Self-contained RNA inhibition with trans-cleaving ribozymes

Zack Field & Ryan Tsoi

University of California

field.zackery@berkeley.edu, ryantsoi@berkeley.edu

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Overview

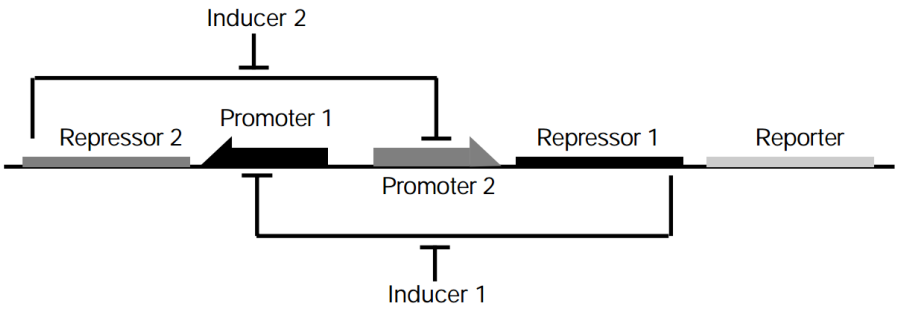
Promise of Synthetic Biology

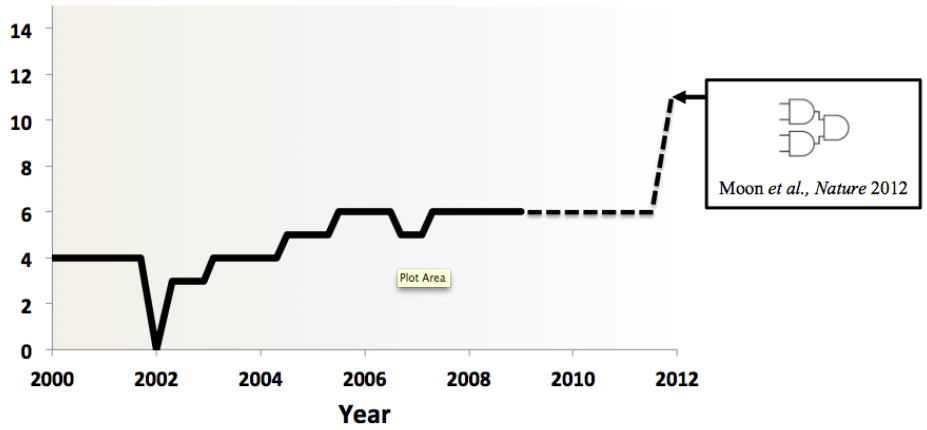
Complexity of eukaryotes $\not\propto$ # of protein coding genes		
Oryza sativa (rice)	470 million	51,00
Gallus gallus (chicken)	1 billion	20,000-23,00
Canis familiaris (dog)	2.4 billion	19,00
Mus musculus (mouse)	2.5 billion	30,00
Homo sapiens	2.9 billion	20,000-25,000

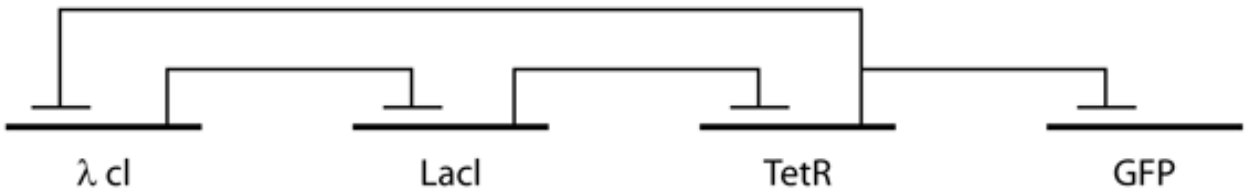
The root of biological complexity is believed to be genetic regulation.
However, the creation of novel protein regulatory elements is too difficult.
Re-writers RNA-world may be the key to getting a handle on regulation.

