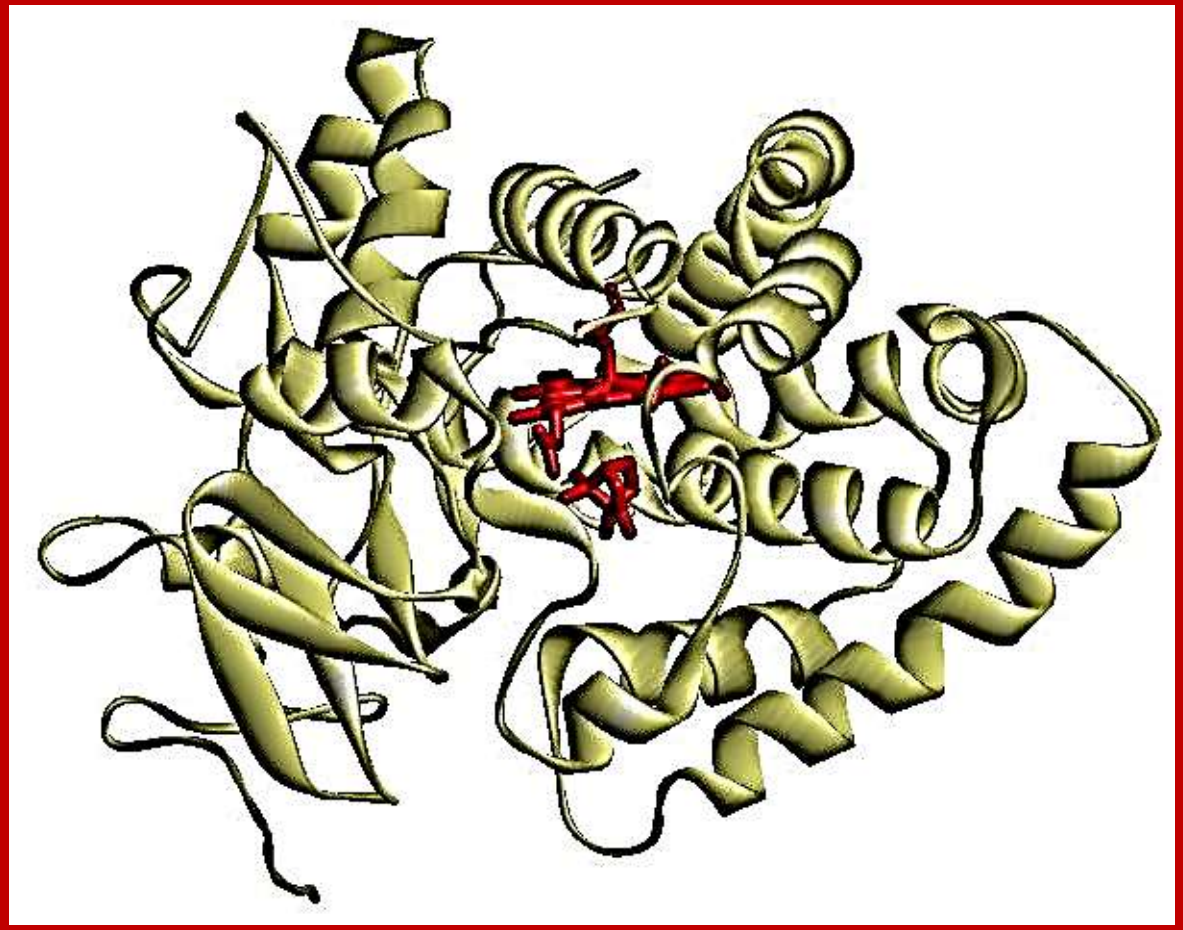
Catalysis

Biological and Biomimetic Catalysis



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Biocatalysis

Biocatalysts:

- Ubiquitous in living systems: enzymes
- Also used outside organisms, *in vitro* in many industrial processes (see *Catalysis*, Table 4.1)

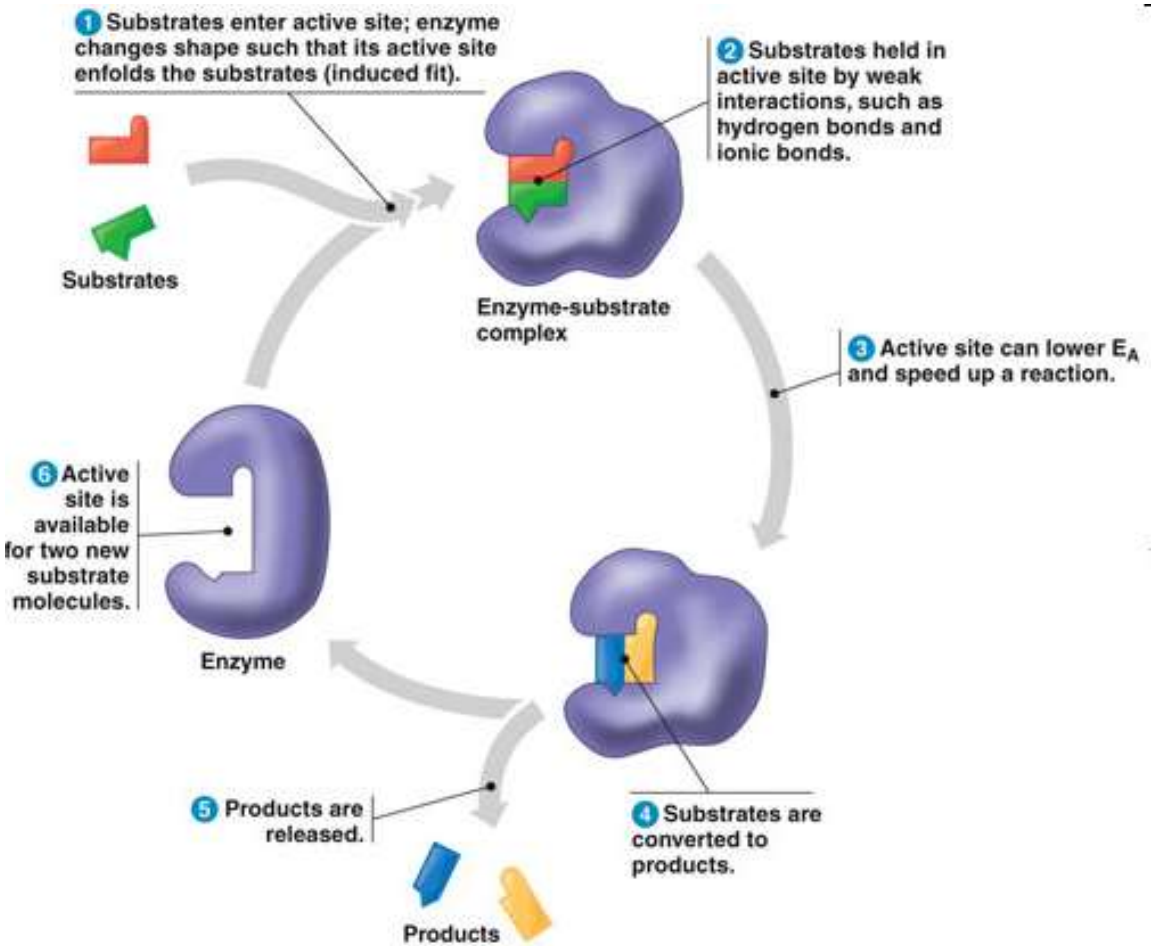
Strengths and weaknesses of enzymes in industrial applications

Strengths	Weaknesses
High selectivities (substrate-, regio-, stereo-, etc.)	Sometimes too selective
Clean reactions, few side products	Product separation
Mild reaction conditions	Often limited to water and ~RT
"Green"	

- Modern protein engineering can modify natural enzymes and turn them into catalysts which can catalyze different reactions and under a variety of conditions

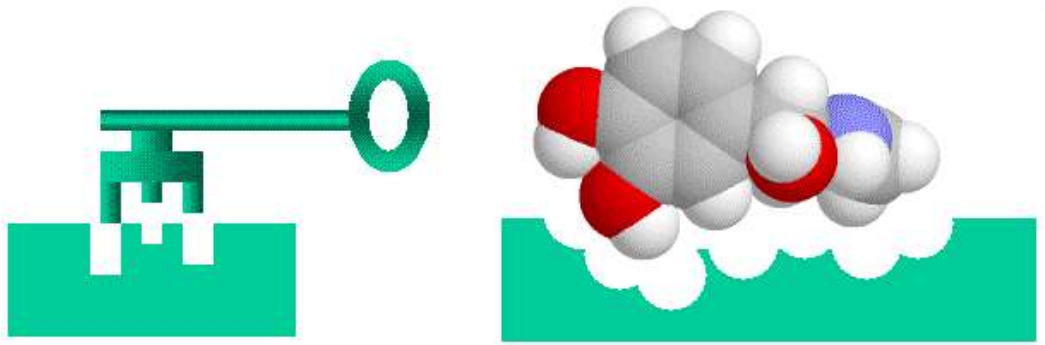
Enzymes

Typical operating mechanism in nature:

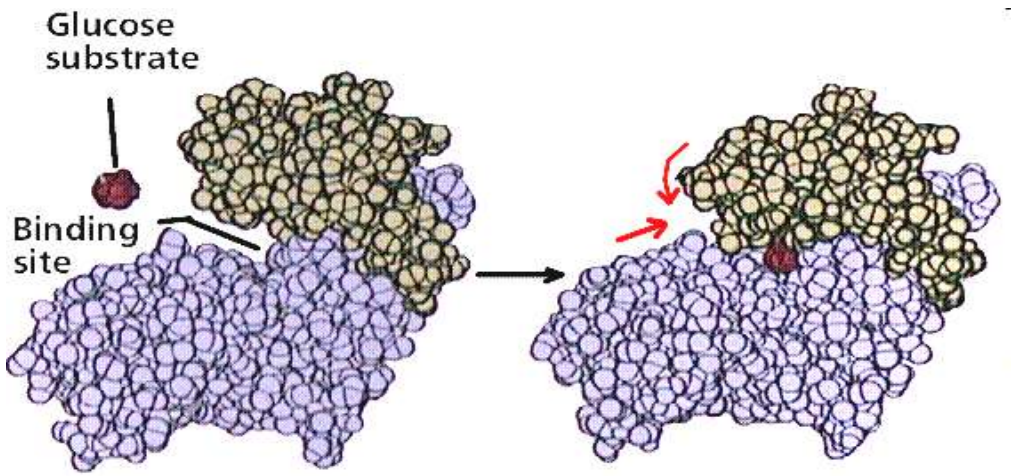


Sometimes > million-fold rate acceleration

Emil Fischer (1894): lock & key principle:



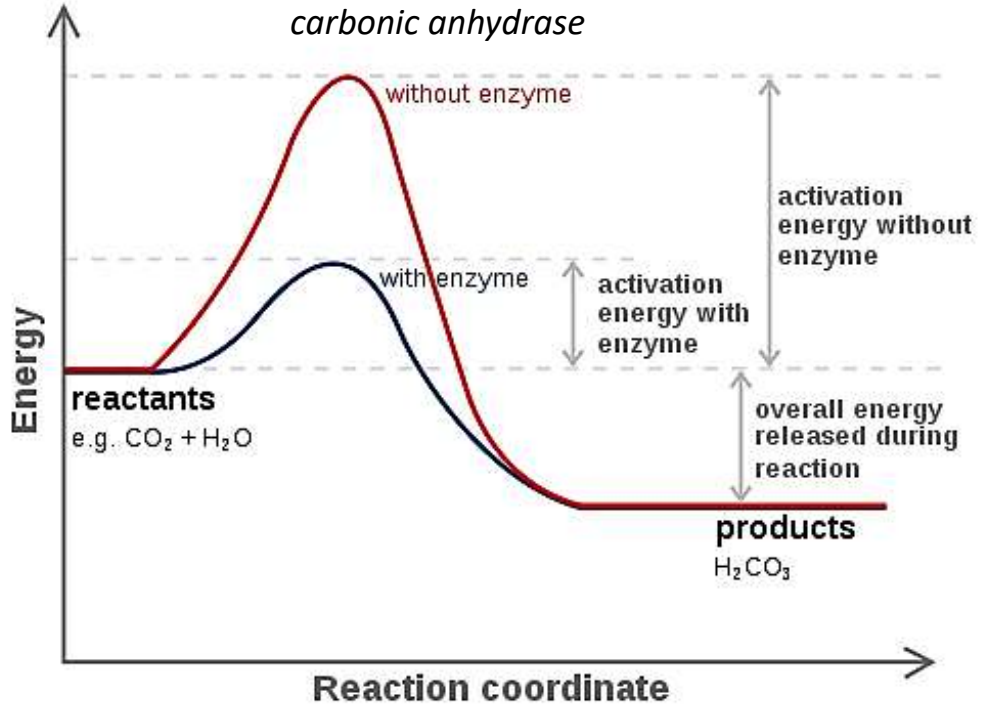
Reality:



Substrate binds via an induced-fit mechanism

Characteristics of enzymes

Linus Pauling (1948): ***"Enzymes are molecules that are complementary in structure to the transition states of reactions that they catalyze"***



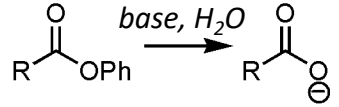
- They stabilize the transition state
- They induce fast reaction rates under mild conditions (atmospheric pressure, close to RT
- Extreme level of molecular recognition: size, shape, chirality
- Each enzyme molecule has a high turnover number in catalysis without being destroyed itself
- Enzymes are subject to competitive inhibition by compounds that bind in the enzyme but do not react themselves

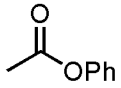
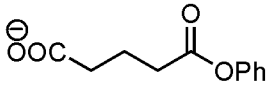
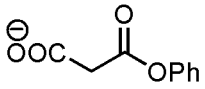
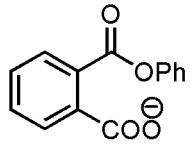
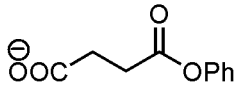
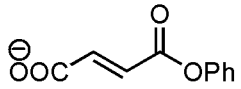
Enzymes

Enzymes lower E^{act} of a reaction

- Docking of substrate(s) in active site can be favourable for enthalpy
  - (1) By avoiding the formation of high-energy intermediates (e.g. localized charges)
  - (2) By activation of a reaction via interactions with amino acids (e.g. H-bonding)
- Docking of substrate(s) in active site can be favourable for entropy
  - (1) By *preorganizing* a substrate in a favorite geometry for an *intra*molecular reaction
  - (2) By *preorganizing* multiple substrates in a favorite orientation for an *inter*molecular reaction

