RNA Thermometers – Analysis and Design

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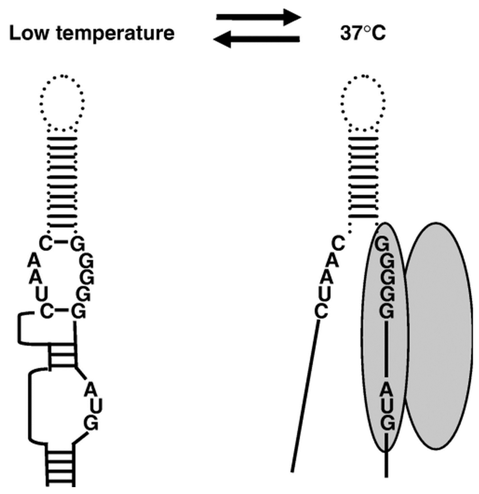
Introduction and Aims

The 5′ untranslated region of the mRNA folds into a structure that blocks ribosome access at low temperatures.

Increasing the temperature gradually shifts the equilibrium between the closed and open conformations towards the open structure in a zipper-like manner, thereby increasing the efficiency of translation initiation.



The main aims of this project are to –

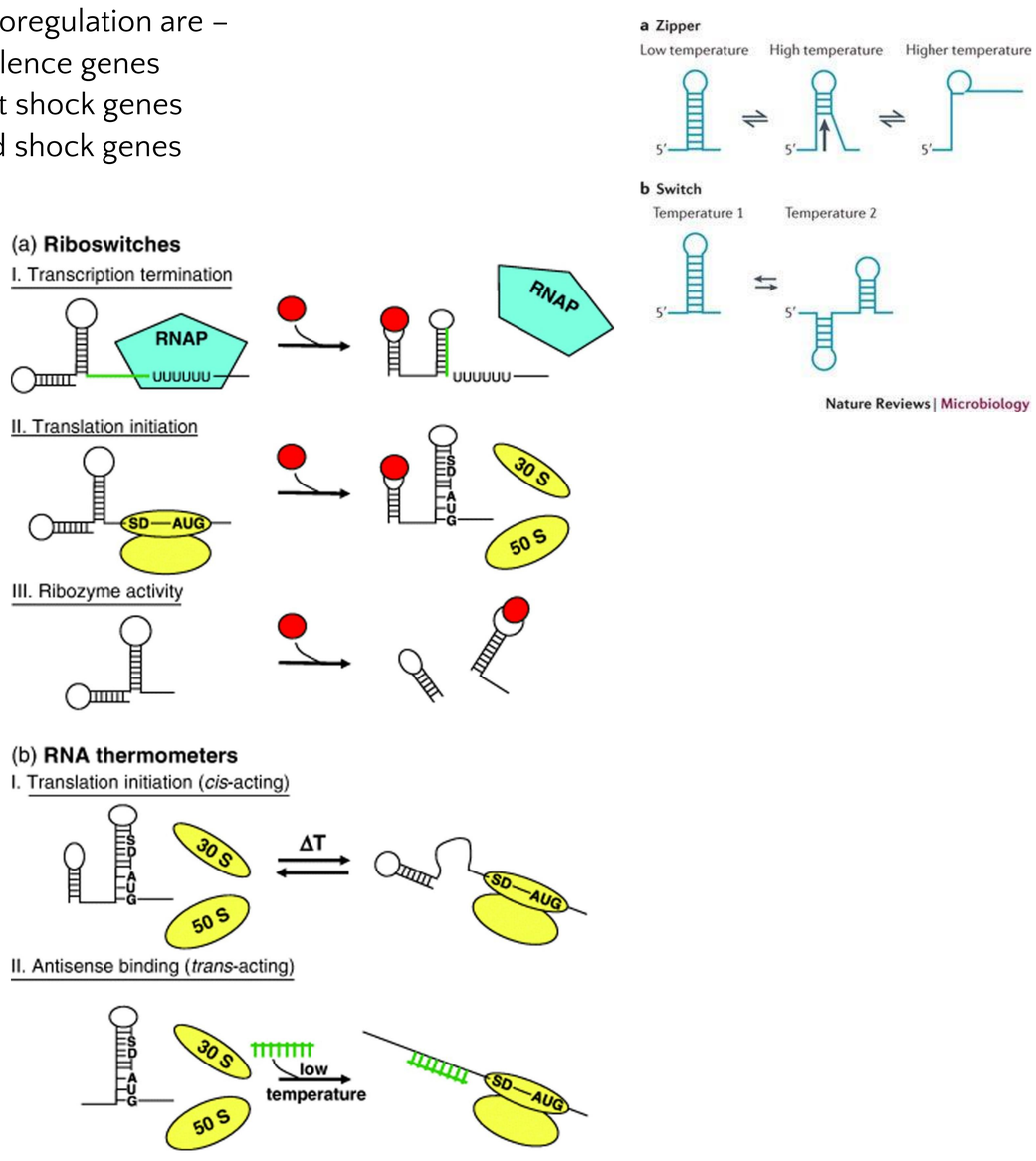
- Characterize the temperature response of natural thermometer and experimentally develop stability profile of naturally existing thermometer.
- Design different circuits that respond to temperature such as bio-molecular circuit where one of the input is temperature which can be further used as a regulating input.
- Develop experimental frameworks for multi-modular circuit design such as computational model assisted and directed evolution.

