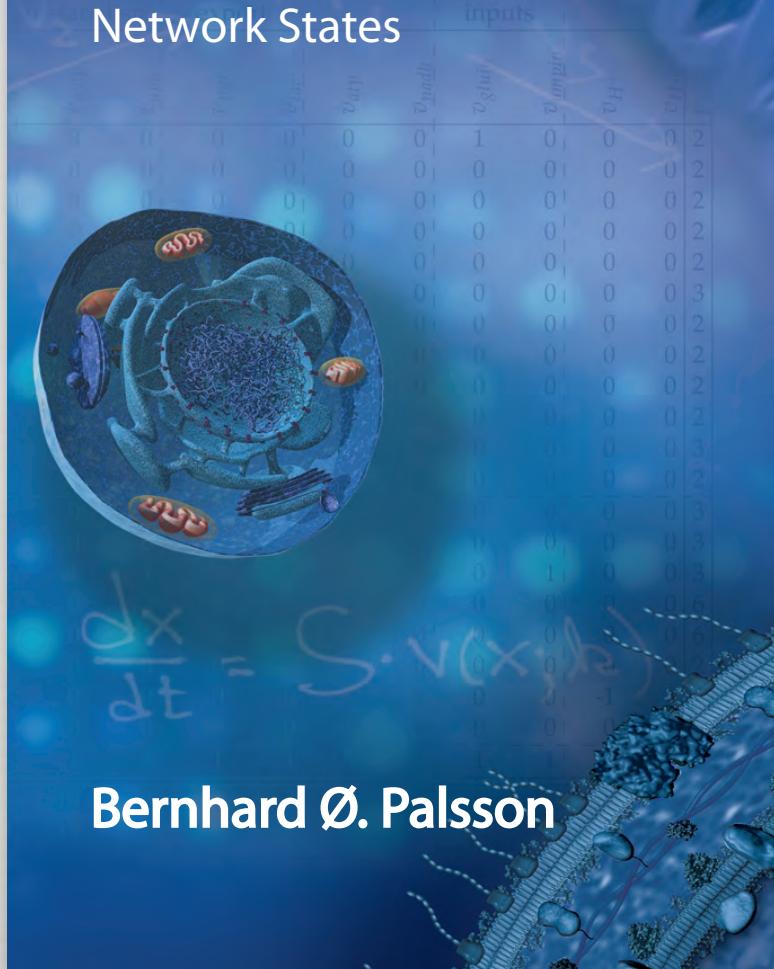


Systems Biology

Simulation of Dynamic Network States



Lecture #5

Enzyme Kinetics

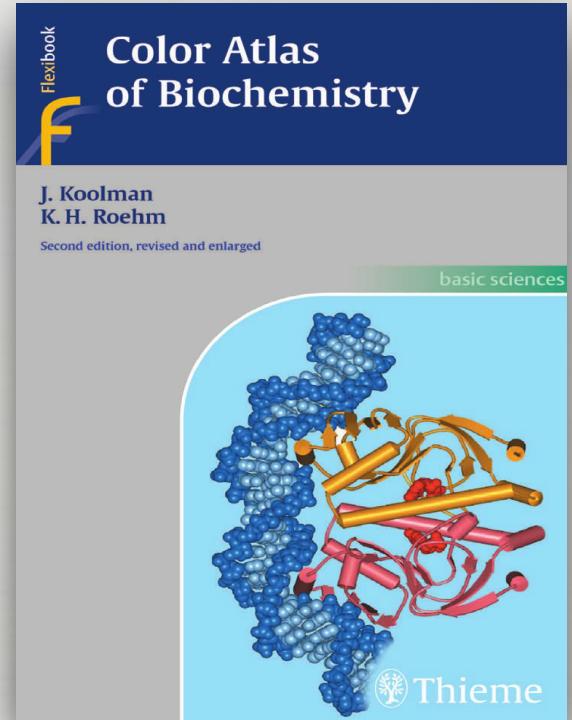
Bernhard Ø. Palsson

Outline

- The principles of enzyme catalysis
- Deriving rate laws for enzyme
- Ex #1: Michaelis-Menten kinetics
- Ex #2: Hill kinetics
- Ex #3: The symmetry model

Some basic information

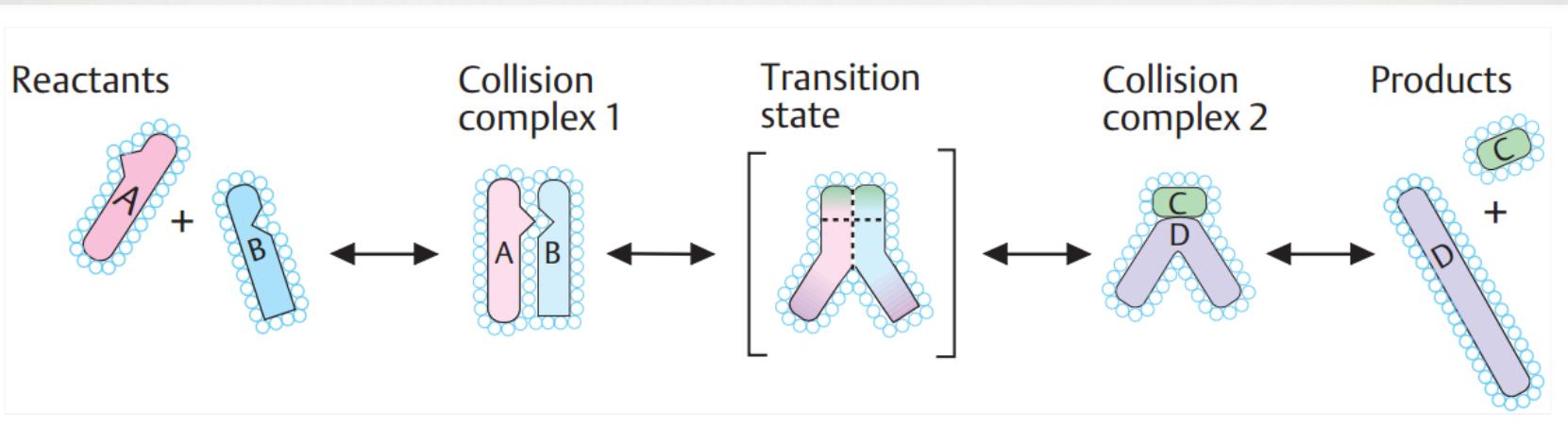
ENZYME CATALYSIS



Accessible from
UCSD IP
addresses:

[https://ebooks.thieme.com/
pdfreader/color-atlas-
biochemistry-3rd-ed](https://ebooks.thieme.com/pdfreader/color-atlas-biochemistry-3rd-ed)

