L5 +6 Enzyme functional nature: Mechanism continued

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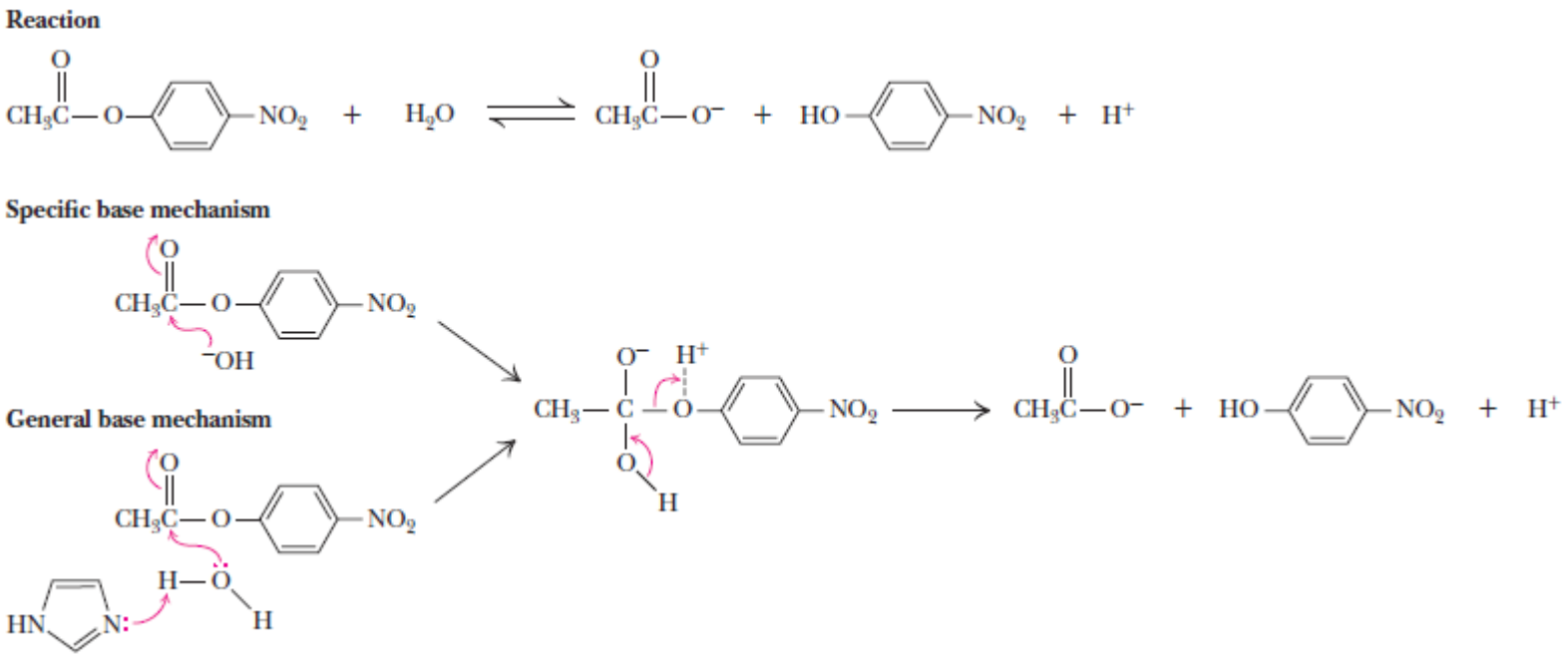
Acid-Base Catalysis occurs by Proton Transfer

General acid catalysis: Proton transfer from an acid lowers the free energy of a reaction's transition state

- Specific base catalysis
- General base catalysis



The enzyme α-glucosidase (EC 3.2.1.20) catalyzes the hydrolysis of maltose into glucose. α -glucosidase is competitively inhibited by the product glucose and inhibited at high maltose concentrations in a partial uncompetitive mode. Determine a kinetic rate expression in terms of the dissociation constants for: the secondary enzyme–substrate complex (K_M), the secondary enzyme–product complex (K_P), the tertiary enzyme–substrate–substrate complex (K_0), and the maximum reaction rates of product formation from the enzyme–substrate active complex (V_{max}) and the enzyme–substrate–substrate partially active complex (V^0_{max}). The molar concentrations of maltose and glucose are [M] and [G], respectively.

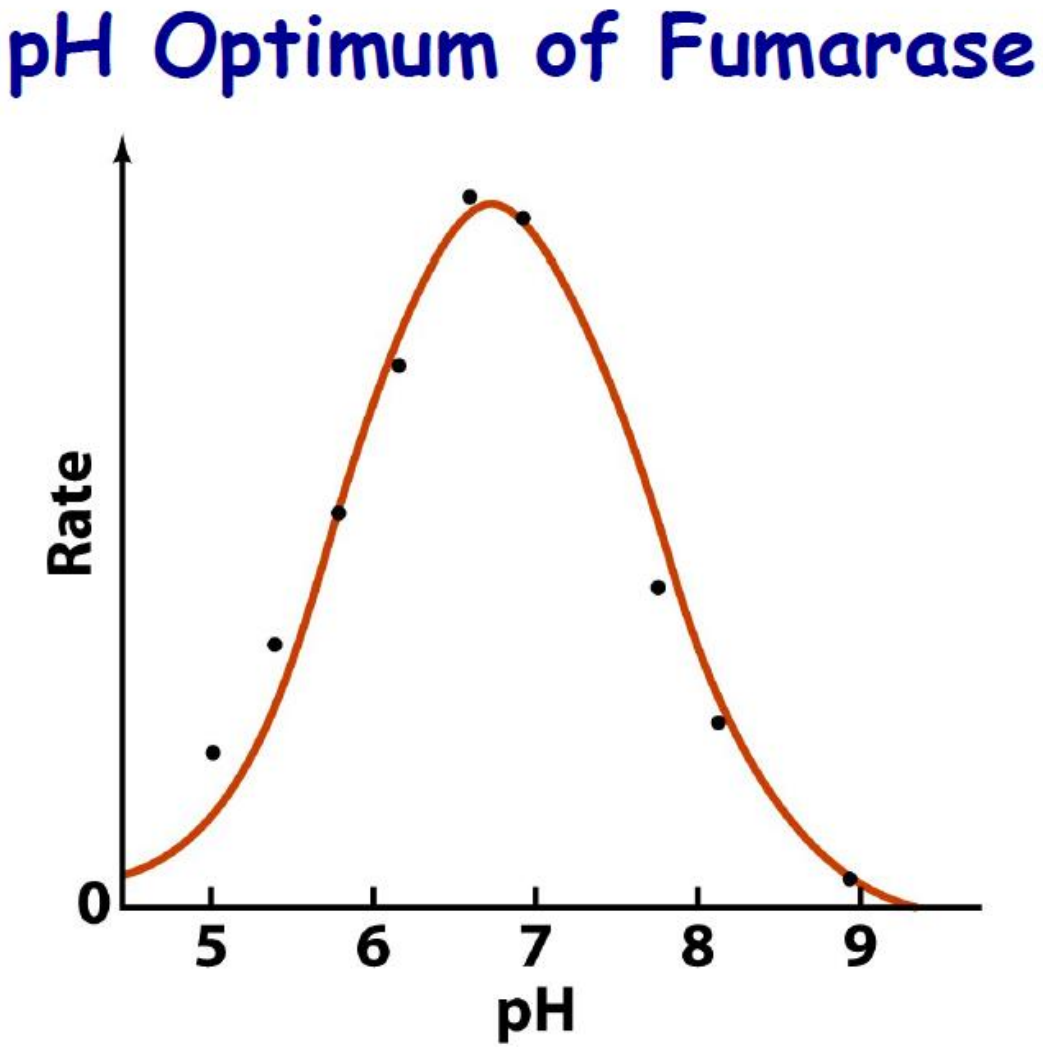
Effects of pH on Enzyme Activity

Most enzymes are active only within a narrow pH range of 5-9.

