CARBONIC ANHYDRASE: Why Zinc?

By: Amir Safi

What Reaction is Catalyzed?

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

- Carbonic Anhydrase Catalyzes the Reaction of water + carbon dioxide to form bicarbonate.

LIFE

Rate of Catalysis

$CO_2 + H_2O = HCO_3^- + H^+$

$k_{uncat} = 10^{-11}\ s^{-1}$

$k_{cat} = 10^{4}\ s^{-1}$

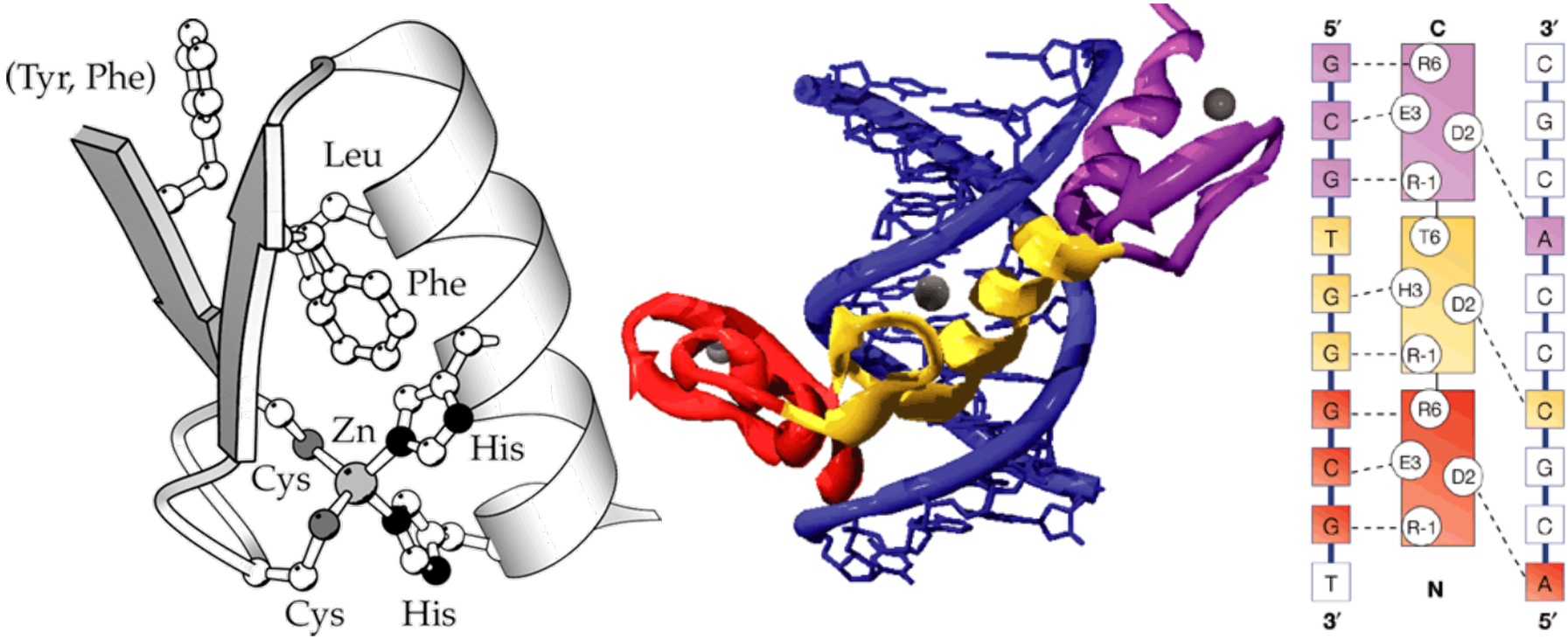
Role of Zn(II) in Proteins

1. Catalytic: involved directly in catalytic steps
(e.g., CA)

2. Structural: required for the stability of a protein structure, or arrangement of an active site
(e.g., CuZnSOD)

3. Regulatory: Zn-free enzyme has activity, addition of Zn^{2+} enhance or inhibit activity
(e.g., zinc finger family of proteins)

Zinc Fingers



Cys_2His_2 zinc fingers are found in 2% of all human genes

Jamieson, Andrew C.; Miller, Jeffrey C.; Pabo, Carl O. Nature Rev. Drug Discovery (2003), 2, 361-368.

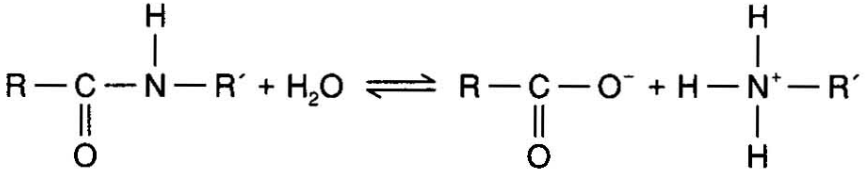
Hydrolytic reactions

Table 2.1
Representative metalloenzymes catalyzing hydrolytic and related reactions.

