Systems Biology of the JAK-STAT signalling pathway

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Outline

- **Systems Biology**
- **JAK-STAT pathway of the Epo receptor**
- **A dynamical model for JAK-STAT pathway**
- **Observing the unobservable**
- *In silico* **biology: Predicting a new experiment**
- **Infering systems' properties**

Enlarging Physics, Math, Engineering

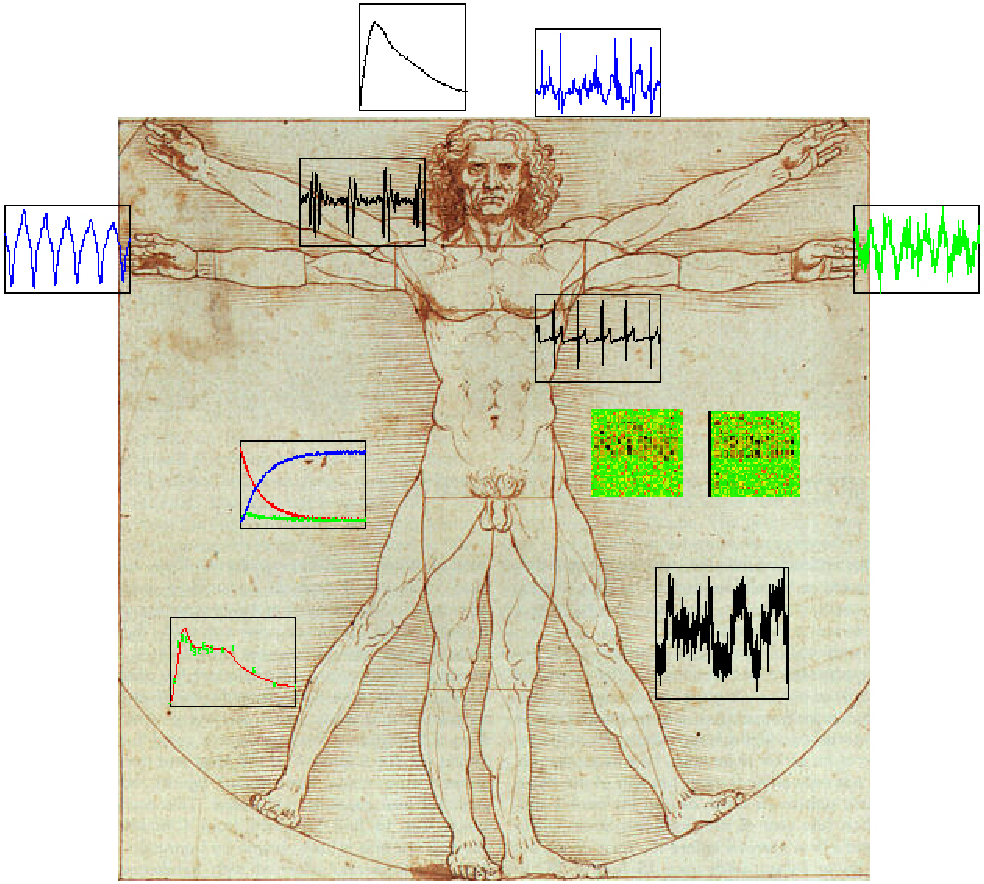
- **Since Newton:**

  Mathematatization of inanimate nature

- **21st century:**

  Additionally: Mathematization of animate nature

Man : A Dynamical System



Diseases caused or expressed by malfunction of dynamical processes

Two Directions in Systems Biology

- **Putting all the omics together**

  So far: large scale, qualitative, static

- **Understanding biomedical networks by data-based mathematical modelling of their dynamical behavior**

  So far: small scale, quantitative, dynamic

Both approaches will converge to: large scale, quantitative, dynamic

Common ground: Investigating networks

Direction II in Systems Biology

Understanding biomedical systems by data-based mathematical modelling of their dynamical behavior

From components and structure to behavior of networks

Systems Biology is based on but more than ...

- **... Mathematical Biology: Data-based**
- **... Bioinformatics: Dynamics**
- **... o.p./g. – o.p.: System**
- **... another omics: Mathematics**

Why Mathematical Modelling in BioMed?

- Make assumptions explicit
- Understand essential properties, failing models
- Condense information, handle complexity
- Understand role of dynamical processes, e.g. feed-back
- Impossible experiments become possible
- Prediction and control
- Understand what is known
- Discover general principles
- "You don't understand it until you can model it"

Why Modelling in Cell Biology?

- Basic Research
  - Genomes are sequenced, but ...
  - ... function determined by regulation
  - Regulation = Interaction & Dynamics
  - Function: Property of dynamic network
  - ”Systems Biology”
- Application
  - Drug development takes 10 years and 1 bn $/€
  - Reduce effort by understanding systems

The (Old) Central Dogma

DNA

$\Downarrow$

RNA

$\Downarrow$

Protein

The (New) Central Dogma

DNA

⇓

RNAs

⇓

Proteins

⇓

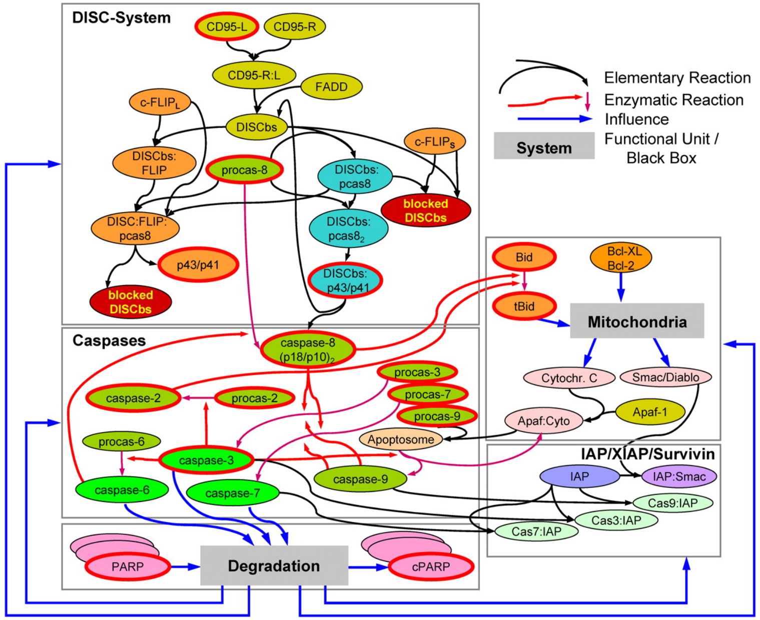
Networks

↙ ↓ ↘

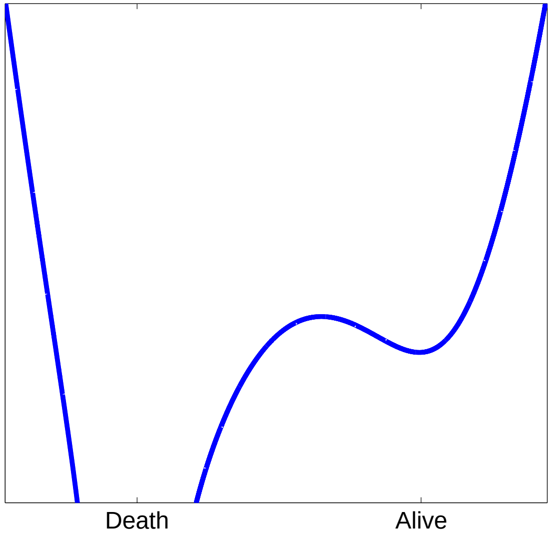
Signalling ↔ **Gene Regulatory** ↔ **Metabolic**

