De novo automated design of small RNA circuits for engineering synthetic riboregulation in living cells

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Presented by

Ryan Keating and Kristine Kim

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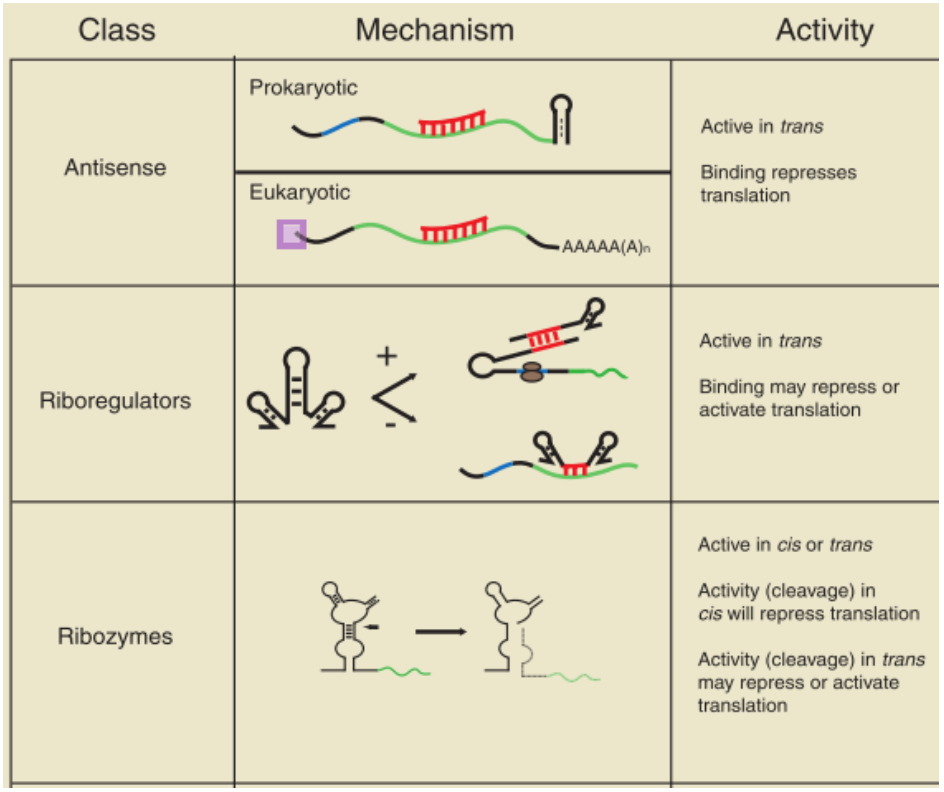
Regulatory RNA interactions



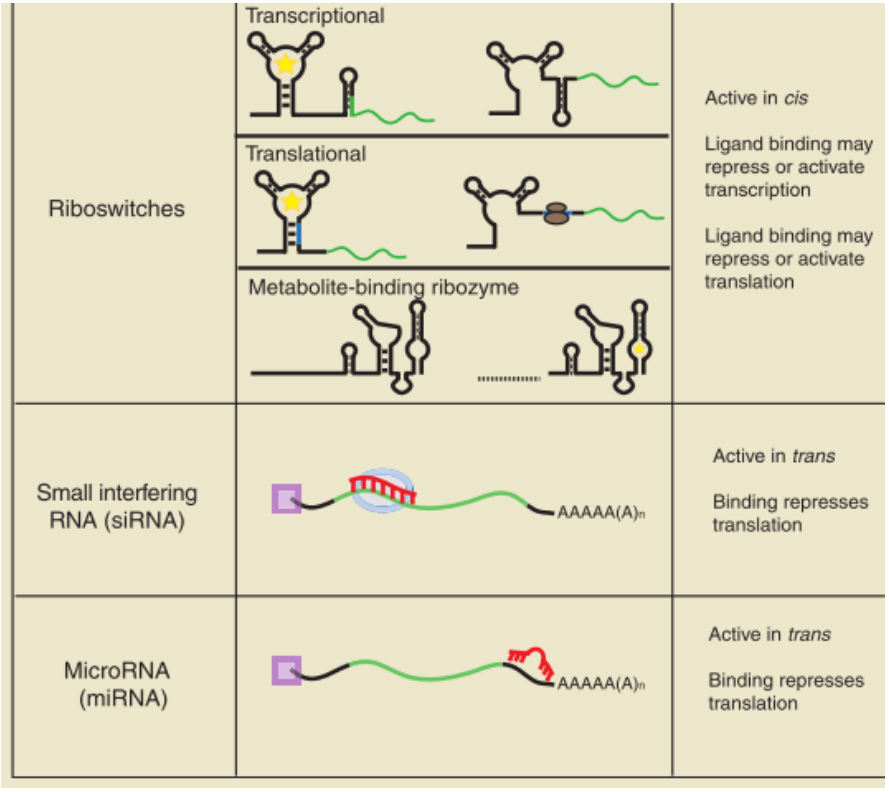


Fig. 1

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RNA's ability to form complex structures that can interact with other RNA mol ecules, proteins, and small molecules enable sophisticated behavior.

Synthetic riboregulator is able to *trans*-activate the translation of a *cis*-repressed gene

