Rutgers 642:613 - Fall 2003

Instructor: Eduardo D. Sontag

Text: Keener & Sneyd, Mathematical Physiology

http://www.math.rutgers.edu/~sontag/613.html

warning: **this is a draft of notes –to be continuously revised!**
tentative **plan for first few weeks:**

- **basic biochemical (including enzymatic) reactions**
  (inhibition, cooperativity, Michaelis-Menten; e.g.'s such as glycolysis; math: time-scale separation (singular perturbations), phase planes, bifurcations, and oscillations - basis for everything else in the course)
- **movement of chemicals inside and in/out of cells**
  (plain and facilitated diffusion, carrier-mediated transport, models of the membrane including the role of ion pumps, an application of the study of cell volume)
- **Hodgkin-Huxley model for neurons**
  (excitability, oscillations; FitzHugh-Nagumo, . . .)
- **calcium dynamics**

Chapter 1 - Reactions

use capital letters $A, B, \ldots$ for names of chemical substances (large molecules such as proteins, or ions, etc)
& corresp. lower-case $a, b, \ldots =$ concentrations
e.g. reaction: one unit of C produced from one A & one B:

$$A + B \xrightarrow{k} C$$

typical modeling assumption is *(ideal) mass action*:
formation rate of $C \propto$ (concentration A)(concentration B):

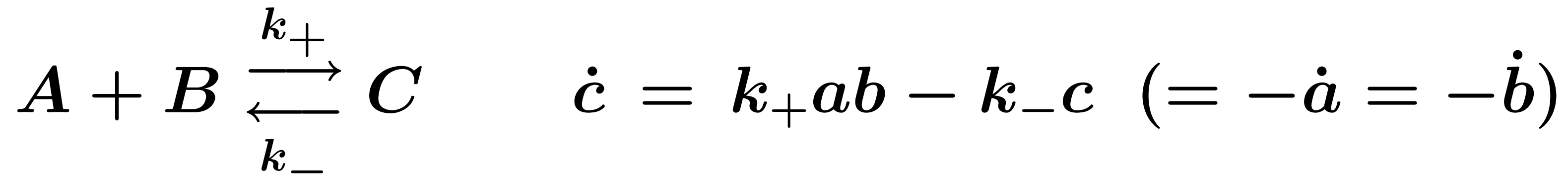
$$\dot{c} = dc/dt = k\,ab$$

and so A and B are being used-up at the same rate:

$$\dot{a} = \dot{b} = -k\,ab$$

collision theory; constant depends on energy needed to bind (press to snap lego pieces), geometry of particles, etc
limitations: *low numbers*: e.g. 2-100 molecules in a cell
$\leadsto$ probabilistic models
saturations: other chemicals needed but in short supply

reversible bimolecular reaction:



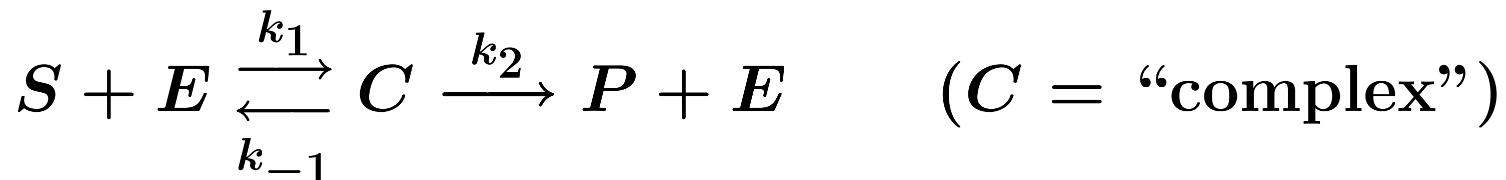
$$A + B \underset{k_-}{\overset{k_+}{\rightleftarrows}} C \qquad \dot{c} = k_+ab - k_-c \;\; (= -\dot{a} = -\dot{b})$$

so at steady state, and using $a + c \equiv$ **const** $\equiv a_0$**, will have:**

$$c = \frac{k_+}{k_-}ab = a_0\frac{b}{K_{\text{eq}} + b} \qquad \left(K_{\text{eq}} = \frac{k_-}{k_+} \;\; \textit{equilibrium const}\right)$$

(e.g. given $c(0) = 0$ **and** $a(0) = a_0$**; and if also given** $b(0) \equiv b + c$**, then may solve (quadratic) for steady state)**

enzymes: **catalysts (proteins usually) facilitate reactions ("substrate into product"); not changed themselves**
e.g. put stress to help break a bond, or bring things together
Michaelis-Menten, 1913: basic mechanism two-step:



$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E \qquad (C = \text{"complex"})$$

(ignoring reverse reaction – removal of P**, or small rate)**

full model of $S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E$ is:

$$\begin{aligned} \dot{s} &= k_{-1}c - k_1 se \\ \dot{e} &= (k_{-1} + k_2)c - k_1 se \\ \dot{c} &= k_1 se - (k_{-1} + k_2)c \\ \dot{p} &= k_2 c \end{aligned}$$

note $\dot{e} + \dot{c} \equiv 0$; so $c + e \equiv e_0$ total enzyme (bound or not)
also, p can be at first ignored (just integrate), so *two ode's*

quasi-steady-state approximation is easier to deal with
and explains observed "saturation" behavior
($\neq$ approx than original MM work, who assumed $\dot{s} = 0$)

time-scale separation is key to approximation
– analyze separately what happens "slowly" vs "quickly"

next we'll spend some time with this,
since similar arguments will occur over and over

first, simple linear example to understand the point;

$$\dot{x} = y$$
$$\dot{y} = (1/\varepsilon)(x - y)$$

where $\varepsilon \approx 0$, with $x(0) = A$, $y(0) = B$
the solution (thanks to Maple) is given by $x(t) =$

$$1/2\left(A + \sqrt{1+4\,\varepsilon}A + 2\,B\varepsilon\right)e^{\left(\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}\frac{1}{\sqrt{1+4\,\varepsilon}}$$
$$-1/2\left(A - \sqrt{1+4\,\varepsilon}A + 2\,B\varepsilon\right)e^{\left(\frac{-1-\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}\frac{1}{\sqrt{1+4\,\varepsilon}}$$

($y(t)$ in next slide); so using expansions:

$$\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon} = 1 - \varepsilon + O(\varepsilon^2)$$
$$\frac{-1-\sqrt{1+4\,\varepsilon}}{2\varepsilon} = -\varepsilon^{-1} - 1 + \varepsilon + O(\varepsilon^2)$$

have for t “not small” $e^{\left(\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t} \approx e^t$, $\quad e^{\left(\frac{-1-\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t} \approx 0$
$\rightsquigarrow$ $x(t) \approx Ae^t$

similarly, $y(t) =$

$$\frac{1}{2\varepsilon}\Big(-1/2\,\left(A+\sqrt{1+4\,\varepsilon}A+2\,B\varepsilon\right)e^{\left(\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}\frac{1}{\sqrt{1+4\,\varepsilon}}$$

$$+1/2\,\left(A+\sqrt{1+4\,\varepsilon}A+2\,B\varepsilon\right)e^{\left(\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}$$

$$+1/2\,\left(A-\sqrt{1+4\,\varepsilon}A+2\,B\varepsilon\right)e^{\left(\frac{-1-\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}\frac{1}{\sqrt{1+4\,\varepsilon}}$$

$$+1/2\,\left(A-\sqrt{1+4\,\varepsilon}A+2\,B\varepsilon\right)e^{\left(\frac{-1-\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}\Big)$$

drop last two terms (dominated by first two for t large) $\rightsquigarrow$

$$\frac{\left(A+\sqrt{1+4\,\varepsilon}A+2\,B\varepsilon\right)e^{\left(\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon}\right)t}}{2\sqrt{1+4\,\varepsilon}}\;\frac{-1+\sqrt{1+4\,\varepsilon}}{2\varepsilon}$$

last is ≈ 1, and so $y(t) \approx Ae^{t}$, same as $x(t)$

(large t; this cannot be true for $t \approx 0$, since $y(0) = B$!)
easier way to see:

equations are

$$\begin{aligned} \dot{x} &= y \\ \dot{y} &= (1/\varepsilon)(x - y) \end{aligned}$$

where $\varepsilon \approx 0$, with $x(0) = A$, $y(0) = B$

now, as $\varepsilon \approx 0$, second ODE is "fast" compared to first,
so view $x(t) \equiv a$ as constant there: $\dot{y} = (1/\varepsilon)(a - y)$

thus $y(t) \to a$ *very fast*, i.e. $y(t) \approx x(t)$
("$y(t)$ tracks $x(t)$")

from the point of view of x, time is passing slowly,
and all *it* sees is $y(t) \approx x(t)$
so x's eqn is $\dot{x} = x$, which has solution $x(0)e^t = Ae^t$

and so, $y(t) \approx x(t) \approx Ae^t$

(*singular perturbation theory* studies this rigorously)

but need to be careful !

$$\dot{x} = y$$
$$\dot{y} = (1/\varepsilon)(x - y)$$

said: "second equation fast compared to first, so assume $x(t)$ is constant in second equation"
but what if y large?

