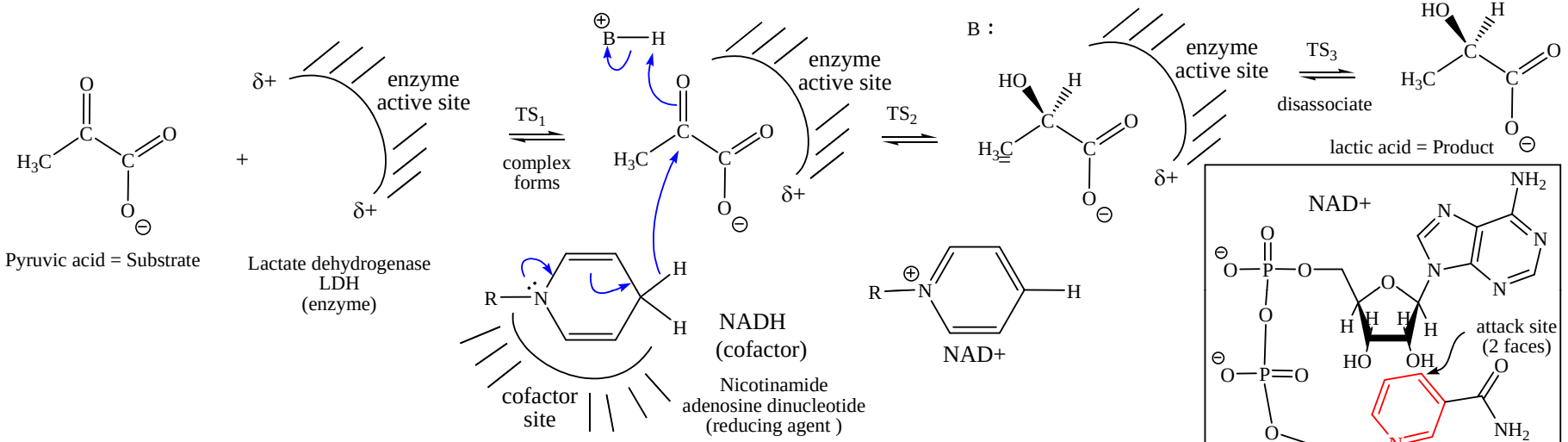
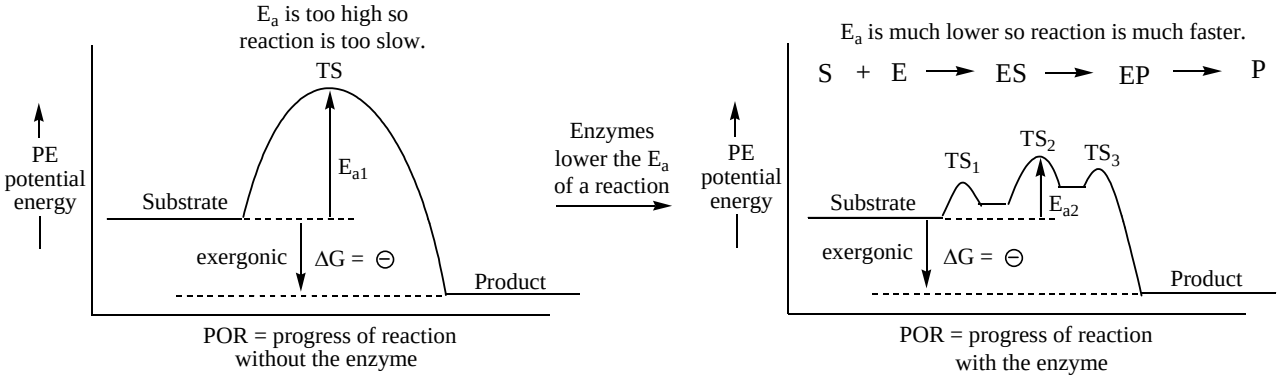
Chapter 3: Enzymes: Structure and Function

Enzymes act as the body's catalysts by complexing the reaction's participants in the correct arrangement to react, lowering the activation energy, E_a, to react, but ΔG stays the same.



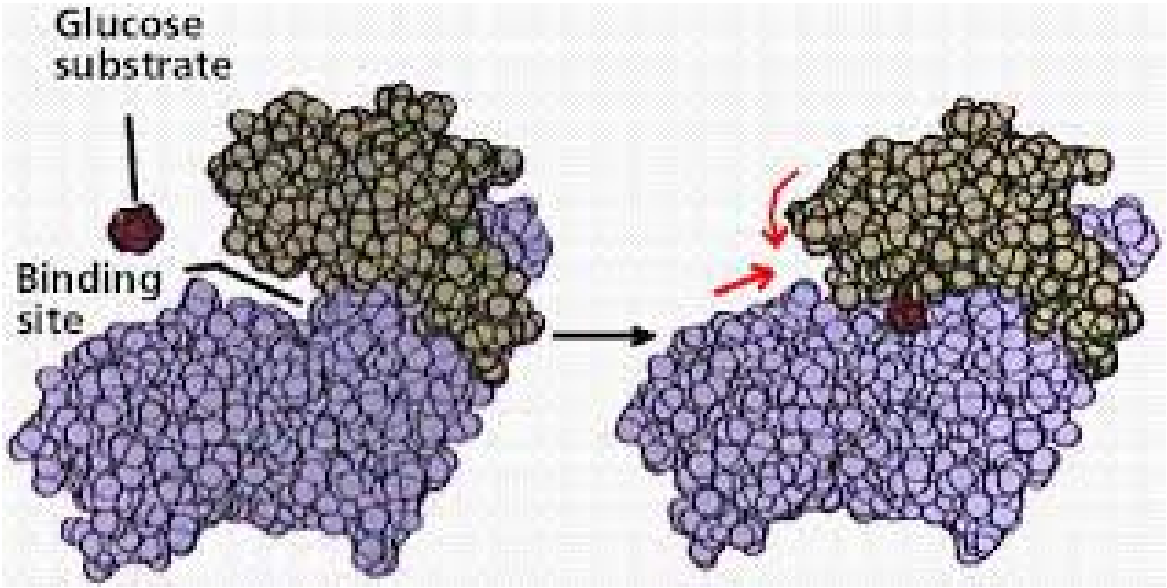


$$\frac{k_{enz}}{k_{no\ enz}} = \frac{10^{\frac{-E_{a2}}{2.3RT}}}{10^{\frac{-E_{a1}}{2.3RT}}} = 10^{\frac{-\Delta E_{a2}}{2.3RT}} = 10^{\frac{-(4-20)}{1.4}} = 10^{11.43} = 3 \times 10^{11}$$

