

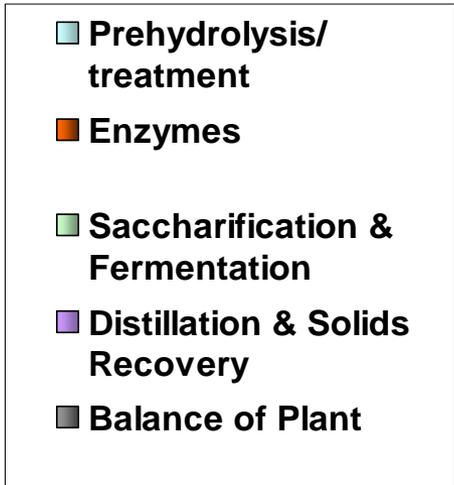
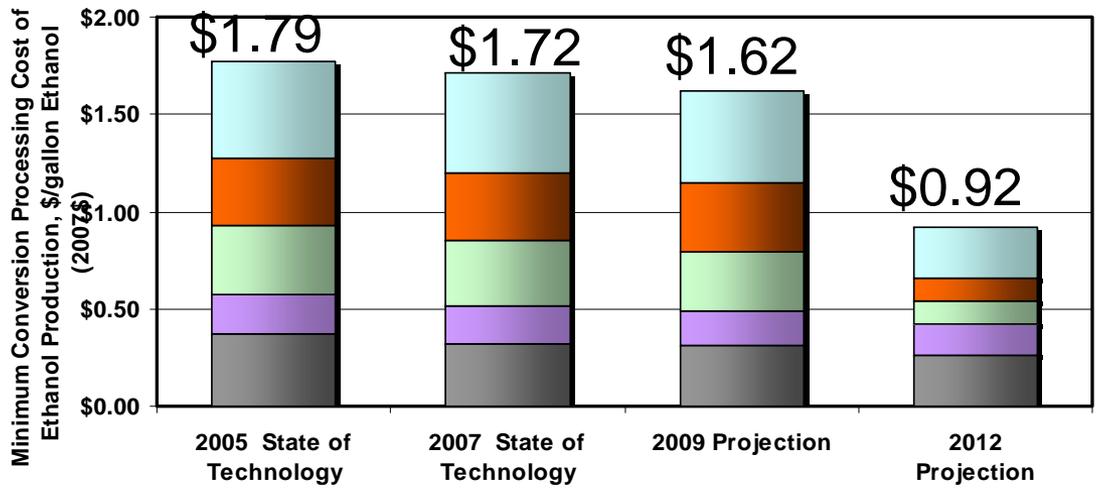
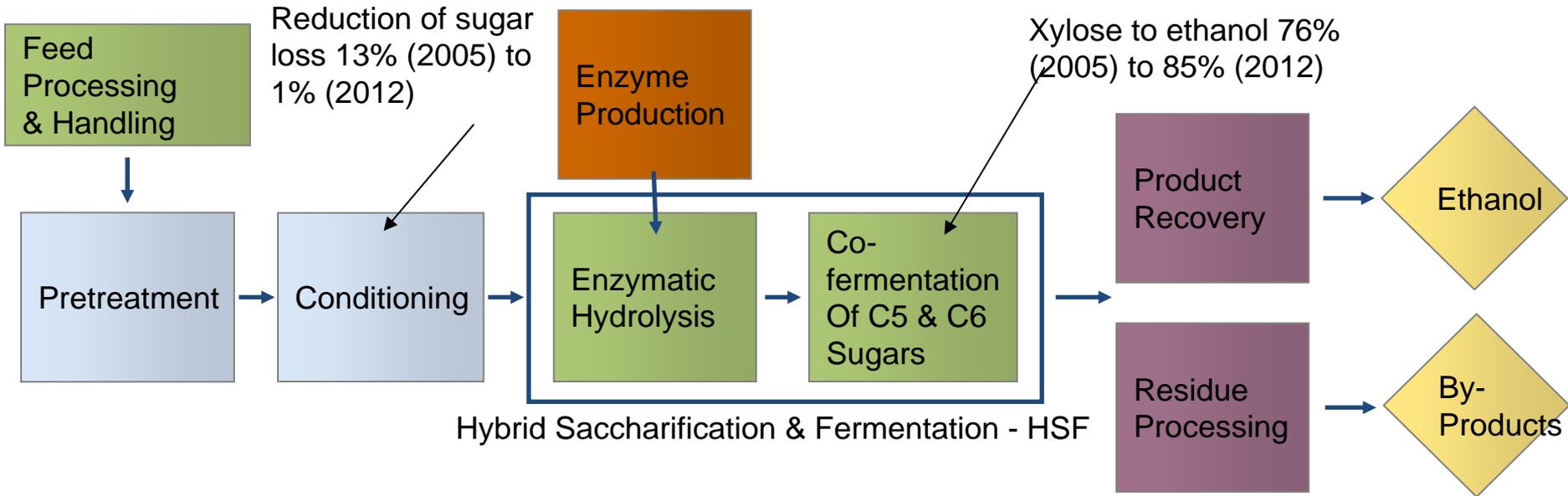
DOE Office of the Biomass Program Enzyme Improvements

