BIOCATLYSTS

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Biocatalysts

A catalyst is a substance which alters to promote the reaction and a substance especially an enzymes that initiates or modifies the rate of a chemical reaction in a living body is termed as biocatalyst

They are enzymes or microbes that initiate or accelerate chemical reactions

What are enzymes	Enzymes are chemical substances which are mostly proteins
	Enzymes catalyze nearly all the biochemical reactions in the living cells
	They have unique three dimensional shapes that fits the shape of reactants
	Enzymes are typically derived from plants, micro-organisms (Yeast, bacteria or fungi) or animal tissue (e.g. protease from pancreases)

How are enzymes made?

- Commercial sources of enzymes are obtained from three primary sources, i.e. animal tissue, plants and microbes
- These naturally occurring enzymes are quite often not readily available in sufficient quantities for food applications or industrial use
- However, by isolating microbial strains that produce the desired enzymes and optimizing the conditions for growth, commercial quantities can be obtained

This artificial method of producing enzyme was earlier known as Fermentation

Today, this fermentation process is carried out in a contained vessel. Once, fermentation is completed, the microorganisms are destroyed; the enzymes are isolated and further processed for commercial use

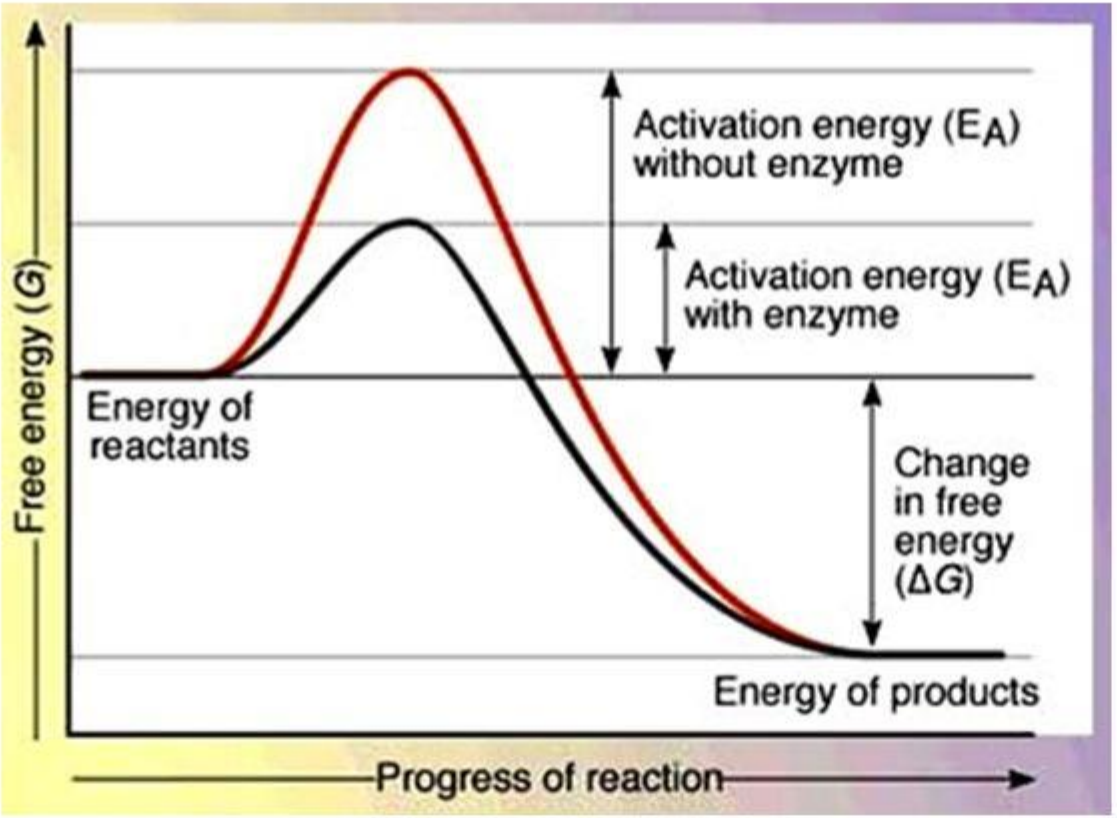
Types of Biocatalysts

Microbes	**e.g. yeast, and other anerobic bacterias**
Lipases	These are the most widely used class of enzymes in organic synthesis, they are preferred widely because of their better stability as compared to others
Proteases	Enzymes which breakdown proteins
Cellulases	Enzymes which breakdown cellulose
Amylases	Which breakdown starch into simple sugars

Why use an enzyme?

- Enzymes speed up chemical reactions in a natural ways
- As they are not alive, they remain as inert mass of proteins
- Enzymes work by weakening bonds which lowers activation energy

Effects of Catalyst on Activation Energy

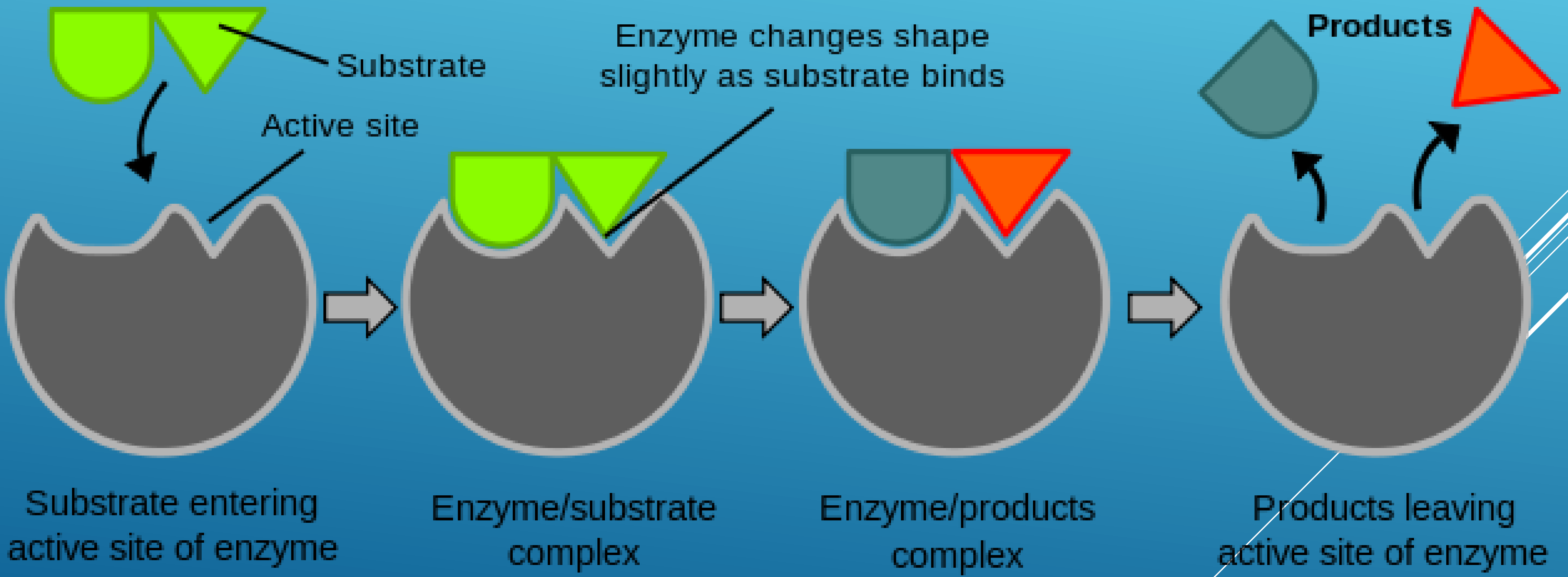


- Enzymes are biological catalyst usually made of proteins.
- Speed reactions by lowering the activation energy of the reaction.

