PHRM 836 September 8, 2015

Enzyme Catalysis: structural basis and energetics of catalysis

Devlin, section 10.3 to 10.5

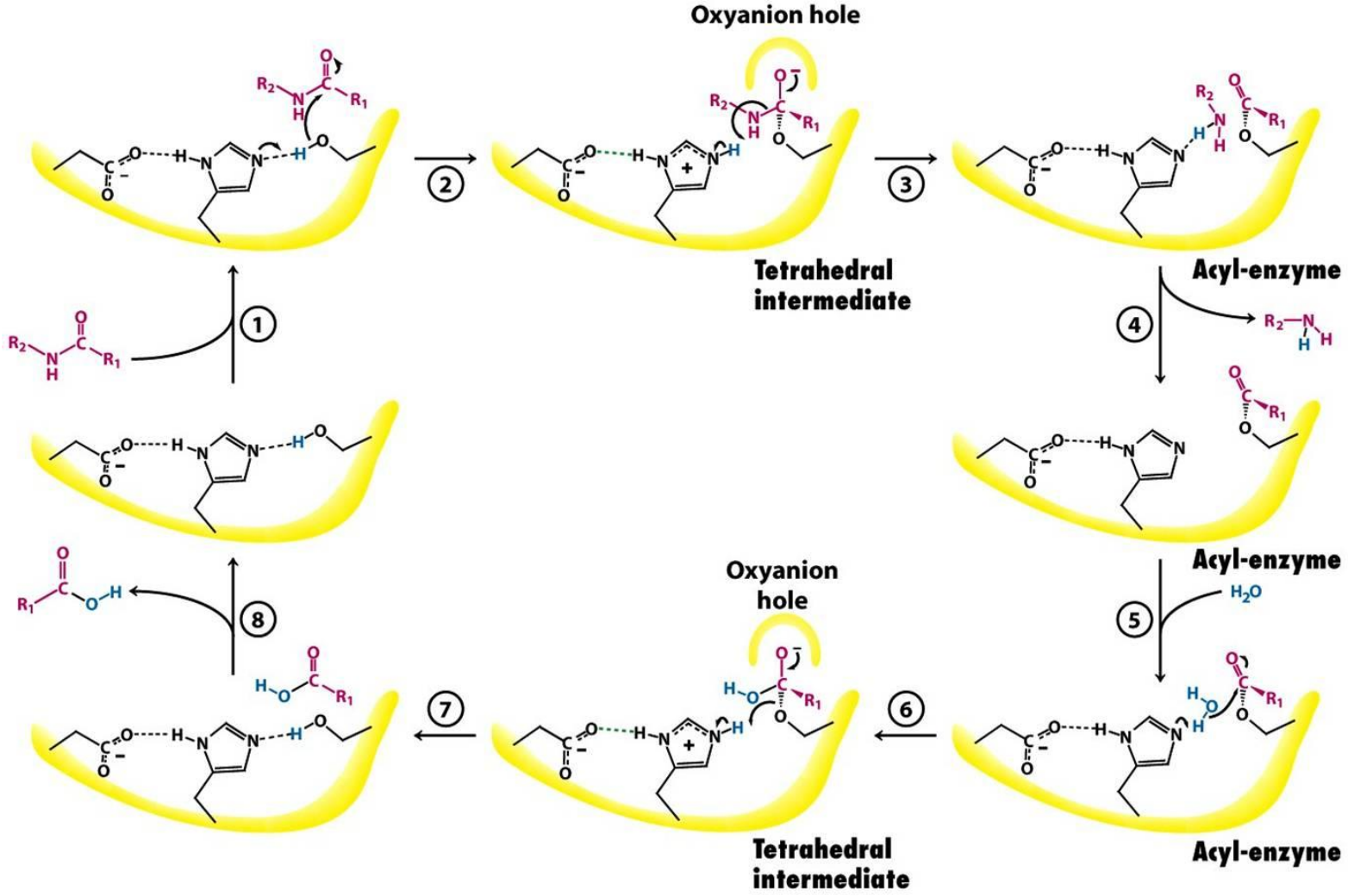
1. Enzyme binding of substrates and other ligands (binding sites, structural mobility)
2. Energetics along reaction coordinate
3. Cofactors
4. Effect of pH on enzyme catalysis

