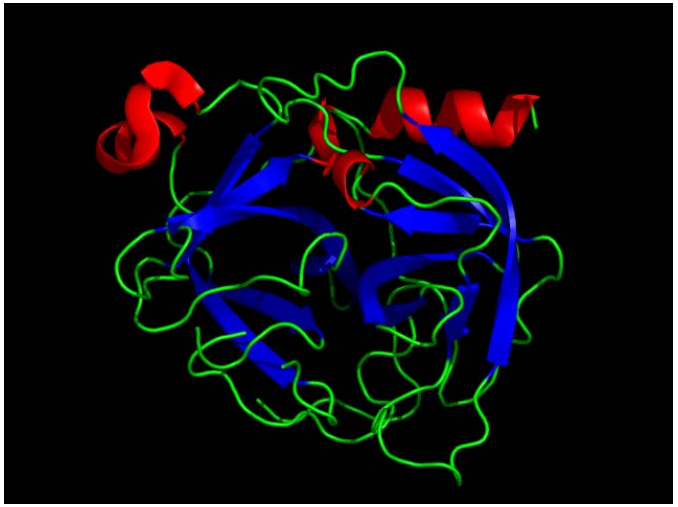
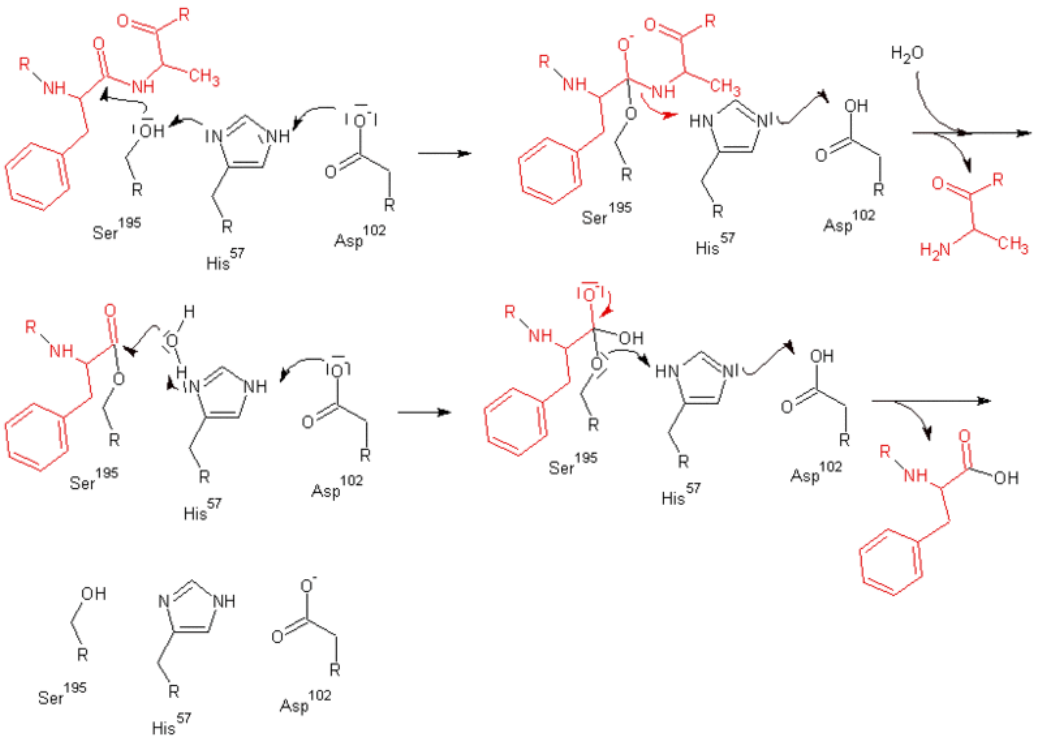
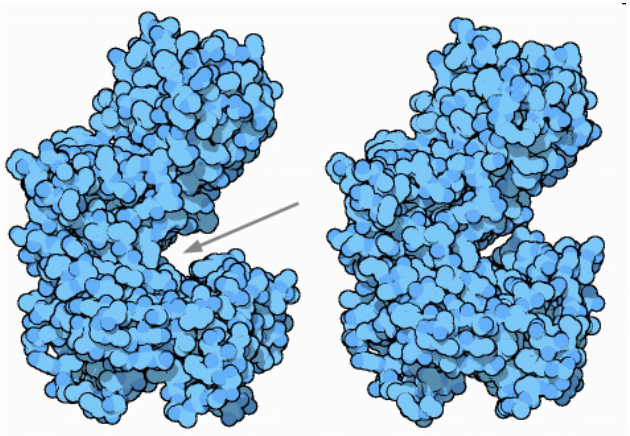
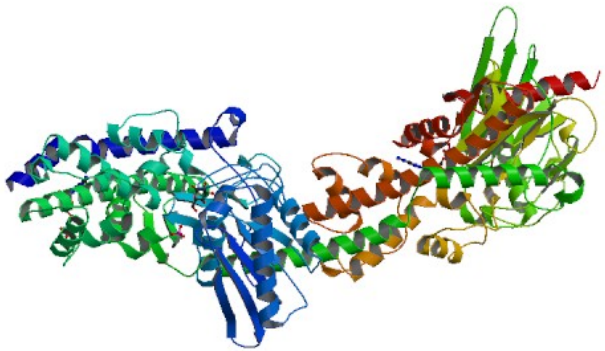
Enzymes and Protein Structure

