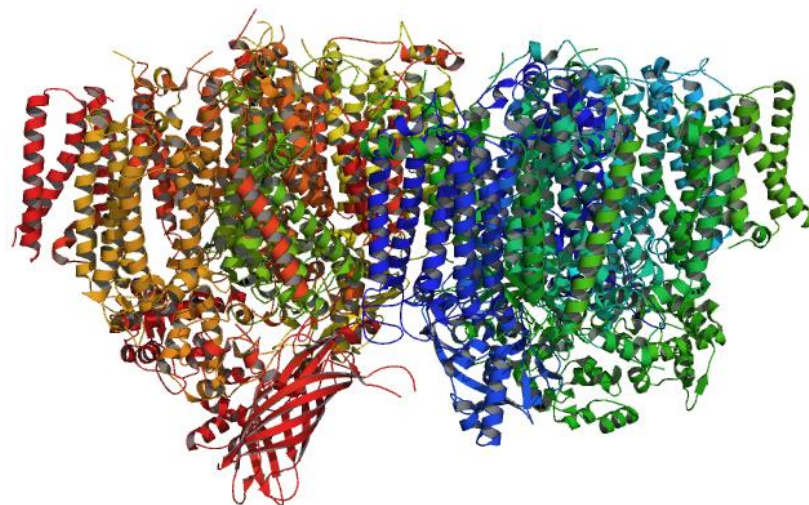
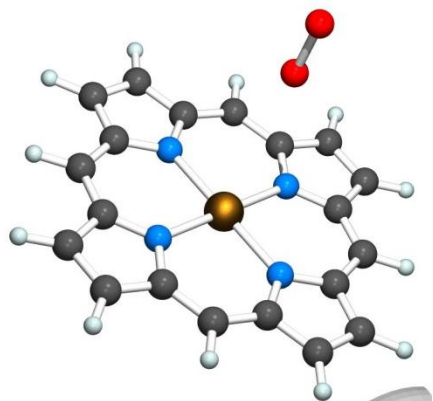
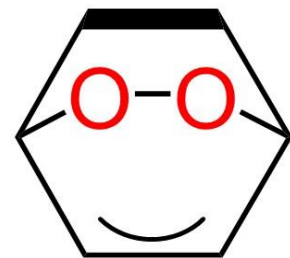


Bioinorganic Chemistry (BIC)

III. Metal-Dependent Hydrolase and Lyase Enzymes



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Department of Chemistry



Review on Part II

Biological ligands for coordination to metals: Generally, **heteroatoms** of **biological molecules** (e.g. proteins, DNA/RNA, cofactor) as potential **ligands** can coordinate to metal: **coordination modes** depends on ligands, metal(s), biological molecules.

Coordination to metal can **sometimes maintain the overall (folded)** structure, but sometimes **only stabilize** its **binding site** in **some** biological molecules.

Storage & Transport of small molecules (e.g. O₂ and metal ions) by some bioinorganic systems can keep **sufficient amounts/distribution** (not too many or few) of the molecules for various biological functionals.

Classification of Enzymes

Enzyme Class	Enzyme Function
EC1 Oxidoreductase	Oxidation–reduction
EC2 Transferase	Transfer of a group from one compound to another
EC3 Hydrolase	Hydrolysis
EC4 Lyase	Nonhydrolytic addition or removal of groups
EC5 Isomerase	Conversion of a substance into an isomeric form
EC6 Ligase	Synthesis of a large molecule from two smaller ones

EC, Enzyme Commission number:

Classification scheme for enzymes based on the **chemical reactions** involved.

Hydrolases

A family of **enzymes** catalyze **hydrolysis** of a chemical bond, in which the **metal** (**cofactor**) acts as a **Lewis acid** generally.



It can be further classified based on the bond involved:

EC 3.1: Ester bonds

EC 3.2: Sugars (DNA glycosylases, glycoside hydrolase)

EC 3.3: Ether bonds

EC 3.4: Peptide bonds (proteases/peptidases)

EC 3.5: C-N bonds other than peptide bonds

EC 3.6: Acid anhydrides

EC 3.7: C-C bonds

EC 3.8: Halide bonds

EC 3.9: P-N bonds

EC 3.10: S-N bonds

Lyases

A family of **enzymes** catalyze **non-hydrolytic addition or removal** of **groups** (non-H₂O).

It can be further classified based on the bond involved:

EC 4.1: C-C Lyases, e.g. decarboxylases, aldehyde lyases & others C-C lyases

EC 4.2: C-O Lyases

EC 4.3: C-N Lyases

EC 4.4: C-S Lyases

EC 4.5: C-Halide Lyases

EC 4.6: P-O Lyases

EC 4.99: Other lyases

- For metal-dependent hydrolases & lyases, a **metal cation**, or sometimes **cations**, (as the cofactor) with a **high Lewis acidity** is important for many hydrolysis & condensation reactions under physiological conditions.

- The general **roles** of the **metal(s)**:

- (1) **activate** a metal-bound **nucleophile** (e.g. ionization of $M-OH_2$ to give a **more reactive** $M-OH$ form),

- (2) **stabilize** a metal-bound **intermediate/product**, or

- (3) sometimes **bring** the **reactant** species in the **active site** (**structural** role).



Zn(II), pK_a : ~8.8; **Mg(II)**, pK_a : ~11.4

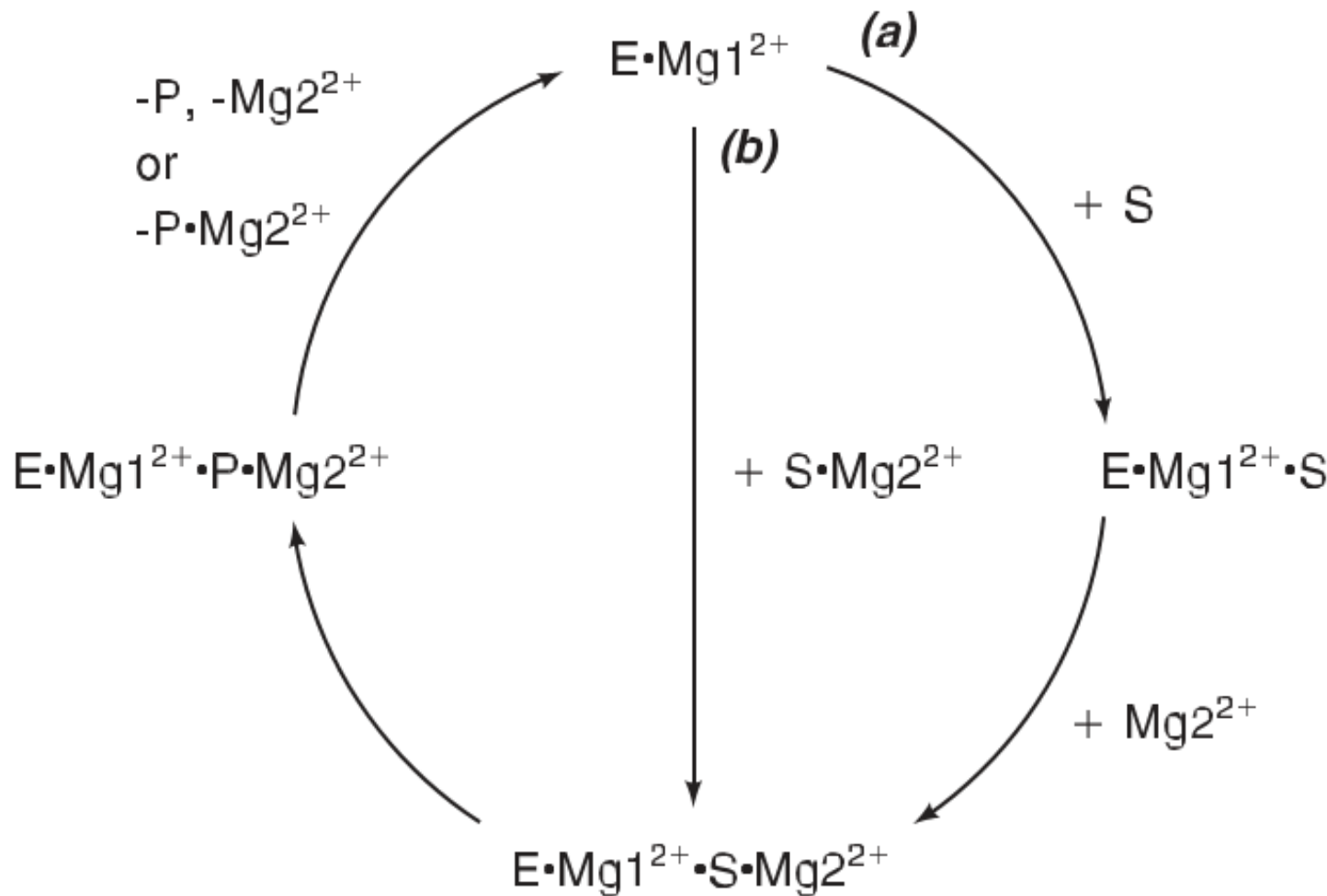
pK_w : 14.0 (25 deg.)

- **Non-redox metals** are widely used in these enzymes to protect sensitive functional groups in proteins and nucleic acids from oxidative damage. Namely, **Zn^{2+} & Mg^{2+} (sometimes Ca^{2+})** are **common metals**.
- **“Intermediate”** Lewis acid **Zn^{2+}** ion center in these enzymes is usually **tetrahedral** & coordinates to **“Intermediate” base histidine (His)** & sometimes to soft cysteine (Cys) or hard carboxylate residues or H_2O .
- Whereas, **harder Mg^{2+}** ion center in these enzymes typically coordinates to **hard carboxylate** residues or **water** molecules (usually **octahedral**).

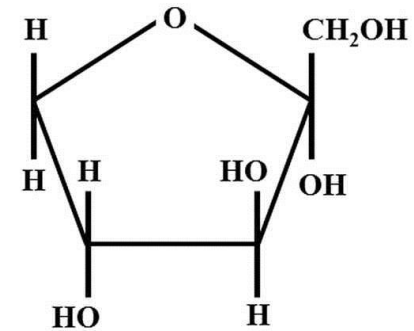
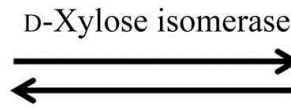
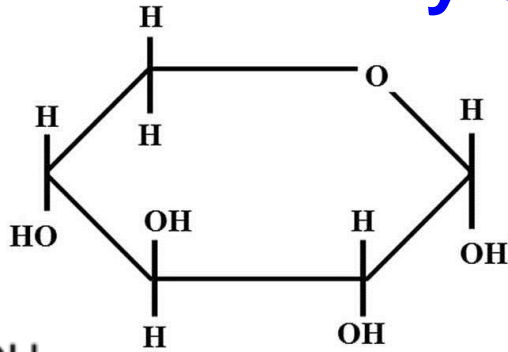
Magnesium (Mg)

Some Mg-dependent enzymes require at least **two** metal-binding sites: (1) an **allosteric regulatory** site modulating either **structure or binding (structural role)**, (2) a **catalytic** site.

**General
mechanistic
features for
transforma-
tions on the
glycolytic
pathway**

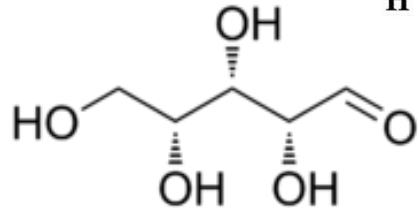


Xylose Isomerase: Mg



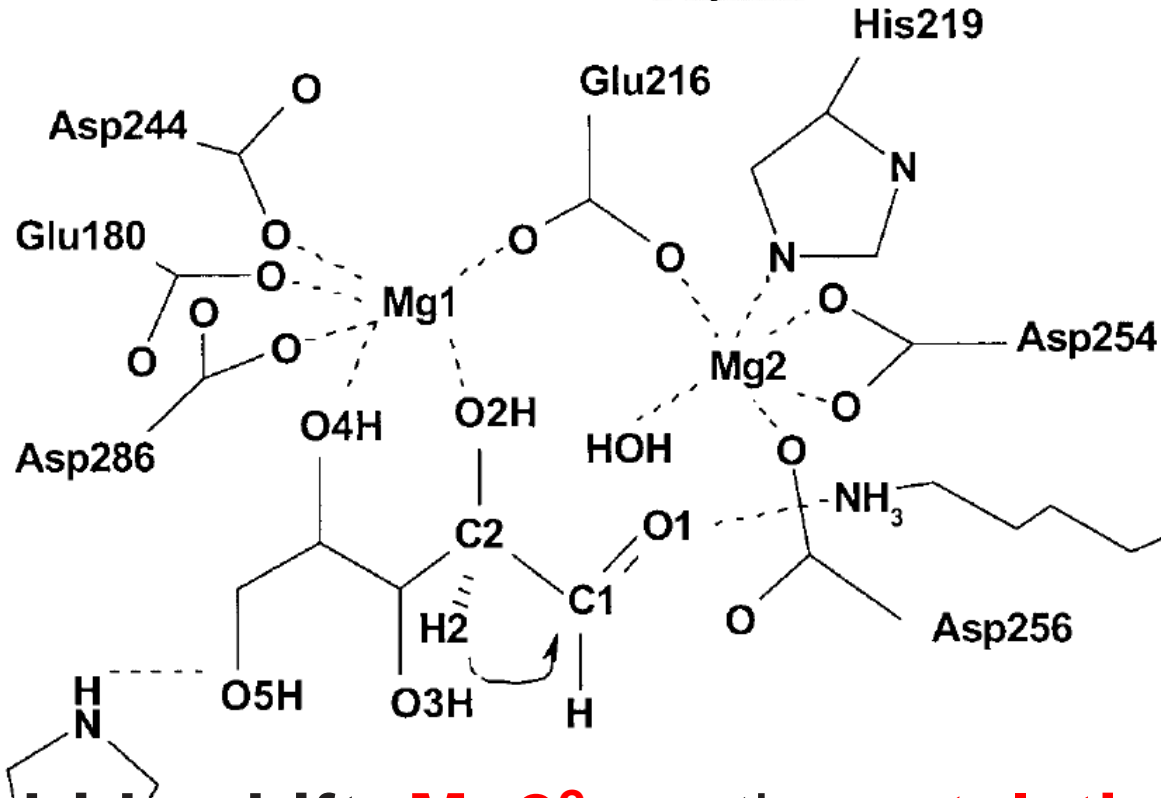
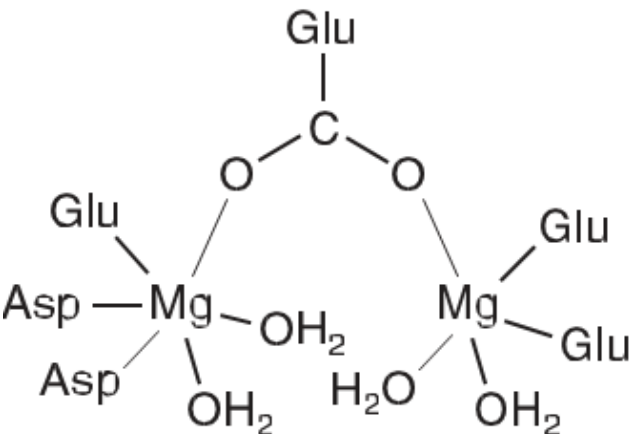
D-Xylose

D-Xylulose



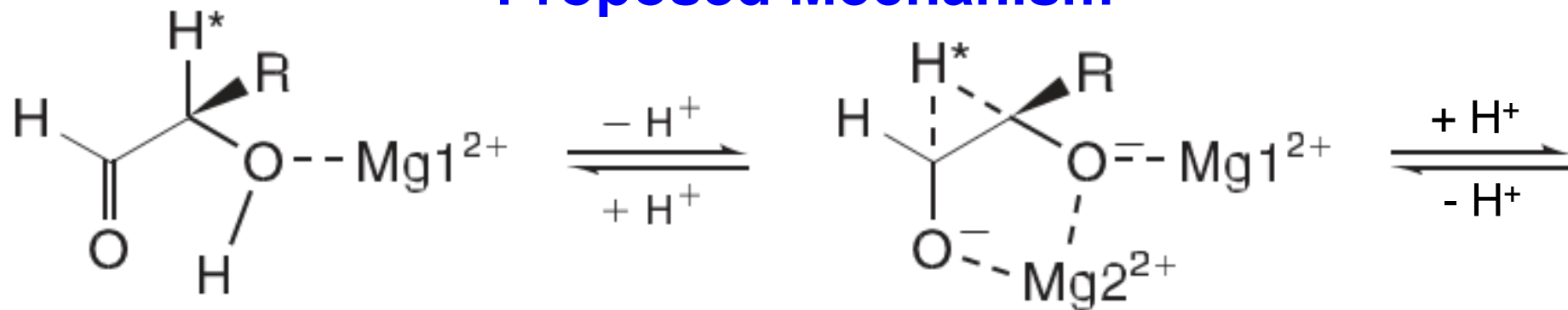
木糖

Open-form



Mg-catalyzed 1,2-hydride shift: **Mg²⁺** as the **catalytic** ion, while **Mg¹⁺** likely plays a **structural** role.

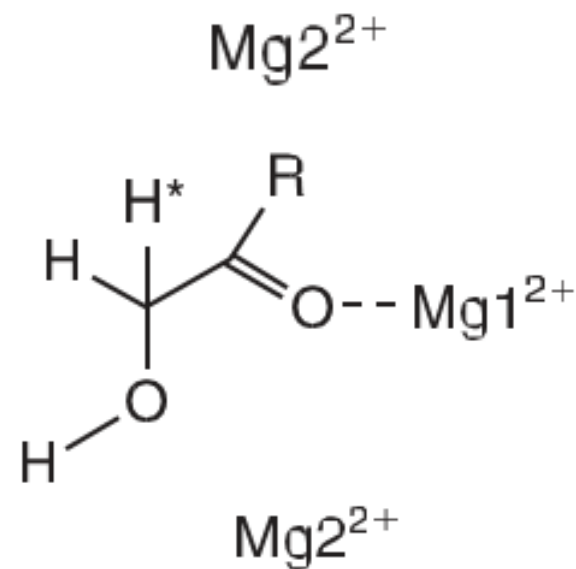
Proposed Mechanism

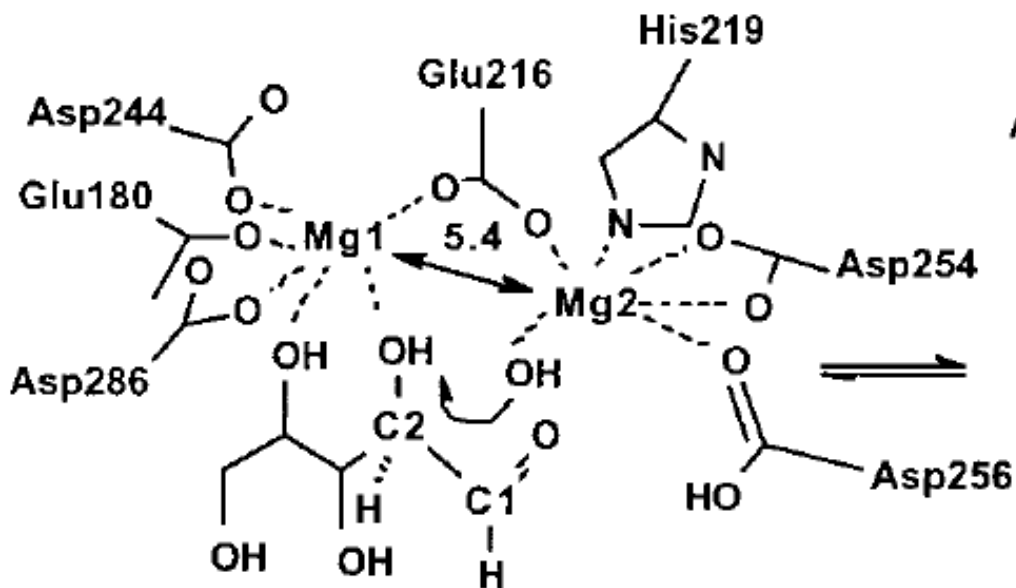


Kinetic results suggests:

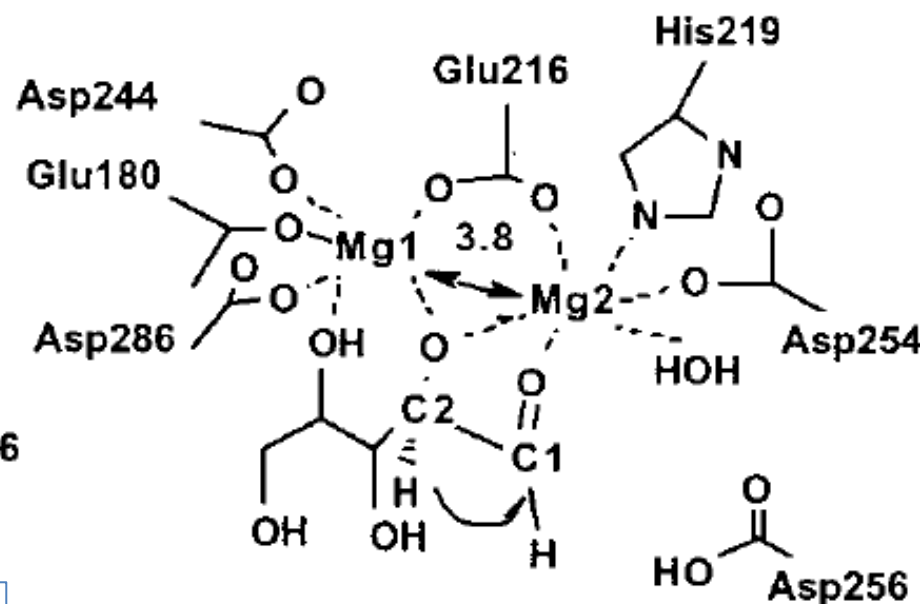
- Mg²⁺ **first binds to site 1** (higher affinity), facilitating **substrate binding & proper orientation** and **stabilizing intermediates**.
- The **second Mg²⁺** then binds, & **initiates the reaction**.

- A Mg²⁺-bound water molecule likely assists proton transfer between the hydroxyl and carbonyl oxygens.

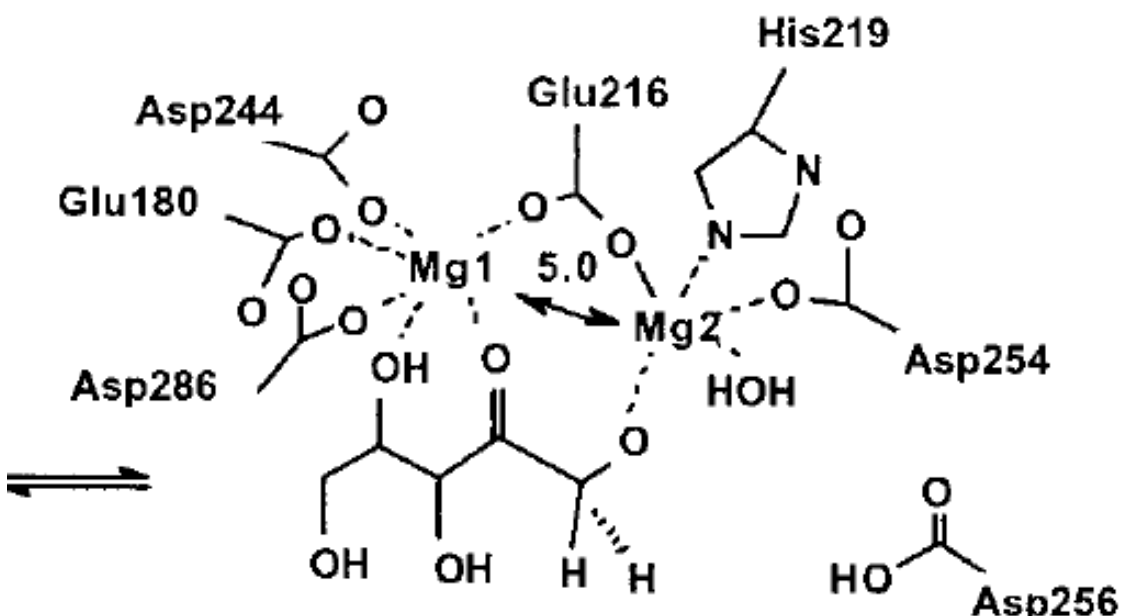




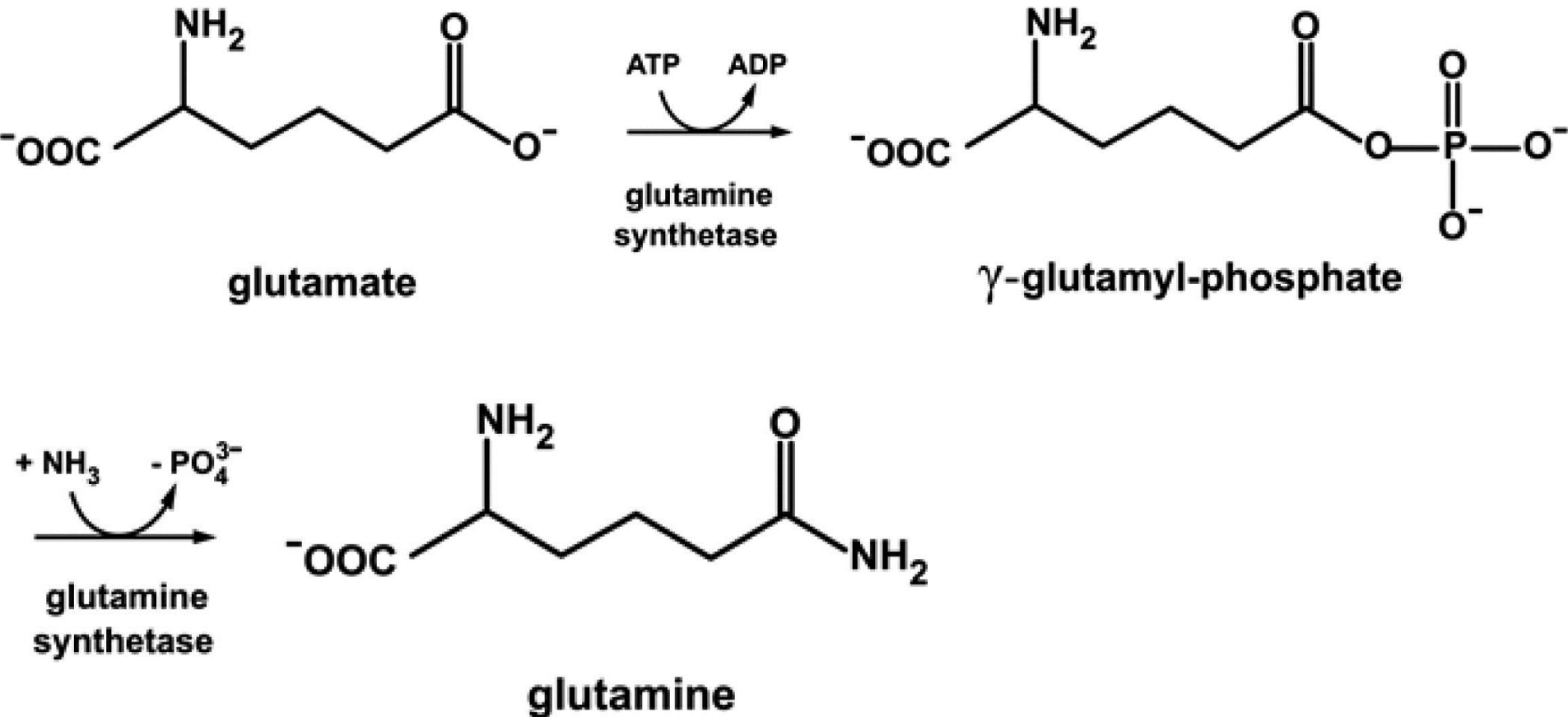
Mg(II)-assisted proton transfer



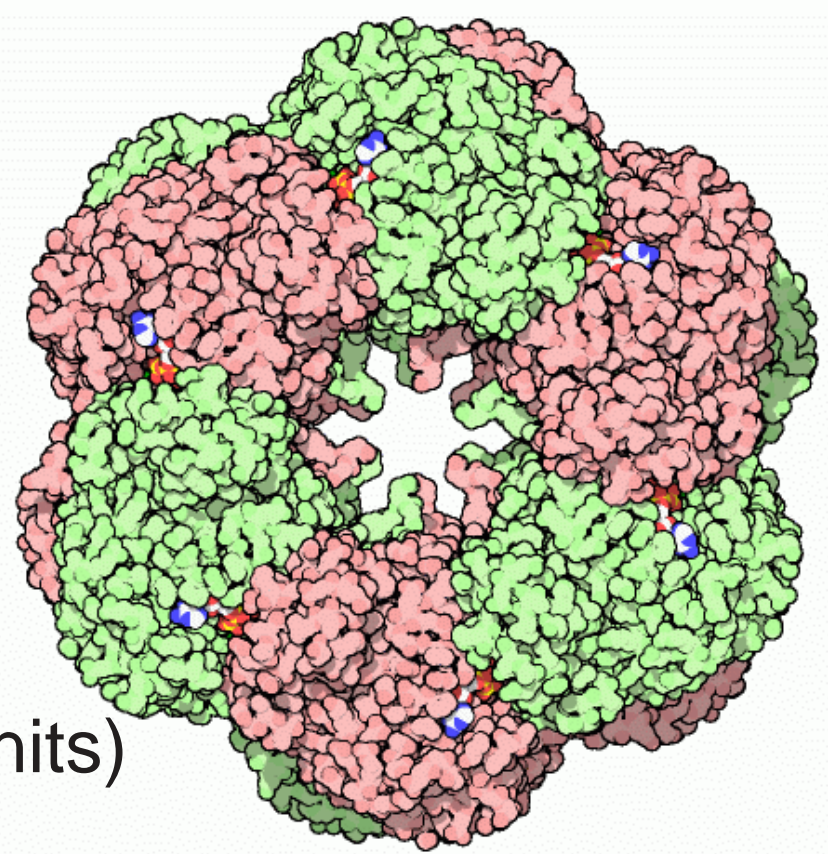
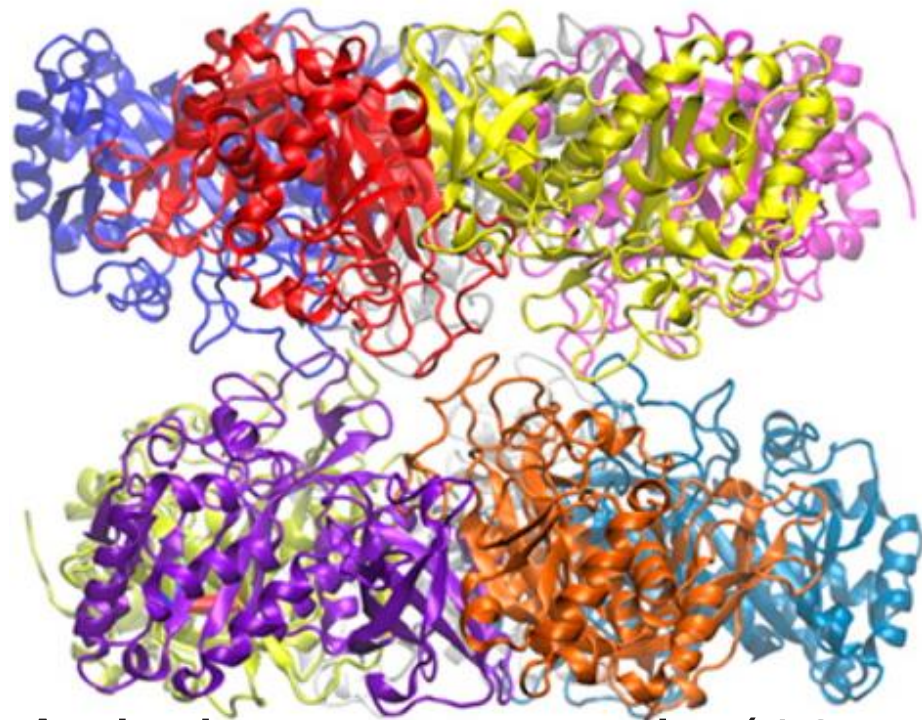
Mg(II)-assisted 1,2 hydride shift



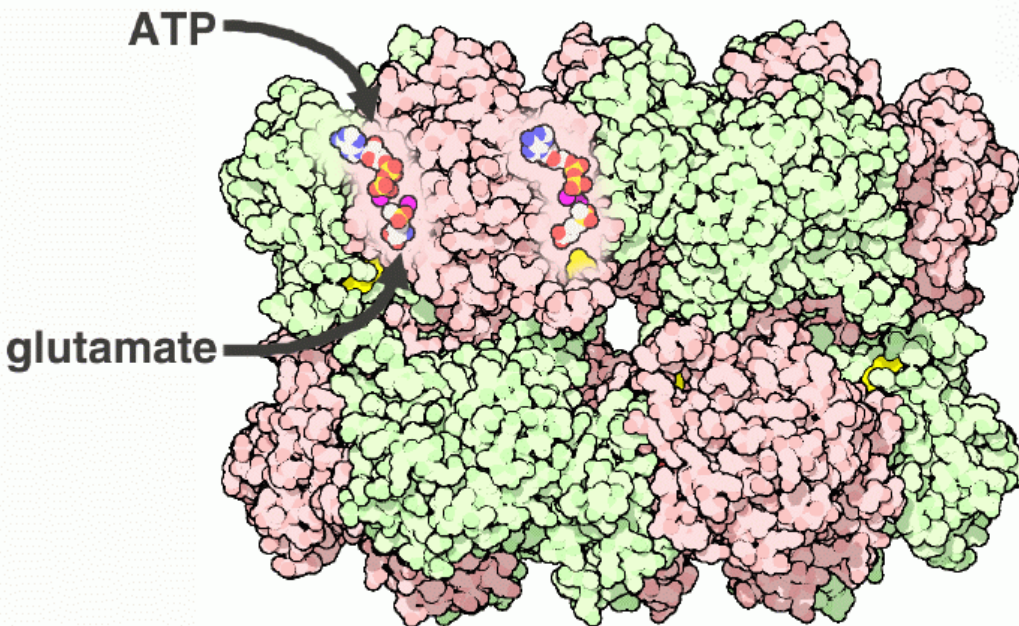
Glutamine Synthetase: Mg

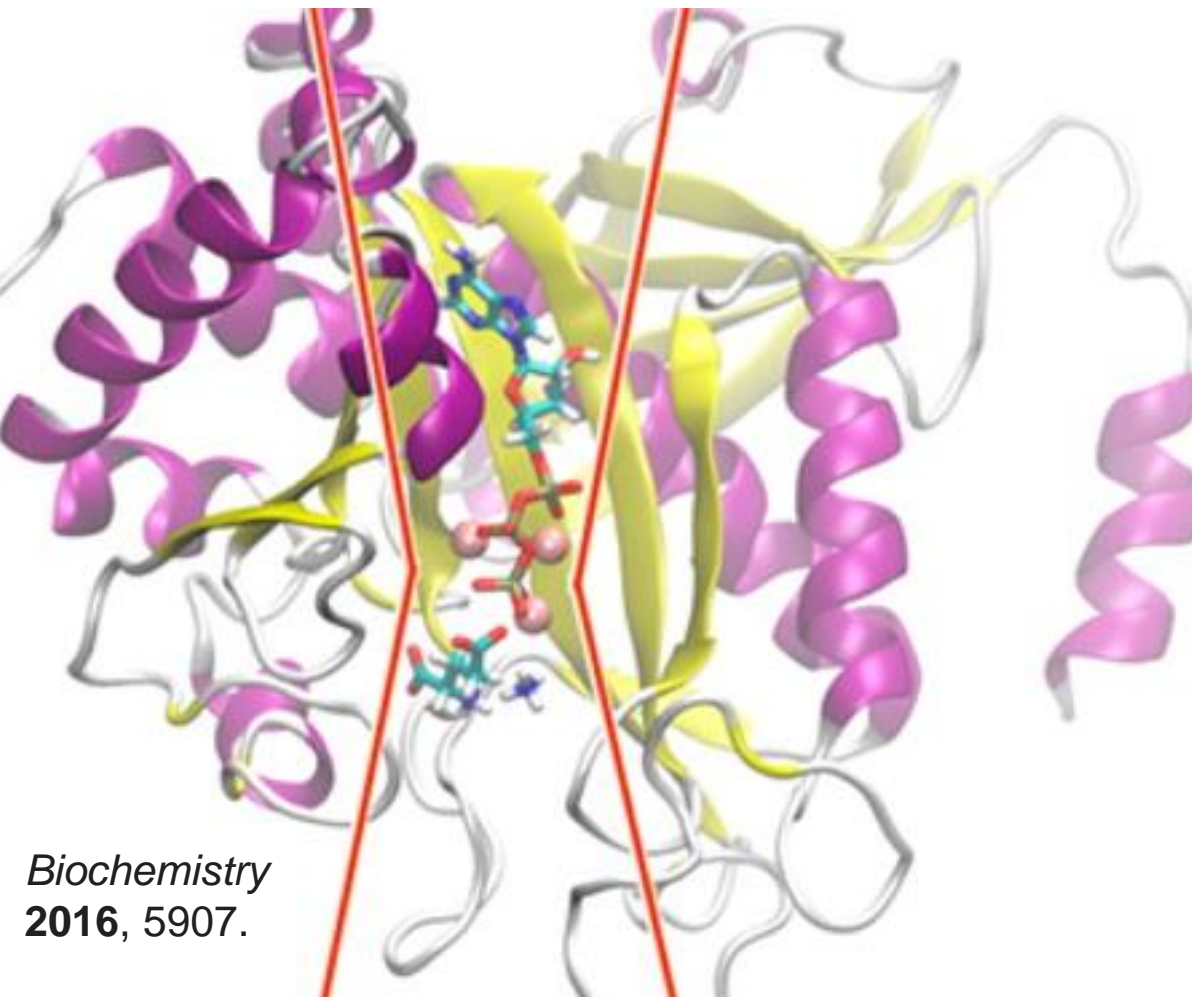


Catalyzes the formation of **glutamine (Gln)** from **glutamate (Glu) & NH_3** with hydrolysis of ATP.