Enzyme catalysis: Review

Devlin sections 10.6 and 10.7

- Definitions of catalysis, transition state, activation energy
- Michaelis-Menten equation
  - Kinetic parameters in enzyme kinetics (k_{cat}, k_{cat}/K_M, *Vmax*, *etc*)
  - Lineweaver-Burk plot
- Transition-state stabilization
- Meaning of proximity, orientation, strain, and electrostatic stabilization in enzyme catalysis
- General acid/base catalysis
- Covalent catalysis

Structure determines enzymatic catalysis as illustrated by this mechanism for ____



Substrate binding by enzymes

- Highly complementary interactions between substrate and enzyme
  - Hydrophobic to hydrophobic
  - Hydrogen bonding
  - Favorable Coulombic interactions
- Substrate binding typically involves some degree of conformational change in the enzyme
  - Enzymes need to be flexible for substrate binding and catalysis.
  - Provides optimal recognition of substrates
  - Brings catalytically important residues to the right position.

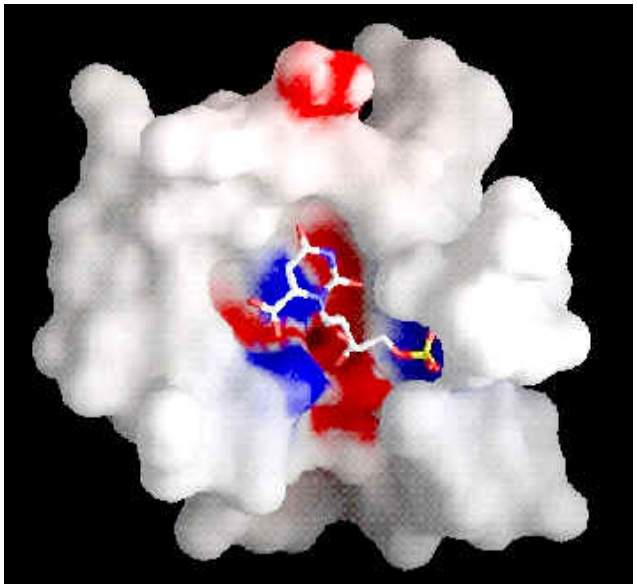


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Figure 10.11 Orotate phosphoribosyltransferase

Induced fit: promoted by rotation around bonds

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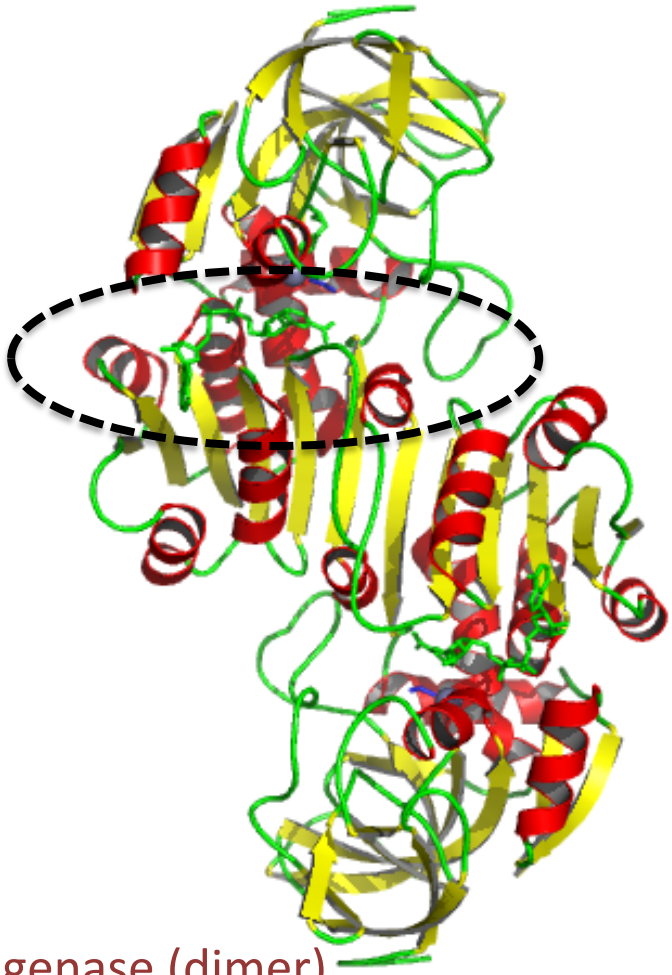
  - Enzymes need to be flexible for substrate binding and catalysis.
  - Provides optimal recognition of substrates
  - Brings catalytically important residues to the right position.
- Substrate binding site is a relatively small region



