

## Enzyme kinetics review: -- 2009 revise



Michaelis Menton form:  $v_0 = v_{MAX}[S]/(K_m+[S])$

where  $v_0$  is initial rate—depends on  $[E_0]$  and  $[S]$ ,

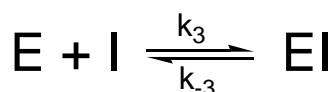
$v_{MAX}$  is  $k_2[E_0]$ , max rate (all  $E_0$  used),

$k_2$  is turnover ( $S \rightarrow P$ )<sub>max</sub> – here rate limit, ignore  $k_{-2}$

$K_m = (k_{-1}+k_2)/k_1 = [E][S]/[ES]$  –dissociation of ES

Inhibition—ties up enzyme concentration:

**Competitive:** (see Engel pp. 710-711)



Two paths for E, less available for making P

$v_0 = k_2[E_0][S]/(\alpha K_M + [S]) = v_{MAX} [S]/(K'_M + [S])$

where  $\alpha = (1+[I]/K_i)$ ,  $K_i = [E][I]/[EI]$  and  $K'_M = \alpha K_M$

no  $[I] \rightarrow$  MM again,  $[I]$  inc.,  $\alpha > 1 \rightarrow$  rate decrease

Lineweaver Burk ( $1/v$  vs.  $1/[S]$ ) different slope,  $K'_M/v_{MAX}$   
same intercept -  $1/v_{MAX}$  and  $v = v_{MAX}/2 \rightarrow [S] = K'_m$

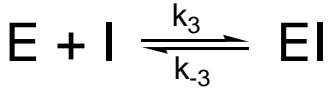
$$1/v = (K'_M/v_{MAX})(1/[S]) + 1/v_{MAX}$$

Conditions:  $I \ll K_i \Rightarrow \alpha \sim 1 \rightarrow$  ordinary M - M

$I \gg K_i \Rightarrow \alpha \sim [I]/K_i = [EI]/[E] \gg 1$

so  $[EI] \gg [E] \rightarrow$  enzyme tied up, not make P

**Non-competitive:** (derive Engel - pp 712-13)



$$v_0 = k_2[E_0][S] / \{ ([S](1+[I]/K_{IS}) + K_m(1+[I]/K_I)) \}$$

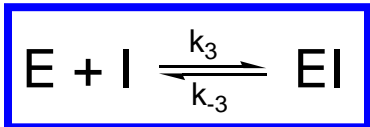
$$= v_{MAX} [S] / (\alpha'[S] + \alpha K_M)$$

where  $\alpha' = (1+[I]/K_{IS})$ ,  $\alpha = (1+[I]/K_I)$ ,  $K_{IS} = [ES][I]/[ESI]$

Again:  $[I]=0 \rightarrow$  MM, as  $[I]$  inc. reaction slows, less P  
 In Lineweaver Burk. both slope and intercept change

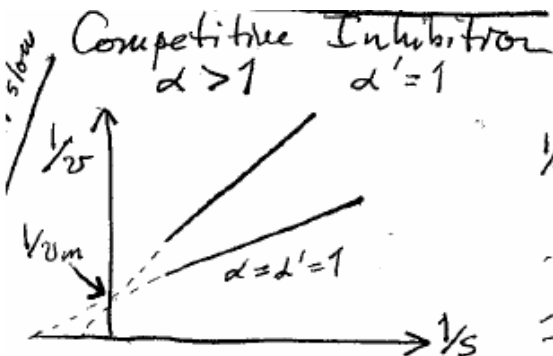
$$1/v = (\alpha K_M / v_{MAX})(1/[S]) + \alpha' / v_{MAX}$$

**Uncompetitive:** drop

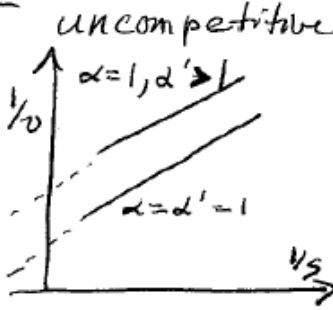


$\rightarrow \alpha = 1, \alpha' > 1,$

slope not changed, but intercept change ( $\alpha' / v_{MAX}$ )



