# Advanced Fuels from Advanced Plants

Jay Keasling, CEO

# Joint BioEnergy Institute (JBEI)
# Six partners – One location

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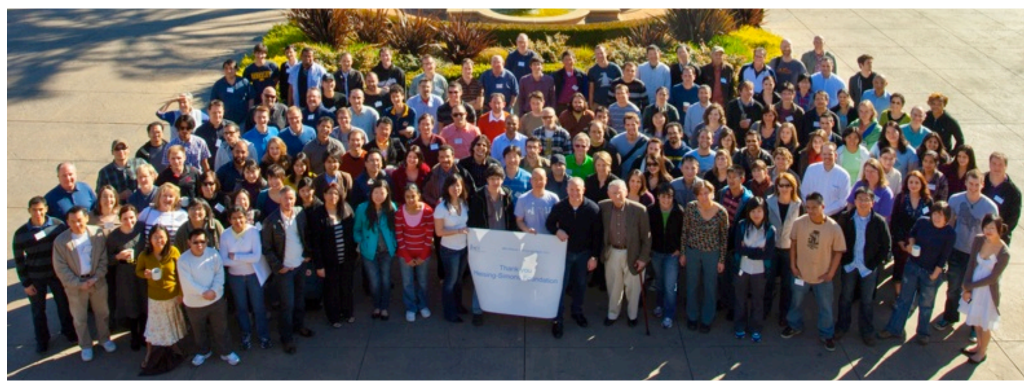

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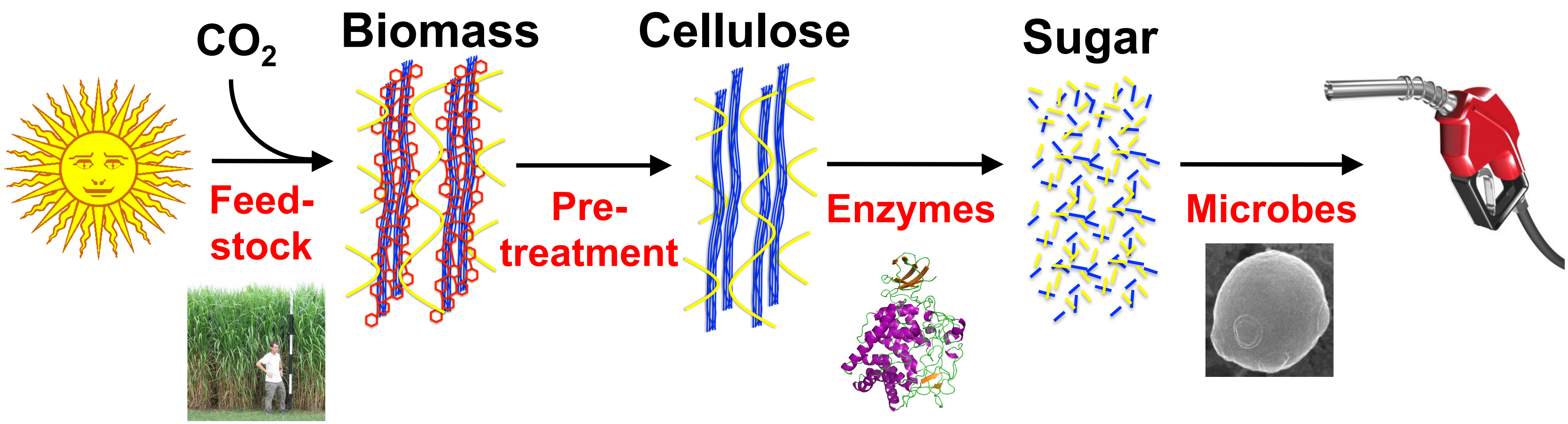
# JBEI provides the basic science for converting biomass to fuels




## Four divisions: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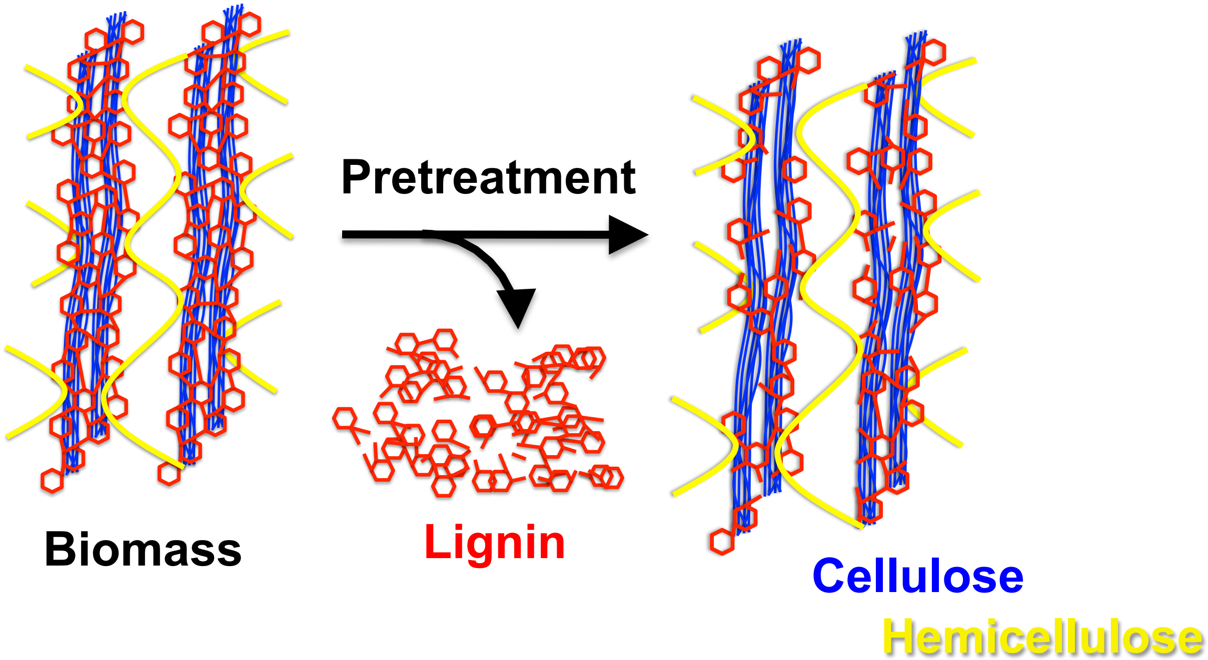
# Lignin recalcitrance





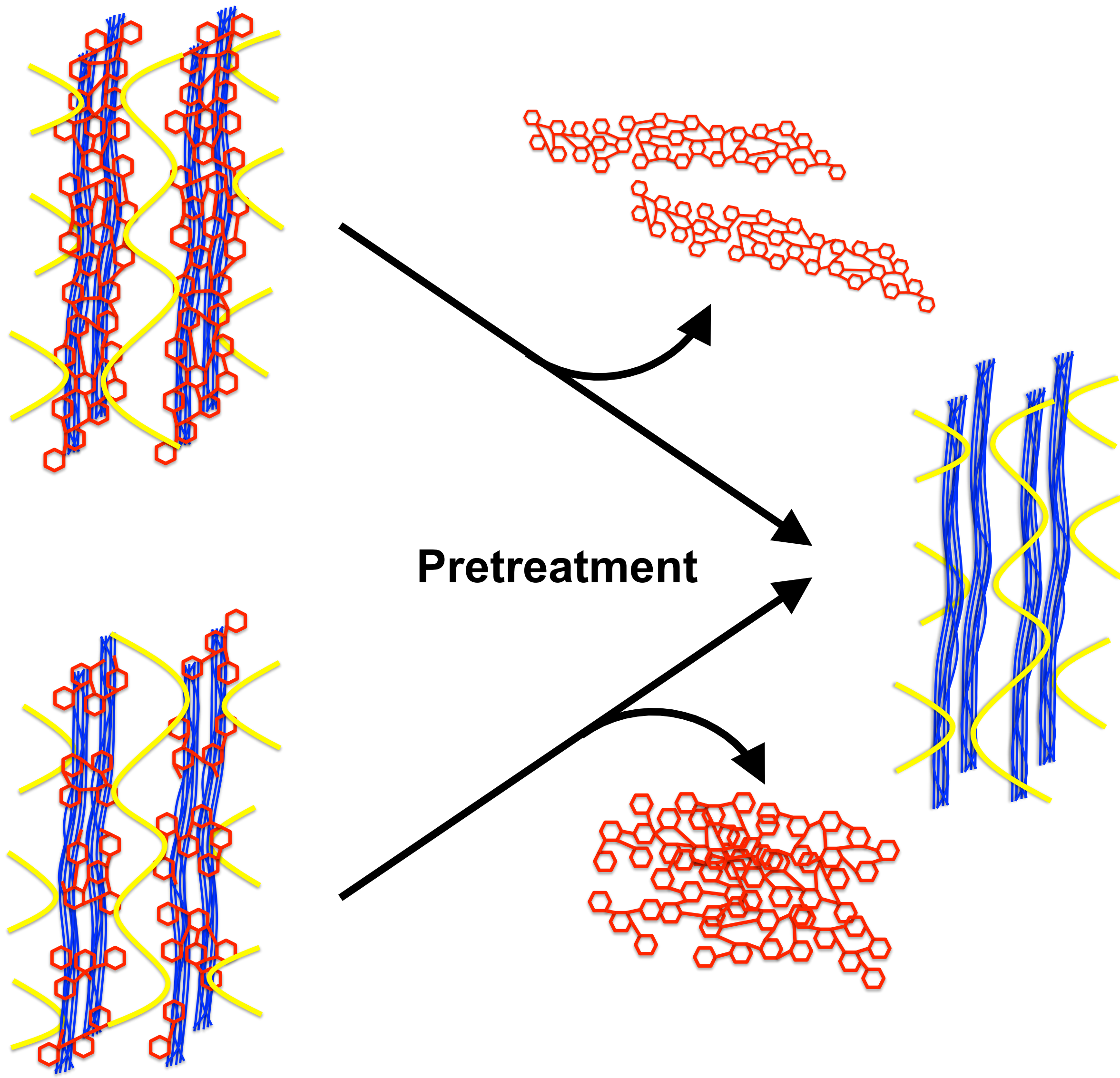

## Challenges

- Cellulose and hemicellulose are occluded by lignin
- Lignin is recalcitrant to depolymerization
- Widely-used pre-treatment processes do not effectively remove lignin

# Two approaches to lignin recalcitrance

**Develop better pretreatment methods**

**Engineer plants to have modified lignin**



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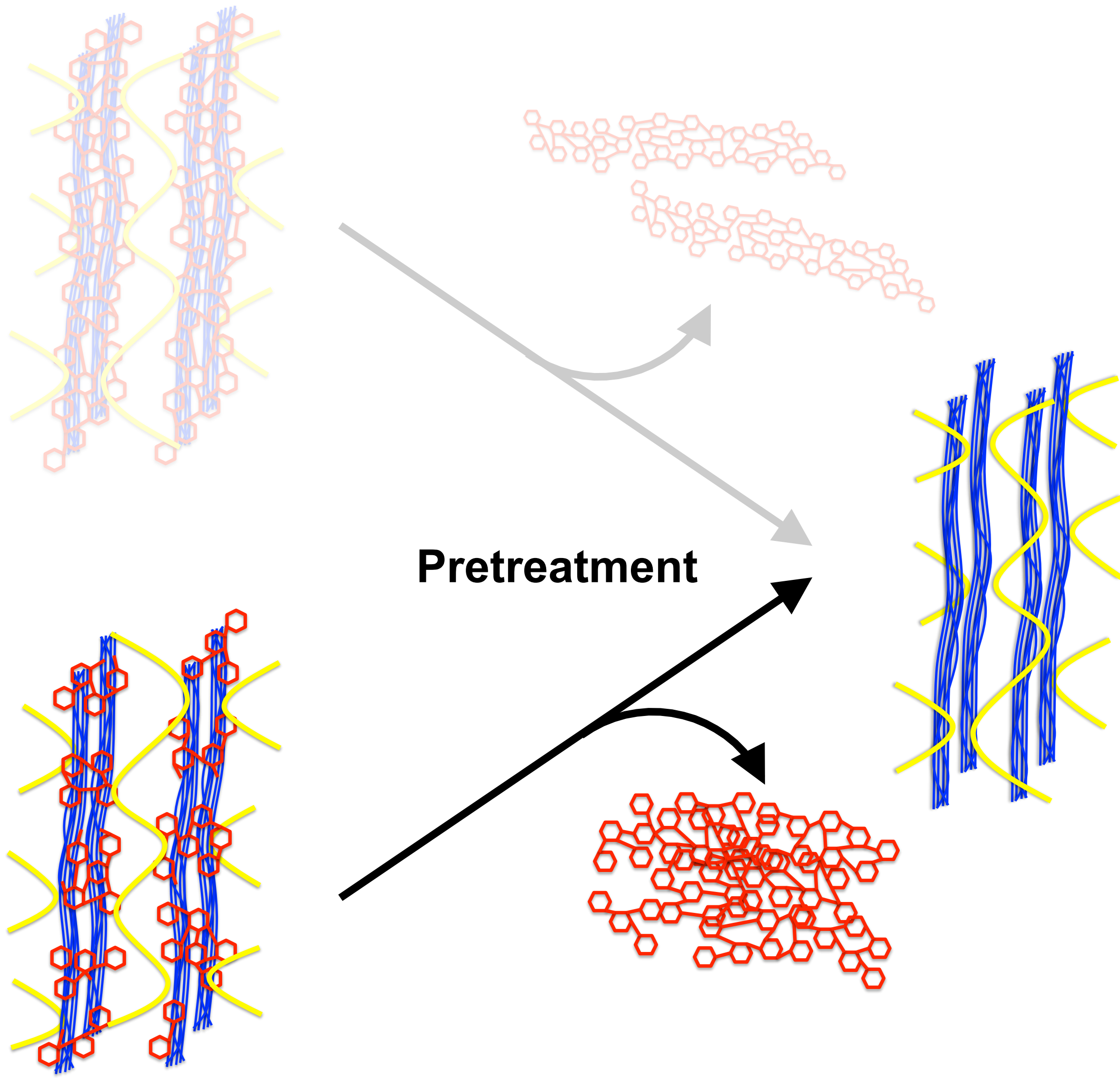
# Two approaches …
# ...plants with modified lignin

**Develop better pretreatment methods**

**Engineer plants to have modified lignin**

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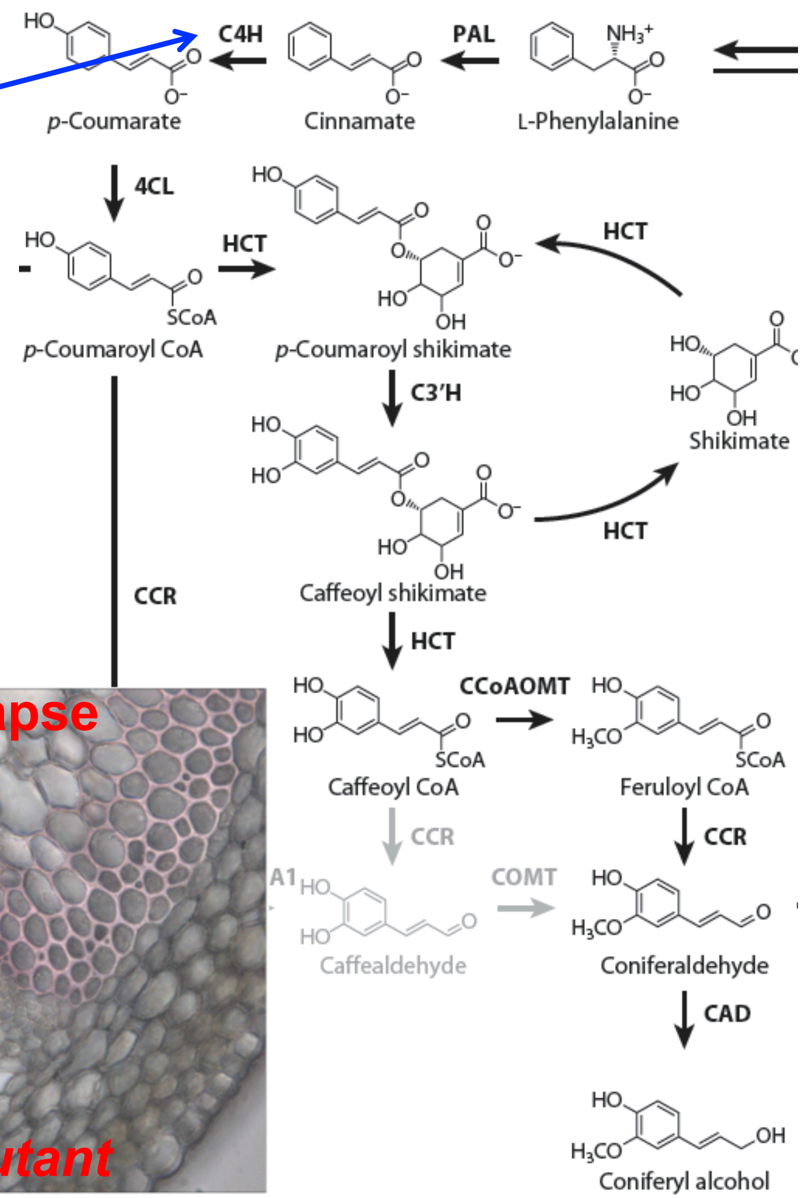
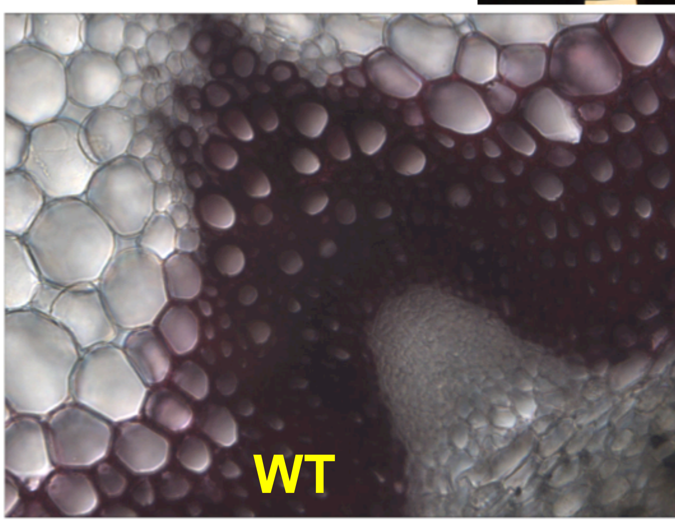
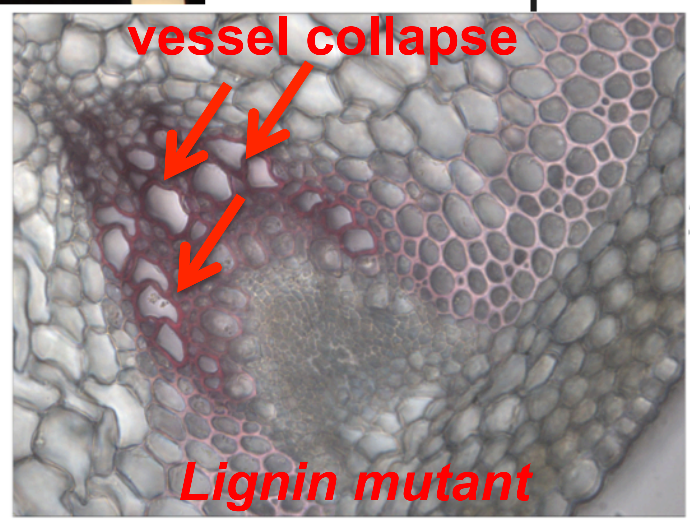
# Some approaches to reducing lignin can be detrimental to growth


