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“Trojan Horse” Strategy For Deconstruction Of Biomass For Biofuels Production

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Problem Overview

- Reducing the dependence on foreign oil is an essential national security need
- DOE has set a goal to replace 30% of transportation fuels by 2025
- Cellulosic biomass is the only viable alternative (50 billion tons/year)
- Cellulosic biomass needs to be deconstructed before it can be fermented
- Deconstruction processes are expensive due to:
  - ~~Pretreatment of biomass~~
  - Costs of microbial enzymes that are produced and added exogeneously
  - The low activity of these enzymes
  - Mass transport rate issues
  - Enzymes are sensitive to inhibitors produced during pretreatment and not compatible with current industrial processes

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
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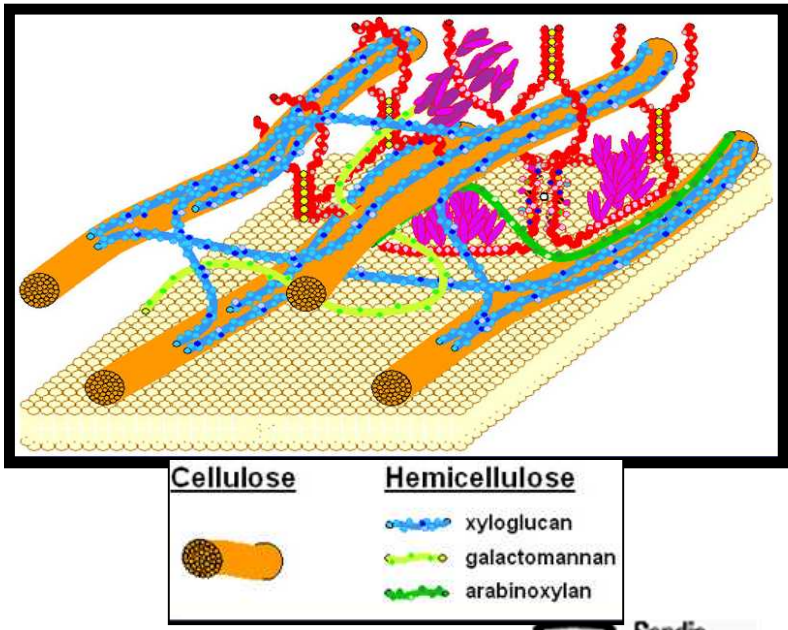
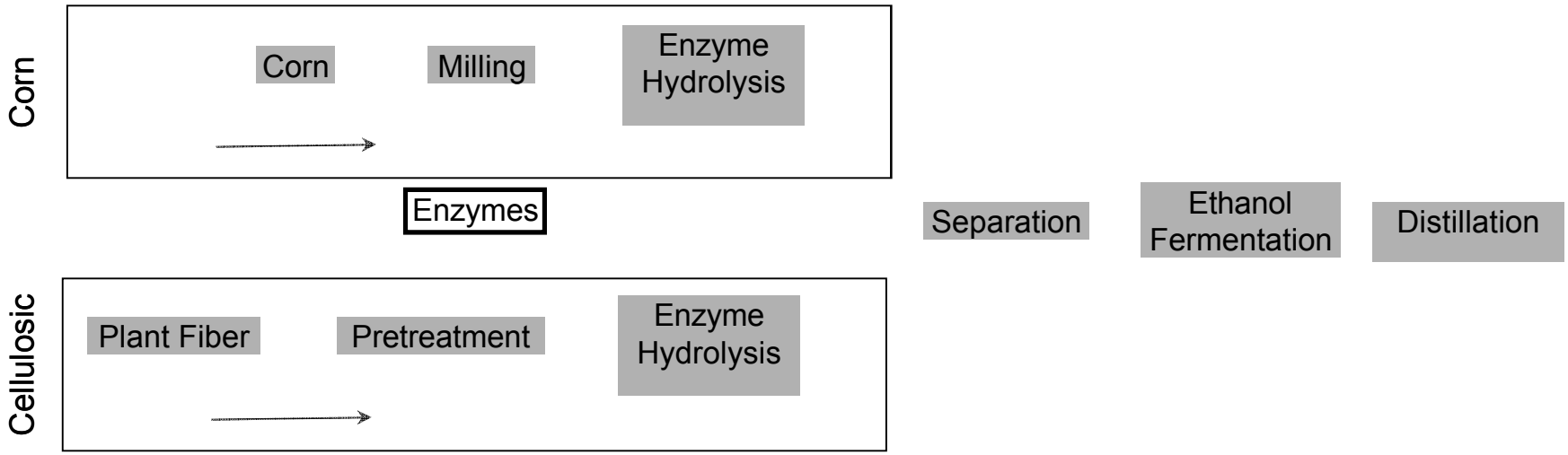


