FUDAN iGem2013

ALeader

Leading the advance of RNA synthetic biology

Guo Hao-tian, Lou ying-qi

Oct 5th, 2013

Our Team

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The RNA engineering issue

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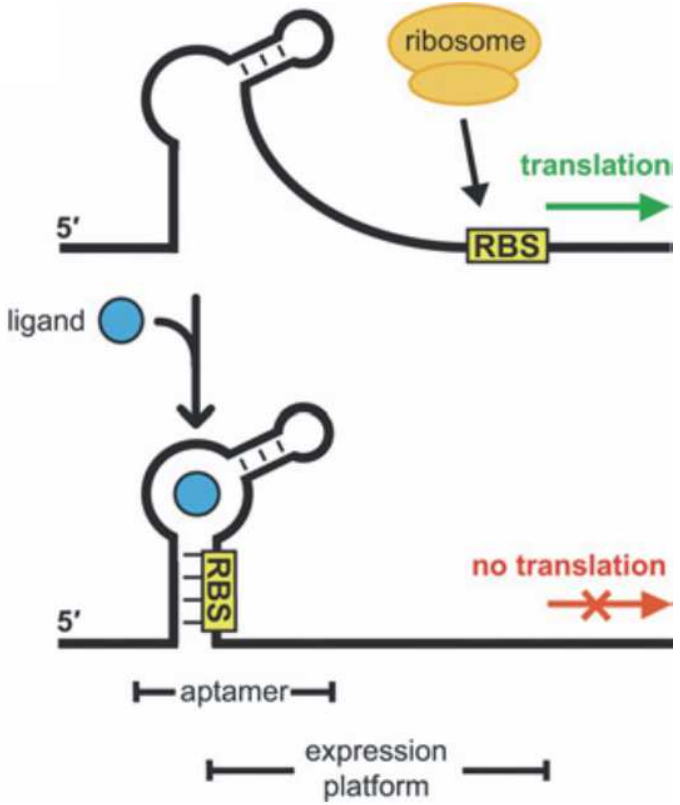
RNA parts are:

Powerful

Programmable

However:

No standard protocol



Kim, J. N.; Breaker, R. R., Biology of the Cell 2008, 100, (1), 1-11.

Our goal

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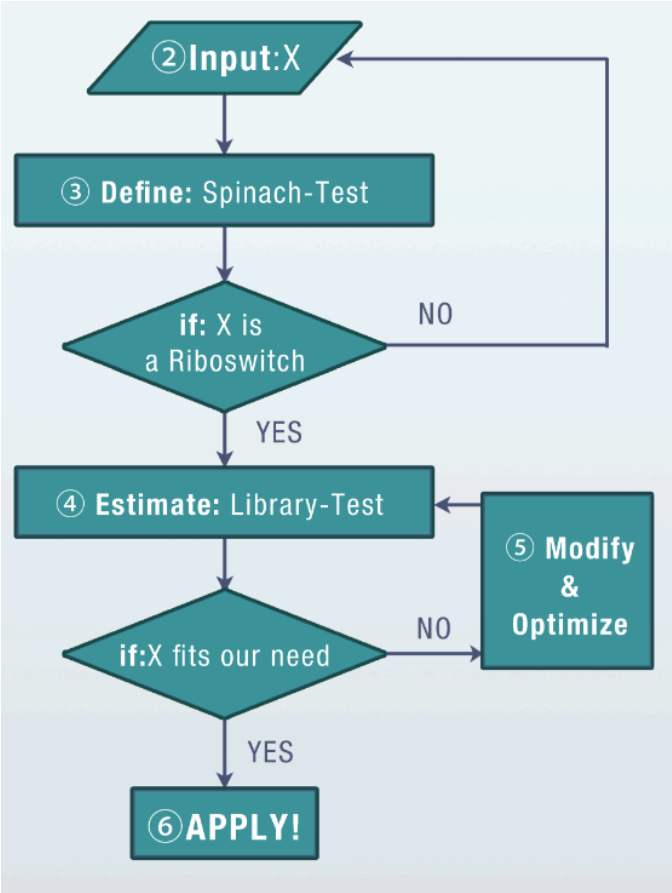
A Standard Protocol For Riboswitches

- For convenience
- For better RNA synthetic biology

Five step process

The first instructions

Tip:
unpublished works are highlighted



Set X = Aleader

Discovered in AAC and AAD

The most complex riboswitch

Input

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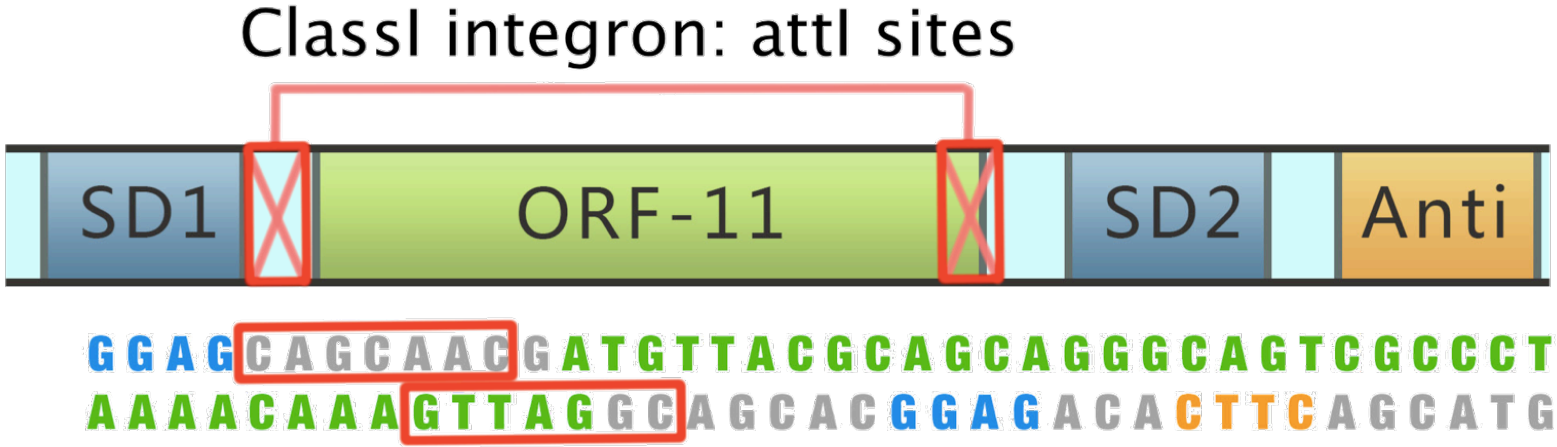
The most complex riboswitch

- Bi-cistron
- ORF-11
- attI sites



Input

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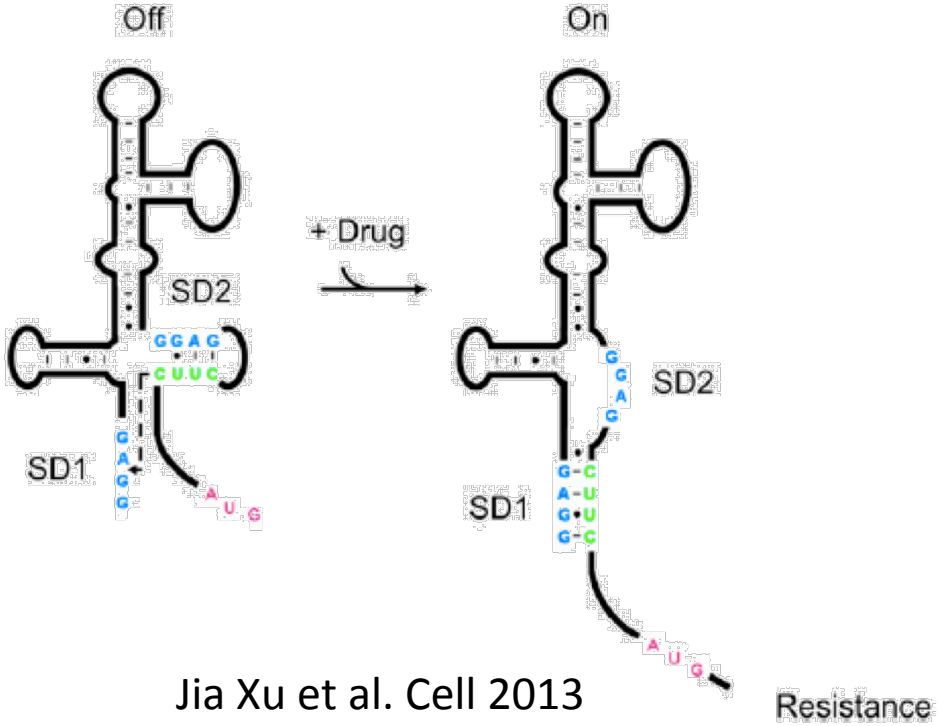
The most complex riboswitch

- Bi-cistron
- ORF-11
- attl sites
- Aptamer



Jia Xu et al. Cell 2013

Definition

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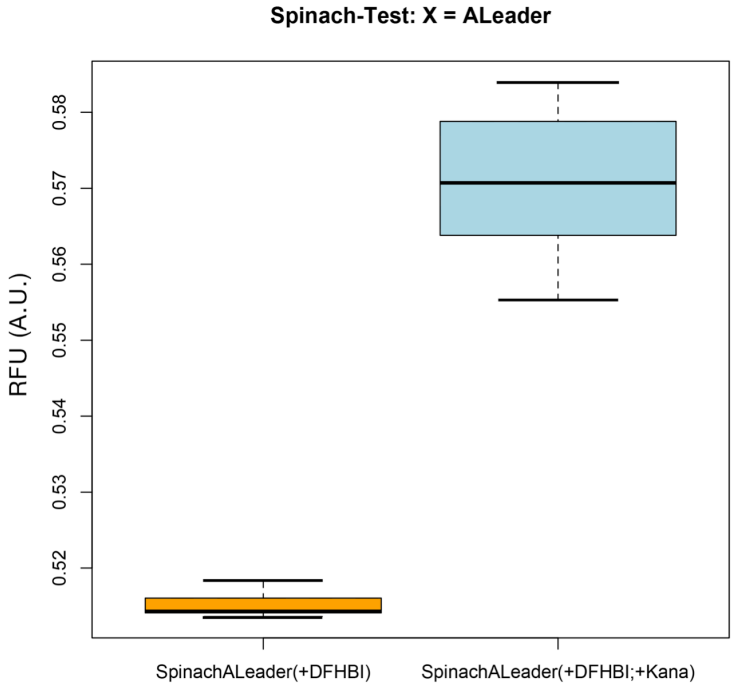
Spinach-Test

- To visualize the in vivo conformational switches
- RNA-mimic GFP: Spinach
- The necessary and replaceable Stem loop 2
- Fusing the Spinach and X



J S Paige et al. Science 2012

Estimation

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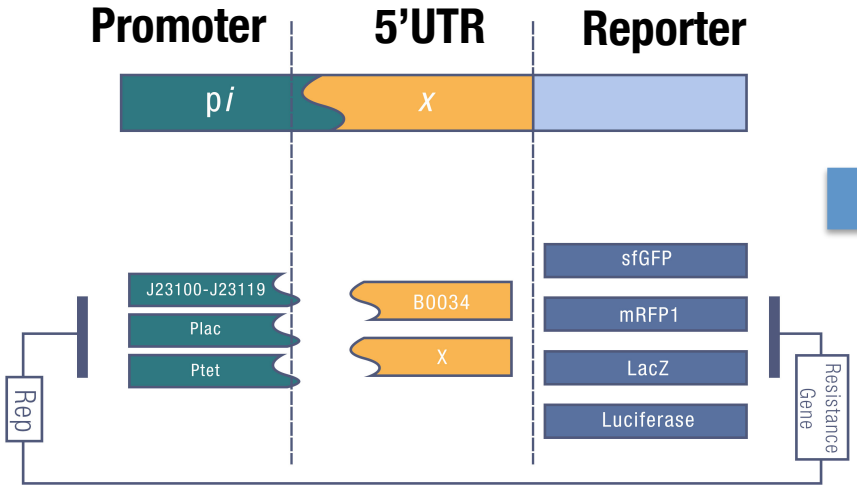
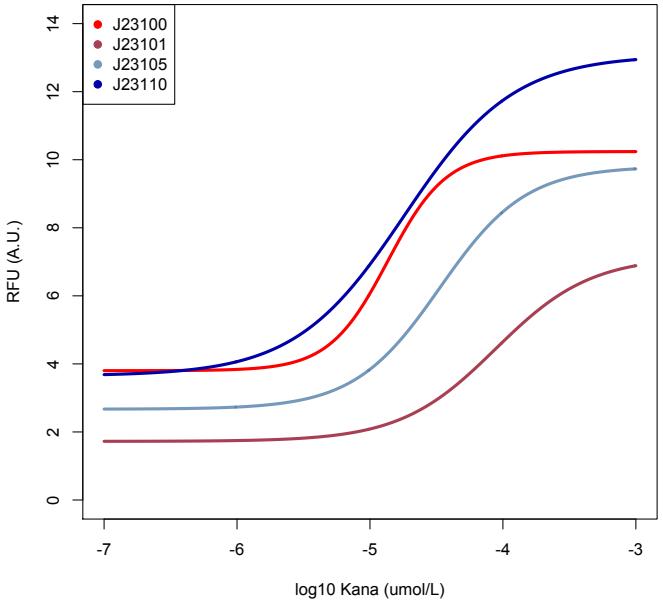
Quantitative estimation

