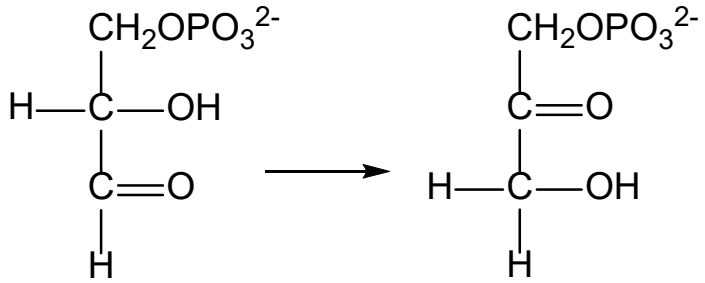
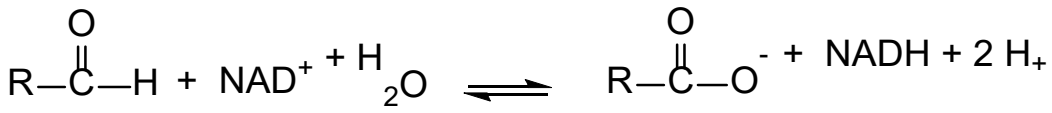
Features of enzymes

1. High reaction rates: 10^6 – 10^{12} x or >) acceleration
2. Mild reaction conditions- water, low temp., low pressure, neutral pH
3. Reaction specificity
   - substrate (reactant), product
4. Capacity for regulation
   - substrates, products, metabolites, other proteins
   - allosteric control
   - covalent modification
   - enzyme synthesis

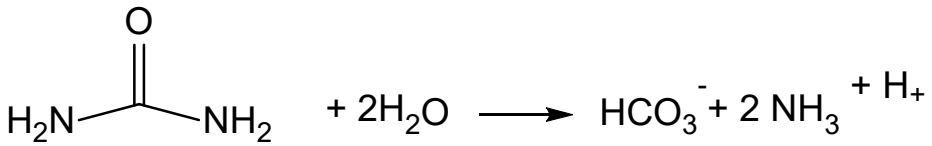
Rate acceleration vs. noncatalyzed rate



~10^9



~10^{10}



~10^{14}

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

~10^7

Carbonic anhydrase

- One of simplest reactions catalyzed in nature
- Occurs nonenzymatically as well
- Why is CA catalysis so important?

Specificity

How do enzymes function

- Bring substrates together in correct orientation for catalysis
- Stabilize transition state of the reaction
- Facilitate bond breakage/formation
- Change the reaction mechanism (not always)
- Add intermediates to the reaction pathway

Binding energy, acid/base catalysis, charge stabilization, covalent catalysis, cofactor involvement

Strategies for enzymatic rate enhancement

1. Proximity/orientation
2. Weak interactions with substrate- “binding energy”
3. Structural flexibility
4. Transition state stabilization
5. General acid/base catalysis
6. Electrostatic interactions
7. Covalent catalysis

General principles of enzyme catalysis

1. Proximity- Enzymes can accelerate a rate simply by holding the two reactants close together in appropriate orientation

   - Intermolecular vs. intramolecular reactions

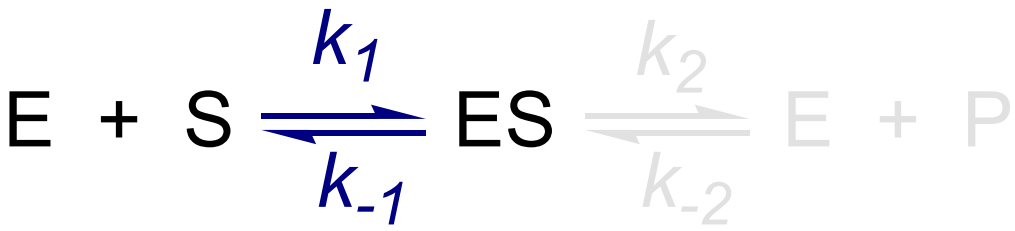
   - Translational, rotational and vibrational degrees of freedom

   - Enzymes are “entropy traps”

Intermolecular vs. intramolecular reactions

Formation of the ES complex: thermodynamic considerations

$$\Delta G = \Delta H - T\Delta S$$



-proximity/orientation: decreases entropy

-noncovalent interactions of E and S: favorable enthalpy change

Binding energy: the energy derived from (noncovalent) enzyme-substrate interactions. The source of free energy to lower A_E of the reaction

Bimolecular reactions: spatial freedom, entropy, and enthalpy

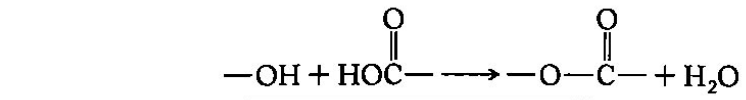
What are units of k_{cat} for a unimolecular, bimolecular, or trimolecular reaction?

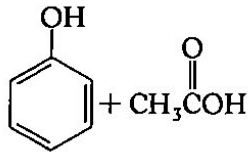
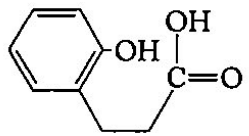
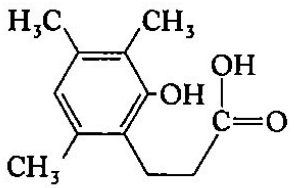
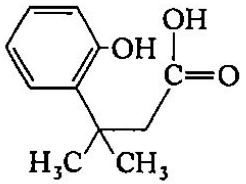
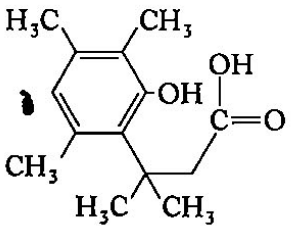
Enzymes as entropy traps: reactions become unimolecular upon binding of substrates

Effective molarity

The hypothetical concentration of one of the reactants of an intermolecular reaction that gives the same rate of reaction as the intramolecular reaction

Table 9.3 *Relative Rates of the Esterification Reaction*

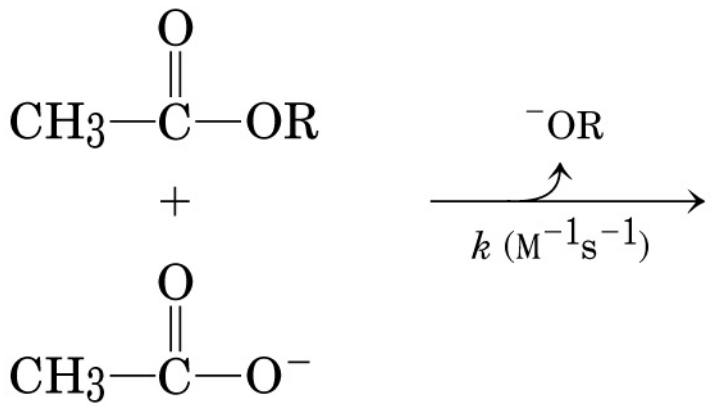


Reactants	Rate constant	Effective concentration of intramolecular groups
	$10^{-10}\ s^{-1}M^{-1}$	—
	$3.2 \times 10^{-6}\ s^{-1}$	$3.2 \times 10^{4}\ M$
	$3.3 \times 10^{-6}\ s^{-1}$	$3.3 \times 10^{4}\ M$
	$3.6 \times 10^{-5}\ s^{-1}$	$3.6 \times 10^{5}\ M$
	$8.5 \times 10^{-2}\ s^{-1}$	$8.5 \times 10^{8}\ M$

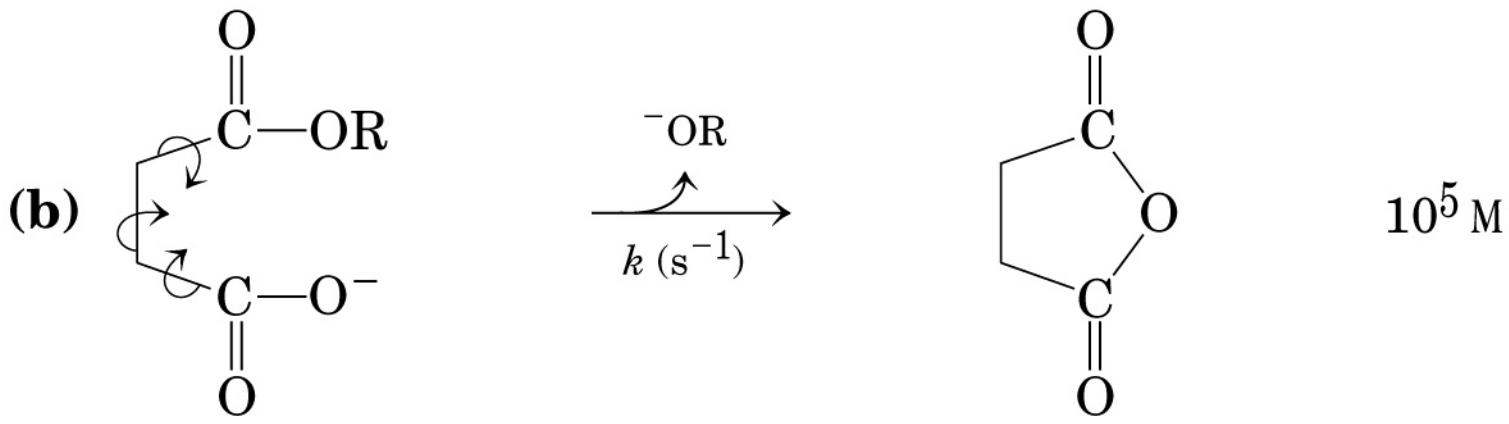
Rate constants for the uncatalyzed reactions in water from S. Milstien and L. A. Cohen, *Proc. Natl. Acad. Sci. USA* 67:1143–1147 (1970).

