# Hybrid models of the cell cycle molecular machinery

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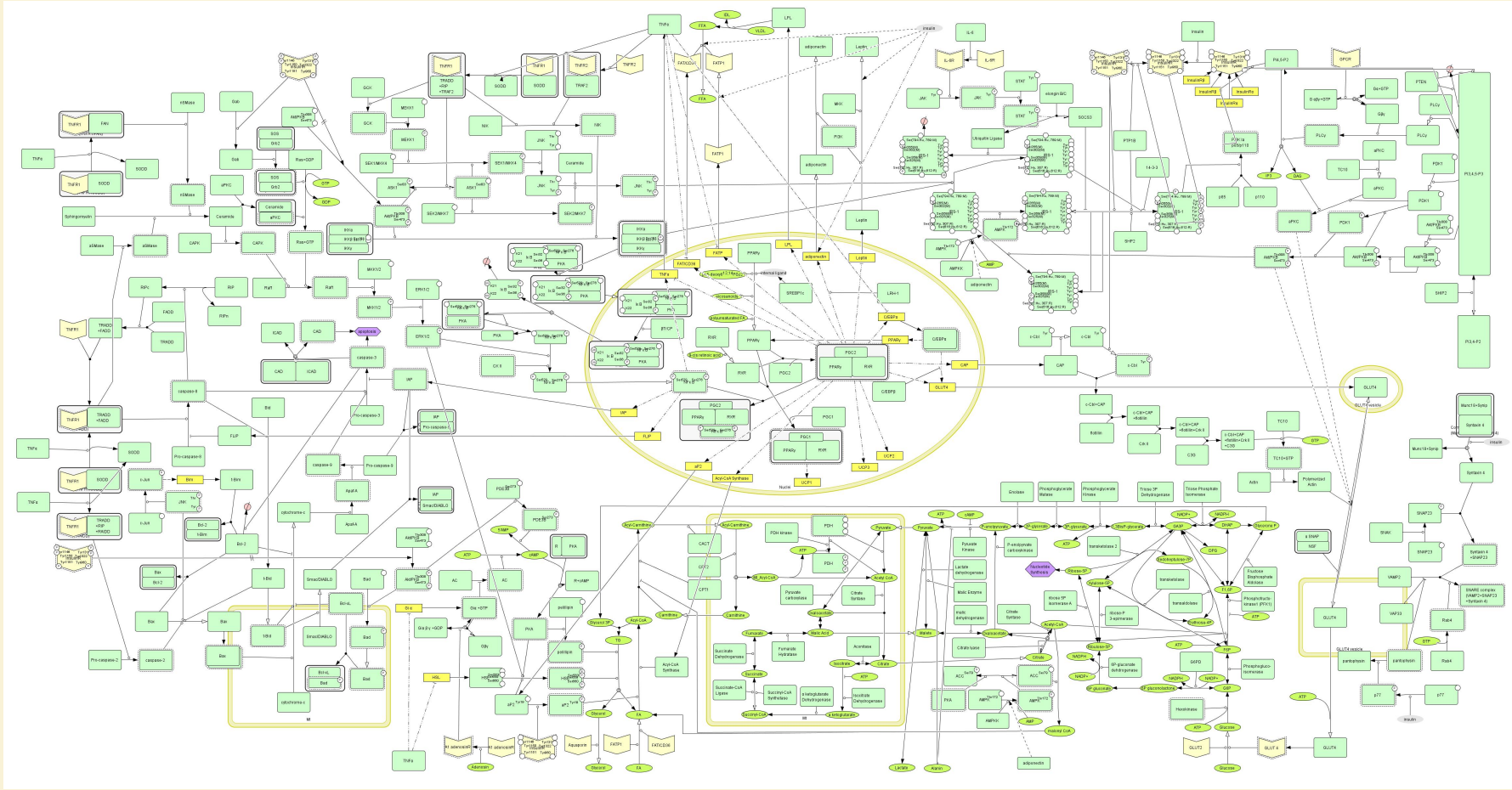
# Outline

- Reduction and hybridization of multi-scale biochemical networks.
- Tropicalization ideas.
- Some general results.
- Application to the cell cycle models.
- Conclusions.

# Biochemical models of the cells

Systems biology: from genes to function. Complex, large scale molecular systems.



# Polynomial differential equations

$$\sum_i \alpha_{ji} A_i \rightleftharpoons \sum_k \beta_{jk} A_k$$

$$R_j(x) = k_j^+ \prod_i x_i^{\alpha_{ji}} - k_j^- \prod_i x_i^{\beta_{ji}}$$

law of mass action

$$\frac{dx_i}{dt} = P_i^+(x) - P_i^-(x)$$

,

$$P_i^{\pm}(x) = \sum_{\alpha \in A_i^{\pm}} a_{i,\alpha}^{\pm} x^{\alpha},\ a_{i,\alpha}^{\pm} > 0, A_i^{\pm} \subset \mathbb{N}^n$$