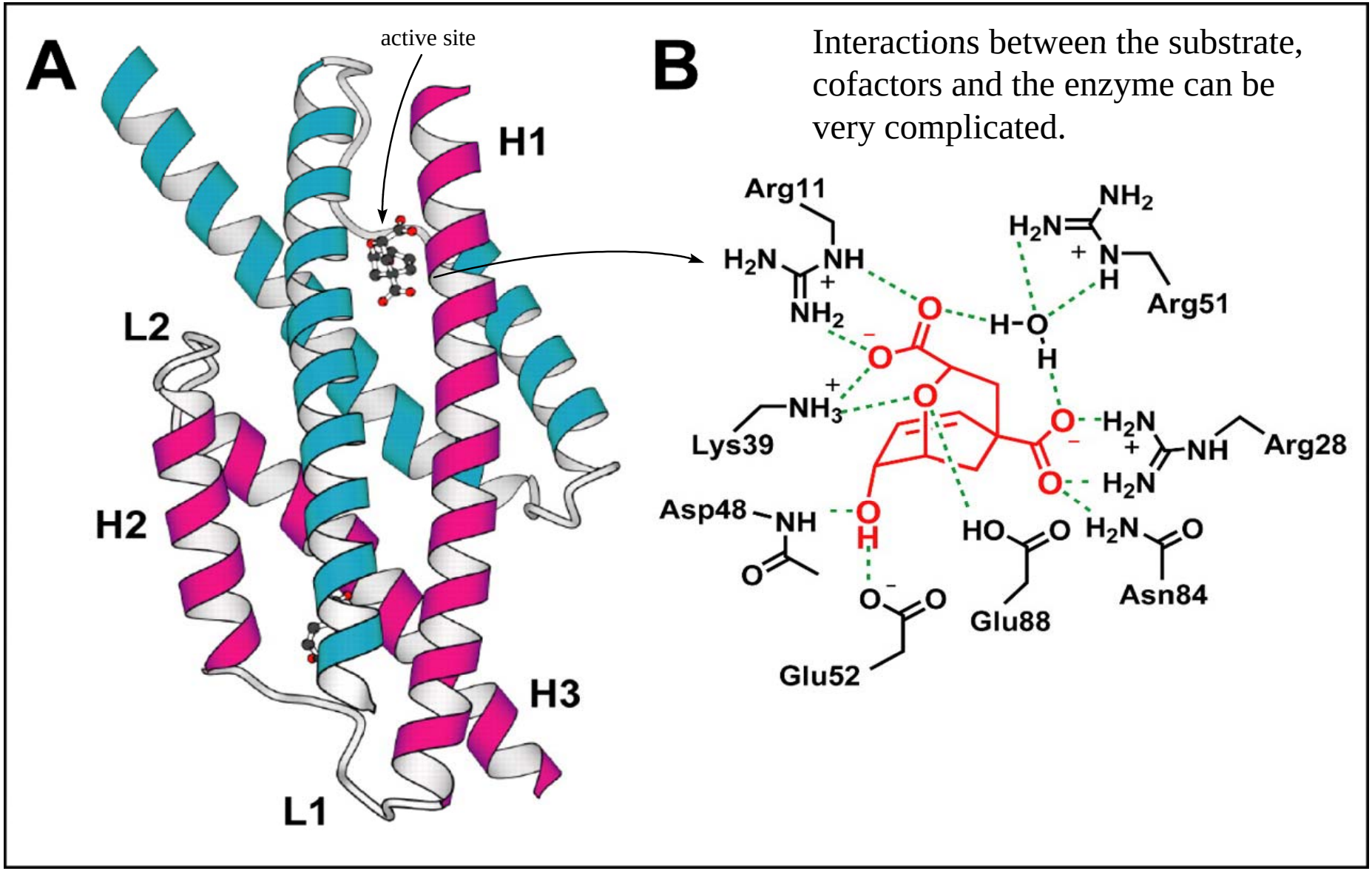
Enzyme catalyzed reactions are always faster. Assumed, E_{a1} = 20 kcal/mole and E_{a2} = 4 kcal/mole.

1. **Provides a reaction surface (the active site)**
2. **Provides a suitable environment (hydrophobic)**
3. **Brings reactants together**
4. **Positions reactants correctly for reaction**
5. **Weakens bonds in the reactants**
6. **Provides acid / base catalysis**
7. **Provides nucleophilic groups**
8. **Stabilises the transition state with intermolecular bonds**

The active site is often a hydrophobic hollow or cleft with key polar (or nonpolar) amino acids in key locations on the enzyme surface that can accept substrates and cofactors. The enzyme contains amino acids that interact with the substrate and cofactor in the usual way (ionic interactions, H bonds, dipole-dipole, dispersion forces and covalent bonds) which all help repeatedly catalyze the reaction (catch and release). It is usually proposed that the transition state complex is stabilized, lowering the activation energy which accelerates the reaction rate. Rather than the old 'lock and key' model, it is proposed that the enzyme and substrate influence one another to form a stronger interaction. This is called the 'induced fit' model.

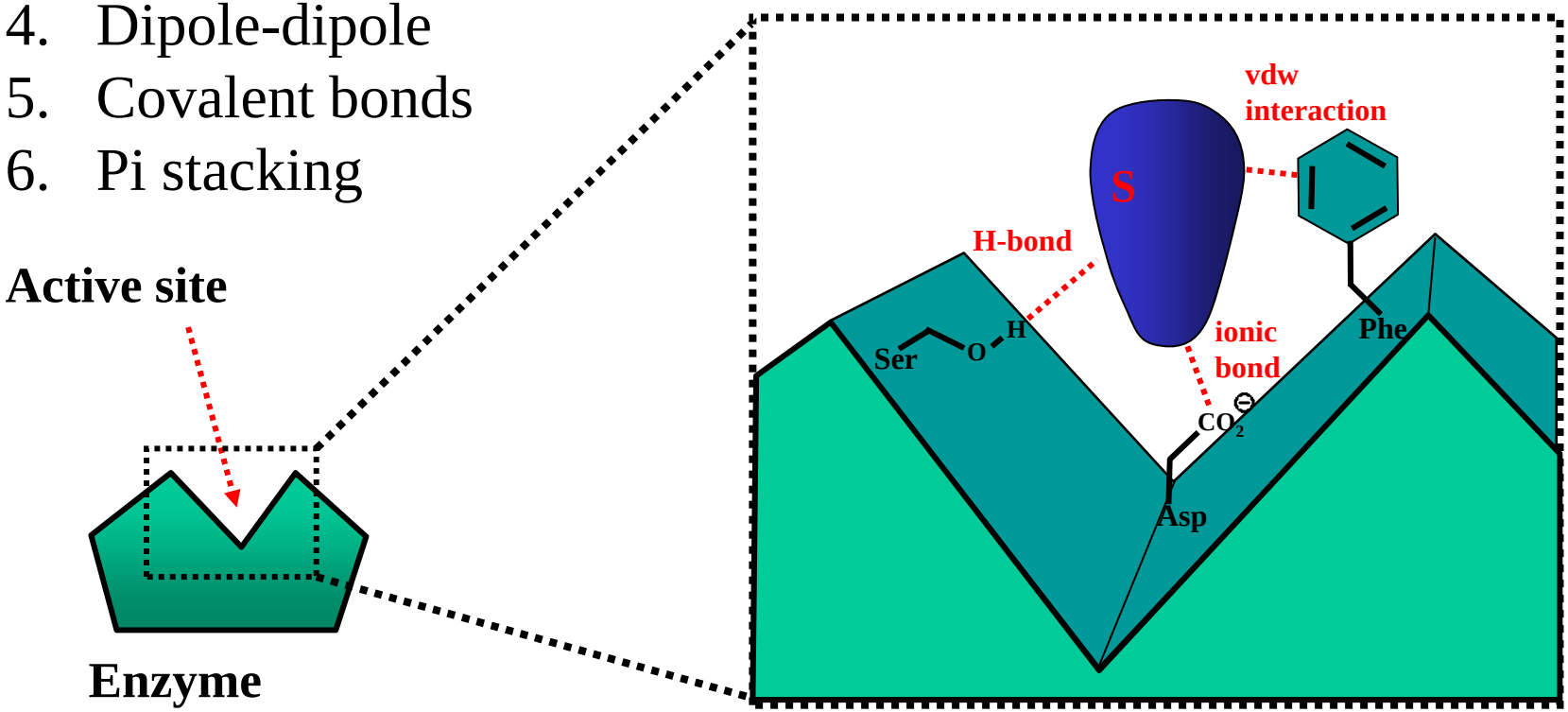


Identification of active sites is crucial in the process of drug discovery. The **3-D structure** of the enzyme is analysed to identify active sites and design drugs which can fit into them. The most common ways to do this are **x-ray crystollography, NMR analysis and computer modeling**. Inhibitors bind to an enzyme's active site and block interaction with natural substrates. Knowing the strength of binding between the active site and an enzyme inhibitor is an important strategy in drug design.

