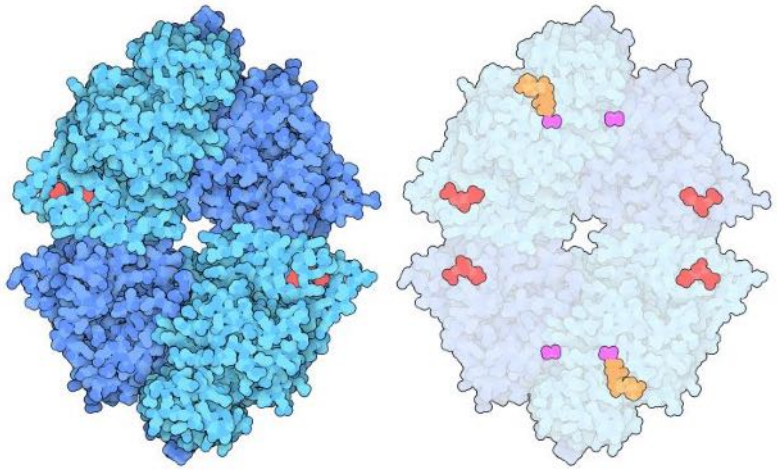
multisubunit enzyme kinetics

basic models of biological networks

regulatory proteins are generally composed of multiple subunits – metabolism, signalling and transcription

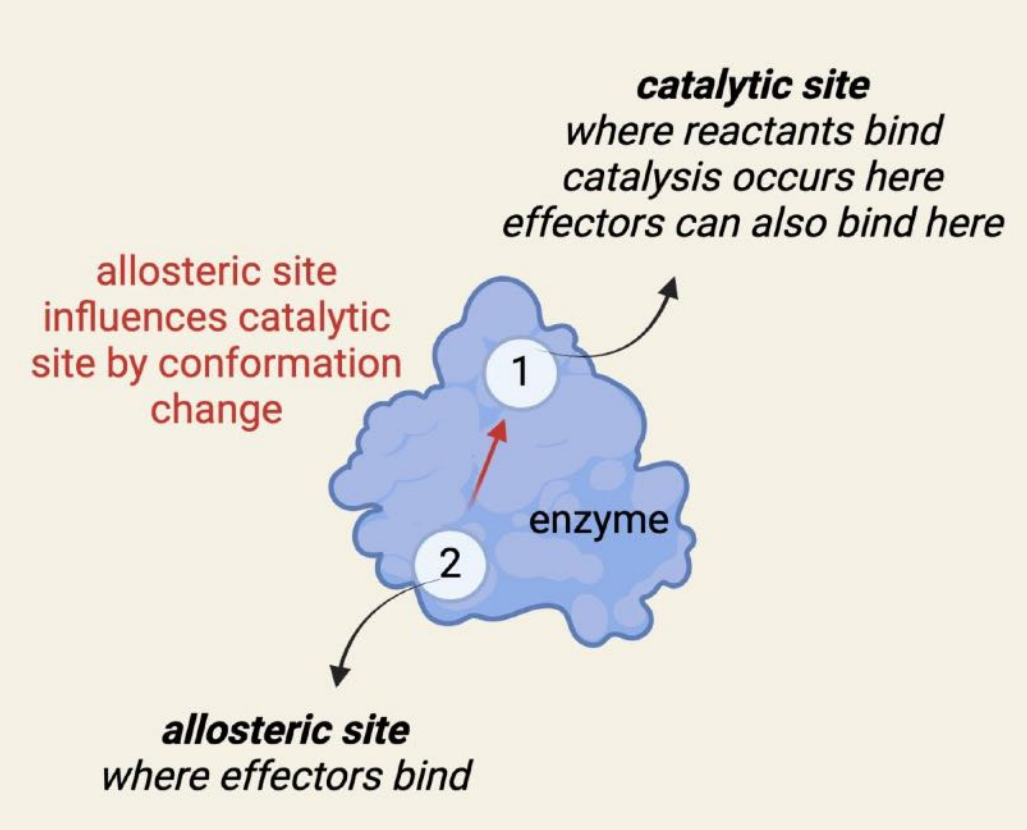
- regulatory proteins in signalling and gene expression
  - transcription factors
  - membrane receptor proteins
- regulatory metabolic enzymes
  - subject to negative and positive feedback regulation



this week: extend previous week's theory to multimeric enzymes

- incorporate into the binding polynomial
  - multiple subunits, each with a catalytic site and one or more allosteric sites
  - conformational changes, between the subunits
  - conformation dependent reactant and effector affinities