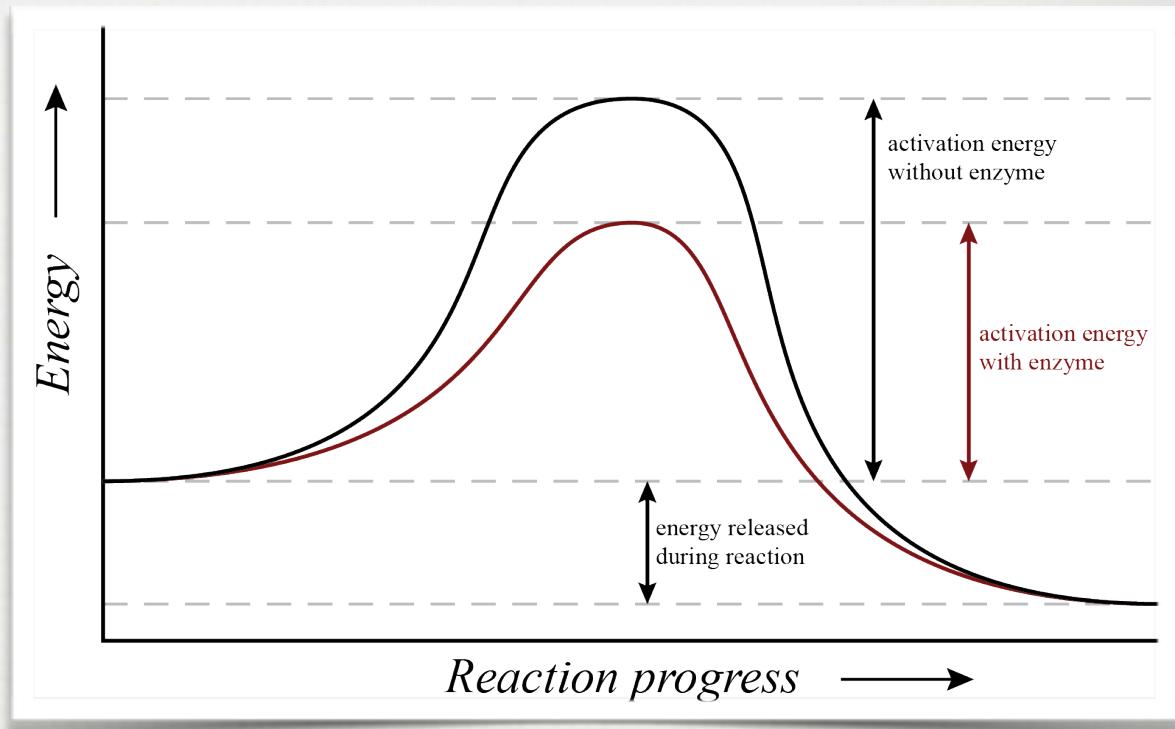
Chemical reactions



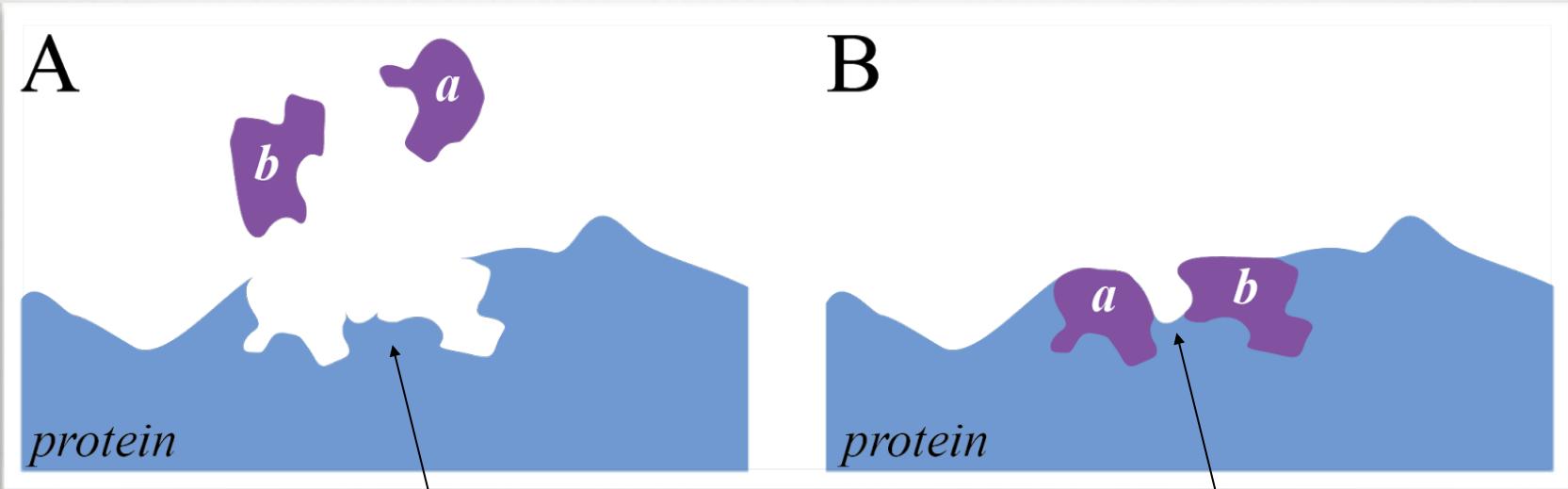
Chemical reactions rely on reactants coming together in solution in a specific way (angle and energy)

Reaction activation energy

Reactions are catalyzed by *enzymes*, specialized proteins that make a process more likely by lowering the activation energy



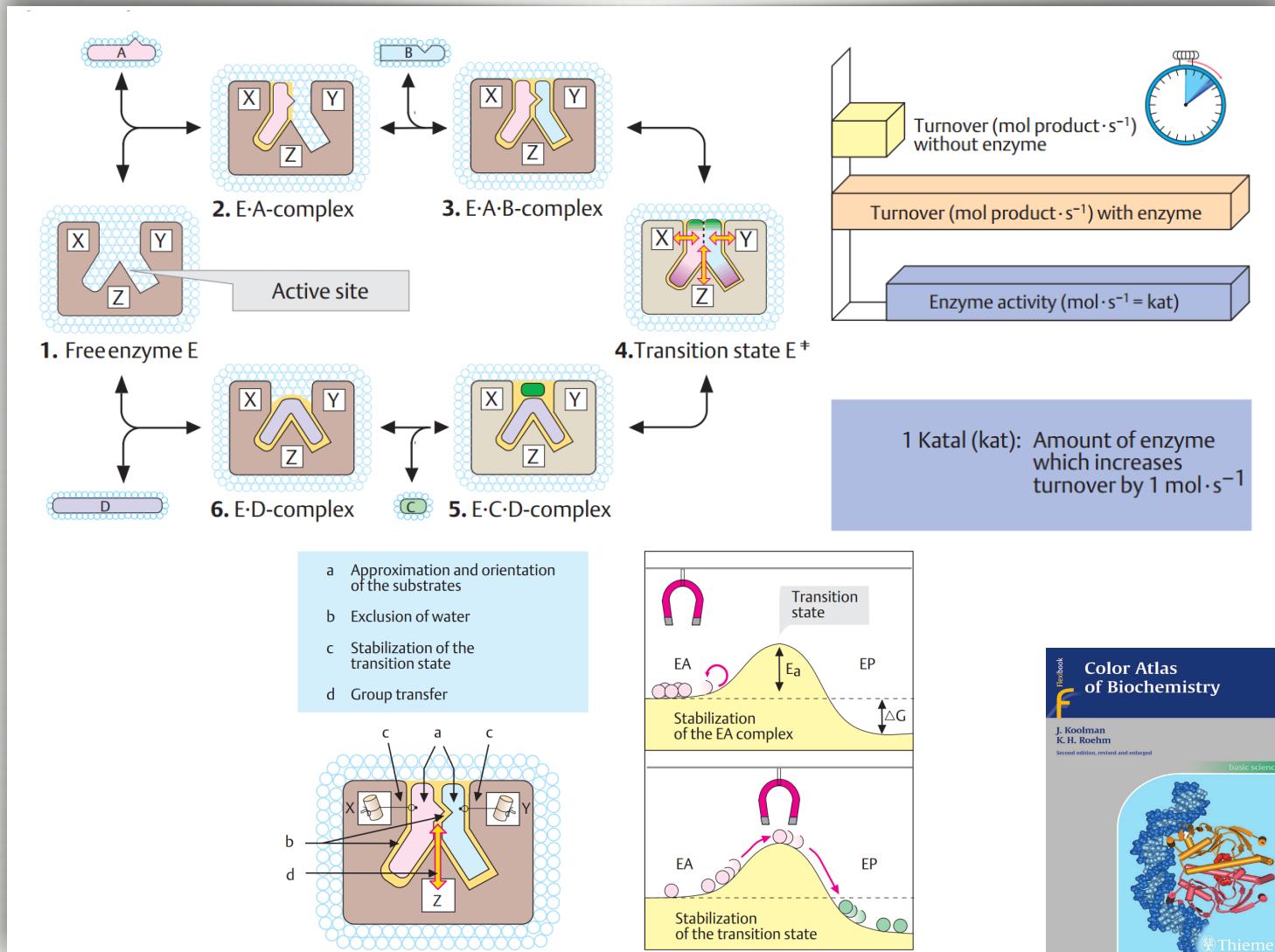
Enzyme catalysis: basics



the surface has
'information' about the
reactants and ...

... the 'right' angle for the
reaction to take place

Enzyme catalysis: basics



Koolman, J. and Roehm, K.H., Color Atlas of Biochemistry, 2nd Ed., Thieme, New York, 2004.

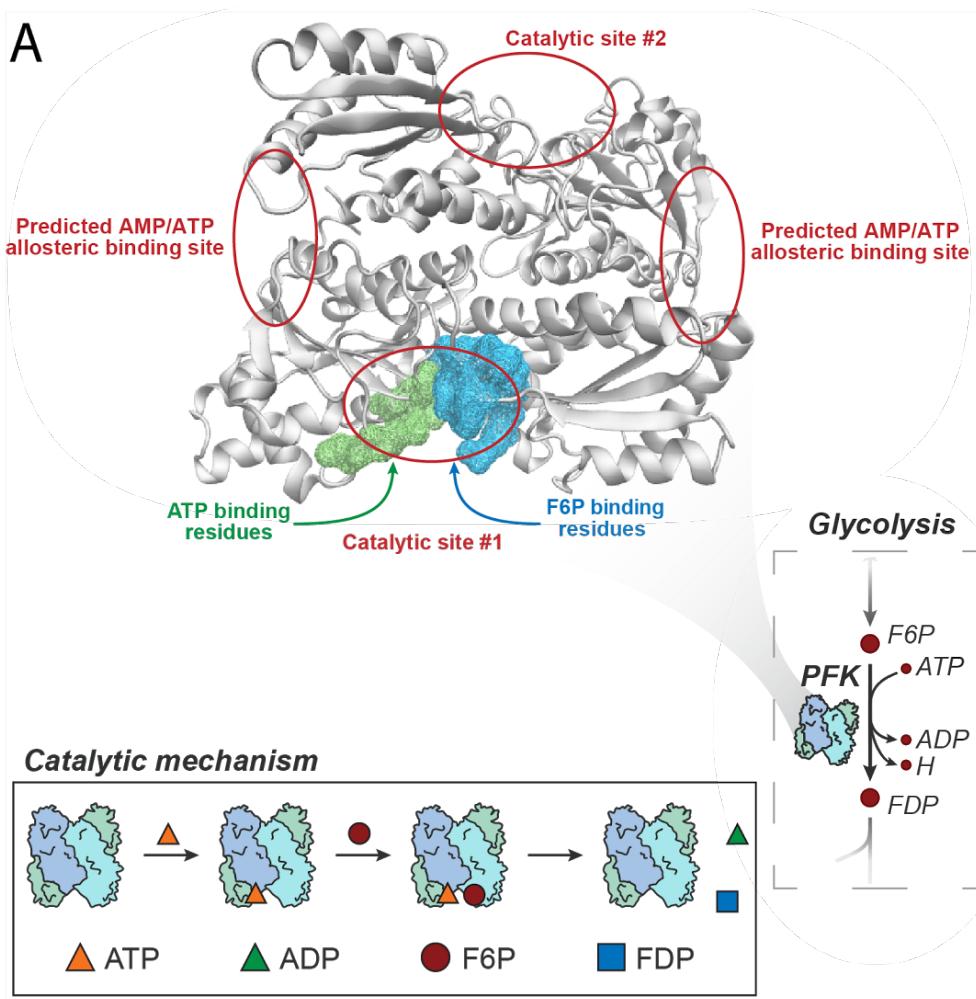
EC Classification of enzymes

Class	Reaction type	Important subclasses
1 Oxidoreductases	<p>○ = Reduction equivalent</p> <p>A_{red} + B_{Ox} ⇌ A_{Ox} + B_{red}</p>	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	<p>A-B + C ⇌ A + B-C</p>	C ₁ -Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	<p>A-B + H₂O ⇌ A-H + B-OH</p>	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	<p>A + B ⇌ A-B</p>	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	<p>A ⇌ Iso-A</p>	Epimerases <i>cis trans</i> Isomerases Intramolecular transferases
6 Ligases ("synthetases")	<p>B + A + XTP ⇌ A-B + XDP</p> <p>X=A, G, U, C</p>	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Linear reaction

