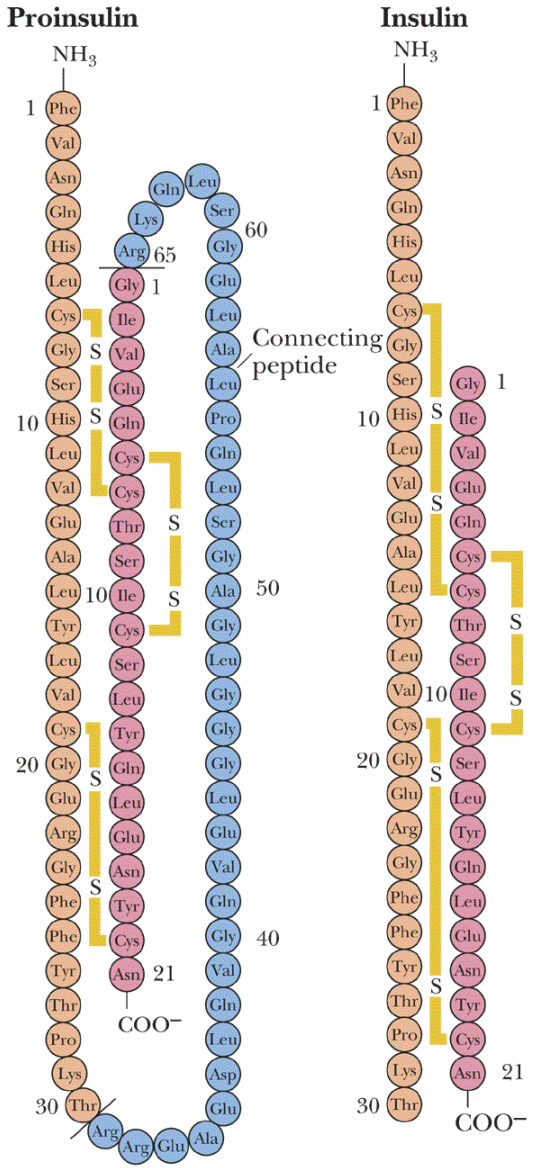
Protease Mechanism and Activation

Zymogens, chymotrypsin, aspartic proteases

Proinsulin

Garrett/Grisham, Biochemistry with a Human Focus
Figure 10.23



Protease Zymogens and Binding Sites

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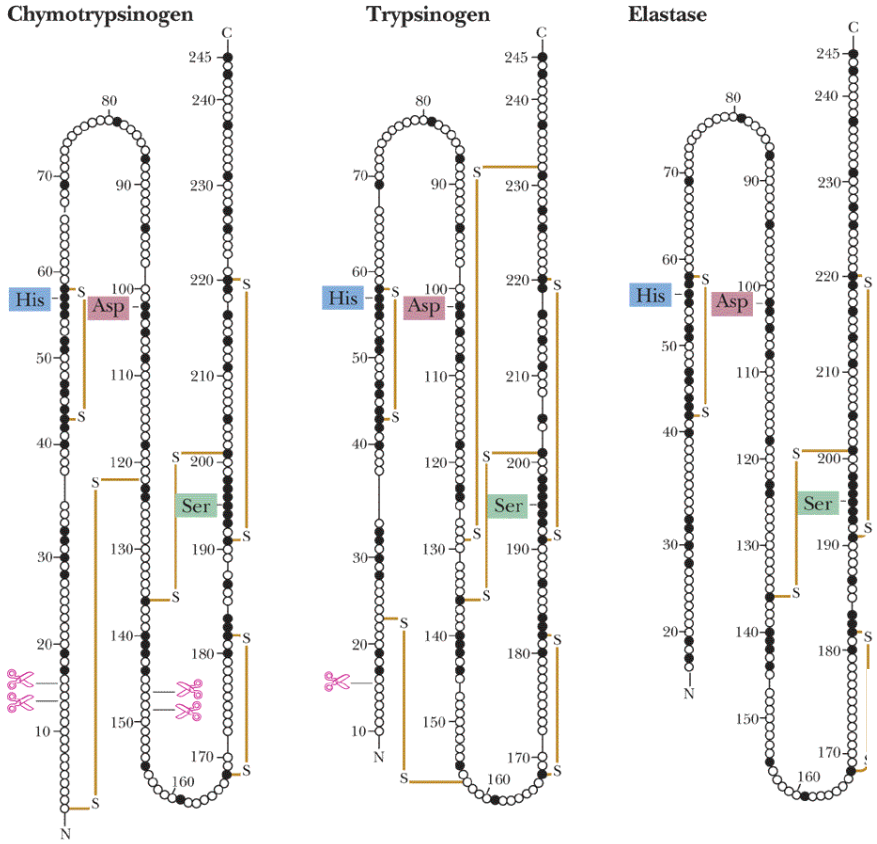
Figure 11.13



His—Asp—Ser

Catalytic Triad

Active site pocket confers specificity

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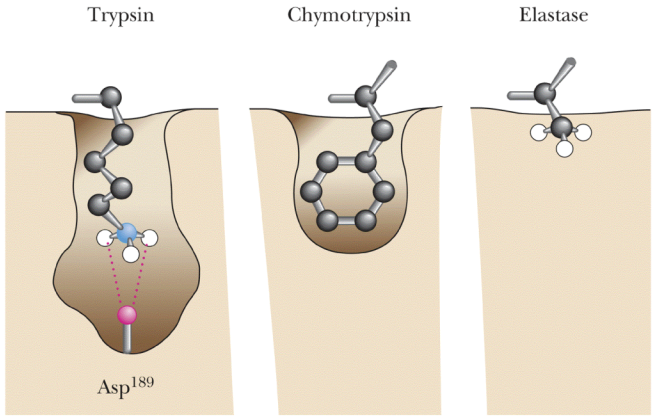
Figure 11.16



Catalytic Triad

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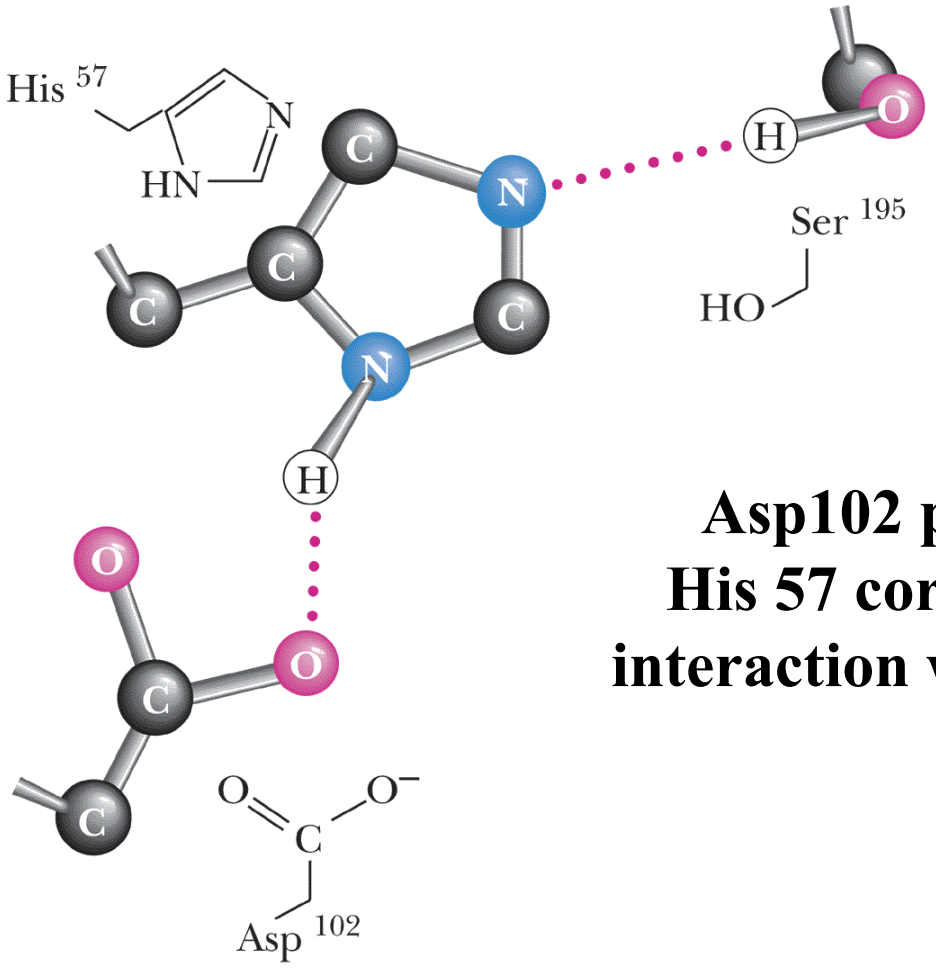
Figure 11.15



Asp102 positions His 57 correctly for interaction with Ser195

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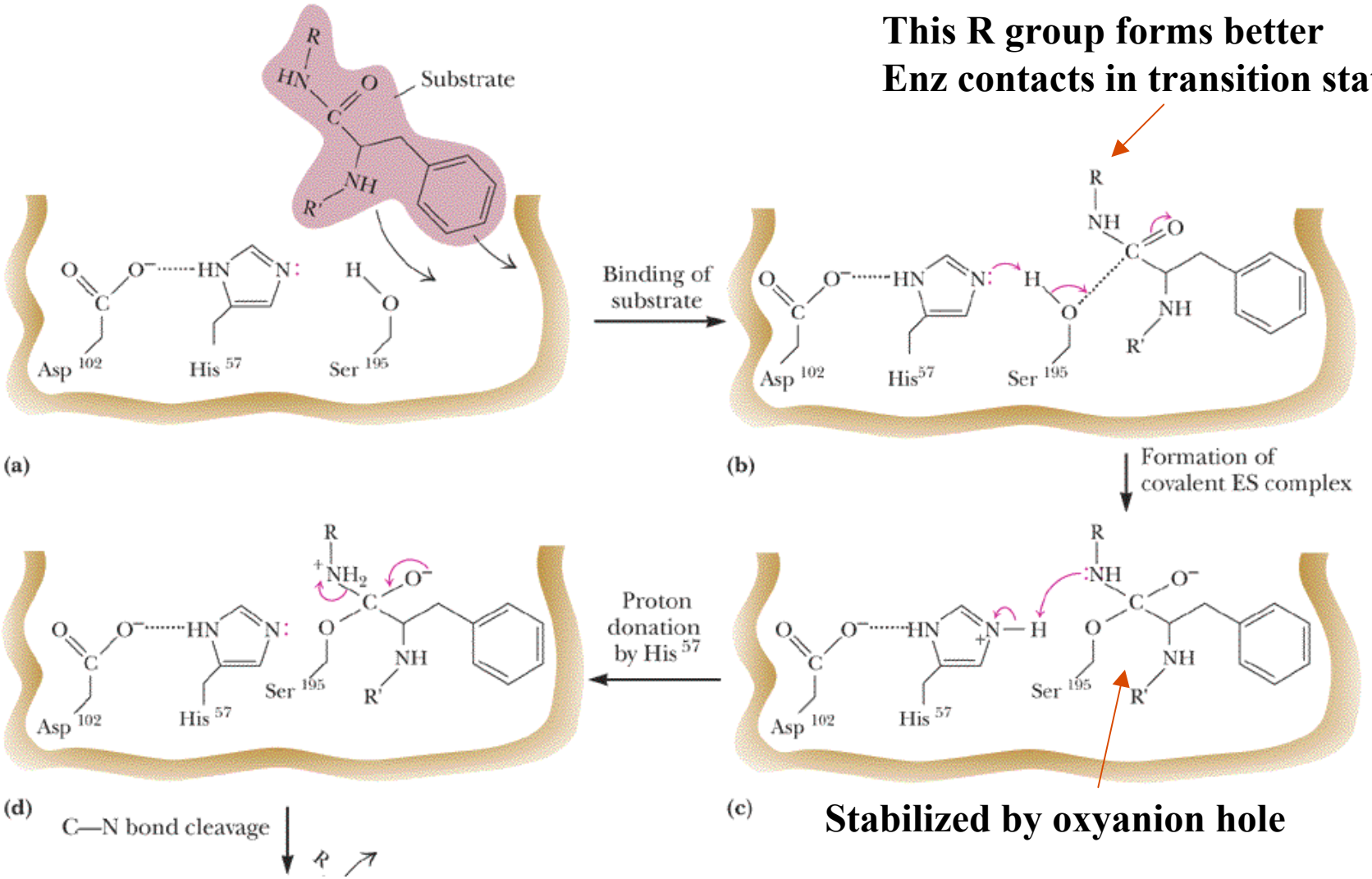
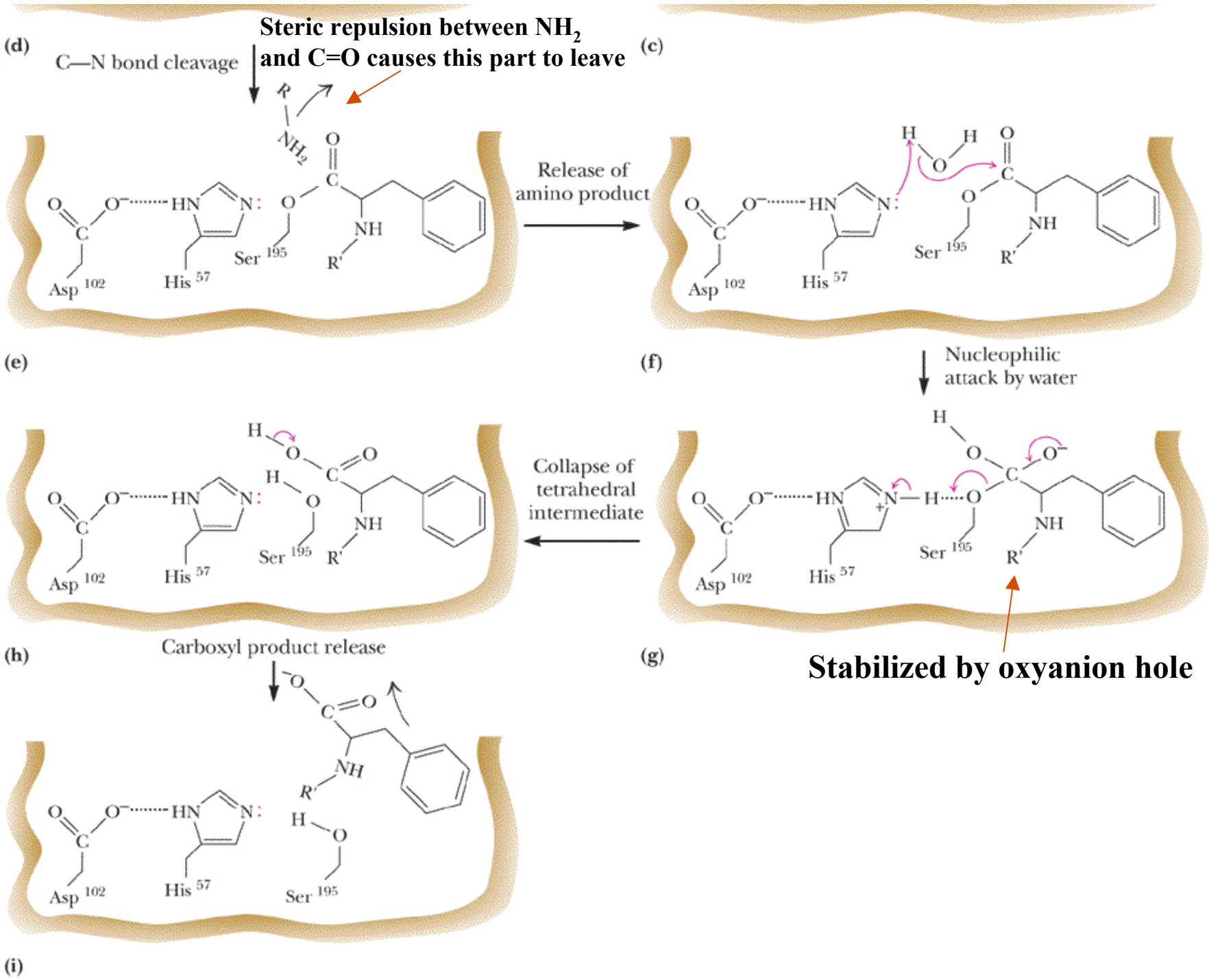
Figure 11.21

Chymotrypsin Mechanism



This R group forms better Enz contacts in transition state.

