Reminder of Course Aims

Course is NOT about memorization, but about training skills, quantitative and critical thinking

- different from most ETH lectures less new knowledge but ability to use existing knowledge
- train to operate independently in “unfamiliar” situations with open end (and less precise) questions
  Keep in mind that it is about concepts and NOT the examples we use, they are for illustration

3. ODE Modeling of Metabolic Pathway

- Relate experimental observations to model behaviors via parameters.
- Understand potential biological problems of metabolic dynamics and how regulation can help.
- Understand basic principles of neg and pos feedback.
- Understand biological challenges arising in bi-directional pathways, example of glycolysis/gluconeogenesis.

Exercise 4

Continuing dynamic analyses, you will be able to:

- understand limitations of simple model to predict response to perturbations, and develop ideas how to overcome the limitations.
- understand effect of basic regulation (neg feedback) on dynamic system - learn to hypothesize and reason on regulatory function.

Exercise 5

build, analyze and simulate a real (bi-directional) pathway: glycolysis.

Systembiologie 551-1174-00L

ODE Modeling of a Metabolic Pathway

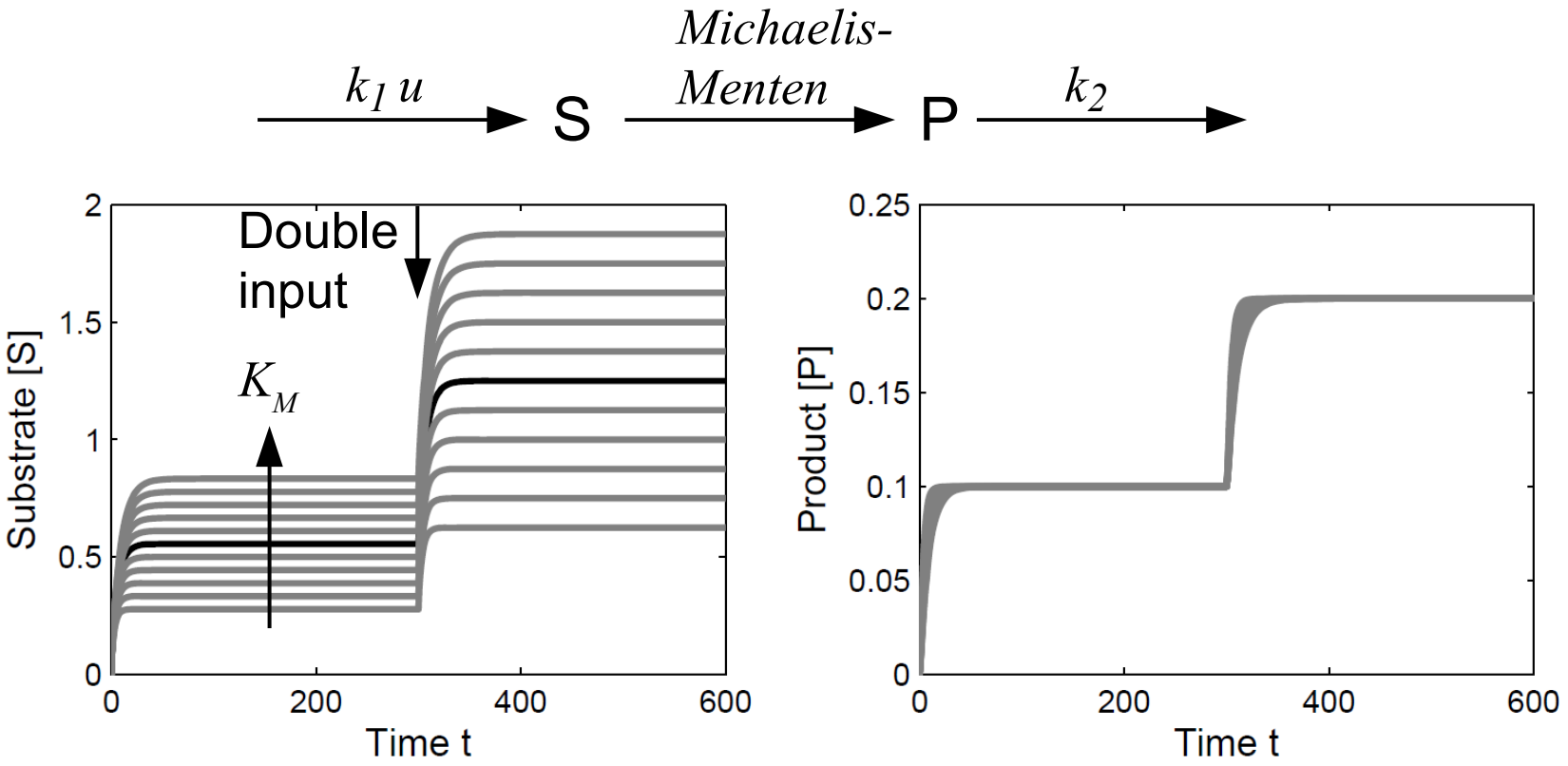
9 March, 2017

Uwe Sauer & Jörg Stelling

Content:

- Concepts: Parameters & model quality (JS)
- Potential biological consequences of metabolic dynamics (US)
- Concepts: Feedback (JS)
- Glycolysis/Gluconeogenesis (US)

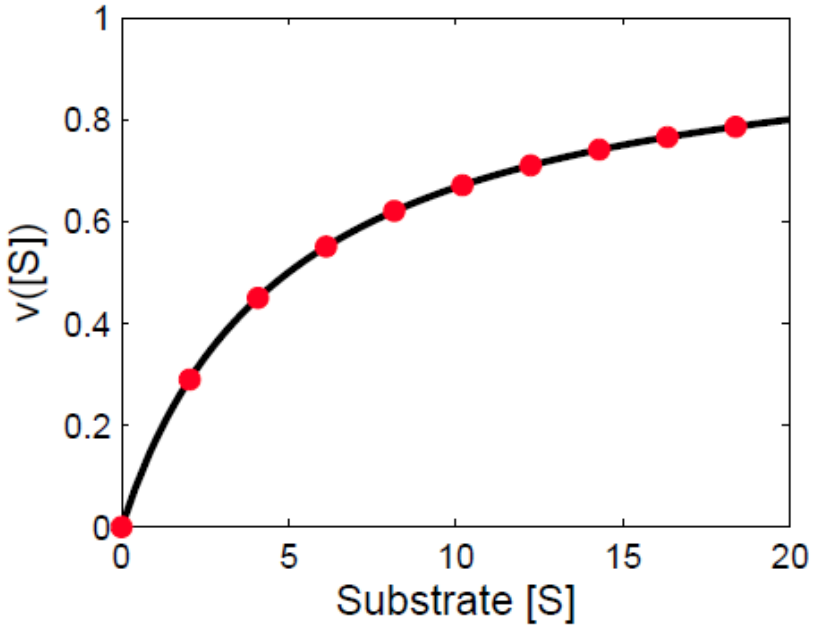
Pathway Dynamics: Example Revisited



- **Parameter choices affect predicted dynamics.**
- Product concentration in steady-state is identical:

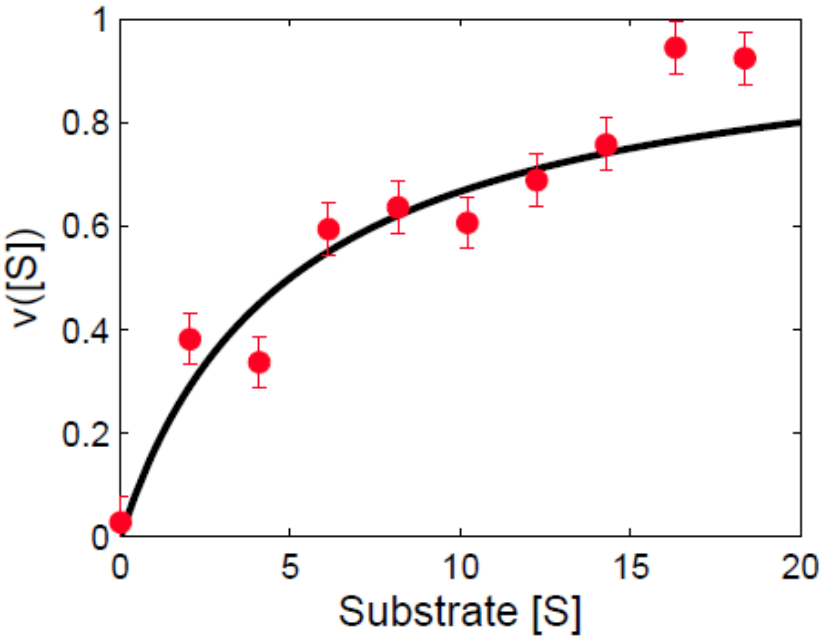
  Fluxes through the pathway have to be equal.

Parameter Values: How to Obtain Them?



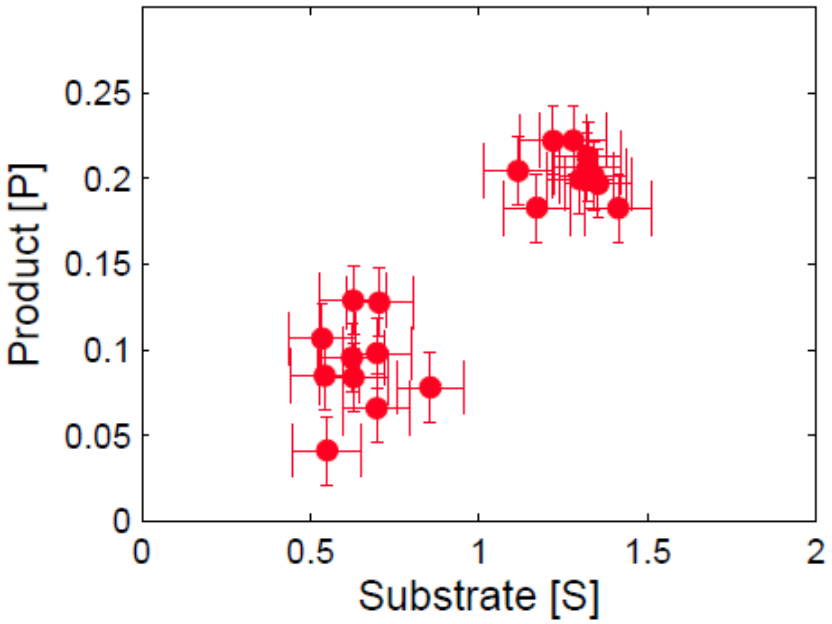
- **Ideal world:** Databases or measure all quantities exactly.

Parameter Values: How to Obtain Them?



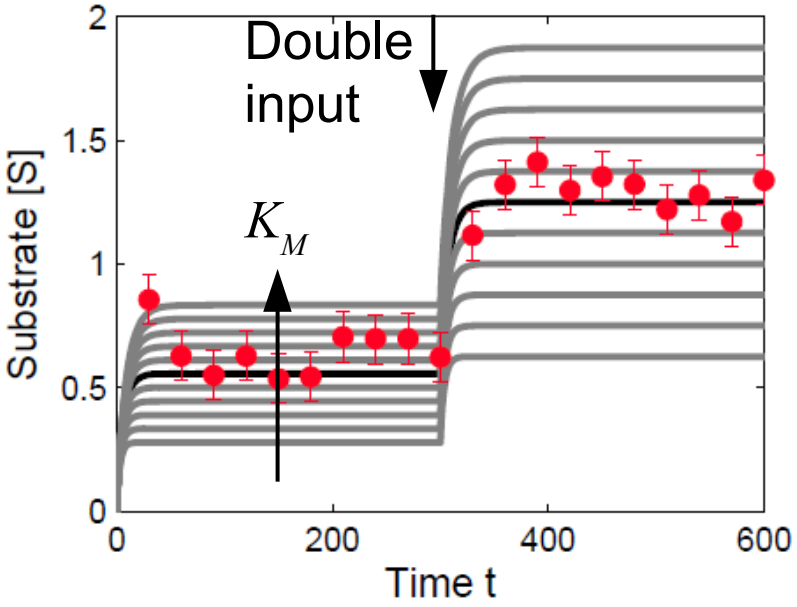
- **Ideal world:** Databases or measure all quantities exactly.
- **Reality:** Measurement errors induce uncertainty.

