Computational Analysis of Metabolic Networks

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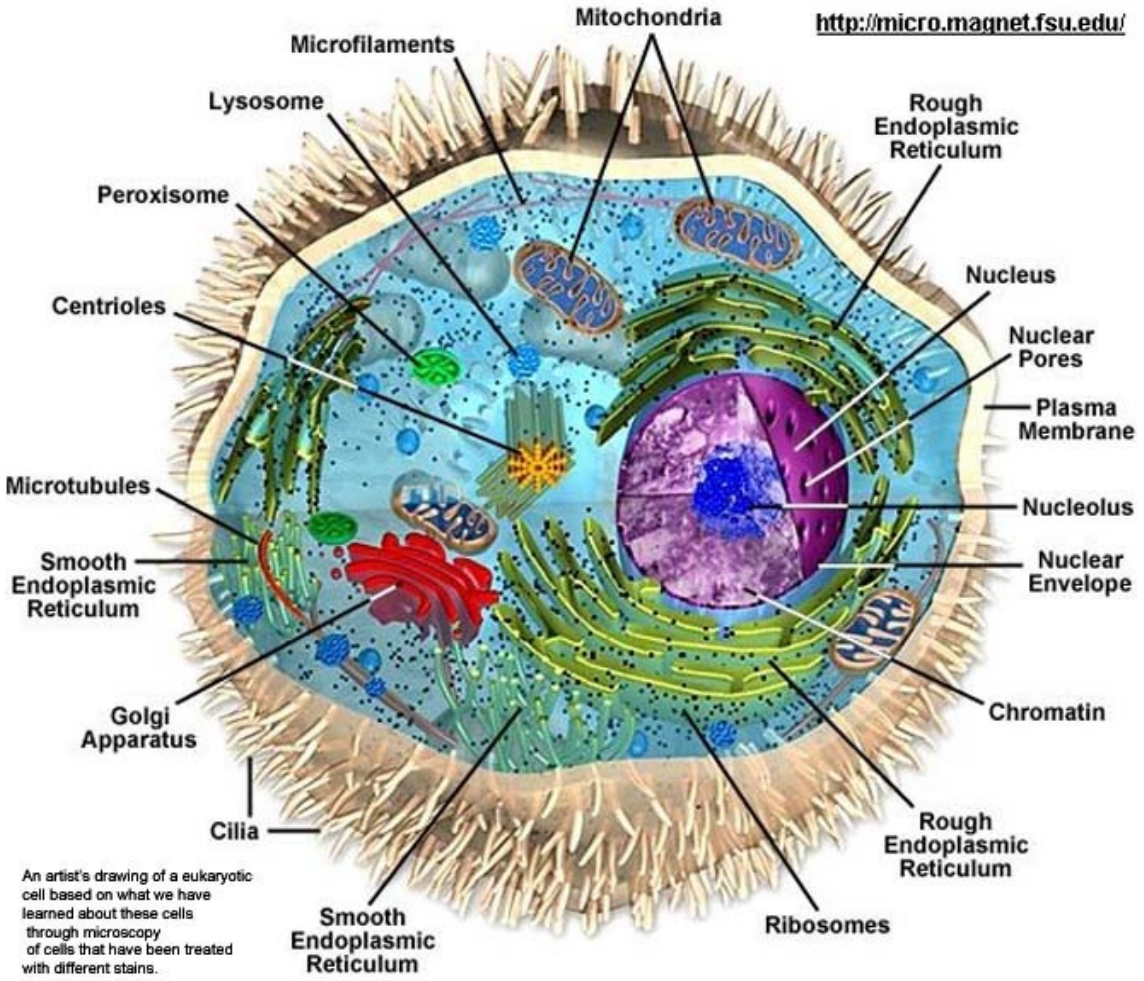
A living cell is a complex dynamic system involving many hierarchies of regulation:

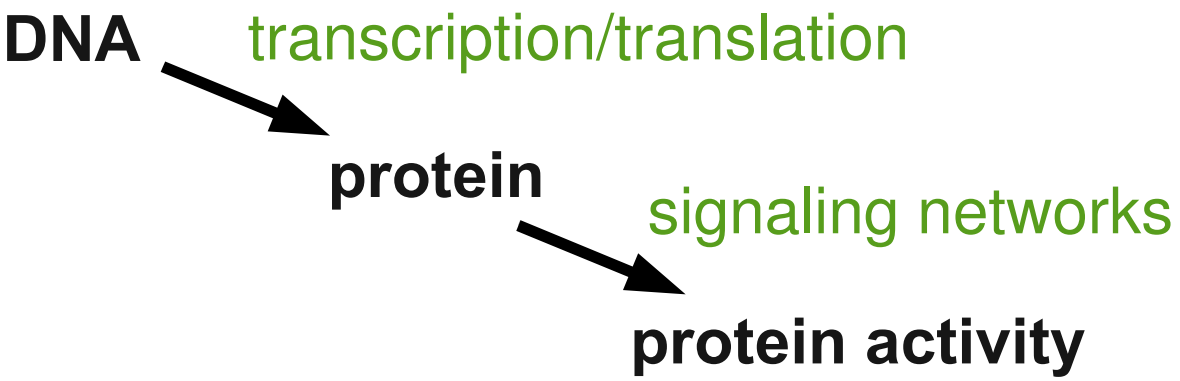


A living cell is a complex dynamic system involving many hierarchies of regulation:

(1) Transcriptional regulation: from DNA to protein

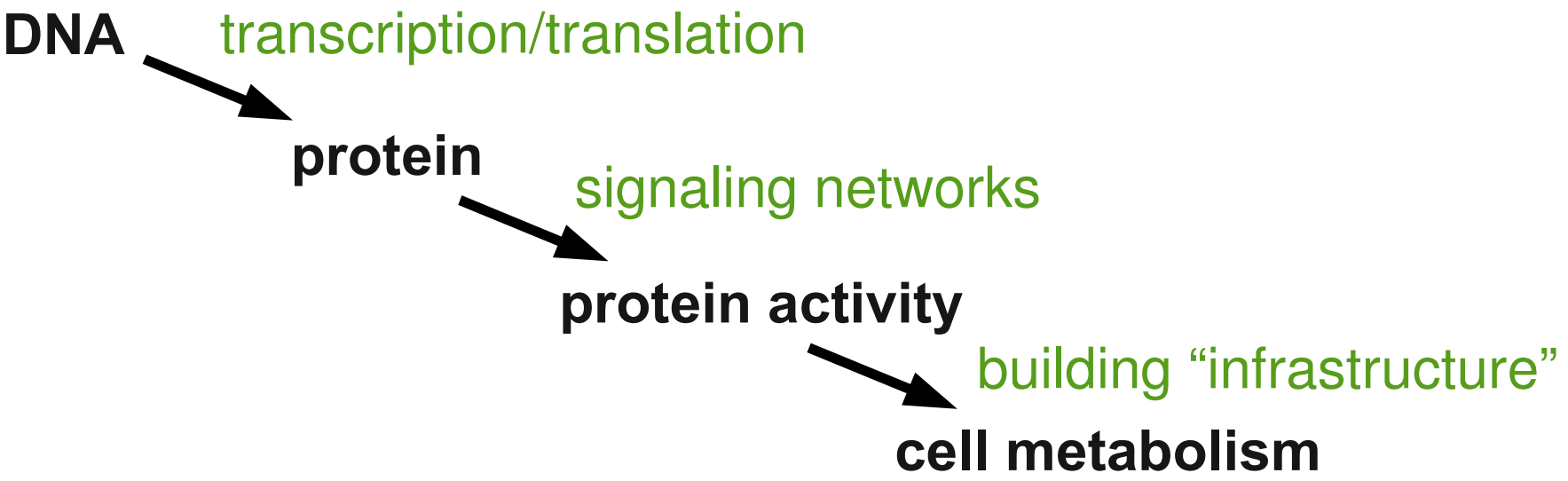
A living cell is a complex dynamic system involving many hierarchies of regulation:

(2) Post-transcriptional regulation: (de-)activation of proteins



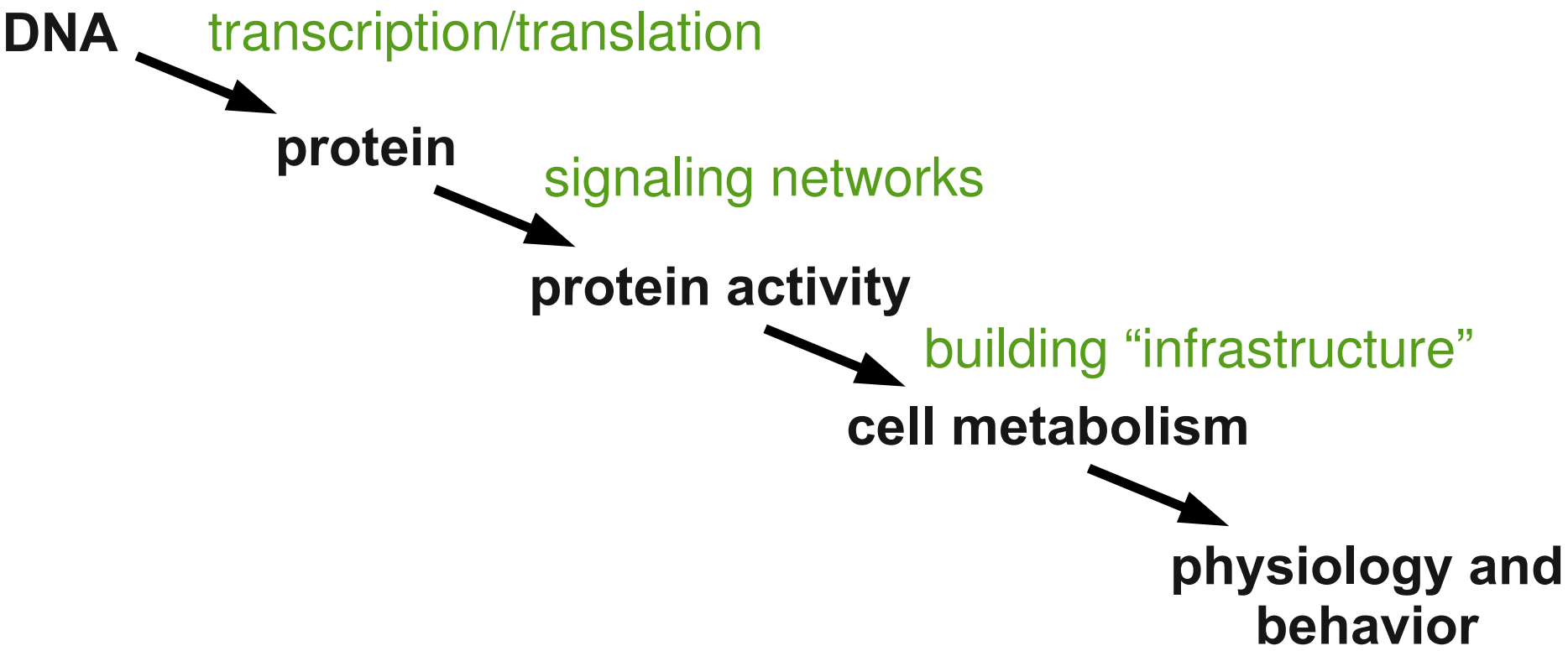
A living cell is a complex dynamic system involving many hierarchies of regulation:

(3) Cellular metabolism: energy and growth



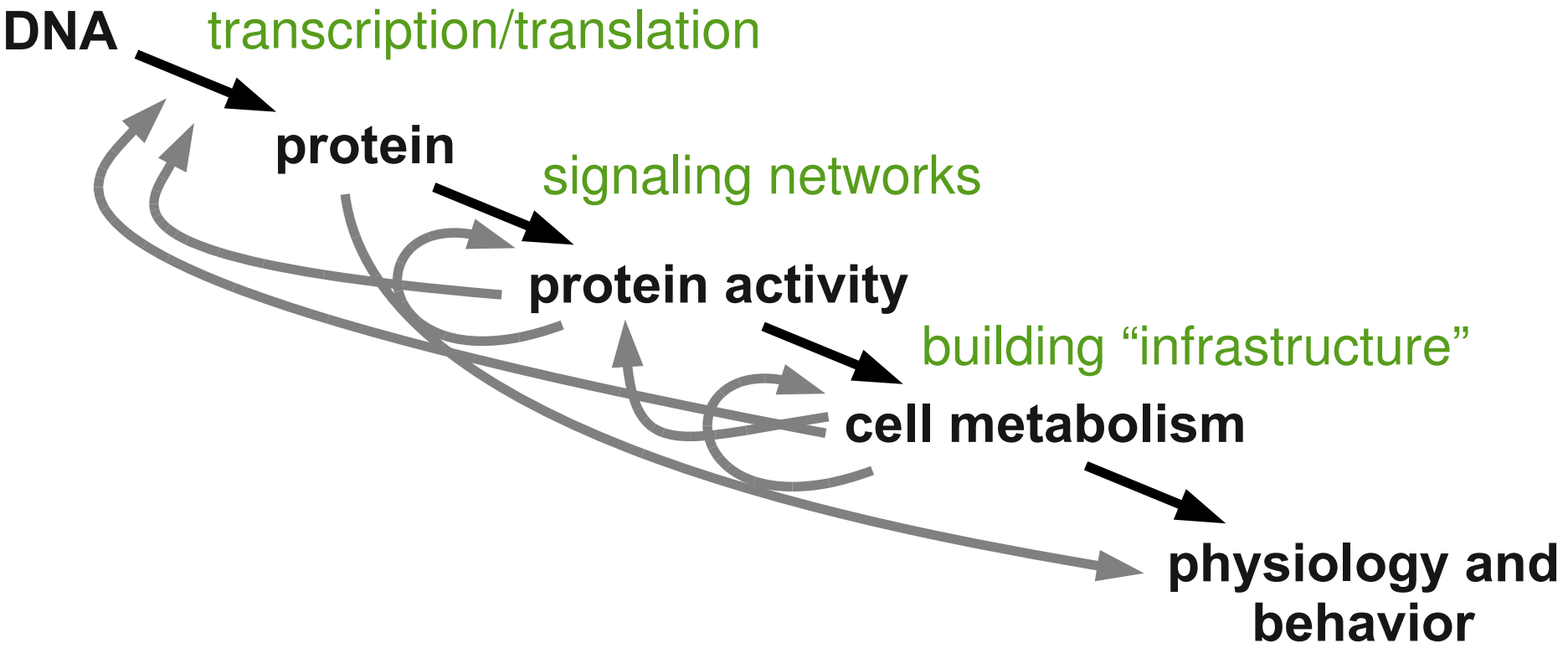
A living cell is a complex dynamic system involving many hierarchies of regulation:

(4) Cellular physiology: division, motility, etc ...



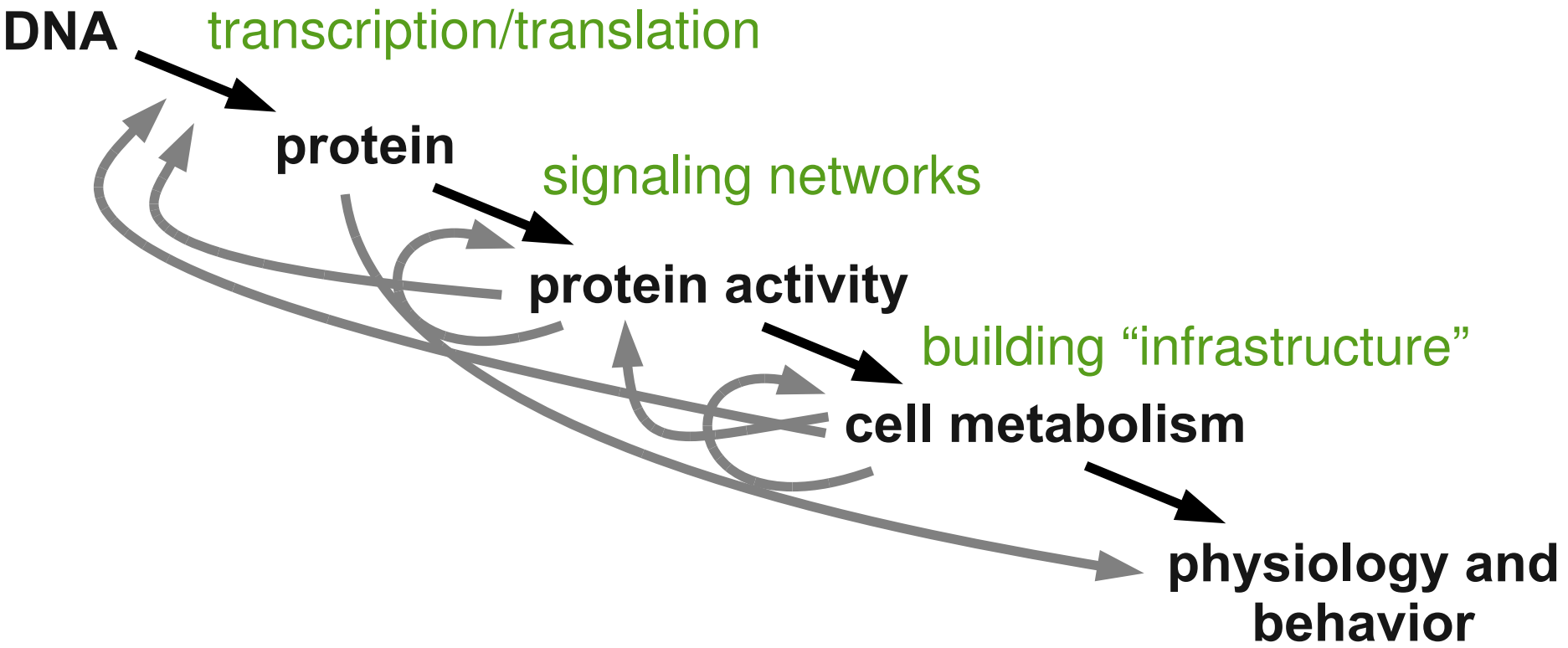
A living cell is a complex dynamic system involving many hierarchies of regulation:

(4) Cellular physiology: division, motility, etc ...



A living cell is a complex dynamic system involving many hierarchies of regulation:

Aim: Computational Modeling of Cellular Processes



Computational modeling as a tool to understand the functioning of cellular interactions?

Three main issues:

(1) Why should one care about modeling?
(2) How should a (good) model be constructed?
(3) And to what end?

The rationale of mathematical modelling

1. **Mathematical Models: A method for representation**
2. **Mathematical Models: A method for deduction**

The rationale of mathematical modelling

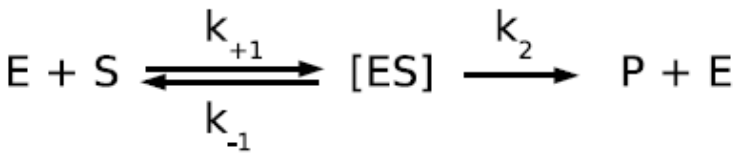
1.

Mathematical Models: A method of representation

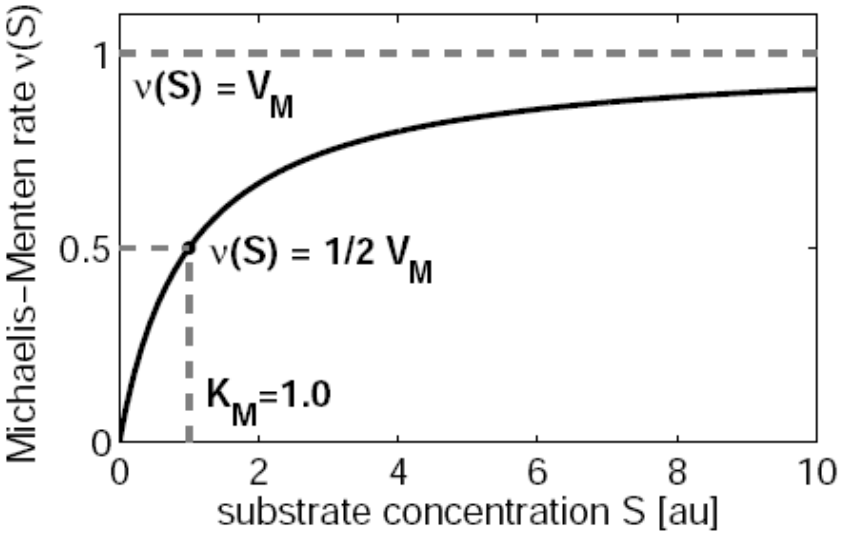
***Transcription* of properties into a formal representation.**

Modeling provides a language for representation.

An example: The basic Michaelis-Menten Scheme



$$\nu(S) = \frac{V_m[S]}{K_M + [S]}$$



The rationale of mathematical modelling

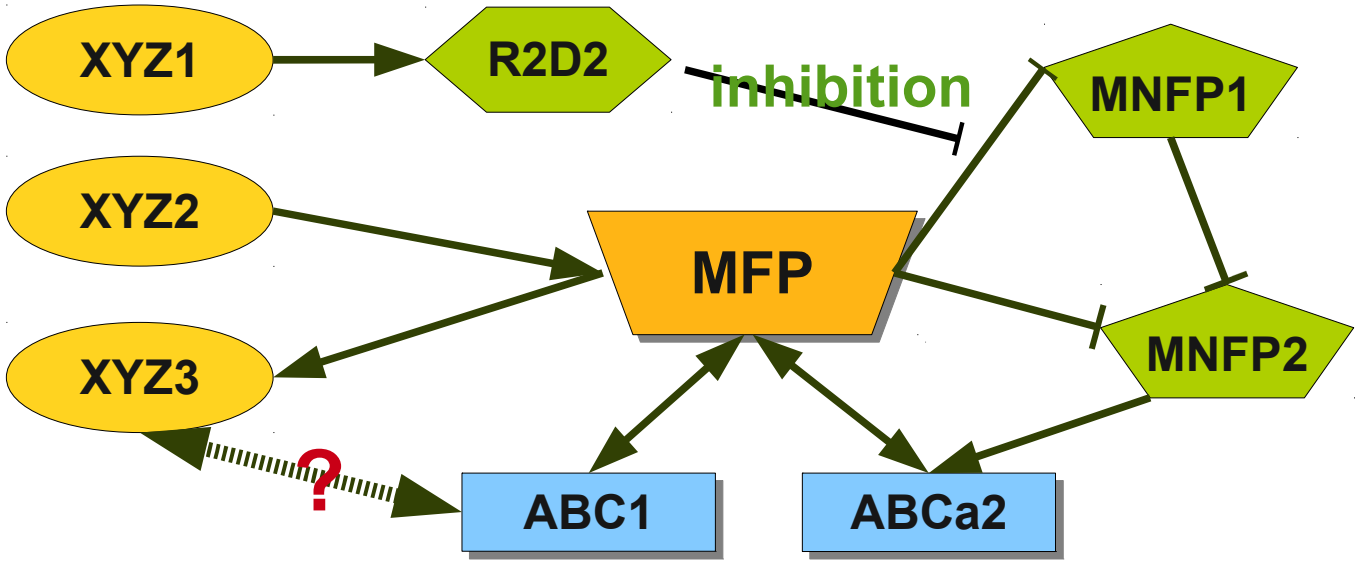
1.

Mathematical Models: A method of representation

***Transcription* of properties into a formal representation.**

Modeling provides a language for representation.

A slightly more complicated example:



Note that models do not need to be 'true'.

The rationale of mathematical modelling

2.

Mathematical Models: A method for deduction
***Translation* into emergent properties at the systems level.**
Mathematical models organize parts into a coherent whole.

Facts often only mean little in isolation, but need to be interpreted in terms of a theory or model.

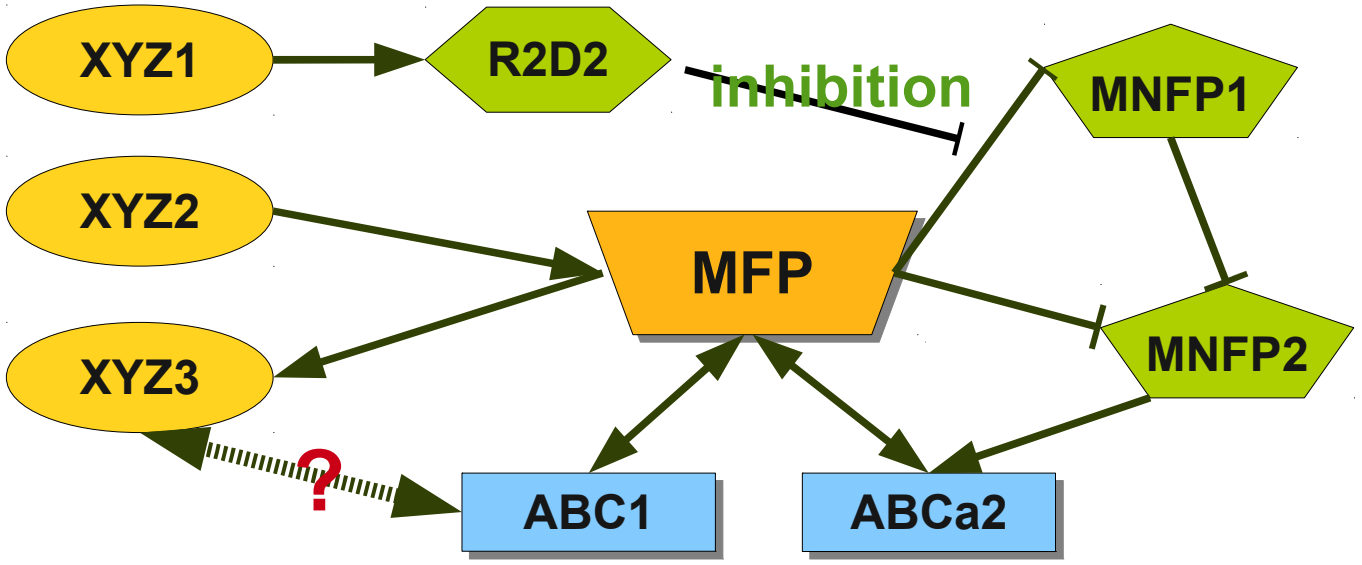
Science is built with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house. — Henri Poincare

The rationale of mathematical modelling

2.

Mathematical Models: A method for deduction
***Translation* into emergent properties at the systems level.**
Mathematical models organize parts into a coherent whole.

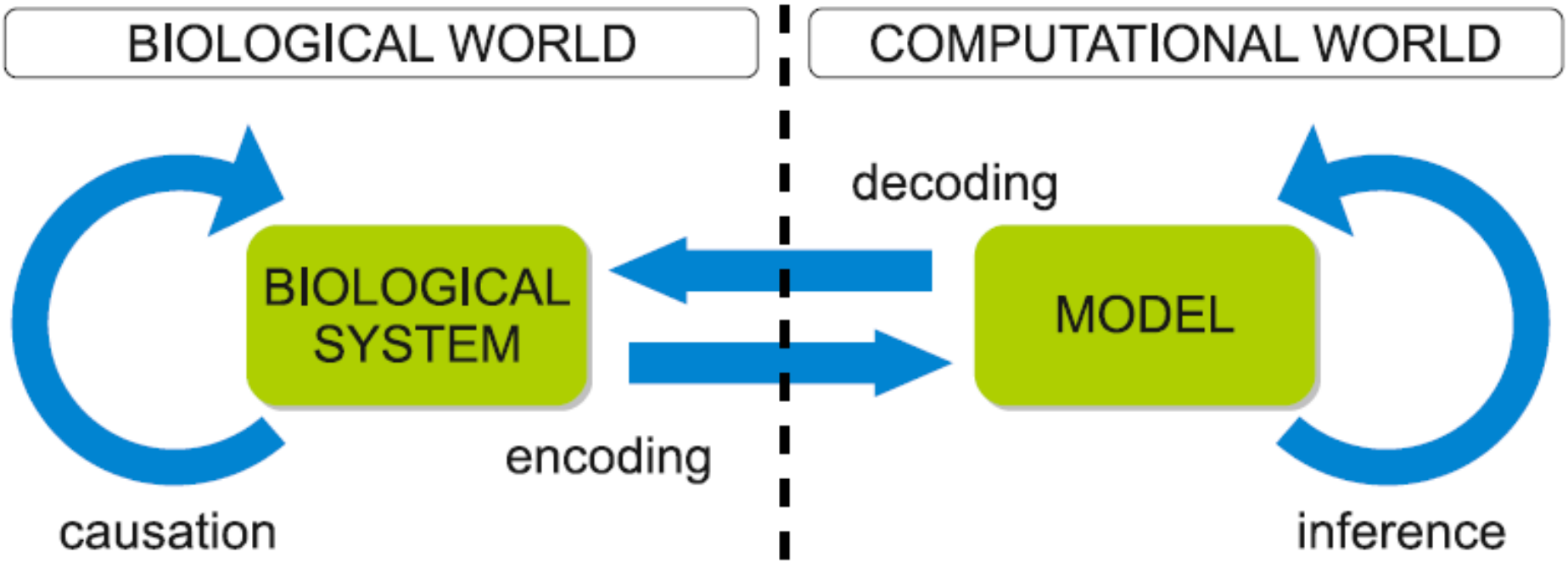
An example: Can this mechanism oscillate?



