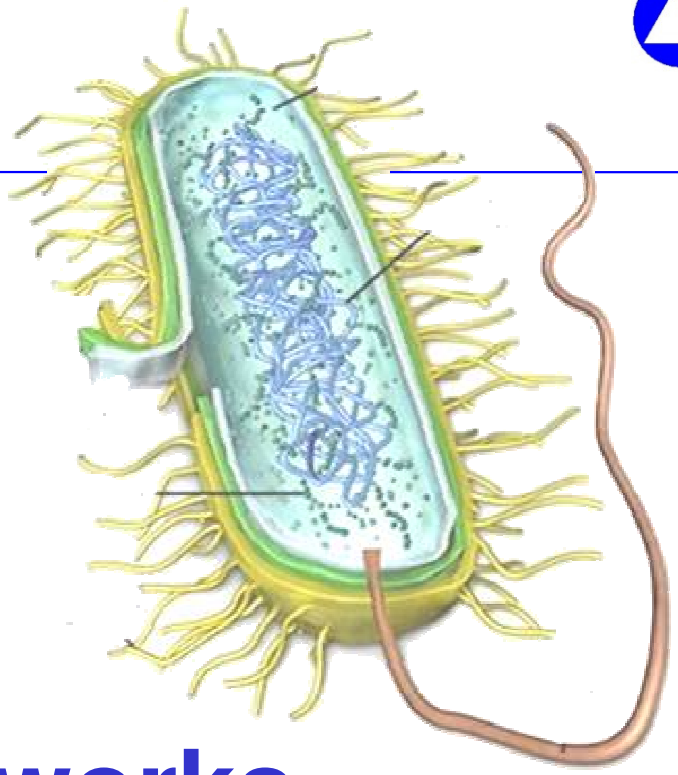
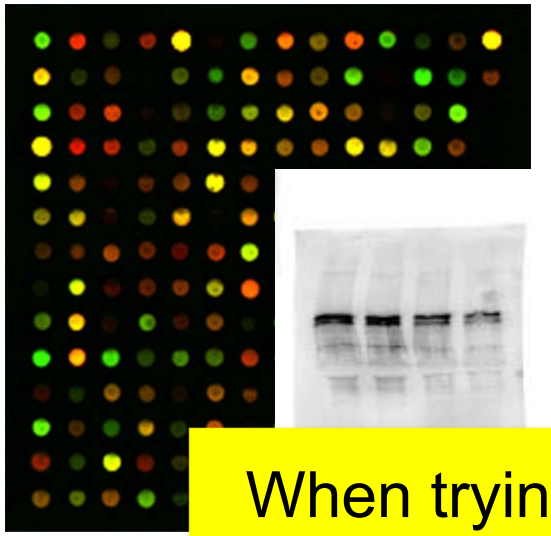
December 15, 2009



Robustness and Bifurcation Analysis of Biochemical Reaction Networks

Frank Allgöwer
Institute for Systems Theory and Automatic Control
University of Stuttgart, Germany

Structural uncertainties – measurement imprecisions



gene expr
data from
array expe

Measurements are often more qualitativ than quantitative in nature.

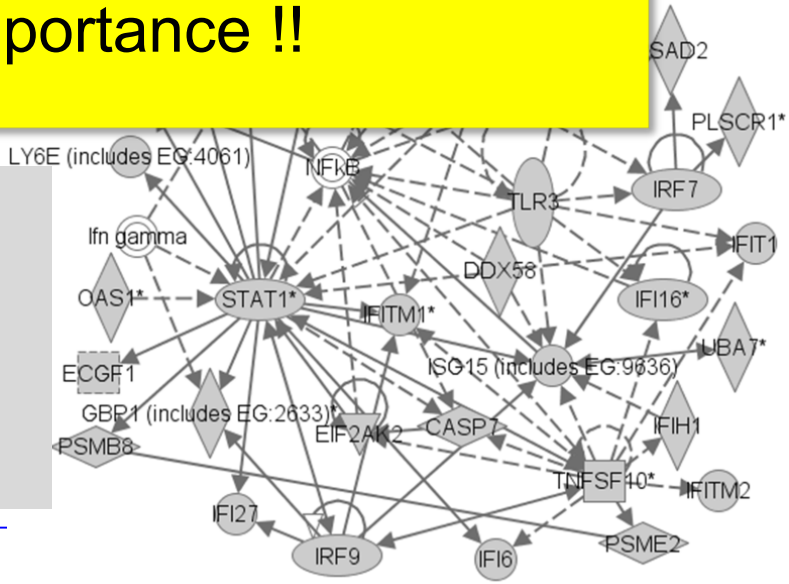
When trying to understand, model, analyse etc. biological systems the consideration of uncertainties is of major importance !!

But in addition, biological knowledge is still rather incomplete.

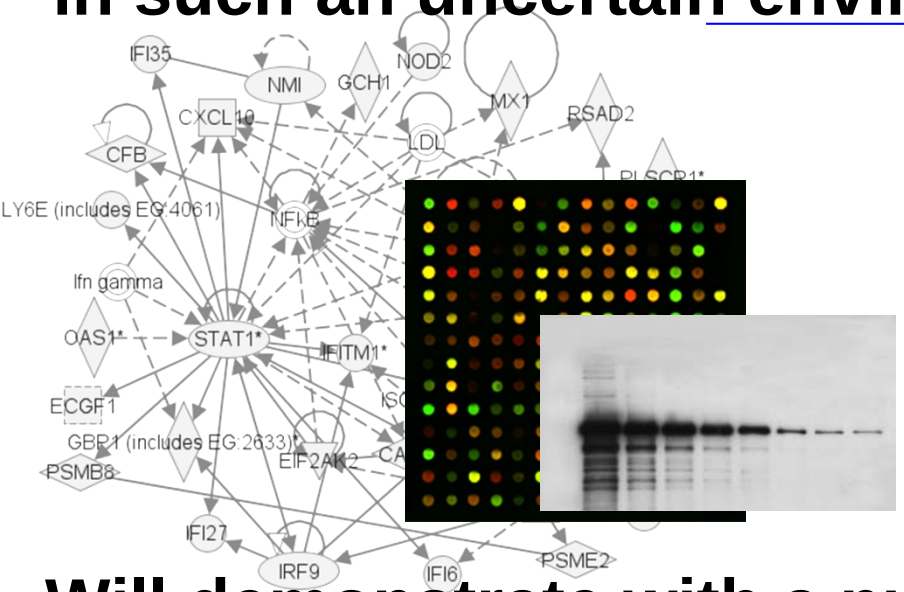
E.g.,

Involved pathways? Involved proteins?

Reaction rates? etc.pp.



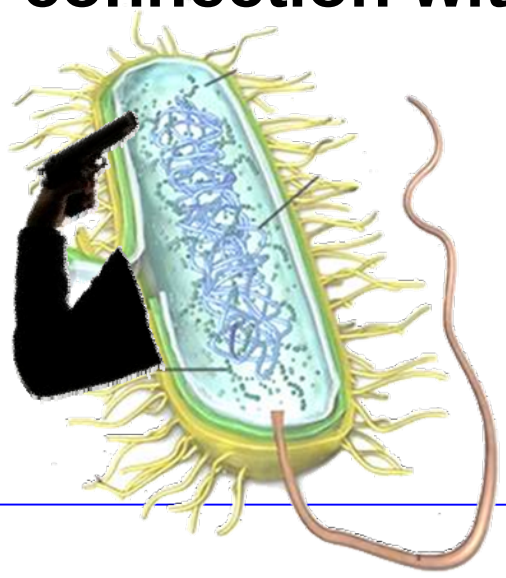
How can systems theoretic methods and tools help in such an uncertain environment?

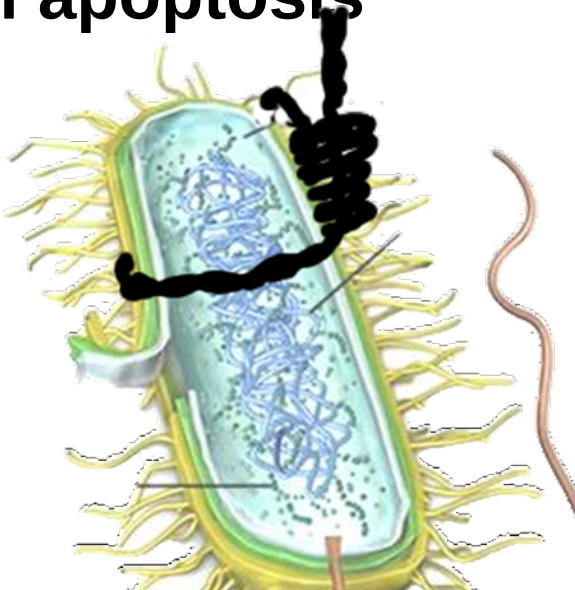


- Mathematical modelling
- Systems analysis
- Model validation/falsification
- Modification of biological function
- …

Will demonstrate with a number of examples (mostly) in connection with apoptosis

Apoptosis – Programmed Cell Death: "suicide" program present in every cell

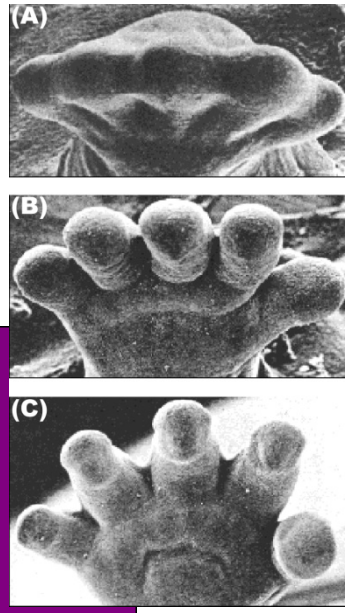


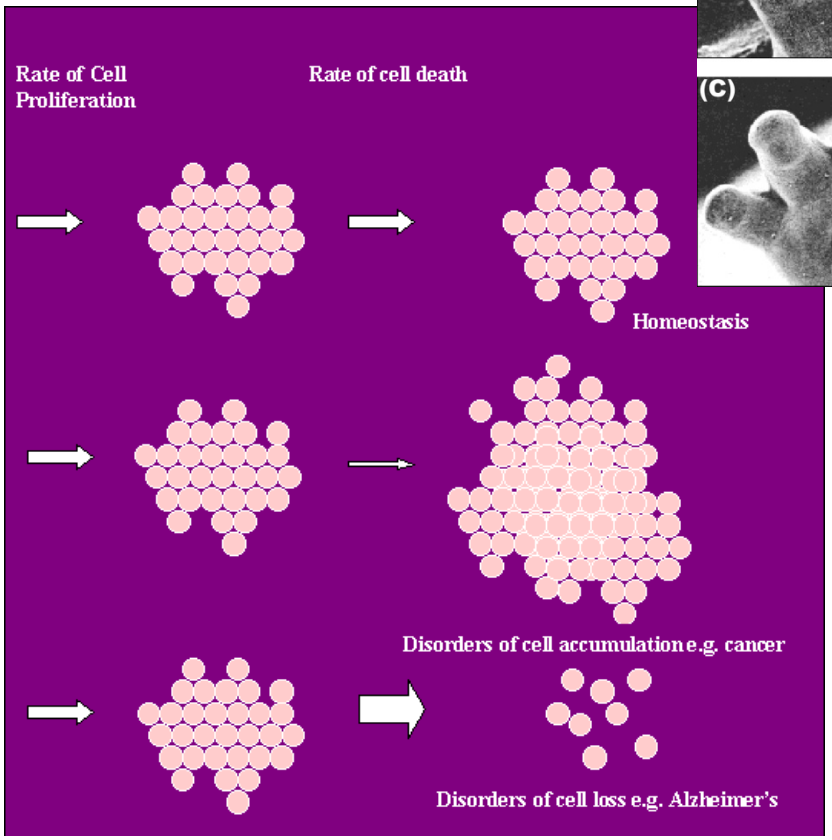


Apoptosis

Apoptosis – Programmed Cell Death

- not by accident but highly organized & **regulated**
- **caspases** at the core of the apoptotic program



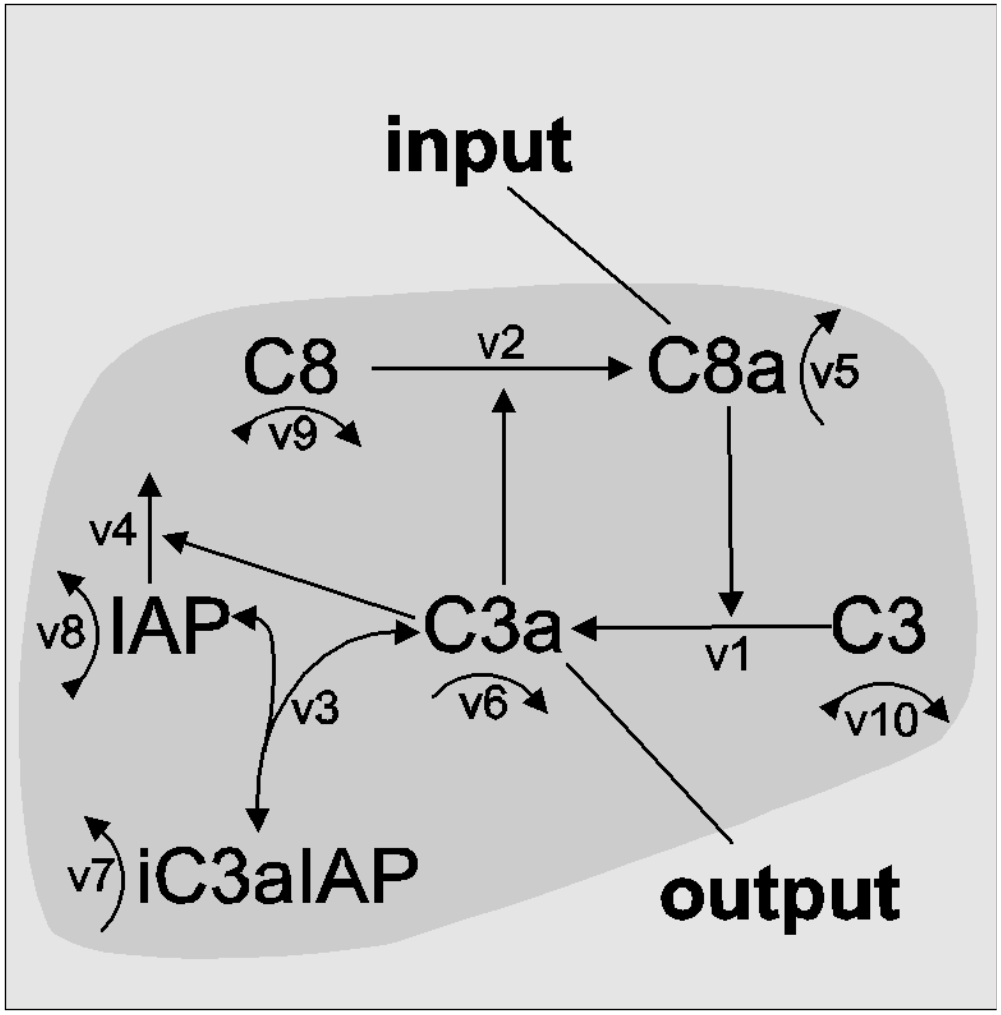


Essential for organism to remove cells that are:

- old
- no longer needed
- potentially harmful (due to mutations or infection)
- **out of control**

10 billion cells made **each day** to balance those dying by apoptosis

A Simple Model of Apoptotic Core Reactions



based on established literature

Input: C8(a)
(activated) initiator caspases

Output: C3(a)
(act.) executioner caspases

IAP: inhibitors of apoptosis proteins

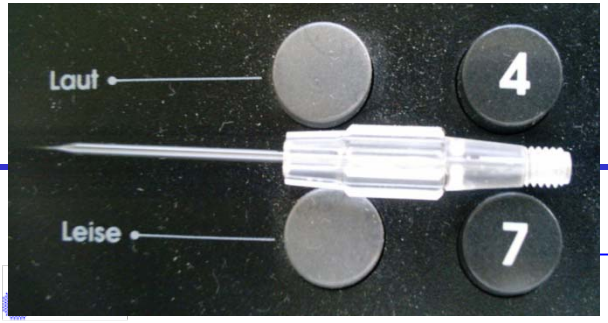
Modelling:

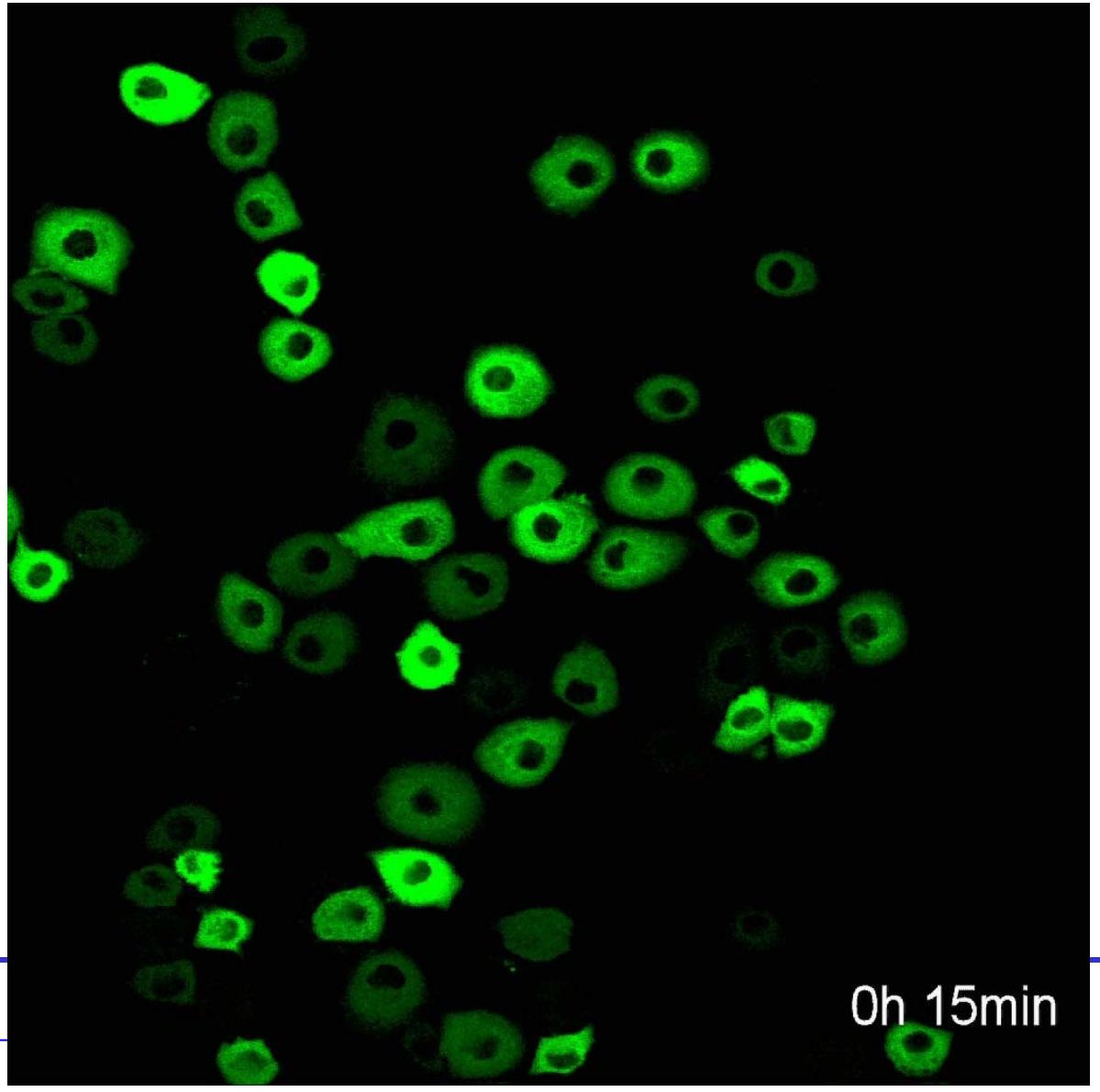
$$v_1 = k_1 \cdot C8a \cdot C3$$
$$\vdots$$
$$\frac{dC3a}{dt} = v_1 - v_3 - v_{v6}$$
$$= k_1 \cdot C8a \cdot C3$$
$$- k_3 \cdot C3a \cdot IAP$$
$$+ k_{-3} \cdot iC3aIAP$$
$$- k_6 C3a$$
$$\vdots$$

Single Cell Experiment

[Kirschbaum and Scheurich]

Central Facility for Microscopy and Image Analysis at University of Stuttgart



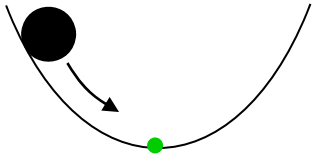


Model validation using simple bifurcation analysis

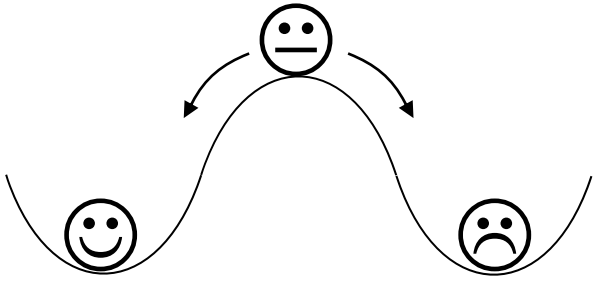
Thomas Eissing

Steady States, Stability and Apoptosis

- Apotosis is no accident!
- “activation energy” required



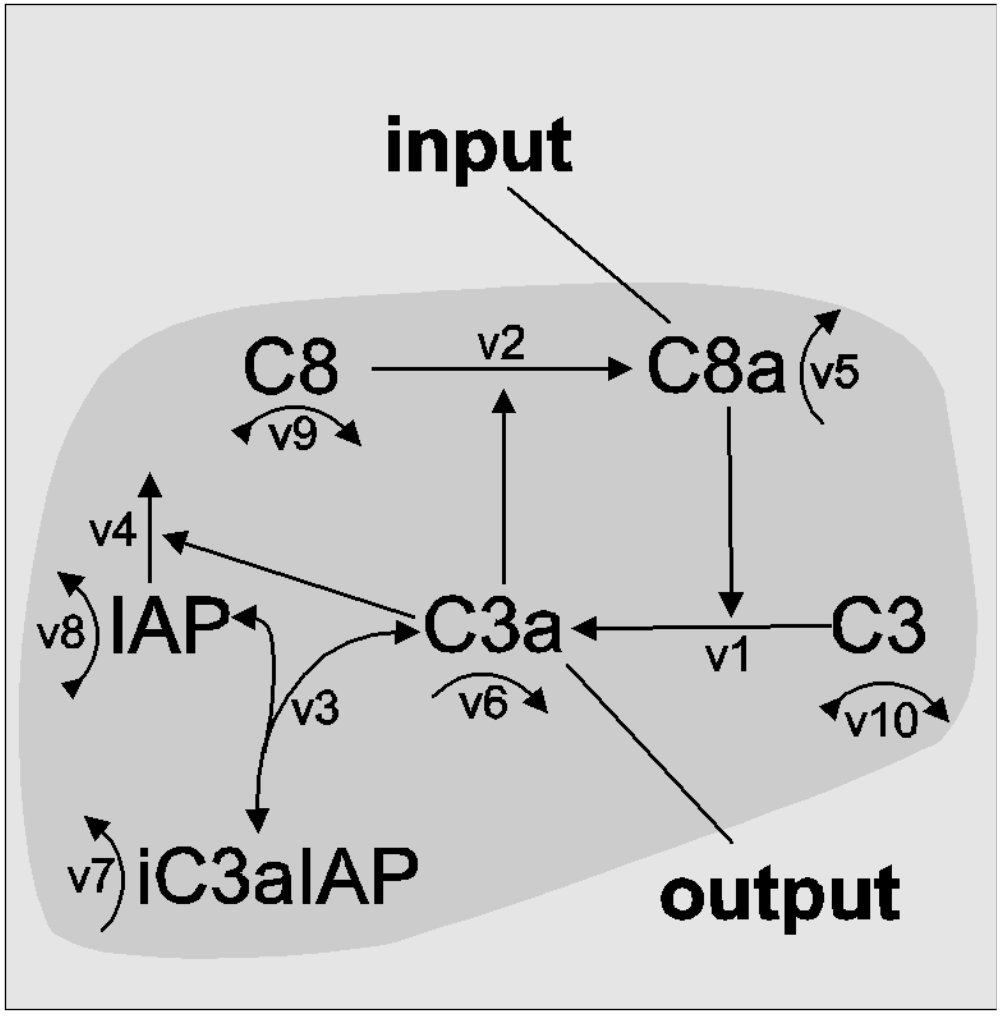
- stable steady state



we require bistability in apoptosis

- bistability in biochemical reaction networks
  [Ferrell,Angeli,Sontag,Lisman,Goldbeter,Kholodenko…]

Can the mathematical model exhibit bistable behavior?



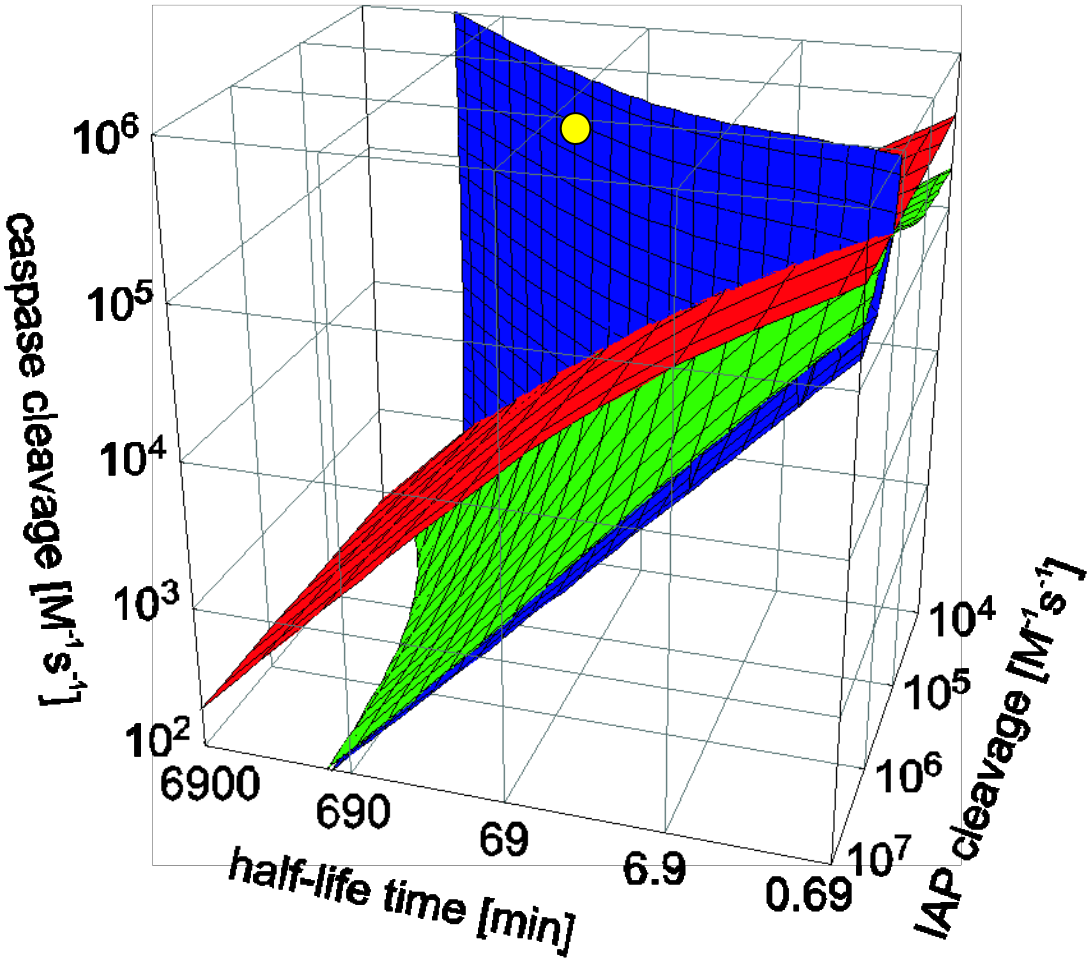
Input: C8(a)
(activated) initiator caspases

Output: C3(a)
(act.) executioner caspases

Model:

$$v_1 = k_1 \cdot C8a \cdot C3$$
$$\vdots$$
$$\frac{dC3a}{dt} = v_1 - v_3 - v_{v6}$$
$$= k_1 \cdot C8a \cdot C3 - k_3 \cdot C3a \cdot IAP + k_{-3} \cdot iC3aIAP - k_6 C3a$$
$$\vdots$$

Parameter Domain for Bistability



- bistability in dependence of parameters connected in a biologically meaningful way
- bistability: below red AND above green AND above blue area

bistability in a small parameter domain far away from literature values

Model Evaluation

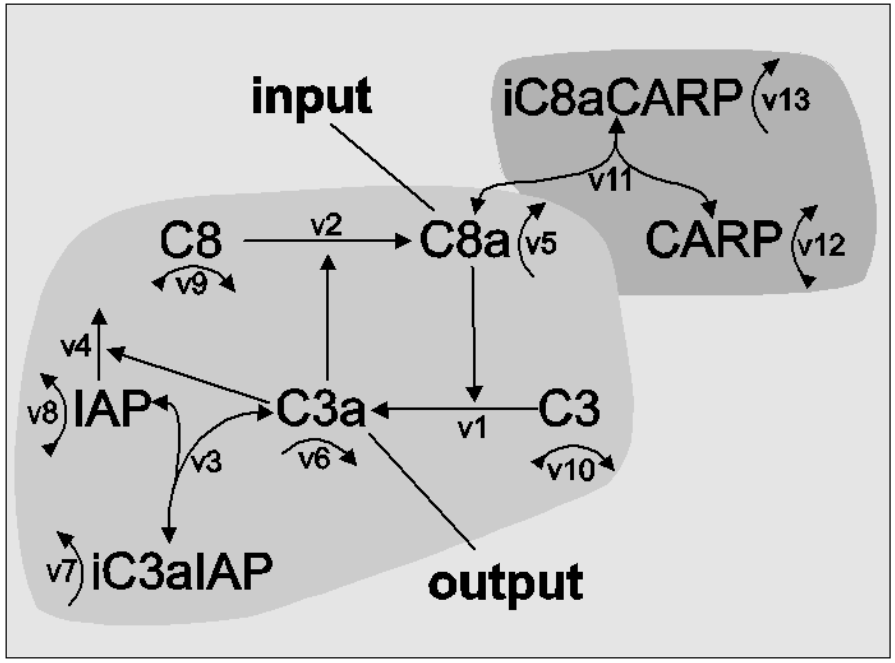
Model analysis reveals:

+ enables a bistable behavior

- parameter ranges not consistent with literature values

How to reconcile this point?

- **model analysis indicates need for control at the level of C8a**
- hypothesis: similar to IAPs
  - BAR (Stegh et al., JBC 2002) binds to and inactivates C8a?
  - extended model: 13 reactions, 8 ODEs
- **McDonald et al.**, PNAS 2004 have **now identified CARPs**



Systems theoretical analysis allows generation/verification/falsification of biological hypotheses

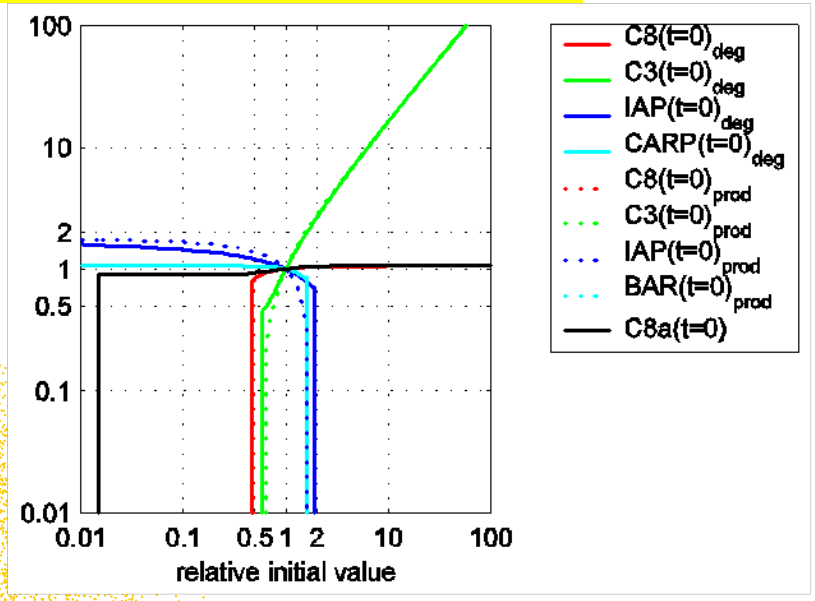
Modelling and Analysis of Apoptosis

Simple systems theoretic methods can be helpful to analyze and refine mathematical models.

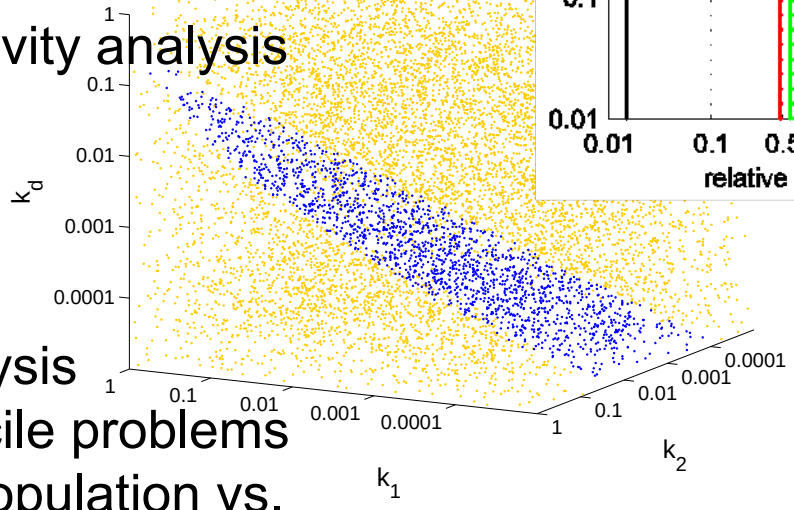
Further analysis:

- Local sensitivity analysis

$$S_{ij} = \frac{p_j}{x_{SSi}} \cdot \frac{x_{SSi}(p_j + \Delta p_j) - x_{SSi}}{\Delta p_j}, \text{ with } \Delta p_j = 1\%$$



- Regional sensitivity analysis



- Stochastic analysis allows to reconcile problems regarding cell population vs. single cells

[Eissing et al. IEEE CCA 2006, JBC 2004, IEE SB 2005]

Summary: Apoptosis Modelling

All analysis results support the hypothesis that the additional regulation of *C8a* is likely to be present in nature.

