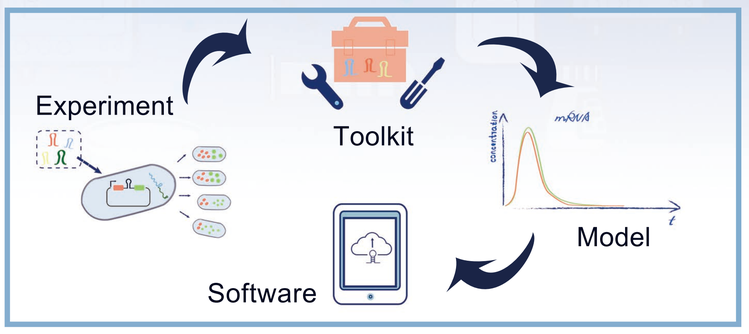
Cistrons Concerto

A pithy regulatory method on post-transcriptional level

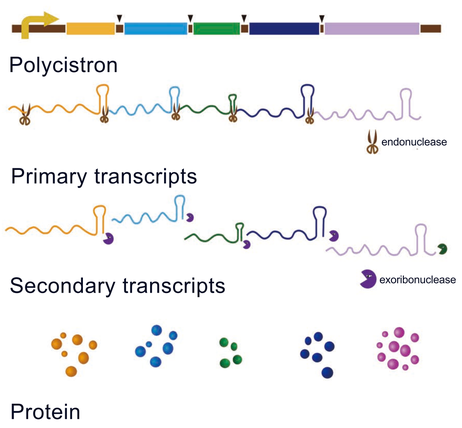
OUC-China

Introduction

- **Our experiments** focus on designing a tightly regulatory element to tune the expression of multiple genes within a polycistron by inserting stem-loops into the intergentic regions. Stem-loops with various folding free energy protect upstream gene in varying degrees, which eventually influences the expression of proteins.
- **Our toolkit** is constructed with various stem-loops to meet future demand after collecting and standardizing these stem-loops.
- **Our software** provides a database of stem-loops that can find out corresponding stem-loops for users when uploading the sequences, which is based on our modeling.



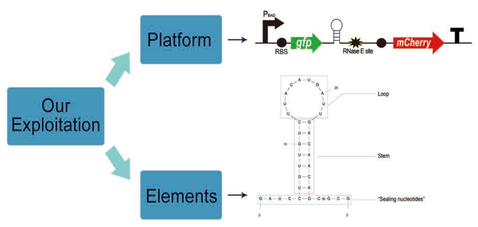
Design



How dose it work?

Diverse stem-loops with different stability at 3' end protect the upstream gene in varying degrees from being degraded by exoribonucleases.

How to test it?

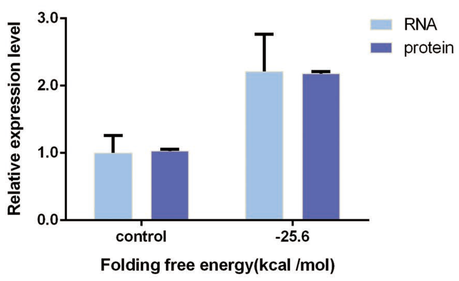


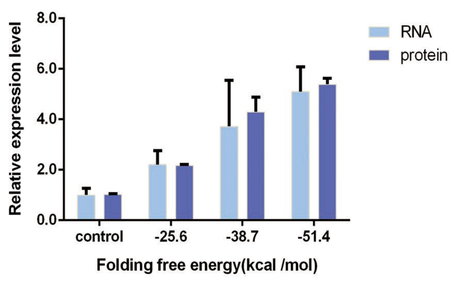
A dual-fluorescent reporter system was constructed with a stem-loop and a endoribonuclease site inserted in the intergenic region between two reporter genes. By measuring the ratio of the upstream to downstream gene, we tested the differential expression caused by stem-loops.

Result

How to prove it?

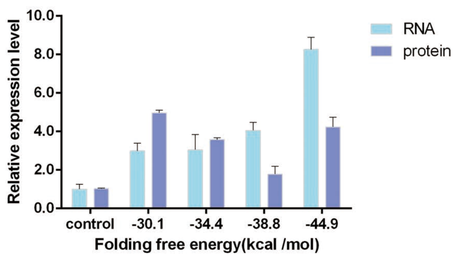
By inserting the native stem-loops that had been previously experimented before by others, we validated the regulatory effect caused by stem-loops both on transcriptional and translational level.

