Reaction networks, stability of steady states, motifs for oscillatory dynamics, and parameter estimation in complex biochemical mechanisms

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Outline

- Reaction network theory and classification as a tool for identification of oscillatory motifs in network's topology
- Cyclic vs competitive autocatalysis as two prevailing motifs in inorganic vs enzyme oscillatory reactions
- Parameter estimation using stoichiometric constraints
- Case studies:
  - CAT-GOX enzyme oscillatory reaction
  - Microbial predator-prey system in bioreactor
  - Carbon-nitrogen metabolism in cyanobacteria
  - Circadian clock in cyanobacteria

Stoichiometric network analysis (SNA)

- one of several approaches to the reaction network theory

  B. L. Clarke, Adv. Chem. Phys. 43, 1 (1980)
  J. Ross, I. Schreiber and M.O. Vlad, Determination of Complex Reaction Mechanisms, Oxford University Press, New York, 2006

  - decomposition of reaction networks into elementary subsystems (extreme currents, elementary/flux modes)
  - simplification of reaction networks
  - linear stability analysis and graphical interpretation
  - identification of positive and negative feedback loops
  - identification of autocatalytic cycles as sources of instabilities
  - combination of autocatalysis and negative feedback leads to bistability or oscillations
  - corroboration or rejection of reaction mechanisms (O.Hadač, I.Schreiber, PCCP 13, 1314, 2011)

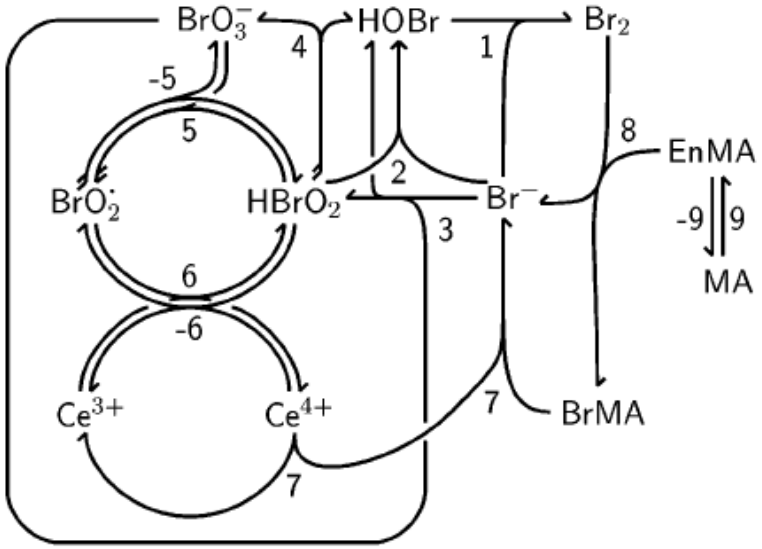
- other approaches: Complex balanced networks (Horn, 1976; Feinberg, 1995)

Example of a graphical representation – network diagram: Belousov-Zhabotinskii reaction

TABLE 4: Reaction Mechanism and Rate Coefficients for a Modified FKN Mechanism of the BZ Reaction[a]

reaction no.	reaction	rate coefficient
(1)	$Br^- + HOBr + H^+ \rightarrow Br_2 + H_2O$	$k_1 = 8 \times 10^9\ M^{-2}\ s^{-1}$
(2)	$Br^- + HBrO_2 + H^+ \rightarrow 2HOBr$	$k_2 = 3 \times 10^6\ M^{-2}\ s^{-1}$
(3)	$Br^- + BrO_3^- + 2H^+ \rightarrow HOBr + HBrO_2$	$k_3 = 2\ M^{-3}\ s^{-1}$
(4)	$2HBrO_2 \rightarrow HOBr + BrO_3^- + H^+$	$k_4 = 3000\ M^{-1}\ s^{-1}$
(5, −5)	$HBrO_2 + BrO_3^- + H^+ \rightleftharpoons 2BrO_2^\bullet + H_2O$	$k_5 = 42\ M^{-2}\ s^{-1}$ $k_{-5} = 4.2 \times 10^7\ M^{-1}\ s^{-1}$
(6, −6)	$Ce^{3+} + BrO_2^\bullet + H^+ \rightleftharpoons Ce^{4+} + HBrO_2$	$k_6 = 8 \times 10^4\ M^{-2}\ s^{-1}$ $k_{-6} = 8.9 \times 10^3\ M^{-1}\ s^{-1}$
(7)	$Ce^{4+} + BrMA \rightarrow Ce^{3+} + Br^- + \text{products}$	$k_7 = 0.5\ M^{-1}\ s^{-1}$
(8)	$Br_2 + EnMA \rightarrow H^+ + Br^- + BrMA$	$k_8 = 6 \times 10^6\ M^{-1}\ s^{-1}$
(9,−9)	$H^+ + MA \rightleftharpoons EnMA + H^+$	$k_9 = 1.3 \times 10^{-2}\ M^{-1}\ s^{-1}$ $k_{-9} = 1.3 \times 10^4\ M^{-1}\ s^{-1}$

[a] MA ≡ $CH_2(COOH)_2$, EnMA ≡ $(OH)_2C{=}CHCOOH$, BrMA ≡ $BrCH(COOH)_2$.



1) Any elementary reaction is drawn as a **multi-headed multi-tailed arrow** oriented from the species entering the reaction to those produced by the reaction

2) **The number of feathers/barbs** at each tail/head **represents the stoichiometric coefficient** of the reactant/product; the order of the reaction is the number of left feathers

Schreiber & Ross, J.Phys.Chem. 2003

Elements of the SNA

B. L. Clarke, Adv. Chem. Phys. 43 1 (1980)

Assume that there are *m* species taking part in *r* chemical reactions so that first *n* species, $n \leq m$, are entering at least one of the reactions

$$\mathrm{R_j}: \sum_{i=1}^{n} \nu_{ij}^{L} X_i \rightarrow \sum_{i=1}^{m} \nu_{ij}^{R} X_i \quad , \qquad j = 1, \cdots, r$$

where $\nu_{ij}^{L} \geq 0$ and $\nu_{ij}^{R} \geq 0$ are, respectively, the left hand and right hand stoichiometric coefficients of species X_i in reaction R_j . The first *n* species are assumed to be reactants or intermediates and the remaining *m* – *n* are inert products.

$\bar{S} = \left\{ \nu_{ij}^{R} - \nu_{ij}^{L} \right\}$ is the ($n \times r$) **stoichiometric matrix**

$X = (X_1, \cdots, X_n)$ is the vector of the chemical species concentrations

$\bar{V}(X) = (\bar{V}_1(X), \cdots, \bar{V}_r(X))$ is the vector of reaction rates

The time evolution of *X* in a flow-through system at constant temperature in a well-stirred reaction cell of constant reaction volume is based on **mass balance equations**:

$$\frac{dX}{dt} = F(X) = \bar{S}\bar{V}(X) + k_0(X_0 - X) = SV(X)$$

S is the extended stoichiometric matrix including inflows and outflows

Stoichiometric constraints: If the rank of S is less than n, there is a nonempty null space of S^T of dimension $d_n = n$ – rank(S) and there are d_n independent linear constraints for X.

Closed systems or subsystems, such as enzyme reactions, may satisfy such constraints

Decomposition of the network into subnetworks

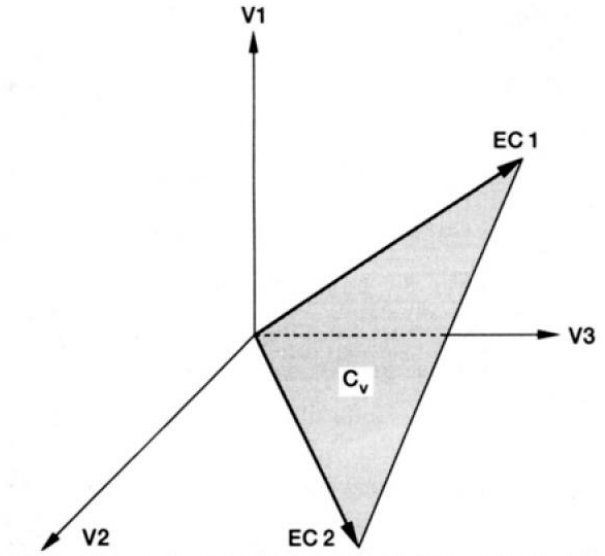
A stationary (or steady) state X_s satisfies the equation:

$$SV(X) = 0$$

Hence $V_s = V(X_s)$ is contained in the **null space of *S*** that has dimension $d_r = r$ – rank(S).

All components of V must be nonnegative numbers which narrows the set of all possible stationary reaction rate vectors V_s (=currents) to an open, convex, **d_r-dimensional cone**, $d_r = r$ – rank(S), in the reaction rate space of all V's

The edges of this steady state cone represent sets of steady states that have minimum possible nonzero elements of V's and define a set of **major subnetworks** (=extreme currents, elementary modes) of the mechanism.

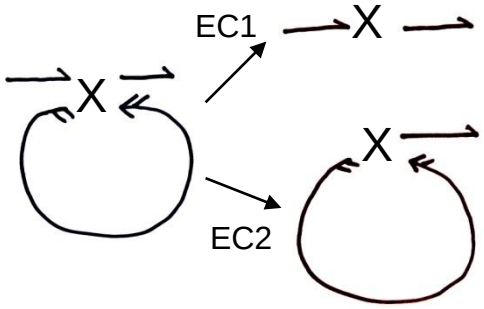


Two-dimensional current cone C_v spanned by two extreme currents EC1 and EC2.
The shaded bounded region of the cone is obtained by applying the normalizing condition $\sum_{k=1}^{K} \alpha_k EC_k \leq 1$

If $EC_{k,}$ $k = 1, \cdots, K$, are vectors pointing along the edges of the cone then any linear combination $\sum_{k=1}^{K} \alpha_k EC_k$ with nonnegative coefficients is again a current.
Conversely, any current V_s can be expressed as a linear combination of extreme currents

Example:

V1 - inflow: → X
V2 - reaction: 2X + R → 3X
V3 - outflow: X →



Stability of stationary states associated with subnetworks

The identification of the edges is useful when examining the stability of the subnetwork at a stationary state X_s. The **Jacobian matrix *J*** of evolution equations at X_s is

$$J = \left.\frac{dF}{dX}\right|_{X=X_s} = S\left.\frac{dV}{dX}\right|_{X=X_s} = S(\operatorname{diag} V_s)\kappa^T(\operatorname{diag} X_s)^{-1}$$

$V_s = \sum_{k=1}^{K} \alpha_k EC_k$ is a linear combination of extreme currents

$\kappa = \{\kappa_{ij}\} = \{\partial \ln V_j(X_s)/\partial \ln X_i\}$ is the **kinetic matrix**

κ_{ij} is the **effective order** of the *j*-th reaction with respect to the *i*-th species; if the reaction rates obey power law kinetics then κ_{ij} is independent of *X*s.

For power law kinetics, **the stability** of the current *V*s **is indicated by principal subdeterminants** β_l of order $l = 1, \cdots, n$ of the matrix

$$B = -S(\operatorname{diag} V_s)\kappa^T$$

Principal subdeterminant of order l contains l diagonal elements of *B*; there is $\binom{n}{l}$ such determinants for each l

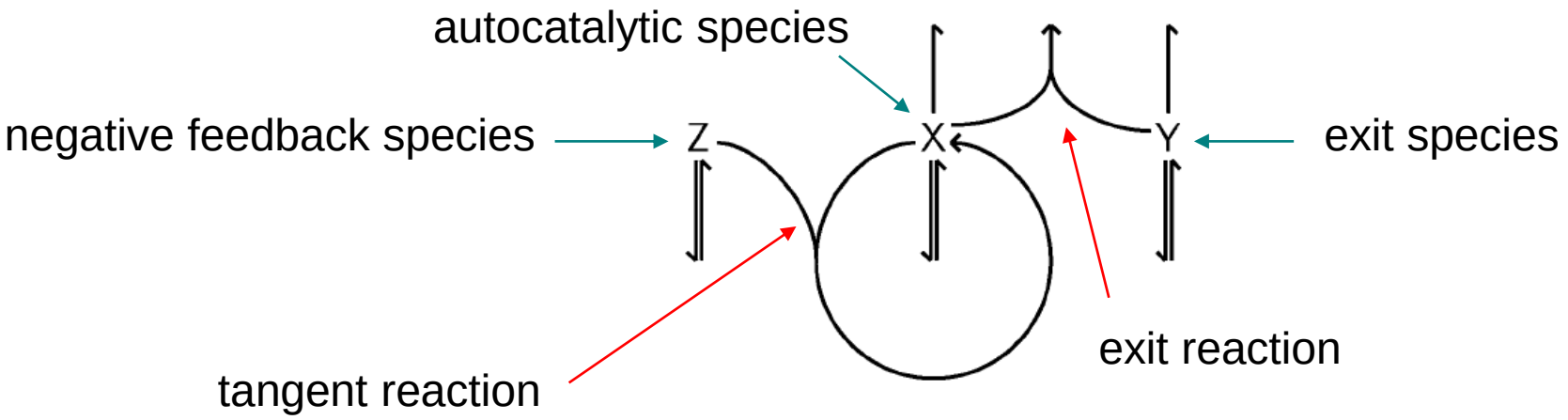
If at least one β_l is negative then at least one eigenvalue of **J is unstable** provided that *l* species associated with β_l are sufficiently small.

Since any current is a linear combination of the extreme currents, the **stability** of the network's steady states **depends on** the stability of its **extreme subnetworks**.

An unstable EC_k induces instability of the entire network if the corresponding α_k is large enough and X_s satisfies the requirement of small concentration of those species for which the corresponding $\beta_l < 0$.

