

# Oscillatory Circuits

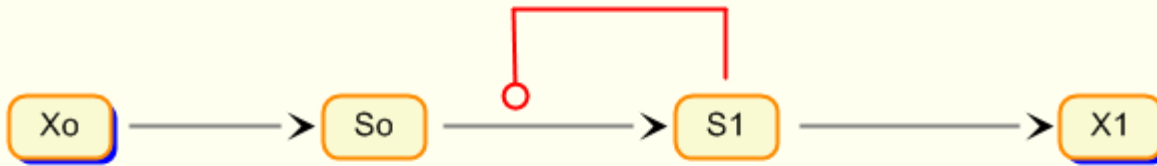
Systems and Synthetic Biology  
Class

# Modifying a Bistable System



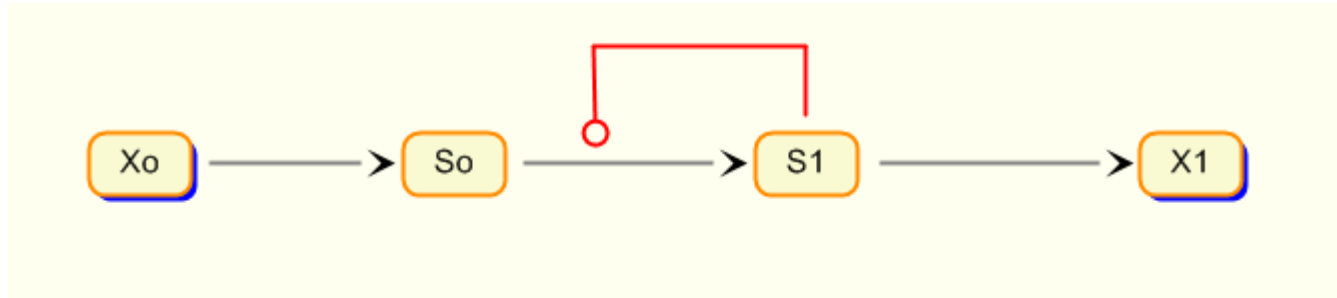
This small network shows bistable behavior

# Modifying a Bistable System



With only the addition of a single reaction, this network will oscillate.

# Modifying a Bistable System to form a Relaxation Oscillator



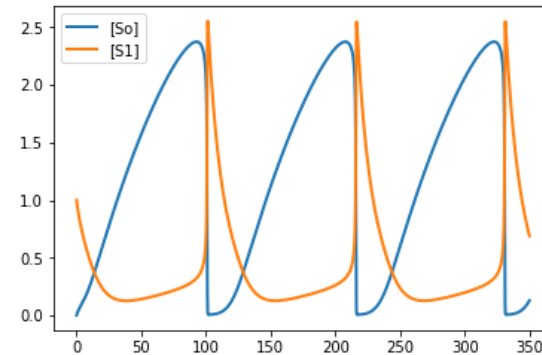
Using the Tellurium Python package at: [tellurium.analogmachine.org](http://tellurium.analogmachine.org)

```
import tellurium as te

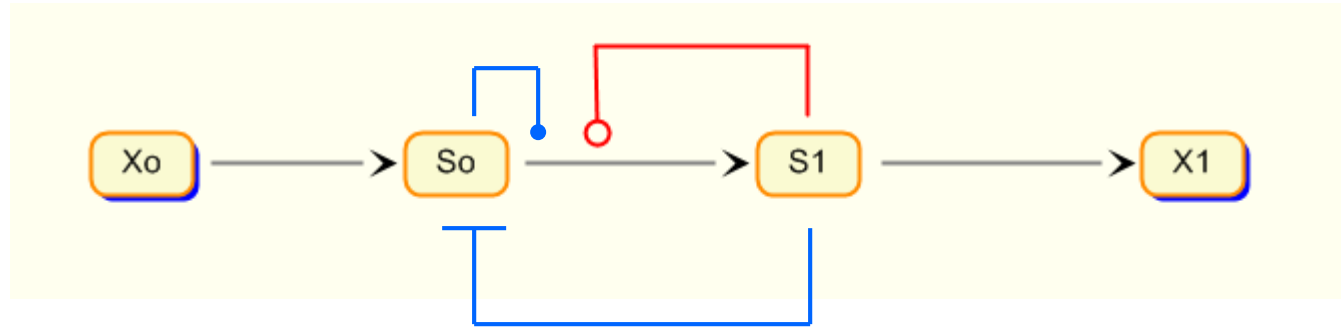
r = te.loada("""
  $Xo -> So;  k0*Xo;
  So -> S1;  k1*So + Vmax*So*S1^n/(15 + S1^n);
  S1 -> $X1;  k2*S1;

  Xo = 1; X1 = 0; S1 = 1; n = 4;
  Vmax = 12; k0 = 0.044;
  k1 = 0.01; k2 = 0.1;
""")

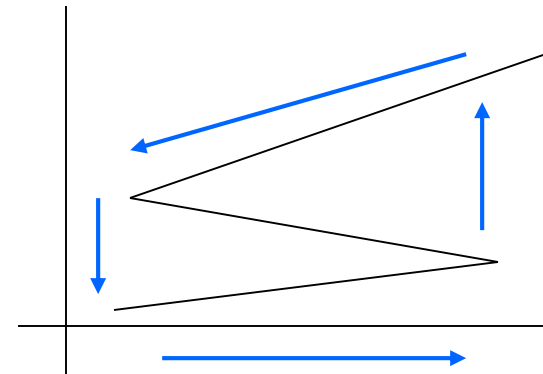
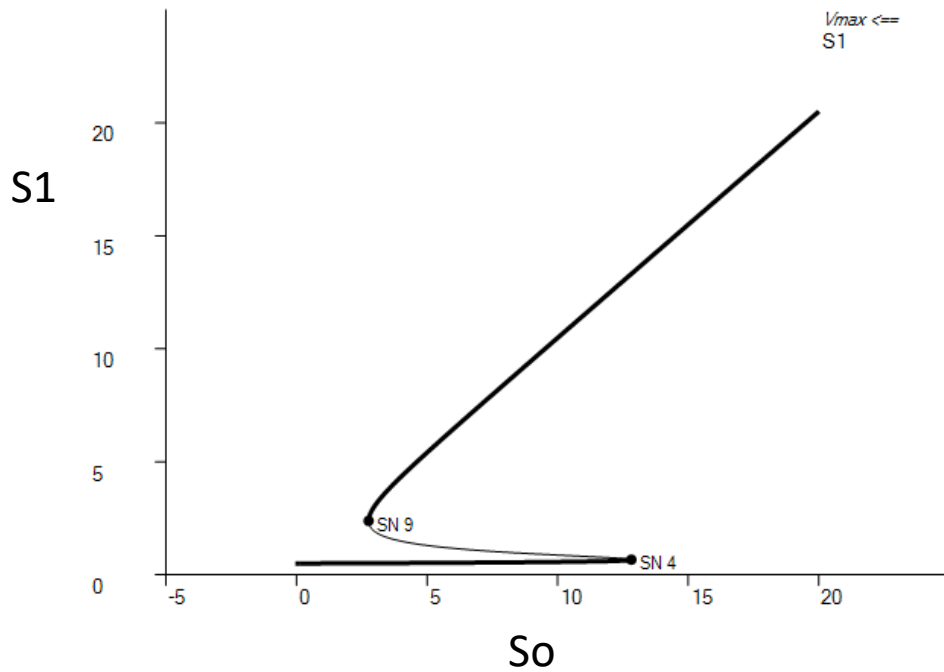
m = r.simulate (0, 300, 1600)
r.plot()
```



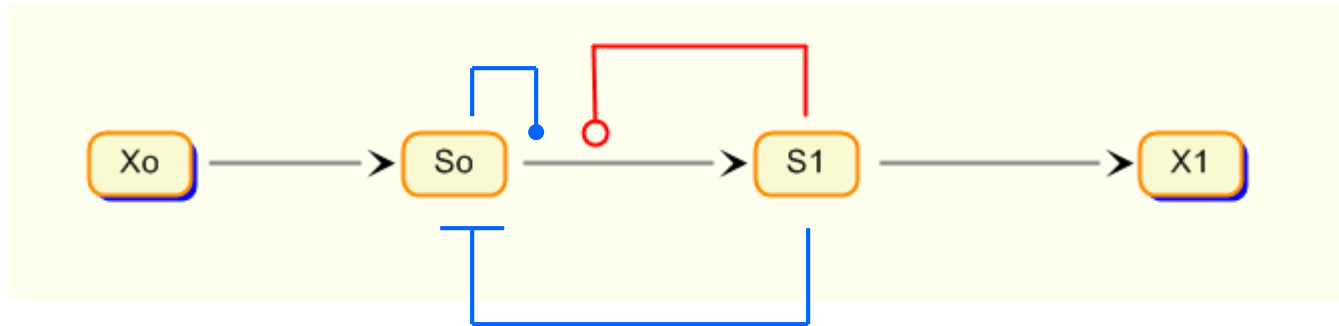
# Relaxation Oscillator



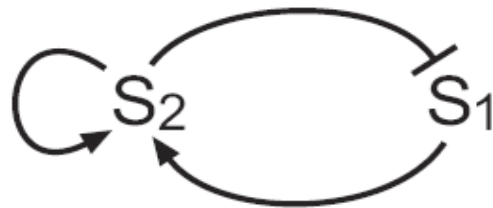
One Parameter Bifurcation Data [1/1]:  $V_{max}$



# Relaxation Oscillator



A relaxation oscillator has two parts, a threshold device, for example a bistable system, and a negative feedback loop.



# Relaxation Oscillator

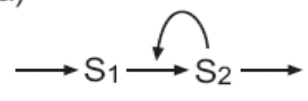

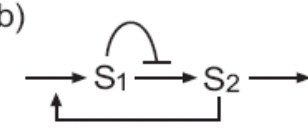
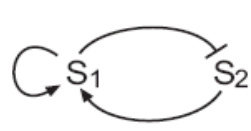
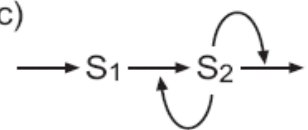

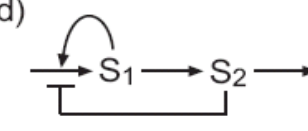
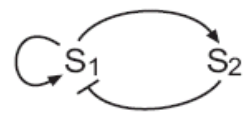
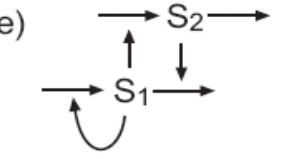
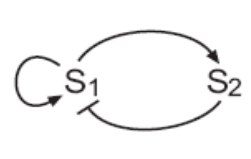
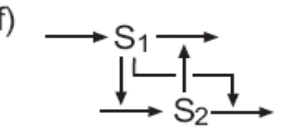
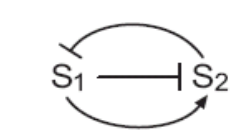
SD = Substrate Depletion

