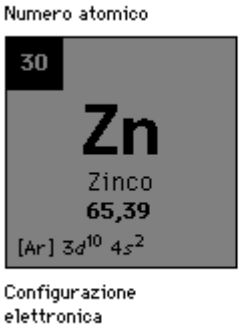
Zinc

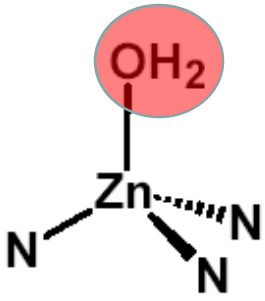


Zinc protein	Molecular mass (kDa)	Ligands	Function
carboanhydrase (CA)	30	3 His 1 H_2O	hydrolysis (12.6)
carboxypeptidase (CPA)	34	2 His 1 η^2-Glu 1 H_2O	hydrolysis (12.2), (12.11)
thermolysin	35	2 His 1 η^2-Glu 1 H_2O	hydrolysis (12.2)
5-aminolevulinic acid dehydratase (ALAD)	8 × 35	8 × { 3 S 1 N/O	condensation (12.18)
alcohol dehydrogenase (ADH)	2 × 40	2 × { 2 Cys 1 His 1 H_2O	oxidation of 1° or 2° alcohols via NAD^+ (12.19)
glyoxalase	2 × 23	2 × { 2 His 2 × { 2 Glu? 2 H_2O	reduction of α-dicarbonyl compounds by glutathione (12.21)
superoxide dismutase (SOD)	2 × 16	2 × { 2 His 1 μ-His^- 1 Asp	disproportionation of $O_2^{\bullet -}$ (10.15)
transcription factors	TFIIIA: 40	n × { 2 His 2 Cys	structural function: formation of specifically folded domains
	GALA: 17	2 × 4 Cys	
insulin hexamer	6 × 6	2 × { 3 His n L	structural function: stabilization of oligomeric storage forms
metallothionein	6	≤7 × 4 Cys	transport and storage protein

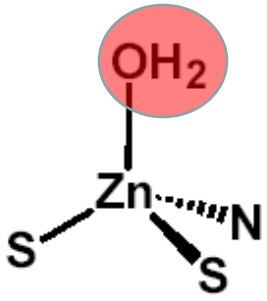
always 4 ligands → tetracoordinate

Zinc Metalloenzymes

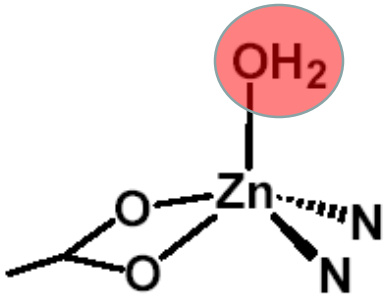
☆ **The unique feature of all of these enzymes is the presence of an activated water molecule bound to Zn(II). The pK_a of metal-free water is 15.7 but can be reduced to 10 in $[Zn(H_2O)_6]^{2+}$ and 7 with three N-donors. This allows for facile ionization of H_2O.**



carbonic anhydrase II



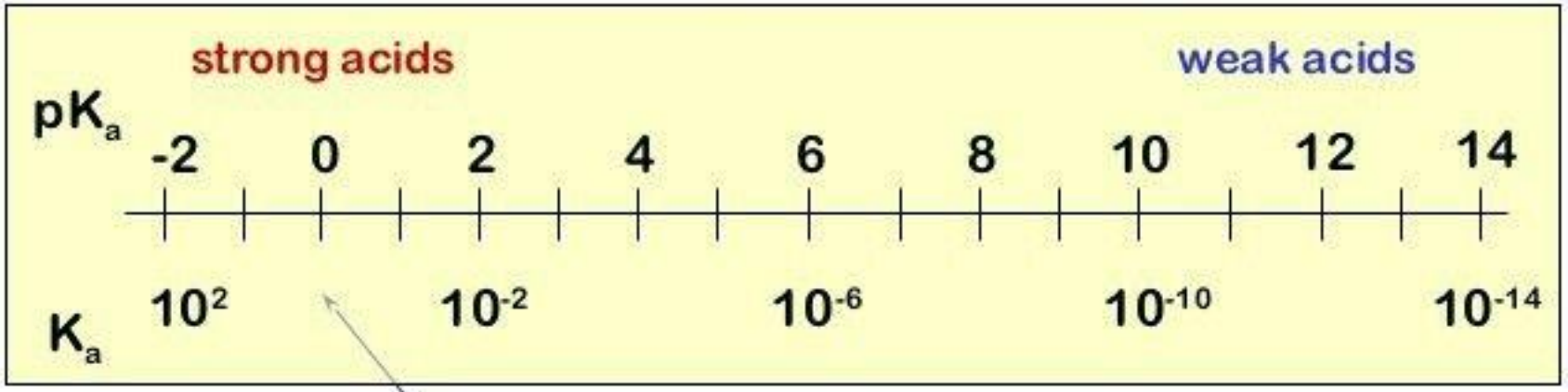
alcohol dehydrogenase



carboxypeptidase A

COMPARISON OF pK_a and K_a VALUES

$$pK_a = -\log K_a$$

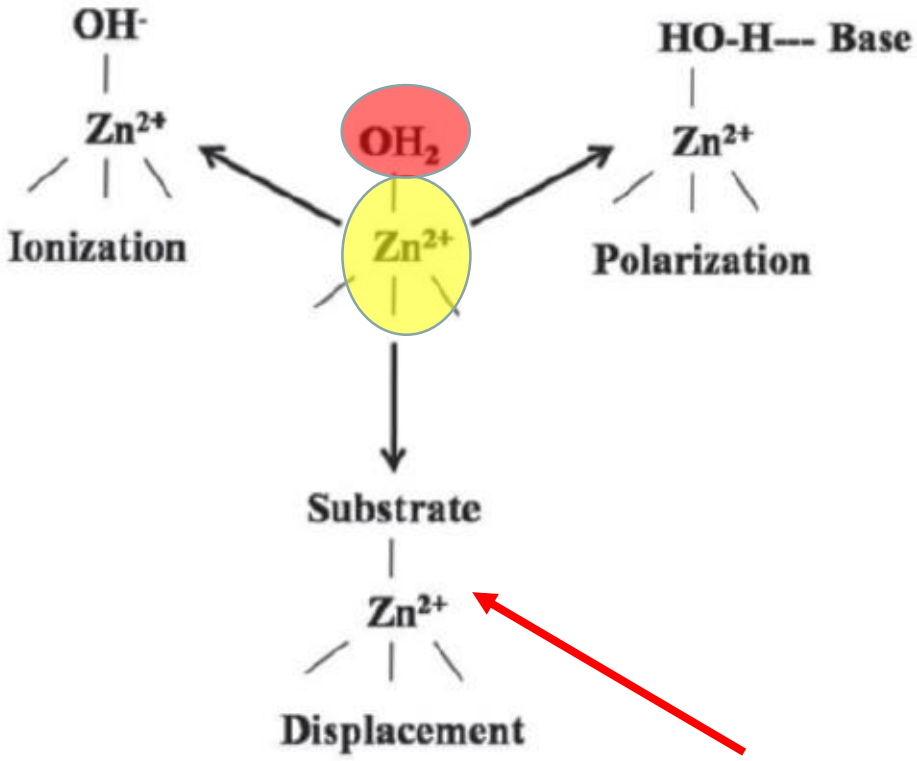


The smaller the value of the pK_a the stronger the acid.

