

MiniCellSim a Tool for Modeling Simple Genetic and Metabolic Regulation Networks

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Web-Page for further information:

<http://www.tbi.univie.ac.at/~pks>

What is systems chemistry?

autocatalysis, template action, self-assembly,
reaction networks, nonlinearity, complexity,

but also with atoms and
molecules that do not occur in
living matter

systems chemistry = systems biology

without genes and the Darwinian mechanism
that, in principle, allows for the reconstruction
of phylogenetic trees

What is systems biology?

In research on the origin of life and cellular organization the scientist has to face the unsolved problem to explain how hierarchical structures, superstructures, and complex dynamics emerge from simple (perhaps yet unknown) elements.



Perhaps we should search for **simple** or **minimal** reaction and regulator **systems** that can be **modelled**, **understood**, **synthesized**, **studied experimentally**, and **made predictable**.



In the domain of molecular genetics and systems biology it is (more or less) well known, how the individual elements (proteins, nucleic acids) work and how the regulatory switches operate, but network dynamics is not at all understood yet.

1. Forward and inverse problems in reaction kinetics
2. Reverse engineering - A simple example
3. Genetic and metabolic networks - MiniCellSim
4. A glimpse of regulation kinetics
5. How do model metabolisms evolve?

- 1. Forward and inverse problems in reaction kinetics**
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Kinetic differential equations

$$\frac{dx}{dt} = f(x;k); x=(x_1,\dots,x_n); k=(k_1,\dots,k_m)$$

Reaction diffusion equations

$$\frac{\partial x}{\partial t} = D \nabla^2 x + f(x;k)$$

Parameter set

$$k_j(T, p, \text{pH}, I, \dots); j=1, 2, \dots, m$$

General conditions: $T, p, \text{pH}, I, \dots$

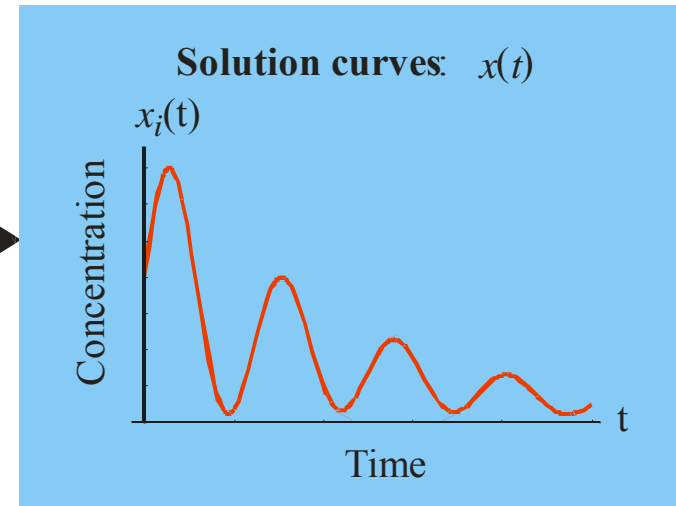
Initial conditions: $x(0)$

Boundary conditions:

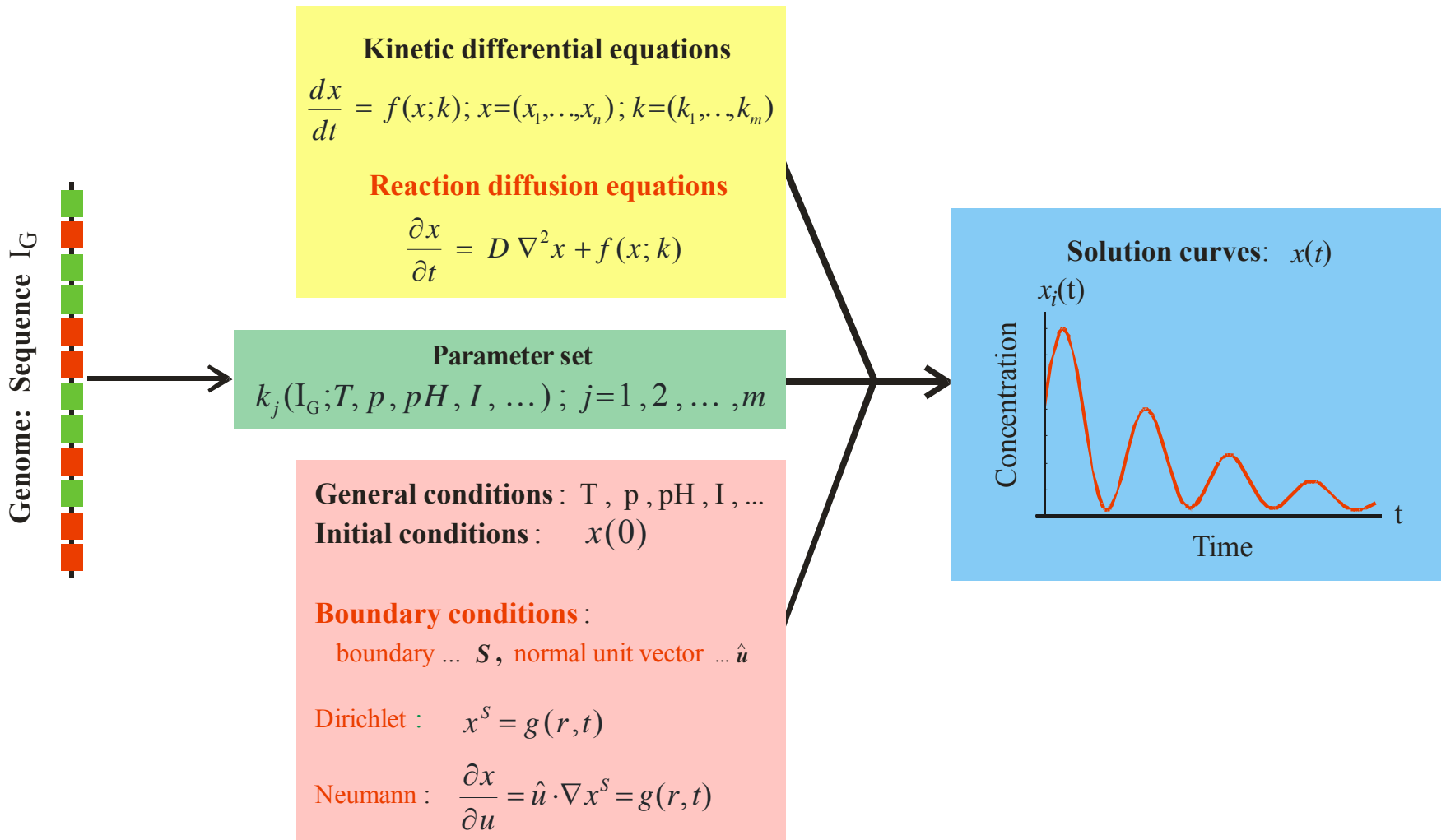
boundary ... S , normal unit vector ... \hat{u}

Dirichlet: $x^S = g(r, t)$

Neumann: $\frac{\partial x}{\partial u} = \hat{u} \cdot \nabla x^S = g(r, t)$



The forward problem of chemical reaction kinetics (Level I)



The forward problem of biochemical reaction kinetics (Level I)

Genome: Sequence I_G

Parameter set
 $k_j(I_G; T, p, pH, I, \dots); j=1, 2, \dots, m$

Kinetic differential equations

$$\frac{dx}{dt} = f(x;k); x=(x_1, \dots, x_n); k=(k_1, \dots, k_m)$$

Reaction diffusion equations

$$\frac{\partial x}{\partial t} = D \nabla^2 x + f(x;k)$$

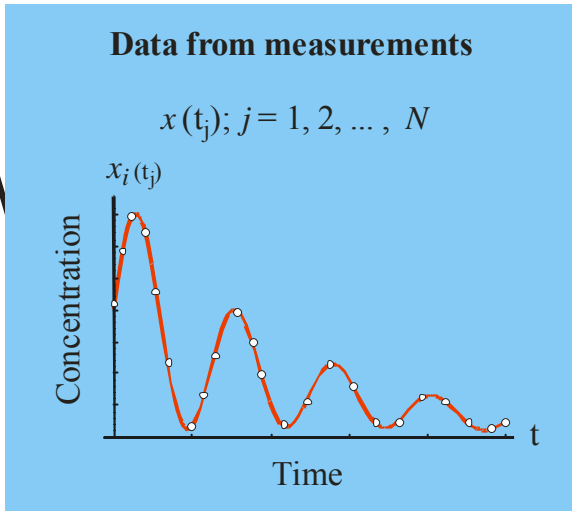
General conditions : T, p, pH, I, \dots
Initial conditions : $x(0)$

Boundary conditions :

boundary ... S , normal unit vector... \hat{u}

Dirichlet : $x^S = g(r, t)$

Neumann : $\frac{\partial x}{\partial u} = \hat{u} \cdot \nabla x^S = g(r, t)$



The inverse problem of biochemical reaction kinetics (Level I)

Genome: Sequence I_G



Kinetic differential equations

$$\frac{dx}{dt} = f(x;k); x=(x_1, \dots, x_n); k=(k_1, \dots, k_m)$$

Reaction diffusion equations

$$\frac{\partial x}{\partial t} = D \nabla^2 x + f(x;k)$$

Parameter set

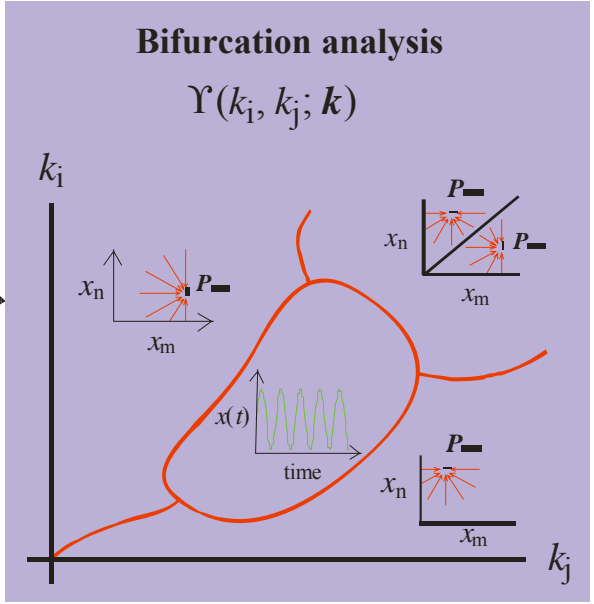
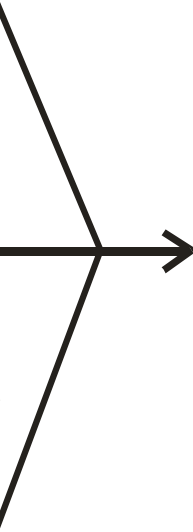
$$k_j(I_G; T, p, pH, I, \dots); j=1, 2, \dots, m$$

General conditions : T, p, pH, I, \dots
Initial conditions : $x(0)$

Boundary conditions :
 boundary ... S , normal unit vector ... \hat{u}

Dirichlet : $x^S = g(r, t)$

Neumann : $\frac{\partial x}{\partial u} = \hat{u} \cdot \nabla x^S = g(r, t)$



The forward problem of bifurcation analysis (Level II)

Genome: Sequence I_G



Parameter set
 $k_j(I_G; T, p, pH, I, \dots); j=1, 2, \dots, m$

Kinetic differential equations

$$\frac{dx}{dt} = f(x;k); x=(x_1, \dots, x_n); k=(k_1, \dots, k_m)$$

Reaction diffusion equations

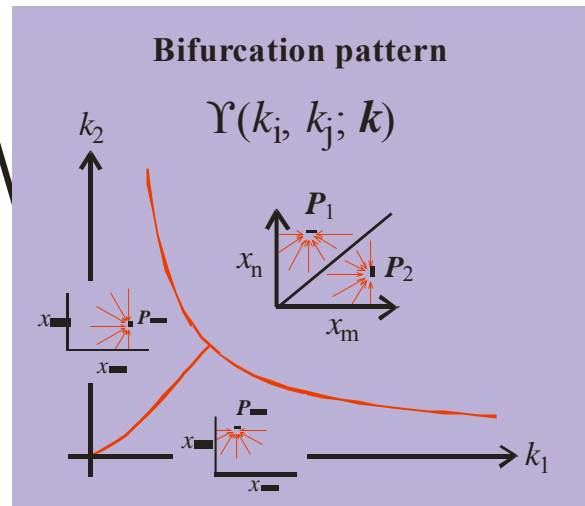
$$\frac{\partial x}{\partial t} = D \nabla^2 x + f(x;k)$$

General conditions: T, p, pH, I, \dots
Initial conditions: $x(0)$

Boundary conditions:
boundary ... S , normal unit vector... \hat{u}

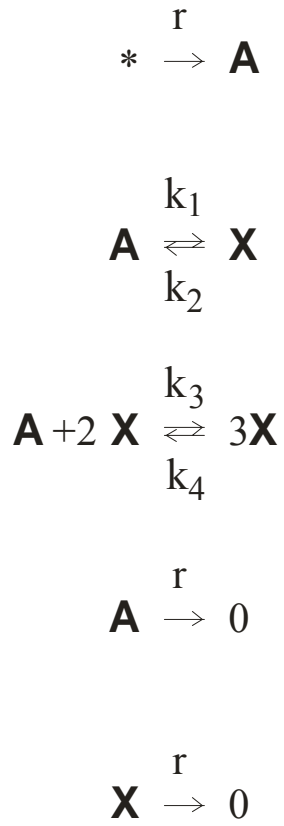
Dirichlet: $x^S = g(r, t)$

Neumann: $\frac{\partial x}{\partial u} = \hat{u} \cdot \nabla x^S = g(r, t)$

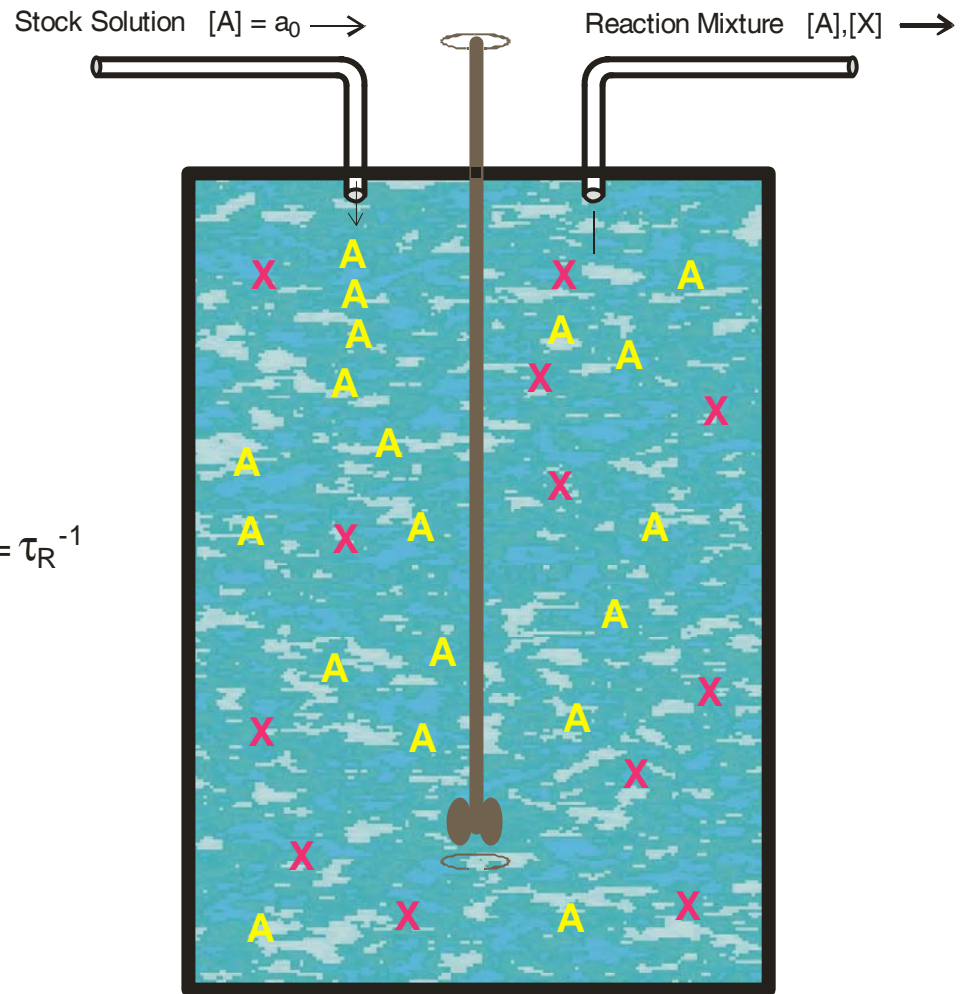


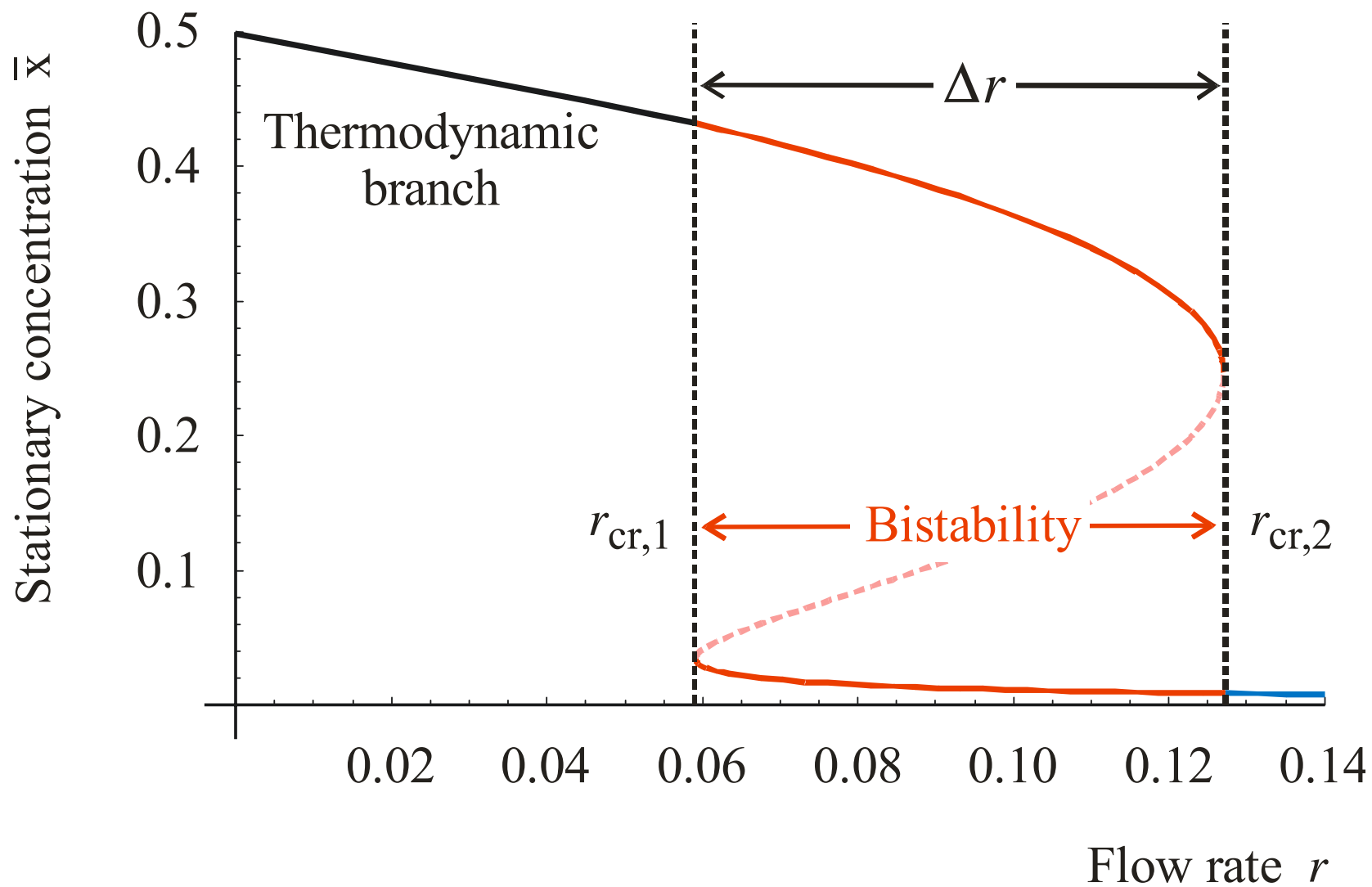
The inverse problem of bifurcation analysis (Level II)