Translation into emergent properties

The rationale of mathematical modelling

The *modeling relation* according to Casti:



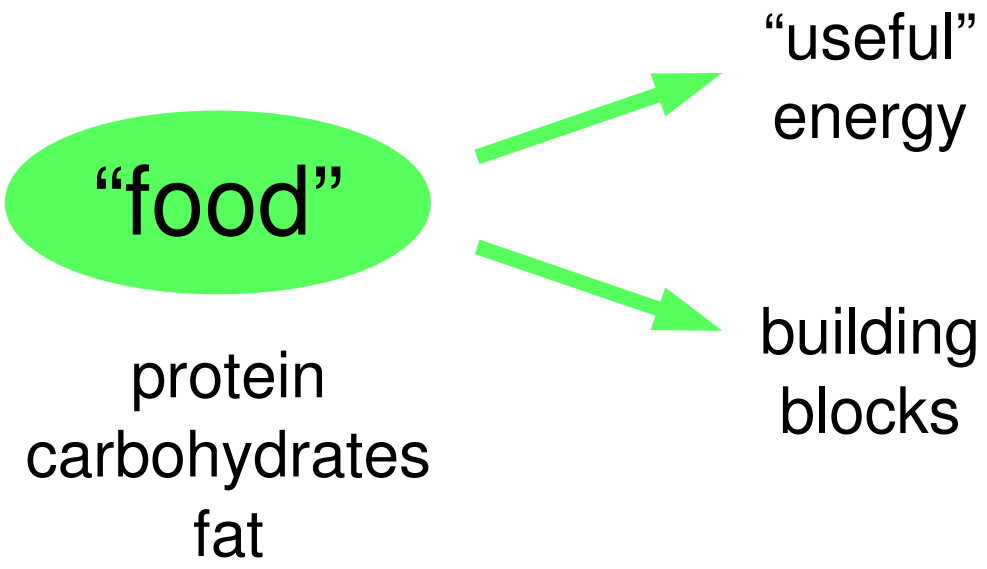
The rationale of mathematical modelling

"There is a zoo of mathematical models in the literature. Many of these appear to have little purpose other than calculating numbers which conform reasonably to experimental data. This is, in itself, not a distinguished endeavor; it is not particularly difficult, and it teaches little. ... **Modeling is relatively meaningless without explicit definition, at the outset, of its purpose**"

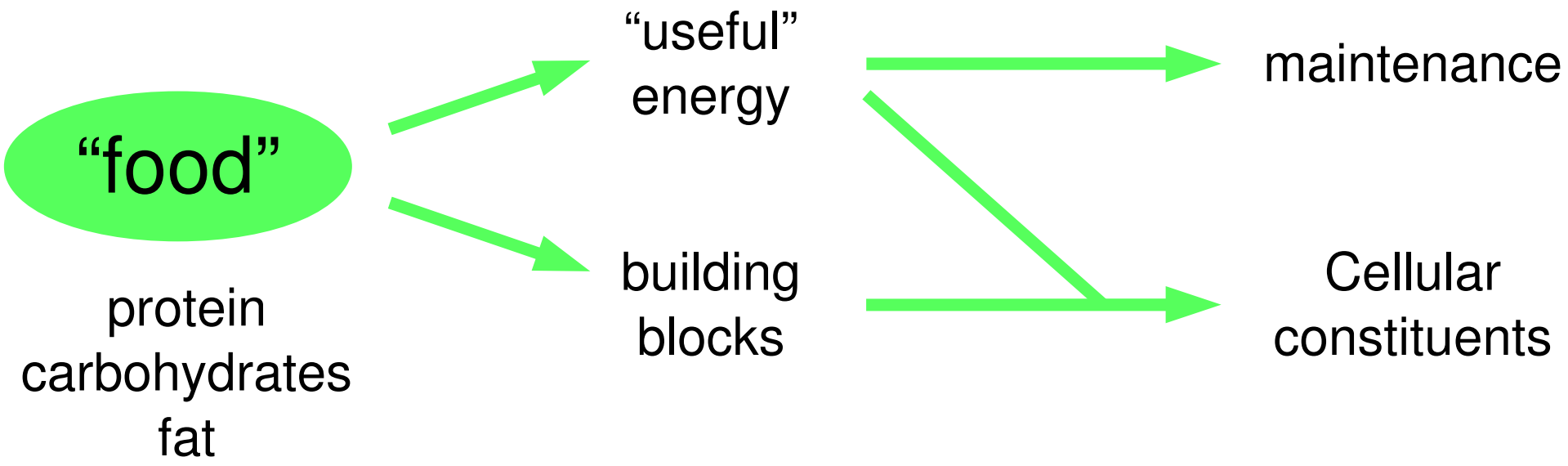
J. E. Bailey (1944-2001)
in Biotechnol. Prog. 14:8-20, 1998

Computational models of metabolic networks

Computational models of metabolic networks



Computational models of metabolic networks



Computational models of metabolic networks

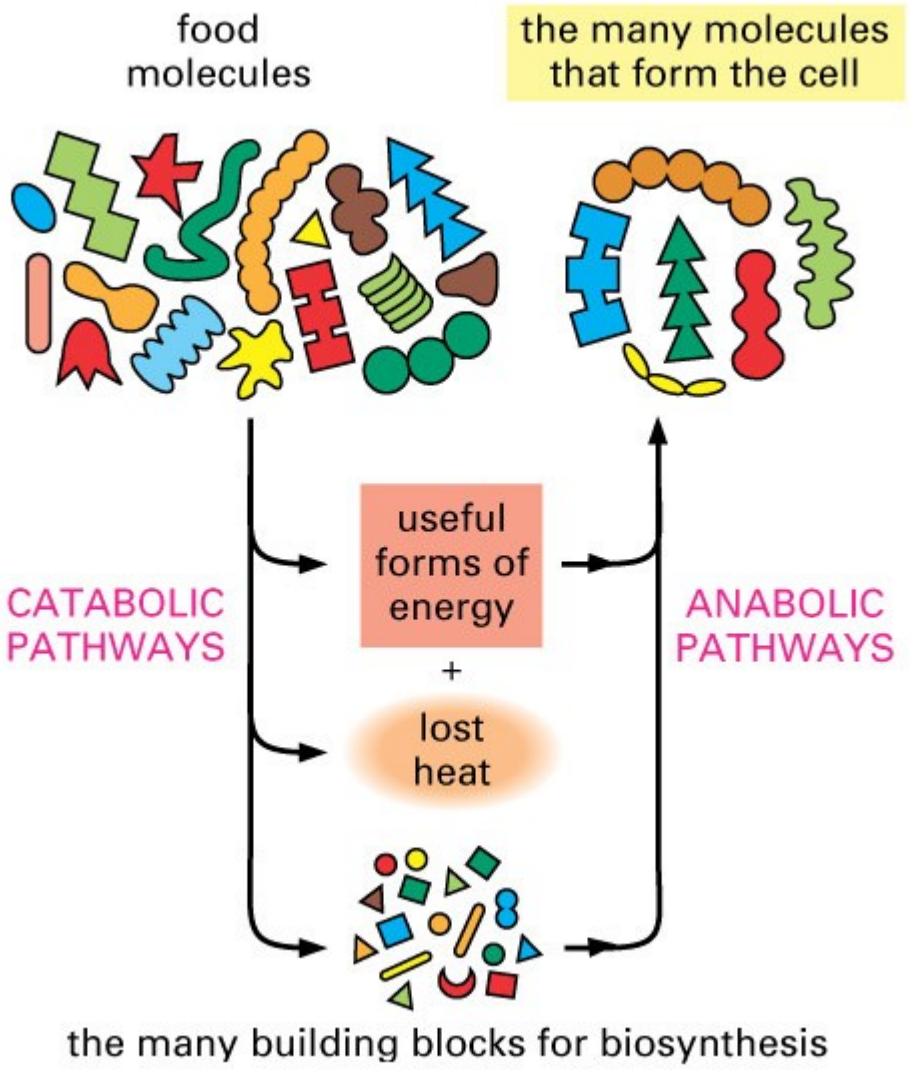
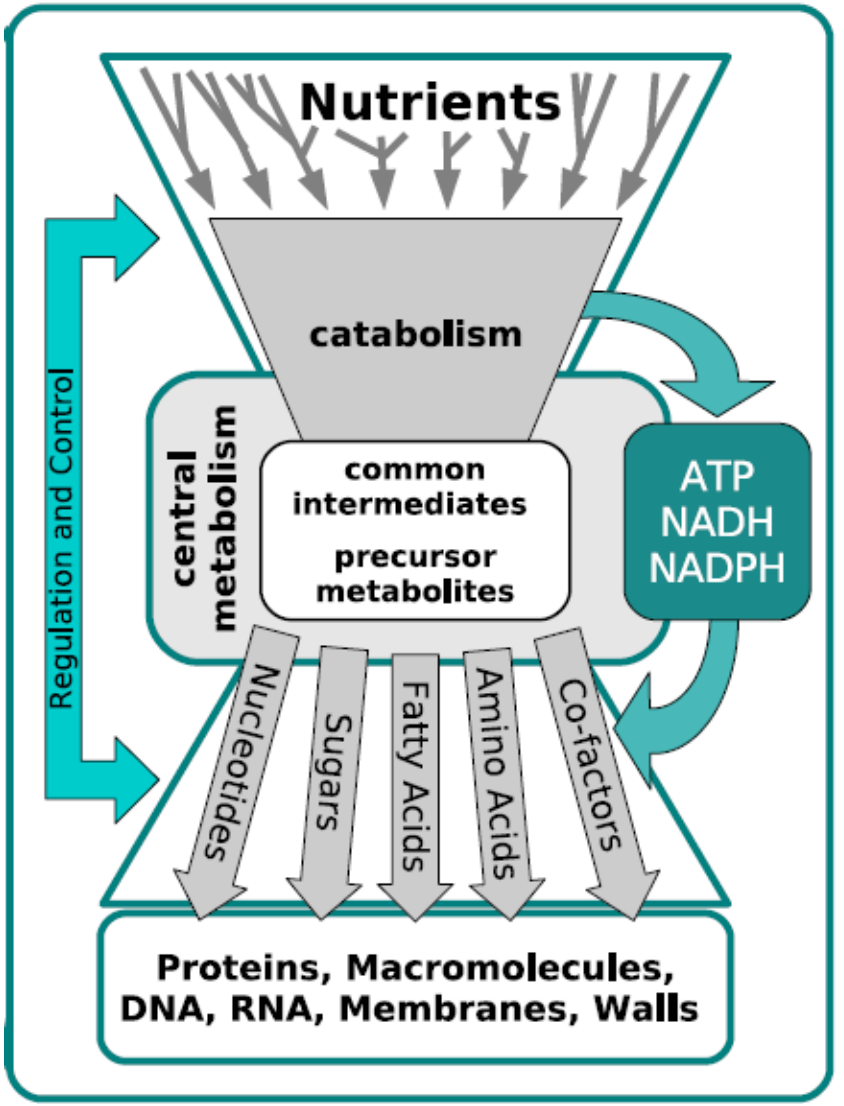


Figure 3-3 Essential Cell Biology, 2/e. (© 2004 Garland Science)

The “bow-tie” structure of metabolism:



The “bow-tie” structure of metabolism:

A Complex Network of Interactions:

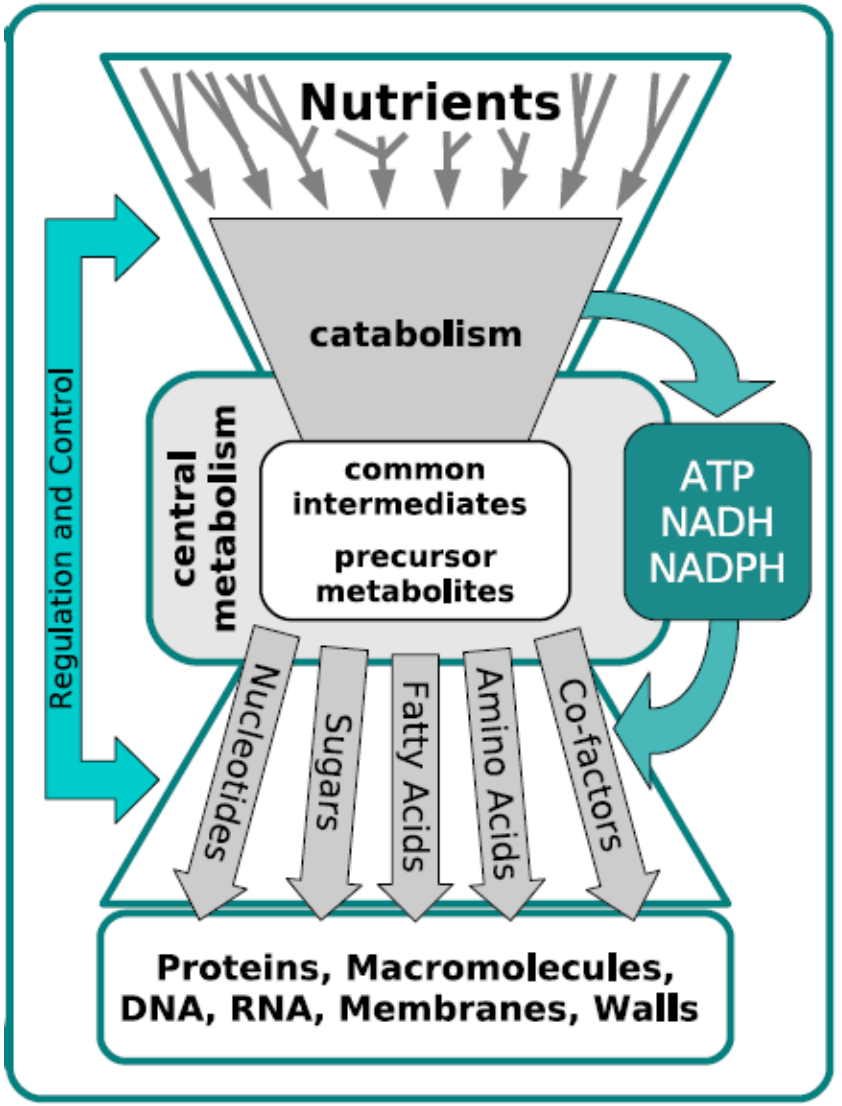
- Biochemical reactions
- Metabolic compounds
- Regulatory interactions

Applications of Relevance:

Biotechnology

Diseases and medical applications

A link from genotype to phenotype



The network of enzyme-catalyzed biochemical reactions:

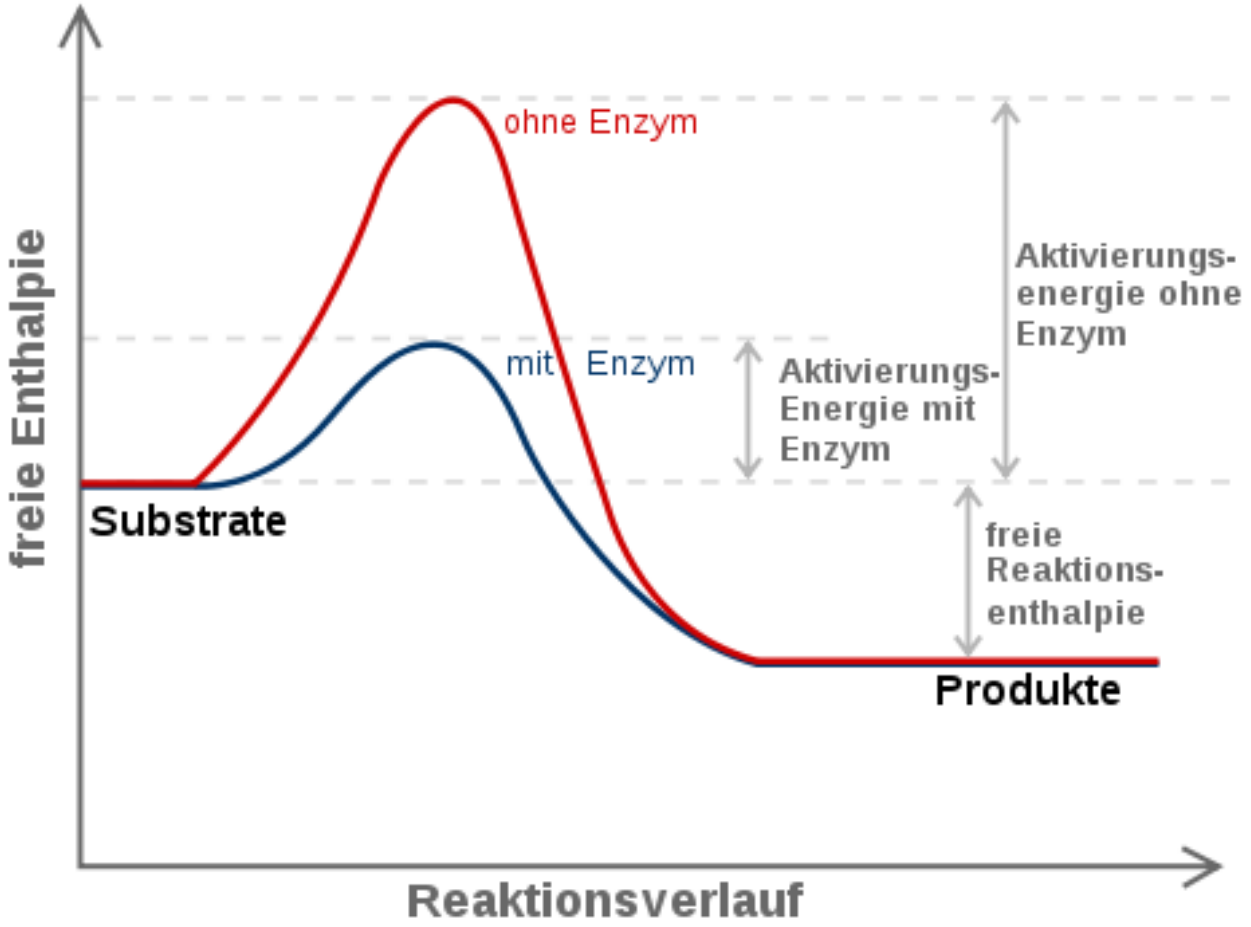
Enzymes are biological molecules that catalyze (i.e., increase the rates of) chemical reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Like all catalysts, enzymes work (only) by lowering the activation energy for a reaction, thus dramatically increasing the rate of the reaction.

from: WIKIPEDIA

Enzyme-catalyzed reactions:

Reaction: **A** ⟶ **B** (exergonic $\Delta G<0$)



Enzyme-catalyzed reactions:

Reaction: **A** ⟶ **B** (exergonic $\Delta G<0$)

Reaction: **C** ⟶ **D** (endergonic $\Delta G>0$)

Enzyme-catalyzed reactions:

Reaction: **A** → **B** (exergonic ΔG<0)

Reaction: **C** → **D** (endergonic ΔG>0)

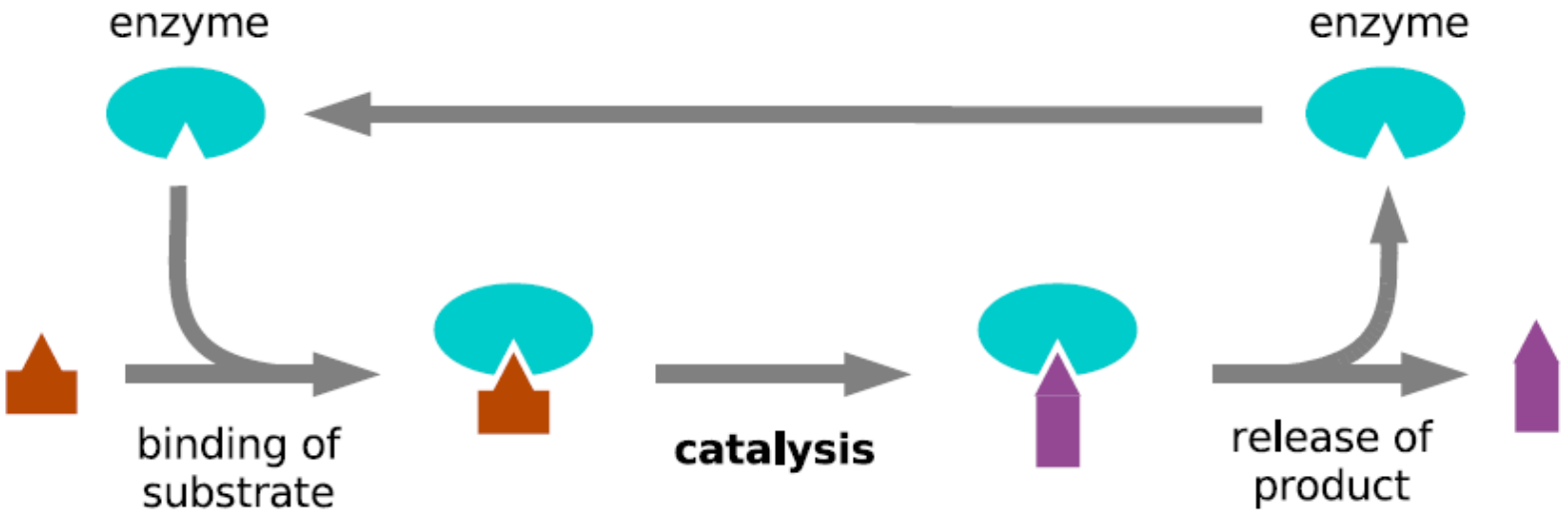
Enzymes can couple processes

Reaction: **C** → **D** (ΔG>0)

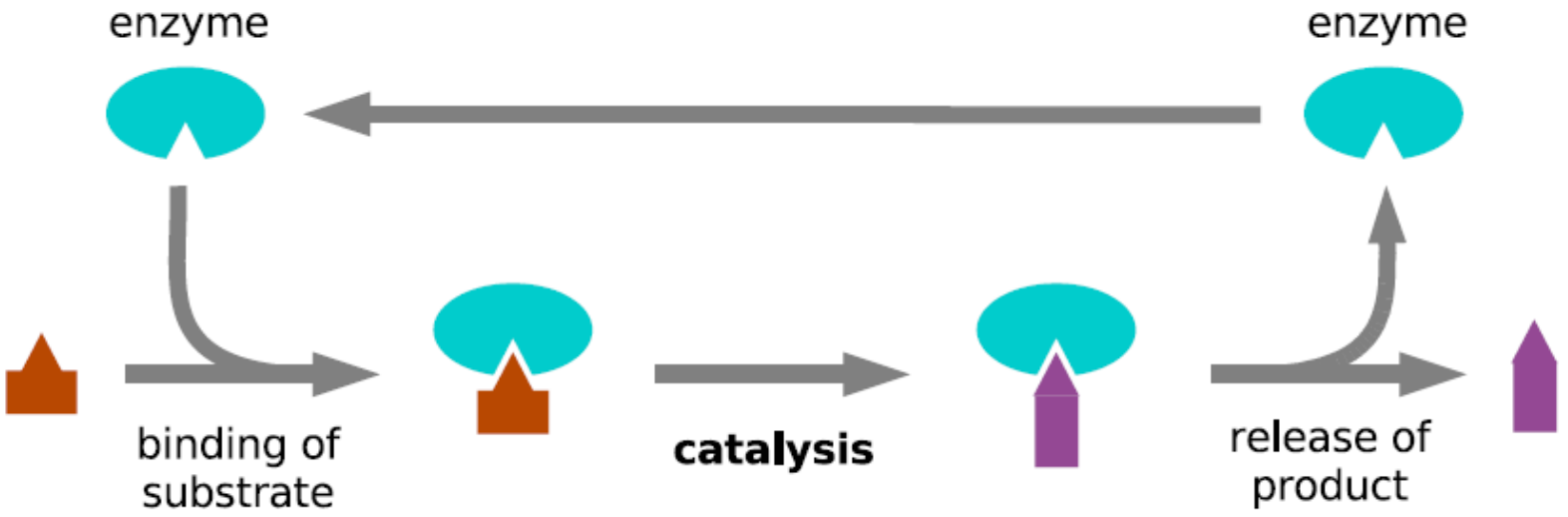
ATP → **ADP + "energy"** (ΔG<0)

C + ATP → **ADP + D** (ΔG<0)

Enzyme mechanisms:

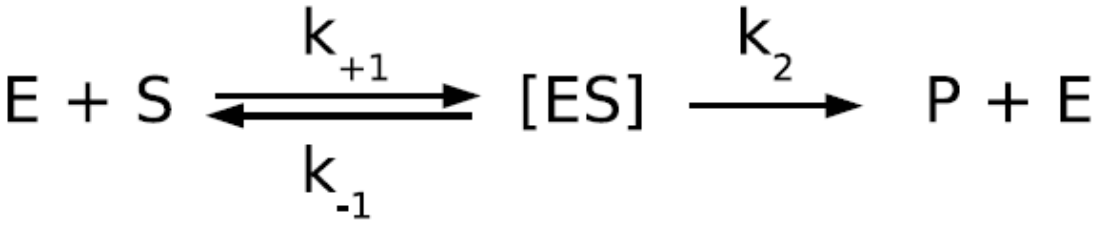


Enzyme mechanisms and rate equations:



Translation into mathematical model: rate equations

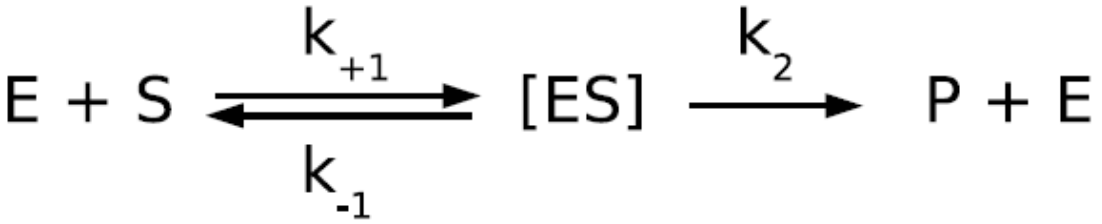
Michaelis-Menten Kinetics:



Translation into mathematical model: rate equations

$$\nu(S) = \frac{V_m[S]}{K_M + [S]}$$

Michaelis-Menten Kinetics:

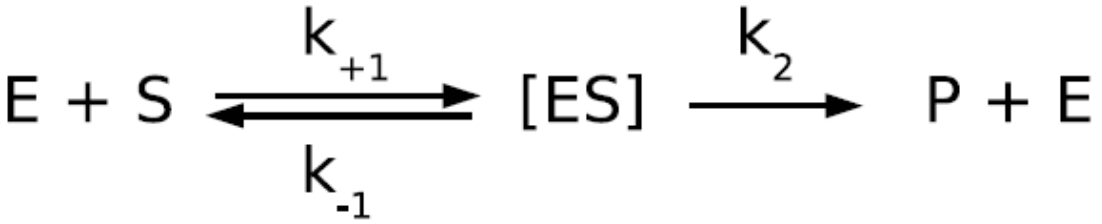


Translation into mathematical model: rate equations

$$\nu(S) = \frac{V_m[S]}{K_M + [S]}$$

Maximal velocity

Michaelis-Menten Kinetics:



Translation into mathematical model: rate equations

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Maximal velocity

Michaelis-Menten constant

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Maximal velocity

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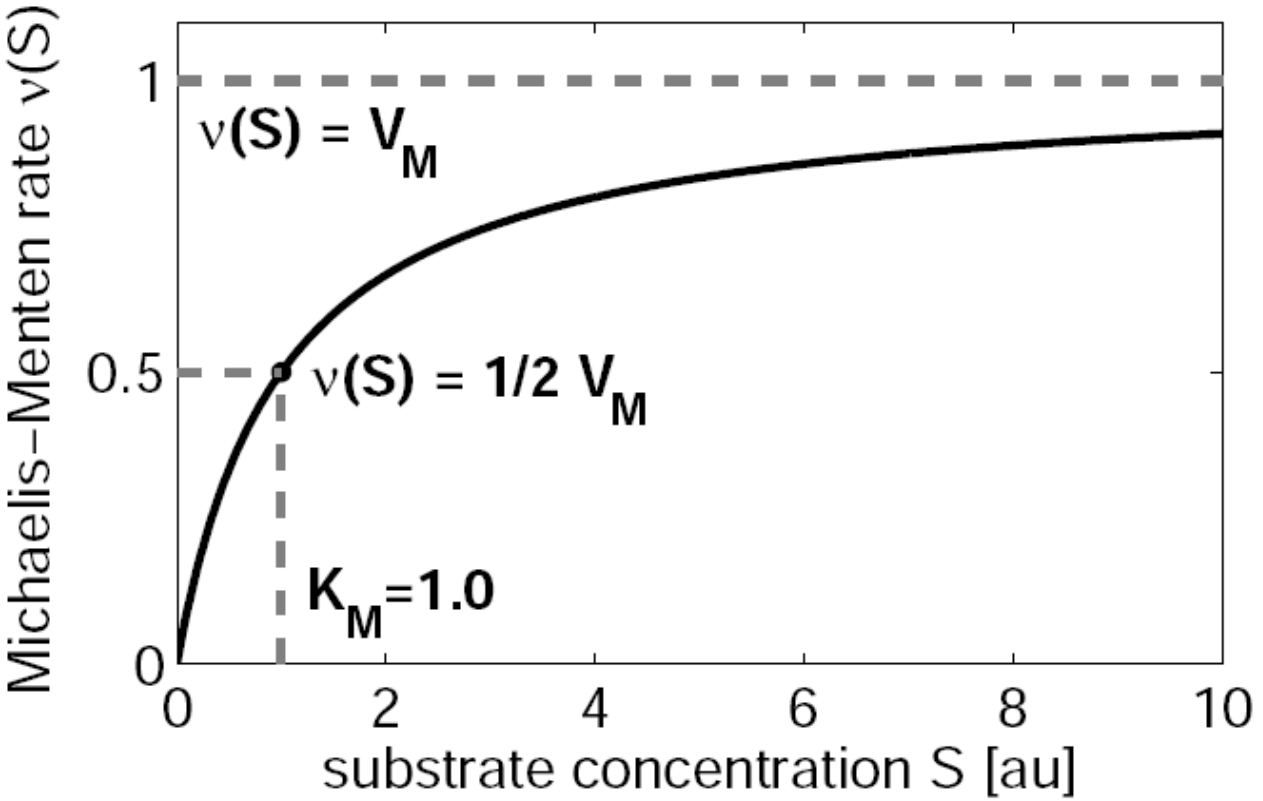
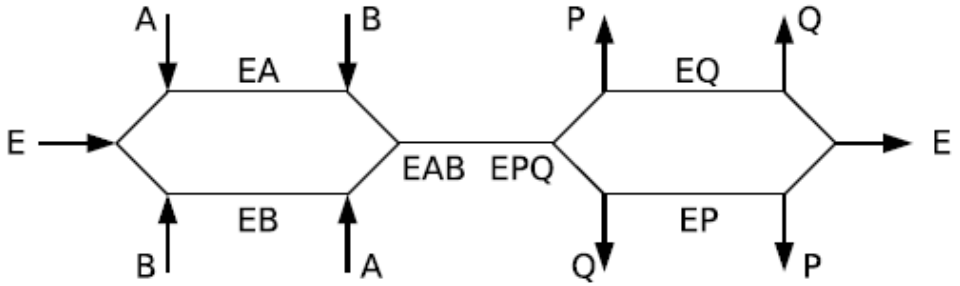