This instability typically involves a **positive feedback loop** associated with **autocatalysis** → convenient graphical identification in the network diagram

The instability arises via: 1) **saddle-node bifurcation** → multiple steady states
2) **Hopf bifurcation** → periodic oscillations (negative feedback required)



Classification of complex oscillatory mechanisms with the use the SNA

M. Eiswirth, A. Freund, J. Ross, Adv. Chem. Phys. 80, 1991
J. Ross, I. Schreiber and M.O. Vlad, Determination of Complex Reaction Mechanisms, Oxford University Press, New York, 2006

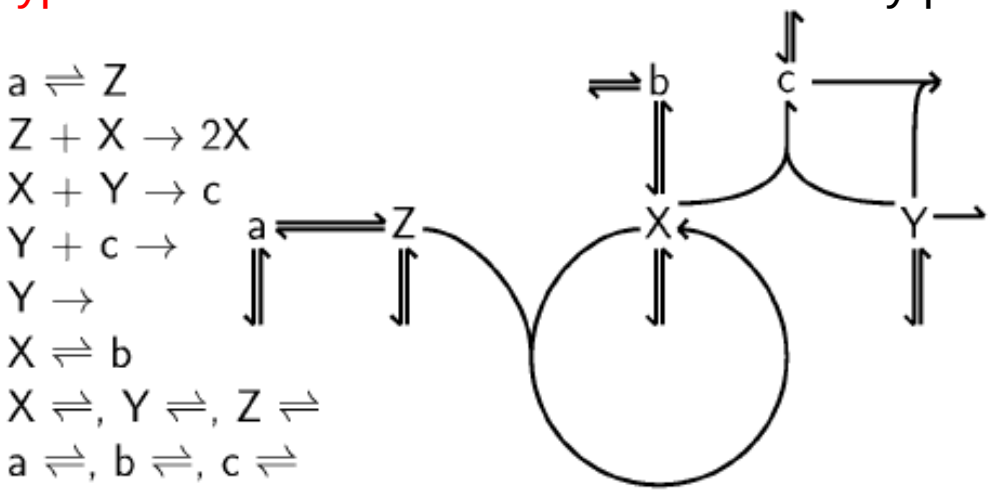
Classification of species:

Essential: must be present for oscillations; if buffered oscillations cease

type X – autocatalytic species – occur on the cycle
type Z – species providing negative feedback
type Y – inhibitory species, take part in the exit reaction
type W – recovery species, products of tangent or exit reactions

Nonessential: can be buffered with no effect on oscillations

type a – reactants with a weak feedback
type b – products with a weak feedback
type c – intermediates not in the oscillatory pathway



Classification of oscillatory reaction mechanisms into categories

Category 1 – order of autocatalysis = order of exit reaction = 1

Category 2 – order of autocatalysis > 1, no exit reaction

category 1B:

$\square + X \xrightarrow{k_1} 2X + 2Z$

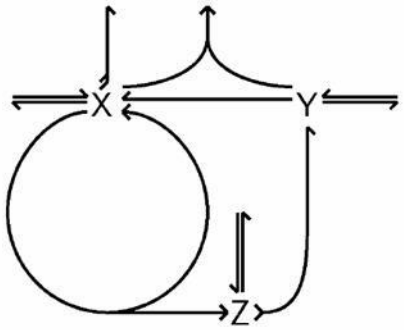
$Z \xrightarrow{k_2} \frac{1}{2}Y$

$Y \xrightarrow{k_3} X$

$X + Y \xrightarrow{k_4}$

$2X \xrightarrow{k_5}$

$X \rightleftharpoons, Y \rightleftharpoons, Z \rightleftharpoons$



category 1CX:

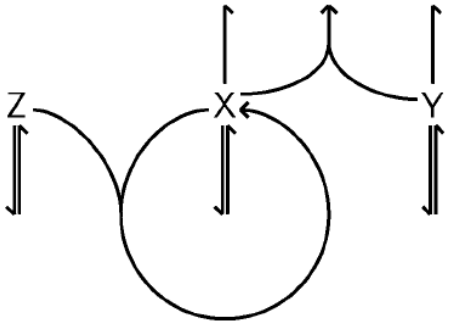
$Z + X \xrightarrow{k_1} 2X$

$X + Y \xrightarrow{k_2}$

$X \xrightarrow{k_3}$

$Y \xrightarrow{k_4}$

$X \rightleftharpoons, Y \rightleftharpoons, Z \rightleftharpoons$



category 1CW:

$Z + X \xrightarrow{k_1} 2X$

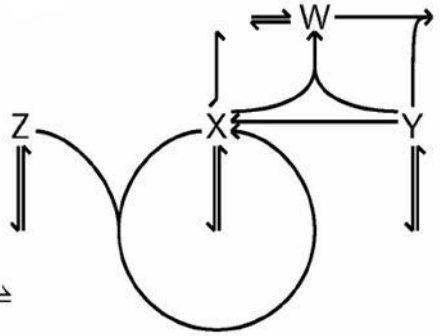
$X + Y \xrightarrow{k_2} 2W$

$Y \xrightarrow{k_3} X$

$Y + W \xrightarrow{k_4}$

$X \xrightarrow{k_5}$

$X \rightleftharpoons, Y \rightleftharpoons, Z \rightleftharpoons, W \rightleftharpoons$



category 2B:

$\square + 2X \xrightarrow{k_1} 3X + Z$

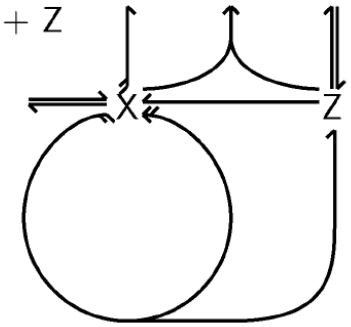
$X + Z \xrightarrow{k_2}$

$Z \xrightarrow{k_3} X$

$2X \xrightarrow{k_4}$

$X \rightleftharpoons$

$Z \rightleftharpoons$



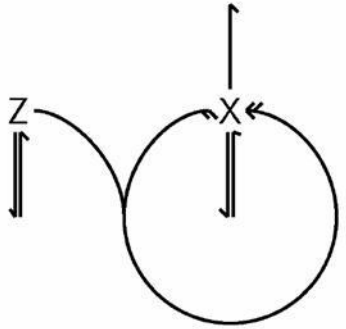
category 2C:

$Z + 2X \xrightarrow{k_1} 3X$

$X \xrightarrow{k_2}$

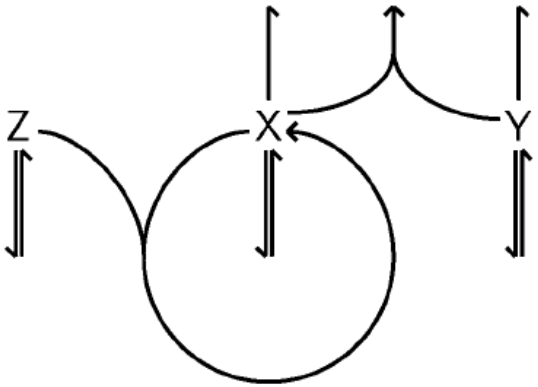
$X \rightleftharpoons$

$Z \rightleftharpoons$



Basic motifs of biochemical bistable and/or oscillatory networks

Oscillatory networks based on cyclic autocatalysis



Less frequent in enzyme reactions, could belong to any category

Examples:

peroxidase-oxidase reaction – oxidation of NADH

single or double back activation – phosphofructokinase in glycolysis

Horse Radish Peroxidase/ Peroxidase-Oxidase

Model A

(Aguda and Clarke, J. Chem. Phys. 87, 1987)

$$H_2O_2 + Per^{3+} \rightarrow coI$$

$$coI + NADH \rightarrow coII + NAD^{\bullet}$$

$$coI + NADH \rightarrow Per^{3+} + NAD^{\bullet}$$

$$coIII + NAD^{\bullet} \rightarrow coI + NAD^{+}$$

$$Per^{3+} + O_2^{\bullet -} \rightarrow coIII$$

$$NAD^{\bullet} + O_2 \rightarrow NAD^{+} + O_2^{\bullet -}$$

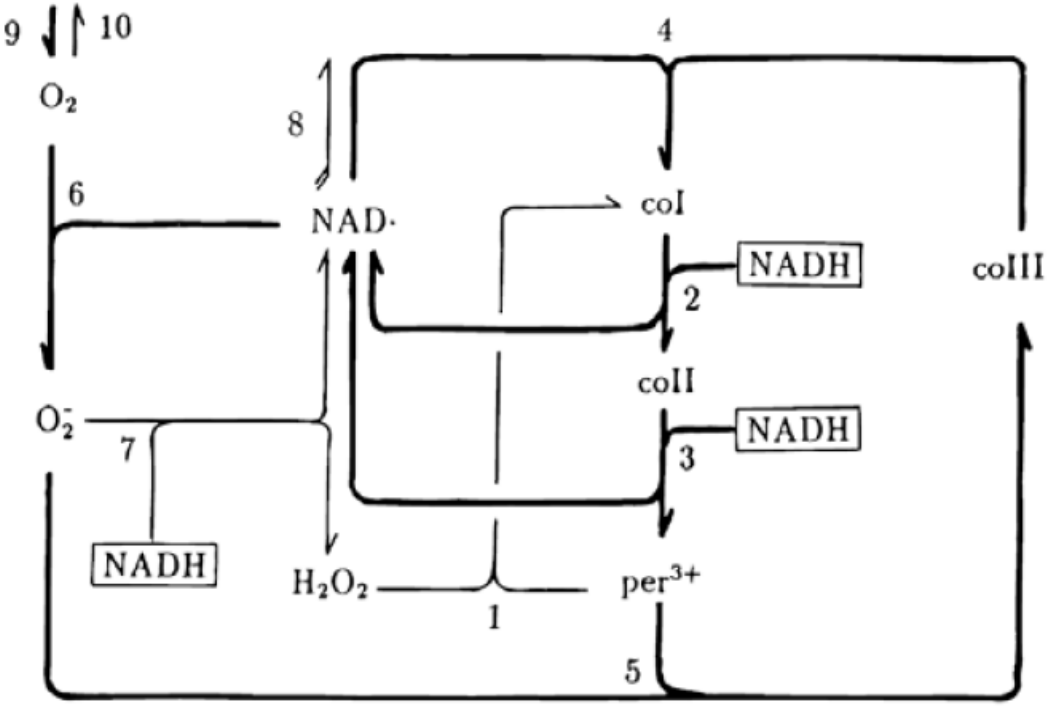
$$NADH + O_2^{\bullet -} + H^{+} \rightarrow H_2O_2 + NAD^{\bullet}$$

$$2\,NAD^{\bullet} \rightarrow (NAD)_2$$

$$\rightarrow O_2$$

$$O_2 \rightarrow$$

network diagram



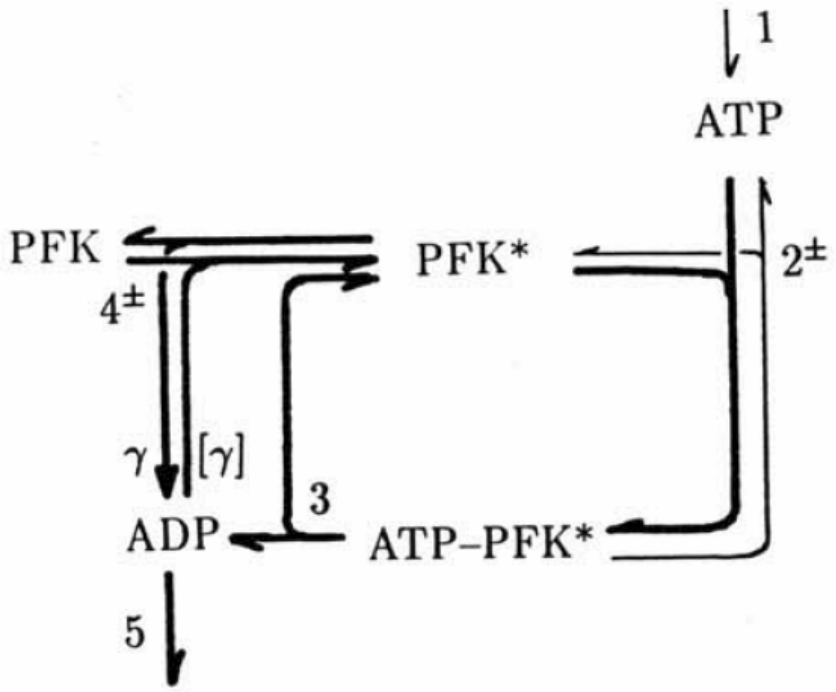
Conclusions based on the model:

Hung, *Schreiber*, *Ross*. J. Phys. Chem, **99**, (1995)

1) mechanism belongs to category 1CW
2) coIII is of type Z
3) O_2 is of type Y
4) coI, coII and $NAD^{\bullet}$ are of type X
5) $O_2^{\bullet -}$ is of type W
6) model is consistent with experiments

Glycolysis

Selkov model – higher order product activation of the enzyme phospho-fructo-kinase PFK by ADP category 2C oscillator

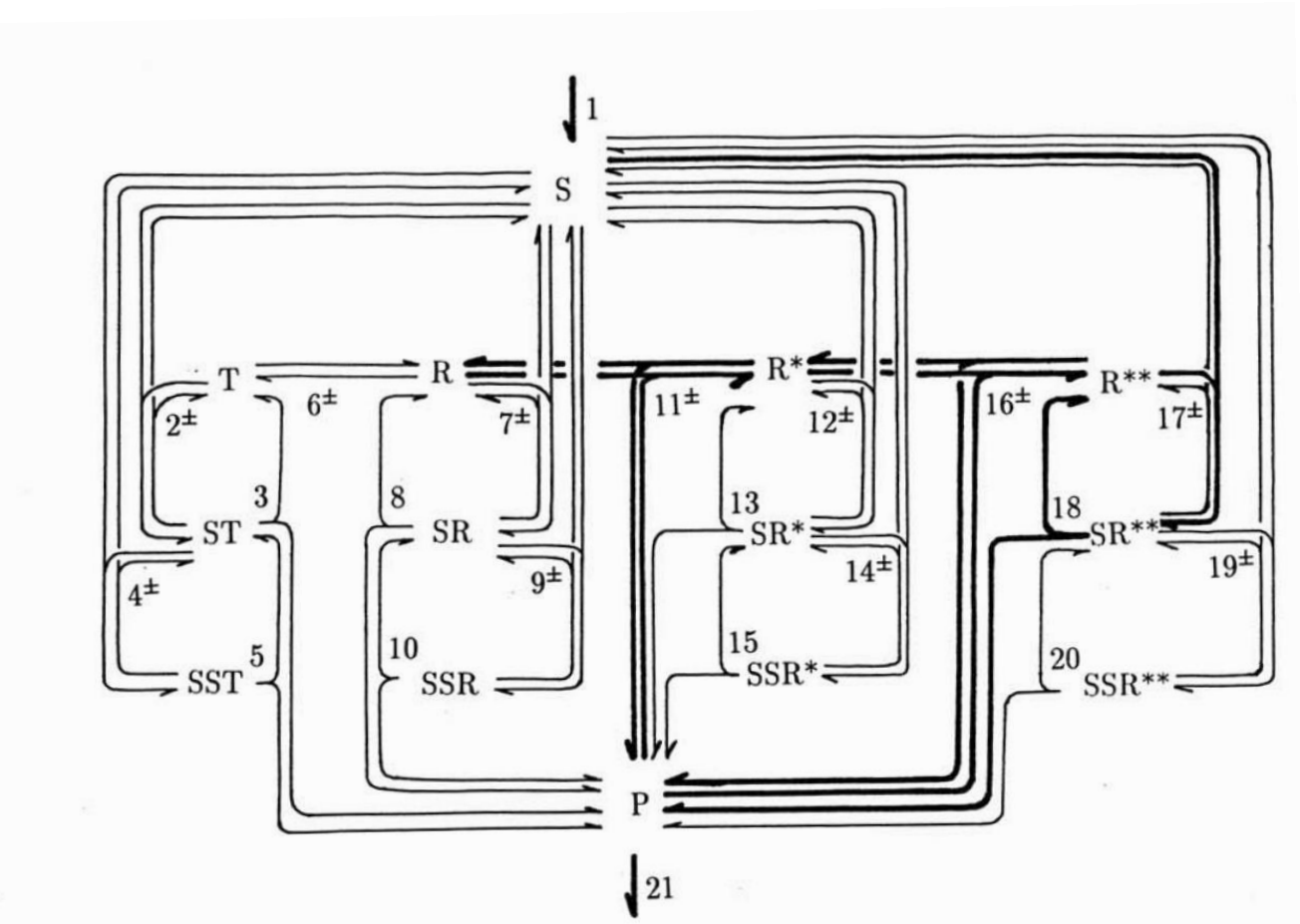


γ - effective order > 1
typically $\gamma = 2$

bistability or oscillations are implied with a first order decomposition of ADP
no exit reaction needed in the removal of ADP (category 2C)

