The journey begins...

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Melbourne '08
BioClock

• Temporal Control in E.coli
• A customizable regulatory system
• One signal switches between multiple states
A binary clock - the original plan

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<th>Red</th>
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<th>Corresponds to Time</th>
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Corresponds to Time: 001 010 011 100 101 110 111

\[ 0 = 2^0 \]
\[ 1 = 2^1 \]
\[ 2 = 2^0 + 2^1 \]
\[ 3 = 2^1 + 2^0 \]
\[ 4 = 2^2 \]
\[ 5 = 2^1 + 2^0 + 2^2 \]
\[ 6 = 2^2 + 2^0 \]
\[ 7 = 2^2 + 2^1 + 2^0 \]
Binary Model - the good and bad

- High multiplicity => greater scope for expansion
- Each “bit” would demand a unique set of biological parts
In comes the Linear Model

- Similar components to Binary Model, but different interactions $\Rightarrow$ translatable
- Proof of principle
Pause for an outline

- Components
  1. Red light sensor
  2. Positive feedback
  3. Riboswitch
- Linear Model Design
- Modelling
- Wetlab
Engineering *Escherichia coli* to see light

These smart bacteria ‘photograph’ a light pattern as a high-definition chemical image.

- Anselm Levskaya*, Aaron A. Chevalier†, Jeffrey J. Tabor†, Zachary Booth Simpson‡, Laura A. Lavery†, Matthew Levy†, Eric A. Davidson‡, Alexander Scouras†, Andrew D. Ellington‡‡, Edward M. Marcotte†‡, Christopher A. Voigt*§
Positive Feedback

- Feedback loop that upregulates itself
Riboswitch

Review
Engineered riboswitches as novel tools in molecular biology
Gesine Bauer, Beatrix Suess *

Linear Model
Step by step...
In the dark
Kick start the system with red light.
System: state 1
Preparing for state 2
State 2
Modelling

JDesigner2 (System Biology Workbench)

Matlab (Mathworks)
Time for hands-on work in the lab - Red light sensor

**Plan of action**

- Make PCB and Cph8 (from Voigt lab, UCSF) into biobricks and test
- Replace OmpC promoter with OmpF
- Using existing biobricks create construct: pLacI-RBS-ho1-RBS-pcyA-RBS-Cph8-Ter-OmpF-RBS-GFP-Ter
Time for hands-on work in the lab- Red light sensor

Progress

• Double transformation of PCB and Cph8 using 3 different antibiotics; used blue-white screening.

• *Expected result*: white colonies in light; blue colonies in dark

• *Observed result*: blue colonies in light and dark
Time for hands-on work in the lab - Red light sensor

Troubleshooting

• Voigt lab had 100W mercury vapour lamp and special incubator; we used an incandescent lamp

=> could be that the light intensity and wavelength were not optimal
Time for hands-on work in the lab

-Positive feedback

Challenges

• Naturally not common occurrence in bacteria
• Need a different one for each component
Time for hands-on work in the lab - Riboswitches

Plan of action

• Use existing biobricks to construct pLacI-key-Ter

• pTet-lock-GFP-Ter-Ter
Colony PCR of key3c+pLacI ligation
XbaI/SpeI double digest of key3c+pLacl ligation
Time for hands-on work in the lab - Riboswitches

Achievements

• New biobrick BBa_K085000

• New biobrick BBa_K085005
Final thoughts...

- Modelling showed that system could work.
- Need to work on input signal
  - Red light
  - Other signals?
- Off protein
- Fitting parts together
Special Thanks!

- Bio21 Institute—Department of Biochemistry and Molecular Biology
  - Heung-Chin Cheng, Paul Gooley, Sally Gras
- The University of Melbourne
- City of Melbourne
- Voigt lab UCSF
- Coburg Senior High School (specially Jenny & Melissa)