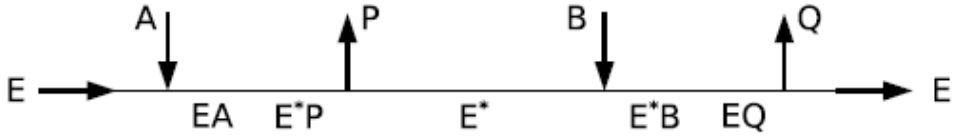
More complicated enzyme-kinetic mechanisms ...

a) Random Bi Bi Mechanism



b) Sequential Bi Bi Mechanism

c) Ping-Pong Mechanism



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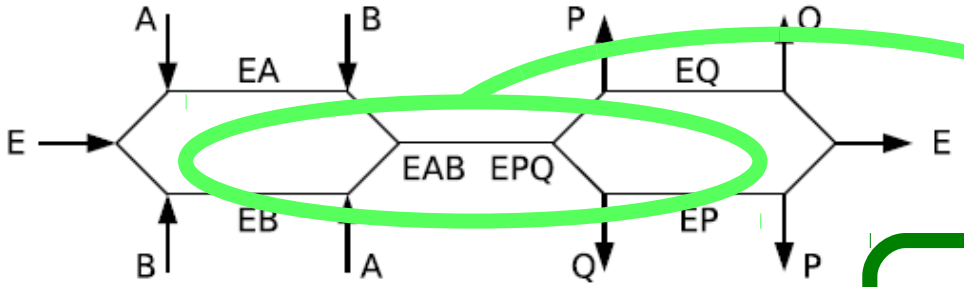
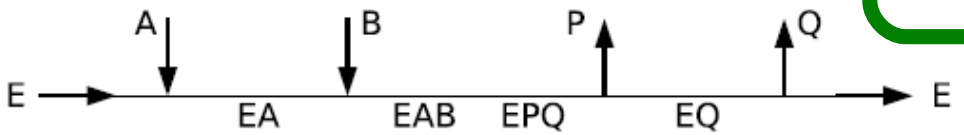


$$\nu_{\mathrm{RandomBiBi}} = \frac{V_{m+}\frac{[A][B]}{K_a K_b} - V_{m-}\frac{[P][Q]}{K_p K_q}}{1 + \frac{[A]}{K_a} + \frac{[B]}{K_b} + \frac{[A][B]}{K_a K_b} + \frac{[P]}{K_q} + \frac{[Q]}{K_q} + \frac{[P][Q]}{K_p K_q}}$$

b) Sequential Bi Bi Mechanism



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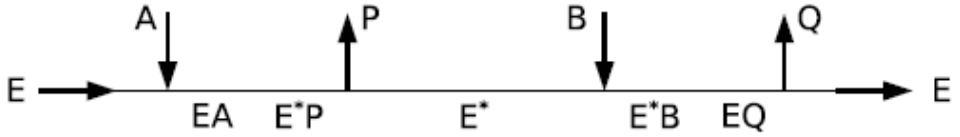
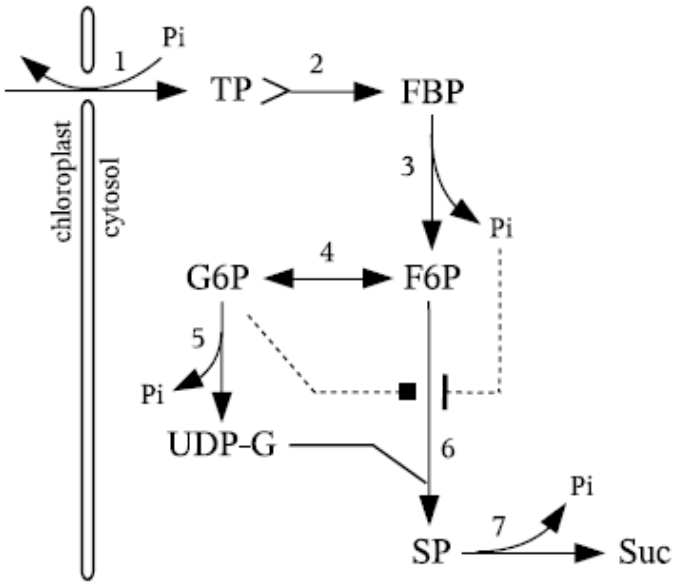


Building a model of cellular metabolism ...

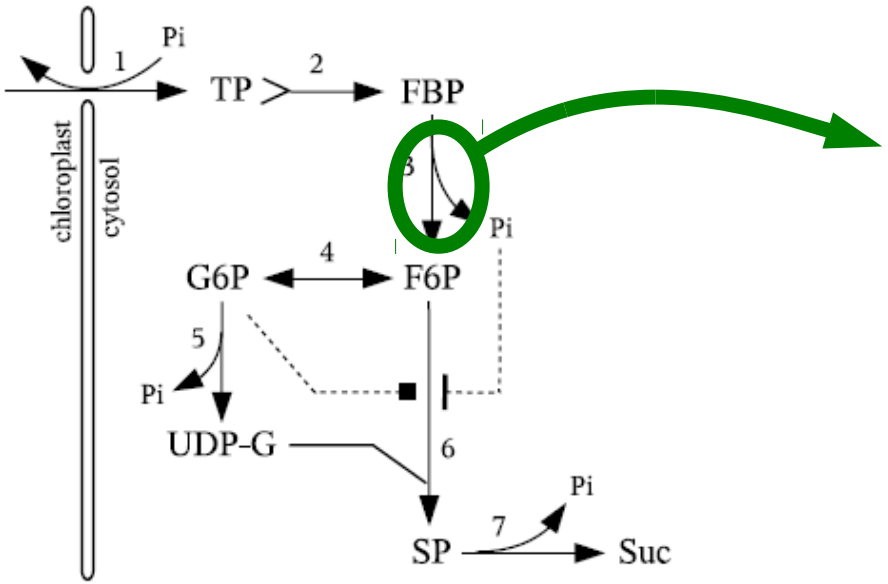
(1) Assemble the building blocks: reactions and metabolites



Building a model of cellular metabolism ...

(1) Assemble the building blocks: reactions and metabolites

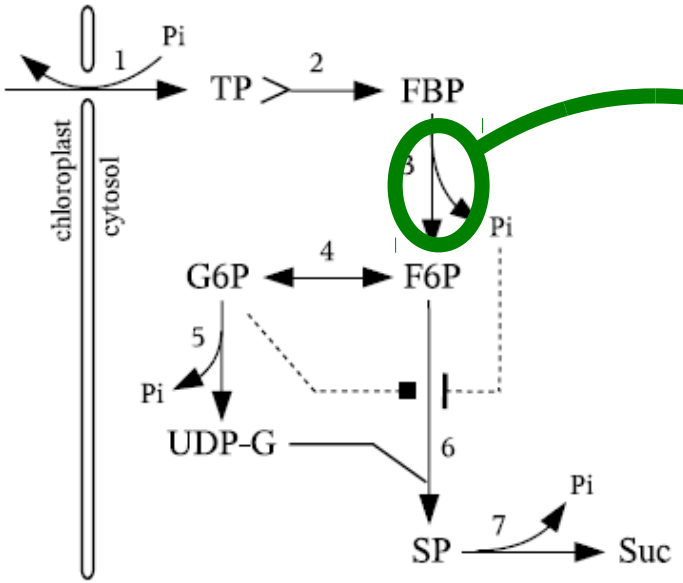
(2) Assign rate equations to each reaction



$$\nu(S) = \frac{V_m[S]}{K_M + [S]}$$

Building a model of cellular metabolism ...

(1) Assemble the building blocks: reactions and metabolites
(2) Assign rate equations to each reaction
(3) Assign values to all parameters



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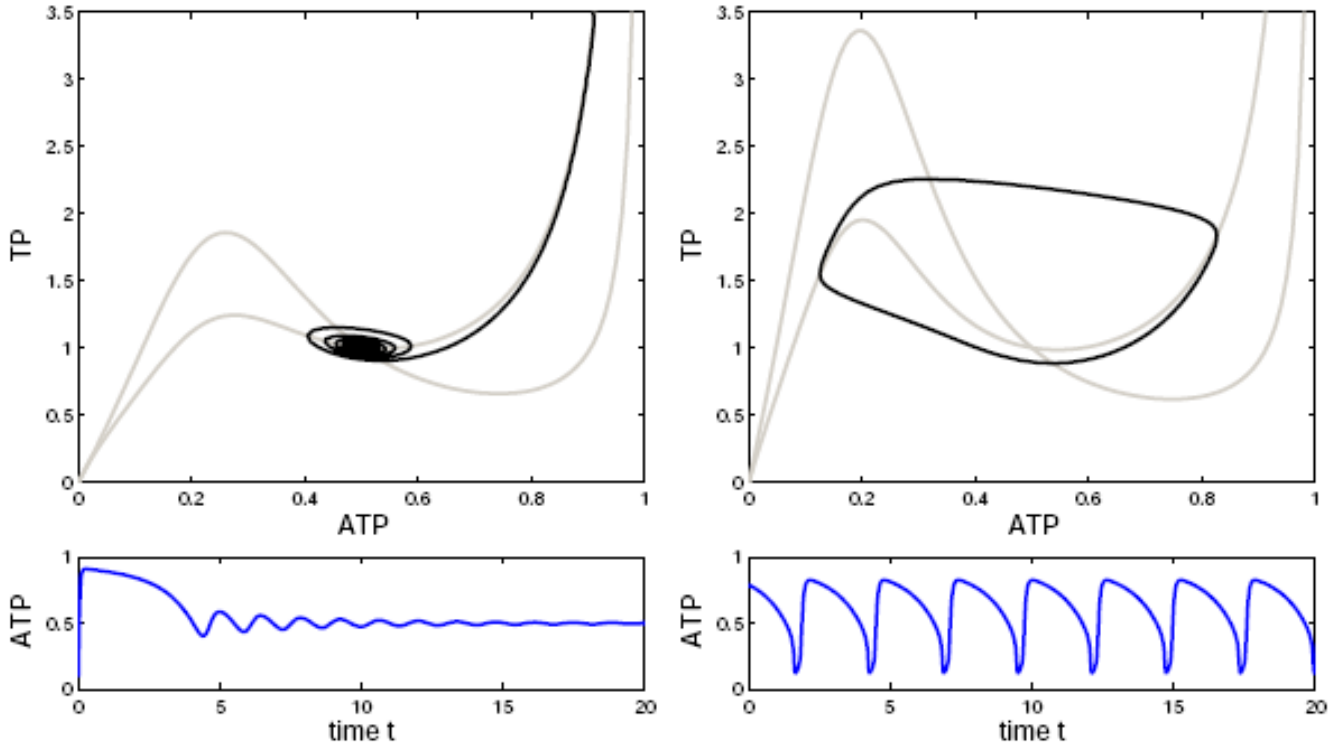
Building a model of cellular metabolism ...

(1) Assemble the building blocks: reactions and metabolites
(2) Assign rate equations to each reaction
(3) Assign values to all parameters
(4) Solve the system numerically



Building a model of cellular metabolism ...

(1) Assemble the building blocks: reactions and metabolites
(2) Assign rate equations to each reaction
(3) Assign values to all parameters
(4) Solve the system numerically
(5) DONE!

Building a model of cellular metabolism ...

(1) Assemble the building blocks: reactions and metabolites
(2) Assign rate equations to each reaction
(3) Assign values to all parameters
(4) Solve the system numerically

Unfortunately not so easy ...
(1) The 'Building blocks' are often unknown.
(2) Parameters are difficult to estimate
(3) Numerically complex

A few words on nomenclature:

Reaction:

$$\text{D-glucose} + \text{ATP} \rightarrow \text{D-glucose-6-phosphate} + \text{ADP}$$

A few words on nomenclature:

Reaction:

$$\text{D-glucose} + \text{ATP} \rightarrow \text{D-glucose-6-phosphate} + \text{ADP}$$

Stoichiometry:

Glc	ATP	G6P	ADP
-1	-1	$+1$	$+1$

Rate equation:

$$\nu_{HK}(\boldsymbol{S}, \boldsymbol{k}) = \frac{V_{\mathrm{m}} \frac{[Glc]}{K_{m,Glc}} \frac{[ATP]}{K_{m,ATP}}}{1 + \frac{[Glc]}{K_{m,Glc}} + \frac{[ATP]}{K_{m,ATP}} + \frac{[Glc]}{K_{m,Glc}} \frac{[ATP]}{K_{m,ATP}}}$$

A few words on nomenclature:

Organize the network into a set of differential equations:

$$\frac{\mathrm{d}S_i(t)}{\mathrm{d}t} = \sum_{j=1}^{r} N_{ij}\nu_j(\mathbf{S}, \mathbf{k})$$

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Organize the network into a set of differential equations:

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Stoichiometric coefficients

A few words on nomenclature:

Organize the network into a set of differential equations:

$$\frac{\mathrm{d}S_i(t)}{\mathrm{d}t} = \sum_{j=1}^{r} N_{ij}\nu_j(\mathbf{S},\mathbf{k})$$

Rate equations

A few words on nomenclature:

Organize the network into a set of differential equations:

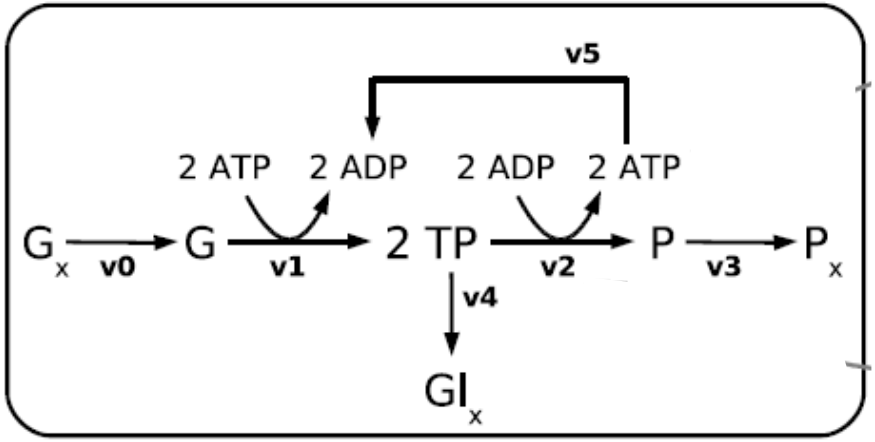
$$\frac{\mathrm{d}S_i(t)}{\mathrm{d}t} = \sum_{j=1}^{r} N_{ij}\nu_j(\mathbf{S},\mathbf{k})$$

In matrix notation:

$$\frac{\mathrm{d}\boldsymbol{S}(t)}{\mathrm{d}t} = \boldsymbol{N}\boldsymbol{\nu}(\mathbf{S},\mathbf{k})$$

Organize the network in a set of differential equations:

A: Reaction scheme



C: Reaction list:

v0: G_x -> G

v1: G+ 2 ATP -> 2 TP + 2 ADP

v2: TP + 2 ADP -> P + 2 ATP

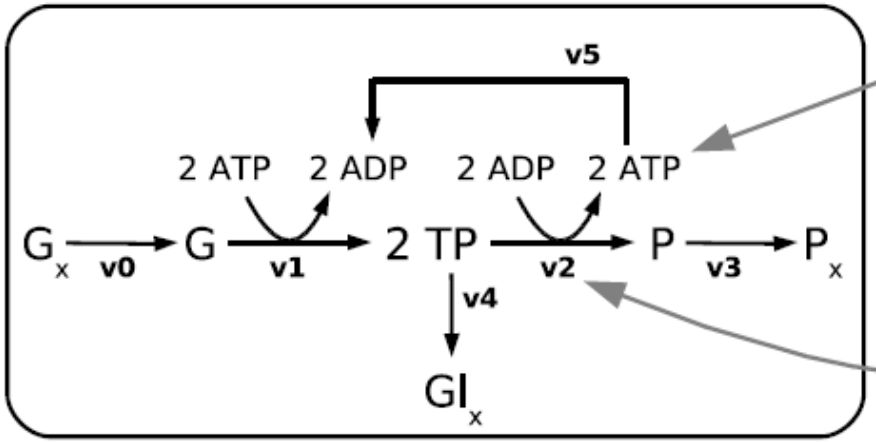
v3: P -> P_x

v4: TP -> GI_x

v5: ATP -> ADP

Organize the network in a set of differential equations:

A: Reaction scheme



B: Stoichiometry

$$N = \begin{array}{c} \begin{matrix} \text{v0} & \text{v1} & \text{v2} & \text{v3} & \text{v4} & \text{v5} \end{matrix} \\ \begin{bmatrix} +1 & -1 & 0 & 0 & 0 & 0 \\ 0 & +2 & -1 & 0 & -1 & 0 \\ 0 & 0 & +1 & -1 & 0 & 0 \\ 0 & -2 & +2 & 0 & 0 & -1 \\ 0 & +2 & -2 & 0 & 0 & +1 \end{bmatrix} \end{array} \begin{matrix} \\ \text{G} \\ \text{TP} \\ \text{P} \\ \text{ATP} \\ \text{ADP} \end{matrix}$$

C: Reaction list:

v0: G_x -> G

v1: G+ 2 ATP -> 2 TP + 2 ADP

v2: TP + 2 ADP -> P + 2 ATP

v3: P -> P_x

v4: TP -> GI_x

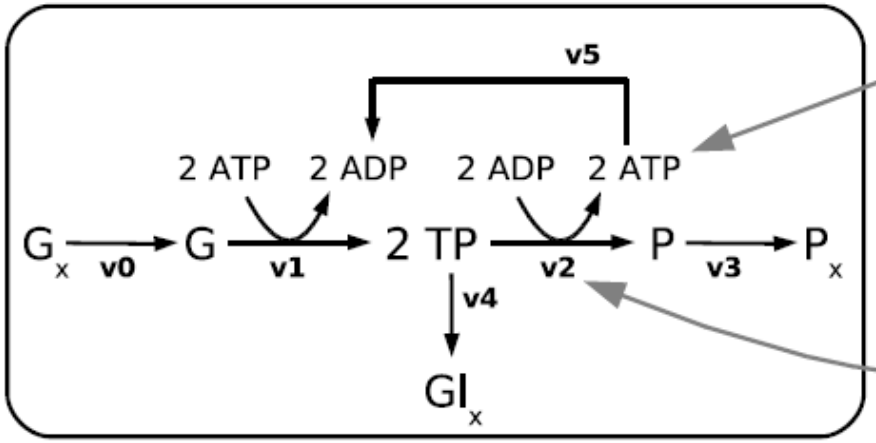
v5: ATP -> ADP

D: The mass-balance equations

$$\frac{\mathrm{d}\boldsymbol{S}(t)}{\mathrm{d}t} = \boldsymbol{N}\boldsymbol{\nu}(\mathbf{S}, \mathbf{k})$$

Organize the network in a set of differential equations:

A: Reaction scheme



B: Stoichiometry

$$N = \begin{array}{c} \begin{matrix} v0 & v1 & v2 & v3 & v4 & v5 \end{matrix} \\ \begin{bmatrix} +1 & -1 & 0 & 0 & 0 & 0 \\ 0 & +2 & -1 & 0 & -1 & 0 \\ 0 & 0 & +1 & -1 & 0 & 0 \\ 0 & -2 & +2 & 0 & 0 & -1 \\ 0 & +2 & -2 & 0 & 0 & +1 \end{bmatrix} \end{array} \begin{matrix} G \\ TP \\ P \\ ATP \\ ADP \end{matrix}$$

C: Reaction list:

v0: G_x -> G

v1: G+ 2 ATP -> 2 TP + 2 ADP

v2: TP + 2 ADP -> P + 2 ATP

v3: P -> P_x

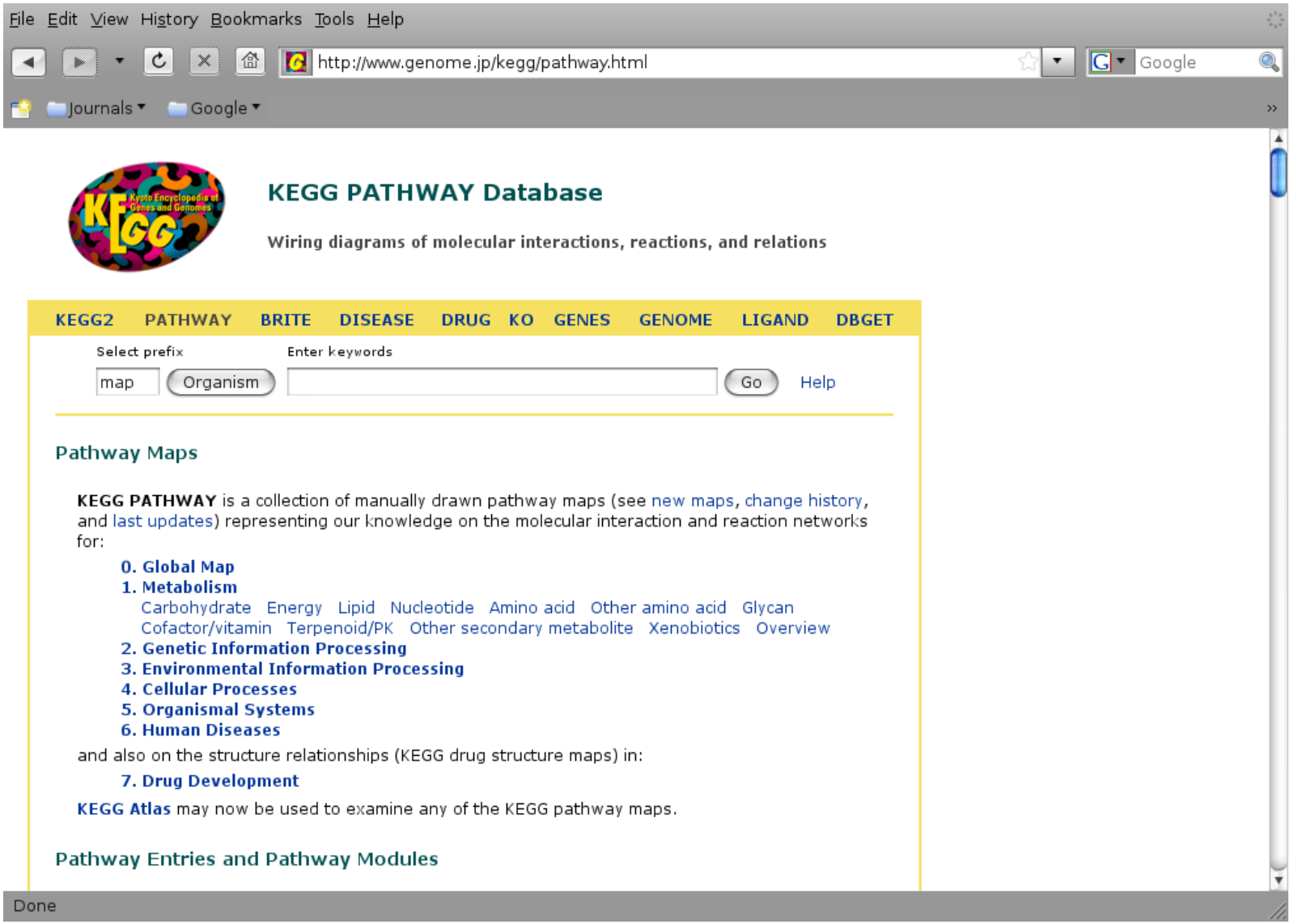
v4: TP -> Gl_x

v5: ATP -> ADP

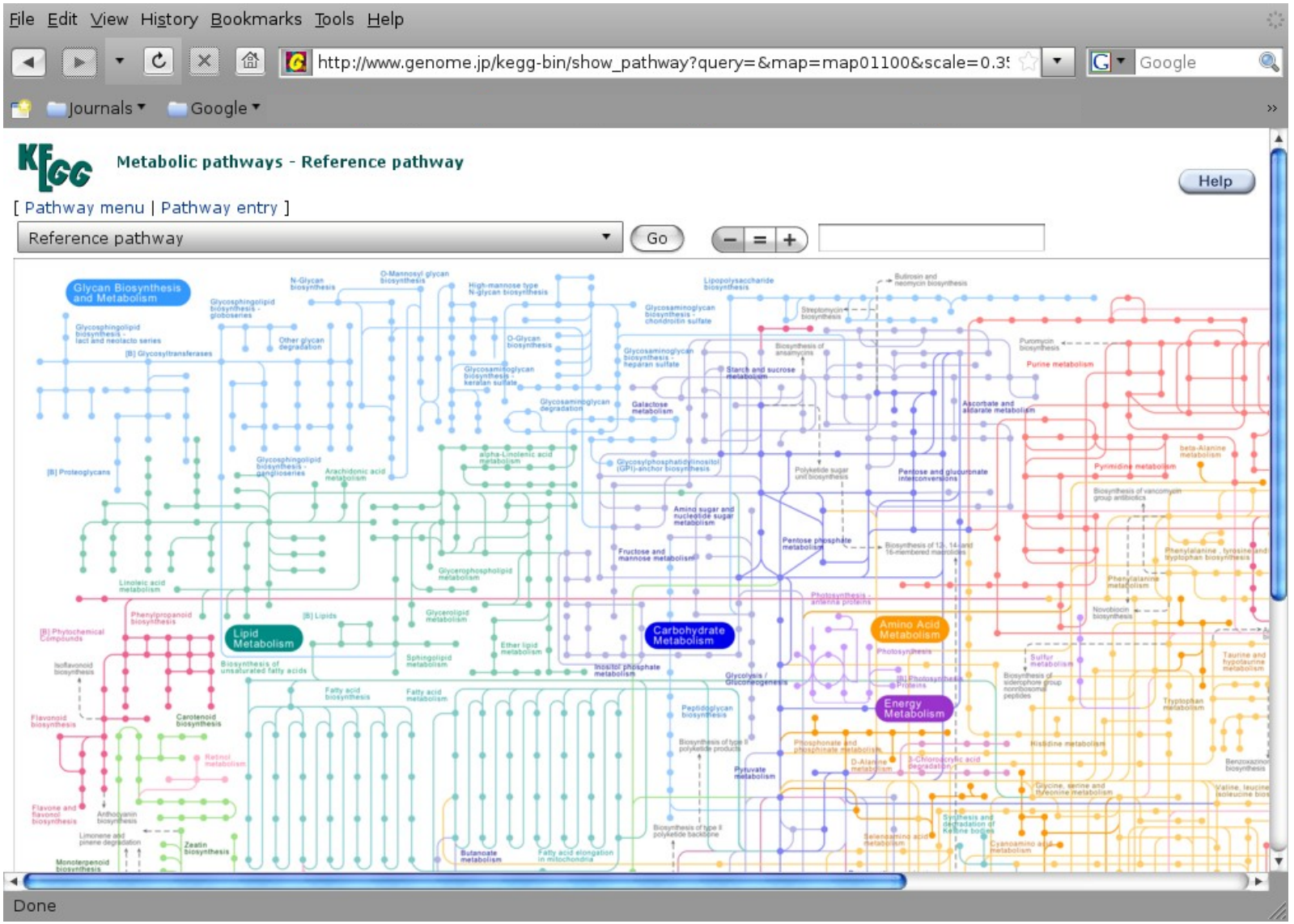
D: The mass-balance equations

$$\frac{d}{dt}\begin{bmatrix} G \\ TP \\ P \\ ATP \\ ADP \end{bmatrix} = \begin{bmatrix} +1 & -1 & 0 & 0 & 0 & 0 \\ 0 & +2 & -1 & 0 & -1 & 0 \\ 0 & 0 & +1 & -1 & 0 & 0 \\ 0 & -2 & +2 & 0 & 0 & -1 \\ 0 & +2 & -2 & 0 & 0 & +1 \end{bmatrix} \cdot \begin{bmatrix} \nu_0 \\ \nu_1 \\ \nu_2 \\ \nu_3 \\ \nu_4 \\ \nu_5 \end{bmatrix}$$

The stoichiometry is increasingly known:



The stoichiometry is increasingly known:



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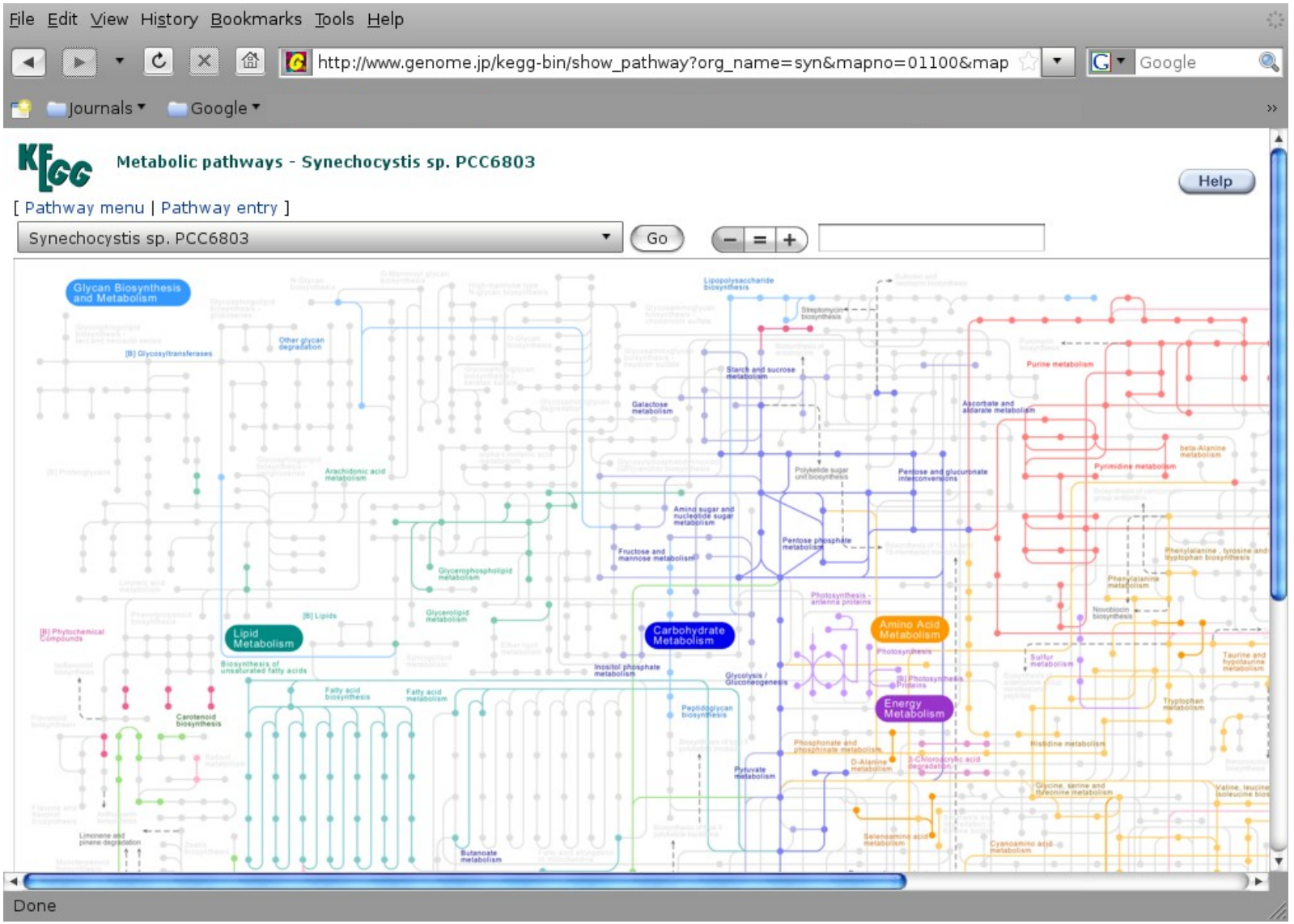


Other resources:

Table 1.3 Useful webpages to assemble information on kinetic models, including pathway databases and model repositories.

