

ALeader

Leading the advance of RNA synthetic biology

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Oct 5th, 2013



Our Team



The RNA engineering issue

A

FUDAN iGem2013

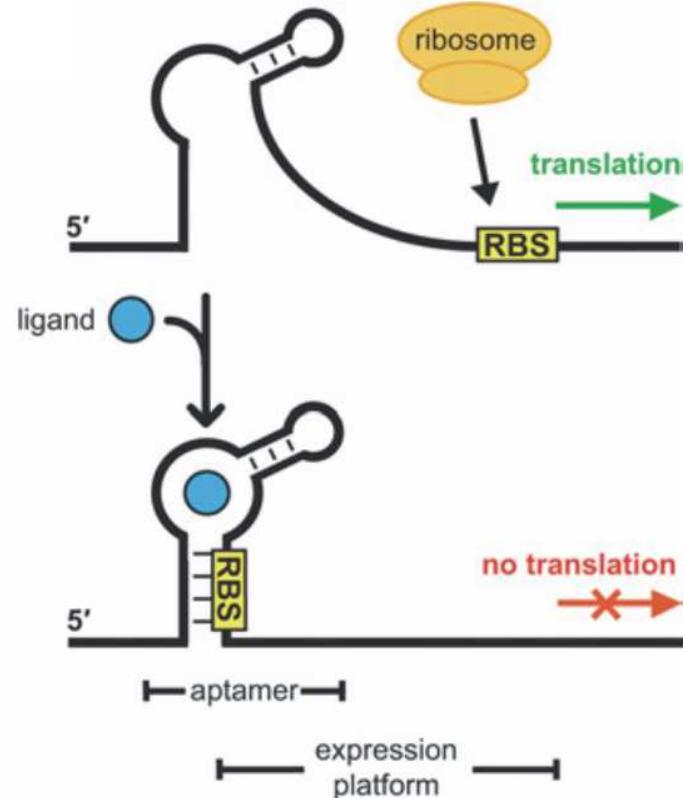
RNA parts are:

Powerful

Programmable

However:

No standard protocol



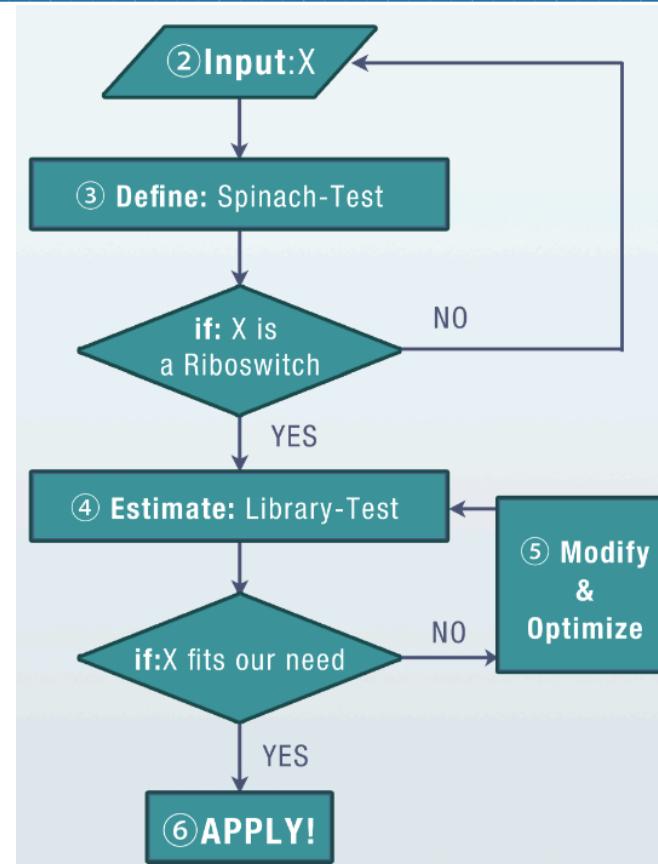
Our goal

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unpublished works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

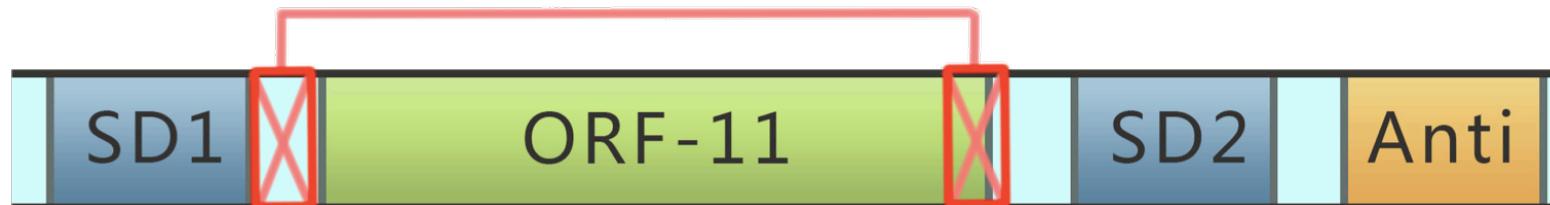
Discovered in AAC and AAD

The most complex riboswitch

The most complex riboswitch

- Bi-cistron
- ORF-11
- attl sites

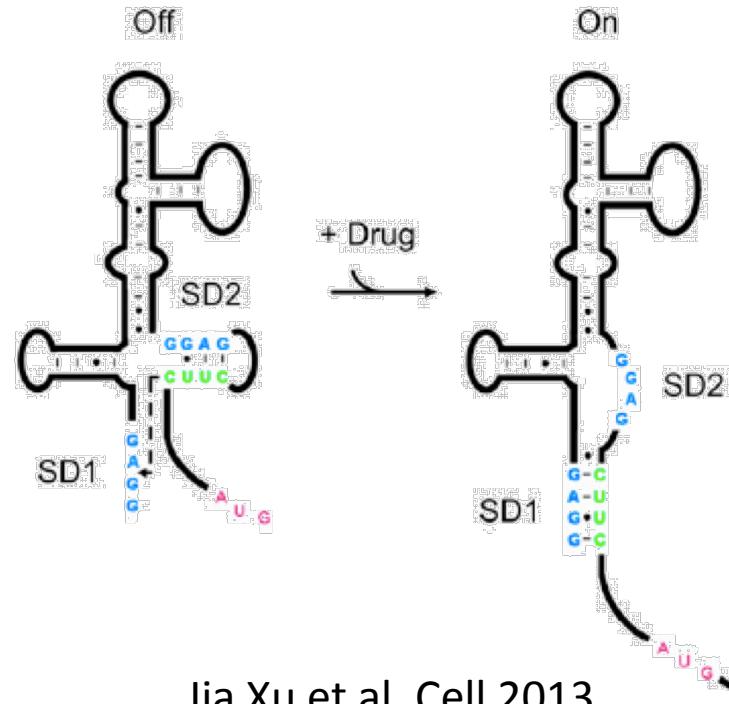
ClassI integron: attl sites



GGAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCT
AAAACAAAGTTAGGCAGCACGGAGACACACTTCAGCATG

The most complex riboswitch

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



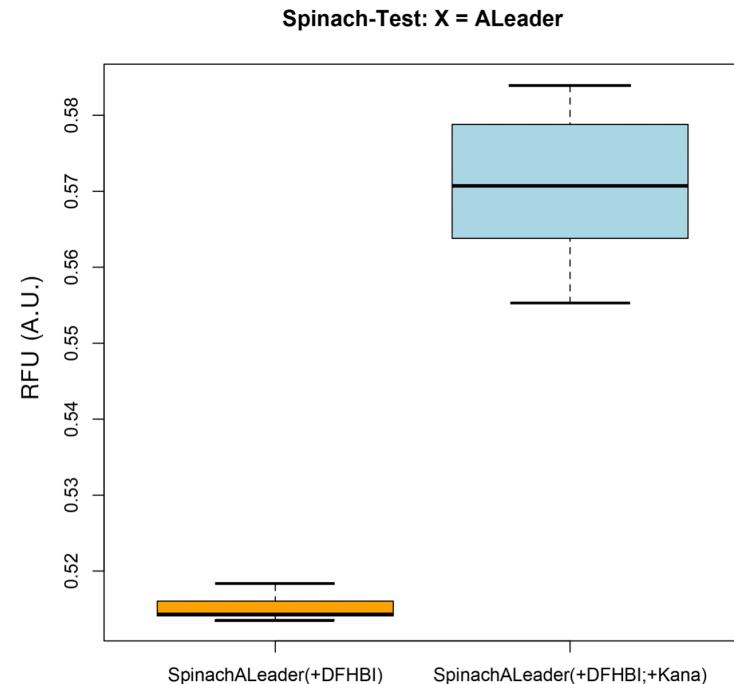
Jia Xu et al. Cell 2013

Resistance

Definition

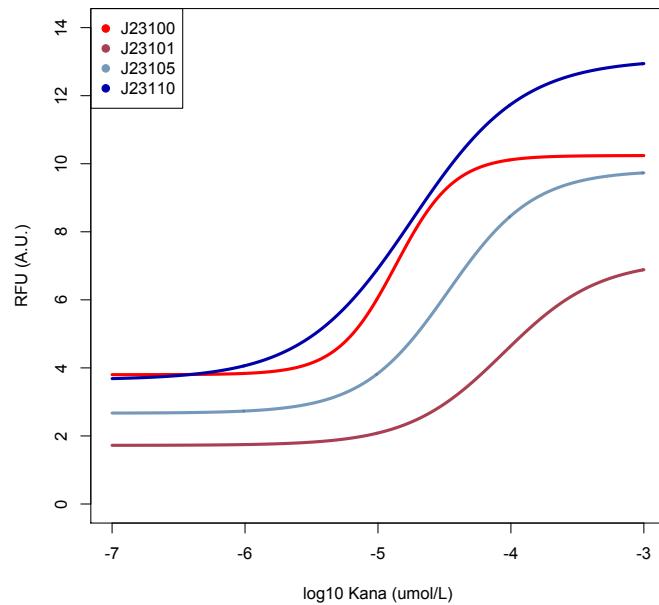
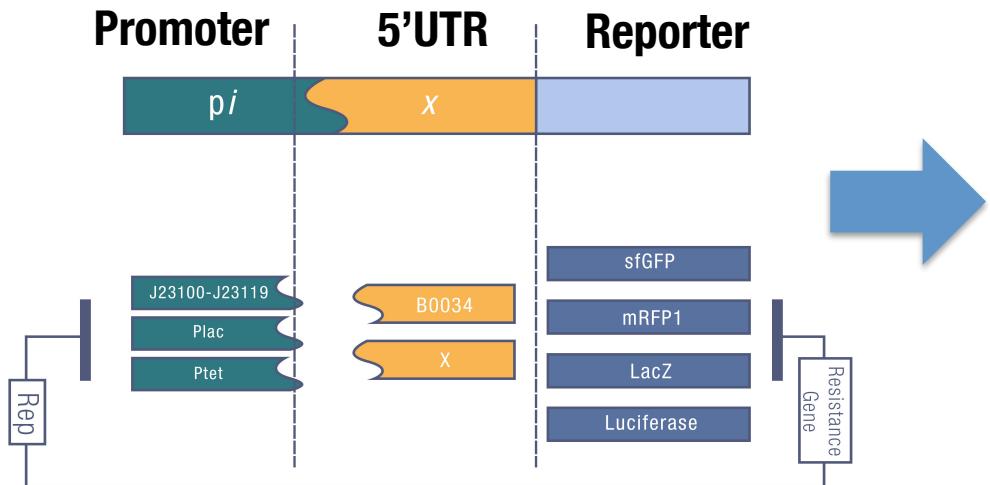
Spinach-Test

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



Estimation

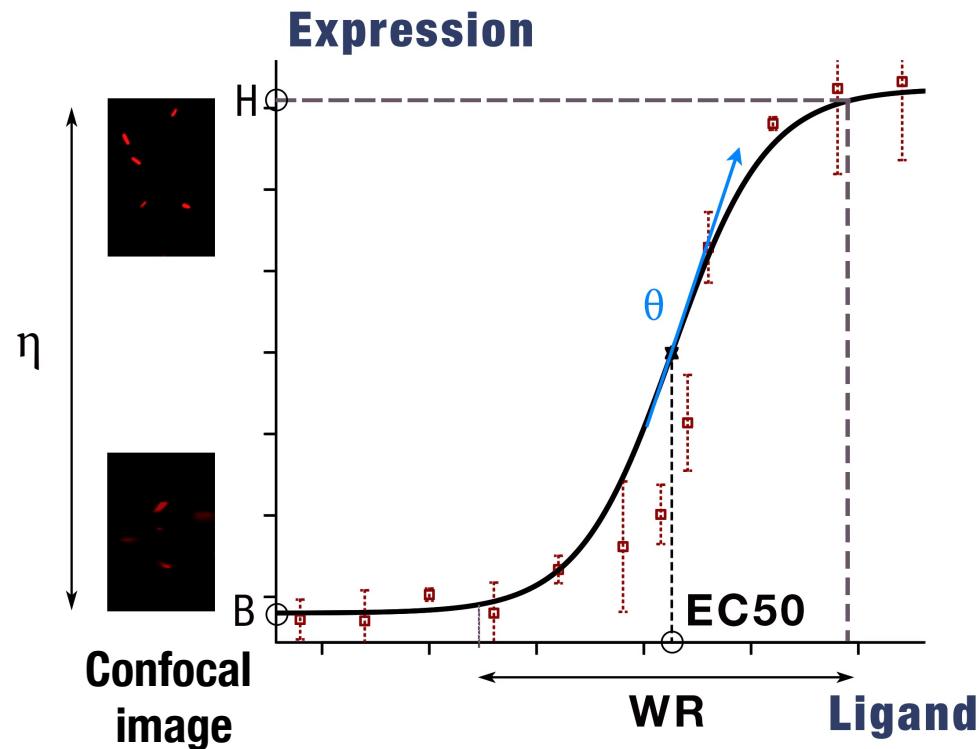
Quantitative estimation based on promoter library