Stabilized by oxyanion hole


(d)
C—N bond cleavage
Steric repulsion between NH_2 and C=O causes this part to leave
(c)
Release of amino product
(e)
(f)
Nucleophilic attack by water
Collapse of tetrahedral intermediate
(h)
(g)
Carboxyl product release
Stabilized by oxyanion hole
(i)
Asp 102
His 57
Ser 195

Oxyanion Hole

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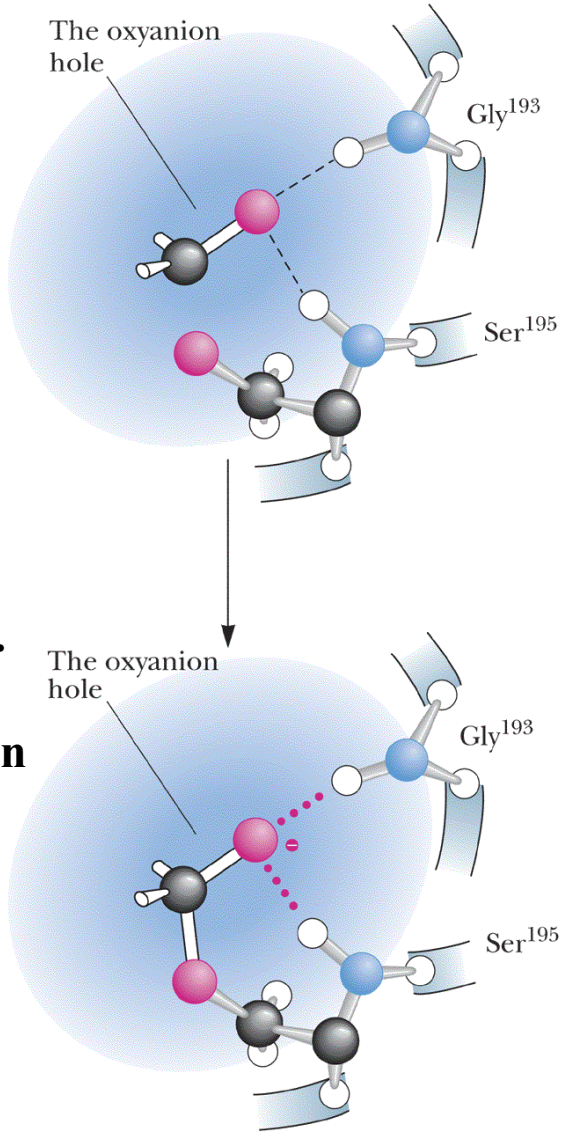
Unnumbered Figure Page 379

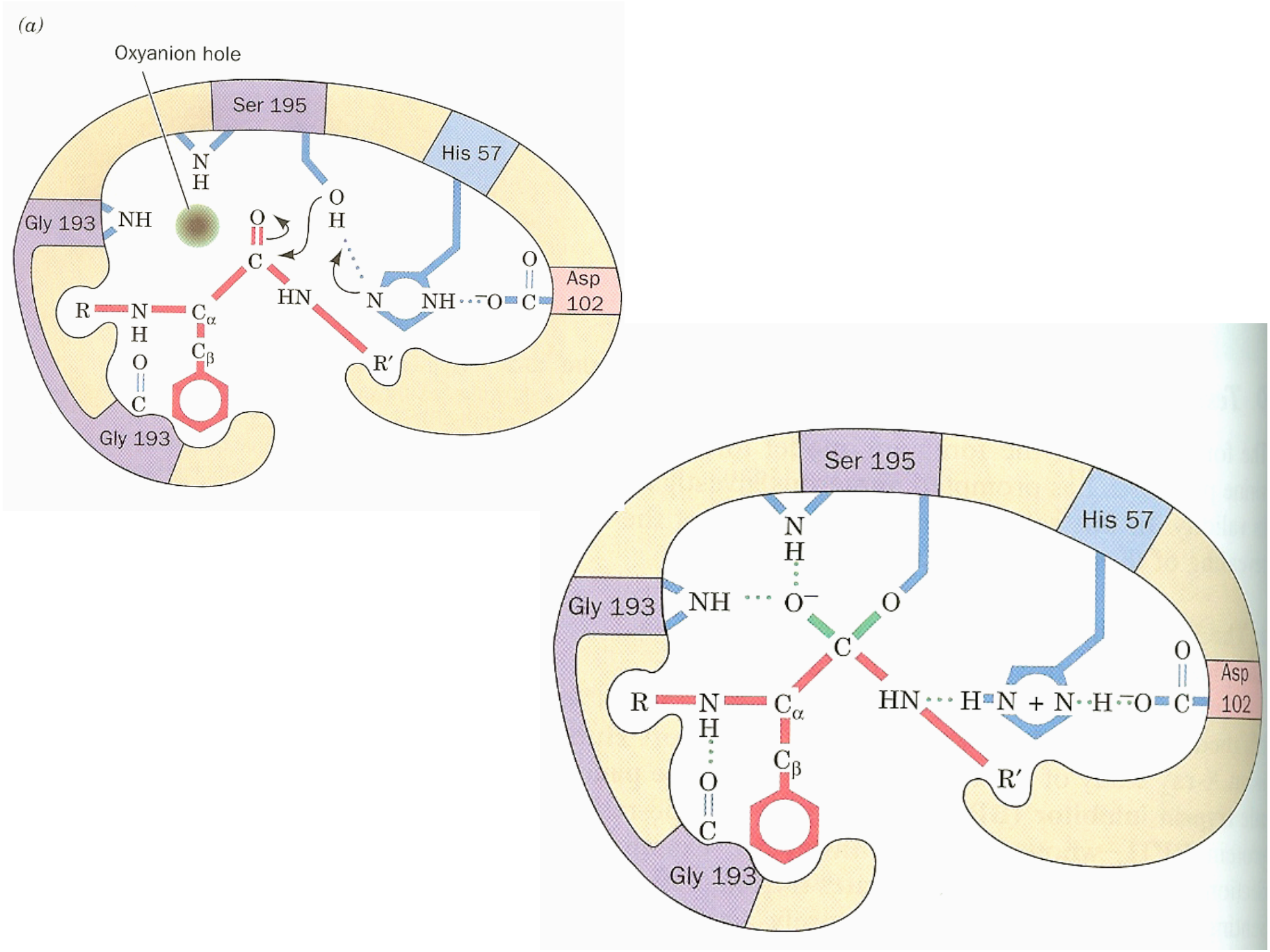
The formation of the tetrahedral intermediate, (c and g), is stabilized by interactions with the Backbone amide N-H groups of Ser195 and Gly193.

Formation of the tetrahedral intermediate increases The interactions between the carbonyl oxygen and The N-H of the backbone for two reasons:

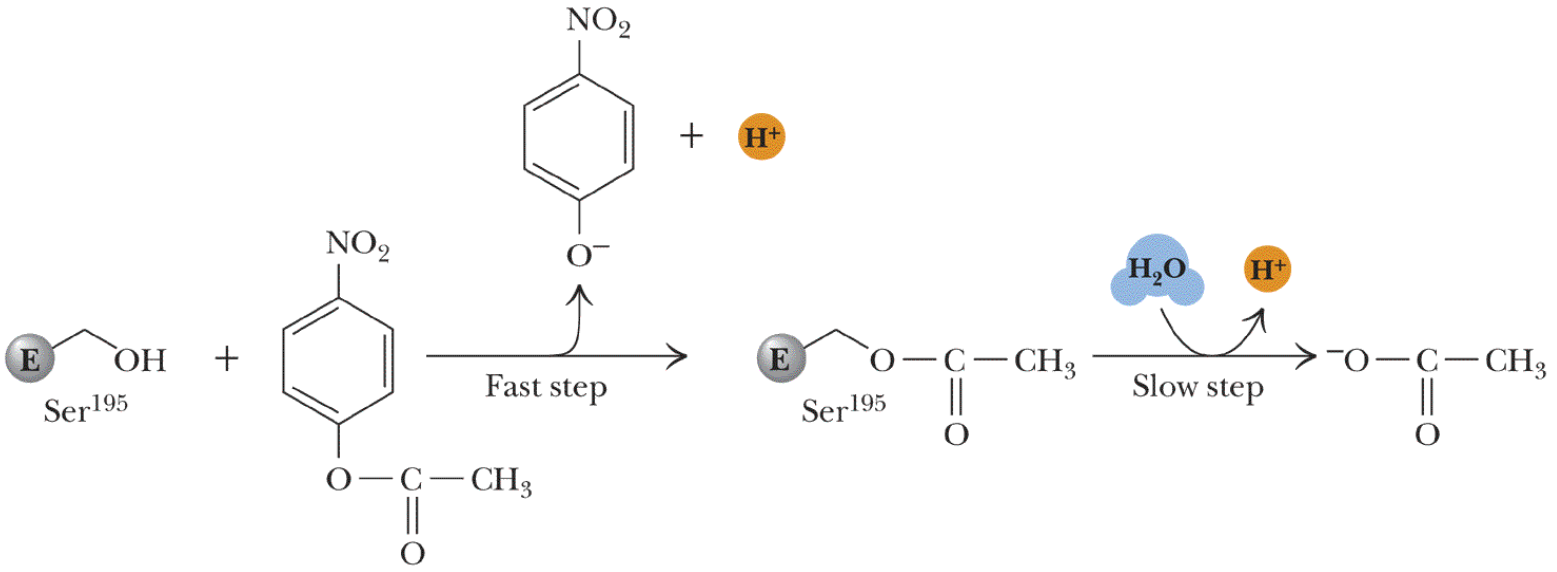
1) C=O→C------O$^-$ the increased bond length of the C-----O- brings the oxygen closer to the N-H groups.

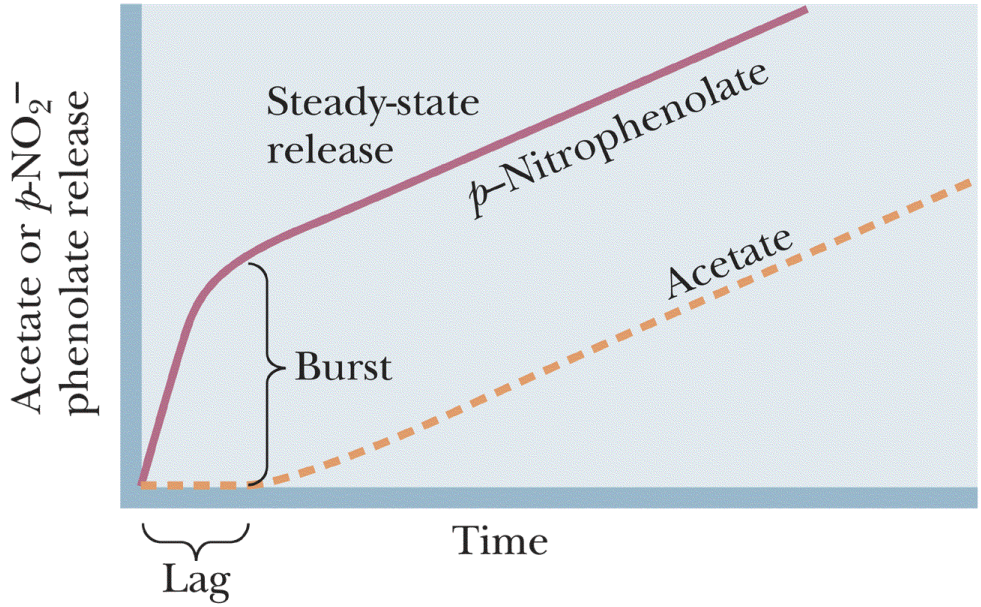
2) Negatively charged O interacts more strongly than Uncharged O in C=O.