We will use pK_a to describe the strengths of acids.
It is a single number, without exponents.

Ionization to form **hydroxide bridge**

polarized by some base to generate **nucleophilic catalysis**

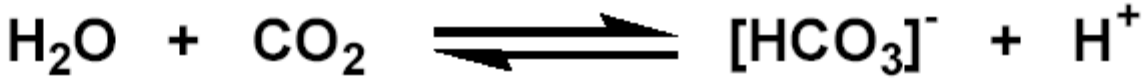


displaced by the substrate

One of the most efficient catalysts known!

Carbonic Anhydrase II

☆ *Carbonic Anhydrase II* **(CAII) is present in red blood cells and catalyzes the reversible hydration of CO_2.**



☆ **This process is slow ($k = 0.037\ s^{-1}$) but is essential for removing CO_2 form actively metabolizing sites.**

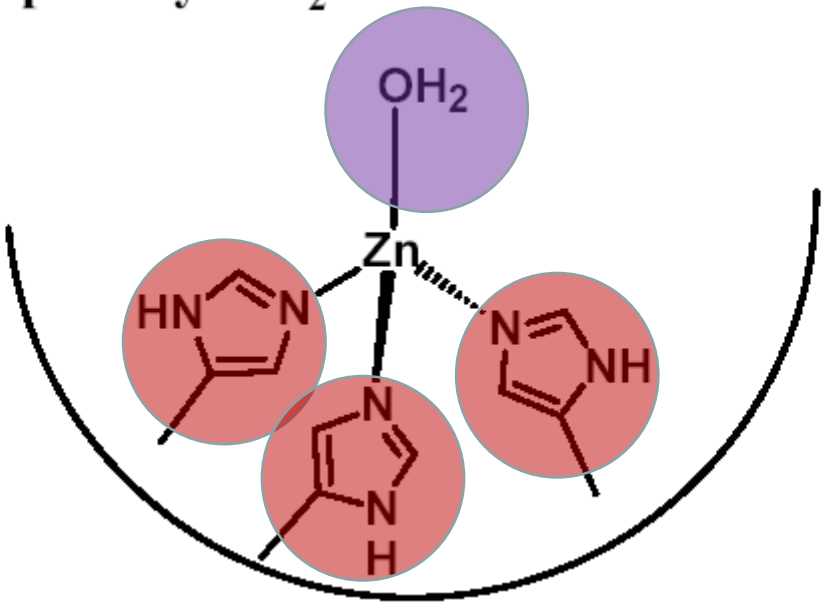
☆ **CAII increases the rate of hydrolysis by a factor of 10^7!!!!!**

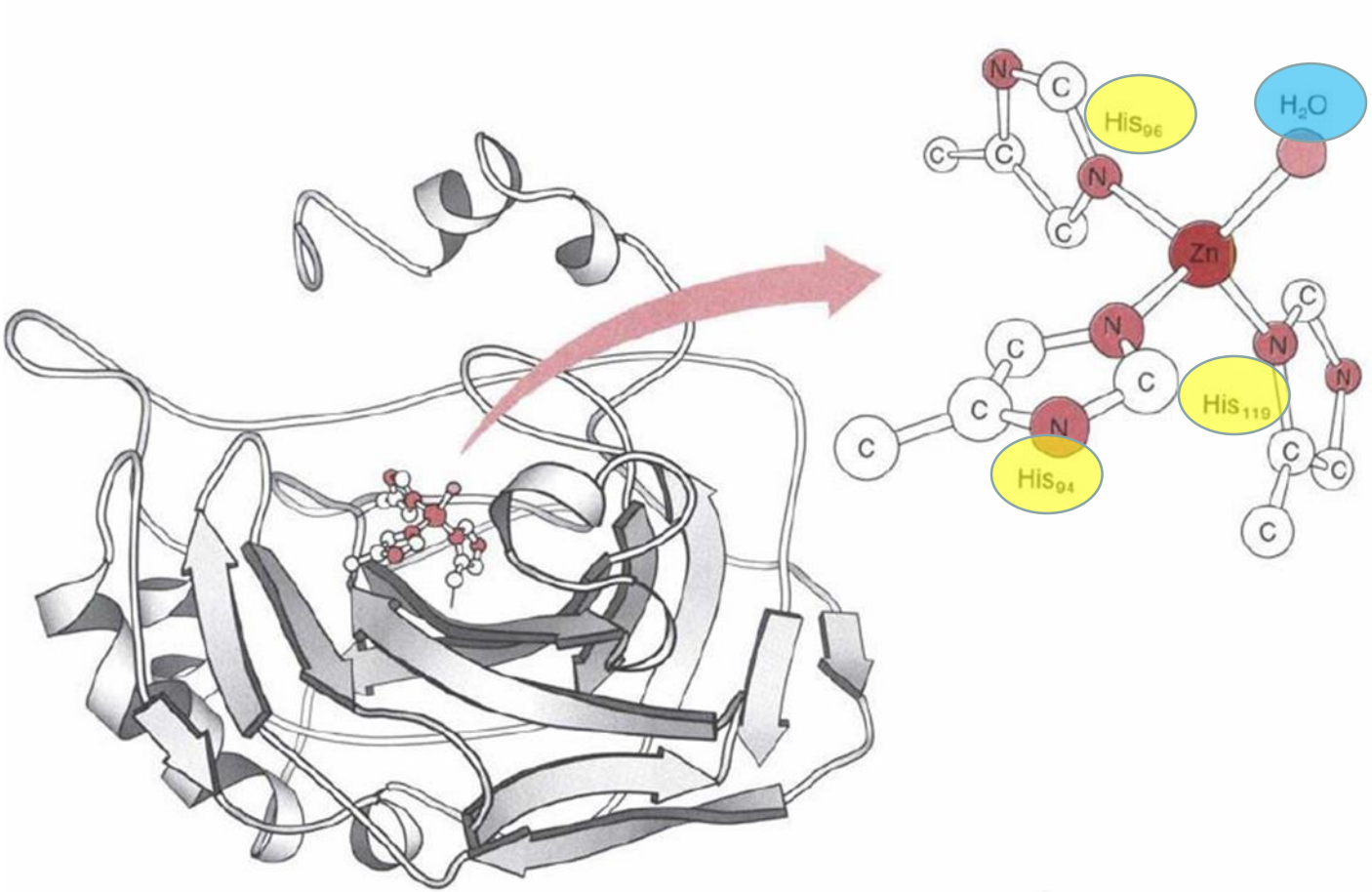
☆ **Can also catalyze hydrolysis of esters and aldehydes**

