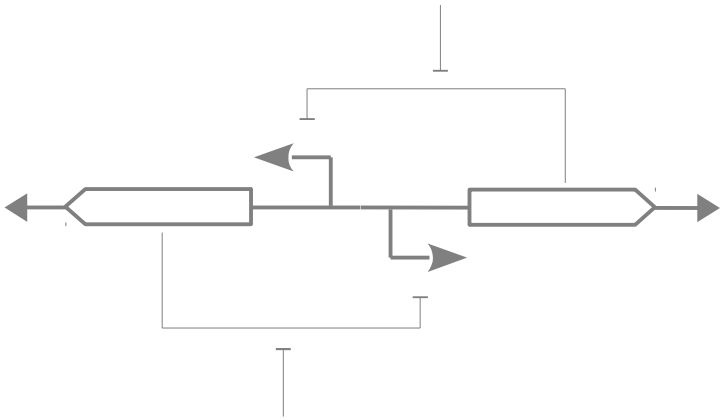
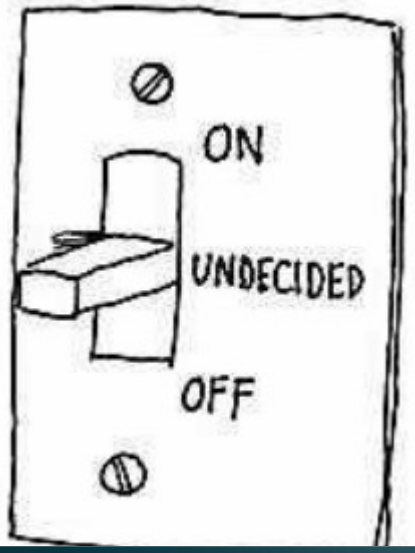
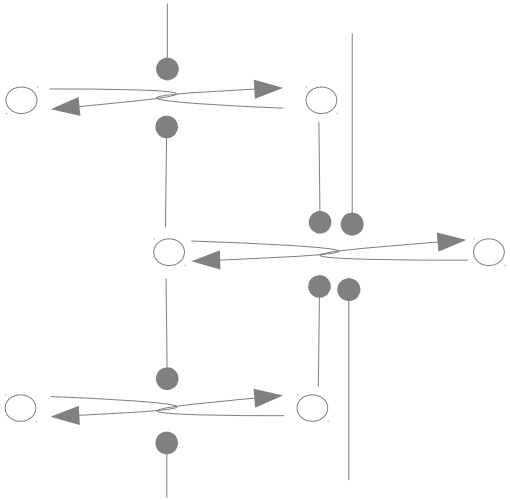


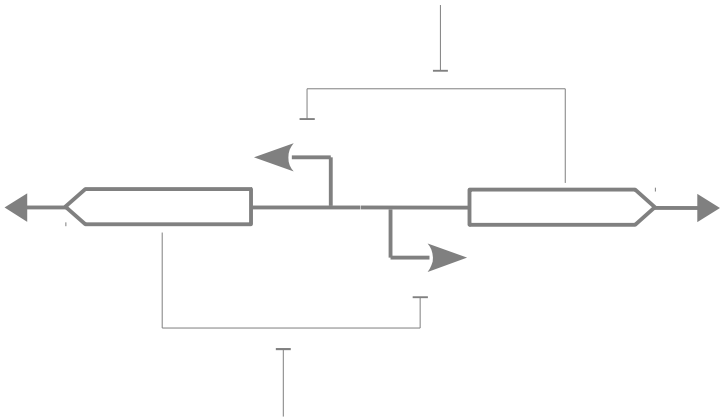


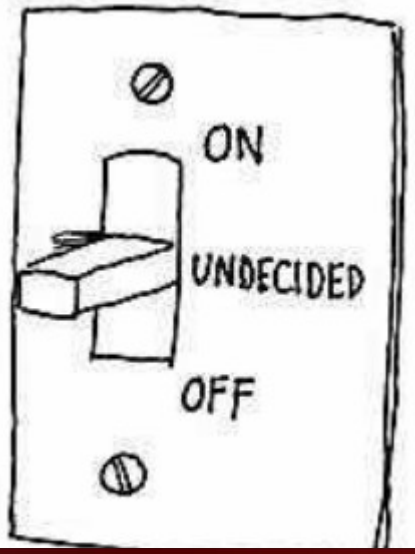


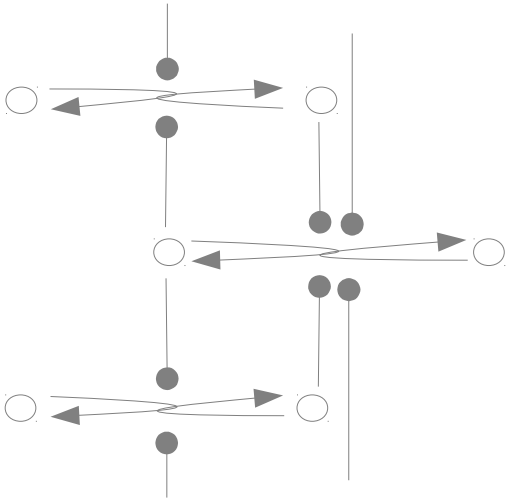
Lessons from Modelling (some) Biological Switches

Harold Fellermann

Senior Research Associate
ICOS Interdisciplinary Computing & Complex Biosystems Research Group
School of Computing Science
Newcastle University







Cybernetics for Synthetic Biological Design

Harold Fellermann

Senior Research Associate
Interdisciplinary Computing & Complex Biosystems
School of Computing Science
Newcastle University

Three Types of Biological Switches

protein-protein interaction switches

(riboswitches)

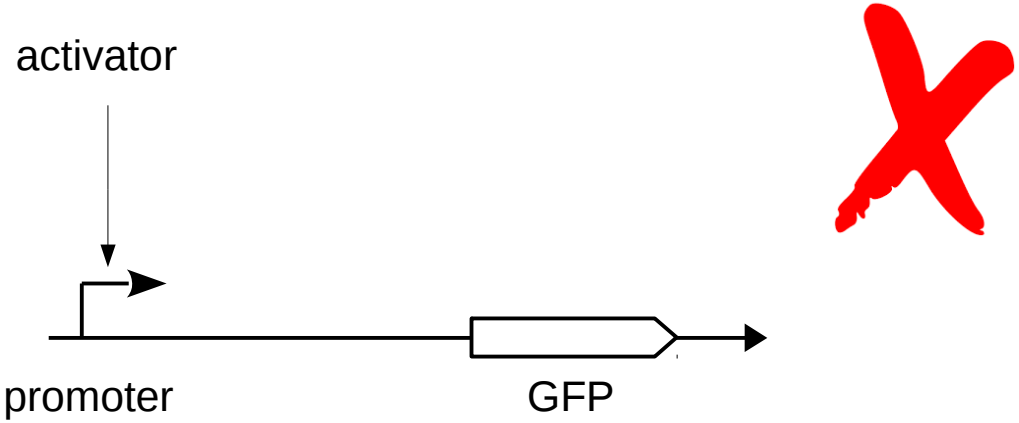
gene-regulated switches

speed

metabolic cost

Let's build a genetic switch

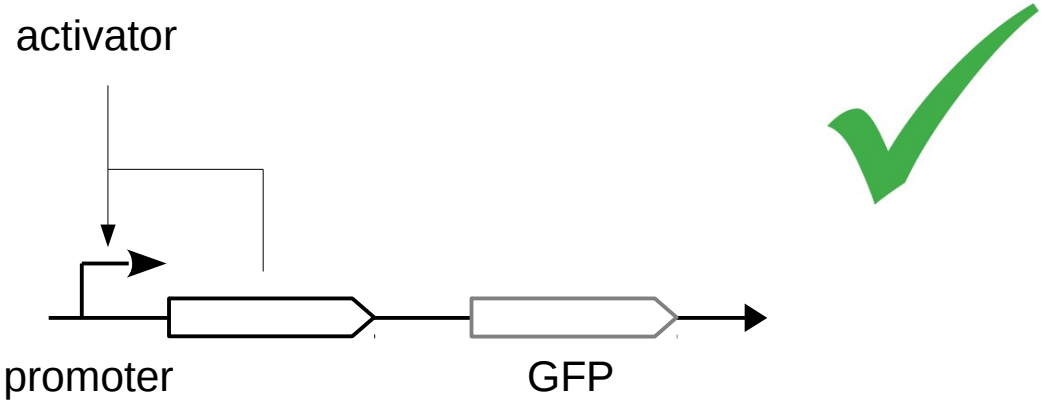
Gene regulators are often referred to as “biological switches” because of their almost discrete state changes.



These are good signal transducers, but not the type of switch I am after.

Let's build a genetic switch

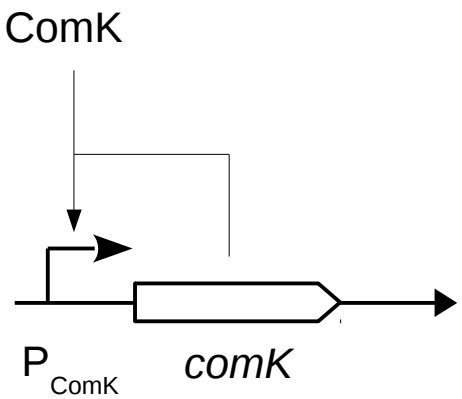
Instead, we want a switch that maintains its state.



This is achieved by auto-inducing one's own activation.

Let's build a genetic switch

Auto-inducing switches
e.g. genetic competence in *B. subtilis*

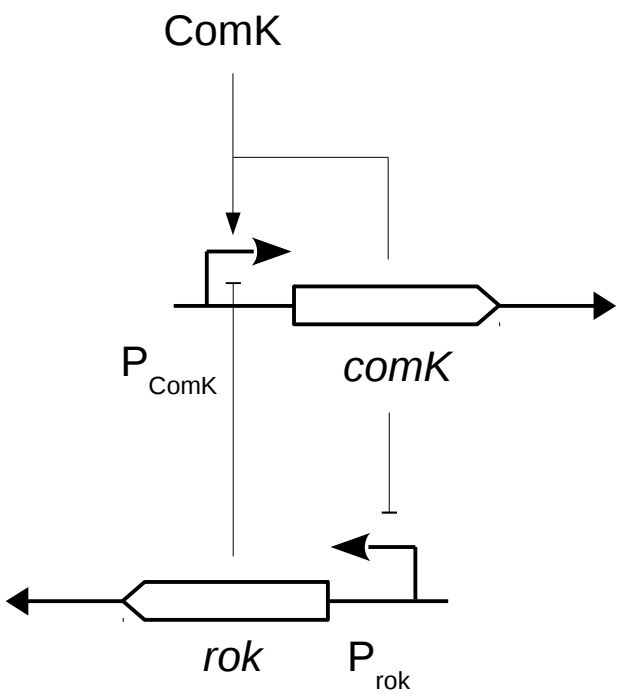


adapted from M. Leisner (2008) *PhD thesis*

Let's build a genetic switch

ComK involves a second regulation loop,

which is a double-repressing feedback:

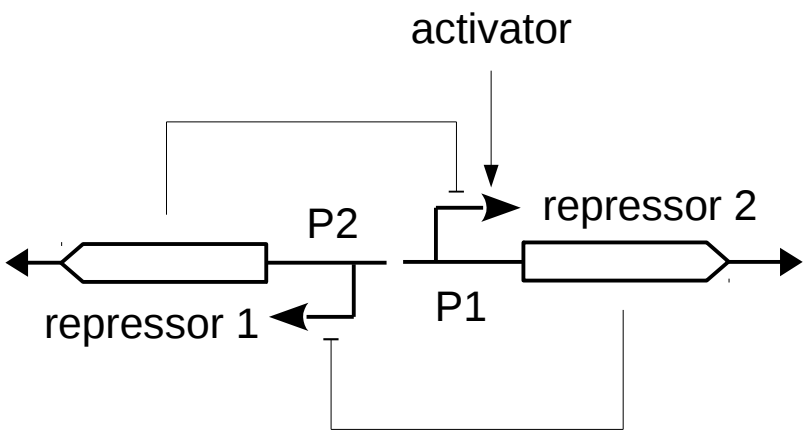


This also resolves to an auto-induction.

adapted from M. Leisner (2008) *PhD thesis*

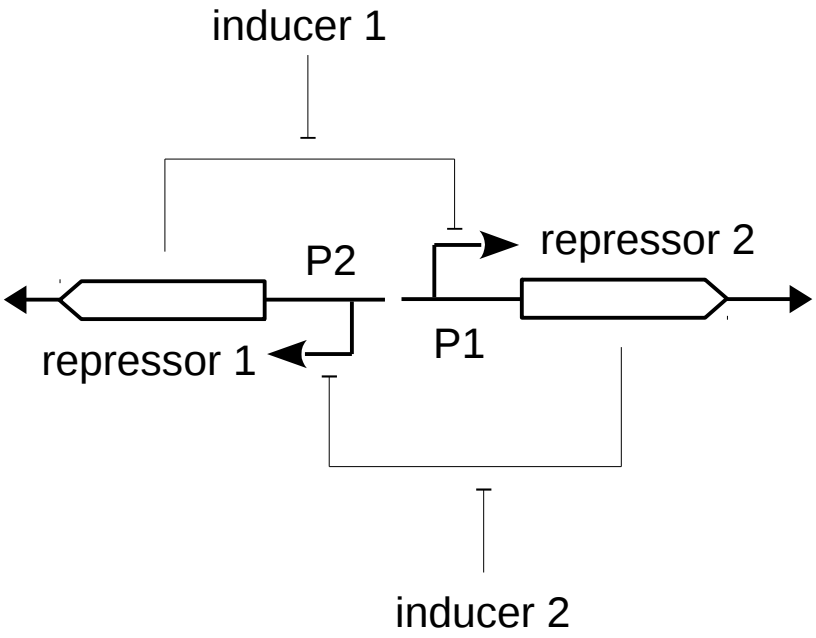
Let's build a genetic switch

So, instead of self-activation, we can repress a repressor:



Let's build a genetic switch

and replace the activators by inducers:

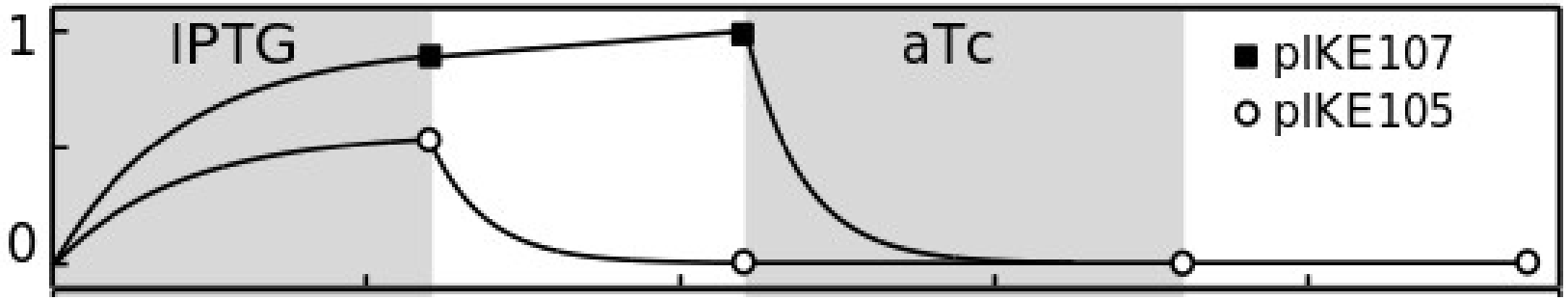


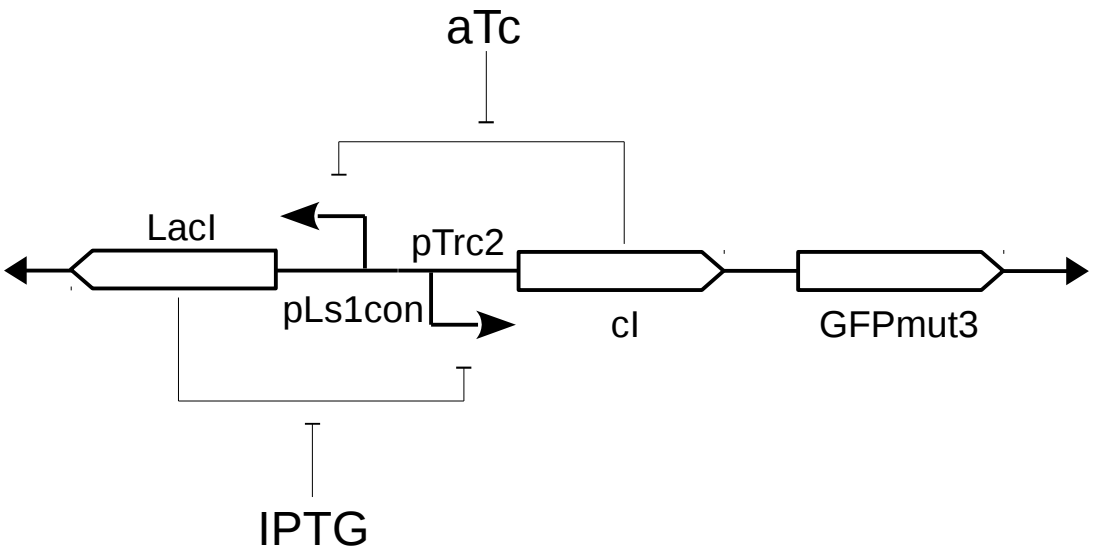
Construction of a genetic toggle switch in *Escherichia coli*

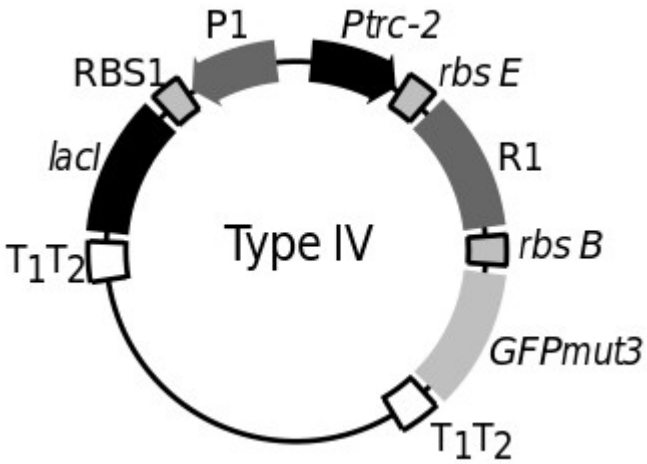
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

** Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

A similar switch regulates lysogenic and lytic state in phage lambda.



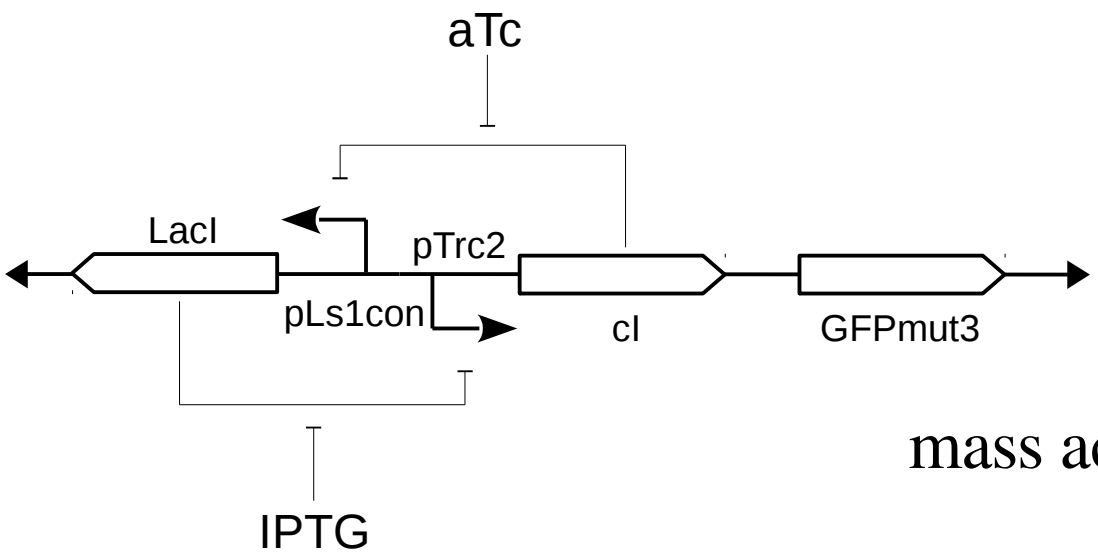




Gardner et al. (2000) *Nature*

A simple model

expression:	pLs1con	→	pLs1con + LacI
regulation:	pLs1con + cI	↔	pLs1con~cI
induction:	cI + aTc	↔	cI~aTc
degradation:	cI	→	
	pTrc2	→	pTrc2 + cI + GFPmut3
same for the	pTrc2 + LacI	↔	pTrc2~LacI
second unit	LacI + IPTG	↔	LacI~IPTG
	LacI	→	
	GFPmut3	→	



11 species
13 reactions
13 rate constants

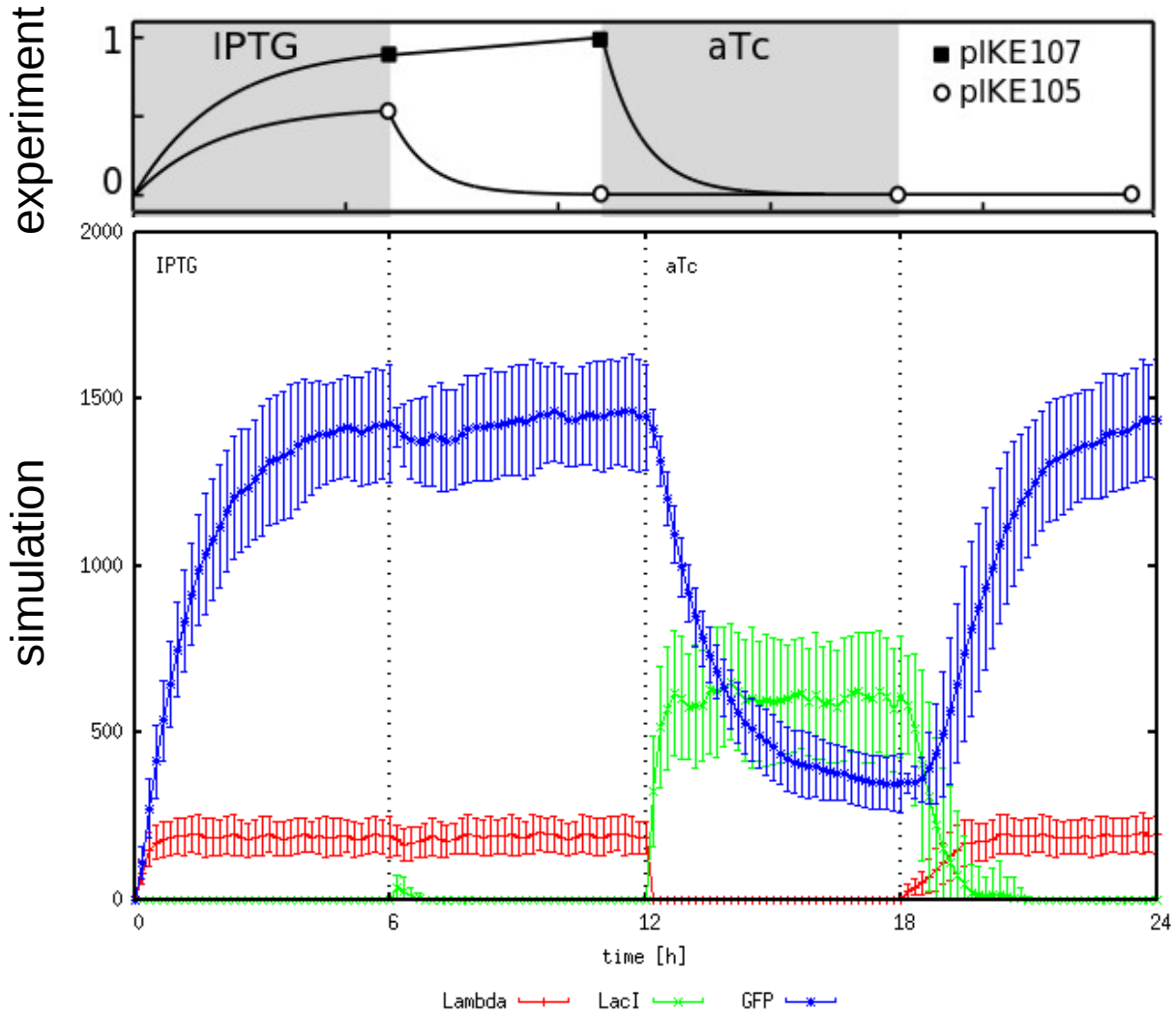
using a simple
mass action kinetics approach

A simple model

Parametrization from literature, data fitting, guessing ...

```
GFP_degradation     : GFPmut3 ->                    0.0132 min-1 // from Gardner data
cI_degradation      : cI ->                         0.1 min-1    // bionumbers
LacI_degradation    : LacI ->                       0.125 min-1  // from Purcell et al.
mRNA_degradation    : mrna - >                      0.263 min-1  // from Semsey et al.

Ptrc2_inhibition    : Ptrc2 + LacI <-> Ptrc2~LacI Kd = 1/170  // iGEM Pico Plumber
Lac_activation      : IPTG + LacI <-> IPTG~LacI   Kd = 1/1200 // iGEM Pico Plumber

PLs1con_inhibition : PLs1con + cI <-> PLs1con~cI
                                                  Kd = 1/10   // blind guess...
cI_activation  : aTc + cI <-> aTc~cI              Kd = 1/1200 // copied from above...

transcription : Ptrc2 -> Ptrc2 + mrna
transcription : PLs1con -> PLs1con + mrna         1 min-1     // guess to obtain ~5
mRNAs

translation   : mrna -> mrna + cI + GFPmut3
translation   : mrna -> mrna + LacI               5 min=1     // guess to obtain
~1000proteins
```

A simple model



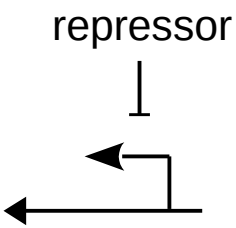
no matter how I tweaked the parameters,
I could not get this model to work ...

How to fix this ...?

Potential strategies to make our model work:

- Fit rate constants against measured data?
- Evolve rate constants using machine learning techniques?
- Define as a satisfiability problem and use some sophisticated logic solver?

Disecting the simple model



expression: $P_{free} \rightarrow P_{free} + X$

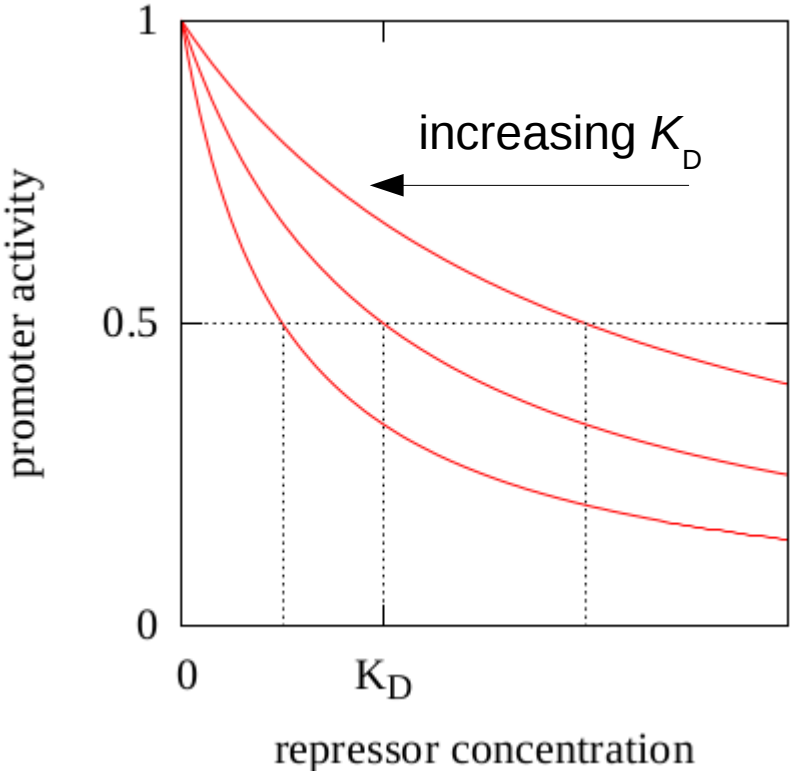
regulation: $\mathbf{P_{free} + R \leftrightarrow P_{blocked}}$