recap last week: monomeric enzymes, binding polynomials per enzyme site



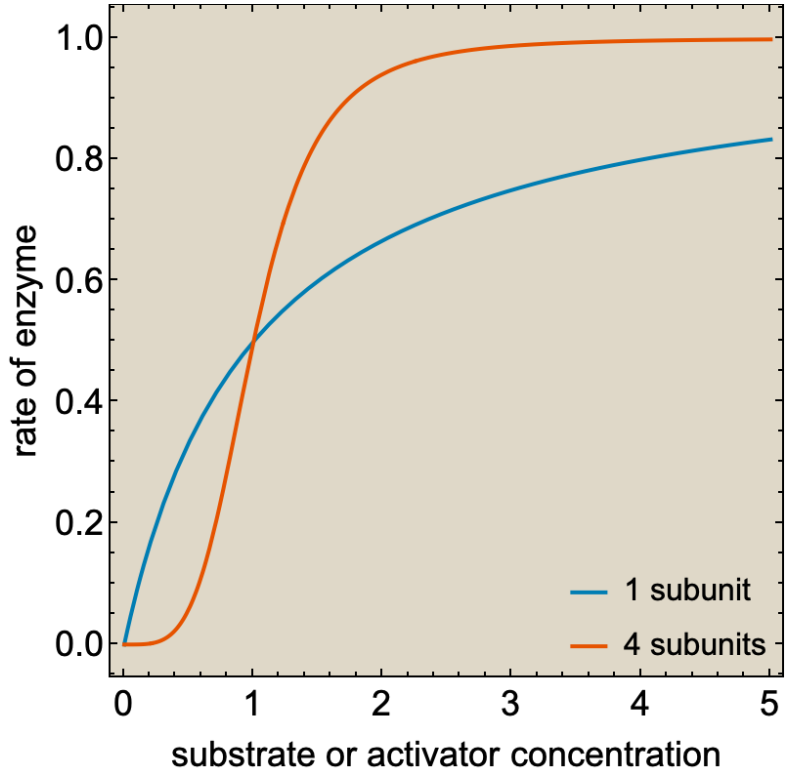
- catalytic site: chemical reaction converting substrates into products or vice versa
- allosteric site: influenced chemical reaction at catalytic site via conformational change
- binding polynomial of enzyme is product of binding polynomials of each site

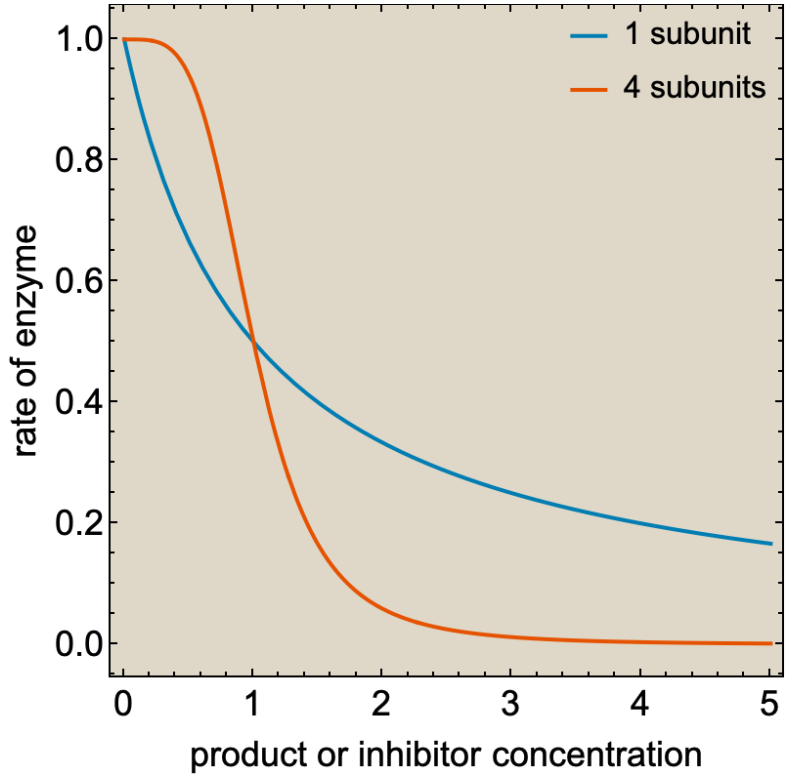
$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)}_{\mathcal{C}} \underbrace{\left(1 + \frac{i}{K_I}\right)}_{\mathcal{A}}$$

- rate of enzyme (e.g. for $A + B \rightleftharpoons P + Q$.)

$$v = k^+ eab - k^- epq = \frac{k^+ e_T \frac{a \cdot b}{K_A K_B} - k^- e_T \frac{p \cdot q}{K_P K_Q}}{\mathcal{B}}$$