Enzyme	Metal(s)	Function
Carboxypeptidase	Zn^{2+}	Hydrolysis of C-terminal peptide residues
Leucine aminopeptidases	Zn^{2+}	Hydrolysis of leucine N-terminal peptide residues
Dipeptidase	Zn^{2+}	Hydrolysis of dipeptides
Neutral protease	Zn^{2+}, Ca^{2+}	Hydrolysis of peptides
Collagenase	Zn^{2+}	Hydrolysis of collagen
Phospholipase C	Zn^{2+}	Hydrolysis of phospholipids
β-Lactamase II	Zn^{2+}	Hydrolysis of β-lactam ring
Thermolysin	Zn^{2+}, Ca^{2+}	Hydrolysis of peptides
Alkaline phosphatase	Zn^{2+}, Mg^{2+}	Hydrolysis of phosphate esters
Carbonic anhydrase	Zn^{2+}	Hydration of CO_2

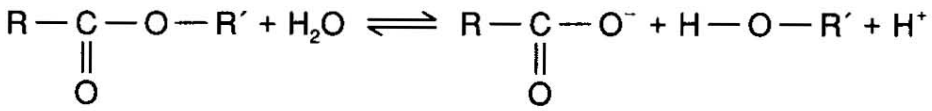
Hydrolytic reactions

peptide hydrolysis



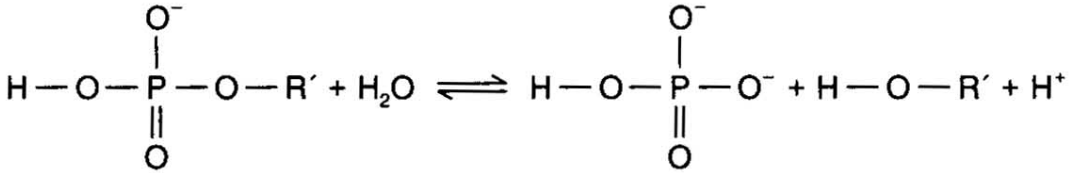
(2.1)

carboxylic ester hydrolysis



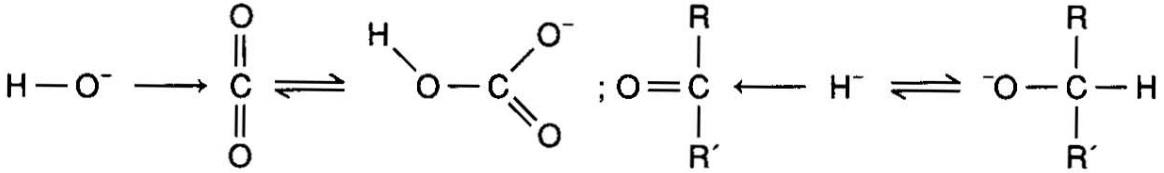
(2.2)

phosphoric ester hydrolysis



(2.3)

nucleophilic addition of OH^- and H^-



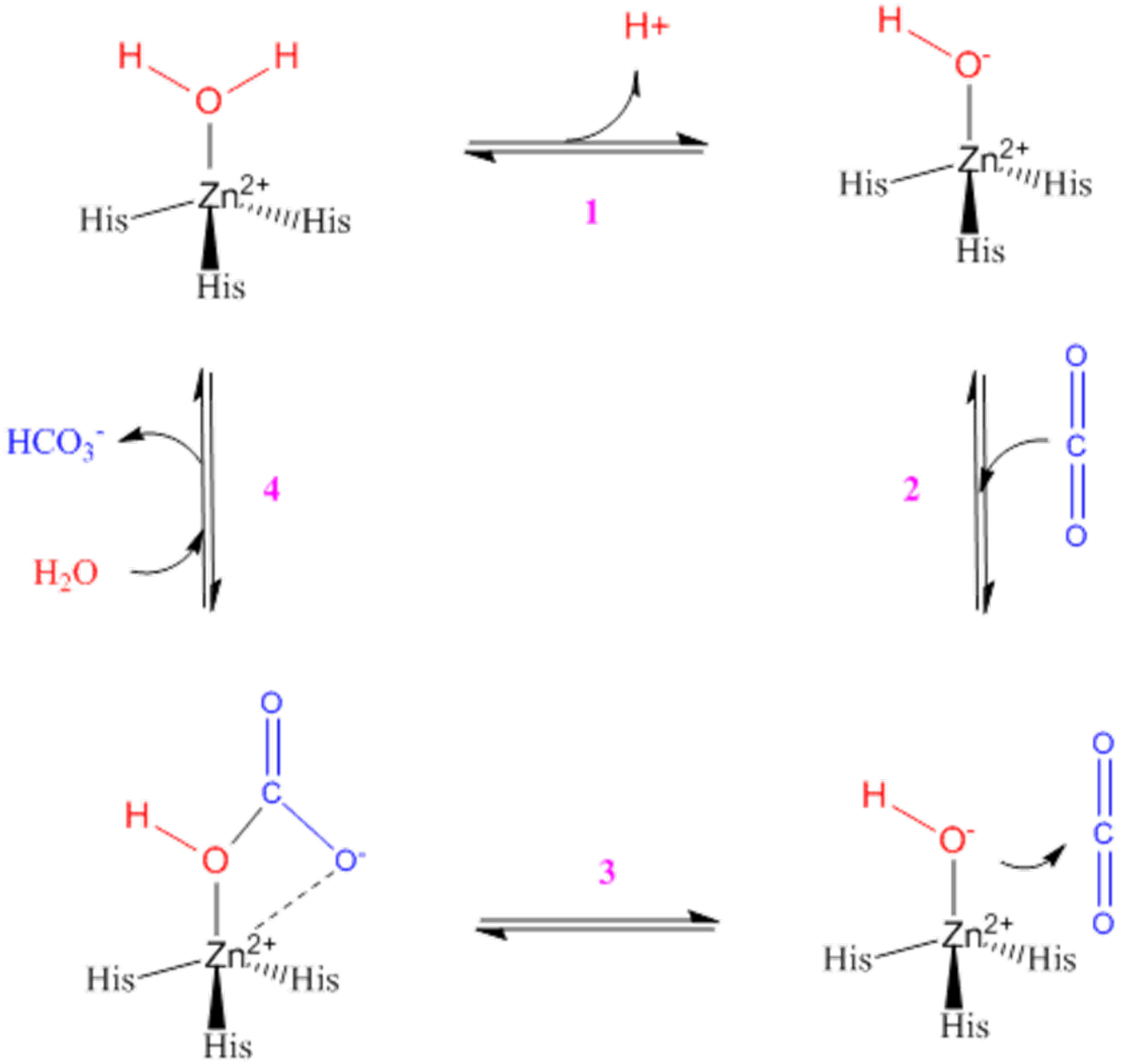
(2.4)