Example of intramolecular vs intermolecular reaction

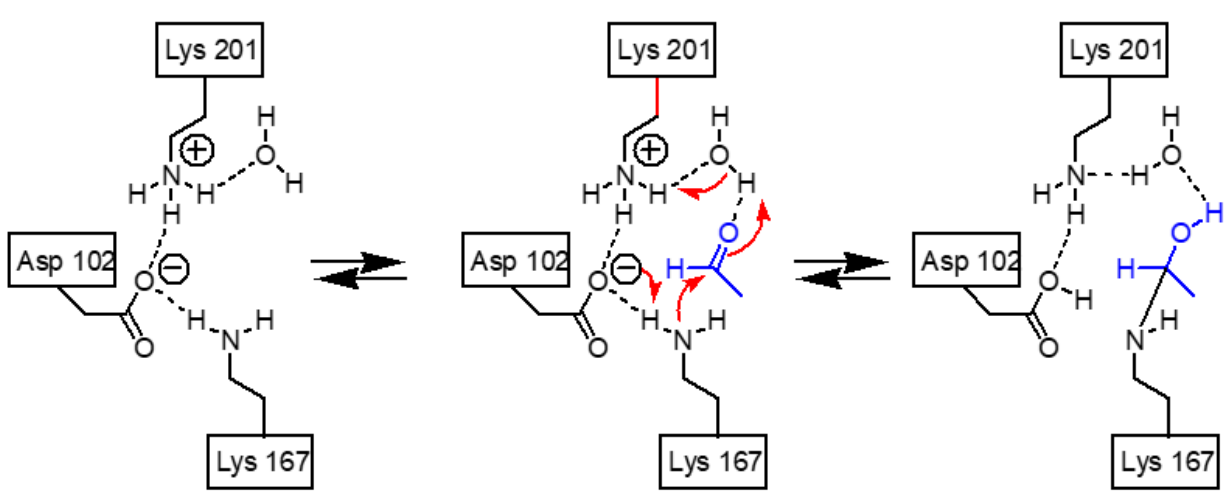


	k_{rel}		k_{rel}
	1		150
	50		1.1×10^6
	23000		1

Examples of mild enzymatic reactions

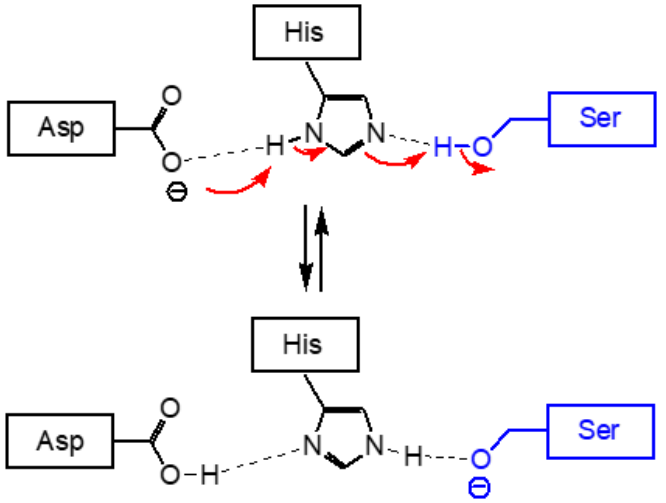
Deoxyribose-phosphate aldolase

First step



- Active site perfectly *preorganized* for binding the substrate
- All participating groups of the enzyme work in tandem; alignment of amino acid residues, substrate, water molecule
- Charges are delocalized via hydrogen bonding
- No oxoanion (pK_a 15) is formed but C=O group is protonated

Serine hydrolases

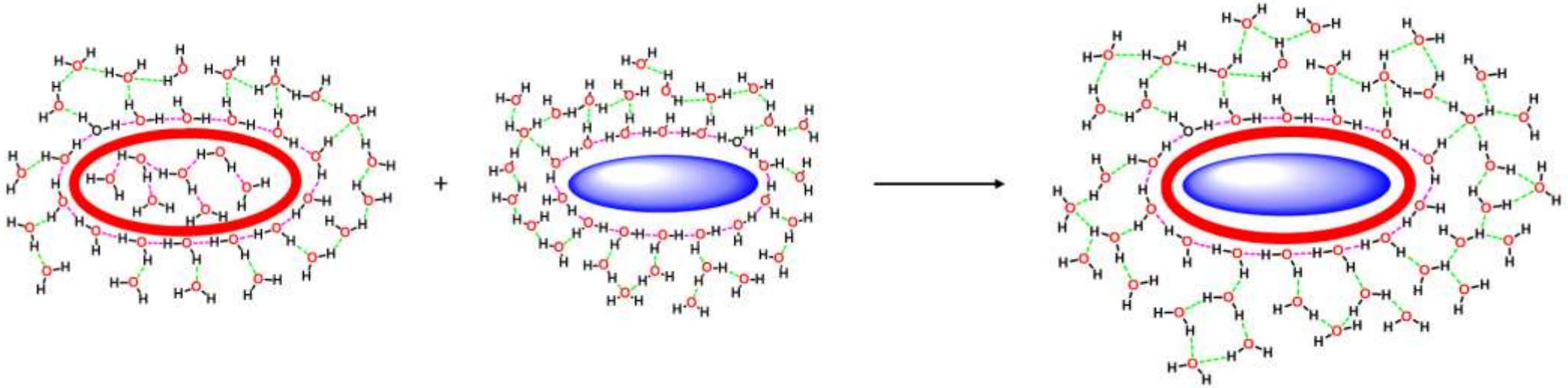


- Deprotonation serine usually requires conc. NaOH
- Preorganized catalytic triad in enzyme
- Negative charges delocalized via hydrogen bonding → deprotonation is mild

Nature's thermodynamic trick: the Hydrophobic Effect

Active sites in enzymes can regulate the amount of water molecules present

- Water molecules that solvate a hydrophobic surface or cavity cannot have ideal hydrogen bonding interactions; they are arranged
- Filling of a hydrophobic cavity in water is favourable: hydrophobic effect



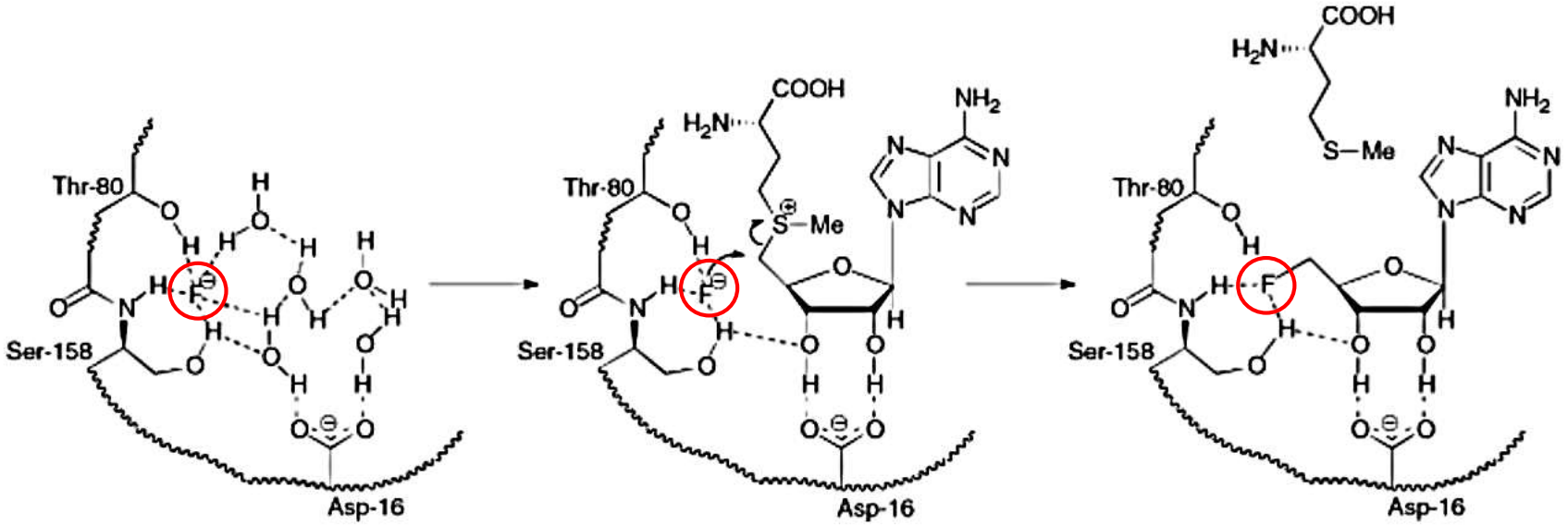
- Enthalpically favourable ($\Delta H < 0$): optimization of hydrogen bonds of *previously arranged* water molecules
- Entropically favourable ($\Delta S > 0$): release of *previously arranged* water molecules

Role of water molecules in an active site

Active sites in enzymes can regulate the amount of water molecules present

- Water molecules may participate in an enzyme-catalyzed reaction
- Alternatively: water molecules may need to be released before an enzyme-catalyzed reaction can take place

Example from a fluorinase:



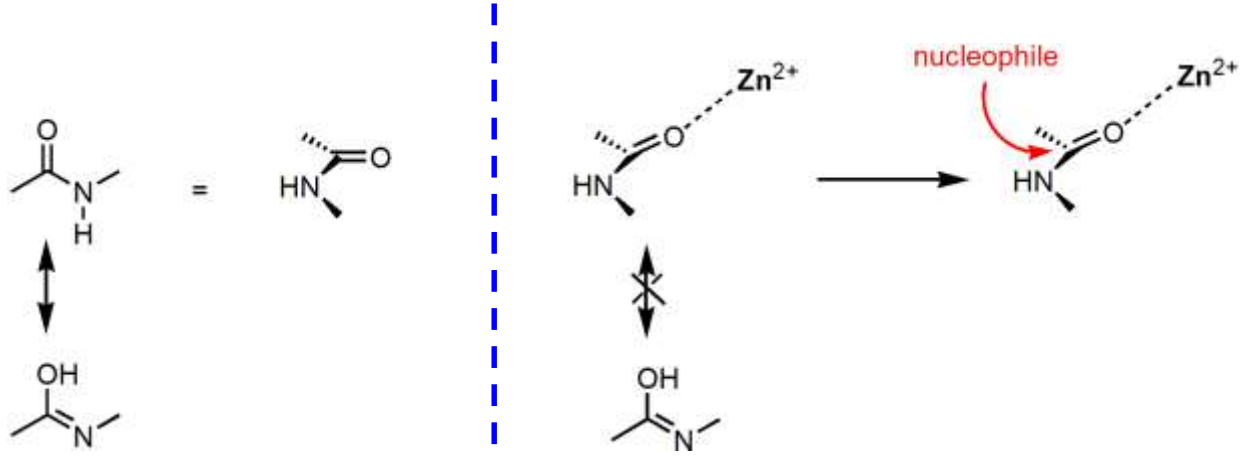
- Hydrated F^- is a poor nucleophile
- Binding of substrate in active site releases all water molecules and turns F^- into strong nucleophile