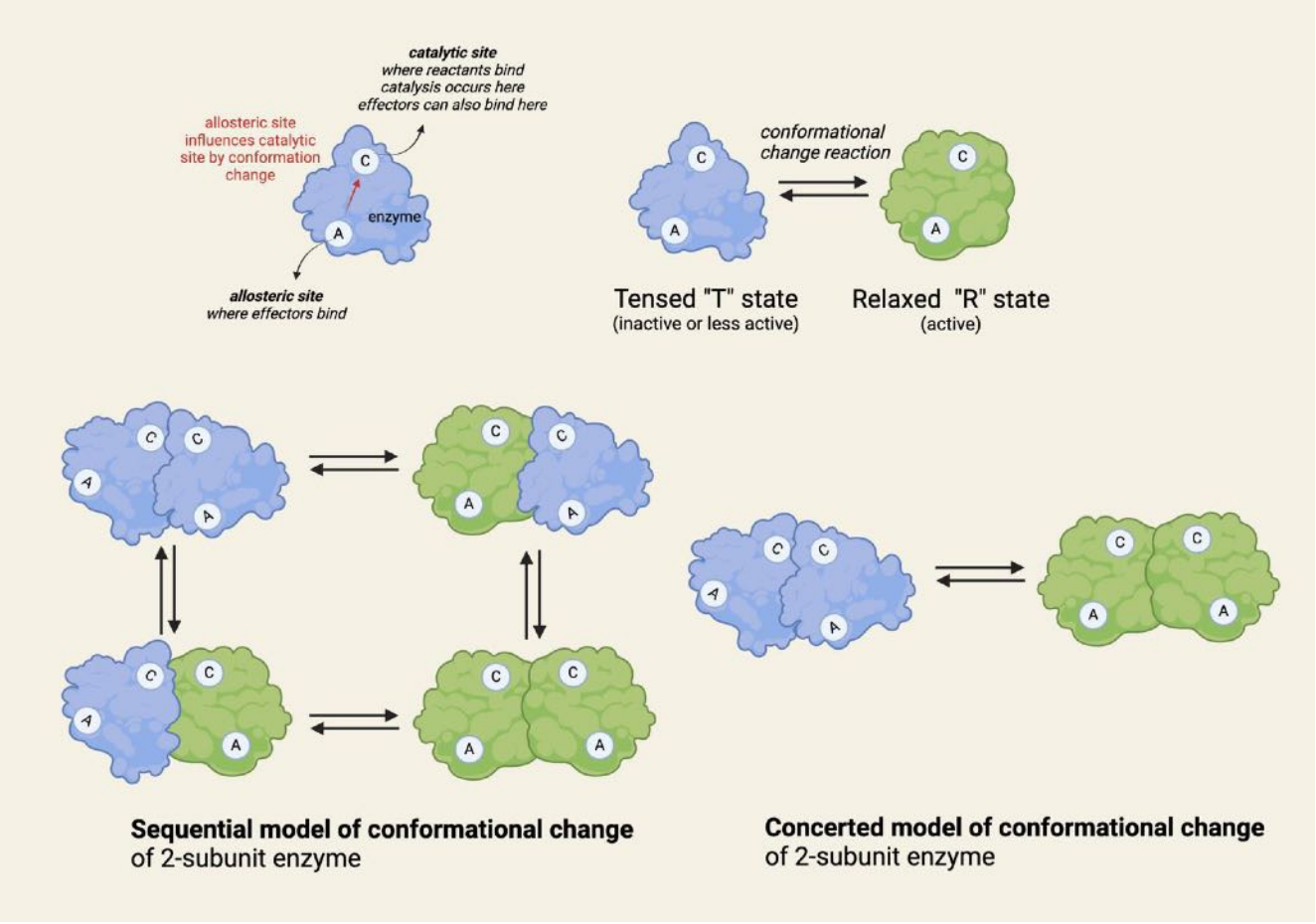
regulatory proteins are generally composed of multiple subunits – sensitising them to reactants or effectors





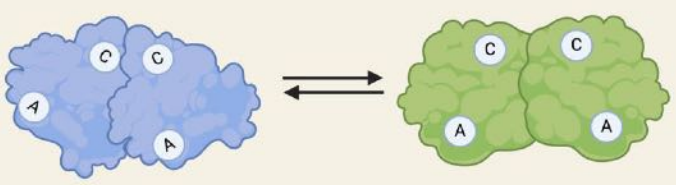
Steeper curves => more sensitive => due to multiple subunits

Cooperative subunits: reactant or effector (de)sensitisation



- relaxed state has either higher affinity for reactants or higher catalytic activity or both.

