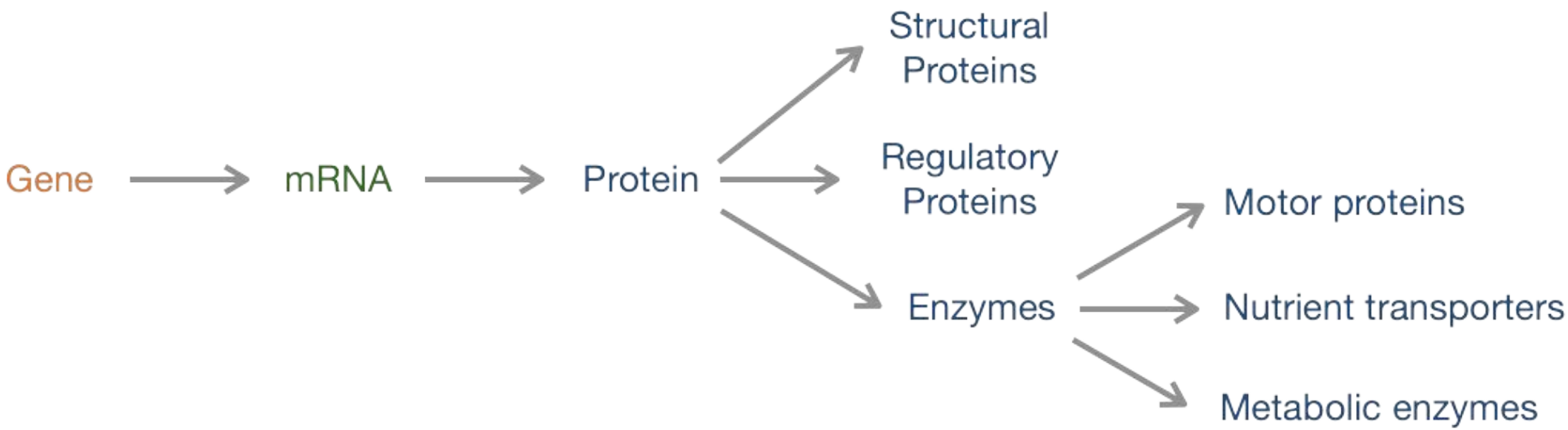
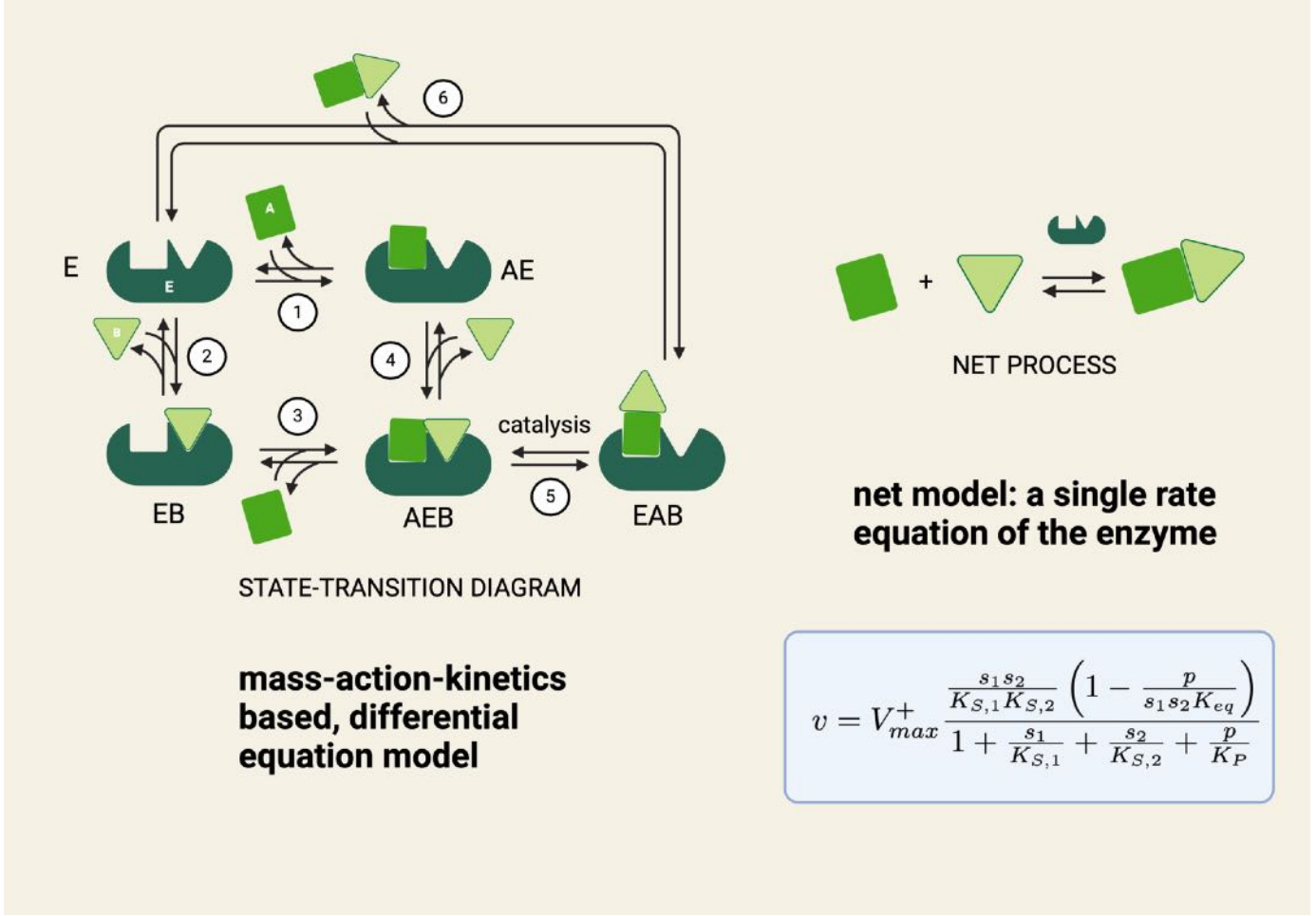
Monomeric/single-subunit Enzyme kinetics