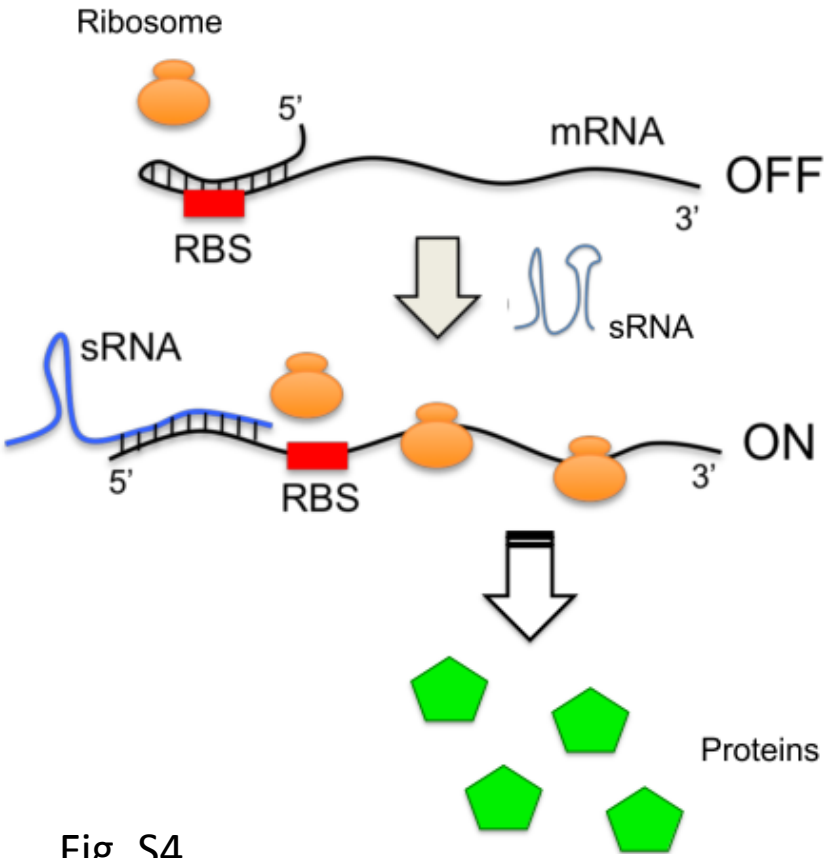


Fig. S4

Thermodynamic scheme of sRNA-mRNA int eraction

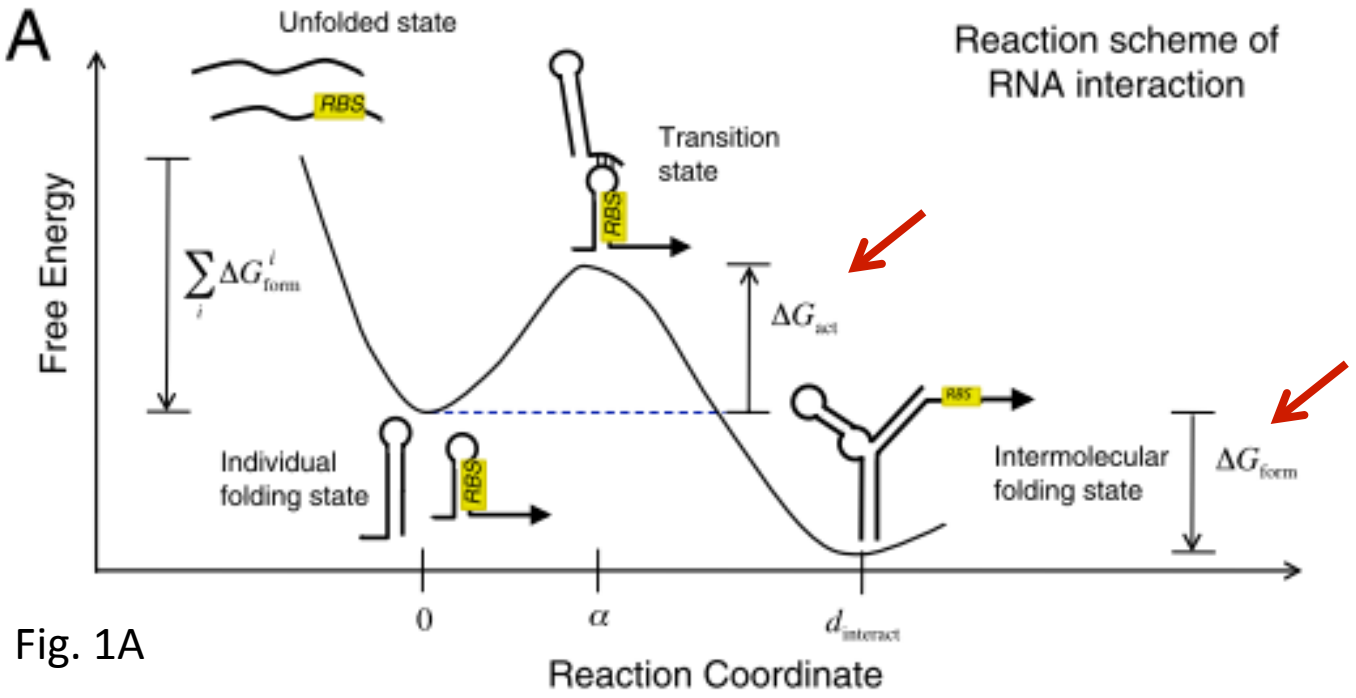


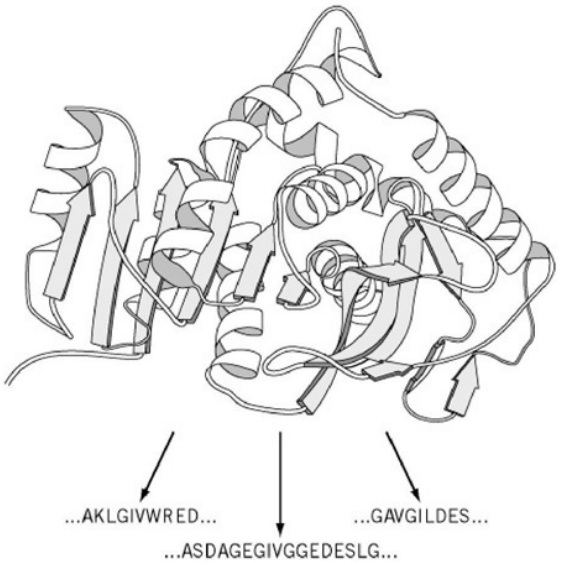
Fig. 1A

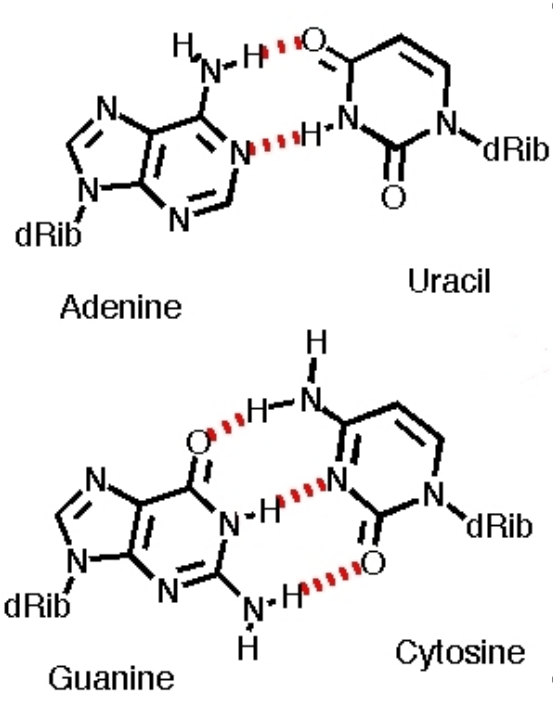
General concepts in their computational approach

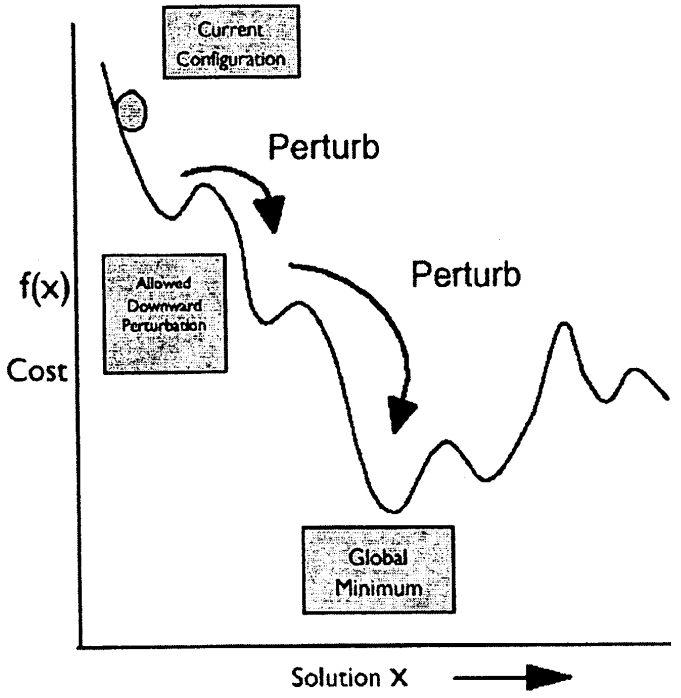
Inverse folding problem

Watson-Crick Base pairing

Monte Carlo Simulated annealing







http://what-when-how.com/molecular-biology/inverse-folding-problem-molecular-biology/

