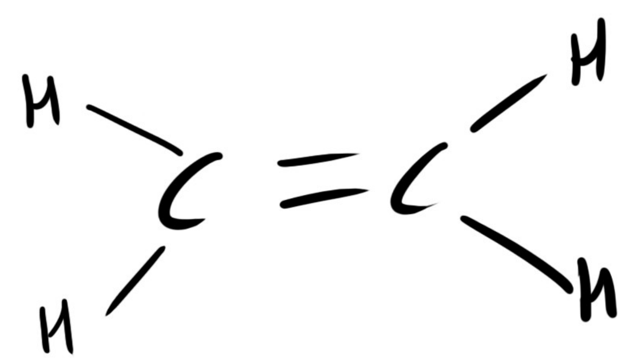
Design and Construction of an Ethylene Generator

Team:Monash_Australia

Ben Porebski, Daniel Buckley, William Yuen, Ricky Wong, Anna Thang
Andrew Perry, Lloyd Low, Ashley Buckle

Introduction

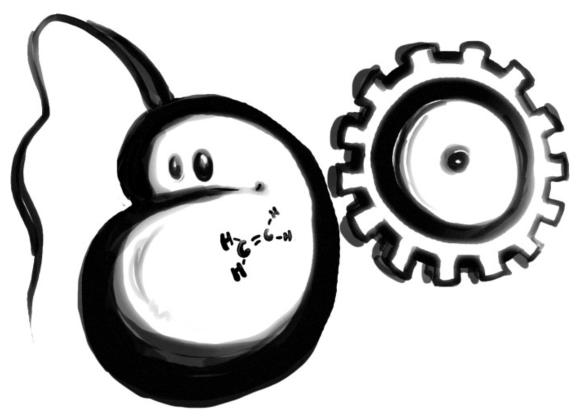
We rely heavily on plastics in our day to day lives – and yet they are currently produced from a non-renewable resource, fossil fuels, in a process requiring large amount of energy. We have found that the majority of plastic products are produced from or derived from the compound Ethylene.



