Self-contained RNA inhibition with trans-cleaving ribozymes

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December 12, 2013

Overview

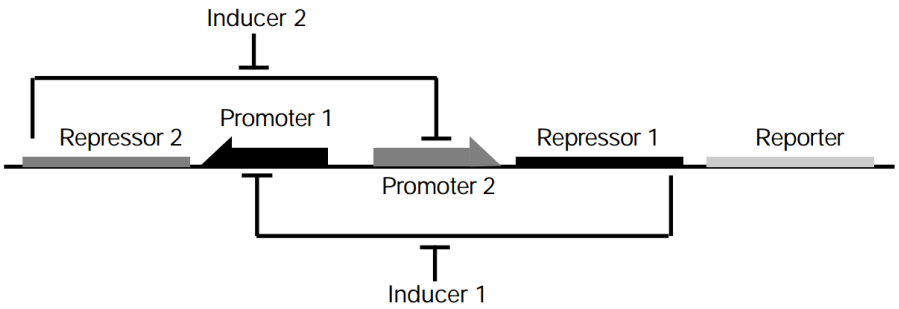
Promise of Synthetic Biology

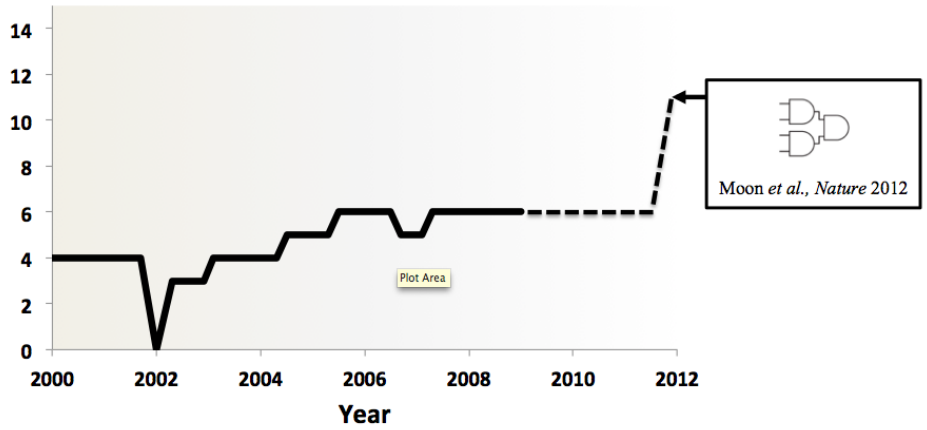
Complexity of eukaryotes $\not\propto$ # of protein coding genes		
Oryza sativa (rice)	470 million	51,00
Gallus gallus (chicken)	1 billion	20,000-23,00
Canis familiaris (dog)	2.4 billion	19,00
Mus musculus (mouse)	2.5 billion	30,00
Homo sapiens	2.9 billion	20,000-25,000

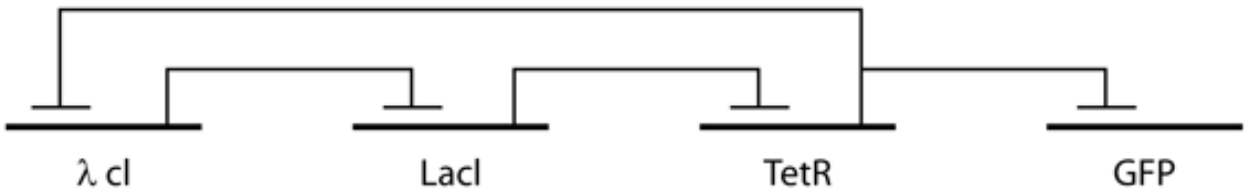
The root of biological complexity is believed to be genetic regulation.
However, the creation of novel protein regulatory elements is too difficult.
Re-writers RNA-world may be the key to getting a handle on regulation.

