RNA Thermometers – Analysis and Design (MI01706)

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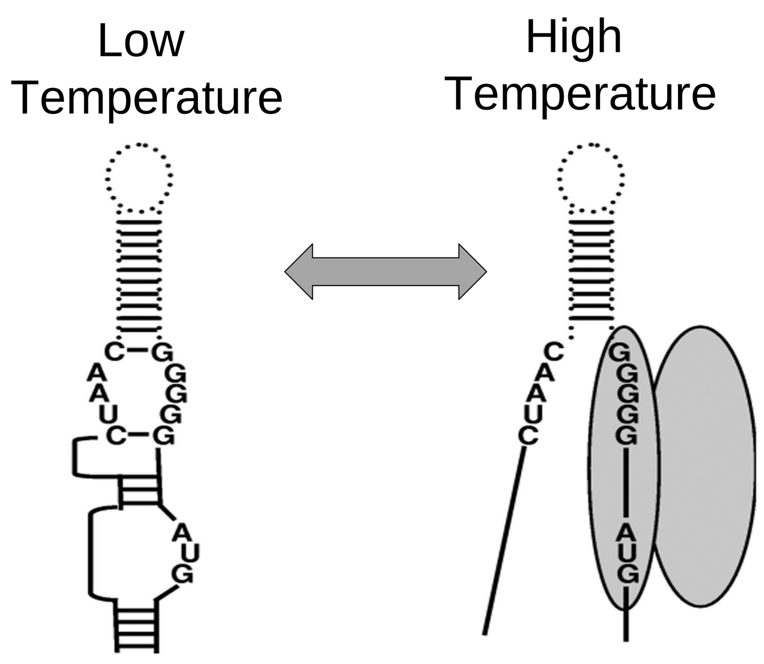
Introduction and Aims

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.



Motivation:

- Temperature is key variable of environment which has global presence.
- Gene expression regulated by combined actions has application in design of temperature compensation in genetic circuits, drug delivery and therapeutic purposes.
- The non-contact regulation with temperature adds the flexibility of turning off which is observed in natural biological system.

Main aims of the project –

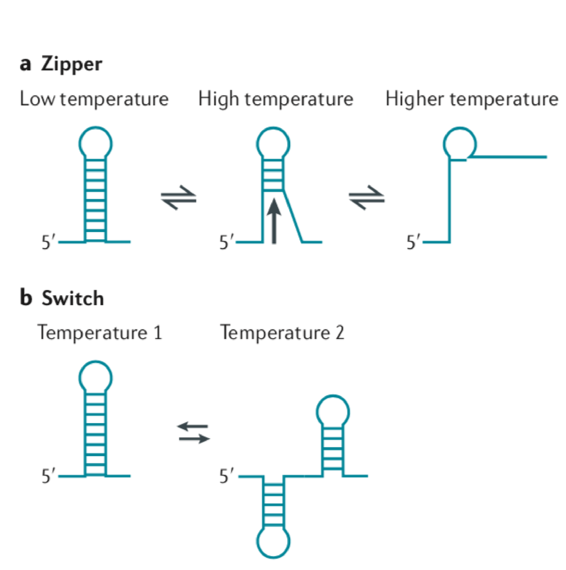
- Characterize the temperature response of natural thermometer and experimentally develop stability profile of naturally existing thermometer.
- Design different circuits that respond to temperature such as bio-molecular circuit where one of the input is temperature which can be further used as a regulating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

Aim 1- Characterization of Natural Thermometers

Bacteria use complex strategies to coordinate temperature-dependent gene expression. Many genes encoding heat shock proteins and virulence factors are regulated by temperature-sensing RNA sequences, known as RNA thermometers (RNATs), in their mRNAs.
Three major temperature responsive gene classes which are prone to thermoregulation are – Virulence genes, Heat shock genes, Cold shock genes.