Examples of Networks I: Apoptosis

Pathway cartoon



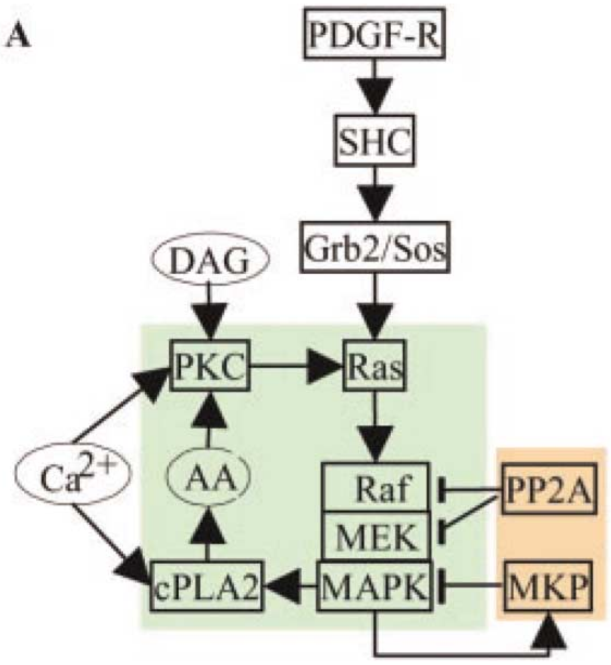
System's behavior



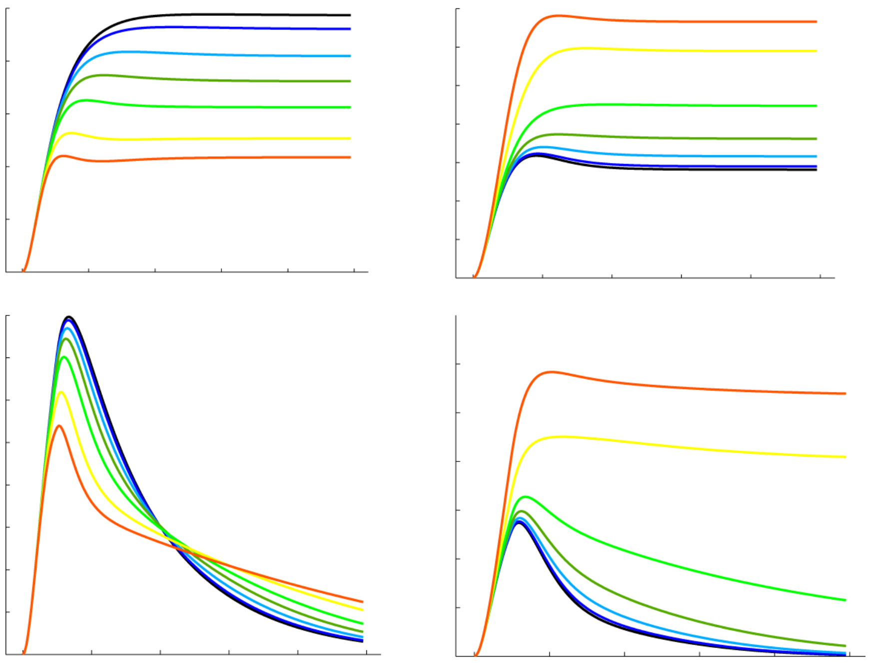
Threshold behavior, one-way bistable

Examples of Networks II: MAP Kinase

Pathway cartoon



System's behavior

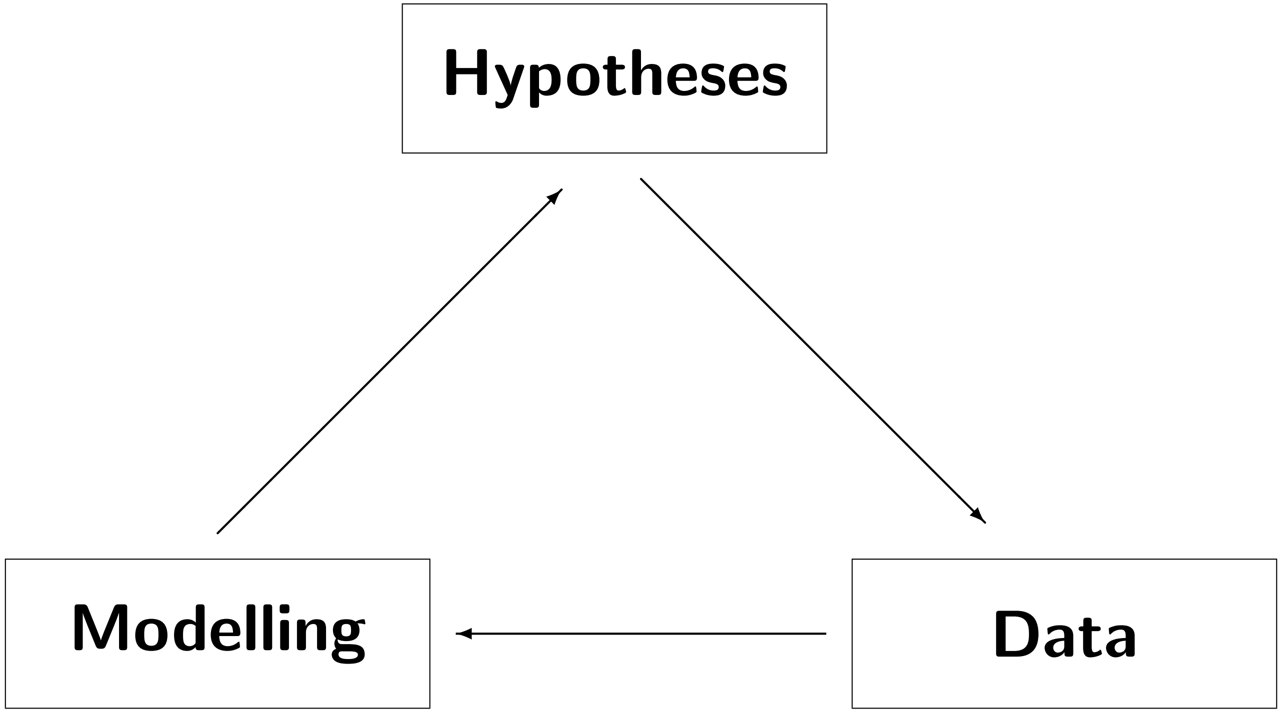


Time scales/parameters important

The Steps of Systems Biology

- **Define the biological question**
- **Modelling**
  - **Experimental design**
  - **Quantitative data**
  - **Parameter estimation**
- **Systems' analysis**
  - **Design priniciples**
  - **Robustness**
- **Applications**
  - **Synthetic biology**
  - **Personalized medicine**

The Systems Biology Cycle: A Process



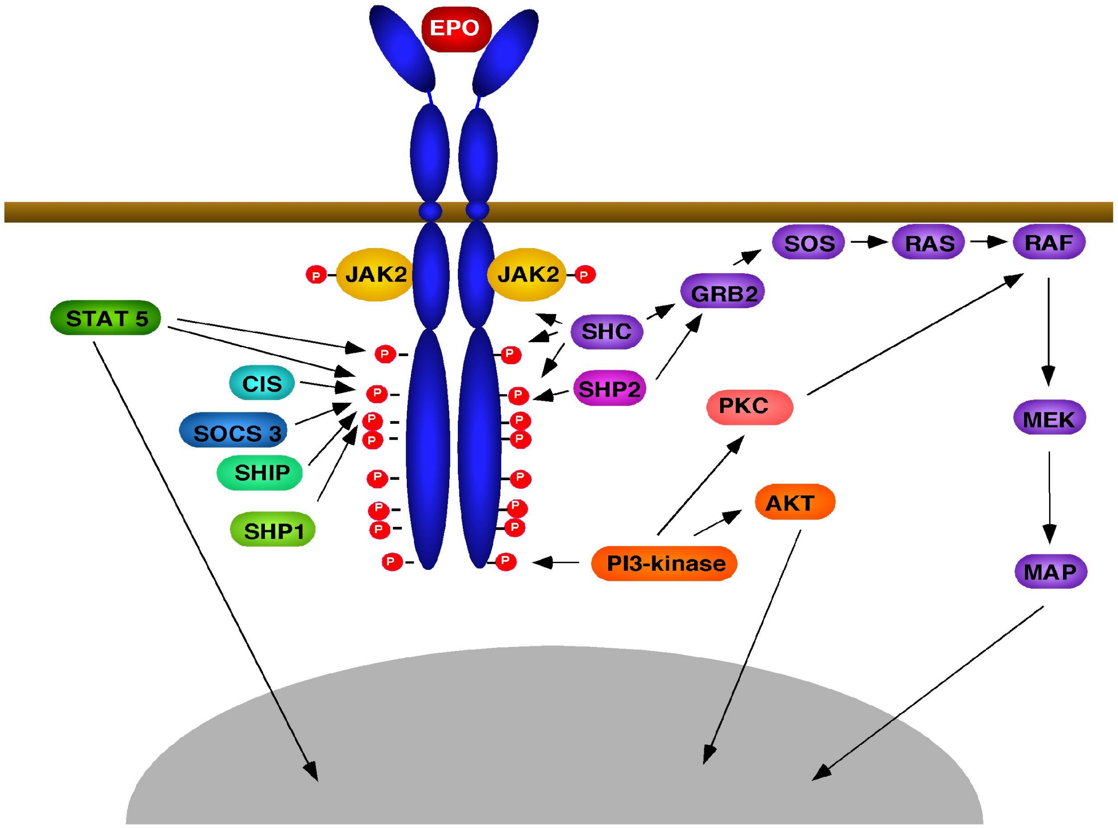
Hypotheses

Modelling

Data

Make the cycle happen: Wet/dry couple projects

Signal transduction through the Erythropoietin receptor (EpoR)



In collaboration with Dr. Ursula Klingmüller

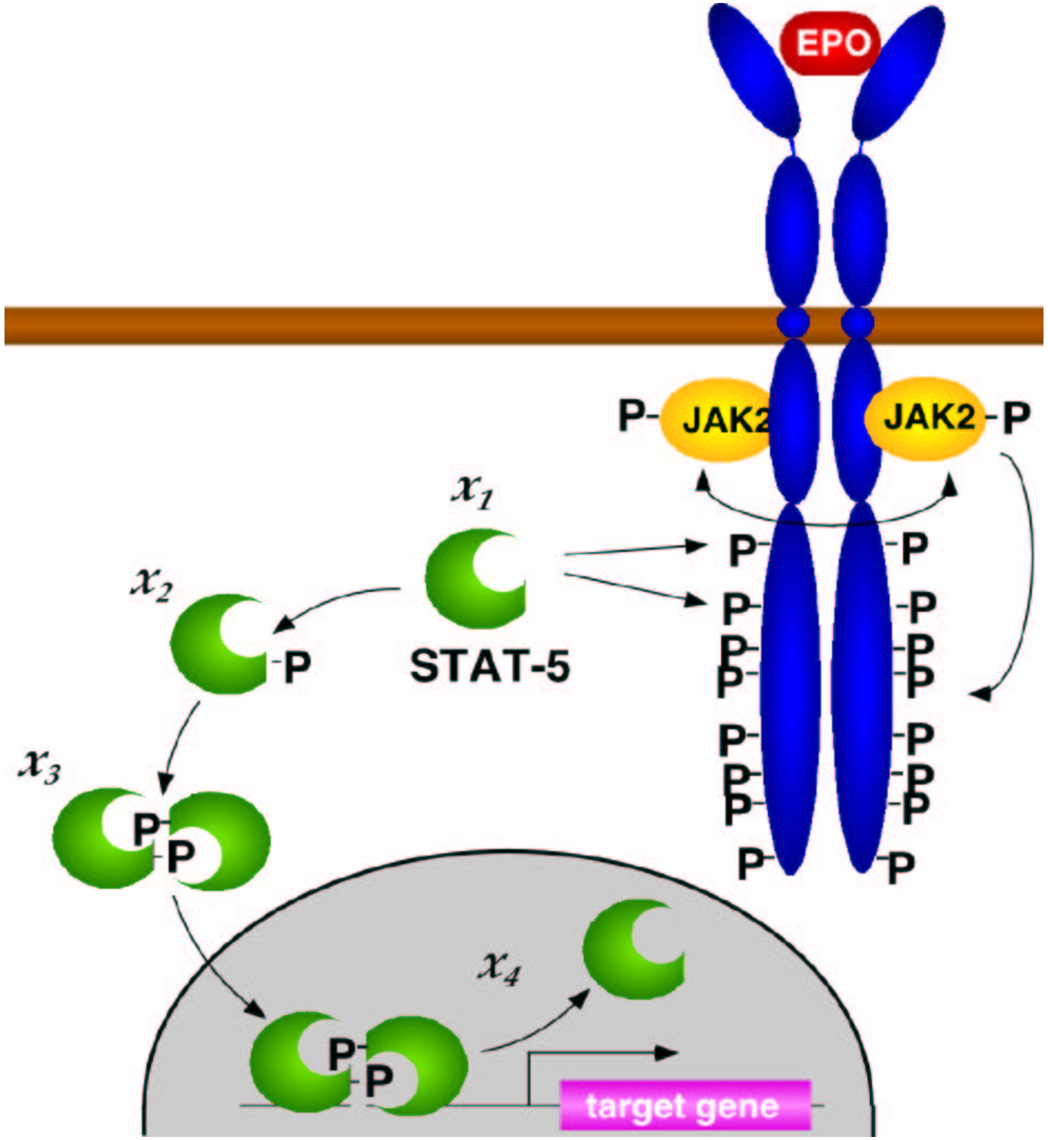
German Cancer Research Centre, Heidelberg