Basic modelling of biological networks

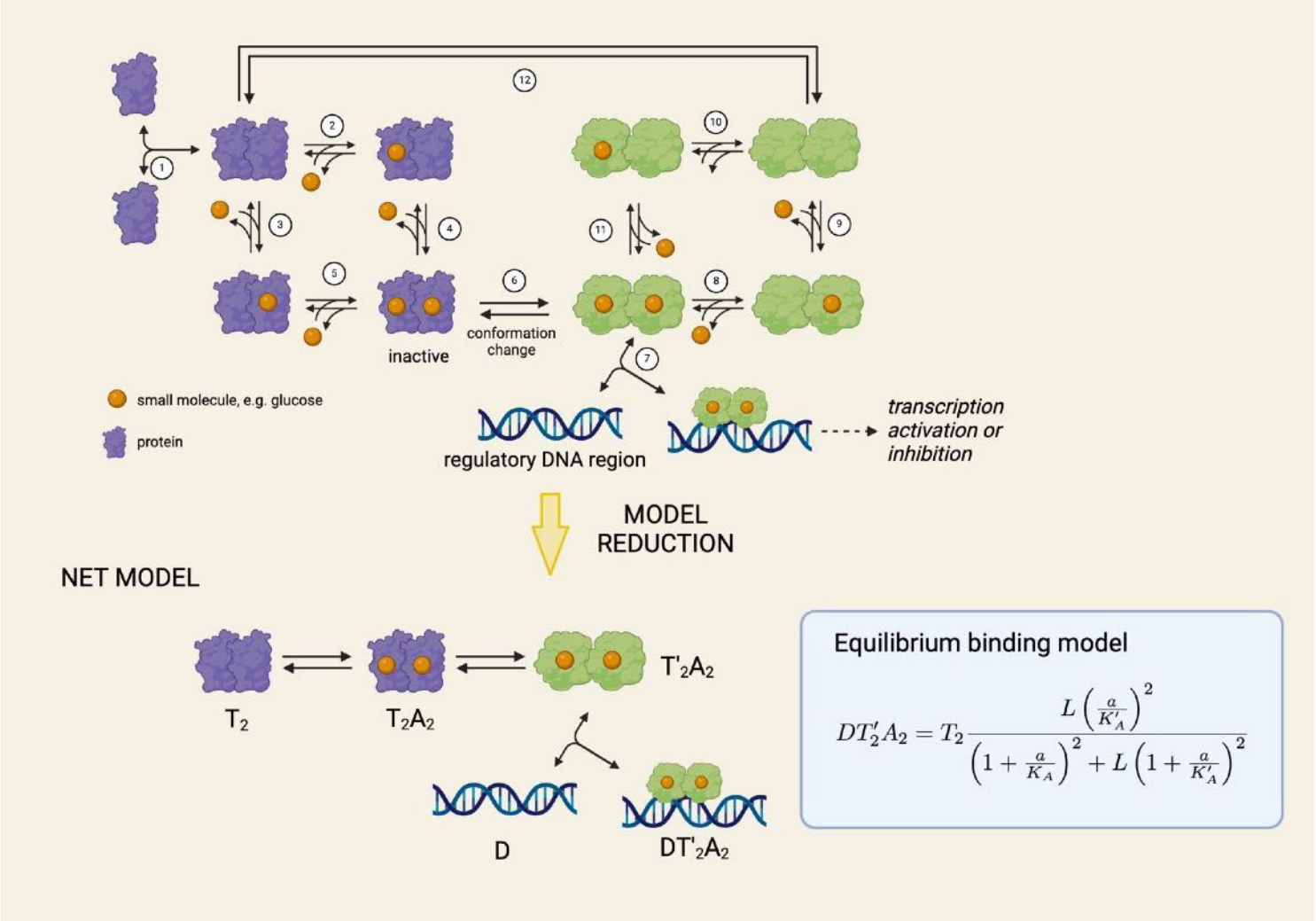
Enzymes


Gene
mRNA
Protein
Structural Proteins
Regulatory Proteins
Enzymes
Motor proteins
Nutrient transporters
Metabolic enzymes

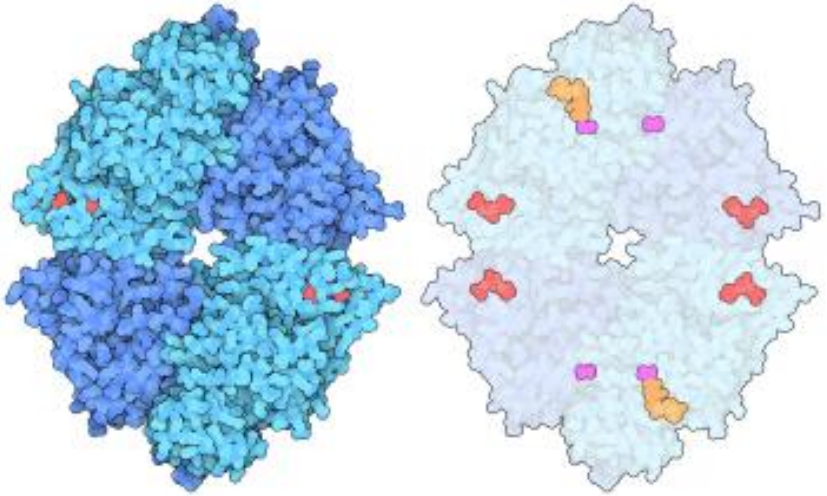
make net models of state-transition diagrams of enzyme catalysis: this week



make net models of state-transition of regulatory proteins: after enzyme kinetics