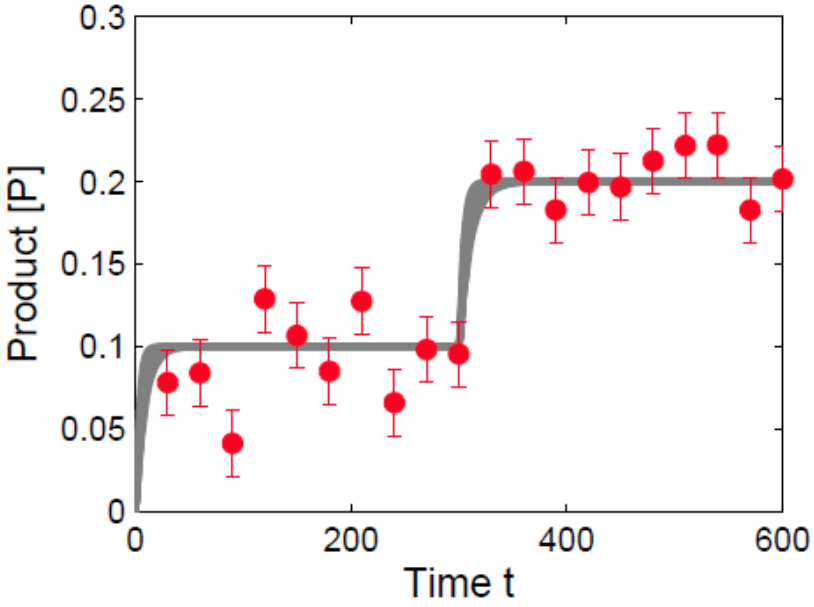
Parameter Values: How to Obtain Them?



- **Ideal world:** Databases or measure all quantities exactly.
- **Reality:** Measurement errors induce uncertainty.
- **Biological reality:** Not all quantities can be measured → We need (the right) models to **estimate** parameters.

Pathway Dynamics: Example With Data

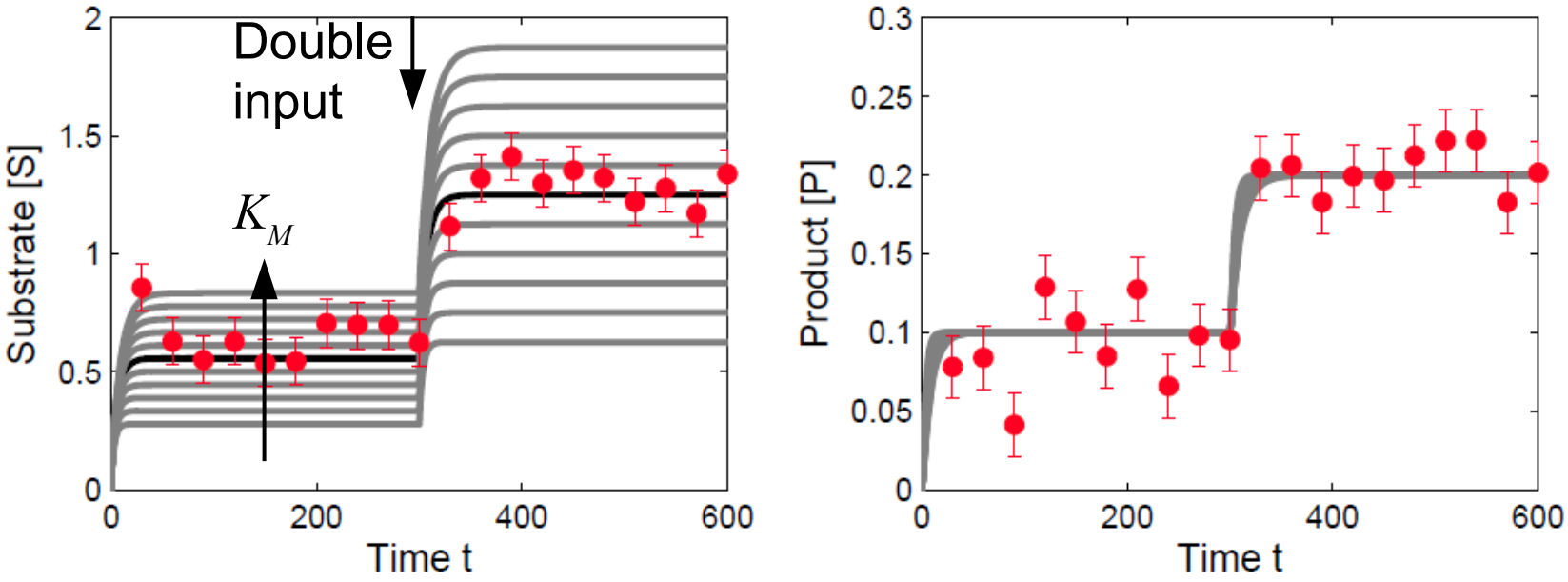




- **Aim:** Select parameter value (for K_M) that best fits data.

Is S or P data more informative for K_M ?

Concept: Measuring Model Quality

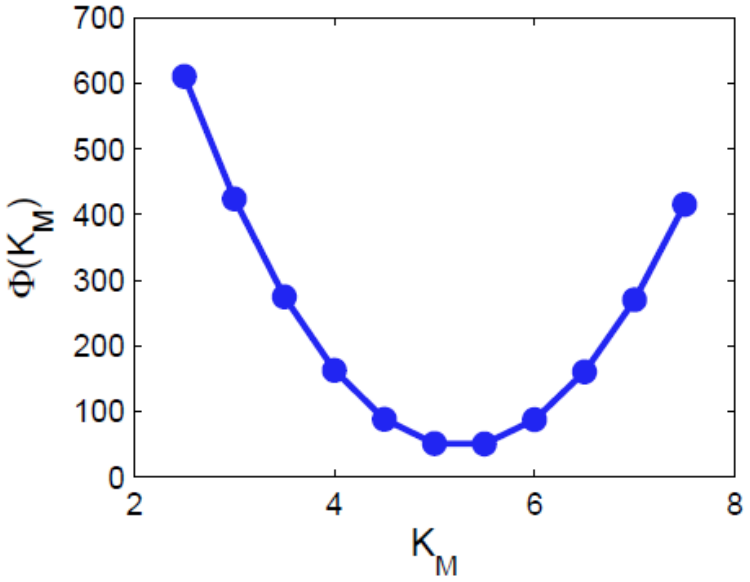


- **Measure parameter quality:** Distance between simulation (x) and experimental (x^E) data, taking measurement error (σ^E) into account:

this formula gives a good approx for K_m

$$\Phi(K_M) = \sum_{i=1}^{N} \left[\frac{x(t_i, K_M) - x^E(t_i)}{\sigma^E(t_i)} \right]^2$$

Concept: Measuring Model Quality



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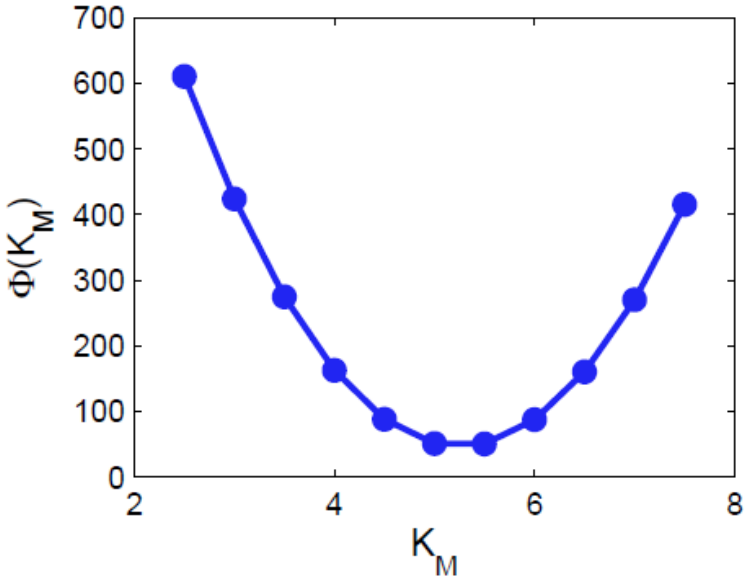
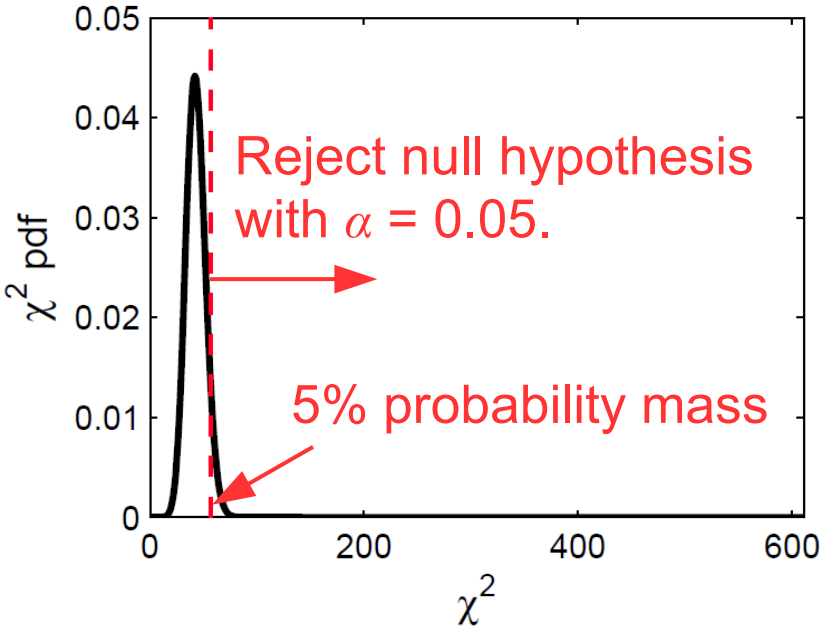
Concept: Measuring Model Quality



data itself is unsure due to background noise and limits of the measuring tools

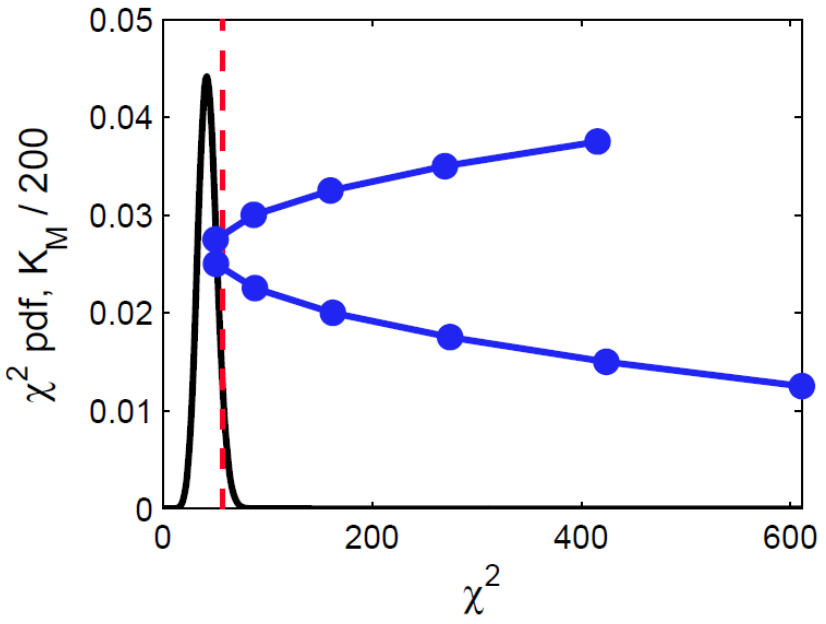
- **Measure parameter quality:** But which distance between model and data is 'small enough'?
- **Answer by statistical test:** Is the devation just caused by measurement noise in the data?

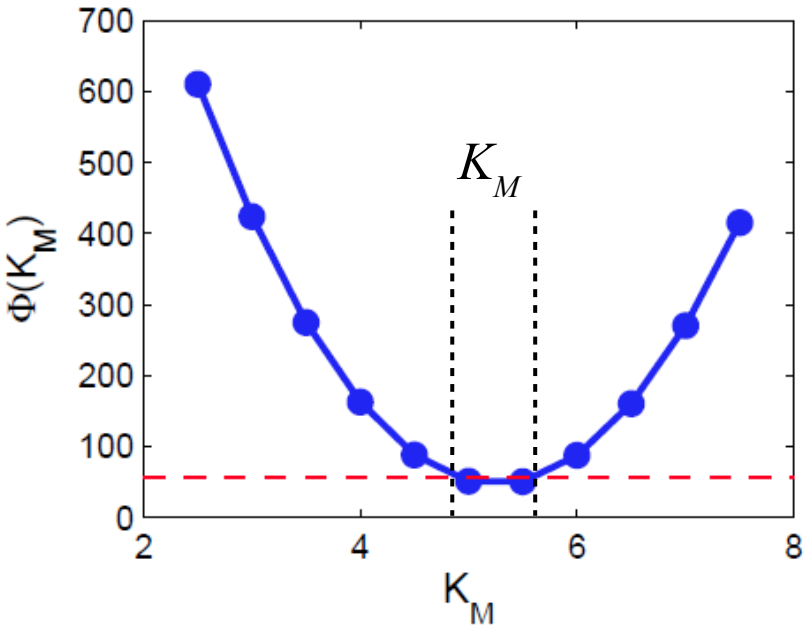
Concept: Test Statistics



- ***χ2*-test:** Null hypothesis = deviation is caused by measurement noise with #data points - #estimated parameters degrees of freedom.
- Select confidence level (α) and compare to distance.

Concept: Uncertain Parameter Values





- ***χ2*-test:** For the chosen confidence level, several parameter assignments are statistically valid.
- **General consequence:** Measurement noise leads to uncertainties in parameter values.