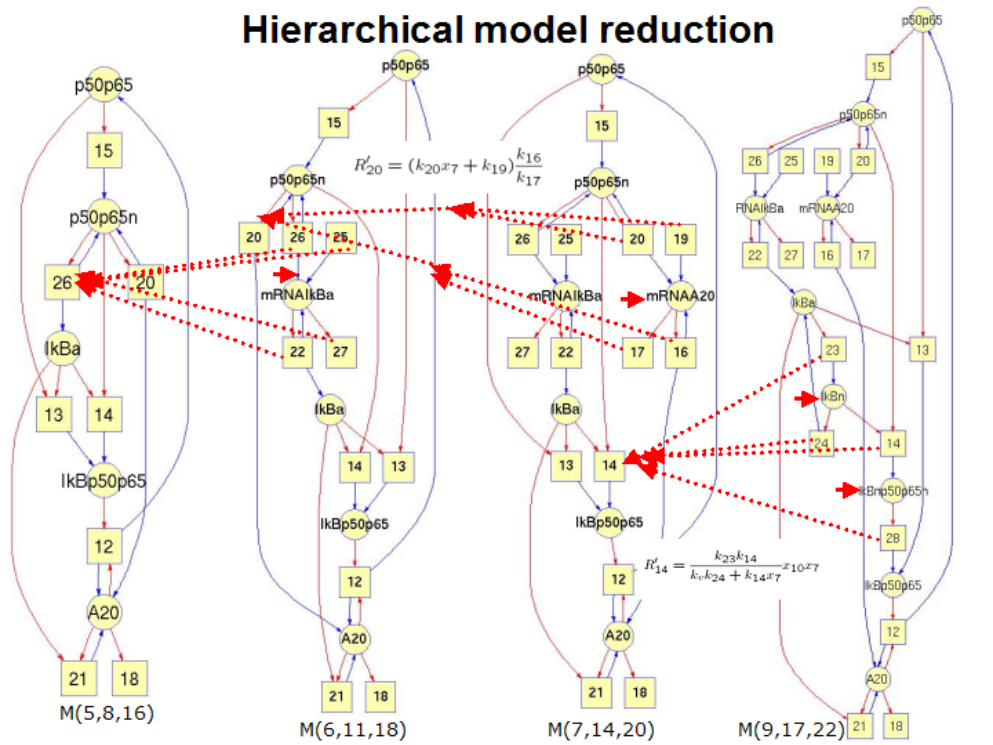
# Main problems in systems biology

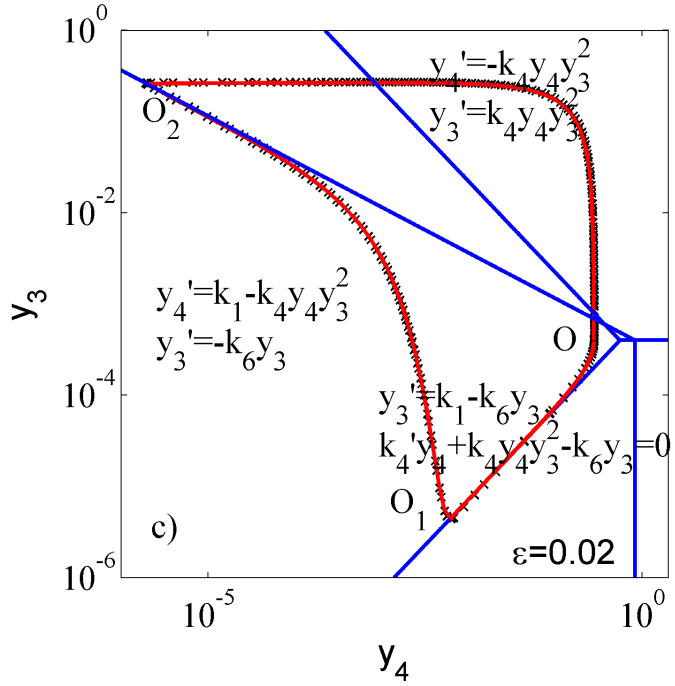
- Compute and control attractors.
- Identify parameters.
- Select models.
- Cope with size and lack of information: $10^2 - 10^3$ variables and parameters, most of them not observed or imprecisely observed.

Our solutions

- Use model reduction: eliminate variables.
- Exploit multi-scaleness of biochemical processes.

# Taming complexity



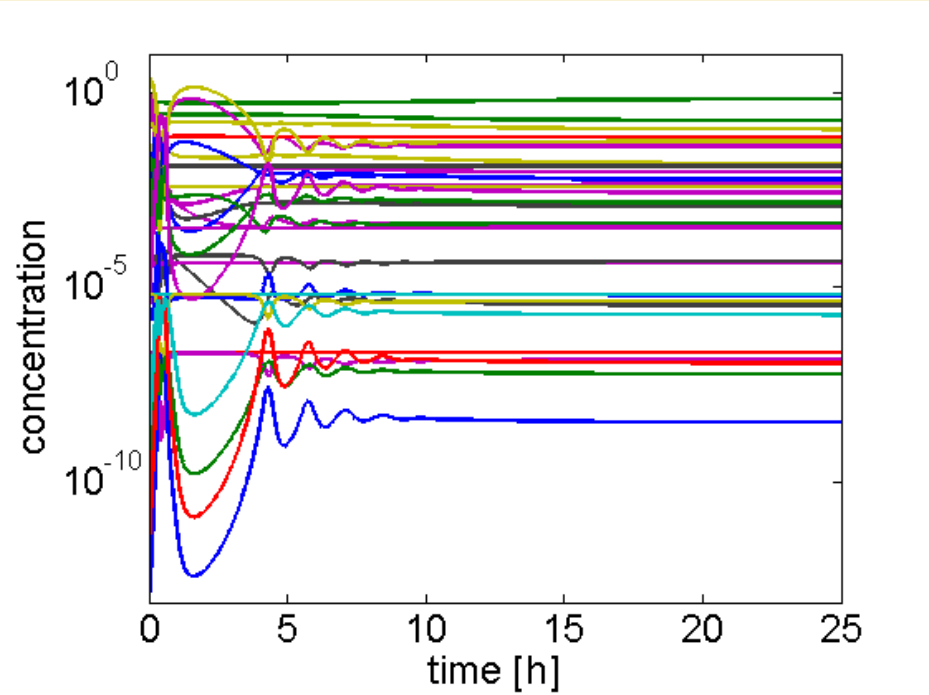

Model reduction: produce models with less variables, equations, parameters; use graph rewriting

Hybridization: use even simpler descriptions piecewisely

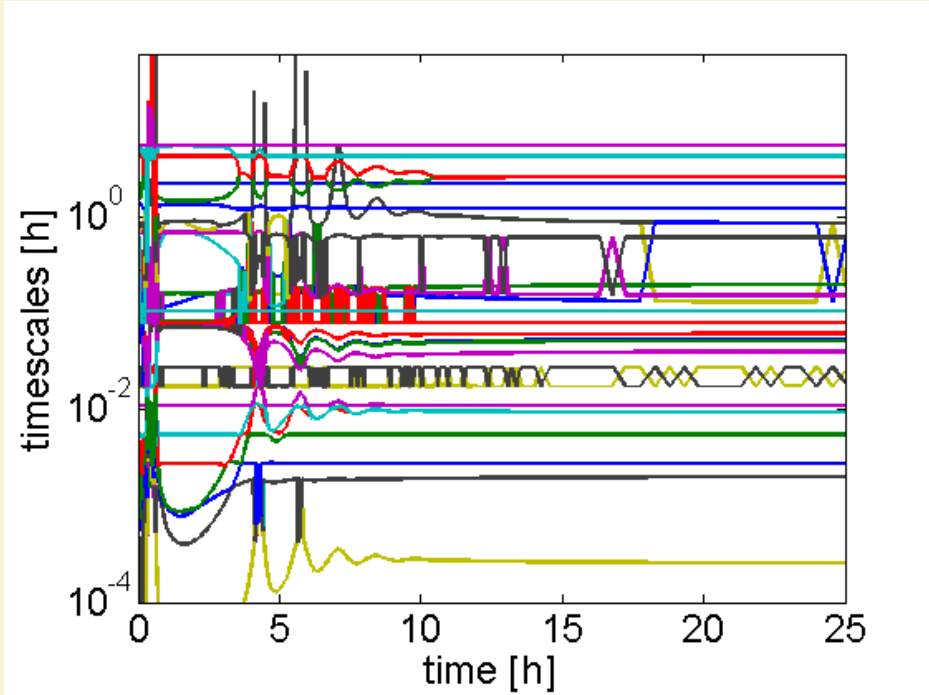
# Multiple scale networks

Molecular networks have many, well separated, time and concentration scales.

Widely distributed concentrations, in log scale




Widely distributed timescales, in log scale




Produced with the NFκB signaling model in Radulescu et al BMC Systems Biol. 2008

Aim: develop simplification methods for multi-scale models with uncertainty.

# How to reduce a network of biochemical reactions? Linear case

- Linear case $\dot{c}_i = \sum_{j, j\neq i}(k_{ij}c_j - k_{ji}c_i)$; find asymptotic approximations for the eigenvalues and eigenvectors.