Bioethanol Production



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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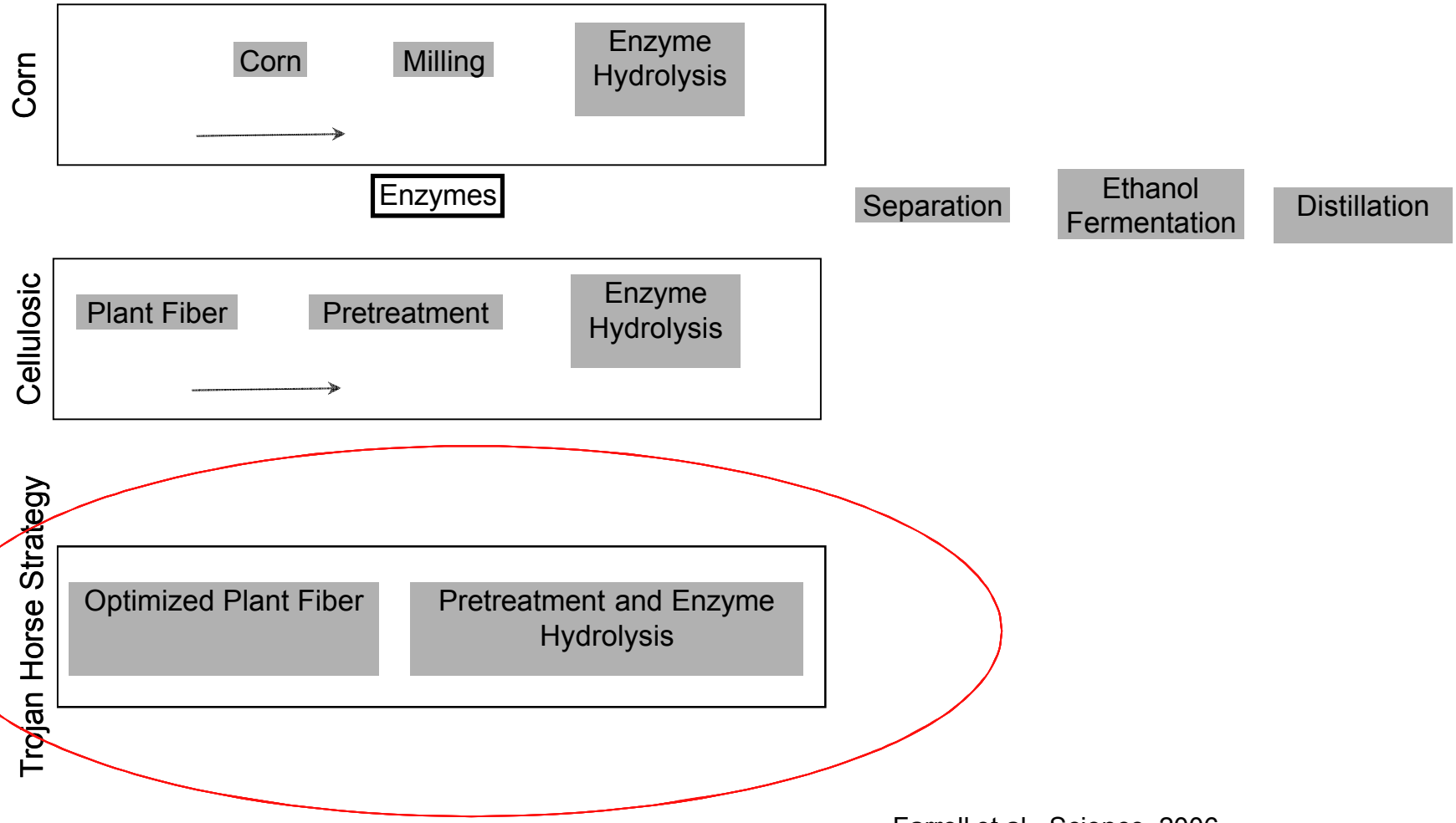
Bioethanol Production



