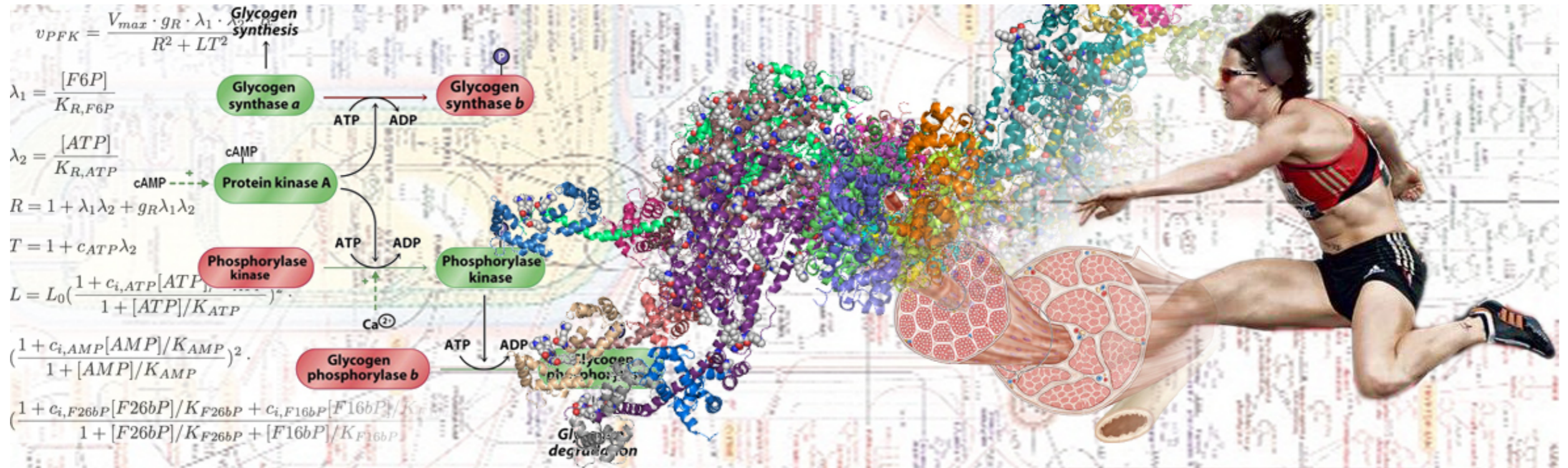


Mini-course: Molecular Systems Biology



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First Lecture: Chemical kinetics

- order of reaction vs molecularity
- direction of reaction, dG
- mass action kinetics
- mass action ratio
- disequilibrium ratio, Γ/Keq
- dG^0 , relation to Keq

Second Lecture: enzyme kinetics

- derivations of rev MM 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.

Derivation irrev MM eq: equilibrium binding



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

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

Derivation irrev MM eq: st st approx



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

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

steady state approximation

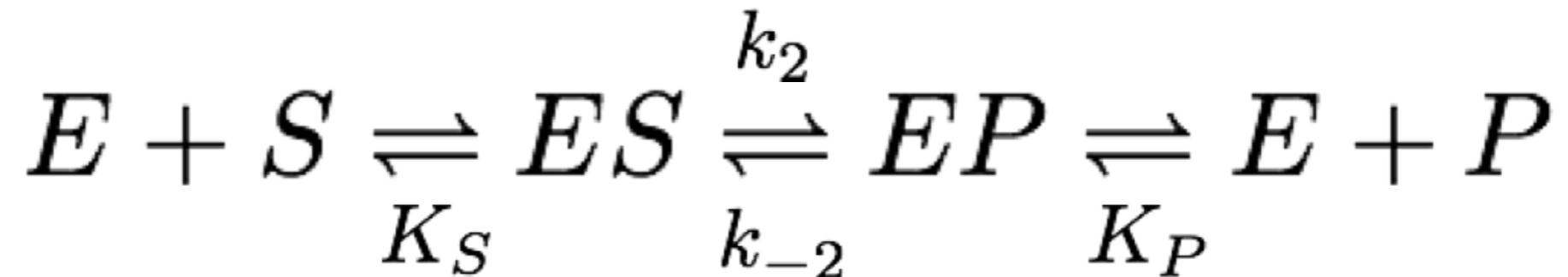
$$0 = k_1 \cdot (e_T - es) \cdot s - k_{-1} \cdot es - k_2 \cdot es$$