Figure from Chreiton, Ch. 9

Proximity and orientation

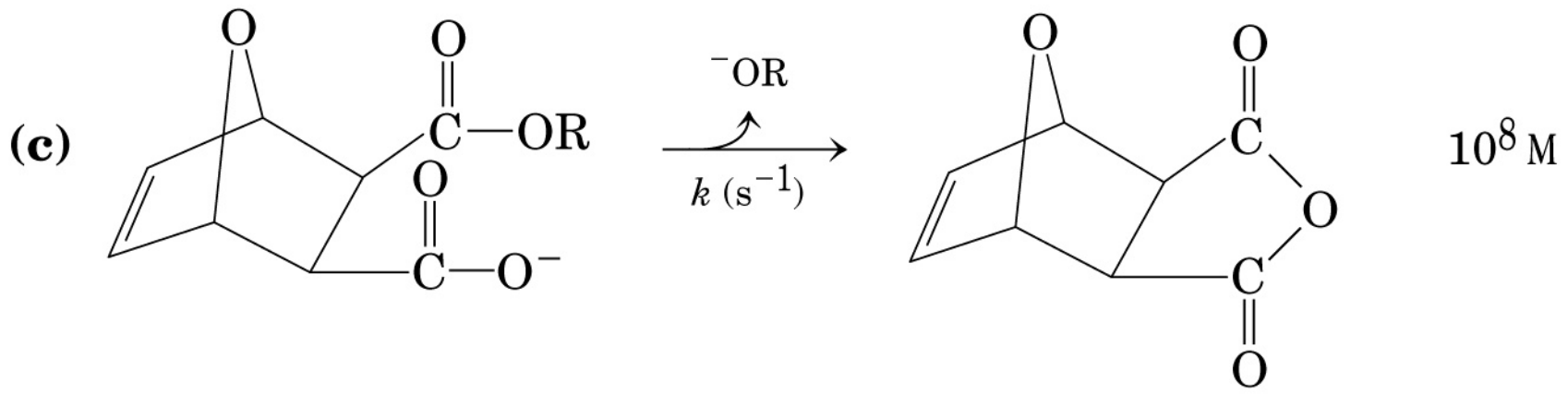
	Reaction	Rate enhancement
(a)		1

Proximity and orientation



S. Ensign principles of enzymatic catalysis

Proximity and orientation



Structural flexibility/induced fit

- e.g. hexokinase

Transition state theory

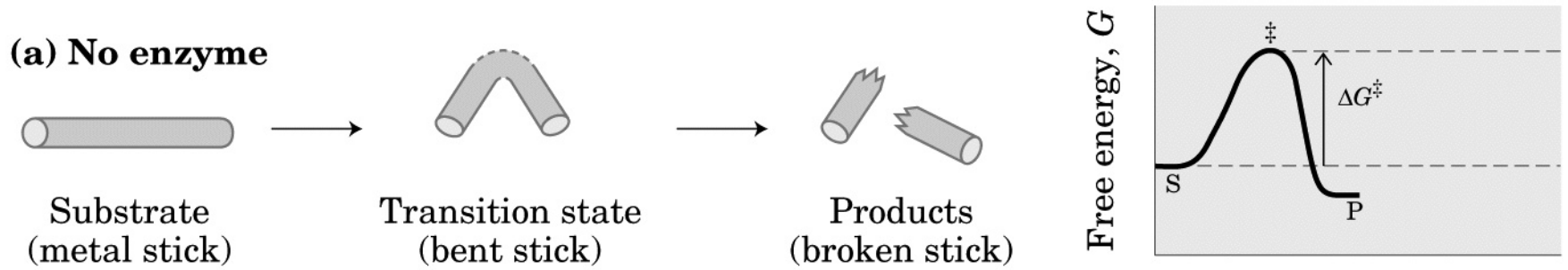


Figure from Lehninger Principles of Biochemistry

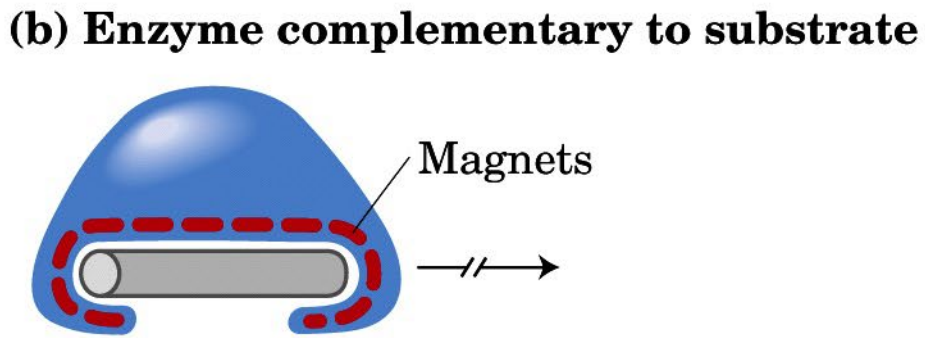
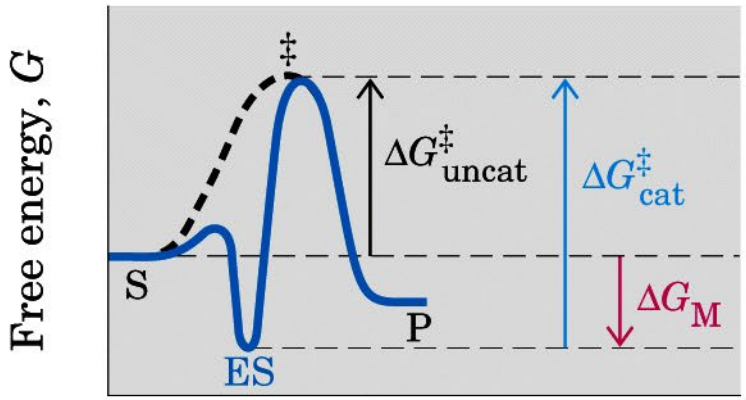
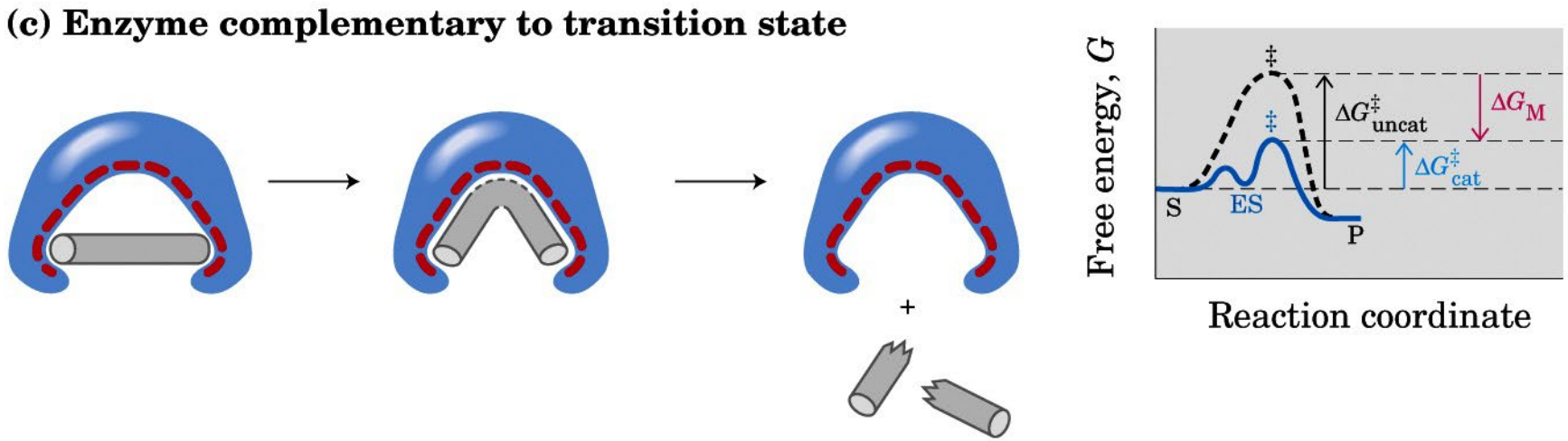