Name	URL
KEGG	http://www.genome.jp/kegg/
BRENDA	http://www.brenda-enzymes.info/
BioModels database	http://www.ebi.ac.uk/biomodels/
JWS Online	http://www.jjj.bio.vu.nl/
CellML	http://www.cellml.org/
SBML	http://www.sbml.org/

Table 1.4 Some selected software packages for kinetic simulations. The list adapted from [9, 217]

Name	URL	Ref
Copasi	http://www.copasi.org	[115]
CellDesigner	http://www.celldesigner.org/	[73]
E-CELL	http://www.e-cell.org/	[172]
Cellware	http://www.cellware.org/	[51]
JDesigner/Jarnac	http://sbw.kgi.edu/sbwWiki/sysbio/jdesigner	
Matlab	http://www.mathworks.com/	
SB Toolbox	http://www.sbtoolbox2.org/	[238]

Modelling metabolic networks

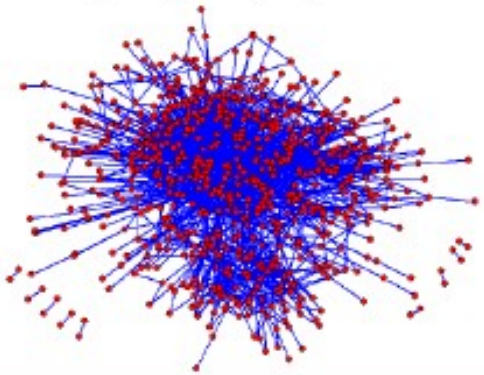
Modelling metabolic networks ... has many facettes:

Size of System

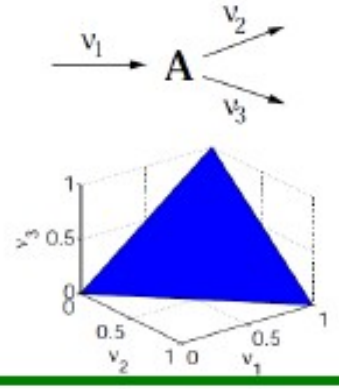
Level of Detail

Qualitative Models ⟷ **Quantitative Models**

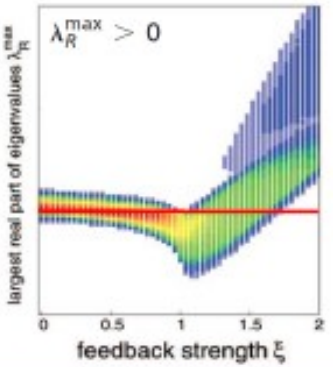
Topological Analysis
- Static description
- No kinetic parameters
- Topological properties



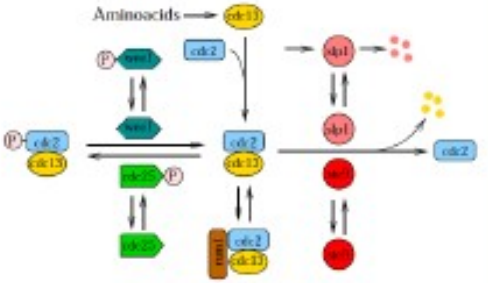
Flux Balance Analysis
- Static description
- No kinetic parameters
- Quantitative predictions



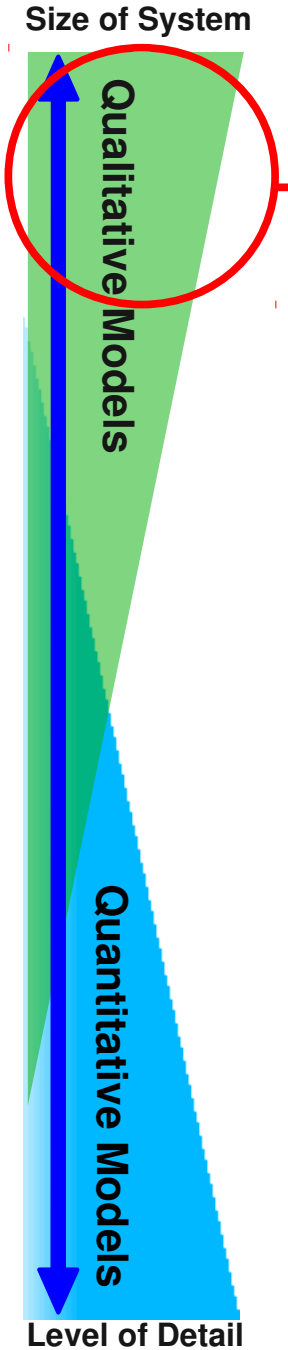
Structural Kinetic Models
- Dynamic description
- No kinetic parameters
- Bifurcation structure



Kinetic Models
- Dynamic description
- Kinetic parameters
- Differential equations



models of metabolic networks



Topological Analysis

- Static description
- No kinetic parameters
- Topological properties

models of metabolic networks

Size of System

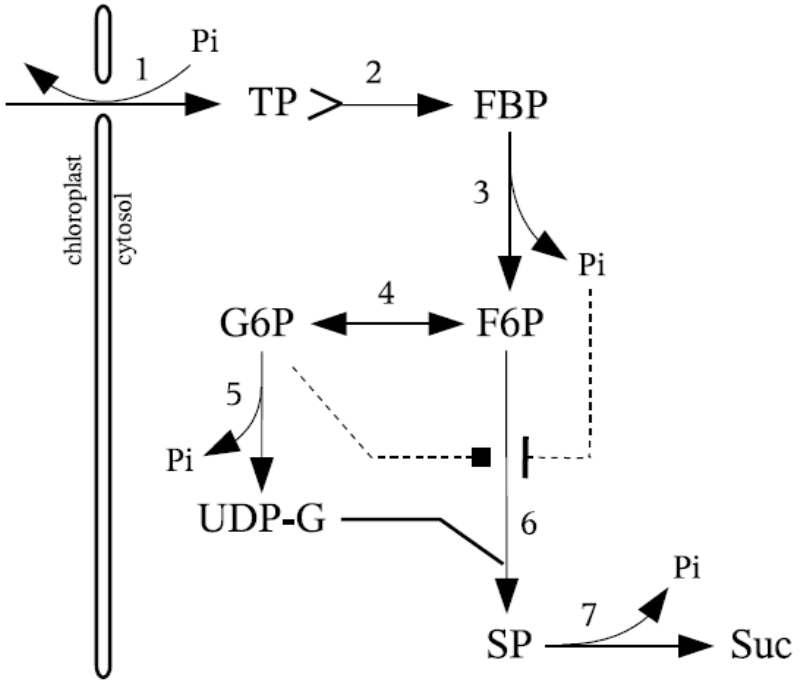
Qualitative Models

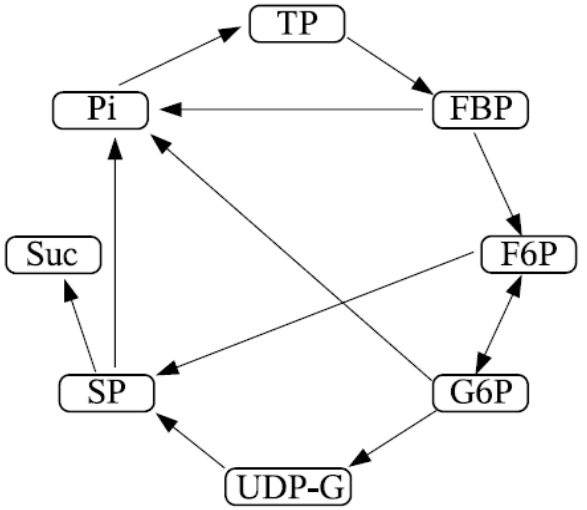
Quantitative Models

Level of Detail

Topological Analysis

- Static description
- No kinetic parameters
- Topological properties





the substrate graph

Size of System

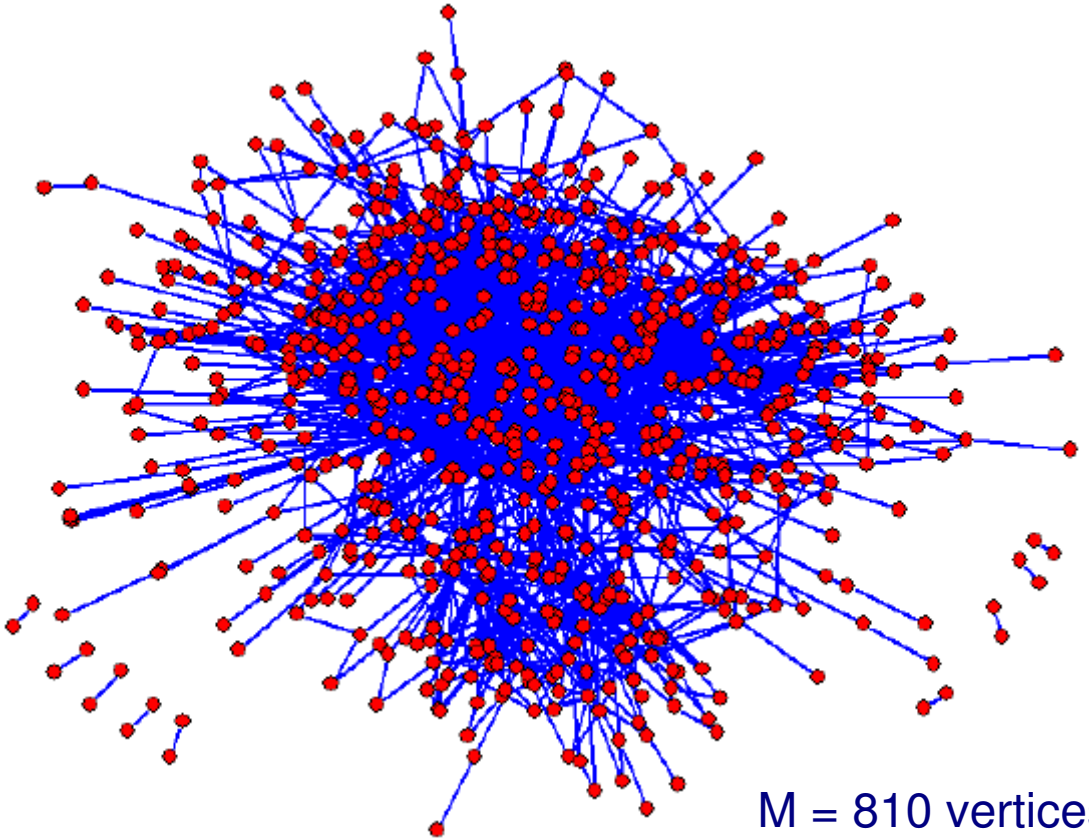
Qualitative Models

Quantitative Models

Level of Detail

Topological Analysis

An example: The *S. cerevisiae* metabolic network



M = 810 vertices (metabolites)
R = 843 reactions
E = 3419 edges

Size of System

Qualitative Models

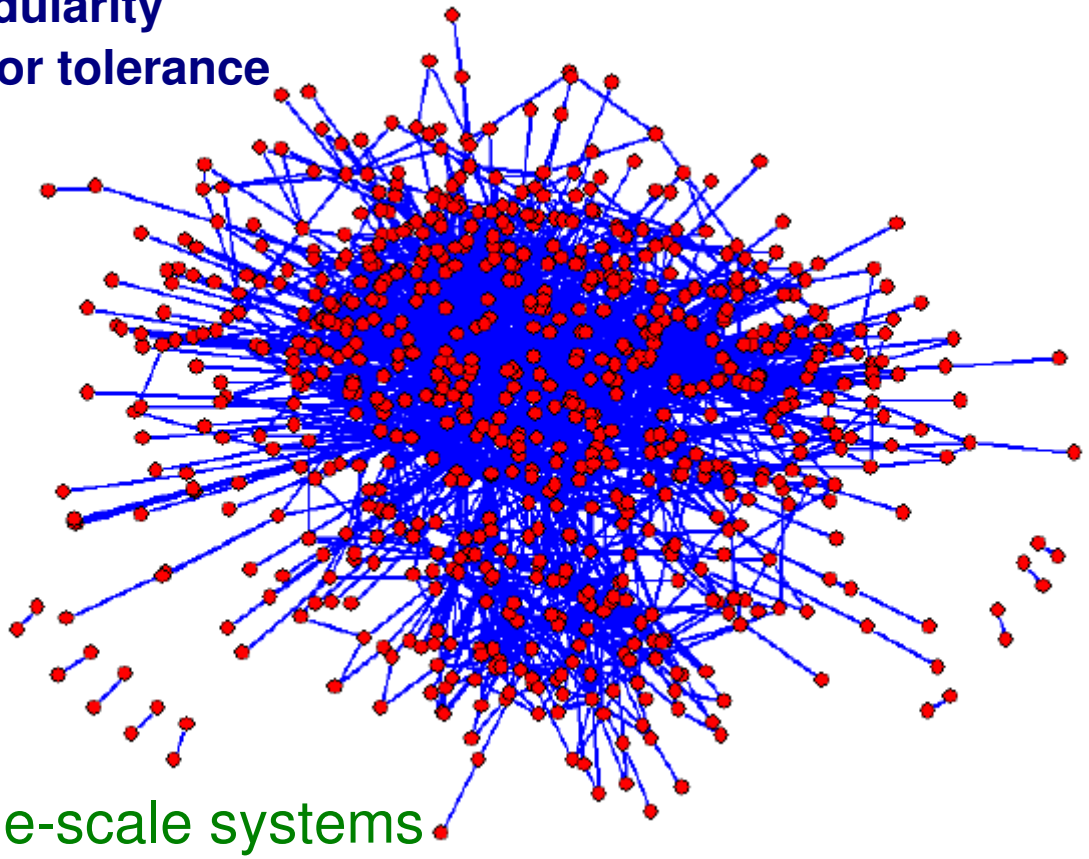
Quantitative Models

Level of Detail

Topological Analysis

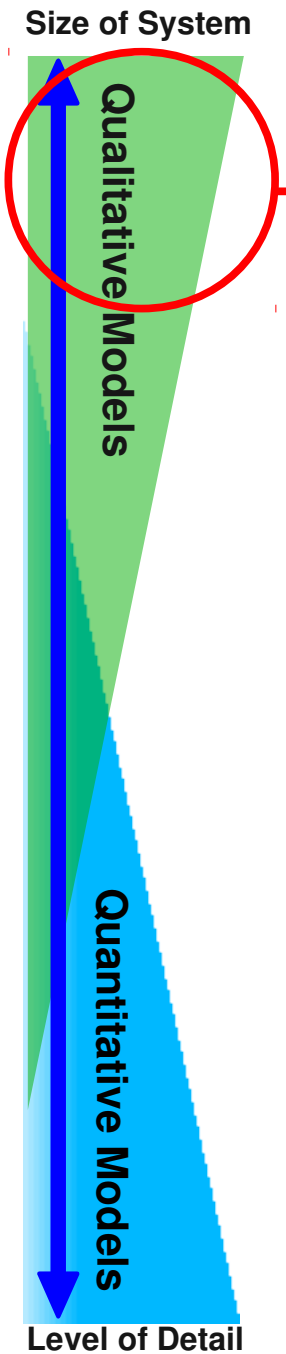
Typical network properties:

- Pathlength and clustering
- Degree distribution
- Hierarchies and modularity
- Robustness and error tolerance



Advantages:

- Applicable to large-scale systems
- Requires only topological information

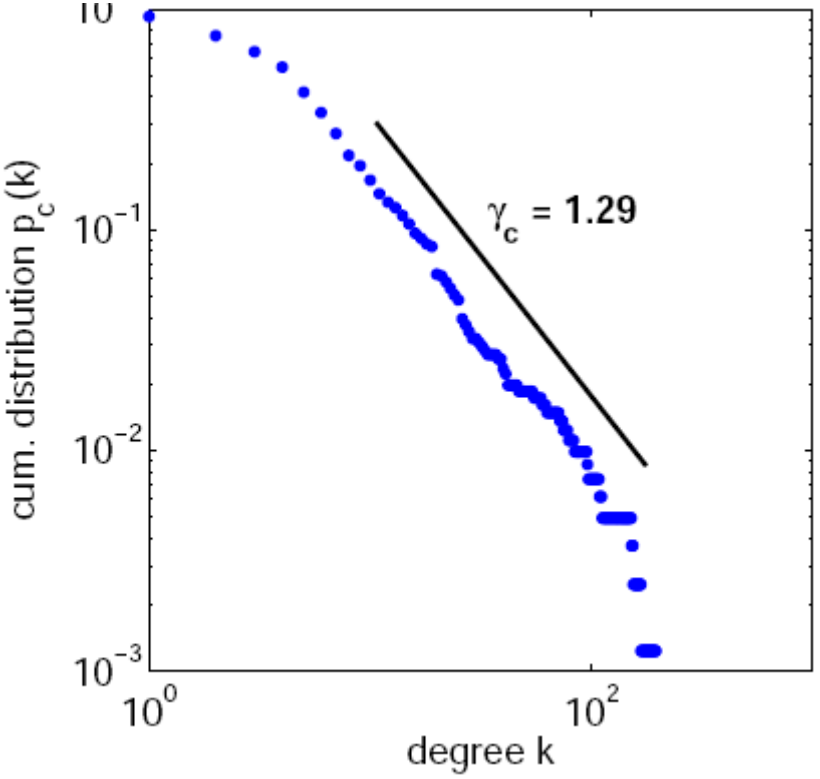


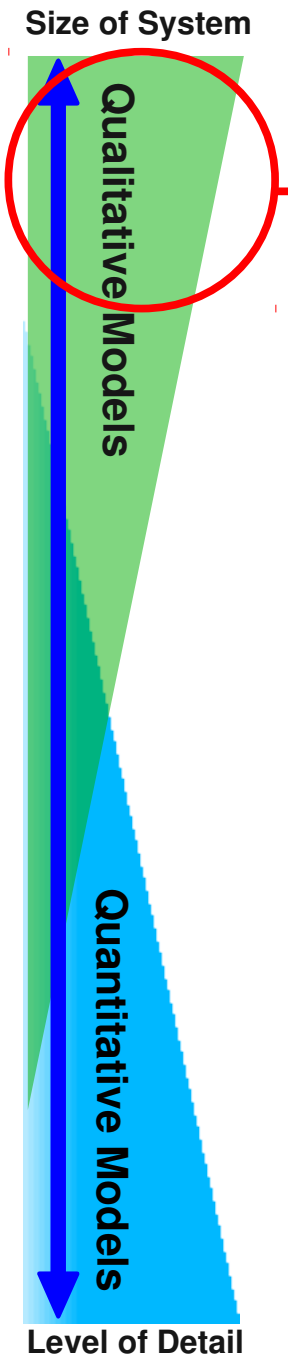
Topological Analysis

Typical network properties:

- Pathlength and clustering
- Degree distribution
- Hierarchies and modularity
- Robustness and error tolerance

the degree distribution





Topological Analysis

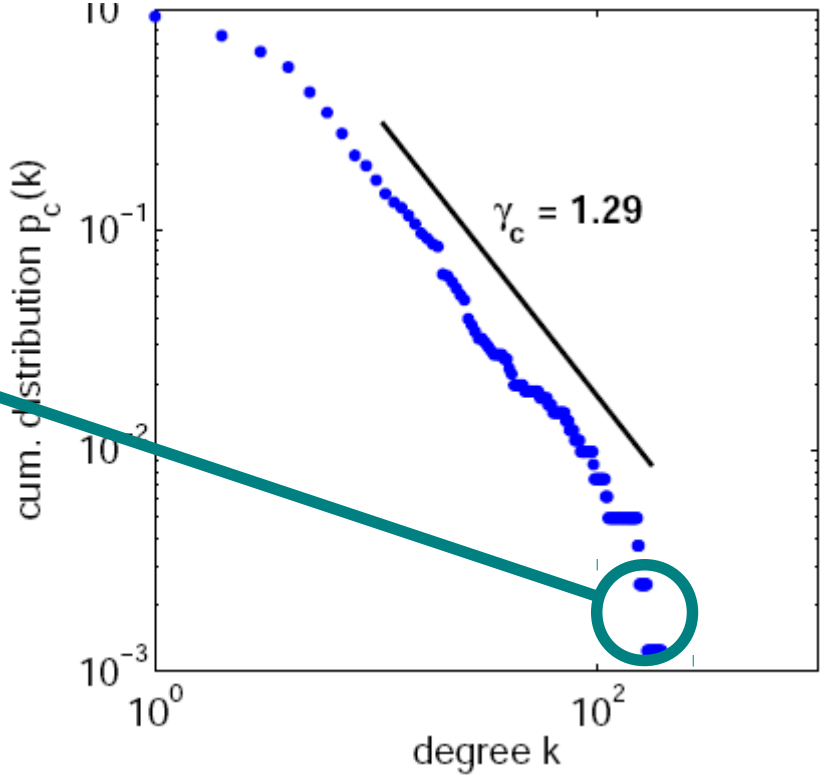
Typical network properties:

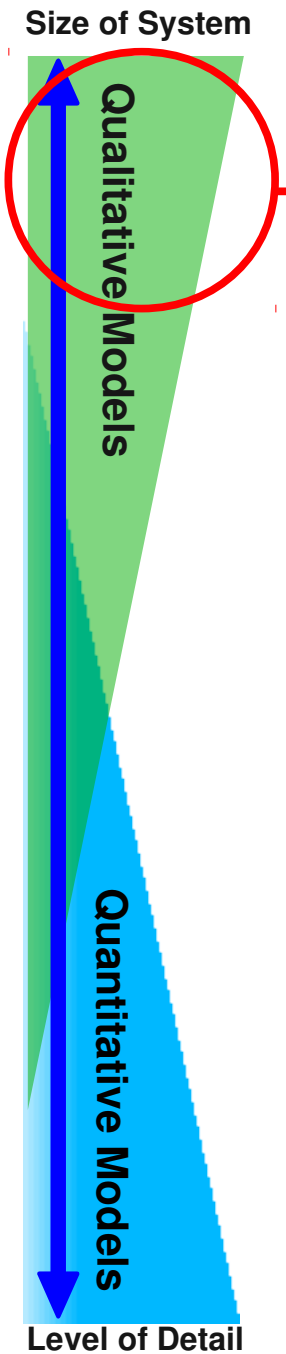
- Pathlength and clustering
- Degree distribution
- Hierarchies and modularity
- Robustness and error tolerance

the degree distribution

highly connected metabolites:

glycolysis, TCA cycle (pyruvate, etc ...)
cofactors (ATP, NAD, etc ...)





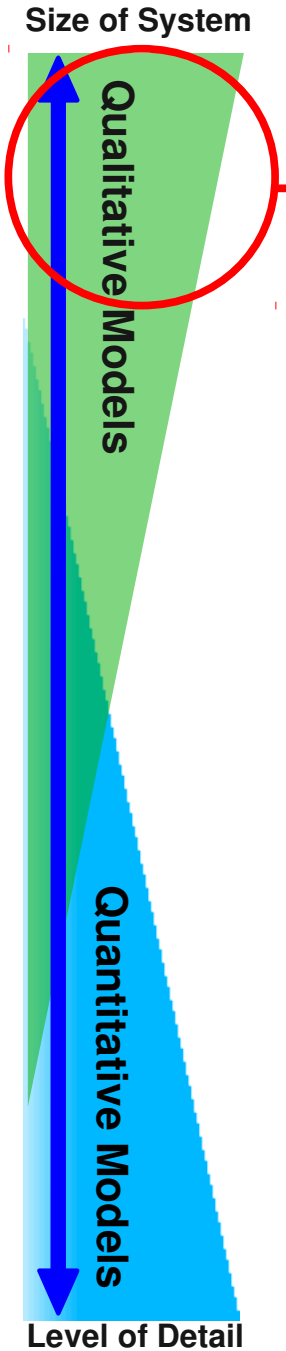
Topological Analysis

Typical network properties:

- Pathlength and clustering
- Degree distribution
- Hierarchies and modularity
- Robustness and error tolerance

highly connected metabolites:

rank	*metabolite*	*reactions*	*rank*	*metabolite*	*reactions*
1	ATP	122	12	S-Adenosyl-L-methionine	16
2	ADP	97	13	L-Glutamine	14
3	NADP+	52	14	beta-D-Fructose 6-phosphate	13
4	NADPH	50	15	GTP	13
5	L-Glutamate	40	16	5-Phospho-α-D-ribose 1-diphosphate	13
6	NAD+	38	17	Pyruvate	13
7	NADH	34	18	L-Aspartate	12
8	AMP	32	19	GDP	12
9	CoA	30	20	S-Adenosyl-L-homocysteine	12
10	2-Oxoglutarate	23	21	Acetate	11
11	Acetyl-CoA	20	⋮	⋮	⋮



Topological Analysis

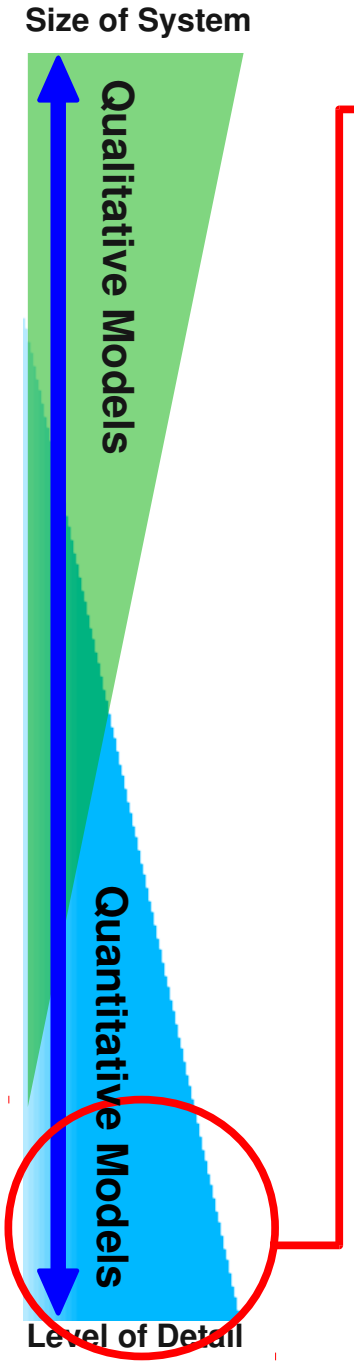
Typical network properties:

- Pathlength and clustering
- Degree distribution
- Hierarchies and modularity
- Robustness and error tolerance

Limitations:

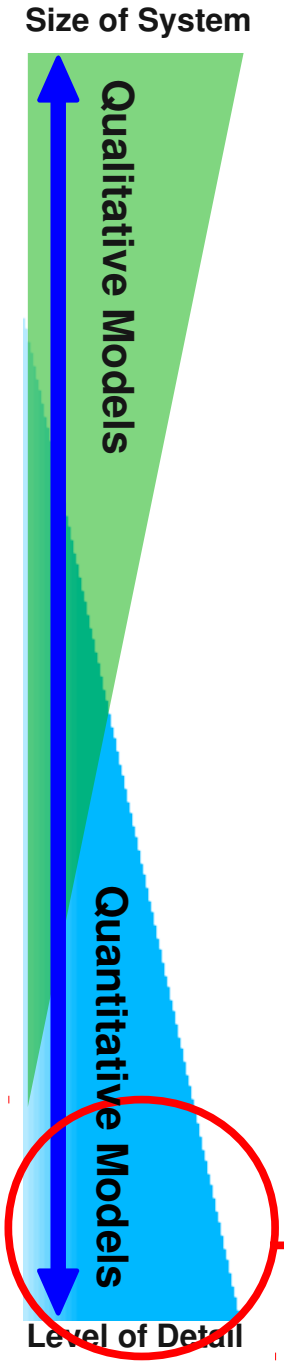
- Only qualitative description
- No dynamic properties
- No specific features of metabolic networks

models of metabolic networks



Explicit Kinetic Models

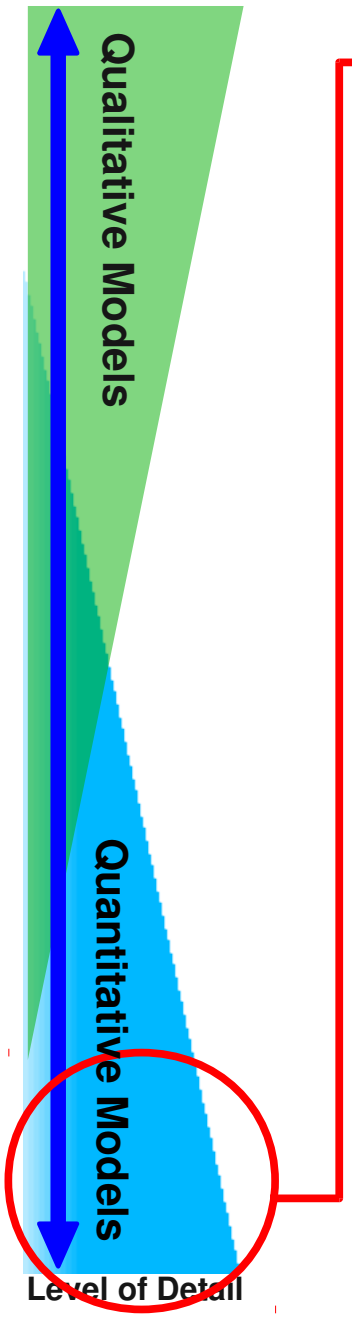
- Dynamic description
- Kinetic parameters
- Differential equations



Explicit Kinetic Models

- Dynamic description
- Kinetic parameters
- Differential equations

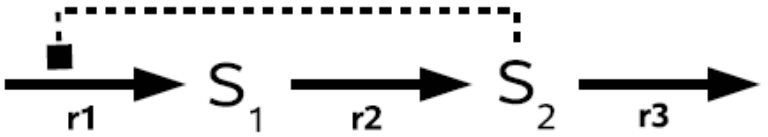
(1) Assemble the “parts list”: reactions and metabolites
(2) Assign rate equations to each reaction
(3) Assign values to all parameters
(4) Solve the system numerically
(5) DONE!