http://www.boc.uu.se/boc14www/res_proj/modulations.html

http://ashakhov.wordpress.com/2011/01/27/simulated-annealing/

Computational design of sRNA circuit

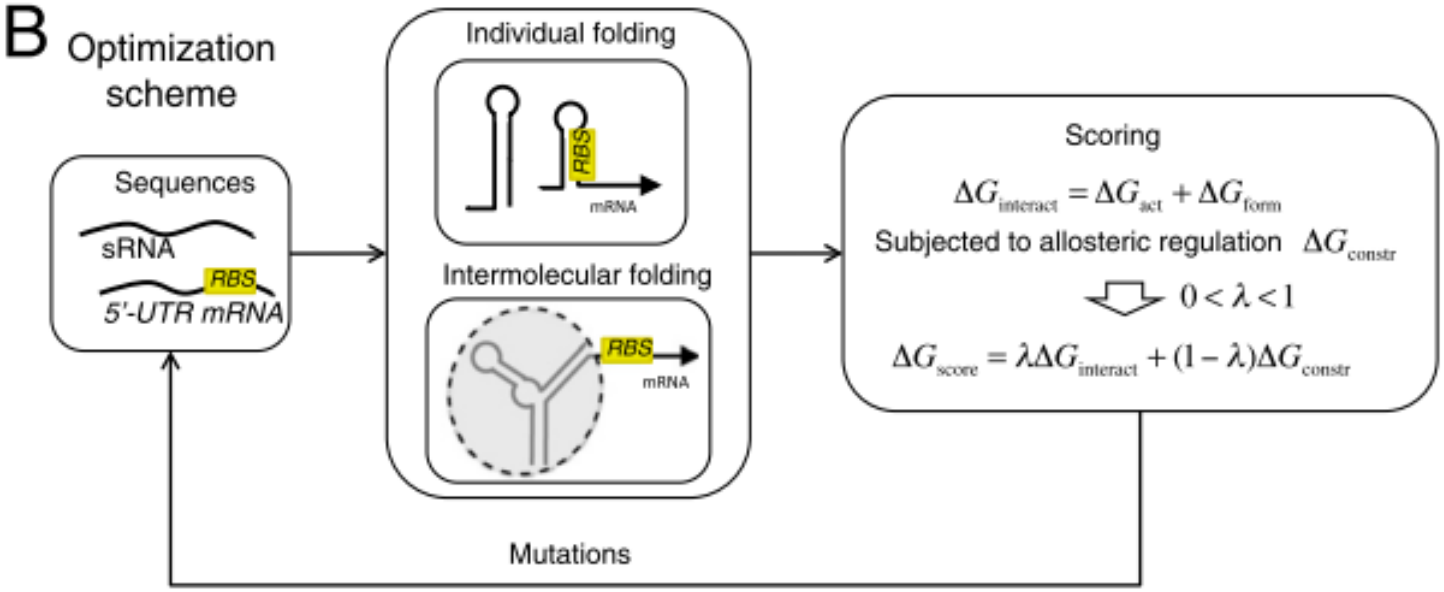


Fig. 1B

1. Choose well-defined structures of single species

2. Algorithm assigns random nucleotides to the sequences of each RNA species

3. Objective function based on MCSA minimizes free energy of complex formation ΔG_{form} and activation energy ΔG_{act}

Engineered RNA devices

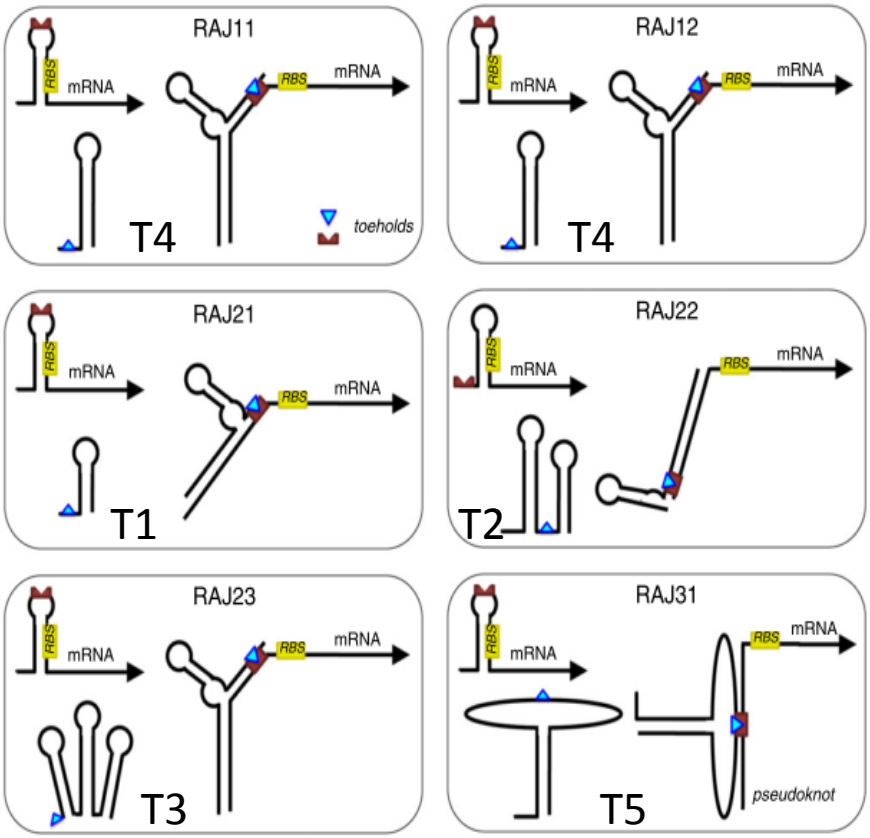


Fig. 2

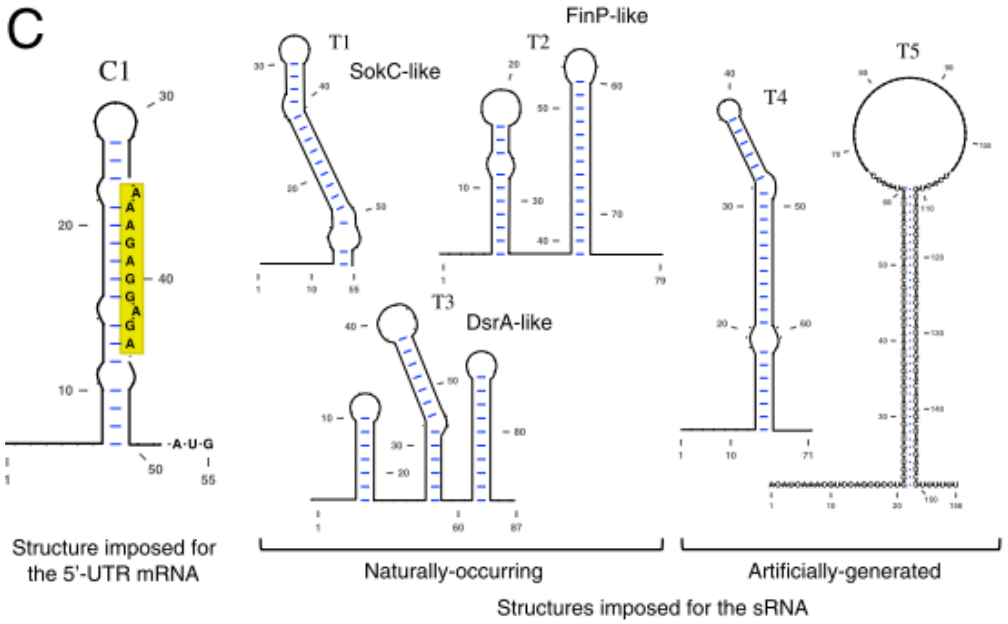


Fig. 1C

- Secondary structures are s pecified for the single RNA species.