AI = Activator-Inhibitor

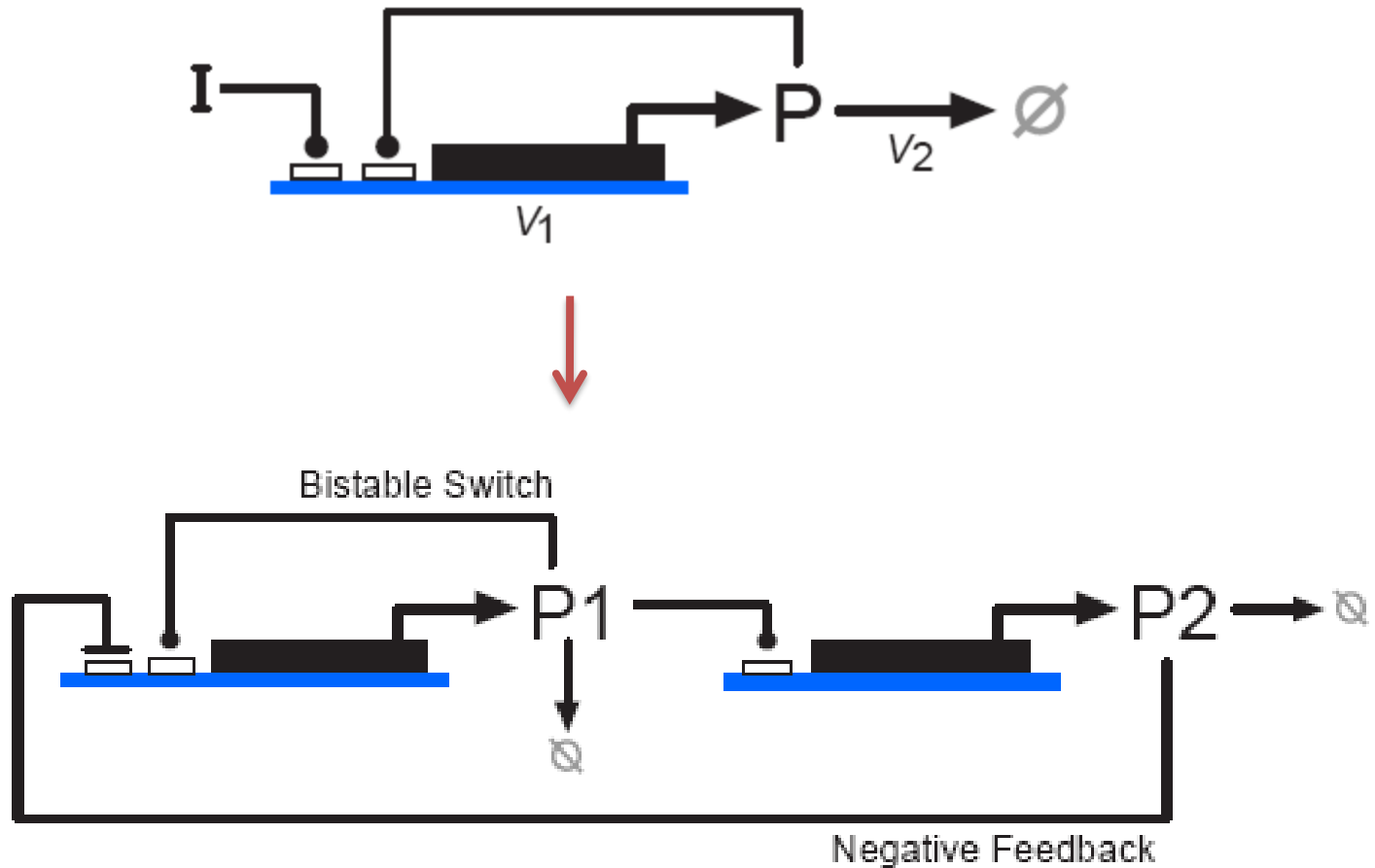
SD/T = Substrate Depletion/  
Toggle.

Classifications  
according to Tyson

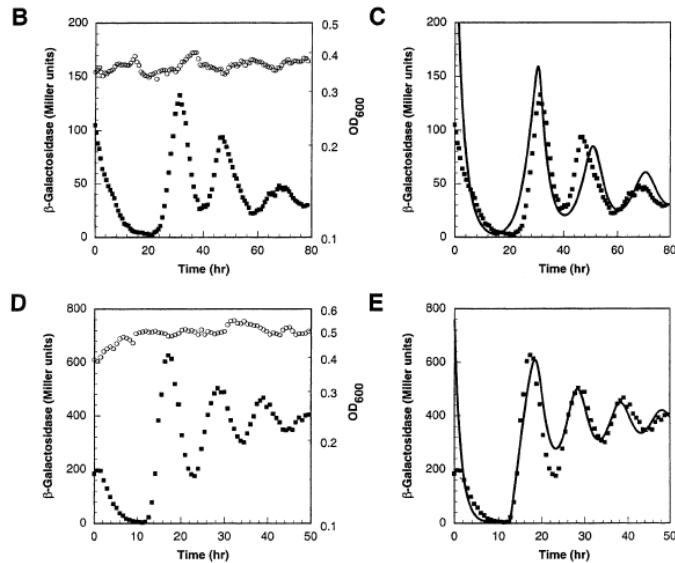
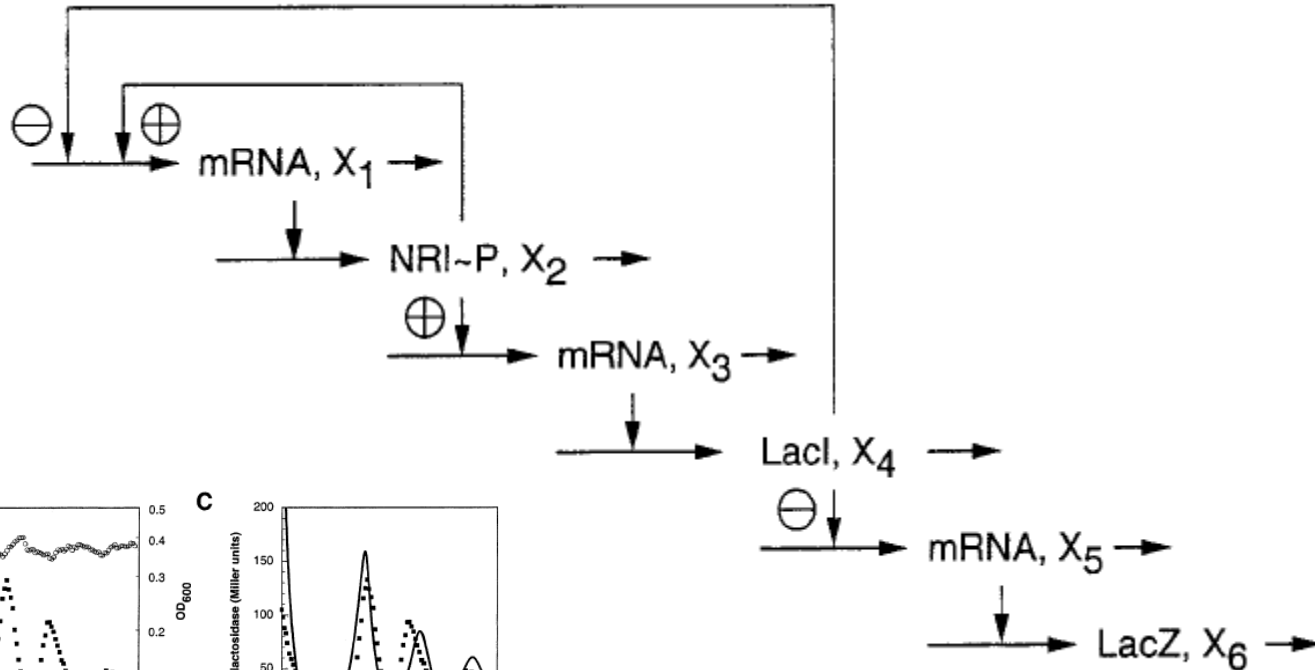
Sniffers, buzzers, toggles and blinkers:  
dynamics of regulatory and signaling  
pathways in the cell John J Tyson, Katherine  
C Chenz and Bela Novak  
Current Opinion in Cel Biology, vol 15,  
221-231 (2003)

Mechanism	Stylized Form	Type
a) 		<b>SD</b>
b) 		<b>SD</b>
c) 		<b>SD</b>
d) 		<b>AI</b>
e) 		<b>AI</b>
f) 		<b>SD/T</b>

# Relaxation Oscillator

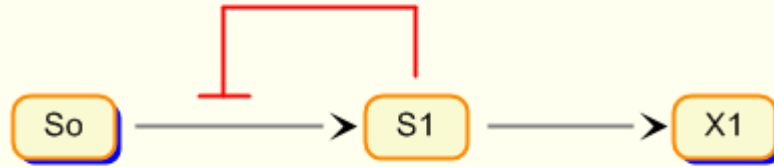


# Synthetic Relaxation Oscillator

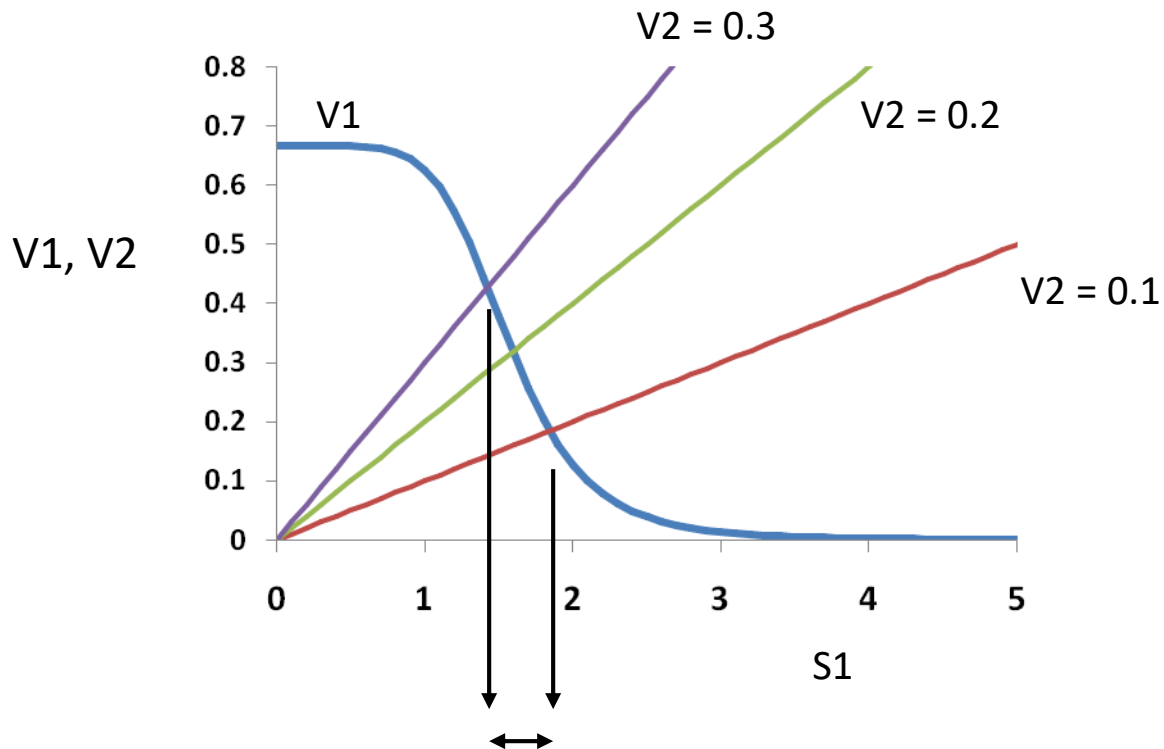
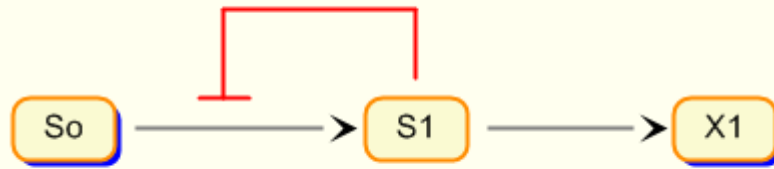


