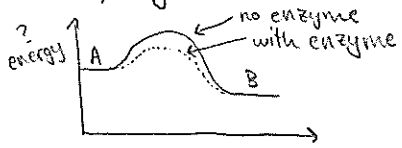
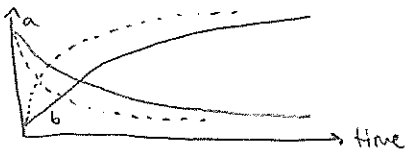
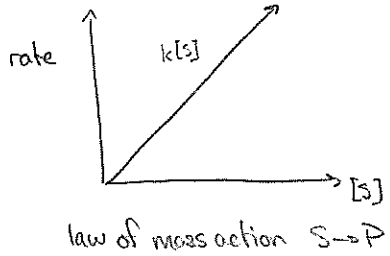
Chapter 3 - Enzyme Kinetics

Enzymes catalyse reactions by binding reactants (called the enzyme's substrate) and aiding in their conversion to the reaction products. They reduce activation energy of reactions but do not alter the reaction equilibrium, eg for $A \rightarrow B$

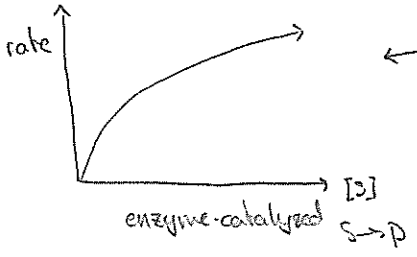




Enzymes are
- efficient (up to 10^7 times increase in reaction rate)
- specific (typically catalyze a single reaction)
- regulated (by binding of other species - biochemical level of control)



law of mass action $S \rightarrow P$



enzyme-catalysed $S \rightarrow P$

← This (nonlinear) hyperbolic kinetic law is a result of saturation of the enzyme pool

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Michaelis-Menten Kinetics

To derive a description of the kinetics of a single-substrate enzyme-catalysed reaction, consider the scheme:

$$S + E \rightleftharpoons C_1 \rightleftharpoons C_2 \rightleftharpoons P + E$$

substrate | free enzyme | enzyme-substrate complex | product

Simplifying assumptions:
1) The reaction happens quickly (compared to binding/unbinding events)
   - Rapid equilibrium approximation
2) Product never binds free enzyme (eg. P is rapidly taken up by downstream processes)

Scheme:

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

$$\frac{d}{dt}s(t) = -K_1 s(t)e(t) + K_{-1}c(t)$$

$$\frac{d}{dt}e(t) = K_{-1}c(t) - K_1 s(t)e(t) + K_2 c(t)$$

$$\frac{d}{dt}c(t) = K_1 s(t)e(t) - K_{-1}c(t) - K_2 c(t)$$

$$\frac{d}{dt}p(t) = K_2 c(t).$$

Conservation: let e_T be the total enzyme concentration then e.g. $e(t) = e_T - c(t)$. Reduced model:

$$\frac{d}{dt}s(t) = -K_1 s(t)(e_T - c(t)) + K_{-1}c(t)$$

$$\frac{d}{dt}c(t) = K_1 s(t)(e_T - c(t)) - K_{-1}c(t) - K_2 c(t)$$

$$\frac{d}{dt}p(t) = K_2 c(t)$$

Now, presume that the enzyme-substrate kinetics are fast compared with the system $S \to P$ kinetics. (Valid if $[S] \gg e_T$; exercise 2.11.) Apply a quasi-steady state approximation:

$$0 = K_1 s(t)(e_T - c^{qss}) - K_{-1}c^{qss} - K_2 c^{qss}$$

$$\Longrightarrow c^{qss} = \frac{K_1 e_T s(t)}{K_{-1} + K_2 + K_1 s(t)}$$

Then, we have

$$\frac{d}{dt}p(t) = \frac{K_2 K_1 e_T s(t)}{K_{-1} + K_2 + K_1 s(t)}$$

$$\frac{d}{dt}s(t) = -\frac{d}{dt}p(t).$$

This is the Michaelis-Menten rate law

$$v = \text{rate of } S \to P = \frac{K_2 K_1 e_T [S]}{K_{-1} + K_2 + K_1 [S]}$$

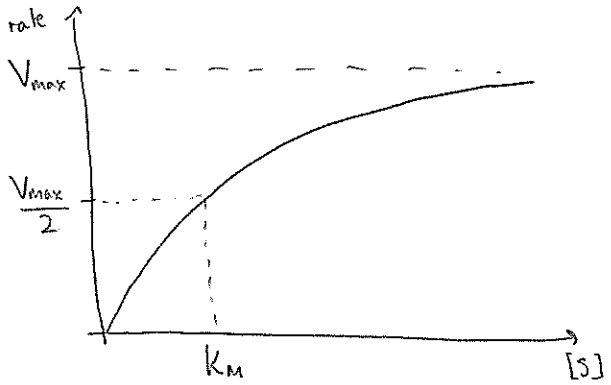
Standard notation:

$$V_{max} = K_2 e_T \text{ (the } \underline{\text{limiting}} \text{ or } \underline{\text{maximal}} \text{ rate)}$$

