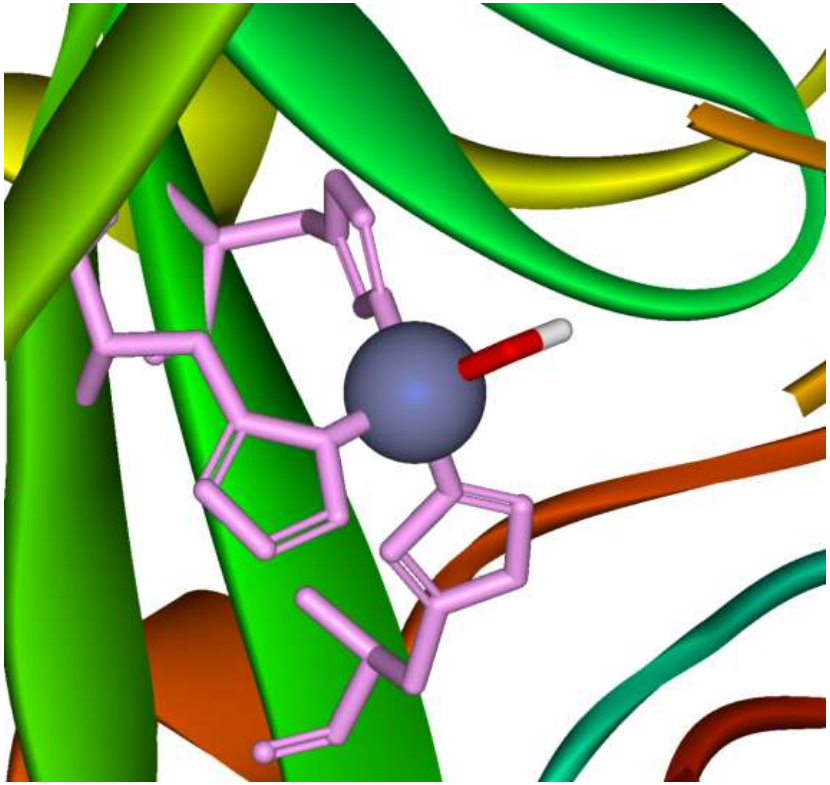
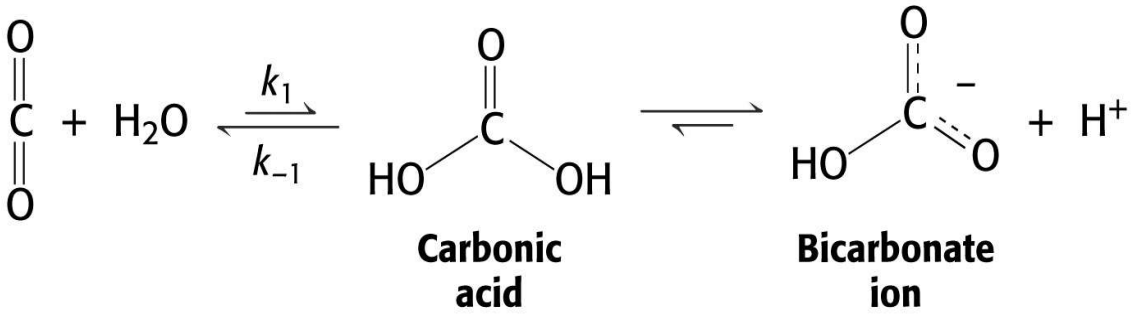
Active site of Carbonic Anhydrase: $[Zn^{II}(His)_3(H_2O)]$



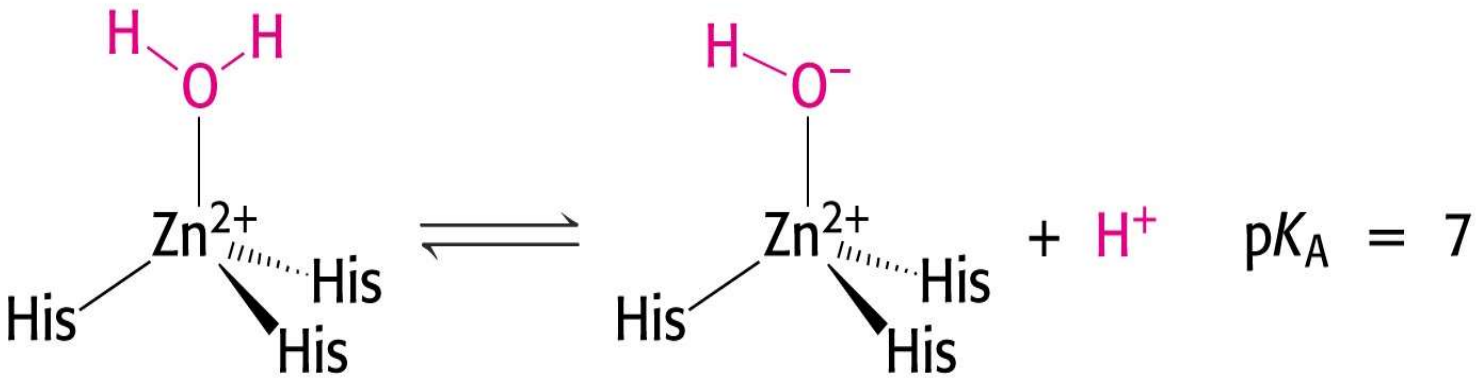
Carbonic anhydrase catalyzes the critically important reaction of hydrating CO_2 to form bicarbonate:



This enzyme enhances the rate of this reaction by more than 10^6! At these rates, the limiting factor is how fast the molecules can diffuse to the active site!

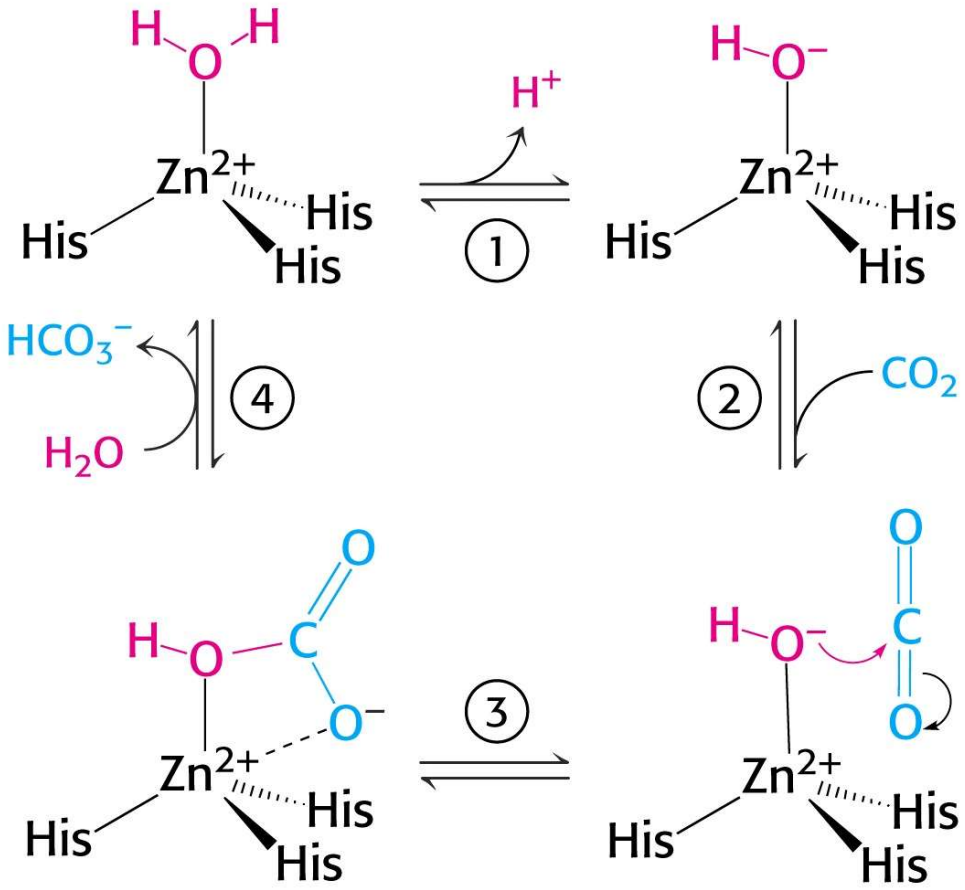
Enzyme Mechanisms – Carbonic Anhydrase

- The binding of water to zinc, reduces the pKa for water from its normal 15.7 down to 7. This allows the formation of the strong hydroxide (HO^-) nucleophile at neutral pH:



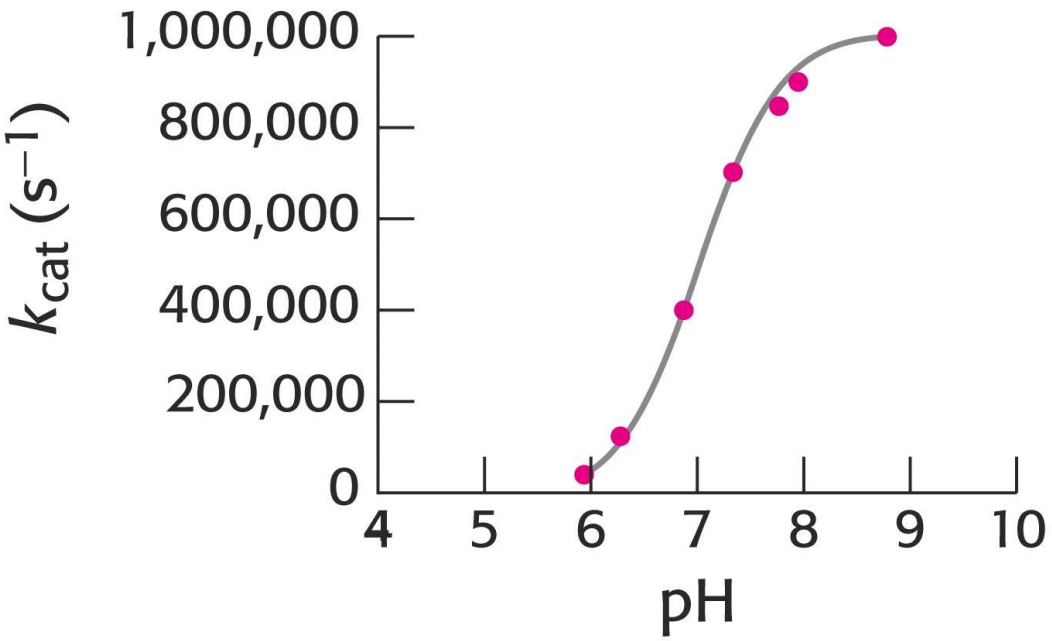
Enzyme Mechanisms – Carbonic Anhydrase

- The enzyme then positions CO_2 for nucleophilic attack by the hydroxide, resulting in the formation of bicarbonate.
- Water then displaces the product, starting the cycle again.



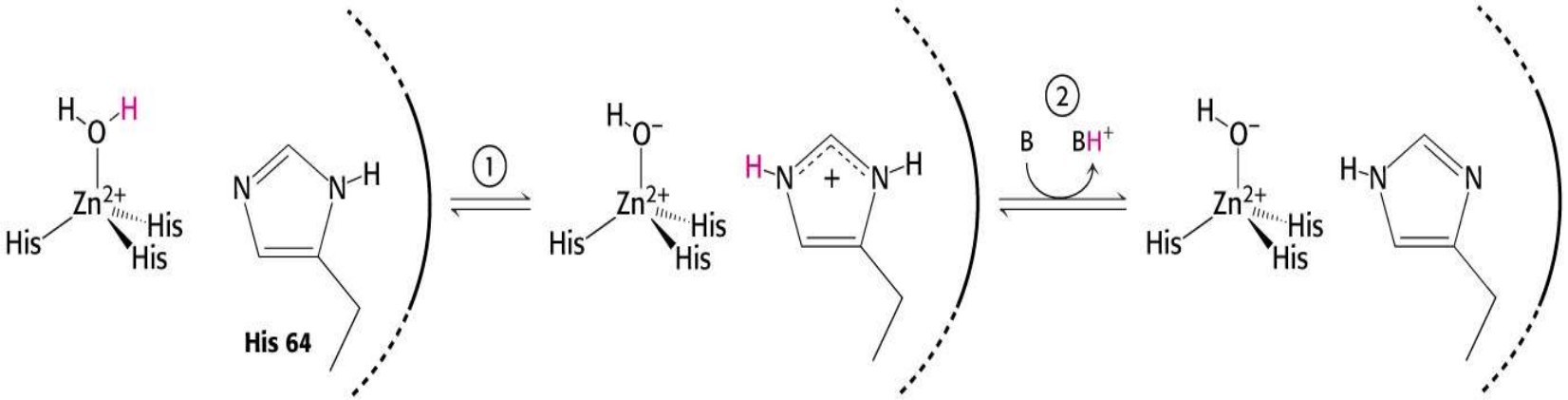
Enzyme Mechanisms – Carbonic Anhydrase

- The pH profile for enzyme activity reveals that below pH=7, the deprotonation of the zinc-bound water can not proceed fast enough to keep up the rate observed at higher pH:



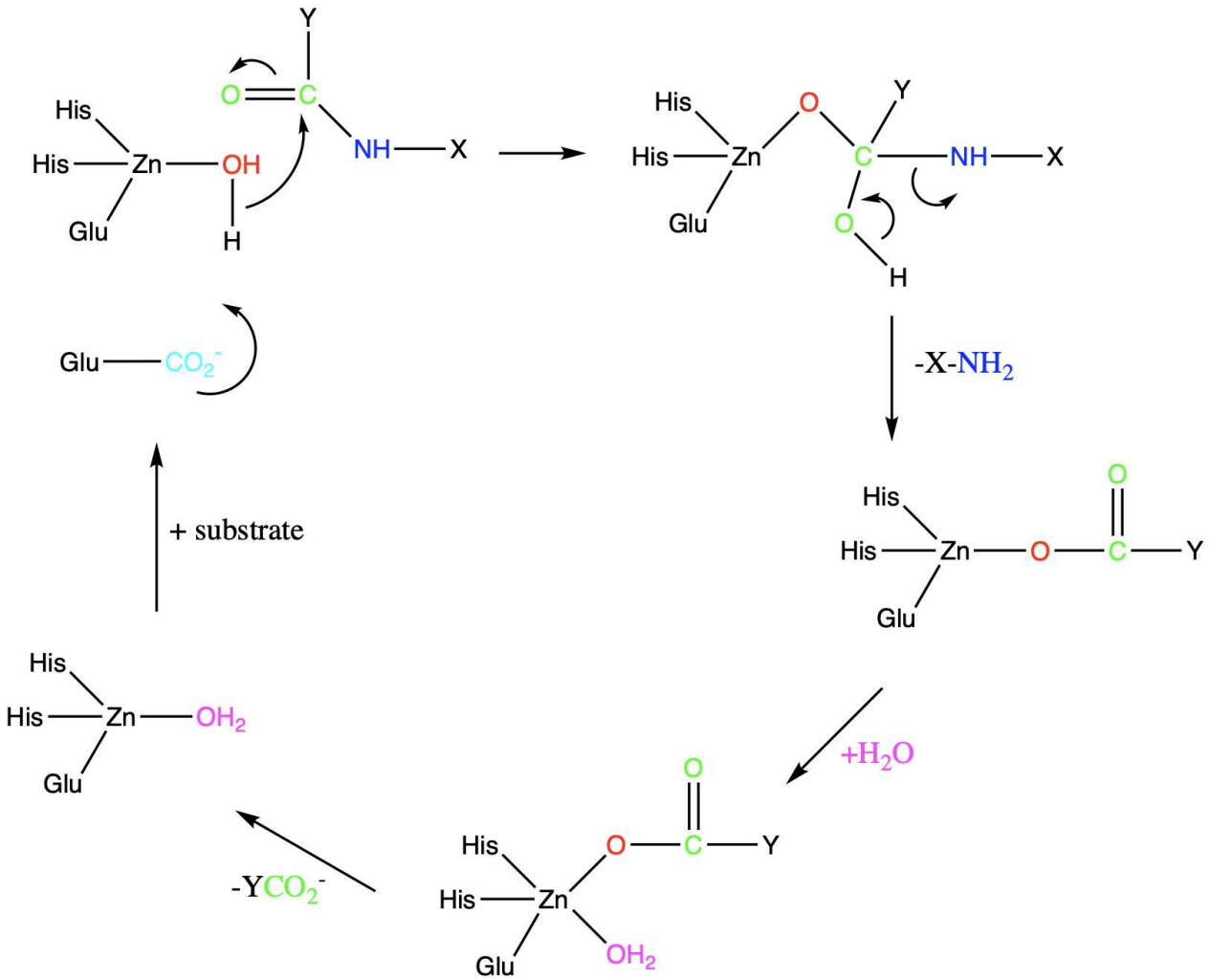
Enzyme Mechanisms – Carbonic Anhydrase

- As the hydroxide ion forms, the exiting hydrogen ion can not diffuse away fast enough to keep up with the exceptional speed of the reaction cycle, so His-64 helps by shuttling it away to the surface of the protein:



- This shifts equilibrium substantially in favor of the hydroxide formation.

Carboxypeptidase



Lessons learnt from natural Hydrolases

A synthetic hydrolase should satisfy the following criteria:

- Fine-tune the pKa of coordinated water (to render it more acidic compared to free water) to provide a metal-bound hydroxide as nucleophile at pH ~7.0.
- For a mononuclear system ---

Two cis labile sites should be available: one to bind the substrate and the other for the generation of hydroxide ion

- For a dinuclear system ---

At one metal center the substrate binds and the nucleophile generated at the other center brings about the nucleophilic attack.