Carbonic Anhydrase II

Description:

- ☆ **Consists of 260 amino acid residues plus one Zn^{2+} ion.**
- ☆ **The active site lies near the bottom of a 15 Å deep cleft.**
- ☆ **A zinc ion coordinated by three N atoms (histidines) with the fourth site occupied by a H_2O molecule.**

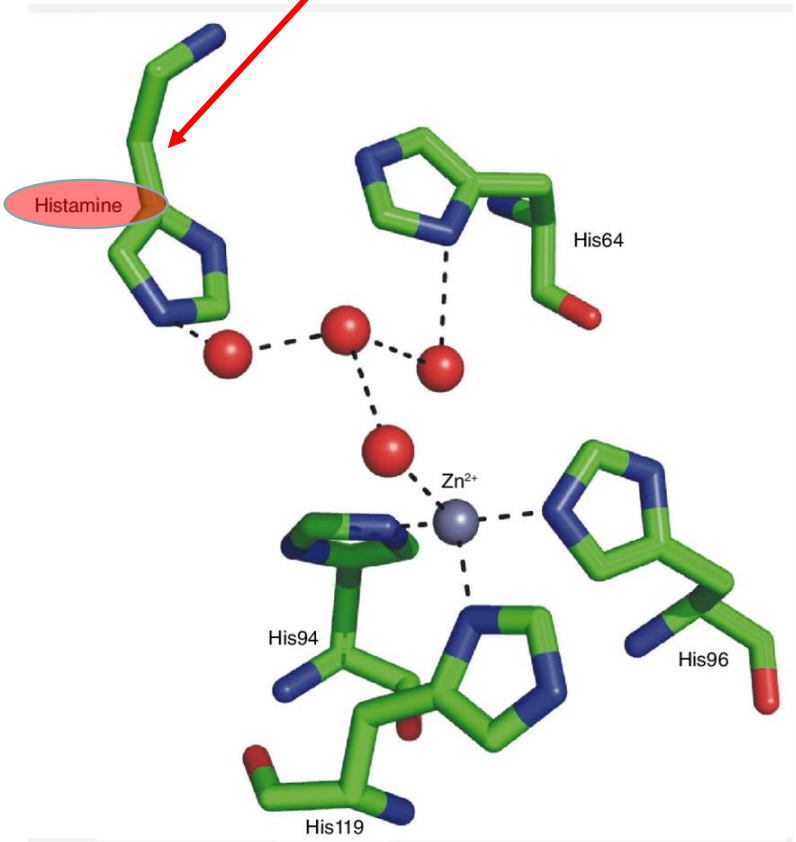



N
C
His96
H2O
C
C
N
C
Zn
C
N
N
C
C
N
His119
C
C
N
His94
C
C
C

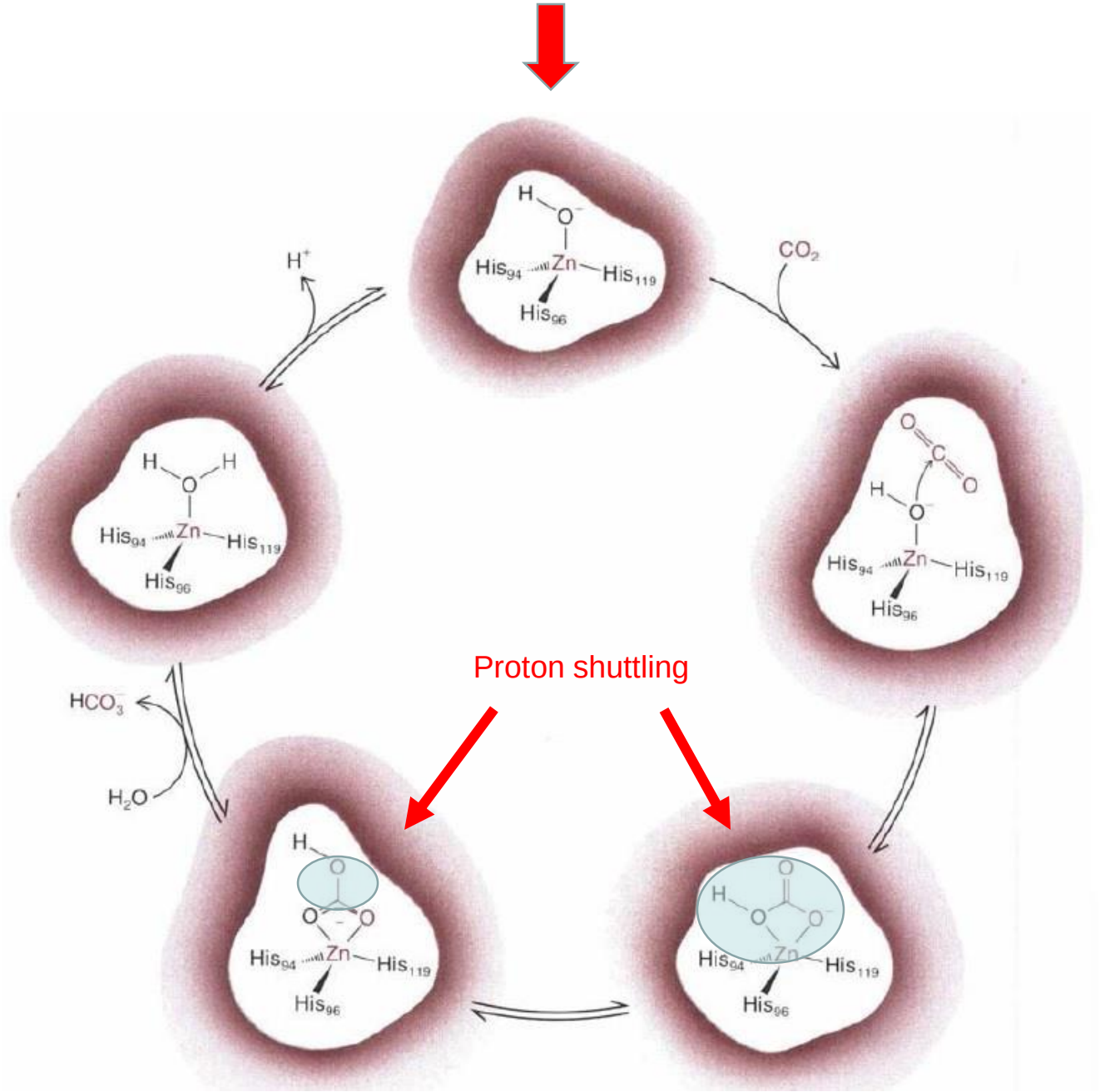
Carbonic Anhydrase II

Hys decarboxylation → Hystamine

- **An ordered array of water molecules in the active site is of special importance**
- **Rate determining step is NOT CO2/HCO3- conversion but rather proton shuttling under participation of amino-acid side chains and water network**