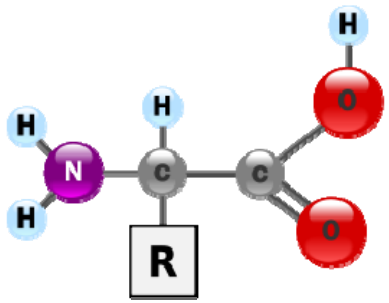


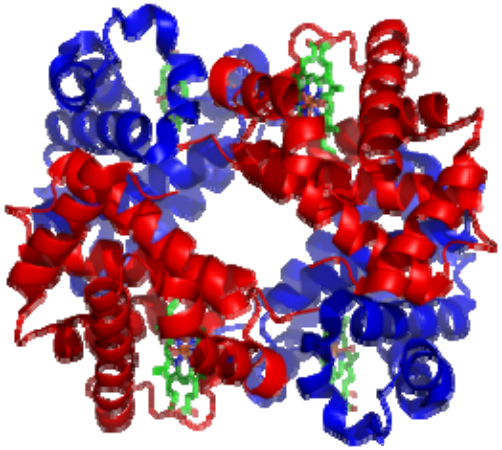


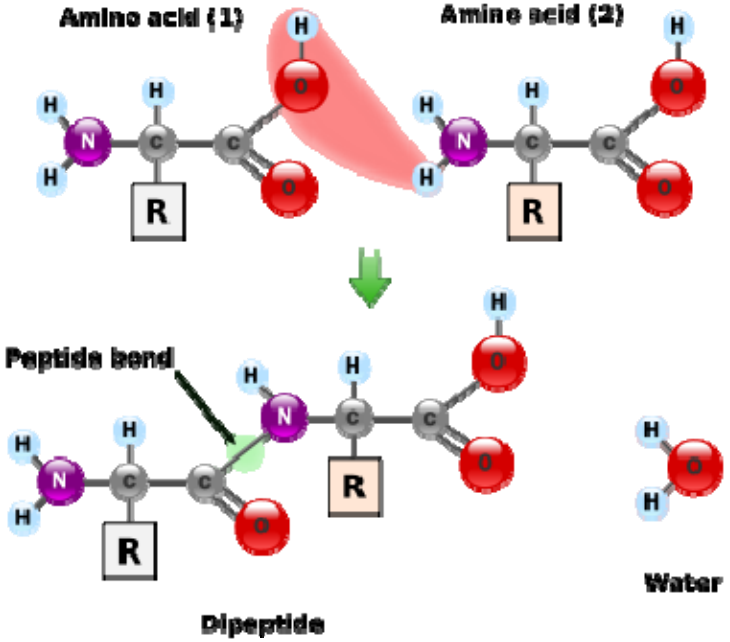




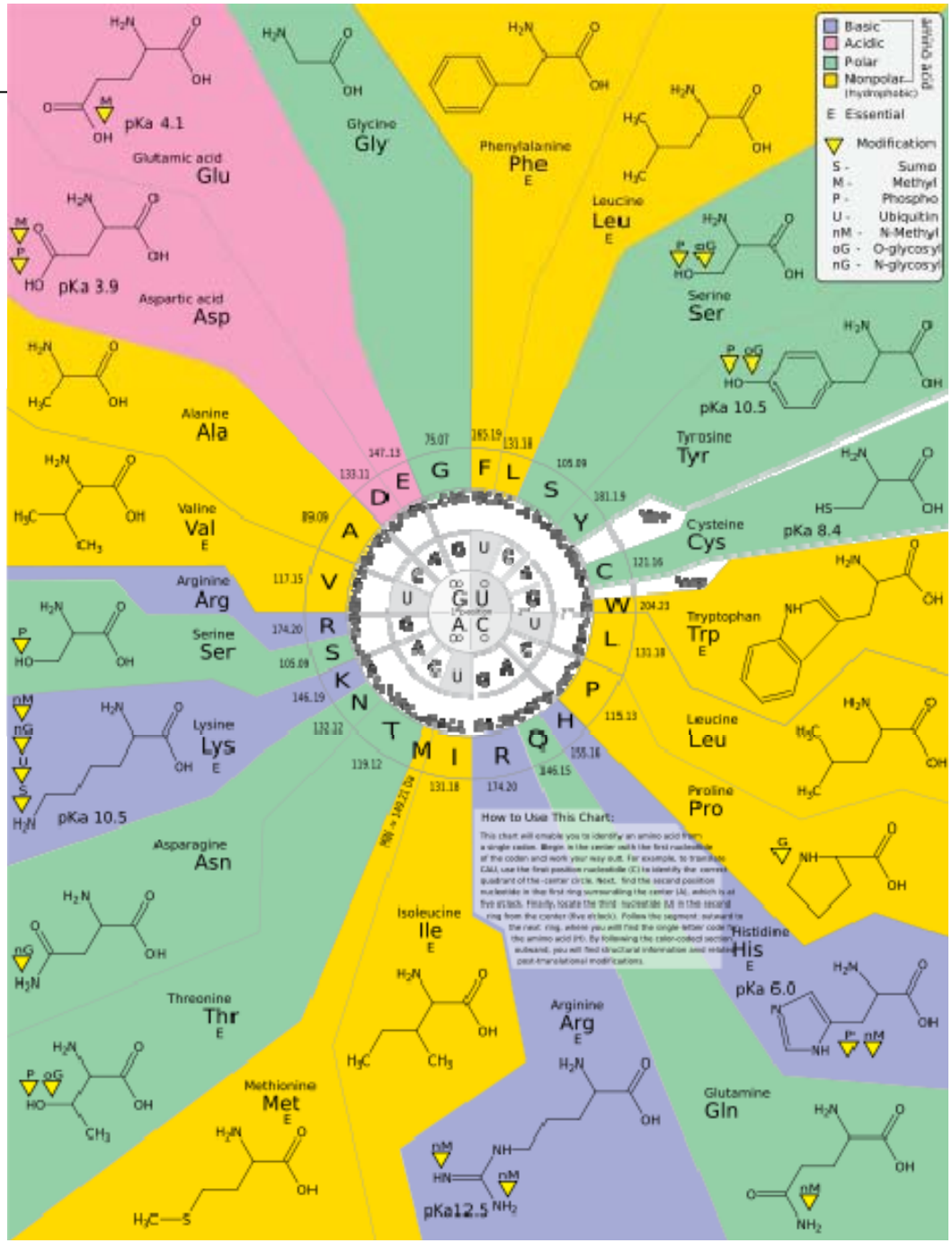
Last Week...


H
H
N
C
C
O
O
H
H
R



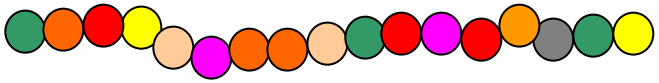

Amino acid (1)
Amine acid (2)
Peptide bond
Dipeptide
Water
R
N
C
O
H

PTM's


Basic
Acidic
Polar
Nonpolar (Hydrophobic)
E Essential
Modification
S - Sumo
M - Methyl
P - Phospho
U - Ubiquitin
nM - N-Methyl
oG - O-glycosyl
nG - N-glycosyl
Glutamic acid Glu
pKa 4.1
Glycine Gly
Phenylalanine Phe E
Leucine Leu E
Aspartic acid Asp
pKa 3.9
Serine Ser
pKa 10.5
Alanine Ala
Tyrosine Tyr
Valine Val E
Cysteine Cys
pKa 8.4
Arginine Arg
Tryptophan Trp E
Serine Ser
Lysine Lys E
pKa 10.5
Leucine Leu
Proline Pro
Asparagine Asn
Isoleucine Ile E
Histidine His E
pKa 6.0
Threonine Thr E
Arginine Arg E
Methionine Met E
Glutamine Gln
pKa12.5
How to Use This Chart:

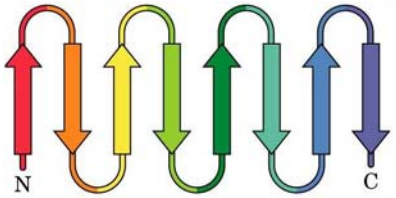
We (Re)Learned About

Primary Structure

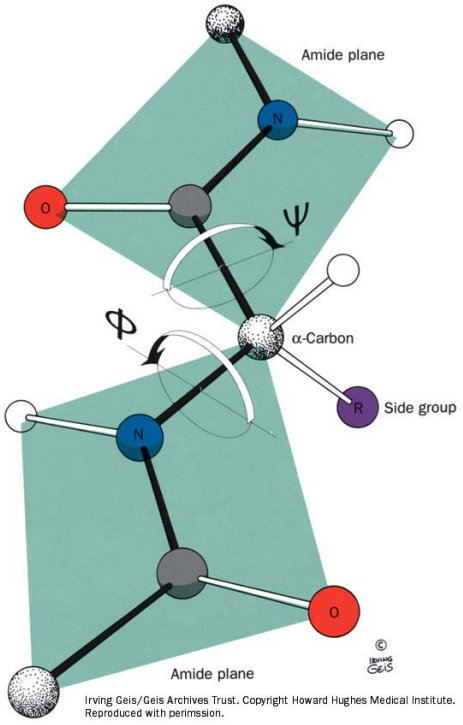


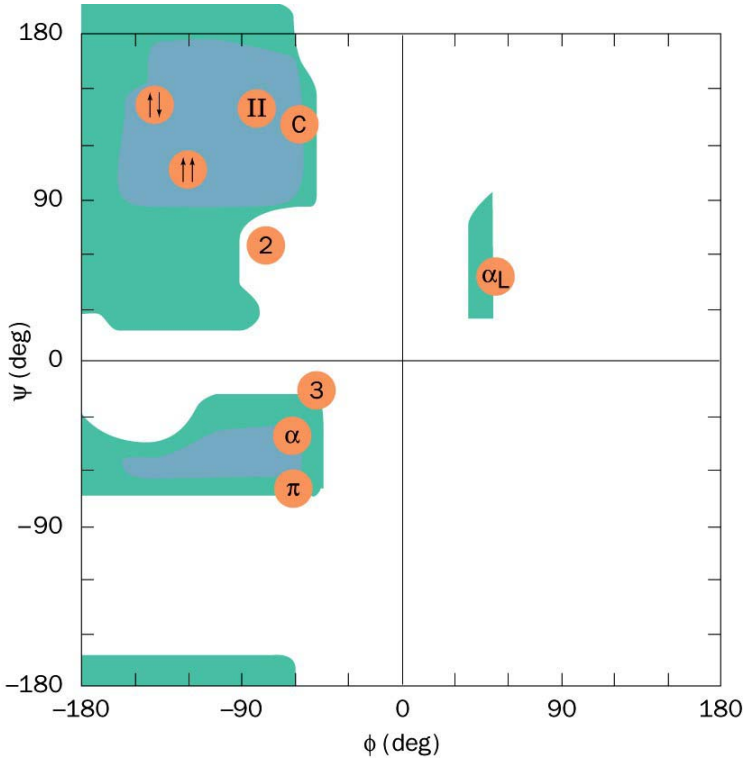
S-Q-D-A-G-M-Q-Q-G-A-D-M-D-Q-V-S-A

And Tertiary Structure



Secondary Structure





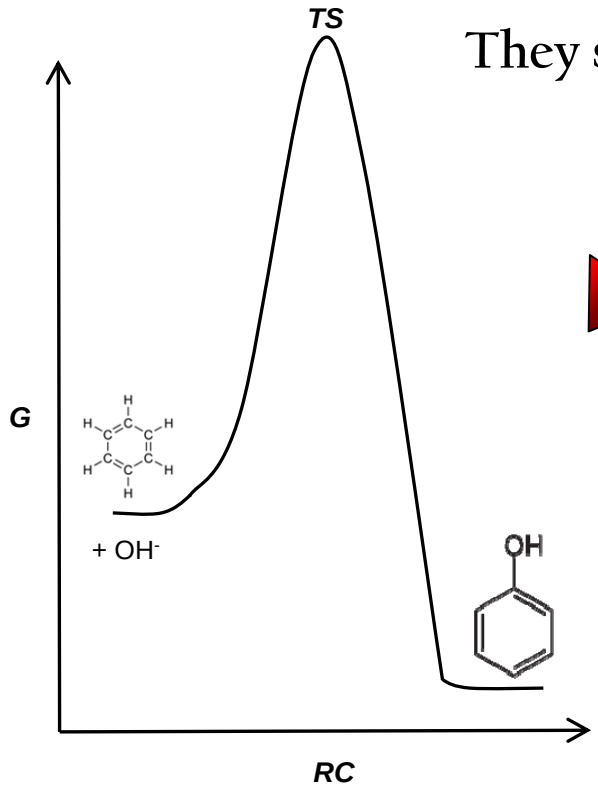
Enzymes

What are these crazy things called 'Enzymes'?

And...

Why do we care about them?

- Enzymes are the catalysts of biology.



They solve this problem

This reaction will go, but it'll take a gazillion years...

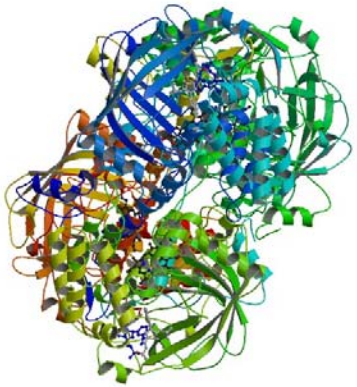
or

You could heat it up to a gazillion degrees

Enzymes Allow…

- Higher reaction rates (same as any catalyst)

- The rates of Enzyme reactions are 10^6 – 10^{12} times those of the corresponding uncatalized reaction…



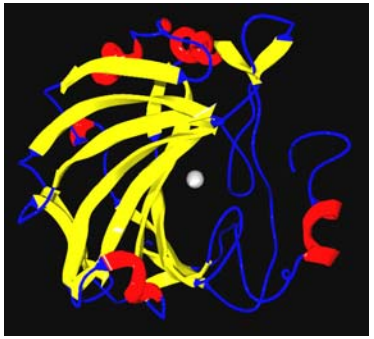
Catalase

$2H_2O_2 \rightarrow 2H_2O + O_2$

Pretty darn fast:

~ $6 * 10^5$ sec^{-1}

- The rates of reaction for the fastest enzymes are diffusion limited, around 10^6 turnovers per second.