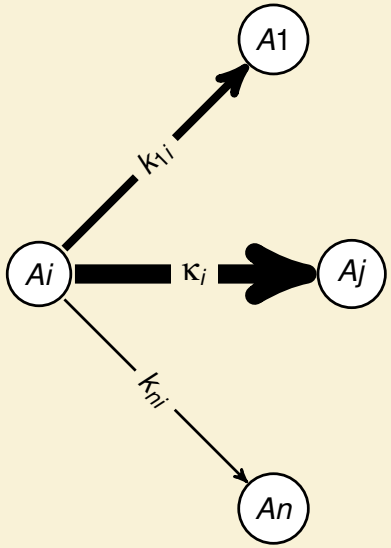
$$c(t) = (l^0, c(0)) + \sum_{k=1}^{n-1} r^k (l^k, c(0)) \exp(-\lambda_k t)$$

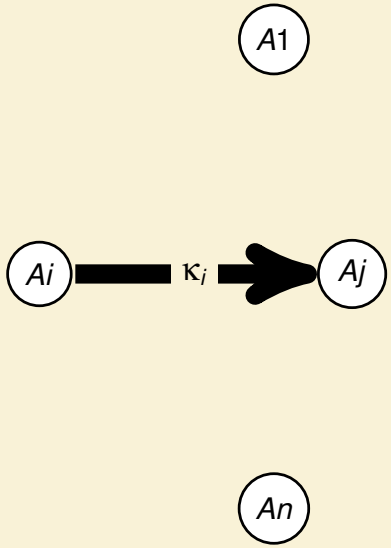
- Well separated constants $k_{l_1} \gg k_{l_2} \gg k_{l_3} \gg \ldots$
- Integer labeled digraphs: reactions are ordered by speed, the lowest order is the most rapid.
- Theorem *(Gorban and Radulescu 2008): the multiscale approximation of an arbitrary linear network with separated constants is an acyclic, deterministic, integer labeled digraph.*

# Auxiliary discrete dynamical systems

For each $A_i$, $\kappa_i = \max_j\{k_{ji}\}$, $\phi(i) = \arg\max_j\{k_{ji}\}$.
$\phi$ determines *auxiliary dynamical system* on a set $\mathcal{A} = \{A_i\}$.

Pruning: keep only the dominating step

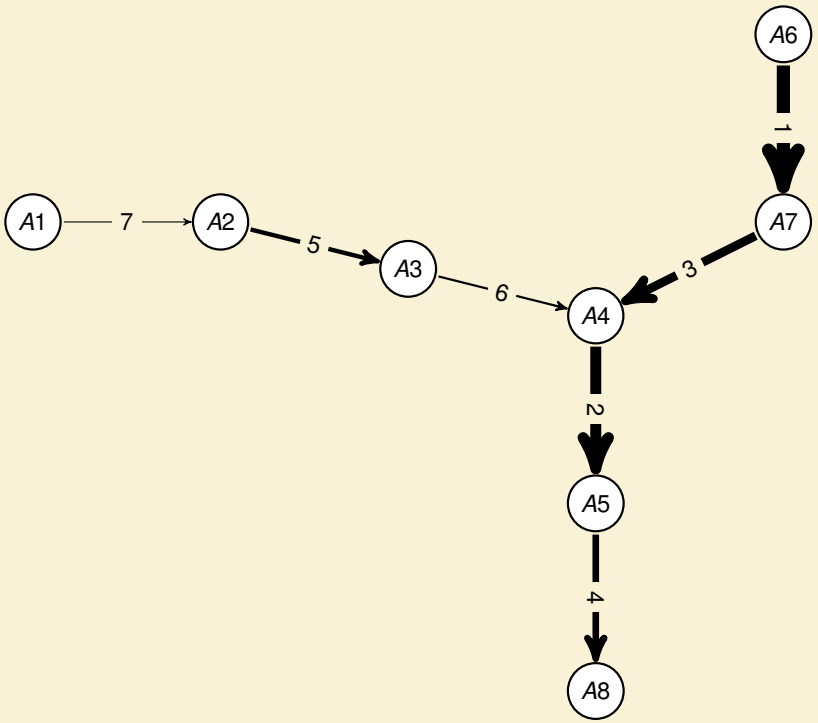



The auxiliary dynamical system is further decomposed into cycles $C_j$ with basins of attraction, $Att(C_j)$: $\mathcal{A} = \cup_j Att(C_j)$.

# 1-st case: acyclic auxiliary dynamic systems

All cycles $C_j$ are point attractors.




$r^i_{\Phi(j)} = \frac{\kappa_j}{\kappa_{\Phi(j)} - \kappa_i} r^i_j$ go along the flow $l^i_j = \frac{\kappa_j}{\kappa_j - \kappa_i} l^i_{\Phi(j)}$ go opposite to the flow.

For instance:

$l^1 \approx (1,0,0,0,0,0,0,0)$

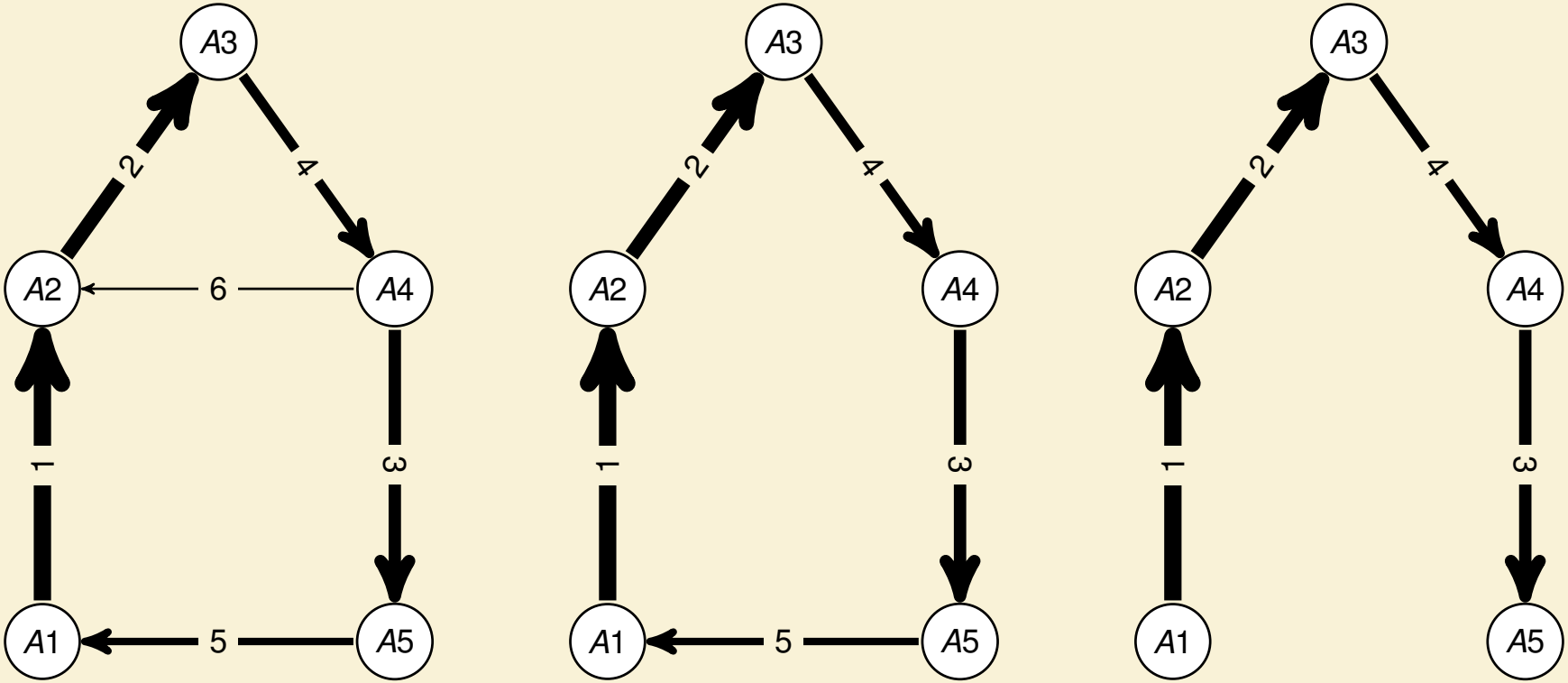
$r^1 \approx (1,0,0,0,0,0,0,-1)$

$l^5 \approx (0,0,0,1,1,1,1,0)$

$r^5 \approx (0,0,0,0,1,0,0,-1)$

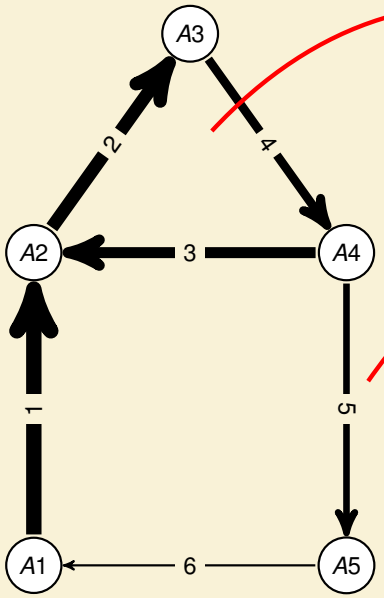
# 2-nd case: $C_j$ are sinks in the initial network

Delete the limiting steps from cycles $C_j$. The obtained *acyclic* reaction network $A_i \to A_{\phi(i)}$ is the right approximation.

