

ALeader

Leading the advance of RNA synthetic biology

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Nov 3rd, 2013





**Ancient
Powerful
Tunable**

Our Team



What's ALeader?

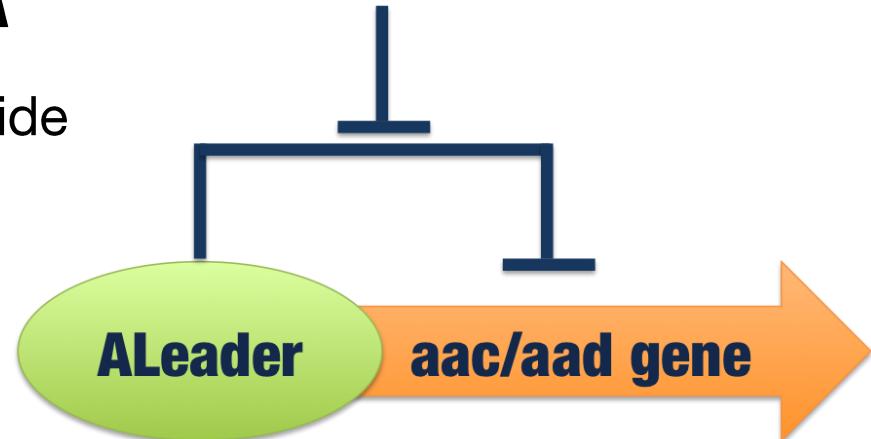


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A conserve Leader RNA

Control the translation of Aminoglycoside antibiotics resistance genes

**Aminoglycoside
Antibiotics**

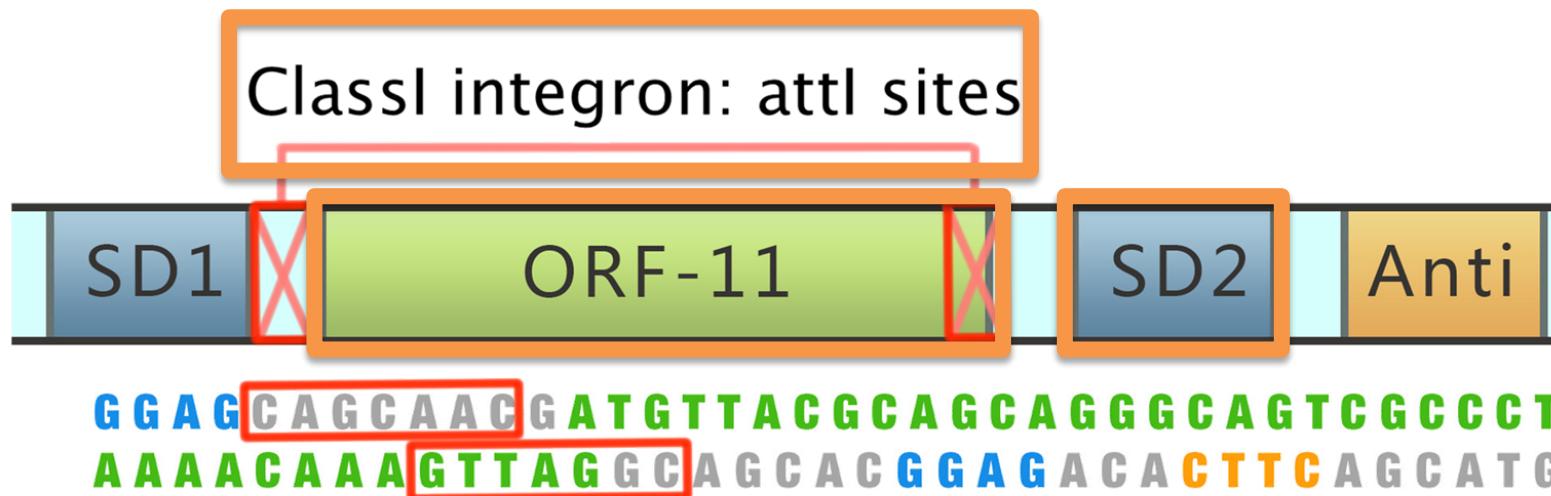


What's ALeader?



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The most complex riboswitch

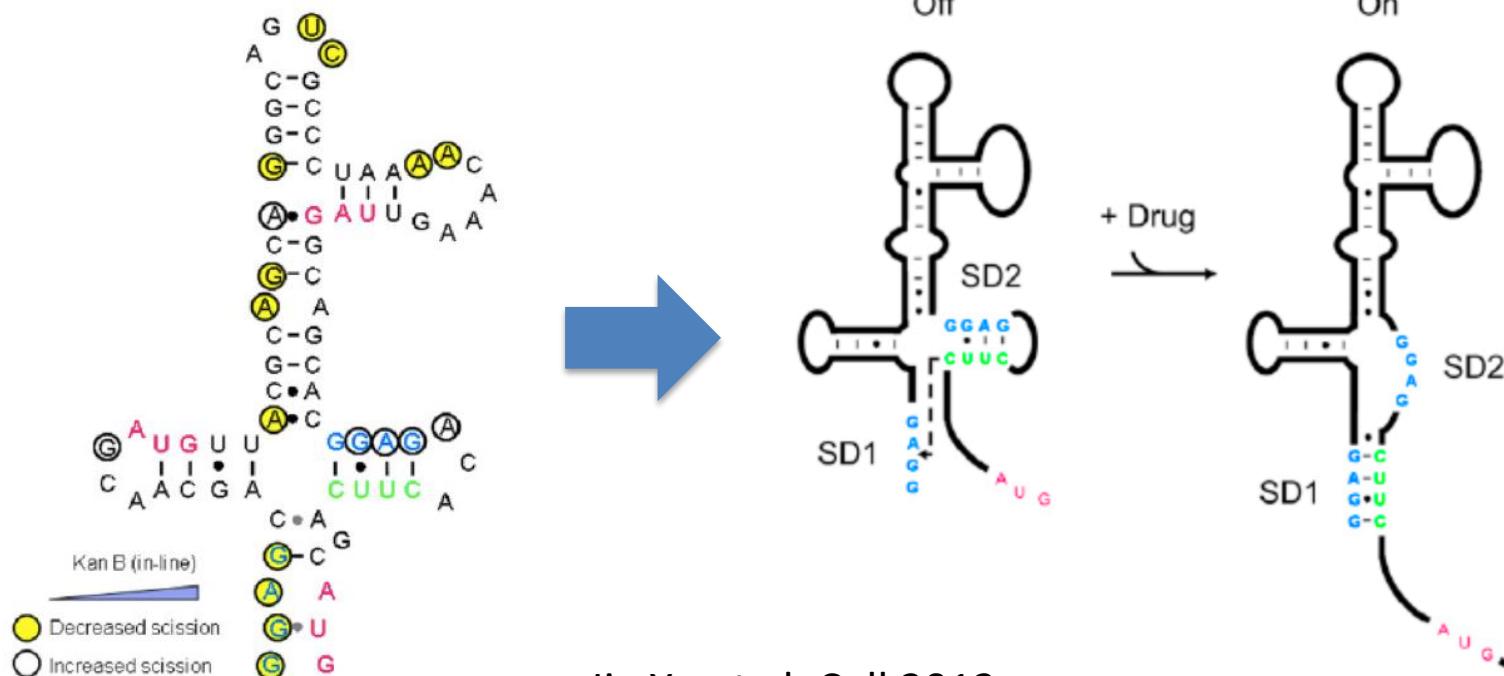


What's ALeader?



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The most complex riboswitch



Jia Xu et al. Cell 2013

Resistance

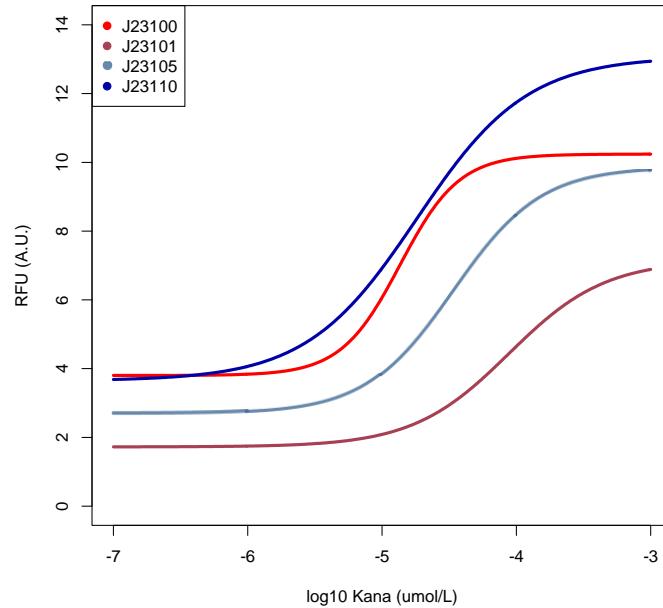
What's ALeader?