(a)
Oxyanion hole
Ser 195
His 57
Asp 102
Gly 193
Gly 193
Ser 195
His 57
Asp 102
Gly 193

Rate-limiting Step





Chymotrypsin Inhibitor

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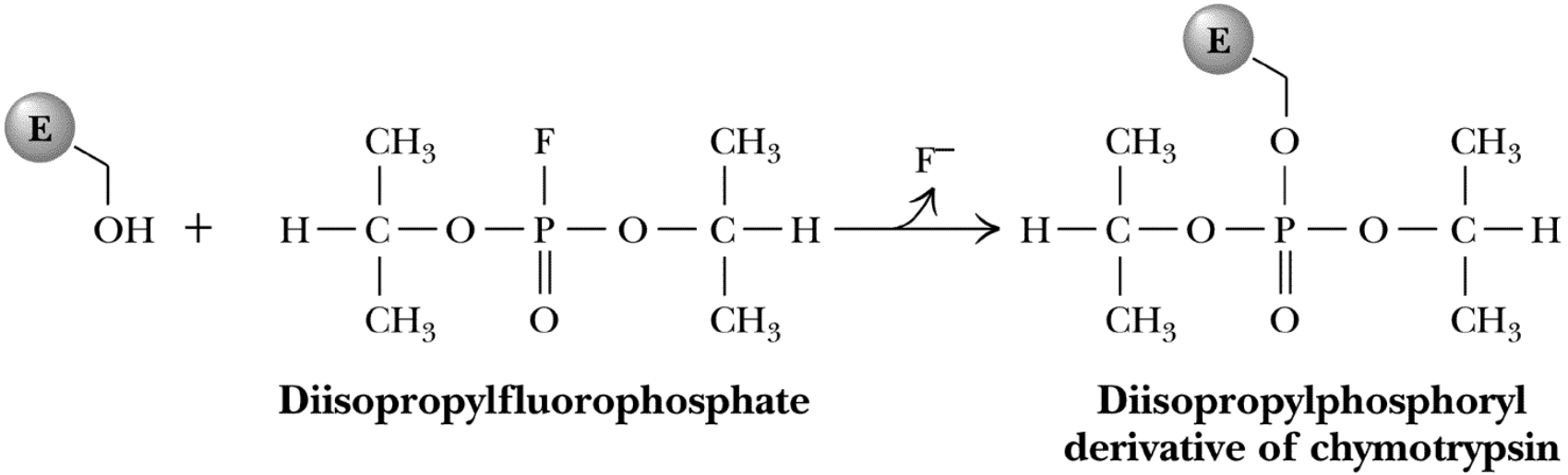
Figure 11.20



Activation of Chymotrypsinogen

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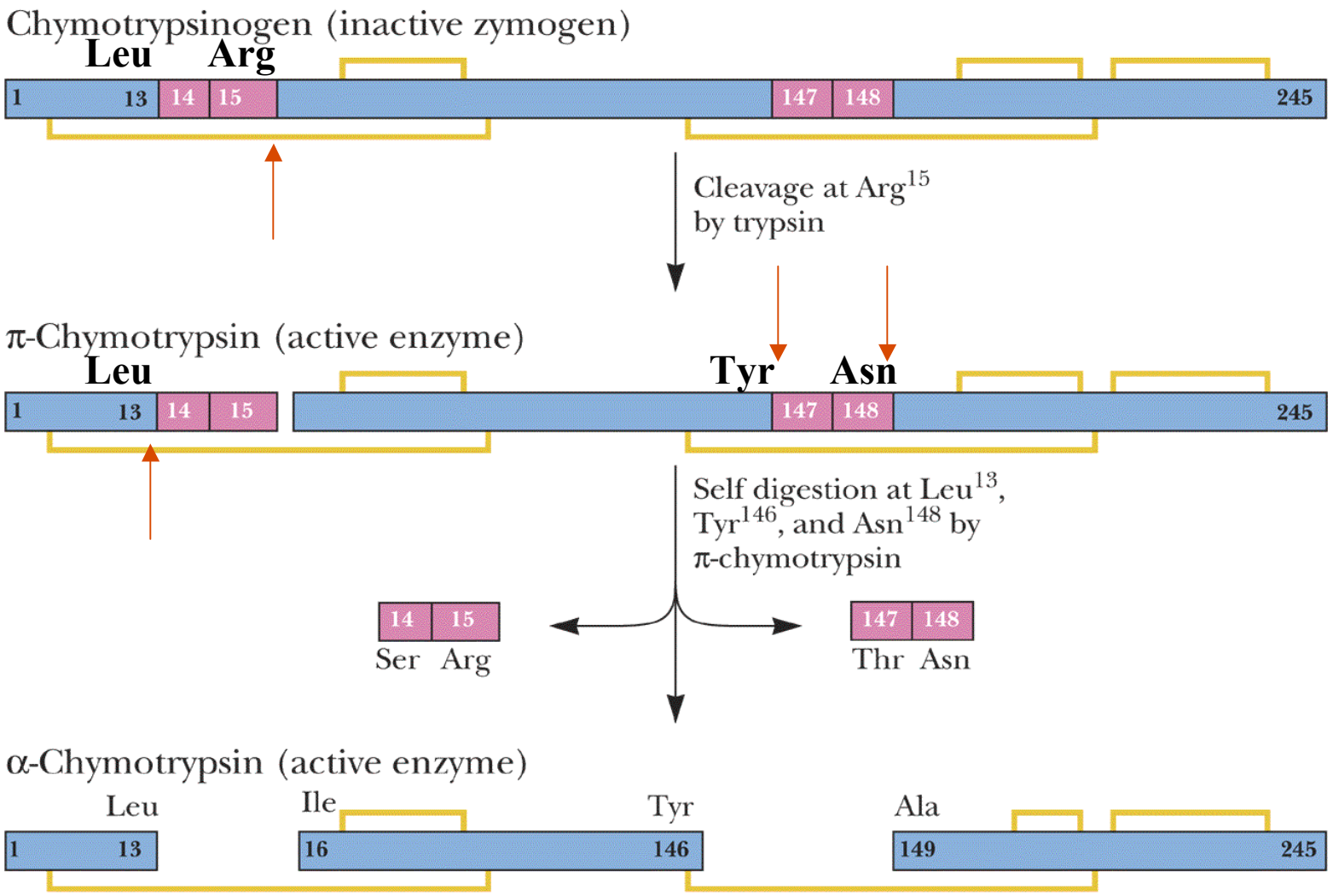
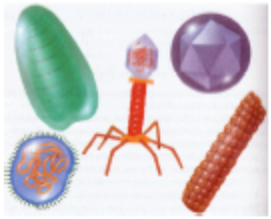
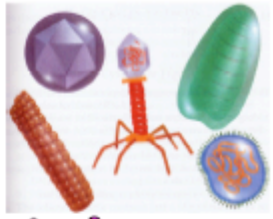
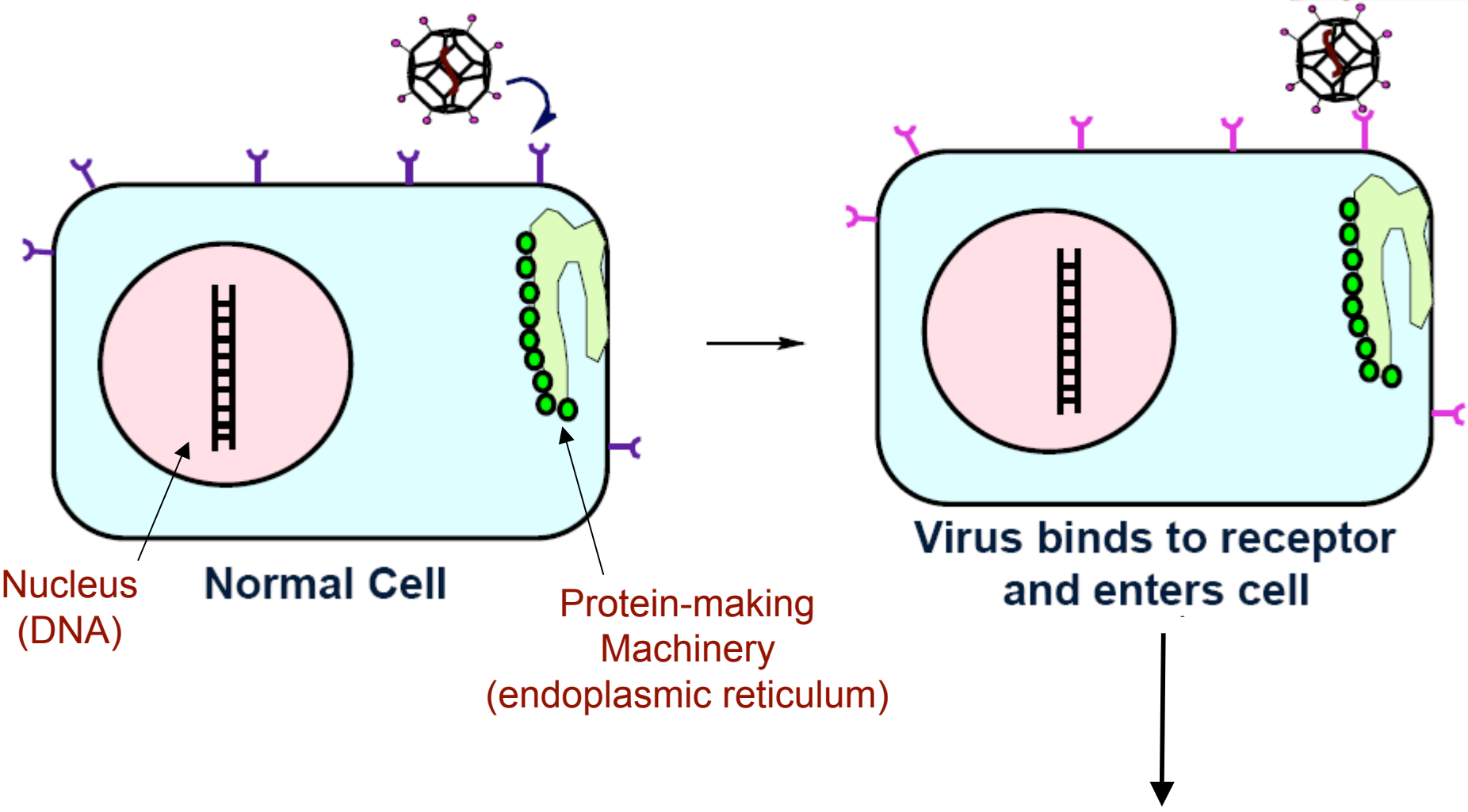
Figure 10.24