Background on Carbonic Anhydrase

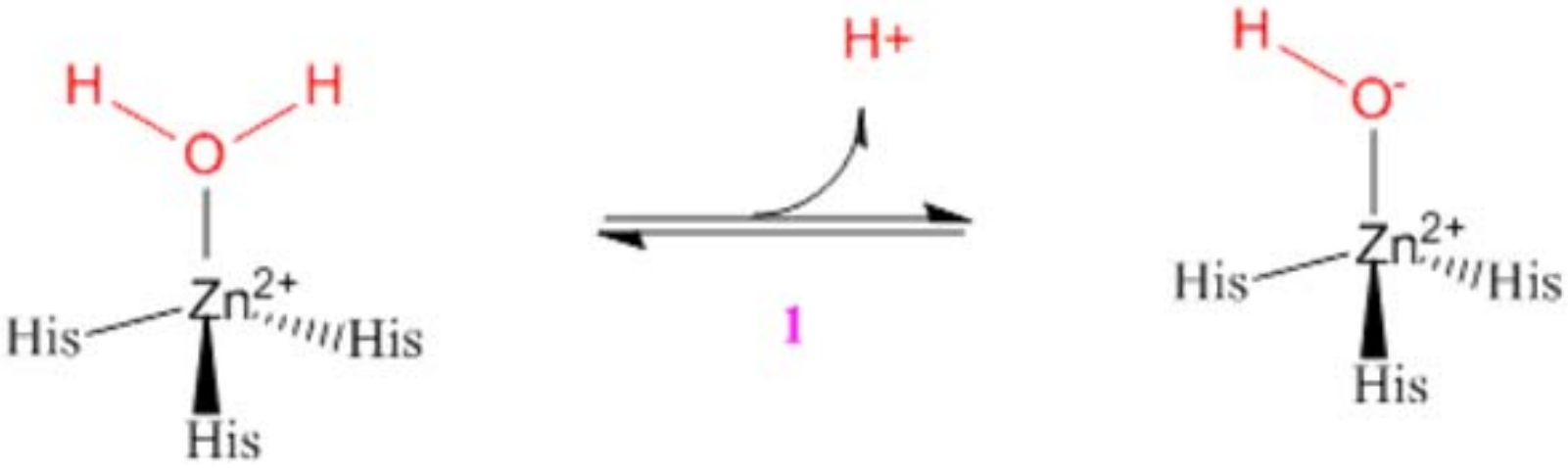
- Carbonic Anhydrase is an enzyme that is located in red blood cells.
- It contains about .31 to .34% zinc.
- This is important because it's the first known direct physiological function of zinc.