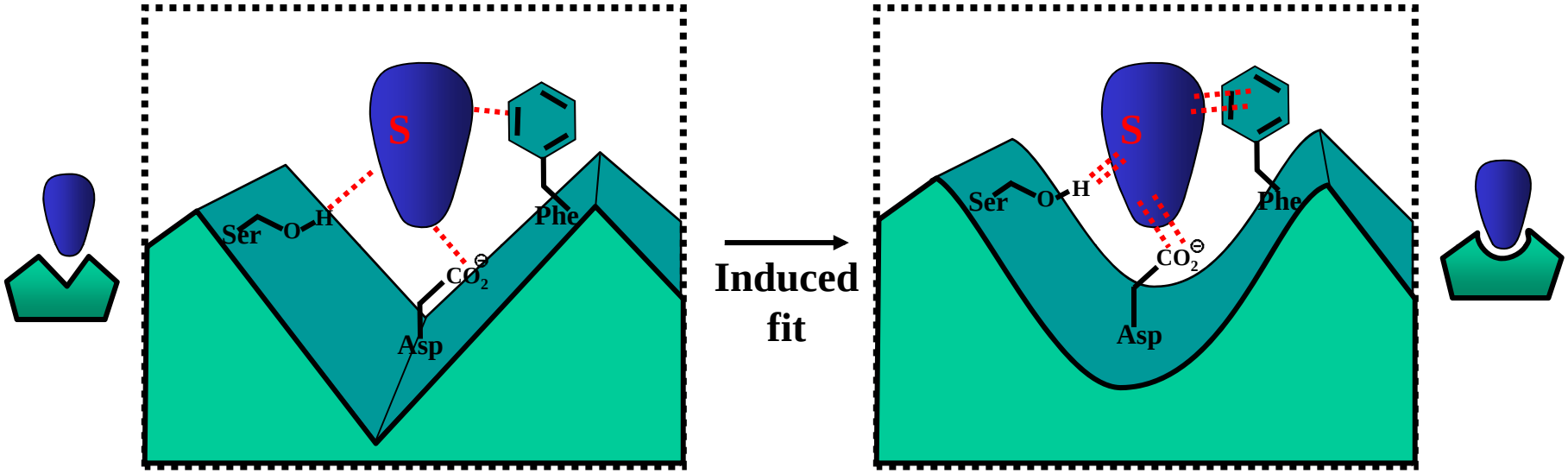

A
active site
H1
L2
H2
H3
L1
B
Interactions between the substrate, cofactors and the enzyme can be very complicated.
Arg11
Arg51
Lys39
Arg28
Asp48
Glu88
Asn84
Glu52

Substrate binding uses the usual forces of interaction.

1. Ionic
2. H-bonding
3. Dispersion forces
4. Dipole-dipole
5. Covalent bonds
6. Pi stacking



Induced fit - active site of the enzyme and the substrate alter shapes to maximise intermolecular bonding.

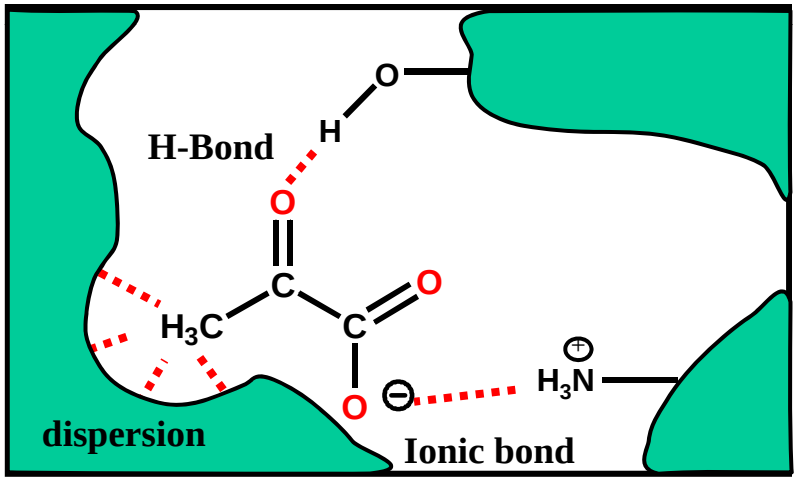


Intermolecular bonds not optimum length for maximum bonding

Intermolecular bond lengths optimised. Susceptible bonds in substrate strained. Susceptible bonds in substrate more easily broken

Binding of pyruvic acid in LDH (lactic dehydrogenase enzyme)

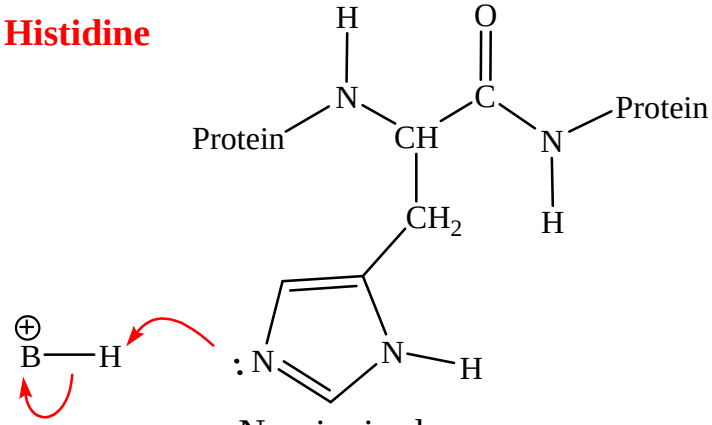
1. Ionic bonding
2. H-bonding
3. Dispersion forces



Catalysis mechanisms – necessary functions

Acid/base catalysis

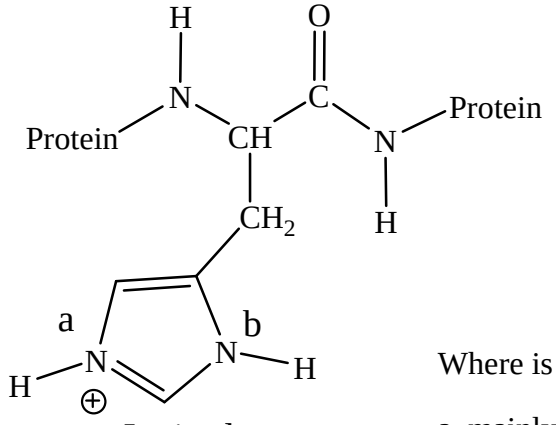
Histidine



Non-ionised
Acts as a basic catalyst
(proton 'sink')

$pK_a \approx 6.0$

B :



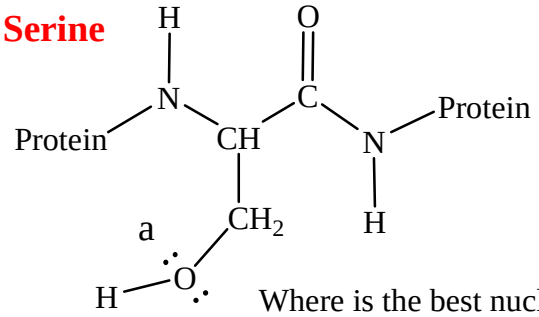
Ionised
Acts as an acid catalyst
(proton source)

Where is the + charge?