Motivation

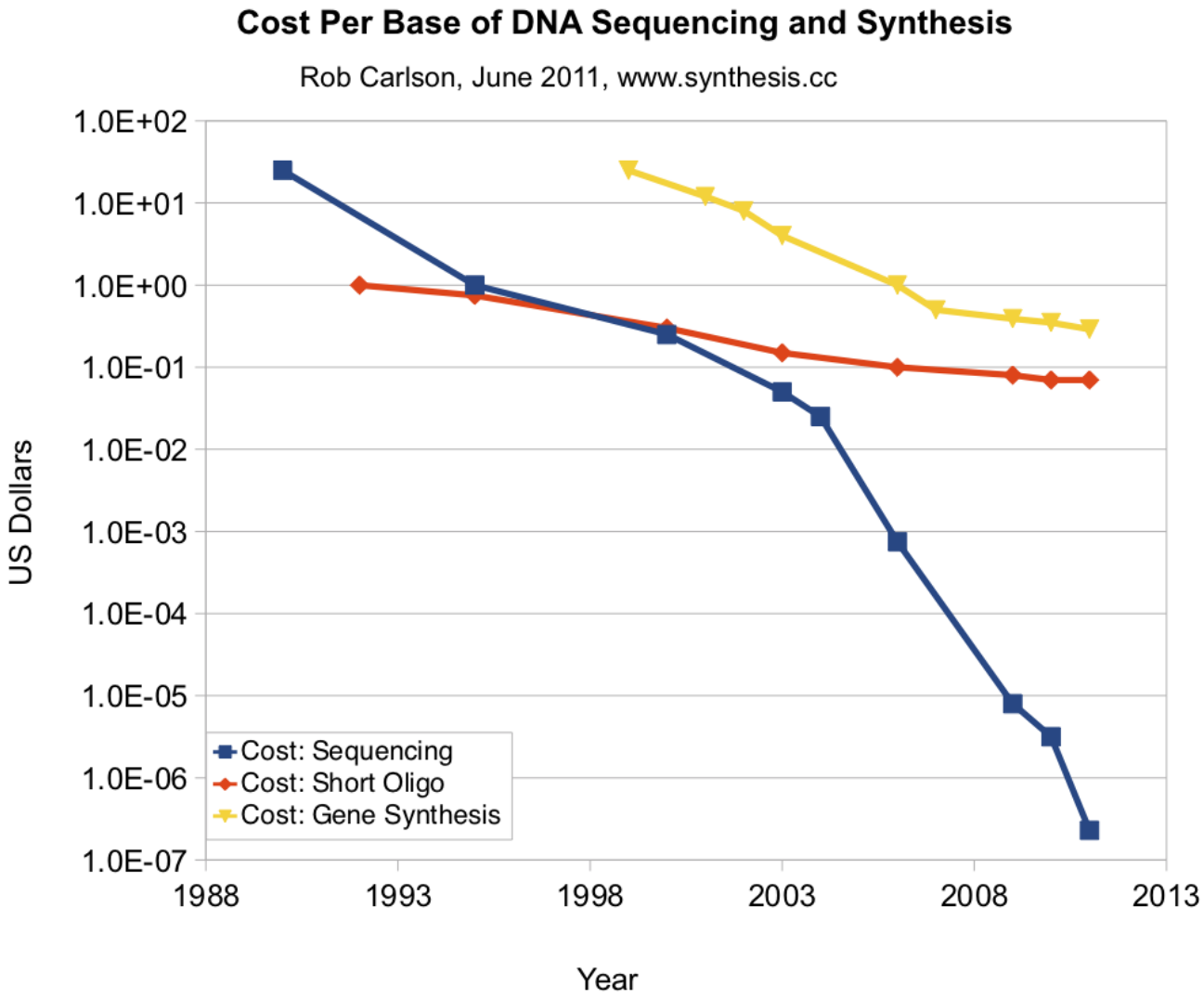
Toggle switch and repressilator in 2000, to now.