A dodecamer protein (12 subunits)



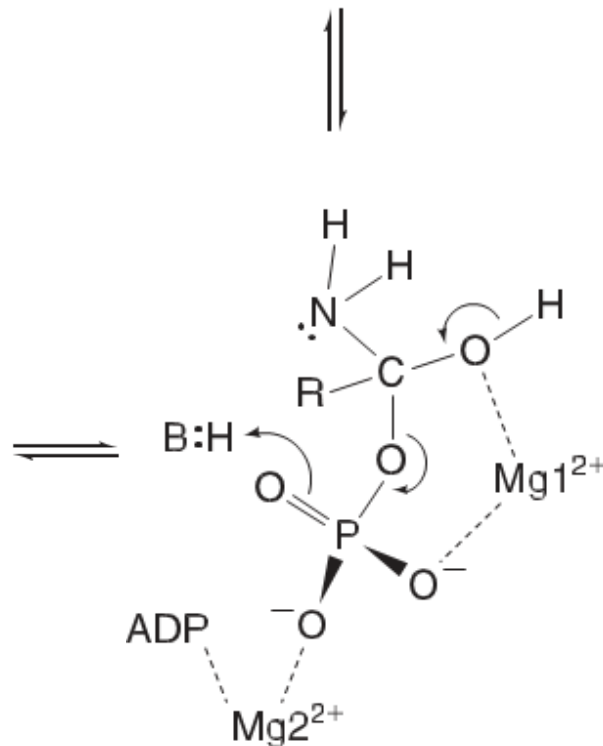
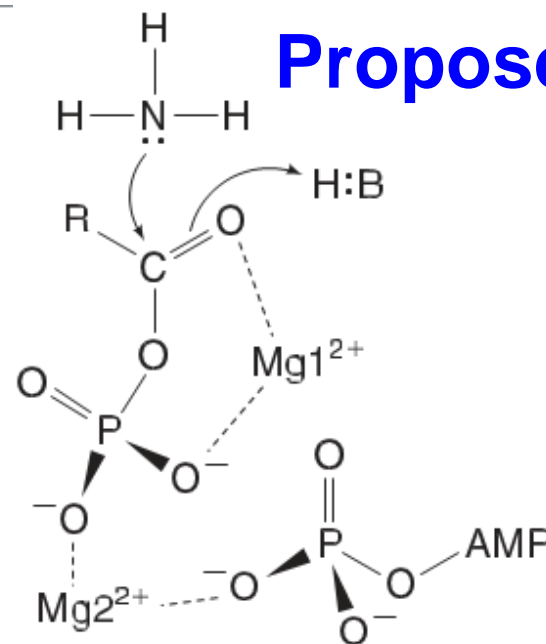
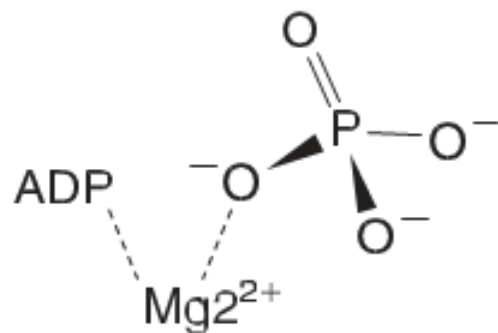
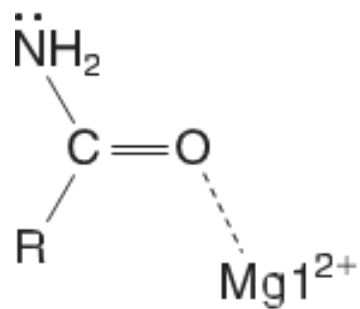
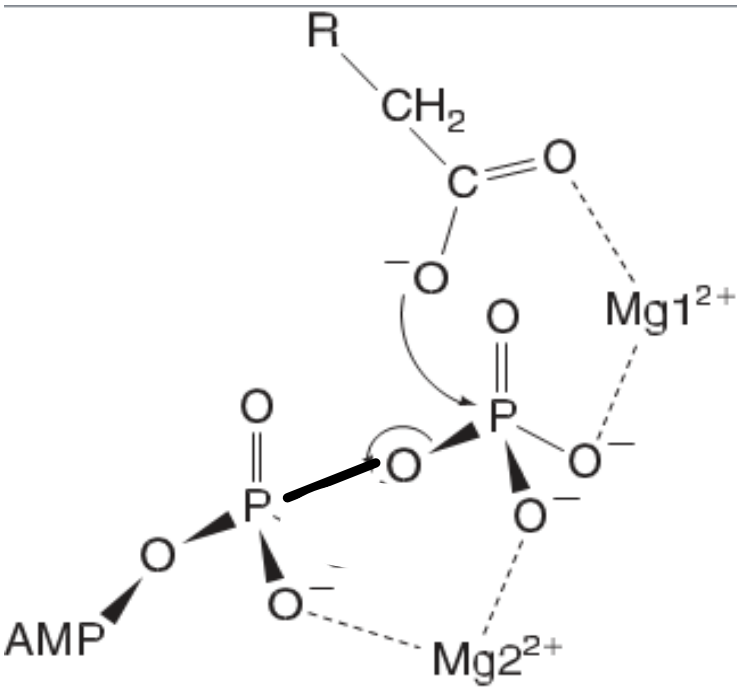


- Crystal structure & kinetic studies: 2 metal-binding sites (high & low affinity) with a Mg-Mg distance (5.8 Å).

Biochemistry
2016, 5907.

- The **high-affinity site (catalytic cofactor)**, while the **weakly** bound ion (possible **binding** of an **Mg-ATP chelate**) in the **low-affinity site**.

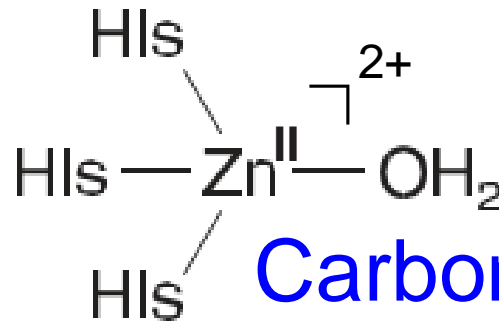
Proposed Mechanism



The catalytic Mg^{2+} ion: a **template** for the reaction (structural role), **electrostatic stabilization** in the transition state, and **Lewis acid** catalysis.

Zinc (Zn)

- pK_a for $Zn^{2+}(aq)$ is $\sim 9-10$, & can drop ~ 7 in an enzyme environment with the **more hydrophobic** environment (favoring reduction in the overall charge).



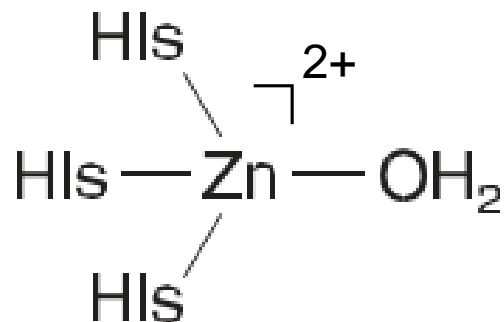
Carbonic anhydrase

- Zn^{2+} readily **activates** bound **water to form $[ZnOH]^+$** (bound HO^- as a Lewis base). Also, H_2O & HO^- bound to Zn^{2+} are **relatively labile** ligands, a requirement for hydrolysis reactions.
- While, Zn^{2+} forms **kinetically inert** bonds to **His** residues.

TABLE 12.1 Coordination Motifs in Catalytic Sites of Some Typical Mononuclear Zinc Enzymes

Carbonic anhydrase	His-X-His-X ₂₂ -His
β-lactamase	His-X-His-X ₁₂₁ -His
Thermolysin	His-X ₃ -His-X ₁₉ -Glu
Carboxypeptidase	His-X ₂ -Glu-X ₁₂₃ -His
Alcohol dehydrogenase	Cys-X ₂₀ -His-X ₁₀₆ -Cys
Alkaline phosphatase	Asp-X ₃ -His-X ₈₀ -His
Adenosine deaminase	His-X-His-X ₁₉₆ -His

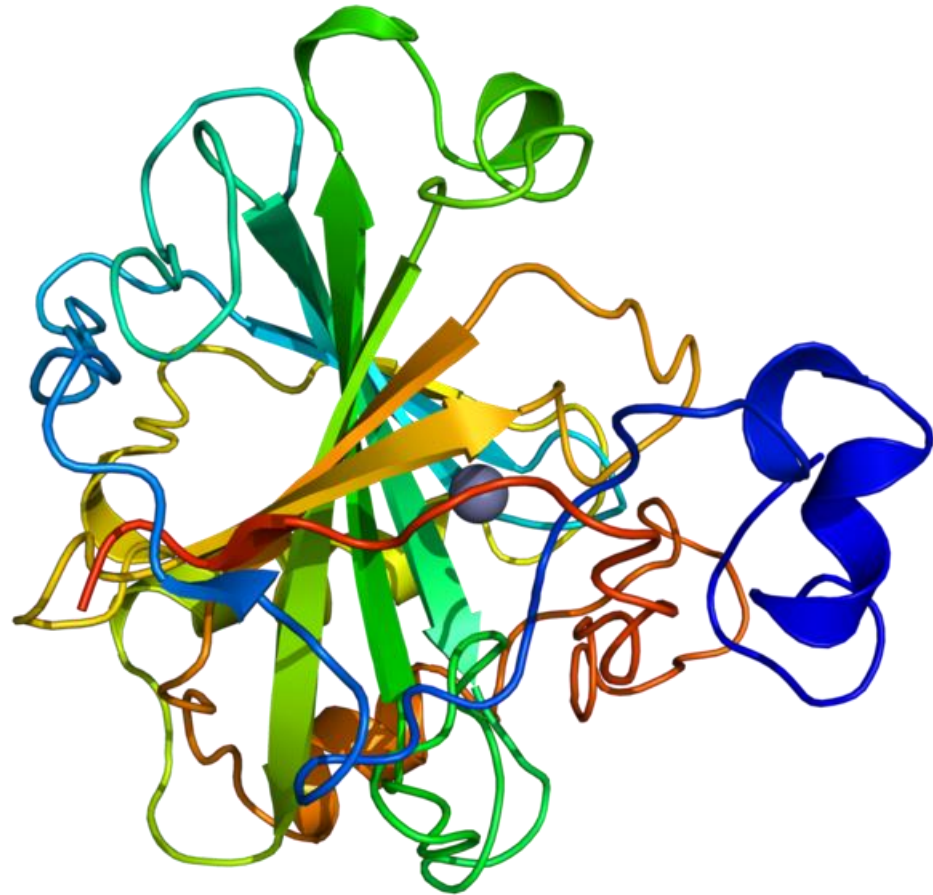
Carbonic anhydrase

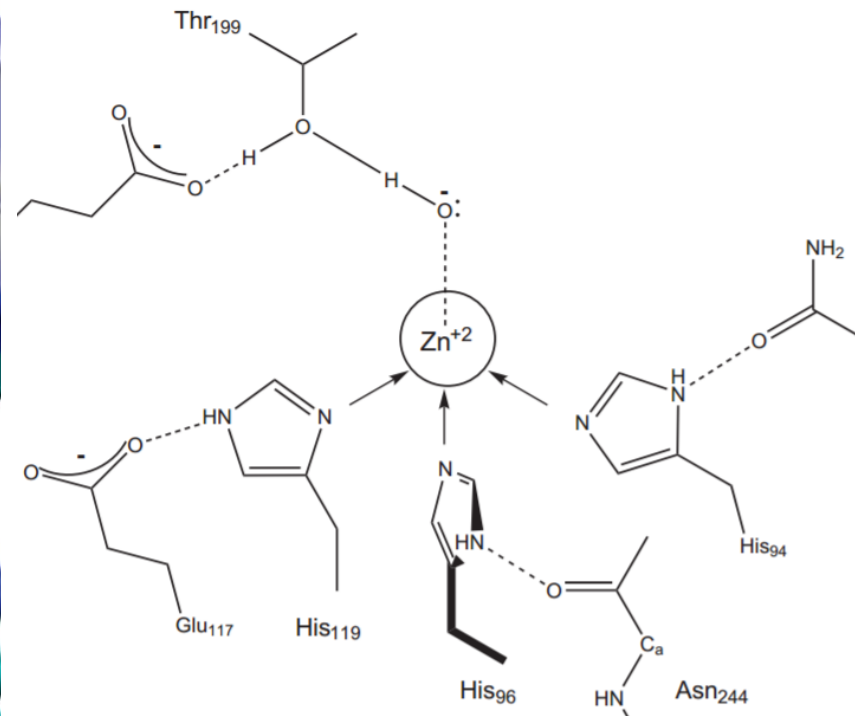
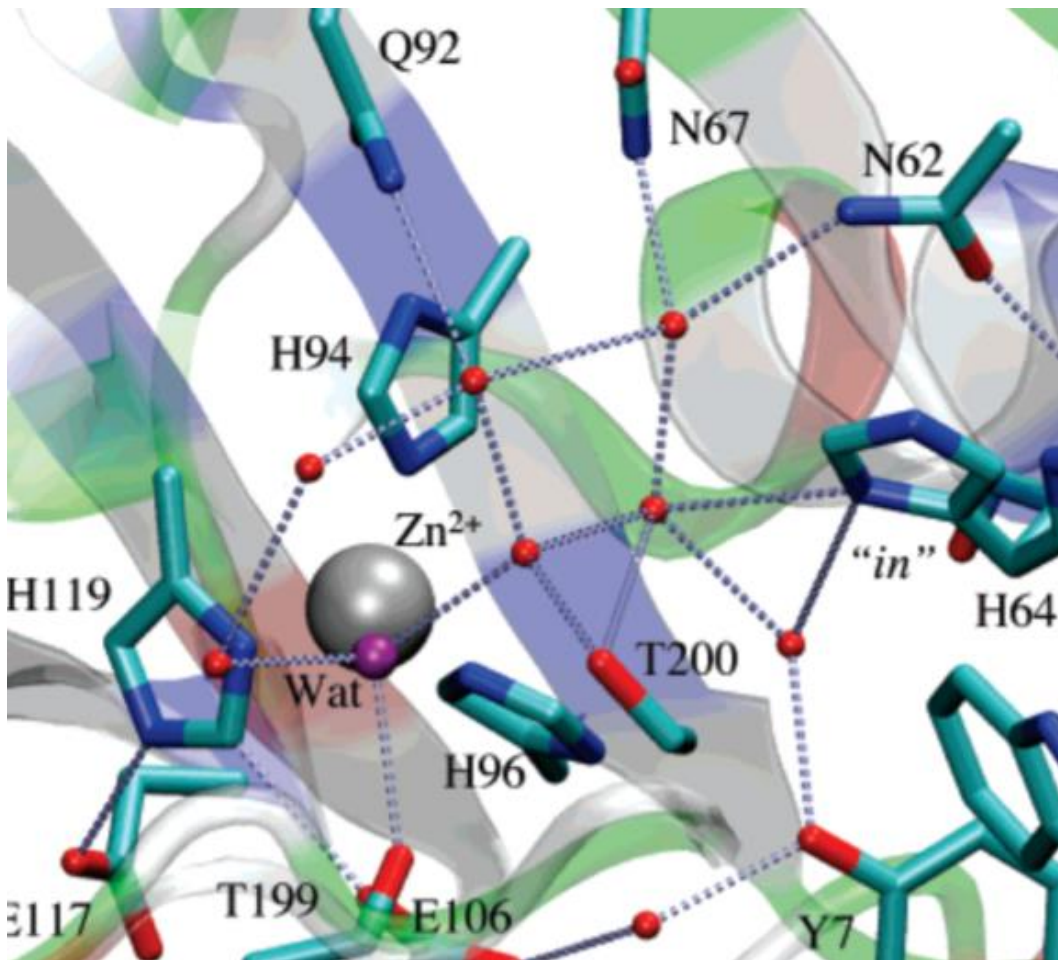


Carbonic Anhydrase (CA): Zn



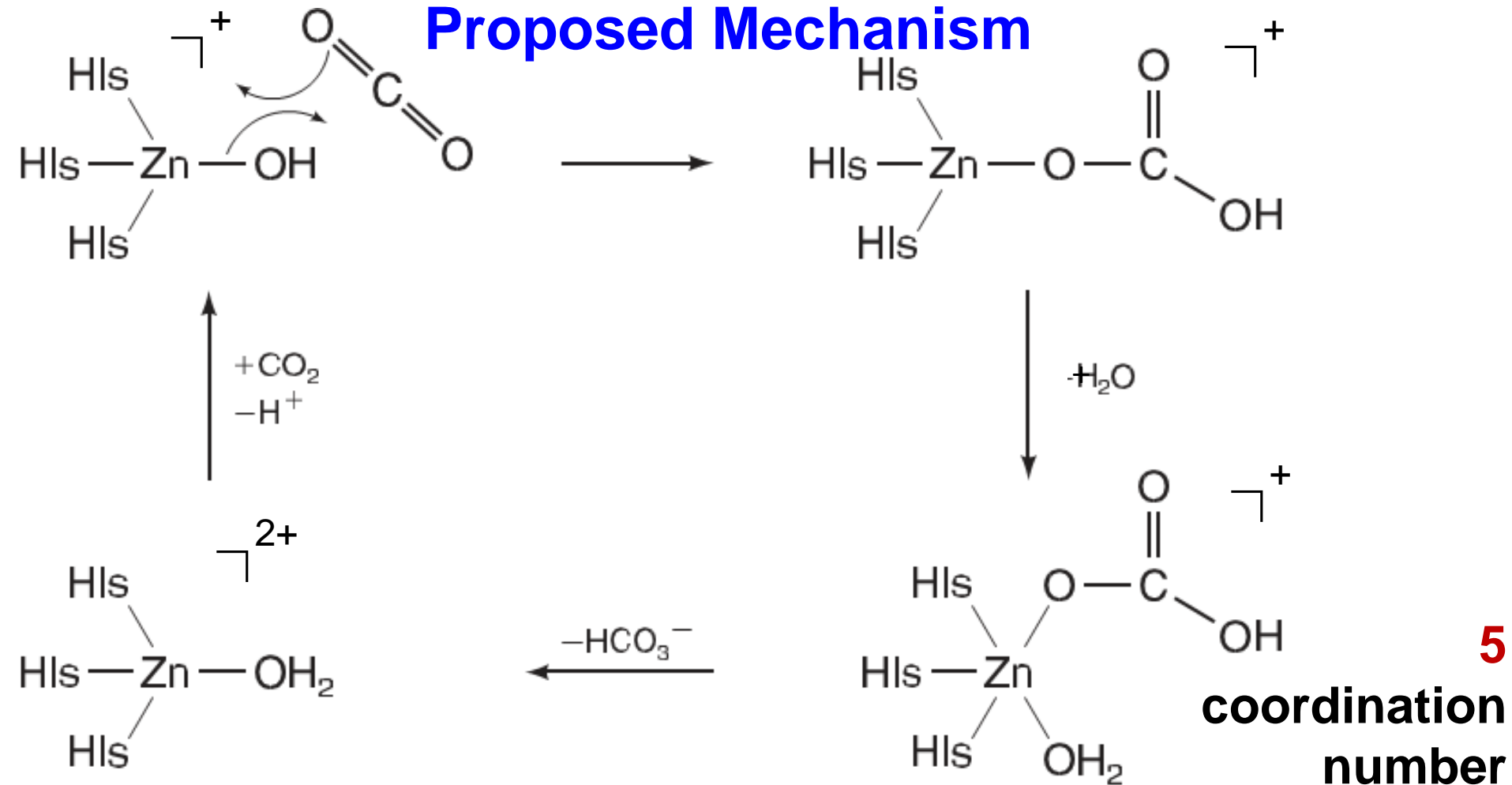
- Catalyzes **hydration of CO_2** , with acceleration of **$\sim 10^7$** relative to the uncatalyzed reaction.
- The first Zn-dependent enzyme to be discovered.
- Prevalent in red blood cells & key in respiration and pH buffering: CA helps transport of CO_2 .





- Zn^{2+} with **3 His** and **1 labile H_2O** (distorted tetrahedron coordination). Water molecules in the active site play key structural & functional roles (proton transfer).

Proposed Mechanism

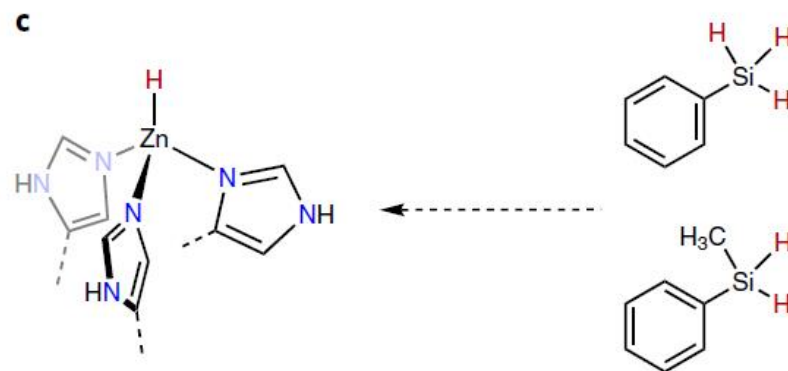
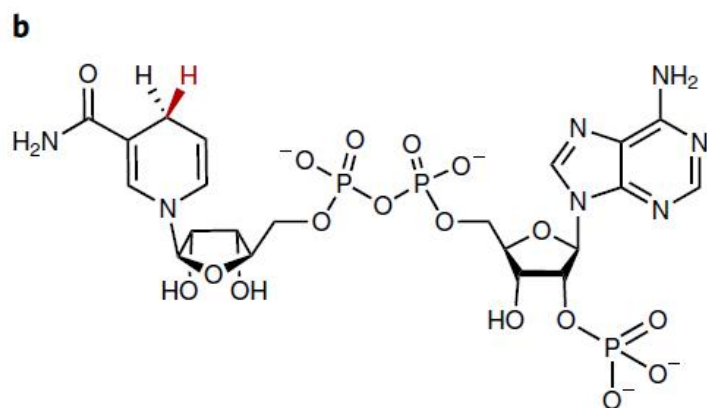
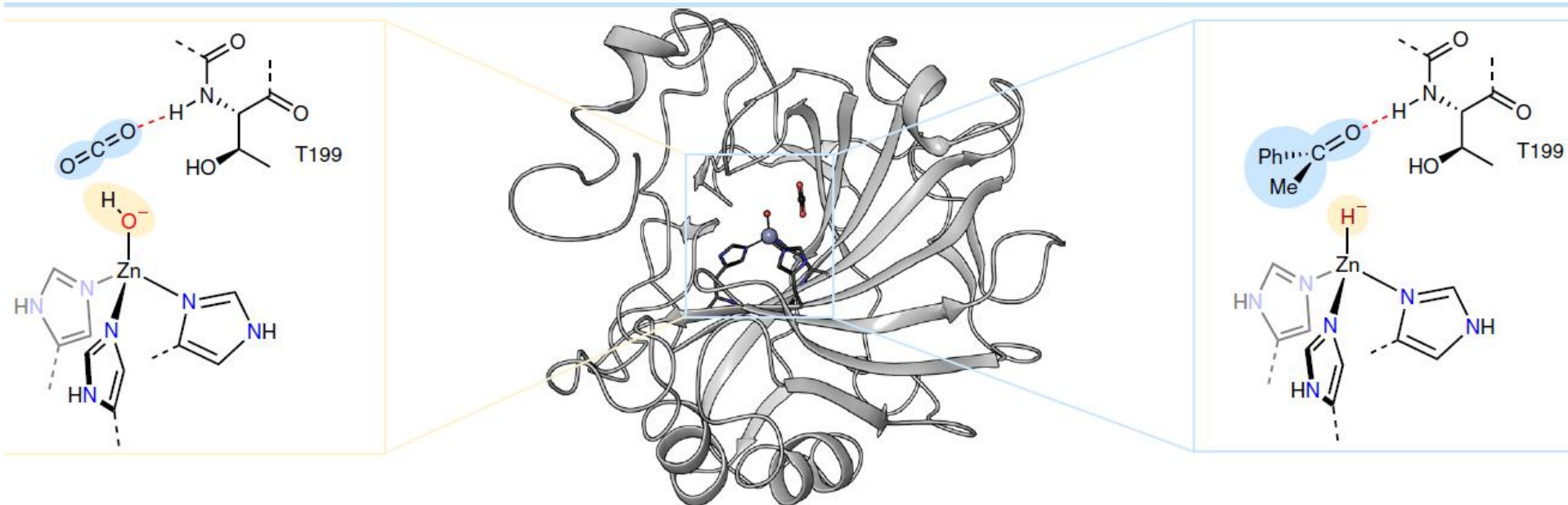


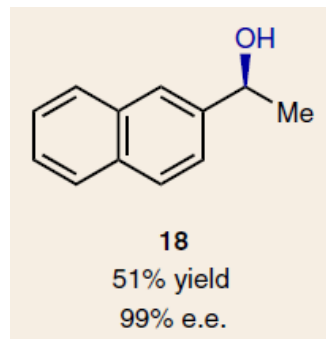
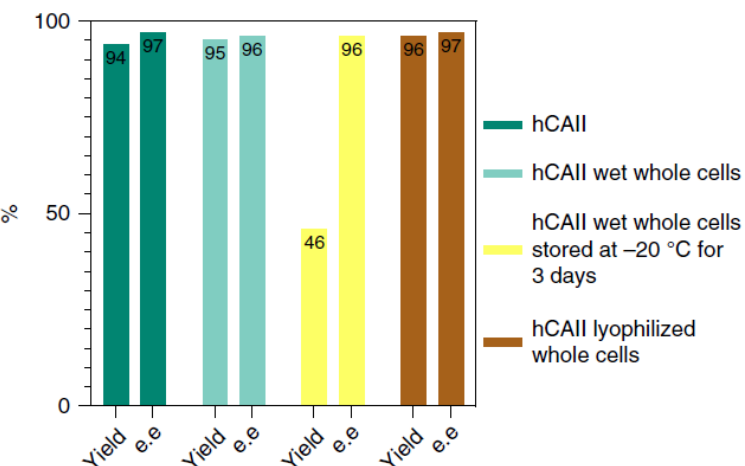
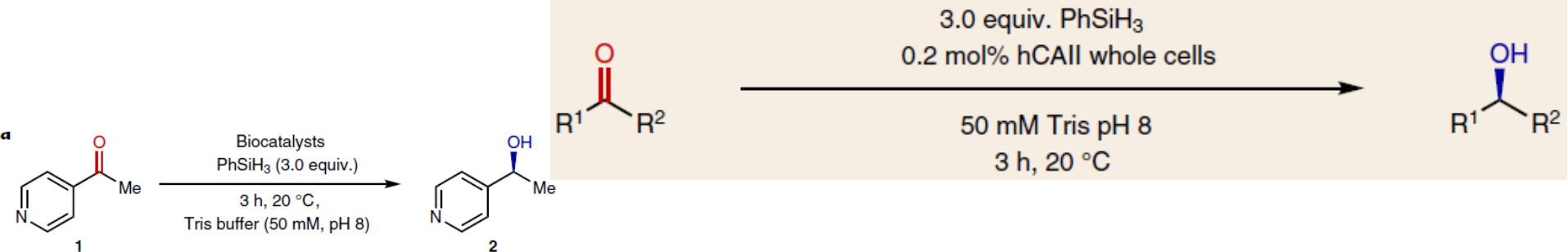
- A **Zn-bound hydroxide** (from **deprotonation**) **attacks** CO_2 . The Zn^{2+} ion also **polarizes the $\text{O}=\text{C}$ bond** and **stabilizes** the developing negative charge (Lewis acid).
- Subsequent uptake of a H_2O & release of HCO_3^- .