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Is ALeader a standard riboswitch?

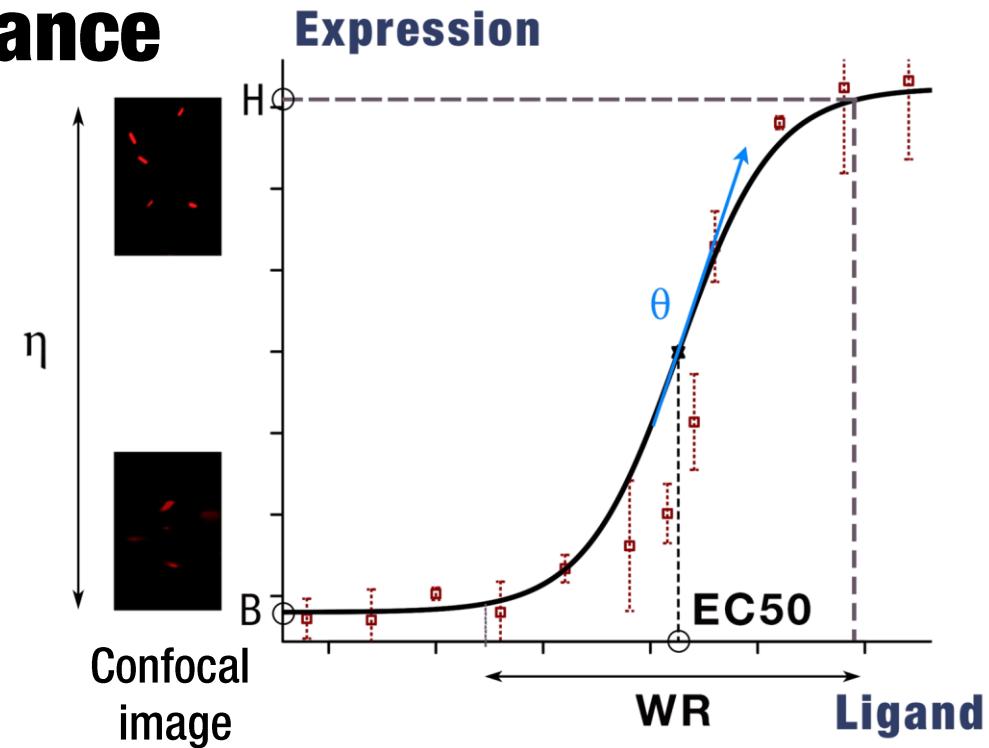
How to modify ALeader?



Our Goal

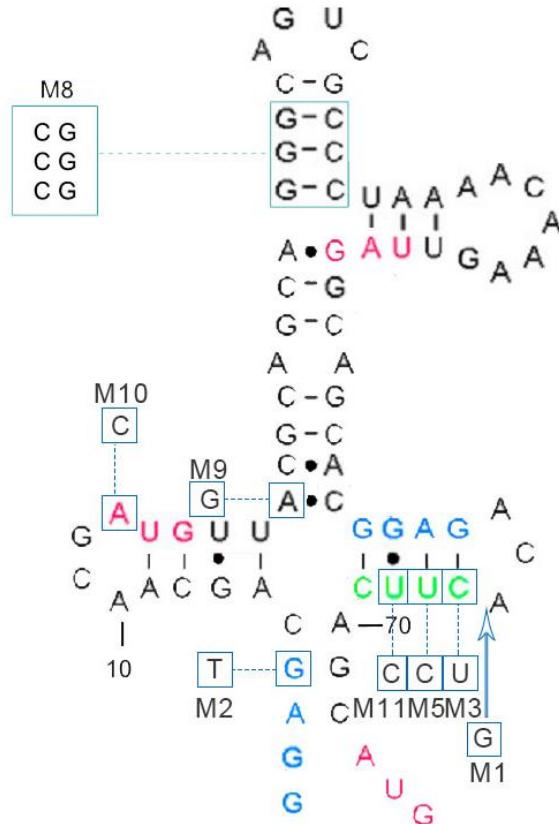
Modifying the performance

- More Standard
- Multi-function
- Lower Basal level
- Higher Highest level
- Higher Dynamic range
- Wider Working range



Mutagenesis

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

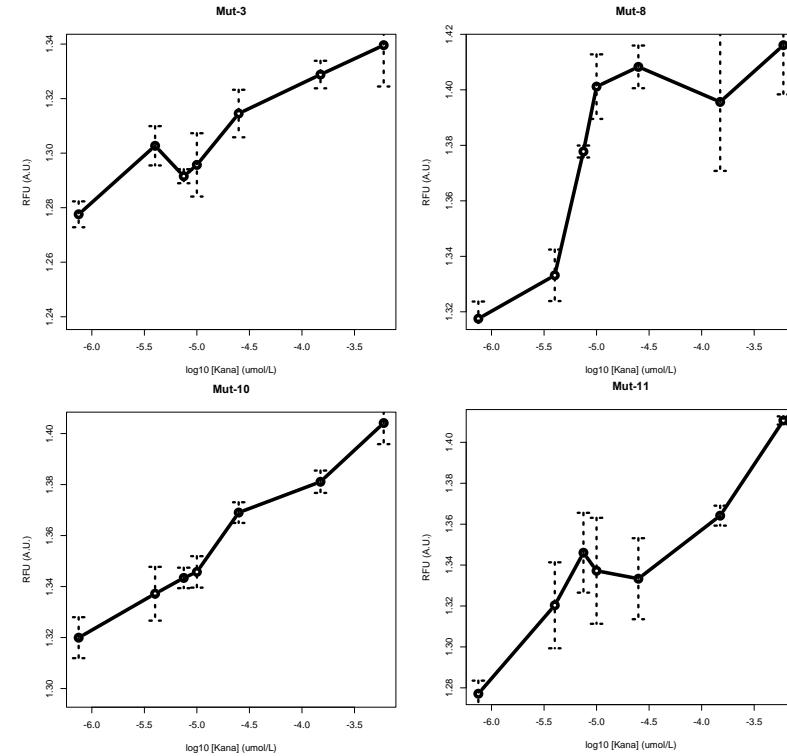


Mutagenesis



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Triphase riboswitch design

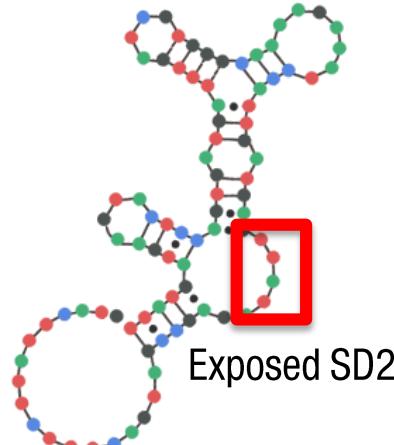


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A competitive sequence for a new conformation

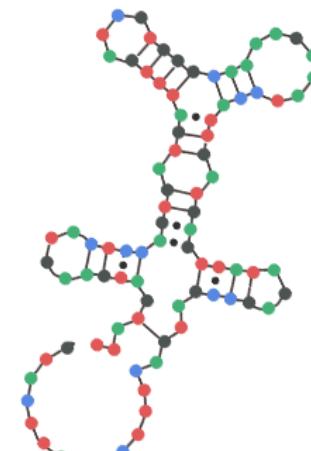
ALeaderT Kana+

Translation on



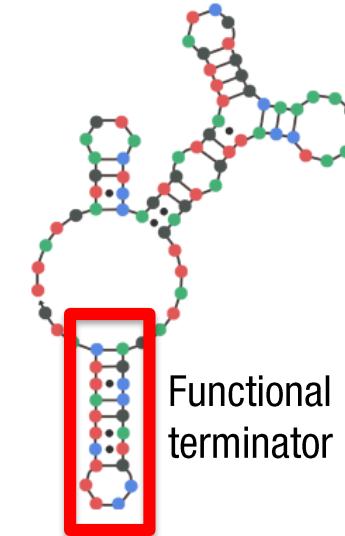
ALeaderT Kana-free

Translation off



ALeaderT Kana-free

Termination on (artificial)