EC # = enzyme
commission #
EC x.x.x.x

Details for specific cases are available



Phosphofructokinase

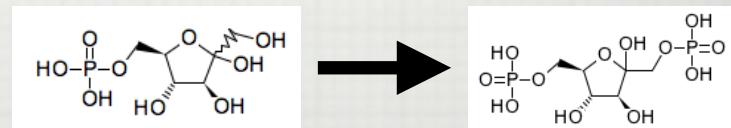
Transferase (phosphotransferase)

EC: 2.7.1.11

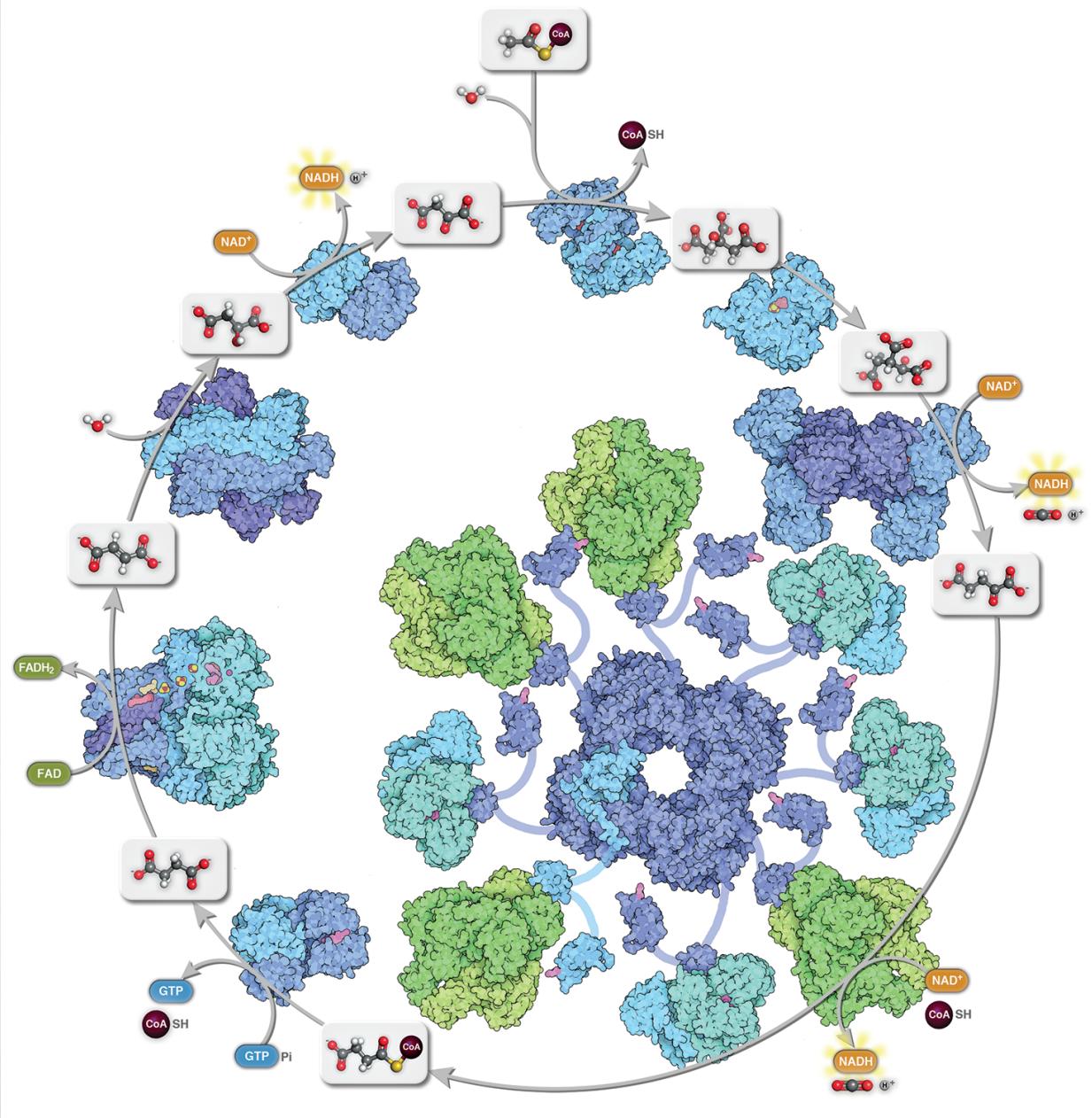
Gene: *pfkP* (there are other isozymes)

Sequence length: 761

PDB ID: 4WL0 (expressed in RBCs)



Enzymes catalyze pathways



Mathematical description of catalytic activity

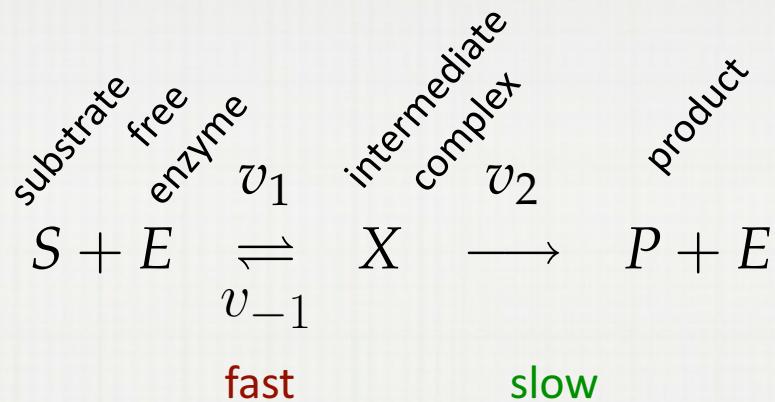
DERIVING RATE LAWS

Deriving Enzymatic Rate Laws from Postulated Reaction Mechanisms

1. Formulate dynamic mass balances on elementary reactions
2. Identify time invariants (Left null space)
3. Reduce to the dynamically independent variables
4. Apply simplifying assumptions:
 - The QSSA or the QEA to reduce the number of independent variables
5. Use numerical integration to determine when the assumptions apply
6. Scale equations and form dimensionless numbers (optional; advanced analysis)

MICHAELIS-MENTEN KINETICS

Michaelis-Menten Reaction Mechanism



$$\frac{d}{dt} \begin{pmatrix} S \\ E \\ X \\ P \end{pmatrix} = \begin{pmatrix} v_1 & v_{-1} & v_2 \\ -1 & 1 & 0 \\ -1 & 1 & 1 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_{-1} \\ v_2 \end{pmatrix} = \begin{pmatrix} -v_1 + v_{-1} \\ -v_1 + v_{-1} + v_2 \\ v_1 - v_{-1} - v_2 \\ v_2 \end{pmatrix}$$

$$\begin{aligned}
 v_1 &= k_1 s e \\
 v_{-1} &= k_{-1}(x) \quad \frac{d}{dt}(e + x) = 0 \\
 v_2 &= k_2(x)
 \end{aligned}$$

$$\begin{aligned}
 e + x &= e_{tot} = e_0 \quad \text{constant} \\
 s + x + p &= s_{tot} = s_0 \quad \text{constant}
 \end{aligned}$$

$$L = \begin{pmatrix} S & E & X & P \\ 0 & 1 & 1 & 0 \\ 1 & 0 & 1 & 1 \end{pmatrix}$$

$\text{rank}(S) = 2$ (dynamic degree of freedom)

$m - r = 4 - 2 = 2$
there are two time invariants

Mass Action Kinetics:

introduction of time-invariants to go from 4 to 2
dynamically independent variables

$$\begin{array}{lcl}
 e = e_0 - x & & \\
 \downarrow & & \downarrow \\
 \frac{ds}{dt} = -v_1 + v_{-1} & = & -k_1 s e + k_{-1}(x) \\
 \frac{de}{dt} = -v_1 + v_{-1} + v_2 & = & -k_1 s e + (k_{-1} + k_2)(x) \\
 \frac{d(x)}{dt} = v_1 - v_{-1} - v_2 & = & k_1 s e - (k_{-1} + k_2)(x) \\
 \frac{dp}{dt} = v_2 & = & k_2(x) \\
 \uparrow & & \\
 p = s_0 - s - x & &
 \end{array}$$

The Quasi-steady State Assumption

Choose independent variables

$$x = \begin{pmatrix} s \\ x \end{pmatrix}$$

ODEs

AEs

$$\begin{aligned}\frac{ds}{dt} &= f_1(s, x) & e = g_1(s, x) \\ \frac{dx}{dt} &= f_2(s, x) & p = g_2(s, x)\end{aligned}$$