degradation: $X \rightarrow$

assuming equilibrium

$$P_{free} + R \rightleftharpoons^{K_D} P_{blocked}$$

$$\frac{[P_{free}]}{[P]_{total}} = \frac{1}{1 + K_D\,[R]}$$

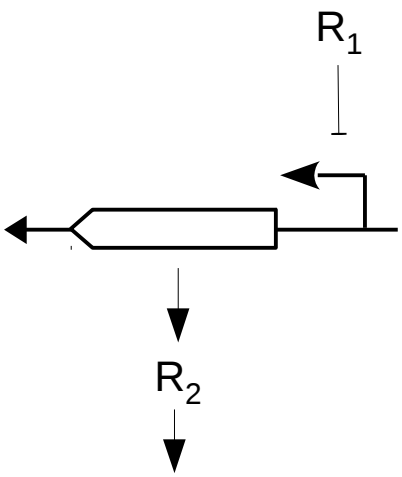


Disecting the simple model

expression: $\mathbf{P_{free}} \rightarrow \mathbf{P_{free} + R_2}$

regulation: $P_{free} + R_1 \leftrightarrow P_{blocked}$

degradation: $\mathbf{R_2} \rightarrow$

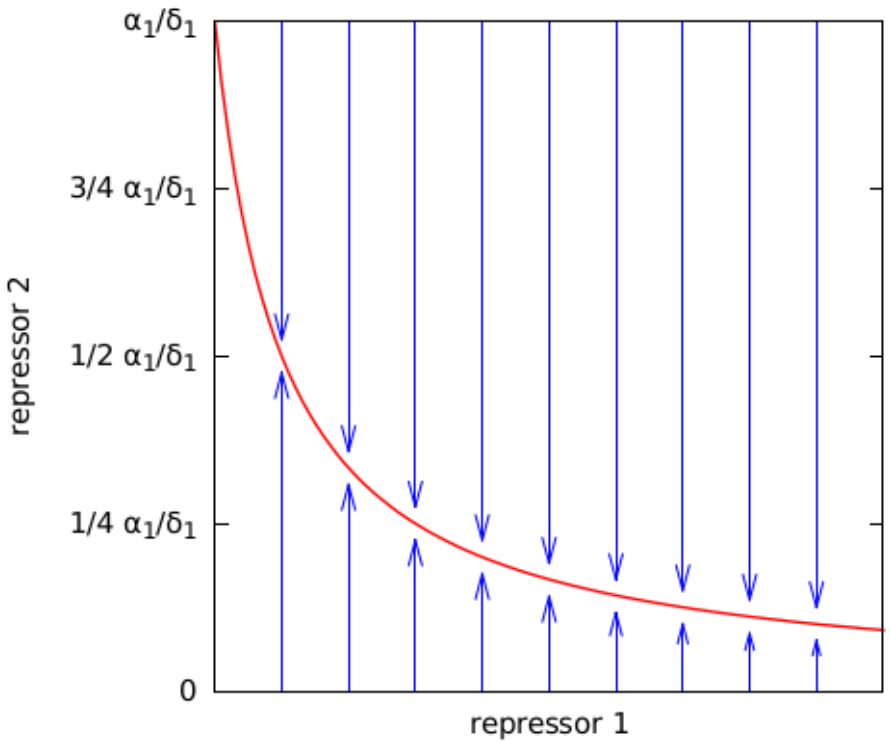


assuming constant $[R_1]$

$$\frac{d}{dt}[R_2] = \alpha_1 \frac{[P_{free}]}{[P]_{total}} - \delta_1 [R_2]$$

$$= \frac{\alpha_1}{1 + K_{D,1}[R_1]} - \delta_1 [R_2]$$

$$[R_2]_{eq} = \frac{\alpha_1}{\delta_1} \frac{1}{1 + K_{D,1}[R_1]}$$

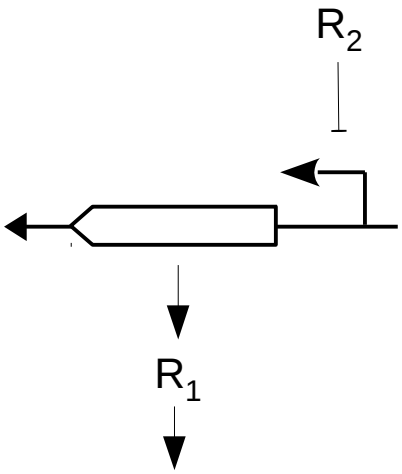


Disecting the simple model

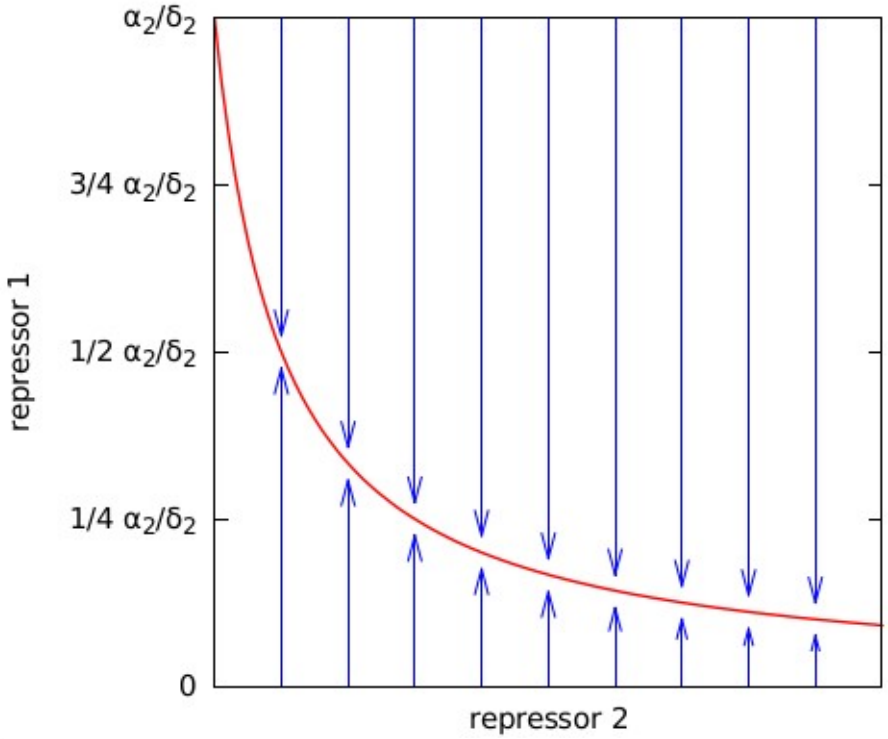
expression: $\mathbf{Q_{free} \rightarrow Q_{free} + R_1}$

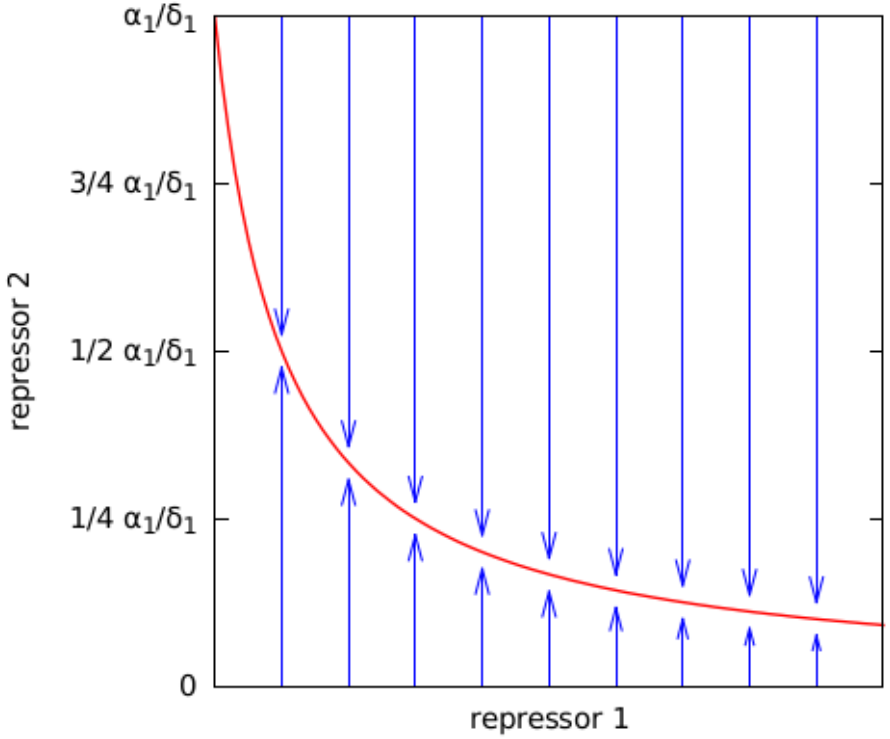
regulation: $Q_{free} + R_2 \leftrightarrow Q_{blocked}$

degradation: $\mathbf{R_1 \rightarrow}$



Same for the second promoter



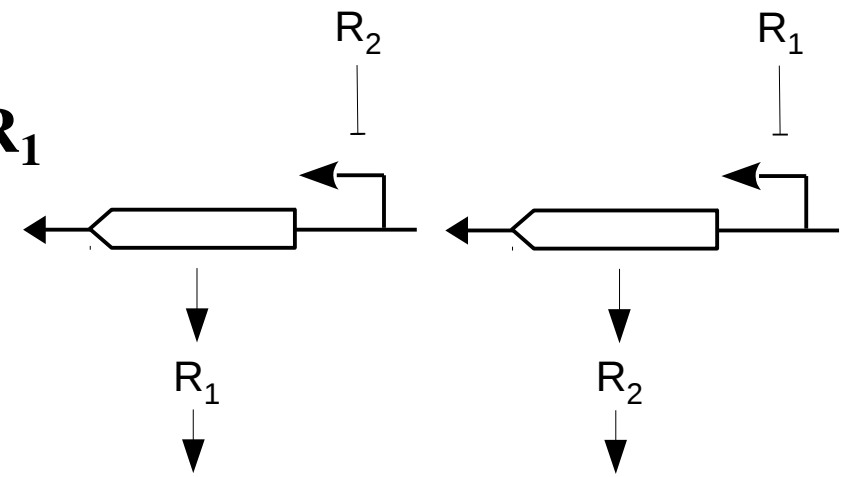


Disecting the simple model

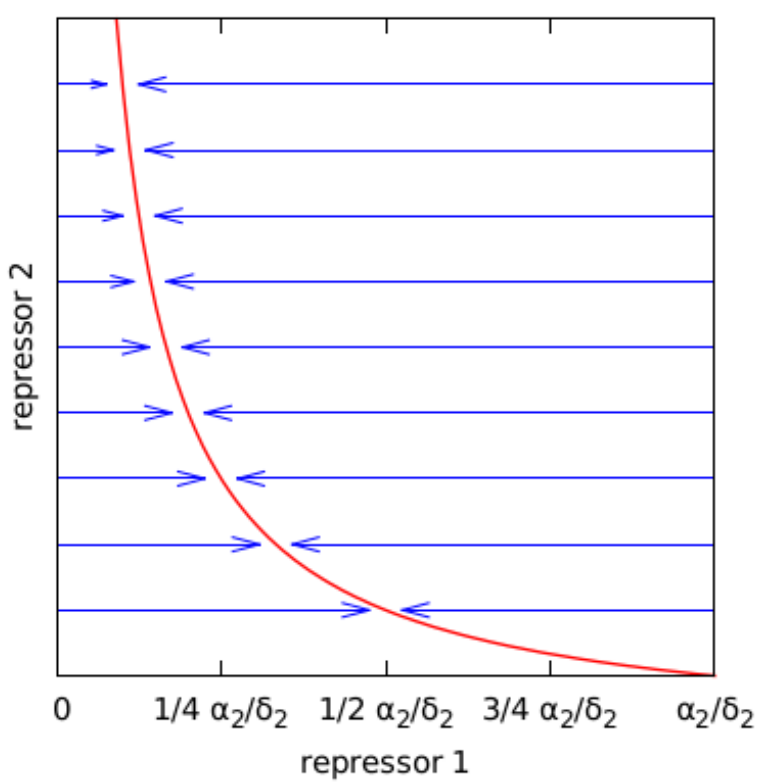
expression: $\mathbf{Q_{free} \rightarrow Q_{free} + R_1}$

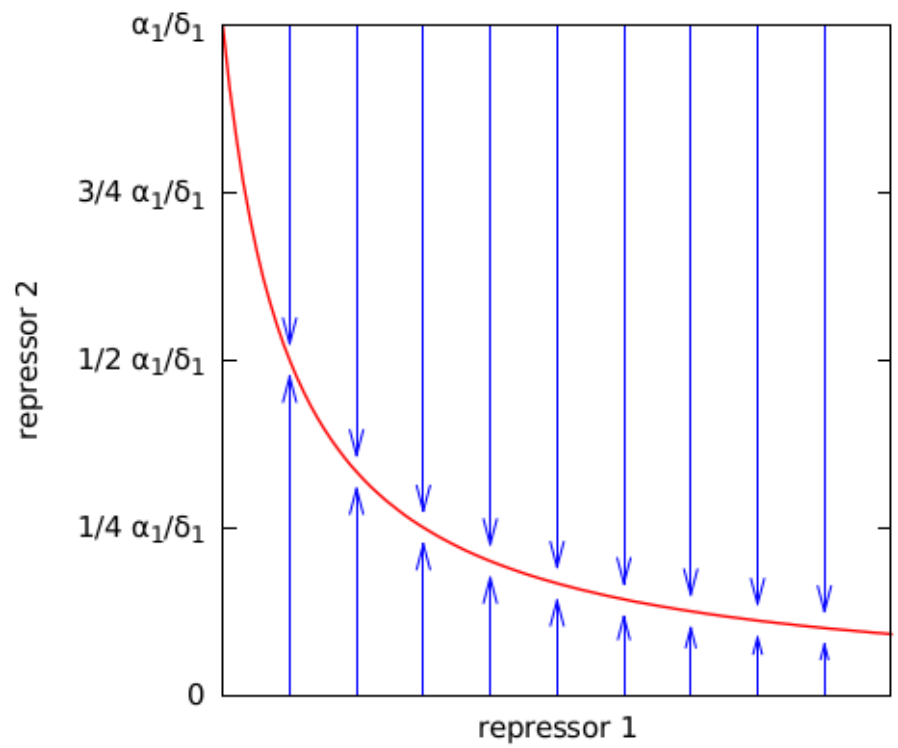
regulation: $Q_{free} + R_2 \leftrightarrow Q_{blocked}$