Glycolysis

Allosteric detailed model – double product activation of PFK by ADP
category 2C oscillator



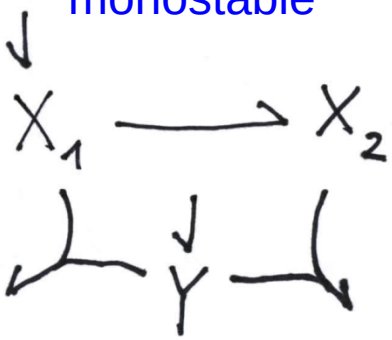
S – ATP
P – ADP
T – inactive enzyme form
R – active enzyme form

Oscillatory networks based on competitive autocatalysis : prevailing case in biochemical/enzyme systems

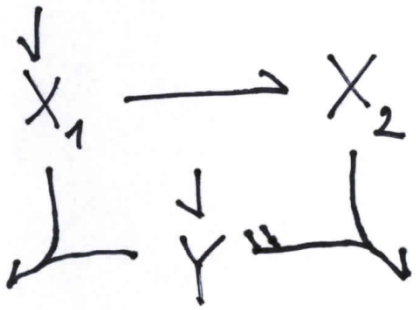
Characteristic topological feature

Second order competitive autocatalysis

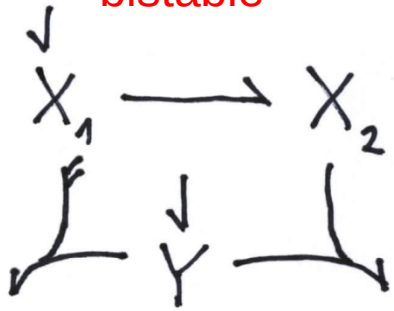
monostable



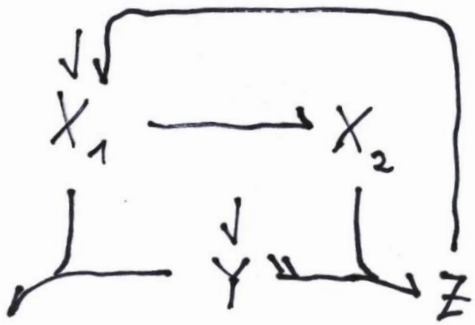
bistable



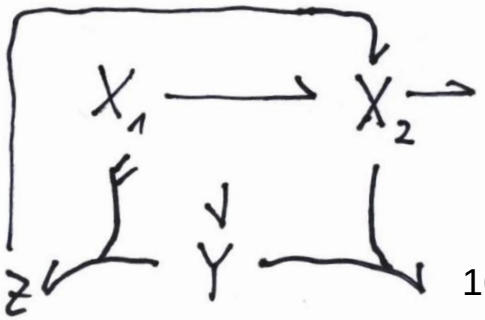
bistable



oscillatory



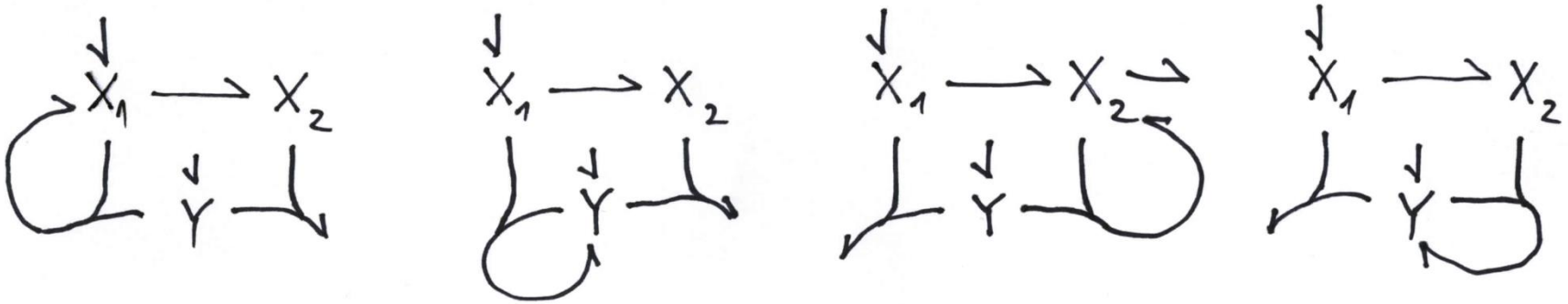
oscillatory



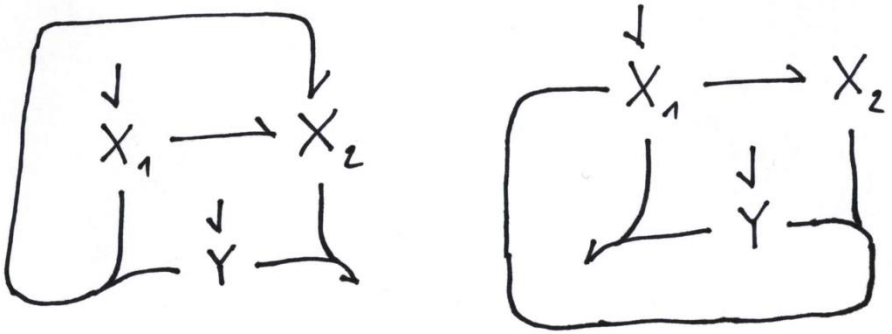
Basic motifs of biochemical bistable and/or oscillatory networks

First order competitive autocatalysis

Networks with a self-regeneration



Networks with a complementary generation

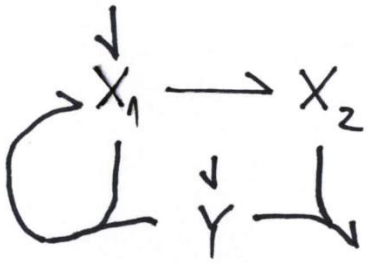


Basic motifs of biochemical bistable and/or oscillatory networks

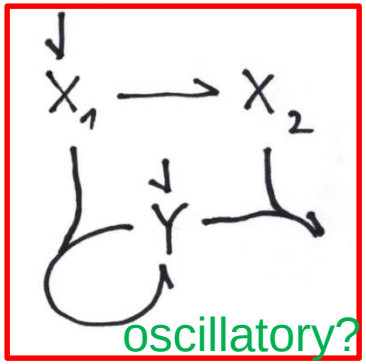
First order competitive autocatalysis

Networks with a single-species loop

monostable

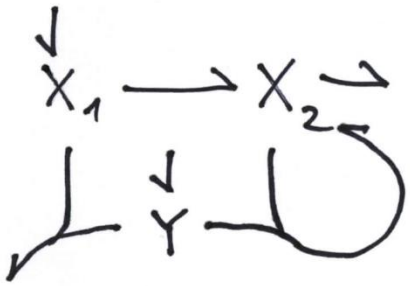


bistable

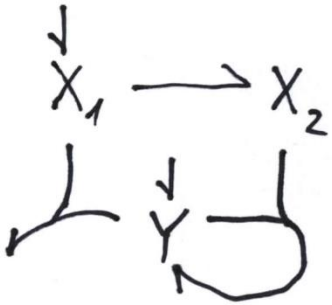


oscillatory?

monostable

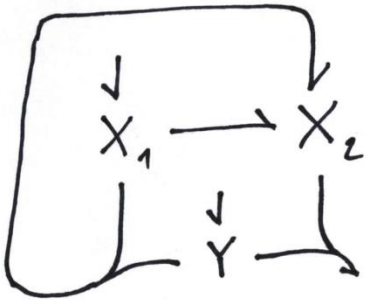


monostable

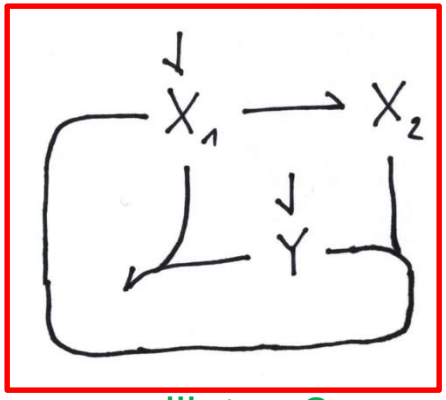


Networks with a two-species loop

monostable

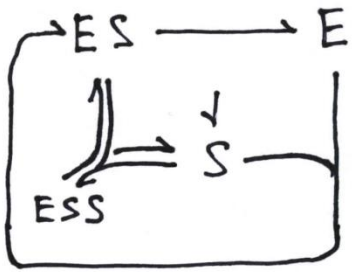


bistable



oscillatory?

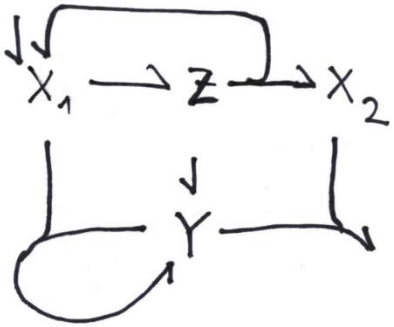
enzyme version –
substrate inhibition



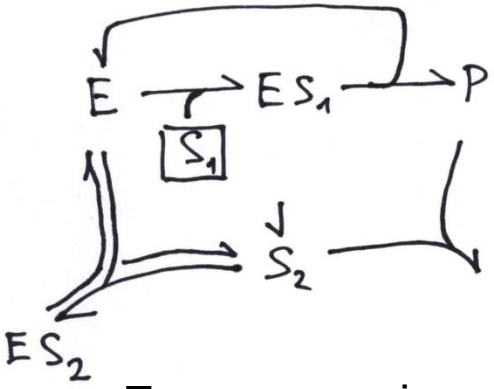
Basic motifs of biochemical bistable and/or oscillatory networks

Oscillatory networks

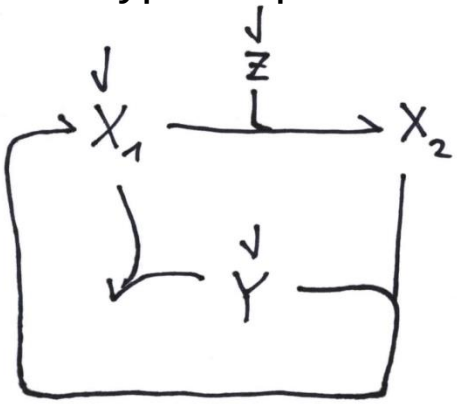
Internal production
of type Z species



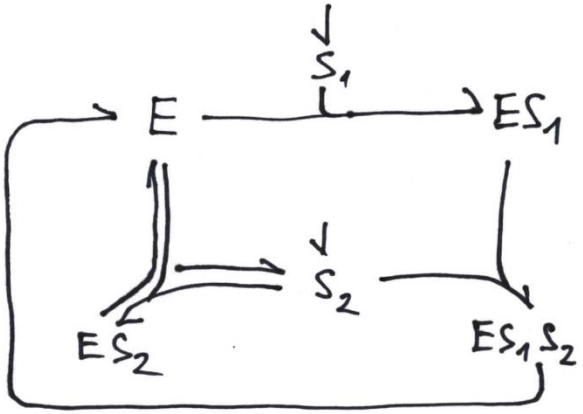
Enzyme version
with reversible recovery of S_2



Flow-limited supply
of type Z species



Enzyme version
with reversible substrate inhibition

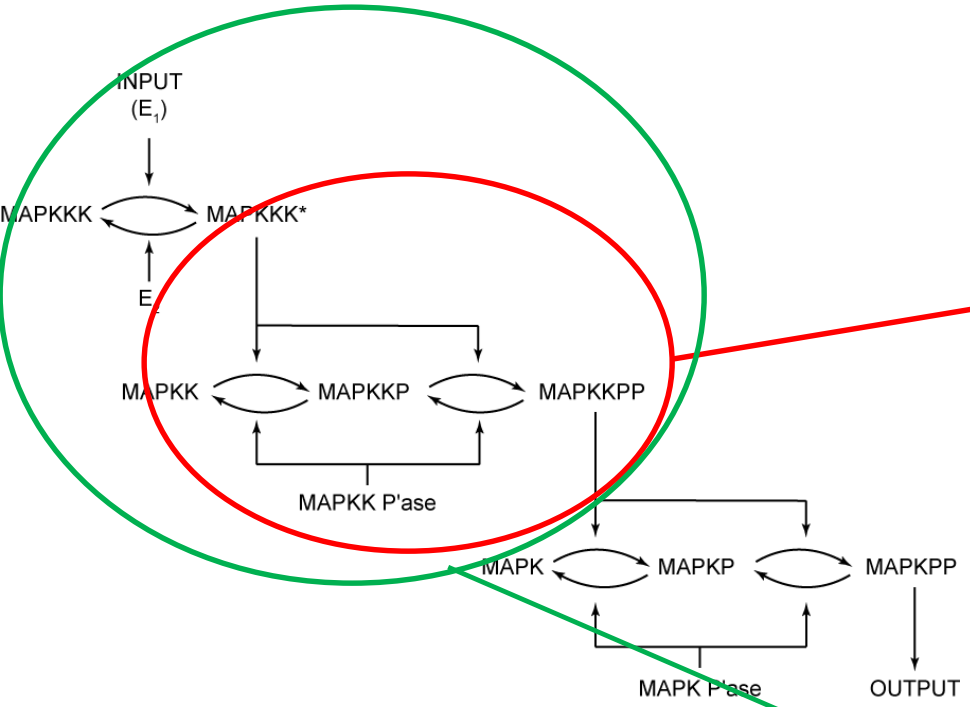


Mitogen Activated Protein Kinases (MAPKs)