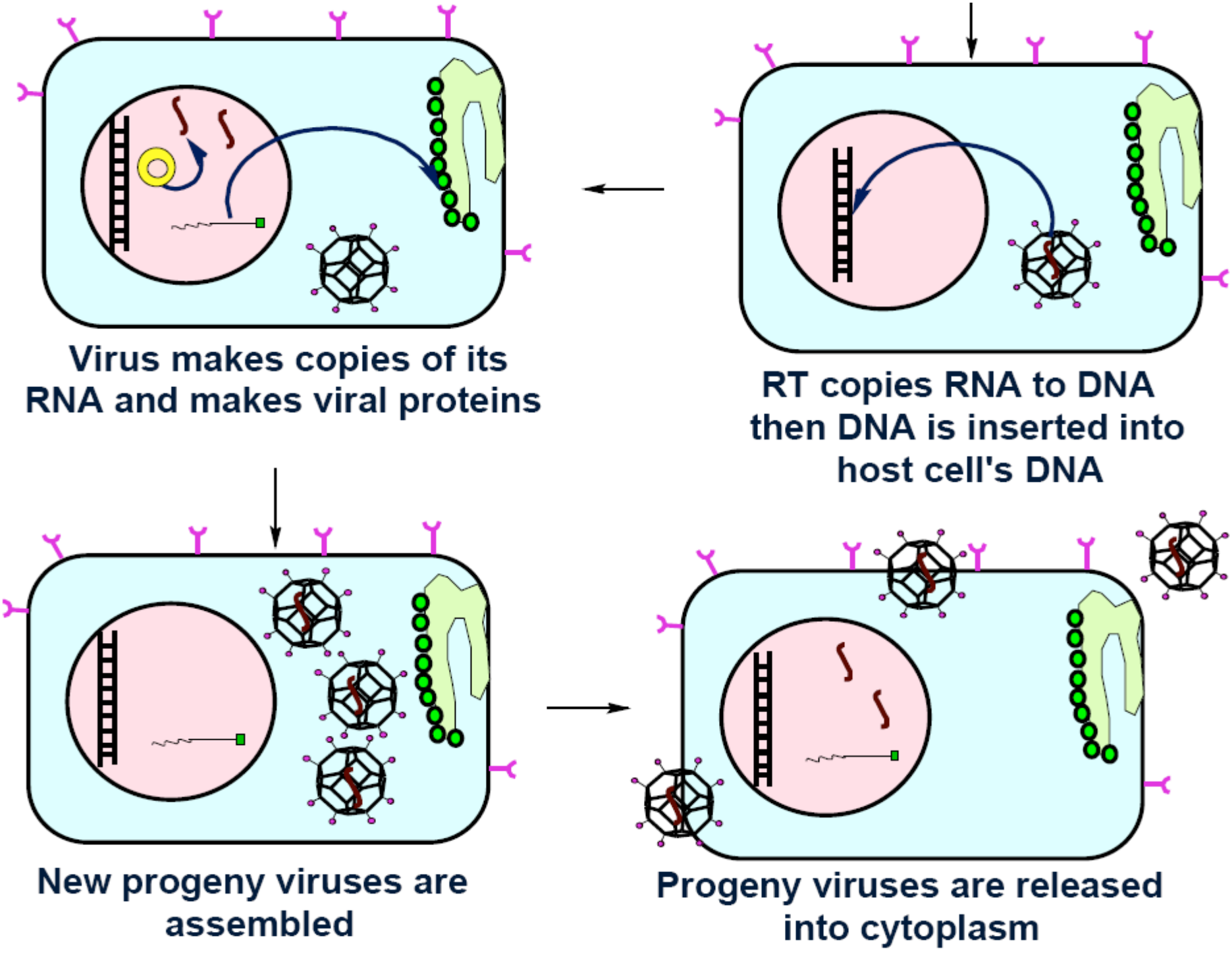




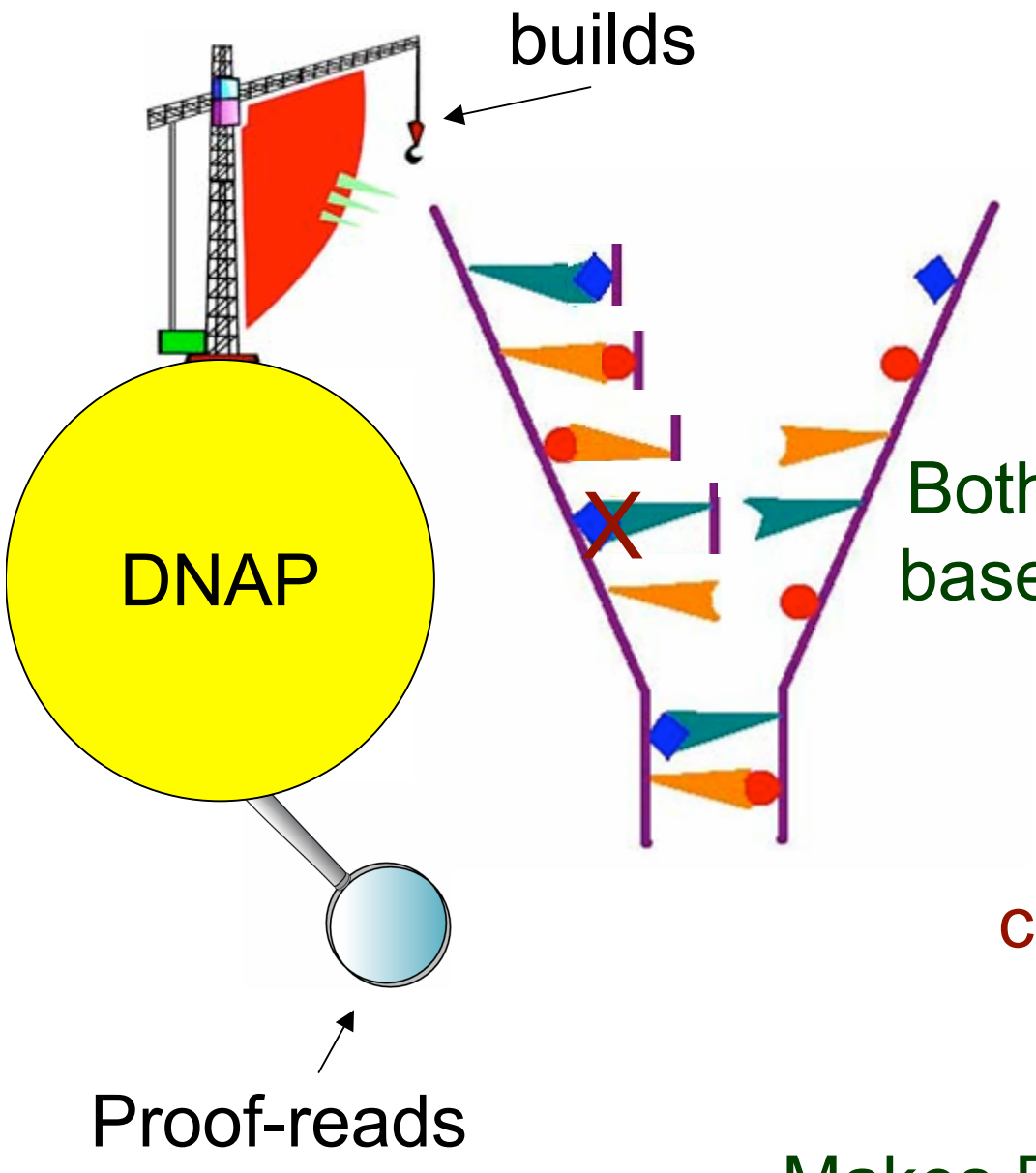
Human Immunodeficiency Virus (HIV)






Virus makes copies of its RNA and makes viral proteins
RT copies RNA to DNA then DNA is inserted into host cell's DNA
New progeny viruses are assembled
Progeny viruses are released into cytoplasm

Human DNA Replication



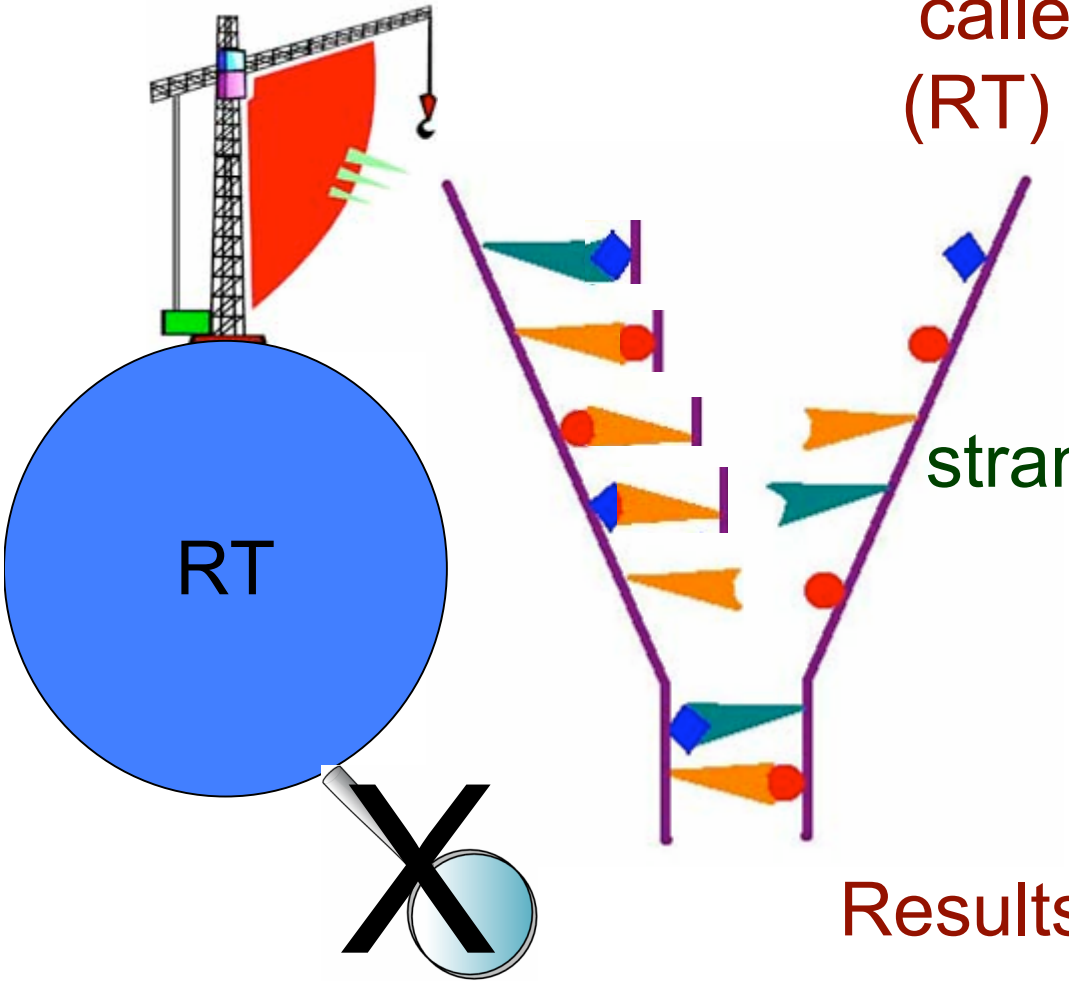
Our cells copy their DNA using an enzyme called DNA polymerase (DNAP)

Both builds DNA by putting in bases that match each strand Thereby making a copy

Also proof-reads each copied base to make sure it is right

Makes DNA copy highly accurate

HIV RNA Conversion



Viruses use an enzyme called Reverse Transcriptase (RT) to copy its RNA into DNA

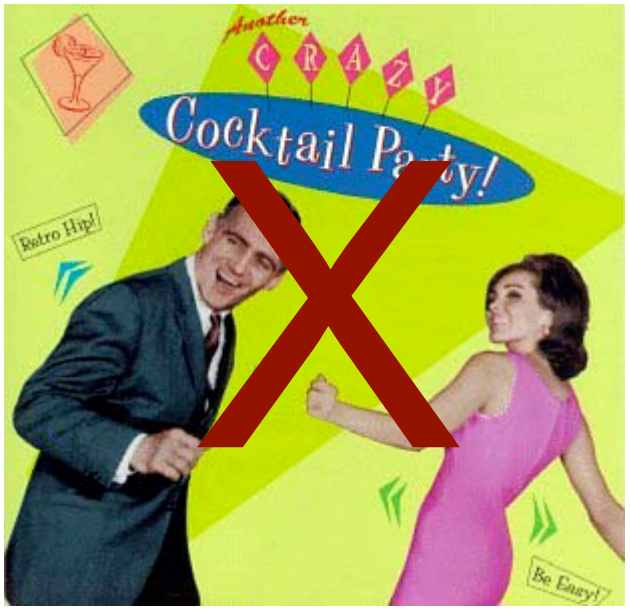
Builds DNA by putting in bases that match each strand thereby making a copy

CANNOT proof-read to make sure right so makes a lot of errors

Results in high rate of mutations

Makes various mutated strains

HIV Cocktail



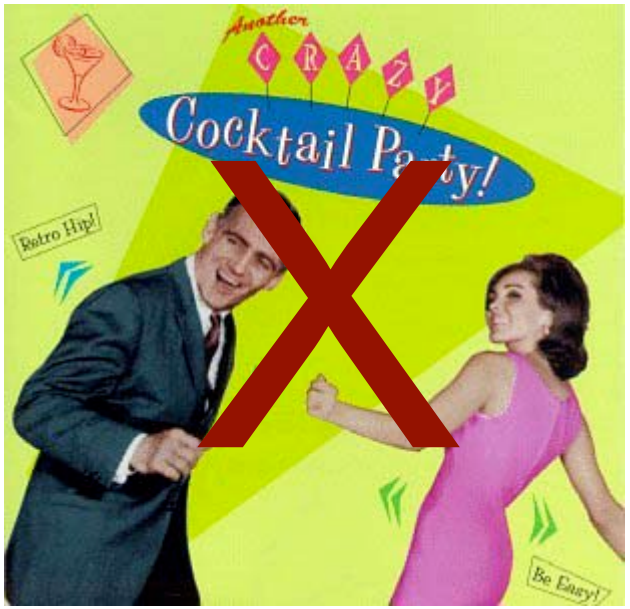
Three enzymes important for HIV to replicate:

Reverse Transcriptase: Copies virus RNA into DNA
Very, Very error prone

Integrase: puts viral DNA copy into host cell's DNA

Protease: Cuts proteins into functional pieces

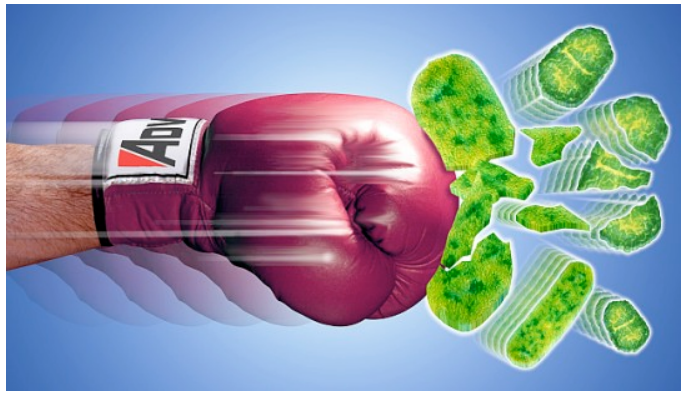
HIV Cocktail



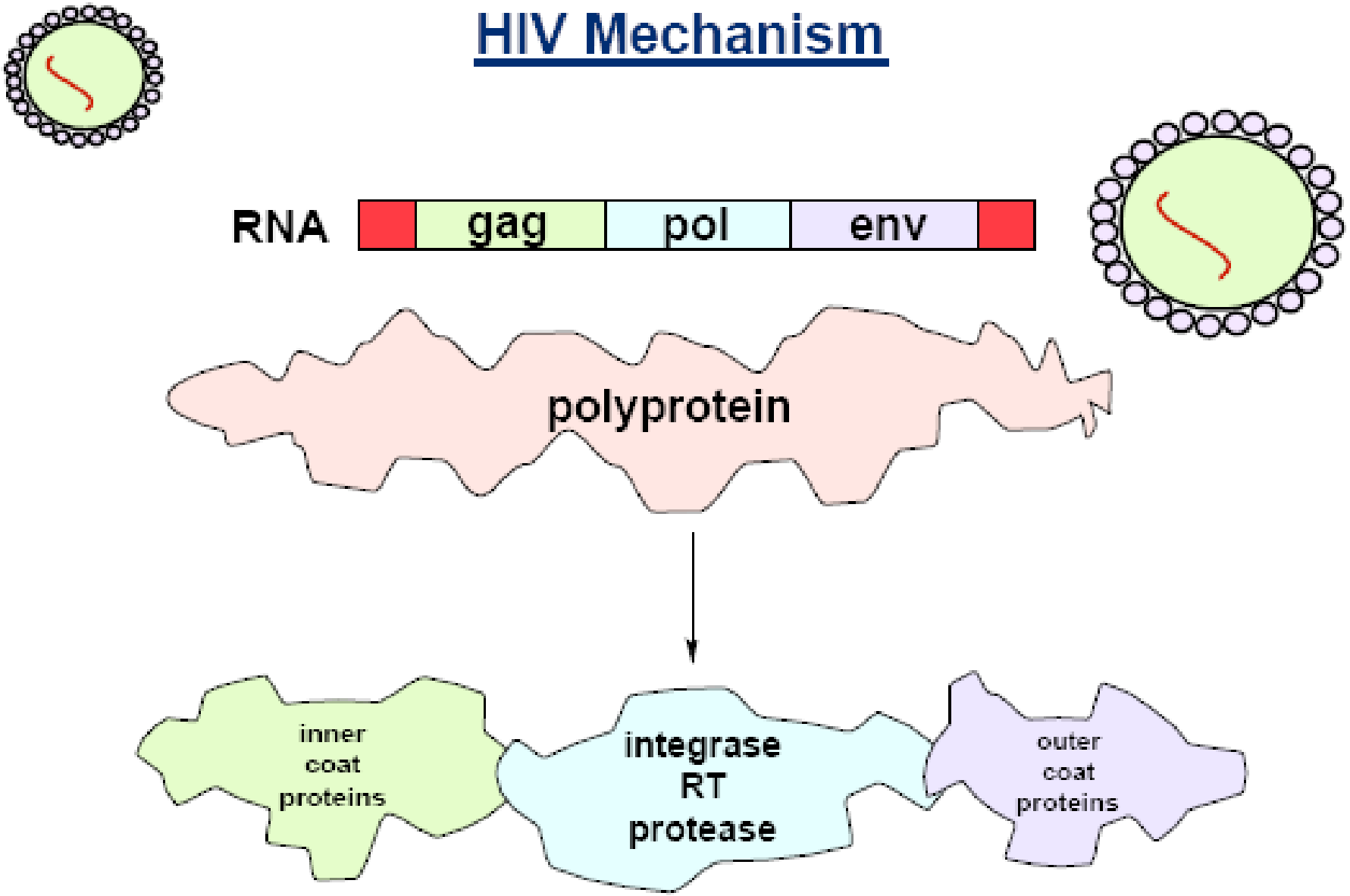
Because HIV can mutate so rapidly
cant just knock out one enzyme
or mutations provide resistance to the drug

So have to knock out more than one (preferably all three)

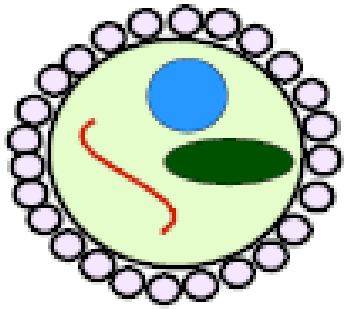
Makes it much less likely that mutations will allow the virus to survive in the presence of many drugs with multiple targets.