Biomass 2010
March 30, 2010

Liz Moore
DOE Golden Field Office

Biochemical Conversion/Enzymatic Hydrolysis

Cost of ethanol production, \$/gallon ethanol



* Conversion costs represented in the figure above are based on conversion of corn stover and equate to an Minimum Ethanol Selling Price \$1.49 in 2012.

Technical Achievements Translate into Cost Savings



	2007 SOT	2008 SOT	2009	2010	2011	2012
Minimum Ethanol Selling Price	\$2.69	\$2.61	\$2.36	\$1.98	\$1.68	\$1.49
Feedstock Contribution (\$/gal)	\$0.97	\$0.90	\$0.74	\$0.65	\$0.60	\$0.57
Conversion Contribution (\$/gal)	\$1.72	\$1.71	\$1.62	\$1.33	\$1.08	\$0.92
Yield (Gallon/dry ton)	72	73	78	83	87	90
Technical Targets						
<i>Feedstock</i>						
Feedstock Cost (\$/dry ton)	\$69.60	\$65.30	\$57.50	\$53.70	\$52.00	\$50.90
<i>Pretreatment</i>						
Solids Loading (wt%)	30%	30%	30%	30%	30%	30%
Xylan to Xylose	75% ^a	75% ^b	80%	85%	88%	90%
Xylan to Degradation Products	13% ^a	11% ^b	8%	6%	5%	5%
<i>Conditioning</i>						
Ammonia Loading (mL of 30wt% per L hydrolyzate)	50	50	50	50	35	25
Hydrolyzate solid-liquid separation	yes	yes	yes	yes	yes	no
Xylose Sugar Loss	2%	2%	2%	2%	1%	1%
Glucose Sugar Loss	1%	1%	1%	1%	1%	0%
<i>Enzymes</i>						
Enzyme Contribution (\$/gal EtOH)	\$0.35	\$0.35	\$0.35	\$0.17	\$0.12	\$0.12
<i>Saccharification & Fermentation</i>						
Total Solids Loading (wt%)	20%	20%	20%	20%	20%	20%
Combined Saccharification & Fermentation Time (d)	7	7	7	5	3	3
Corn Steep Liquor Loading (wt%)	1%	1%	1%	1%	0.6%	0.25%
Overall Cellulose to Ethanol	85%	85%	85%	85%	85%	85%
Xylose to Ethanol	76%	80%	80%	80%	85%	85%
Minor Sugars to Ethanol	0%	0%	40%	80%	85%	85%

Enzyme Solicitation



Enzyme Solicitation (up to \$33.8 million) issued in FY07

Objective: Creating highly effective, inexpensive enzyme systems for commercial biomass hydrolysis; second phase: cellulase development with cost-sharing industry partners

Goal: Utilize pretreated corn stover and switch grass to maximize production of glucose and xylose yields and develop a more robust enzyme to reduce costs

Awardees: Danisco (Genencor), DSM, Novozymes, Verenium

Award Duration: 2008-2011

Recipients are participating in a technical and economic validation process with DOE and NREL, as defined in the Funding Opportunity Announcement (FOA).