The Ethylene molecule

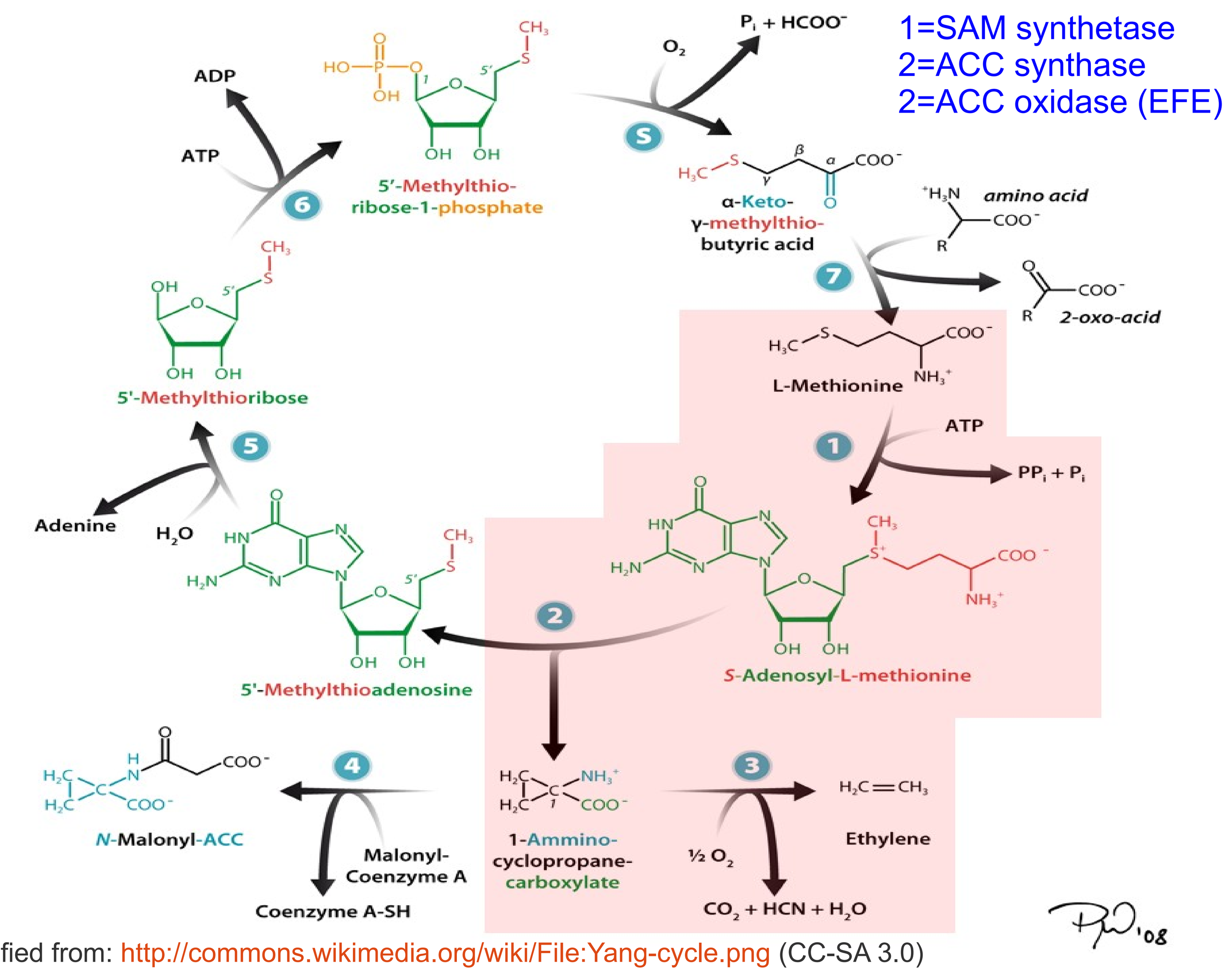
A steam cracking tower

The problem: Ethylene is currently produced in an energy intensive process called steam cracking where large hydrocarbons from oil or natural gas are heated to roughly 900 °C and saturated with steam and subsequently cooled to -157 °C to be compressed and distilled multiple times. This process has a large carbon footprint. To solve this, we have found that plants can produce ethylene from the amino acid methionine at room temperature with renewable and readily available compounds at a much lower carbon cost.



Aim: Construct an ethylene generation device in an *Escherichia coli* chassis to produce ethylene using the enzymes involved in plant ethylene biosynthesis.

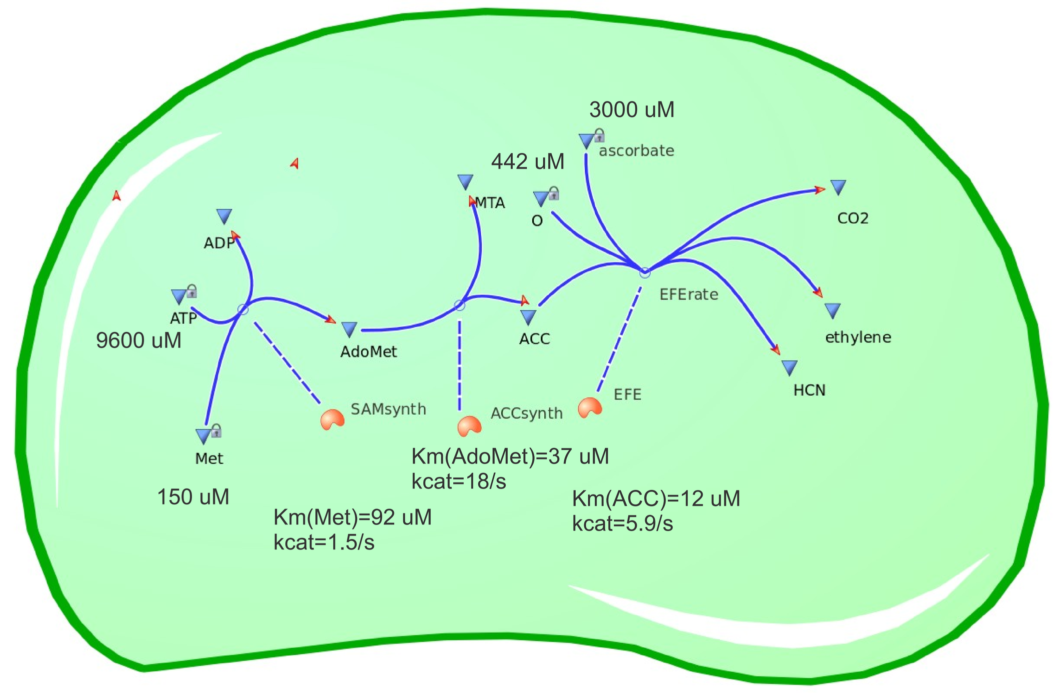
Ethylene biosynthesis



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

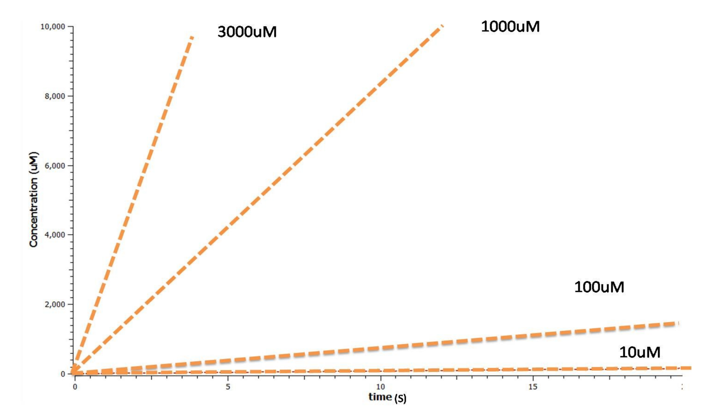
Ethylene biosynthesis occurs in plants through the Yang cycle, also known as the methionine cycle.