HIV Mechanism



Protease excises itself and then cleaves the other proteins from polyproteinthereby producingactive virusparts.



Each virus is then packed with:

1) Reverse Transcriptase
2) Integrase

Why not packed with protease?

Aspartic Protease Mechanism

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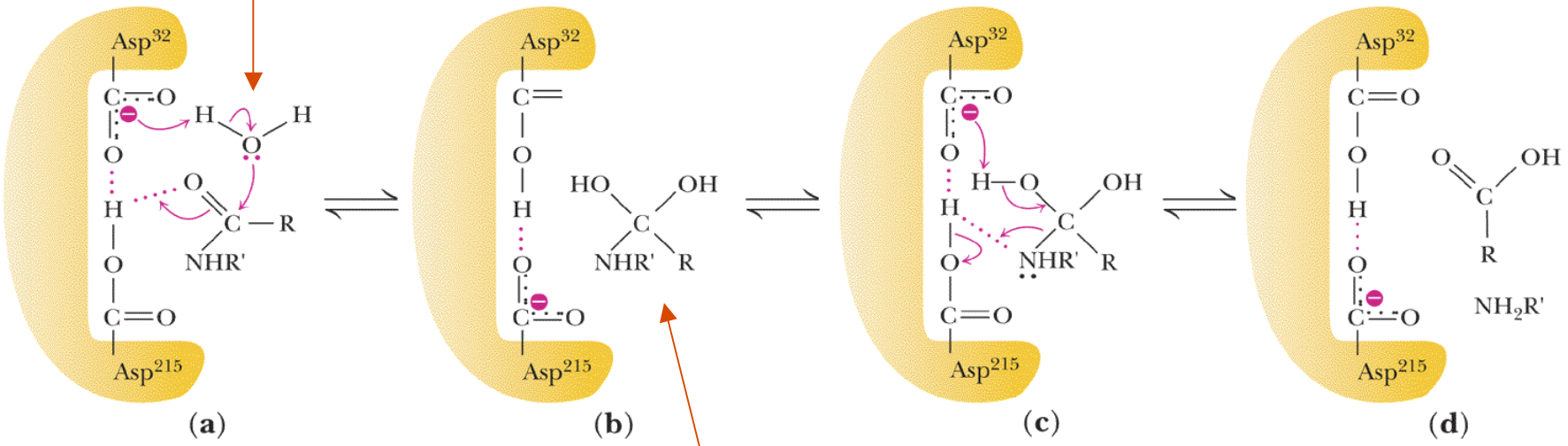
Figure 11.23

Catalytic Water



One proton is shared between Asp215 and Asp32.

Tetrahedral Intermediate

HIV Protease Inhibitors

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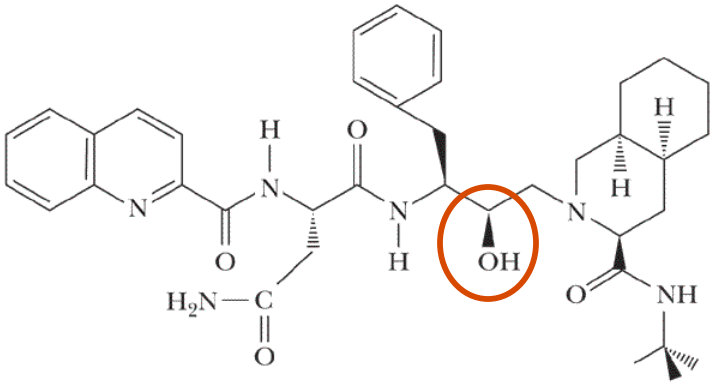
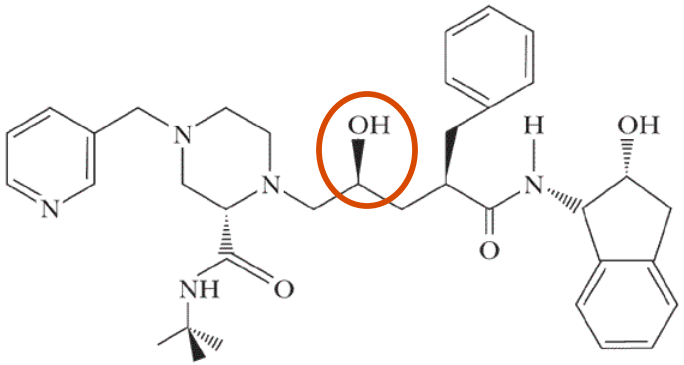
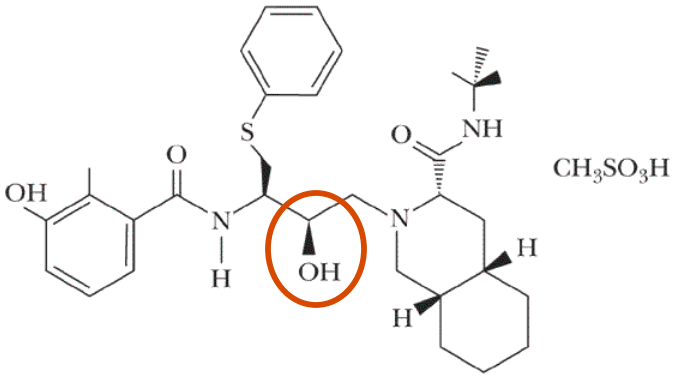


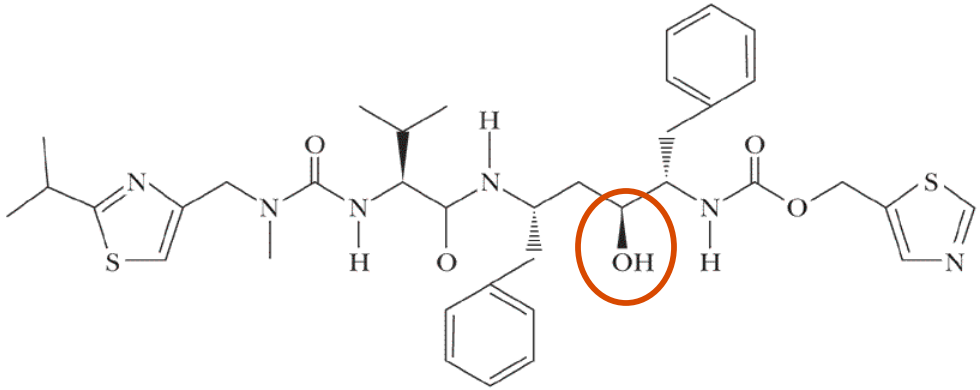
Invirase (Saquinavir)



Crixivan (Indinavir)



Viracept (Nelfinavir mesylate)



Norvir (Ritonavir)

Major Components of Blood Clotting

1) Intact Endothelial Cells

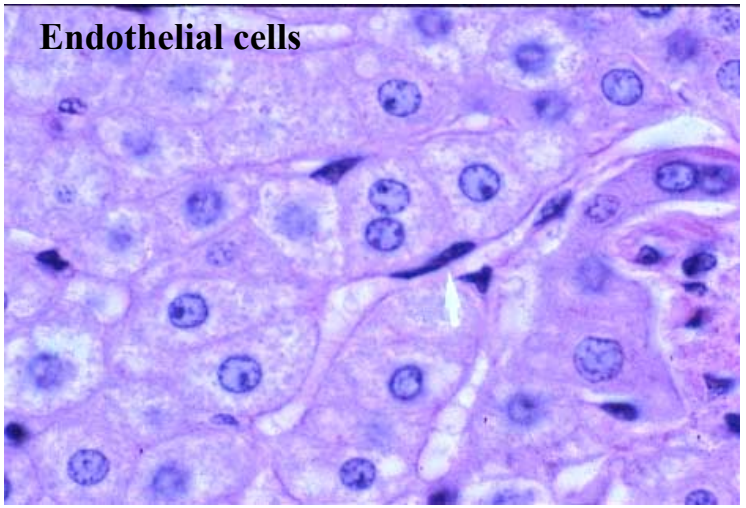
- inhibit blood clotting

- surface not conducive to clot formation

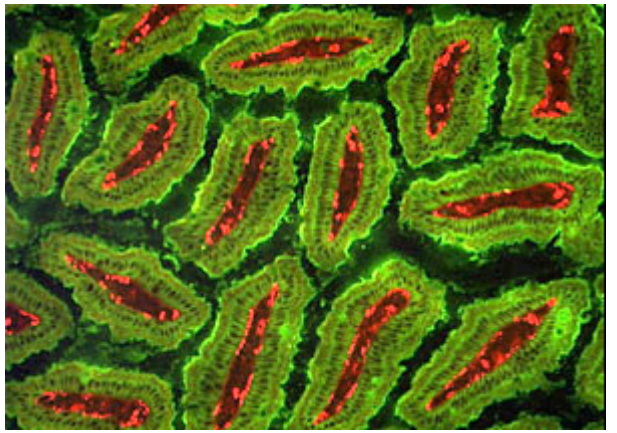
- display membrane proteins that inhibit clotting

- store von Willebrand factor in cytop. granules. Constituitively expressed and secreted into circulation or subendothelium. (20% made by platelets, rest endothelium)

- make prostacyclin ~ inhibits platelet aggregation



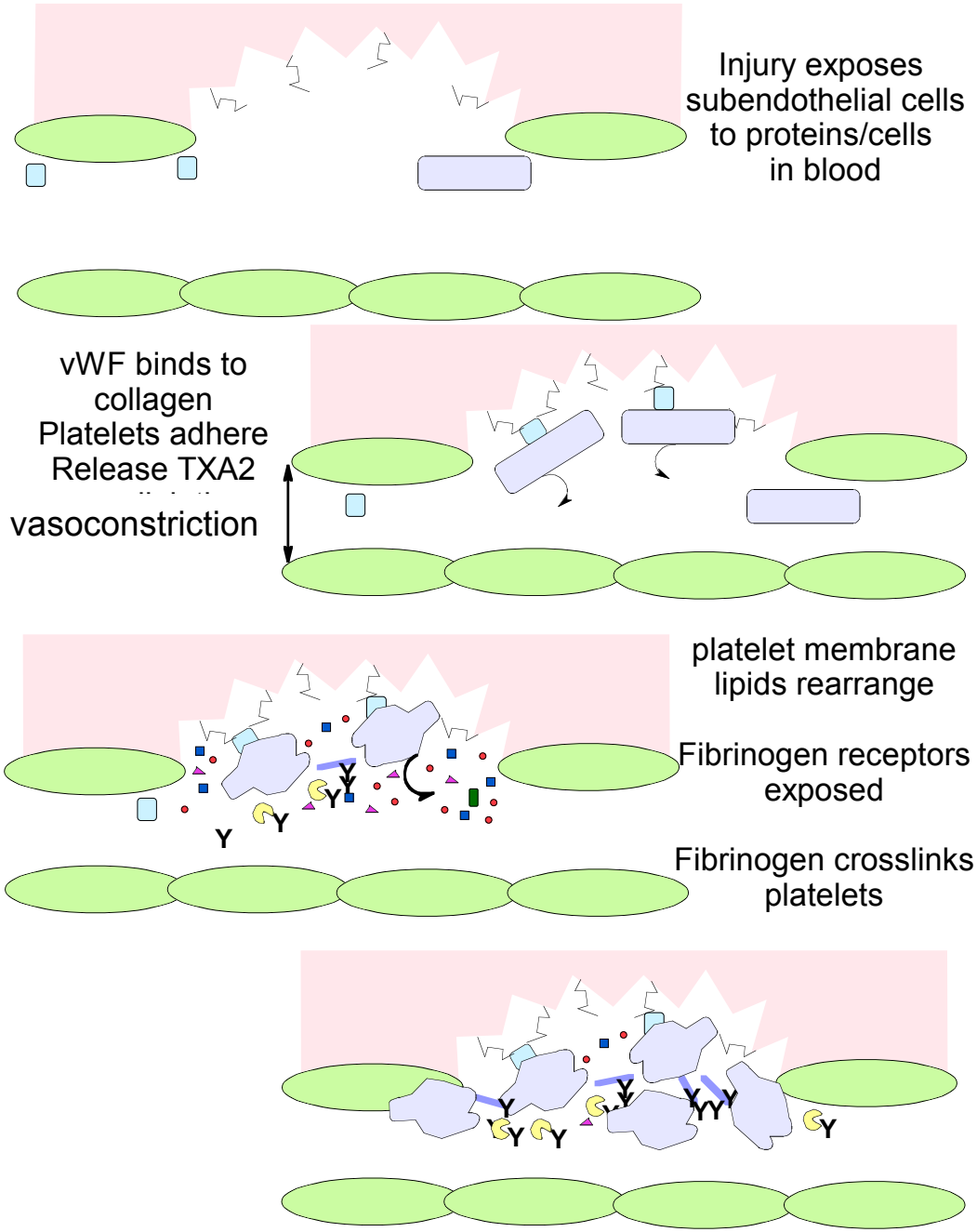
http://www.meddean.luc.edu/lumen/MedEd/orfpath/murali.htm



http://www.vet.uga.edu/vpp/CLERK/anderson/

Endothelial cells with stored vWF (red)

Blood Clotting Initiation



2) Subendothelial Cells

- contain membrane prots and extracellular matrix prots (collagen) that normally do not contact blood

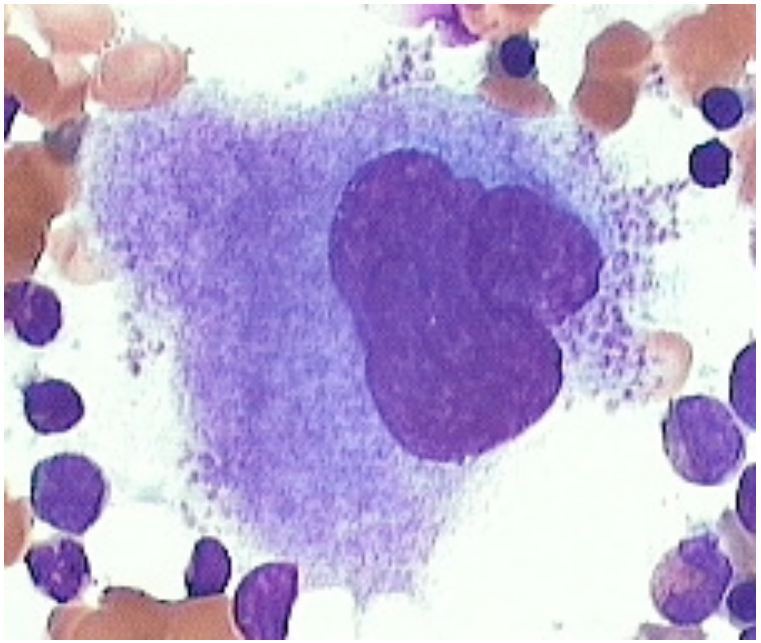
- When exposed after injury, platelets aggregate at the site by mediation of von Willebrand factor (vWF) that binds to both platelet receptors and collagen/subendo cells

