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Polymers in the Liver - Metabolism and Regulation

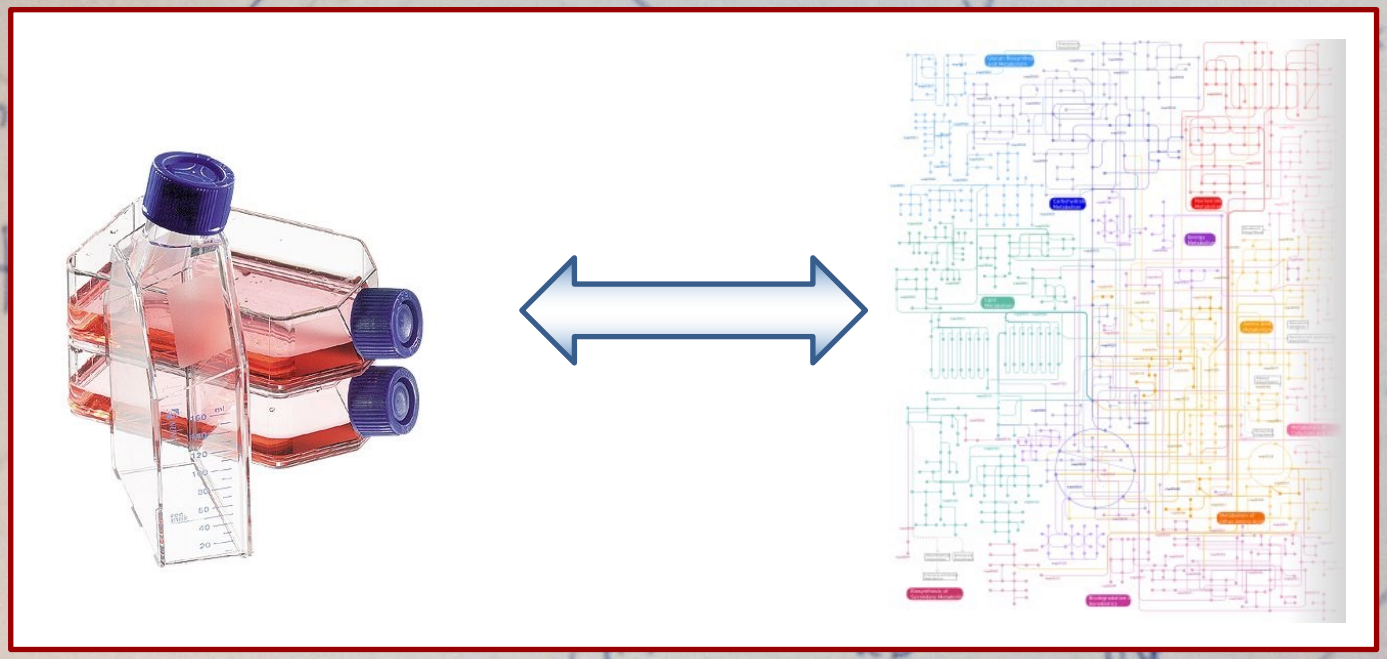
Systems Biology Centre GRONINGEN

Dynamic modelling of metabolism

Model construction and enzyme kinetics

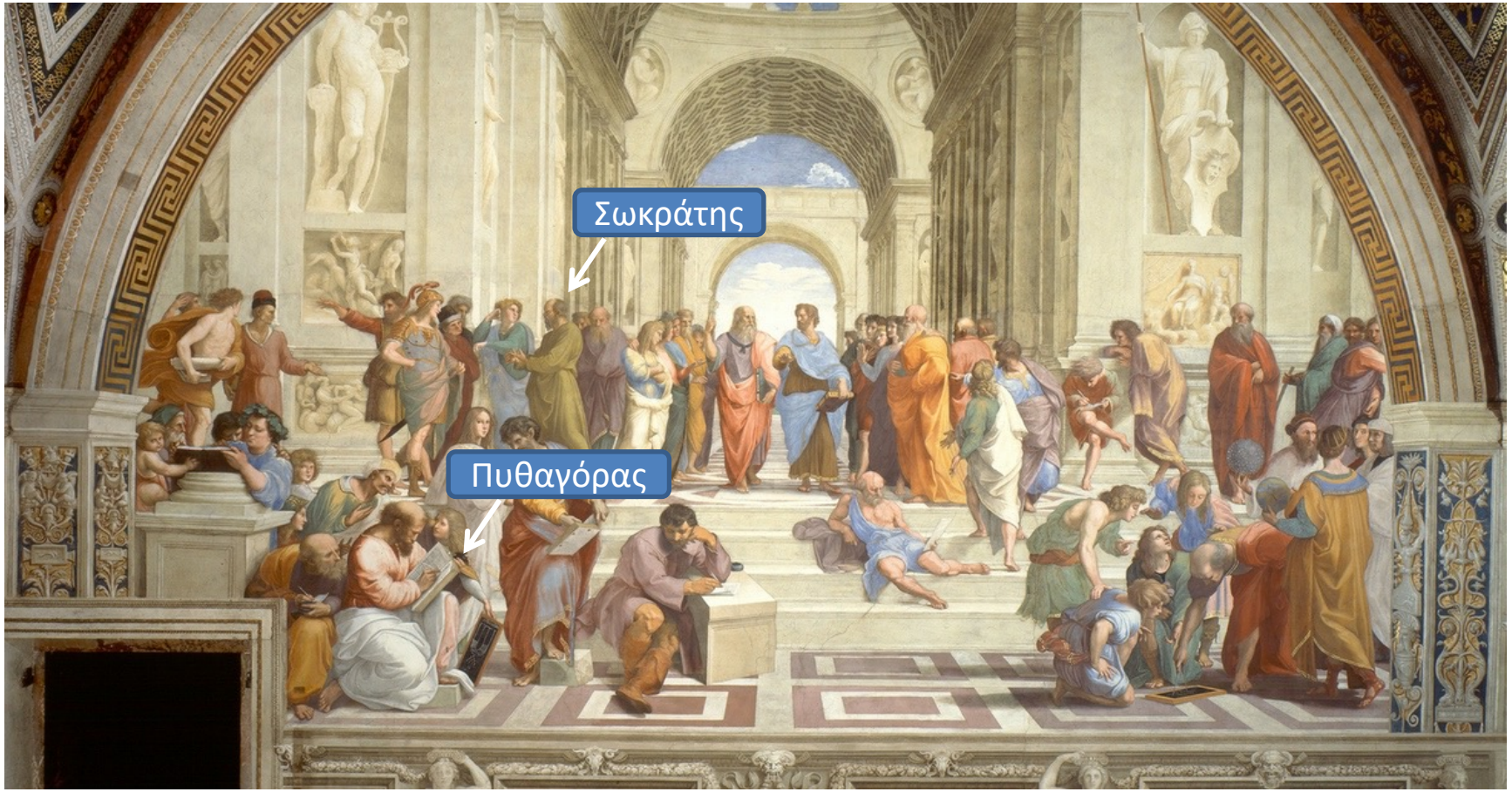
rijksuniversiteit groningen

umcg



Barbara M. Bakker *University Medical Centre Groningen, University of Groningen*

We are all here to learn and discuss!



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The Menu

Principles of dynamic modelling

a. Model construction

b. Enzyme kinetics

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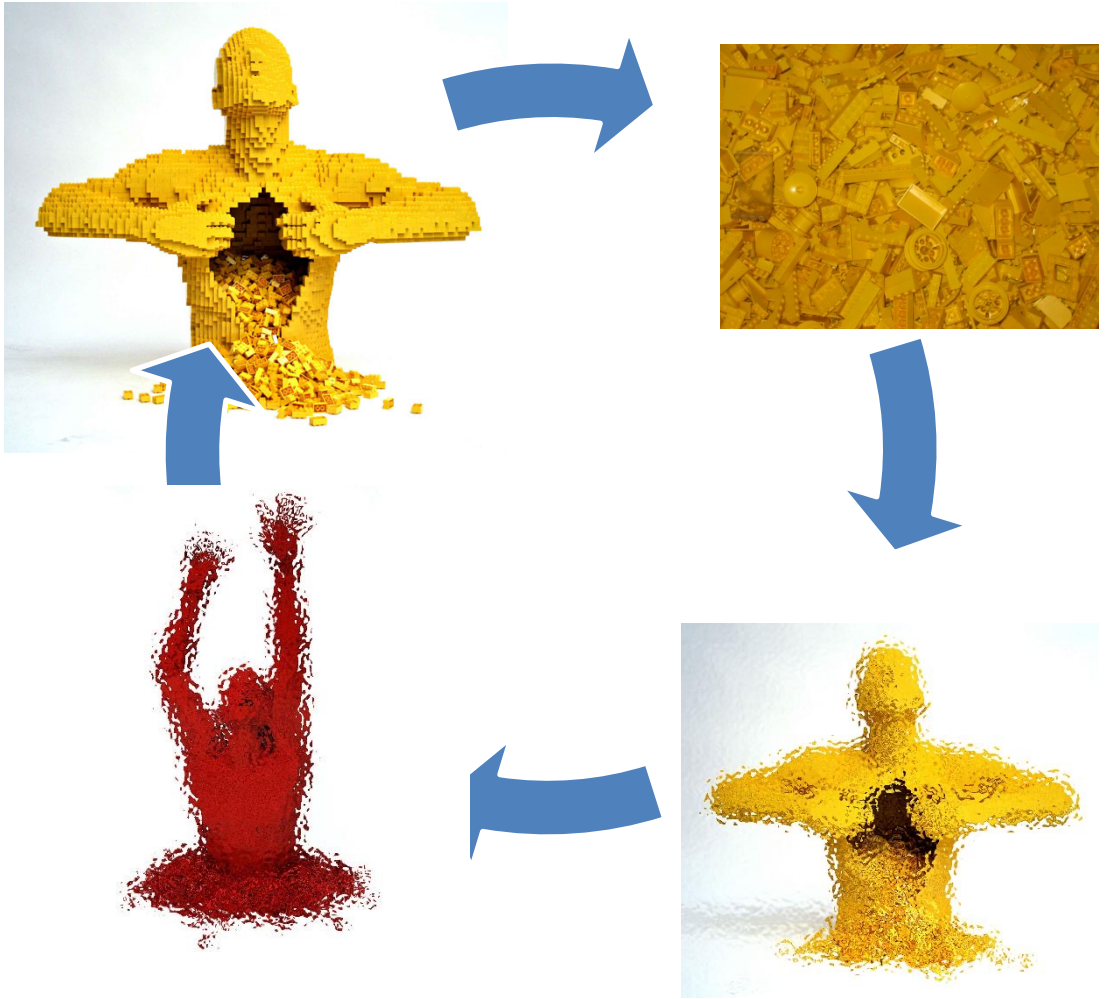
The Systems Biology cycle



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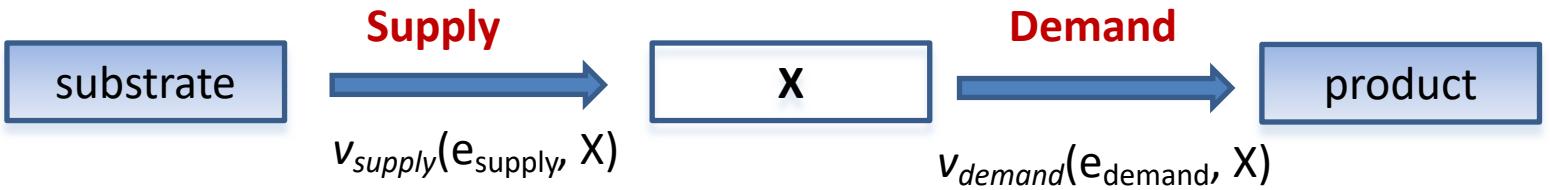
Computational modelling (dynamic)



Simulating concentration of metabolite X in time

$$d[X]/dt = v_{supply}(e_{supply}, X) - v_{demand}(e_{demand}, X)$$

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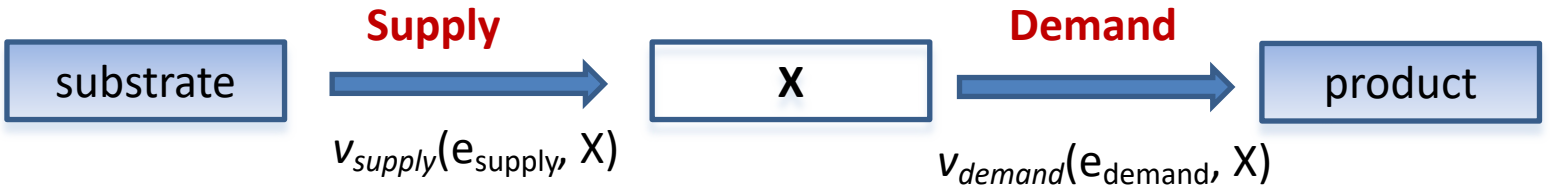
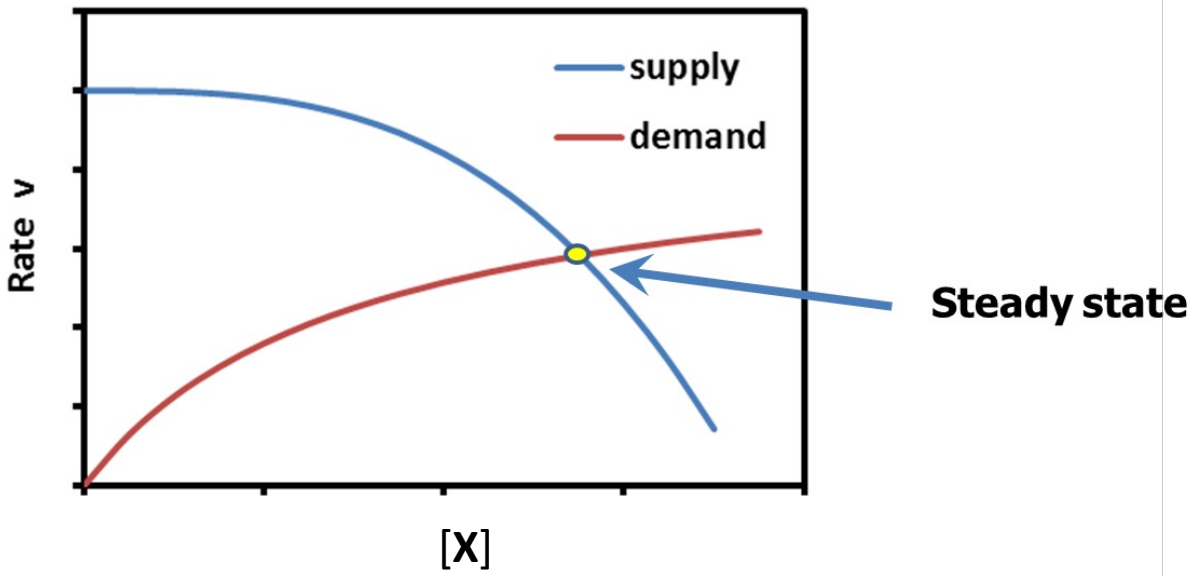
Computational modelling (steady state)





