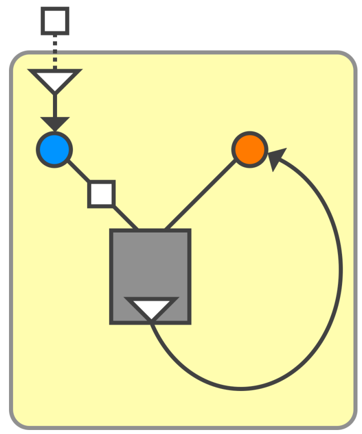
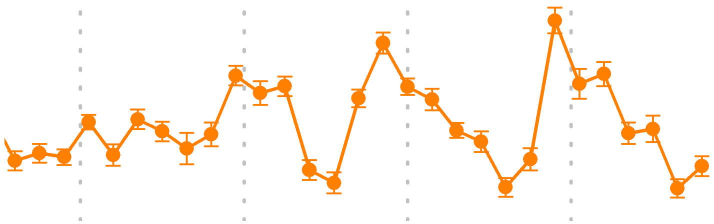
The watchmaker's apprentice:

Building a synthetic genetic oscillator with parts borrowed from nature

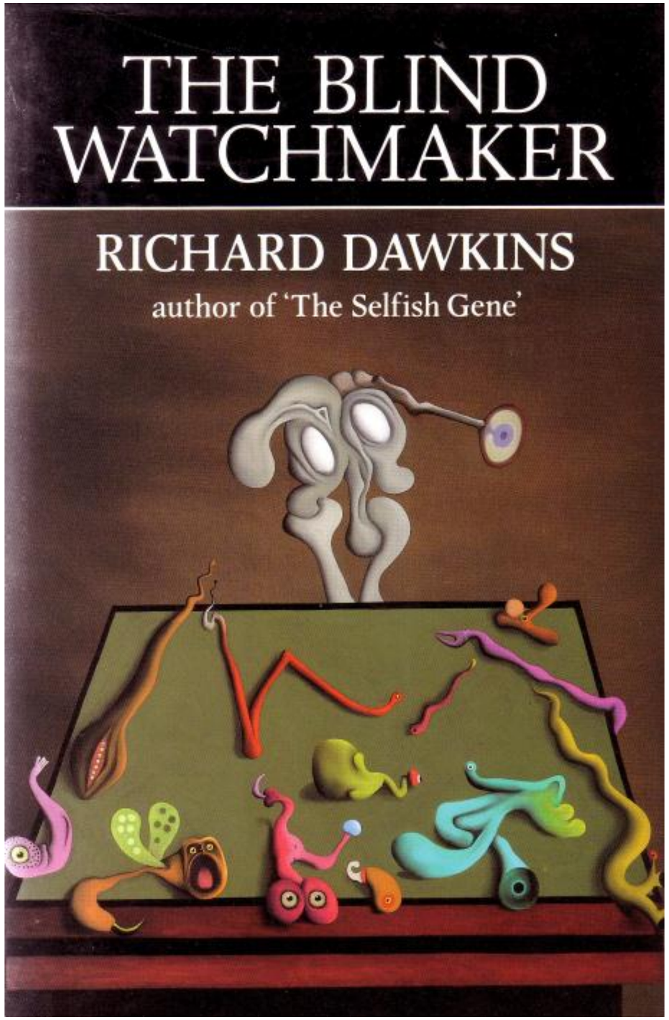




Mukund Thattai
Simons Centre for the Study of Living Machines
NCBS/TIFR

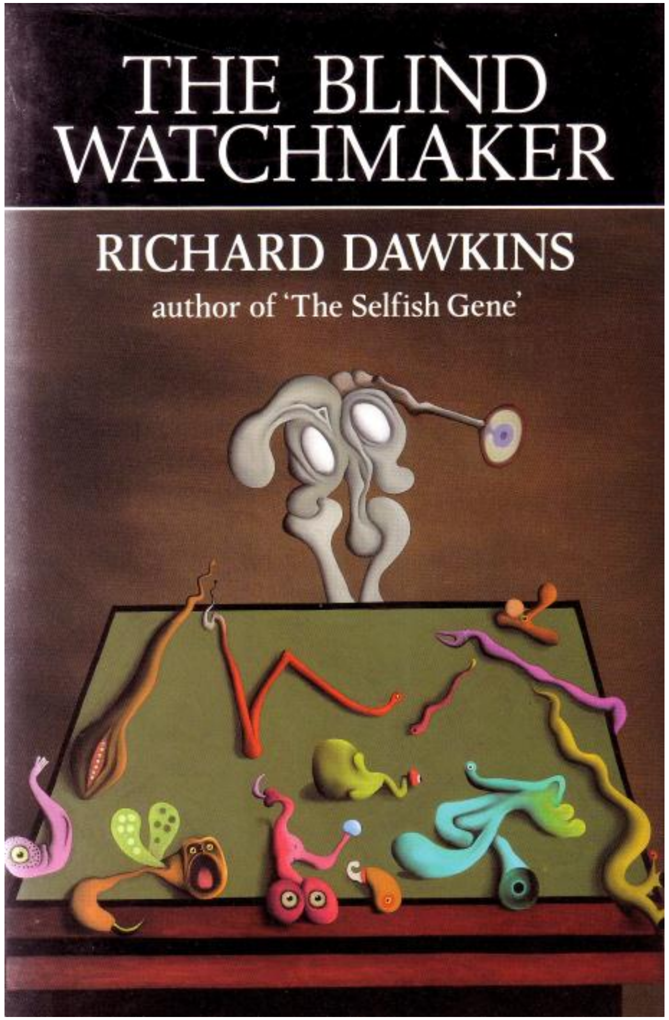
September 2013

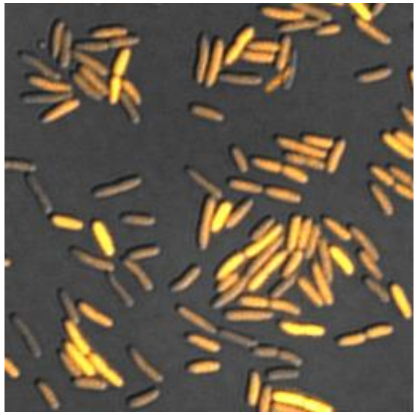
The blind watchmaker generates complexity through the undirected processes of mutation and selection...



The blind watchmaker generates complexity through the undirected processes of mutation and selection...

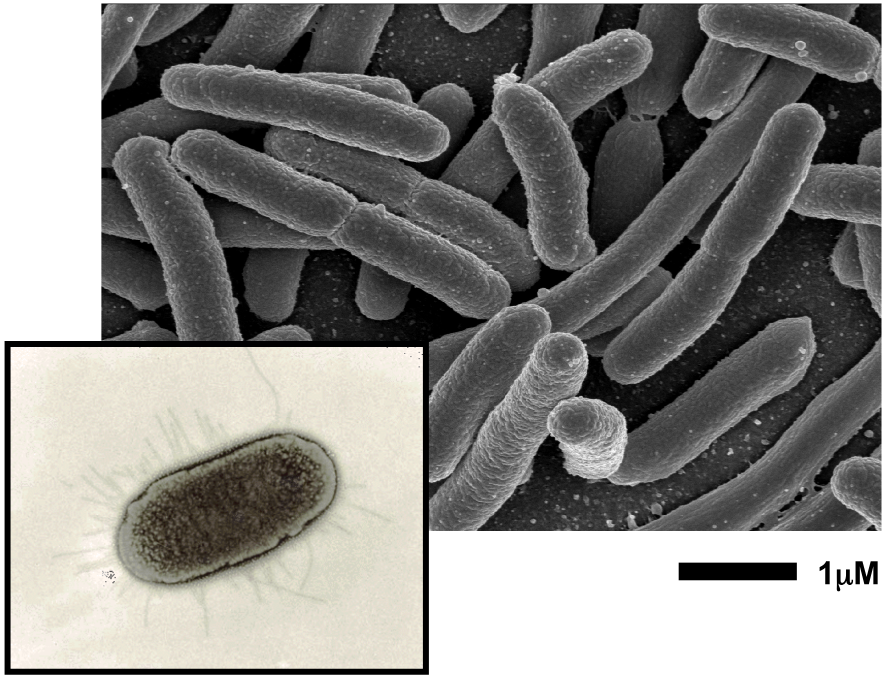
... while the watchmaker's apprentice has a design in mind, and uses the complex parts invented by nature to realize it





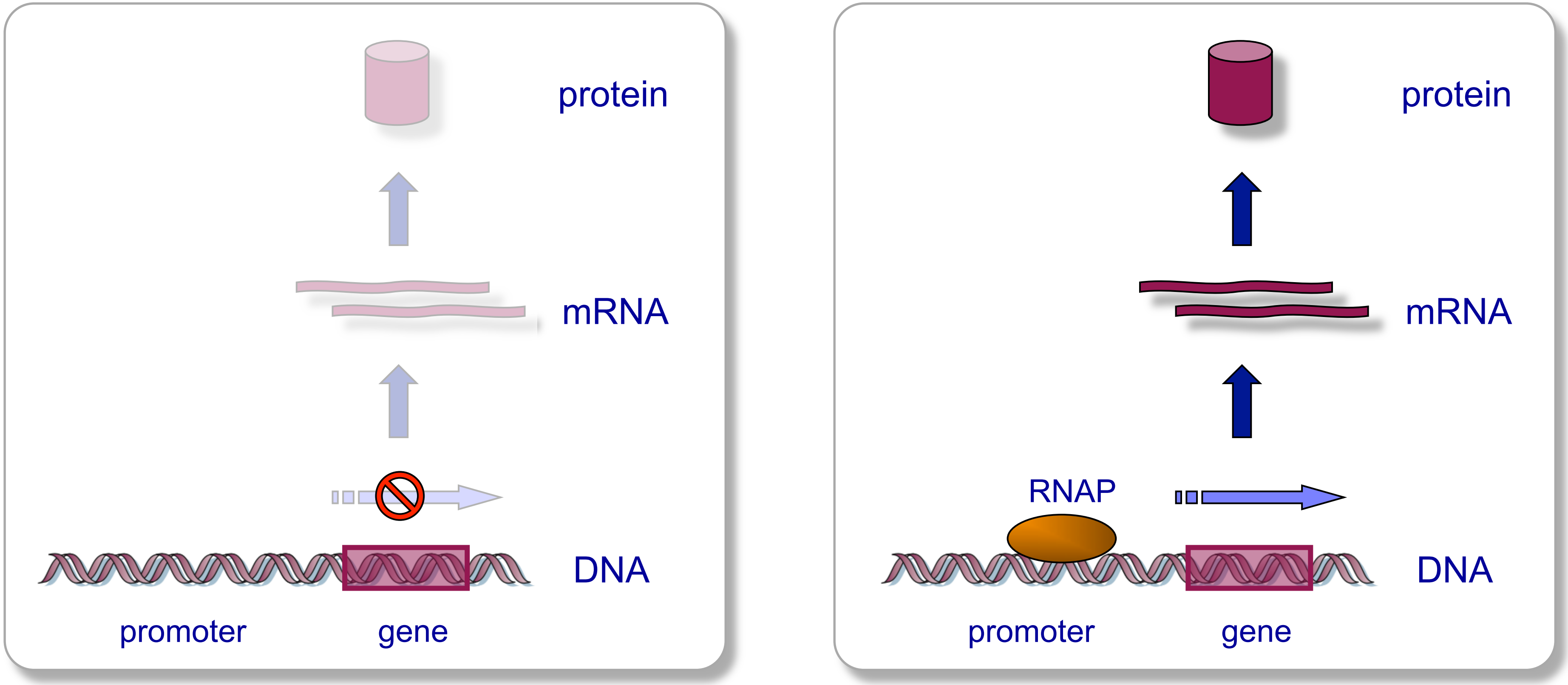
A brief introduction to genetic networks

The *E. coli* genome contains about 4000 genes...

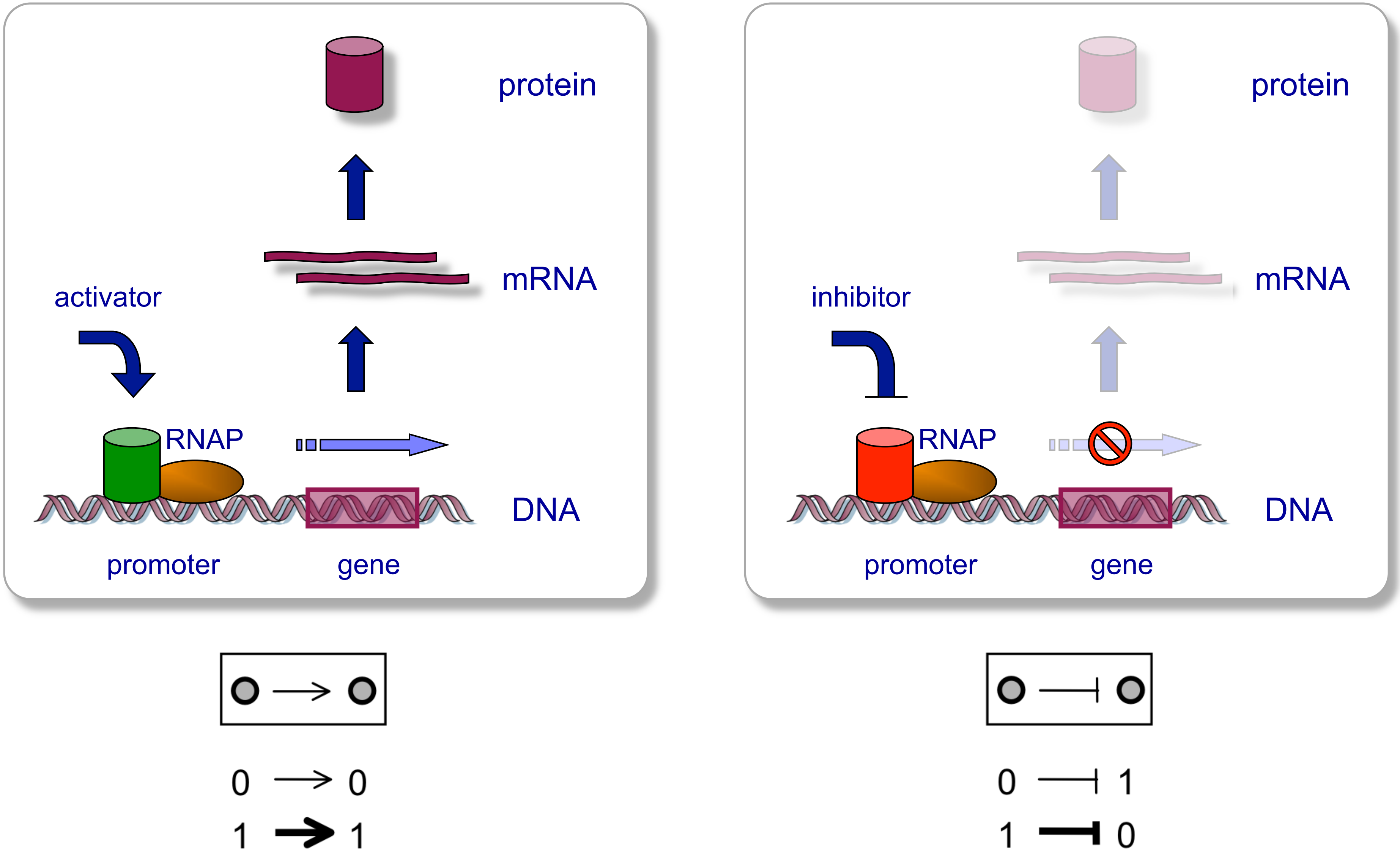


... and the human genome contains about 24000 genes

Genes can be turned on and off, allowing combinatorial complexity



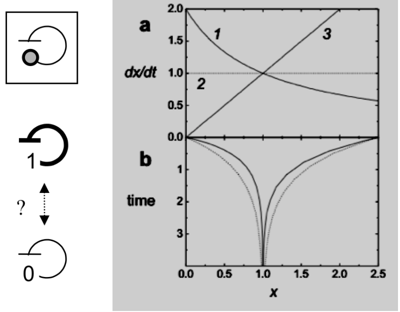
Genes can be turned on and off, allowing combinatorial complexity