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

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

reversible form of MM eq

Equilibrium binding assumption



Dissociation constants K_S and K_P

$$K_S = \frac{e \cdot s}{es}$$

$$K_P = \frac{e \cdot p}{ep}$$

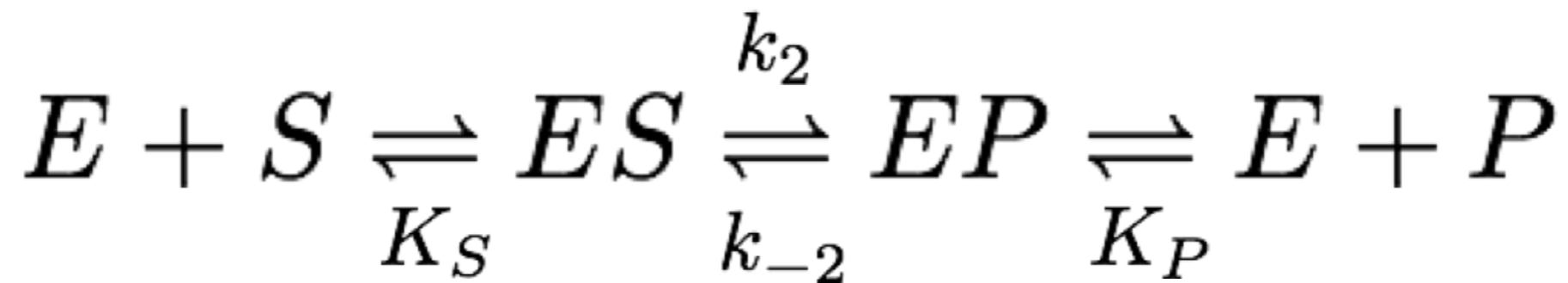
$$es = \frac{e \cdot s}{K_S}$$

$$ep = \frac{e \cdot p}{K_P}$$

Conservation relation

$$e_T = e + es + ep$$

reversible form of MM eq



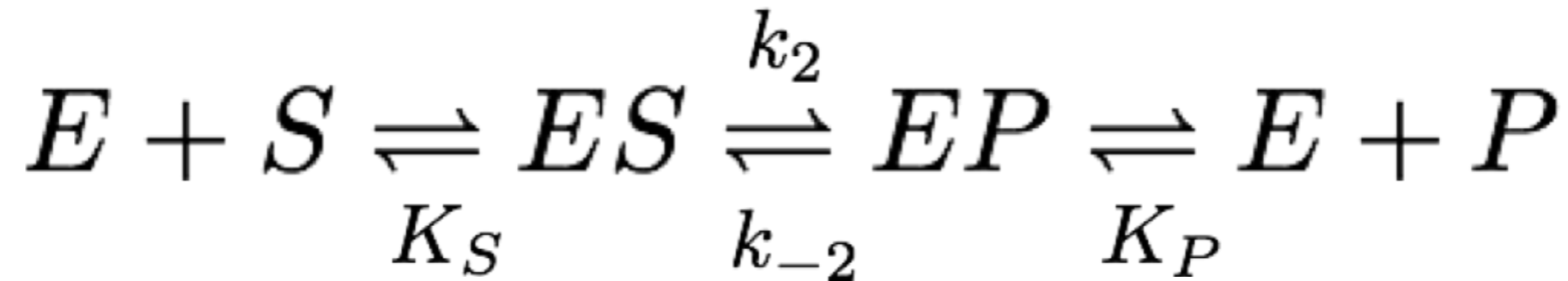
Fraction of enzyme in ES form, Y_{ES} :