Kinetic Modelling



Used the program Tinkercell to create a simple model of the device.

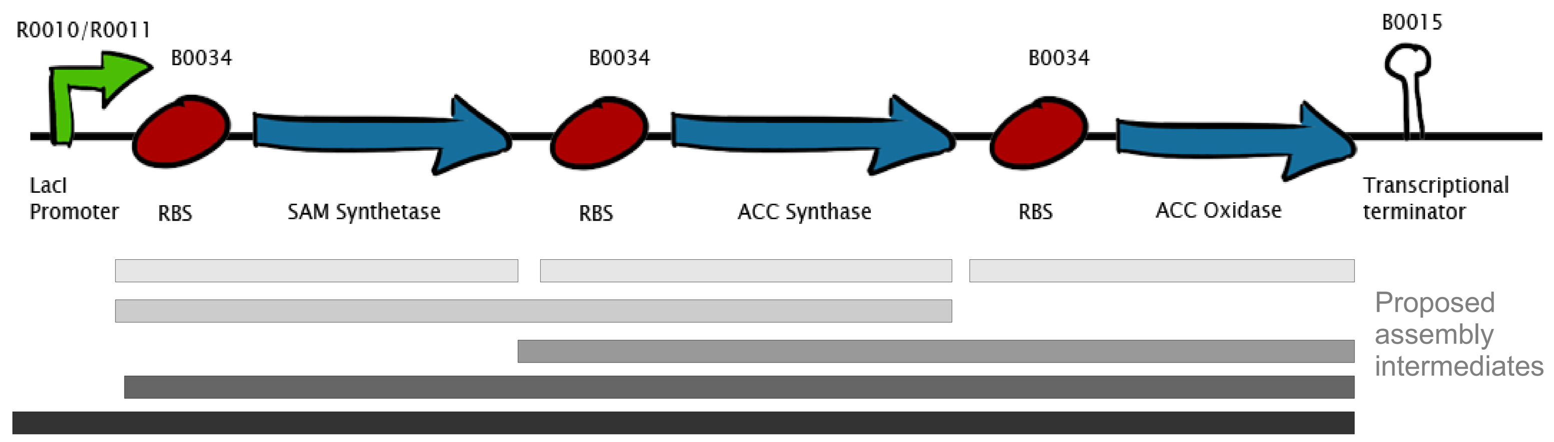
Estimated ethylene output

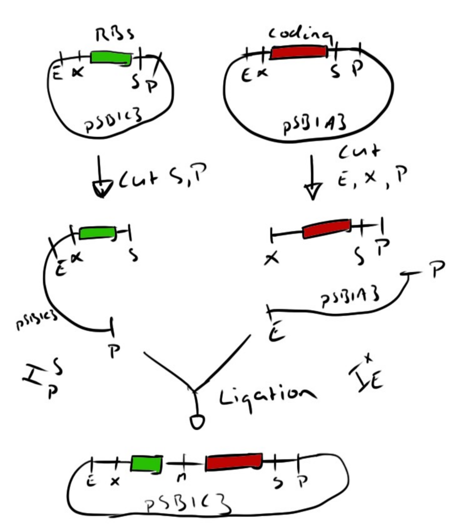


Used Michaelis Menton Kinetics to model the rate of reaction

Fixed input and enzyme concentrations

Design & Assembly





Alternative RBS assembly method

- Design uses Lac inducible promoter (R0011) and strong ribosome binding sites(RBS) (B0034)
- Initially tried 3A Assembly procedure
- Experienced problems with attaching RBS to coding sequences
  - RBS sequence too small?
  - RBS lost?
- Developed alternative assembly procedure (See left) to try and compensate for the possibility of the RBS being lost
- Successfully cloned SAM Synthetase from *E. coli* genomic DNA
  - Shipped as part K417000

Future Work

- Finish construction of our design
- Measure ethylene output via Gas Chromatography
- HCN removal via the enzyme Cyanide Dihydratase
- Reconstruct entire Yang's Cycle to recycle methionine
- Optimise ethylene output via alternative RBSs, promoters
- Measure enzyme concentration, steady state

MONASH University