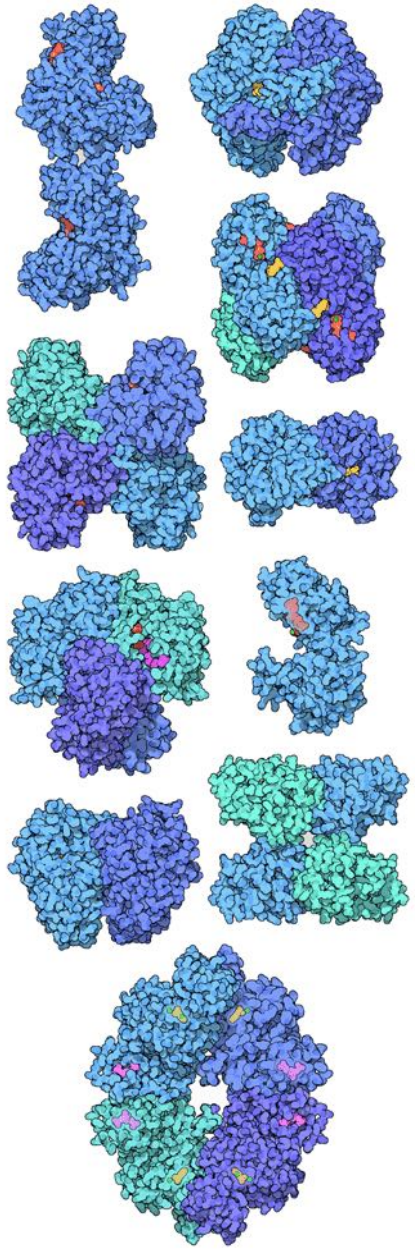


Enzymes: monomers (this week) and multimers (next week)



pyruvate kinase:

1. 4 subunits, a multimer
2. different binding sites on a single subunit
3. conformational changes occur when those sites are occupied
4. regulatory and catalytic sites
5. multimers are enzyme subject to regulation by feedforward and feedback loops
6. more sensitive to regulators than monomer (single subunit enzymes)