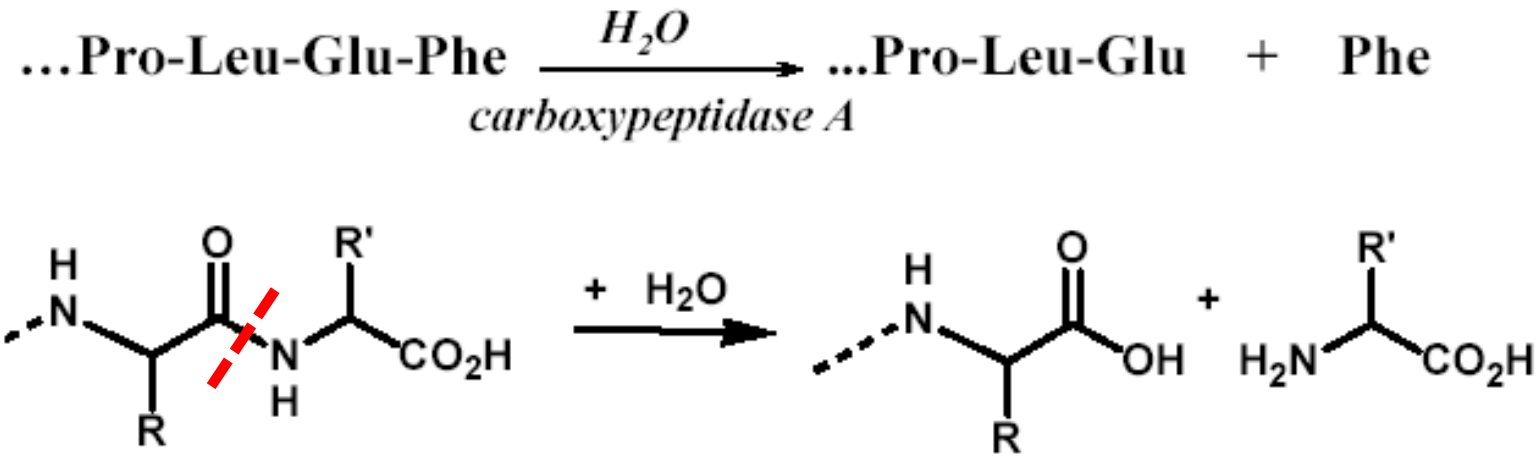


The Zn(II) ion, its ligands, His64 and the water molecules (red spheres) involved in the carbonic anhydrase activation mechanism are shown [7].


H+
CO2
HCO3−
H2O
Proton shuttling
His94
Zn
His119
His96

Carboxypeptidase A

☆ ***Carboxypeptidase A*** **(CPA) is a pancreatic enzyme that cleaves the carboxyl terminal amino acid from a peptide chain by hydrolyzing the amide linkage. There is a high selectivity for substrates with large terminal aliphatic or phenyl substituents**



Carboxypeptidase A

☆ ***Basic Description:***

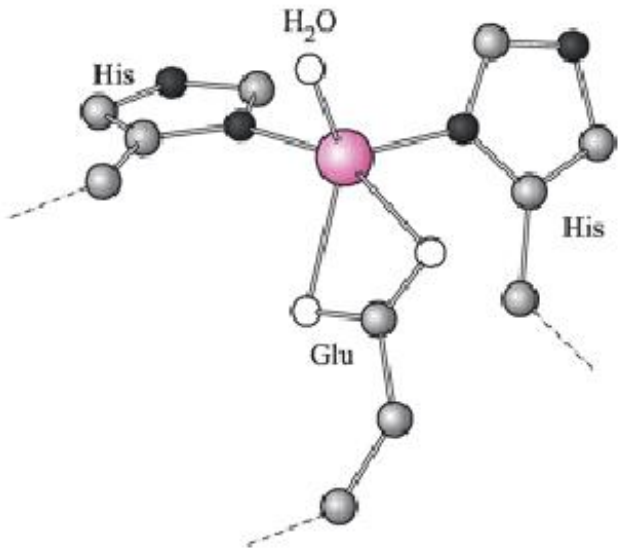
- **Consists of 307 amino acid residues plus one Zn^{2+} ion**
- **MW of *ca.* 34,600**
- **Roughly egg-shaped with approx. dimensions 50 Å x 38 Å**

Water ~ 20 -30 $Å^3$

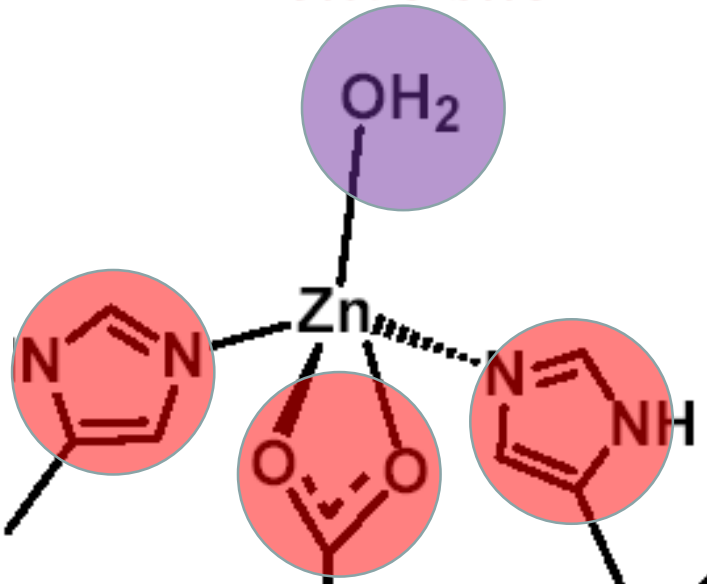
☆ ***Active Site***

- **A cleft on one side contains the Zn^{2+} ion = *active site***
- **The zinc ion is coordinated by two N atoms (histidines) and two O atoms (glutamate) in the protein chain; the fifth site contains H_2O.**

