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Strategy For Deconstruction Of Biomass For Biofuels Production

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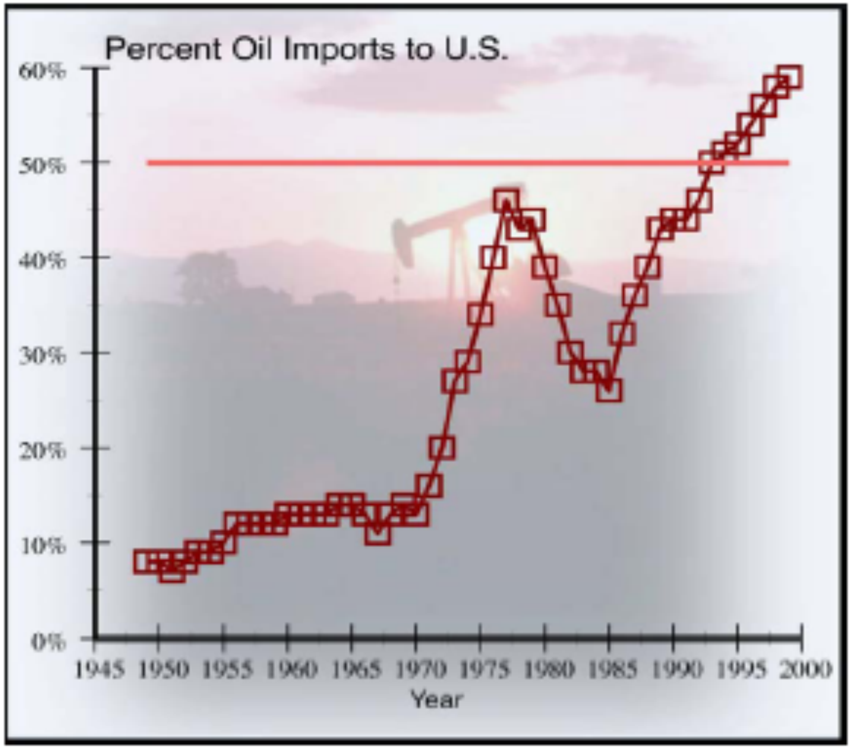
Presentation Outline

- Background and Motivation
- JBEI - Joint BioEnergy Institute
- Consolidated Bioprocessing Drivers for Ethanol
- Enzyme Engineering Efforts
- Trojan Horse Strategy
- Summary and Next Steps

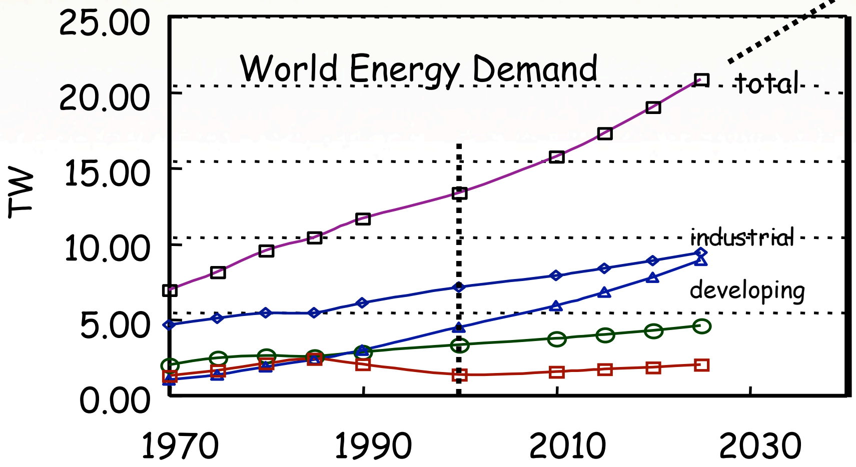
Problem Overview

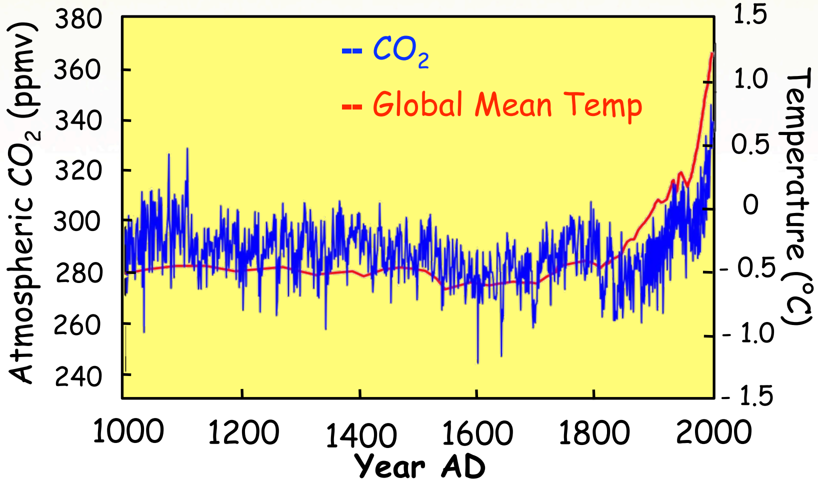
- Dependence on foreign oil is a national security risk and DOE has set a goal to replace 30% of transportation fuels by 2025
- Environmental impact of petroleum based fuels is also a national risk
- Alternative fuels and energy production mechanisms are desirable as a means of reducing this dependence



- Although bio-derived energy alone will not be the single replacement strategy, it can be a part of a diversified approach to energy generation and sustainability.
- → Biofuels can be produced domestically

Energy Security and Environmental Concerns





- Growing demand for energy and finite availability of traditional energy feedstocks (oil and gas) motivates the consideration of alternative fossil feedstocks (tar sands, shale, coal) for the short term
- Biomass conversion offers the possibility of a sustainable source of fuel

Biofuels: Motivation

- Bio-derived liquid fuels address two significant national risks:
    1) Dependence on foreign oil
        → Biofuels can be produced domestically
    2) Climate impact of CO_2 emissions from fossil fuels
        → Biofuels are potentially carbon neutral