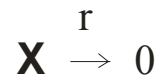
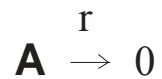
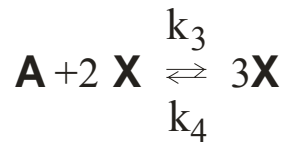
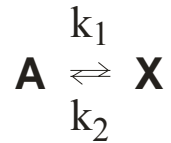
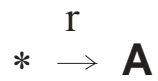
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Flow rate $r = \tau_R^{-1}$



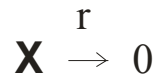
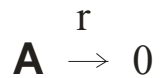
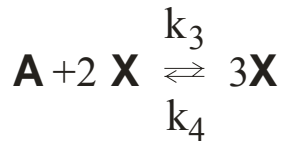
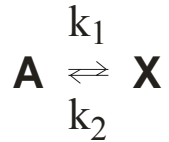
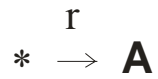




Kinetic differential equations:

$$\frac{d[\mathbf{A}]}{dt} = \frac{da}{dt} = r(a_0 - a) - (k_1 + k_3 x^2) a + (k_2 + k_4 x^2) x$$

$$\frac{d[\mathbf{X}]}{dt} = \frac{dx}{dt} = -r x + (k_1 + k_3 x^2) a - (k_2 + k_4 x^2) x$$



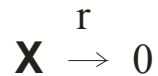
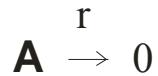
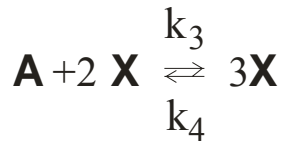
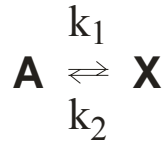
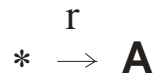
Kinetic differential equations:

$$\frac{d[\mathbf{A}]}{dt} = \frac{da}{dt} = r(a_0 - a) - (k_1 + k_3 x^2) a + (k_2 + k_4 x^2) x$$

$$\frac{d[\mathbf{X}]}{dt} = \frac{dx}{dt} = -r x + (k_1 + k_3 x^2) a - (k_2 + k_4 x^2) x$$

Steady states:

$$\bar{x}^3 (k_3 + k_4) - \bar{x}^2 k_3 a_0 + \bar{x} (k_1 + k_2 + r) - k_1 a_0 = 0$$



Kinetic differential equations:

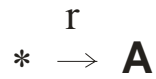
$$\frac{d[\mathbf{A}]}{dt} = \frac{da}{dt} = r(a_0 - a) - (k_1 + k_3 x^2) a + (k_2 + k_4 x^2) x$$

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Steady states:

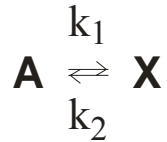
$$\bar{x}^3 (k_3 + k_4) - \bar{x}^2 k_3 a_0 + \bar{x} (k_1 + k_2 + r) - k_1 a_0 = 0$$

$$k_1 = k_2 = \alpha, k_3 = k_4 = 1: \quad 2\bar{x}^3 - \bar{x}^2 a_0 + \bar{x} (r + 2\alpha) - \alpha a_0 = 0$$

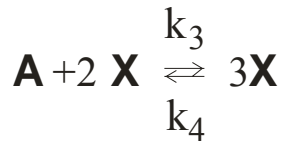


Kinetic differential equations:

$$\frac{d[\mathbf{A}]}{dt} = \frac{da}{dt} = r(a_0 - a) - (k_1 + k_3 x^2)a + (k_2 + k_4 x^2)x$$

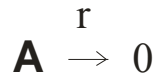


$$\frac{d[\mathbf{X}]}{dt} = \frac{dx}{dt} = -r x + (k_1 + k_3 x^2)a - (k_2 + k_4 x^2)x$$



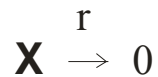
Steady states:

$$\bar{x}^3(k_3 + k_4) - \bar{x}^2 k_3 a_0 + \bar{x}(k_1 + k_2 + r) - k_1 a_0 = 0$$

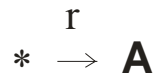


$$k_1 = k_2 = \alpha, k_3 = k_4 = 1: 2\bar{x}^3 - \bar{x}^2 a_0 + \bar{x}(r + 2\alpha) - \alpha a_0 = 0$$

Polynomial discriminant of the cubic equation:

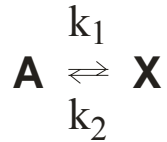


$$216D = r^3 + r^2 \left(6\alpha - \frac{a_0^2}{8}\right) + r(12\alpha^2 - 5\alpha a_0^2) + 8\alpha^3 + 4\alpha^2 a_0^2 + \frac{\alpha a_0^4}{2} = 0$$

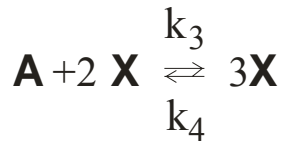


Kinetic differential equations:

$$\frac{d[\mathbf{A}]}{dt} = \frac{da}{dt} = r(a_0 - a) - (k_1 + k_3 x^2)a + (k_2 + k_4 x^2)x$$

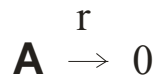


$$\frac{d[\mathbf{X}]}{dt} = \frac{dx}{dt} = -r x + (k_1 + k_3 x^2)a - (k_2 + k_4 x^2)x$$



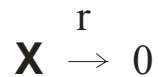
Steady states:

$$\bar{x}^3(k_3 + k_4) - \bar{x}^2 k_3 a_0 + \bar{x}(k_1 + k_2 + r) - k_1 a_0 = 0$$



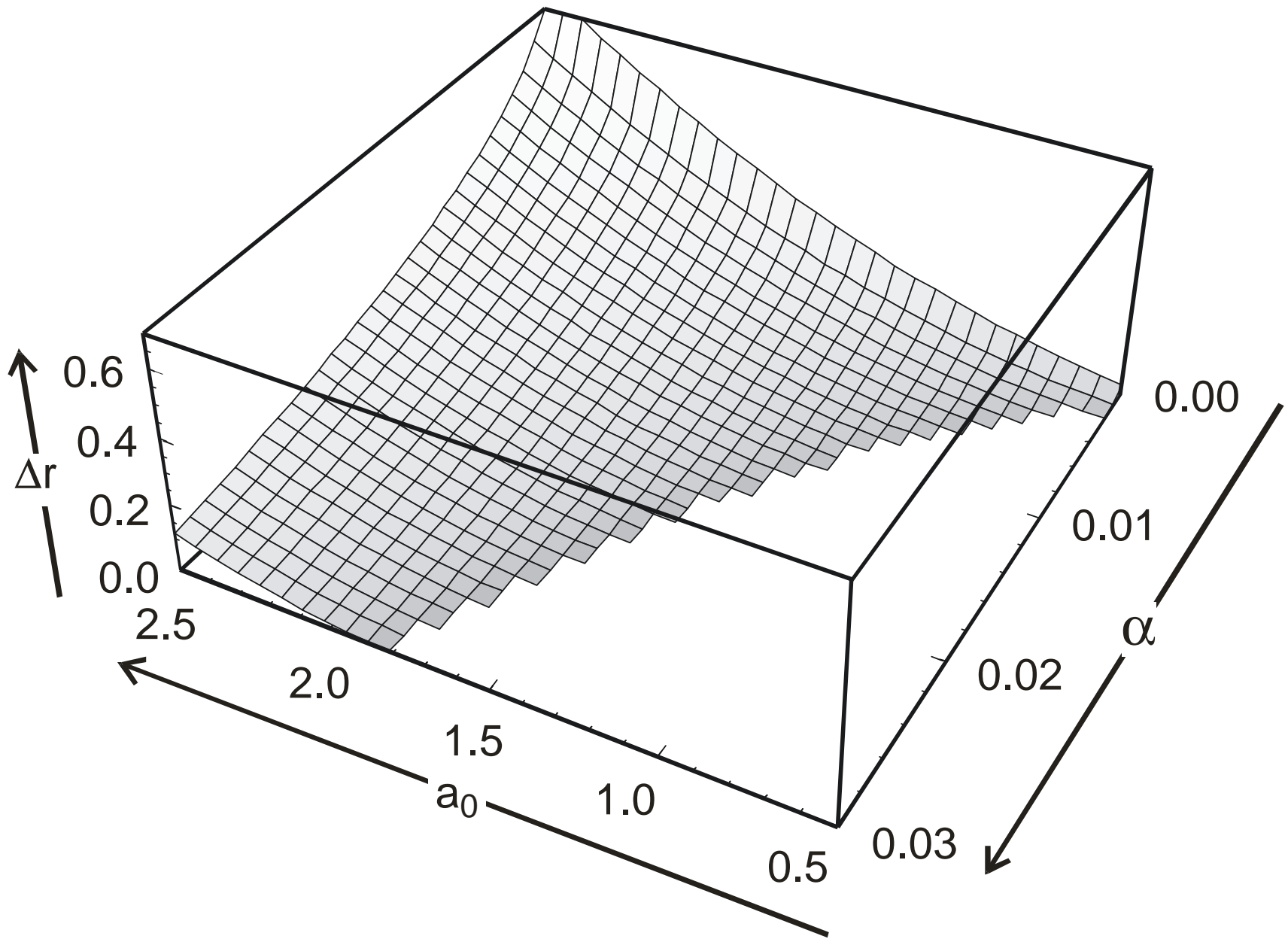
$$k_1 = k_2 = \alpha, k_3 = k_4 = 1: 2\bar{x}^3 - \bar{x}^2 a_0 + \bar{x}(r + 2\alpha) - \alpha a_0 = 0$$

Polynomial discriminant of the cubic equation:



$$216D = r^3 + r^2 \left(6\alpha - \frac{a_0^2}{8}\right) + r(12\alpha^2 - 5\alpha a_0^2) + 8\alpha^3 + 4\alpha^2 a_0^2 + \frac{\alpha a_0^4}{2} = 0$$

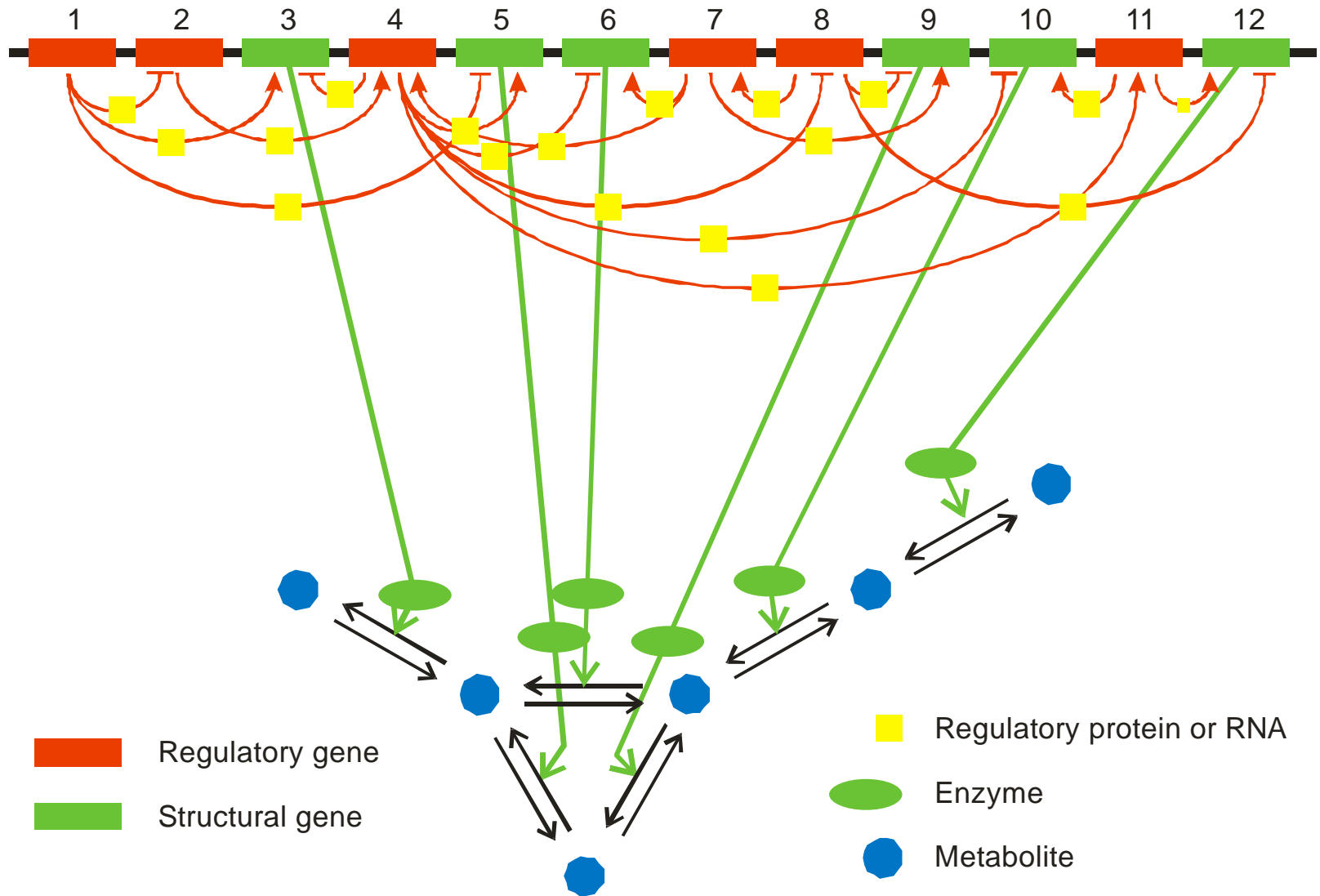
$D < 0$: 3 roots $r_1, r_2,$ and $r_3, 2$ are positive $\Rightarrow \Delta r = r_1 - r_2$



Range of hysteresis as a function of the parameters a_0 and α

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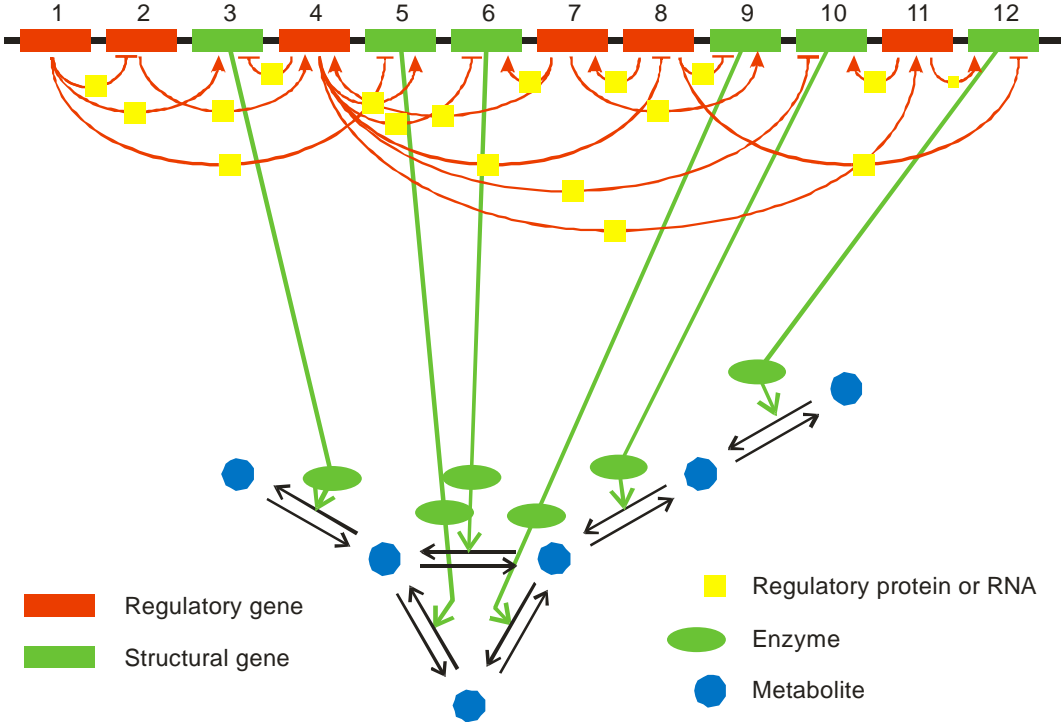
A model genome with 12 genes



Sketch of a genetic and metabolic network

Genetic regulatory network

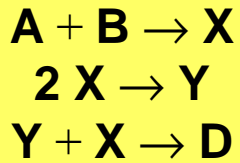
A model genome with 12 genes



Metabolic network

Proposal of a new name:

Genetic and metabolic network



$$\begin{aligned}
 \frac{da}{dt} &= \frac{db}{dt} = -k_1 ab \\
 \frac{dx}{dt} &= k_1 ab - k_2 x^2 - k_3 xy \\
 \frac{dy}{dt} &= k_2 x^2 - k_3 xy \\
 \frac{dd}{dt} &= k_3 xy
 \end{aligned}$$