Carboxypeptidase A



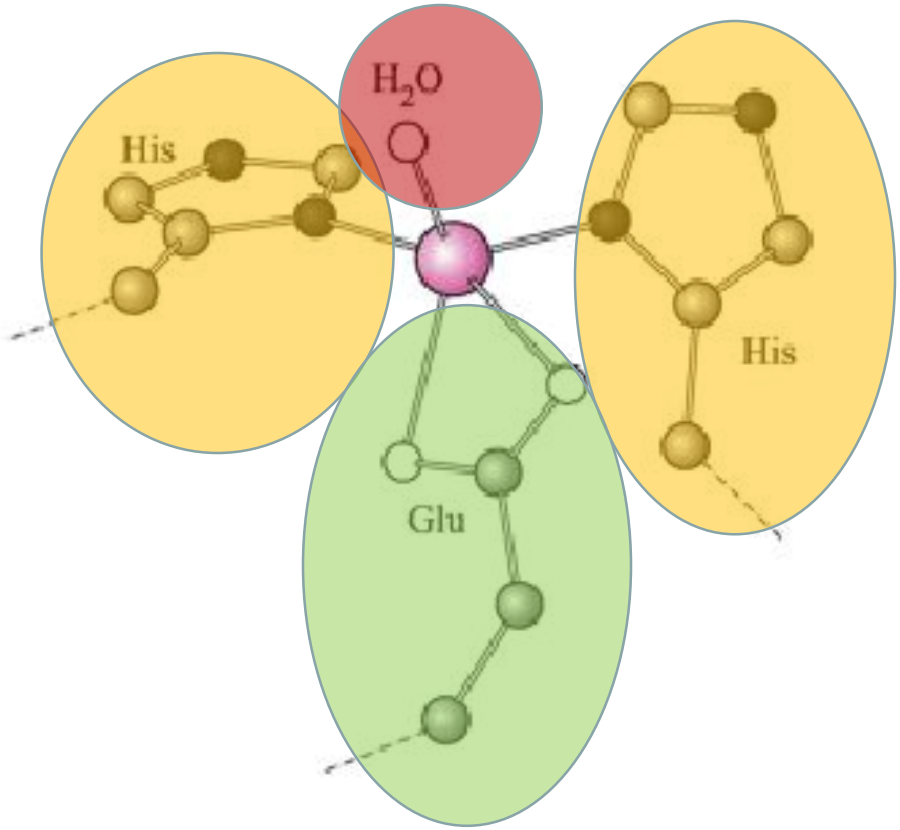
Active site



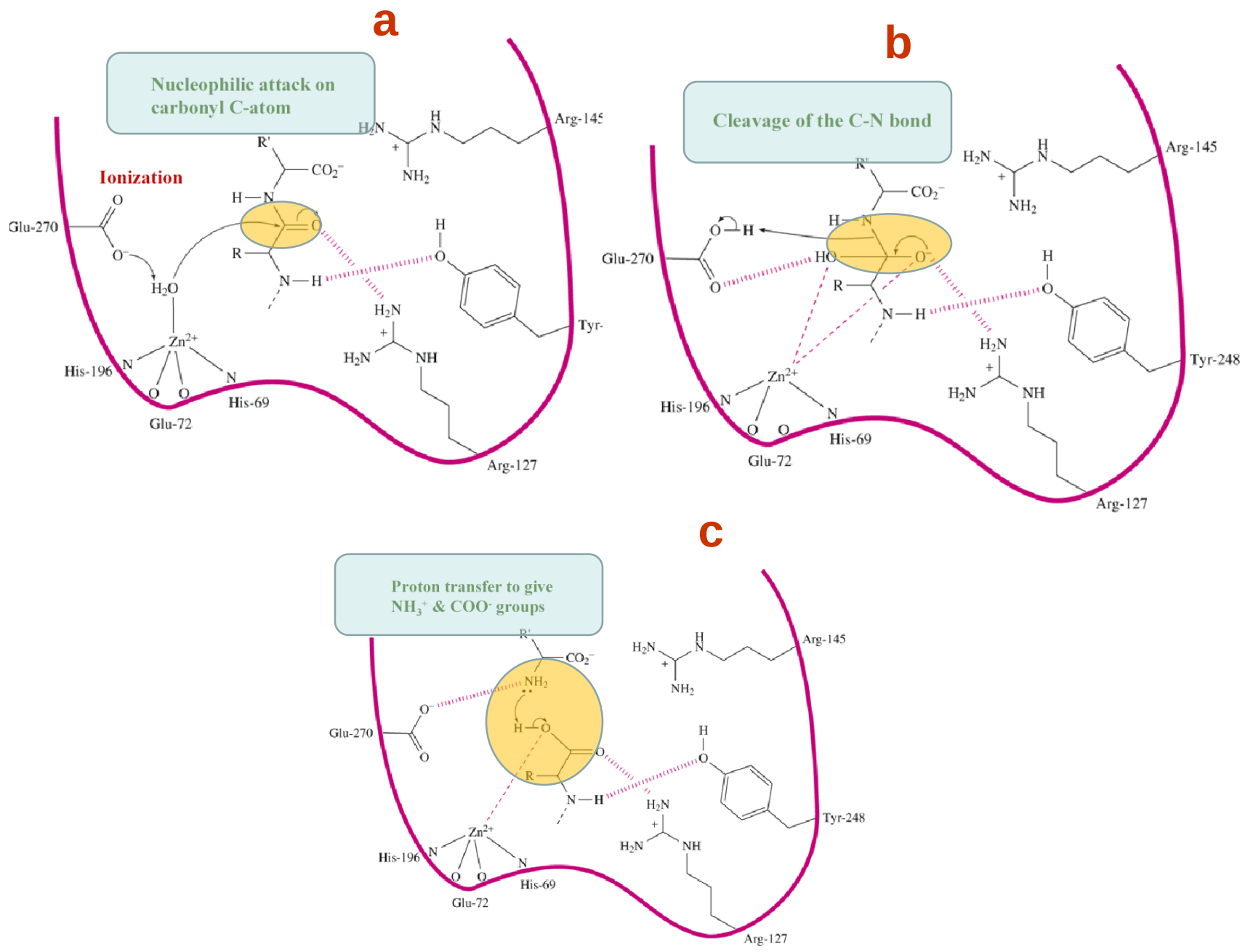
Histidine-196

Histidine-69

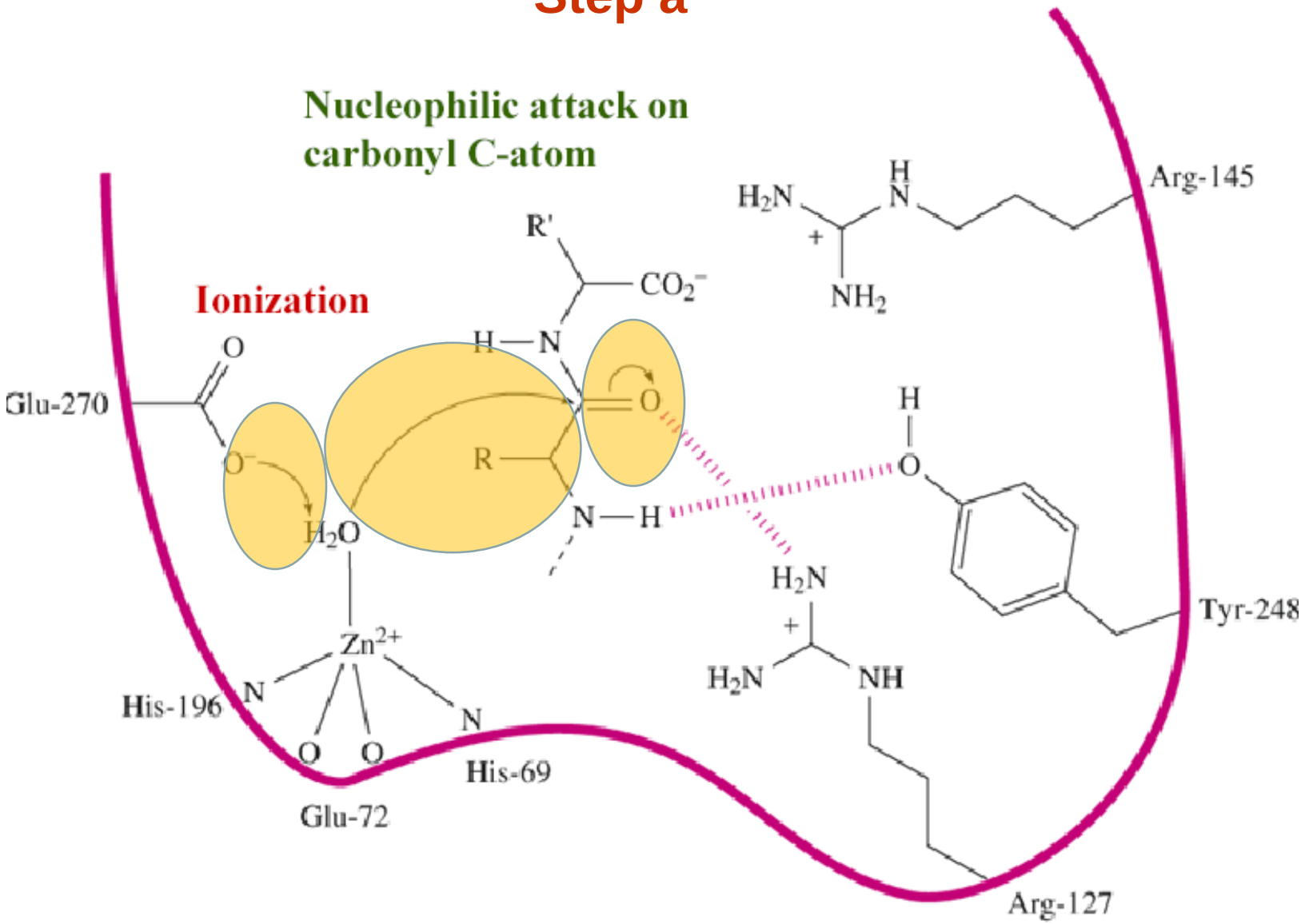
Glutamate-72



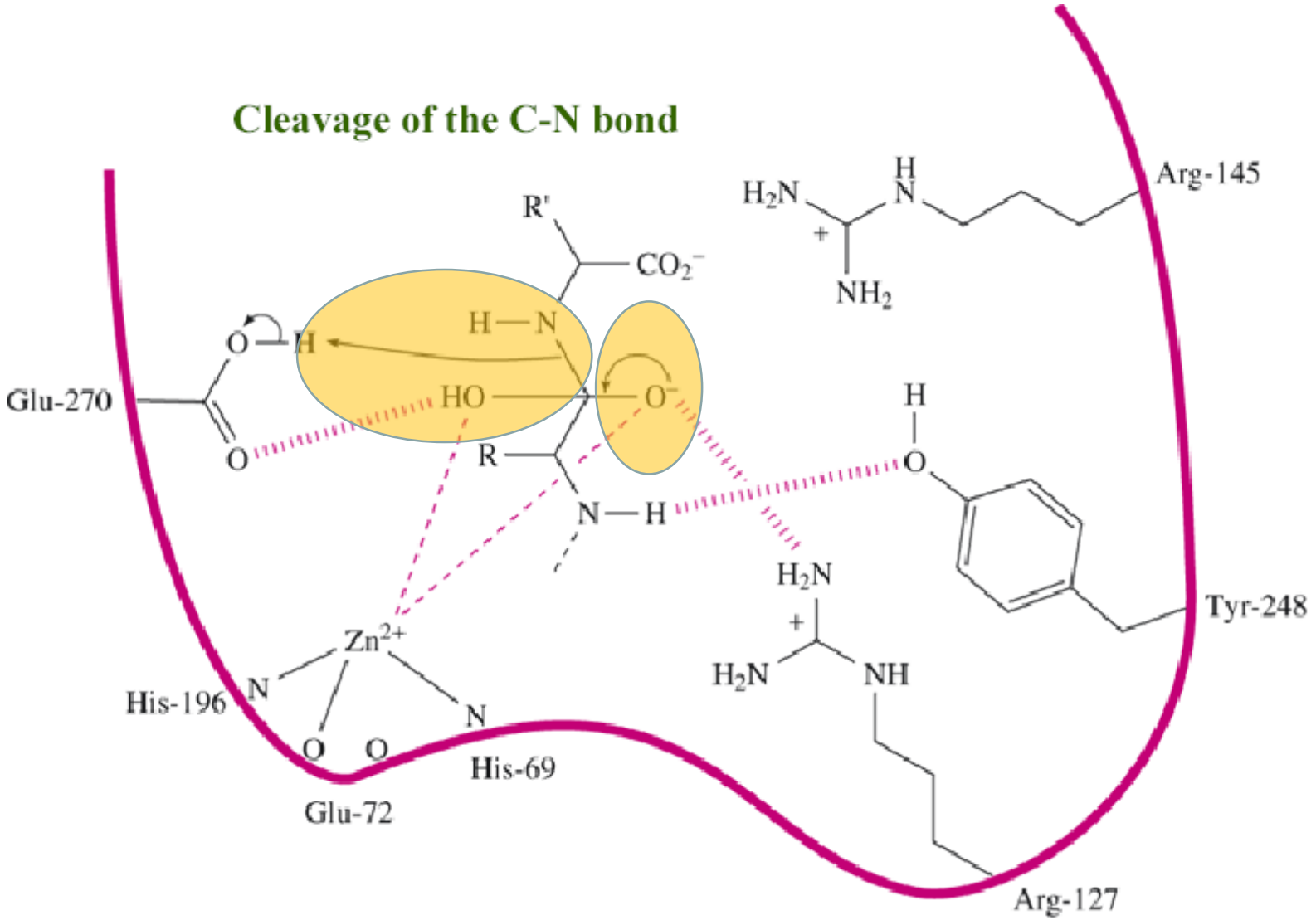