Reaction rates exhibit bell-shaped curves in dependence of pH (reflects ionization state of important residues)

pH optimum gives information about catalytically important residues, if 4/5 -> Glu, Asp; 6->His, 10->Lys

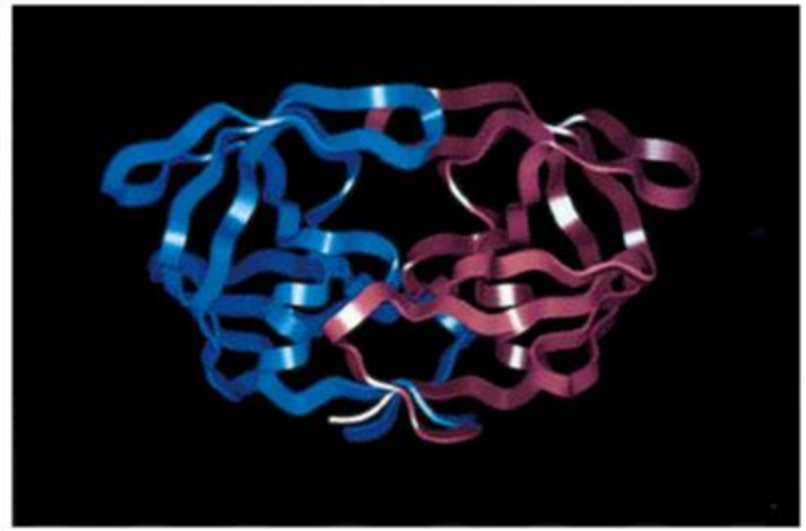
pK of residues can vary depending on chemical environment +/- 2



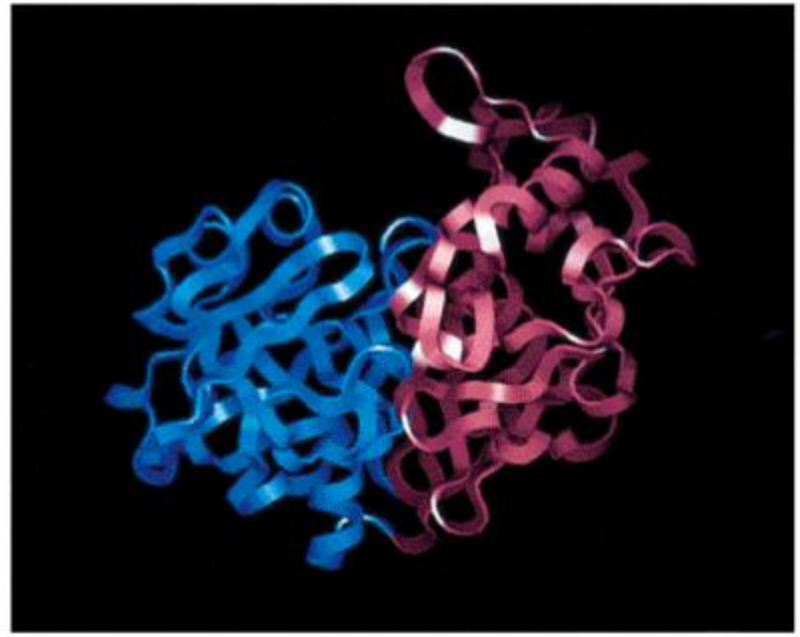
The Aspartic Proteases

Pepsin, chymosin, cathepsin D, renin and HIV-1 protease

- All involve two Asp residues at the active site
- Two Asps work together as general acid-base catalysts
- Most aspartic proteases have a tertiary structure consisting of two lobes (N-terminal and C-terminal) with approximate two-fold symmetry
- HIV-1 protease is a homodimer



(a)

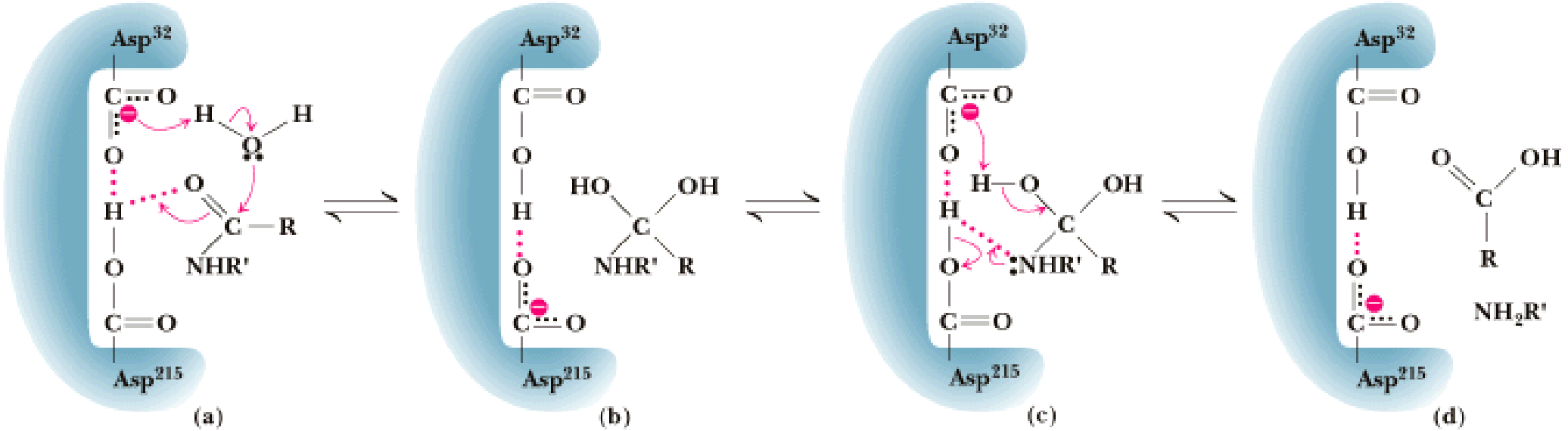


(b)

