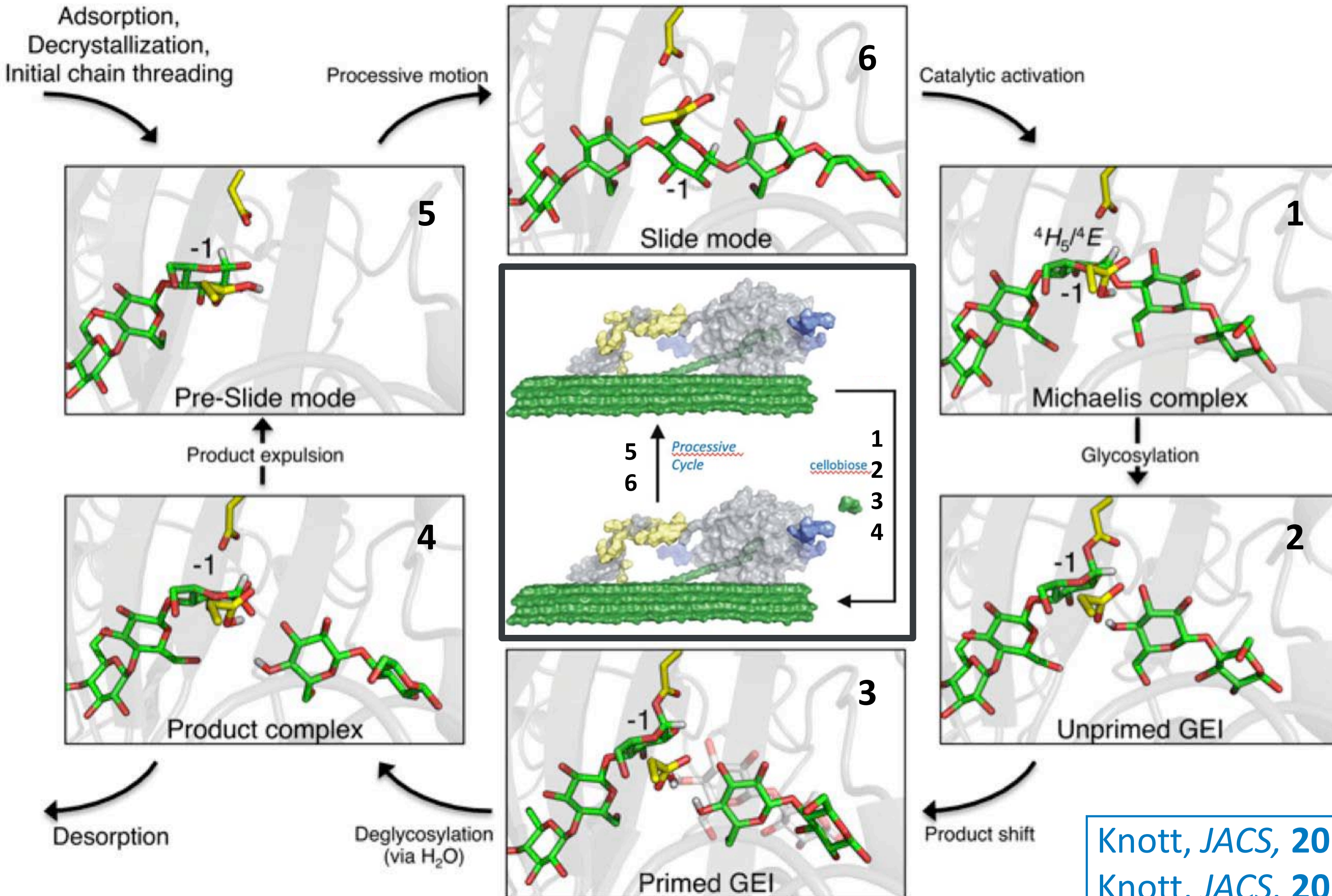
- Glycosylation and CBM NMR structure



Chen, et al. PNAS 2014
Happs, et al., In prep for JBC 2015

Cel7A Processive Mechanism

FY14 Completed Milestone



Knott, *JACS,* **2014**
Knott, *JACS,* **2013**
Beckham, JBC, 2014

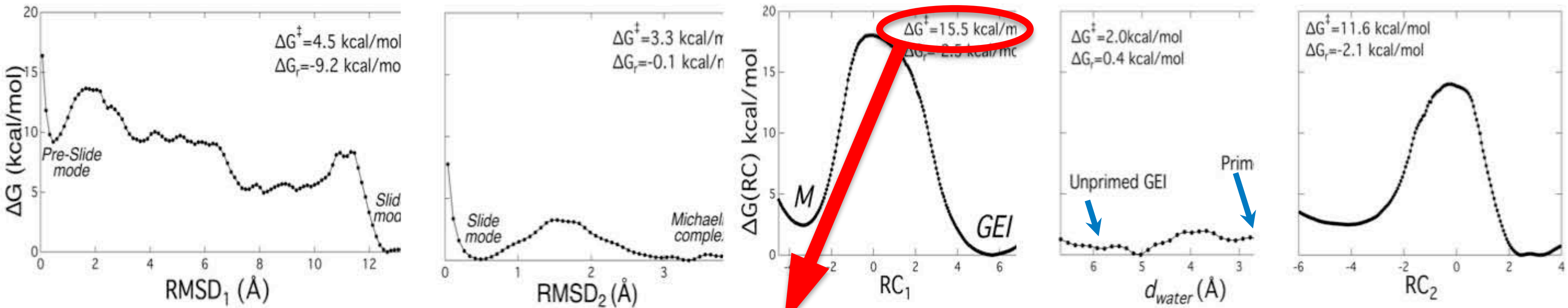
Rate-Limiting Step Determined

FY14 Completed Milestone

Processive motion is NOT rate-limiting.

Glycosylation step is the rate-limiting step.