All glycolytic enzymes

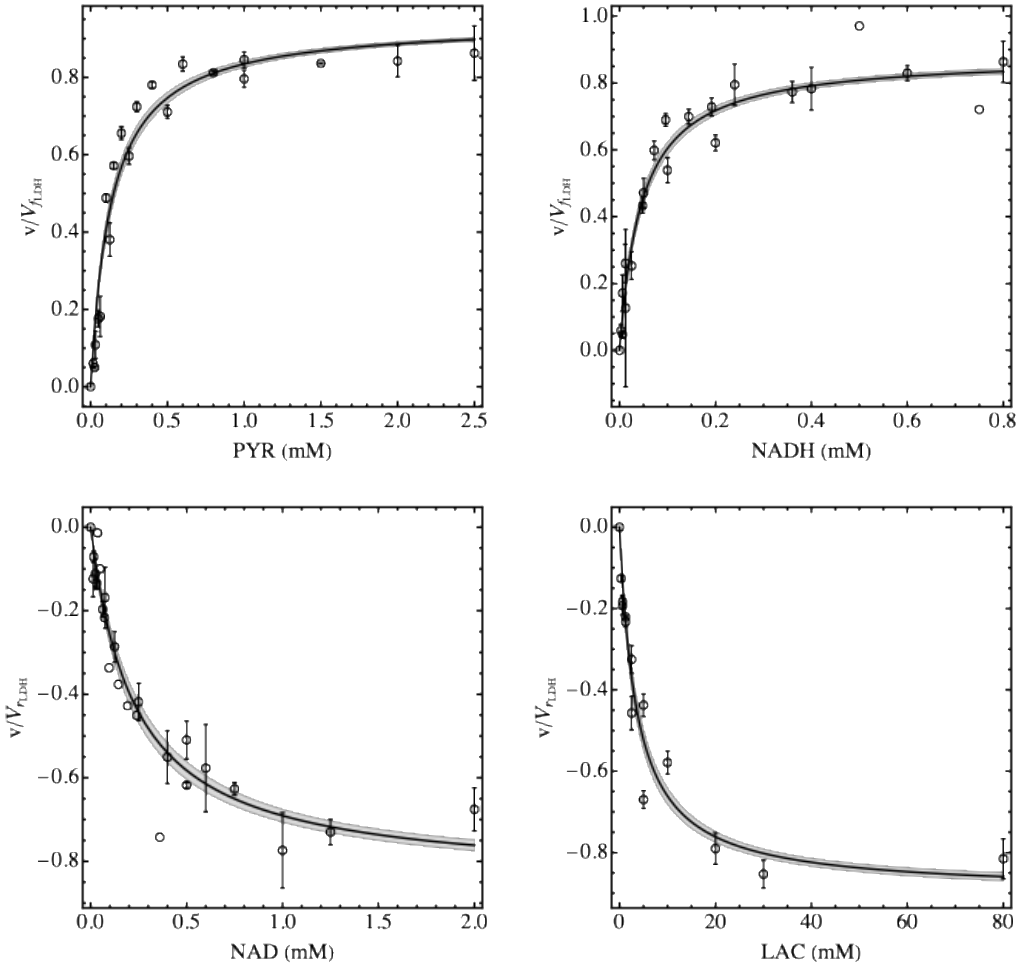
Enzyme kinetics: experimental example

- Lactate dehydrogenase
- Important in cancer cells, Warburg effect
- NADH + PYRUVATE ⇋ NAD +LACTATE
- Activity measured in cell free extract
- Rate equation to be fitted to data:

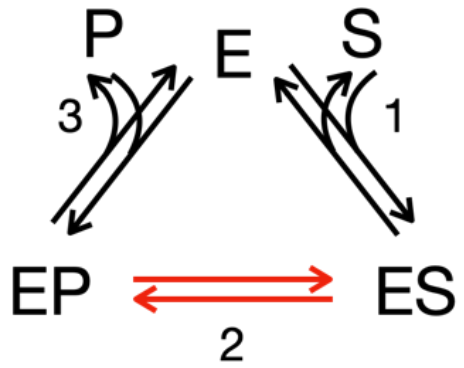
$$v = V_{MAX}^{+} \frac{\frac{nadh \cdot pyr}{K_{NADH} K_{PYR}} - V_{MAX}^{-} \frac{nad \cdot lac}{K_{NAD} K_{LAC}}}{\left(1 + \frac{nadh}{K_{NADH}} + \frac{nad}{K_{NAD}}\right)\left(1 + \frac{pyr}{K_{PYR}} + \frac{lac}{K_{LAC}}\right)}$$

- And for us to derive, our first net model of a state-transition diagram of an enzyme.

- Question: what would be a possible state-transition diagram forlactate dehydrogenase?



Derivation of enzyme kinetics: equations



state-transition diagram

(un)binding reactions
catalysis reaction

$$E + S \underset{}{\overset{1}{\rightleftharpoons}} ES$$
$$ES \overset{2}{\rightleftharpoons} EP$$
$$EP \overset{3}{\rightleftharpoons} E + P.$$

reactions

$$v_1 = k_1^+ \cdot e \cdot s - k_1^- \cdot es$$
$$v_2 = k_2^+ \cdot es - k_2^- \cdot ep$$
$$v_3 = k_3^+ \cdot ep - k_3^- \cdot e \cdot p.$$

mass-action kinetics
rate equations

$$\frac{ds}{dt} = -v_1$$
$$\frac{de}{dt} = -v_1 + v_3$$
$$\frac{des}{dt} = v_1 - v_2$$
$$\frac{dep}{dt} = v_2 - v_3$$
$$\frac{dp}{dt} = v_3.$$