e.g. if $y(0) = B = \varepsilon^{-1} \gg 1$, then $\dot{x}(0) = y(0) = B$ is large, ***so x changes fast too*****; in fact, $X(t) \not\approx Ae^{t}$, but $\approx$**

$$\frac{A + \sqrt{1+4\,\epsilon}A + 2\,B\epsilon}{2\sqrt{1+4\,\epsilon}}e^{t} \approx \frac{A + \sqrt{1+4\,\epsilon}A + 2}{2\sqrt{1+4\,\epsilon}}e^{t} \approx (A{+}1)e^{t}$$

e.g.: $\varepsilon = 0.01$, $B = 100$, $A = 1$, solution (to two decimals)

is $X(t) = 1.97e^{0.99t} - 0.97e^{-100.99t}$ vs $X(t) = e^{0.99t}$

$\therefore$ need to consider orders of everything appearing in eqs ...also, magnitudes depend on units... "nondimensionalize"

back to $S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} P + E$ ($e = e_0 - c$ & ignore p):

$$\begin{aligned} \dot{s} &= k_{-1}c - k_1 s(e_0 - c) \\ \dot{c} &= k_1 s(e_0 - c) - (k_{-1} + k_2)c \end{aligned}$$

enzymes typically at concentrations $\ll$ substrates
so assume $e(0) = e_0 = \varepsilon \ll 1$, so also $c = e_0 - e$ is small

but everything else (substrate concentration, kinetic const)
is "$O(1)$" (not too small and not too large)
(see book for more careful nondimensional analysis)

write $x = \frac{c}{\varepsilon}$ and express eqns in terms of s, x (instead of c)
so that everything but ε is $O(1)$ (typical $\varepsilon \approx 10^{-7}$ to 10^{-2})

$$\begin{aligned} \dot{s} &= \varepsilon \left[(k_{-1} + k_1 s)\, x - k_1 s \right] \\ \dot{x} &= k_1 s - (k_1 s + k_{-1} + k_2)\, x \qquad \text{(use that } \dot{x} = \dot{c}/\varepsilon\text{)} \end{aligned}$$

so to analyze what happens for t *large*, let $\tau = \varepsilon t$
(e.g. view in hours τ instead of seconds t when $\varepsilon = 1/3600$)

using $\frac{ds}{d\tau} = \frac{1}{\varepsilon}\frac{ds}{dt}$ and $\frac{dx}{d\tau} = \frac{1}{\varepsilon}\frac{dx}{dt}$ leads to eqs using τ as time:

$$\begin{aligned} ds/d\tau &= [(k_{-1} + k_1 s)x - k_1 s] \\ \varepsilon\, dx/d\tau &= k_1 s - (k_1 s + k_{-1} + k_2)x \end{aligned}$$

so setting $\varepsilon = 0$ (think of second equation converging fast to equilibrium, too fast to be "noticed" in the time scale τ), obtain algebraic equality $k_1 s - (k_1 s + k_2 + k_{-1})x = 0$, i.e.

$$x = \frac{s}{s + K_{\mathrm{m}}}, \quad K_{\mathrm{m}} = \frac{k_{-1} + k_2}{k_1}$$

so (substitute and simplify) $ds/d\tau = -k_2 s/(s + K_{\mathrm{m}})$, so:

$$\dot{s} \;=\; \varepsilon\left(\frac{ds}{d\tau}\right) \;=\; -\frac{V_{\max}\, s}{s + K_{\mathrm{m}}} \qquad (V_{\max} = k_2\,\varepsilon)$$

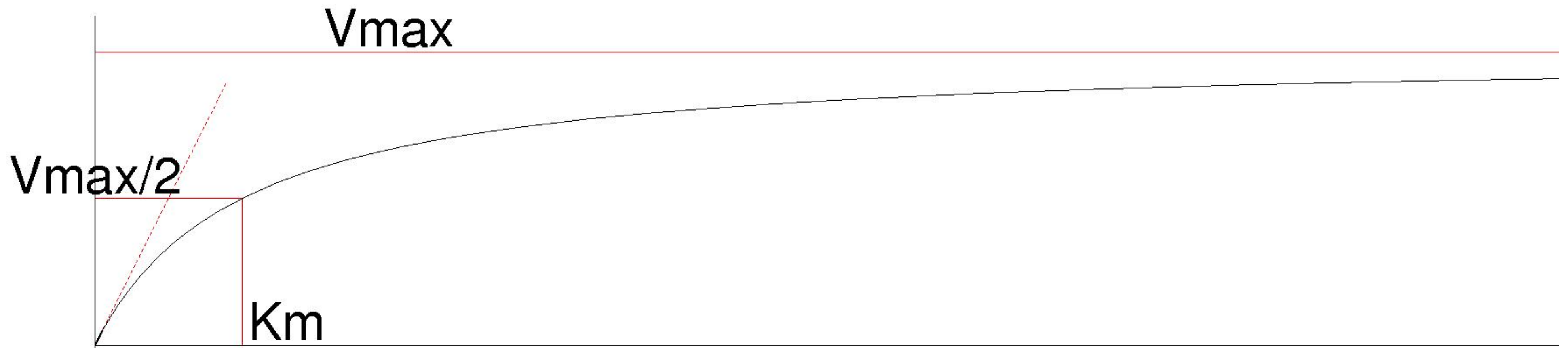
and, since $\dot{p} = k_2 c = k_2 \varepsilon x = V_{\max} x$,

$\dot{p} = \dfrac{V_{\max}\, s}{s + K_{\mathrm{m}}}$ *Michaelis-Menten rate of product formation*

$$\therefore\; \frac{1}{\dot{p}} = \frac{s + K_{\mathrm{m}}}{V_{\max}\, s} = \frac{1}{V_{\max}} + \frac{K_{\mathrm{m}}}{V_{\max}} \cdot \frac{1}{s} \qquad (\text{linear } 1/\dot{p} \text{ vs } 1/s)$$

"Lineweaver-Burk plot" to estimate params (regression)

$$\frac{V_{\max} s}{s + K_{\mathrm{m}}}$$



slope at $s=0$ is $V_{\max}/K_{\mathrm{m}}$, half of max rate reached at $s=K_{\mathrm{m}}$, asymptotic value (max reaction rate) is $V_{\max}$

(note: orig. M&M deriv ($\dot{s}=0$) gives k_{-1}/k_1 instead of K_{m})

this was all obtained for slow time scale; instead,

$$\dot{s} = \varepsilon \left[(k_{-1} + k_1 s)\, x - k_1 s \right]$$
$$\dot{x} = k_1 s - (k_1 s + k_{-1} + k_2)\, x$$

for original time scale and t small, as $\varepsilon \approx 0$, $s(t) \approx s(0)$ for t "not large", so in this *initial layer* near time $t=0$, $\dot{x} = k_1 s(0) - (k_1 s(0) + k_2 + k_{-1}) x$ (linear ODE, can solve)

approx. vs exact. Schnell & Mendoza, "Closed form solution for time dependent enzyme kinetics," J Theor Biology 1997 (187):207-212. (That paper obtains a solution for $s(t)$ using "Lambert W function" (inverse of $ye^y = x$; separation of vars) The same result can be obtained by simply asking Maple to solve the equation (!).) Graph has time scaled to $(0, 1)$