Merits of Biocatalysts

Biocatalyst- enabled mfg.	Chemistry based mfg.
1. Biocatalysts can operate at or near room temperature and pressure.	1.Chemistry based cannot operate at or near room temperature and pressure.
2. They often use manufacturing equipment that is less complex and expensive to build and operate.	2. They often use manufacturing equipment that is more complex and expensive to build and operate.
3. Biocatalyst-enabled processes can create products of higher quality as compared to chemistry-based manufacturing processes	3. Chemistry-based processes can create products of higher quality as compared to Biocatalyst-enabled processes
4. They are pollutionless and easily decomposable at any stage	4. They cant be easily disposed
5. They cannot be practically used in industries.	5. They can be practically used in industries.

Action of Enzyme



What Affects catalyst Activity?

- Three factors:

1. Environmental Conditions

2. Cofactors and Coenzymes

3. Enzyme Inhibitors

1. Environmental Conditions

1. **Extreme Temperature are the most dangerous**

   - high temps may denature (unfold) the enzyme.

2. **pH** (most like 6 - 8 pH near neutral)

3. **Ionic concentration** (salt ions)

2. Cofactors and Coenzymes

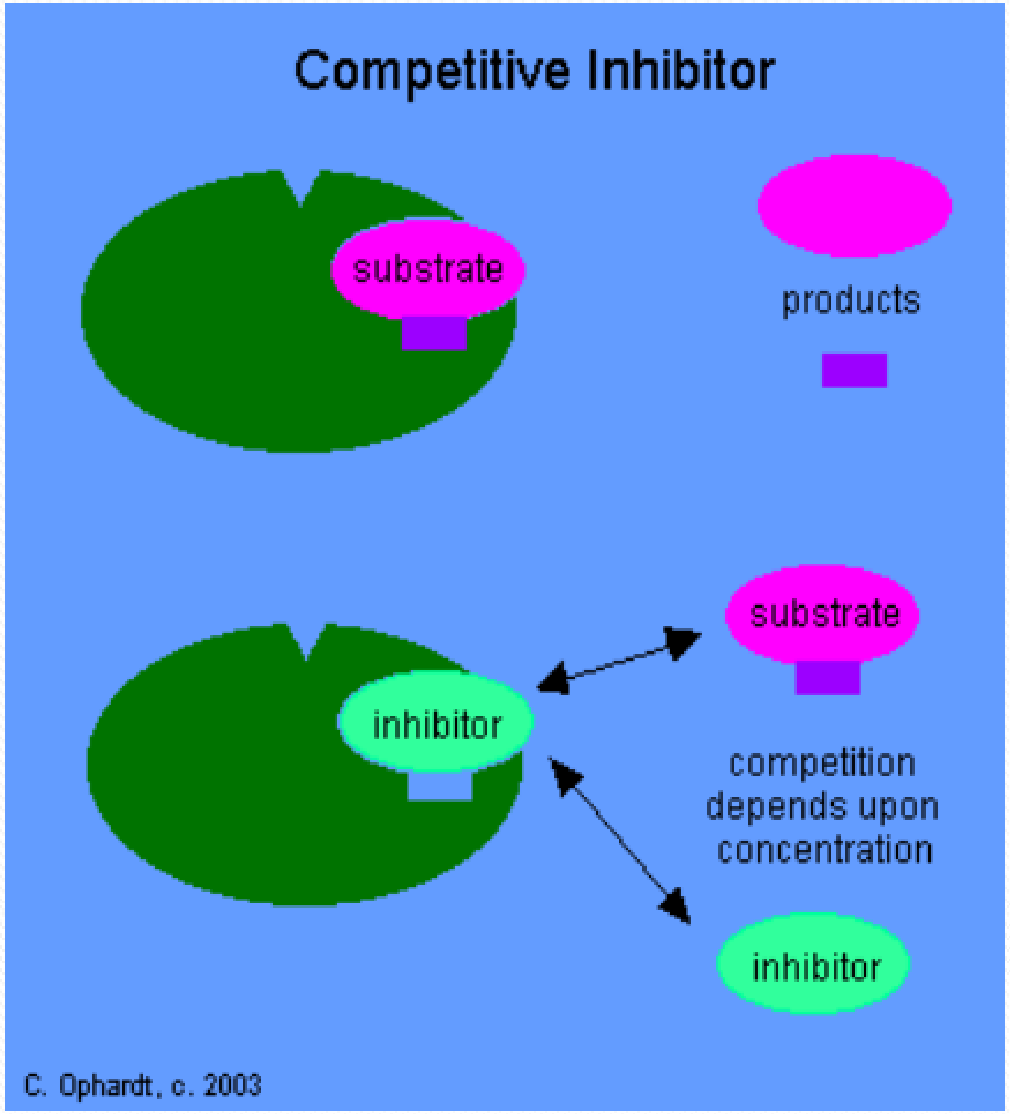
- Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes need for proper enzymatic activity.

- Example:

  Iron must be present in haemoglobin in order for it to pick up oxygen.

3. Enzyme Inhibitors

- Inhibitors are the one which do not enter the active site, but bind to another part of the enzyme causing the enzyme to change its shape, which in turn alters the active site.



Biocatalyst enzymes in human body: Digestive Enzymes

Our food is made up of:

- 1. Carbohydrates: Bread, Pasta, Potato
- 2. Protein: Meat, Nuts, Lentils
- 3. Fats: Butter, Milk

- Our body needs to digest them and turn them into a form that can be absorbed into the blood and used by cells.
- Enzymes make this possible.

Reaction for breakdown of Carbohydrates

Amylase + Starch → Maltose

Enzyme (in saliva)

(in pasta, bread)
(carbohydrate)

(a form of sugar that can)
(be absorbed by the body)