• A
• T
• C
• G

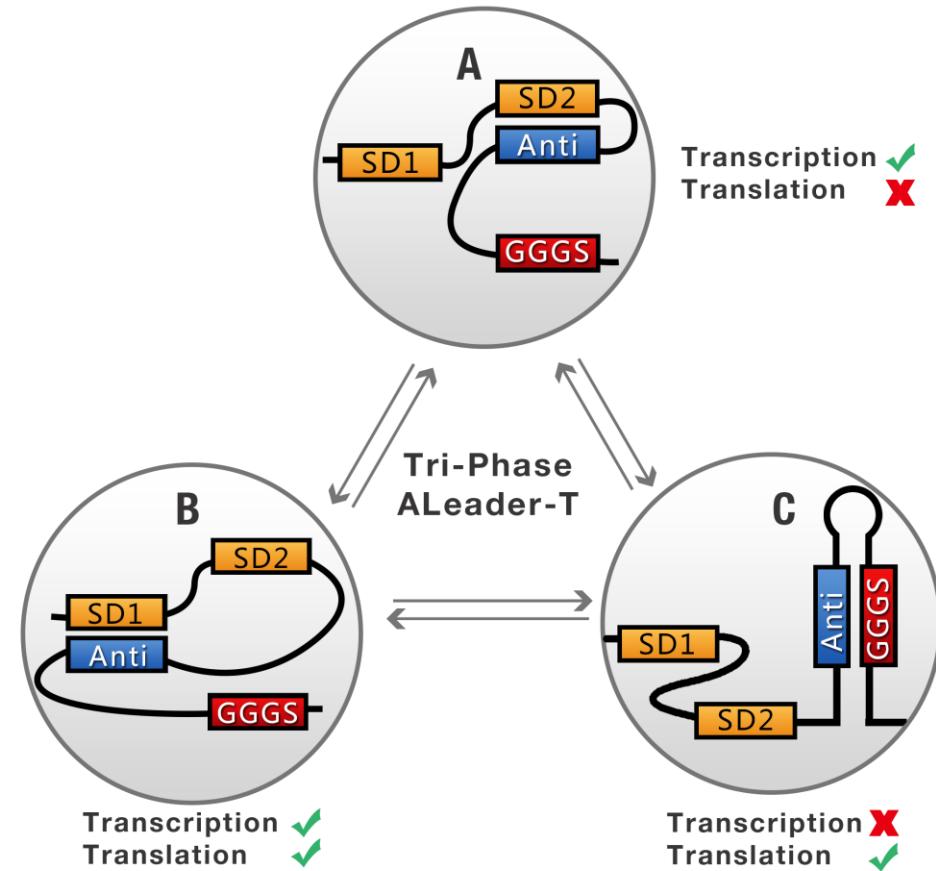
Triphase riboswitch design

A

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Three conformations:

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

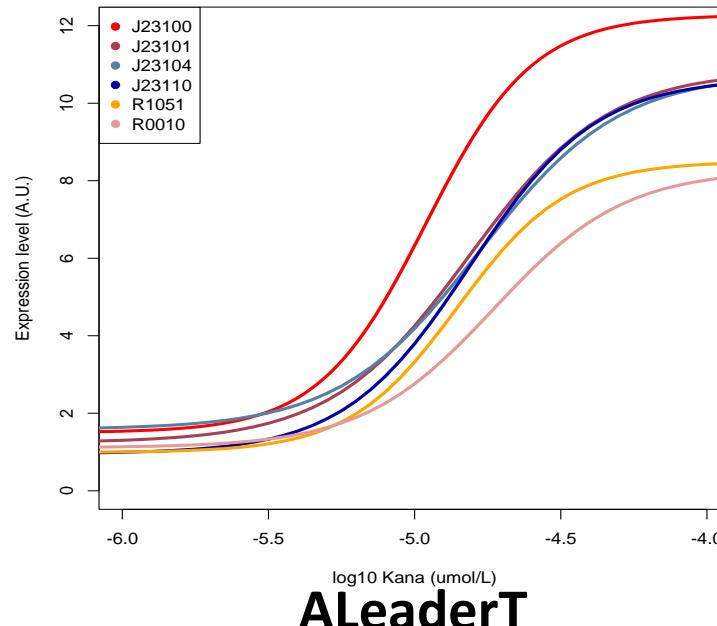
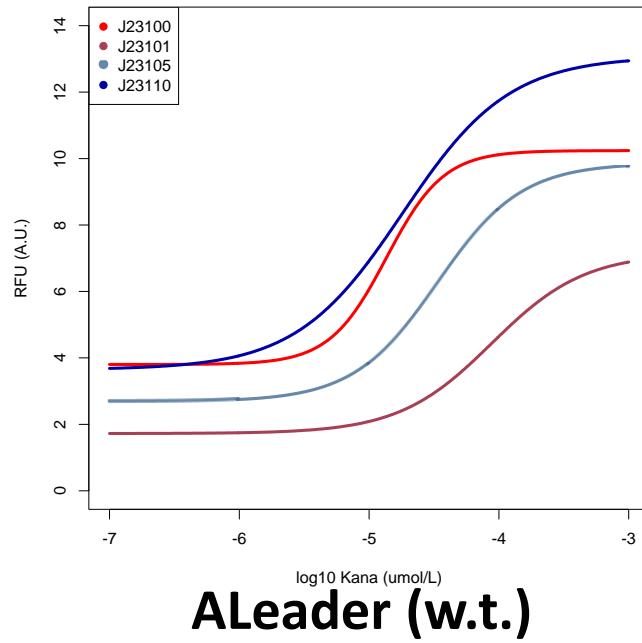


Triphase riboswitch design



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More Standard with Higher Robustness

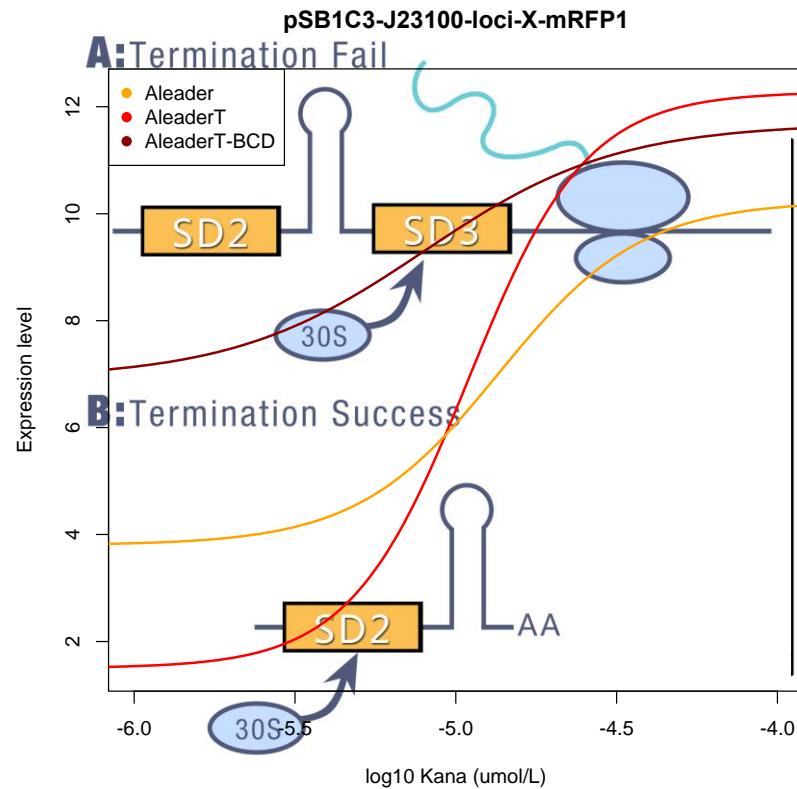
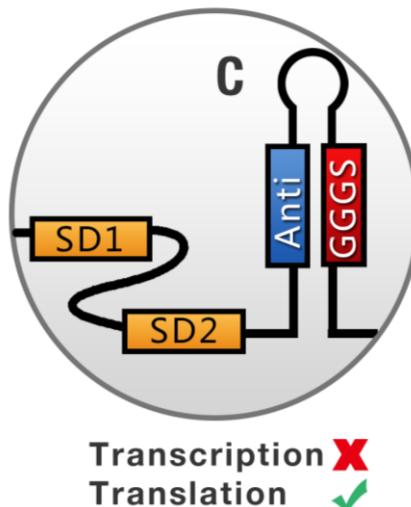


Triphase riboswitch design



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Dual-acting:
termination and translation