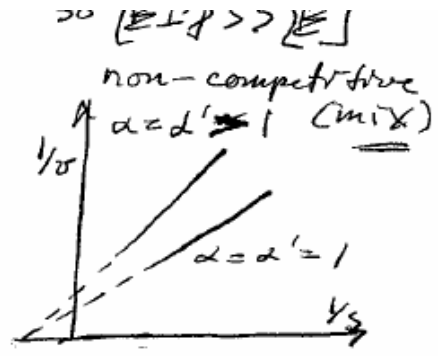
as described above



here no EI form ( $\alpha = 1$ )  
 but ESI form ( $\alpha' > 1$ )

as described above

here no EI form ( $\alpha = 1$ )  
 but ESI form ( $\alpha' > 1$ )



here both EI  
 + ESI form

here both EI  
 and ESI form

## Inhibition hugely important –

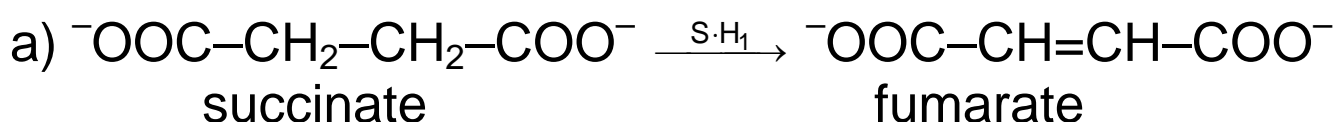
most biochemistry processes need enzymes →

- need specific reaction to go under control
- need operate with low concentration enzyme
- need fast response to S perturbation - on/off

**control** – bio feedback/signaling– gone wrong – “sick”

Drugs/pharma intercede → inhibit

See dihydrofolate reductase example (Engle pp. 713-14)  
or e.g. succinic dehydrogenase



if add malonate:  $\text{}^{-}\text{OOC}-\text{CH}_2-\text{COO}^{-}$

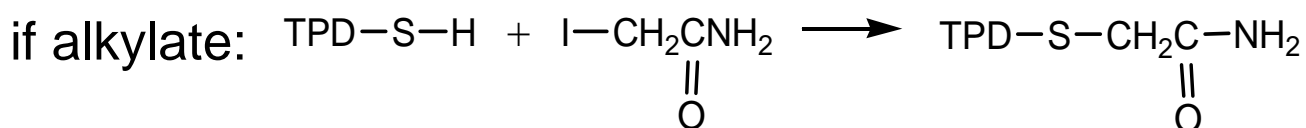
has similar shape, 2  $\text{}^{-}\text{COO}^{-}$  groups – binds active site  
but **cannot dehydrogenate** (not form C=C)

so stays on enzyme: EI forms, **ties up E**

inhibit reaction of succinate: less ES

like case (a) — **Competitive inhibition**

b) Triosephosphate dehydrogenase - Cys in active site

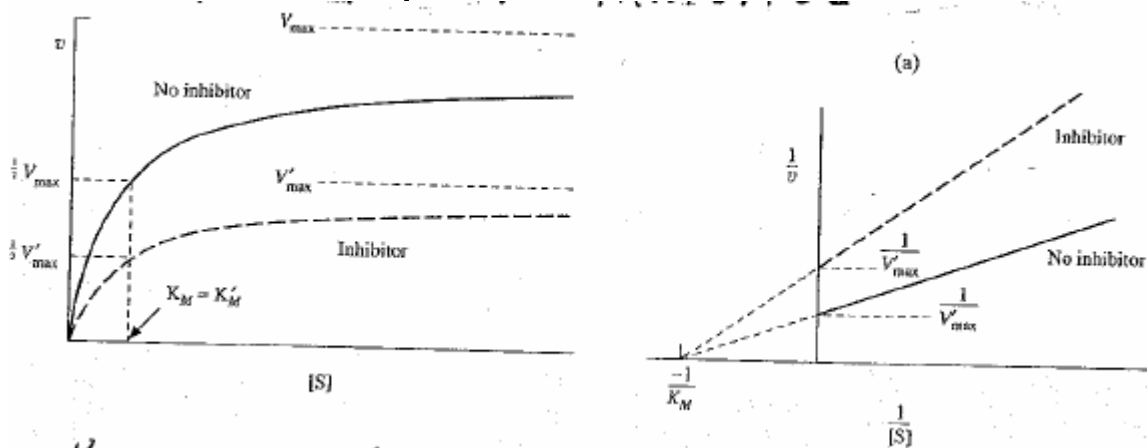


This enzyme now lost to reaction inhibition (**no reversal**)

but  $K_M$  same,  $v_{\text{max}}$  reduced (**recall slope  $K_m/v_{\text{max}}$** )