based on promoter library





Estimation

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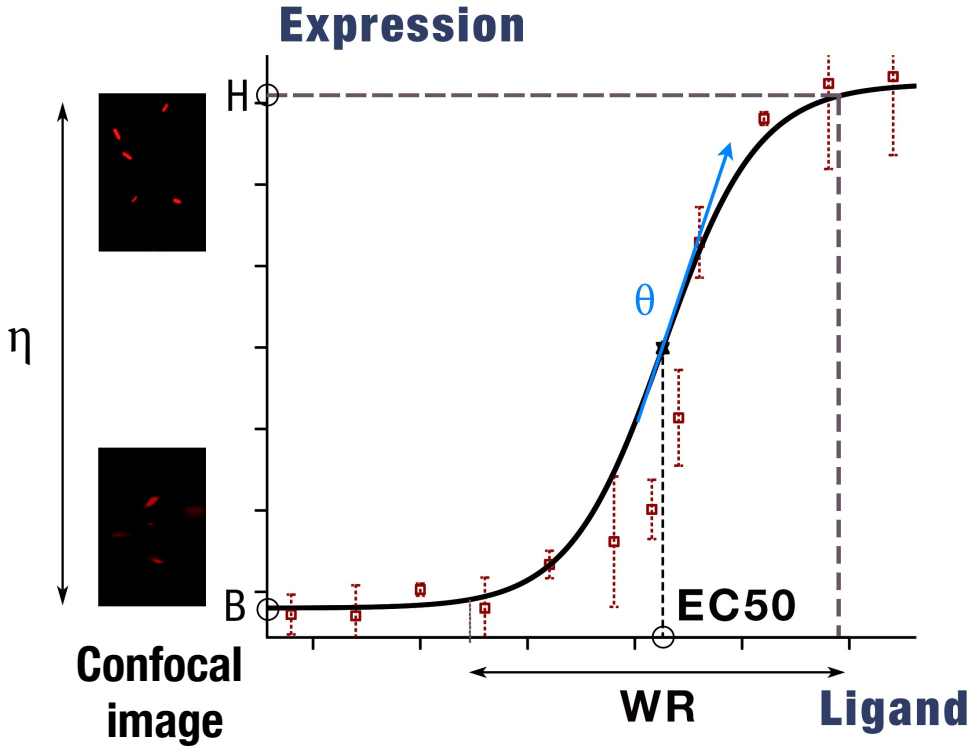
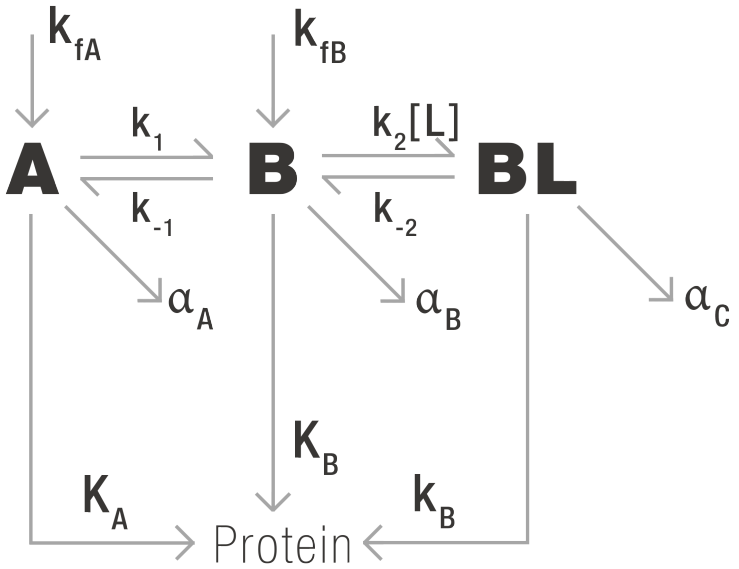
Response Curve Analysis

- **Dose-response curve**
- **Performance descriptor**
  - Basal level
  - Highest level
  - Dynamic range
  - Working range



The traditional method: Mutagenesis

based on the design principle of riboswitch



$$P = \frac{k_f}{a_p} \frac{K_A K_1 + K_B(1 + K_2 L)}{a_1 K_1 + a_2(1 + K_2 L)}$$

P: protein production
kf: transcriptional initiation rate
K_A,K_B: representative regulatory activities of conformation A and B
a_1,a_2,a_p: degradation rate of conformation A and B, and protein
K_1: partitioning constant
K_2: the aptamer association constant
L: ligand concentration

Modification & Optimization

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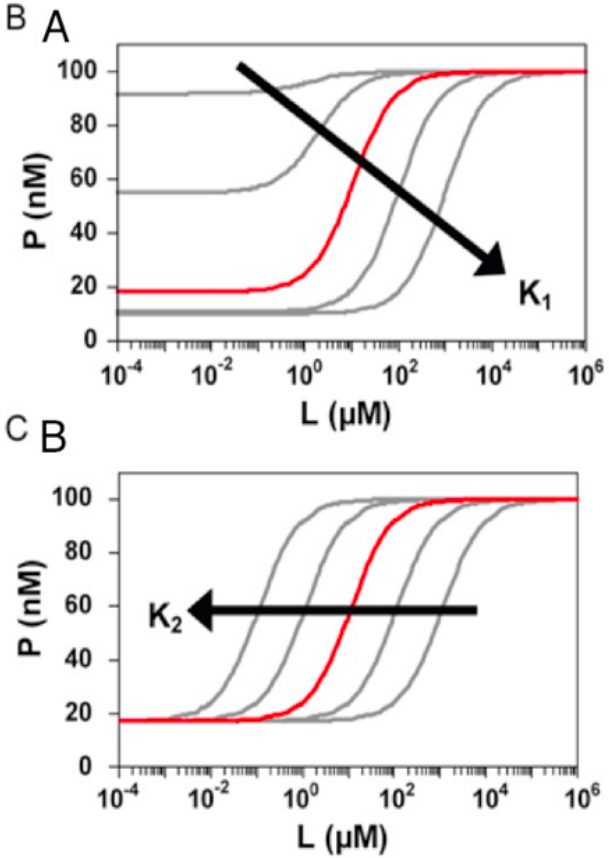
Modeling of the riboswitches

- Increasing the relative stability of conformation A

- Increasing the binding affinity



Modification & Optimization

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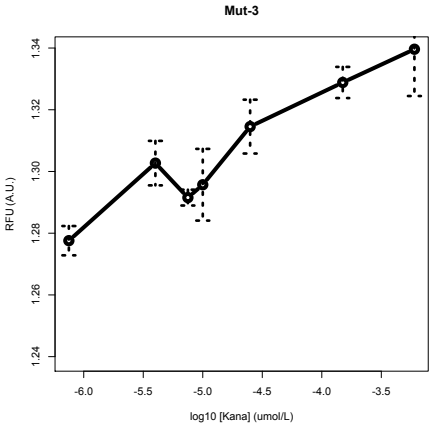
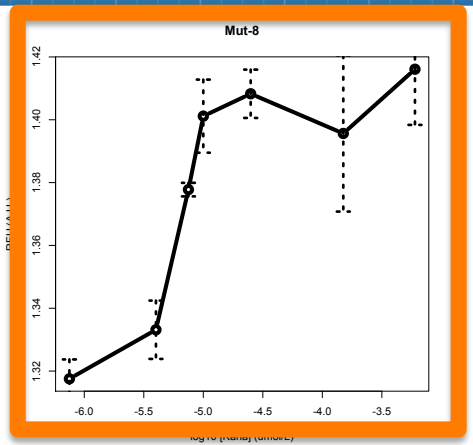
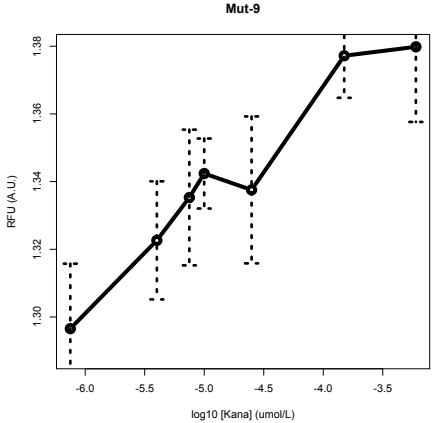
Practice:

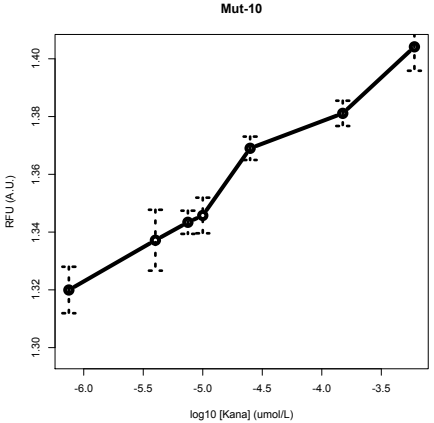
Mutants of Aleader

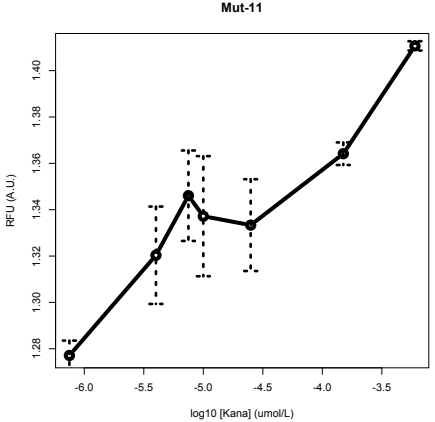
- We mutated the ALeader
- And estimate the mutants











Modification & Optimization

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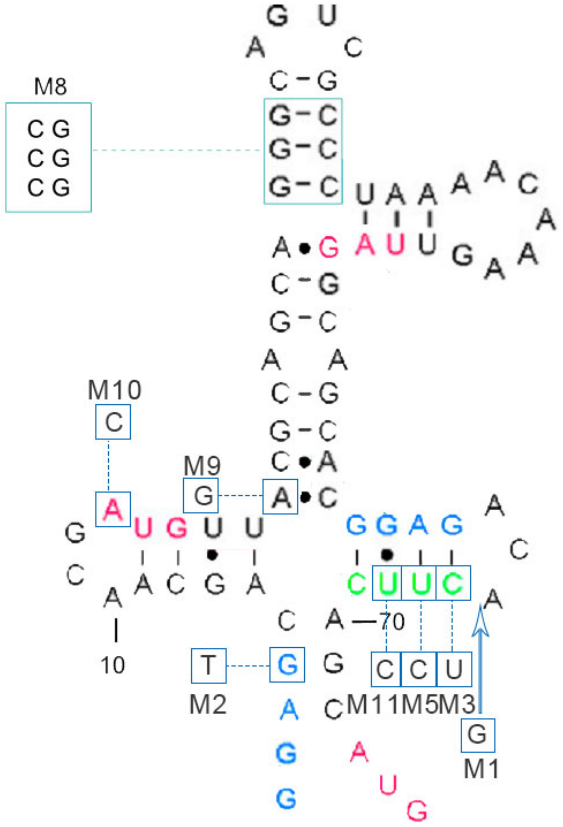
If mutants fits our need?

- For such a conserve riboswitch
- Unworkable

Mutants	Position	Function	Designed feature	Result
Mark1	66insC	SD2	K1 increase	Function failed
Mark2	2G>U	SD1	K1 decrease	Function failed
Mark3	66C>U	AntiSD	K1 altere	DR=1.10
Mark5	67U>C	AntiSD	K1 altere	Function failed
Mark8	13A>C	Start codon	K2 altere	DR=1.09
Mark9	18A>G	Ligand binding session	K2 altere	DR=1.12
Mark10	26G>C,27G>C,28G>C, 35C>G,36C>G,37>G	Ligand binding session	K2 altere	DR=1.10
Mark11	68U>C	AntiSD	K1 altere	DR=1.11



Modification & Optimization

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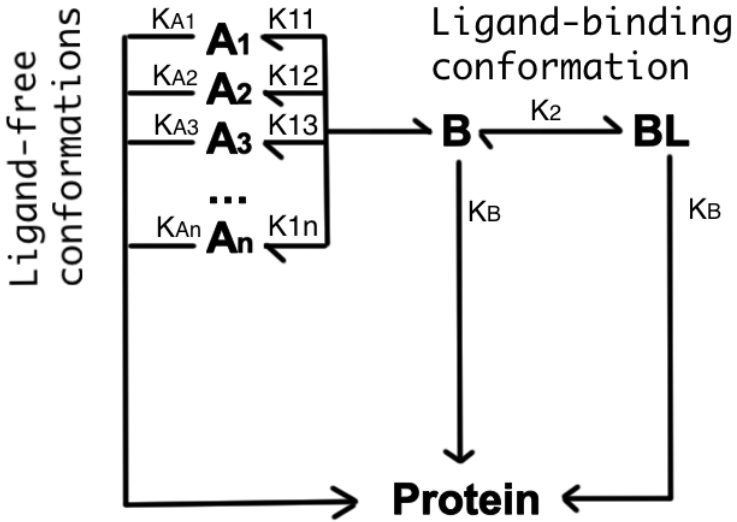
Our strategy:

Multi-phase riboswitch



$$P = \frac{k_f}{a_p} \frac{\sum_{i=1}^{n} K_{Ai} K_{1i} + K_B(1 + K_2 L)}{\sum_{i=1}^{n} a_{1i} K_{1i} + a_2(1 + K_2 L)}$$

P: protein production
kf: transcriptional initiation rate
K_{Ai},K_B: representative regulatory activities of conformation Ai and B
a_{1i},a_2,a_p: degradation rate of conformation Ai and B, and protein
K_{1i}: partitioning constant between conformation Ai and B
K_2: the aptamer association constant
L: ligand concentration

Modification & Optimization

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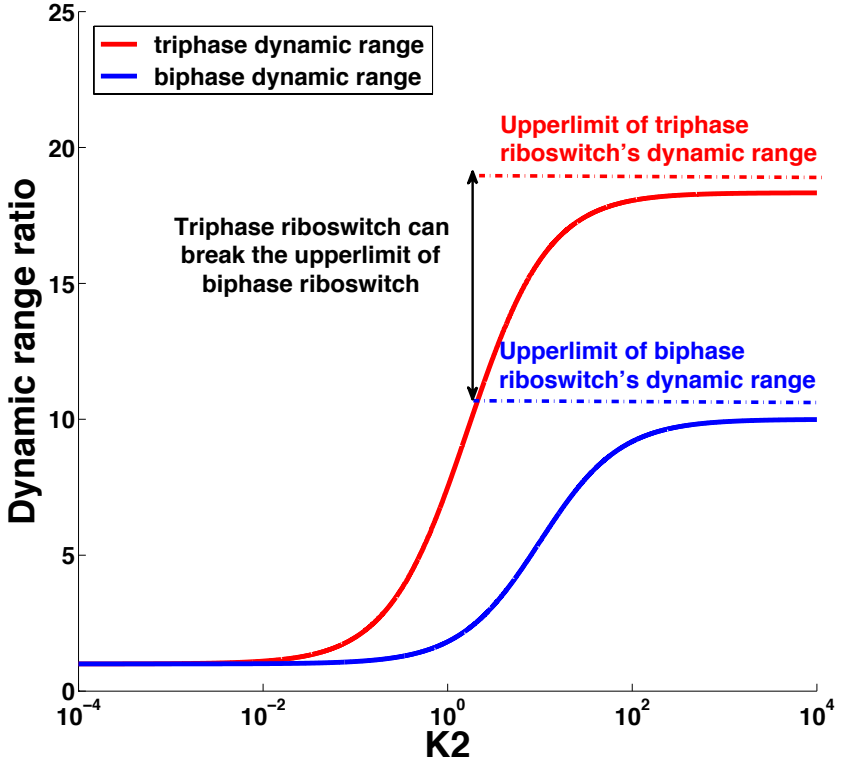
Why it is good?