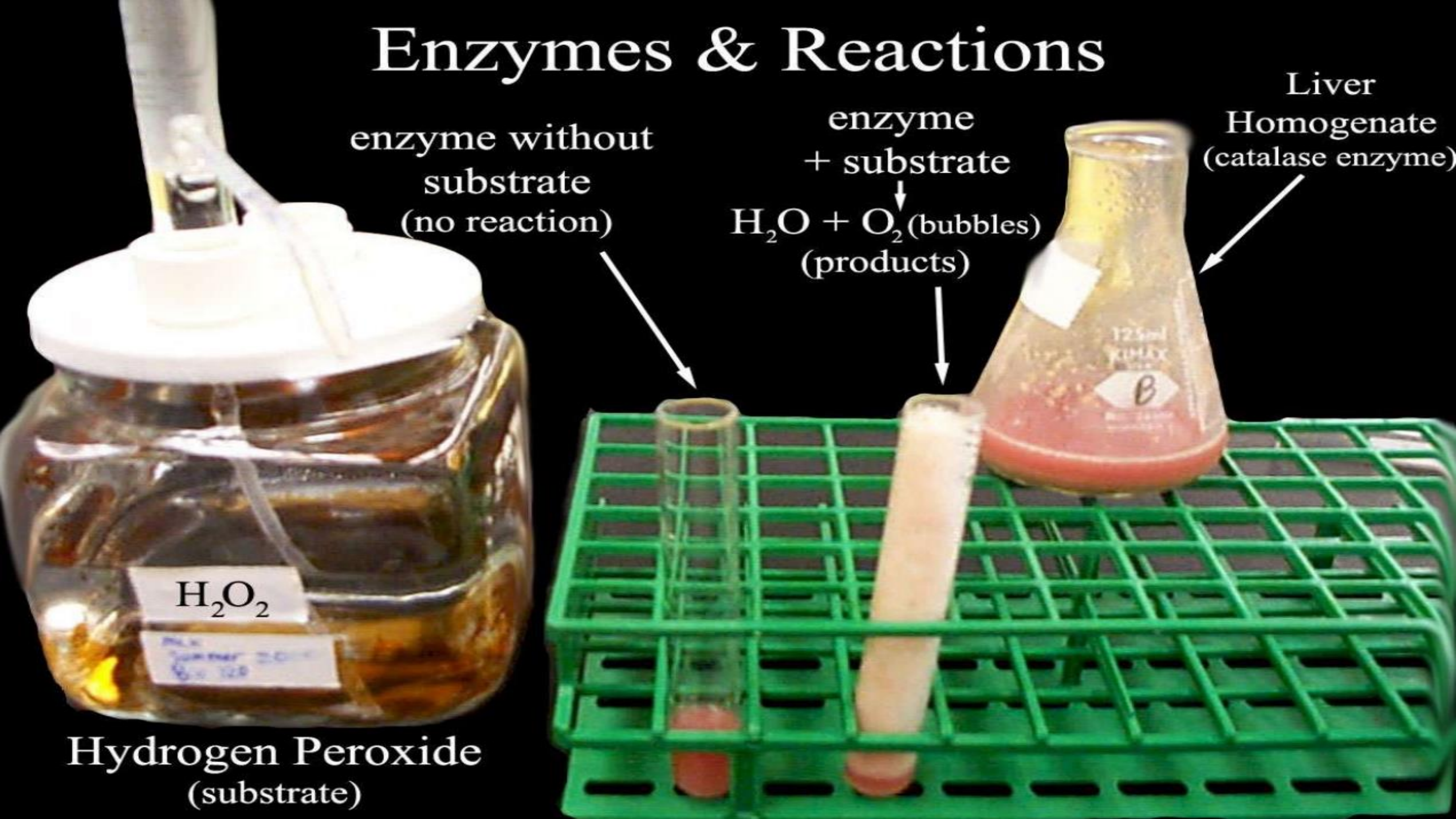
Reaction for breakdown of proteins

- Protease + Proteins → Amino Acids
- Enzyme (in stomach) (eg. In meat, nuts) (the body can absorb them and use them)

Reaction for breakdown of fats

Lipase enzyme	+	Fats	$\longrightarrow$	Fatty Acids + Glycerol
(secreted by pancreas)		(Butter, Milk)		(can be absorbed into body)

Enzymes & Reactions



DNA replication

- DNA replication is a biological process that occurs in all living organisms.
- The process starts when one double-stranded DNA molecule produces two identical copies of the molecule.
- **DNA polymerase** are a family of Enzyme that carry out all forms of DNA replication

Applications and uses

- Biocatalysts are used in Food industries for processing.
- It is used in diagnostic tests
- Biocatalysts are the most important aspect in molecular biology(DNA replication, cloning).

  For e.g. Polymerases (to polymerize)

  Restrictases(to cut the DNA strands)

  DNA ligases(to bind the DNA strands).

Demerits of Biocatalysts:

- Enzymes require narrow operation parameters.
- Enzymes display their highest catalytic activity in water.
- Enzymes may cause allergies

Medical applications of biocatalysts:

- Development of medical applications for enzymes have been at least as extensive as those for industrial applications

- For example, pancreatic enzymes have been in use since the nineteenth century for the treatment of digestive disorders.

- The most successful applications are extracellular: in the removal of toxic substances and the treatment of life-threatening disorders within the blood circulation.

Diagnostic catalysts

Diagnostic enzymes are used in diagnosis of various disorders in human body

Disorders	Enzyme
Heart attack	LDH(Lactatae Dehydrogenase)
Liver(pH disorder)	Alkaline phosphatase
Diabetes(blood and urine test)	Amylase, Lipase

Some important therapeutic enzymes

Enzyme	Use
Asparaginase	Leukaemia
Collagenase	Skin ulcers
Glutaminase	Leukaemia
Hyaluronidasea	Heart attack
Lysozyme	Antibiotic

Conclusion:

- These examples are just a few of the many ways commercial enzymes touch our lives. They are tools of nature that help provide everyday products in an environmentally conscious manner. Current commercial use of enzymes, together with new applications, will continue to play an important role in maintaining and enhancing the quality of life we enjoy today while protecting the environment for generations to come.

Metalloproteins : Functions in Biological Chemistry

- Catalysis of hydrolysis and dehydration by zinc enzymes: Carbonic anhydrase

- Catalysis of electron transfer reactions: Cytochromes, non-heme-iron-enzymes, blue Cu-proteins, iron-sulfur proteins

- Transport of atom groups (e.g., O_2): Hemoglobin, Hemerythrin, Hemocyanin)

- Signal transduction: Calmodulin (Ca^{2+}-binding regulatory protein)

Some fundamental metal sites in metalloproteins

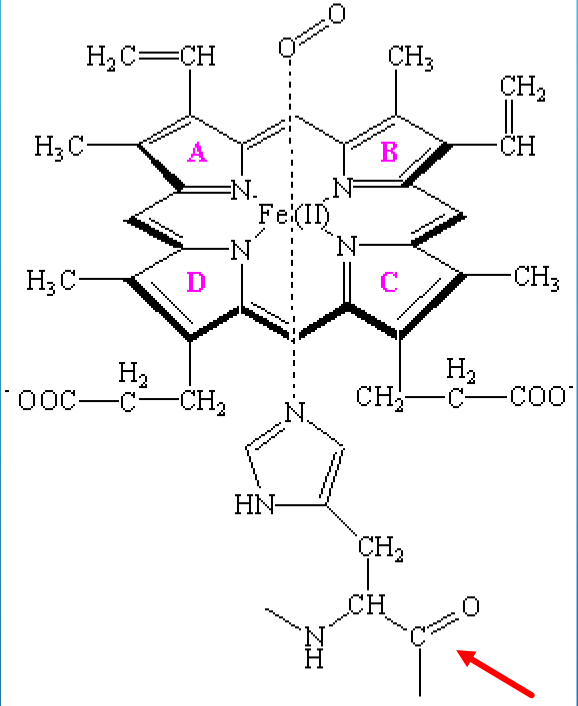
Metal site	Function
1. Metal complexes of porphyrins and corrins	
- Iron porphyrins	
= Hemoglobin & Myoglobin	O_2 transport
= Cytochromes	Redox catalysts
- Vitamin B_{12} = Cobalt corrinoid	Radical catalyst Methyltransferase
2. Bridged bimetallic complexes	
- Fe_2 clusters	
= Hemerythrin	O_2 transport
= Methane Monooxygenase	Hydroxylase
= Ribonucleotide Reductase RR2	Radical generation
- Cu_2 clusters	
= Hemocyanin	O_2 transport

Some fundamental metal sites in metalloproteins

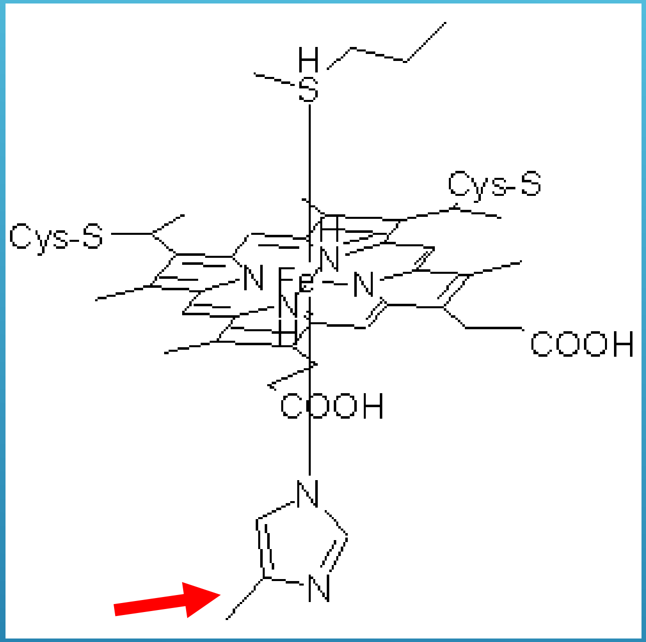
- Mn_2 clusters	
= O_2-evolving complex	Photosystem II
= Mn-Catalase	H_2O_2 disproportionation
- Zn_2 clusters	
= Zinc aminopeptidases	Peptide cleavage
- Ni_2 clusters	
= Urease	Hydrolysis of urea
3. Fe-S clusters	Electron transfer
4. Mo-protein	Xanthine oxidase
3. Zinc fingers	DNA binding