Example: Michaelis-Menten Parameters

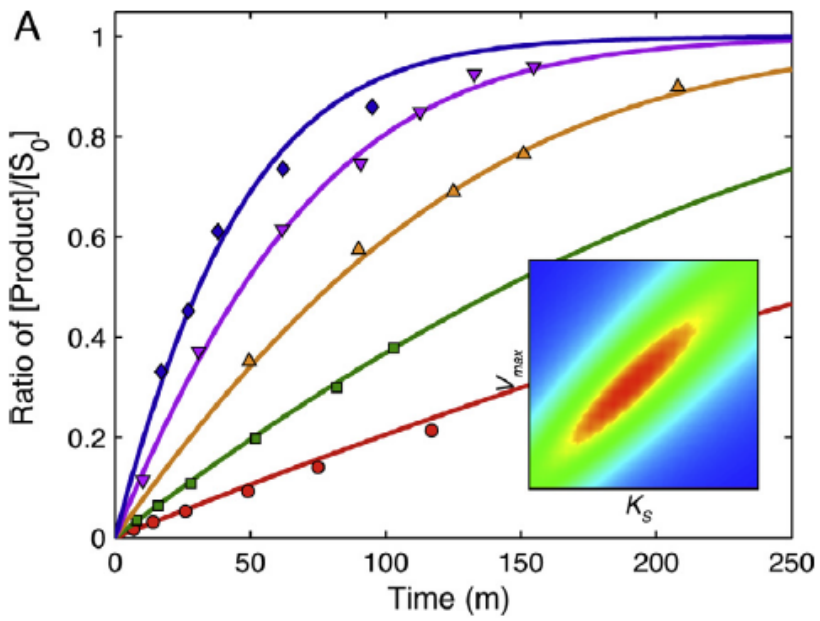
Die Kinetik der Invertinwirkung.

Von

L. Michaelis und Miß Maud L. Menten.

(Eingegangen am 4. Februar 1913.)

$$v = \frac{v_{max}[S]_0}{[S]_0 + K_M}$$



K. A Johnson, *FEBS Letters* 587: 2753 (2013).

- v_{max}/K_M estimation with pen & paper (1913): 0.045 ± 0.003 min^{-1}
- v_{max}/K_M estimation by computation (2013): 0.046 ± 0.001 min^{-1}

Summary: Teaching Goal I

- Parameter values often have to be estimated indirectly experimental data using a model.
- Model behaviors and experimental data can be related by measuring the distance between both.
- This distance depends on parameter values - ‘good’ parameter values show a low distance.
- With a statistically defined distance threshold, we obtain parameter estimates and their uncertainties.

Systems Biology 551-1174-00L

ODE Modeling of Metabolic Pathways

9 Mar, 2017

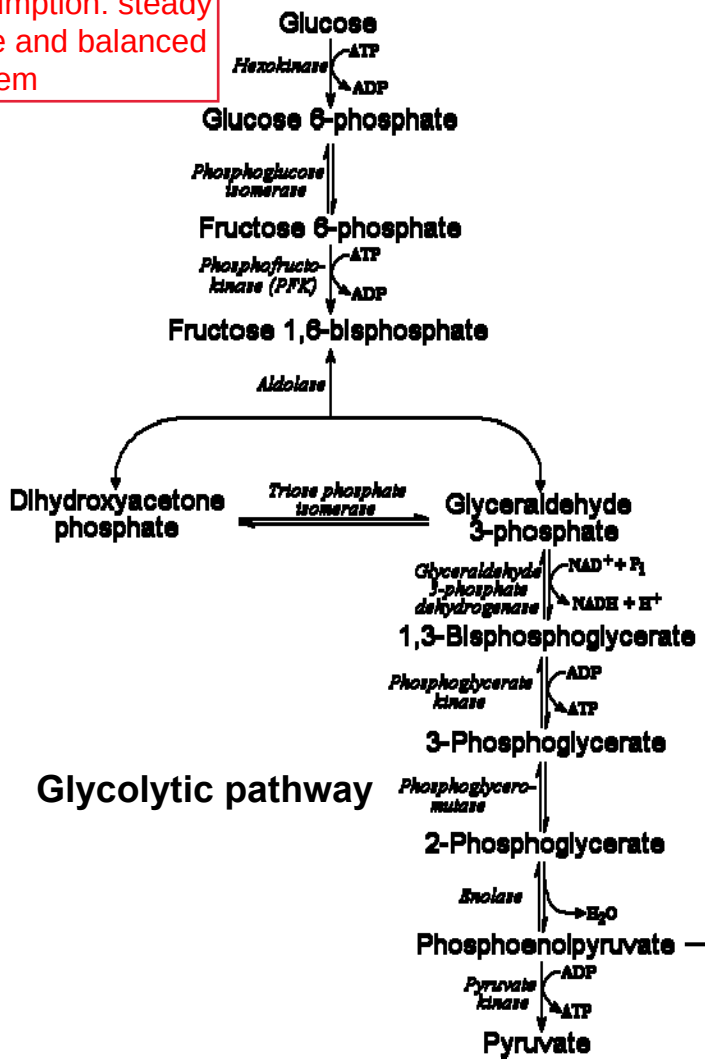
Uwe Sauer, Institute of Molecular Systems Biology

Content:

- Concepts: Parameters & model quality (JS)
- **Biological consequences of metabolic dynamics (US)**
- Concepts: Feedback for simple systems (JS)
- Glycolysis/gluconeogenesis (US)

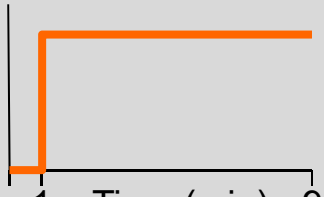
Metabolite Dynamics Upon Perturbation

assumption: steady state and balanced system



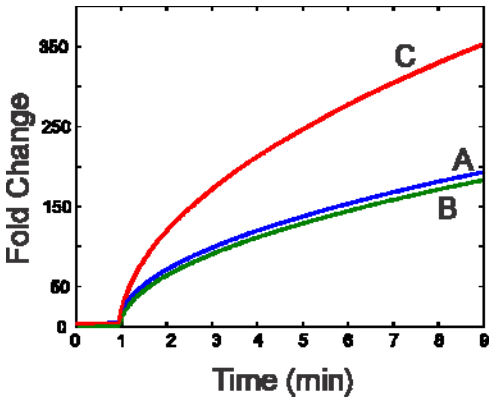
Glycolytic pathway

6-fold step increase of INFLUX to A

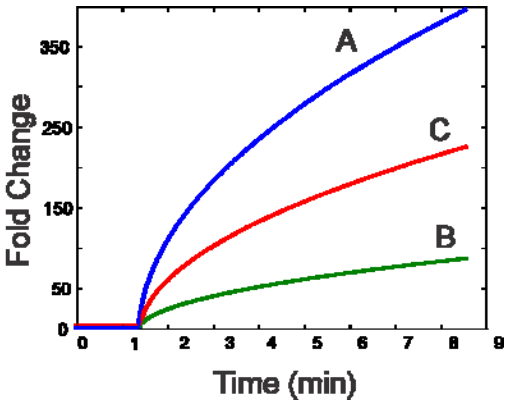


Parameter set 1

V_{max} and K_m of E_1 and E_2 similar



Parameter set 2



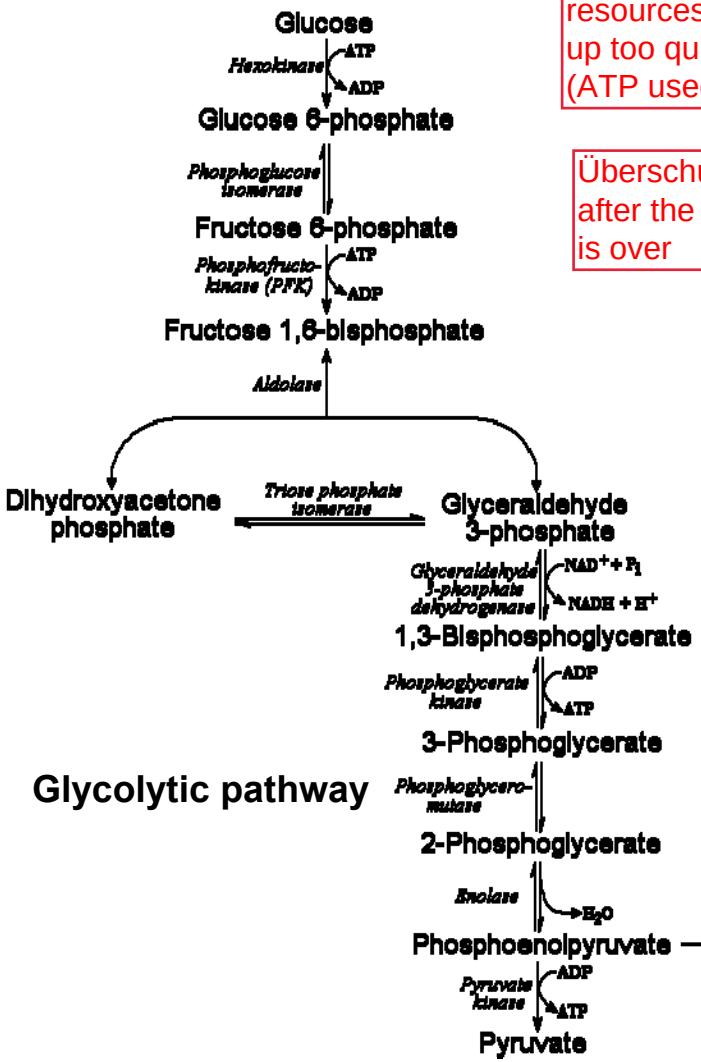
K_m of E_1, 40 times larger than in set 1

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C$$

E_1 is NOT saturated

no steady state in both cases

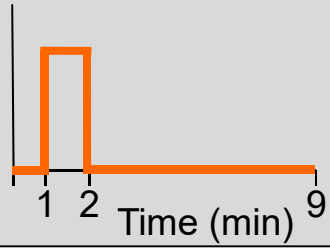
Metabolite Dynamics Upon Perturbation



problems: toxicity, resources are used up too quickly (ATP used up)

Überschuss even after the pulse (1s) is over

6-fold pulse-like increase of INFLUX to A



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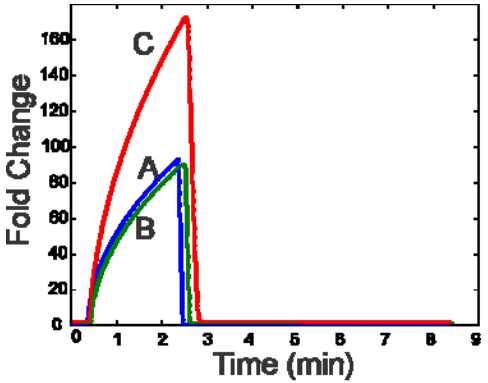
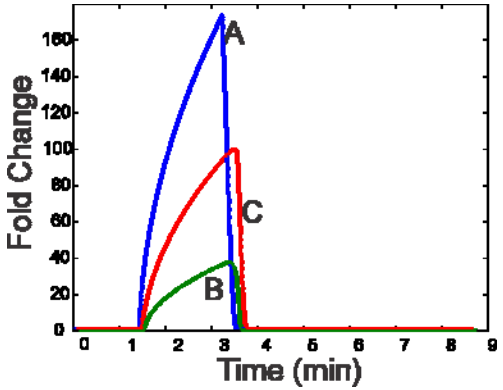


Parameter set 2



K_m of E_1, 40 times larger than in set 1

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C$$

What are potential problems for the cell ?

Consequences of Metabolite Dynamics for the Network

- Consequences of unregulated pathways:

  long time until steady state
  overshoot in metobolie [conc]

- Potential problems of such overshoots ?

  metabolite toxicity
  influence other reactions where it participates
  could drain cell of ATP, phosphates etc

In exercise you will explore how S and P inhibition can alleviate such problems.

How can cells avoid/reduce such problems ?

implement regulation

Achieving appropriate dynamic responses to perturbations is a general control problem for all biological systems.