“Enhancing Cellulase Commercial Performance for the Lignocellulosic Biomass Industry”

- DOE Value: 8.4M
- Cost Share: 13.4M

- Partners/Subcontractors: NREL



GENENCOR[®]

A Danisco Division

Danisco - Project Goals and Objectives

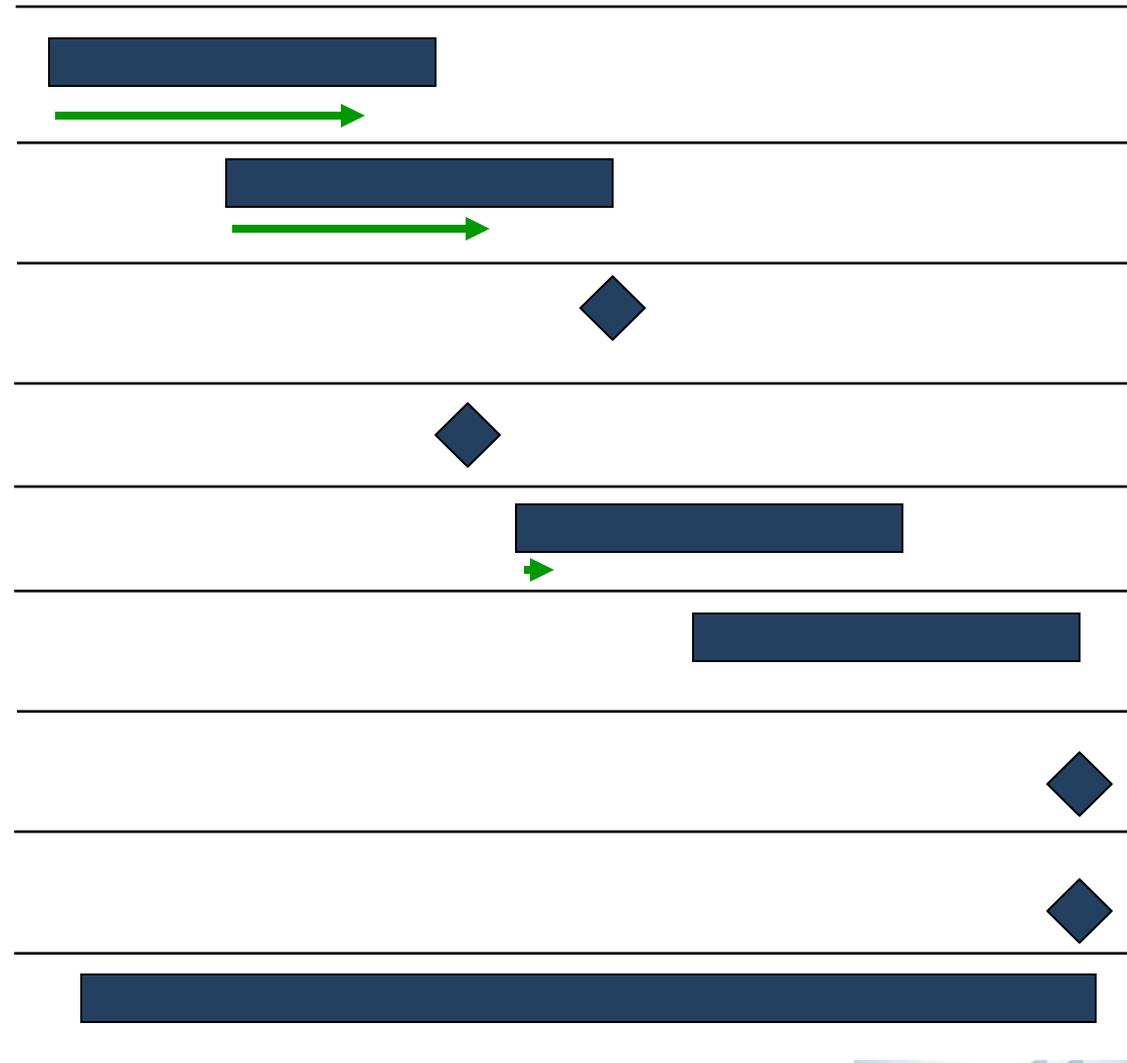


- Enhance relevant properties of selected cellulolytic enzymes to improve the performance and lower the dose of the whole cellulase mix for saccharification of dilute Acid Pretreated Cornstover.
- Using our proprietary protein engineering methods to identify enzyme variants with improved performance in the critical parameters of high efficiency, low protein loading, and tolerance to the real-world process conditions.
- Our end goal is a cocktail of enzymes optimized for efficient and cost-effective saccharification performance under real-world process-relevant conditions.
- Final Targets: Enzyme Load: 3 g / kg cellulose