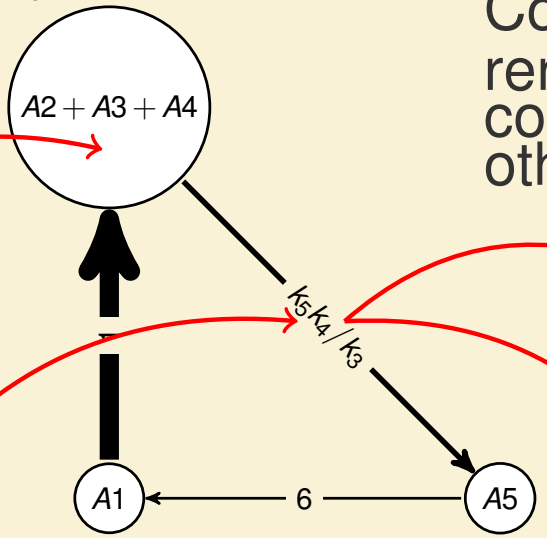


# 3-rd case: some of $C_j$ are not sinks

Slow reactions leave the cycle.



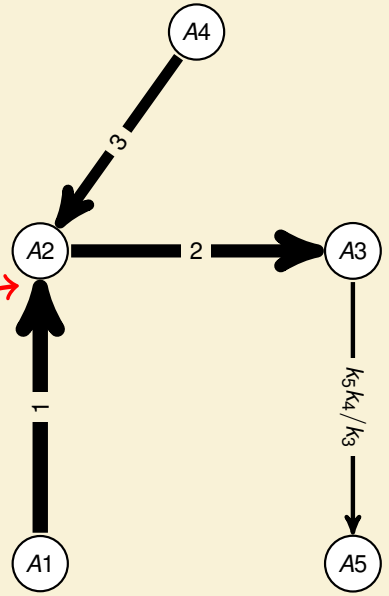

Pool species in cycles;




Renormalize constants of outgoing slow reactions.

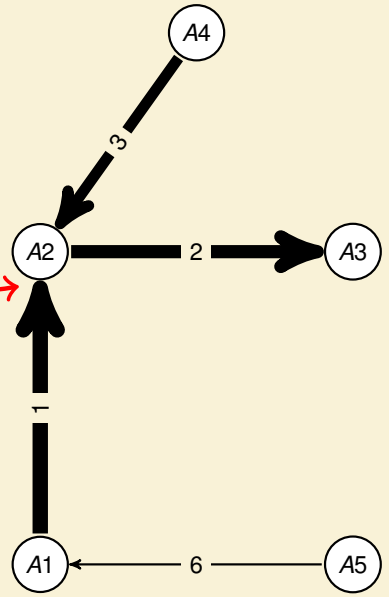
Compare renormalized constants to other constants;

$k_5k_4/k_3 > k_6$




$k_5k_4/k_3 < k_6$

Prune, restore cycles



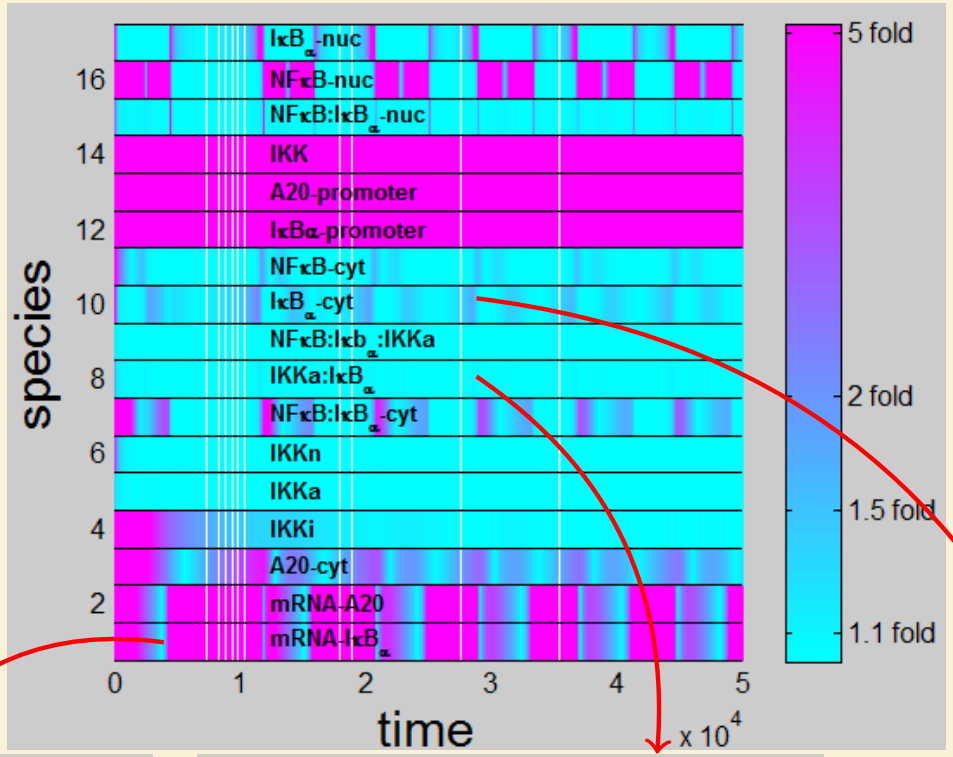
# How to reduce a network of biochemical reactions? Nonlinear case

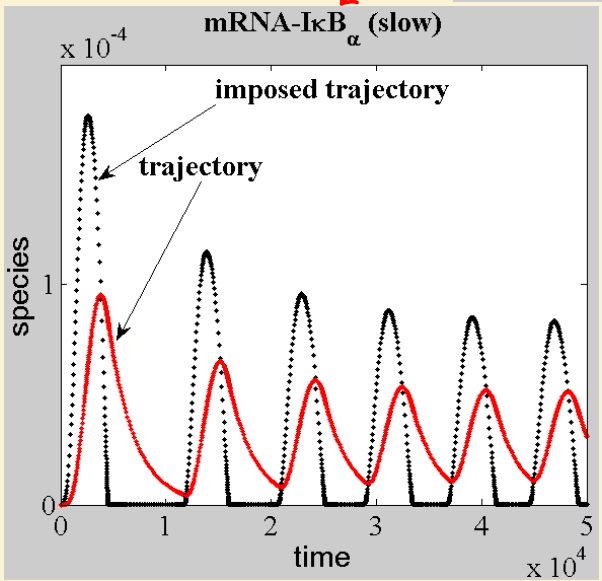
- Exploit some form of local equilibrium: quasi-steady state approximation, quasi-equilibrium.
- Given $\boldsymbol{c}(t)$ solution of $\frac{d\boldsymbol{c}}{dt} = \boldsymbol{f}(\boldsymbol{c})$, the imposed trajectory of the $i$-th species is a solution $c_i^*(t)$ of