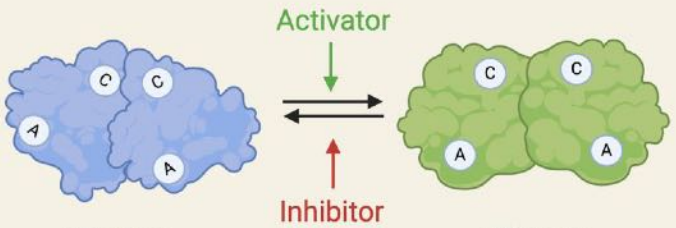
Cooperative subunits: reactant or effector (de)sensitisation



Spontaneous conformational change

Conformation equilibrium constant

$$L = \frac{\text{concentration of tensed subunits}}{\text{concentration of relaxed subunits}}$$



"T" state "R" state

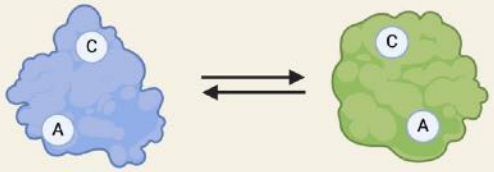
Spontaneous conformational change +
Effector-induced conformation change
(Effector then binds to allosteric site)

Conformation equilibrium constant

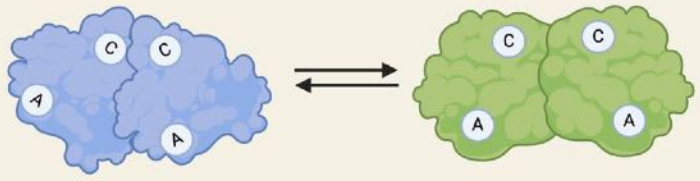
$$L = \frac{\text{concentration of tensed subunits}}{\text{concentration of relaxed subunits}} = L' \frac{1 + \frac{i}{K_I}}{1 + \frac{a}{K_A}}$$

multimeric enzymes: binding polynomials

$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)}_{\mathcal{C}} \underbrace{\left(1 + \frac{i}{K_I}\right)}_{\mathcal{A}}$$

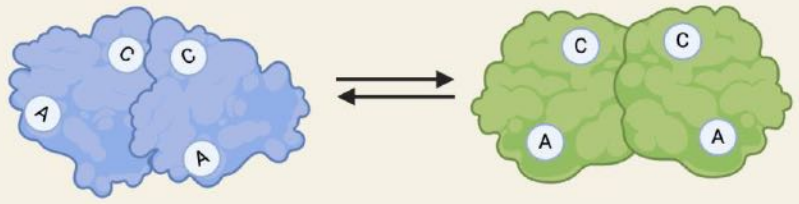


$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)}_{\mathcal{C}_\mathcal{R}} \underbrace{\left(1 + \frac{i}{K_I}\right)}_{\mathcal{A}_\mathcal{R}} + L \underbrace{\left(1 + \frac{a}{K'_A} + \frac{a \cdot b}{K'_A K'_B} + \frac{p}{K'_P} + \frac{p \cdot q}{K'_P K'_Q}\right)}_{\mathcal{C}_\mathcal{T}} \underbrace{\left(1 + \frac{i}{K'_I}\right)}_{\mathcal{A}_\mathcal{T}}$$

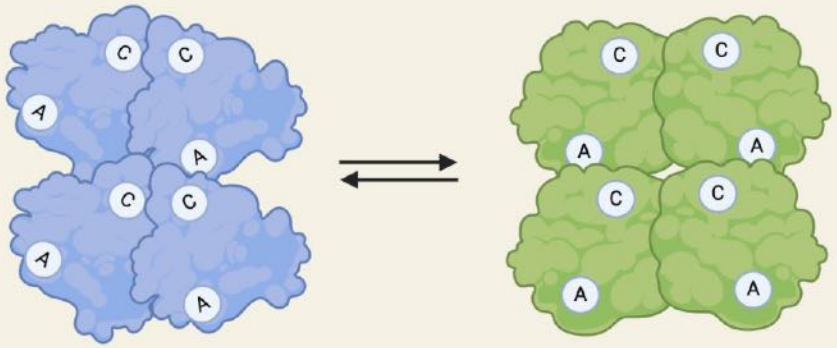


$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)^2}_{(\mathcal{C}_\mathcal{R})^2} \underbrace{\left(1 + \frac{i}{K_I}\right)^2}_{(\mathcal{A}_\mathcal{R})^2} + L \underbrace{\left(1 + \frac{a}{K'_A} + \frac{a \cdot b}{K'_A K'_B} + \frac{p}{K'_P} + \frac{p \cdot q}{K'_P K'_Q}\right)^2}_{(\mathcal{C}_\mathcal{T})^2} \underbrace{\left(1 + \frac{i}{K'_I}\right)^2}_{(\mathcal{A}_\mathcal{T})^2}$$