(Bonawitz and Chapple, 2010)



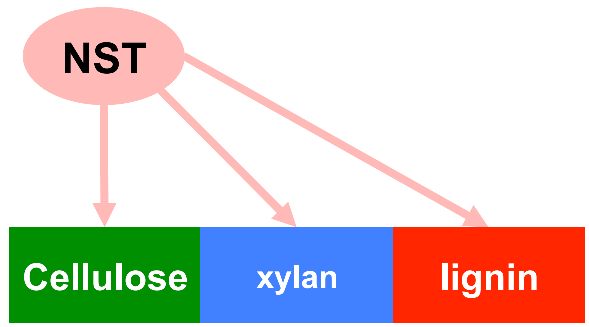



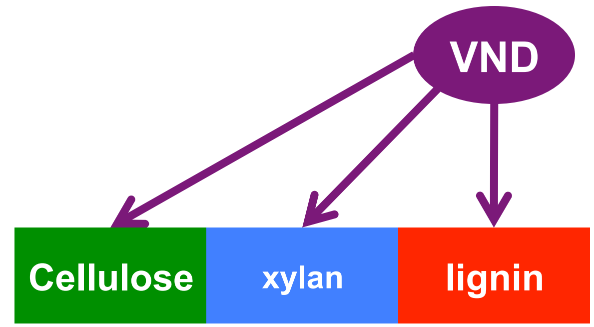
# Controlling the lignin biosynthetic pathway

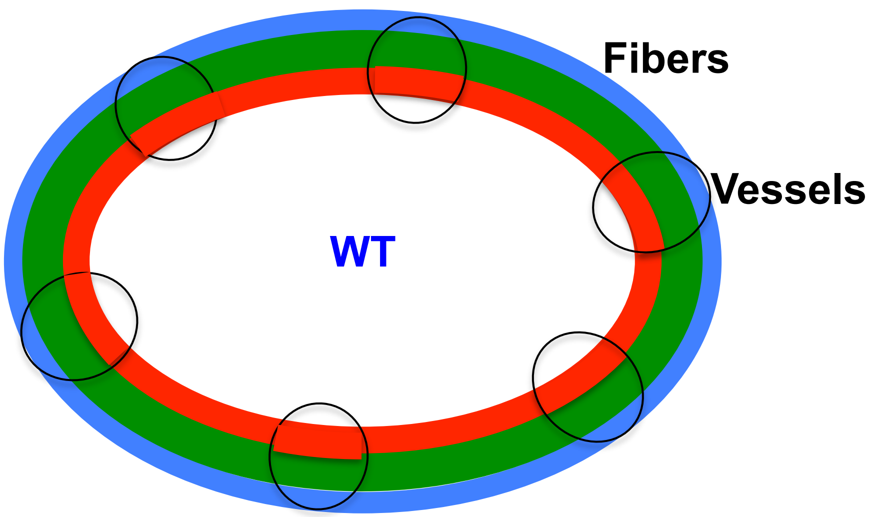
**Wild type**

**Fibers**

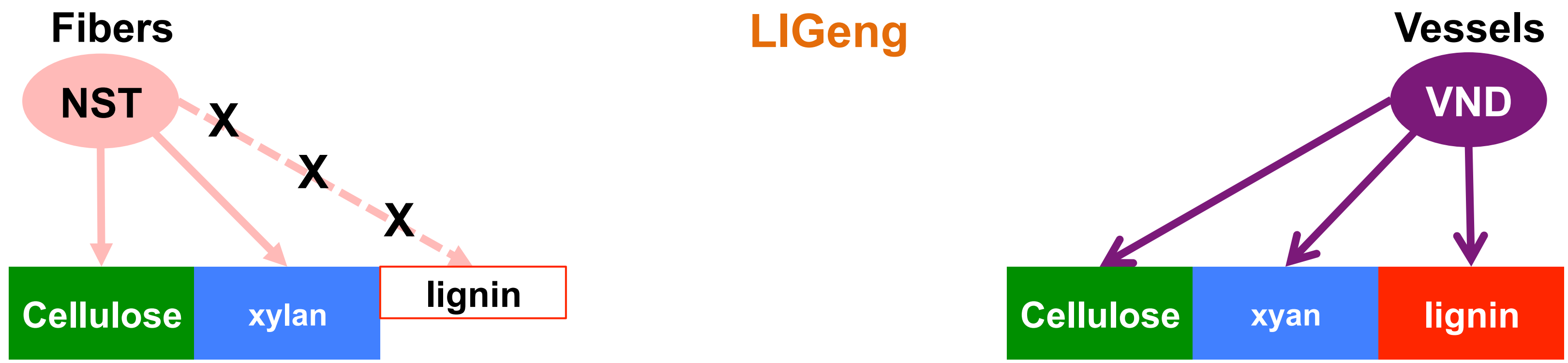


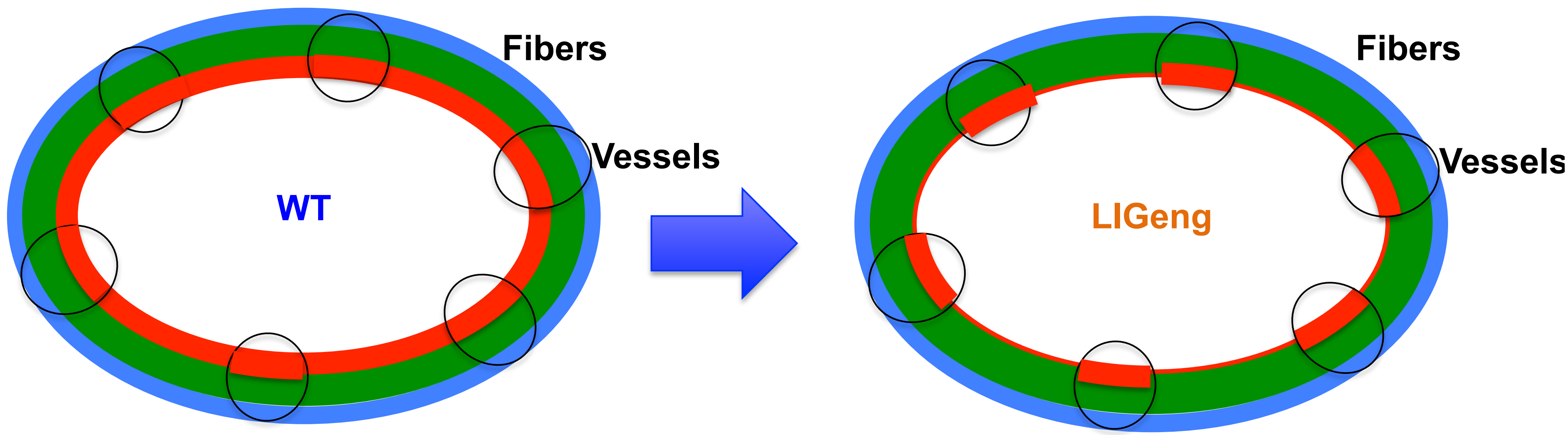

**Vessels**



# Controlling the lignin biosynthetic pathway



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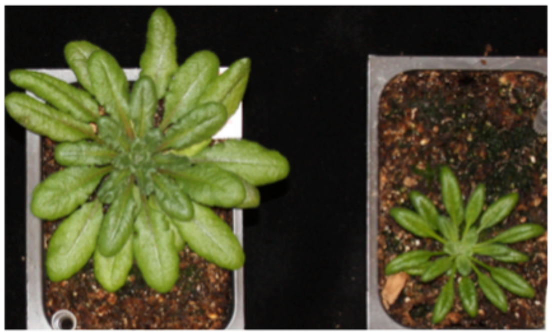
# Controlling the lignin biosynthetic pathway



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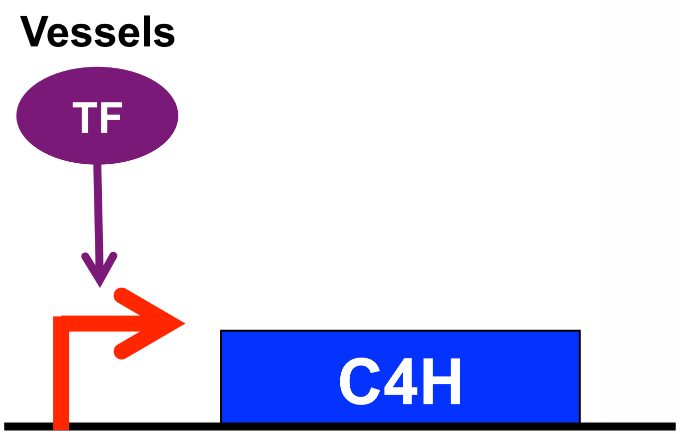
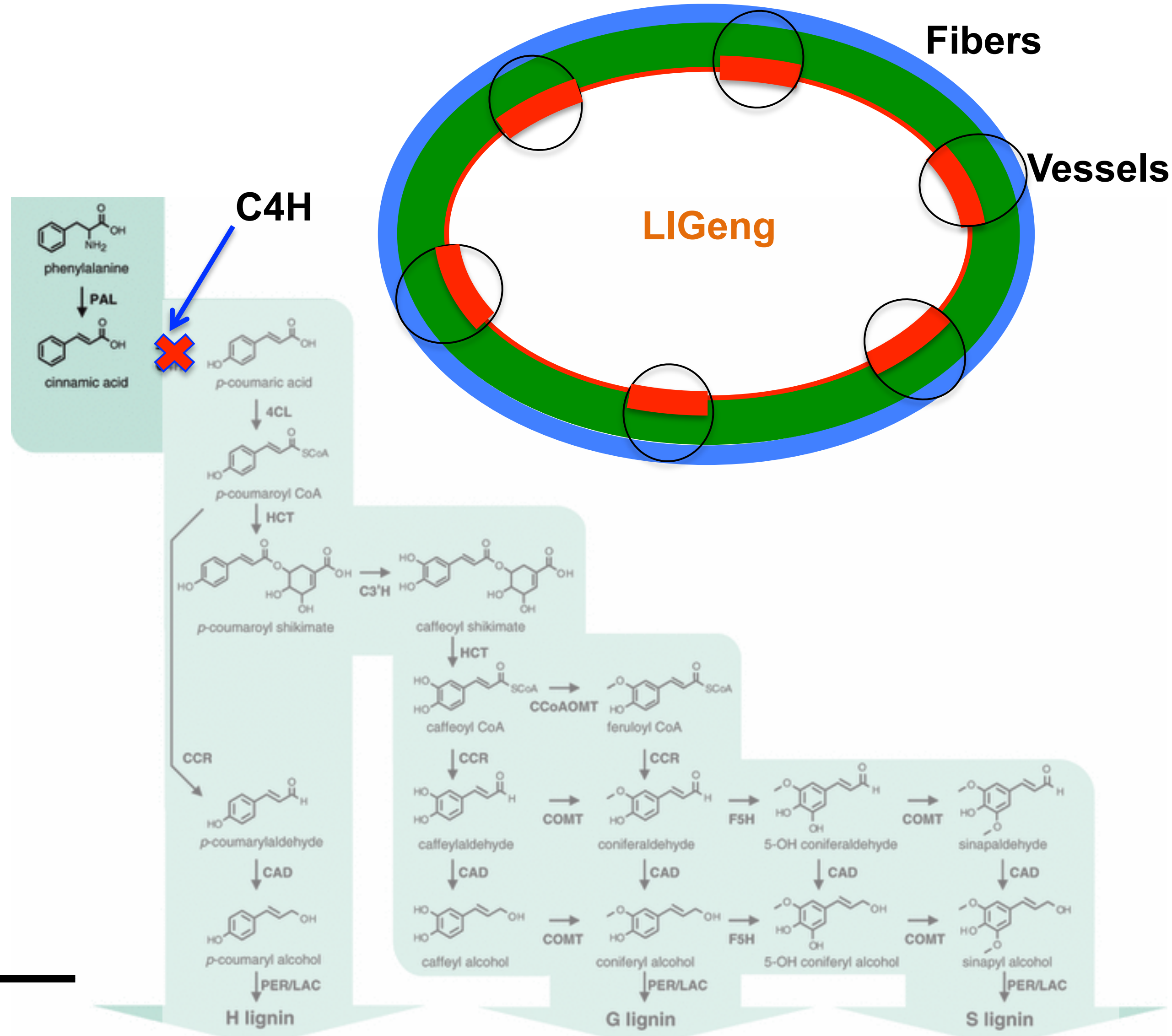
Lignin mutant




pVND

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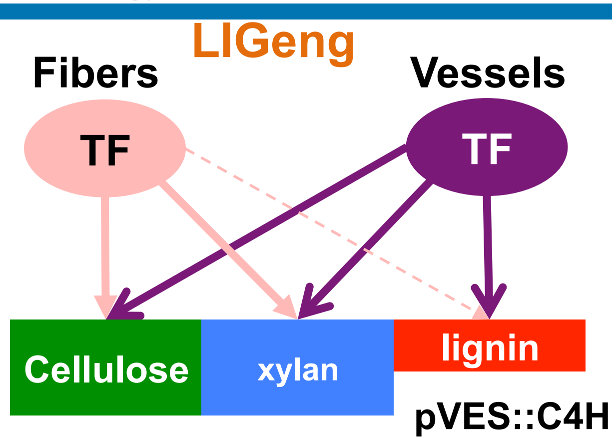
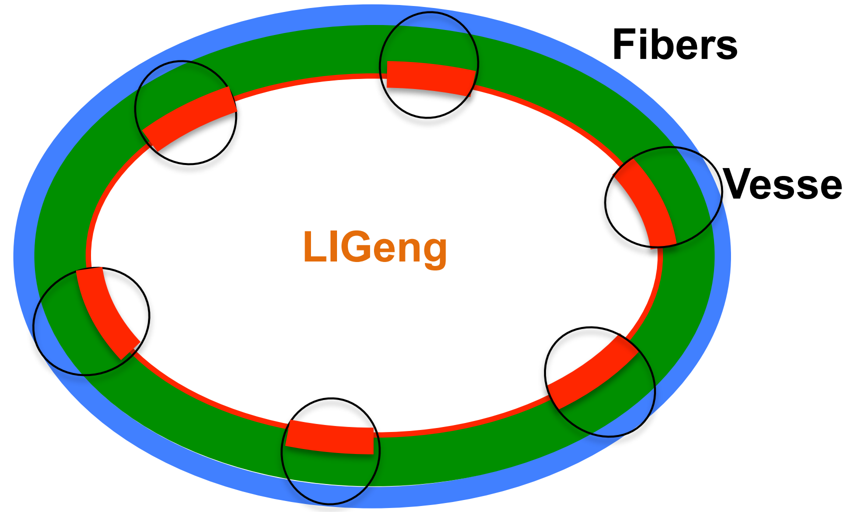
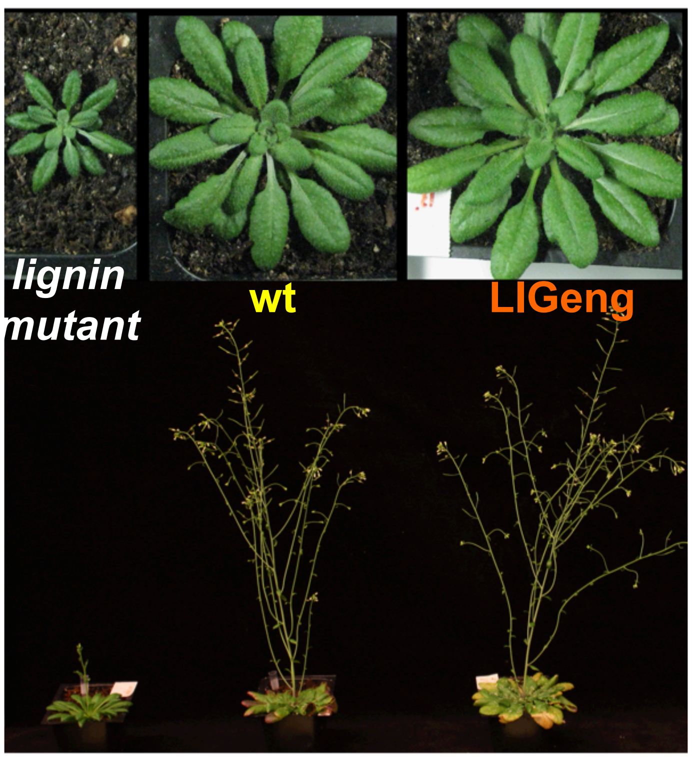
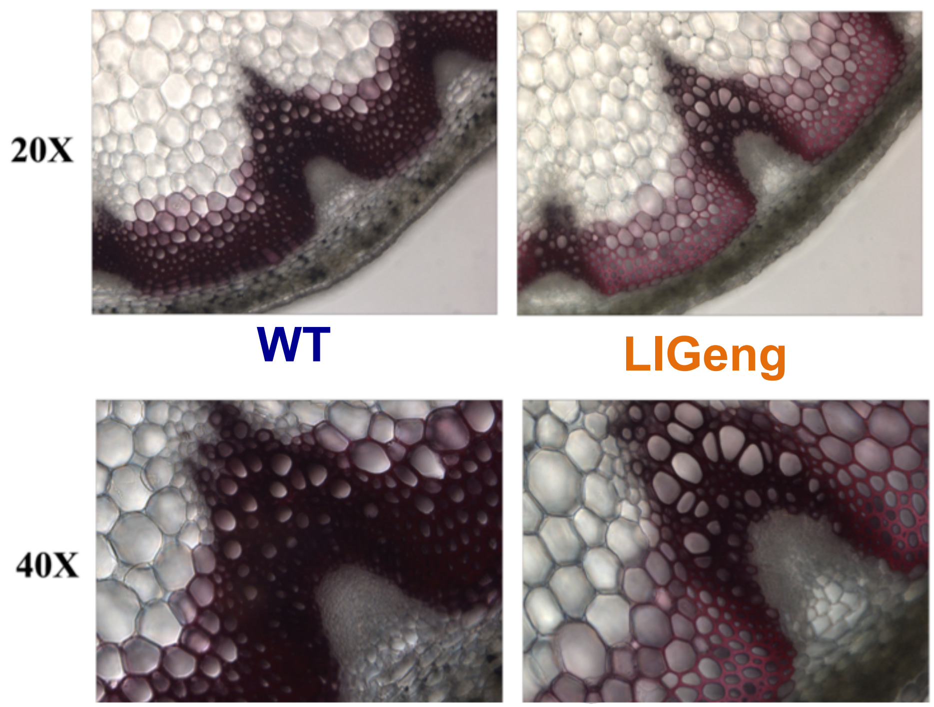
# Engineering lignin deposition in vessels