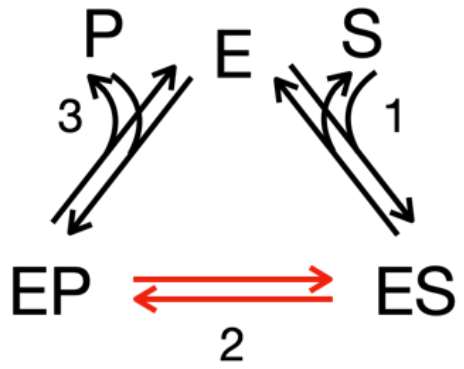
differential equations

- aim: make net model
- enzyme kinetics derivation
- outcome:

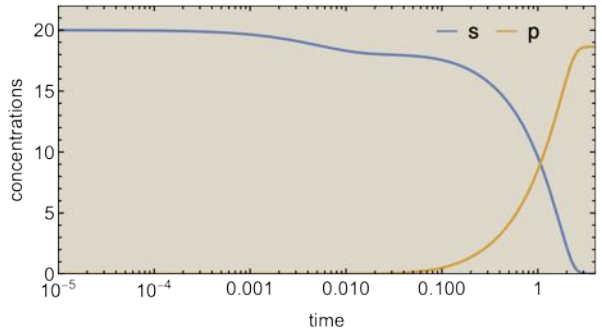
$$v = \frac{V_{max}^{+} \frac{s}{K_S} - V_{max}^{-} \frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

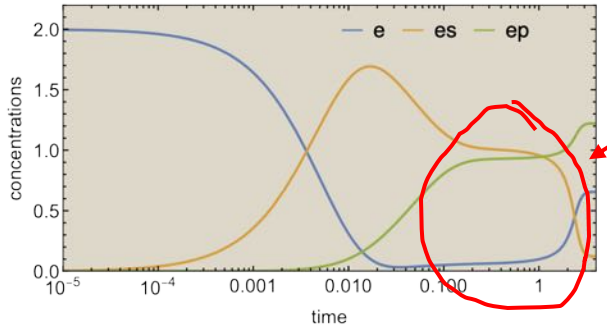
net model

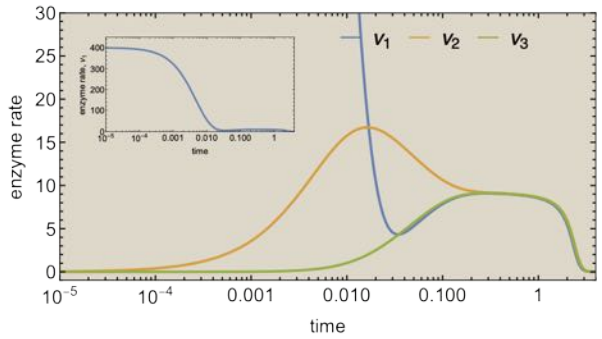
Derivation of enzyme kinetics: emergence of quasi-steady state of enzyme species