Abiotic reduction of ketones with silanes catalysed by carbonic anhydrase through an enzymatic zinc hydride

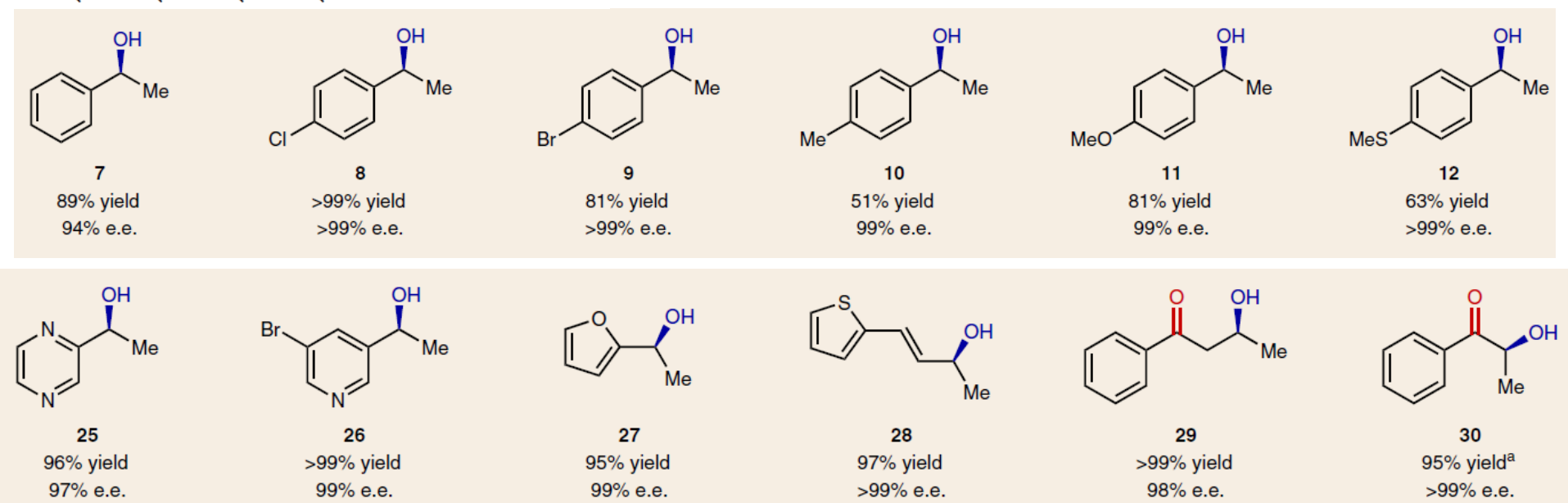
Nat. Chem. **2021**, 312

Pengfei Ji¹, Jeeyoung Park¹, Yang Gu¹, Douglas S. Clark^{1,2} and John F. Hartwig¹✉

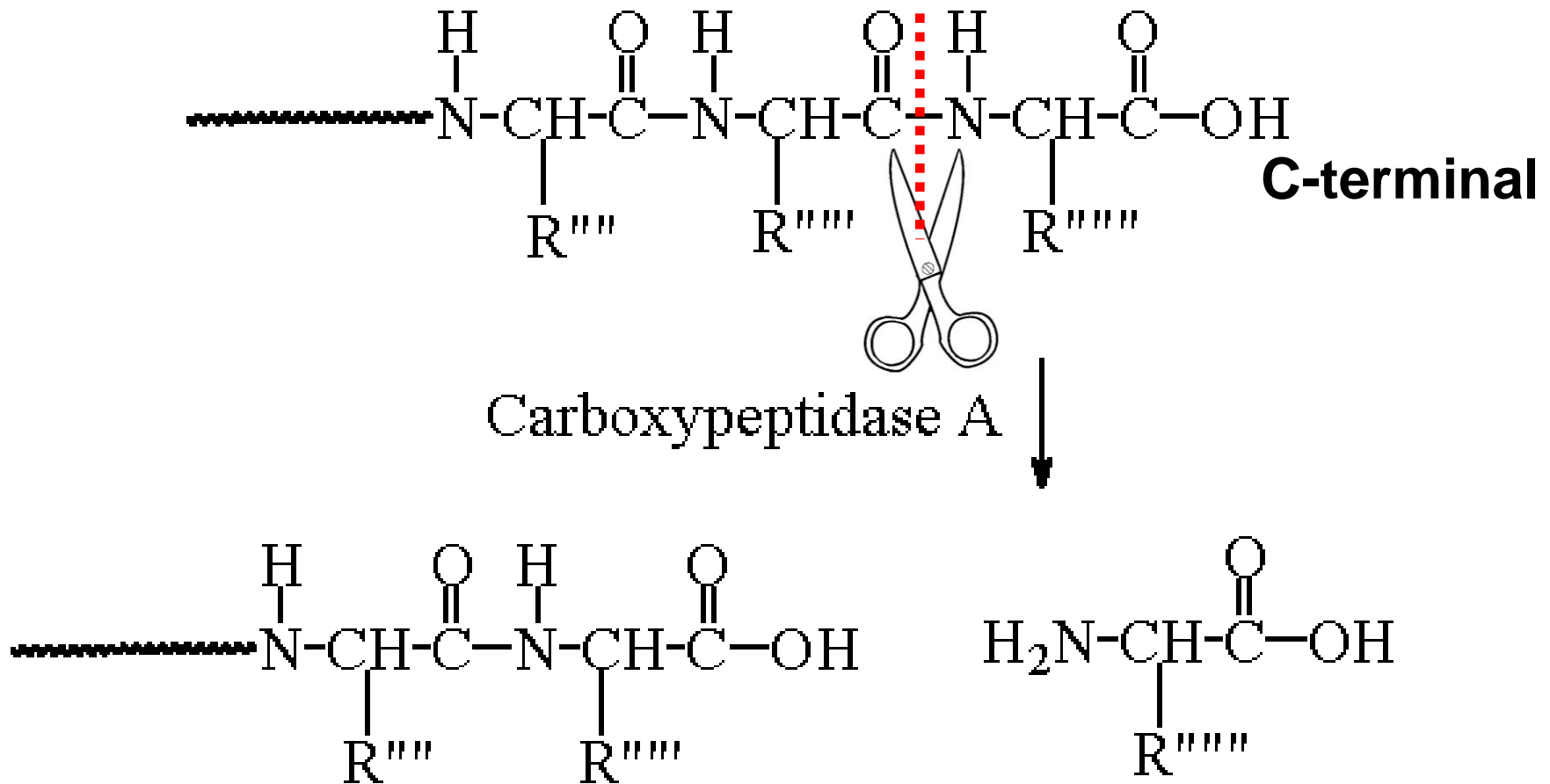




Nat. Chem. **2021**, 312

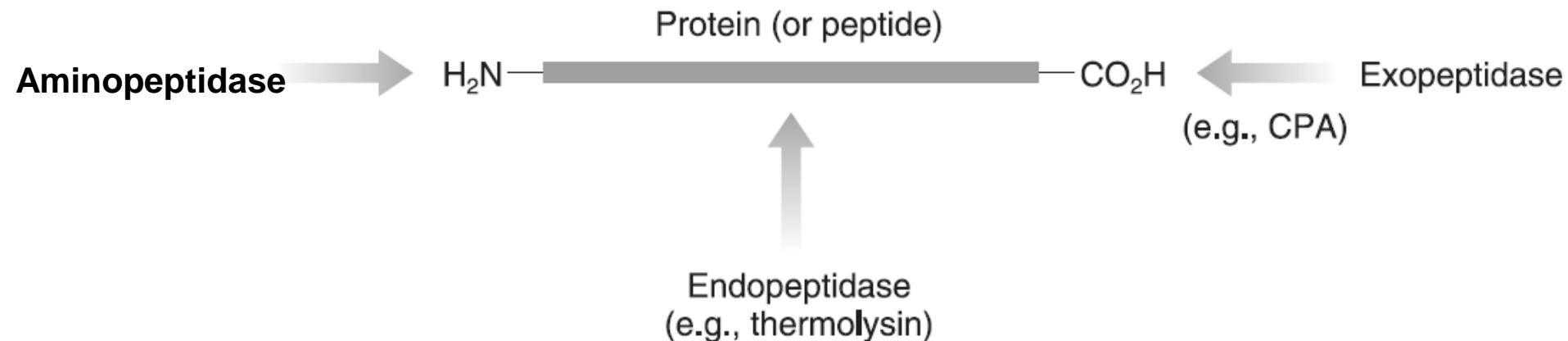
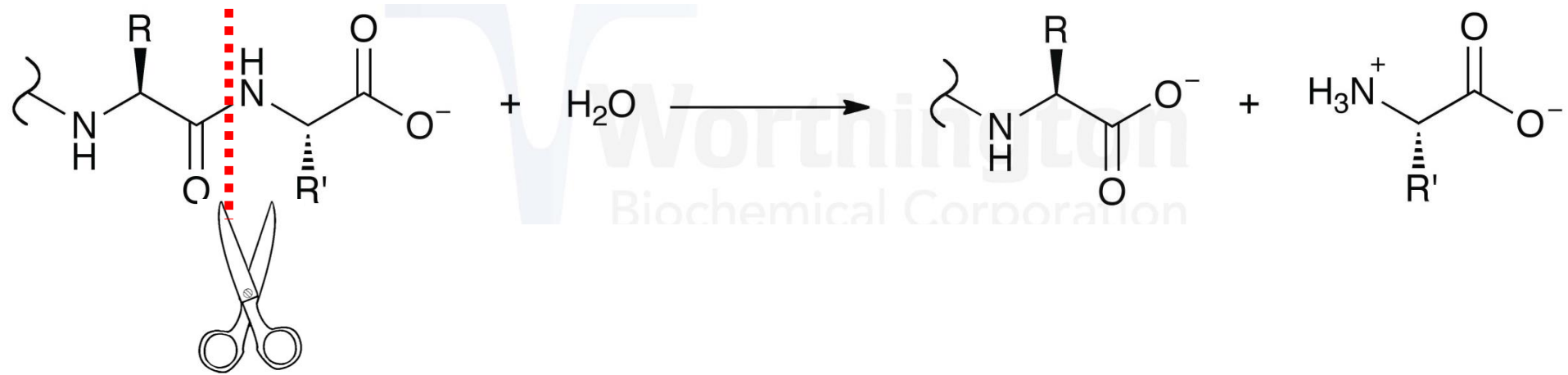


Carboxypeptidase: Zn

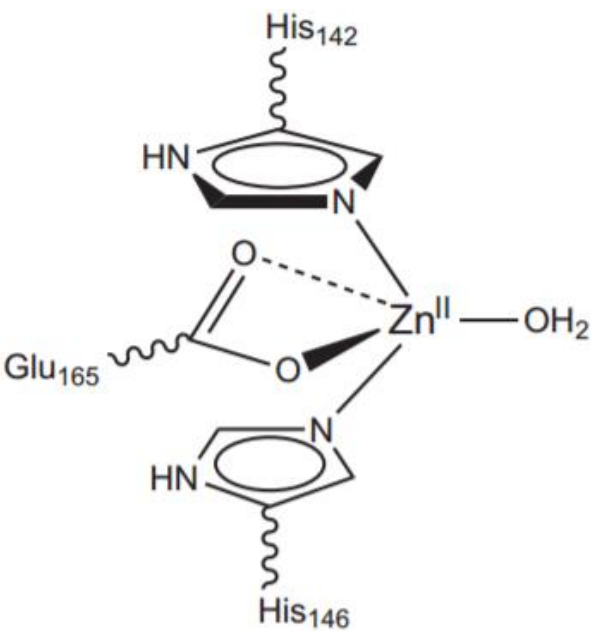


- **Carboxy**peptidase A (CPA): a digestive enzyme that **hydrolyzes**/cleaves a **peptide bond of residues** with aromatic or aliphatic side-chains at the **carboxy**-terminal (**C-terminal**) of a protein.

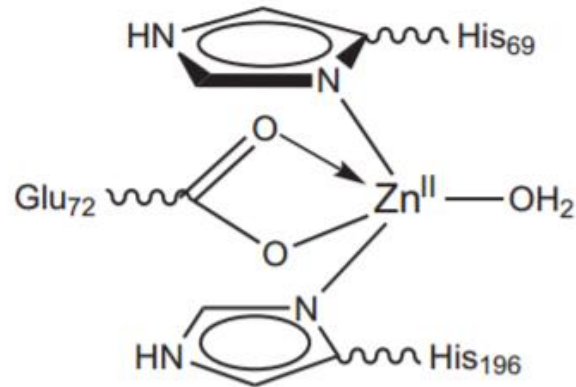
- Carboxypeptidase B (CPB) is similar to CPA, but it favors to cleave a peptide bond of residues with **basic residues** (Arg, Lys).



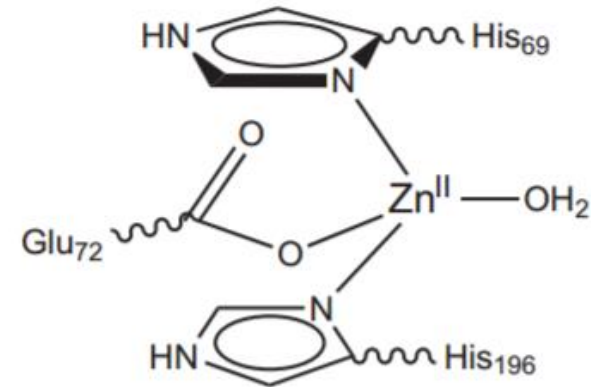
Active sites



thermolysin



carboxypeptidase A

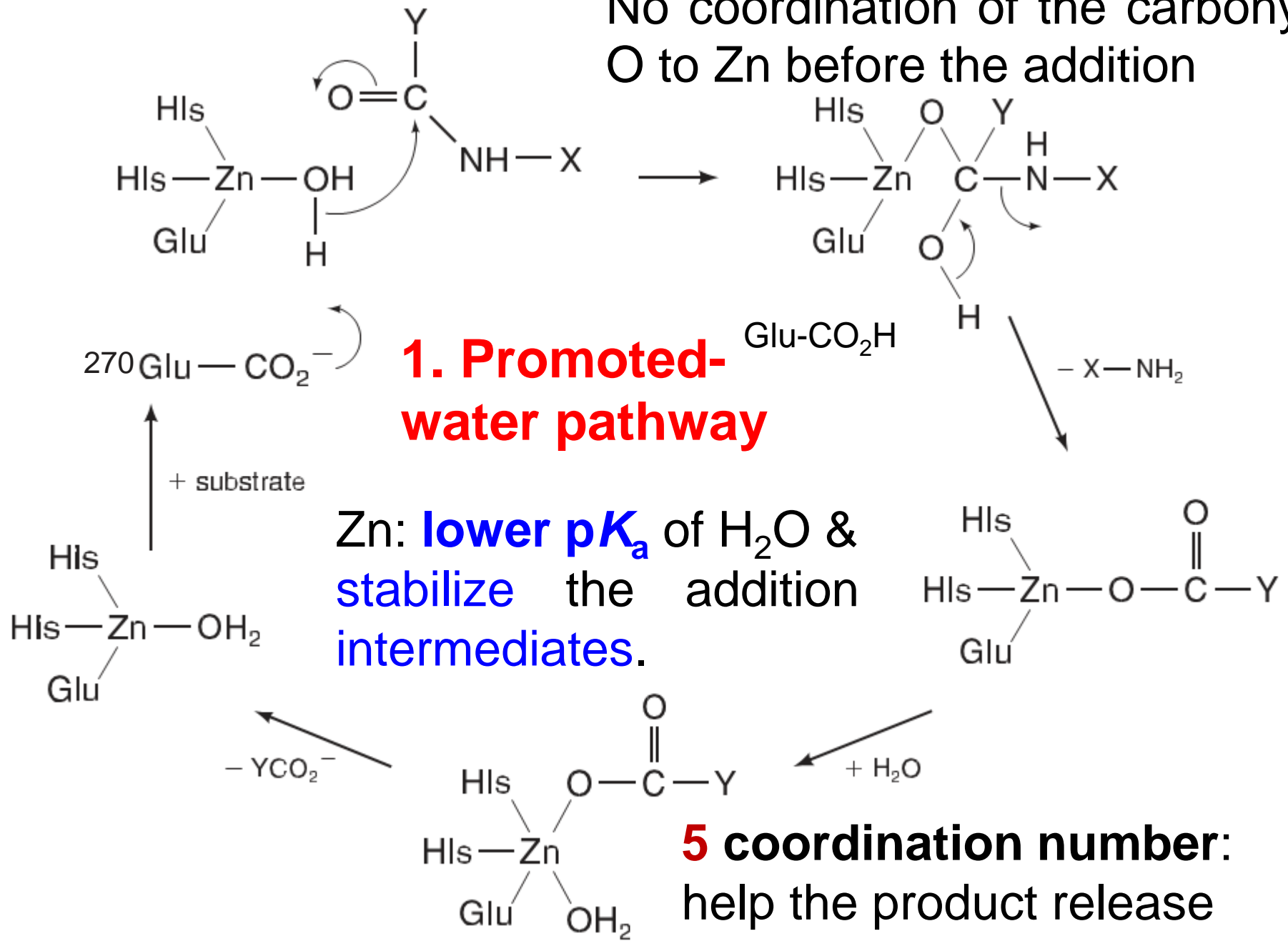


carboxypeptidase B

- The Zn ion in CPA: tetrahedral with 2 **His** ligands, 1 bidentate **Glu** & 1 **H₂O**, which is hydrogen bonded to Ser197 and Glu270.
- In the presence of the substrate, the bound Glu shifts to monodentate coordination.
- The enzyme can also hydrolyze esters.

Two Proposed Mechanisms

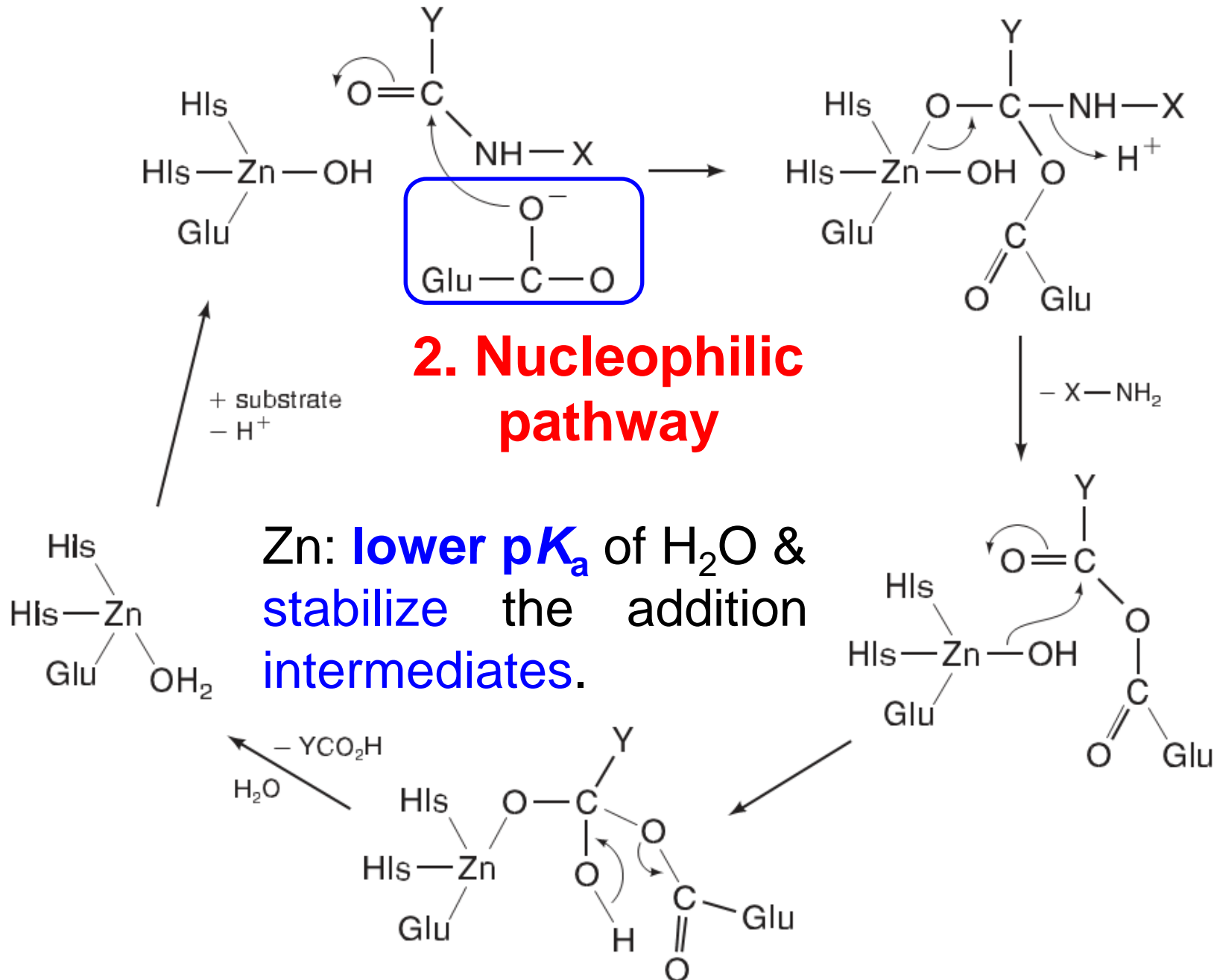
No coordination of the carbonyl O to Zn before the addition



Form an **anhydride** intermediate

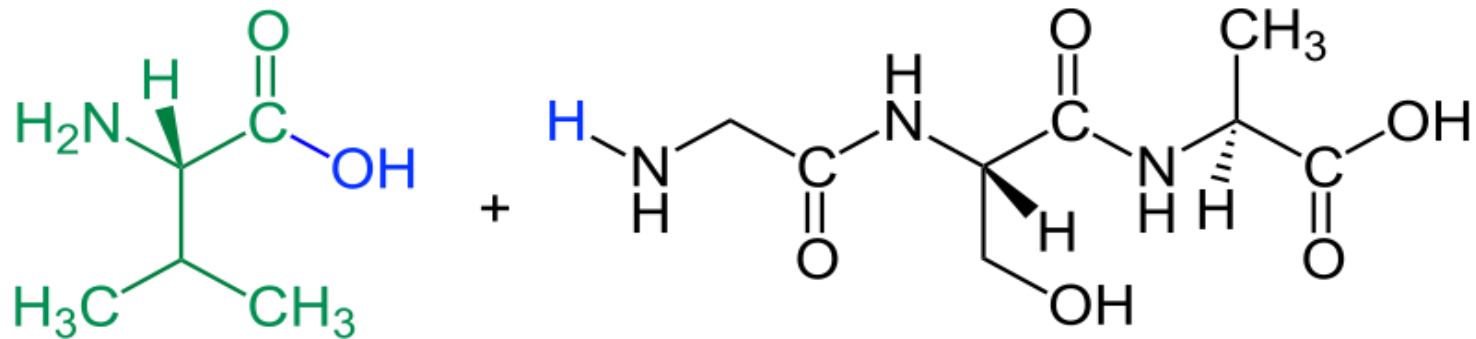
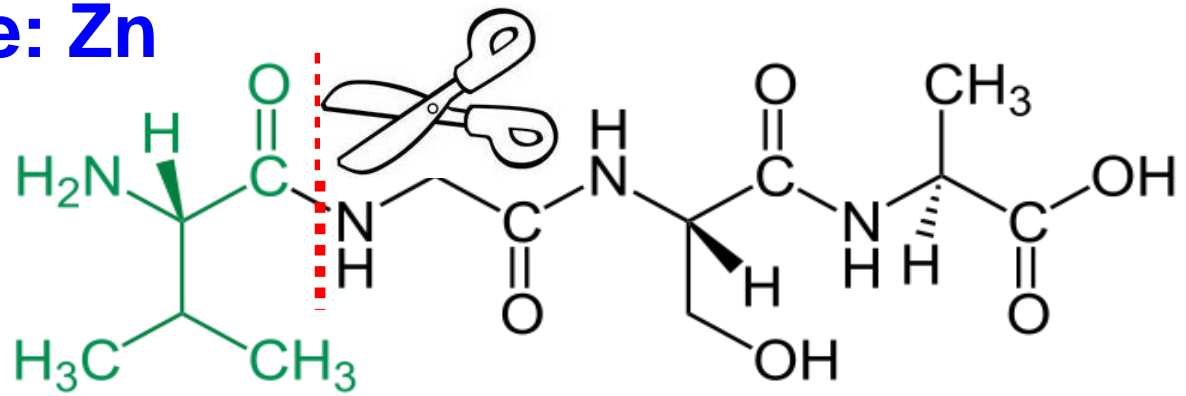
2. Nucleophilic pathway

Zn: **lower pK_a** of H_2O & **stabilize** the addition intermediates.



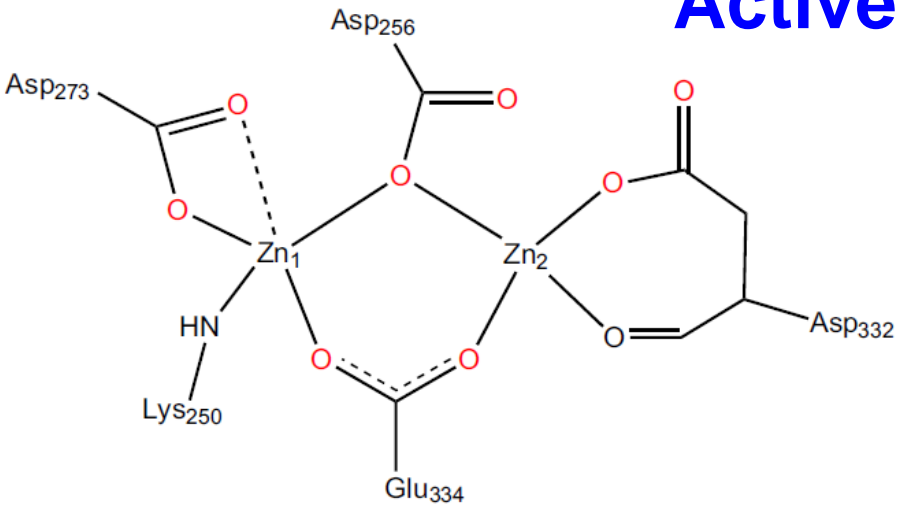
Aminopeptidase: Zn

N-terminal

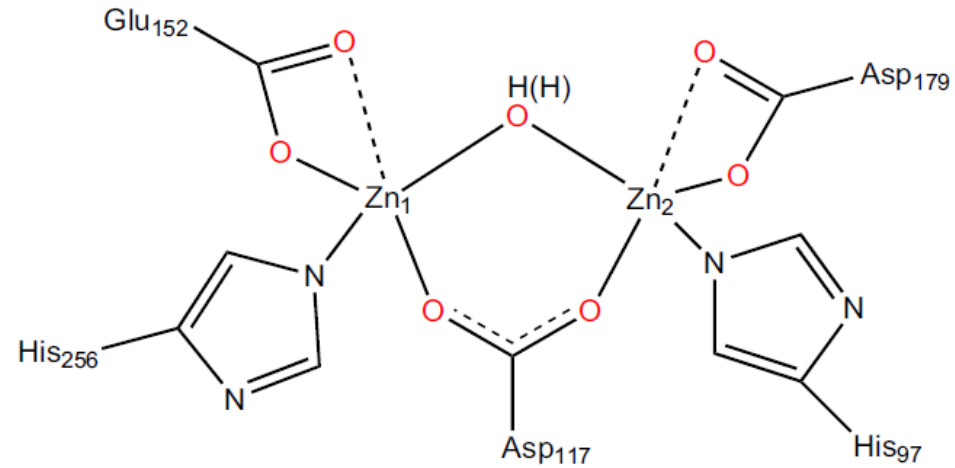


- **Hydrolyzes**/cleaves a **peptide bond of residues** at the **amino-terminal (N-terminal)** of a protein. **A dinuclear Zn active site** was found & both Zn ions are required with different roles.

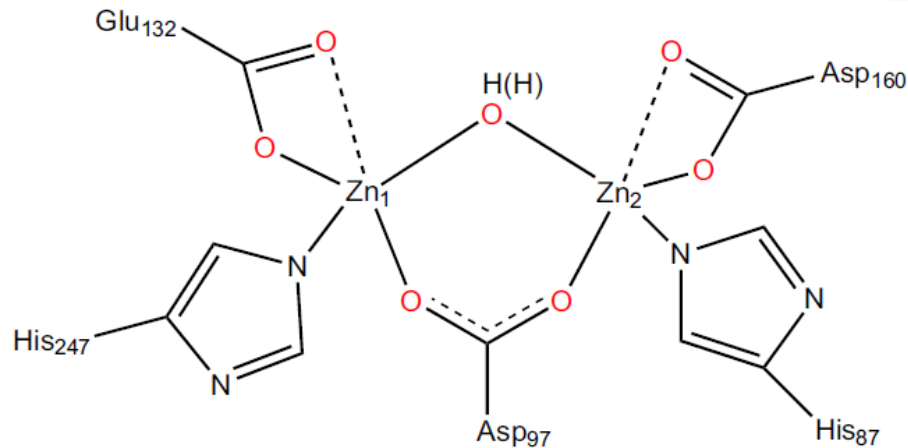
Active Sites



BILAP



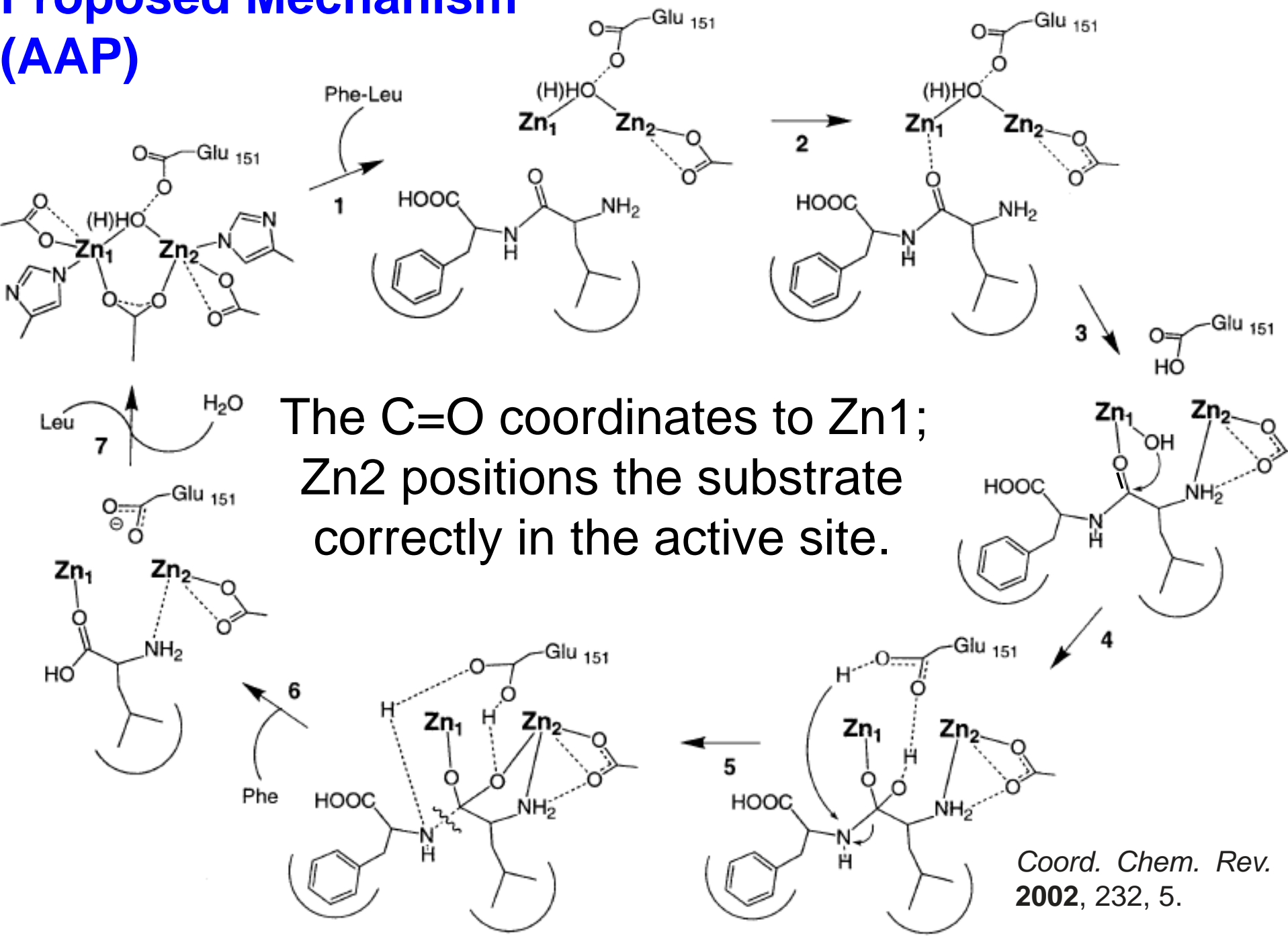
AAP



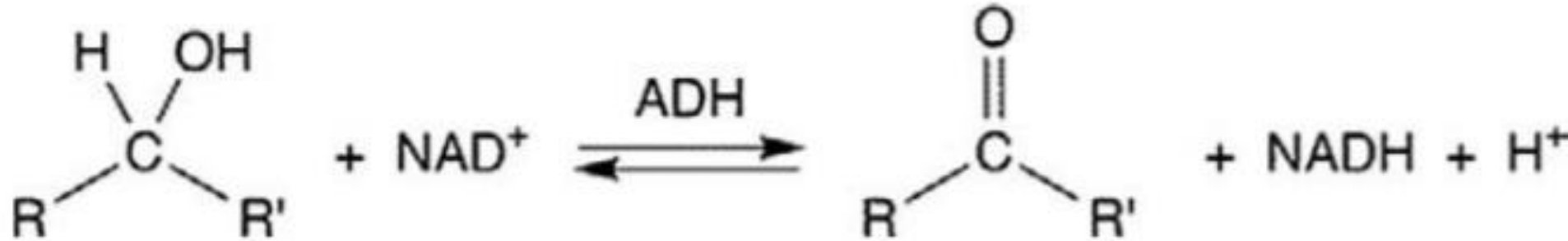
SAP

- The first group: leucine aminopeptidase from bovine lens; the second group: leucine aminopeptidases AAP from *Aeromonas proteolytica* and SAP from *Streptomyces griseus*.