Alcohol dehydrogenase (dimer)

- Ethanol → acetaldehyde
- NADH, Zn cofactors

Transition-state binding vs substrate binding

- Enzyme must bind substrate, transition state and product.
- Tight binding to substrate or product slows overall reaction by increasing the height of the barrier to TS* or product dissociation, respectively.
- Tight binding to TS* speeds the reaction.

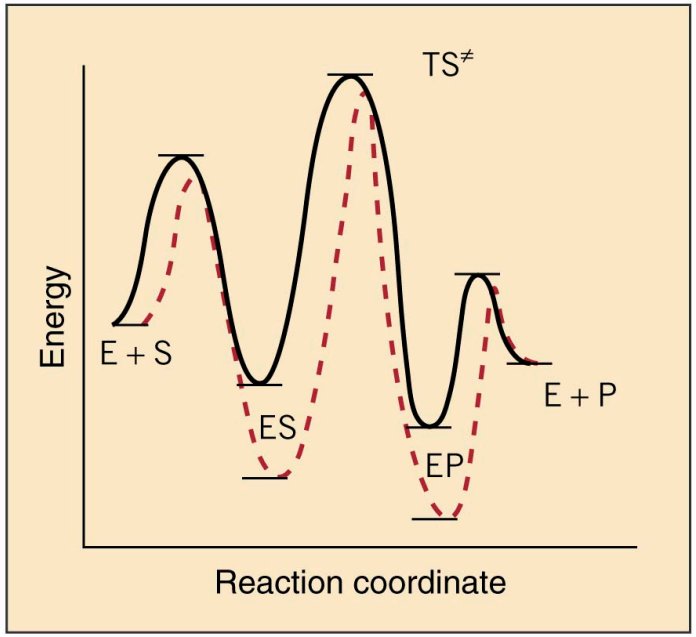
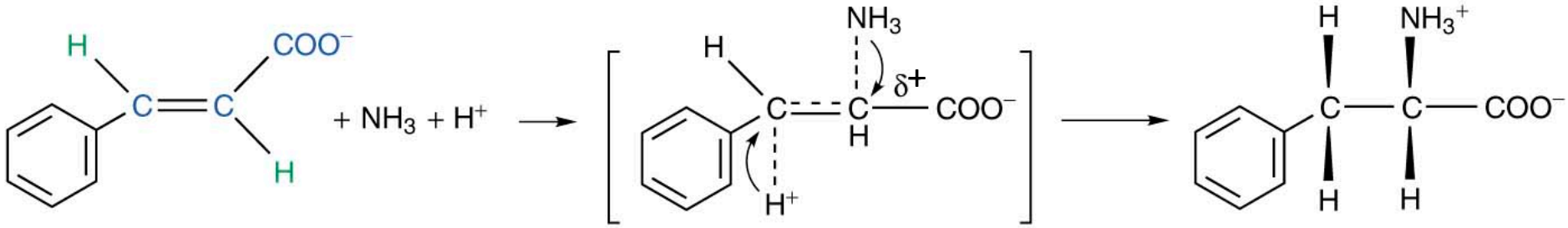


Figure 10.15

Transition states

- Rate enhancement of a chemical reaction by transition state stabilization.
- Partial charges occur frequently in transition states.