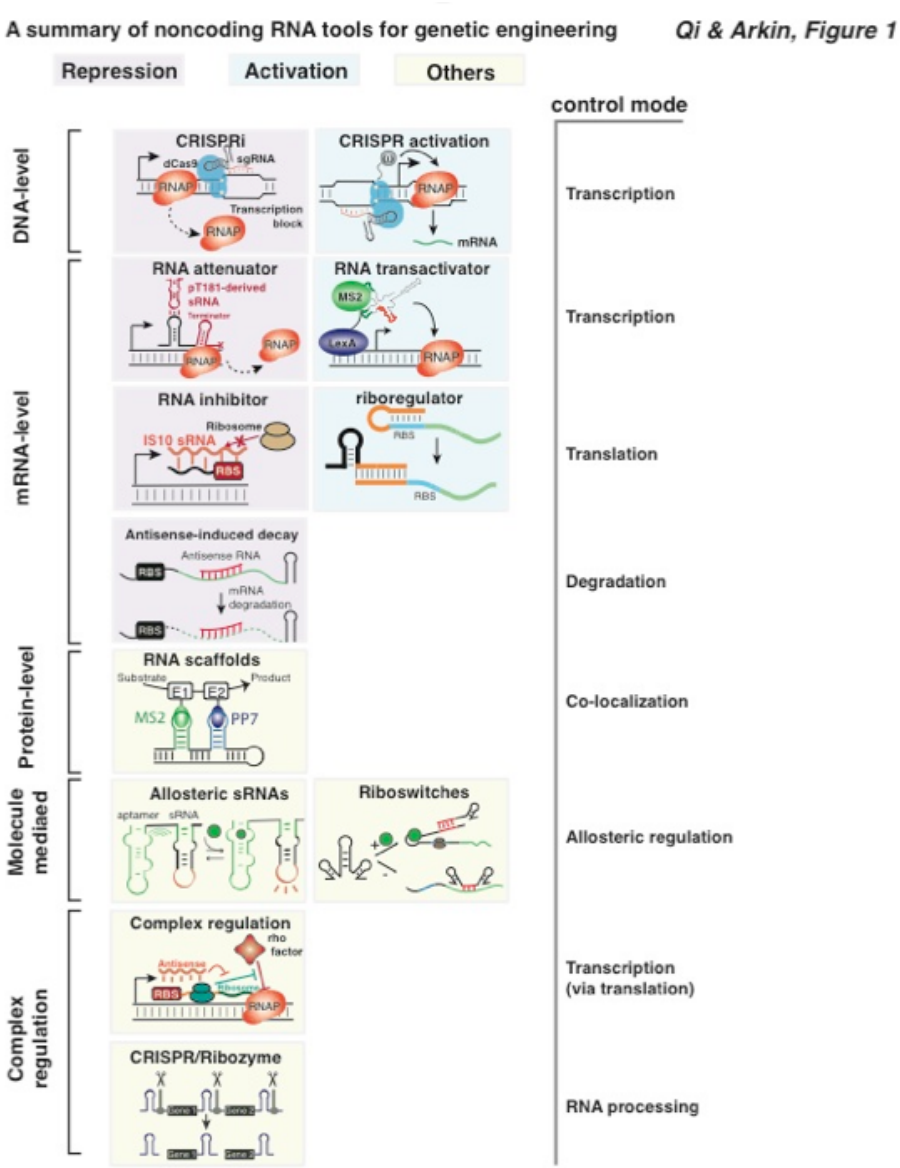
Exponential decrease in the cost of enabling technologies should result in exponential growth of circuit complexity.