Some fundamental metal sites in metalloproteins

→ anchoring points to protein



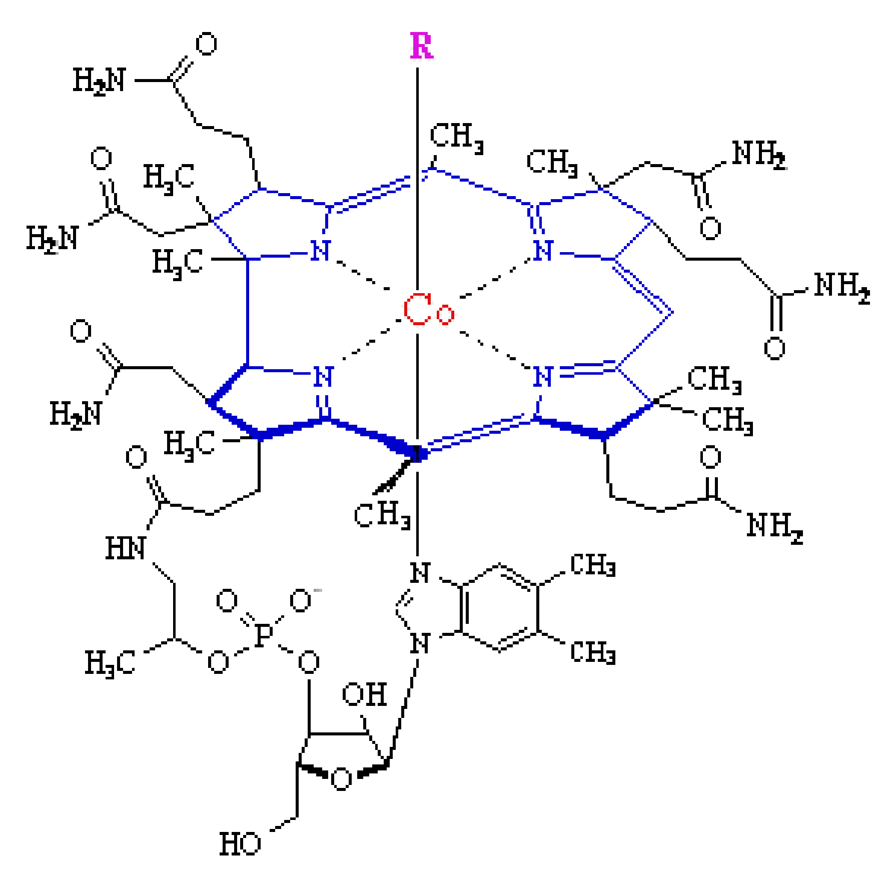
Hemoglobin/myoglobin



Cytochrome c (involved in respiratory chain)

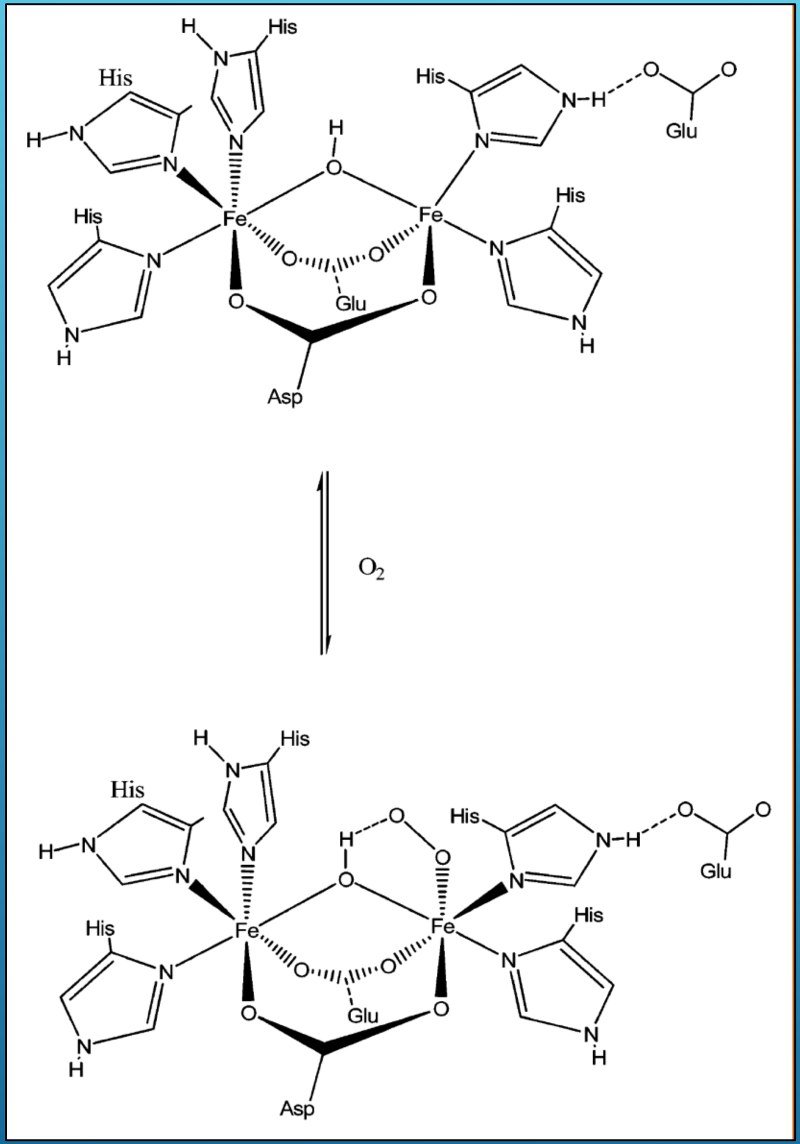
Some fundamental metal sites in metalloproteins

R= 5‘-Ado coenyzme B_{12}

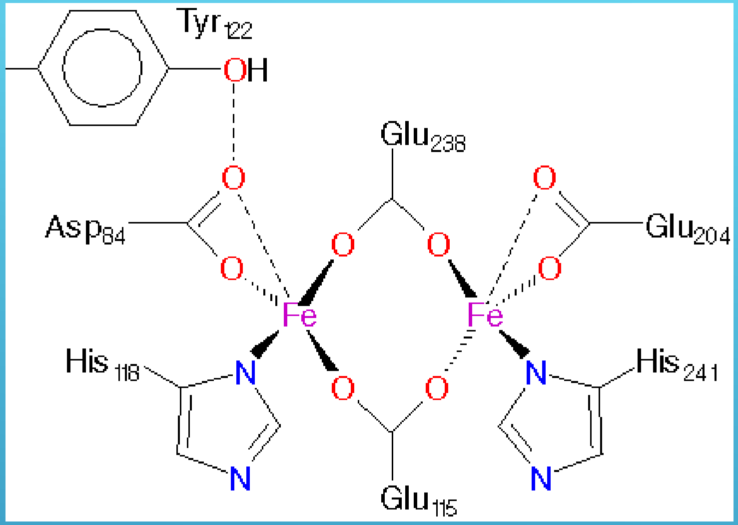


- Organometallic compound (M-C bond)
- 9 chiral centers

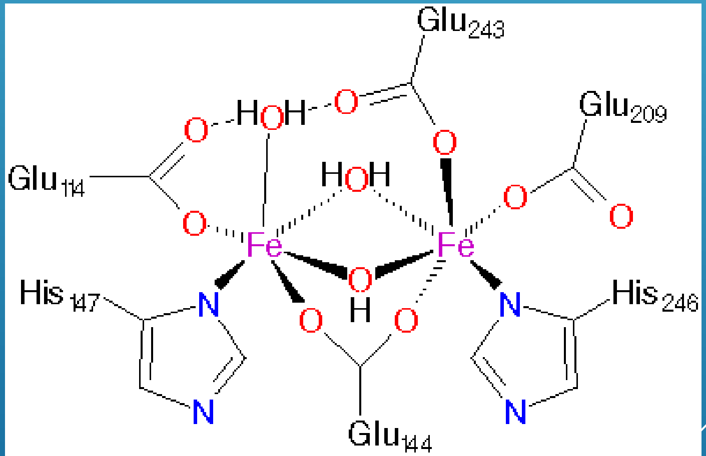
Some fundamental metal sites in metalloproteins