Motivation

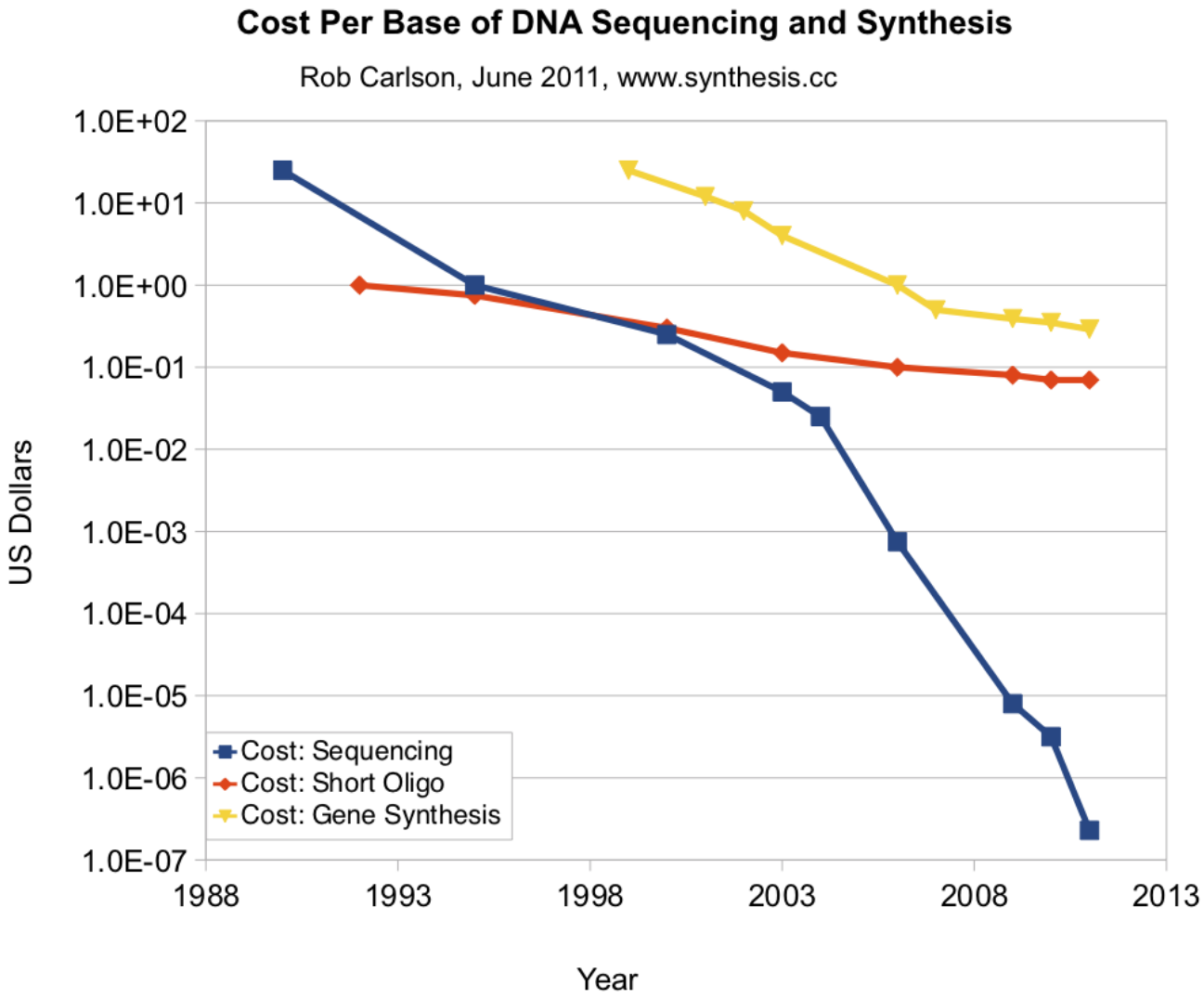
Toggle switch and repressilator in 2000, to now.