- Current market for biomass (agriculture-derived) fuels:
    - Ethanol (e.g., from corn) : ~5 billion gal/yr in 2006
    - Compare to 140 billion gal/yr for petroleum gasoline
- Biohydrogen from algae and other microorganisms
- Biodiesel (e.g., from soy beans): ~300 million gal/yr
    - Compare to 62 billion gal/yr for petroleum diesel fuel
    - Potential market for up to 1 billion gal/yr domestic production from vegetable oils with room for further enhancements

JBEI leverages the Bay Area biotech and high tech industry

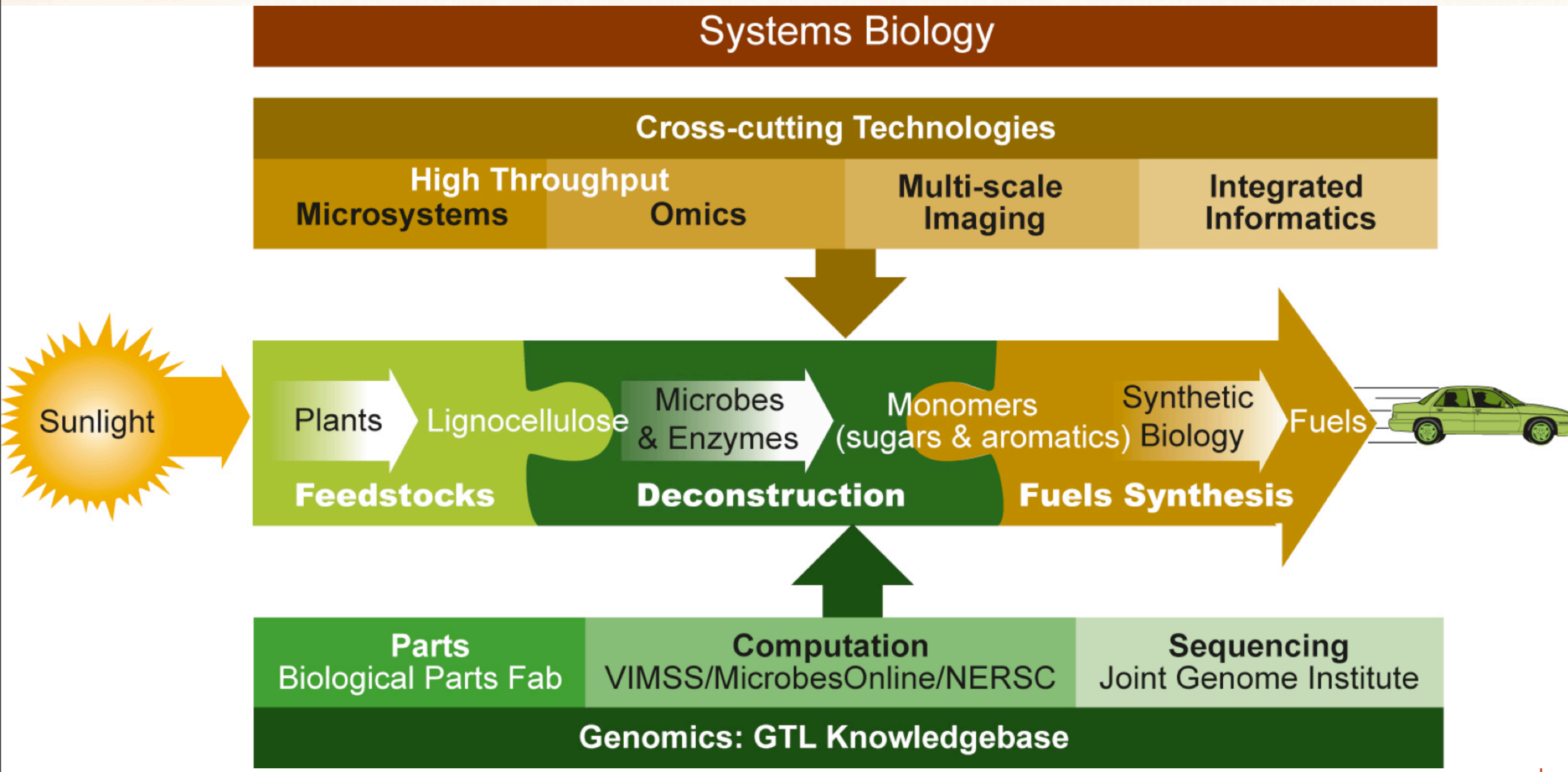
Vibrant industries grow around intellectual centers

- Silicon Valley and Biotech Industry around UCB, UCSF, Stanford
- Bay Area and CA becoming centers for renewable energy

Benefits of JBEI location in Bay Area and CA

- Intellectual environment
- Recruiting
- Commercialization

JBEI: an interlocking approach



JBEI Impact

- Elucidate & modify plant cell wall structure and synthesis
    - New generation of energy crops
- Efficient, cost-effective routes for deconstruction of lignocellulose
    - Engineered enzymes and deconstruction processes
- Engineered organisms for scalable production of ethanol and next generation biofuels
- Enabling and integrating technologies for bioenergy research
- Integrated science & technology to transform the U.S. biofuels industry

Joint BioEnergy Institute (JBEI)

- JBEI is an alliance between three national laboratories, industry, major universities, and federal agencies
- Develop the basic science and establish a technology toolkit to produce an array of biofuels and high value chemicals from biomass
- CEO/PI: Jay Keasling, LBNL
- Create unique infrastructure and disruptive technologies that enable researchers and companies to rapidly develop new biofuels and scale-up production to meet US transportation needs
- Advance new technologies and rapidly transition them to the commercial sector to seed a new national biofuels industry
- Education component

