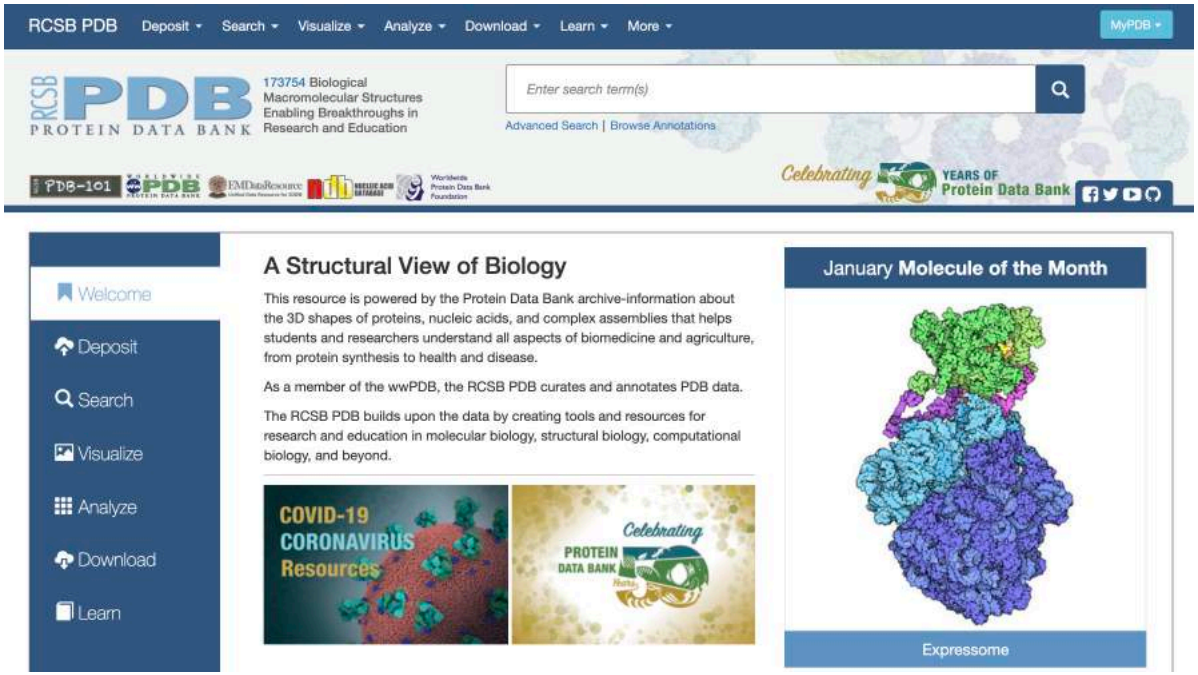
Protein structure and function

Max Perutz John Kendrew

Solved the structures of haemoglobin and myoglobin

Advanced reading

https://www.rcsb.org/



AlphaFold: Using AI for scientific discovery

https://deepmind.com/blog/article/AlphaFold-Using-AI-for-scientific-discovery

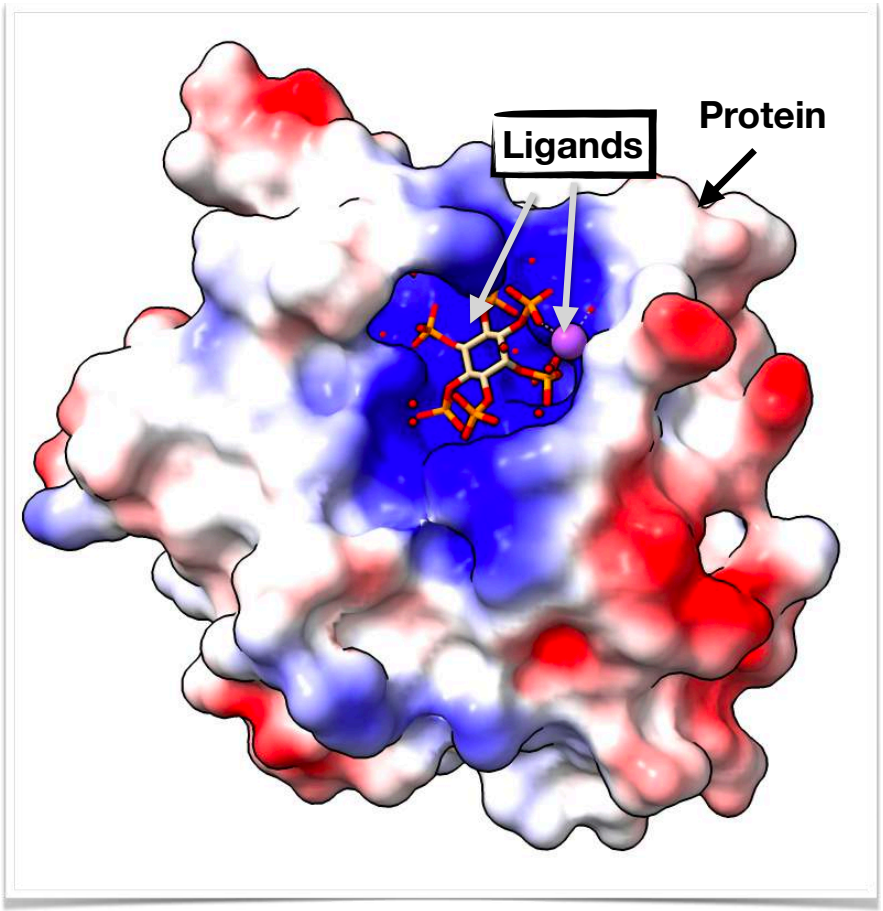
Protein misfolding and diseases

https://www.nature.com/scitable/topicpage/protein-misfolding-and-degenerative-diseases-14434929/

Intrinsically disordered proteins (IDPs)

Ligands