degradation: $\mathbf{R_1 \rightarrow}$



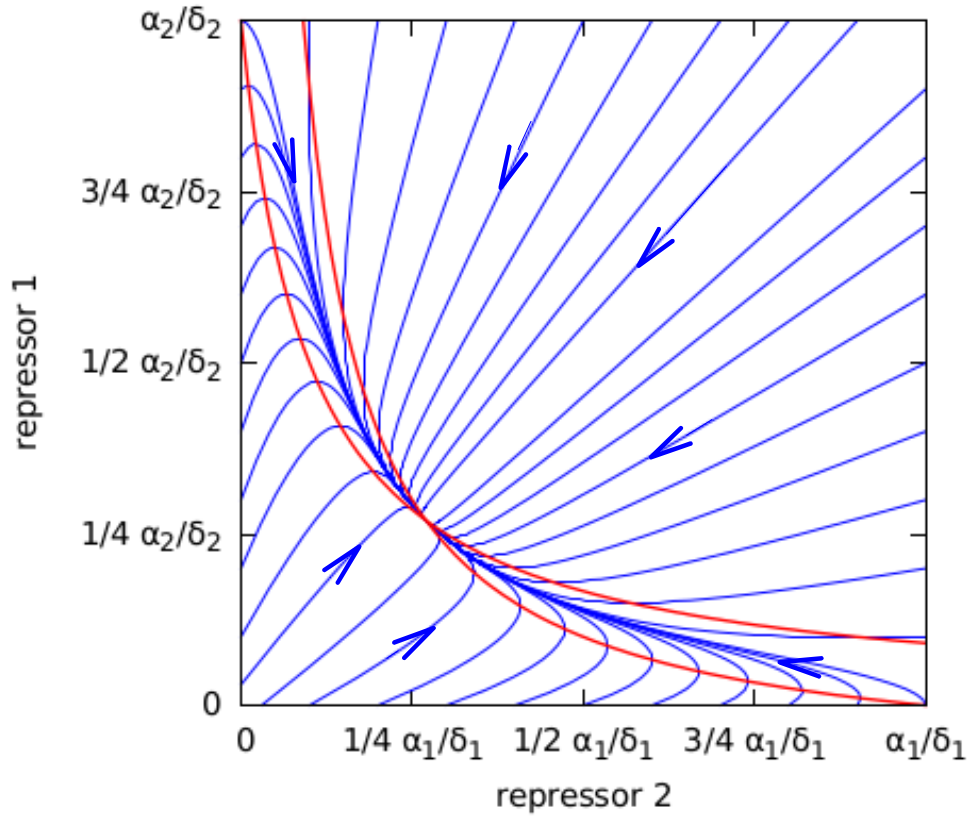
Same for the second promoter





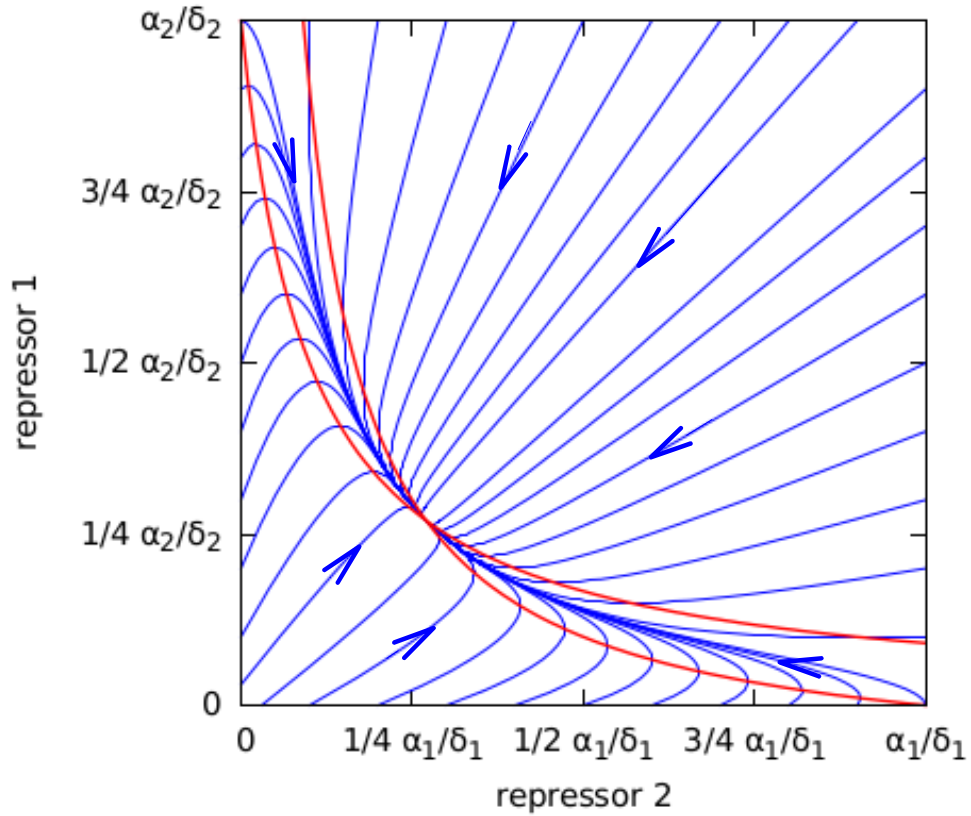
The simple model in phase space

- For each (R_1,R_2) combination, we draw an arrow to where the dynamics will lead in infinitesimal time.
- The crossing of the red lines (nullklines) defines the steady state (here attractor).



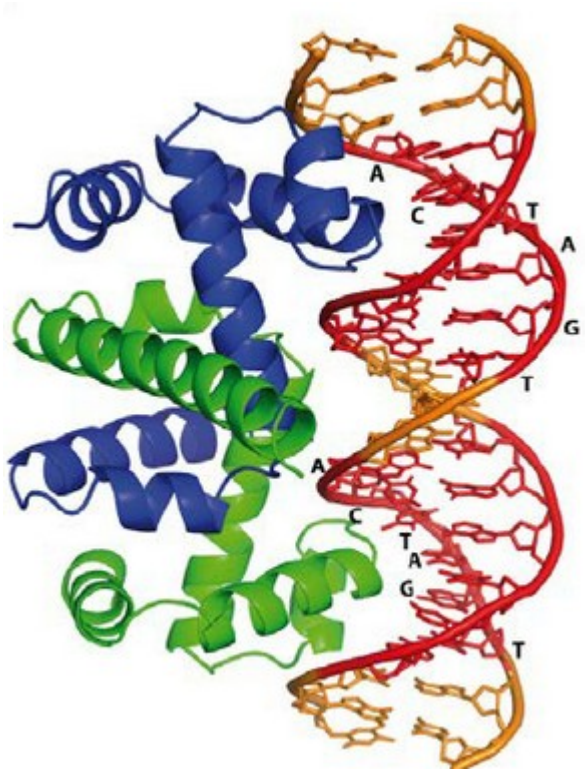
The simple model in phase space

- It exhibits a *single* steady state.
- This is why the model is *not bistable*.
- One can proof that ***no parametrization*** can make this model bistable!

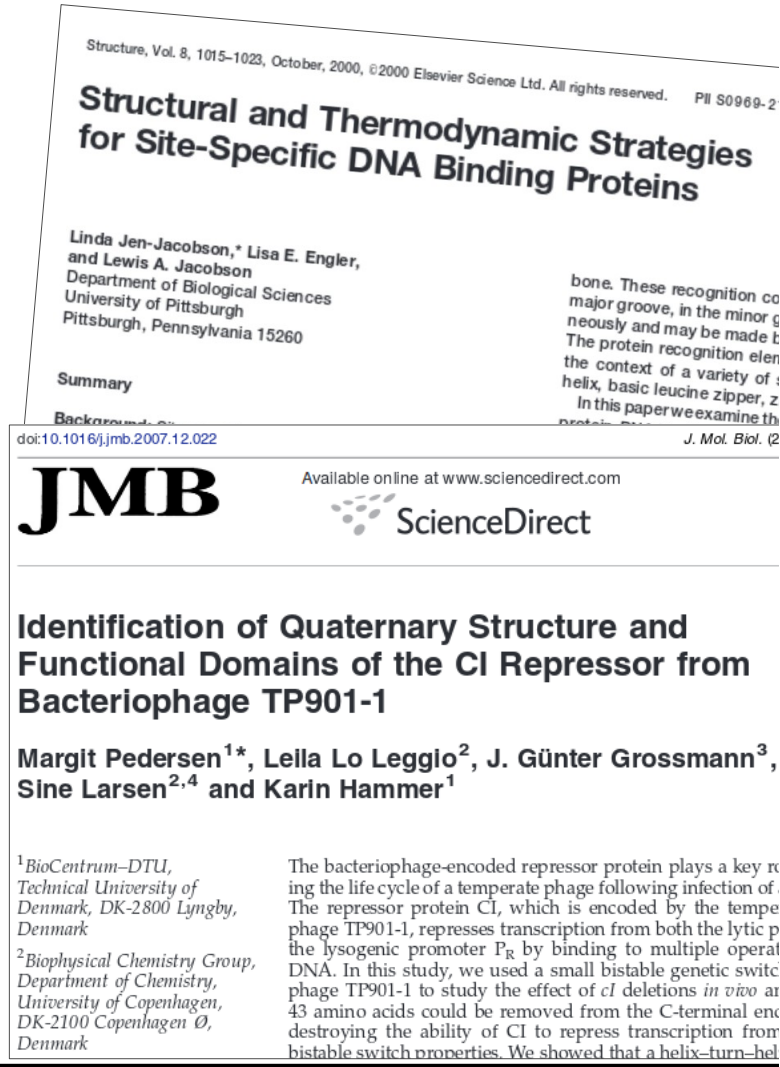


So how to fix this then...?

Repressors are usually cooperative;
they form multimers, e.g. dimers or tetramers



LexA repressor – DNA interaction



Structure, Vol. 8, 1015–1023, October, 2000, ©2000 Elsevier Science Ltd. All rights reserved. PII S0969-2

Structural and Thermodynamic Strategies for Site-Specific DNA Binding Proteins

Linda Jen-Jacobson,* Lisa E. Engler, and Lewis A. Jacobson
Department of Biological Sciences
University of Pittsburgh
Pittsburgh, Pennsylvania 15260

Summary

bone. These recognition co
major groove, in the minor g
neously and may be made b
The protein recognition elem
the context of a variety of s
helix, basic leucine zipper, z
In this paper we examine th

doi:10.1016/j.jmb.2007.12.022 J. Mol. Biol. (2

JMB

Available online at www.sciencedirect.com
ScienceDirect

Identification of Quaternary Structure and Functional Domains of the CI Repressor from Bacteriophage TP901-1

Margit Pedersen[1]*, Leila Lo Leggio[2], J. Günter Grossmann[3], Sine Larsen[2,4] and Karin Hammer[1]

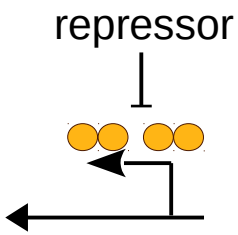
[1]*BioCentrum–DTU, Technical University of Denmark, DK-2800 Lyngby, Denmark*

[2]*Biophysical Chemistry Group, Department of Chemistry, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark*

The bacteriophage-encoded repressor protein plays a key ro
ing the life cycle of a temperate phage following infection of
The repressor protein CI, which is encoded by the tempe
phage TP901-1, represses transcription from both the lytic p
the lysogenic promoter P_R by binding to multiple operat
DNA. In this study, we used a small bistable genetic switc
phage TP901-1 to study the effect of *cI* deletions *in vivo* an
43 amino acids could be removed from the C-terminal end
destroying the ability of CI to repress transcription from
bistable switch properties. We showed that a helix–turn–hel

The corrected model

Let's take the multimerization into account:



expression: $P_{free} \rightarrow P_{free} + R_2$

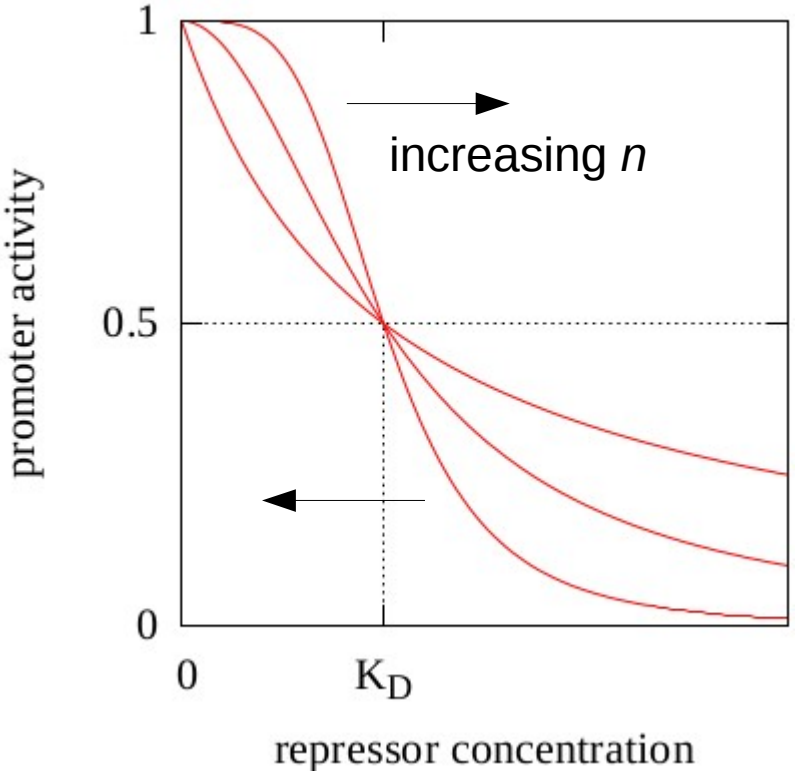
regulation: $P_{free} + \boldsymbol{n}\, R_1 \leftrightarrow P_{blocked}$

degradation: $R_2 \rightarrow$

This gives an inflection
in the promoter response curve.

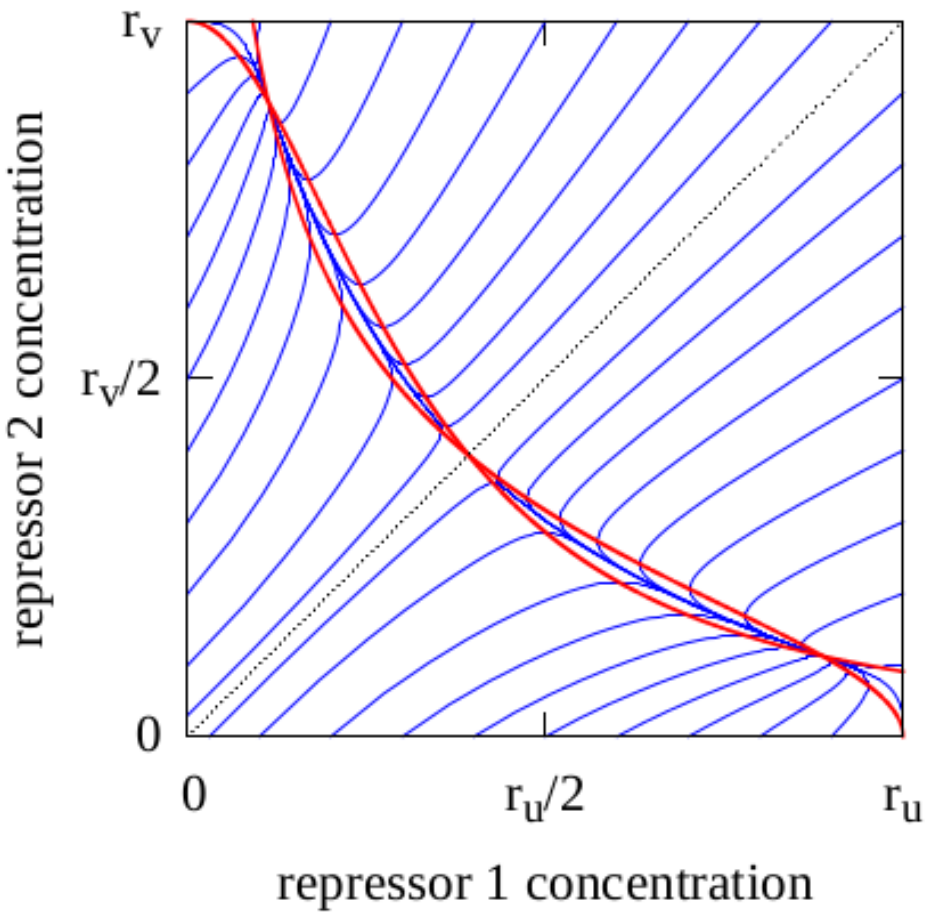
$$\frac{[P_{free}]}{[P]_{total}} = \frac{1}{1 + K_D\,[R]^n}$$

