Systembiologie 551-1174-00L

ODE Modeling of Enzyme Kinetics

2 March, 2017

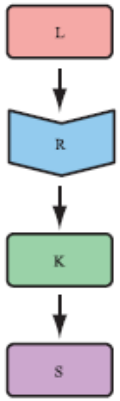
Uwe Sauer & Jörg Stelling

Content:

- Finding the appropriate modeling approach (US)
- Steady state vs. dynamics (US)
- From reaction kinetics to pathway dynamics (JS)
- Example: Substrate inhibition (JS)

Dynamic Analysis: Approach

Pictogram



Reaction list

$R+L \underset{k_{r1}}{\overset{k_{f1}}{\rightleftharpoons}} LR^*$

$LR^*+K \underset{k_{r2}}{\overset{k_{f2}}{\rightleftharpoons}} LR^*K$

$LR^*K \xrightarrow{k_{f3}} LR^*+K^*$

$K^*+S \underset{k_{r4}}{\overset{k_{f4}}{\rightleftharpoons}} K^*S$

$K^*S \xrightarrow{k_{f5}} K^*+S^*$

Your choice

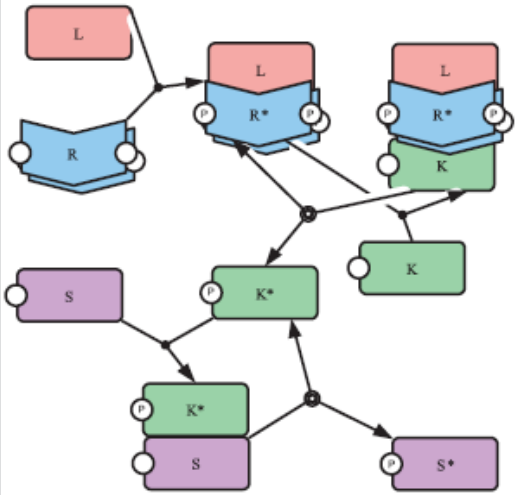
Approximations

if $[S]_0 >> [K^*]_0$: $\frac{d[K^*S]}{dt} \approx 0$

$$\frac{d[K^*]}{dt} = k_{f3}[LR^*K] + k_{f5}[K^*S] - \frac{k_{f5}[K^*]_0[S]}{\left(\frac{k_{r4}+k_{f5}}{k_{f4}}\right)+[S]}$$

$$\frac{d[S^*]}{dt} = \frac{k_{f5}[K^*]_0[S]}{\left(\frac{k_{r4}+k_{f5}}{k_{f4}}\right)+[S]}$$

Pathway diagram



Mass-action kinetics

$$\frac{d[R]}{dt} = -k_{f1}[L][R] + k_{r1}[LR^*]$$

$$\frac{d[LR^*]}{dt} = k_{f1}[L][R] - k_{r1}[LR^*] - k_{f2}[LR^*][K] + k_{r2}[LR^*K] + k_{f3}[LR^*K]$$

$$\frac{d[LR^*K]}{dt} = k_{f2}[LR^*][K] - k_{r2}[LR^*K] - k_{f3}[LR^*K]$$

$$\frac{d[K]}{dt} = -k_{f2}[LR^*][K] + k_{r2}[LR^*K]$$

$$\frac{d[K^*]}{dt} = k_{f3}[LR^*K] - k_{f4}[K^*][S] + k_{r4}[K^*S] + k_{f5}[K^*S]$$

$$\frac{d[S]}{dt} = -k_{f4}[K^*][S] + k_{r4}[K^*S]$$

$$\frac{d[K^*S]}{dt} = k_{f4}[K^*][S] - k_{r4}[K^*S] - k_{f5}[K^*S]$$

$$\frac{d[S^*]}{dt} = k_{f5}[K^*S]$$

Figure from: Aldridge et al. (2006) Nature Cell Biology 8: 1195.

ODE Models: General and 'Biological' Form

$$\frac{d\,\boldsymbol{x}(t)}{dt} = \boldsymbol{f}\left(\boldsymbol{x}(t), \boldsymbol{p}, \boldsymbol{u}(t)\right) \quad , \quad \boldsymbol{x}(t_0) = \boldsymbol{x_0}$$

- System of ordinary, first-order, linear or nonlinear differential equations (ODEs) characterized by:
  - Right hand sides $\boldsymbol{f}(\boldsymbol{x}(t),\boldsymbol{u}(t),\boldsymbol{p})$ = reaction rates.
  - System states $\boldsymbol{x}(t)$ = dynamic concentrations.
  - Parameters $\boldsymbol{p}$ = kinetic constants.
  - Inputs $\boldsymbol{u}(t)$ = external manipulations.
  - Initial conditions $\boldsymbol{x}(t_0)$ = initial system state.