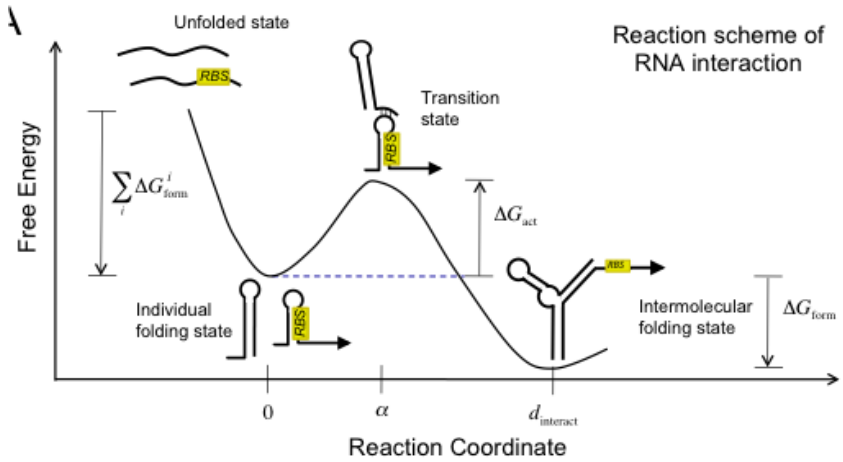


Pitfalls in current promoter-repressor pair design

- Orthogonal - Limited number of repressors (until very recently)
- Predictable - Gene circuit evolves away
- Safe - shRNA toxicity in gene therapy
- Reliable - 40 hour toggle switch breakdown
- Designable - Protein structure prediction too difficult
- Composable - Unpredictable behavior when juxtaposed

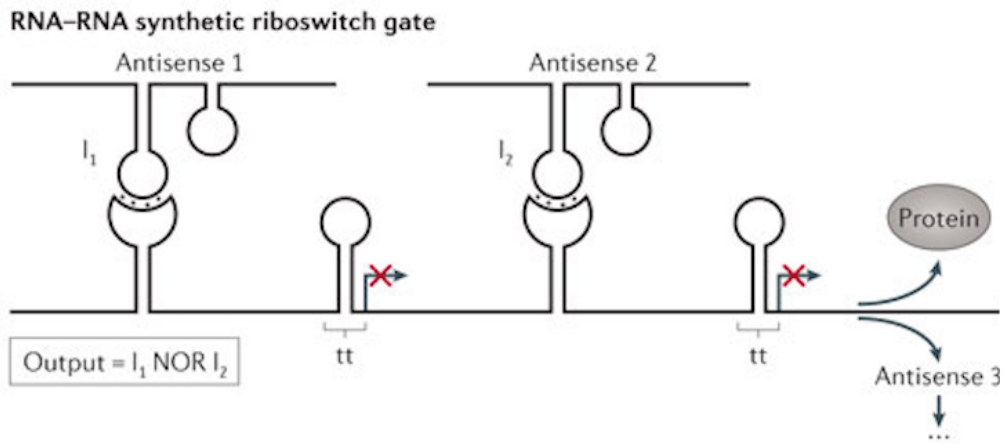
Signal Transduction with RNA, possible tools





Choice of trans-cleaving hammerhead ribozymes

Self-contained mechanism of RNA degradation
Composable and functionally complete: NOR gate



Base-pairing rather than aptamer coupling for ease of rational design
Watson Crick base pairing dominates free energy minimization

General riboregulation model

Stochastic model - Gillespie algorithm

Algorithm 1 Gillespie

Inputs:
Set of M reactions; $R_i = (c_i) i \in 1, \ldots, M$
Initial population sizes, *endtime*
Output: Catalog of Molecular events
while $\tau <$ *endtime* **do**
  $a_0 = 0$
  for $i = 1$ to M **do**
    $a_i = h_i c_i$, where h_i is the amount of reactant
    $a_0 += a_i$
  end for
  $(\tau, \mu) \leftarrow P(\tau, \mu)$
  updatePopulation(R_μ)
end while

trans-cleaving ribozyme model and kinetics

From Samarsky *et al.*

$$A_{DNA} \xrightarrow{k_{tc_a}} A_{RNA}\ ,\ B_{DNA} \xrightarrow{k_{tc_b}} B_{RNA}$$