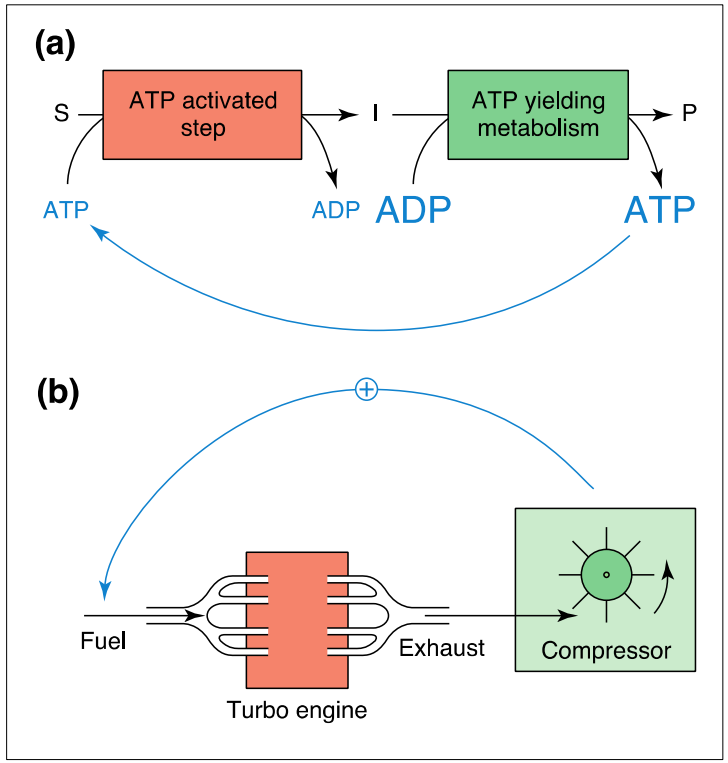
- avoid extreme responses
- quickly achieve a new steady state
- respond flexibly at a time scale that matters
- remain robust to different types, intensities and frequencies of perturbation.
- …….

Just like in technical control systems (eg air condition), biological control systems are not perfect. In the following we'll discuss an example: Turbo Design

Dynamic Problems of Pathways with Turbo-Design

- many catabolic pathways begin with an ATP-requiring activation reaction
- net ATP generation occurs further down the pathway
- biologically useful when there is a continuous supply of substrate (eg in higher organisms)
- *perturbation: what happens upon substrate fluctuations?*

Teusink et al. 1998 Trends Biochem. Sci

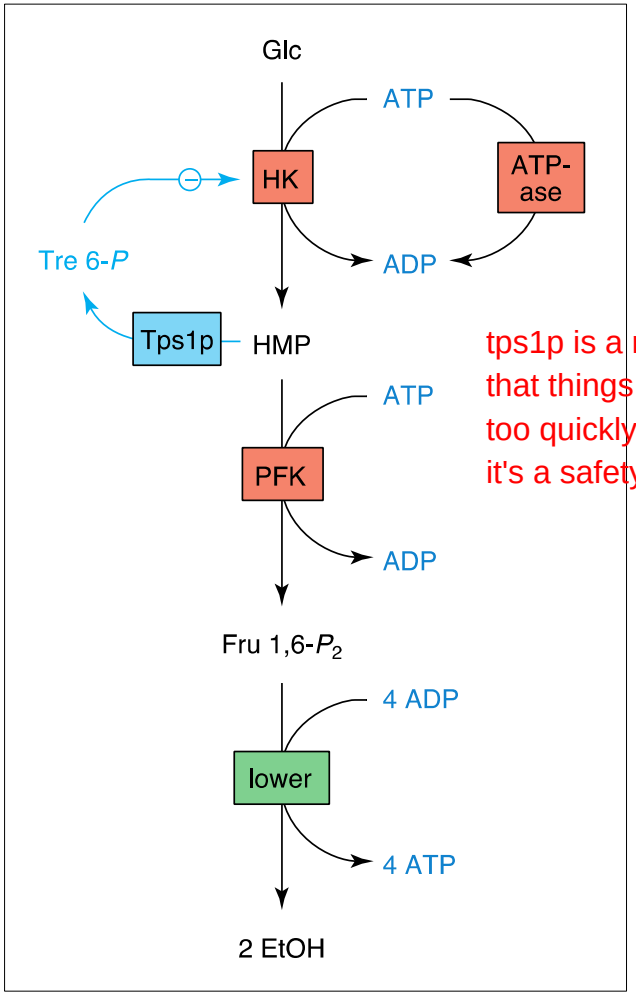


Comparison of an activated catabolic pathway with a turbo engine. (a) General scheme for a catabolic pathway in which the first step involves coupling of ATP hydrolysis to activation of a substrate (S). Downstream, the conversion of an intermediate (I) to a product (P) generates a surplus of ATP. (b) Schematic representation of a turbo engine, in which exhaust gases are used to increase the influx of fuel.

Turbo-Design: Example Glycolysis

pool of ATP drops and the next reaction cant even start due to little ATP present (lots of ADP)

- In bacteria and yeasts, sudden excess of substrate supply can lead to **substrate-accelerated death** (eg maltose supply in *S. cerevisiae*)
- With glucose it does not occur, unless *tps1* is deleted, which encodes the trehalose-6-P synthetase
- Although trehalose-6-P has no role in glycolysis, one function of Tps1-p is to inhibit the first step of glycolysis to reduce the initial flux (ie **guard** the initiation of glycolytic flux)



Schematic representation of the core model of glycolysis. In the model the lower part of glycolysis is represented as a single step. Glc, glucose; HMP, hexose monophosphate; Fru 1,6-P2, fructose 1,6-bisphosphate; Tre 6-P, trehalose 6-phosphate; EtOH, ethanol; HK, hexokinase; PFK, phosphofructokinase; Tps1p, Tre 6-P synthase; lower, lower part of glycolysis.

Teusink et al. 1998 Trends Biochem. Sci

tps1p is a regulating step so that things are not used up too quickly.
it's a safety system.

Turbo-Design Glycolysis: What is the Problem?

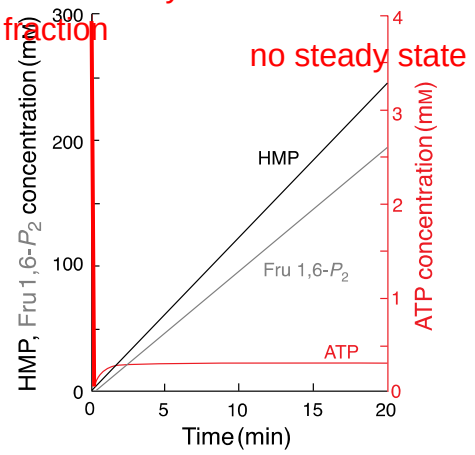
Time course simulations with a kinetic model of yeast glycolysis of a glucose pulse at T_0 (only the core reactions shown on the figure before)

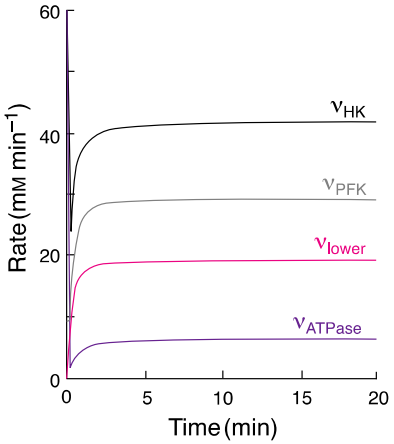
Unguarded glycolysis

ATP drops extremely, then recovers only a small fraction

trapped in low ATP state!

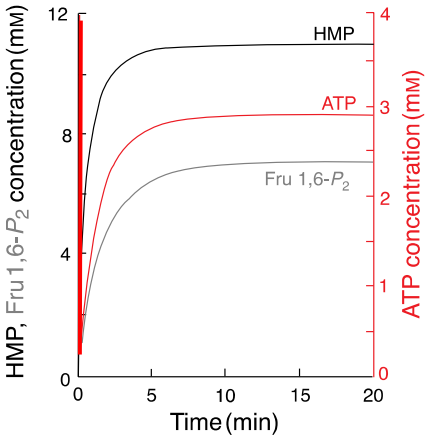
no steady state



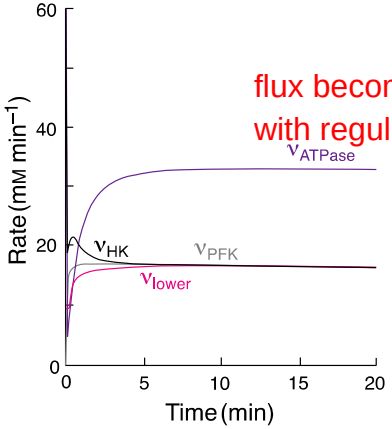


Guarded glycolysis

these find their steady state elater on



flux becomes balanced with regulation



Guarded means a feedback inhibition of hexokinase by hexose-P (for example through the trehalose-6-P loop)

Teusink et al. 1998
Trends Biochem. Sci

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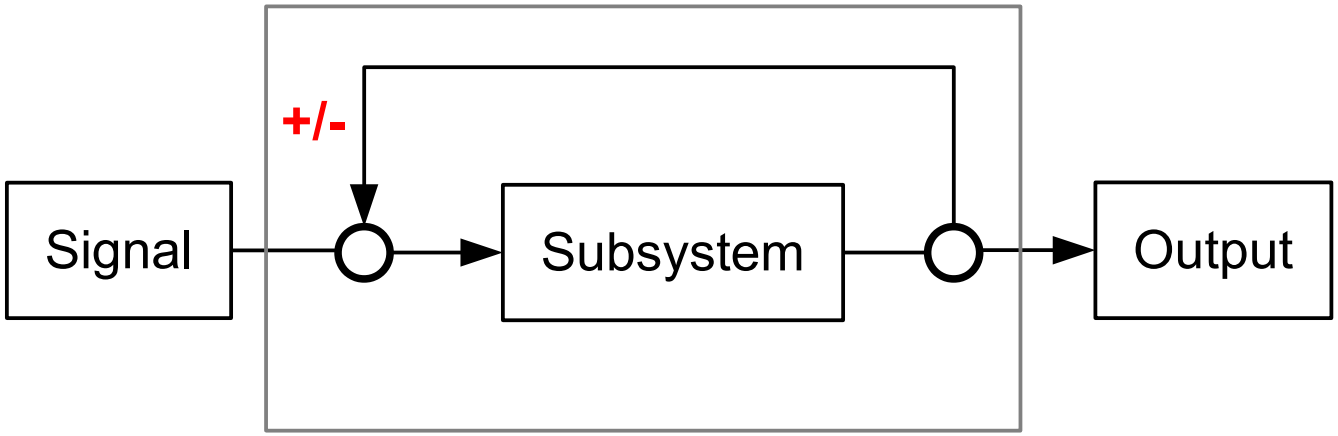
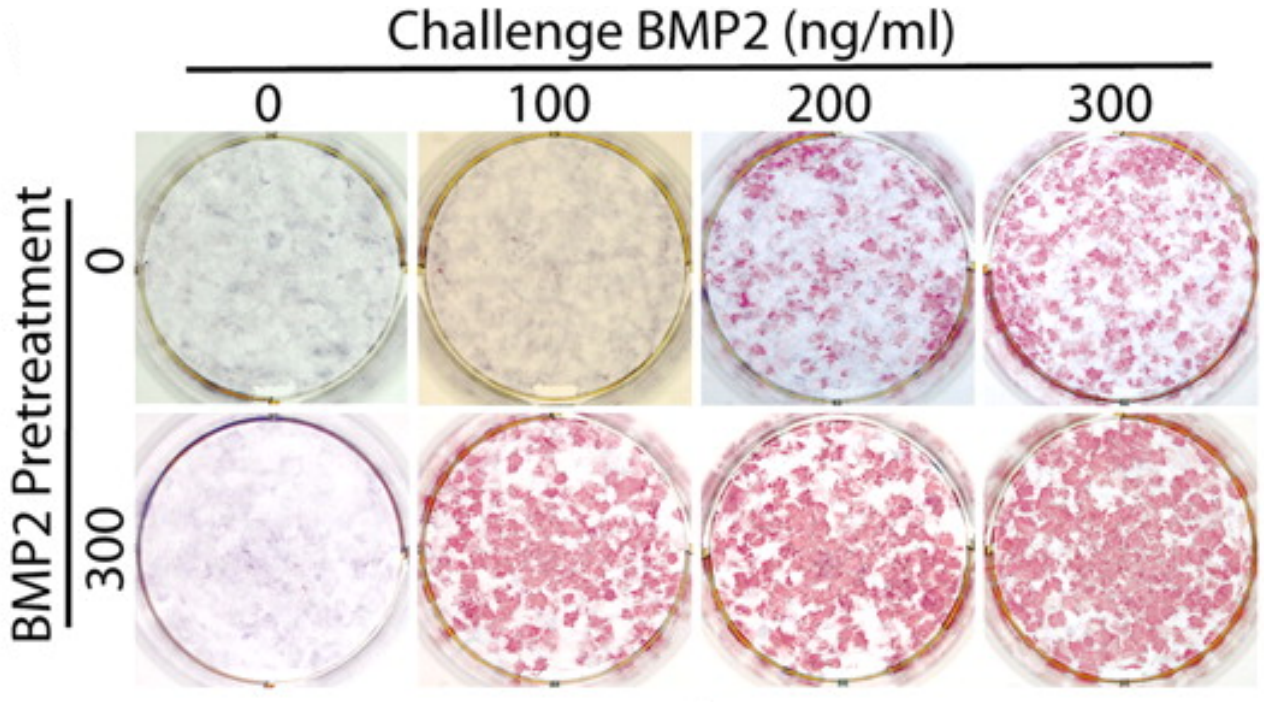
Concept: Feedback Systems



- **Circular patterns of interactions can establish feedback loops with positive or negative net effect.**
- Intertwined feedback loops → **Complex dynamics.**

Example: Memory in Cell Differentiation



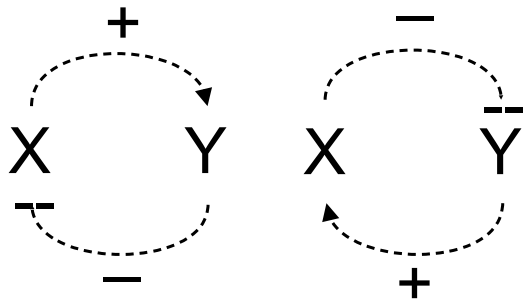
From: Wang et al. (2009) PNAS 106: 6638-6643.