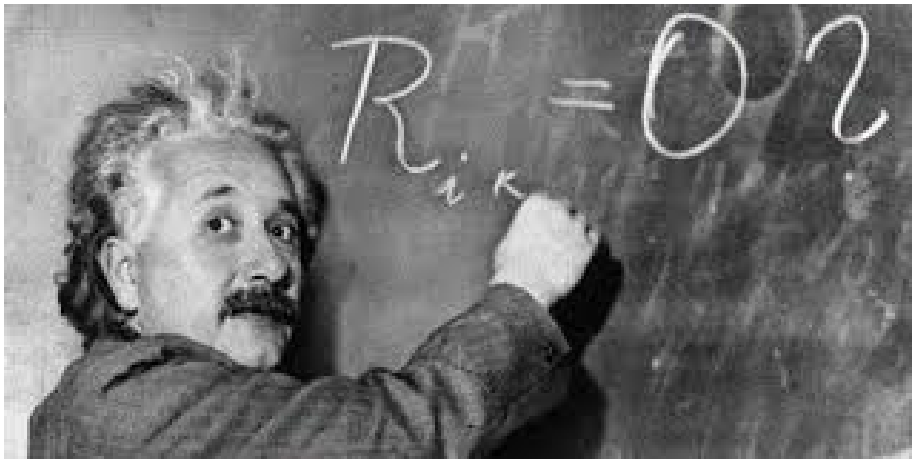
ODE Models: Solution

$$\frac{d\,\boldsymbol{x}(t)}{dt} = \boldsymbol{f}\left(\boldsymbol{x}(t), \boldsymbol{p}, \boldsymbol{u}(t)\right) \quad , \quad \boldsymbol{x}(t_0) = \boldsymbol{x_0}$$

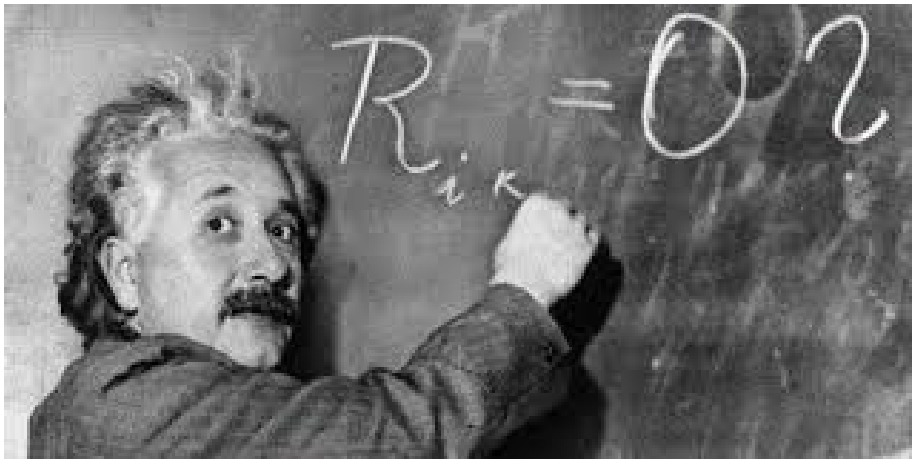
- Fundamentally: Existence and uniqueness of solution of finding $\boldsymbol{x}(t)$ with given initial condition $\boldsymbol{x_0}$ is guaranteed.

- Three possible "solution" methods:
  - **Analytical** → Only applicable for simple systems.
  - **Numerical** → Simulation (nearly) always possible.
  - **Graphical** → Qualitative analysis methods.

"Everything should be made as simple as possible ..."



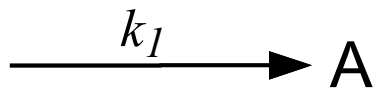
"Everything should be made as simple as possible, but not simpler."



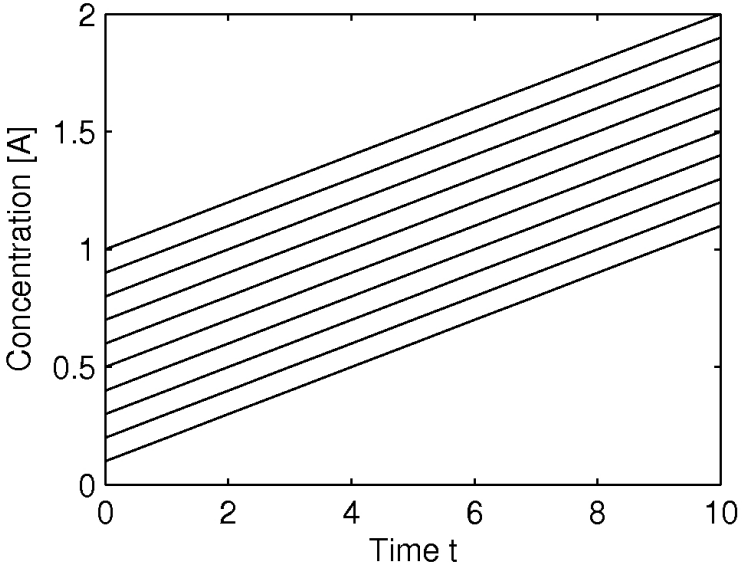
ODE Models: Elementary Reactions

- Constant production: $\xrightarrow{k_1}$ A
- Linear degradation: A $\xrightarrow{k_1}$
- Dimerization: A + B $\xrightarrow{k_1}$ A·B
- Monomolecular conversion: A $\xrightarrow{k_1}$ B
- Bimolecular conversion: A + B $\xrightarrow{k_1}$ C

Elementary Reactions: Constant Production

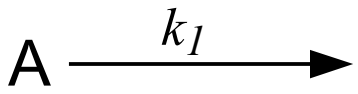


$$\frac{d[A]}{dt} = k_1 \quad \Rightarrow \quad [A] = [A]_0 + k_1 \cdot t$$



- Constant synthesis, for example, by considering only metabolite production, not degradation.
- Analytic solution → From initial concentration $[A]_0$, linear increase of $[A]$ without bound over time.