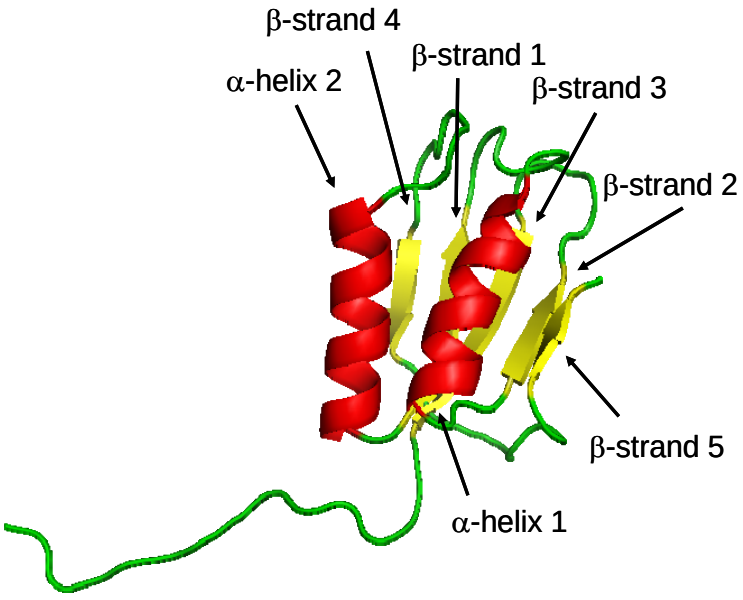


$$CO_2 + H_2O \rightarrow^{\text{Carbonic anhydrase}} HCO_3^- + H^+$$

Enzymes Allow…

- Milder reaction conditions (same as any catalyst, but better)

  - We won't have to heat the reaction up to a gazillion degrees to make it go at a reasonable pace

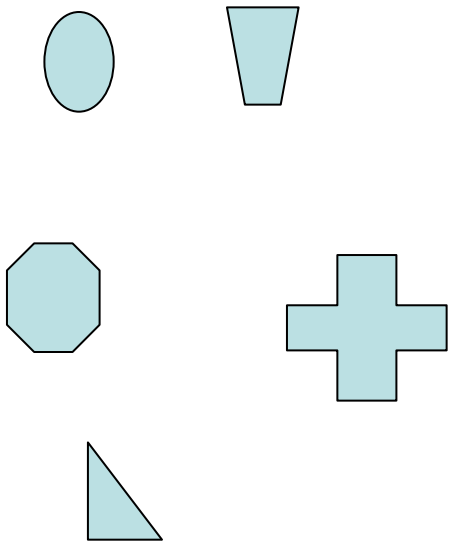


- Enzymes from hythermophiles work well at around 85°C, but not at room temp!!

Acylphosphatase from *Sulfolobus solfataricus*

Enzymes Allow…

- Reaction Specificity (better than inorganic catalysts)



- Can't really do this with an inorganic catalyst. A set of similar molecules will likely all be reactive…

Enzymes Allow…

- Capacity for Regulation (way better than inorganic catalysts)

- For inorganic catalysts, you can really only interfere with their activity by using competitive inhibitors or permanent covalent deactivation

  E.g. you can 'poison' a platinum catalyst surface with SiO_2, presumably by bridging the tetrahedral coordination sites.

- Enzymes can be controlled by:

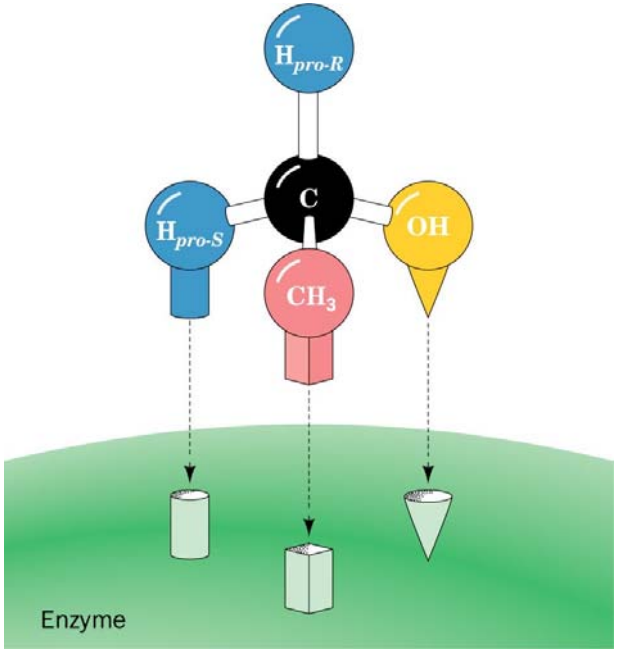
  - Expression levels
  - (ir)Reversible covalent modification (i.e. PTMs)
  - Non-covalent 'competitive' inhibition
  - Non-competitive 'allosteric' inhibition
  - Un-competitive inhibition

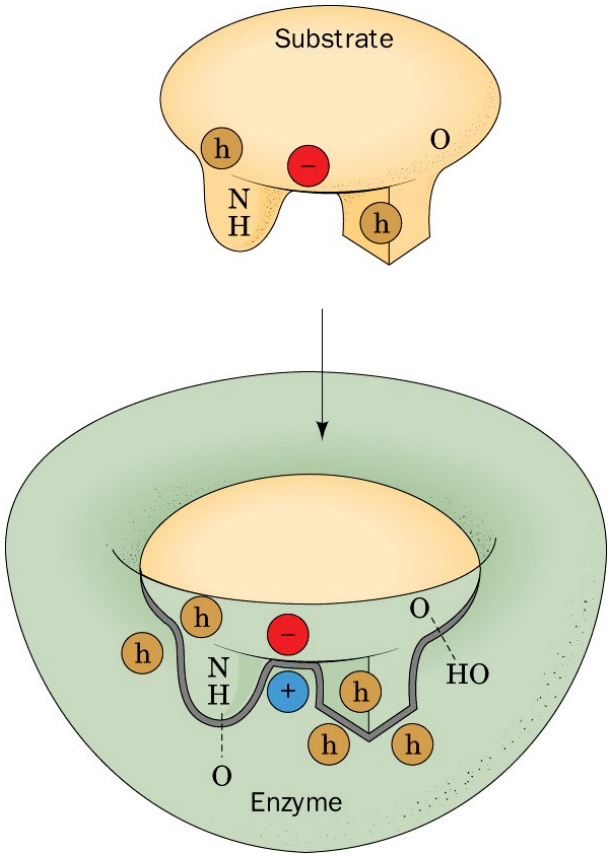
The 'Lock and Key' Model

- The lock and key model is almost completely useless, but it does express two important, basic concepts about enzymes:

- Geometric and Chemical Specificity

- Geometric Specificity extends to the steroisomer: L for Amino Acids and D for sugars

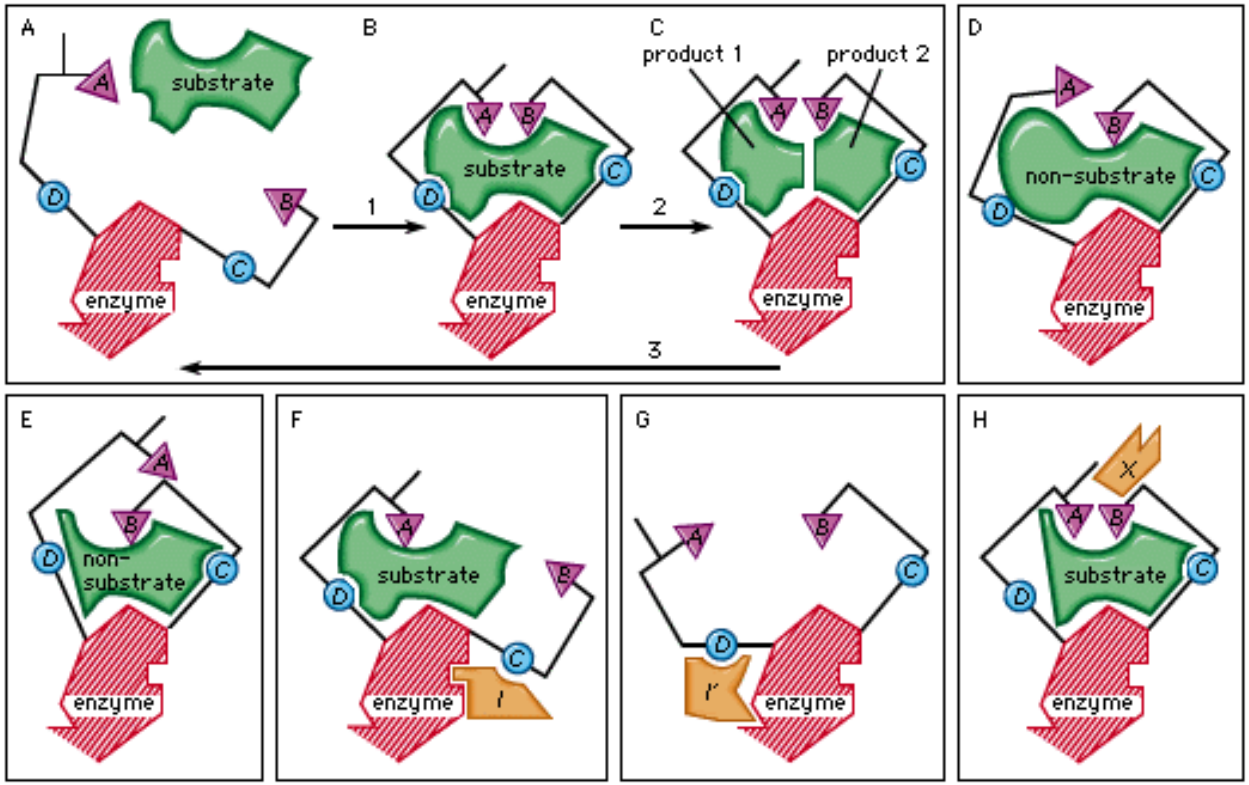




Why the Lock and Key Model is Useless

- So the Enzyme and Substrate structures are complimentary... So I can predict the structure of a substrate or an inhibitor by *wedging small molecules into the enzyme structure*: Rational Drug Design