- The First
- The Best
  - Always better
  - Breaking through the upper limit
  - Integrating the multi-functions

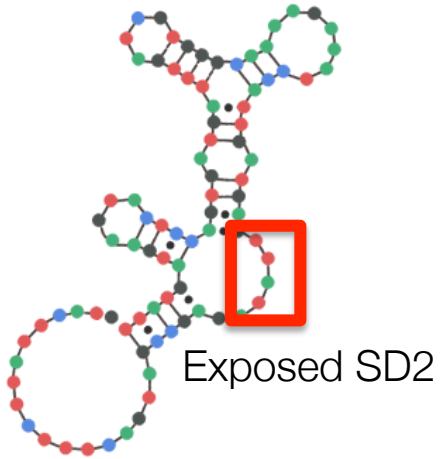


Practice: ALeaderT

Make an artificial conformation by adding a competitive sequence

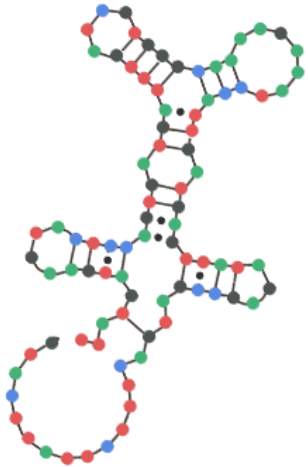
ALeaderT Kana+

Translation on



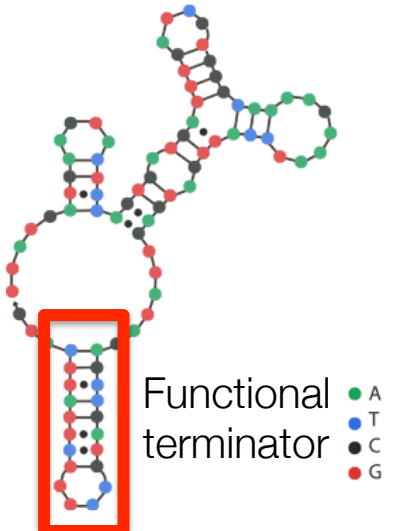
ALeaderT Kana-free

Translation off



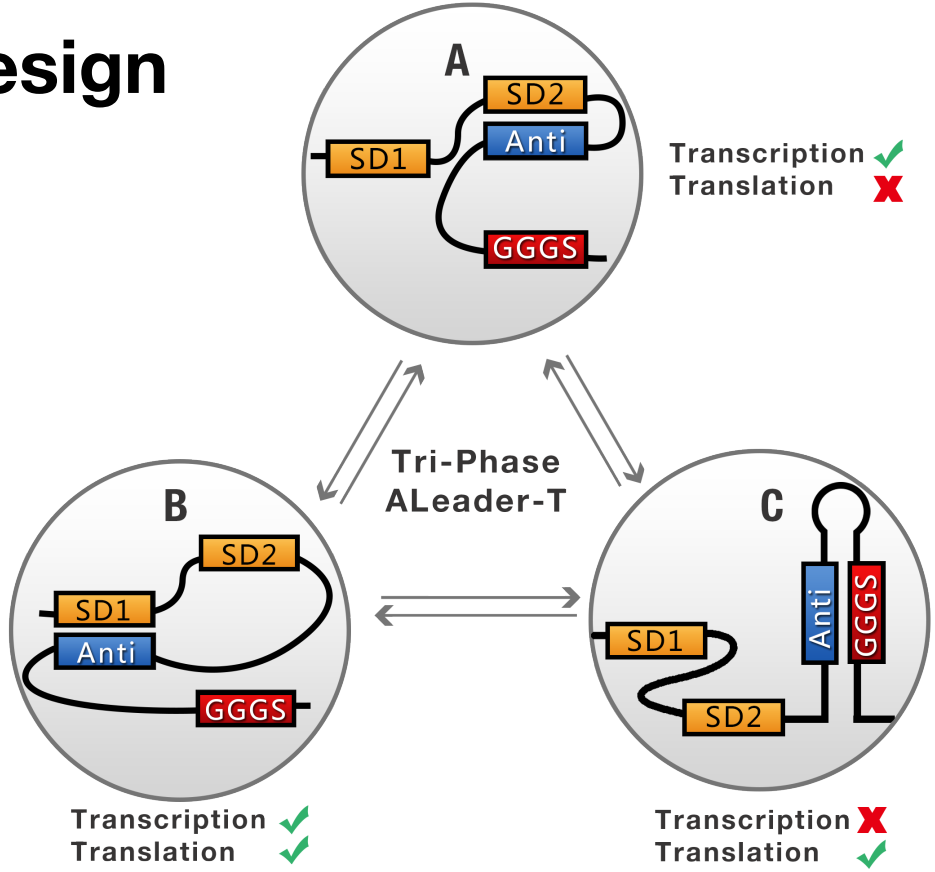
ALeaderT Kana-free

Termination on (artificial)



Practice: ALeaderT Design

- Three states:
- Ligand-free
  - A: Translation repression
  - B: Termination
- Ligand-binding
  - C: Translation initiation



Modify and Optimize

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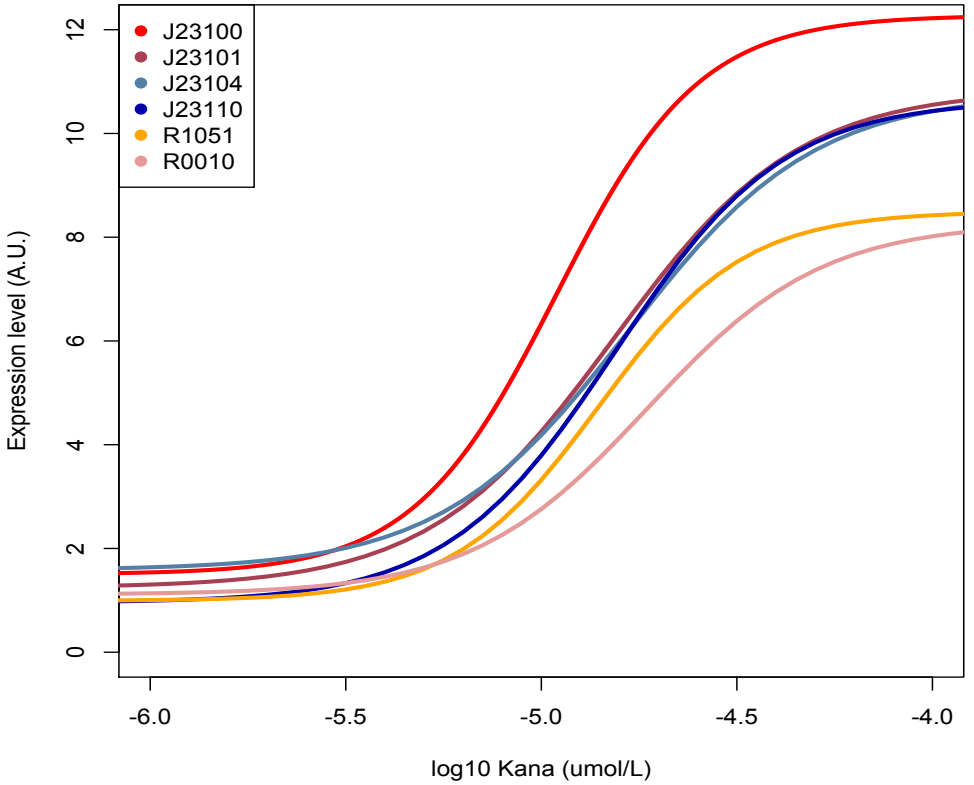
With

a promoter library and mRFP1



Modify and Optimize

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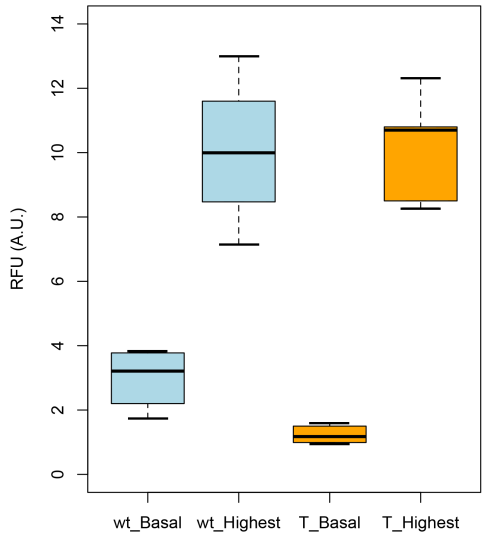
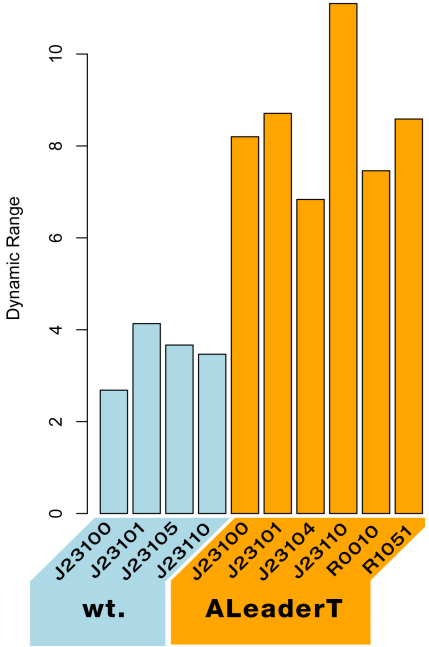
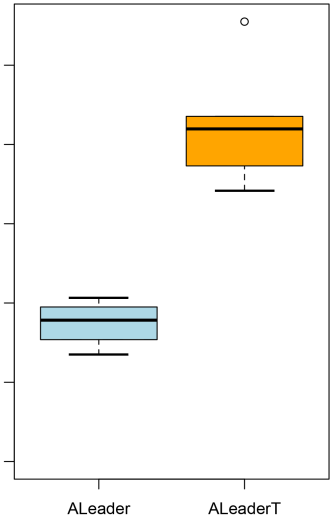
If ALeaderT fits our need?

- lower basal level and higher dynamic range
- lower noise







Modify and Optimize

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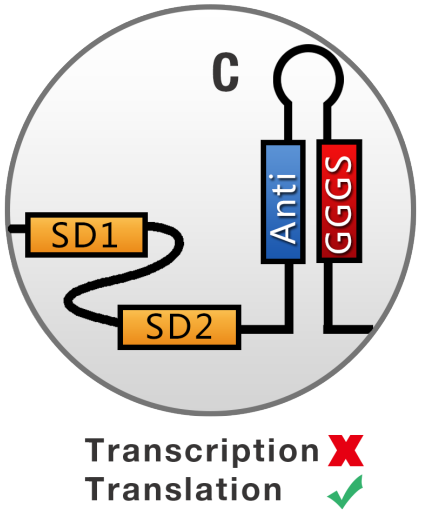
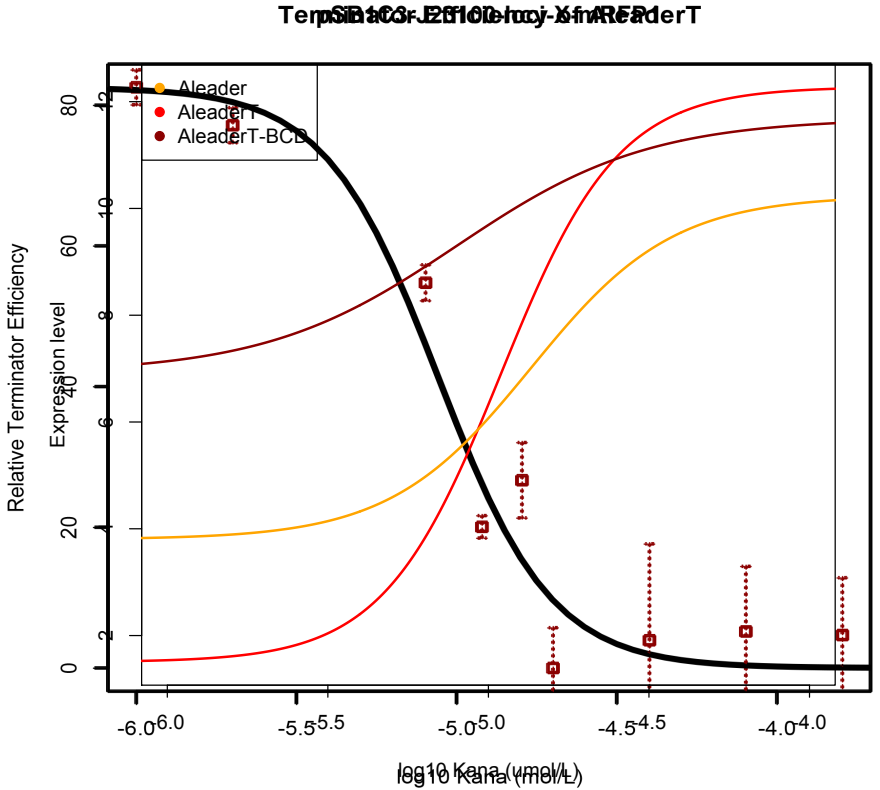
- **ALeaderT integrates the terminator and translational element**