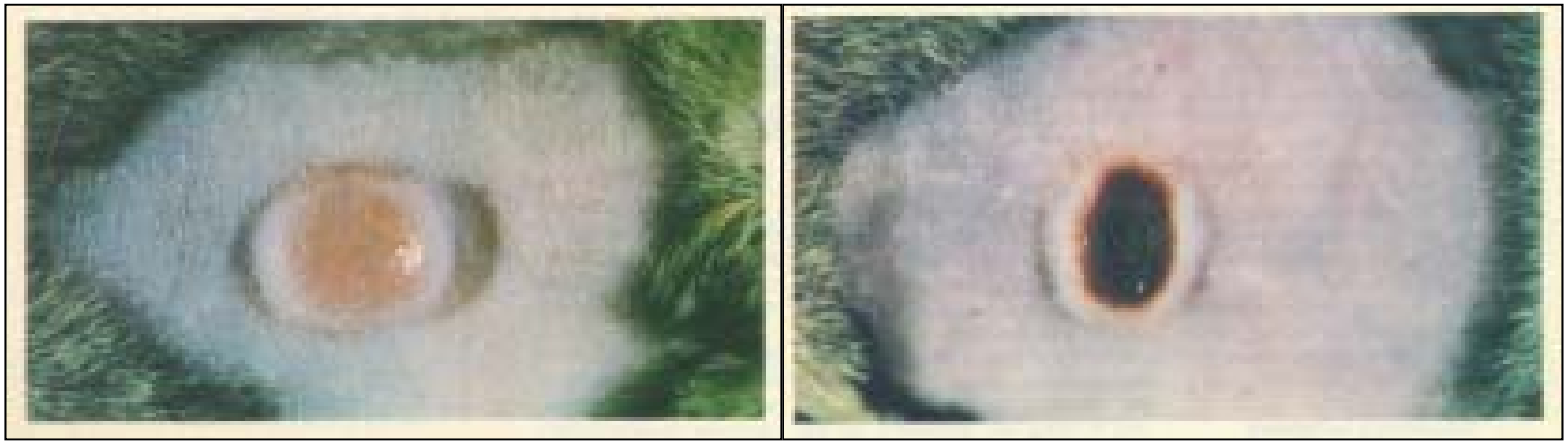
Observation: Application of fairly standard tools, applied in a smart way, allows to get meaning- and useful results.

Can be done this way, because model complexity is fairly limited in this example.

But, biological systems are in general rather complex …

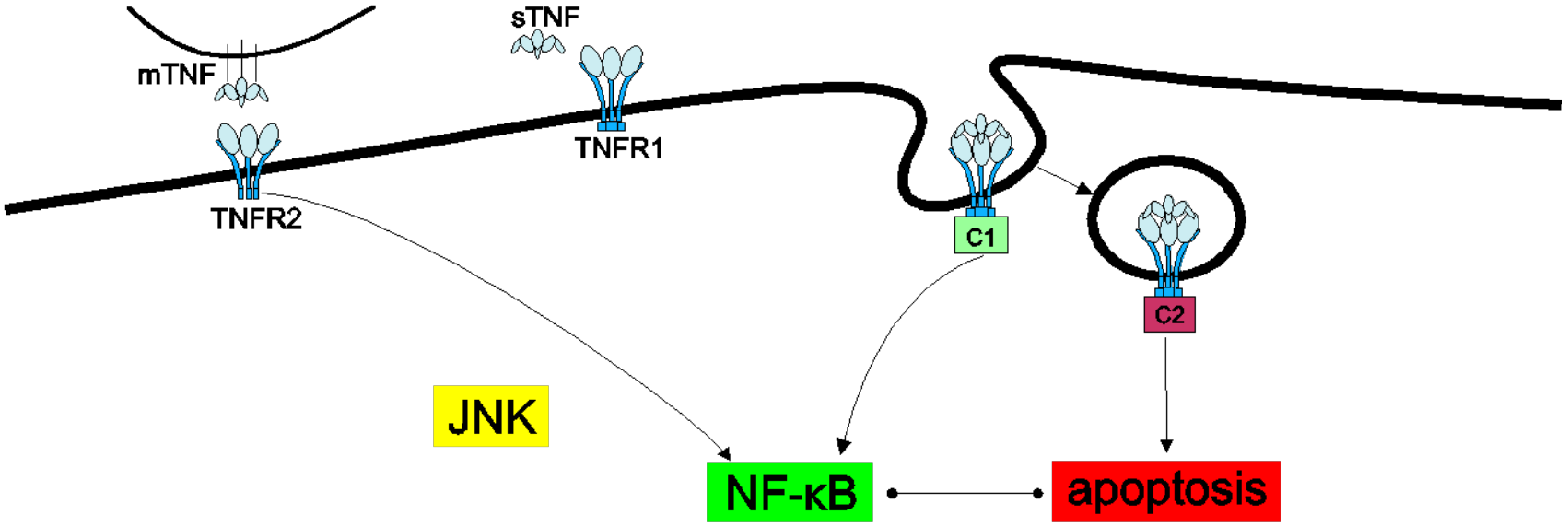
Antitumoral Effects of Tumor Necrosis Factor *in vivo*

A single injection of TNF induces a hemorrhagic necrosis of the tumor



Old, Scientific American 1988

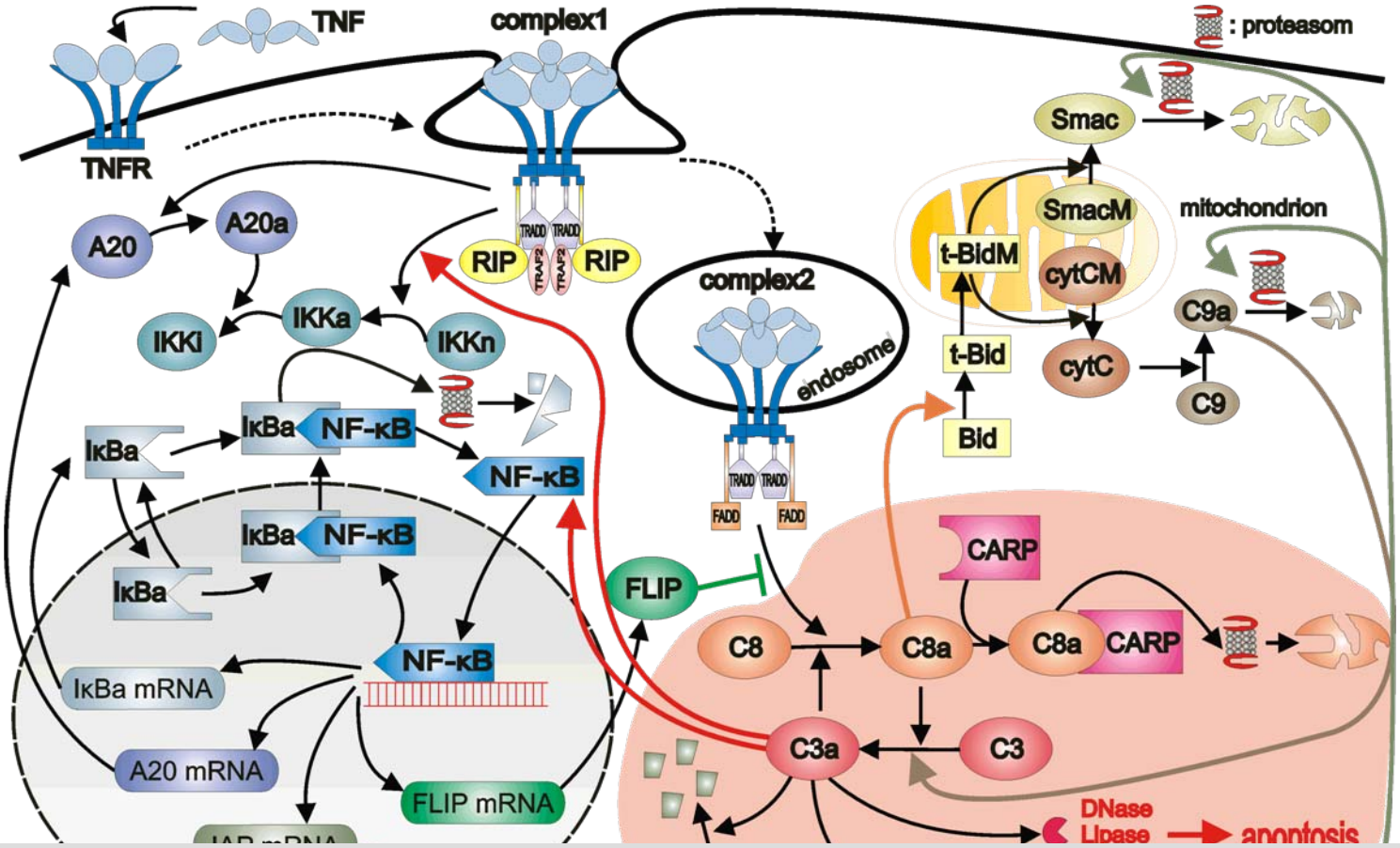
TNF Signaling Pathways



many pathways with input TNF have an effect on apoptosis:

- NF-κB: inflammation, anti-apoptotic
- Apoptosis: sacrifice the cell
- JNK: pro- and anti-apoptotic functions reported
- …

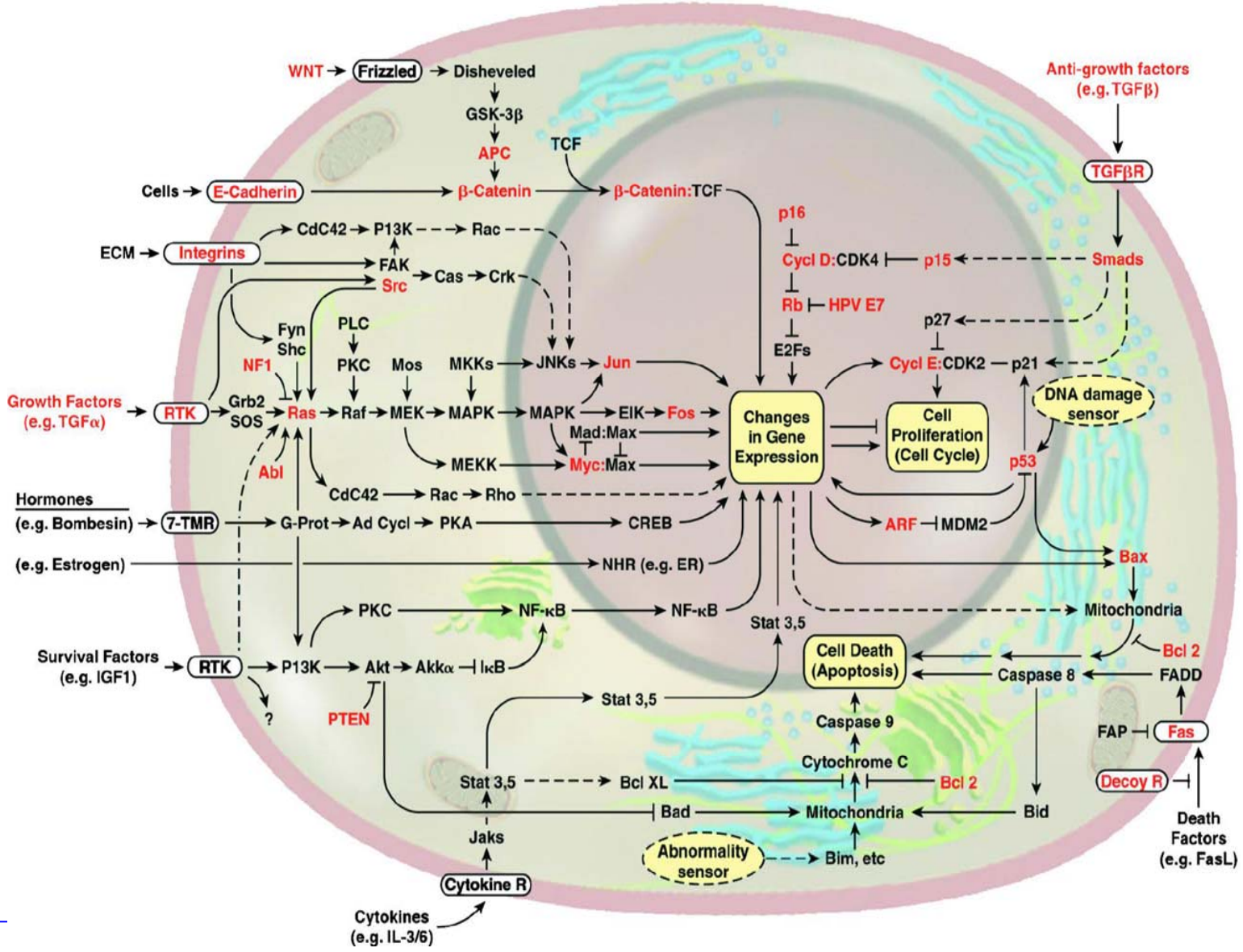
TNF Signaling Pathways



Model for combined system:

- 74 biological reactions (interactions, expression, degradation)
- 41 molecules
- 148 parameters (reaction rates) *[Schliemann et al. DA 2006]*

… and more complex

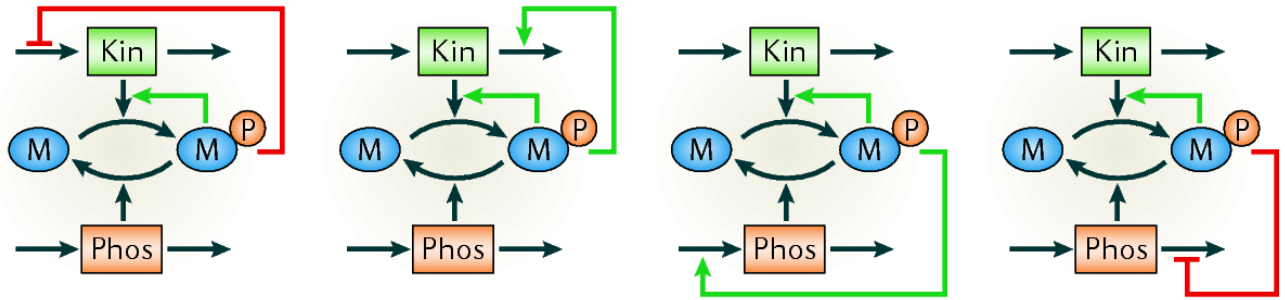


Bifurcation search in high-dimensional parameter space

Steffen Waldherr

Feedback circuits and dynamical behaviour

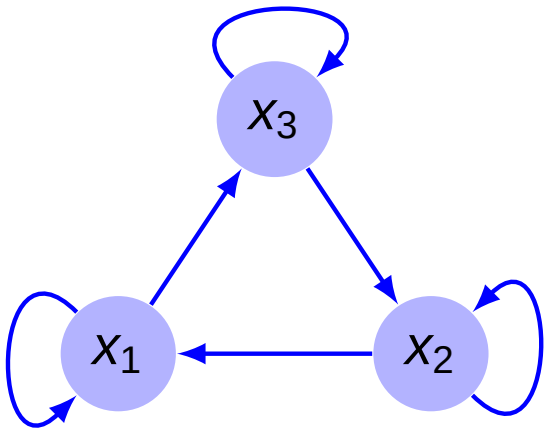
- Feedback is abundant in signal transduction networks



Kholodenko, Nature Rev. Mol. Cell Biol. 2006

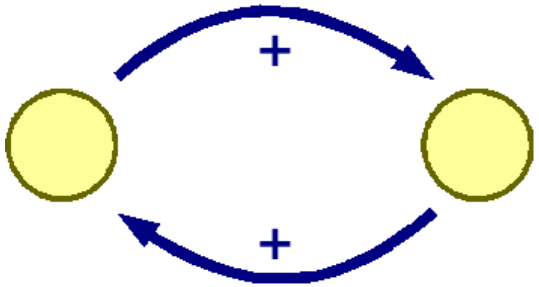
- Defining feedback circuits via the interaction graph
  - ODE $\dot{x} = F(x)$ for biochemical network
  - Jacobian matrix $\frac{\partial F}{\partial x}$ gives interaction graph

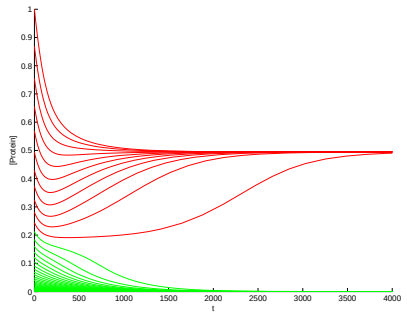
$$\begin{aligned}\dot{x}_1 &= a(x_2) - d(x_1)\\ \dot{x}_2 &= a(x_3) - d(x_2)\\ \dot{x}_3 &= a(x_1) - d(x_3)\end{aligned} \qquad \hat{=}$$



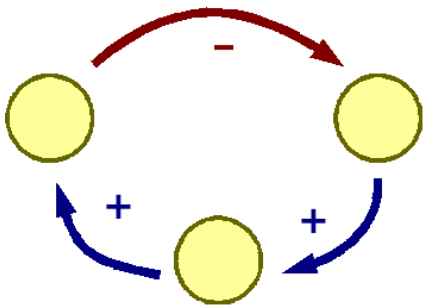
Roles of feedback circuits

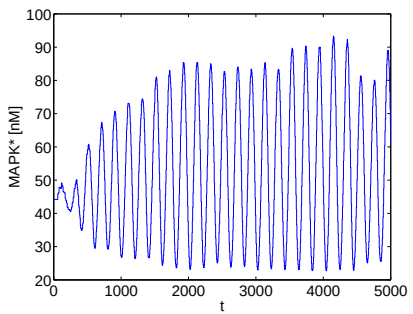
- Positive feedback enables bistability (switching).





- Negative feedback enables sustained oscillations.





Observation

- Feedback circuits enable complex dynamical behaviour.
- Parameter values are relevant for behaviour.

