CATALYSIS

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SUSTC

Enzymes accelerate the rate of a chemical reaction without changing its overall equilibrium

- Kinetics
  - Reaction rate
- Thermodynamics
  - Gibbs free energy
- Equilibrium
  - Equilibrium constant (*K*)
  - $\Delta G = -RT\ln K$
  - $k_{forward}/k_{reverse}$
    - Increase both reaction rates

Comparison of Uncatalyzed and Catalyzed Rates for Some Enzymatic Reactions

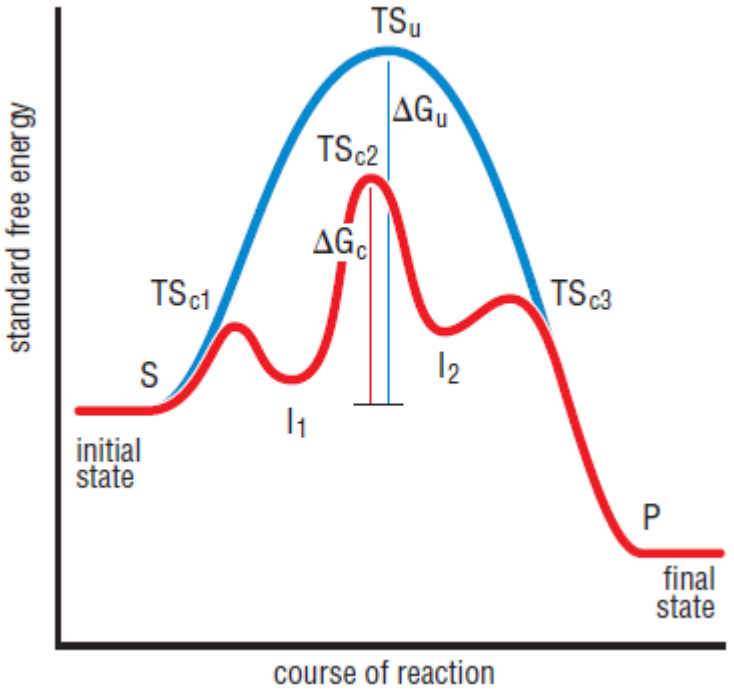
Enzyme	Nonenzymatic rate k_{non} (s^{-1})	Enzymatic rate k_{cat} (s^{-1})	Rate acceleration k_{cat}/k_{non}
Cyclophilin	2.8×10^{-2}	1.3×10^{4}	4.6×10^{5}
Carbonic anhydrase	1.3×10^{-1}	10^{6}	7.7×10^{6}
Chymotrypsin	4×10^{-9}	4×10^{-2}	10^{7}
Triosephosphate isomerase	6×10^{-7}	2×10^{3}	3×10^{9}
Fumarase	2×10^{-8}	2×10^{3}	10^{11}
Adenosine deaminase	1.8×10^{10}	370	2.1×10^{12}
Urease	3×10^{-10}	3×10^{4}	10^{14}
Alkaline phosphatase	10^{-15}	10^{2}	10^{17}
ODCase	2.8×10^{-16}	39	1.4×10^{17}

Catalysis usually requires more than one factor

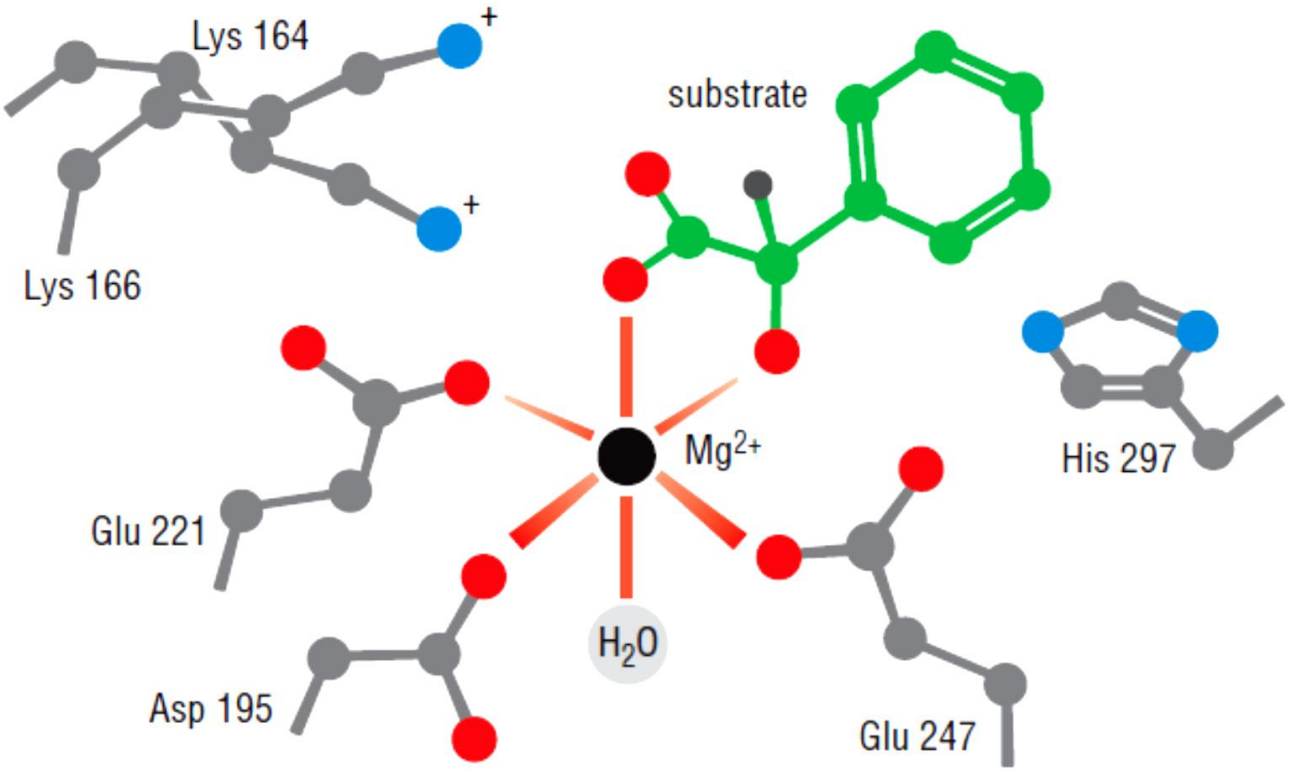
- No unique secret to enzymatic catalysis but a combination of several simple contributory factors
  - Particular combination vary from enzyme to enzyme
- Physical factors
  - Physical properties of enzyme structure
  - The ability to orient the ligand precisely relative to catalytic residues in the active site
- Chemical factors
  - Chemical properties of residues and/or cofactors
  - The abilities to
    - stabilize unstable chemical species by weak interactions
    - polarize bonds
    - form covalent adducts

