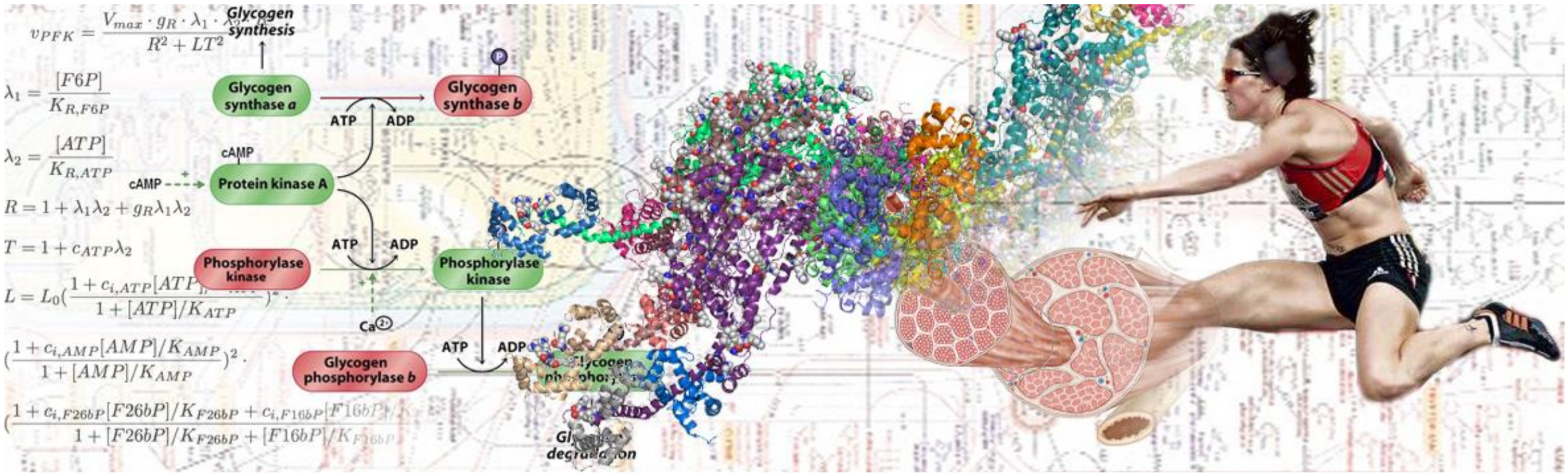
Mini-course: Molecular Systems Biology



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First Lecture: Chemical Kinetics

- order of reaction vs molecularity
- direction of reaction, ΔG
- mass action kinetics
- mass action ratio
- disequilibrium ratio, Γ/K_{eq}
- ΔG^0, relation to K_{eq}

Second Lecture: Enzyme Kinetics

- derivations of reversible Michaelis-Menten kinetic equation using equilibrium binding model
- Haldane relation
- characteristics of enzyme kinetics
- types of inhibition

Michaelis-Menten kinetics

Michaelis Menten equation describes enzyme activity in absence of product:

$$v = \frac{V_m \cdot s}{s + K_m} = \frac{V_m \cdot \frac{s}{K_m}}{1 + \frac{s}{K_m}}$$

This equation is often used for the description of initial rates of in vitro enzyme activities, but generally does not work for coupled reactions due to the presence of product in such systems.

Deriving a kinetic rate equation: equilibrium binding approach

1. Write down mechanism
2. Write down conservation equation for enzyme species
3. Assume equilibrium binding of substrates and products
4. Express bound enzyme species in terms of free enzyme, [S] and [P], and their dissociation constants
5. Work out fractional saturation with S and P
6. Solve $v = k_f[ES] - k_r[EP]$