⇒ like case (c) — **non-competitive**  $\alpha, \alpha' > 1$

## Plot for non-competitive inhibition



$K_M$  same,  $V_{max}$  reduced

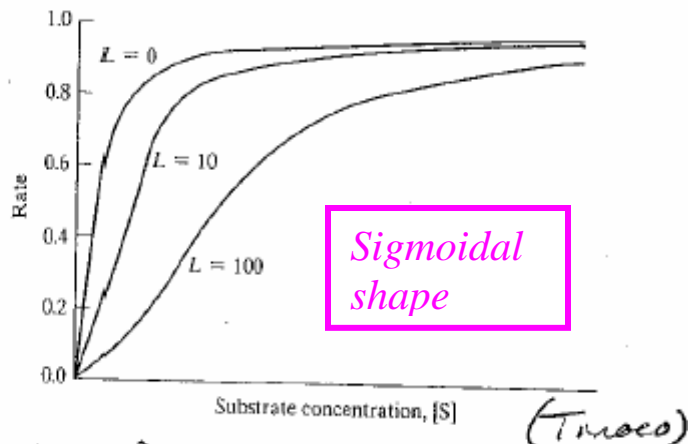
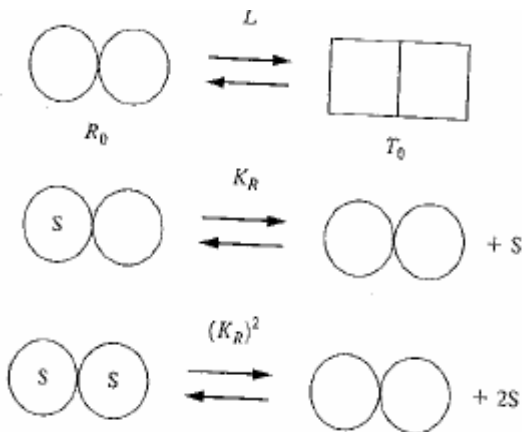
**Allosterism** → binding substrate to one site/subunit affect binding/release at 2nd or 3rd ...

e.g. hemoglobin – 4 subunits, each bind  $O_2$  → cooperate

MWC model → each subunit has 2 forms  
different binding efficiency

R – relaxed – O -binds better

T – tense -  - no substrate:  $L = [T_0]/[R_0]$  equilibrium



Solve mechanism use pre-equilibrium:

$$v = v_{\max} \gamma (1 + \gamma) / \{ (1 + \gamma)^2 + L \}$$

$$\gamma = [S] / K_R$$

$$L = [T_0] / [R_0]$$

$L = 0 \rightarrow$  no T  $\rightarrow v = v_{\max} \gamma / (1 + \gamma)$  this is M-M

L increase  $\rightarrow$  sigmoidal shape in rate vs. [S]

$\rightarrow$  binding S convert T  $\rightarrow$  R  $\Rightarrow$  max rate constant  
analogous to increasing enzyme  $[E_0]$ , **only R binds**

Many topics in biochemistry have time/rate dependence

- a) diffusion  $\rightarrow$  rate material moves in medium  
faster motion more “friction” affect equilibrium  
 $\Rightarrow$  separations like – electrophoresis – HPLC  
 $\Rightarrow$  pharmacological – cross blood-brain barrier  
– drug absorption
- b) charge transfer – many reactions are redox move  
charge in enzyme -- special method-- Marcus theory  
  
also move charge in cell through  $\text{Na}^+$  or  $\text{K}^+$  channel  
signaling, neurobiology, muscle activation, etc.
- c) Photobiology – transfer of energy after excitation  
light absorption and re-emission (fluorescence)  
structural changes, energetics, signaling