state-transition diagram

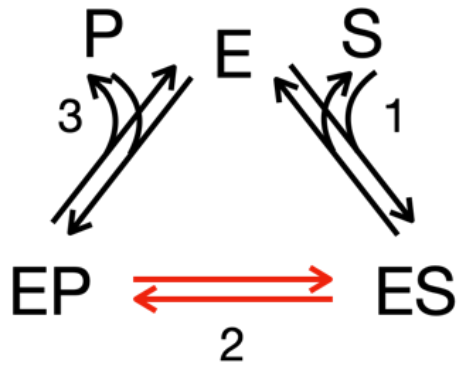




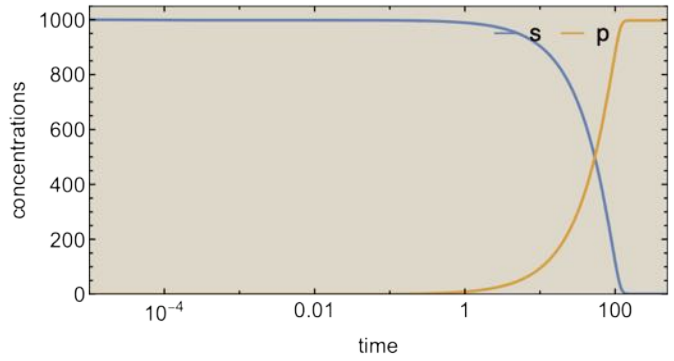


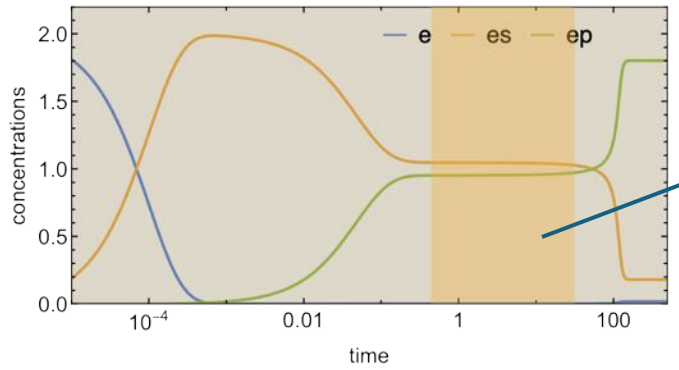
time window
with enzyme-species concentrations
constant: quasi-steady state
I.e. de/dt ≅ 0, des/dt ≅ 0, dep/dt≅ 0

Derivation of enzyme kinetics: emergence of quasi-steady state of enzyme species, due to substrate excess

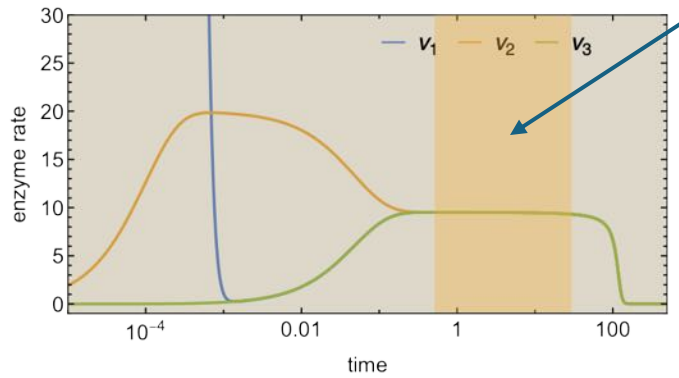


state-transition diagram





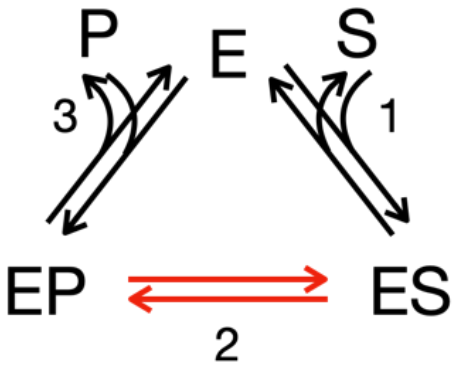
quasi-steady state of enzyme-species concentrations



therefore rates constant!

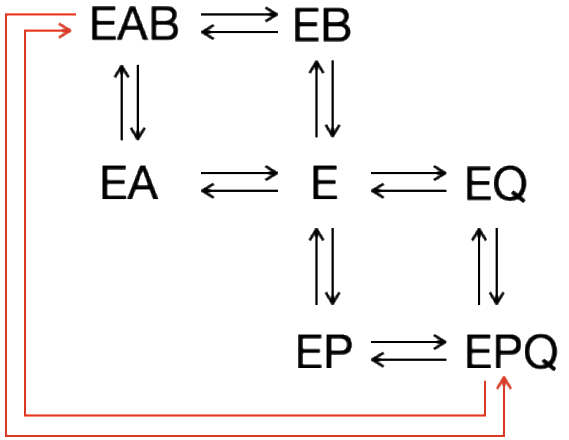
derivation of enzyme kinetics: net model is valid in quasi-steady state regime