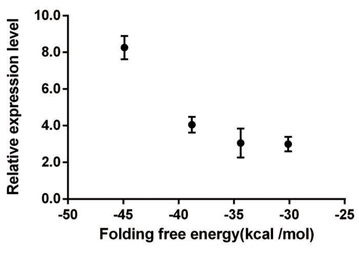




Anything more?

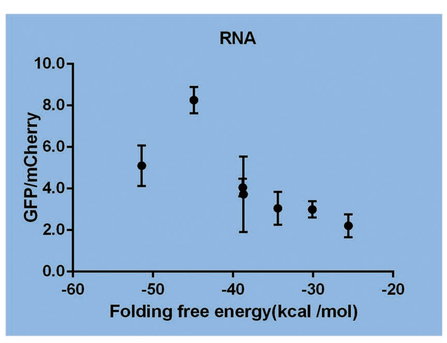
Our designed stem-loops were also tested with the conclusion that stem-loops, as a novel regulatory element, functioned well with predictable behaviors.

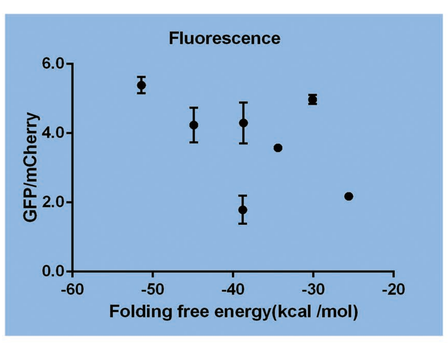




What conclusions can we draw?

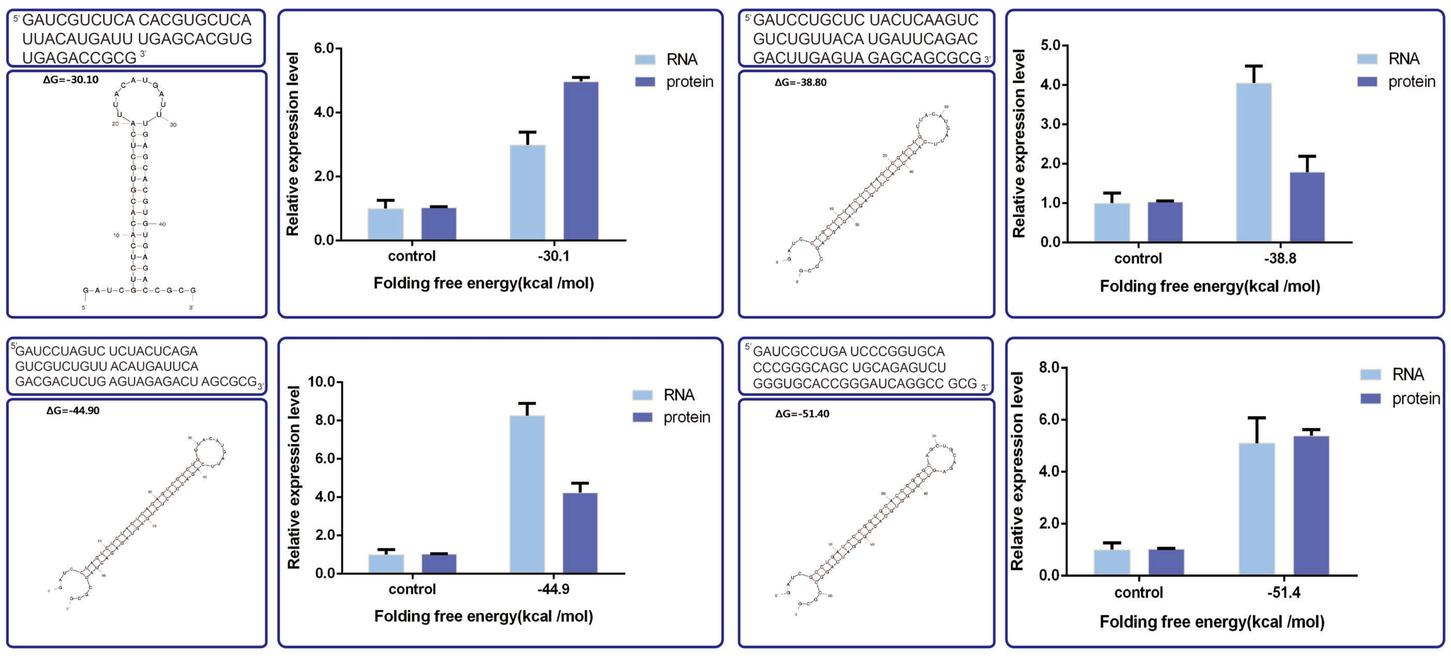
Relative expression level is related to the folding free energy of stem-loops both on transcriptional and translational level.