Kinetic information allows to predict steady-state flux and concentration

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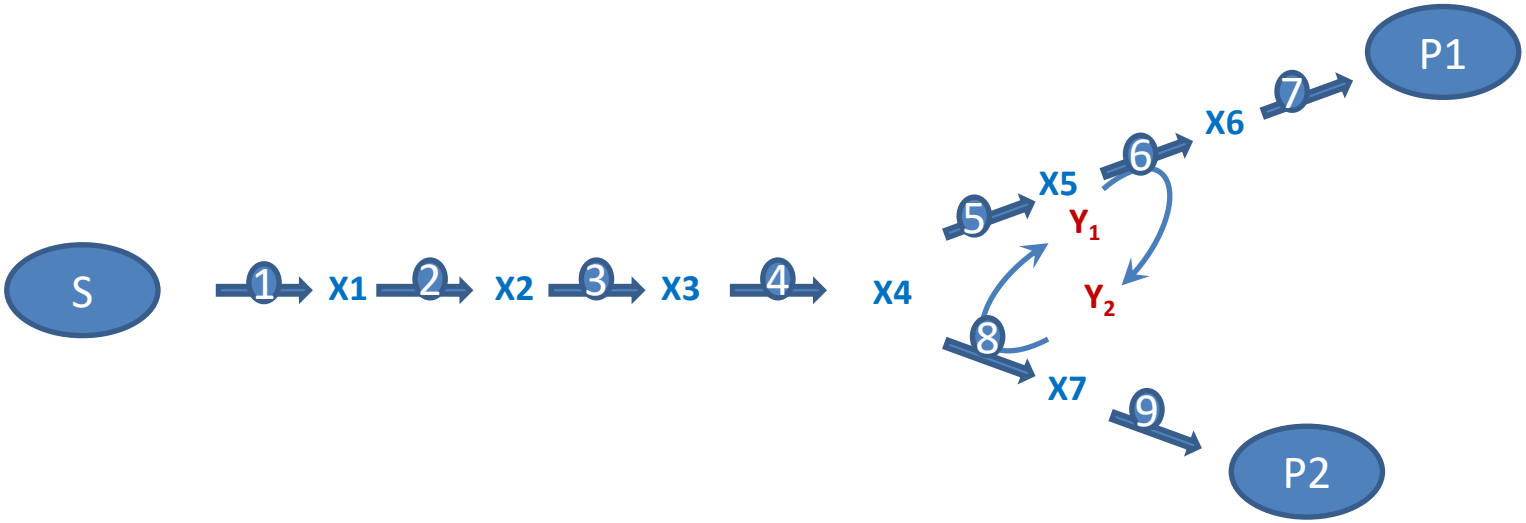
Dynamic models: the challenge



$d[X_1]/dt = v_1 - v_2$

…

…

$d[X_4]/dt = v_4 - v_5 - v_8$

…

$d[Y_1]/dt = -v_5 + v_8$

$d[Y_2]/dt = +v_5 - v_8$

Velocities (**v**) depend on concentrations (**X**)

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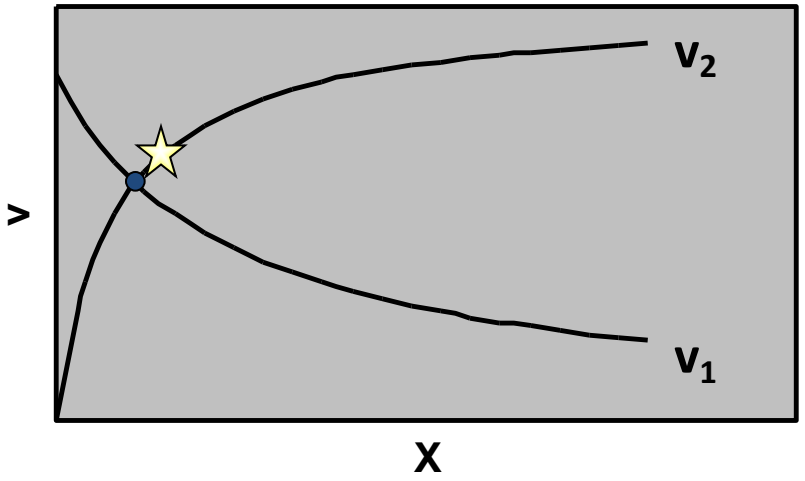
Steady state

Metabolite X is at steady state if the rate of enzyme 1 (v_1) equals the rate of enzyme 2 (v_2) → Production equals consumption.



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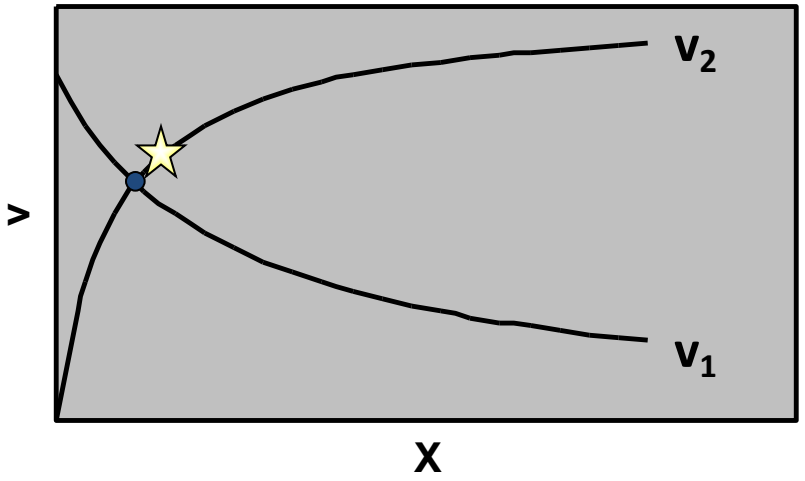
What is required to obtain a steady state?

1. Concentrations of S and P are constant or external source / sink
2. Stability of the steady state



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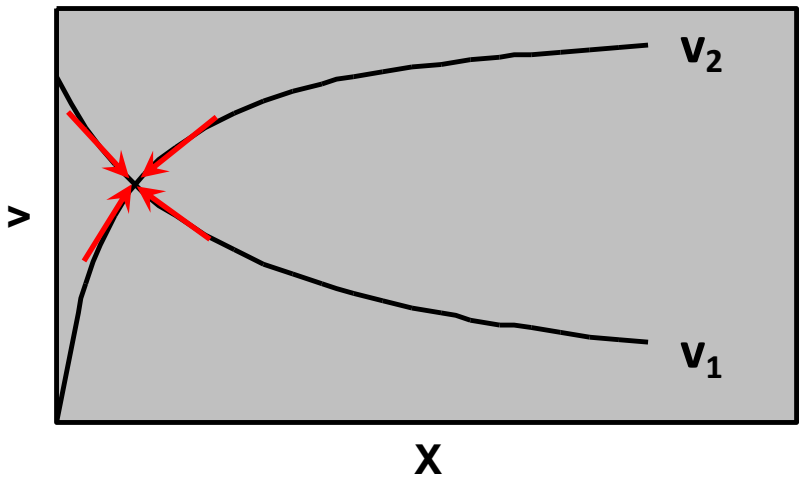
Stability of a steady state

A stable steady state:



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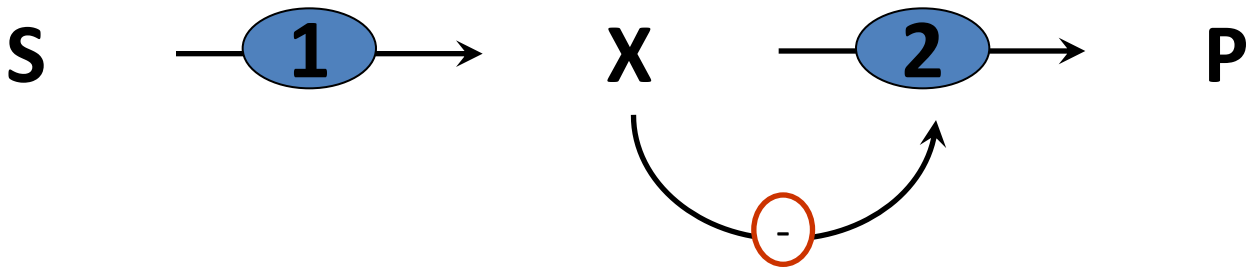
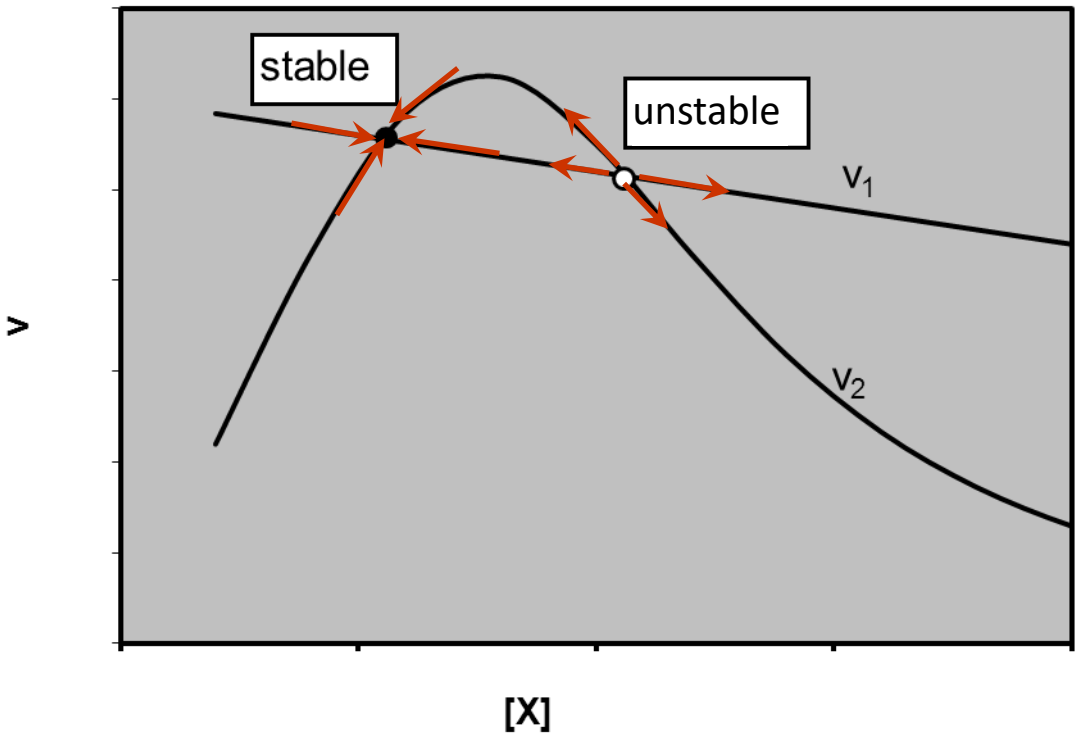
(In)stability of a steady state