Characterization of Natural Thermometers

Structured regions in bacterial mRNAs can act as thermos sensors, known as RNA thermometers (RNATs), that control translation efficiency of the transcript.
Some RNATs act like zippers that open and close, in a reversible manner, according to the ambient temperature. These RNATs control heat shock and virulence genes.
Escherichia coli uses a cascade of hierarchically organized RNATs to monitor any harmful temperature upshifts and to induce the production of protective heat shock proteins when required.

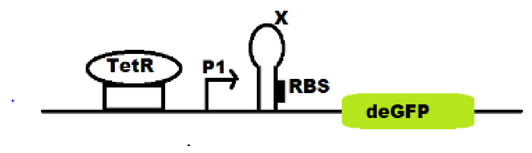
Three major temperature responsive gene classes which are prone to thermoregulation are –

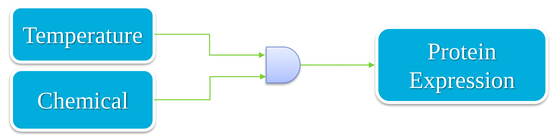
- Virulence genes
- Heat shock genes
- Cold shock genes



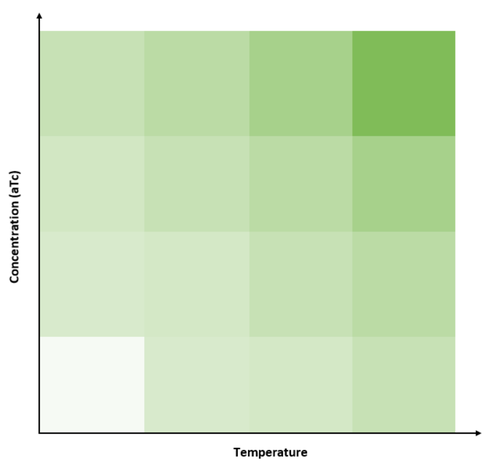
Design of temperature sensitive circuits

An AND gate has been designed which takes one chemical and one temperature as input and gave gene expression as output.

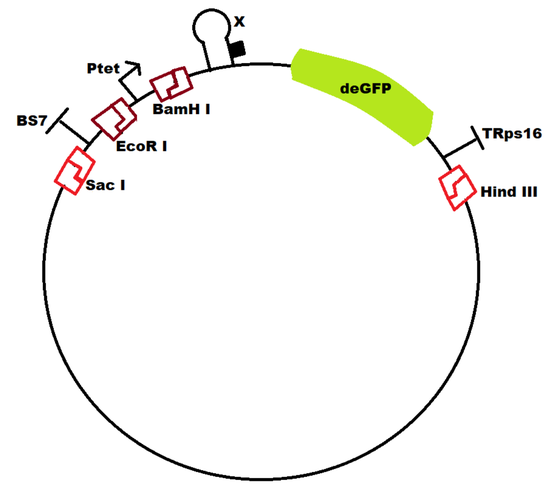




Schematic of AND Gate. Ptet promoter is activated by presence of high concentration of aTc, which is acting as the chemical based input.



Expected Results



Experimental framework for multi-modular circuit design

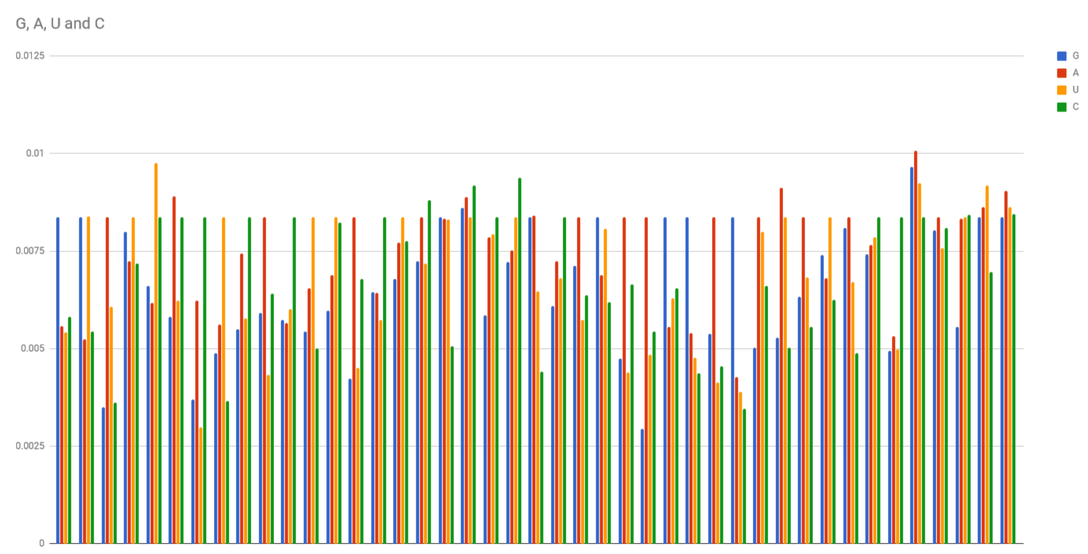
NUPACK, a web application for the analysis and design of nucleic acid structures, devices, and systems, was used for analysing the sequence :

GGAUCCCUCA CUUACUAGUC UGCAGAAGGA GAUAUACCCA UGG

It enabled analysis and design of the equilibrium base-pairing properties of one or more test tubes of interacting nucleic acid strands.
A list of 130 sequences were generated using the base sequence, modifying one base pair at a time.

The sensitivity (the slope) was plotted, in the temperature range of 24-42, vs Position of Base.

The best sensitivity sequence was sent for synthesis



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Ackowledgements

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