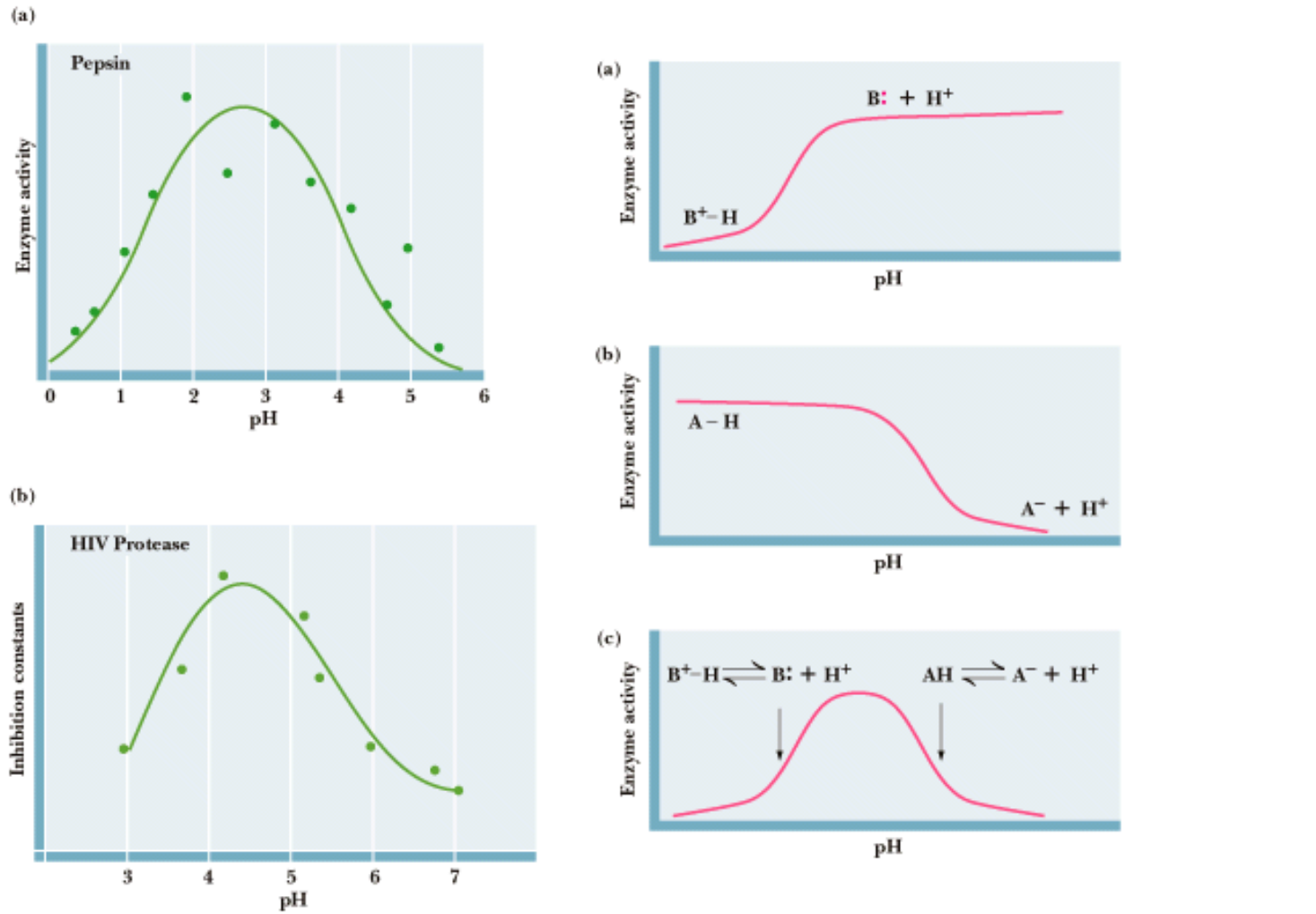
Aspartic Protease Mechanism

The pK_a values of the Asp residues are crucial

- One Asp has a relatively low pK_a, other has a relatively high pK_a
- Deprotonated Asp acts as general base, accepting a proton from HOH, forming OH^- in the transition state
- Other Asp (general acid) donates a proton, facilitating formation of tetrahedral intermediate

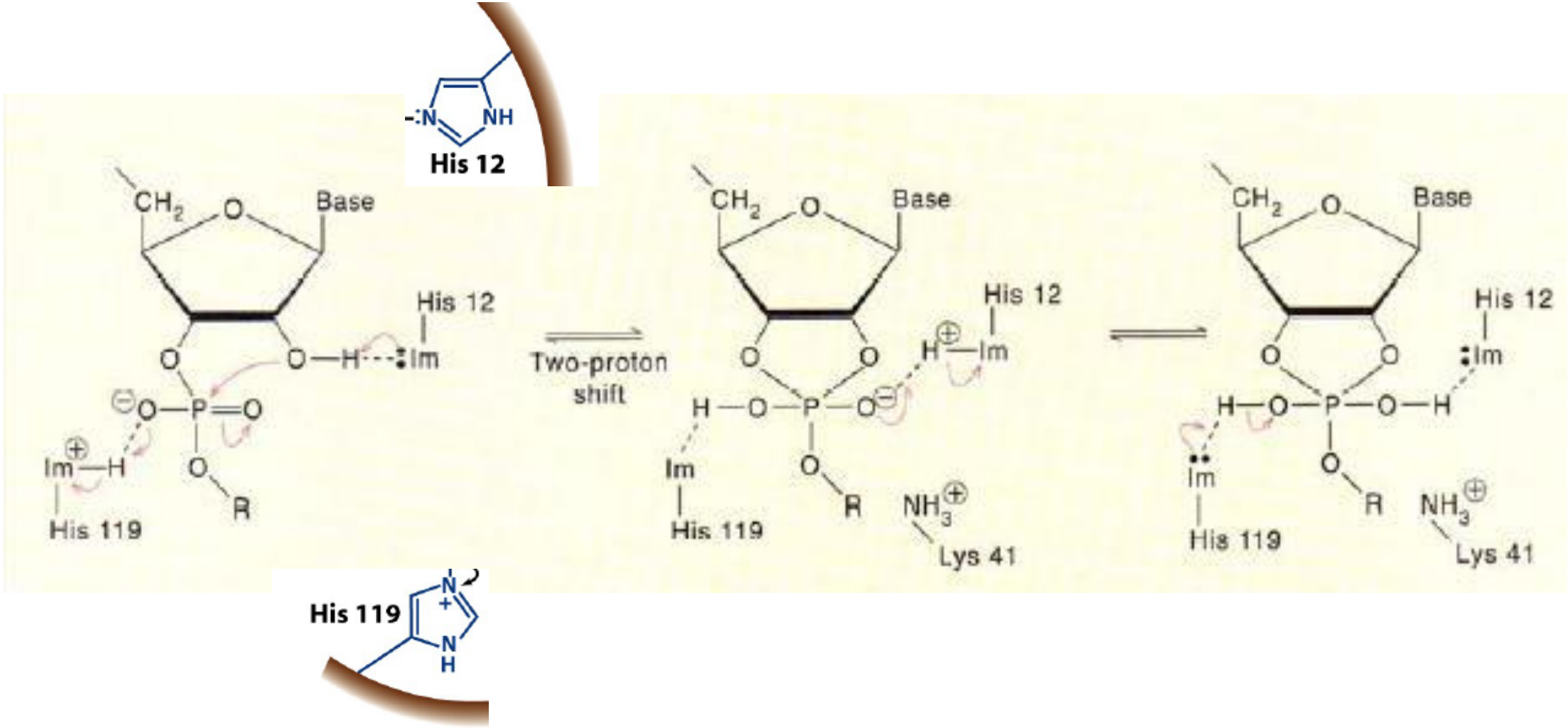

Asp32
Asp215
NHR'
R
HO
OH
NH2R'
(a)
(b)
(c)
(d)