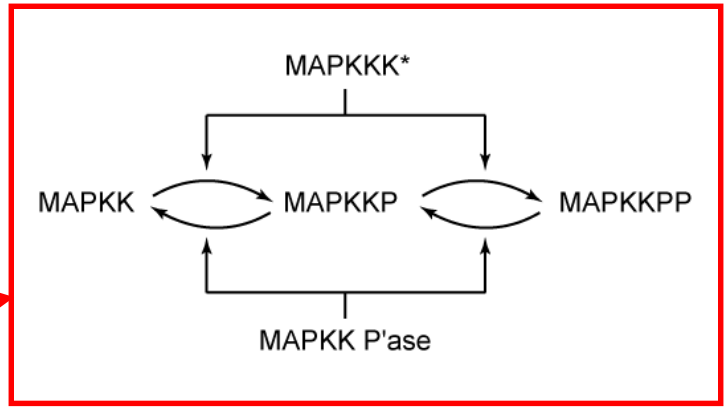
Three-stage phosphorylation/dephosphorylation of enzymes, transcription factors, proteins

Huang-Ferrell model of MAPK cascade

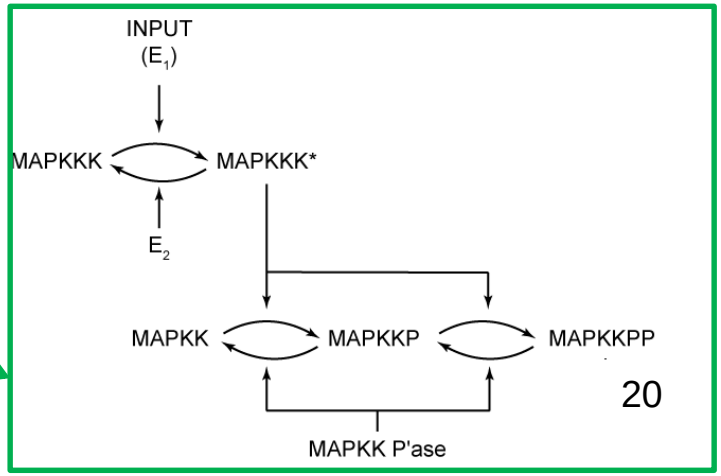
Huang & Ferrell, PNAS **93**, 19, 1996



Bistable subsystem



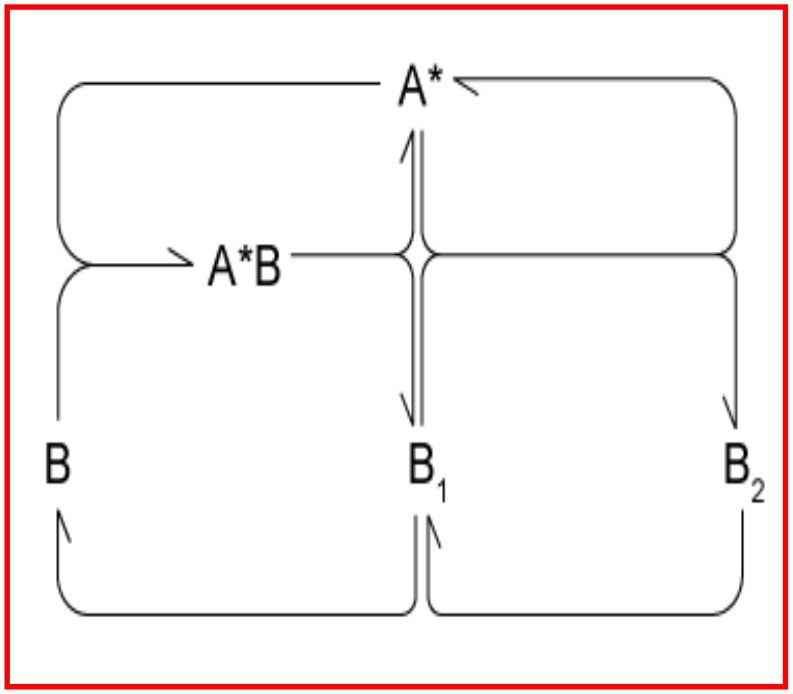
Oscillatory subsystem



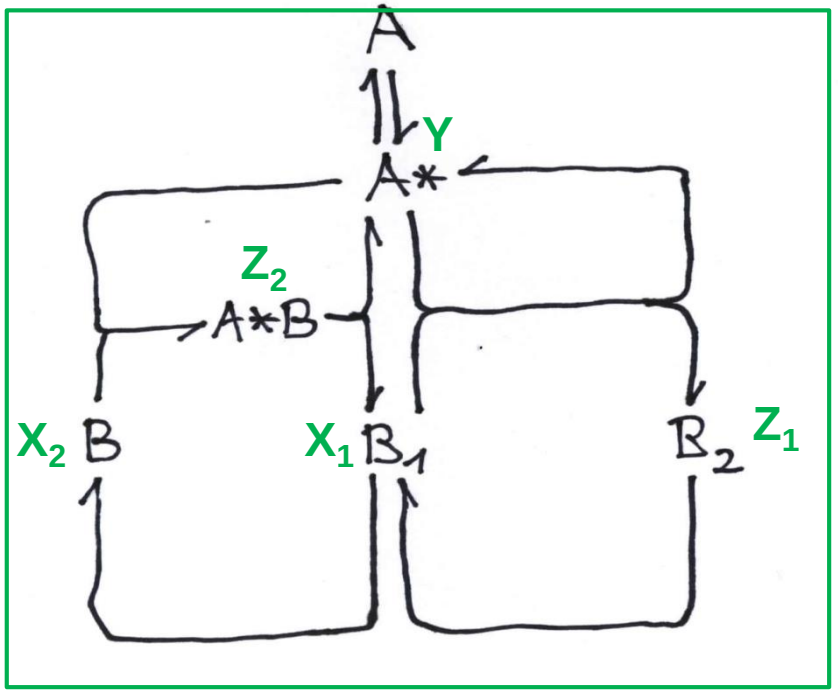
Minimal bistable/oscillatory MAPK network

Hadac, Nevoral, Pribyl, Schreiber, *PLoS ONE* **12(6)**: e0178457 (2017)

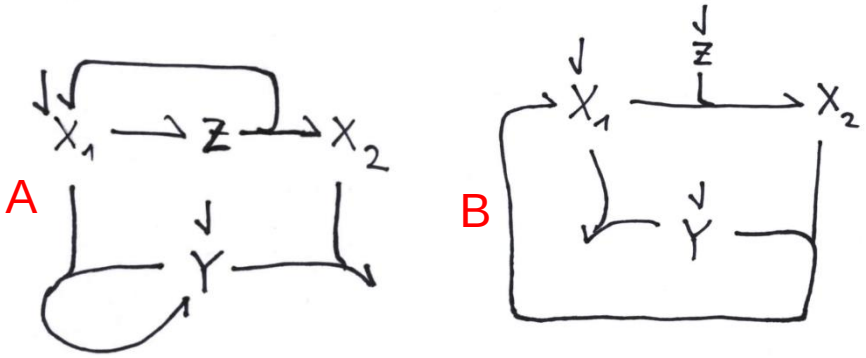
bistable



oscillatory **a distinct motif**

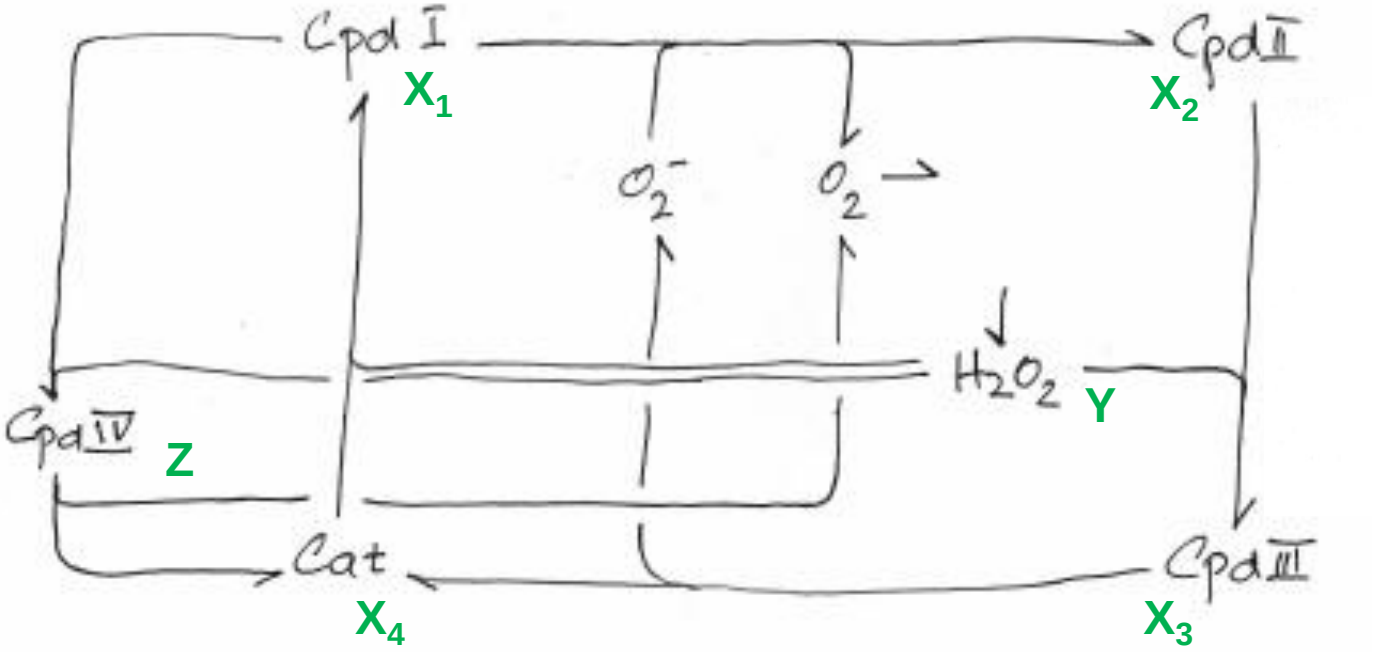


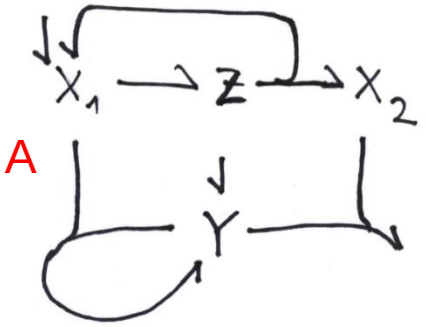
Compared to previous two motifs:



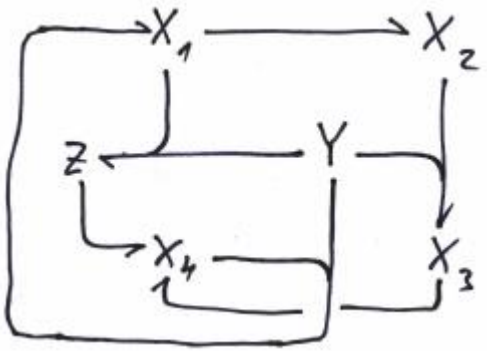
Catalase – hydrogen peroxide reaction

Membrane reactor: *T. Hideshima and T. Inoue, Biophys. Chem. (1997), S. Sasaki et al., Electroanalysis (2004), Cip, Schreiberova, Schreiber, Russ. J. Phys. Chem. (2011), Muzika, Jurasek ,Schreiberova, Schreiber, J Phys Chem A **121**, (2017)*



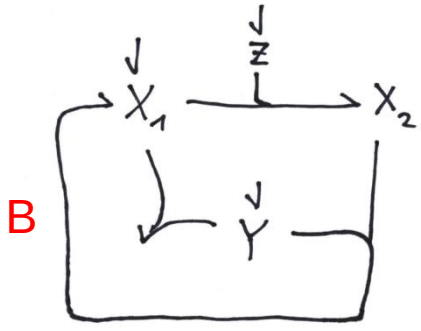


Basic motif:



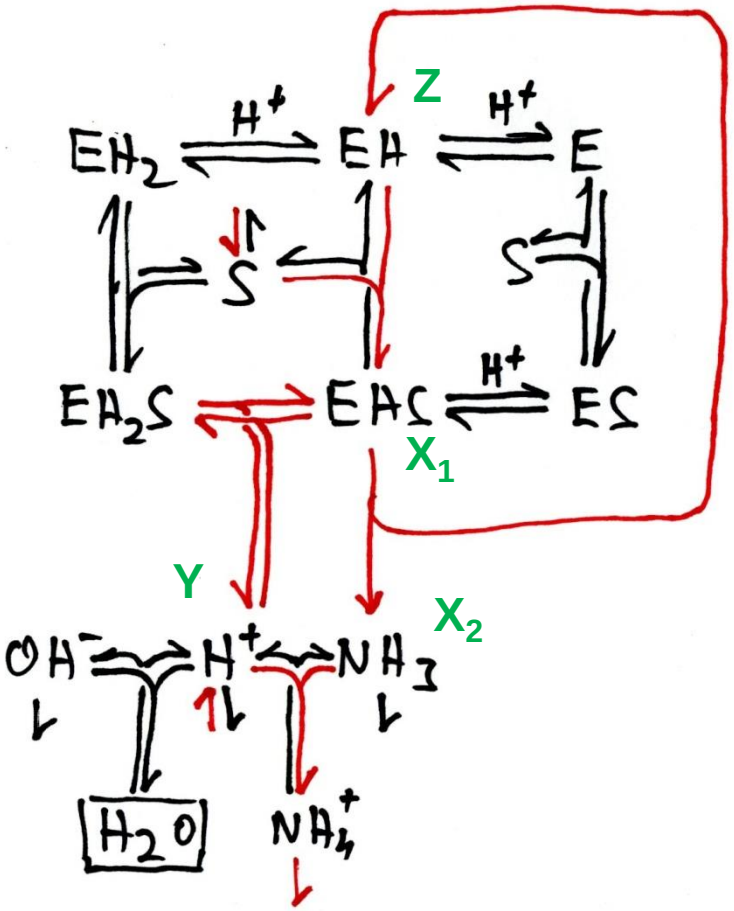
Related to B but Z is internally produced plus there are three reactions competing for Y