JBEI at a glance

Three National Laboratories
- LBNL
- LLNL
- SNL

Three Universities
- UC Berkeley
- UC Davis
- Stanford

Three Scientific Divisions
- Feedstocks
- Deconstruction
- Fuels Synthesis

One Technology Division

A Single Facility

Bay area location
Leverages local biotechnology companies

Ethanol Production Today

- Primary mode of fuel ethanol production: corn kernel (starch) wet and dry milling
  - 4.9-5.2 billion gallons produced in 2006
  - Took 13 years to reach 1 billion gallon production levels
- New mandate: double the amount of ethanol blended with gasoline by 2012
- 76 corn ethanol refineries under construction (112 in place already)
- Food vs. fuel – corn prices have spiked because of increased demand
  - $4 per bushel at the peak, highest in ten years
  - Will result in higher prices across the board for associated products (meat, etc.)
- Must develop alternative sources of feedstocks and processing to meet US goals and needs
- Ethanol derived from cellulosic material is the most viable alternative
  - Believed to cost 5x more today to establish a cellulosic biorefinery

Cellulosic Biomass

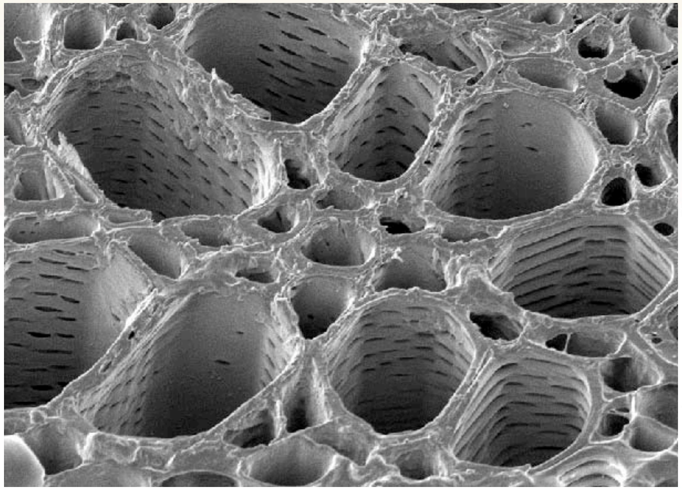
- Cellulose is the most abundant component of plant biomass

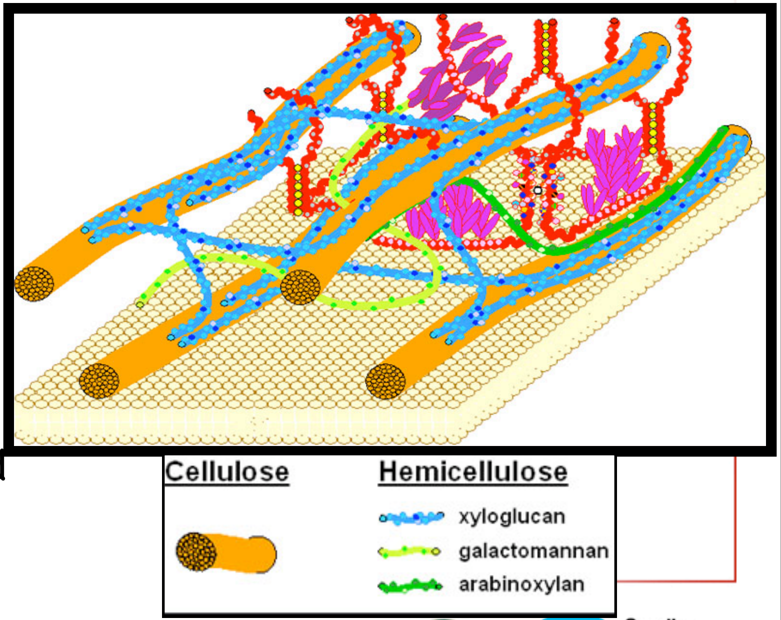
- Cellulose is synthesized as individual molecules (linear chains) and aggregates with the assistance of hemicellulose and lignin resulting in protofibrils which are packed into larger units (microfibrils), eventually forming cellulose fibers

- The chains are stiffened by inter and intra chain bonding and antiparallel orientation

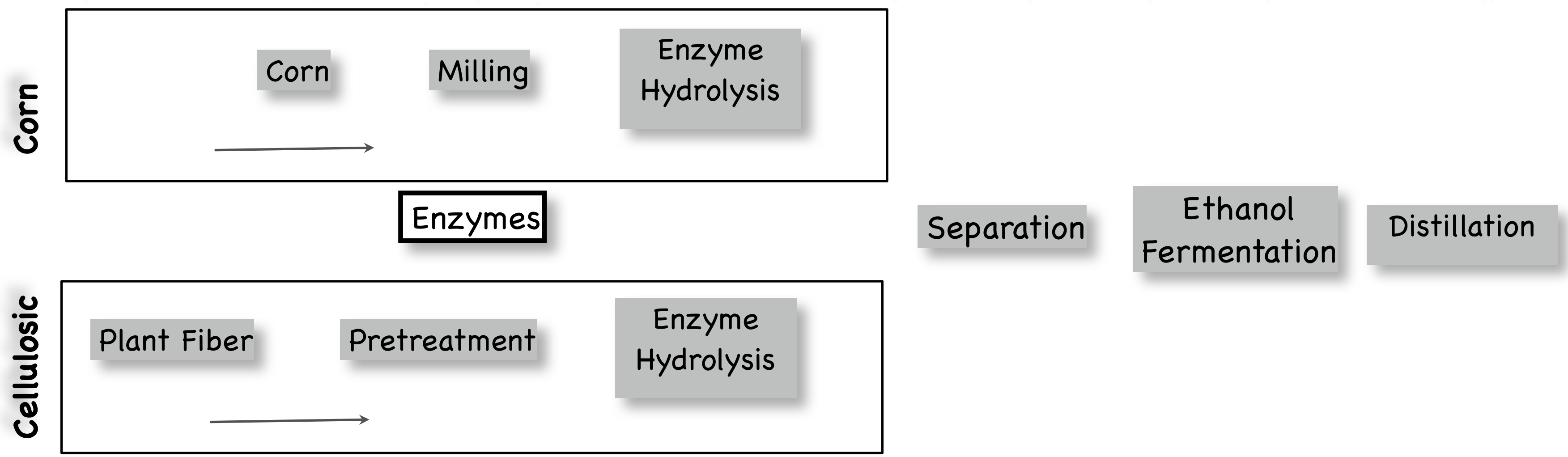
- Common Deconstruction Processes:

- Steam explosion, dilute acid treatment followed by washing to remove hemicellulose and lignin followed by cellulose hydrolysis by enzyme systems





Bioethanol Production



Continued research and innovation has brought pretreatment and enzyme costs down from $5 to $0.50/gallon (but only for limited feedstocks) but costs are still too high

Farrell et al., Science 2006
Ragauskas et al., Science 2006

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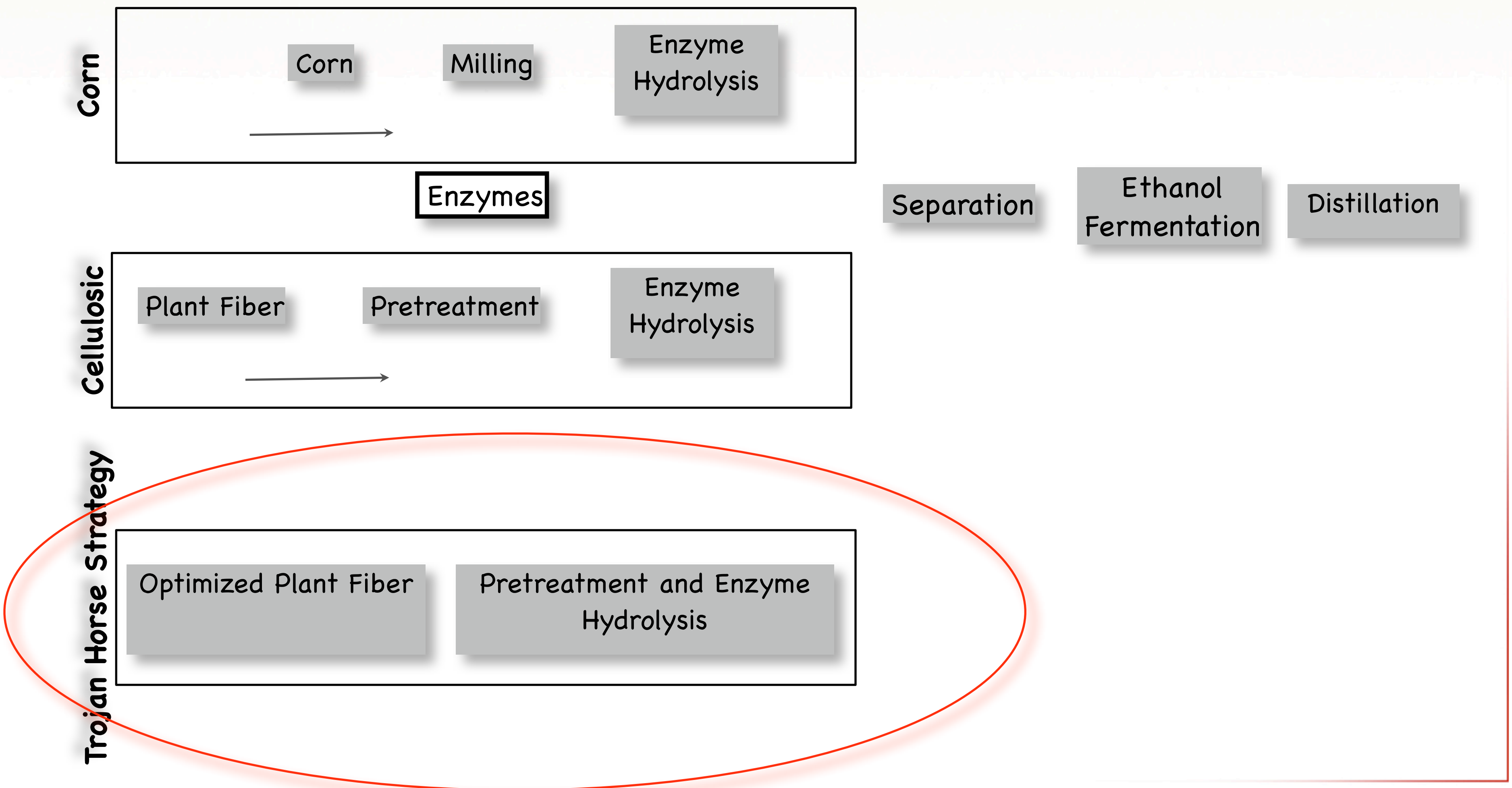
Bioethanol Production



Farrell et al., Science, 2006
Ragauskas et al., Science 2006

Current Industrial Enzymes

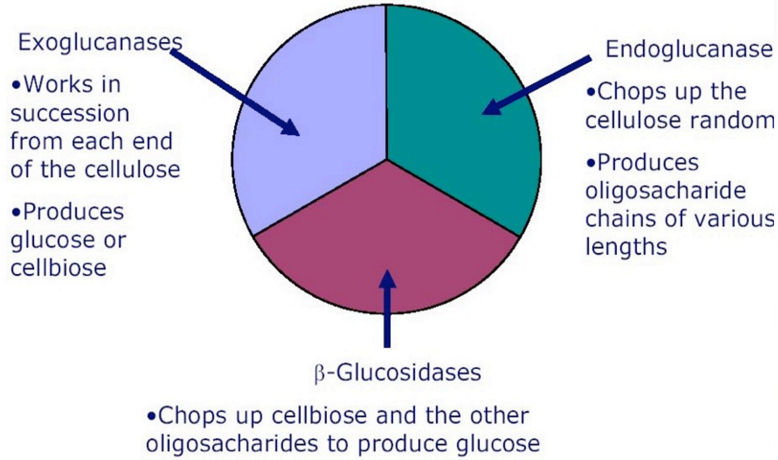
- Cellulosic enzyme system consists of three major components (fungal systems)

