UOTTAWA IGEM 2014
ENGINEERING FATE
Cellular Decision making is the process by which cells assume unique, functionally different states.
CELLULAR DECISION MAKING

\[ P(E|S) = \frac{P(S|E)P(E)}{P(S)} \]

Perkins, T., and Swain, P. 2009
According to Huang 2009, stem cell differentiation could be controlled by a **tri-stable switch**.
ACHEEIVMENTS

- Designed a tri-stable switch network
- Engineered a novel repression strategy
- Participated in the Measurement Interlab Study
- Collaborated with teams
- Designed deterministic and stochastic models

![Diagram](attachment:image.png)
- Extremely well characterized
- Robust transformation protocols
- Large networks
METHODOLOGY: FLOW CYTOMETRY

Flow Cytometry

Sheath fluid

Sample (stained cells in suspension)

Nozzle

Hydrodynamic Focusing
Cells pass through in 'single file'

Fluorescence emitted from stained cells detected

Forward and side scattered light from all cells detected

Laser light source
WHAT IS A TRI-STABLE SWITCH?
GEV AND RTTA

<table>
<thead>
<tr>
<th>GEV</th>
<th>rtTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Estradiol</td>
<td>Anhydrotetracycline (aTc)</td>
</tr>
<tr>
<td>Gal4 Binding Sites</td>
<td>TetR Binding sites</td>
</tr>
</tbody>
</table>
DUAL INPUT PROMOTERS

Activating Sites (Gal4)

Repessing Sites (tetR)

TATA box

Native GAL1 Promoter

Ellis et al. 2009
DUAL INPUT PROMOTERS

Ellis et al. 2009
REPRESSION BY HINDRANCE

Protein Complex

Activator Binding Sites

TATA box
Protein Complex

10 Base pairs
Gal4 Binding Sites

Tet Binding Sites

pGALtx Strong Repression

GFP

[aTc (ng/μL)]

[Estradiol (nM)]
REPRESSION BY HINDRANCE

Tet Binding Sites  Gal4 Binding Sites

Promoter Activity (GFP)

Estradiol (nM)  aTc (ng/mL)
- Multiple designs
- Still under construction

**Design 2**

```
Selection Cassette  Term.  pGEVtx  GEV  Term.  pTRE  BFP
<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| Selection Cassette  Term.  pTREgx  rTTA  Term.  pGEV  GFP
|                |        |       |     |     |     |     |
| Selection Cassette  Const.  rtTA  Term.  Const.  GEV  Term.  |
```
PROMOTER CHARACTERIZATION

pGALtx Weak Repression

pGALtx Strong Repression

GFP

[Estradiol (nM)]

[aTc (ng/μL)]

GFP

[aTc (ng/μL)]

[Estradiol (nM)]
PROMOTER CHARACTERIZATION

pTRE (4 sites)

pTREgx (2 sites)
Our lab received a part from a colleague in the United States. Although no patent exists for this part, the US lab offered it exclusively to our research team (as per email correspondence). Could our team submit this genetic sequence to the Registry of Standard Parts, which would make it available to all iGEM teams?
<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Designer</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K1400000</td>
<td>Regulatory</td>
<td>PTRE(4)GX Dual input promoter. Activation at tetO binding sites, repression at gal4 sites.</td>
<td>Dylan Siriwardena</td>
<td>431</td>
</tr>
<tr>
<td>BBa_K1400001</td>
<td>Regulatory</td>
<td>PTRE(2)GX Dual input promoter. Activation at tetO binding sites, repression at gal4 sites.</td>
<td>Dylan Siriwardena</td>
<td>430</td>
</tr>
<tr>
<td>BBa_K1400002</td>
<td>Regulatory</td>
<td>PGAL Modified native pGAL</td>
<td>Dylan Siriwardena</td>
<td>478</td>
</tr>
<tr>
<td>BBa_K1400003</td>
<td>Regulatory</td>
<td>pTre(4) Single input tet responsive promoter</td>
<td>Dylan Siriwardena</td>
<td>436</td>
</tr>
<tr>
<td>BBa_K1400004</td>
<td>Regulatory</td>
<td>pGALtx Dual input promoter. Activation at gal4 binding sites, repression at tetO sites.</td>
<td>Dylan Siriwardena</td>
<td>474</td>
</tr>
<tr>
<td>BBa_K1400005</td>
<td>Regulatory</td>
<td>pTRE(2) Single input tetr responsive promoter</td>
<td>Dylan Siriwardena</td>
<td>440</td>
</tr>
</tbody>
</table>
## INTERLAB STUDY