Hill equation



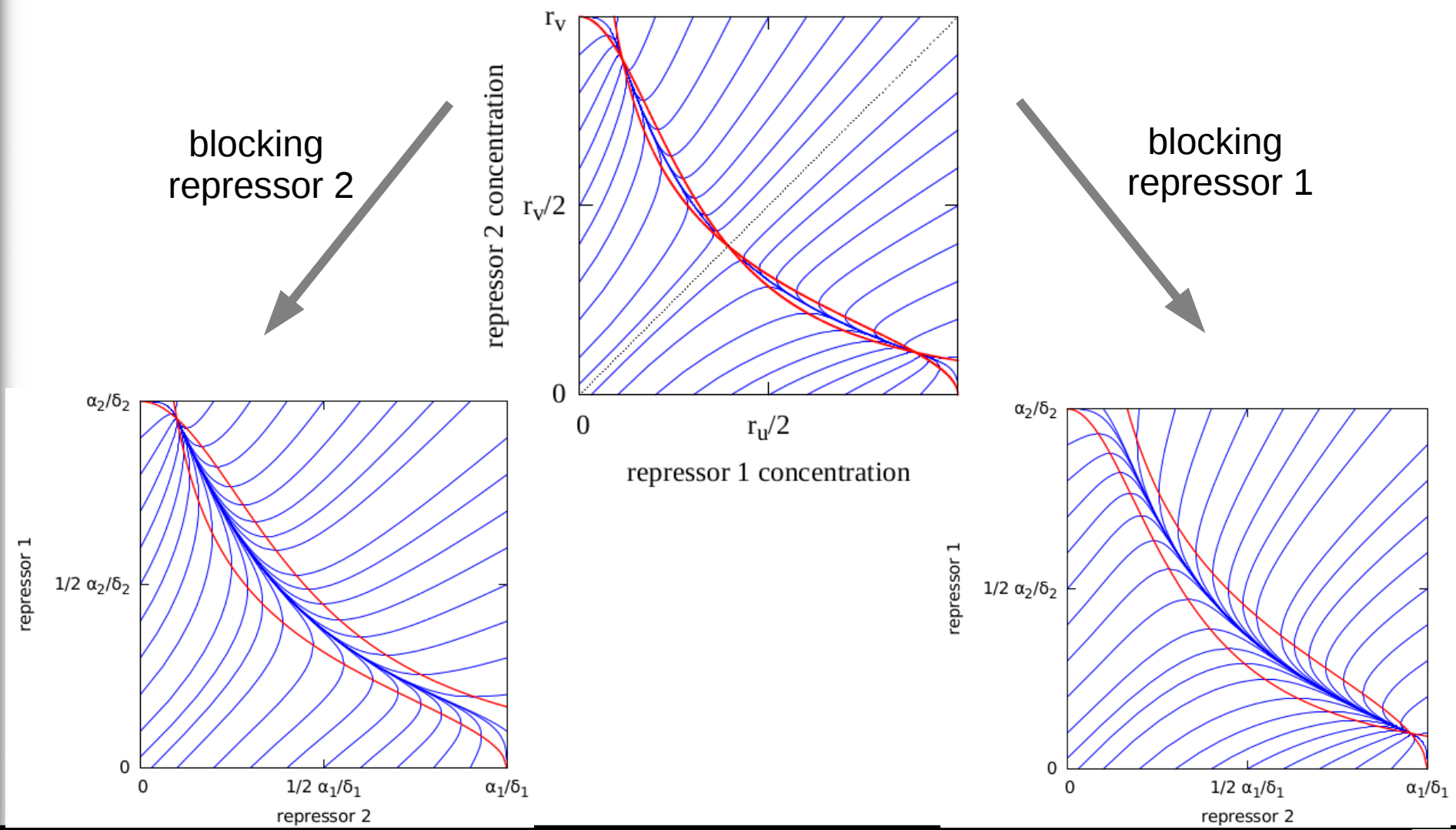
The corrected model in phase space

Now, the phase space diagram can exhibit *two* separate stable states.

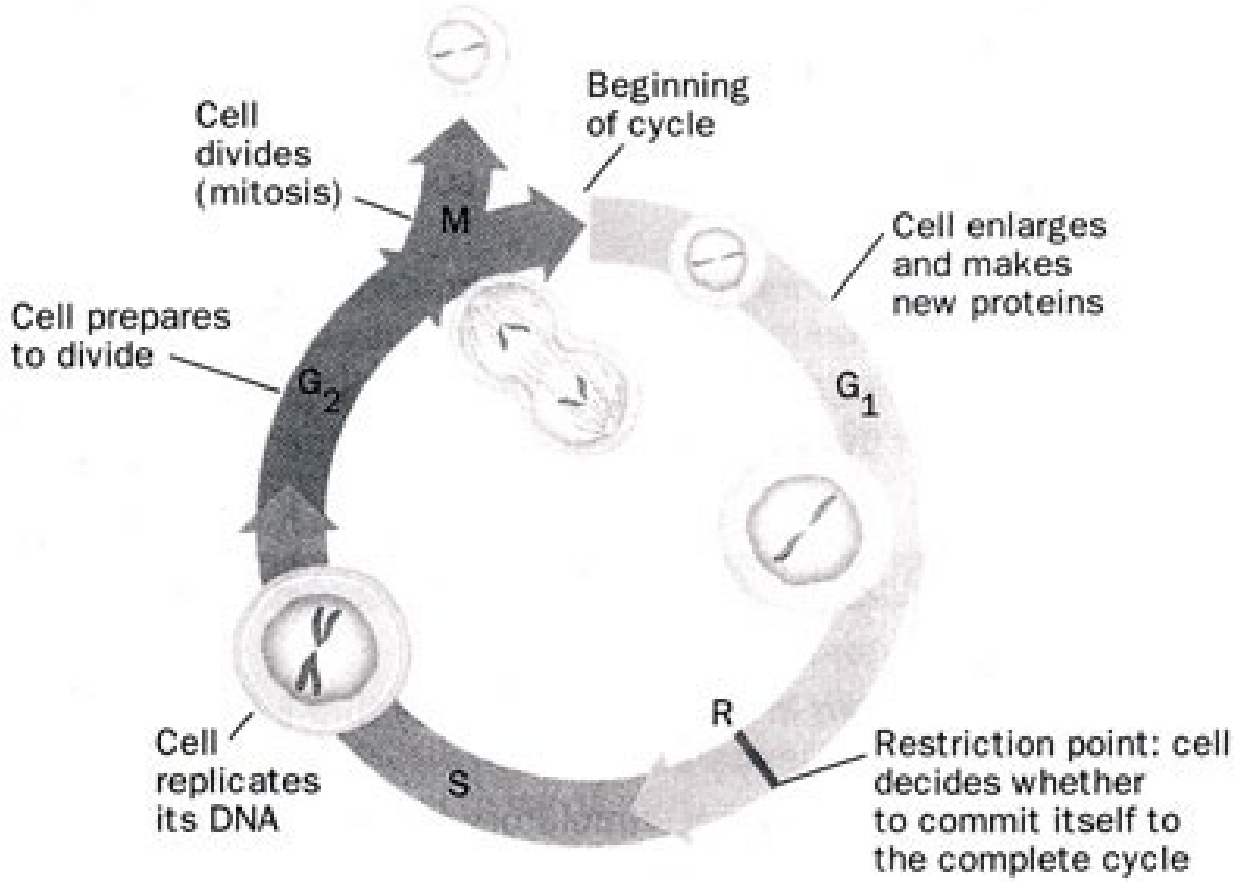


The corrected model in phase space

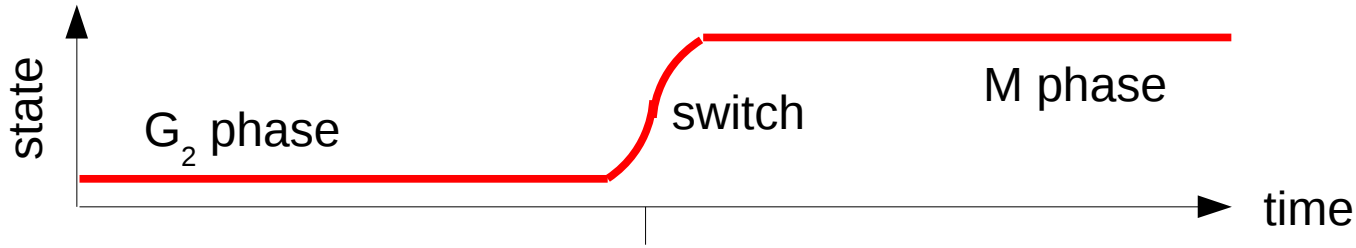
Adding inducers moves nullklines until one steady state disappears:



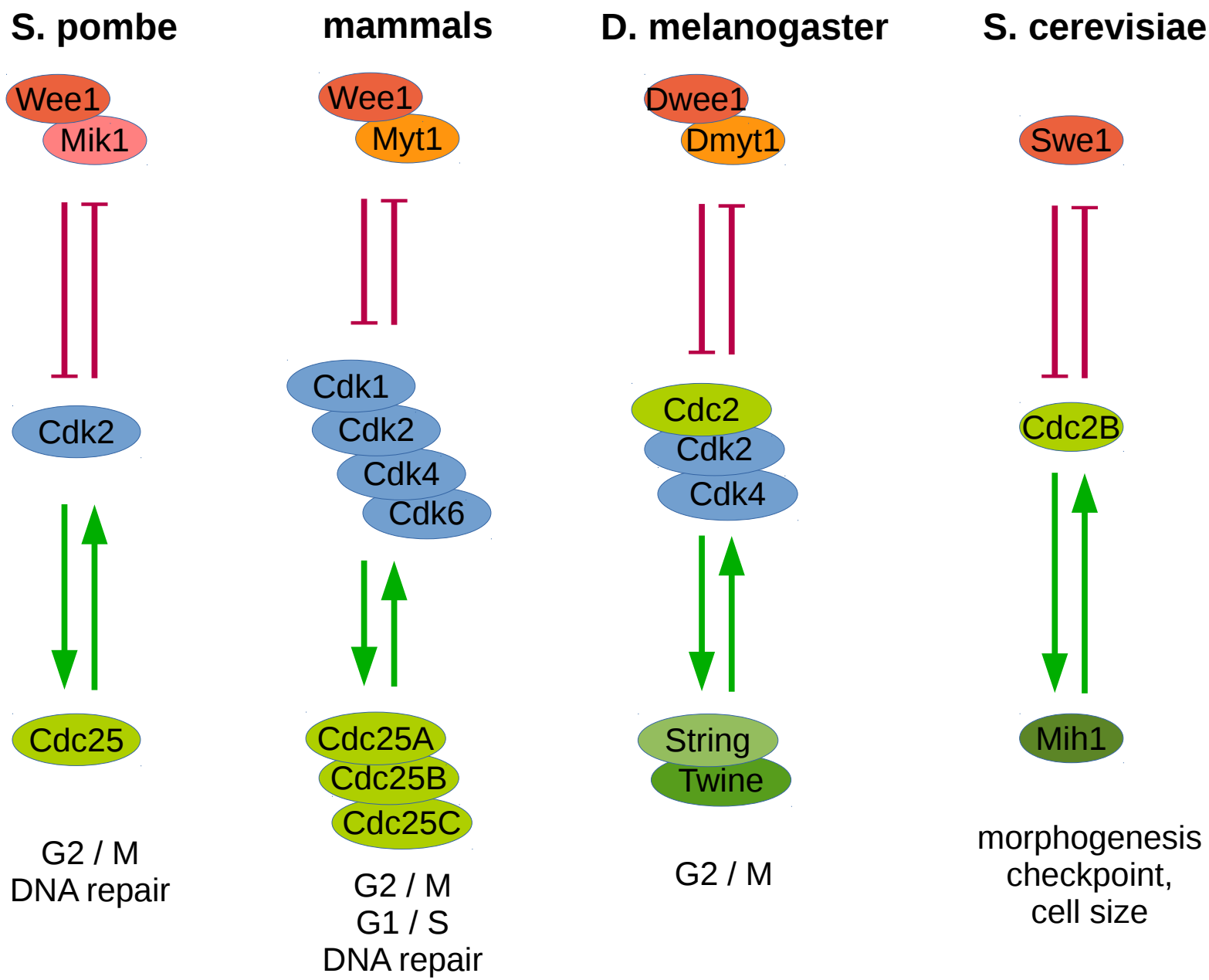
The cell cycle and its check points



Phases are guarded by check points

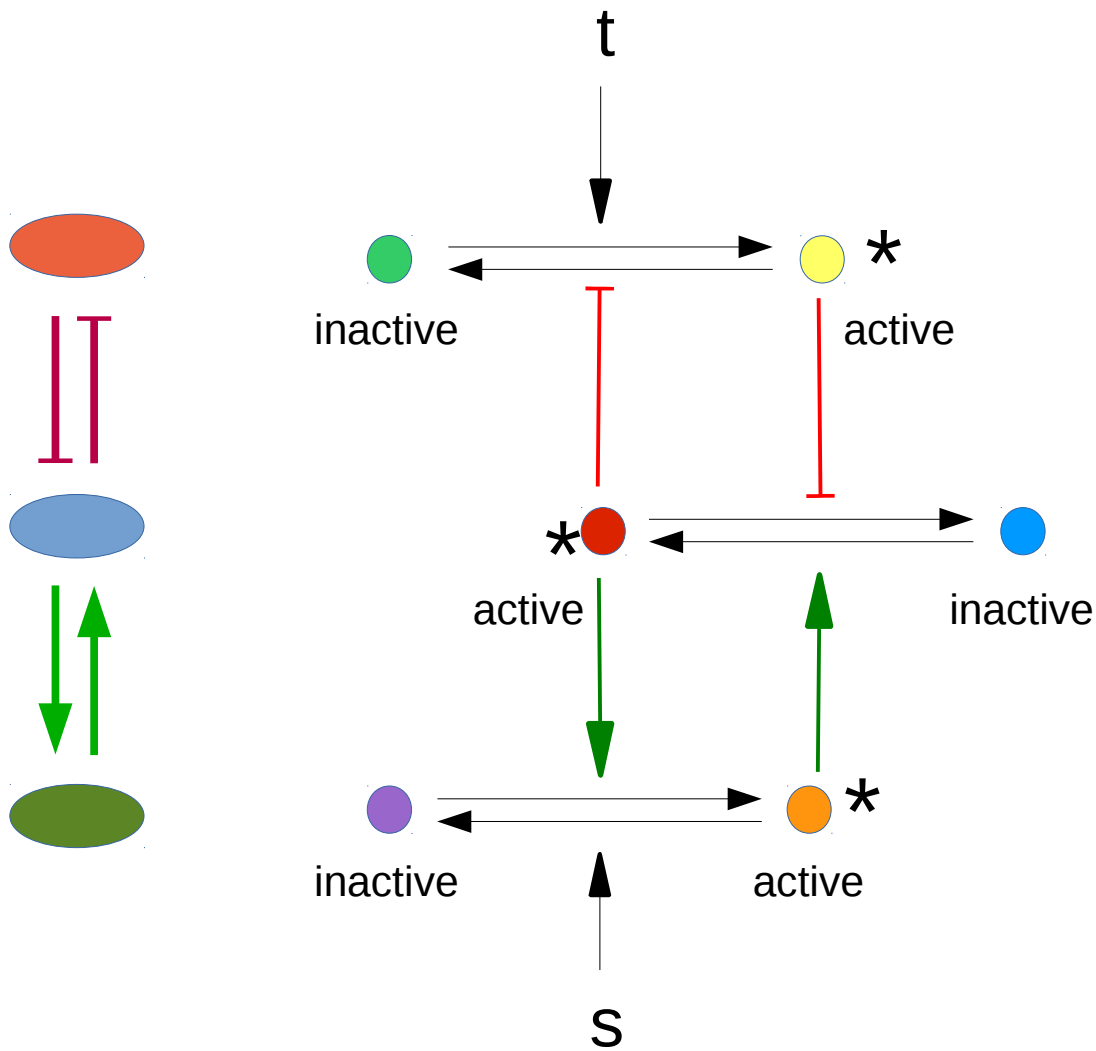


Conserved protein-protein interactions



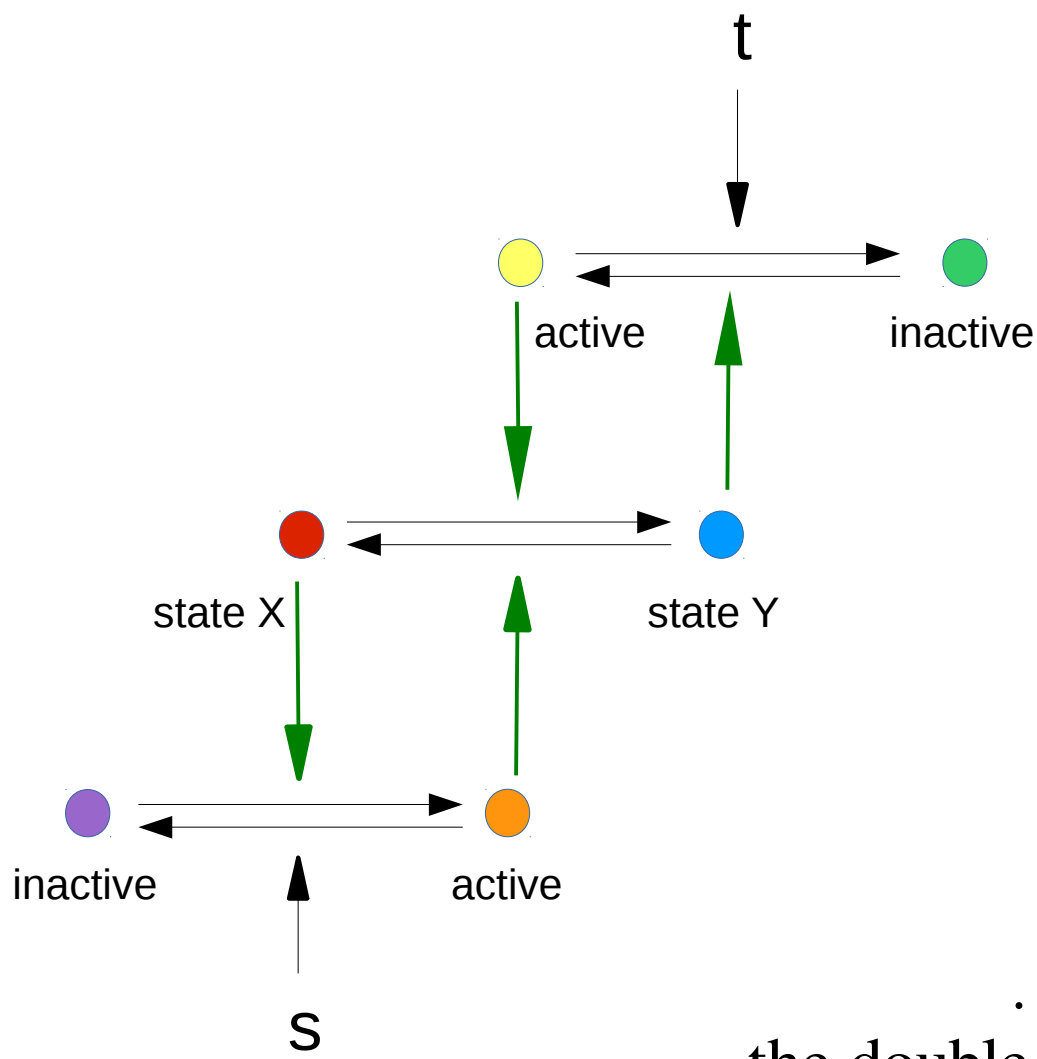
adapted from Harashima et al. *Trends in Cell Biology* 23(7):345 2013

Untangling the diagrams



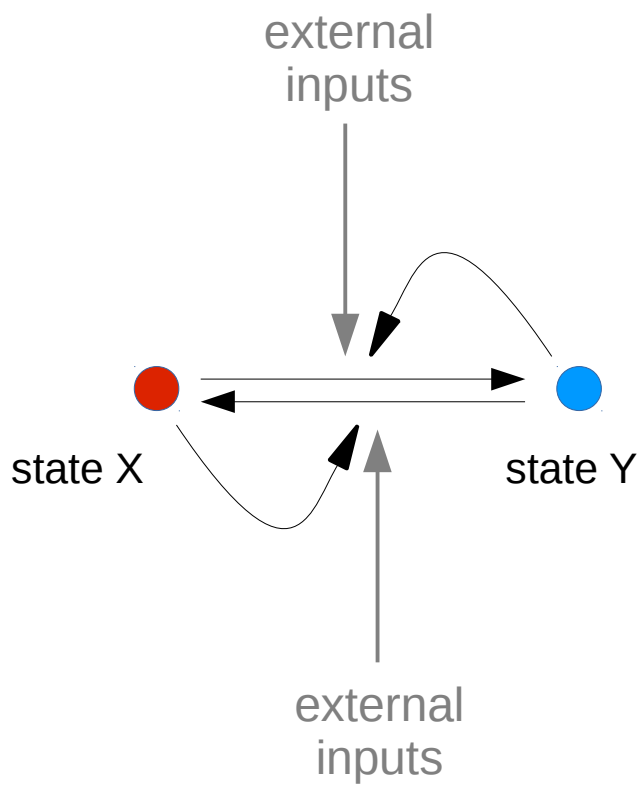
Let's distinguish "active" and "passive" forms of the protein.

Untangling the diagrams



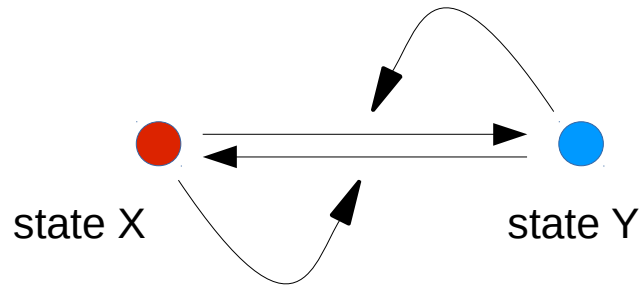
… and replace
the double-negative by a
double-positive feedback loop.

Untangling the diagrams



We are only really interested
in one of the molecules.
(the others are determined by the state of this one).

A simple model

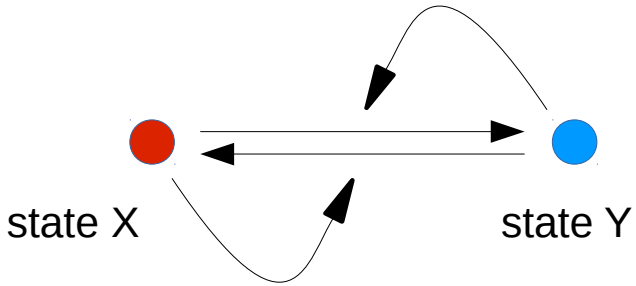


$$X + Y \rightarrow 2\,X$$
$$Y + X \rightarrow 2\,Y$$

For purpose of illustration no parameters.

Using a simple mass action kinetics approach.

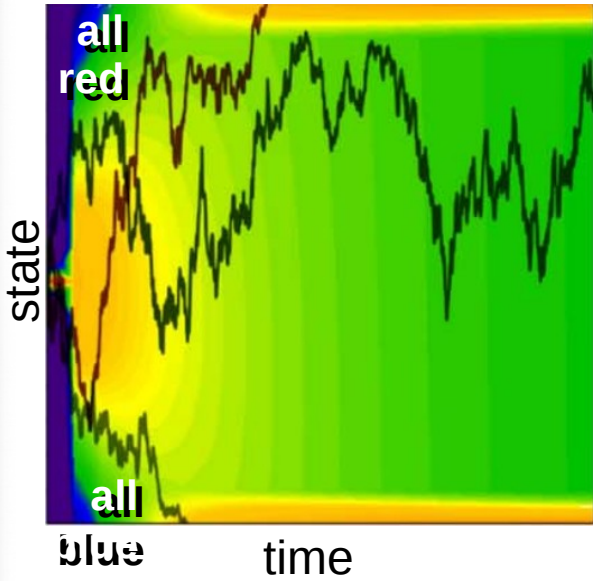
A simple model

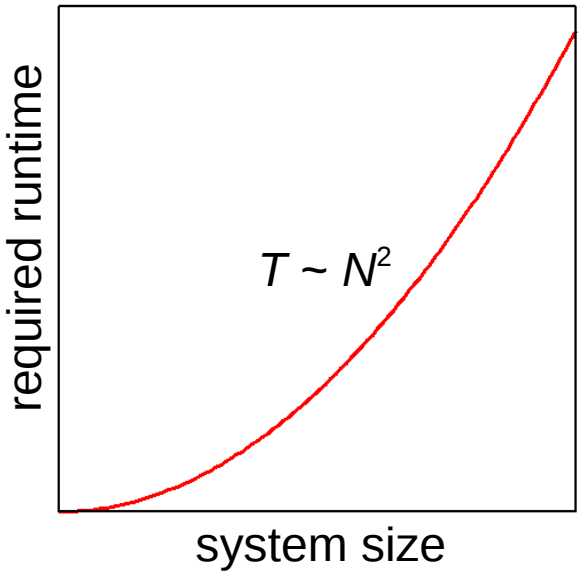


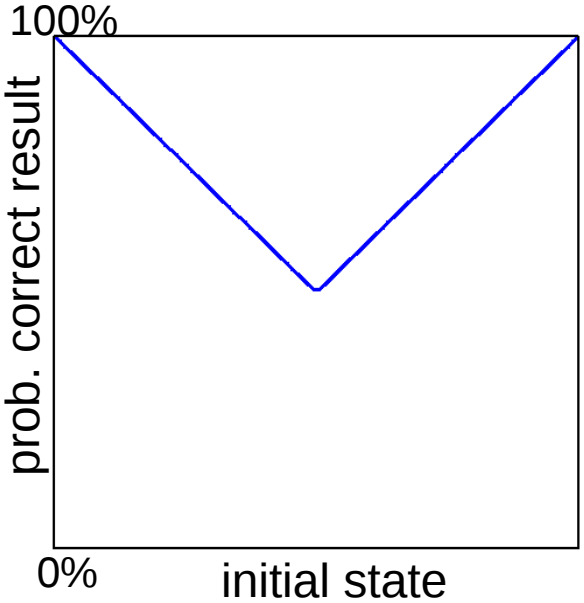
$$X + Y \rightarrow 2\,X$$
$$Y + X \rightarrow 2\,Y$$

$$\dot{x} = \dot{y} = xy - yx = 0$$

This is very bad switch:



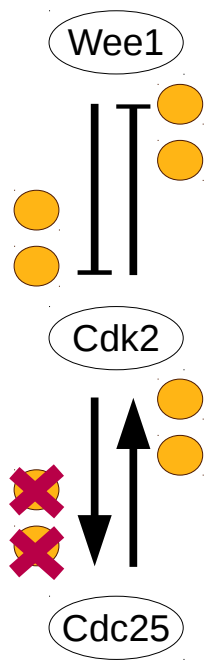


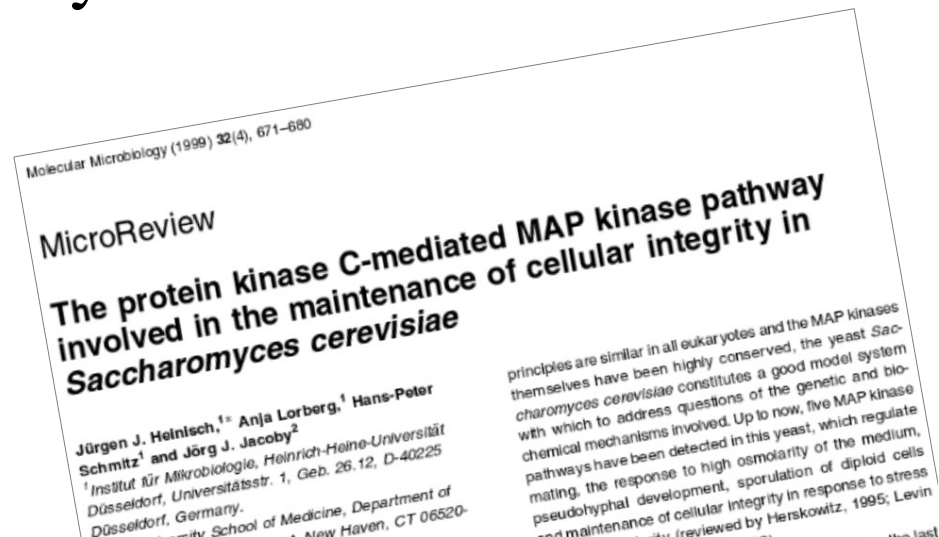


How to fix this ...?

Turns out that the molecules involved in the cell cycle switches are *dual-specific* serine-tyrosine kinases.

They phosphorylate their targets on *two* sites:





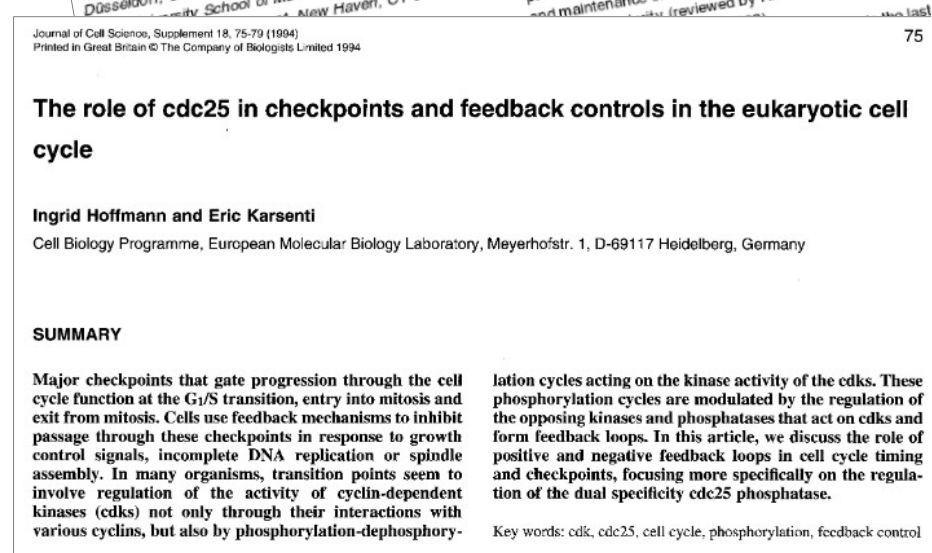
Molecular Microbiology (1999) 32(4), 671–680

MicroReview

The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in *Saccharomyces cerevisiae*

Jürgen J. Heinisch,[1*] Anja Lorberg,[1] Hans-Peter Schmitz[1] and Jörg J. Jacoby[2]
[1] Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb. 26.12, D-40225 Düsseldorf, Germany.
School of Medicine, Department of New Haven, CT 06520-

principles are similar in all eukaryotes and the MAP kinases themselves have been highly conserved, the yeast *Saccharomyces cerevisiae* constitutes a good model system with which to address questions of the genetic and biochemical mechanisms involved. Up to now, five MAP kinase pathways have been detected in this yeast, which regulate mating, the response to high osmolarity of the medium, pseudohyphal development, sporulation of diploid cells and maintenance of cellular integrity in response to stress (reviewed by Herskowitz, 1995; Levin



Journal of Cell Science, Supplement 18, 75-79 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

The role of cdc25 in checkpoints and feedback controls in the eukaryotic cell cycle

Ingrid Hoffmann and Eric Karsenti
Cell Biology Programme, European Molecular Biology Laboratory, Meyerhofstr. 1, D-69117 Heidelberg, Germany

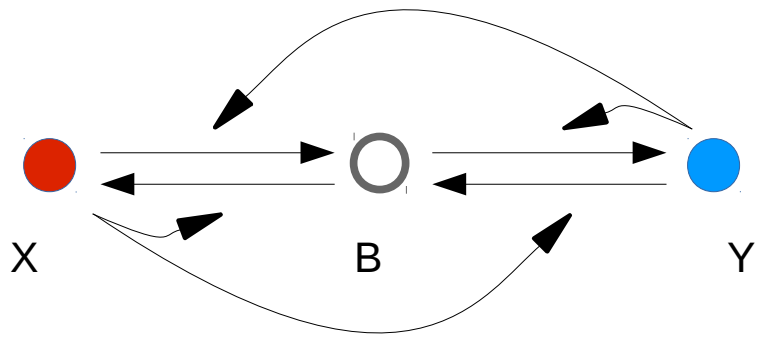
SUMMARY

Major checkpoints that gate progression through the cell cycle function at the G_1/S transition, entry into mitosis and exit from mitosis. Cells use feedback mechanisms to inhibit passage through these checkpoints in response to growth control signals, incomplete DNA replication or spindle assembly. In many organisms, transition points seem to involve regulation of the activity of cyclin-dependent kinases (cdks) not only through their interactions with various cyclins, but also by phosphorylation-dephosphorylation cycles acting on the kinase activity of the cdks. These phosphorylation cycles are modulated by the regulation of the opposing kinases and phosphatases that act on cdks and form feedback loops. In this article, we discuss the role of positive and negative feedback loops in cell cycle timing and checkpoints, focusing more specifically on the regulation of the dual specificity cdc25 phosphatase.