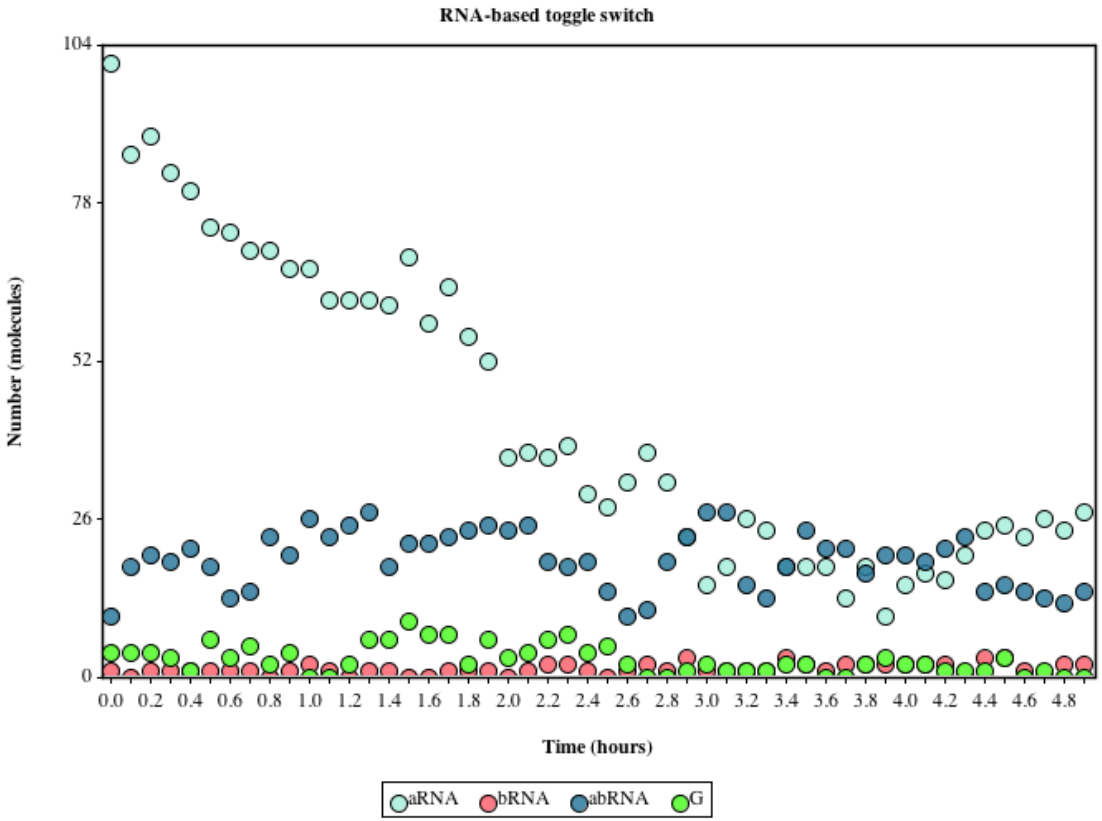
$$A_{RNA} + B_{RNA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A_{RNA} \cdot B_{RNA} \xrightarrow{k_2} A_{RNA} \cdot F_1 \cdot F_2 \xrightarrow{k_3} A_{RNA} + F_1'$$