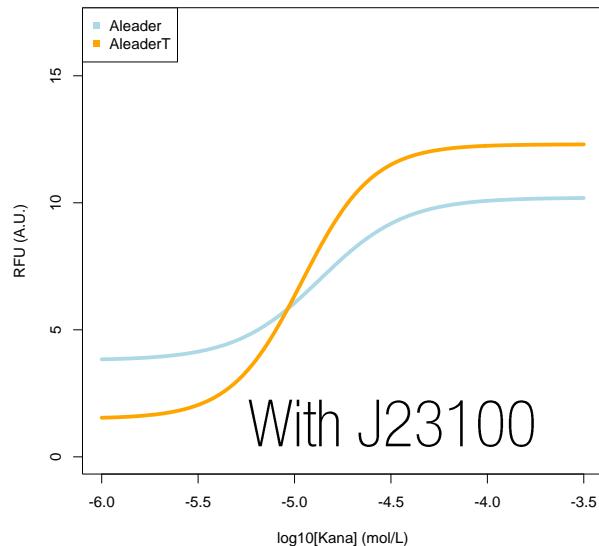


Triphase riboswitch design

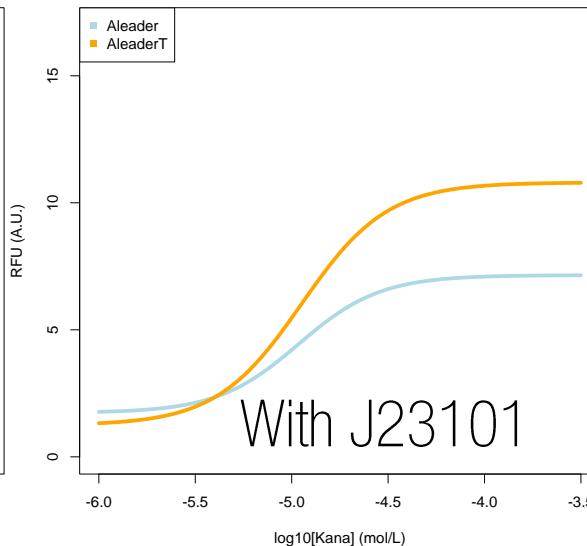


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- The Higher highest level and the Lower basal level
- Lead to the higher dynamic range



With J23100



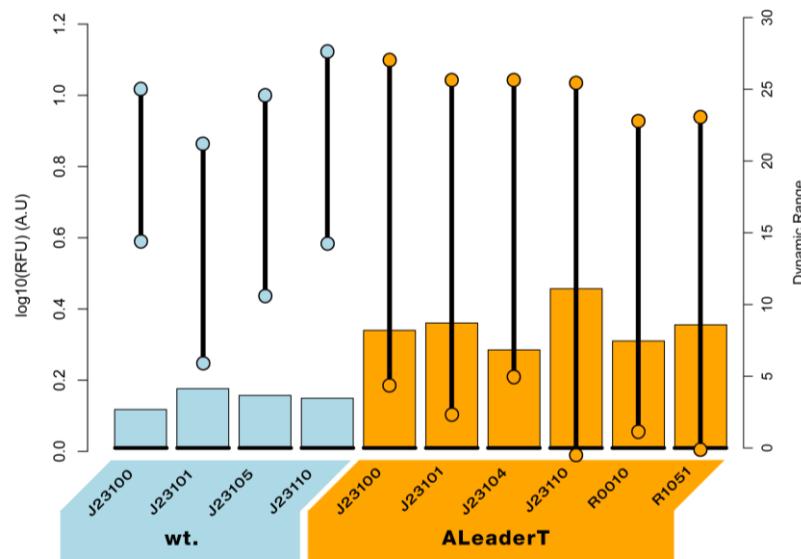
With J23101

Triphase riboswitch design



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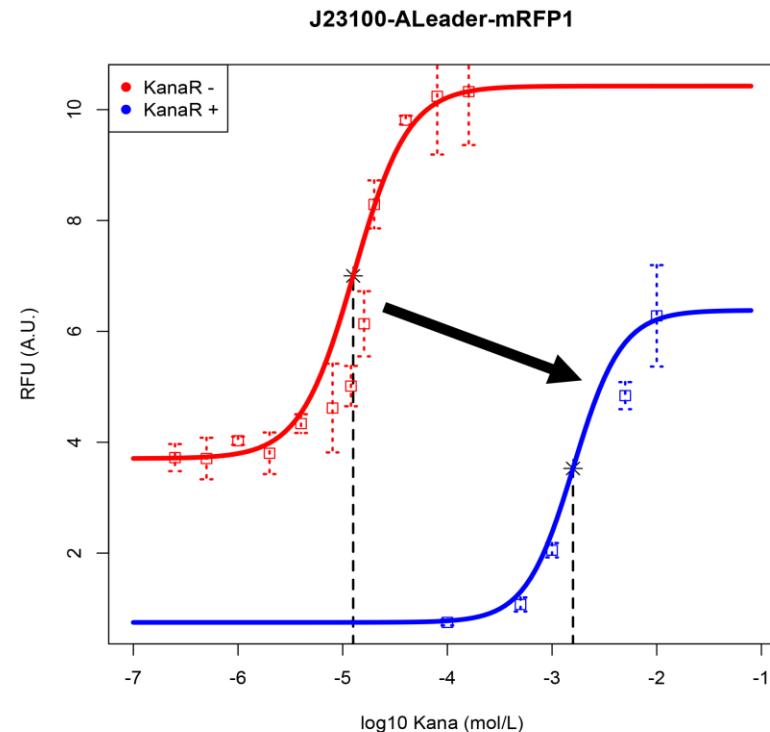
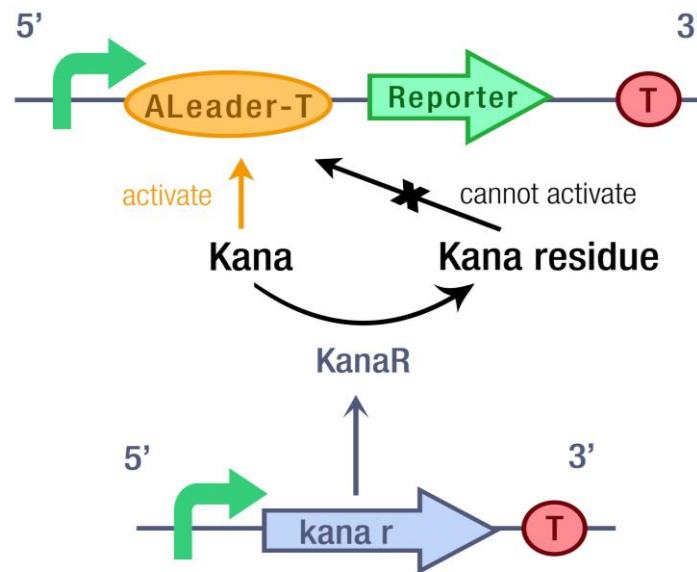
- The Higher highest level and the Lower basal level
- Lead to the higher dynamic range



Environment Transformer



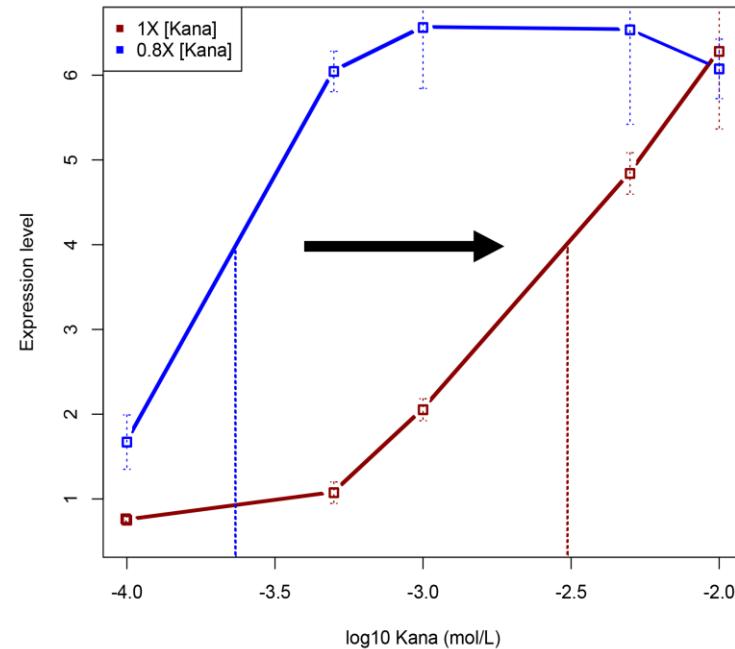
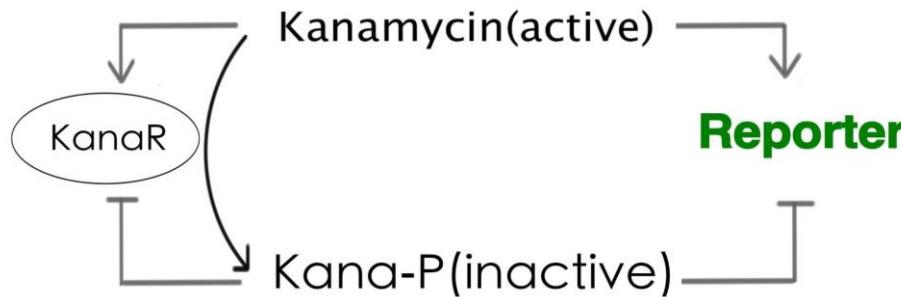
Manage the working range rather than extend it