Reactant

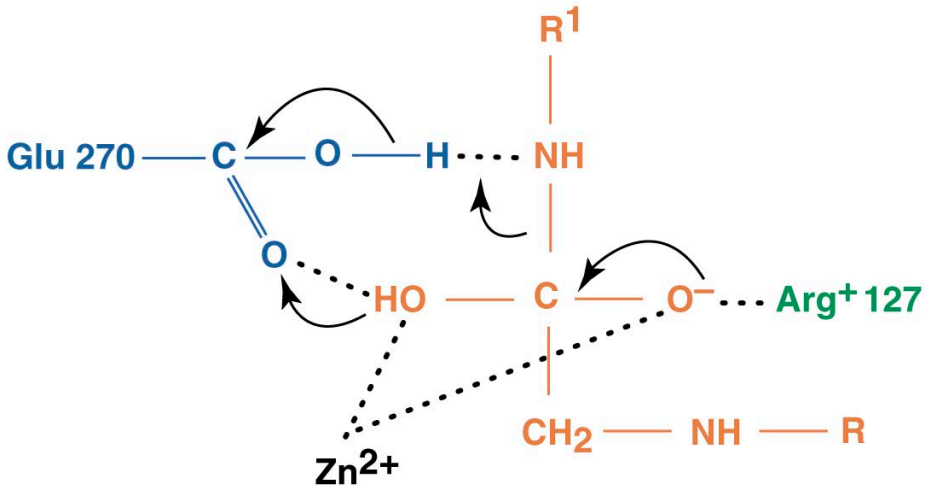
Transition state

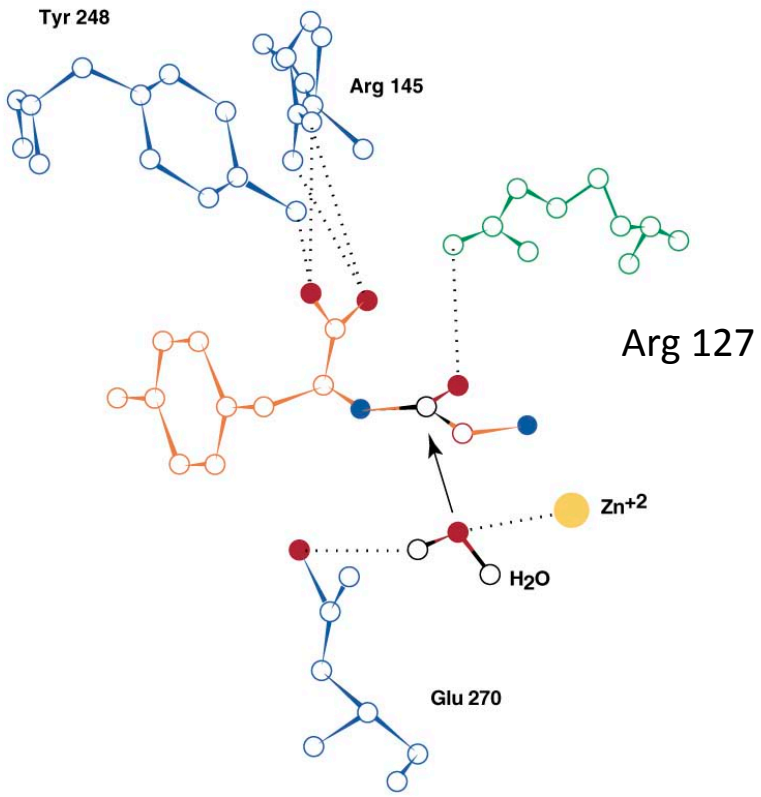
Product

chemical intermediate; very short-lived; high energy

Figure 10.13

Transition state stabilization: an illustration





Environment optimal for reaction!

Figure 10.39, 10.40

Cofactors and coenzymes

1. Non-protein small molecules required for function of some enzymes
2. Organic cofactors are also called coenzymes or prosthetic groups.
   - Many (not all) are derivatives of vitamins
   - For some enzymes, are chemically modified during the reaction
   - Function: hydride or electron transfer; group transfer
3. Inorganic cofactors
   - Metal ions
   - Metal clusters
   - Function: polarize bonds; coordination; metal reduction/ oxidation

More information: http://en.wikipedia.org/wiki/Cofactor_(biochemistry)

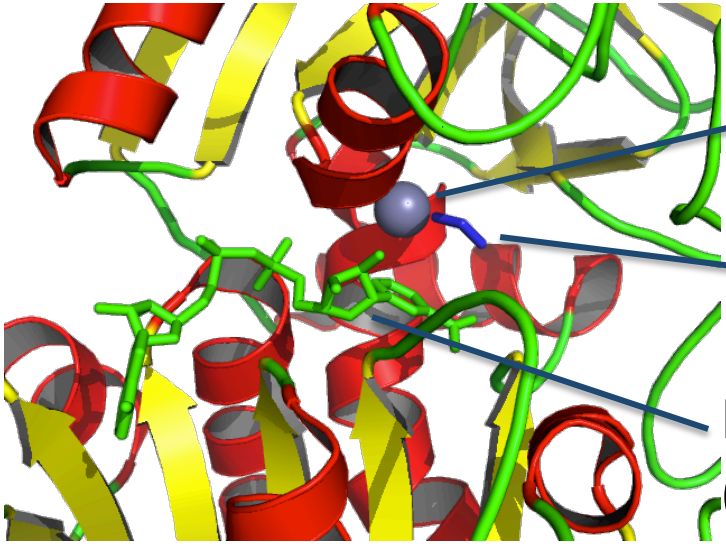
Examples of important organic cofactors or coenzymes