Elementary Reactions: Linear Degradation

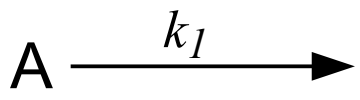


$$\frac{d[A]}{dt} = -k_1[A] \quad \Rightarrow \quad \frac{d[A]}{[A]} = -k_1\,dt$$

$$\int_{[A]_0}^{[A]} \frac{d[A]}{[A]} = -k_1 \int_0^t dt \quad \Rightarrow \quad [A] = [A]_0 \cdot e^{-k_1 \cdot t}$$

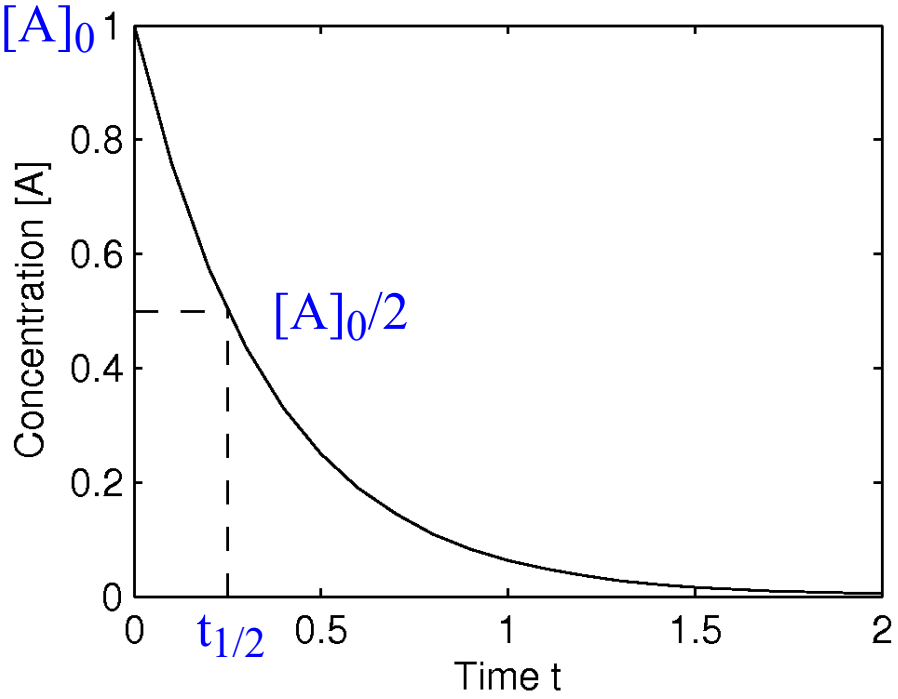
- Degradation processes depend only on concentration of degraded substance → Solve by separating variables.
- Biological example where only degradation is relevant?

Elementary Reactions: Linear Degradation



$$[A] = [A]_0 \cdot e^{-k_1 \cdot t}$$

$$t_{1/2} = \frac{\ln(2)}{k_1}$$

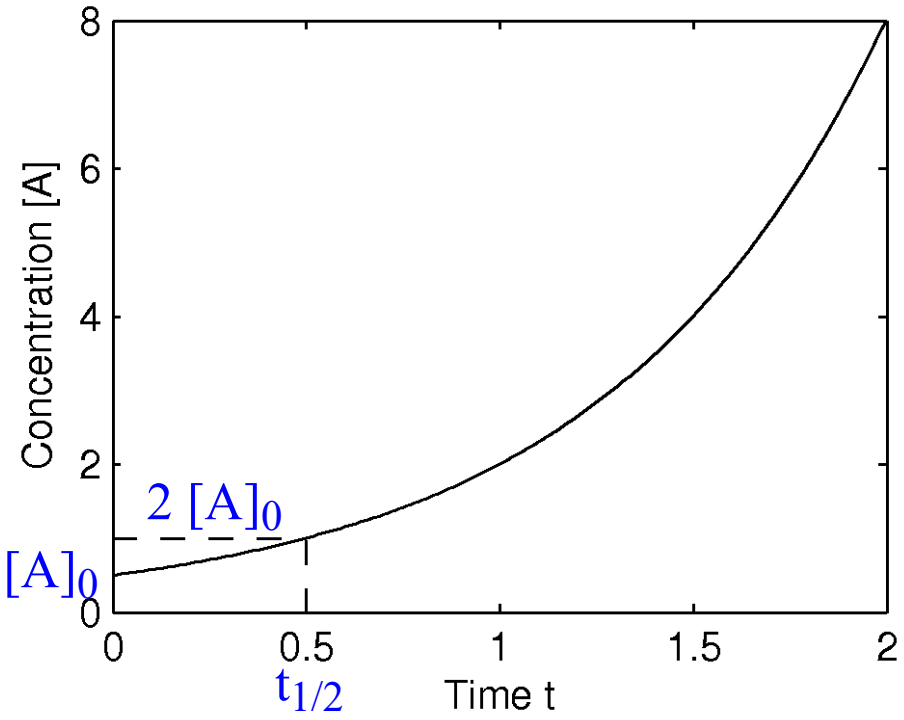


- Characteristic half-life $t_{1/2}$ (time-independent).
- Determination of kinetic constant k_1 via log(measured concentration time course).

Elementary Reactions: Autocatalysis

$$[A] = [A]_0 \cdot e^{+k_1 \cdot t}$$

$$t_{1/2} = \frac{\ln(2)}{k_1}$$



- Identical approach for autocatalytic production.
- Unbounded explosion → Doubling time $t_{1/2}$.
- Biological example for exponential proliferation?