Shift and Deglycosylation are NOT rate-limiting.



15.5 kcal/mol

Transition state theory (TST)

$$k=(k_B T/h)(\kappa)(\exp(-\Delta G^{\ddagger})/k_B T)$$

$k = 10.8\ s^{-1}$

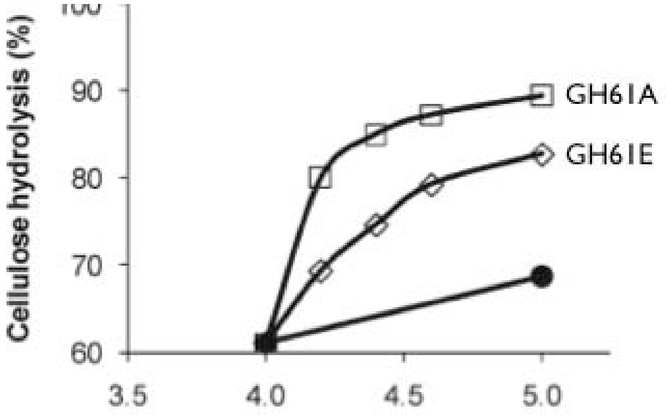
$k = 7.1 \pm 3.9\ s^{-1}$

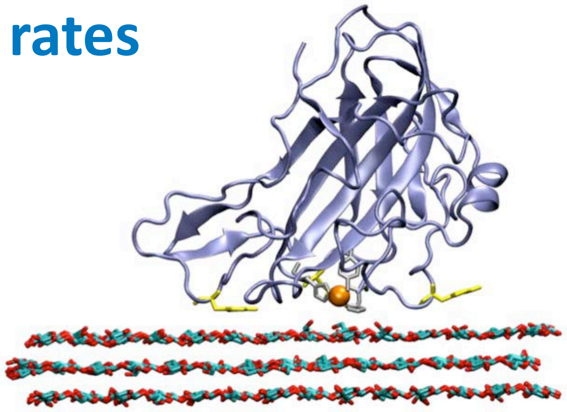
3 – Tech Accomp Task 1: LPMO/GH61

FY14 Completed Milestone

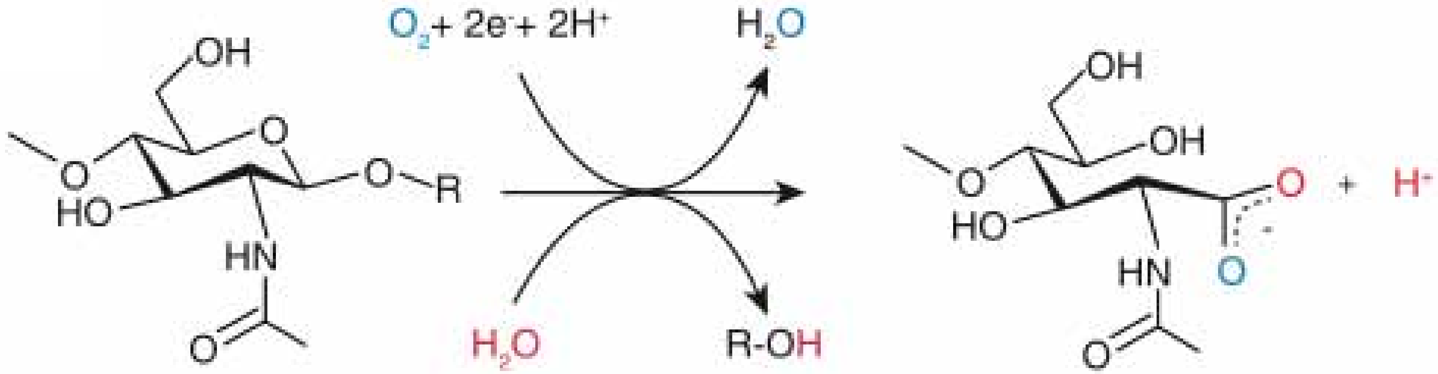
Lytic Polysaccharide Mono Oxygenase (LPMO)   GH61 is a LPMO