Estimation

Response Curve Analysis

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



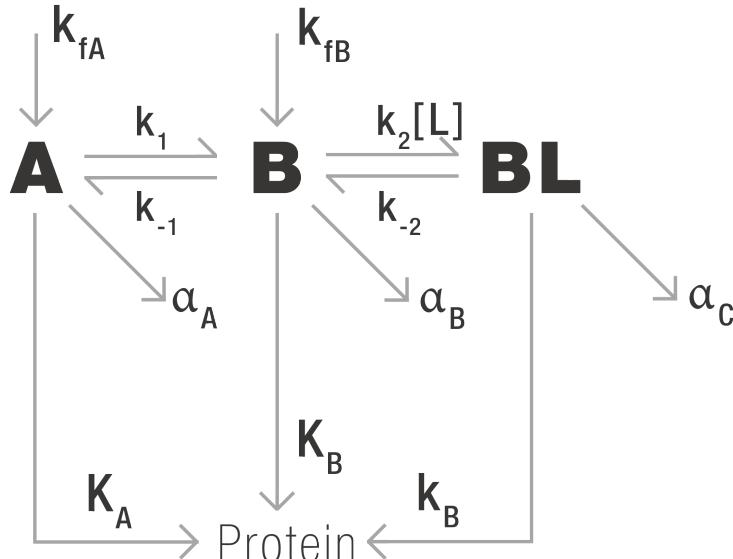
Modification & Optimization



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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

k_f : 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

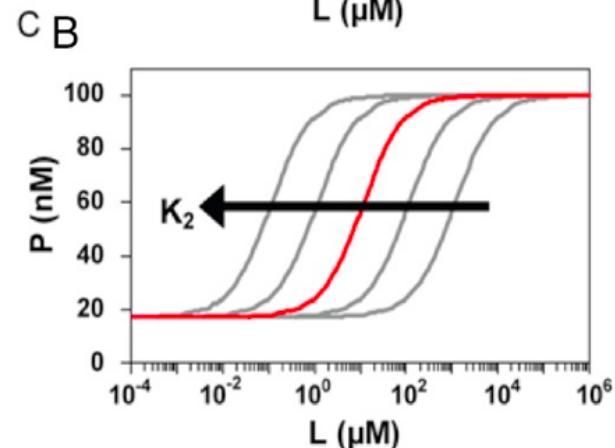
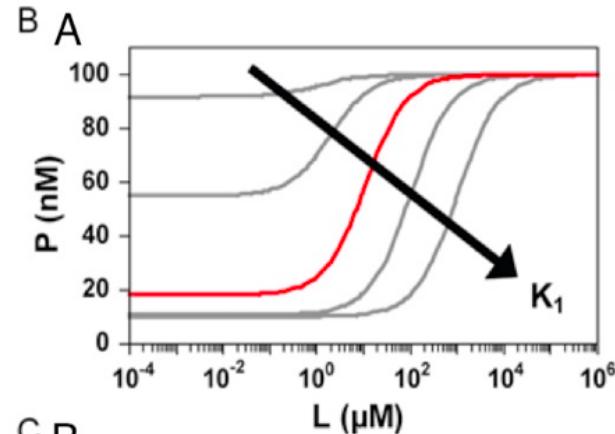
Modification & Optimization



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Modeling of the riboswitches

- Increasing the relative stability of conformation A
- Increasing the binding affinity



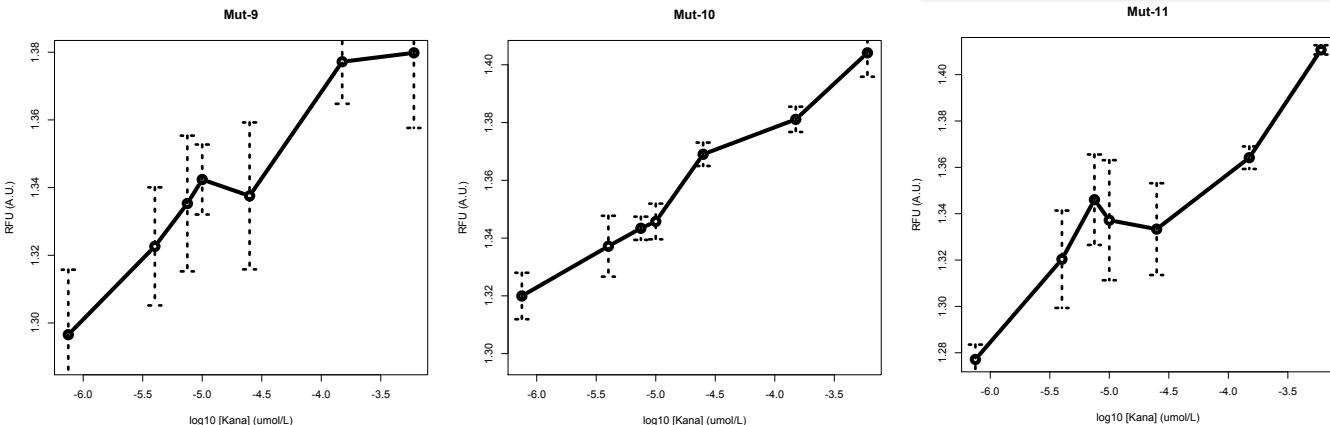
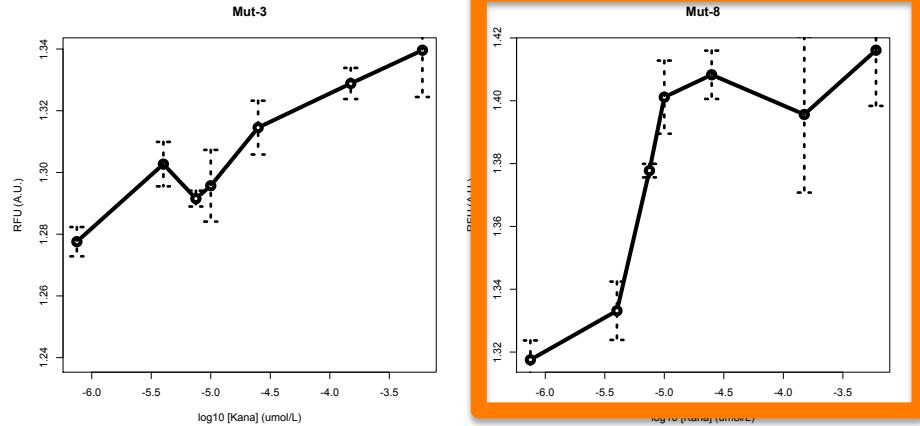
Modification & Optimization



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Practice: Mutants of Aleader

- We mutated the ALeader
- And estimate the mutants



Modification & Optimization

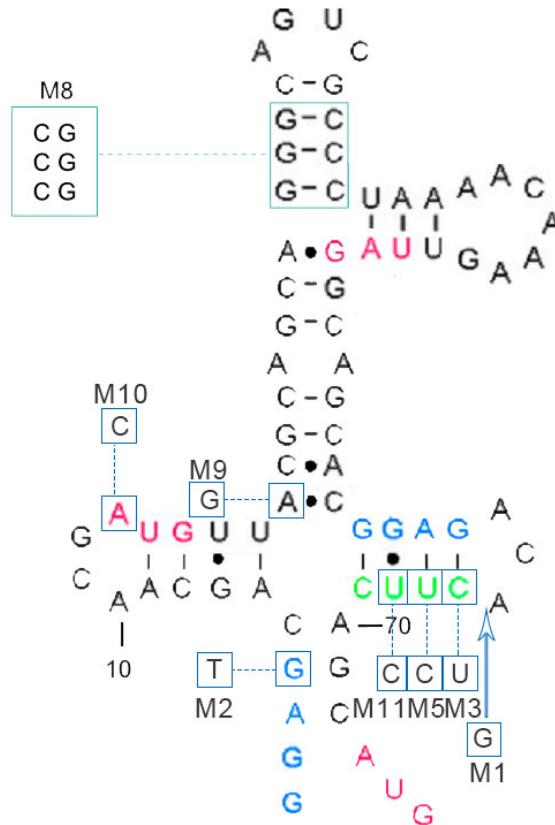


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If mutants fits our need?

- 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 altered	DR=1.10
Mark5	67U>C	AntiSD	K1 altered	Function failed
Mark8	13A>C	Start codon	K2 altered	DR=1.09
Mark9	18A>G	Ligand binding session	K2 altered	DR=1.12
Mark10	26G>C,27G>C,28G>C, 35C>G,36C>G,37>G	Ligand binding session	K2 altered	DR=1.10
Mark11	68U>C	AntiSD	K1 altered	DR=1.11



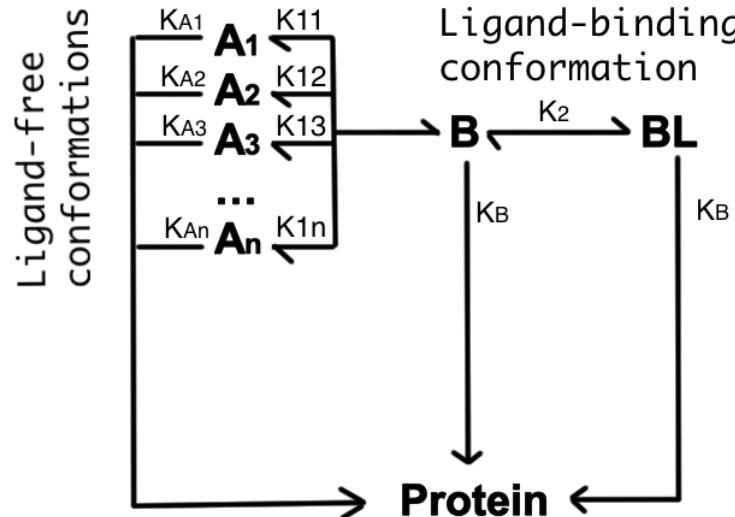
Modification & Optimization



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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}, a₂, a_p: degradation rate of conformation Ai and B, and protein

K_{1i}: partitioning constant between conformation Ai and B

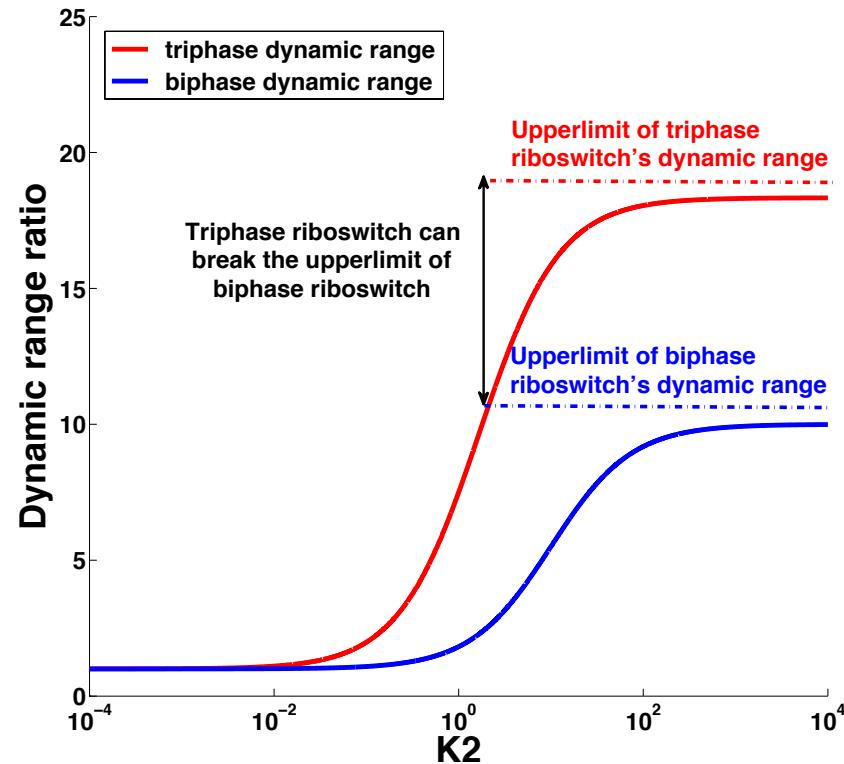
K₂: the aptamer association constant

L: ligand concentration



Why it is good?