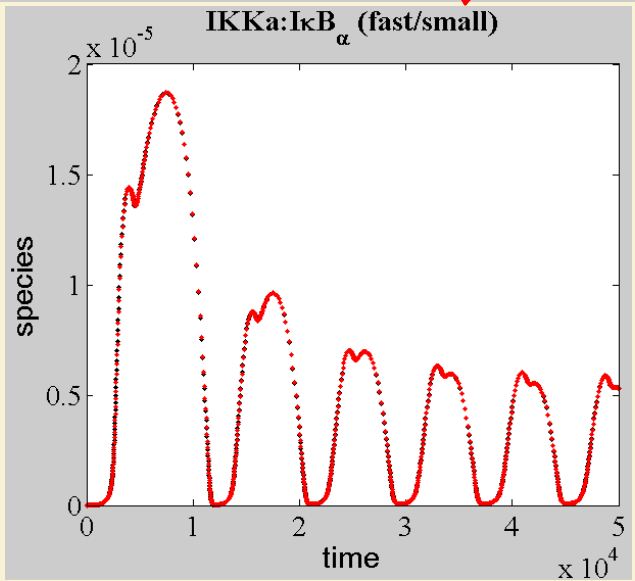
$$f_i(c_1(t), \ldots, c_{i-1}(t), c_i^*(t), c_{i+1}(t), \ldots, c_n(t)) = 0$$

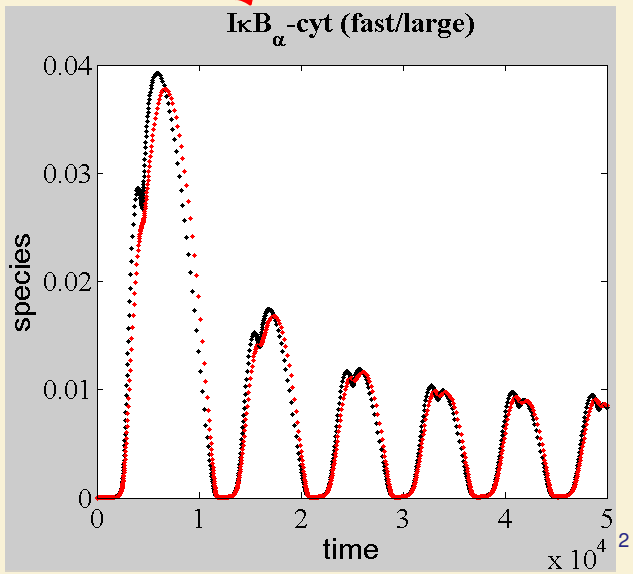
  We say that a species $i$ is slaved if the distance between the trajectory $c_i(t)$ and the imposed trajectory $c_i^*(t)$ is small for some time interval $I$,
  $sup_{t\in I}|log(c_i(t)) - log(c_i^*(t))| < \delta$, for some $\delta > 0$ sufficiently small.
- At least two conditions lead to slaved species: quasi-stationarity, quasi-equilibrium.

# Slaved species - NFκB model



# Small concentration slaved species - quasi-steady state approximation

Example: Michaelis-Menten mechanism.

$$S + E \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} SE \xrightarrow{k_2} P + E$$

QSS approximation (Briggs-Haldane): *quasi-steady state species are low concentration, fast species.*

Pooling of reactions.

$$S \xrightarrow{R(S,E_{tot})} P$$

$$\frac{dP}{dt} = -\frac{dS}{dt} = k_2 ES,\ k_1 S.(E_{tot} - E) = (k_{-1} + k_2)ES$$

$$R(S, E_{tot}) = k_2 E_{tot}.S/(k_m + S)$$

# Small concentration slaved species - quasi-equilibrium approximation

QE approximation (Michaelis-Menten): *quasi-equilibrium reactions are fast, reversible reactions.*

Pooling of species. $S_{tot} = S + ES$, $E_{tot} = E + ES$.

$$S + E \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} SE \xrightarrow{k_2} P + E,\ S_{tot} \xrightarrow{R(S_{tot}, E_{tot})} P$$

$$\frac{dP}{dt} = -\frac{dS_{tot}}{dt} = k_2 ES,\ k_1(S_{tot} - ES).(E_{tot} - ES) = k_{-1} ES$$

$$R(S_{tot}, E_{tot}) = \frac{2k_2 E_{tot} S_{tot}}{(E_{tot} + S_{tot} + \frac{k_{-1}}{k_1})(1 + \sqrt{1 - \frac{4E_{tot} S_{tot}}{(E_{tot} + S_{tot} + k_{-1}/k_1)^2}})}$$

# Graph rewriting operations - pooling and pruning of reactions and species

*Let $S^f$ be the stoichiometric matrix of the fast subsystem: fast reactions for QE; reactions producing or consuming fast species for QSS.*

Pooling

Reaction pools for QSS, satisfy $S^f\gamma = 0$: elementary modes.

Species pools for QE, satisfy $b^T S^f = 0$: conservation laws.

Impose minimality conditions $R(\gamma') \subset R(\gamma) \Rightarrow \gamma' = 0 \vee \gamma' = \gamma$.

Pruning

Quasi-steady state species and quasi-equilibrium reactions can be pruned.

Algebraic conditions

Find approximations to algebraic equilibrium equations: Newton-Puisseux series.

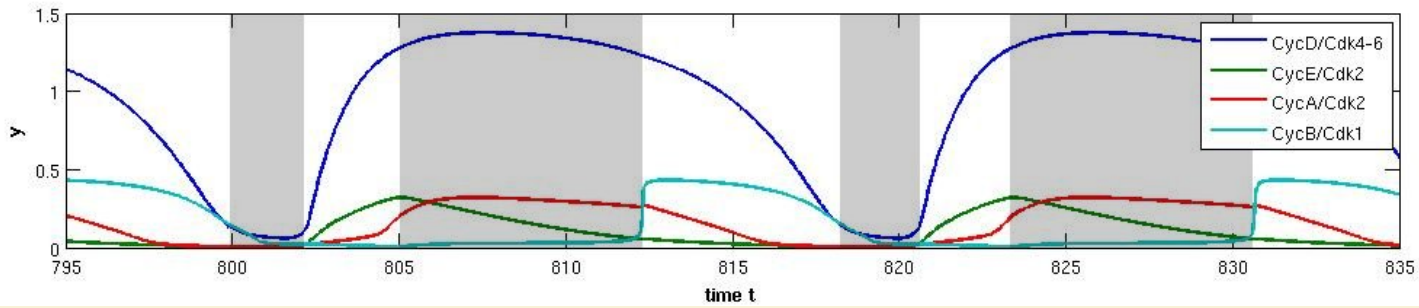
# Hybridization

- The set of slaved species can change in time.
- Rate functions can be approximated by monomial functions.

$$VX/(K+X) = \begin{cases} V/KX, & X << K \\ V, & X >> K \end{cases}$$

- This leads to piecewise smooth hybrid models.

$$\frac{dx_i}{dt} = \sum_{k=1}^{N} s_k a_k x^{\alpha_k} - \sum_{l=1}^{M} \tilde{s}_l b_k x^{\beta_k}, \quad s_k, \tilde{s}_l \in \{0,1\}$$




Hybrid cell cycle models (Noel et al 2011, 2012).