Our strategy：Triphase riboswitch

Summary – the advantages of riboswitch

- Easily designed
- Decreasing the basal level
- Improving the dynamic range
- Reducing the noise
- Integrating the functions

Modify and Optimize

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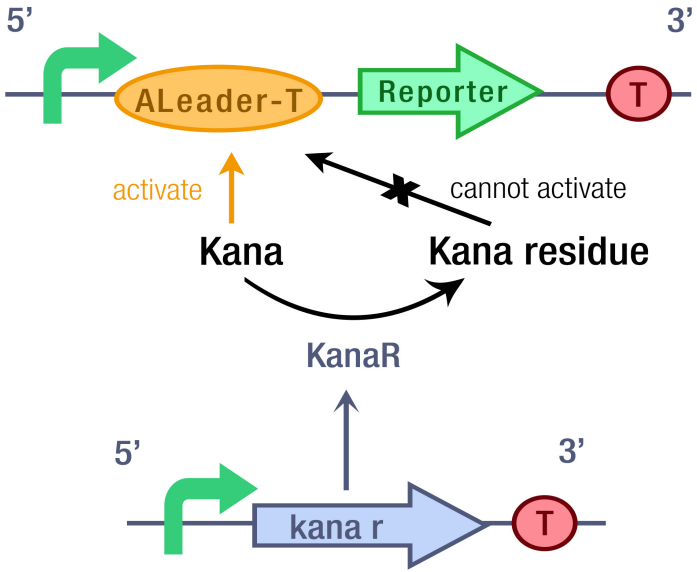
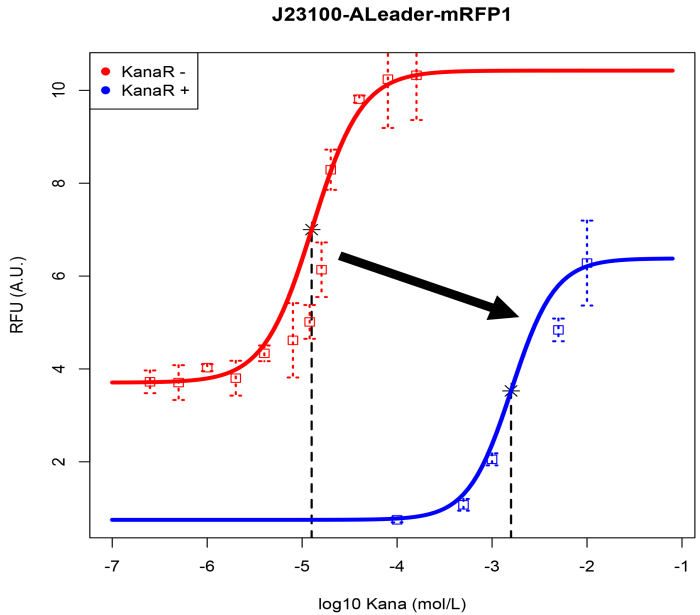
- **Environment Transformer**
  - Manage the working range rather than extend it





Modify and Optimize

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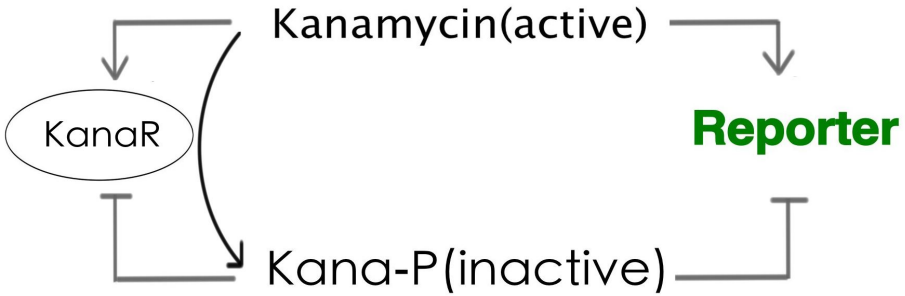
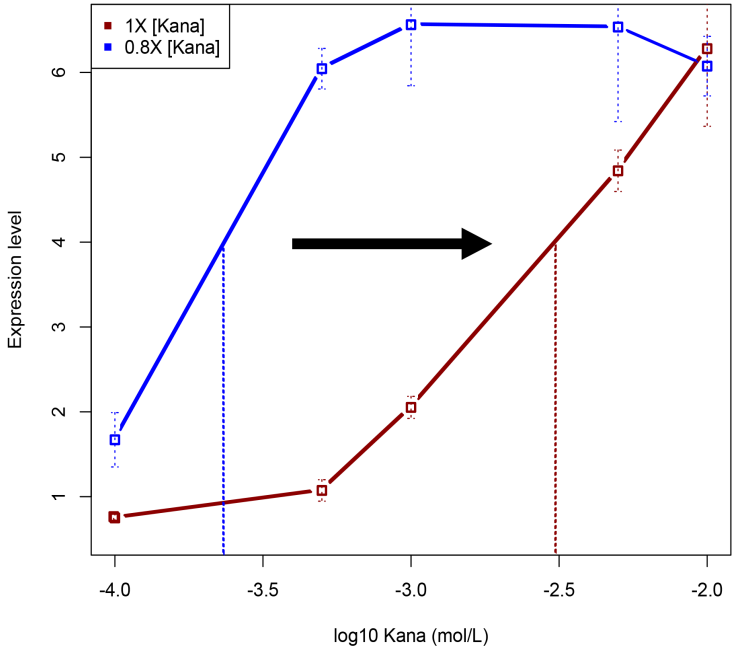
- **Auto-regulated Transformer**





Modify and Optimize

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- **Summary:**
  - Adding competitive sequence
    - ➔ **Triphase riboswitch design**
  - Expressing an enzyme
    - ➔ **Environ-Transformer**
  - Site-directed mutagenesis

Apply

- **What about the Kanamycin detector?**
  - High dynamic range
  - Wide working range

Kanamycin detector = ALeaderT + KanaR

User guides for Application

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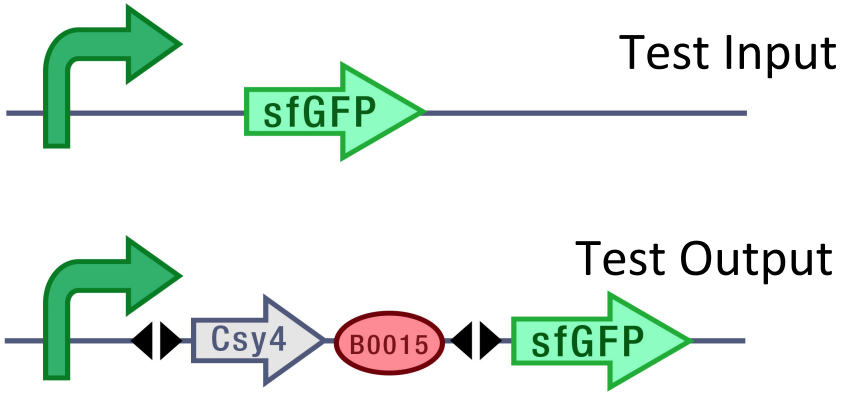
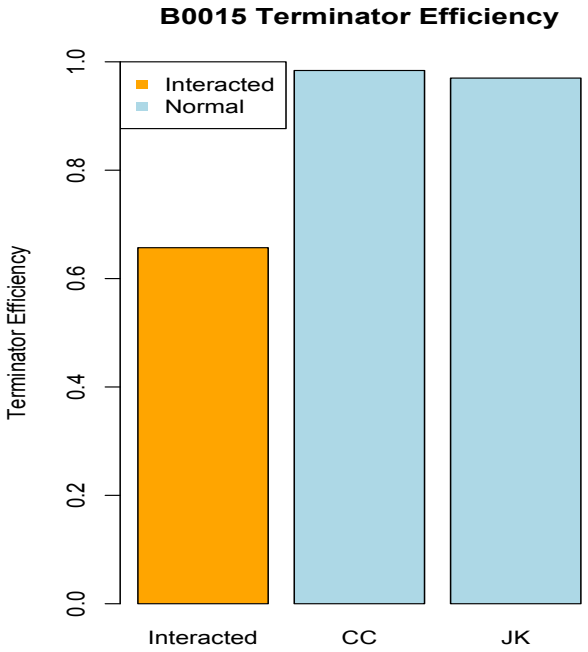
To Users:

Be careful of RNA interaction

Interfered terminator





Future works

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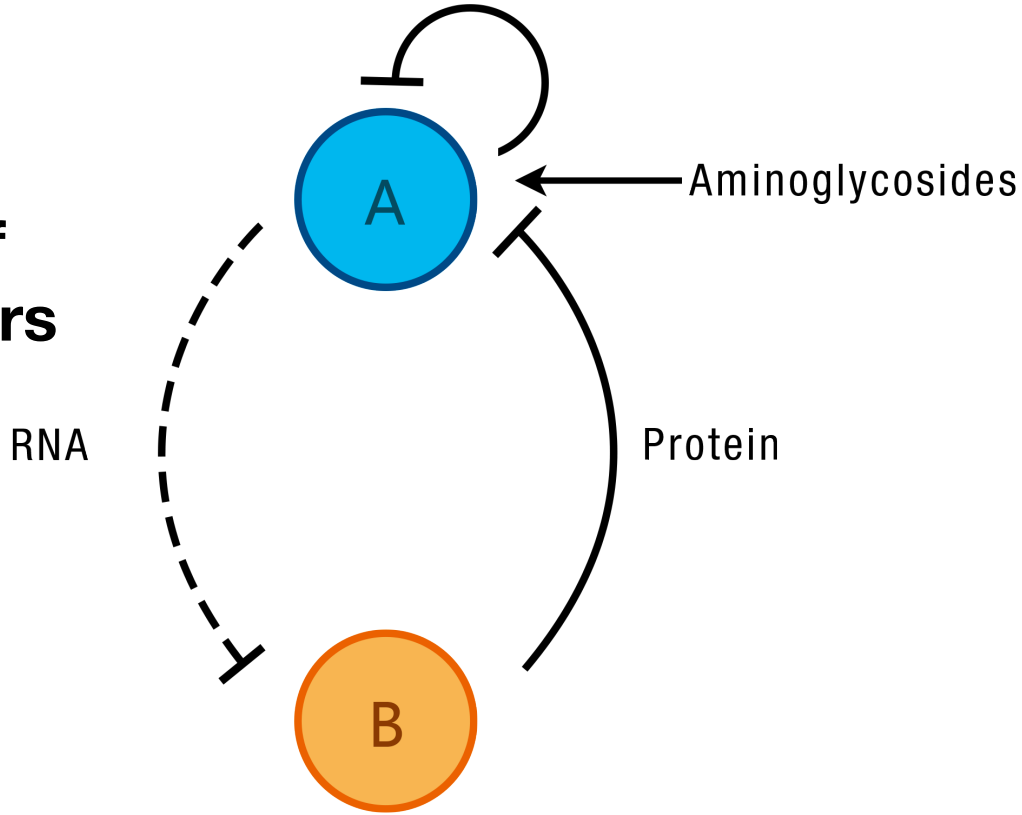
The different behaviors of RNA and protein regulators in the networks



Human Practice

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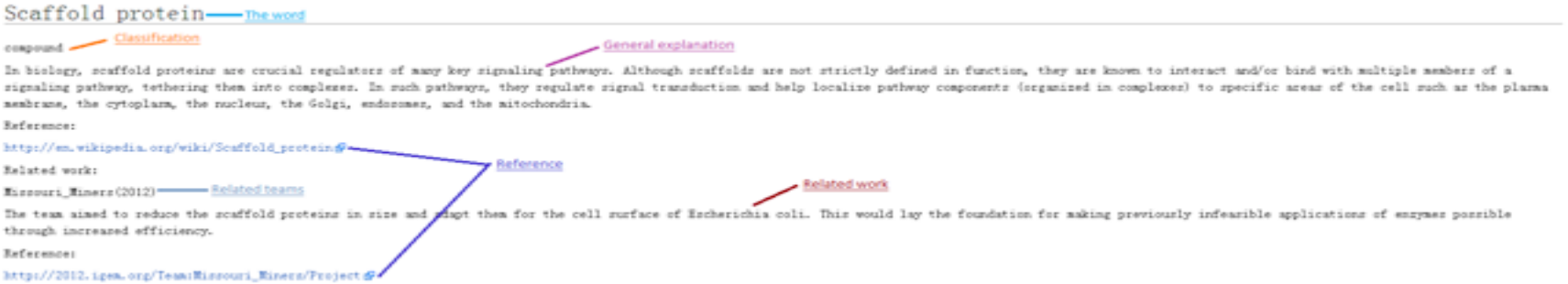
- **iGEMcyclopedia 2.0**
  - For fresh iGEMers
  - For better communication



Human Practice

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Activities at Fudan and Communications with other teams