Cell, Vol. 113, 597–607, May 30, 2003, Development of Genetic Circuitry Exhibiting Toggle Switch or Oscillatory Behavior in *Escherichia coli*, Mariette R. Atkinson Michael A. Savageau Jesse T. Myers and Alexander J. Ninfa

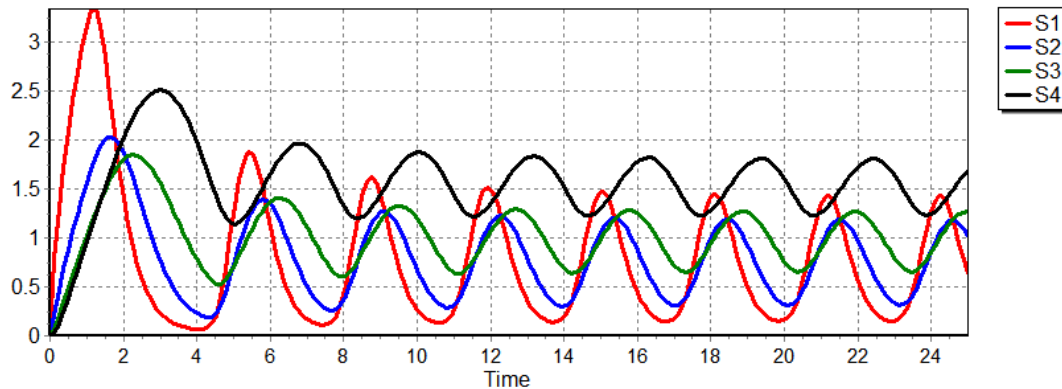
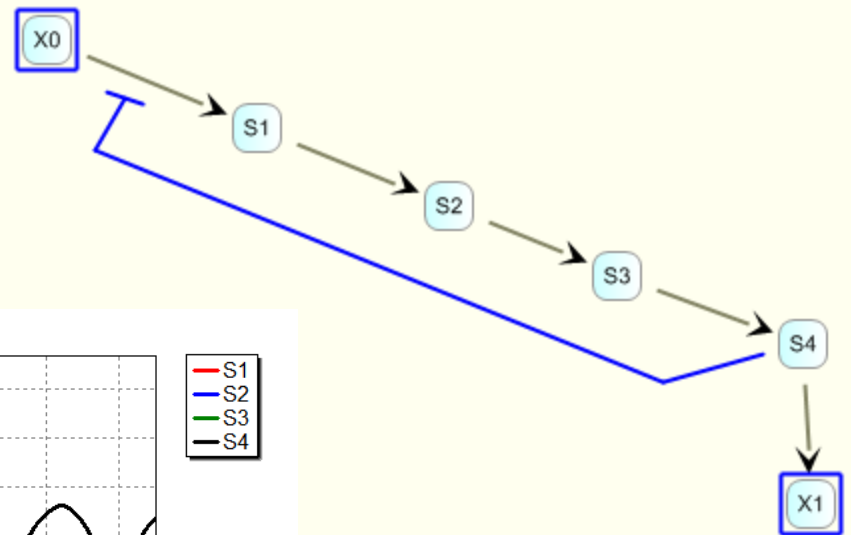
# Negative Feedback



# Negative Feedback



# Negative Feedback: Phase Shift Oscillator



If the signal takes too long to make the appropriate adjustment, the system can go out of phase and begin to spontaneously oscillate.

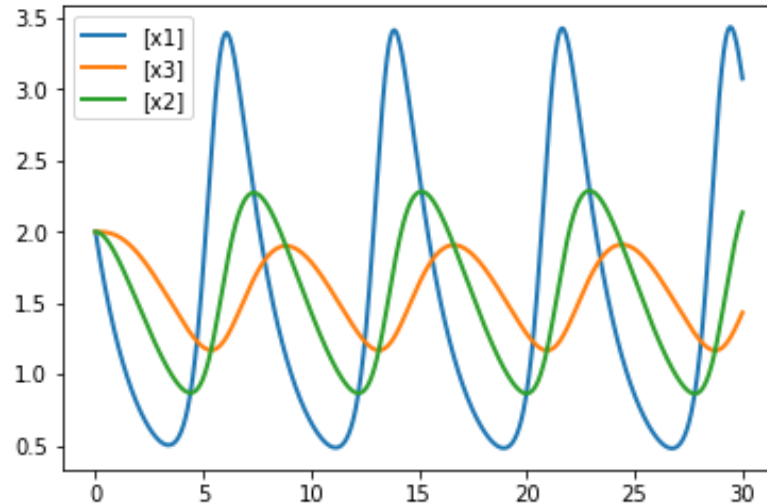
# Negative Feedback Phase Shift Oscillator

```
import tellurium as te

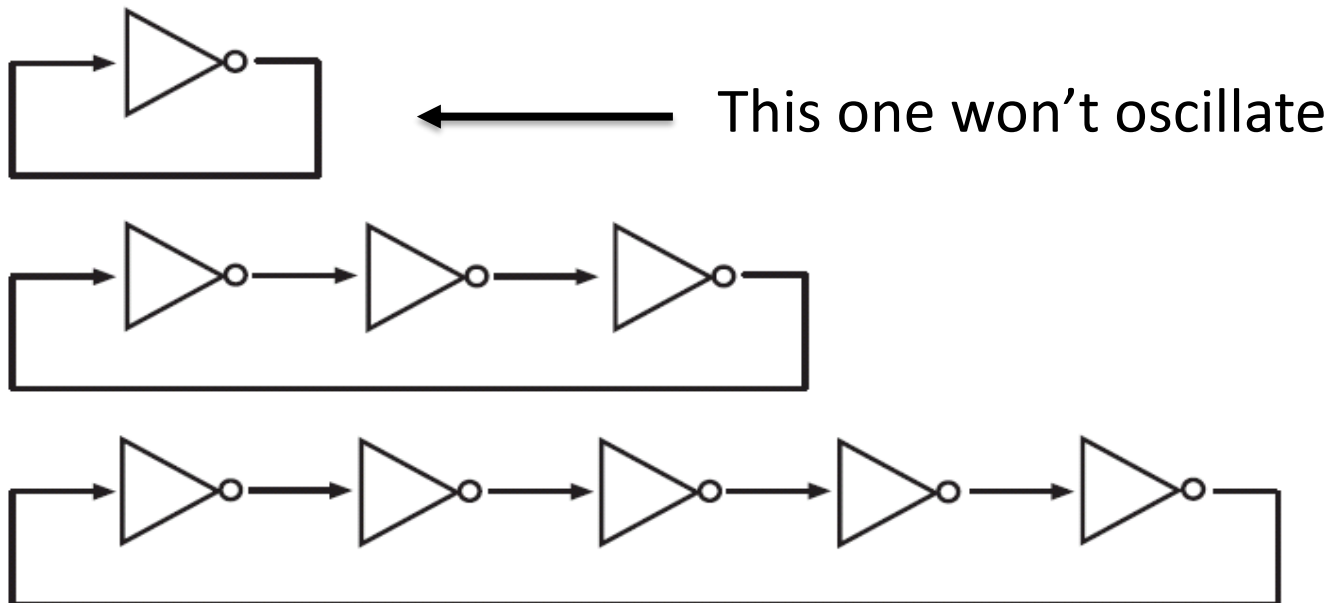
r = te.loada("""
    J1: -> x1; vm/(km + x3^n);
    x1 -> x2; k1*x1
    x2 -> x3; k2*x2
    x3 ->;    k3*x3;

    k1 = 0.5; k2 = 0.5; k3 = 0.5;
    n = 10;
    x1 = 2; x2 = 2; x3 = 2
    vm = 18; km = 0.5;
""")

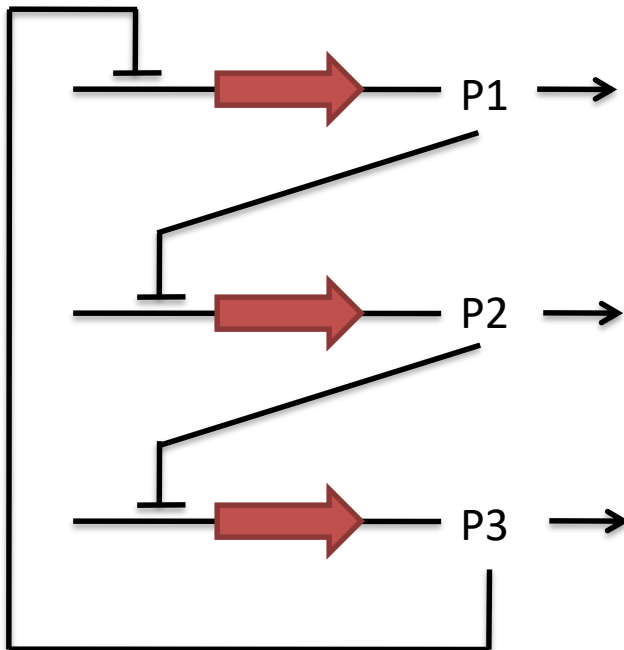
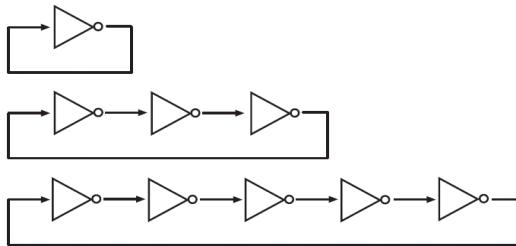
m = r.simulate (0, 30, 1000)
r.plot()
```



# Ring Oscillator



# Ring Oscillator – Jarnac Model



```
import tellurium as te
```

```
r = te.loada("""
```

```
J1: -> x1; vm1/(km1 + x3^n);
```

```
x1 -> ; k1*x1;
```

```
J3: -> x2; vm2/(km2 + x1^n);
```

```
x2 -> ; k2*x2;
```

```
J4: -> x3; vm3/(km3 + x2^n);
```

```
x3 -> ; k3*x3;
```

```
k1 = 0.5; k2 = 0.5; k3 = 0.5;
```

```
n = 4; vm1 = 5; vm2 = 5; vm3 = 5;
```

```
km1 = 0.5; km2 = 0.5; km3 = 0.5;
```

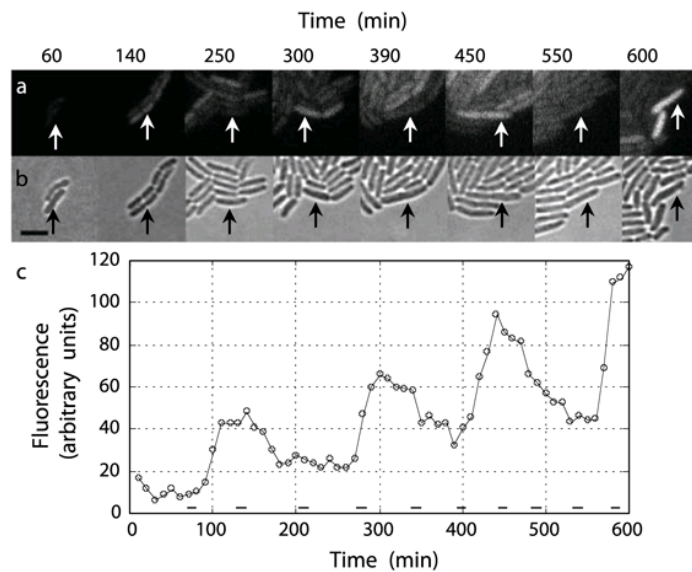
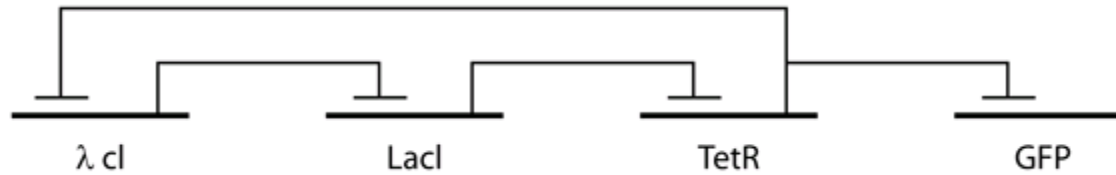
```
s1 = 0.6; x1 = 1; x2 = 1; x3 = 2
```

```
""")
```

```
m = r.simulate (0, 40, 100)
```