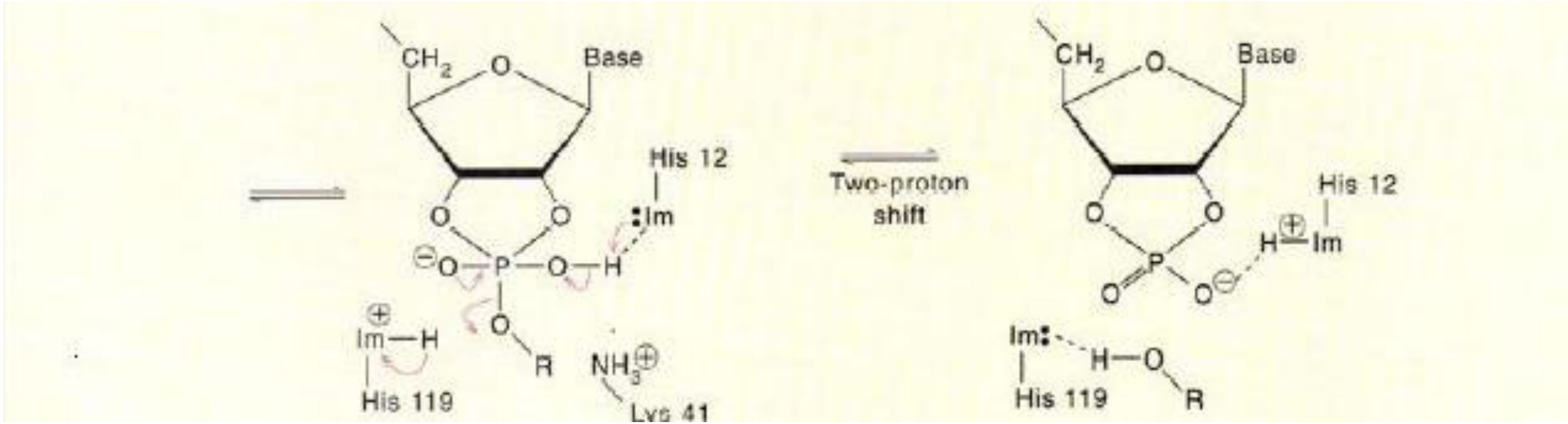
Garrett & Grisham: Biochemistry, 2/e
Unnumbered Figure p.525



RNase A is an acid-base catalyst

Bovine pancreatic RNase A: Digestive enzyme secreted by pancreas into the small intestine
2',3' cyclic nucleotides isolated as intermediates pH-dependence indicates 2 important His, 12, 119
that act in a concerted manner as general acid and base catalysts to catalyze a two-step reaction



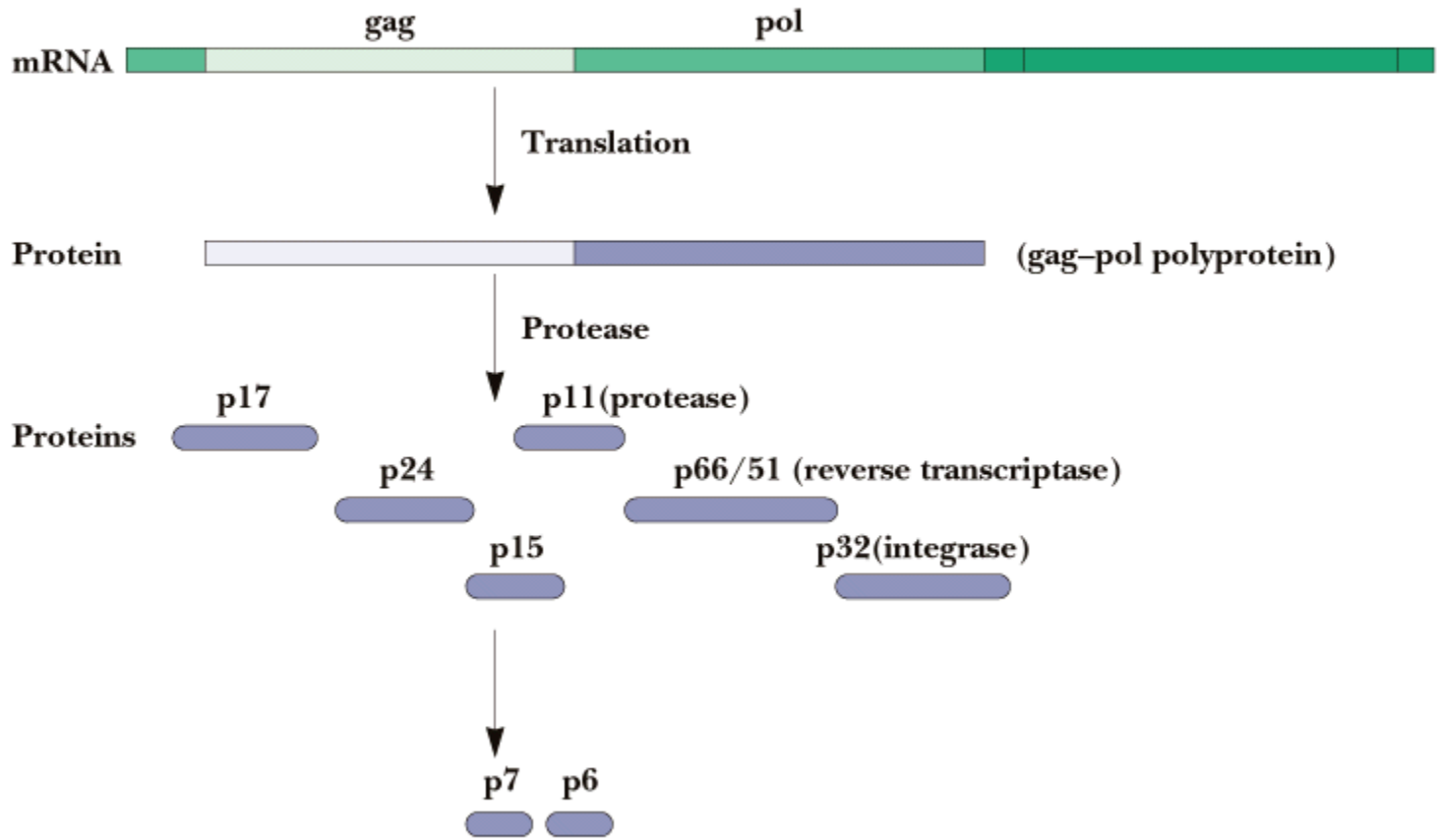

CH2
O
Base
His 12
Im
O
O
O
P
O
H
Im
H
His 119
R
NH3
Lys 41
Two-proton
shift
CH2
O
Base
His 12
Im
H
O
O
P
O
O
Im
H
O
His 119
R

HIV-1 Protease

A novel aspartic protease

- HIV-1 protease cleaves the polyprotein products of the HIV genome
- This is a remarkable imitation of mammalian aspartic proteases
- HIV-1 protease is a homodimer - more genetically economical for the virus
- Active site is two-fold symmetric
- Two Asp residues - one high pK_a, one low pK_a

Garrett & Grisham: Biochemistry, 2/e
Figure 16.28



Therapy for HIV?

Protease inhibitors as AIDS drugs

- If the HIV-1 protease can be selectively inhibited, then new HIV particles cannot form
- Several novel protease inhibitors are currently marketed as AIDS drugs
- Many such inhibitors work in a culture dish
- However, a successful drug must be able to kill the virus in a human subject without blocking other essential proteases in the body

Garrett & Grisham: Biochemistry, 2/e
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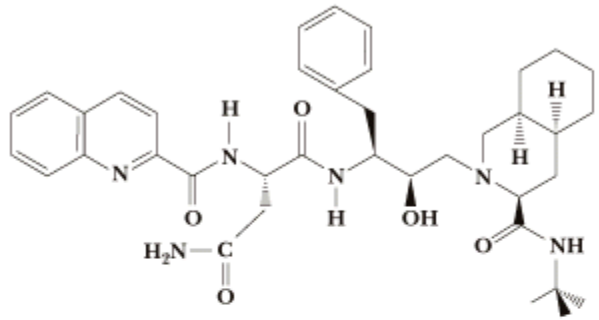
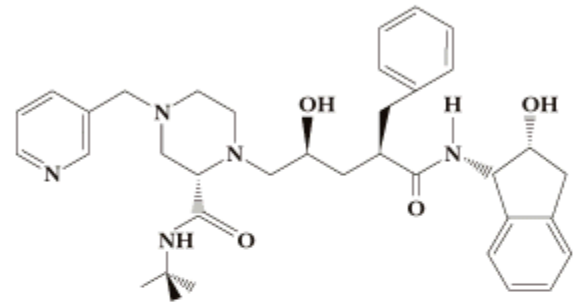
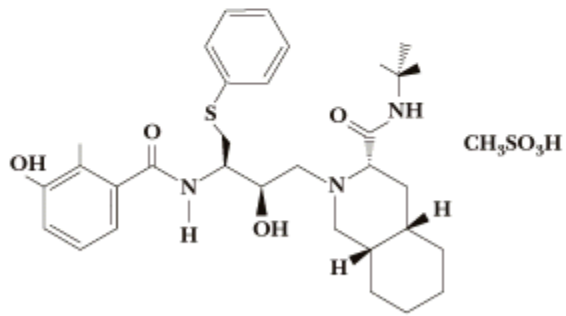