Key words: cdk, cdc25, cell cycle, phosphorylation, feedback control

A better model

We introduce an inactive intermediate state between the two others:



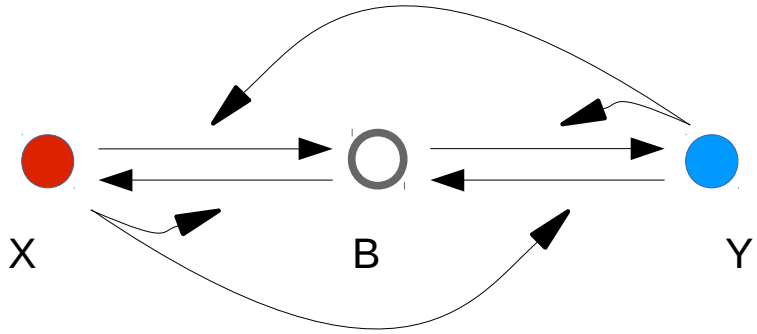
$$X + B \rightarrow 2\,X$$
$$X + Y \rightarrow X + B$$
$$Y + X \rightarrow Y + B$$
$$Y + B \rightarrow 2\,Y$$

$$\dot{x} = x\,(b\text{-}y)$$

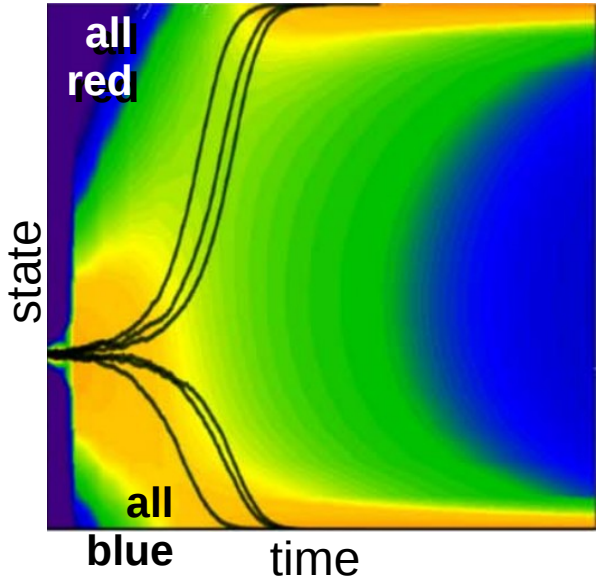
$$\dot{y} = y\,(b\text{-}x)$$

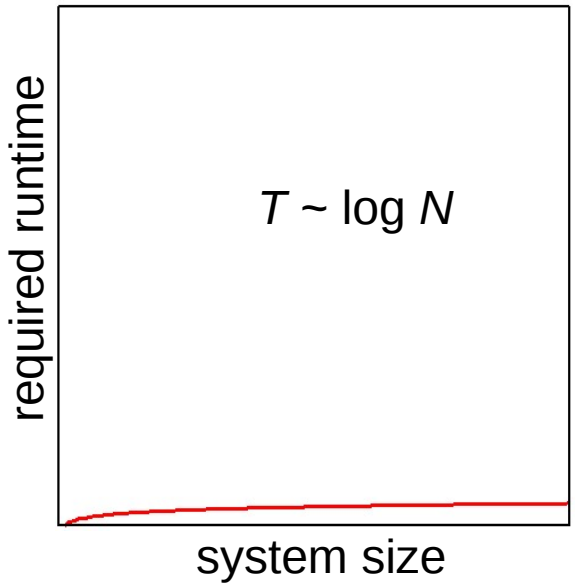
$$\dot{b} = 2\,xy - b(x+y)$$

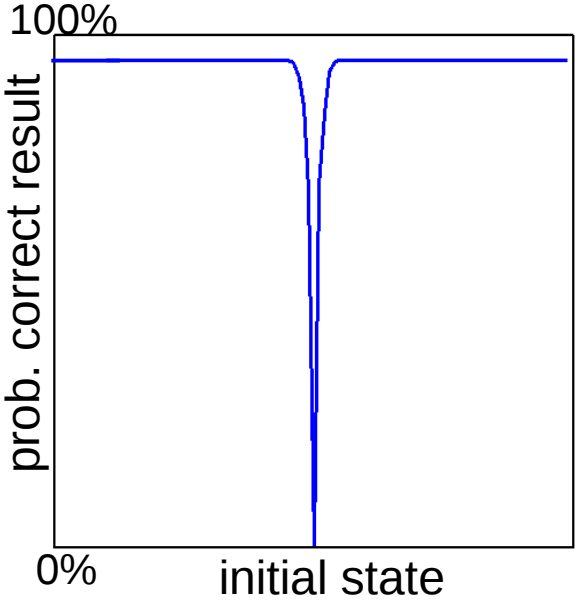
A better model



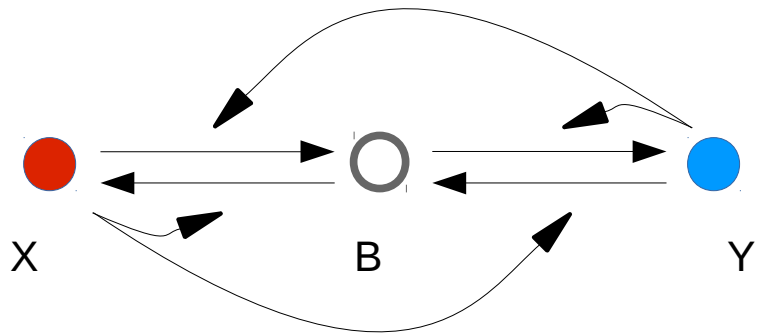
This is an optimal switch:







A better model



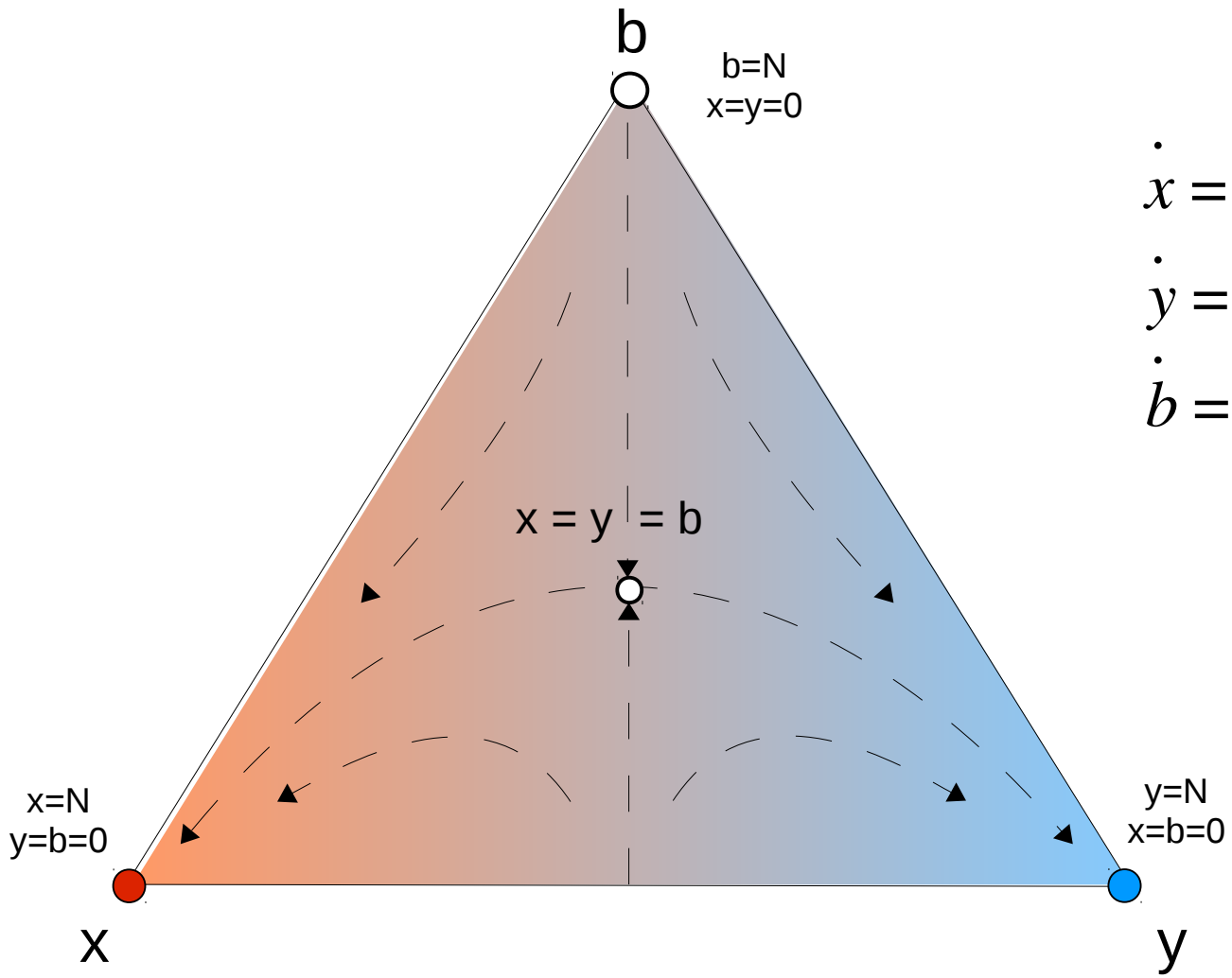
$$X + B \rightarrow 2\,X$$
$$X + Y \rightarrow X + B$$
$$Y + X \rightarrow Y + B$$
$$Y + B \rightarrow 2\,Y$$

$$\dot{x} = x\,(b\text{-}y)$$

$$\dot{y} = y\,(b\text{-}x)$$

$$\dot{b} = 2\,xy - b(x+y)$$

A better model in phase space



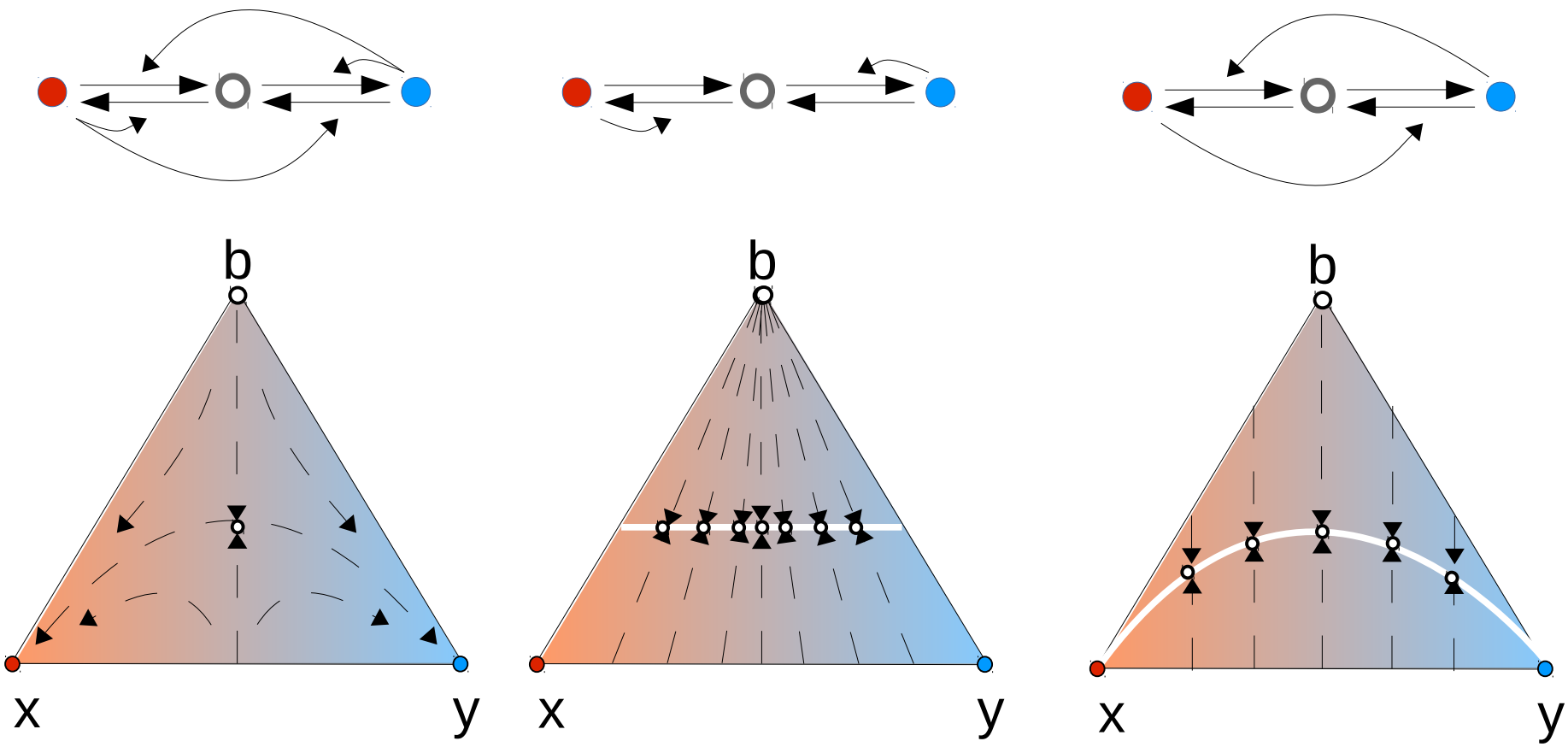
$$\dot{x} = x\,(b\text{-}y)$$

$$\dot{y} = y\,(b\text{-}x)$$

$$\dot{b} = 2\,xy - b(x+y)$$

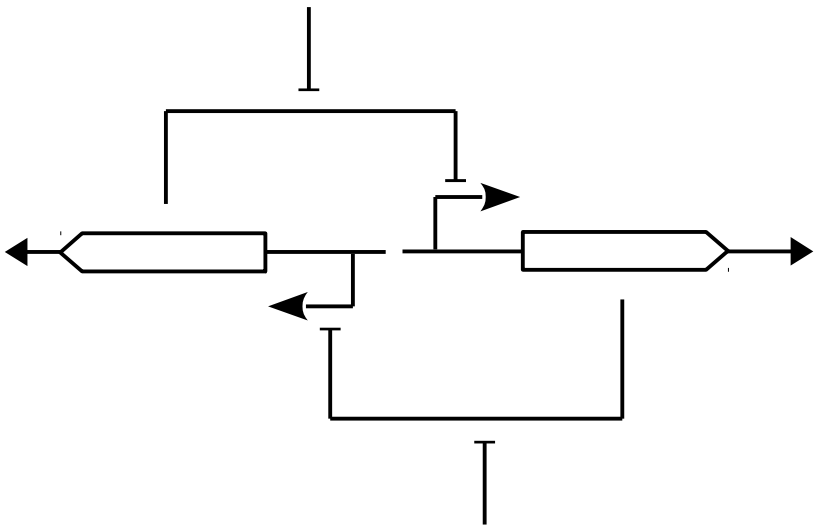
A better model

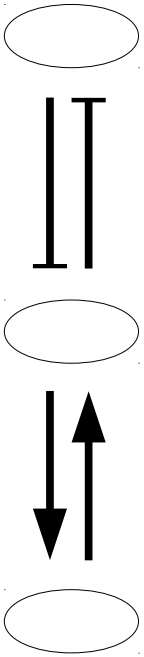
Variations of the model in phase space



What have we learned?