Proposed Mechanism (AAP)

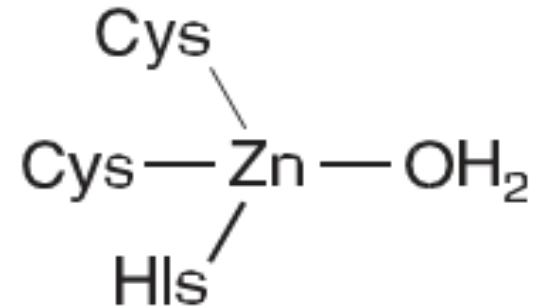


Liver Alcohol Dehydrogenase (LADH): Zn

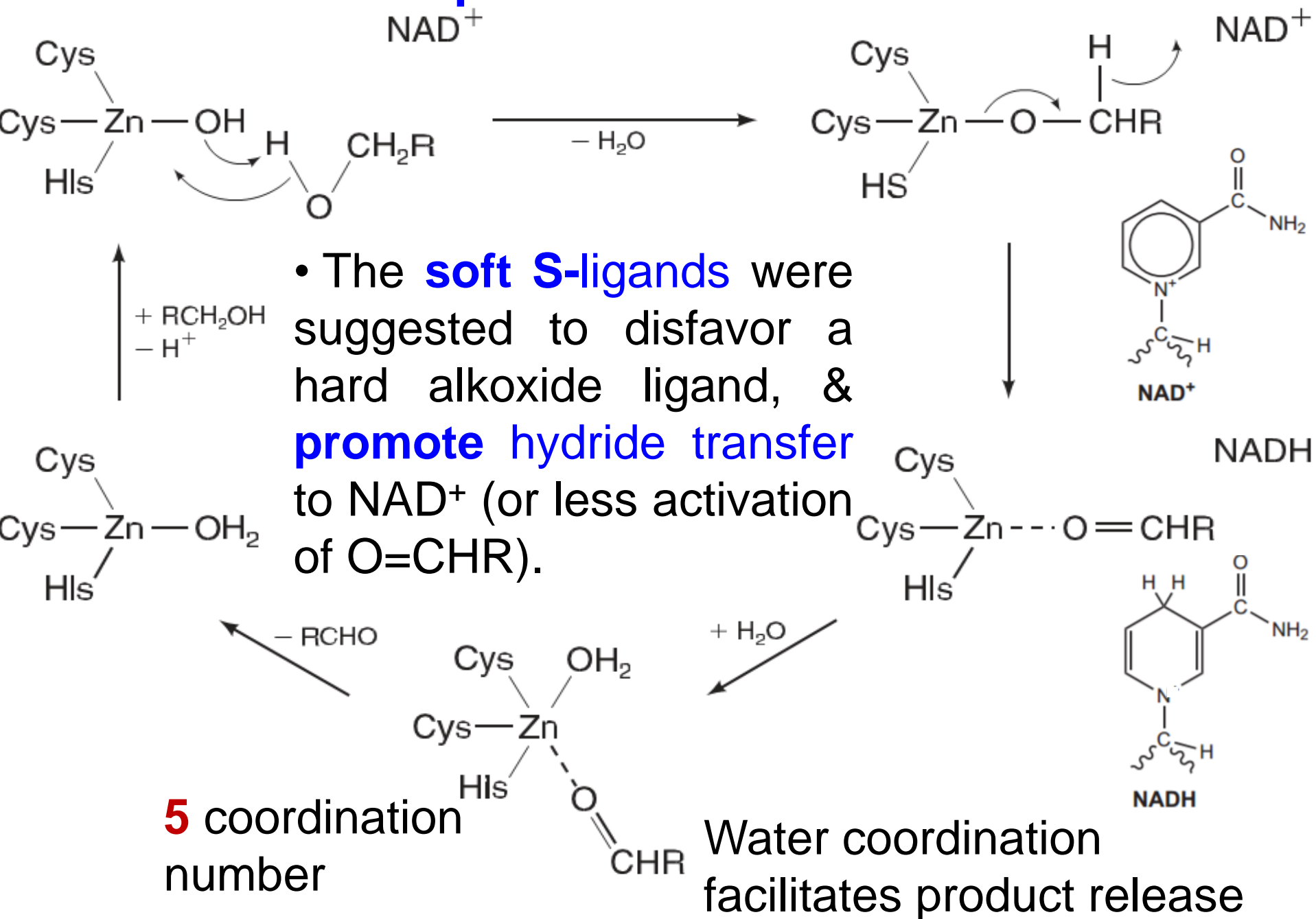


- Formally, LADH catalyzes an oxidation of a primary or secondary **alcohol** to give an **aldehyde** or **ketone**, respectively, through **hydride transfer to NAD⁺**.

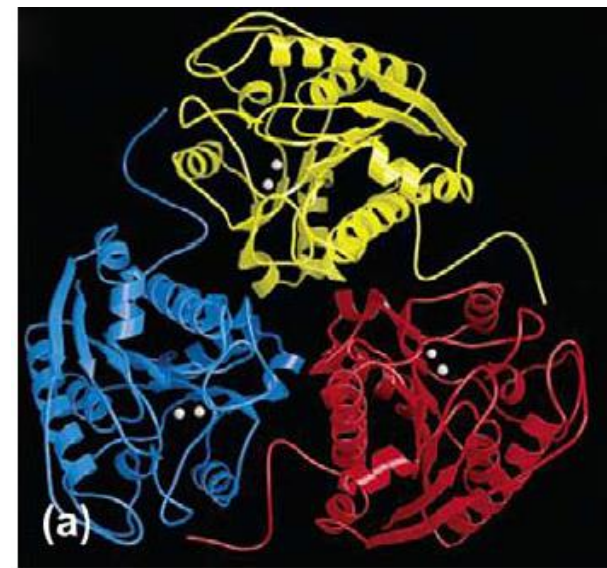
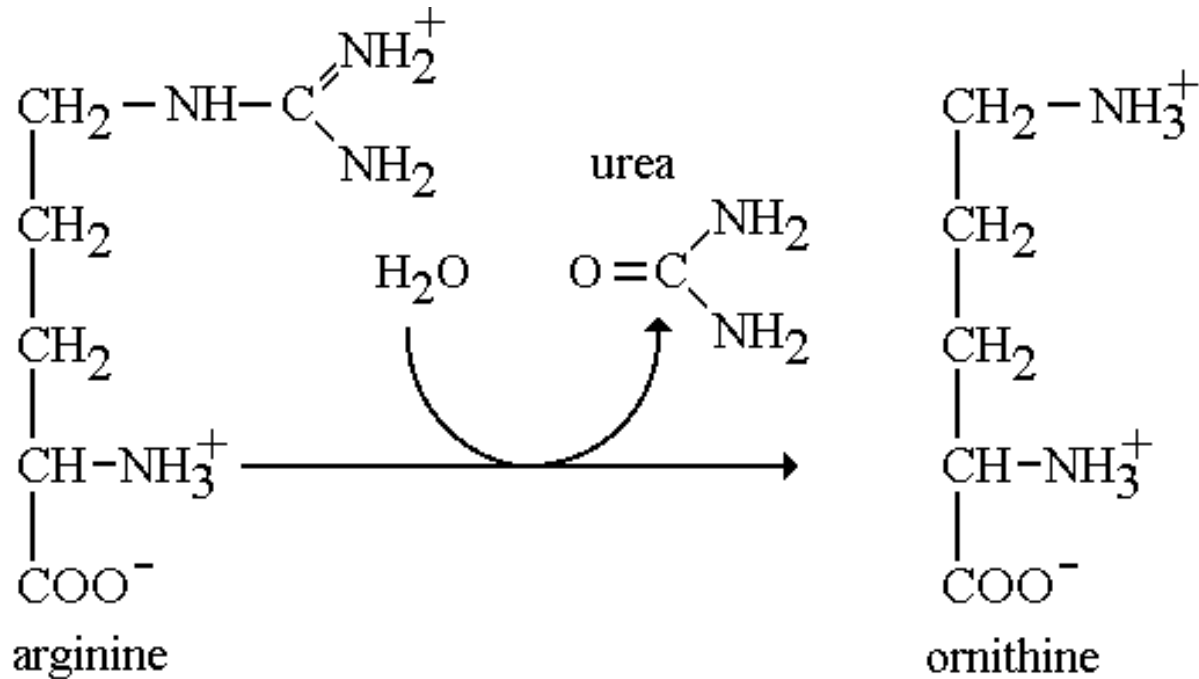
- LADH in mammalian liver are dimeric proteins with **2 Zn²⁺ ions** in each subunit: one is **catalytically active** (coordinating with 1 **His**, 2 (**soft**) **Cys** & 1 **H₂O**) & the other Zn plays **a structural role** (with 4 Cys).



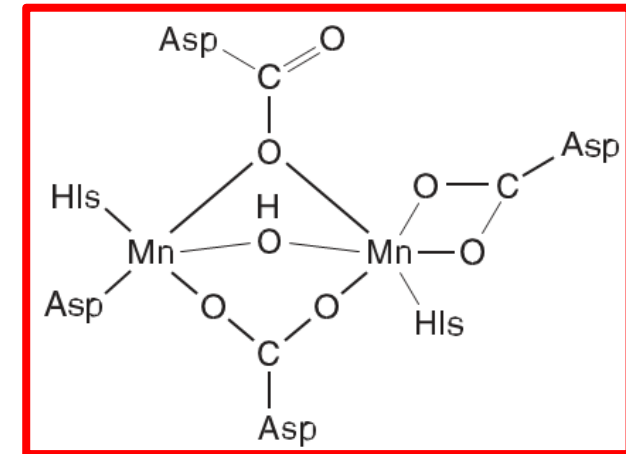
Proposed Mechanism



Arginase: Mn

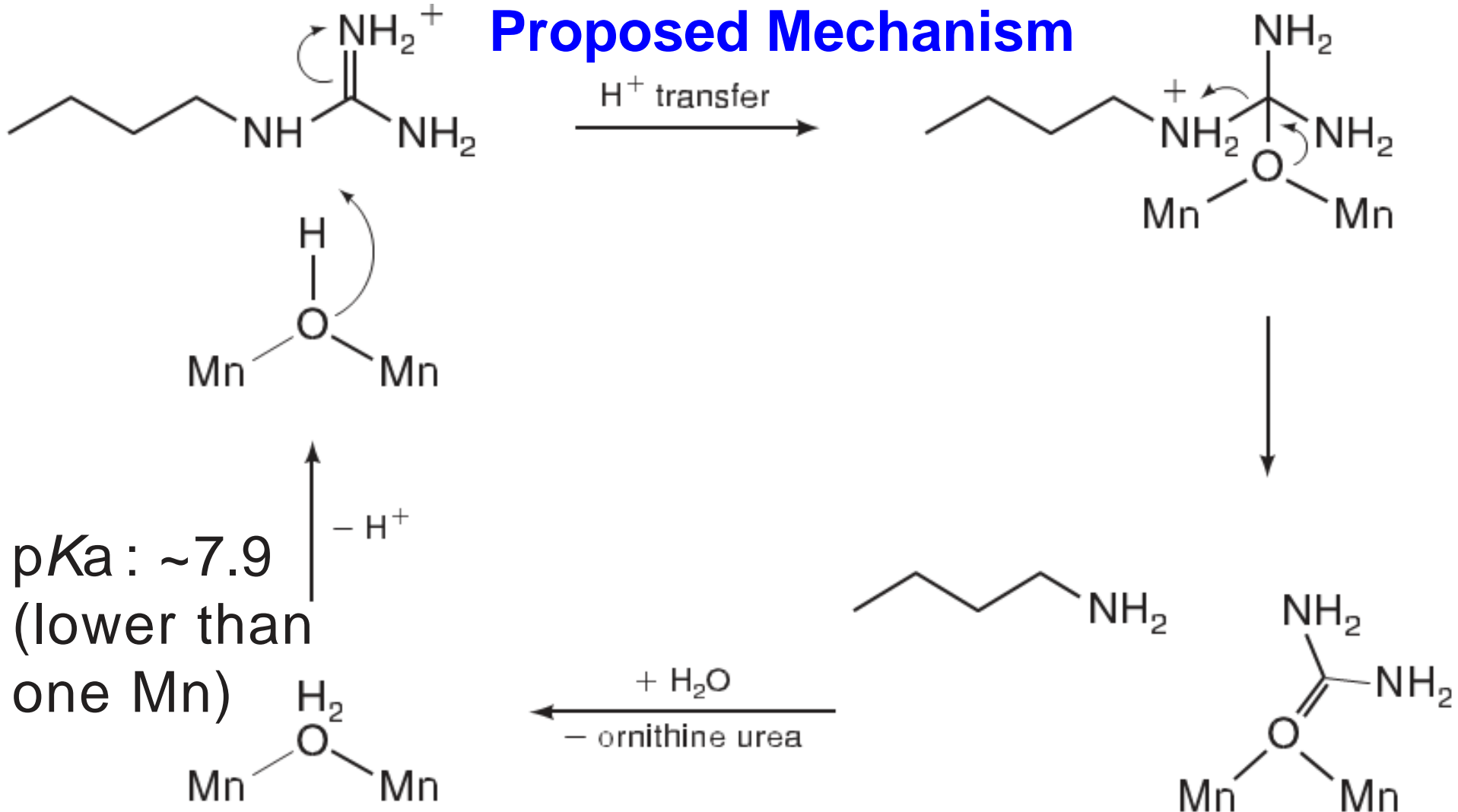


- Catalyzes hydrolysis of **arginine** to give **urea** & **ornithine** (the final step in the urea cycle).
- A trimer protein; 2 Mn ions has 3 bridging ligands (a bidentate Asp, a monodentate Asp and a hydroxide) in the active site.



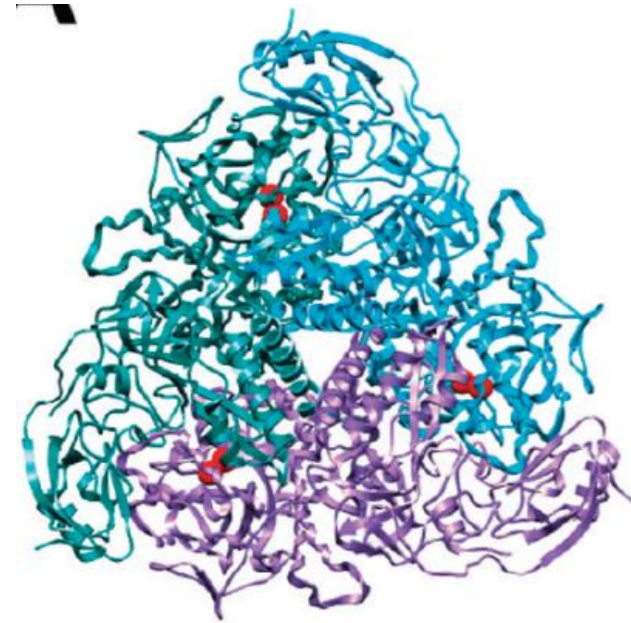
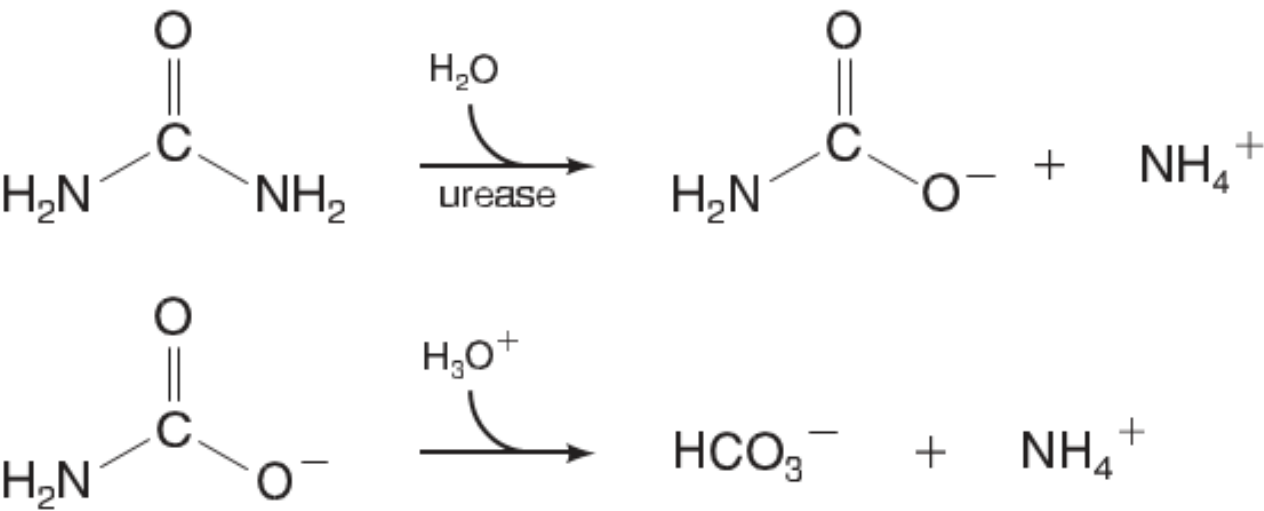
Active Site
(Mn-Mn: ~3.3 Å)

Proposed Mechanism



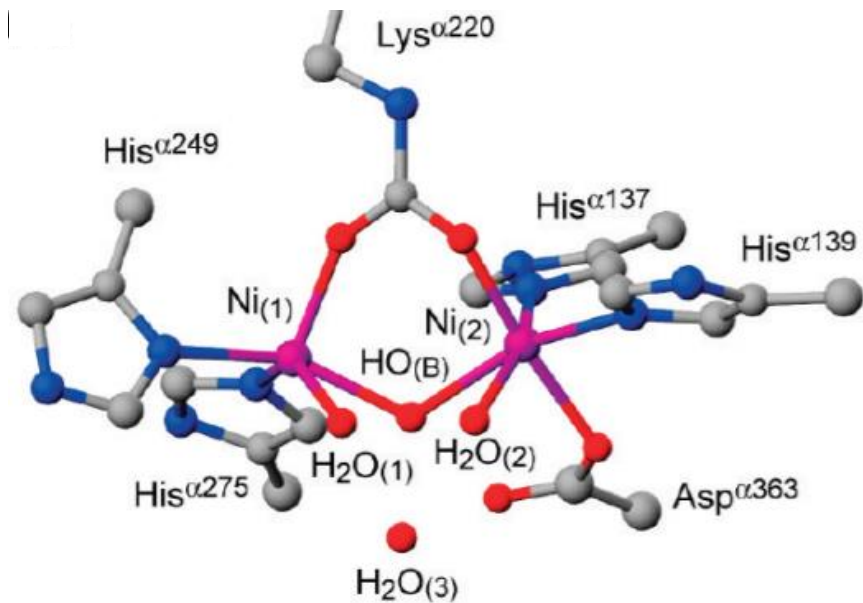
- The substrate does not bind directly to the metal cofactors. Why? Why not proton transfer to the terminal N atom?

Urease: Ni

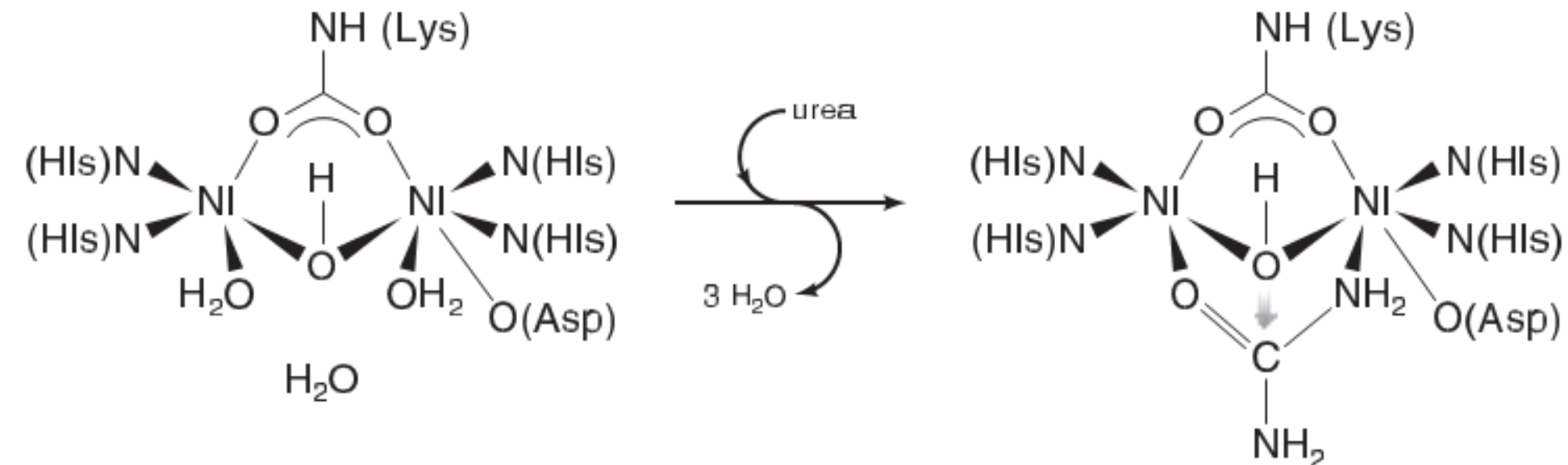


- Hydrolyzes **urea** to give **NH₃** & **carbamate**, which decomposes to give another NH₃ & **HCO₃⁻** (key role in nitrogen metabolism in plants & microbes).

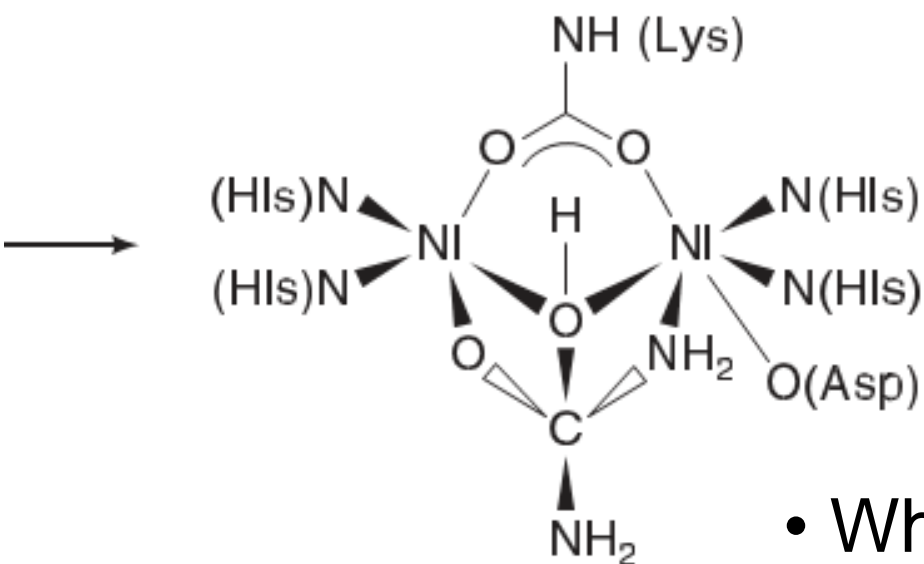
- It was the **first Ni enzyme** & contains **two Ni ions**.



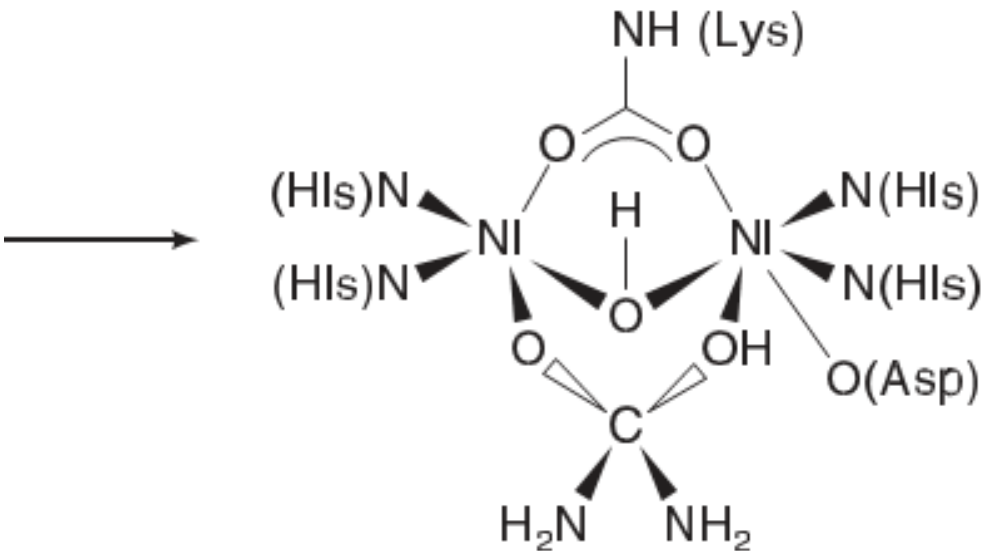
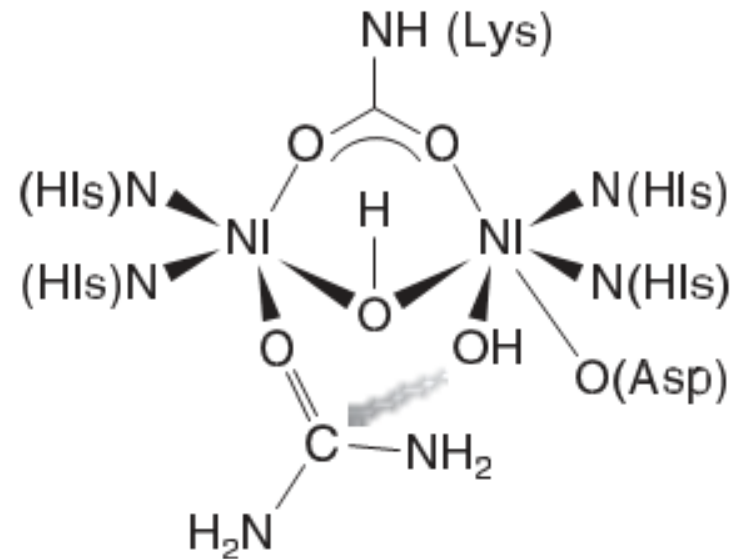
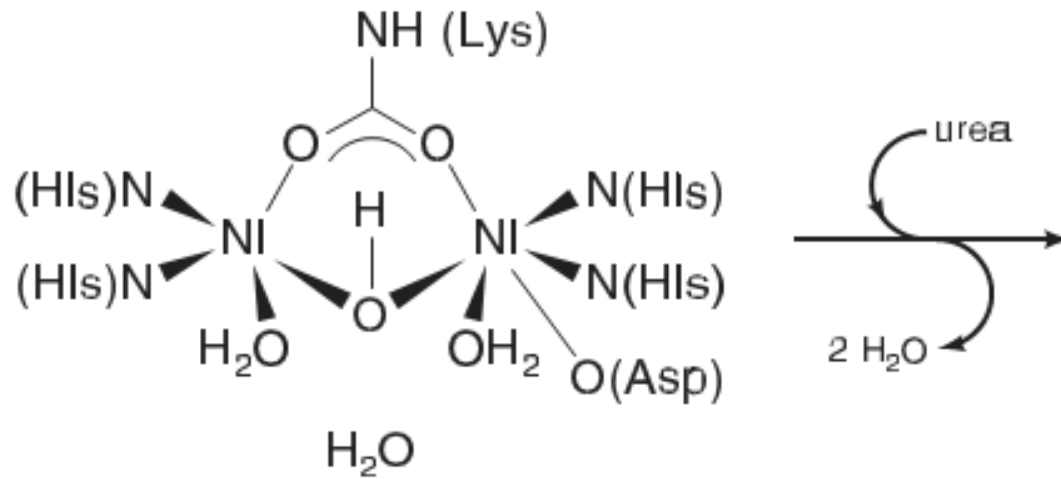
Two Proposed Mechanisms



1. A pathway via the **bridging hydroxide** as the nucleophile



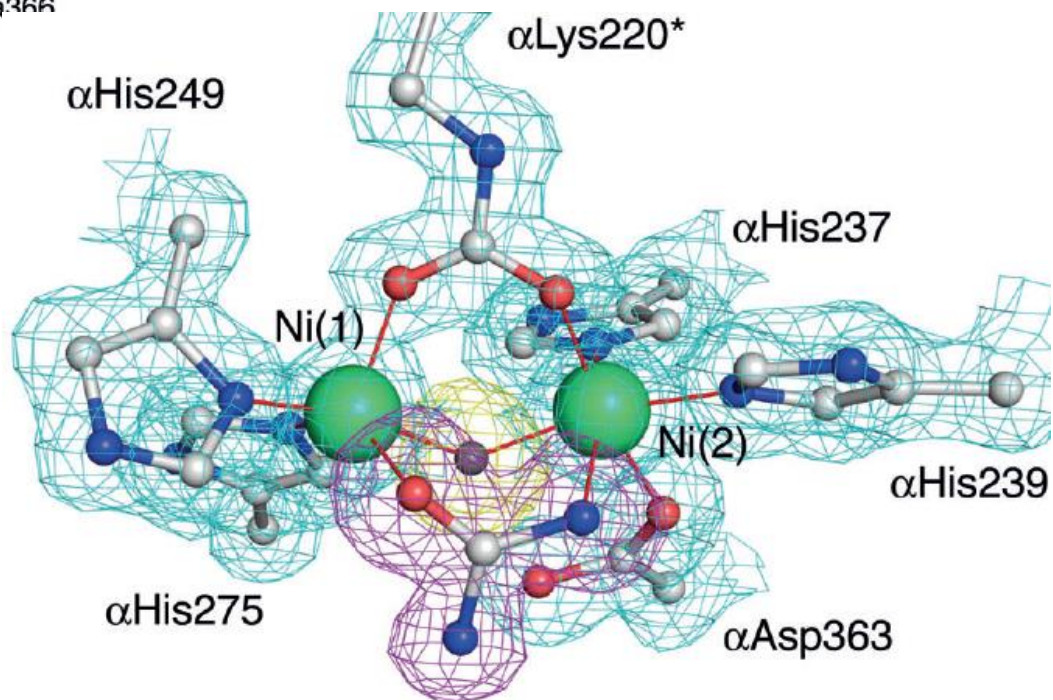
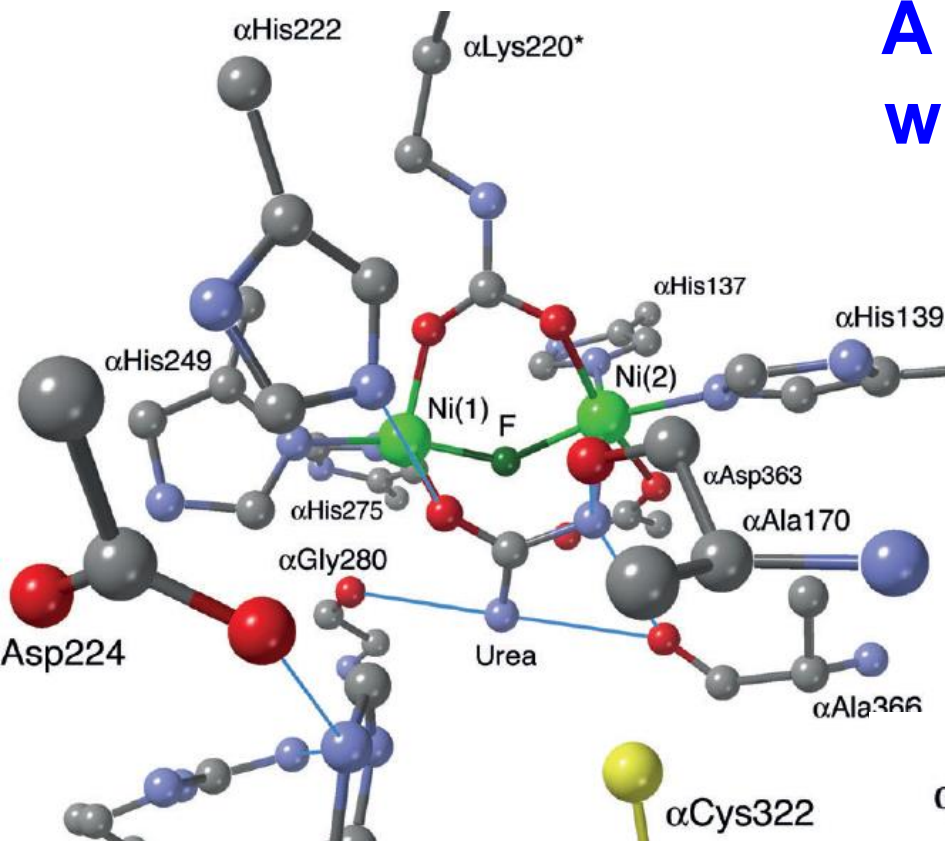
- Why the urea O preferentially coordinates to the left Ni center?