Genes can be connected into networks with useful dynamical properties

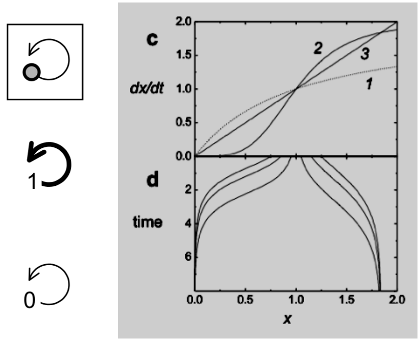
Noise reduction

Negative feedback

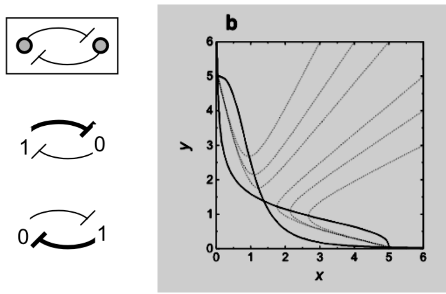


Memory

Positive feedback

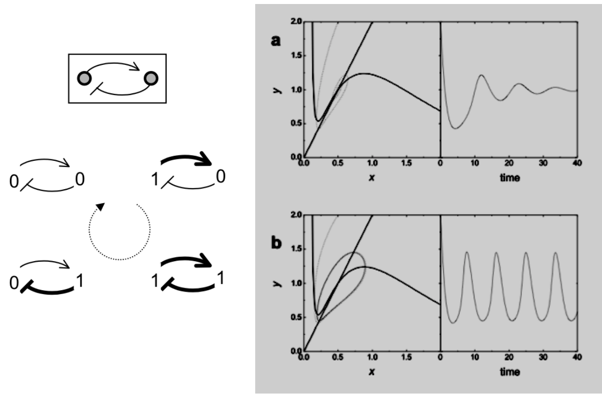


Flip-flop

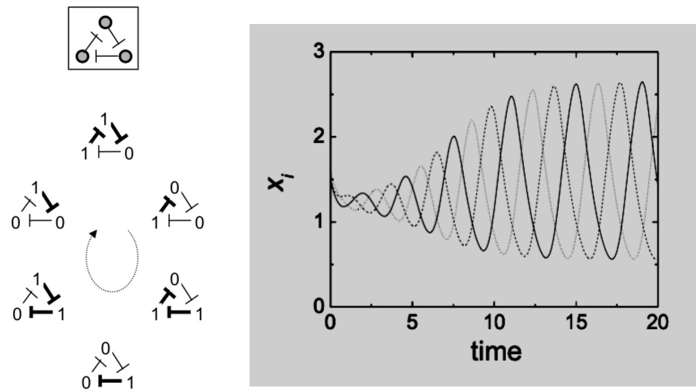


Oscillations

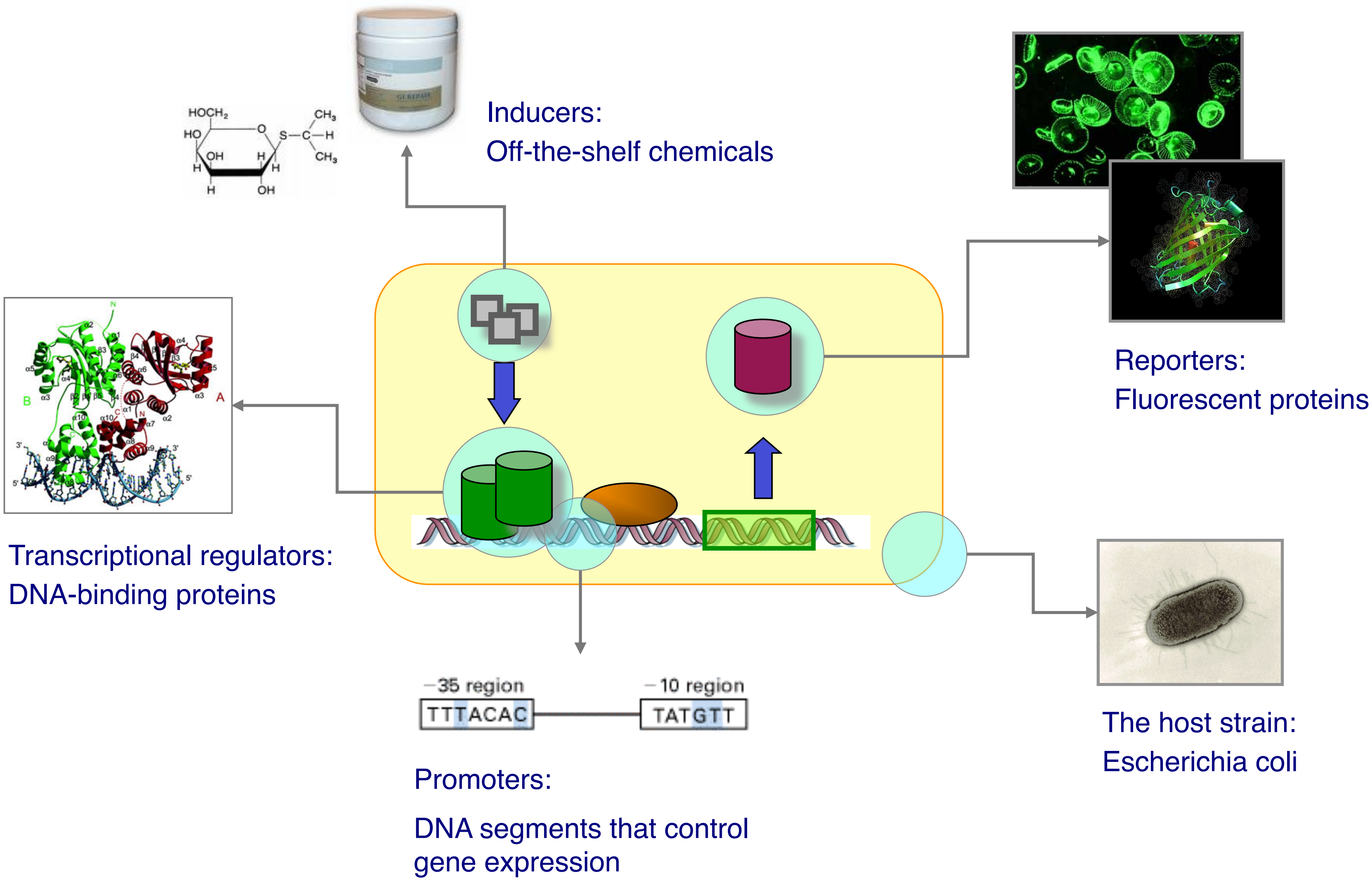
Hysteretic oscillator



Ring oscillator

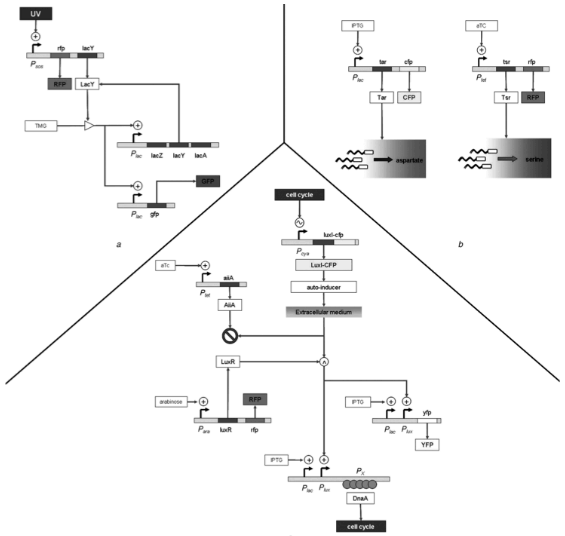


We can build networks from basic parts...



... but getting things to work is hard!

In principle, UV exposure would trigger transient expression of lacY, and the resulting TMG uptake would turn on lac expression. **In practice, the design did not function as expected.** We soon realised that the presence of extraneous lacI binding sites on the plasmid backbone had perturbed the system.



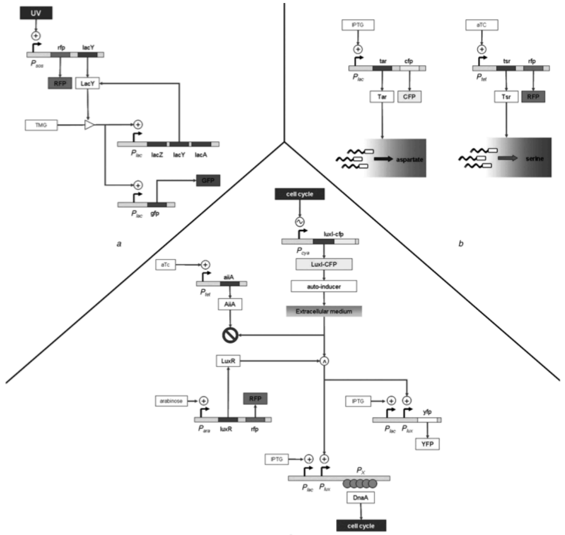
However, imple- menting such a gradient turned out to be far from trivial. **Moreover, we were not able to rescue chemotaxis in the knockout strain.**

As expected, the RFP control signal did not oscillate. **However, synchronisation could not be achieved** because the presence of DnaA boxes did not seem to affect cell-cycle progression.

S. Dabholkar, M. Thattai. Brainstorming biology. IET Synth. Biol. 1, 17-20 (2007)

... but getting things to work is hard!

In principle, UV exposure would trigger transient expression of lacY, and the resulting TMG uptake would turn on lac expression. **In practice, the design did not function as expected.** We soon realised that the presence of extraneous lacI binding sites on the plasmid backbone had perturbed the system.



However, imple- menting such a gradient turned out to be far from trivial. **Moreover, we were not able to rescue chemotaxis in the knockout strain.**

As expected, the RFP control signal did not oscillate. **However, synchronisation could not be achieved** because the presence of DnaA boxes did not seem to affect cell-cycle progression.

METHODOLOGY

Introduction of customized inserts for streamlined assembly and optimization of BioBrick synthetic genetic circuits

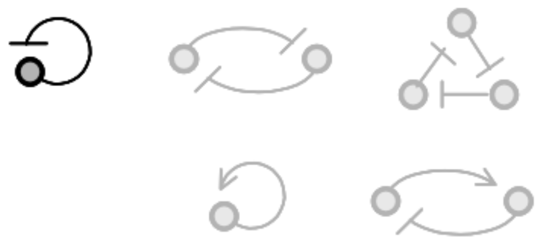
Julie E Norville[1,2,3*], Ratmir Derda[4,5,6], Saurabh Gupta[2], Kelly A Drinkwater[1,2], Angela M Belcher[2,3], Andres E Leschziner[7], Thomas F Knight Jr[1,8]

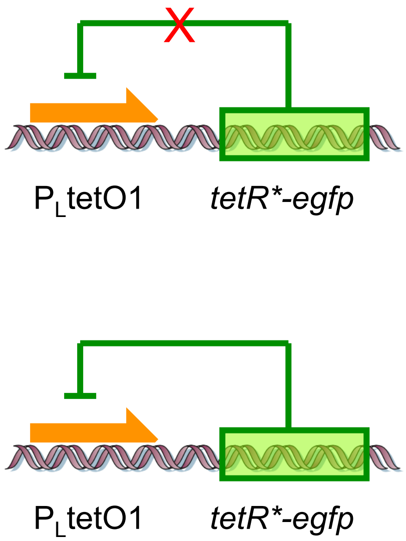
BioBrick standard biological parts are freely available to researchers through the Registry of Standard Biological Parts [42]. Although BioBricks have been used to construct a large variety of genetic circuits [12,43-54], these **circuits often require optimization [44,55-59]** and currently, there is no standard methodology for optimizing BioBrick circuits.