Hemerythrin

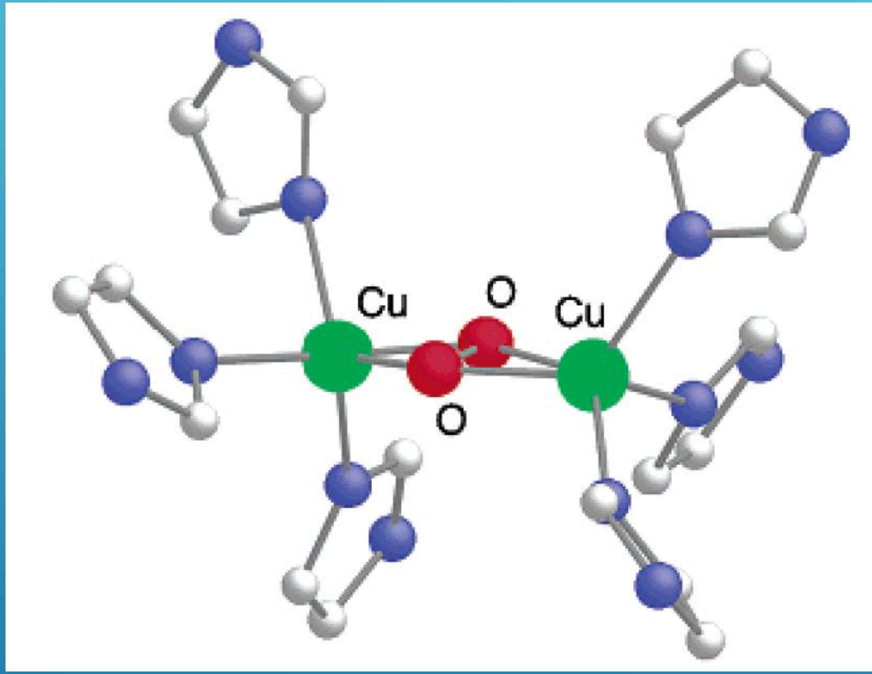


Ribonucleotide reductase R2 unit

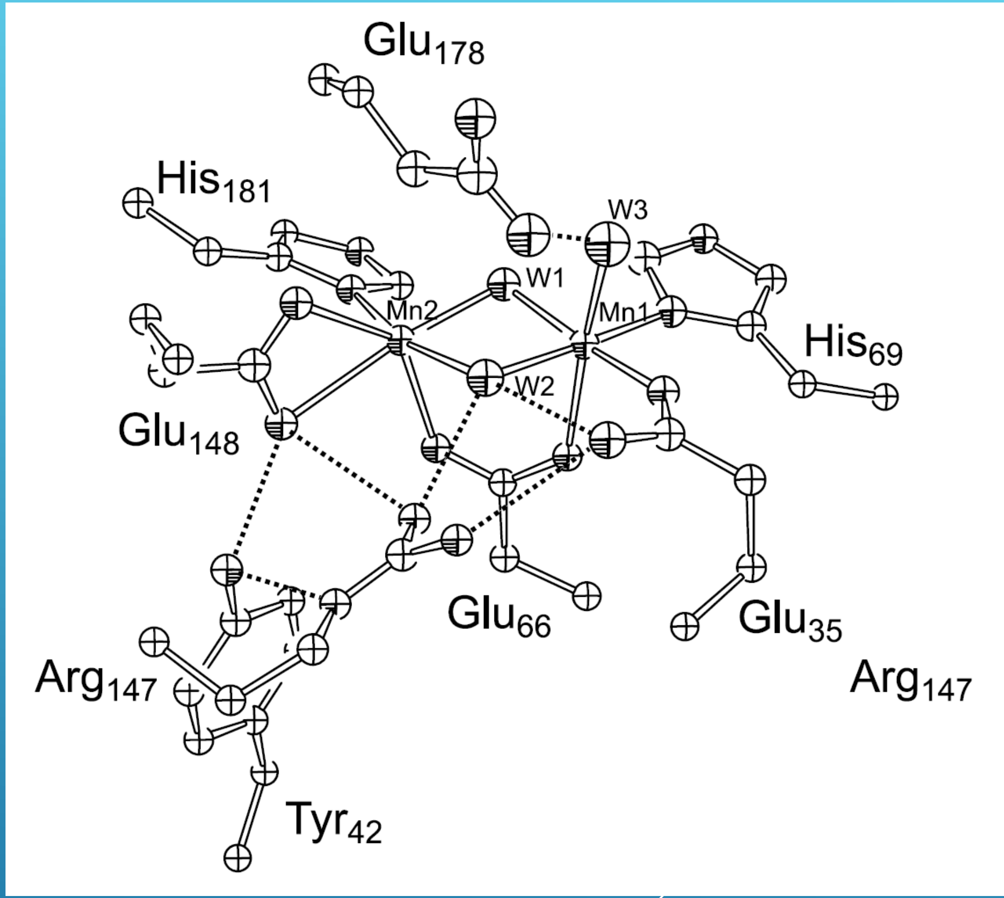


Methane monooxygenase hydroxylase protein

Some fundamental metal sites in metalloproteins

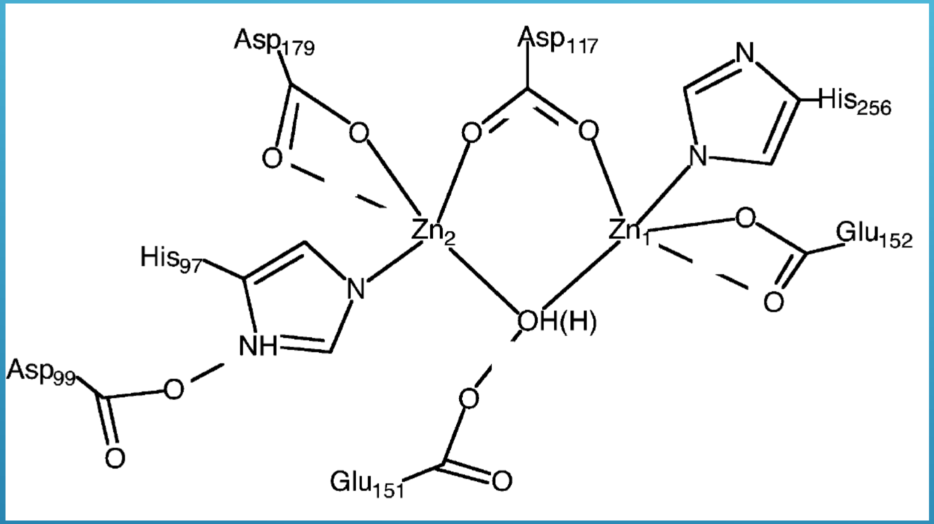


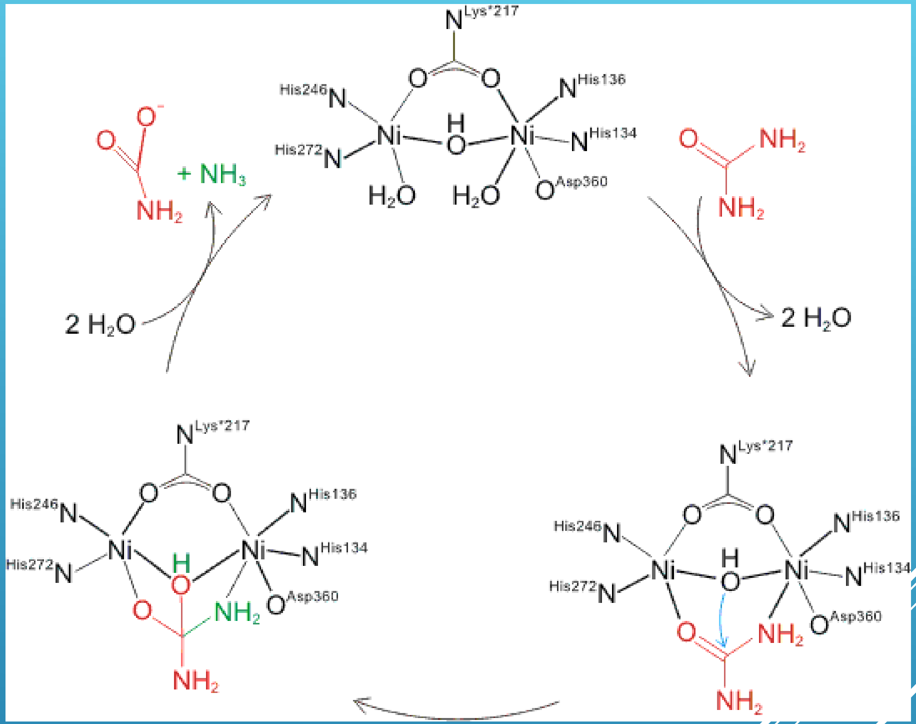
Hemocyanin (oxygen transport)
Cuff et al.,J.Mol.Biol.1998



Mangenese catalase
(Whitaker et al., Eur. J. Biochem. 2003, 270, 1102-1116)