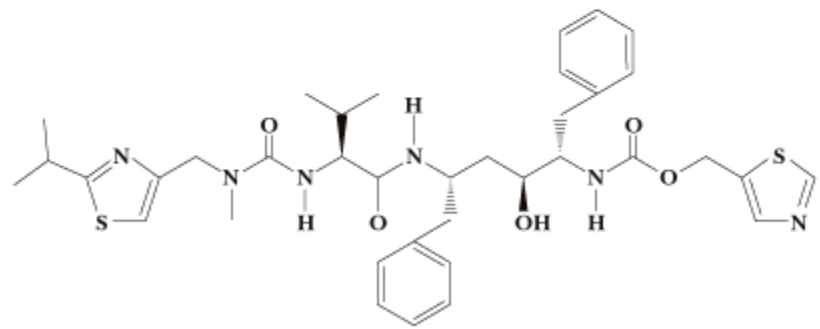
Invirase (Saquinavir)



Crixivan (Indinavir)



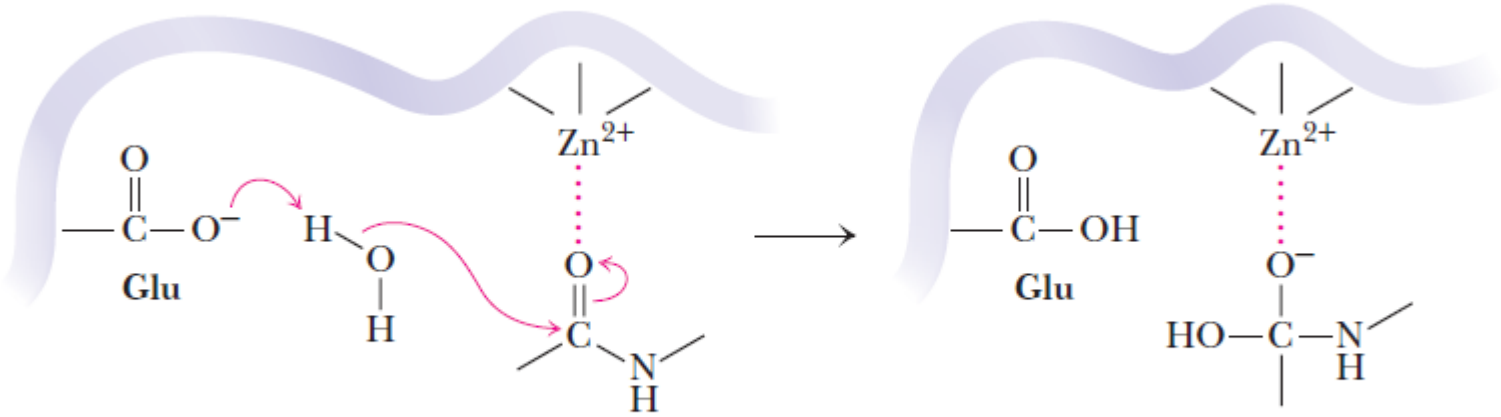
Viracept (Nelfinavir mesylate)



Norvir (Ritonavir)

Saunders College Publishing

Metal Ion Catalysis



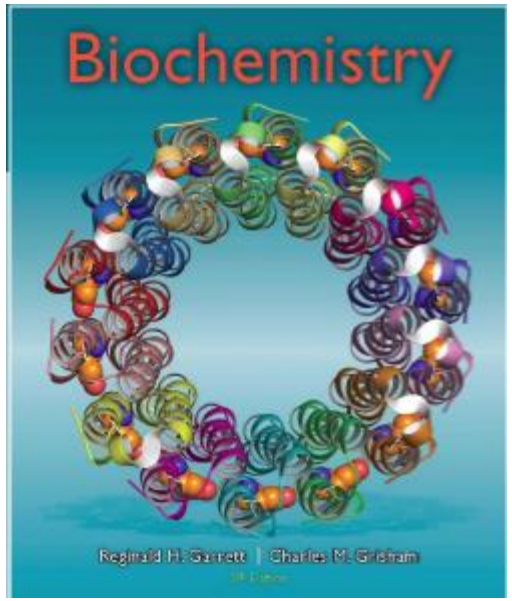
Initial step of thermolysin reaction

One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts, stabilizing the increased electron density or negative charge that develop during reaction

Another potential function of metal ions is to provide a powerful nucleophile at neutral pH.

Enzyme regulation

Chapter 15, Biochemistry



Reginald Garrett & Charles Grisham • University of Virginia

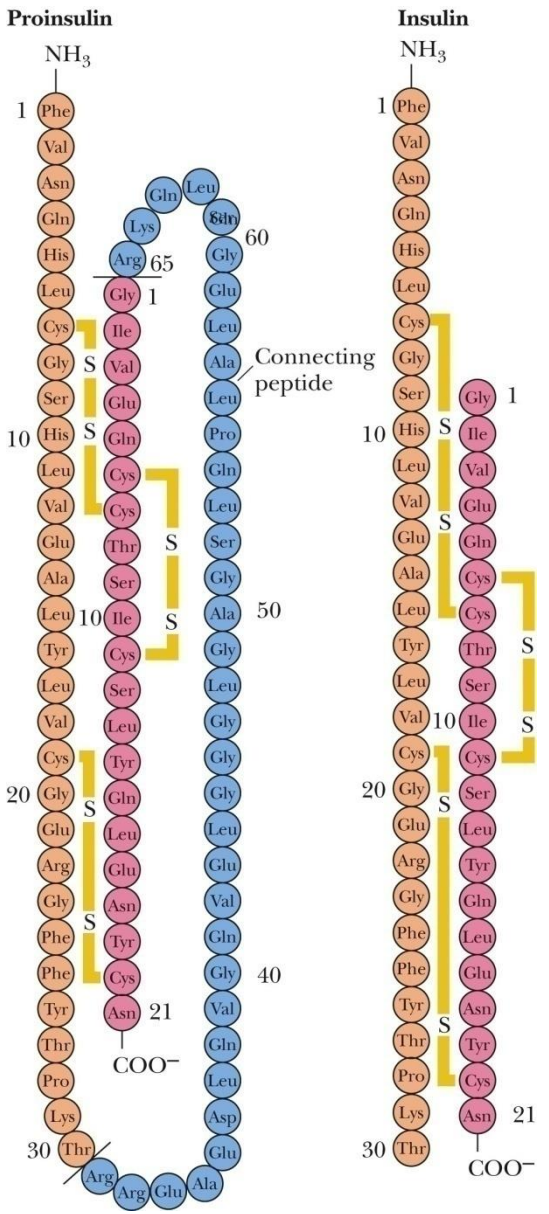
Different kinds of regulation

- Product inhibition
- Substrate/co-factor availability
- Gene controls induction/repression
- allosteric regulation
- Covalent modification
- Zymogens/Isozymes