With association of Bertalanffy

With iGEM2013_USTC CHINA

Human Practice

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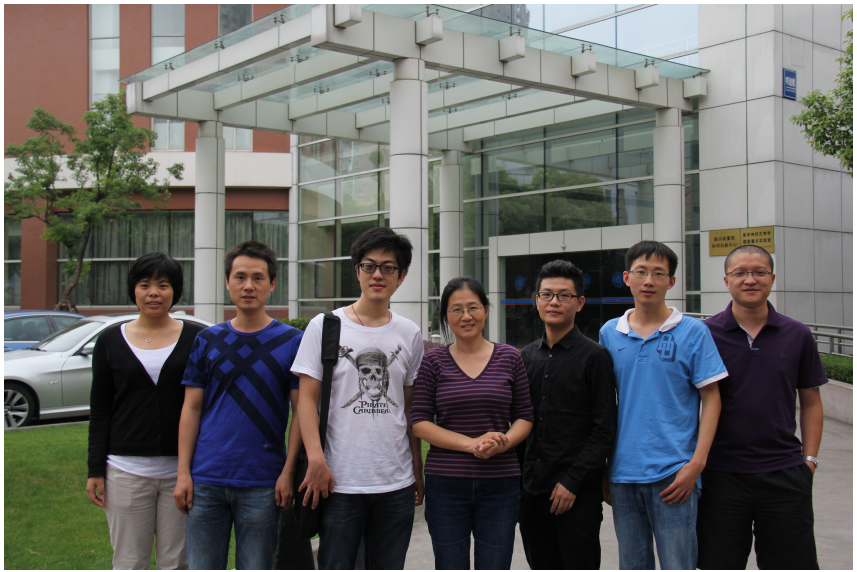
- **Spread the idea of iGEM**
  - The communication with Prof Chen and Prof Murchie's Lab



Judge Form

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- Best Human Practice:
  iGEMpedia 2.0
- Best Biobrick Measurement approach:
  interacted terminator measurement
- Best New standard:
  the standard protocol of riboswitch engineering
- Best Model:
  the theoretical study of triphase riboswitch

- Best new Biobrick part, Natural:
  BBa_K1100000 (ALeader)
- Best new Biobrick part, Engineered:
  BBa_K1100002 (ALeaderT-BCD),
  BBa_K1100014(insulator-ALeaderT),
  BBa_K1100011(interacted B0015 measurement)
- Most improved registry part:
  BBa_B0015 (measured by BBa_K1100011)
- Best Part collection:
  BBa_K1100140-150 (ALeaderT library)

Sponsor and Acknowledgement

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- School of life science, Fudan University
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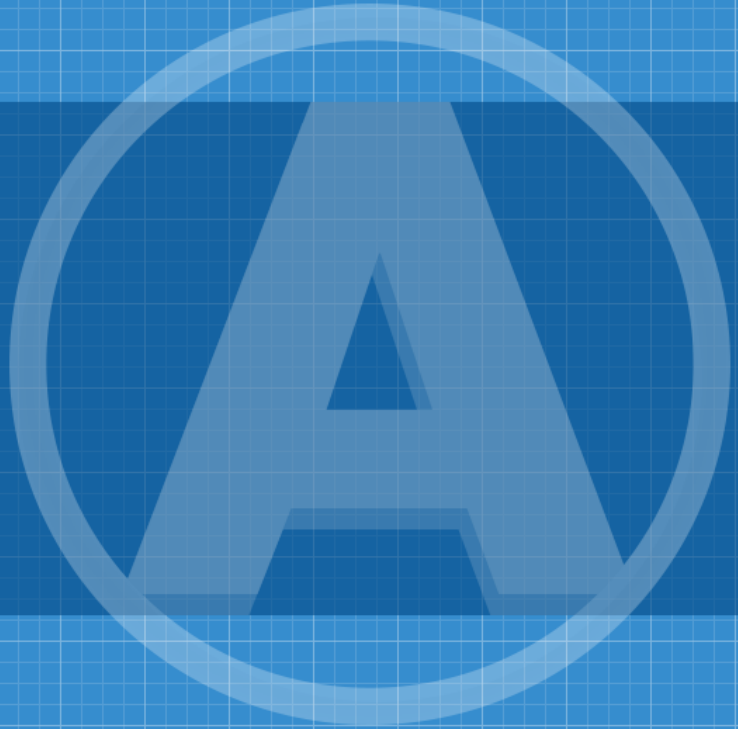
- Prof. Zhong Jiang, School of Life Science, Fudan University
- Prof. Yang Ji, School of Life Science, Fudan University
- Prof. Lv Hong, School of Life Science, Fudan University Associate Prof.
- Yu Yao, School of Life Science, Fudan University Associate
- Prof. Ding Yu, School of Life Science, Fudan University
- Dr. Qi Lei, Center for system and Synthetic Biology, UCSF

Thank you

Q&A time



Our strategy:

Triphase riboswitch

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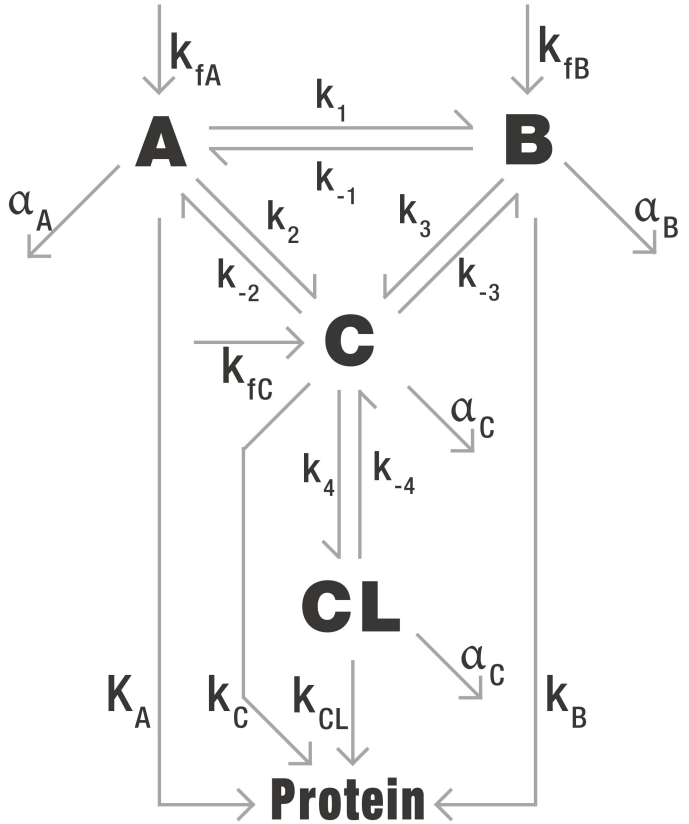

Supplementary

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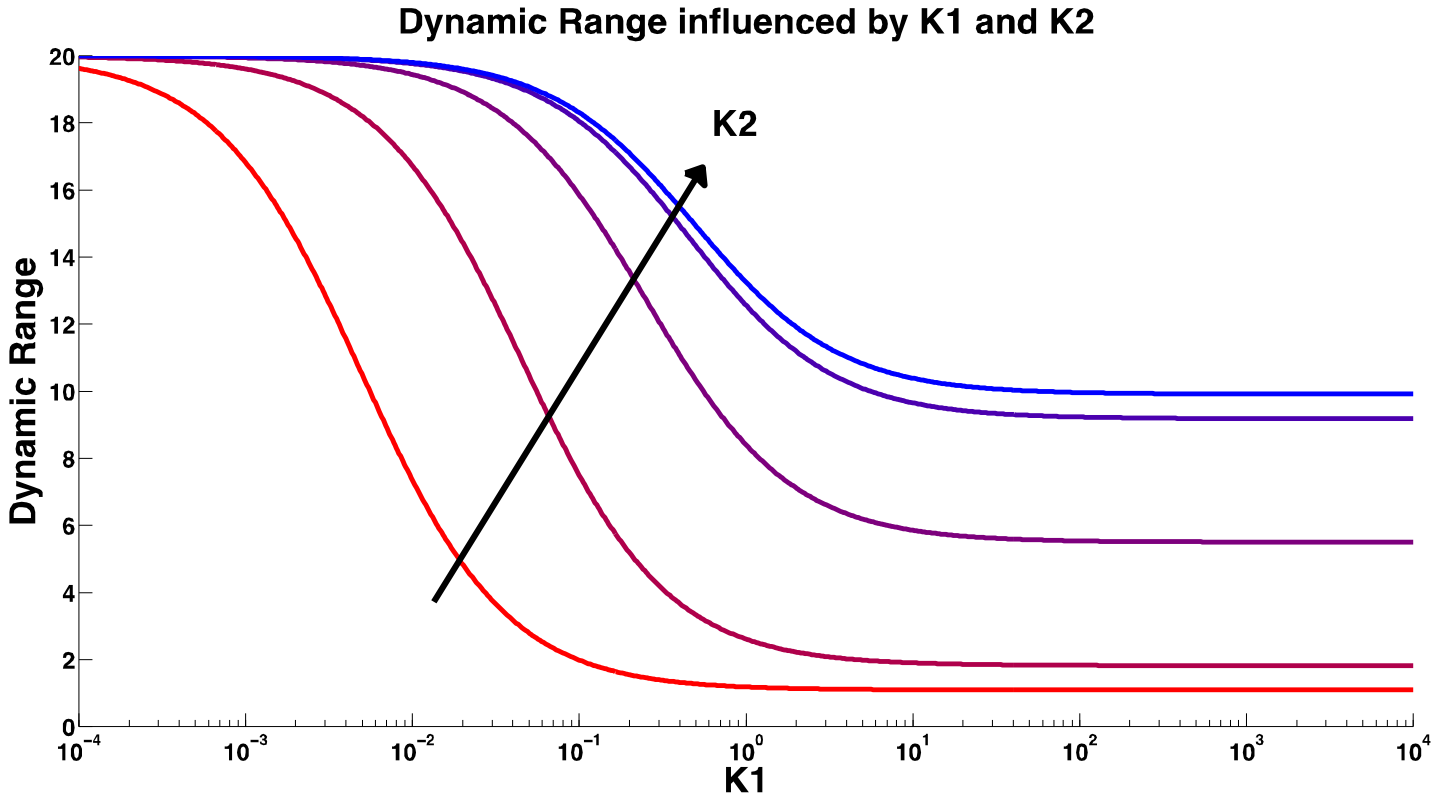

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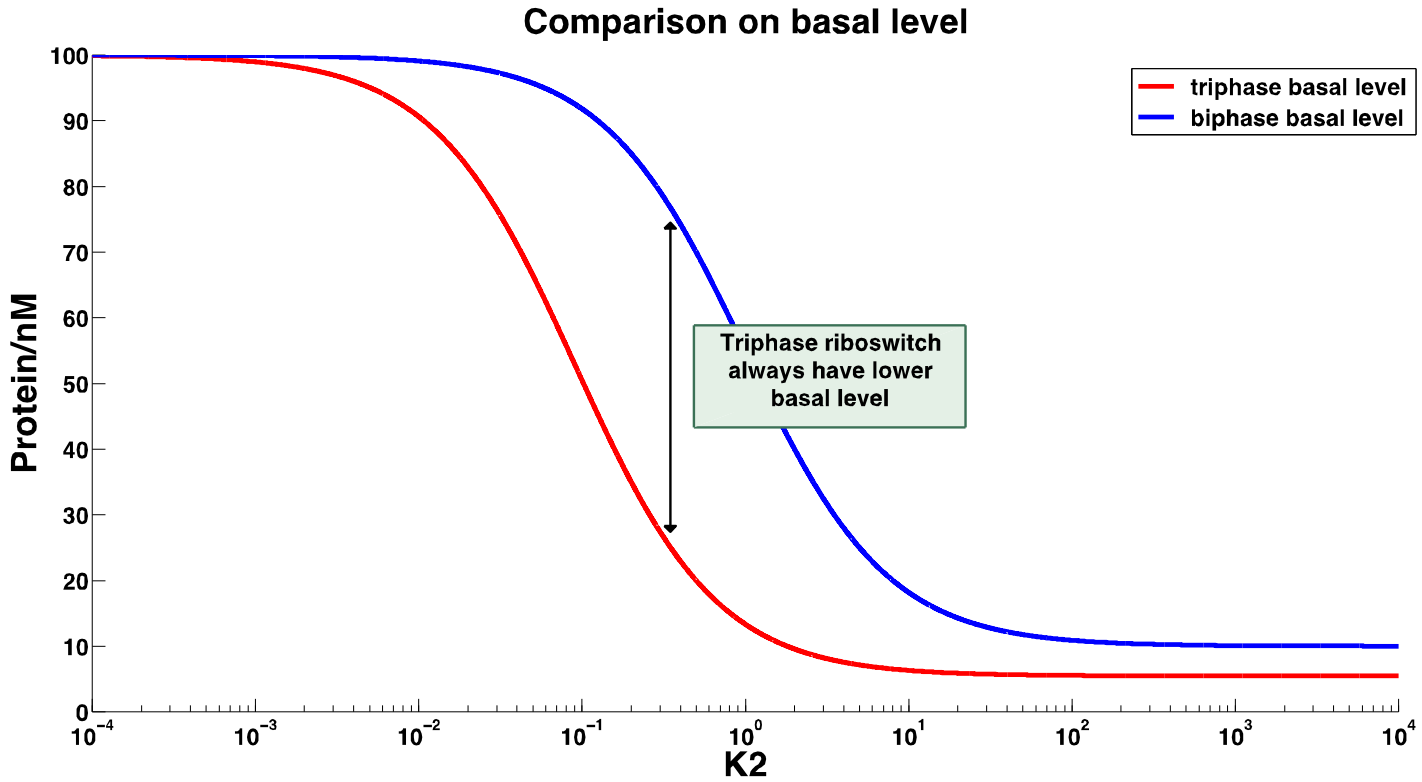