Environment Transformer



- Auto-regulated Transformer



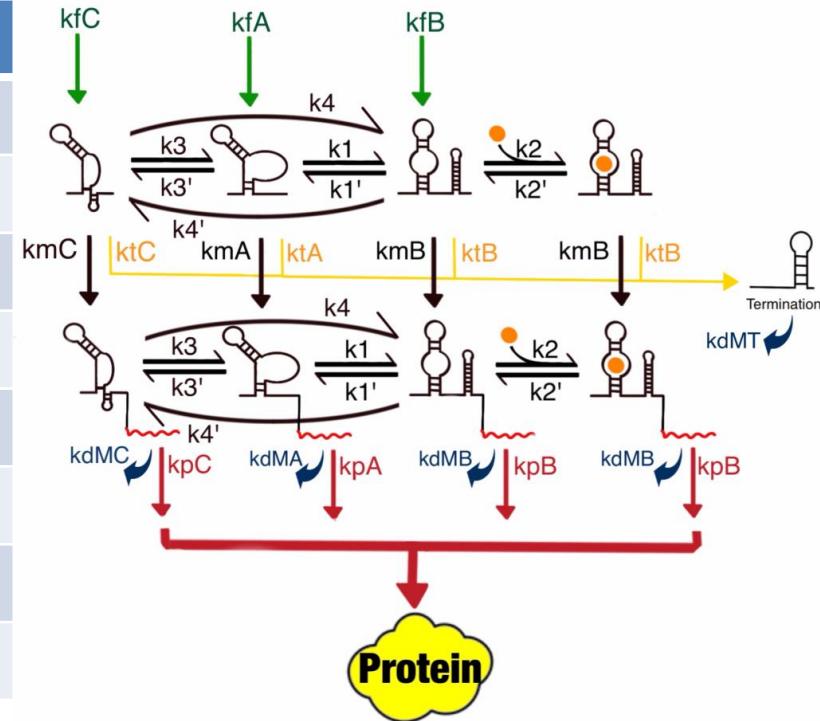
Modeling



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A description of the Triphase

Processes	Unit	Range(log 10)
mRNA initiating	M/s	-13 to -8
Conformation switching	1/s	-3 to 3
Binding Ligand	1/M·s	3 to 8
Releasing Ligand	1/s	-3 to 3
Extension frequency	1/s	-3 to -1
mRNA degradation	1/s	-5 to -2
Translation	1/s	-4 to 1
Protein degradation	1/s	-5 to -2

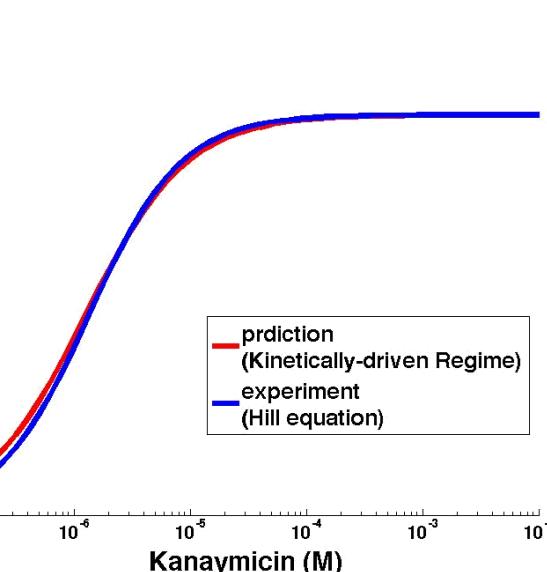
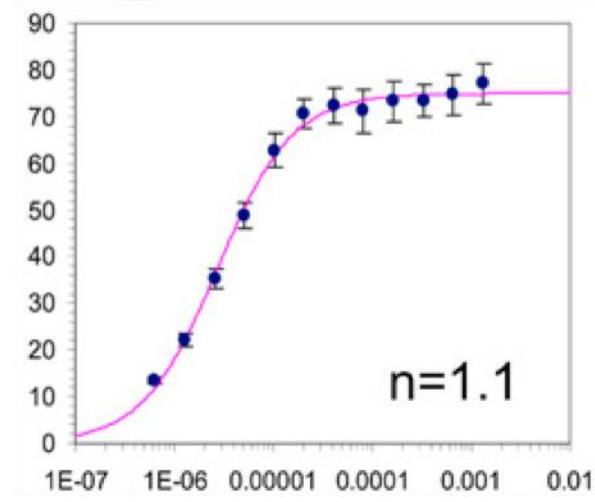


Modeling

The modeling of ALeader fits the SPR signal test

Conformati on	ΔG°
B (Binding)	-7.8
A (Free)	-4.3

Calculated by RNA fold



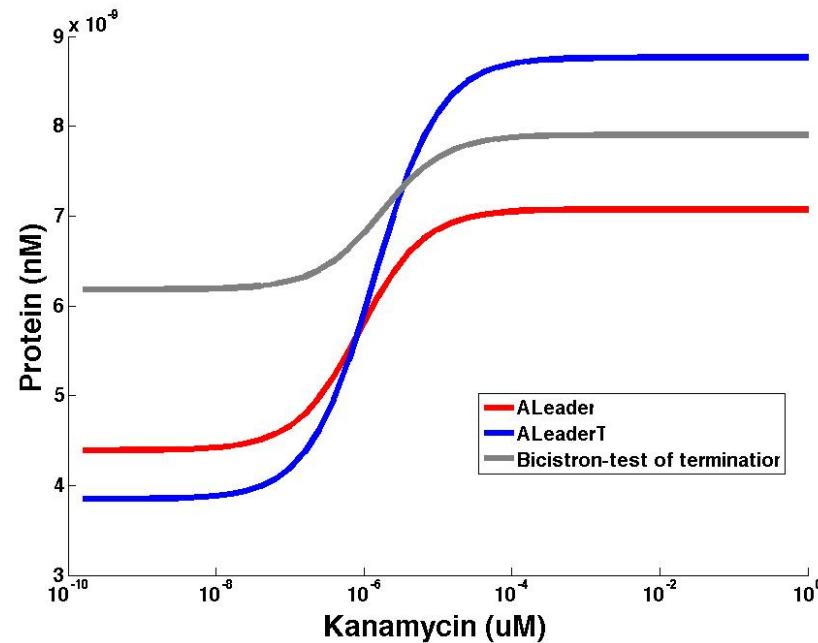
Modeling

Modeling of the ALeaderT shows the similar regulatory pattern

Our prediction:

Low switching rate

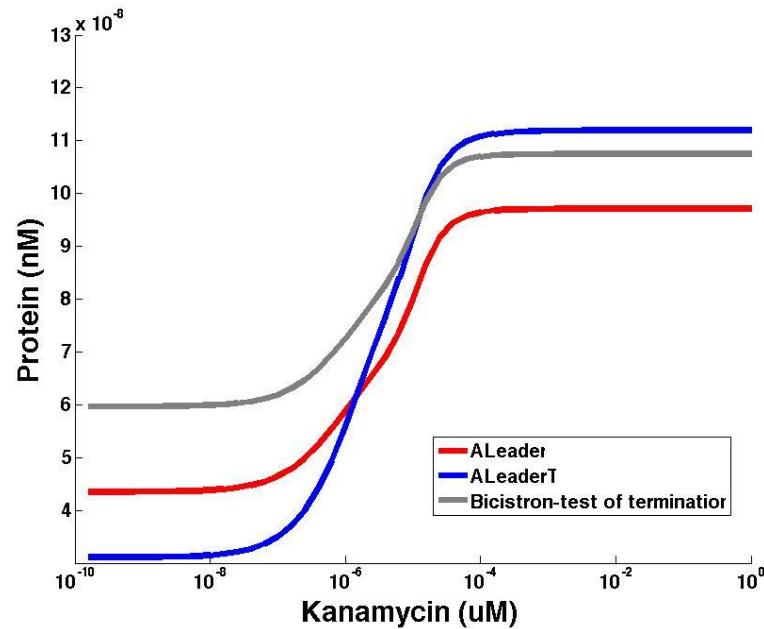
Ribosome impact on SD1?