Reducing activation-energy barrier by catalysis

- Activation energy
  - The energy required to overcome the free energy barrier (activation-energy barrier) for a chemical reaction
  - The higher this barrier, the slower the reaction
  - Ground state
    - Substrate bound state
- Transition state
  - The top of the activation-energy barrier
  - Chemical species that exists for about the time required for a single atomic vibration to occur (~10^{-15} s)
  - Lowering the free energy of the transition state to lower the barrier
- Intermediate
  - A different path to cause the reaction by adding more free-energy “hills”
    - Each hill has much lower barrier
  - Local “valleys”
  - Metastable molecules



Active-site geometry



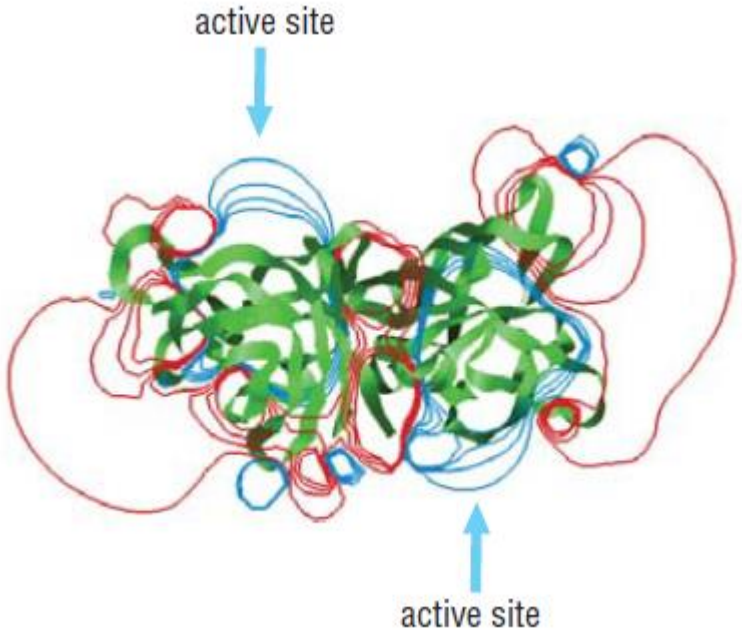
The active site of mandelate racemase

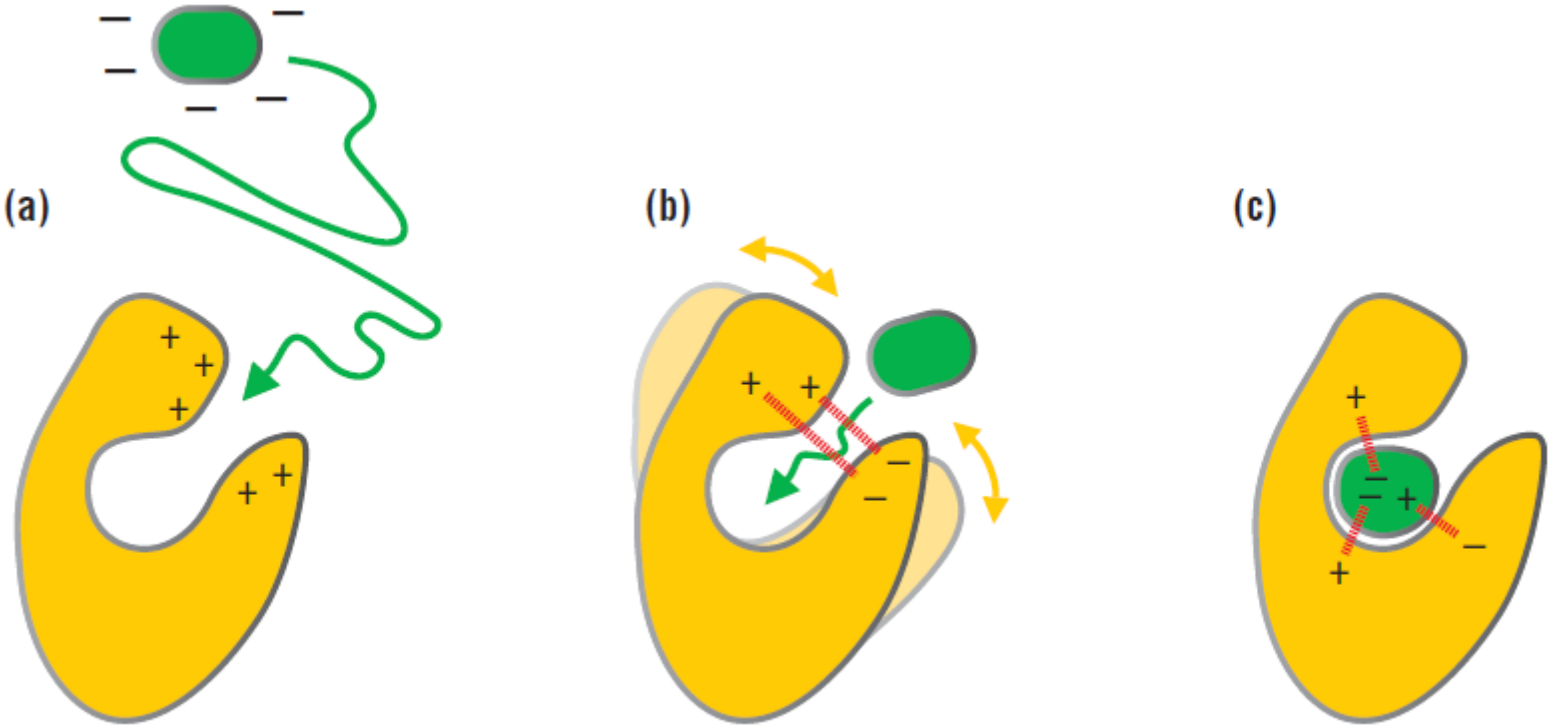
The enzyme/substrate interaction

- The interaction between substrate and the active site of an enzyme
  - The formation of an enzyme–substrate complex is the first step in enzymatic reaction
  - Usually noncovalent
  - Specificity is determined by
    - the close fit of the substrate within the active-site pocket
    - van der Waals interactions between nonpolar groups combined with complementary arrangements of polar and charged groups
- The binding affinity between substrate/product and enzyme
  - K_d is ~10^{-3} M to 10^{-9} M
  - Too high binding affinities reduce catalytic efficiency

The enzyme/substrate interaction

- Most biological molecules are charged
- The binding of substrate to the enzyme is guided by polar and charged groups
  - Electrostatic potential on the surface



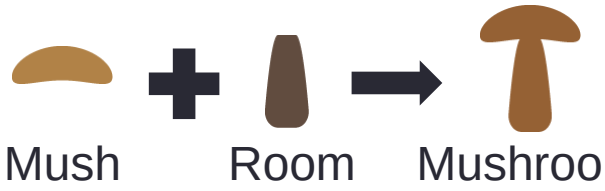


Gated binding

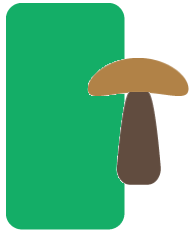
The different ways in which electrostatic interactions can influence the binding of a ligand to a protein