always 4 ligands → tetracoordinate


a
Nucleophilic attack on carbonyl C-atom
Ionization
Glu-270
Arg-145
Tyr-
His-196
His-69
Glu-72
Arg-127
b
Cleavage of the C-N bond
Glu-270
Arg-145
Tyr-248
His-196
His-69
Glu-72
Arg-127
c
Proton transfer to give NH3+ & COO- groups
Glu-270
Arg-145
Tyr-248
His-196
His-69
Glu-72
Arg-127

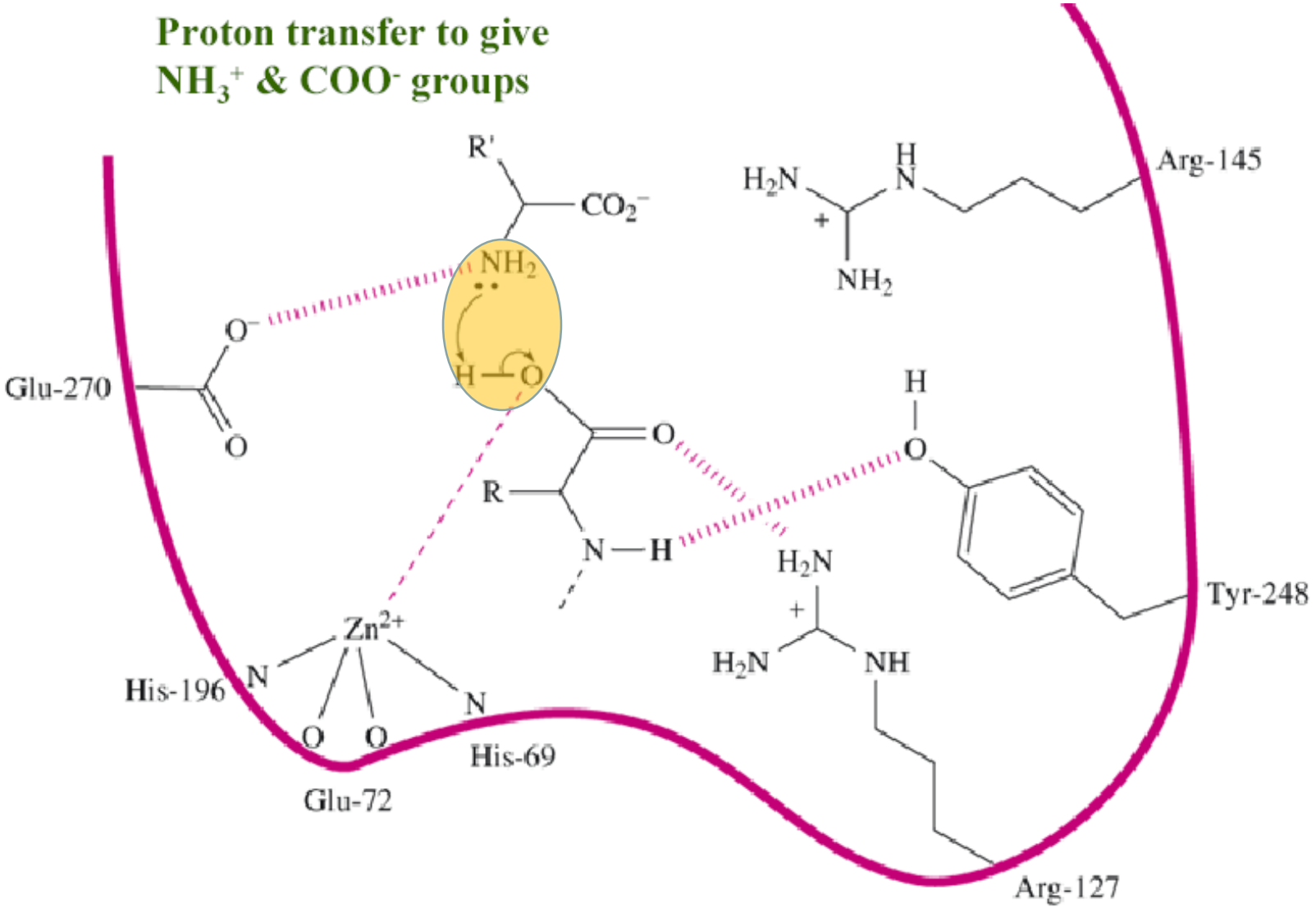
Step a


Nucleophilic attack on carbonyl C-atom
Ionization
Glu-270
Arg-145
Tyr-248
Arg-127
His-196
His-69
Glu-72
R'
CO_2^-
H_2O
Zn^{2+}