- RBS nucleotides sequence i s maintained fixed

A closer look at device RAJ11

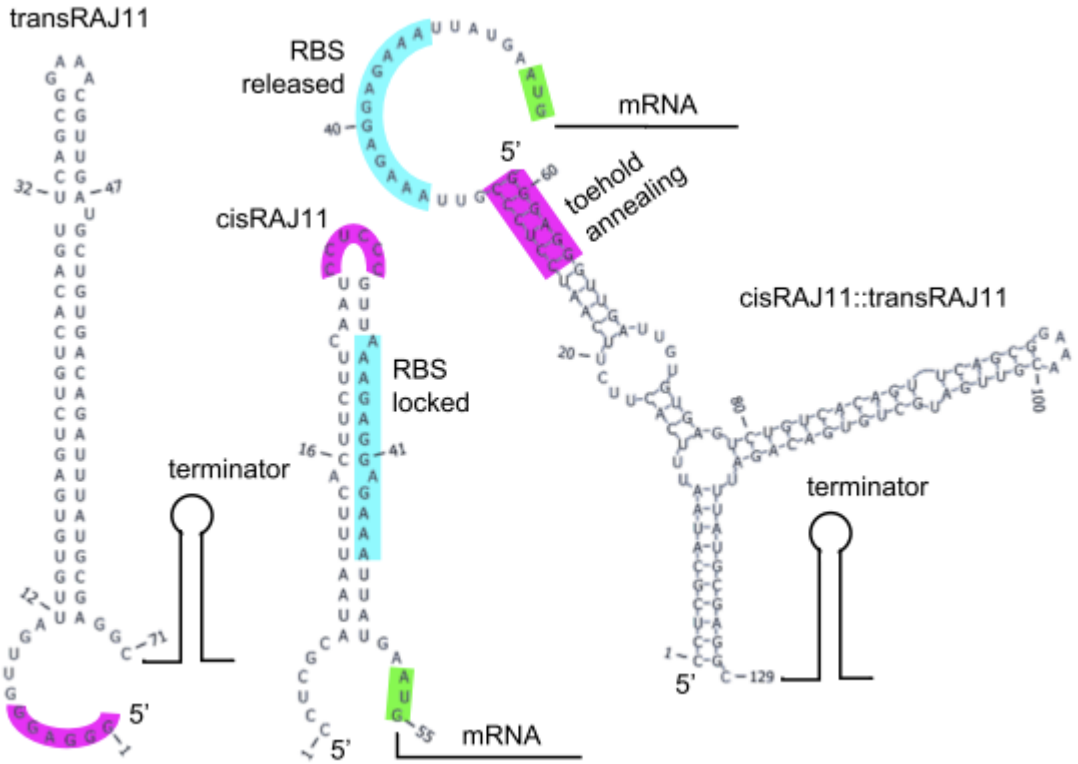


Fig. S6 Conformational change induced by the riboregulator releases the RBS to activate translation

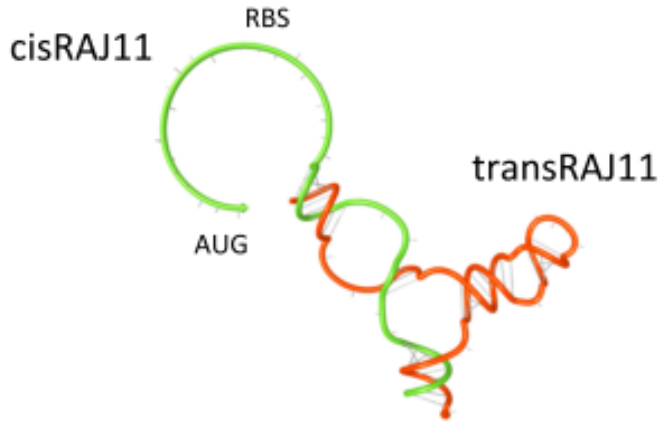


Fig. S7 3D helical structure of RAJ11

The making of a device

- Both cis and trans elements were expressed in a single pl asmid, but in opposite direc tions
- Each device was added to th e plasmid using restriction e nzymes
- TetO and LacO promoters, in duced by aTc and IPTG

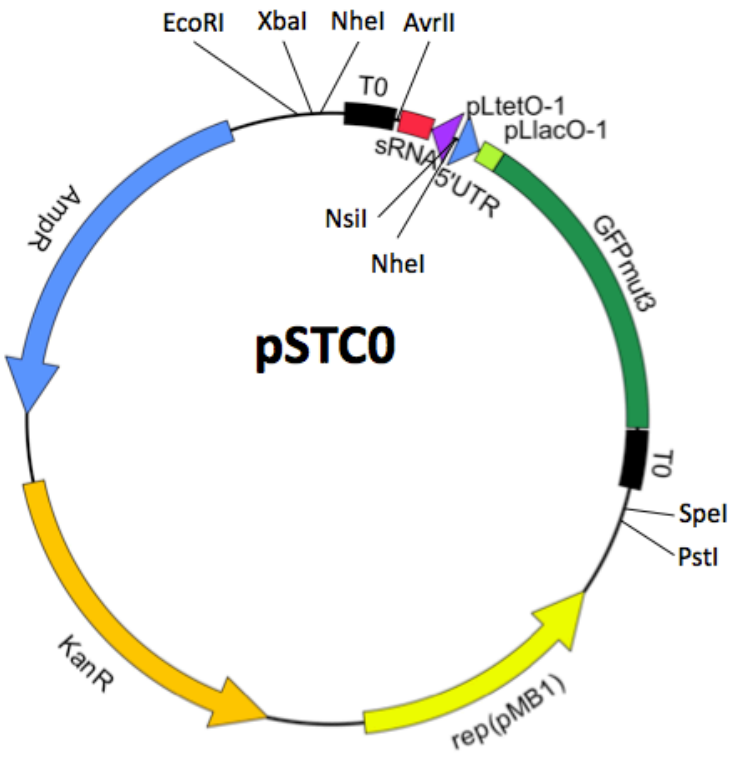


Figure S2.