Destabilization of a bound substrate

Stabilization of the transition state can be reached by destabilization (or: activation) of the substrate

- Interactions with certain parts of the active site may activate chemical bonds of a molecule, e.g. by bending or stretching them

Example: amide activation in carboxypeptidase

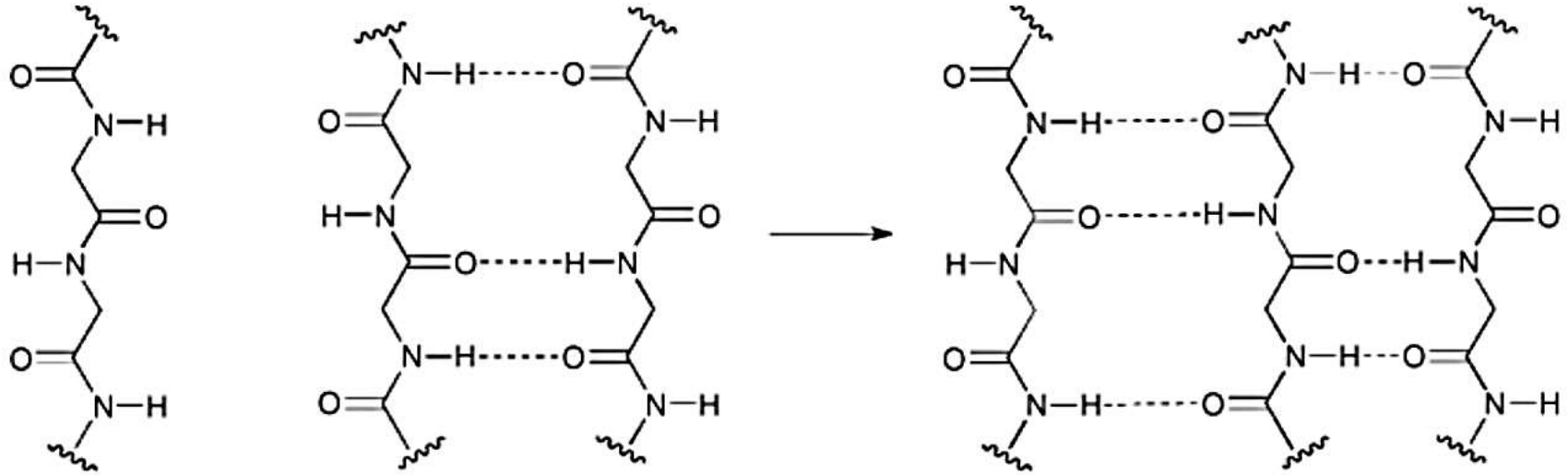


Role of enzyme protein structure on an active site

Binding of substrate via induced fit may effect protein structure far from the active site

- Remote amino acid fragments may bend towards active site and even assist in reaction
- Remote amino acid fragments may stabilize transition state of reaction in active site

Example:

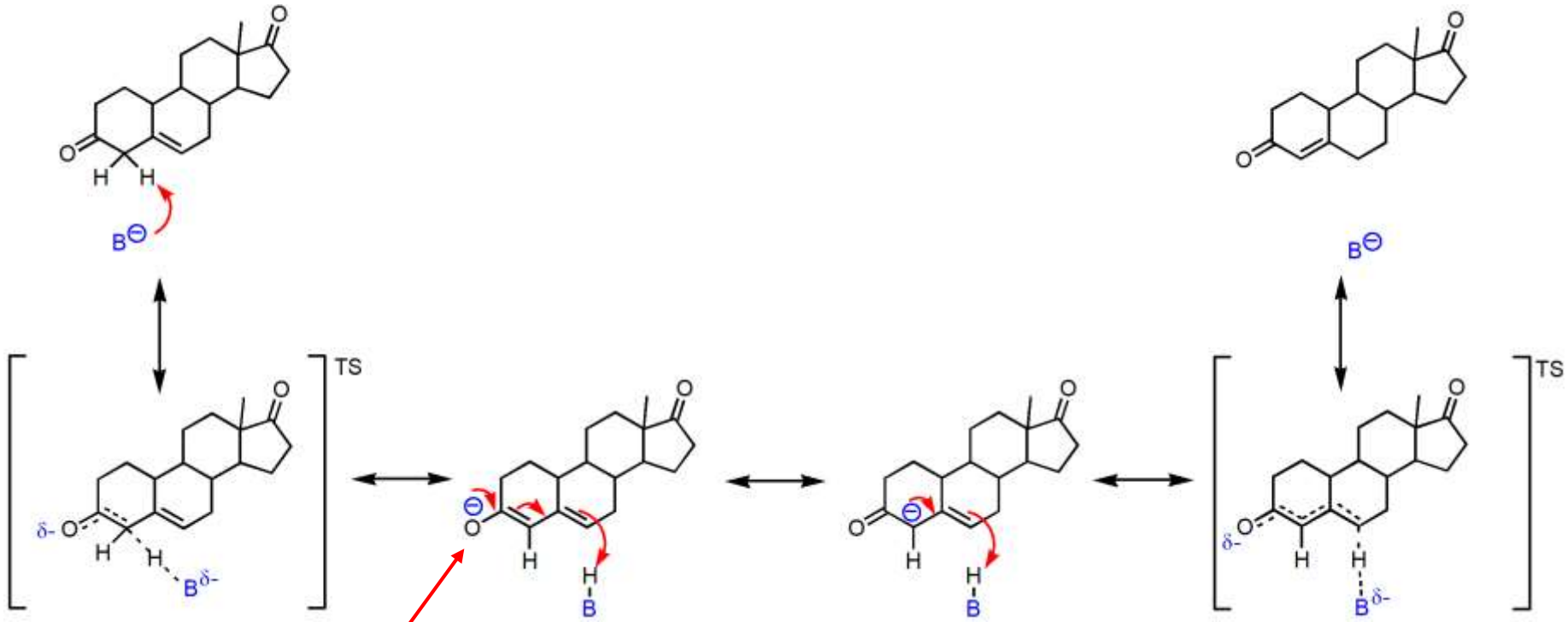


Enzyme with unoccupied active site
(which is remote from this structure)

Enzyme with transition state arrangement in active site; H-bonds stabilize protein structure

Charge stabilization

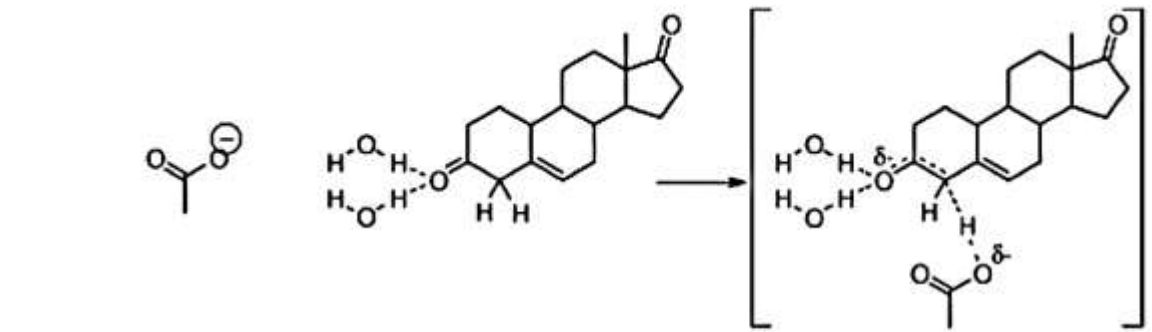
Base-catalyzed isomerization of a carbon-carbon double bond