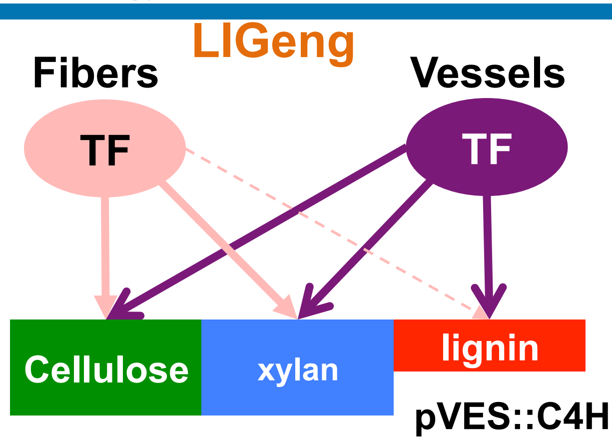


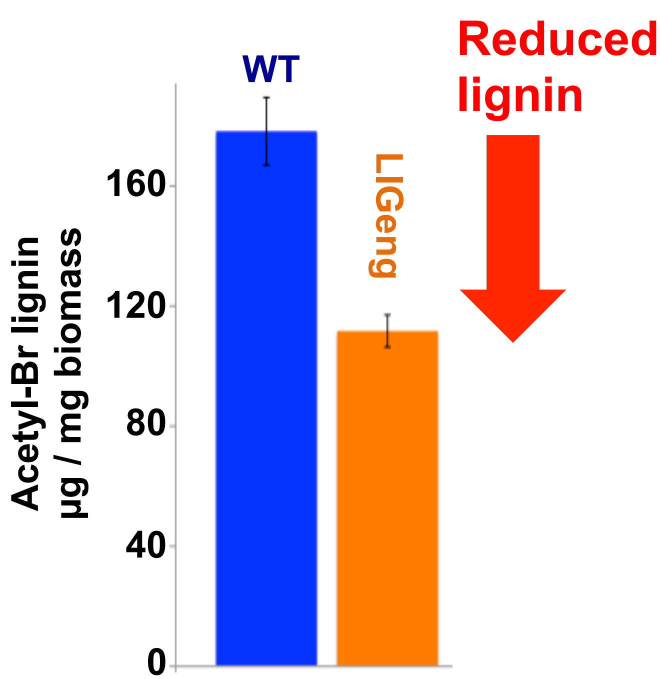



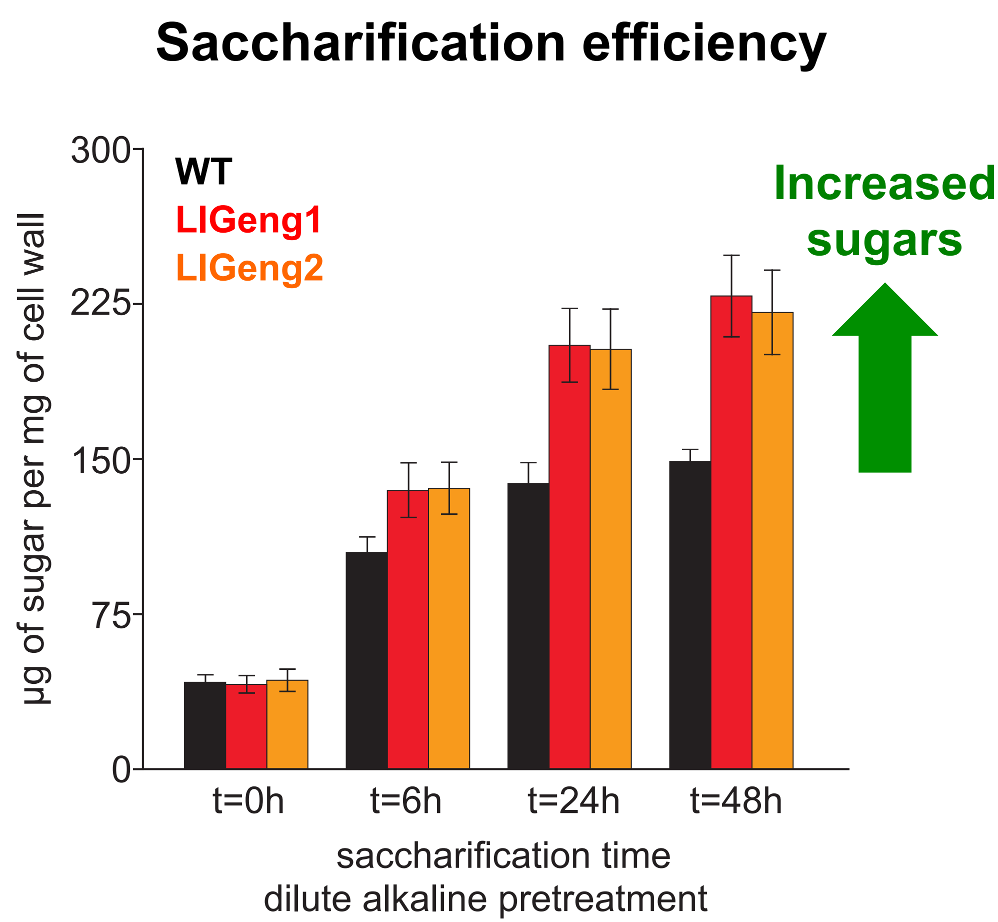



# Reduced lignin and increased sugar release

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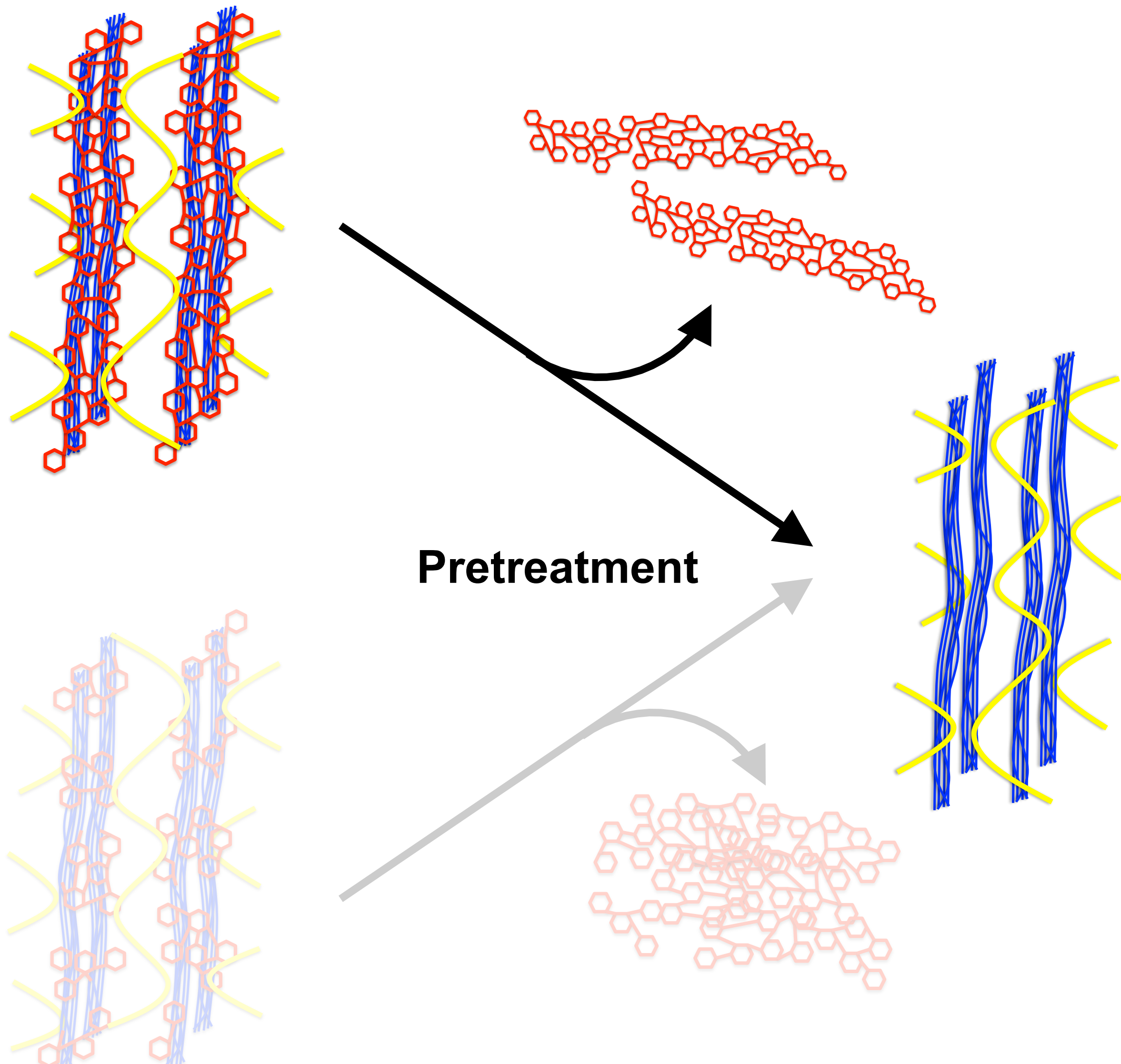
# Two approaches …
# ...better pretreatment methods

**Develop better pretreatment methods**

**Engineer plants to have modified lignin**

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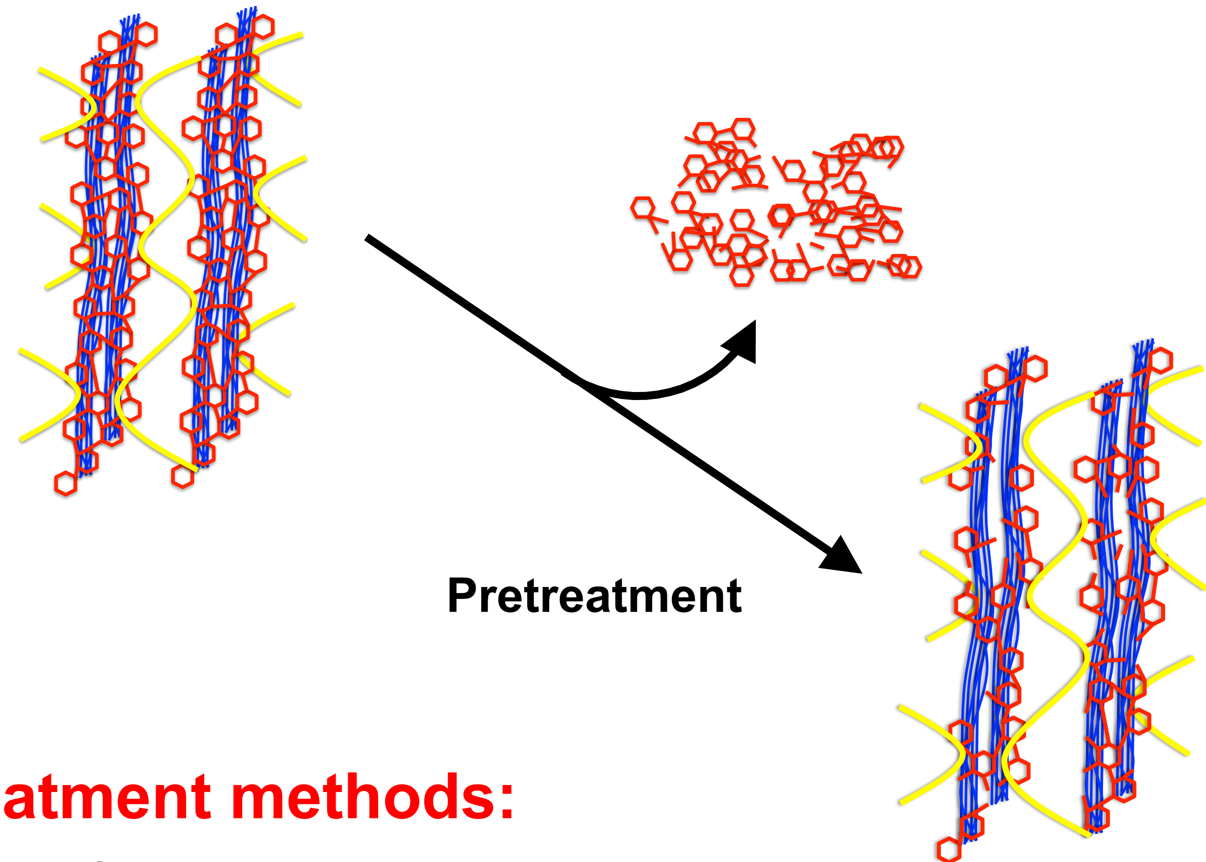
# Traditional pretreatment methods

**Develop better pretreatment methods**




## Traditional pretreatment methods:

- Involve extremes of temperatures, pH, pressure
- Dilute acid, base, lime
- Produce cellulose/hemicellulose contaminated with lignin and various by-products

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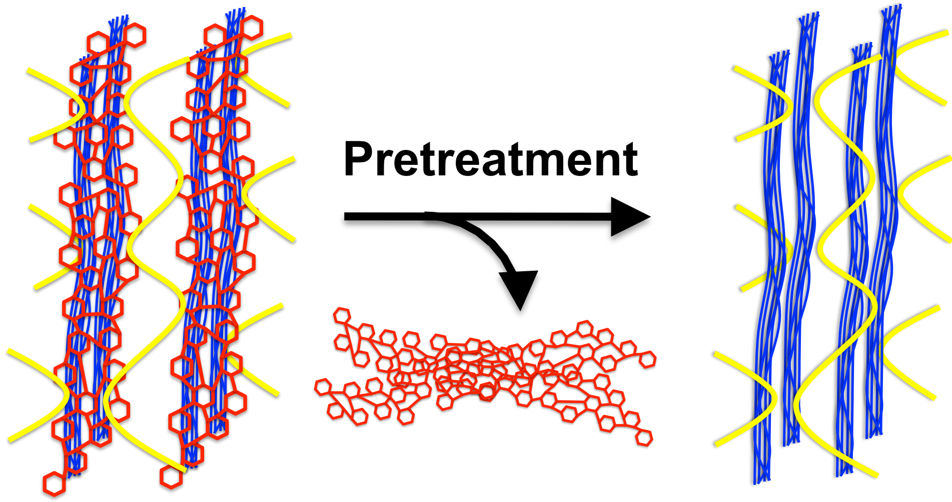
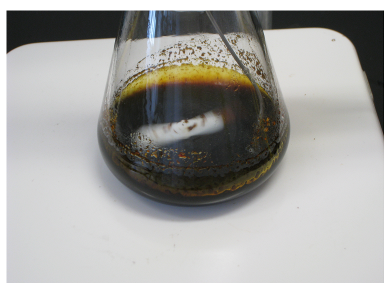
# Ionic liquids for pretreatment