- The function of nearly all proteins depends on their ability to reversibly bind other molecules referred to as **ligands**
  - The transient nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic circumstances.
  - Ligands can be small molecules (O_2), metal ions, other proteins, nucleic acids etc.



https://www.rbvi.ucsf.edu/

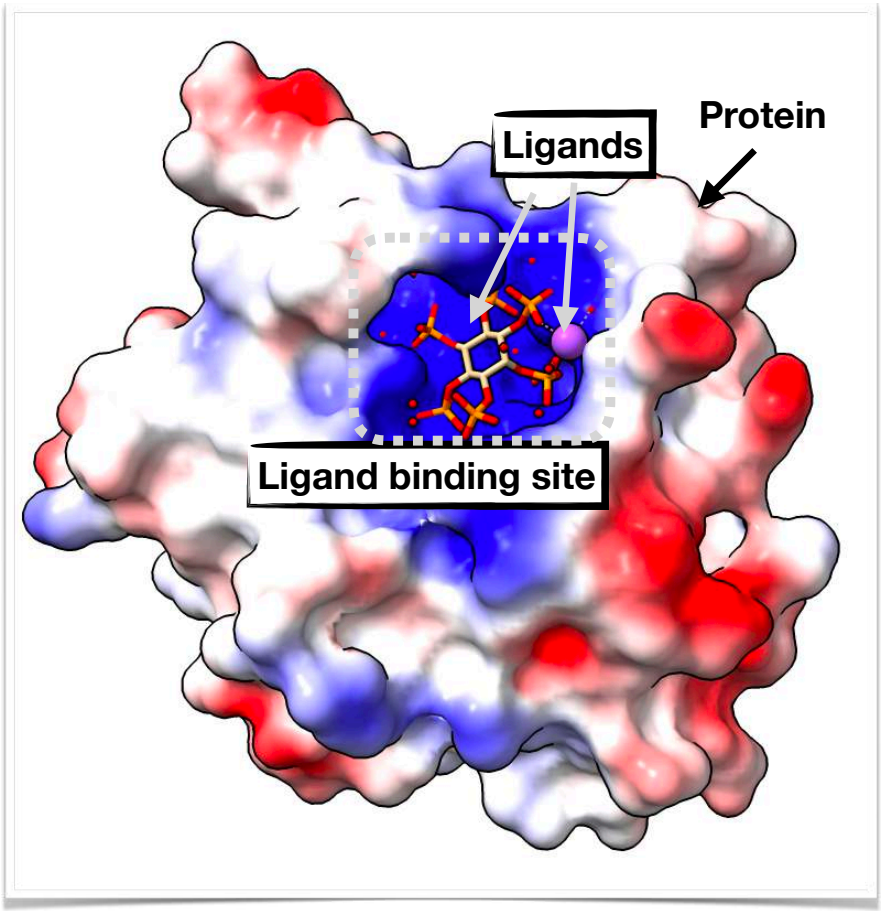
Ligand binding site

- **Ligand-binding sites** on proteins and the corresponding ligands are chemically and topologically complementary. Multiple noncovalent bonds mediate interactions between protein and ligand.