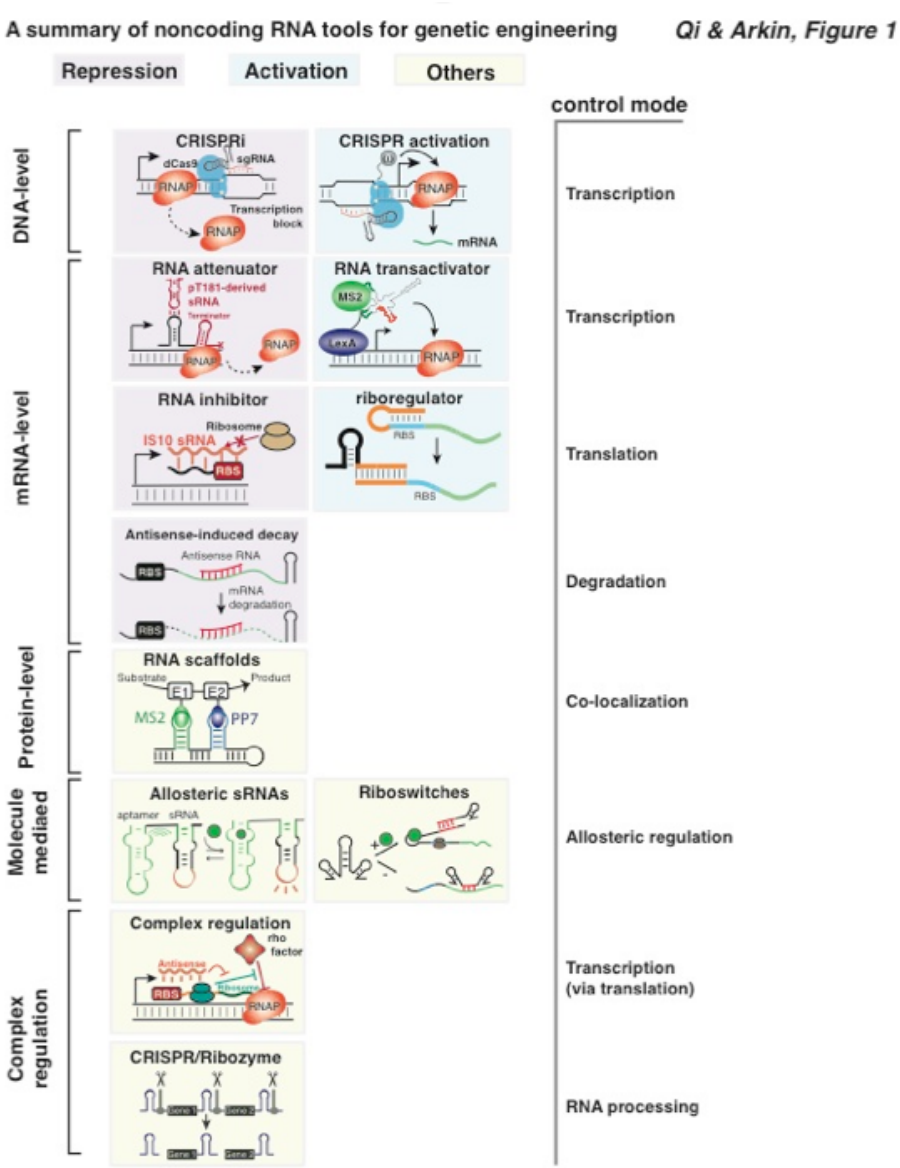
Exponential decrease in the cost of enabling technologies should result in exponential growth of circuit complexity.