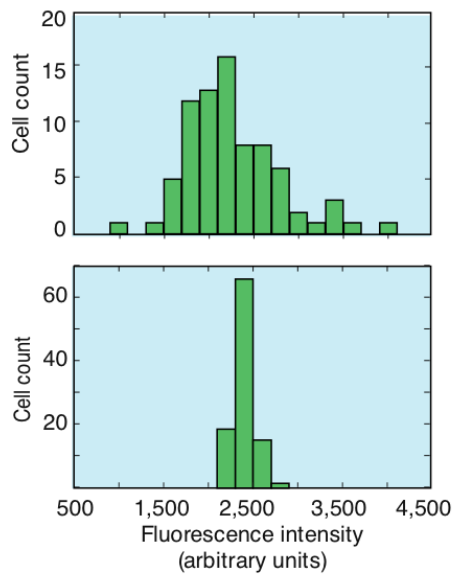
📖 S. Dabholkar, M. Thattai. Brainstorming biology. IET Synth. Biol. 1, 17-20 (2007)

Negative feedback

Noise reduction





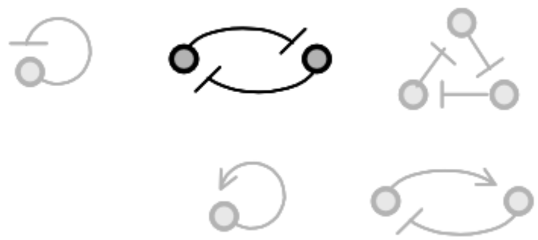


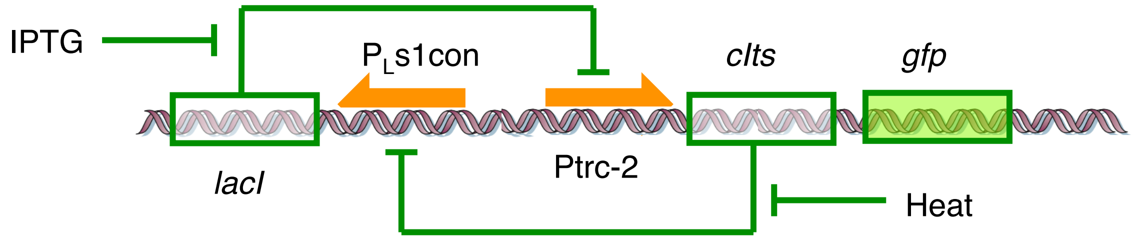
Figures adapted from Hasty et al, 2002

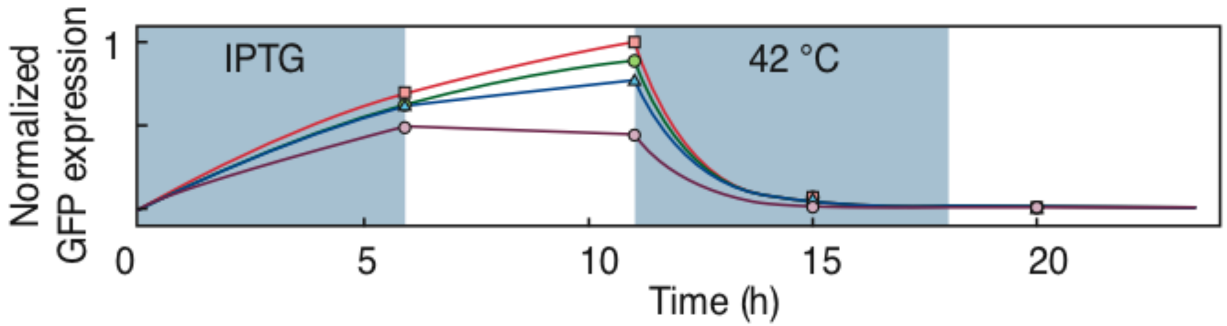
Becskei & Serrano, Nature, 2000

Flip flop

Memory





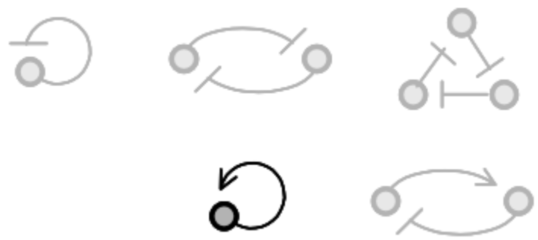


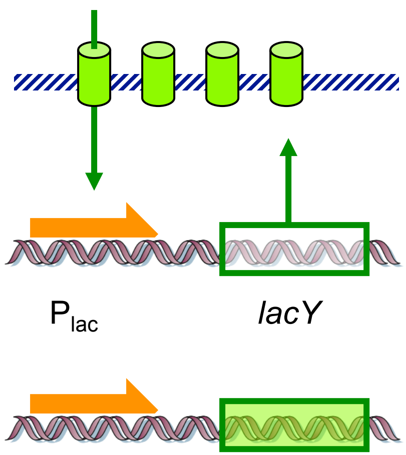
Figures adapted from Hasty et al, 2002

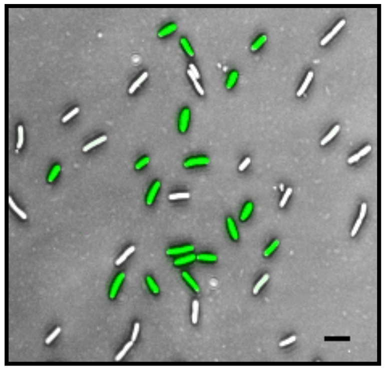
Gardner et al., Nature, 2000

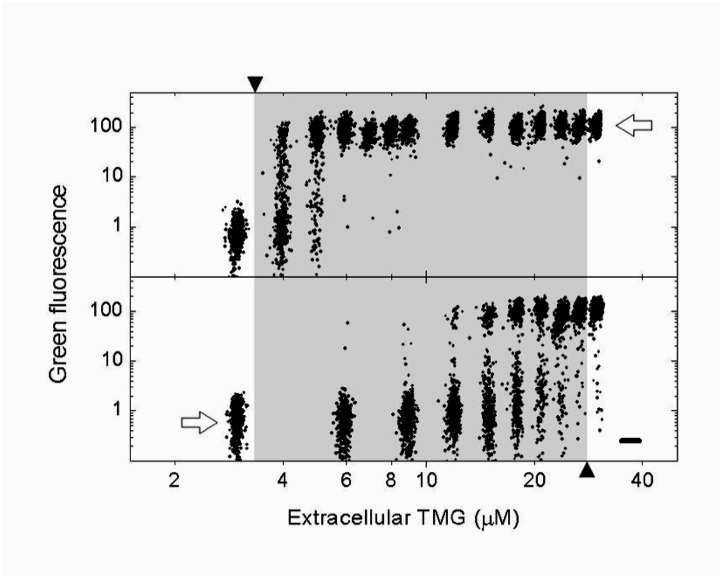
Positive feedback

Memory





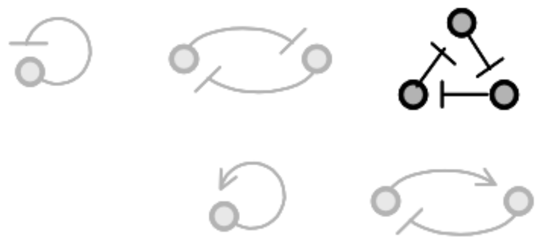


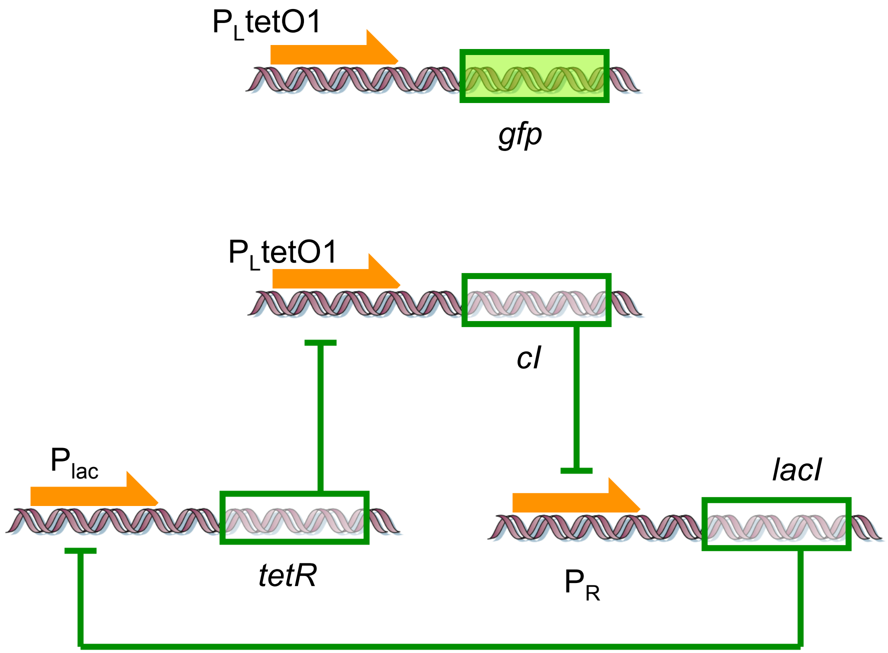


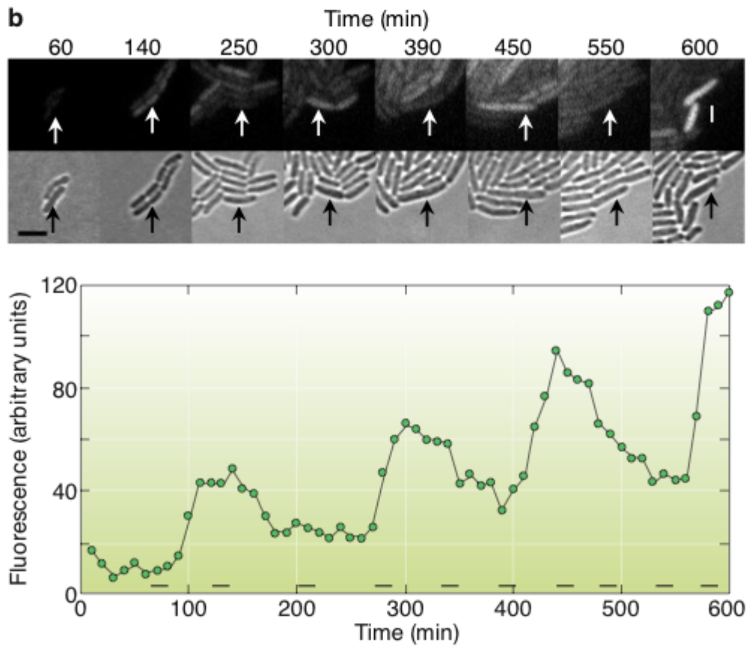
Ozbudak & Thattai et al., Nature, 2004

Ring oscillator

Oscillations





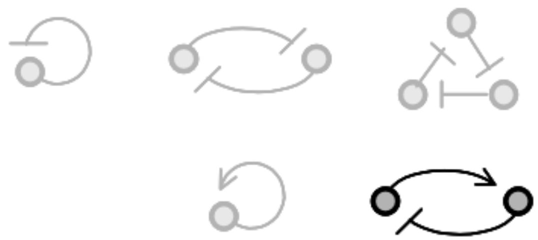


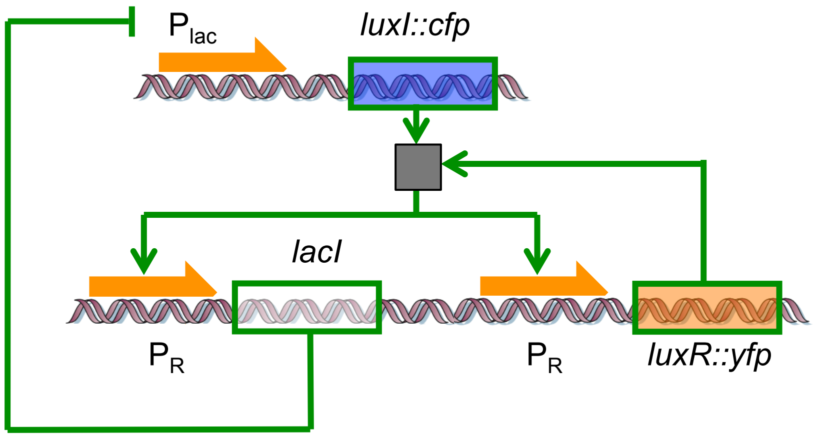
📖 Figures adapted from Hasty et al, 2002