Figure from Lehninger Principles of Biochemistry



The enzyme active state should be more complementary to the structure of the transition state than to the structure of the substrate (or product)



Don’t underestimate the importance of the weak interactions between enzyme and substrate!
(Jencks, the Circe Effect)

The rate acceleration for some enzymes is derived solely from binding energy

Active site residues and groups

- Weak interactions to stabilize/destabilize substrate, intermediates, transition state, product (enthalpic role)
- Functional groups involved in the reaction chemistry (catalytic role): transition state stabilization, changing reaction pathway

Roles of functional groups at the active site

Strategy: induce electron density changes in substrates/intermediates/transition states to facilitate bond cleavage and formation

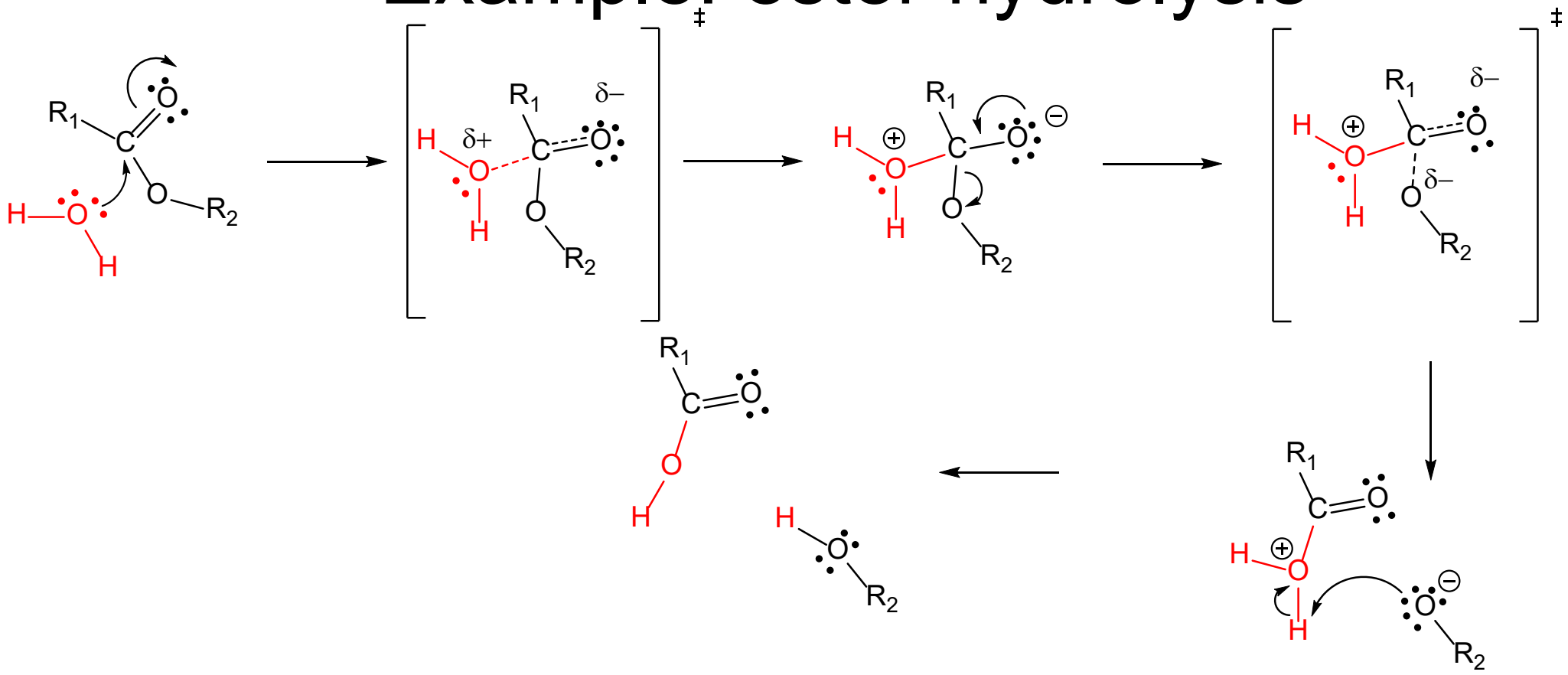
- general acids/bases
- electrostatic interactions
- covalent adducts

Roles of functional groups at the active site

Strategy: induce electron density changes in substrates/intermediates/transition states to facilitate bond cleavage and formation

- general acids/bases
- electrostatic interactions
- covalent adducts

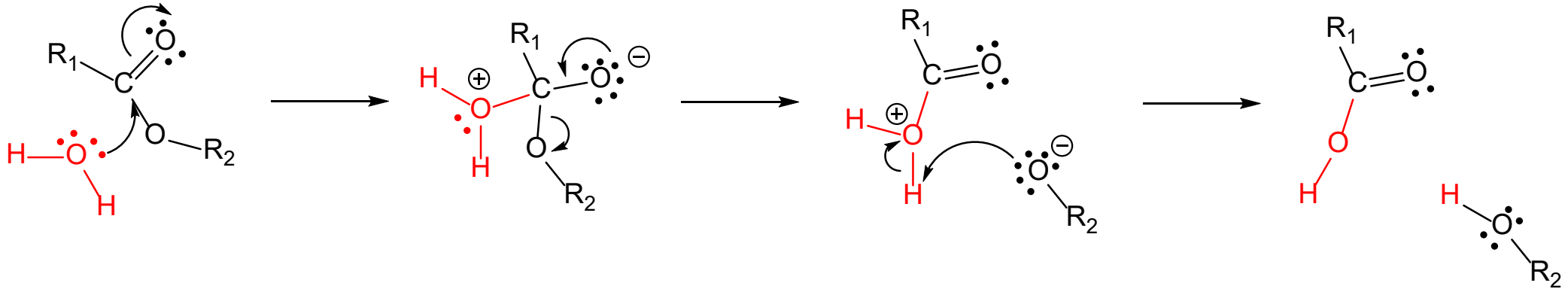
Example: ester hydrolysis



Note: showing the lone pairs of electrons on reactants, products, and intermediates is optional when drawing mechanisms

Transition state (TS) vs. intermediate (I)

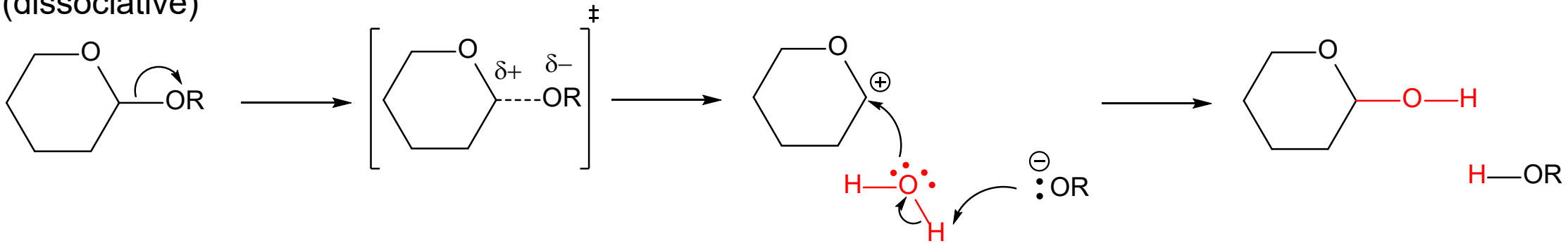
- TS: dashed lines showing bonds forming/breaking
- Intermediate: Solid lines showing bonds resulting from the step involving the TS
- When drawing a mechanism, it is common not to show the transitions states
- The electron pushing in a step represents the transition state involved in that step