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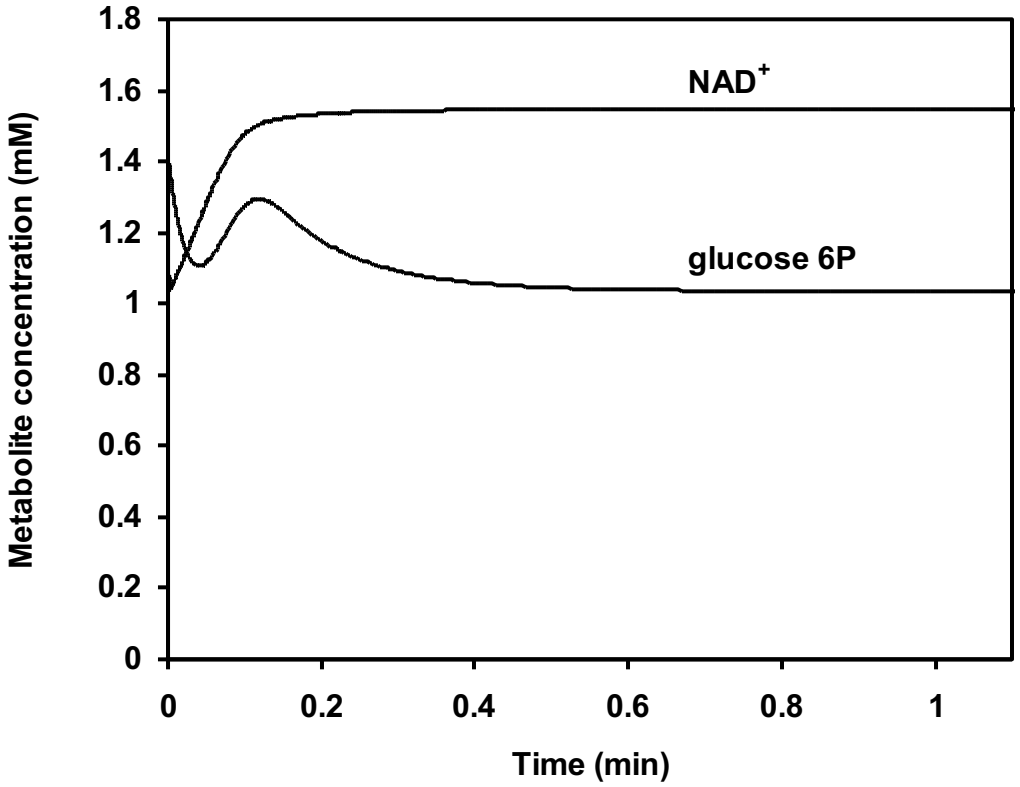
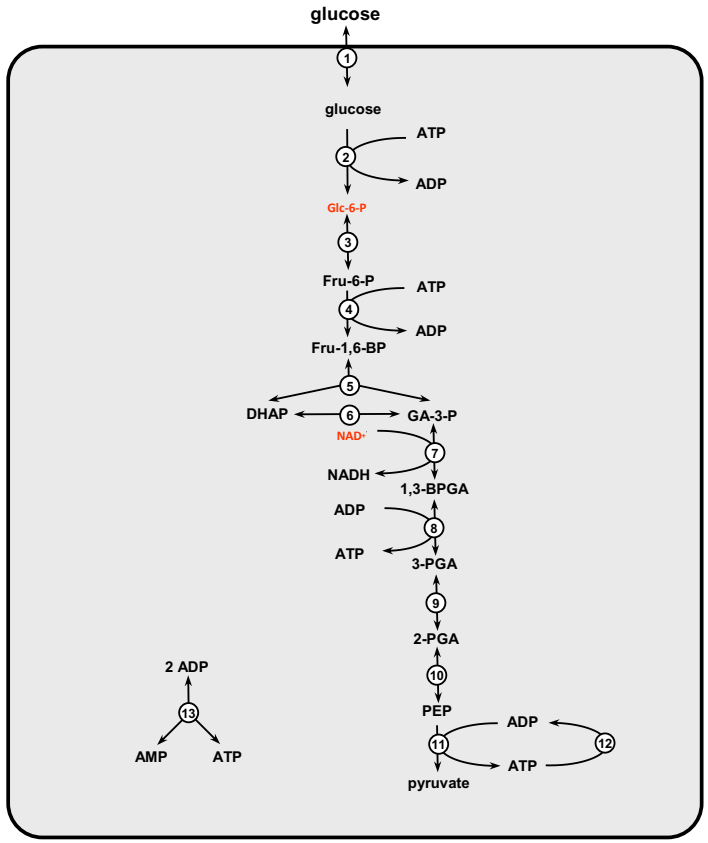
Dynamic model





To simulate (predict) how concentrations and rates in a pathway behave in time; and in which steady state they may settle.

Teusink (2000) Eur J Biochem. 267, 513-5329 (simulated at www.jjj.bio.vu.nl)

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Construction of a computer model

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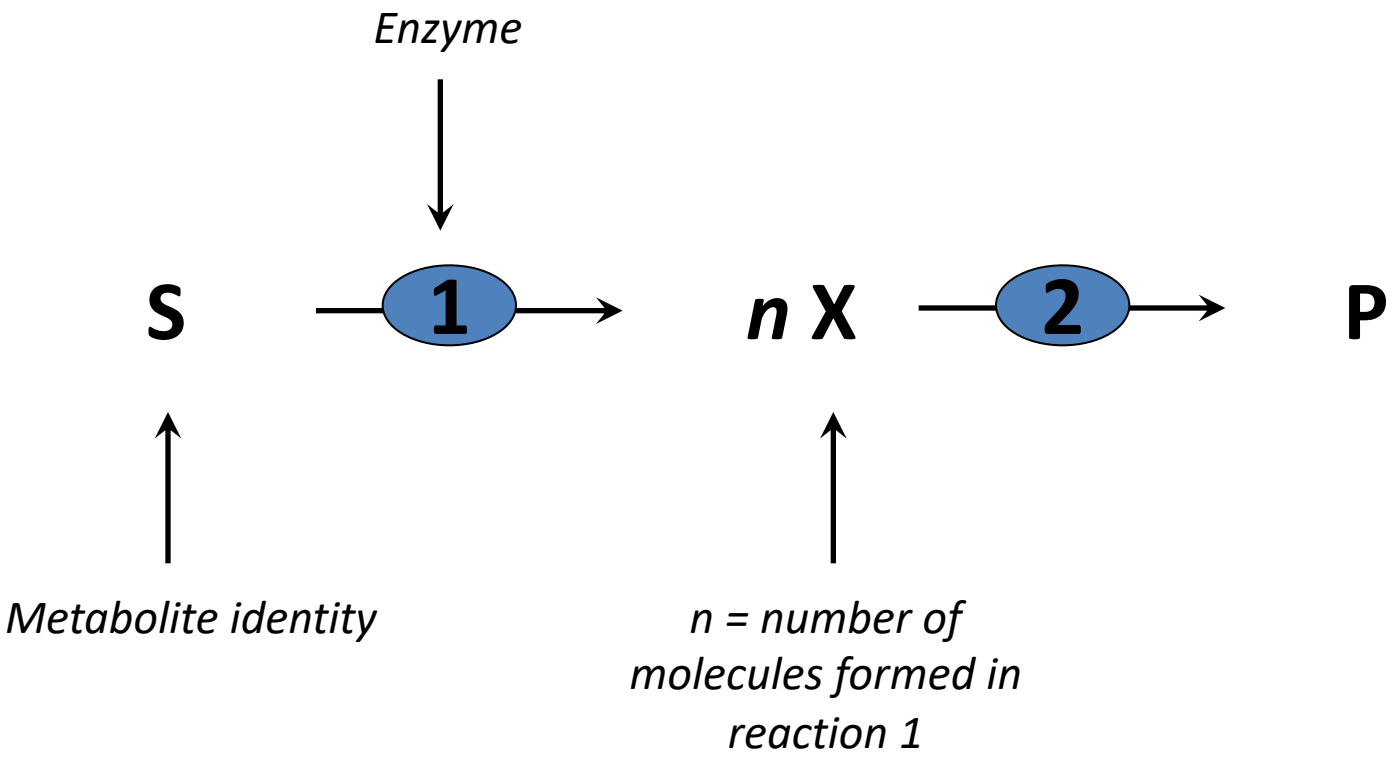
Pathway stoichiometry



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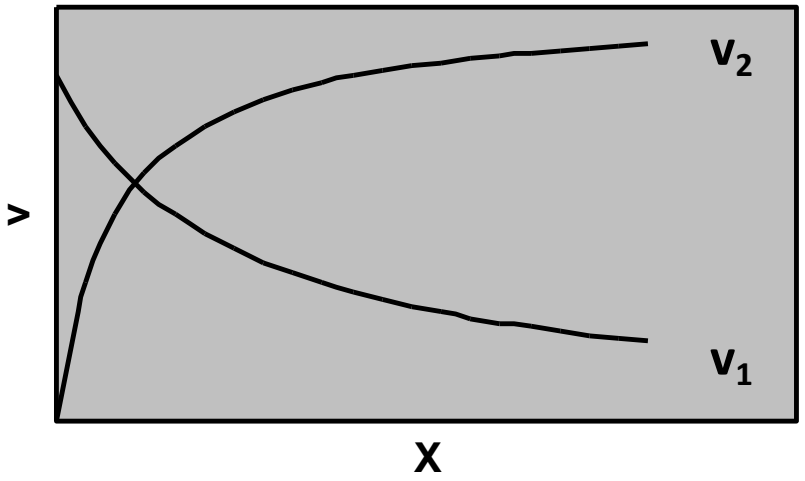
Enzyme kinetics

rate of enzyme 1 = $v_1(e_1, S, X)$

rate of enzyme 2 = $v_2(e_2, X, P)$



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Prediction of dynamics

$$d[X]/dt = v_1(e_1, S, X) - v_2(e_2, X, P)$$

→ *Prediction of X(t) and v(t) at given S, P, e_1 and e_2*

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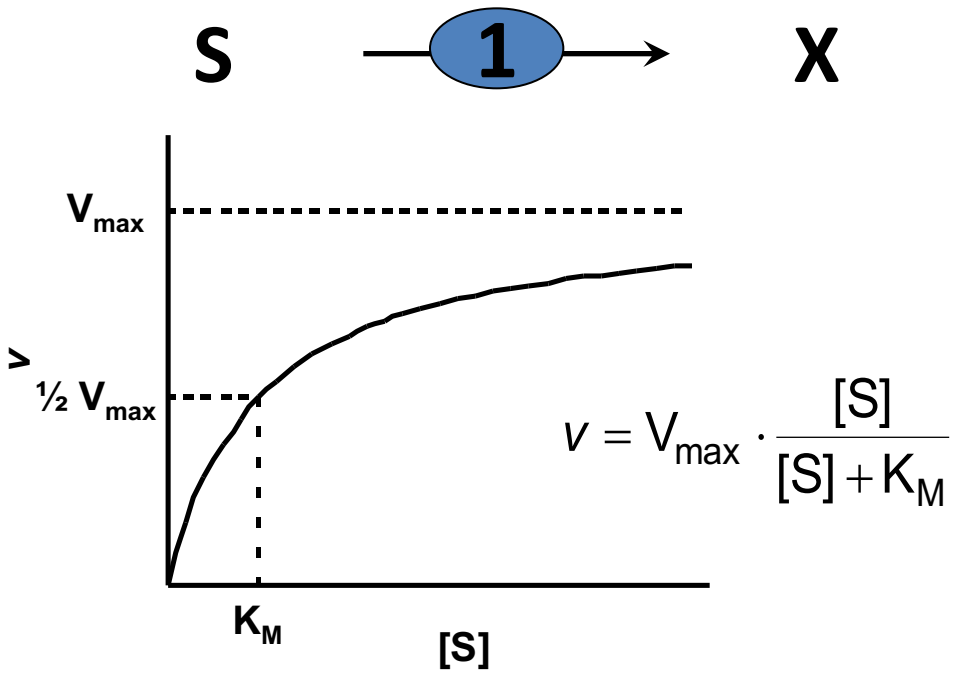
Rate equations



Classical Michaelis-Menten kinetics

If [S] >> K_M, then $v = V_{max}$.

If [S] = K_M, then $v = ½ V_{max}$.

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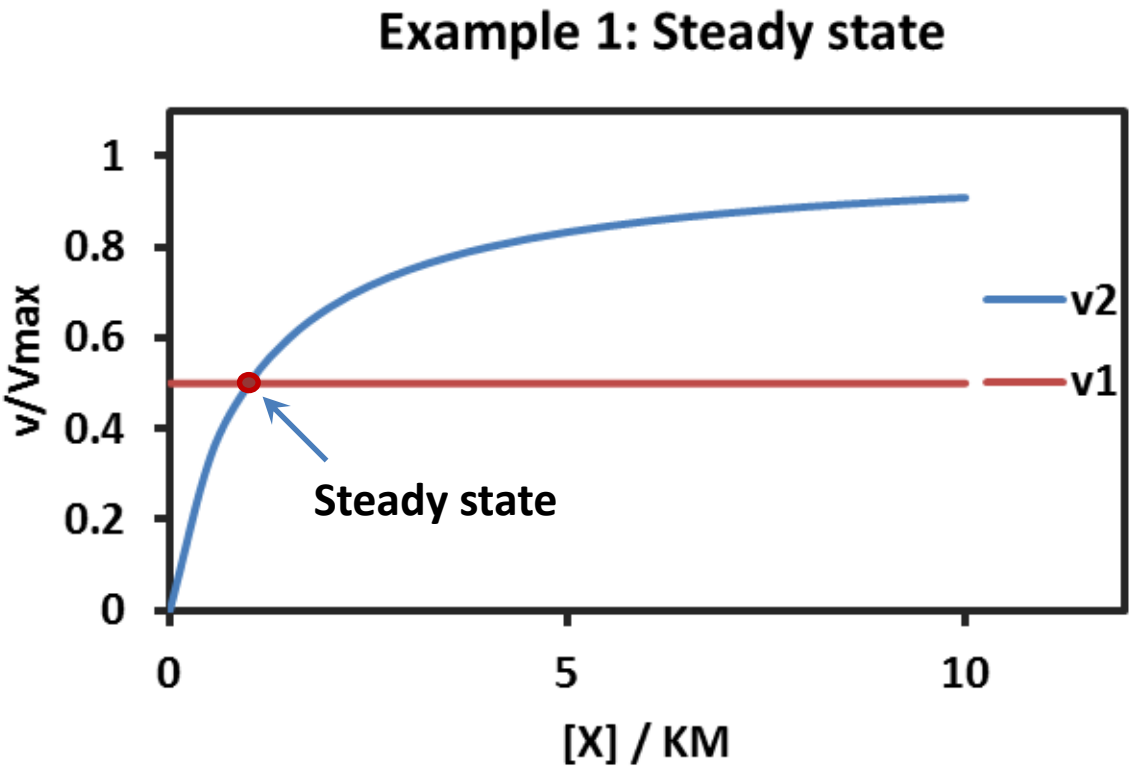
Classical MM kinetics in a pathway