Epo

Epo = Erythropoietin

- **Hormone produced by kidneys**
- **Turns erythroid progenitor cells into red blood cells**
- **Well known to Tour de France cyclists**

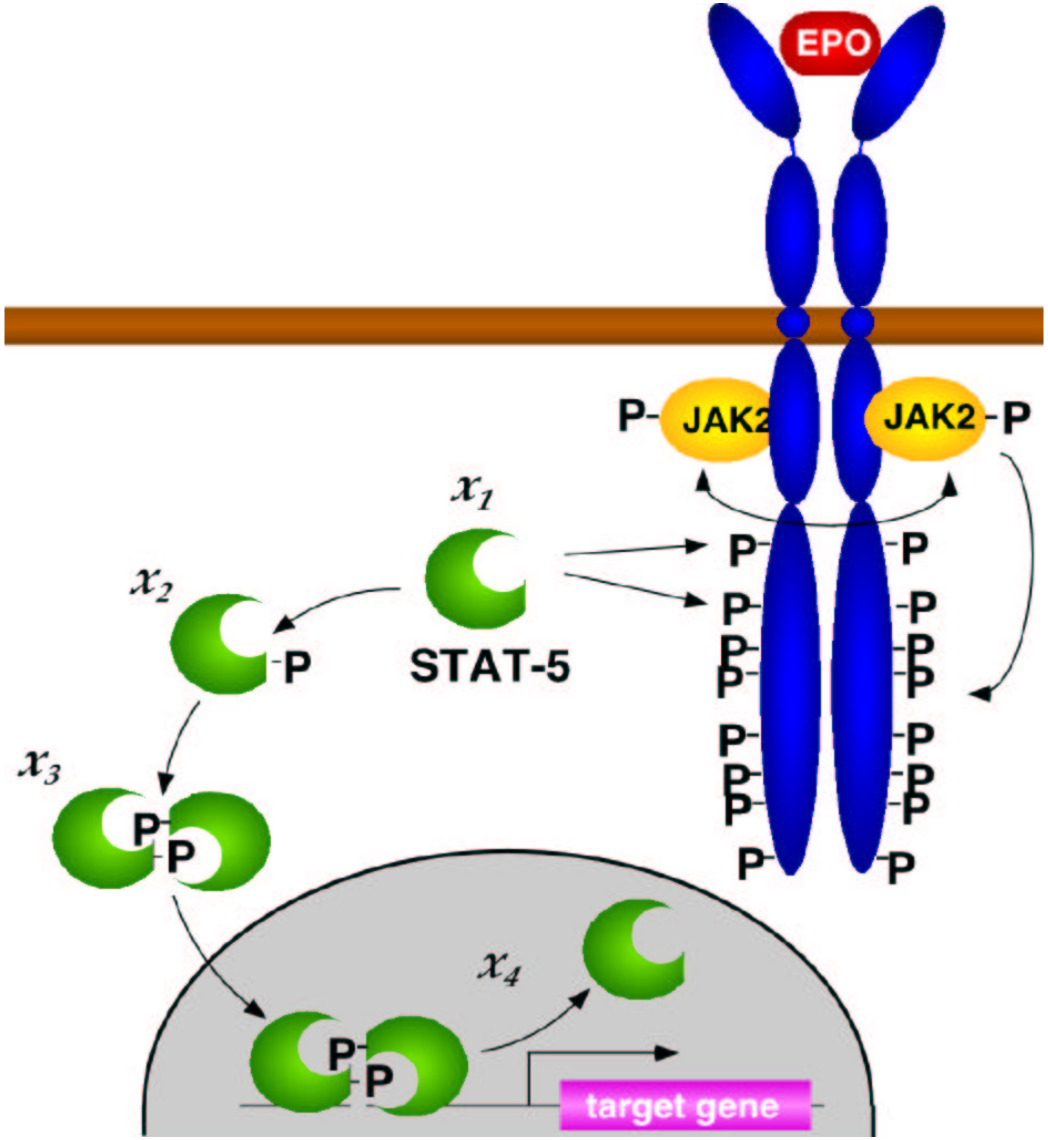
JAK – STAT Pathway



The Program

- Translate the cartoon in (differential) equations
- Measure protein dynamics
- Estimate parameters in equations
- Test and refine the mathematical model
- Predict the outcome of new experiments
- Use the model: E.g. identify potential drug targets

JAK – STAT Pathway



From Chemical Reactions ...

$$\mathbf{STAT5 + EpoR_A \;\rightarrow\; STAT5 - P}$$

$$\mathbf{STAT5 - P + STAT - P \;\rightarrow\; STAT5 - P = STAT5 - P}$$

$$\mathbf{STAT5 - P = STAT5 - P \;\rightarrow\; STAT5 - P = STAT5 - P_{nuc.}}$$

… to Mathematical Equations

$$\begin{aligned}
\dot{x}_1 &= -p_1 x_1 \mathrm{EpoR_A} \\
\dot{x}_2 &= p_1 x_1 \mathrm{EpoR_A} - p_2 x_2^2 \\
\dot{x}_3 &= \frac{1}{2} p_2 x_2^2 - p_3 x_3 \\
\dot{x}_4 &= p_3 x_3
\end{aligned}$$

Measurements

- $\mathbf{y_1(t)}$ **: Phosphorylated STAT-5 in the cytoplasm**

$$\mathbf{y_1(t) = p_5(x_2(t) + 2\,x_3(t))}$$

- $\mathbf{y_2(t)}$ **: All STAT-5 in the cytoplasm**

$$\mathbf{y_2(t) = p_6(x_1(t) + x_2(t) + 2\,x_3(t))}$$

- $\mathbf{y_3(t)}$ **: Activation of the epo receptor**

$$\mathbf{y_3(t) = p_7\,EpoR_A(t)}$$

Simulation vs. Data-Based Modeling I

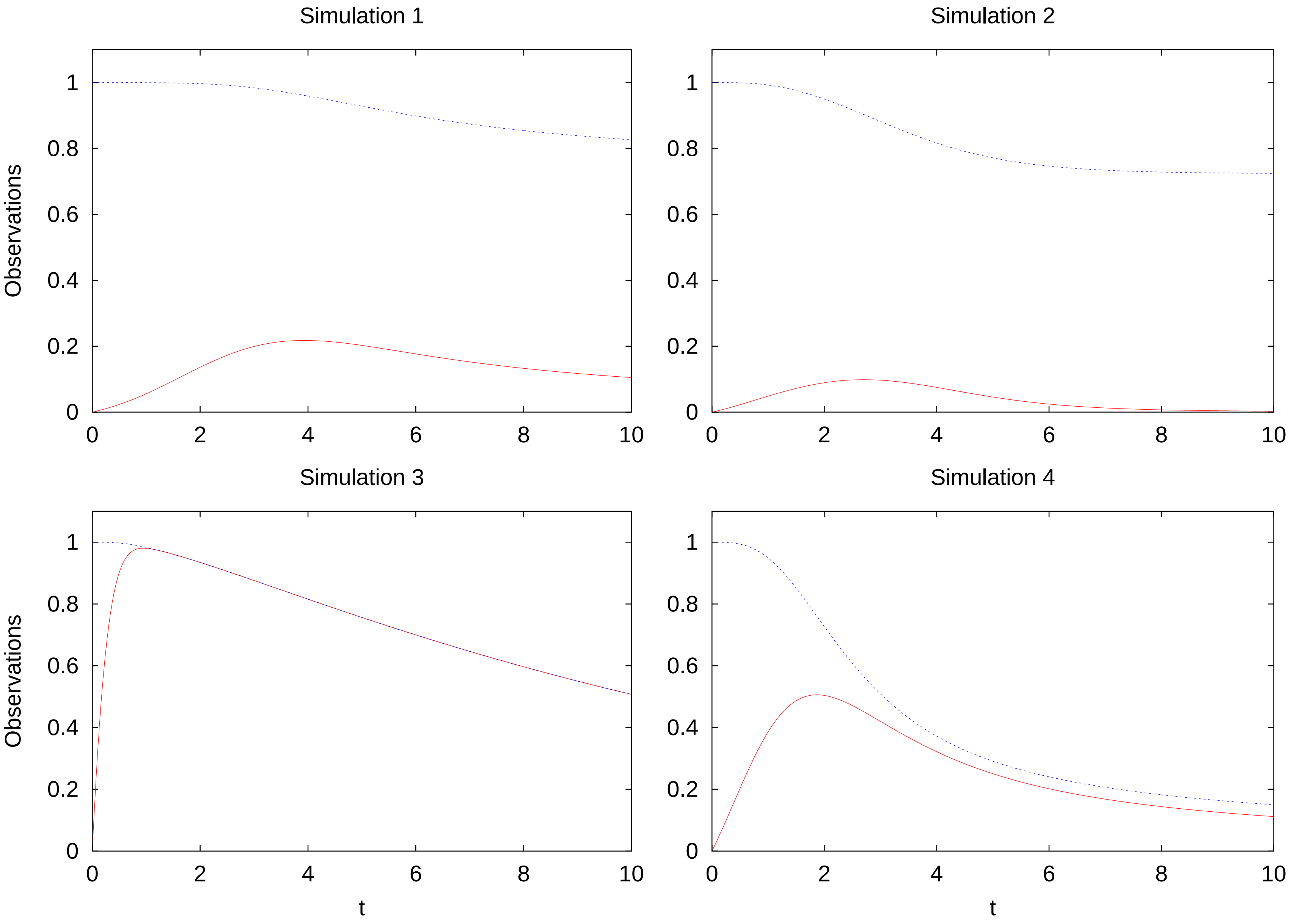
Model comprises:

- **Structure of the equations (the cartoon)**
- **Values of the parameters**

Simulation:

- **Structure from pathway cartoon**
- **Parameters from**
  - **Independent measurements**
  - **Literature**
  - **Educated guesses**

Simulations



Simulation vs. Data-Based Modeling II

Simulation dilemma:

If discrepancies between experiment and model

- **Wrong structure or wrong parameters ?**

Data-based modeling:

- **Structure from pathway cartoon**
- **Parameters estimated from data**

If discrepancies:
Think about the cartoon ! Learn biology !