Derivation of irreversible Michaelis-Menten equation: equilibrium binding

$$E + S \rightleftharpoons ES \rightarrow E + P$$

$$K_S = \frac{e \cdot s}{es}; es = \frac{e \cdot s}{K_S} \qquad Y_{ES} = es/e_T; \frac{e \cdot s/K_S}{e + e \cdot s/K_S}$$

$$v = k_2 \cdot es = k_2 \cdot e_T \frac{s/K_S}{1 + s/K_S} = V_{MAX} \cdot \frac{s/K_S}{1 + s/K_S}$$

Deriving a kinetic rate equation: steady-state approximation

1. Write down mechanism
2. Write down conservation equation for enzyme species
3. Assume steady state for bound enzyme species
4. Express bound enzyme species in terms of free enzyme, [S] and [P], and the rate constants of the mechanism
5. Work out fractional saturation with S and P
6. Solve $v = k_f[ES] - k_r[EP]$

Derivation of irreversible Michaelis-Menten equation: steady-state approximation

$$E + S \rightleftharpoons ES \rightarrow E + P$$

$$d(e)/dt = -k_1 \cdot e \cdot s + k_{-1} \cdot es + k_2 \cdot es$$
$$d(es)/dt = k_1 \cdot e \cdot s - k_{-1} \cdot es - k_2 \cdot es$$

steady state approximation

$$0 = k_1 \cdot (e_T - es) \cdot s - k_{-1} \cdot es - k_2 \cdot es$$

$$es = \frac{k_1 \cdot e_T \cdot s}{k_1 \cdot s + k_{-1} + k_2} = \frac{e_T \cdot s}{s + \frac{k_{-1}+k_2}{k_1}}$$

$$v = k_2 \cdot es = \frac{k_2 \cdot e_T \cdot s}{s + \frac{k_{-1}+k_2}{k_1}} = \frac{V_{MAX} \cdot s}{s + K_M}$$

Reversible form of the Michaelis-Menten equation

Equilibrium binding assumption

$$E + S \underset{K_S}{\rightleftharpoons} ES \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} EP \underset{K_P}{\rightleftharpoons} E + P$$

Dissociation constants K_S and K_P

$$K_S = \frac{e \cdot s}{es} \qquad K_P = \frac{e \cdot p}{ep}$$

$$es = \frac{e \cdot s}{K_S} \qquad ep = \frac{e \cdot p}{K_P}$$

Conservation relation

$$e_T = e + es + ep$$

Reversible form of the Michaelis-Menten equation (2)

$$E + S \underset{K_S}{\rightleftharpoons} ES \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} EP \underset{K_P}{\rightleftharpoons} E + P$$

Fraction of enzyme in ES form, Y_{ES}:

$$Y_{ES} = \frac{es}{e_T} = \frac{e \cdot s}{K_S} / (e + \frac{e \cdot s}{K_S} + \frac{e \cdot p}{K_P}) = \frac{s}{K_S} / (1 + \frac{s}{K_S} + \frac{p}{K_P})$$

Fraction of enzyme in EP form, Y_{EP}:

$$Y_{EP} = \frac{ep}{e_T} = \frac{e \cdot p}{K_P} / (e + \frac{e \cdot s}{K_S} + \frac{e \cdot p}{K_P}) = \frac{p}{K_P} / (1 + \frac{s}{K_S} + \frac{p}{K_P})$$

Reversible form of the Michaelis-Menten equation (3)

$$E + S \underset{K_S}{\rightleftharpoons} ES \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} EP \underset{K_P}{\rightleftharpoons} E + P$$

$$v = k_2 \cdot es - k_{-2} \cdot ep$$

$$v = \frac{k_2 \cdot e_T \cdot \frac{s}{K_S}}{1 + \frac{s}{K_S} + \frac{p}{K_P}} - \frac{k_{-2} \cdot e_T \cdot \frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

$$v = \frac{V_{mf} \cdot \frac{s}{K_S} - V_{mr} \cdot \frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