Steady state
v1 = v2
[X] = constant

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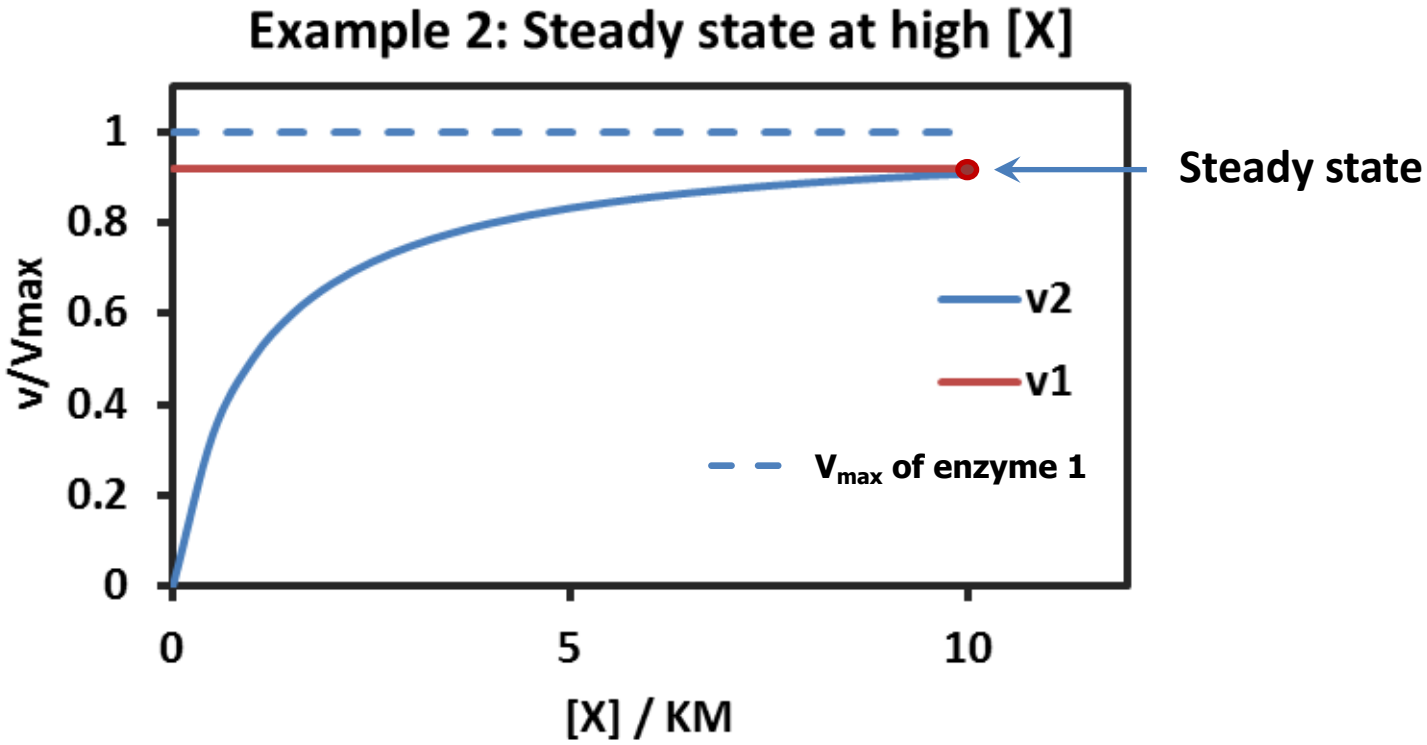
Classical MM kinetics in a pathway



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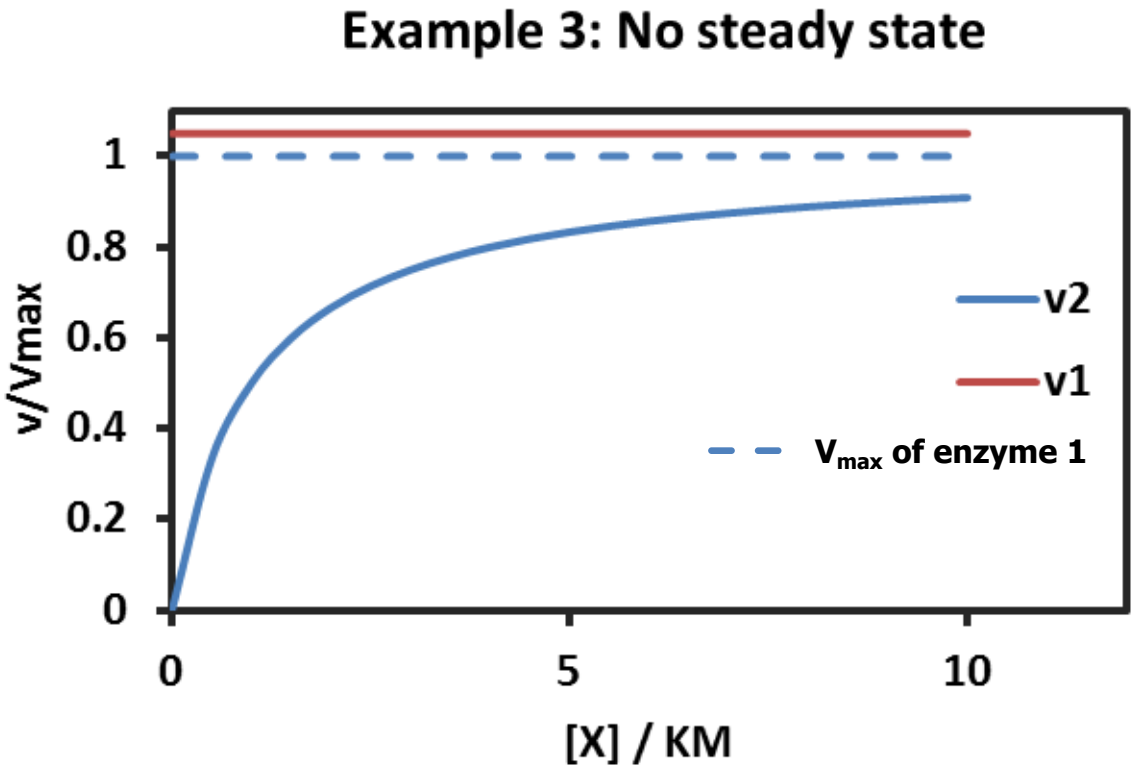
Classical MM kinetics in a pathway



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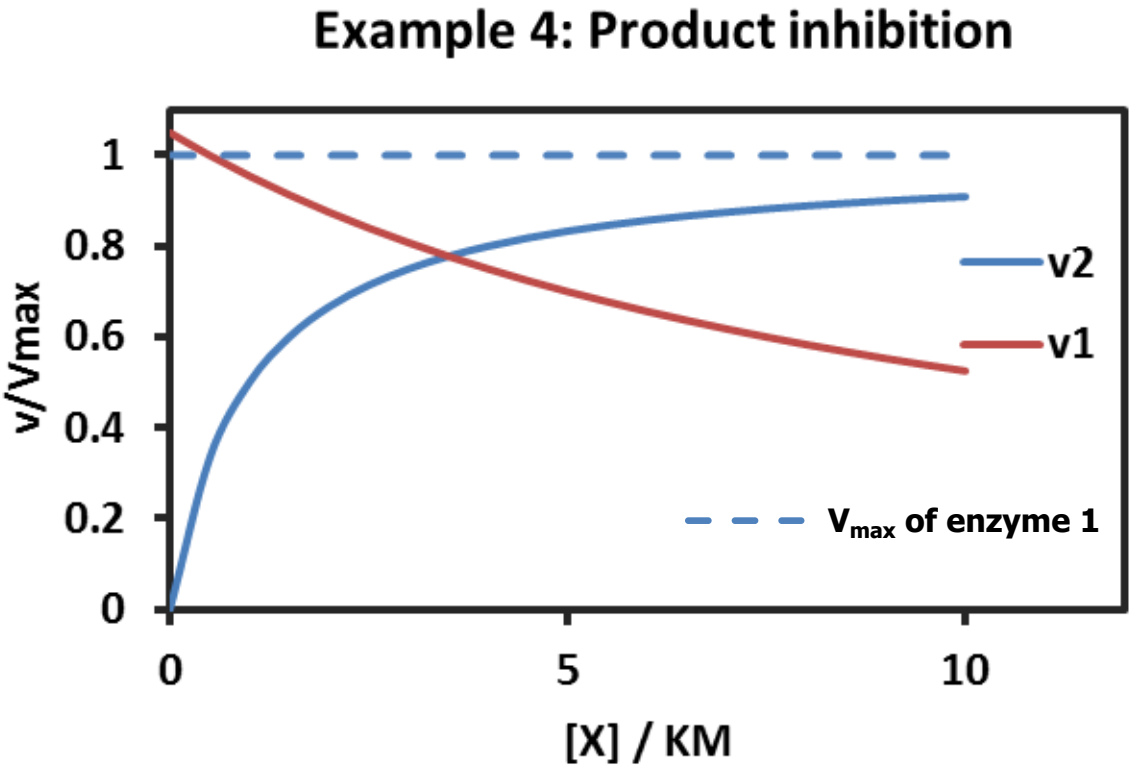
Product inhibition 'saves' the steady state



Enzymes 'communicate' via metabolite concentrations.

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Reversible enzyme reactions (1)

$$v = \frac{V_{+max} \cdot \frac{[S]}{K_{MS}} - V_{-max} \cdot \frac{[P]}{K_{MP}}}{1 + \frac{[S]}{K_{MS}} + \frac{[P]}{K_{MP}}}$$

$V_{+max} = k_2 \cdot [E_{tot}]$ $\quad K_{MS} = (k_{-1} + k_2) / k_1$

$V_{-max} = k_{-1} \cdot [E_{tot}]$ $\quad K_{MP} = (k_{-1} + k_2) / k_{-2}$

The equation if symmetrical, like the reaction scheme.

An increase of the enzyme concentration leads to a proportional increase of V_{+max} and V_{-max} (→ effect of gene expression)

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Reversible enzyme reactions (2)

$$v = \frac{V_{+max} \cdot \frac{[S]}{K_{MS}} - V_{-max} \cdot \frac{[P]}{K_{MP}}}{1 + \frac{[S]}{K_{MS}} + \frac{[P]}{K_{MP}}}$$

If [P] = 0, the equation reduces to the original Michaelis-Menten equation:

$$v = \frac{V_{+max} \cdot \frac{[S]}{K_{MS}}}{1 + \frac{[S]}{K_{MS}}} = \frac{V_{+max} \cdot [S]}{K_{MS} + [S]}$$

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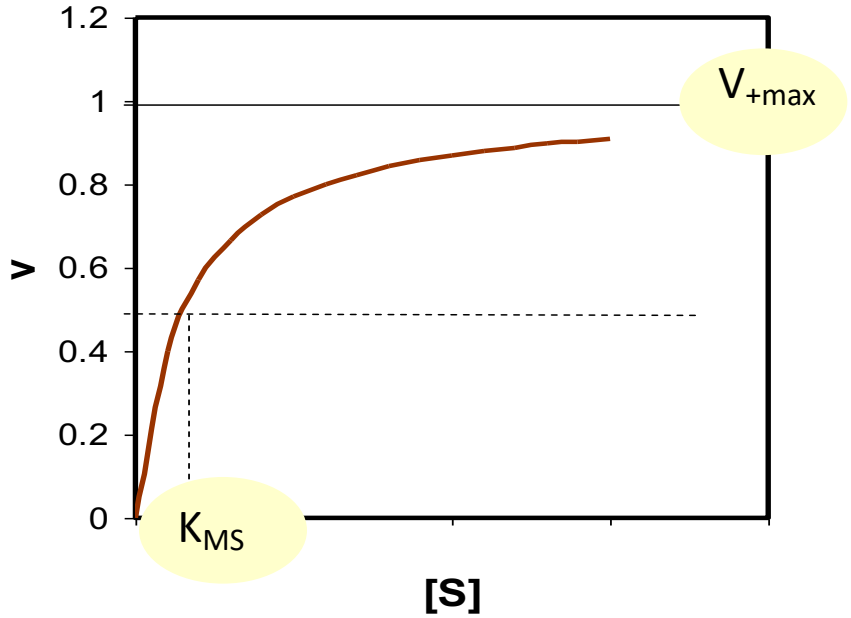
Reversible enzyme kinetics (2)



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$$v = \frac{V_{+max} \cdot \frac{[S]}{K_{MS}}}{1 + \frac{[S]}{K_{MS}}} = \frac{V_{+max} \cdot [S]}{K_{MS} + [S]}$$

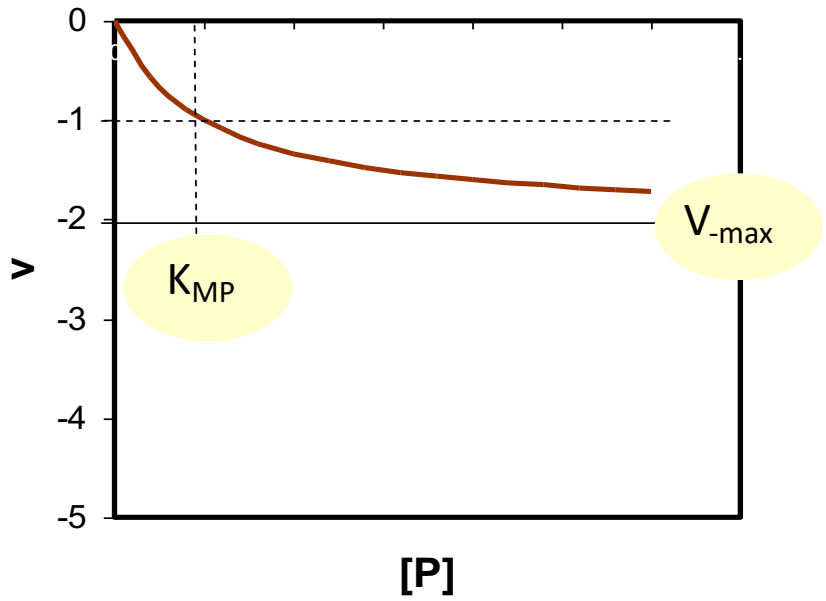
Reversible enzyme kinetics (3)

$$v = \frac{V_{+max} \cdot \frac{[S]}{K_{MS}} - V_{-max} \cdot \frac{[P]}{K_{MP}}}{1 + \frac{[S]}{K_{MS}} + \frac{[P]}{K_{MP}}}$$