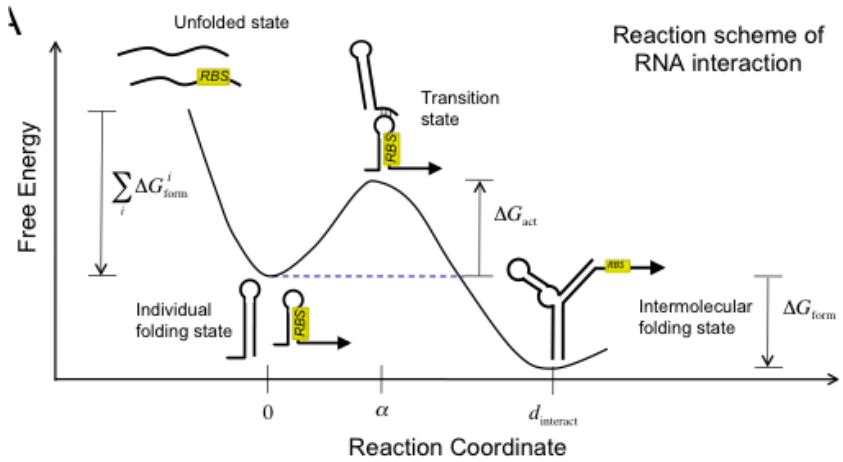


Pitfalls in current promoter-repressor pair design

- 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
- Cooperativity - Unpredictable behavior when juxtaposed

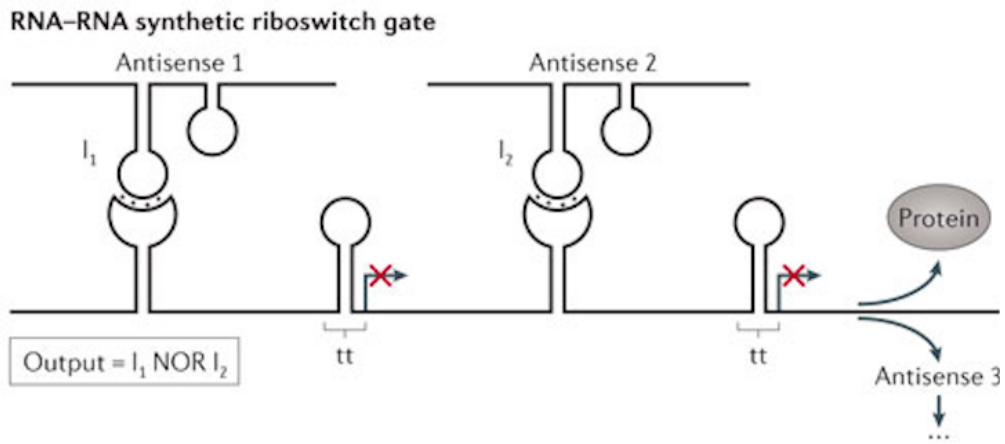
Signal Transduction with RNA, possible tools





Choice of trans-cleaving hammerhead ribozymes

Self-contained mechanism of RNA degradation
Composable and functionally complete



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 and $avg\_Prob[] = c$; $R_i = (c_i) i \in 1, \ldots, M$
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.*

