EscheRatio coli

Stanford iGEM 2010
We created two new tools

Ratiometric Sensor

iGEM Twitter Community
Inspired by Stanford iGEM 2009
Ratios are important in biology

Hormones

Developmental Biology

Metabolic Pathways
Components and Qualities of a Ratiometric Sensor

Sensor 1

Sensor 2

Signal Integrator

Output
Components and Qualities of a Ratiometric Sensor

- Sensor 1
- Sensor 2
- Signal Integrator
- Output

• Modular
Components and Qualities of a Ratiometric Sensor

- Modular
- Autonomous
Components and Qualities of a Ratiometric Sensor

- Sensor 1
- Sensor 2
- Signal Integrator
- Output

- Modular
- Autonomous
- Orthogonal
Ratiometric sensors have two kinds of behavior

**Linear**
- Opposing Enzymes
  - Oxidation/Reduction
  - DNA Methylation/Demethylation
  - Kinase/Phosphatase

**Ultrasensitive**
- Stoichiometric Inhibition
  - sRNA/mRNA
Predicted behavior for the kinase/phosphatase system is linear
Ratiometer takes PoPS inputs and creates PoPS outputs

Kinase/Phosphatase Ratiometer

Input 1

Input 2

Output (In1/In2)
Ratios are computed by phosphorylation of the transcription factor.
Components are orthogonal

Input 1

PoPS

pknH

K353005

PknH

Input 2

PoPS

embR

K353007

EmbR

K353008

MstP

Output

(In1/In2)

PoPS

+
Well-characterized inputs and outputs were chosen.
sRNA Modeling

\[[\text{sRNA}] + [\text{mRNA}]\]

\[[\text{sRNA} \times \text{mRNA}]\]

\text{mRNA Free}

\text{mRNA Total}

\[0\]

\[1\]
Differentiator takes PoPS inputs and creates RiPS outputs
Ratios are computed by sRNA inhibition
Redundancy allows accurate measurement
RSID's designed for modularity
Well-characterized inputs and outputs were chosen
Twitter provides useful communication functions
Created multi-lingual video tutorials to encourage membership
Established a Twitter List as a platform for communication

Twitter profile pictures of iGEM Teams.
iGEM teams utilized Twitter to share knowledge

**Newcastle_iGEM** @camgem Have you guys been using Gibson to ligate BioBricks at all?
1:03 PM Aug 14th via web

**camgem** @Newcastle_iGEM We tried it out once in our first week, but we've only just ordered most oligos. Why do you ask?
6:30 PM Aug 14th via web

**Newcastle_iGEM** @camgem We got some weird results on Friday. We'll see what happens on Monday :)
5:21 AM Aug 15th via web

**camgem** @Newcastle_iGEM Good luck! We'll let you know if we have issues too.
7:37 AM Aug 15th via web
Kinase system parts built
sRNA system parts built and characterized
sRNA system produces and inhibits GFP

AHL Isoclines

GFP Fluorescence vs. Arabinose (M)

Decreasing AHL (sRNA)
sRNA system measures ratios

Decreasing AHL (sRNA)
Ratio is not consistently detected at saturating [AHL]
Ratio is not consistently detected at very low \([AHL]\).
sRNA system consistently measures a ratio

Ratio ~ $5 \times 10^{-2} - 5 \times 10^{-3}$
Accomplishments

- Designed two ratiometric sensor systems.
- Submitted 12 new parts to the registry.
- Built and characterized a functional ratiometric sensor.
- Established an iGEM community on the Twitter network.
Two new tools in the toolbox!
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