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



Modification & Optimization



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Practice: ALeaderT

Make an artificial conformation by adding a competitive sequence

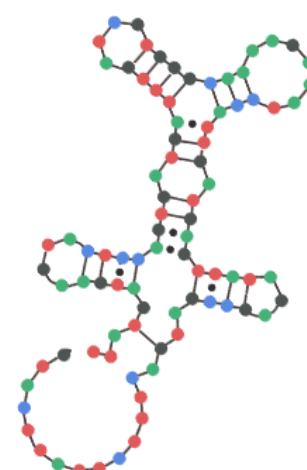
ALeaderT Kana+

Translation on



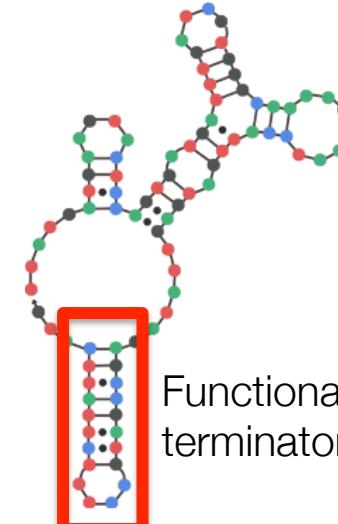
ALeaderT Kana-free

Translation off



ALeaderT Kana-free

Termination on (artificial)



Functional
terminator

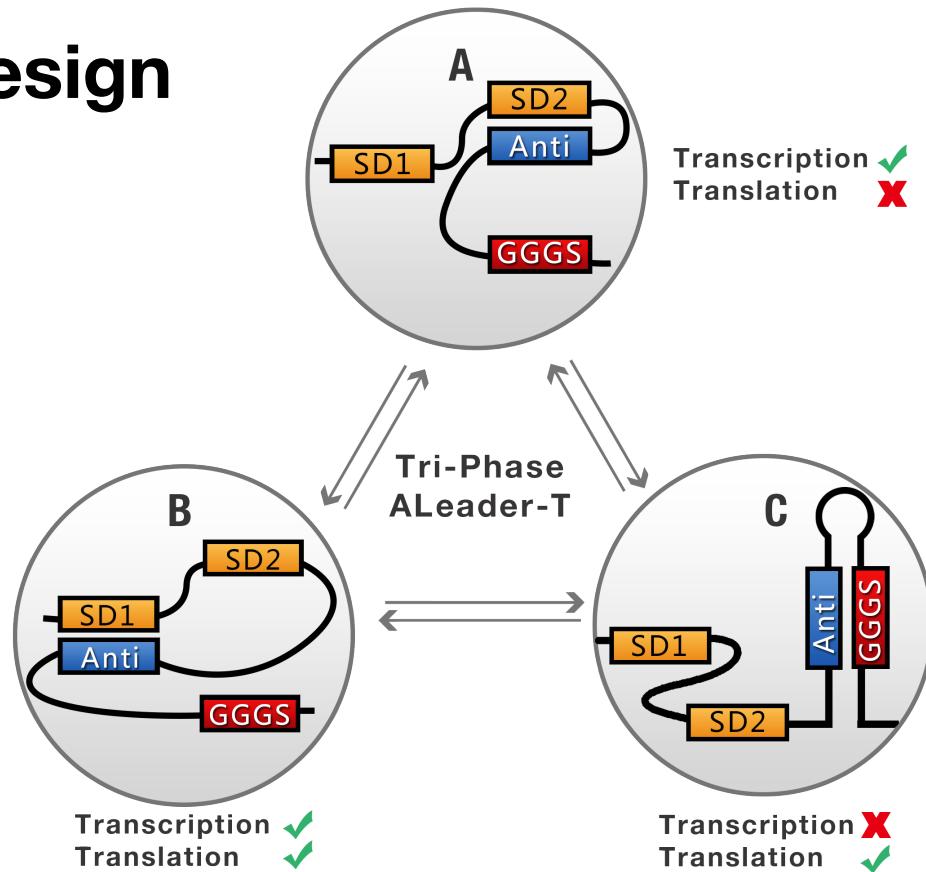
● A
● T
● C
● G

Modification & Optimization



Practice: ALeaderT Design

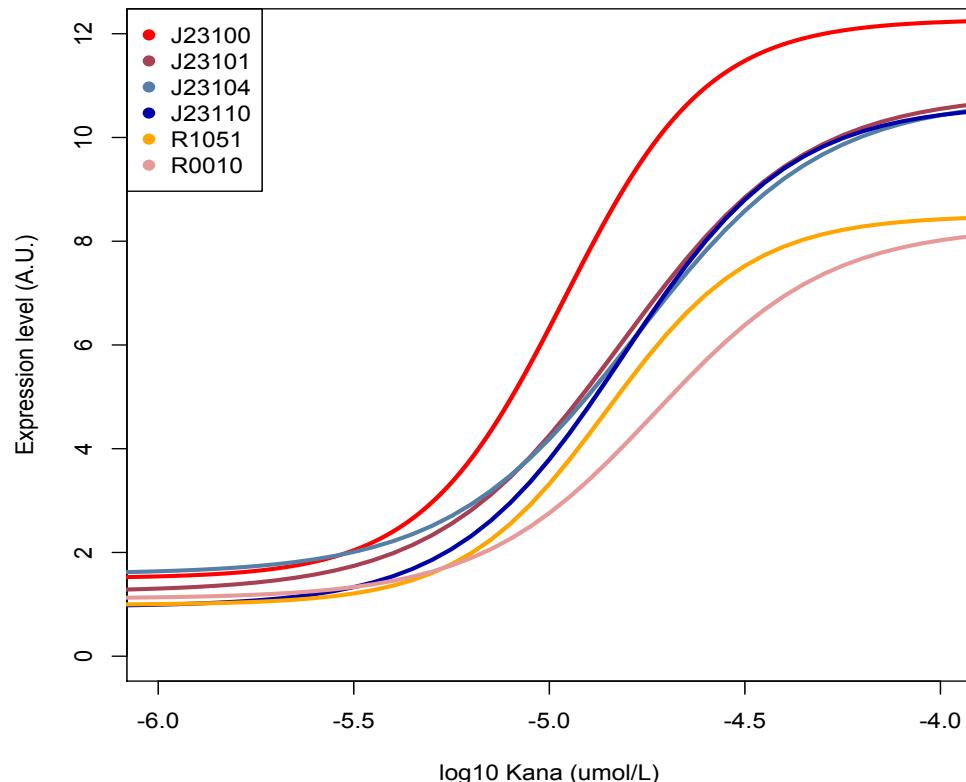
- Three states:
- Ligand-free
 - A: Translation repression
 - B: Termination
- Ligand-binding
 - C: Translation initiation



Modify and Optimize



With
a promoter library
and mRFP1



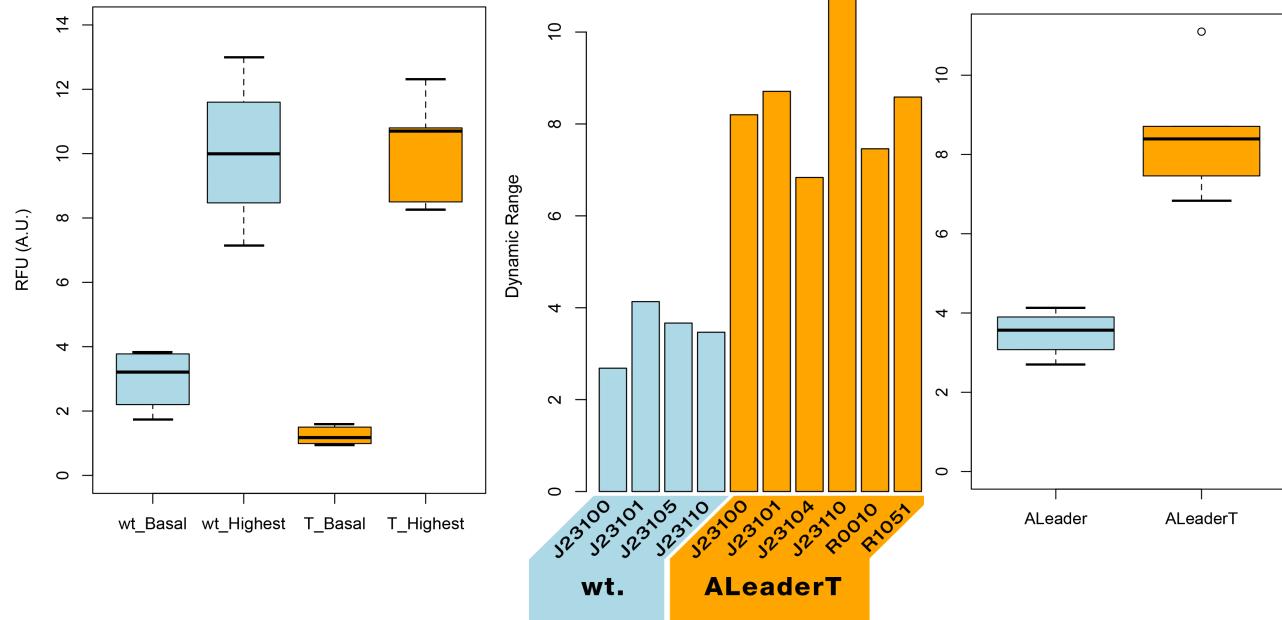
Modify and Optimize



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If ALeaderT fits our need?

- lower basal level and higher dynamic range
- lower noise

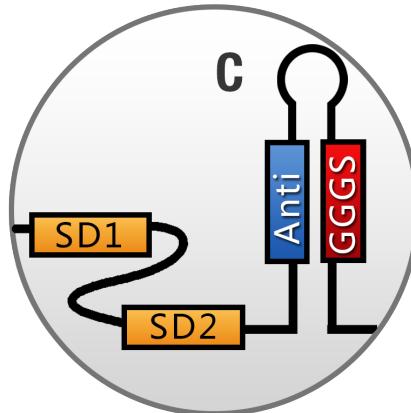


Modify and Optimize

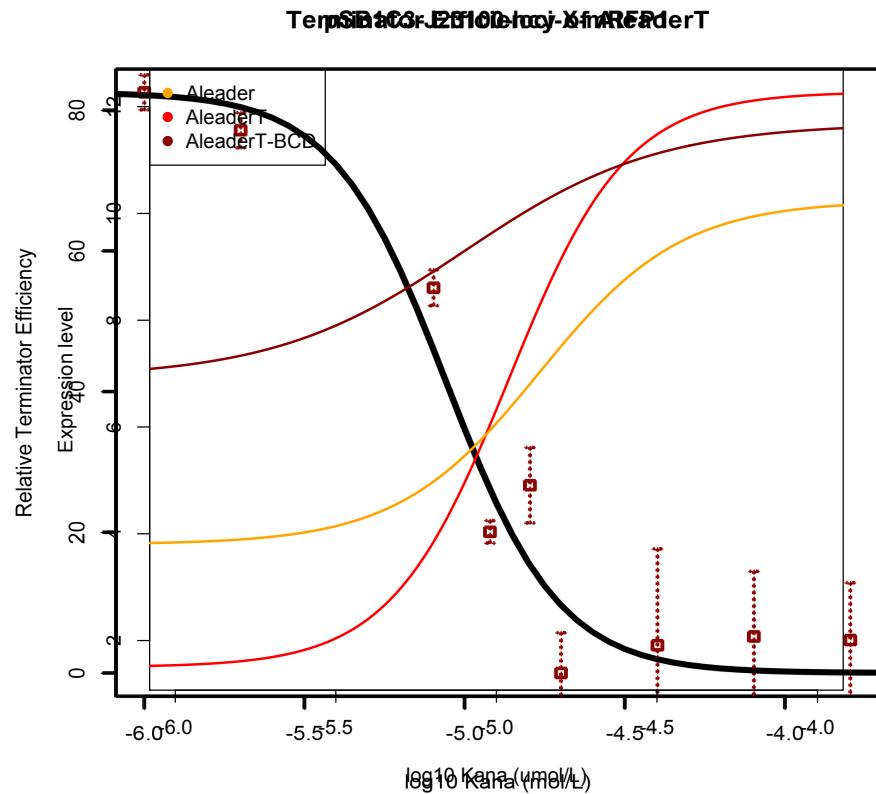


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- **ALeaderT integrates the terminator and translational element**



Transcription **X**
Translation **✓**





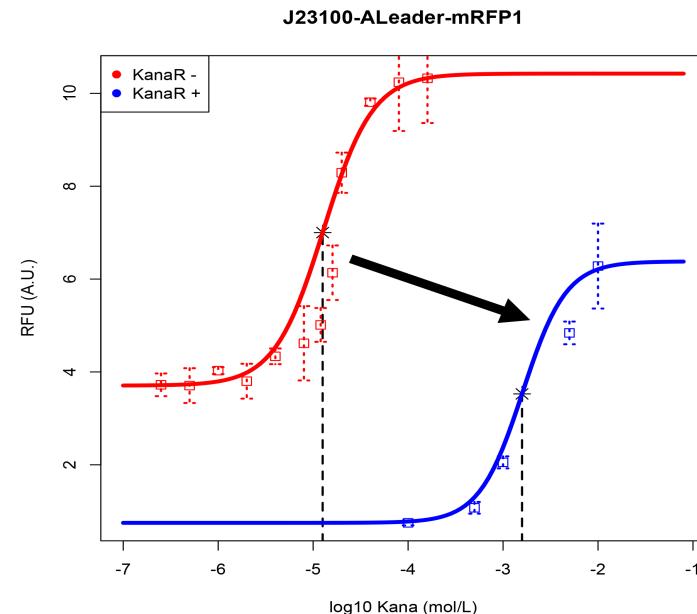
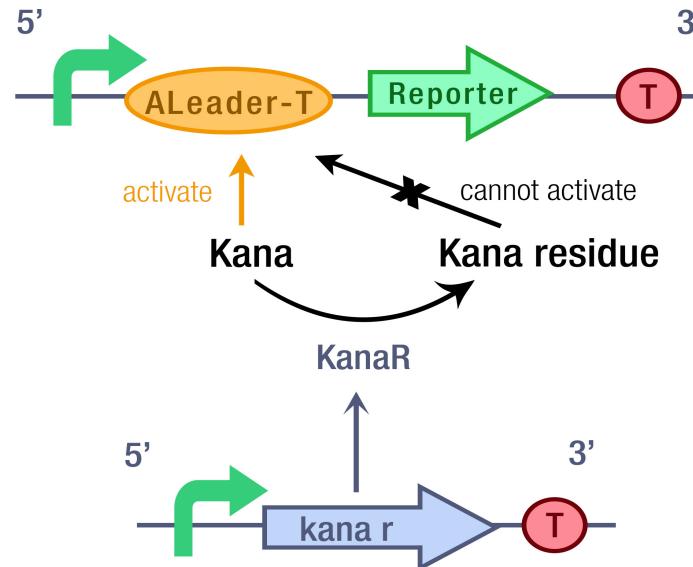
Our strategy: Triphase riboswitch