# Tropical ideas

Systems biology models satisfy:

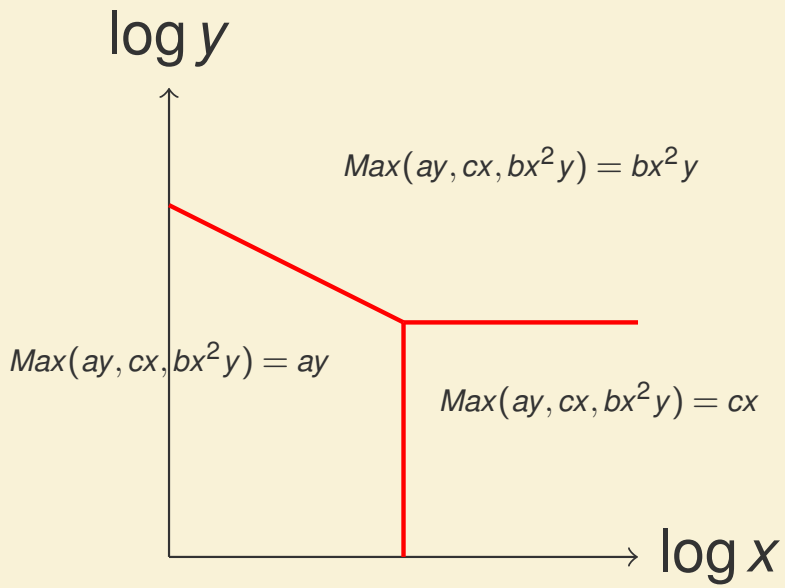
- vectors fields of ODE models are polynomials or ratios of multivariate polynomials $P_i(x) = \sum_{\alpha \in A} a_\alpha x_2^{\alpha_2} \dots x_n^{\alpha_n}$.
- reduction methods exploit dominance relations between monomial rate terms.
- the dominant (reduced) subsystem depends on the time-scale, it can change with time (hybrid models).

Tropical (idempotent) analysis offers convenient solutions to:

- solve systems of polynomial equations $P_i(x) = 0$ with separated monomials.
- what about ODE systems $\frac{dx_i}{dt} = P_i(x)/Q_i(x), 1 \leq i \leq n$, with separated monomials?

# Litvinov-Maslov tropicalization

Heuristics: replace multivariate polynomials in rhs of ODEs by piece-wise smooth, max-plux polynomials.
$\sum_{\alpha\in A} a_\alpha x^\alpha \to exp[max_{\alpha\in A}\{log(a_\alpha) + < log(x), \alpha >\}]$.
The tropical manifold is the set of points where max-plus polynomials are not smooth.




The tropical manifold of the polynomial $ay + cx + bx^2y$ on "logarithmic paper".

# Tropicalized systems

$$\frac{dx_i}{dt} = DomP_i^+(\boldsymbol{x}) - DomP_i^-(\boldsymbol{x})$$

two terms tropicalization (Savageau 2009)

$$\frac{dx_i}{dt} = DomP_i(\boldsymbol{x})$$

complete, or one term tropicalization (Noel et al 2011)

where $Dom\{a_{i,\alpha}\boldsymbol{x}^{\alpha}\}_{\alpha\in A_i} =$
$sign(a_{i,\alpha_{max}})exp[max_{\alpha\in A_i}\{log(|a_{i,\alpha}|)+ < \boldsymbol{u}, \alpha >\}]$.
$\boldsymbol{u} = (logx_1, \ldots, logx_n)$, and $a_{i,\alpha_{max}}$, $\alpha_{max} \in A_i$ denote the coefficient of the monomial for which the maximum is attained.

# Is the heuristics justified?

$$\frac{dx_i}{dt} = P_i(x,\varepsilon) = \sum_{j=1}^{M} M_{ij}(x,\varepsilon), \quad M_{ij} = P_{ij}(\varepsilon)x^{\alpha_{ij}} \tag{1}$$

$P_{ij}(\varepsilon) = \varepsilon^{\gamma_{ij}}\bar{P}_{ij}$, where $\gamma_{ij} \neq \gamma_{i'j'}$ for $(i,j) \neq (i',j')$.

Definition. *The system* (1) *is permanent, if there are two constants* $C_- > 0$ *and* $C_+ > 0$ *such that*

$$C_- < \bar{x}_i(t) < C_+, \quad \textit{for all } t > T_0(x(0)) \textit{ and for every } i,$$

*where* $x_i = \varepsilon^{a_i}\bar{x}_i$. $C_\pm$ *and* $T_0$ *are uniform in* $\varepsilon$ *as* $\varepsilon \to 0$.

# Is the heuristics justified?

Proposition *Assume that system* (1) *is permanent. Let* $x$, $\hat{x}$ *be the solutions to the Cauchy problem for (1) and the complete (or two terms) tropicalization, respectively, with the same initial data:*

$$x(0) = \hat{x}(0).$$

*Then the difference* $y(t) = x(t) - \hat{x}(t)$ *satisfies the estimate*

$$|y(t)| < C_1 \varepsilon^{\gamma} \exp(bt), \quad \gamma > 0,$$

*positive constant* $C_1, b$ *is uniform in* $\varepsilon$. *If the original system (1) is structurally stable in the domain* $\Omega_{C_-,C_+} = \{x : C_- < |x| < C_+\}$, *then the corresponding tropical systems are also permanent and there is an orbital topological equivalency* $h_\varepsilon$ *between the trajectories* $x(t)$ *and* $\bar{x}(t)$ *of the corresponding Cauchy problems. The homeomorphism* $h_\varepsilon$ *is close to the identity as* $\varepsilon \to 0$.

# Tropical equilibration

Rescale $x_i = \varepsilon^{a_i}\bar{x}_i$, get $\frac{d\bar{x}_i}{dt} = \sum_i \varepsilon^{\mu_i(j)} F_i(\bar{\mathbf{x}})$, $\quad F_i = p_{ij}\bar{\mathbf{x}}^{\alpha^{ij}}$, where

$$\mu_i(j) = \gamma_{ij} + \sum_{l=1}^{n} \alpha_l^{ij} a_l$$

Tropical equilibration problem. There are $(j(i), k(i))$, such that $j(i) \neq k(i)$,

$$\mu_i = \mu_i(j(i)) = \mu_i(k(i)) > \mu_i(l) \quad \textit{for all } l \neq j(i), k(i)$$

This is a hard problem.