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

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

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

reversible form of MM eq

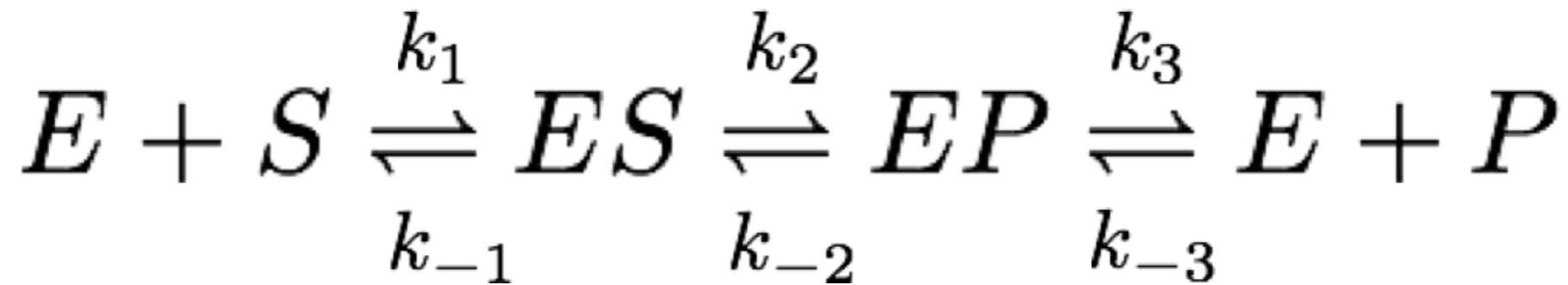


$$v = k_2 \cdot es - k_{-2} \cdot ep$$

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

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

reversible form of MM eq: st st approximation



$$\frac{de}{dt} = -v_1 + v_3 = 0$$

$$\frac{des}{dt} = v_1 - v_2 = 0$$

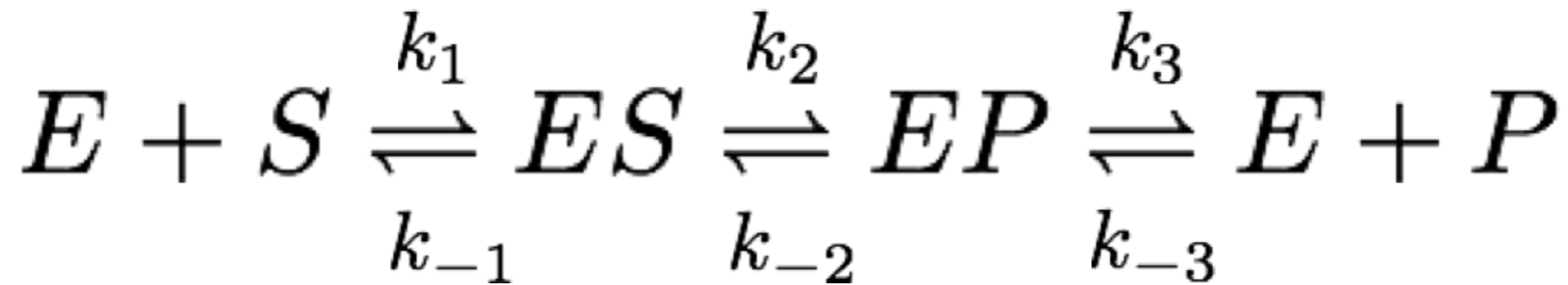
$$\frac{dep}{dt} = v_2 - v_3 = 0$$

$$e_{tot} = e + es + ep$$

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

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

reversible form of MM eq: st st approximation



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

$$\frac{v}{e_{tot}} = \frac{\overbrace{k_1^+ k_2^+ k_3^+}^{num_1} s - \overbrace{k_1^- k_2^- k_3^-}^{num_2} p}{\underbrace{k_1^- k_2^- + k_1^- k_3^+ + k_2^+ k_3^+}_{const} + \underbrace{k_1^+ (k_2^- + k_2^+ + k_3^+)}_{coef_s} s + \underbrace{k_3^- (k_1^- + k_2^- + k_2^+)}_{coef_p} p} \quad (4.27)$$