http://www.pdb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb49_1.html

Carbon Anhydrase Mechanism



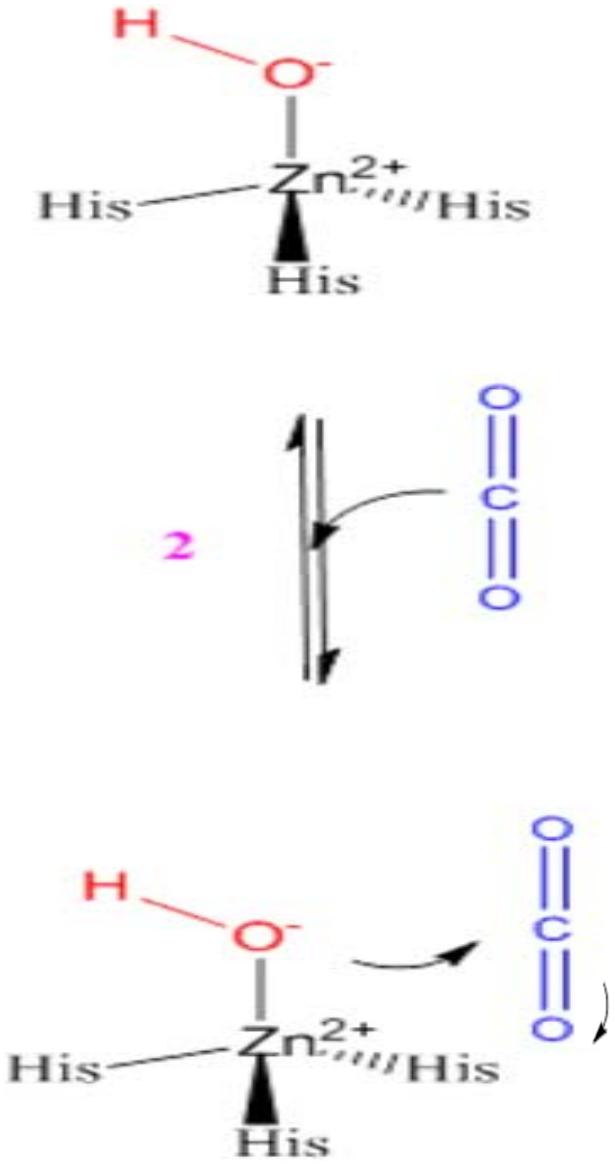
Step 1: deprotonation



1. Berg, Jeremy M. (**2007**). Biochemistry, 6th Ed.

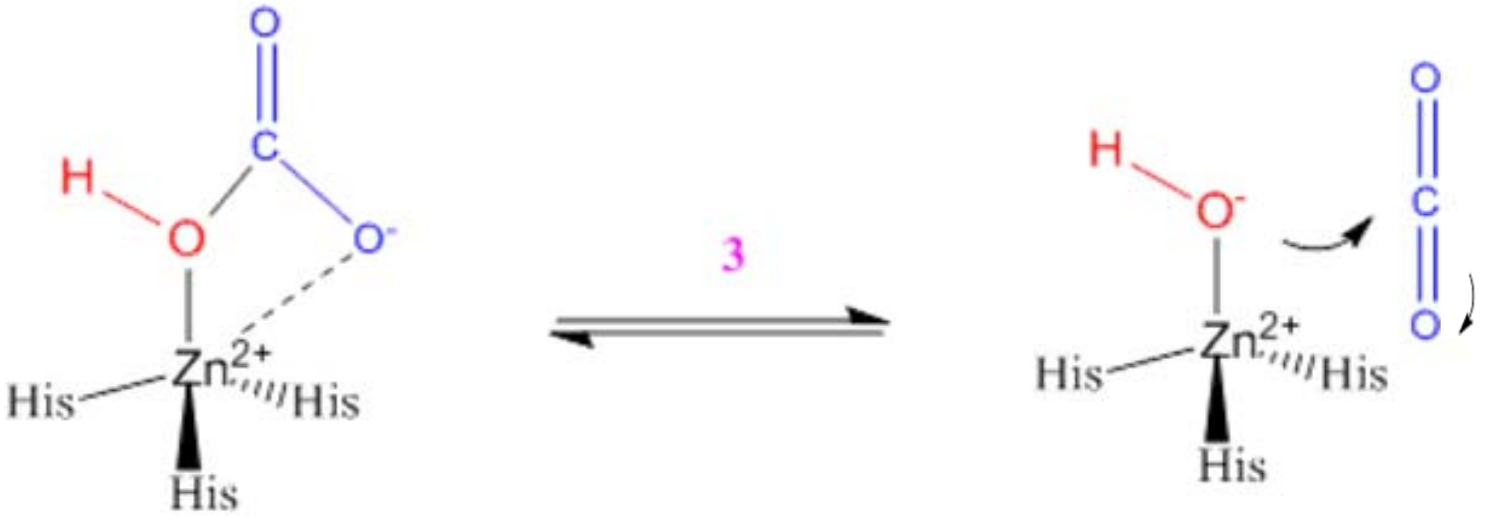
Step 2: Carboxylation

- The anionic oxygen then performs a nucleophilic attack on the carbon.

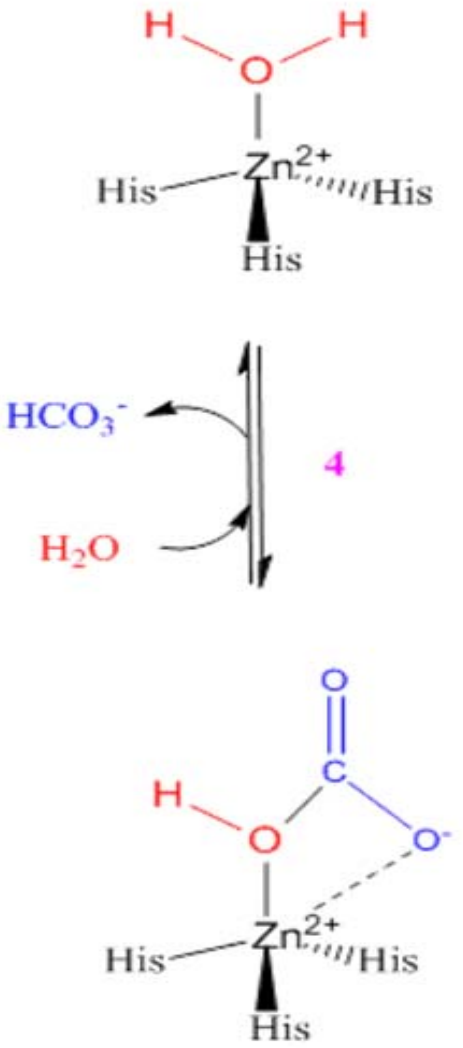


Step 3: Formation of Ring Structure

Anionic oxygen forms a bond with Zn^{2+} to make a ring-like resonance.