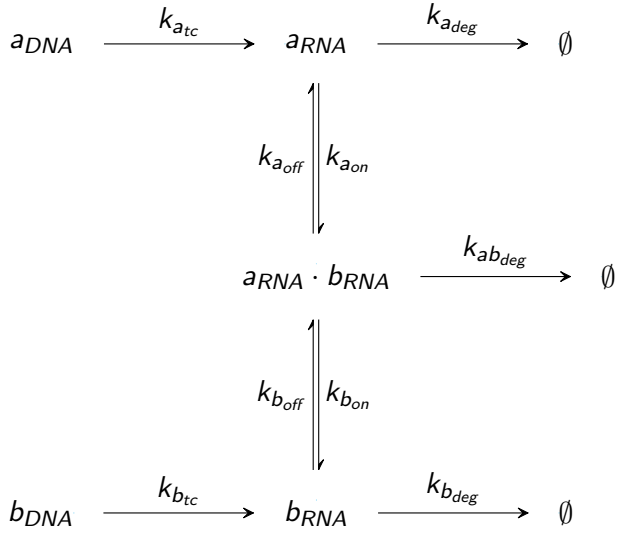
All species undergo degradation at some rate k_{deg}.

Approximated by MM kinetics
Fraction of substrate/time: $k_{obs} = k_2 \times [A_{RNA} \cdot B_{RNA}]/[B_{RNA_0}]$
$k_1 >> k_{-1}$, and tunable.
$k_3 >> k_2 >> k_{deg}$ by single orders of magnitude.

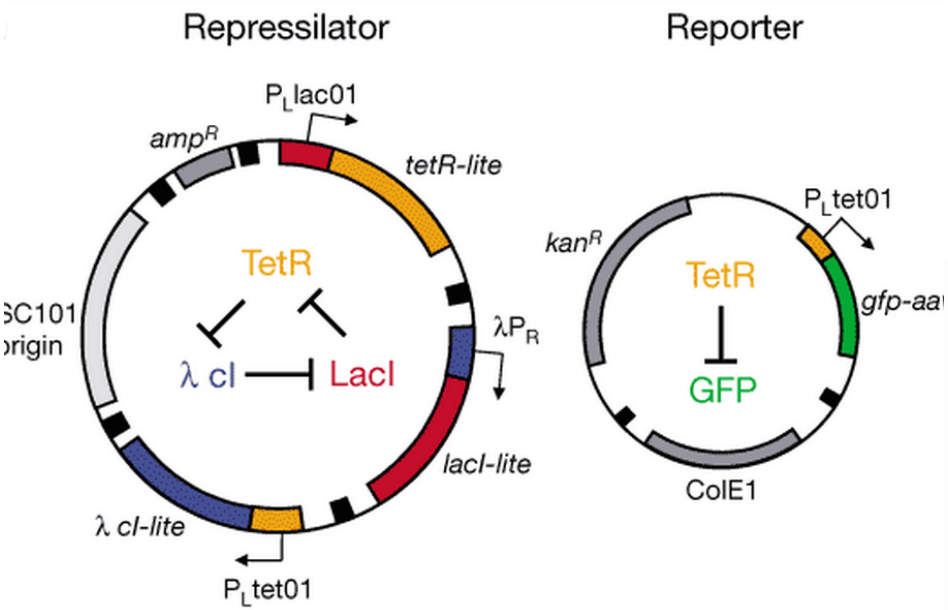
Toggle switch model riboregulation

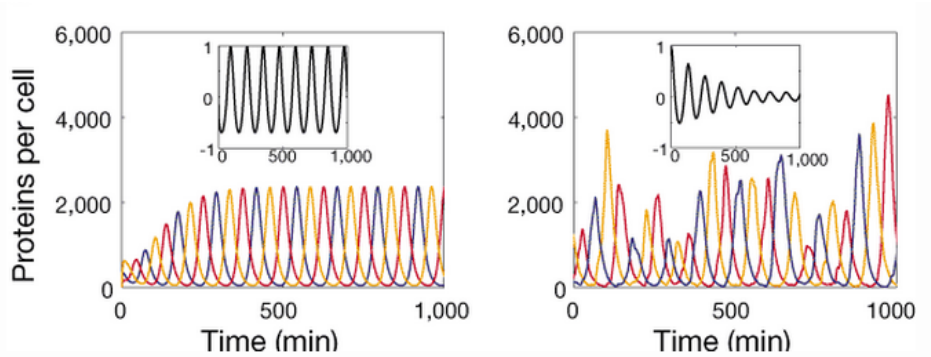
No possible bistable point. First limitation.