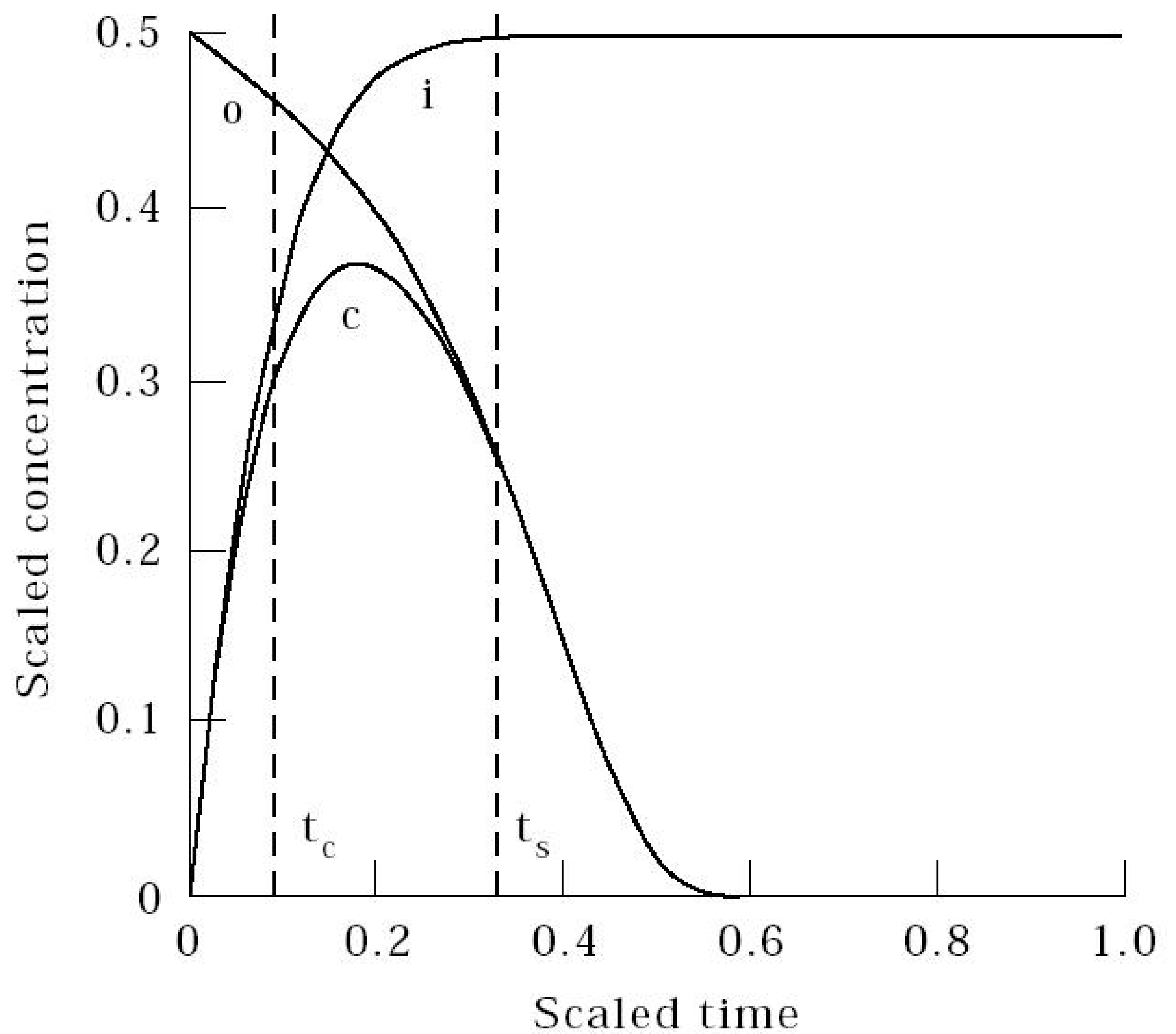


FIG. 3. Comparison of the present closed-form solution for the complex concentration (curve c) with those obtained by singular perturbation methods to order $[E_0]/[S_0]$. It may be appreciated that the agreement with the inner solution (curve i) during the fast transient ($t < t_C$) and with the outer solution (curve o) for $t > t_S$ is very good. However, data from the perturbation methods are noticeably different in the important transition interval $t_C < t < t_S$.

Mechanisms for Enzyme Inhibition/Activation

cellular control built from feedforward and feedback loops

enzyme activation/inhibition modulates "gain" of processes
[but also: irreversible inhibitors: poisons (e.g. nerve gases)]

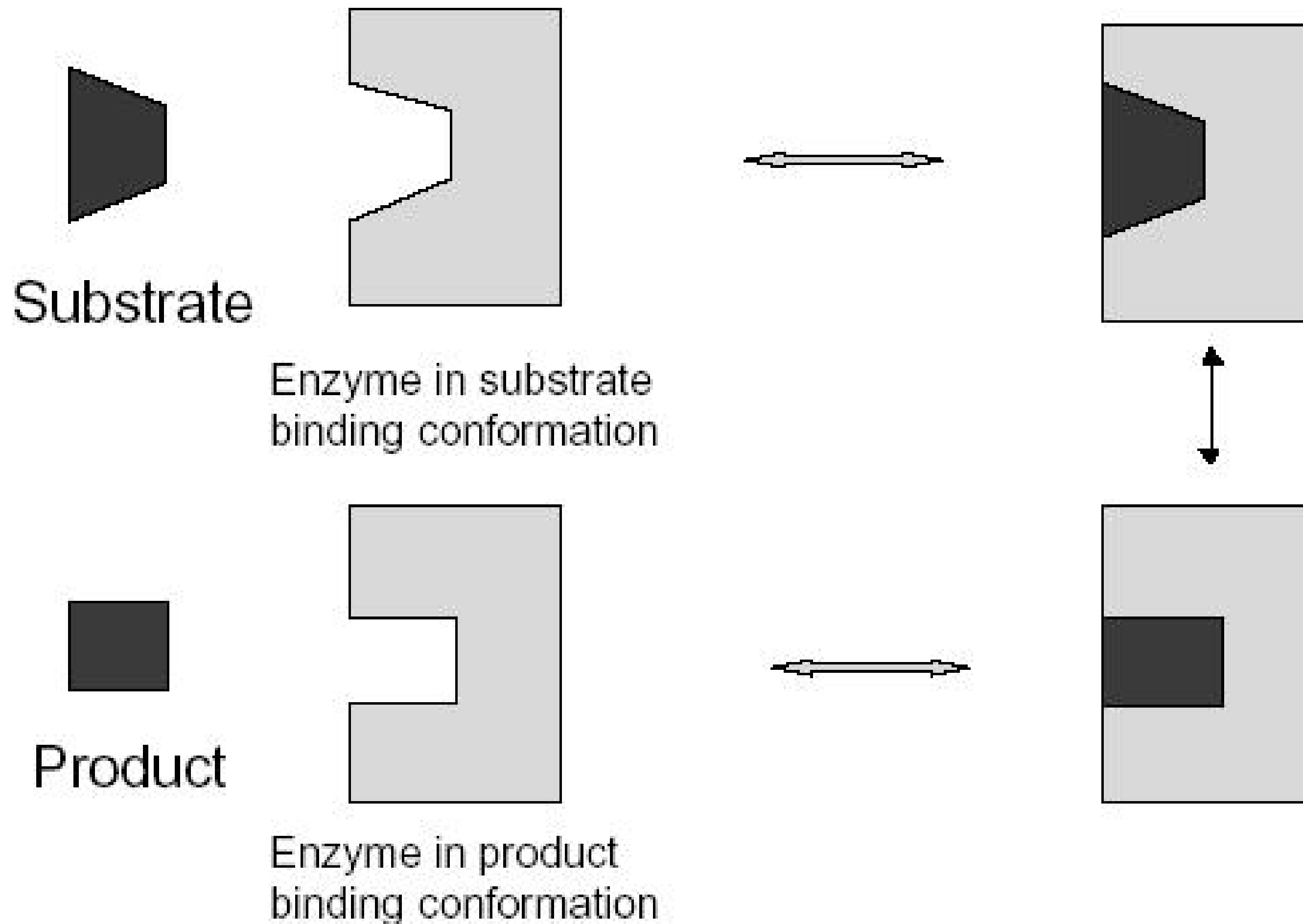
size of enzyme $\gg$ size of substrate
one or more *binding sites* for *ligands* - "lock and key" fit

***competitive inhibition:* occupies & prevent binding**

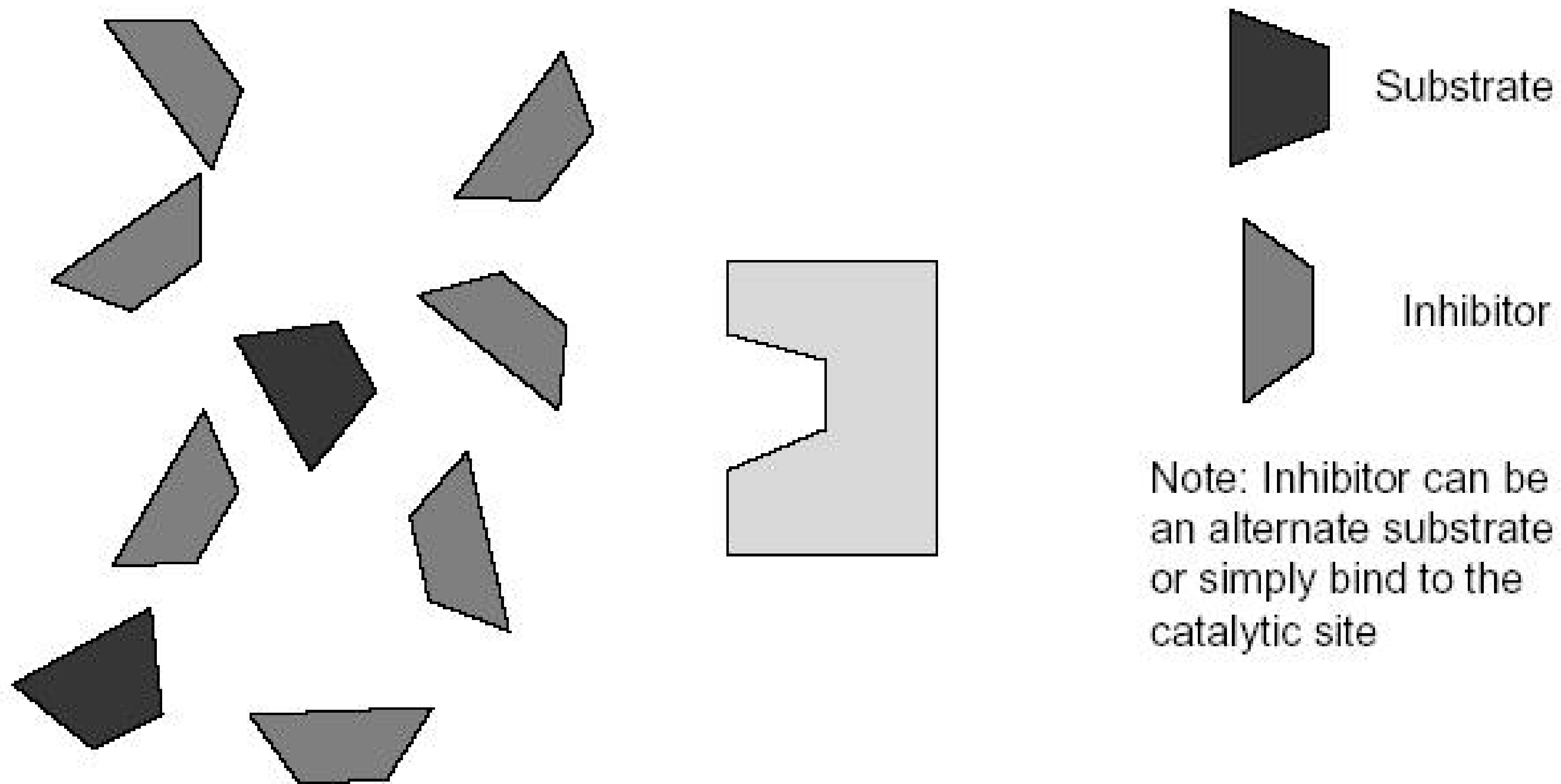
alternative: bind at *effector* ("*regulatory*" "*allosteric*") site different from *active site* at which catalytic activity occurs

changes shape of protein - *allosteric inhibition* or *activation*

Merriam-Webster: allosteric: "all+steric"; steric: relating to or involving the arrangement of atoms in space; ="solid" in Greek