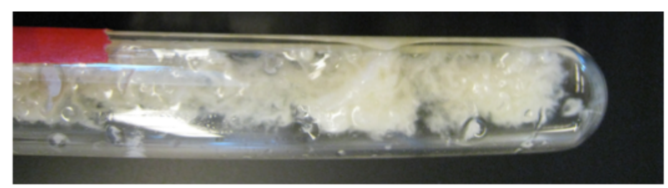

**Ionic liquids**

**Biomass**

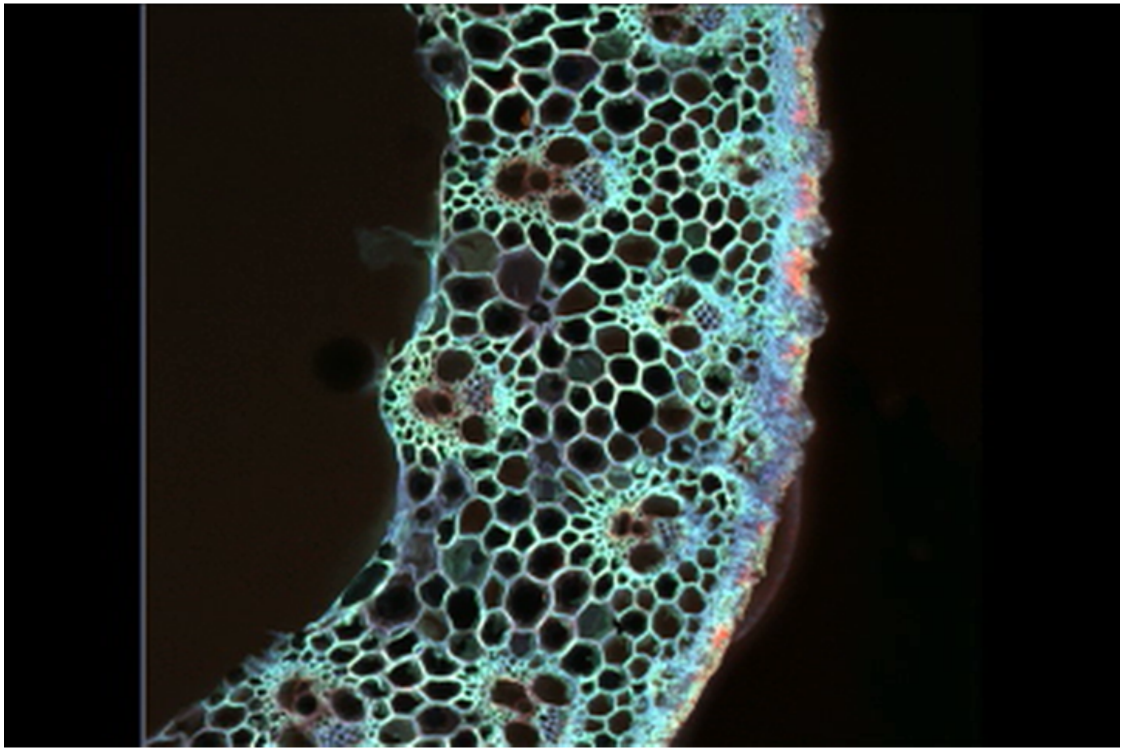
**Mix**
**Heat**
**Stir**



**Add**
**Anti-solvent**

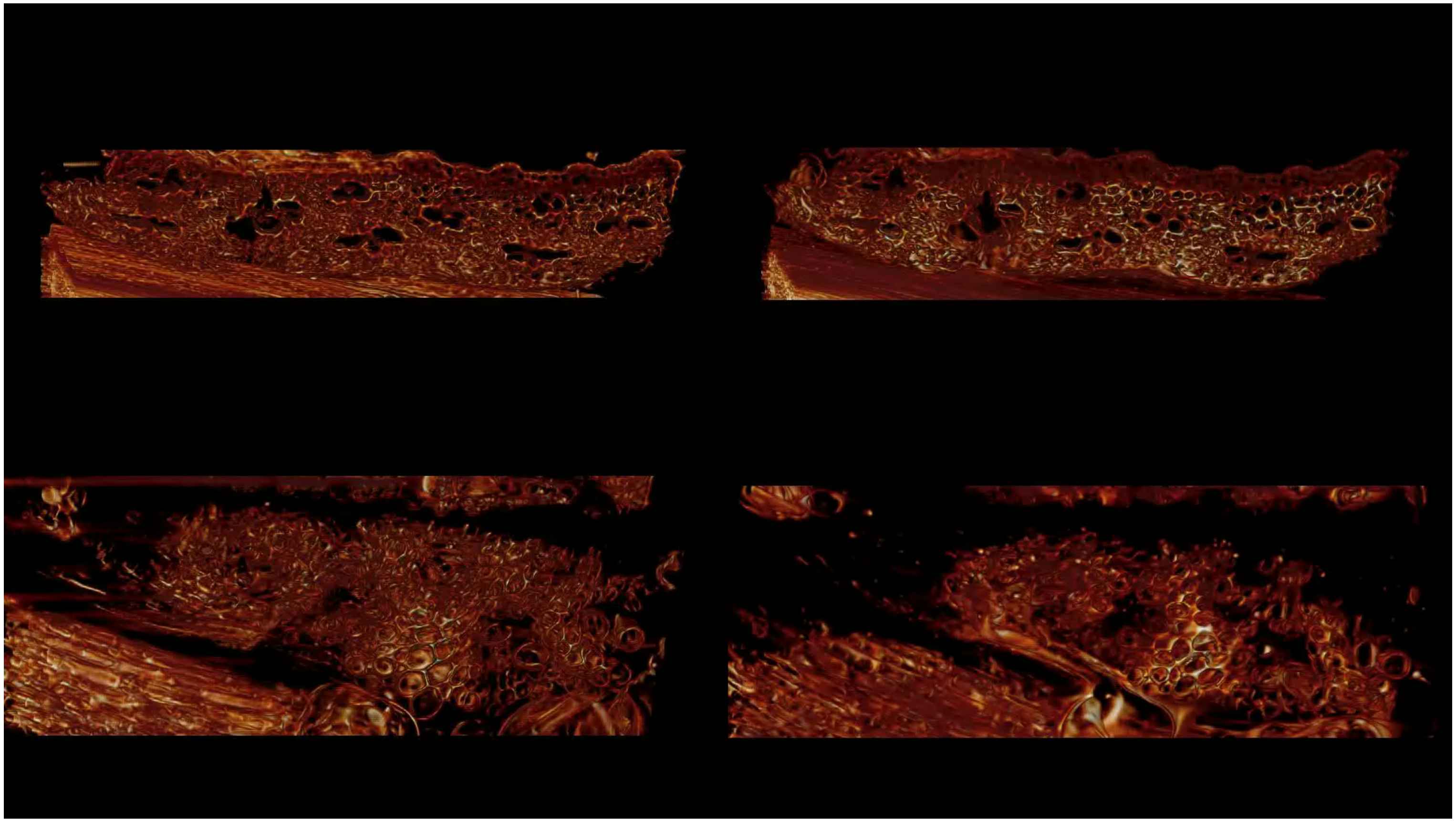


# Switchgrass undergoing IL pretreatment



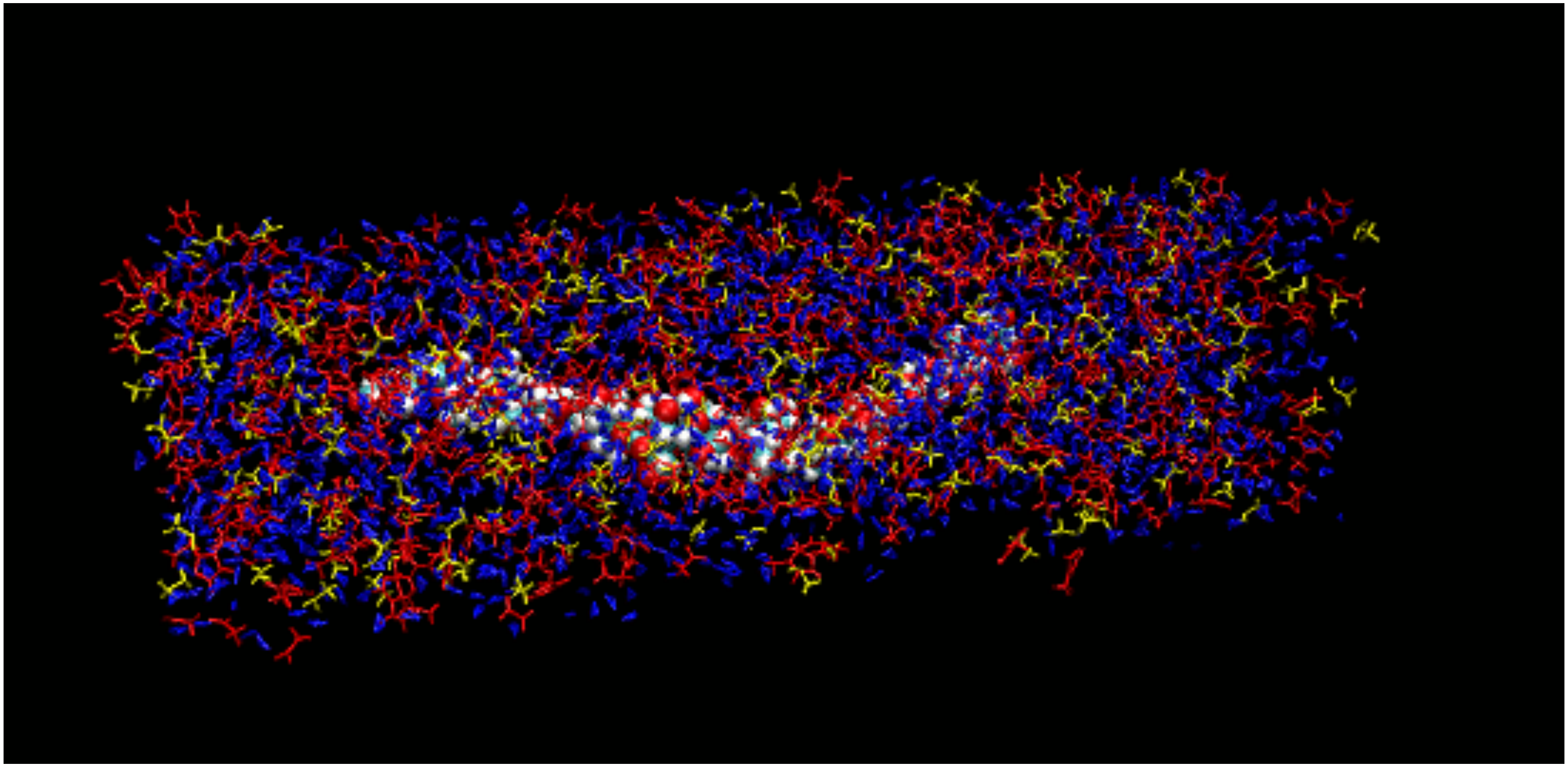
- **[$C_2$mim][OAc], 120°C**
- **In situ studies using bright field microscopy**
- **Complementary Raman and fluorescence studies indicate that lignin is solvated first, then cellulose**

# 3D images of switchgrass in IL



- **Images taken at NCXT @ ALS (Larabell, LeGros, Parkinson)**

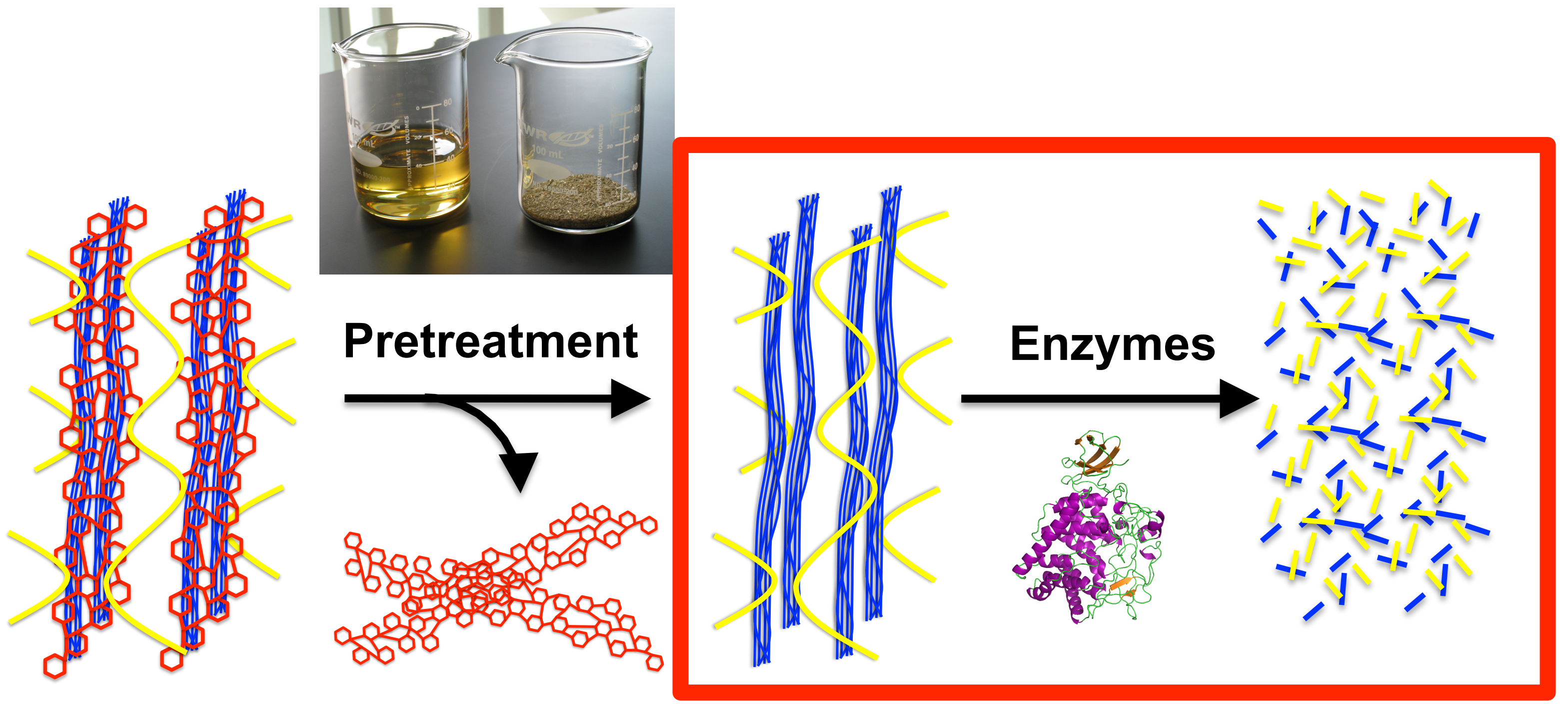
# Molecular dynamics simulations of cellulose in ionic liquids



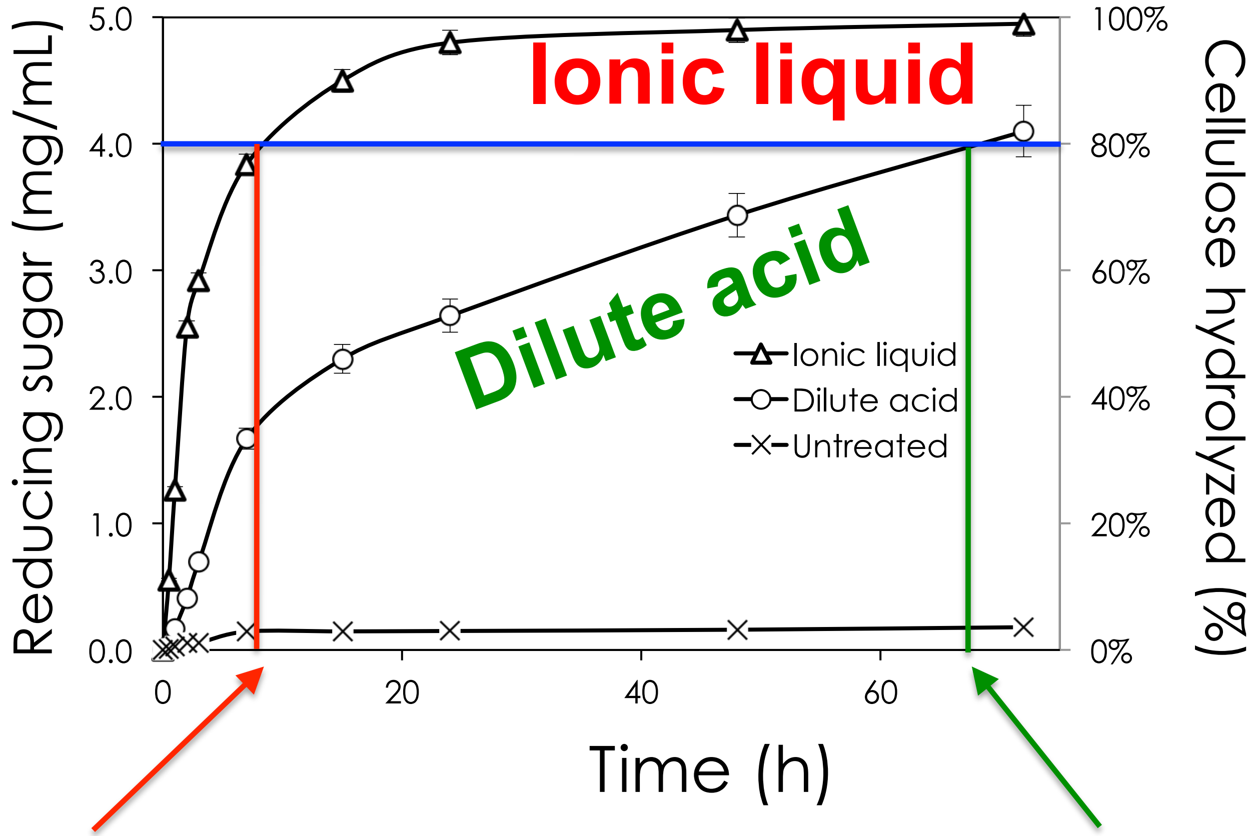
- **20-mer of cellulose; [$C_2$mim][OAc]/$H_2O$ (w/w) = 50/50**
- **MD simulations conducted at NERSC**

**Liu et al., *Journal of Physical Chemistry* (2011), 115(34), 10251-10258.**

# Does pretreatment with ionic liquids improve cellulose hydrolysis?



# Ionic liquid pretreated biomass is hydrolyzed more quickly by enzymes




**Ionic liquid pretreatment: 80% hydrolysis in 7 hours**

**Dilute acid pretreatment: 80% hydrolysis in 70 hours**

***Novozymes commercial cellulase cocktails***

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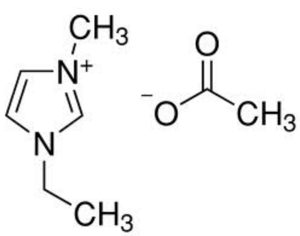
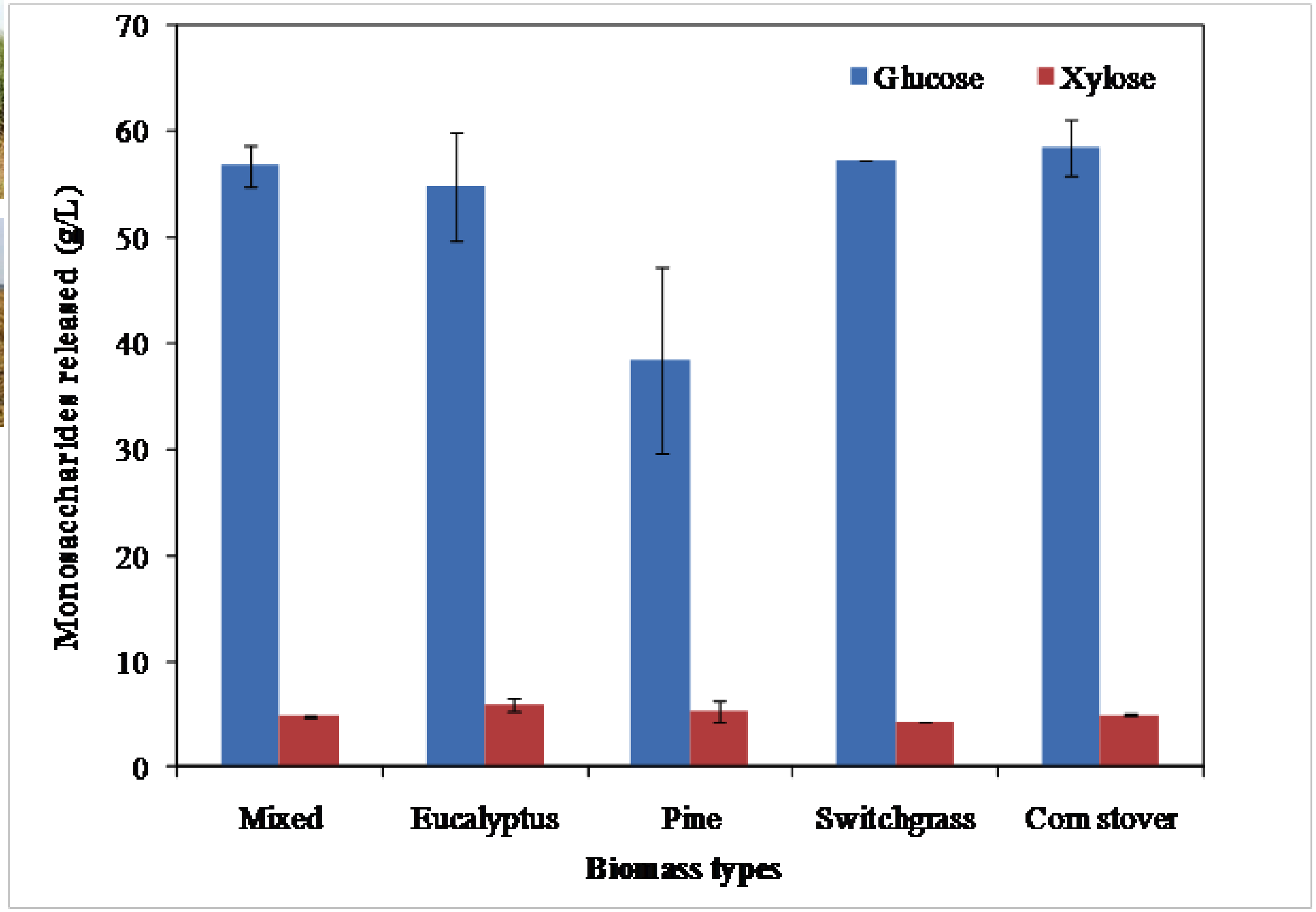
# IL pretreatment of mixed feedstocks