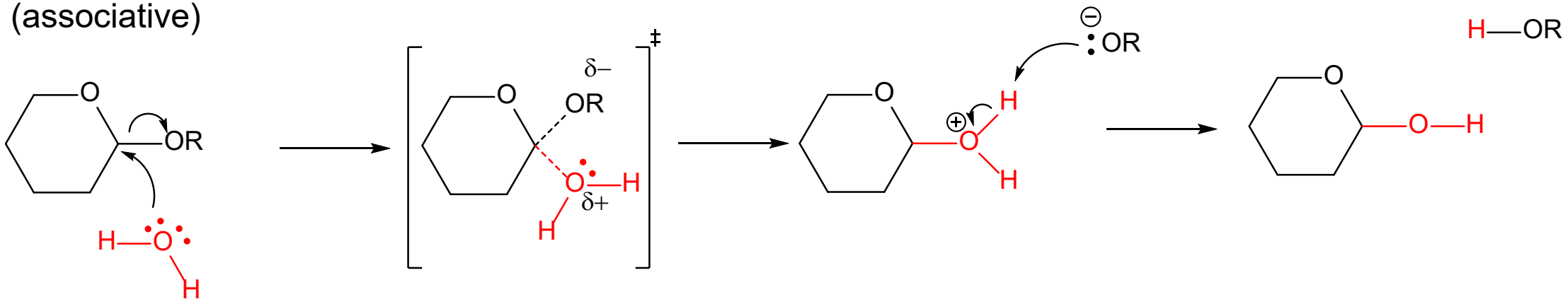
Note: showing the lone pairs of electrons on reactants, products, and intermediates is optional when drawing mechanisms

Transition state (TS) vs. intermediate (I)

S_N1(dissociative)



S_N2 (associative)



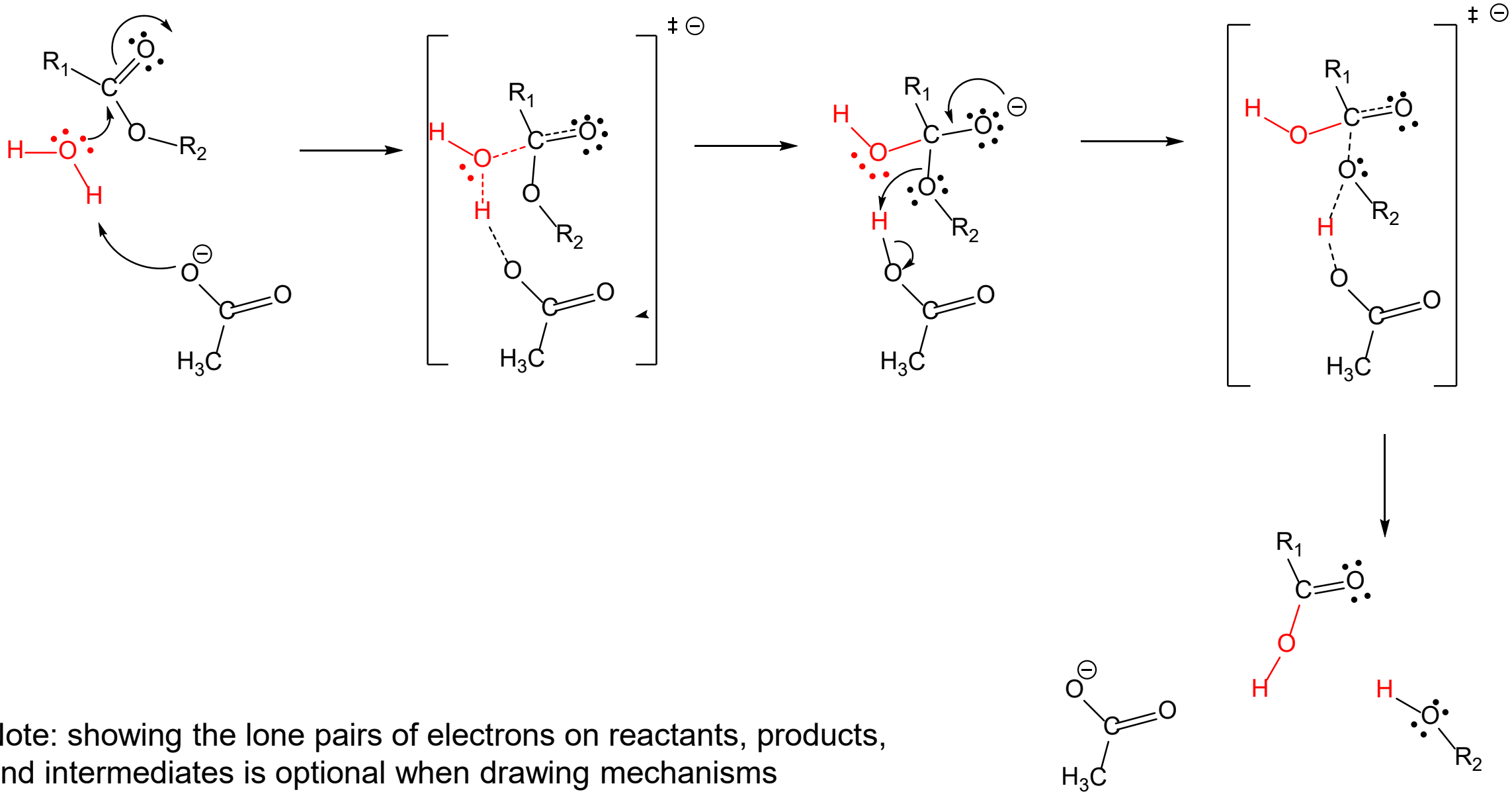
Functional groups: general acid/base catalysis

- make a potentially reactive group more reactive by increasing its intrinsic electrophilic or nucleophilic character via proton addition or removal

- General acid: A molecule other than water that serves as a proton donor

- General base: A molecule other than water that serves as a proton acceptor

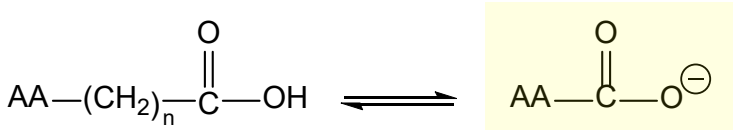
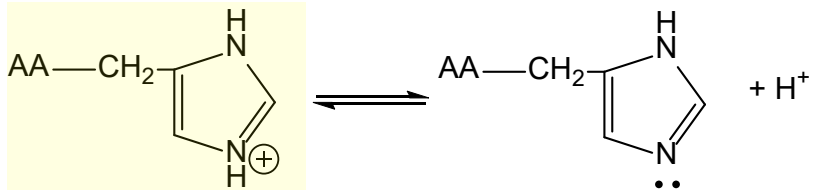
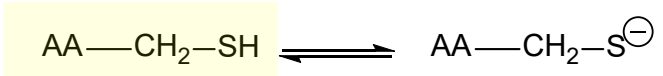
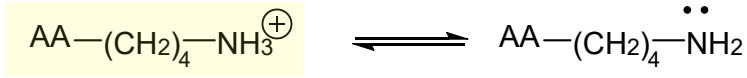
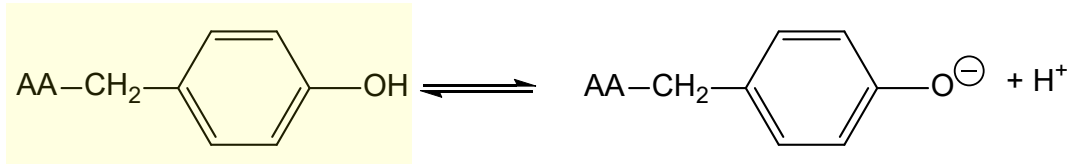
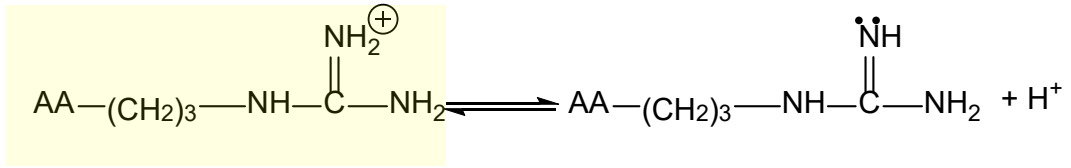
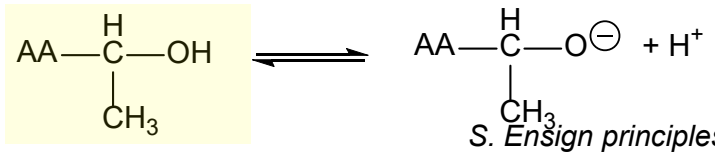
Acid-base Example: ester hydrolysis