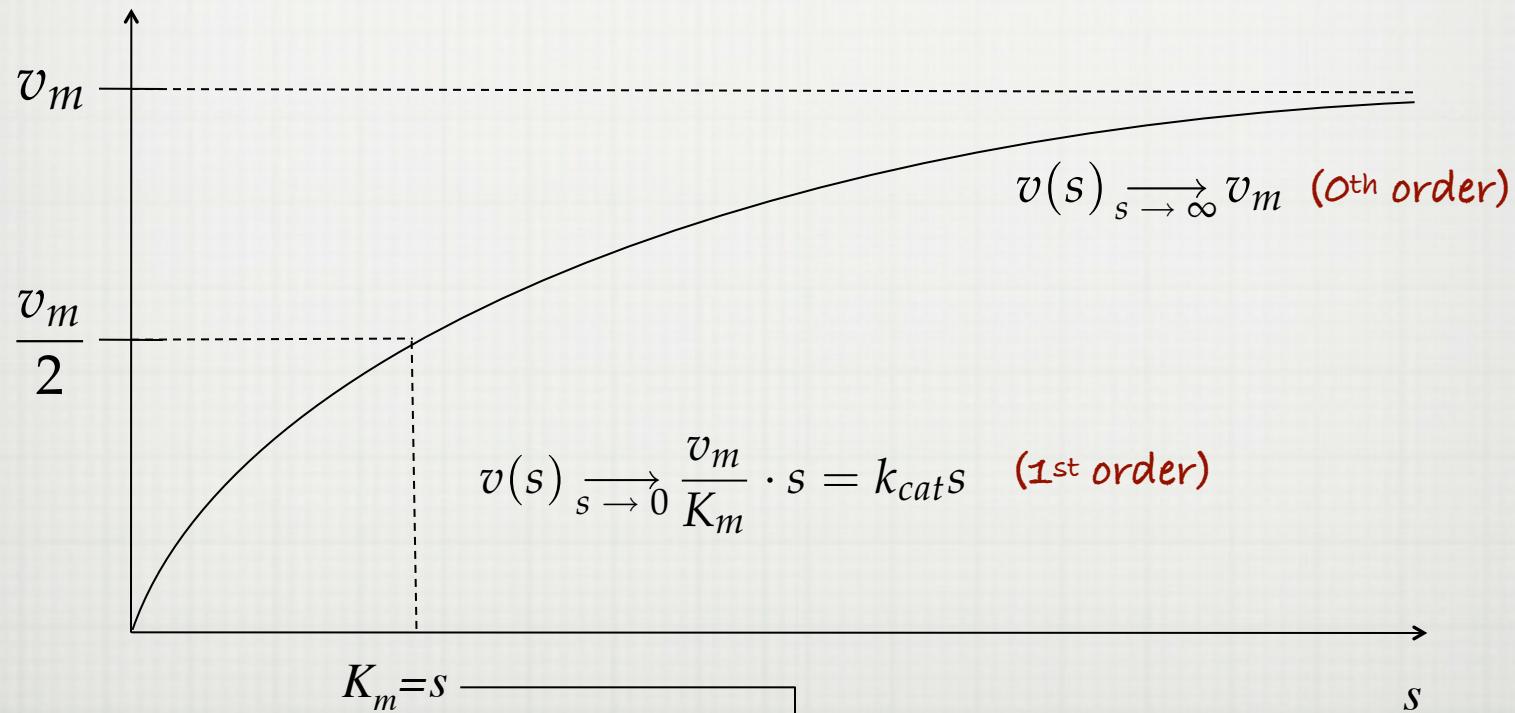
Applying the QSSA

$$\frac{dx}{dt} = 0 ; f_2(s, x) = 0 ; \text{ calc } x = f_3(s)$$

$$\frac{ds}{dt} = f_1(s, f_3(s)) = \frac{\overbrace{-k_2 e_0 s}^{\mathcal{V}_m}}{\underbrace{\frac{k_{-1} + k_2}{k_1} + s}_{K_m}} = \frac{-\mathcal{V}_m s}{K_m + s}$$

The Famous MM-equation

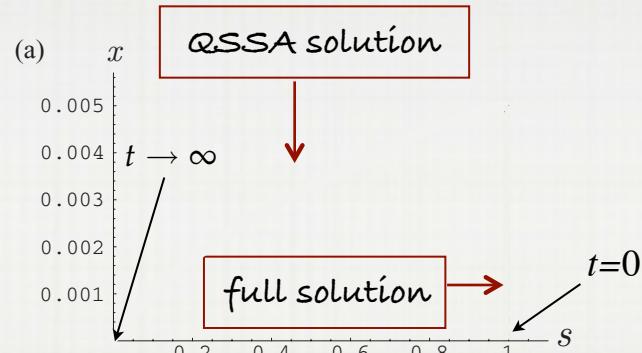
The Michaelis-Menten Rate Law



$$v(s) = \frac{-ds}{dt} = \frac{v_m s}{K_m + s} = \frac{v_m s}{s + s} = \frac{v_m s}{2}$$

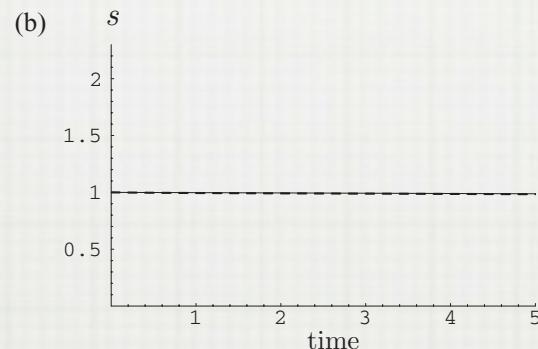
Michaelis-Menten Mechanism: dynamic simulation

phase portrait

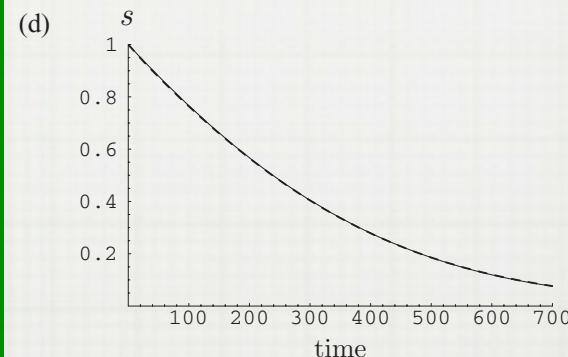


$$\begin{array}{ll} k_2 & = k_{-1} \\ 100e_0 & = K_m \\ s_0 & = K_m \end{array}$$

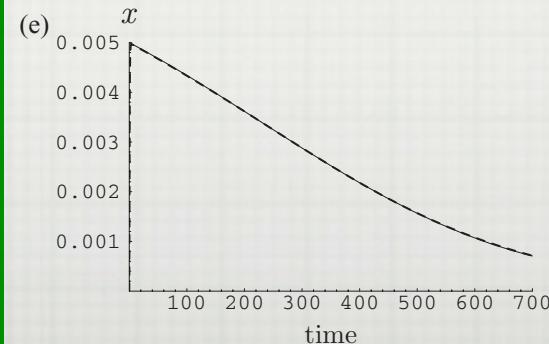
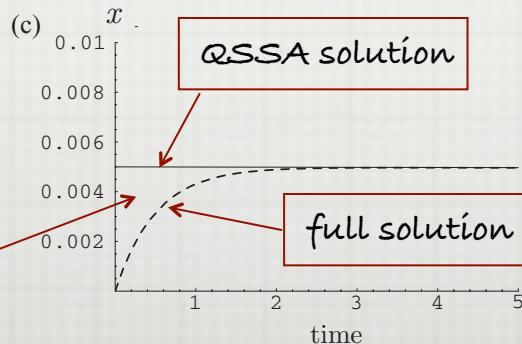
*fast
response*



*slow
response*



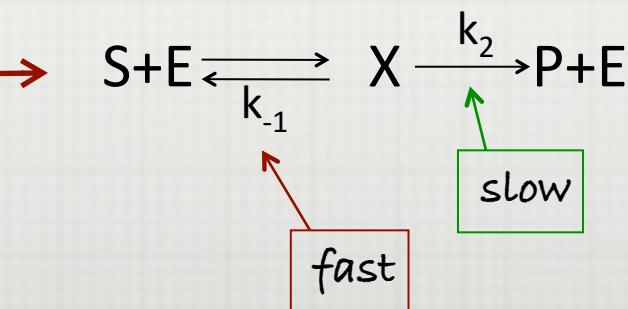
*Difference
is the error*



Applicability of the QEA, QSSA

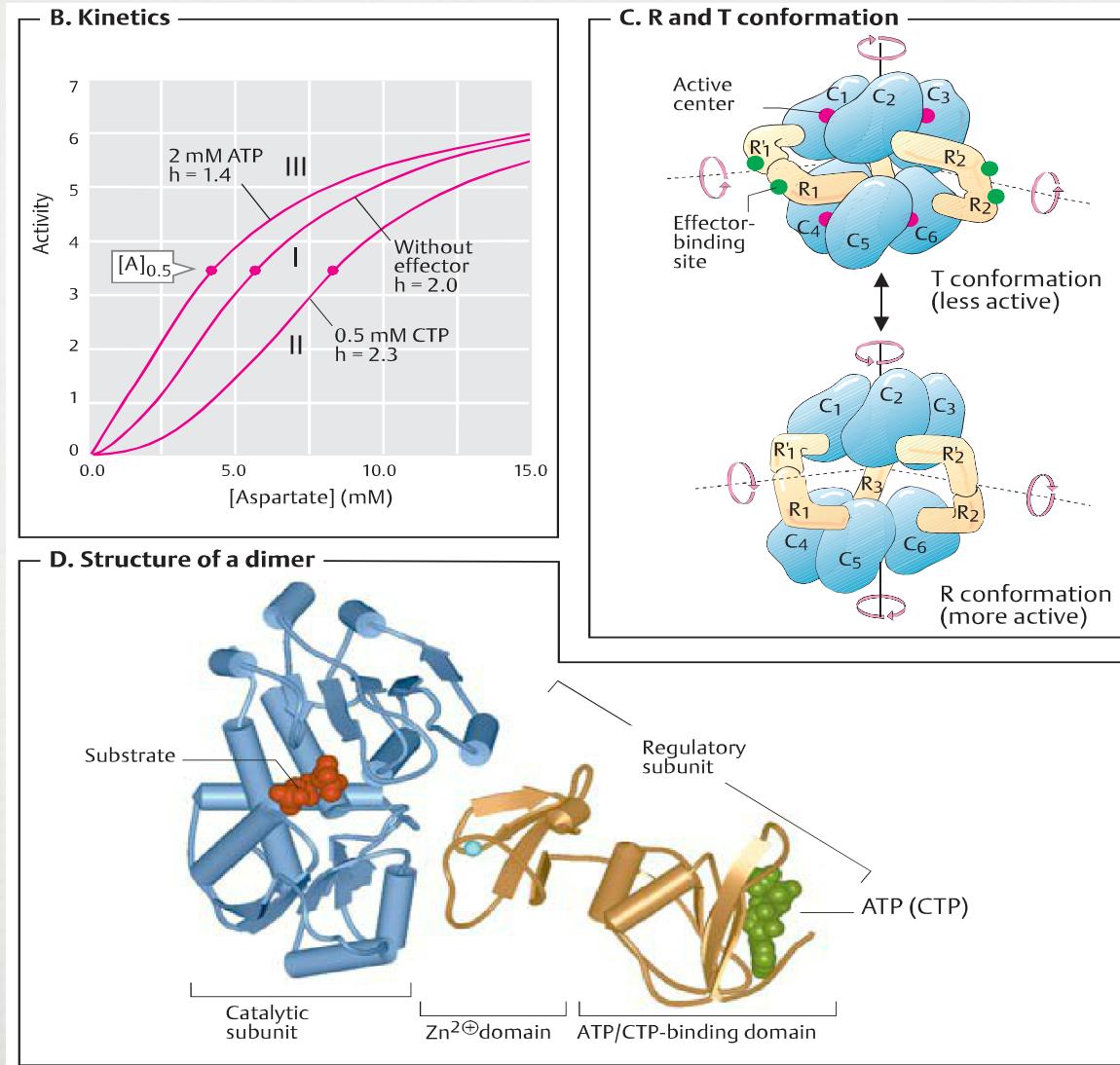
- When $k_2 \ll k_{-1}$ then the QEA works
- When $e_0 \ll K_m$ then the QSSA works
- When $K_m \ll s$ then the QSSA works

$$k_2 \ll k_{-1}$$

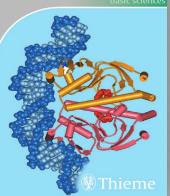


(Chem. Eng. Sci., 42, 447-458.)