- A given protein may have separate binding sites for several different ligands.

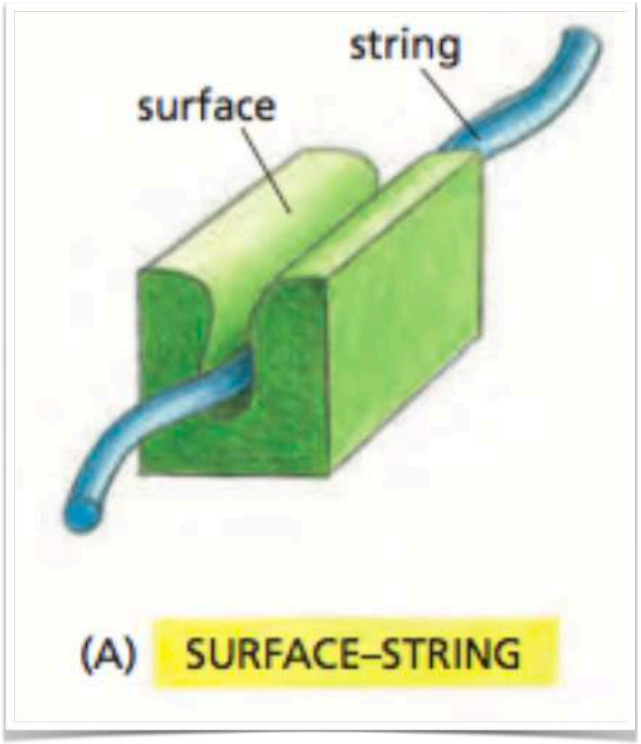
  - The **affinity** of a protein for a particular ligand refers to the strength of binding

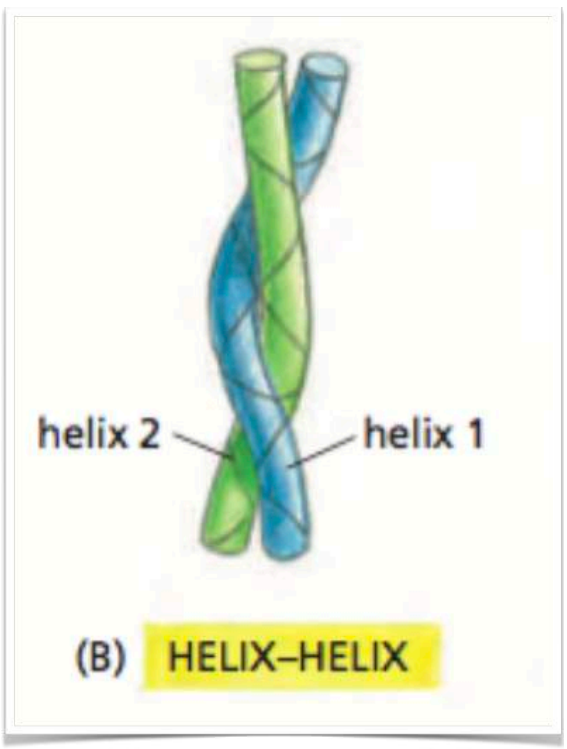
  - The **specificity** of a protein for a particular ligand refers to the preferential binding of one or a few closely related ligands