Cis repression of the RBS is very effective.

- In the cis repressed form, the RBS site is blocked
- Repressed to 1-4% of maximal output!

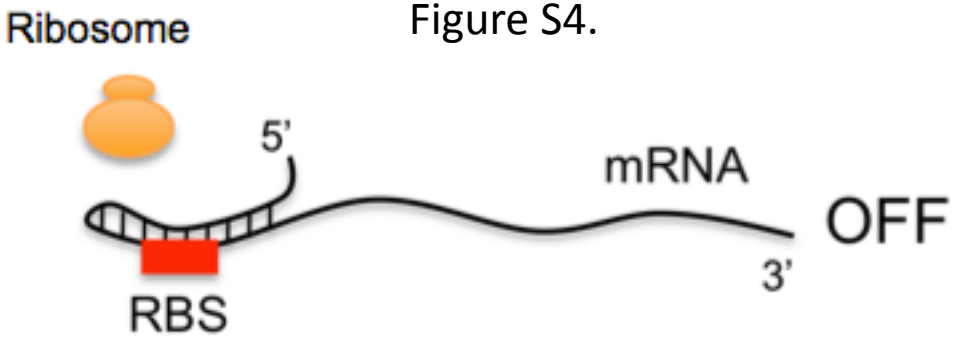


Figure S4.

5'-UTR mRNA
(cis repressing element)

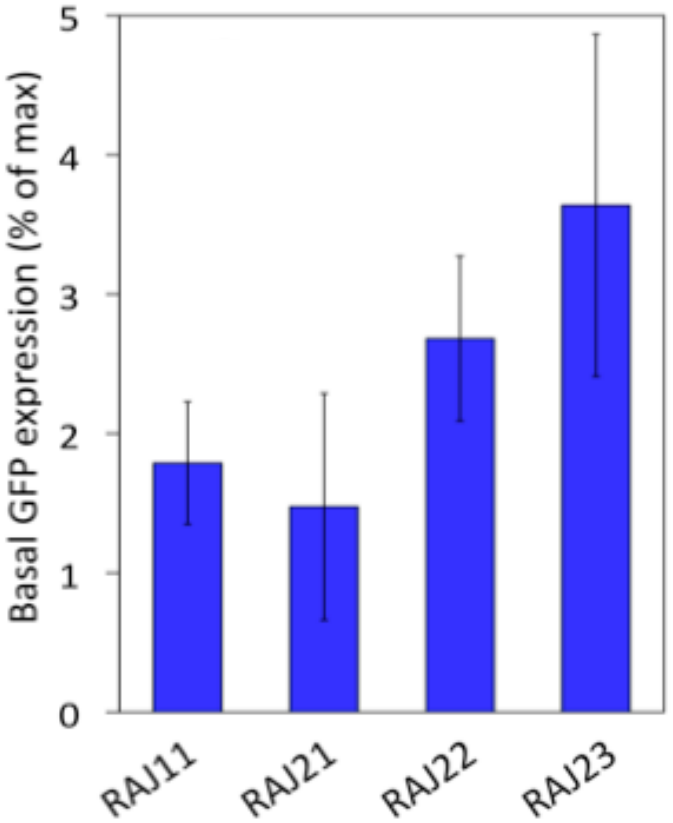


Figure S13.

Measuring device response

- Maximum activation fold of 11.2
- 4/6 devices had activation fold l ower than 5

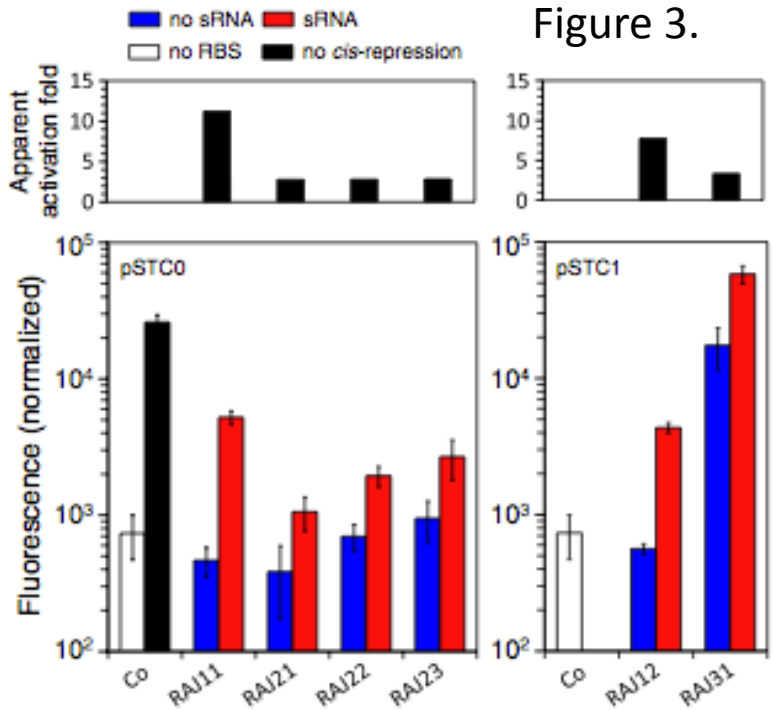
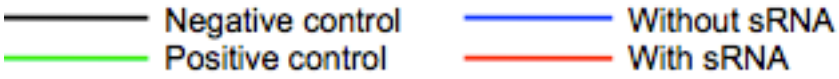


Figure 3.

Measuring device response

- Maximum activation fold of 11.2
- 4/6 devices had activation fold lower than 5



Flow Cytometry Data
Negative control: no RBS
Positive control: open RBS

Figure 3.