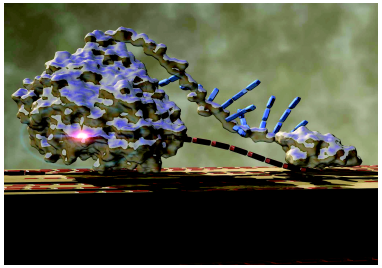
- The mode of action of each of these being:

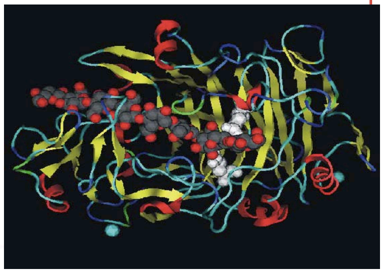
- (1) Endo-glucanase (EC 3.2.1.4), "random" cleavage of cellulose chains yielding glucose and cello-oligo saccharides

- (2) Exo-glucanase (EC 3.2.1.91), exo-attack on cellulase from ends with cellobiose as the primary structure

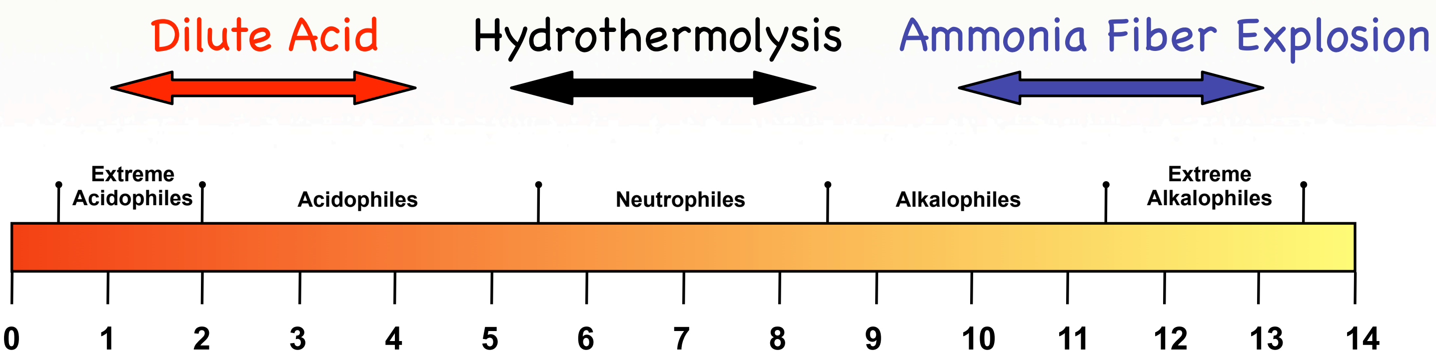
- (3) ß-glucosidase (EC 3.2.1.21), hydrolysis of cellobiose and other oligosacharides to glucose







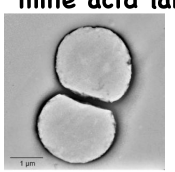
Need: Development of Enzymes Robust for Consolidation with Pretreatment

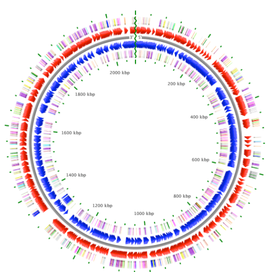


Acidic

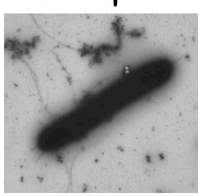
Basic

mine acid lake pH <2





Soda lake pH>10



Picrophilus torridus

able to grow around pH 0 (and even at negative pH values)

Alkaliphilus transvaalensis

pH range 8.5-12.5 (optimum pH 10.0)

OEM Source: Extremophiles

Habitats that are not conducive to DNA, RNA and protein stability are not conducive for life

Micro-organisms that live optimally at relatively extreme levels of acidity, salinity, temperature or pressures discovered through bio-prospecting

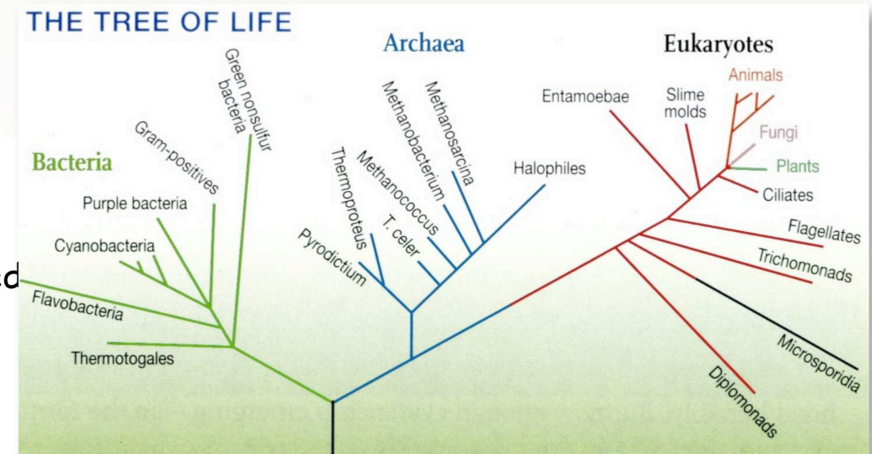
Enzymes isolated from these organisms are used in some industrial manufacturing processes (e.g. lipase, Dnase, Rnase)