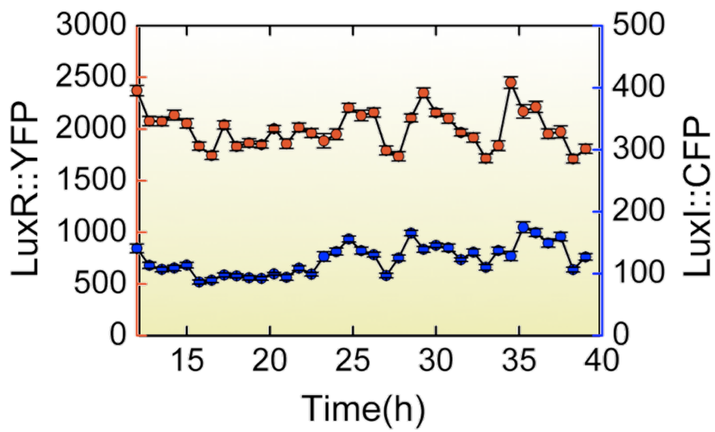
📖 Elowitz & Leibler, Nature, 2000

Hysteretic oscillator

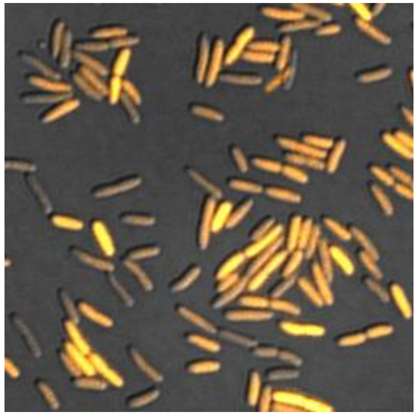
Synchronized oscillations







Rai et al., PLoS Comp Biol, 2012

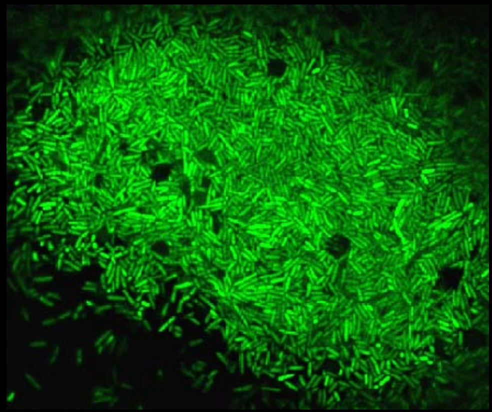


The basic parts:
Borrowing from a cell
communication system

Bacteria can change their behaviour depending on local cell density

Density-dependent bioluminescence in *Vibrio harveyi*

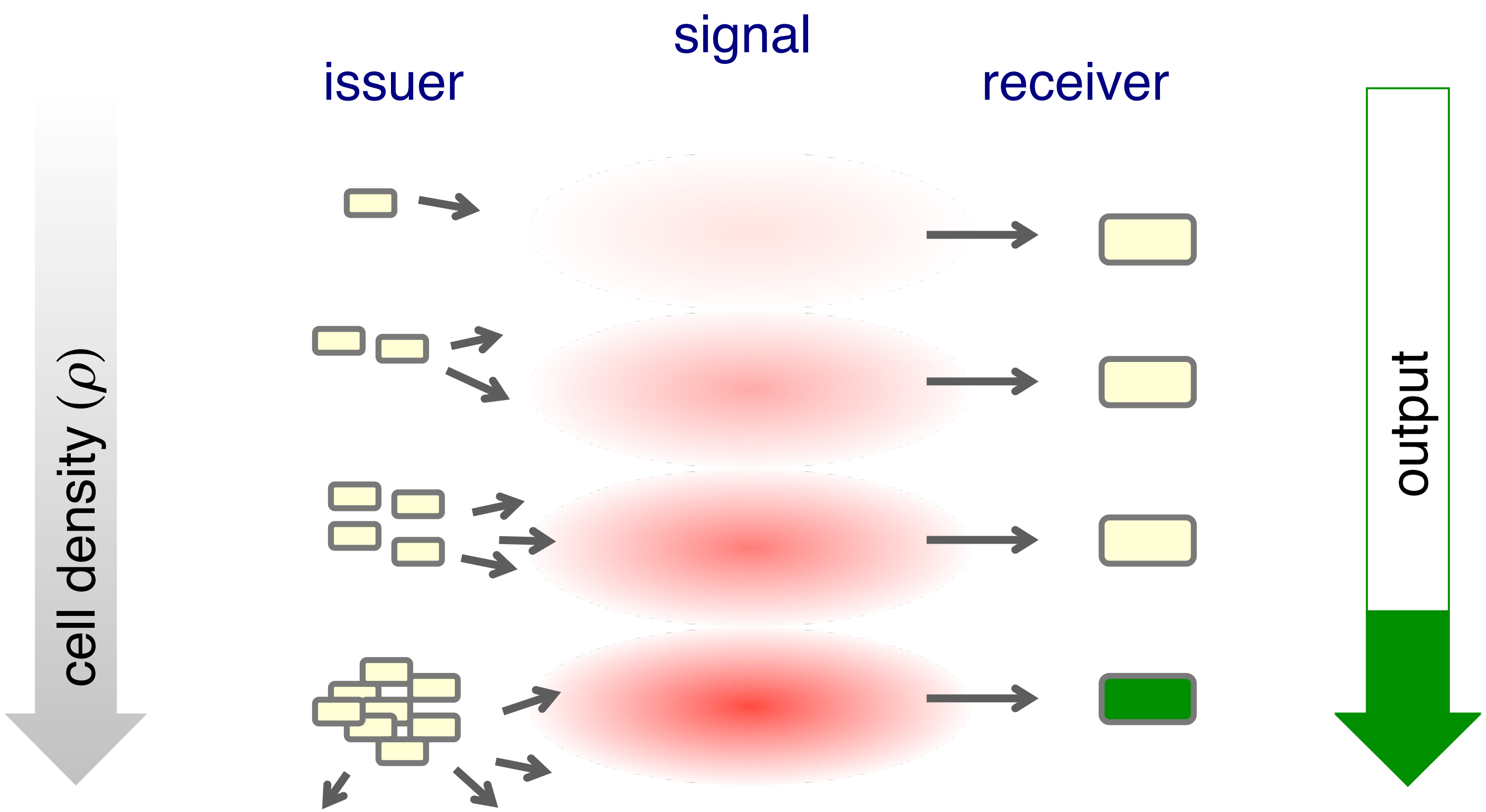
Symbiosis between bioluminescent *Vibrio fischeri* and the hawaiian bobtail squid



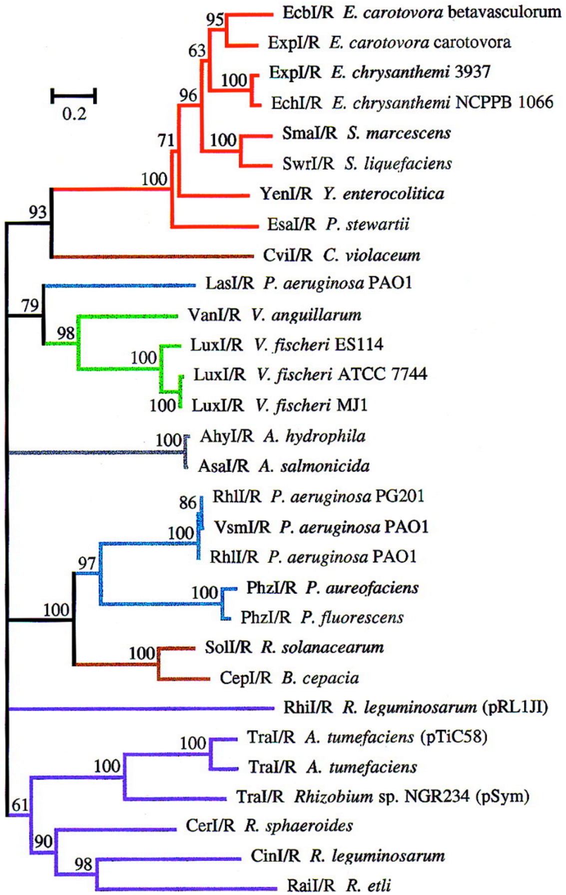
Quorum sensing during biofilm formation in *Pseudomonas aeruginosa*

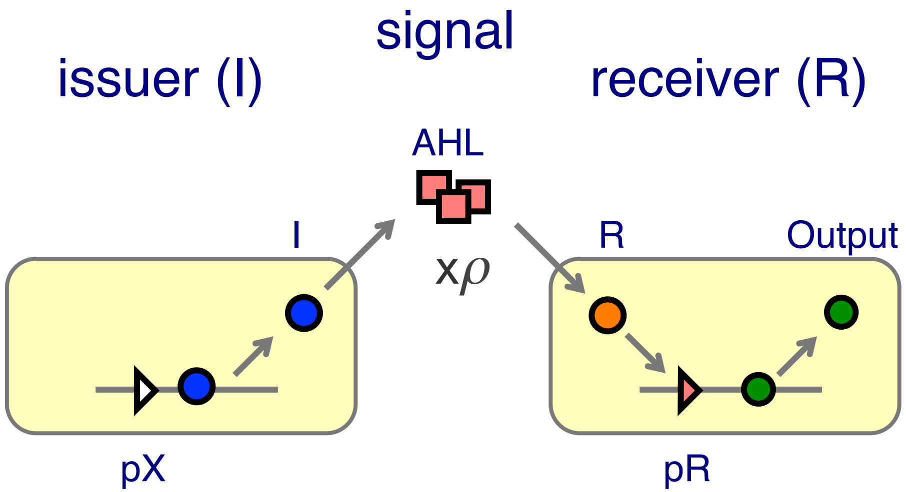
- MJ McFall-Ngai & EG Ruby, University of Hawaii
- F Ausubel, MGH
- MB Miller & BL Bassler, 2001

“Quorum sensing” is a form of chemical cell-to-cell communication



Quorum-sensing systems are built from a handful of conserved molecular components

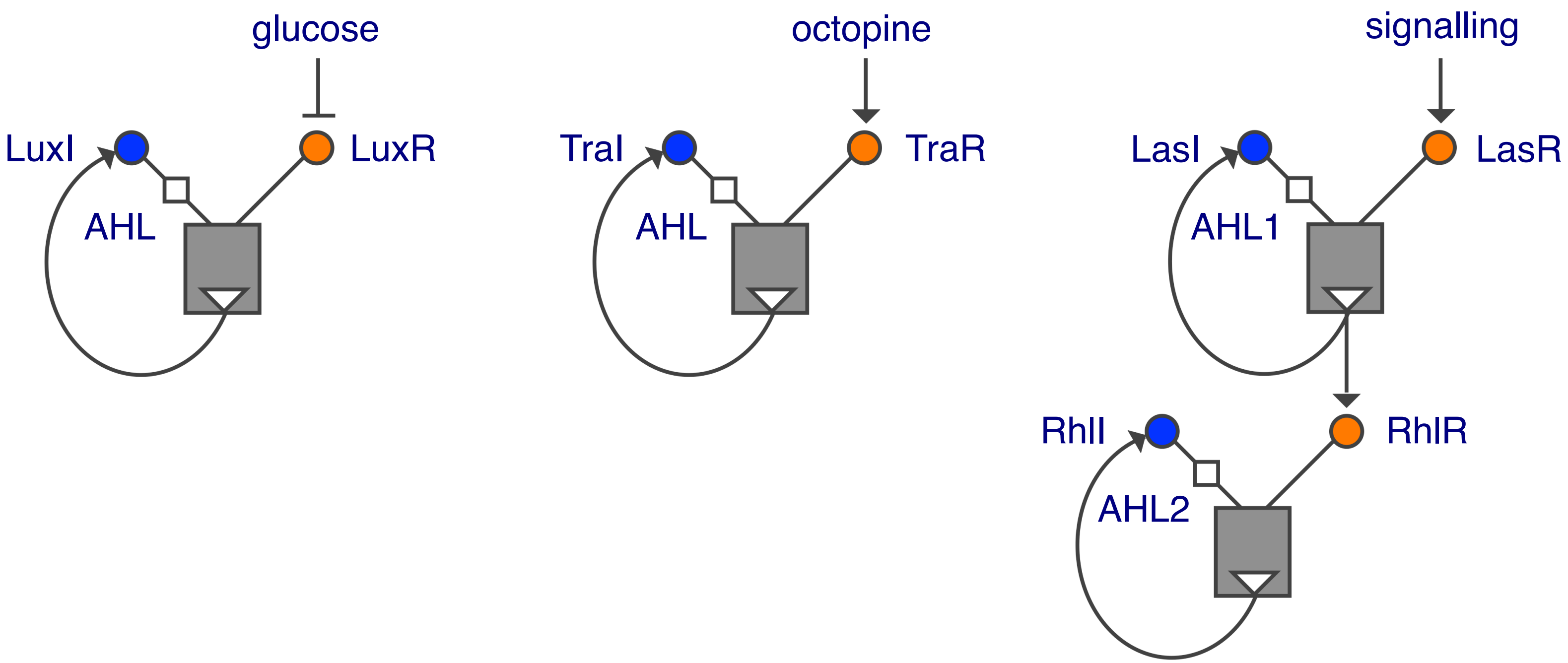


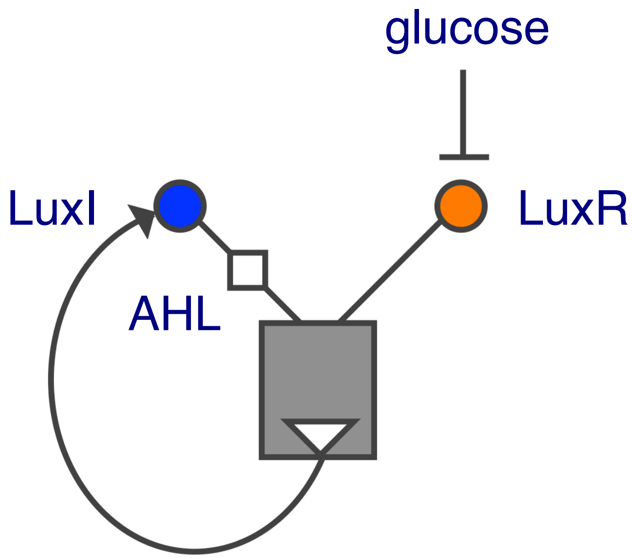


📖 Gray and Garey, 2001; Vannini et al., 2002

A topological puzzle:
All characterized natural QS systems use I-feedback

Species:	*Vibrio fischeri*	*Agrobacterium tumefaciens*	*Pseudomonas aeruginosa*
Niche:	marine endosymbiont	plant pathogen	human pathogen
Process:	bioluminescence	plasmid conjugation	virulence; biofilm formation



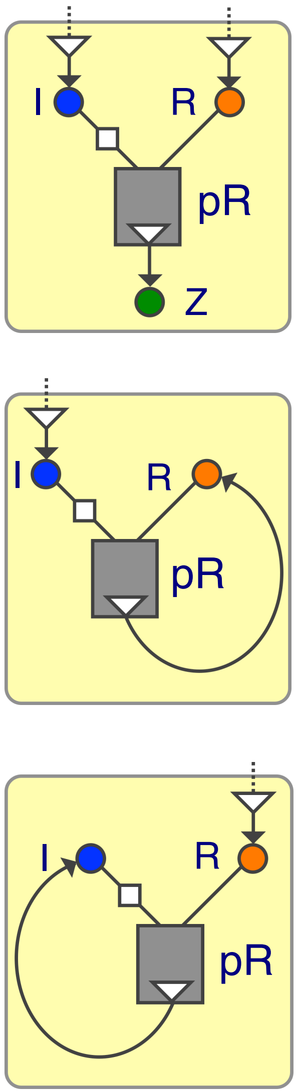


What is happening inside the biochemical black box?

What is the role of transcriptional feedback?

Why is one feedback topology preferred over another?