The loop breaking approach

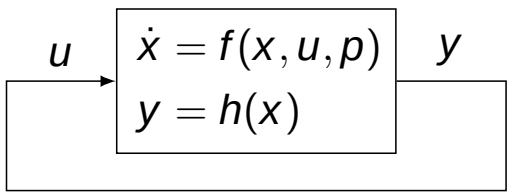
- ODE model for signalling network: $\dot{x} = Sv(x,p) = F(x,p)$

Loop breaking definition

A *loop breaking* is a pair (f,h) such that

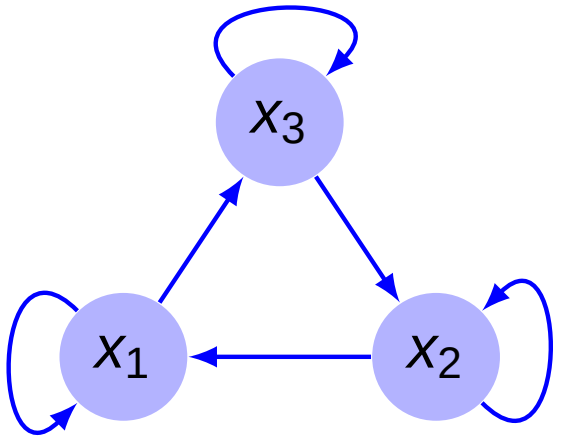
$$F(x,p) = f(x,h(x),p)$$

- Open loop system: $\dot{x} = f(x,u,p)$
  $y = h(x)$
- Closing the loop: $u = h(x)$
  $\Rightarrow$ we recover the closed loop system



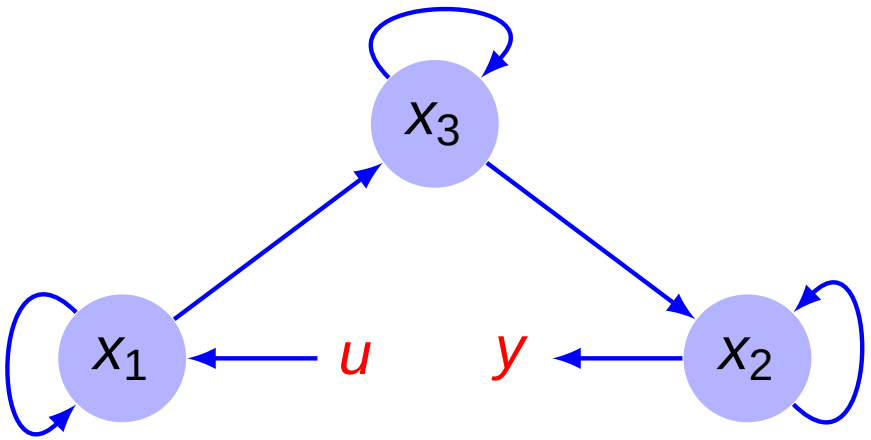
Loop breaking: example

$$\dot{x}_1 = a(x_2) - d(x_1)$$
$$\dot{x}_2 = a(x_3) - d(x_2)$$
$$\dot{x}_3 = a(x_1) - d(x_3)$$



Loop breaking: choosing $y = x_2$

$$\dot{x}_1 = a(u) - d(x_1)$$
$$\dot{x}_2 = a(x_3) - d(x_2)$$
$$\dot{x}_3 = a(x_1) - d(x_3)$$
$$y = x_2$$



What is it good for?

Loop breaking benefit

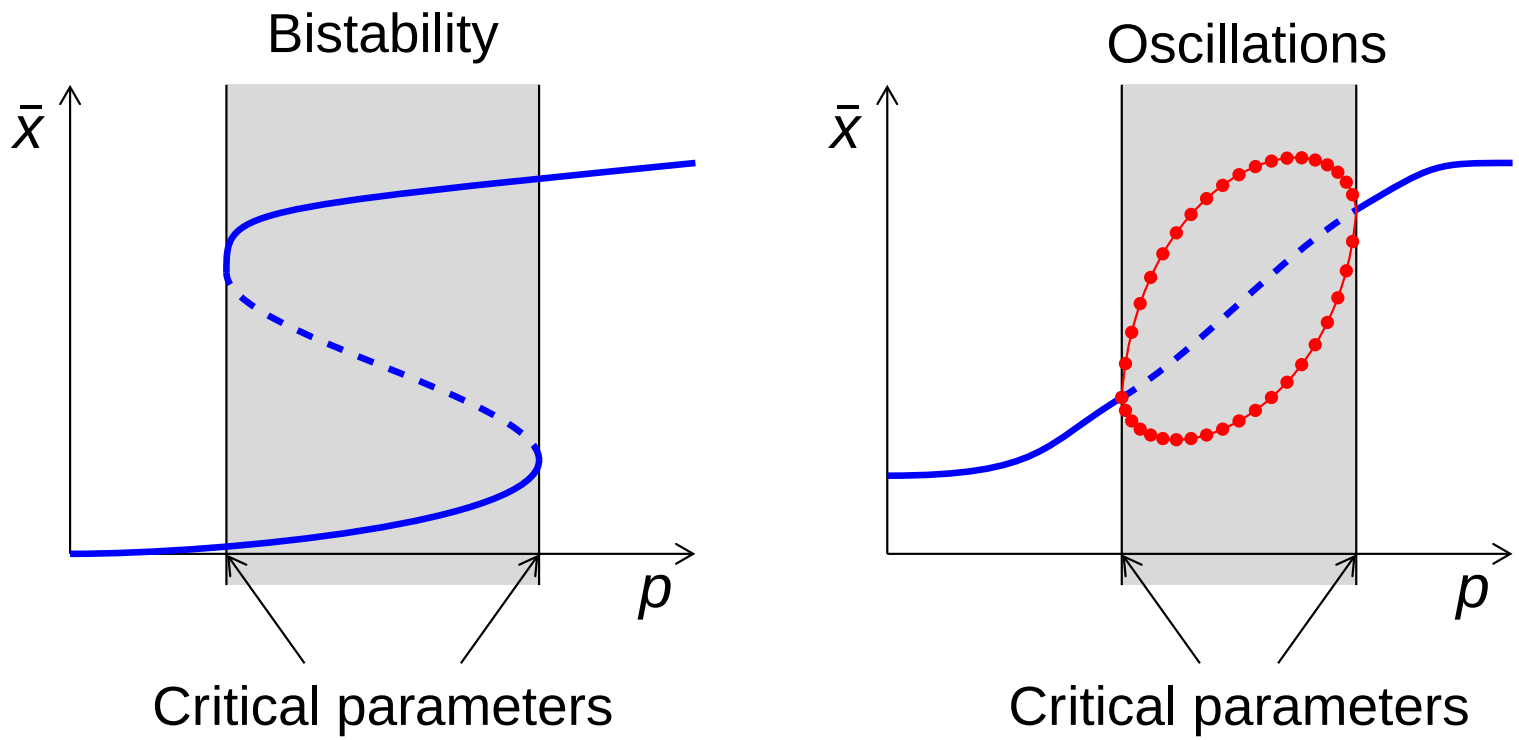
- Control theory methods for input–output systems can be used
- Dynamical behaviour is less complex for open loop system
  (e.g. no oscillations are possible)
- Loop breaking point may have biological meaning

Application

Bifurcation search in high-dimensional parameter space

- Bifurcation = qualitative change of dynamical behaviour
  - From non-oscillatory to oscillatory behaviour
  - From single stationary state to multi-stationarity

Changes in dynamical behaviour



Complex dynamical behaviour is coupled to unstable stationary points.

On the border of stability

- Change stability of stationary point $\bar{x}$ ($F(\bar{x}, p) = 0$).
- Find critical parameters in high-dimensional parameter space.

Problem statement

- Given ODE model of signalling network $\dot{x} = F(x, p)$,
- given preliminary parameters p_1 and stationary point $\bar{x}_1$,
- find parameters p_2 and stationary point $\bar{x}_2$ such that stability is different for $\bar{x}_1$ and $\bar{x}_2$.

- Critical parameter vector p_c on any path between p_1 and p_2.

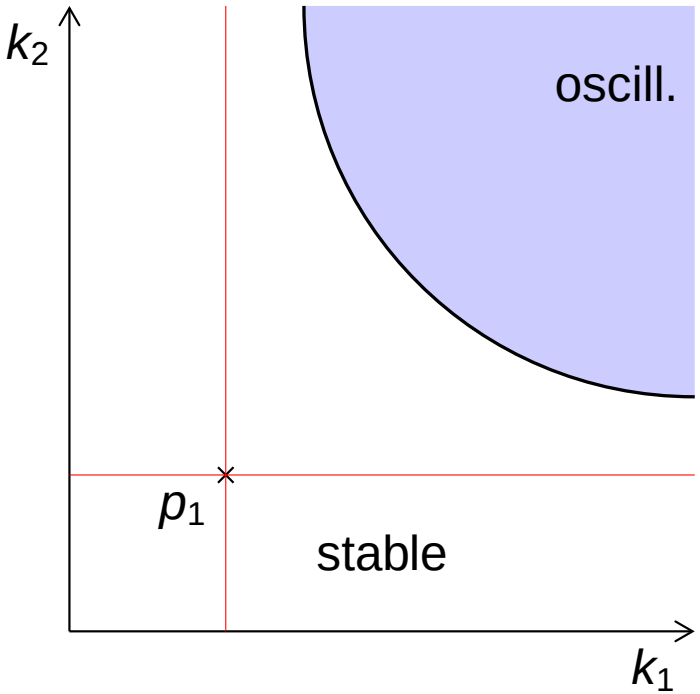
Classical approach

Vary one parameter at a time and do bifurcation analysis

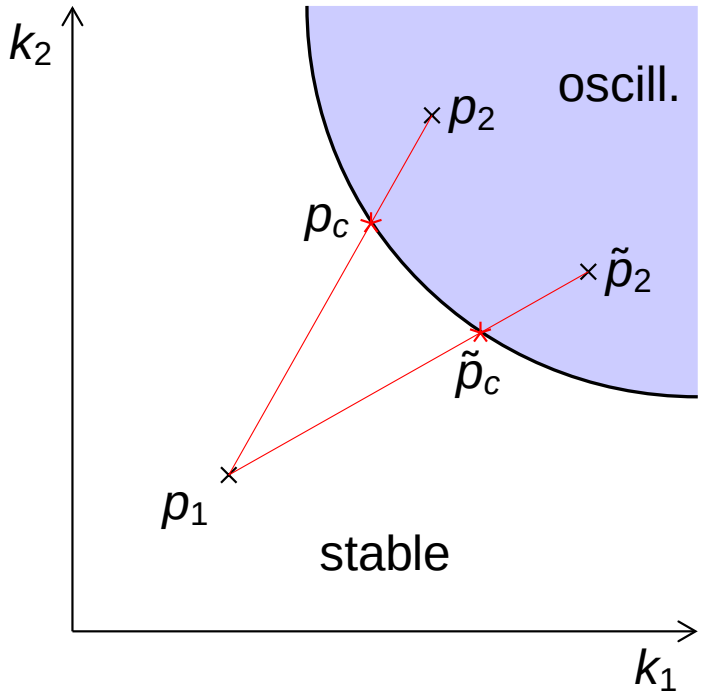
numerically via e.g. continuation methods

Our approach: multi-parametric variations

Classical approach



Our approach

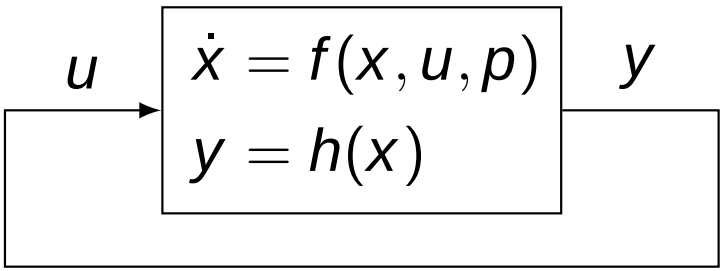


Characteristics (problems)

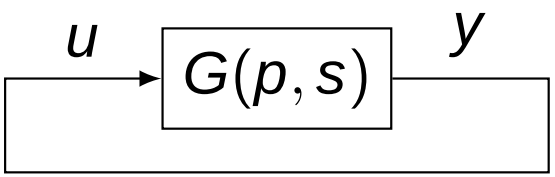
- critical parameter vector p_c is not unique
- different dynamic regimes may exist

Control engineering approach

- Use loop breaking to reformulate problem



- Linear approximation around stationary point $\bar{x}(p)$ and Laplace transformation to frequency domain



Result of reformulation

- Transfer function $G(s,p)$ describes **open loop** characteristics
- Control theory (e.g. Nyquist criterion) can be used to check local stability of the **closed loop** system

Theoretical results

- Define critical frequencies ω_c: $G(p, j\omega_c) \in \mathbb{R}$ (polynomial equation for ω_c) $\Rightarrow$ solution branches $\omega_c(p)$

Theorem

- There exists a critical parameter vector p_c, if and only if there exist parameters p_1 and p_2 such that

$$G(p_1, j\omega_c(p_1)) \leq 1 \leq G(p_2, j\omega_c(p_2)).$$

- In this case, $j\omega_c(p_c)$ is an eigenvalue of the closed loop system.

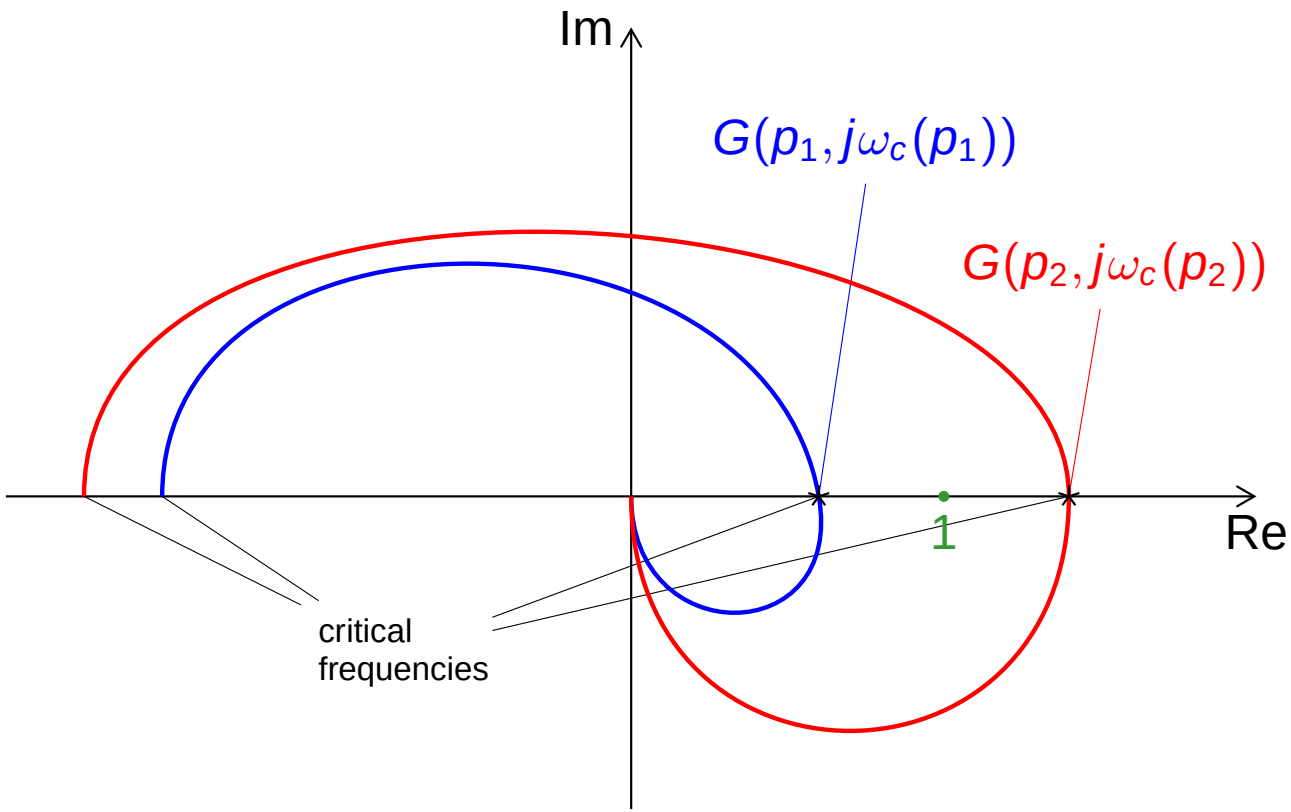
- Some technical assumptions required, but not very restrictive.

Remarks

- One of p_1, p_2 given by preliminary parameters.

Illustration in the Nyquist plot

- Plot of $G(p, j\omega)$ for $0 \leq \omega < \infty$ in the complex plane



$\Rightarrow$ $\bar{x}(p_1)$ and $\bar{x}(p_2)$ have different stability properties

Numerics: iterative parameter search

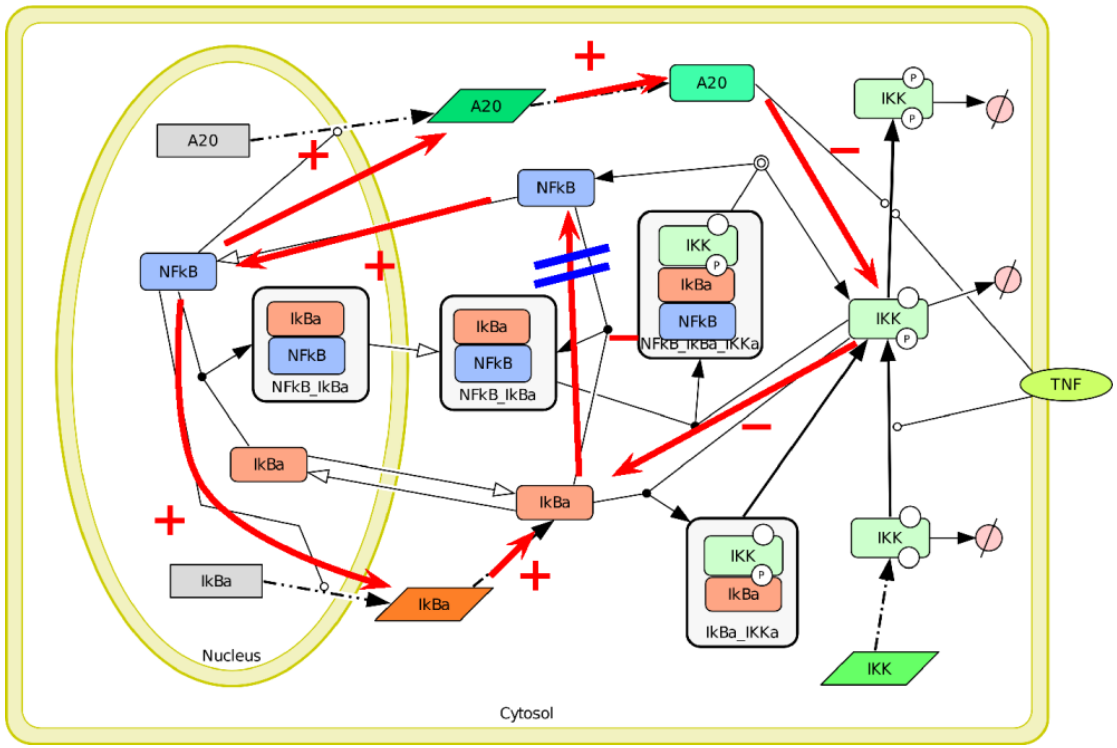
- Given p_1 where $G(p_1, j\omega_c(p_1)) < 1$,
  find p_2 such that $G(p_2, j\omega_c(p_2)) > 1$
- Approach: Track a path p_μ in parameter space such that $G(p_\mu, j\omega_c(p_\mu))$ changes in the desired way
- Basically continuation, but use gradient of $G(p, j\omega_c(p))$ to decide which direction in parameter space to go

Iterative algorithm

1. *Parameter update:* Change parameters along the gradient of $G(p, j\omega_c(p))$ to change $G(p, j\omega_c(p))$ towards the point 1.
2. *Stationary state tracking:* Solve $F(\bar{x}, p) = 0$ and recompute new $\omega_c(p)$
3. *Iterate* or finish if 1 has been crossed

Works well for medium-sized systems.