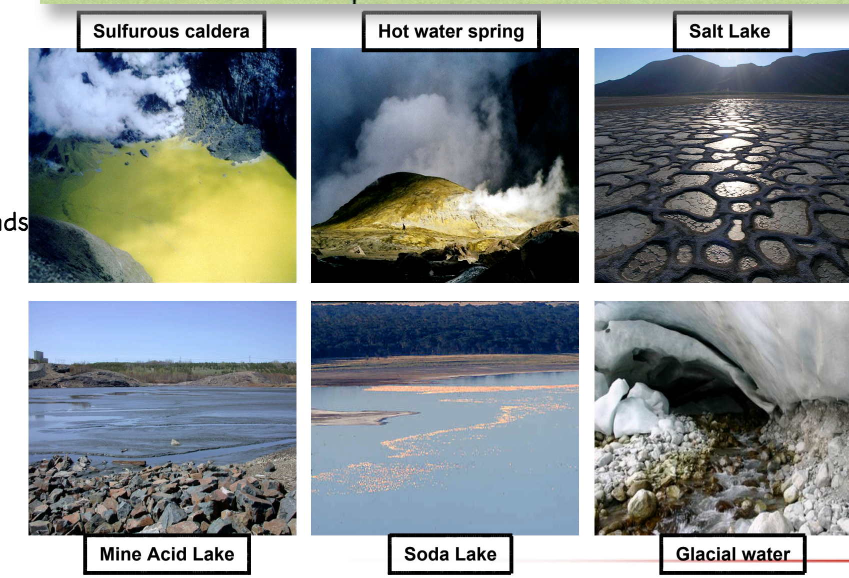
Sulfolobus solfataricus – volcanic fields -Iceland, Solomon Islands

Alicyclobacillus acidocaldarius – volcanic fields -Iceland, Solomon Islands

Pyrococcus families – hydro-thermal vents

Enzymes have no detectable activity activity below 50 ^{o}C and exhibit only 5% activity at 55 ^{o}C





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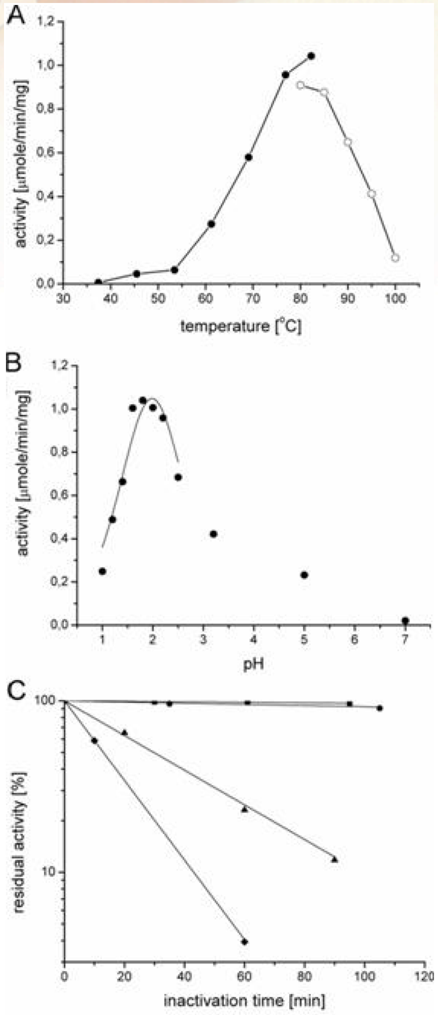
Activity and Stability Profile of SSO 1949

adapted from Huang et al. (Biochem. J., 2005)



(A) The cellulolytic activity of SSO1949 was assayed at various temperatures.

(B) The pH optimum of SSO1949 as determined at 80 °C. The pH profile displays a bell-shaped behavior typical for active sites with protonable groups.

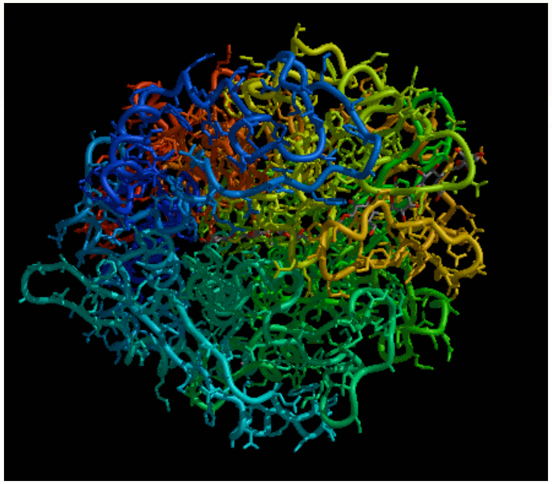
(C) SSO1949 was preincubated for up to 2 h at various temperatures before the activity was determined at the optimal conditions (pH 1.8, 80 °C). SSO1949 resists a 2 h incubation at pH 1.8 at 80 °C and 85 °C. At 90 °C and 95 °C the half-life of the enzyme decreases to 30 and 13 min respectively.

Properties of the enzyme- temp optimum of 80 °C, pH optimum of ~ 1.8 and thermostability make it an attractive target for enzymatic deconstruction of cellulosic biomass and of interest for enzyme engineering for enhanced catalytic activity

Designing a Superior Enzyme

- Enzymes are highly specialized in terms of activity, operating conditions, and functionality developed through evolution.
- When they are taken out of their normal working environment, they typically suffer in terms of activity and durability (industrial processes, fuel cells, pharmaceuticals).
- Additionally, some of the chemical and physical prerequisites for full enzyme activity may be deleterious for our process goals.
- We can modify enzymes at the genetic level to try and improve their performance for our applications – glucose production and substrate activity.
- Drawback: we may actually destroy what we are attempting to improve.

Using Computational Modeling to Engineer Superior Enzymes



MKREIEWNAIIELGVRPMSLKYGRDTIVEVDLNAVKHNVKEFKKRVNDENIAMMAAVKAN
GYGHGAVEVAKAAIEAGINQLAIAFVDEAIELREAGINVPILILGYTSVAAAEEAIQYDV
MMTVYRSEDLQGINEIANRLXKKAQIQVKIDTGMSRIGLQEEEVKPFLEELKRMEYVEVV
GMFTHYSTADEIDKSYTNMQTSLFEKAVNTAKELGIHIPYIHSSNSAGSMEPSNTFQNMV
RVGIGIYGMYPSKEVNHSVVSLQPALSLKSKVAHIKHAKKNRGVSYGNTYVTTGEEWIAT
VPIGYADGYNRQLSNKGHALINGVRVPVIGRVCMDQLMLDVSKAMPVQVGDEVVFYGKQG
EENIAVEEIADMLGTINYEVTCMLDRRIPRVYKENNETTAVVNILRKN

Computational Simulations of mutations (3D)

- Fast calculations can quickly screen all combinations of mutations "in silico"
- Molecular Dynamics simulations of top contenders for more accurate predictions
- assumes the protein retains the same overall fold.