TABLE 10.3 • Coenzymes

Coenzyme	*Vitamin*	*Reaction Mediated*
Biotin	Biotin	Carboxylation
Cobalamin (B_{12})	Cobalamin (B_{12})	Alkylation
Coenzyme A	Pantothenate	Acyl transfer
Flavin coenzymes	Riboflavin (B_2)	Oxidation–reduction
Lipoic acid		Acyl transfer
Niacin coenzymes	Niacin	Oxidation–reduction
Pyridoxal phosphate	Pyridoxine (B_6)	Amino group transfer
Tetrahydrofolate	Folic acid	One-carbon group transfer
Thiamin pyrophosphate	Thiamin (B_1)	Carbonyl transfer

More information: http://en.wikipedia.org/wiki/Cofactor_(biochemistry)

Enzyme active sites: cofactors bind as substrates



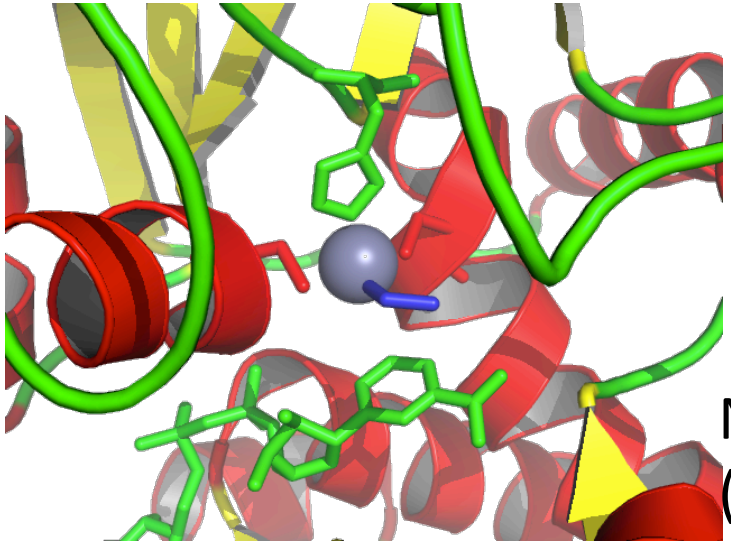
Alcohol dehydrogenase (dimer)

- Ethanol → acetaldehyde
- Uses zinc and NAD

Active Site

http://www.rcsb.org/pdb/101/motm_disscussed_entry.do?id=1adc#.Ujb9HgFU9us.email

Enzyme active sites: exquisite spatial complementarity (*i.e.* stereochemistry)



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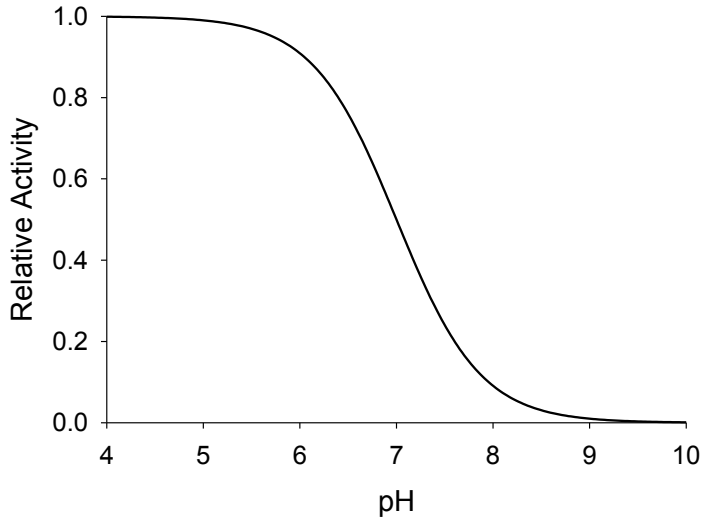
Effect of pH on enzyme catalysis

- When the rate-limiting component of the catalytic process involves a titratable residue, the measured activity of the enzyme depends on pH and the ionization status of that residue. → Highest enzymatic activity occurs with the proper ionization state.
- Example below for general acid involvement in the catalytic step:
  - Enz—AH (activite) ⇔ Enz—A^- (inactive)
  - Enzyme activity = 50% maximum activity when pH = pK_a of the general acid



pK_a = ?
Is the proper ionization state protonated or unprotonated?