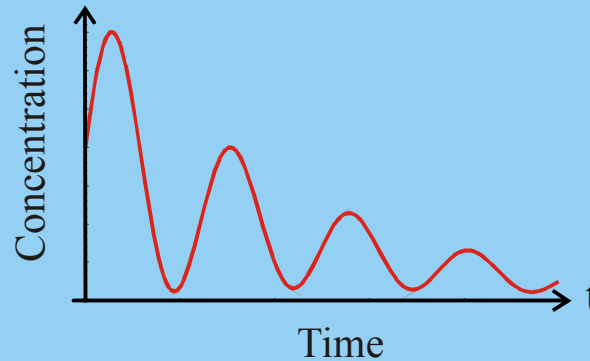
Stoichiometric equations

SBML – systems biology markup language

Kinetic differential equations

ODE Integration by means of *CVODE*

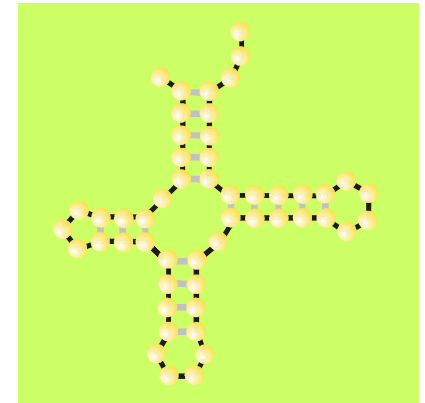
$x_i(t)$ Solution curves



Sequences

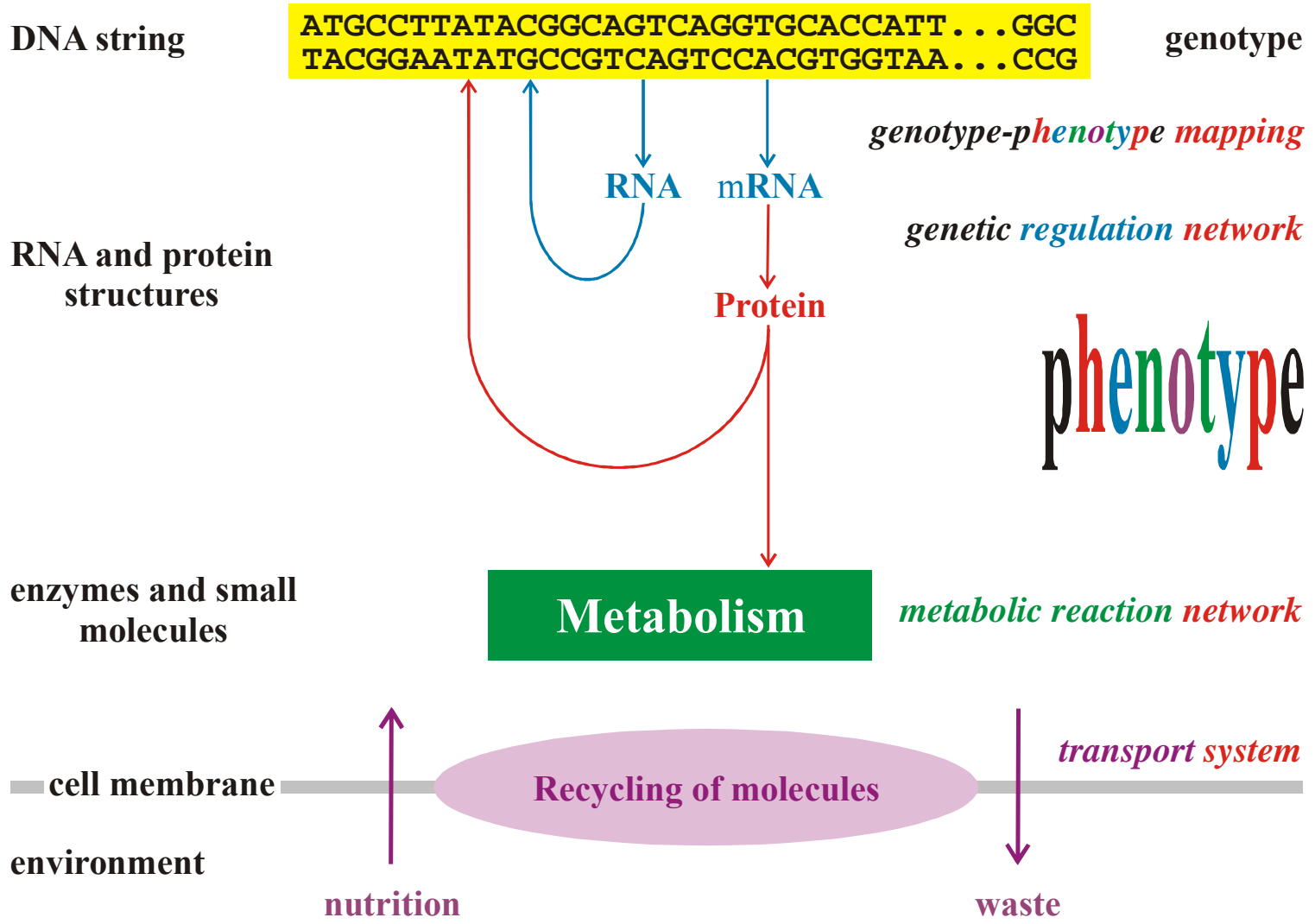
Vienna RNA Package

Structures and kinetic parameters

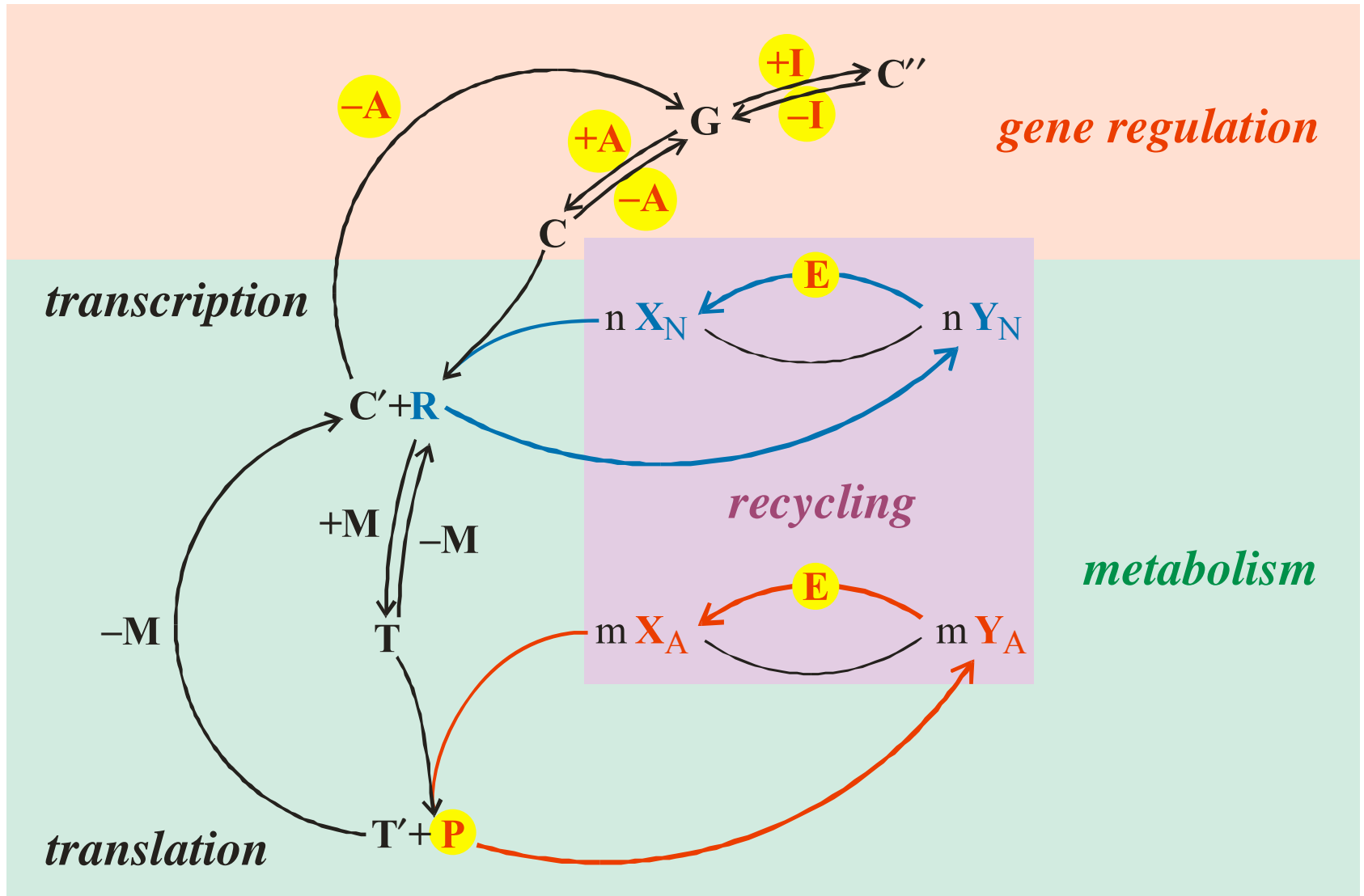


The elements of the simulation tool MiniCellSim

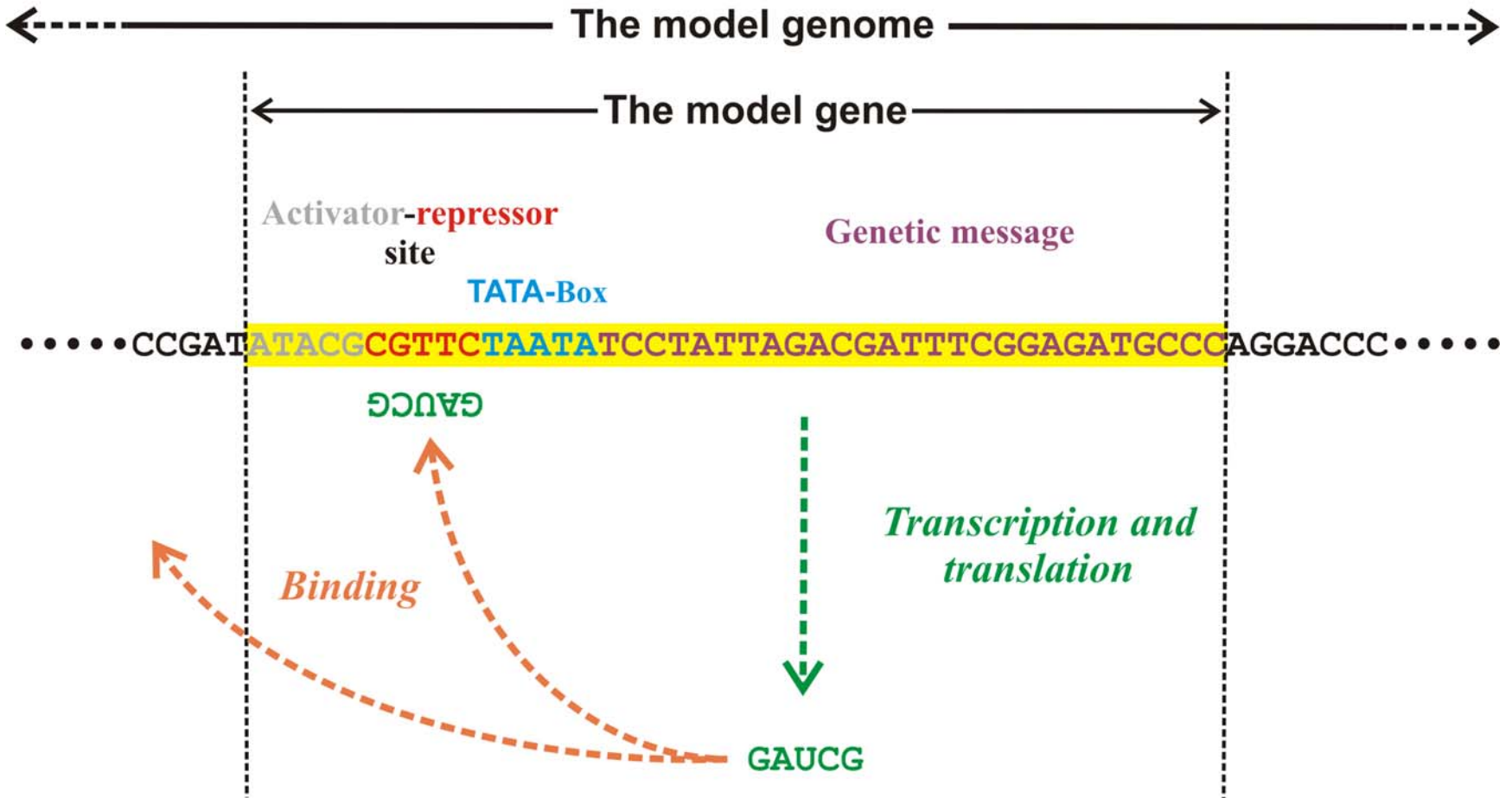
SBML: Bioinformatics **19**:524-531, 2003; *CVODE: Computers in Physics* **10**:138-143, 1996



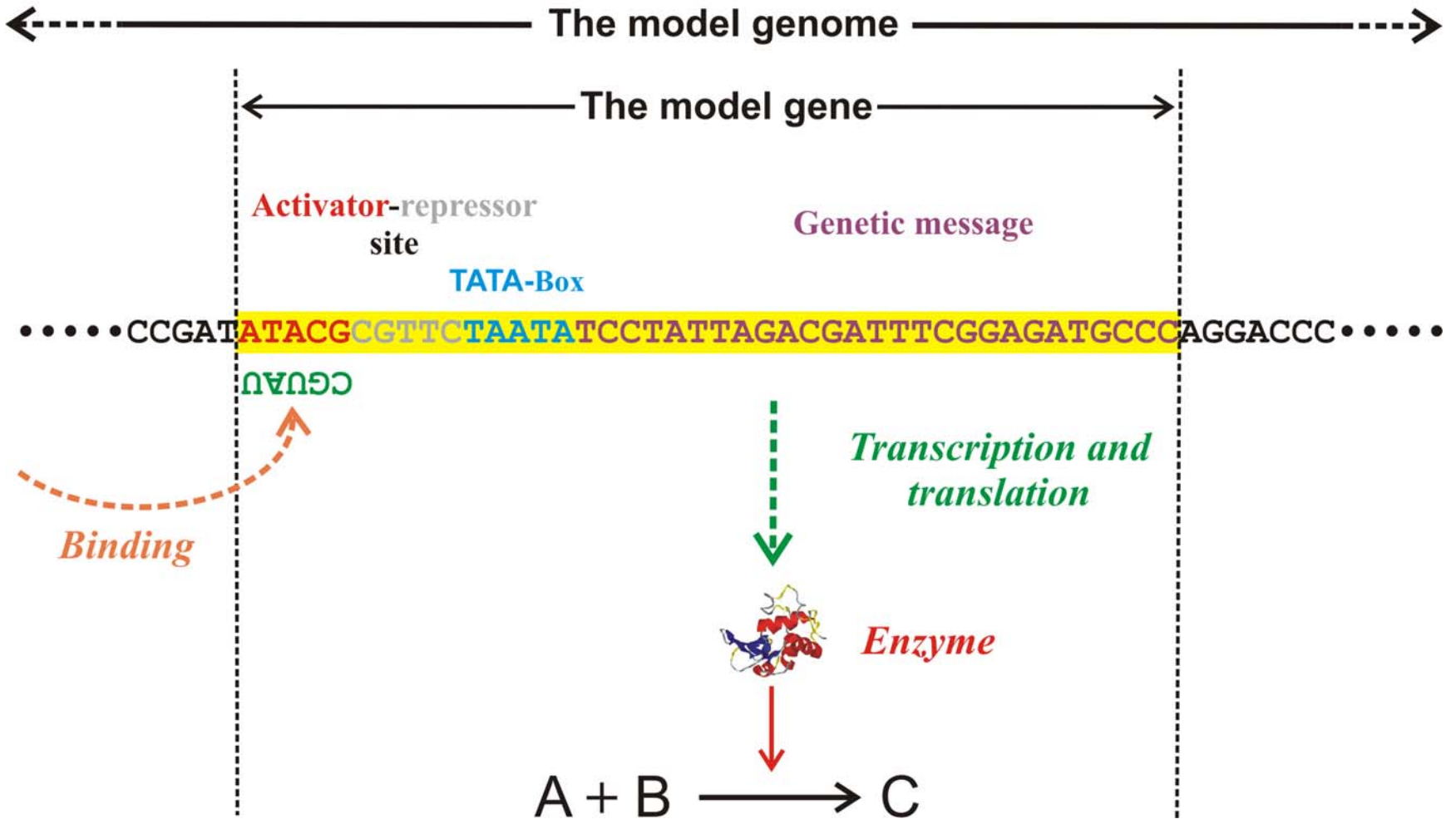
The regulatory logic of MiniCellSym



The chemical reaction dynamics of MiniCellSym

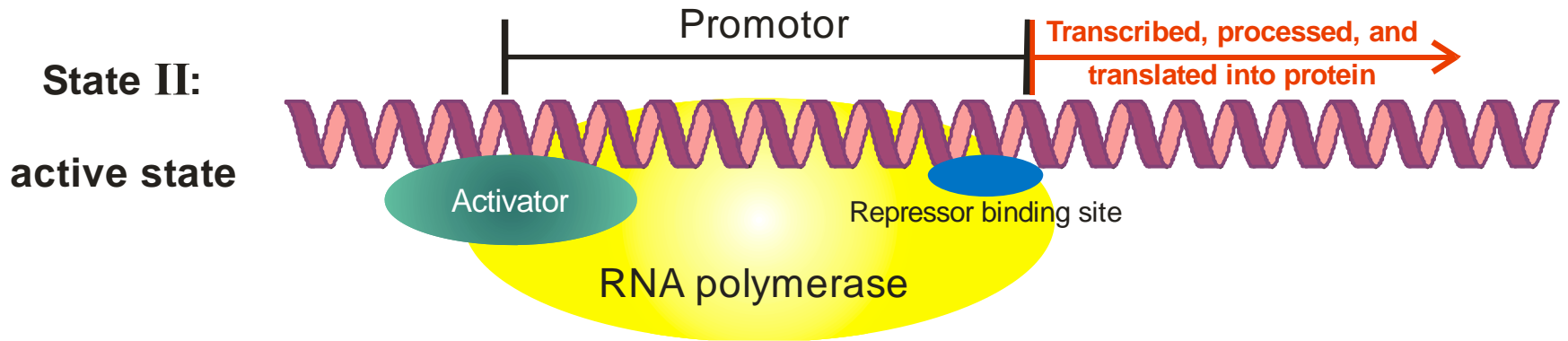
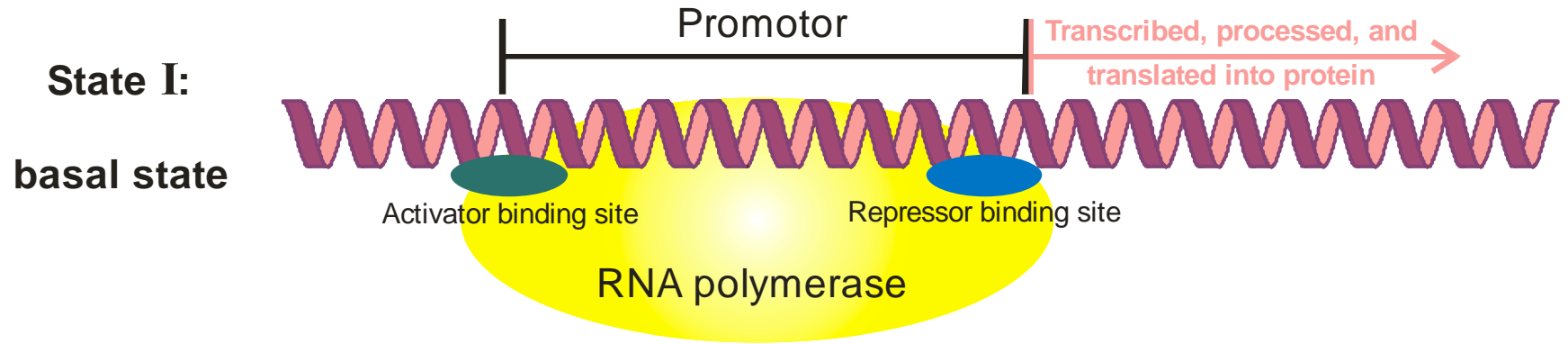