Inferring feedback behavior from feedforward data



Feedforward

$$\frac{1}{\gamma_Z}\frac{dY_Z}{dt} = Q_Z f(\mu\rho\bar{Y}_I, \bar{Y}_R) - Y_Z$$

extract biochemistry (two regulated inputs)

R-feedback

α

$$\frac{1}{\gamma_R}\frac{dY_R}{dt} = Q_R f(\mu\rho\bar{Y}_I, Y_R) - Y_R$$

I-feedback

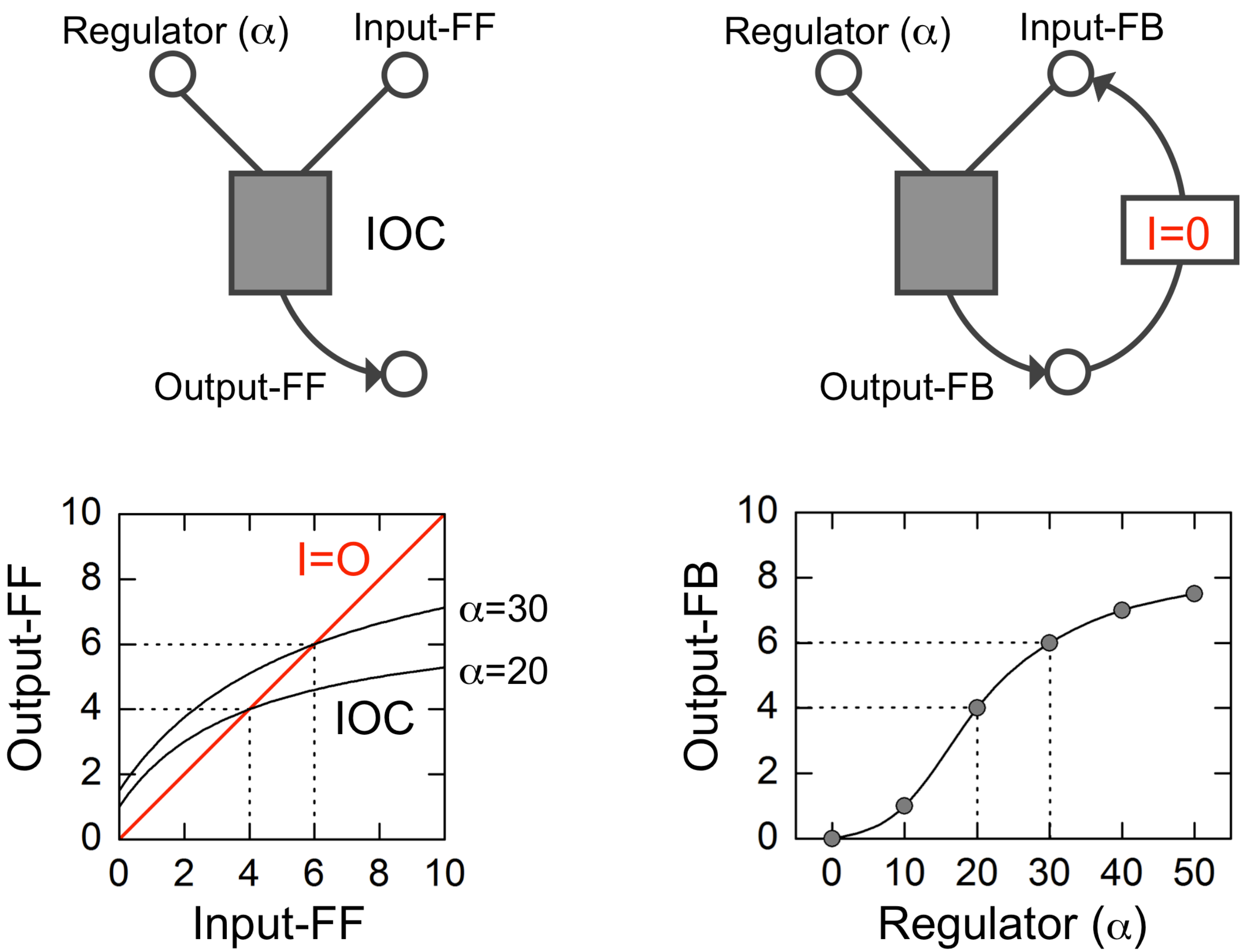
α

$$\frac{1}{\gamma_I}\frac{dY_I}{dt} = Q_I f(\mu\rho Y_I, \bar{Y}_R) - Y_I$$

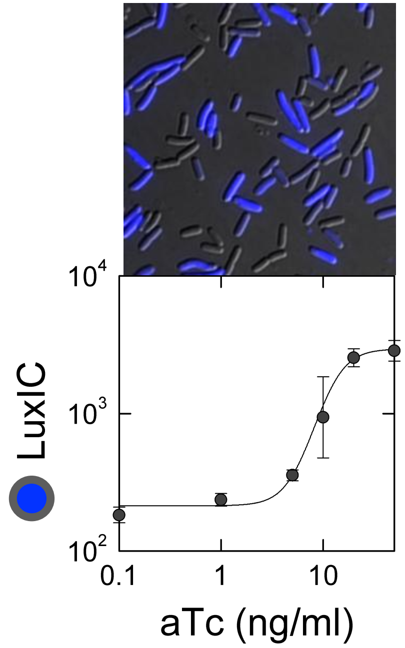
predict feedback responses (single regulated input)

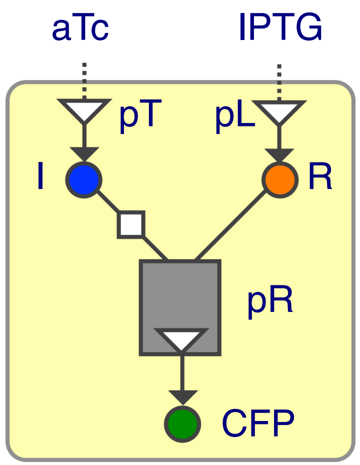
Rai et al., PLoS Comp Biol, 2012

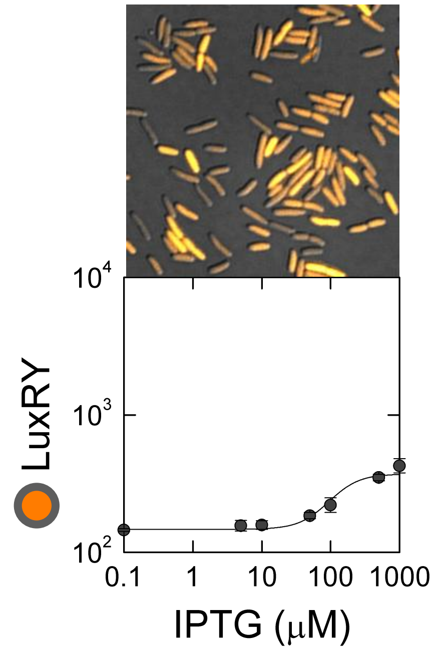
Inferring feedback behavior from feedforward data

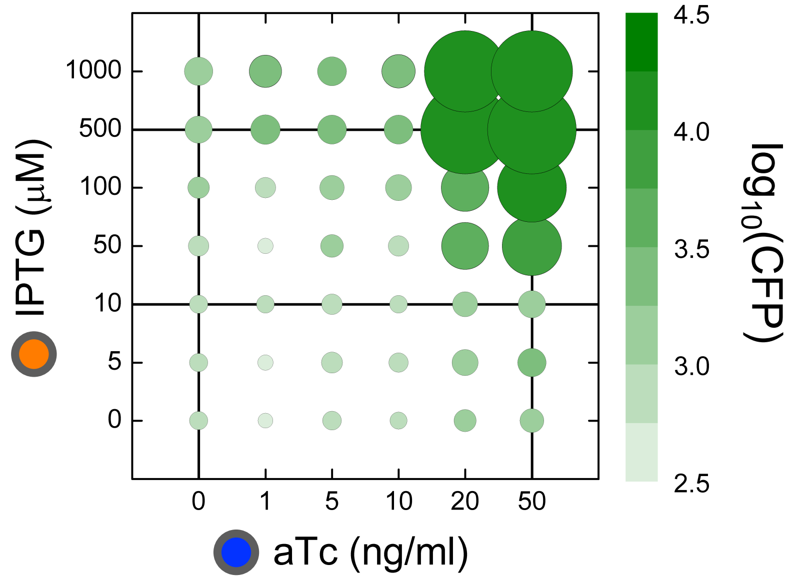


Extracting biochemistry: the feedforward response



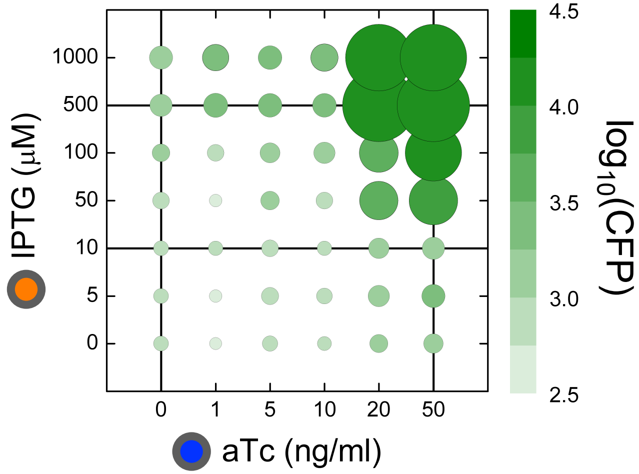






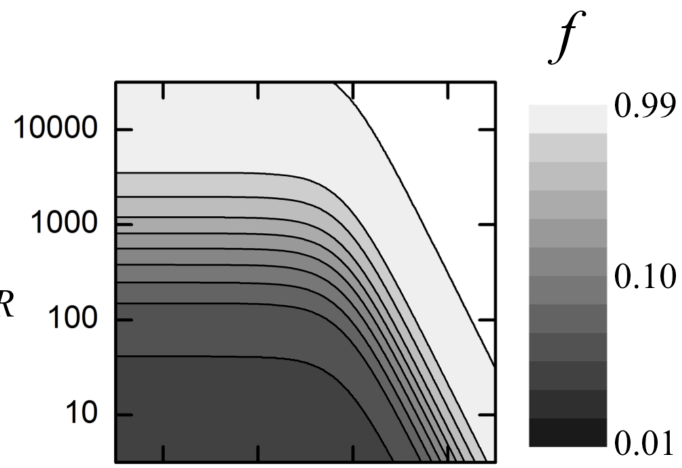
Rai et al., PLoS Comp Biol, 2012

The 2-D promoter logic function of pR



$$f(\mu\rho Y_I, Y_R) = \beta + (1-\beta)\frac{Y_R^n(\tilde{\delta} + (\tilde{\mu}\rho Y_I)^m)}{1 + Y_R^n(\tilde{\delta} + (\tilde{\mu}\rho Y_I)^m)}$$