Elementary Reactions: Dimerization

$$A + B \xrightarrow{k_1} A{\cdot}B$$

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k_1[A][B] \;,\; \frac{d[A\cdot B]}{dt} = +k_1[A][B]$$

- Dynamic system in three variables (species).
- Simplification by exploiting mass conservation:

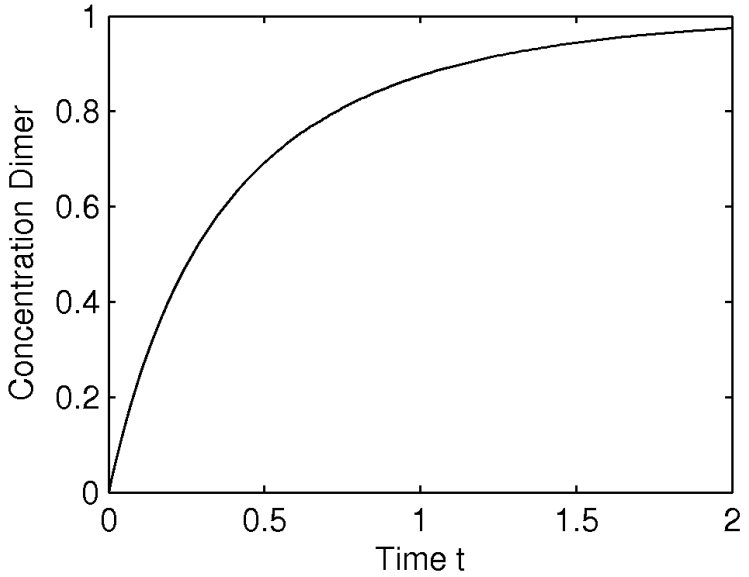
$$[A] = [A]_0 - [A\cdot B] \;,\; [B] = [B]_0 - [A\cdot B]$$

- What entities could A and B be, biologically?

Elementary Reactions: Dimerization

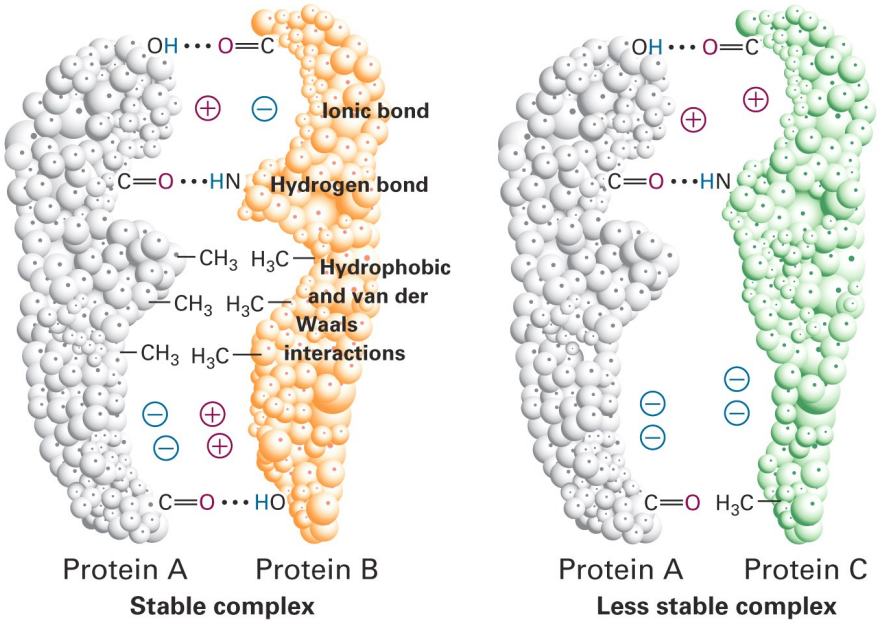
$$A + B \xrightarrow{k_1} A \cdot B$$

$$[A \cdot B] = \frac{[A]_0[B]_0 \cdot \left(1 - e^{-k_1\left([A]_0 - [B]_0\right)t}\right)}{[A]_0 - [B]_0 e^{-k_1\left([A]_0 - [B]_0\right)t}}$$



- Saturation: Asymptotic complex concentration equals initial concentration of component in short supply ($[B]_0$).

Composite Reaction: Reversible Dimerization



$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A\cdot B$$

$$\frac{d[A\cdot B]}{dt} = +k_1[A][B] - k_{-1}[A\cdot B]$$

H. Lodish et al., Molecular Cell Biology, 5th ed., 2004.

- Reversible dimerization reaction: Steady state solution

$$\frac{d[A\cdot B]}{dt} = 0 \quad \Rightarrow \quad [A\cdot B]_{ss} = \frac{k_1}{k_{-1}}$$

- Biochemical assays: Dissociation constant $K_D = \dfrac{k_{-1}}{k_1}$

Composite Reactions: Production & Degradation

$$\frac{d[A]}{dt} = +k_1 \cdot u - k_2[A]$$

- Production of A controlled by input signal u, coupled to linear degradation of component A.
- Example: Controlled gene expression and degradation of mRNA.
- How do parameters influence the dynamics?

Composite Reactions: Production & Degradation

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

- Steady state ($t \rightarrow \infty$) is proportional to input u:

$$[A]_{ss} = \frac{k_1 \cdot u}{k_2}$$

- **Dynamics determined by degradation only.**

Reaction Kinetics: Pathway Dynamics

- Enzyme kinetic rate laws **approximate** dynamics in larger reaction networks (in contrast to detailed mass-action kinetics).