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

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Team Members

Blake Simmons - Biomicrofuel cell, enzyme design, deconstruction of biomass

Jeri Timlin - image analysis

Micheal Sinclair - image analysis

Masood Hadi - enzymology, protein production/characterization, plant molecular biology/biotechnology

Rajat Sapra - extremophile microbiologist, metabolic engineering

Yooli Light - protein production/characterization, molecular biology

Professor Dr. Anne Britt - Vice Chair of Plant Biology, UC Davis

>20 publications, Arabidopsis thaliana transformation

UCDAVIS AGGIES

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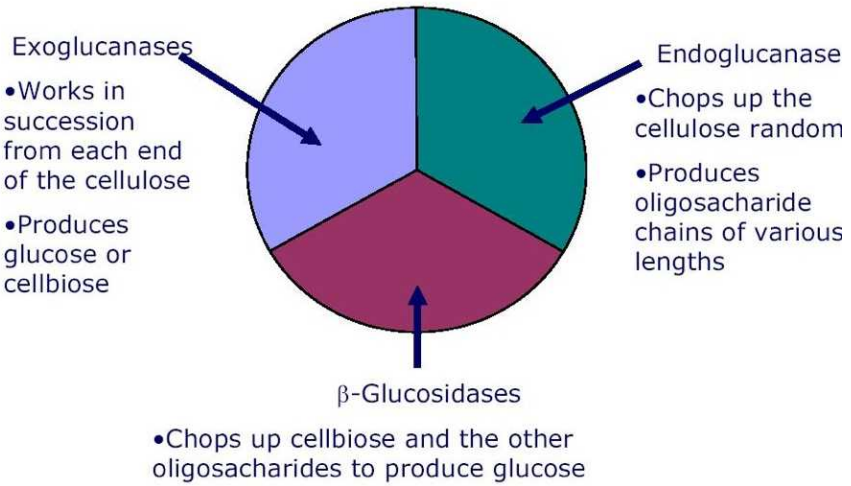
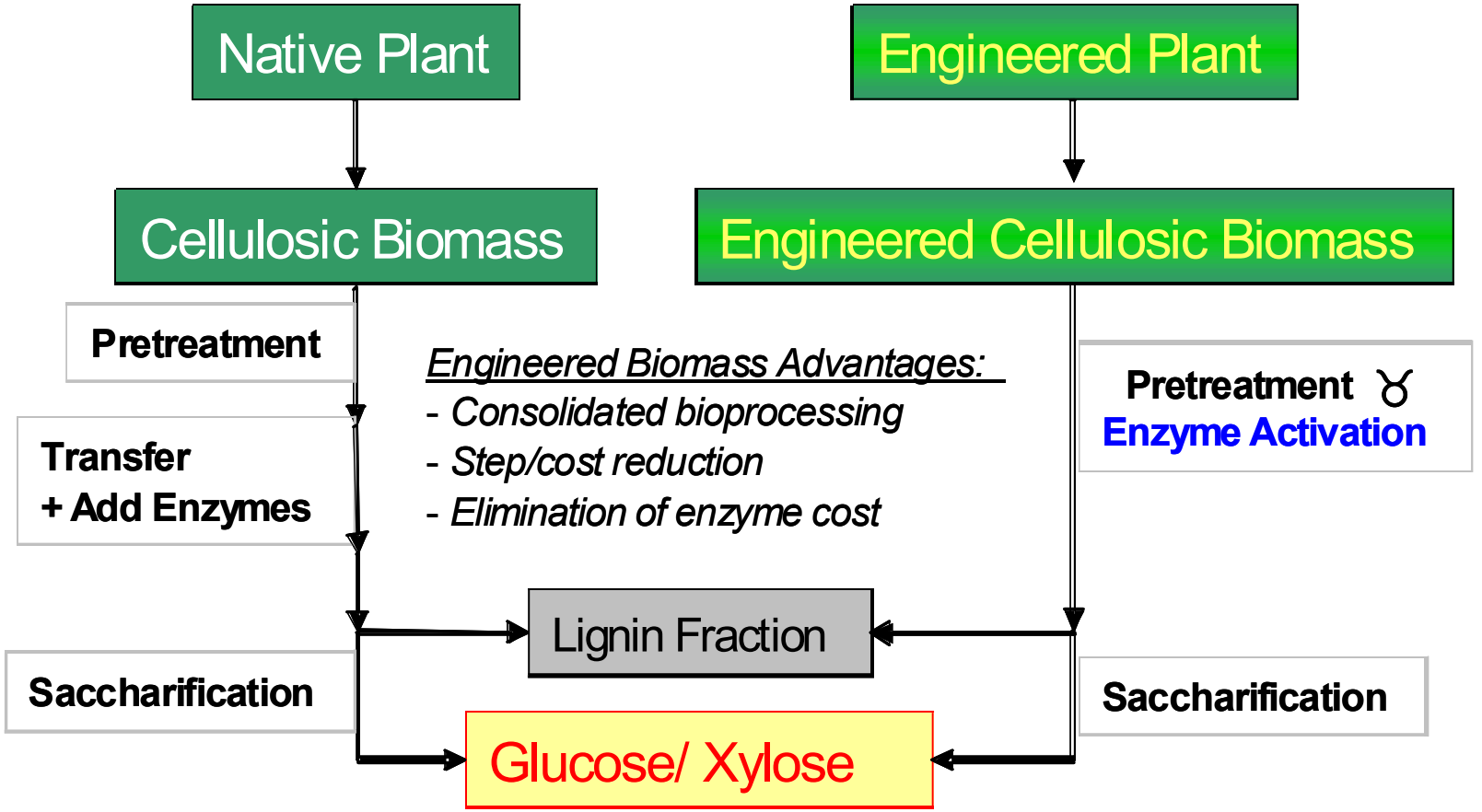
Current Industrial Enzymes