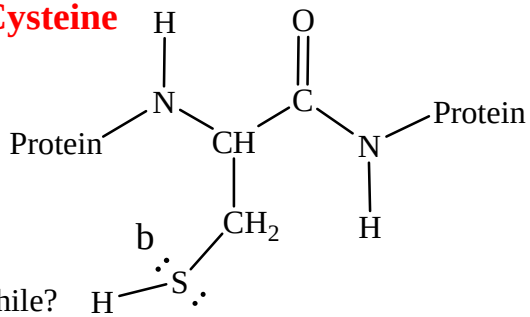
a. mainly on a
b. mainly on b
c. on both a and b

Nucleophilic residues

Serine



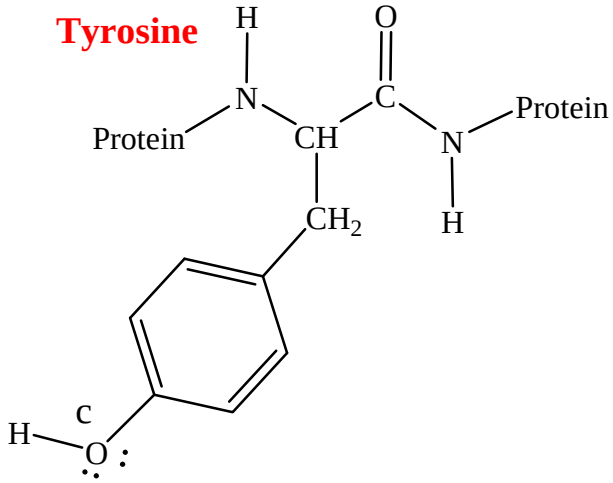
Cysteine



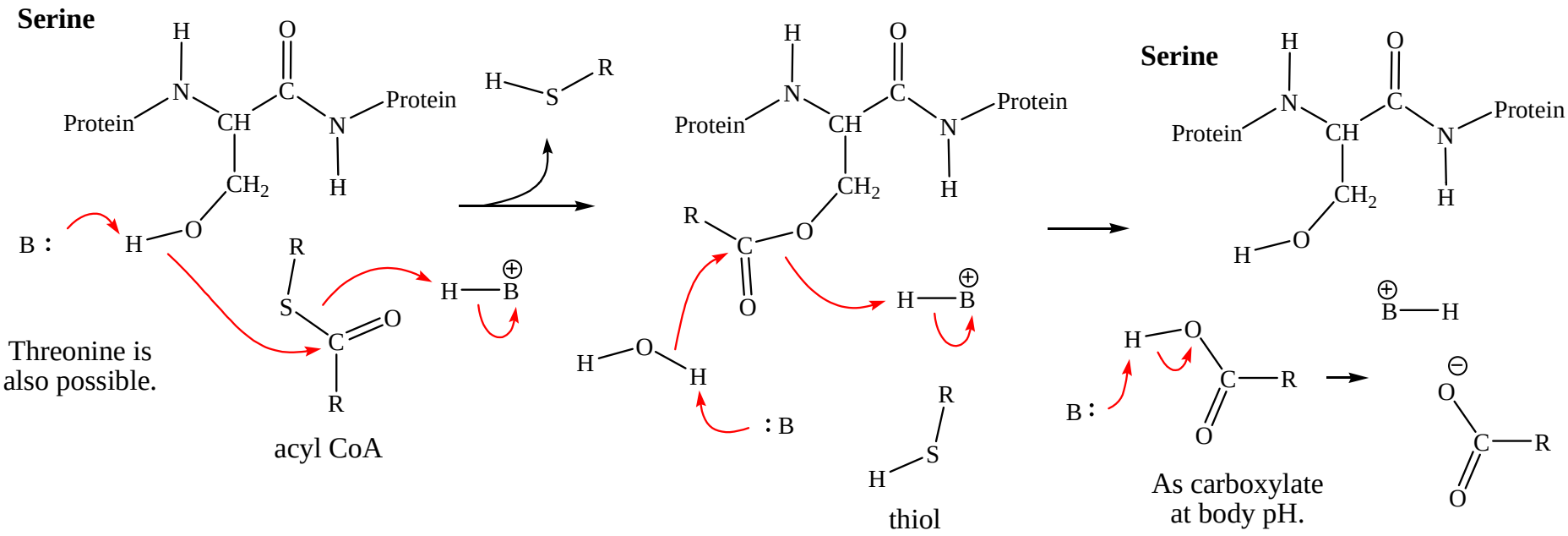
Where is the best nucleophile?