novozymes
Rethink Tomorrow

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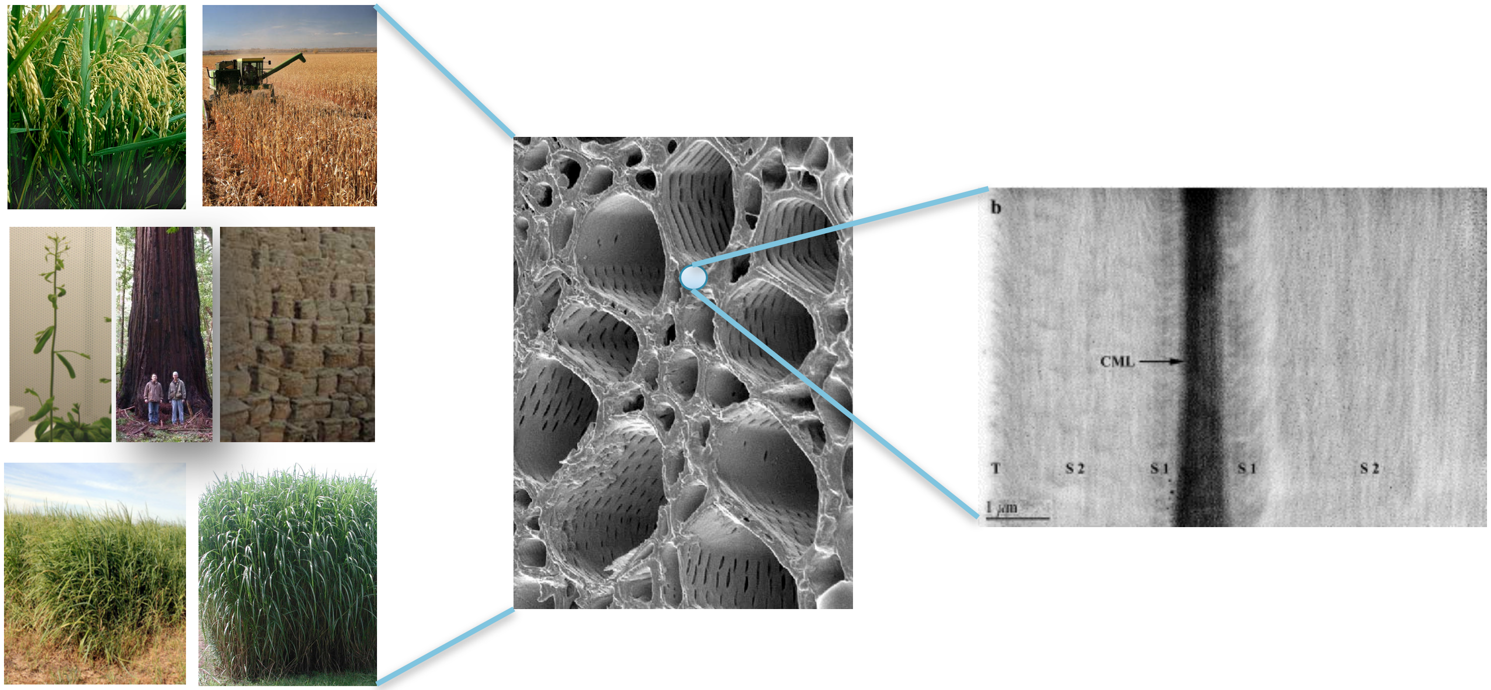
# Engineering cell wall deposition in fibers



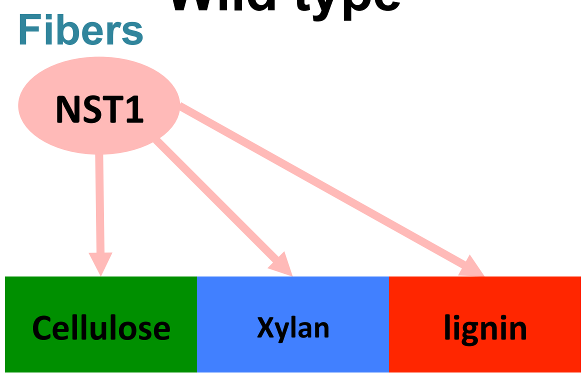

## Challenges

- High-density biomass would reduce transport costs and increase fuel yields
- More sugar but less lignin is preferable

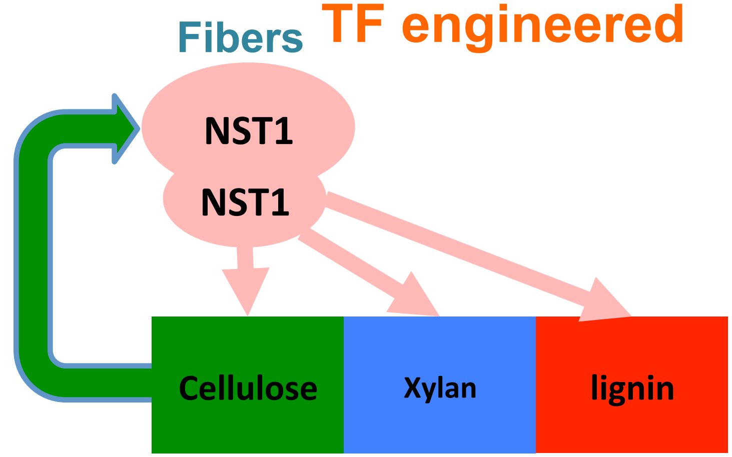
# Engineering cell wall deposition in fibers - the positive feedback loop

**Increase of secondary cell wall biosynthesis in fiber cells**

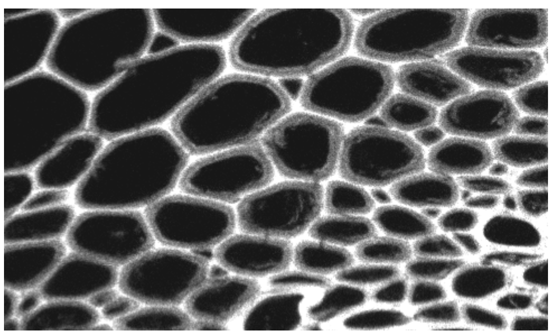
**Wild type**

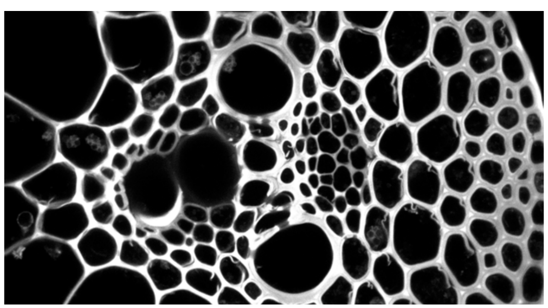



**TF engineered**




**before**





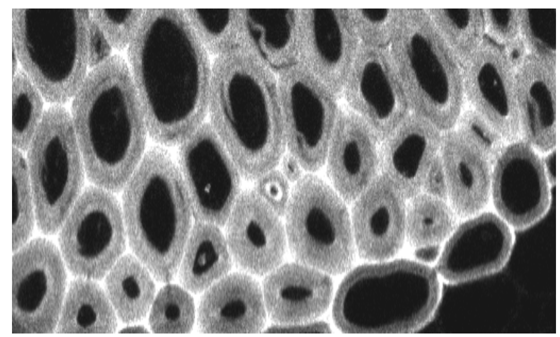
**interfascicular fibers**

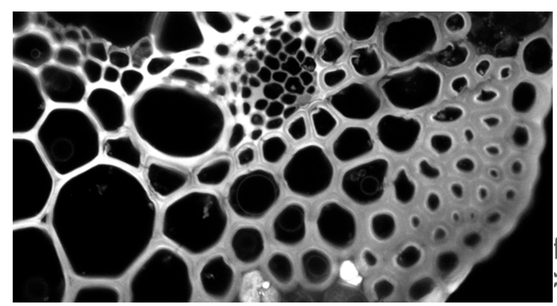
in *Arabidopsis* (dicots)

in *Brachypodium* (grasses)

Yang, Mitra *et al.*, *in prep*

**after**





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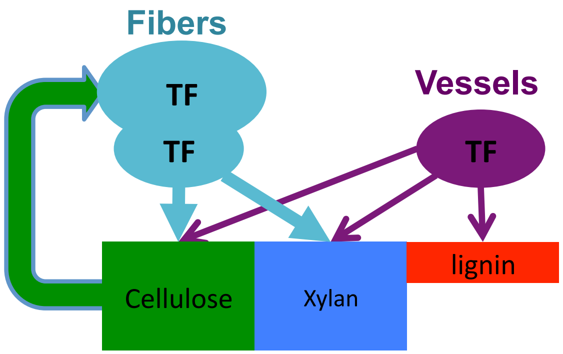
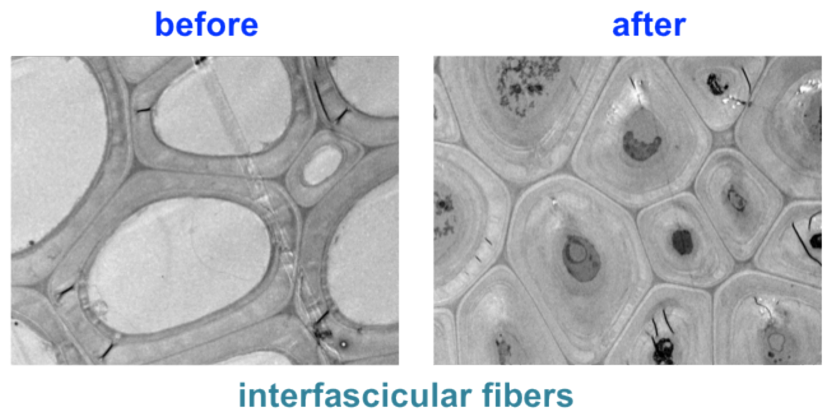
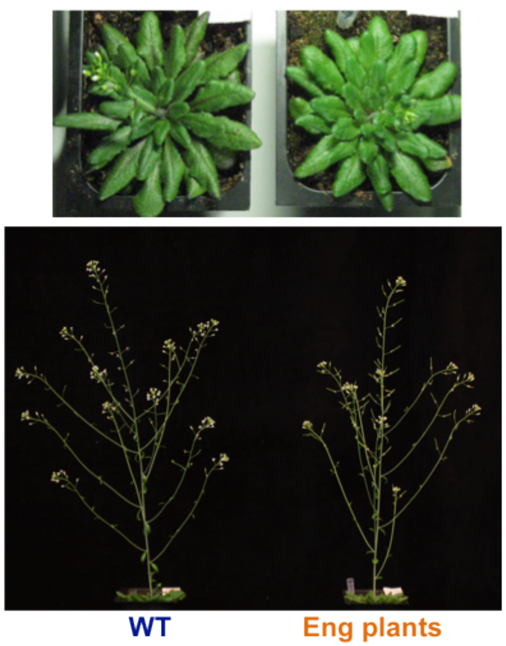
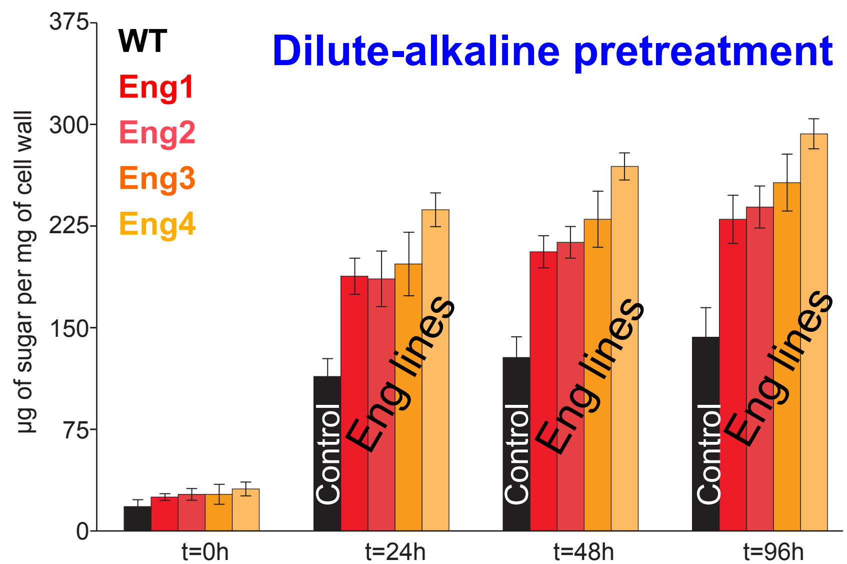
# Stacking technologies: Engineered plants with low lignin and high cell wall deposition

## Lignin and Fiber engineered




**Increased sugars**

**Yang, Mitra *et al.*, *submitted***

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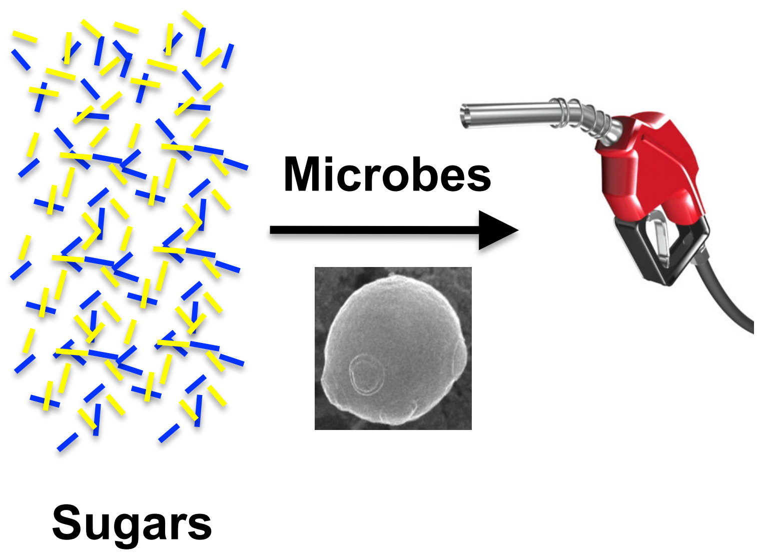
# Some key challenges in converting lignocellulosic biomass to fuels




## Challenges

- Biofuels are needed for all kinds of engines, particularly diesel and jet engines
- Many fuel-producing organisms can only utilize a fraction of the sugars from biomass

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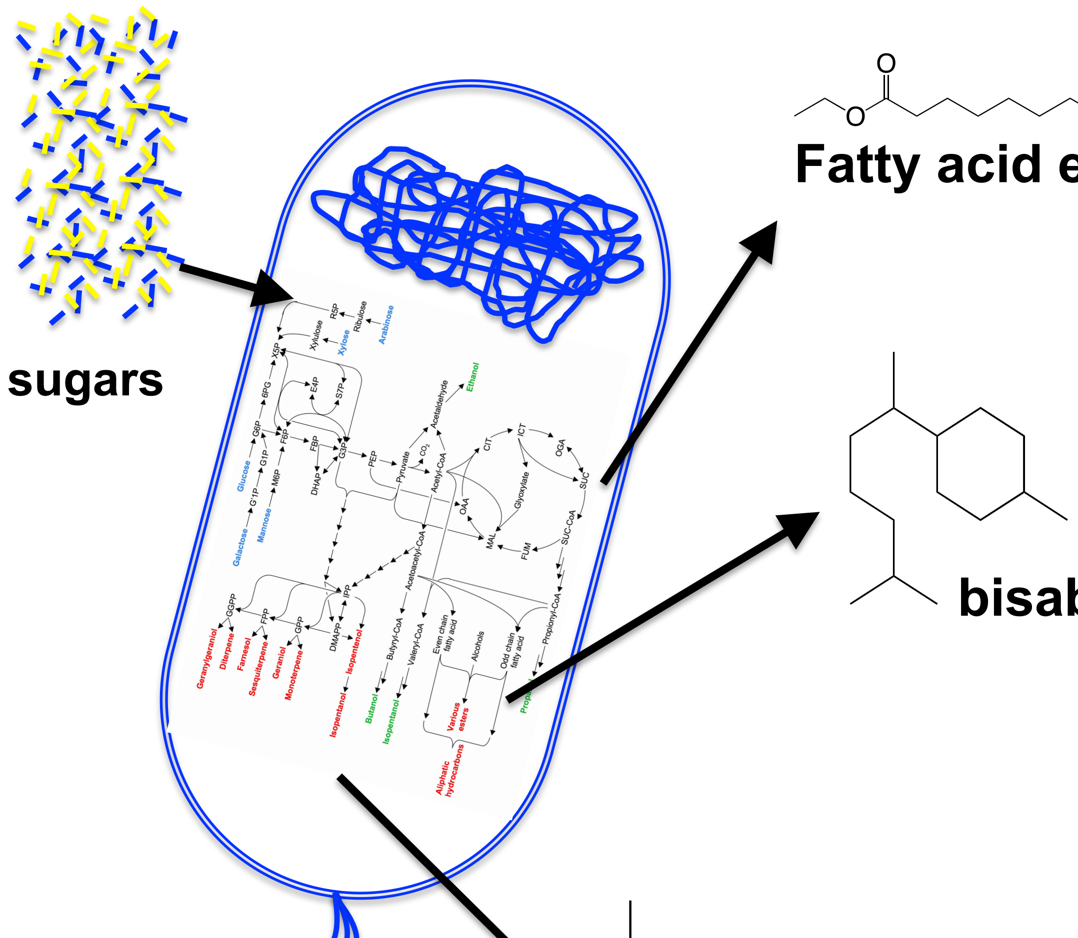
# Advanced biofuels from biomass