Reactive groups in enzyme active sites

- Reactive groups in enzyme active sites are optimally positioned to interact with the substrate
  - Folding energy pay the cost of positioning the groups
- Residues of active site increase the probability of productive collisions between two reacting molecules
  - Two molecules must collide reactive side-to-reactive side

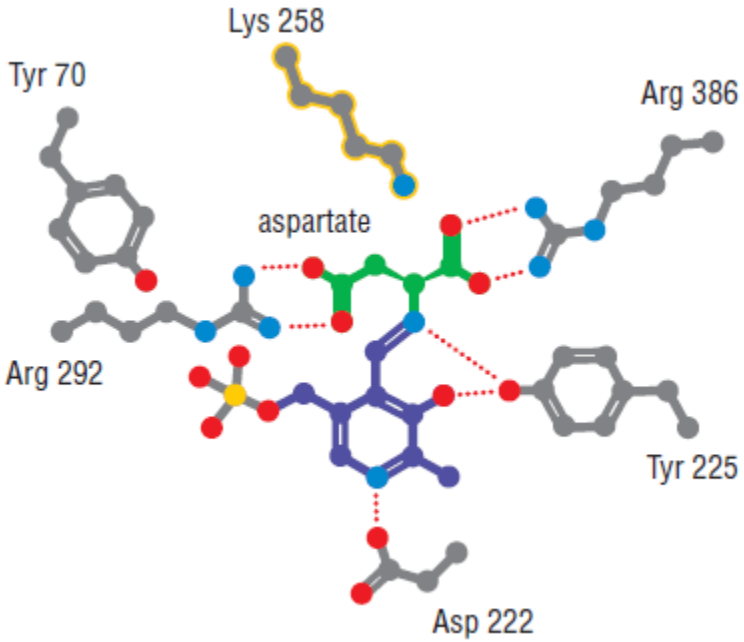


In free solution



Orientated by enzyme

Functional roles of active site



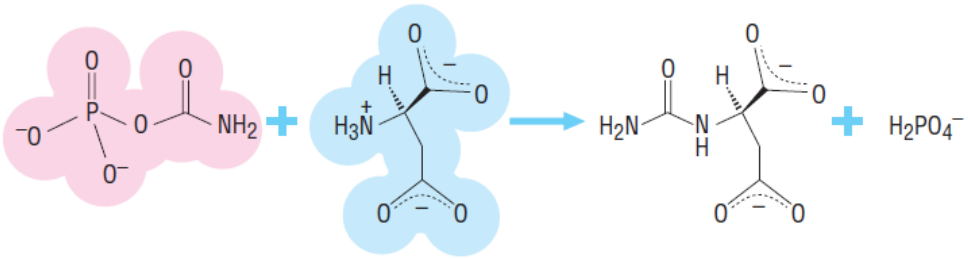
The active site of *E. coli* aspartate aminotransferase. Mutation of arginine 292 to aspartic acid produces an enzyme that prefers arginine to aspartate as a substrate.

- Enzyme active sites consist of a specificity sub-site and a reaction sub-site
- Specificity sub-site
  - Using polar and nonpolar groups to make weak interactions with the substrate
- Reaction sub-site
  - carry out the chemistry
- Design new catalysts in medicine and industry

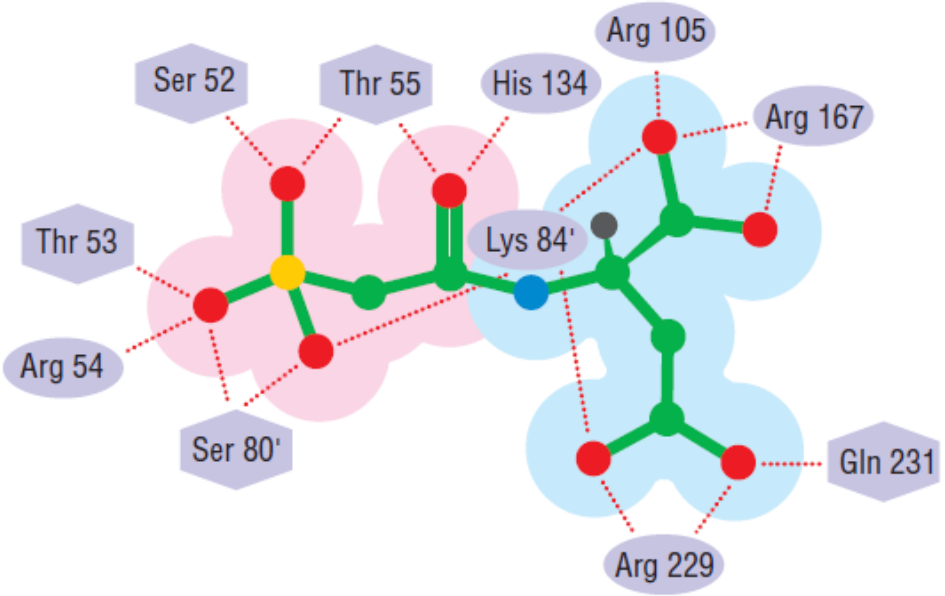
Promoting proximity

- Proximity effect
- Similar reactions will occur far faster if the reaction is intra-molecular
- Orientation also counts
- More than the entropic change

Promoting proximity



Catalysis of the reaction of carbamoyl phosphate and aspartate by the enzyme aspartate transcarbamoylase depends on holding the substrates in close proximity and correct orientation in the active site