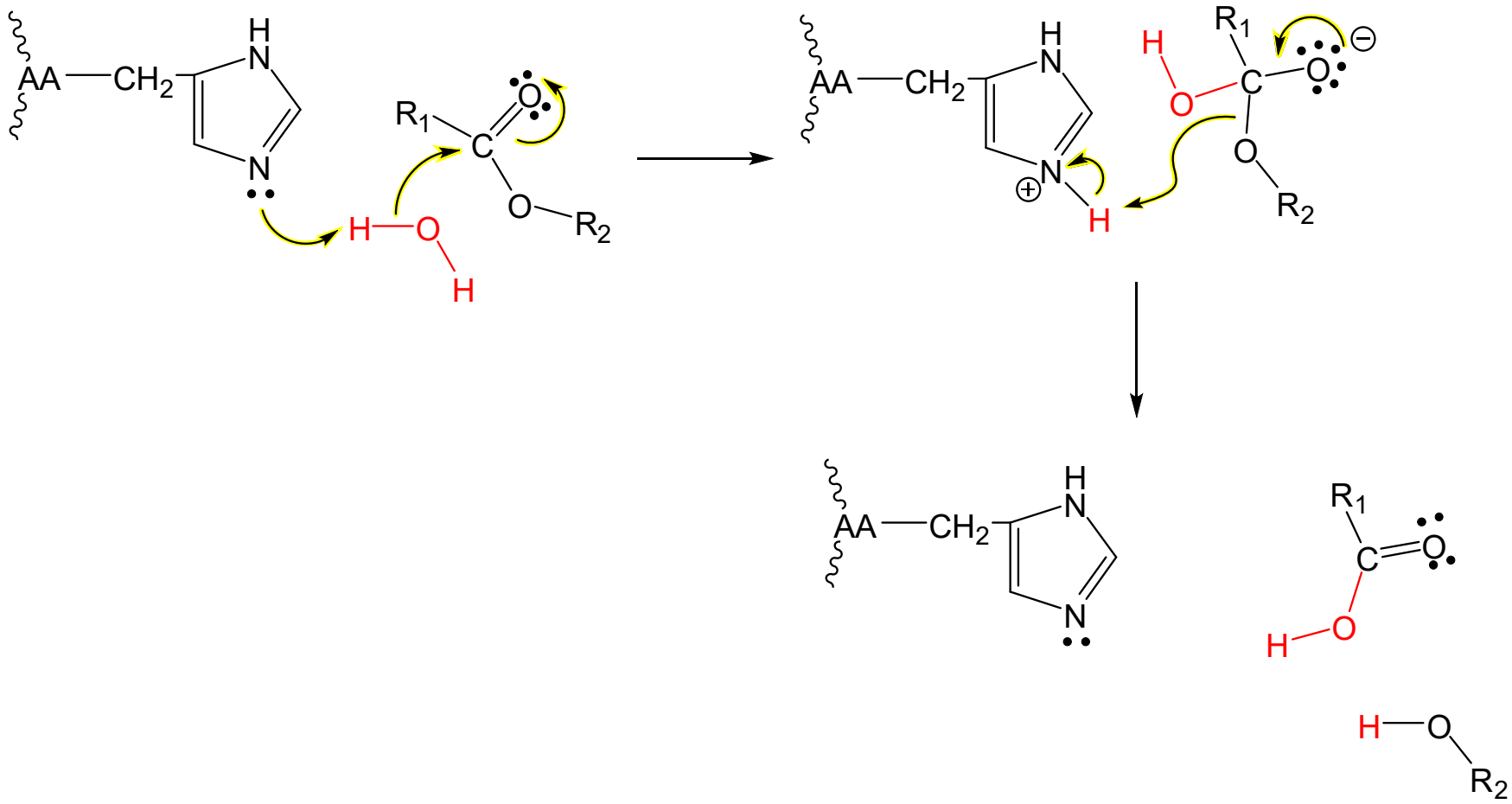
Note: showing the lone pairs of electrons on reactants, products, and intermediates is optional when drawing mechanisms

Amino acid residues with side chains that have acid/base properties

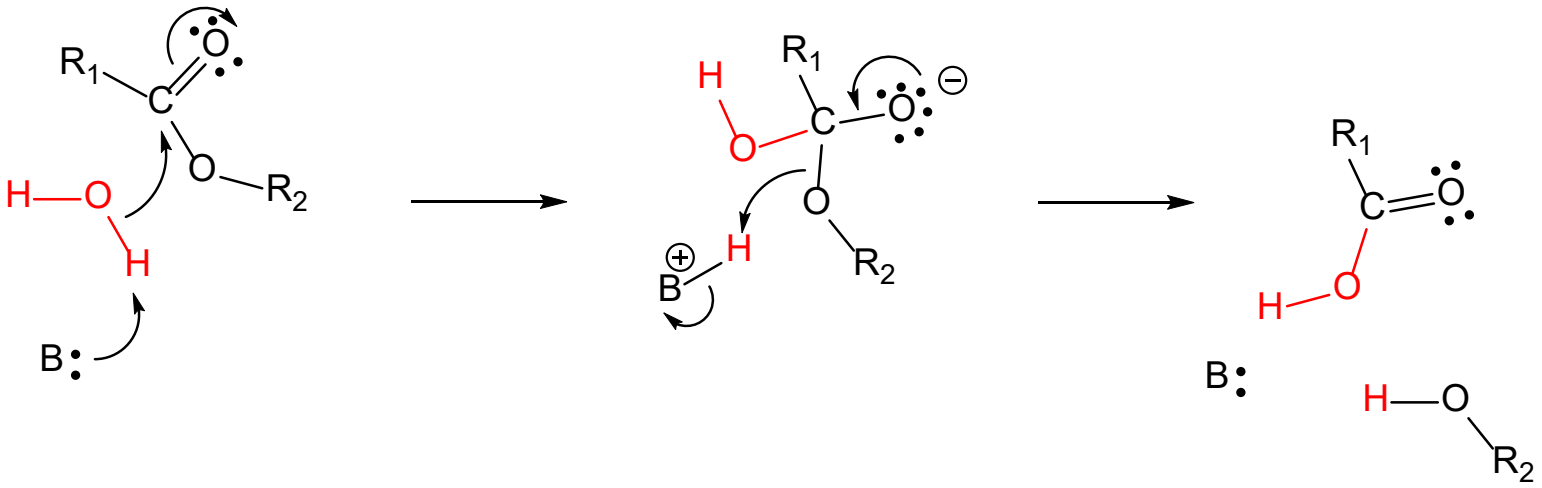
Which amino acid is best suited to serve as a general acid/base at physiological pH values?

pK_a free AA	
Asp 3.65, Glu 4.25	
6.0	
8.5	
10.5	
10.5	
10.8	
13	
13	

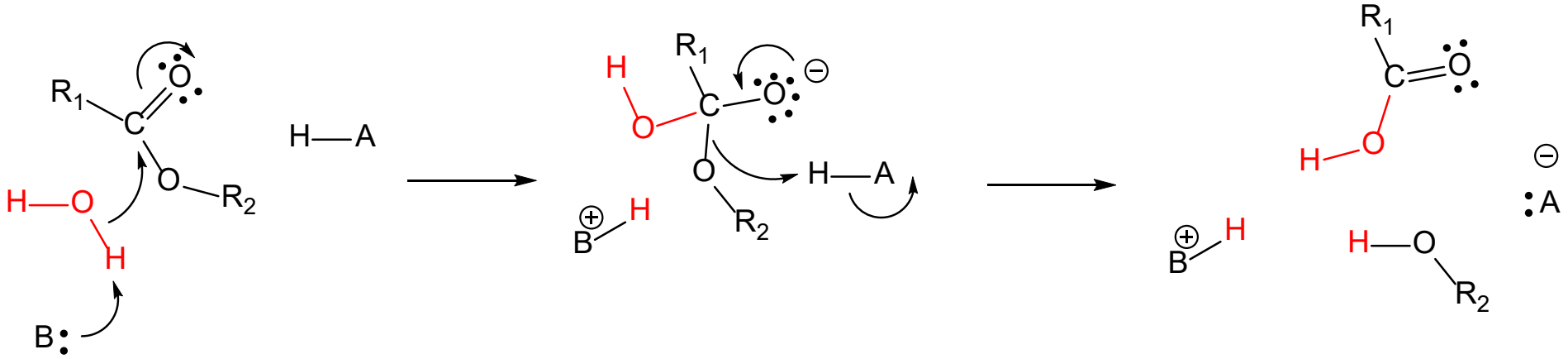
Histidine serving as both a general acid and base (one base mechanism)



Abbreviating general acids and bases when drawing reaction mechanisms (one base mechanism)