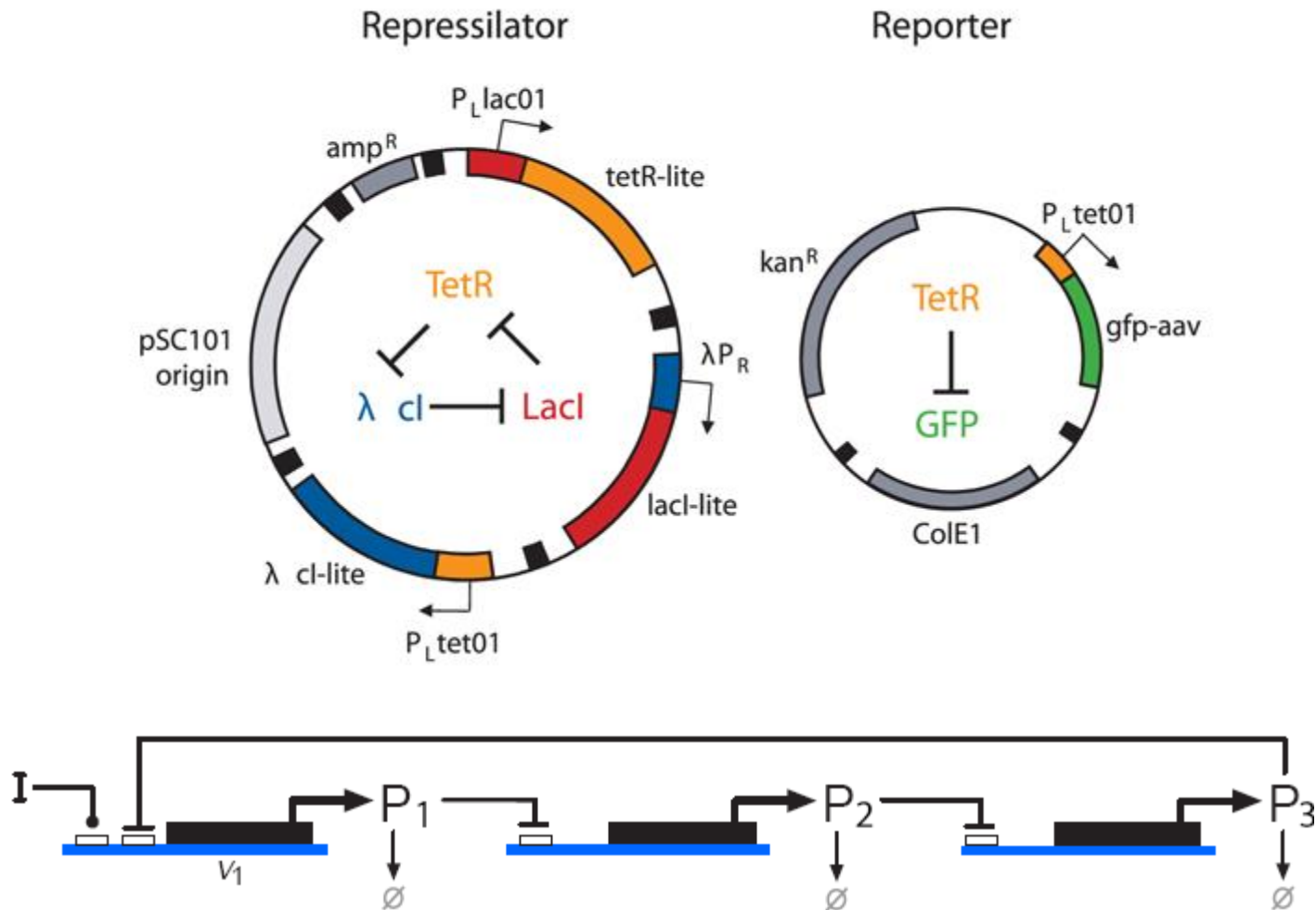
```
r.plot()
```

# Ring Oscillator: Repressilator



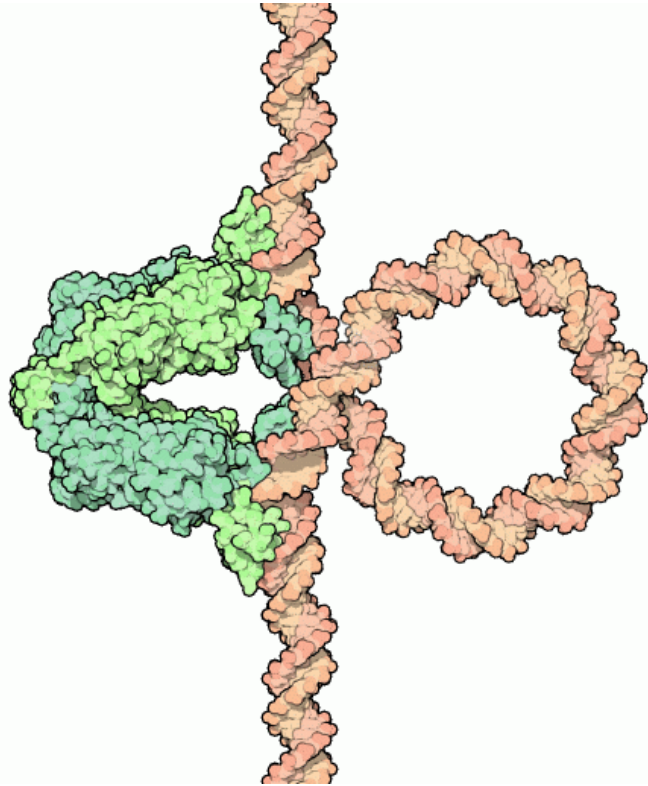
[A synthetic oscillatory network of transcriptional regulators](#) Michael B. Elowitz and Stanislas Leibler Nature 403, 335-338(20 January 2000)

# Ring Oscillator: Repressilator

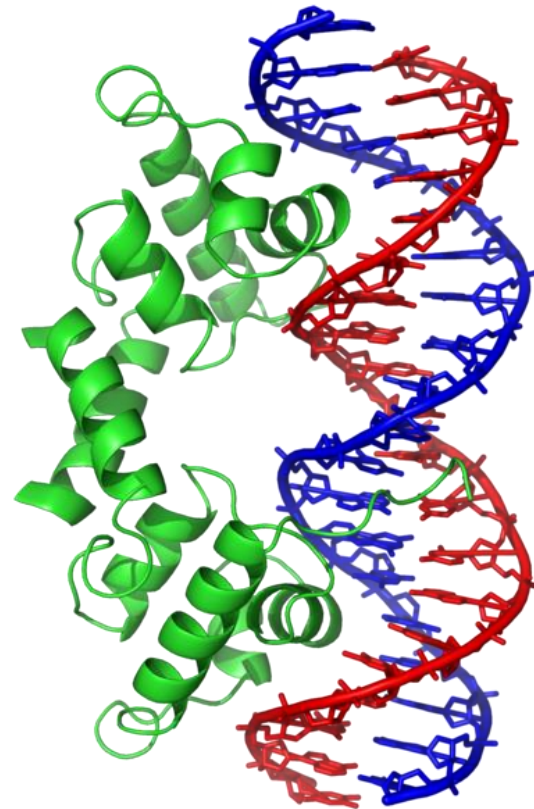


[A synthetic oscillatory network of transcriptional regulators](#) Michael B. Elowitz and Stanislas Leibler Nature 403, 335-338(20 January 2000)

# We'll Talk About Parts in the Next Class



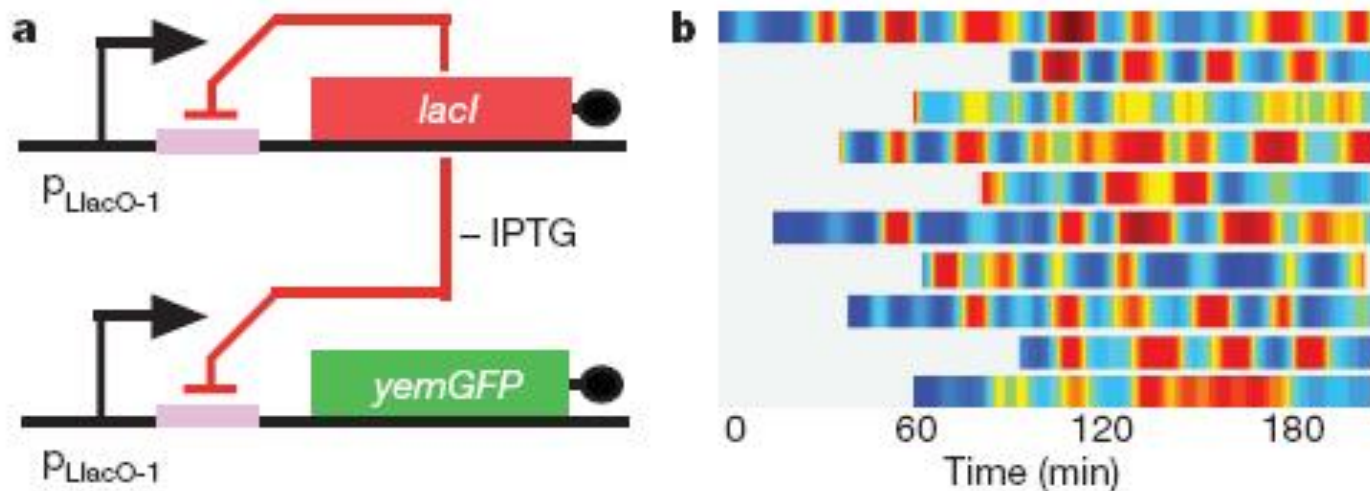
LacI Repressor



Lambda cI Repressor

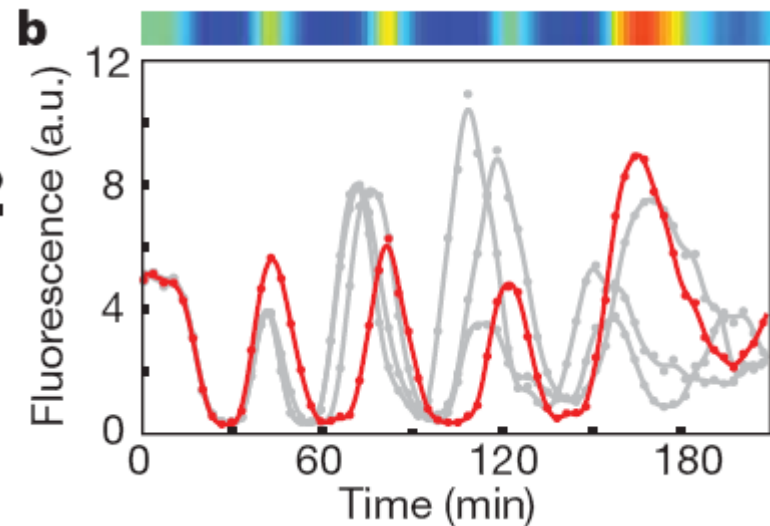
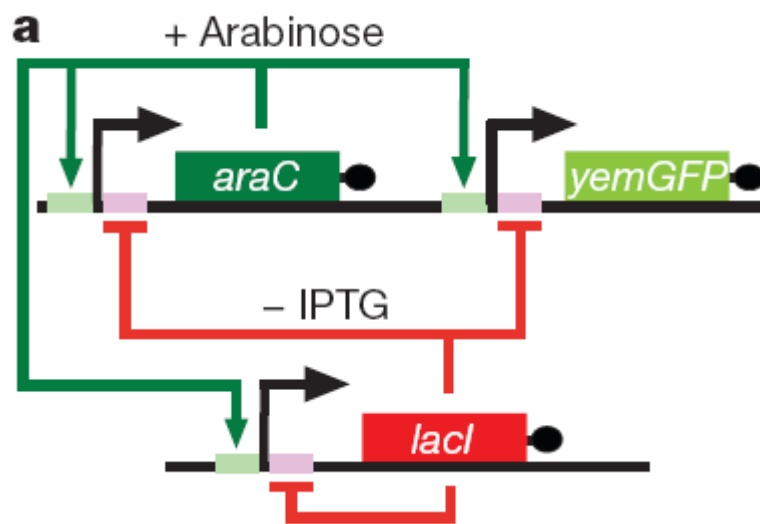
# Synthetic Oscillators: Feedback Oscillator

Ideally Oscillators should be studied in single cells.