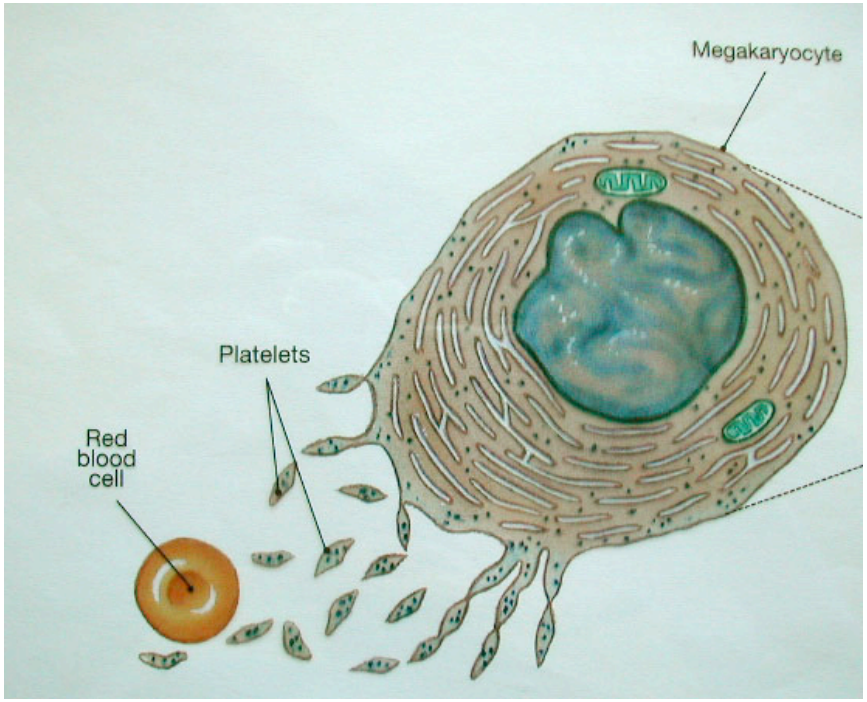
- vWF is a large, multimeric protein with subunits of 225 kD each

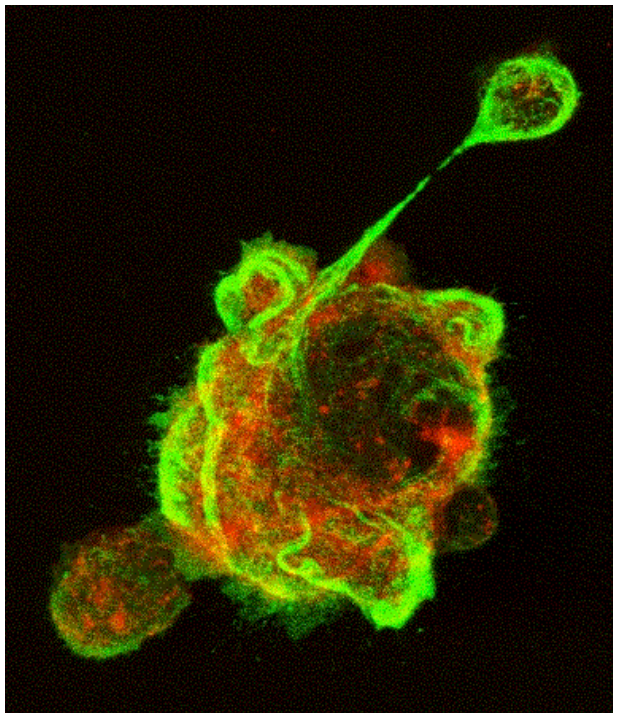
3) Platelets

- unpigmented, enucleated cells that are fragments of larger progenitor cells called megakarocytes (bone marrow).

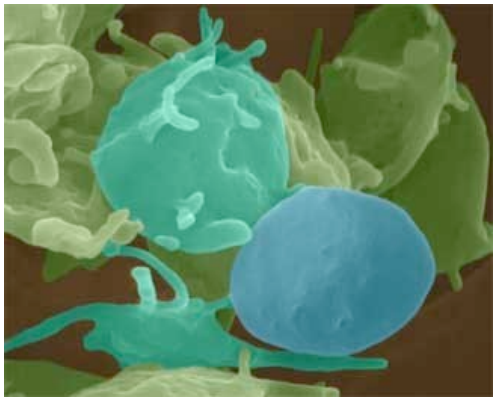
- Once bound release TxA2 and seratonin (and more!) that induce vasoconstriction to reduce blood flow and increase platelet aggregation.



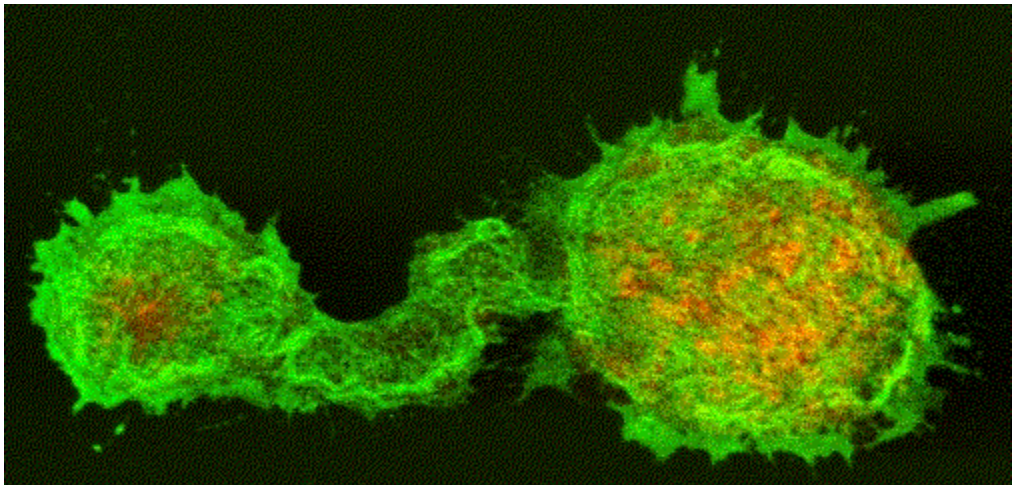




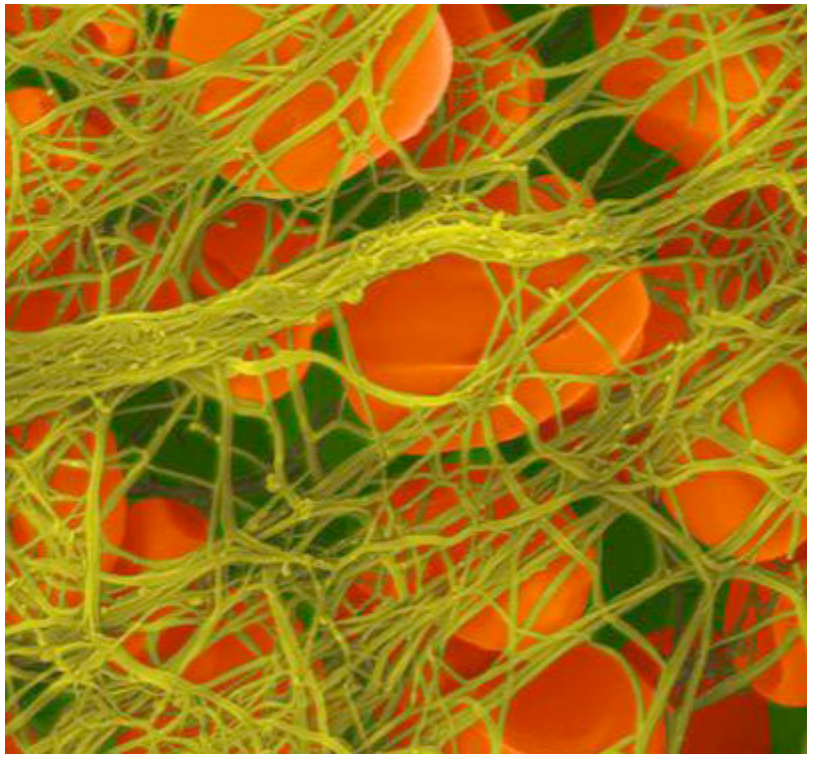
Platelets bleb off of megakarocytes



Platelets



4) Clotting Factors



- Soluble plasma proteins

- most made in liver

- most are serine proteases
  and circulate as
  zymogens

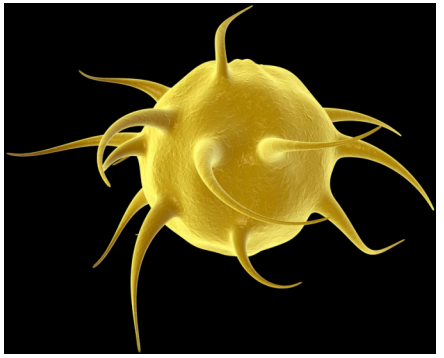
- cascade in which clotting factors are activated by
  selective proteolytic cleavage must have Ca^{2+}

Factor Number	Common Name
I	Fibrinogen
II	Prothrombin
III	Tissue Factor
IV	Ca2+
Va	Proaccelerin
VII	Proconvertin
VIII	Antihemophilic Factor
IX	Christmas Factor
X	Stuart Factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin Stabilizing Factor

* Step 1: Platelet Aggregation

- Platelet adhesion is mediated by vWF. This activates platelets causing release of TxA2

- During activation, a receptor for fibrinogen becomes exposed on the platelet membrane.



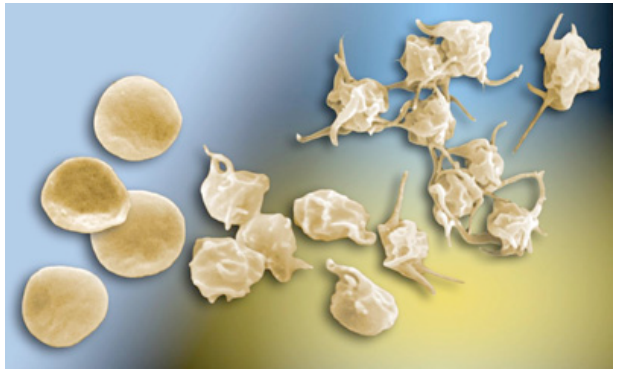
- Activated platelets release:

α-granules:
- Fibrinogen
- vWF
- Factor V
- Factor VIII
- Platelet derived growth factor (PDGF) ~ promotes healing
- Platelet factor IV – prevents formation of active thrombin inhibitor from heparin and anti-thrombin III.

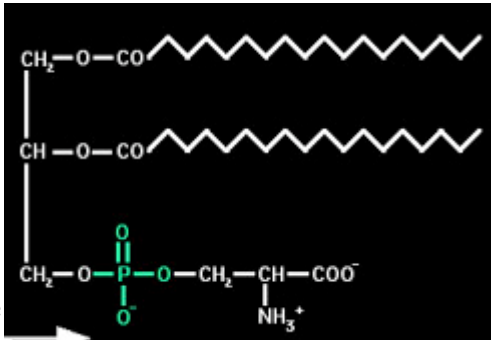
dense core granules:
- ADP/ATP
- Seratonin
- Ca^{2+}

Platelet activation also induces large morphological changes

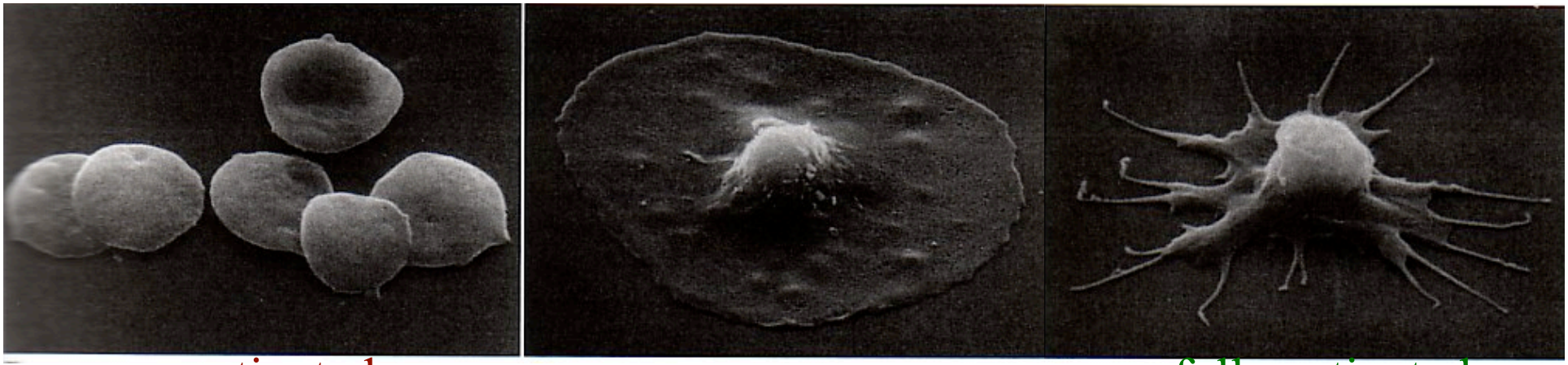
- membrane lipids rearrange



-phosphatidyl serine which is usually on the
inner membrane of the platelet,
flips out to outer membrane where it plays a role
in binding prothrombin.