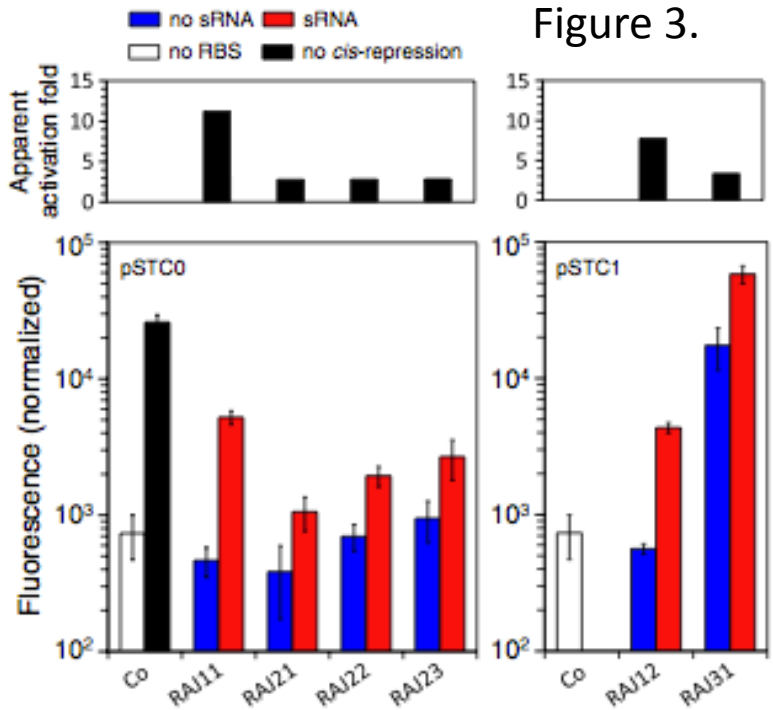
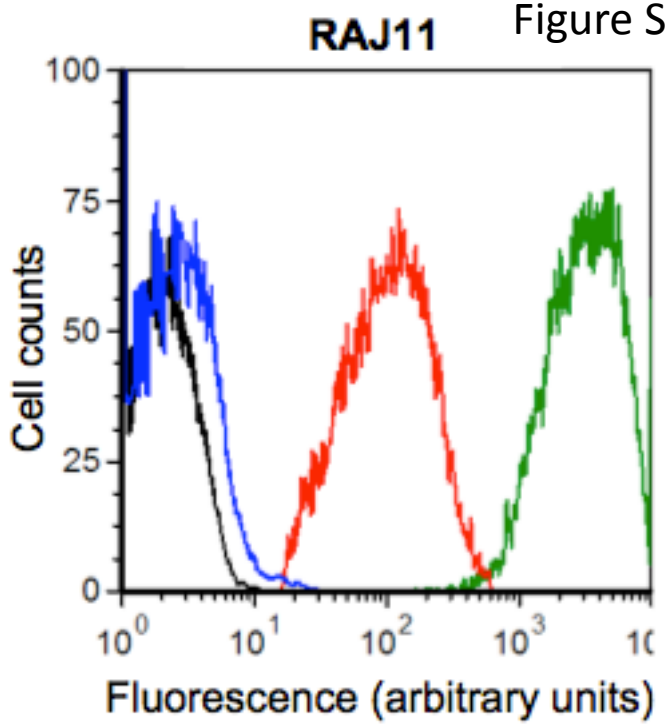


Figure S15.



Measuring device response

- Fluorometry and flow cytometry were used to measure GFP fluorescence independently

- Results varied by scale, but both methods increased together with high correlation

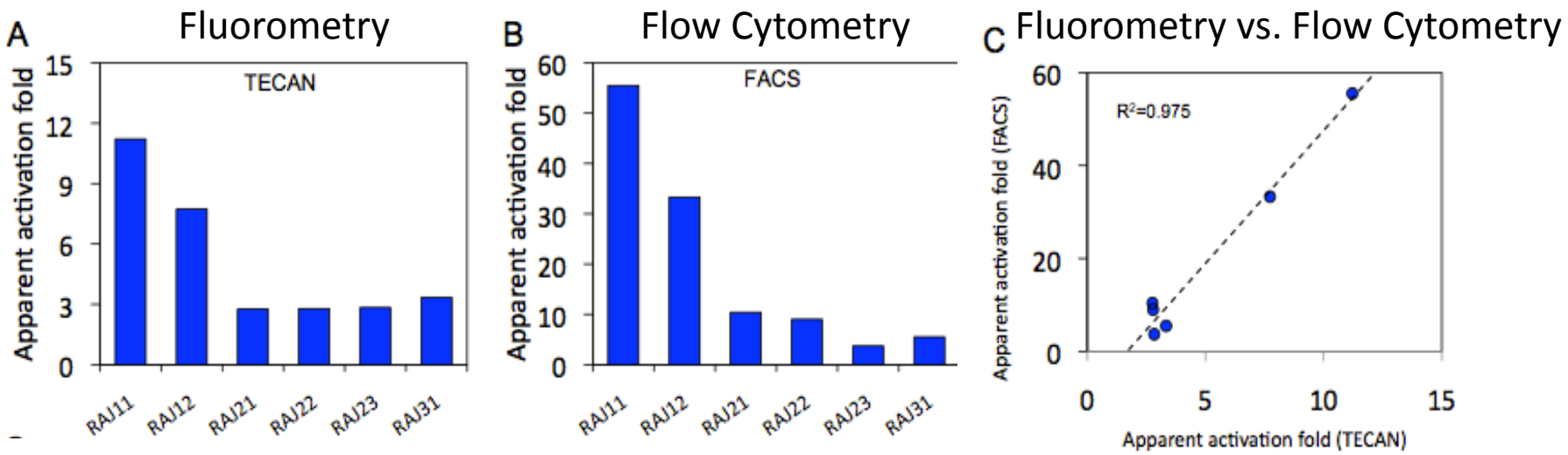


Figure S12.

The six devices were orthogonal *in vivo*.

- Computational prediction of orthogonality at 1 µM and 100 µM initial conc

Figure 4B.

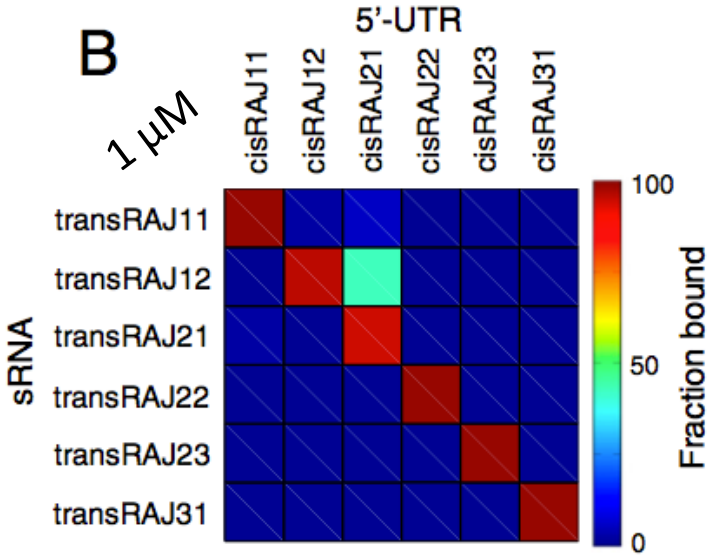
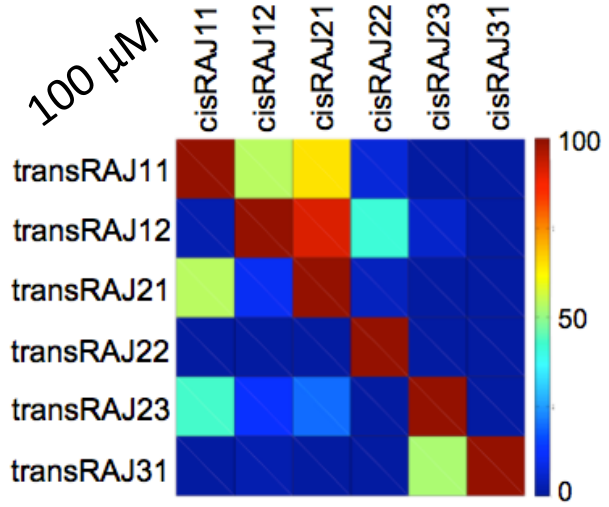


Figure S11.



The six devices were orthogonal *in vivo*.

- Computational prediction of orthogonality at 1 µM and 100 µM initial conc
- Experimental validation displayed minimal cross-talk
- If RAJ11 and RAJ21 had the greatest cross-talk, why not validate using those elements?