(pic from Rick Kahn, Emory U Biochem BAHS 501, 2001)



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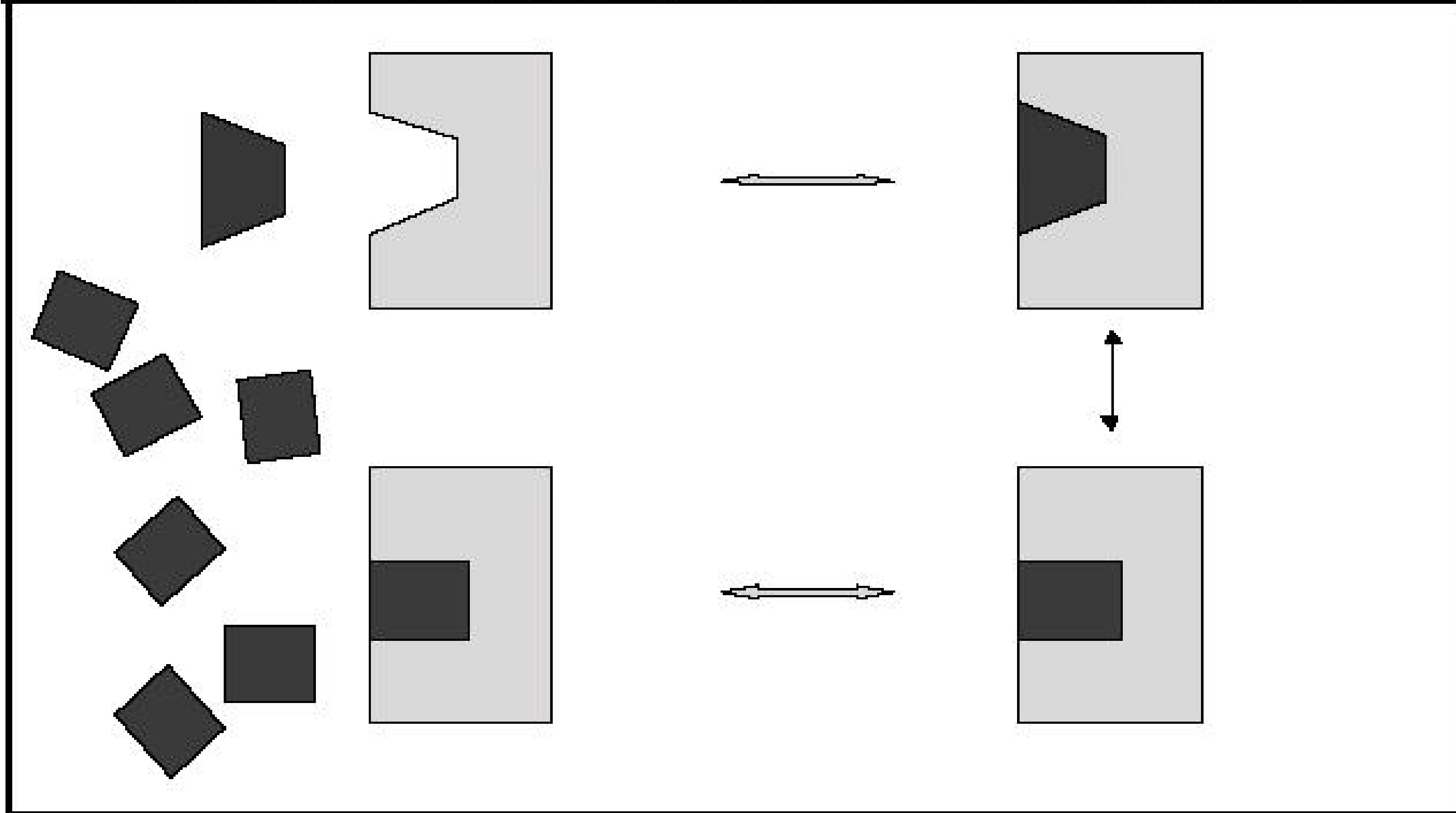

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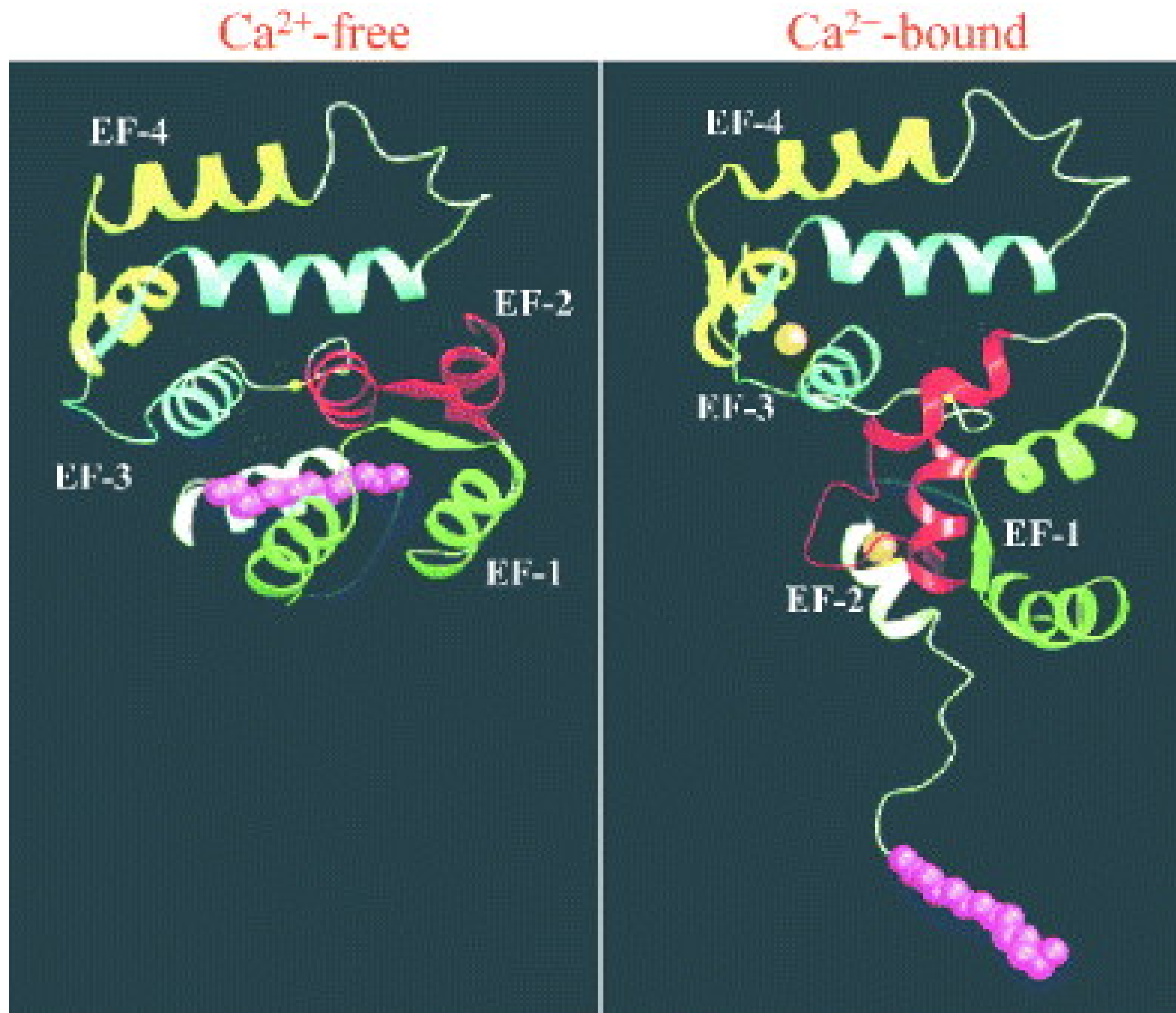
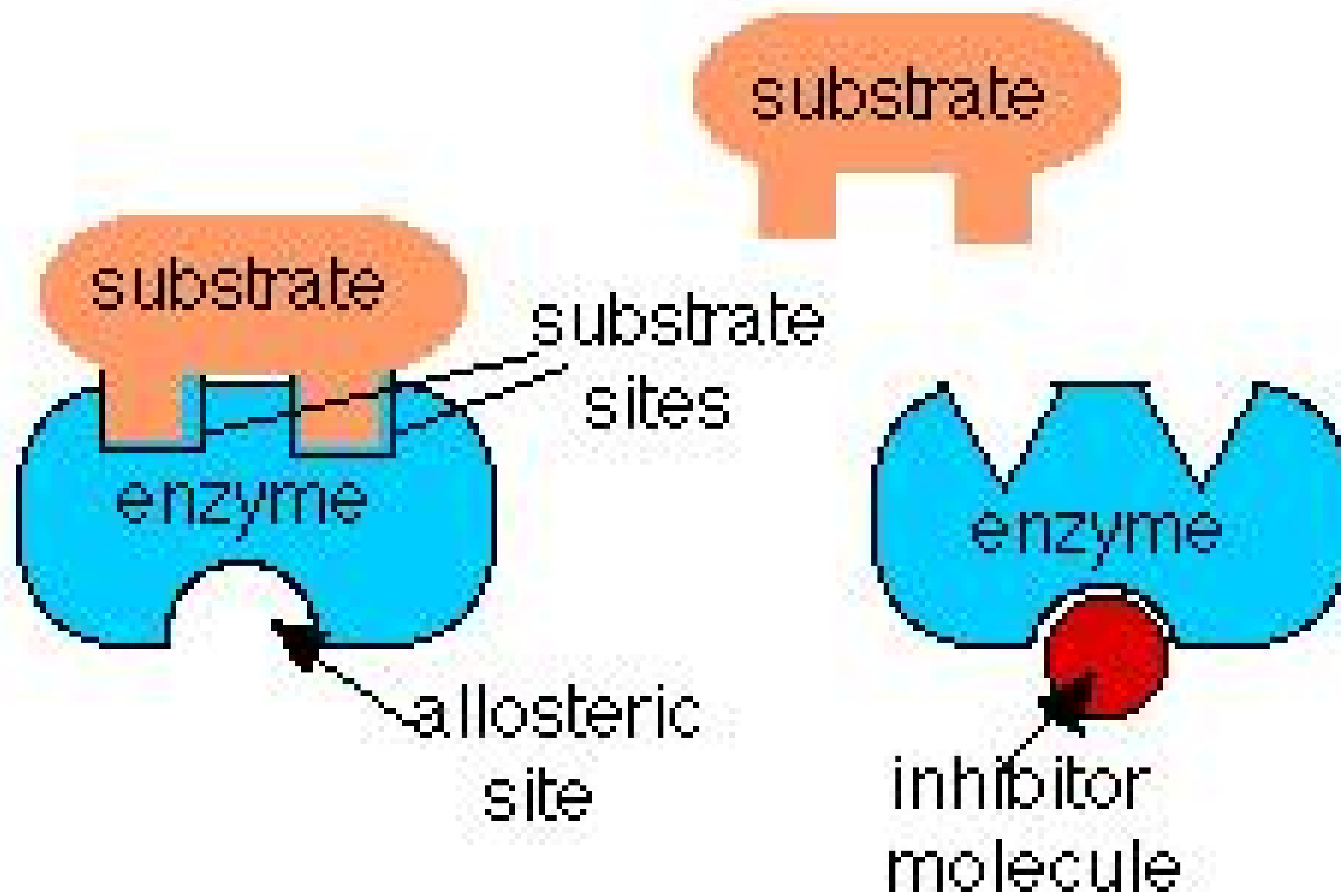
Conformation changes due to binding also important, e.g.:

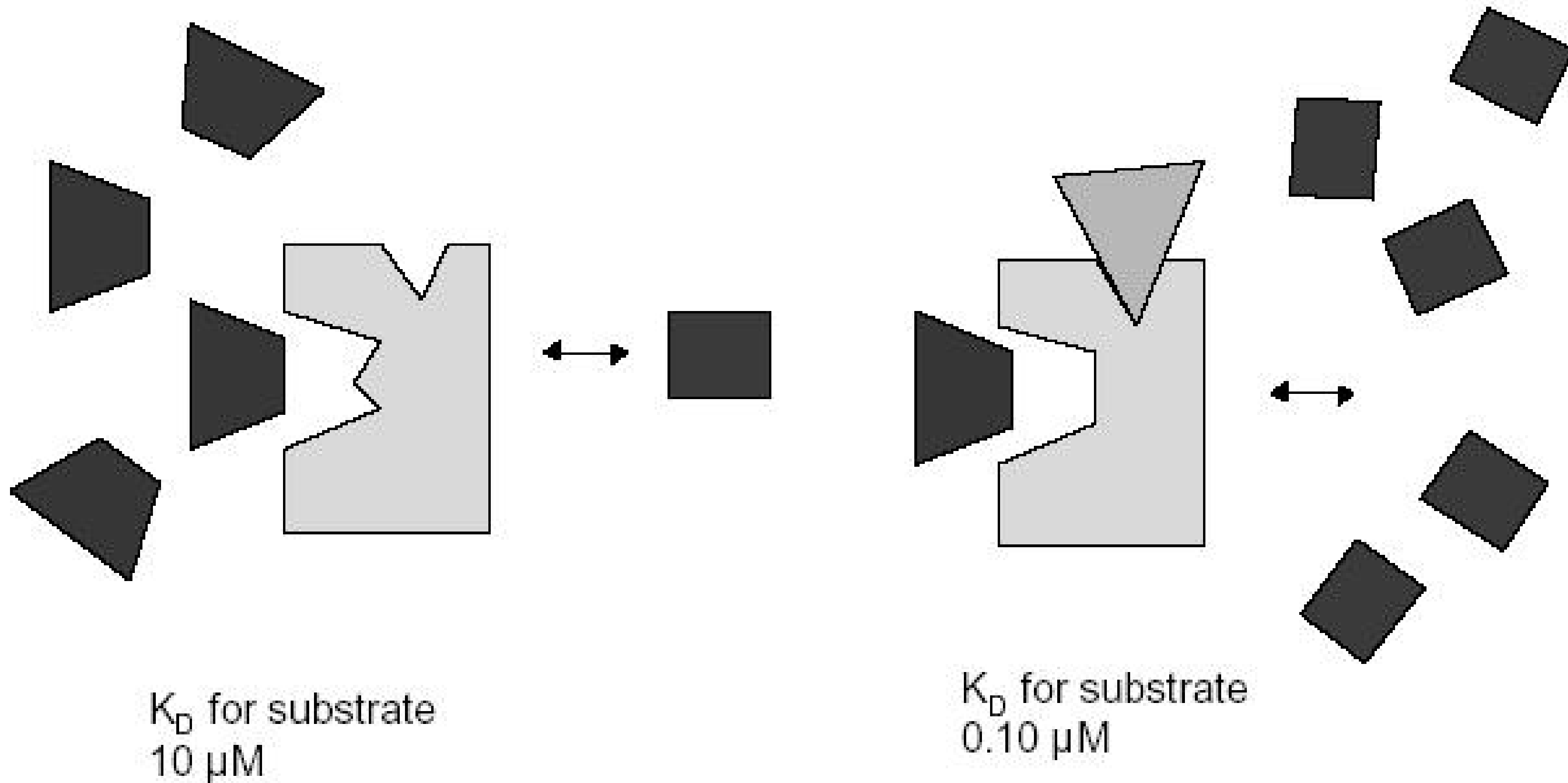


Ca^{2+} binds (at two sites) to recoverin, inducing shape change

recoverin is a protein that acts as calcium sensor in retinal rod cells;
it controls the lifetime of photoexcited rhodopsin by inhibiting rhodopsin kinase http://www.biochemj.org/

Allosteric Inhibition





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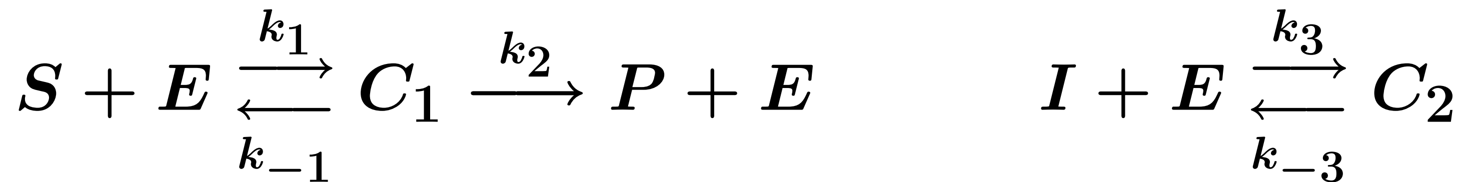
Competitive Inhibition

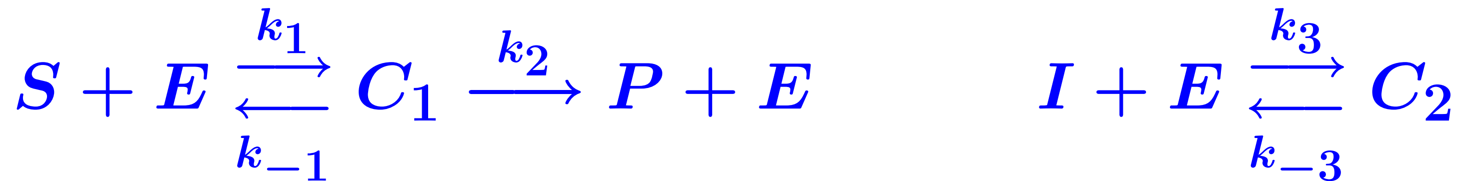


C_1 is complex as before; but catalytic reaction cannot proceed if inhibitor I bound to active site (complex C_2)

$$\begin{aligned} \dot{s} &= k_{-1}c_1 - k_1 se \\ \dot{e} &= (k_{-1}+k_2)c_1 + k_{-3}c_2 - k_1 se - k_3 ie \\ \dot{c}_1 &= k_1 se - (k_{-1}+k_2)c_1 \\ \dot{c}_2 &= k_3 ie - k_{-3}c_2 \\ \dot{i} &= k_{-3}c_2 - k_3 ie \\ \dot{p} &= k_2 c_1 \end{aligned}$$

note $c_1 + c_2 + e \equiv e_0$ total enzyme (bound or not)
and also $i + c_2 \equiv \text{const}$, so eliminate e, i

as earlier, first ignore $\dot{p}$, so three ode's
quasi-steady-state: do $c_i = \varepsilon x_i$, $\tau = \varepsilon t$, set $\varepsilon = 0 \ldots \rightsquigarrow$