- Example Michaelis-Menten kinetics (lecture 1):

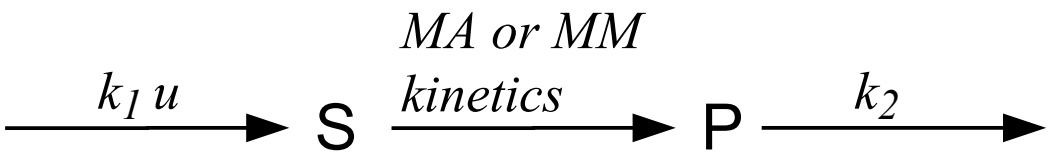
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \xrightarrow{k_2} E + P$$

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

- Predicted dynamics in networks depend on the exact kinetics used → Linear pathway example.

Reaction Kinetics: Pathway Dynamics

- **Example:** Chain of consecutive reactions:

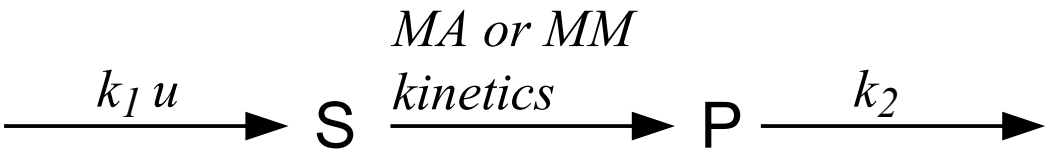


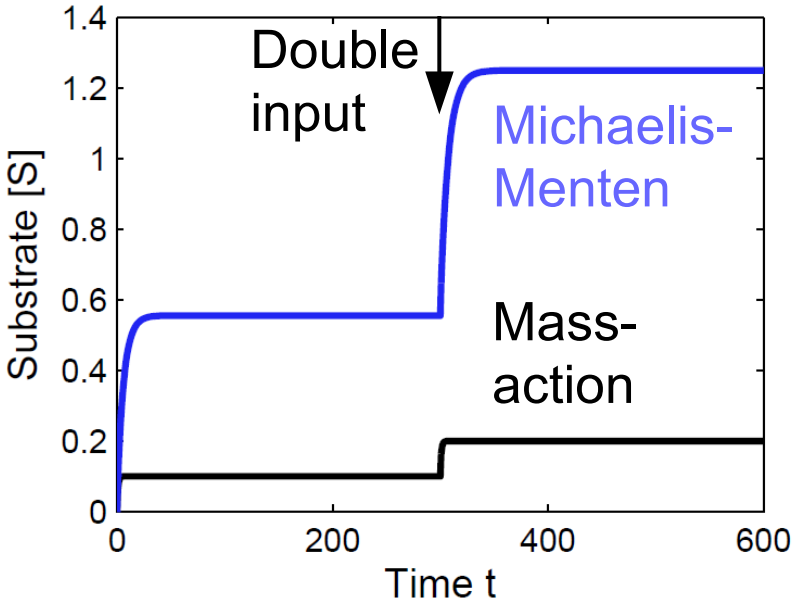
- For the S→P conversion assume either mass-action kinetics: $v_{MA}([S]) = v_{max}[S]$

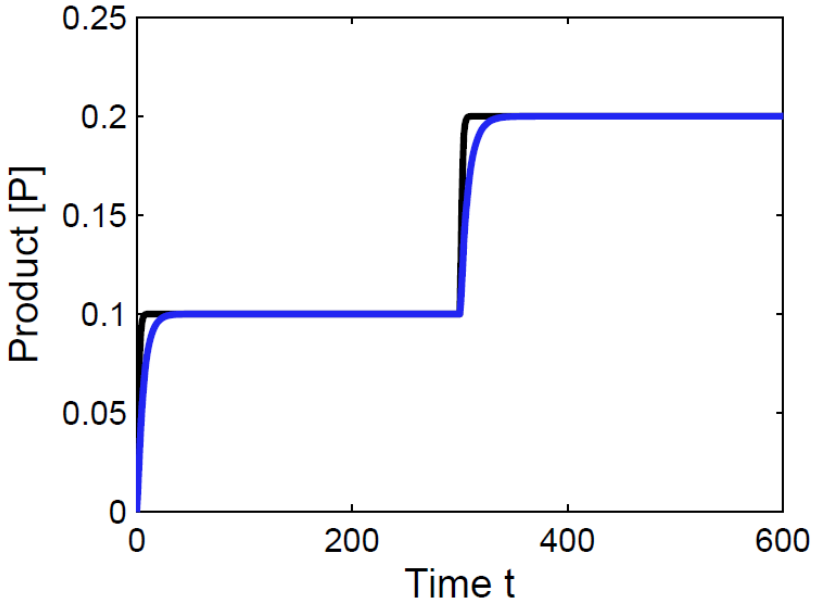
  or Michaelis-Menten kinetics: $v_{MM}([S]) = \frac{v_{max}[S]}{[S]+K_M}$

- Impact on the dynamic / steady-state behavior?

Reaction Kinetics: Pathway Dynamics







- Assumed kinetics influence predictions of dynamics and of steady-state substrate concentration.
- Same product concentration in steady-state. (Why?)