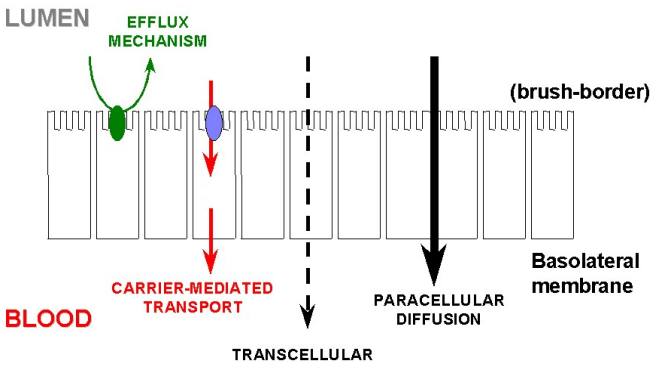
Activate the substrate towards nucleophilic attack and/or stabilization of transition state.

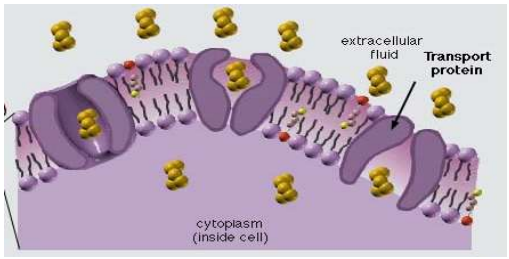
- Release the product(s) at a reasonable rate

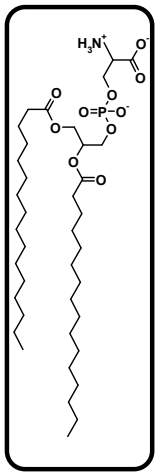
Drug Chemistry: Metals in medicine

Absorption Mechanisms

- **Transcellular absorption**
  - Main route for most oral drugs
  - Drug must be in solution at cell surface
  - pKa important - drug must be unionised
  - Lipophilicity important - ideal log D 1-4
  - H-bonds - solvation shell needs dispersing
  - Lipinski's 'Rule of 5'
- **Paracellular absorption**
  - Drug passes through gaps between cells
  - Inefficient – pores have << surface area than cellular surface
  - Restricted to low MW hydrophilic molecules
- **Active Transport**
  - Drugs carried through membrane by a transporter – requires energy
  - Many transporters exist for nutrient molecules, eg glucose, amino acids
  - SAR specific – few drugs absorbed by this route







Phosphatidylserine

Bulk physical properties

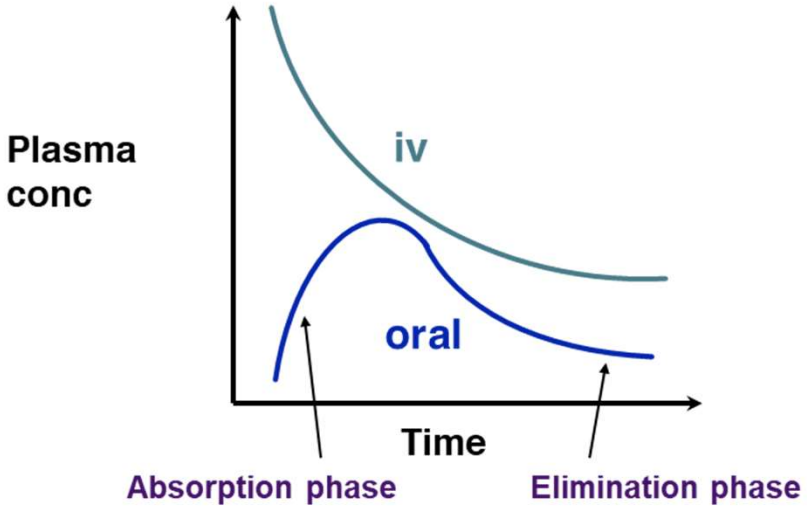
When a compound is nearing nomination for entry to clinical trials, we need to look at:

- Solubility, including in human intestinal fluid
- Hygroscopicity, i.e. how readily a compound absorbs water from the atmosphere
- Crystalline forms – may have different properties
- Chemical stability (not a physical property! Look at stability to pH, temperature, water, air, etc)

How can these be altered?

- Different counter ion or salt
- Different method of crystallisation

Oral Dosing - Bioavailability



Upon oral dosing of a drug, there is an initial increase in the systemic concentration of the drug, as it is absorbed from the gut.

As absorption is completed and the compound is eliminated from the body, the concentration of drug decreases over time.

Oral Bioavailability (F%) is defined as:

The fraction of the dose which makes it to the systemic circulation (i.e. survives 1st pass metabolism).

$$F = \frac{\textbf{AUC after an oral dose}}{\textbf{AUC after an equivalent iv dose}}$$

Limiting factors include: Chemical instability, eg acid sensitive compound in the stomach; Incomplete absorption - solubility, formulation; Gut wall metabolism - labile functional groups