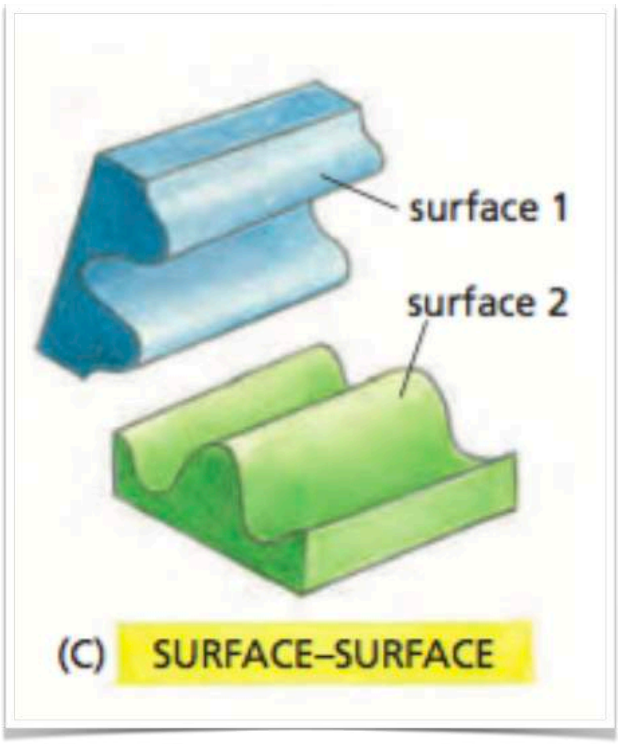


https://www.rbvi.ucsf.edu/

Protein-protein interactions







Enzymes: Biological catalysts

A special case of protein-ligand interactions

Discovery of enzymes

Early enzymologists

- **Biological catalysis** was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach.
- In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by “ferments.”
- Buchner identified that fermentation can occur from cell-free extracts of yeast.
- Frederick W. Kühne later gave the name **enzymes** (from the Greek enzymos, “leavened”) to the molecules detected by Buchner.

Eduard Buchner

F.W. Kuhne

What are enzymes?

Biological catalysts

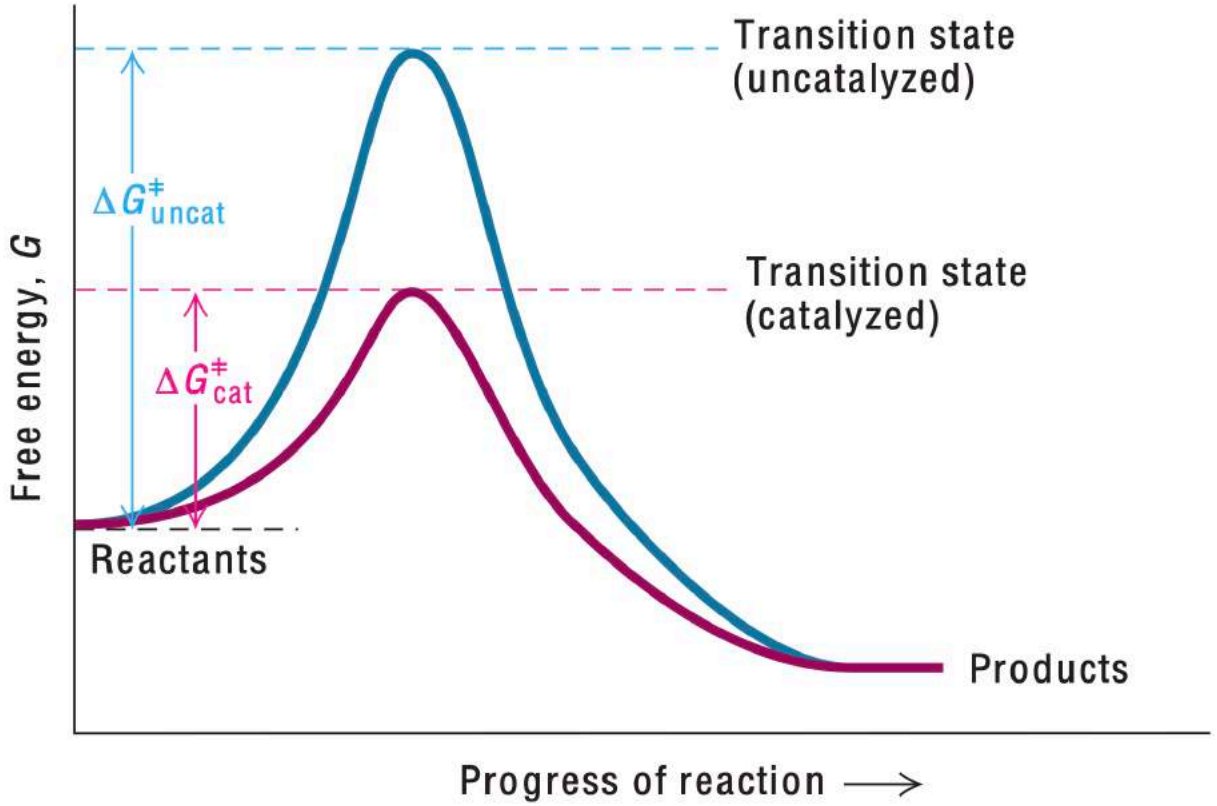
- The two fundamental conditions of life are:
  - Ability to self-replicate
  - Ability to catalyze chemical reactions efficiently and selectively
- Most reactions while thermodynamically favorable are extremely slow under the mild temperature and pH of living systems.
- **Enzymes are highly efficient and specific biological catalysts that have evolved to function under physiological conditions**
- All enzymes are proteins with a few exceptions.