- Human bone marrow cells exhibit memory of bone morphogenetic protein 2 (BMP2) pretreatment.

What could be mechanisms for memory?

Two Basic Types of Feedback Systems

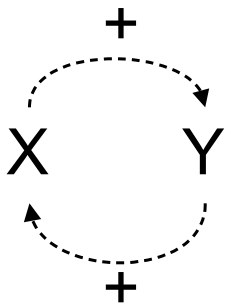
Negative Feedback



X activates Y, Y inhibits/inactivates X

vixe versa

Positive Feedback

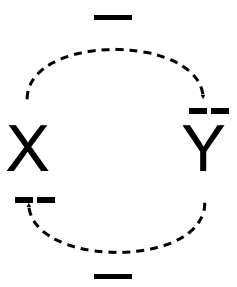


X activates Y, Y activates X

Turbo example from before, e.g.

≃

Mutual Antagonism

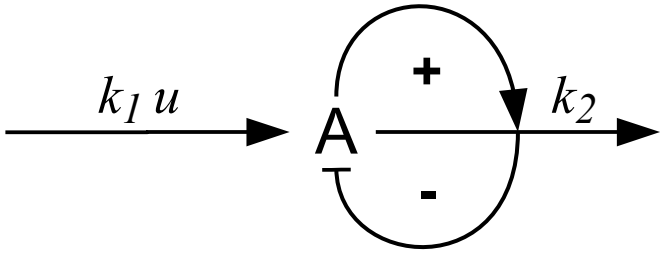


X inhibits Y, Y inhibits X

- Patterns of interactions between two components

  → **Qualitatively different feedback structures.**

Negative Feedback: Production-Degradation



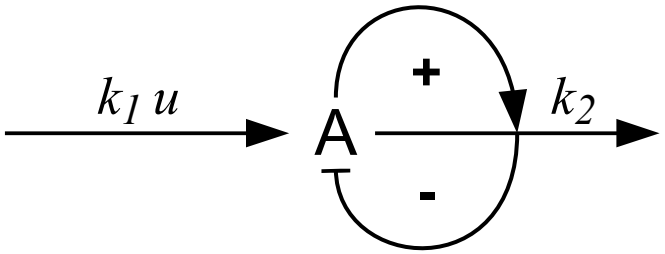
$$\frac{d[A]}{dt} = +k_1 \cdot u - k_2[A] \quad \Rightarrow \quad [A] = \frac{k_1 \cdot u}{k_2}\left(1 - e^{-k_2 \cdot t}\right)$$

- Increased A accelerates degradation, leading to reduced concentration of A → **Negative feedback**.
- From analytic solution: After perturbation, the system will return to (a) steady-state again → **Homeostasis**.

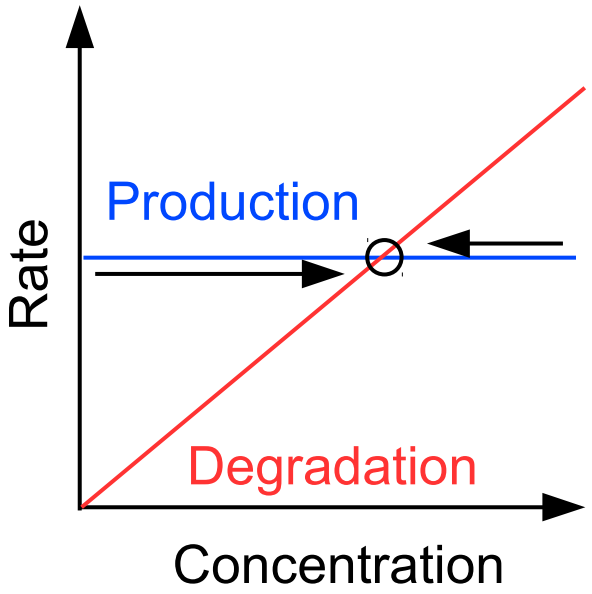
Can we analyze the behavior graphically?

Negative Feedback: Production-Degradation

mass action law assumed

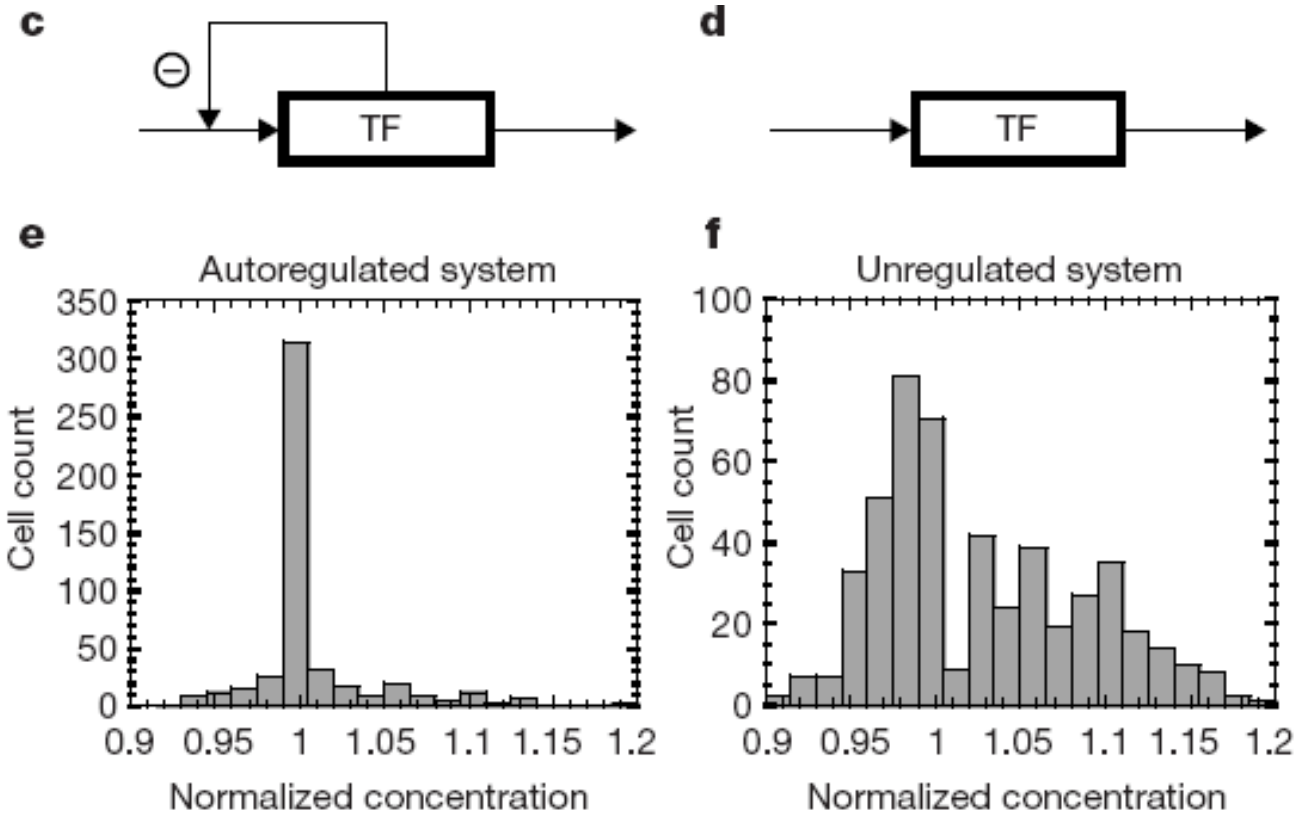


tthe system will reach a steady state through negative feedback eventually. degradation will rise until it crosses production and it is in homeostasis



- Increased A accelerates degradation, leading to reduced concentration of A → **Negative feedback**.
- Graphically: After perturbation, the system will return to (a) steady-state again → **Homeostasis**.

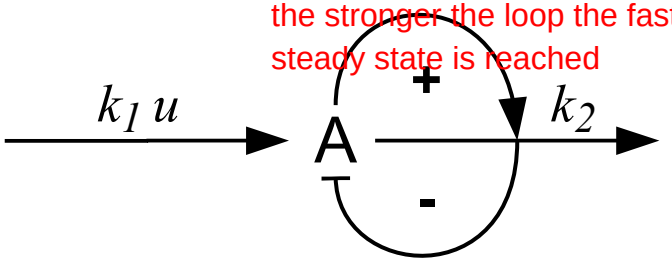
Negative Feedback: Example #1



From: Becskei & Serrano (2000) Nature 405: 591-593.

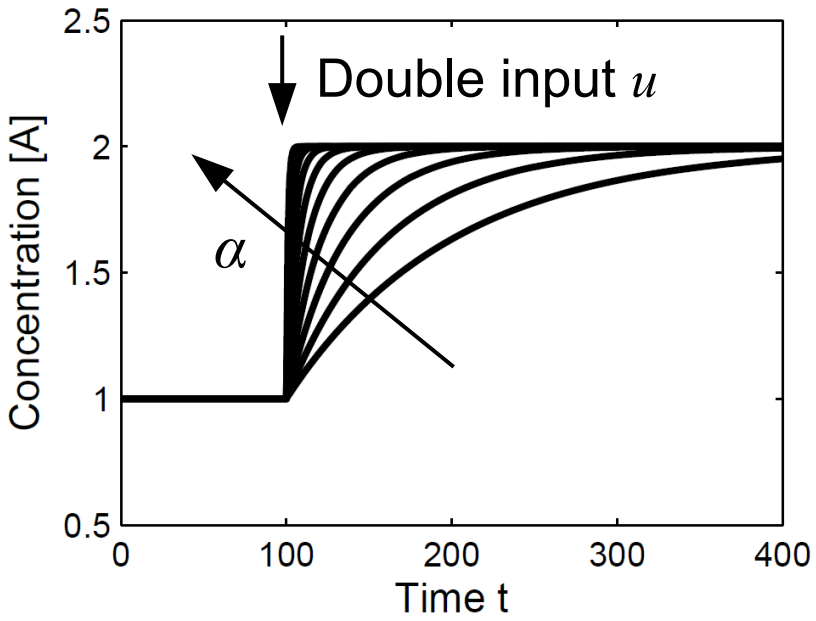
Negative Feedback: Production-Degradation

alpha moderates the strength
of the feedback below.
the stronger the loop the faster the
steady state is reached



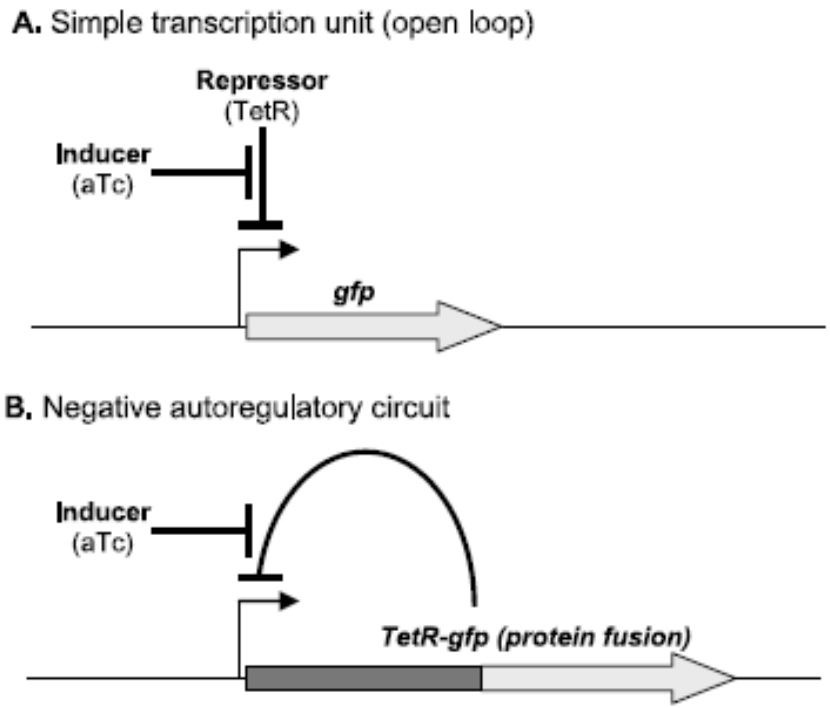
$$\frac{d[A]}{dt} = \alpha\left(k_1 \cdot u - k_2[A]\right)$$