Has HUGE effect on hydrolysis rates





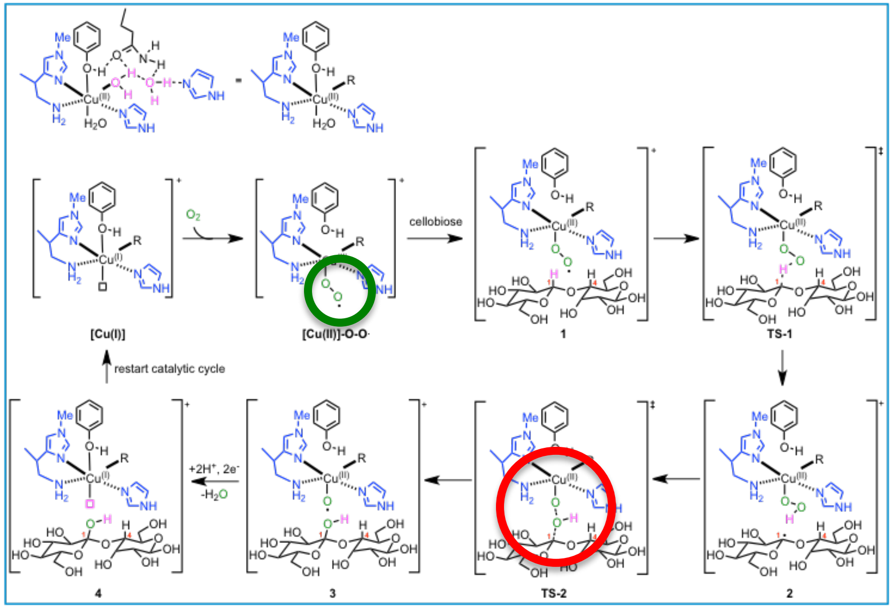
M. Wu, et al, *JBC* **2013**



3 – Tech Accomp Task 1: LPMO/GH61

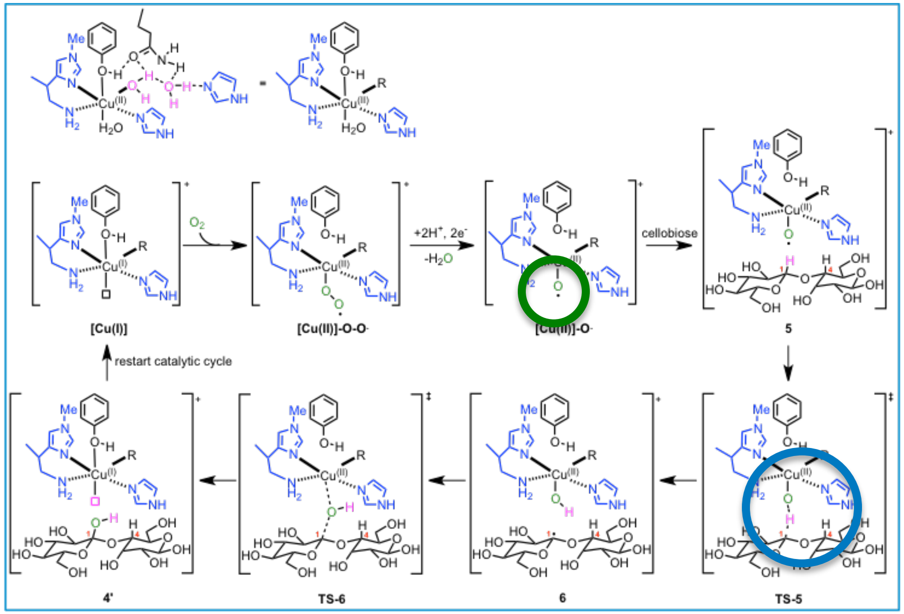
FY14 Completed Milestone

Proposed Mechanism 1



47.5 kcal/mol Barrier

Proposed Mechanism 2



15.9 kcal/mol Barrier

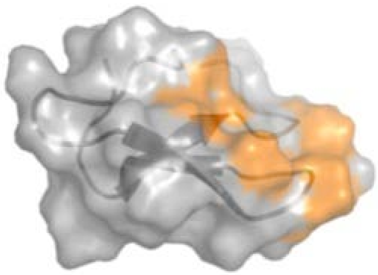
Kim, et al., **PNAS, 2014**

Significance of Accomplishments Task 1

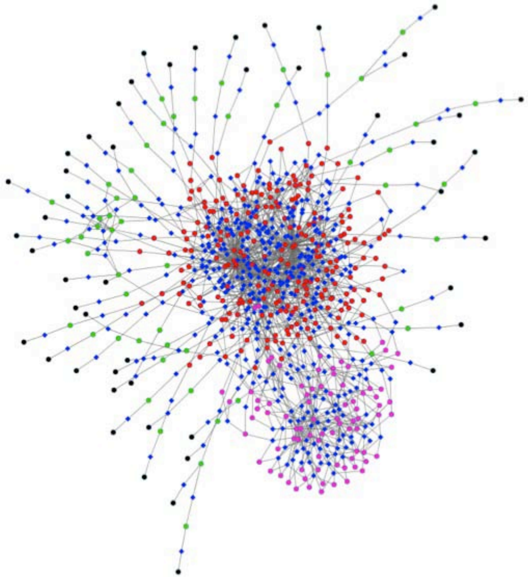
- Mechanisms
  - **Understand** the molecular mechanism of action
  - **Know what the barriers are**
  - **Eliminate incorrect mechanisms**
  - Correctly estimated the speed of action of Cel7A
  - Have applied these methods to other cocktail cellulases
  - Contributed to design of improved cellulases (2.5.4.100)
- Other Cellulase accomplishments
  - CBM-1 glycosylated **NMR structures solved**
  - Binding function of Linker correctly **predicted**
  - Flexibility of linker predicted
  - Huge binding function of CBM glycosylation provides new **design principle: Glycosylation** (not just AA sequence).
- **Insight and Understanding Achieved**
- **Prediction of properties**
- **Design Principles**