# Tropical equilibration

Lemma1. *We have permanency only if we have tropical equilibration of at least two terms, one positive (production), the other negative (degradation).*

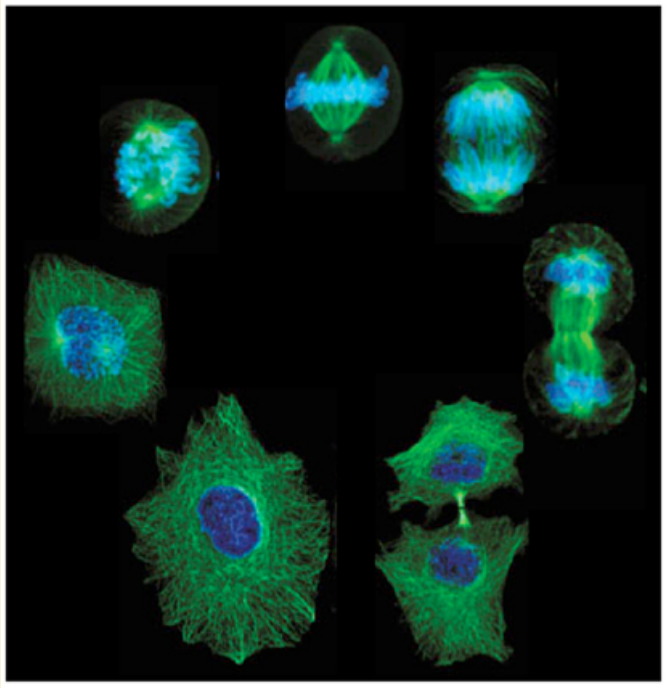
Lemma2. *In the case of tropical equilibration of two terms (toric systems), and if* $0 = \mu_1 < \mu_2 < \ldots < \mu_{n-1} < \mu_n$ *(total separation) each variable follows*

$$\frac{dx}{dt} = b_1(z)x^{\beta_1} - b_2(z)x^{\beta_2}, \quad b_1, b_2 > 0,$$

*where z is a slow function of time and we have permanency iff i)* $0 < \beta_1 < \beta_2$, *or ii)* $\beta_1 < \beta_2 < 0$, *or iii)* $\beta_1 < 0, \quad \beta_2 > 0$.

Theorem. If the above conditions are satisfied, then the system has an attractive invariant manifold.

# An example: cell cycle model

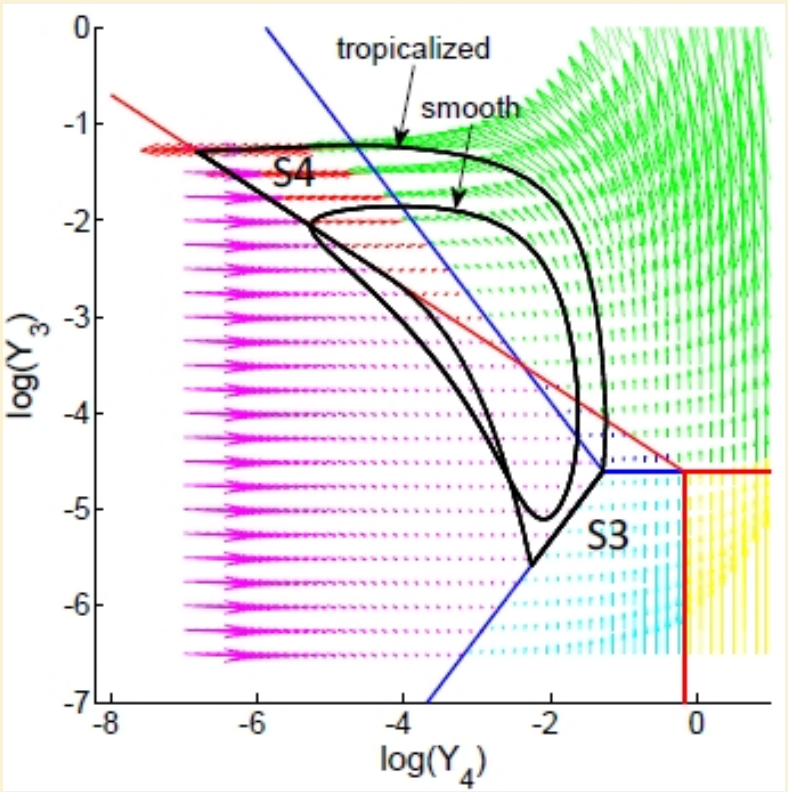


- Cell proliferation is regulated by cyclic activity of cyclins
- Tumors result from uncontrolled cell proliferation
- A simple model, Tyson 91

$$
\begin{aligned}
y_1' &= \varepsilon^{-3} k_9 y_2 - \varepsilon^{-6} k_8 y_1 + k_6 y_3, \\
y_2' &= \varepsilon^{-6} k_8 y_1 - \varepsilon^{-3} k_9 y_2 - \varepsilon^{-2} k_3 y_2 y_5, \\
y_3' &= \varepsilon^{2} k_4' y_4 + \varepsilon^{-2} k_4 y_4 y_3^2 - k_6 y_3, \\
y_4' &= -\varepsilon^{2} k_4' y_4 - \varepsilon^{-2} k_4 y_4 y_3^2 + \varepsilon^{-2} k_3 y_2 y_5, \\
y_5' &= \varepsilon^{2} k_1 - \varepsilon^{-2} k_3 y_2 y_5.
\end{aligned}
$$

$y_1$ cdc2, $y_2$ p-cdc2, $y_3$ cyclin-p:cdc2 complex (active MPF), $y_4$ cyclin-p:cdc2-p complex (inactive MPF), $y_5$ cyclin.

# An example: cell cycle model




- the smooth and tropicalized trajectories are qualitatively similar, but one does not have convergence when $\varepsilon \to 0$.
- $y_3$, $y_4$ are excitable, not equilibrated in the same time.
- the differences are due mainly to “wall crossing”.

# Tropical equilibrations

Rescale $y_i = \varepsilon^{a_i}\bar{y}_i$ and get

$$
\begin{aligned}
\bar{y}_1' &= \varepsilon^{-3+a_2-a_1}k_9\bar{y}_2 - \varepsilon^{-6}k_8 y_1 + k_6\varepsilon^{a_3-a_1}\bar{y}_3,\\
\bar{y}_2' &= \varepsilon^{-6+a_1-a_2}k_8\bar{y}_1 - \varepsilon^{-3}k_9\bar{y}_2 - \varepsilon^{-2+a_5}k_3\bar{y}_2\bar{y}_5,\\
\bar{y}_3' &= \varepsilon^{2+a_4-a_3}k_4'\bar{y}_4 + \varepsilon^{-2+a_3+a_4}k_4\bar{y}_4\bar{y}_3^2 - k_6\bar{y}_3,\\
\bar{y}_4' &= -\varepsilon^2 k_4'\bar{y}_4 - \varepsilon^{-2+2a_3}k_4\bar{y}_4\bar{y}_3^2 + \varepsilon^{-2+a_2+a_5-a_4}k_3\bar{y}_2\bar{y}_5,\\
\bar{y}_5' &= \varepsilon^{2-a_5}k_1 - \varepsilon^{-2+a_2}k_3\bar{y}_2\bar{y}_5.
\end{aligned}
$$

From the equilibration of $y_1$, $y_2$, $y_5$ we find
$a_1 = 3, \quad a_2 = 0, \quad a_5 = 4.$

# Tropical equilibrations

Rescaled equations

$$\begin{aligned}
\bar{y}_1' &= \varepsilon^{-6}(k_9\bar{y}_2 - k_8\bar{y}_1) + k_6\varepsilon^{-1}\bar{y}_3,\\
\bar{y}_2' &= \varepsilon^{-3}(k_8\bar{y}_1 - k_9\bar{y}_2) - \varepsilon^{2}k_3\bar{y}_2\bar{y}_5,\\
\bar{y}_5' &= \varepsilon^{-2}(k_1 - k_3\bar{y}_2\bar{y}_5).
\end{aligned}$$

Provided that $\bar{y}_2 > \delta > 0$ there is a locally attractive invariant manifold satisfying $\bar{y}_2\bar{y}_5 = k_1/k_3 + O(\varepsilon^2)$. This allows reducing the system from 5 to 2 equations.

