

# Self-contained RNA inhibition with trans-acting ribozymes

Zack Field & Ryan Tsoi

University of California

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

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## 4 Second Section

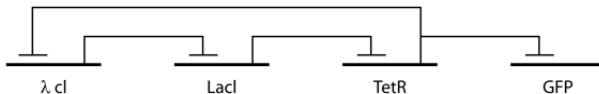
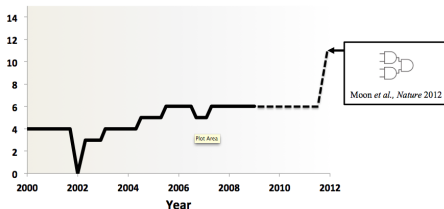
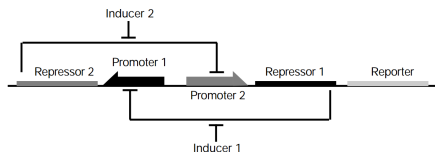
# Promise of Synthetic Biology

Complexity of eukaryotes $\approx$ # 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 complexity is believed to be the regulation of these genes. 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

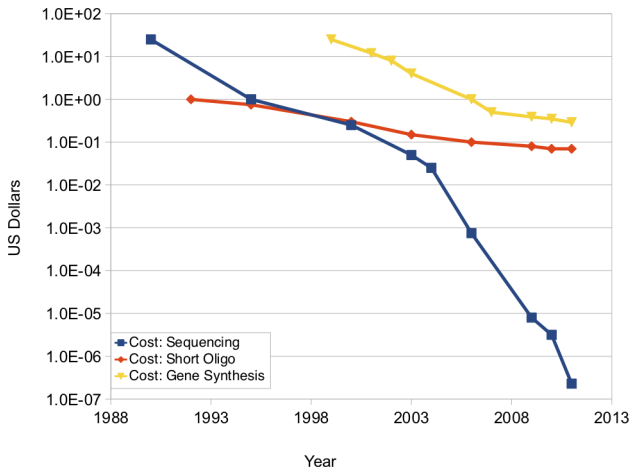
Toggle switch and repressilator in 2000 (images).



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

### Cost Per Base of DNA Sequencing and Synthesis

Rob Carlson, June 2011, [www.synthesis.cc](http://www.synthesis.cc)



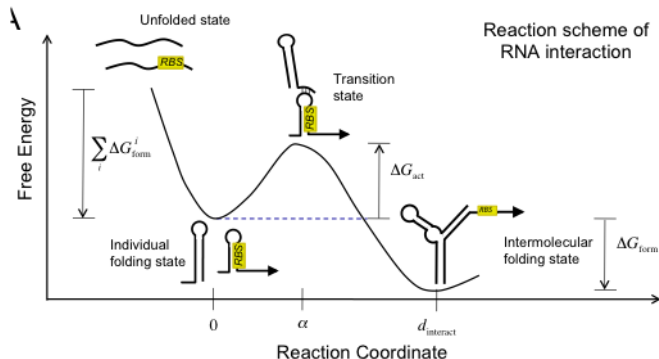
## Pitfalls in current promoter-repressor pair design

- 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

# Types of riboregulation

Why shRNA sucks, because of cleaving mechanism. But this can be avoided.

Self-contained action. removes some dependencies.



# Choice of trans-acting ribozymes

Proof of functional completeness

Include reasoning for not having aptamer effected ribozymes. More difficult to design and predict than simple oligonucleotide effectors.

Watson Crick base pairing dominates free energy minimization



# General riboregulation model

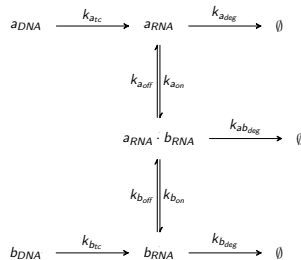
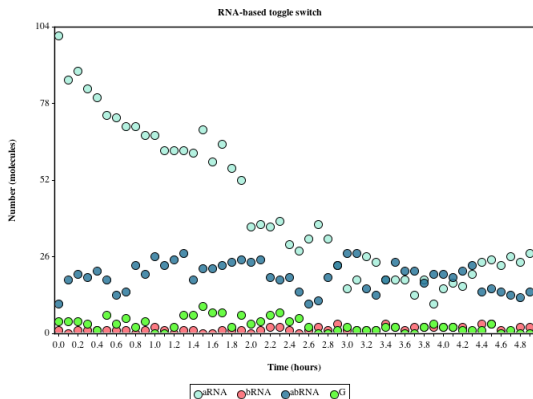
Stochastic model - Gillespie algorithm

The probability

Rate of trans cleaving

# Toggle switch model riboregulation

No possible bistable point. First limitation.



# Parameter Comparison

# Implications and further work

If these

- Pray, L. A.; <http://www.nature.com/scitable/topicpage/eukaryotic-genome-complexity-437>
- Arkin, A. and Weiss, Ron; Principles of Synthetic Biology Fall 2013; Lecture 3
- Carlson, Rob; DNA cost curves; <http://www.synthesis.cc/2011/06/new-cost-curves.html>
- Stanton, B.C. et. al.; *Genomic Mining of prokaryotic repressors for orthogonal logic gates*; <http://www.nature.com/nchembio/journal/vaop/ncurrent/full/nchembio.1411.html>
- Martin, J.N., et. al.; Lethal toxicity caused by expression of shRNA in the mouse striatum: implications for therapeutic design; <http://www.nature.com/gt/journal/v18/n7/full/gt201110a.html>
- Bongarets; *GFP as a Marker for Conditional Gene Expression in Bacterial Cells* <http://www.ifr.ac.uk/Safety/molmicro/pubs/bongaerts2002.pdf>
- Anderson, J.C.; *org.devicecourse Gillespie module*
- Gillespie, Daniel T. (1977). *Exact Stochastic Simulation of Coupled*

# Blocks of Highlighted Text

## Block 2

Pellentesque sed tellus purus. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos himenaeos. Vestibulum quis magna at risus dictum tempor eu vitae velit.

## Block 3

Suspendisse tincidunt sagittis gravida. Curabitur condimentum, enim sed venenatis rutrum, ipsum neque consectetur orci, sed blandit justo nisi ac lacus.

## Heading

- 1 Statement
- 2 Explanation
- 3 Example

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Integer lectus nisl, ultricies in feugiat rutrum, porttitor sit amet augue. Aliquam ut tortor mauris. Sed volutpat ante purus, quis accumsan dolor.

# Table

Treatments	Response 1	Response 2
Treatment 1	0.0003262	0.562
Treatment 2	0.0015681	0.910
Treatment 3	0.0009271	0.296

Table : Table caption



# Theorem

Theorem (Mass–energy equivalence)

$$E = mc^2$$

## Example (Theorem Slide Code)

```
\begin{frame}  
\frametitle{Theorem}  
\begin{theorem}[Mass--energy equivalence]  
$E = mc^2$  
\end{theorem}  
\end{frame}
```

# Figure

Uncomment the code on this slide to include your own image from the same directory as the template .TeX file.

An example of the `\cite` command to cite within the presentation:

This statement requires citation [Smith, 2012].

# References



John Smith (2012)

Title of the publication

*Journal Name* 12(3), 45 – 678.

# The End