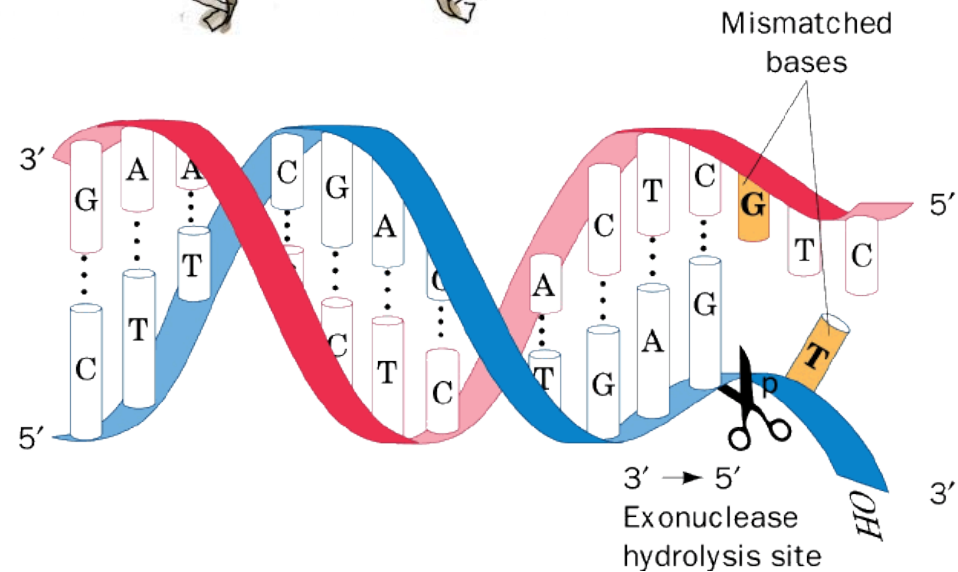
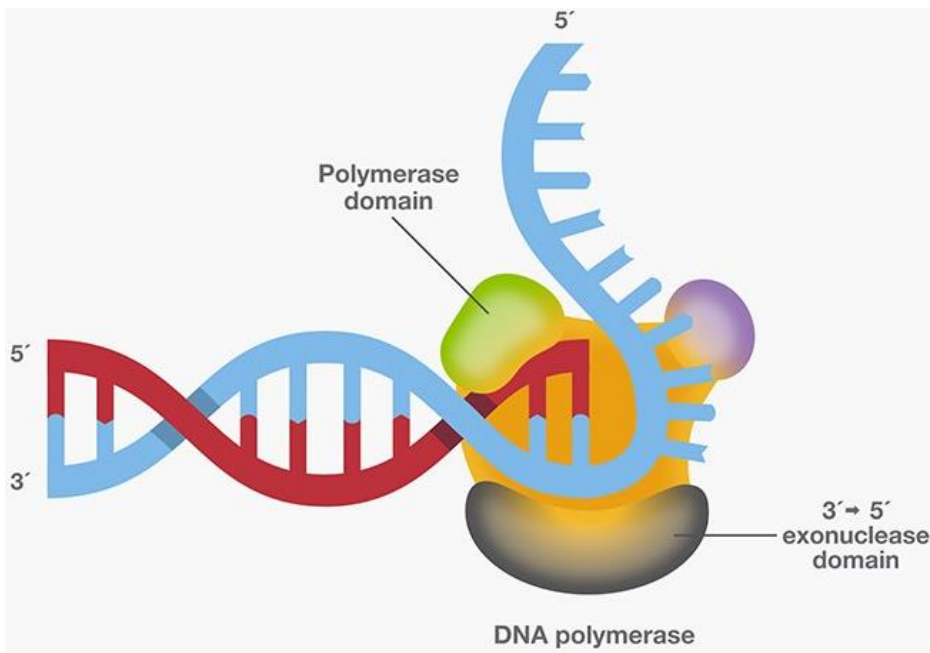
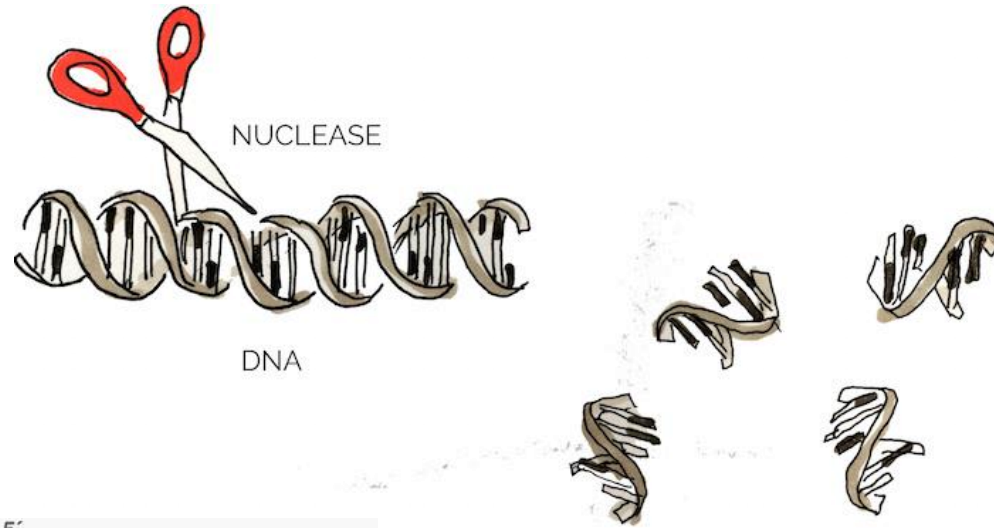
2. A pathway via the **terminal** **hydroxide** as the nucleophile

A Recent Crystal Structure with the Bridging F Ligand

That supports the pathway 1 (via the bridging OH as the nucleophile)



Hydrolysis or Condensation Reactions with Nucleic Acids



Hydrolysis or Condensation Reactions with Nucleic Acids

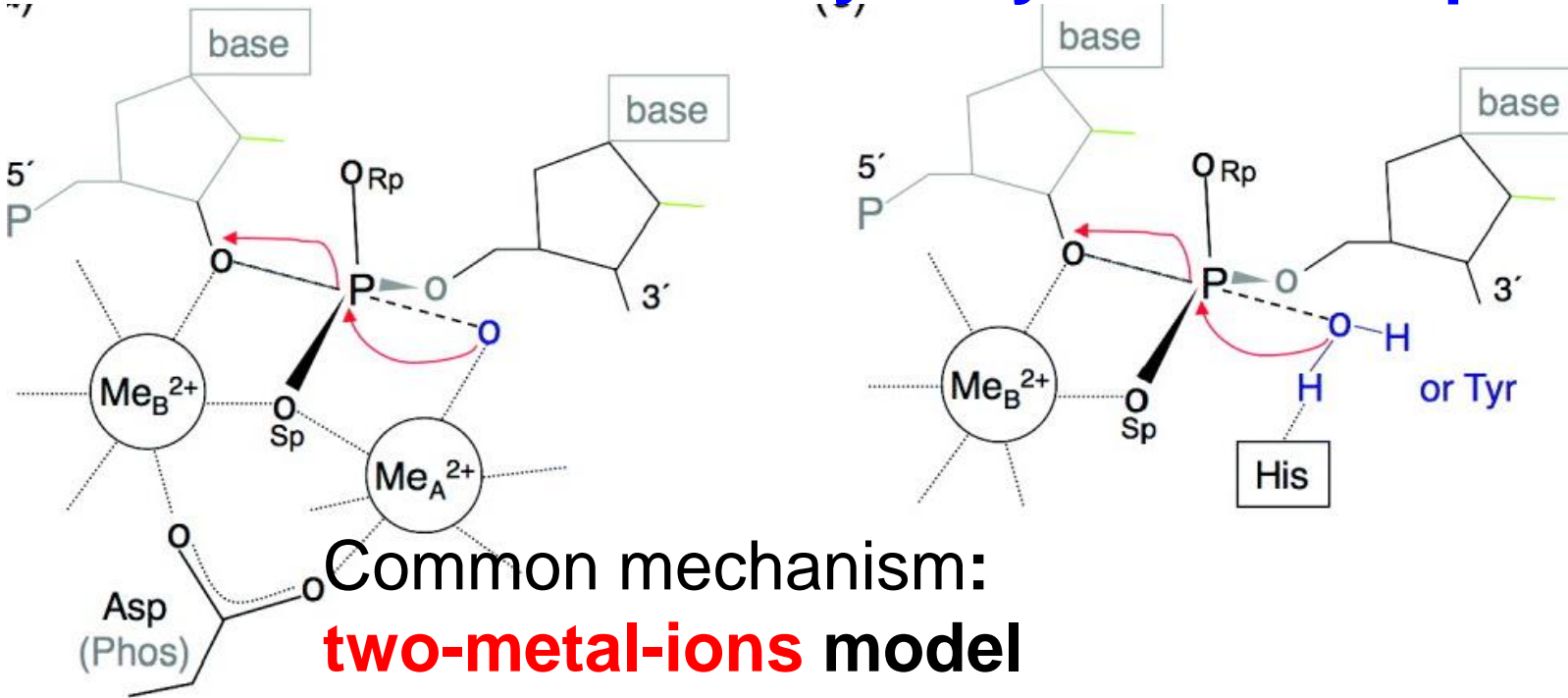
- Almost all these enzymes **require divalent metal ion** cofactor to promote activity. **Mg ion** (relatively labile) is typically used, while **Ca & Zn ions** are also used in some cases.

Ion	Coordination Numbers	Geometry ^a	Radius (Å)	Ligand P
Mg ^{2+ b}	6	oct	0.65	O; (H ₂ O)
Mn ²⁺	5, 6	dist oct, sq pyr	0.85	O, N,
Co ²⁺	4, 5, 6	dist oct, tet, sq pyr	0.81	N, S
Zn ²⁺	4, 5	tet, sq pyr	0.79	O, N, S

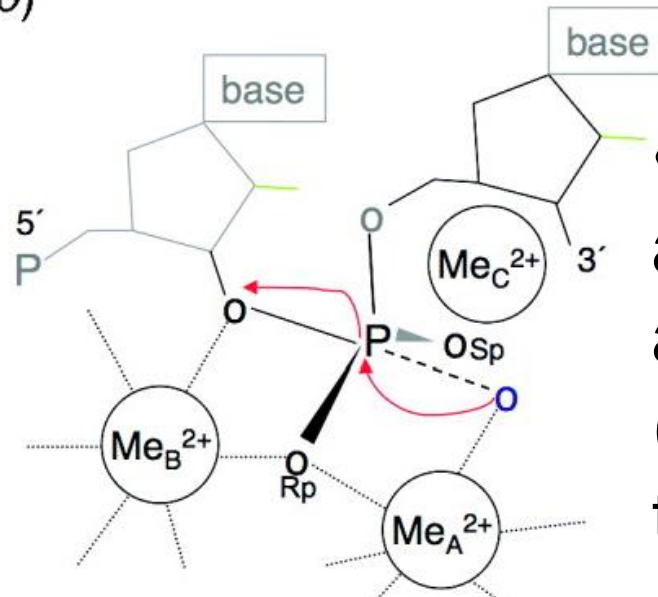
^a Octahedral = oct, distorted octahedral = dist oct, square pyramidal = sq pyr, tetrahedral = tet.

^b Maguire, M. E. and Cowan, J. A., “Magnesium Chemistry and Biochemistry”, *Biometals* **15**, 203–210 (2002).

3 Generic Models for Hydrolysis of Phosphodiester

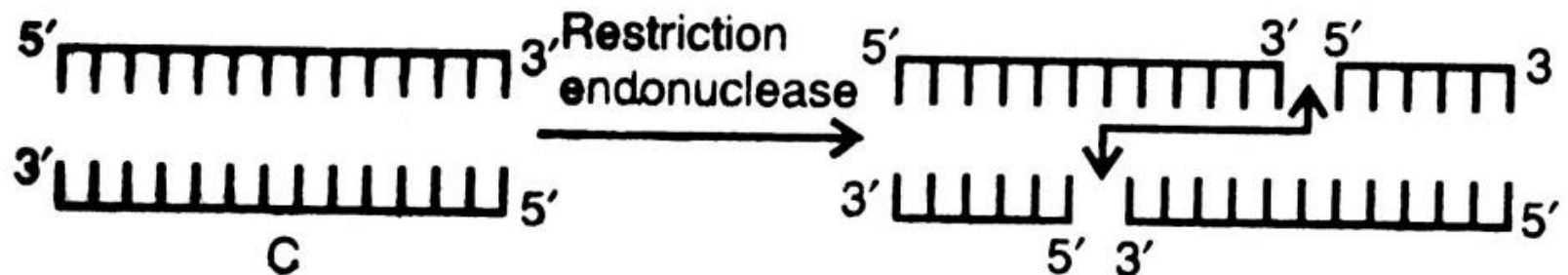
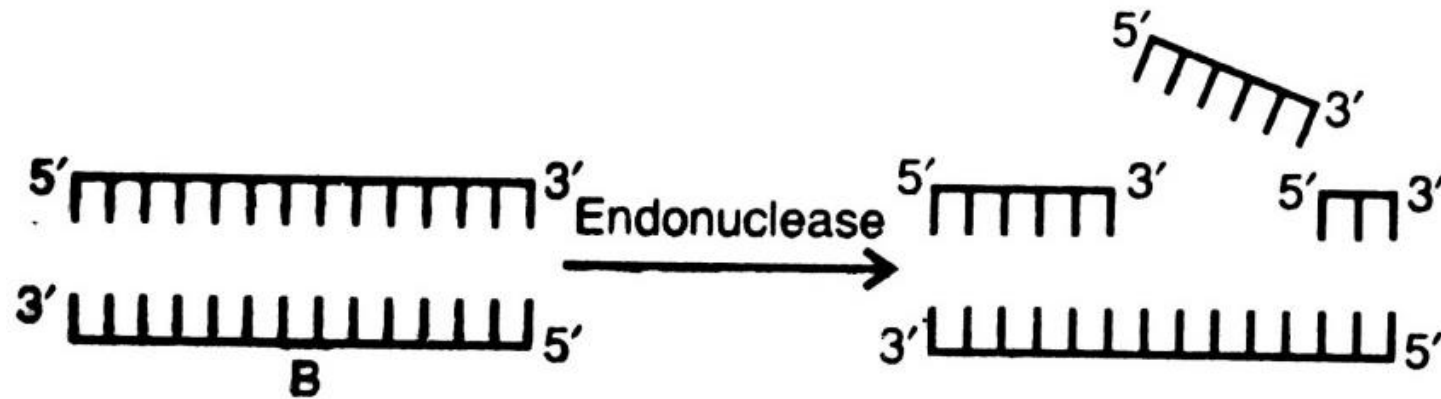
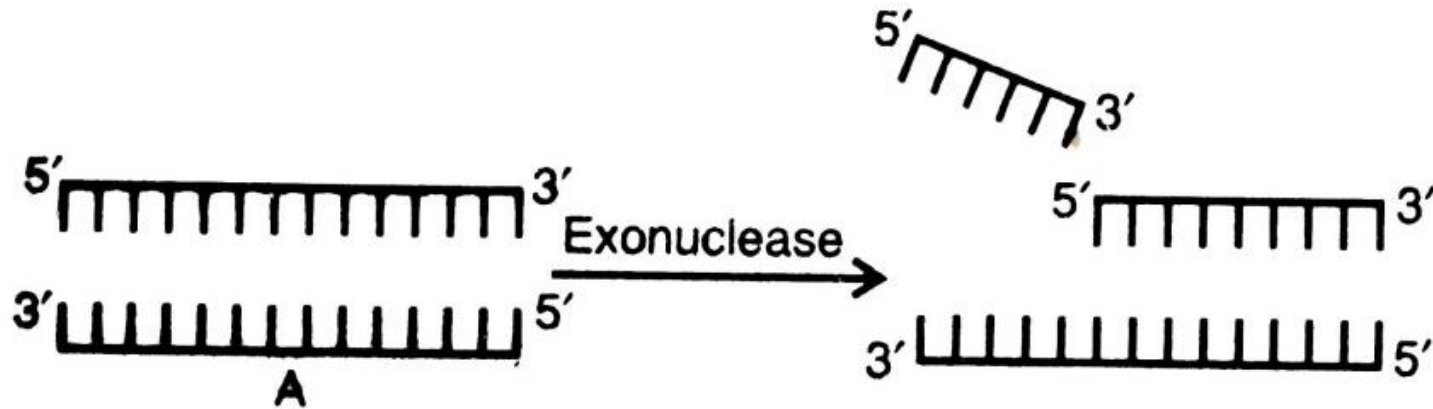


(b)



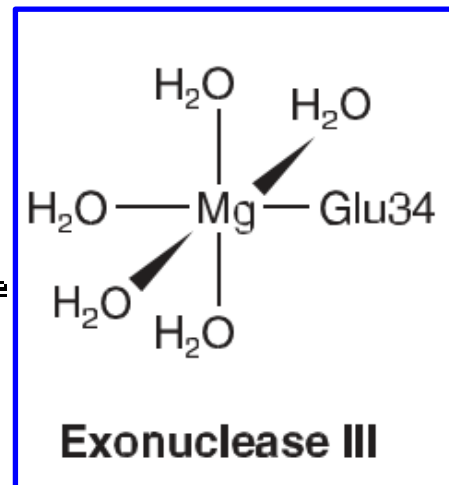
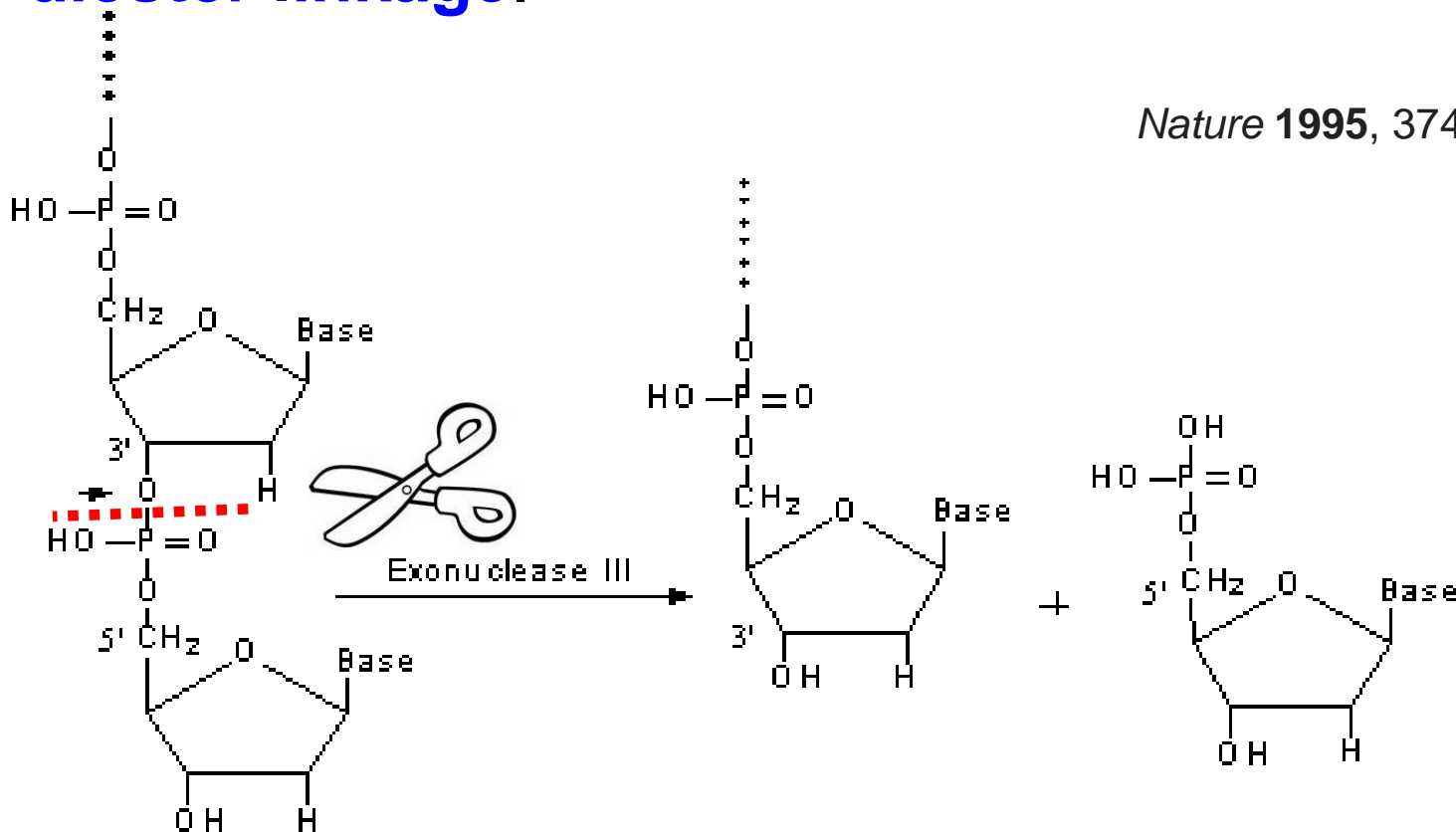
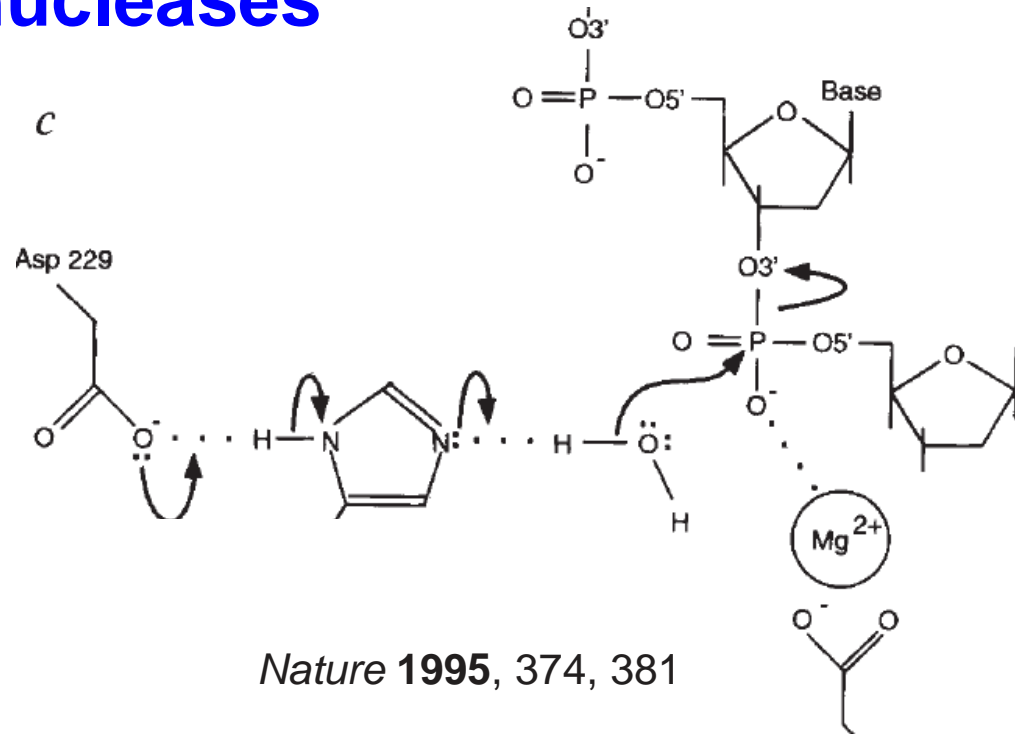
- Mg as a cofactor or a Mg-NTP complex as the substrate (NTP: nucleotide triphosphate).

Nucleases (cleavage of poly-nucleotide chain)

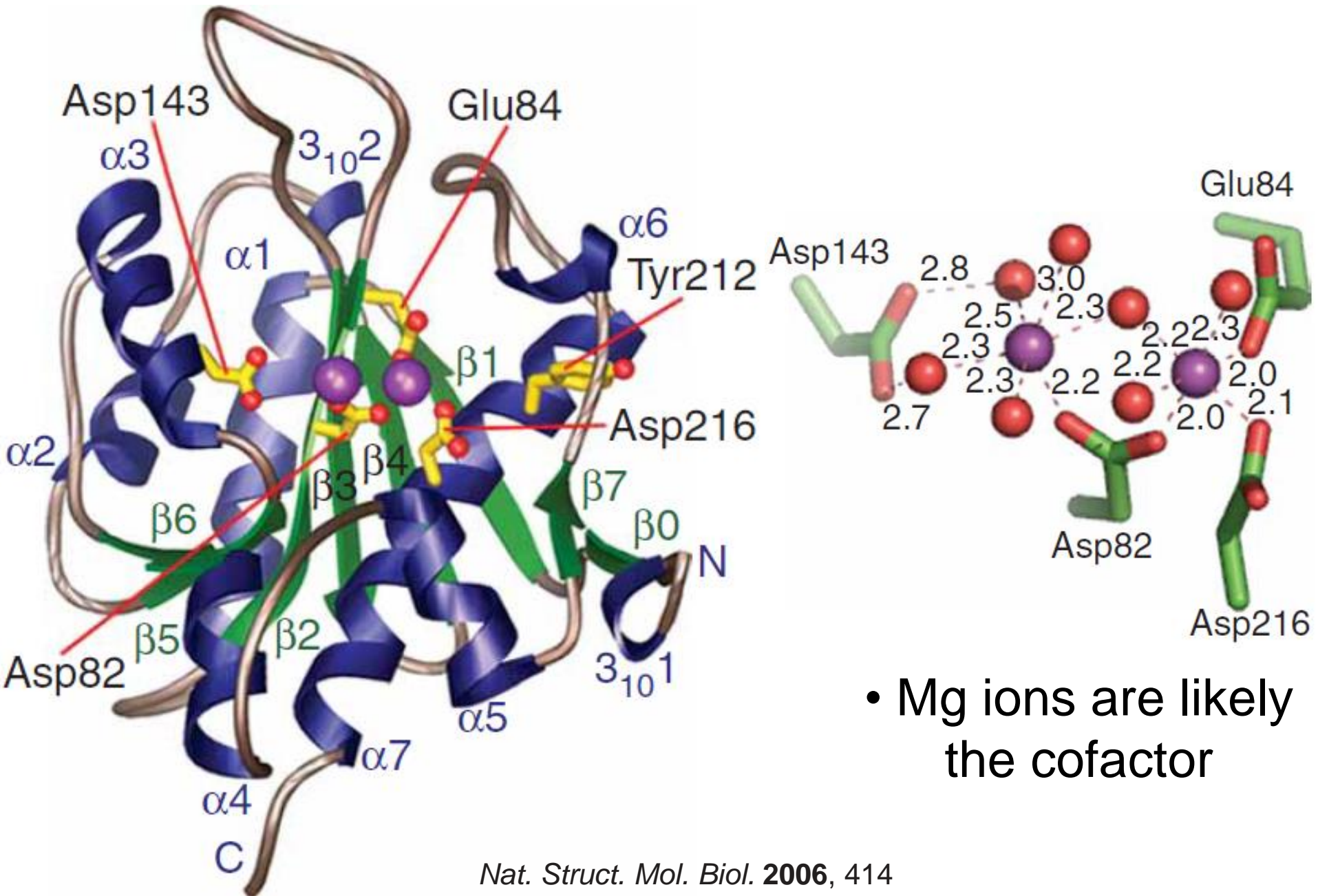


Exonucleases

- **Cleave** fragments from the **end** of a **poly-nucleotide chain** (DNA or RNA) by **hydrolysis** of the **terminal phosphodiester linkage**.

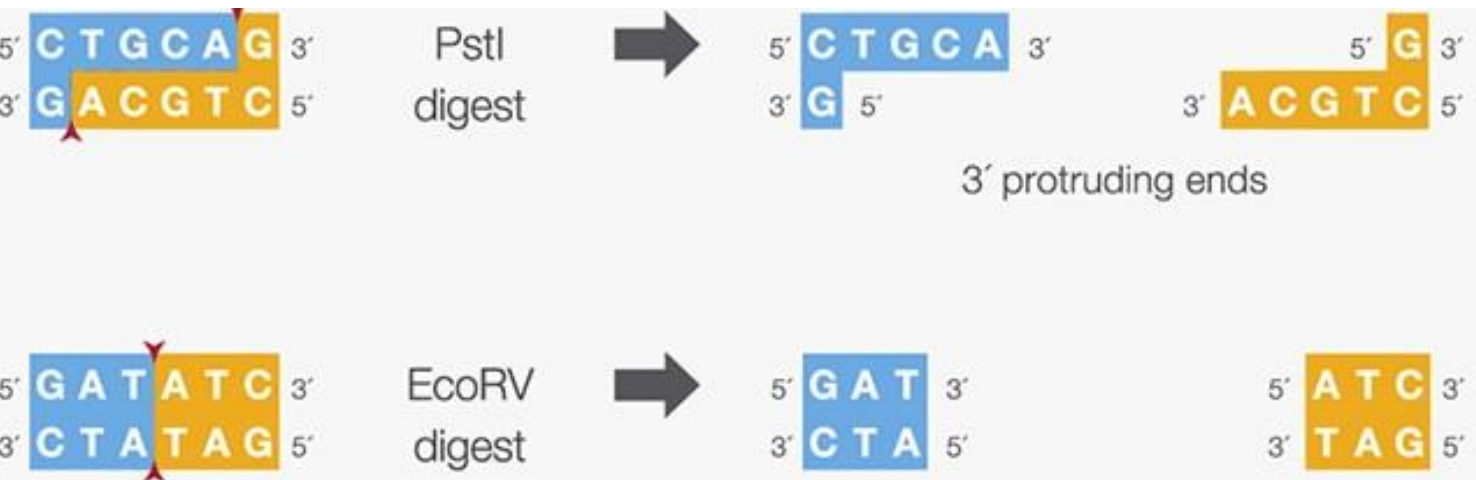
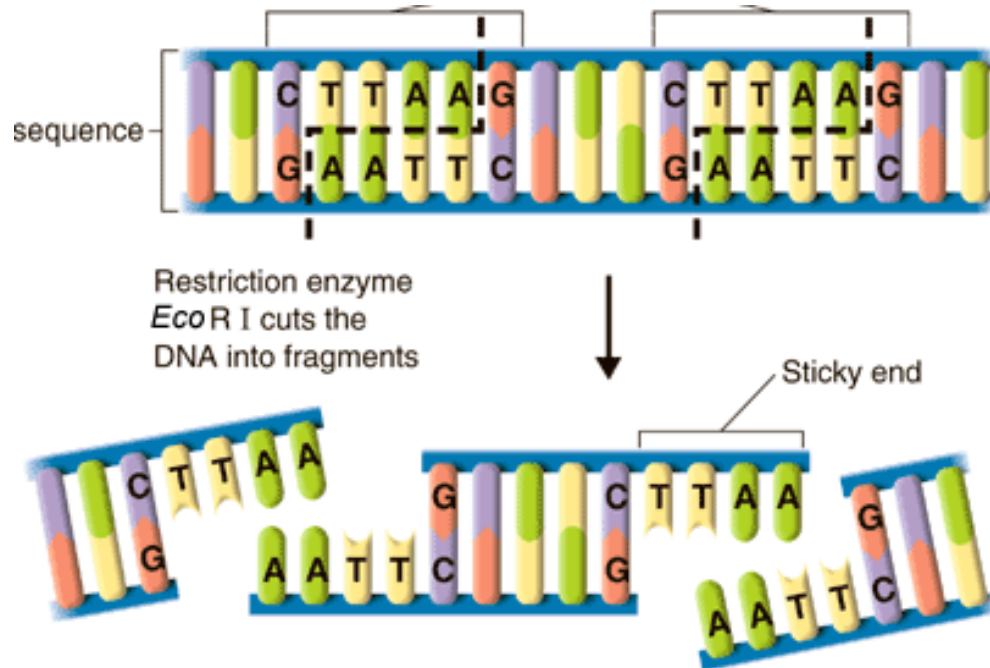


Crystal Structure of WRN Exonuclease

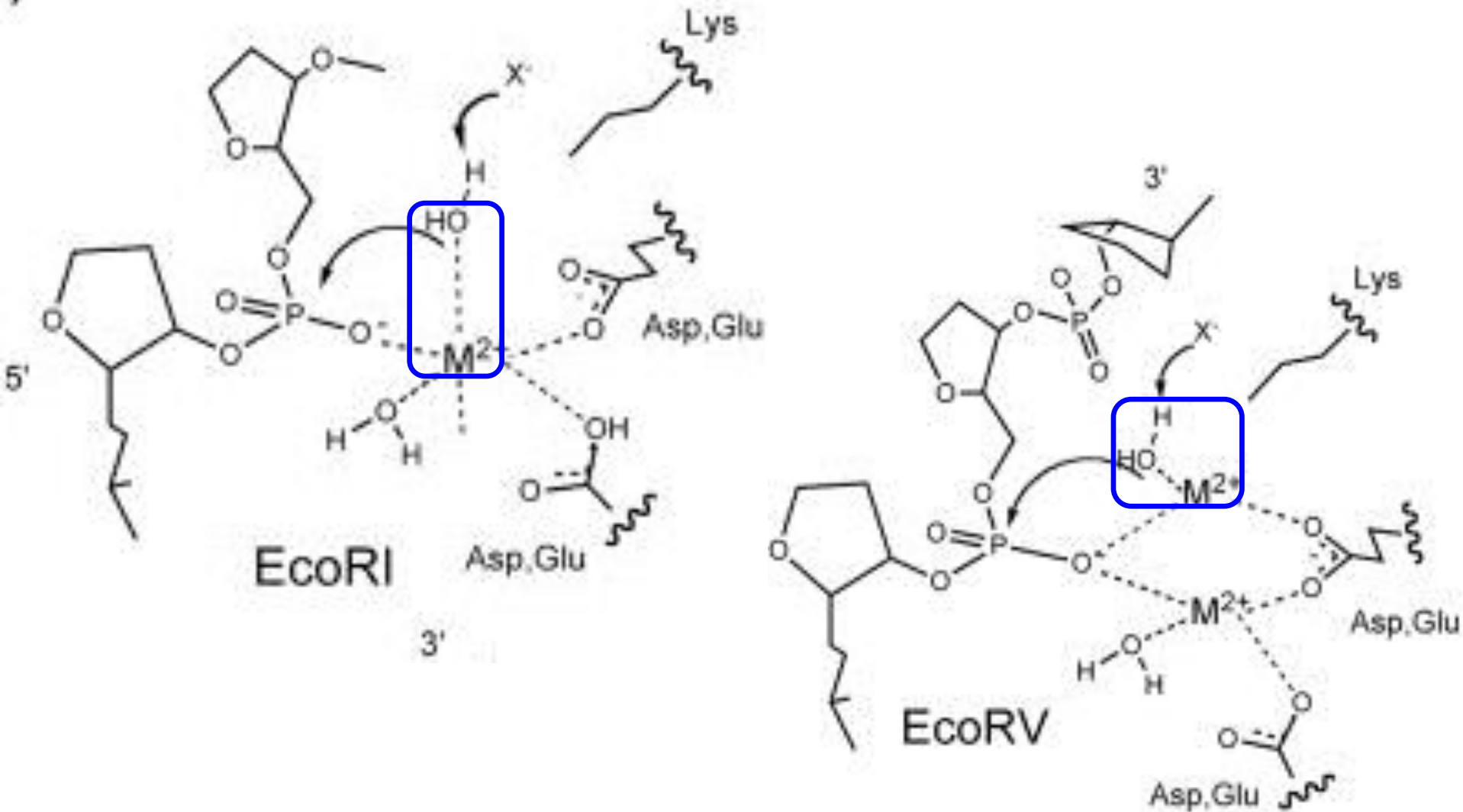


Endonucleases

- **Endo**nucleases **cleave** the phosphodiester bond **within** a polynucleotide chain.
- **Restriction** endonucleases cleave only at **very specific nucleotide sequences** (Most restriction enzymes are endonucleases).