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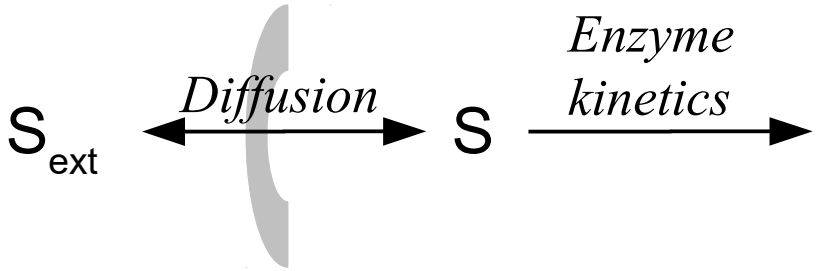
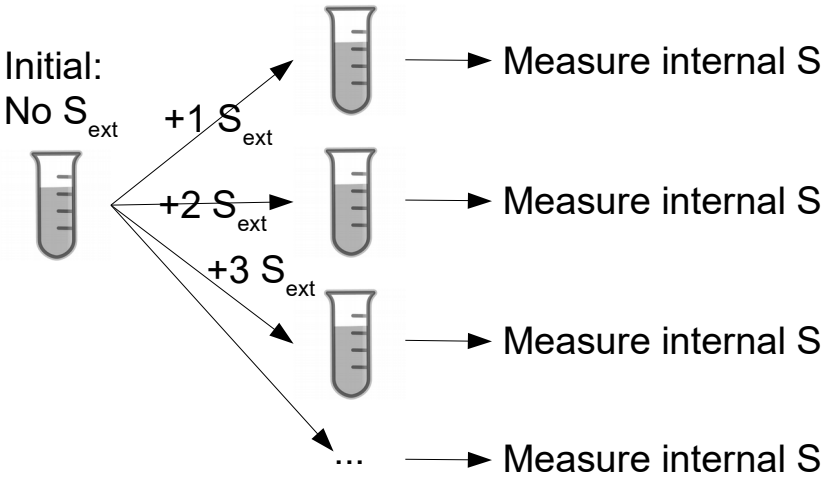
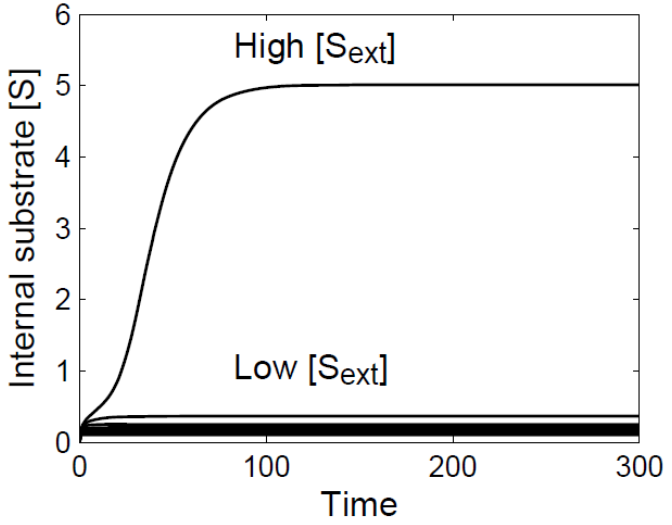
- From reaction kinetics to pathway dynamics (JS)
- Example: Substrate inhibition (JS)

Example: Pathway Dynamics



- Extracellular substrate S_{ext} is transported and converted.
- How can you explain the experimental data shown below?





Reaction Kinetics: Substrate Inhibition

- Substrate inhibition:
  A reactant (substrate) causes a *decrease* in the rate of product formation as its concentration *increases*.

- Estimated to occur in ~20% of enzymes.

A short list of enzymes that are subject to substrate inhibition

4-hydoxyphenylpyruvate hydroxylase	kynunrenine aminotransferase
acetylcholinesterase	lactate dehydrogenase
adenosine 5′-pyrophosphate sulfurylase	L-amino acid oxidase
adenosine kinase	lipoxygenase
adenylate cyclase	malate dehydrogenase
aldehyde dehydrogenase	*N*-methyl transferase
alanine aminopeptidase	nucleotidediphosphate kinase
alcohol dehydrogenase	*O*-acetylserine sulfhydrolase
aldehyde dehydrogenase	octopine dehydrogenase
aldose reductase	PAPS synthetase
alkaline phosphatase	phenol sulfotransferase
aminoacylase-I	prenyltransferase
aminoimidazolecarboximide ribotide	purine nucleoside phosphorylase
transformylase	pyrophosphatase
arylamidase	pyruvate decarboxylase
aspartate transcarbamylase	pyruvate kinase
carboxypeptidase	ribonuclease A
cholinesterase	ribonuclease T1
citrate synthase	ribonuclease T2
cytochrome P450 (some)	ribonucleoside diphosphate reductase
diamine oxidase	serine hydroxymethyltransferase
diphospoglyceromutase	sucrose-6-glycosyltransferase
DNA-methyltransferase	sulfotransferases
enolase	trannsglucosyl-amylase
esterase	tRNA nucleotidyltransferase
formyltetrahydrofolate synthase	trypsin
fructose-I,6-bisphosphatase	tryptophan hydroxylase
galactosyltransferase	tyrosine hydroxylase
gentamycin acetyltransferase	urease
glutamate dehydrogenase	uridine kinase
glutathione reductase	xanthine oxidase
glycerol-3-phosphate dehydrogenase	α-D-galactosidase
HIV1-reverse transcriptase	α-glucosidase
isocitrate dehydrogenase	β-fructofuranosidase
	β-hydroxysteroid dehydrogenase

Figure from: M.C. Reed et al. (2010) Bioessays 32: 422.