Some fundamental metal sites in metalloproteins

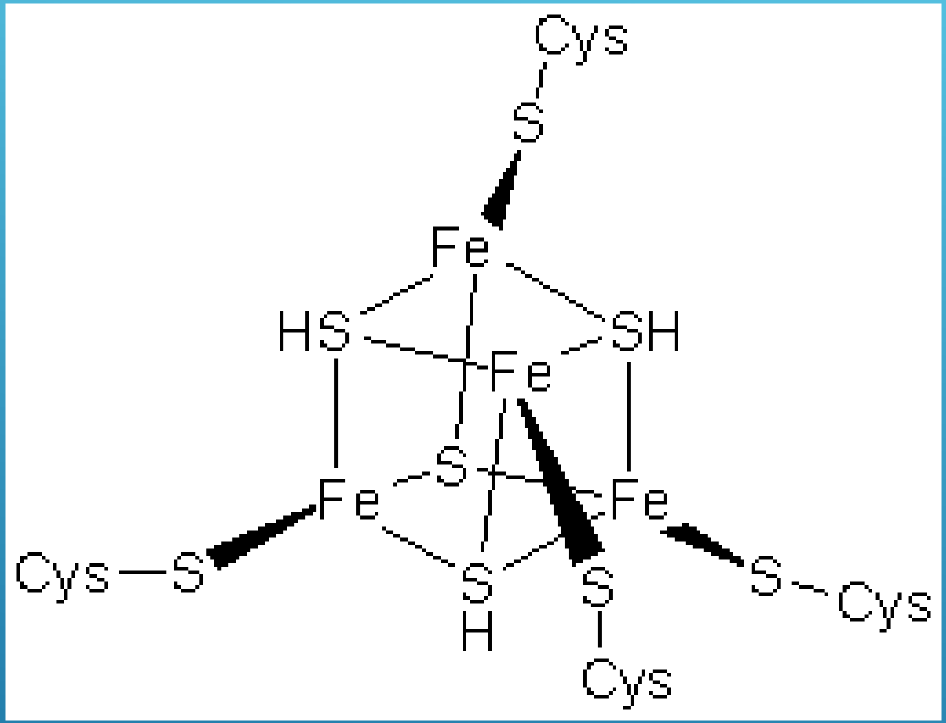




Aminopeptidase from Aeromanas proteolytica
(Stamper et al., Biochemistry 2004, 43, 9620-9628)

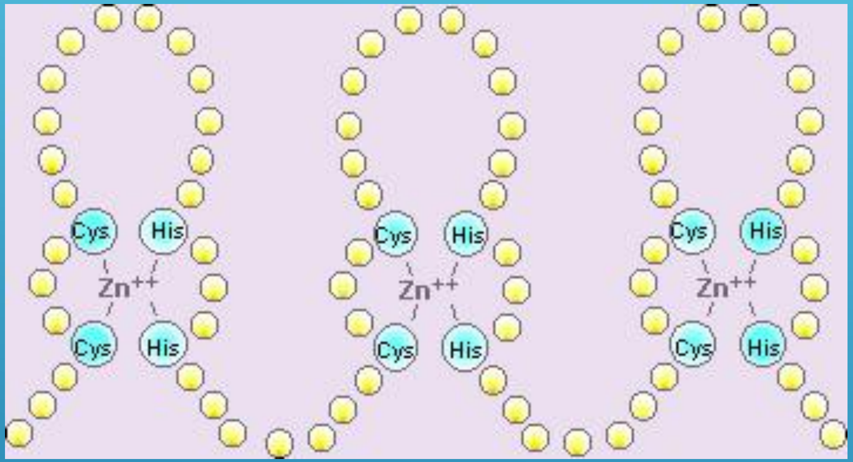
Urease: catalytic cycle
http://www.cup.uni-muenchen.de/ac/kluefers/homepage/L_bac.html

Some fundamental metal sites in metalloproteins



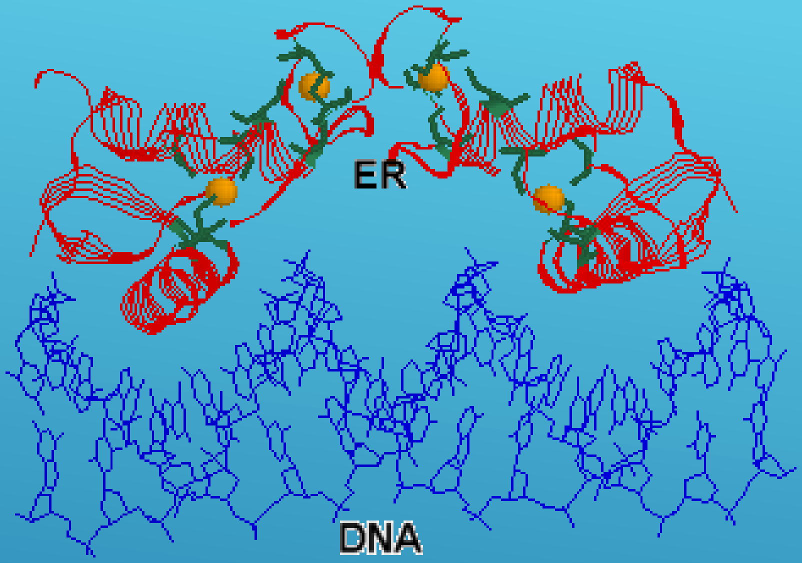
4Fe-4S cluster
http://www.steve.gb.com/science/enzymes.html