Summary – the advantages of riboswitch

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

Modify and Optimize

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

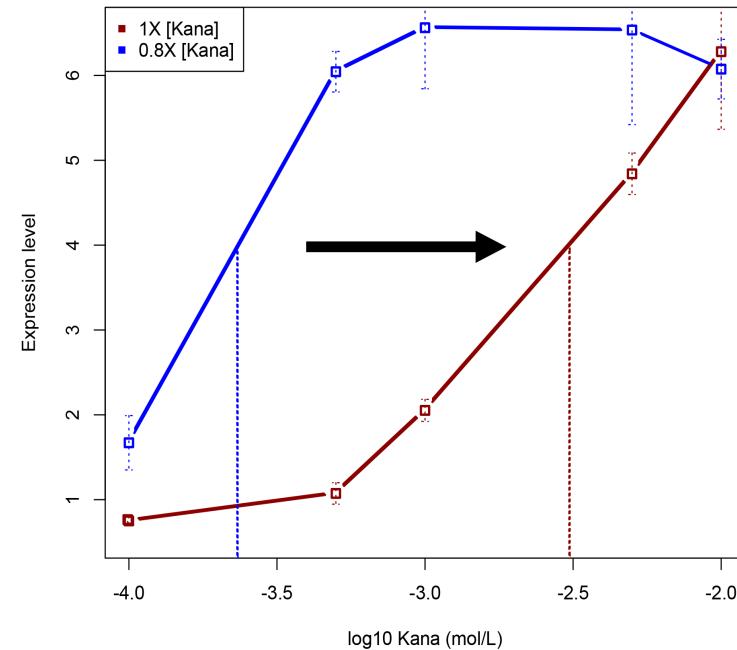
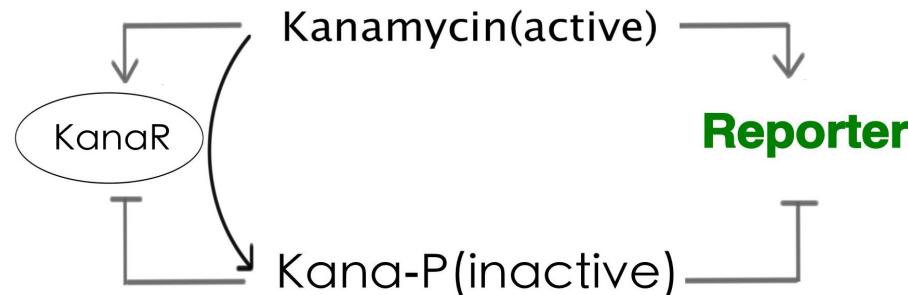


Modify and Optimize



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- Auto-regulated Transformer



Modify and Optimize



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



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

Kanamycin detector = ALeaderT + KanaR

User guides for Application

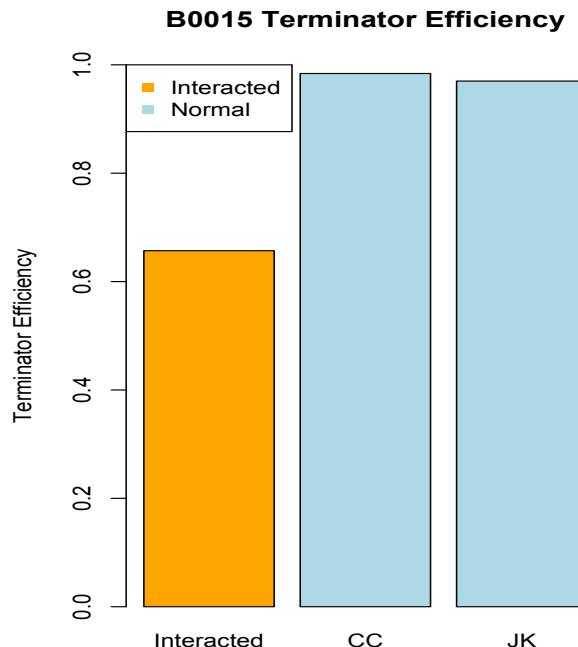
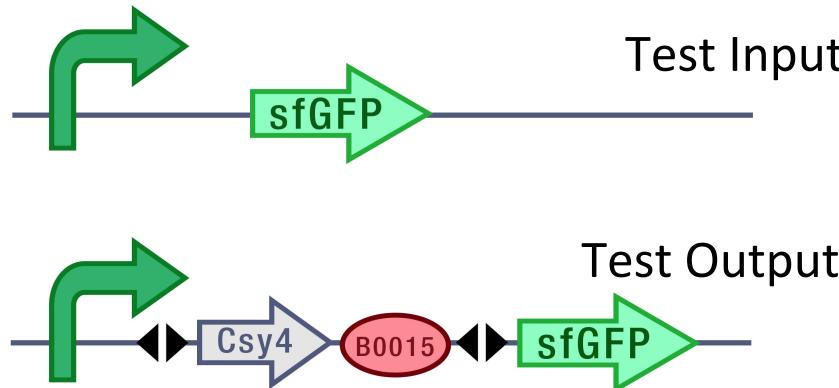


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To Users:

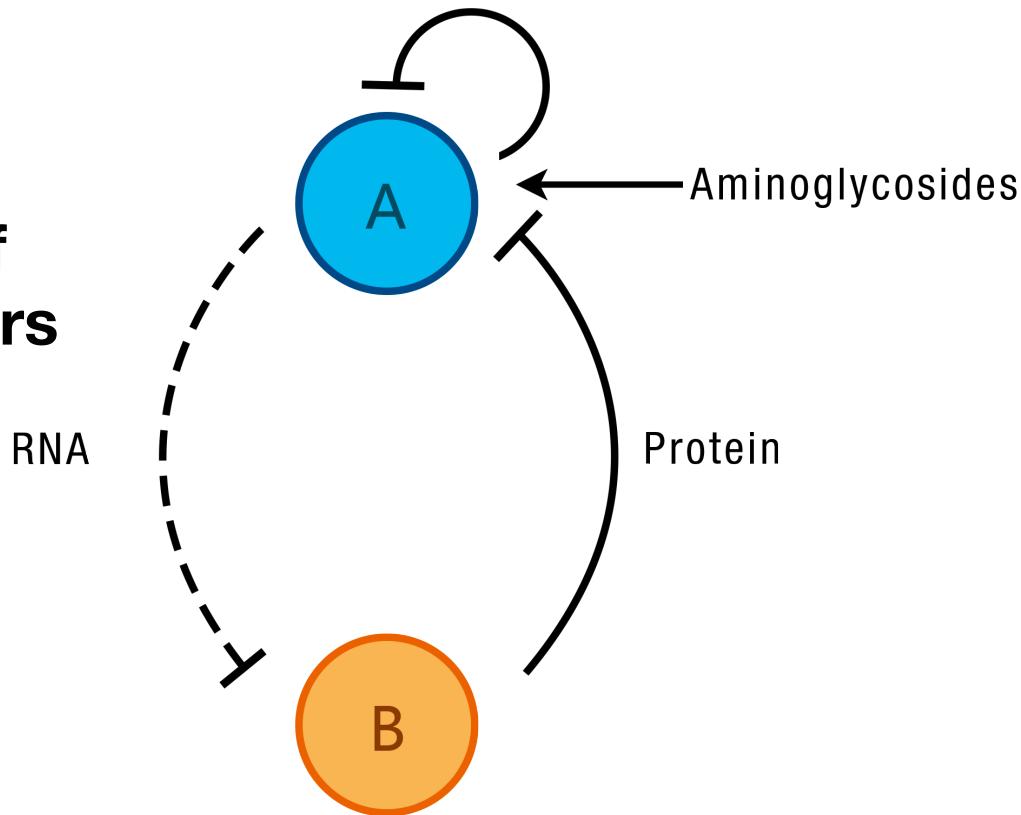
Be careful of RNA interaction

Interfered terminator



Future works

The different behaviors of RNA and protein regulators in the networks



Human Practice



• iGEMcyclopedia 2.0

- For fresh iGEMers
- For better communication

Scaffold protein — The word

compound — Classification

In biology, scaffold proteins are crucial regulators of many key signaling pathways. Although scaffolds are not strictly defined in function, they are known to interact and/or bind with multiple members of a signaling pathway, tethering them into complexes. In such pathways, they regulate signal transduction and help localize pathway components (organized in complexes) to specific areas of the cell such as the plasma membrane, the cytoplasm, the nucleus, the Golgi, endosomes, and the mitochondria.

Reference:

http://en.wikipedia.org/wiki/Scaffold_protein

Related work:

Missouri_Miners (2012) — Related teams

The team aimed to reduce the scaffold proteins in size and adapt them for the cell surface of *Escherichia coli*. This would lay the foundation for making previously infeasible applications of enzymes possible through increased efficiency.

Reference:

http://2012.igem.org/Team:Missouri_Miners/Project

The diagram illustrates the structure of an iGEMcyclopedia entry. At the top left, there is a link labeled "Scaffold protein — The word". A blue arrow points from this link to the text "In biology, scaffold proteins are crucial regulators of many key signaling pathways...". Below this, there is a link labeled "compound — Classification", which has an orange arrow pointing to it. Further down, there is a link labeled "Reference:", with a blue arrow pointing to the URL "http://en.wikipedia.org/wiki/Scaffold_protein". To the right of the main text, there is a pink arrow pointing to the text "General explanation". At the bottom, there is a link labeled "Related work:", with a red arrow pointing to the text "The team aimed to reduce the scaffold proteins in size and adapt them for the cell surface of *Escherichia coli*...". Below this, there is another link labeled "Reference:", with a blue arrow pointing to the URL "http://2012.igem.org/Team:Missouri_Miners/Project".

Human Practice



Activities at Fudan and Communications with other teams



With association of Bertalanffy

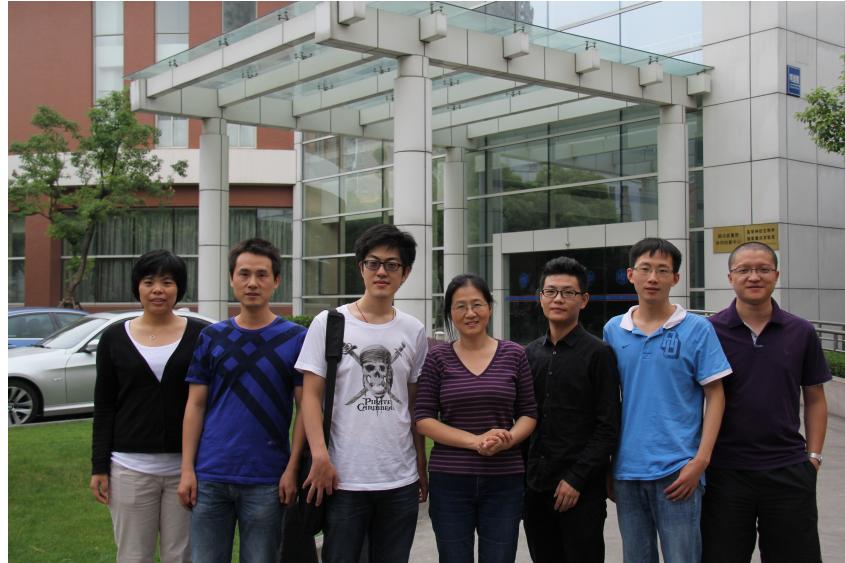


With iGEM2013_USTC CHINA

Human Practice



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



Judge Form



- 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

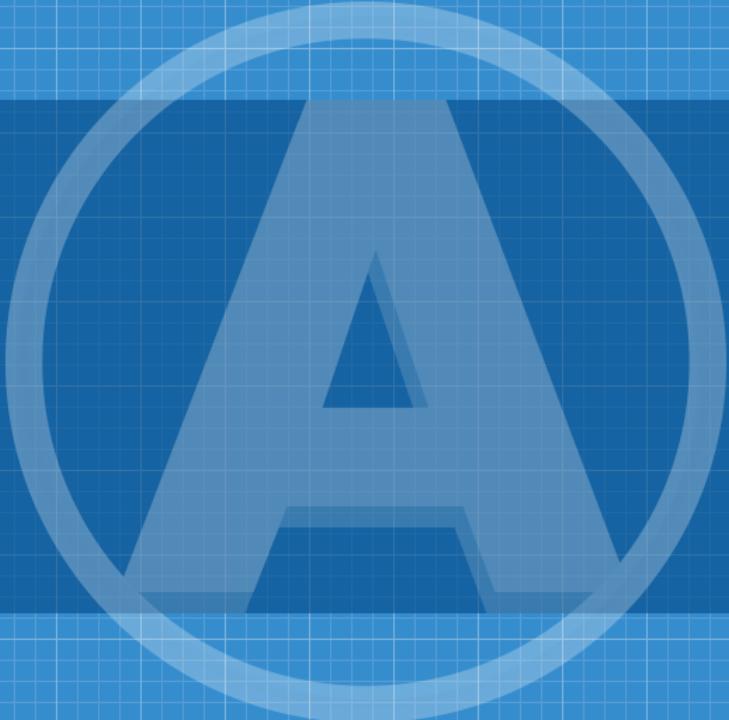


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- School of life science, Fudan University
- Prof. Zhao Guoping, School of Life Science, Fudan University
- Prof. Dongrong Chen and Prof. Alastair I.H. Murchie's Lab, IBS Fudan University
- Prof. Lu Daru, School of Life Science, Fudan University
- 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



