Make it or Break it:

Diesel Production

and Gluten Destruction

the Synthetic Biology Way
Washington iGEM 2011 Objectives

- Diesel Production
- Gluten Destruction
- iGEM Toolkits
- Community Outreach
Making it: work on diesel production

Diesel Production

Gluten Destruction

iGEM Toolkits

Community Outreach
Society is dependent upon limited petroleum reserves.
Integrating CO$_2$ into fuel production

CO$_2$ → Combustion → Petroleum Reserves
Biofuels are renewable, yet also inefficient, incompatible, and corrosive.
An ideal fuel is both renewable and efficient.
Microbial production of alkanes from fatty acids

Cellular Fatty Acid Biosynthesis

Acyl-ACP Reductase

Aldehyde DeCarbonylase

AAR

ADC

The basic alkane production pathway

Strong constitutive promoter

RBS

ADC

RBS

AAR

Bba_K590026

Bba_K590031

Bba_K590032
Analyzing alkane production with GCMS

Gas Chromatograph

Mass Spectrometer
Analyzing alkane production with GCMS

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Mass Spectrometer
Analyzing alkane production with GCMS

Gas Chromatograph

Mass Spectrometer

Experimental Spectrum

Reference Spectrum
ADC expression alone is not sufficient for hydrocarbon production.
AAR expression results in production of a C14 alcohol
Alkane production from the PetroBrick

We converted sugar into diesel!
Initial alkane yield

Optimization essential for BioBricks
Optimized alkane yield

Achieved 171 mg/L diesel yield
Filling Out the Alkane Profile
Even Chain Length Production

- Standard Fatty Acid 2 Carbon Starter Unit
  *(E. coli FabH)*

$$\text{CoA}$$

- Alternative Fatty Acid 3 Carbon Starter Unit
  *(B. subtilis FabH2)*

$$\text{CoA}$$

- Synthesized FabH2 Gene
- Cloned into a 3K3-Lac Inducible Vector (aka the *FabBrick*)
Looking for C16 Alkane Production

Expected C16 Elution Time
Looking for C16 Alkane Production
Looking for C16 Alkane Production

Relative Abundance

Uninduced
Looking for C16 Alkane Production

Relative Abundance

C16 Alkane

Induced
GCMS Confirms C16 Alkane Production

Experimental Spectrum

C16 Reference Spectrum

Relative Abundance

Induced
Looking for C14 Alkane Production

- C14 Alkane
- C15 Alkane
- C14 Alcohol
- C17 Alkene

Relative Abundance

- C13 Alkane
- Expected C14 Elution Time

Graph showing relative abundance over time.
GCMS Confirms C14 Alkane Production

Experimental Spectrum

C14 Reference Spectrum

Relative Abundance

8.5  8.6  8.7  8.8  8.9  9.0

minutes
First report of recombinant *even* chain length Alkane Production!
System Modularity

- Alternative Chassis
- Enzyme Localization
- System Optimization
- Decarbonylase Redesign
- Branched Alkanes
- Alternative Aldehyde
From making it to breaking it: gluten destruction

- Diesel Production
- Gluten Destruction
- iGEM Toolkits
- Community Outreach
Gluten intolerance is a common and difficult problem.
Indigestible gluten peptides trigger an immune response.
Indigestible gluten peptides trigger an immune response.
A protein therapeutic is in clinical trials... but is has low activity at pH 4

- SC PEP from *Sphingomonas capsulata*
- Low activity in acidic conditions
- High activity on PQLP
Kumamolisin is optimal at pH 4... but it has unknown activity on PQLP

- Endopeptidase from Alicyclobacillus sendaiensis
- High activity in acidic conditions
- Unknown activity on PQLP
We used computational tools to redesign Kumamolisin for PQLP.

Native active site

Mutate

G319S variant

To download your copy of Foldit, go to http://Fold.It
To test our designs, we developed a whole cell lysate assay.

Over 100 novel mutants tested!
Activity of mutants from whole cell lysate screen

Fold Change from Native Kumamolisin

- N291D
- D169G
- G319S, D358G, D368H

Native Kumamolisin
The top mutants were purified and characterized: over 10-fold improvement.
Greater than 100-fold improvement with a combinatorial mutant

$118 \times \text{activity}$

$k_{\text{cat}}/K_M = 3.9 \times 10^6 \text{M}^{-1}\text{s}^{-1}$

$784 \times \text{better than what’s in clinical trials}$

$1 \times \text{activity}$

$k_{\text{cat}}/K_M = 3.3 \times 10^4 \text{M}^{-1}\text{s}^{-1}$

$0.15 \times \text{activity}$

$k_{\text{cat}}/K_M = 5.0 \times 10^3 \text{M}^{-1}\text{s}^{-1}$
UW iGEM Objectives

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Gibson Assembly Toolkit

Gibson Assembly

Gibson Assembly Tool

Standard Biobrick Vectors

RFC 21 (BglBrick)

…CTGCGCCGAGATCT…

…AACAGGGTTCTCGAG…

pGBA plasmids

pGBA 1A3
pGBA 1C3
pGBA 3K3
pGBA 4A5
pGBA 4C5

“plasmids for Gibson Assembly”
Improved cloning efficiency with new pGA vectors

GFP

pSB1A3

pSB Vector Cloning

GFP

pGA1A3

pGA Vector Cloning

Gibson Assembly Efficiencies

pSB: 12%

pGA: 99%

Efficiency
Project goal: **Build magnetic* E. coli – “MagnetoColi”**

*Thanks to the Komeili lab (Berkeley) for the AMB-1 strain and a genome prep!*
Magnetosome Toolkit

BioBricked 18 genes from mamAB operon

Scaffold Polymerization

Vesicle Formation

Biomineralization
MamK expression

Scaffold Polymerization

0 mM IPTG
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Showing synthetic biology to the community!

Engineering Discovery Days

Bennett Elementary School
Teaching the community about cloning!
Teaching the community about cloning!

Completed Cell with New Vector!
Washington iGEM 2011
Accomplishments

- Made diesel from sugar
- Engineered enzyme to improve activity 100 fold
- Gibson Assembly Toolkit
- Magnetosome Toolkit
- Showed community synthetic biology is awesome
Biobricks Submitted

Gluten Destruction: 5

Diesel Production: 39

Toolkits: 21

KumaMax: 1

Total Biobricks: 65!!!
Our Volunteer Army

**Diesel Production**
- Austin Moon
- Benjamin Mo
- Casey Ager
- Chris Choe
- David Zong
- Elaine Lai
- Emily Yang
- Juhye An
- Lei Zheng
- Marika Cheng
- Matthew Harger
- Seth Sagulo
- Emily Yang

**Gluten Destruction**
- Angus Toland
- Daniel Hadidi
- Liz Stanley
- Sarah Wolf
- Sean Wu
- Sydney Gordon

**Toolkits**
- Alicia Wong
- Michael Brasino
- Rashmi Ravichandran

**Outreach**
- Andrew Mak
- Cindy Wu

**Advisors**
- Aaron Chevalier
- Chris Eiben
- Ingrid Swanson Pultz
- Jeremy Mills
- Justin Siegel
- Matt Smith
- Rob Egbert

**Professors**
- David Baker
- Eric Klavins
- Herbert Sauro
Thank you!
pGA Vector fluorescence

- **pGA4A5**
- **pGA3K3**
- **pGA1A3**
- **pGA1C3**

GFP fluorescence (AU)

- $10^3$
- $10^4$
- $10^5$
- $10^6$