Danisco - Progress to Date



Set 1: Make Enzyme Variants
Analyze/ Evaluate Performance / Impact of Set 1 variants
Deliverable: Identify Economic benefit of Set 1 winner
Identify next Enzyme Set
Set 2: Make Enzyme variants
Analyze / Evaluate Performance / Impact of Set 2 variants
Deliverable: Identify Economic benefit of Set 2 winner
Deliverable: Identify Economic benefit of combined winners of Sets
Systems Characterization

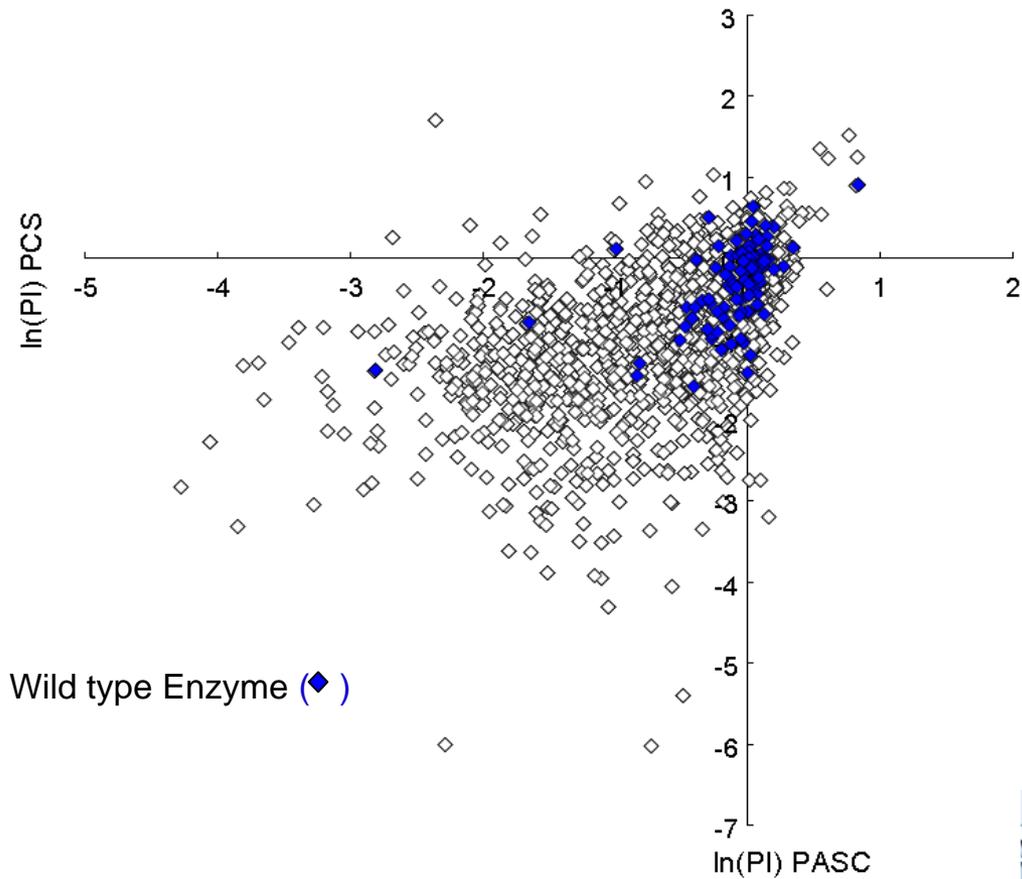


Danisco - Progress to Date



- Evaluated >100 sites for each of two different enzymes by Site Evaluation Libraries, generating about 200,000 data points per enzyme.
- Identified multiple sites for each enzyme with increased performance.
- Generated combinatorial libraries for each enzyme; screening the CLs and evaluating winners from the screens

Results sample:
There is a correlation of
variant performance between
activity assays





“Development of a Commercial Enzyme System For Lignocellulosic Biomass Saccharification”

- DOE Value: 7.4M
- Cost Share: 7.8M

Unlimited. **DSM**

- Partners: ABNT/SNL/LANL

DSM - Project Goals and Objectives



- Evolution and development of cellulolytic enzymes system to reach enzyme efficiency mandates set by DoE Biomass MYPP.
- Production of cellulase enzymes on pilot plant scale supporting a cost structure commensurate with *an on-site production* model by combining the following improvements
 - *in secreted enzymes expression titers,*
 - *doubling the specific activity of the cellulolytic enzymes*
 - *reducing the dosage needed for LC biomass saccharification.*
 - *Whole broth product*
- Demonstration of biomass saccharification yield performance on C6 and C5 monomeric sugars at or above 90%
- Successful application test with the evolved LC hydrolyzing enzymes system at a pilot plant scale reaching enzyme cost contribution of \$0.10/gallon of cellulosic ethanol production.