Explicit Kinetic Models

- Dynamic description
- Kinetic parameters
- Differential equations

A simple Example:



$$\frac{\mathrm{d}\mathbf{S}(t)}{\mathrm{d}t} = \mathbf{N}\,\boldsymbol{\nu}(\mathbf{S},\mathbf{k})$$

$$\frac{d}{dt}\begin{bmatrix} S_1(t) \\ S_2(t) \end{bmatrix} = \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \cdot \begin{bmatrix} \nu_1(\mathbf{S},\mathbf{k}) \\ \nu_2(\mathbf{S},\mathbf{k}) \\ \nu_3(\mathbf{S},\mathbf{k}) \end{bmatrix}$$

Size of System

Qualitative Models

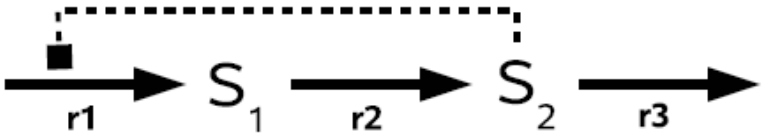
Quantitative Models

Level of Detail

Explicit Kinetic Models

- Dynamic description
- Kinetic parameters
- Differential equations

A simple Example:



$$\frac{\mathrm{d}\mathbf{S}(t)}{\mathrm{d}t} = \mathbf{N}\,\boldsymbol{\nu}(\mathbf{S},\mathbf{k})$$

$$\begin{bmatrix} \nu_1(\mathbf{S},\mathbf{k}) \\ \nu_2(\mathbf{S},\mathbf{k}) \\ \nu_3(\mathbf{S},\mathbf{k}) \end{bmatrix}$$

A typical rate equation:

$$r_{\mathrm{PGK}} = \frac{r_{\mathrm{PGK}}^{\max}\left(C_{\mathrm{adp}}C_{\mathrm{pgp}} - \frac{C_{\mathrm{atp}}C_{3\mathrm{pg}}}{K_{\mathrm{PGK,eq}}}\right)}{\left(K_{\mathrm{PGK,adp}}\left(1+\frac{C_{\mathrm{atp}}}{K_{\mathrm{PGK,atp}}}\right)+C_{\mathrm{adp}}\right)\left(K_{\mathrm{PGK,pgp}}\left(1+\frac{C_{3\mathrm{pg}}}{K_{\mathrm{PGK,3pg}}}\right)+C_{\mathrm{pgp}}\right)}$$

Example from Chassagnole (2002)

Size of System

Qualitative Models

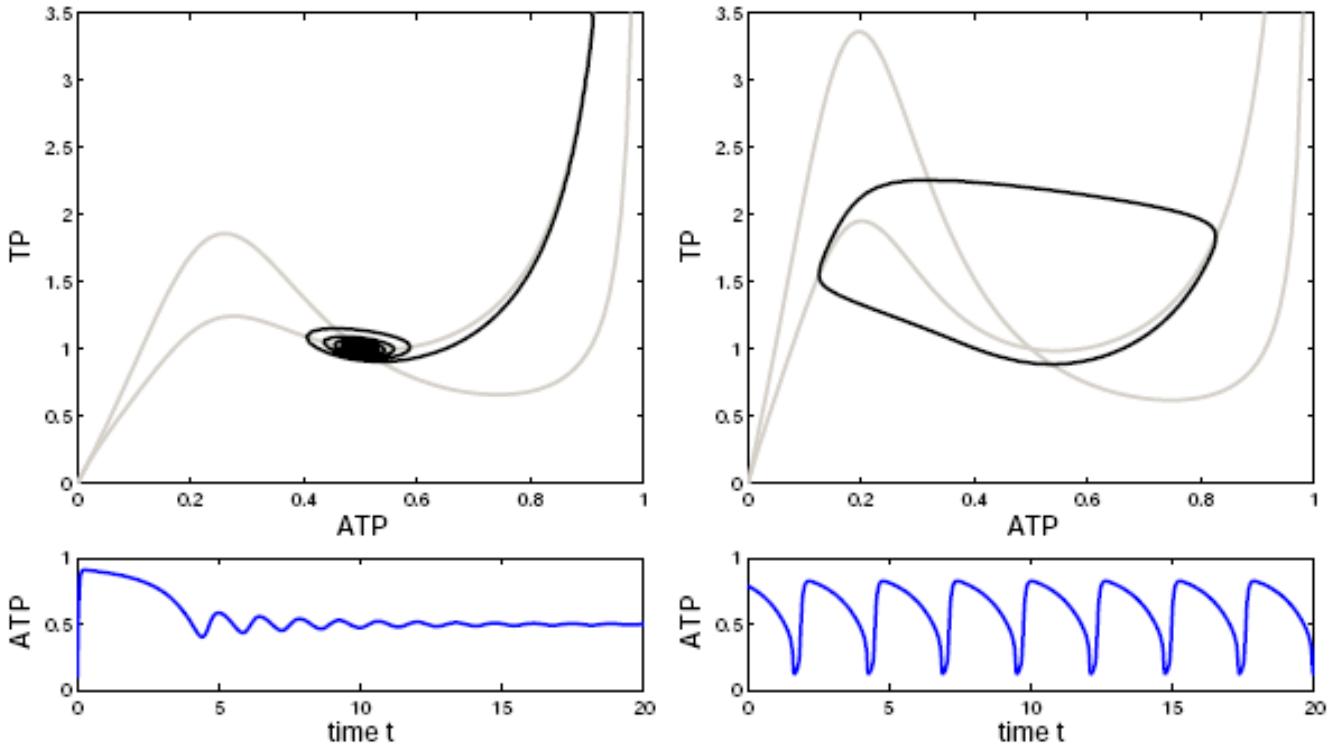
Quantitative Models

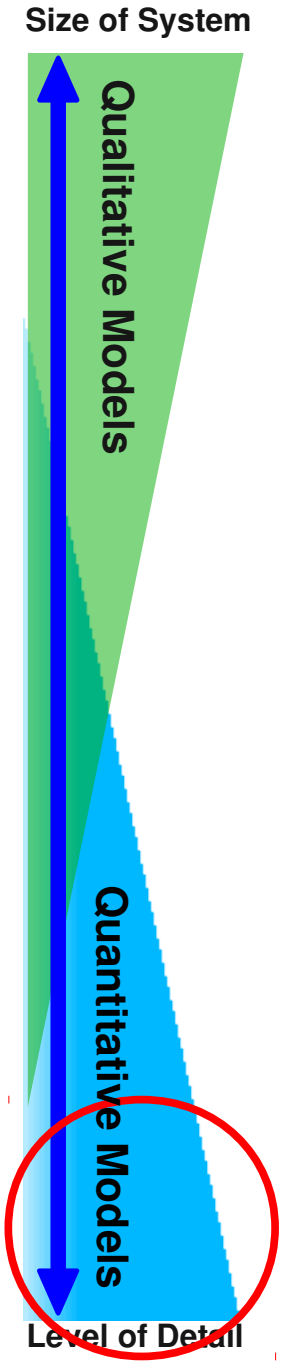
Level of Detail

Explicit Kinetic Models

- Dynamic description
- Kinetic parameters
- Differential equations

Then solve system computationally:





A more complex example: Identify best (putative) drug targets in a model of human metabolism.

By E. Murabito (Manchester)

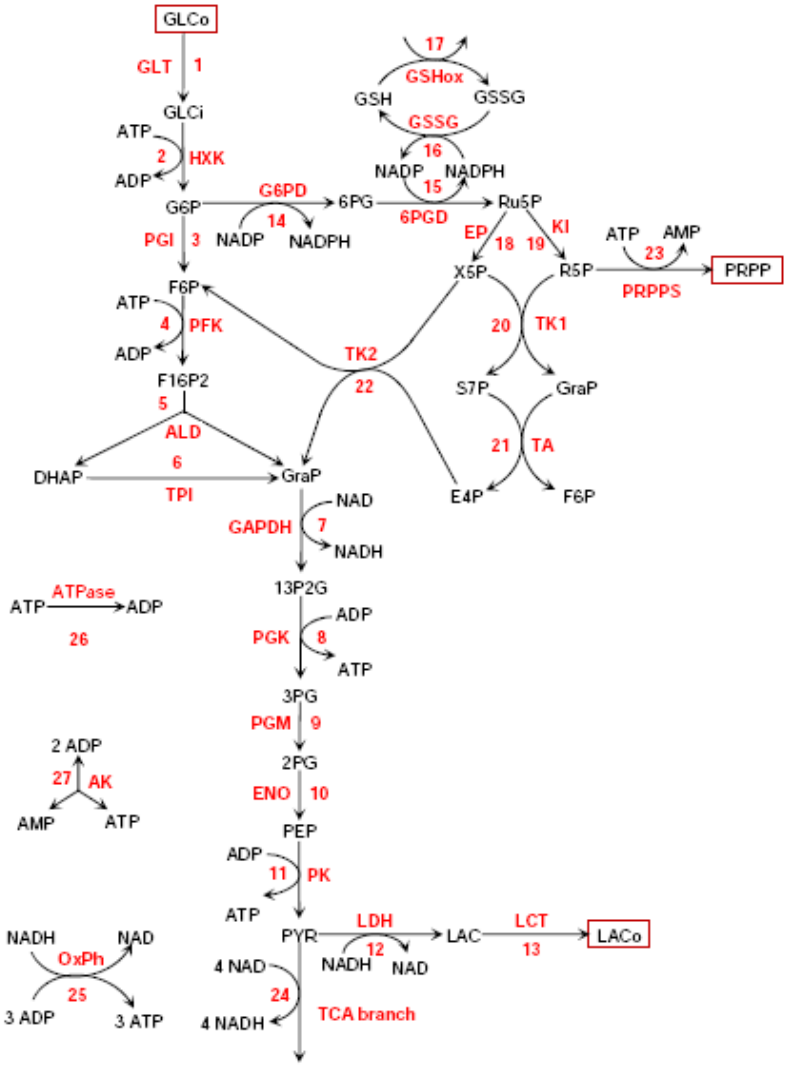
Size of System

Qualitative Models

Quantitative Models

Level of Detail

A more complex example: Identify best (putative) drug targets in a model of human metabolism.



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Size of System

Qualitative Models

Quantitative Models

Level of Detail

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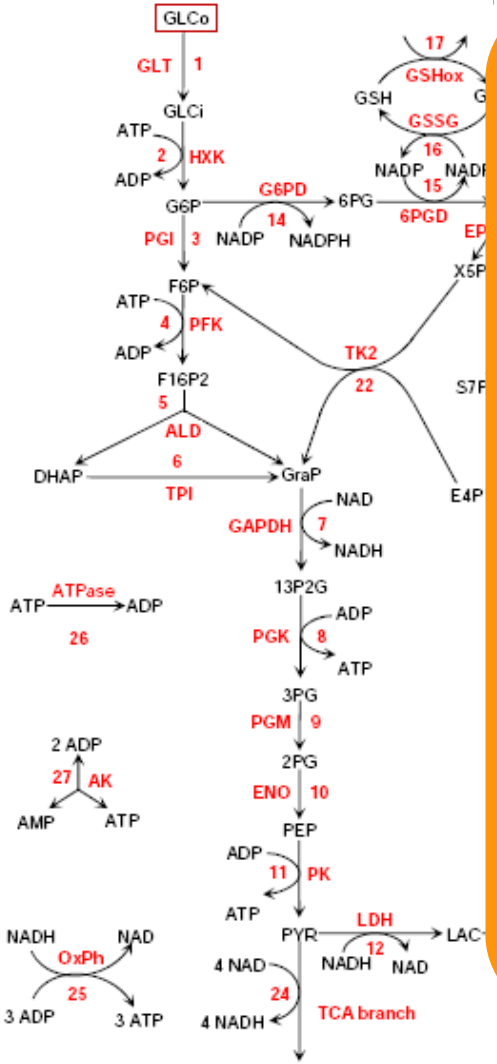
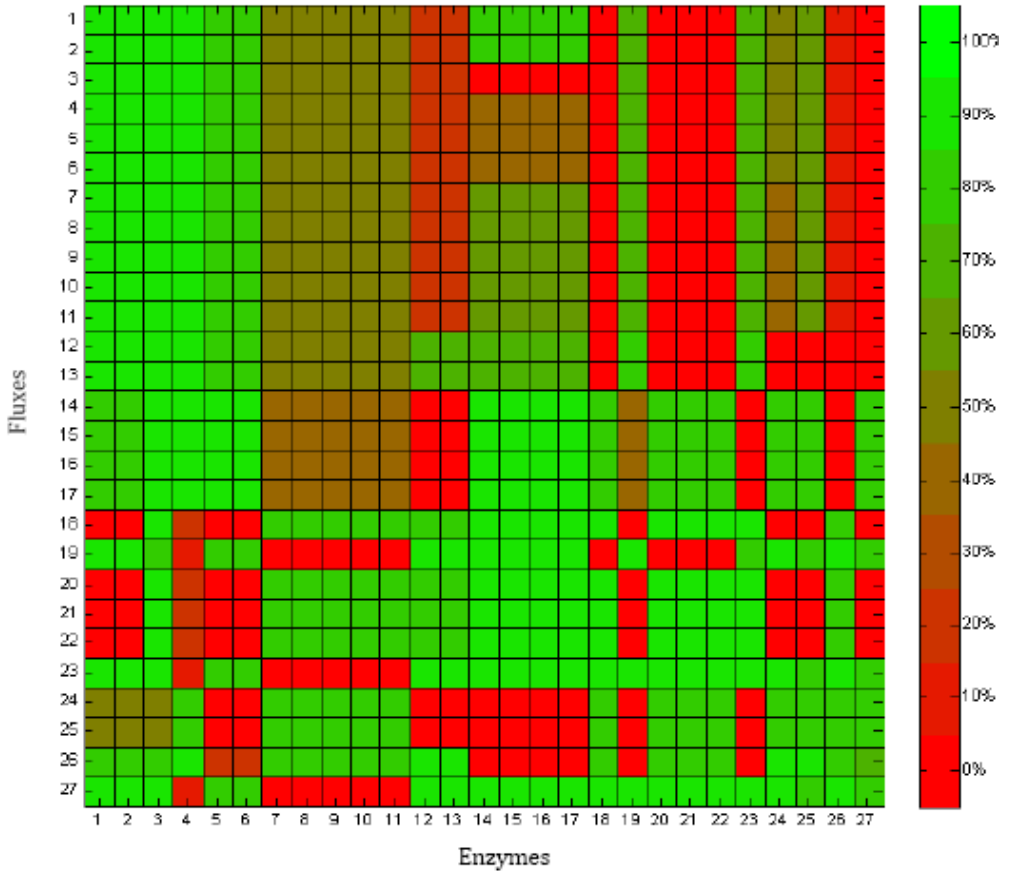
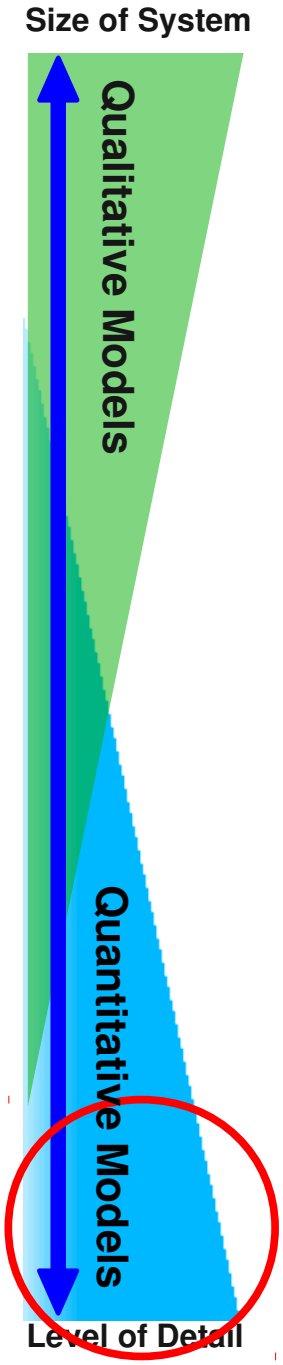


Matrix of control coefficients



By E. Murabito (Manchester)

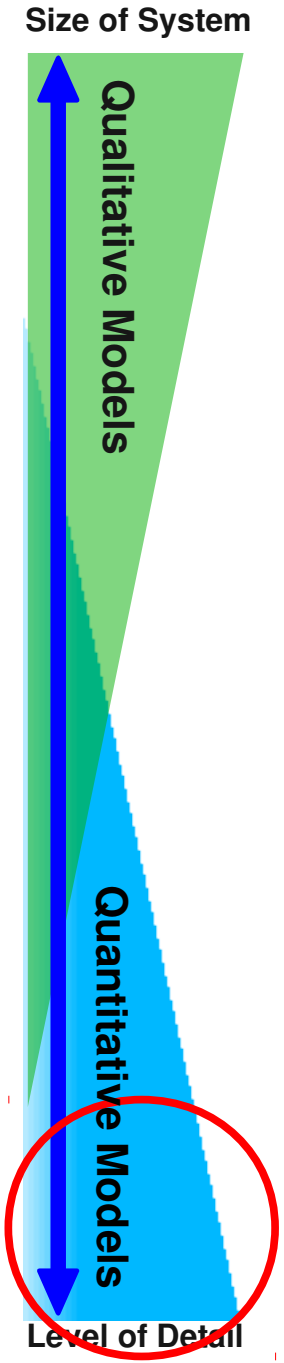


A more complex example: Identify best (putative) drug targets in a model of human metabolism.

Advantages

A large number od potential targets can be tested quickly
No ethical problems
Fast results
Cheap

By E. Murabito (Manchester)



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But:
Current models not predictive yet
Too many unknown parameters
Too many unknown interactions

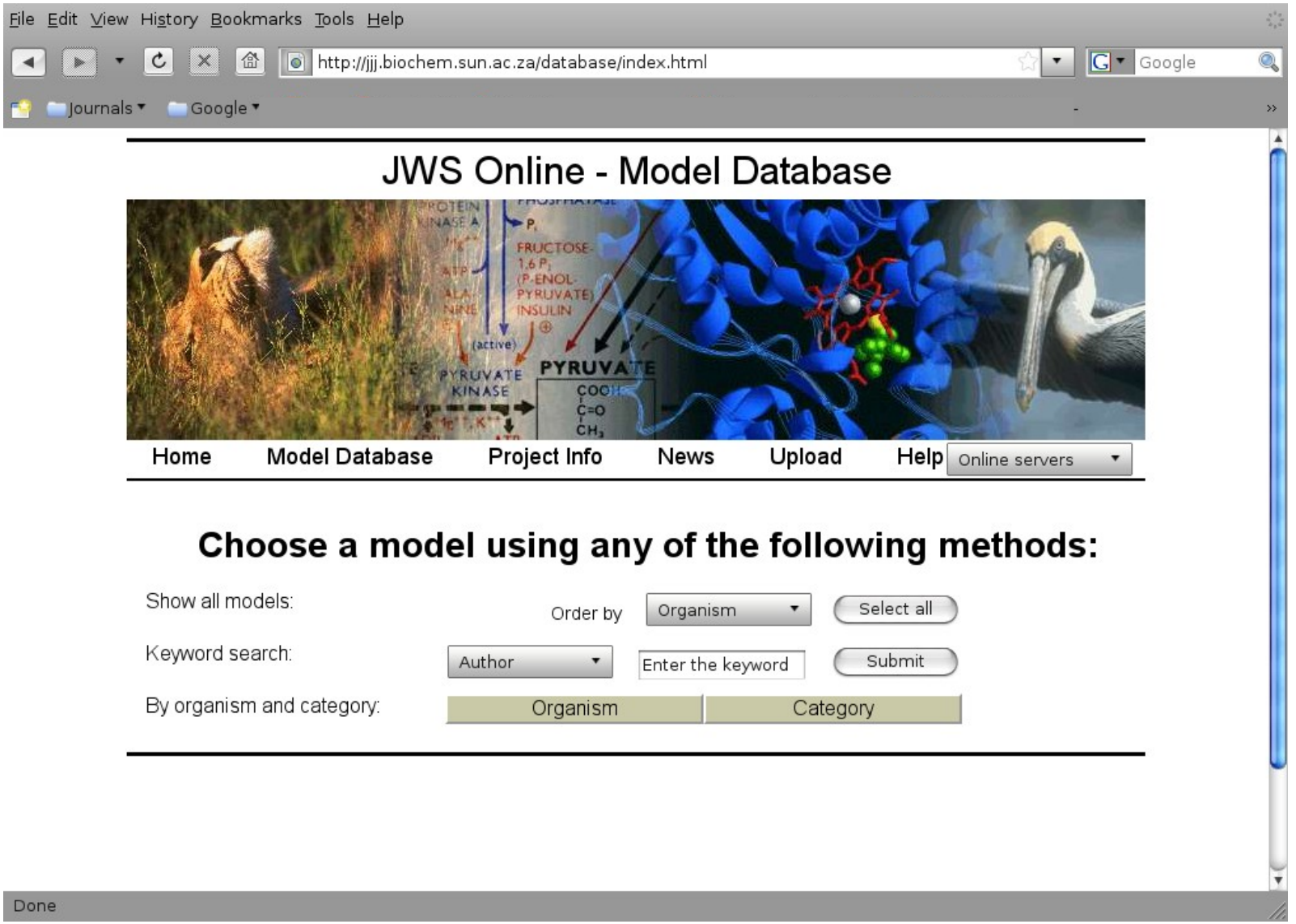
Size of System

Qualitative Models

Quantitative Models

Level of Detail

See repositories for explicit kinetic models:



Size of System

Qualitative Models

Quantitative Models

Level of Detail

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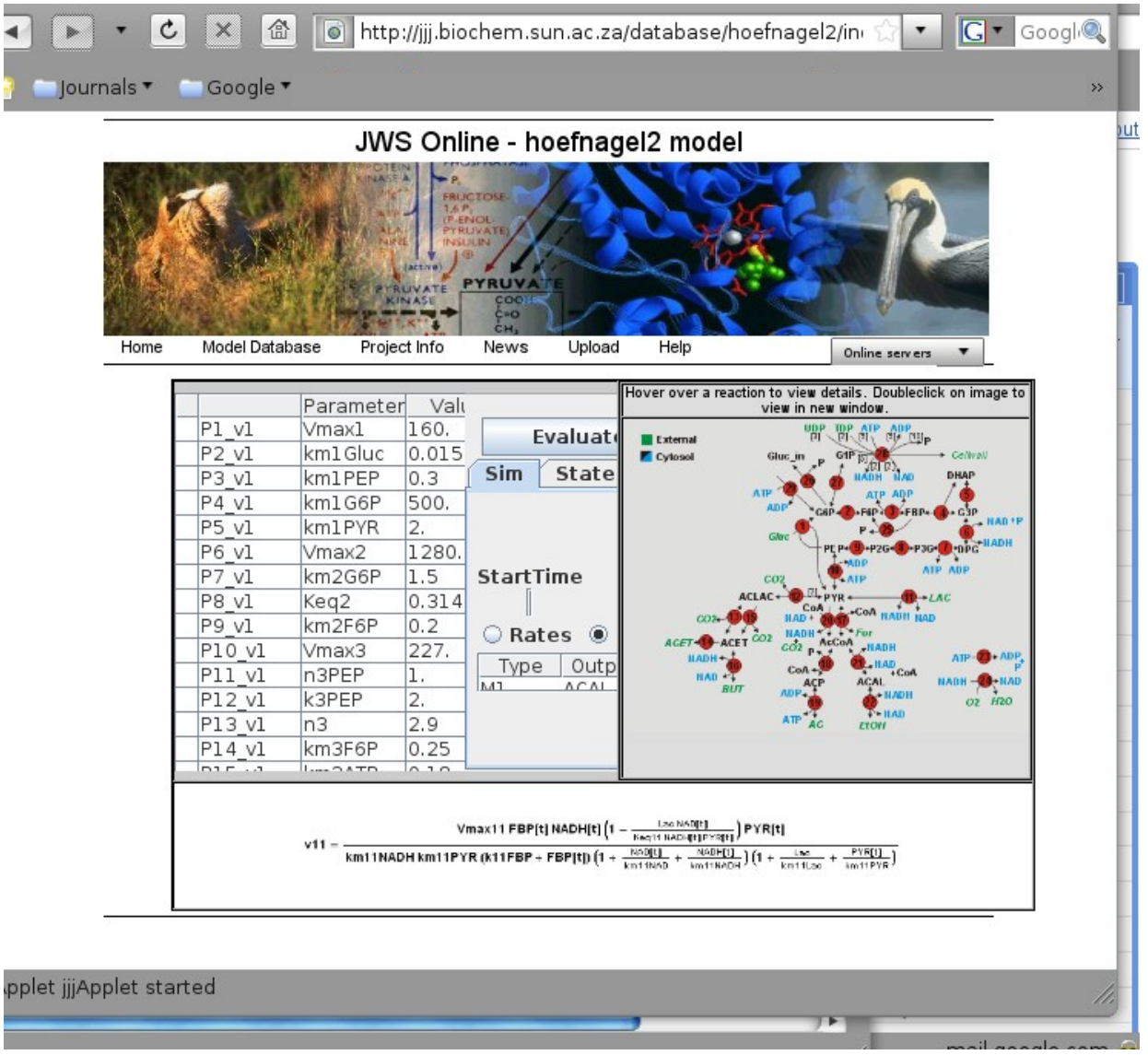