The model regulatory gene in MiniCellSim

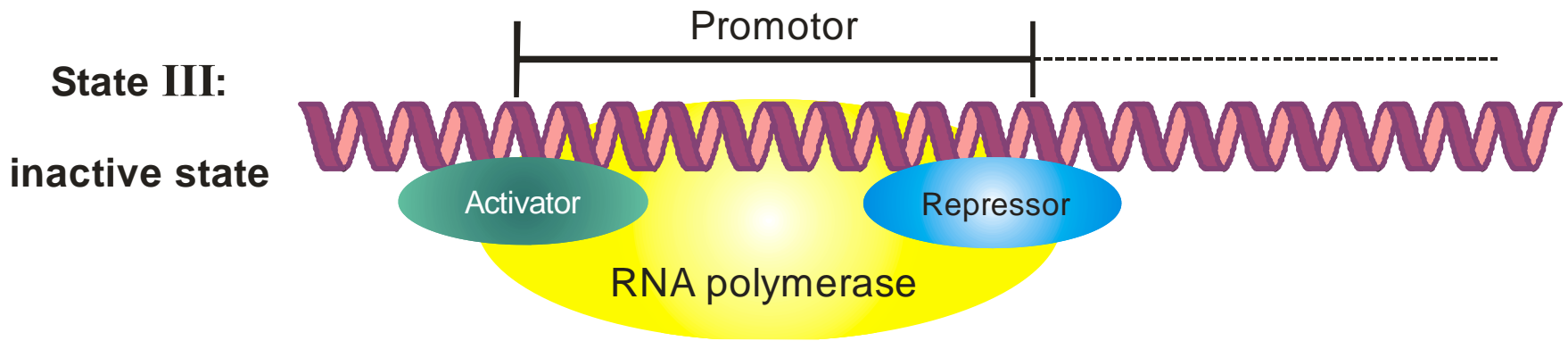
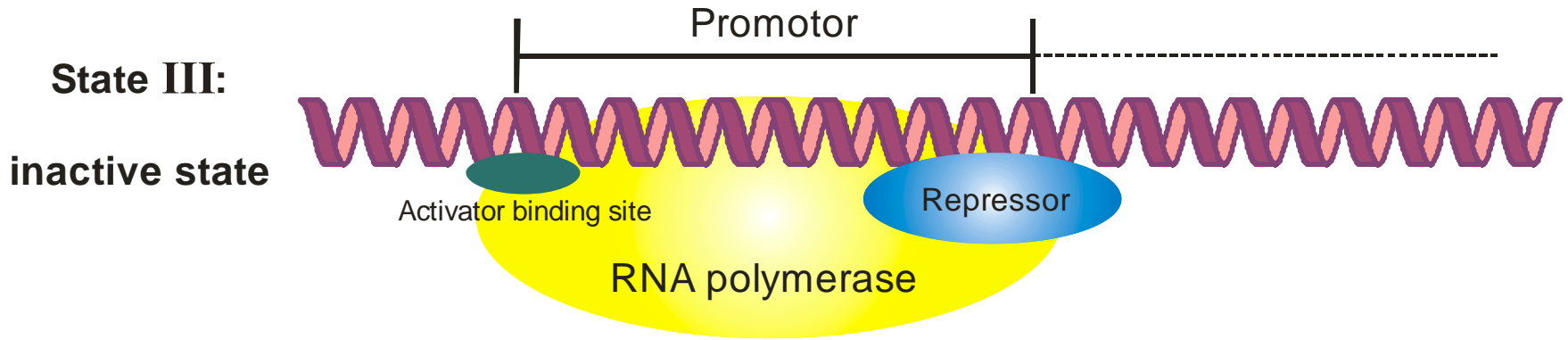


The model structural gene in MiniCellSim

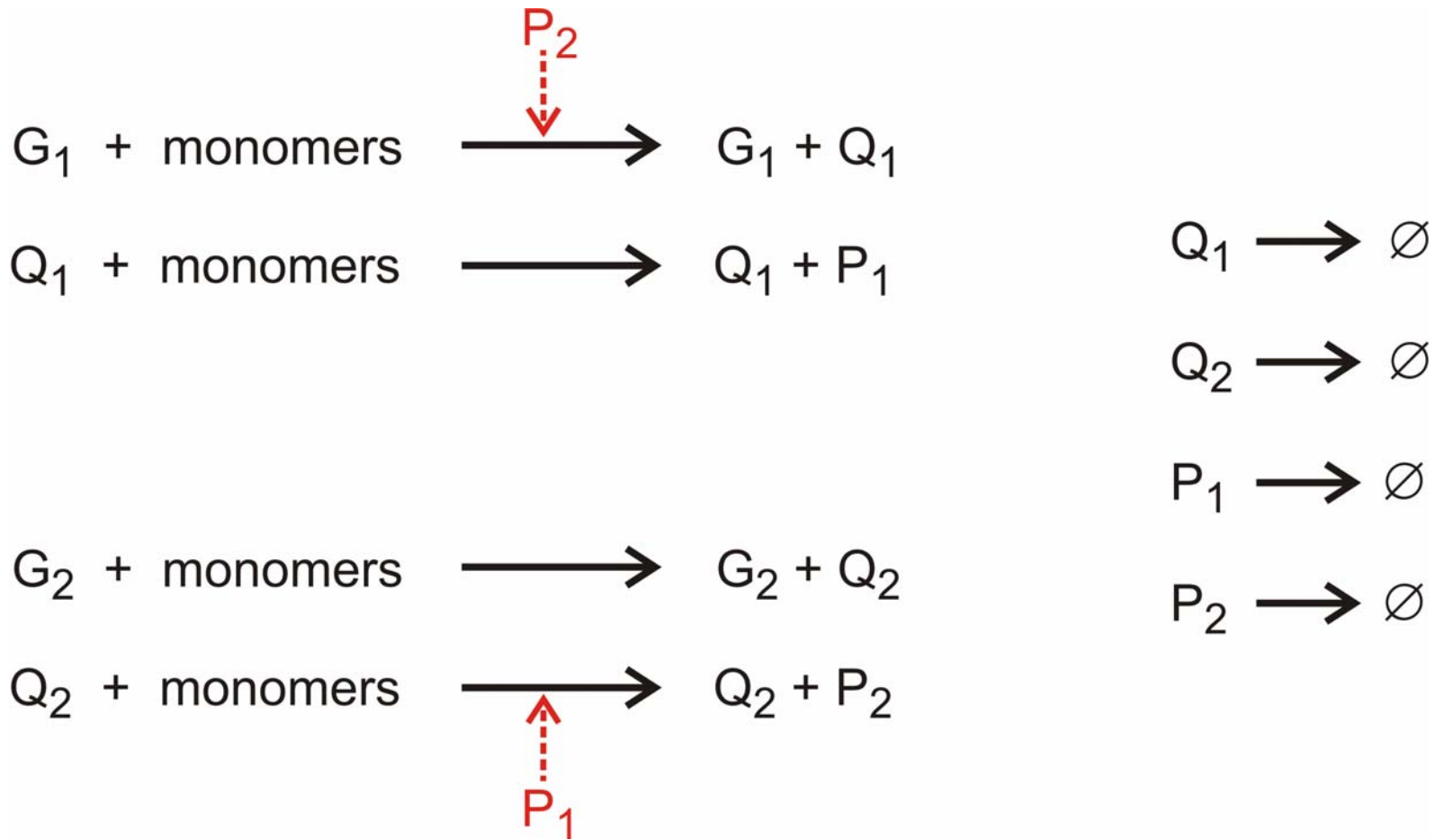
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Active states of gene regulation



Inactive states of gene regulation

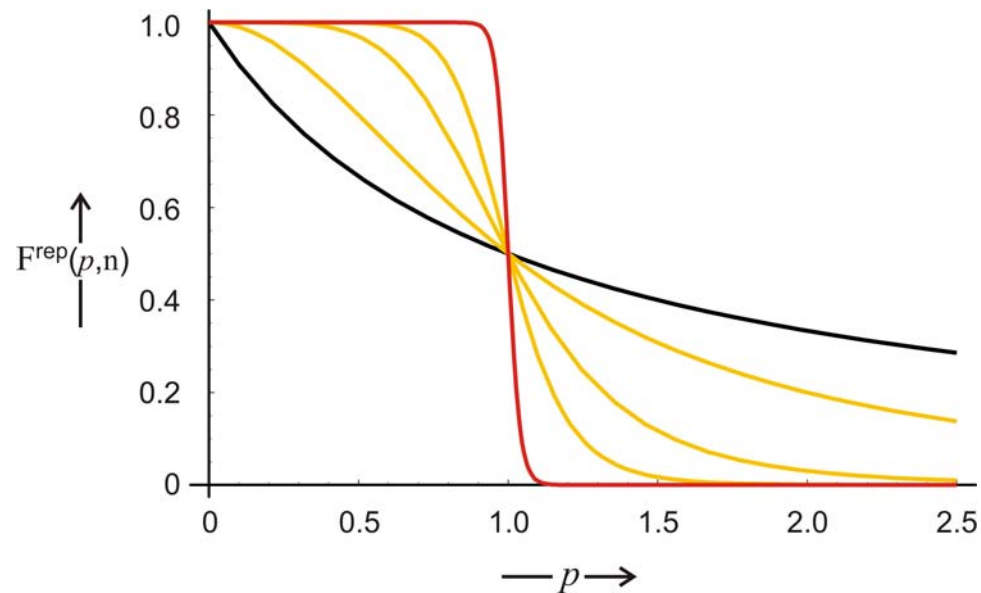
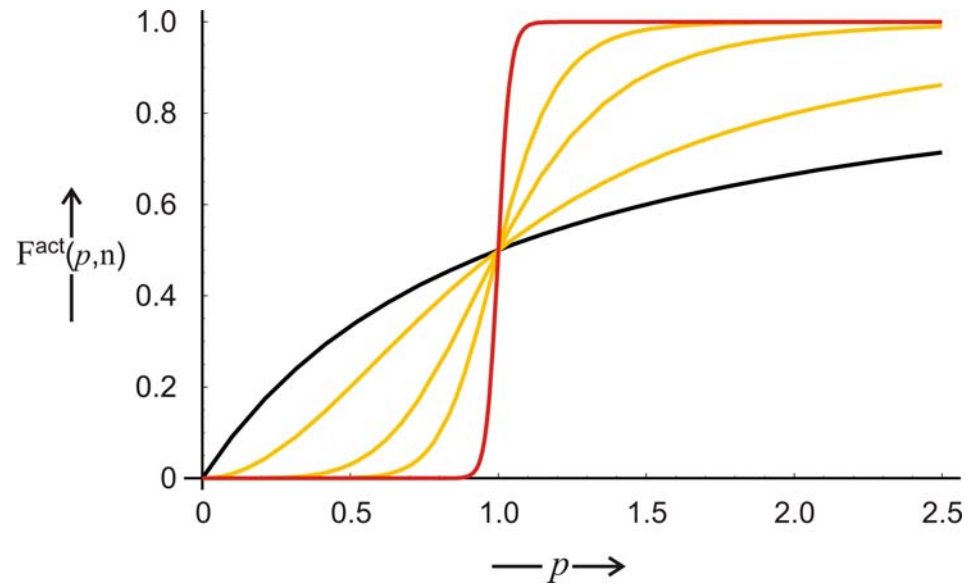


Cross-regulation of two genes

Activation: $F_i(p_j) = \frac{p_j^n}{K + p_j^n}$

Repression: $F_i(p_j) = \frac{K}{K + p_j^n}$

$i, j = 1, 2$



Gene regulatory binding functions

$$[G_1]=[G_2]=g_0=\text{const.}$$

$$[Q_1]=q_1, [Q_2]=q_2,$$

$$[P_1]=p_1, [P_2]=p_2$$

$$\text{Activation: } F_i(p_j) = \frac{p_j^n}{K + p_j^n}$$

$$\text{Repression: } F_i(p_j) = \frac{K}{K + p_j^n}$$

$$i, j = 1, 2$$

$$\frac{dq_1}{dt} = k_1^Q F_1(p_2) - d_1^Q q_1$$

$$\frac{dq_2}{dt} = k_2^Q F_2(p_1) - d_2^Q q_2$$

$$\frac{dp_1}{dt} = k_1^P q_1 - d_1^P p_1$$

$$\frac{dp_2}{dt} = k_2^P q_2 - d_2^P p_2$$

$$\text{Stationary points: } \bar{p}_1 - \mathcal{G}_1 F_1(\mathcal{G}_2 F_2(\bar{p}_1)) = 0, \bar{p}_2 = \mathcal{G}_2 F_2(\bar{p}_1)$$

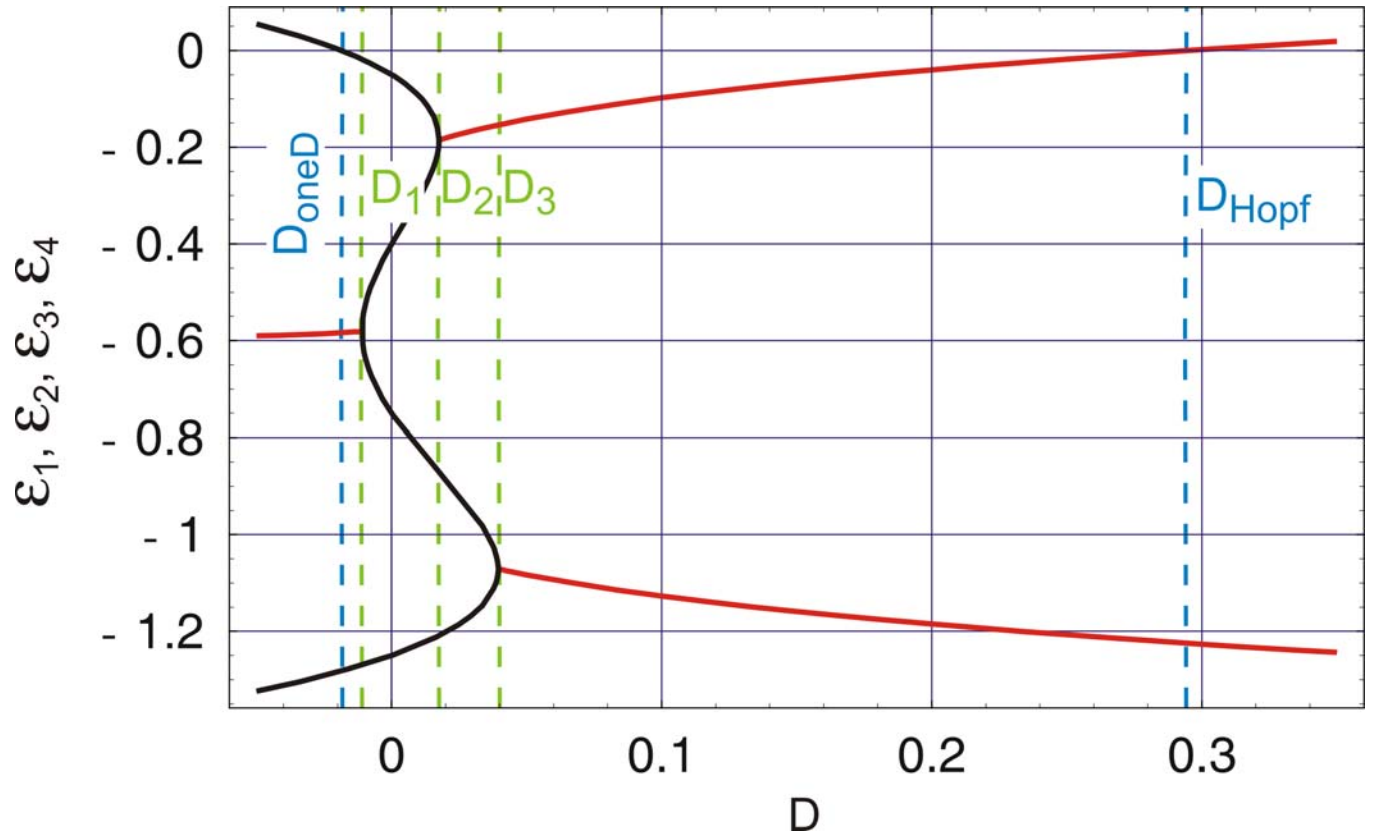
$$\mathcal{G}_1 = \frac{k_1^Q k_1^P}{d_1^Q d_1^P}, \mathcal{G}_2 = \frac{k_2^Q k_2^P}{d_2^Q d_2^P}$$

Qualitative analysis of cross-regulation of two genes

Eigenvalues of the Jacobian of the cross-regulatory two gene system

$$(\varepsilon + d_1^Q)(\varepsilon + d_2^Q)(\varepsilon + d_1^P)(\varepsilon + d_2^P) + D = 0$$

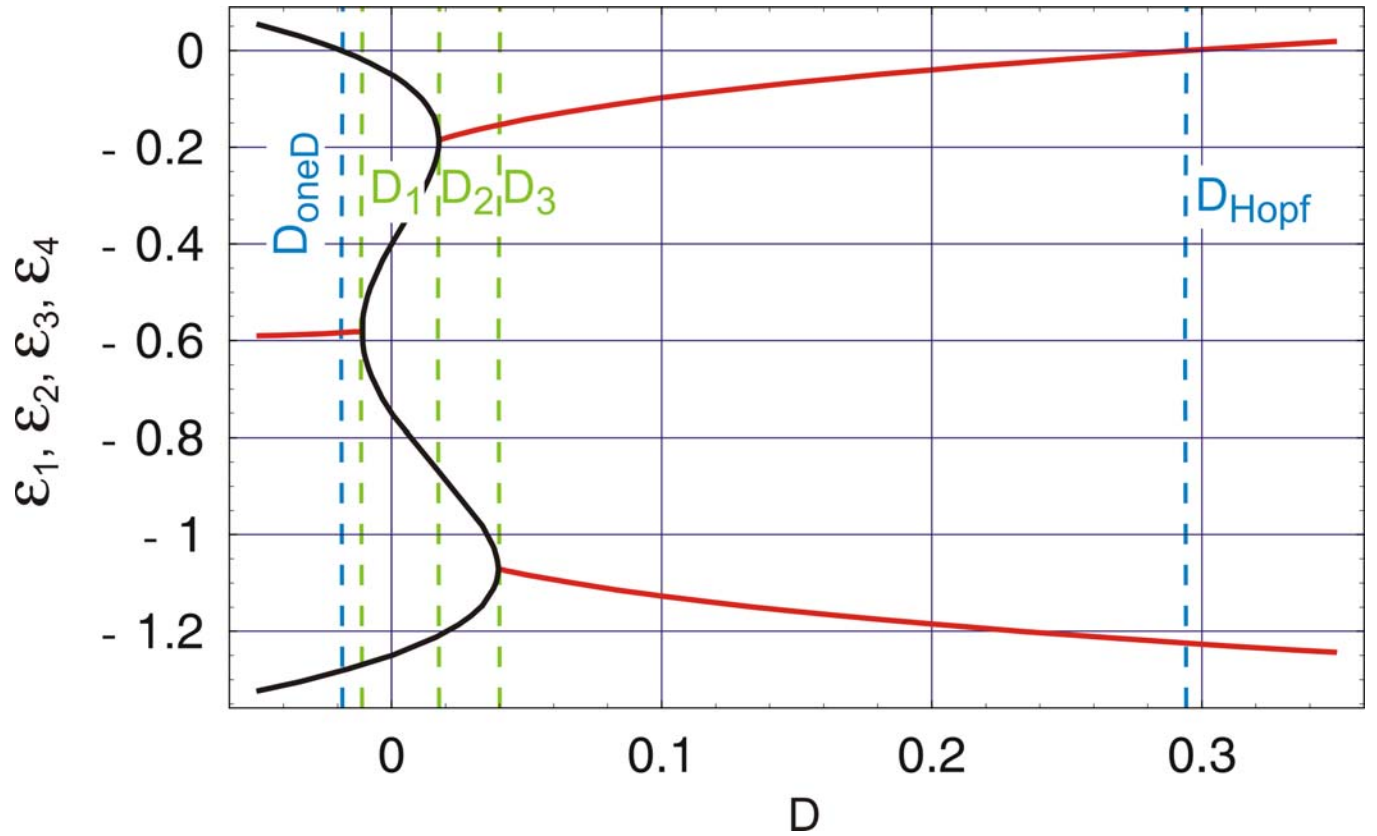
$$D = -k_1^Q k_2^Q k_1^P k_2^P \Gamma(\bar{p}_1, \bar{p}_2)$$



$$(\varepsilon + d_1^Q)(\varepsilon + d_2^Q)(\varepsilon + d_1^P)(\varepsilon + d_2^P) + D = 0$$