DSM - Technical Progress [Oct'08 – Mar'10]



- *Technical Accomplishments*

- 2.75x improvement in enzyme expression achieved at lab scale
 - Development of a thermally stable and acidic pH optimum cellulase cocktail with multiple benefits
- Development of robust and cost effective fermentation process
- Improvement in enzyme efficiency
- Completed genomes sequencing and harvest
- Status of Progress in task, subtask or milestone as of Dec., 2009
 - 41 green, 7 yellow
 - 13 patent applications filed and 3 in progress





“Project Decrease: Development of a Commercial-Ready Enzyme Application System for Ethanol”

- DOE Value: 12.3M
- Cost Share: 12.3M

- Partners: PNNL, Universités Aix- Marseille, France, Cornell University.

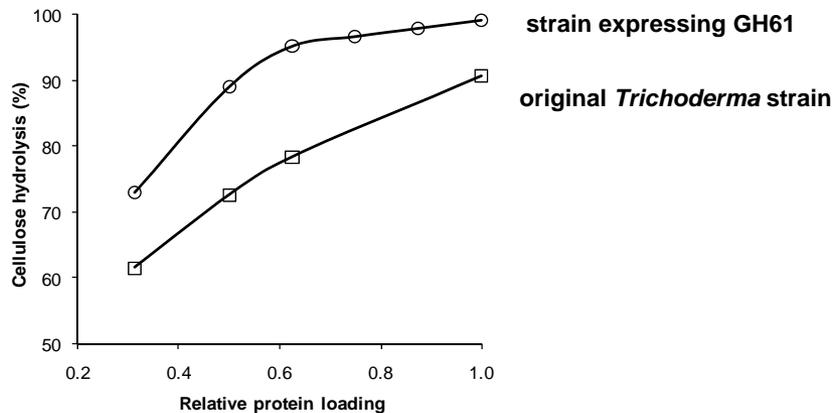


Novozymes - Project Goals and Objectives

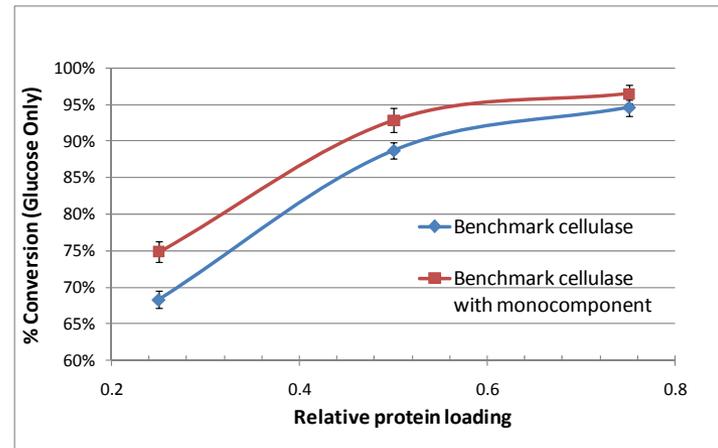


- Improve (and/or replace) current advanced biomass enzyme components in order to increase overall specific performance and allow for a two-fold reduction in total protein loading required to hydrolyze pretreated lignocellulosic biomass to fermentable sugars
- The improved proteins will provide the basis for an enzyme system which Novozymes intends to deploy commercially, within 3 years following the close of this 2.5 year project (project ends Apr 2011)

Optimize existing components
e.g., Proprietary GH61 component



Identify novel synergistic proteins



Novozymes' path toward commercially viable bioconversion

2006-2007

Celluclast®
Novozym®188

2008

Experimental
Cellulase
blends

2009

Cellic CTec
Cellic HTec

2010

Cellic 2

2011+

Further
improved
enzymes



“Commercialization of Customized Cellulase Solutions for Biomass Saccharification”

- DOE Value: 8.5M
- Cost Share: 8.5M

- Partners: None



Verenium - Program Overview



Program Goal:

- Using Verenium's enzyme discovery, evolution and heterologous expression technologies, develop a commercial cellulase product with robust, highly active enzymes resulting in at least a 4-fold reduction in enzyme cost