Size of System

Qualitative Models

Quantitative Models

Level of Detail

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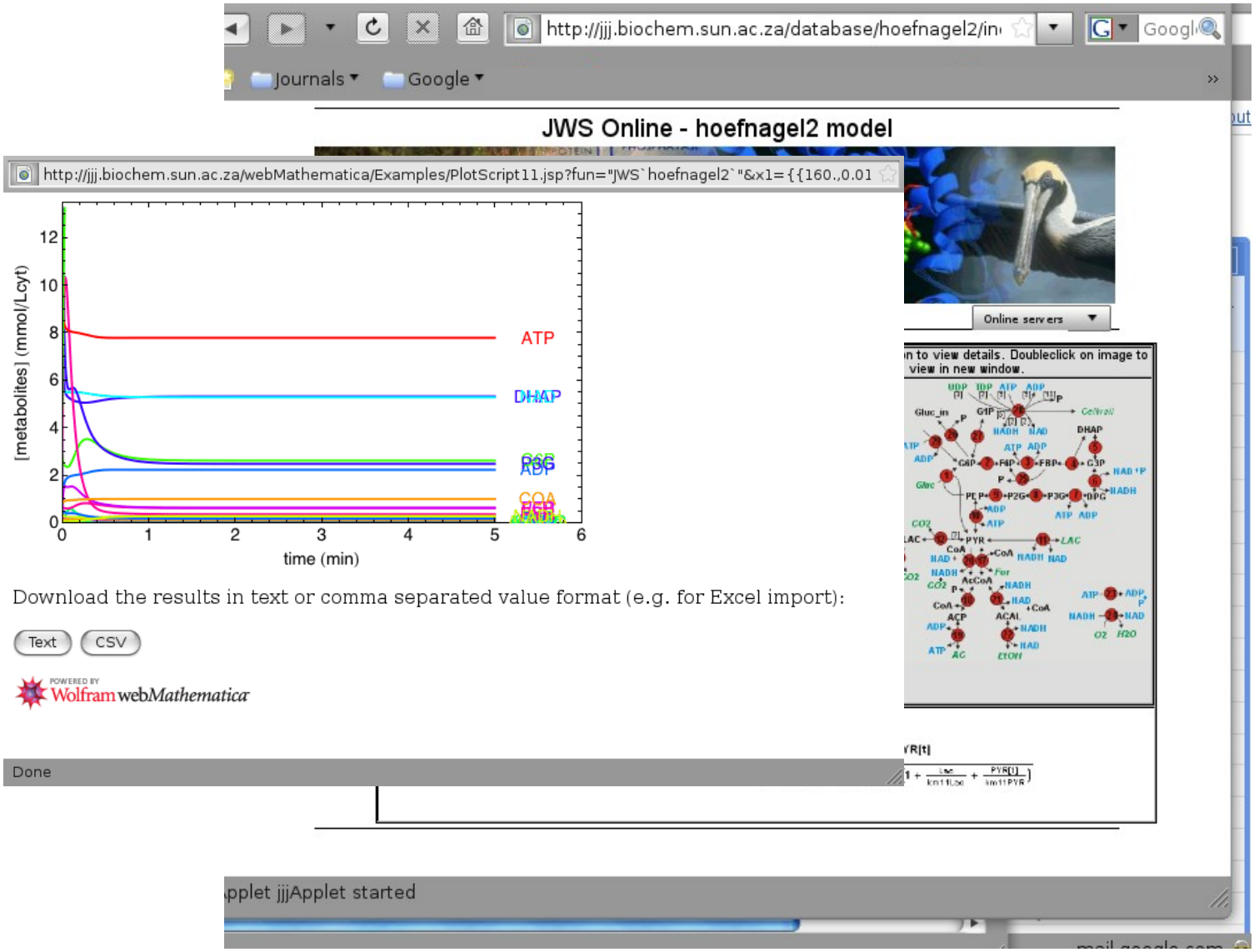

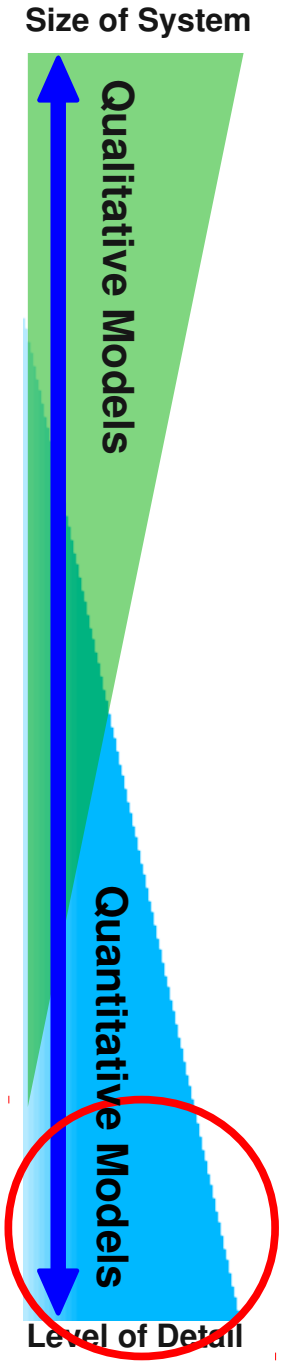

See repositories for explicit kinetic models:

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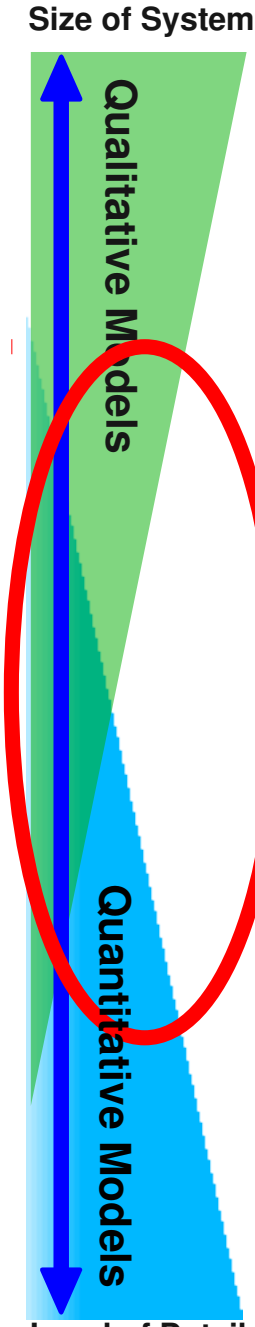
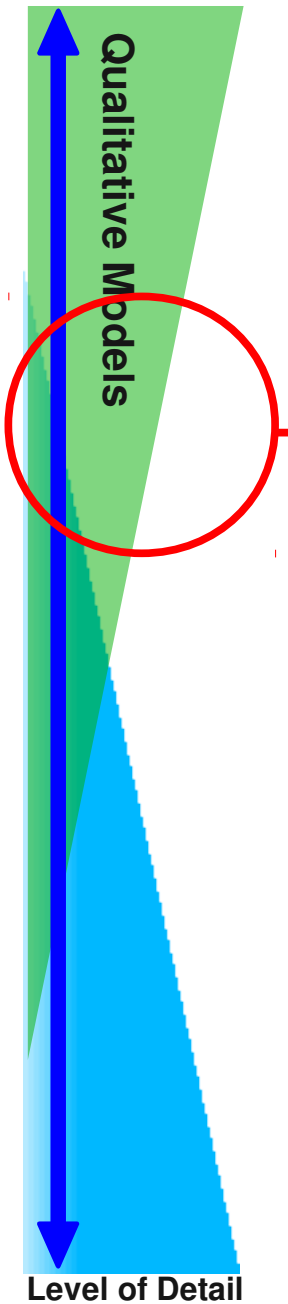
models of metabolic networks



Most research now on the intermediate level!



Stoichiometric Analysis (Flux Balance Analysis)

- Static description
- No kinetic parameters
- Quantitative predictions

Exploits constraints in flux distribution

$$\mathbf{N}\boldsymbol{\nu}(\mathbf{S}^0, \mathbf{k}) = \mathbf{0}$$

Size of System

Qualitative Models

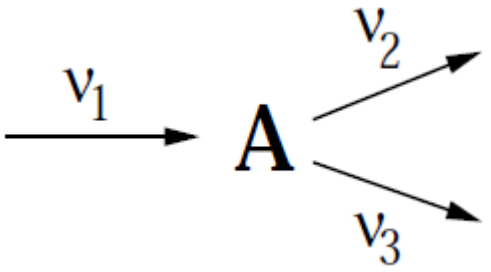
Level of Detail

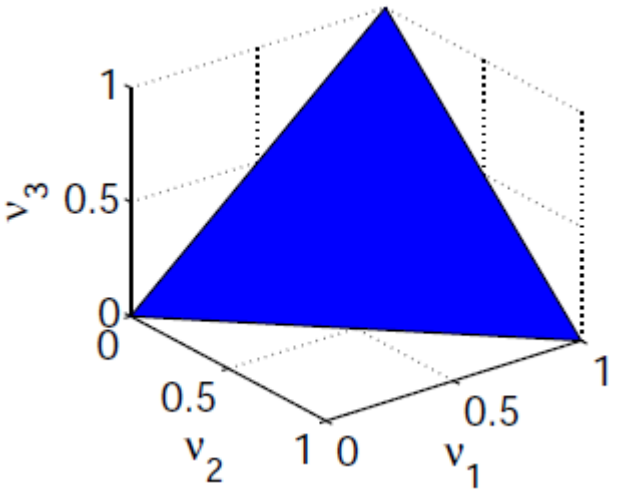
Stoichiometric Analysis (FBA)
- Static description
- No kinetic parameters
- Quantitative predictions

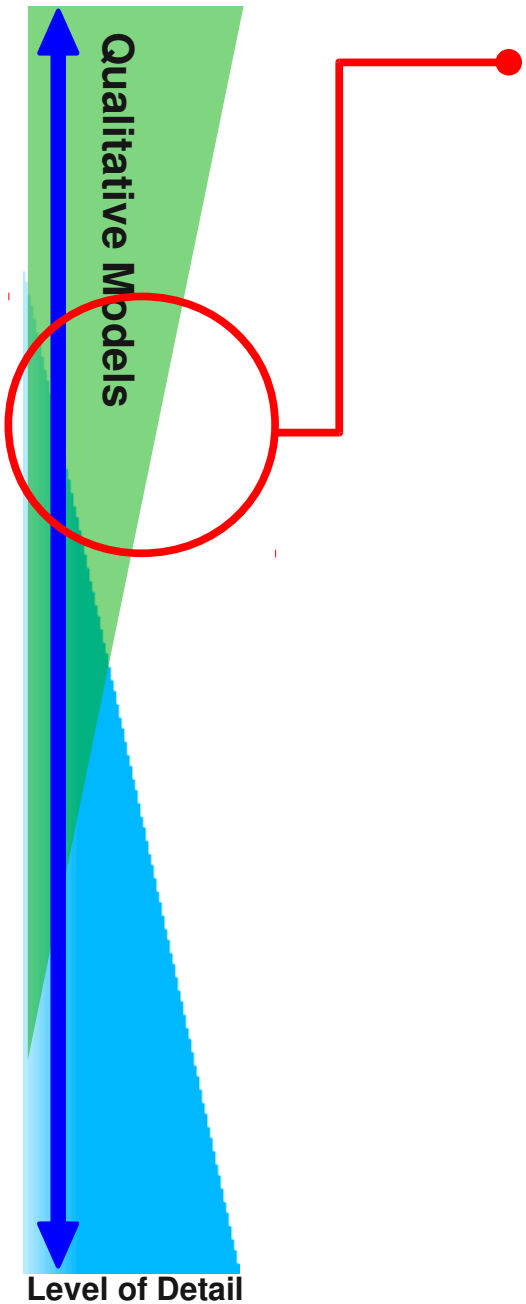
Exploits constraints in flux distribution

$$\mathbf{N}\boldsymbol{\nu}(\mathbf{S}^0, \mathbf{k}) = \mathbf{0}$$

A simple Example:





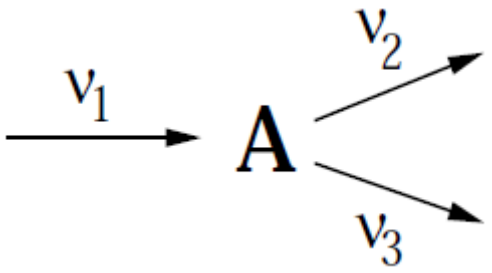


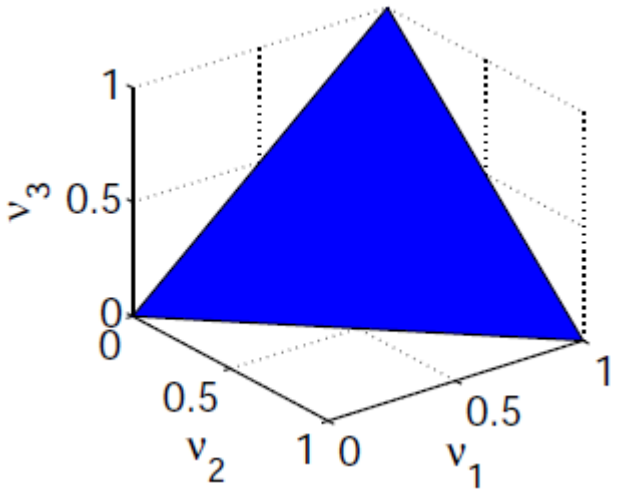
Stoichiometric Analysis (FBA)
- Static description
- No kinetic parameters
- Quantitative predictions

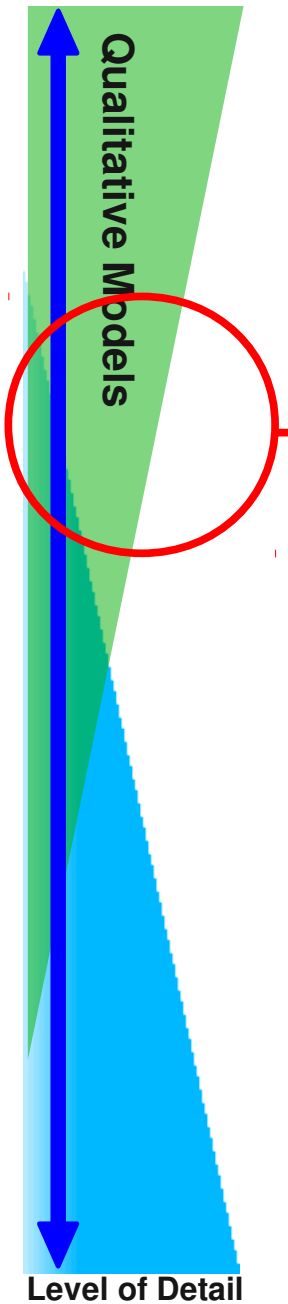
Exploits constraints in flux distribution

In general only a small fraction of fluxes need to be known:
independent = (number fluxes) – (number metabolites)

A simple Example:







Stoichiometric Analysis (FBA)

- Static description
- No kinetic parameters
- Quantitative predictions

Exploits constraints in flux distribution

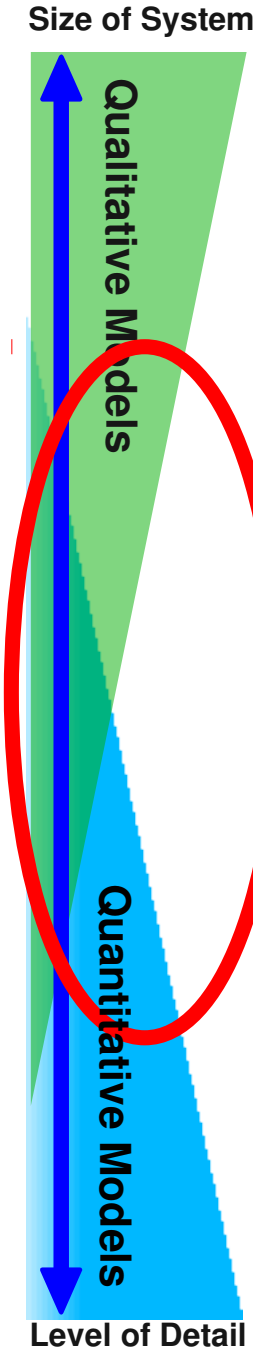
$$\mathbf{N}\boldsymbol{\nu}(\mathbf{S}^0, \mathbf{k}) = \mathbf{0}$$

The *S. cerevisiae* metabolic network

M = 810 vertices (metabolites)
R = 843 reaction rates

Optimize according to an objective function

Probably the best approach to date ...



Most research now on the intermediate level!

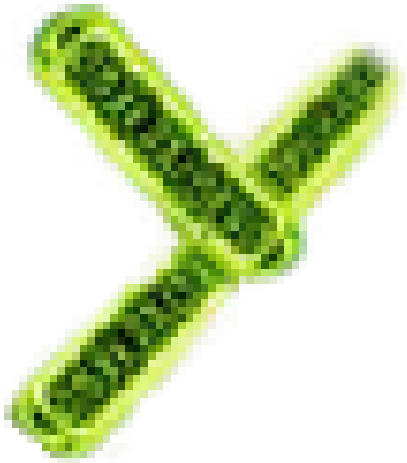
Keep the advantages of topological analysis

No explicit enzyme-kinetic rate equations
No knowledge of kinetic parameters
Computationally feasible for large networks

Extend with features of explicit kinetic models

Include quantitative description of dynamics
Include feedback regulation into the description

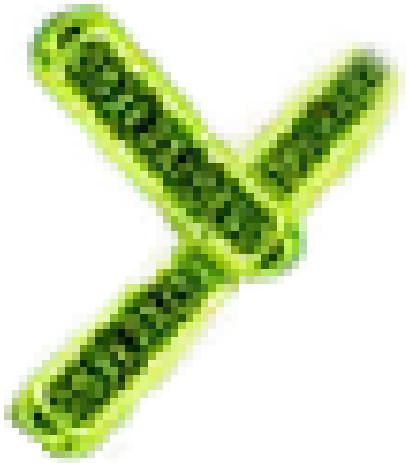
Research at HU-Berlin/ITB: Systems Biology of Cyanobacterial Biofuel Production



Research at HU-Berlin/ITB: Systems Biology of Cyanobacterial Biofuel Production

Fossil fuels (mainly petroleum, coal, natural gas) account for 80%-90% of the current world energy demand.

Fossil fuels account for 95% of transportation fuels.



Fossil fuels (mainly petroleum, coal, natural gas) account for 80%-90% of the current world energy demand.

Fossil fuels account for 95% of transportation fuels.

Alternatives are urgently needed!

Wishlist: Renewable, cheap, abundant, biodegradable, etc ...

Biofuel production from biomass

a) vegetable oil/biodiesel (soybean, palm, rapeseed)
b) bioalcohol from sugar cane and starch (corn, maize)

+ some others (charcoal, biogas, etc ...)

palm plantation (wiki)

maize (wiki)

Biofuel production from biomass

a) vegetable oil/biodiesel (soybean, palm, rapeseed)
b) bioalcohol from sugar cane or starch (corn, maize)

Some Issues:

- High energy input in production, low net yield
- Food vs. fuel issues: direct competition with food crops
- Requires food crops (starch, e.g. sugarcane, maize, etc ...)
- Requires large amounts of arable land
- Requires large amounts fresh water
- Little potential for increase in production

So what to do?
Alternatives are needed!

Biofuel production from cellulosic biomass

Better and cheaper biomass: non-edible parts of plants:
Woodchips (e.g. poplar), fuel crops, grass, corn stover ...

A DIFFICULT AND EXPENSIVE PROCESS (AS YET)

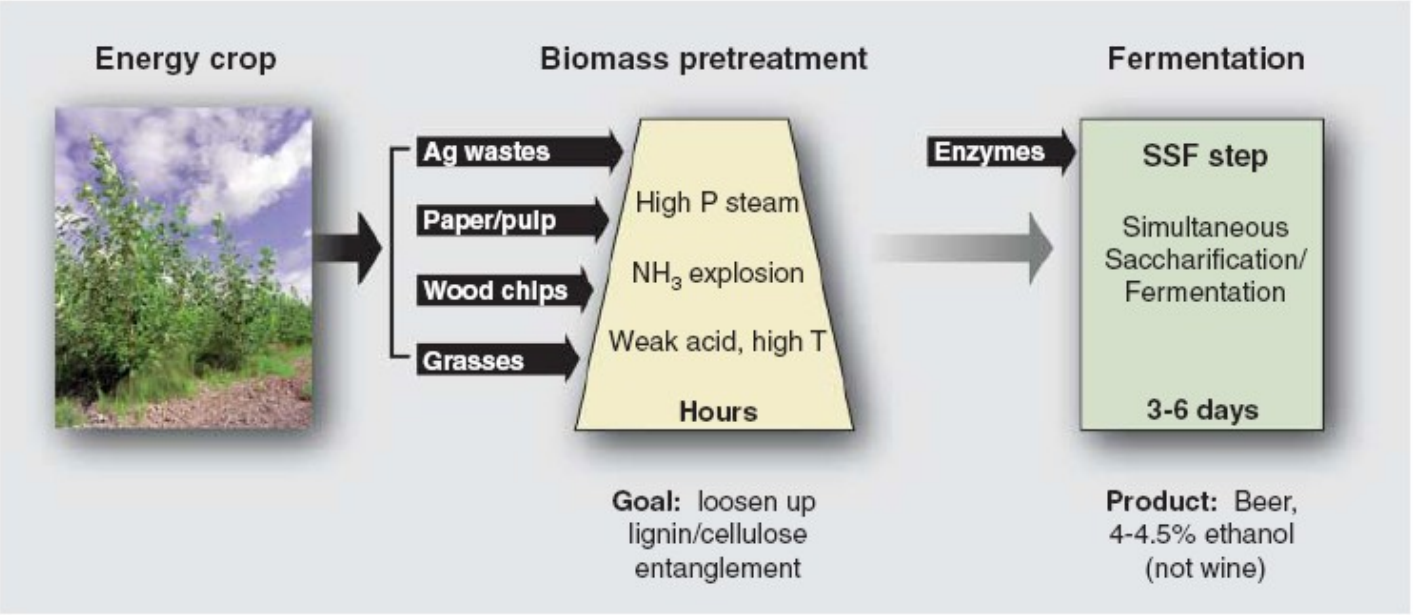


Fig. 1. Schematic of the overall conversion process of an energy crop to ethanol.

"biomass recalcitrance": Plant cell walls naturally resist decomposition from microbes and enzymes

Biofuel production from cellulosic biomass

Better and cheaper biomass: non-edible parts of plants:
Woodchips (e.g. poplar), fuel crops, grass, corn stover ...

But considerable potential within the next decade(s)!

PERSPECTIVES

Challenges in Engineering Microbes for Biofuels Production

Gregory Stephanopoulos

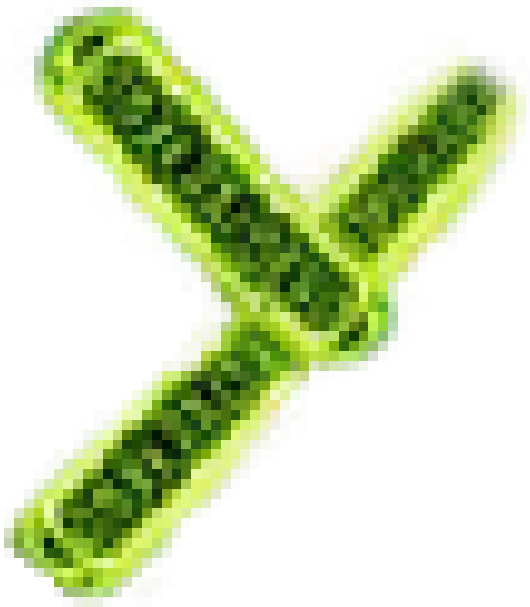
SCIENCE VOL 315 9 FEBRUARY 2007

Switchgrass (wiki):

Possible Improvements:

Manipulate cellusosic content in plants
Enzymatic pretreatment (funghi)
Better conversion of 5-carbon sugars
Higher ethanol resistance

Alternative: Cyanobacteria as a source of biofuels



Alternative: Cyanobacteria as a source of biofuels

Photosynthetic cyanobacteria: An alternative to fossil fuels

They live from nothing but sun and air

High yield and fast growth rates (up to 6h division time)

Straightforwardly cultivable (algae farms)

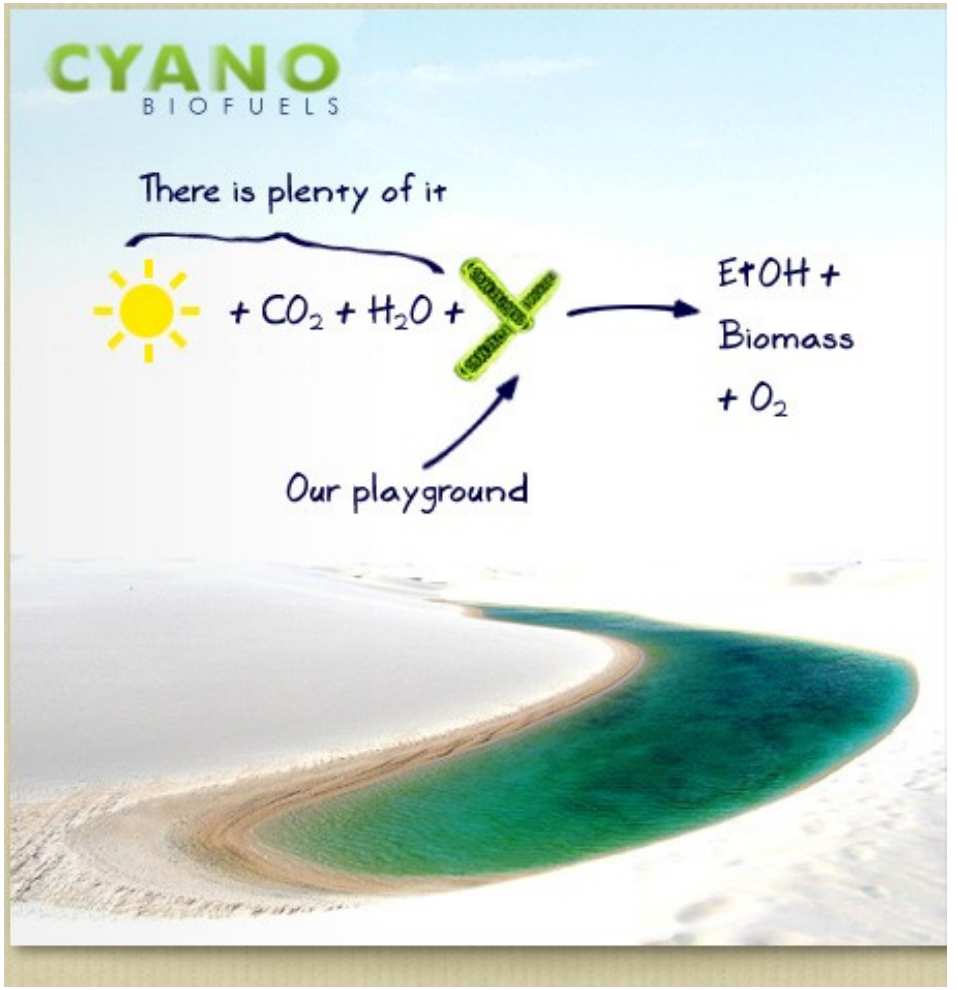
Little arable land required

Can be cultivated in seawater

An open pond Spirulina farm:

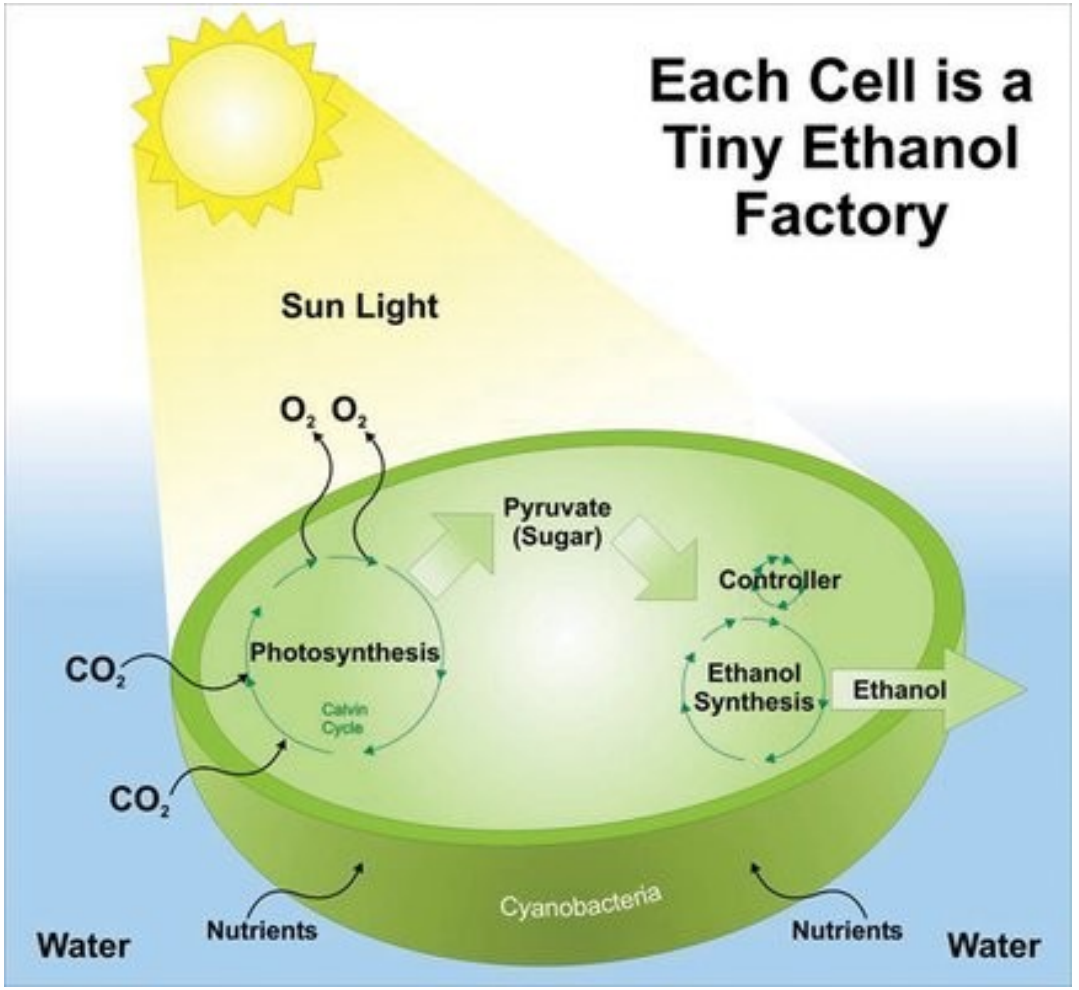
FORSYS-Partner: Systems Biology of Cyanobacterial Biofuel Production