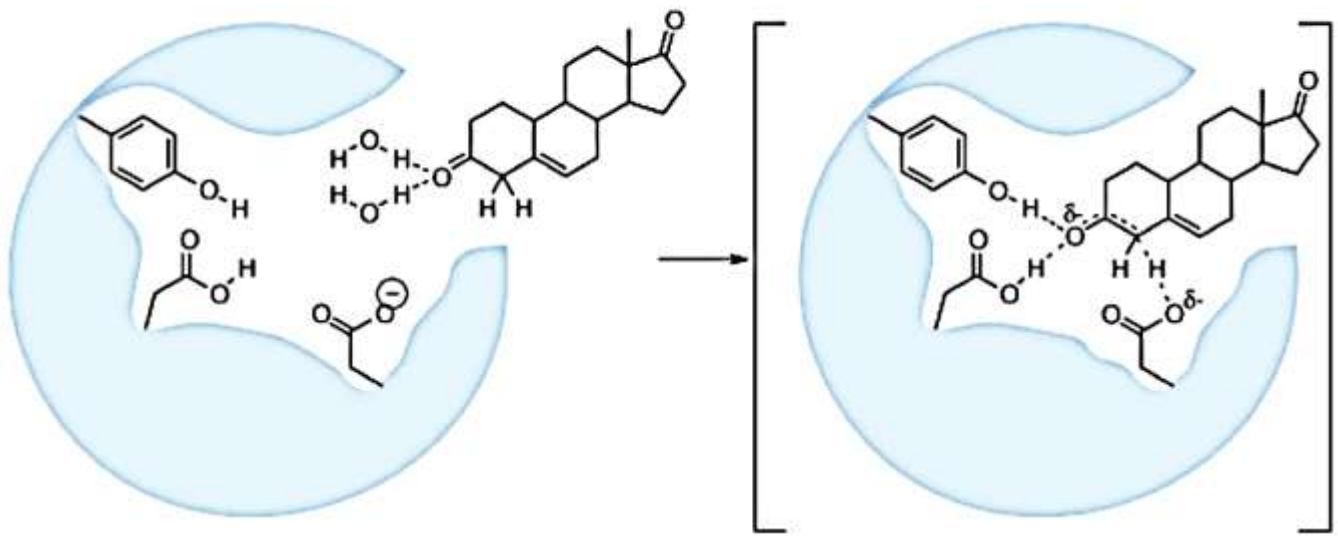
If B^- = CH_3COO^- in H_2O: k_{rel} = 1

Charge stabilization

Isomerization of a carbon-carbon double bond catalyzed by the enzyme *ketosteroid isomerase*



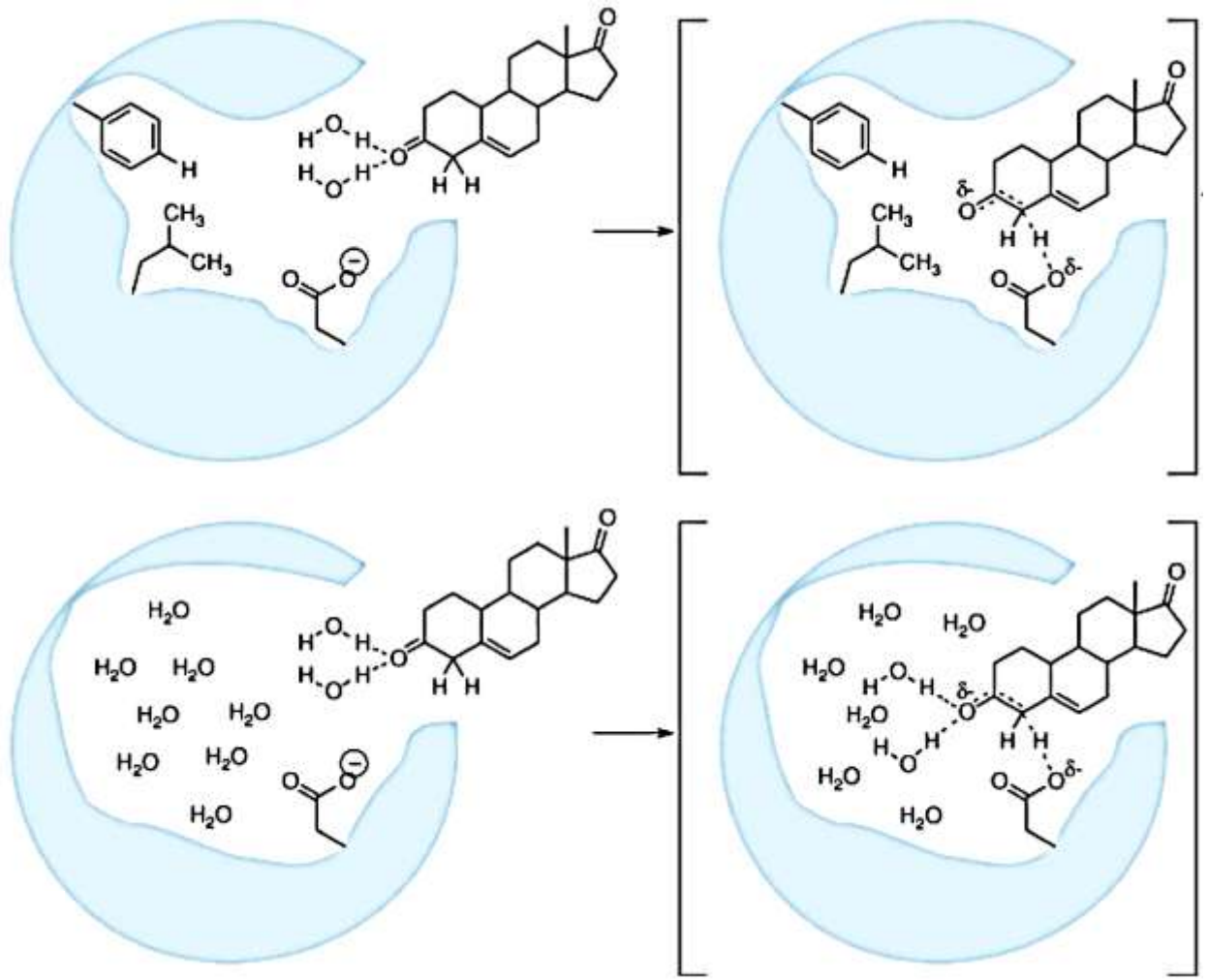
$k_{rel} = 1$



$k_{rel} \approx 10^{12}$

Charge stabilization

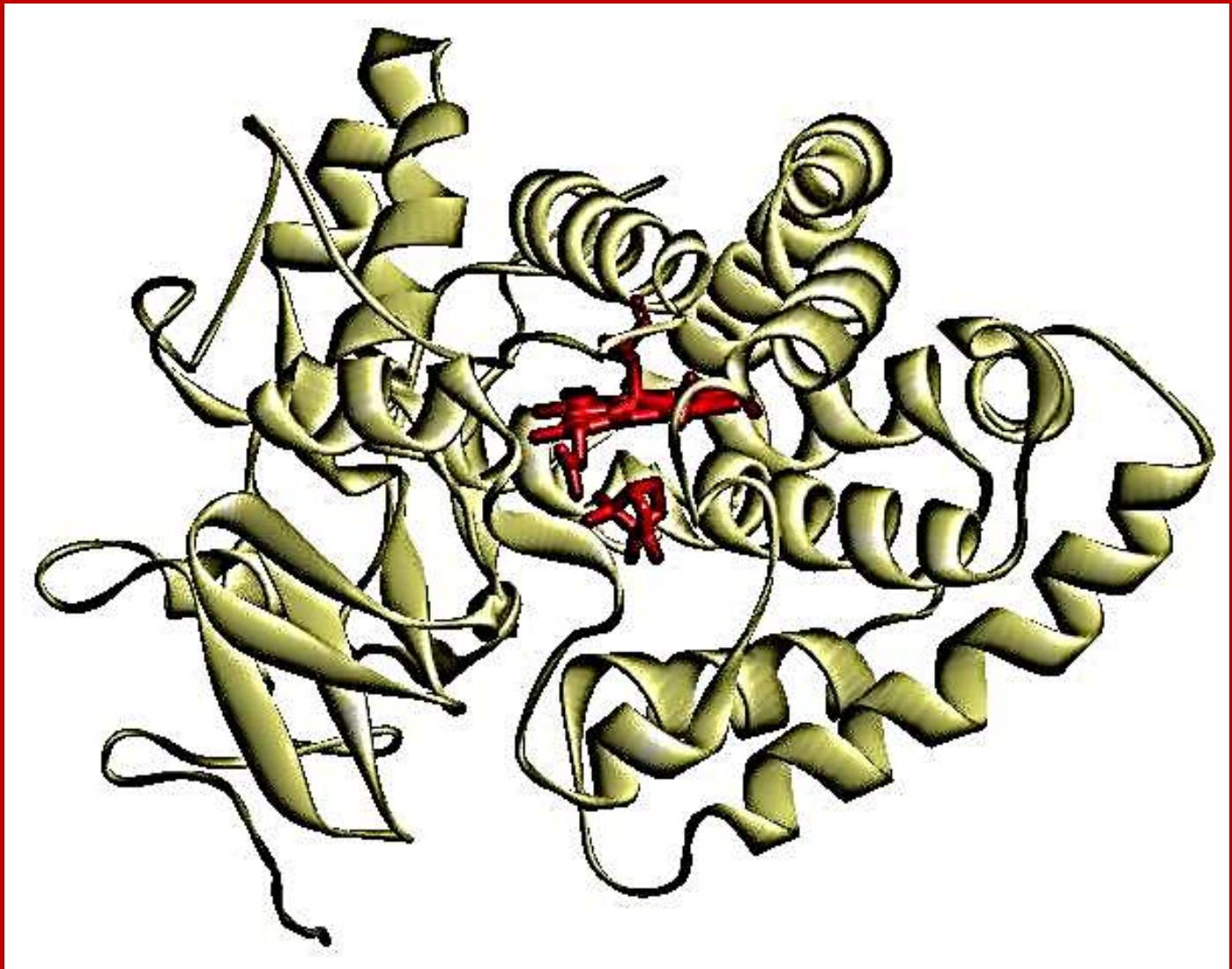
Isomerization of a carbon-carbon double bond catalyzed by the enzyme *ketosteroid isomerase*