Parameter Estimation in Nonlinear Partially Observed Noisy Dynamical Systems

Dynamics:

$$\dot{\vec{x}} = \vec{f}(\vec{x}, \vec{p})$$

Observation:

$$\vec{y}(t_i) = \vec{g}(\vec{x}(t_i), \vec{p}) + \vec{\epsilon}(t_i), \qquad \vec{\epsilon}(t_i) \sim N(0, \Sigma_i)$$

Log-Likelihood:

$$E = \chi^2(\vec{p}, \vec{x}(t_0)) = \sum_{i=1}^{N} \sum_{j=1}^{M} \left(\frac{(y_j^D(t_i) - g_j(\vec{x}(t_i; \vec{p}, \vec{x}(t_0))}{\sigma_{i\,j}} \right)^2$$

Really Good Data

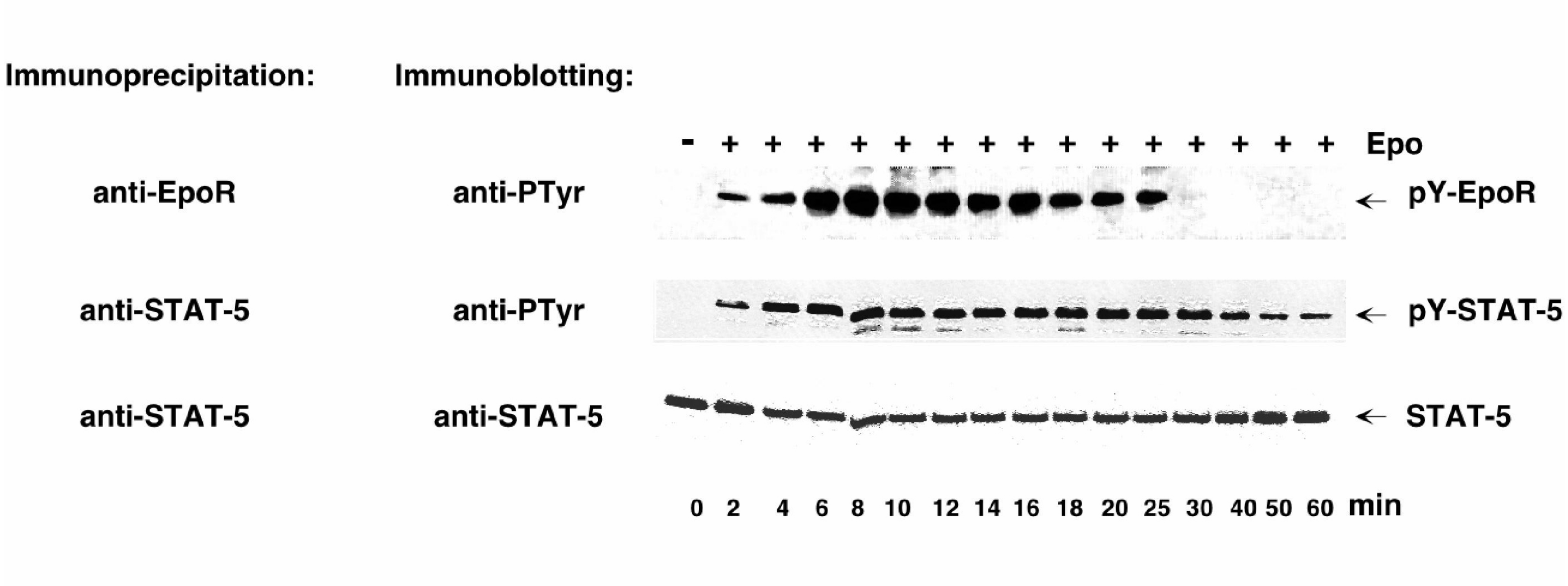
"What makes you feel good ?"

"Good data."

"What makes you feel really good ?"

"Really good data !"

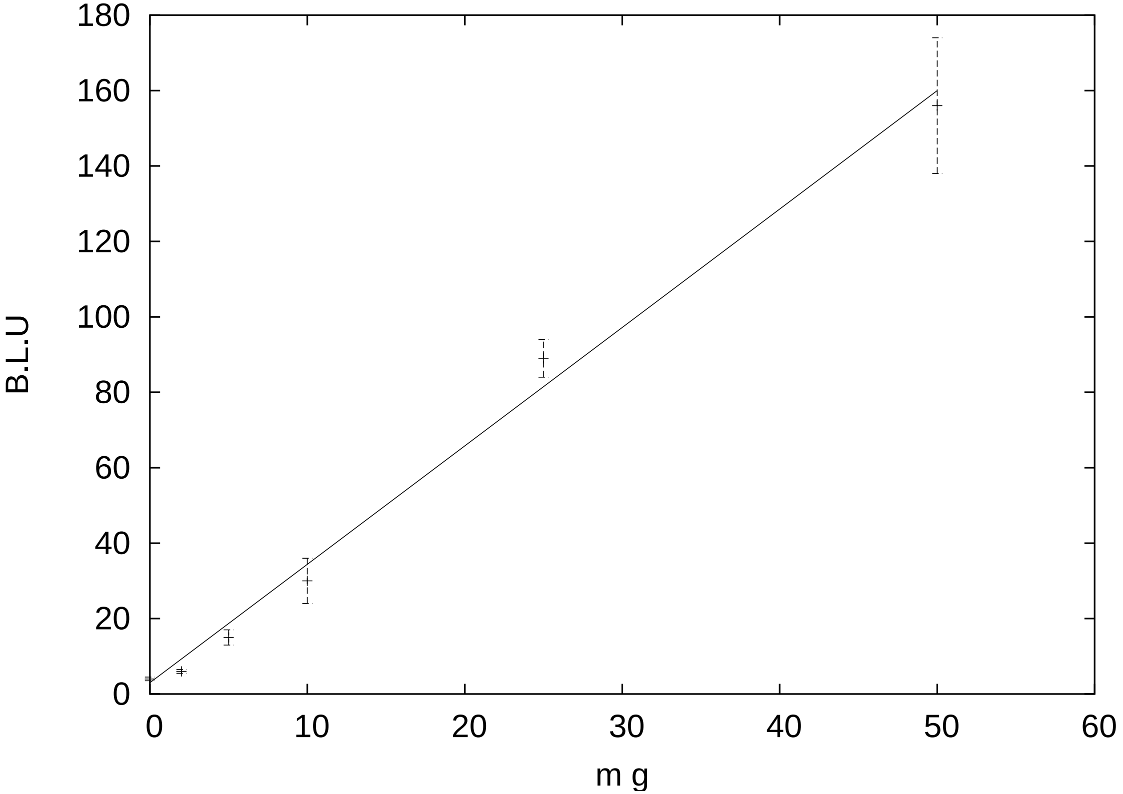
Quantitative Immunoblotting



M. Schilling et al.: Quantitative data generation for Systems Biology. IEE Proc. Sys. Bio. 152, 193, 2005

M. Schilling et al.: Computational processing and error reduction strategies for standardized quantitative data in biological networks. FEBS J. 272, 6400, 2005