Some fundamental metal sites in metalloproteins



Coordination of zinc in a zinc finger

Estrogen receptor
mechanism

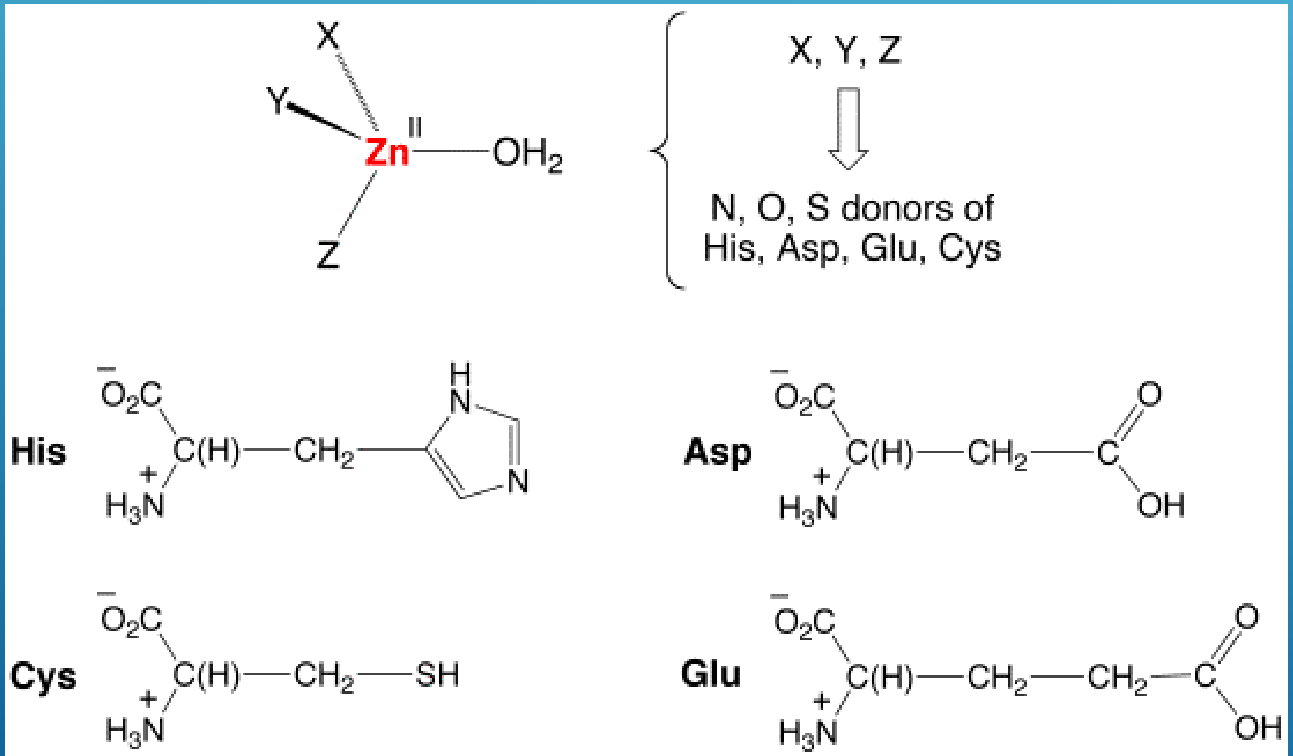


Zinc finger of the estrogen receptor
is responsible DNA-binding

Zinc finger: http://www.infobiogen.fr/services/chromcancer/Deep/TranscripFactorsID20043.html
Zinc finger of estrogen receptor: http://www.web-books.com/MoBio/Free/Ch4F2.htm
Estrogen receptor mechanism
http://www.cancer.gov/cancertopics/understandingcancer/estrogenreceptors/

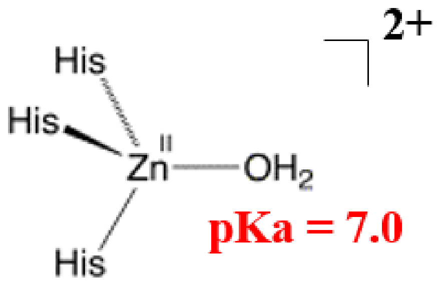
Mononuclear zinc enzymes: Carbonic anhydrase

Zinc is essential to all forms of life, with an average adult human containing ~3 g of zinc. The influence of Zn derives from its presence in enzymes. An understanding of the roles that Zn plays in biological systems requires a detailed appreciation of how the chemistry of Zn is modulated by its coordination environment. The most common structural motif in Zn enzymes is one in which a tetrahedral Zn center is attached to the protein backbone by three amino acid residues, with the fourth site being occupied by the catalytically important water (or hydroxide) ligand. Importantly, His binds to metals as a neutral molecule, whereas Cys, Asp, and Glu bind after deprotonation, as Cys^-, Asp^-, and Glu^- anions.



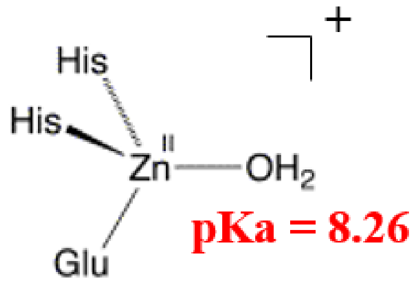
http://www.columbia.edu/cu/chemistry/groups/parkin/zinc.html