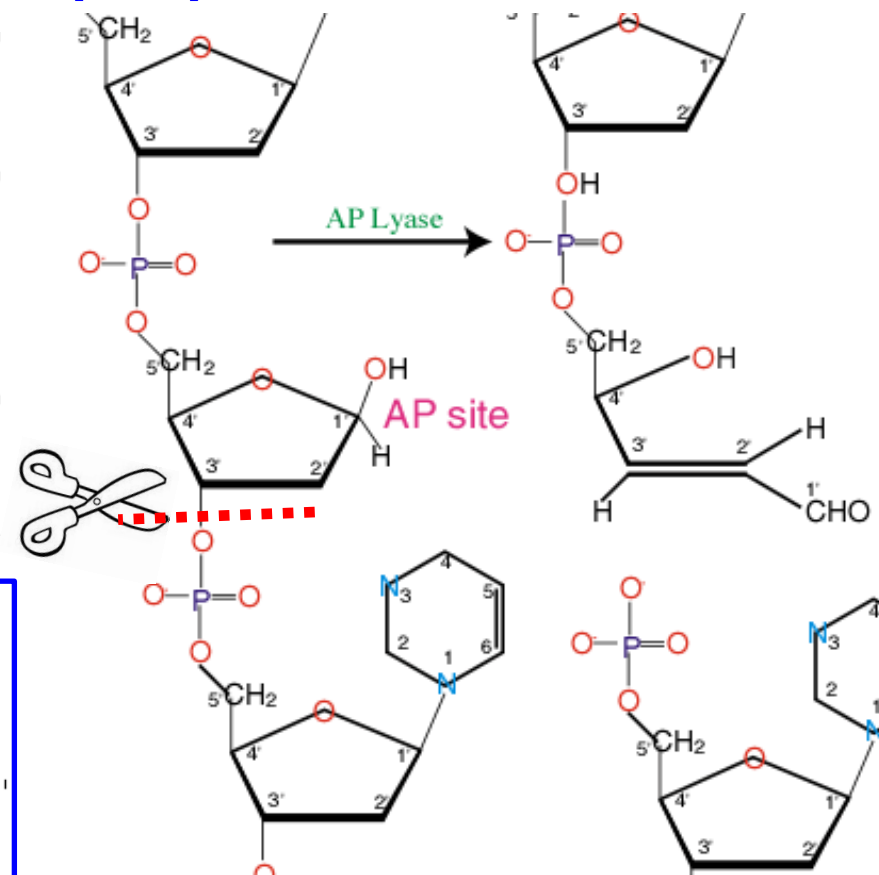
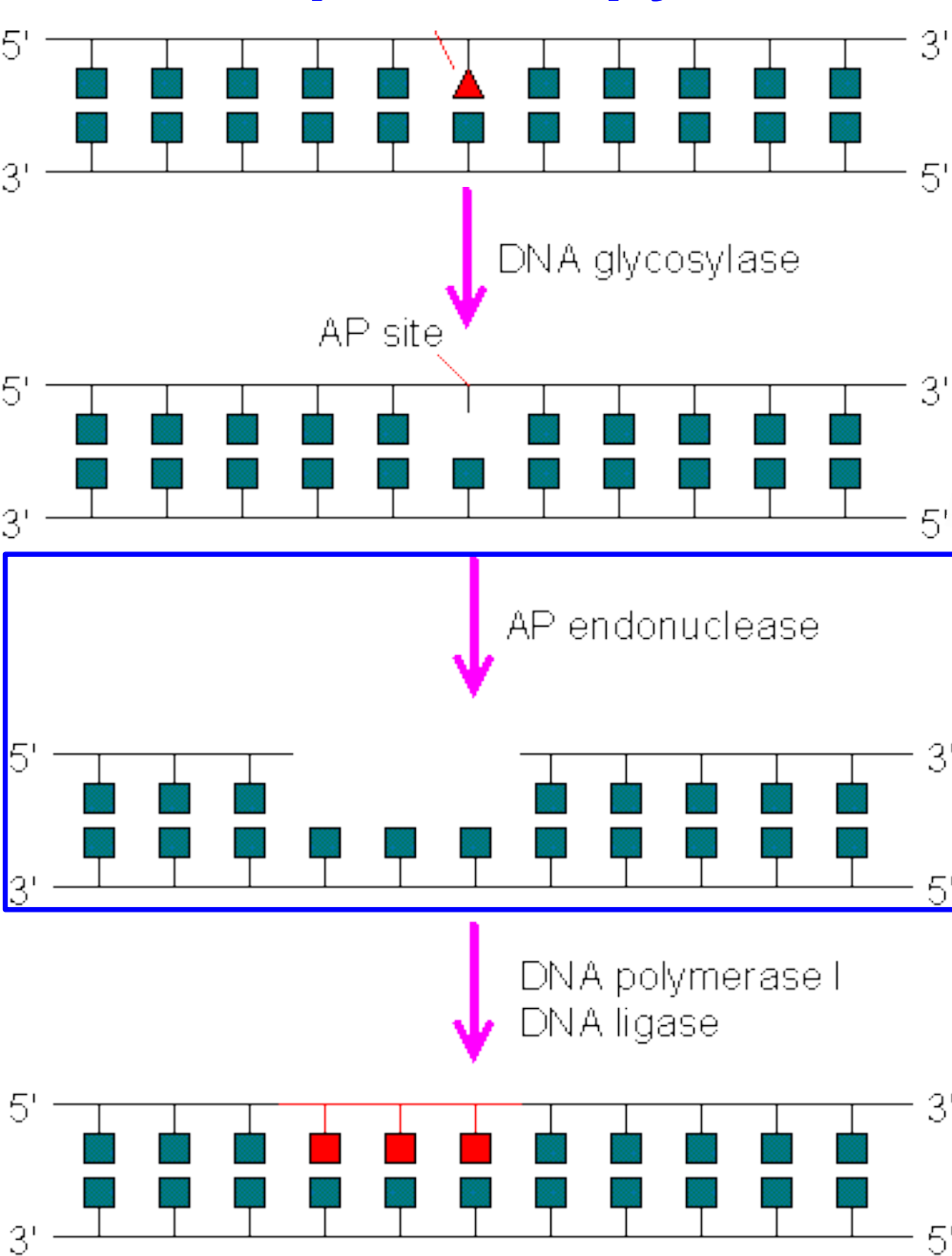


Restriction Endonucleases



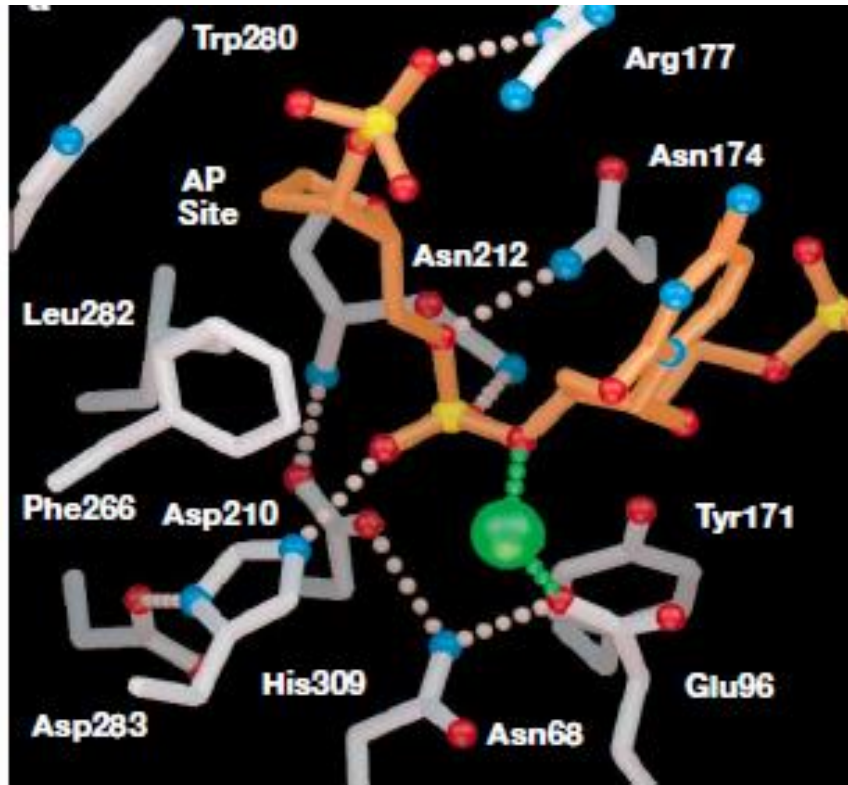
- A few **Mg**-dependent nucleases: restriction endonucleases. Some uncertainty about the **metal cofactor stoichiometry**, although an **inner-sphere pathway** seems to be likely.

Apurinic/apyrimidinic (AP) Endonuclease

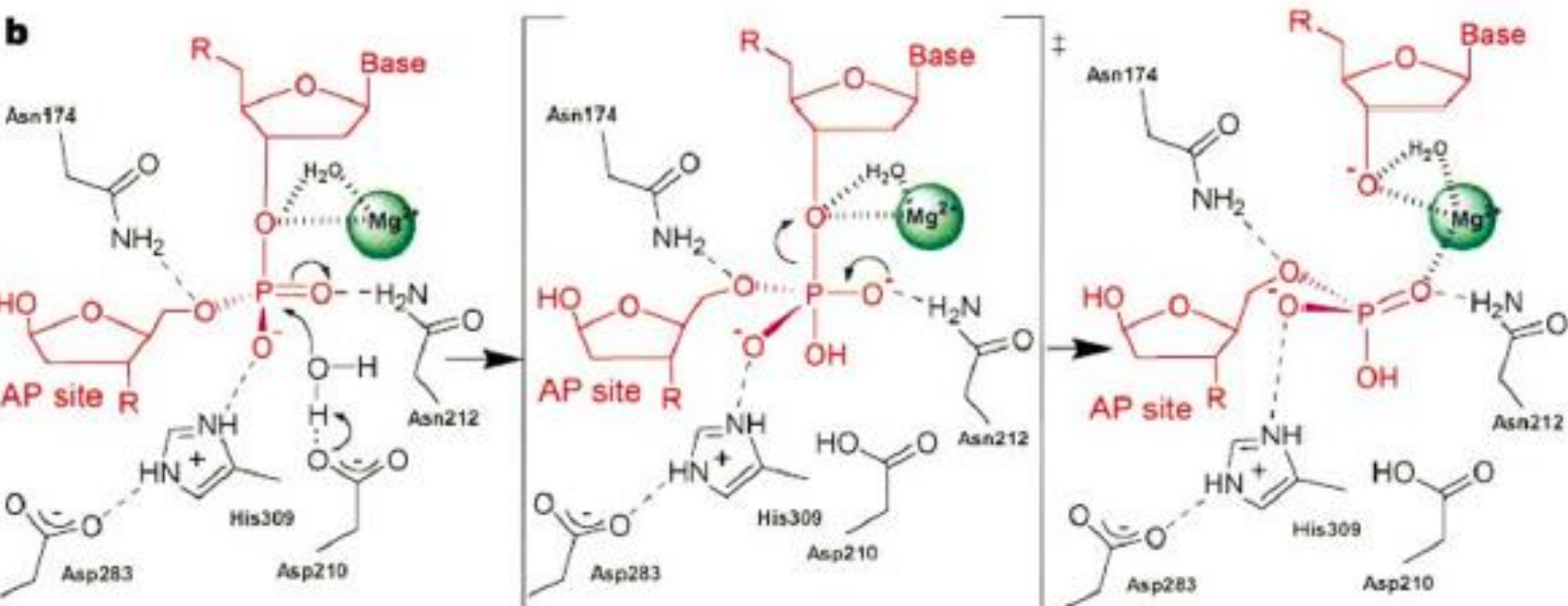


- Catalyzes cleavage of **DNA at AP sites** for repair of damaged or mismatched nucleotides in DNA.

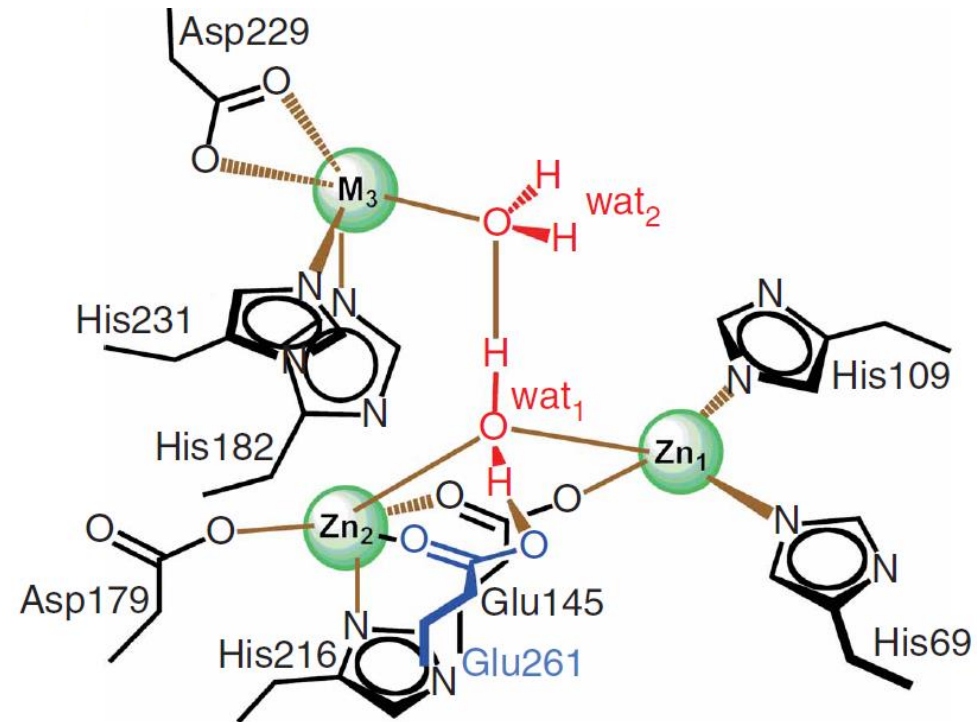
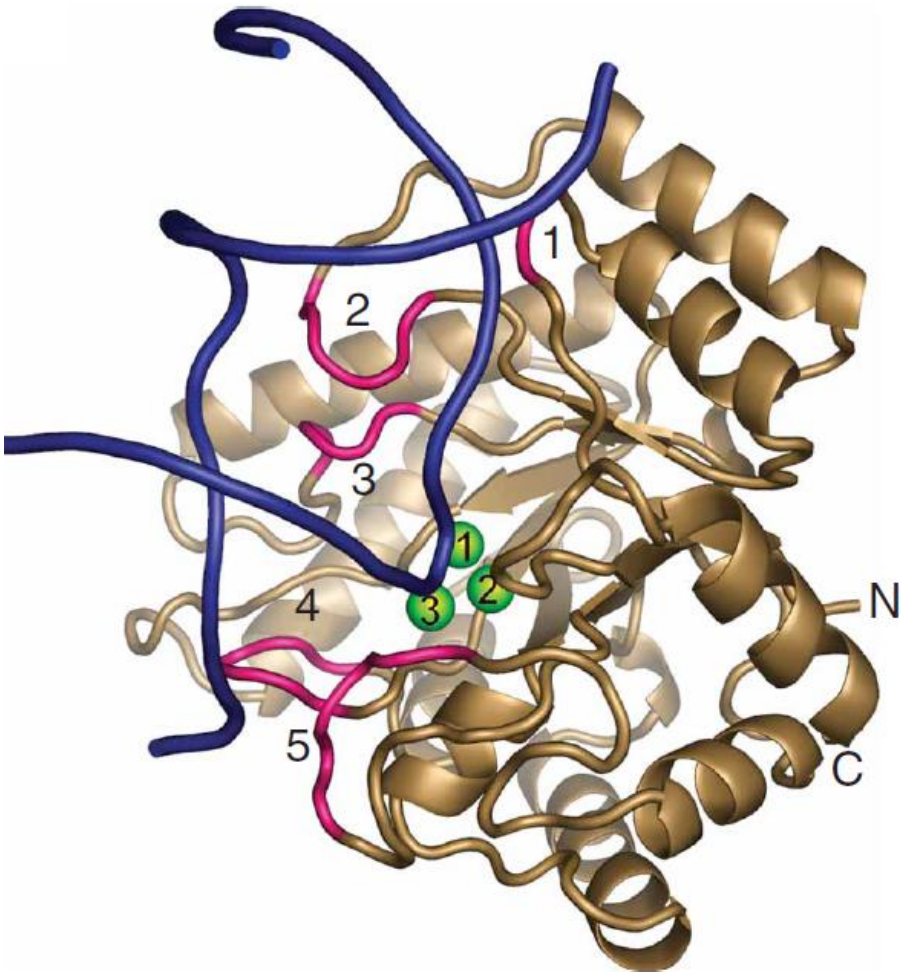
Human base excision repair enzyme APE1



Nature 2000,
403. 451

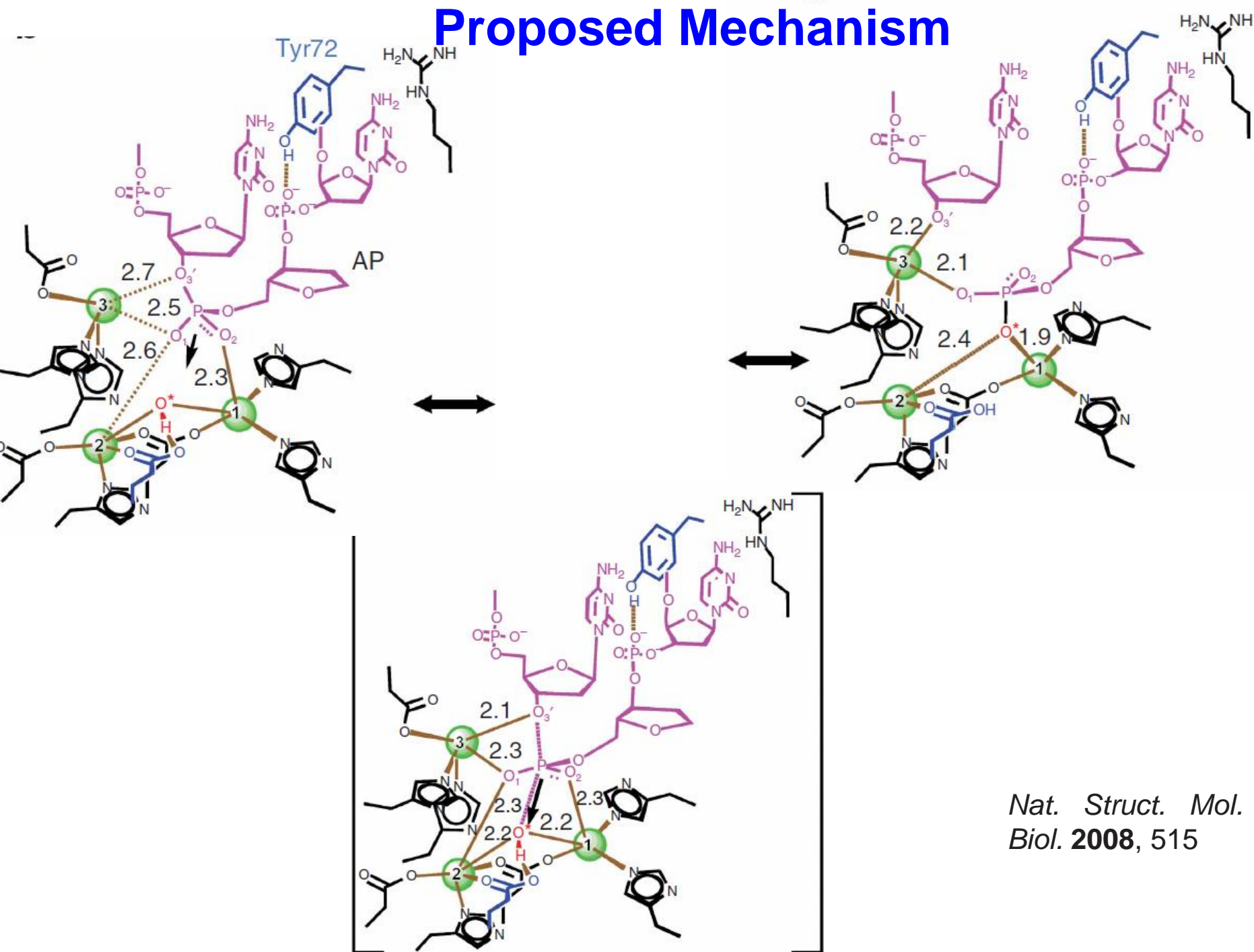


E. Coli Endonuclease IV



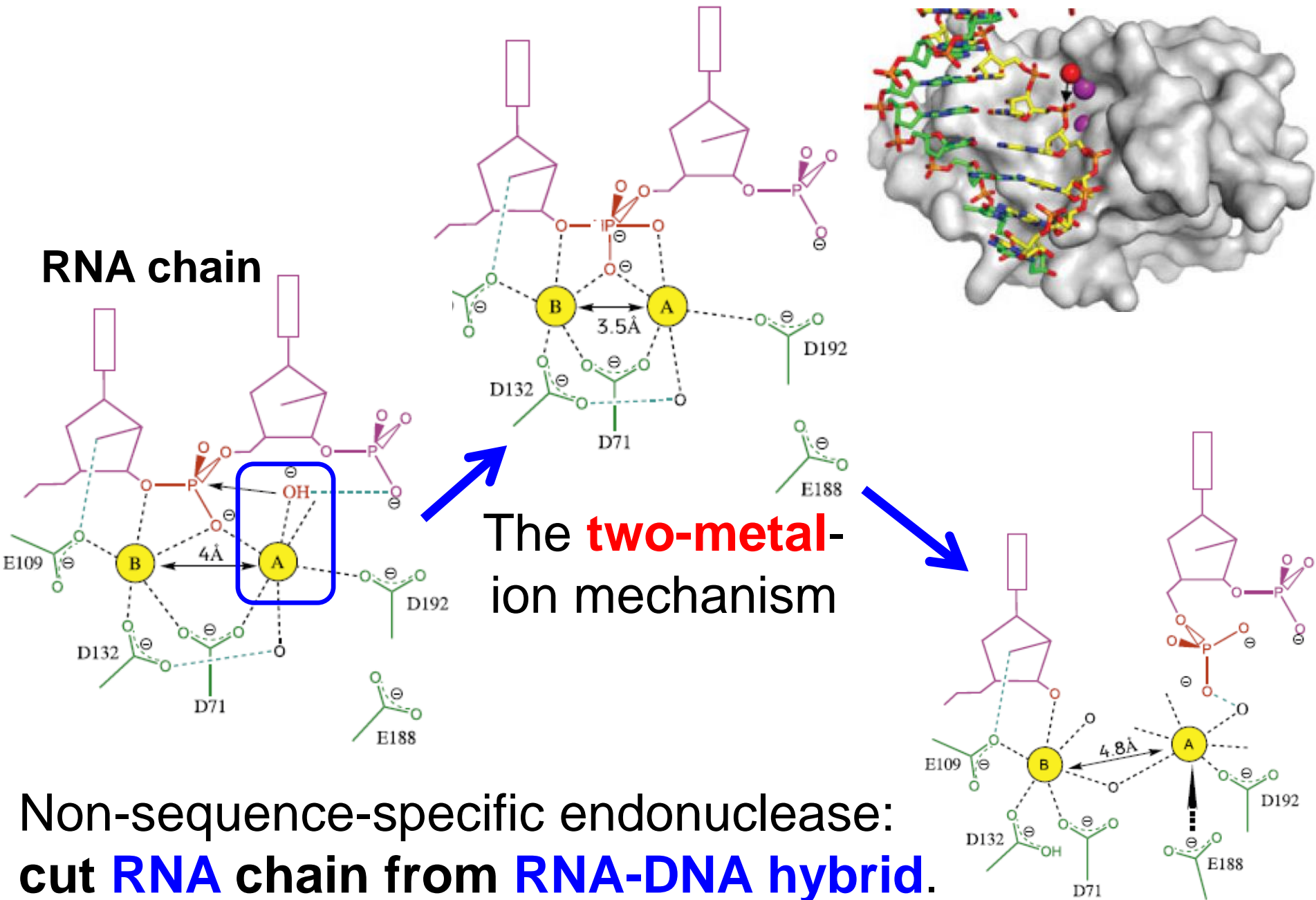
The **three-metal (Zn)** ion active site

Proposed Mechanism

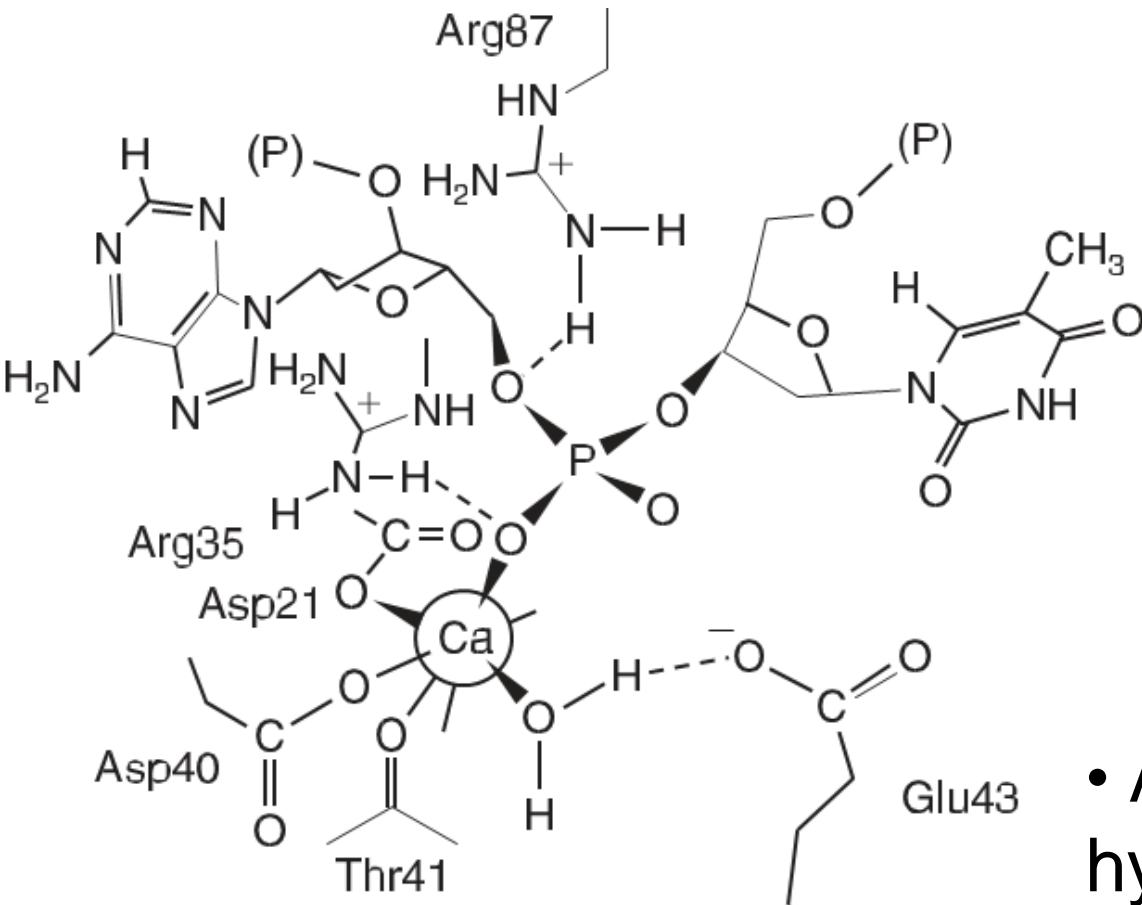


Nat. Struct. Mol. Biol. **2008**, 515

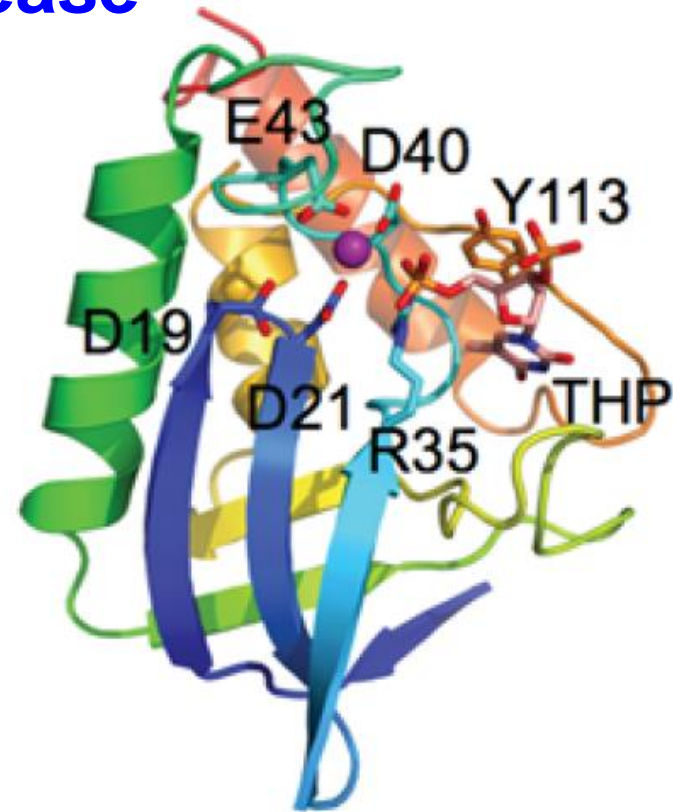
RNase H (Ribonuclease H)



Staphylococcal Nuclease

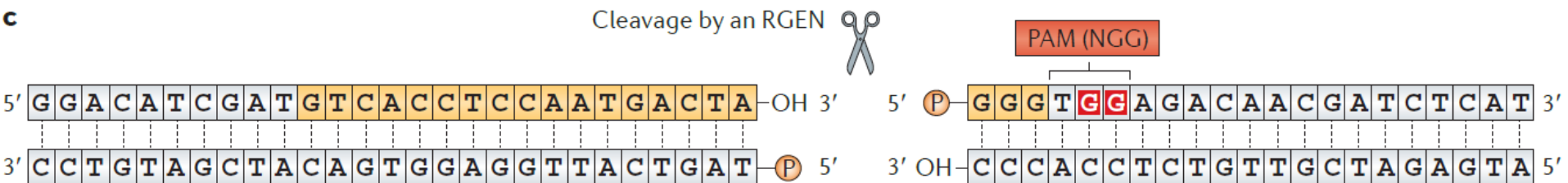
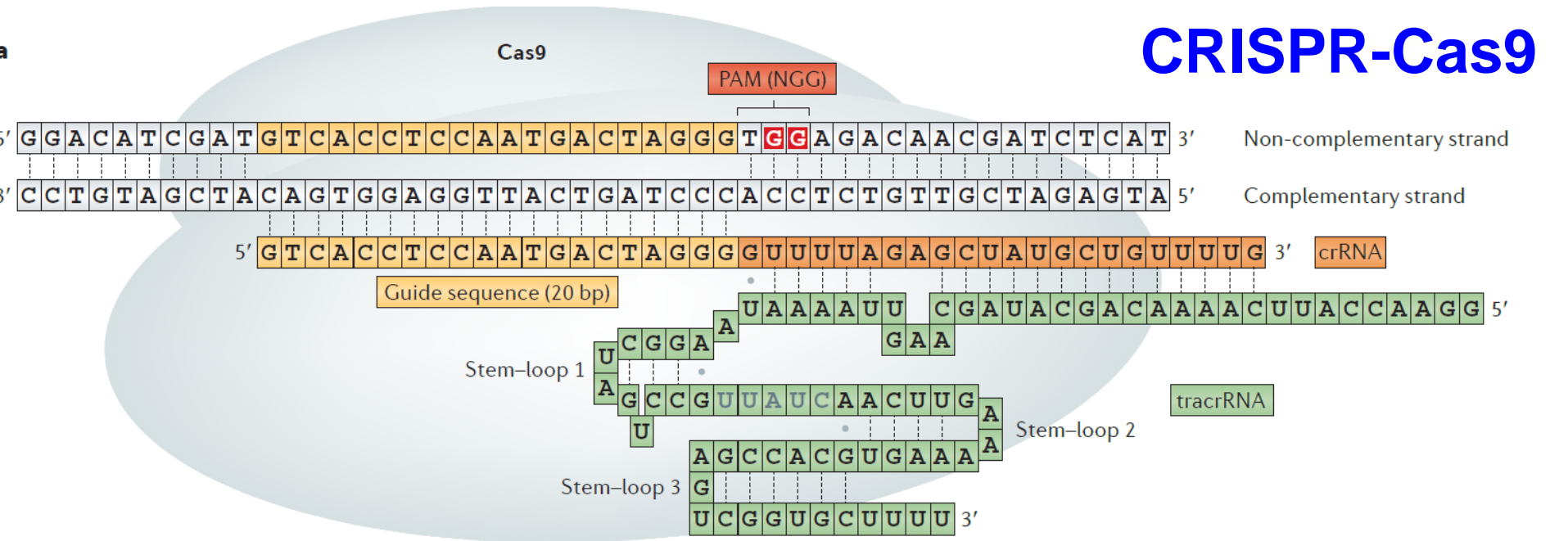


The active site with an inhibitor thymidine-3',5'-bisphosphate



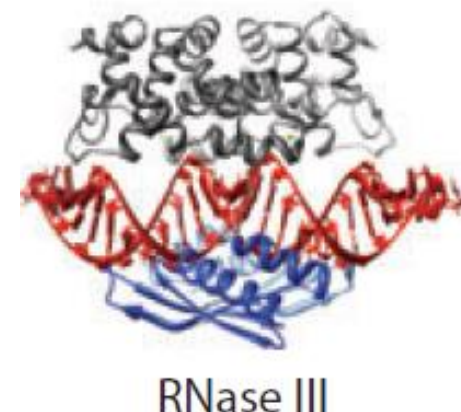
- A Ca^{2+} enzyme hydrolyzes DNA or RNA.
- Ca^{2+} binds to the phosphodiester & stabilizes the leaving group after hydrolysis.