A fast, robust and tunable synthetic gene oscillator. Jesse Stricker, Scott Cookson, Matthew R. Bennett, William H. Mather, Lev S. Tsimring & Jeff Hasty. *Nature* advance online publication 29 October 2008

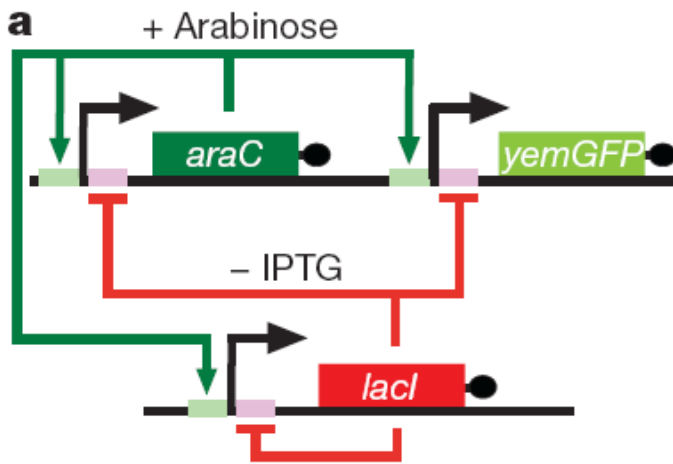
# Synthetic Oscillators: Relaxation Oscillator



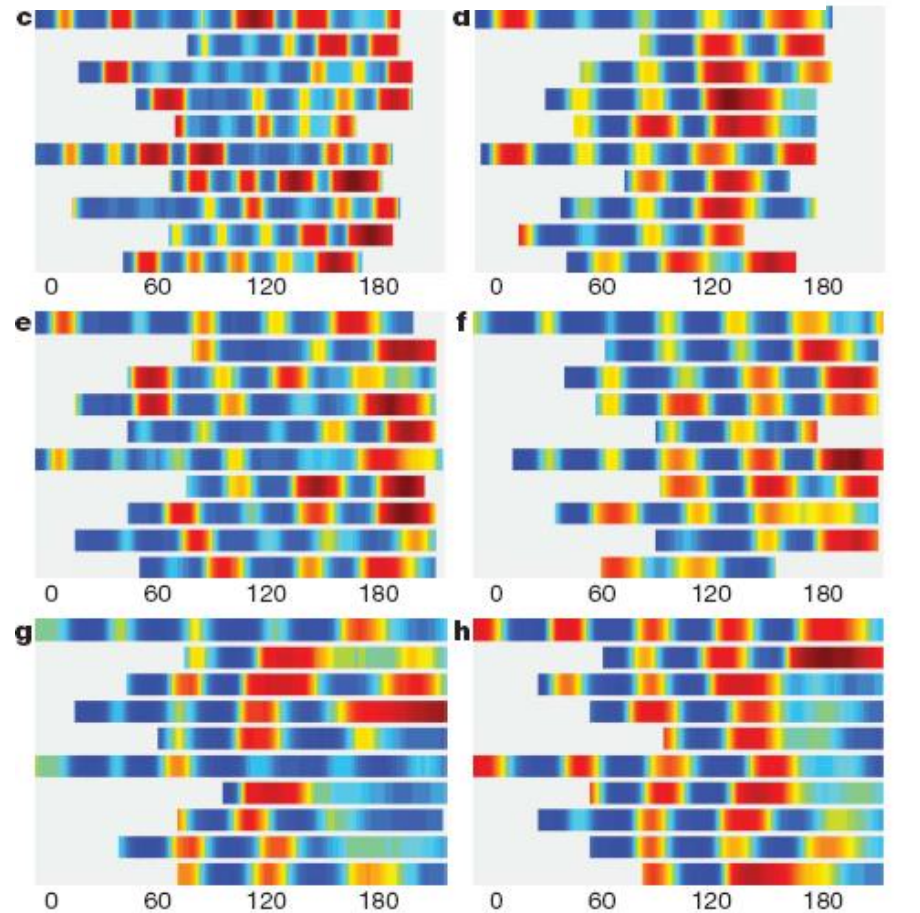
A fast, robust and tunable synthetic gene oscillator. Jesse Stricker, Scott Cookson, Matthew R. Bennett, William H. Mather, Lev S. Tsimring & Jeff Hasty. *Nature* advance online publication 29 October 2008

# Synthetic Oscillators: Relaxation Oscillator

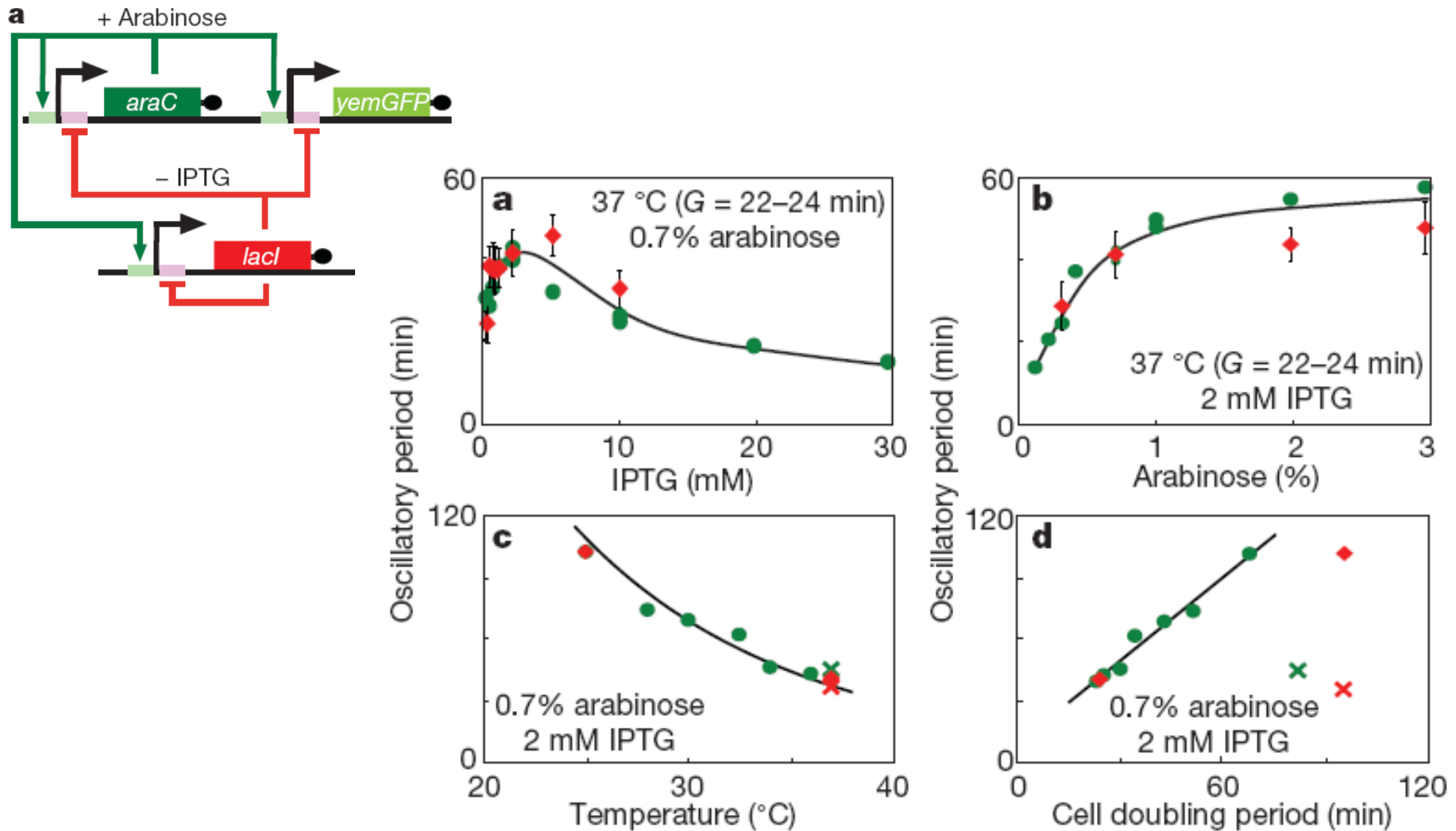
arbitrary units. **c–h**, Single-cell density map trajectories for various IPTG conditions (**c**, 0 mM IPTG; **d**, 0.25 mM; **e**, 0.5 mM; **f**, 1 mM; **g**, 2 mM; **h**, 5 mM).