Reaction Kinetics: Substrate Inhibition

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \xrightarrow{k_2} E + P$$

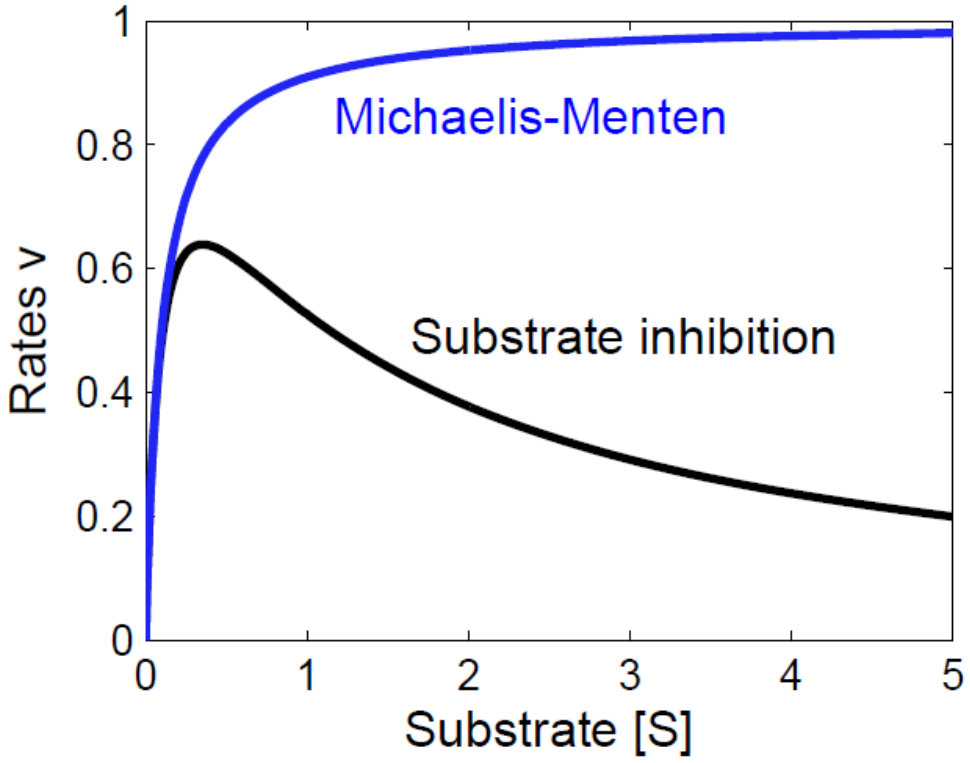
$$E{\cdot}S + S \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E{\cdot}S{\cdot}S$$

- Binding of second substrate molecule → 'Unproductive' enzyme-substrate complex, inhibition constant $K_I = \frac{k_{-3}}{k_3}$

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

- Inhibition term has effect at *high* substrate concentrations.

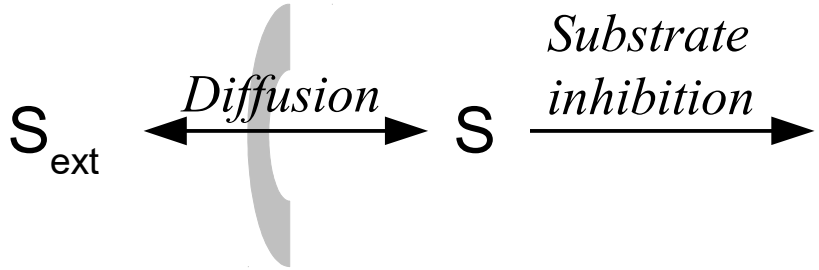
Reaction Kinetics: Substrate Inhibition



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

- Inhibition term has effect at *high* substrate concentrations.

Substrate Inhibition: Pathway Dynamics

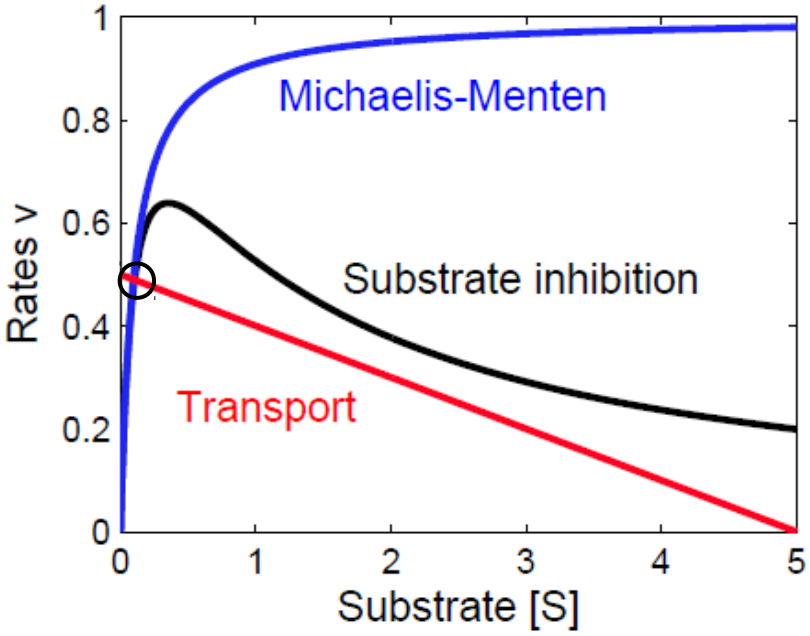


- Extracellular substrate S_{ext} is transported and converted.
- Assume diffusion as a transport mechanism with rate