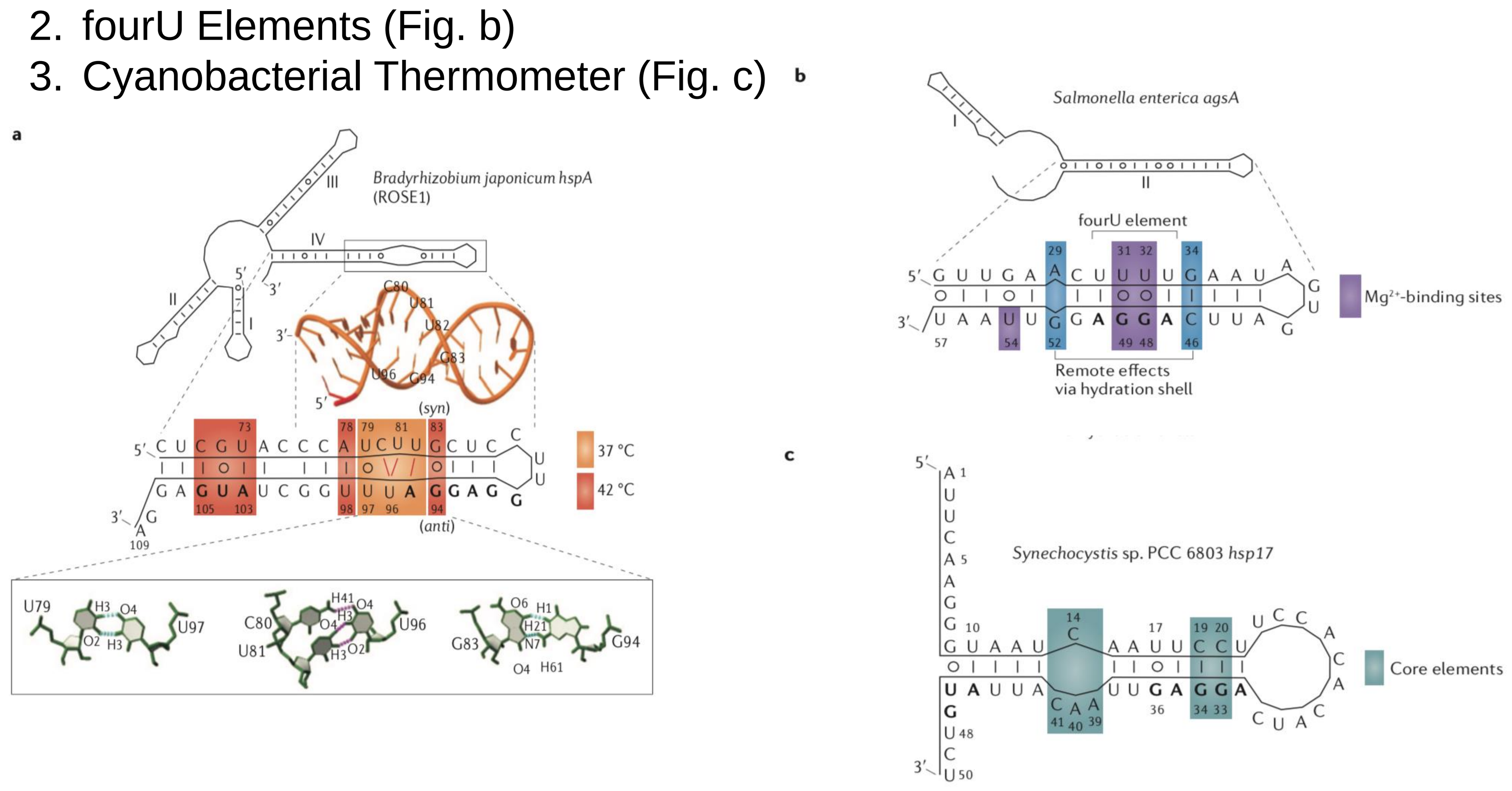
There are two major classes of naturally occurring RNA thermometers.

- RNA Zippers
- RNA Switches



To study the general principles for sequences of RNATs, three classes were reviewed :

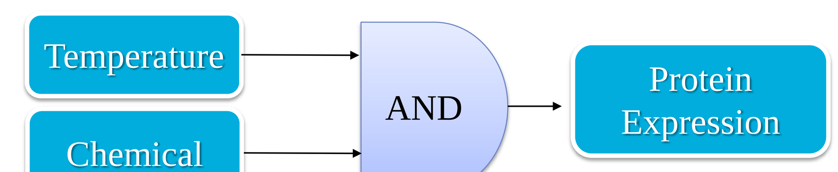
1. ROSE Elements (Repression of heat shock gene expression) (Fig. a)
2. fourU Elements (Fig. b)
3. Cyanobacterial Thermometer (Fig. c)