If [S] = 0, the equation also reduces to the original Michaelis-Menten equation, but in the reverse direction and as a function of [P]:

$$v = -\frac{V_{-max} \cdot \frac{[P]}{K_{MP}}}{1 + \frac{[P]}{K_{MP}}} = -\frac{V_{-max} \cdot [P]}{K_{MP} + [P]}$$

Reversible enzyme kinetics (3)



If [S] = 0, the equation also reduces to the original Michaelis-Menten equation, but in the reverse direction and as a function of [P]:

$$v = -\frac{V_{-max} \cdot \frac{[P]}{K_{MP}}}{1 + \frac{[P]}{K_{MP}}} = -\frac{V_{-max} \cdot [P]}{K_{MP} + [P]}$$

The Haldane relation

$$v = \frac{V_{+max} \cdot \frac{[S]}{K_{MS}} - V_{-max} \cdot \frac{[P]}{K_{MP}}}{1 + \frac{[S]}{K_{MS}} + \frac{[P]}{K_{MP}}}$$

Equation 1

At thermodynamic equilibrium: $[P]/[S] = K_{eq}$ AND $v = 0$

It follows that: $K_{eq} = \frac{V_{+max}}{K_{MS}} \cdot \frac{K_{MP}}{V_{-max}}$ Equation 2 (the Haldane relation)

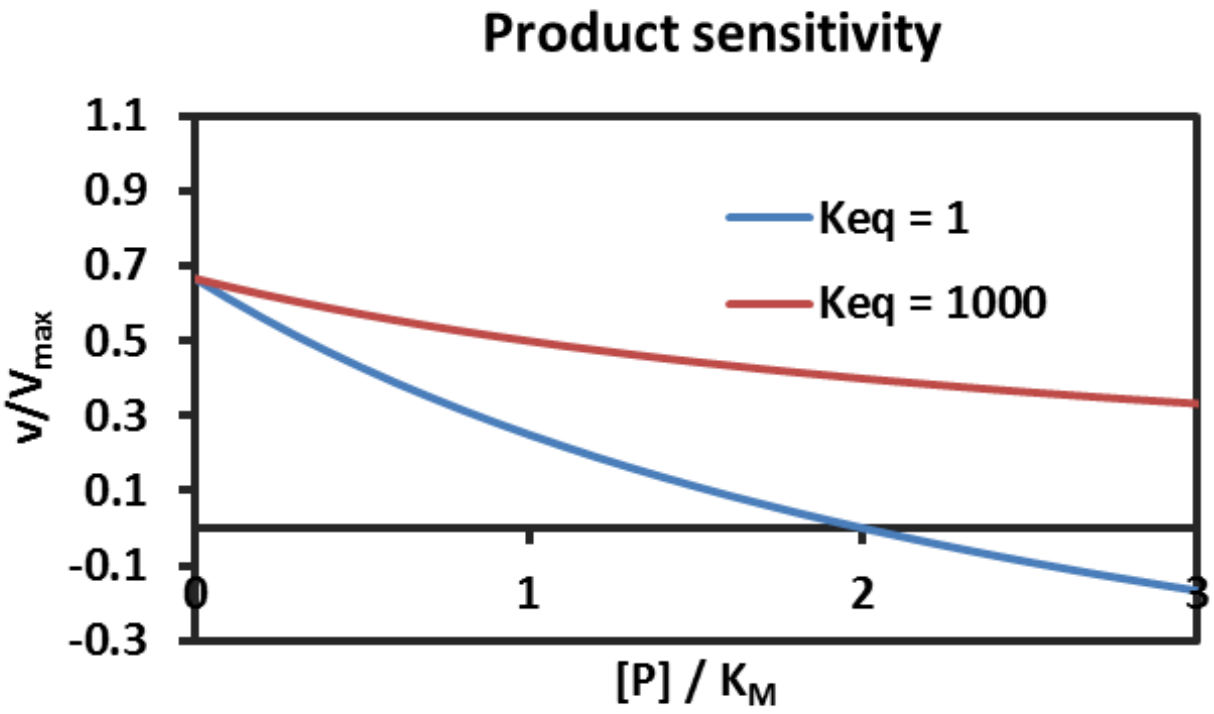
$$v = \frac{V_{+max} \frac{[S]}{K_{MS}} \left(1 - \frac{[P]}{[S]} \Big/ K_{eq}^{\star}\right)}{1 + \frac{[S]}{K_{MS}} + \frac{[P]}{K_{MP}}}$$

Equation 3

This is another way of writing the above discussed 'reversible Michaelis-Menten' equation.

Product sensitivity

$$v = \frac{V_{+\max}\dfrac{[S]}{K_{MS}}\left(1-\dfrac{[P]}{[S]}\Big/K_{eq}\right)}{1+\dfrac{[S]}{K_{MS}}+\dfrac{[P]}{K_{MP}}}$$



Full-scale kinetic model

$d[\text{glucose}]/dt = v_1(e_1, \mathbf{X}) - v_2(e_2, \mathbf{X})$

$d[\text{Glc6P}]/dt = v_2(e_2, \mathbf{X}) - v_3(e_3, \mathbf{X})$

$d[\text{Fru6P}]/dt = v_3(e_3, \mathbf{X}) - v_4(e_4, \mathbf{X})$

....

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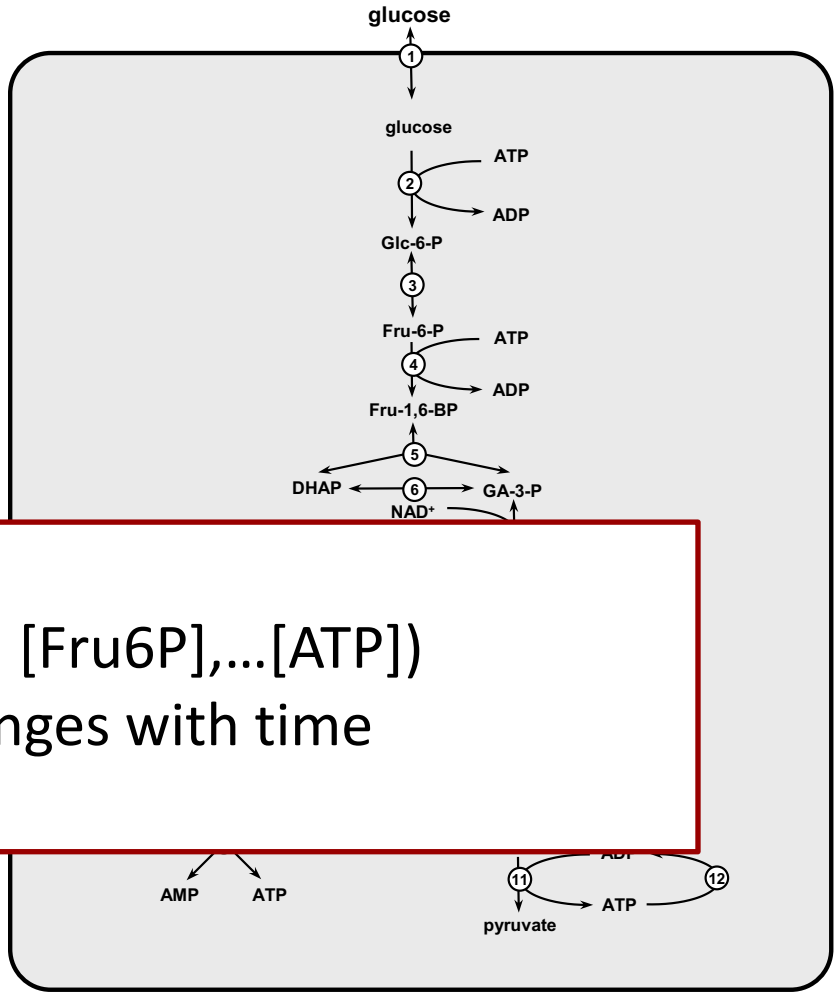
X = ([glucose], [Glc6P], [Fru6P],…[ATP])
Calculate how **X** changes with time



$d[\text{ATP}]/dt = -v_2(e_2, \mathbf{X}) - v_4(e_4, \mathbf{X}) + v_8(e_8, \mathbf{X}) + v_{11}(e_{11}, \mathbf{X}) - v_{12}(e_{12}, \mathbf{X}) + v_{13}(e_{13}, \mathbf{X})$

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Numerical solution

$d[\text{glucose}]/dt = v_1(e_1, \mathbf{X}) - v_2(e_2, \mathbf{X})$

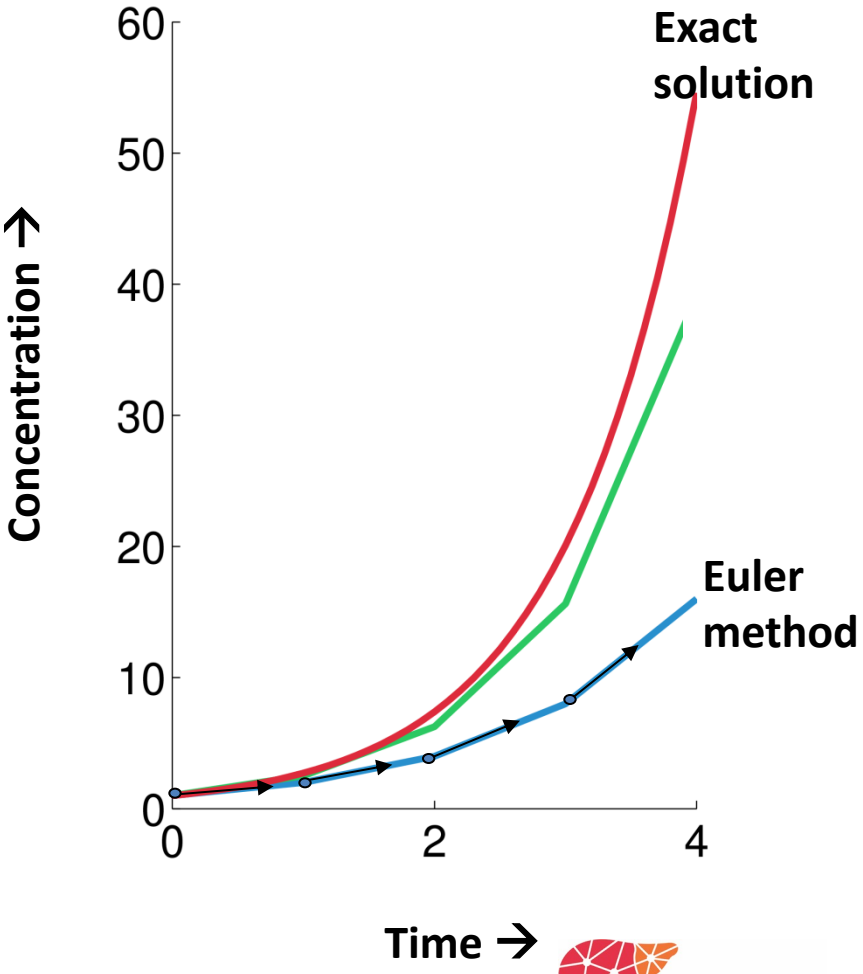
$d[\text{Glc6P}]/dt = v_2(e_2, \mathbf{X}) - v_3(e_3, \mathbf{X})$

…

Each rate may depend on multiple concentrations, which in turn depend on multiple rates

Euler approximation

- Calculate rates at time *t*
- Predict concentrations at next time point
- Recalculate rates
- Iterative procedure → computer power!



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Solvers (non-exhaustive)

Euler: *one step method*

Runge-Kutta: *takes intermediate steps*

Adams: *uses information from previous steps*

} **'Non-stiff' problems**

BDF (backward differentiation formula) /Gear — **'Stiff' problems**

LSODA: *switches during the simulation between Adams (non-stiff, fast) and BDF (stiff, more expensive in computer time, more stable)*

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Finding the steady state

$$d[X]/dt = v_1(e_1, S, X) - v_2(e_2, X, P)$$

At steady state $d[X]/dt = 0$

→Find X for which: $v_1(e_1, S, X) - v_2(e_2, X, P) = 0$

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Steady state of the full-scale kinetic model

$d[\text{glucose}]/dt = v_1(e_1, \mathbf{X}) - v_2(e_2, \mathbf{X}) = 0$

$d[\text{Glc6P}]/dt = v_2(e_2, \mathbf{X}) - v_3(e_3, \mathbf{X}) = 0$

$d[\text{Fru6P}]/dt = v_3(e_3, \mathbf{X}) - v_4(e_4, \mathbf{X}) = 0$

....