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

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

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

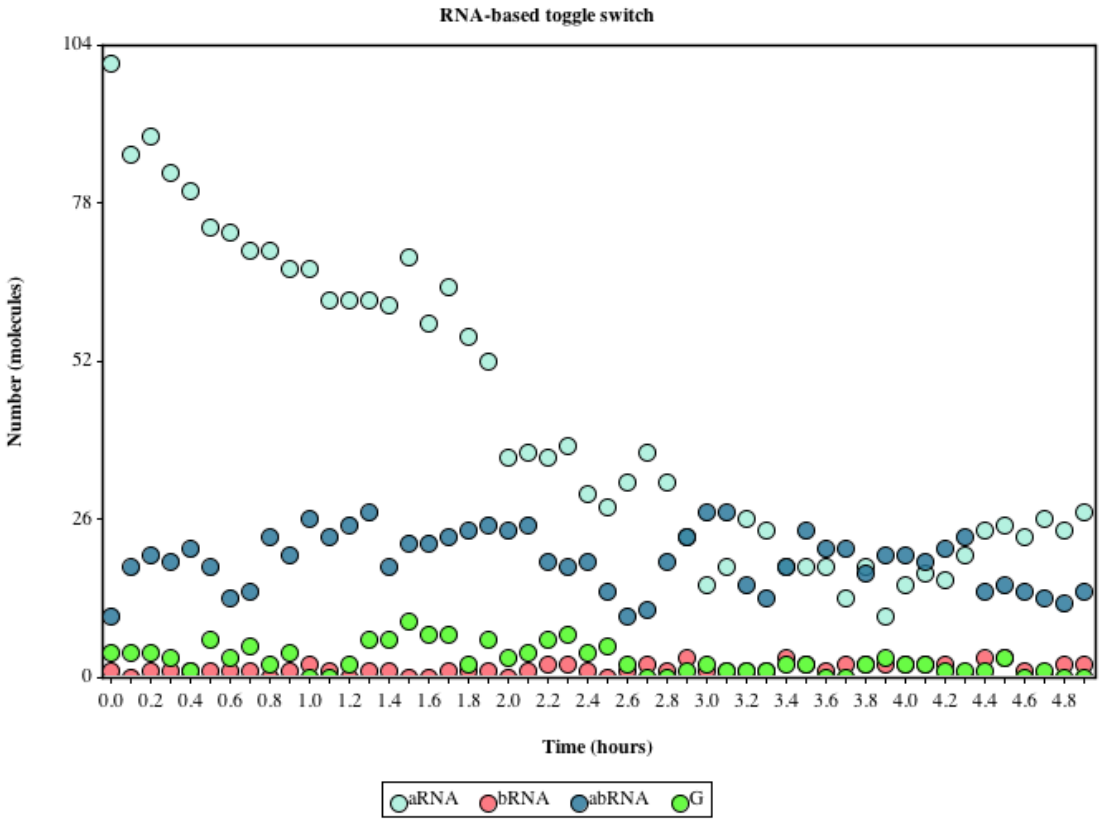
MM kinetics

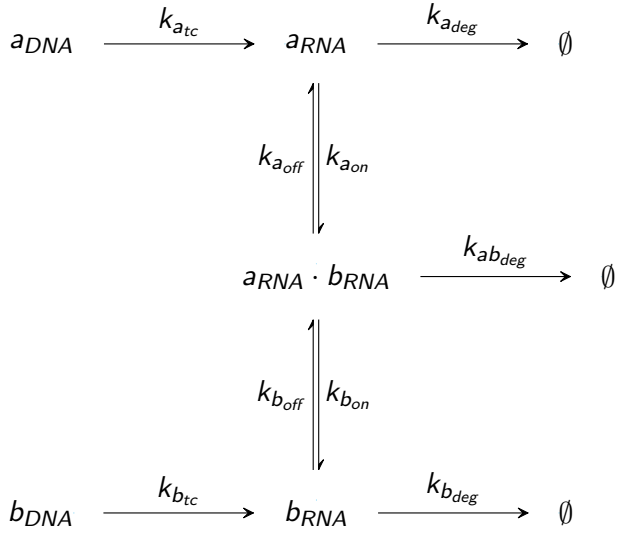
Fraction of substrate/time: $k_{obs} = k_2 \times [A_{RNA} \cdot B_{RNA}]/[B_{RNA_0}]$

$k_3 >> k_2 >> k_{deg}$ by single orders of magnitude.