multimeric enzymes: binding polynomials



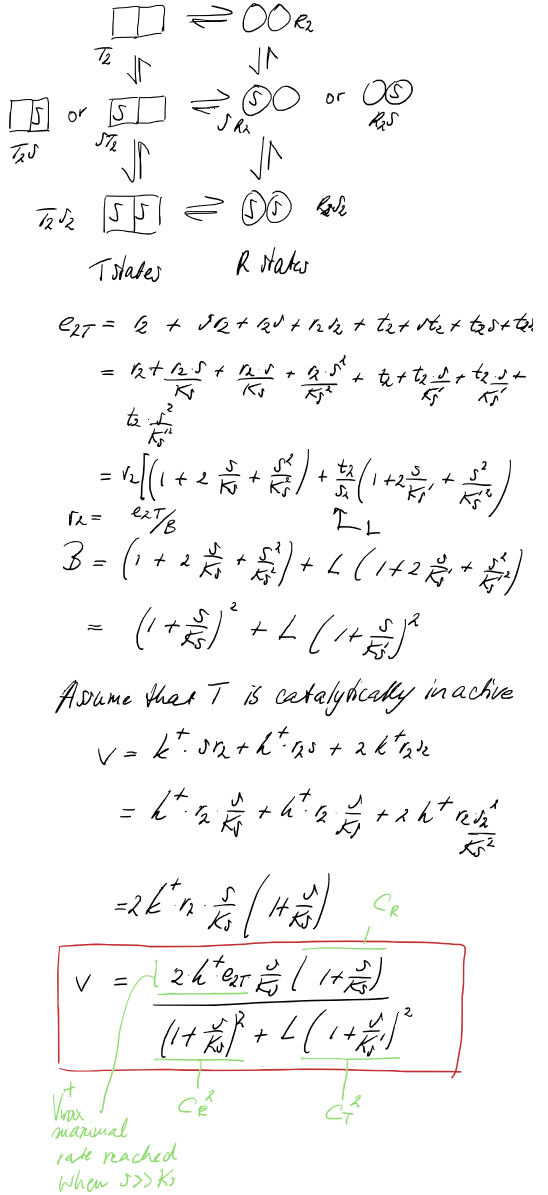
$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)^2}_{(\mathcal{C}_\mathcal{R})^2} \underbrace{\left(1 + \frac{i}{K_I}\right)^2}_{(\mathcal{A}_\mathcal{R})^2} + L \underbrace{\left(1 + \frac{a}{K'_A} + \frac{a \cdot b}{K'_A K'_B} + \frac{p}{K'_P} + \frac{p \cdot q}{K'_P K'_Q}\right)^2}_{(\mathcal{C}_\mathcal{T})^2} \underbrace{\left(1 + \frac{i}{K'_I}\right)^2}_{(\mathcal{A}_\mathcal{T})^2}$$



$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)^4}_{(\mathcal{C}_\mathcal{R})^4} \underbrace{\left(1 + \frac{i}{K_I}\right)^4}_{(\mathcal{A}_\mathcal{R})^4} + L \underbrace{\left(1 + \frac{a}{K'_A} + \frac{a \cdot b}{K'_A K'_B} + \frac{p}{K'_P} + \frac{p \cdot q}{K'_P K'_Q}\right)^4}_{(\mathcal{C}_\mathcal{T})^4} \underbrace{\left(1 + \frac{i}{K'_I}\right)^4}_{(\mathcal{A}_\mathcal{T})^4}$$

Exercise:

- derive the binding polynomial for a
  - two subunit enzyme,
  - with two conformations,
  - catalysing the reaction $S \rightleftharpoons P$
- derive the rate equation



General case: n subunits, two conformations

Rate equation:

$$v = \frac{V_M^+ \prod_i \frac{s_i}{K_i} {\mathcal{C}_\mathcal{R}}^{n-1} \left(1 - \frac{\prod_j p_j}{\prod_i s_i K_{eq}}\right)}{{\mathcal{C}_\mathcal{R}}^n + L \frac{{\mathcal{A}_\mathcal{T}}^n}{{\mathcal{A}_\mathcal{R}}^n} {\mathcal{C}_\mathcal{T}}^n}, \quad \mathcal{B} = {\mathcal{C}_\mathcal{R}}^n + L \frac{{\mathcal{A}_\mathcal{T}}^n}{{\mathcal{A}_\mathcal{R}}^n} {\mathcal{C}_\mathcal{T}}^n$$

$n =$ number of subunits

$\mathcal{C}_\mathcal{R} =$ binding polynomial of catalytic site in the R state

$\mathcal{C}_\mathcal{T} =$ binding polynomial of catalytic site in the T state

$\mathcal{A}_\mathcal{R} =$ binding polynomial of allosteric site in the R state

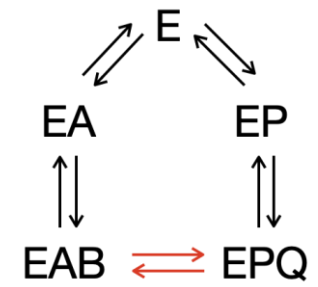
$\mathcal{A}_\mathcal{T} =$ binding polynomial of allosteric site in the T state

$V_M^+ =$ maximal forward rate $= n \cdot k^+ \cdot e_{n,T}$

$K_{eq} =$ equilibrium constant of the reaction

Exercise:

1. Consider an enzyme without allosteric site, set n to 1, choose the affinities of the T and R state the same. What happens?
2. Consider the following mechanism



for an enzyme without any allosteric sites and 3 subunits. Give the rate equation.