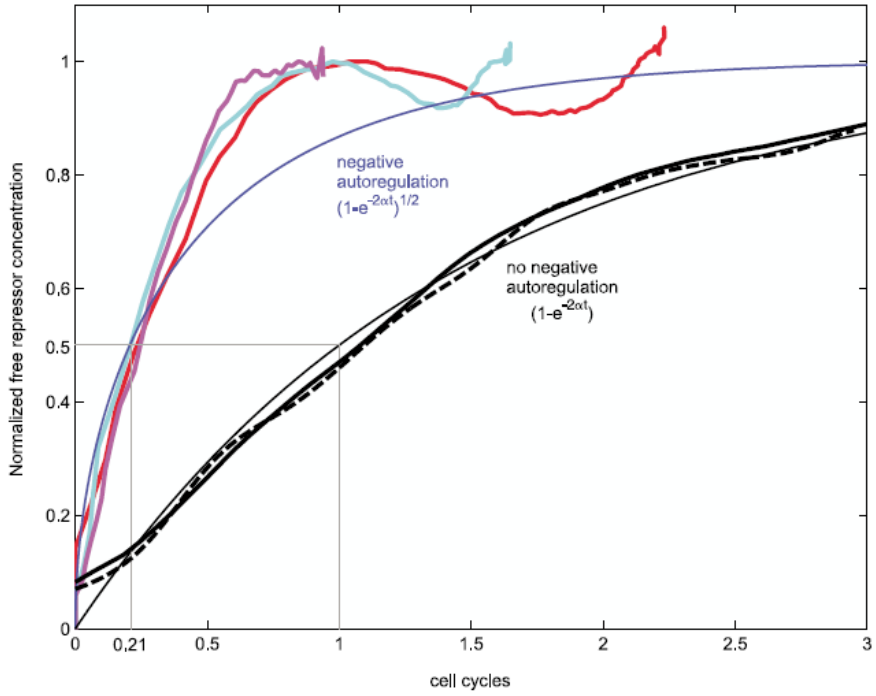
alpha changes how quickly steady state is reached



- Assume modified feedback and identical steady-state by scaling of both rates with factor α.
- Increased feedback gain → **Faster responses.**

Negative Feedback: Example #2





From: Rosenfeld et al. (2002) J. Mol. Biol. 323: 785-793.

Negative Feedback: Functions

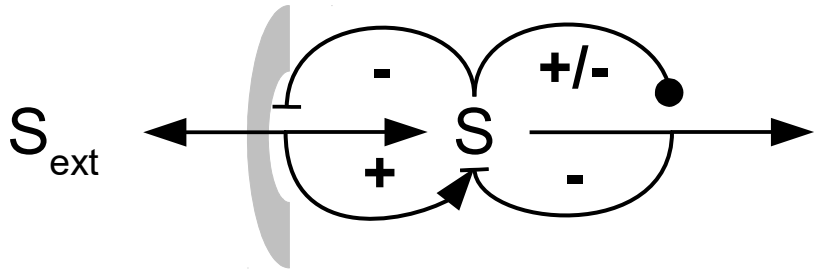
- **Simple negative feedback systems:**
  - Eventually approaching steady state.
  - Existence of a unique steady state.

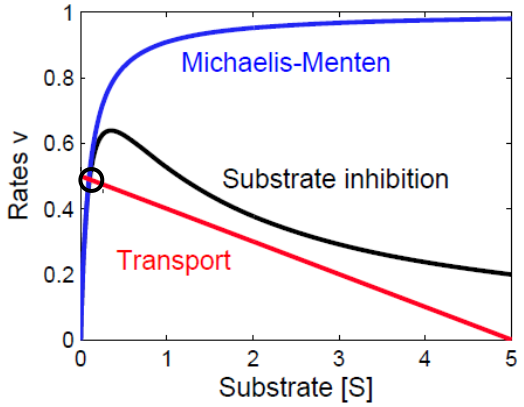
- **Functions in biological networks:**
  - Set point regulation → Homeostasis.
  - Perturbation rejection, fast responses.

Biological examples?

glycolysis

Positive Feedback in Substrate Inhibition





- Diffusion of extracellular substrate S_{ext} with rate:

$$v_{Transport}\left(\left[S_{ext}\right],\left[S\right]\right) = D\cdot\left(\left[S_{ext}\right]-\left[S\right]\right)$$

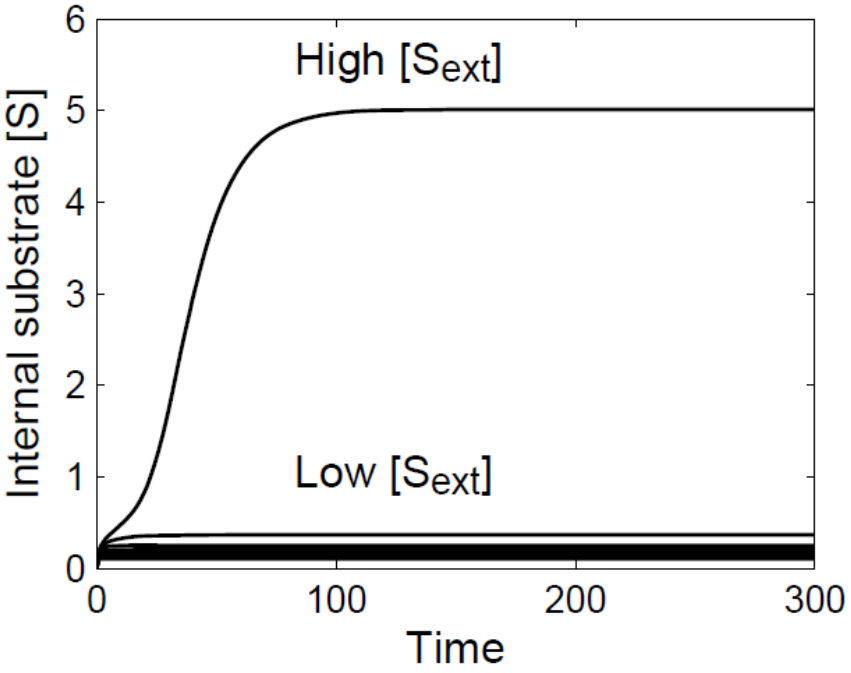
and degradation of substrate with substrate inhibition:

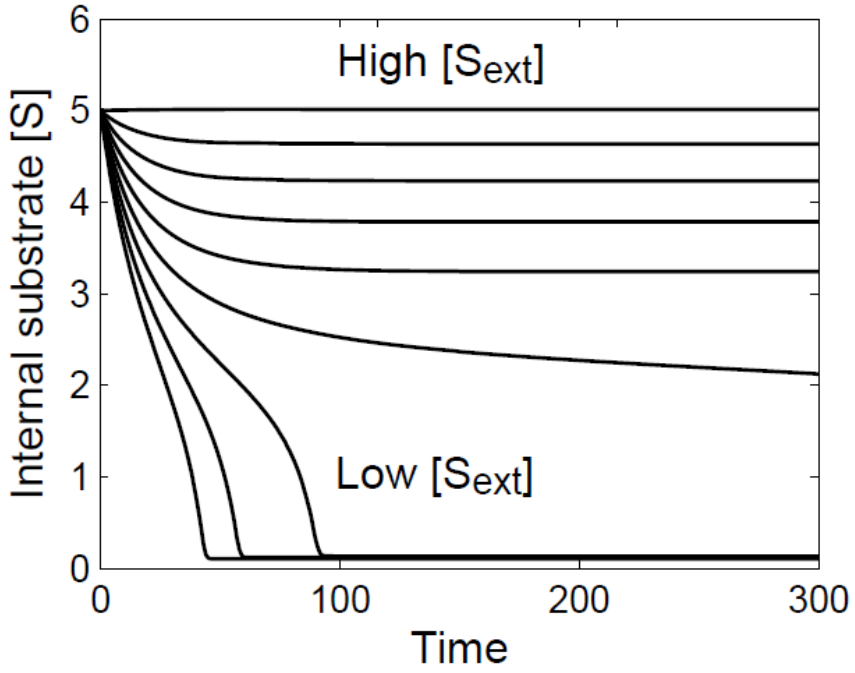
$$v_{Degradation}\left(\left[S\right]\right) = \frac{v_{max}\left[S\right]}{\left[S\right]+K_M+\frac{\left[S\right]^2}{K_I}}$$

- **Positive feedback possible** (depending on state).

Substrate Inhibition: Dynamic Memory

AB are different

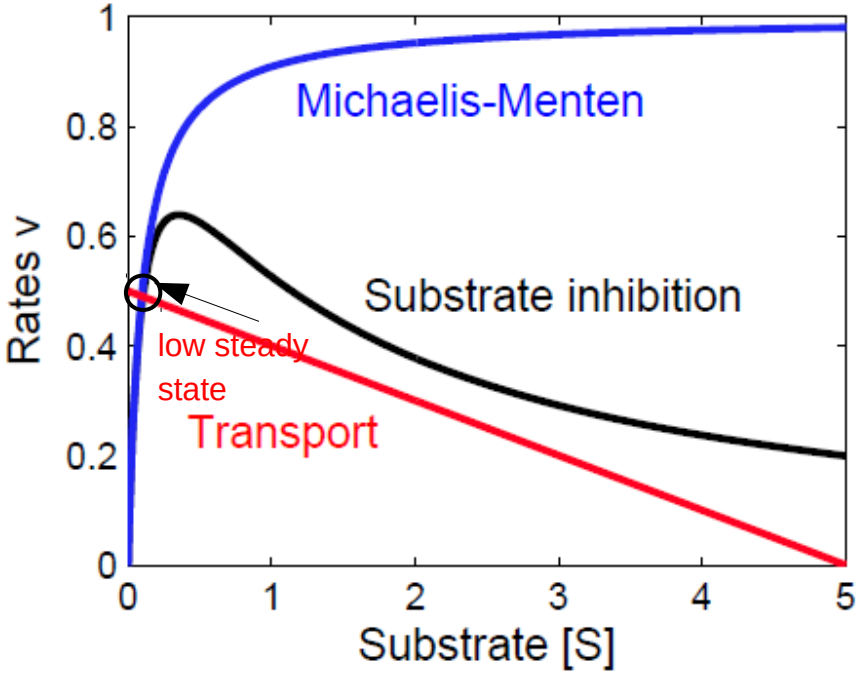


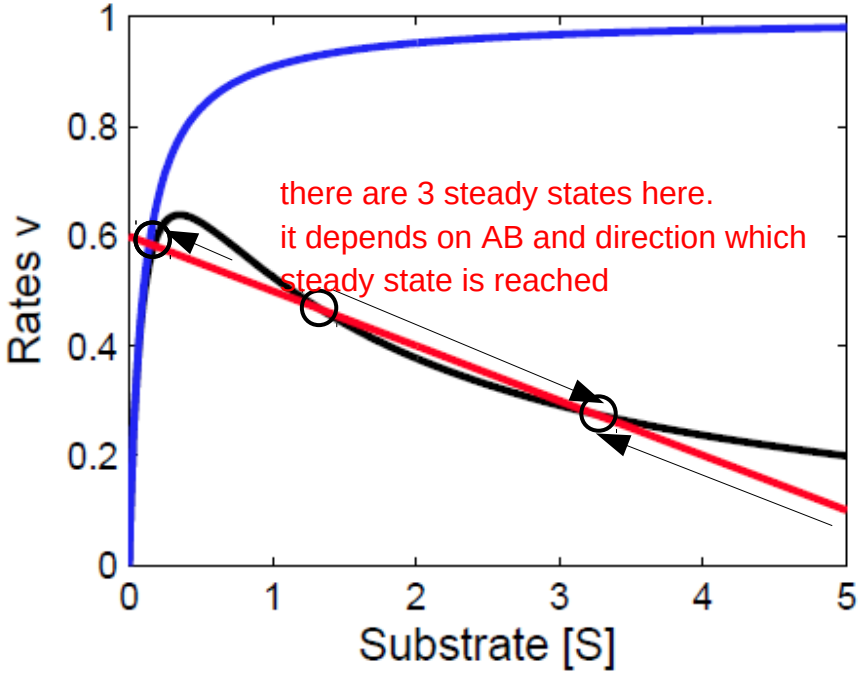


- For the same (constant) external substrate concentrations and low or high initial internal state:

  The system 'remembers' its internal state.