Supplementary



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Our strategy:

Triphase riboswitch

$$P = \frac{k_f}{a_p} \frac{K_A K_2 + K_B K_3 + K_C (1 + K_4 L)}{K_A K_2 + K_B K_3 + K_C (1 + K_4 L)}$$

P: protein production

k_f: transcriptional initiation rate

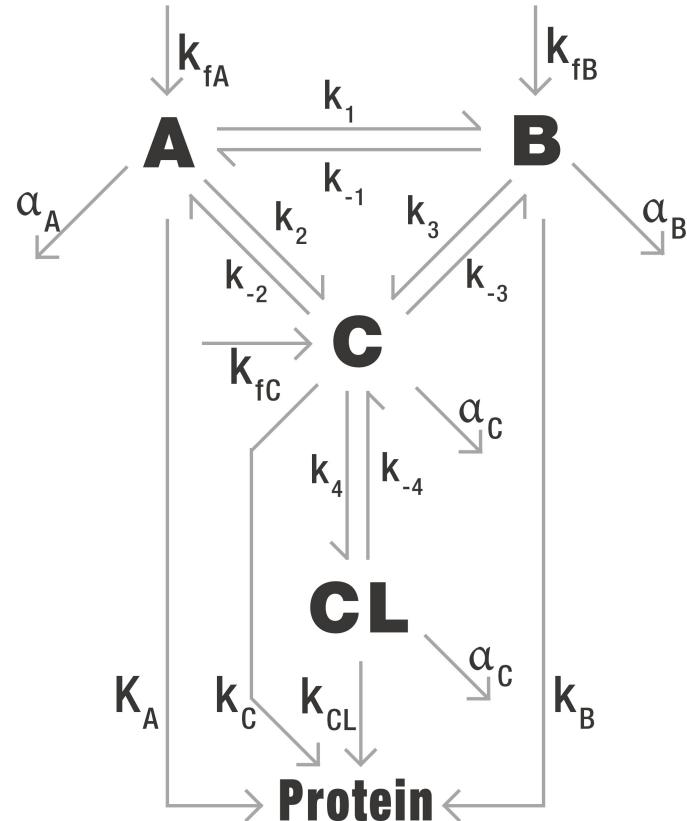
K_A, K_B, K_C: representative regulatory activities of conformation A, B and C

a₁, a₂, a₃, a_p: degradation rate of conformation A, B, C and protein

K₁, K₂, K₃: partitioning constant between conformation A and B, A and C, B and C

K₄: the aptamer association constant

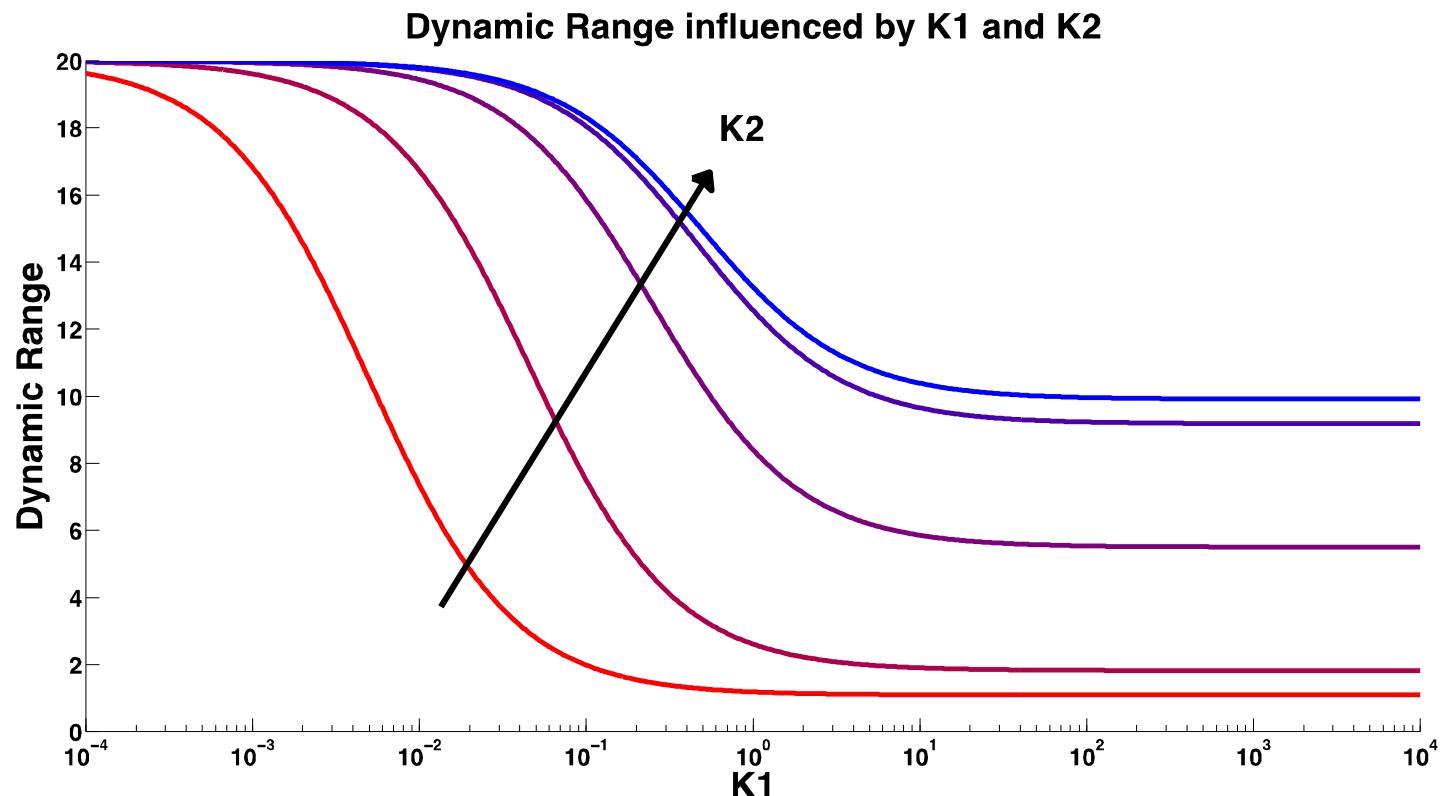
L: ligand concentration



Supplementary



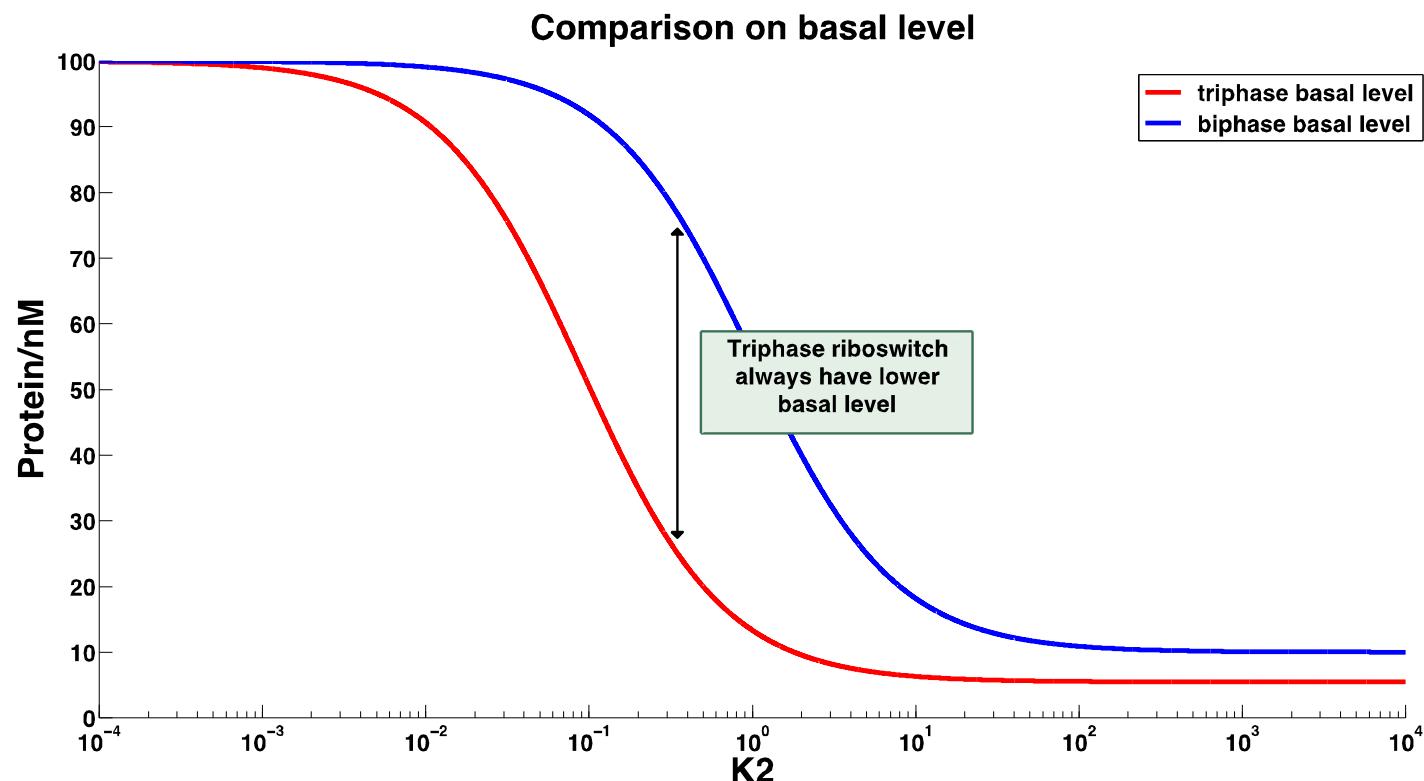
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Supplementary