...Actually not so much. Why? ...Because the enzyme *moves*!



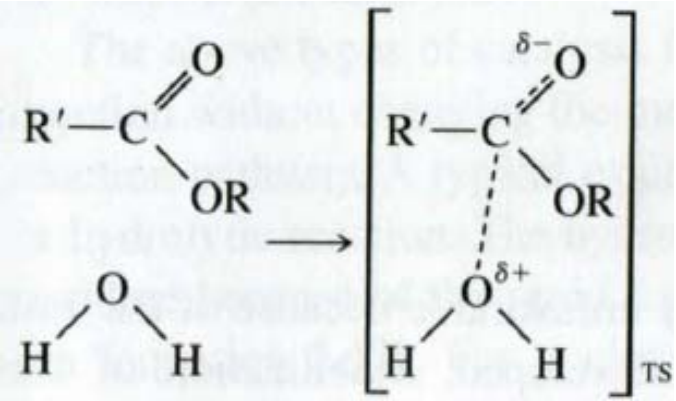
Enzymes: The Ultimate Catalysts

- Enzymes are almost always better than a comparable inorganic or small molecule catalyst

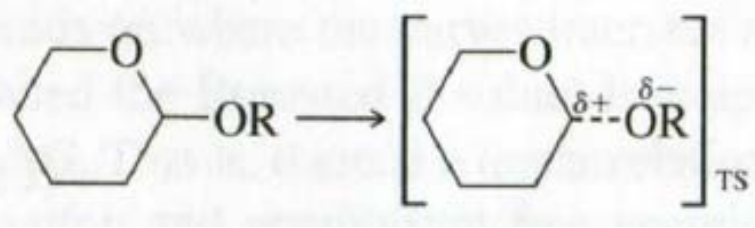
But why?

The Chemistry of Catalysis

- Catalysis is all about stabilization of the Transition State



Attack by Water on an Ester

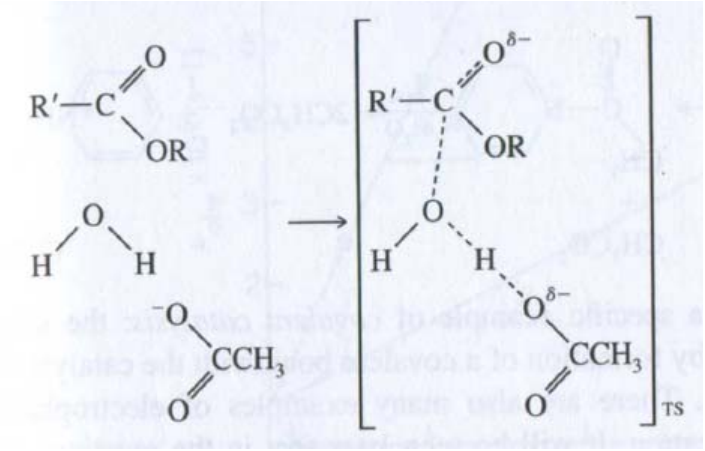


Hydrolysis of an Acetal

- Both of these reactions involve very unfavorable charge separation in the Transition State

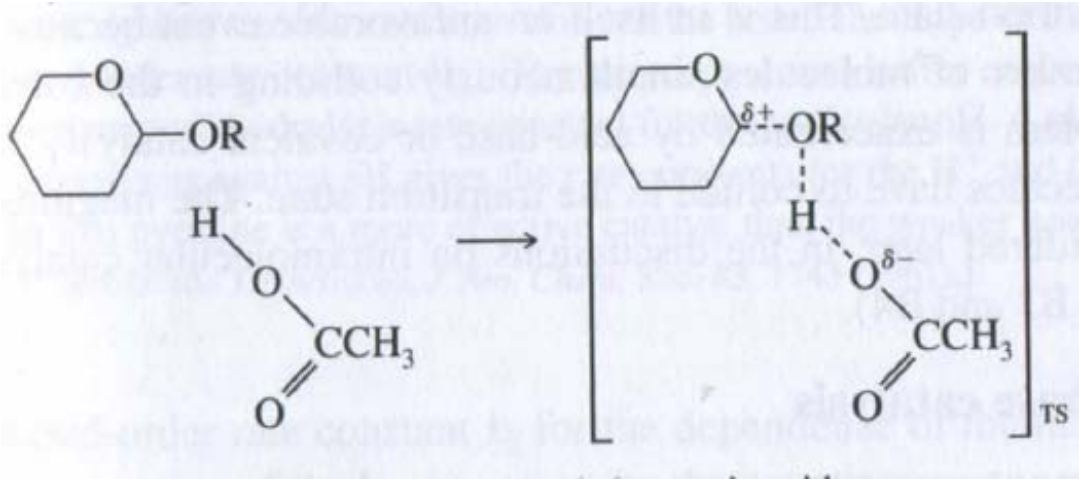
The Chemistry of Catalysis

- In many cases catalysts work by stabilizing this charge buildup



- General Base Catalysis by acetate ion

- General Acid Catalysis by Acetic Acid



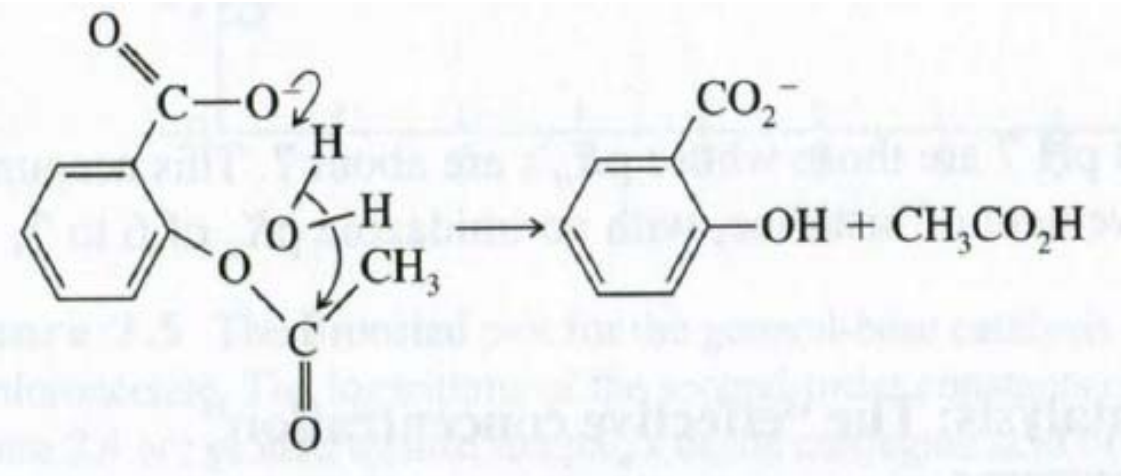
Enzymes and Effective Concentration

- Effective concentration is a measure of concentration based on the proximity of reactive groups

- You can either have a solution that is highly concentrated (i.e. water = 55 M)

or

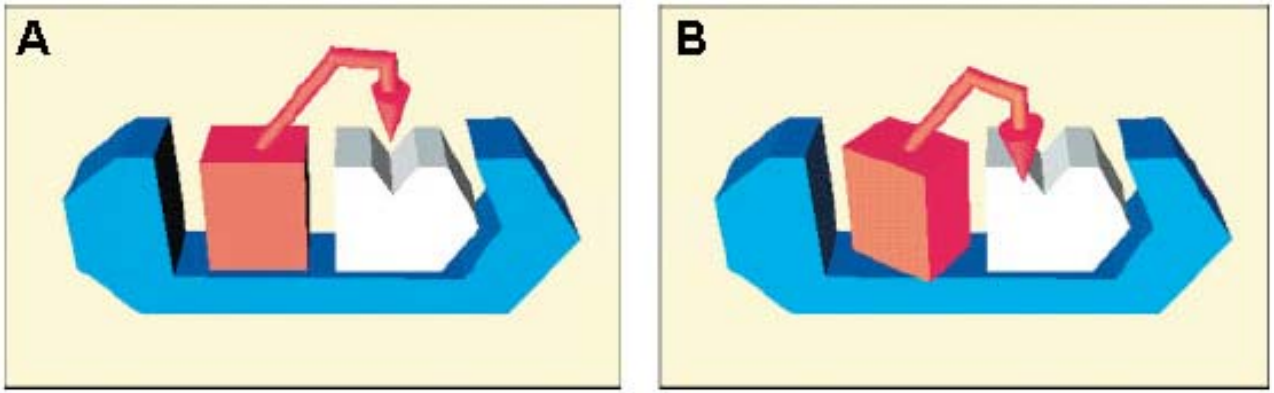
- You can can have two groups on the same molecule!