CRISPR-Cas9



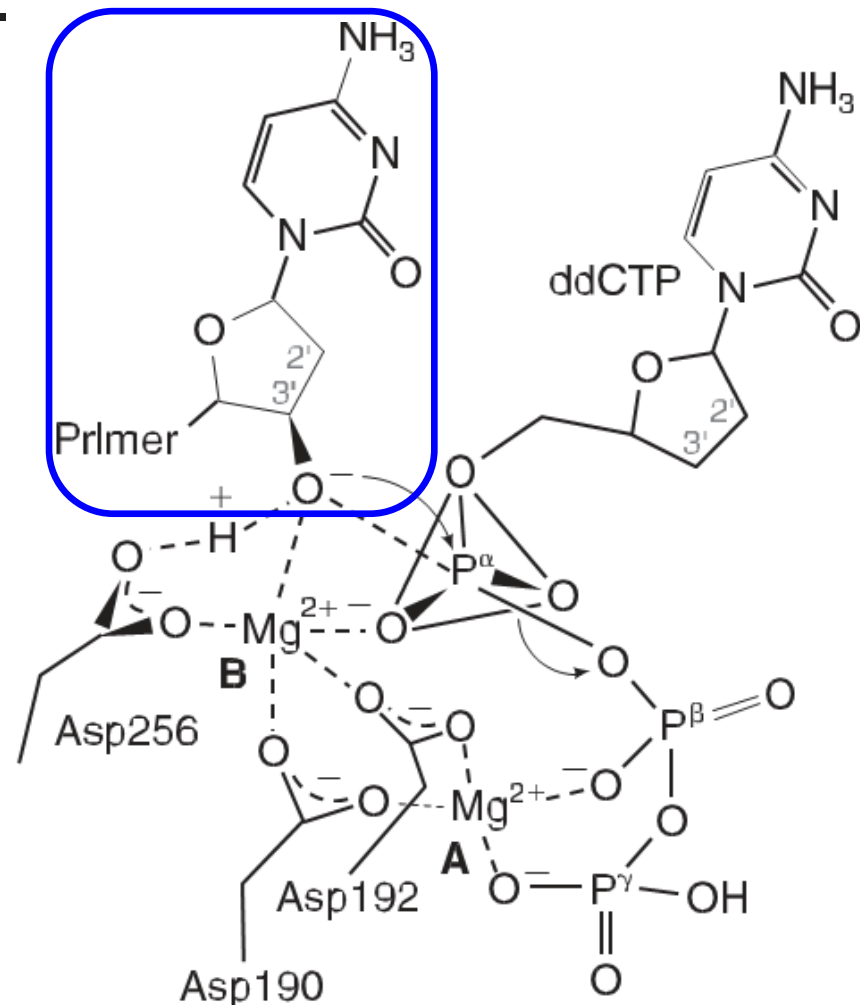
- CRISPR (clustered regularly interspaced short palindromic repeat)-associated protein 9 (Cas9)
- RGENs (RNA-guided engineered **nucleases**); crRNA (CRISPR RNA); tracrRNA (a trans-activating crRNA)

Nat. Rev. Genet. **2014**, 321;
Annu. Rev. Biochem. **2013**, 237



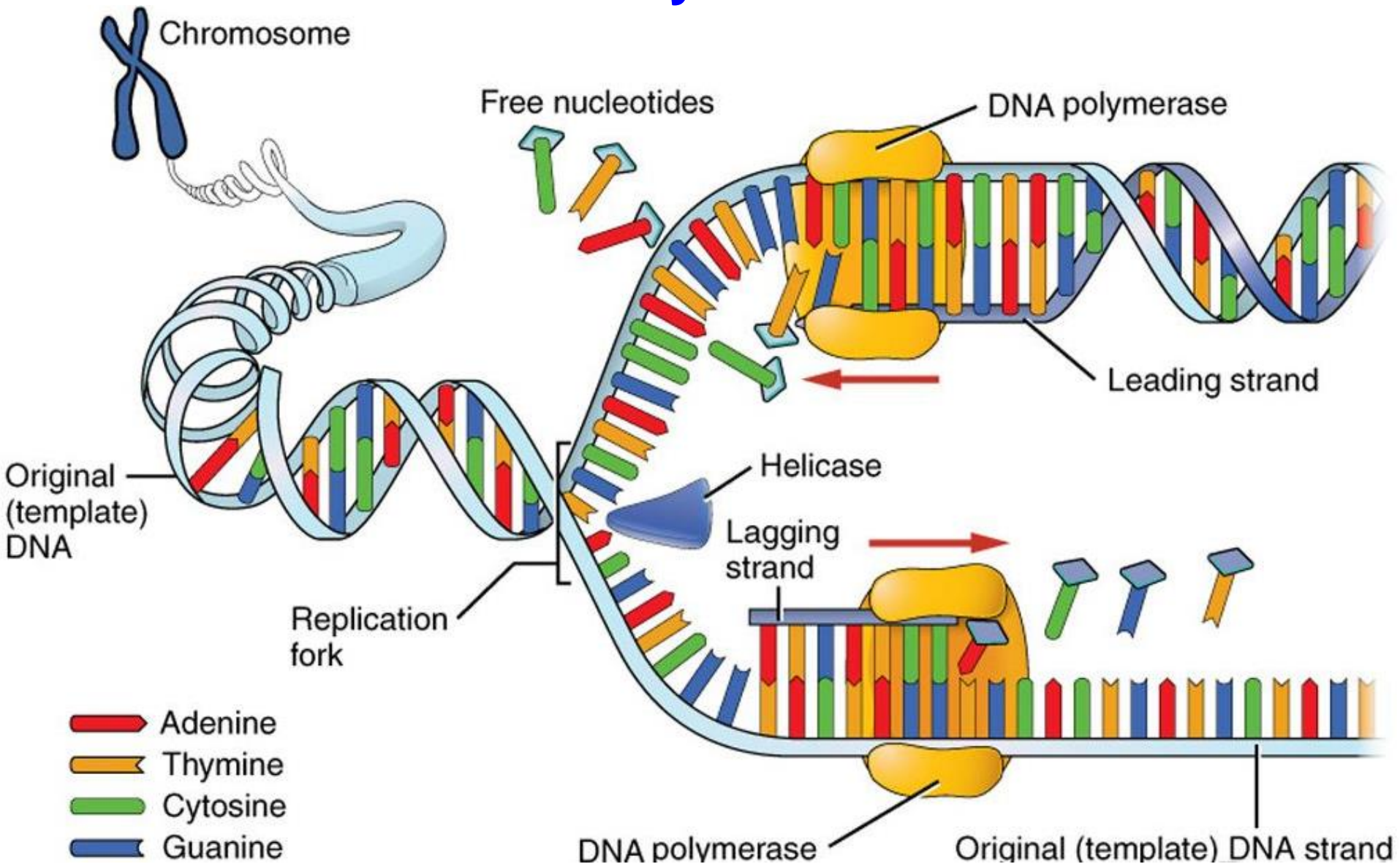
Polymerases

- Catalyzes replication & synthesis of DNA or RNA strands.
- Typically require **2 metals** (usually **Mg²⁺**), & **2 bridging Asp groups**.
- Acidic active-site residues can interact with the **Mg-phosphate chelate** to promote nucleophilic attack at the phosphate.
- Both **metals** help **stabilize** the negative charge on the 5-coordinate **transition state** & the **leaving group** during phosphoryl or nucleotidyl transfer reactions.



Active site of DNA polymerase β

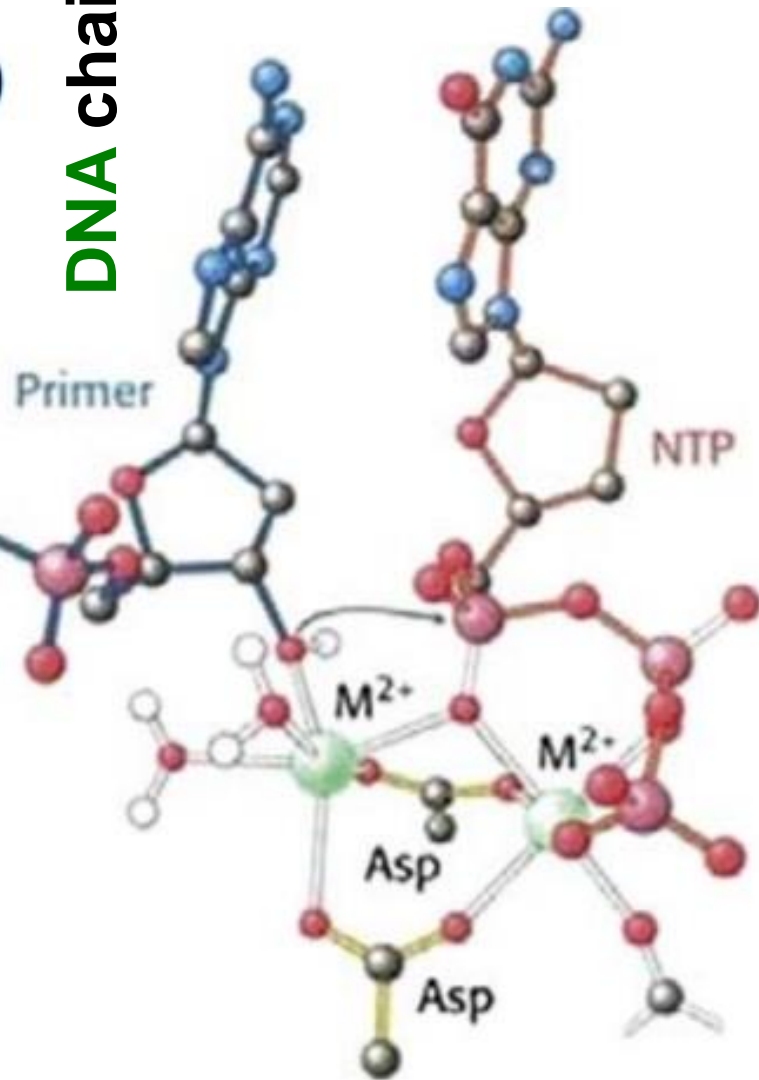
DNA Polymerase



- Catalyzes **synthesis of DNA** from deoxyribonucleotides

DNA chain

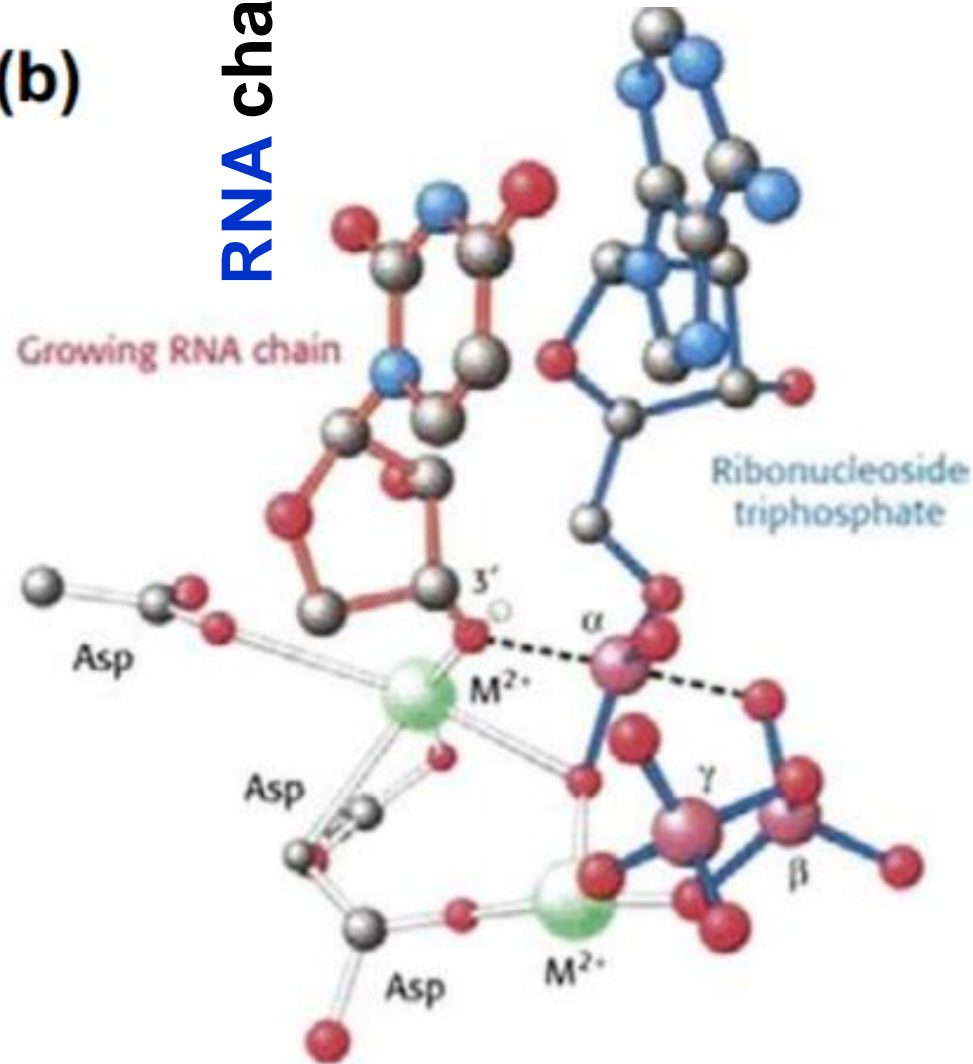
DNA Polymerase



(b)

RNA chain

RNA Polymerase



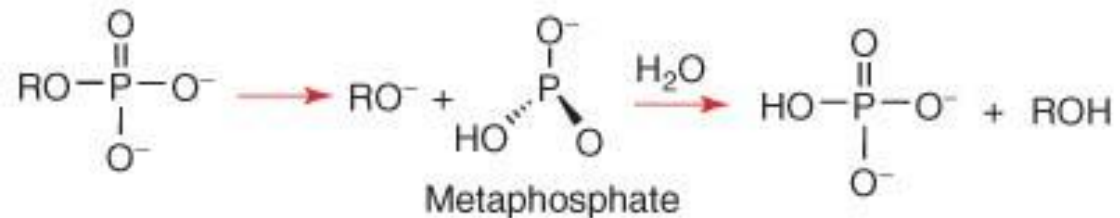
- (left) **DNA polymerase** catalyzed phosphodiester bond formation (Mg^{2+}); (right) Transition-state model for phosphodiester bond formation in **RNA polymerase**.

Phosphoryl Transfers

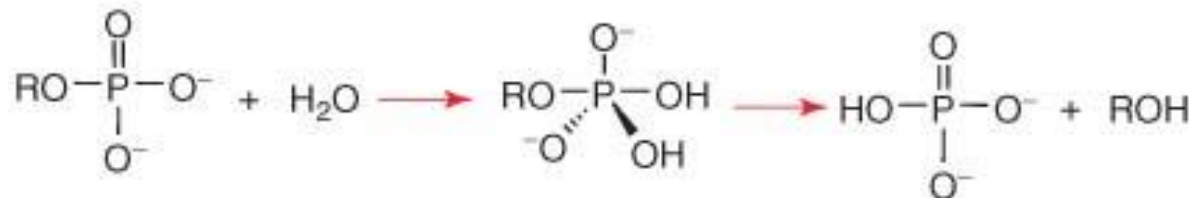
- Enzymes that catalyze **phosphorylation** of substrates (usually Ser, Thr, or Tyr residues) typically use **Mg²⁺-chelates** to facilitate nucleophilic attack at the phosphate.
- Hydrolysis of **simple** phosphate esters is often catalyzed by enzymes with **transition-metal cofactors**, e.g. alkaline phosphatase & purple acid phosphatase.

3 possible mechanisms

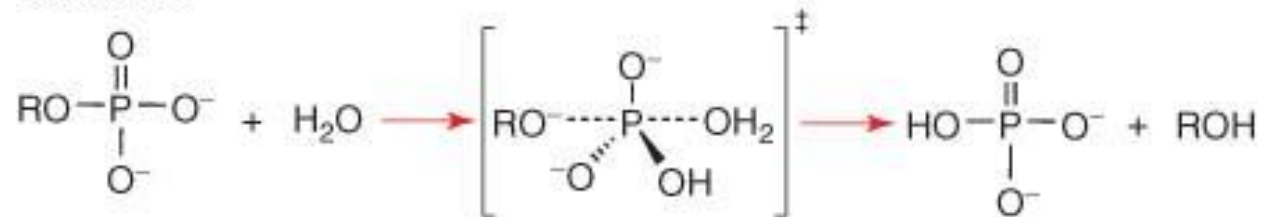
Dissociative



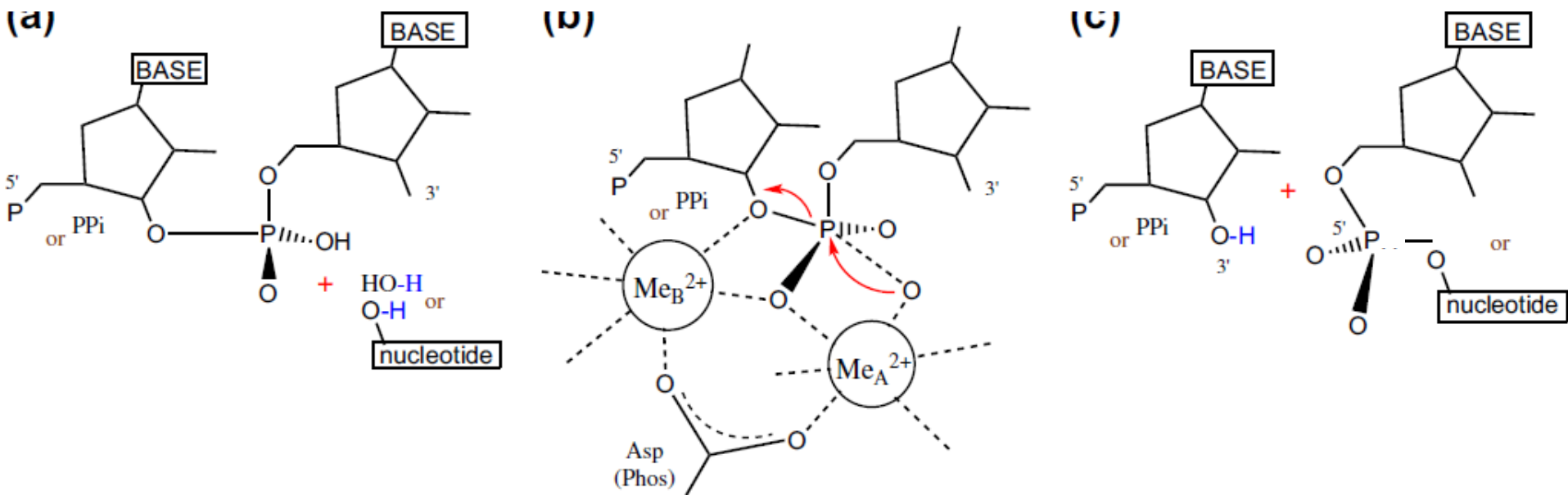
Associative



Concerted



2-Metal-ion-dependent Phosphoryl Transfer Reaction

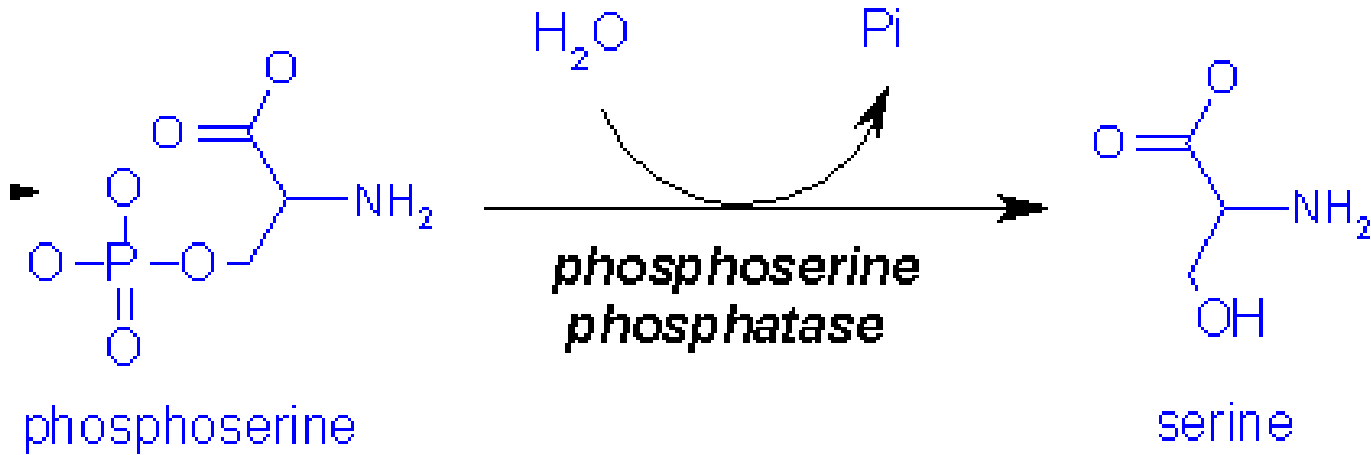


(a) **Substrates:** phosphate & water (or RO-H).

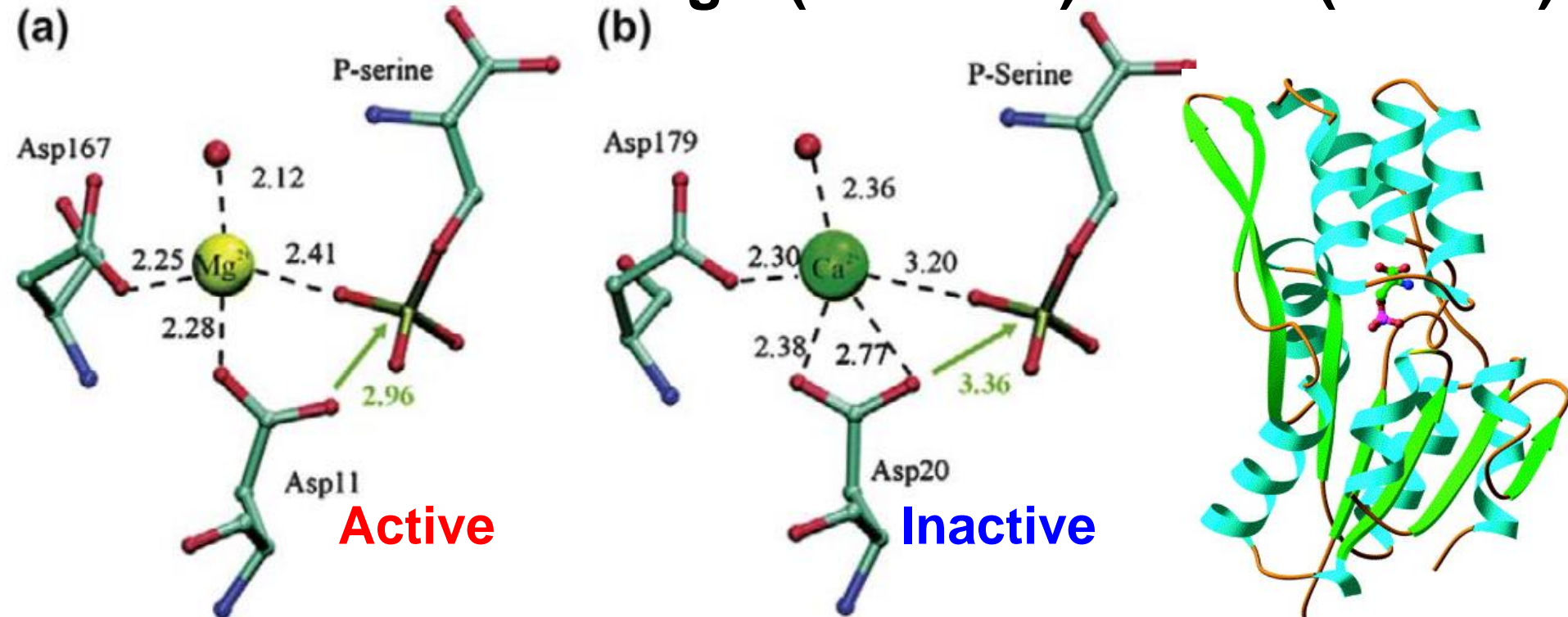
(b) **Intermediate:** the **2 metals** are always **coordinated** by **oxygen of the phosphate** and a conserved **Asp**.

(c) **Products:** a new phosphoryl bond is formed between the nucleophile and phosphate.

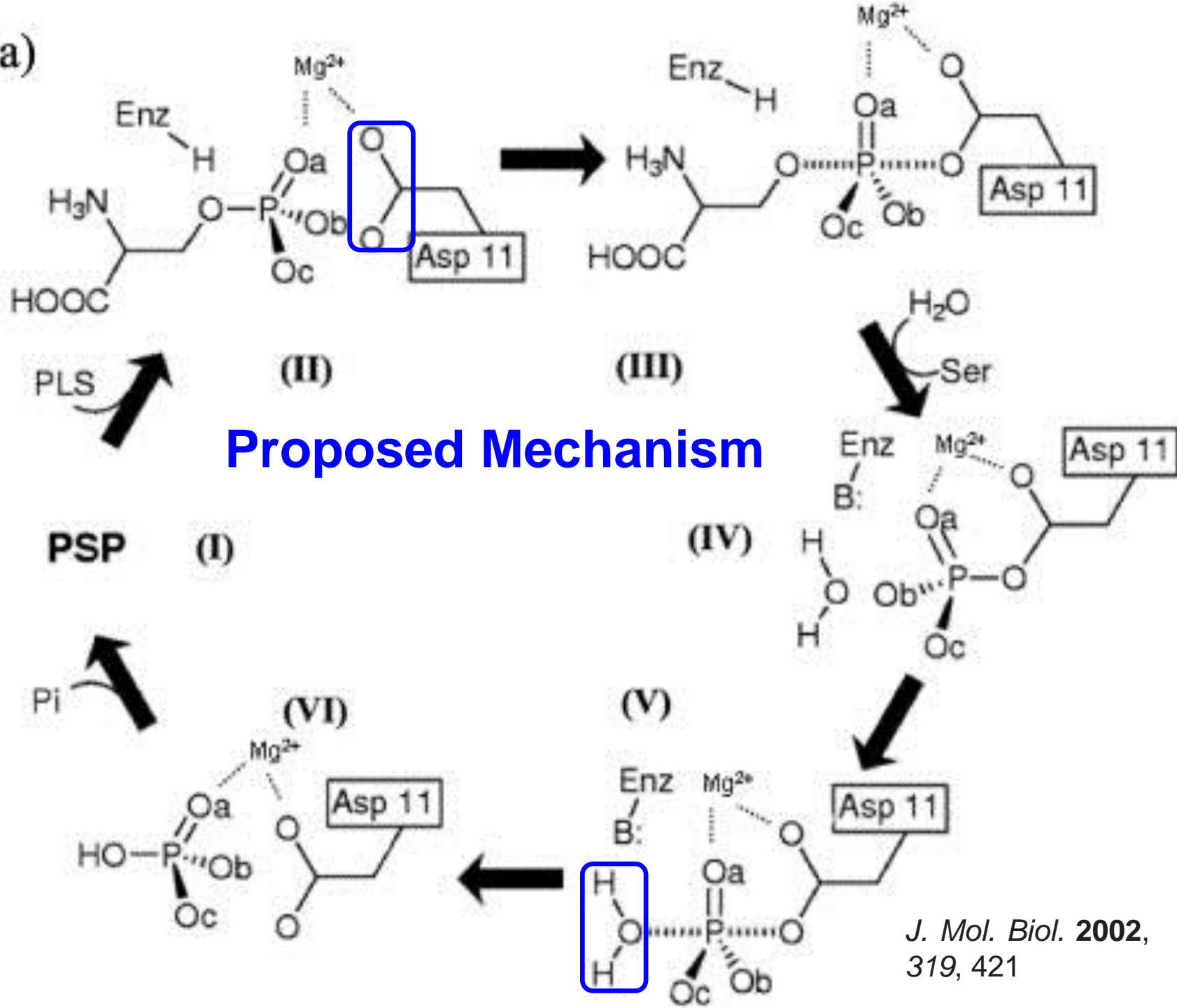
Phosphoserine Phosphatase (PSP)



Active site with one Mg^{2+} (bacterial) or Ca^{2+} (human)



(a)



E. coli Alkaline Phosphatase



Phosphate monoester

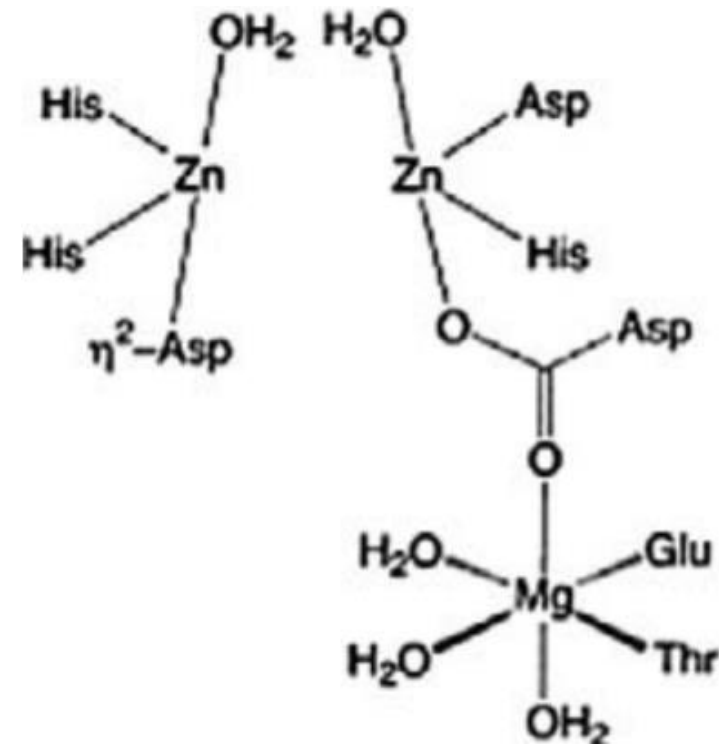
or R'OH
(instead of H₂O)

Alcohol

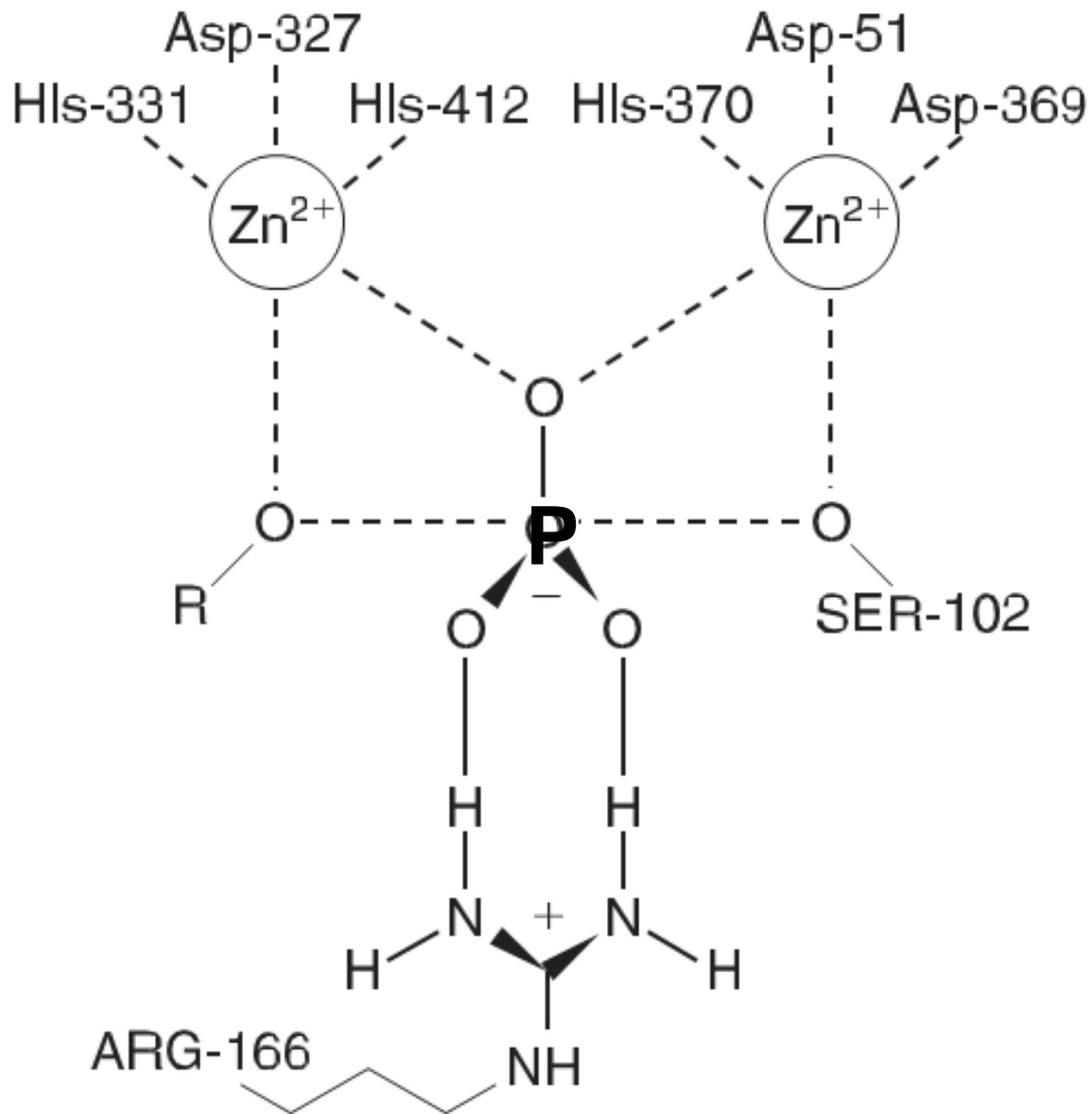
Phosphate
O=P(OH)₂ (OR')
phosphotransferase

- Catalyzes removal of the phosphate groups.