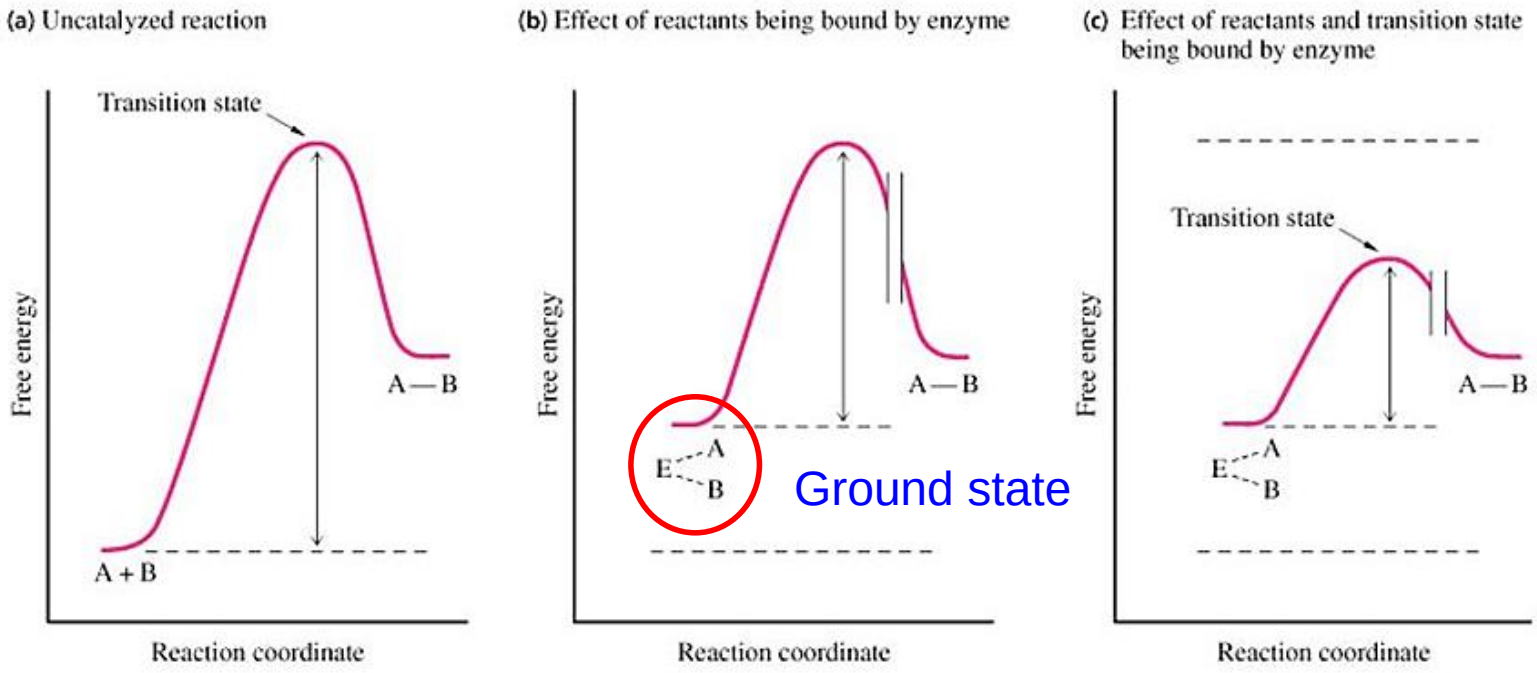


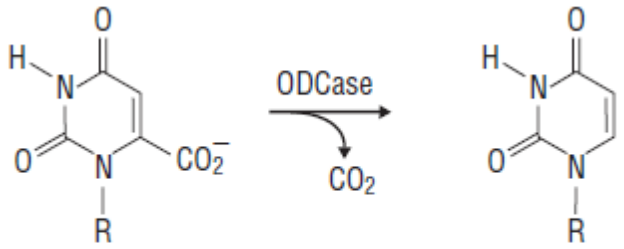
The inhibitor, PALA (*N*-phosphonoacetyl-L-aspartate), mimics the bindings of both substrates to the active site

- Binding of substrates in the correct orientation significantly contributes to the catalytic efficiency
- Multi-substrate reaction
- Holding the substrate close to each other in the proper orientation may be all that is needed to facilitate the appropriate chemistry
  - The substrate molecules are intrinsically reactive

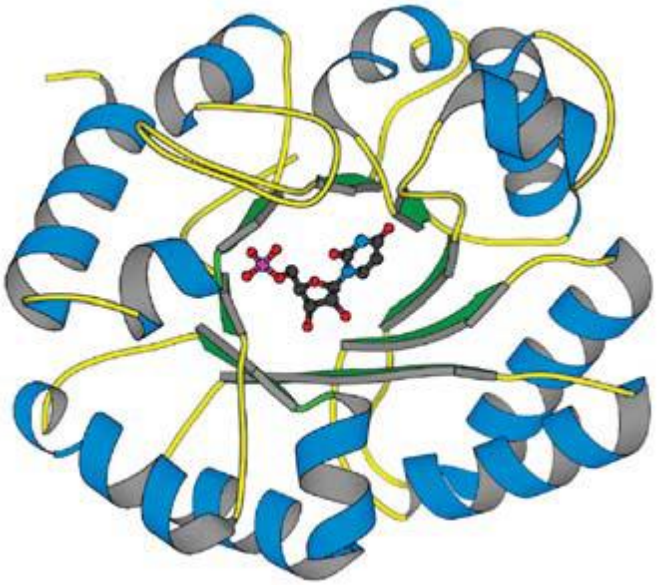
Destabilization of ground states

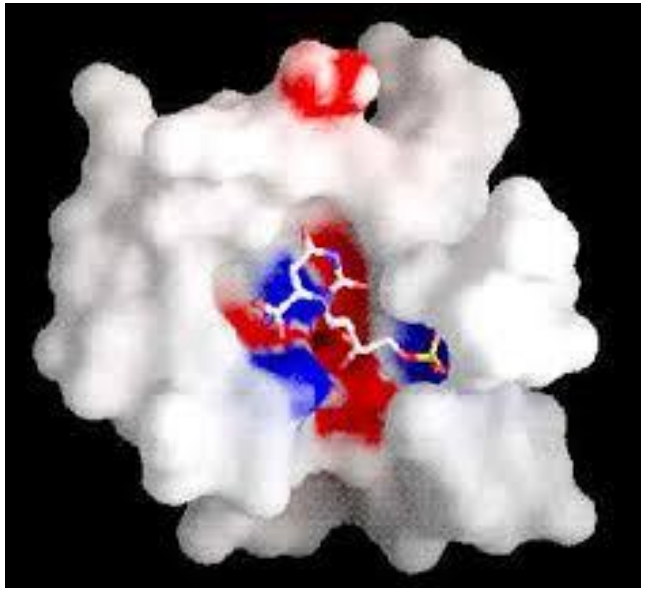
- The bound substrate is in a less stable conformation
  - Relative to standard state
- Lifting the free-energy hill towards the transition state





OMP UMP

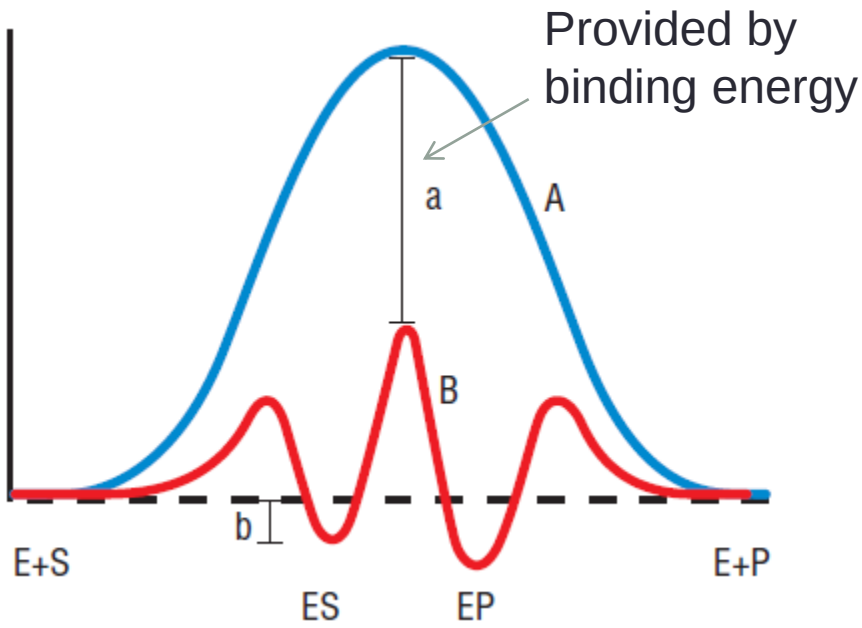




The enzyme orotidine 5’-monophosphate decarboxylase (ODCase) catalyzes the transformation of orotidine 5’-monophosphate to uridine 5’-monophosphate