NFκB signalling

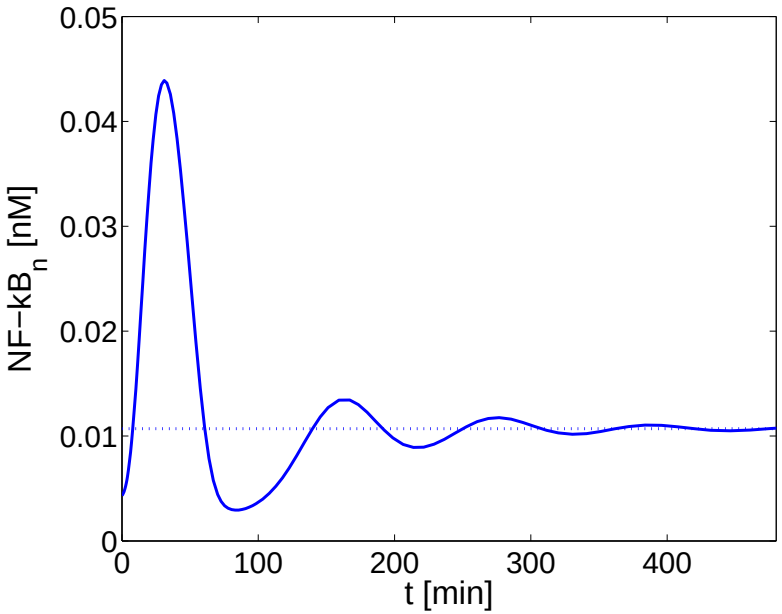


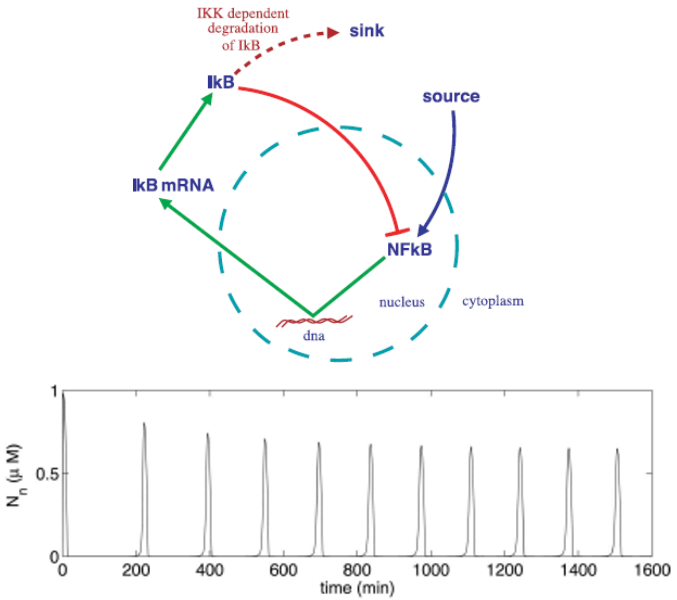
Model summary

- 14 dynamic state variables, 25 reaction parameters
- Model from Lipniacki *et al.*, Journal of Theoretical Biology 2004

Analysis of dynamical behaviour in NFκB signalling

- The original model is globally stable with damped oscillations.
- A simplistic model without the outer feedback loop has sustained, spiky oscillations.





Krishna et al., PNAS 2006

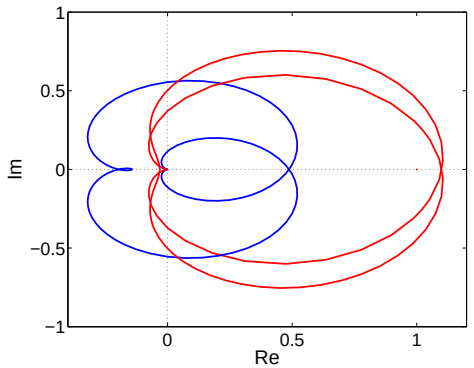
Questions

- Can the more complex model also show oscillations?
- If yes, which parameter changes are required?

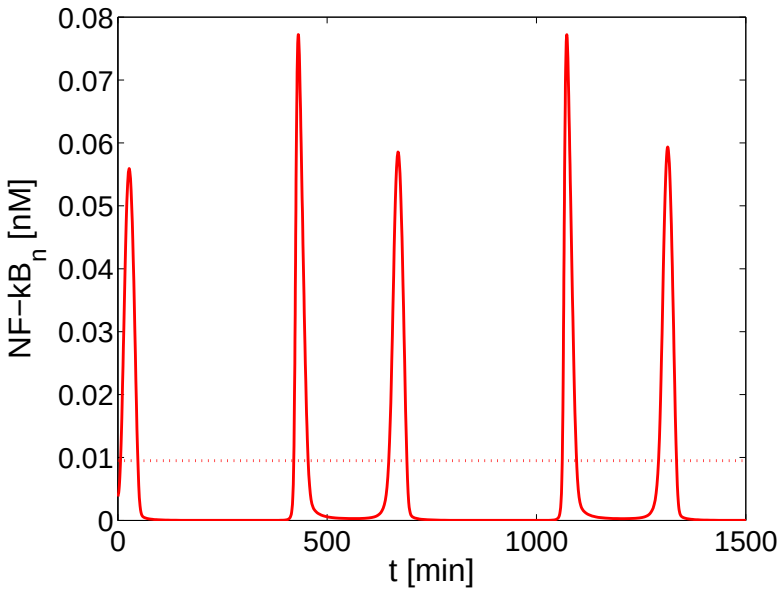
Application of loop breaking to NF-κB signalling

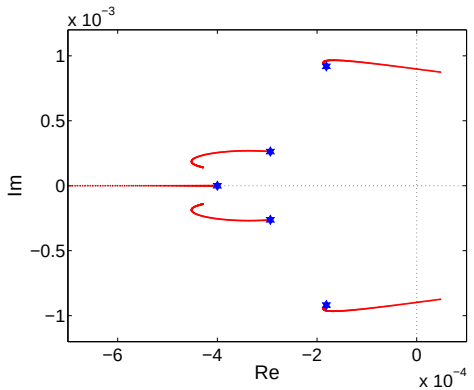
- Loop breaking point is chosen such that the two major feedback circuits are broken simultaneously.
- Finds parameter point such that the equilibrium becomes unstable with imaginary eigenvalues ($\omega_c = 9 \cdot 10^{-4} \frac{1}{\mathrm{s}}$).
- Suggested main parameter variations:
  - Increase turnover of NF-κB target genes involved in the feedback circuits by factors 7 and 3.
  - Increase turnover of the NF-κB activating kinase IKK by factor 3.

Results: oscillations in NFκB



Nyquist curves





Closed loop eigenvalues

Results

- Sustained oscillations are possible with physiological parameter values
- Additional negative feedback circuit does not necessarily destroy spiky oscillations

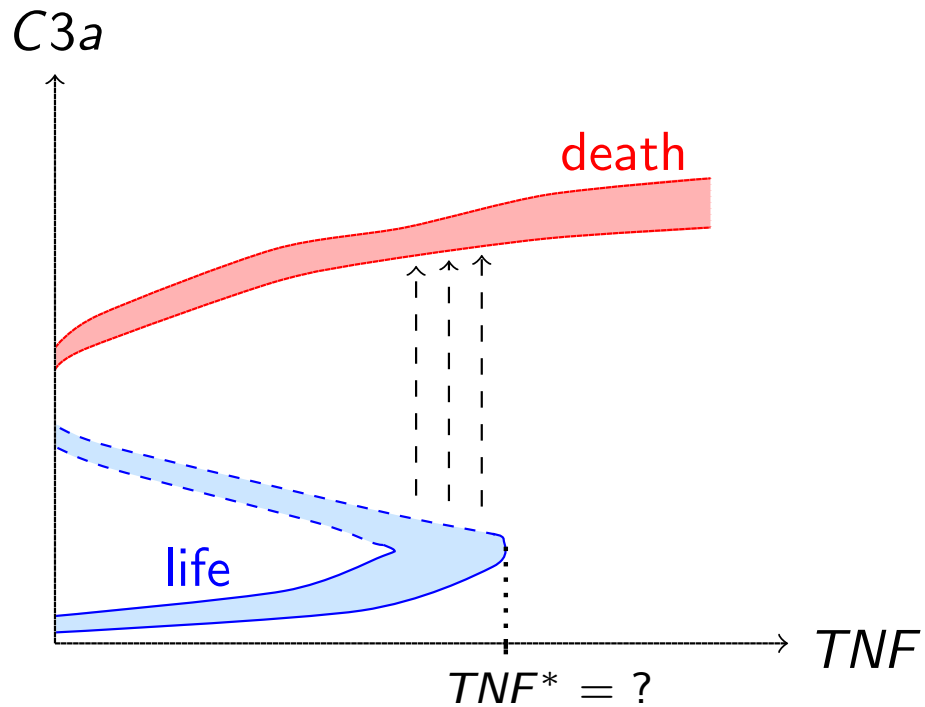
Global sensitivity analysis under uncertainty

Jan Hasenauer

Motivation

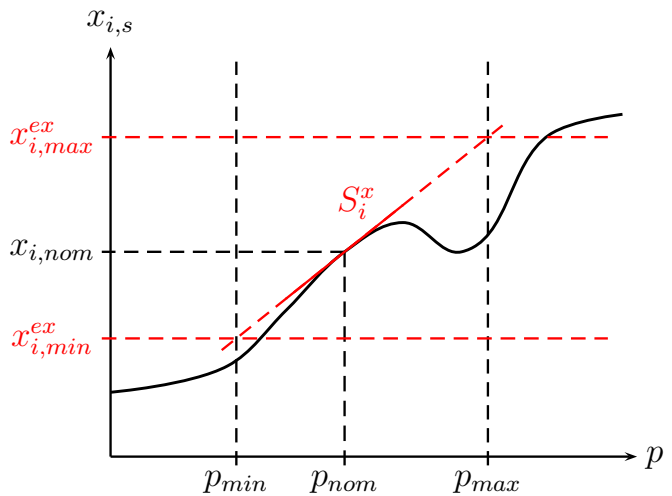
Question: Which TNF concentration is required to force all cancer cells into apoptosis?

Answer: TNF^*



Problem: Cancer cells show high mutation rates and are hence uncertain systems.

Motivation: Local vs. Global Uncertainty Analysis



General Problem:

Chemical and biochemical signalling pathways often show large modelling uncertainties.

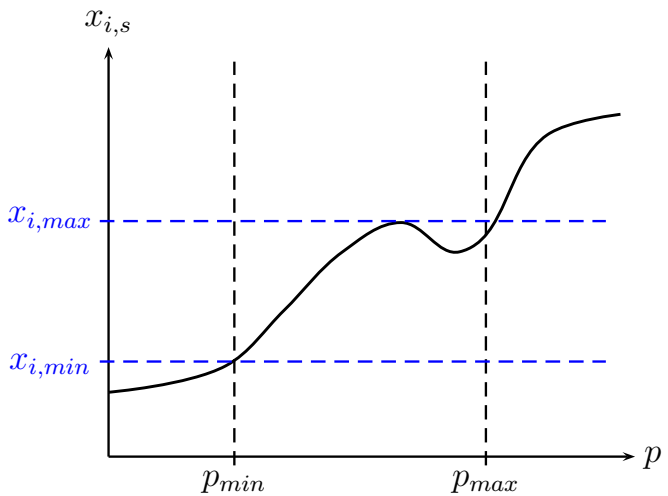
Basic Question:

How do uncertainties influence the predictions?

Uncertainty analysis for the steady states

- Local analysis: approximation at the nominal values
  $\Rightarrow$ boundaries obtained by extrapolation.
- Global analysis: computation for a parameter set, $\mathcal{P} = [p_{min}, p_{max}]$
  $\Rightarrow$ boundaries hold for $p \in \mathcal{P}$.

Motivation: Local vs. Global Uncertainty Analysis



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System Class: Biochemical Reaction Networks

System class

We consider systems of ordinary differential equation,

$$\dot{x} = S \cdot v(x, p), \qquad x(0) = x_0,$$

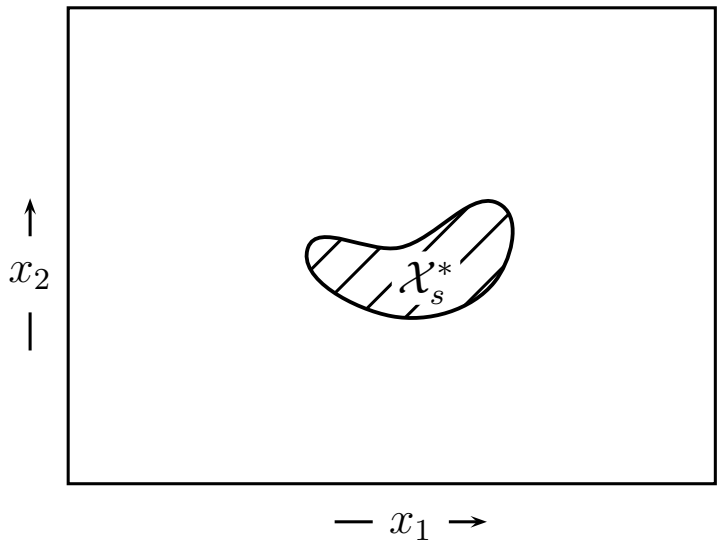
where $x \in \mathbb{R}^n$ is the concentration vector, $S \in \mathbb{R}^{n \times m}$ the stoichiometric matrix, $v(x, p) \in \mathbb{R}^m$ the reaction flux vector and $p \in \mathbb{R}^q$ the vector of independent parameters.

Steady state

The steady states x_s of such systems are defined by

$$0 = S \cdot v(x_s, p).$$

Problem Description

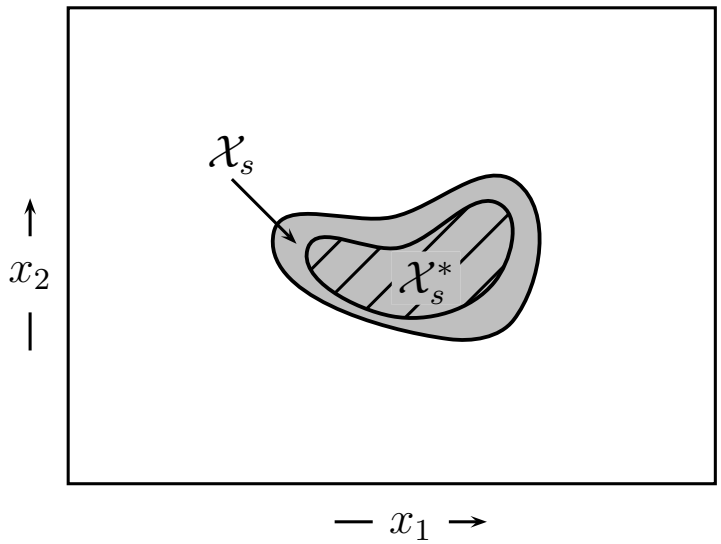


Problem: Compute set of feasible steady states

Compute for a given set $\mathcal{P} \subset \mathbb{R}^q$ the smallest subset $\mathcal{X}_s^* \subset \mathbb{R}^n$ of the state space, which contains all solutions x_s of $0 = S \cdot v(x_s, p)$, for $p \in \mathcal{P}$. Hence

$$\mathcal{X}_s^* = \{x_s \in \mathbb{R}^n | \exists\, p \in \mathcal{P} : S \cdot v(x_s, p) = 0\}\,.$$

Problem Description



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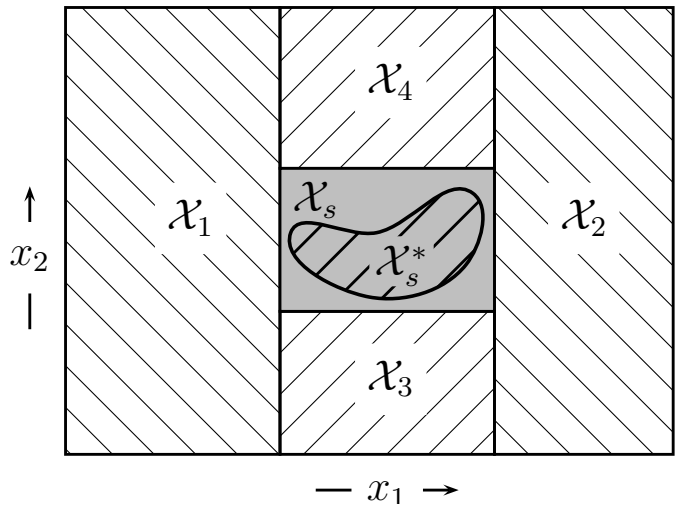
$$\mathcal{X}_s \supset \{x_s \in \mathbb{R}^n | \exists\, p \in \mathcal{P} : S \cdot v(x_s, p) = 0\}\,.$$

$\mathcal{X}_s$ as an outer approximation of $\mathcal{X}_s^* \iff \mathcal{X}_s \supset \mathcal{X}_s^*$

Formulation as Feasibility Problem



$\mathcal{X}_s^*$ = set of feasible steady states of an uncertain system (in general not computable analytically!)