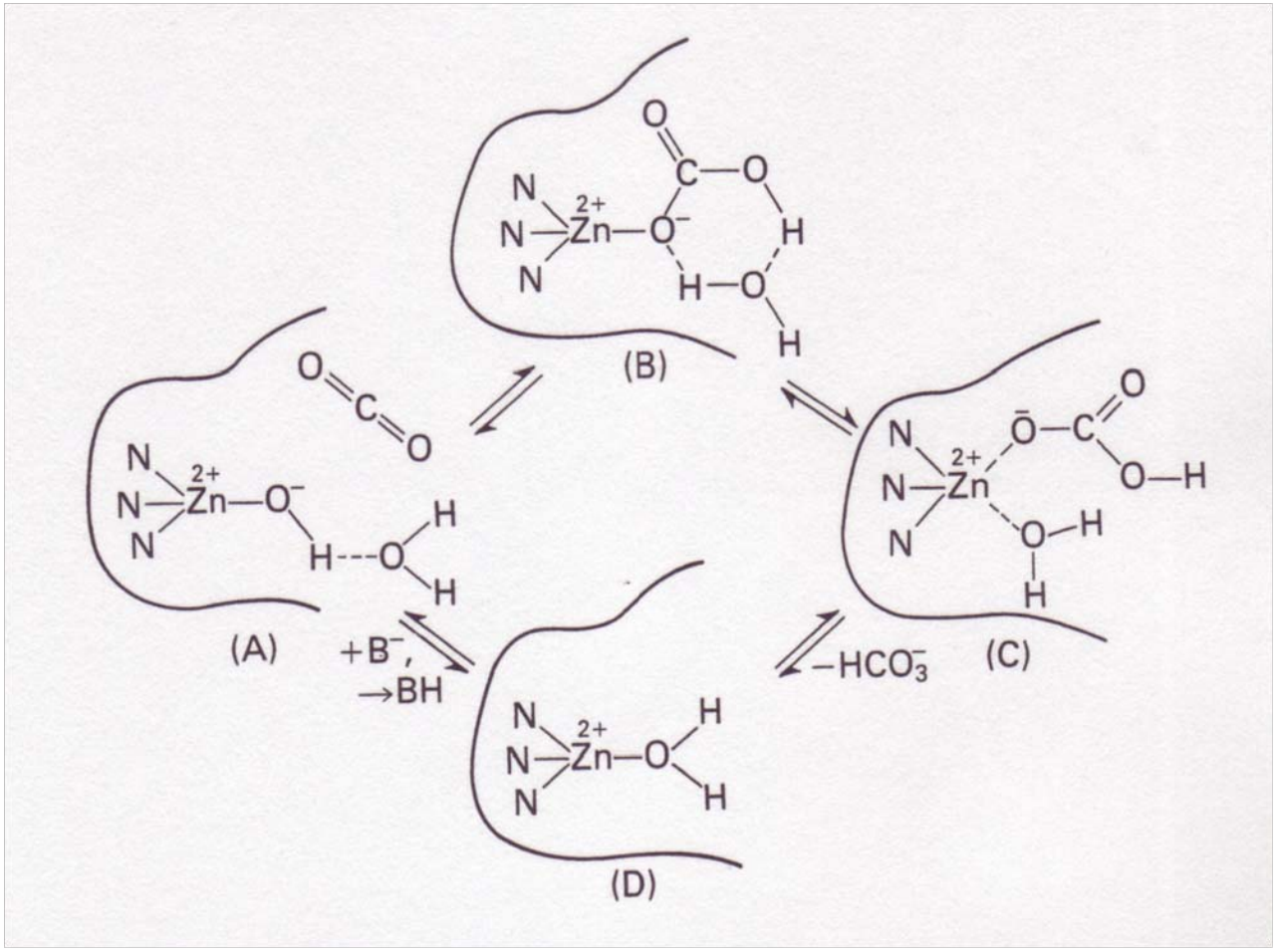


Step 4: Formation of Bicarbonate



- Addition of water displaces the bicarbonate ion.

Carbonic Anhydrase Mechanism



BIG QUESTION:

WHY DOES CARBON ANHYDRASE USE ZINC?

History of Zinc

- Zinc compounds have been used for at least 2,500 years
- Zinc derived its name, supposedly, from the German word *Zinke*, which means 'tooth-like' or 'pointed'.
- Metallic zinc was first produced in India sometime in the 1400's by heating the mineral calamine ($ZnCO_3$) with wool.
- Zinc was rediscovered by Andreas Sigismund Marggraf in 1746 by heating calamine with charcoal.