Rates of reactions with or without enzymes

Enzyme	Uncatalyzed rate (k_{un}, s^{-1})	Catalyzed rate (k_{cat}, s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Enzymes as biocatalysts

Enzymes enhance reaction rates by lowering activation energies

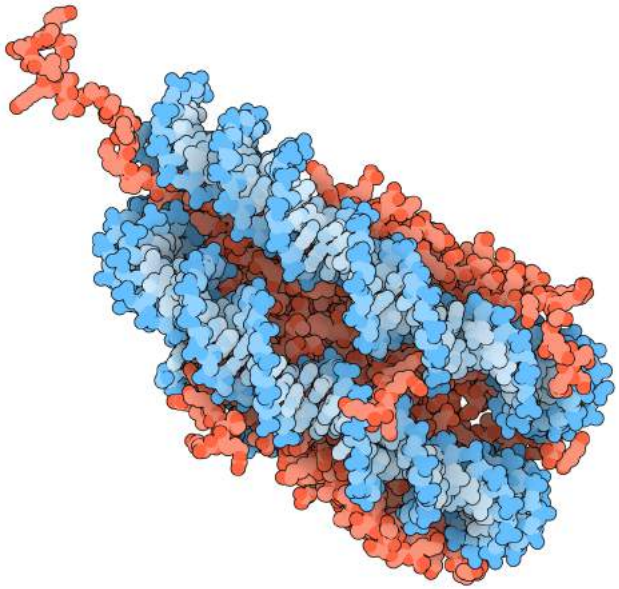


Protein conformation and enzyme function

- **Substrate specificity:** Enzymes are highly specific both in the reactions that they catalyze and in their choice of reactants, which are called **substrates**
- An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions.
  - In contrast to uncatalyzed reactions, side reactions leading to by-products (wasteful) are rare in enzyme-catalyzed reactions.
- Catalytic activity depends on the integrity of enzyme's native protein conformation.
  - if an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost.
  - if an enzyme is broken down into its component amino acids, its catalytic activity is always destroyed.

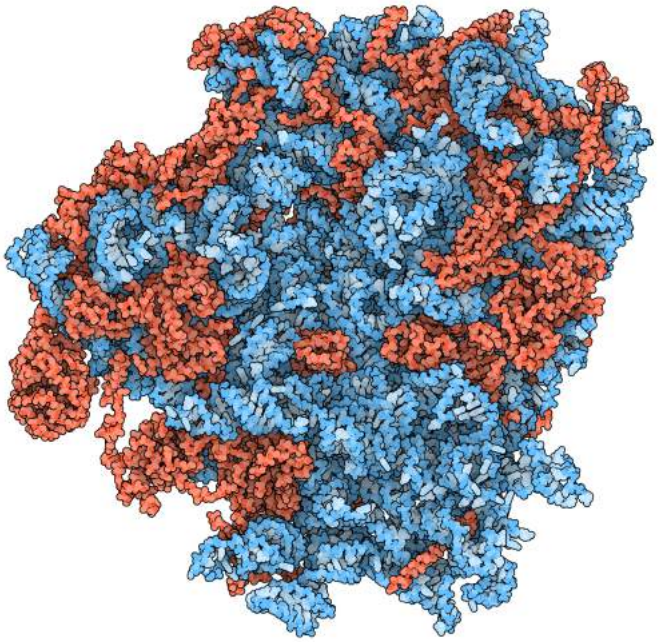
Primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity

Which of these is an enzyme?