→ a distinct motif

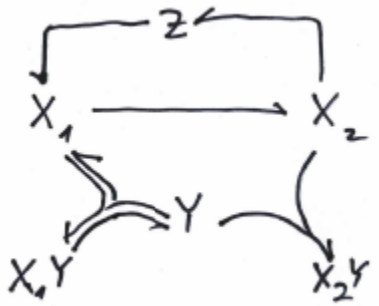


Urea-urease reaction

Bistability - Hu et al., J. Phys. Chem., 2010, Muzika et al. Phys. Chem. Commun., 2014
Oscillations - Muzika, Ruzicka, Schreiberova, Schreiber, Phys. Chem. Chem. Phys. 2019

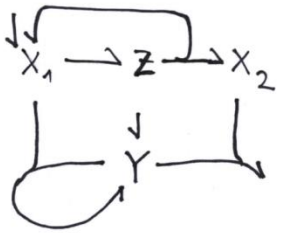


Basic motif:

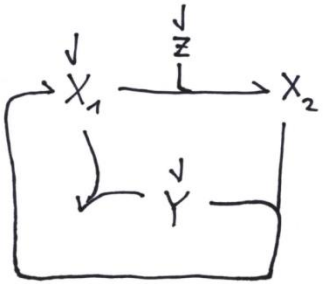


Compared to previous two motifs:

A



B



Constrained stoichiometric analysis – parameter estimation

Radojkovic, Schreiber, Phys. Chem. Chem. Phys, 2018

The dimensional evolution equations based on mass balances in a stirred reactor:

$$\frac{d\mathbf{X}}{dt} = \mathbf{S}\mathbf{v}(\mathbf{X})$$

can be transformed to a dimensionless form using a simple scaling

$$x_i = \frac{X_i}{X_{scl}}, \quad \tau = \frac{t}{T_{scl}} \quad \text{and} \quad \mathrm{v}_i = \frac{V_i}{V_{scl}}, \qquad \text{where} \qquad V_{scl} = X_{scl} / T_{scl}$$

$$\frac{d\mathbf{x}}{d\tau} = \mathbf{N}\mathbf{v}(\mathbf{x}), \qquad \mathrm{x} = (x_1, \cdots, x_n), \quad \mathrm{v} = (\mathrm{v}_1, \cdots, \mathrm{v}_m), \qquad \mathbf{N} \quad \text{is} \quad (n \times m)$$

The reaction rates are assumed to obey mass action kinetics

$$\mathrm{v}_j(\mathbf{x}) = k_j \prod_{i=1}^{n} x_i^{\kappa_{ij}} = k_j \, \bar{\mathrm{v}}_j, \; \kappa_{ij} = \frac{\partial \ln \mathrm{v}_j}{\partial \ln x_i} \quad \text{is reaction order}$$

k_j is rate coefficient $\qquad$ $\bar{\mathrm{v}}_j$ is reduced reaction rate

In vector notation: $\mathbf{v}(\mathbf{x}) = diag(\mathbf{k})\bar{\mathbf{v}}(\mathbf{x}), \quad \mathbf{k} = (k_1, \cdots, k_m)$

,

Constrained stoichiometric analysis – parameter estimation

The steady state rate vector is confined to the right null space of **N**

$$\mathbf{N}\mathbf{v}(\mathbf{x}) = 0$$

The set of elementary subnetworks (or extreme currents) are edges of the reaction rate cone are arranged as columns of the matrix

$$\mathbf{E} = (\mathbf{E}_1, \cdots, \mathbf{E}_f)$$

Any steady state reaction rate vector is a non-negative linear combination

$$\mathbf{v}_s = \mathbf{E}\boldsymbol{\alpha}, \quad \boldsymbol{\alpha} = (\alpha_1, \cdots, \alpha_f), \quad \alpha_k \geq 0$$

The evolution equations can be rewritten in terms of convex parameters $\boldsymbol{\alpha}$ and $\mathbf{x}_s$

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}) = \mathbf{N}\mathbf{v}(\mathbf{x}) = \mathbf{N}diag(\mathbf{k})\bar{\mathbf{v}}(\mathbf{x}) = \mathbf{N}diag(\mathbf{E}\boldsymbol{\alpha})(diag\,\bar{\mathbf{v}}(\mathbf{x}_s))^{-1}\bar{\mathbf{v}}(\mathbf{x})$$

Jacobian at $\mathbf{x}_s$ is

$$\mathbf{J} = \left.\frac{d\mathbf{f}}{d\mathbf{x}}\right|_{\mathbf{x}=\mathbf{x}_s} = \mathbf{N}\left.\frac{d\mathbf{v}}{d\mathbf{x}}\right|_{\mathbf{x}=\mathbf{x}_s} = \mathbf{N}(\text{diag}\ \mathbf{v}_s)\mathbf{K}^T(\text{diag}\ \mathbf{x}_s)^{-1} = -\mathbf{V}(\text{diag}\ \mathbf{x}_s)^{-1} = -\mathbf{V}(\text{diag}\ \mathbf{h})$$

($\mathrm{K} = \{\kappa_{ij}\}$ is kinetic matrix)

,

Constrained stoichiometric analysis – parameter estimation

Constraint equations: a suitable subset of $\mathbf{E}\boldsymbol{\alpha} = \mathbf{v}_s$ for which we know the reaction rates from known k_i's and x_i's determined experimentally or known from literature. As a reference point we use conditions at a Hopf bifucation.

Procedure toward constructing a set of constraint equations:

a) Analyze principal minors of V for each edge/face of the cone and identify unstable subnetworks

b) Determine unstable dominant subnetwork leading to a Hopf bifurcation (with the help of Routh-Hurwitz criterion if necessary)

c) Introduce:

$$\boldsymbol{\alpha} = \left(\boldsymbol{\alpha}^{(uds)}, \boldsymbol{\alpha}^{(uv)}\right)$$

$$\mathbf{x} = \left(\mathbf{x}^{(fv)}, \mathbf{x}^{(uv)}, \mathbf{x}^{(iv)}\right)$$

$$\mathbf{k} = \left(\mathbf{k}^{(fv)}, \mathbf{k}^{(uv)}, \mathbf{k}^{(iv)}\right)$$

fv= fixed/known value, uv=unknown value, iv=indeterminate value,
uds = unstable dominant subnetwork – a bifurcation parameter

d) Given the known values $\mathbf{k}^{(fv)}$ and $\mathbf{x}^{(fv)}$ choose a subset $\mathbf{v}^{(fv)}$ of $\mathbf{v}_s$ which is known and a subset $\mathbf{v}^{(uv)}$ where $\mathbf{k}^{(uv)}$ and/or $\mathbf{x}^{(uv)}$ are linear unknowns

,

Constrained stoichiometric analysis – parameter estimation

By leaving out irrelevant rows the reduced matrix **E** becomes $\hat{\mathbf{E}} = \left[\hat{\mathbf{E}}^{(uds)}, \hat{\mathbf{E}}^{(uv)}\right]$

and by rearranging $\mathbf{E}\boldsymbol{\alpha} = \mathbf{v}_s$ the constraint equations are:

$$\begin{bmatrix} & 0 & 0 \\ \hat{\mathbf{E}}^{(\mathbf{uv})} & \mathbf{A} & 0 \\ & 0 & \mathbf{B} \end{bmatrix} \begin{bmatrix} \boldsymbol{\alpha}^{(\mathbf{uv})} \\ \mathbf{x}^{(\mathbf{uv})} \\ \mathbf{k}^{(\mathbf{uv})} \end{bmatrix} = \begin{bmatrix} \mathbf{v}^{(\mathbf{fv})} \\ 0 \\ 0 \end{bmatrix} - \hat{\mathbf{E}}^{(\mathbf{uds})}\boldsymbol{\alpha}^{(\mathbf{uds})}$$

where the matrix **A** involves known parts of rates where $\mathbf{x}^{(uv)}$ is linear uknown and **B** involves reduced rates for steps where $\mathbf{k}^{(uv)}$ is (linear) unknown

,

Constrained stoichiometric analysis – parameter estimation

In many reactions, especially those with immobilized enzymes, there are concentration constraints:

$$\mathbf{C}\mathbf{x} = \mathbf{x}^{tot}$$

there are *n* - *d* such equations, where *n* is the number of species and *d* is the rank of **N**

The matrix **C** can be determined as a set of edges (arranged in rows) spanning the non-negative cone in left null space of **N**: $0 = \mathbf{C}\mathbf{N}$

The relevant subset of concentration equations for which $\mathbf{x}^{tot}$ is known is:

$$\hat{\mathbf{C}}^{(fv)}\mathbf{x}^{fv} + \hat{\mathbf{C}}^{(uv)}\mathbf{x}^{uv} = \hat{\mathbf{x}}^{tot}$$

Upon adding the conservation constraint to stoichiometric constraints:

$$\begin{bmatrix} & 0 & 0 \\ \hat{\mathbf{E}}^{(uv)} & \mathbf{A} & 0 \\ & 0 & \mathbf{B} \\ 0 & \hat{\mathbf{C}}^{uv} & 0 \end{bmatrix} \begin{bmatrix} \boldsymbol{\alpha}^{(uv)} \\ \mathbf{x}^{(uv)} \\ \mathbf{k}^{(uv)} \end{bmatrix} = \begin{bmatrix} \mathbf{v}^{(fv)} \\ 0 \\ 0 \\ \hat{\mathbf{x}}^{tot} \end{bmatrix} - \begin{bmatrix} \hat{\mathbf{E}}^{(uds)} & \boldsymbol{\alpha}^{(uds)} \\ \hat{\mathbf{C}}^{(fv)} & \mathbf{x}^{(fv)} \end{bmatrix}$$

,

Constrained stoichiometric analysis – parameter estimation

Method of solving the constraint equations:

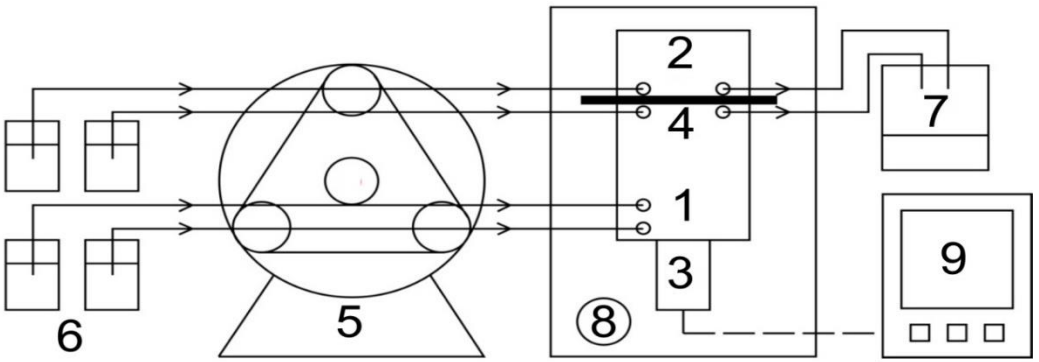
- Number of constraint equations is typically significantly lower than the number of unknowns because the number *f* of the involved edges (and therefore number of unknown α_k's) is much higher than the number of equations for which the reaction rate is given
- Consequently the constraint equations are underdetermined and do not have a unique solution
- The problem is solved by finding an optimal solution via linear programming - criterion for selecting a unique solution is based on proximity of the dominant subnetwork for the Hopf bifurcation, which is achieved by minimizing the function

$$f\left(\boldsymbol{\alpha}^{(\mathrm{uv})}, \mathbf{x}^{(\mathrm{uv})}, \mathbf{k}^{(\mathrm{uv})}\right) = \sum_{k=1}^{\dim(\boldsymbol{\alpha}^{uv})} \alpha_k^{(uv)}$$

- For a chosen $\boldsymbol{\alpha}^{(udc)}$ the linear programming problem is solved and eigenvalues of the Jacobian are determined. As $\boldsymbol{\alpha}^{(udc)}$ is increased, the coupling of the unstable dominant subnetwork becomes stronger until a Hopf bifurcation occurs and the set of rate coefficients $\mathbf{k}^{(uv)}$ determined at this point is taken as the parameter estimate.