<table>
<thead>
<tr>
<th>Construct</th>
<th>Arithmetic mean</th>
<th>Standard dev.</th>
<th>Mean fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>J23101</td>
<td>143.8629</td>
<td>15.11104</td>
<td>0.75833</td>
</tr>
<tr>
<td>J23115</td>
<td>3.73</td>
<td>1.108355</td>
<td>0.019662</td>
</tr>
<tr>
<td>I20260</td>
<td>17.72222</td>
<td>1.266035</td>
<td>0.093417</td>
</tr>
</tbody>
</table>

**Fraction of positive control fluorescence**

![Graphs of J23101, J23115, and I20260](image)
pGaltx_IGEM1

\[
ff = \epsilon + \text{zmax} \left( \frac{k1.m1.}{k1.m1+x.m1} \right) \left( \frac{y.n2.}{k2.n2+y.n2} \right)
\]
\[
\frac{d}{dt}\text{ DETERMINISTIC MODEL}
\]

\[
\begin{align*}
rtT a + aT c & \rightarrow rtT a^* \\
GEV + \beta - estradiol & \rightarrow GEV^*
\end{align*}
\]
RELATIVE THEORETICAL PARAMETERS
EXPERIMENTAL PARAMETERS

Phase Diagram: Design Two Multiplicative

$r_{T:a}$ [molecules] vs. $GEV$ [molecules]
EXPERIMENTAL PARAMETERS
STOCHASTIC DESIGN

10 β-estradiol: 1 aTc

1 β-estradiol: 1 aTc

1 β-estradiol: 10 aTc
- Designed a tri-stable switch network
ACHIEVEMENTS

- Engineered a novel repression strategy

Diagram:
- Protein Complex
- 10 Base pairs
- Participated in the Measurement Interlab Study
Helped the University of Waterloo with construction

Sent Queens University a transcriptional activator (GEV)
Characterized and improved multiple promoters
Investigated opinion on intellectual property law

How do we encourage collaboration between American and Canadian synthetic biologists?

Most Common Answers:
1. International committees/conferences
2. Collaborative grants
3. Open-access data base of genetic sequences
4. Minimization of legal issues
- Designed deterministic and stochastic models
Phase Diagram: Design Two Additive
FUTURE DIRECTIONS

NITROGEN - PHOSPHOROUS SENSING CELL

NITROGEN EXPRESSING CELL

PHOSPHOROUS EXPRESSING CELL
ACKNOWLEDGEMENTS

- Dr. Mads Kaern
- Ian Roney
- Vaibhav Gupta
- Kaern Lab members
- University of Waterloo iGEM Team
Team successful registered
Judging form complete and submitted
Wiki for the uOttawa iGEM team is up and running
Project description is a clear outline of the student work and is distinguishable from the work of others
Documentation of new standard BioBrick aspect of the project completed and submitted to the iGEM registry

New BoBrick Part, designed by the team, is validated experimentally and works!
New BioBricks are characterized
New BioBricks are submitted!
iGEM projects involve and integrate important questions, and this consideration is described

We collaborated with other registered iGEM teams
OUTREACH

Lego synthetic gene network activity

DNA Extraction
Design 1
Design 2

ADE2 U  KanMX  rtACT1  pGEVtx  GEV  tPGK1  pTRE  BFP  ADE2 D

ADE4 U  NatMX  rtACT1  pTREgx  rTTA  tPGK1  pGEV  GFP  ADE4 D

Gal U  His  pmrp7  rtTA  tPGK1  pmrp7  GEV  tPGK1  Gal D
PROMOTER ANALYSIS

2 Sites vs 4 Sites

pGaltx

Gal4 Binding Sites  Tet Binding Sites

[Graphs showing promoter activity (GFP) vs [aTc](ng/mL) and [Estradiol](nM).]
SELF-ACTIVATION

![Graph showing the effect of Estradiol (nM) on GFP. The graph indicates an increase in GFP with increasing Estradiol levels, peaking at approximately 25 units around 1000 nM before plateauing.](image-url)
THE ‘STATE’ OF AFFAIRS
BIFURCATION ANALYSIS