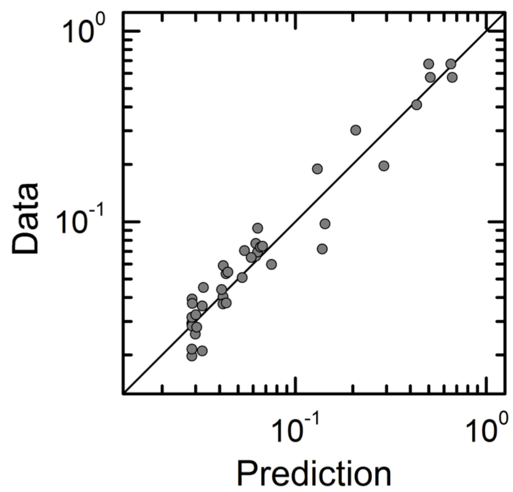
n: Hill coefficient of LuxR-DNA binding



Y_R

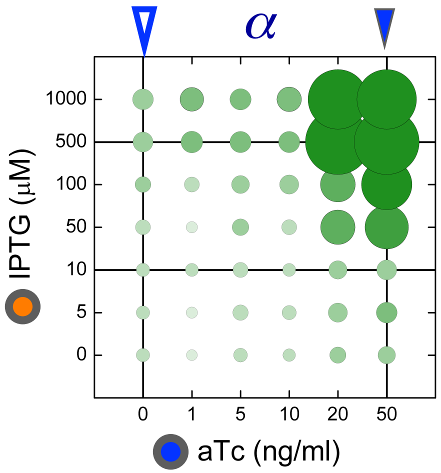
Y_I

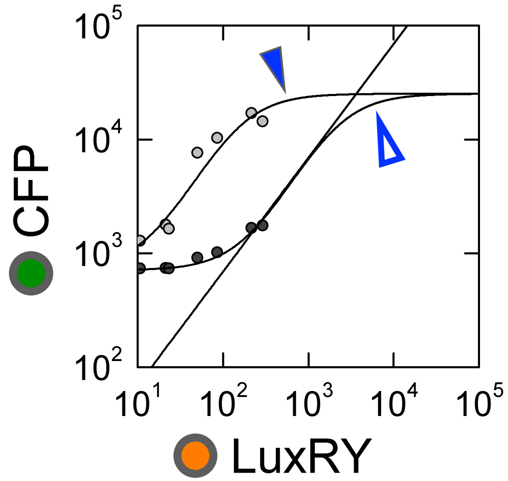
5 parameter fit to data

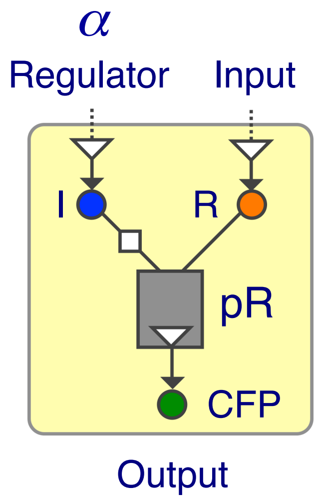


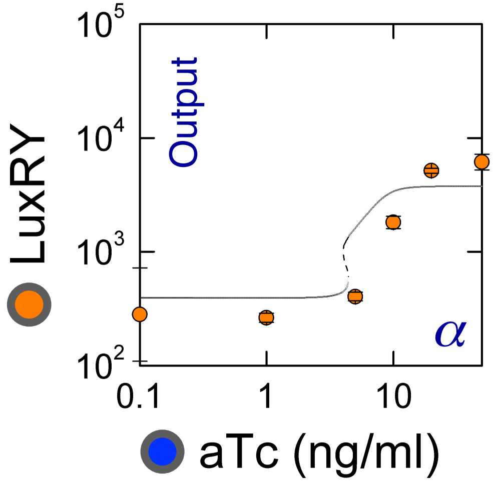
Thattai, Phil. Trans. Roy. Soc. A, 2012

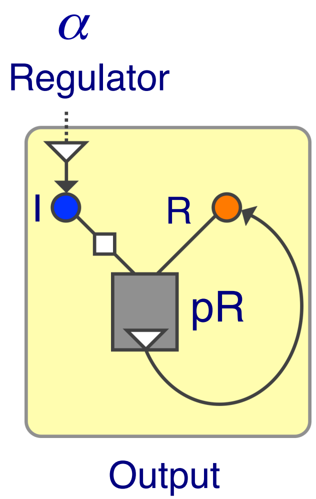
R-feedback response: monostable, smooth





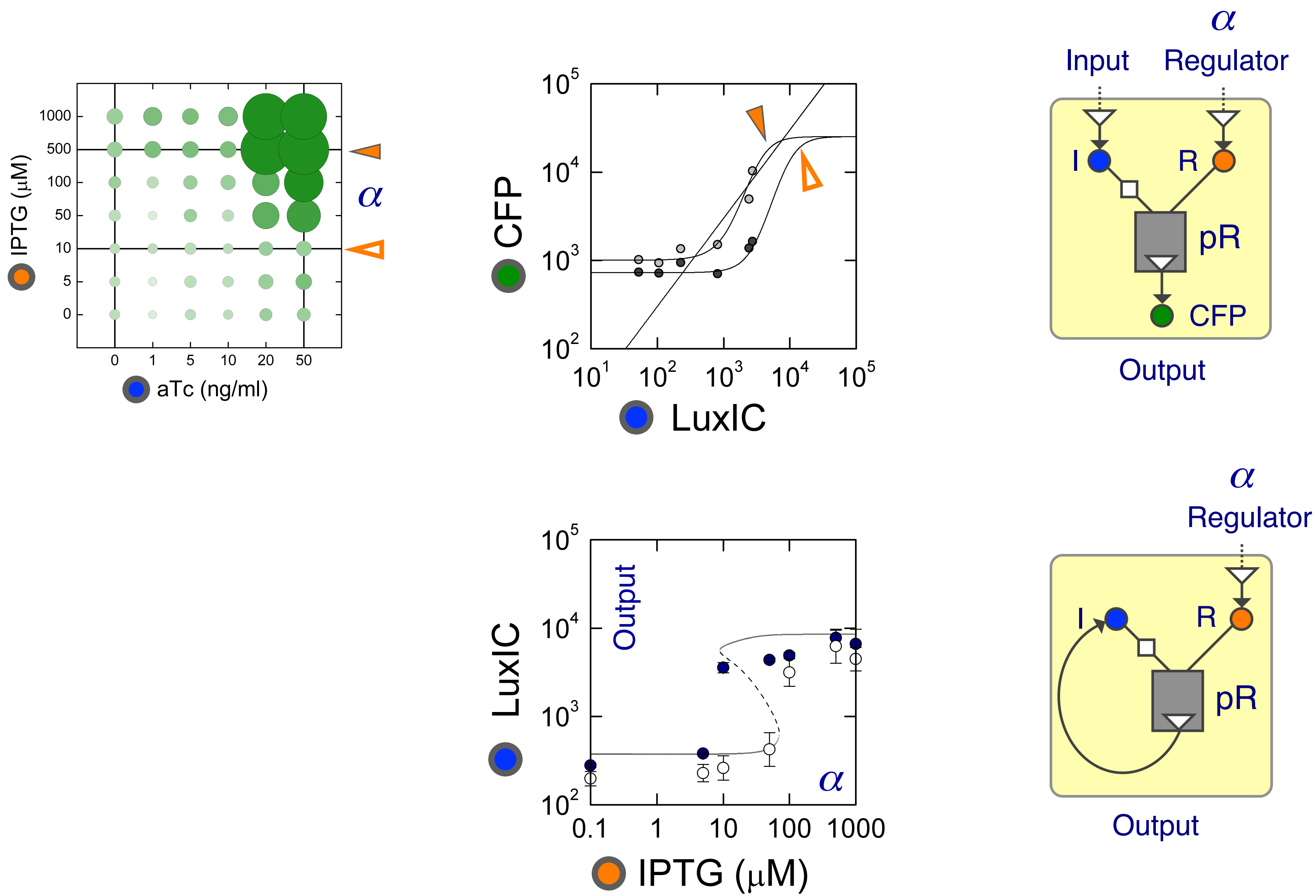






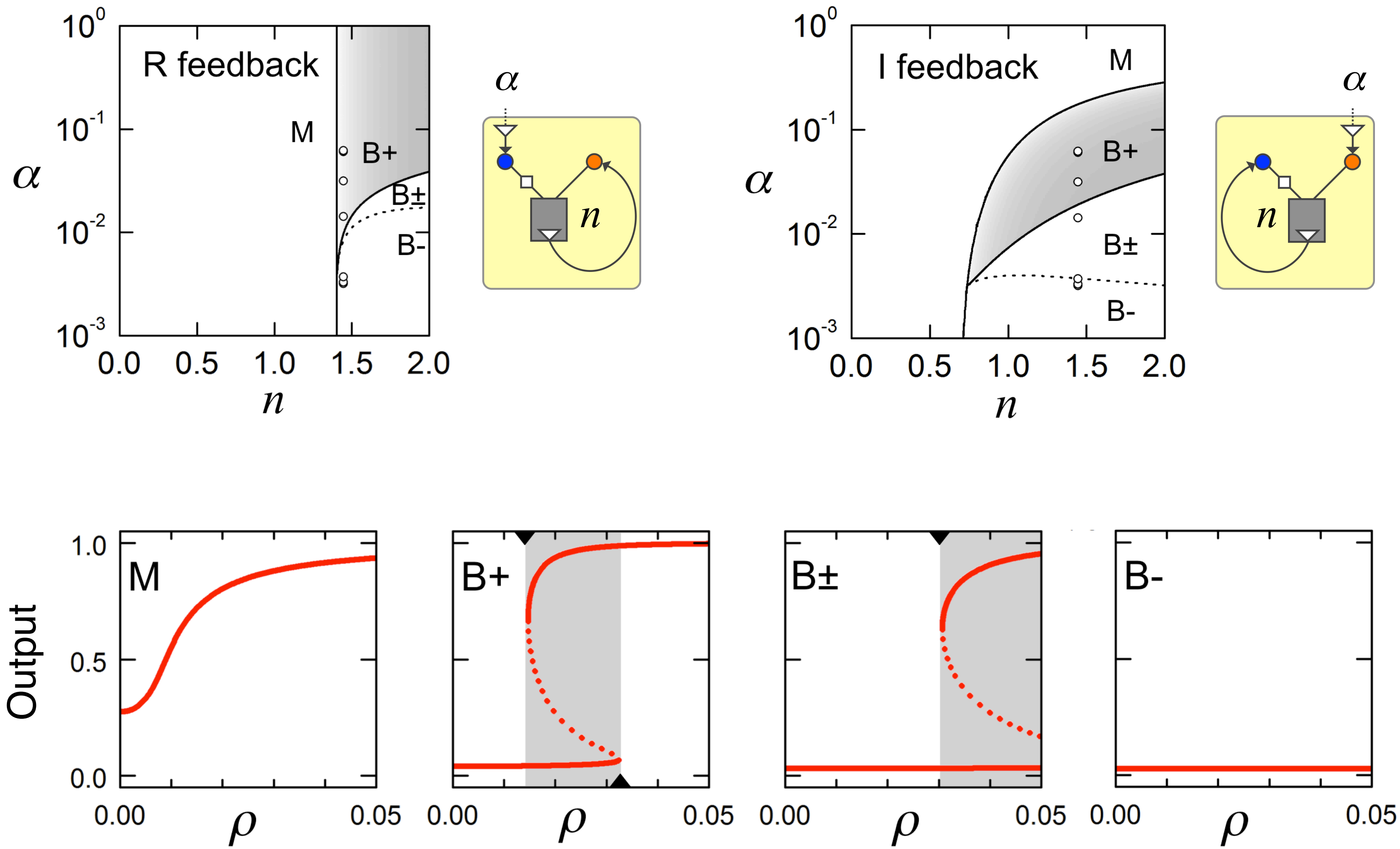
📖 Rai et al., PLoS Comp Biol, 2012

I-feedback response: hysteretic, switch-like

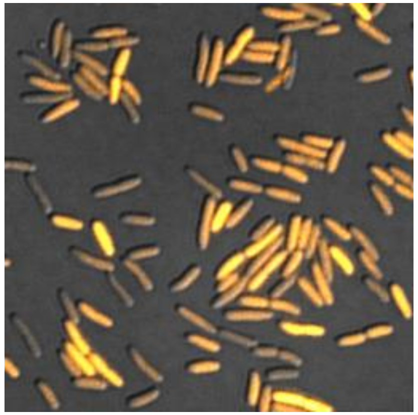


Rai et al., PLoS Comp Biol, 2012

Phase diagram of density-dependent responses

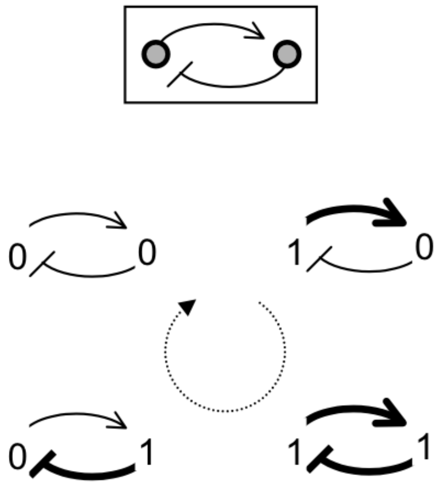


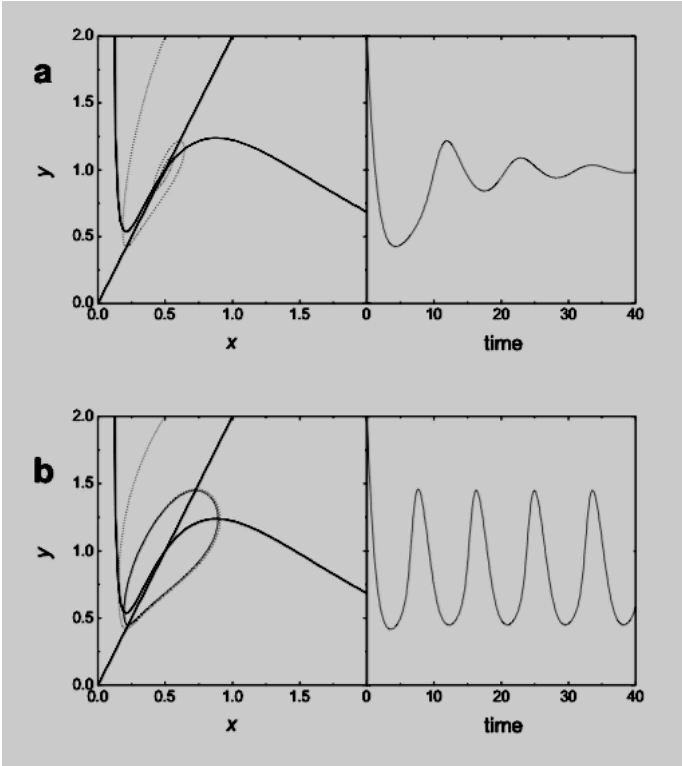
Rai et al., PLoS Comp Biol, 2012



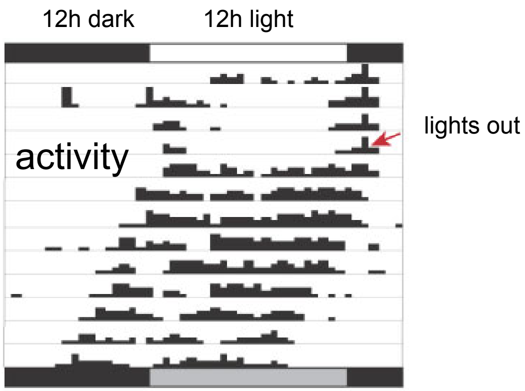
Endgame:
Building an oscillator from the bottom up

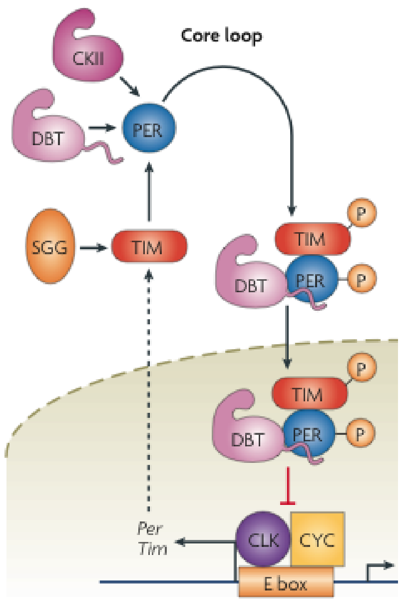
The “hysteretic oscillator” motif: negative feedback with delay



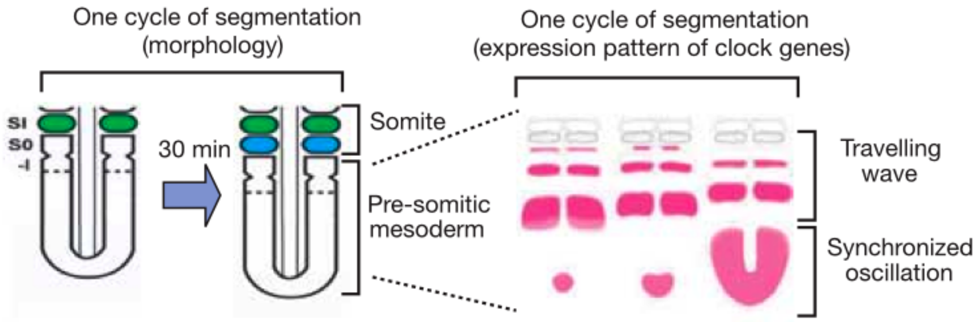


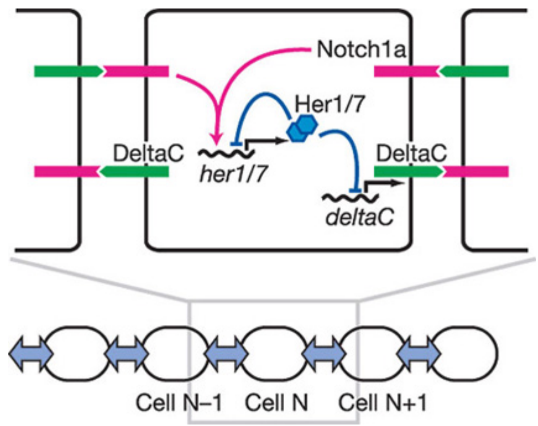
Drosophila circadian clock





Zebrafish segmentation clock





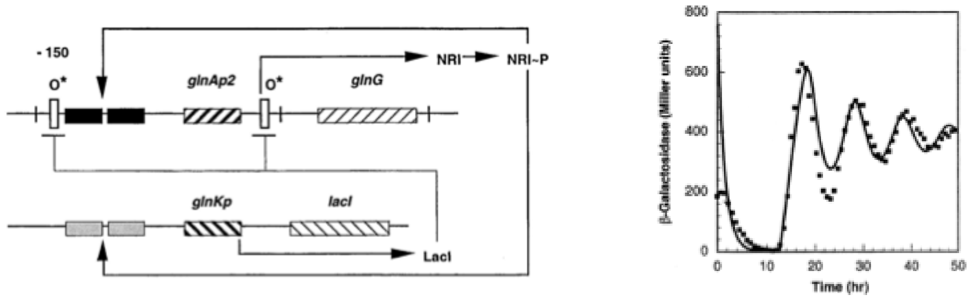
[synchronized]