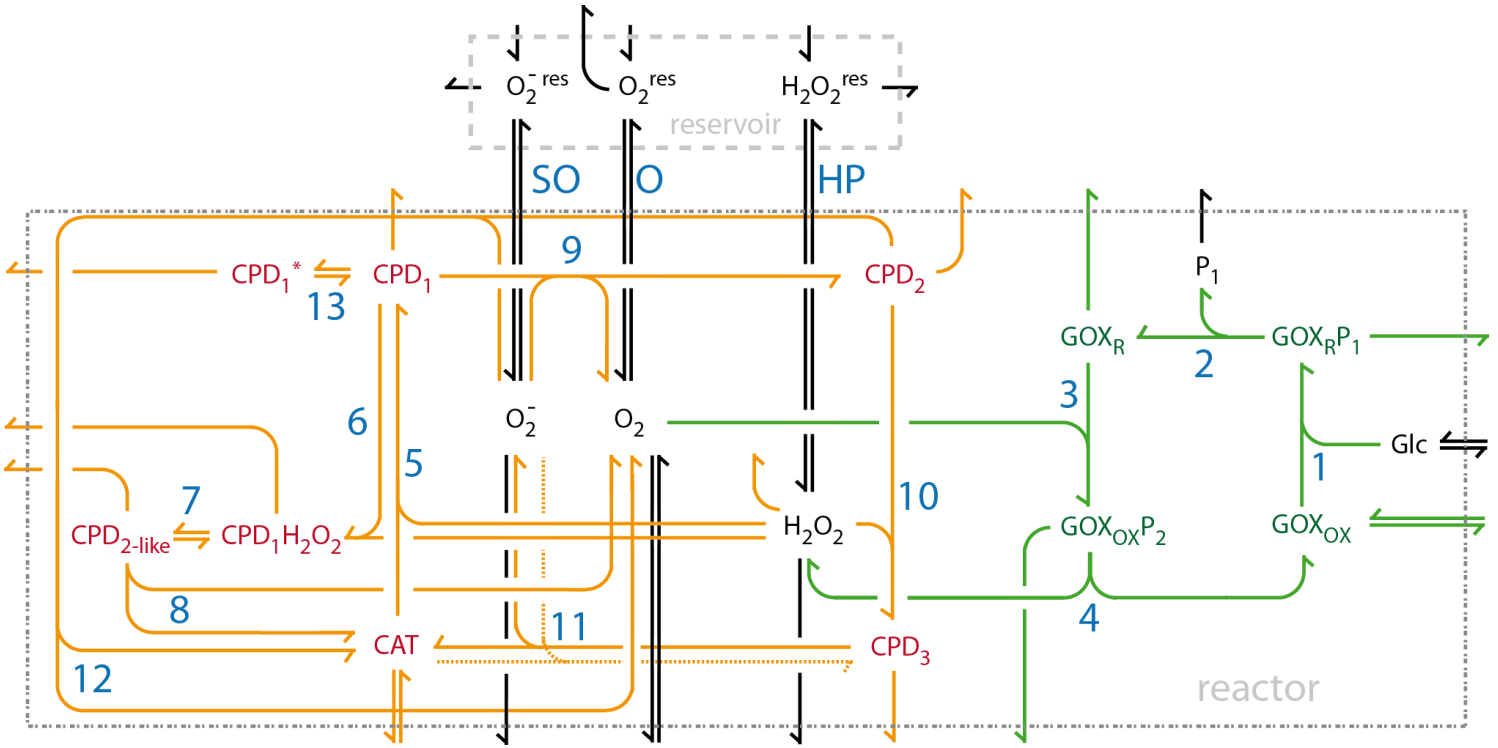
Parameter estimation: Application to catalase – glucose-oxidase reaction

*Muzika, Jurasek ,Schreiberova, Schreiber, J Phys Chem A **121**, (2017)*



Experimental setup:
1 – reactor
2 – reservoir
3 – DO probe
4 – membrane
5 – peristaltic pump
6 – stock solutions
7 – waste
8 – magnetic stirrer
9 – HI 5421 DO-meter

Reaction network diagram corresponding to the experimental setup



list of species :

1 Glc
2 GODox
3 GODrP1
4 GODr
5 GODoxP2
6 H2O2
7 O2
8 O2-
9 CAT
10 CpdI
11 CpdIH202
12 CpdII
13 CpdIII
14 O2res
15 H2O2res
16 CpdI*
17 O2-res
18 Cpl2like

Red – unknown concentrations of species or rate coefficients

Blue – known/measured values of concentrations or rate coefficients (including flow rate, membrane permeabilities)

Green – estimated values of concnetrations → educated guess guided by the SNA

list of (pseudo) reactions :

1 Glc + GODox --> GODrP1 — gox reactions
2 GODrP1 --> GODr
3 GODr + O2 --> GODoxP2
4 GODoxP2 --> GODox + H2O2

5 H2O2 + CAT --> CpdI — cat reactions
6 CpdI + H2O2 --> CpdIH2O2
7 Cpd2like --> O2 + CAT
8 CpdI + O2- --> CpdII + O2
9 CpdII + H2O2 --> CpdIII
10 CpdIII --> CAT + O2-
11 CpdII + O2- + 2H+ --> CAT + O2 + H2O
28 O2- + CAT --> CpdIII
29 CpdI --> CpdI*
30 CpdI* --> CpdI
33 CpdIH2O2 --> Cpd2like
34 Cpd2like --> CpdIH2O2

12 --> Glc 13 Glc --> — reactor in/out
14 --> O2 15 O2 -->
16 H2O2 -->
17 O2- -->

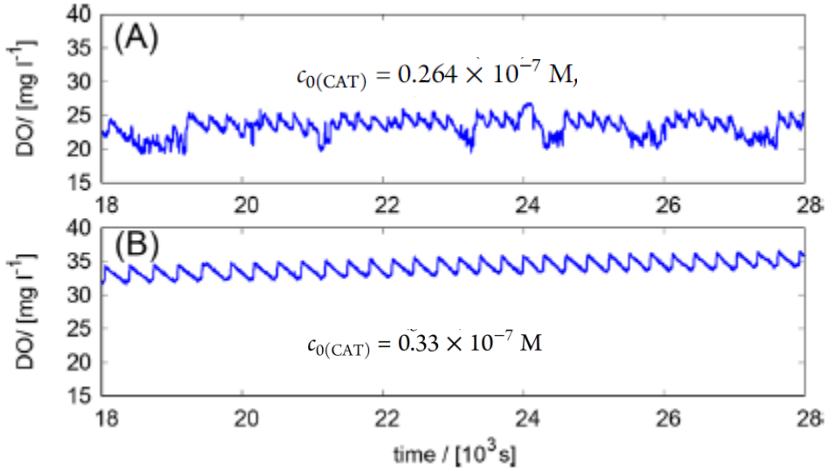
18 --> eps2*O2res 19 eps2*O2res --> — reservoir in/out
20 --> eps2*H2O2res 21 eps2*H2O2res -->
31 --> eps2*O2-res 32 eps2*O2-res -->

22 1/eps1*H2O2res --> H2O2 — exchange across membrane
23 H2O2 --> 1/eps1* H2O2res
24 1/eps1*O2res --> O2
25 O2 --> 1/eps1*O2res
26 1/eps1*O2-res --> O2-
27 O2- --> 1/eps1*O2-res

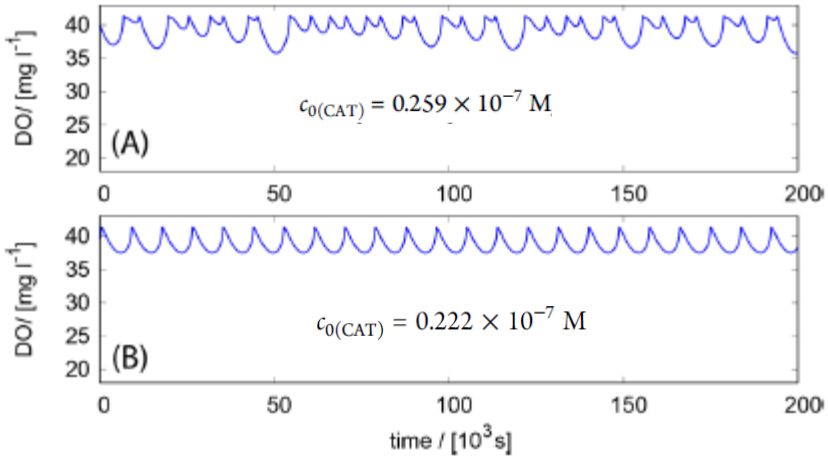
Experimental recording of dissolved oxygen concentration in the reactor vs simulation

$c_{0(CAT)} = 0.66\times10^{-7}$ M $c_{0(GOX)} = 3.9\times10^{-6}$ M $c_{0(glucose)} = 2\times10^{-3}$ M $c_{0(H_2O_2)} = 0.667$ M $\mathrm{pH}_{\mathrm{buffer}} = 5.8$ $k_0 = 1.12\times10^{-4}$ s^{-1}

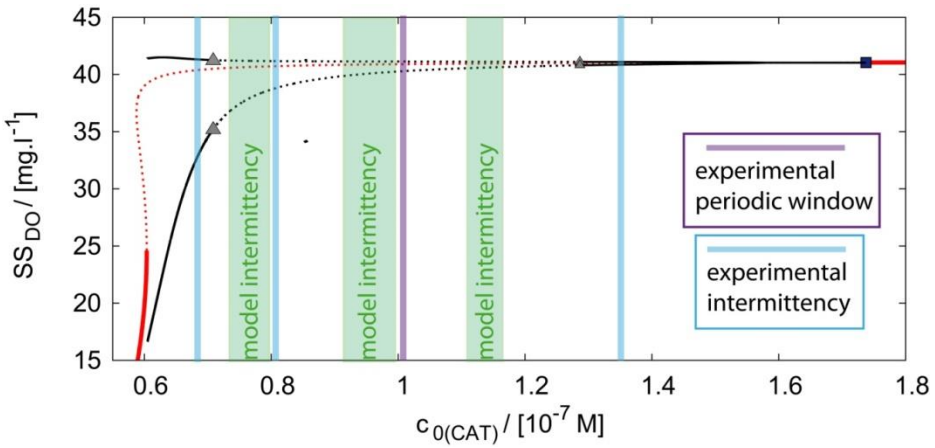
Measured dynamics



Simulated dynamics



Bifurcation diagram calculated from the model superimposed with experimental observations.
curves: DO vs inlet concentration of catalase,
red curve – steady state, black curve – minima and maxima of oscillations,
full line – stable, dashed line – unstable, square – Hopf bifurcation point, triangle – period doubling point.



Transformation of a non-power-law network into a power-law network: predator –prey system in flow stirred bioreactor/chemostat

$$\frac{dS}{dt} = D(S_0 - S) - \frac{1}{Y_{S/X_B}} \frac{\mu_S S}{K_s + S} X_B$$

$$\frac{dX_B}{dt} = \frac{\mu_S S}{K_s + S} X_B - \frac{1}{Y_{X_B/X_P}} \frac{\mu_B X_B}{K_B + X_B} X_P - DS$$

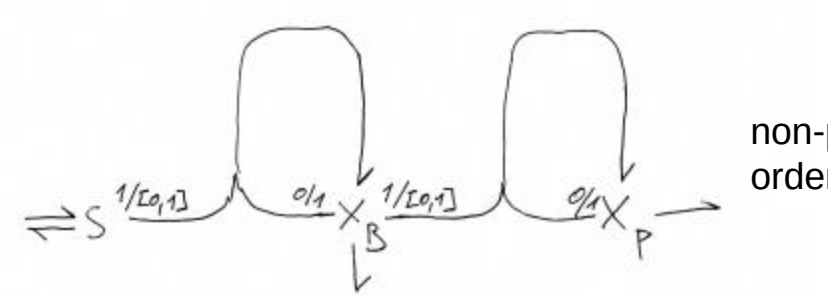
$$\frac{dX_P}{dt} = \frac{\mu_B X_B}{K_B + X_B} X_P - DP$$

Ali et al., Ecological Modelling 259 (2013) 10– 15

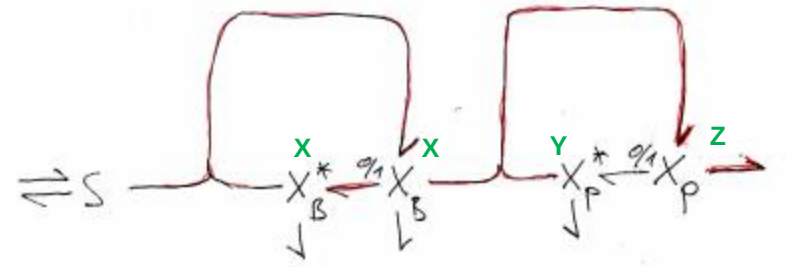
S substrate

X_B population of bacteria (e.g. Azotobacter)

X_P population of predator (e.g. protozoans)



non-power-law, variable effective reaction order of S, difficult to analyze by SNA



power law
red – unstable subnetwork
(autocatalytic cycle + exit reaction)

Carbon-nitrogen metabolism in cyanobacteria in chemostat
(under constant light, decoupled from circadian clock)

Grimaud et al., Ecological Modelling 291 (2014) 121– 133

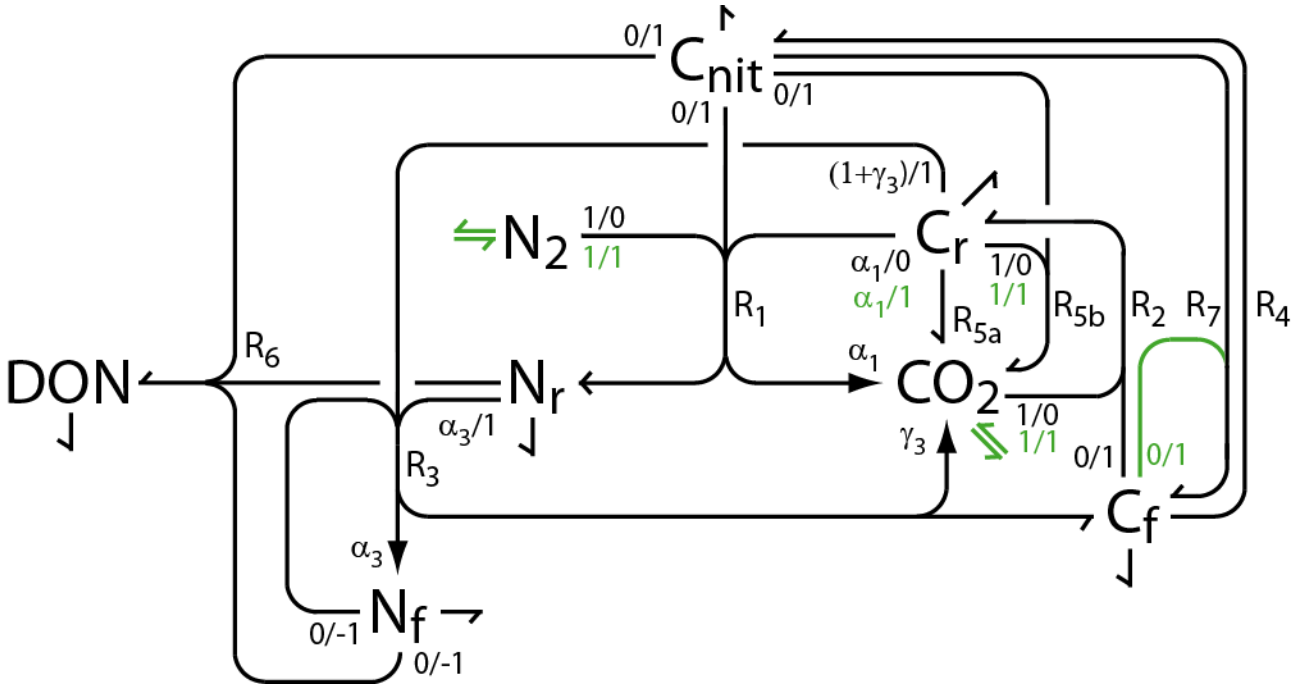
$$\dot{C}_r = r_2 \frac{\bar{I}(t)}{\bar{I}(t) + K_I + (\bar{I}^2/K_{il})} C_f - \left[(r_3 + \gamma_3)\frac{N_r}{N_f} + r_5 + D \right] C_r - \alpha_1 r_1 C_{nit} - \lambda_5 \frac{\rho_2}{C_f} C_{nit}$$

$$\dot{C}_f = r_3 C_r \frac{N_r}{N_f} - (r_4\phi(t) + D)C_f + r_7 C_{nit}$$

$$\dot{N}_r = r_1 C_{nit} - \left(\alpha_3 r_3 \frac{C_r}{N_f} + \lambda_6 \frac{\rho_1}{N_f} + D \right) N_r$$

$$\dot{C}_{nit} = r_4\phi(t) C_f - (r_7 + D) C_{nit}$$

C_f	Functional carbon pool
C_r	Carbohydrates pool
C_{nit}	Nitrogenase pool
N_r	Nitrogen storage pool
N_f	Functional nitrogen pool