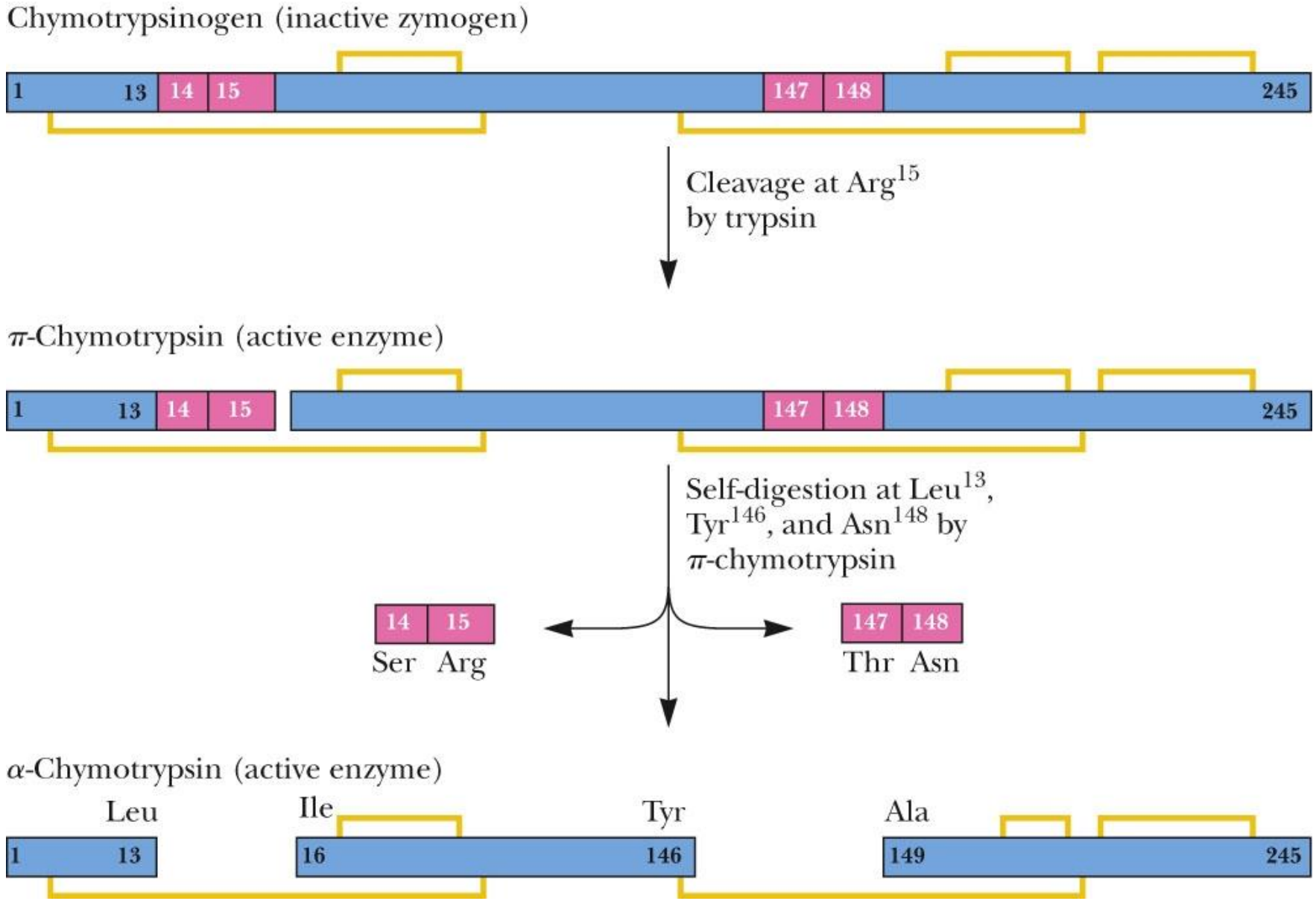
Zymogens are inactive precursors of enzymes. Typically, proteolytic cleavage produces the active enzyme

Example 1

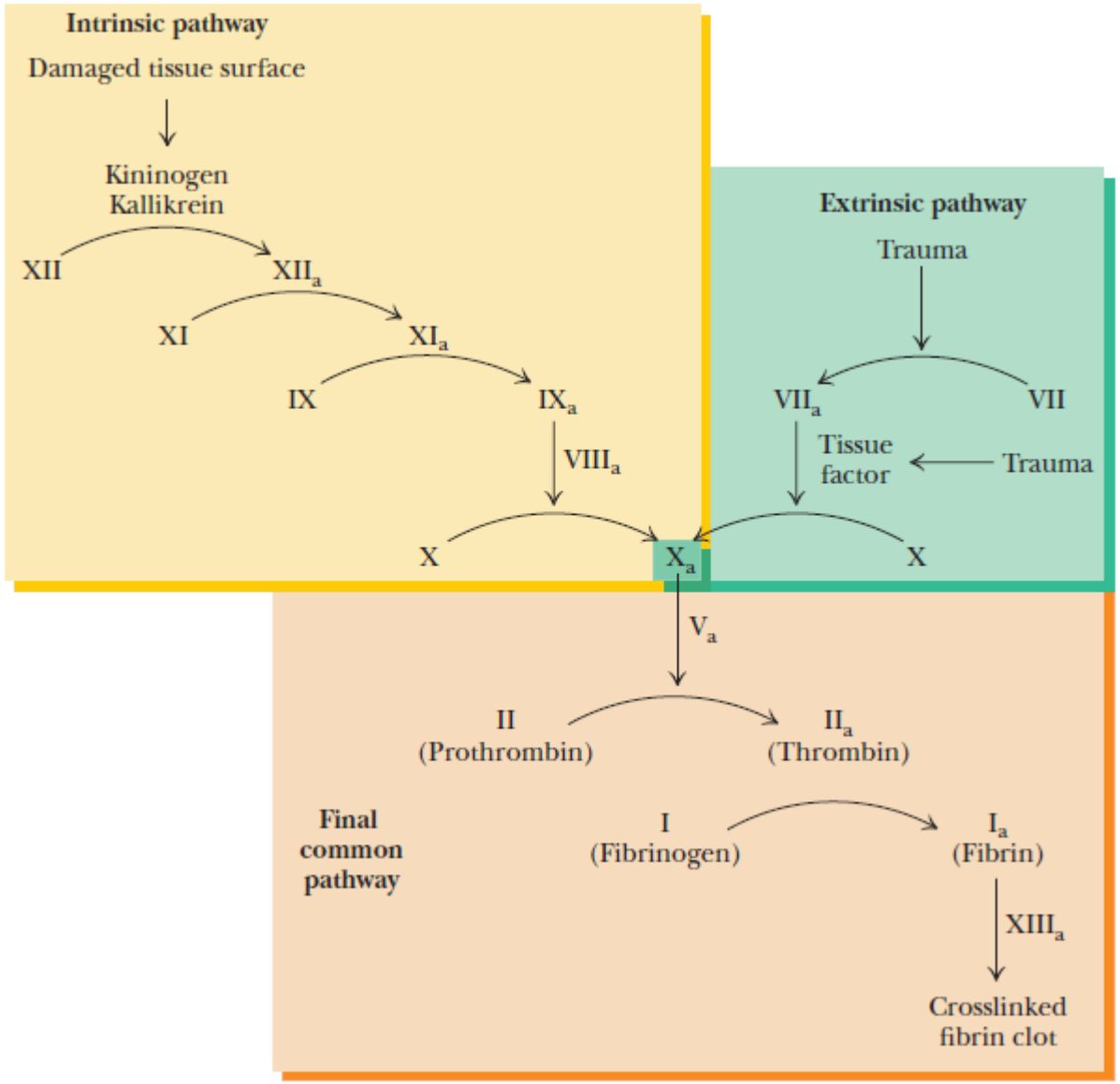
Proinsulin is an 86-residue precursor to insulin