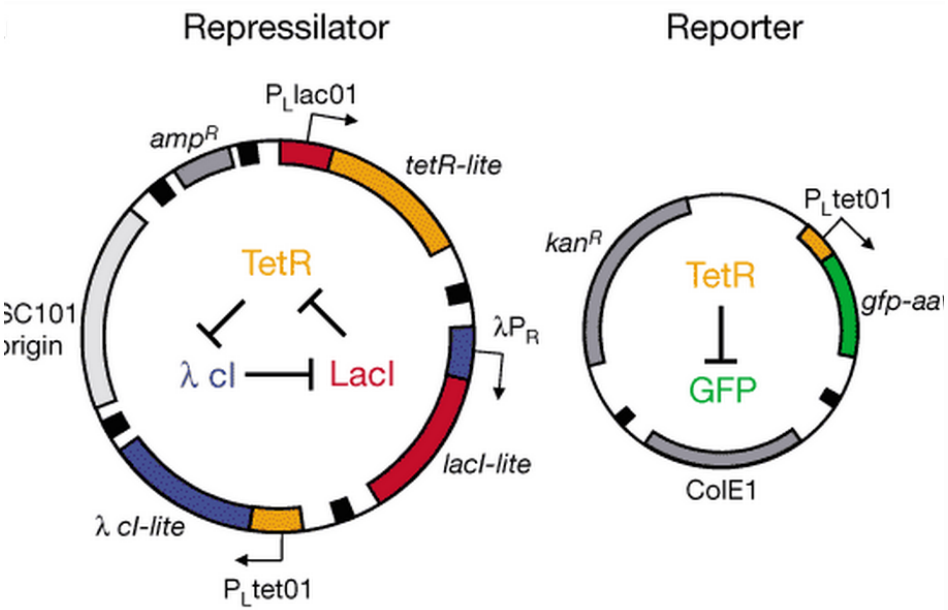
Toggle switch model riboregulation

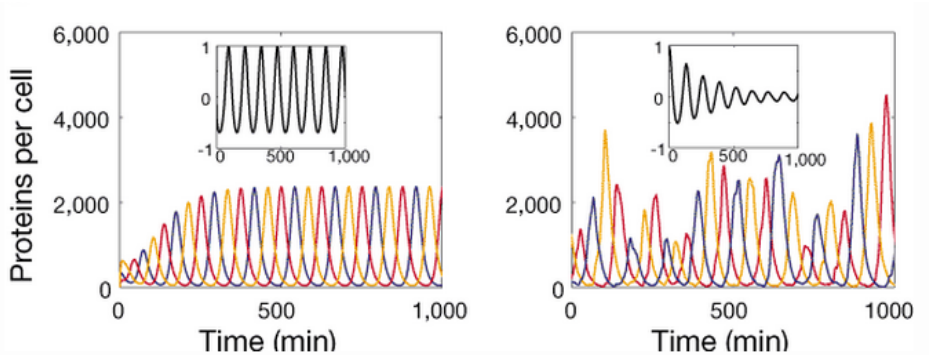
No possible bistable point. First limitation.