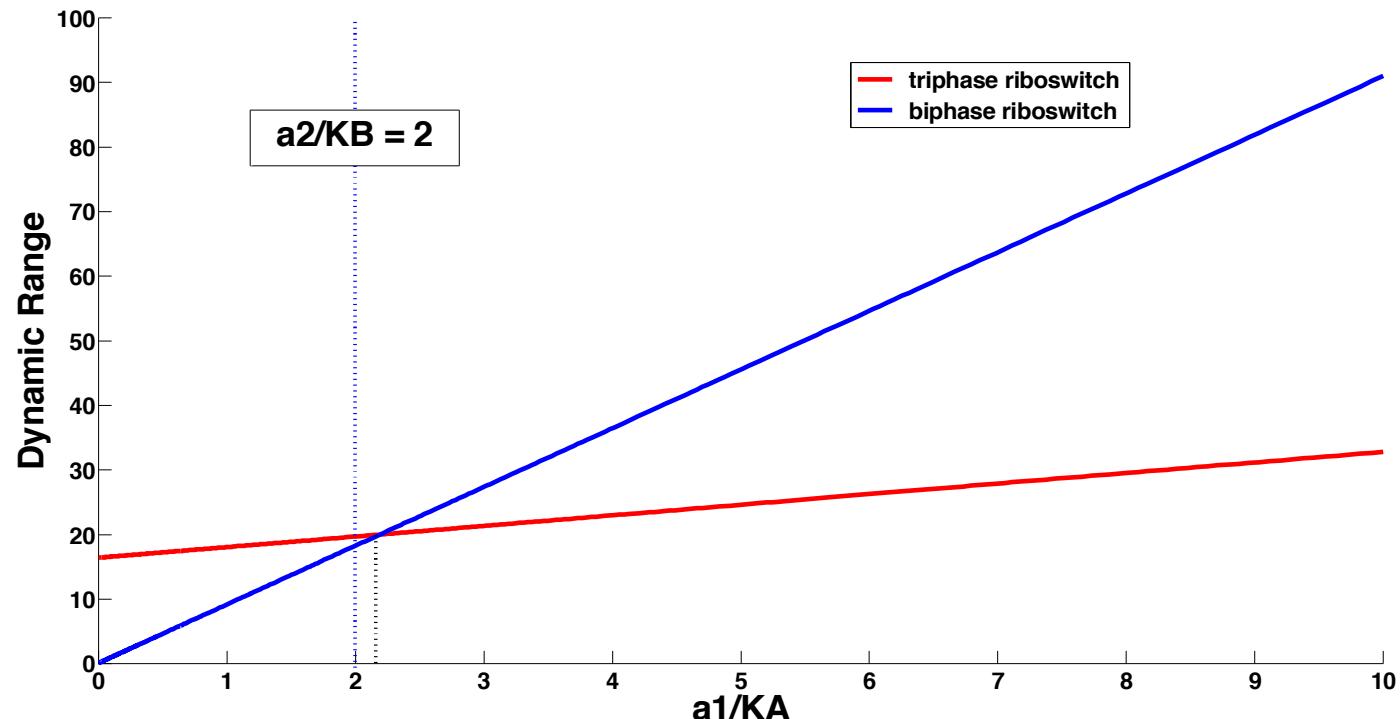
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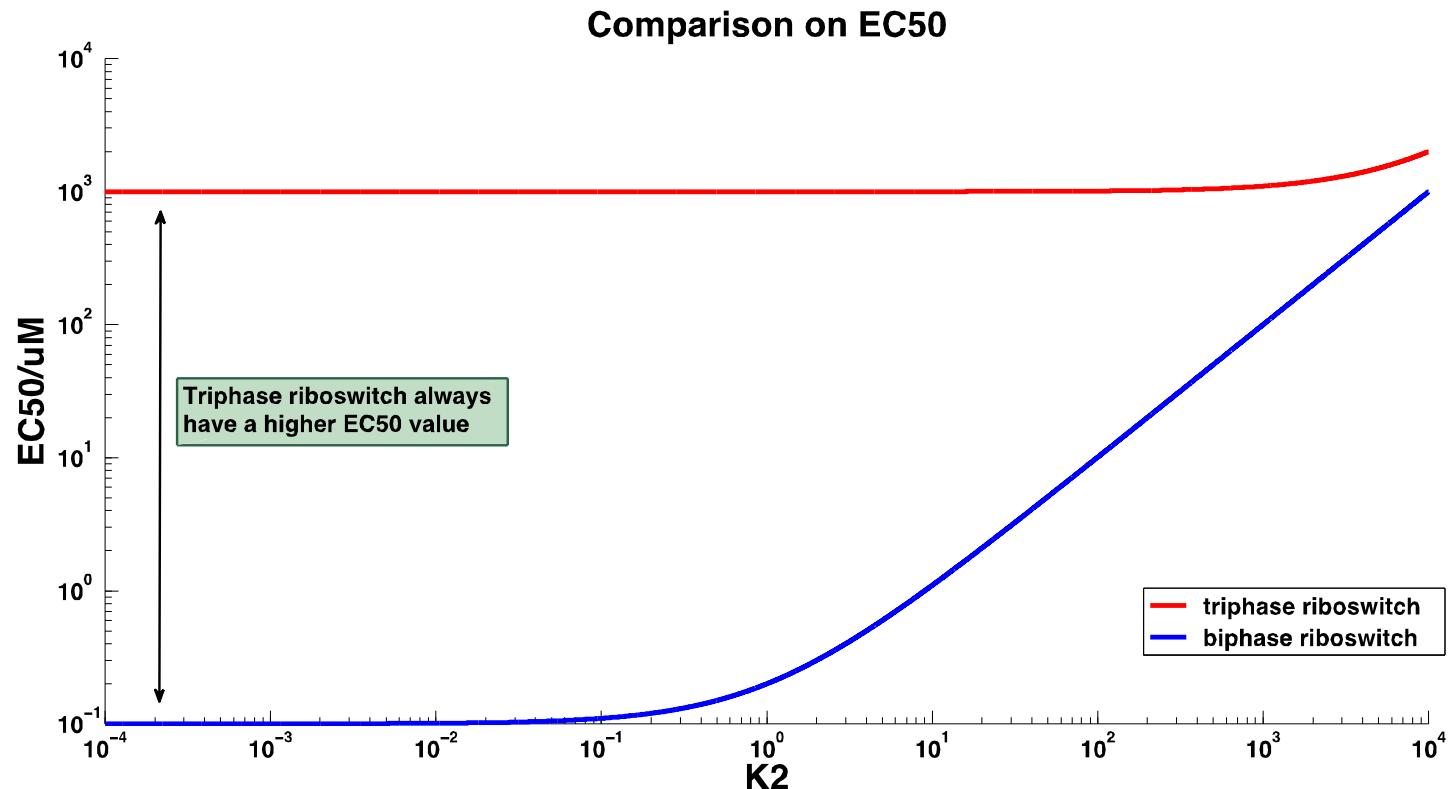
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Supplementary



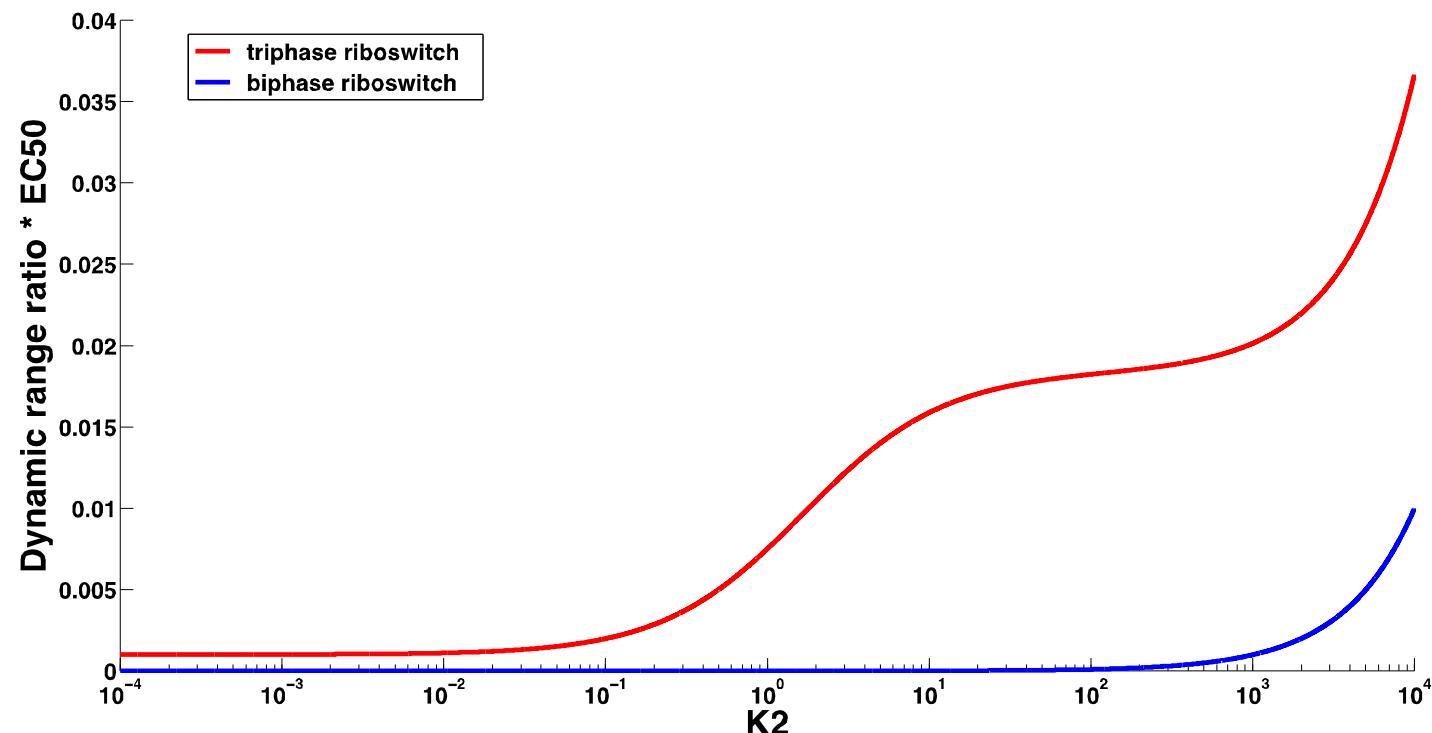
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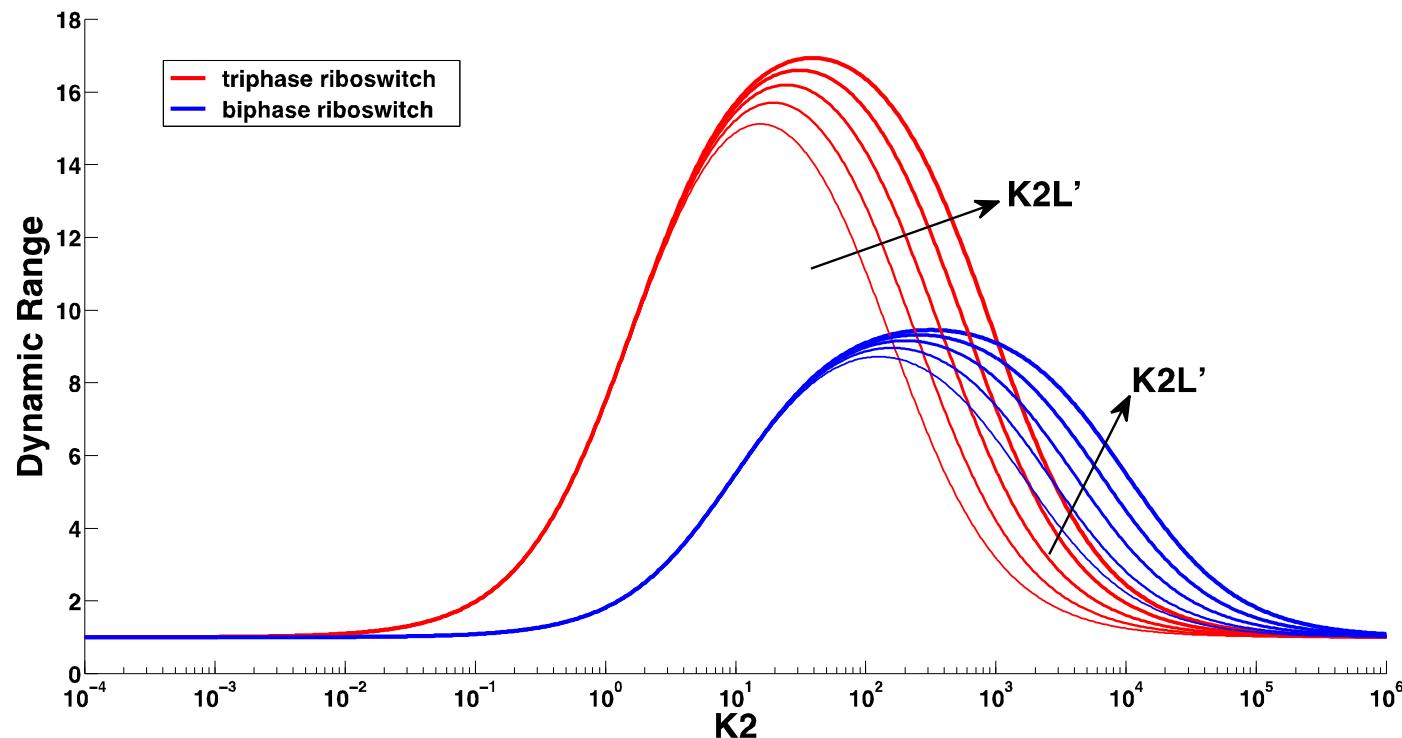
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Supplementary