Autocatalyzed hydrolysis of Aspirin

Enzymes and Orbital Steering

- The much maligned theory of orbital steering is that on an enzyme reactive groups can be precisely positioned relative to each other to maximize orbital overlap:



Cartoon depiction of orbital overlap in isocitrate dehydrogenase

SCIENCE | VOL. 277 | 11 JULY 1997

- Orbital overlap in the transition state is helpful, but not critical. Error allowed without significant change: ~ 10° or so.

Electrostatics and Enzyme Catalysis

- Water obscures electrostatic catalysis!

Strength of charge (eV)

$$E = \frac{e_1 e_2}{rD}$$

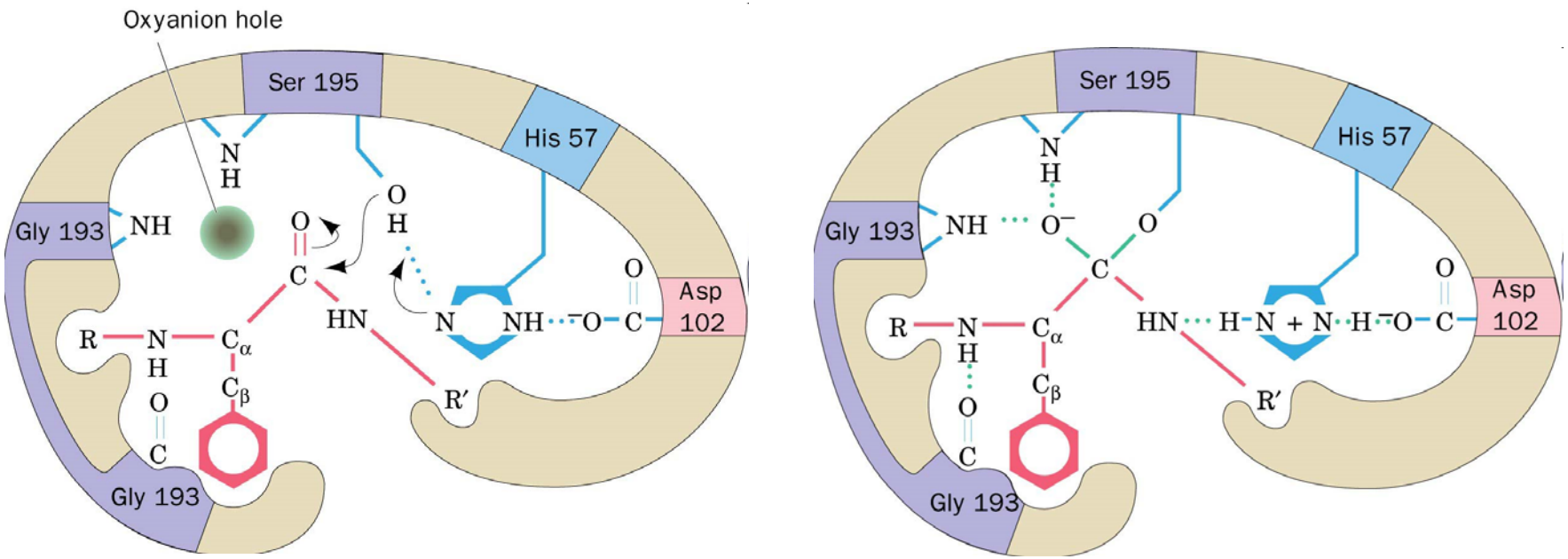
The Dielectric Constant of the medium

distance between charges

- Enyzmes can create a nice, cozy hydrophobic environment to promote Electrostatic Catalysis

Common Kinds of Catalysis

- **Covalent** Nucleophilic



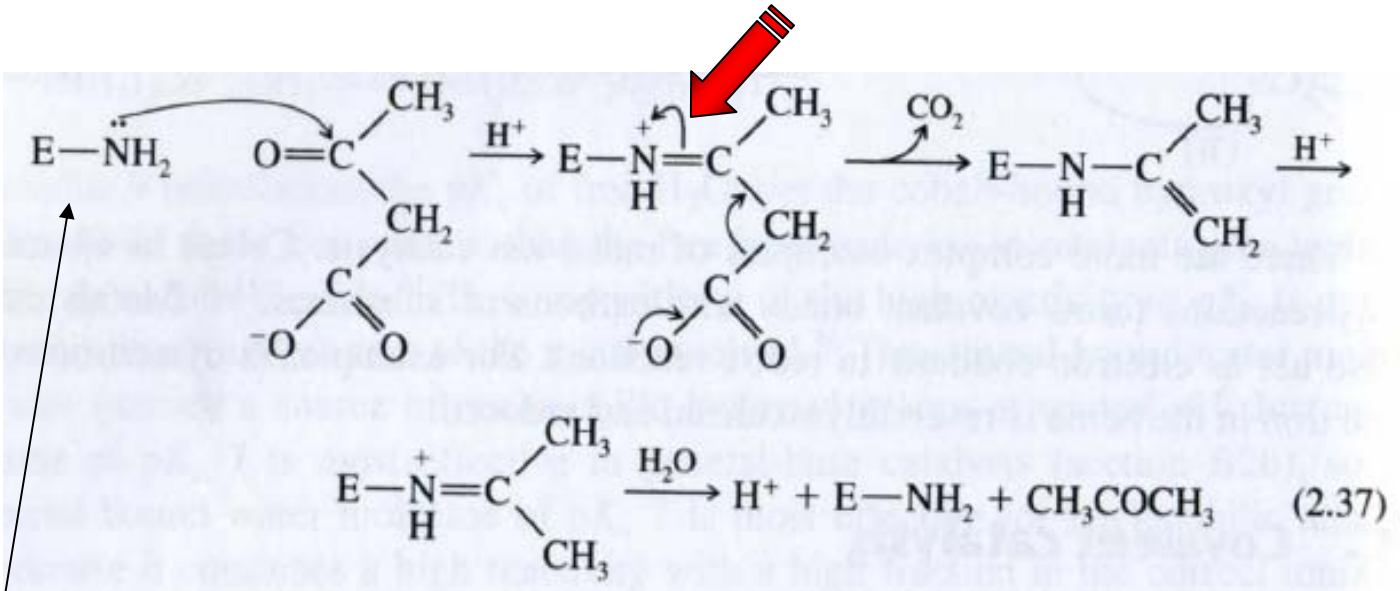
- The most famousest of all enzymatic mechanisms: The Chymotrypsin 'ping-pong'

Nucleophilic Hydrolysis is Pretty Common!

Nucleophile	Enzyme	Intermediate
—OH (serine)	Serine proteases	Acylenzyme
	Alkaline phosphatases, phosphoglucomutase	Phosphorylenzyme
—OH (threonine)[a]	Proteasome, amidases	Acylenzyme
OH^- (zinc-bound)	Carbonic anhydrase, liver alcohol dehydrogenase	—
—SH (cysteine)	Thiol proteases, glyceraldehyde 3-phosphate dehydrogenase	Acylenzyme
$—CO_2^-$ (aspartate)	ATPase (K^+/Na^+, Ca^{2+})	Phosphorylenzyme
$—NH_2$ (lysine)	Acetoacetate decarboxylase, aldolase, transaldolase, pyridoxal enzymes	Schiff base
	DNA ligase	Adenylenzyme (phosphoamide)
Imidazole (histidine)	Phosphoglycerate mutase, succinyl-CoA synthetase, nucleoside diphosphokinase, histone phosphokinase, acid phosphokinase	Phosphorylenzyme
—OH (tyrosine)	Glutamine synthetase	Adenylenzyme
	Topoisomerases	Nucleotidylenzyme (phosphotyrosine)

Common Kinds of Catalysis

- **Covalent** Electrophilic (Schiff Base)

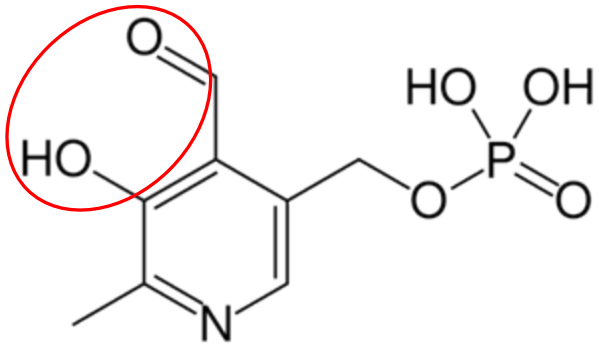


Acetoacetate decarboxylase mechanism

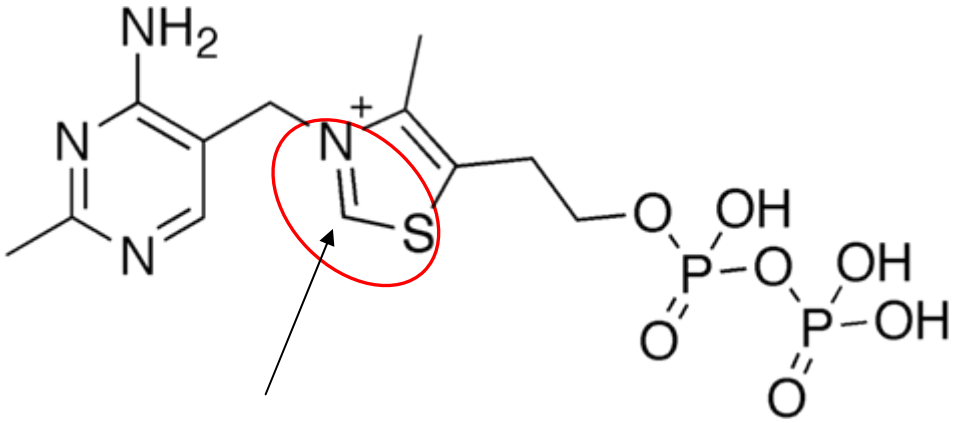
Lysine residue (not the Schiff Base)