📖 Gallego & Virshup, Nat. Rev. Mol. Cell Biol., 2007

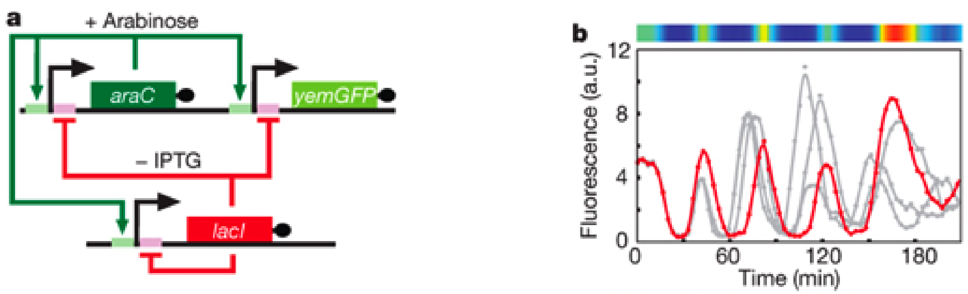
📖 Panda et al., Nature, 2002

📖 Horikawa et al., Nature, 2006

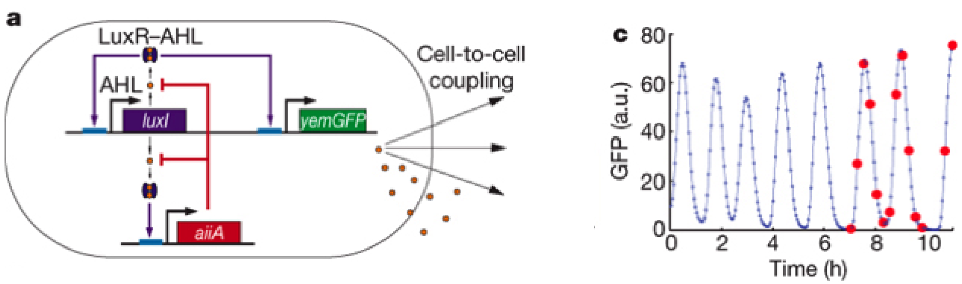
Synthetic oscillators



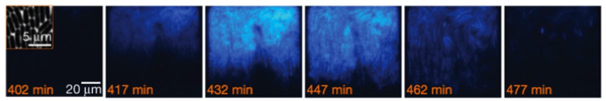
📖 Atkinson et al., Cell, 2003



📖 Stricker et al., Nature, 2008

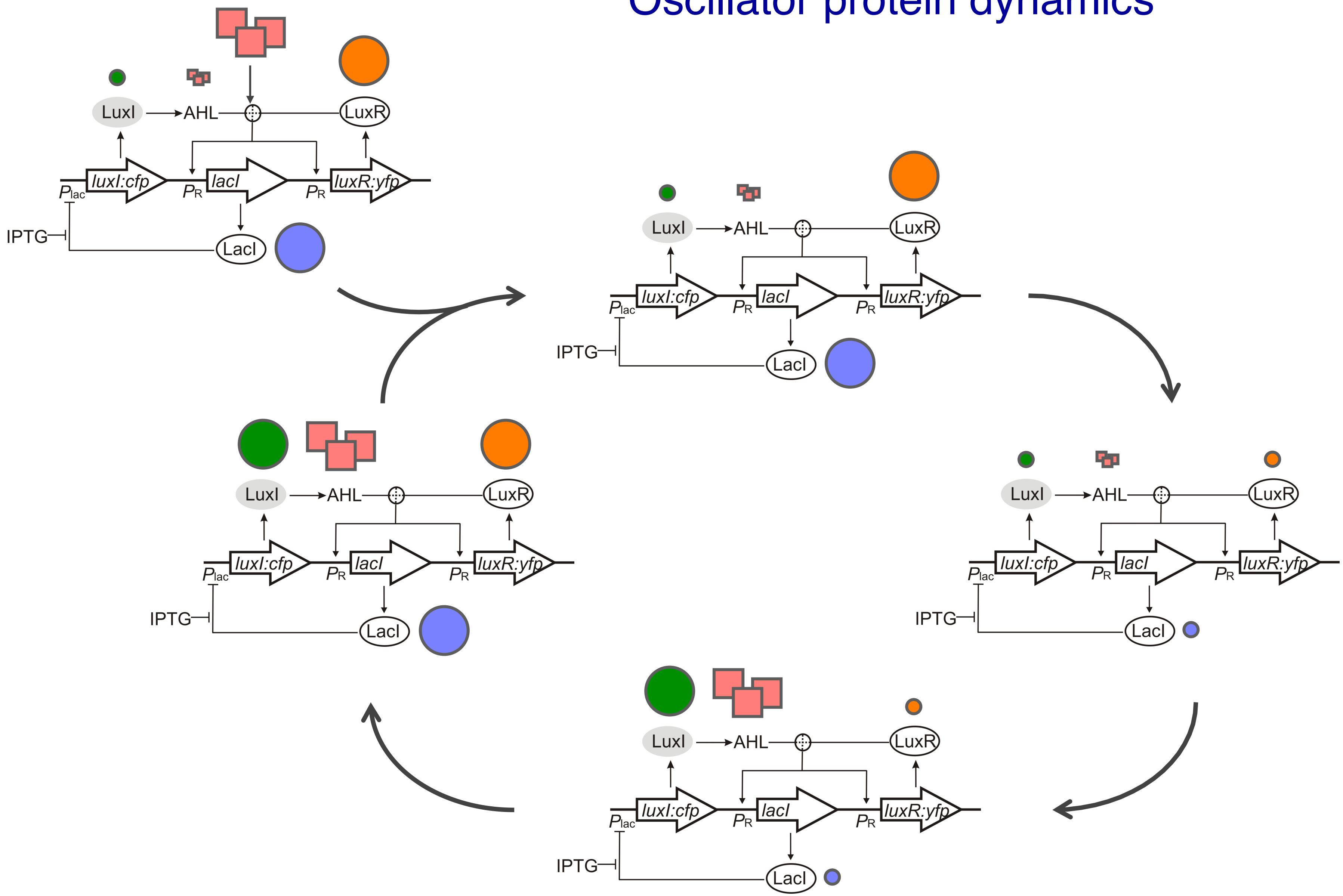


📖 Danino et al., Nature, 2010

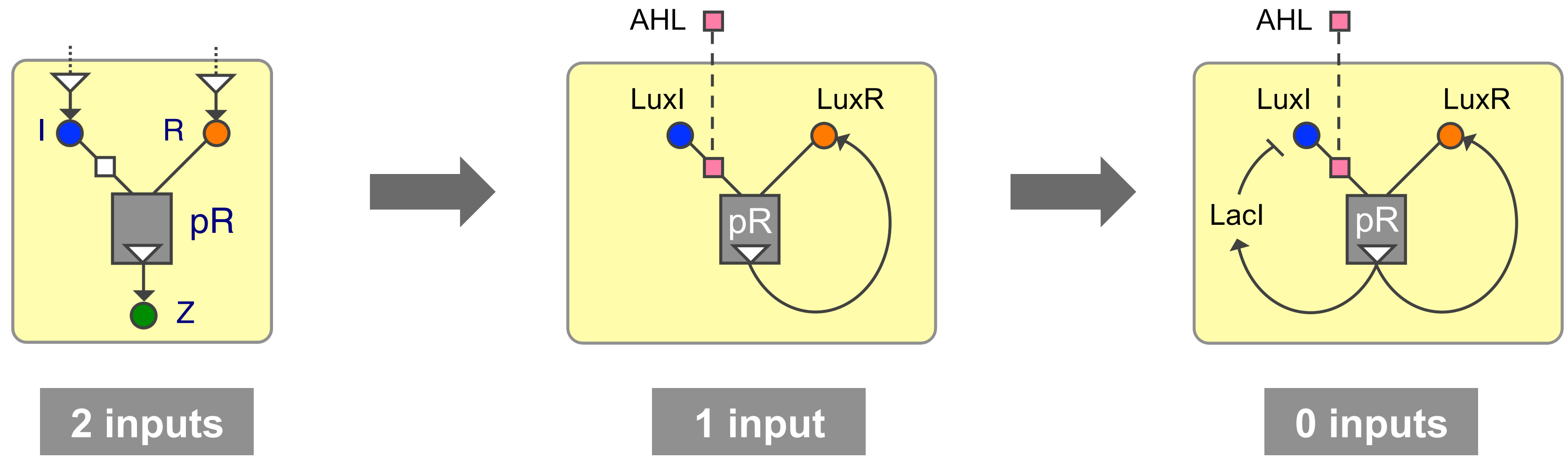


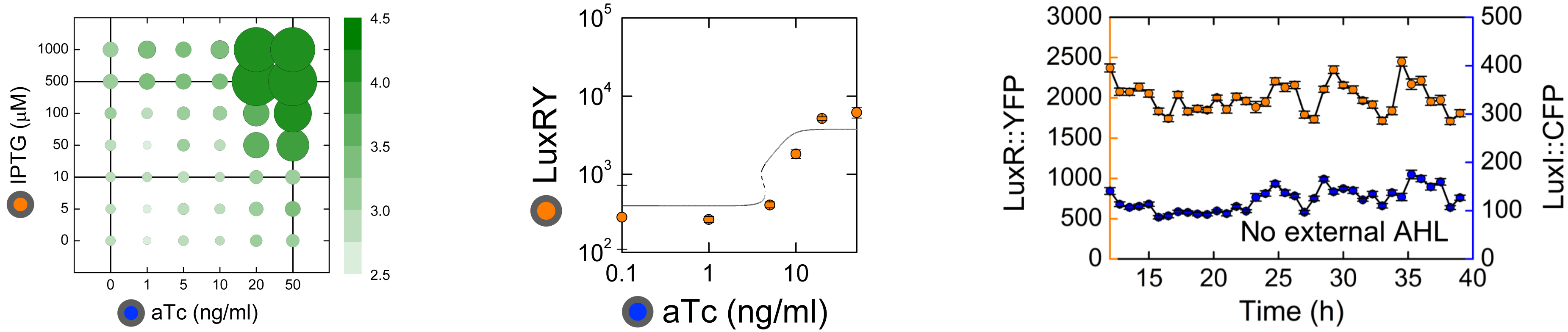
[synchronized]

Oscillator protein dynamics

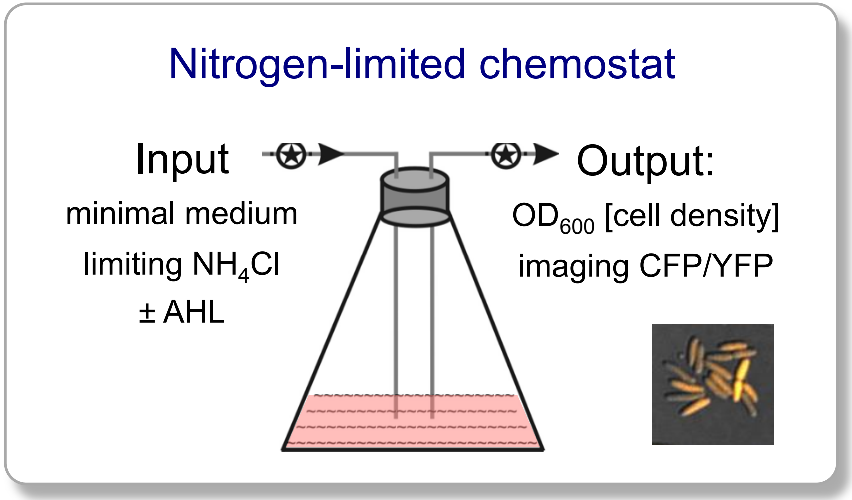


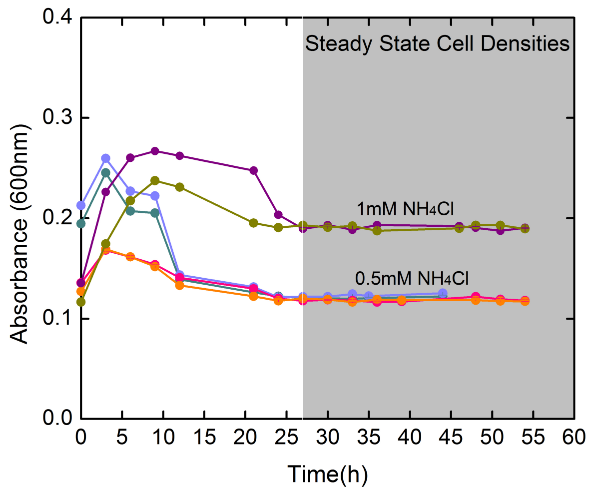
Roadmap: putting parts together to make the oscillator