Modeling

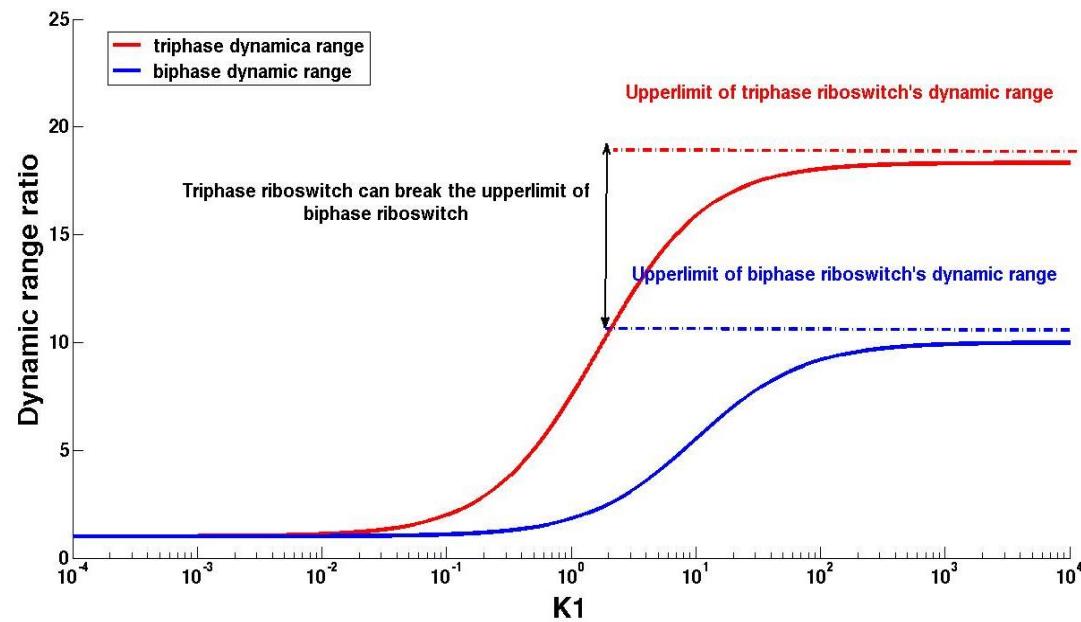
Modeling including the ribosomes is more accurate

(High/Basal)	Exp.	model
ALeader	10.2/3.8	9.7/4.3
ALeaderT	12.3/1.5	11.2/3.1
BCD	11.7/6.9	10.7/6.0



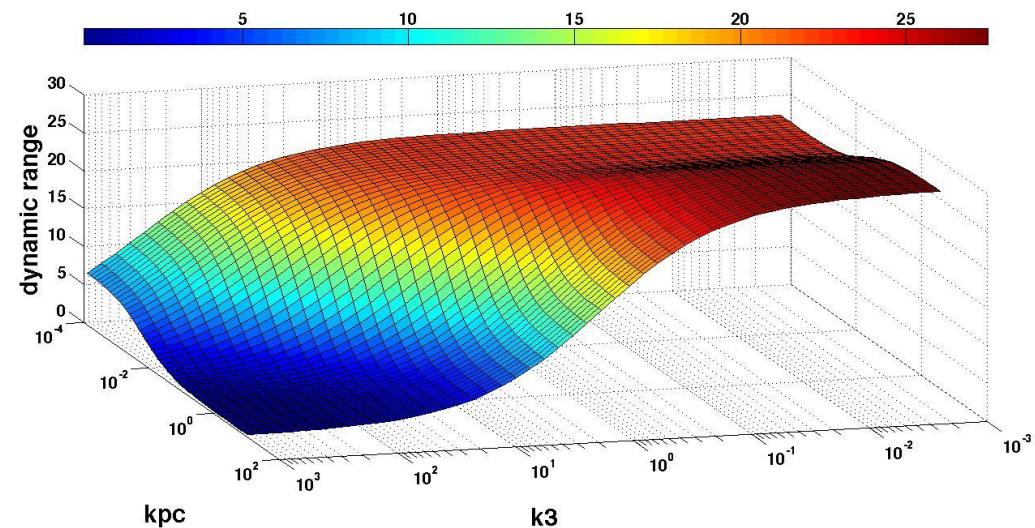
The General Triphase Riboswitch Theory

Break through
the upper limit



The General Triphase Riboswitch Theory

Effective Tuning &
Safer Tuning



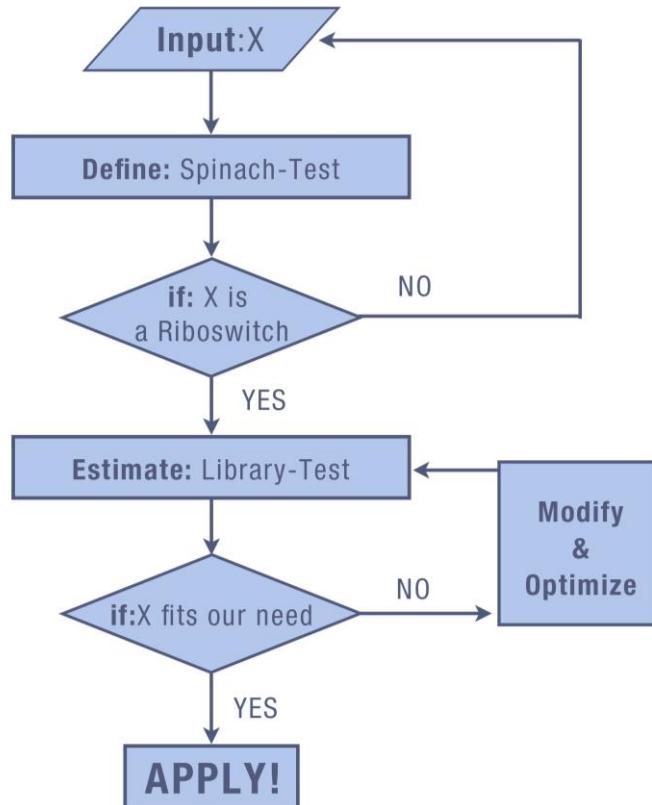
New Standard



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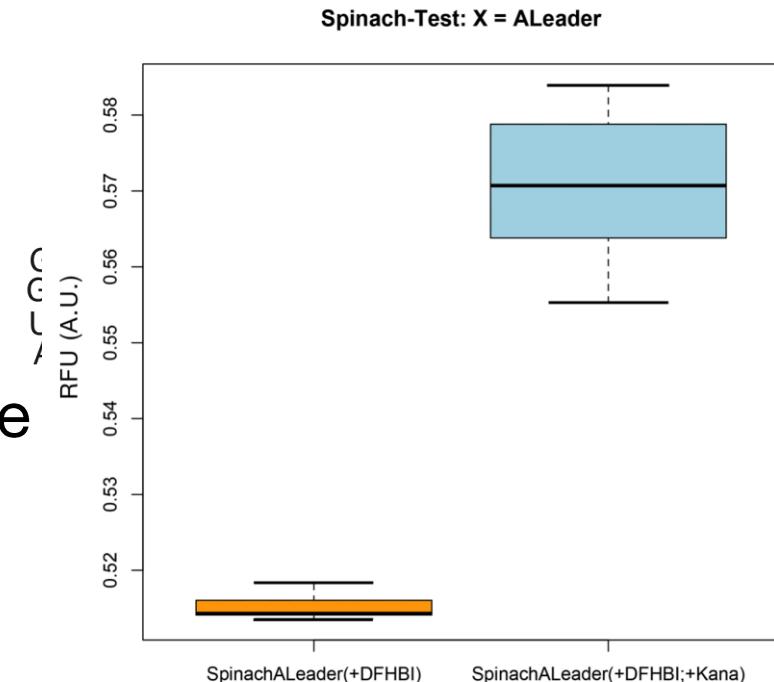
A Five-step General Protocol For Riboswitches Engineering

1. Input the interest sequence
2. Define the conformation switching
3. Estimate the dose-response curve
4. Modify and optimize the performance
5. Apply it in your projects!