Stabilization of transition states

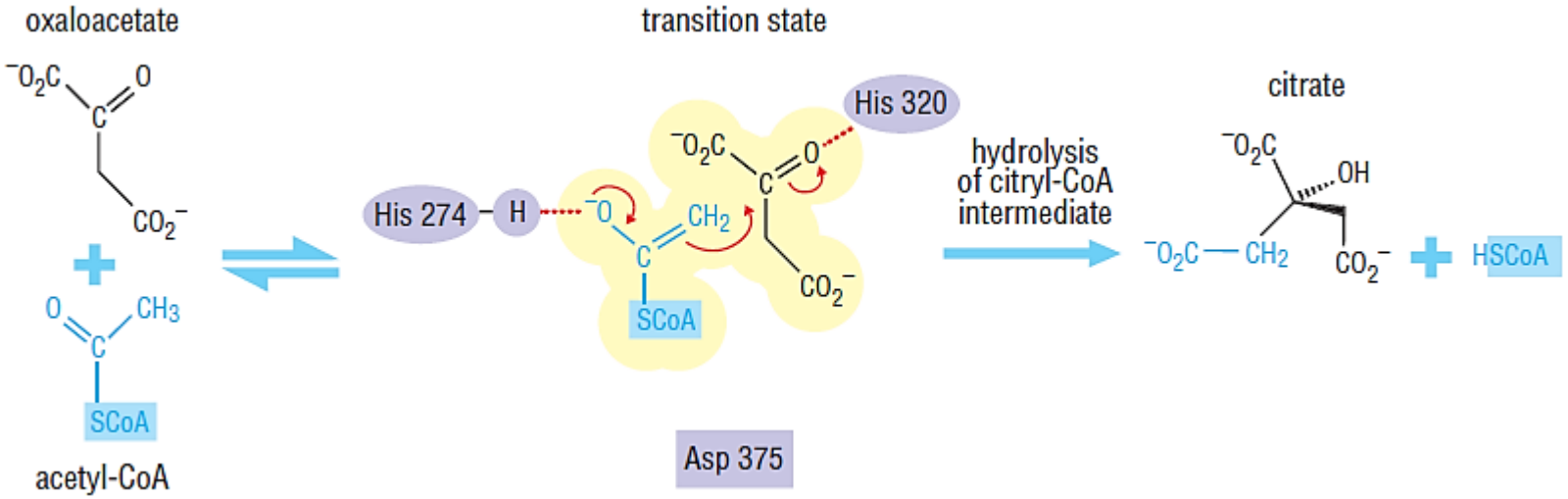
- Different binding energies between transition and ground states
  - The transition state can be bound more tightly than the ground state
- Enzyme active sites develop chemical environment favor for the transition states
  - Stereochemistry
  - Charge



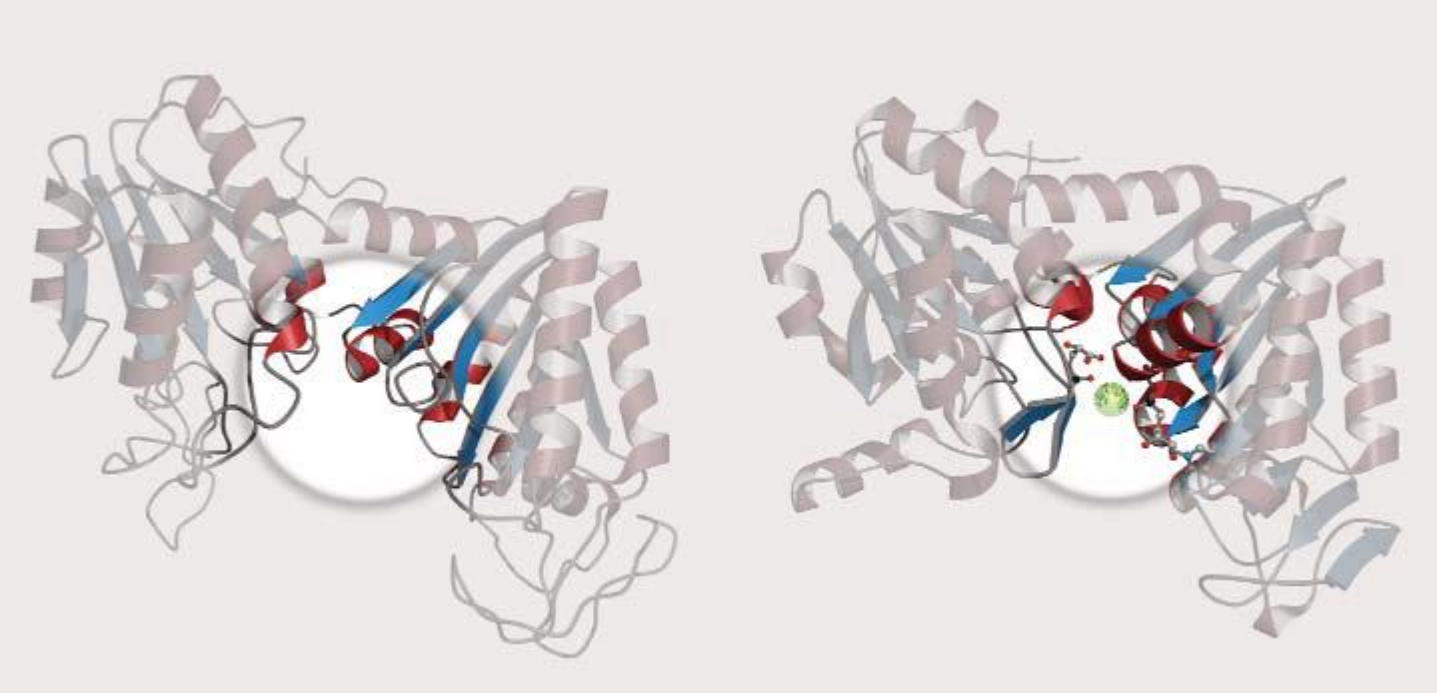
A hypothetical case in which substrate and product are of equal energy, showing the effect of binding energy on enzyme catalysis

Stabilization of transition states

- The sources of the binding energy required to reduce that free-energy difference are the weak interactions
  - Better/more weak interactions for transition states
- Steric effect (shape complementarity)
  - Active sites fit transition states sterically better



The active site of citrate synthase stabilizes a transition state with a different geometry from that of the substrate



Phosphoglycerate kinase (PGK) undergoes a conformational change in its active site after substrate binds to stabilize the transition state.