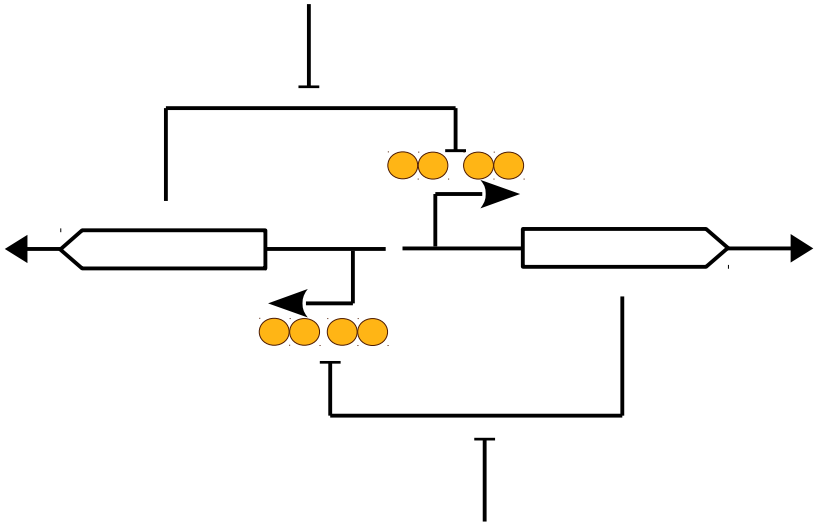
These schematics are not sufficient specifications of the system:



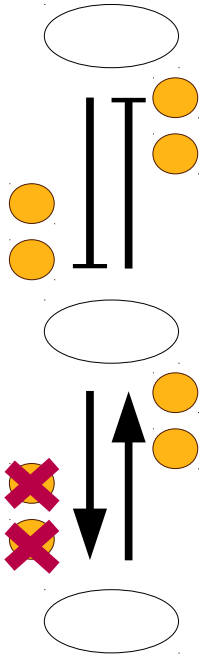


What have we learned?

These would be much more informative:



indicating repressor cooperativity

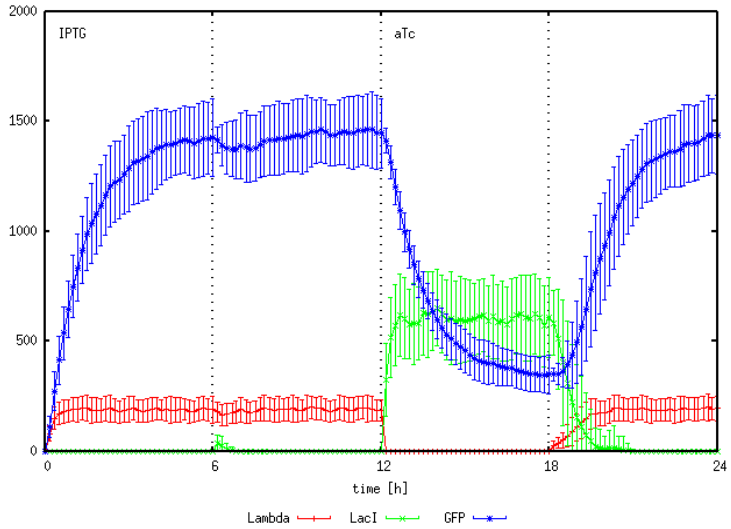


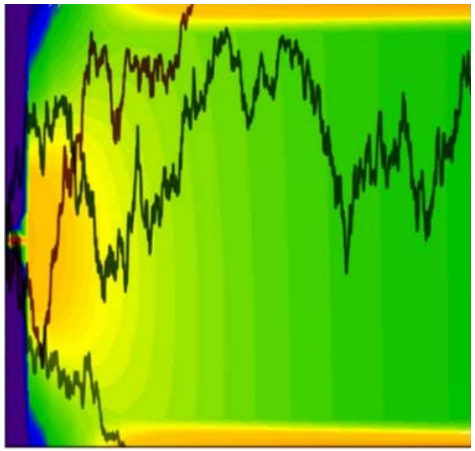
indicating (de)phosphorylations

but are used much less often...

What have we learned?

Trajectories show *how* a system works

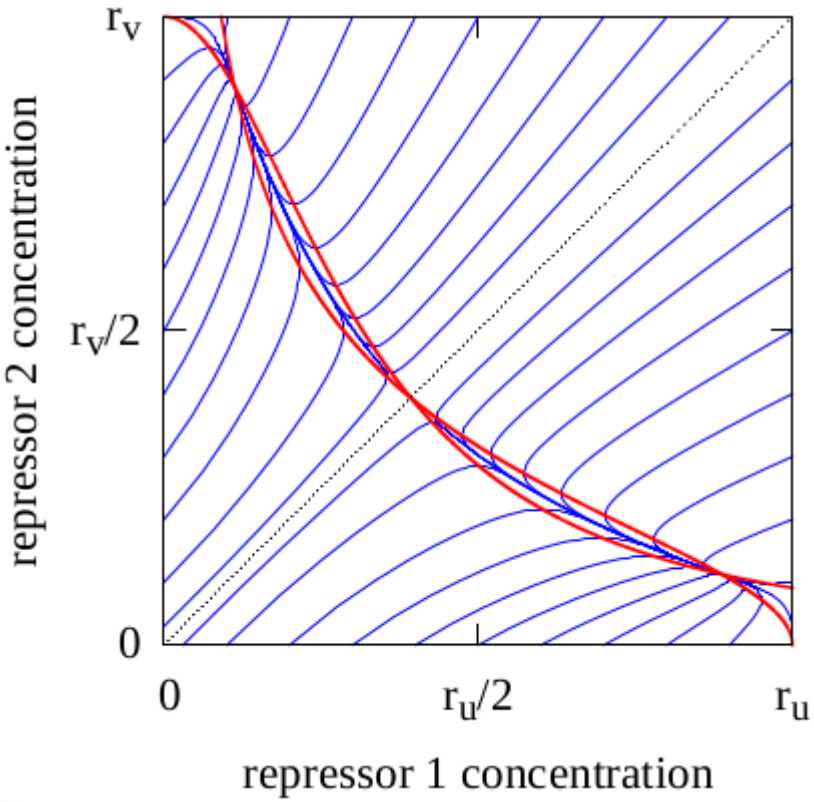


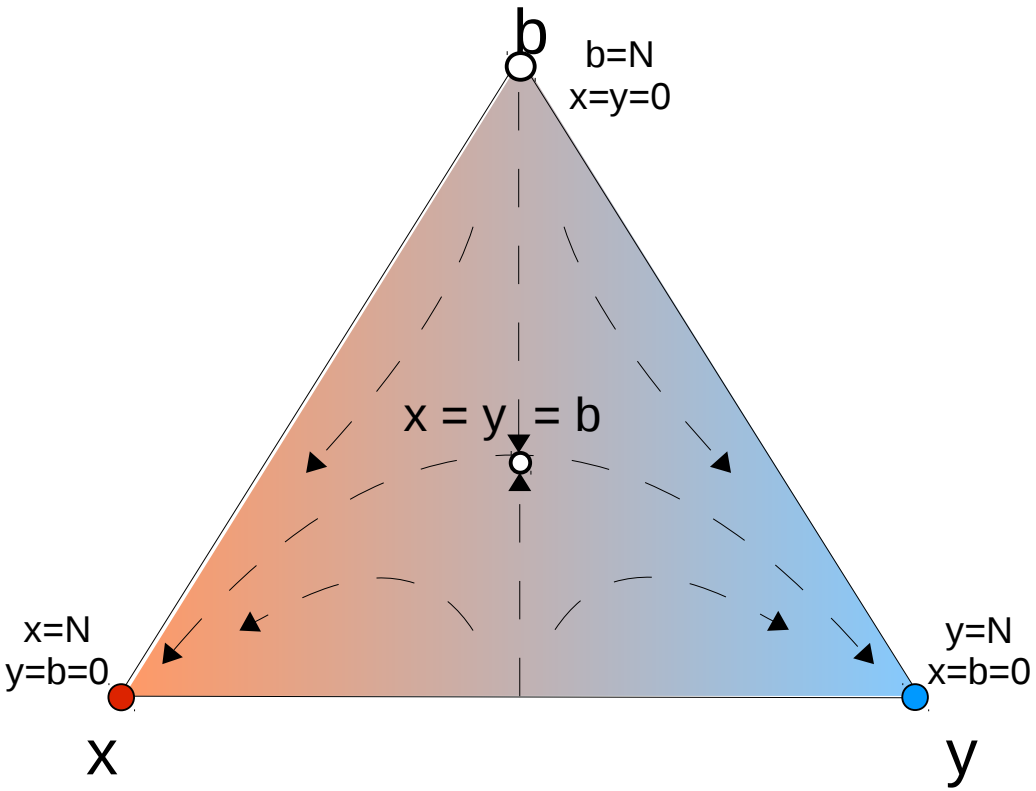


but not *why* it works...

What have we learned?

State space diagrams show exactly *how* the system works and *why*:

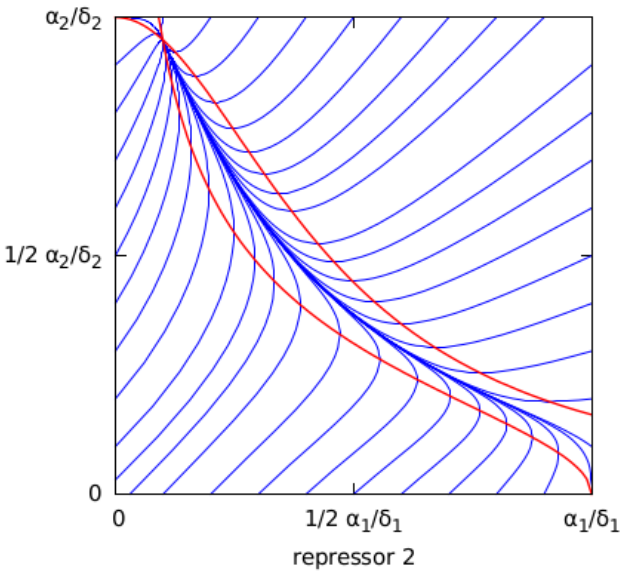




Using phase space diagrams in SB design

- Could we implement programs that calculate and show the phase space while we design SB circuits?

- When given some system equations, one can automatically *construct* the phase space diagram



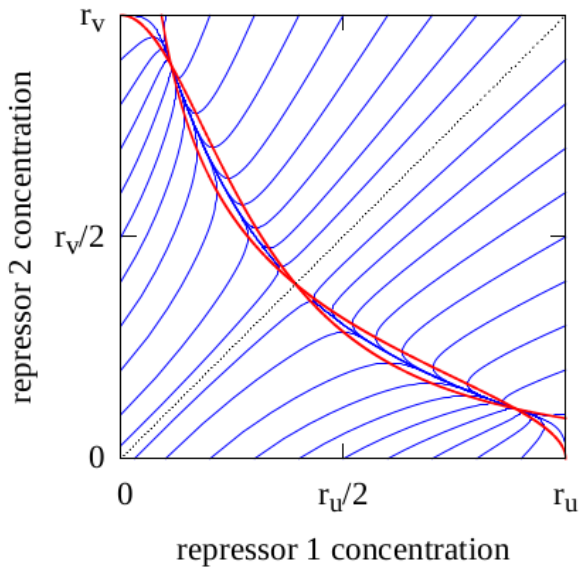
- Algorithms exist to *interpret* the phase diagram

- Some efford is needed to *extract* meaningful manifolds

1st example: 12 species → 2 dim. state space
2nd example: 9 species → 2 dim. state space

Using phase space diagrams in SB design

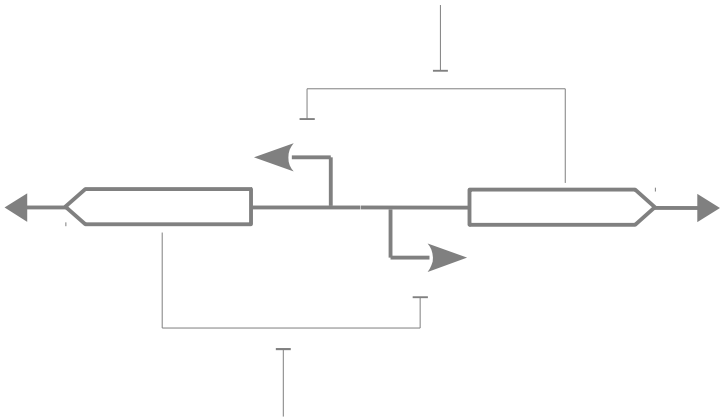
- Could we use phase spaces to *specify* an SB circuit?

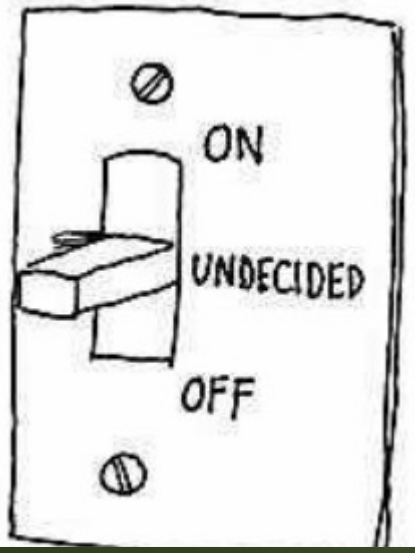


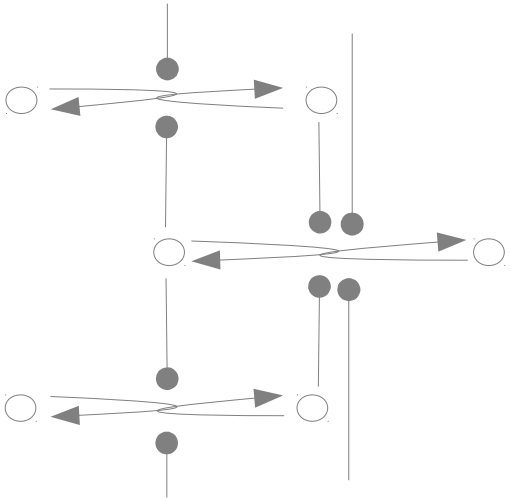
"I require
- a two-protein system
- with two separated stable nodes.
- when induced with IPTG, only one node should remain stable,
- when induced with aTc, only the other node should remain stable."

- Could we *automatically infer* circuits from phase space specifications?

This might be more tricky... but interesting :-)







Thanks for your attention

Questions ?