Nucleosome: binds and compacts the organism's genome

- Protein-ligand interaction?
- Enzyme-substrate interaction?



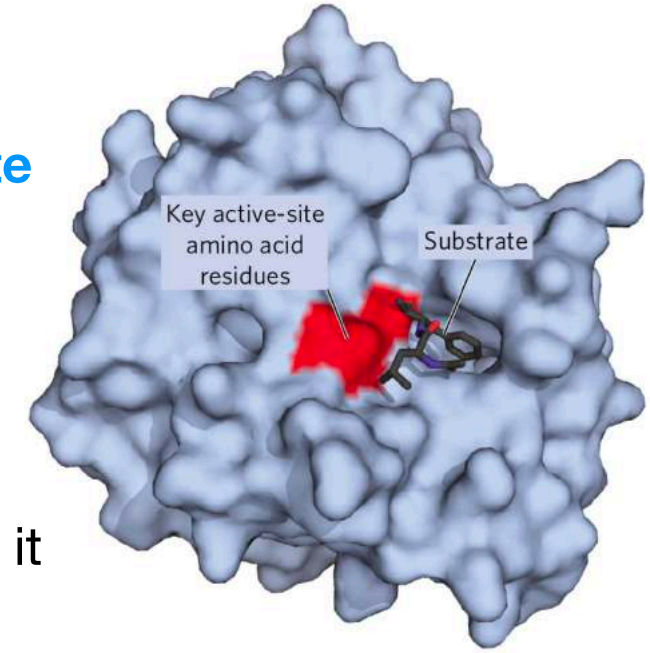
Ribosome: links amino acids during protein synthesis

- Protein-ligand interaction?
- Enzyme-substrate interaction?

Protein conformation and enzyme function

Concept of active site

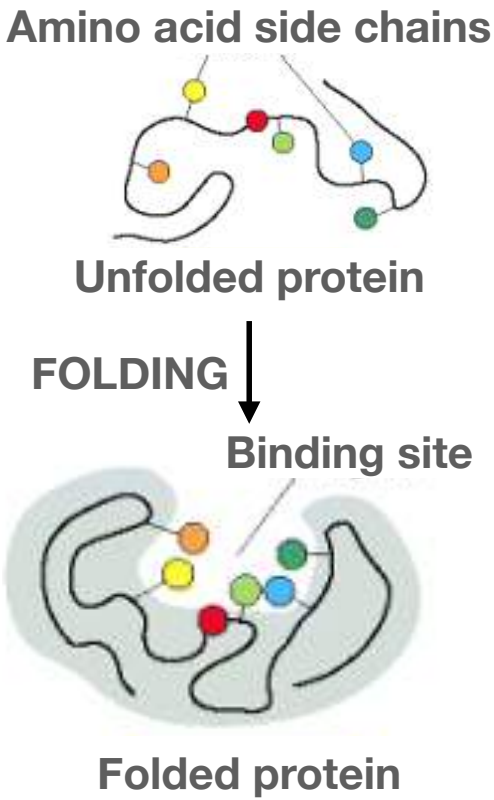
- Enzyme-catalyzed reactions takes place within the confines of a pocket on the enzyme called the **active site**
- Specific amino acid residues within the surface of the active site mediate interactions that help:
  1. **binding of the substrate**
  2. **catalyze its chemical transformation to product**
- Often, the active site encloses a substrate, sequestering it completely from solution.