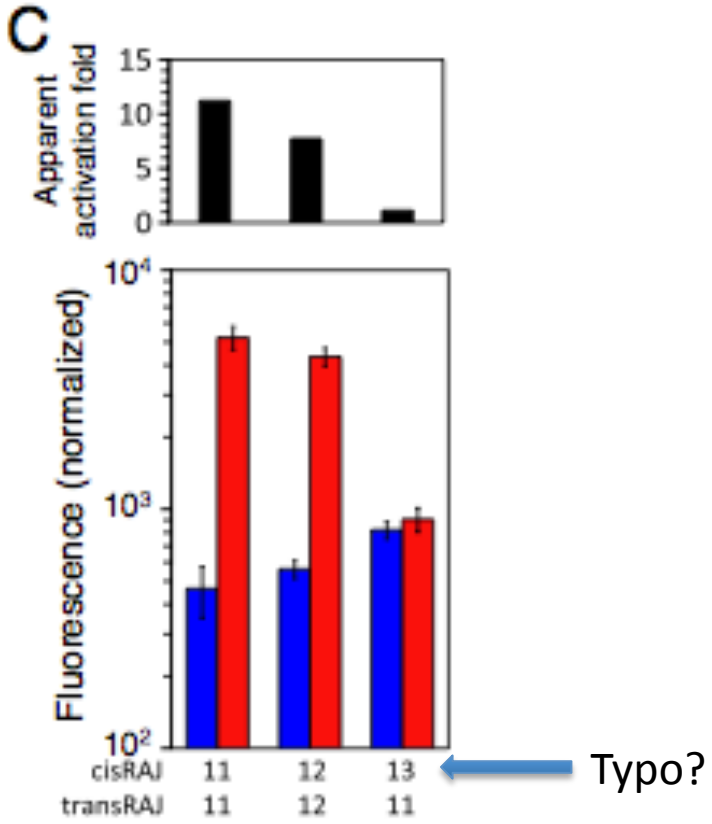
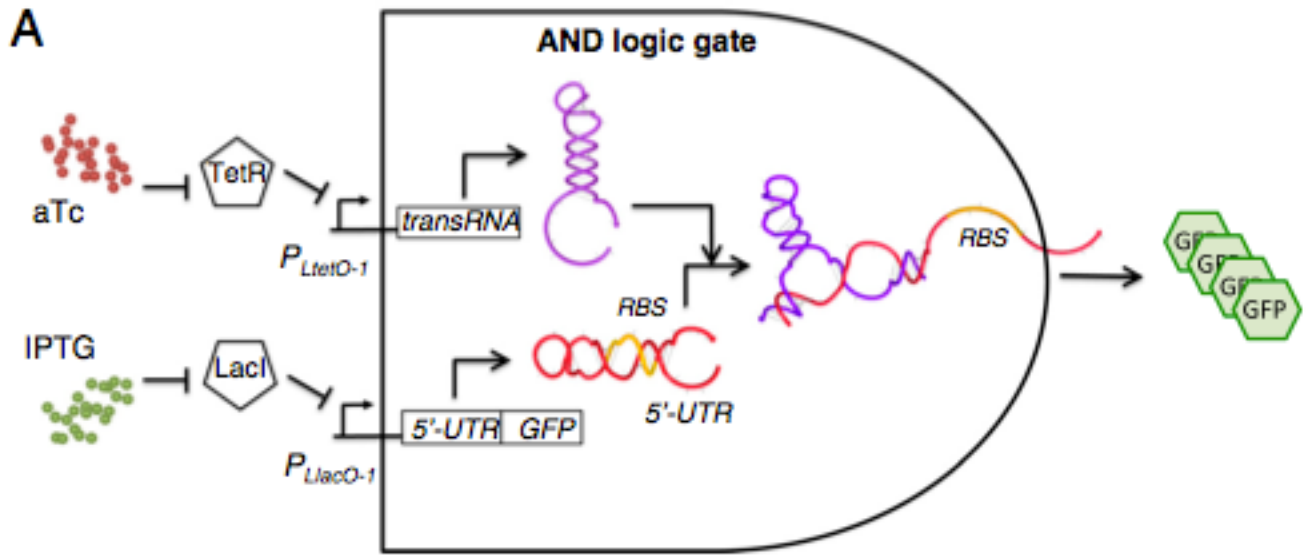


Figure 4C.

RAJ11 device successfully used in an AND gate.

- aTc and IPTG used as system inducers.
  - Sound familiar?
- Gate activation quantified by fluorescence

Figure 5A.



RAJ11 device successfully used in an AND gate.

- RAJ11 exhibits AND gate functionality, as tested by fluorometry
- A computational transfer function was computed based on model equation and experimental results.

Figure 5B.

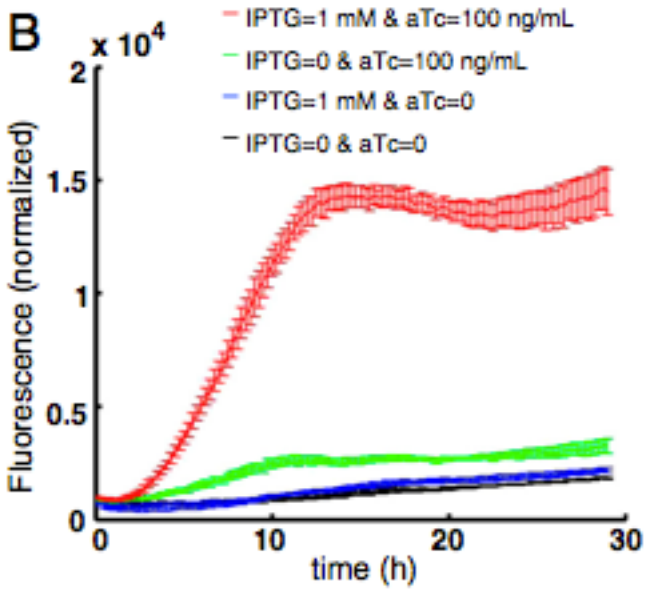
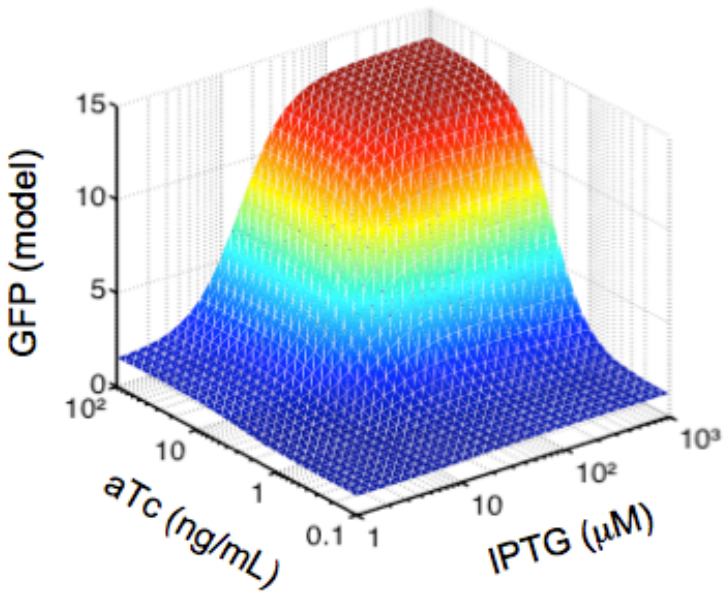


Figure S20.