A fast, robust and tunable synthetic gene oscillator. Jesse Stricker, Scott Cookson, Matthew R. Bennett, William H. Mather, Lev S. Tsimring & Jeff Hasty. *Nature* advance online publication 29 October 2008

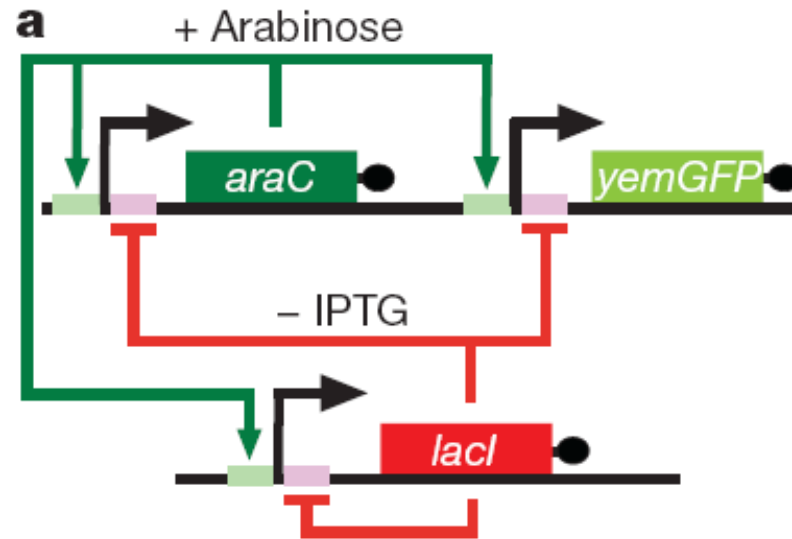


# Synthetic Oscillators: Relaxation Oscillator



**Figure 2 | Robust oscillations.** a–c, Oscillatory periods on transects with

# Synthetic Oscillators: Movies



<http://www.nature.com/nature/journal/vaop/ncurrent/supinfo/nature07389.html>

# Synthetic Oscillators: Mammalian

Vol 457 | 5 January 2009 | doi:10.1038/nature07616

nature

LETTERS

## A tunable synthetic mammalian oscillator

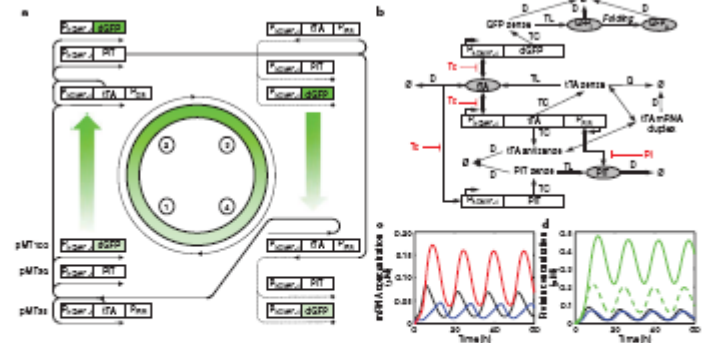
Marcel Tigges<sup>1</sup>, Tatiana T. Marquez-Lago<sup>1,2,3</sup>, Jörg Stelling<sup>1,2,3</sup> & Martin Fussenegger<sup>1</sup>

*Nature* **457**, 309–312 (15 January 2009) doi:10.1038/nature07616; Received 26 July 2008; Accepted 4 November 2008

**A tunable synthetic mammalian oscillator** Marcel Tigges, Tatiana T. Marquez-Lago, Jörg Stelling & Martin Fussenegger

Autonomous and self-sustained oscillator circuits mediating the periodic induction of specific target genes are minimal genetic time-keeping devices found in the central and peripheral circadian clocks<sup>1,2</sup>. They have attracted significant attention because of their intriguing dynamics and their importance in controlling critical repair<sup>3</sup>, metabolic<sup>4</sup> and signalling pathways<sup>5</sup>. The precise molecular mechanism and expression dynamics of this mammalian circadian clock are still not fully understood. Here we describe a synthetic mammalian oscillator based on an auto-regulated sense-antisense transcription control circuit encoding a positive and a time-delayed negative feedback loop, enabling autonomous, self-sustained and tunable oscillatory gene expression. After detailed systems design with experimental analyses and mathematical modelling, we monitored oscillating concentrations of green fluorescent protein with tunable frequency and amplitude by time-lapse microscopy in real time in individual Chinese hamster ovary cells. The synthetic mammalian clock may provide an insight into the dynamics of natural periodic processes and foster advances in the design of post-thetic networks in future gene and cell therapies.

Synthetic gene circuits that emulate the expression dynamics of living systems provide new insights into the connectivity of genes and proteins in the post-genomic era<sup>6</sup> and they advance our understanding of complex control networks. Circadian pacemakers<sup>7,8</sup> are of particular interest because they coordinate many periodic physiological activities. The mammalian circadian clock consists of a central pacemaker in the suprachiasmatic nuclei of mammalian brains<sup>9</sup>, with subsidiary oscillators in most peripheral cell types<sup>10,11</sup>. In contrast to neurons in the suprachiasmatic nuclei, peripheral oscillations damp rapidly when disconnected from remote control by the suprachiasmatic nuclei<sup>12</sup>. However, both oscillators rely on a very similar gene circuitry that involves a set of transcription repressors (CRY and PER) and activators (BMAL1 and CLOCK) connected by mutual feedback<sup>13</sup>. Previously designed simple synthetic gene networks in bacteria showed self-sustained<sup>14</sup>, damped<sup>15</sup> or metabolically controlled oscillations<sup>16</sup>, but those oscillations lacked robustness and/or tunability. In mammalian cells, even synthetic clock replicas using natural components and network design have not provided oscillating transgene expression<sup>17</sup> as observed for reporter genes plugged

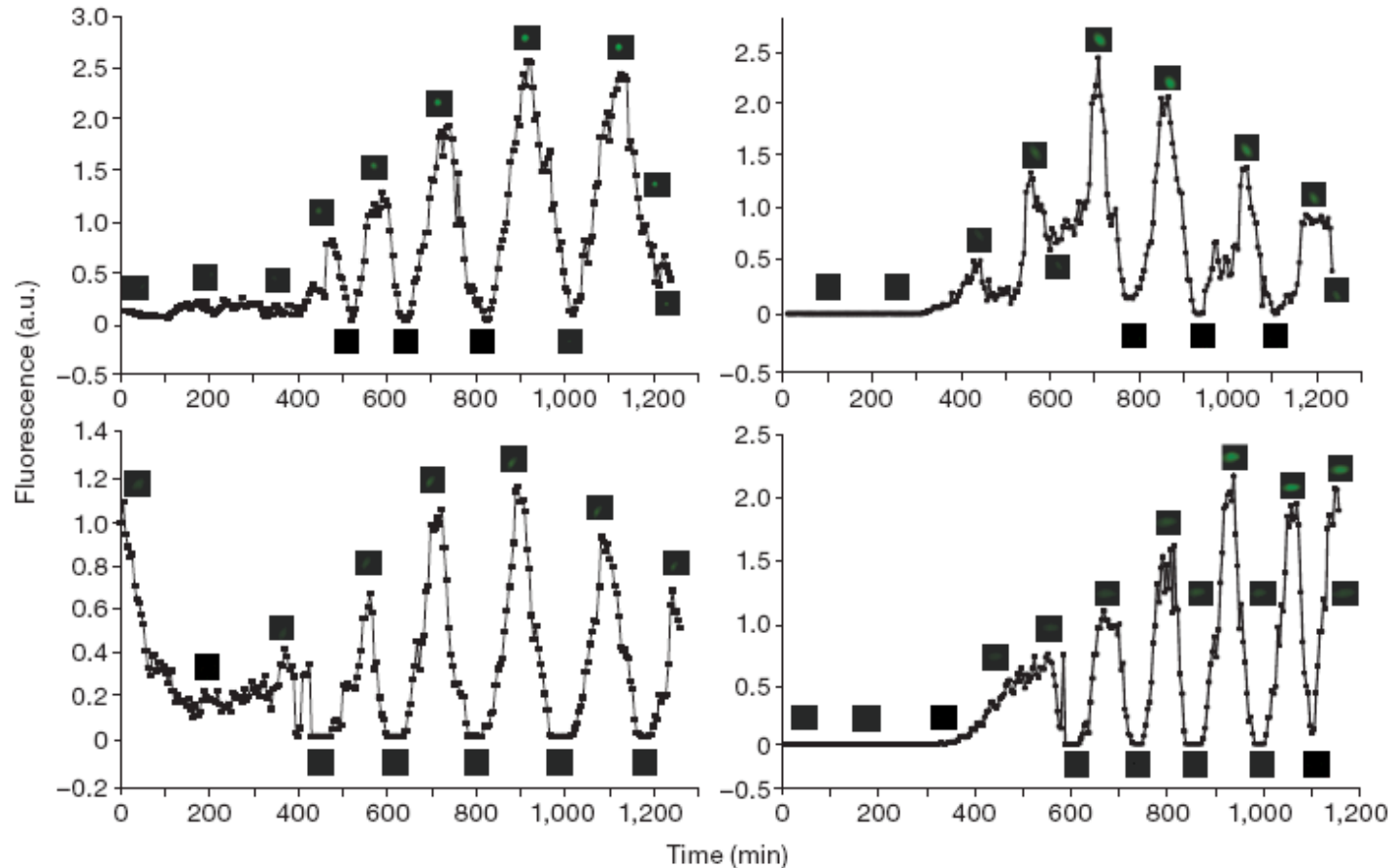


**Figure 1** Mammalian clock components and predicted oscillation dynamics. **a**, Core mammalian oscillator. An auto-regulated  $P_{Ub^{TM}}$ -driven ITA transcription triggers an increasing expression of sense ITA ( $pMT35$ ),  $Ub^{TM}$ -GFP ( $pMT100$ ) and dPTT ( $pMT36$ ) (1). As  $Ub^{TM}$ -GFP and dPTT levels reach a peak (2), PTT transiently induces  $P_{ub}$ -driven ITA anti-sense expression (3), resulting in a gradual decrease in sense ITA, PTT and  $Ub^{TM}$ -GFP (4). **b**, Intracellular processes considered in the mathematical model. Abbreviation and symbols are as follows: a single-headed arrow is, irreversible

reaction; a double-headed arrow is, reversible reaction;  $\emptyset$ , inhibitor of degradation process; dGFP, destabilized GFP; TC, transcription; P, protein synthesis; GFP, unfolded inactive GFP; GFP\*, folded active GFP; TC, transcription; TL, translation; D, degradation; c, d, Model predictions for the reference parameters  $\tau$  (plasmid ratio 1:1:1, no an-thibiotic) with mRNA concentrations (c) (black, ITA; blue, PTT; red, ITA-sense-antisense duplex) and protein concentrations (d) (black, ITA; blue, PTT; dashed green, unfolded GFP; solid green, active GFP).

<sup>1</sup>Department of Biosystems Science and Engineering, ETH Zurich, Mattenstrasse 26, CH-8050 Basel, Switzerland; <sup>2</sup>Institute of Computational Science and <sup>3</sup>Swiss Institute of Bioinformatics, ETH Zurich, CH-8092 Zurich, Switzerland

# Synthetic Oscillators: Mammalian



*Nature* **457**, 309-312 (15 January 2009) doi:10. 1038/nature07616; Received 26 July 2008;  
Accepted 4 November 2008

**A tunable synthetic mammalian oscillator** Marcel Tigges, Tatiana T. Marquez-Lago, Jörg Stelling  
& Martin Fussenegger

# Natural Oscillators

1. Circadian rhythms (eg *Drosophila*, 24 hour period, feedback oscillator)
2.  $\text{Ca}^{++}$  Oscillations
3. Glycolytic Oscillations\* (relaxation oscillator)
4. Signaling Pathway Oscillations (P53, ERK, NF- $\kappa$ B)
5. Cell Cycle (relaxation oscillator)
6. Synchronous Rhythmic Flashing Of Fireflies
7. Segmentation during development
8. Many examples of chemical oscillators (mostly relaxation oscillators)
9. ....

\* During growth phase on glucose and ethanol, starve yeast of glucose, add cyanide and glucose, the glycolytic pathway will oscillate (NAD/NADH, ATP/ADP)

Buck, John; "Synchronous Rhythmic Flashing of Fireflies. II," *Quarterly Review of Biology*, 63:265, 1988



# Oscillatory Systems

## Natural Oscillatory Networks

Resilient circadian oscillator revealed in individual cyanobacteria Irina Mihalcescu, Weihong Hsing & Stanislas Leibler, *Nature* **430**, 81-85 (1 July 2004)

Hoffmann, A., Levchenko, A., Scott, M.L. and Baltimore, D. (2002) *Science* 298, 1241–1245 The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation.

Oscillations and variability in the p53 system. Naama Geva-Zatorsky et al, *Molecular Systems Biology* **2**  
Article number: 006.0033 doi:10.1038/msb4100068

Shih YL, Le T, Rothfield L: Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. *Proc Natl Acad Sci USA* 2003, 100:7865-7870.