http://www.buzzle.com/articles/history-of-zinc.html

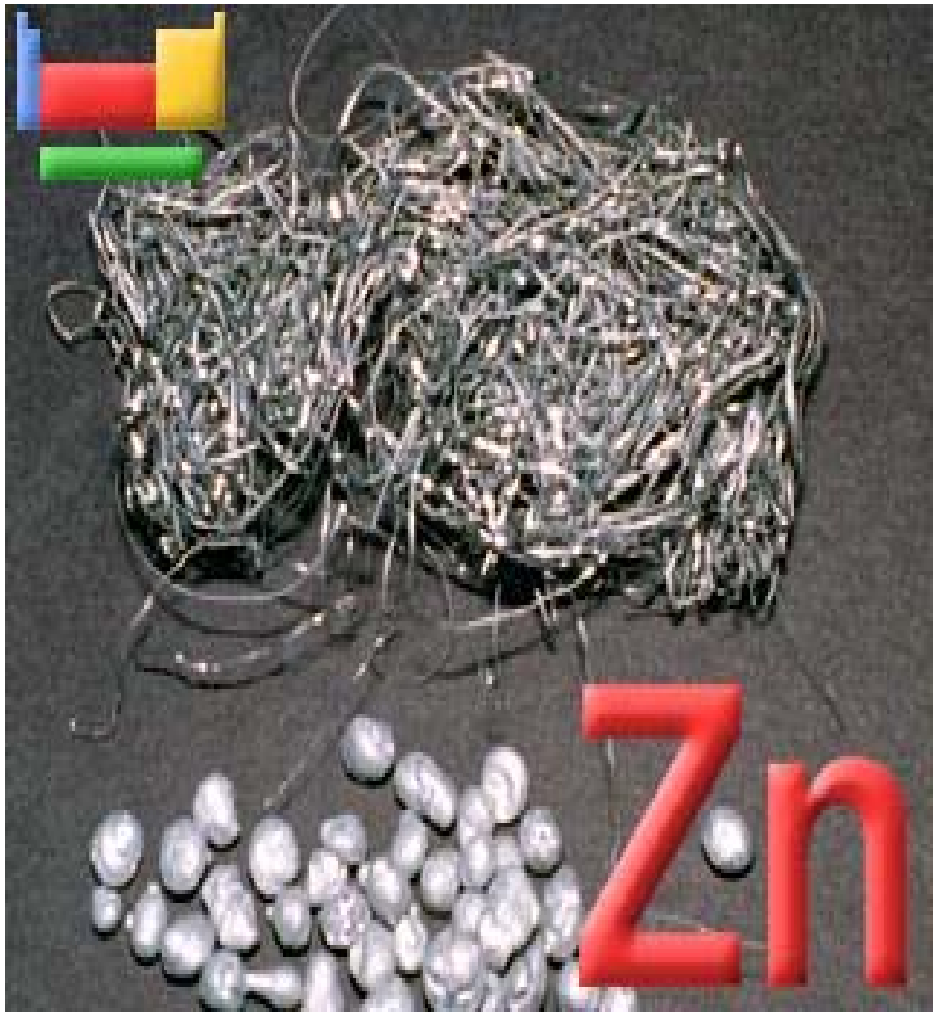
Fe, Zn, Cu: the most common transition metal ions in biology

ELEMENT	Sea Water (M) x 10^-8	Human Body (M) x 10^-8
Fe	.005-2	2230
Zn	8	1720
Cu	1	1650

Properties of Zinc

- 2+ is the only accessible oxidation state (d^{10})
- Binds both hard and soft donors
- Tetrahedral geometry preferred
- 5-6 CN are accessible
- Good Lewis acid because high charge/radius ratio

Zinc is d^{10}



- Zinc Ion is colorless
- Not Redox Active

Hard/ Soft Acid Base Theory

- Good Lewis acid because high charge/radius ratio

Borderline

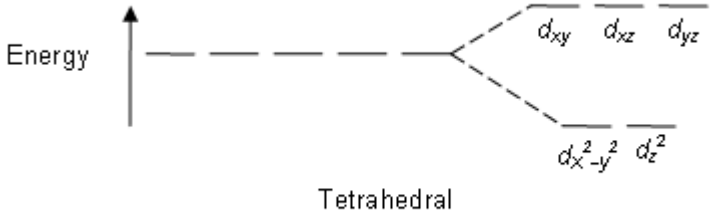
Metals:

Fe^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}

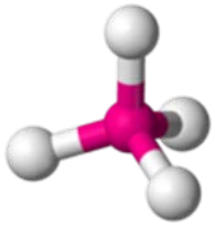
Ligands:

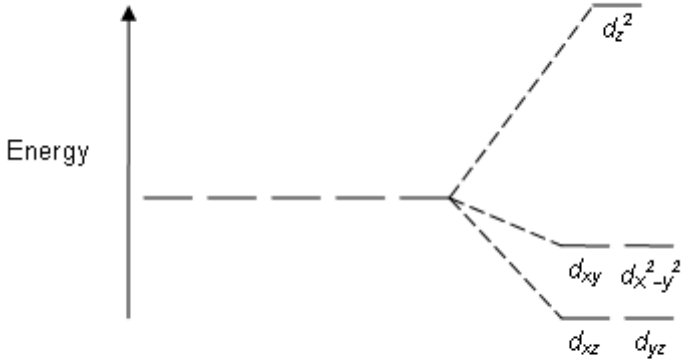
SR^-, his, X^-, RCO_2^-

Zinc Geometry and d orbital Splitting diagrams.

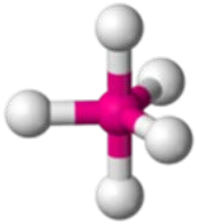


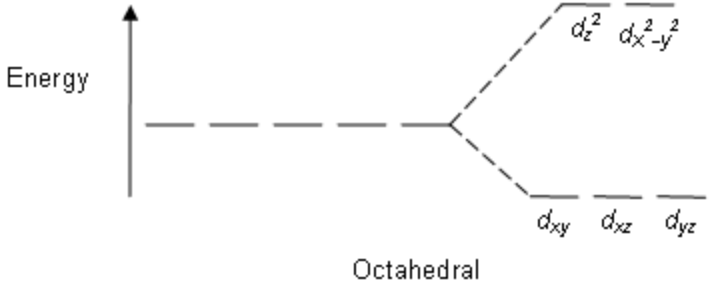
Tetrahedral





Trigonal bipyramidal





Octahedral

RECAP

Why Zinc:

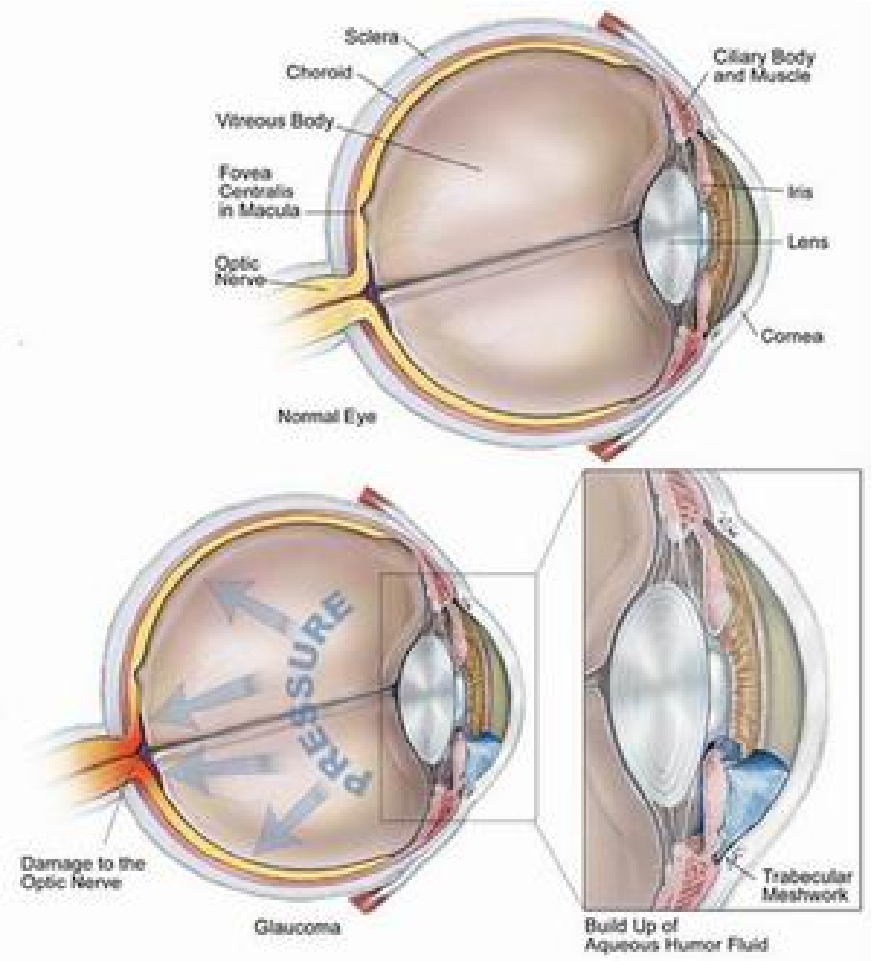
1. 2+ is the only accessible oxidation state (d^{10})
   - No complications from redox activity.