The 'Other' Electrophilic Catalysts

- **Since there aren't many Amino Acids that make good electrophiles, many electrophilic catalysis enzymes use co-enzymes:**



Pyridoxal phosphate
(decarboxylases)

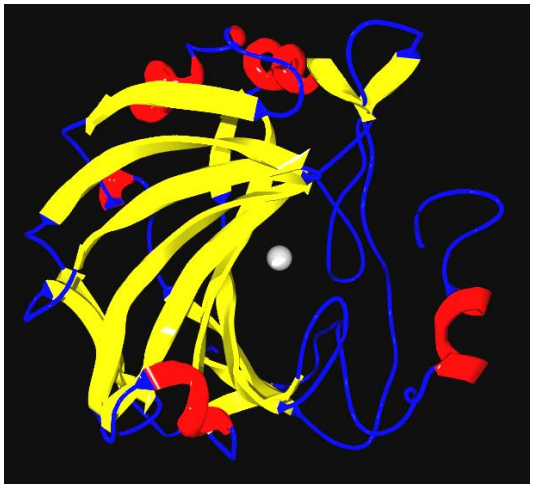


Stable C-!!

Thiamine
Pyrophosphate
(dehydrogenases)

Common Kinds of Catalysis

- **Metalloenzymes**

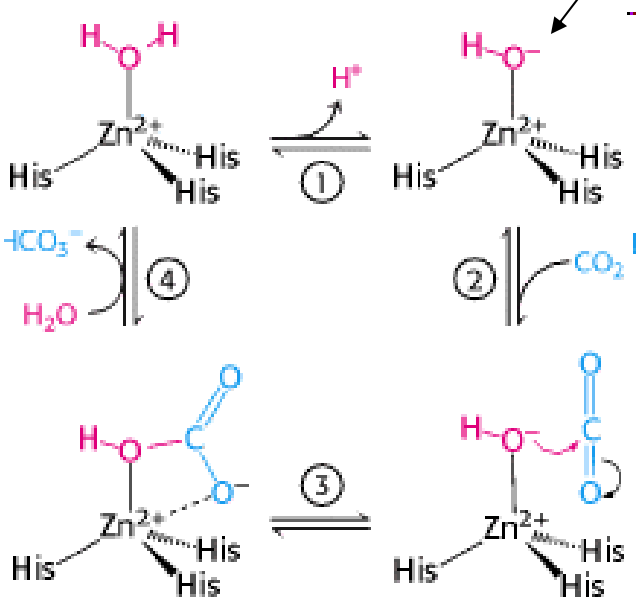


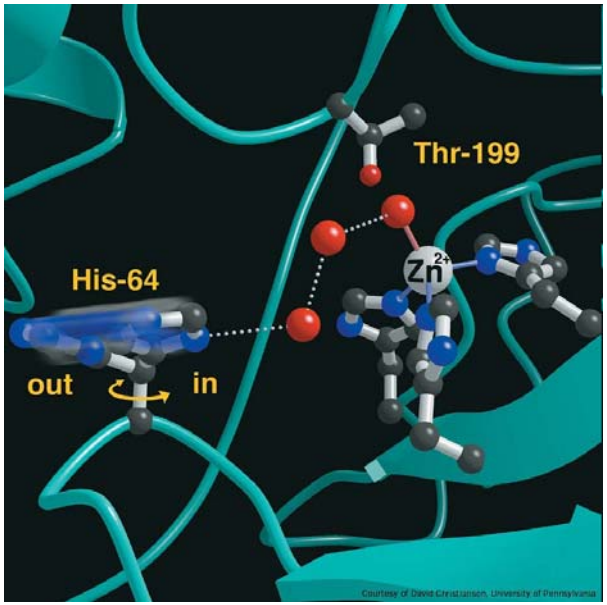
Carbonic anhydrase

A stable hydroxyl group… at neutral pH!

Can use

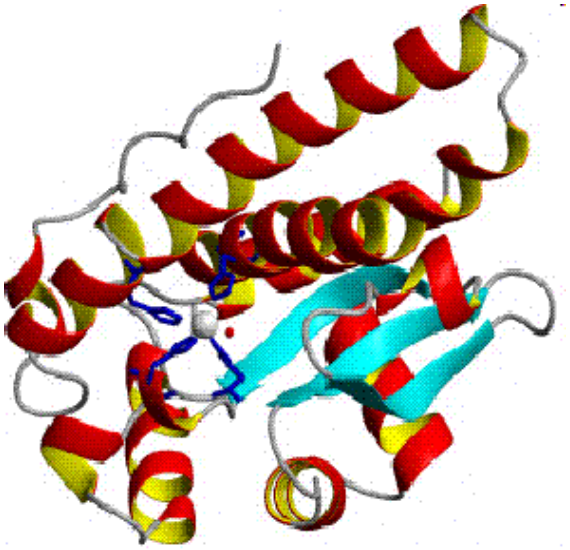
- Zn^{2+}
- $Fe^{2+/3+}$
- Cu^{2+}
- Co^{3+}
- Mn^{2+}