3. Add an allosteric site to which an inhibitor binds. Give suitable binding polynomials for the allosteric R and T state.

General case: n subunits, two conformations

Rate equation:

$$v = \frac{V_M^+ \prod_i \frac{s_i}{K_i} {\mathcal{C}_\mathcal{R}}^{n-1} \left(1 - \frac{\prod_j p_j}{\prod_i s_i K_{eq}}\right)}{{\mathcal{C}_\mathcal{R}}^n + L \frac{{\mathcal{A}_\mathcal{T}}^n}{{\mathcal{A}_\mathcal{R}}^n} {\mathcal{C}_\mathcal{T}}^n}, \quad \mathcal{B} = {\mathcal{C}_\mathcal{R}}^n + L \frac{{\mathcal{A}_\mathcal{T}}^n}{{\mathcal{A}_\mathcal{R}}^n} {\mathcal{C}_\mathcal{T}}^n$$

n = number of subunits

$\mathcal{C}_\mathcal{R}$ = binding polynomial of catalytic site in the R state

$\mathcal{C}_\mathcal{T}$ = binding polynomial of catalytic site in the T state

$\mathcal{A}_\mathcal{R}$ = binding polynomial of allosteric site in the R state

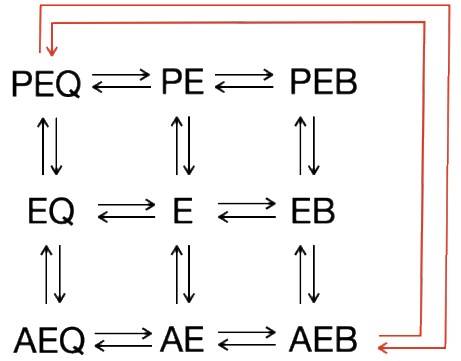
$\mathcal{A}_\mathcal{T}$ = binding polynomial of allosteric site in the T state

V_M^+ = maximal forward rate $= n \cdot k^+ \cdot e_{n,T}$

K_{eq} = equilibrium constant of the reaction

Exercise:

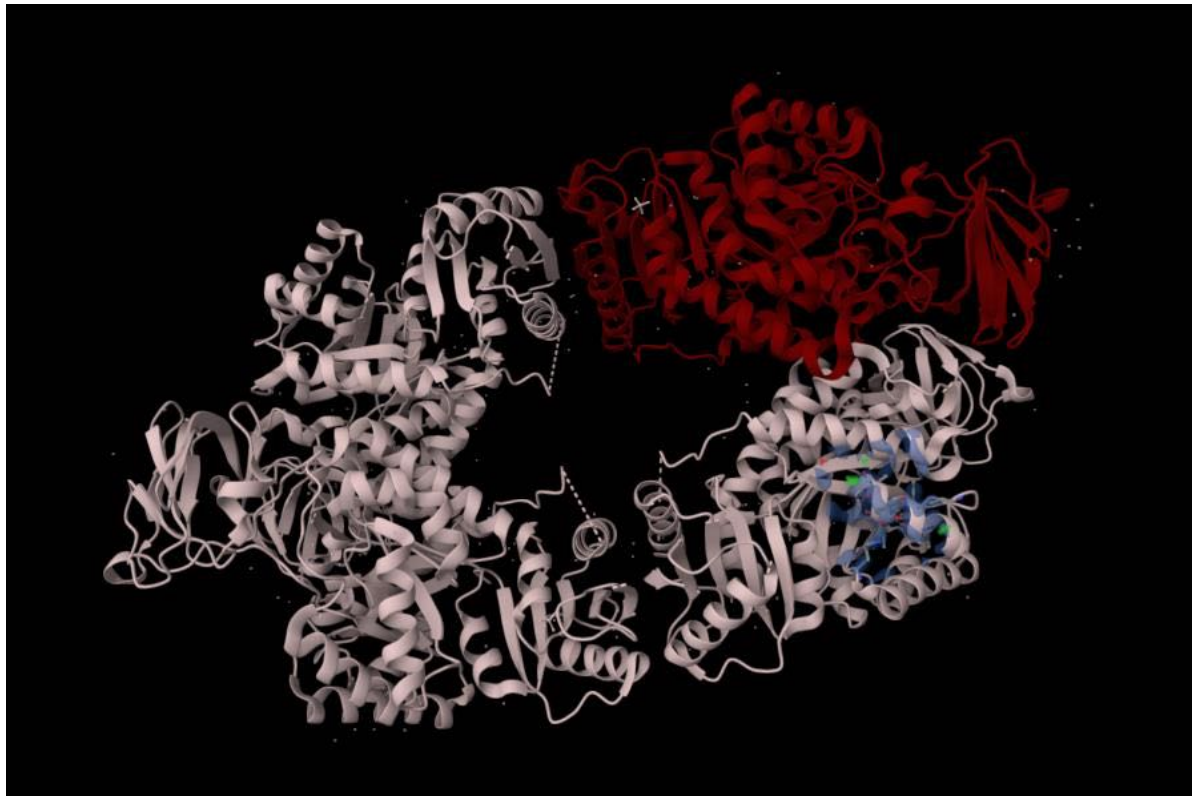
1. For which value of L does the enzyme not have a T state? What happens to the rate equation in this case?
2. Say the equilibrium constant is very high. Is the enzyme now still reversible? Is the enzyme now still inhibited by the concentrations of its products?
3. Consider n = 4 and this mechanism