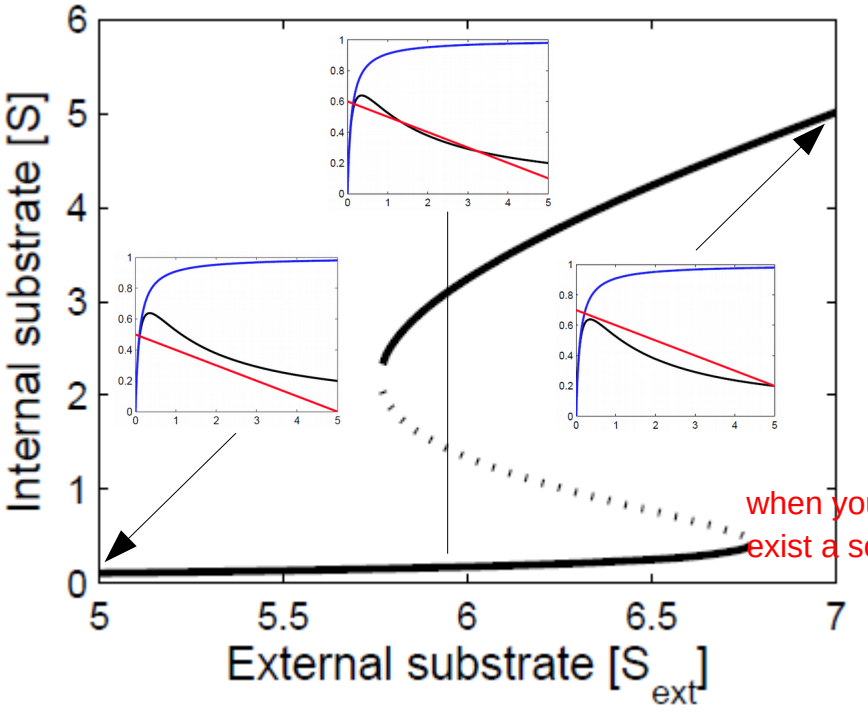
Substrate Inhibition: Dynamic Memory





- **Explanation:** For certain external substrate concentrations more than one steady-state exists
  → Path depends on the initial internal substrate.

Substrate Inhibition: Dynamic Memory



when you go over 6.8, there doesnt exist a solution anymore

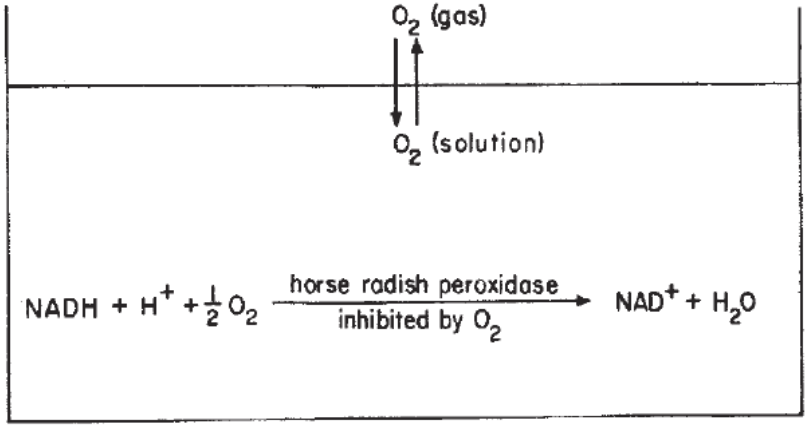
- Qualitatively different internal steady-states are possible, for the same external conditions → **Memory through a purely dynamic process.**

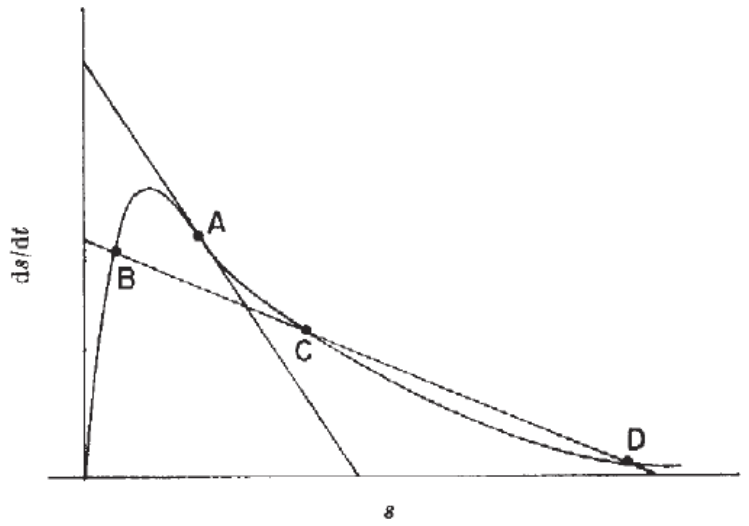
Similarities to turbo design?

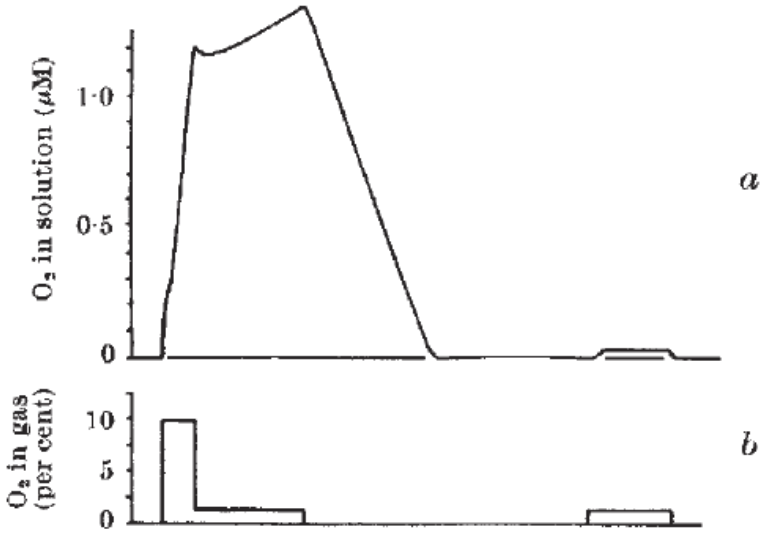
Example #1: Substrate Inhibition Kinetics

Bistability caused by Substrate Inhibition of Peroxidase in an Open Reaction System

NATURE, VOL. 217, MARCH 16, 1968

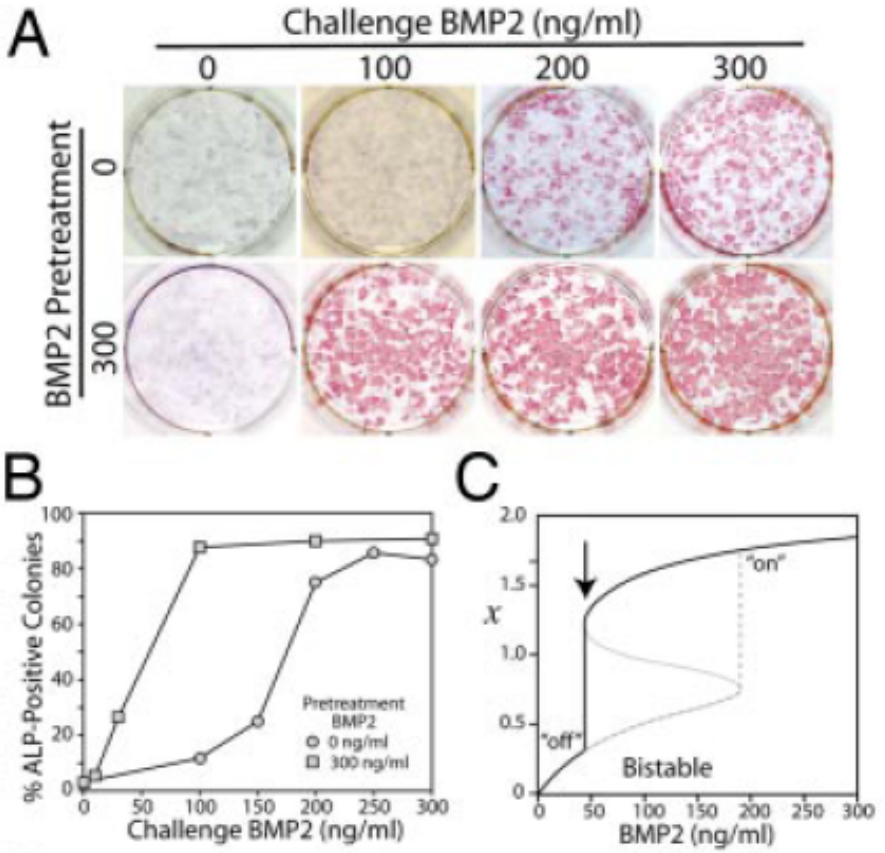






- **Example:** Horseradish peroxidase with substrate inhibition.

Example #2: Memory in Cell Differentiation



From: Wang et al. (2009) PNAS 106: 6638-6643.

- **Example:** Human bone marrow cells exhibit memory of bone morphogenetic protein 2 (BMP2) treatment.

Positive Feedback: Functions

- **'Simple' positive feedback systems:**
  - Multiple steady states may be possible.
  - Phenomenon in nonlinear systems: Memory.

- **Functions in biological networks:**
  - Discrete decisions from continuous signals.
  - Possibly irreversible decisions (development).

How can decisions become irreversible?

Summary: Teaching Goal III

- Negative and positive feedback have distinct functions such as homeostasis vs. memory.

- Dyanmic behaviors can be analyzed graphically by plotting rates as a function of states and reasoning about the the impact of rates on states.

- Memory can be established dynamically, provided that more than one steady-state exists in a system.

Systems Biology 551-1174-00L

ODE Modeling of a Metabolic Pathway

9 Mar, 2017

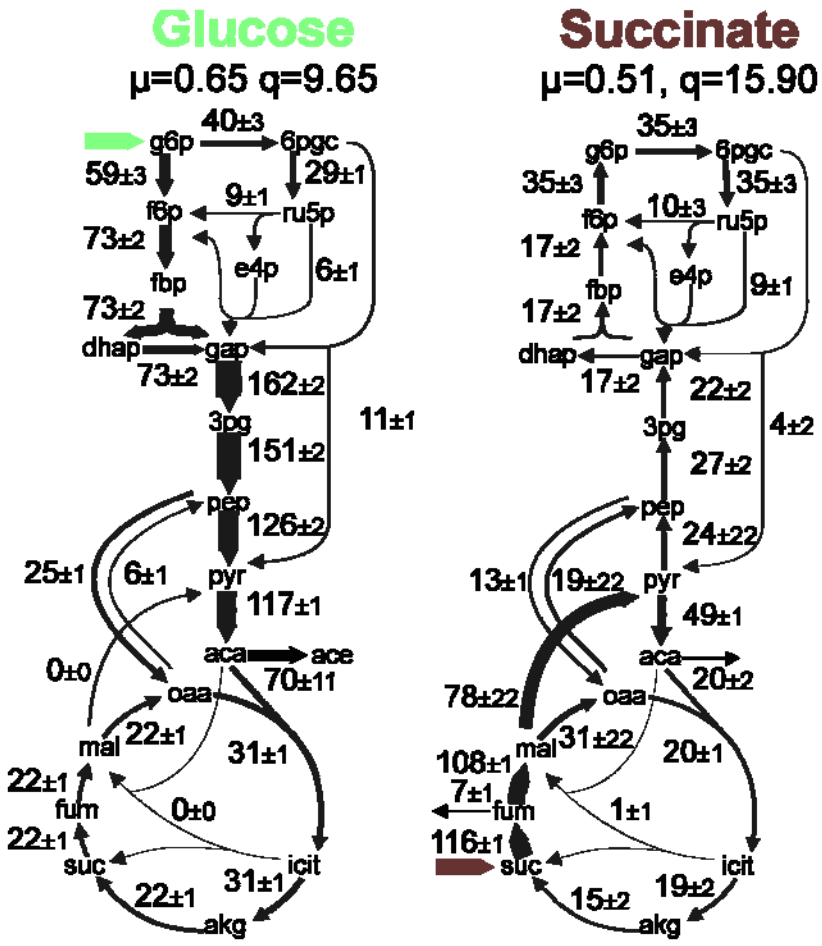
Uwe Sauer, Institute of Molecular Systems Biology

Content:

- Concepts: Parameters & model quality (JS)
- Biological consequences of metabolic dynamics (US)
- Concepts: Feedback for simple systems (JS)
- **Glycolysis/Gluconeogenesis (US)**