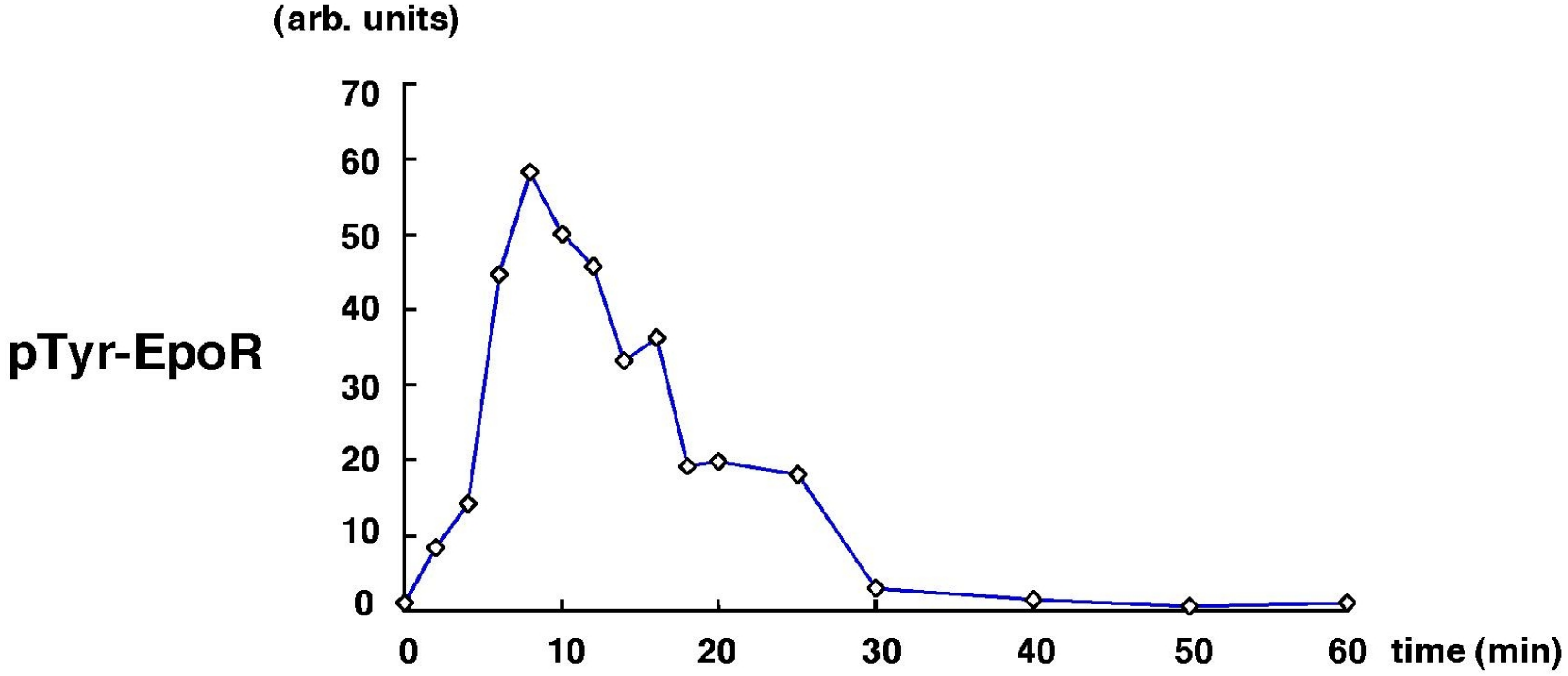
Really Good Data



$g(x)$ is linear

The data

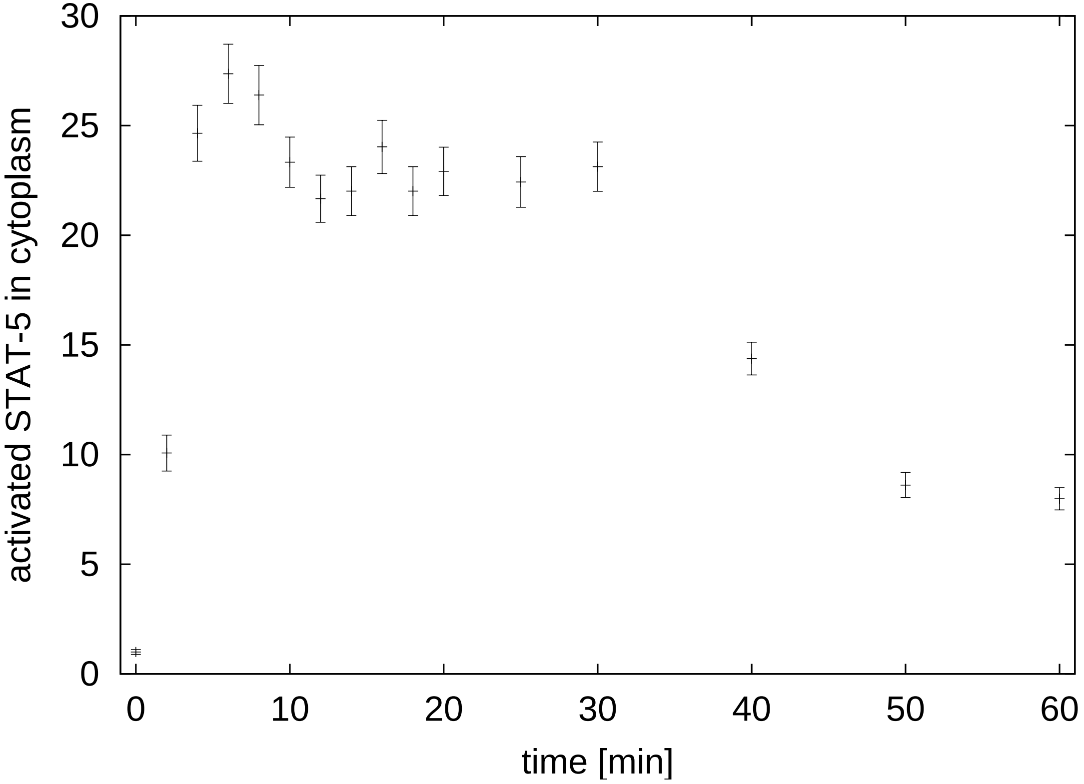
Activation of the epo receptor :



Maximum at 8 min

The data

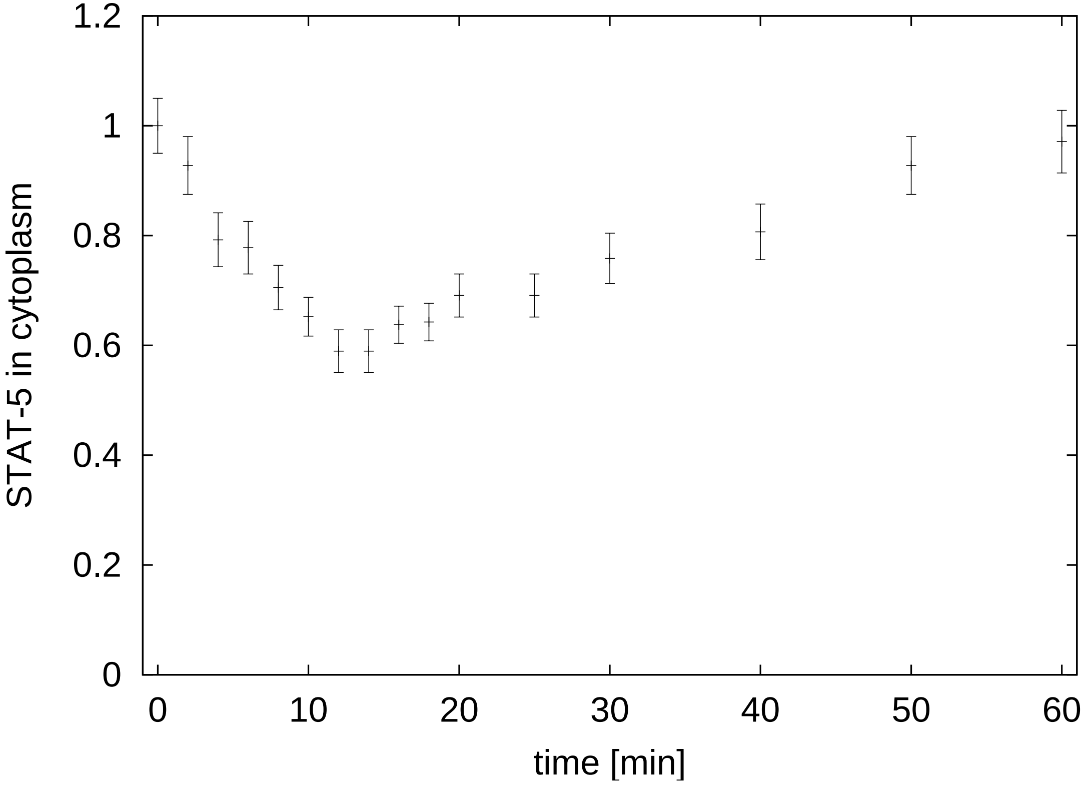
Phosphorylated STAT-5 in cytoplasm :



Plateau from 10 to 30 min

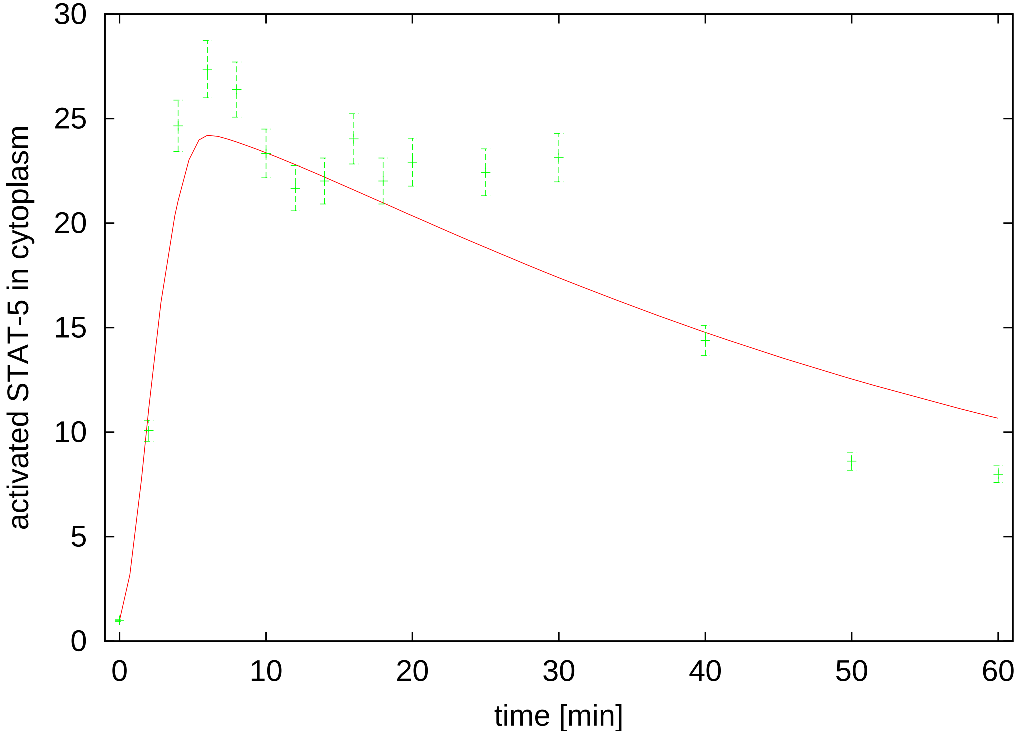
The data

All STAT-5 in cytoplasm :