$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} P + E \qquad I + E \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2$$

$$c_1 = \frac{K_\mathrm{i} \varepsilon s}{K_\mathrm{m} i + K_\mathrm{i} s + K_\mathrm{m} K_\mathrm{i}} \qquad \left(K_\mathrm{m} = \frac{k_{-1} + k_2}{k_1} \right)$$

$$c_2 = \frac{K_\mathrm{m} \varepsilon i}{K_\mathrm{m} i + K_\mathrm{i} s + K_\mathrm{m} K_\mathrm{i}} \qquad \left(K_\mathrm{i} = \frac{k_{-3}}{k_3} \right)$$

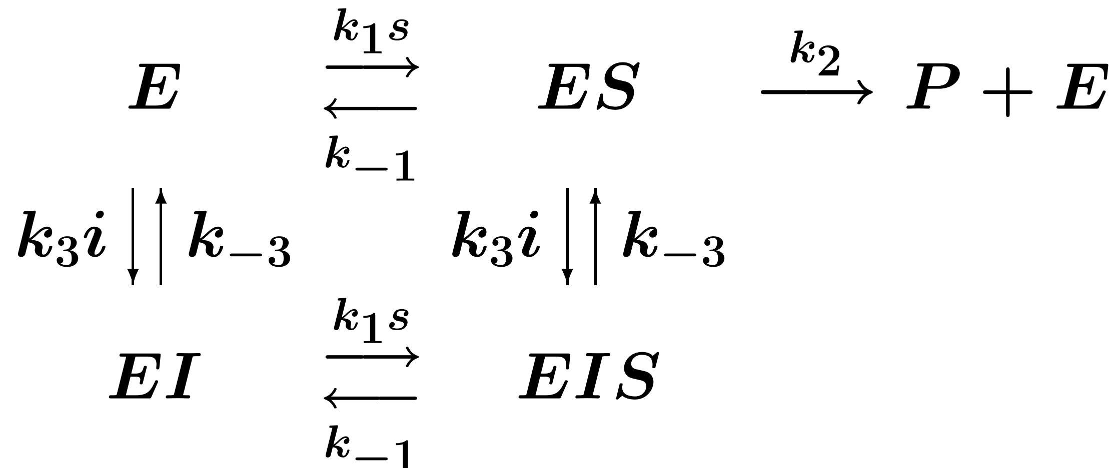
so, using $\dot{p} = k_2 c_1$, and again with $V_\mathrm{max} = k_2\,\varepsilon$

$$\dot{p} = \frac{V_\mathrm{max}\, s}{s + K_\mathrm{m}(1 + i/K_\mathrm{i})} \qquad \text{compare: } \dot{p} = \frac{V_\mathrm{max}\, s}{s + K_\mathrm{m}}$$

so the rate of production of product $\dot{p}(t)$
is smaller than if there had been no inhibition,
given the same amount of substrate $s(t)$
(smaller for $i \gg 1$, $k_3 \gg 1$, $k_3 \ll 1$)

but for $s \approx \infty$ still same $\dot{p} = V_\mathrm{max}$
("so much s that i doesn't get chance to bind & block")

Allosteric Inhibition



EI denotes complex of enzyme and inhibitor, etc

shorthand: "$EI \underset{k_{-1}}{\overset{k_1 s}{\rightleftarrows}} EIS$" really means $EI + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EIS$, etc

assuming binding of S or I to E independent

but product can only be formed (& released) if I not there

Homework (#2, p.30): do q-s-s approx to obtain a formula:

$$\dot{p} = \frac{V_{\max}}{1 + i/K_{\mathrm{i}}} \cdot \frac{s^2 + as + b}{s^2 + cx + d} \qquad \text{(for some } a = a(i), \ldots)$$

so max rate (large s) goes ↘, compared w/competitive inh

intuition: i can bind, so max throughput affected

Cooperativity

if multiple binding sites in enzyme, and
binding additional substrate molecules helped by previous
(classical example: oxygen/hemoglobin)

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} P + E \qquad S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \xrightarrow{k_4} P + C_1$$

(so product can be formed from either site)

$$\begin{aligned}
\dot{s} &= k_{-1}c_1 - k_1 se + k_{-3}c_2 - k_3 sc_1 \\
\dot{e} &= (k_{-1} + k_2)c_1 - k_1 se \\
\dot{c}_1 &= k_1 se - (k_{-1} + k_2)c_1 + (k_4 + k_{-3})c_2 - k_3 sc_1 \\
\dot{c}_2 &= k_3 sc_1 - (k_4 + k_{-3})c_2 \\
\dot{p} &= k_2 c_1 + k_4 c_2
\end{aligned}$$

note $c_1 + c_2 + e \equiv e_0$ total enzyme (bound or not)
as earlier, first ignore $\dot{p}$, so three ode's
quasi-steady-state: do $c_i = \varepsilon x_i$, $\tau = \varepsilon t$, set $\varepsilon = 0 \ldots \rightsquigarrow$

$$c_1 = \frac{K_2 \varepsilon s}{K_1 K_2 + K_2 s + s^2} \qquad \left(K_1 = \frac{k_{-1} + k_2}{k_1} \right)$$

$$c_2 = \frac{\varepsilon s^2}{K_1 K_2 + K_2 s + s^2} \qquad \left(K_2 = \frac{k_4 + k_{-3}}{k_3} \right)$$

$$\Rightarrow \ \dot{p} = k_2 c_1 + k_4 c_2 = \frac{(k_2 K_2 + k_4 s)\, \varepsilon s}{K_1 K_2 + K_2 s + s^2}$$

e.g.: independent identical binding: $k_1{=}2k_3, k_{-3}{=}2k_{-1}, k_4{=}2k_2$ (two sites, so double probab of binding/unbinding S or P)

$$\dot{p} = 2\frac{k_2 \varepsilon s}{K + s} \qquad \left(K = \frac{k_{-1} + k_2}{k_3} \right) \quad \text{twice, as expected}$$

more interesting: if $K_2 \ll 1$ and $1 \ll K_1$ (e.g. $k_3 \gg 1$, $k_1 \ll 1$)

$$\dot{p} = \frac{V_{\mathrm{max}} s^2}{K_{\mathrm{m}}^2 + s^2} \qquad \left(K_{\mathrm{m}} = \sqrt{K_1 K_2}\,, \ \ V_{\mathrm{max}} = k_4 \varepsilon \right)$$

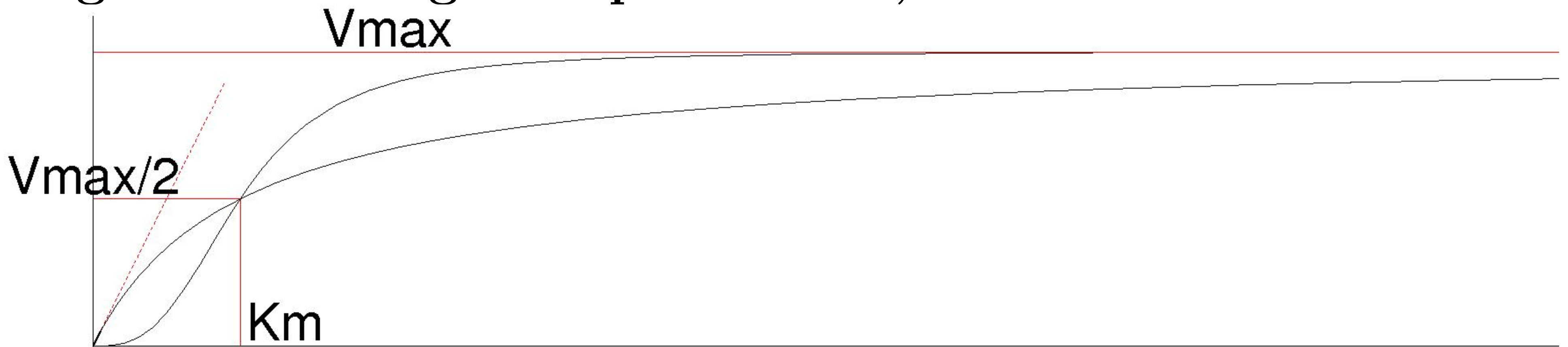
more generally:

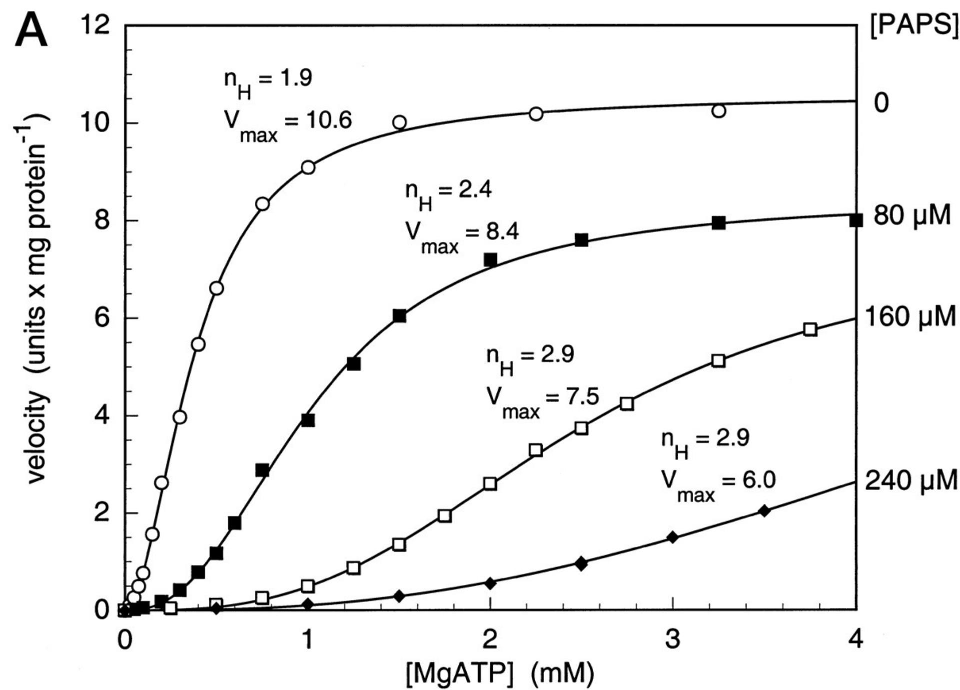
$$\dot{p} = \frac{V_{\max} s^n}{K_{\mathrm{m}}^n + s^n} \qquad n = \text{“\textit{Hill coefficient}”}$$

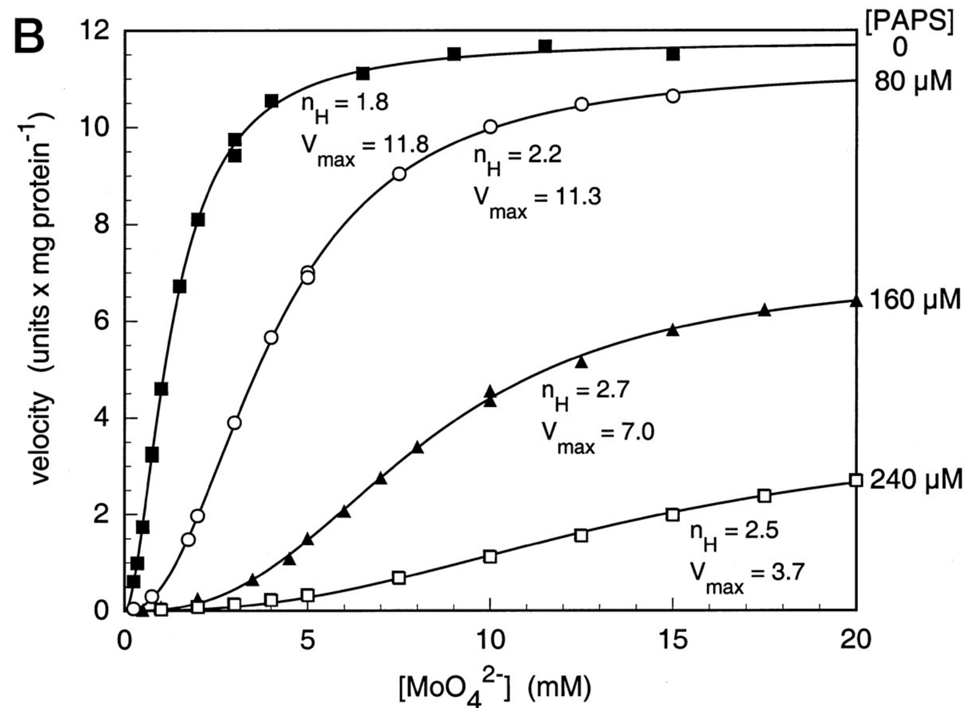
n **may be fractional (different limits; non-independence, etc)**
experimental determination of $V_{\max}, n, K_{\mathrm{m}}$**: observe that**

$$n \ln s = n \ln K_{\mathrm{m}} + \ln\left(\frac{\dot{p}}{V_{\max} - \dot{p}}\right)$$

so plot $\ln\left(\frac{\dot{p}}{V_{\max}-\dot{p}}\right)$ **vs** $\ln s$**, look at slope & intersects**
note graph of $f(s) = \frac{V_{\max} s^n}{K_{\mathrm{m}}^n + s^n}$ **= graph when** n**=1, after change of vars** $s \mapsto s^n$ **(contract if** $s \approx 0$**, expand if** $s \gg 0$**), so max at** $V_{\max}$**, half-max at** K_{m}**; for** $n > 1$**:** $f'(0) = 0$ **so “sigmoidal” – e.g. compare** $n = 1, 3$**:**







experimental example of co-operativity; fits to various Hill curves that arise due to allosteric affects (which change cooperativity)

from Ian J. MacRae et al., "Induction of Positive Cooperativity by Amino Acid Replacements within the C-terminal Domain of Penicillium chrysogenum ATP Sulfurylase," J. Biol. Chem., Vol. 275, 36303-36310, 2000

(intracellular reaction in the incorporation of inorganic sulfate into organic molecules by sulfate assimilating organisms; the allosteric effector is "PAPS")

(= 3'-phosphoadenosine-5'-phosphosulfate)

velocity curves of C509S vs (a) MgATP and (b) MoO_4^2

E.g. of oscillations: glycolysis

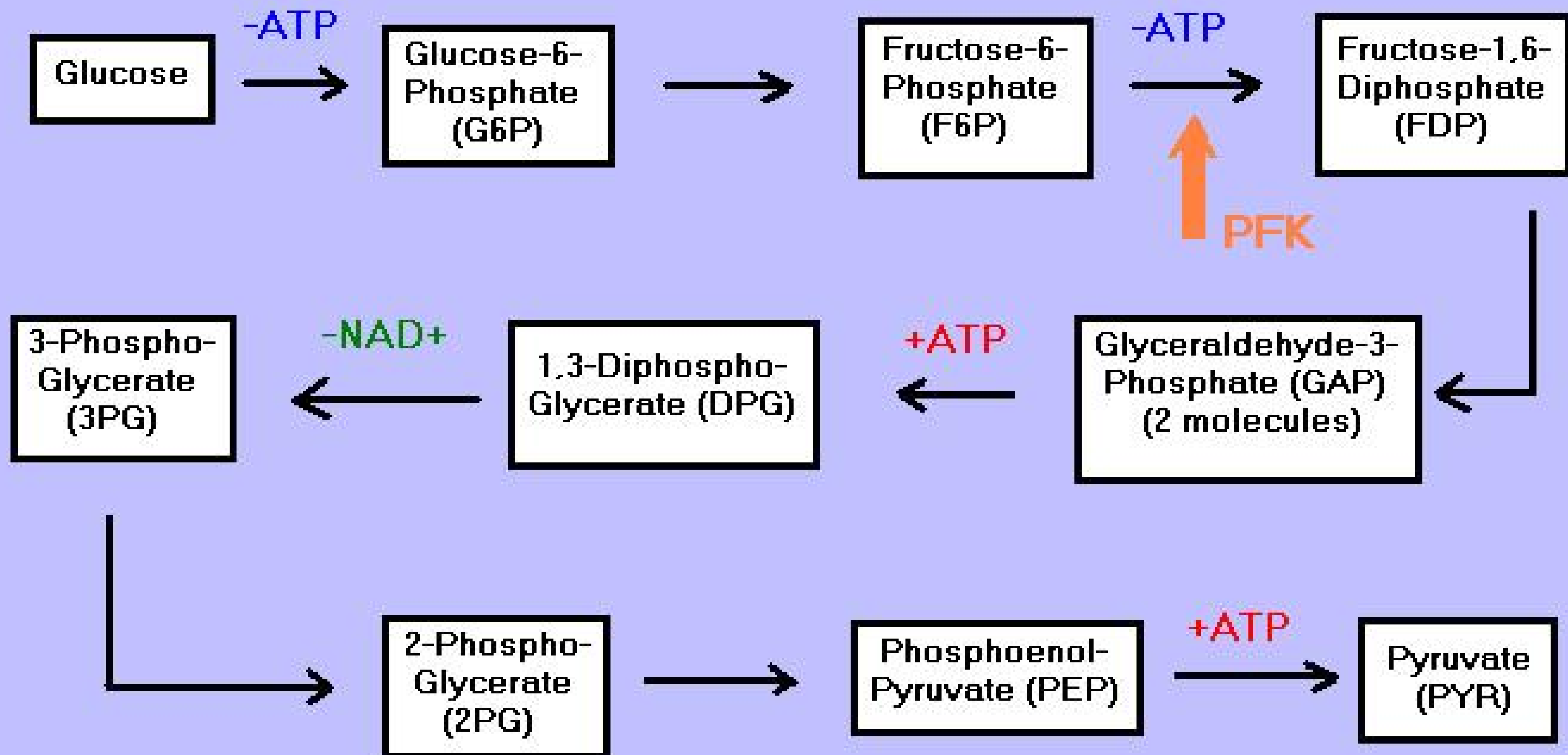
adenosine **tri**phosphate (ATP) = "energy currency" of cell
obtained by ***phosphorylation*** (adding phosphate group HPO_4^{2-})
to adenosine **bi**phosphate (ADP) or **mono**phosphate (AMP)$_{(+2)}$

energy stored in the covalent bonds between phosphates:
natural repulsion of negatives needs to be overcome
–think of compressing a spring and "clicking" it on position–
hydrolysis: energy released: water cleaves bond ATP→ADP

glycolysis: process whereby metabolic energy (ATP)
is produced via conversion of glucose (G) to pyruvate (PYR)

process entails multiple reactions, each of which is catalyzed
by its own enzyme: "the glycolytic pathway"