3 – Technical Accomplishments Task 2

- Lignin binding of Cellulases



- Metabolic model for *T. reesei*

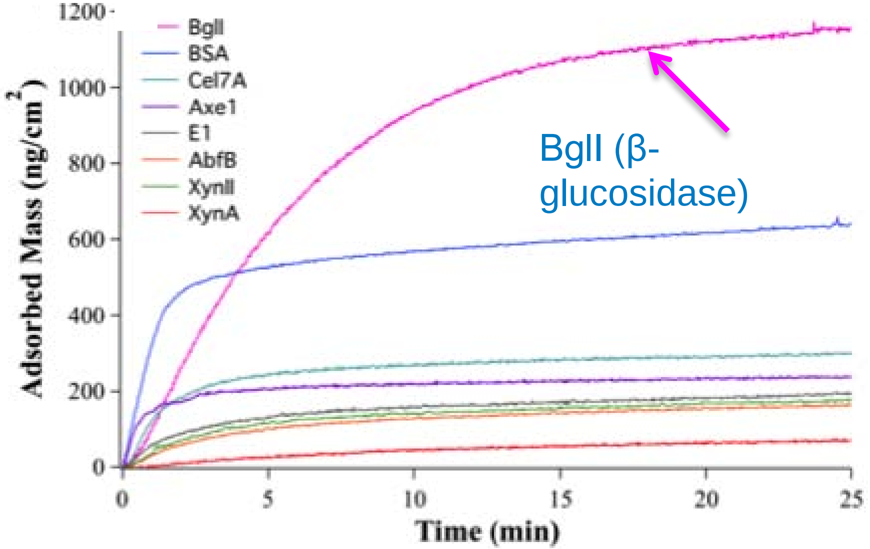


Task 2 Cellulase Binding to Lignin

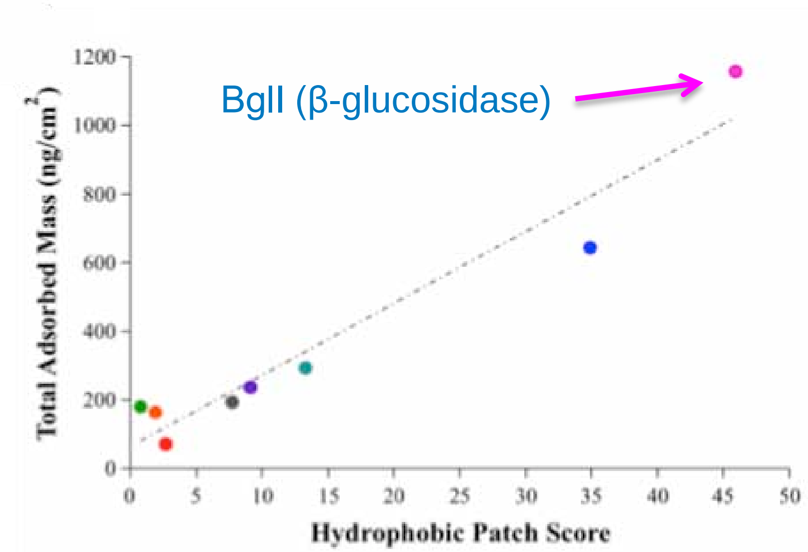
FY14 Completed Joint Milestone

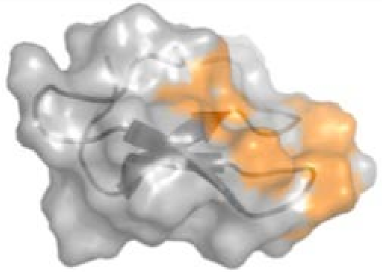
Hydrophobic patch scores correlate with enzyme adsorption

Mass Adsorbed in Quartz-Crystal MicroBalance