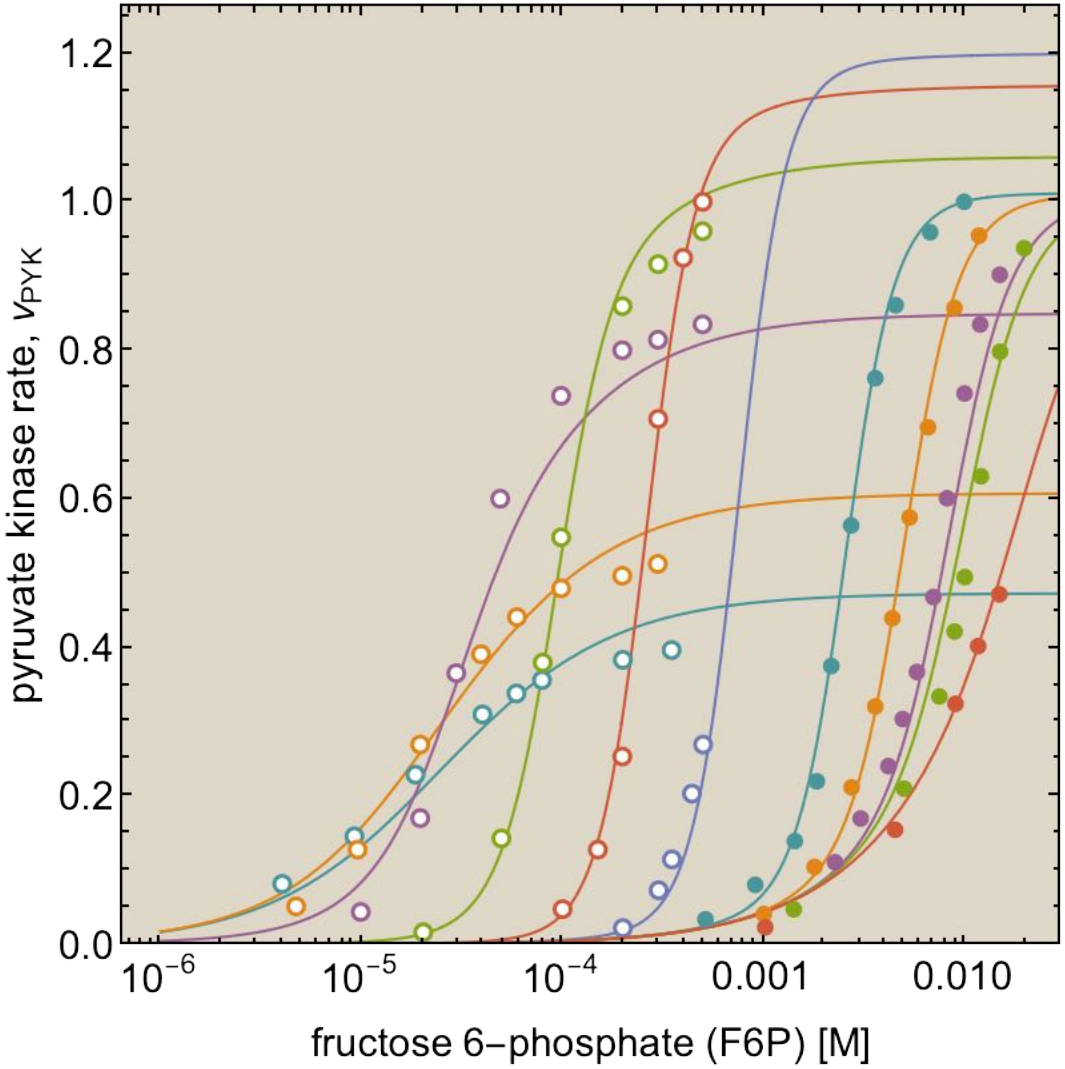
Give the binding polynomial and rate equation for an enzyme without allosteric sites.