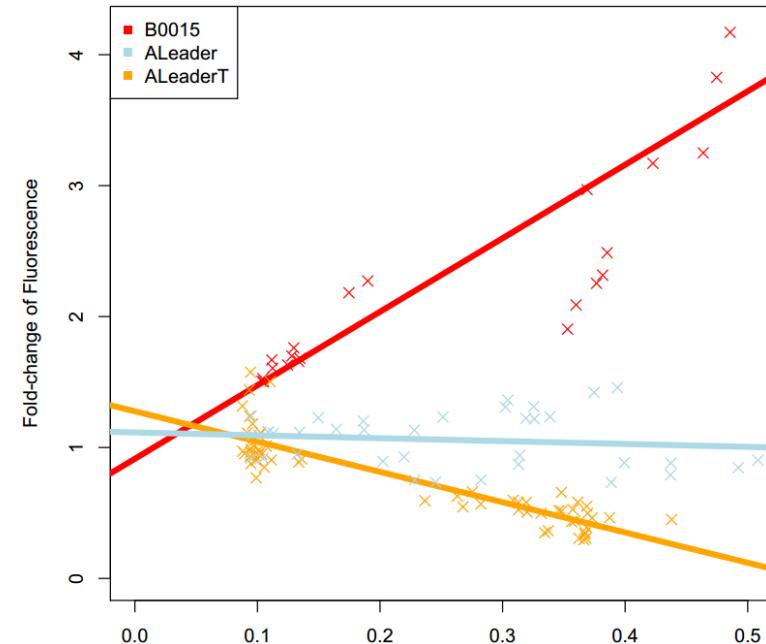
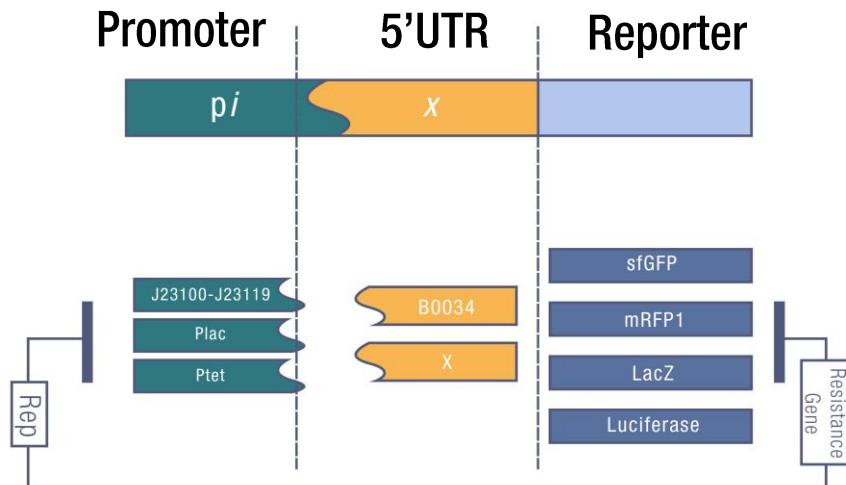
Spinach-Test

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



Quantitative estimation

based on promoter library



Modify and Optimize

- **Methods:**
 - Adding competitive sequence
 - ➔ Triphase riboswitch design (for dynamic range)
 - Expressing an enzyme
 - ➔ Environ-Transformer (for working range)
 - And some proper site-directed mutagenesis

Advices for Users

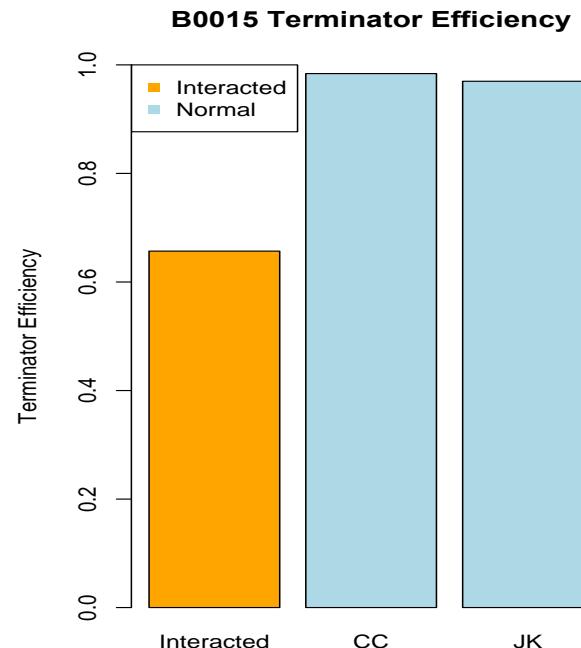
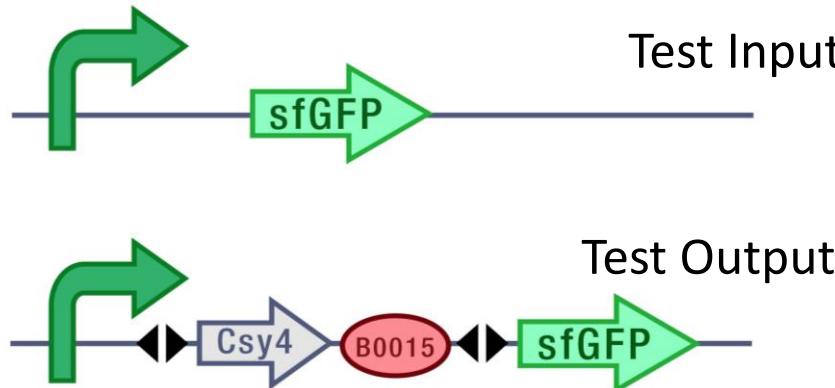


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To Users:

Be careful of RNA interaction

Interfered terminator

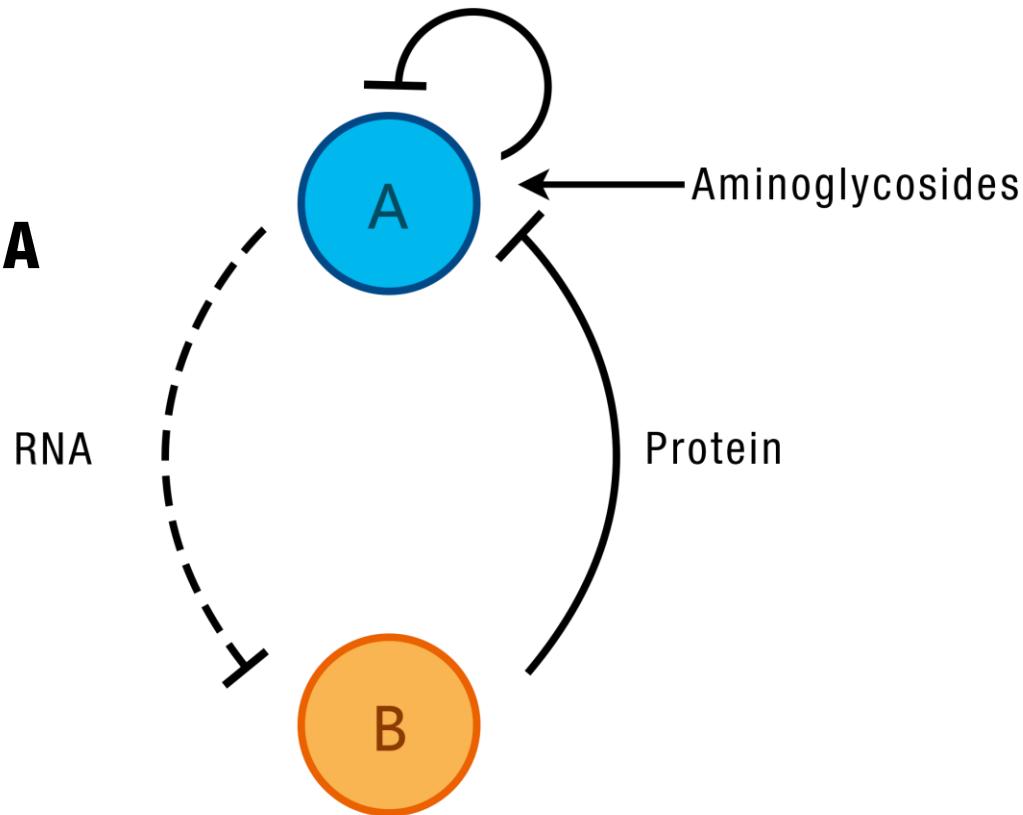


Advices for Users



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**The different behaviors of RNA
and protein regulators in the
networks**