Regulatory Enzymes



Koolman, J. and Roehm, K.H., Color Atlas of Biochemistry, 2nd Ed., Thieme, New York, 2004.

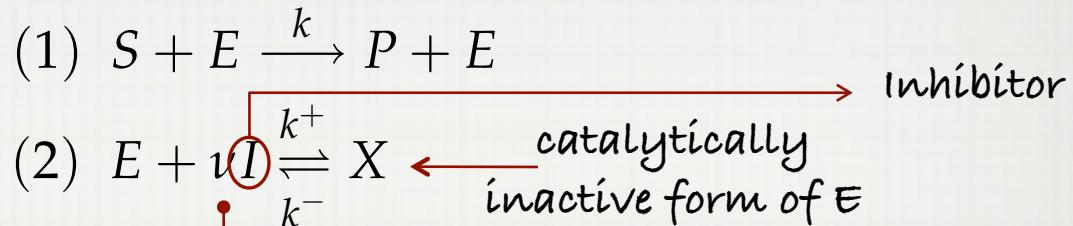


Originally used to describe oxygen binding to hemoglobin

HILL KINETICS

Hill Kinetics

1. Reaction mechanism



2. Mass balance

$$e_t = e + x$$

“degree of cooperativity”, rarely an integer due to lumping effect of reaction (2)

$\nu_{\text{Hb}} \sim 2.3-2.6$,
also called the Hill coefficient

3. QEA on reaction (2)

$$k^+ e i^\nu = k^- x$$

$$x = \frac{k^+}{k^-} e i^\nu = e \left(\frac{i}{K_i} \right)^\nu$$

conservation quantity

“per site” binding constant

4. Reaction rate $v = kse = f(s, i)$

$$K_i = \left(\frac{k^-}{k^+} \right)^{\frac{1}{\nu}}$$

Applying Simplifying Assumptions

Mass balance:

$$e + x = e_t$$

$$e + e \left(\frac{i}{K_i} \right)^\nu = e_t$$

QEA:

$$x = e \left(\frac{i}{K_i} \right)^\nu$$



$$e = \frac{e_t}{1 + (i/K_i)^\nu}$$

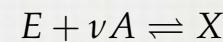
Add e to the rate law

inhibition

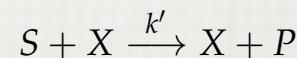
$$v = ks \cdot e$$

$$= \frac{kse_t}{1 + \left(\frac{i}{K_i} \right)^\nu}$$

activation



(e) (a) (x)