a. serine oxygen
b. cysteine sulfur
c. tyrosine oxygen
d. all are similar

Tyrosine

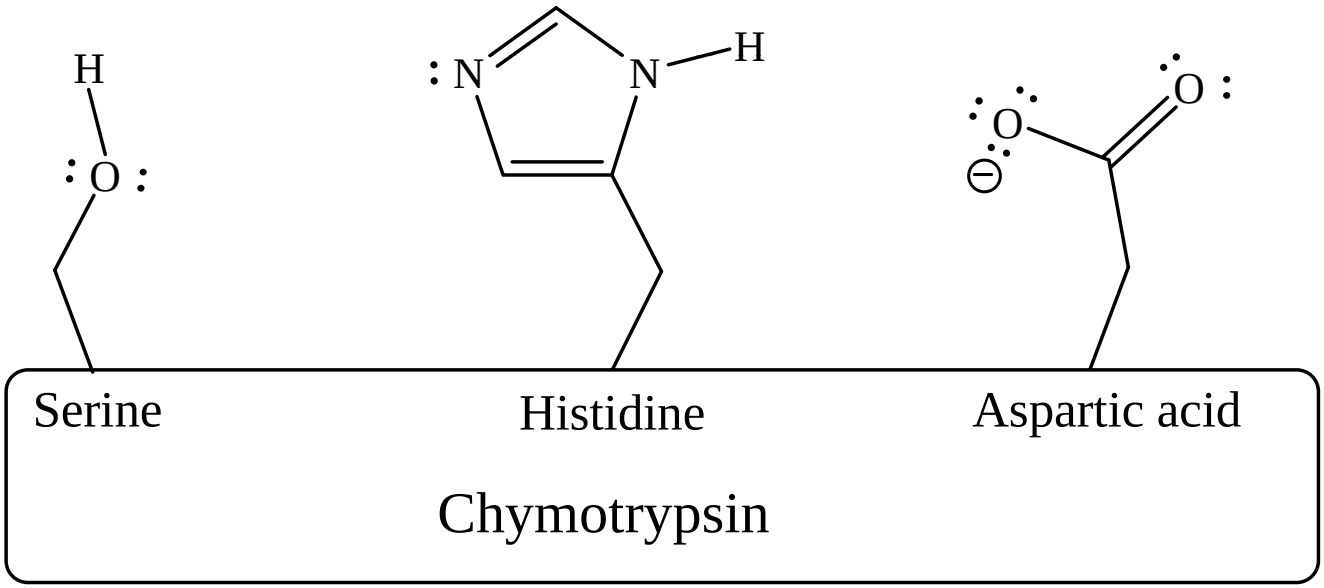


Serine acting as a nucleophile

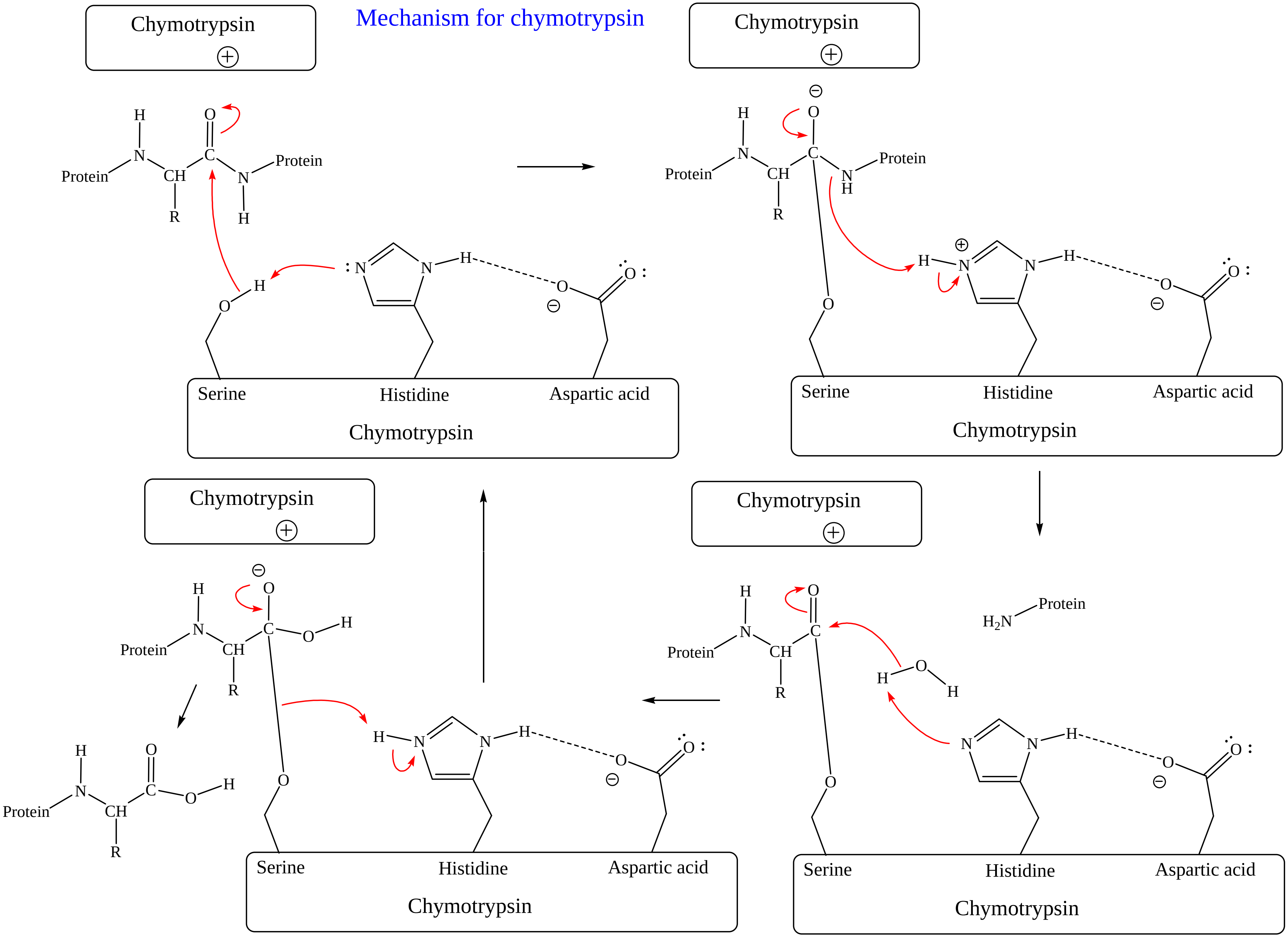


Mechanism for chymotrypsin uses 3 amino acids at the active site

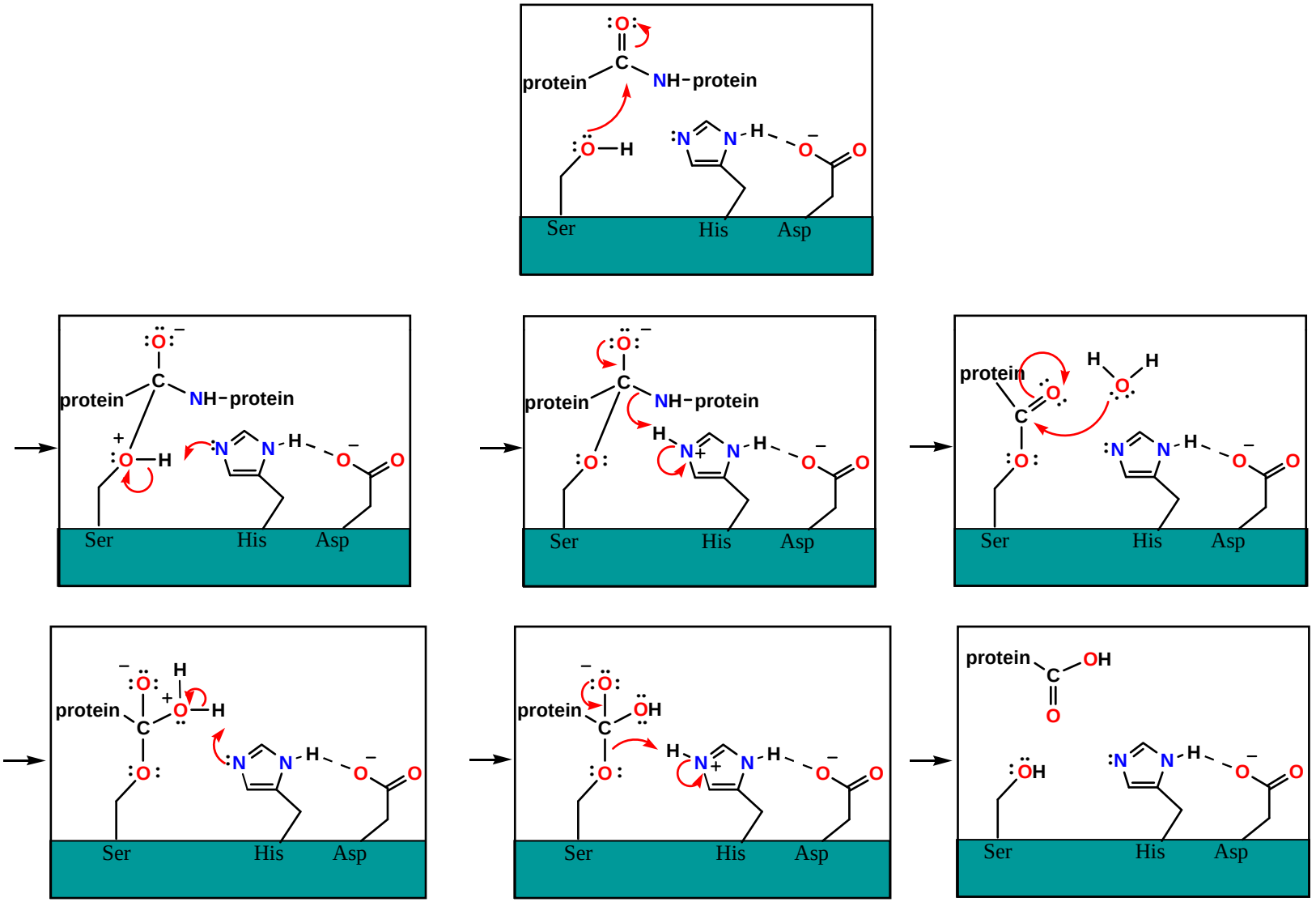
Catalytic triad of serine, histidine and aspartate