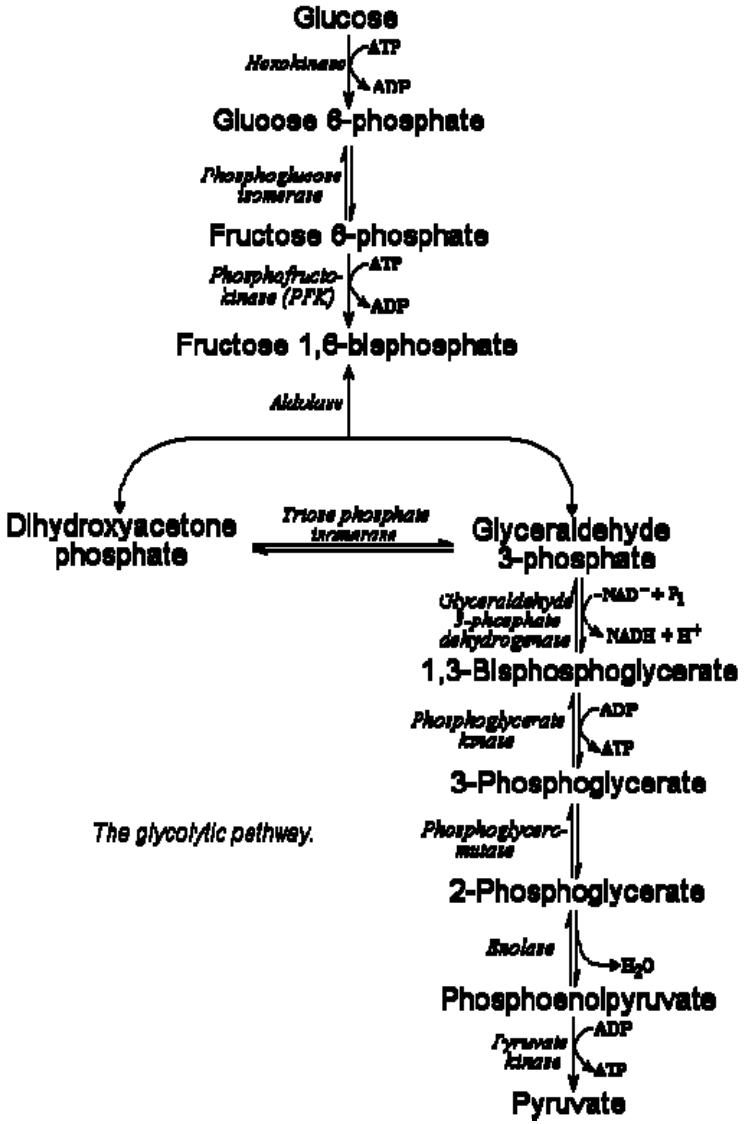
Central Metabolism Must Be Flexible

- To accommodate different carbon inputs
- Is amphibolic; ie serves both catabolic and anabolic functions
- Provide suitable supply of ATP, redox cofactors, and carbon precursors for different cellular needs (eg rapid growth, survival, stress responses etc)
- **Is therefore highly interconnected and can operate in multiple directions**
- In the following example we focus on the specific flexibility problem of **changing flux direction** in a pathway



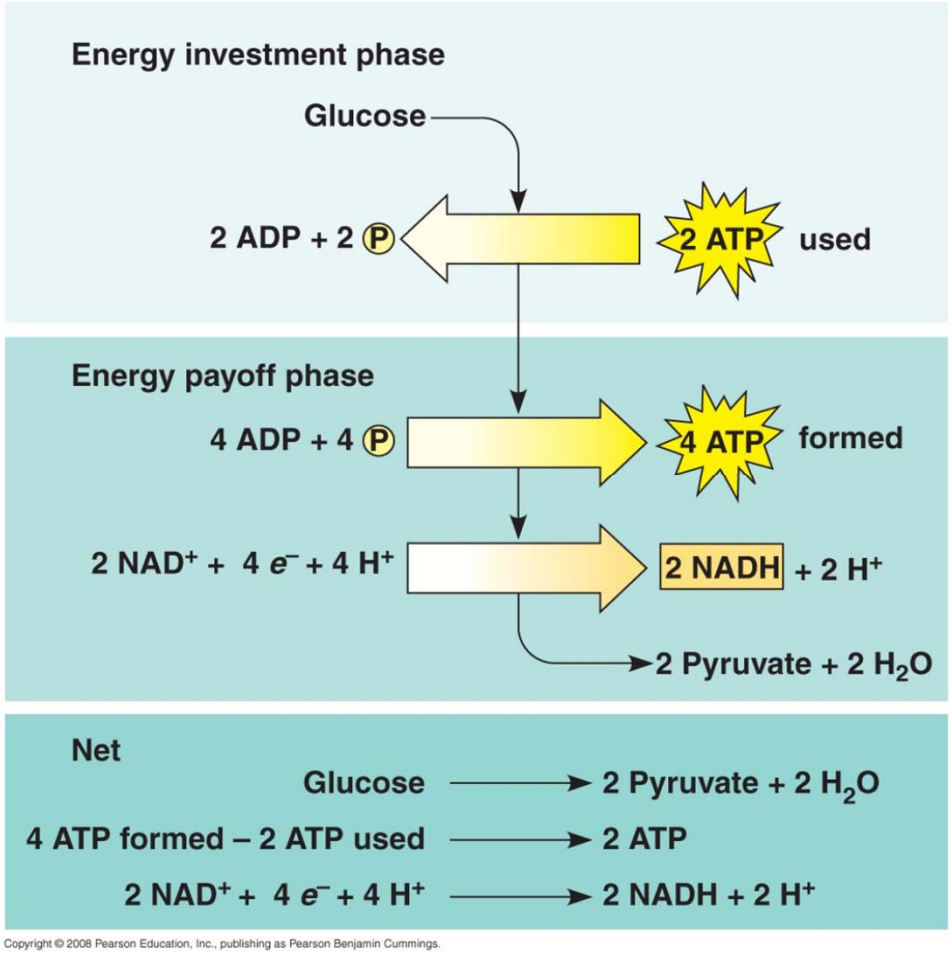
Glycolysis

Perhaps THE most central pathway throughout all species



The glycolytic pathway.

Function: catabolism of hexoses



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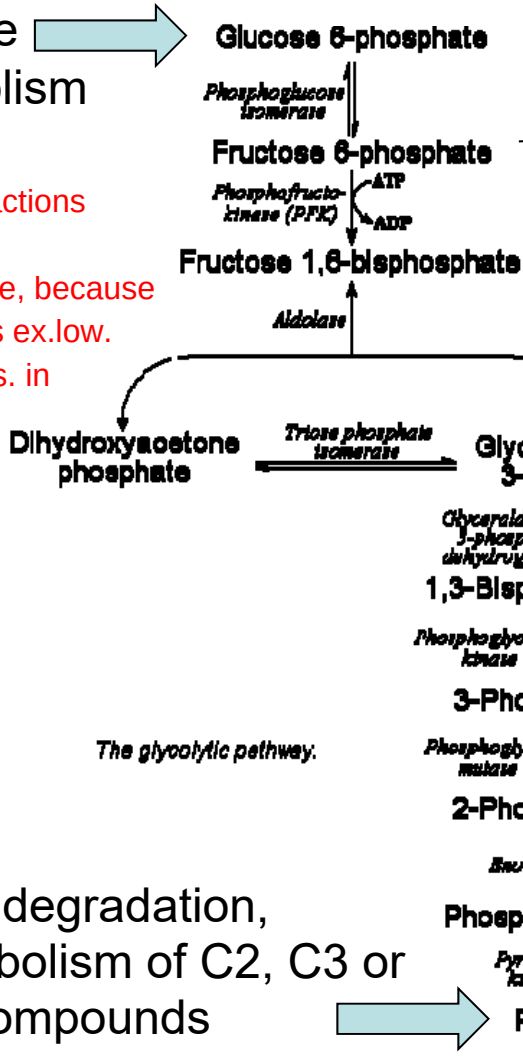
Why is that necessary?

Glycolysis - Gluconeogenesis

Except for 2, all reactions are freely reversible

Glucose metabolism



The glycolytic pathway.

why are the two reactions irreversible?
Practiclly irreversible, because K for ATP to ADP is ex.low. Realistically irrevers. in the cell.

Fructose-6-P

Phospho-fructokinase

ATP

ADP

irreversible

P_i FBPase

Fructose-1,6-BP

glycolytic

gluconeogenic

PEP

irreversible too.

ADP

ATP

Pyruvate-kinase

PP_i PEP synthetase

ATP

Pyruvate

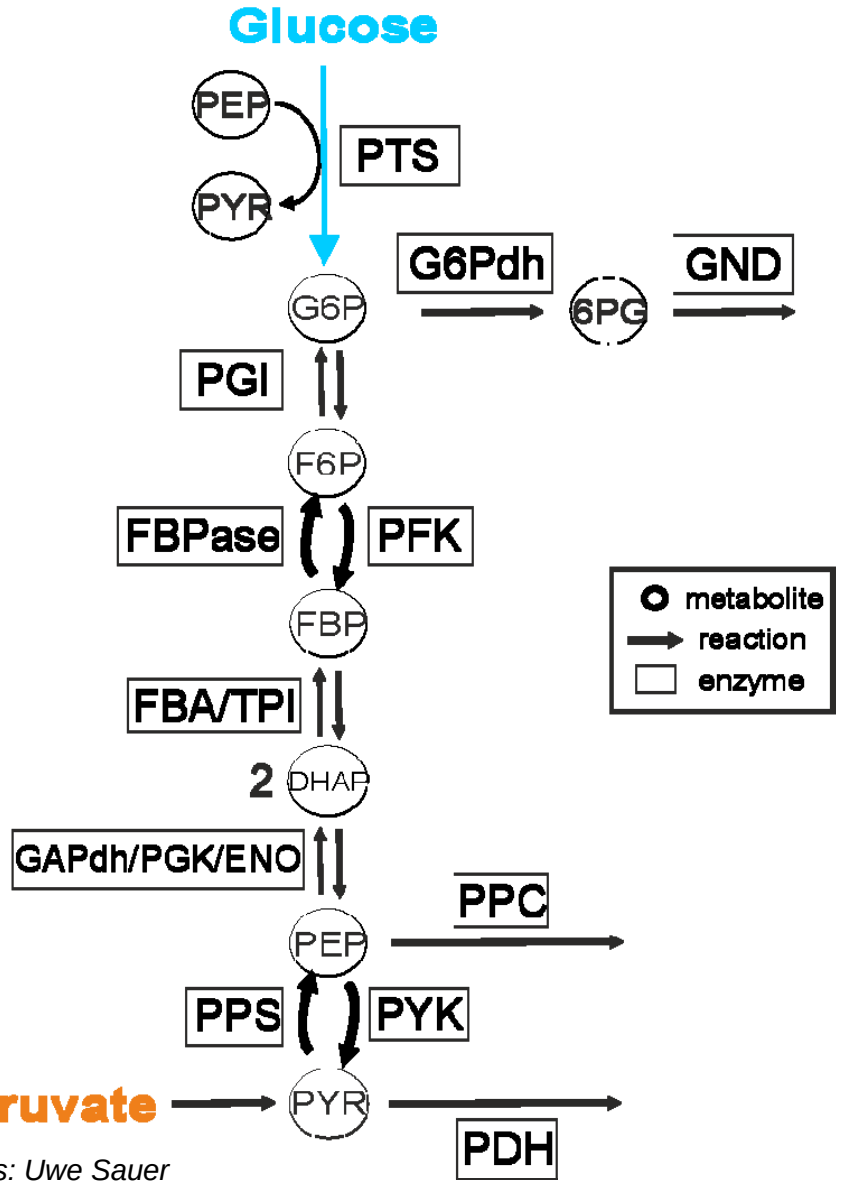
Lipid degradation, metabolism of C2, C3 or C4 compounds

Which Challenges Arise When Pathways Must Operate in 2 Directions ?

direction determined by metabolite conc.
genetic regulation necessary to reduce protein coststst
when conditions change alternative reactions are necessary, but genetic reg. take times
simultaneous presence/activity of enzymes cataliyzing opposing reactions leads to futile cycling

Simplified Model of Glycolysis/Gluconeogenesis

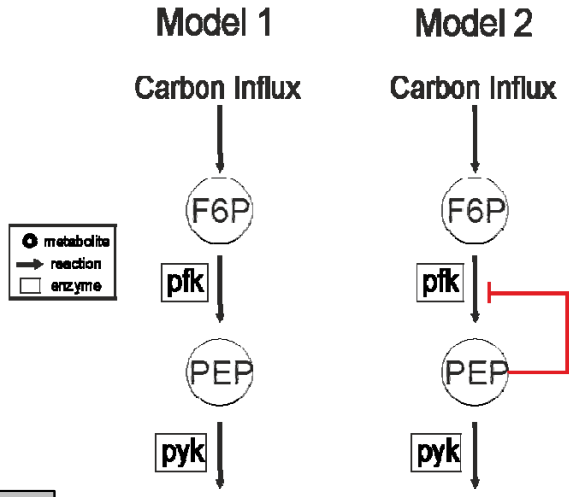
An abstraction of the whole system. ***What is missing?***



Exercise 4: Two Reaction Model

Greatly simplified model(s) of glycolysis

- 2 key (irreversible) reactions of glycolysis
- PEP (nearly) at end of glycolysis, a high energy compound
- PEP is generally positioned at a metabolic decision point



Phosphofructokinase
Pyruvate kinase

Goal of exercise 4:

- Formulate and implement a simple kinetic model of 2 metabolic reactions (with and w/o regulation)
- Implement and simulate models. Study system behavior under different parameters and perturbations.
- Understand why a biological system would need to develop such feedback?
- What does feedback achieve in this case?

Teaching Goals Lecture 3

- Understand potential biological problems of metabolic dynamics and how regulation can help.

  Generally explain problems arising from metabolite dynamics. Be able to explain on an example.

- Understand basic principles of neg and pos feedback.

  Identify types of feedback. Be able to analyze effects of feedback graphically.

- Understand biological challenges arising in bi-directional pathways, example of glycolysis/gluconeogenesis.

  Be able to rationalize and explain need for feedback on a reversible linear pathway

- Relate experimental observations to model behaviors via parameters.

  Explain measures and statistics to compare models to data. Rationalize uncertainty in parameters.