$\mathcal{X}_i$ = set for which infeasibility certificates can be computed

$\mathcal{X}_s$ = obtained outer approximation of $\mathcal{X}_s^*$

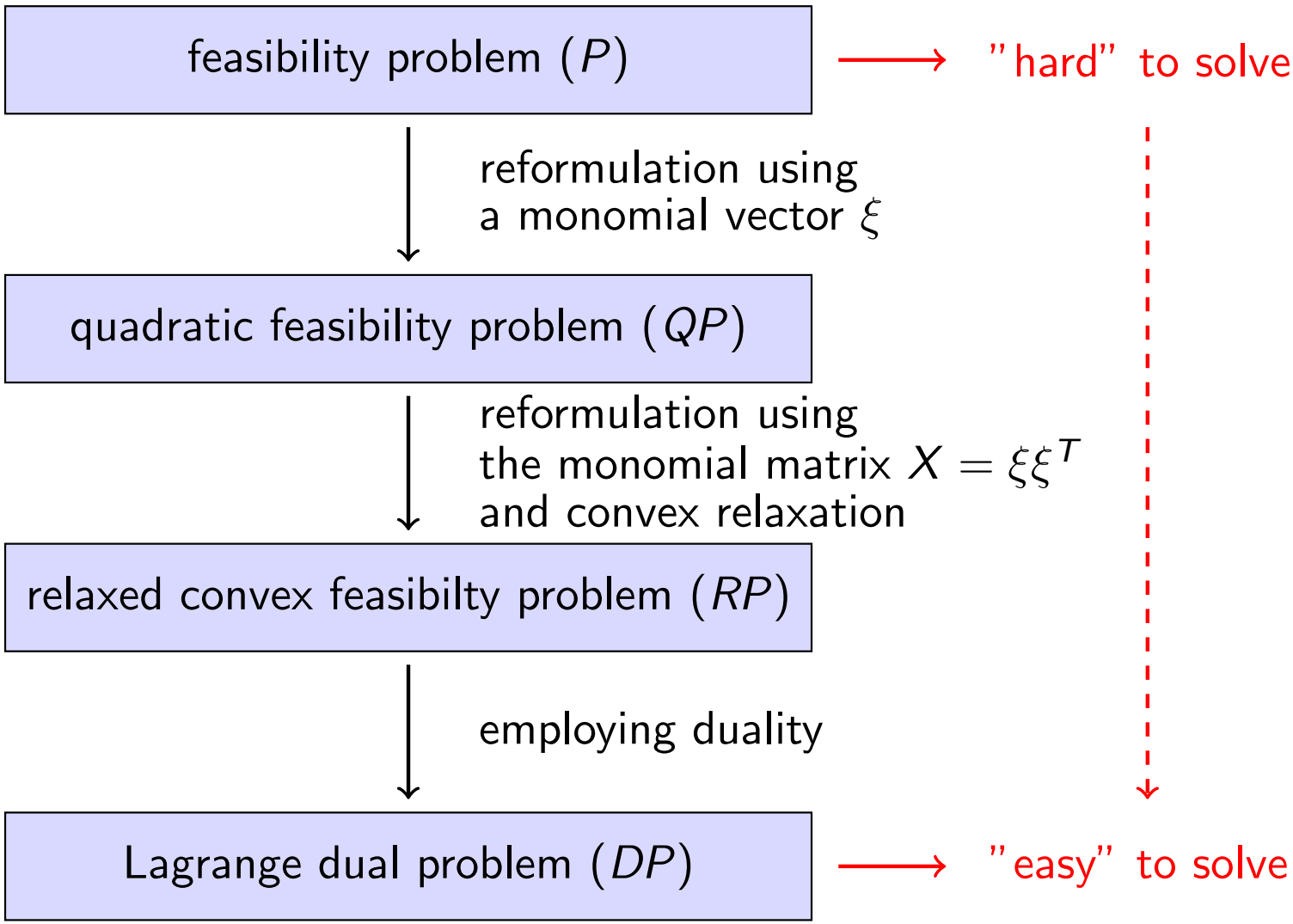
Feasibility problem

- verification that a set $\mathcal{X}$ can not contain steady states
- feasibility problem:

$$(P): \begin{cases} \text{find} & x \in \mathcal{X},\ p \in \mathcal{P} \\ \text{such that} & S \cdot v(x,p) = 0. \end{cases}$$

(P) infeasible $\Longleftrightarrow$ $\mathcal{X}$ does not contain steady states for $p \in \mathcal{P}$.

Summary: Reformulation of the Feasibility Problem



Quadratic Decomposition

Reformulation of steady state condition

- Assumption: $v_j(x,p) = r_j \prod_{k=1}^{n} x_k^{\sigma_{jk}} \quad j = 1,\ldots,m$
- Steady state definition: $0 = f(x,p) = S \cdot v(x,p)$
$$\iff \quad 0 = f_i(x,p) = \xi^T Q_i \xi, \quad i = 1,\ldots,n,$$
- Monomial vector: $\xi^T = (1, p_1, \ldots, p_q, x_1, \ldots, x_n, p_1 x_1, \ldots) \in \mathbb{R}^\kappa$

Quadratic feasibility problem

$$(QP): \begin{cases} \text{find} & \xi \in \mathbb{R}^\kappa \\ \text{subject to} & \xi^T Q_i \xi = 0 \quad i = 1,\ldots,n \\ & B\,\xi \geq 0 \\ & \xi_1 = 1. \end{cases}$$

in which $x \in \mathcal{X}$, $p \in \mathcal{P} \iff B\,\xi \geq 0$ with $B = B(\mathcal{X},\mathcal{P})$.

(QP) infeasible $\iff$ (P) infeasible.

Convex Relaxation

Feasibility problem

- Symmetric monomial matrix: $X = \xi\xi^T$
- Feasibility problem in X:

$$(\widetilde{QP}): \begin{cases} \text{find} & X \in \mathcal{S}^\kappa \\ \text{subject to} & tr(Q_i X) = 0 \quad i = 1, \ldots, n \\ & BXe_1 \geq 0 \\ & tr(e_1 e_1^T X) = 1 \\ & rank(X) = 1 \end{cases}$$

in which $e_1 = [1,\ 0,\ \ldots,\ 0]^T$

Convex Relaxation

Relaxed feasibility problem

- Symmetric monomial matrix: $X = \xi\xi^T$
- Feasibility problem in X:

$$(RP): \begin{cases} \text{find} & X \in \mathcal{S}^\kappa & \\ \text{subject to} & tr(Q_i X) = 0 & i = 1, \ldots, n \\ & BXe_1 \geq 0 & \\ & tr(e_1 e_1^T X) = 1 & \\ & \cancel{rank(X) = 1} & X \succcurlyeq 0 \end{cases}$$

in which $e_1 = [1,\ 0,\ \ldots,\ 0]^T$

Effect of relaxation

(RP) infeasible $\Longrightarrow$ (QP) infeasible $\Longleftrightarrow$ (P) infeasible.

Lagrange Dual Problem

Lagrange dual problem

$$(DP): \begin{cases} \text{maximize} & \nu_1 \\ \text{subject to} & e_1 \lambda_1^T B + e_1^T \lambda_1^T B^T + \lambda_2 \\ & + \nu_1 \, e_1 e_1^T + \sum_{i=1}^{n} \nu_{2,i} \, Q_i = 0 \\ & \lambda_1 \geq 0, \; \lambda_2 \succcurlyeq 0 \end{cases}$$

- Lagrange multipliers: $\lambda_1 \in \mathbb{R}^{2(\kappa-1)}$, $\lambda_2 \in \mathcal{S}^{\kappa}$, $\nu_1 \in \mathbb{R}$ and $\nu_2 \in \mathbb{R}^n$
- (DP) is a semidefinite optimization problem
  $\Longrightarrow$ efficiently solvable

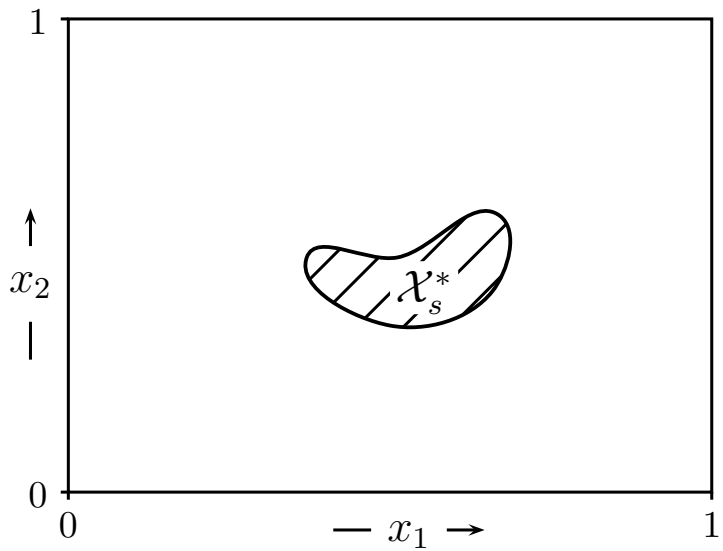
Theorem

(DP) unbounded above $\Longrightarrow$ (RP) infeasible $\Longrightarrow$ (P) infeasible.
$\Longrightarrow$ *analysis of (DP) to verify that $\mathcal{X}$ can not contain steady states.*

Computation of the Set of Feasible Steady States

Algorithm

- computation of $\mathcal{X}_s$ based on a bisection algorithm
- in each bisection step the matrix $B(\mathcal{X}, \mathcal{P})$, with $B\,\xi \geq 0$, is modified
- lower and upper bounds for all state variables known initially



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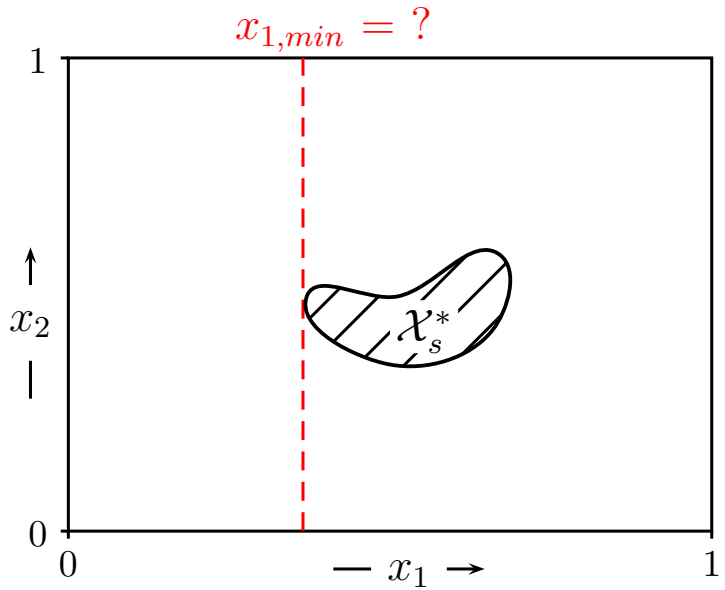

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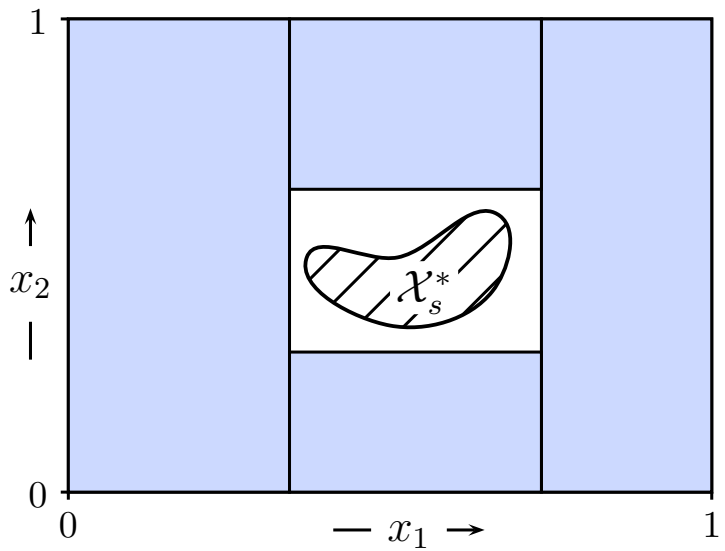
Computation of other boundaries



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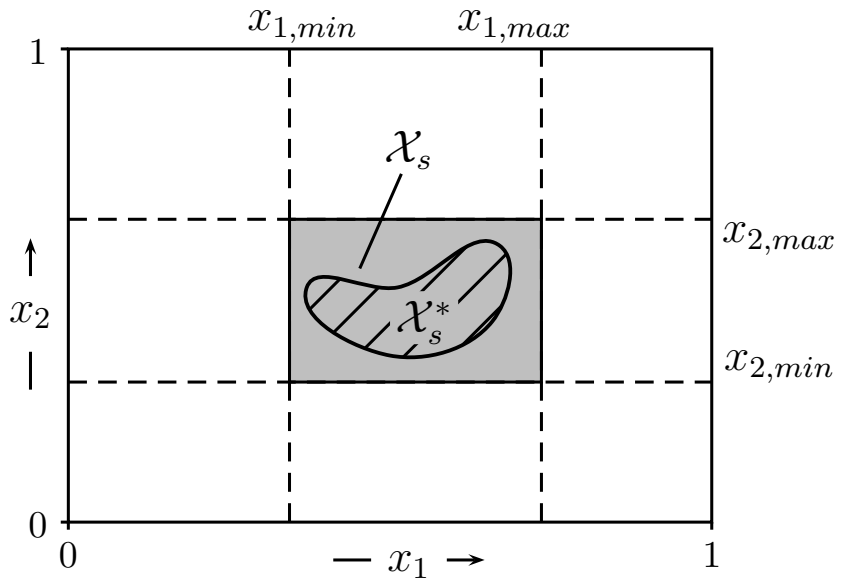
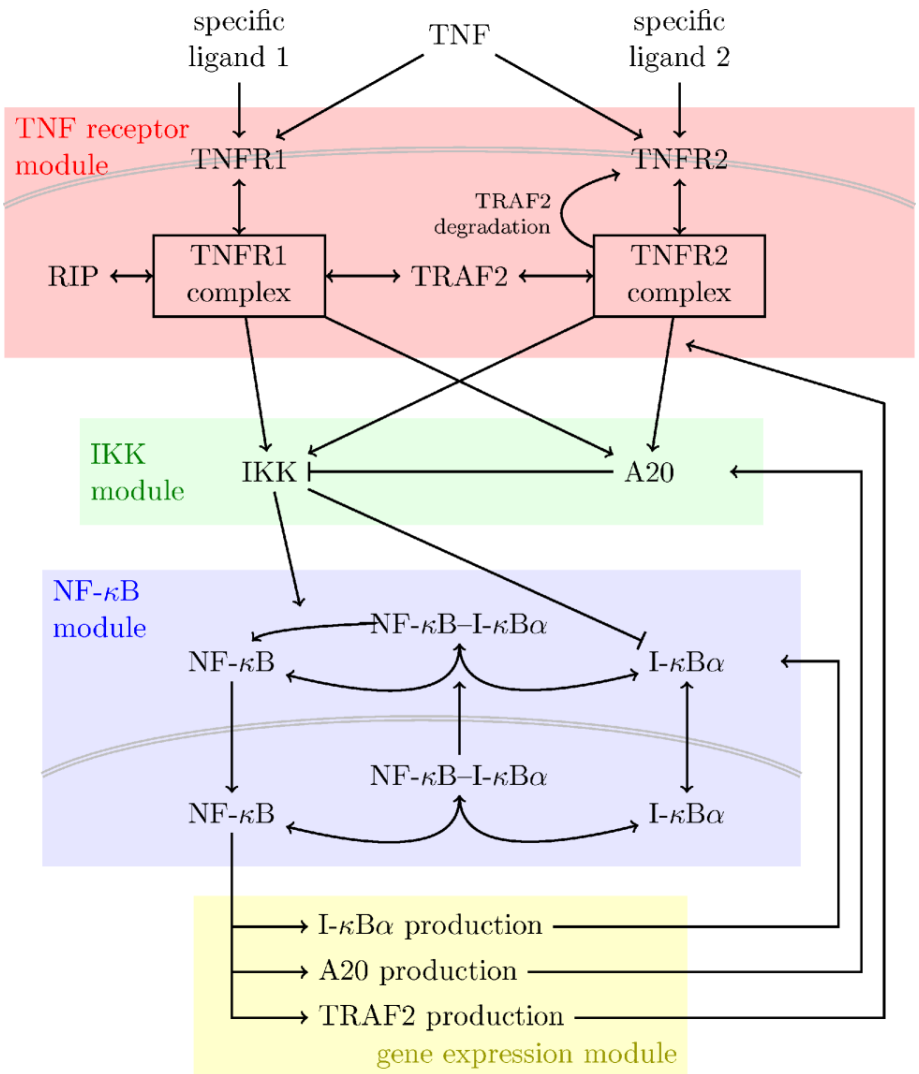