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

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

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

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

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

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 form of MM eq

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

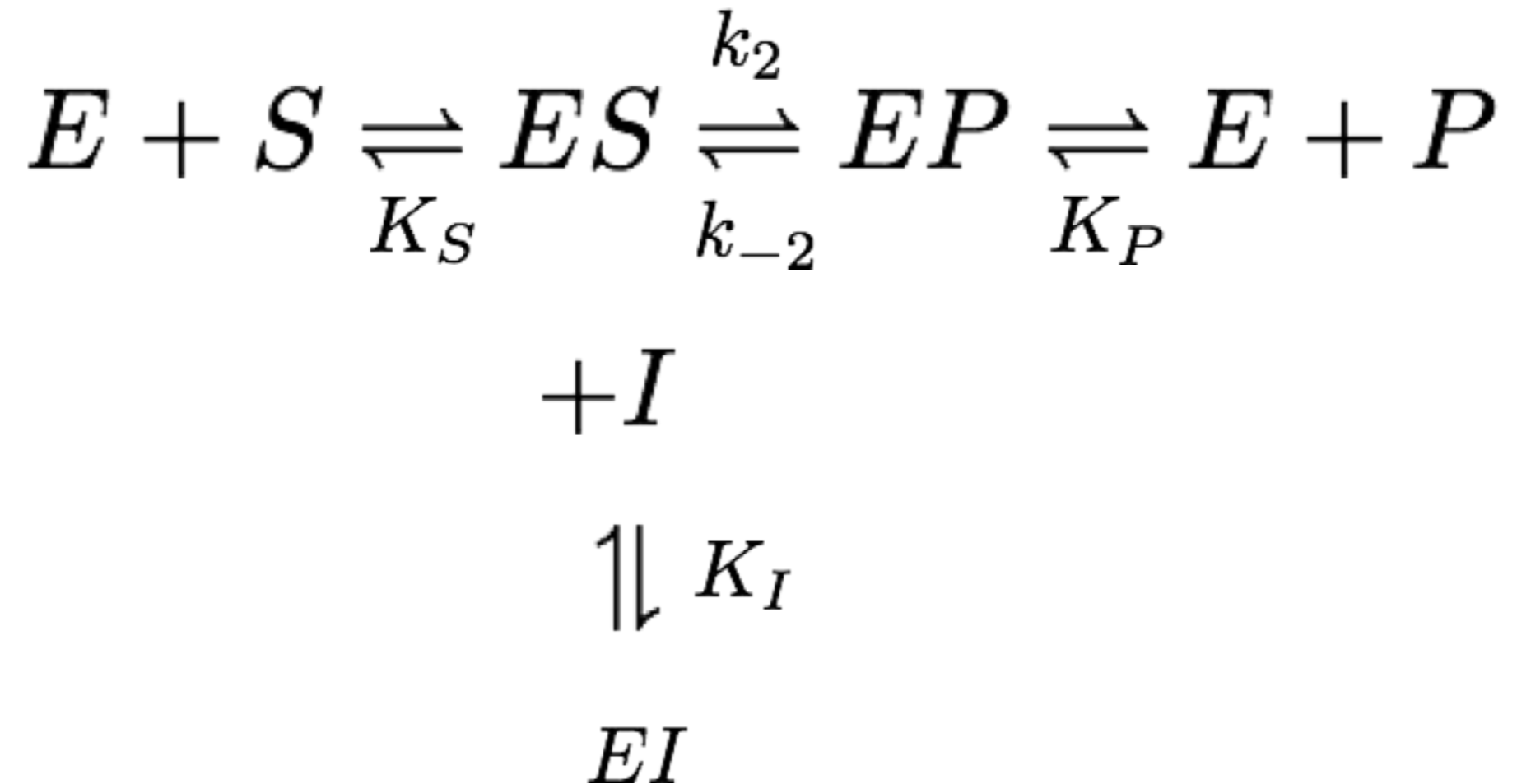
substituting V_{mr} with the Haldane relation:

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

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

Try it yourself

Derive the rate equation for an enzyme inhibitor with the following mechanism



Multiple sub-units: cooperativity

In enzymes consisting of more than one sub-unit, the binding of substrate to a sub-unit 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 eq

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

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

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

reduce the above equation by substituting, alpha = 1, h=1, p=0.

Experimental data II

For an enzyme catalyzed, chemical reaction;
 $A \leftrightarrow B$ 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:

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

$V_{max,f}$ 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:

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

Which can be solved (e.g. express V_m in terms of K_a using the first equation and substitute this expression in the second equation): $V_{max,f}=10$ mM/s, $K_a=0.1$ mM

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

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

$V_{max,r}=33.33$ mM/s, $K_b=1$ mM

Haldane relation

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