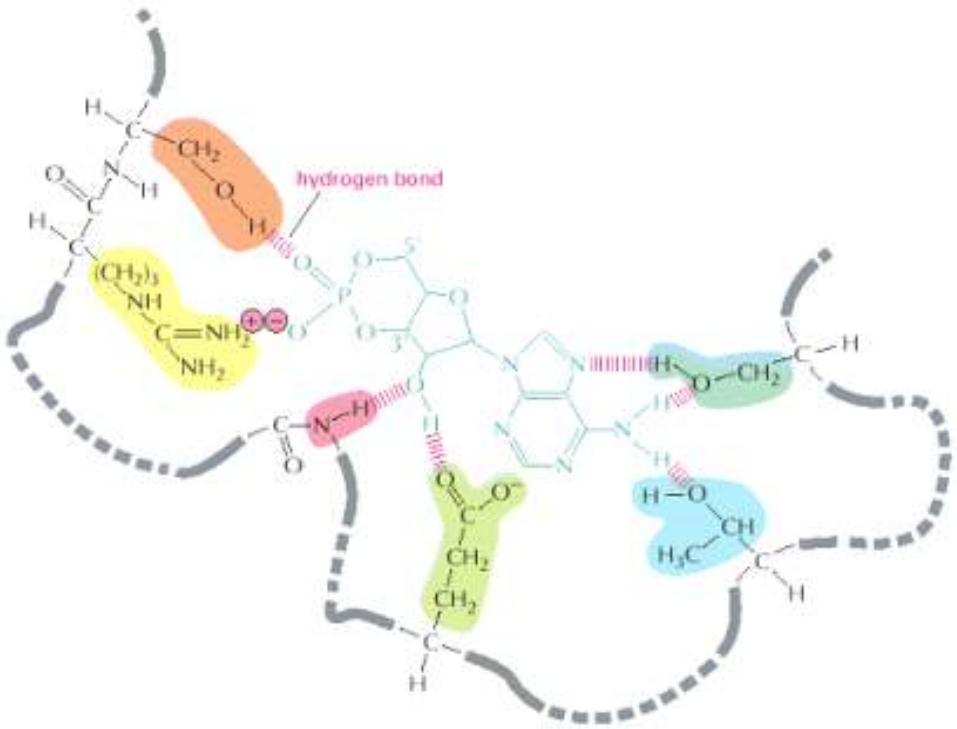


Some enzymes are components of multienzyme complexes in which reactants are channeled from one enzyme to another, never entering the bulk solvent.

Active site: Location of enzyme catalysis

The amino acids composing the active site are not necessarily adjacent in the amino acid sequence but are brought into proximity in the native conformation.





Some types of enzymes

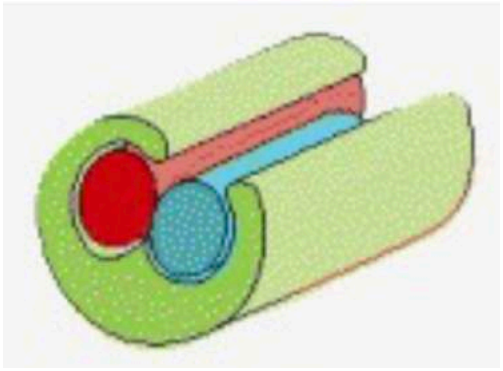
Enzyme names typically end in “-ase,” with the exception of some enzymes, such as pepsin, trypsin, thrombin and lysozyme that were discovered and named before the convention became generally accepted at the end of the 19th century.

- **Hydrolases** - general term for enzymes that catalyze a hydrolytic cleavage reaction.
  - **Nucleases** - break down nucleic acids by hydrolyzing bonds between nucleotides.
  - **Proteases** - break down proteins by hydrolyzing bonds between amino acids.
- **Synthases** - general name used for enzymes that synthesize molecules in anabolic reactions by condensing two smaller molecules together.
- **Isomerases** - catalyze the rearrangement of bonds within a single molecule.
- **Polymerases** - catalyze polymerization reactions such as the synthesis of DNA and RNA.
- **Kinases** - catalyze the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins.
- **Phosphatases** - catalyze the hydrolytic removal of a phosphate group from a molecule.
- **Oxido-Reductases** - general name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often called oxidases, reductases, and dehydrogenases.

Cofactors

- The catalytic activity of many enzymes depends on the presence of small molecules termed **cofactors**, although the precise role varies with the cofactor and the enzyme.
- An enzyme without its cofactor is referred to as an **apoenzyme**
- The complete, catalytically active enzyme is called a **holoenzyme**
  - **Holoenzyme=Apoenzyme+cofactor**
- Cofactors can be subdivided into two groups:
  - Metals (magnesium, iron, calcium etc.)
  - Small organic molecules (coenzymes: biotin, NAD+)

Strategies of enzyme catalysis



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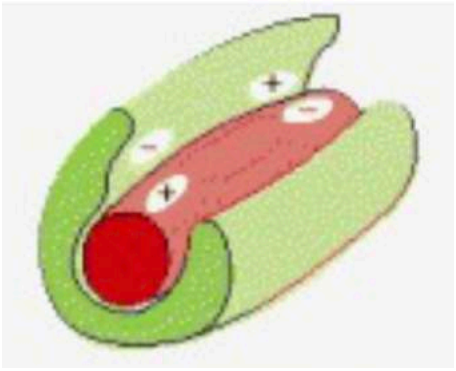