Protein Adsorbed vs. Hydrophobic Patch Score





Exposed hydrophobic patch

Big Patch = High Score
Small Patch = Low Score

Sammond, et al *JBC* **2014**

Significance

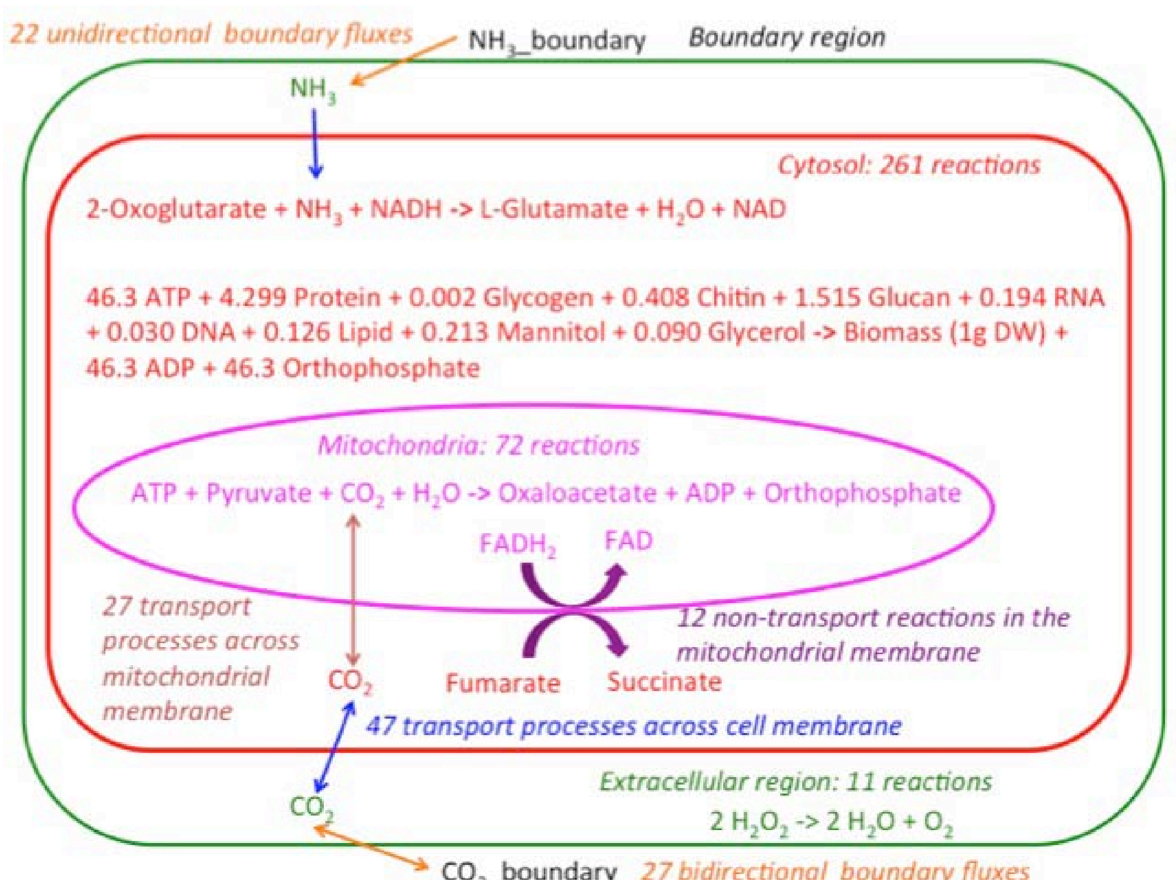
- Bg-I is the predominant enzyme binding to lignin
- Identifies enzymes most susceptible to lignin binding
- Design tool for decreasing lignin binding.