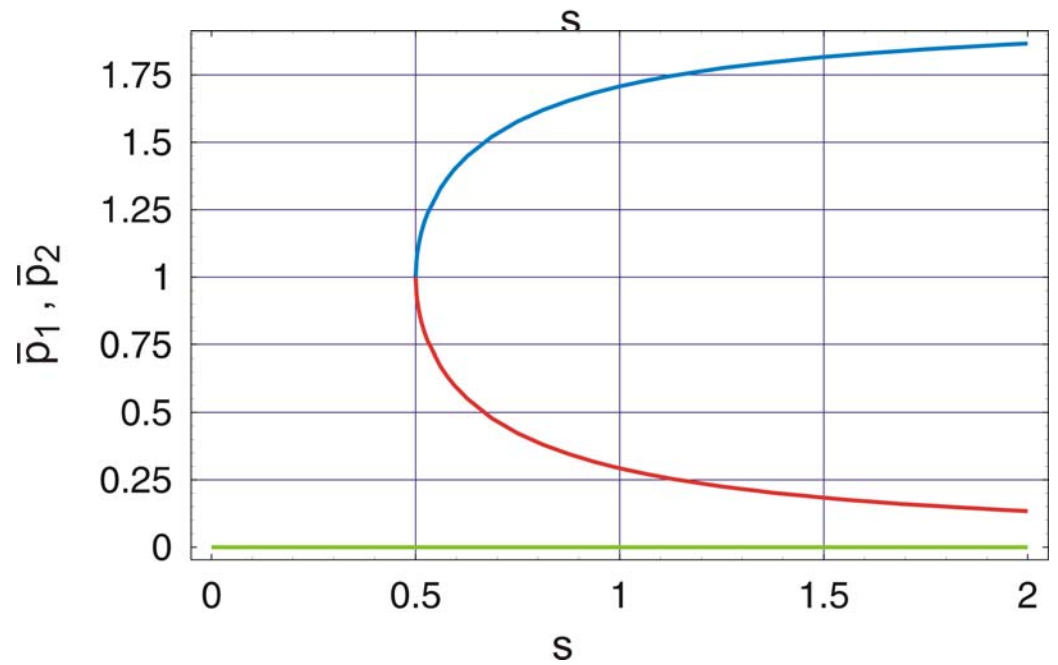
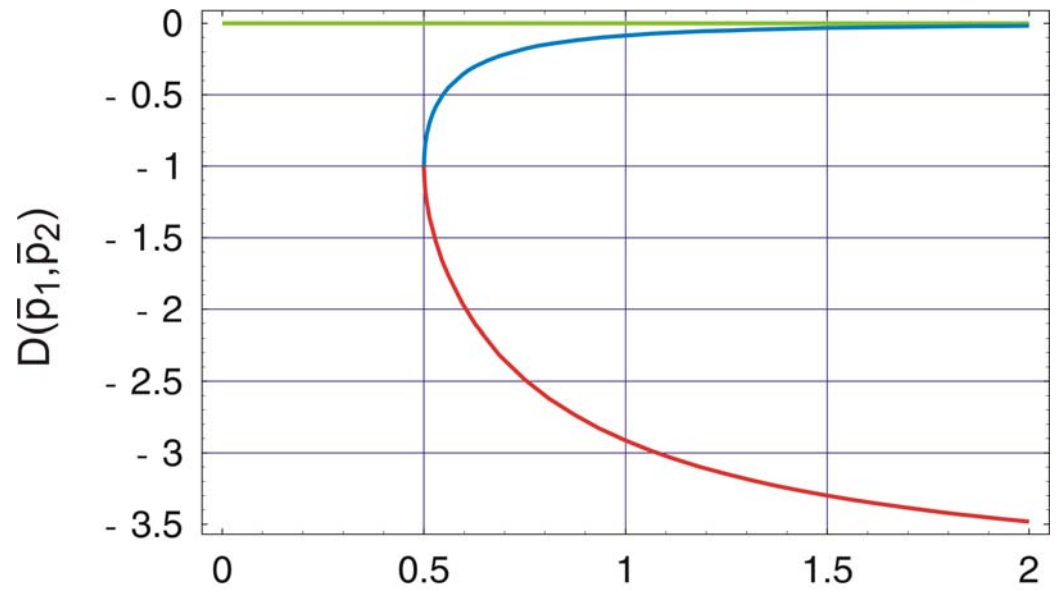
Eigenvalues of the Jacobian of the cross-regulatory two gene system

$$D = -k_1^Q k_2^Q k_1^P k_2^P \Gamma(\bar{p}_1, \bar{p}_2)$$

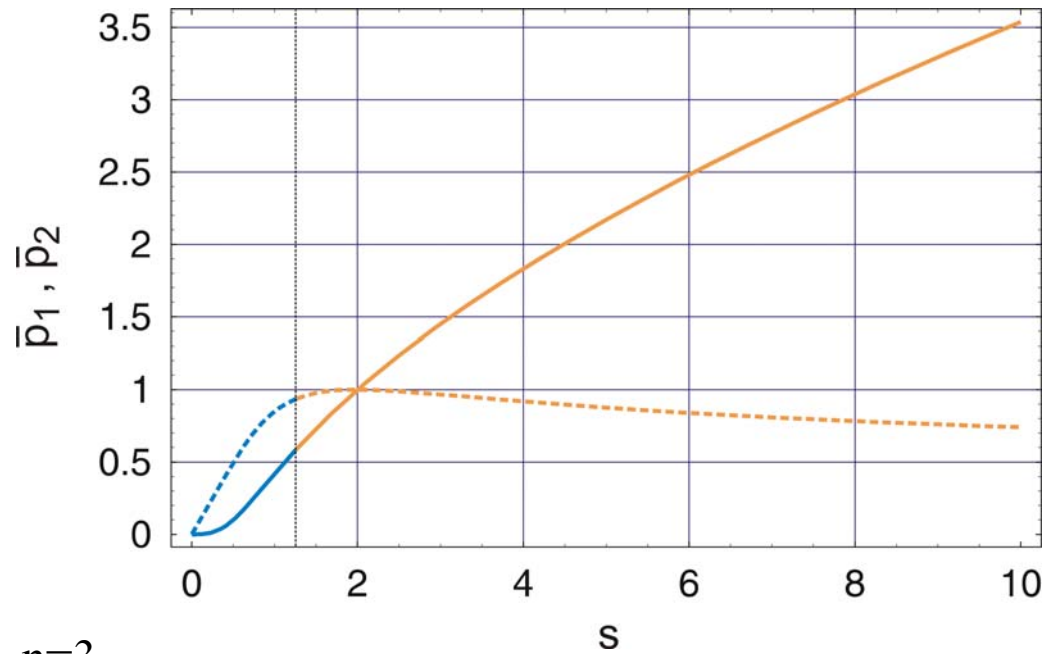
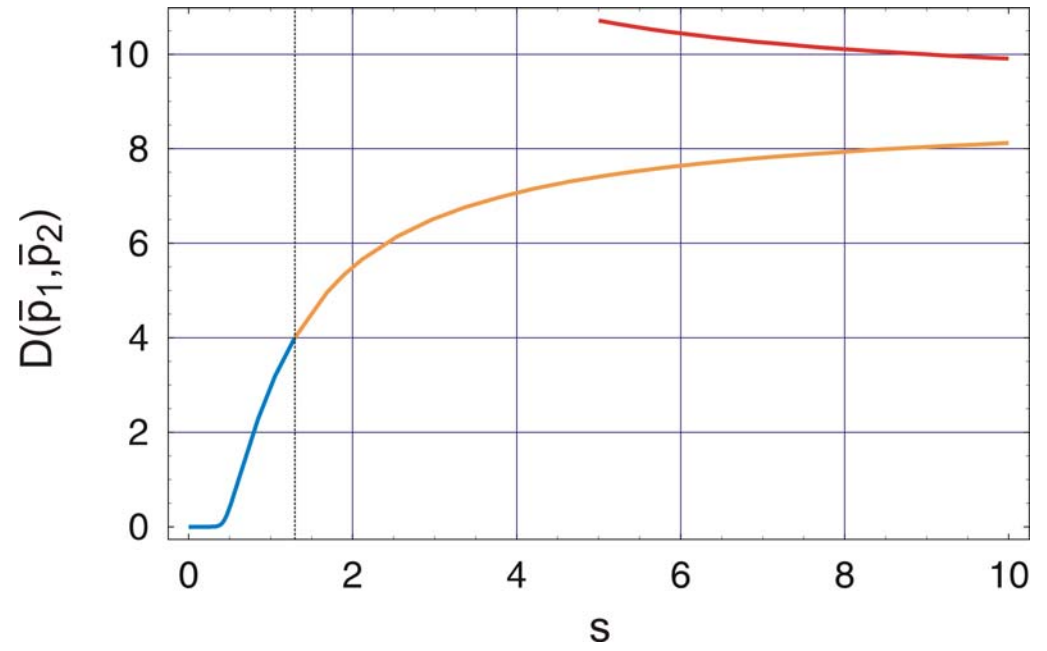


$$D_{\text{OneD}} = -d_1^{\text{Q}} d_2^{\text{Q}} d_1^{\text{P}} d_2^{\text{P}}$$

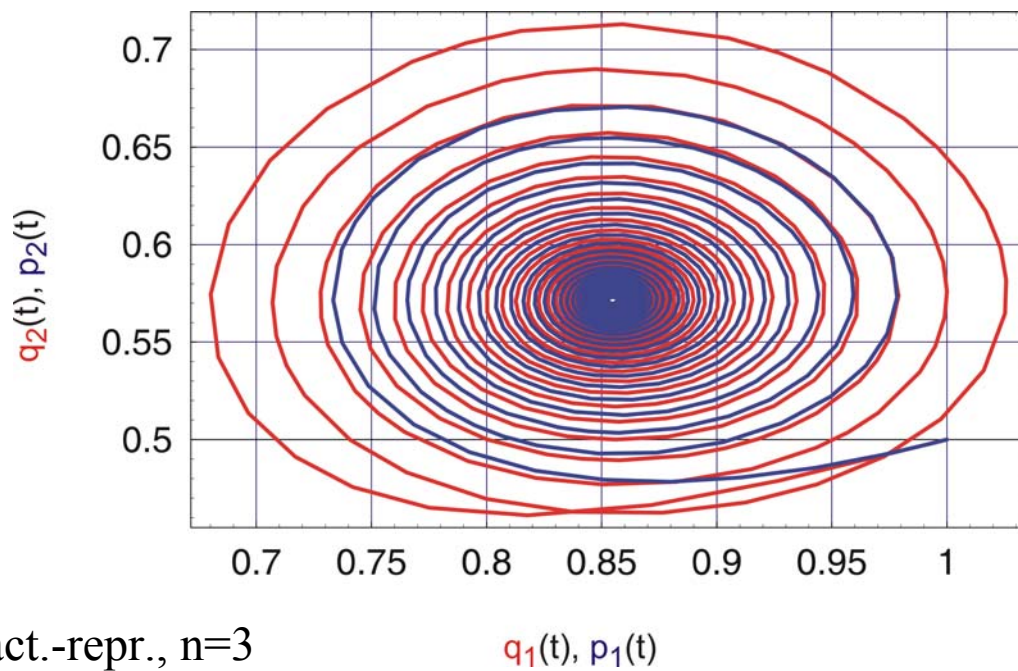
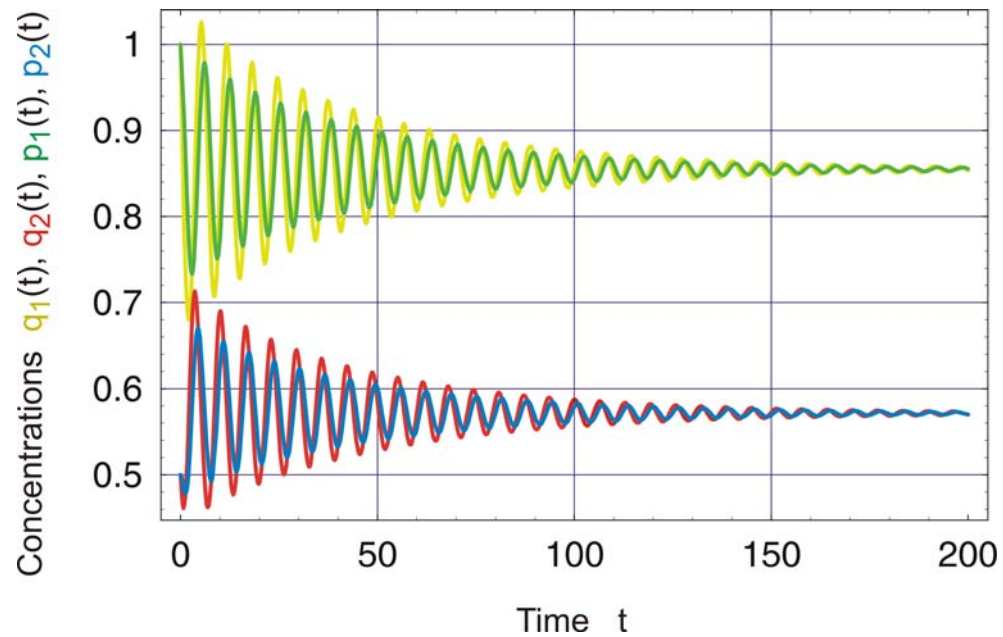
$$D_{\text{Hopf}} = \frac{(d_1^{\text{Q}} + d_2^{\text{Q}})(d_1^{\text{Q}} + d_1^{\text{P}})(d_1^{\text{Q}} + d_2^{\text{P}})(d_2^{\text{Q}} + d_1^{\text{P}})(d_2^{\text{Q}} + d_2^{\text{P}})(d_1^{\text{P}} + d_2^{\text{P}})}{(d_1^{\text{Q}} + d_2^{\text{Q}} + d_1^{\text{P}} + d_2^{\text{P}})^2}$$



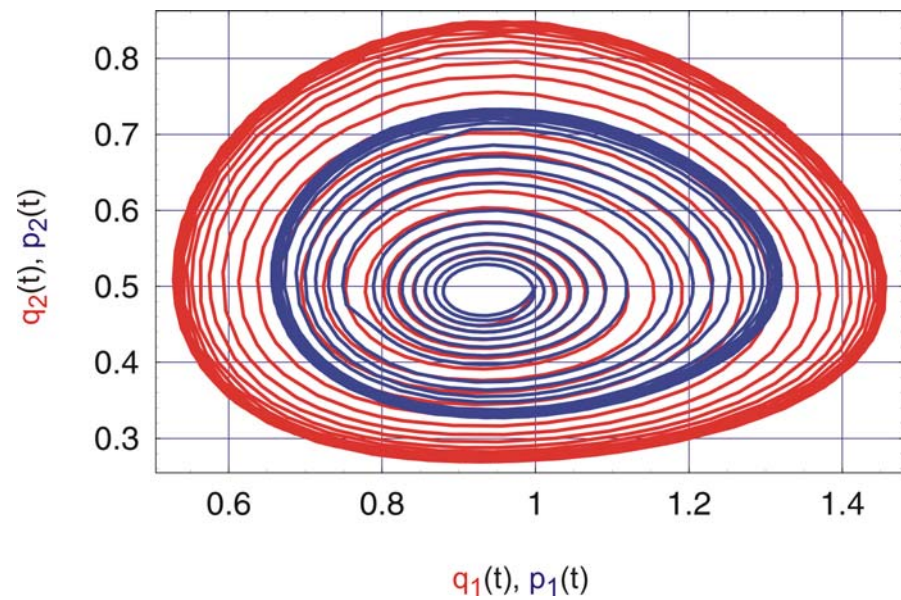
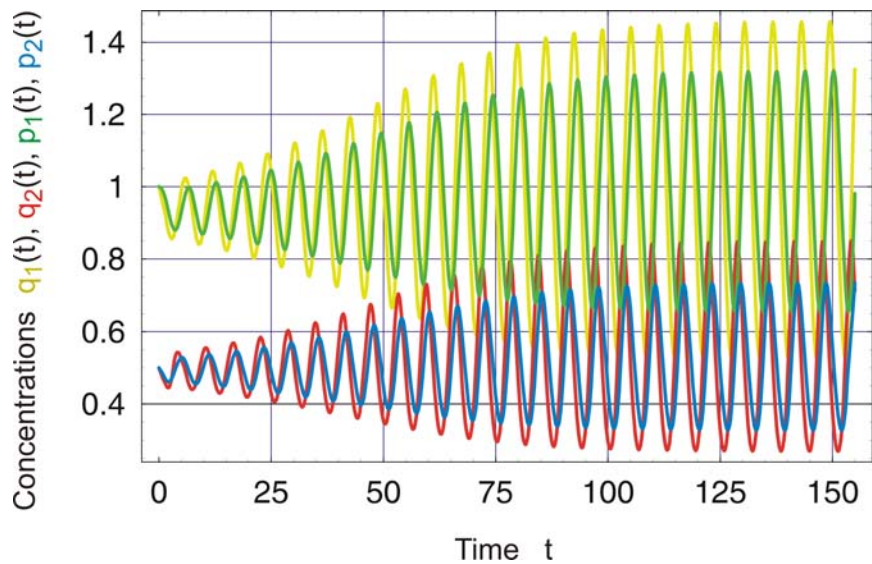
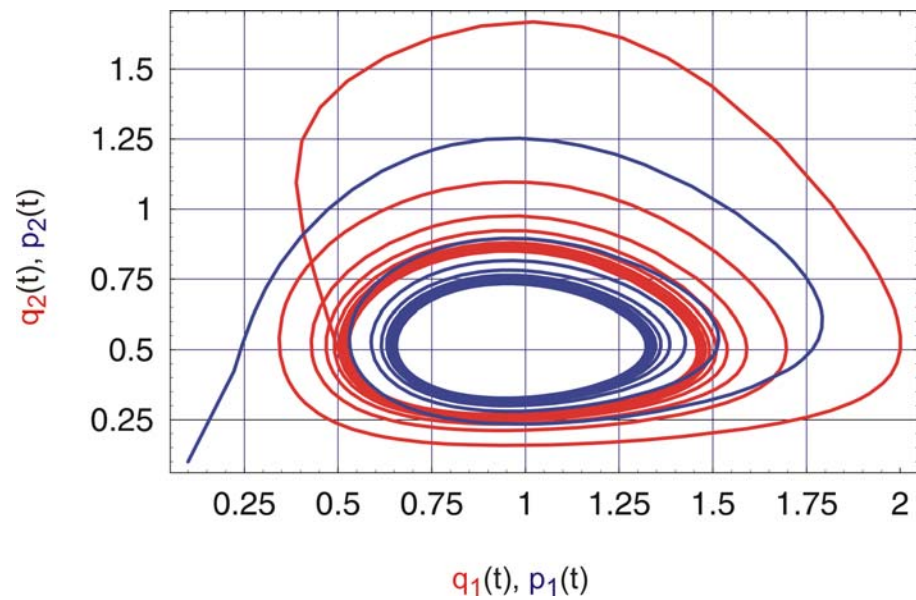
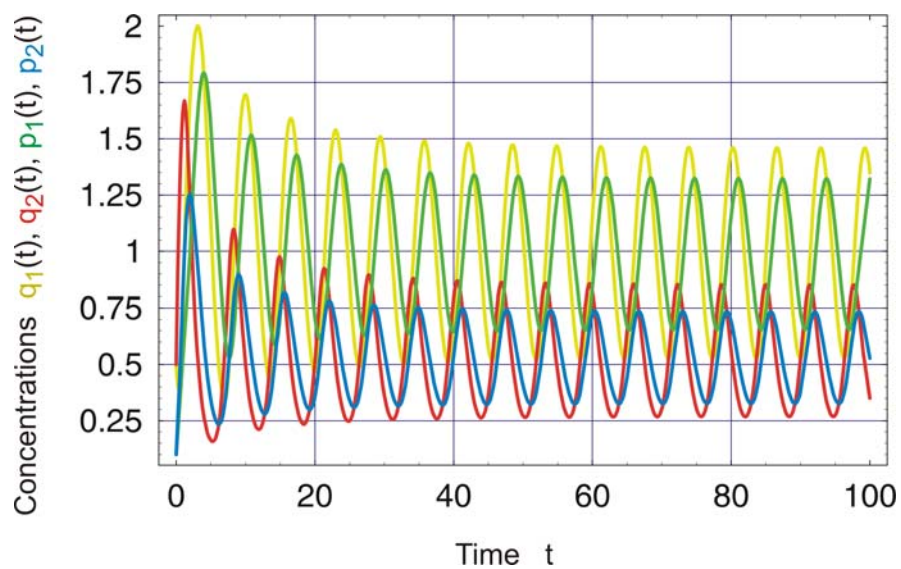
Regulatory dynamics at $D \leq 0$, act.-act., $n=2$