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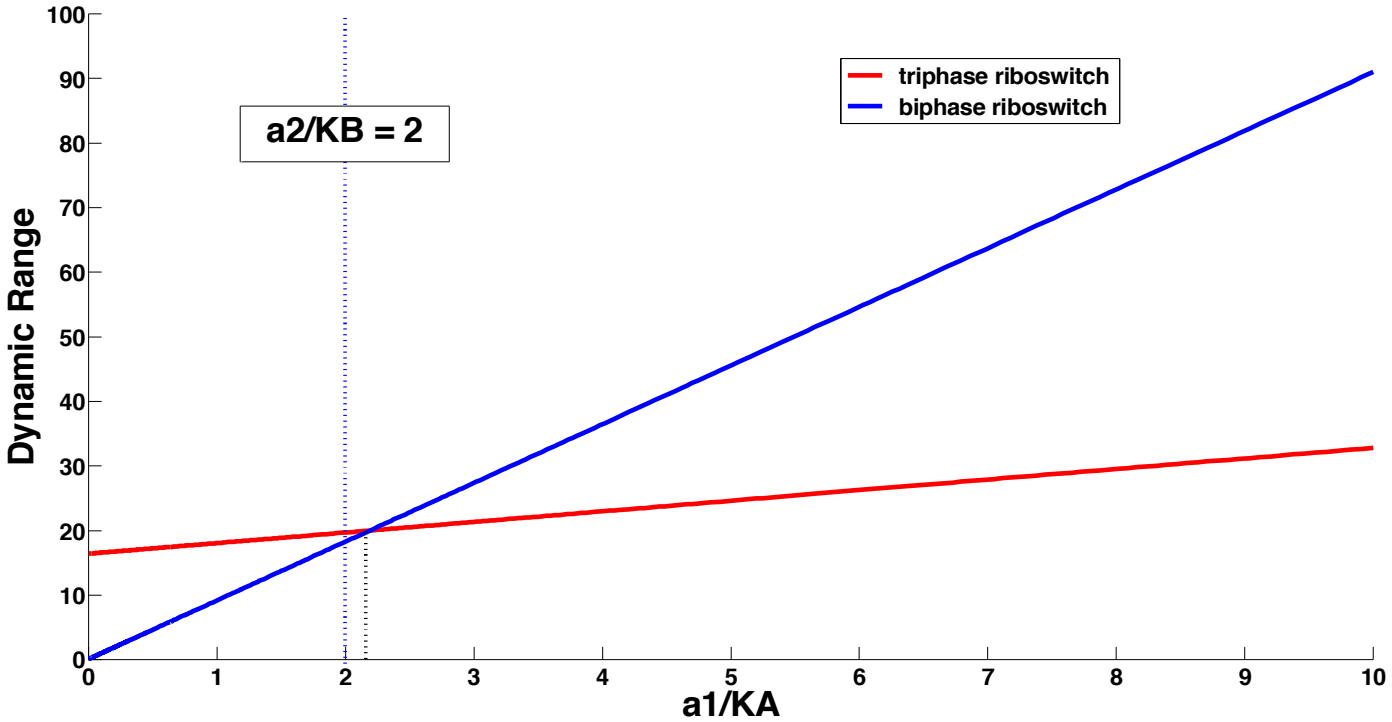

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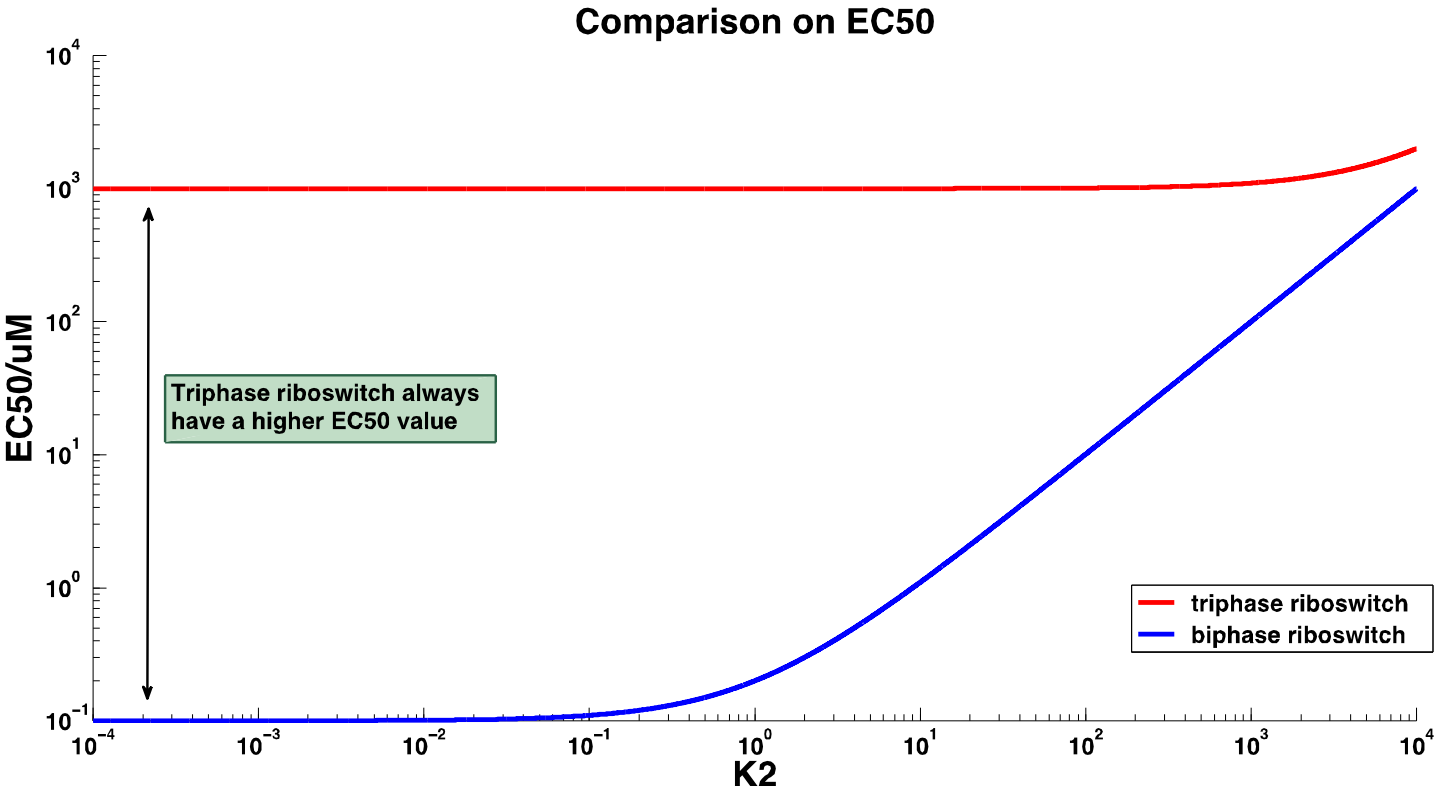
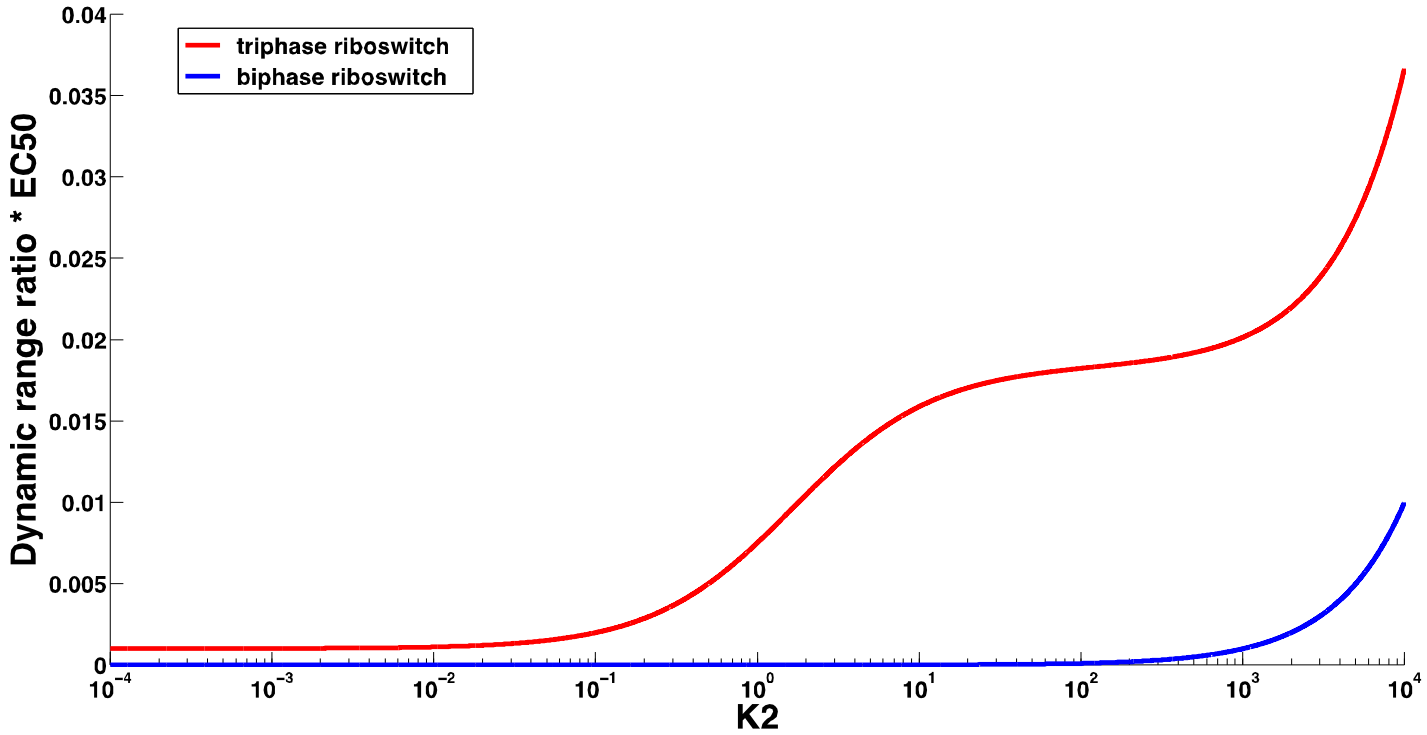


Supplementary

FUDAN iGem2013



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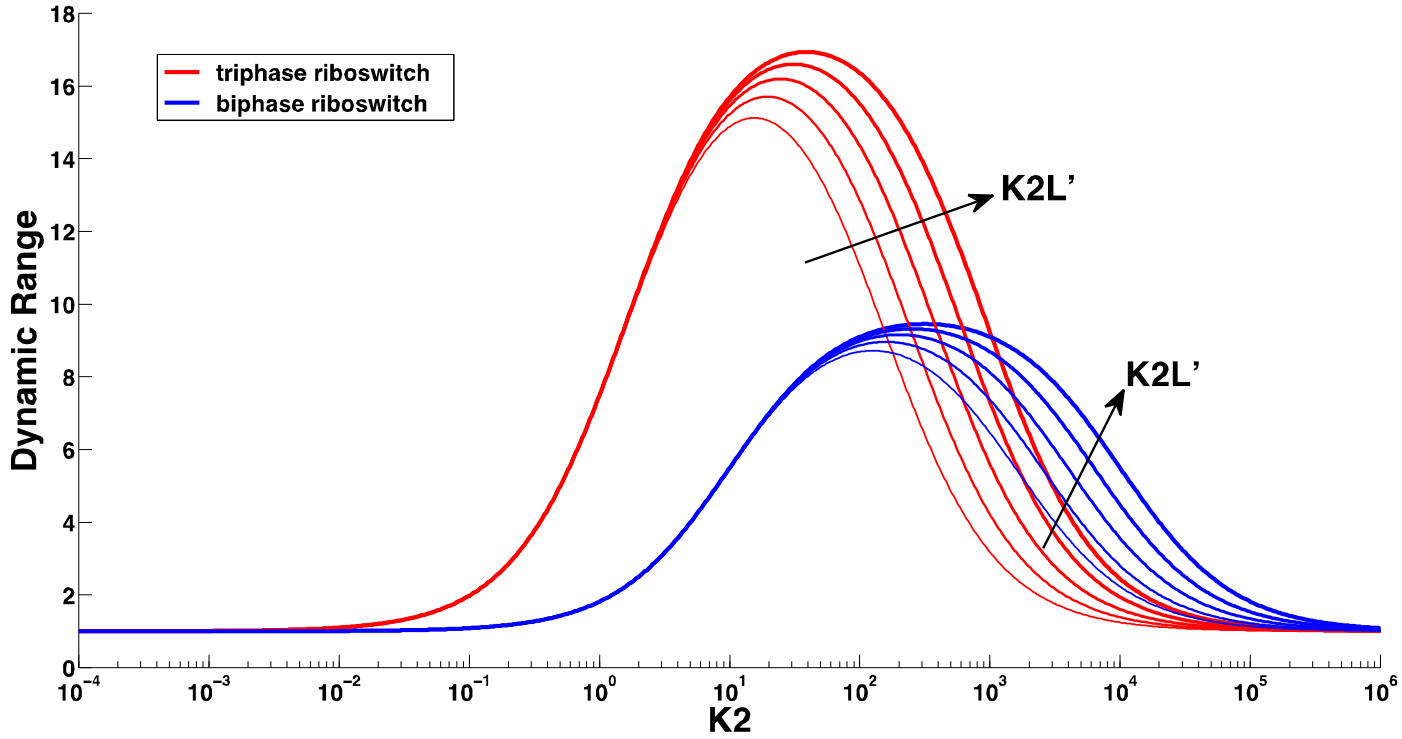