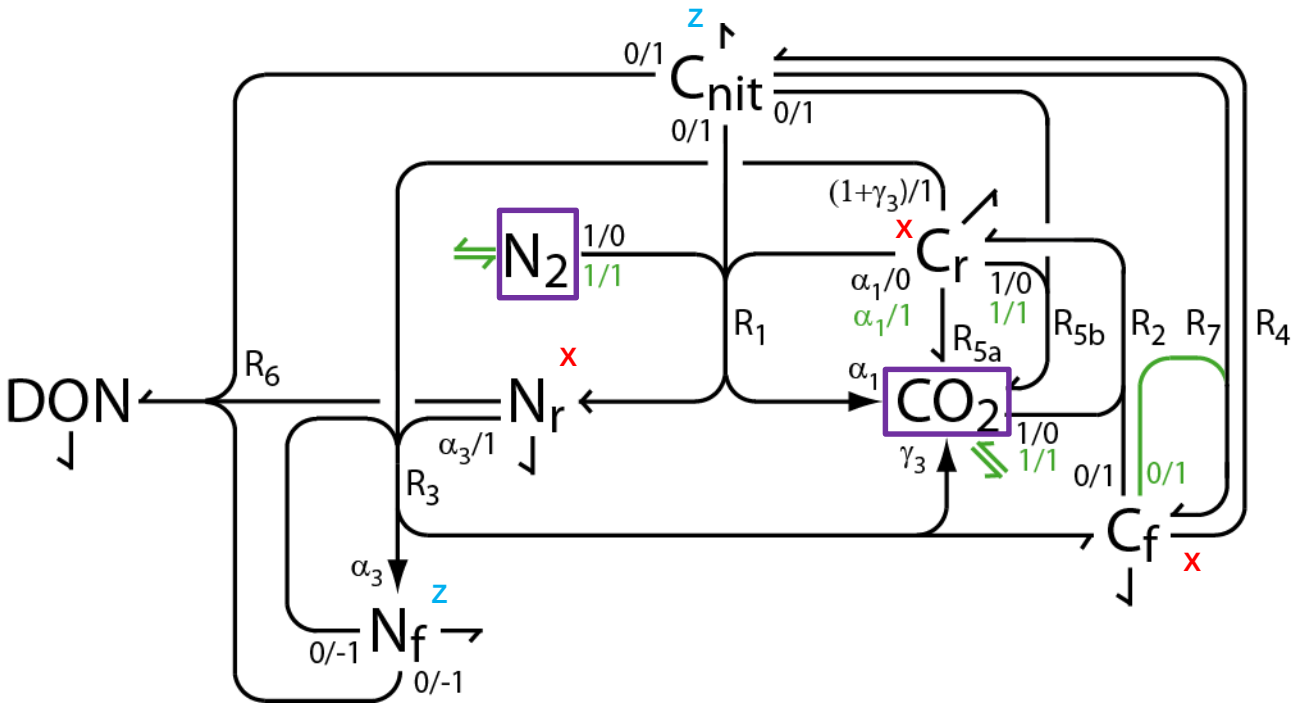
ratio s/o = stoichiometry/order

Black – original formulation
Green – modified/extended formulation

CN metabolism in cyanobacteria

IS THERE A POSITIVE FEEDBACK? IS THE INSTABILITY OSCILLATORY?

Červený, J; Šalagovič, J.; Muzika, F; Šafránek, D; Schreiber, I., in Cyanobacteria: From Basic Science to Applications, Elsevier, 2019



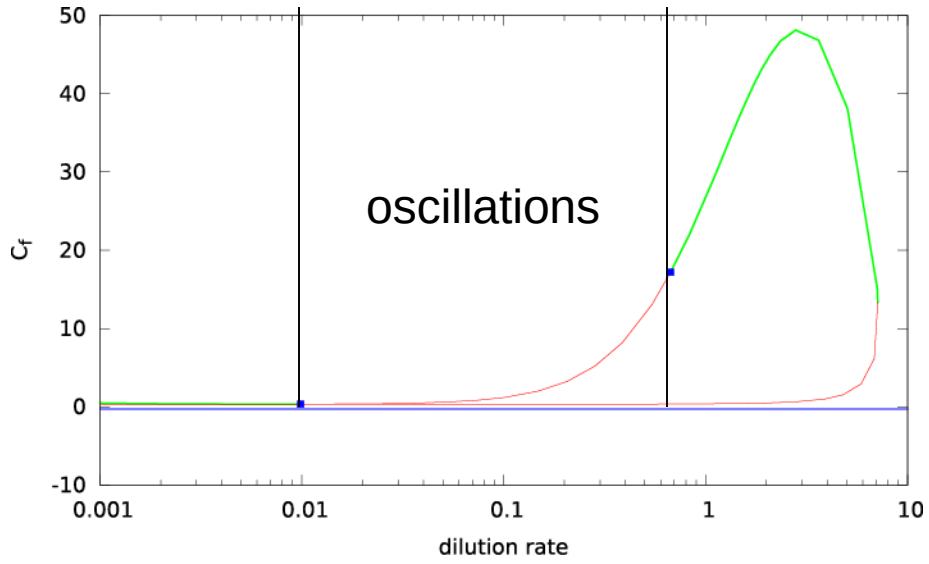
SNA + subsequent bifurcation analysis for the system with extended setting and N_2 and CO_2 in pool condition:

- There are two similar unstable subnetworks: a) network involving R1,R2,R3 and outflow of C_f and N_f
  b) network involving R1,R2,R3,R4 and outflow of C_{nit}

Both involve nitrogen fixation (R1) and photosynthesis (R2) and both yield the instability as follows:

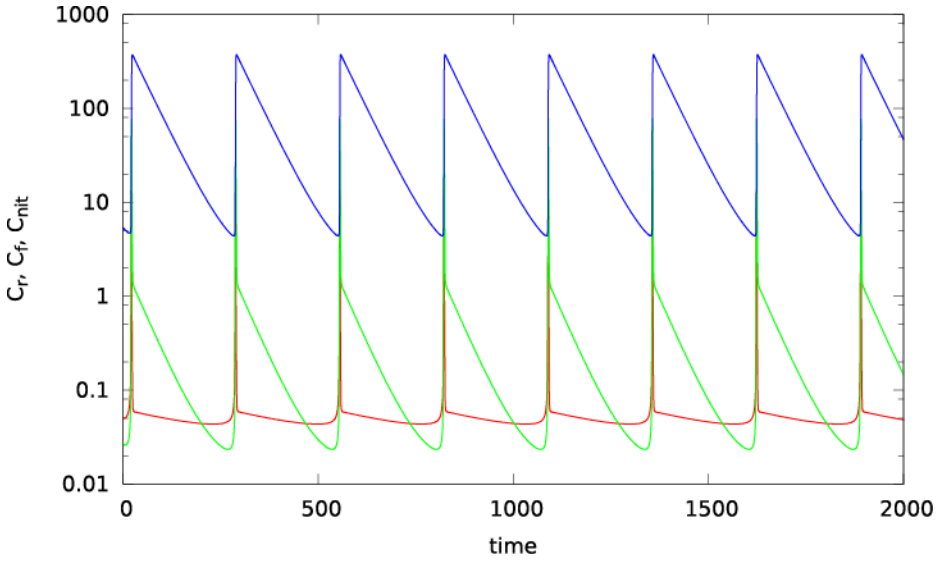
- There are three autocatalytic (type X) species C_r, N_r C_f forming a cycle R1→R3→R2→R1
- There are two negative feedback (type Z) species C_{nit} , N_f regulating R1 and R3, respectively giving rise to oscillations via Hopf bifurcation;
- Oscillations are stable and occur in a wide range of parameters
- There are also stable stationary states – a trivial one (zero biomass) and a positive one (becomes unstable via Hopf bifurcation)

CN metabolism in cyanobacteria
bifurcation behavior and oscillatory dynamics



Dependence of stationary state on dilution rate:

- Green – stable positive stationary state
- Red – unstable positive stationary state
- Blue – stable zero stationary statenetwork
- Square – oscillatory instability (Hopf bifurcation) delimiting region of oscillatory dynamics
- System is bistable



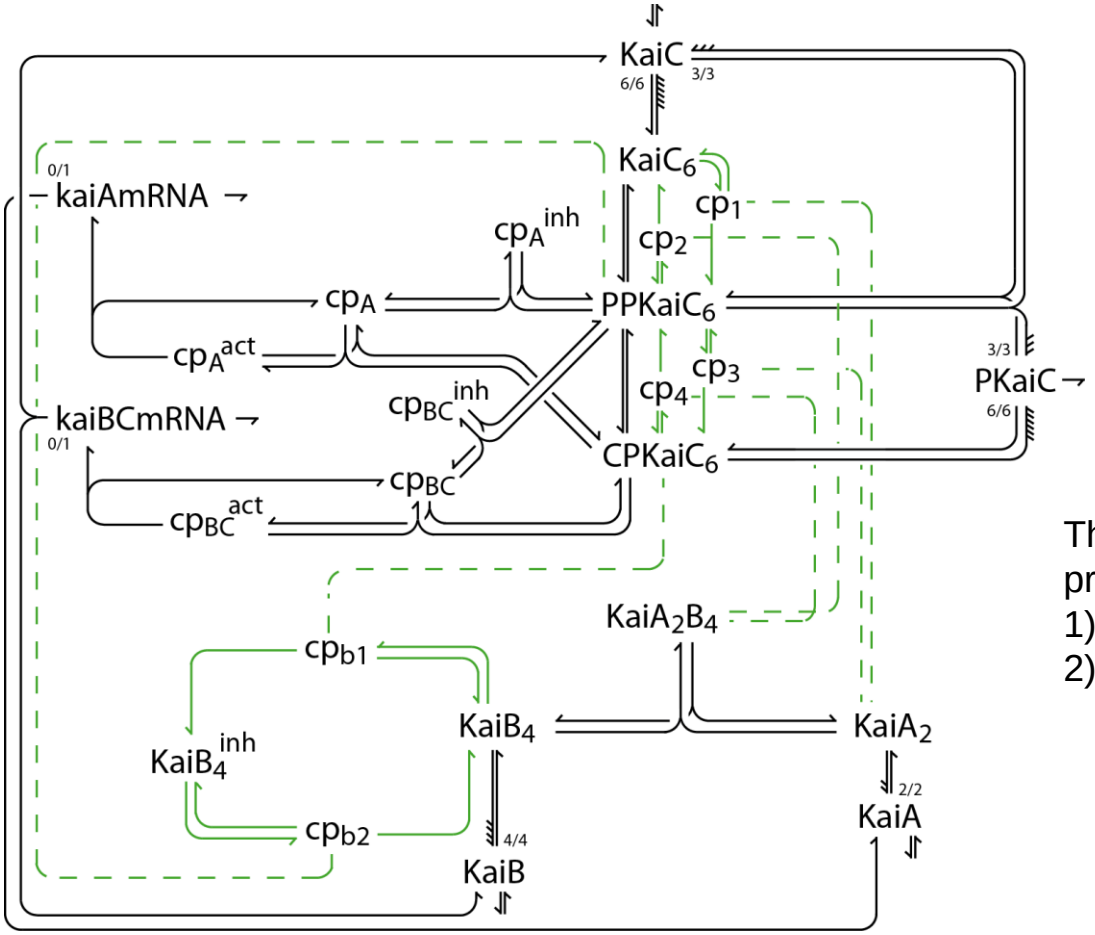
Oscillatory waveform (for D=0.1):

- Red – C_r (type X)
- Green – C_f (type X)
- Blue – C_{nit} (type Z)
- C_r and C_f are almost in-phase
- C_{nit} is phase delayed