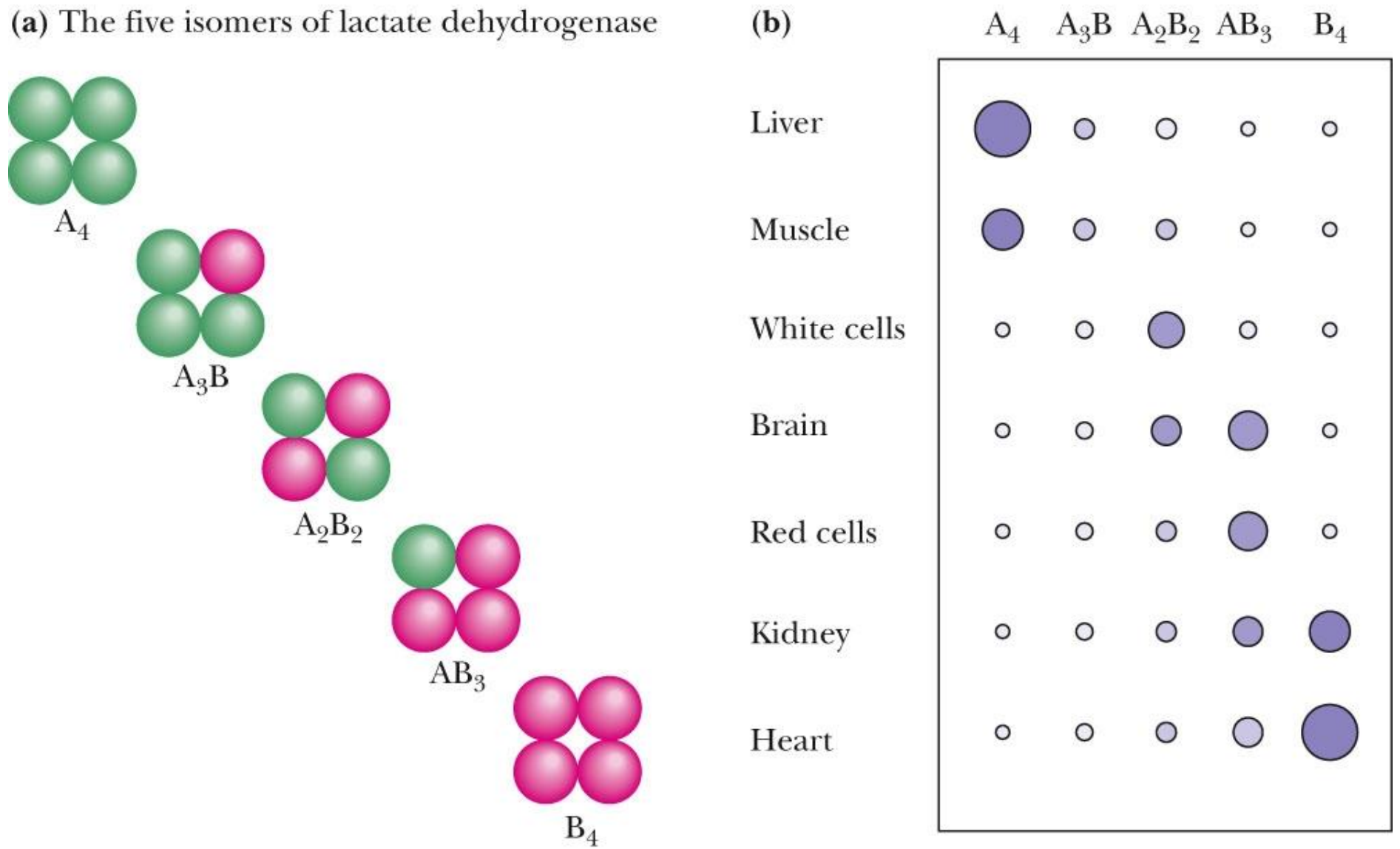
Example 2


Chymotrypsinogen (inactive zymogen)
1
13
14
15
147
148
245
Cleavage at Arg15 by trypsin
π-Chymotrypsin (active enzyme)
1
13
14
15
147
148
245
Self-digestion at Leu13, Tyr146, and Asn148 by π-chymotrypsin
14
15
Ser Arg
147
148
Thr Asn
α-Chymotrypsin (active enzyme)
Leu
Ile
Tyr
Ala
1
13
16
146
149
245

Blood clotting is regulated by
Series of zymogen



Isozymes Are Enzymes With Slightly Different Subunits



The isozymes of lactate dehydrogenase (LDH).

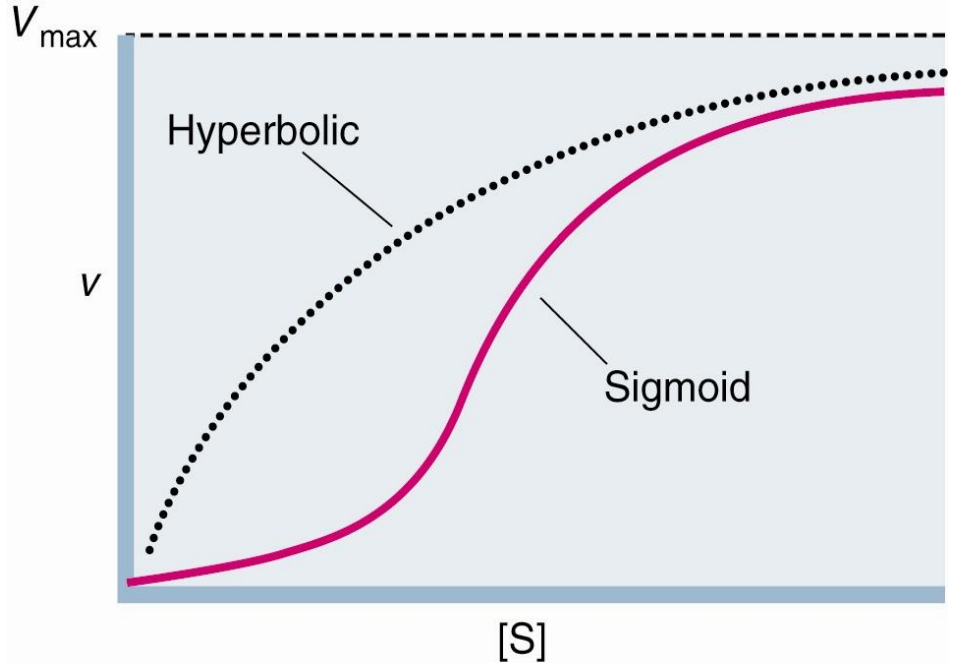
Allosteric Regulation?