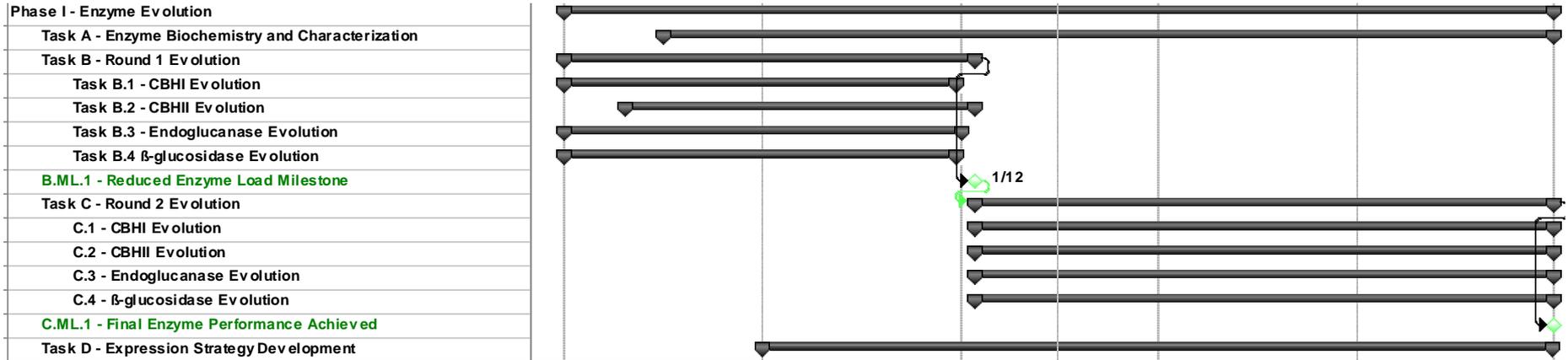
Phase I: Enzyme Evolution (Grant Funded)

- By December 31st, 2010: Demonstrate 4-fold reduction in enzyme dose under relevant process conditions
- End of activities funded under grant award

Phase II: Expression and Commercialization (Currently Unfunded)

- Develop commercial production strains for evolved enzyme cocktail
- Demonstrate performance and economics at Verenium's pilot and demonstration scale facilities
- Due to budget constraints, the original scope of the grant award was limited to Phase I activities. The Phase II expression activities are currently outside the scope of the award.

Verenium - Program Overview

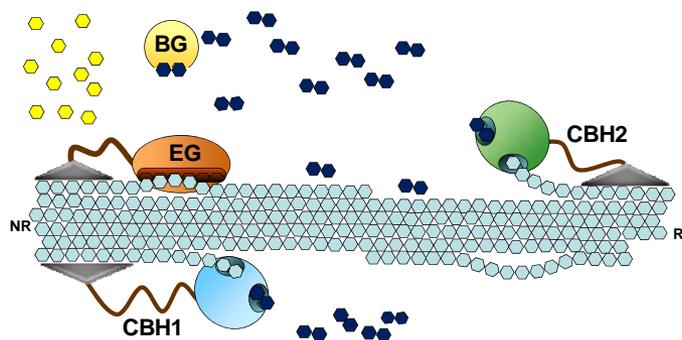


Program managed by detailed WBS, deliverables, milestones

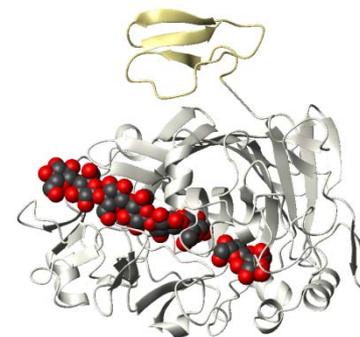
- 1st round of evolution completed
- 2nd round of evolution to be completed by Dec 31st, 2010

Performance improvement will be measured as the amount of enzyme required to meet target cellulose conversion under defined process conditions

Verenium - Program Status



Minimal 4 enzyme cocktail (E4)
required for cellulose digestion



Structure of CBHI Enzyme

- **Round 1 evolution:**
 - Discovered >50 beneficial mutations for each member of the E4 cocktail
 - Best single mutants reduce cellulase dose up to 2x
- **Round 2 evolution:**
 - Combinations of individual mutations are proving to be additive
 - On track to achieve 4x dose reduction by end of 2010