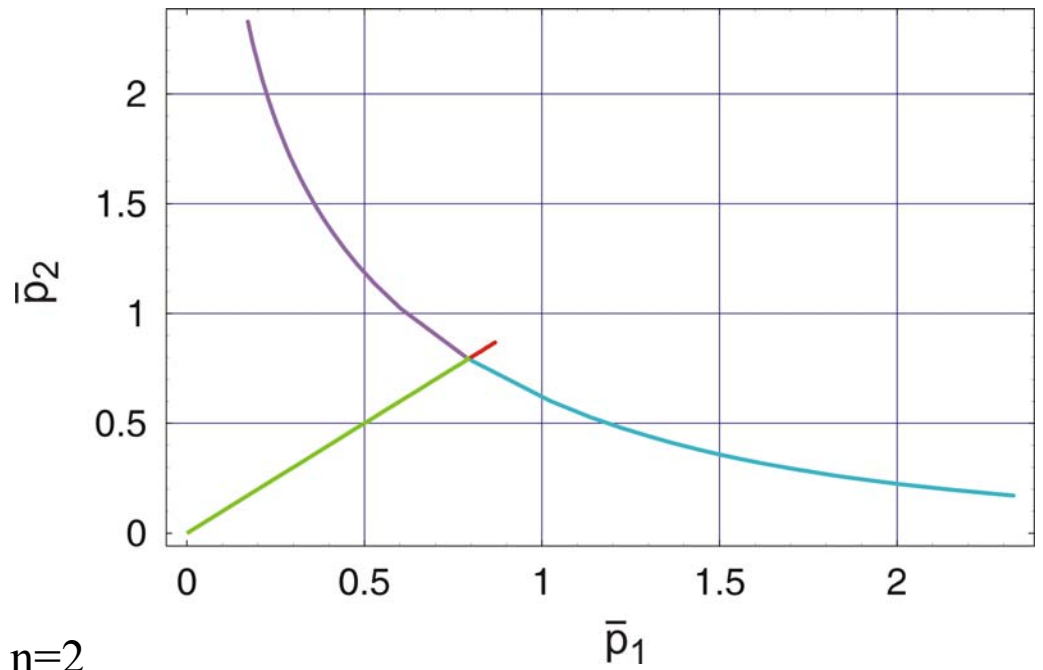
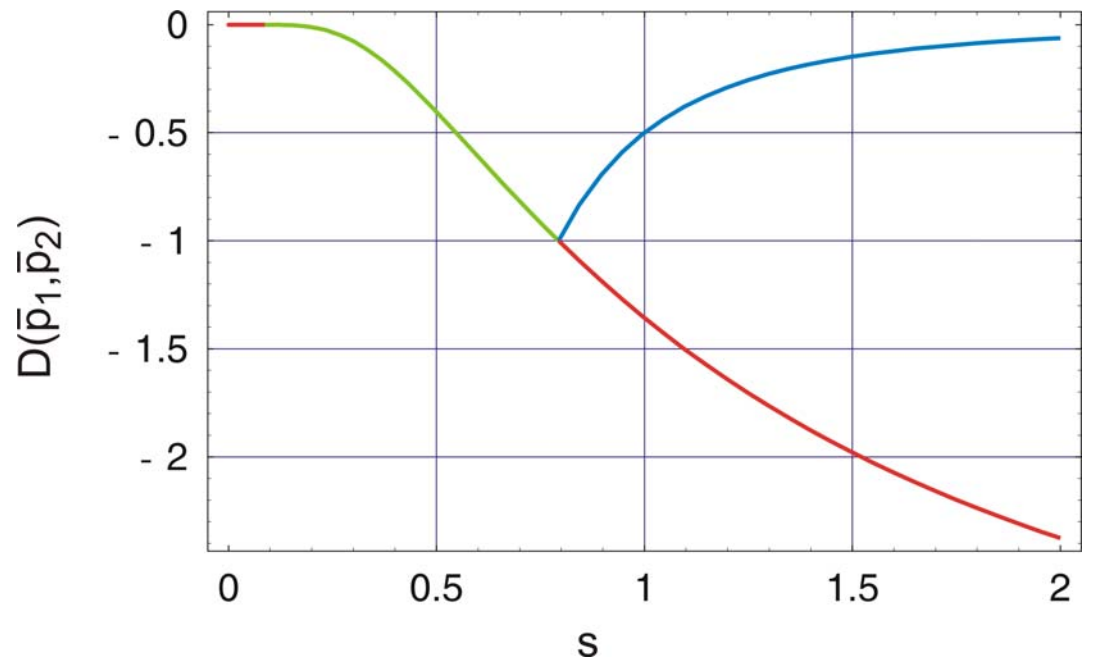
Regulatory dynamics at $D \geq 0$, act.-rep., $n=3$



Regulatory dynamics at $D < D_{\text{Hopf}}$, act.-repr., $n=3$



Regulatory dynamics at $D > D_{\text{Hopf}}$, act.-repr., $n=3$



Regulatory dynamics at $D \leq 0$, rep.-rep., $n=2$

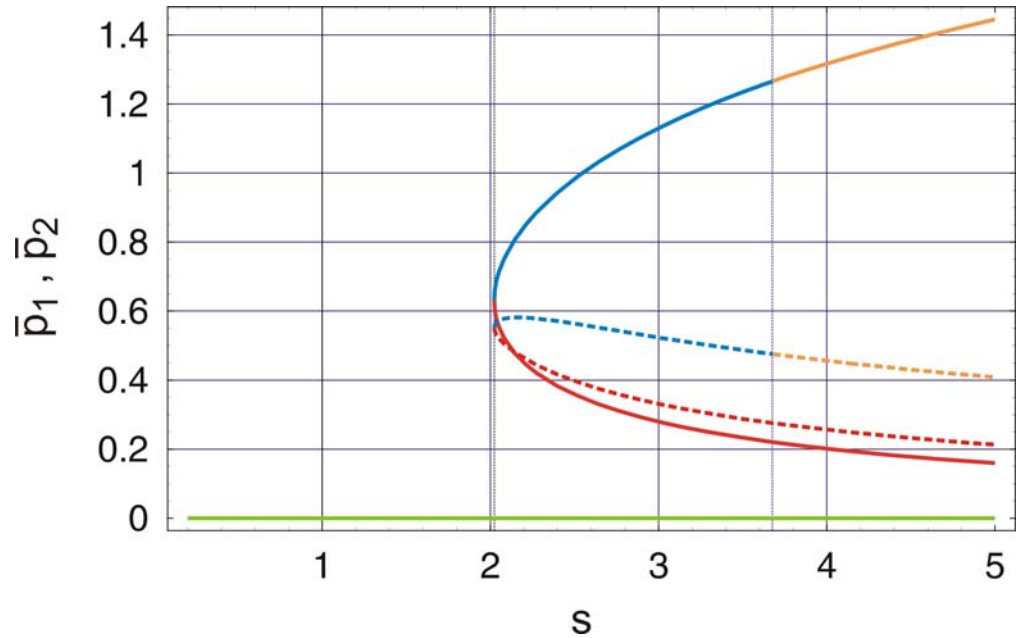
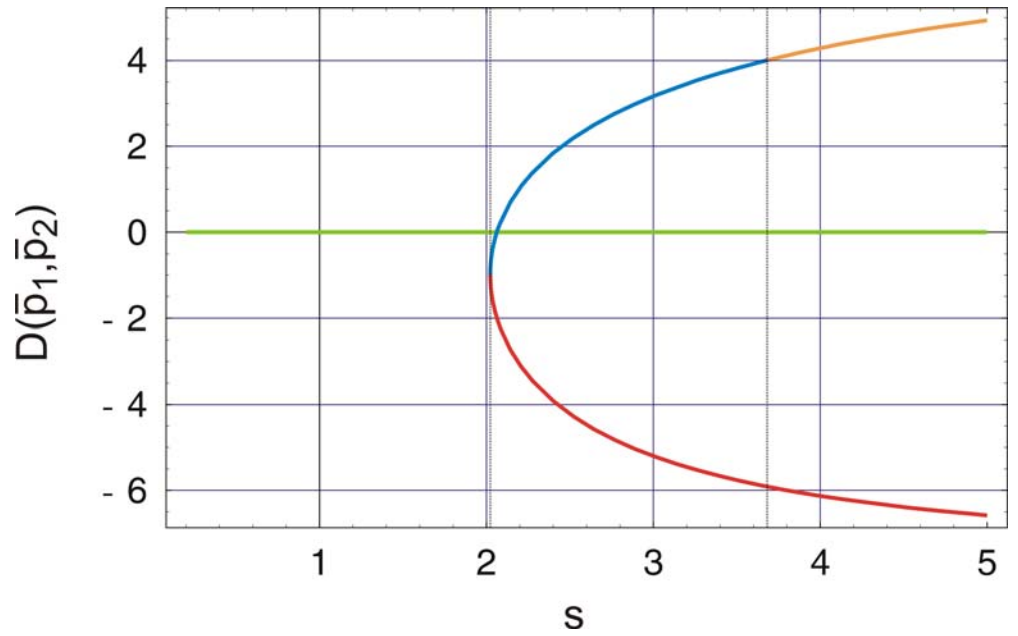
Hill coefficient: n	Act.-Act.	Act.-Rep.	Rep.-Rep.
1	S , E	S	S
2	E , B(E,P)	S	S , B(P ₁ ,P ₂)
3	E , B(E,P)	S , O	S , B(P ₁ ,P ₂)
4	E , B(E,P)	S , O	S , B(P ₁ ,P ₂)

$$\text{Activation: } F_i(p_j) = \frac{p_j^n}{K + p_j^n}$$

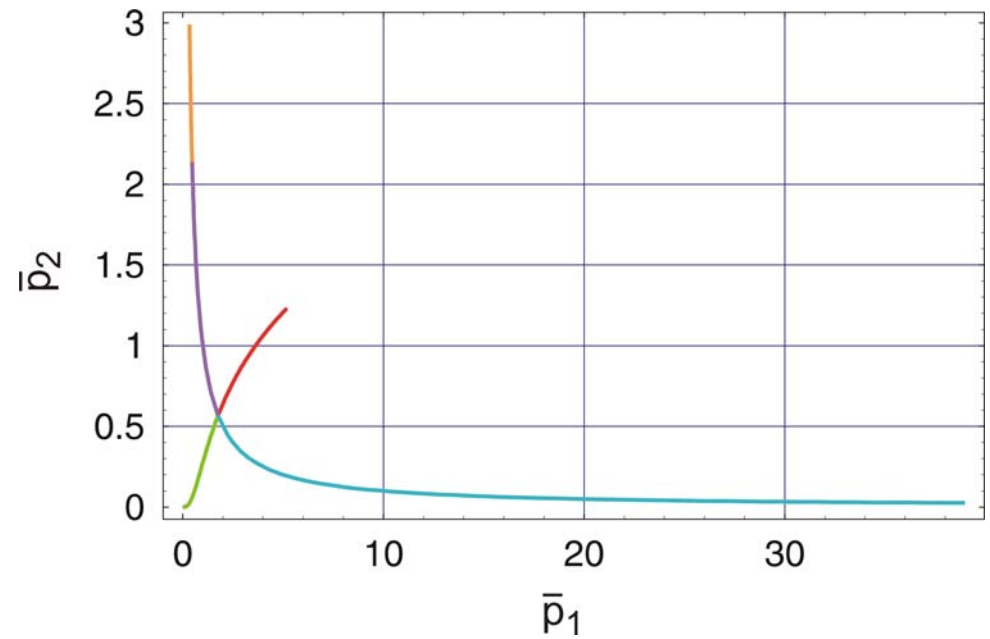
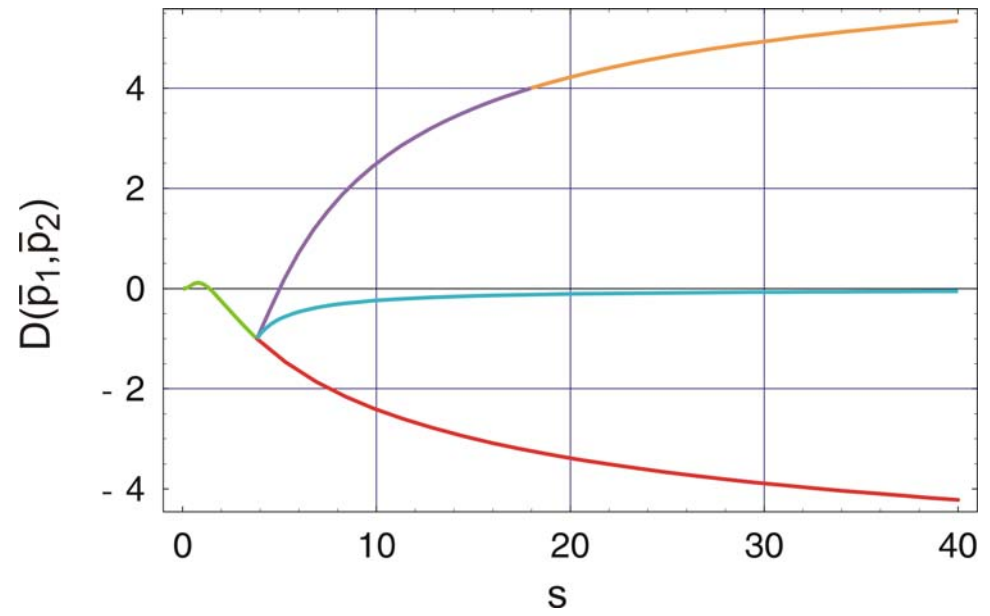
$$\text{Repression: } F_i(p_j) = \frac{K}{K + p_j^n}$$

$$\text{Intermediate: } F_i(p_j) = \frac{p_j^m}{\kappa_1 + \kappa_2 p_j + \kappa_3 p_j^2 + \dots + p_j^n}$$

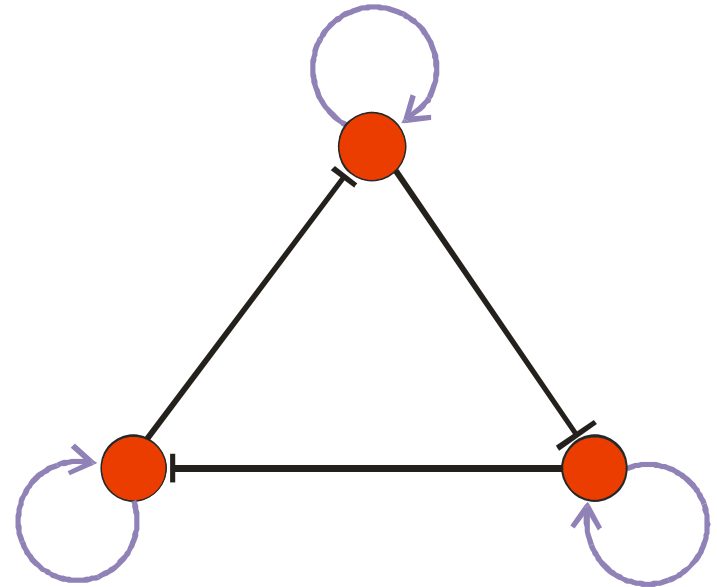
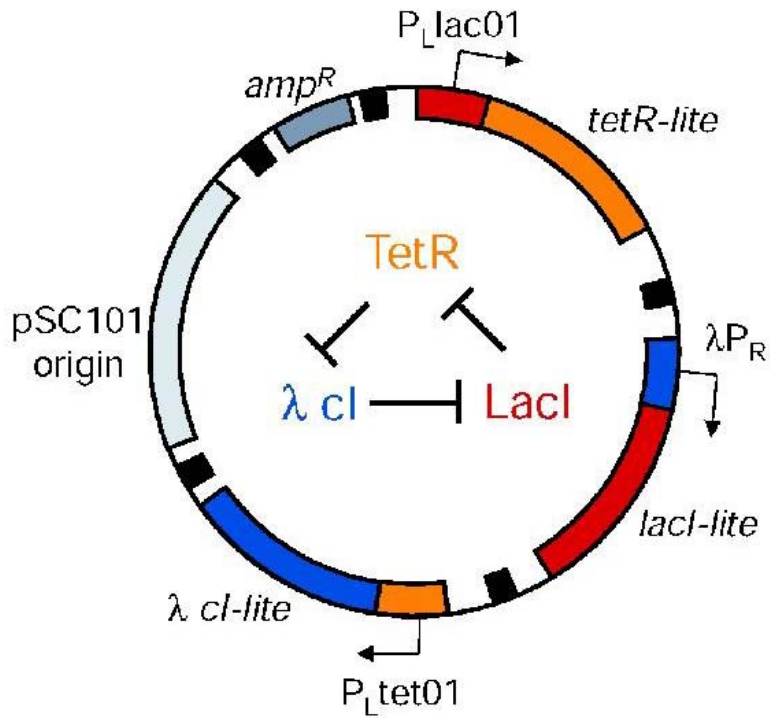
$$i, j = 1, 2; \quad 1 \leq m \leq n-1$$