Reduce Bg-I binding to improve enzyme performance in current cocktails

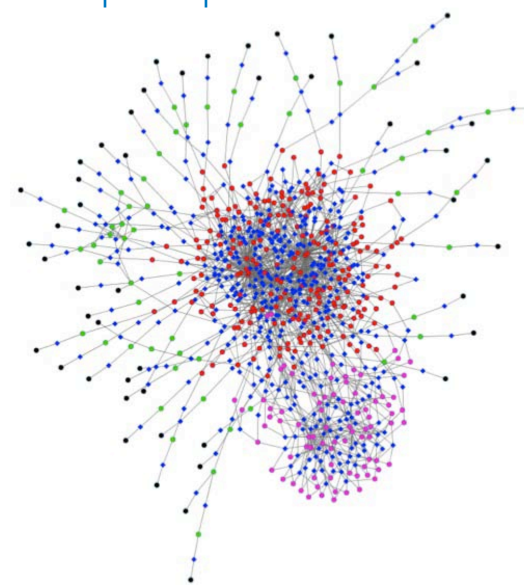
Task 2: Metabolic model of *T. reesei*

FY14 Completed Milestone

- ***Trichoderma reesei:*** Important industrial enzyme source
- **First central carbon metabolic model for *T. reesei***
- Model for optimizing growth and increasing product yields

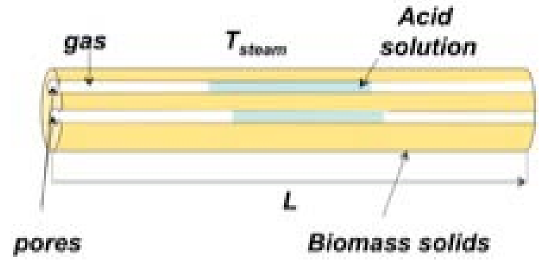


4	Compartments
413	Metabolites (species)
479	Reactions
75	Pathways
258	Enzymatic activities

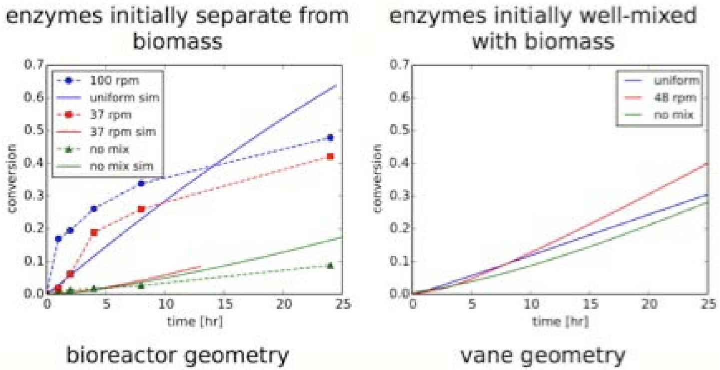


3 – Technical Accomplishments Task 3

- Multi-physics modeling of dilute-acid pretreatment (in subsequent slide)



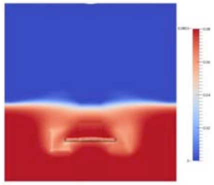
- Population-balance modeling of enzymatic hydrolysis kinetics



Predictive for digestion of different mixtures of crystallinity

- Coupled CFD and kinetics of enzymatic hydrolysis

Predictive of reactor performance for different configurations, initial conditions, etc.