## Single molecule enzymology

It is popular now to try to study single molecules as they undergo some process, enzyme reaction is a good one

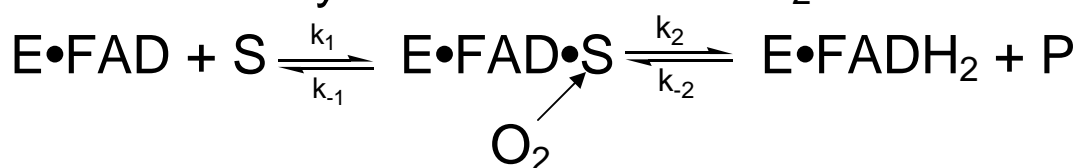
We discuss look at average of many molecule sample  
How differ if look at 1 molecule, enzyme turns over  
can follow rate of individual steps

Idea—fluorescent enzyme, changes with substrate

Trap enzyme so dilute yet not move, add substrate  
Signal will stay on various times, depending on turnover

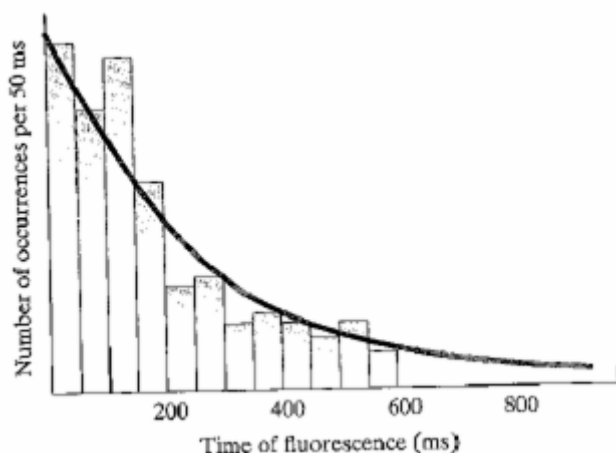
Cholesterol oxidase, S=cholesterol, P=oxidized cholest.

Active site has flavin adenine dinucleotide (FAD)  
catalyse oxidation with O<sub>2</sub>



Observe on/off signal for varying amount of time

Plot number of times within  
t → t+Δt → histogram



Results—the various  
enzyme molecules fit MM  
but had different rates,  
could be due to different  
conformers—**from Tinoco**

## Photobiology:

We will return to basics of **absorption** and **fluorescence** at end of course — in the spectroscopy section

**idea:** light consists of photons  $E=h\nu=hc/\lambda$

where  $\nu$  – frequency of light,  $\lambda$  – wavelength

transmitted energy:  $I_t = I_0(10)^{-\epsilon bc}$  - intensity is flux:  $\text{cm}^{-2}\text{s}^{-1}$

$b$  – path in cm,  $c$  – concentration (molar),  $\epsilon$  - absorptivity

amount absorbed is then:  $I_{\text{abs}} = I_0(1 - (10)^{-\epsilon bc})$  note:  $(10)^{-\epsilon bc} = e^{-2.3\epsilon bc}$

if  $c$  is low =  $[A]$ , then  $I_{\text{abs}} \sim I_0(2.3)\epsilon b[A]$   $e^{-x} = 1 - x + x^2/2 - \dots$

excitation rate:  $r = d[A]/dt = I_{\text{abs}}/b \rightarrow 1000\text{cm}^3/\text{L}$