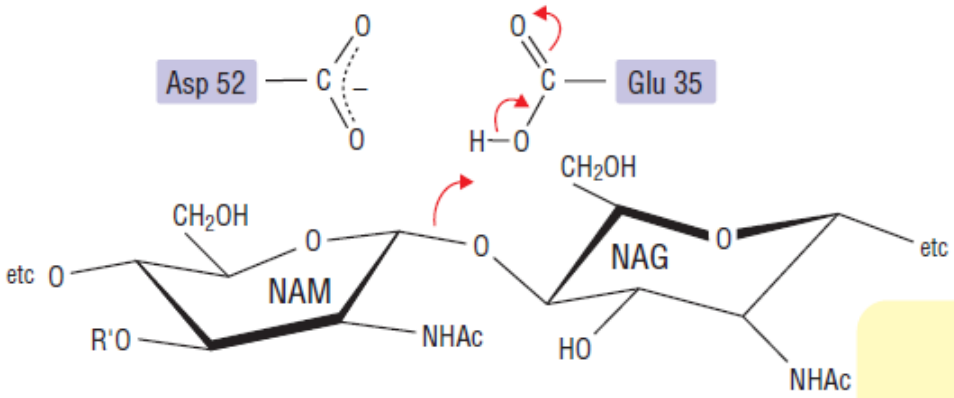
Exclusion of water

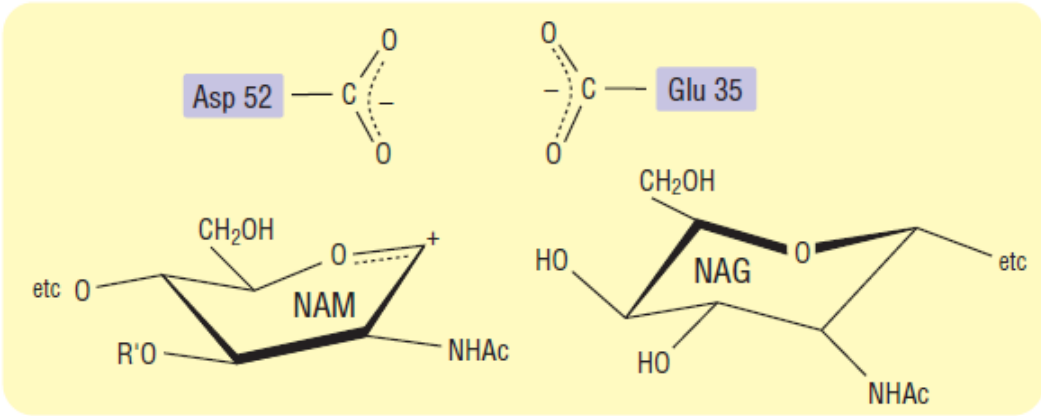
- Many enzyme active sites protect their substrates from water during catalysis
  - Active sites must be accessible to substrates
  - Enzymes stay in aqueous system

- What drives the motions?
  - The movements of the lid are driven by the thermal motions that apply to all molecules in solution
- What makes the lid swing open once a reaction ends?
  - Not clear
- How fast does the lid move?
  - Not very fast, a few Å every minisecond

Active-site chemistry

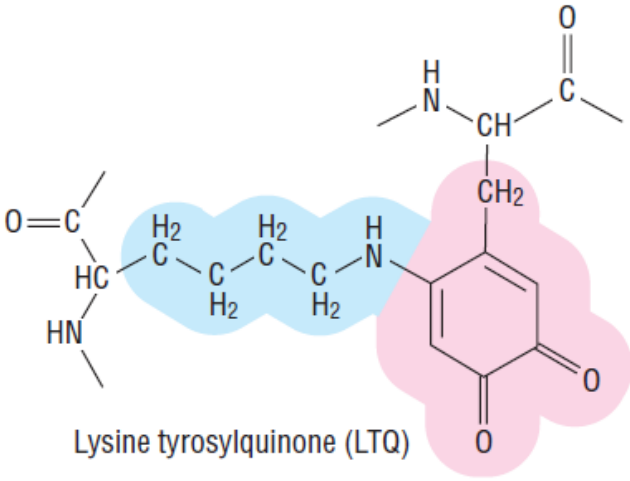
- Active sites promote acid-base catalysis
  - Proton transfer
- pKa of catalytic groups at active sites
  - The ability to donate or accept protons



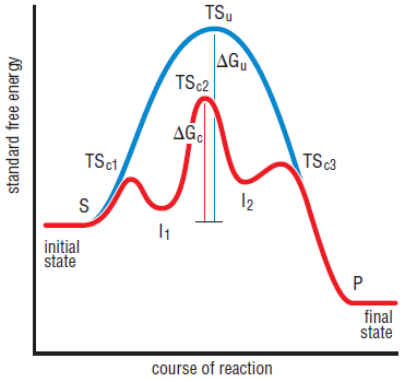


Cofactors

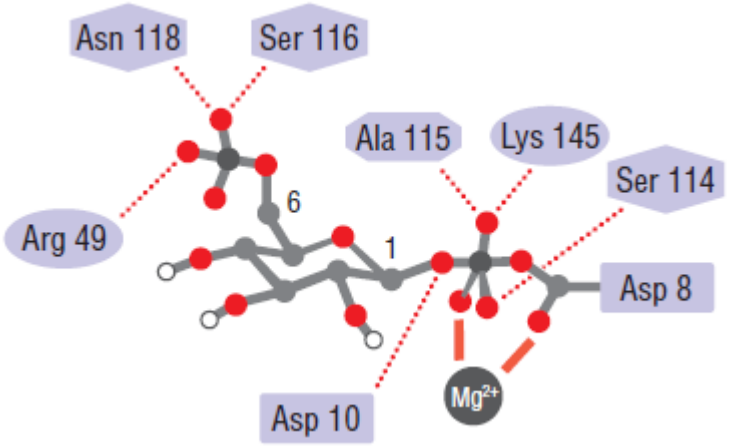
- Many active sites use cofactors to assist catalysis
  - Non-amino-acid cofactors
  - Providing chemical species that are not found in amino-acid sidechains
  - Allowing specialized chemical functions
- Coenzymes
  - Cofactors that are organic compounds and assist catalysis
  - CoA, Heme, Vitamin, …
- Metal-ion cofactors
  - Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, K^{+}, …
- Modified amino-acid chains