Regulatory dynamics, int.-act., $m=2$, $n=4$

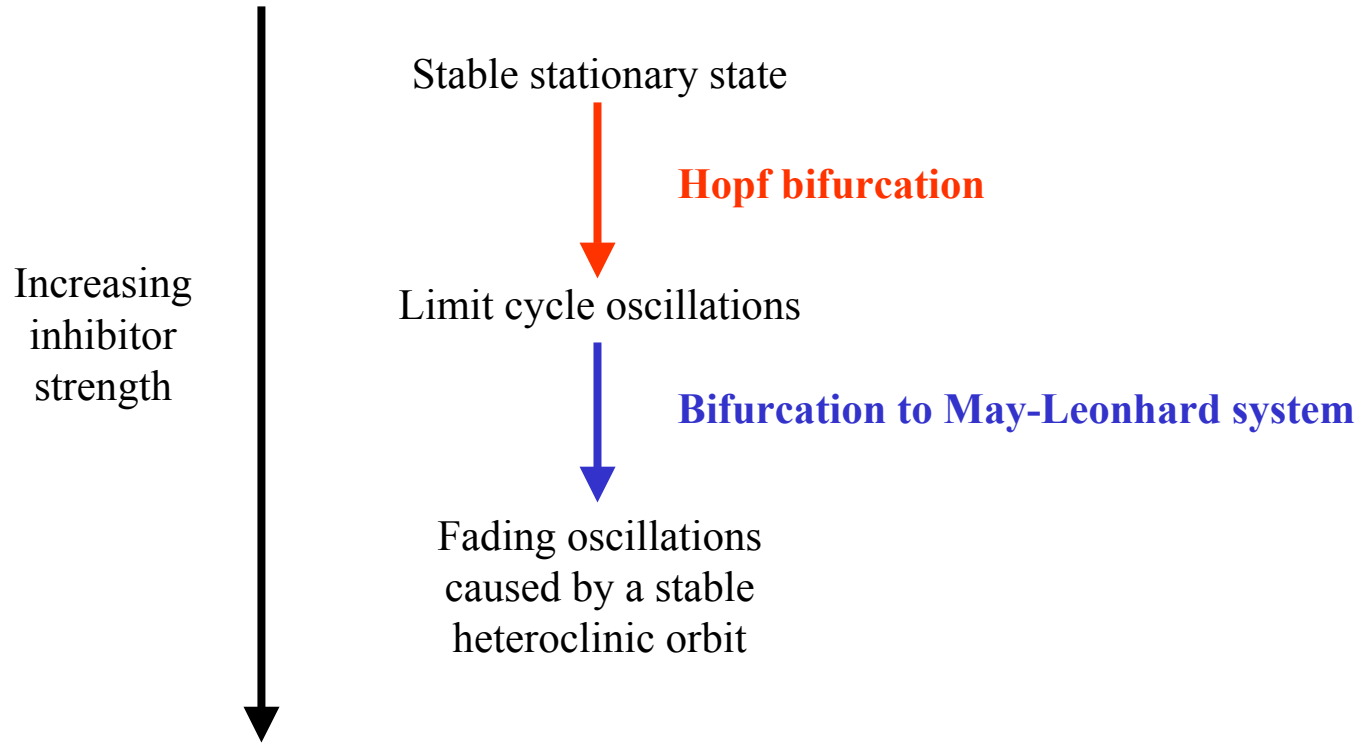


Regulatory dynamics, rep.-int., $m=2$, $n=4$

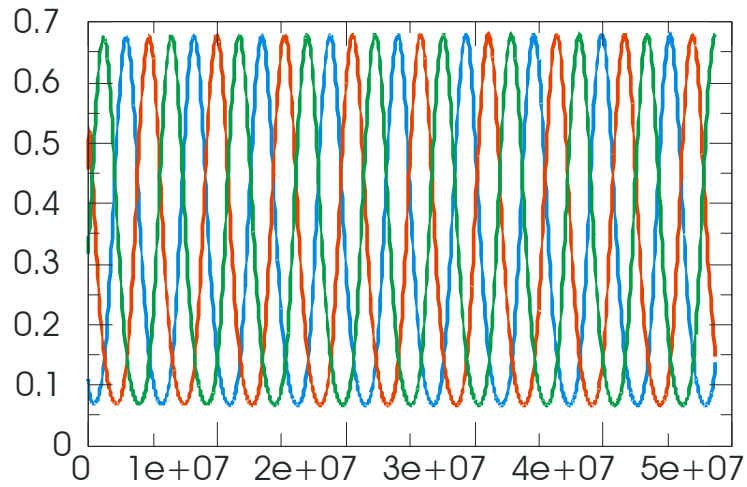


An example analyzed and simulated by MiniCellSim

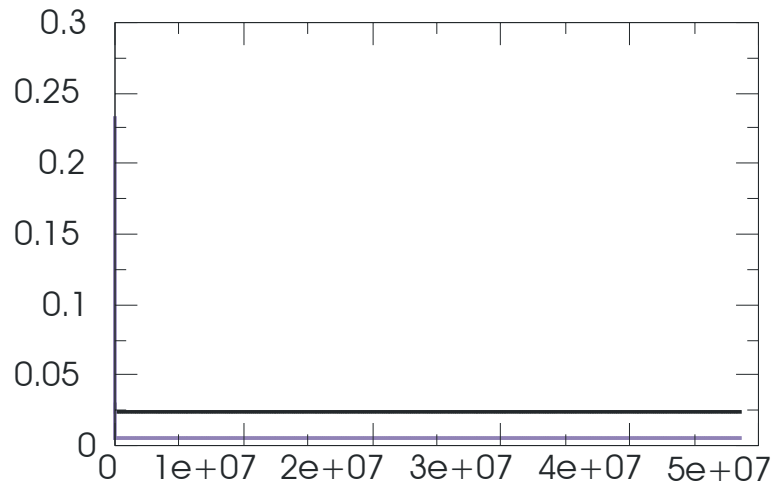
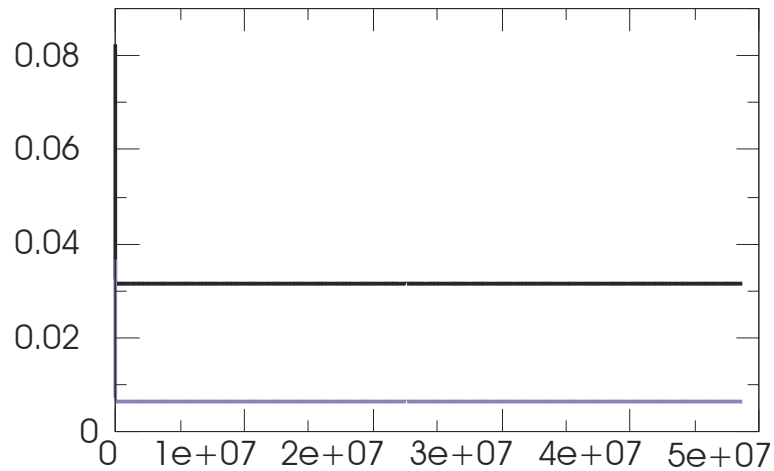
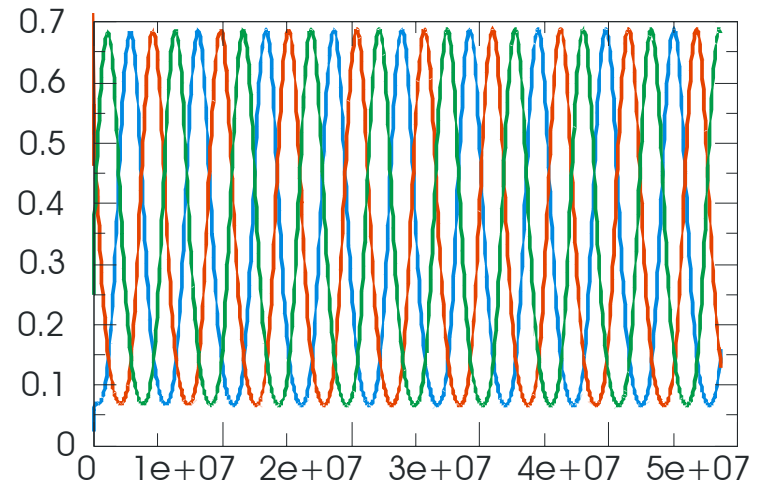
The repressilator: M.B. Elowitz, S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature* **403**:335-338, 2002



Proteins

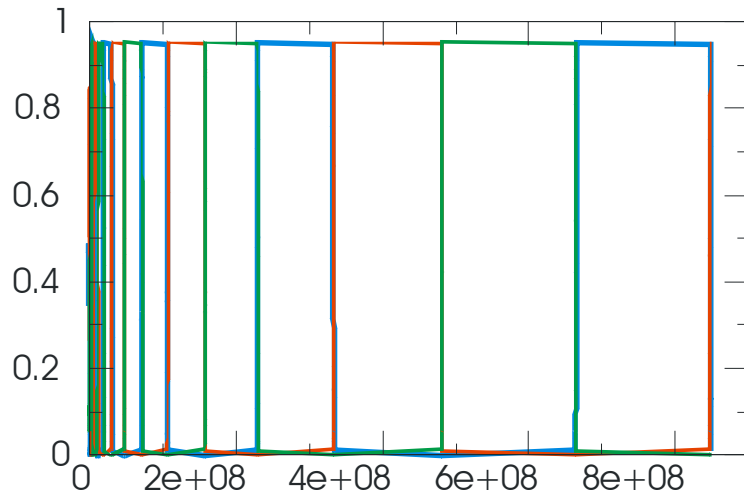


mRNAs

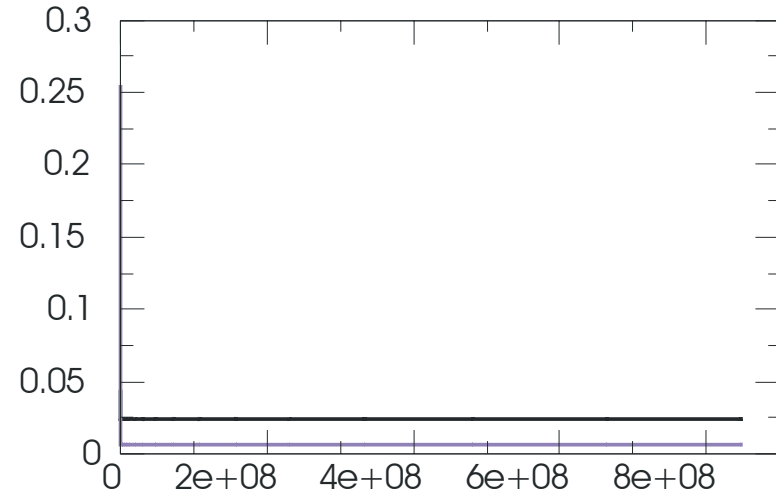
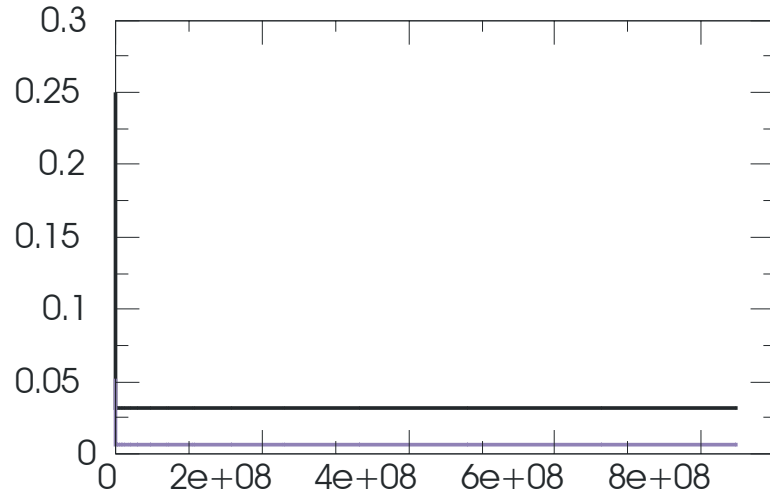
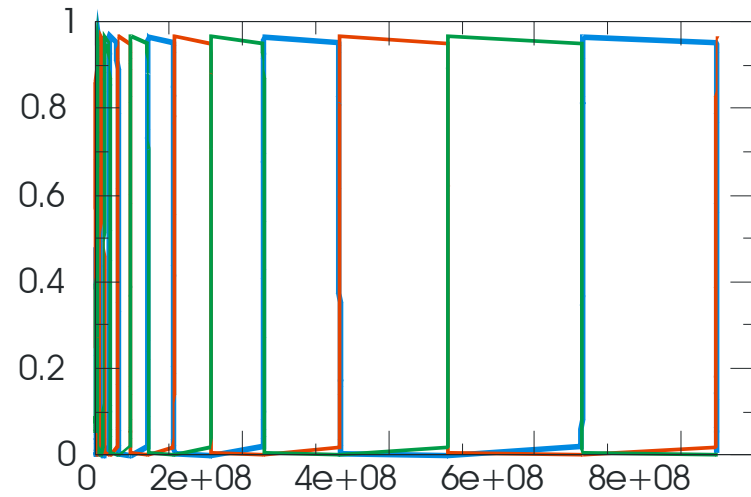


The repressilator limit cycle

Proteins

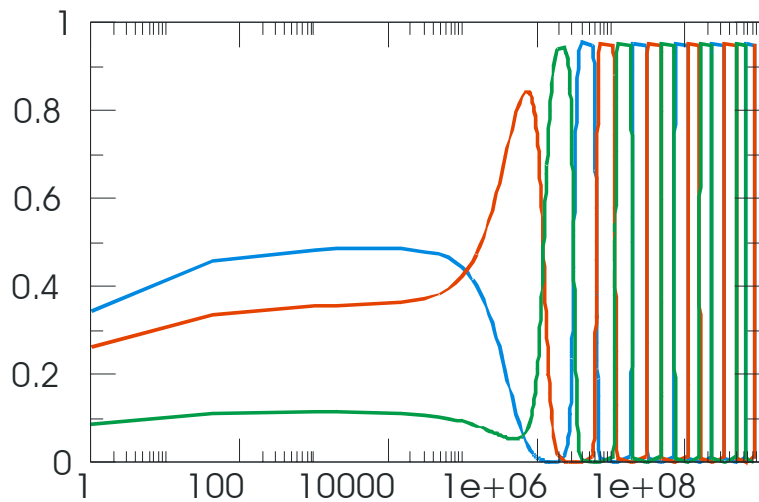


mRNAs

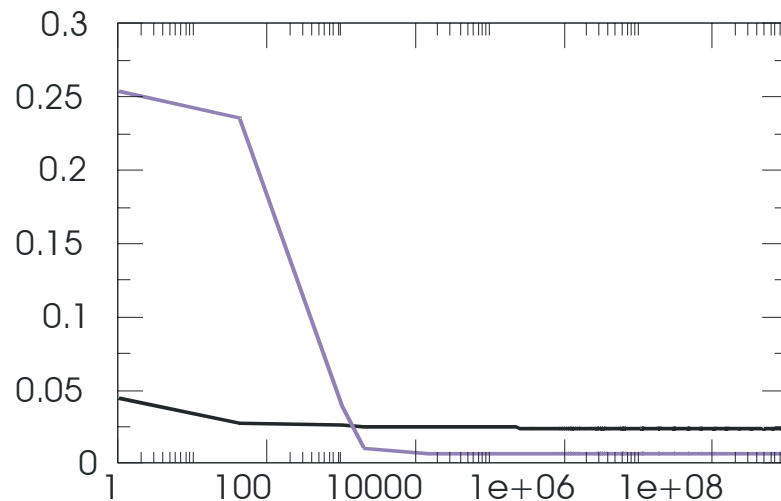
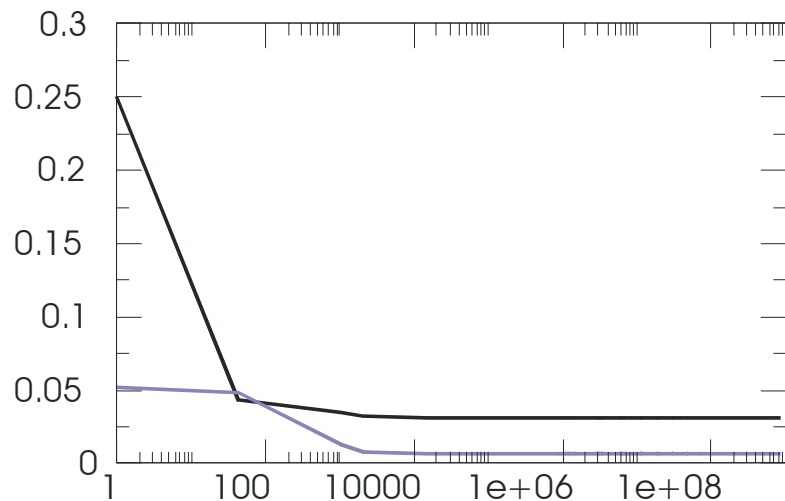
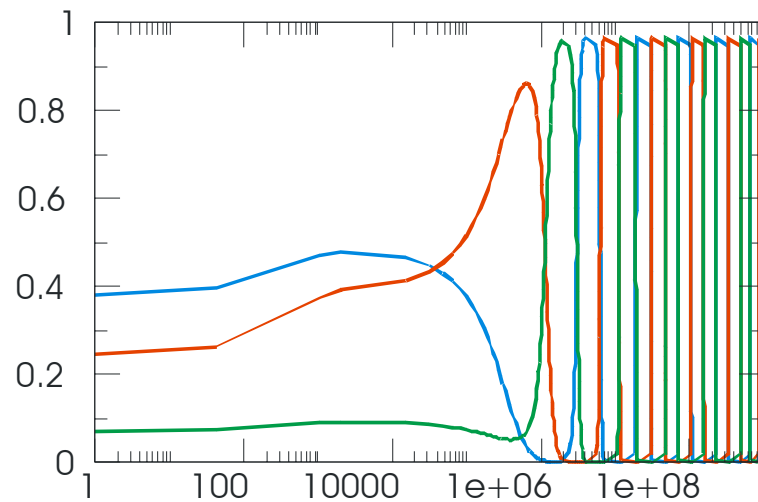