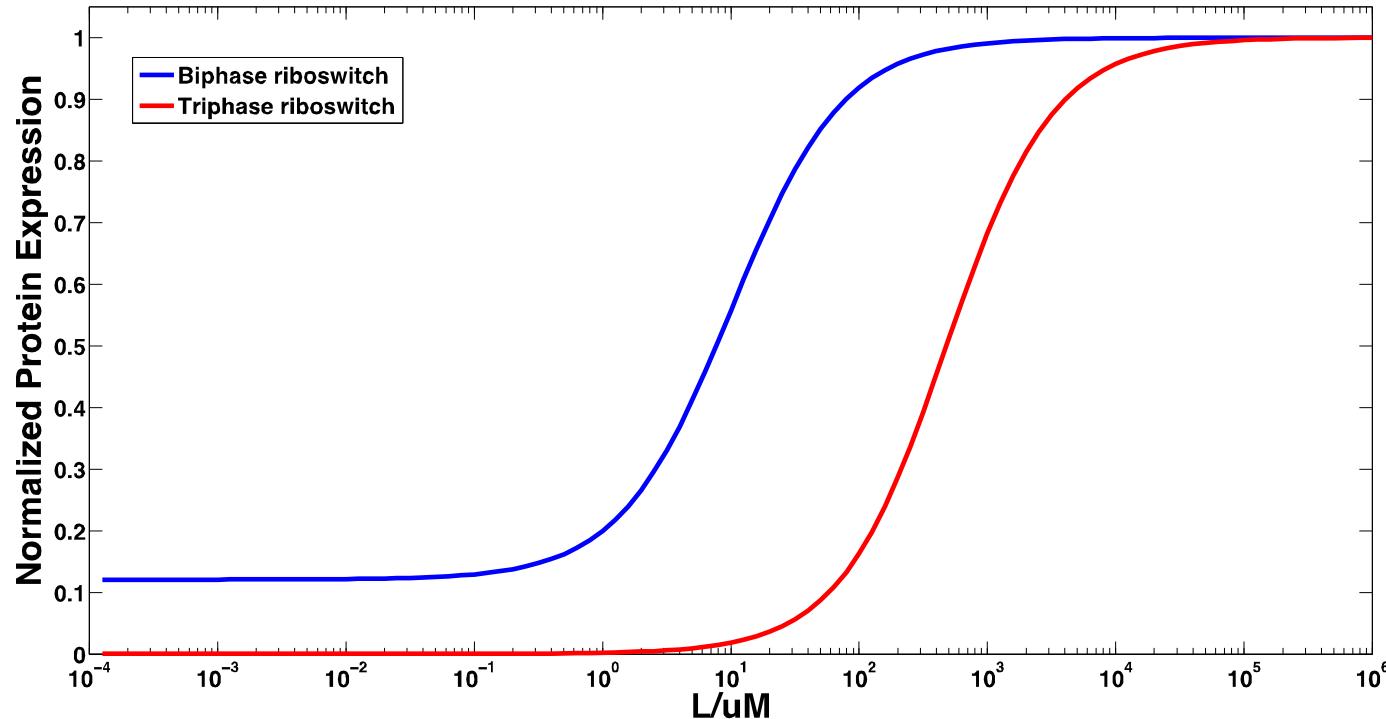
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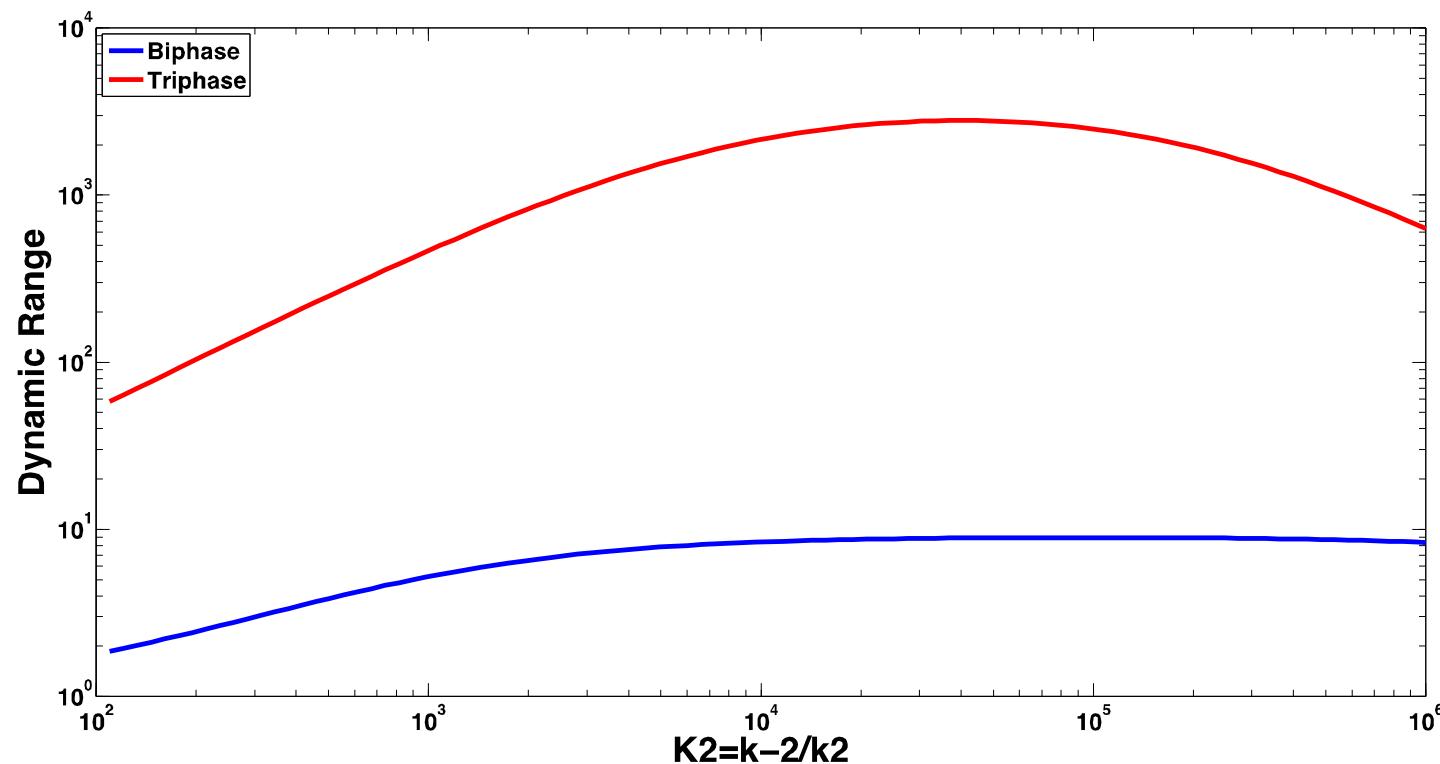
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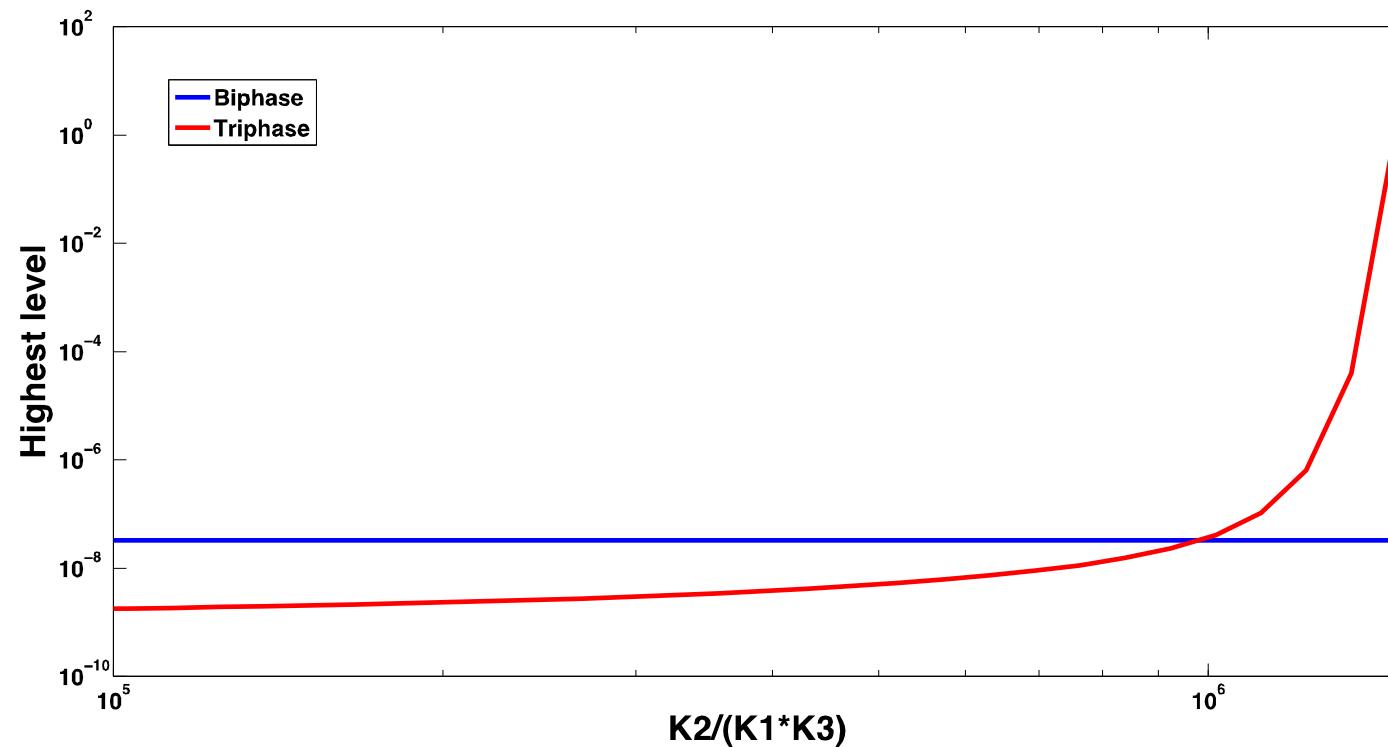
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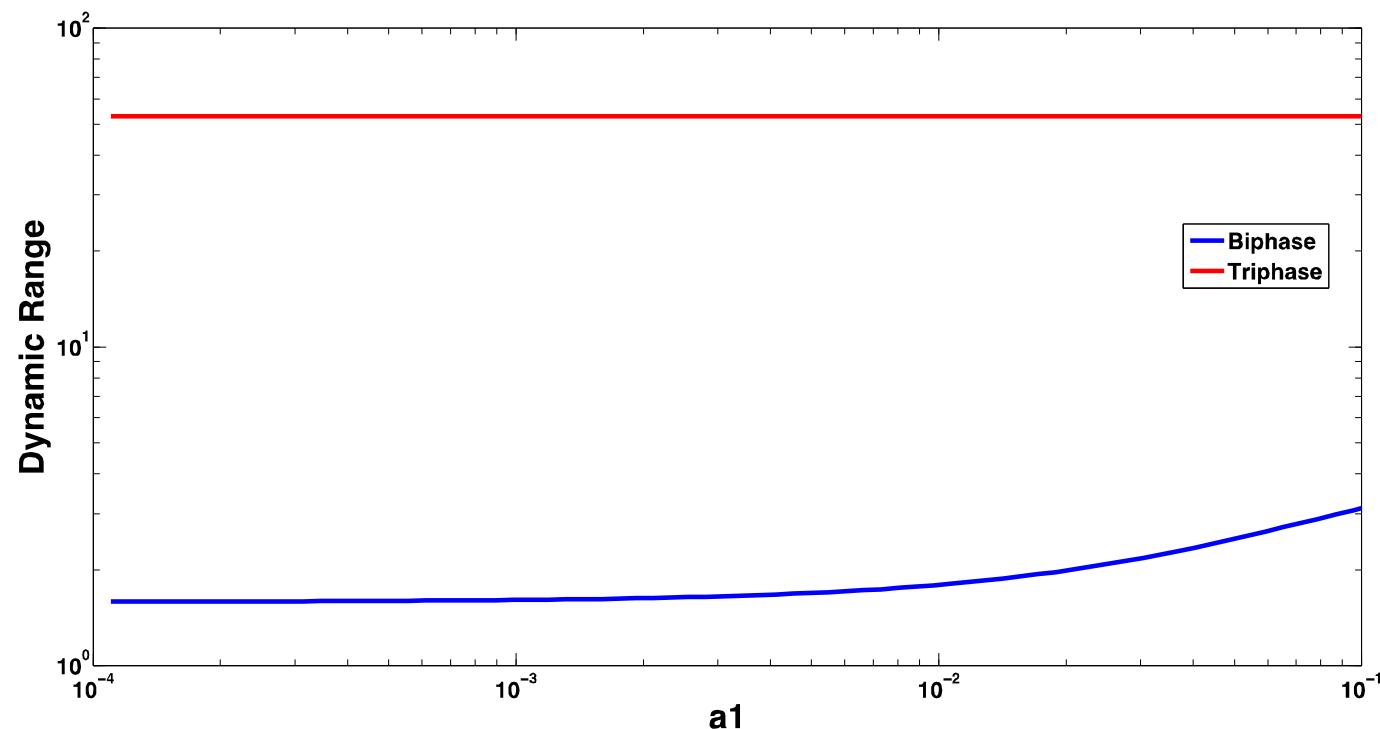
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Supplementary



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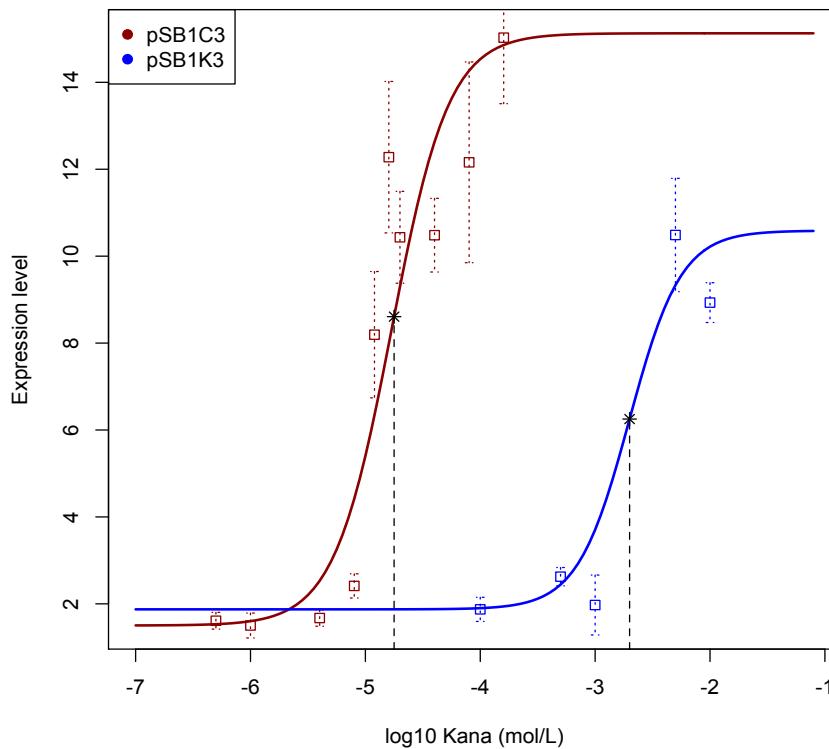