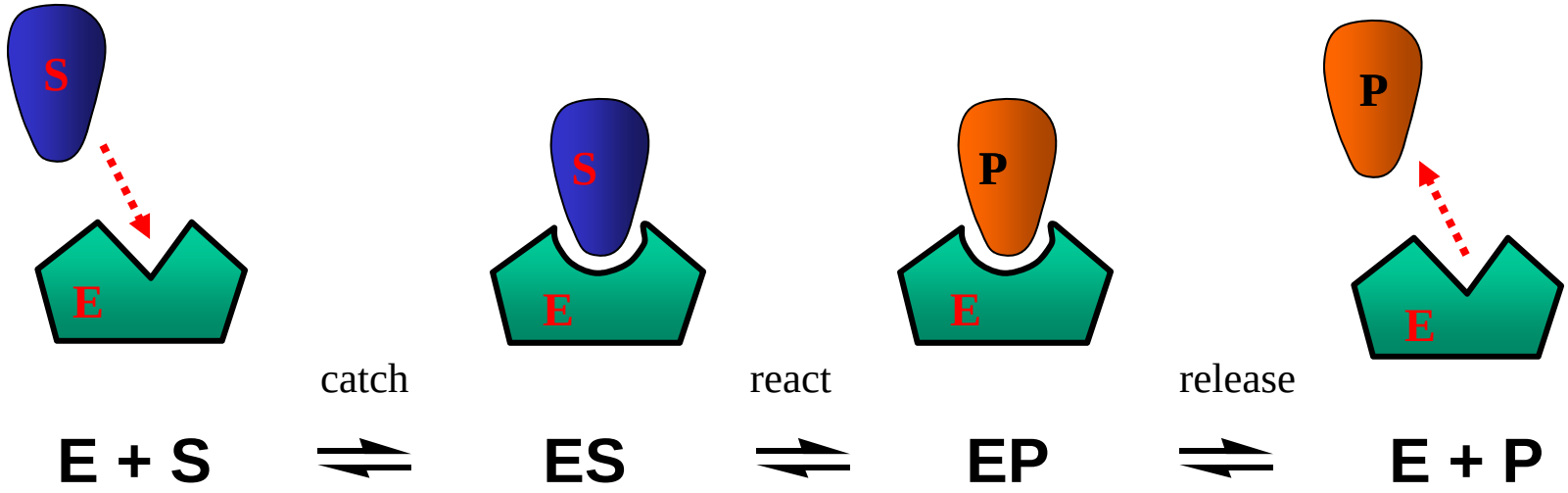
Mechanism for chymotrypsin



Mechanism for chymotrypsin



Overall Process of Enzyme Catalysis

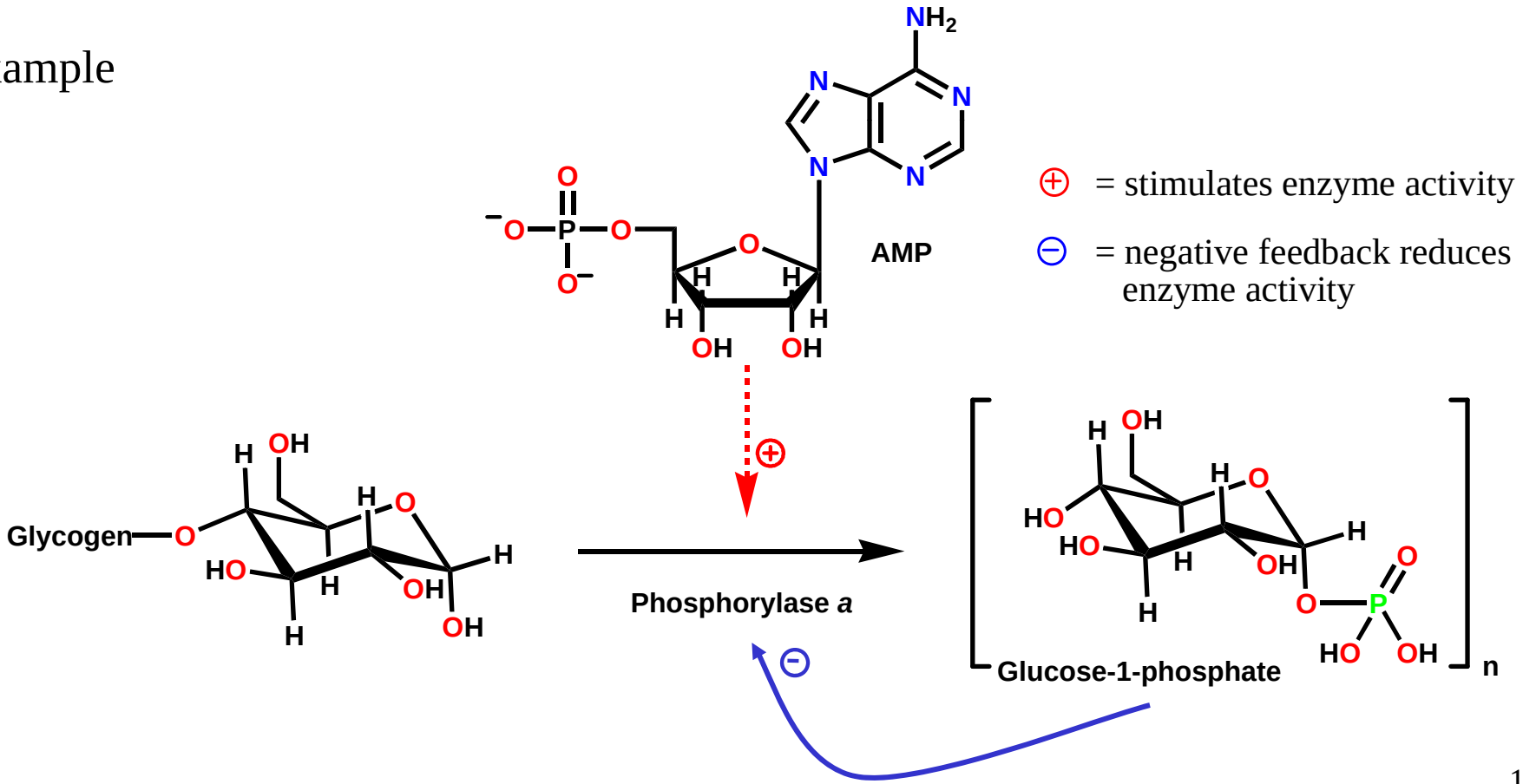


1. Binding interactions must be strong enough to hold the substrate sufficiently long for the reaction to occur
2. Interactions must be weak enough to allow the product to depart
3. Interactions stabilize the transition state, lowering E_a
4. Designing molecules with stronger binding interactions results in enzyme inhibitors which block the active site