Computational Transfer Function



Strengths

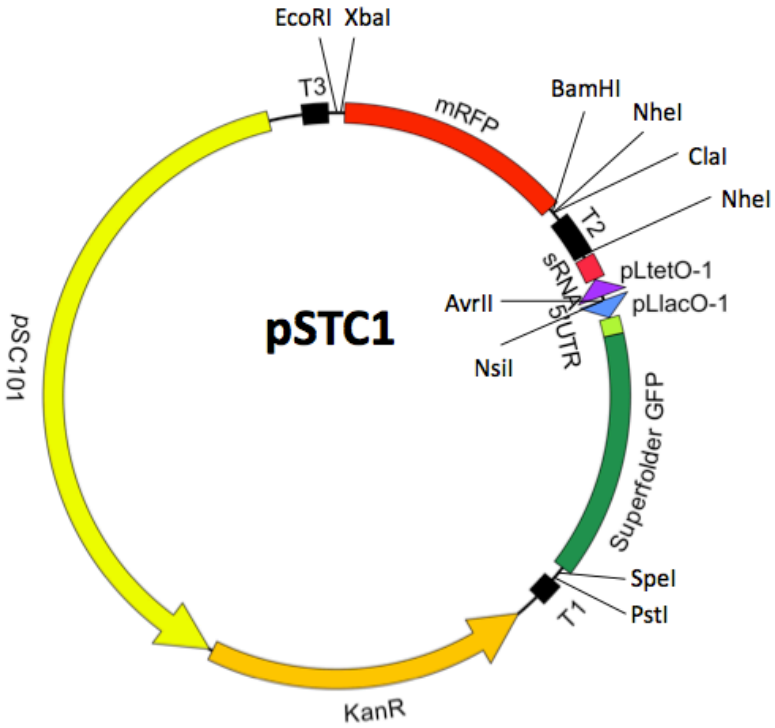
- Engineer devices using a systematic algorithm!
- Work done in context of cell
  - Predetermined structures known to be stable *in vivo*
- Both cis and trans elements expressed on same plasmid
  - sRNA and mRNA transcribed locally
- RNA regulated gates faster than protein regulated gates
- Implement the device on your favorite gene!

Discussion

- Key assumptions
  - Modeling using only base pair interactions, not secondary structure
  - Ignores kinetics of folding process
  - Initial interactions between sRNA and mRNA caused by unpaired nucl eotides only

- Weaknesses
  - AND gate governed by the same two molecules in previous papers (aTc and IPTG)
  - Premature / inefficient transcription may occur
  - Was the algorithm successful?
  - Poor presentation of ideas in paper

Questions?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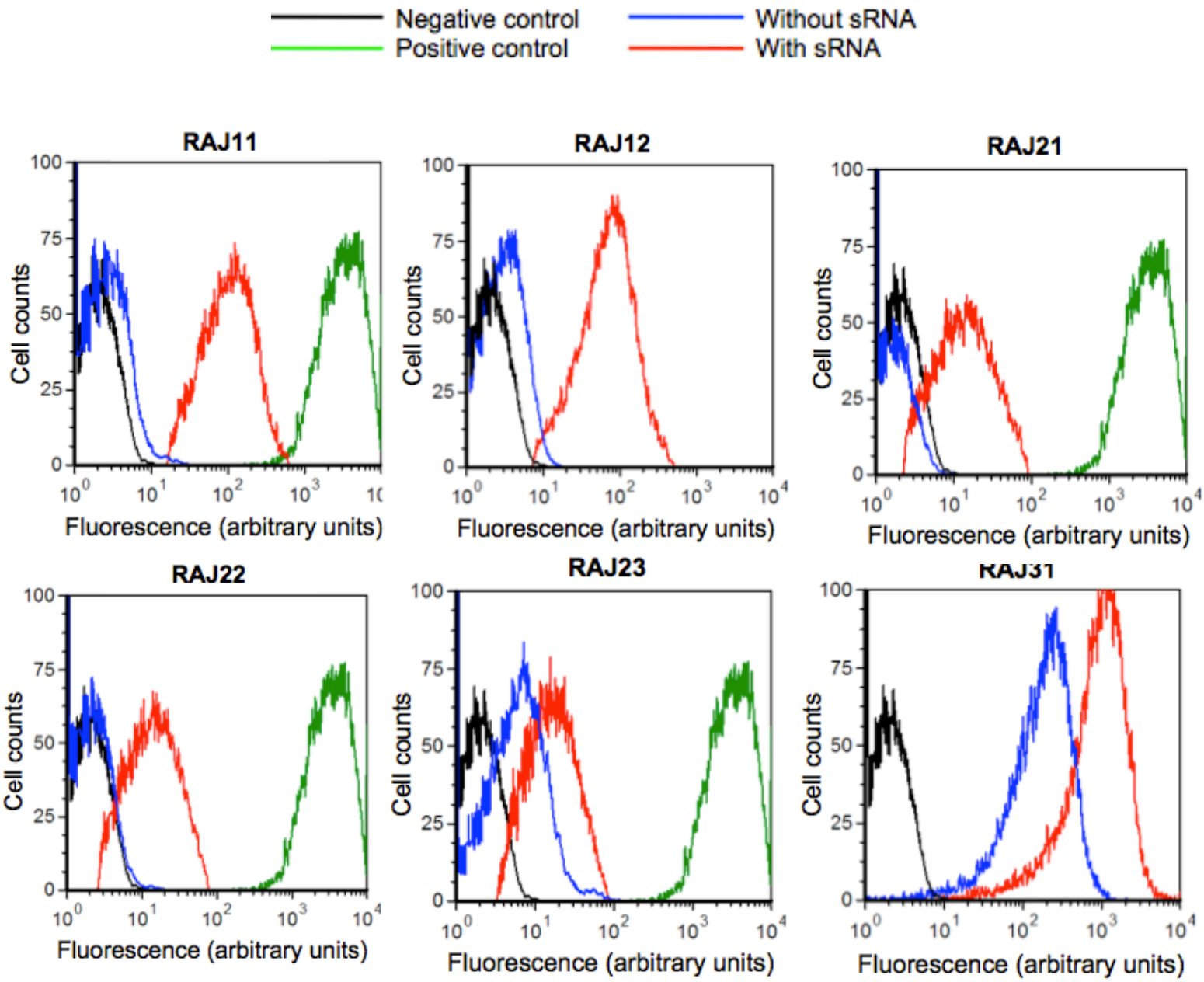
EcoRI
XbaI
T3
mRFP
BamHI
NheI
ClaI
NheI
T2
pLtetO-1
pLlacO-1
sRNA
5'UTR
AvrII
NsiI
Superfolder GFP
SpeI
PstI
T1
KanR
pSC101


Negative control
Positive control
Without sRNA
With sRNA
RAJ11
RAJ12
RAJ21
RAJ22
RAJ23
RAJ31
Cell counts
Fluorescence (arbitrary units)