Abbreviating general acids and bases when drawing reaction mechanisms: two base mechanism

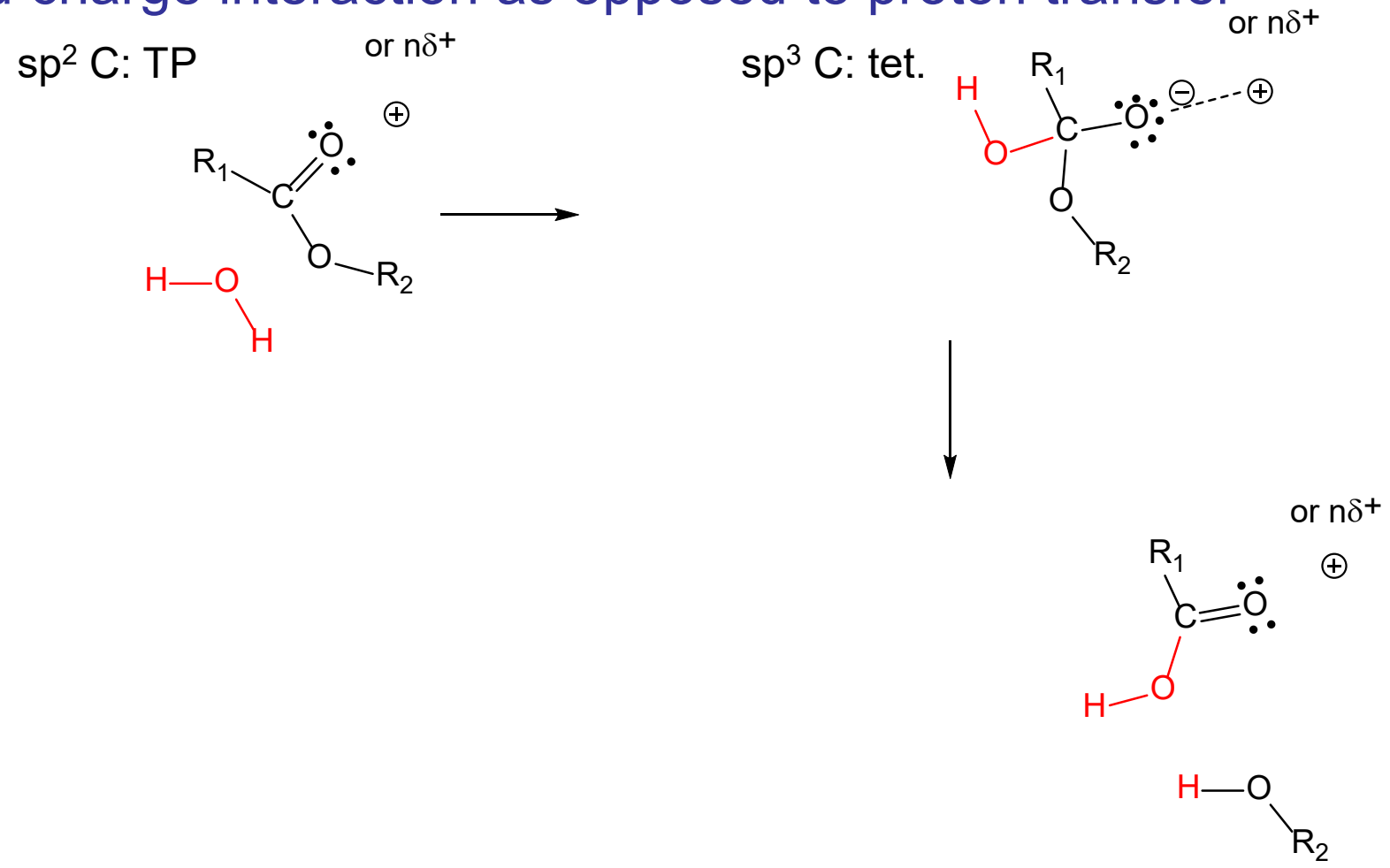


Examples we will look at in more detail where histidine functions as a general acid/base:

- Serine protease (hydrolase)
- Ribonuclease (phosphodiesterase)

Functional groups: electrostatic interactions

- Stabilize the distribution of electrical charge in transitions state(s) or high energy intermediate(s)
- A fixed charge interaction as opposed to proton transfer



Electrostatic interactions: Metal ion catalysis

- 1/3 of enzymes require a metal
- Roles:
  - Bind and orient substrate(s)
  - Mediate oxidation-reduction reactions
  - Electrostatically stabilize or shield negative charges

Charge stabilization vs. charge shielding

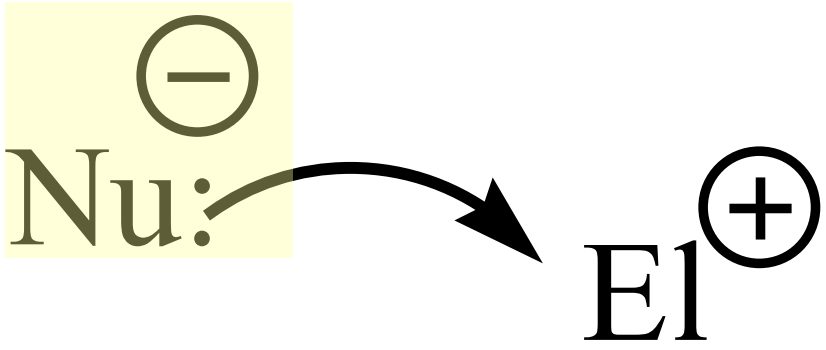
Metals can lower the pK_a of water

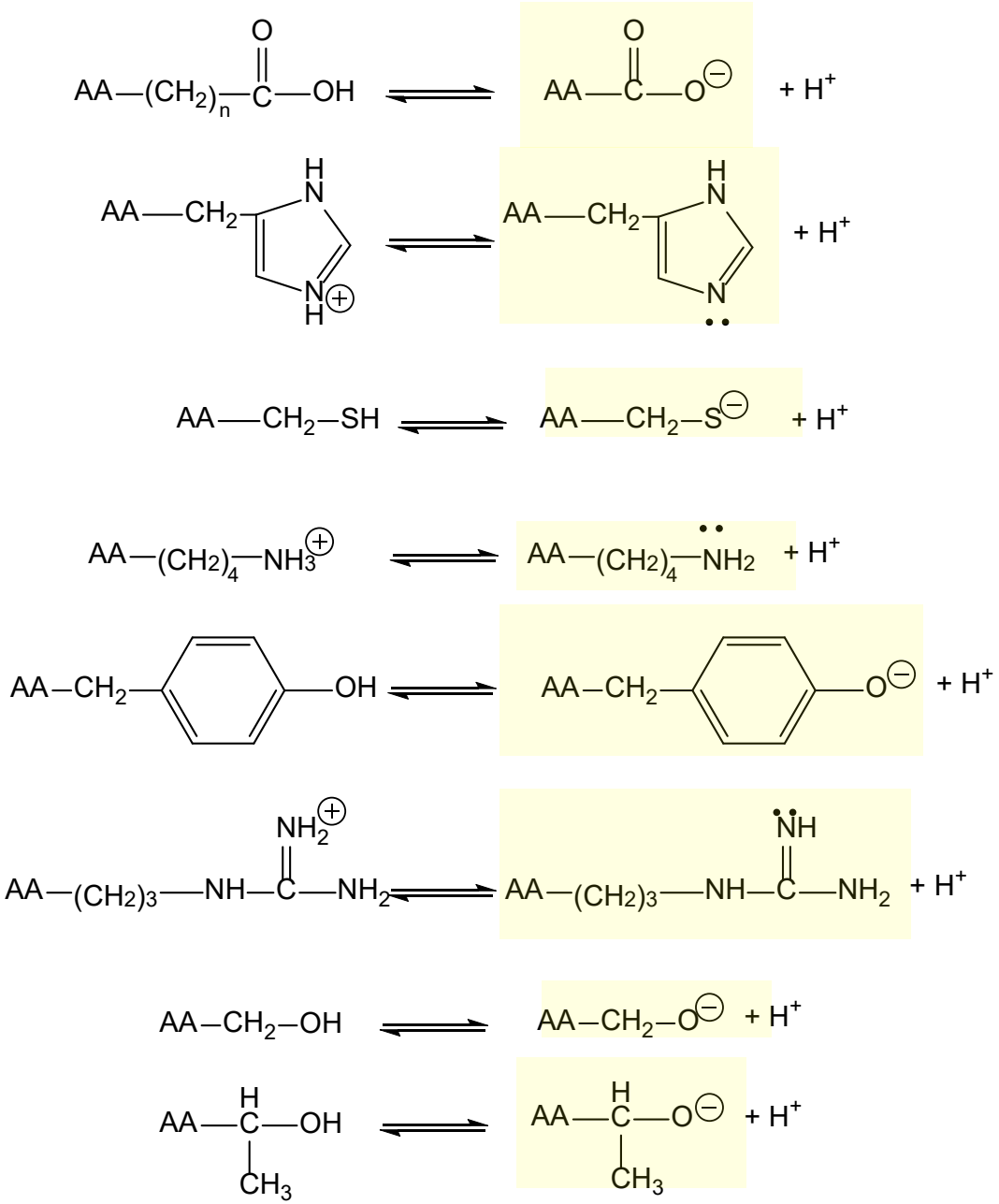
(Carbonic anhydrase mechanism)