(http://bill.srnr.arizona.edu/NLBchemd/NLBchemdOverview.html)



glucose → two GAP molecules; net gain of two ATP's

Oscillations

***damped* oscillations in glycolysis reported by Duysens and Amesz [1957]**

***sustained* oscillations:**

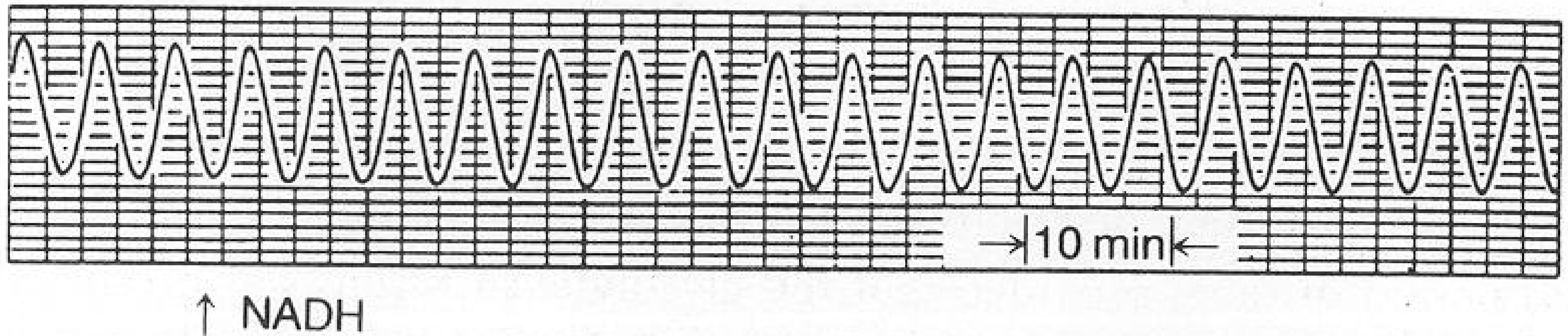


Fig. 2.2. Sustained oscillations in an extract of the yeast *Saccharomyces carlsbergensis* utilizing trehalose as the glycolytic substrate. The slow degradation of this substrate gives rise to regular oscillations that can be maintained for more than 100 cycles (Pye, 1971). The oscillations are recorded by measuring the fluorescence of the glycolytic intermediate, NADH.

http://bill.srnr.arizona.edu/classes/496y/Glycolysis/Glycol03.gif

Why?

physiological significance unclear; suggested possibilities:

- **circadian rhythms**
- **facilitate alternation between biochemical pathways**
- **increase efficiency of glycolysis itself**
- **drive pulsatile secretion of insulin in pancreas**
- **cause arrhythmia in cardiac cells**

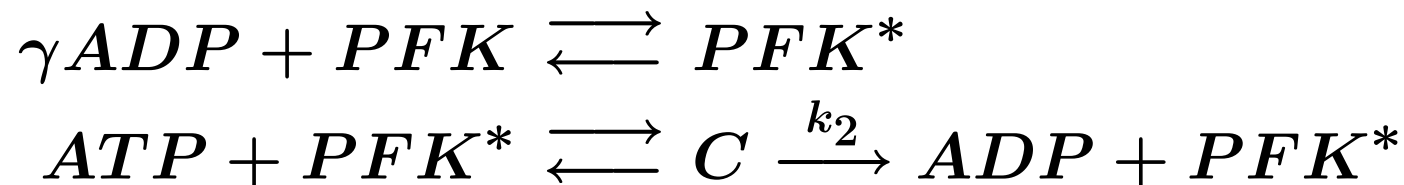
Golbeter: ***alternatively, glycolytic oscillations may be an epiphenomenon, a necessary consequence of the regulatory properties of PFK and without functional significance***

negative feedback for regulation may produce oscillations - shower temperature control example; also:
current work in control theory: "Bode integral formula"

simplified model (Sel'kov 68; mod: Goldbeter & Lefever 72) step: phosphofructokinase (PFK) catalyzes conversion of fructose-6-phosphate (F6P) to fructose-1, 6-diphosphate (FDP)

will assume that PFK (enzyme) works as catalyst when in "active" form PFK* (bound with γ molecules of ADP)

(in fact: there is allosteric inhibition of PFK by ATP, which is in turn removed by AMP, but since 2ADP$\leftrightarrow$ATP+AMP, and AMP removes the ATP from the PFK allosteric site, same effect)



$$\gamma ADP + PFK \rightleftharpoons PFK^*$$
$$ATP + PFK^* \rightleftharpoons C \xrightarrow{k_2} ADP + PFK^*$$

and also constant supply $\xrightarrow{\nu_1}$ATP and removal of ADP

conservation of total enzyme $\varepsilon \ll 1$, nondimensionalize, etc

(in book, E=PFK, ES_2^γ=PFK*, S_1=ATP, S_2=ADP, $S_1ES_2^\gamma$=C)

Applying the law of mass action to the Sel'kov kinetic scheme, we find five differential equations for the production of the five species $s_1 = [S_1], s_2 = [S_2], e = [E], x_1 = [ES_2^\gamma], x_2 = [S_1ES_2^\gamma]$:

$$\frac{ds_1}{dt} = \nu_1 - k_1 s_1 x_1 + k_{-1} x_2, \tag{1.58}$$

$$\frac{ds_2}{dt} = k_2 x_2 - k_3 s_2^\gamma e + k_{-3} x_1 - \nu_2 s_2, \tag{1.59}$$

$$\frac{dx_1}{dt} = -k_1 s_1 x_1 + (k_{-1} + k_2) x_2 + k_3 s_2^\gamma e - k_{-3} x_1, \tag{1.60}$$

$$\frac{dx_2}{dt} = k_1 s_1 x_1 - (k_{-1} + k_2) x_2. \tag{1.61}$$

The fifth differential equation is not necessary, because the total available enzyme is conserved, $e + x_1 + x_2 = e_0$. Now we introduce dimensionless variables $\sigma_1 = \frac{k_1 s_1}{k_2 + k_{-1}}, \sigma_2 = (\frac{k_3}{k_{-3}})^{1/\gamma} s_2, u_1 = x_1/e_0, u_2 = x_2/e_0, t = \frac{k_2 + k_{-1}}{e_0 k_1 k_2} \tau$ and find

$$\frac{d\sigma_1}{d\tau} = \nu - \frac{k_2 + k_{-1}}{k_2} u_1 \sigma_1 + \frac{k_{-1}}{k_2} u_2, \tag{1.62}$$

$$\frac{d\sigma_2}{d\tau} = \alpha \left[u_2 - \frac{k_{-3}}{k_2} \sigma_2^\gamma (1 - u_1 - u_2) + \frac{k_{-3}}{k_2} u_1 \right] - \eta \sigma_2, \tag{1.63}$$

$$\epsilon \frac{du_1}{d\tau} = u_2 - \sigma_1 u_1 + \frac{k_{-3}}{k_2 + k_{-1}} \left[\sigma_2^\gamma (1 - u_1 - u_2) - u_1 \right], \tag{1.64}$$

$$\epsilon \frac{du_2}{d\tau} = \sigma_1 u_1 - u_2, \tag{1.65}$$

where $\epsilon = \frac{e_0 k_1 k_2}{(k_2 + k_{-1})^2}, \nu = \frac{\nu_1}{k_2 e_0}, \eta = \frac{\nu_2 (k_2 + k_{-1})}{k_1 k_2 e_0}, \alpha = \frac{k_2 + k_{-1}}{k_1} (\frac{k_3}{k_{-3}})^{1/\gamma}$. If we assume that ϵ is a small number, then both u_1 and u_2 are "fast" variables and can be set to their quasi-steady values.

for (nondimensionalized) ATP and ADP concentrations, resp.:

$$\dot{\sigma}_1 = \nu - f(\sigma_1, \sigma_2) \qquad (0 \le \nu \le 1)$$
$$\dot{\sigma}_2 = \alpha f(\sigma_1, \sigma_2) - \eta\sigma_2$$

where

$$f(\sigma_1, \sigma_2) = \frac{\sigma_1 \sigma_2^\gamma}{\sigma_1 \sigma_2^\gamma + \sigma_2^\gamma + 1}$$

see separate file: find nullclines and steady state
linearize at steady-state; char eqn = $\lambda^2 - H +$ positive
so stable if $H < 0$, unstable if $H > 0$
at $H = 0$ have imaginary roots; with a little more work:
supercritical Hopf bifurcation (small periodic orbit)

$H(0) \equiv \eta(\gamma - 1) > 0$ provided $\gamma > 1$ & $H(1) = -\eta < 0$

so $\exists$ switch (periodic orbit for parameters < bifurcation)
(ν small if e.g. k_2 large, so lots of ADP produced, or ν_1 small
(little ATP produced) – "positive feedback" effect larger

Goldbeter-Lefever model: better agreement with experiments: *two* bifurcation points. See Golbeter's book for many examples!)

Suggested problems:

- page 30, problem 1
- page 30, problem 2
- page 31, problem 4, parts (a) and (b)
- page 31, problem 5
- page 31, problem 7
- a bit harder(?): page 32, problem 10; also page 31, problem 6