Step b

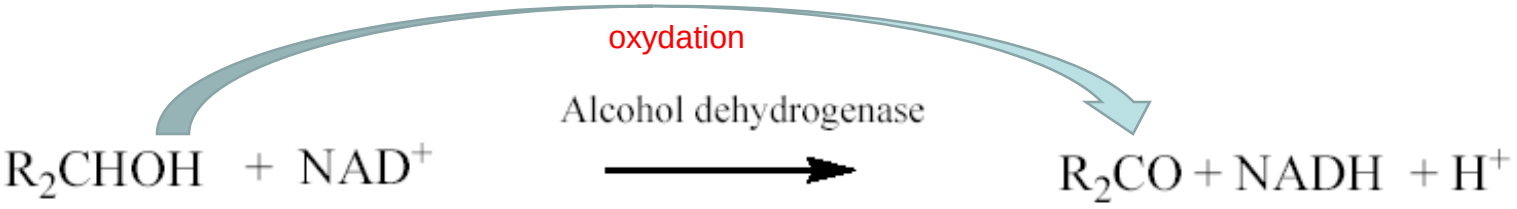

Cleavage of the C-N bond
Arg-145
Glu-270
Tyr-248
His-196
His-69
Glu-72
Arg-127

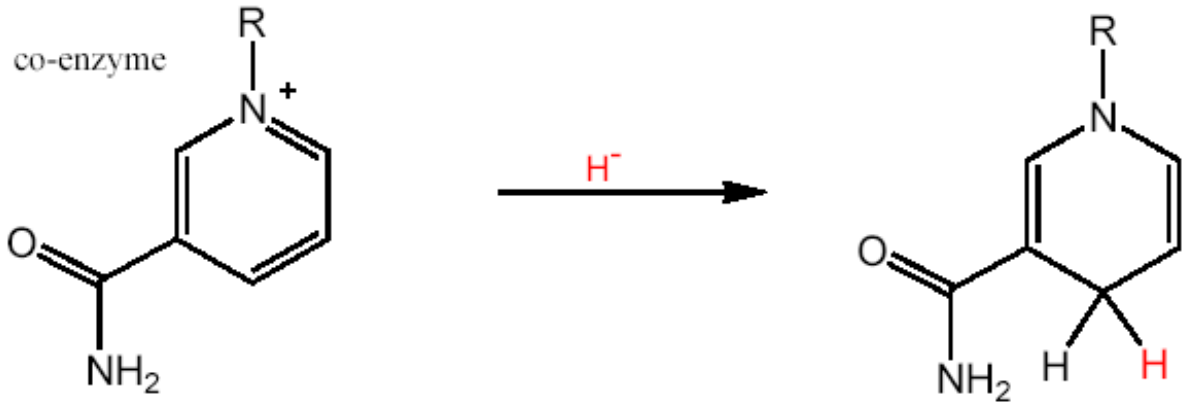
Step c


Proton transfer to give
NH3+ & COO- groups
R'
CO2−
NH2
H2N
H
N
+
NH2
Arg-145
O−
Glu-270
O
H
O
O
R
N
H
H
O
Tyr-248
Zn2+
His-196
N
N
O
O
Glu-72
His-69
H2N
+
H2N
NH
Arg-127

Alcohol dehydrogenase

Alcohol dehydrogenase converts alcohols to aldehydes or ketones





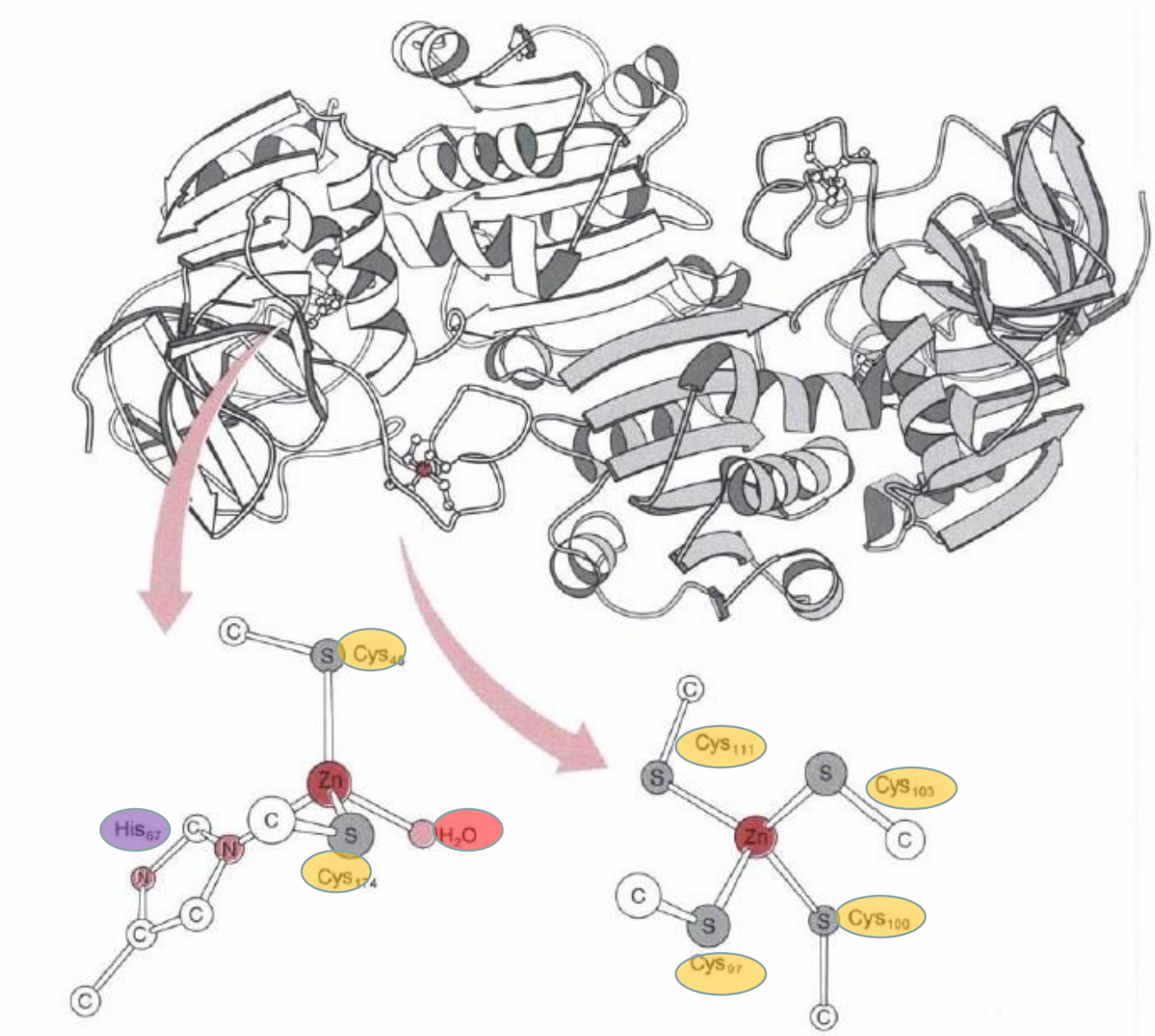
Nicotinamide Adenine Dinucelotide

NADH

Alcohol dehydrogenase

☆ Liver alcohol dehydrogenase comprises two 40-kD single polypeptide sub-units, each of which contains two zinc ions. One Zn atom is in the catalytic site which also binds NAD+. The other Zn atom plays a structural role binding four cysteinate residues.

☆In the oxidation of alcohol, two hydrogen atoms are removed - one to the 4-position of NAD+ and the other as a proton. The transfer to NAD+ is generally thought to be a hydride transfer.



catalytic

structural (inaccesible to solvents)