Example: pyruvate kinase from *E. coli*

- Enzyme in glycolysis
- Catalyzes a two substrate, two product reaction
  $PEP + ADP \leftrightharpoons PYR + ATP$
  PEP = phospoenolpyruvate, PYR = pyruvate
- activated by F6P (metabolite in glycolysis; https://ecocyc.org/pathway?orgid=ECOLI&id=GLYCOLYSIS&detail-level=2)
- see: https://www.uniprot.org/uniprotkb/P0AD61/entry for structure and 4 subunits (https://ecocyc:org/gene?orgid=ECOLI&id=PKI-MONOMER)



Example: pyruvate kinase from *E. coli*

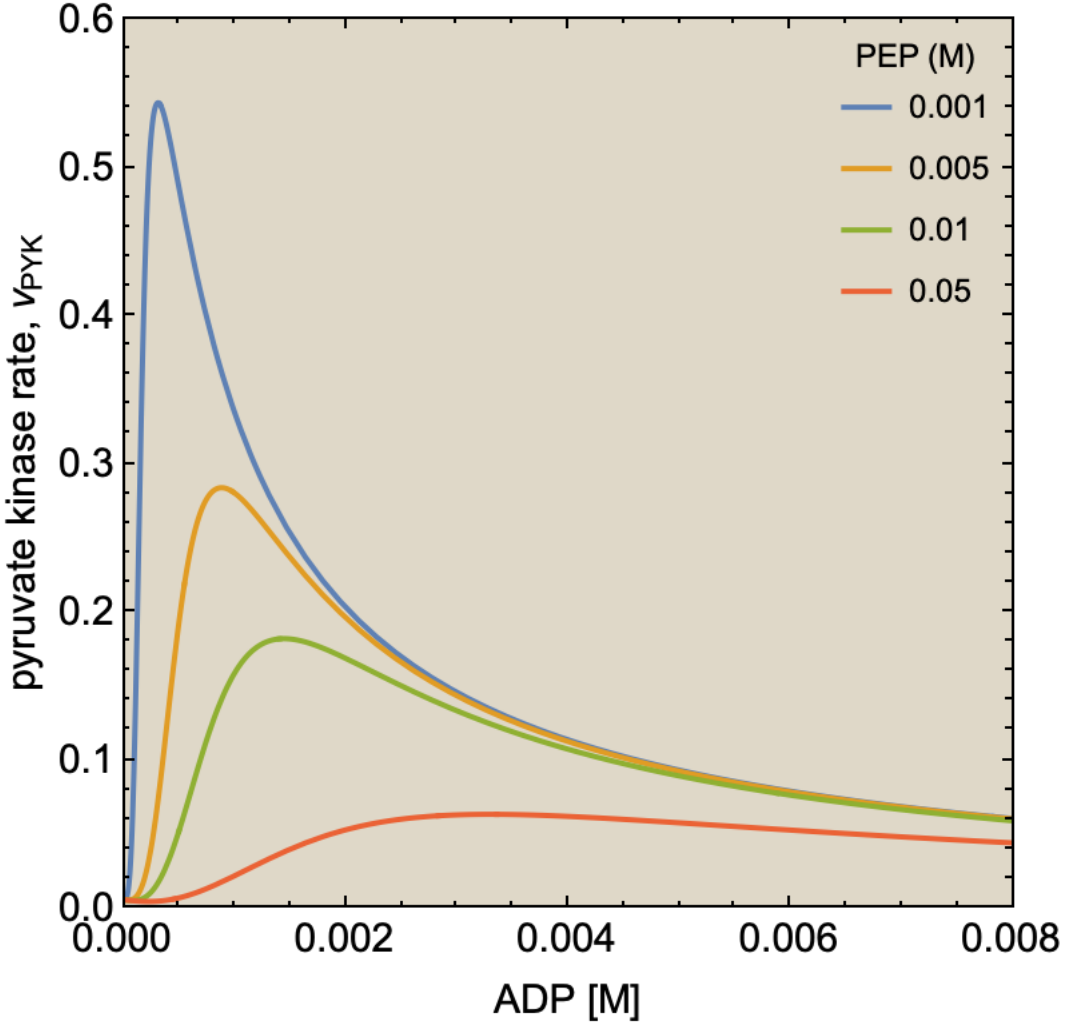


$$PEP + ADP \leftrightharpoons PYR + ATP$$

Note that F6P is an activator of the reaction
Its rate activation depends on the concentration of the substrates.

Substrate variation:
Open symbols variation of ADP (fixed PEP)
Closed symbols variation of PEP (fixed ADP)

Example: pyruvate kinase from *E. coli*



$$PEP + ADP \leftrightharpoons PYR + ATP$$

F6P is both an activator and inhibitor!