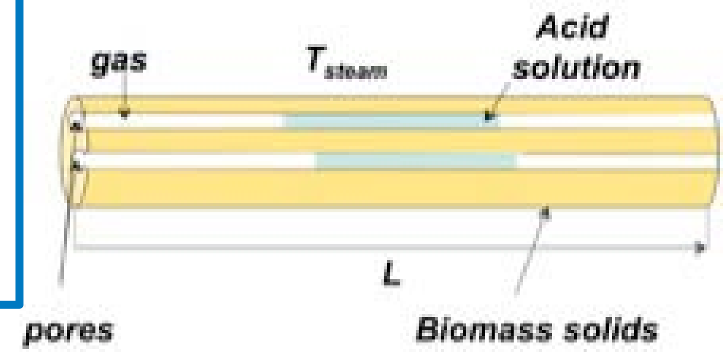


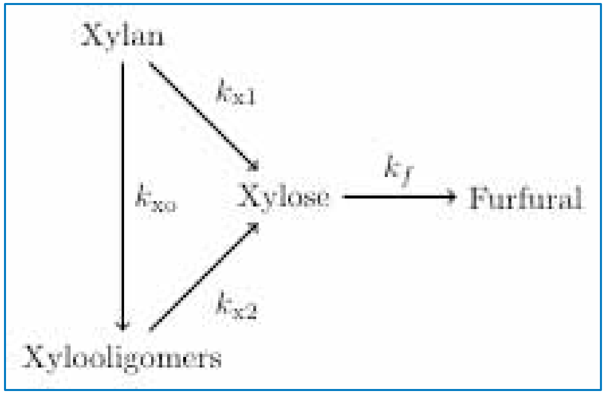
Task 3 : Multi-physics modeling of dilute-acid pretreatment

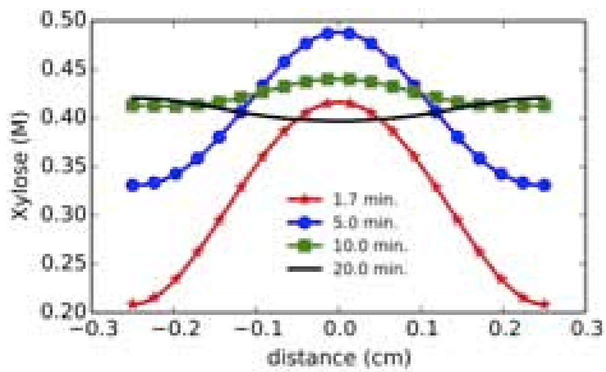
FY14 Completed Milestone

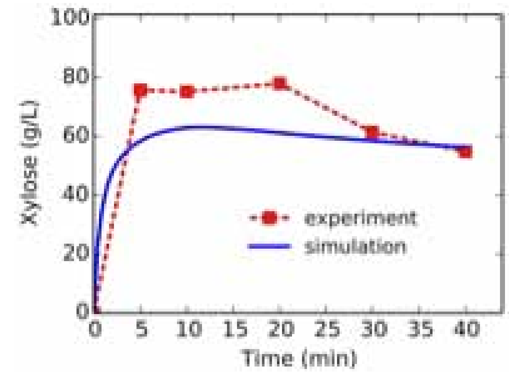
High-Solids Pretreatment/particles with voids

- Coupled reaction kinetics, steam-water phase transition, and mass and energy transport of Particles contain solid, liquid, and gas phases
- Substrate and Product profiles are predicted
- Simulated product yield match experimental data









Predicts effects on pretreatment due to:

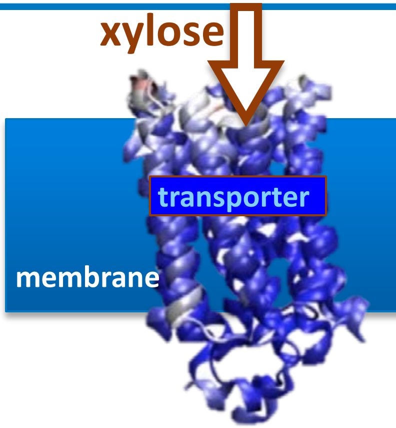
- **initial *water content***
- **time**
- **temperature**
- **acid loading on pretreatment**

Sitaraman, et al., *Chemical Engineering Journal,* **2015**

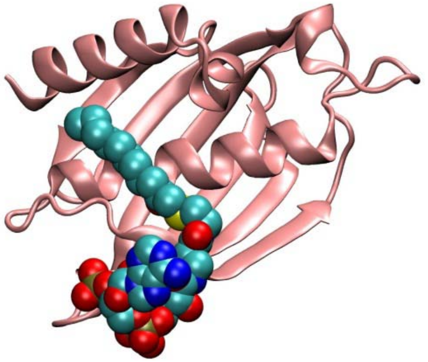
4 – Relevance

- **26 publications** in high-impact journals (PNAS, JACS, JBC, …)
- New **Insight** and **Understanding:** enzymes, metabolism, processes.
- **Higher performing cellulases** - collaboration with Enzyme Design project (Himmel)
- **New methods of enzyme design for current and future targets**.
- FY15/16/17 Milestones to design non-glucose-inhibited sugar transporters, the **2022 mixed-stream C5/C6 biological upgrading becomes feasible**.
- Thioesterase selectivity (Milestone) will add the ability to selectively produce **hydrocarbons of desired length** at the biological sugar-upgrading stage (2017 and 2022).
- **Metabolic models** to produce **high-valued coproducts** for 2017.
- Reactor design principles and models provide more accurate estimates for costs in TEA analysis
- **Reactor models for aerobic fermentation** can be the determining factor for the feasibility of full-scale aerobic industrial processes.