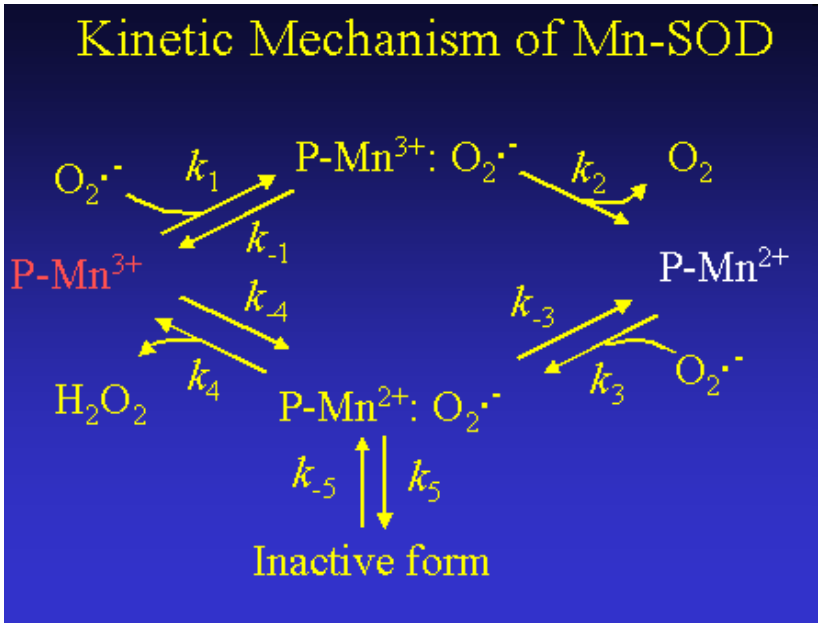


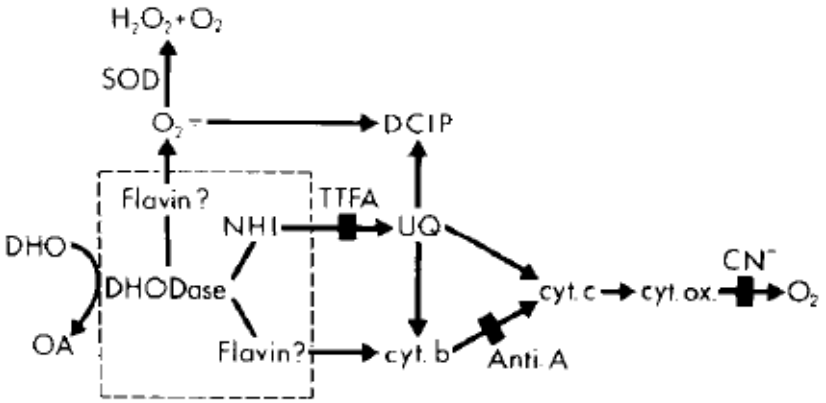


Metalloenzymes

Superoxide Dismutase



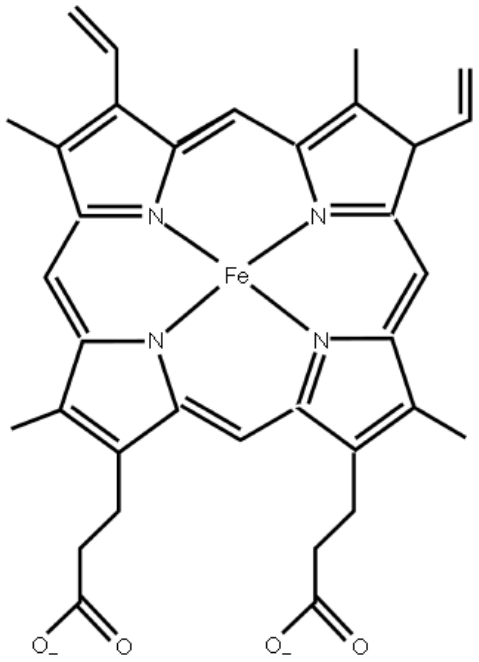


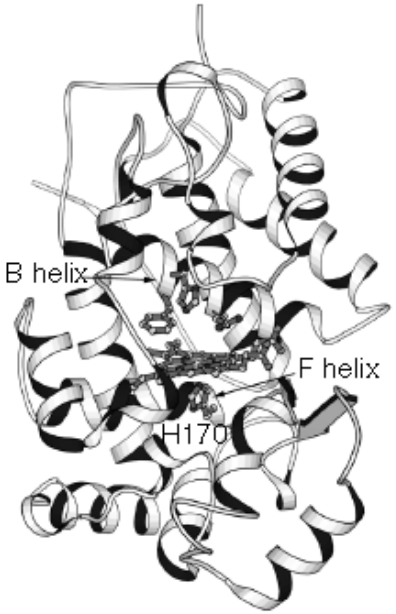


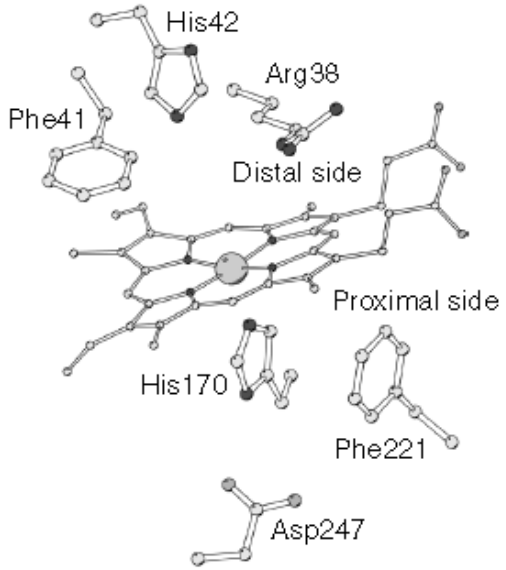
JBC, Vol. 250, No. 11, June 10, pp. 4322-4326, 1975

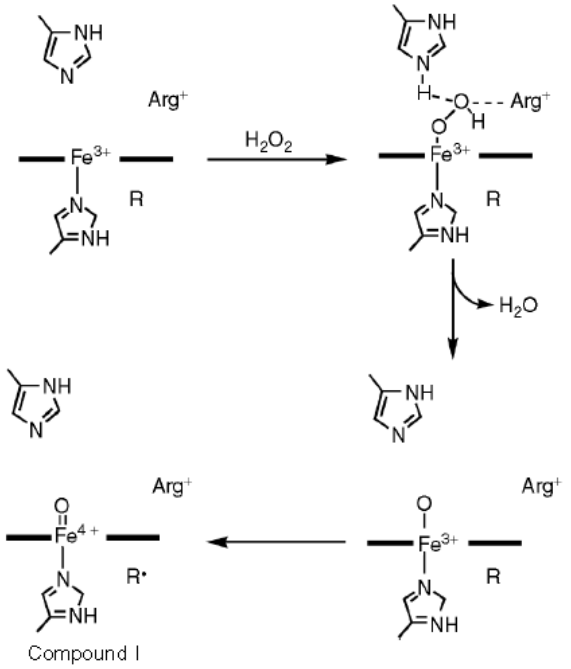
Heme Enzymes

Heme



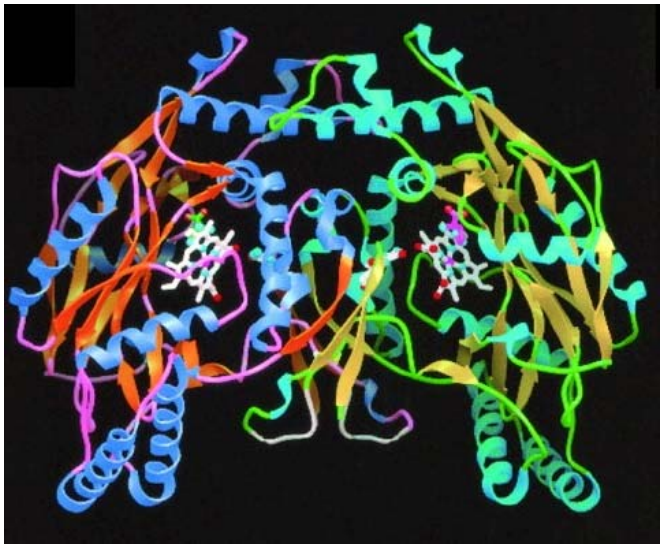


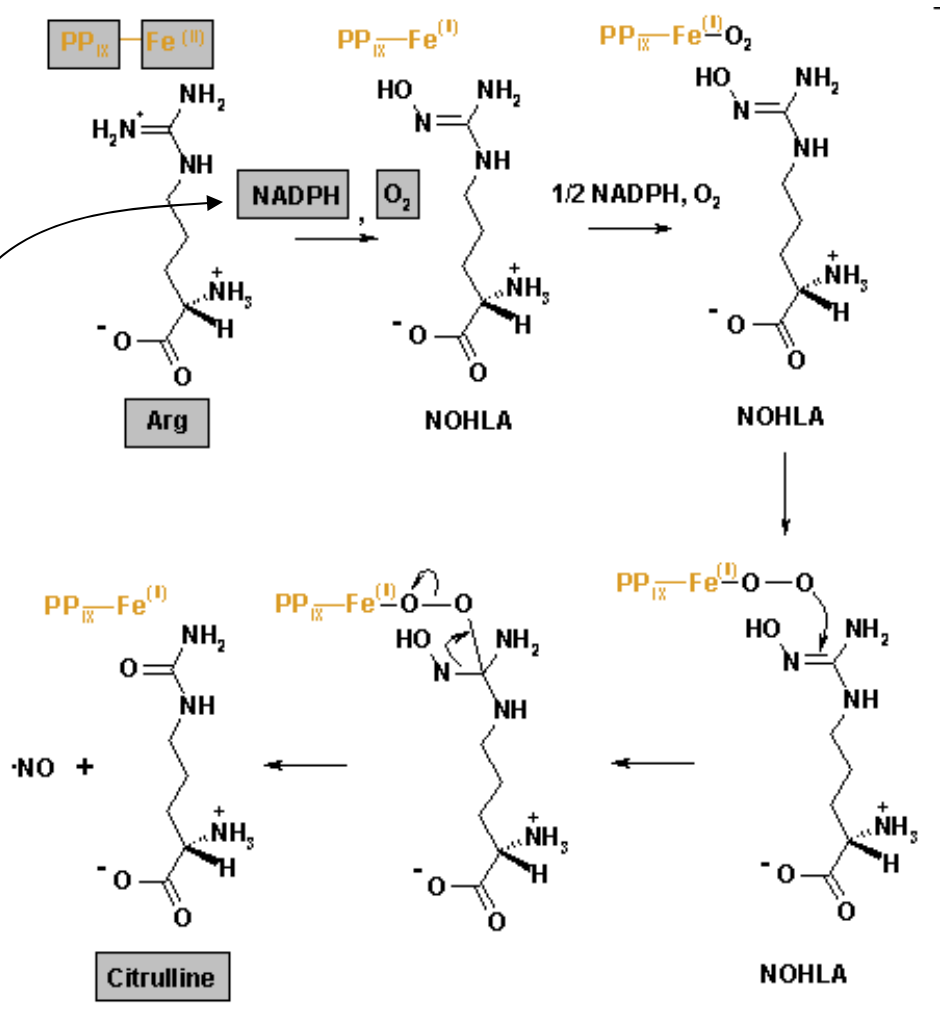




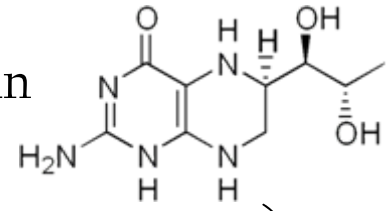
Horseradish peroxidase

The Nitric Oxide Synthases (NOS)