$\Longrightarrow$ lower and upper bounds for all state variables

TNF-Induced Anti-Apoptotic Signalling

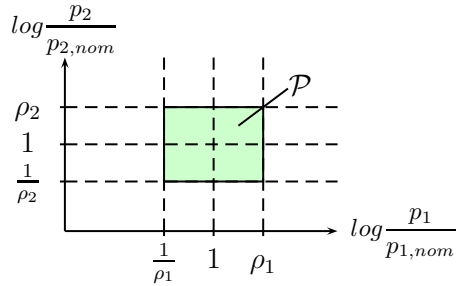


Schematic of antiapoptotic signalling pathway

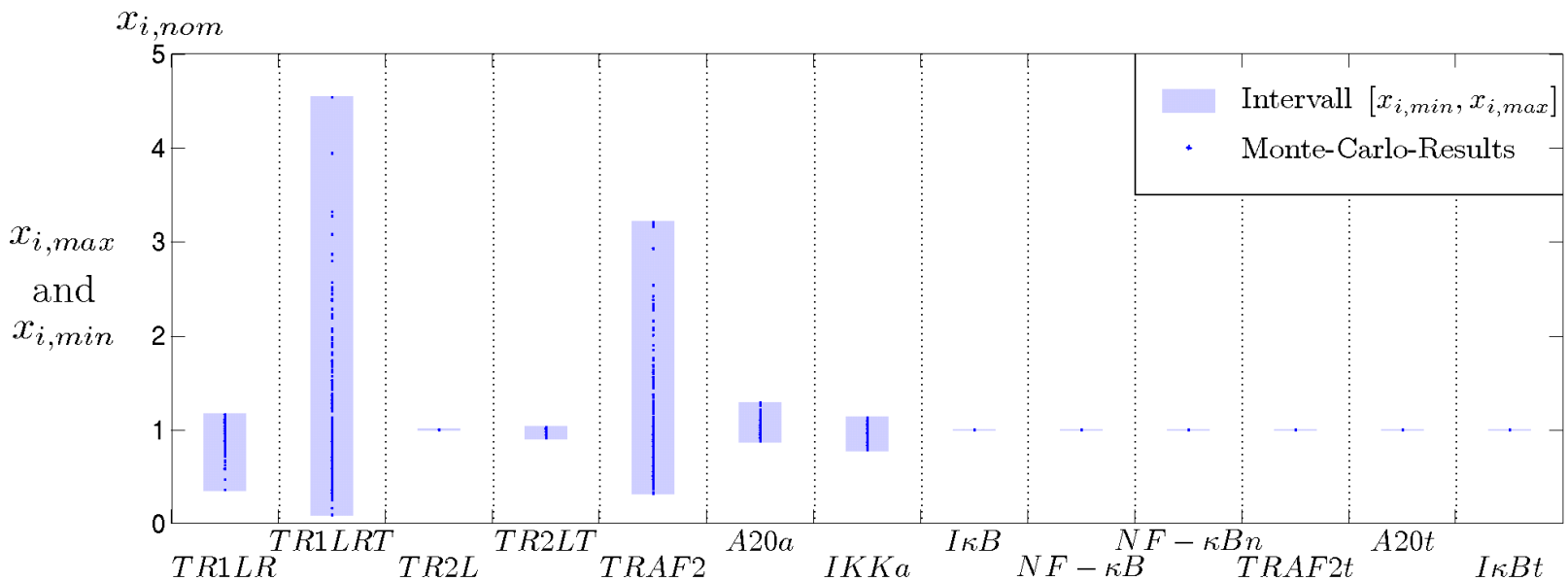
- Biological relevance:
  - apoptosis
  - proliferation
  - inflammation
- Components:
  - *TNF*-receptors
  - *NF-κB* signalling pathway
- Model:
  - 24 state variables
  - 56 parameter
- Inputs:
  - *TNF*1
  - *TNF*2
- Output:
  - *NF-κBn*

Computation of $\mathcal{X}_s$

- Parameter uncertainties of factors:
  $$\rho^T = (\rho_1, \ldots, \rho_q)$$
- Parameter set $\mathcal{P}$ is a hyperrectangle

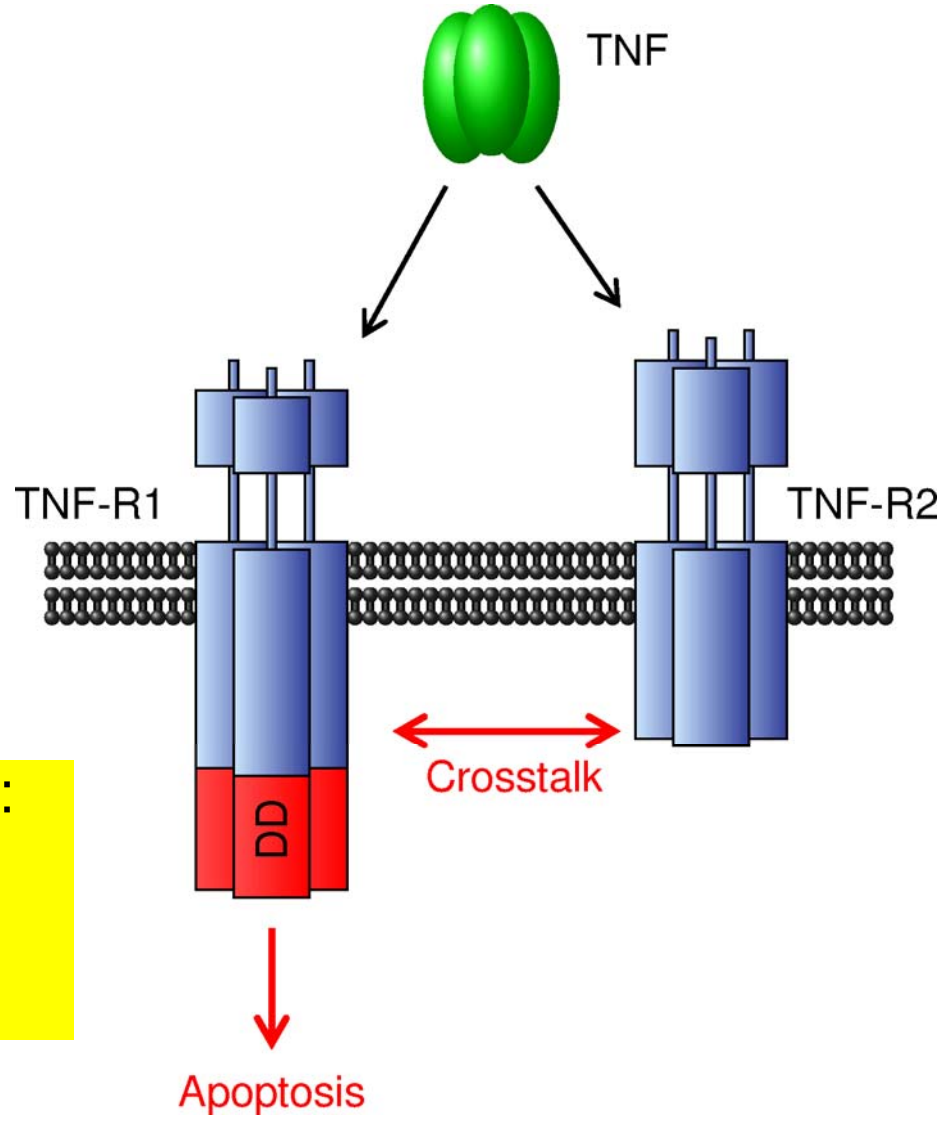


Set of feasible steady states for a variation: $\rho^T = (2, 2, 2, 2)$



The TNF receptor network

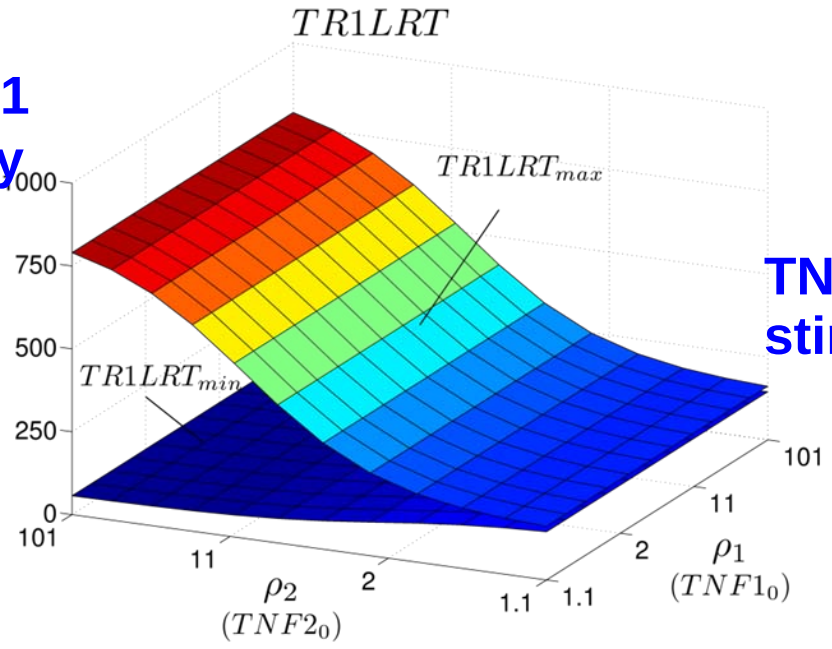
- Two types of receptors for TNF ligand: TNF-R1, TNF-R2
- Crosstalk via the adaptor protein TRAF2
- Apoptosis depends on activity of **TNF-R1** complex



Goal for global sensitivity analysis: Evaluate effect of variations in **receptor-specific stimulation** on TNF-R1 activity

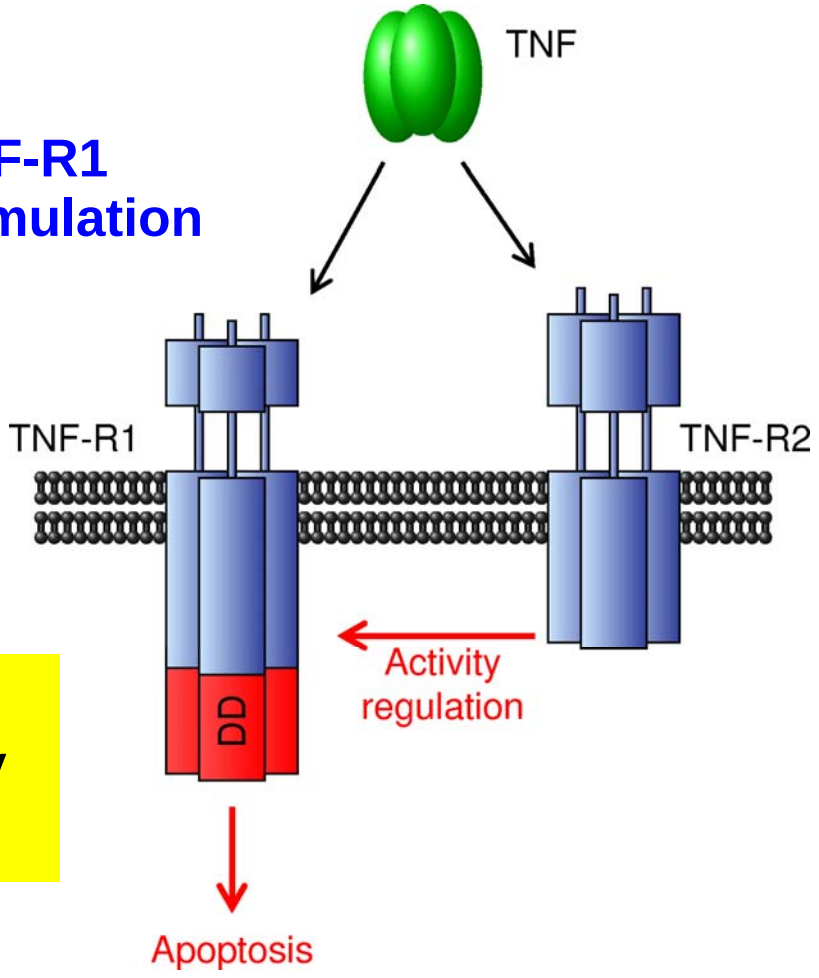
Global analysis of TNF stimulus variations

TNF-R1 activity



TNF-R1 stimulation

TNF-R2 stimulation

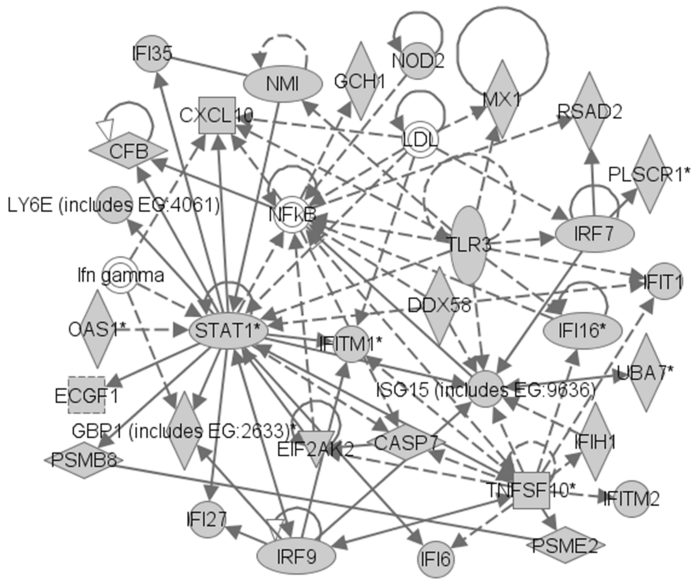


Model prediction:
Receptor 2 regulates activity of receptor 1

Model validation/falsification under uncertainty

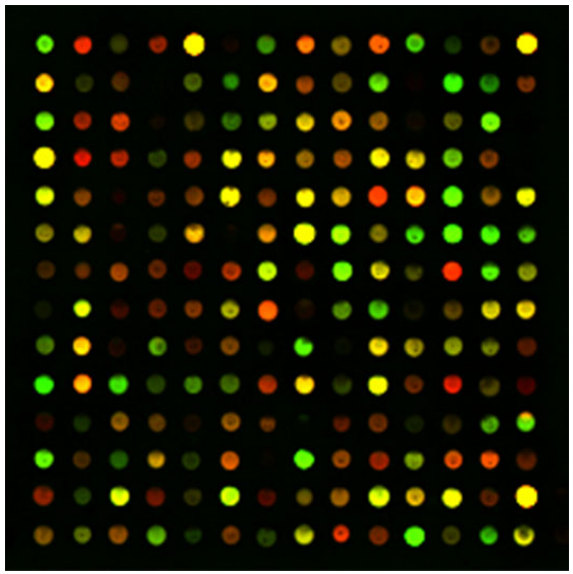
Christian Breindl

Motivation



Zhu et al., *BMC Bioinformatics* 2008

?



Biological hypothesis

Experimental observations of distinct operation modes

Qualitative model description

Qualitative measurements

Can the biological hypothesis explain the experimental observations?

Requirements for the Modeling and Analysis Framework

- Focus on gene regulation networks
- Representation of the knowledge about the biological system
  - Knowledge about / Hypothesis on interaction structure
  - Large uncertainties about reaction kinetics
- Represention of measurements of protein concentrations
  - Often only qualitative information possible,
    e.g. high or low, on or off
  - Focus on steady state behavior, especially multistability

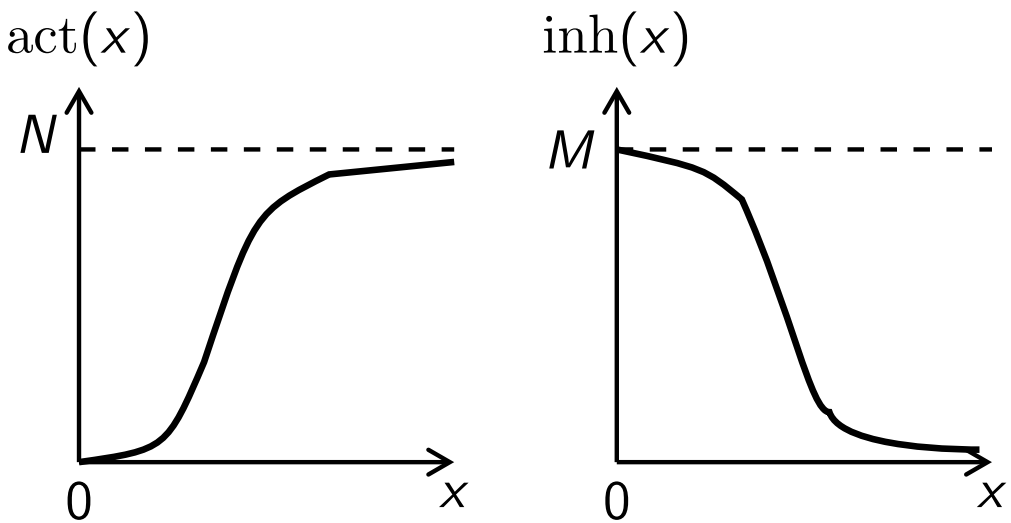
Goal

Introduction of a modeling and analysis framework which is appropriate to

- Represent the available biological knowledge and measurements
- Answer to question wether model structure and qualitative measurements are consistent

Modeling framework

- Proteins are assumed to have linear degradation rates
- A protein can either activate or inhibit the production of another protein $\Rightarrow$ use of general activation and inhibition functions
- Kinetic uncertainties $\Rightarrow$ exact shapes need not be specified



Model equations

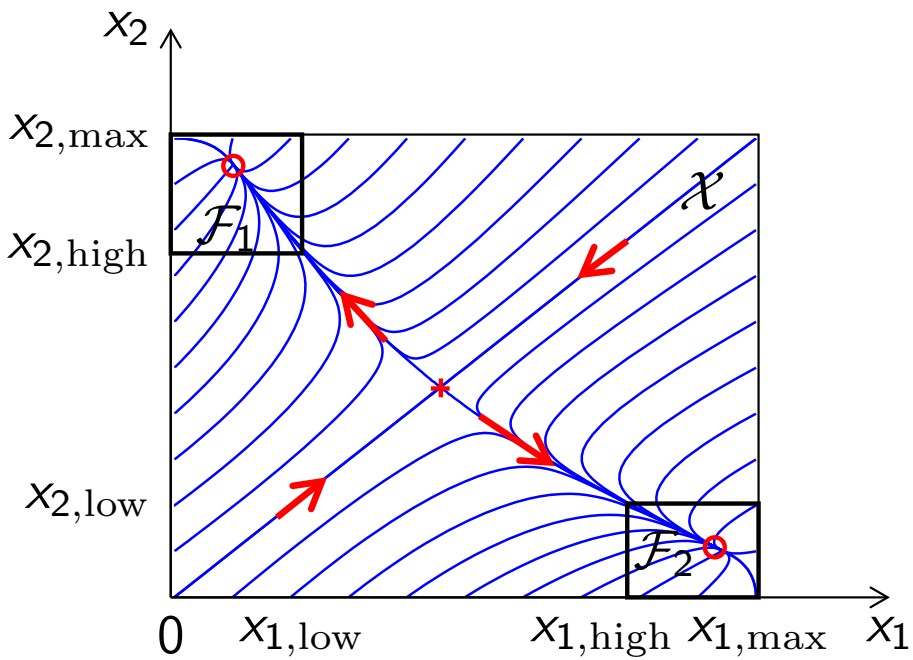
$$\dot{x}_i = -k_i \cdot x_i + f_i(x), \quad i = 1, \ldots, n$$

where $f_i(x)$ are composed of activation and inhibition functions.