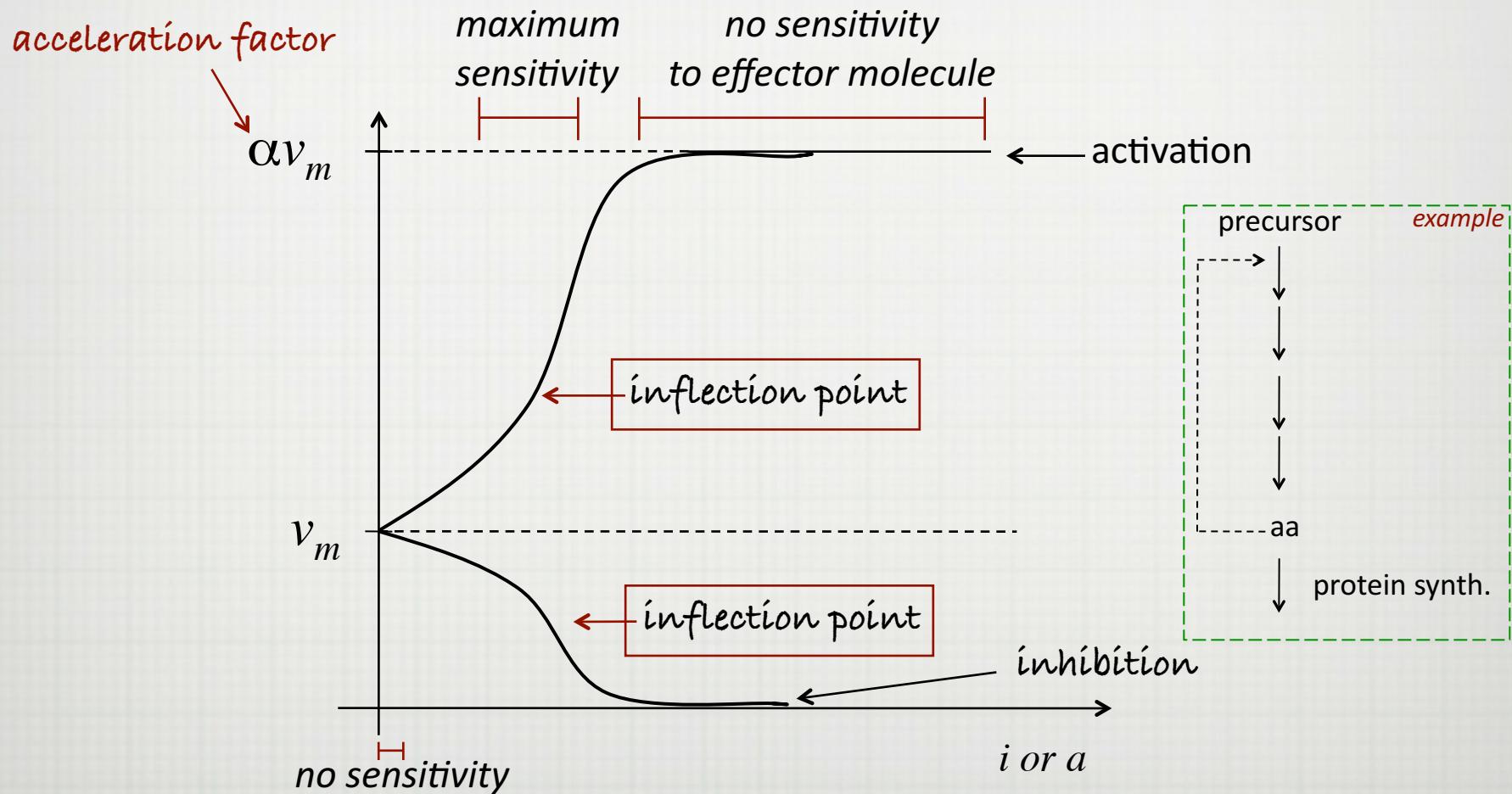


$$v = kse_t \left(\frac{1 + \alpha(a/K_a)^\nu}{1 + (a/K)^\nu} \right)$$

a : concentration of A

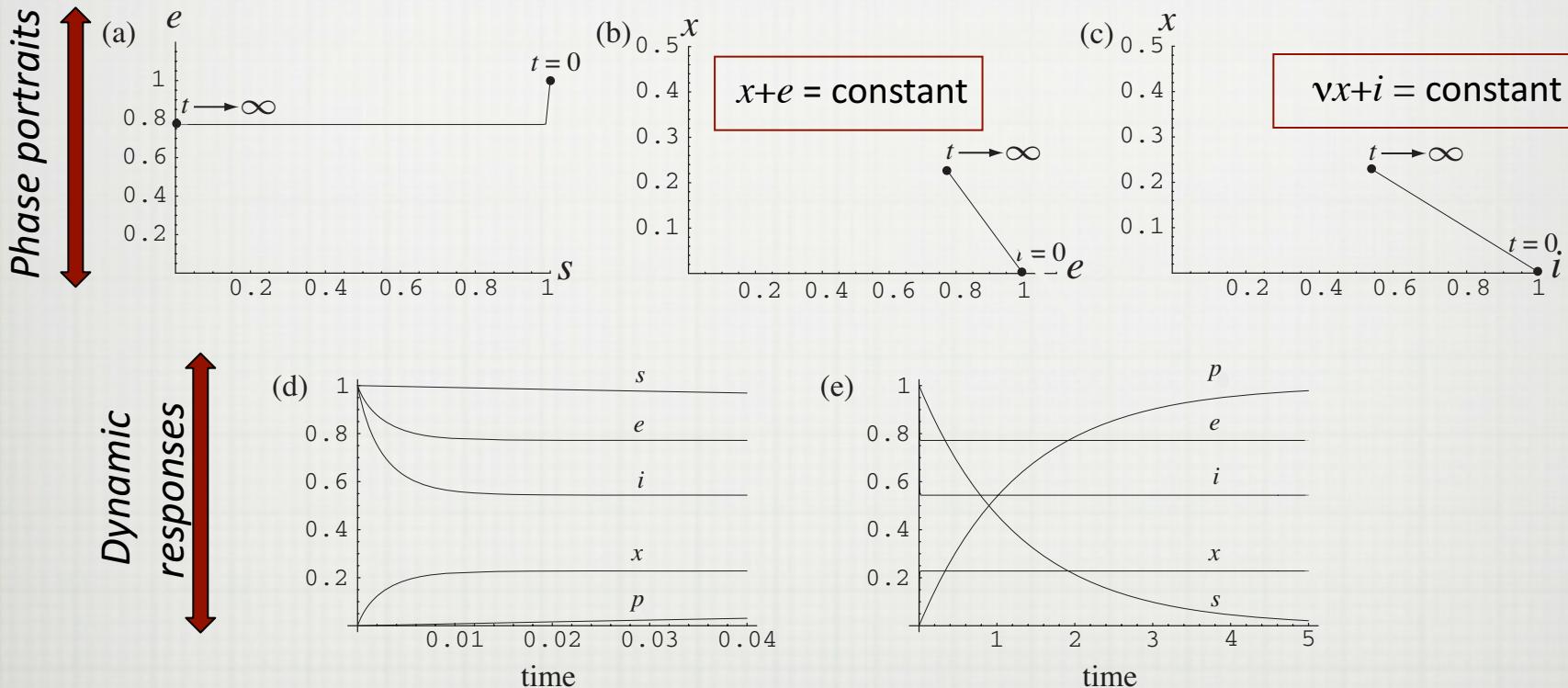
$$\alpha = k'/k$$

Graphical Representation



Dynamic Simulation of Hill Kinetics

$k_i^+ = k_i^- = 100$,
 $k = 1$, $\nu = 2$, $x_0 = 0$
and $e_0 = s_0 = i_0 = 1$



fast

distribution of enzyme states

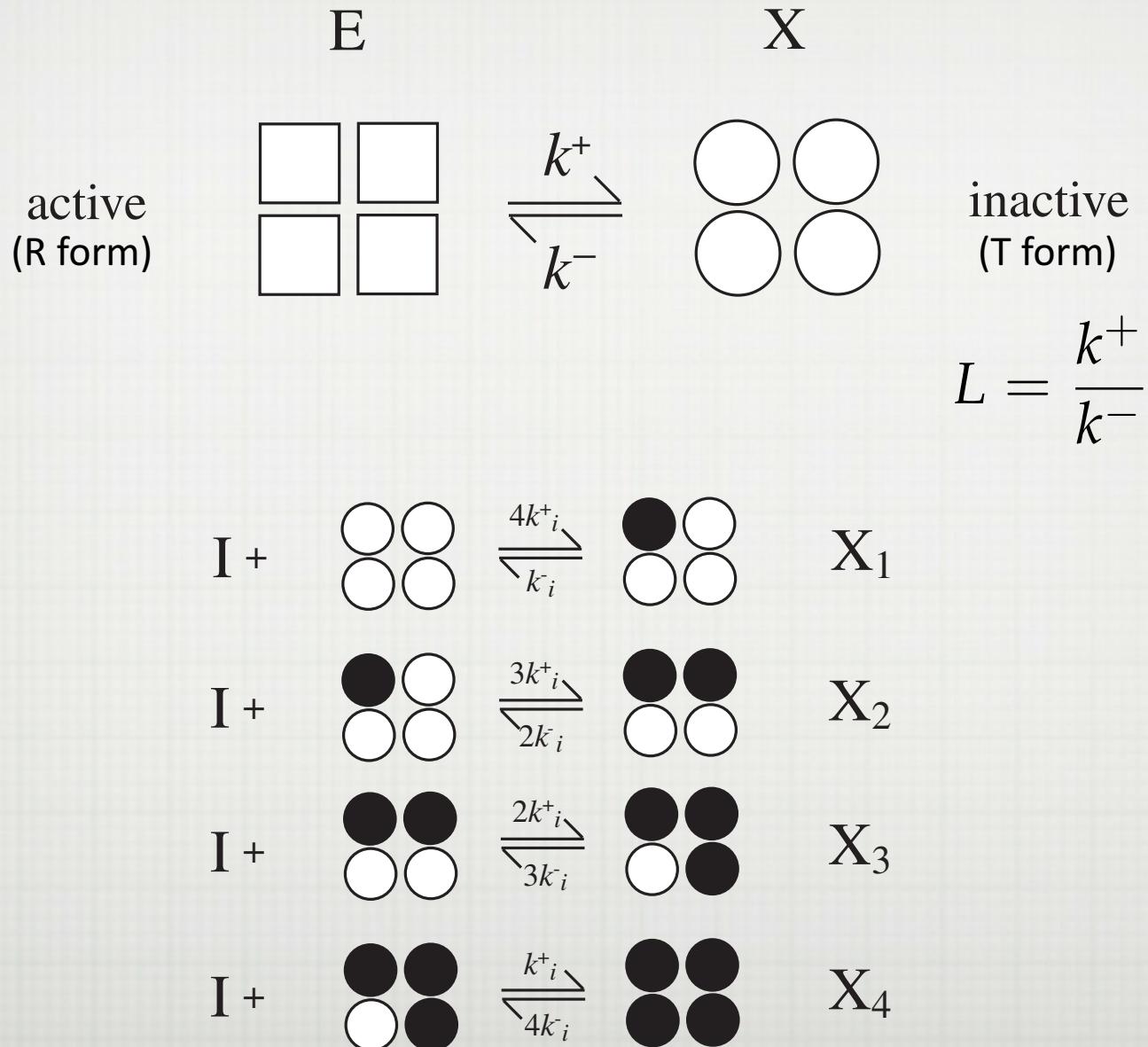
slow

catalysis

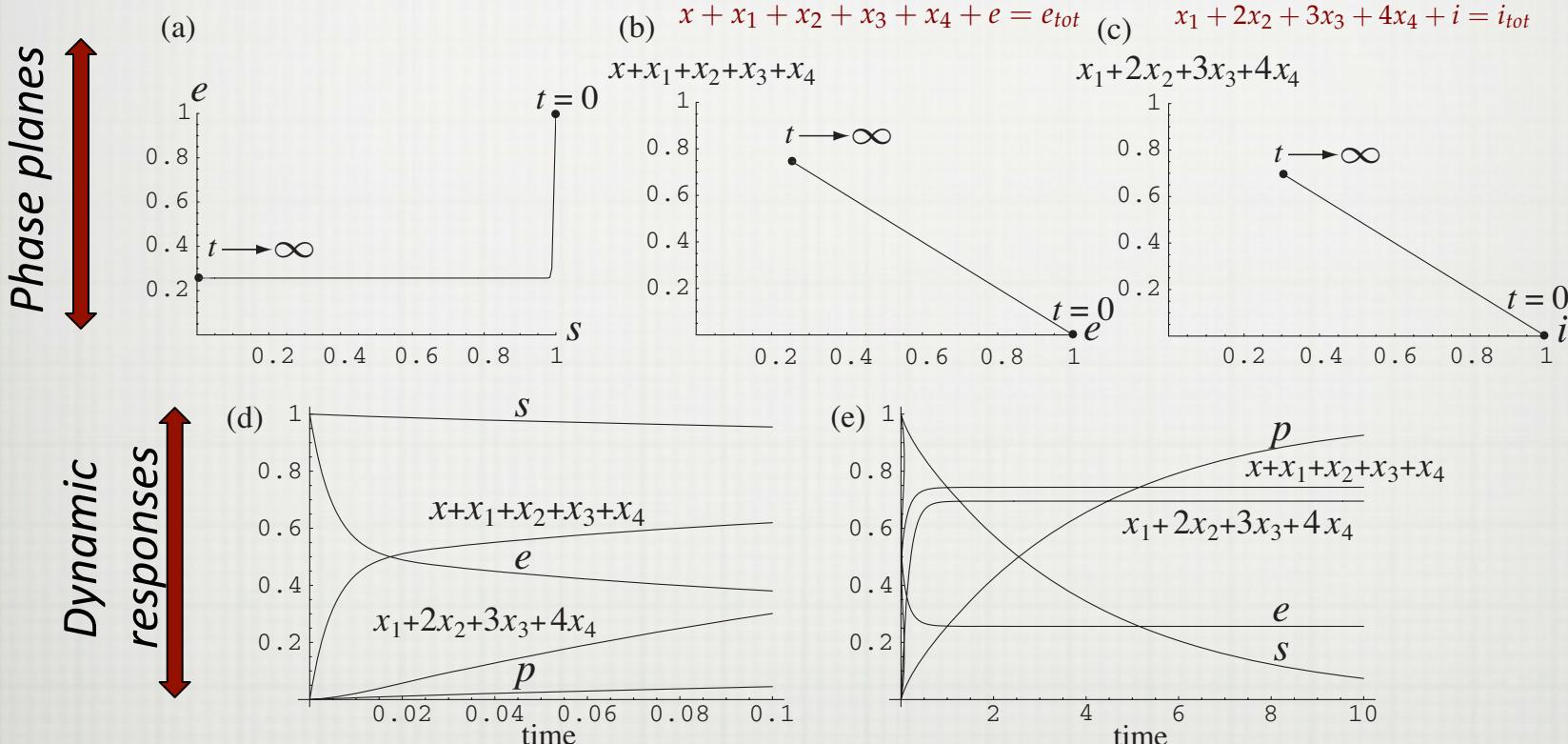
And now, chemically realistic mechanisms

THE SYMMETRY MODEL

The Symmetry Model



Dynamic Response of the Symmetry Model



$k^+ = k^- = 100$, $k_i^+ = k_i^- = 100$, $k = 1$,
 $\nu = 4$, $x_0 = x_{1,0} = x_{2,0} = x_{3,0} = x_{4,0} = 0$
and $e_0 = s_0 = i_0 = 1$

fast

distribution of
enzyme states

slow

catalysis

Deriving the Rate Law

Mass balance $e_0 = e + x + x_1 + x_2 + x_3 + x_4$

QEA

$$4k_i^+ ix = k_i^- x_1 \Rightarrow x_1 = 4x(i/K_i) = 4x \underbrace{(i/K_i)}_{\text{red bracket}}$$

$$3k_i^+ ix_1 = 2k_i^- x_2 \Rightarrow x_2 = \frac{3}{2} \underbrace{x_1}_{\text{red box}} (i/K_i) = 6x \underbrace{(i/K_i)^2}_{\text{red bracket}}$$

$$2k_i^+ ix_2 = 3k_i^- x_3 \Rightarrow x_3 = \frac{2}{3} \underbrace{x_2}_{\text{red box}} (i/K_i) = 4x \underbrace{(i/K_i)^3}_{\text{red bracket}}$$

$$k_i^+ ix_3 = 4k_i^- x_4 \Rightarrow x_4 = \frac{1}{4} \underbrace{x_3}_{\text{red box}} (i/K_i) = x(i/K_i)^4$$