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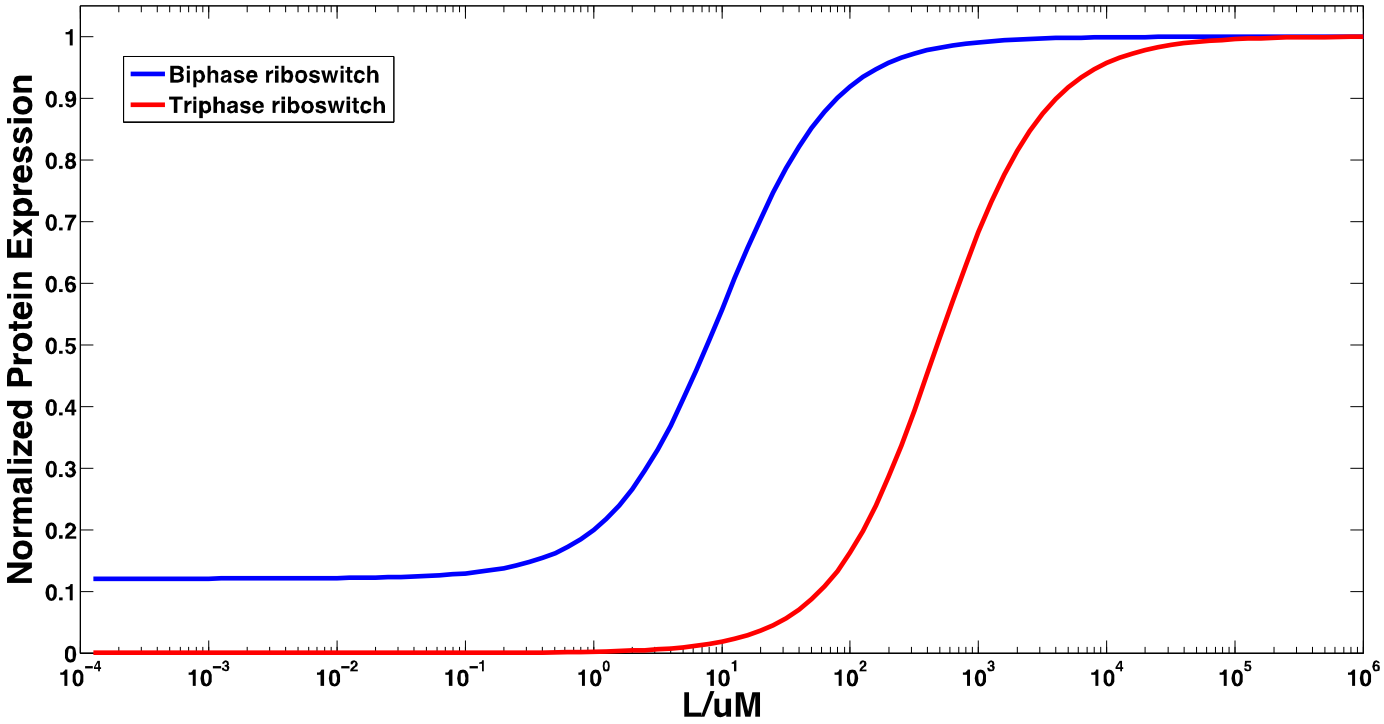

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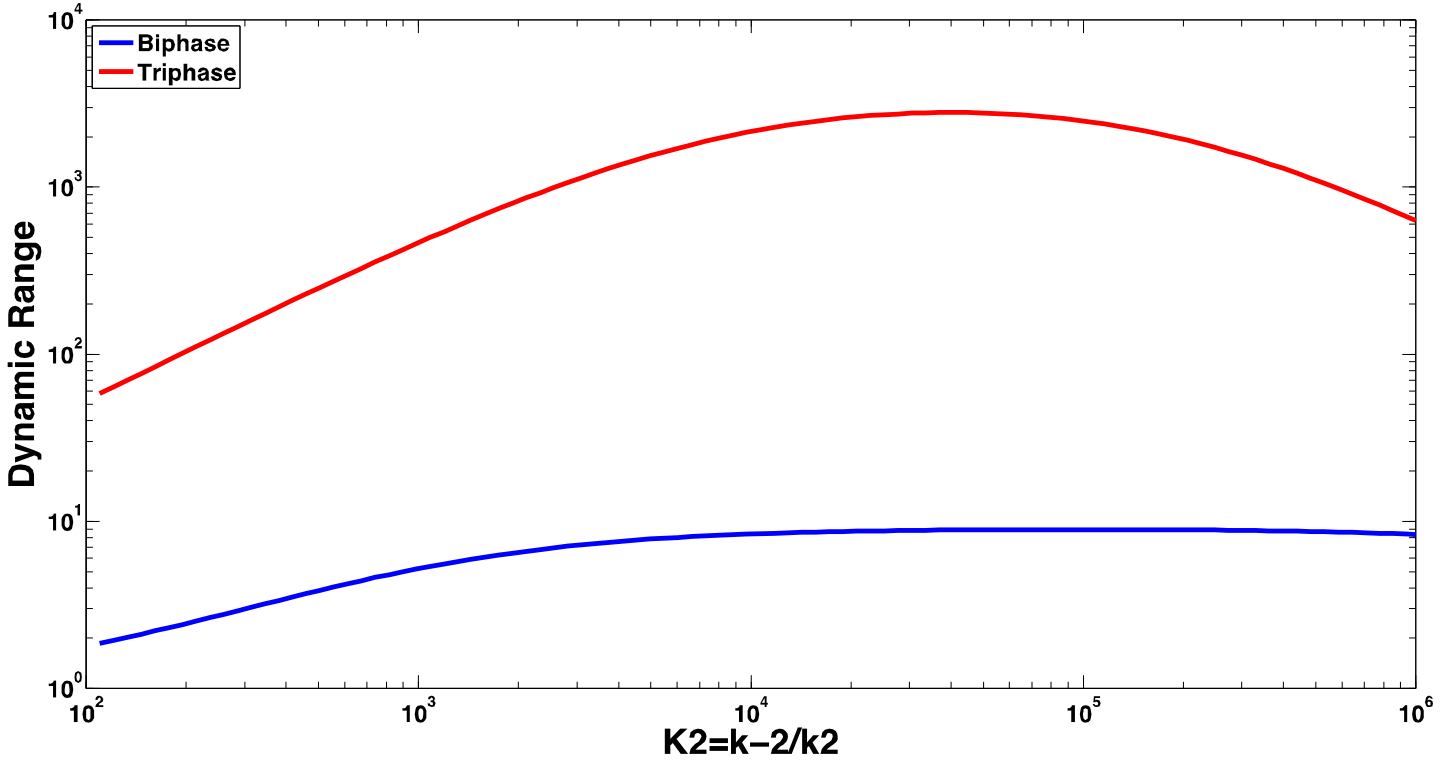

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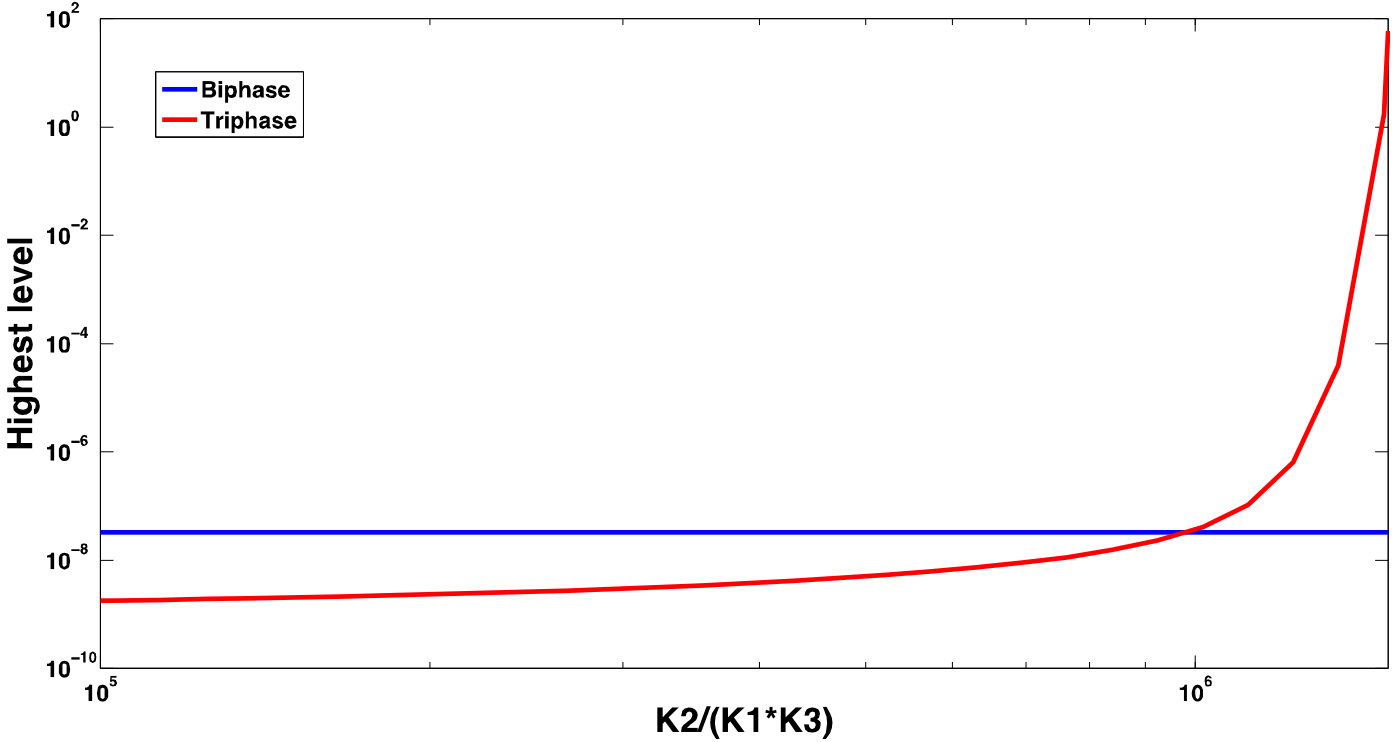

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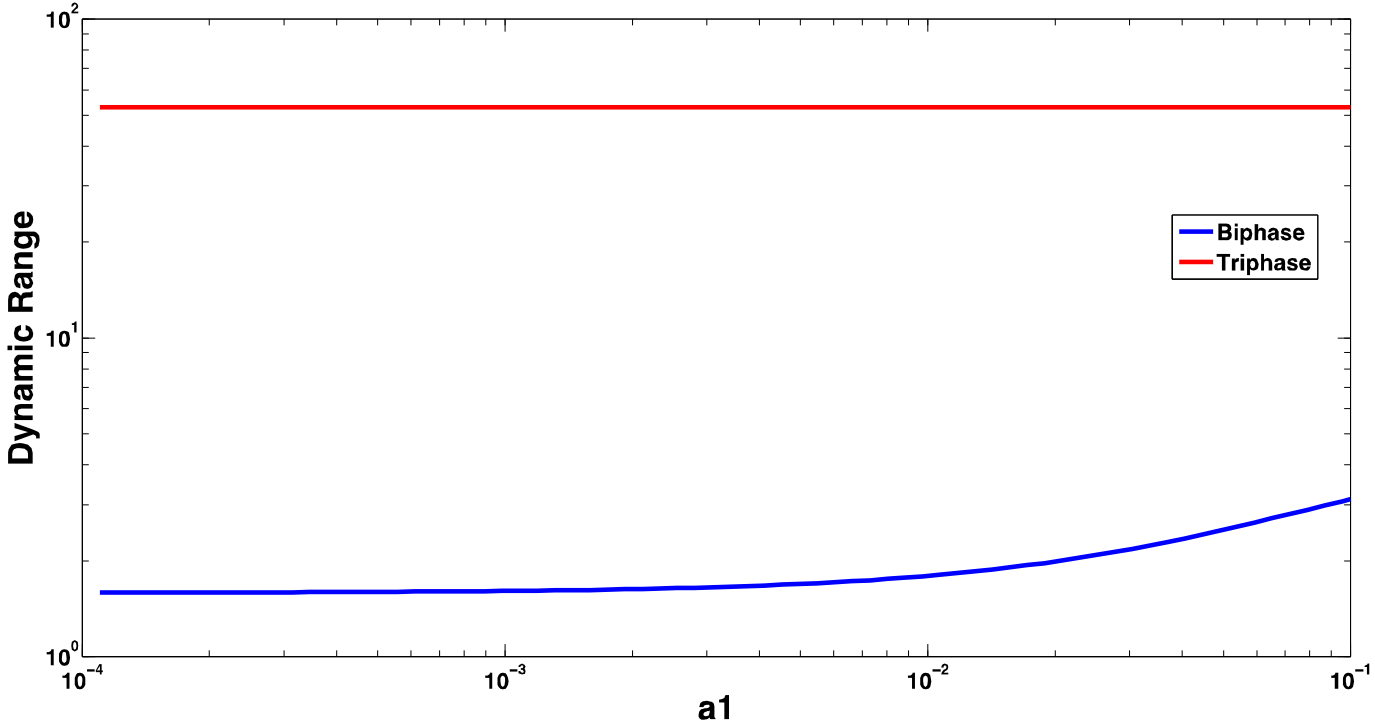

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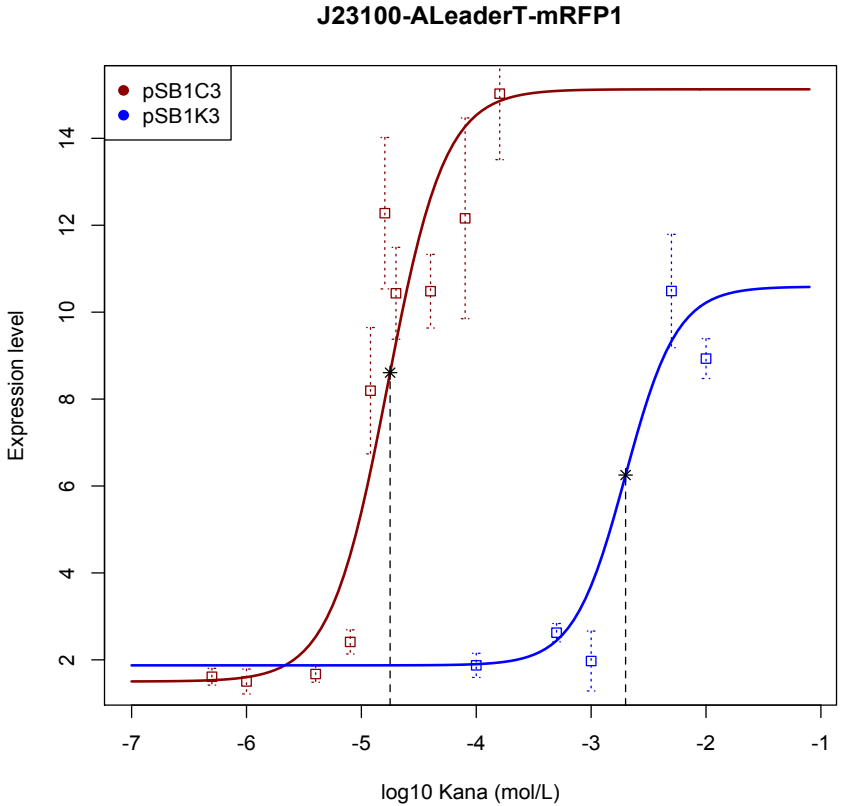

