Monash University iGEM 2010
The design and construction of an ethylene generator
Ethylene

- Simple organic molecule – C$_2$H$_4$
- Precursor to many plastics
  - Commonly polymerised
Ethylene

Low Density Polyethylene (LDPE)
Ethylene

High Density Polyethylene Plastic
Ethylene

- Not limited to polymersation
- Can undergo further modification or used as is
Ethylene

Polyethylene terephtalate (PET)
Ethylene

Polystyrene (Packing foam)
Ethylene

Polyvinyl Chloride (PVC)
Product examples

Ethylene Oxide
- Disinfectant
Product examples

Ethylene Glycol
- Anti-freeze
Product examples

Anaesthetic

- 85% Ethylene : 15% O2
Product examples

Welding gas
Ethylene production

- Most widely produced organic compound in the world
  - Over 112 million tons produced in 2009

- Produced by ‘steam cracking’
  - Large hydrocarbons
  - Crude oil, natural gas
  - Heated to ~900 °C
  - Saturated with steam
  - Cooled to -157 °C
  - Repeated compression and distillation
Ethylene production

- World scale plant uses 3 compressors
  - Totalling to 67,000 kW
  - 1 hour produces 48 metric tons of CO2 in electrical generation*

- This excludes:
  - Mining of crude oil/natural gas
  - Transportation
  - Environmental impact

*mCO2 = 718g/kWh
Aim

Genetically engineer *Escherichia coli* to produce ethylene at:

- Roughly room temperature
- Low cost
- Low energy requirements and
- Using renewable organic feed-stocks
Project

- Increased production and reliance on ethylene
  - Non-renewable resource

- Biosynthetic production of ethylene

- Along side recycling programs
  - Reduction of environmental impact/damage
  - Reduction of CO2 footprint associated with production

- Can be inserted back into petrochemical facilities
Yang Cycle

**Ethylene Biosynthesis in Plants**

**Enzymes**

1. SAM synthetase
2. ACC synthase
3. ACC oxidase
4. ACC N-malonyl-transferase
5. MTA nucleosidase
6. MTR kinase
7. Transaminase
8. Spontaneous reaction

**Abbreviations**

- ATP: Adeninnucleotidtriphosphate
- ADP: Adeninnucleotididiphosphate
- ACC: 1-Aminocyclopropane-carboxylate
- HCN: Hydrocyanide acid
- MTA: 5'-Methythioadenosin
- MTR: 5'-Methythioribose
- PP: Diphosphate (Pyrophosphate)
- P: Phosphate
- SAM: 5'-Adenosyl-L-methionine

**Sources**


Modified from: http://commons.wikimedia.org/wiki/File:Yang-cycle.png (CC-SA 3.0)
Yang Cycle

Enzymes of interest
1 – SAM Synthetase
2 – ACC Synthase
3 – ACC Oxidase

Ascorbate
Project
Design of protein coding sequences

Enzymes of interest

- SAM Synthetase
- ACC Synthase
- ACC Oxidase

SAM Synthetase :: Cloned from *E. coli*

ACC Synthase :: Apple gene, codon optimized for *E. coli*

ACC Oxidase :: Tomato gene, codon optimized for *E. coli*

Removed of illegal restriction sites to ensure RFC 10 compliant
SAM Synthetase – primer design

Forward primer: gtttcttcgaattcgcggccgttctagatggcaaaacaccttttacgtccgag
Reverse primer: gtttcttcctgcagcggccgctactagtaattacctcagccgcgcacatcgc

Paste in sequence below to design primers to BioBrick it.
Primers are generated with a melting temperature greater than 55°C and have a G/C on 3’- end.

For more information, see this page. For problems with this tool, contact technical support
SAM Synthetase amplification

PCR amplified from *E. coli* genomic DNA

~1.2 kbp
SAM Synthetase sequencing
### ACC Synthetase

**EC 4.4.1.14 - 1-aminocyclopropane-1-carboxylate synthase**

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## ACC Oxidase

**BRENDA**

The Comprehensive Enzyme Information System

**EC 1.14.17.4 - aminocyclopropanecarboxylate oxidase**

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Design
Assembly strategy

E = EcoRI-HF
X = XbaI
S = SphI
P = PstI
M = Mixed site
Design
Kinetic Modelling

The Tinkercell design
Aims of Modelling

- To determine the maximum output of ethylene
- Started with a simple model and advanced to a more detailed model
Model 1: Fixed enzyme & input concentrations
Model 1: Fixed Enzyme Concentrations

- Concentrations of 10 – 3000 µM
  
  (Sakakibara et al., Nature, 2008)

- Michaelis-Menten equation used

\[
\text{rate} = \frac{k_{\text{cat}} \times [\text{substrate}] \times [\text{enzyme}]}{K_m + [\text{substrate}]}
\]
Assumptions

- The best value (Km, kcat etc) was always chosen to estimate maximum ethylene output
- The Km with respect to the rate limiting substrate was used
- No product inhibition eg. HCN or CO2
- Enzymes were present in a steady state concentration 1:1:1 ratio
- All substrate concentrations (ATP, Methionine etc) were kept constant
Information on EC 4.4.1.14 - 1-aminocyclopropane-1-carboxylate synthase:

Mark a special word or phrase in this record:

Select one or more organisms in this record:

- All organisms
- Ananas comosus
- Antirrhinum majus
- Arabidopsis sp.
- Arabidopsis thaliana

Show additional data
- Do not include text mining results
- Include AMENDA (text mining) results\textsuperscript{new} (more...)
- Include FREnda results\textsuperscript{new} (AMENDA + additional results, but less precise; more...)

Please login to have access to the AMENDA and FREnda data

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<th>EC NUMBER</th>
<th>COMMENTARY</th>
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http://www.brenda-enzymes.info
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<td>EFE turnover rate (kcat)</td>
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Ethylene Production at different concentrations of Enzymes
Model 2: Transcription + translation
Model 2: Transcription+ translation

- Rate of transcription and translation changes concentration of enzymes
- Ultimately changes the ethylene output
- 1:1:1 ratio of enzymes due to same transcription rate
- Assume the Promoter is fully activated all the time
Elowitz Repressilator

http://www.ebi.ac.uk/biomodels-main/BIOMD0000000012
mRNA Synthesis

Time Course Simulation

Concentration (μM)

Time (s)
Translation rate of all three enzymes
Ethylene Production at Floating Enzyme Values

Time Course Simulation

Concentration (μM)

Time (s)
Modelling conclusions

• Framework for understanding the device
• Created a simple and a more detailed model
• Require measured mRNA & protein levels from the device to generate an accurate model
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Future development

- Finish device
  - measure ethylene output using gas chromatography
- Alternative RBS optimisation
- HCN detoxification
- Ascorbate dependence
- Explore gas phase signalling
- Complete the Yang cycle
- Large scale industrial production
Special Thanks

Ashley Buckle  Andrew Perry  Lloyd Low

Anna Thang  David 'The Texan' Hoke

Lecturers and professors at Monash University who have supported the project

Jeremy Nagel  Scarlette Baccini
Sponsors

MONASH University

MR. GENE

GENEART

YARRA VALLEY GRAMMAR