Victoria BC
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Signal Integration:
Applications of RNA
Riboregulator Capabilities
Our Team

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In British Columbia, Canada
Overview

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When RNA has self-complementary sequence, it can fold back on itself and form a hairpin loop.

If the hairpin hides the Shine-Dalgarno sequence in the ribosome binding site, ribosome binding is inhibited.

The secondary structure of mRNA can then directly control the rate of protein expression.
The hairpin will persist until forced to unfold by outside influences. We explored two such mechanisms:

- Increasing temperature will melt the hydrogen bonds between base pairs that form the hairpin stem

- An additional piece of RNA which overlaps the complementary region will cause the hairpin to unravel, rather like a key will position the tumblers of a lock and let it open
These mechanisms formed the basis for our main projects:

• An RNA thermometer

• An RNA lock and key
RNA Thermometer

• An RNA hairpin will unfold when exposed to temperatures past the melting point for the sequence.

• This permits the temperature-sensitive expression of the downstream gene.

• The TUDelft 2008 iGEM team retrieved natural RNA thermometers from three species, then sequenced and redesigned them to test for a modified temperature range.
RNA Thermometer

Their characterization found the 32°C thermometer variant to be the most effective.

BBa_K115017
(32 degrees Celsius)
RNA Thermometer - Applications

• When working with bacteria, this would be a useful added feature that would indicate what temperature they were grown at.

• This would be an easy way to confirm that your culture did not go beyond the minimum or maximum you intended.

• Another way to use this system is to apply it to any protein you would like to regulate by temperature.

• This is essentially a switch that is easier to administrate than a chemical trigger.
Biothermometer

• We designed both a complex (three states corresponding to three colours), and a simple system (binary states corresponding to on/off colour) for a biothermometer.

• Our resources inspired us to start our lab work with the simple system.
The complete system was designed to show three colours for three different temperature states.

These three temperature ranges would be defined by two RNA thermometer parts, one for 32°C and one for 37°C.

A system of promoters, repressors and activators would then effect the regulation of the fluorescent proteins CFP, GFP and RFP.
Biothermometer - Complex

LacI Promoter → CFP → LacI Repressor
LacI Promoter → CFP → LuxR Activator → cI Repressor
LacI Promoter → CFP → LuxR Activator → cI Repressor

LuxR Activator → GFP → cI Repressor
LuxR Activator → GFP → cI Repressor

LasR Promoter → RFP
LasR Promoter → RFP

38°C
34°C
30°C
At each temperature a different set of regulators is active.
Biothermometer - Simple

• The simple system placed the fluorescent protein directly under the control of the ribothermometer.

• These systems are designed to repress fluoresce at low temperatures.

K235036 – red fluorescence is produced above 32°C.

K235037 – green fluorescence is produced above 32°C.
Biothermometer - Simple

K235036

32 °C ➔ RFP ➔ 32 °C

Promoter

K235037

32 °C ➔ GFP ➔ 32 °C

Promoter

34
Biothermometer - Simple

- The inverse system would have placed the fluorescent protein under the control of the lactose promoter, and put the LacI protein under the control of the ribothermometer.

- This system should only fluoresce at temperatures below 32°C, or when IPTG is present.

- We were able to complete a lactose controlled GFP, although we had not yet added temperature sensitivity.

K235032- Lactose promoter regulates green fluorescence.
Biothermometer Testing

• Early tests had shown that we had BioBricks which produced functional GFP and RFP.

• We cultured *E. coli* that contained our temperature sensitive parts K235036 and K235037, as well as ones containing the lactose controlled K235032 both with and without IPTG.

• These were grown overnight at 30°C, 34°C, 37°C, and 42°C, along with positive and negative controls.

• The next day fluorescence microscopy was used to test for fluorescence levels.
Biothermometer Results

- The lactose-controlled \textbf{K235032} fluoresced \textcolor{green}{green} at levels proportional to growth rate.

- Neither \textbf{K235036} nor \textbf{K235037} showed any fluorescence at any temperature.

\textbf{K235032} at 37°C
• Ribolock hairpins prevent translation until the presence of a key.
Background - Ribolock and Key

• A ribokey is a sequence complementary to the lock that binds and exposes the RBS.
Biothermometer - Ribolock

• In theory, there is nothing preventing a ribolock from acting like a ribothermometer, as both are hairpin structures.

• Our intent was to test the repression capability of a ribolock by culturing *E. coli* encoding ribolock-controlled GFP at room temperature, 30°C, 37°C, and 45°C, and measuring the resultant fluorescence.
Background - Ribolock and Key

• A ribolock/ribokey system is most useful when two different promoters control the production of ribolocked mRNA and of the ribokey.

• The Berkeley 2006 iGEM team began with the system produced by Collins et al., then redesigned the lock and key sequences to increase the efficiency.
**Ribolock NAND Gate**

- We designed a NAND (Not AND) gate that utilised the ribolock functionality without the temperature sensitivity.

- It would express RFP controlled by a λcl promoter with repression regulated by the arabinose promoter and the lactose promoter.

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>IPTG</th>
<th>RFP produced</th>
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<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>Red</td>
</tr>
<tr>
<td>No</td>
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<td>Yes</td>
<td>No</td>
<td>Red</td>
</tr>
<tr>
<td>Yes</td>
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Ribolock NAND Gate

- pLac
- pAra
- pλcl
We designed multiple systems utilizing the regulatory capabilities of mRNA hairpin loops; however, our limited funding encouraged us to pursue a project with less complexity.

We assembled two biothermometers, unfortunately we did not have time to troubleshoot the constructs when they failed to express fluorescence.

We were able to produce a working GFP under the control of a lactose promoter.
Future Directions

• Completing assembly of a ribolocked fluorescent protein and characterizing its temperature sensitivity.

• Re-assembling the biothermometer systems, with sequencing to ensure accuracy.

• Modifying the NAND gate prototype to improve ease of assembly, and construction of the gate.
References


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Questions?

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