5 – Future Work (3 Examples)



- ***Xylose Transporter Design:*** Understand and eliminate C6 inhibition in collaboration with "2.4.3.102 Targeted Microbial Development" to **enable mixed-stream upgrading of sugars**.



- ***Hydrocarbon enzyme design*** *(Go/No-Go 9/03/2015):* Enhanced hydrocarbon production and selectivity by thioesterase and decarboxylase design as part of metabolic pathways in an industrially relevant organisms.

- ***Aerobic Fermentation CFD modeling*** *(Go/No-Go 3/31/2016):* A validated model of aerobic fermentation at scale for improved reactor design and inclusion in TEA analysis.

Summary

- Addressing 7 of the 11 Barriers in the 2014 MYPP
- Actively working toward 2017 and 2022 Goals
- Added new insights and understanding to complex processes
- Large collaborative effort internal to NREL and external
- Large publication record in high-profile journals

Acknowledgements

NREL Team
Michael Crowley lead Task 1
Yannick Bomble lead Task 2
Jonathan Stickel lead Task 3
Gregg Beckham Cellulase Superstar
Brandon Knott Cellulase Mechanism
Seonah Kim LPMO Mechanism (QM)
Christina Payne Enzyme Thermodynamics
Lintao Bu Cellulase Studies
Deanne Sammond Lignin Binding, Rosetta
Heather Mayes Sugar Conformations (QM)
Laura Berstis QM, Electron Transfer
Antti-Pekka Hynninen HPC performance
Renee Happs CBM structure (NMR)

NREL Experimental
Michael Himmel, Steven Decker, Michael Resch, Michael Guarnieri, Jeff Linger

Resources
NREL Peregrine Compute Resource
NSF XSEDE Computer Resource

Collaborators:
Jerry Stahlberg, Mats Sandgren
John McGeehan
Vincent Eijsink
Charles Brooks III
Michael Shirts
Zhongping Tan
Andreas Goetz
Courtney Taylor
Claire McCabe
Jhih-Wei Chu

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Publications 2014-2015

1. C. M. Payne *et al.*, Fungal Cellulases. *Chemical Reviews* **115**, 1308-1448 (2015).
2. H. Sitaraman *et al.*, Multiphysics modeling and simulation of high-solids dilute-acid pretreatment of corn stover in a steam-explosion reactor. *Chemical Engineering Journal* **268**, 47-59 (2015).
3. P. N. Ciesielski *et al.*, Biomass Particle Models with Realistic Morphology and Resolved Microstructure for Simulations of Intraparticle Transport Phenomena. *Energy & Fuels* **29**, 242-254 (2015).
4. S. Kim, J. Ståhlberg, M. Sandgren, R. S. Paton, G. T. Beckham, Quantum mechanical calculations suggest that lytic polysaccharide monooxygenases use a copper-oxyl, oxygen-rebound mechanism. *Proceedings of the National Academy of Sciences* **111**, 149-154 (2014).
5. L. Chen *et al.*, Specificity of O-glycosylation in enhancing the stability and cellulose binding affinity of Family 1 carbohydrate-binding modules. *Proceedings of the National Academy of Sciences* **111**, 7612-7617 (2014).
6. H. B. Mayes, L. J. Broadbelt, G. T. Beckham, How sugars pucker: electronic structure calculations map the kinetic landscape of five biologically paramount monosaccharides and their implications for enzymatic catalysis. *Journal of the American Chemical Society* **136**, 1008-1022 (2014).
7. B. C. Knott, M. F. Crowley, M. E. Himmel, J. Ståhlberg, G. T. Beckham, Carbohydrate–protein interactions that drive processive polysaccharide translocation in enzymes revealed from a computational study of cellobiohydrolase processivity. *Journal of the American Chemical Society* **136**, 8810-8819 (2014).
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9. T. Ito *et al.*, Crystal structure of glycoside hydrolase family 127 β-l-arabinofuranosidase from Bifidobacterium longum. *Biochemical and biophysical research communications* **447**, 32-37 (2014).
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