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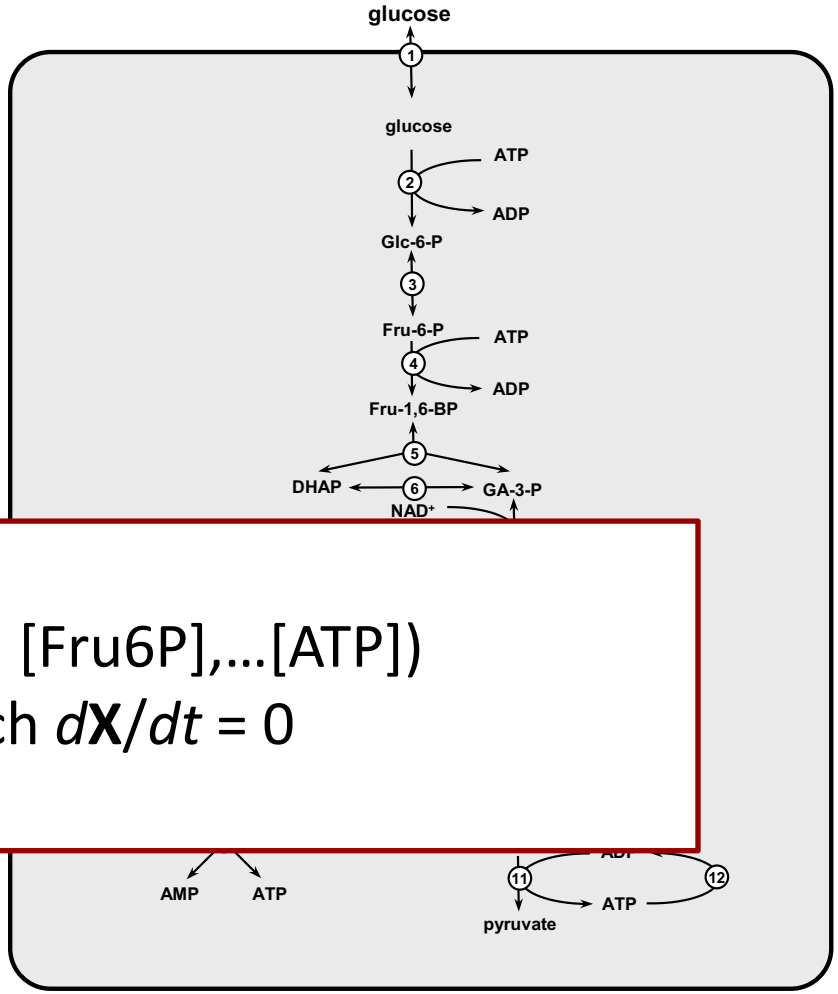

X = ([glucose], [Glc6P], [Fru6P],…[ATP])

→ Find **X** for which $d\mathbf{X}/dt = 0$

$d[\text{ATP}]/dt = -v_2(e_2, \mathbf{X}) - v_4(e_4, \mathbf{X}) + v_8(e_8, \mathbf{X}) + v_{11}(e_{11}, \mathbf{X}) - v_{12}(e_{12}, \mathbf{X}) + v_{13}(e_{13}, \mathbf{X}) = 0$

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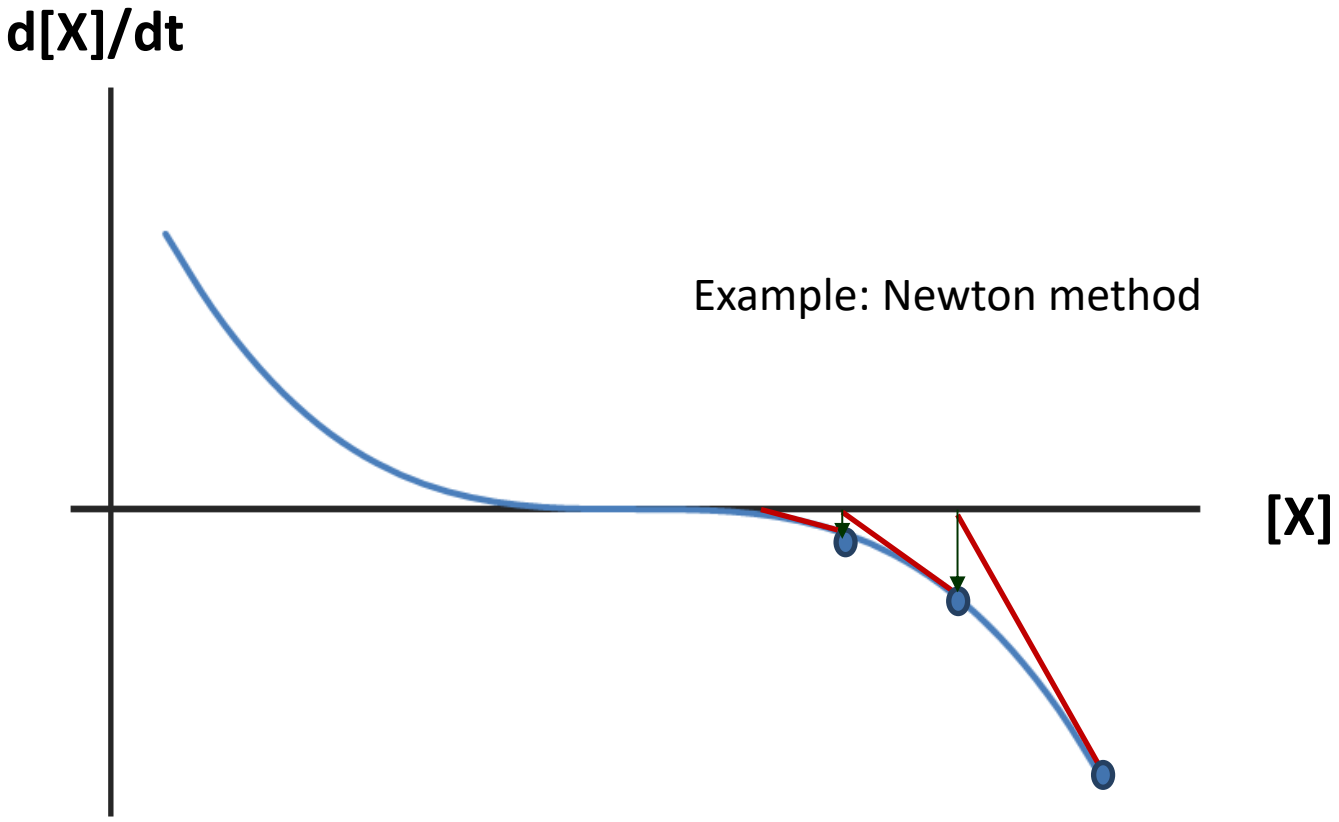
Numerical root finding algorithm



- **X** = (X_1, X_2, …X_n) → many dimensions
- Iterative procedure → computer power

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Enzyme kinetics

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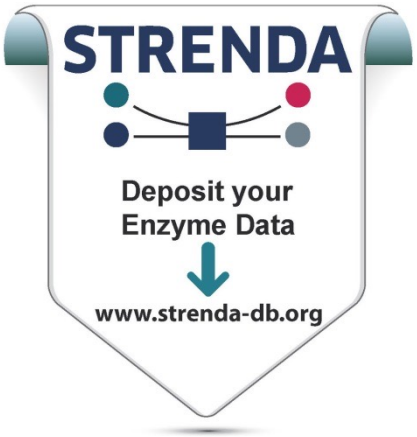
Model parameters

For a metabolic model typically:

- Kinetic parameters: V_{max}, K_m, …
- Equilibrium constants
- Enzyme concentrations
- Compartment volumes
- Conserved moieties (*e.g.* [ATP] + [ADP] + [AMP] = constant)

Enzyme kinetic databases



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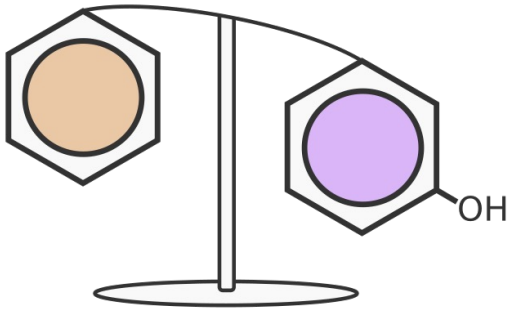
Equilibrium constants



eQuilibrator

http://equilibrator.weizmann.ac.il/

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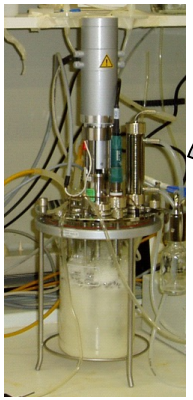
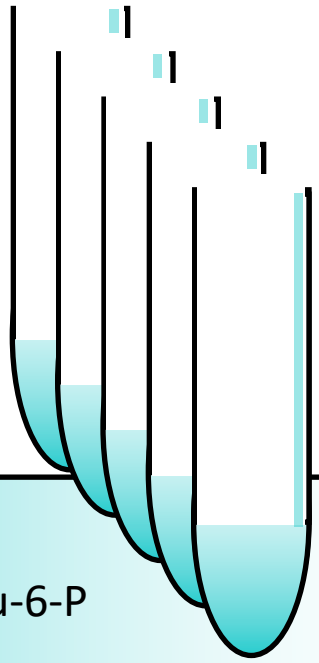
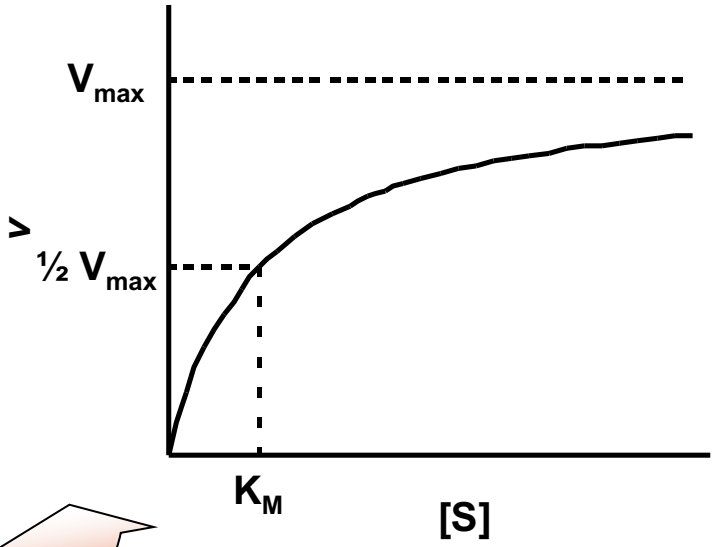
1. Independent biochemical analysis



Enzyme purification





Variation of substrate concentration

glc-6-P → fru-6-P

$$v = \frac{V_{\max} \cdot [\mathrm{Glc6P}] / K_{Glc6P} \cdot \left(1 - \Gamma / K_{eq}\right)}{1 + [\mathrm{Glc6P}] / K_{glc6P} + [\mathrm{Fru6P}] / K_{fru6P}}$$

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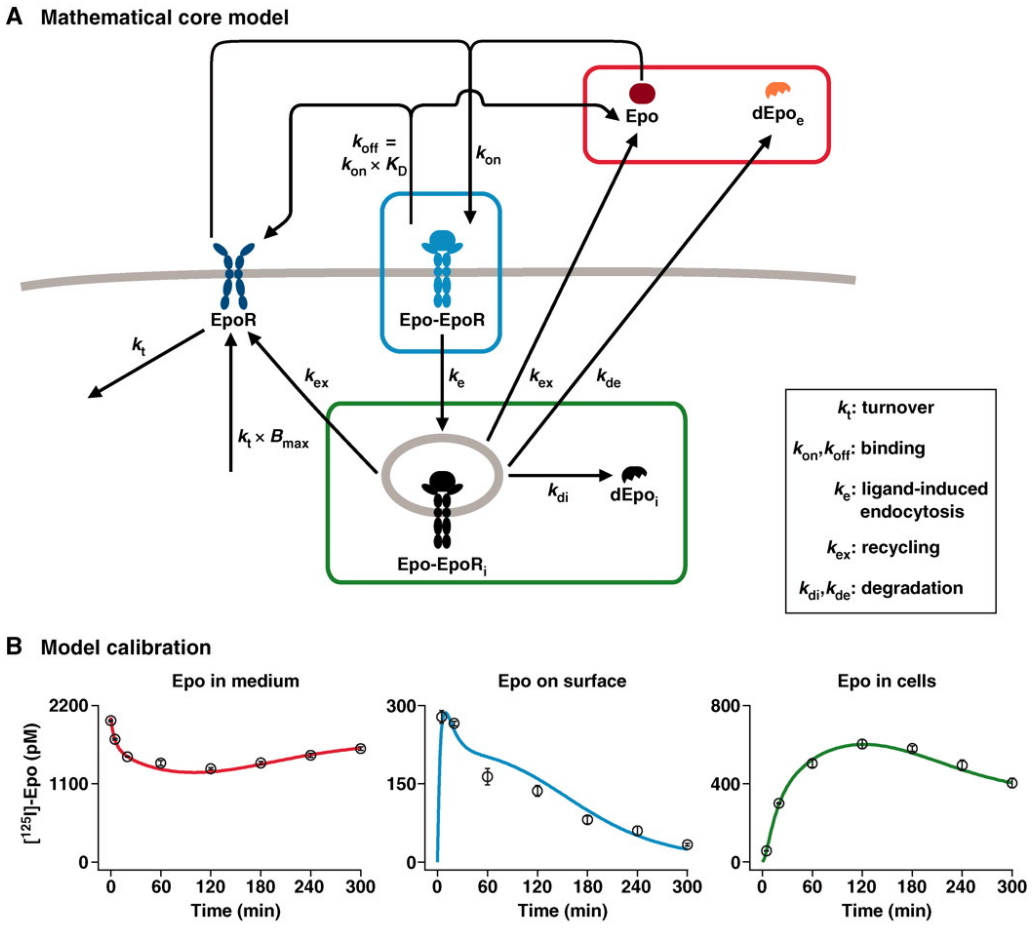
2. Parameter fitting

Dynamic modeling of the EpoR system.