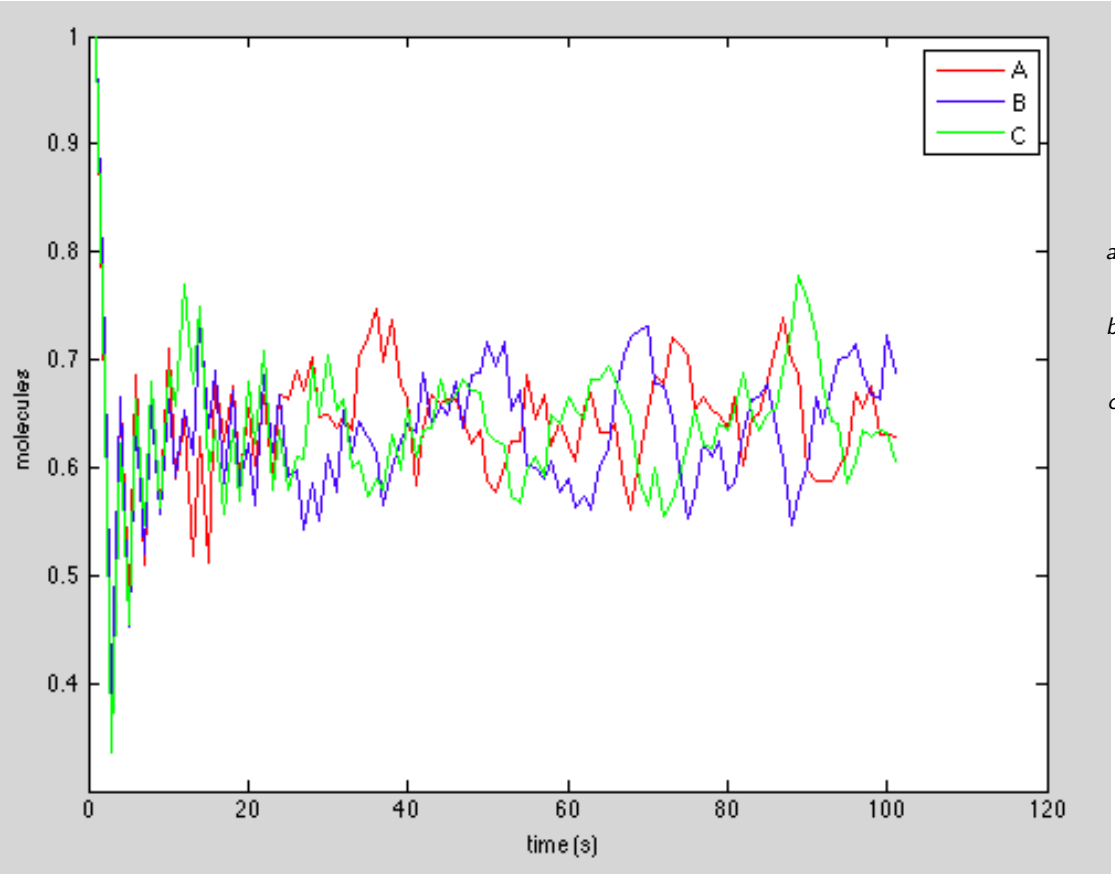


Classic Repressilator





Riboregulatory Repressilator



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

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

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

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

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

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

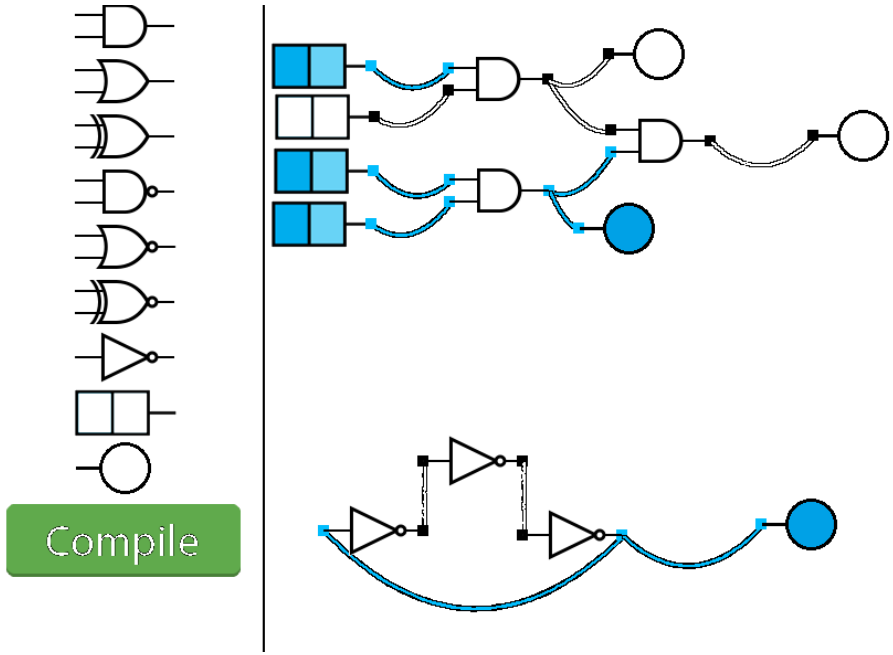
Repressilator Comparison

Rate of oscillation
Tunable:

- promoter strength
- strength of binding
- cleavage rate - fairly fixed
- degradation rate

Implications and further work

Biocircuit-Design Automation,
Biocompiler



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Sources

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Multiple Columns

Heading

1. Statement
2. Explanation
3. Example

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