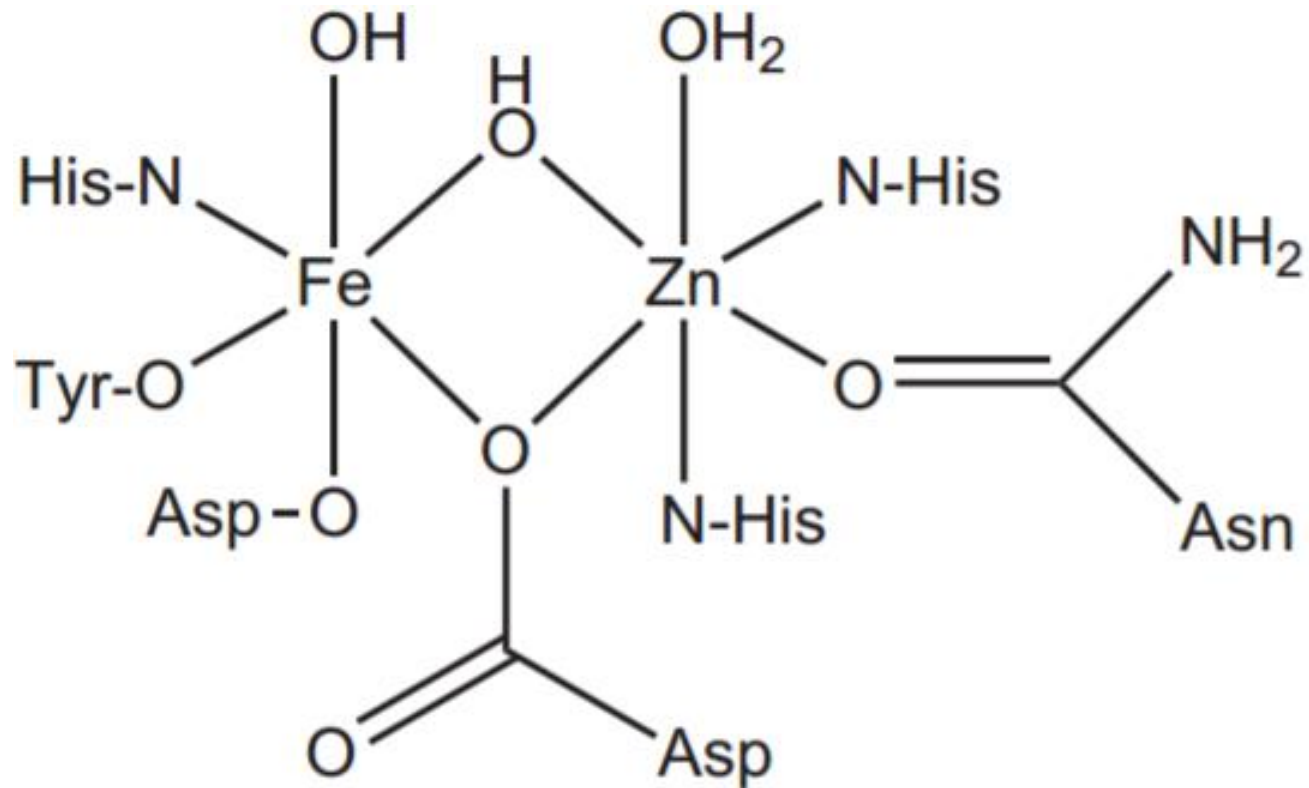
- The **optimal pH** for activity: ~8 (**basic**) → a low pK_a values for the Zn-bound water.



Proposed Mechanism

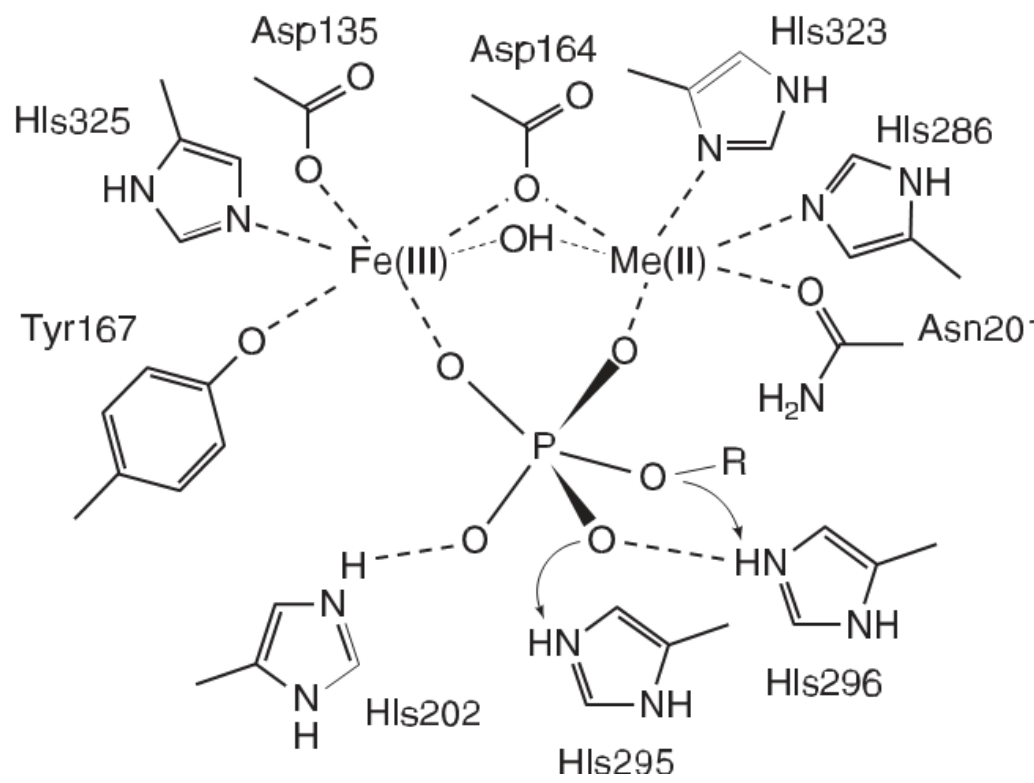
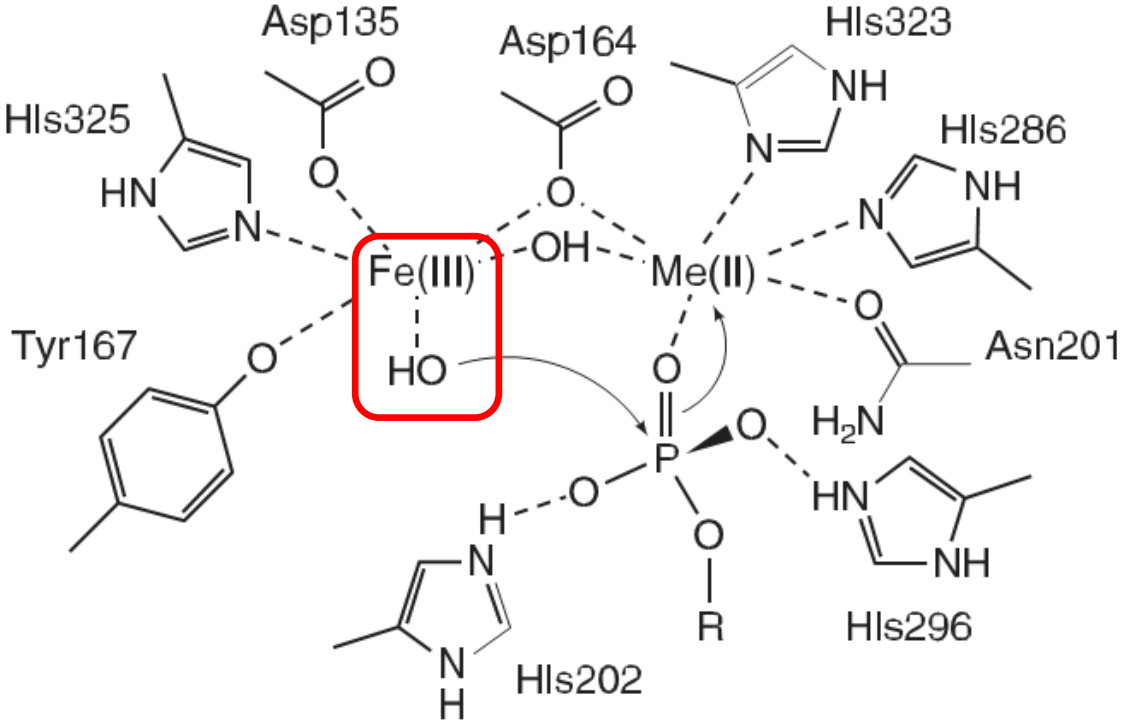


Kidney Bean Purple Acid Phosphatase

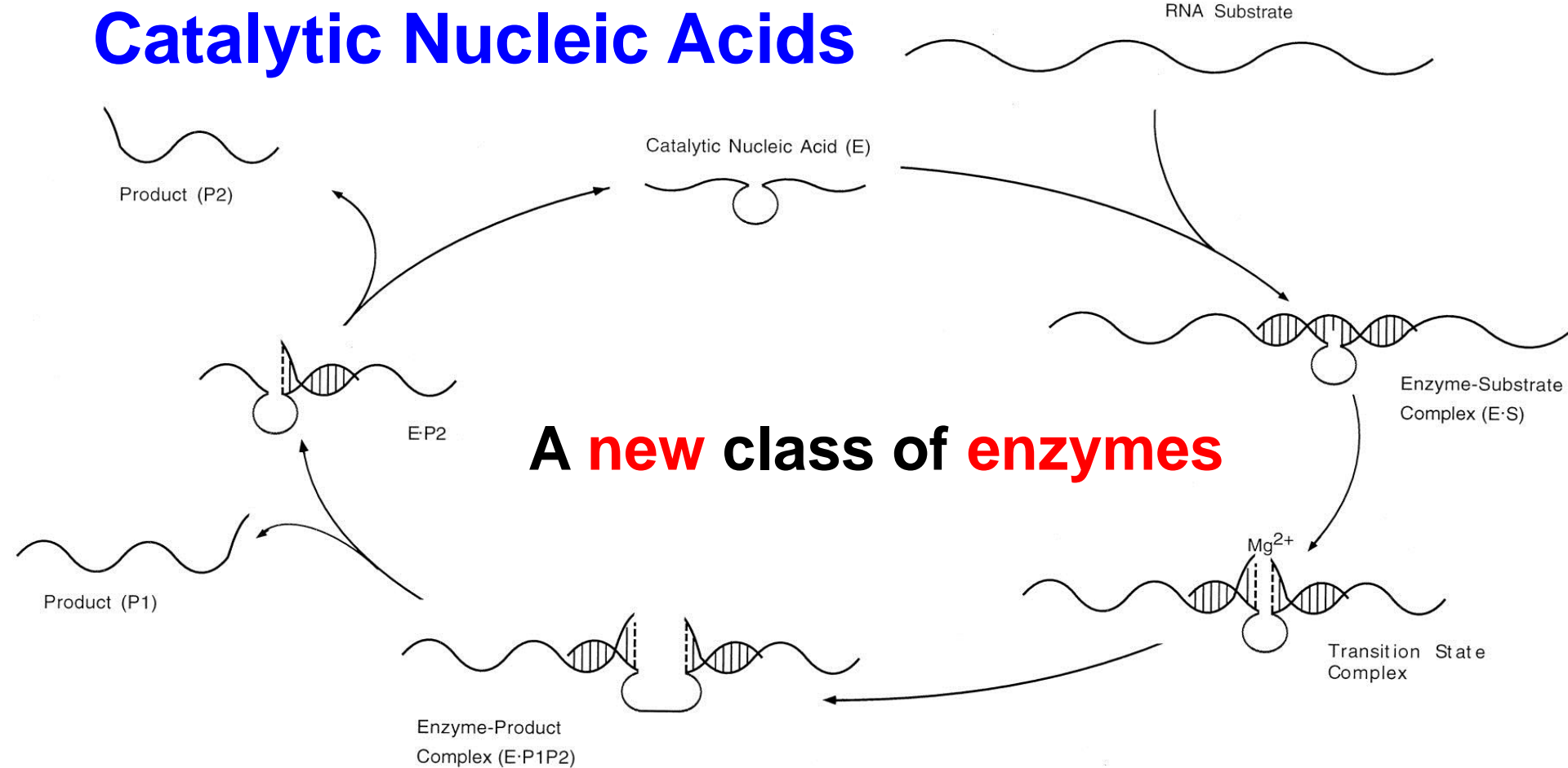


- The **optimal pH** for activity: **acidic** → the high pK_a values for the bound water.
- The substrates in acid phosphatases are **possibly less reactive** than alkaline phosphatase → require **stronger Lewis acid (Fe(III))**.

Proposed Mechanism



Catalytic Nucleic Acids



A new class of enzymes

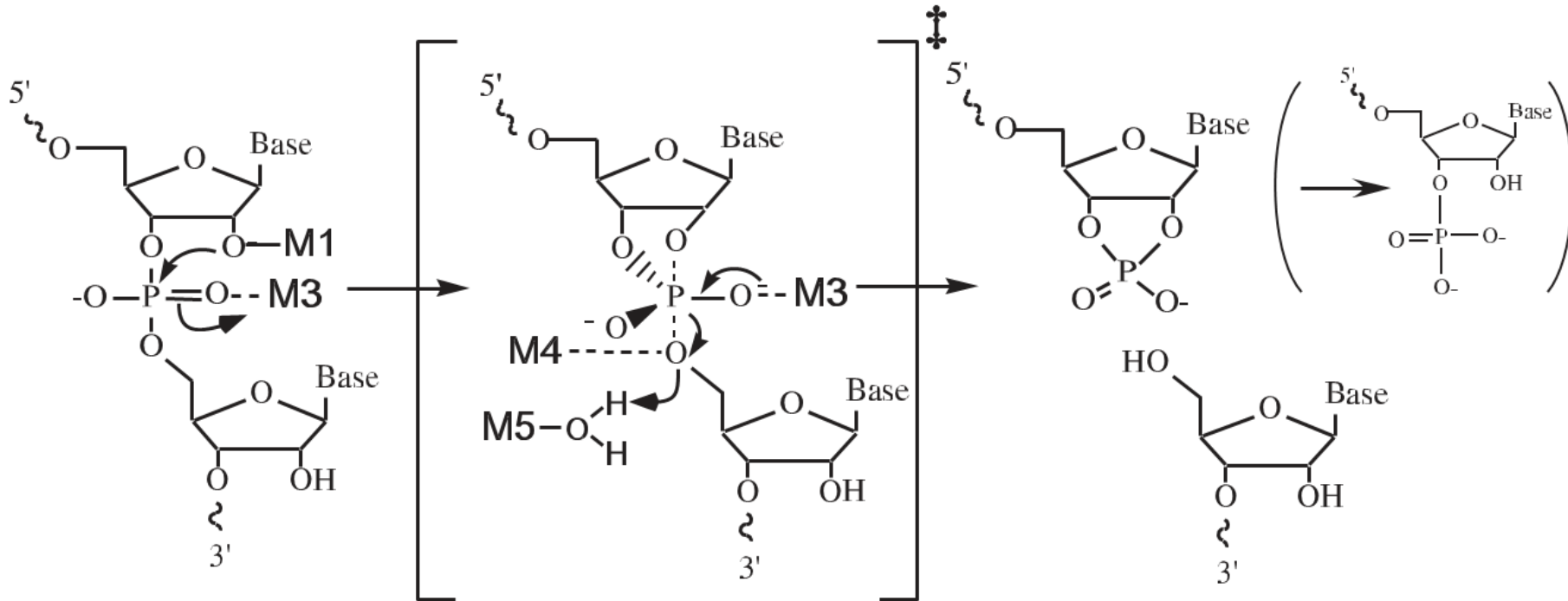
- Some **RNA molecules** were found to have **enzymatic activities** in 1982 (so-called catalytic RNA, **ribozymes**, RNA enzymes, or RNAzymes).
- **Single-stranded DNA molecules** were also found to **catalyze RNA cleavage** in 1994 (so-called deoxyribozymes, DNA enzymes or DNAzymes).

Reactions Catalyzed by Catalytic Nucleic Acids^a

Reaction ^b	Catalytic Activity					
	Enzyme ^c	k_{cat} (min ⁻¹)	K_{m} (μM)	$k_{\text{cat}}/k_{\text{u}}$		
Phosphoester transfer	R-nat	0.1	1×10^{-3}	10^{11}	(R-nat): catalytic RNA derived from naturally occurring sources.	
	R-lab	0.3	0.02	10^{13}		
Phosphoester cleavage	R-nat	1	0.05	10^6		
	R-lab	0.1	0.03	10^5		
	D-lab	3	8×10^{-4}	10^6		
Polynucleotide ligation	R-nat	4	3	10^6		
	R-lab	100	9	10^9	(R-lab): catalytic RNA obtained by in vitro selection.	
	D-lab	0.04	100	10^4		
Polynucleotide phosphorylation	R-lab	0.3	40	$> 10^5$		
Mononucleotide aminoacylation	R-lab	0.3	5×10^3	$> 10^7$		
Polynucleotide aminoacylation	R-lab	1	9×10^3	10^6		
Aminoacyl ester hydrolysis	R-lab	0.02	0.5	10		
Aminoacyl transfer	R-lab	0.2	0.05	10^3		
Amide bond cleavage	R-lab			10^2		
Amide bond formation	R-lab	0.04	2	10^5		
Peptide bond formation	R-lab	0.05	200	10^6		
N-Alkylation	R-lab	0.6	1×10^3	10^7		
S-Alkylation	R-lab			10^3		
Oxidative DNA cleavage	R-lab			$> 10^6$	(D-lab): catalytic DNA obtained by in vitro selection.	
Biphenyl rotation	R-lab	3×10^{-5}	500	10^2		
Porphyrin metallation	R-lab	0.9	10	10^3		
	D-lab	0.2	3×10^3	10^3		
Diels–Alder cycloaddition	R-lab	> 0.1	> 500	10^3		

2 Classes of Catalytic Nucleic Acids for Hydrolysis

Class A

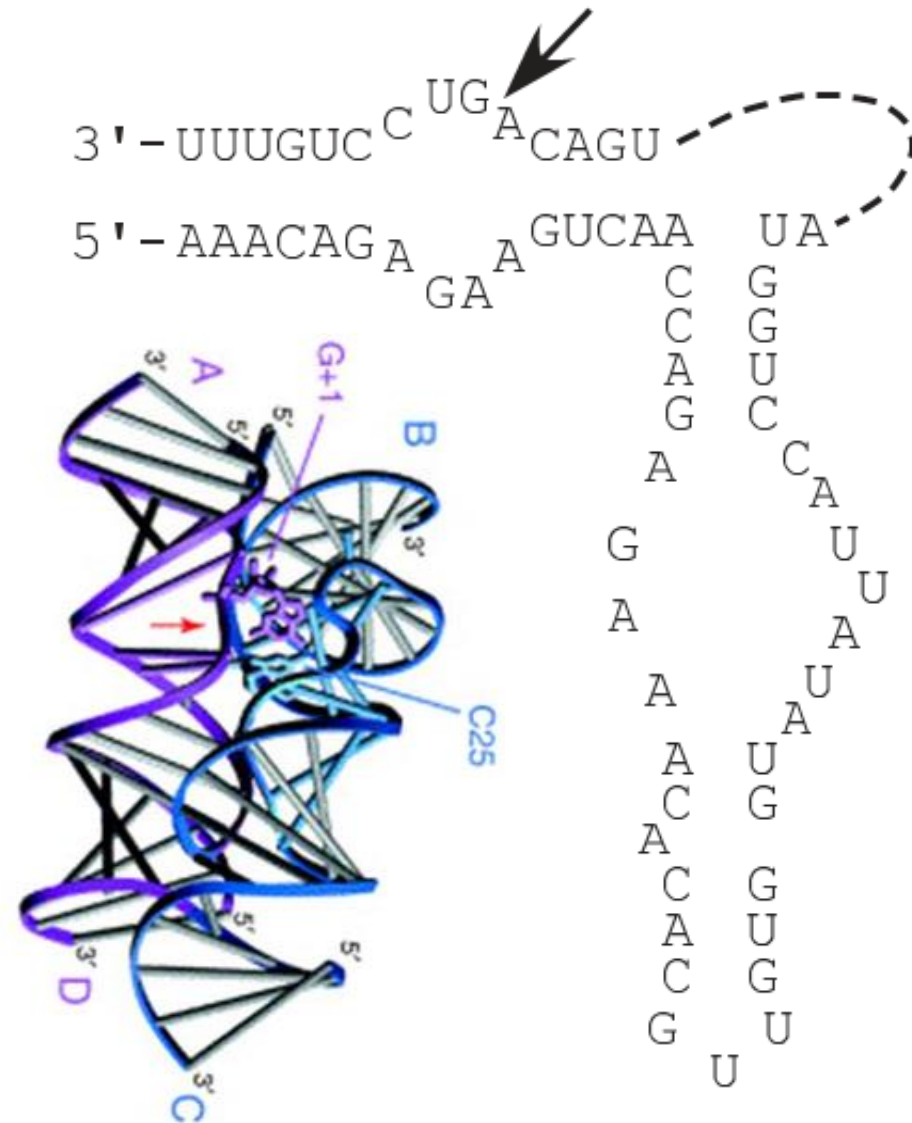


- They are typically **small** (< 200 bases).
- **M1 metal** activates an **internal** 2'-OH for **nucleophilic** attack at the phosphorus of a phosphodiester bond & form **2',3'-cyclic phosphate** & **5'-OH termini** (e.g. in hammerhead, hairpin, hepatitis delta virus (HDV)).

2 Examples of Sequence of Catalytic RNA

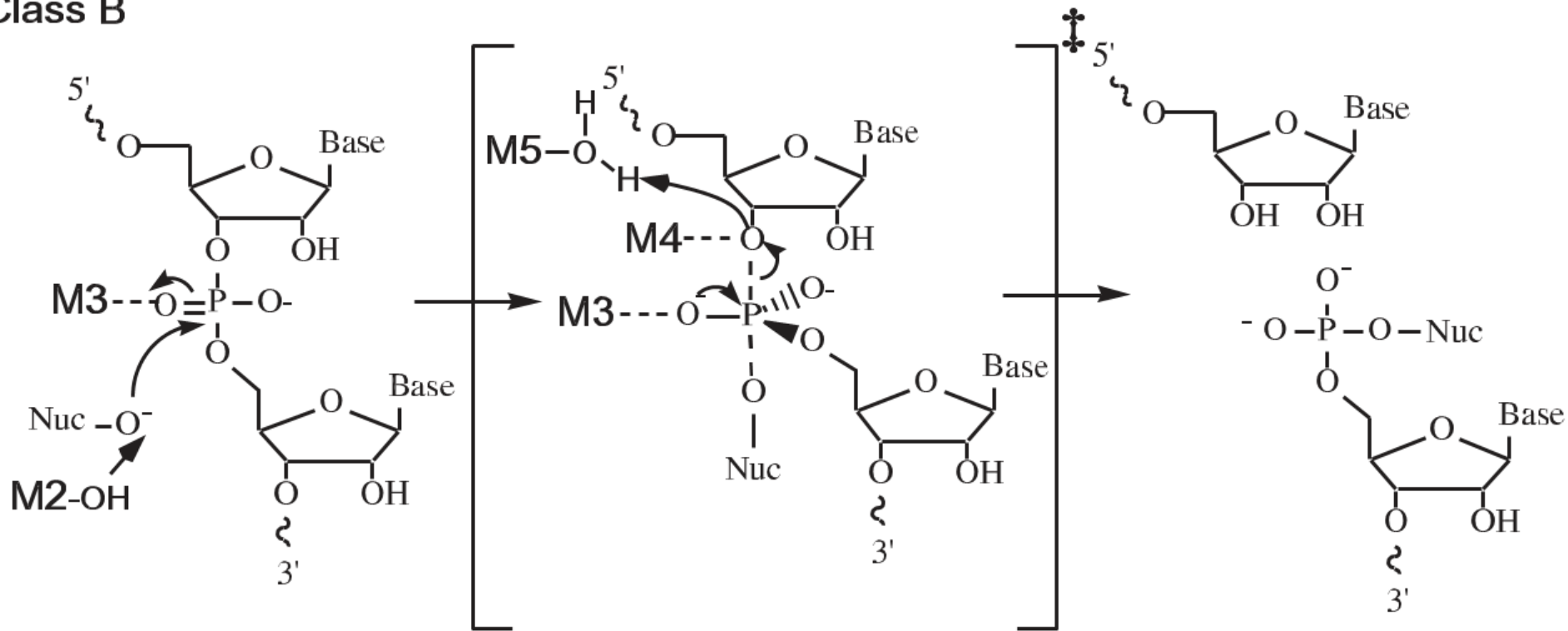


Hammerhead Ribozyme



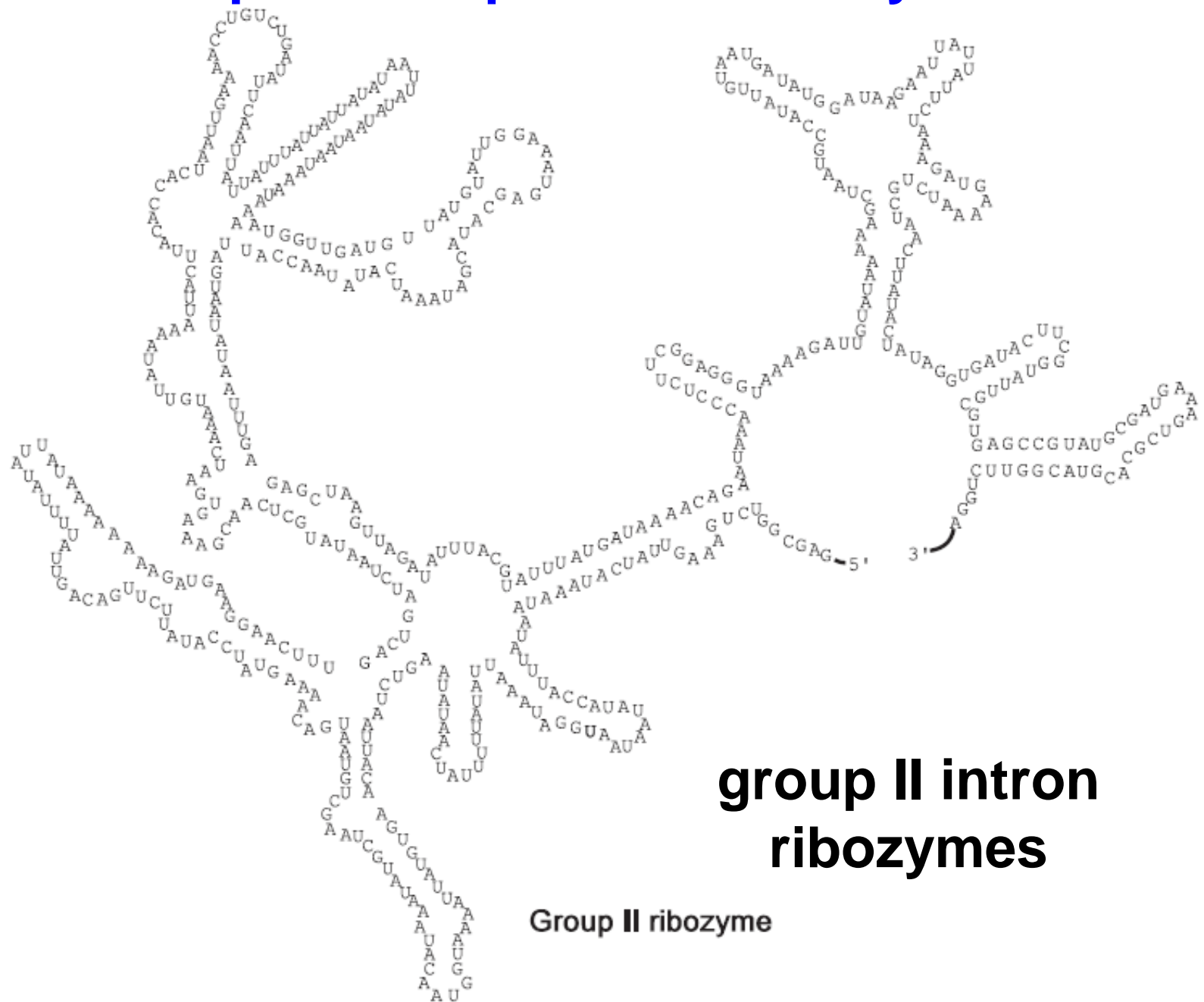
Hairpin Ribozyme

Class B



- These catalytic nucleic acids are **larger** (> 400 bases, e.g. ribonuclease P, group I and group II intron ribozymes).
- They use an **external nucleophile**, e.g. an activated nucleotide or water, to attack the adjacent phosphodiester to form 3'-OH and 5'-phosphate termini.

One Example of Sequence of Catalytic RNA



Catalytic Nucleic Acids	Functional	Nonfunctional
Hammerhead ^b	Mg ²⁺ , Mn ²⁺ , Ca ²⁺ , Cd ²⁺ , Co ²⁺	Ba ²⁺ , Sr ²⁺ , [Cr(NH ₃) ₆] ³⁺ , Pb ²⁺ , Zn ²⁺ , Tb ²⁺ , Eu ²⁺
Hairpin ^b	All tested, including [Cr(NH ₃) ₆] ³⁺	
Hepatitis δ virus ^b	Mg ²⁺ , Mn ²⁺ , Ca ²⁺ , Sr ²⁺	Cd ²⁺ , Ba ²⁺ , Co ²⁺ , Pb ²⁺ , Zn ²⁺
<i>Neurospora</i> VS ^b	Mg ²⁺ , Mn ²⁺ , Ca ²⁺	
RNase P ^b	Mg ²⁺ , Mn ²⁺ , Ca ²⁺	Sr ²⁺ , Ba ²⁺ , Zn ²⁺ , Co ²⁺ , Cu ²⁺ , Fe ²⁺ , Ni ²⁺
<i>Tetrahymena</i> Group I ^b	Mg ²⁺ , Mn ²⁺	Ca ²⁺ , Sr ²⁺ , Ba ²⁺ , Zn ²⁺ , Co ²⁺ , Cu ²⁺
<i>Tetrahymena</i> Group II ^b	Mg ²⁺	Ca ²⁺ , Mn ²⁺

● **Mg²⁺, Mn²⁺, or Ca²⁺**: generally essential for the catalytic function of most catalytic nucleic acids.

● **Structural role**: facilitate **folding** to **stable tertiary** structures by **charge neutralization** with the **anionic** phosphodiester **backbones**.

● **Catalytic role**: increase **nucleophilicity** of the nucleophile or **stabilize the negative charge** in transition states & products.

Key Summary

Hydrolase: enzymes catalyze **hydrolysis** of a chemical bond.

General roles of the metal(s):

1. **Structural** role: **bring** the **substrate** into the active site & **orient** the substrate properly for the reactions.
2. **Catalytic** roles: as a **Lewis acid** to **activate** a metal-bound **nucleophile** (forming **reactive** M-OH or M-OR), **stabilize** the negative charge in **transition states** & leaving group/products.
3. **Non-redox Zn^{2+} & Mg^{2+}** are commonly used. The **two metal ions** are often required for the reactions.

Catalytic nucleic acids: a new class of enzymes.

**Thank You for Your
Attention!
Any Questions?**