$$= I_0(2303)\epsilon b[A]/b$$

integrate rate:  $[A] = [A_0]\exp[-I_0(2303)\epsilon t]$  - 1<sup>st</sup> order

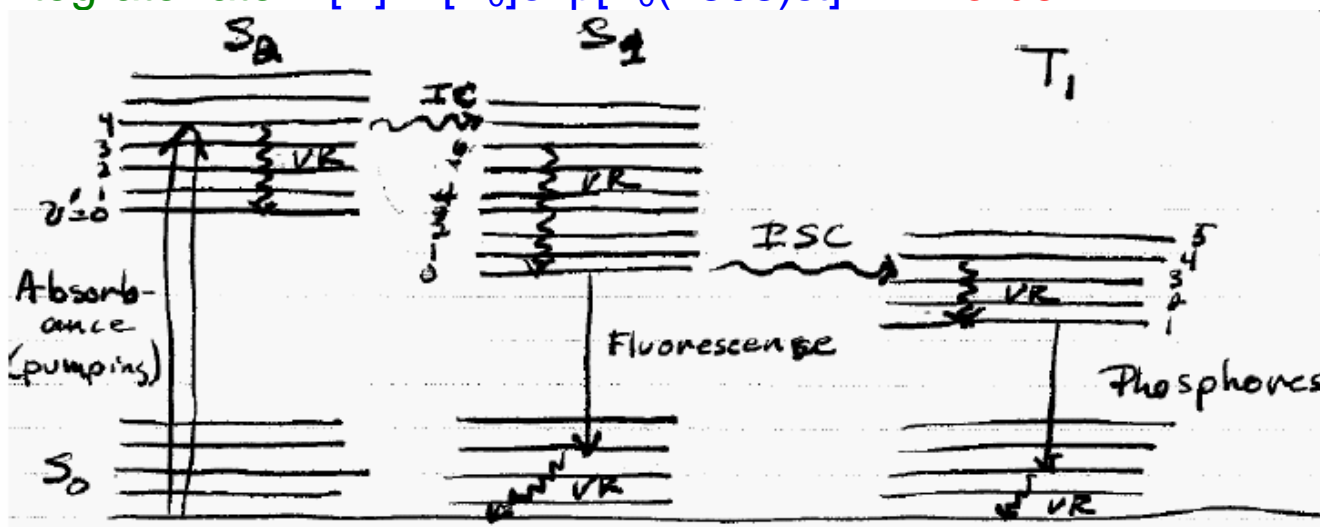


TABLE 26.1 Photophysical Reactions and Corresponding Rate Expressions

Process	Reaction	Rate
Absorption/excitation	$S_0 + h\nu \rightarrow S_1$	$k_a[S_0]$ ( $k_a$ )
Fluorescence	$S_1 \rightarrow S_0 + h\nu$	$k_f[S_1]$
Internal conversion	$S_1 \rightarrow S_0$	$k_{ic}[S_1]$
Intersystem crossing	$S_1 \rightarrow T_1$	$k_{isc}[S_1]$
Phosphorescence	$T_1 \rightarrow S_0 + h\nu$	$k_p[T_1]$
Intersystem crossing	$T_1 \rightarrow S_0$	$k_{isc}^T[T_1]$

Jablonski diagram, like a kinetic mechanism, follow energy

Engel p. 718-19, alternate view, kinetic analogy Table 26.1

**Quenching**—provide alternate energy path, like parallel mech.  
reduce fluorescence – view as yield:

$$I_f^0/I_f = 1 + (k_q/k_f)[Q] \text{ - Stern-Volmer plot: } I_f^0/I_f \text{ vs } [Q]$$

$$\text{Lifetime: } 1/\tau_f = k_f + k_q[Q]$$

**Diffusion** – Engel Chap 24, Tinoco, Chapter 6

Analysis of gas collisions will yield the gas law

$$PV = n R T = N/N_0 R T$$

but in form:  $PV = 1/3 N m \langle u^2 \rangle \rightarrow$  mean velocity

since Kinetic Energy  $\sim 1/2 m v^2 \sim 1/2 m \langle u^2 \rangle$

$$PV = 2/3 n (N_0 \cdot 1/2 m \langle u^2 \rangle) \quad \leftarrow PV=nRT \rightarrow \underline{1/2}$$

$$\underline{m\langle u^2 \rangle = 3kT}$$

T – measure average Kinetic Energy

$$RT = 2/3 \langle U_{tr} \rangle \leftarrow \text{average trans energy}$$

[in solution and big molecules  $\rightarrow$  vibration and rotation  
 $\rightarrow$  collisions

$\Rightarrow$  temperature  $\rightarrow$  average kinetic energy]