Combine

$$\begin{aligned} e_0 &= e + x + 4x(i/K_i) + 6x(i/K_i)^2 + 4x(i/K_i)^3 + x(i/K_i)^4 \\ &= e + x(1 + (i/K_i))^4 \quad \text{where } x = Le \\ &= e(1 + L(1 + (i/K_i)))^4 \end{aligned}$$

Deriving the Rate Law (Con't)

$$v = ks \cdot e$$
$$e = \frac{e_0}{1 + L(1 + (i/K_i))^4}$$
$$v(s, i) = \frac{kse_0}{1 + L(1 + (i/K_i))^4}$$

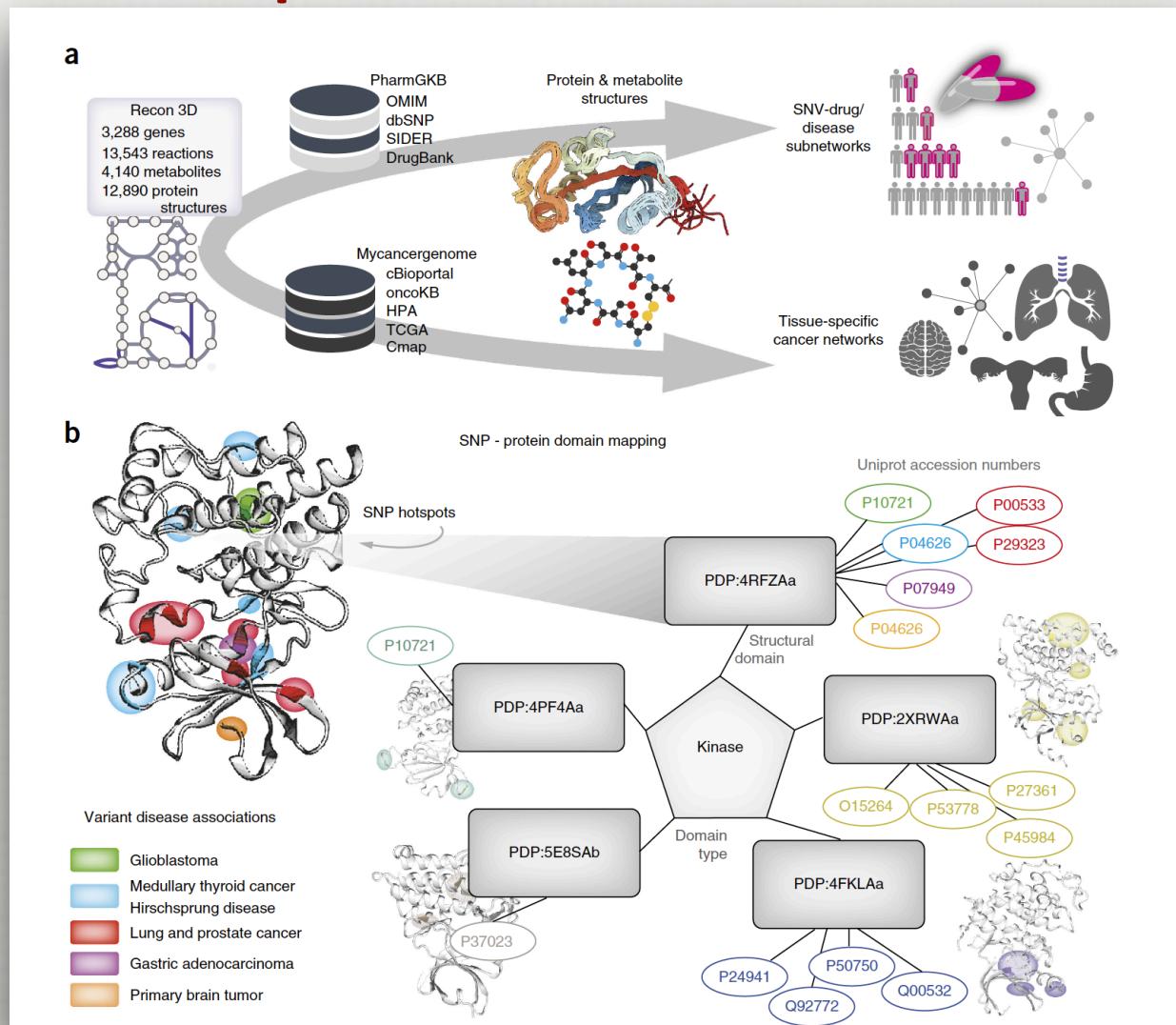


Can derive similar equations for activators
and substrates

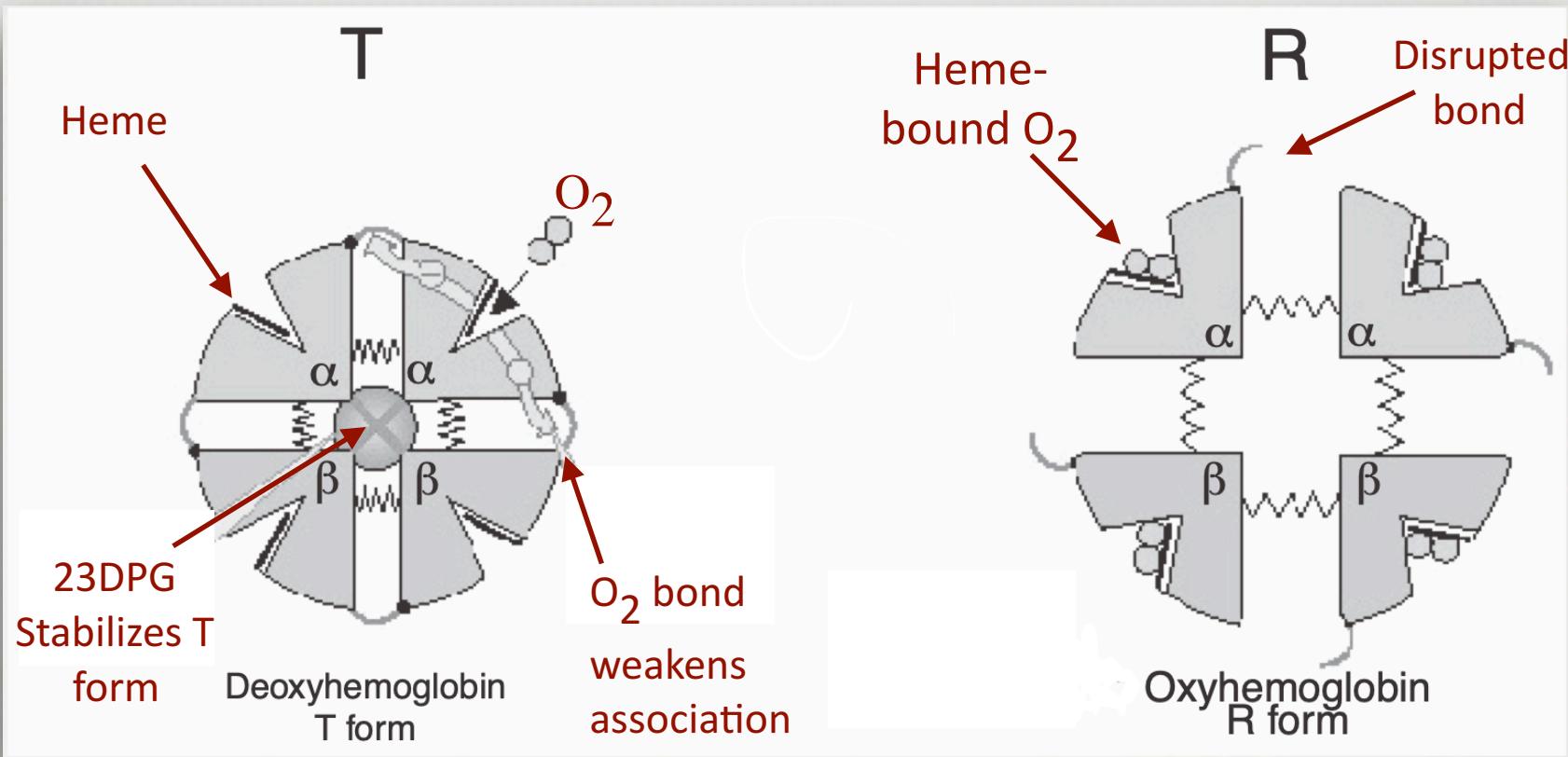
In the age of large-scale computations

FULL KINETIC DESCRIPTIONS

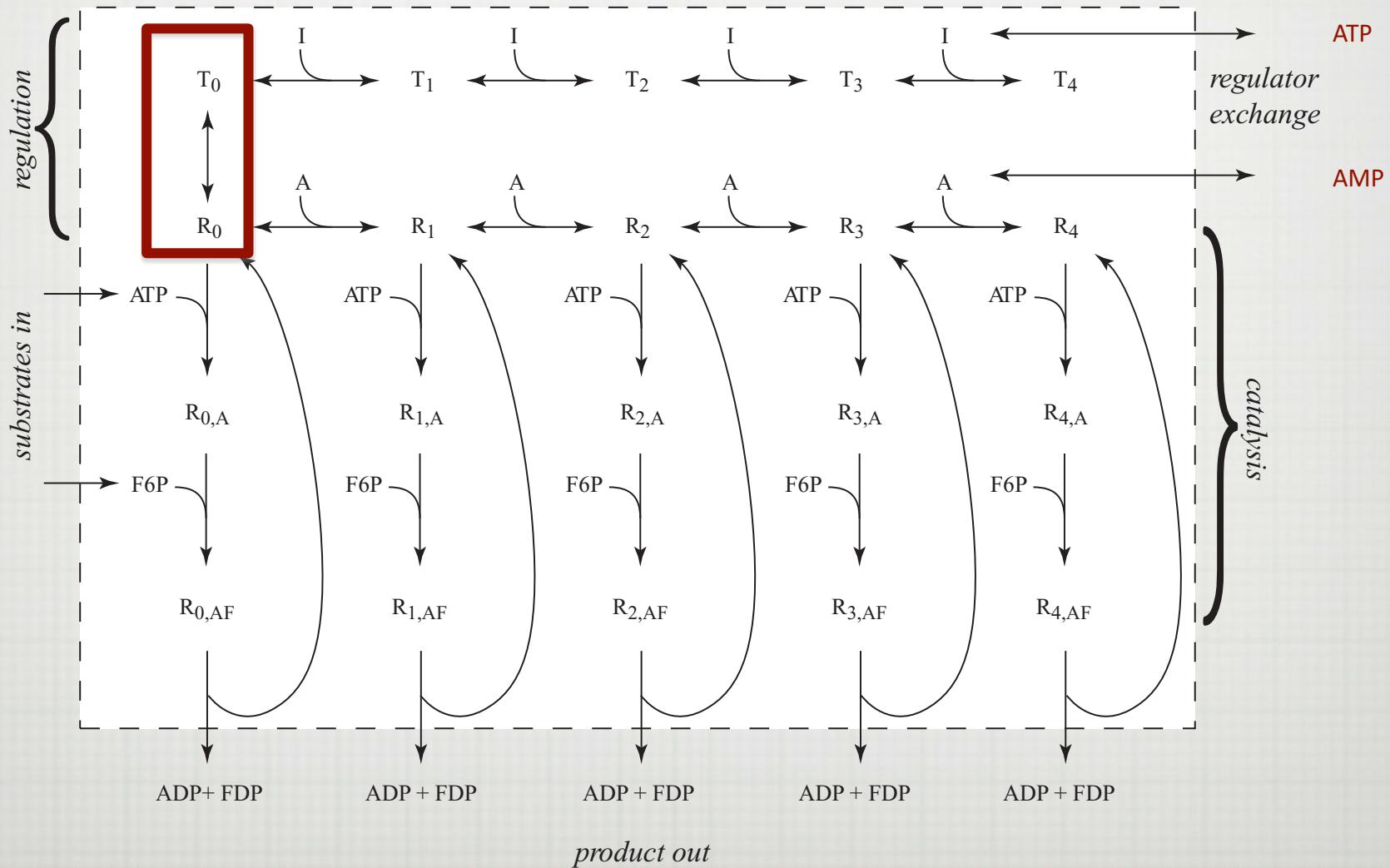
Details for specific cases are available

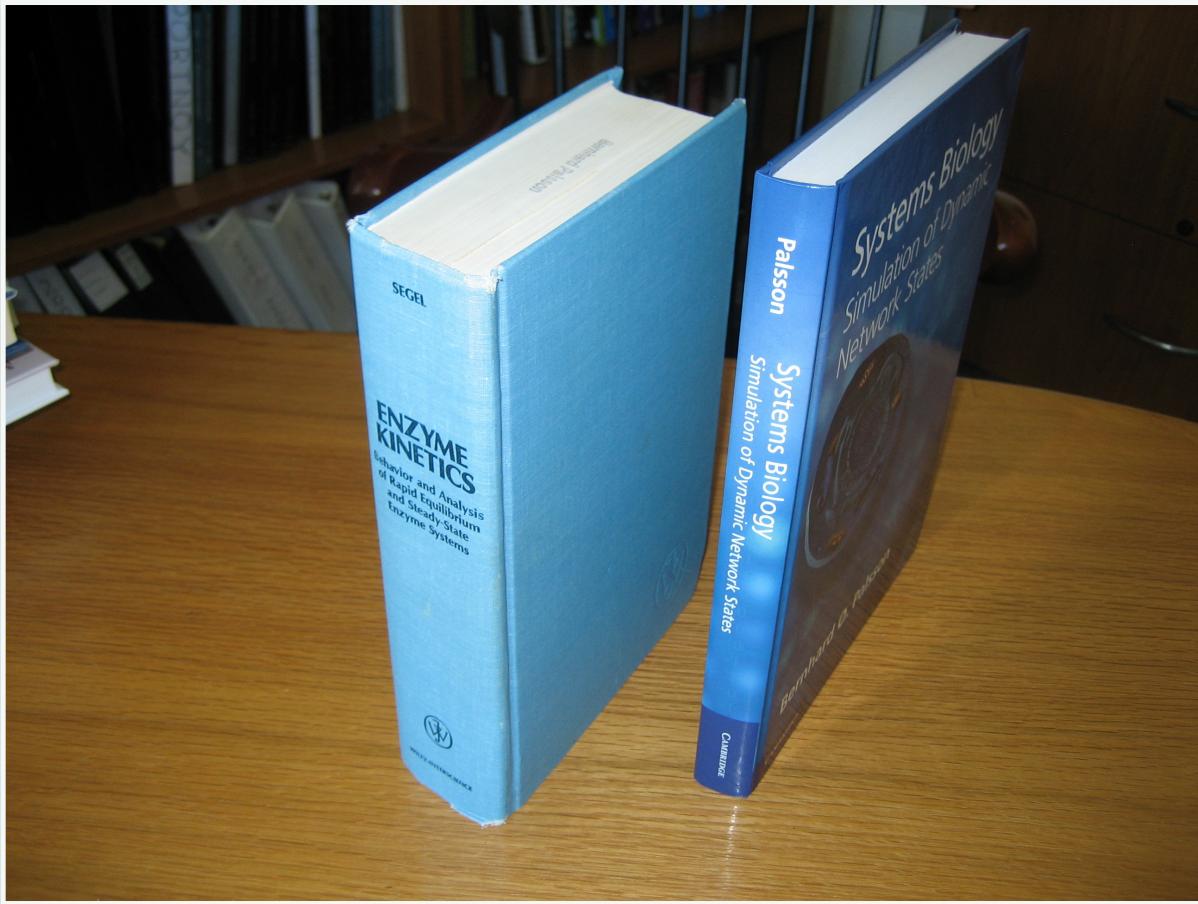


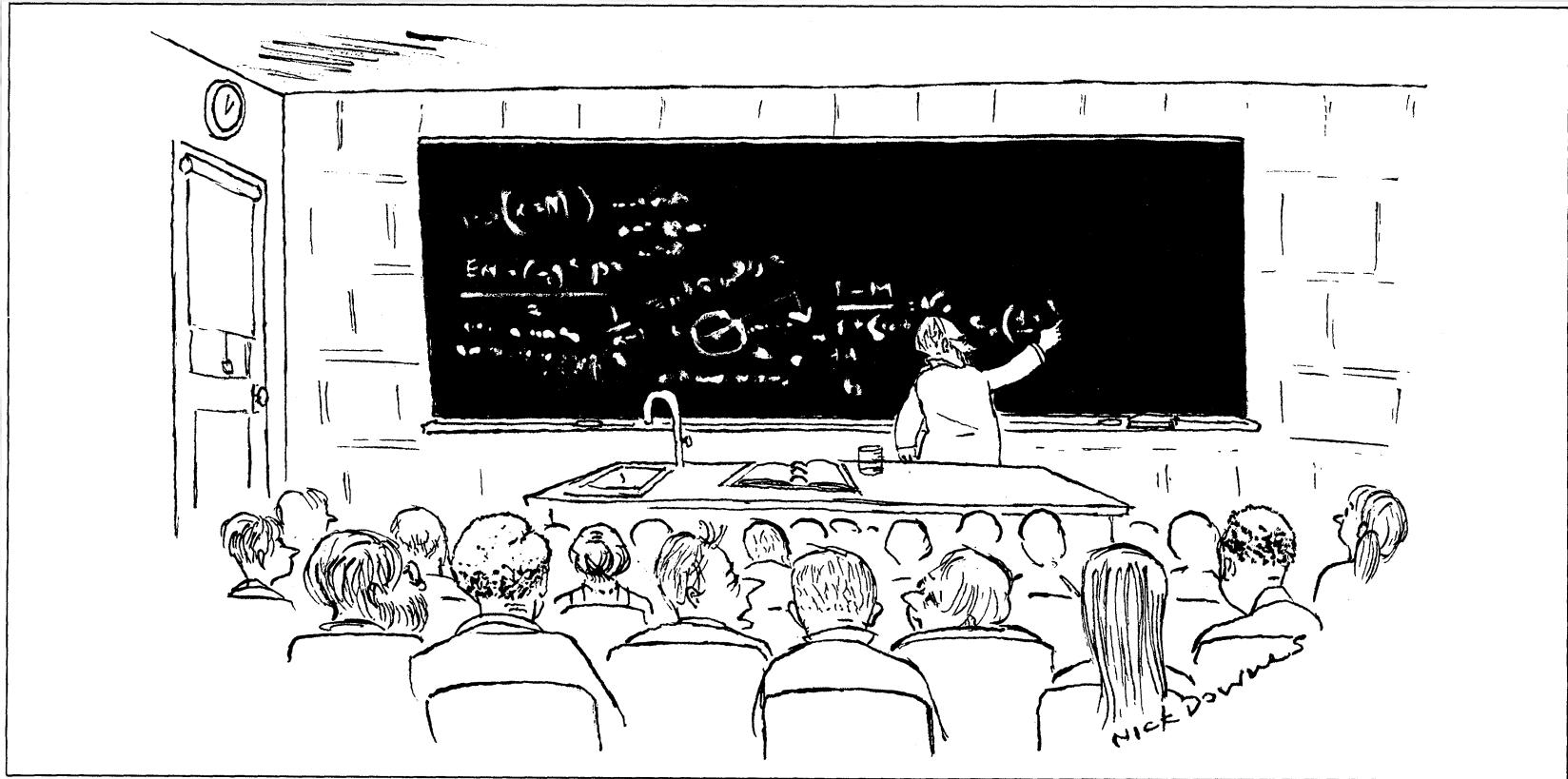
Hemoglobin, Ch 13



Enzyme Modules, Ch 14







"I think I'm beginning to grasp the concept of infinity."

Summary

- Enzymes are highly specialized catalysts that accelerate reaction rates
- Reaction mechanisms are formulated for the chemical conversions carried out by enzymes in terms of elementary reactions.
- Rate laws for enzyme reaction mechanisms are derived based on simplifying assumptions.
- Two simplifying assumptions are commonly used: the quasi-steady state (QSSA) and the quasi-equilibrium assumptions (QEA).
- The validity of the simplifying assumptions can be determined using scaling of the equations followed by mathematical and numerical analysis.
- A number of rate laws have been developed for enzyme catalysis and for the regulation of enzymes. Only three reaction mechanisms were described in this chapter.