2. Binds both hard and soft donors

3. Tetrahedral geometry preferred/ 5-6 CN are accessible

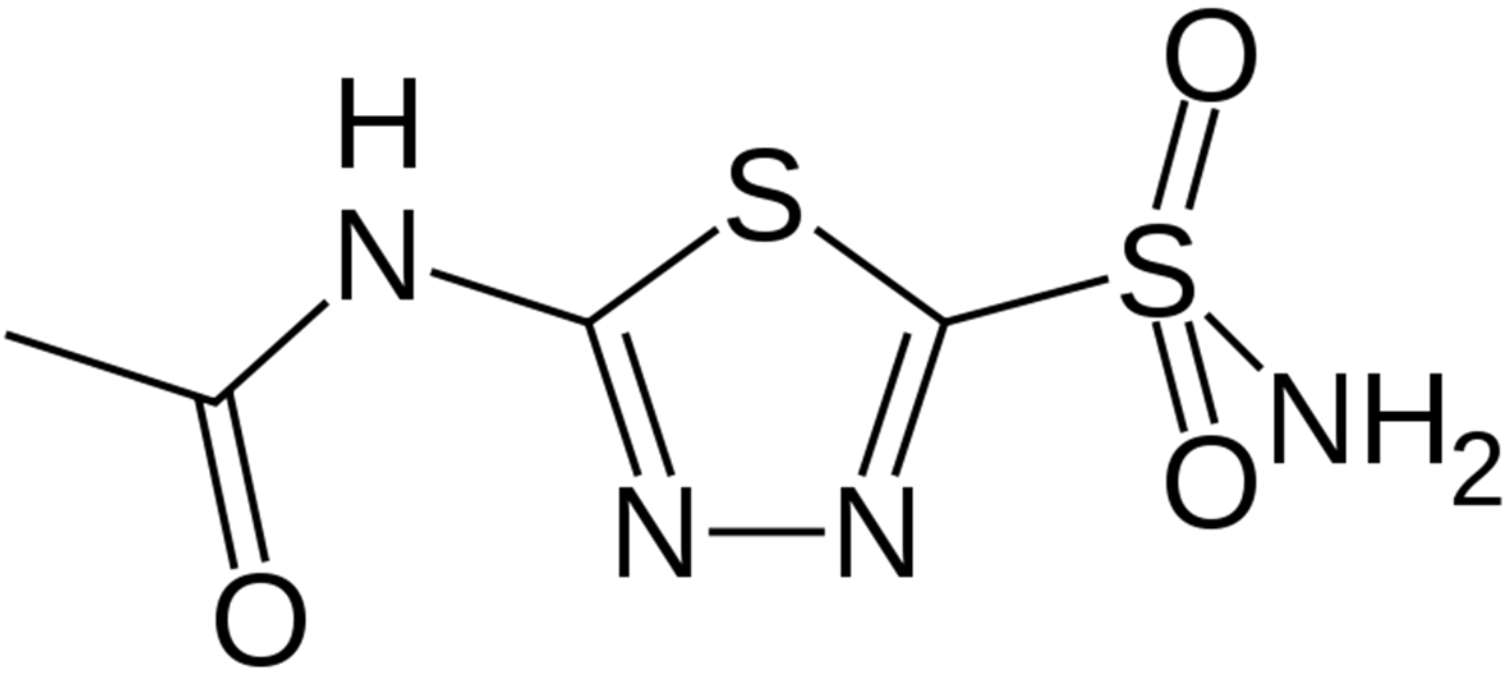
Medical Application:
Treat Glaucoma

Carbonic anhydrase inhibitors are applicable during emergencies because they are able to rapidly reduce the pressure that has built up inside the eye by 40% to 60%. The less fluid that is allowed to build up yields less pressure behind the eye.



http://www.pdb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb49_2.html

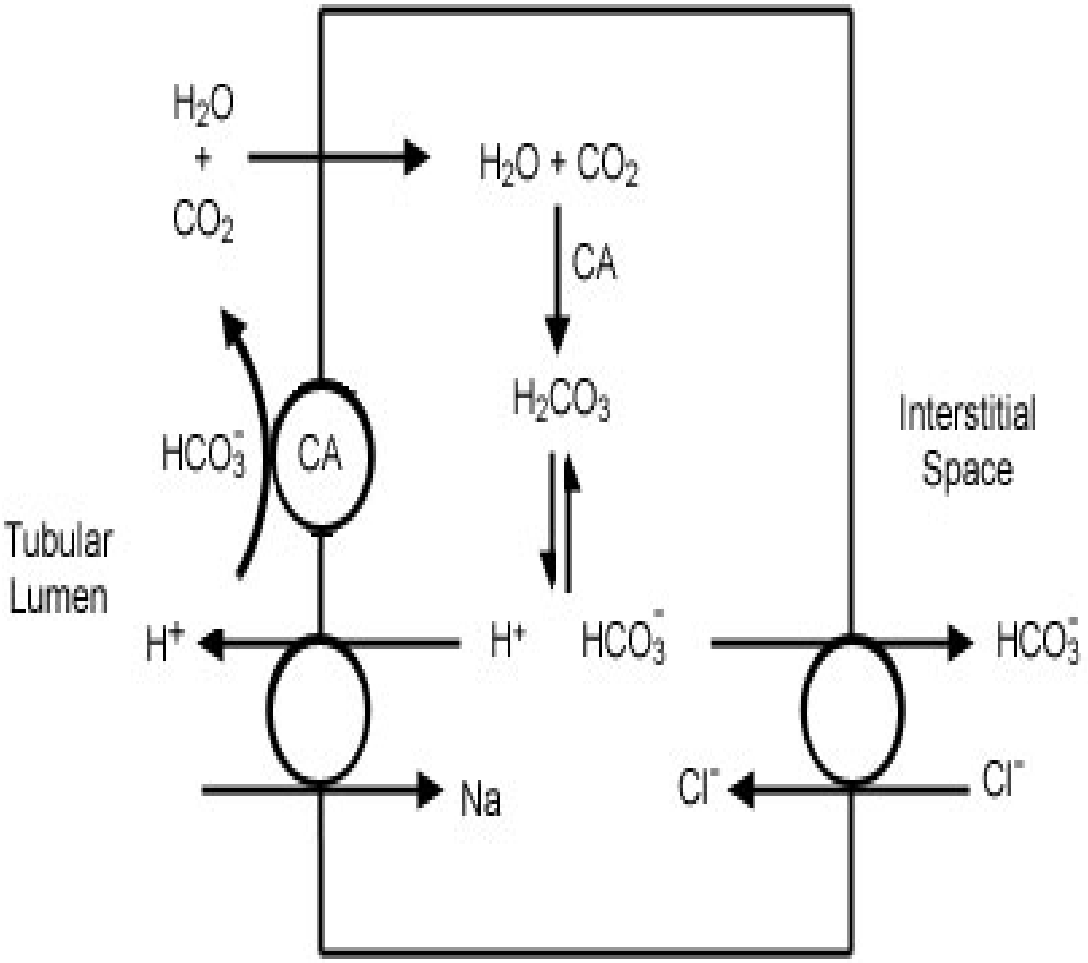
Acetazolamide



Diuretic: Diuretics are drugs that increase the rate of urine flow.
Sold as the drug Diamox

http://jpet.aspetjournals.org/content/115/3/251.abstract

Carbonic Anhydrase Inhibition



Bad News or Good News

Glaucoma:

- The mechanism of action to lower pressure is not known. The route of administration can be oral, intravenous, topical, or inhaled by smoking, and it dictates many of the behavioral and physiologic consequences. Of these routes, smoking has the more rapid onset of psychoactive and other pharmacologic effect

Is Zinc Boring?

http://www.youtube.com/watch?v=99wPiMb-k0o