- quasi-steady state assumption
  - assume differential equations for enzyme species constant
- quasi-equilibrium assumption
  - assume differential equations for enzyme species constant
  - and, assume that (un)binding rates are zero (equilibrium) (catalysis rate not; so catalysis rate is assumed to be the rate limiting step)



mechanism for

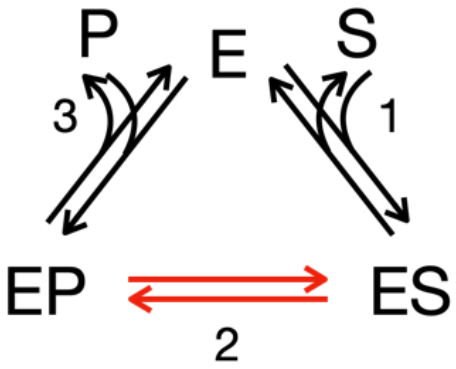
$A + B \rightleftharpoons P + Q$.



(un)binding reactions

catalysis reaction

we focus on the quasi-equilibrium assumption



- Assume quasi-steady state of enzyme species
- Assume (un)binding reactions as fast and catalysis slow, such that (un)binding reactions are (close to) zero and catalysis reaction not.
- Then:

$$\text{Enzyme rate: } v_2 = k_2^+ es - k_2^- ep$$

$$\text{(un)binding reaction rates at equilibrium: } v_1 = 0,\ v_2 = 0$$

$$\text{Equilibrium conditions } K_1 = K_S = \frac{e \cdot s}{es},\quad K_3 = K_P = \frac{e \cdot p}{ep}$$

$$\text{conservation of total } e_T = e + es + ep = e + \frac{e \cdot s}{K_S} + \frac{e \cdot p}{K_P}$$

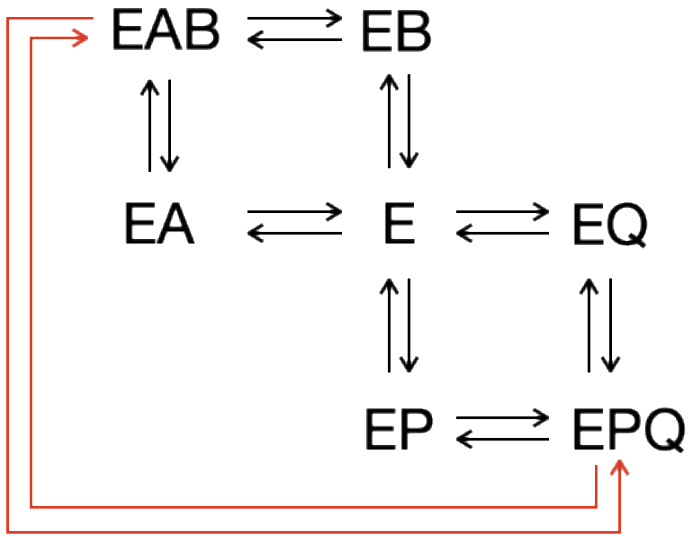
$$\text{solve for e: } e = e_T \frac{1}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

$$\text{solve for es and ep: } es = e_T \frac{\frac{s}{K_S}}{1 + \frac{s}{K_S} + \frac{p}{K_P}},\quad ep = e_T \frac{\frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

$$\text{Enzyme rate equation: } v_2 = k_2^+ es - k_2^- ep = \frac{k_2^+ e_T \frac{s}{K_S} - k_2^- e_T \frac{p}{K_P}}{1 + \frac{s}{K_S} + \frac{p}{K_P}}$$

Do the same for:

$$A + B \rightleftharpoons P + Q.$$



Do the same for:

$$A + B \rightleftharpoons P + Q.$$