Functional groups: Covalent catalysis

- Rate enhancement through the transient formation of enzyme-substrate covalent bond(s)
- Changes the reaction pathway to speed up the reaction (adds intermediate(s))
  - Increase substrate nucleophilicity
  - Increase substrate electrophilicity

Potential nucleophiles in enzymatic catalysis



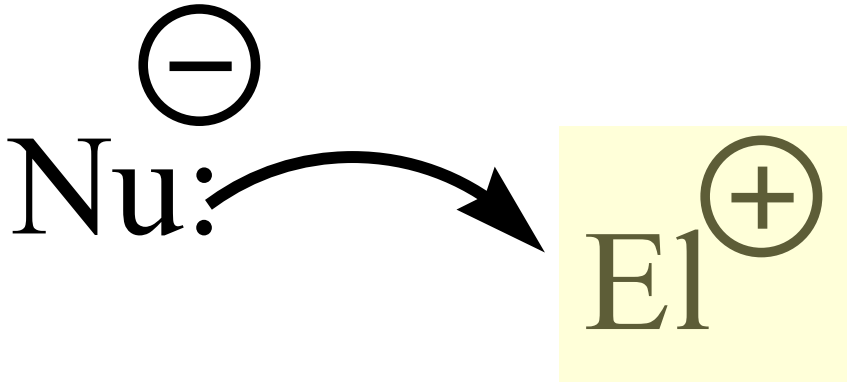


S. Ensign principles of enzymatic catalysis

Classic examples of covalent catalysis we will look at in more detail later (time permitting)

- Serine protease (hydrolase)
- Aldolase
- Glyceraldehyde-3-phosphate dehydrogenase

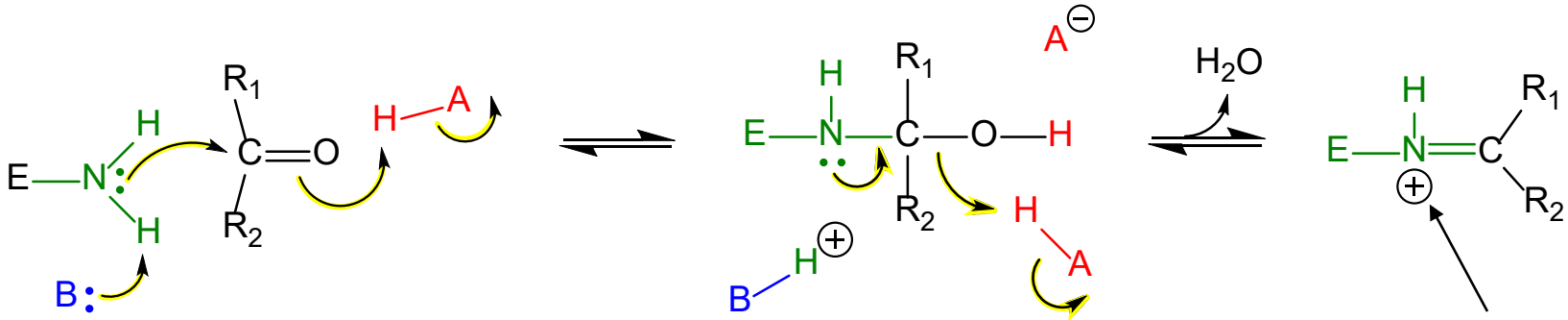
Potential electrophiles in enzymatic catalysis?



- No natural unmodified amino acids are good electrophiles
- A schiff base is a good electrophile and can be formed from lysine
- Some coenzymes are used as electrophiles (thiamine pyrophosphate, pyridoxal phosphate)
- Metal ions, as Lewis acids, can be good electrophiles

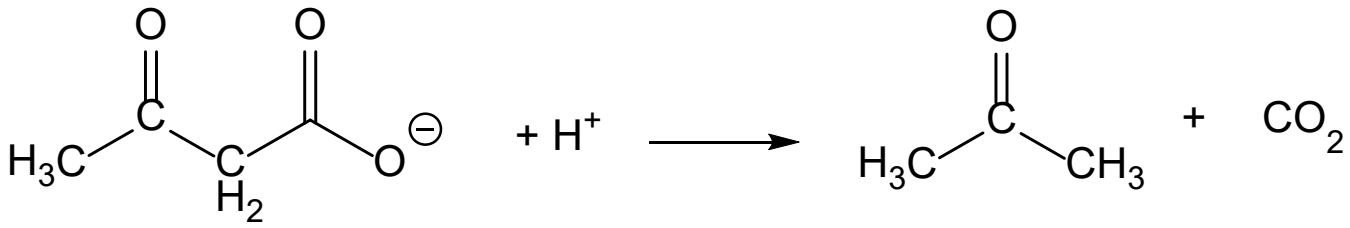
Schiff Base formation

- Note importance of general acid/base catalysis



An electron sink that reduces the high energy enolate character of intermediates

Example: Schiff Base formation in acetoacetate decarboxylase

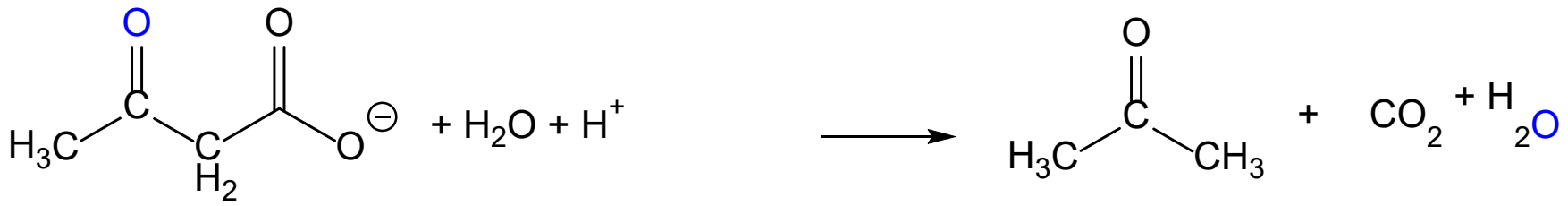


- Nonenzymatic mechanism for decarboxylation (slow):
- Resonance stabilization of the anion:
- Thermodynamic barrier for nonenzymatic decarboxylation?
- pK_a acetone: 19.3

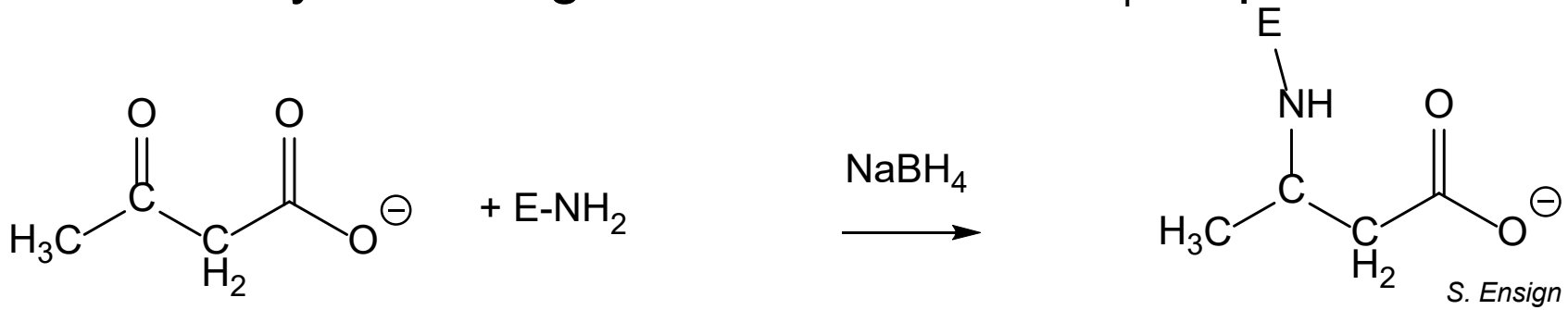
Experimental observations, acetoacetate decarboxylase