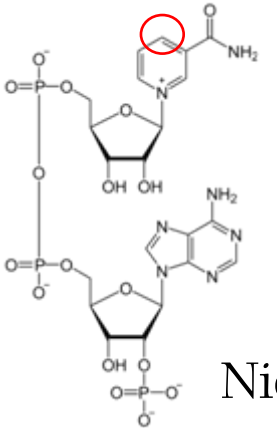


Tetrahydrobiopterin



FMN

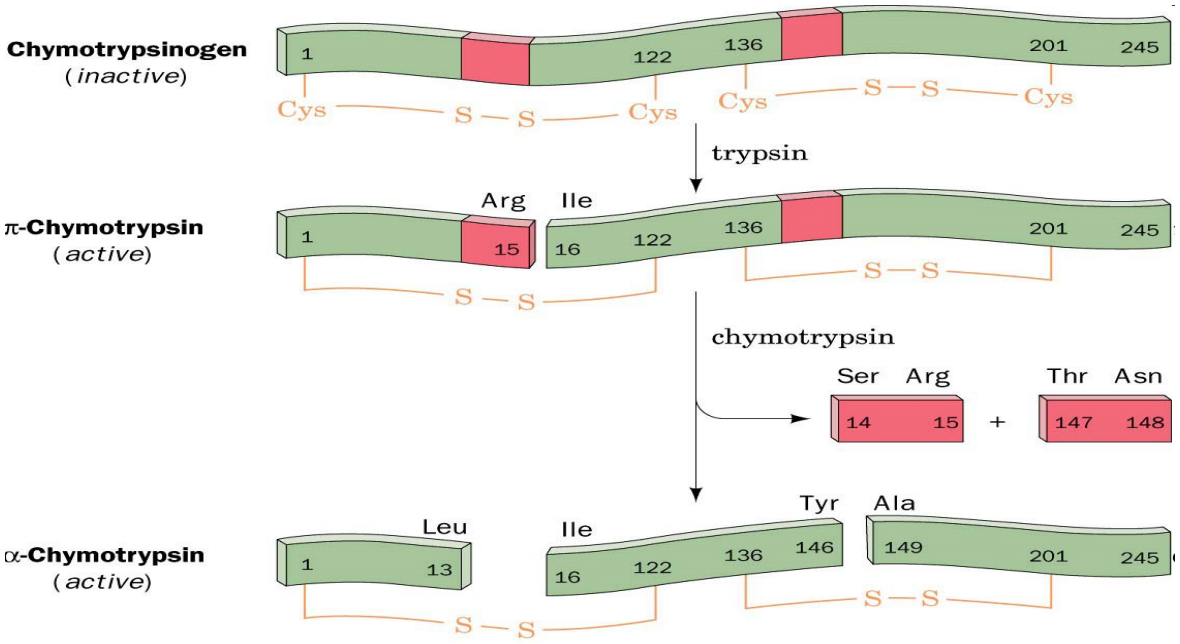
NADPH → NADP⁺ + e⁻



Nicotinamide adenine dinucleotide phosphate

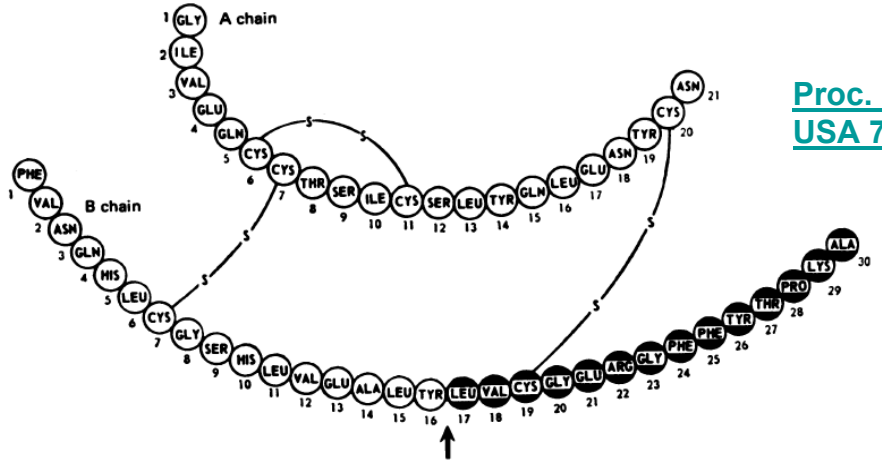
Zymogens (pro-enzymes)

- Some enzymes are not 'born' active, they have to be cut first:



- Many proteins of the coagulation pathway

Insulin (yes, I know it's not an enzyme)



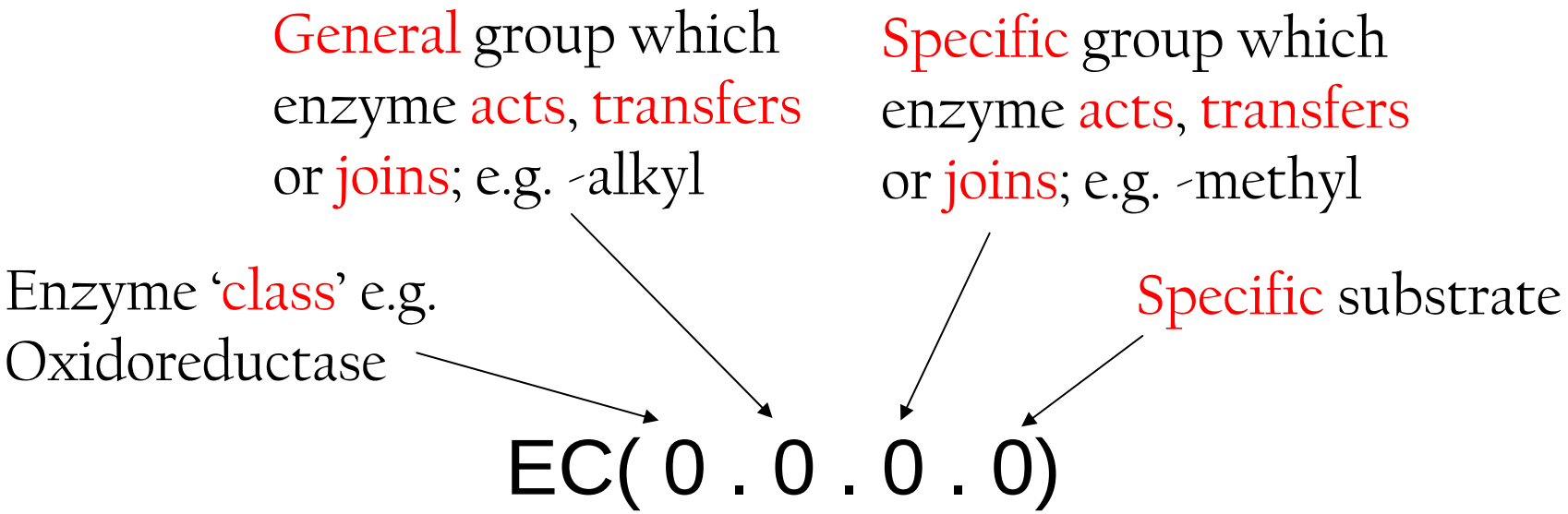
Proc. Nati. Acad. Sci. USA 76 (1979)

Types of Enzymes

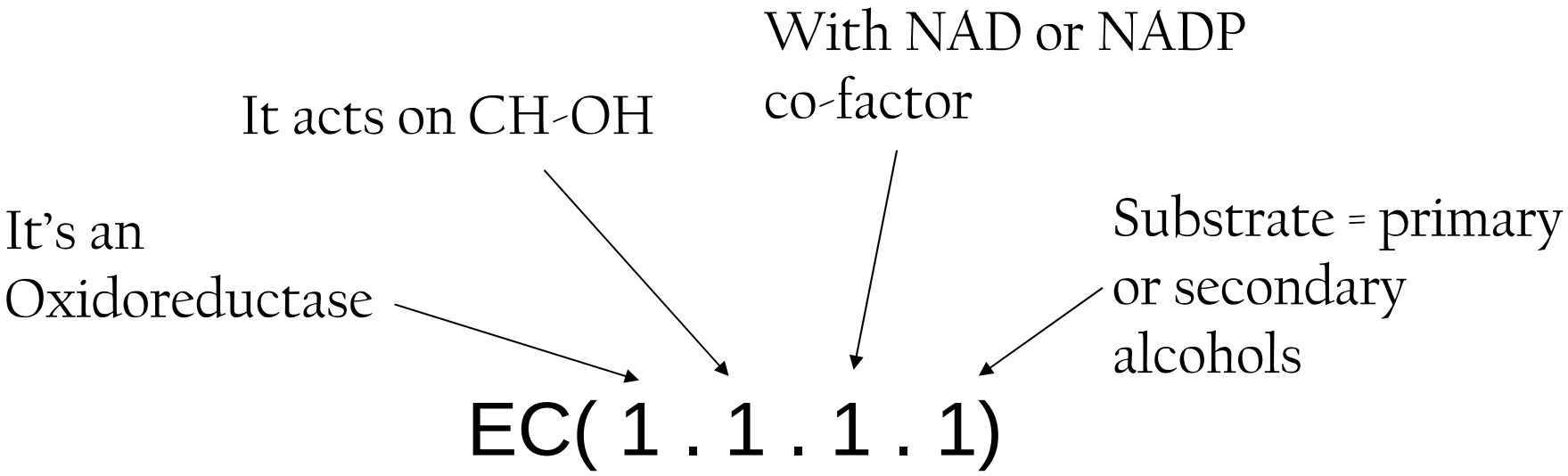
- Enzyme types by reaction catalyzed:
  - Oxidoreductase (oxidation/reduction, e.g. HRP)
  - Transferase (transfer of a reactive group, e.g. DNA methyltransferase)
  - Hydrolase (hydrolysis of a bond, e.g. proteases)
  - Lyase (non-oxidative, non-hydrolytic bond making/breaking, e.g. adenylyl cyclase (cAMP))
  - Isomerase (isomerization, e.g. prolyl isomerase)
  - Ligases (linkage of molecules *using energy from NTP*, e.g. Succinyl-CoA synthetase)

Naming the Enzymes

- In 1955, the 'International Union of Biochemistry' formed a 'Commission on Enzyme Nomenclature'
- In 1961 commission released it's first Enzyme Nomenclature Guide with 712 enzymes
- By 1992, there were 3,196 enzymes
- It works like this:



Example



It's Alcohol Dehydrogenase

Enzymes, the Web and You

- Useful Websites:
  - BRENDA (http://www.brenda-enzymes.org/): all you ever wanted to know about any enzyme... ***and more***!
  - Expasy (http://www.expasy.org/): mostly useful as a link to BRENDA!
  - NIST database (http://xpdb.nist.gov/enzyme_thermodynamics/enzyme_thermodynamics_data.html): Thermochemical data on Enzyme reactions
  - Kegg and ERGO: Genome databases
  - PDB.org (www.pdb.org): Protein structures!!

The End...