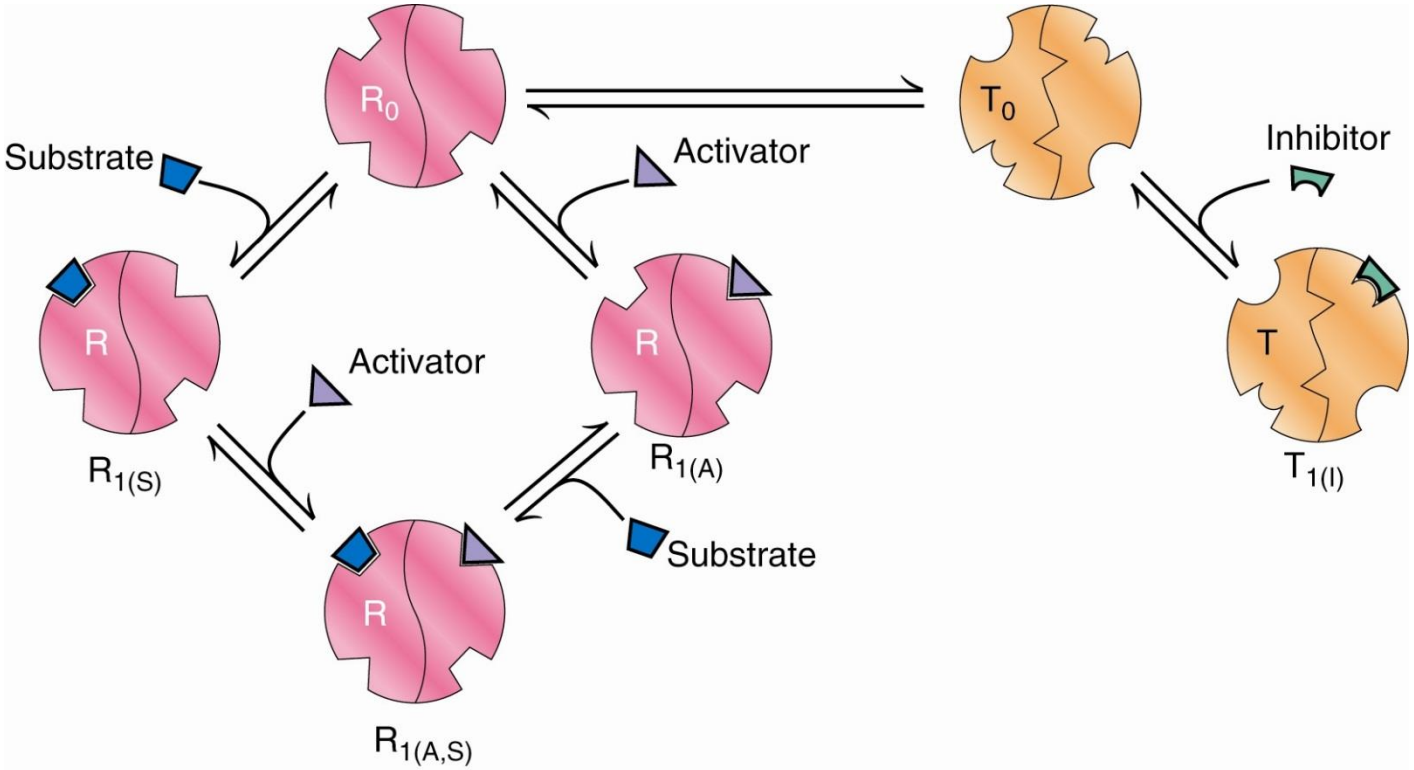
Action at "another site"

Enzymes situated at key steps in metabolic pathways are modulated by allosteric effectors

These effectors are usually produced elsewhere in the pathway

Effectors may be feed-forward activators or feedback inhibitors
Kinetics are sigmoid ("S-shaped")





Effects of I:

$I + T_0 \longrightarrow T_{1(I)}$

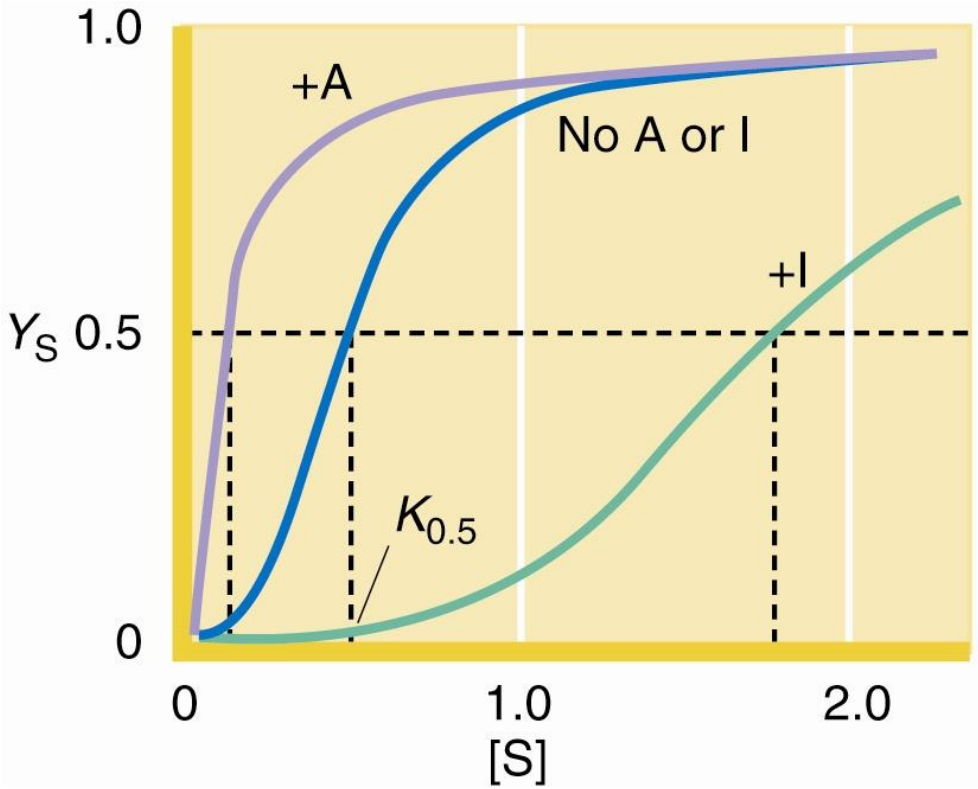
Increase in number of T-conformers (decrease in R_0 as $R_0 \longrightarrow T_0$ to restore equilibrium)

Thus, I inhibits association of S and A with R by lowering R_0 level. I increases cooperativity of substrate saturation curve.

Cooperativity is achieved because S binding increases the population of R, which increases the sites available to S

Monod, Wyman, Changeux (MWC) Model:

- Allosteric proteins can exist in two states: R (relaxed) and T (taut)
- In this model, all the subunits of an oligomer must be in the same state
- T state predominates in the absence of substrate S
- S binds much tighter to R than to T



Effects of A:

$A + R_0 \rightarrow R_{1(A)}$

Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

(1) More binding sites for S made available.

(2) Decrease in cooperativity of substrate saturation curve.

Covalent Modification Regulate the Activity of Enzymes?

Phosphorylation:

- Enzyme activity can be regulated through reversible phosphorylation
- This is the most prominent form of covalent modification in cellular regulation
- Phosphorylation is accomplished by protein kinases
- Each protein kinase targets specific proteins for phosphorylation
- Phosphoprotein phosphatases catalyze the reverse reaction – removing phosphoryl groups from proteins
- Kinases and phosphatases themselves are targets of regulation

- Protein kinases phosphorylate Ser, Thr, and Tyr residues in target proteins
- Kinases typically recognize specific amino acid sequences in their targets
- In spite of this specificity, all kinases share a common catalytic mechanism based on a conserved core kinase domain of about 260 residues (see Figure 15.9)
- Kinases are often regulated by **intrasteric control**, in which a regulatory subunit (or domain) has a **pseudosubstrate sequence** that mimics the target sequence, minus the phosphorylatable residue

Thank you