V Becker et al. Science 2010;328:1404-1408

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Modelling ‘schools’

1. **Independent biochemical measurement of parameters**

Biochemical school, accessible parameters (e.g. enzyme kinetics),

Number of parameters too large for fitting, aim of the model

2. **Parameter fitting**

Engineering school, parameters not directly accessible (e.g. protein-protein interactions in signalling, number of parameters small)

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Model validation

Classical example: glycolysis in bakers' yeast

- **Compare new data to independent model prediction**
- **Independent biochemical analysis of parameters**
- **Special feature: the assay medium mimicked the cytosolic environment of the cells**

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Testing Biochemistry Revisited: How *In Vivo* Metabolism Can Be Understood from *In Vitro* Enzyme Kinetics

Karen van Eunen[1,2,3], José A. L. Kiewiet[1,2], Hans V. Westerhoff[1,2,4,5], Barbara M. Bakker[1,2,3]*

1 Department of Molecular Cell Physiology, VU University Amsterdam, Amsterdam, The Netherlands, 2 Kluyver Centre for Genomics of Industrial Fermentation, Delft, The Netherlands, 3 Department of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, 4 Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary BioCentre, The University of Manchester, Manchester, United Kingdom, 5 Synthetic Systems Biology, Netherlands Institute for Systems Biology, University of Amsterdam, Amsterdam, The Netherlands

Van Eunen (2012) PLoS Comp Biol

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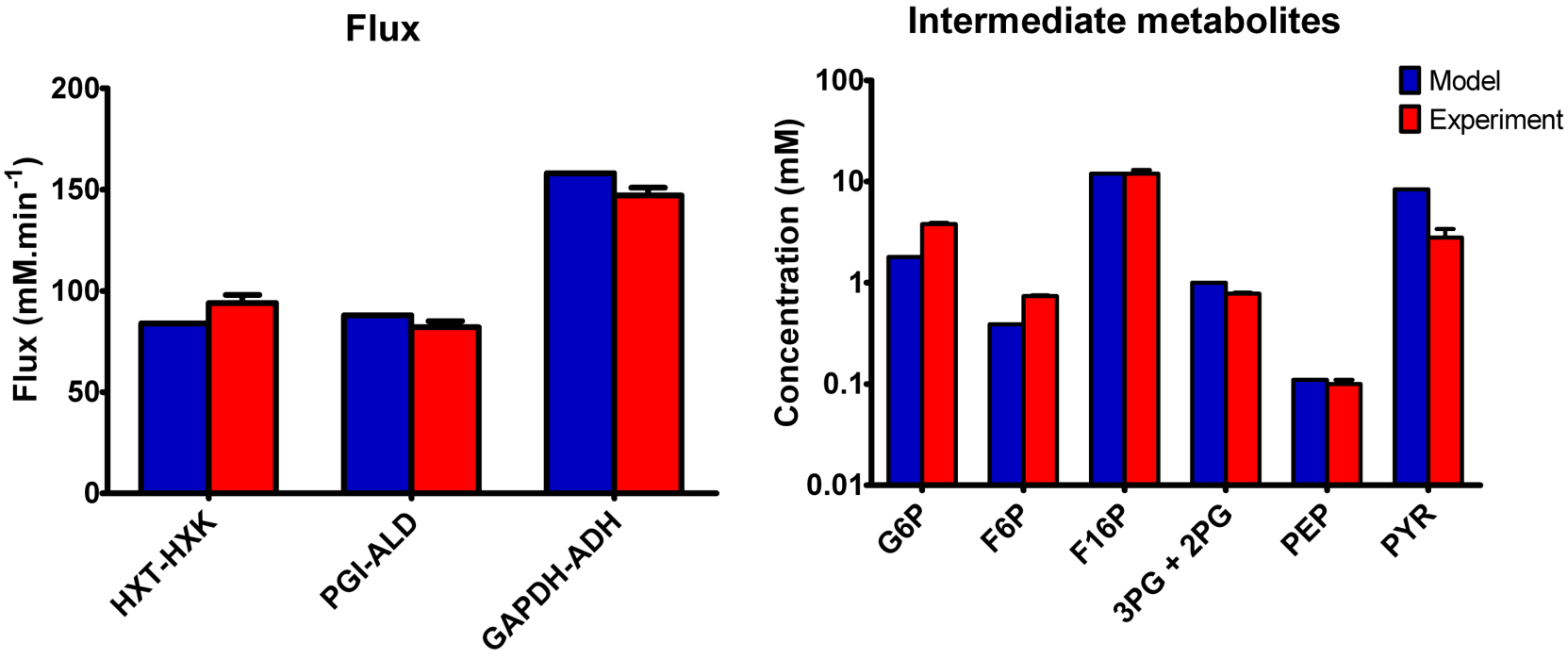
Model validation

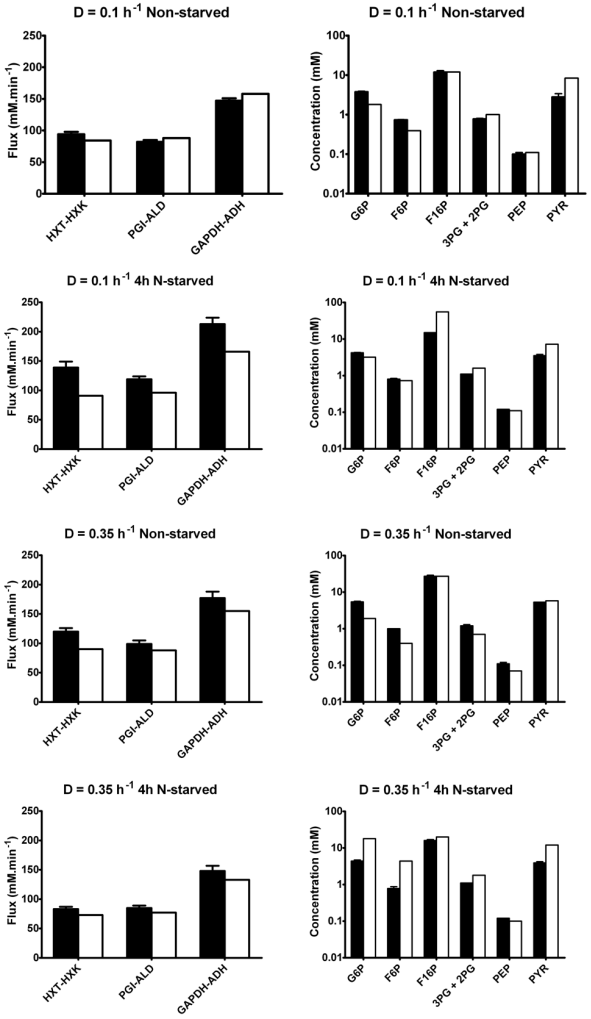
Classical example: glycolysis in bakers' yeast



- V_{max} values measured in samples from yeast chemostat culture D=0.1 h^{-1}
- → inserted in model → prediction of metabolite concentrations and fluxes
- Independent measurement of metabolite concentrations and fluxes

Model validation

Classical example: glycolysis in bakers' yeast



Experiment

Model

The model gives reasonable predictions for 4 independent culture conditions

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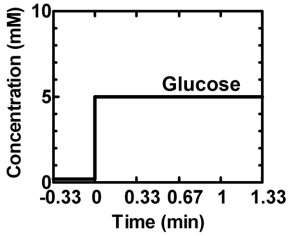
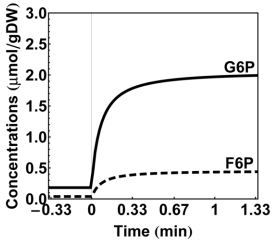
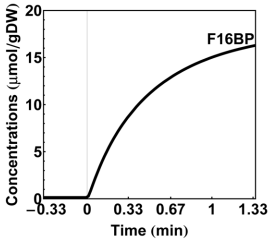
Model validation

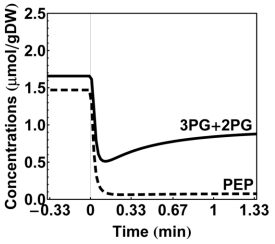
Classical example: glycolysis in bakers' yeast

Simulation

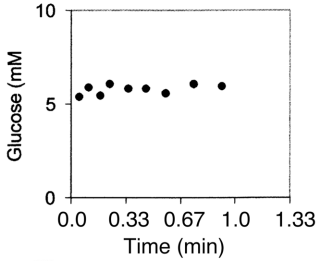


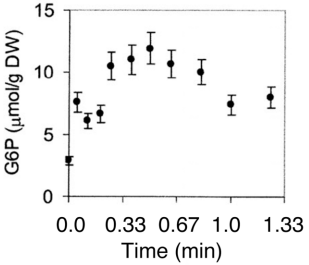


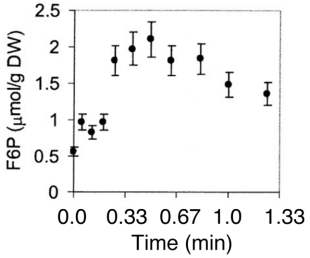


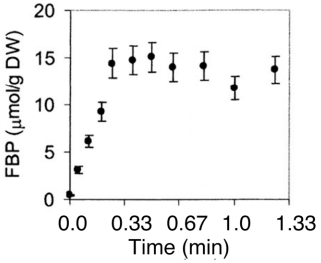


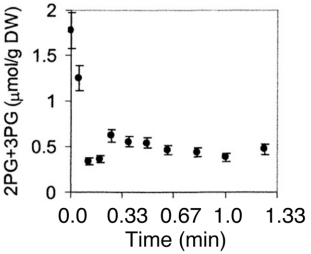
Experiment

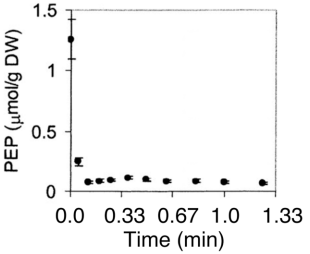








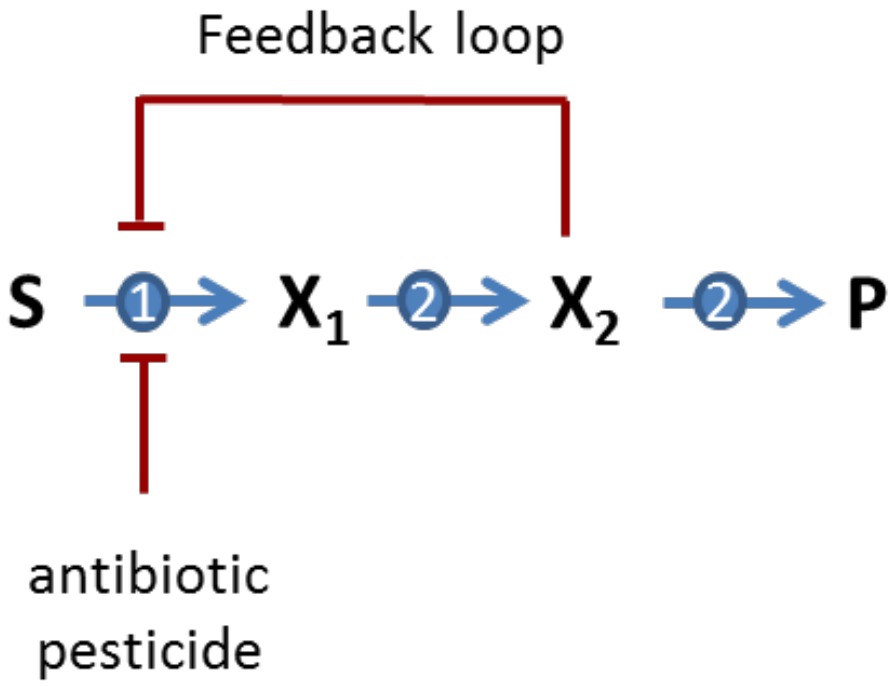




Experimental data were taken from Visser *et al.* 2004, Biotechnol. Bioeng.

… and under dynamic conditions

Enzyme inhibition



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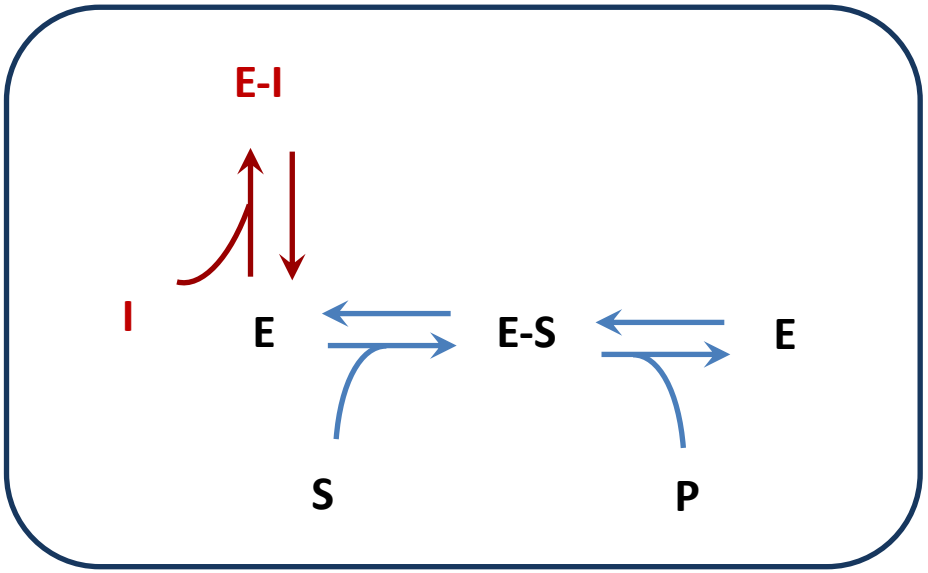
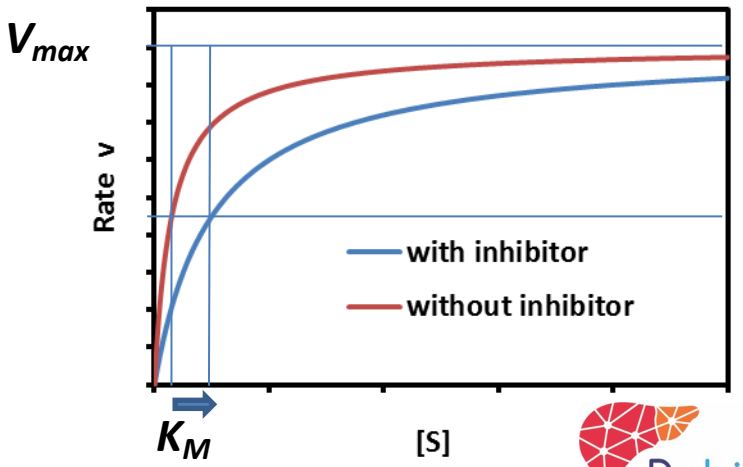
Competitive enzyme inhibition



$$v = \frac{V_{+\max}\dfrac{[\mathrm{S}]}{K_{\mathrm{MS}}}\left(1-\dfrac{[\mathrm{P}]}{[S]}\Big/K_{eq}\right)}{1+\dfrac{[\mathrm{S}]}{K_{\mathrm{MS}}}+\dfrac{[\mathrm{P}]}{K_{\mathrm{MP}}}+\dfrac{[I]}{K_I}}$$