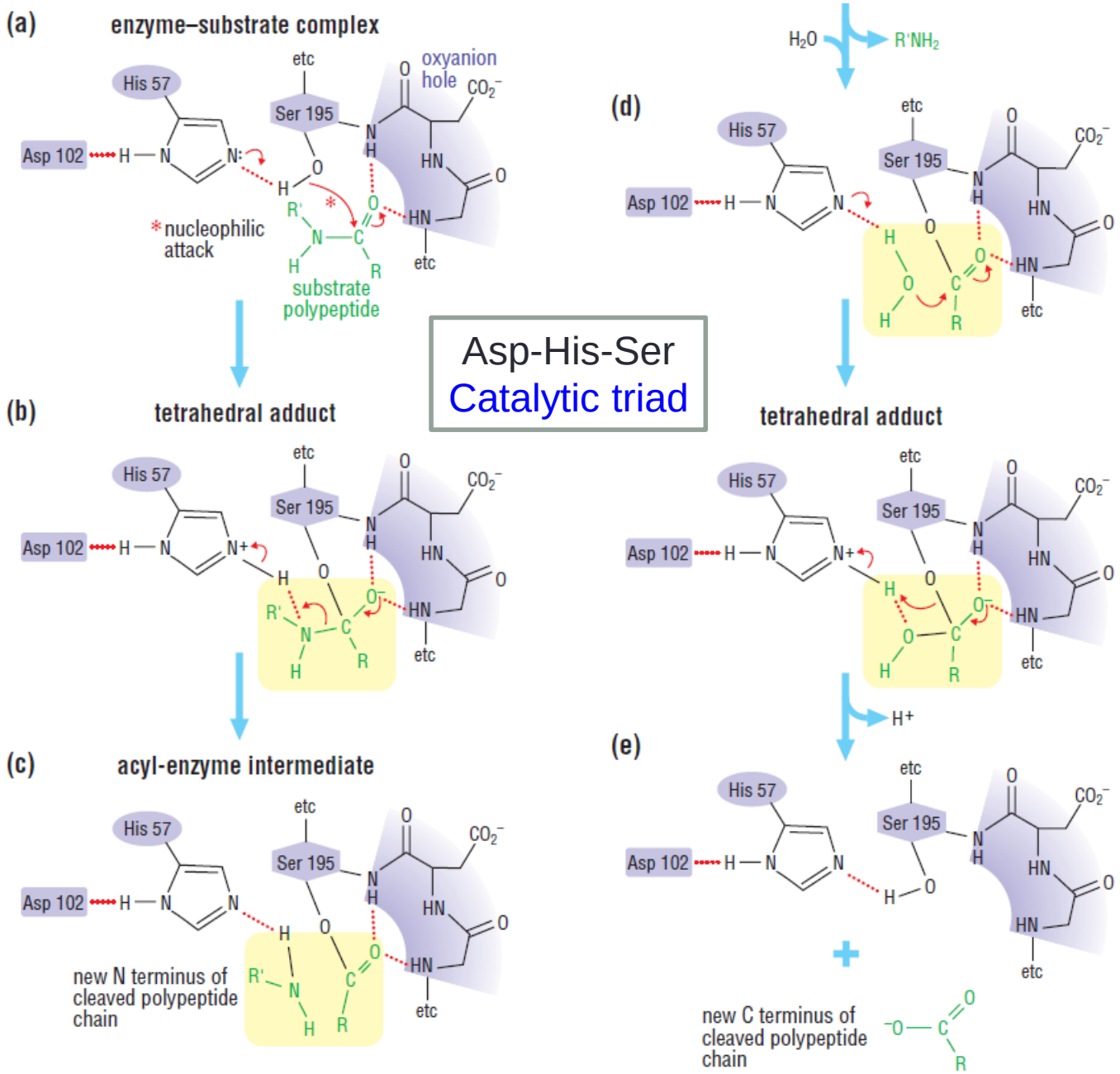
Multi-step reactions



- Some active sites employ multi-step mechanisms
- The reaction is broken up into a number of steps
- Each step has a lower-energy transition state
- Each step "product" is a relatively unstable reaction intermediate
- Kinases and phosphatases use two-step strategy in phosphoryl-group transfer
  - A catalytic residue mediate the phosphoryl-group transfer by forming a covalent bonded intermediate



The phosphoenzyme–substrate intermediate in the active site of betaphosphoglucomutase

