Towards engineering a Biothermometer
Delft University of Technology

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Team and Instructors

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**Modeling:**
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**Lab work:**
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Ruud Jorna

**Ethics:**
Steven Flipse
Presentation outline

• Background & Basics
• Design
• Laboratory work
• Modeling
• Ethics
• Summary
The project is approached as two separate modules
  • Sensor: RNA thermometer
  • Reporter: isoprenoid biosynthesis pathway

Potential applications
  • Monitoring temperature during fermentation
  • Temperature inducible protein expression
RNA thermometers

- Functionality
  - Post-transcriptional regulation
  - The hairpin masks ribosome binding site

Design
Turning RNA thermometers into BioBricks

- 3 known thermometers\(^2\) turned into BioBricks
- Secondary structure important for functionality
- Adjustment for scar

Design

Changing the switching temperature

- Stability profile for 37°C (*RNAfold*)

- Similar profile used to design 32°C and 27°C RNA thermometers
Towards engineering a Biothermometer

Software

- Software tool written
- Produces RNA sequences of hairpin structures with a given stability profile
- Can be used to design RNA thermometers
Lab work on RNA thermometers

• Assemble BioBricks to make working constructs

• Testing sensitivity
  • Replacing the isoprenoid biosynthesis pathway with luciferase
  • Generate a protocol to measure luciferase (luminescence)
    • Correct luciferase measurements for total protein content
Experimental approach

• Temperature sensitive device (BBa_K115035)

• Reference device (BBa_K115012)
Experimental procedure

- Grow cultures at different temperatures
- Lyse cells
- Measure
  - total protein content
  - luciferase activity

- Problems in cell lysis
  - Lysis buffer – interferes with protein content
  - Protein precipitation – not reproducible
  - Fastprep – denatures luciferase
  - Beadbeater – not reproducible
  - Sonication – denatures some luciferase but reproducible
Results

- **BBa_K115035 is a working device (32°C switch)**

<table>
<thead>
<tr>
<th></th>
<th>20°C</th>
<th>30°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1.00</td>
<td>2.44</td>
<td>4.66</td>
</tr>
<tr>
<td>SEM ± 2</td>
<td>0.30</td>
<td>1.04</td>
<td>2.18</td>
</tr>
<tr>
<td>BBa_K115035</td>
<td>1.00</td>
<td>1.92</td>
<td>17.62</td>
</tr>
<tr>
<td>SEM ± 2</td>
<td>0.10</td>
<td>0.36</td>
<td>7.52</td>
</tr>
</tbody>
</table>

- At 30°C a **1.92** fold increase (Ref: 2.44)
- At 37°C a **17.62** fold increase (Ref: 4.66)
Color Pathway

- 14 enzymes involved
- *Escherichia coli* and *Saccharomyces cerevisiae* genes for FPP production
- Three enzymes obtained in the laboratory:
  - *atoB* — thiolase
  - *idi* — isopentenyl-PP isomerase
  - *ispA* — FPP synthase
- FPP to colorant(s) → Biobricks by Edinburgh 2007

Color Pathway

Geranylgeranyl diphosphate

\[ \text{phytoene synthase} \]

\[ \text{phytoene desaturase} \]

Lycopene (Red)

\[ \text{lycopene B-cyclase} \]

B-carotene (Orange)

\[ 3,3'-B\text{ ionone hydroxylase} \]

Zeaxanthin (Yellow)

• FPP to colorant(s) → Biobricks by Edinburgh 2007
Modeling

• Input:
  • Temperature
  • Challenge: Find appropriate model describing temperature sensitivity

• Output:
  • Color
  • Challenge: Define parameter spaces yielding stable dynamic system

**TABLE**

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Color</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Lycopene</td>
</tr>
<tr>
<td>B-Carotene</td>
<td>B-Carotene</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>Zeaxanthin</td>
</tr>
</tbody>
</table>

**Model**

- Input: Temperature
- Output: Color, Lycopene, B-Carotene, Zeaxanthin
Modeling

• Enzyme production: Hill type
  • $m$, $\alpha$, and $K$ are estimated by using luciferase assay results

Without inhibition

$$[e] = \alpha \frac{T^m}{T^m + K^m} + 1$$

With inhibition

$$[e] = \alpha \frac{T^m}{T^m + K^m \frac{1}{T^p + K^p_i}} + 1$$
Modeling

- The results show a switch behavior for the model
  - Parameters estimated by Genetic Algorithm

The results for the switch at 30°C is:

\[
\frac{e_2}{e_0} = 2 \times 10^{40} \frac{T^{25}}{T^{25} + 28.78^{25}} \frac{1}{T^{25} + 39.25^{25}} + 1
\]
Modeling

• Sensitivity analysis
  • The sensitivity analysis on the last four products show the effect of the parameter on the model

• Bifurcation analysis
  • The bifurcation analysis shows the stability for all estimated parameters
Ethics

• Goal
  • Understanding ethical issues in Synthetic Biology and within iGEM

• Why?
  • Ethical issues important to consider, also during design

• Report
  • “LIFE to LEGO – SynthEthics in the TU Delft iGEM project”
    • Ethical reflections for participants in the open source synthetic biology
      based iGEM competition
Ethical issues integrated

- Value sensitive design
  - Consider ethical issues in biological design, from the beginning of the project

- Literature survey – Macro ethics
  - Learn which ethical issues play in Synthetic Biology

- Questionnaire – Micro ethics
  - Learn participants' opinions
  - Generate awareness, reflect on ethical issues

- Question of the week
  - Stimulate discussion on ethical issues relevant to the project
The issues identified

- General ethical issues in synthetic biology
  - Biosafety
  - Biosecurity
  - Naturalness
  - Patenting

- Ethical issues in iGEM specifically
  - Value of Life
  - Intellectual Property

- BioTerror
- BioError
- Usefulness
- Open source
- Commercialization
- Responsibility
- Applied or fundamental science?
- Misuse
Summary

• Software to design RNA thermometers developed

• RNA thermometer (BBa_K115035) confirmed

• Three genes of color pathway cloned

• Mathematical models describing the biothermometer generated

• More awareness on ethical issues by:
  • Implementing value sensitive design
  • Interviews
  • Discussing ethical questions
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Sponsors

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Questions?
The last four products