Examples of Zinc Enzymes and Proteins



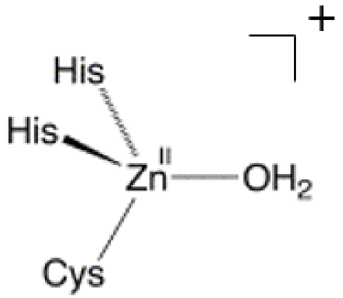
[NNN]Zn^{II}–OH_2

Carbonic Anhydrase



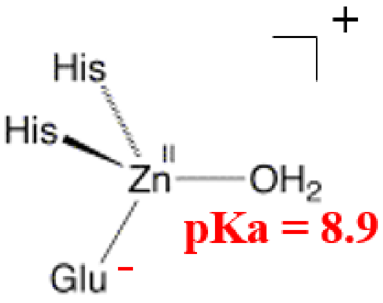
[NNO]Zn^{II}–OH_2

Thermolysin



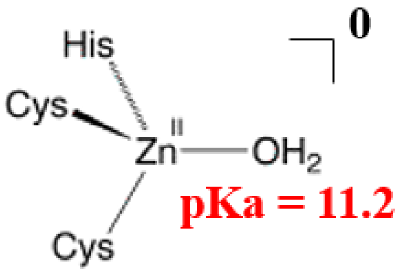
[NNS]Zn^{II}–OH_2

Bacteriophage T7 Lysozyme



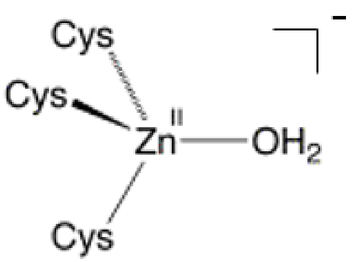
[NNO]Zn^{II}–OH_2

Carboxypeptidase A



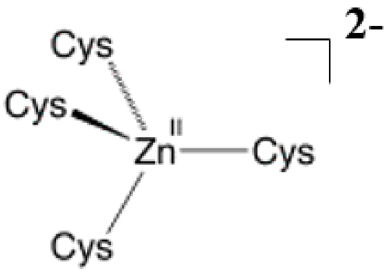
[SSN]Zn^{II}–OH_2

Liver Alcohol Dehydrogenase



[SSS]Zn^{II}–OH_2

5-Aminolevulinate Dehydratase



[SSSS]Zn^{II}

Ada DNA Repair Protein

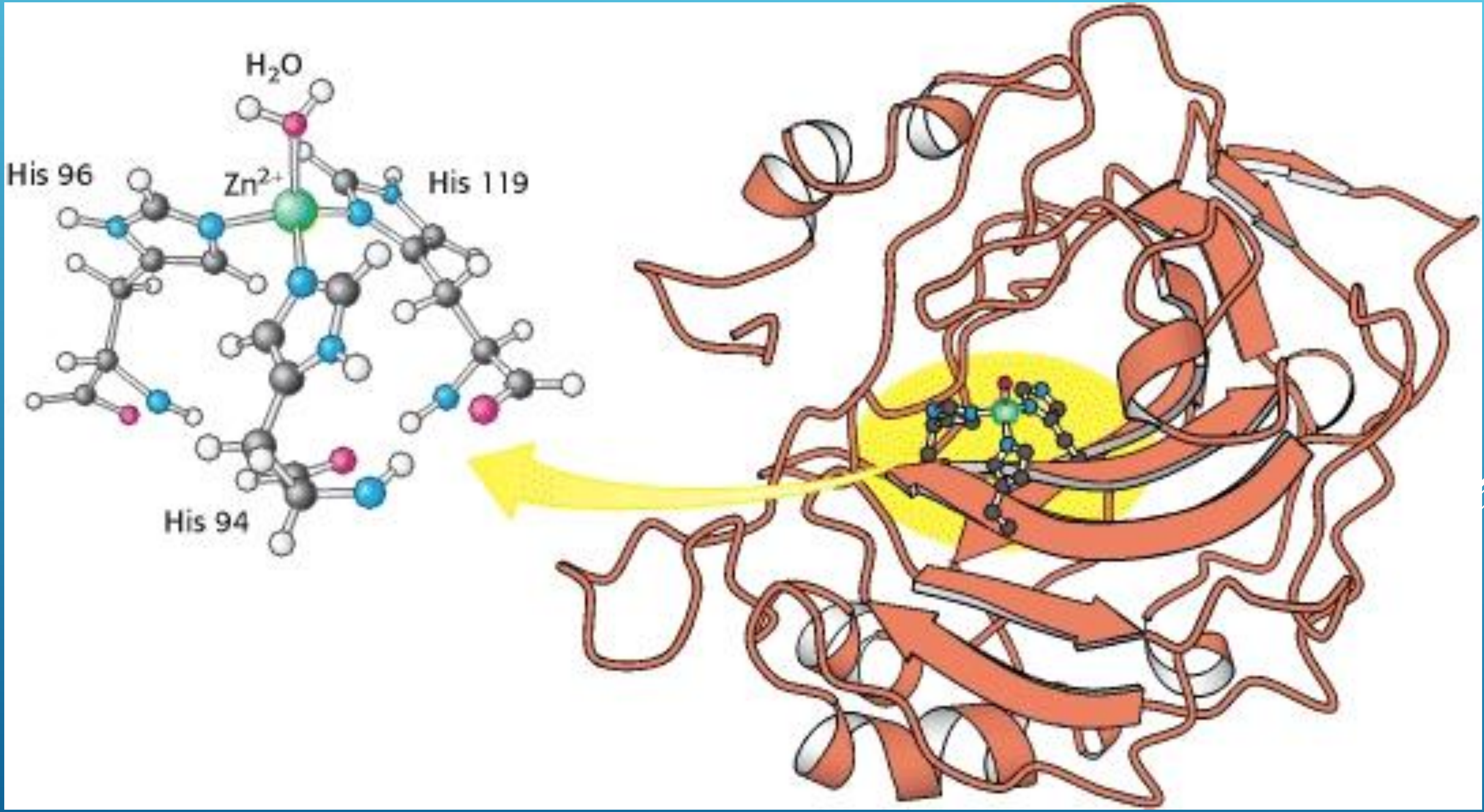
P.Andersson et al, Eur. J. Biochem. 113, 425-433 (1981)
W.N.Lipscomb, N. Sträter, Chem. Rev. 96, 2375-2433 (1996).

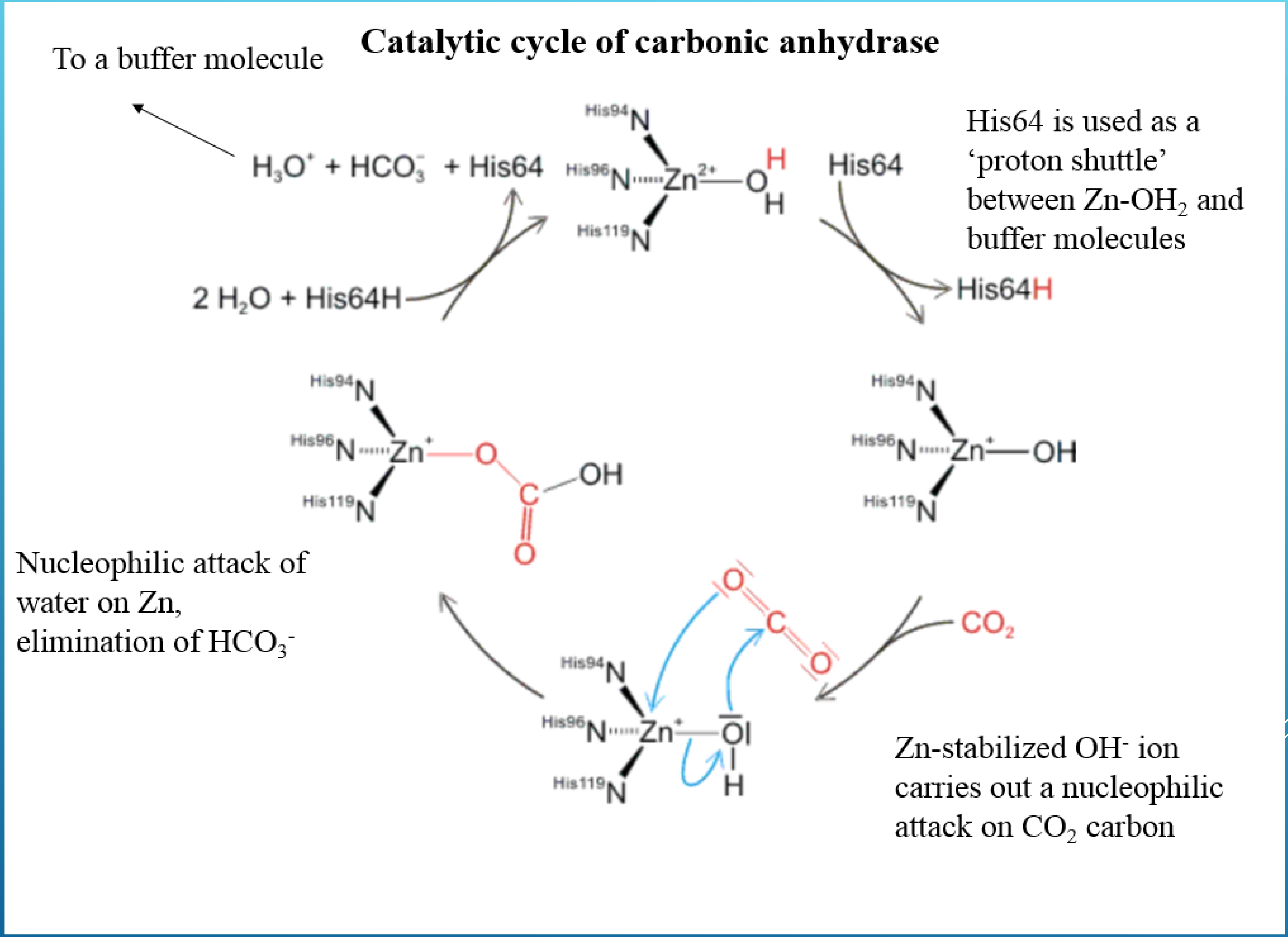
Carbonic anhydrase is a zinc-containing enzyme that catalyses the reversible hydration of carbon dioxide:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

In the absence of a catalyst, this hydration reaction proceeds with an effective first-order rate constant of ~0.01 s^{-1} at 37°C, pH 7. This is too slow for physiological processes. For example, CO_2 must be almost instantaneously converted into HCO_3^- in muscles to be transported in the blood. Conversely, HCO_3^- in the blood must be dehydrated to form CO_2 for exhalation as the blood passes through the lungs. Carbonic anhydrases accelerate CO_2 hydration dramatically. The most active enzymes, typified by human carbonic anhydrase II, hydrate CO_2 at rates as high as $kcat = 10^6\ s^{-1}$, or a million times a second.

Carbonic anbydrase is a monomeric ~29 kD protein consisting of ~260 amino acids. Zn2+ in the active site is coordinated by three histidine residues and a H2O/OH- ligand.




Catalytic cycle of carbonic anhydrase
To a buffer molecule
H3O+ + HCO3- + His64
His64 is used as a 'proton shuttle' between Zn-OH2 and buffer molecules
His94N
His96N
His119N
Zn2+
His64
His64H
2 H2O + His64H
Zn+
OH
CO2
Nucleophilic attack of water on Zn, elimination of HCO3-
Zn-stabilized OH- ion carries out a nucleophilic attack on CO2 carbon

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- http://www.sc.ehu.es