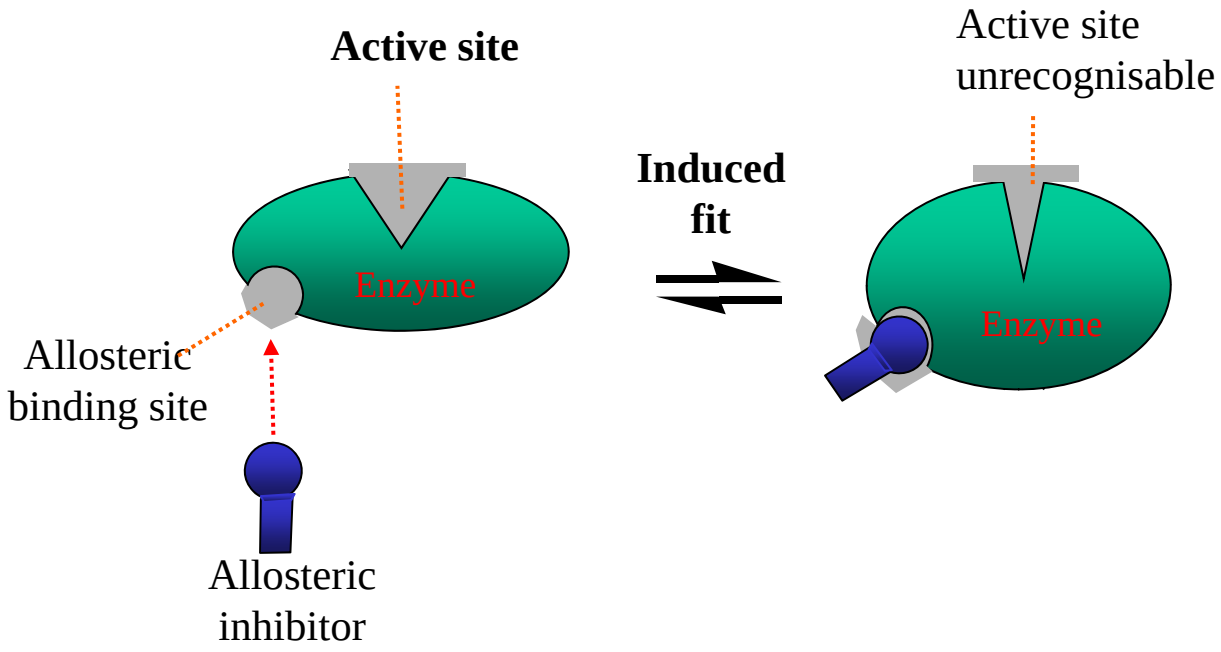
Regulation of Enzymes

1. Many enzymes are regulated by agents within the cell
2. Regulation may enhance or inhibit the enzyme
3. The products of some enzyme-catalysed reactions may act as inhibitors
4. Often they bind to a binding site called an allosteric binding site

Example

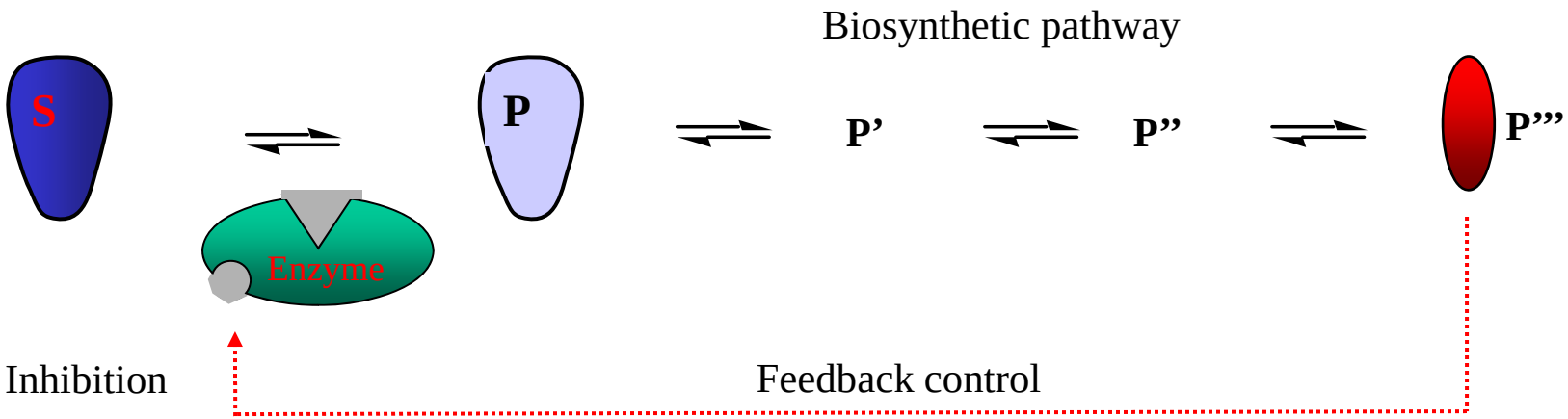


Regulation of Enzymes



1. Inhibitor binds reversibly to an allosteric binding site (molecule near end of pathway
2. Intermolecular bonds are formed (the usual kinds)
3. Induced fit of allosteric inhibitor alters the shape of the enzyme
4. Active site is distorted and is not recognised by the substrate (catalysis slows or stops)
5. Increasing substrate concentration does not reverse inhibition
6. Inhibitor differs in structure to the substrate (different enzyme location)