Human Practice



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Human Practice

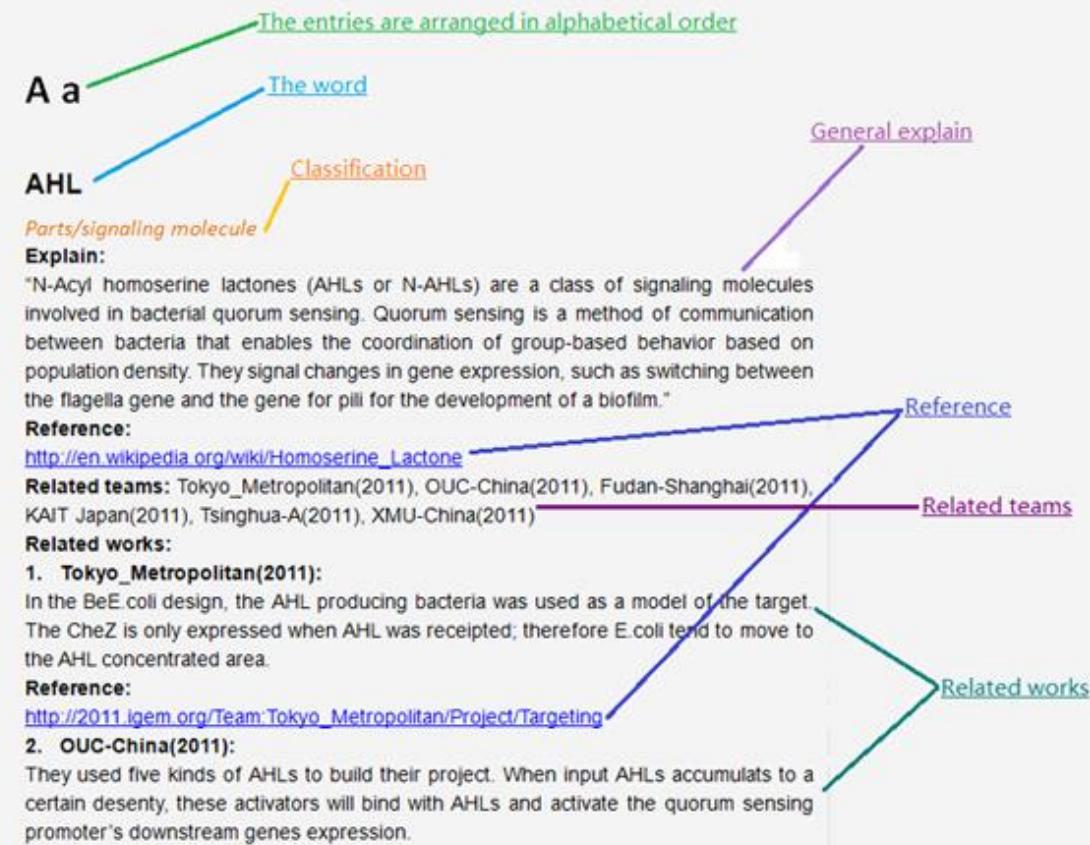


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iGEMcyclopedia 2.0

For fresh iGEMers

For better communication



Human Practice



Activities at Fudan and Communications with other teams



With association of Bertalanffy



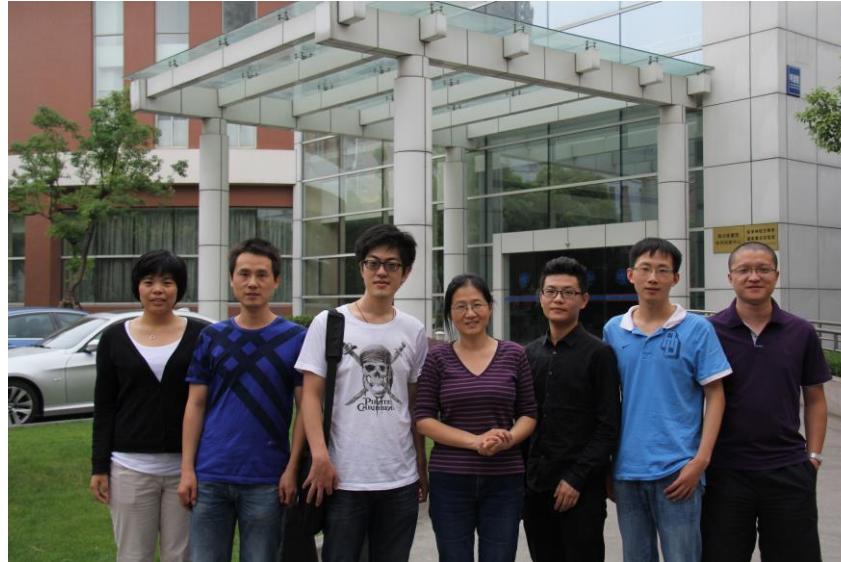
With iGEM2013_USTC CHINA

Human Practice



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



Sponsor and Acknowledgement

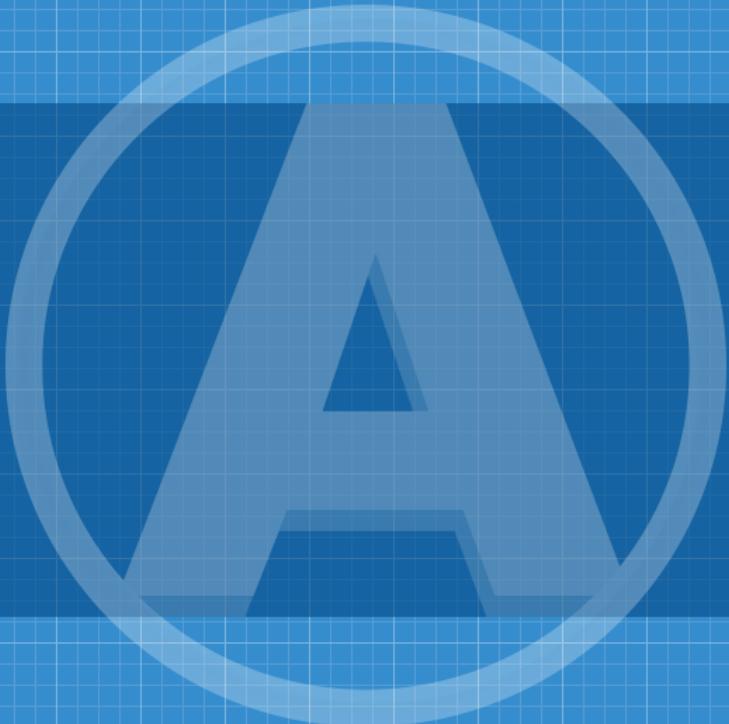


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- School of life science, Fudan University
- Prof. Zhao Guoping, School of Life Science, Fudan University
- Prof. Dongrong Chen and Prof. Alastair I.H. Murchie's Lab, IBS Fudan University
- Prof. Lu Daru, School of Life Science, Fudan University
-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



ODE Model



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$$\frac{dA}{dt} = k_{fA} - (k_1 + k_3 + k_m)A + k_1'B + k_3'C$$

$$\frac{dB}{dt} = k_{fB} + k_1A - (k_1' + k_4' + k_2L + k_m)B + k_2'B_L + k_4C$$

$$\frac{dB_L}{dt} = k_2LB - (k_2' + k_m)B_L$$

$$\frac{dC}{dt} = k_{fC} + k_3A + k_4'B - (k_3' + k_4 + k_m)C$$

$$\frac{dA_m}{dt} = k_{MA}A - (k_1 + k_3 + k_{dMA})A_m + k_1'B_m + k_3'C_m$$

$$\frac{dB_m}{dt} = k_{MB}B + k_1A_m - (k_1' + k_4' + k_2L + k_{dMB})B_m + k_2'B_{Lm} + k_4C_m$$

$$\frac{dB_{Lm}}{dt} = k_{MB}B_L + k_2LB_m - (k_2' + k_{dMB})B_{Lm}$$

$$\frac{dC_m}{dt} = k_{MC}C + k_3A_m + k_4'B_m - (k_3' + k_4 + k_{dMC})C_m$$

$$\frac{dT}{dt} = k_{tA}A + k_{tB}(B + B_L) + k_{tC}C - k_{dT}T$$

$$\frac{dP}{dt} = k_{pA}A_m + k_{pB}(B_m + B_{Lm}) + k_{pC}C_m - k_{dp}P$$

Conformation	ΔG°
B (Natural Binding)	-7.8
A (Natural Free)	-4.3
C (Artificial Free)	-11.80

Partitioning Parameter	No ribosome Influence
K1	1.5234
K3	0.4309
K4	0.6564