# Tropical equilibrations

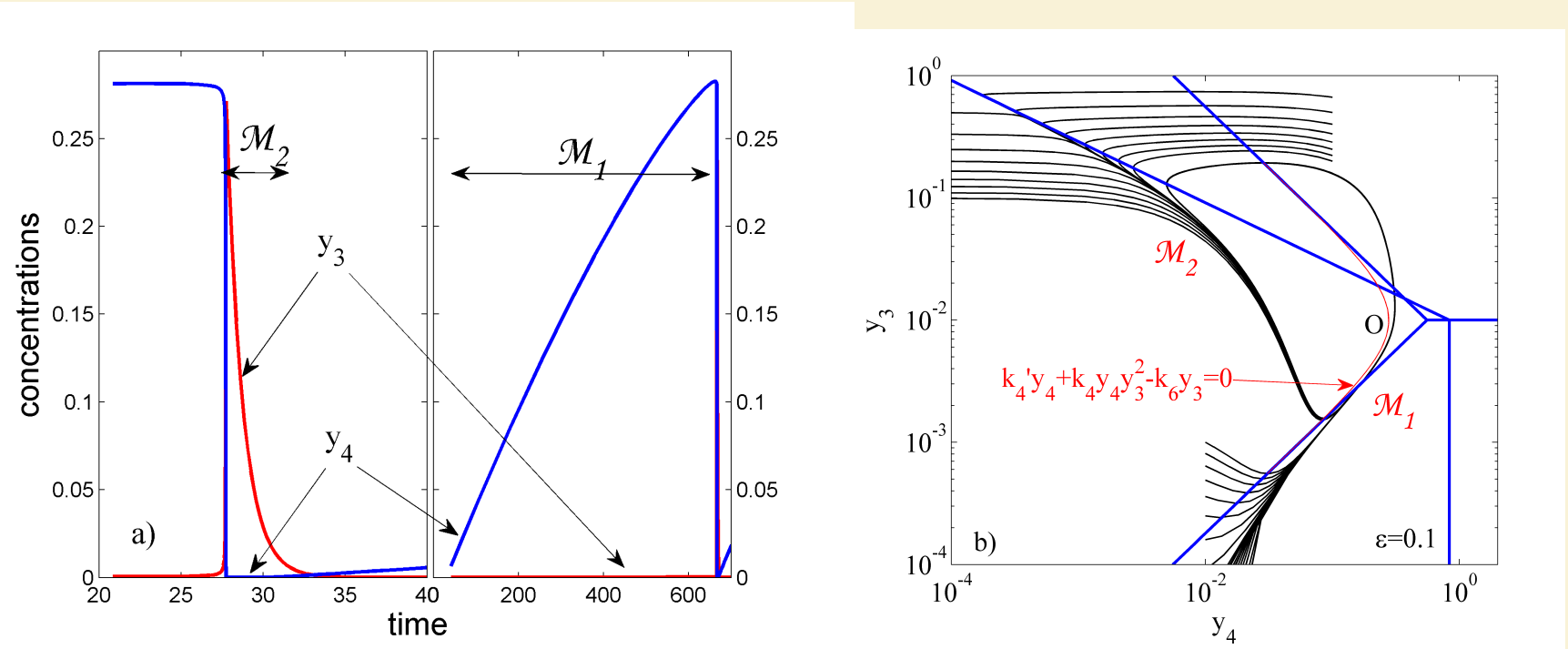
if $y_3$ is equilibrated, then $a_3 = 2, \quad a_4 = 0$ and

$$\bar{y}_3' = k_4'\bar{y}_4 + k_4\bar{y}_4\bar{y}_3^2 - k_6\bar{y}_3,\ \bar{y}_4' = \varepsilon^2(-k_4'\bar{y}_4 - k_4\bar{y}_4\bar{y}_3^2 + k_1).$$

if $y_4$ is equilibrated, then $a_3 = 0, \quad a_4 = 4$ and

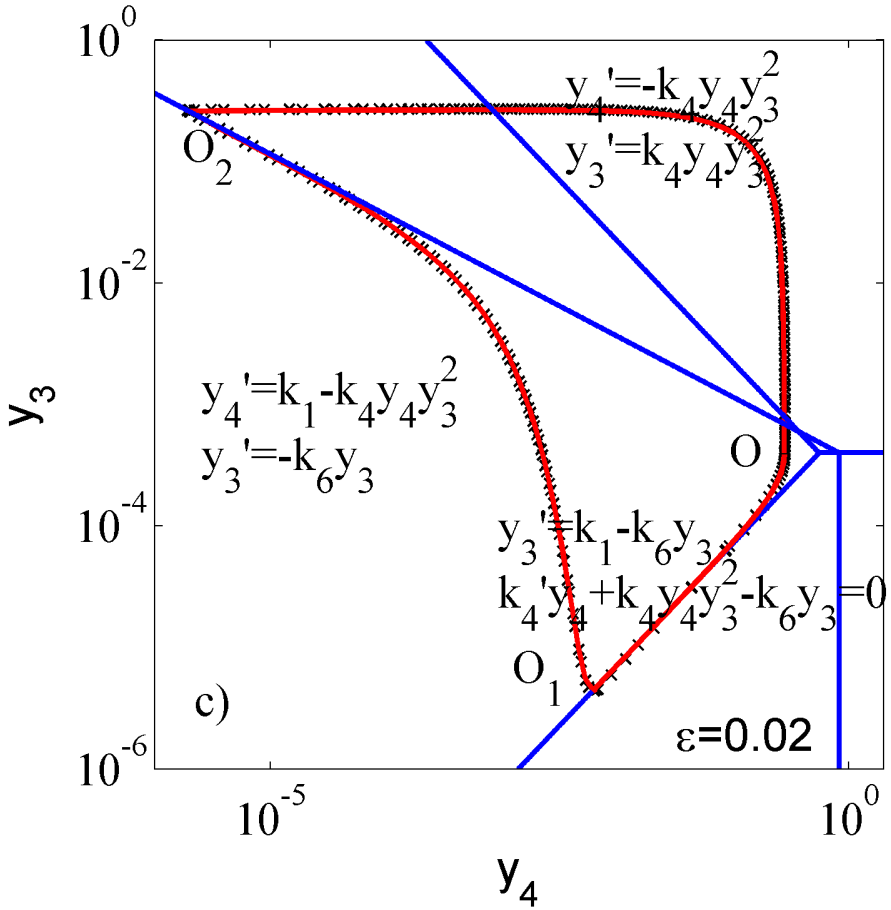
$$\tilde{y}_3' = \varepsilon^6 k_4'\tilde{y}_4 + \varepsilon^2 k_4\tilde{y}_4\tilde{y}_3^2 - k_6\tilde{y}_3,\ \tilde{y}_4' = -\varepsilon^2 k_4'\tilde{y}_4 + \varepsilon^{-2}(-k_4\tilde{y}_4\tilde{y}_3^2 + k_1).$$

# Tropical equilibrations




Tropical equilibrations correspond to locally attractive invariant manifolds and slower parts of the cycle.

# Hybrid dynamics




The rapid parts of the cycle are described by the one term or two terms tropicalization. The cycle can be decomposed into three modes.

# Conclusions

- Network with many, well separated, time scales, can be reduced to simpler networks, in a way that does not depend on the exact values of the parameters, but on their order relations.
- Tropical analysis is the natural framework for unifying various approaches to this problem and provides some heuristic, generally valid for permanent systems.
- For excitable oscillators such as the cell cycle, the reduced model is hybrid. Tropical equilibrations allow to identify the slow parts of the cycle and the tropicalization provides the description of the fast parts.
- However, a fully tropical general theory of ODEs is still missing...work in progress.

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