Regulation of Enzymes

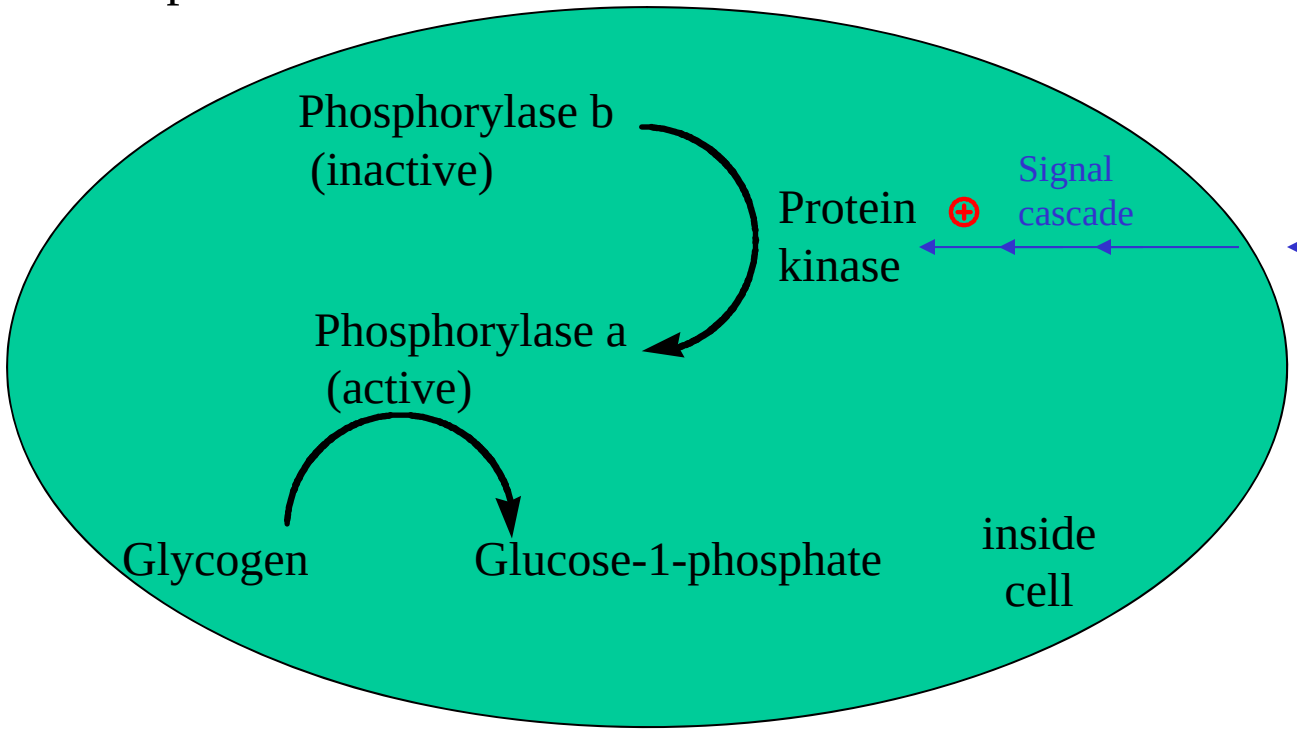


Enzymes with allosteric sites are often at the start of a biosynthetic pathway. The enzyme is controlled by the final product of the pathway. The final product binds to the allosteric site and switches off the enzyme as it builds up in concentration.

Regulation of Enzymes

1. External signals can regulate the activity of enzymes (e.g. neurotransmitters or hormones)
2. Chemical messenger initiates a signal cascade which activates enzymes called protein kinases
3. Protein kinases phosphorylate target enzymes to affect activity

Example



Adrenaline, outside cell, reacts with different types of cells in different ways.

Enzyme kinetics can be used to study factors important to enzyme behavior.

Michaelis-Menton derivation

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

$\frac{dP}{dt} = v_o = k_2 [ES]$ ← rate of product formation

assume: $k_{-1} >> k_2$

assume: $[S] >> [E]$ so $[ES] \approx$ constant = steady state assumption

$\frac{d[ES]}{dt} = 0 = k_1[E][S] - k_{-1}[ES] - k_2 [ES]$

$k_1[E][S] = k_{-1}[ES] + k_2 [ES] = (k_{-1} + k_2)[ES]$ (rearranged)

$E_{total} = [E_T] = [E] + [ES]$

$[E] = [E_T] - [ES]$

$[ES] = [E_T] - [E]$

$k_1([E_T] - [ES])([S]) = (k_{-1} + k_2)[ES]$ ← substitution: $[E] = [E_T] - [ES]$

$k_1 [E_T][S] - k_1 [ES][S] = (k_{-1} + k_2)[ES]$

$k_1 [E_T][S] = k_1 [ES][S] + (k_{-1} + k_2)[ES]$

$\left(\frac{1}{k_1}\right) \times \left[k_1 [E_T][S] = k_1 [ES][S] + (k_{-1} + k_2)[ES]\right]$

$\frac{(k_1 [E_T][S])}{(k_1)} = \frac{k_1 [ES][S] + (k_{-1} + k_2)[ES])}{(k_1)}$

(continued on next slide)

$$[E_T]\,[S] = \frac{(k_1[S] + k_{-1} + k_2)}{k_1}\,[ES]$$

$$[ES] = \frac{k_1}{(k_1[S] + k_{-1} + k_2)}\,[E_T]\,[S] \quad \text{(rearranged)}$$

$$\left(\frac{(1/k_1)}{(1/k_1)}\right)\frac{k_1}{(k_1[S] + k_{-1} + k_2)} = \frac{1}{([S]) + \dfrac{(k_{-1} + k_2)}{k_1}}$$

defined $K_M = \dfrac{(k_{-1} + k_2)}{k_1}$

algebra (substitution)

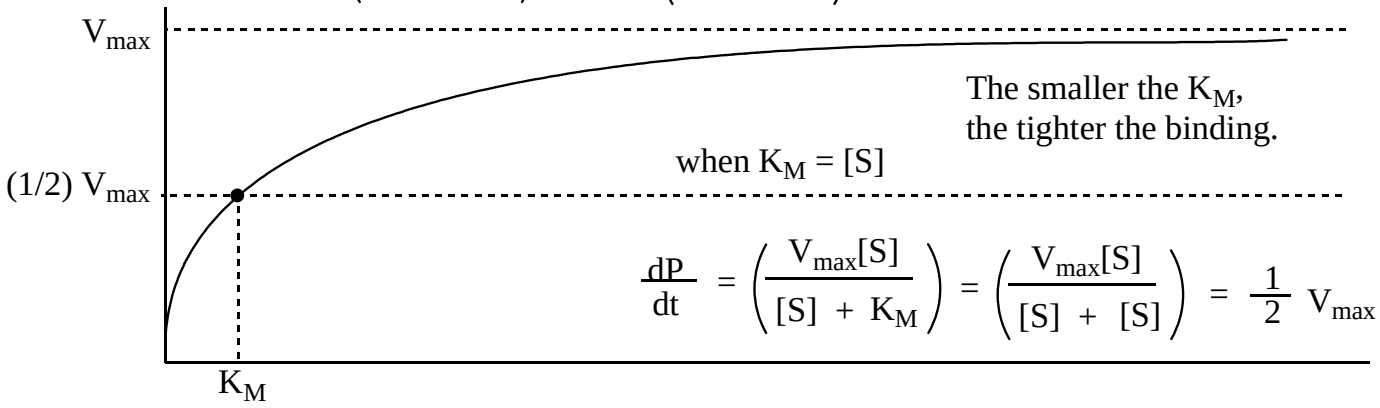
$$= \frac{1}{[S] + K_M}$$

$$[ES] = \left(\frac{1}{[S] + K_M}\right)[E_T]\,[S]$$

$$\frac{dP}{dt} = \nu_o = k_2\,[ES] = k_2\left(\frac{[E_T]\,[S]}{[S] + K_M}\right) = \left(\frac{V_{max}[S]}{[S] + K_M}\right)$$

← **Michaelis-Menton Eq.** $V_{max} = k_2\,[E_T]$

$$\frac{dP}{dt} = k_2\left(\frac{[E_T][S]}{[S] + K_M}\right) = \left(\frac{V_{max}[S]}{[S] + K_M}\right)$$ ← Michaelis-Menton Eq. $V_{max} = k_2[E_T]$



defined

$$K_M = \frac{(k_{-1} + k_2)}{k_1}$$

$$K_M \approx \frac{\text{unbound}}{\text{bound}}$$

Another way to plot the data (inverse).

$$\frac{1}{\frac{dP}{dt}} = \frac{1}{\left(\frac{V_{max}[S]}{[S] + K_M}\right)} = \frac{[S] + K_M}{V_{max}[S]} = \frac{K_M}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

initial rate

Lineweaver-Burk Plots

$$\frac{1}{\nu_o} = \frac{K_M}{V_{max}}\left(\frac{1}{[S]}\right) + \left(\frac{1}{V_{max}}\right)$$

$$y = \frac{1}{\nu_o} \qquad y = m\,(x) + b$$