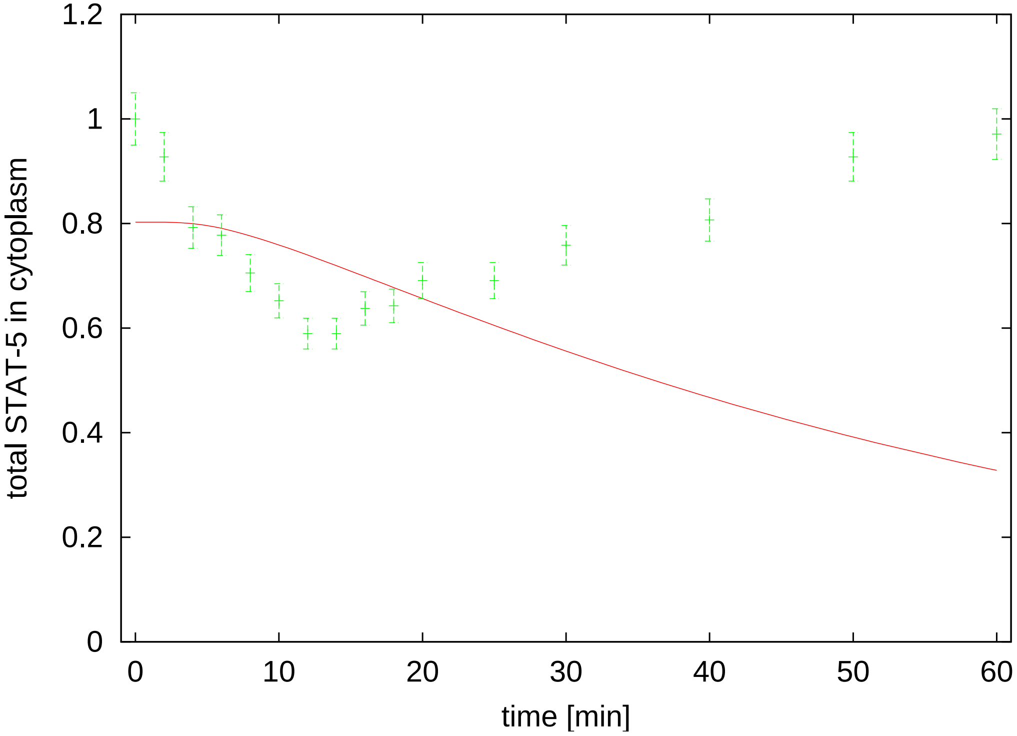
First results

Phosphorylated STAT-5 in cytoplasm :

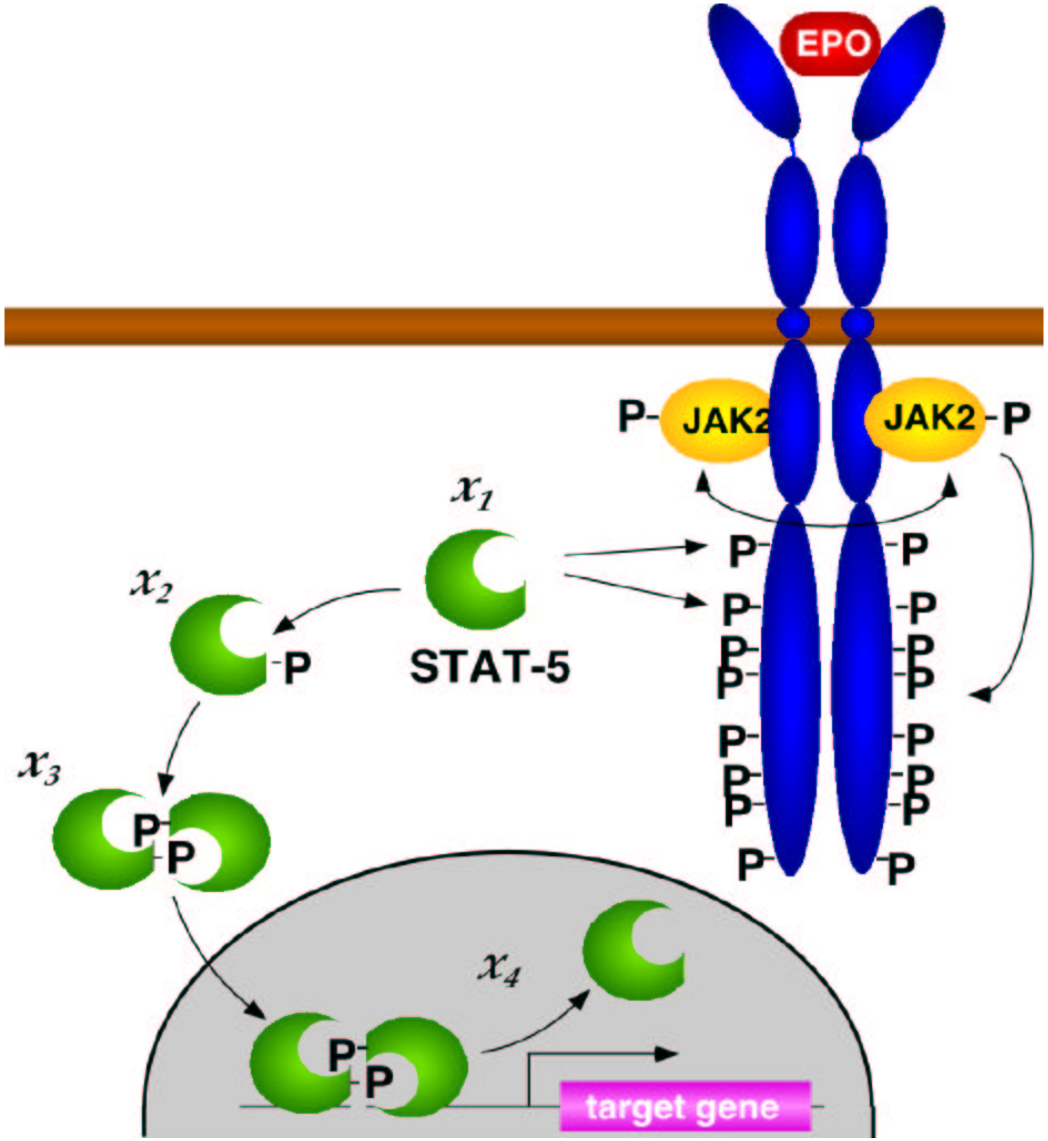


First results

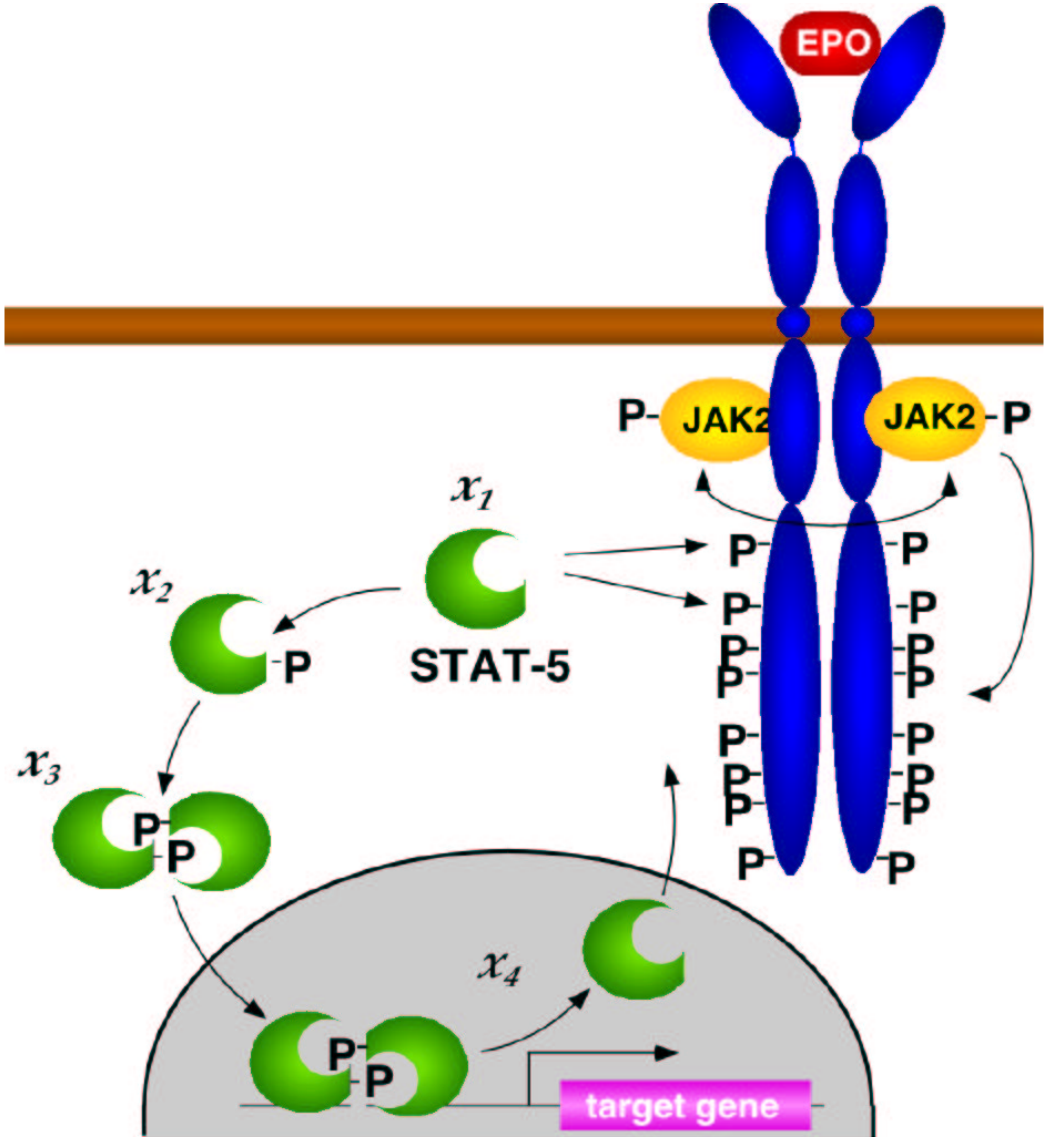
All STAT-5 in cytoplasm :