$k_{rel} \approx 10^4 - 10^7$

$k_{rel} \approx 10^9$

Some enzymes and their mechanisms

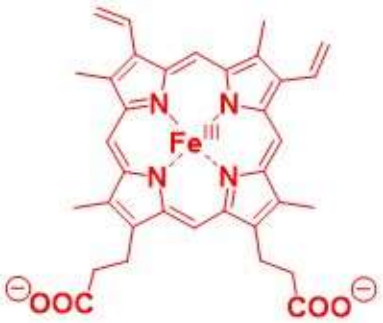


Peroxidases

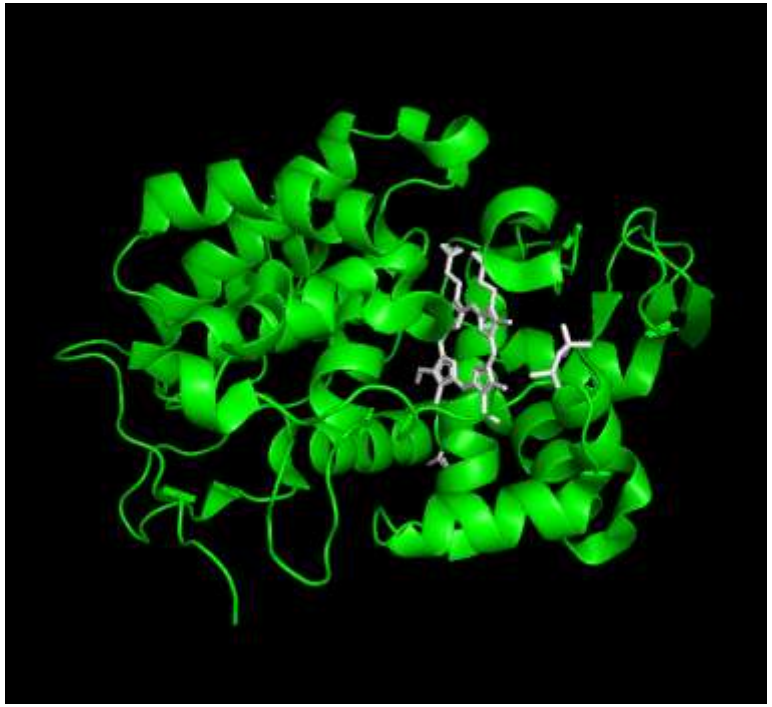
Enzymes that catalyze the reduction of hydrogen peroxide

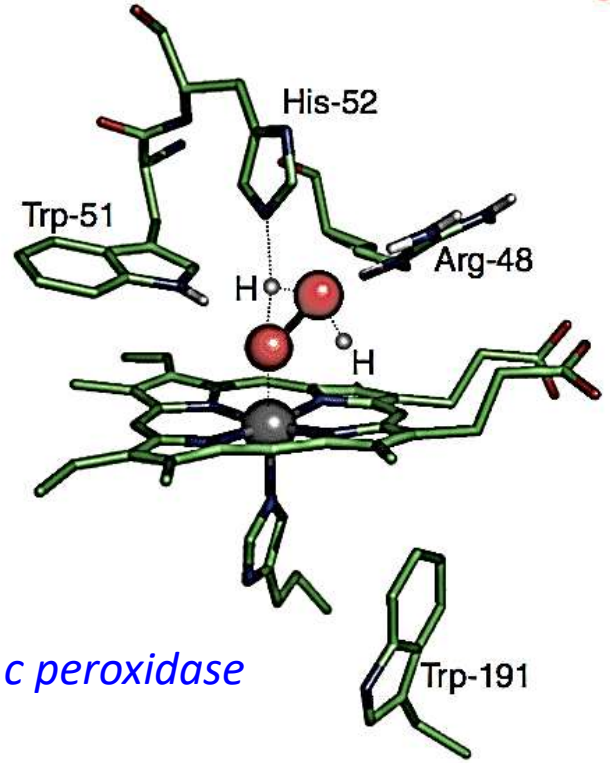
$$H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O$$

- Main players: horseradish peroxidase (HRP), cytochrome c peroxidase (CcP) and catalase
- Active sites contain heme (iron porphyrin)