Cyano Biofuels GmbH
Dan Kramer
Karl Ziegler
Biochemistry (HU-Berlin)
Wolfgang Lockau
Yvonne Zilliges
Genetics (HU-Berlin)
Thomas Boerner
Jan-Christoph Kehr
Modelling (ITB, HU-Berlin)
Ralf Steuer
Henning Knoop
Microbiology (U. Giessen)
Annegret Wilde
and FRISYS (Freiburg)
Wolfgang Hess



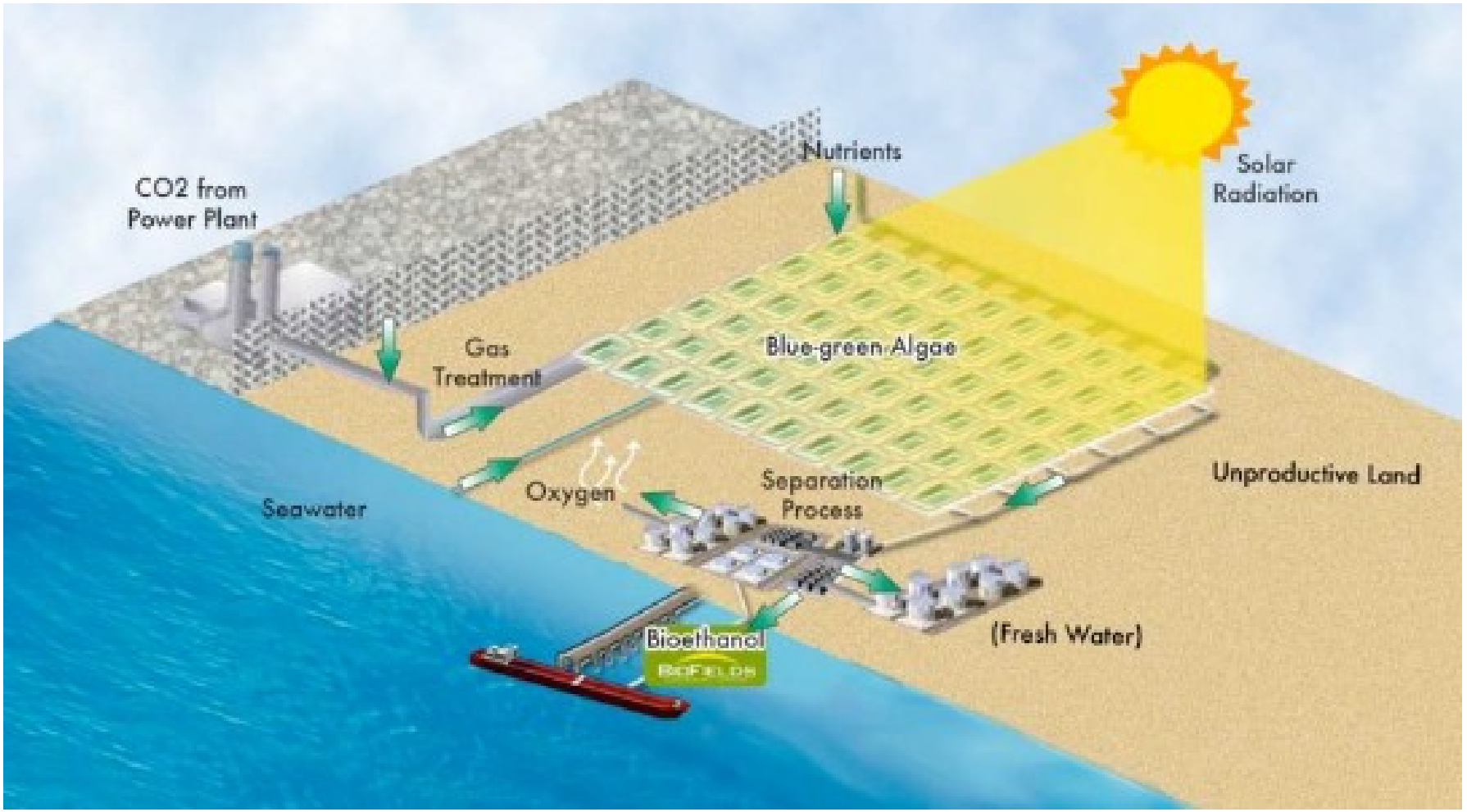
Plot courtesy of CYANO BIOFUELS

FORSYS-Partner: Systems Biology of Cyanobacterial Biofuel Production



Plot courtesy of Algenol

FORSYS-Partner: Systems Biology of Cyanobacterial Biofuel Production

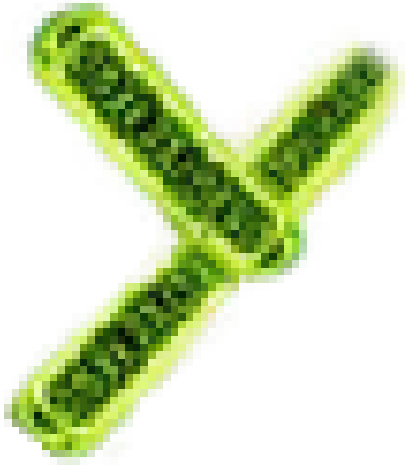


Plot courtesy of Algenol

FORSYS-Partner: Systems Biology of Cyanobacterial Biofuel Production

Plot courtesy of http://www.algenolbiofuels.com/

FORSYS-Partner: Systems Biology of Cyanobacterial Biofuel Production



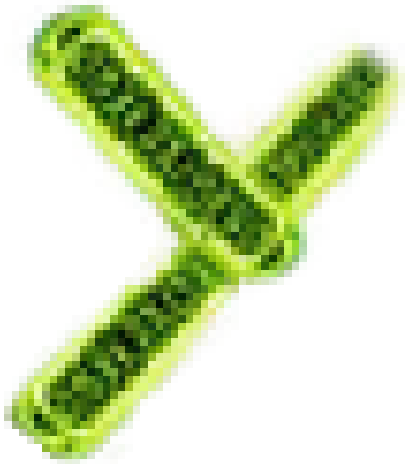
The modelling perspective

Progress in strain improvement will depend on the development of theoretical methods that facilitate the elucidation of mechanisms and the identification of genetic targets for modification (*).

(*) from: Exploiting biological complexity for strain improvement through systems biology, G. Stephanopoulos, H. Alper & J. Moxley, Nature Biotechnology 22, 1261 – 1267, 2004

FORSYS-Partner: Systems Biology of Cyanobacterial Biofuel Production

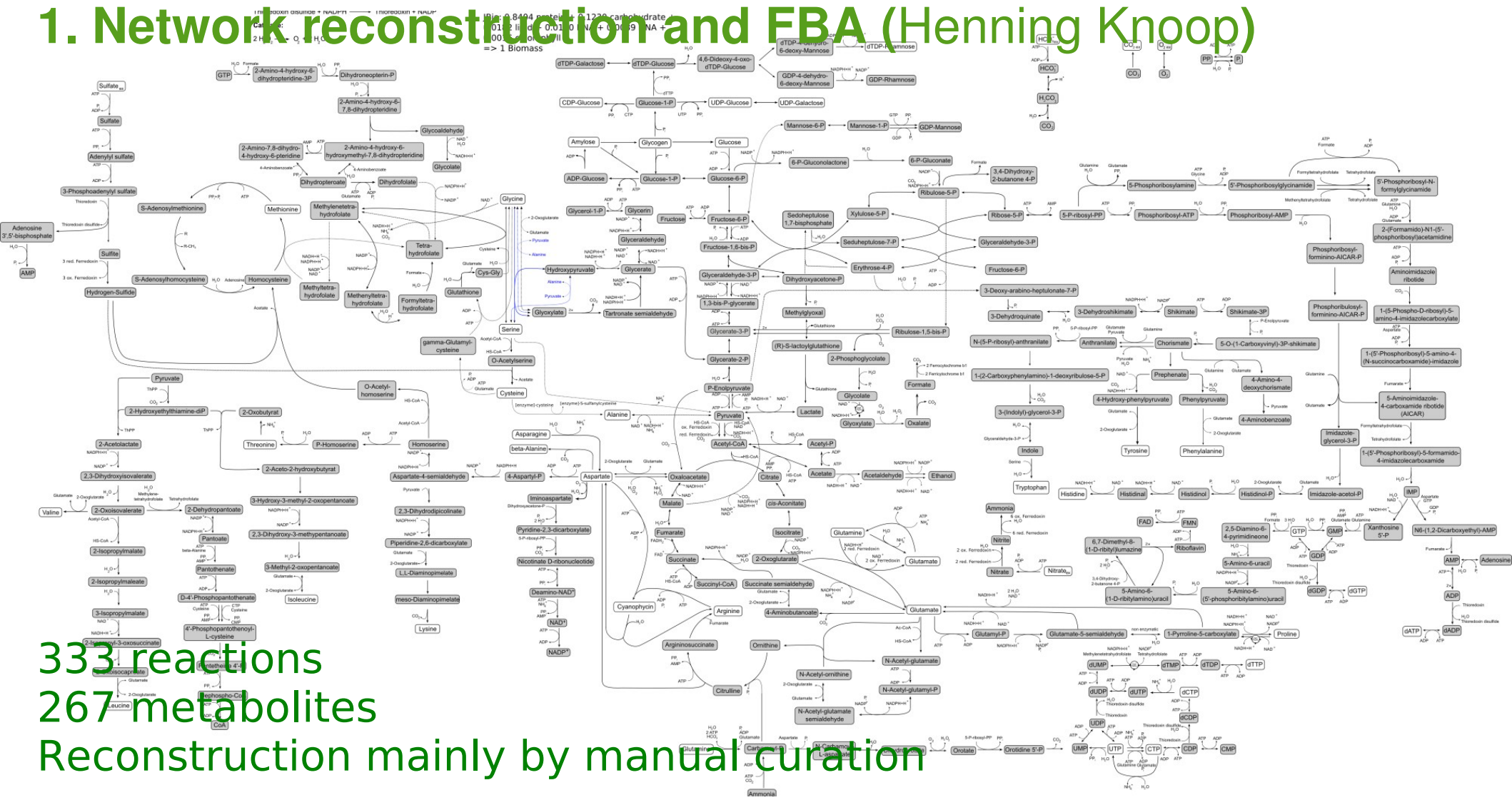
What can be, if anything, achieved by mathematical modelling?



1. Network reconstruction and FBA
2. Towards large-scale kinetic models
3. Integrative models: The regulation of metabolism
4. Predicting biotechnological modifications

Understanding cellular metabolism

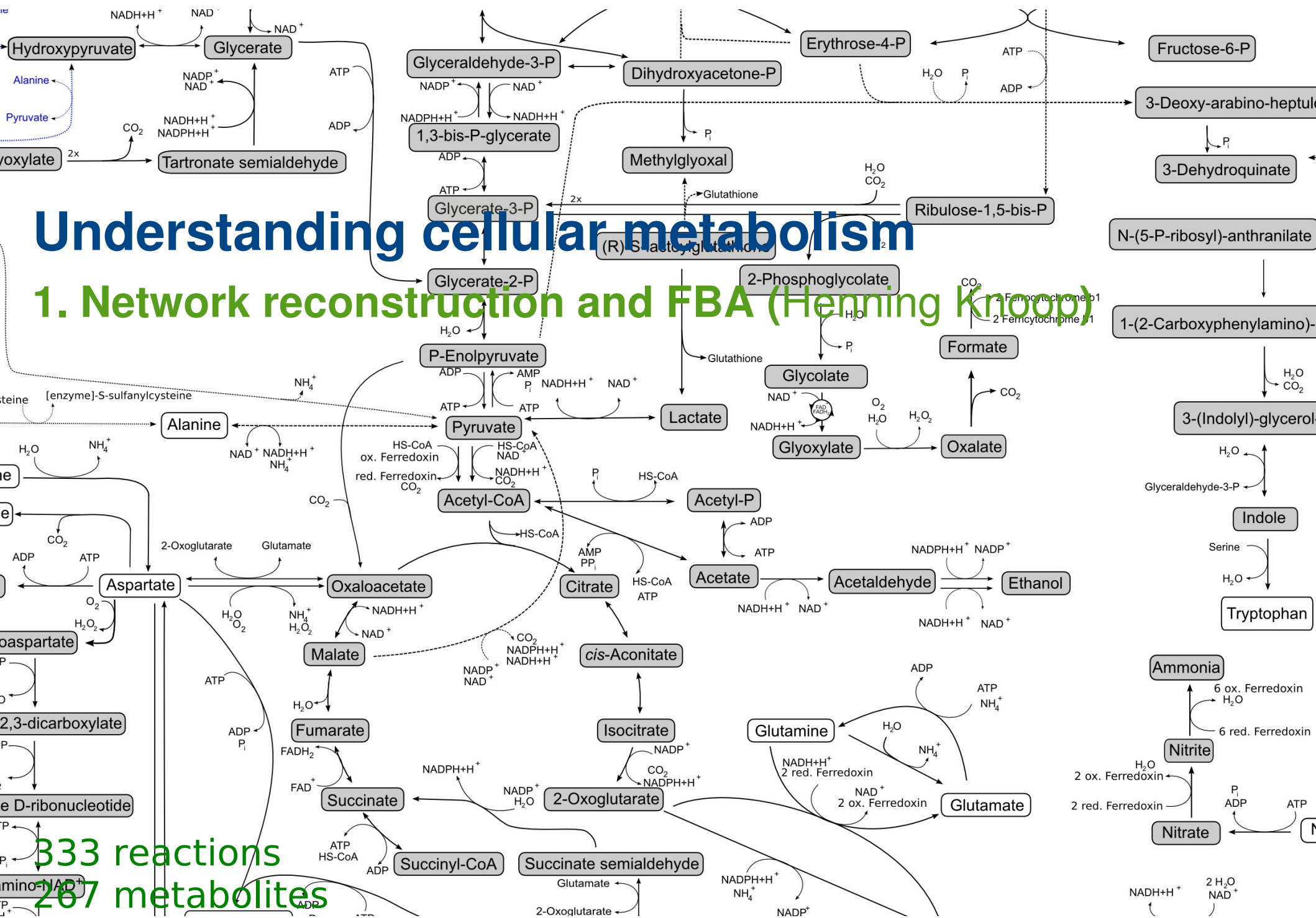
1. Network reconstruction and FBA (Henning Knoop)



333 reactions

267 metabolites

Reconstruction mainly by manual curation



Understanding cellular metabolism

1. Network reconstruction and FBA (Henning Knoop)

333 reactions

267 metabolites

Reconstruction mainly by manual curation

End of Part II

Hands on examples

Hands on examples

1. Numerical Integration in matlab

```
ODE23TB Solve stiff differential equations, low order method.
ODEXTEND  Extend solution of initial value problem for differential equations.
ODE23  Solve non-stiff differential equations, low order method.
ODE45  Solve non-stiff differential equations, medium order method.
ODE113  Solve non-stiff differential equations, variable order method.
ODE15I  Solve fully implicit differential equations, variable order method.
ODE15S Solve stiff differential equations and DAEs, variable order method.
ODE23S  Solve stiff differential equations, low order method.
ODE23T  Solve moderately stiff ODEs and DAEs, trapezoidal rule.
```

Hands on examples

1. Numerical Integration: Runge-Kutta algorithm

```
>> help ode45
 ODE45  Solve non-stiff differential equations, medium order method.
    [T,Y] = ODE45(ODEFUN,TSPAN,Y0) with TSPAN = [T0 TFINAL] integrates the
    system of differential equations y' = f(t,y) from time T0 to TFINAL with
    initial conditions Y0. Function ODEFUN(T,Y) must return a column vector
    corresponding to f(t,y). Each row in the solution array Y corresponds to
    a time returned in the column vector T. To obtain solutions at specific
    times T0,T1,...,TFINAL (all increasing or all decreasing), use
    TSPAN = [T0 T1 ... TFINAL].
```

Hands on examples

1. Numerical Integration: a simple example

```
function [dxdt]=VerySimple(t,x);
% file: VerySimple.m
% Stellt die Funktion dxdt = -kx zur Verfuegung
k=1;
dxdt = -k*x;
```

then:

```
[t,x]=ode45('VerySimple',[0 1],1);
plot(t,x,'o');
```

Hands on examples

1. Numerical Integration: a simple example

```
function [dxdt]=VerySimple(t,x);
% file: VerySimple.m
% Stellt die Funktion dxdt = -kx zur Verfuegung
k=1;
dxdt = -k*x;
```

then:

```
[t,x]=ode45('VerySimple',[0 1],1);
plot(t,x,'o');
```

Hands on examples

1. Numerical Integration: the Lorenz System

$$\frac{dx}{dt} = \sigma(y(t) - x(t))$$
$$\frac{dy}{dt} = -x(t)\,z(t) + rx(t) - y(t)$$
$$\frac{dz}{dt} = x(t)\,y(t) - bz(t)$$

Hands on examples

1. Numerical Integration: the Lorenz System

```
function [fx]=LorenzSys(t,x);
% file: LorenzSys.m
% Lorenz System
r=45.92;
b=4.0;
sig=16.0;

dxdt = sig*(x(2)-x(1));
dydt = -x(1)*x(3)+r*x(1)-x(2);
dzdt =  x(1)*x(2)-b*x(3);

% Rueckgabe: Spaltenvektor fx
fx = [dxdt ; dydt ; dzdt ];
```

Hands on examples

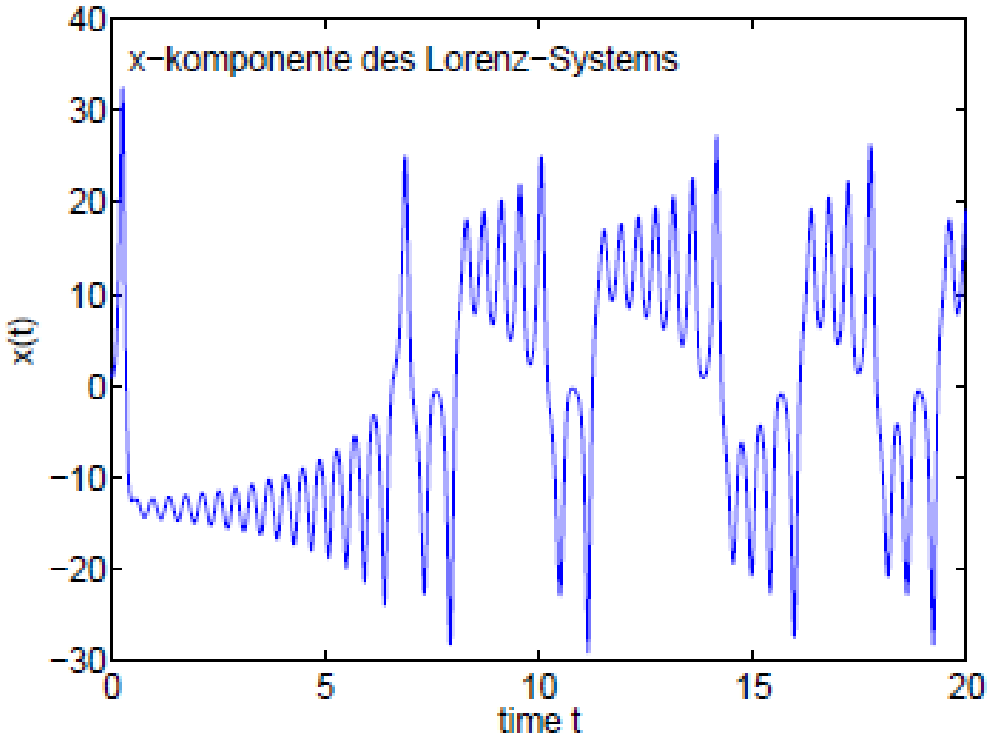
1. Numerical Integration: the Lorenz System

```
function [fx]=LorenzSys(t,x);
% file: LorenzSys.m
% Lore
r=45.9
b=4.0;
sig=16

dxdt = sig*(x(2)-x(1));
dydt = -x(1)*x(3)+r*x(1)-x(2);
dzdt =  x(1)*x(2)-b*x(3);

% Rueckgabe: Spaltenvektor fx
fx = [dxdt ; dydt ; dzdt ];
```

```
x0=[1 1 1];
[t,x]=ode45('LorenzSys',[0 20],x0);
```

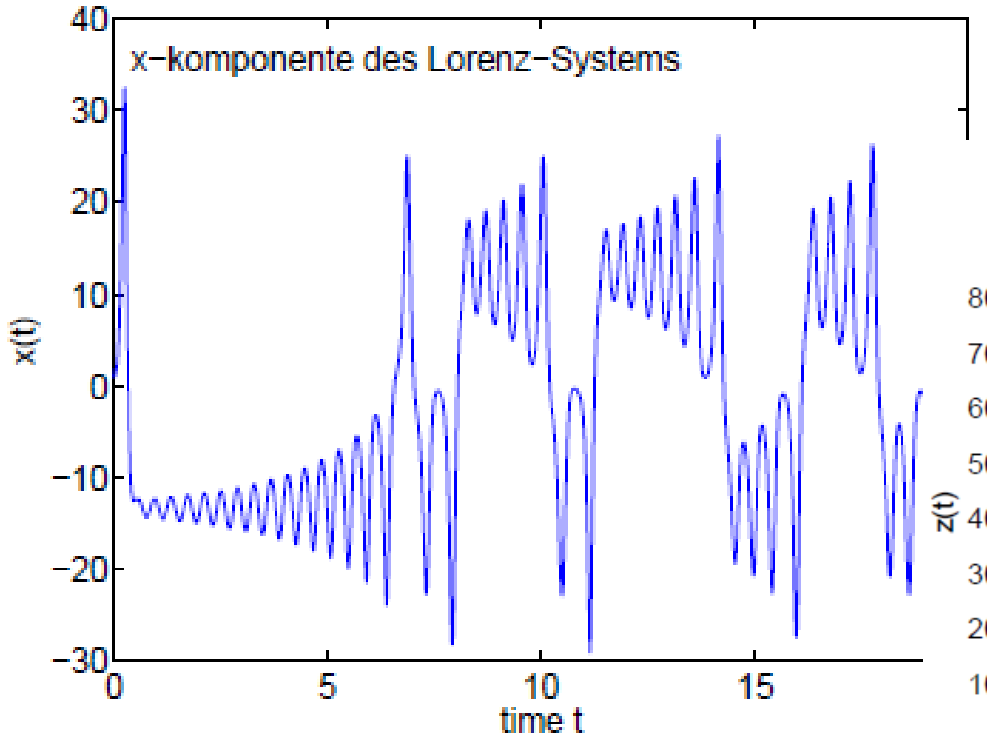
Hands on examples

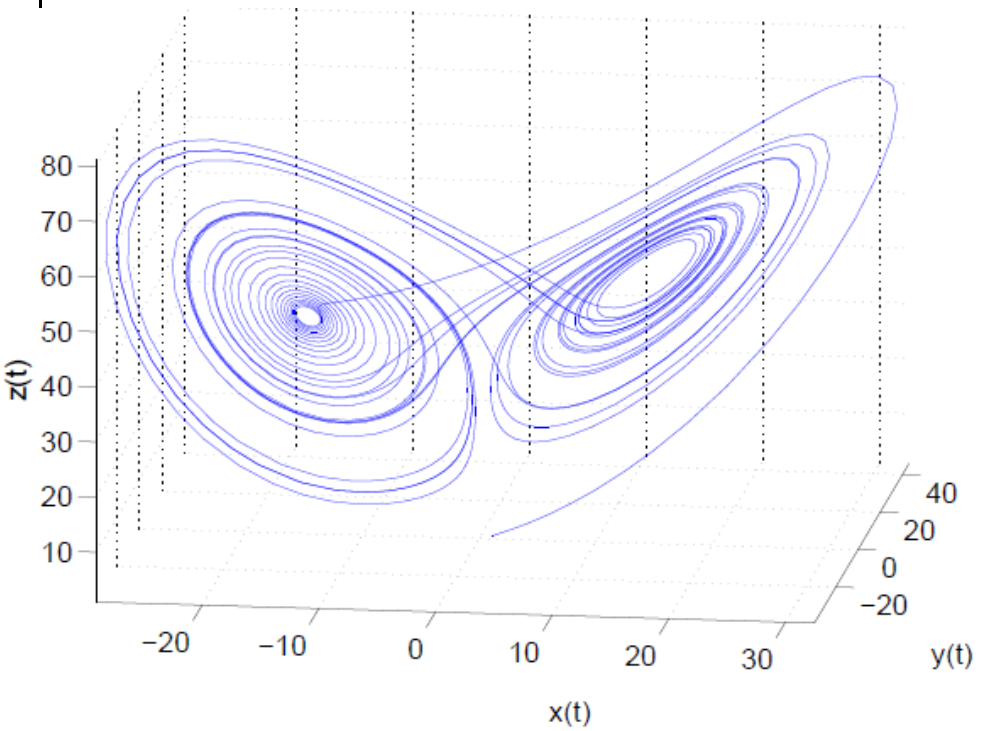
1. Numerical Integration: the Lorenz System



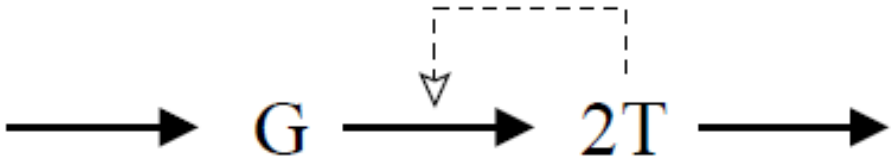
Hands on examples

1. Numerical Integration: the Lorenz System





Hands on examples: a metabolic pathway



$$\dot{G} = V_{in} - k_1 G T$$

$$\dot{T} = 2k_1 G T - k_p \frac{T}{K_M + T}$$

[8]M. Bier, B. Bakker, H. Westerhoff, *How Yeast Cells Synchronize their Glycolytic Oscillations: A Pertubation Analytic Treatment*, Biophysical Journal, Vol. 78, (2000), 1087-1093.