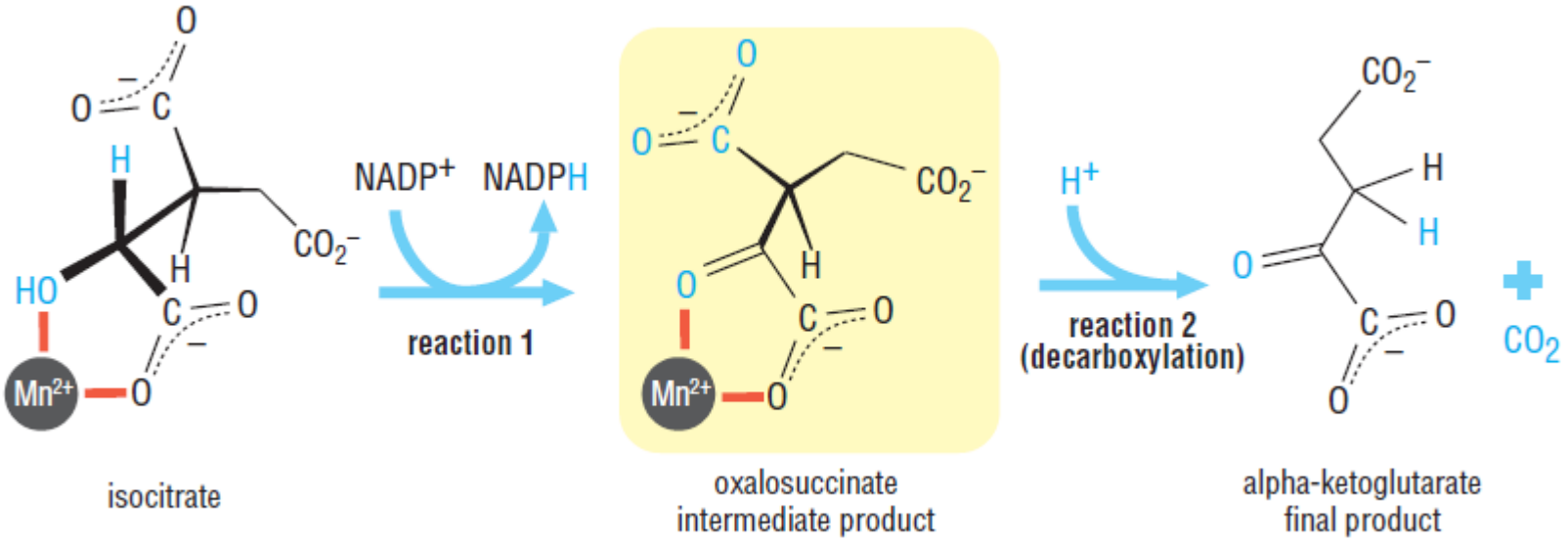


Asp-His-Ser
Catalytic triad

Peptide hydrolysis catalyzed by the serine protease chymotrypsin

Multifunctional enzymes

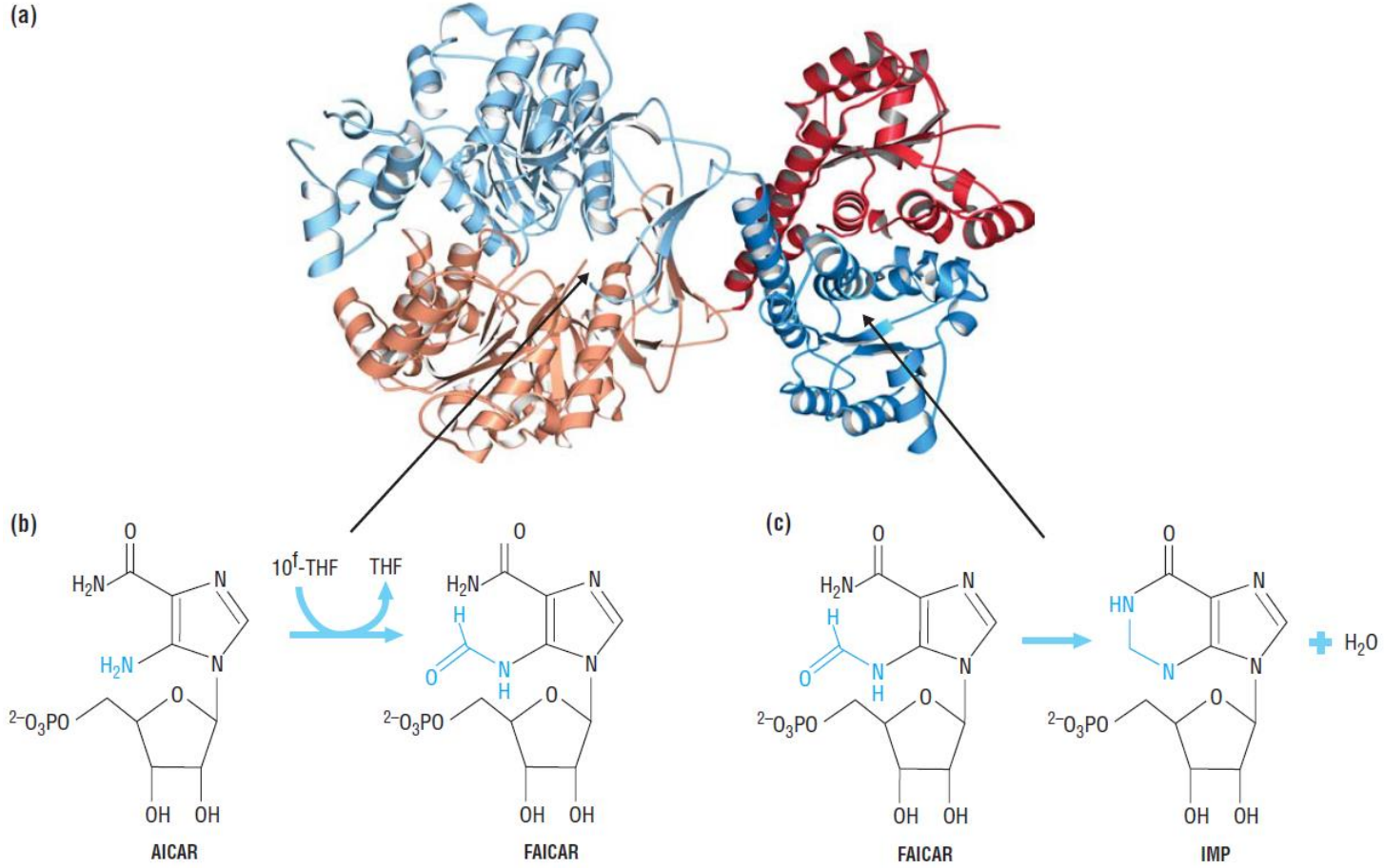
- Some enzymes can catalyze more than one reaction
  - Bifunctional or multifunctional (more than two reactions) enzymes
- Some bifunctional enzymes have only one active site



The reaction catalyzed by isocitrate dehydrogenase

Multifunctional enzymes

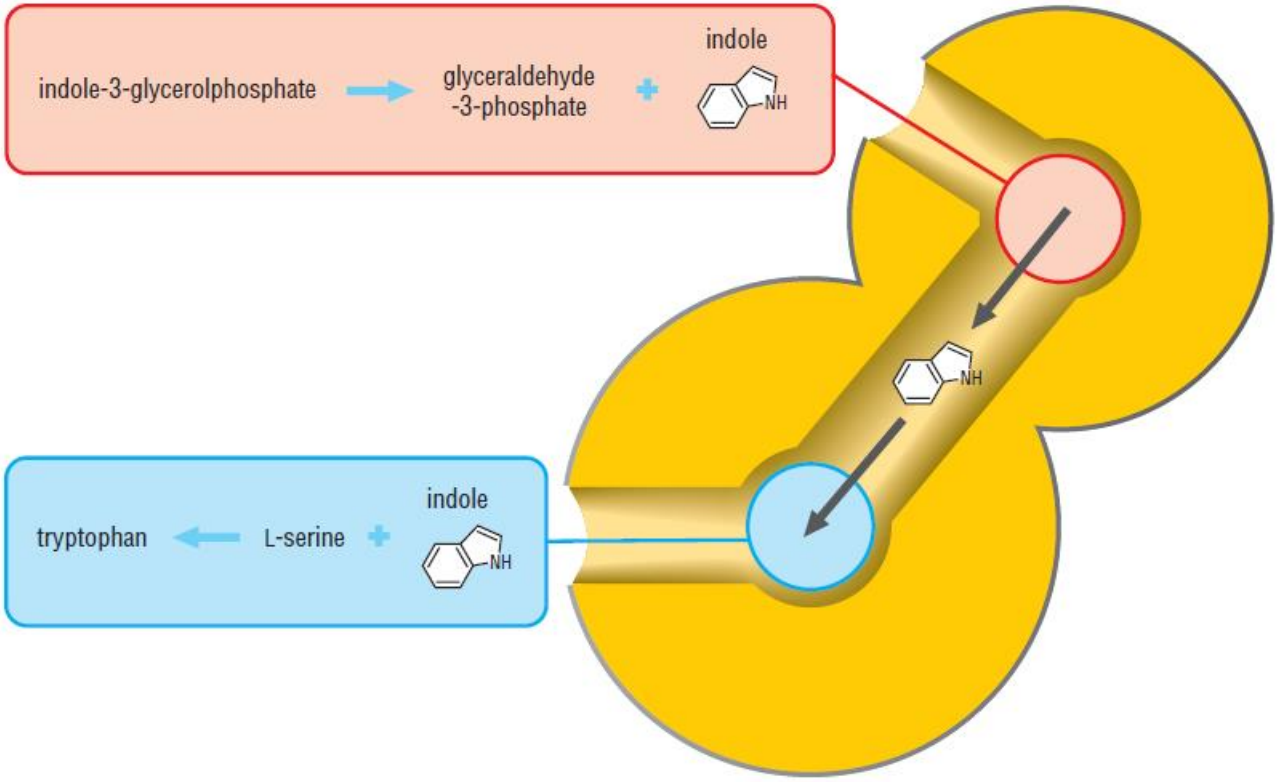
- Some bifunctional enzymes contain two active sites



AICAR transformylase-IMP cyclohydrolase

Multifunctional enzymes

- Some bifunctional enzymes shuttle unstable intermediates through a tunnel connecting the active sites



The two active sites of the bifunctional enzyme tryptophan synthase are linked by an internal channel

Three consecutive reactions are catalyzed by the three active sites of carbamoyl phosphate synthetase, shuttling intermediates over huge distances