Reversible Michaelis-Menten: Steady-state approximation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

$$\begin{aligned}
\frac{de}{dt} &= -v_1 + v_3 = 0 \\
\frac{des}{dt} &= v_1 - v_2 = 0 \qquad\qquad e_{tot} = e + es + ep \\
\frac{dep}{dt} &= v_2 - v_3 = 0
\end{aligned}$$

$$\begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} -k_1^+ s - k_3^- p & k_1^- & k_3^+ \\ k_1^+ s & -k_1 - k_2^+ & k_2^- \\ 1 & 1 & 1 \end{pmatrix} \begin{pmatrix} e \\ es \\ ep \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ -e_{tot} \end{pmatrix} \quad (4.25)$$

$$\begin{pmatrix} e \\ es \\ ep \end{pmatrix} = \begin{pmatrix} -k_1^+ s - k_3^- p & k_1^- & k_3^+ \\ k_1^+ s & -k_1 - k_2^+ & k_2^- \\ 1 & 1 & 1 \end{pmatrix}^{-1} \begin{pmatrix} 0 \\ 0 \\ -e_{tot} \end{pmatrix} \quad (4.26)$$

Reversible Michaelis-Menten: Steady-state approximation (2)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

$$\begin{pmatrix} e \\ es \\ ep \end{pmatrix} = \begin{pmatrix} -k_1^+ s - k_3^- p & k_1^- & k_3^+ \\ k_1^+ s & -k_1 - k_2^+ & k_2^- \\ 1 & 1 & 1 \end{pmatrix}^{-1} \begin{pmatrix} 0 \\ 0 \\ -e_{tot} \end{pmatrix} \tag{4.26}$$

$$\frac{v}{e_{tot}} = \frac{\overbrace{k_1^+ k_2^+ k_3^+}^{num_1} s - \overbrace{k_1^- k_2^- k_3^-}^{num_2} p}{\underbrace{k_1^- k_2^- + k_1^- k_3^+ + k_2^+ k_3^+}_{const} + \underbrace{k_1^+ (k_2^- + k_2^+ + k_3^+)}_{coef_s} s + \underbrace{k_3^- (k_1^- + k_2^- + k_2^+)}_{coef_p} p}$$

$$V_{MAX}^+ = \frac{num_1}{const} \qquad K_{MS} = \frac{const}{coef_s}$$

$$V_{MAX}^- = \frac{num_2}{const} \qquad K_{MP} = \frac{const}{coef_p}$$

$$v = \frac{V_{MAX}^+ \frac{s}{K_{MS}} - V_{MAX}^- \frac{p}{K_{MP}}}{1 + \frac{s}{K_{MS}} + \frac{p}{K_{MP}}}$$

Haldane relation

$$v = \frac{V_{mf} \cdot \frac{s}{K_S} - V_{mr} \cdot \frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

at equilibrium $v = 0$:

$$V_{mf} \cdot \frac{s_{eq}}{K_S} = V_{mr} \cdot \frac{p_{eq}}{K_P}$$

leading to the Haldane relation (not limited to eq. state)

$$\frac{V_{mf} \cdot K_P}{V_{mr} \cdot K_S} = \frac{p_{eq}}{s_{eq}} = K_{eq}$$

$$\frac{V_{mf} \cdot K_P}{K_{eq} \cdot K_S} = V_{mr}$$

Reversible Michaelis-Menten: second form (in terms of K_{eq})

$$v = \frac{V_{mf} \cdot \frac{s}{K_S} - V_{mr} \cdot \frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

substituting V_{mr} with the Haldane relation:

$$v = \frac{V_{mf} \cdot \frac{s}{K_S}(1 - \frac{p}{s \cdot K_{eq}})}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

$$v = \frac{V_{mf} \cdot \frac{s}{K_S}(1 - \frac{\Gamma}{K_{eq}})}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

Try it yourself!