Transduction of Intracellular and Intercellular Dynamics in Yeast Glycolytic Oscillations, Wolf et al, *Biophys J*, 78, 1145-1153 (2000)

**Review:** Oscillations in cell biology Karsten Kruse and Frank Julicher, *Current Opinion in Cell Biology* 2005, 17:20–26

# Oscillatory Systems

## Synthetic Oscillatory Networks

Elowitz, M. B. & Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* 403, 335–338 (2000).

Atkinson, M. R., Savageau, M. A., Myers, J. T. & Ninfa, A. J. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. *Cell* 113, 597–607 (2003).

A synthetic gene–metabolic oscillator  
Eileen Fun et al, *Nature*, 435, 118-122 (2005)

An excitable gene regulatory circuit induces transient cellular differentiation, *Nature* **440**, 545-550 (23 March 2006) , Gürol M. Süel et al

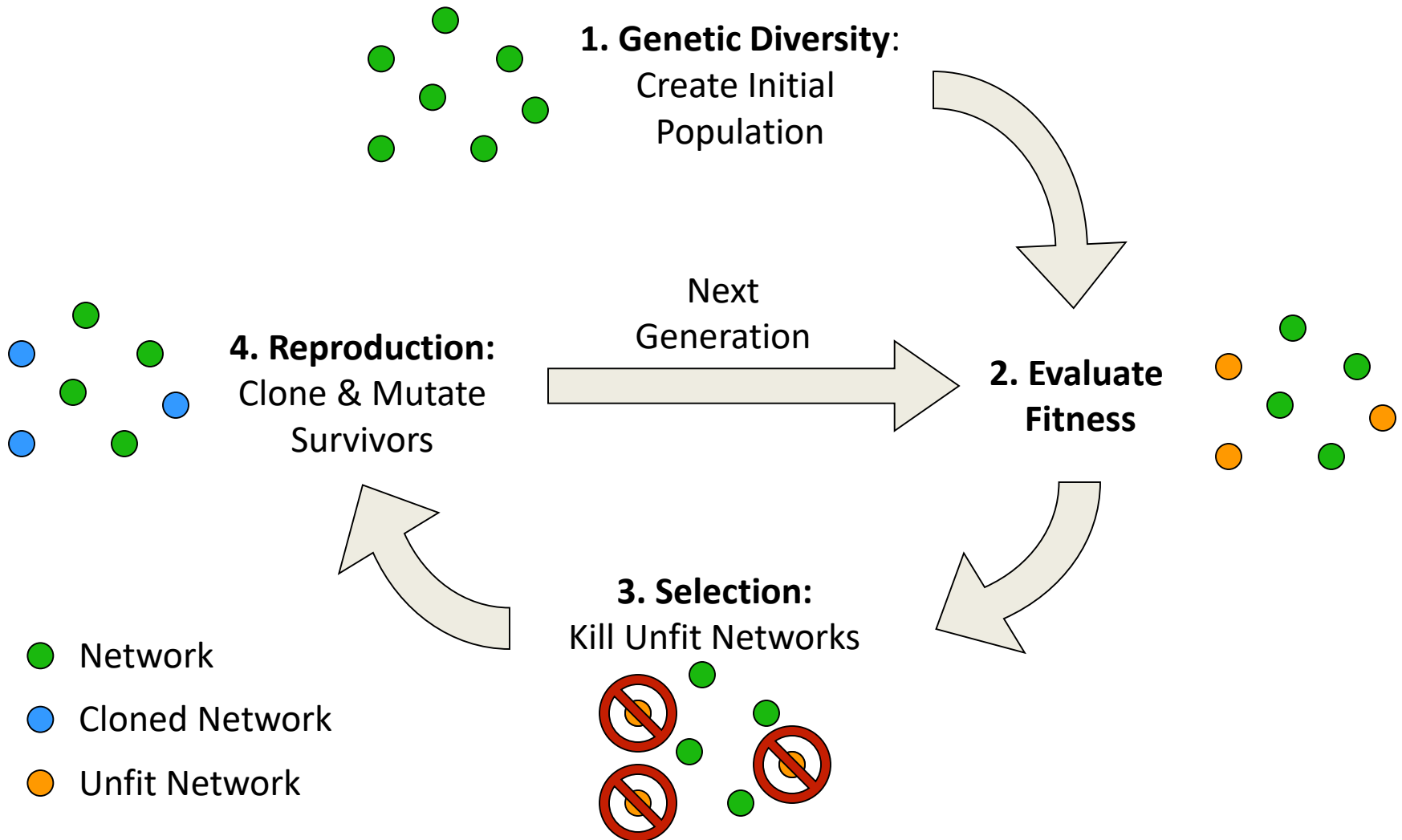
Elowitz, M. B. & Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* 403, 335–338 (2000).

*Nature* **457**, 309-312 (15 January 2009) doi:10.1038/nature07616; Received 26 July 2008; Accepted 4 November 2008 **A tunable synthetic mammalian oscillator**  
Marcel Tigges· Tatiana T. Marquez-Lago, Jörg Stelling & Martin Fussenegger

A fast, robust and tunable synthetic gene oscillator. Jesse Stricker, Scott Cookson, Matthew R. Bennett, William H. Mather, Lev S. Tsimring & Jeff Hasty. *Nature* advance online publication 29 October 2008

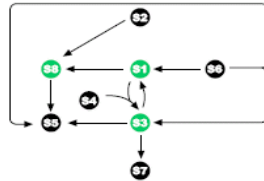
**Review:** Oscillations in cell biology Karsten Kruse and Frank Julicher, *Current Opinion in Cell Biology* 2005, 17:20–26

# Evolving Oscillators in silico

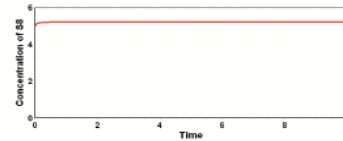


# Evolution of an Oscillator

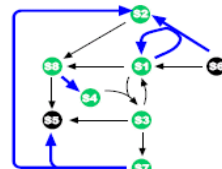
I. After one Generation



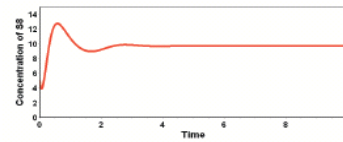
Response is flat



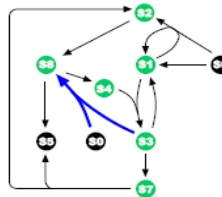
II. After 10 Generations



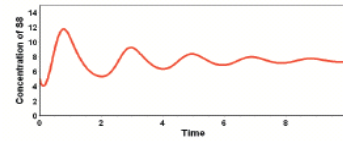
Beginning of Oscillatory Behavior



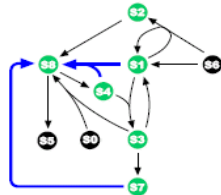
III. After 50 Generations



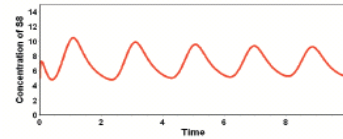
Damped Oscillations



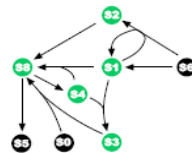
IV. After 100 Generations



Sustained Oscillations

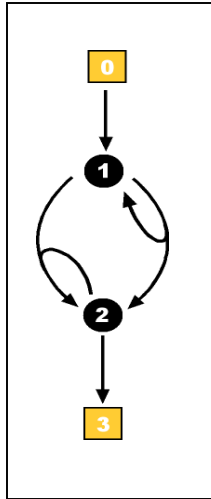


VI. Core Oscillatory Network

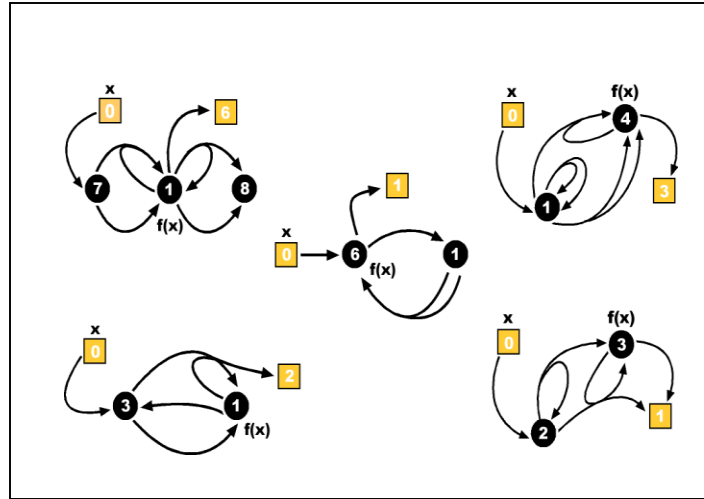


# Selection of Evolved Networks

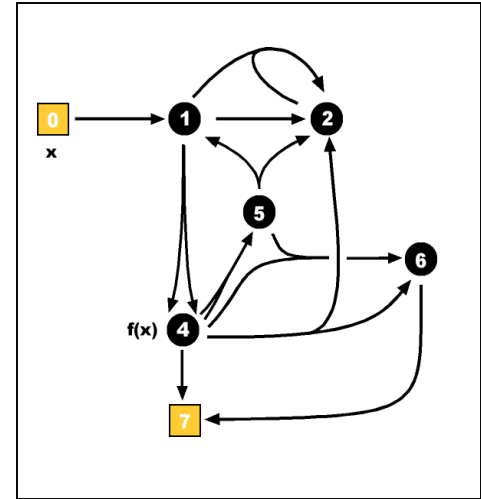
## Mathematical Functions



Quadratic



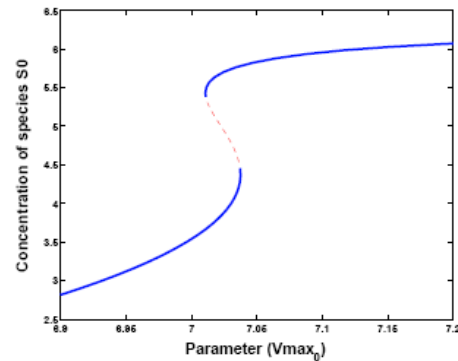
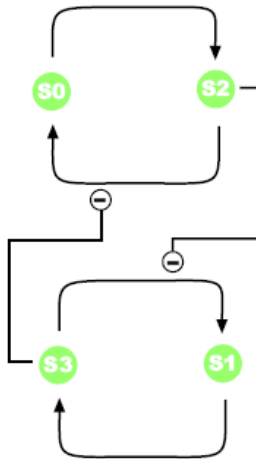
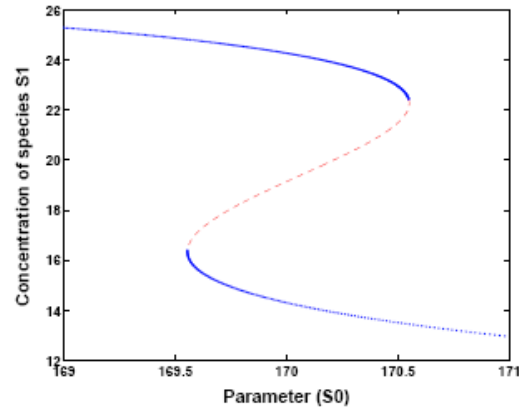
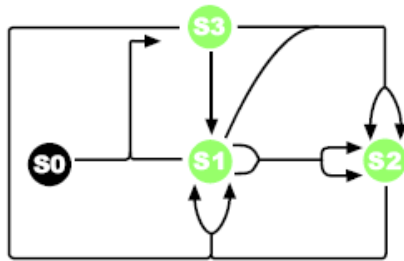
Square Root