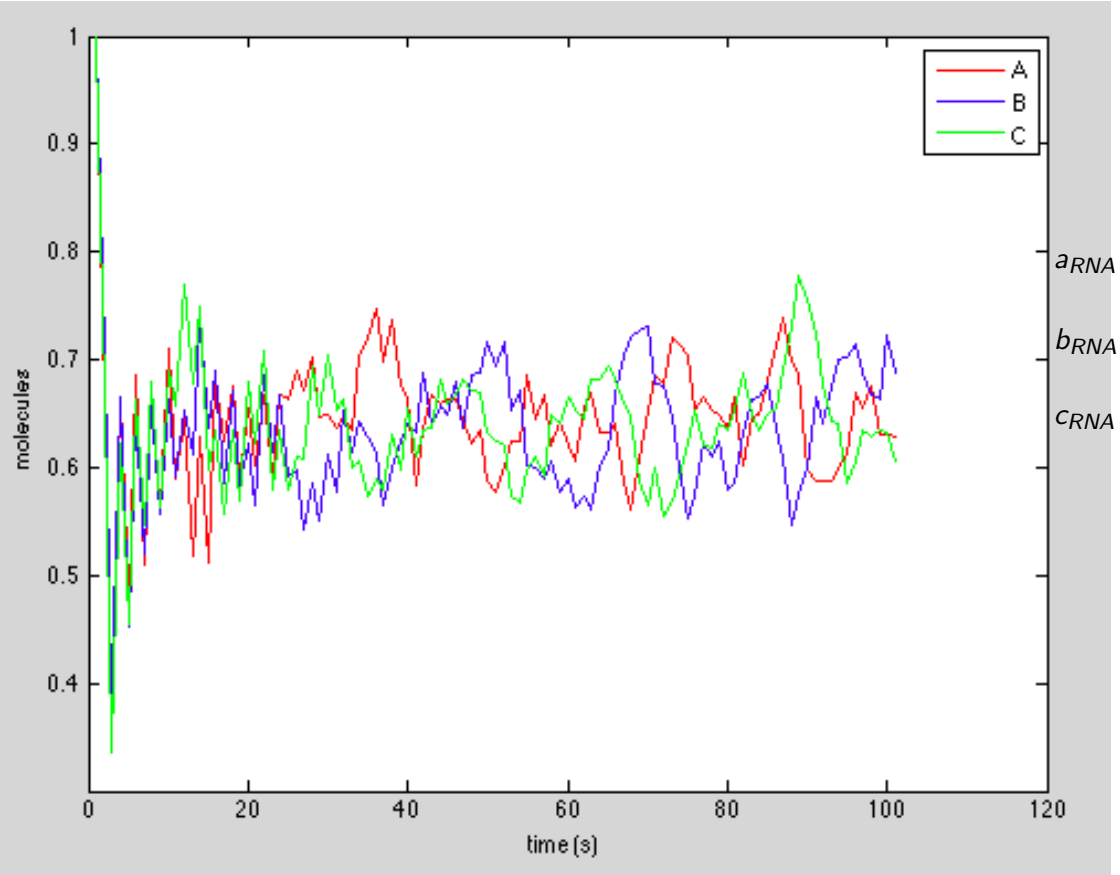


Classic Repressilator





Riboregulatory Repressilator



$$a_{DNA} \xrightarrow{k_{a_{tc}}} a_{RNA} \xrightarrow{k_{deg_a}} \emptyset$$

$$b_{DNA} \xrightarrow{k_{b_{tc}}} b_{RNA} \xrightarrow{k_{deg_b}} \emptyset$$

$$c_{DNA} \xrightarrow{k_{c_{tc}}} c_{RNA} \xrightarrow{k_{deg_c}} \emptyset$$

$$a_{RNA} + b_{RNA} \underset{k_{off_{ab}}}{\overset{k_{on_{ab}}}{\rightleftharpoons}} a_{RNA} \cdot b_{RNA} \xrightarrow{k_{cleave_a}} a_{RNA}$$