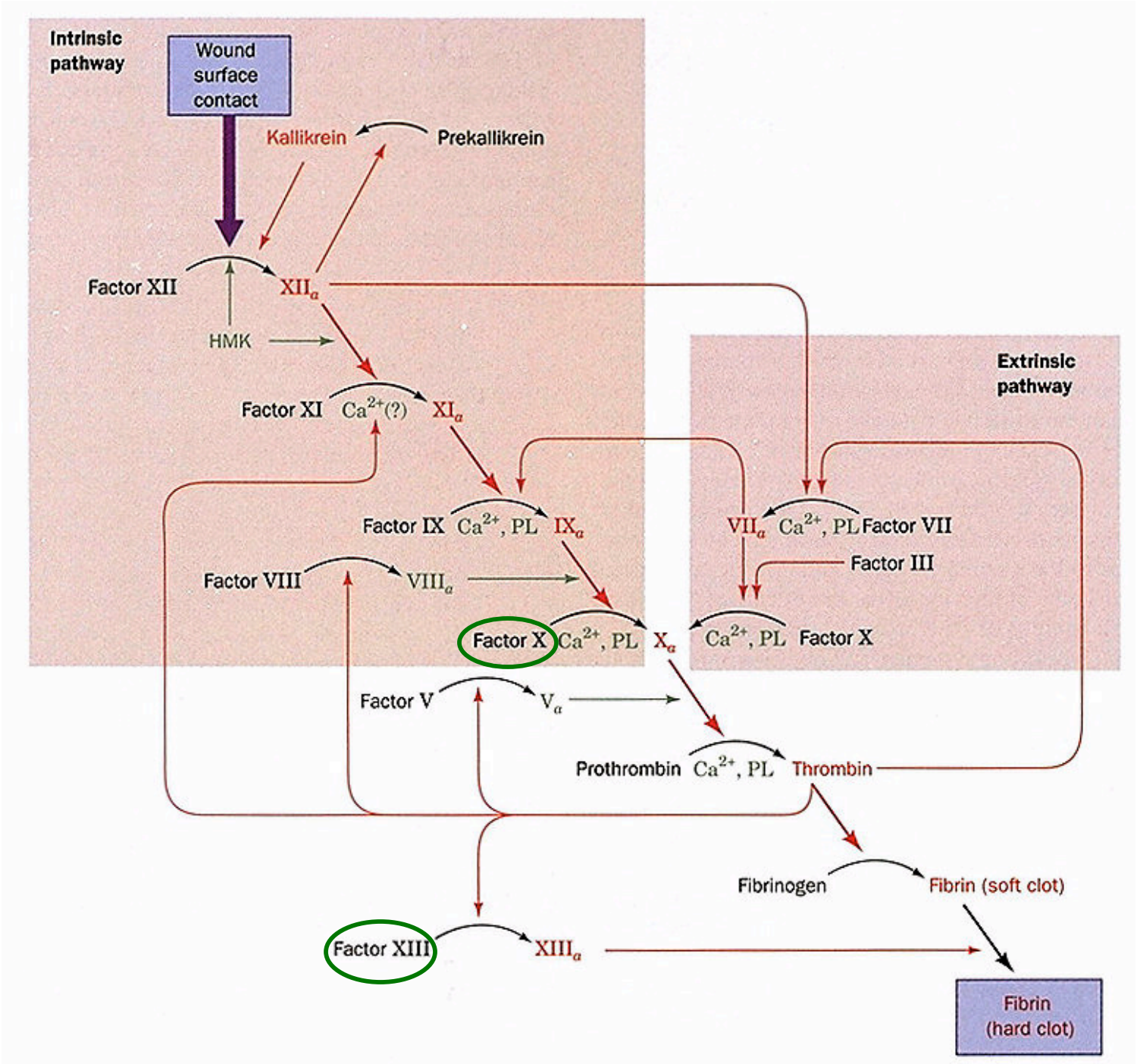


phosphatidyl serine



unactivated

fully activated


Intrinsic pathway
Wound surface contact
Kallikrein
Prekallikrein
Factor XII
XII_a
HMK
Factor XI
Ca^{2+}(?)
XI_a
Extrinsic pathway
Factor IX
Ca^{2+}, PL
IX_a
VII_a
Ca^{2+}, PL
Factor VII
Factor III
Factor VIII
$VIII_a$
Factor X
Ca^{2+}, PL
X_a
Ca^{2+}, PL
Factor X
Factor V
V_a
Prothrombin
Ca^{2+}, PL
Thrombin
Fibrinogen
Fibrin (soft clot)
Factor XIII
$XIII_a$
Fibrin (hard clot)

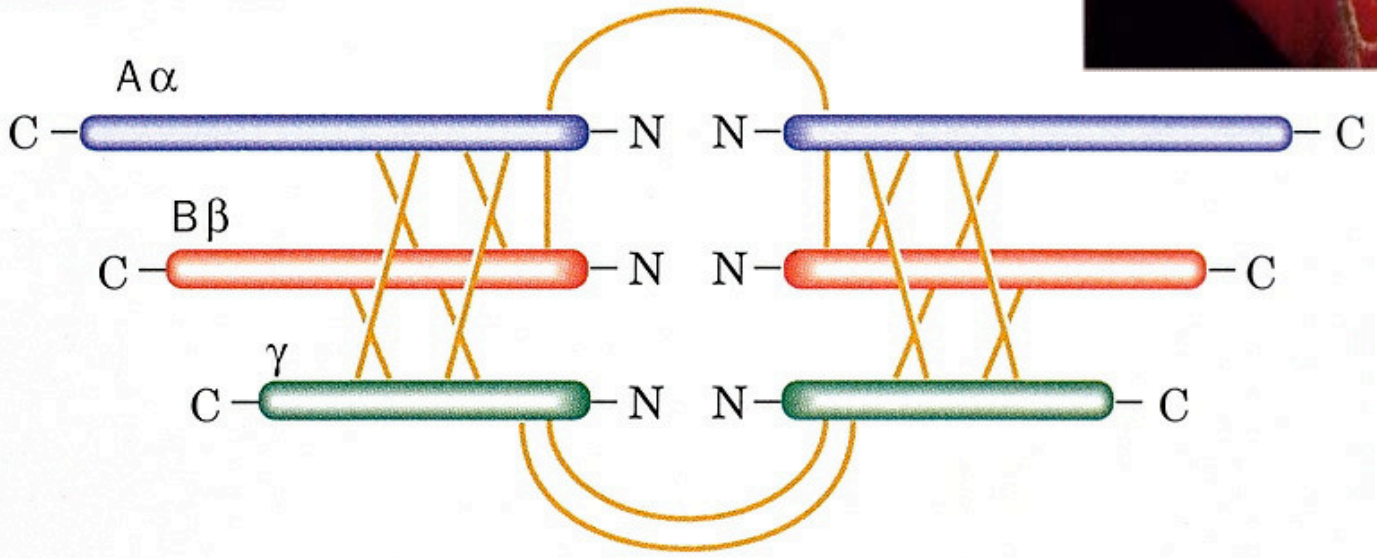
Step 2: Fibrin is formed by cleaving fibrinogen (factor I)

- Thrombin catalyzes the conversion of fibrinogen to fibrin
- Fibrinogen is 2-3% of plasma protein
- Fibrinogen has 3 pairs of non-identical subunits

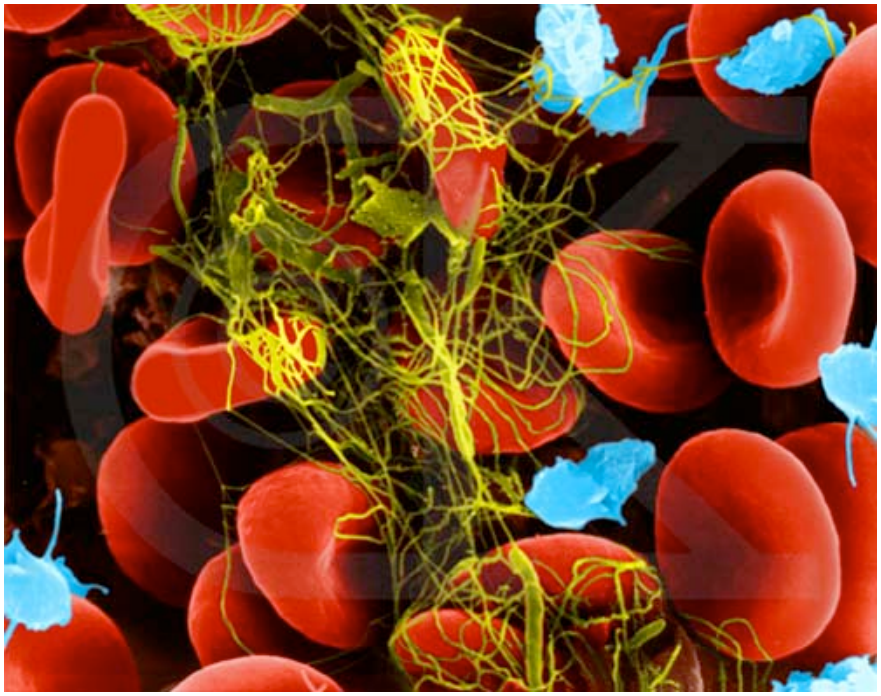
  Aα (610 res) Bβ (461 res) γ (411 res)

-Also two pairs of N-linked oligosaccharides

-A and B refer to parts of α and β released upon thrombin cleav. A (20 aa) ; B (18 aa)



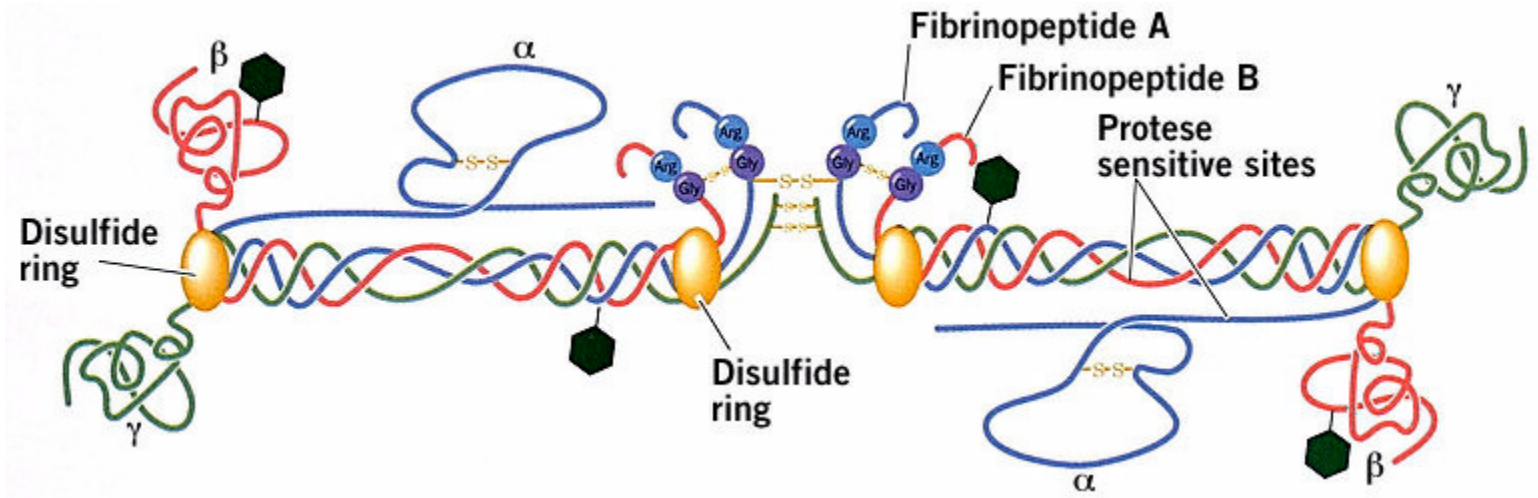
Once cleaved, fibrin is insoluble



So how do A and B confer solubility?

1) A & B mask sites that mediate aggregation

2) A & B are highly anionic and so repel other fibrinogen molecules

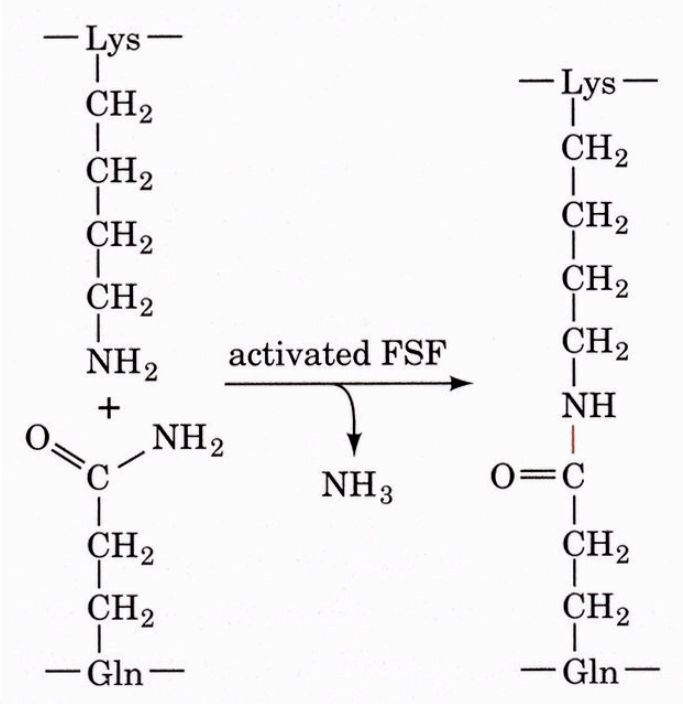


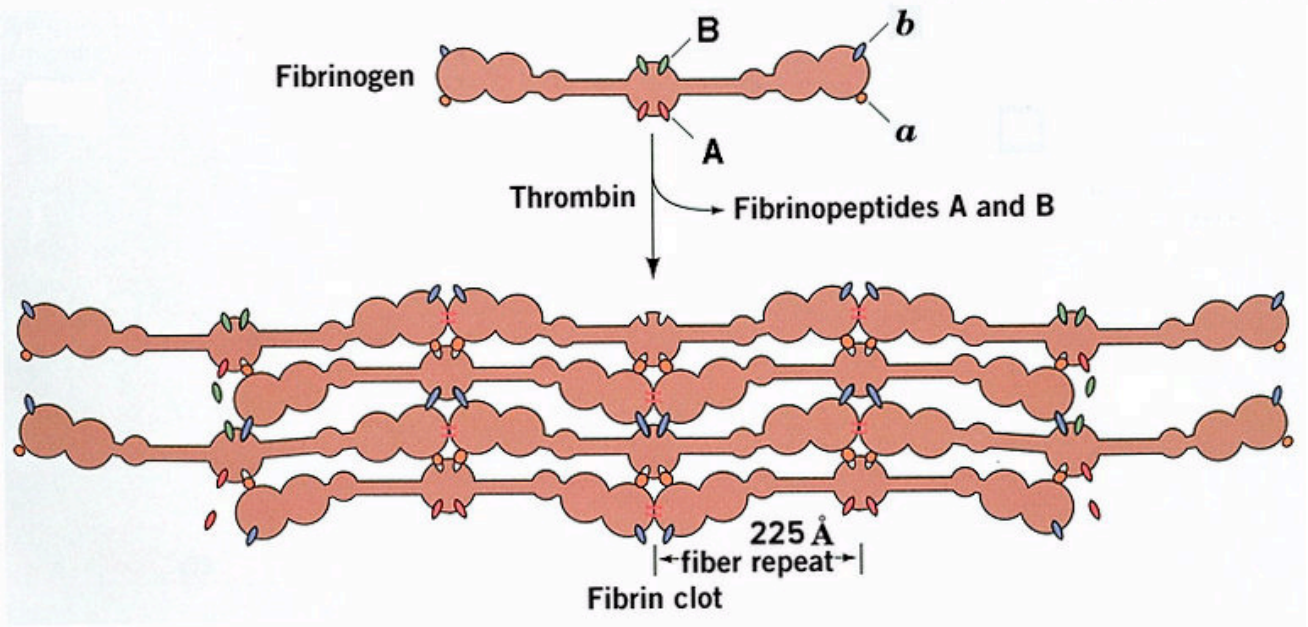
*Step 3: Conversion of soft fibrin clots to hard clots

- Involves crosslinking of neighboring fibrin molecules

- Catalyzed by fibrin-stabilizing factor (fsf or factor XIIIa)

- Joins C-term segments of γ chains by forming isopeptide bonds between Gln on one γ and a Lys on the other.