Supplementary



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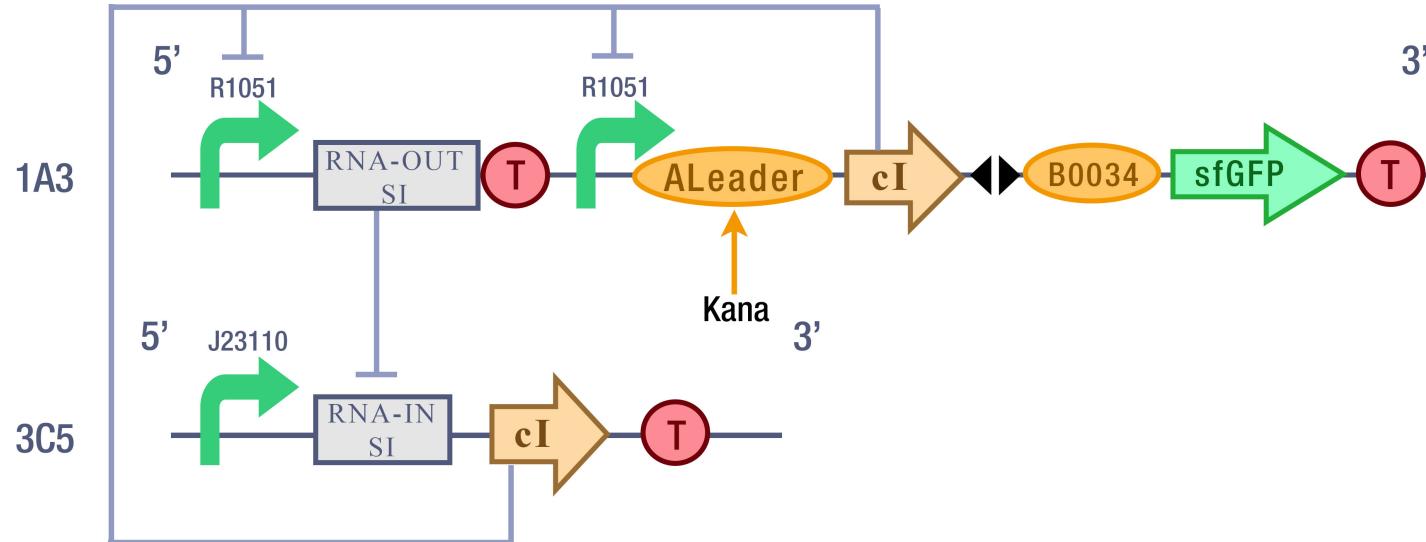
J23100-ALeaderT-mRFP1



Supplementary



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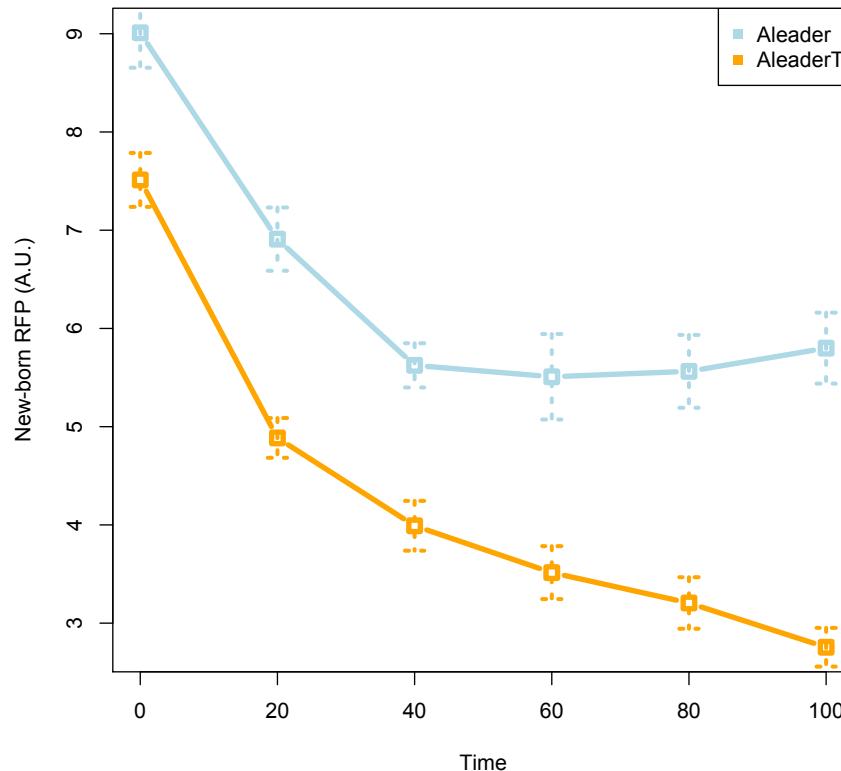


Supplementary



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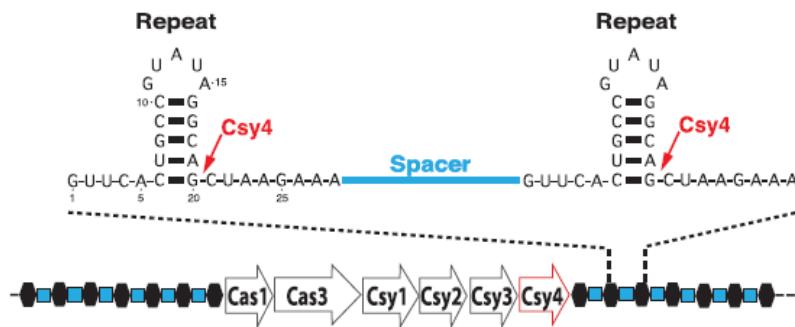
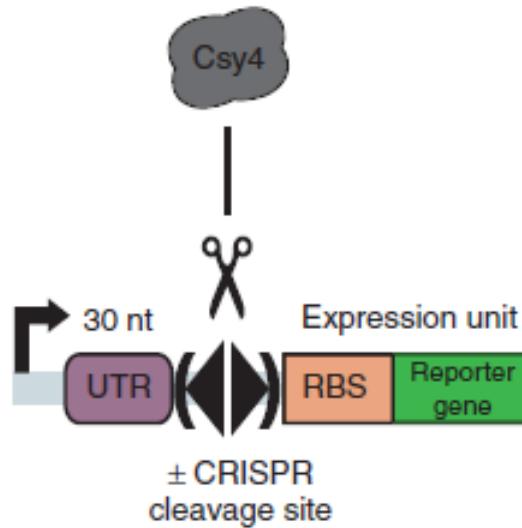
J23100-X-mRFP1



Supplementary



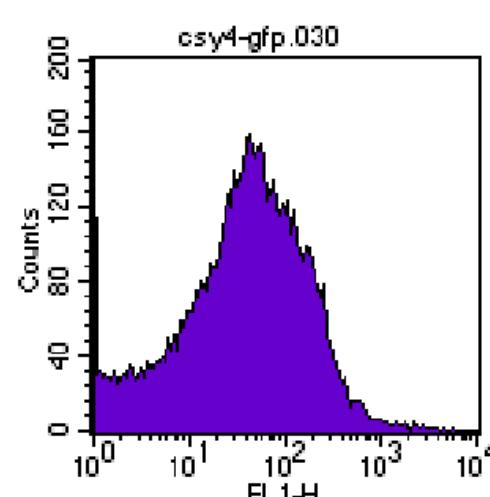
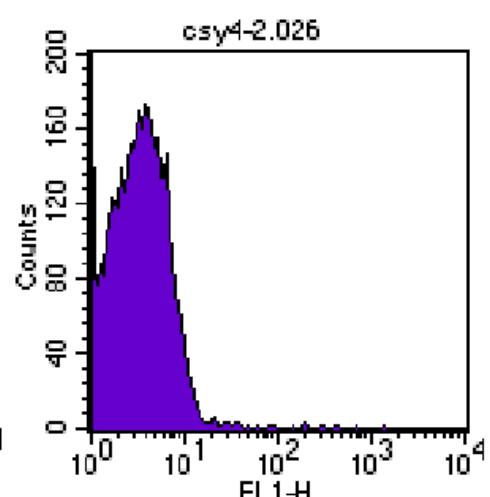
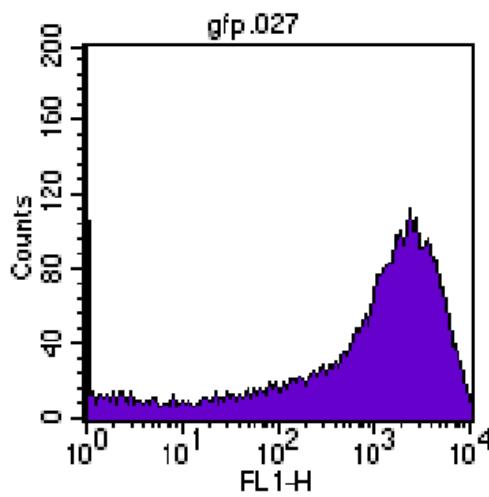
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Supplementary

A

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File: gfp.027

Geo Mean
243.23

File: csy4-2.026

Geo Mean
2.91

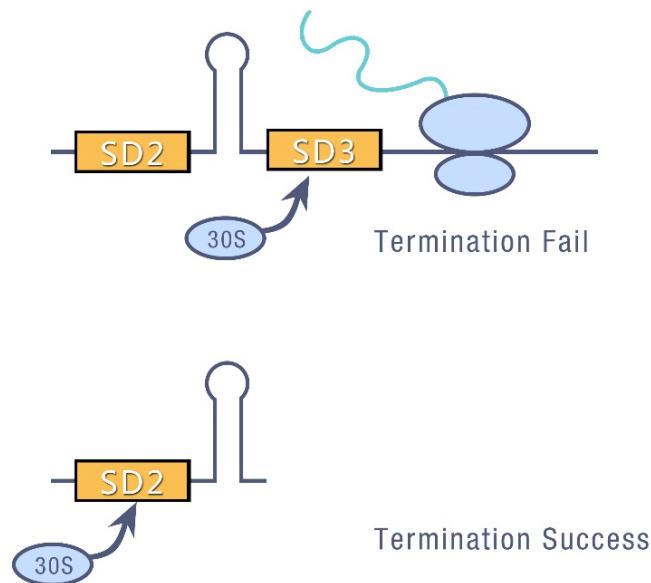
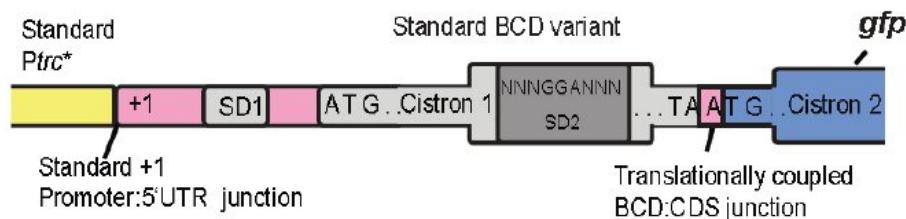
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Geo Mean
22.75

Supplementary



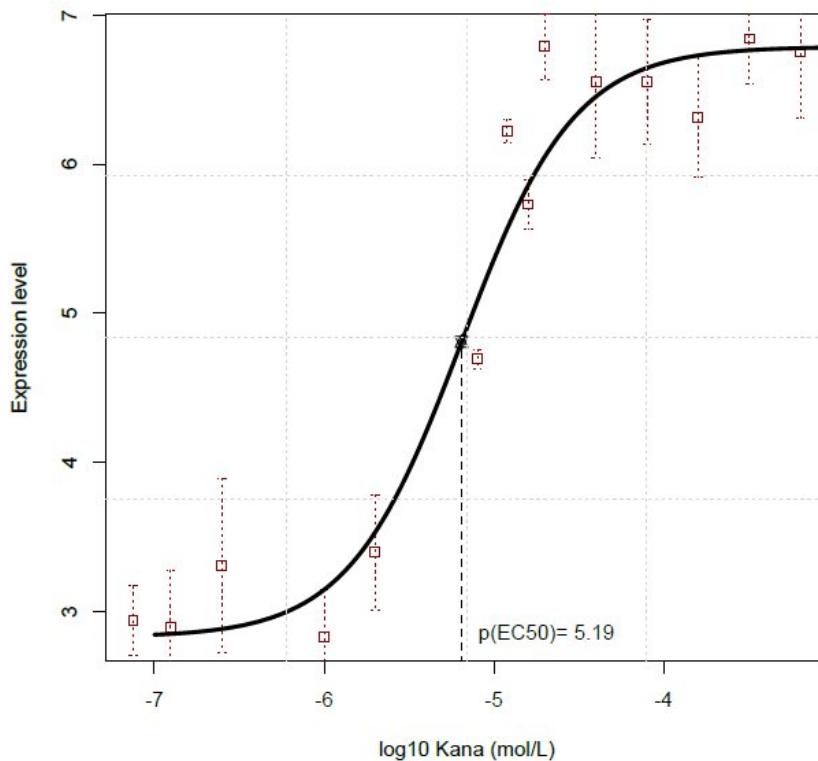
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Supplementary



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Supplementary



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Test of Spinach with DFHBI