$$b_{RNA} + c_{RNA} \underset{k_{off_{bc}}}{\overset{k_{on_{bc}}}{\rightleftharpoons}} b_{RNA} \cdot c_{RNA} \xrightarrow{k_{cleave_b}} b_{RNA}$$

$$c_{RNA} + a_{RNA} \underset{k_{off_{ca}}}{\overset{k_{on_{ca}}}{\rightleftharpoons}} c_{RNA} \cdot a_{RNA} \xrightarrow{k_{cleave_c}} c_{RNA}$$

Repressilator Comparison

Rate of oscillation
Tunable:

- promoter strength - well characterized process
- strength of binding - determined by W-C Base-pairing
- cleavage rate - fairly fixed
- degradation rate - introduction of cleavage sites

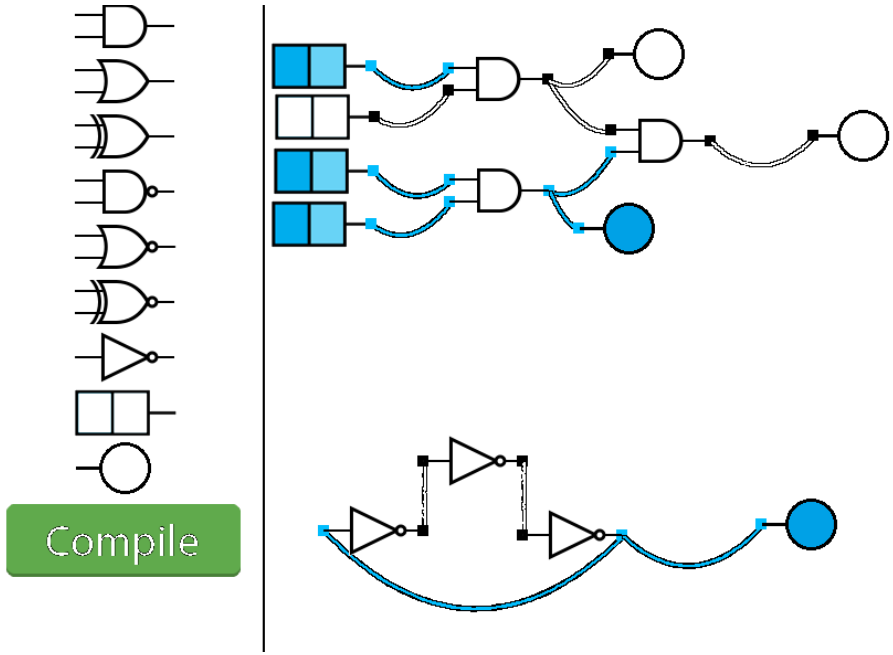
Conclusions

Character of trans-cleaving ribozymes

- Orthogonal - determined by WC Base-pairing
- Predictable - less stress on cells, cleaving reaction well characterized
- Safe - mRNA is less persistent, and less chemically reactive
- Reliable - still possible for cell to mutate
- Designable - minimization of free energy based on WC Base-pairing
- Composable - Unpredictable behavior when juxtaposed

Further work

Biocircuit-Design Automation,
Biocompiler



Thanks!

Thanks to Sergey and Jeremy for their feedback and help throughout the course
Thanks to Adam and Ron for all your time and for giving us a solid synbio foundation
And thanks to Leslie for working out the logistics

Sources

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