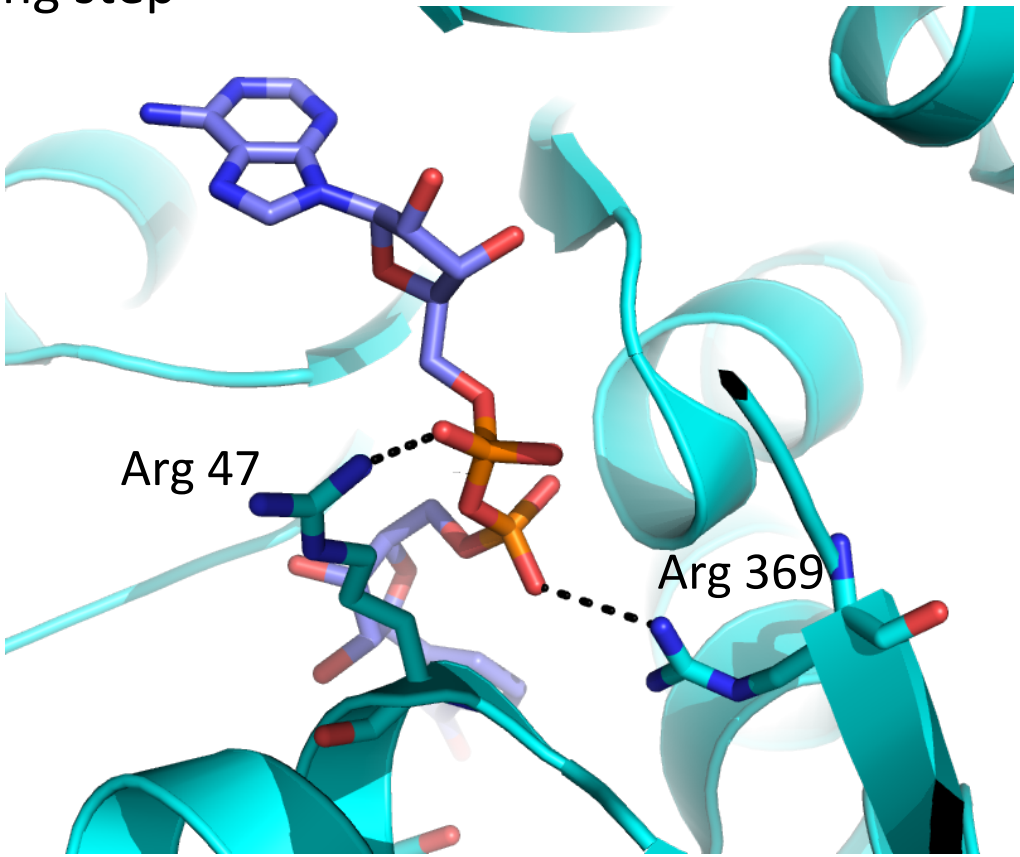
Effect of pH on enzyme catalysis

Example of pH dependence due to binding

- Alcohol dehydrogenase (ADH) has 3 isoforms with different pH optima
- NADH release is the rate-limiting step

ADH isoform	pH optima	Res 47	Res 369
β1	10	Arg	Arg
β2	8.5	His	Arg
β2	7.0	Arg	Cys

Explain the basis for this pH dependence (Clinical Correlation 10.6)



Summary of Enzyme Catalysis

- The function of enzymes is intimately linked to their structure
  - Specificity for substrate, cofactors (induced changes in structure)
  - Stabilization of the transition state, which defines enzyme catalysis
- The pH dependence of catalysis derives from interactions of titratable groups formed in the rate-limiting steps of the reaction mechanism, whatever that step may be (bond making/breaking; product release; substrate binding, etc).