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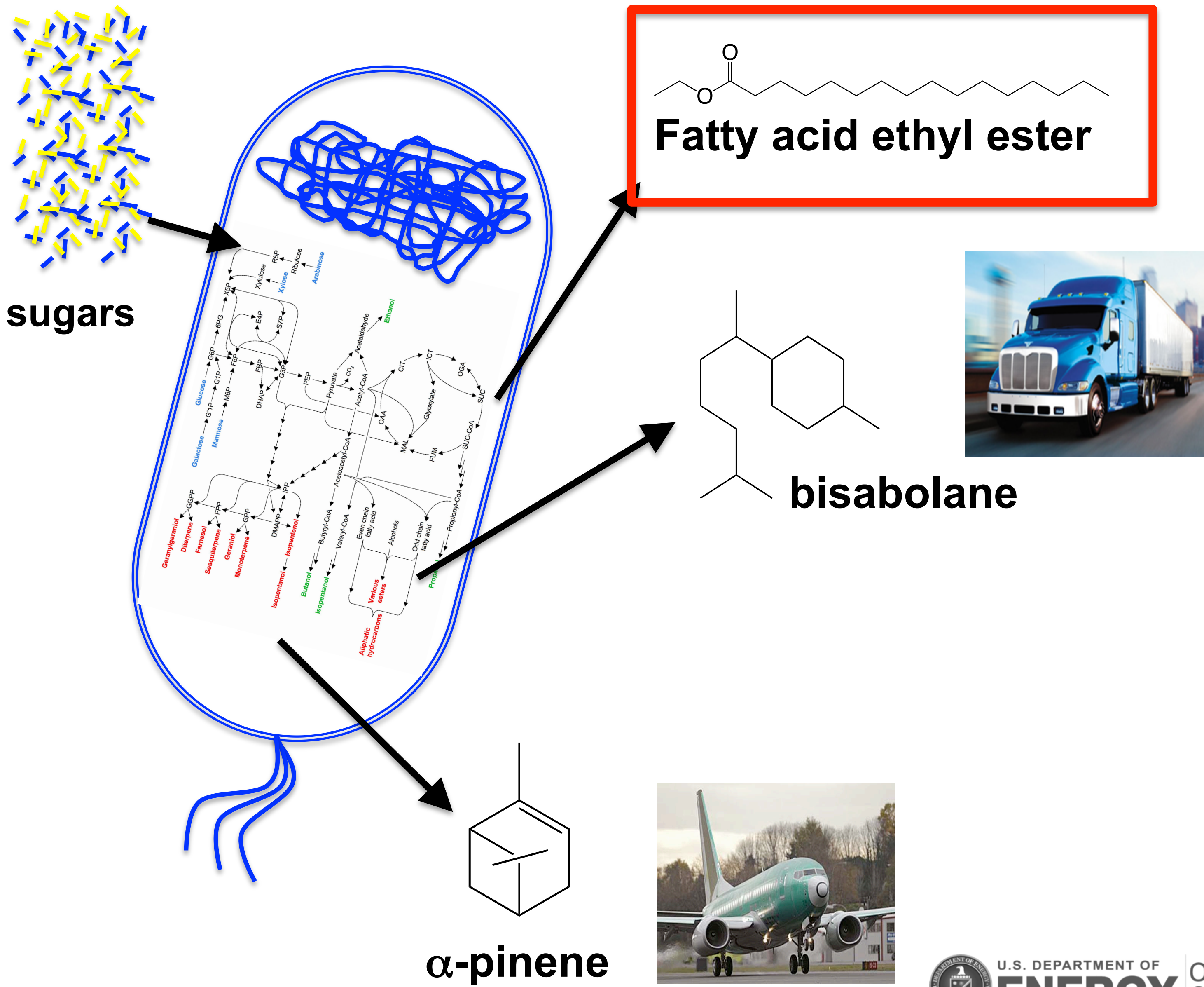
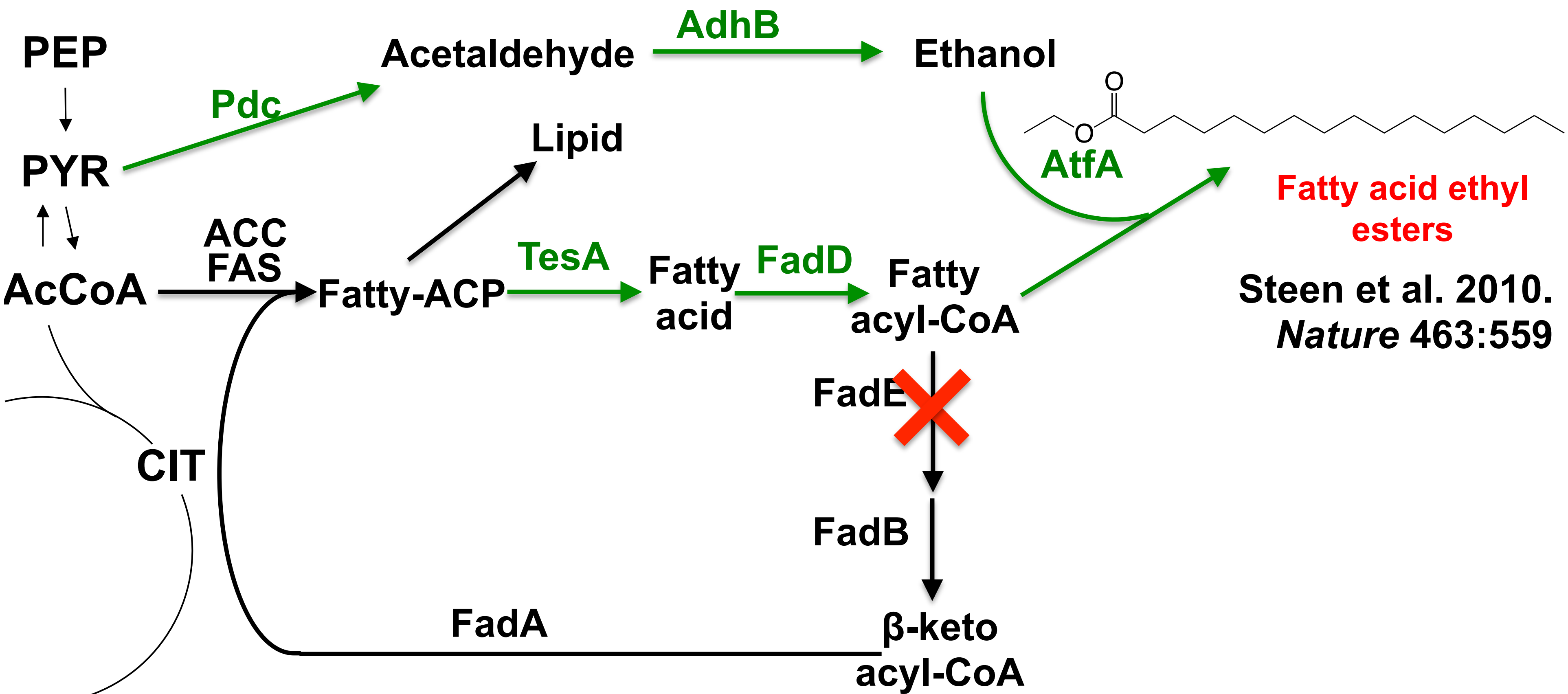
# Advanced biofuels from biomass

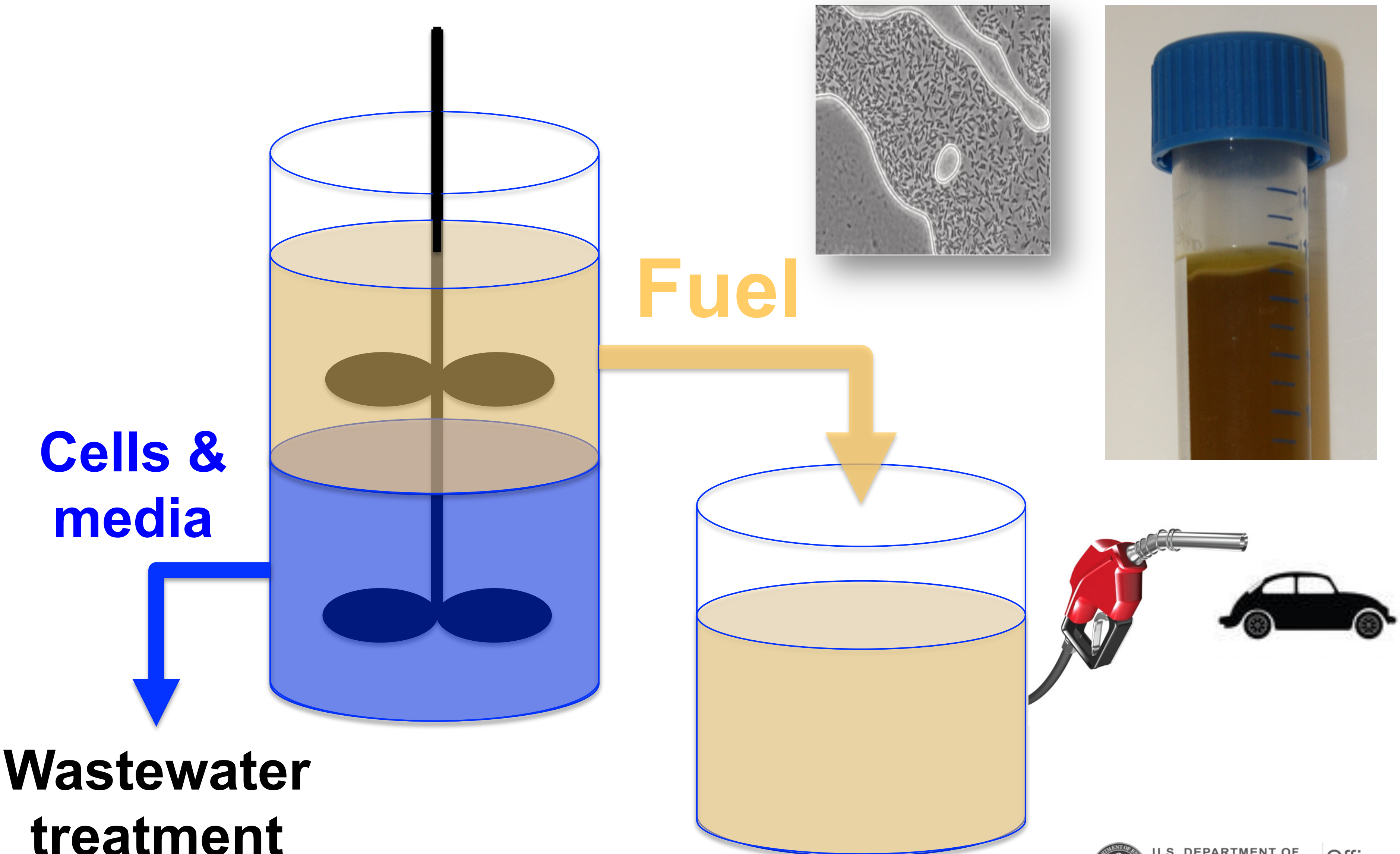


# Fatty acid ethyl esters




Steen et al. 2010. *Nature* 463:559

# Phase separation allows simple purification of fuel

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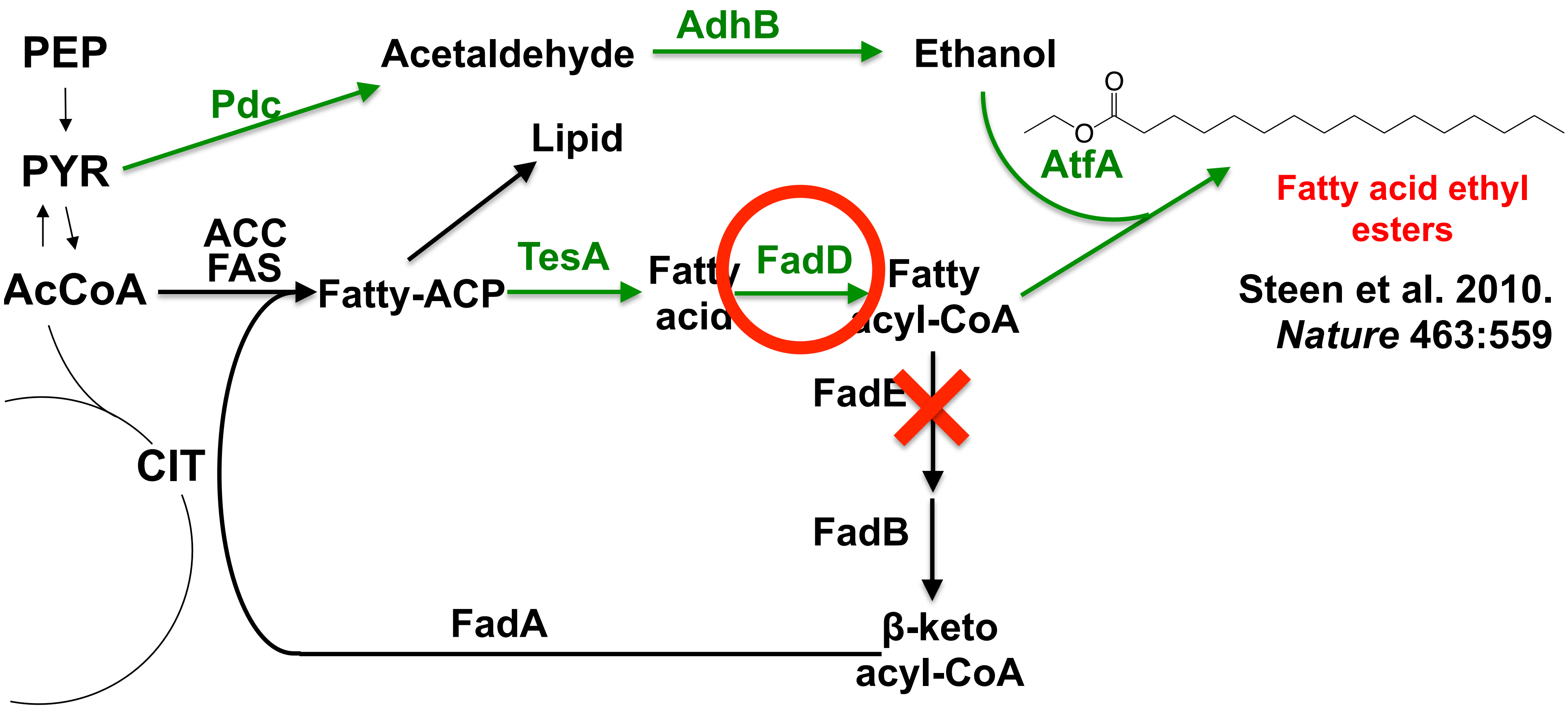
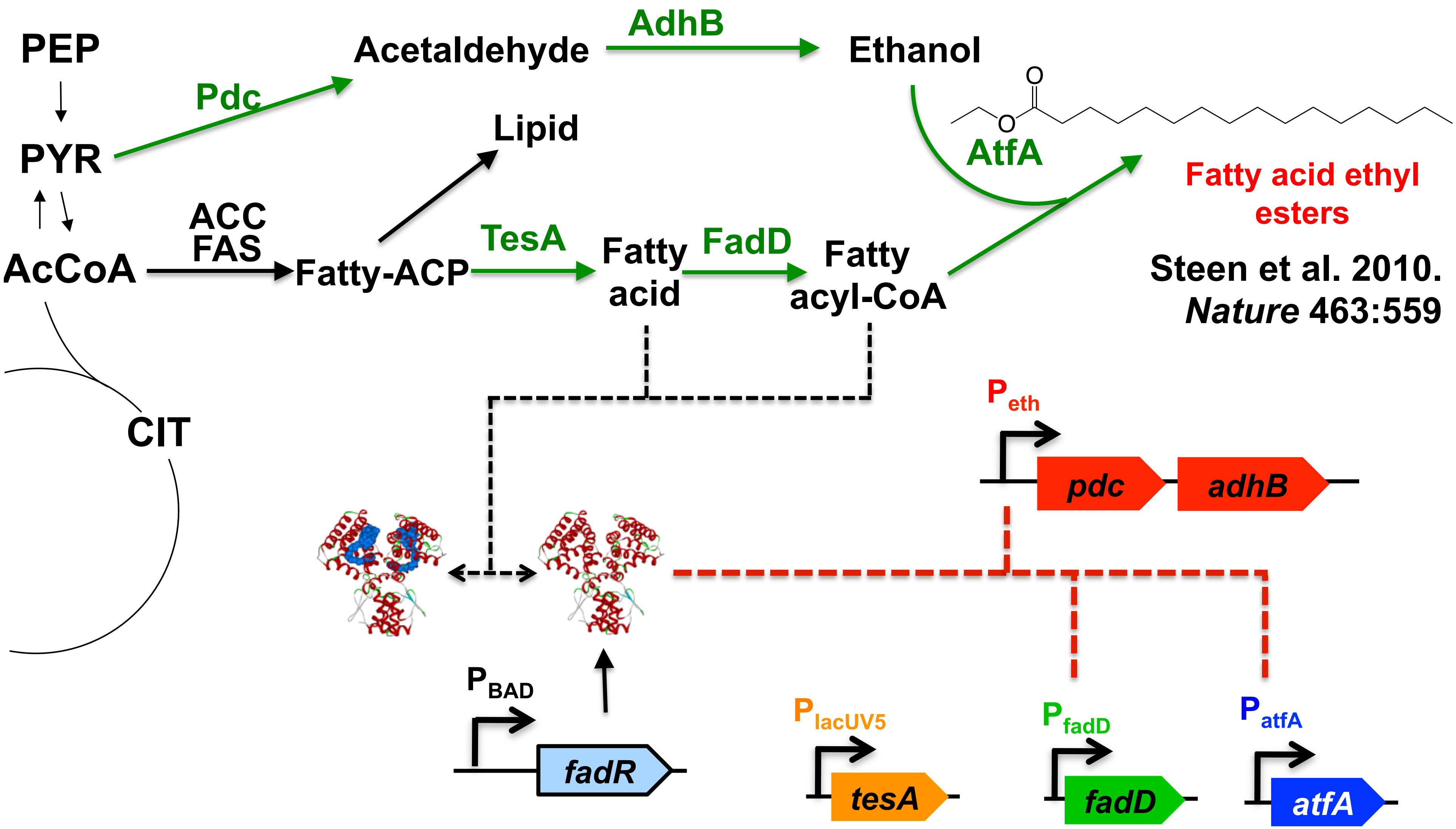
# Strain instabilities limit scale-up