Heme

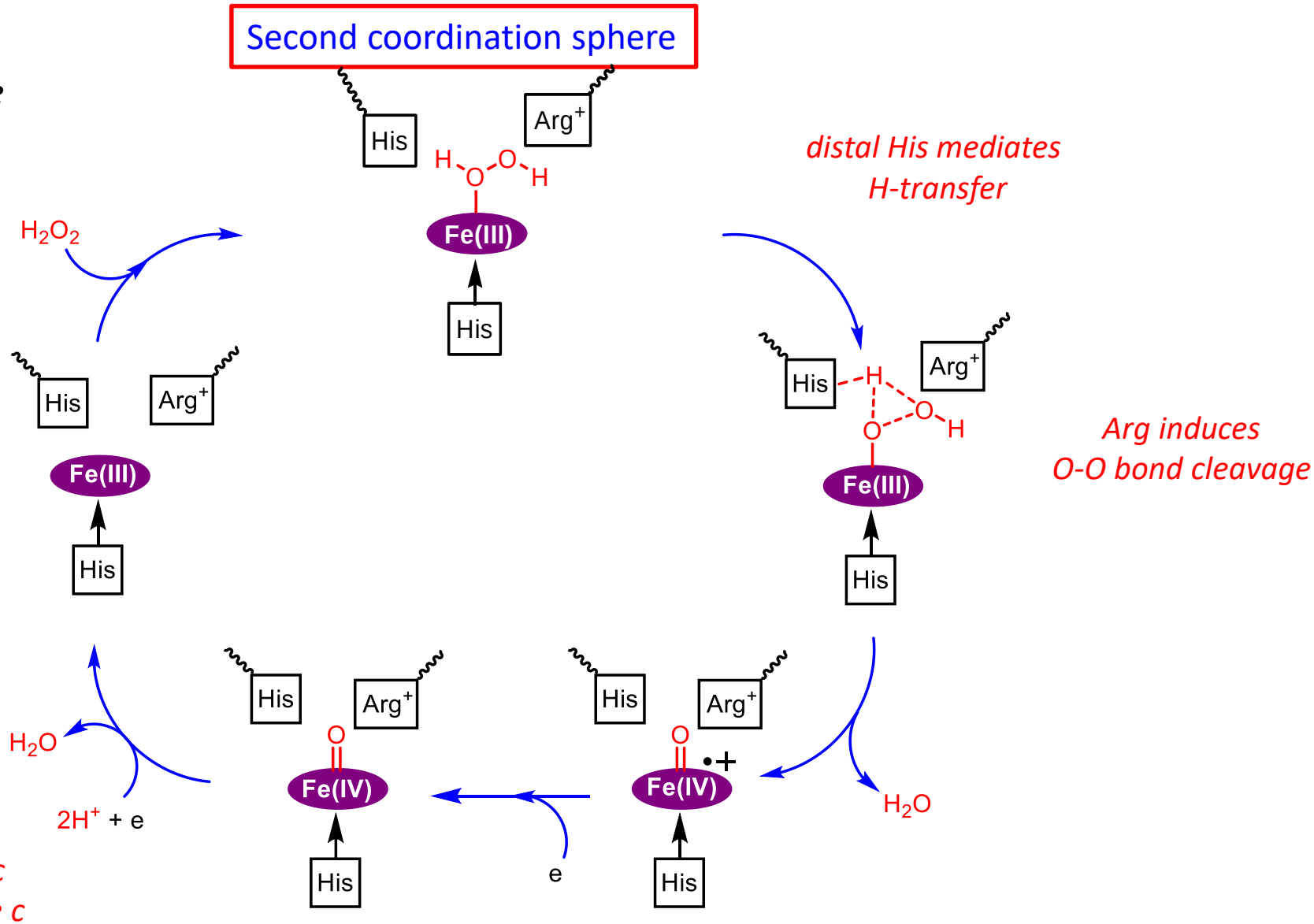




cytochrome c peroxidase

Cytochrome c peroxidase

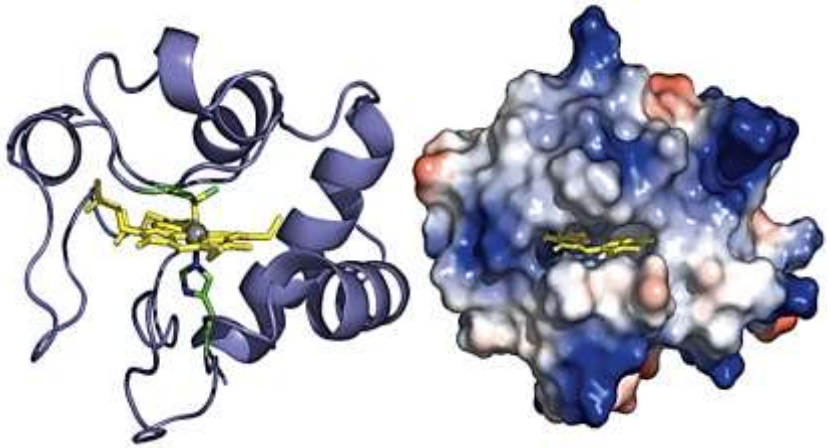
Catalytic cycle



electrons supplied by organic compounds or by cytrochrome c

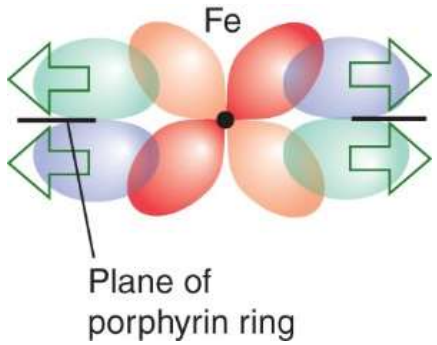
Electron supply

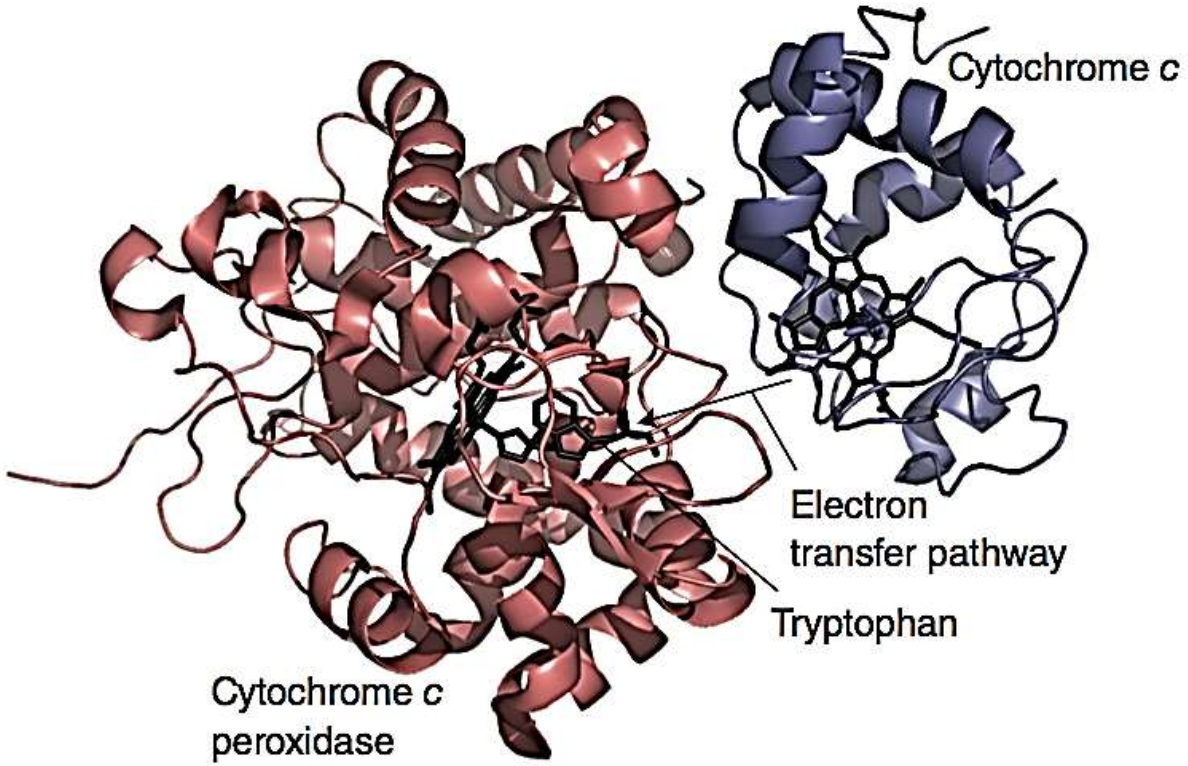
Example: supply by cytochrome C



Pattern of negative (red) and positive (blue) charges on protein surface recognized by redox partners

- Efficient outer sphere electron transfer because metal d-orbitals effectively extend to the edge of the porphyrin





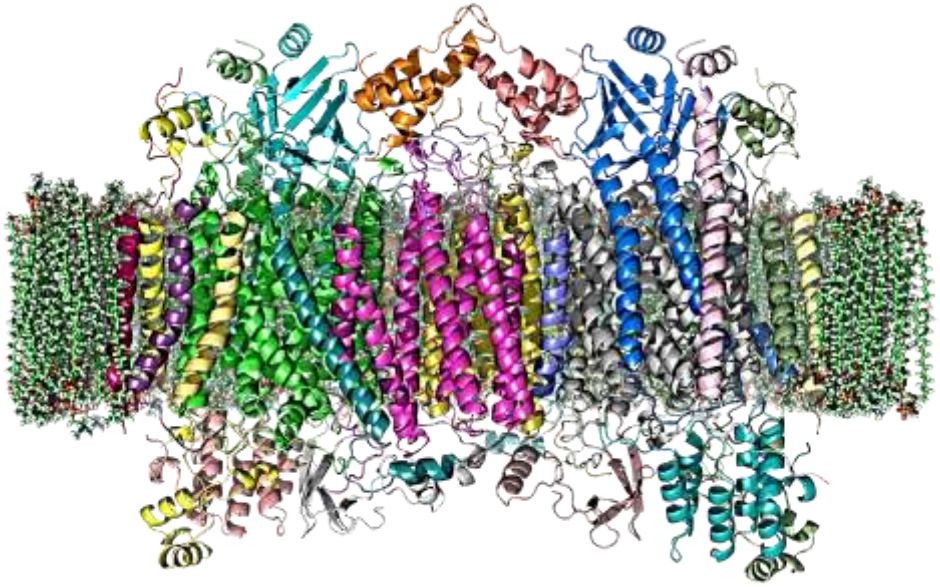
- Outer sphere electron transfer (electron tunneling) between the enzyme and the cofactor protein
- Docking expels water molecules and favors electron transfer