Representation of measurements

- An operation mode corresponds to a stable steady state and can be characterized by a forward-invariant set
- The projections of these sets onto the coordinate axes are intervals of the form

$$\mathcal{I}_{x,\text{low}} = [0, x_{\text{low}}]$$
$$\mathcal{I}_{x,\text{high}} = [x_{\text{high}}, x_{\text{max}}]$$



Mutual inhibition network: $\dot{x}_1 = -k_1 \cdot x_1 + \text{inh}_1(x_2)$

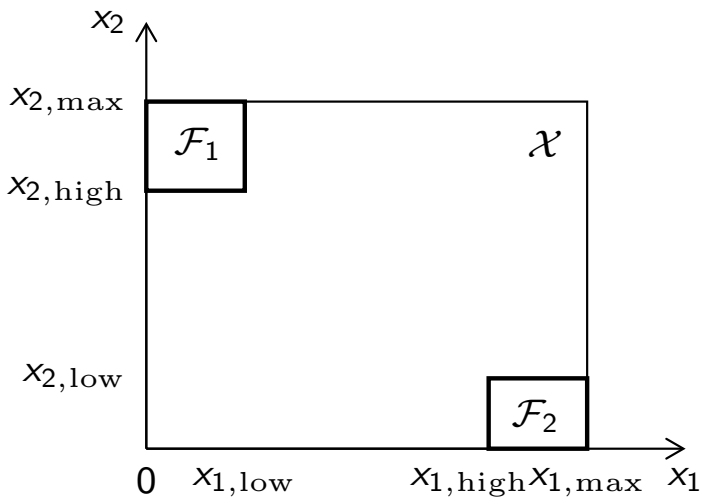
$\dot{x}_2 = -k_2 \cdot x_2 + \text{inh}_2(x_1)$

Example: Mutual inhibition network

- $\mathcal{F}_1 = [0, x_{1,\text{low}}] \times [x_{2,\text{high}}, x_{2,\max}]$, $B(\mathcal{F}_1) = (0, 1)$
- $\mathcal{F}_2 = [x_{1,\text{high}}, x_{1,\max}] \times [0, x_{2,\text{low}}]$, $B(\mathcal{F}_2) = (1, 0)$

Exemplary problem formulation

- Experimental observations



- Two alternative hypothesis:

$$\begin{aligned} \dot{x}_1 &= -k_1 \cdot x_1 + \text{inh}_1(x_2) & \qquad \dot{x}_1 &= -k_1 \cdot x_1 + \text{inh}_1(x_2) \\ \dot{x}_2 &= -k_2 \cdot x_2 + \text{inh}_2(x_1) & \qquad \dot{x}_2 &= -k_2 \cdot x_2 + \text{act}_2(x_1) \end{aligned}$$

- Which model can explain the observations?

Mathematical problem formulation

Experimental observations

- m distinct operation modes
- Forward invariant sets $\mathcal{F}_i$, $i = 1, \ldots, m$
- According Boolean lists $B(\mathcal{F}_i)$, $i = 1, \ldots, m$

Validation problem

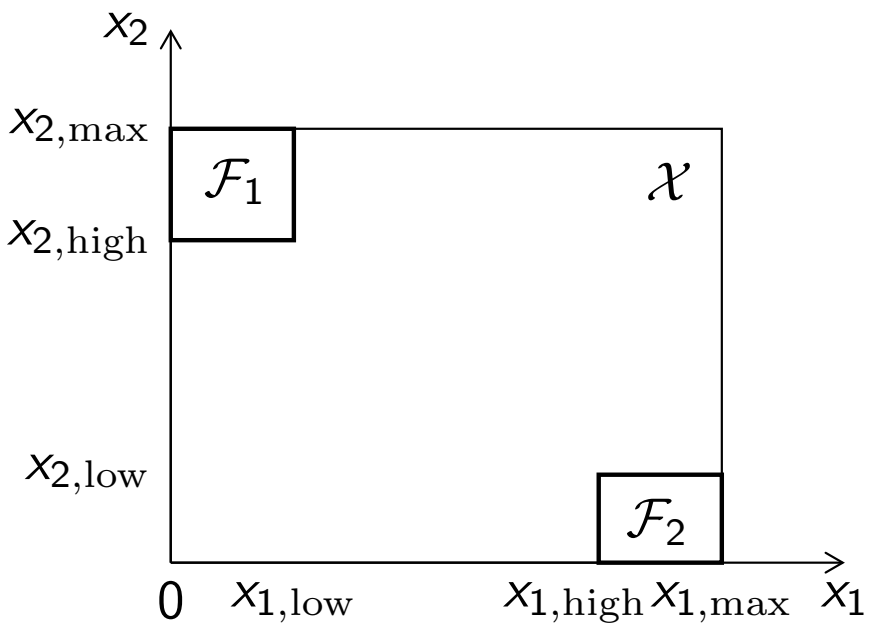
For the **given model structure**, do there exist **activation and inhibition functions** such that the model **exhibits** m **forward-invariant sets** $\tilde{\mathcal{F}}_i$ which lie **qualitatively at the same positions** as the sets $\mathcal{F}_i$, i.e.

$$B(\mathcal{F}_i) = B(\tilde{\mathcal{F}}_i) \qquad i = 1, \ldots, m$$

Solving the problem - Concept of compatible intervals

Idea: Consider only one differential equation at a time, e.g.

$$\dot{x}_1 = -k_1 \cdot x_1 + \mathrm{inh}_1(x_2)$$



Def.: compatible intervals (sloppy)

$\mathcal{I}_{x_2}$ is compatible with $\mathcal{I}_{x_1}$ for this differential equation

$\Rightarrow$

$\exists \mathrm{inh}_1(x_2)$ such that

x_1 cannot leave $\mathcal{I}_{x_1}$ as long $x_2 \in \mathcal{I}_{x_2}$

More generally

Given intervals $\mathcal{I}_{x_i}$ for all proteins x_i. If all intervals are compatible for all equations, the set $\mathcal{F} = \mathcal{I}_{x_1} \times \ldots \times \mathcal{I}_{x_n}$ is forward-invariant.

Solving the problem - Decision rules

- Decision rules allow to decide which types of intervals can be compatible for an equation $\dot{x}_i = -k_i \cdot x_i + f_i(x)$

Definition of a decision rule (sloppy)

Example equation: $\dot{x}_1 = -k_1 \cdot x_1 + \mathrm{inh}_1(x_2)$

- A decision rule for this equation is an equation for the Boolean values of the x_1- and x_2-intervals
- If $B(\mathcal{I}_{x_1})$ and $B(\mathcal{I}_{x_2})$ fulfill the Boolean equation, these two intervals are compatible

Example

For the equation: $\dot{x}_1 = -k_1 \cdot x_1 + \mathrm{inh}_1(x_2)$ there is the rule

$$B(\mathcal{I}_{x_1}) \sim \text{not } B(\mathcal{I}_{x_2})$$

Therefore, e.g. $\mathcal{I}_{x_{1,\mathrm{low}}}$ and $\mathcal{I}_{x_{2,\mathrm{high}}}$ are compatible ($0 =$ not 1).

Solving the problem - Decision rules

- Decision rules allow to decide which types of intervals can be compatible for an equation $\dot{x}_i = -k_i \cdot x_i + f_i(x)$
- Systematic enumeration of all possible rules for arbitrarily complex production terms $f_i(x)$ is possible with only few "building blocks"

Building blocks for arbitrary $f_i(x)$

1.	$f_i = \mathrm{act}(x_j) \cdot \mathrm{act}(x_k)$
i)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$ and $B(\mathcal{I}_{x_k})$
ii)	$B(\mathcal{I}_{x_i}) \sim 0$

2.	$f_i = \mathrm{act}(x_j) \cdot \mathrm{inh}(x_k)$
i)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$ and (not $B(\mathcal{I}_{x_k})$)
ii)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$
iii)	$B(\mathcal{I}_{x_i}) \sim 0$

3.	$f_i = \mathrm{inh}(x_j) \cdot \mathrm{inh}(x_k)$
i)	$B(\mathcal{I}_{x_i}) \sim$ (not $B(\mathcal{I}_{x_j})$) and (not $B(\mathcal{I}_{x_k})$)
ii)	$B(\mathcal{I}_{x_i}) \sim$ (not $B(\mathcal{I}_{x_j})$) or (not $B(\mathcal{I}_{x_k})$)
iii)	$B(\mathcal{I}_{x_i}) \sim$ not $B(\mathcal{I}_{x_j})$
iv)	$B(\mathcal{I}_{x_i}) \sim$ not $B(\mathcal{I}_{x_k})$
v)	$B(\mathcal{I}_{x_i}) \sim 1$

4.	$f_i = \mathrm{act}(x_j) + \mathrm{act}(x_k)$
i)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$ and $B(\mathcal{I}_{x_k})$
ii)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$ or $B(\mathcal{I}_{x_k})$
iii)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$
iv)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_k})$
v)	$B(\mathcal{I}_{x_i}) \sim 0$

5.	$f_i = \mathrm{act}(x_j) + \mathrm{inh}(x_k)$
i)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$ and (not $B(\mathcal{I}_{x_k})$)
ii)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$ or (not $B(\mathcal{I}_{x_k})$)
iii)	$B(\mathcal{I}_{x_i}) \sim B(\mathcal{I}_{x_j})$
iv)	$B(\mathcal{I}_{x_i}) \sim$ not $B(\mathcal{I}_{x_k})$
v)	$B(\mathcal{I}_{x_i}) \sim 0$
vi)	$B(\mathcal{I}_{x_i}) \sim 1$

6.	$f_i = \mathrm{inh}(x_j) + \mathrm{inh}(x_k)$
i)	$B(\mathcal{I}_{x_i}) \sim$ (not $B(\mathcal{I}_{x_j})$) and (not $B(\mathcal{I}_{x_k})$)
ii)	$B(\mathcal{I}_{x_i}) \sim$ (not $B(\mathcal{I}_{x_j})$) or (not $B(\mathcal{I}_{x_k})$)
iii)	$B(\mathcal{I}_{x_i}) \sim$ not $B(\mathcal{I}_{x_j})$
iv)	$B(\mathcal{I}_{x_i}) \sim$ not $B(\mathcal{I}_{x_k})$
v)	$B(\mathcal{I}_{x_i}) \sim 1$

Solving the problem - Decision rules

Summary of the method

Validation problem can be translated into a combinatorial one and solved algorithmically: **Find a valid rule for every differential equation that can explain all steady states**

Computational complexity

- each protein has maximally k regulating proteins
- number of dynamical equations n

$$\Rightarrow \textbf{maximum complexity is } n \cdot 6^{k-1}$$

- complexity is linear in system size n !!

Example 1: Mutual inhibition network

Alternative model equations

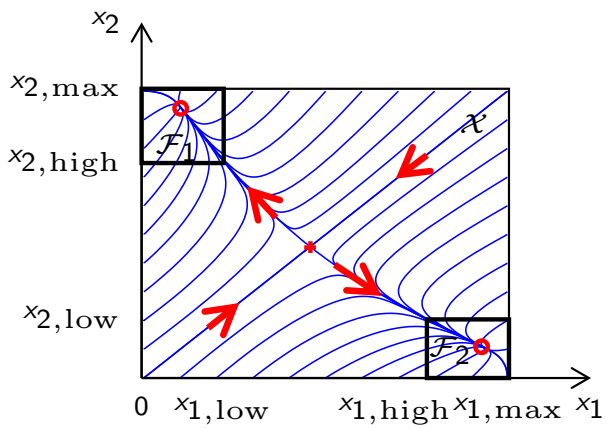
$$\dot{x}_1 = -k_1 \cdot x_1 + \mathrm{inh}_1(x_2)$$
$$\dot{x}_2 = -k_2 \cdot x_2 + \mathrm{inh}_2(x_1) \;/\; \mathrm{act}_2(x_1)$$

Observed steady states

$$B(\mathcal{F}_1) = (0,1)$$
$$B(\mathcal{F}_2) = (1,0)$$

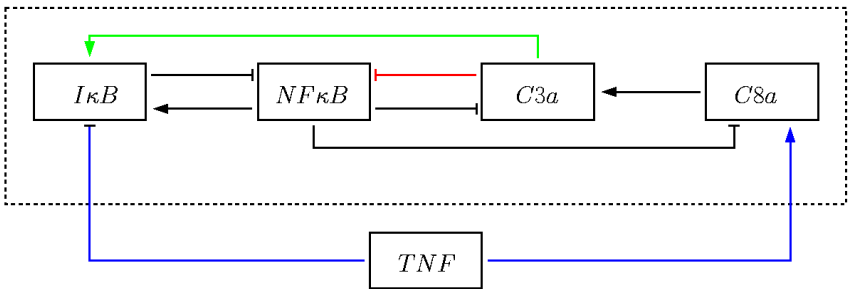
- The mutual inhibition network can be validated because of the following valid rules

$$B(\mathcal{I}_{x_1}) \sim \text{not } B(\mathcal{I}_{x_2})$$
$$B(\mathcal{I}_{x_2}) \sim \text{not } B(\mathcal{I}_{x_1})$$



- The second hypothesis can be falsified as no valid rules exist that can explain the observations

Example 2: Apoptosis Signaling Network



Example ODE:

$$\frac{d[C3a]}{dt} = -k([C3a]) + \mu([NF\kappa B]) \cdot \nu([C8a])$$

Desired behavior in absence of TNF:

- A stable living state S1 with high $[NF\kappa B]$ and low $[C3a]$
- A stable apoptotic state S2 with low $[NF\kappa B]$ and high $[C3a]$

Application of the presented method shows:

- There exist biologically reasonable concentrations for $[I\kappa B]$ and $[C8a]$ and
- activation and inhibition functions such that
- S1 and S2 can be reproduced by the model

Summary

- Method intended as a first qualitative validation for poorly understood gene regulation networks
- Focus on multistability, not on dynamical behavior
- Efficient algorithm to solve the qualitative validation problem with respect to multistability (complexity $\mathcal{O}(n \cdot 6^{k-1})$)
- Not a Boolean approach, only analysis is performed with Boolean rules
- Proved valuable for a number of applications (apoptosis, lactose utilization network of E. coli, ...)
- It is not necessary to construct and simulate different models, algorithm can already give a yes or no answer
- Only qualitative result

Conclusions of Talk

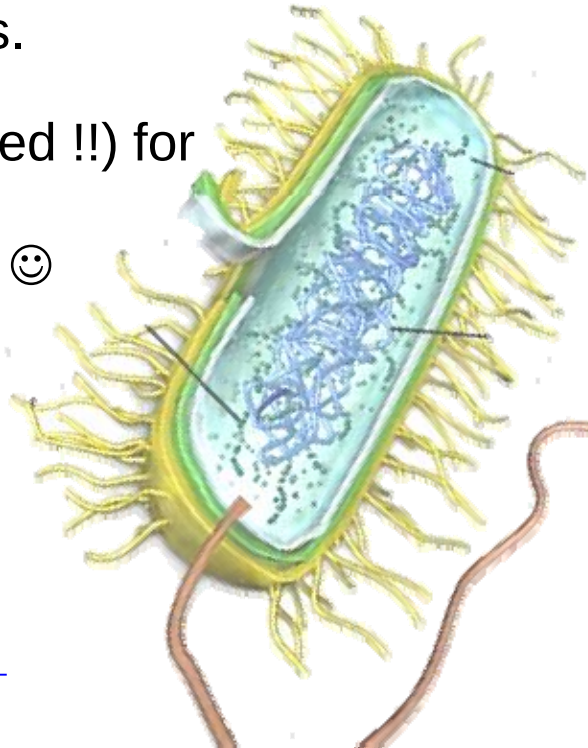
When investigating biological systems typically significant uncertainties have to be taken into account.

Systems and control theory provides many methods and tools that allow to deal with uncertainty.

Showed with a number of examples that these methods are indeed useful when investigating biological systems.

The good news: There is the possibility (and the need !!) for developing many new systems theoretic methods.

Systems biology is a “bonanza” for systems people ☺



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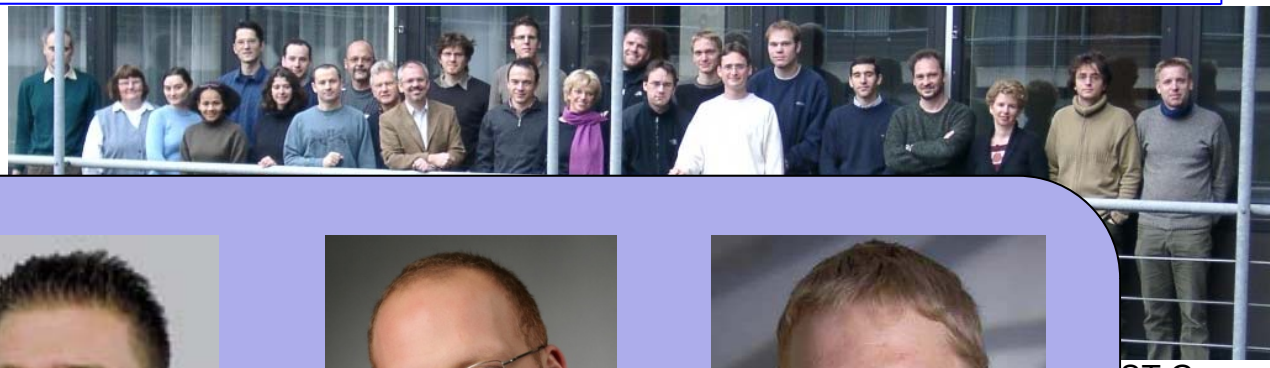

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