Steady state when rtTA starts low and GEV starts high

Steady state when rtTA starts low and GEV starts high
\[ \frac{d}{dt} GEV = a + \frac{(\frac{c}{K} (GEV \ast beta))^n}{1 + (\frac{c}{K} (GEV \ast beta))^n} \left( \frac{1}{1 + (\frac{c}{K} (rtTa \ast aTc))^n} \right) - k \ast GEV \]

\[ \frac{d}{dt} rtTa = a + \frac{(\frac{c}{K} (rtTa \ast aTc))^n}{1 + (\frac{c}{K} (rtTa \ast aTc))^n} \left( \frac{1}{1 + (\frac{c}{K} (GEV \ast beta))^n} \right) - k \ast rtTa \]
\[ \begin{align*} 
\frac{d}{dt} \text{GEV} &= a + b_1 \left( \frac{\left( \frac{c}{K} \text{GEV} \times b \times a \right)^n}{1 + \left( \frac{c}{K} \text{GEV} \times b \times a \right)^n} \right) \left( \frac{1}{1 + \left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n} \right) - k \times \text{GEV} \\
\frac{d}{dt} \text{rt} \times a &= a + b_1 \left( \frac{\left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n}{1 + \left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n} \right) \left( \frac{1}{1 + \left( \frac{c}{K} \text{GEV} \times b \times a \right)^n} \right) - k \times \text{rt} \times a \\
\frac{d}{dt} \text{GEV} &= a + d_1 \left( \frac{\left( \frac{c}{K} \text{GEV} \times b \times a \right)^n}{1 + \left( \frac{c}{K} \text{GEV} \times b \times a \right)^n} \right) + b_1 \left( \frac{1}{1 + \left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n} \right) - k \times \text{GEV} \\
\frac{d}{dt} \text{rt} \times a &= a + d_1 \left( \frac{\left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n}{1 + \left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n} \right) + b_1 \left( \frac{1}{1 + \left( \frac{c}{K} \text{GEV} \times b \times a \right)^n} \right) - k \times \text{rt} \times a \\
\frac{d}{dt} \text{GEV} &= a + b_1 \left( \frac{\left( \frac{c}{K} \text{GEV} \times b \times a \right)^n}{1 + \left( \frac{c}{K} \text{GEV} \times b \times a \right)^n} + \left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n \right) - k \times \text{GEV} \\
\frac{d}{dt} \text{rt} \times a &= a + b_1 \left( \frac{\left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n}{1 + \left( \frac{c}{K} \text{rt} \times a \times Tc \right)^n} + \left( \frac{c}{K} \text{GEV} \times b \times a \right)^n \right) - k \times \text{rt} \times a 
\end{align*} \]
BIFURCATION ANALYSIS

Steady state when rTA starts high and GEV starts low

Steady state when rTA starts high and GEV starts low
BIFURCATION ANALYSIS

Steady state when rtTA starts low and GEV starts very low

Steady state when rtTA starts low and GEV starts very low
PTRE (4 SITES)
**DIFFERENTIAL EQUATIONS**

**ACTIVATION:**
\[ \frac{dy}{dt} = k' + k \left( \frac{x^n}{K^n + x^n} \right) \]

**REPRESSION:**
\[ \frac{dy}{dt} = k' + k \left( \frac{K^n}{K^n + x^n} \right) \]

**SYSTEM:**
\[ A = a + b \left( \frac{\left( K^*y(1) + y(3) \right)^n}{(1) + \left( K^*y(1) + y(3) \right)^n} \right) \times \left( \frac{1}{(1) + \left( K^*y(2) + y(4) \right)^n} \right) - k*y(1); \]
\[ B = a + b \left( \frac{\left( K^*y(2) + y(4) \right)^n}{(1) + \left( K^*y(2) + y(4) \right)^n} \right) \times \left( \frac{1}{(1) + \left( K^*y(1) + y(3) \right)^n} \right) - k*y(2); \]
CURVE FITTING
\[ \frac{dy}{dt} = k' + k \left( \frac{x^n}{K^n + x^n} \right) \]

\[ y = 0.5198 + 24.1339 \left( \frac{x^{1.6168}}{x^{1.6168} + 293.2010^{1.6168}} \right) \]

\[ y = 0.3225 + 22.0956 \left( \frac{x^{1.6619}}{x^{1.6619} + 204.9485^{1.6619}} \right) \]

\[ y = 0.2931 + 3.3717 \left( \frac{x^{1.1519}}{x^{1.1519} + 606.0607^{1.1519}} \right) \]
**Multiplicative Regression:**

\[ z = z_1 + z_{\text{max}} \cdot \left( \frac{k_1 n_1}{k_1 n_1 + x n_1} \right) \cdot \left( \frac{y n_2}{k_2 n_2 + y n_2} \right) \]

- \( aTc \) (repression)
- Beta estradiol (activation)
Promoter: pTreGx

\[ z = 0.3551 + 61.6556 \cdot \left( \frac{3847.5 \cdot 0.3581}{3847.5 \cdot 0.3581 + x \cdot 0.3581} \right) \cdot \left( \frac{y^{0.9887}}{2803.8 \cdot 0.9887 + y^{0.9887}} \right) \]