Model of circadian clock in cyanobacteria

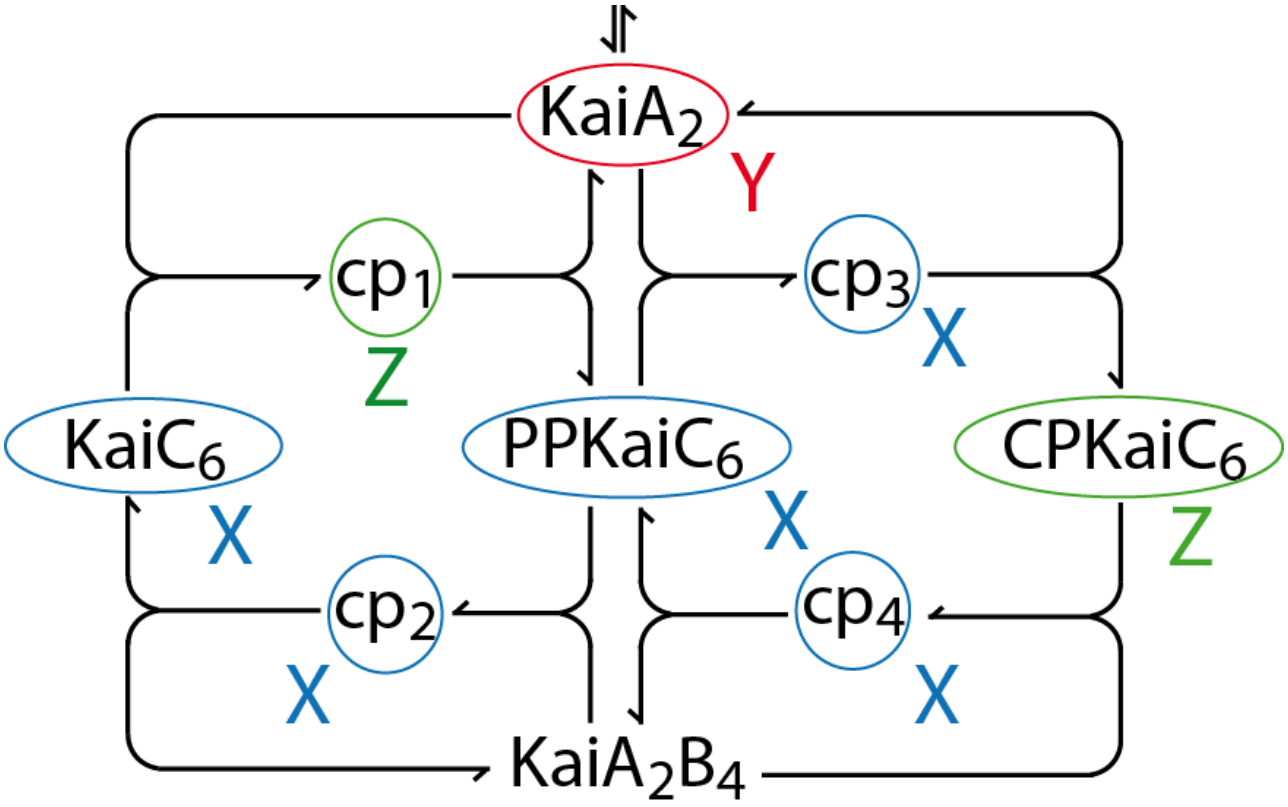
Miyoshi et al., J. Biol. Rhythms 22 (2007) 69– 80
Červený et al., in Cyanobacteria: From Basic Science to Applications, Elsevier, 2019

An simple KaiA,KaiB,KaiC model characterized by KaiC hexamers being in three possible states:
1) unphosphorylated hexamer (KaiC6), composed of 6 unphosphorylated KaiC proteins
2) partially phosphorylated hexamer (PPKaiC6), composed of 3 phosphorylated KaiC proteins (PKaiC) and 3 unphosphorylated monomers (KaiC)
3) completely phosphorylated hexamer (CPKaiC6), composed of 6 phosphorylated KaiC proteins (PKaiC)



There are two major unstable subnetworks providing oscillations:
1) phosphorylation subnetwork
2) transcriptional subnetwork

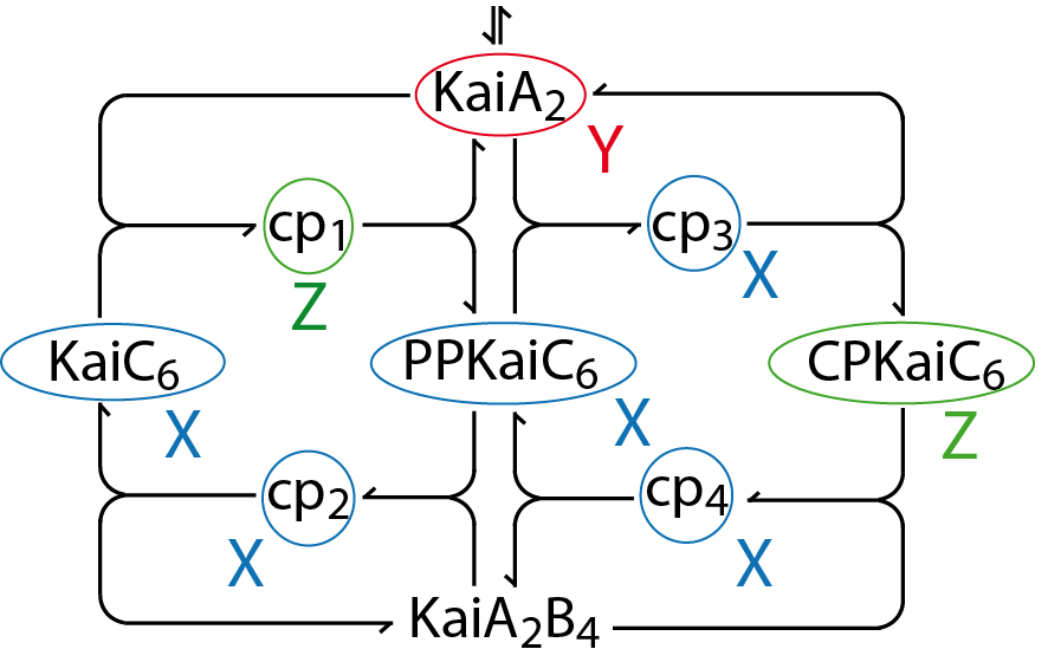
Model of circadian clock in cyanobacteria – phosphorylation subnetwork



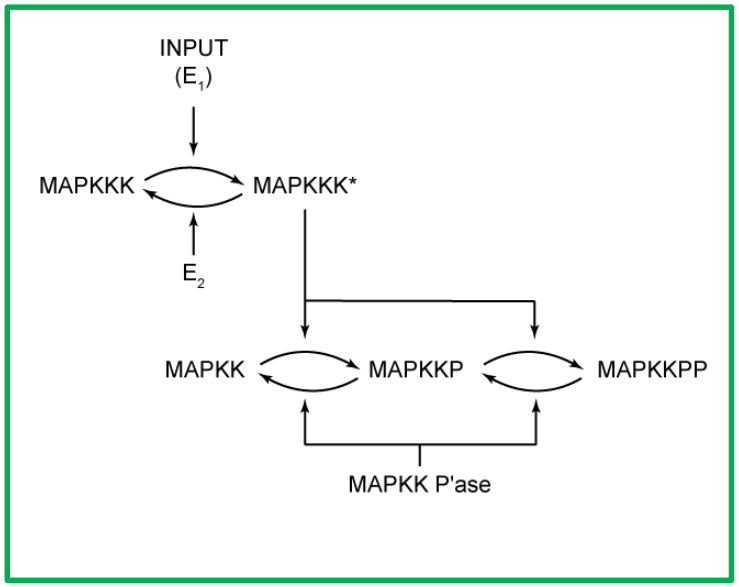
Equivalent to stage 1&2 MAPK network !

Model of circadian clock in cyanobacteria – phosphorylation subnetwork

Phosphorylation network



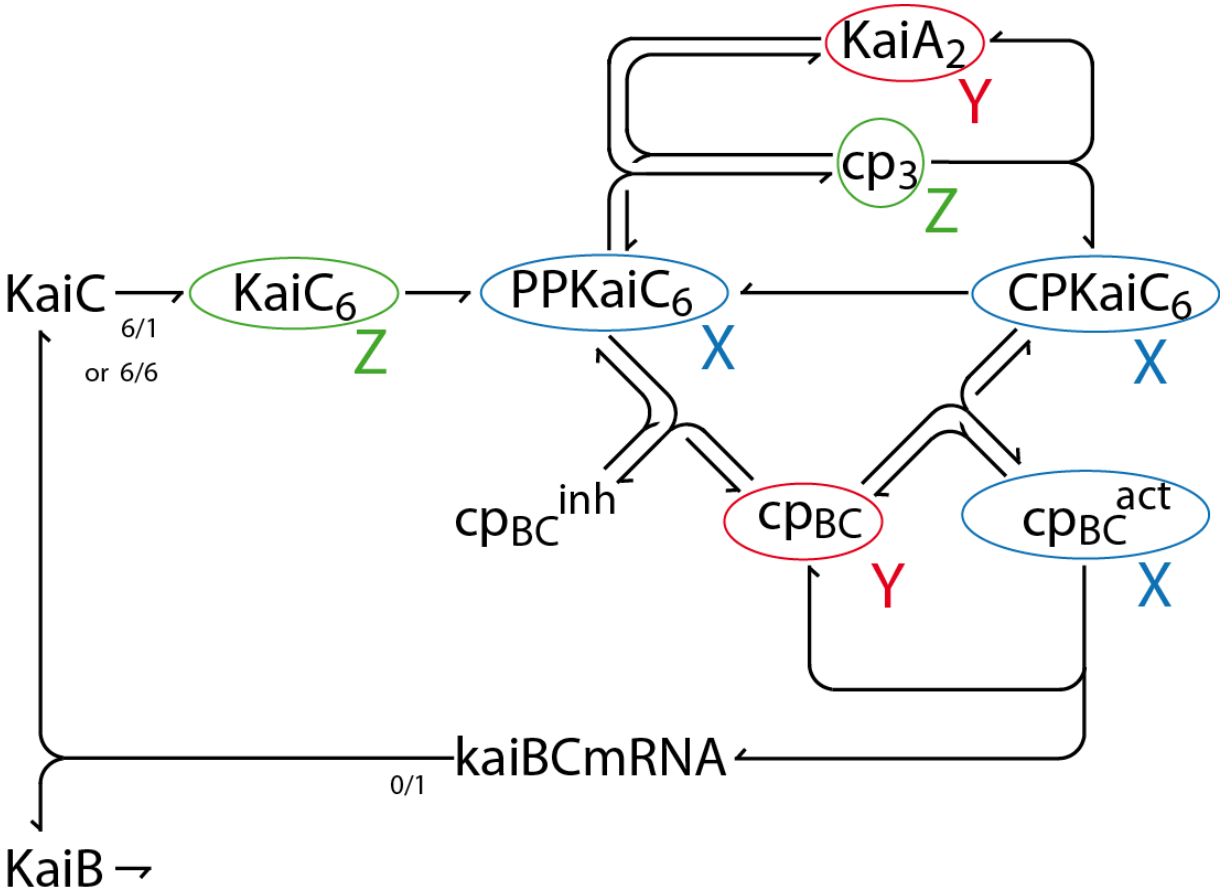
Stage 1&2 MAPK network



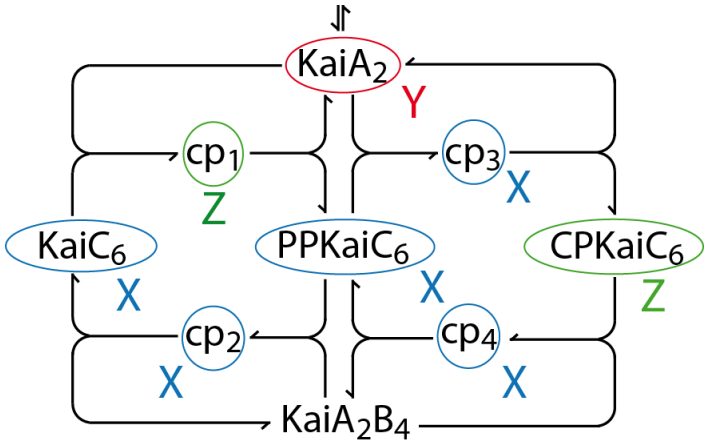
Model of circadian clock in cyanobacteria – transcriptional subnetwork

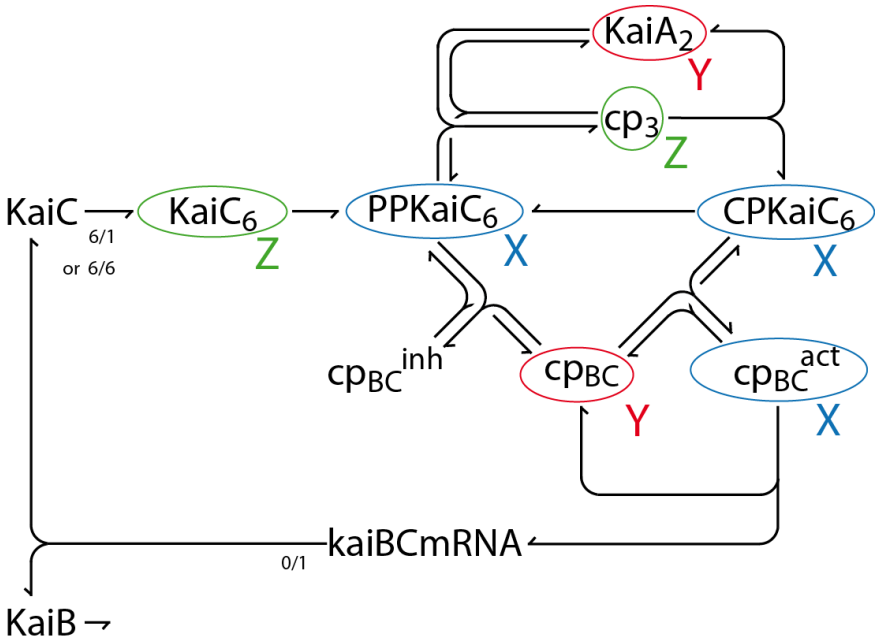
$$v_{ts1} = k_{a1} \frac{k_{bts1}[RNAP]}{1 + k_{bts1}[RNAP]} \frac{[positive\ regulator]}{[negative\ regulator]}$$

$$v_{ts2} = k_{a2} \frac{k_{bts2}[RNAP]}{1 + k_{bts2}[RNAP]} \frac{[positive\ regulator]}{[negative\ regulator]}$$



Role of species in both subnetworks compared





1)The roles of KaiA2 and PPKaiC6 are unchanged

2)The roles of KaiC6 and CPKaiC6 are reversed

3) The roles of CPKaiC6 and the activated complex cp3 are reversed

Differences in oscillatory phase shifts - ?experimentally detectable?

Summary

- Chemical oscillators are based either on an autocatalytic cycle (typical in inorganic oscillators, such as BZ reaction) or on competitive autocatalysis (CA) (ubiquitous among enzyme-catalyzed networks)
- SNA is a convenient tool for identifying sunbnetworks that have potential for oscillations/bistability → classification of (bio)chemical oscillators according to oscillatory prototypes/motifs
- Stoichiometric network analysis can be adapted for estimating unknown rate coefficients when experimental data at (or near) a Hopf bifurcation/fold bifurcation are provided
- By applying the parameter estimation method to cat-gox oscillatory system we find semiquantitative agreement between the model and experiments
- SNA is useful for mass action system → rational polynomial rate expression must be transformed to m.a. → network has to be extended to a set of quasielementary steps → predator-prey example
- Modelling of cyanobacteria rhythmic dynamics → oscillatory subnetworks in the CN metabolism model and in a circadian clock model have been identified