The repressilator heteroclinic orbit

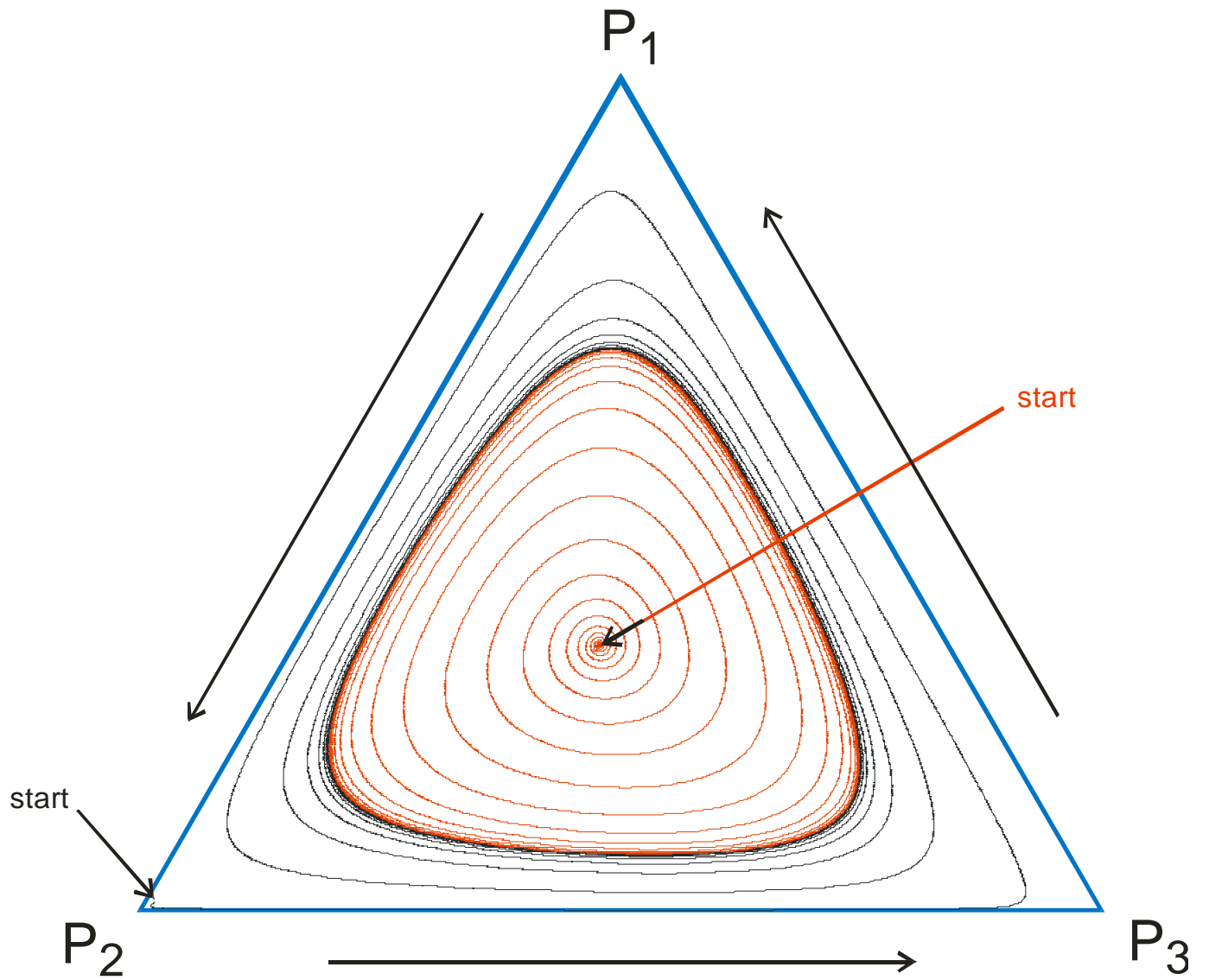
Proteins



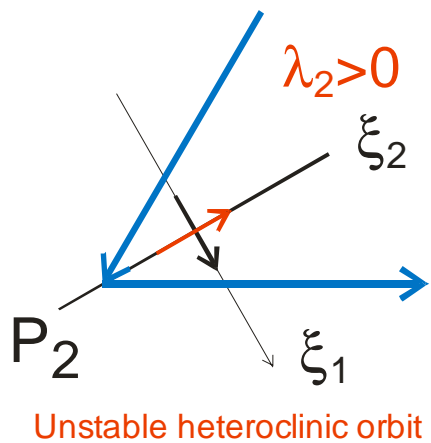
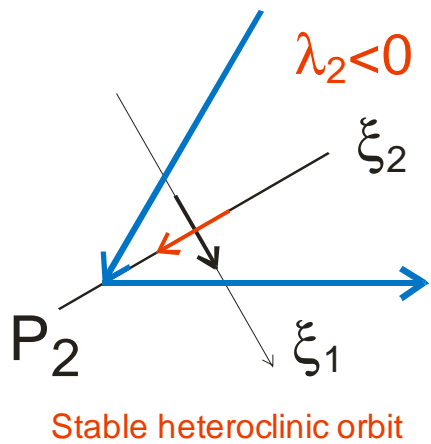
mRNAs



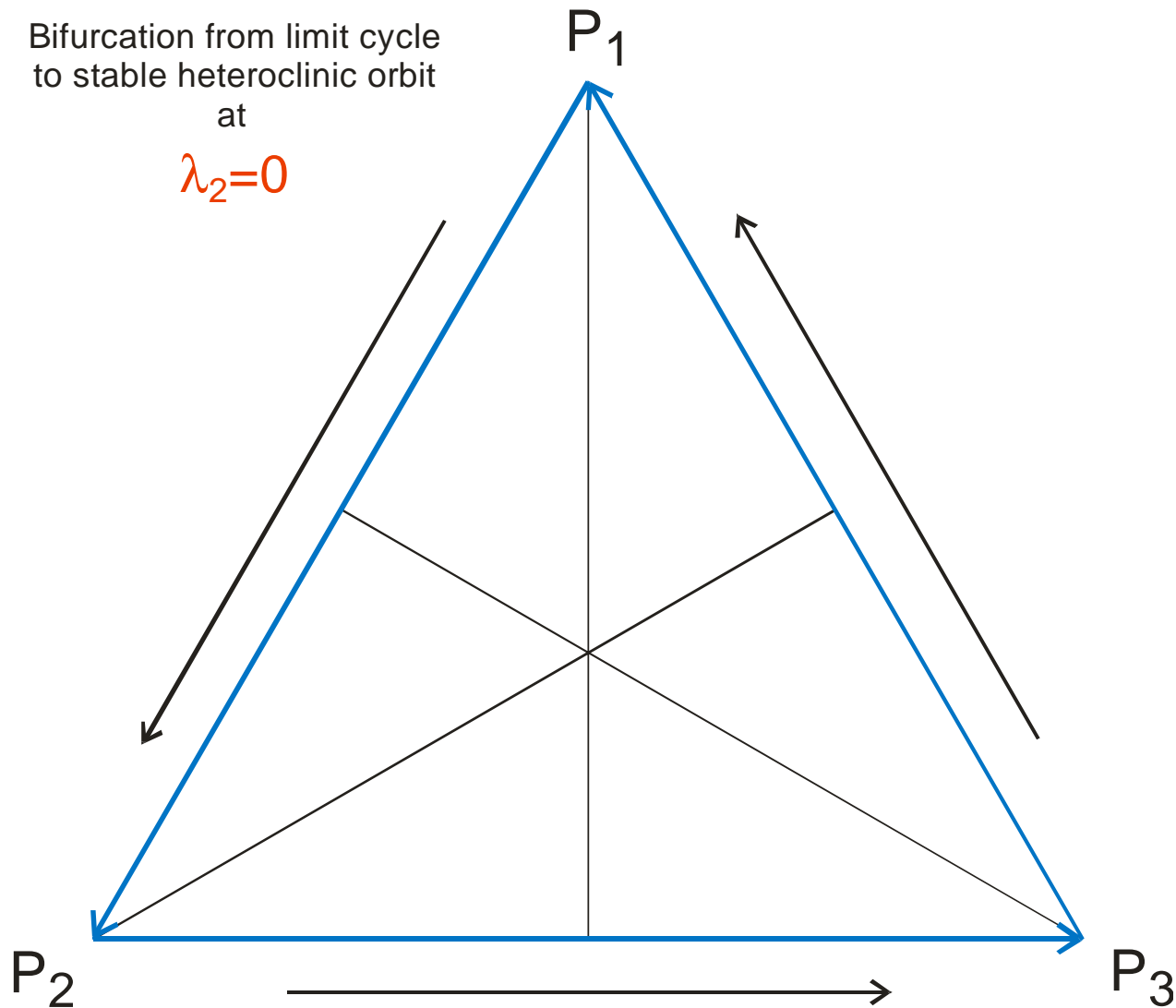
The repressilator heteroclinic orbit (logarithmic time scale)



The repressilator limit cycle



Bifurcation from limit cycle
to stable heteroclinic orbit
at
 $\lambda_2 = 0$



The repressilator heteroclinic orbit

1. Forward and inverse problems in reaction kinetics
2. Reverse engineering - A simple example
3. Genetic and metabolic networks - MiniCellSim
4. A glimpse of regulation kinetics
5. **How do model metabolisms evolve?**

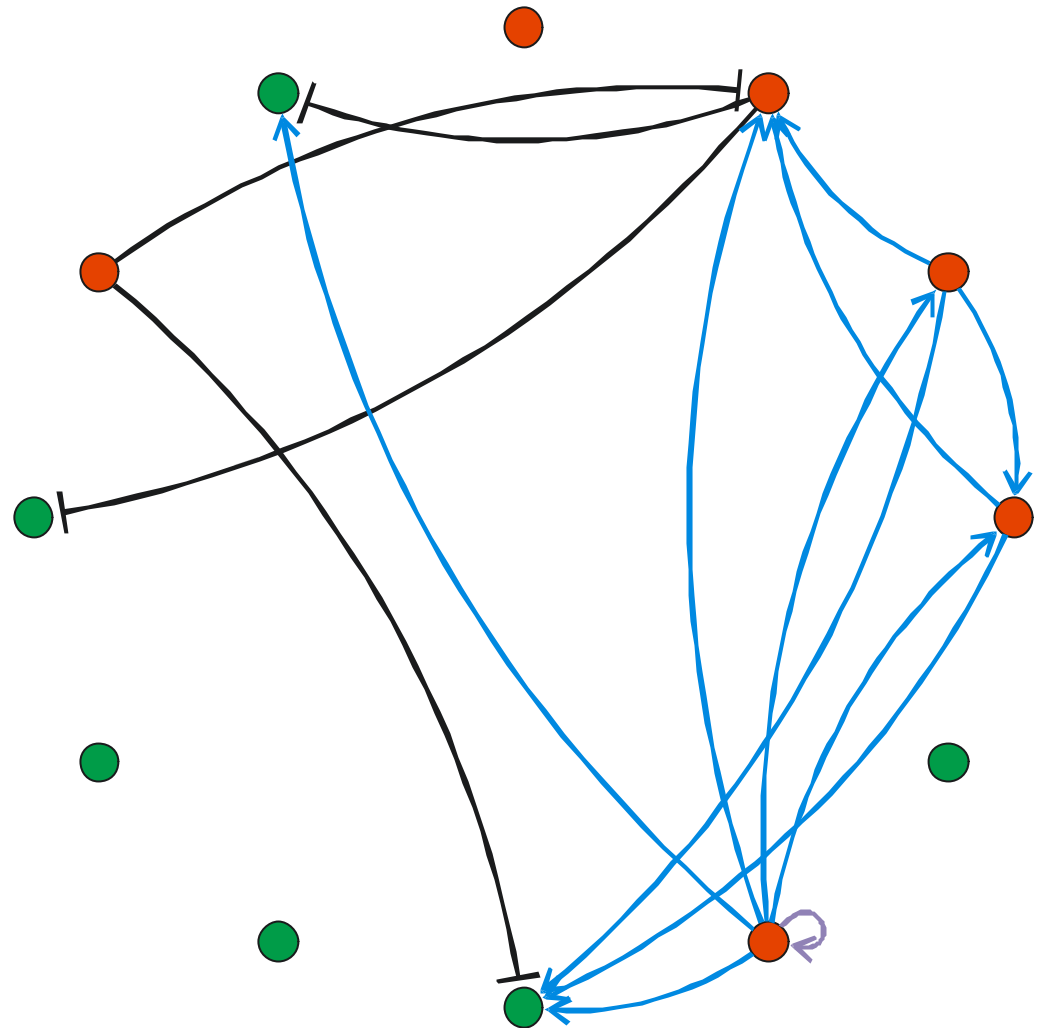
Evolutionary time: 0000

Number of genes: 12

06 structural + 06 regulatory

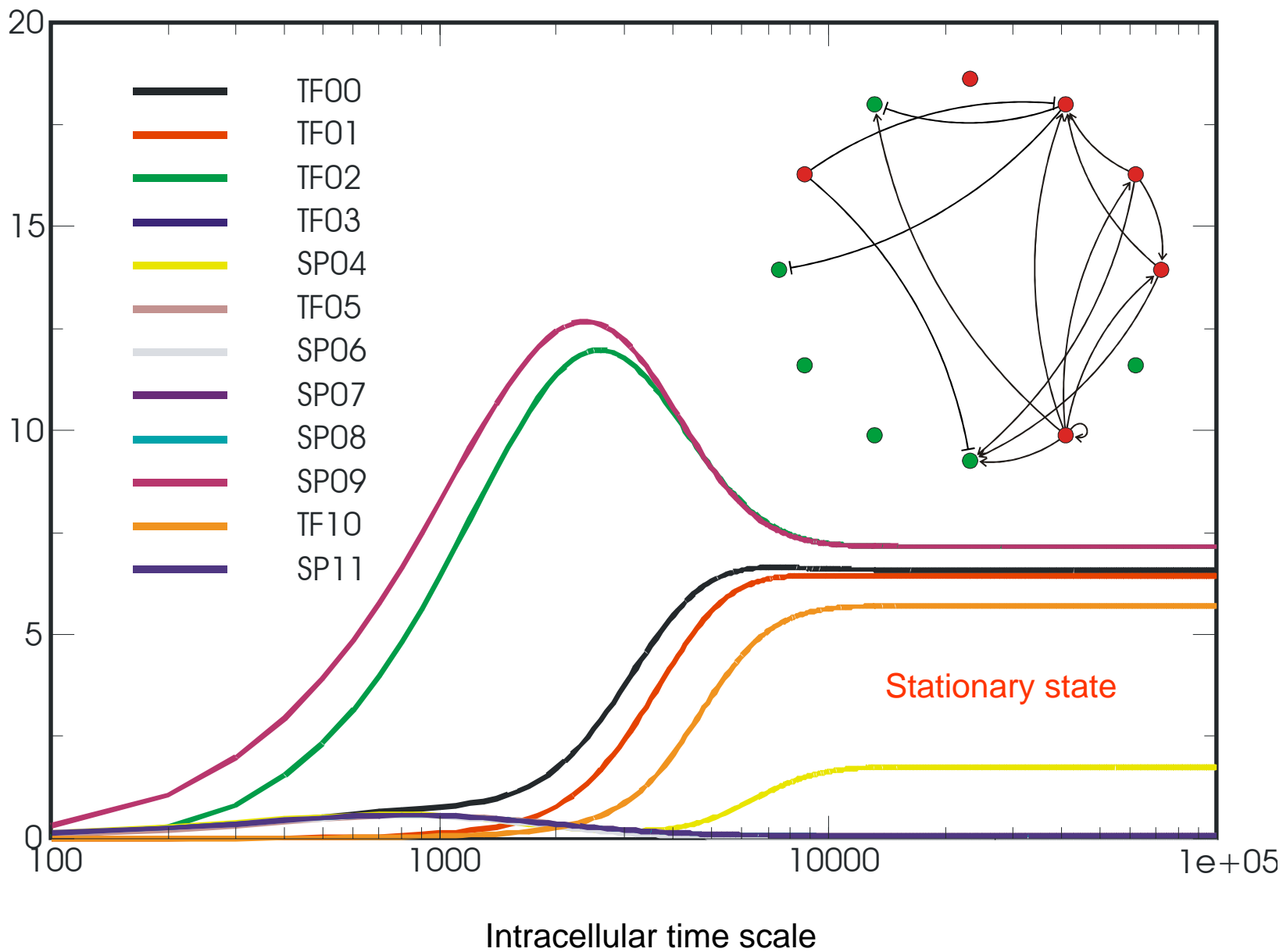
Number of interactions: 15

04 inhibitory + 10 activating +
+ 1 self-activating



A genabolic network formed from a genotype of $n = 200$ nucleotides

Evolutionary time scale [generations]: 0000 initial network



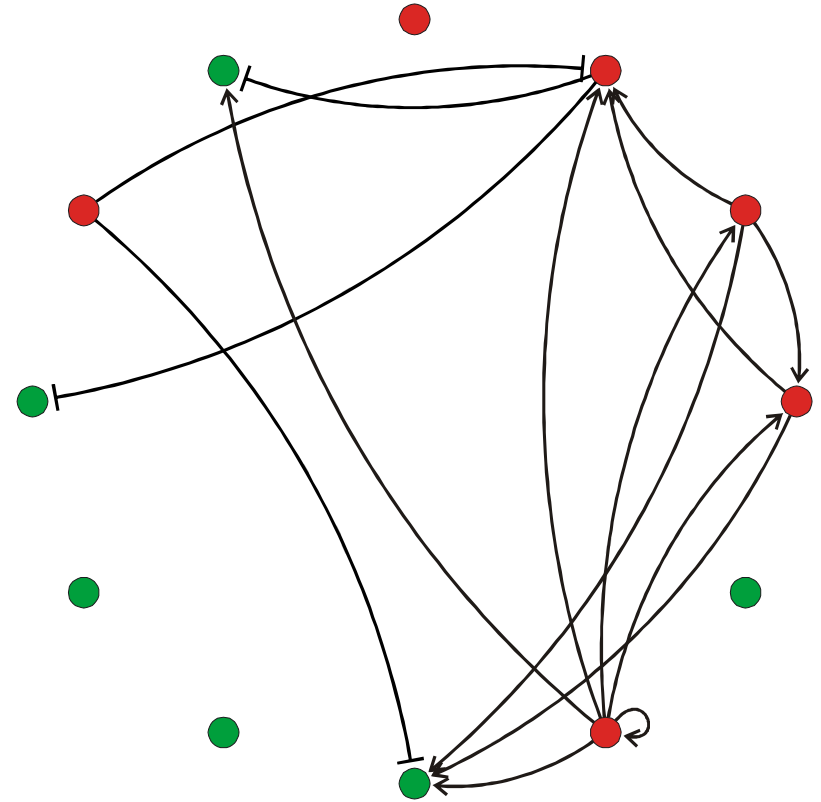
Evolution of a genabolic network:

Initial genome: random sequence of length $n = 200$,
AUGC alphabet

Gene length: $n = 25$

Simulation with mutation rate: $p = 0.01$

Evolutionary time unit \gg intracellular time unit



Number of genes: total / **structural genes** **regulatory genes**

Genes 12/08 Time 0000

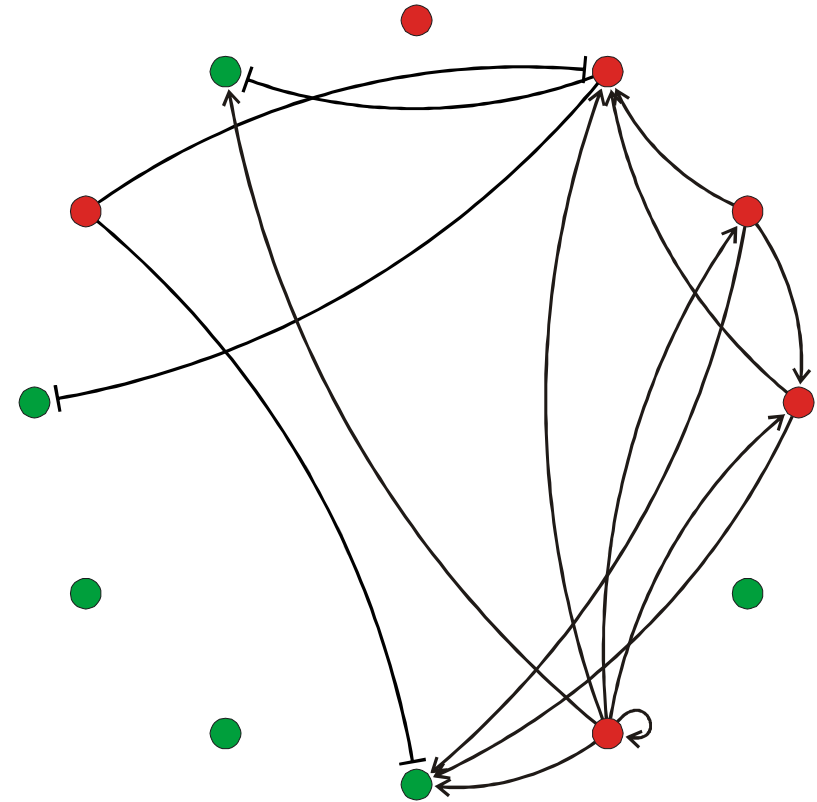
Evolution of a genabolic network:

Initial genome: random sequence of length $n = 200$,
AUGC alphabet

Gene length: $n = 25$

Simulation with mutation rate: $p = 0.01$

Evolutionary time unit \gg intracellular time unit



Recorded events:

- (i) Loss of a gene through corruption of the start signal “TA” (analogue of the “TATA Box”),
- (ii) creation of a gene,
- (iii) change in the edges through mutation-induced changes in the affinities of translation products to the binding sites, and
- (iv) change in the class of genes (tf \leftrightarrow sp).

Statistics of one thousand generations

Total number of genes: 11.67 ± 2.69

Regulatory genes: 5.97 ± 2.22

Structural genes: 5.70 ± 2.17

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Universität Wien

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