JAK – STAT Pathway



Model Extension

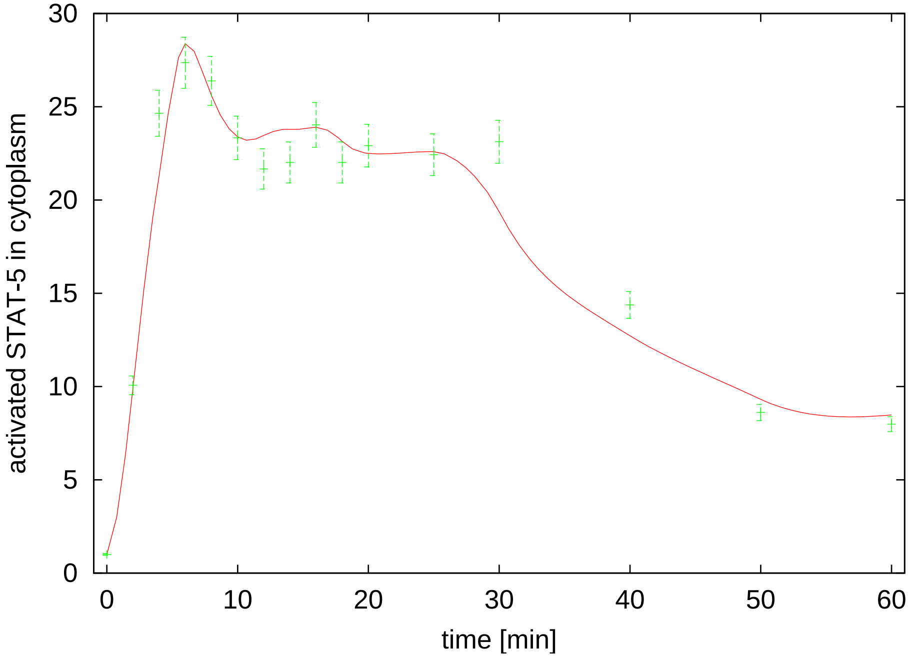


Second try

$$\begin{aligned}
\dot{\mathbf{x}}_1 &= 2\mathbf{p}_4\mathbf{x}_3^{\tau} - \mathbf{p}_1\mathbf{x}_1\mathbf{EpoR}_A \\
\dot{\mathbf{x}}_2 &= \mathbf{p}_1\mathbf{x}_1\mathbf{EpoR}_A - \mathbf{p}_2\mathbf{x}_2^2 \\
\dot{\mathbf{x}}_3 &= \frac{1}{2}\mathbf{p}_2\mathbf{x}_2^2 - \mathbf{p}_3\mathbf{x}_3 \\
\dot{\mathbf{x}}_4 &= \mathbf{p}_3\mathbf{x}_3 - \mathbf{p}_4\mathbf{x}_3^{\tau}
\end{aligned}$$

Results

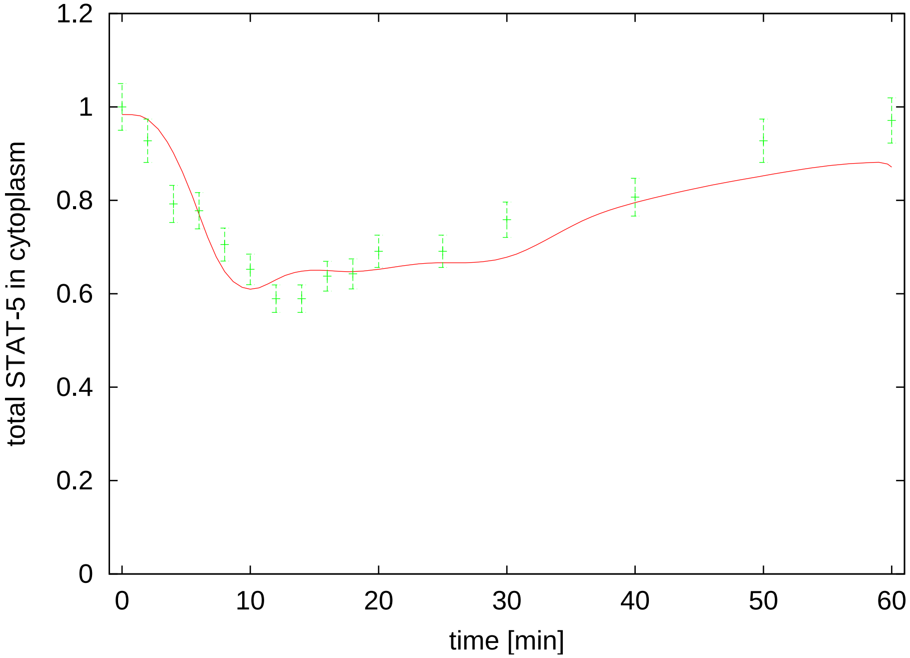
Phosphorylated STAT-5 in cytoplasm :



Sojourn time in nucleus $\tau \approx 6$ min

Results

All STAT-5 in cytoplasm :



Observing the unobservable

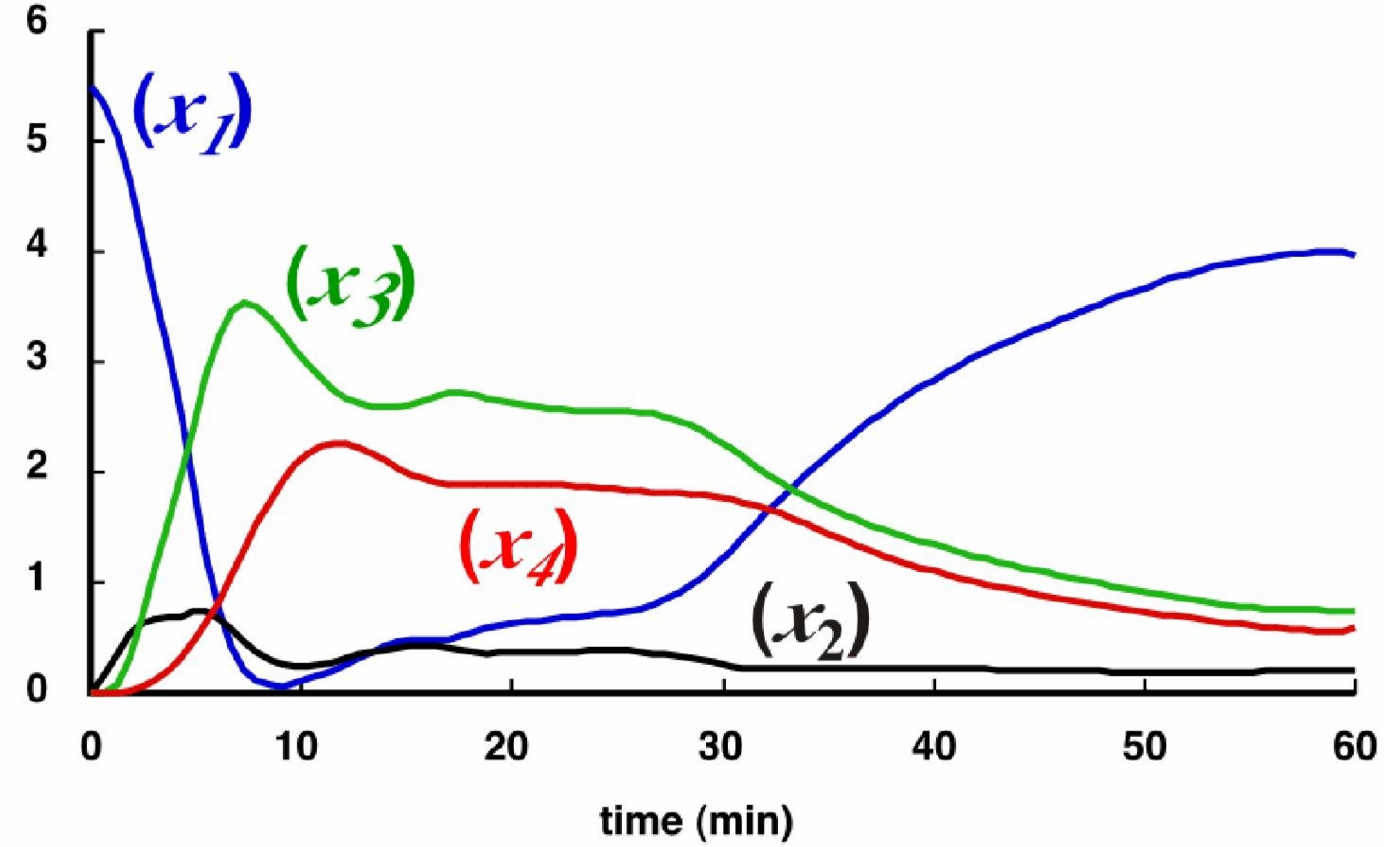
Simulating the fitted model :
Access to dynamic variables x_i

- **Unphophorylated STAT-5 is limiting factor**

- **Experimental fact:**
  Phosphorylated monomeric STAT-5 is hard to measure

  Explanation by the model:
  It is rapidly processed into dimeric STAT-5

Observing the unobservable: The Individual Players


6
5
4
3
2
1
0
(x_1)
(x_3)
(x_4)
(x_2)
0
10
20
30
40
50
60
time (min)

In silico Biology: Impossible Experiments

”What happens if ... ?” Investigations

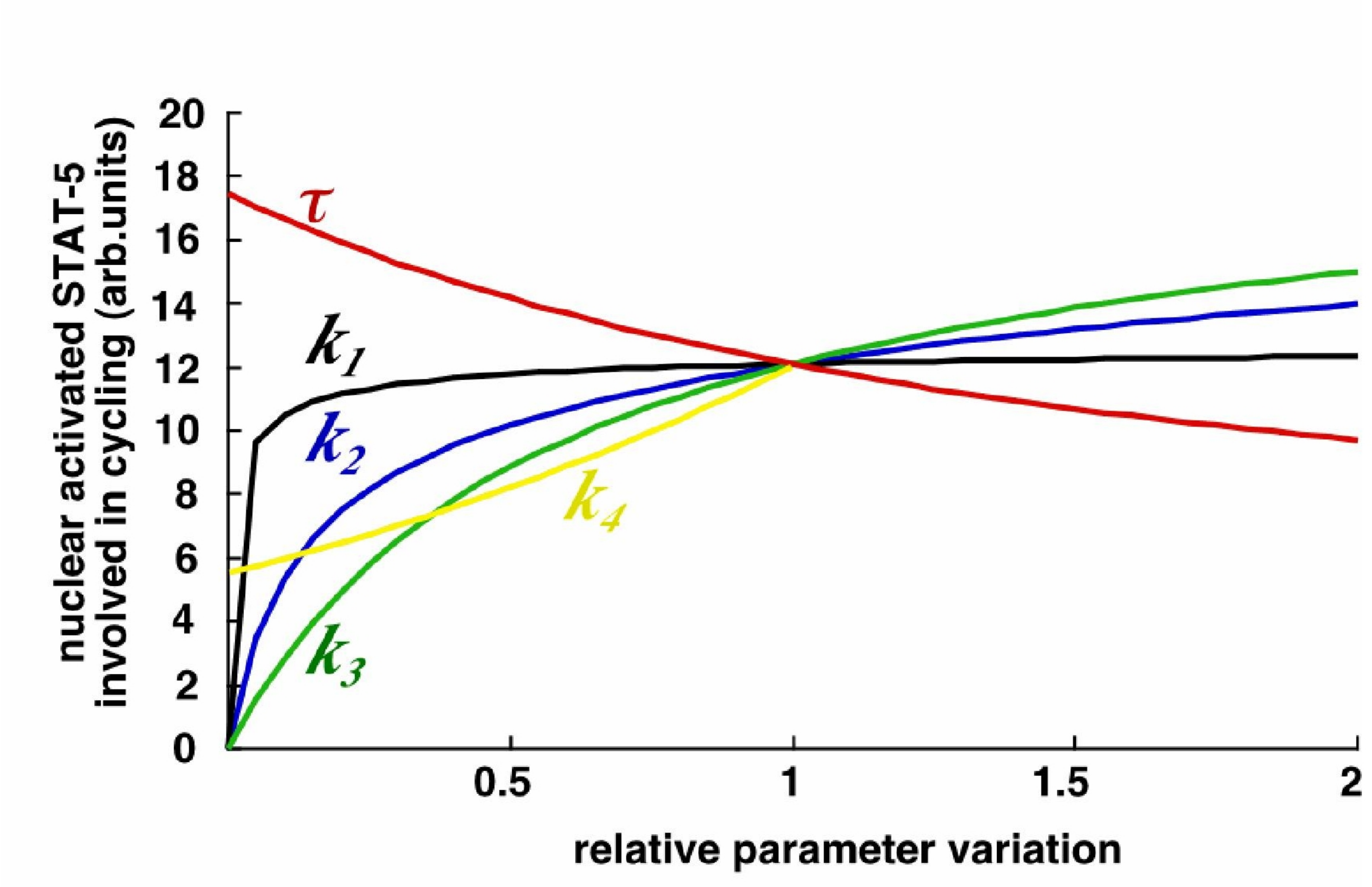
Sensitivity analysis:

- **Change parameters in the model**
- **Calculate the transcriptional yield**

Perspective:

Identification of potential targets for medical intervention

Sensitivity Analysis



Prediction of New Experiment

- Result of sensitivity analysis:

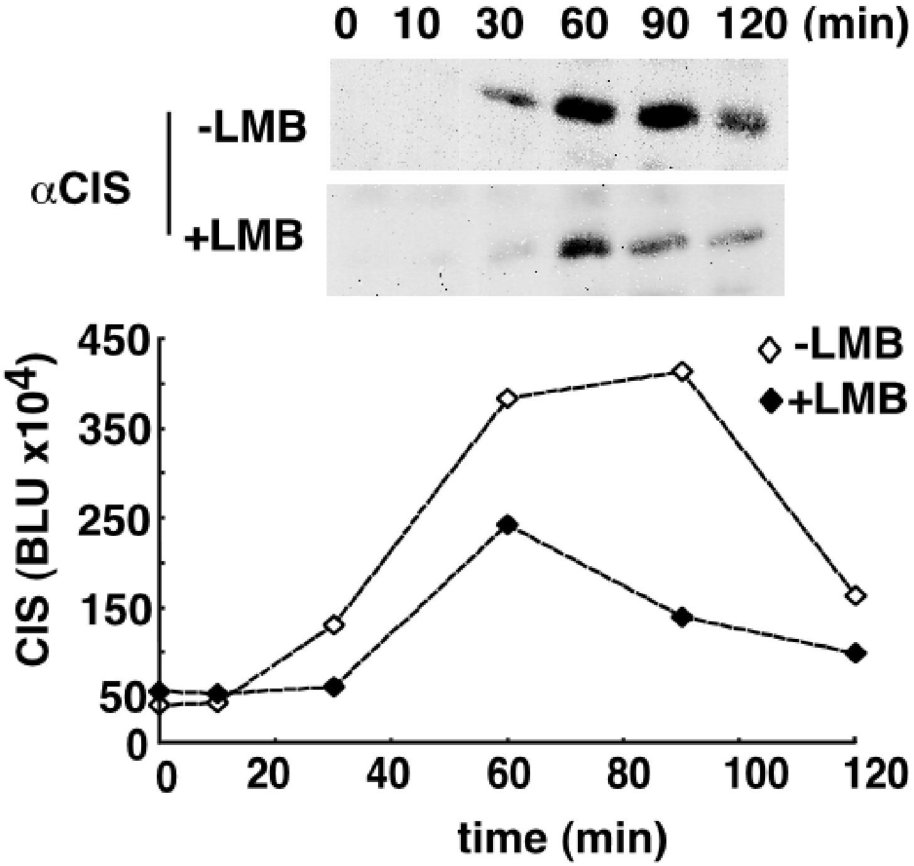
  Transcriptional yield is most sensitive to nuclear shuttling parameters.

- Setting nuclear export to zero

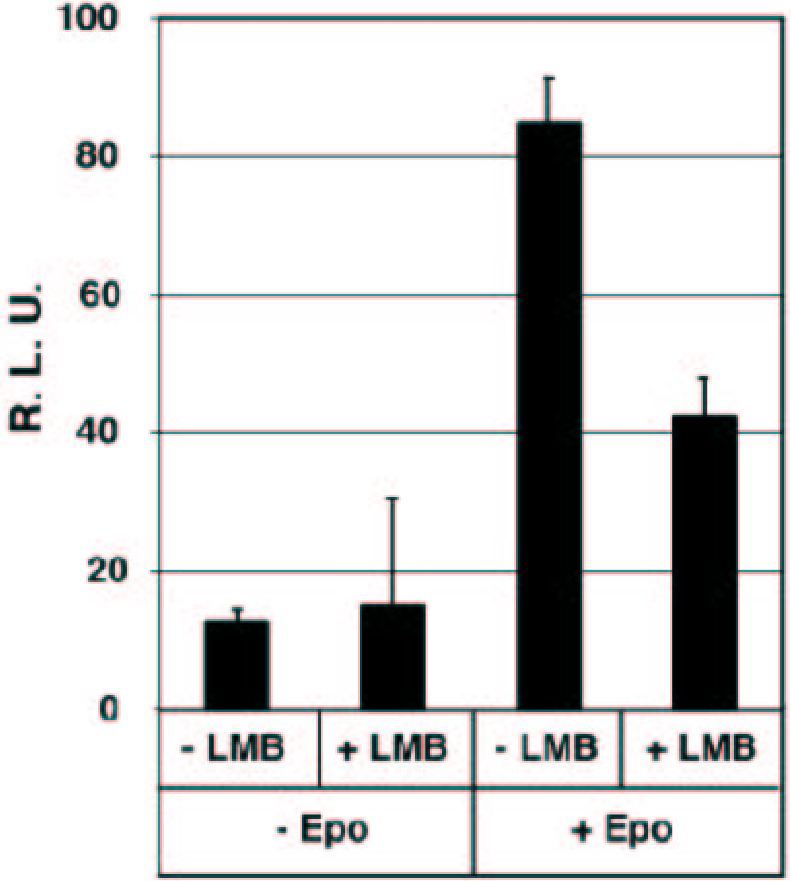
  $\Longrightarrow$ Only one cycle : Only 50 % efficiency

- Blocking nuclear export by leptomycin B confirms prediction.

Experimental Confirmation of Prediction



Experimental Confirmation of Prediction



Why Cycling ?

- **Optimal use of limited pool of STAT-5**
- **Continuous monitoring of receptor activity :**

Systems' property: "Remote Sensor"

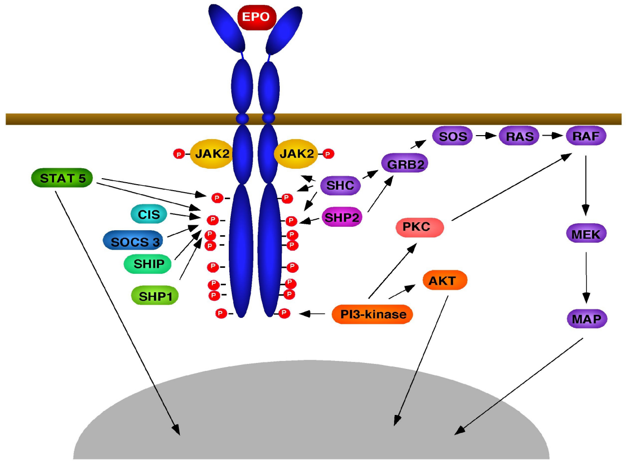
Swameye et al. Proc. Natl. Acad. Sci. 100, 2003, 1028-1033

"All models are wrong ..."

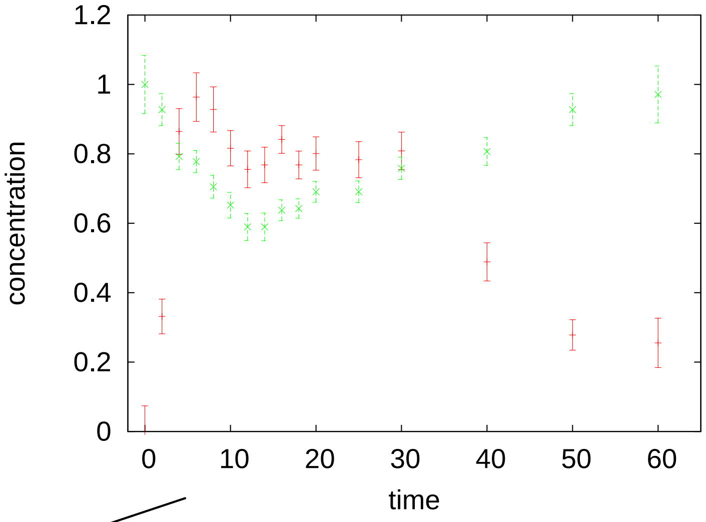
- No scaffolding for receptor–STAT-5 interaction, 200 eqs.
- Spatial effects, ODE vs. PDE
- Stochastic effects
- Data averaged over 10^6 cells

"... but some are useful"

- Captures the main effects
- Makes testable prediction



Signal transduction through the Erythropoietin receptor (EpoR)



$$\dot{\vec{x}} = \vec{f}(\vec{x},\vec{p})$$

In silico **biology**

Test the prior knowledge

Understanding systems' properties

Identification of potential drug targets

Acknowledgements

Theoretical side	Experimental side
	DKFZ, Heidelberg
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	Ursula Klingmüller