- 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



Trojan Horse Strategy



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

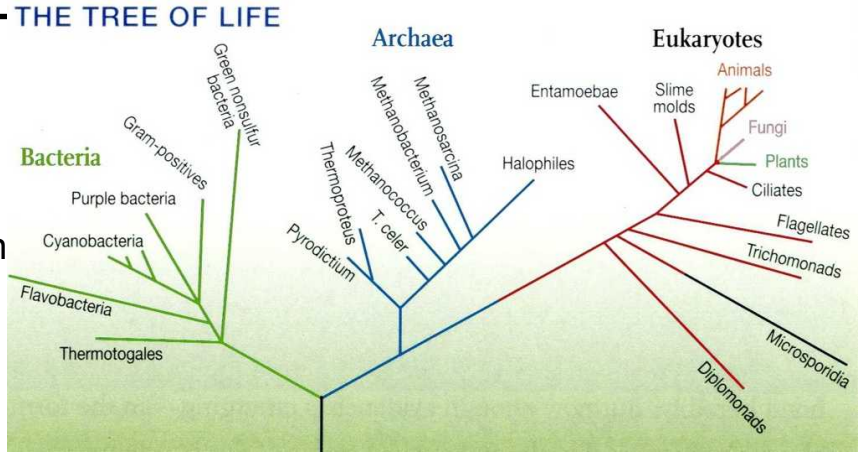
Enzymes isolated from these organisms are used in some industrial manufacturing processes (e.g. lipase, Dnase, Rnase)

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 ^{0}C and exhibit only 5% activity at 55 ^{0}C