Statistical Analysis on Protein Sequences (2D)

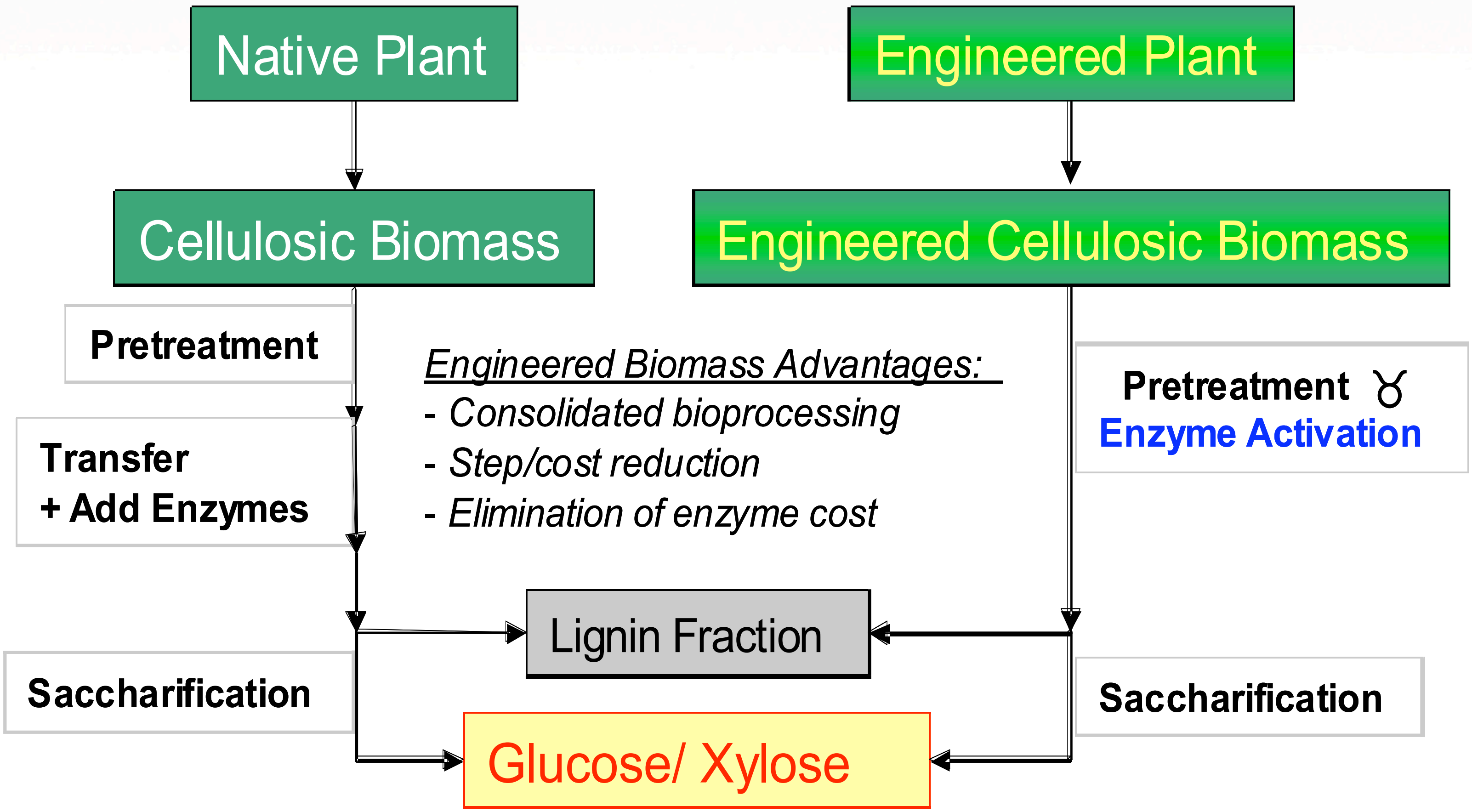
- Analyze all known variants of the mutants
- Use Sandia's **signature** descriptor to identify optimal consensus sequences

Improve catalytic properties (V_{max}, pH opt)

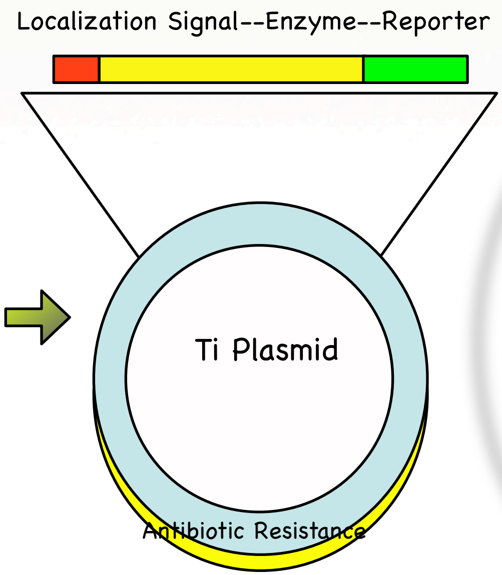
Modify substrate specificity and binding affinity

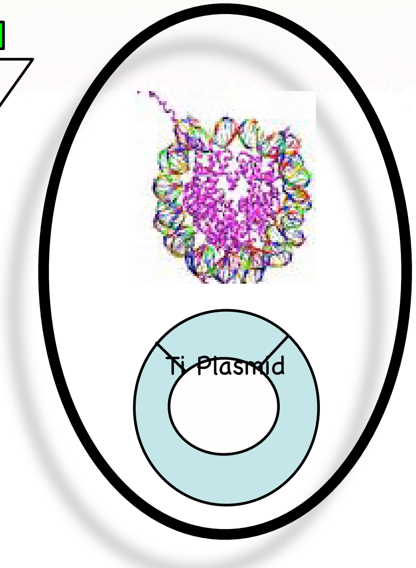
Improve thermostability (T_{opt})