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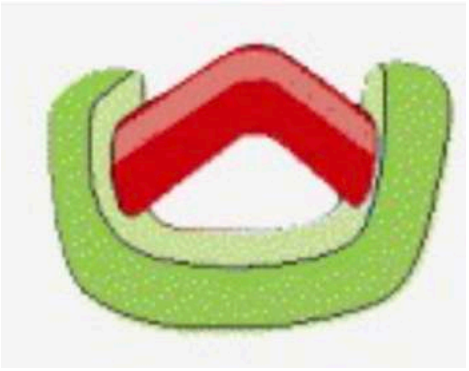


3

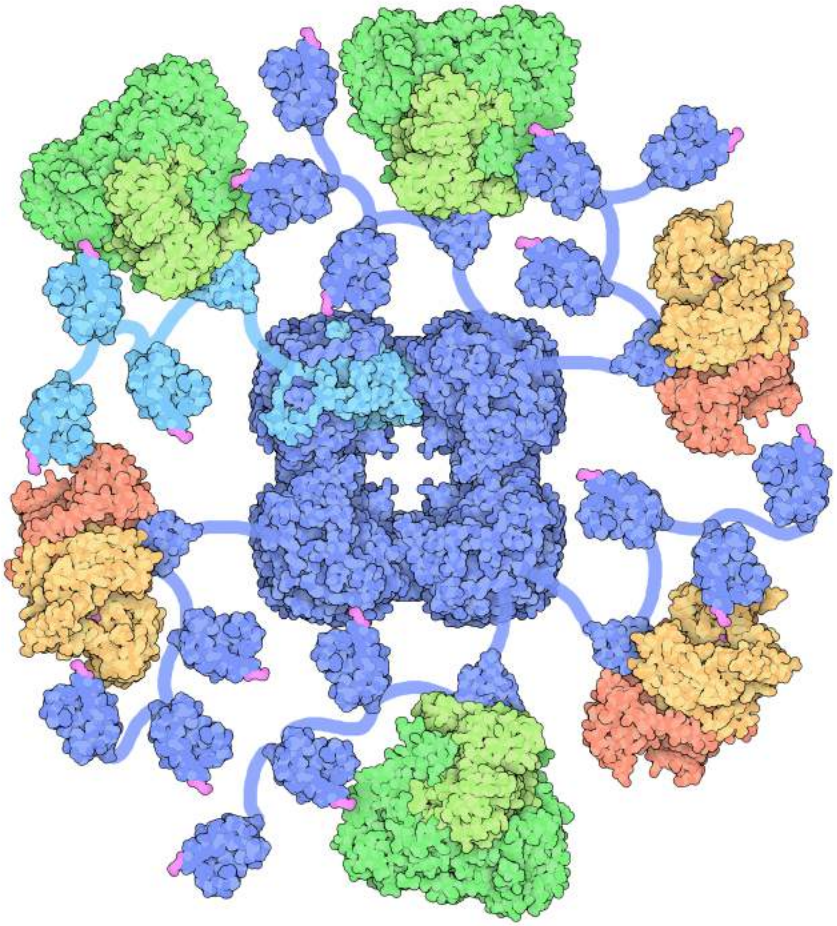
1. **Holding substrates together in a precise alignment**: Orients substrates to encourage a reaction

2. **Charge stabilization of reaction intermediates**: binding of substrate to enzyme rearranges electrons in substrate creating partial -ve and +ve charges that favor a reaction

3. **Altering bond angles in the substrate to increase the rate of a particular reaction**: Enzyme strains the bound substrate forcing it toward a transition state to favor a reaction

Multienzyme complexes

- The efficiency of **enzymes** in accelerating chemical reactions is crucial to the maintenance of life.
- Multienzyme complexes help to increase the rate of cell metabolism
- Reaction rates can be increased without raising substrate concentrations by bringing the various enzymes involved in a reaction sequence together to form a large protein assembly known as a **multienzyme complex**



PYRUVATE DEHYDROGENASE COMPLEX

- **3 enzymes; multiple copies**
- **5 co-enzymes**