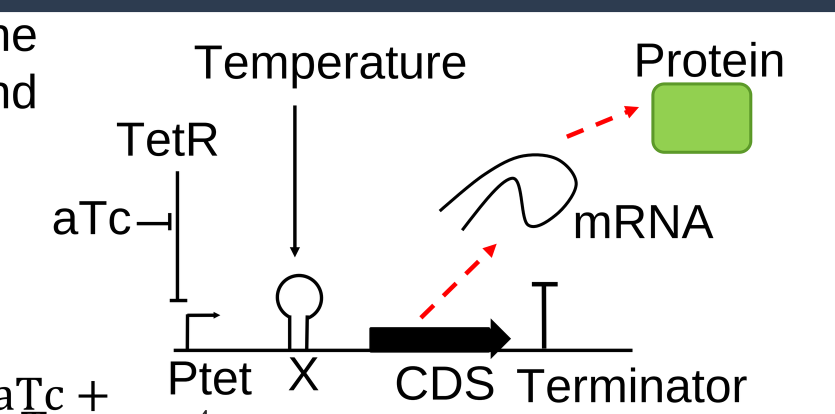


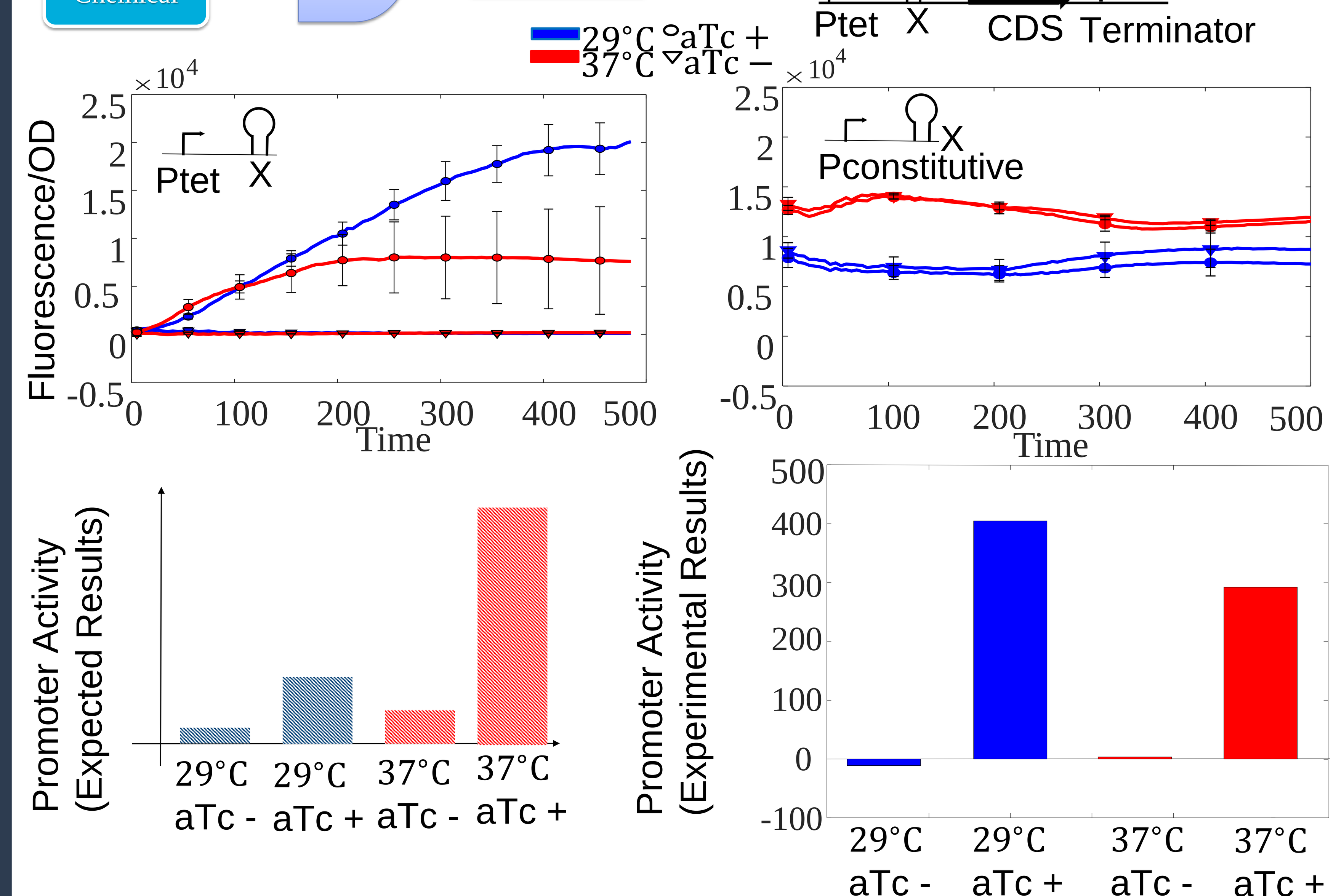
Future Work: Sequences have been designed and in the process of synthesis

Aim 2- Design Of Temperature Sensitive Circuits

Designed combinatorial action of gene expression with a Chemical Inducer and Temperature (Please see [3])







- aTc input dominates the temperature noted from the GFP/OD traces
- At high aTc induction levels the thermometer action is effectively inverted from activation to repression in presence of temperature

Future Work :
To compare the response to a circuit with TetR/Ptet system in the absence of Thermometer and a circuit with absence of both TetR/Ptet system and thermometer.