$$K_M = \frac{K_{-1} + K_2}{K_1} \text{ (the } \underline{\text{half-saturating}} \text{ or } \underline{\text{Michaelis}} \text{ constant)}$$

rate of $S \rightarrow P$

$$v = \frac{V_{max}[S]}{K_M + [S]}$$



Exercise: reversible Michaelis-Menten kinetics

$$\text{rate } v = \frac{V_f[S] + V_r[P]}{1 + K_1[S] + K_2[P]}$$

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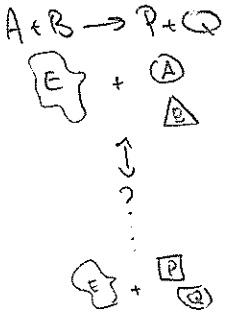
Two-substrate reactions

Most enzyme-catalysed reactions involve more than one substrate. The two-substrate reaction $A + B \rightarrow P + Q$ can be catalysed through a number of possible mechanisms.



1) Compulsory order mechanism
   eg. A binds first

$$E + A \rightleftharpoons EA$$
$$EA + B \rightleftharpoons EAB \rightarrow E + P + Q$$

2) Random order mechanism
   either can bind first

3) Double-displacement (or ping-pong) mechanism

$$A + E \rightleftharpoons AE \rightarrow P + E^*$$
$$E^* + B \rightleftharpoons BE^* \rightarrow E + Q$$

A "deposit" a group on E, which is then transferred to B. In each case, a quasi-steady state assumption applied to the enzyme complex gives rate

$$v = \frac{V_{max}[A][B]}{K_A K_B + K_B[A] + K_A[B] + [A][B]}.$$

Regulation of Enzyme Activity

Competitive inhibition

A competitive inhibitor mimics the enzyme substrate but undergoes no reaction. The scheme is

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P$$
$$I + E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_I$$

Model: the dynamics of the complexes are

$$\frac{d}{dt}c(t) = k_1 s(t) e(t) - (k_{-1} + k_2) c(t)$$
$$\frac{d}{dt}c_I(t) = k_3 i(t) e(t) - k_{-3} c_I(t)$$

Substituting

$$e(t) = \underset{\text{total}}{e_T} - c(t) - c_I(t)$$

and then applying quasi-steady state assumption to both complexes.

$$0 = k_1 s(e_T - c - c_I) - (k_{-1} + k_2) c$$
$$0 = k_3 i(e_T - c - c_I) - k_{-3} c_I$$

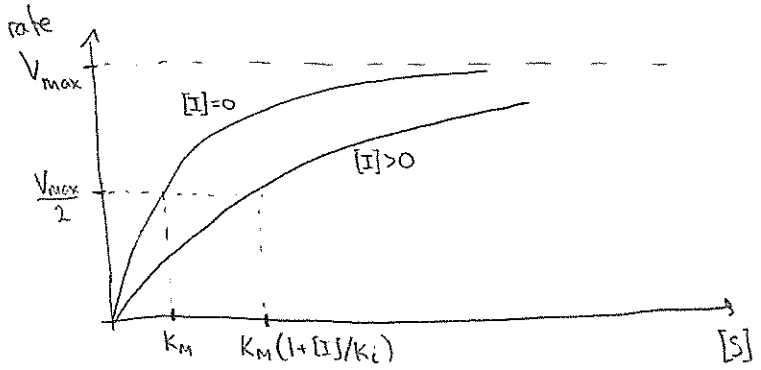
Result (exercise):

$$c^{qss} = \frac{k_i e_T s}{K_M k_i + K_M i + k_i s}, \quad K_M = \frac{k_{-1} + k_2}{k_1}, \quad k_i = \frac{k_{-3}}{k_3}.$$

Then the rate of formation of product is $k_2 c^{qss}$;

$$v = k_2 c = \frac{V_{max} s}{K_M\left(1 + \frac{i}{k_i}\right) + s}, \quad V_{max} = k_2 e_T, \quad s = [S], \quad i = [I].$$

Conclusion: maximal rate is unchanged, but half-maximal concentration is increased.



Allosteric Regulation

An allosteric regulator binds at a site distinct from the active site and alters the enzyme's character.

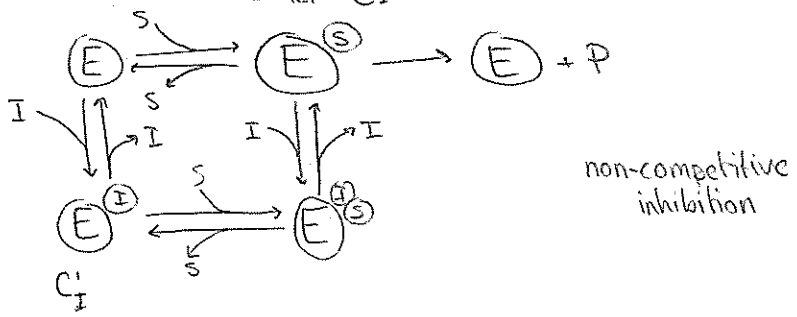
One possible mechanism:

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

$$I + E \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_I^1$$

$$I + C \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_I^2$$

$$S + C_I^1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_I^2$$



non-competitive inhibition

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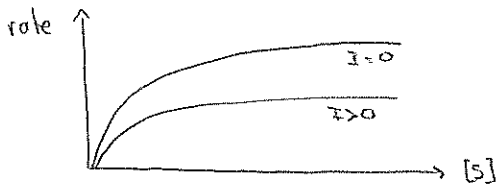
GSSA applied to complexes:

$$\text{rate} = \frac{V_{max}}{1 + 1/K_i} \frac{S}{K_M + S}$$



Cooperativity: mechanism by which the binding of one molecule to a protein affects the binding affinity of others (eg. allostery)

Canonical example: hemoglobin - a tetramer, 4 oxygen binding sites

Framework:

a ligand-protein binding event

protein E
ligand X

$$E + X \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} EX$$

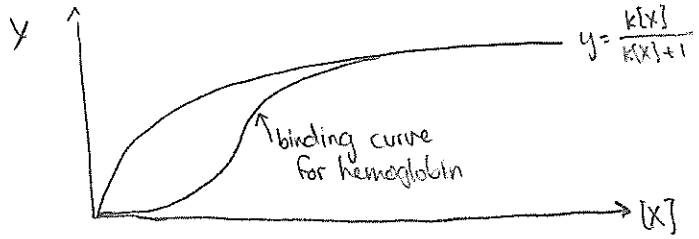
The fractional occupancy of E is

$$y = \frac{\text{number of occupied binding sites}}{\text{total number of binding sites}} = \frac{[EX]}{[EX]+[E]}$$

In steady-state $[EX] = k[E][X]$ where $K = K_1/K_{-1}$, the <u>association constant</u>

Then

$$y = \frac{k[E][X]}{k[E][X]+[E]} = \frac{k[X]}{k[X]+1}$$



E hemoglobin
X O_2

$$E + X \underset{K_{-1}}{\overset{4K_1}{\rightleftharpoons}} EX$$
$$EX + X \underset{2K_{-2}}{\overset{3K_2}{\rightleftharpoons}} EX_2$$
$$EX_2 + X \underset{3K_{-3}}{\overset{2K_3}{\rightleftharpoons}} EX_3$$
$$EX_3 + X \underset{4K_{-4}}{\overset{K_4}{\rightleftharpoons}} EX_4$$

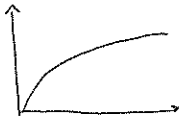
Fractional saturation

$$y = \frac{[EX] + 2[EX_2] + 3[EX_3] + 4[EX_4]}{4([E] + [EX] + [EX_2] + [EX_3] + [EX_4])}$$

If the binding events are identical and independent,

$$K_1 = K_2 = K_3 = K_4, \quad K_{-1} = K_{-2} = K_{-3} = K_{-4}$$

→ standard hyperbolic binding curve



$$y = \frac{4K[X]}{K[X]-1}$$

More generally,

$$y = \frac{B_1[X] + 3B_1B_2[X]^2 + 3B_1B_2B_3[X]^3 + B_1B_2B_3B_4[X]^4}{1 + 4B_1[X] + 6B_1B_2[X]^2 + 4B_1B_2B_3[X]^3 + B_1B_2B_3B_4[X]^4}$$

where

$$B_i = \frac{K_i}{K_{-i}}.$$

If the final binding event has high affinity compared to the three (strong separativity) then $B_4 \gg B_1, B_2, B_3$. Then

$$y = \frac{B_1 B_2 B_3 B_4 [X]^4}{1 + B_1 B_2 B_3 B_4 [X]^4}.$$

Generalized by a Hill function

$$y = \frac{K[X]^n}{1 + K[X]^n}. \quad \left(\text{or } \frac{[S]^n}{K^n + [S]^n}\right)$$

Used to fit sigmoidal behaviours. Here n is the Hill coefficient, which characterizes steepness.

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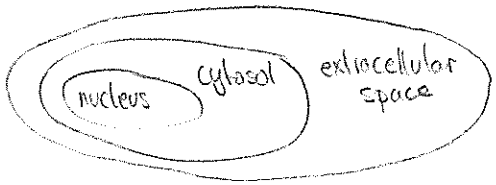
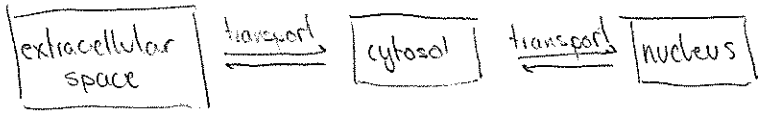
Compartmental Modelling

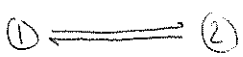
Well-mixed compartments, eg





species S. $[S]_e$ $[S]_c$ $[S]_n$

Simplest model of transport: Diffusion



rate of transport of of material is $D([S_1] - [S_2])$

$$\frac{d}{dt}[S_2]V_2 = D([S_1] - [S_2]) \Rightarrow \frac{d}{dt}[S]_2 = \frac{D([S_1] - [S_2])}{V_2}$$