Hands on examples: a metabolic pathway

```
function [dSdt]=BierModell(t,S);
% file: BierModell.m
% function [dSdt]=BierModell(t,S);
%
% definiert das Glycolyse-Modell von Bier et al. (2000)
%

% Die Parameter
Vin=0.36;
k1=0.02;
kp=6.0;
Km=13.0;

% Die Variablen
G = S(1);
T = S(2);

% Die Gleichungen
dSdt(1) = Vin - k1*G*T;
dSdt(2) = 2*k1*G*T - kp*T/(Km+T);
dSdt=dSdt'; % funktion muss spalten-vektor zurueckgeben
```

Hands on examples: a metabolic pathway

```
function [dSdt]=BierModell(t,S);
% file: BierModell
% function [dSdt
%
% definiert das
%

% Die Parameter
Vin=0.36;
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dSdt=dSdt'; % funktion muss spalten-vektor zurueckgeben
```
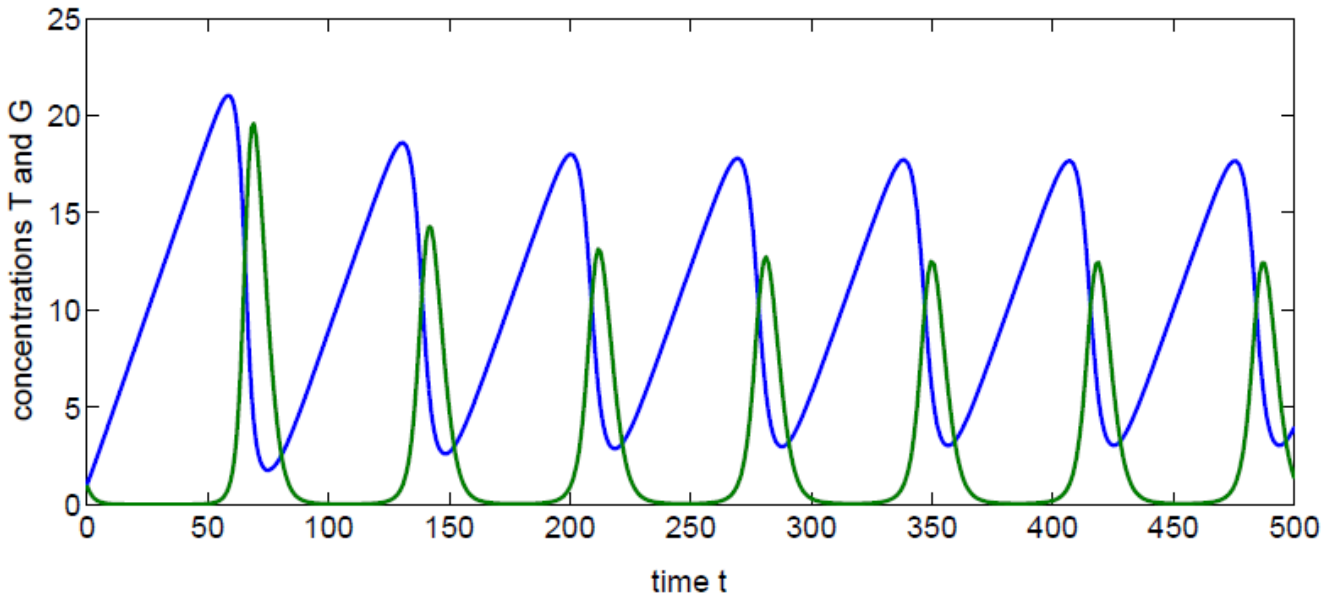
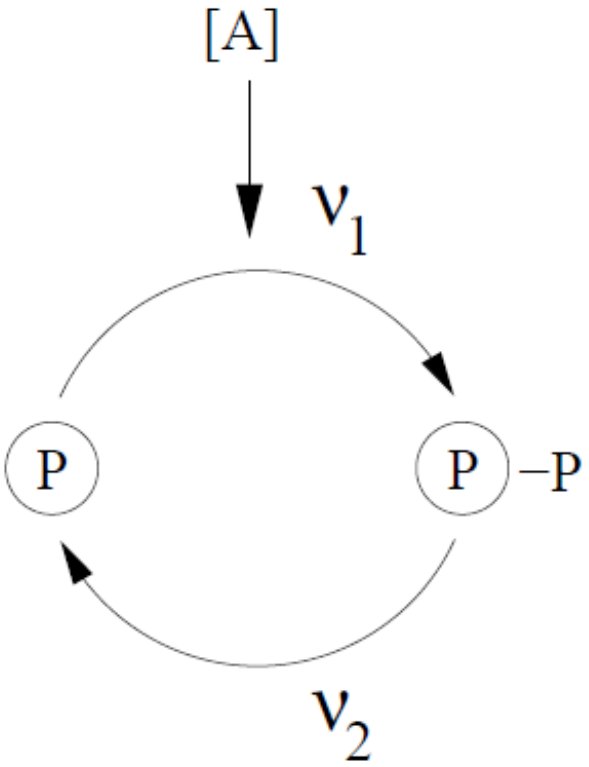
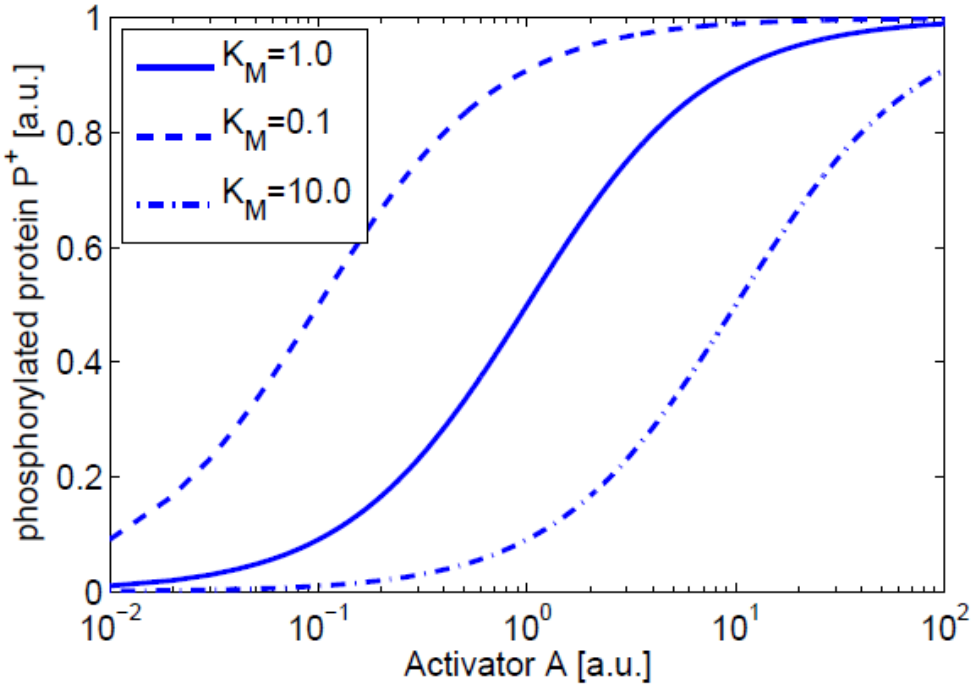

```
S0 = [ 1 1];  % Anfangsbedingungen festlegen
[t,S]=ode45('BierModell',[0 500],S0);
plot(t,S);
```

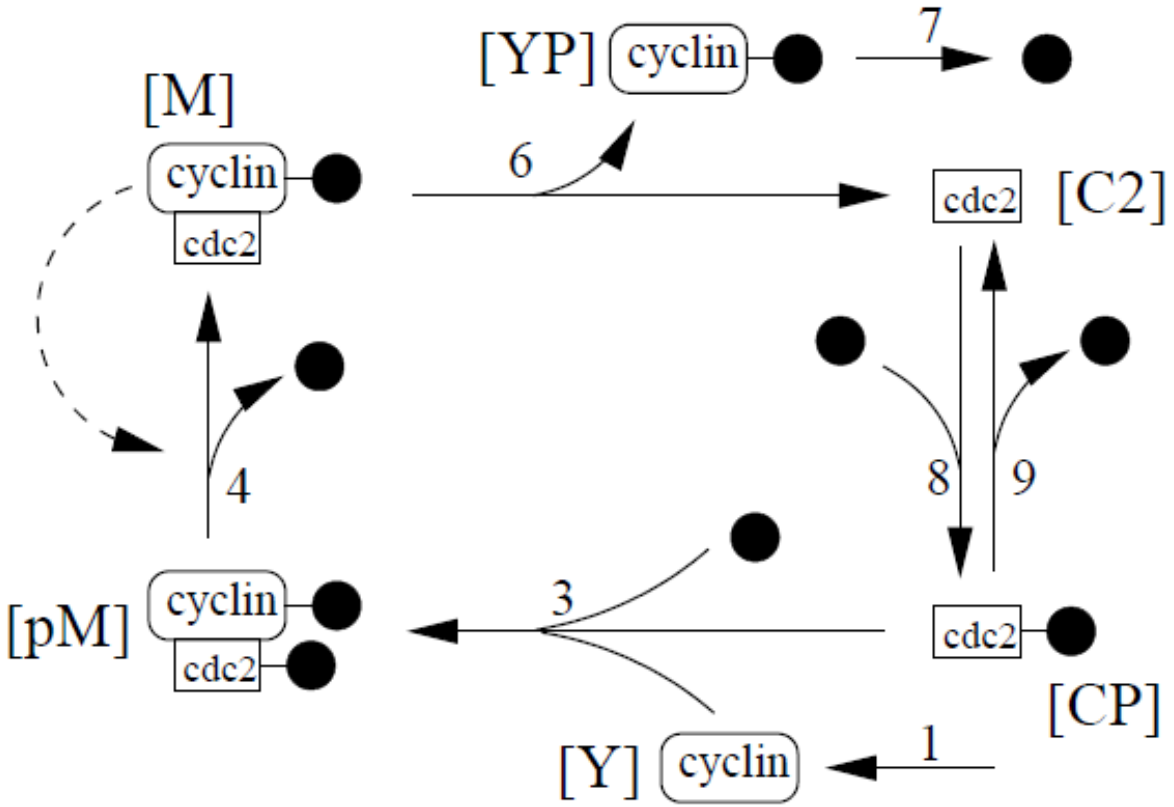


Hands on examples: signal transduction



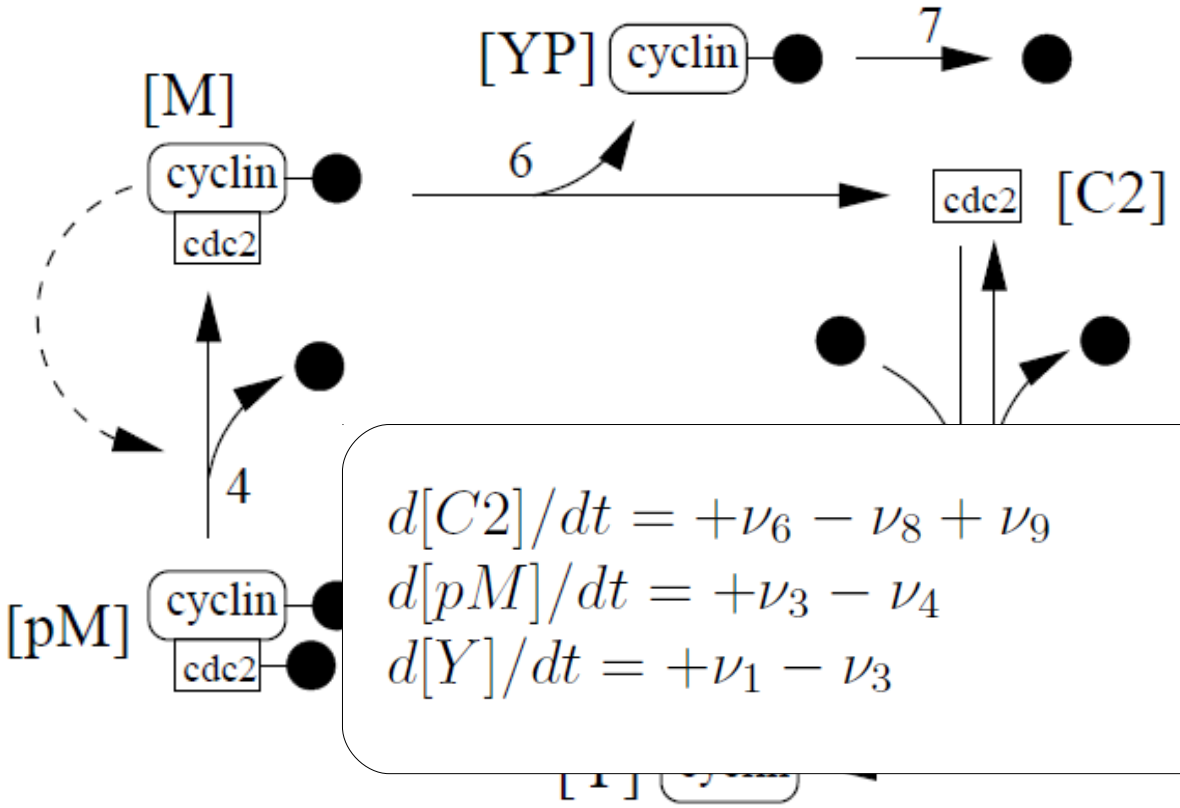


Hands on examples: a model of the cell cycle



J. J. Tyson, *PNAS* **88**, 7328-7332 (1991)

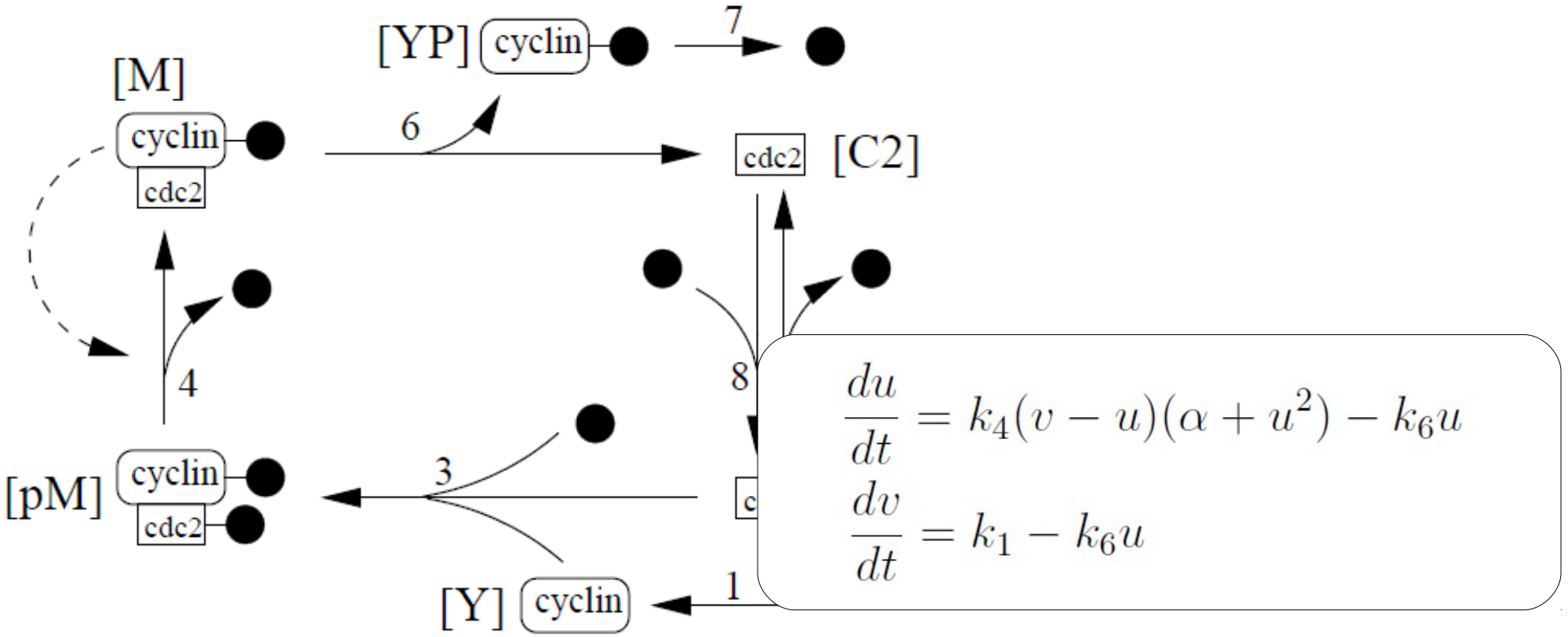
Hands on examples: a model of the cell cycle



$$d[C2]/dt = +\nu_6 - \nu_8 + \nu_9 \qquad d[CP]/dt = +\nu_8 - \nu_9 - \nu_3$$
$$d[pM]/dt = +\nu_3 - \nu_4 \qquad d[M]/dt = +\nu_4 - \nu_6$$
$$d[Y]/dt = +\nu_1 - \nu_3 \qquad d[YP]/dt = +\nu_6 - \nu_7$$

J. J. Tyson, *PNAS* **88**, 7328-7332 (1991)

Hands on examples: a model of the cell cycle



$$\frac{du}{dt} = k_4(v-u)(\alpha+u^2) - k_6 u$$

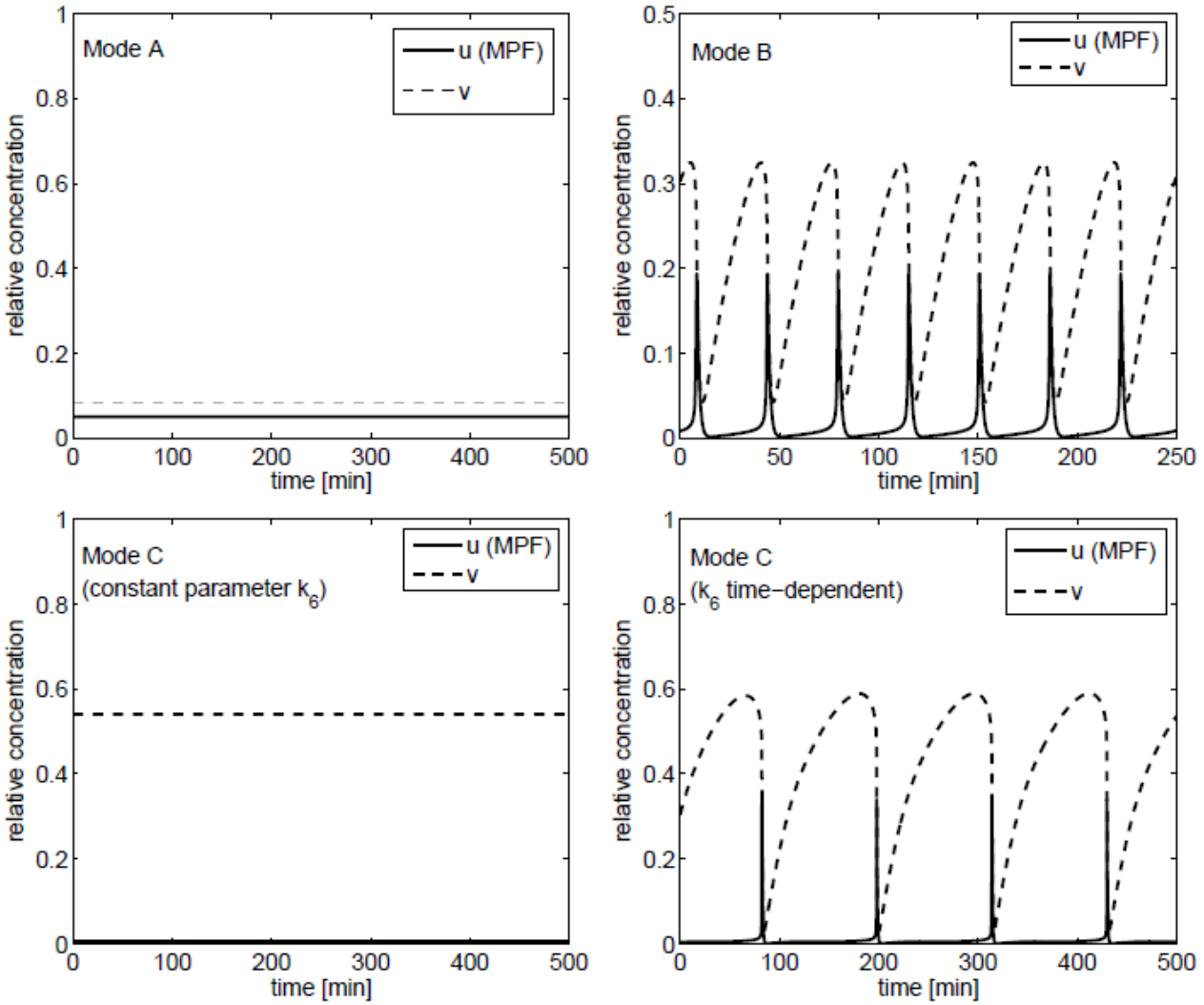
$$\frac{dv}{dt} = k_1 - k_6 u$$

J. J. Tyson, *PNAS* **88**, 7328-7332 (1991)

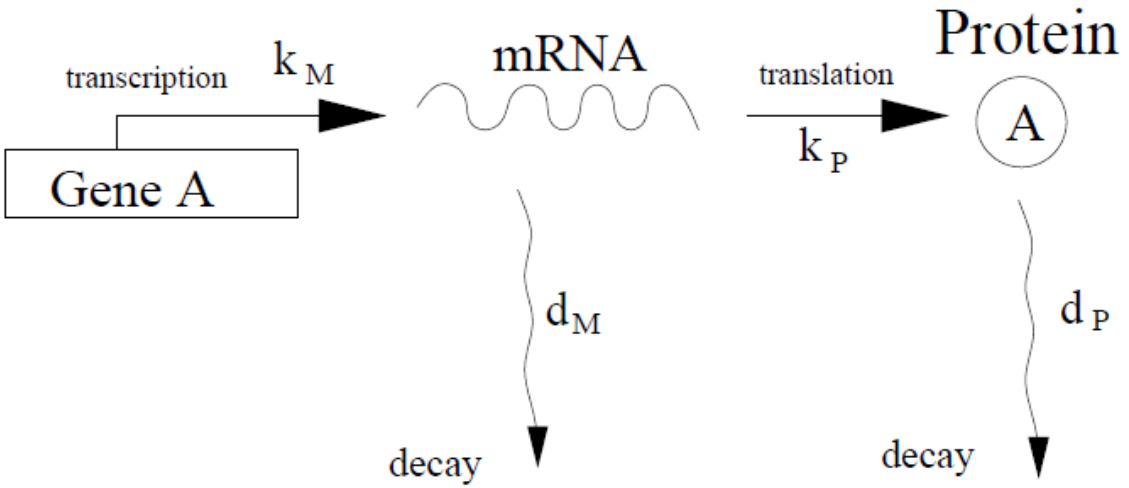
Hands on examples: a model of the cell cycle

```
function dSdt = Tyson(t,S)
% file: Tyson.m
% Regulationschema fuer den Zell Zyklus
% nach J. J. Tyson, PNAS 88, 1991

% die Variablen
  u = S(1);  % [M]/[CT] 'MPF-Aktiviatet'
  v = S(2);

% die Parameter
k1=0.015;
k4=180;
k4s=0.018;
k6=1.0;

a = k4s/k4;

dSdt(1,1) = k4*(v-u)*(a+u^2) - k6*u;
dSdt(2,1) = k1 - k6*u;
```

Hands on examples: a model of the cell cycle



Gene expression as a stochastic process



$(M, A) \rightarrow (M + 1, A)$ Transcription of a mRNA

$(M, A) \rightarrow (M - 1, A)$ Decay of a mRNA

$(M, A) \rightarrow (M, A + 1)$ Translation of a protein

$(M, A) \rightarrow (M, A - 1)$ Decay of a protein

Gene expression as a stochastic process

$$\frac{d[M]}{dt} = k_M - \delta_M[M] \qquad \frac{d[A]}{dt} = k_A[M] - \delta_A[A]$$

$$[M^0] = \frac{k_M}{\delta_M} \qquad [A^0] = \frac{k_A}{\delta_A}\frac{k_M}{\delta_M} = \frac{k_M}{\delta_A}\, b$$

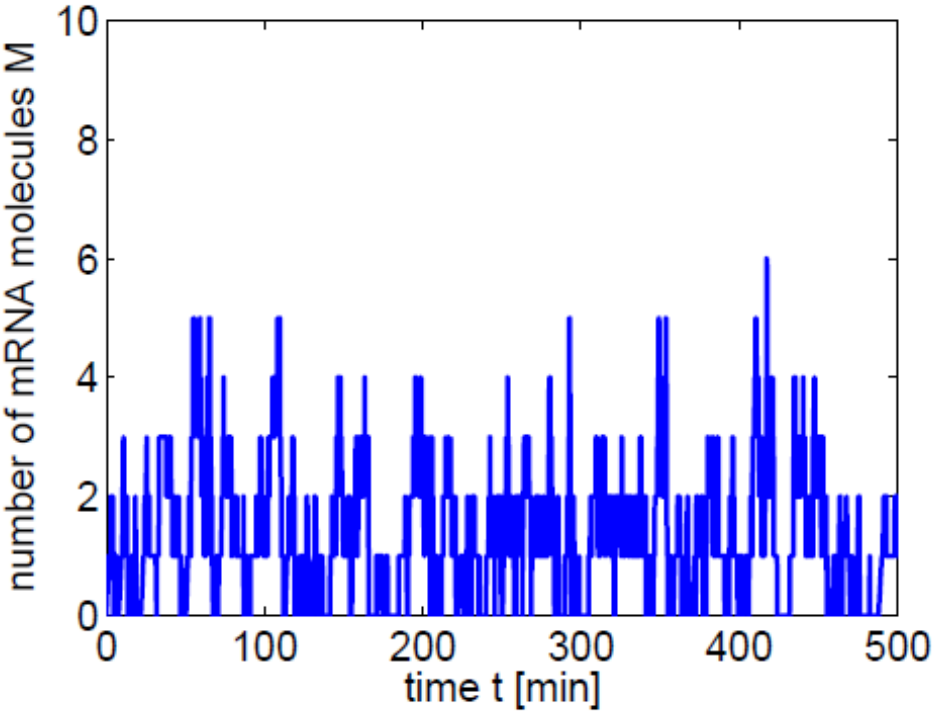
Gene expression as a stochastic process

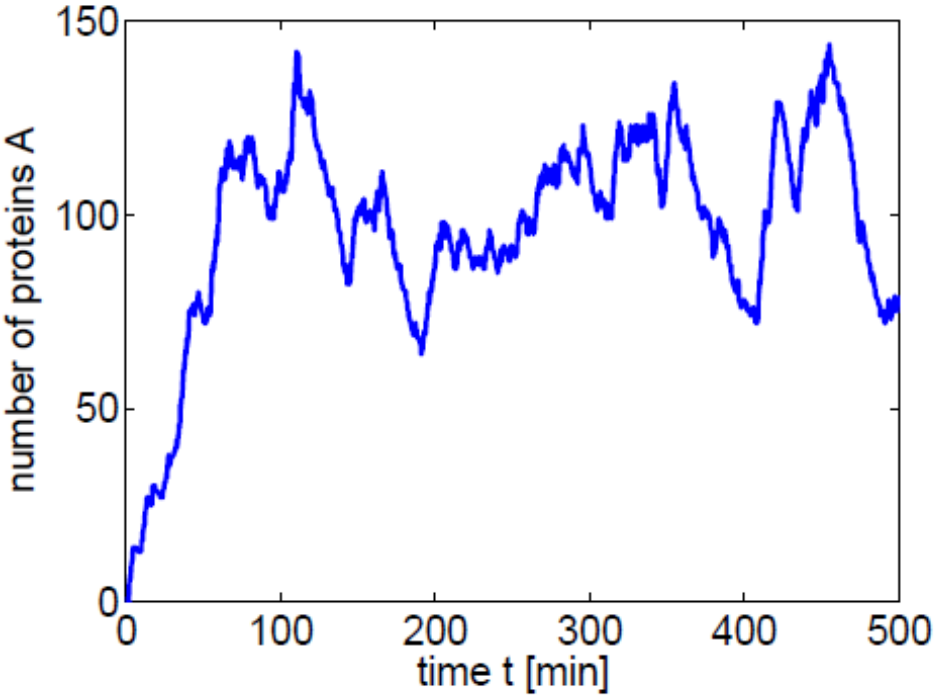
```
function S = StochExpression(zeit,OMEGA)
% file: StochExpression.m
% Stochastische Simulation der Gen Expression
%
if (nargin<2) , OMEGA=1; if (nargin<1), zeit=100; end;end;

% Parameter
b = 5.0;
A0 = 100;
dm = 0.35;
da = 0.2;

ka = b*dm;
km = A0*da/b;

% Anfangsbedingungen
M=0;
A=0;

t=0;
ix=1;
```

Gene expression as a stochastic process

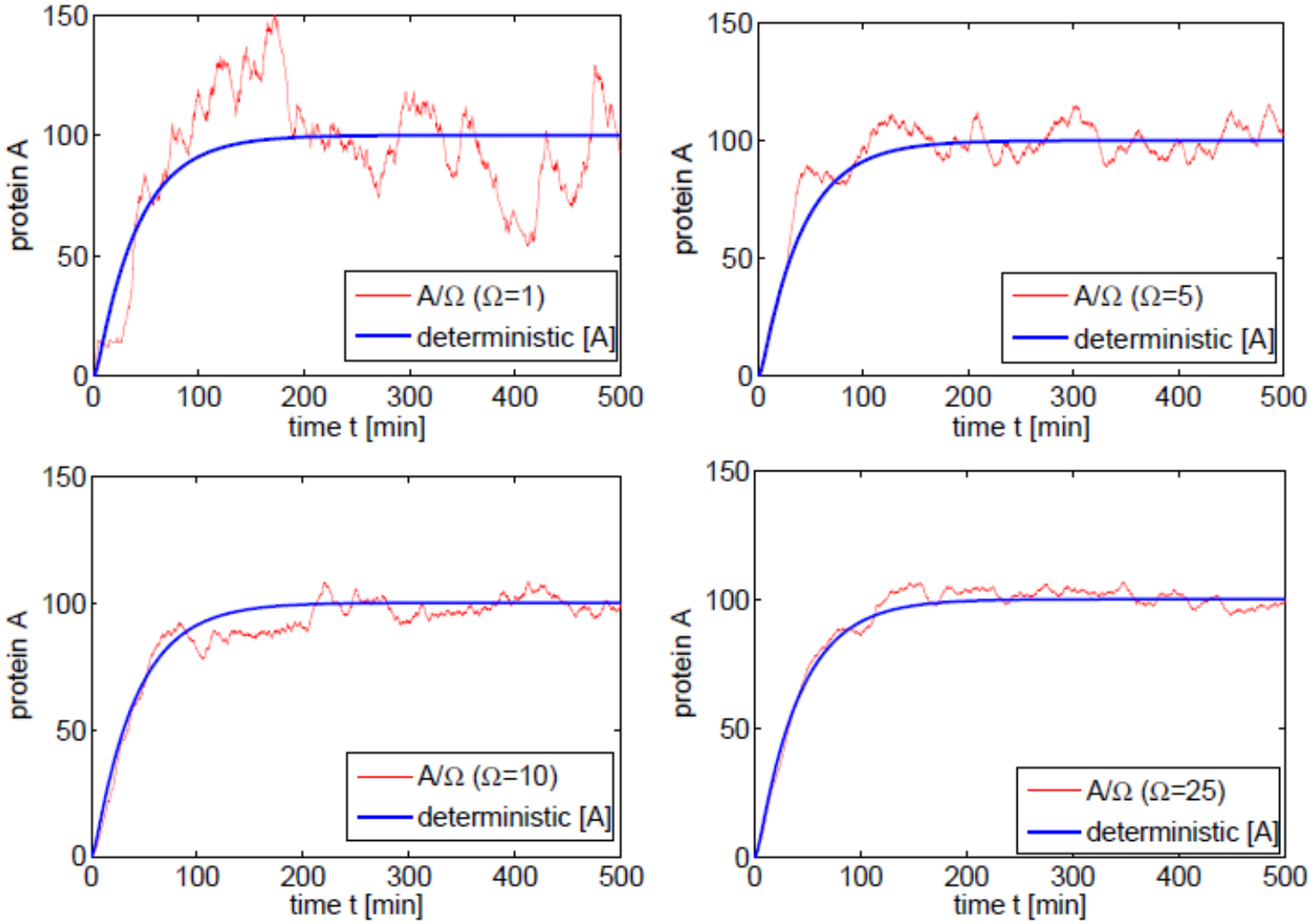
```
% Simulation
while (t<zeit);
   S(ix,:)=[t M/OMEGA A/OMEGA]; ix=ix+1;
   % Berechnung der Raten fuer alle Uebergaenge
   w1 = OMEGA*km;
   w2 = dm*M;
   w3 = ka*M;
   w4 = da*A;

   rate=w1+w2+w3+w4;

   % Ziehe Zeitschritt dt   */
   dt = -log(1-rand)/rate;
   t = t + dt;

   rate=rate*rand;
   rate = rate - w1 ; if (rate < 0.0), M=M+1; continue; end;
   rate = rate - w2 ; if (rate < 0.0), M=M-1; continue; end;
   rate = rate - w3 ; if (rate < 0.0), A=A+1; continue; end;
   rate = rate - w4 ; if (rate < 0.0), A=A-1; continue; end;
end;
```

Gene expression as a stochastic process





Gene expression as a stochastic process



The end