Aim 3- Computational Sensitivity Analysis RNA Thermometer

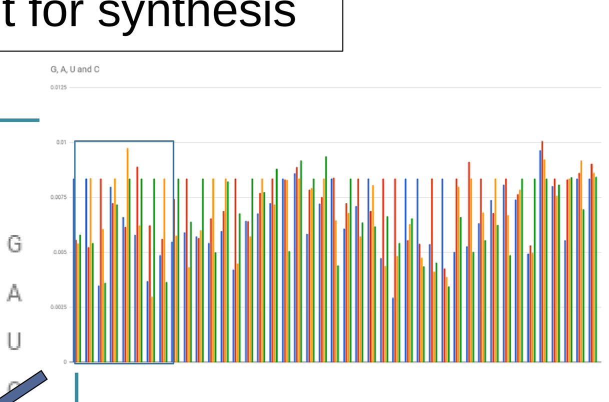
NUPACK, a web application for the analysis and design of nucleic acid structures, devices, and systems, used for analysing the sequence :

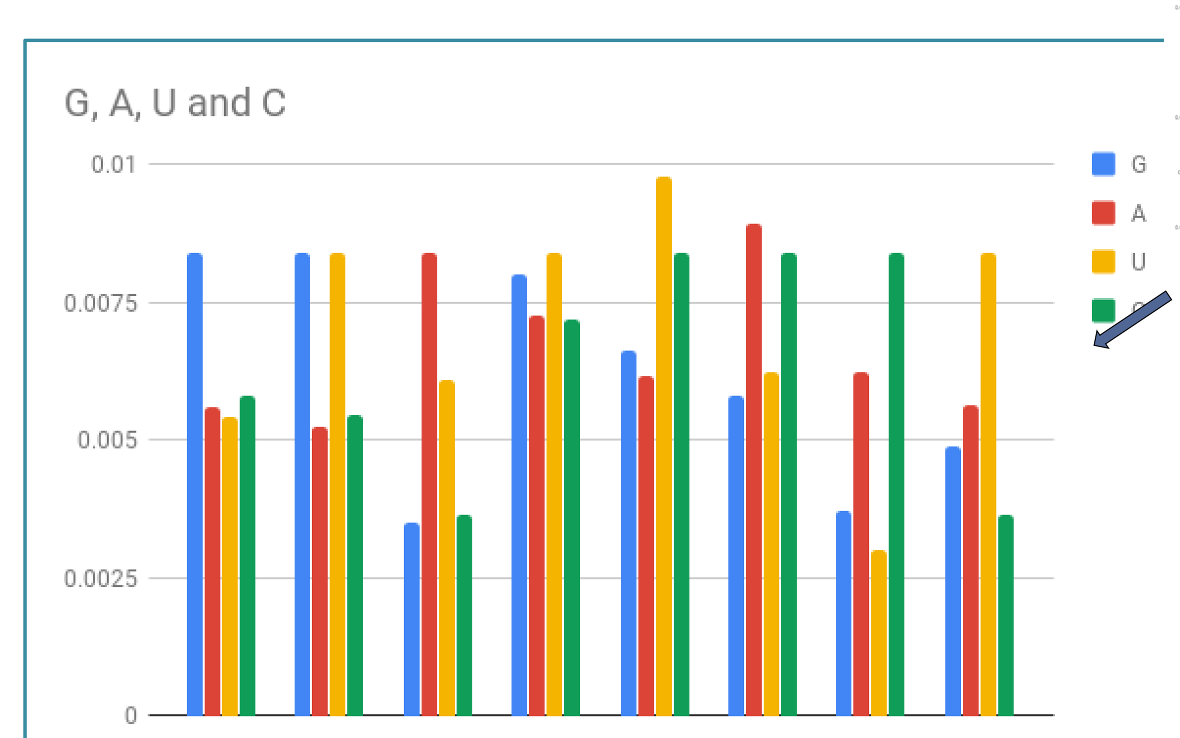
GGAUCCCUCA CUUACUAGUC UGCAGAAGGA GAUAUACCCA UGG

List of 130 sequences generated using the base sequence, modifying one base pair at a time.

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

The best sensitivity sequence to be sent for synthesis





Future Work :
Measurement protocols being characterized, Sequences have been designed.

References

[1] Kortmann J, et. al., "Bacterial RNA thermometers: molecular zippers and switches," Nat Rev Microbiol., vol. 10 no. 4 pp. 255-65, 2012

[2] Meyer S, et. al., "Characterizing the Structure-Function Relationship of a Naturally Occurring RNA Thermometer," ACS Biochem.,Vol. 56 no. 51, pp. 6629-6638, 2017

[3] H. Jia, et. al., "Temperature sensitive protein expression in protocells," Chem. Comm., vol. 55, no. 45, pp. 6421–6424, 2019

Acknowledgements

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* is pursuing the project as a BTP project