Oxidases

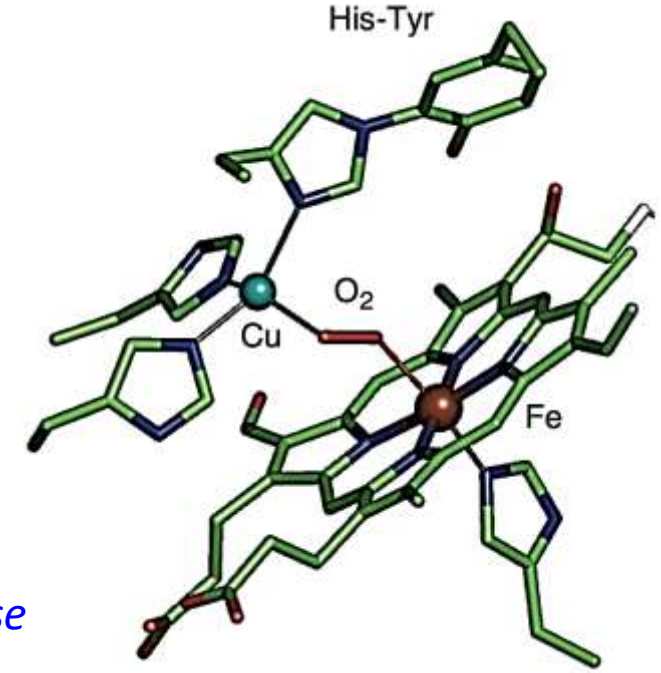
Membrane-bound enzymes that catalyze the reduction of oxygen

$$O_2 + 8H^+ (\text{inside}) + 4e \rightarrow 2H_2O + 4H^+ (\text{outside})$$

- Main player: cytochrome c oxidase
- Active site contains a dinuclear heme (iron porphyrin) / copper-His_3 complex
- Acts as catalyst *and* proton pump

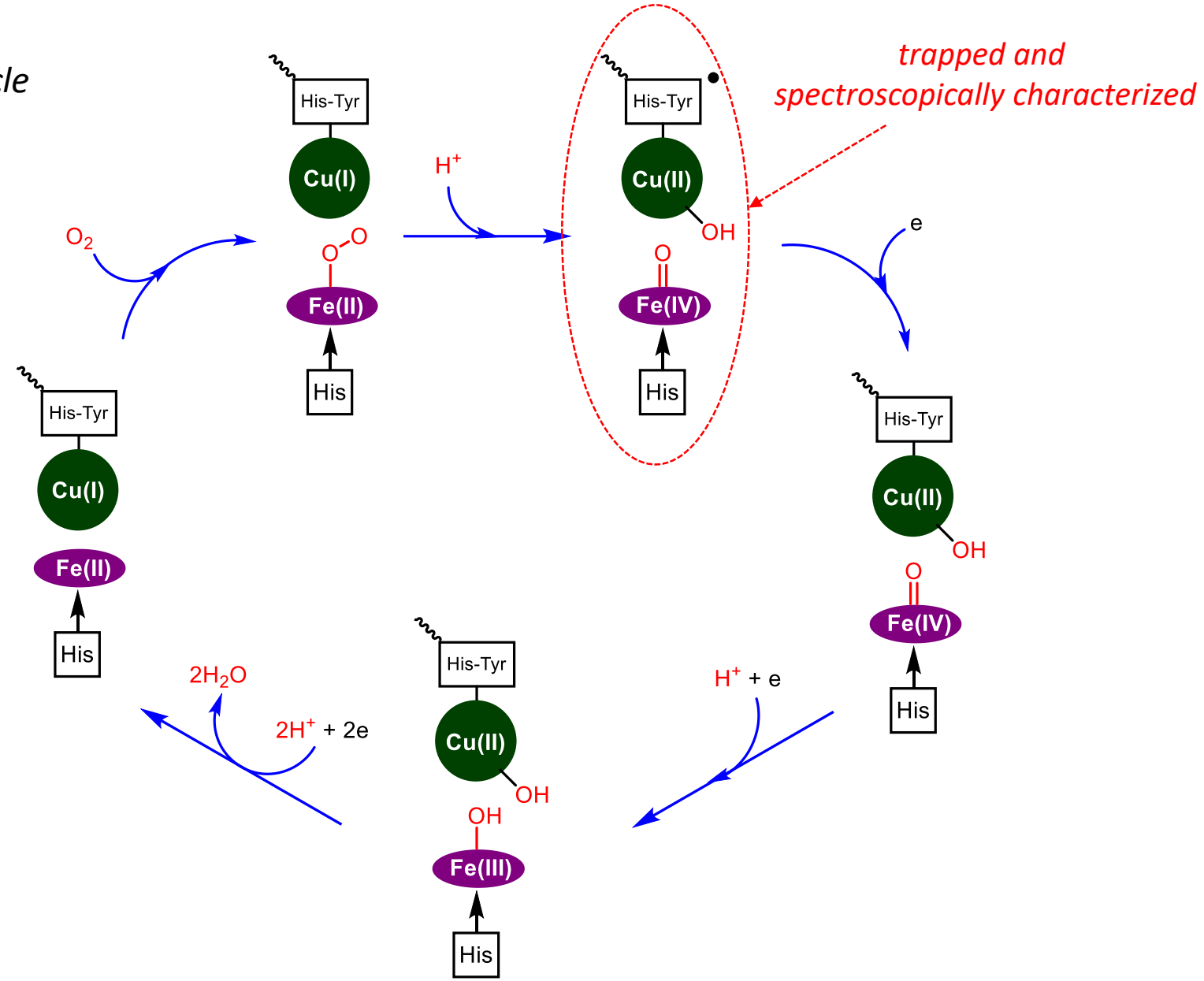


cytochrome c oxidase



Cytochrome c oxidase

Catalytic cycle



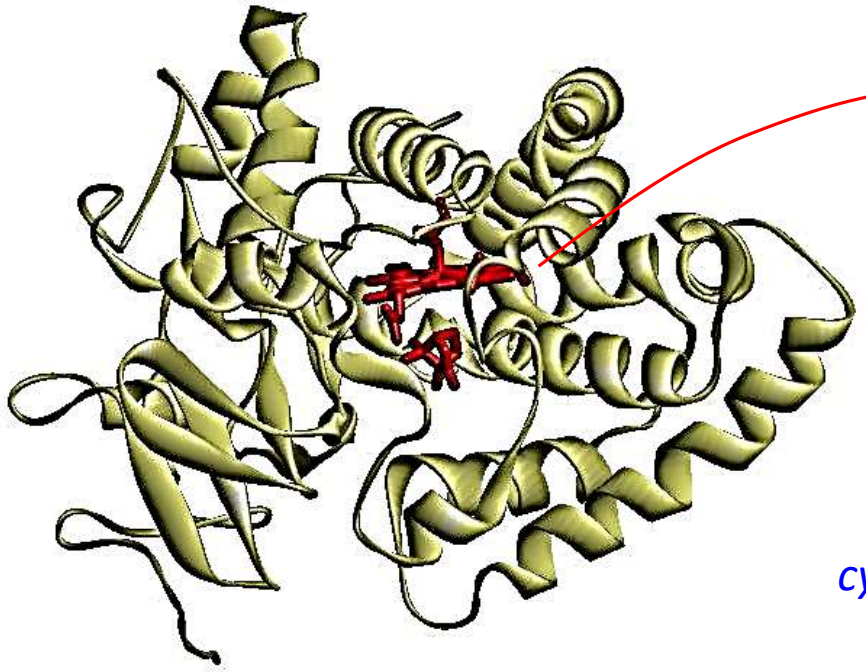
Oxygenases

Enzymes that catalyze the insertion of oxygen atoms into organic substrates

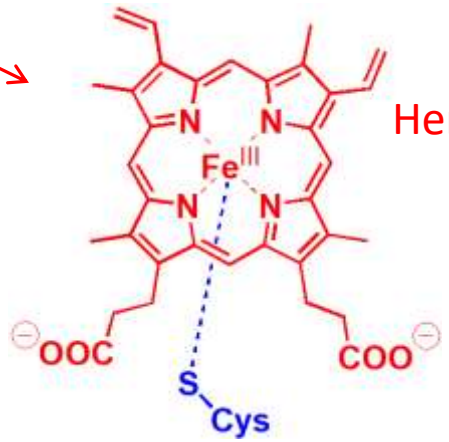
- Monooxygenases insert one O-atom of O_2, dioxygenases insert both O-atoms

$$R\text{-}H + O_2 + 2H^+ + 2e \rightarrow R\text{-}OH + H_2O$$

- Monooxygenases also catalyze epoxidation of alkenes
- Main players: cytochrome P450, methane monooxygenase



cytochrome P450



Heme cofactor