Derive the rate equation for an enzyme acted on by an inhibitor I, with the following mechanism:

$$E + S \underset{K_S}{\rightleftharpoons} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{K_P}{\rightleftharpoons} E + P$$

$$\begin{array}{c} +I \\ \Updownarrow \; K_I \\ ESI \end{array}$$

Multiple subunits: Cooperativity

In enzymes consisting of more than one subunit, the binding of substrate to a subunit can influence the binding of substrate to the other subunit(s). This effect is called cooperativity and can be positive or negative.

Rate equations for enzymes showing cooperativity, typically have the (s/K_s) term taken to a power h (h>1, positive; h<1, negative cooperativity). The well known sigmoidal saturation curve is typical for positive cooperativity.

Hill-equation:

$$v = \frac{V_m \cdot \left(\frac{s}{K_S}\right)^h}{1 + \left(\frac{s}{K_S}\right)^h}$$

Other binding site: allostery

Some enzymes have other binding sites in addition to the the active site. Binding to such sites can effect the activity of the enzyme. This effect is called allostery, the effect can be positive or negative.

Reversible Hill equation

A relatively simple kinetic equation that can accommodate cooperativity and allosteric effectors is the reversible Hill equation:

$$v = \frac{V_{mf} \cdot \frac{s}{K_S}(1 - \frac{\Gamma}{K_{eq}})(\frac{s}{K_S} + \frac{p}{K_P})^{h-1}}{(\frac{s}{K_S} + \frac{p}{K_P})^h + \frac{1+(x/K_x)^h}{1+\alpha(x/K_x)^h}}$$

with X as allosteric effector; if α<1 then X is an inhibitor, if α>1 an activator.

Reduce the above equation by substituting: α=1, *h*=1, *p*=0.

Analysis of experimental data

For an enzyme catalyzed, chemical reaction, A ⟷ B, the following initial rates were obtained (a and b given at t = 0):

v	a	b
(mM/s)	(mM)	(mM)
9.9	10	0
7.86	10	5
9.8	5	0
6.01	10	10
-30.3	0	10
-32.68	0	50

Assume a random order mechanism and rapid equilibrium binding of substrate and product.

Calculate:

- V_{mf}
- V_{mr}
- K_a
- K_b
- K_{eq}

The calculations shown here are examples; there are several correct ways to come to the answer.

Random order mechanism, rapid equilibrium binding; the reversible MM equation can be used:

$$v = \frac{Vm \cdot \frac{a}{K_a} \cdot (1 - \frac{\Gamma}{K_{eq}})}{1 + \frac{a}{K_a} + \frac{b}{K_b}}$$

$V_{max,f}$ and K_a

Both parameters can be calculated from the 2 incubations without b added at t=0. In absence of b the above equation reduces to the irreversible MM equation. Using the irreversible MM equations the 2 incubations lead to 2 equations with 2 unknowns:

$$v = \frac{Vm \cdot \frac{a}{K_a}}{1 + \frac{a}{K_a}}; 9.9 = \frac{Vm \cdot \frac{10}{K_a}}{1 + \frac{10}{K_a}}; 5.95 = \frac{Vm \cdot \frac{5}{K_a}}{1 + \frac{5}{K_a}}$$

Which can be solved (e.g. express Vm in terms of Ka using the first equation and substitute this expression in the second equation): Vmax,f=10 mM/s, Ka=0.1 mM

$V_{max,r}$ and K_b Same as for Vmaxf and Ka but now using the 2 incubations without a:

$$v = -\frac{Vmr \cdot \frac{b}{K_b}}{1 + \frac{b}{K_b}}; -30.30 = -\frac{Vmr \cdot \frac{10}{K_b}}{1 + \frac{10}{K_b}}; -32.68 = -\frac{Vmr \cdot \frac{50}{K_b}}{1 + \frac{50}{K_b}}$$

Vmax,r=33.33 mM/s, Kb=1 mM

Haldane relation

$$K_{eq} = \frac{V_{mf} \cdot K_b}{V_{mr} \cdot K_a}; \quad 3 = \frac{10 \cdot 1}{33.33 \cdot 0.1}$$