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

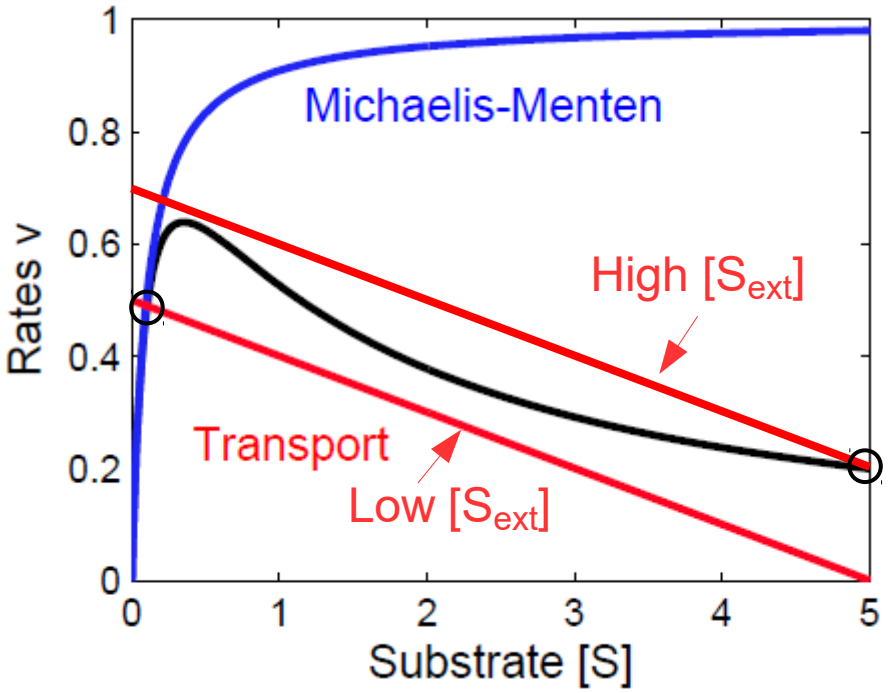
and diffusion constant $D \rightarrow$ Resulting pathway dynamics?

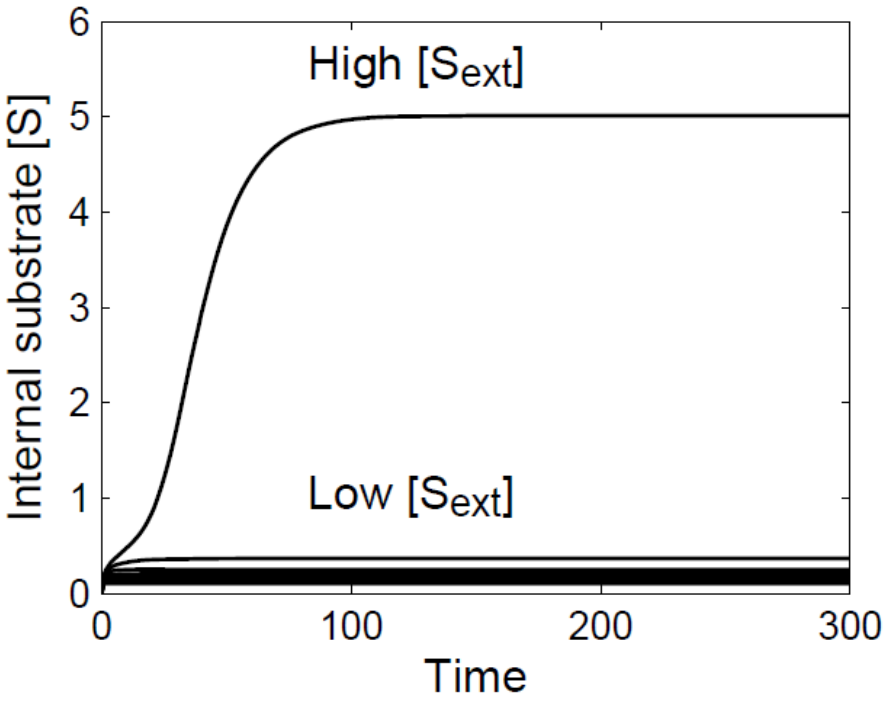
Substrate Inhibition: Pathway Dynamics



- For a **fixed** external substrate concentration: High internal substrate → Transport rate < conversion rate until rates are balanced (and vice-versa).
- **Example of a qualitative (graphical) analysis.**

Substrate Inhibition: Pathway Dynamics





- Start at $[S_{ext}] = 0$, then set to variable (but time-constant) $[S_{ext}]$: Rates eventually balance, but strong **'switch'** in internal substrate concentration.

Summary: Teaching Goal III

- Pathway maps can be translated to dynamic models with biochemical reaction networks as an intermediary step.

- Dynamic models can be developed at different levels of detail → Mass-action vs. enzyme kinetics.

- Choices in kinetic assumptions influence the predicted dynamics → Linear reaction pathway.

Summary: Teaching Goal IV

- Even networks of only two reactions can show non-intuitive dynamic behavior where the exact kinetics matter (or not) → Linear reaction pathway.

- Dynamic system responses are hard to predict without formal analysis → Changes vs. constant behaviors in linear reaction pathway; Switch-like response to external input changes in substrate-inhibition system.