Sulfurous caldera


Hot water spring


Salt Lake


Mine Acid Lake

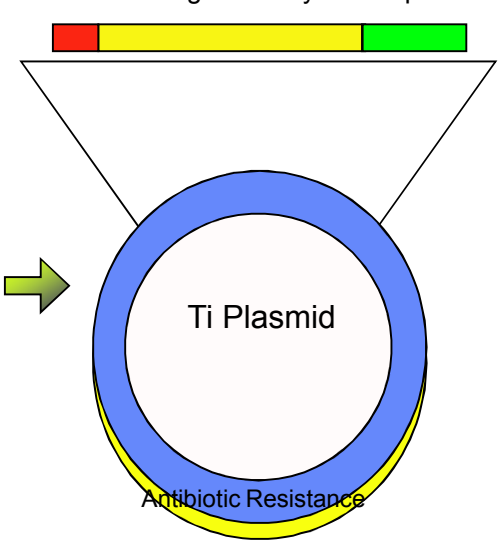

Soda Lake

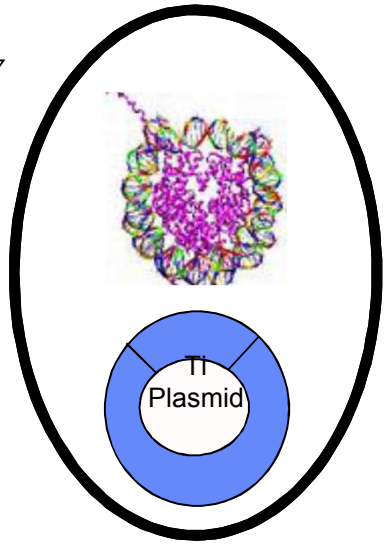

Glacial water

Actuator Construction

Agrobacterium"Gene Ferry" Mediate Plant Transformation

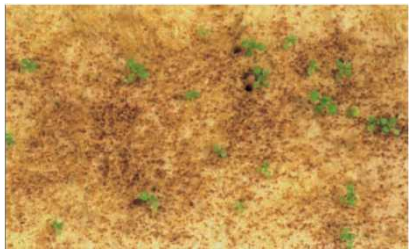
Localization Signal--Enzyme--Reporter





~ 70-85% transformation efficiency

Grow Seeds



Antibiotic Selection

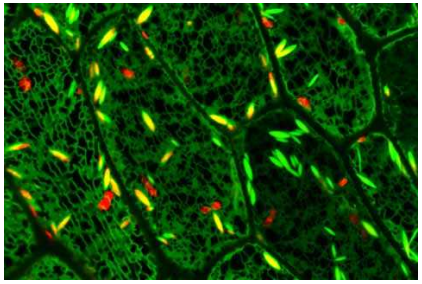
Hadi et al., 2002, PCR

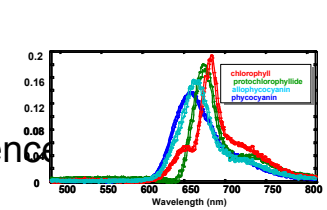
~30K reports, Vain, P., 2005, Nature

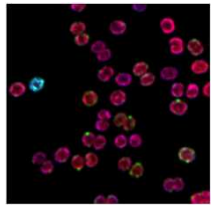
BT Cotton, Roundup Soybean

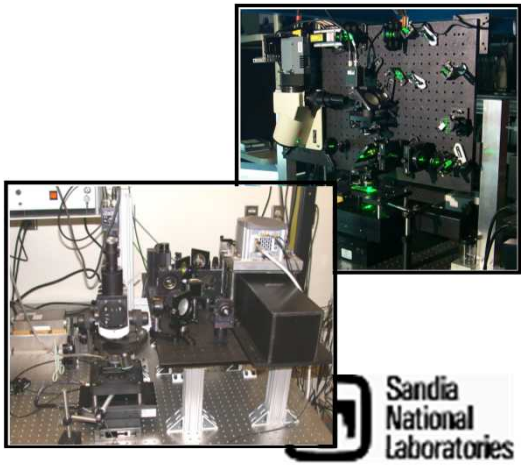
Assay -testing of the actuator

- 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









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Risks

- Technical
  - Tissue specific localization
  - Reduced activity or no activity
  - Over-expression problems

- Biosafety
  - Using Arabidopsis - a well characterized model system
  - University collaborators with all necessary regulatory approvals and years of experience
  - Vegetative parts will be shipped to Sandia for analysis
  - Field test model plant/tissue will not be a food source for any specie
    - Male sterile

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

- Poplar, Willow
- Agricultural companies e.g. Monsanto, Mendel biotech, Amyris biotech etc.

- “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