Toolkit

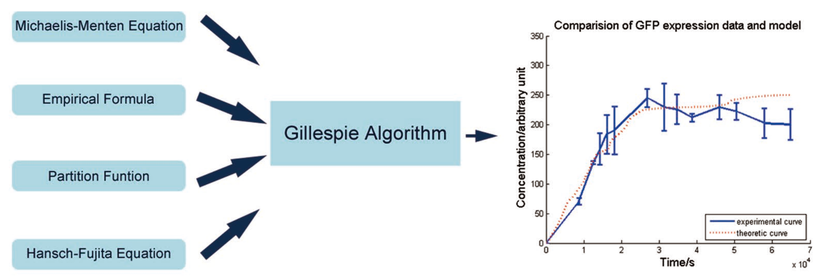
We constructed a toolkit of experimented stem-loops with detailed descriptions, containing specific sequence, folding free energy as well as secondary structure predicted by mfold. All the stem-loops are ranked by a gradient of folding free energy. In order to get more available parts and improve our system, we are supposed to design more stem-loops to correct our results and expand our toolkit for a broader use. Therefore, a software was built to help extend our measuring. In addtion, we have standardized our stem-loops and submitted them as parts to iGEM registry.



from Mfold ΔG: kcal/mol

Model

In our modeling, we validated the effectiveness of initial designs. Firstly, we analyzed the whole system and found 10 reactions in total, including the transcription, the hydrolysis process, the translation and the degradation.In order to obtain a quantitative relationship between the free energy and the expression level, we utilized the Gillespie Algorithm. The parameters' verification in the algorithm seeks to various methods. Subsequently, we assessed simulated results with rigorous statistical methods and compared them to our experimental one. In a conclusion, we believe this model is helpful for our understanding towards our system and further evaluate our project's potential capacity.



Software

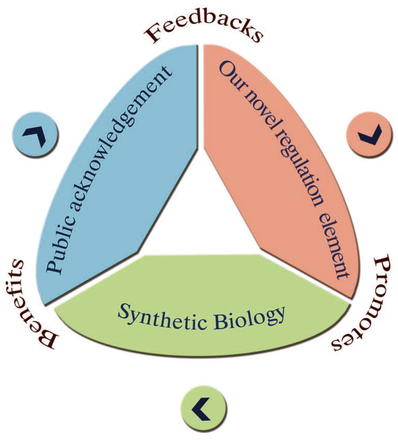
We have developed our own software which provides a database of stem-loops. When users upload their own sequence, the software can find out corresponding stem -loops and store them into the database. Moreover, when users need proteins to be expressed in specific quantity, such as inputting the ratio of two different proteins, the software can provide suitable stem-loops according to the ratio. The relationship between the quantitative expression of mRNA and the folding free energy is based on our model. We believe that our toolkit will play a part in prokaryotic regulation field.

HERE IS OUR QR CODE.

Human Practice

This year our team devoted to a novel regulation element, which made great sense in synthetic biology, but might be a little obscure for those who were unfamiliar with this field. Our regulatory element worked out through synthetic biology and then was acknowledged by the public. Our work includes:

- **Communicating and Improving**: Consult several experts of synthetic biology and improve our design according to the feedbacks.
- **Investigating and Promoting**: Investigate the popularity and public perception of our design as well as synthetic biology.
- **Potential Application**: Do practical research and look for chances to optimize industrial production.



References

1. Smolke, C.D. and J.D. Keasling, Effect of gene location, mRNA secondary structures, and RNase sites on expression of two genes in an engineered operon. Biotechnol Bioeng, 2002. 80(7): p. 762-76.
2. Pfleger, B.F., et al., Combinatorial engineering of intergenic regions in operons tunes expression of multiple genes. Nat Biotechnol, 2006. 24(8): p. 1027-32.
3. Xu, C., et al., Cellulosome stoichiometry in Clostridium cellulolyticum is regulated by selective RNA processing and stabilization. Nat Commun, 2015. 6: p. 6900.

Acknowledgements:

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