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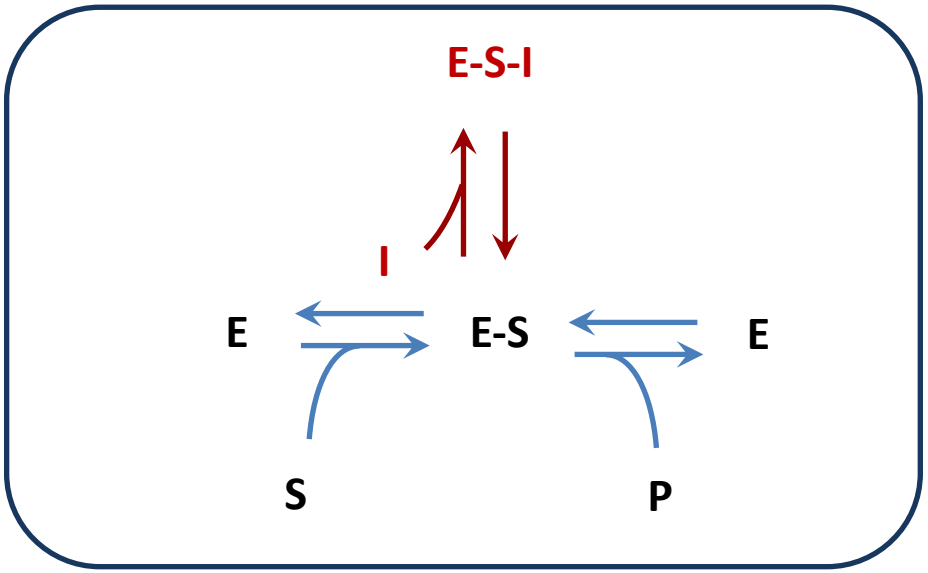
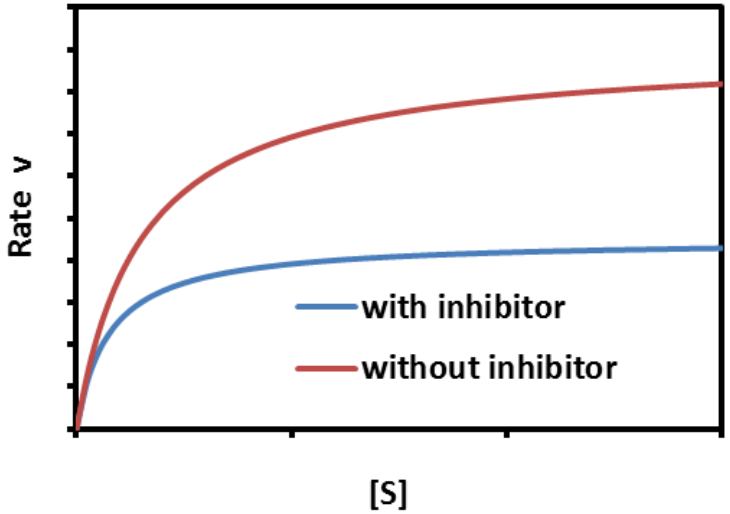
Uncompetitive enzyme inhibition





$$v = \frac{V_{\max,app}\dfrac{[S]}{K_{MS,app}}\left(1-\dfrac{[P]}{[S]}\Big/K_{eq}\right)}{1+\dfrac{[S]}{K_{MS,app}}+\dfrac{[P]}{K_{MP,app}}}$$

$V_{max,app} = V_{max}/(1+[I]/K_i)$

$K_{MS,app} = K_{MS}/(1+[I]/K_i)$

$K_{MP,app} = K_{MP}/(1+[I]/K_i)$

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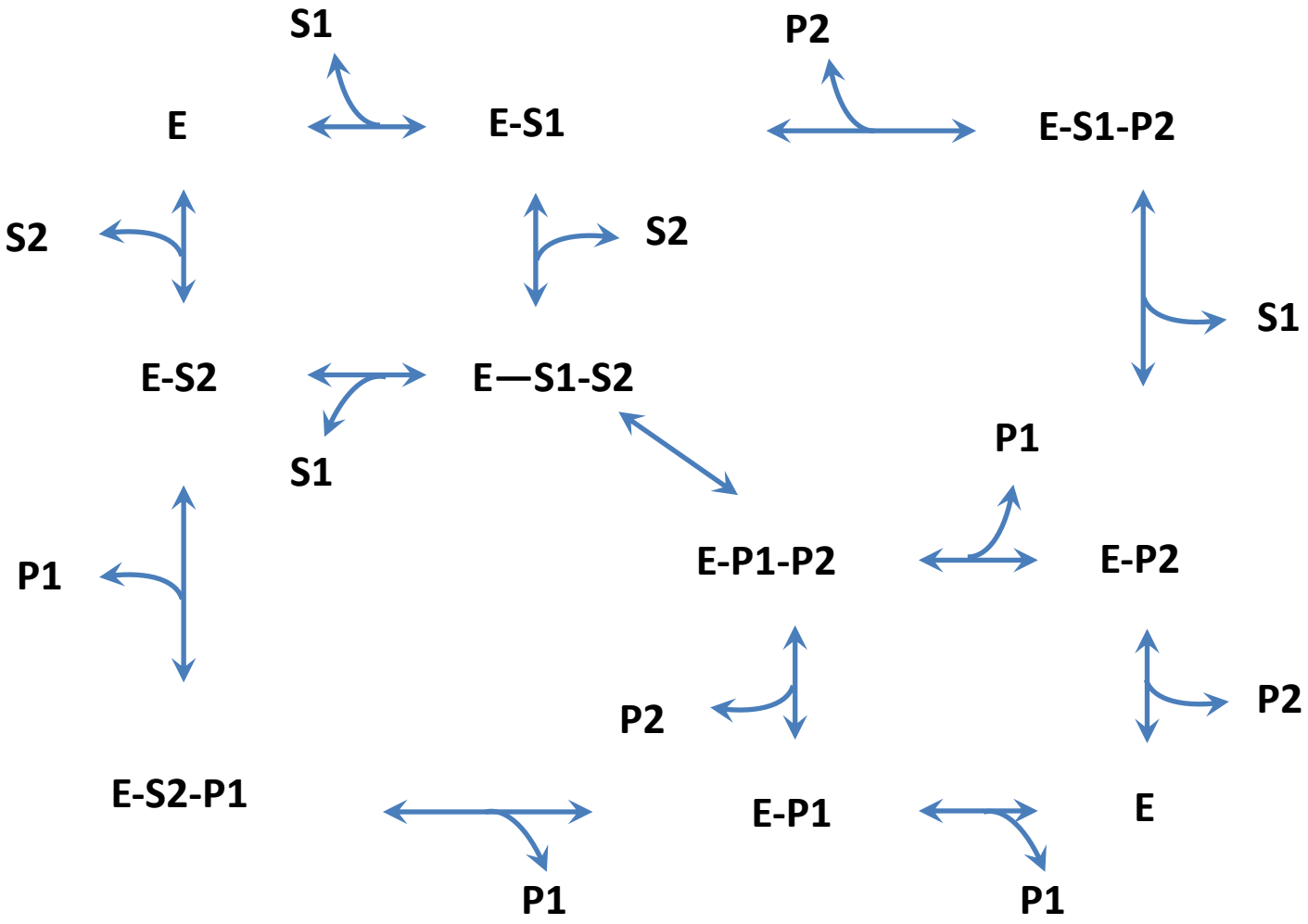
2-substrate 2-product reaction



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2-substrate 2-product reaction

$S_1 + S_2 \xrightarrow{2} P_1 + P_2$

$$v = \frac{V_{+\max} \frac{[S_1]}{K_{MS_1}} \cdot \frac{[S_2]}{K_{MS2}} \left(1 - \frac{[P_1] \cdot [P_2]}{[S_1] \cdot [S_2]} \Big/ K_{eq} \right)}{\left(1 + \frac{[S_1]}{K_{MS1}} + \frac{[P_1]}{K_{MP1}} \right) \cdot \left(1 + \frac{[S_2]}{K_{MS2}} + \frac{[P_2]}{K_{MP2}} \right)}$$

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2-substrate 2-product reaction with competitive inhibitor

competitive inhibitor

$$v = \frac{V_{+\max} \frac{[S_1]}{K_{MS_1}} \cdot \frac{[S_2]}{K_{MS2}} \left(1 - \frac{[P_1]\cdot[P_2]}{[S_1]\cdot[S_2]} \Big/ K_{eq}\right)}{\left(1 + \frac{[S_1]}{K_{MS1}} + \frac{[P_1]}{K_{MP1}} + \frac{[I]}{K_I}\right) \cdot \left(1 + \frac{[S_2]}{K_{MS2}} + \frac{[P_2]}{K_{MP2}}\right)}$$

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Model databases

Biomodels

JWS online

Both deliver models in the SBML format (systems biology markup language)

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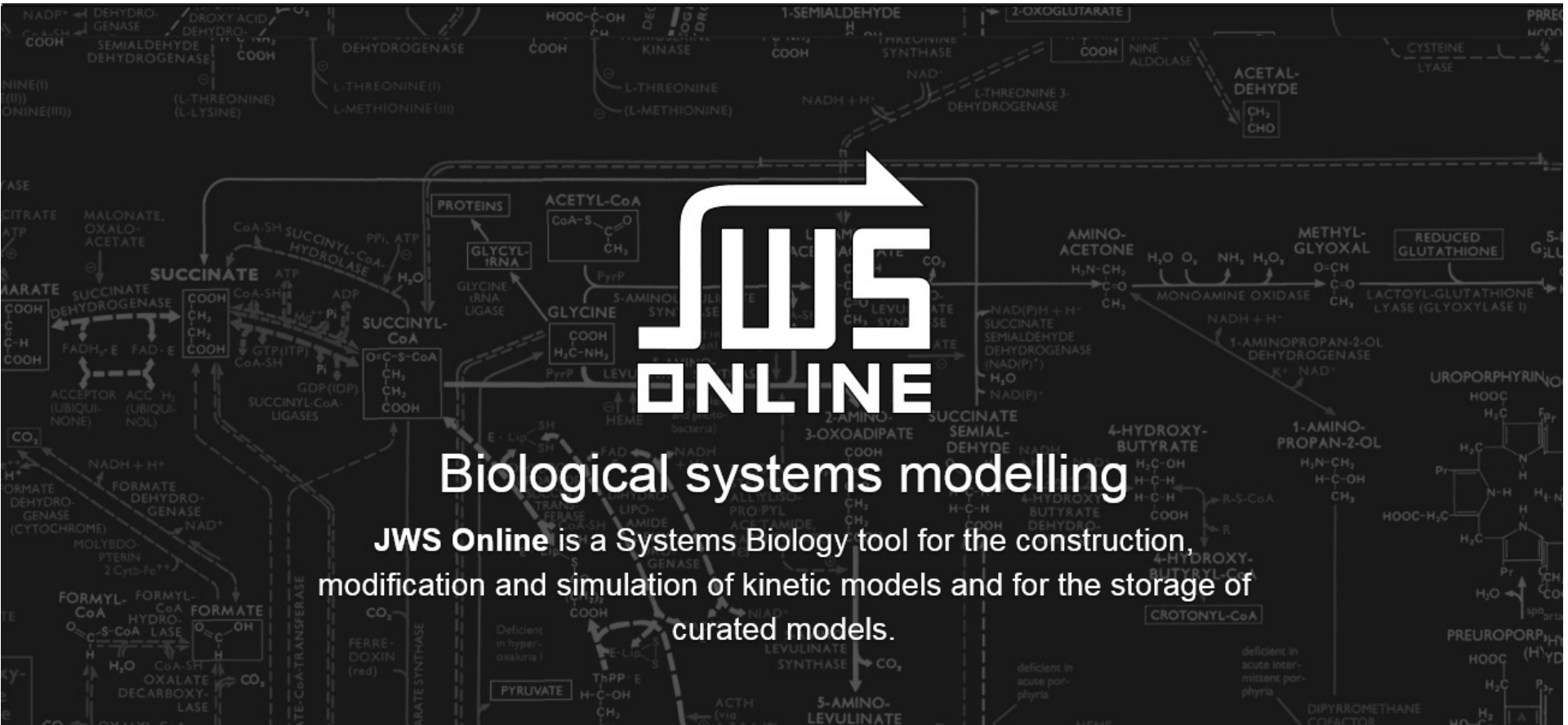
Implementation in JWS Online

Interactive modelling database at:

http://jjj.biochem.sun.ac.za/



Tutorial!