Trojan Horse Deconstruction Strategy



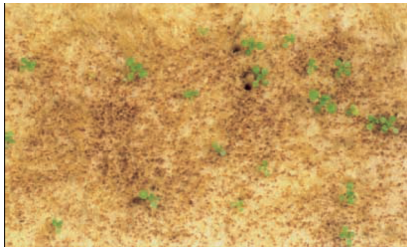
Actuator Construction Agrobacterium "Gene Ferry" Mediate Plant Transformation





~ 70-85% transformation efficiency

Grow Seeds



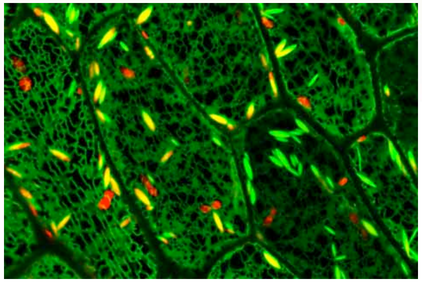
Antibiotic Selection

Hadi et al., 2002, PCR

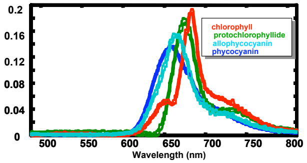
~30K reports, Vain, P., 2005, Nature

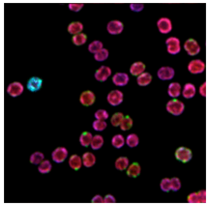
BT Cotton, Roundup Soybean

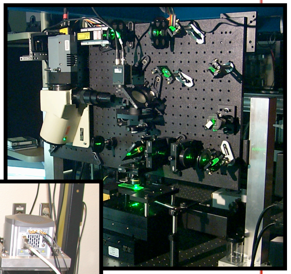
Assay -testing of the actuator

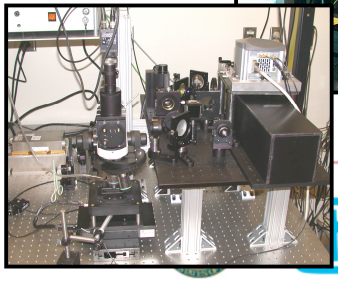


- Actuator Expression
  - RNA analysis
    - Northern, RT PCR
- Actuator Activity
  - Western
  - Enzymatic activity (in vitro)
  - Enzyme compartmentalization using imaging
    - 2D -entire leaves, stems, coarser
    - 3D high resolution, with in the apoplast
    - Multivariate analysis will enable separating overlapping florescence
    - Em Immunolabelling
- Cellulose hydrolysis assay
  - In vitro assays
  - In situ assay -AFM









Developing Engineered Bioenergy Crops

- Improved understanding of all cell wall synthesizing and modifying enzymes in the model systems selected: rice and Arabidopsis

- Transgenic plants with optimized cell wall composition for deconstruction

- Translate genetic developments from model plant systems to proposed bioenergy crops

Rice → **Switchgrass**

Arabidopsis → **Poplar**

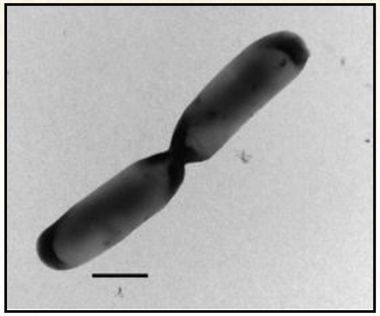
Switchgrass

Poplar

Future Directions

- Rice straw is over half of the worlds cultivated biomass (Kim and Dale 2004)
- Rice straw is the major lignocellulose material in California ~ 2.2 tons/acre (~600K acres planted in CA 2006)
- Crude protein per acre -110 kg
- Soluble @ 40% per acre -44 kg
- Enzyme @ 5% -2.2 kg/acre, extraction and processing costs $0
- ~ 65 gallon/ton biomass

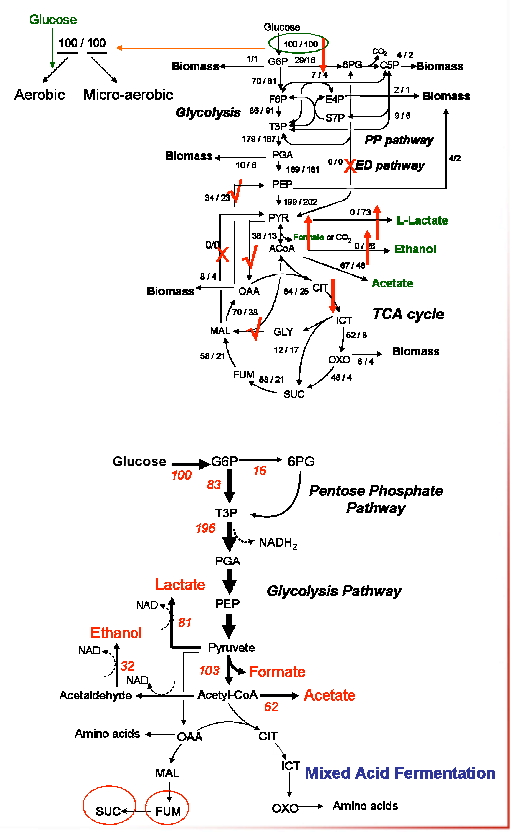
Next Generation Biofuels



- Organisms engineered to produce and withstand high concentrations of biofuels and by-products formed during deconstruction and fermentation
    - Geobacillus thermoglucosidasius M10EXG, Sapra et al.,
- Develop pathways for production of future biofuels
    - Sequence and regulatory information for metabolic pathways producing next generation biofuels

•In silico Metabolic Engineering: Flux Balance Analysis, Stoichiometric Flux Analysis

- Sale et al.,



- “The stone age ended not for a lack of stones, and the oil age will end, but not for the lack of oil”,

founding architect of OPEC

- Collaborators:
- R.Sapra
- J. Timlin
- M. Sinclair
- B. Simmons
- A. Britt
- P. Ronald
- M. Whalen

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