Steen et al. 2010. *Nature* 463:559

***fadD* was unstable and limited process scale-up**

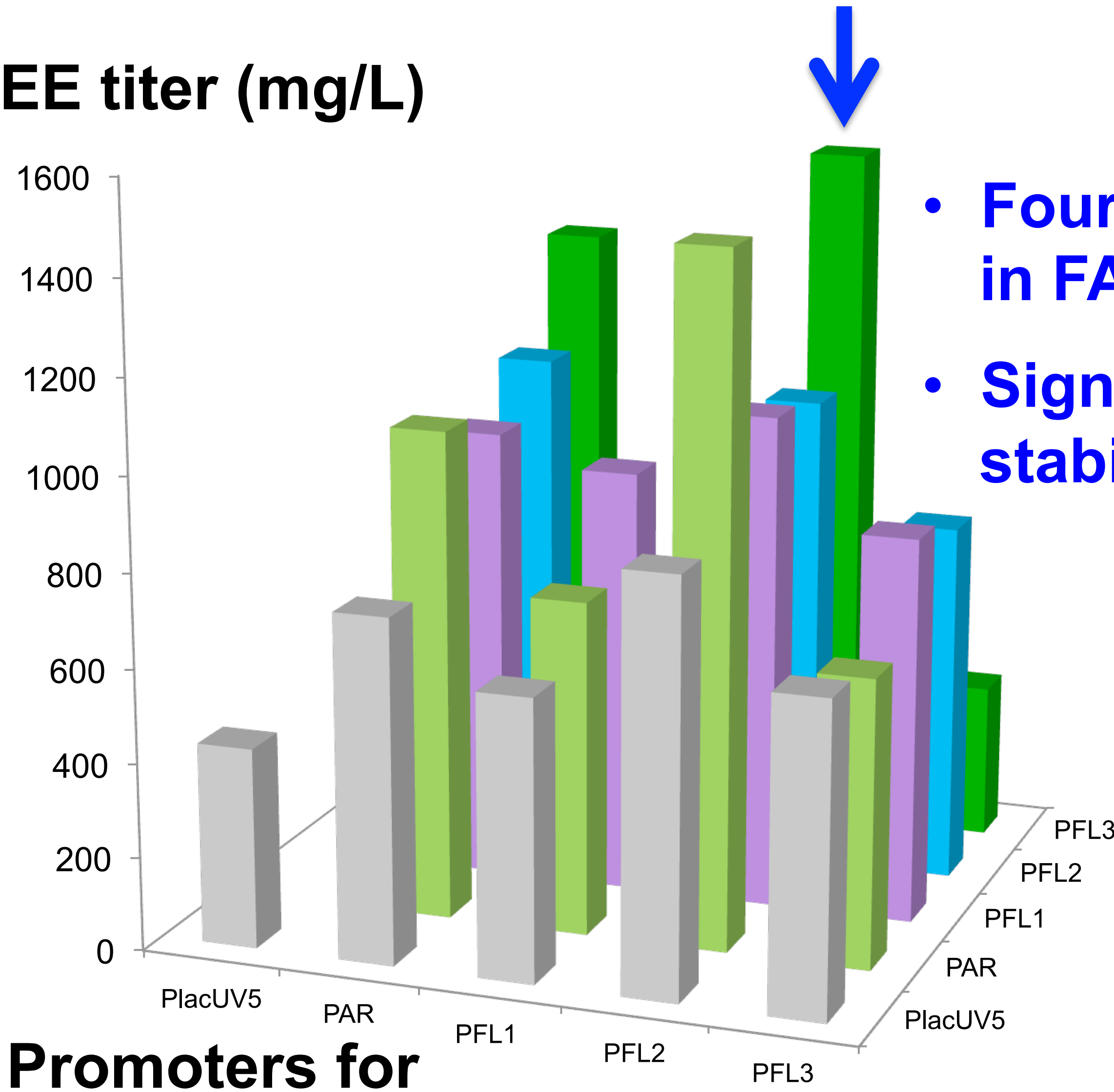
# Strain instabilities limit scale-up




Steen et al. 2010. *Nature* 463:559

# FadR regulation improves FAEE production

**FAEE titer (mg/L)**



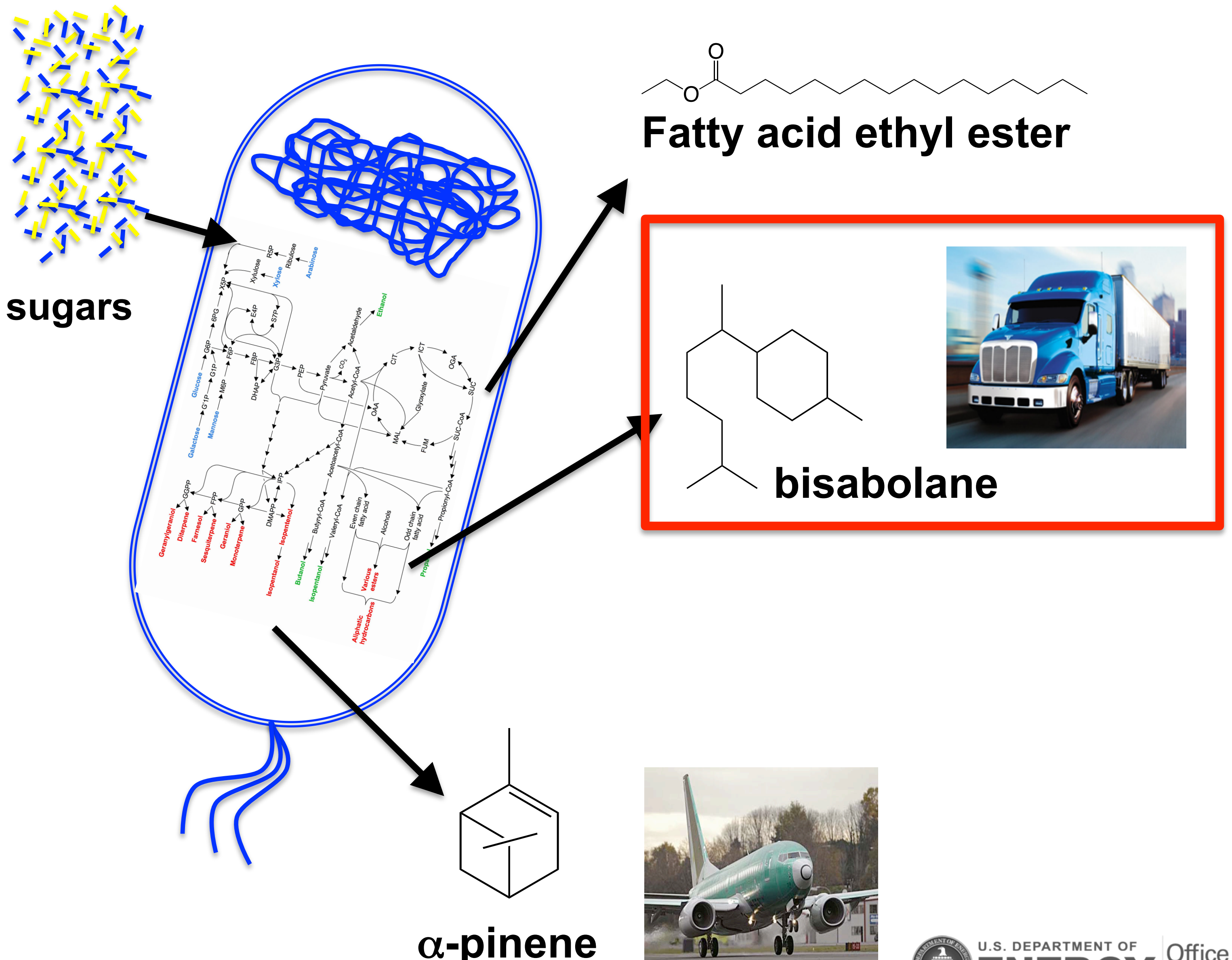

- **Four-fold improvement in FAEE production**
- **Significantly improved stability of host**

**Promoters for *atfA***

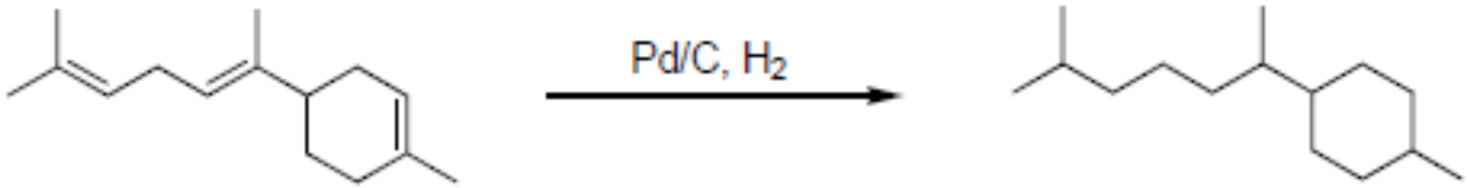
**Promoters for ethanol production**

Zhang et al. 2012 *Nat. Biotechnol.* **30**:354

# Advanced biofuels from biomass



# Bisabolane has many qualities of No. 2 diesel

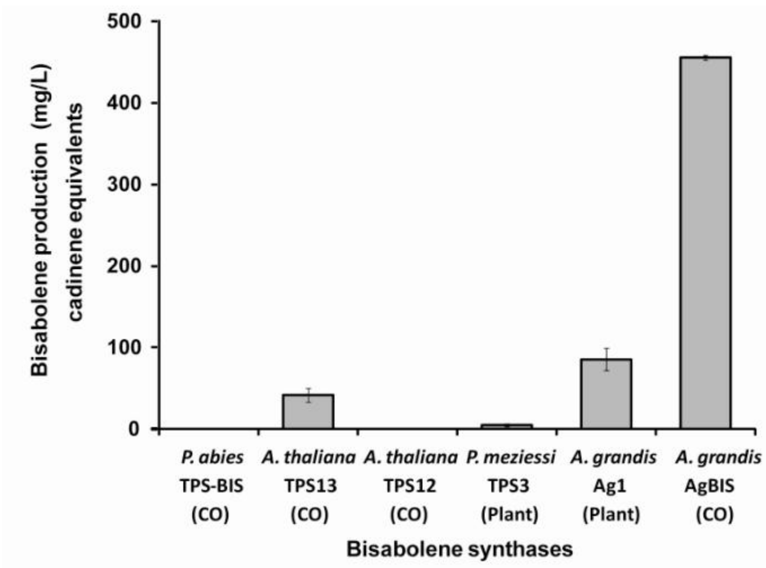


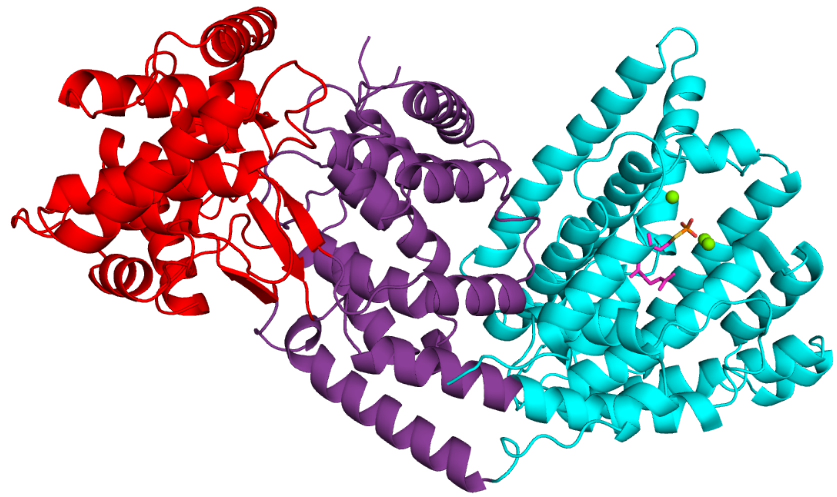

**Bisabolene** **Bisabolane**

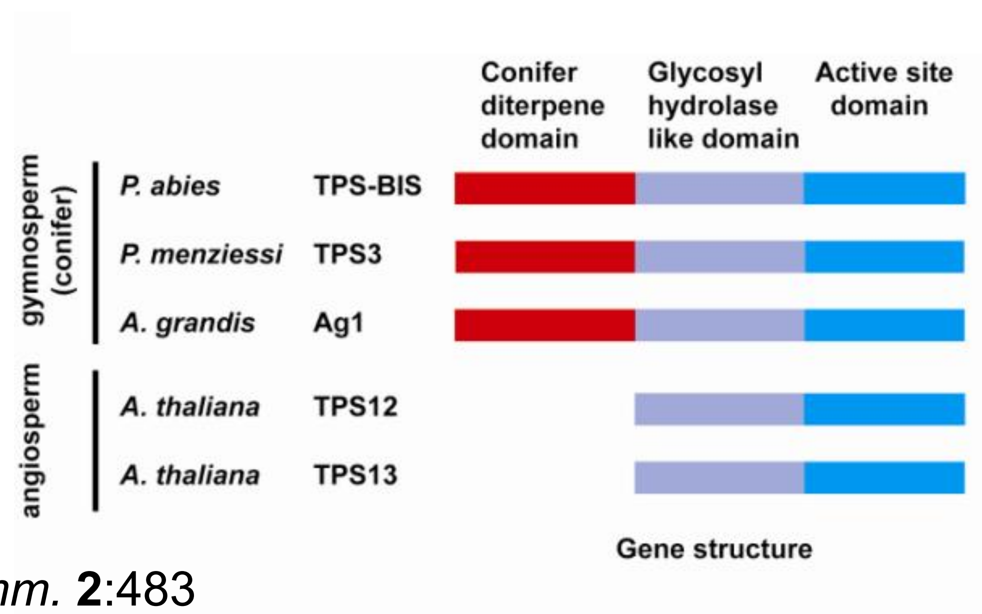
| | No 2 Diesel | Bisabolane |
|---|---|---|
| **Cetane No.** | 41.6 | 41.9 |
| **Freeze pt.** | N/a | <-81C |
| **Cloud pt.** | -21C | <-78C |
| **Density** | 864.6 g/L | 819 g/L |

Peralta-Yahya et al. 2010 *Nature Comm.* **2**:483

# Finding the best bisabolene synthase






Peralta-Yahya et al. 2010 *Nature Comm.* **2**:483

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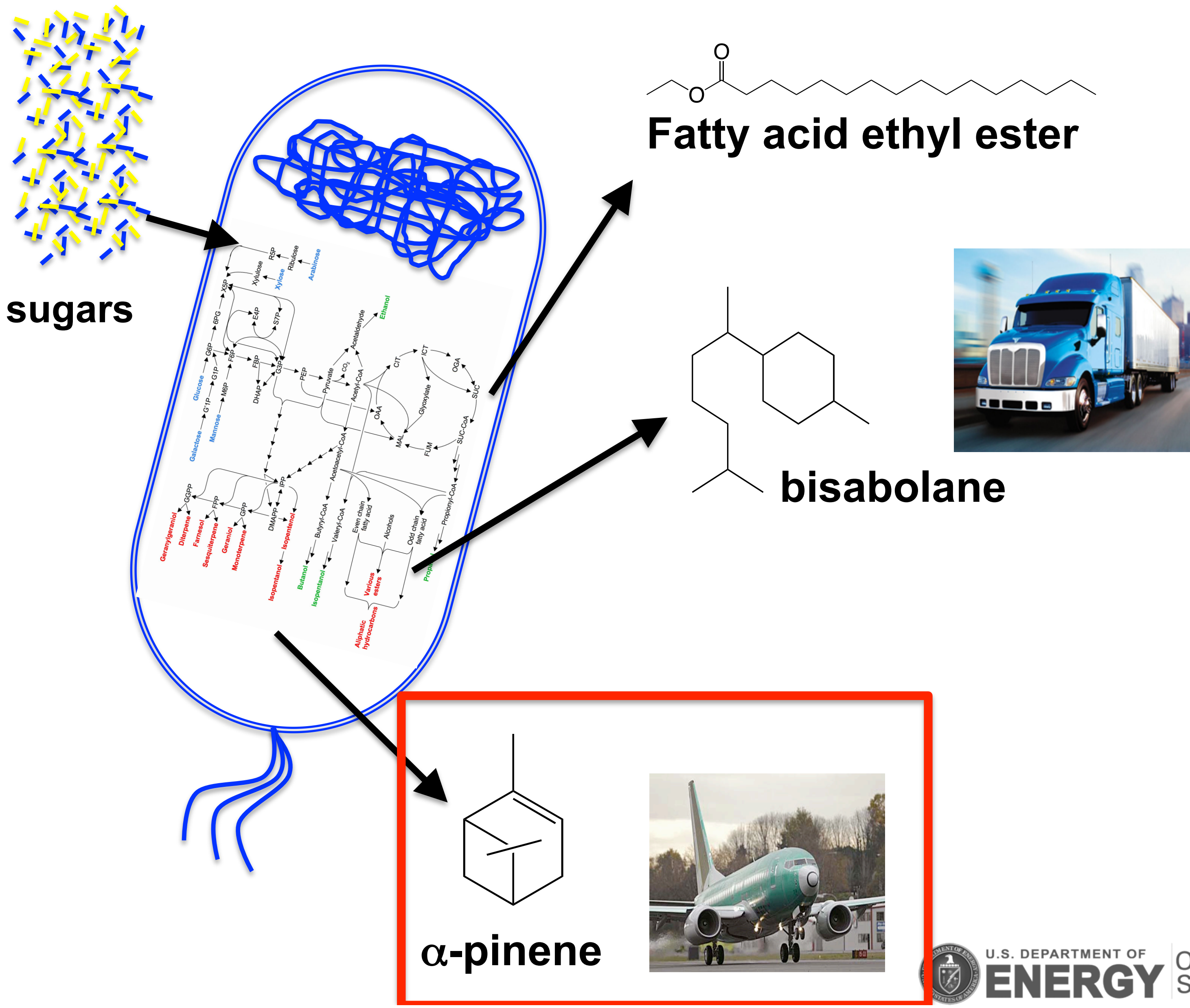
# Advanced biofuels from biomass