Cubic

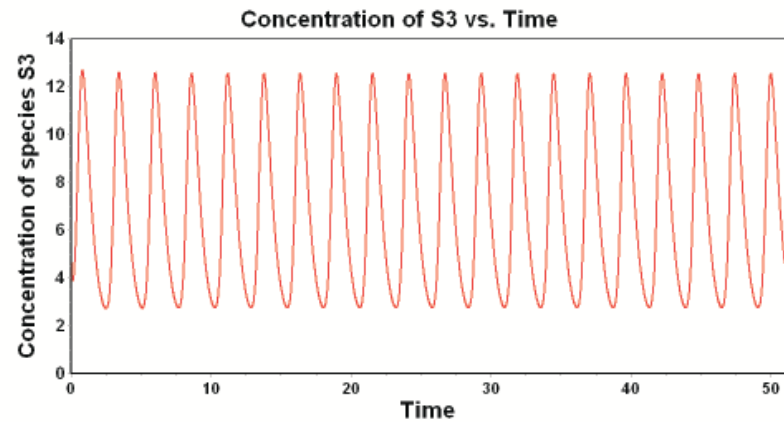
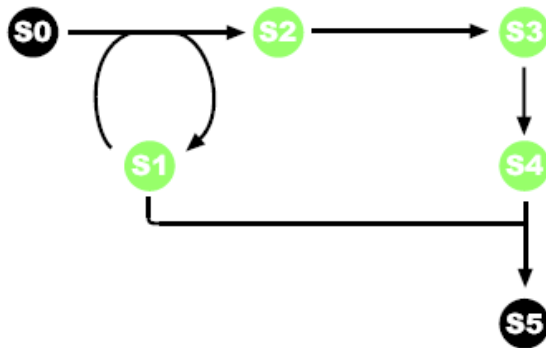
# Selection of Evolved Networks

## Bistable Networks



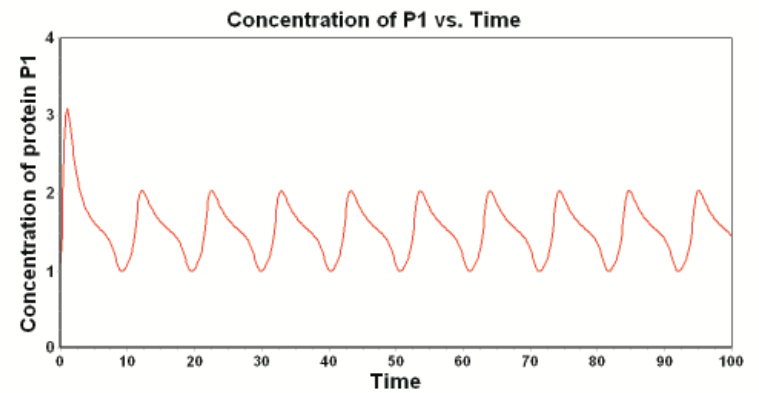
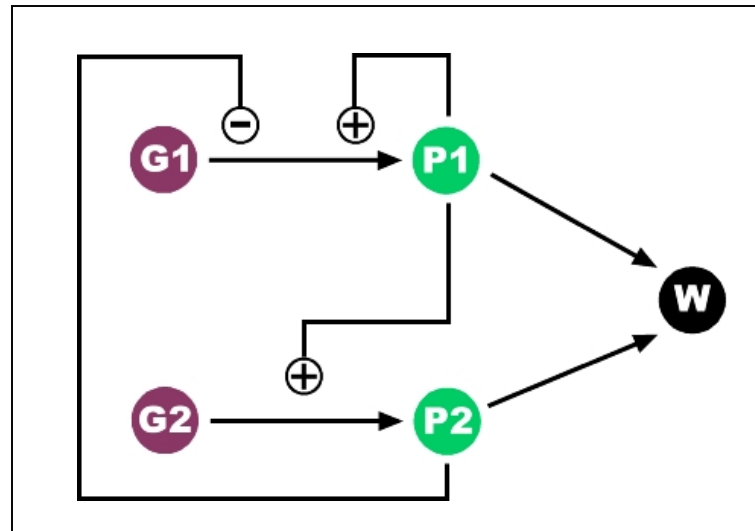
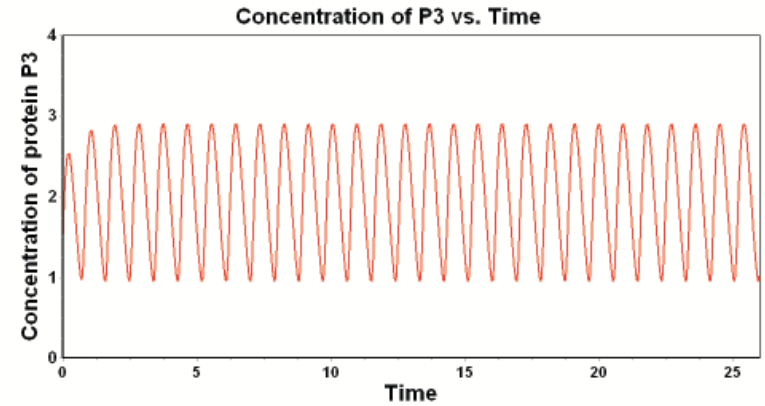
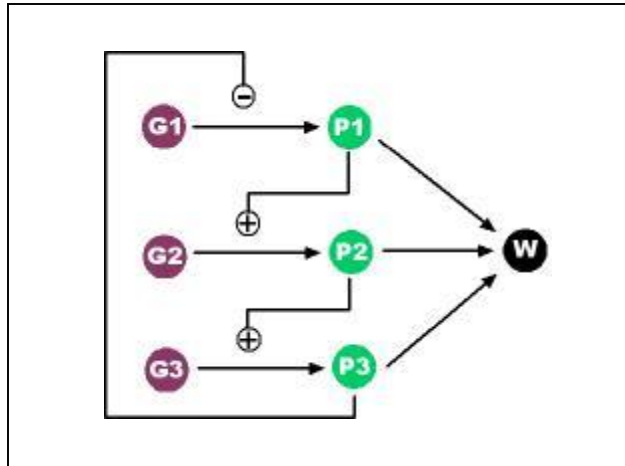
# Selection of Evolved Networks

## Oscillators



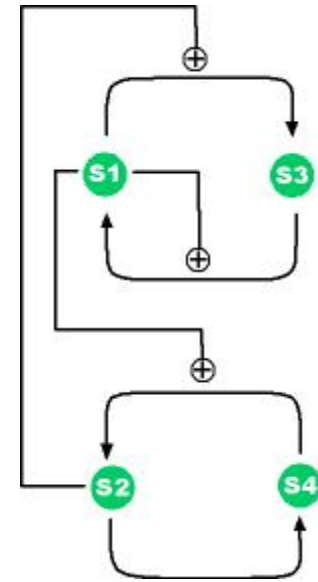
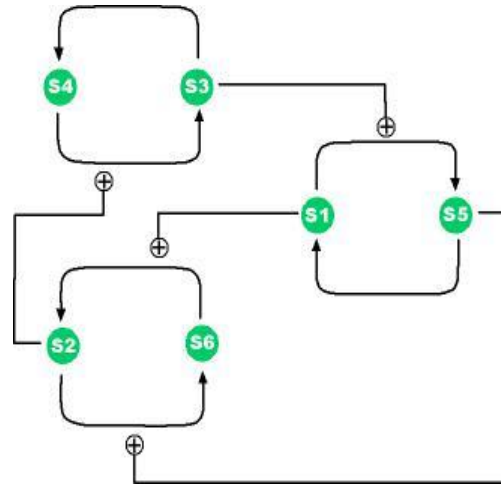
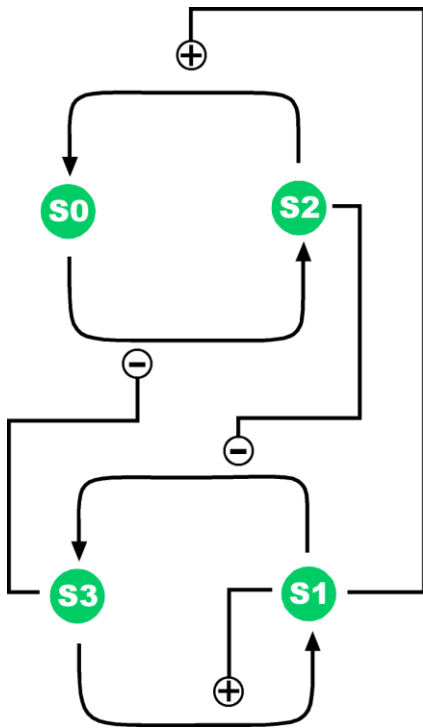
# Selection of Evolved Networks

## Oscillators



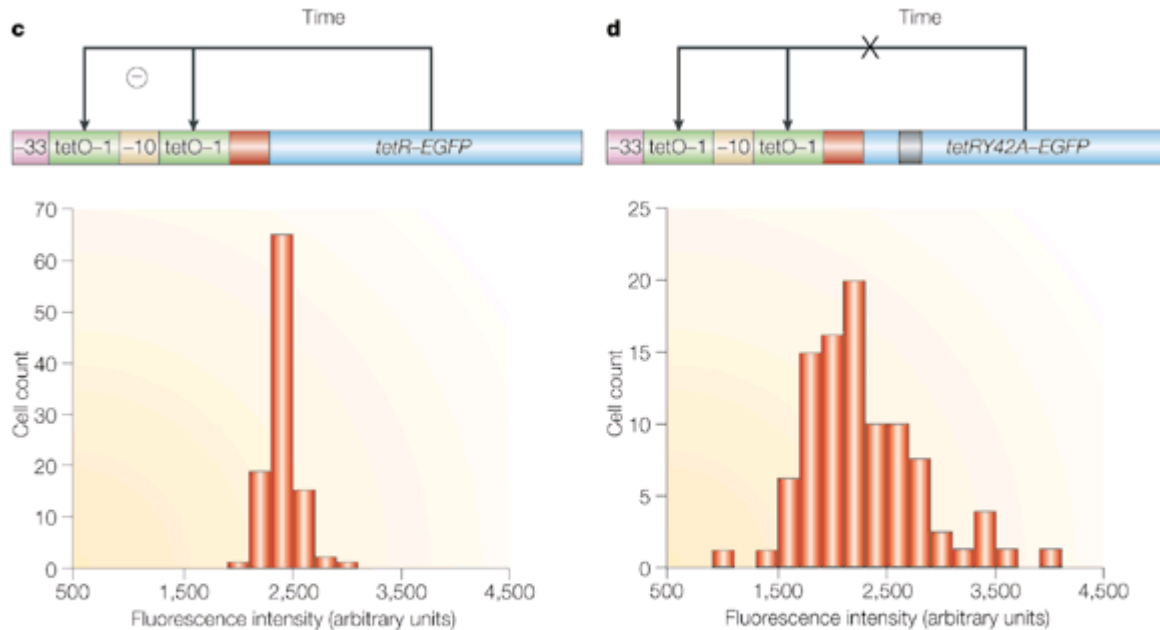
# Selection of Evolved Networks

Oscillators



# Negative Feedback

Reduction in noise due to negative feedback.



Nature Reviews | Genetics

Review: Computational studies of gene regulatory networks: in numero molecular biology Jeff Hasty, David McMillen, Farren Isaacs & James J. Collins  
Nature Reviews Genetics 2, 268-279 (April 2001)

Original Paper: Becskei, A. & Serrano, L. Engineering stability in gene networks by autoregulation. Nature 405, 590– 593 (2000).