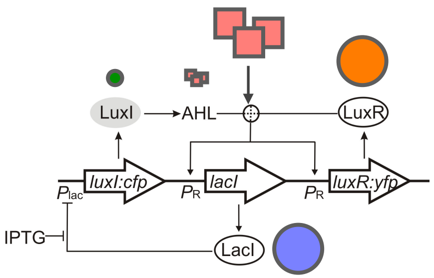




Experiments: The synchronization protocol



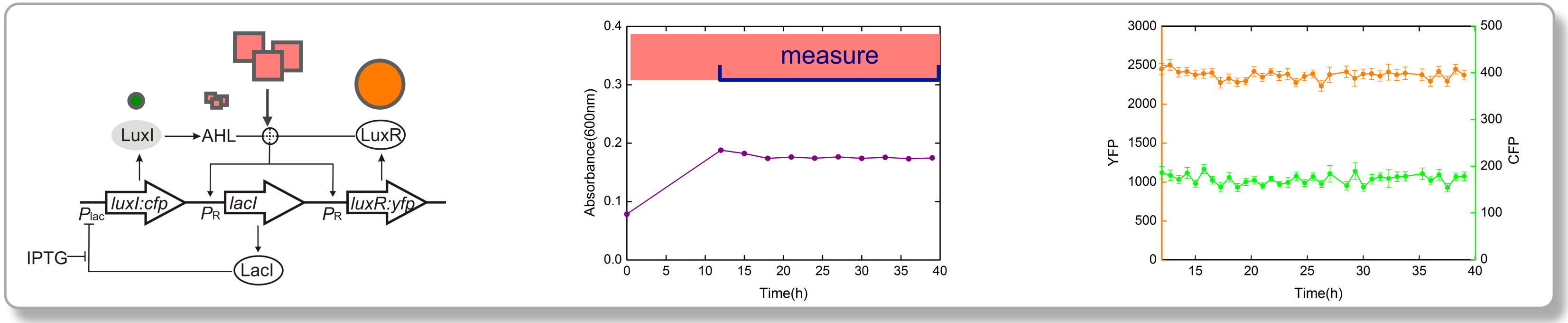


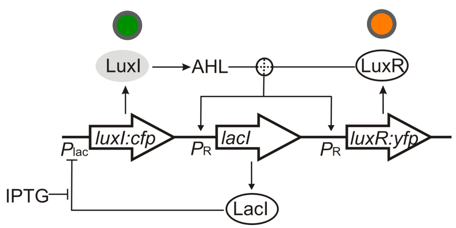


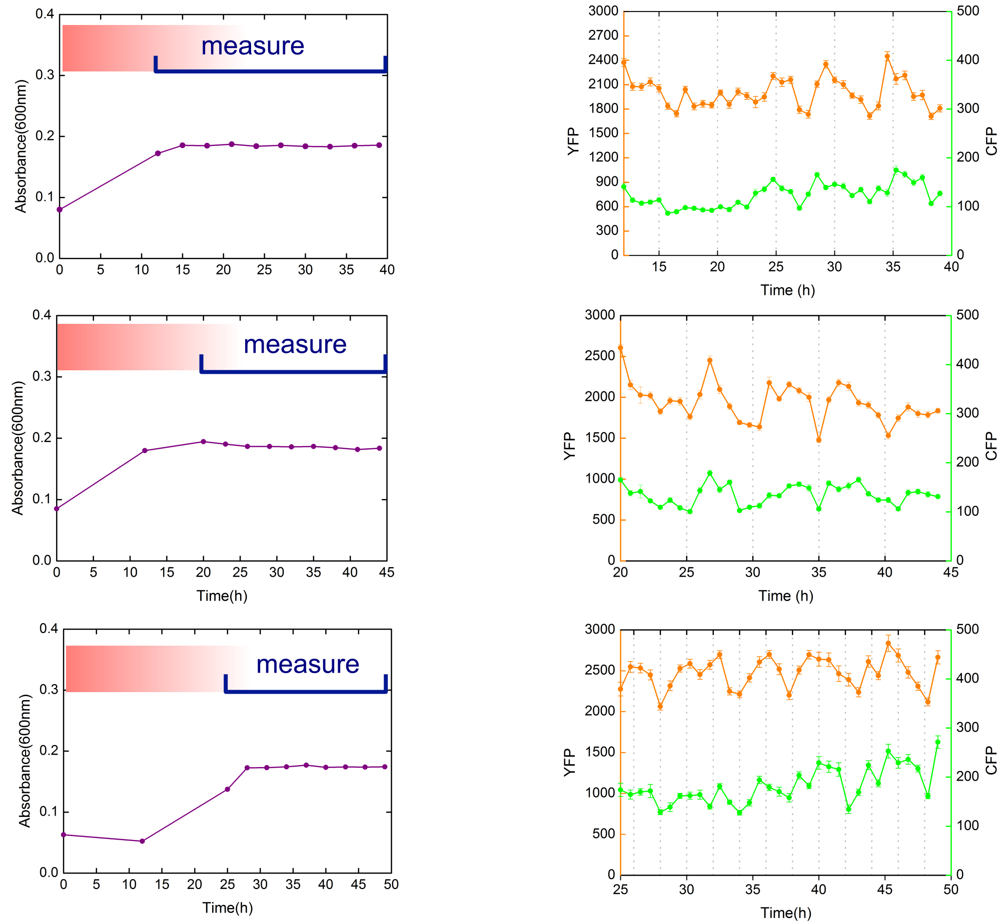
Control: continuous AHL — measure

Test: Synchronize with 12h AHL — measure

Experiments: Synchronized oscillations over 24h

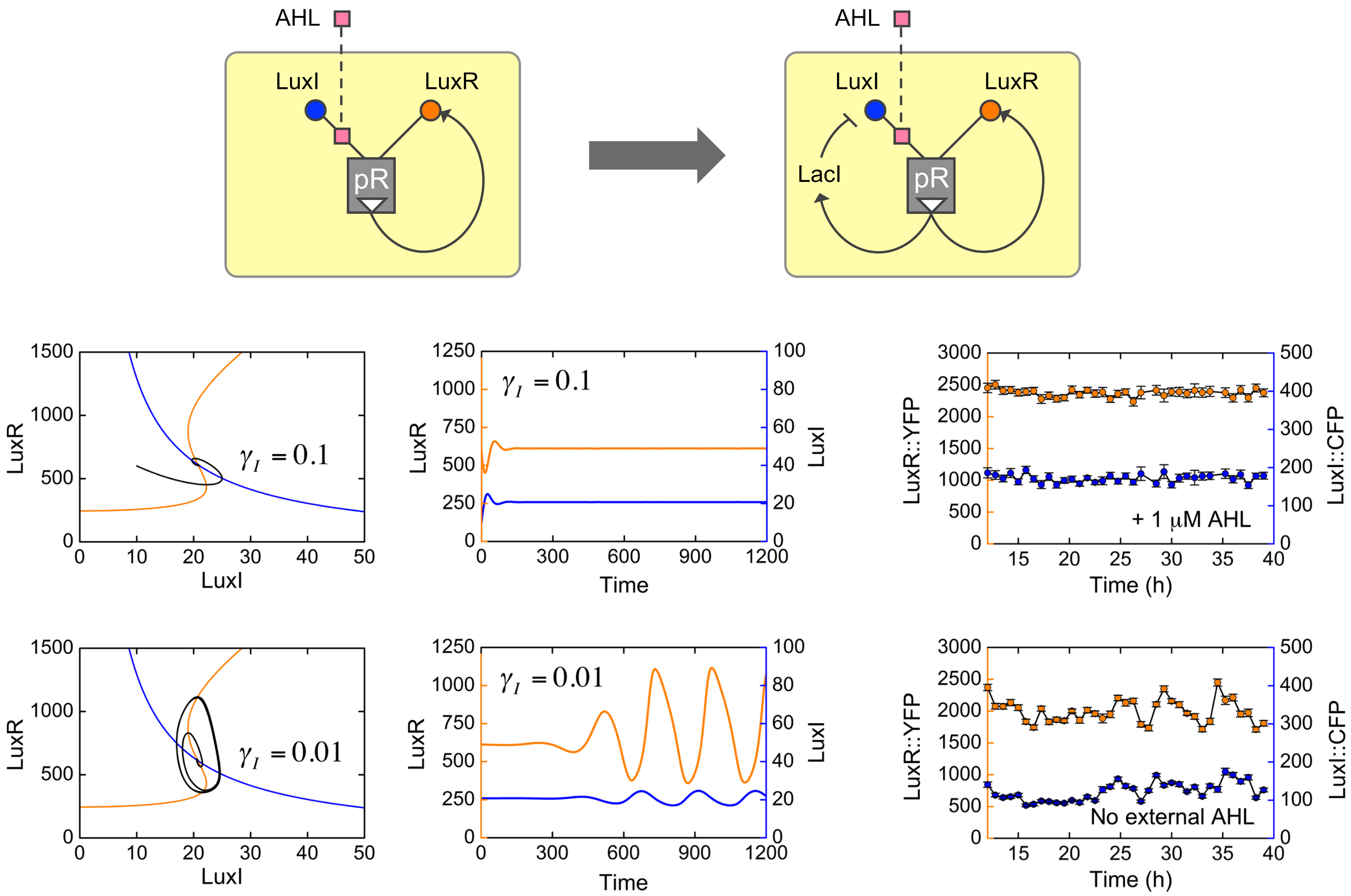




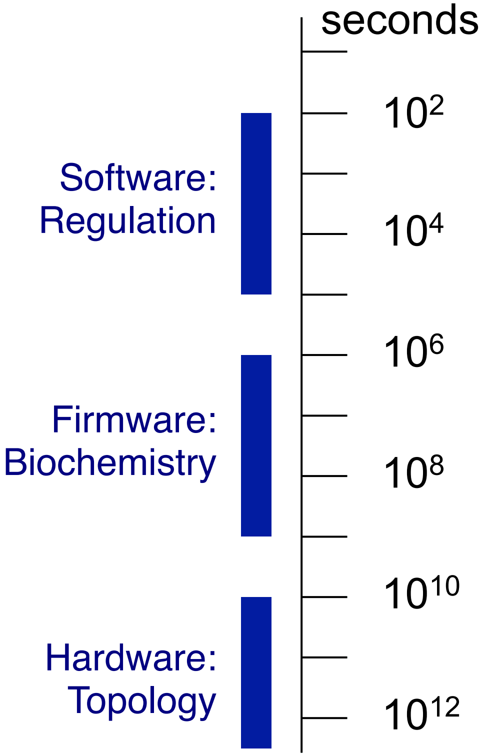


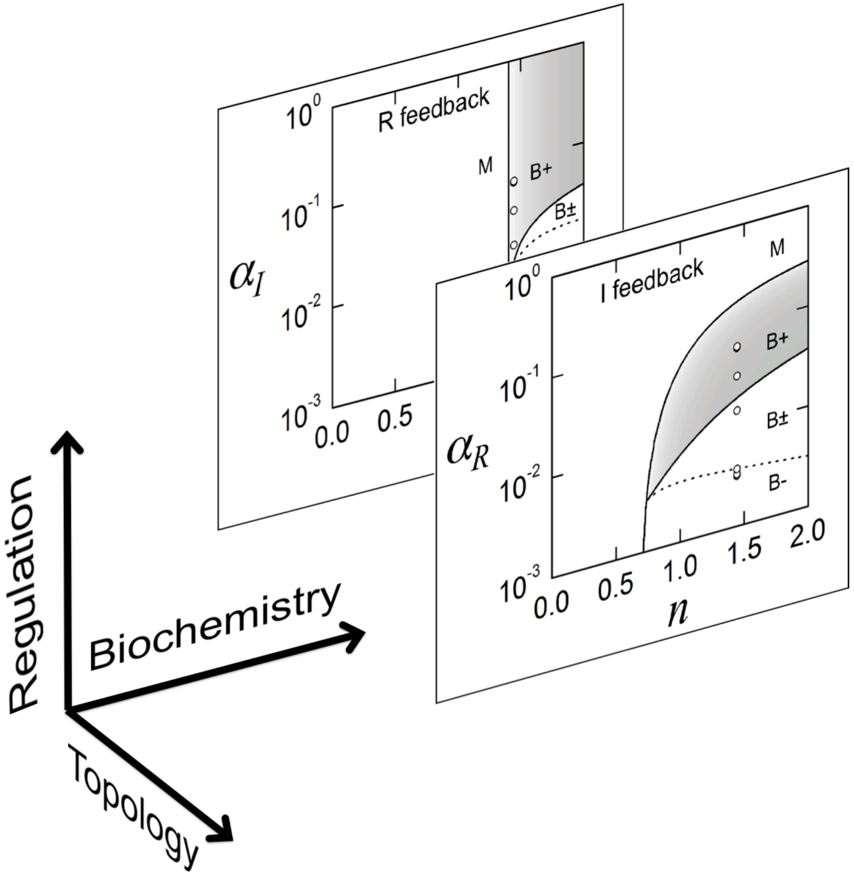
⌛ Herioc experiments by Navneet Rai

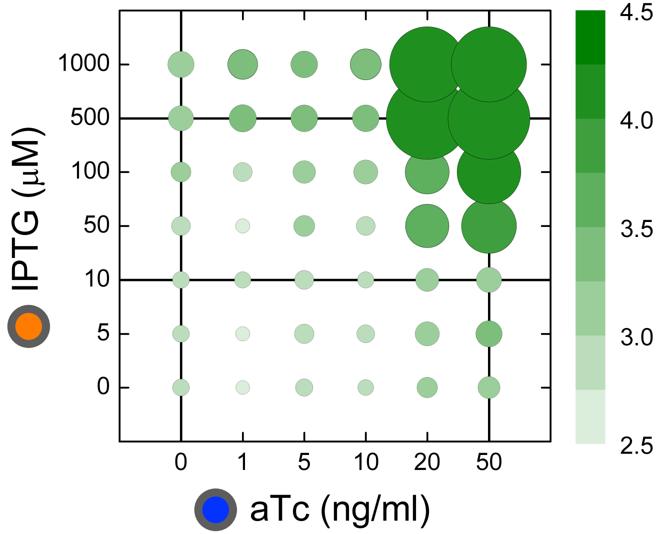
Promoter logic explains oscillations in dual feedback system



The regulation / biochemistry / topology hierarchy







Acknowledgements

Navneet Rai, Rajat Anand, Sugat Dabholkar
KV Venkatesh (ChemE, IIT Bombay)

Undergraduates:
Krishna Ramkumar (IIT Bombay), Sushant More (IISER Pune), Varun Sreenivasan (St. Xavier's Bombay), Shashanka Kundu (St. Stephen's), Vini Gautam (Delhi University), Senthil Kumar (PSG Coimbatore)

Further reading:

> Rai, N. et al., Prediction by promoter logic in bacterial quorum sensing. PLoS Comp. Biol. 8, e1002361 (2012).
> Thattai, M. Using topology to tame the complex biochemistry of genetic networks. Phil. Trans. Roy. Soc. A 20110548 (2012).

ncbs
thattai@ncbs.res.in