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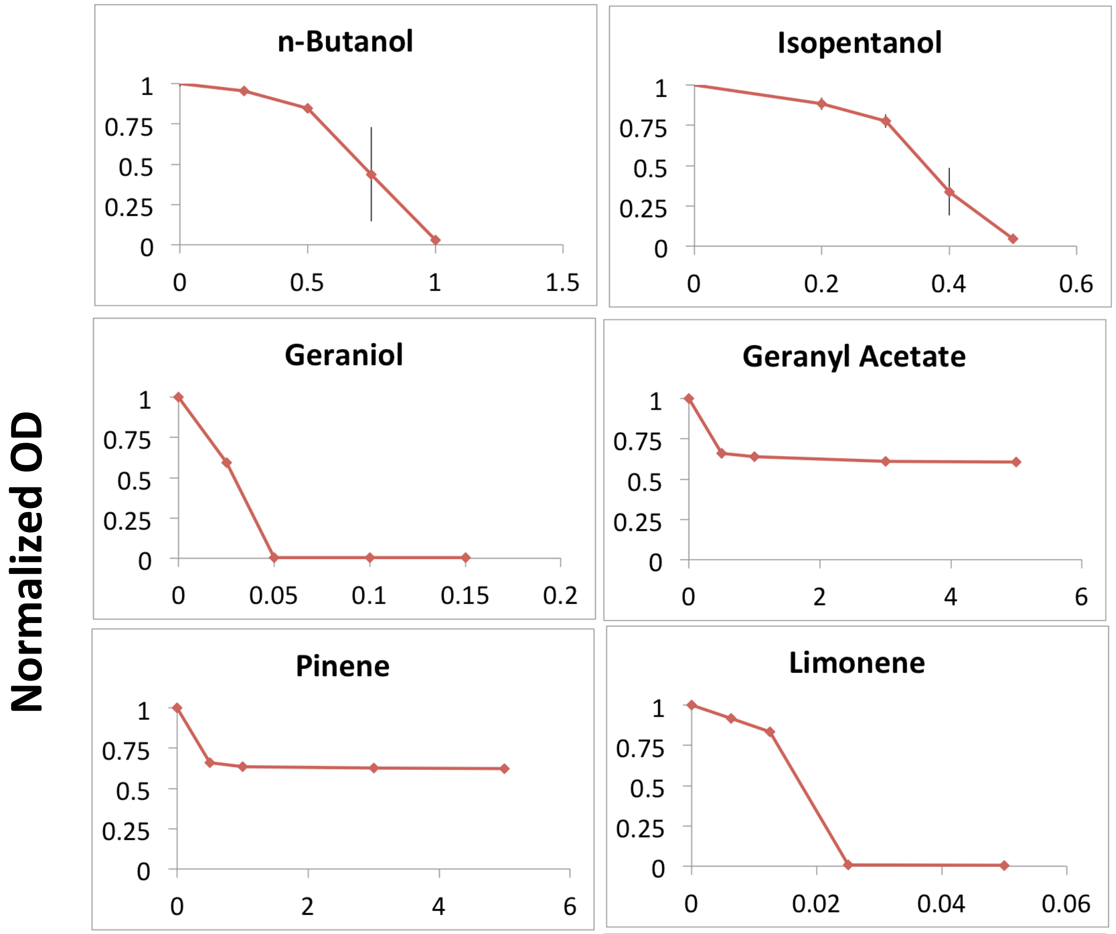
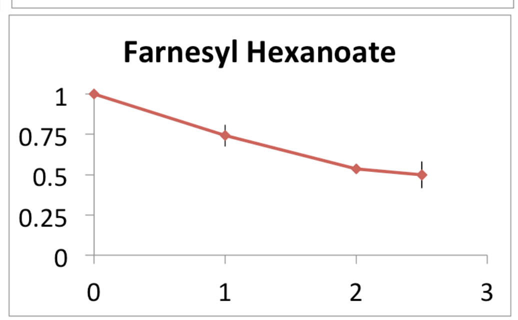
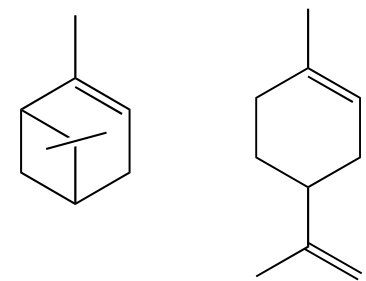
# Toxic final products



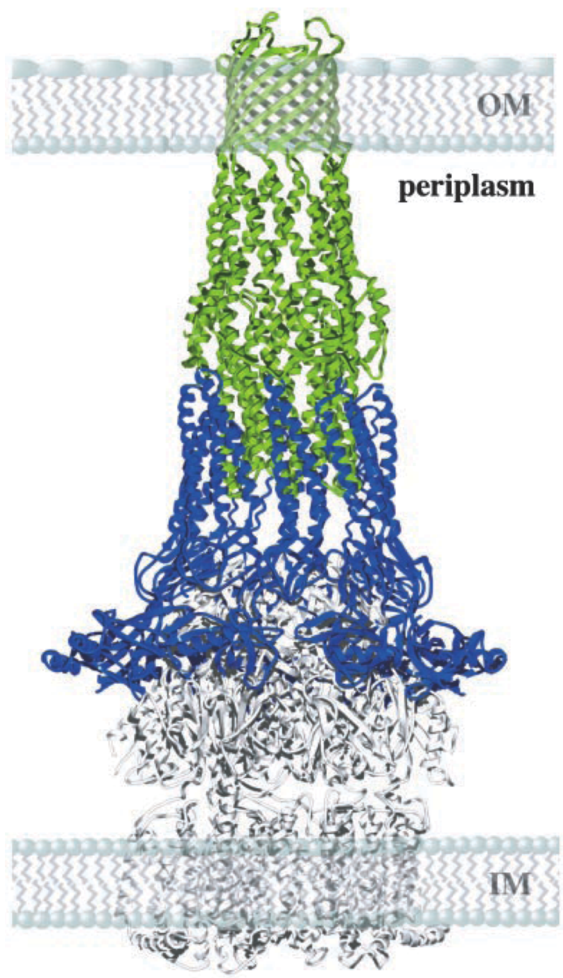

***E. coli***
**M9 Minimal Media**
**(vol/vol)**

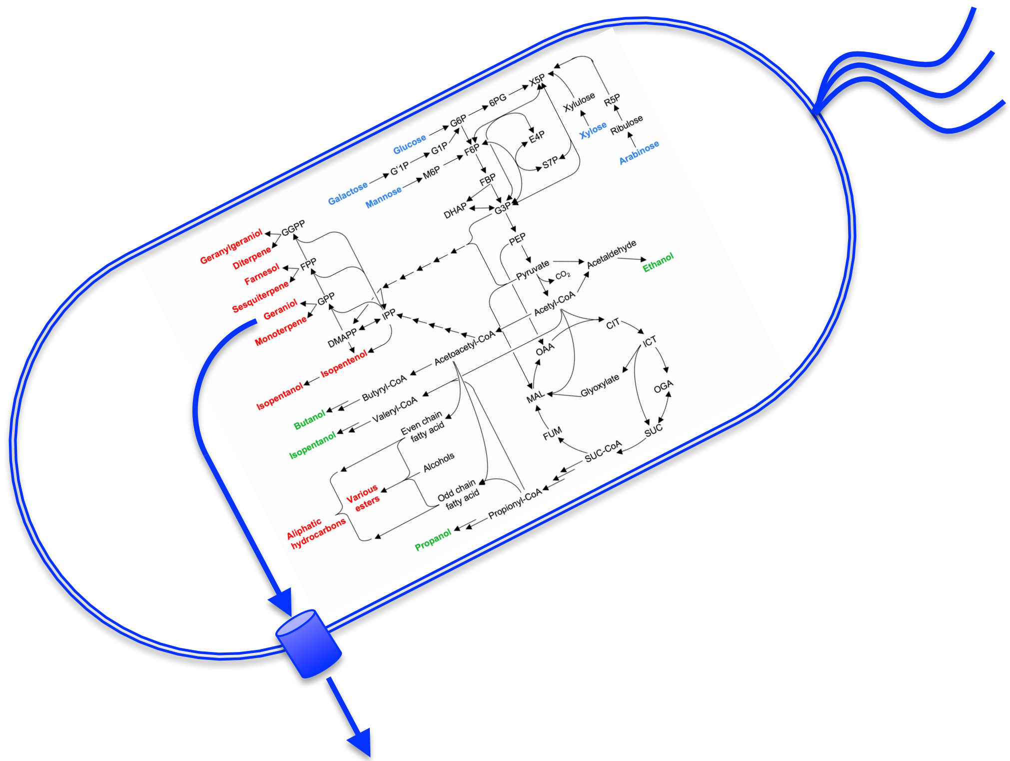


**α-pinene** **limonene**

Dunlop et al. 2011. *Mol. Sys. Biol.* **7**:487.

# Bioprospecting for solvent resistance pumps



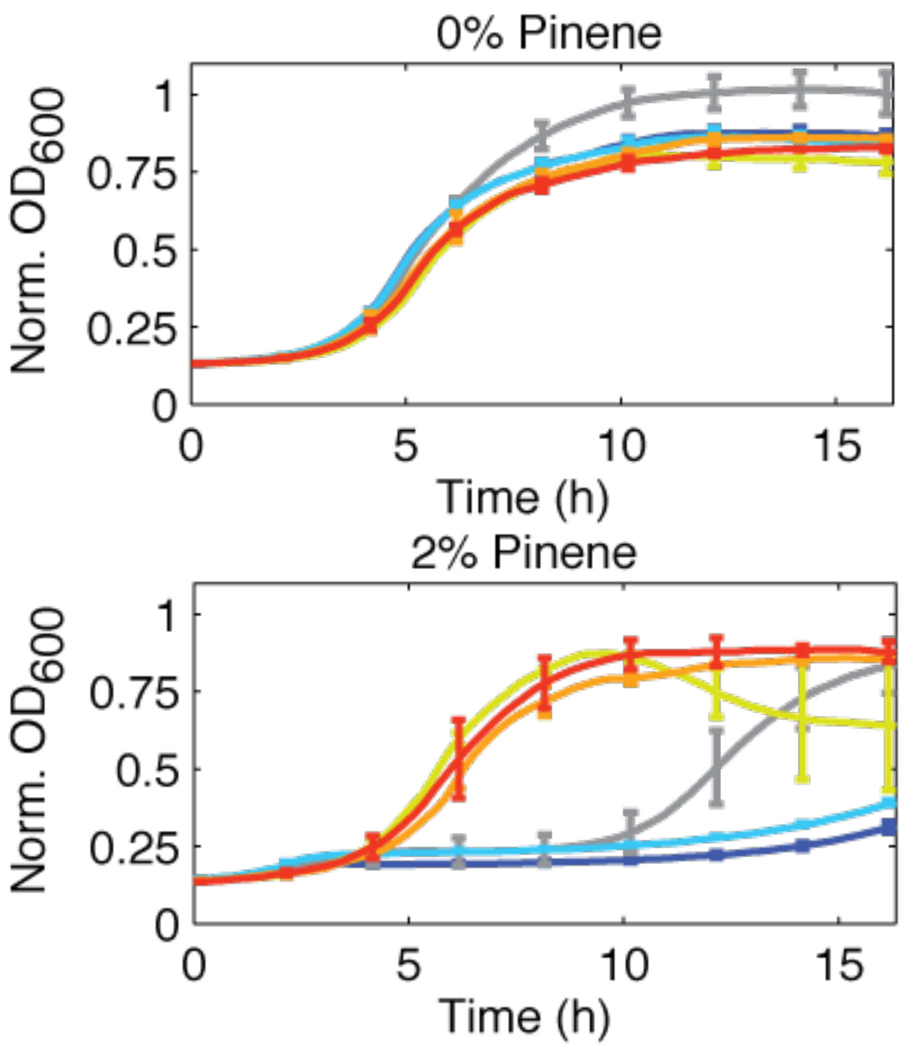



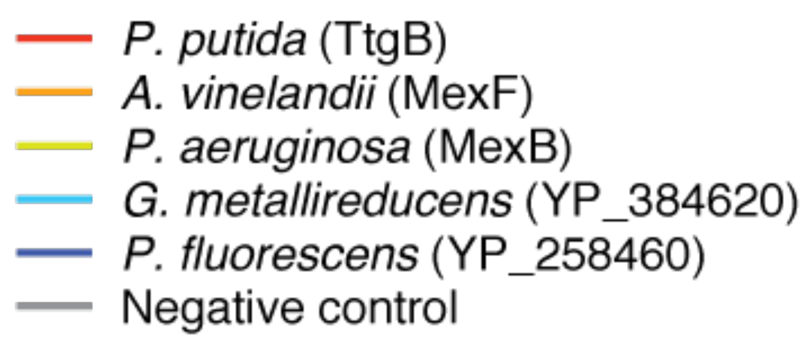
**Many different pumps in many microbes**

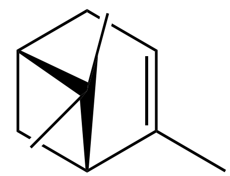
**How do we find the right ones?**

Dunlop et al. 2011. *Mol. Sys. Biol.* **7**:487.

# Engineering pinene tolerance into *E. coli*



**α-pinene**

Dunlop et al. 2011. *Mol. Sys. Biol.* **7**:487.

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# Interactions with industry

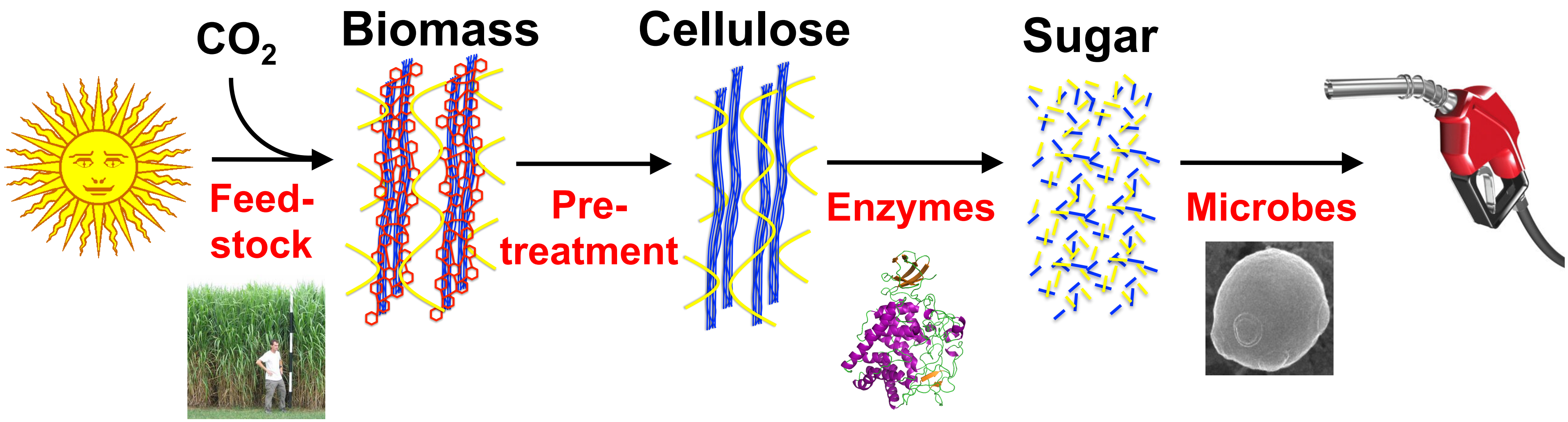
BOEING

bp

StatoilHydro

alloportis
an evolution in biofuels

novozymes
Rethink Tomorrow




Mendel
BIOTECHNOLOGY

ceres

CYTEC

ARBORGEN

PlumCreek

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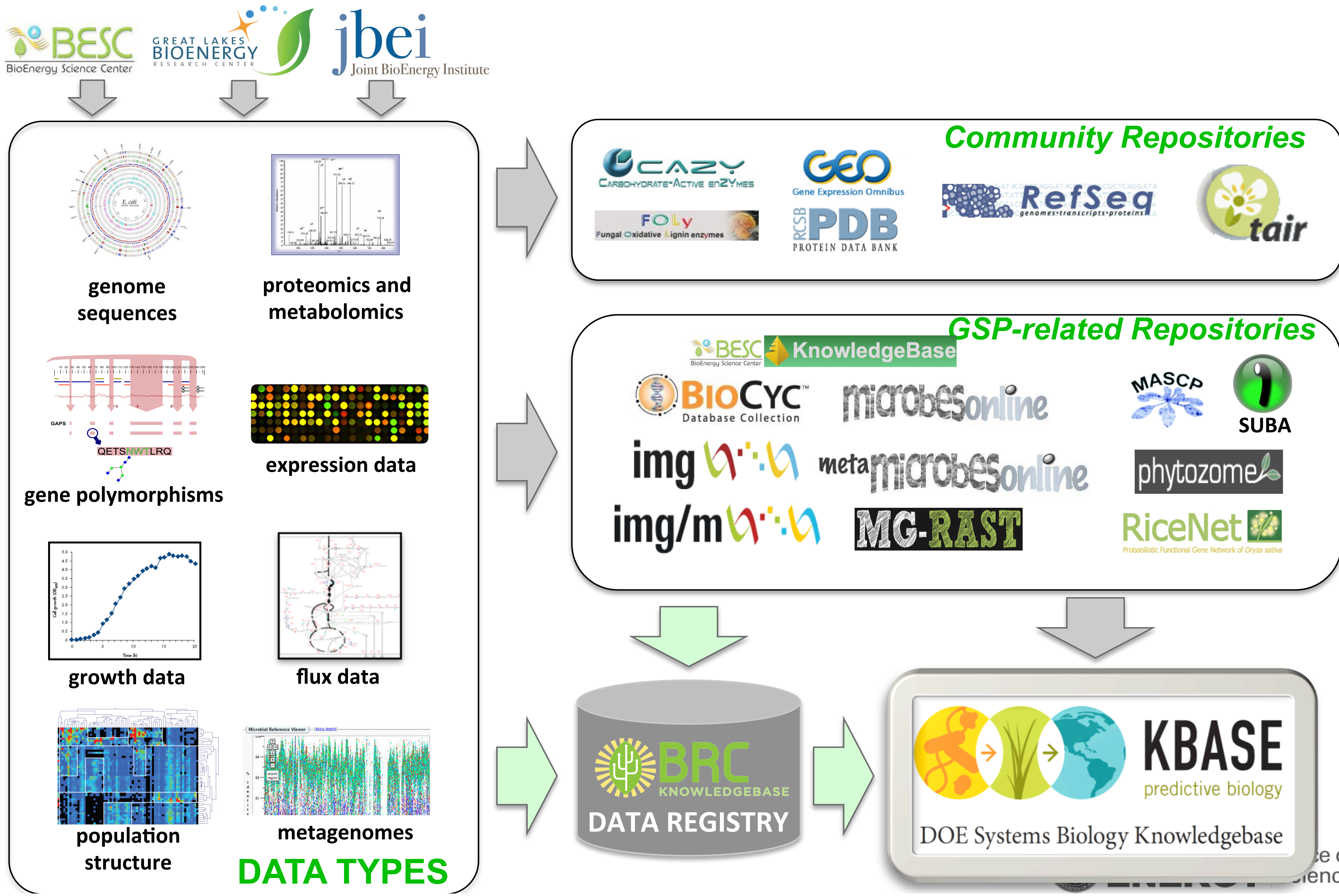
# KBASE and BRC Interactions



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# Thanks to


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