- Carry out reaction in ^{18}O-labelled H_2O: acetone product has ^{18}O in carbonyl O

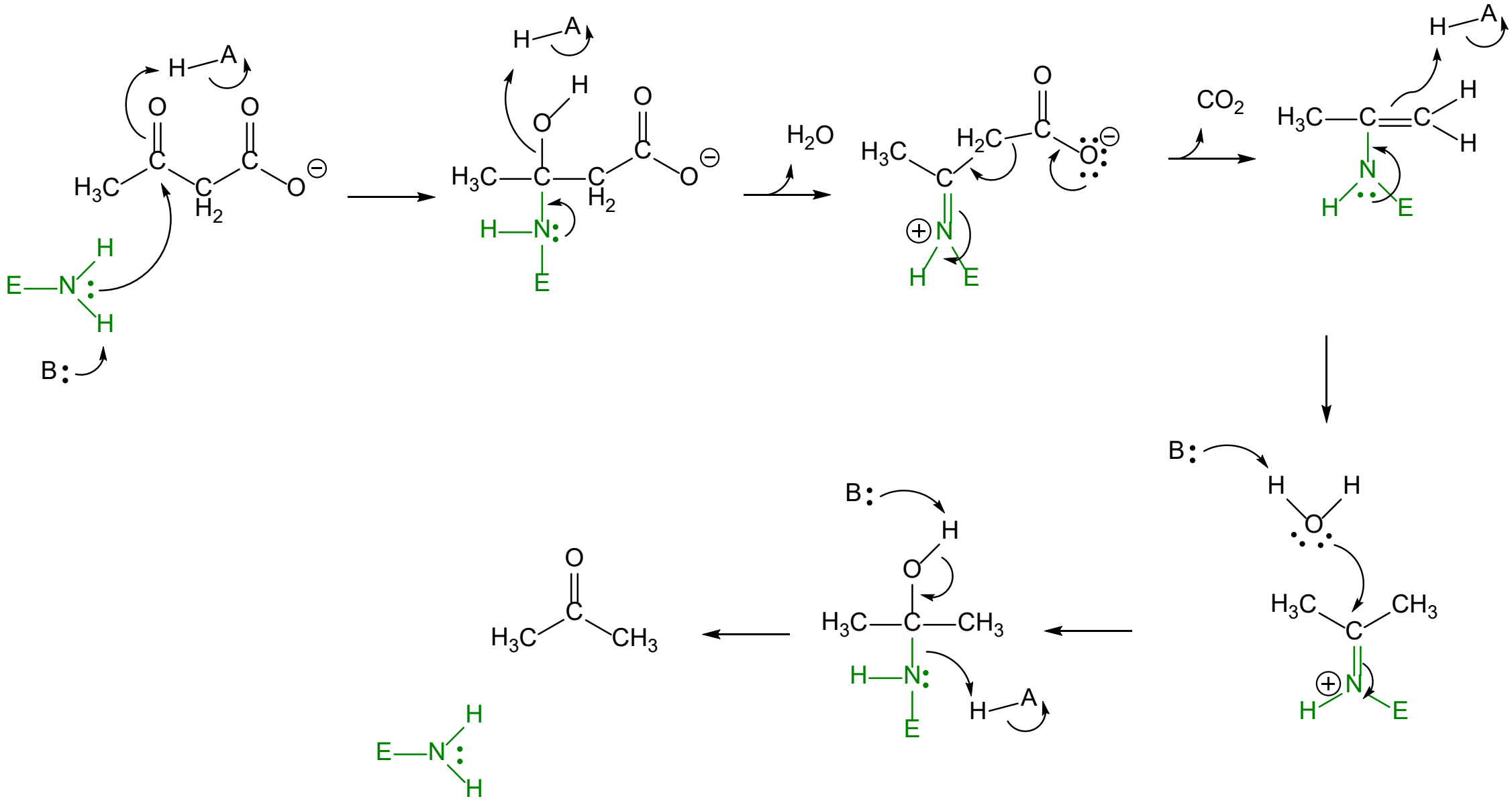
- Carry out reaction with acetoacetate labelled with ^{18}O in carbonyl O: no ^{18}O in acetone product



- Reduce enzyme during turnover with $NaBH_4$: trap a covalent adduct w/K115



Mechanism for acetoacetate decarboxylase



Roles of functional groups at the active site

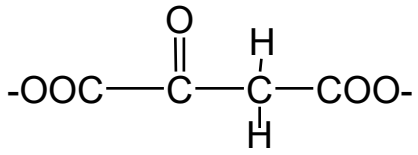
Strategy: induce electron density changes in substrates/intermediates/transition states to facilitate bond cleavage and formation

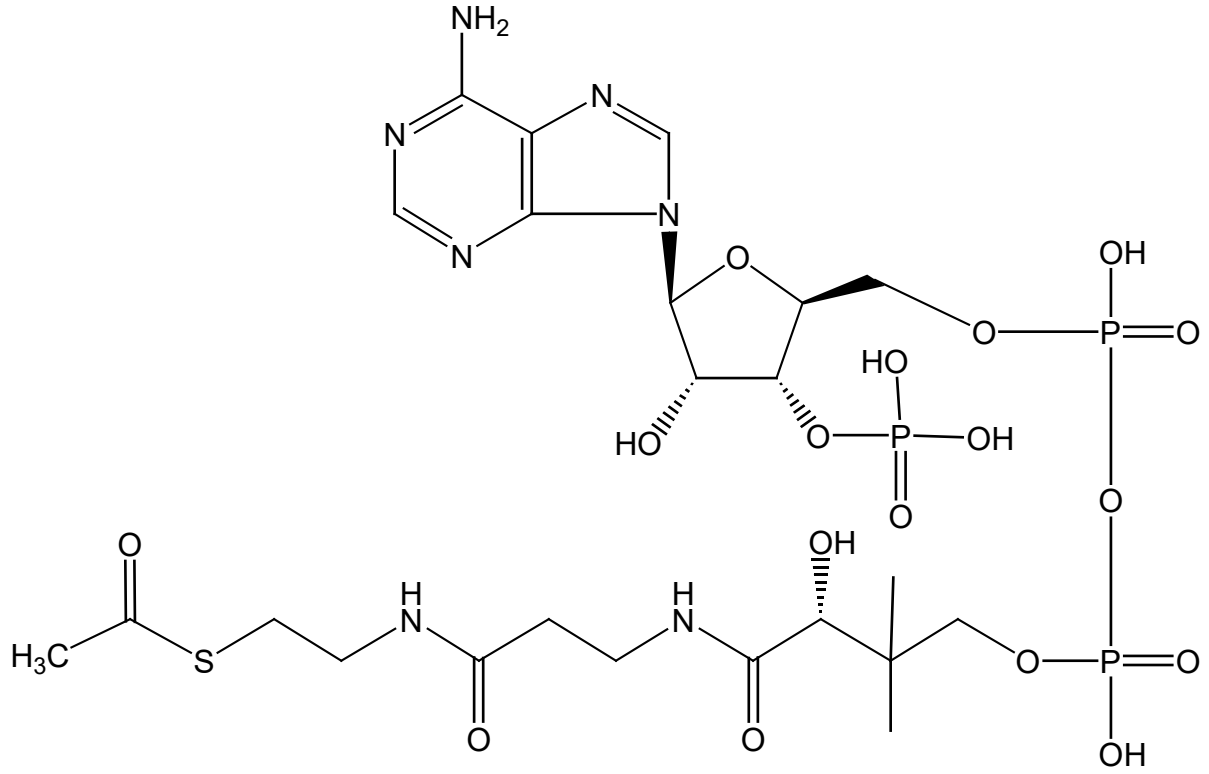
- general acids/bases
- electrostatic interactions
- covalent adducts

Case studies that highlight principles of rate acceleration

- Tyrosyl-tRNA synthetase: all rate acceleration comes from binding energy!
- Serine proteases (e.g. chymotrypsin)
  - Binding energy, general acid/base catalysis, covalent catalysis, transition state stabilization
- Ribonuclease A
- Enzymes of glycolysis and the citric acid cycle (examples to be chosen based on lecture time remaining)

Why are enzymes big? Consider citrate synthase





Transition state theory

- Transition state analogs
- Catalytic antibody design

Why are enzymes big? (cont.)

- Define a specific active site
- Substrate channels
- Subunit interactions
- Regulatory control
- Membrane-bound?
- In vivo interactions (sequential reactions, multiprotein complexes)