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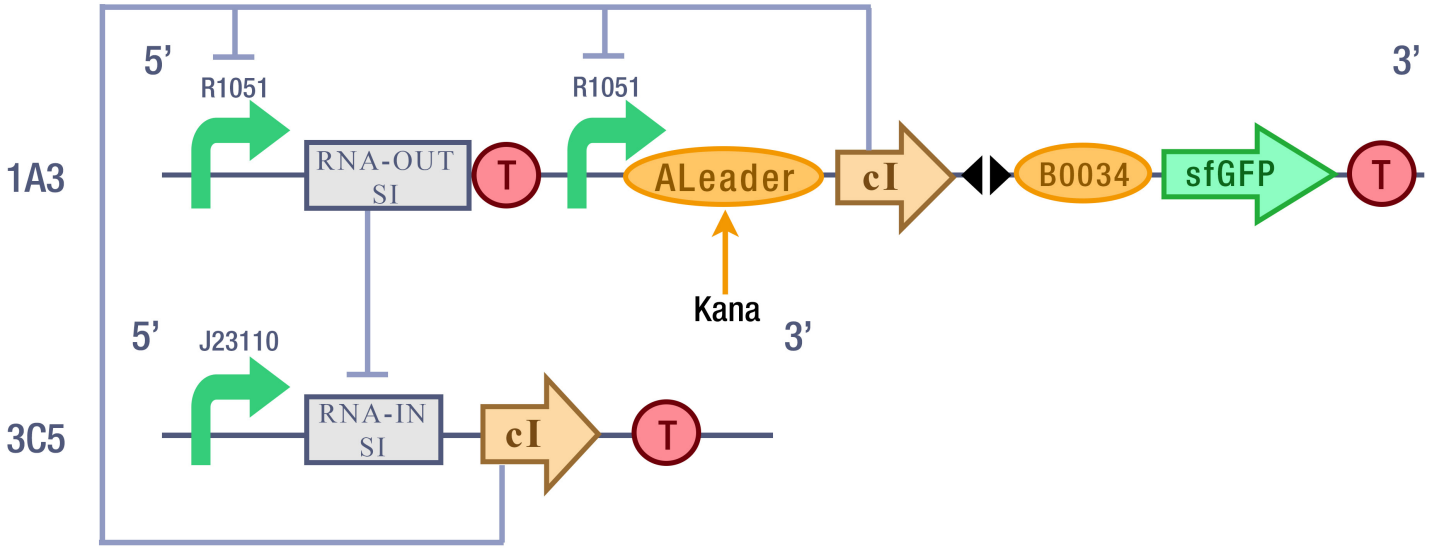

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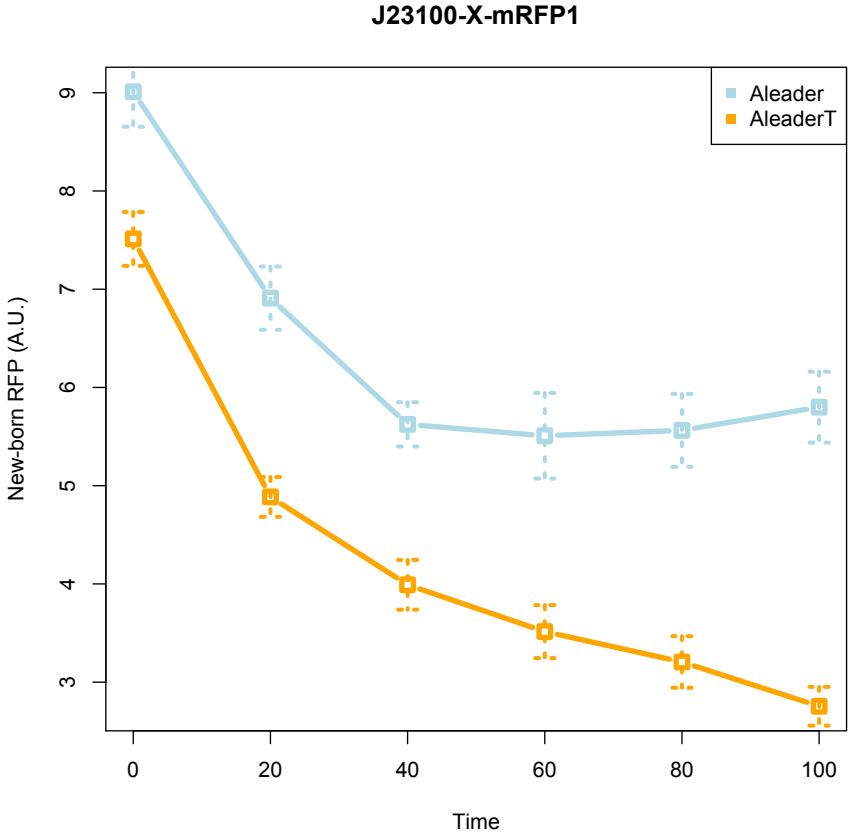

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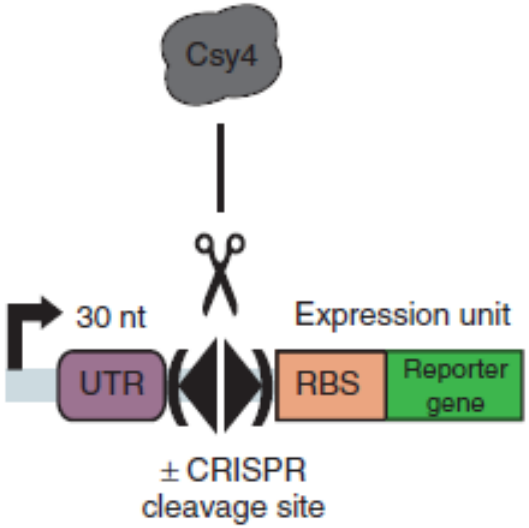

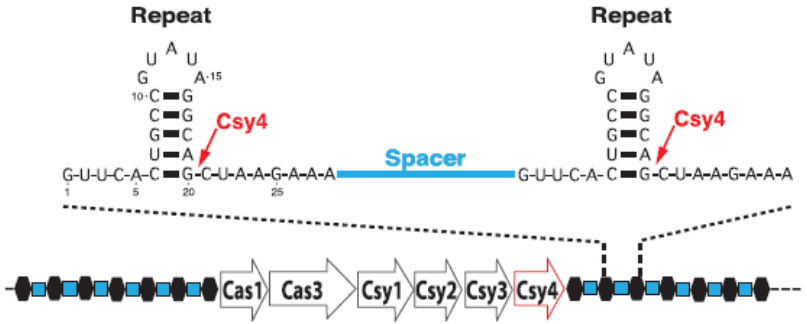

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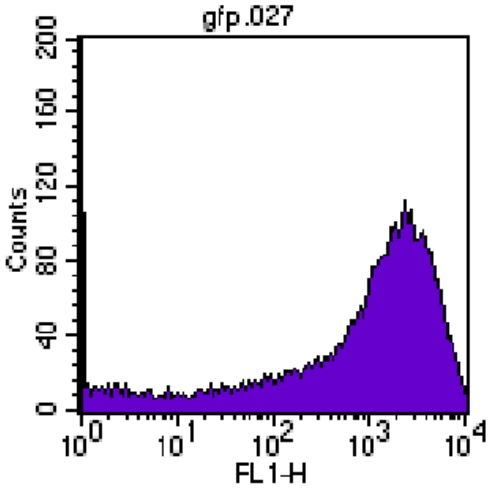

File: gfp.027

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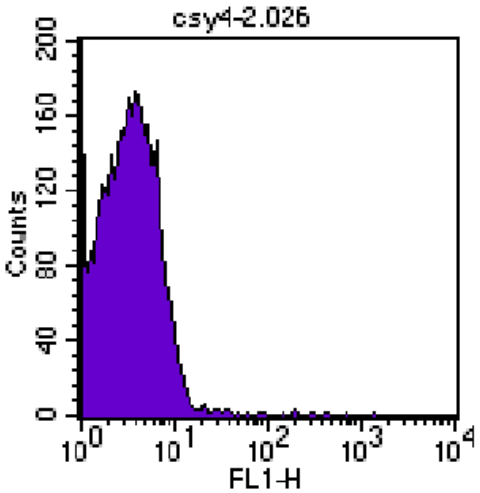
243.23



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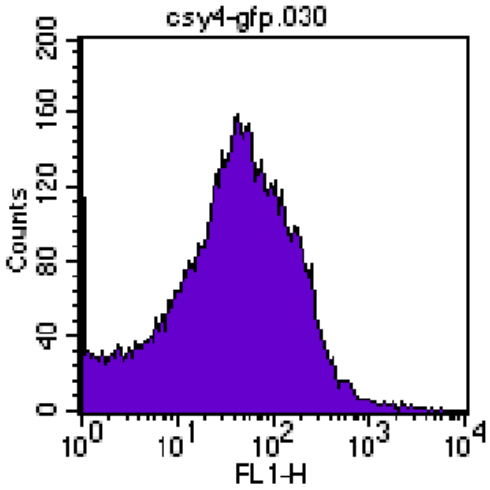
Geo Mean
2.91



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Geo Mean
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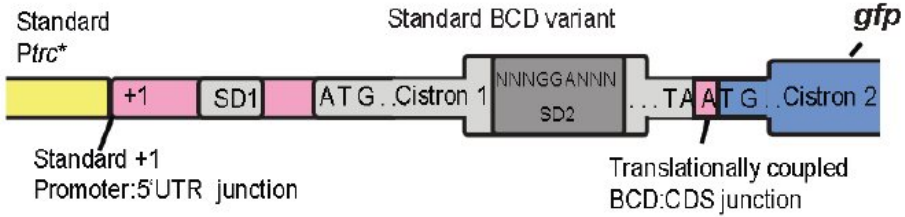
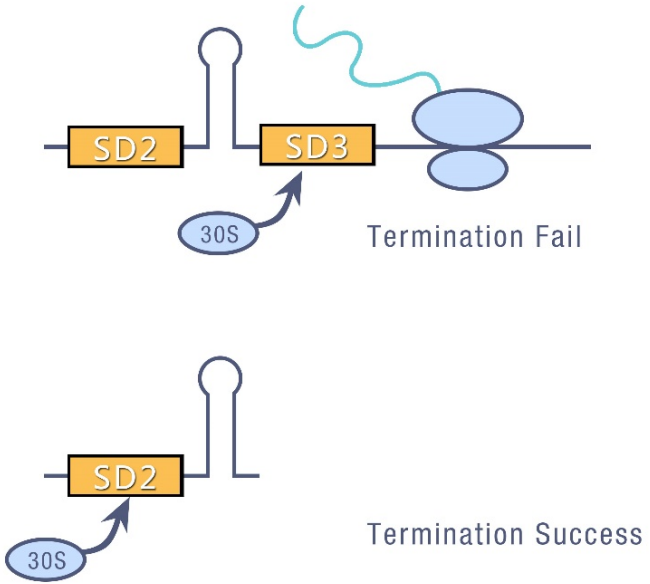
Supplementary





Supplementary

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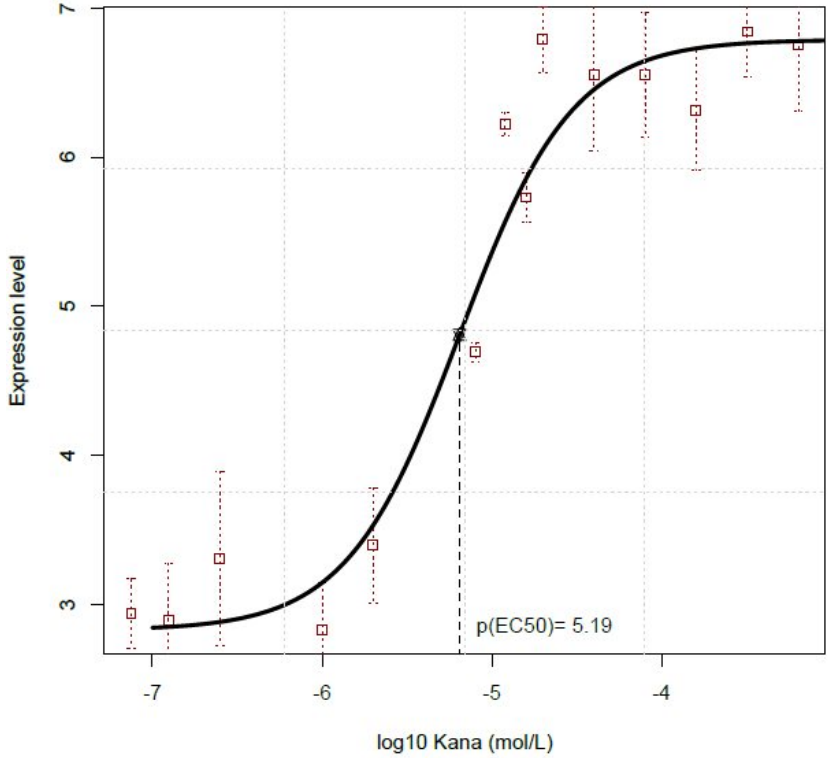

Supplementary

FUDAN iGem2013