Distribution of energies relate to

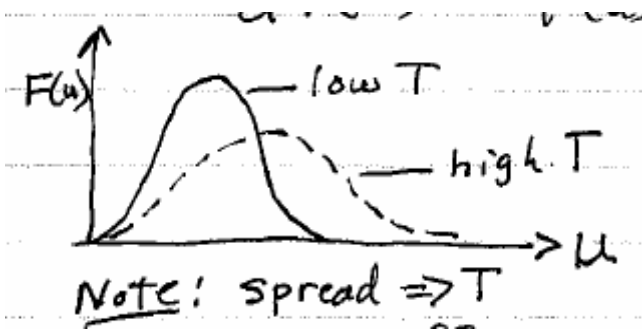
Probability of any one  $E_i$ , discrete  $E_i$

$$\text{Boltzmann: } P_j = N_j/N = g_j e^{-E_j/kT} / \sum_i g_i e^{-E_i/kT}$$

each weight by  $e^{-E_i/kT}$

Maxwell-Boltzmann velocity distribution – continuous

$$dP(u) = F(u)du = 4\pi (m/2\pi kT)^{3/2} u^2 e^{-mu^2/2kT}$$



can be used to describe collisions and other properties of gases

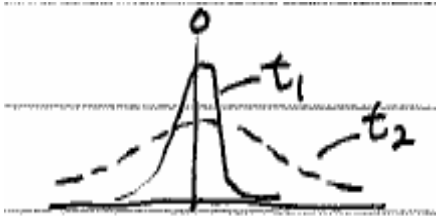
- Liquids → Diffusion better model
- $J_x$  flow of concentration through area

Fick 1st  $J_x = -D (dc/dx)$  - need concentration gradient  
if  $dc/dx$  – constant → flow

Fick 2<sup>nd</sup>  $(\partial c/\partial t) = D (\partial^2 c/\partial x^2)$  time dependent –  
gradient vary w/x

D – determine experimentally from Fick's law ( $\text{cm}^2 \text{s}^{-1}$ )

relates to distributions:  $D = \langle x^2 \rangle / 2t$



x – displacement from initial position  
reverse – time to travel distribution  
(average)

$D = kT/f = f/6\pi \eta r$   $f$  – frictional coefficient (Einstein)

$f = 6\pi \eta r$   $\eta$  – viscosity ,  
 $r$  – radius (assume spherical)

⇒ could measure size from D

shape means correct  $f \rightarrow f/f_0$  increase more 0

Sedimentation – diffuse in medium – gravity drive

→ can be molecule or cellular component (big)  
gravity ↓ bouyant force keep up ↑ (velocity depend)

$$F = mg - mV_2 \rho g \uparrow - f v = m \partial v / \partial t : \text{friction slows}$$

$\rho =$  density of medium,  $V_2 =$  partial specific vol.

Centrifuge increase g – gravity acceleration effect = a

$$a = \omega^2 x \quad \omega - \text{frequency}, x - \text{distribution from center}$$

terminal velocity becomes:  $U_t = m(1 - V_2 \rho) \omega^2 x / f$

sedimentation coefficient:  $s = U_t / \omega^2 x = m(1 - V_2 \rho) / f$

combine with diffusion  $f = kT/D$

Mass:  $M = RTs/D(1 - V_2 \rho) \rightarrow$  in molecular weight

Electrophoresis – charge molecules move in field

$$u = ZeE/f$$

$u/E$  – electrophoretic mobility

$D$  – diffusion constant small molecule  $\sim 10^{-5} \text{ cm}^2 \text{ s}^{-1}$

large molecule – smaller/slower

H-ion – can hop in water

Shows up in pre-exponential  $M + N \xrightleftharpoons[k_d]{k_d} (M N)^* \rightarrow P$

$$A_d = 4\pi r_{MN} (D_M + D_N) N_0 / 1000 N_0 \quad \text{Avogadro \& (1000 cm}^3 \rightarrow \text{L)}$$

$r_{MN}$  – encounter distance  $\sim$  few Å  
 $\sim 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$  for  $r \sim 4 \times 10^{-8} \text{ cm}$ ,  $D \sim 1.5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$

max rate if  $E_a \sim 0$

See Tinoco Table 7.5 / pg. 374 – typical rates

ions can be faster big molecule slower / even with enzyme