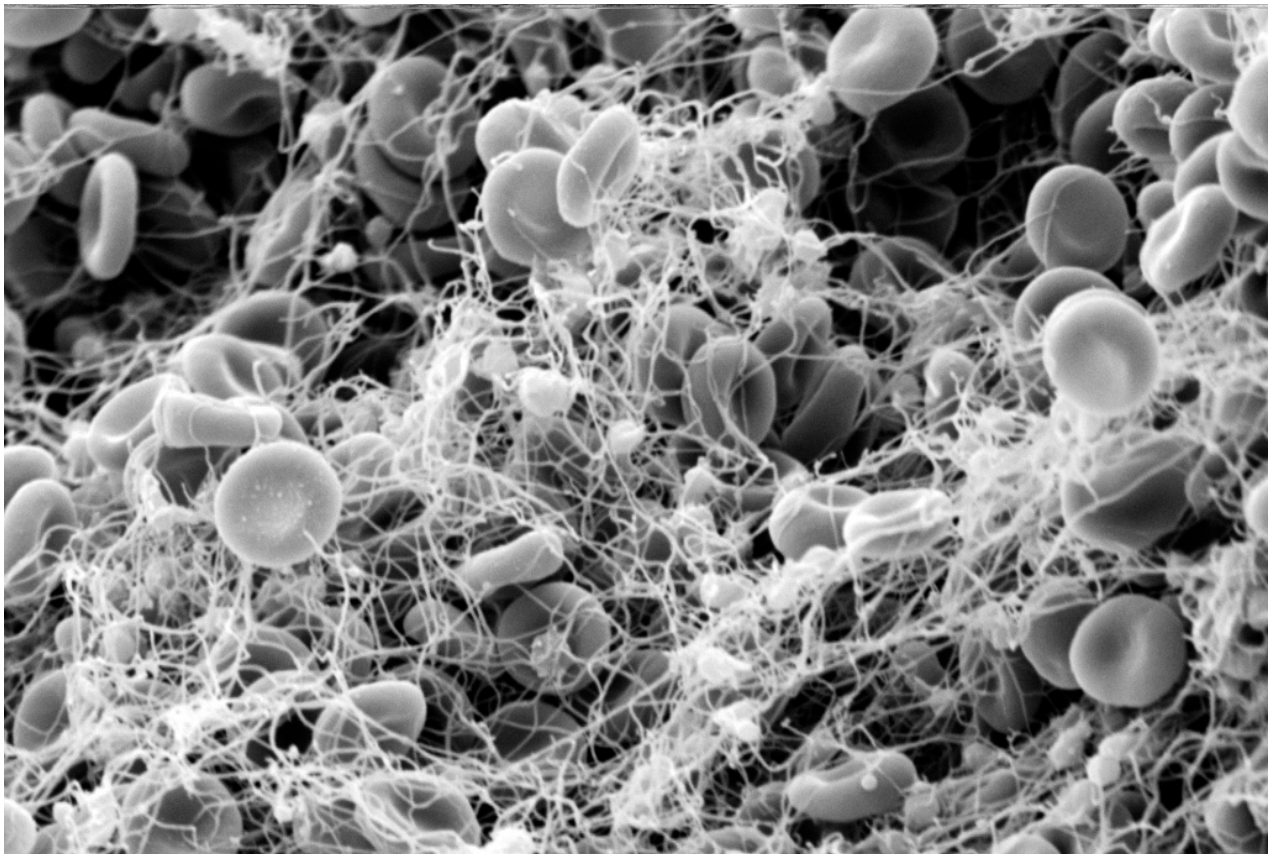


- α are linked also but slower

- If decreased FSF, have increased bleeding

- FSF in both platelets and plasma occur as zymogen activated by thrombin

- Thrombin cleaves an Arg-Gly bond near the N-terminus of FSF

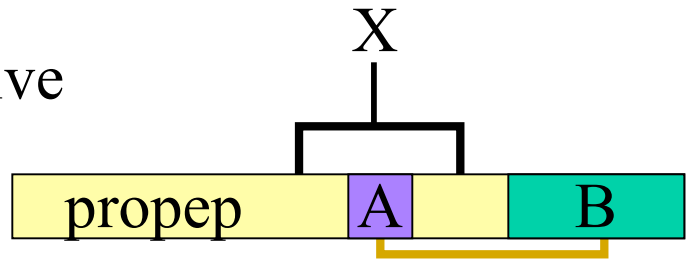


Overview of Thrombin Activation

Thrombin needs to active only locally, at site of injury

Circulates in plasma as prothrombin – single chain of 582 aa
(almost twice the size of active thrombin)

Monomer in zymogen but a dimer when active
A chain (36aa) and B chain (259aa)
connected by disulfide bond

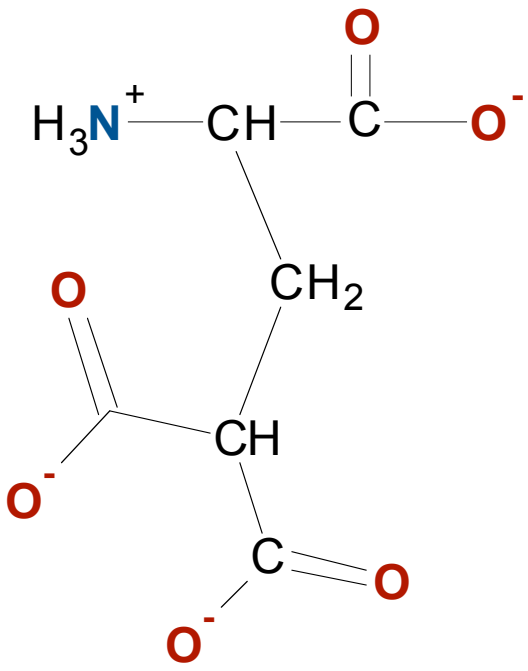


Prothrombin is cleaved by factor X twice
Arg271 – Thr 272 releases N-term propeptide
Arg320 – Ile 321 separates A and B chains

Second cleavage activates, allowing an ion pair
NH_4^+ of Ile321 and Asp524

Propeptide of Prothrombin

- Propeptide has 3 domains



1) N-term 40 residue Gla domain
   - Gla = γ carboxyglutamate

   - Strong Ca^{2+} chelator

   - Bind Ca $^{2+}$ that mediates interaction with phospholipid membrane of platelet

$H_3\overset{+}{N}$–^{1}Ala – Asn – Thr – Phe – Leu – Gla – Gla – Val – Arg – Lys ^{10}–

^{11}Gly – Asn – Leu – Gla – Arg – Gla – Cys – Val – Gla – Gla ^{20}–

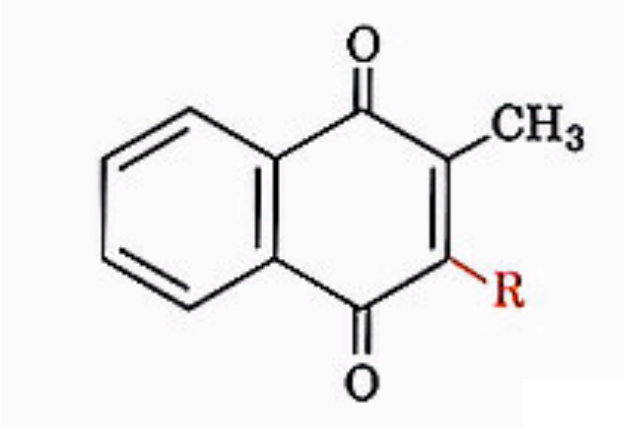
^{21}Thr – Cys – Ser – Tyr – Gla – Gla – Ala – Phe – Gla – Ala ^{30}–

^{31}Leu – Gla – Ser – Ser – Thr – Ala – Thr – Asp – Val – Phe ^{40}–

Vitamin K as a Cofactor

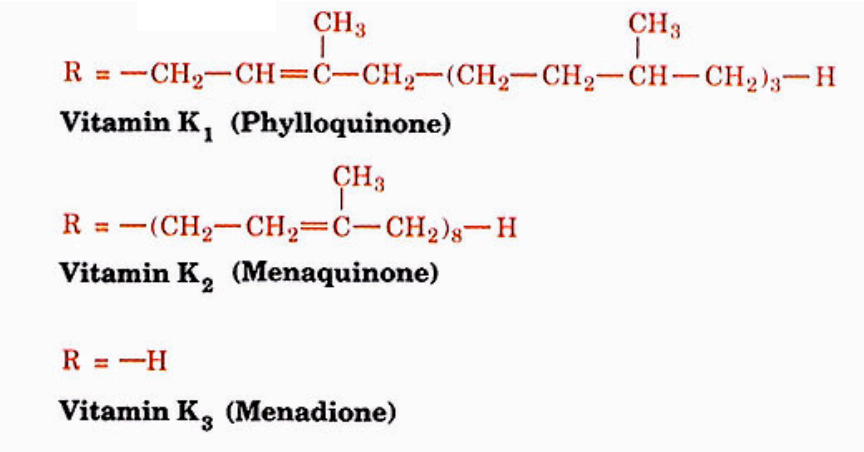
- Vit K is essential cofactor for proper prothrombin synthesis

- Must have in diet or no clotting



- Prothrombin is made in liver

- Made without Vit K but only 1-2% active. Reason is that Vit K is a necessary cofactor for post-translational modification of prothrombin in ER



-Needed for Glu conversion to Gla

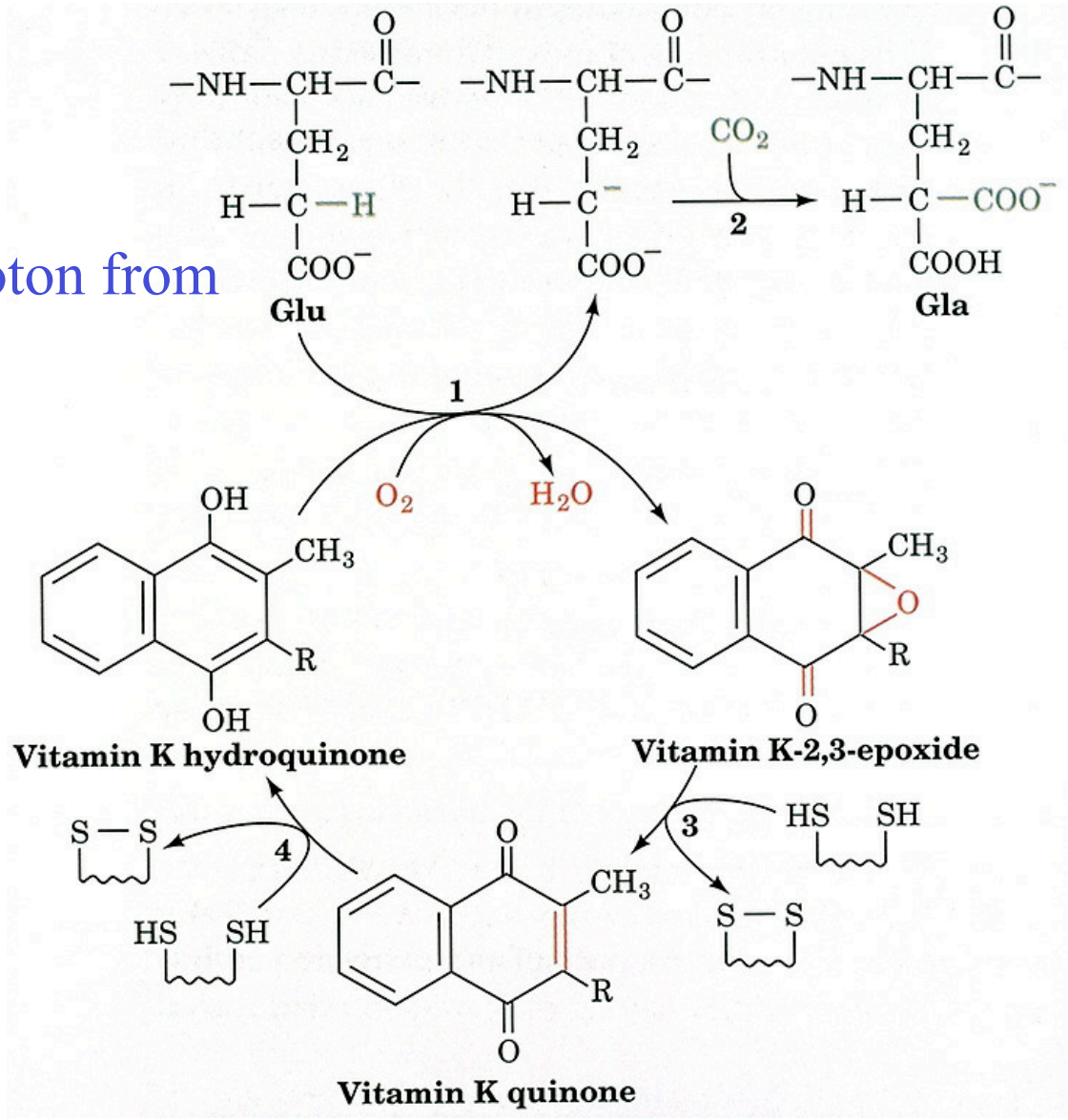
Vitamin K cofactor in Glu to Gla conversion

Conversion of Glu to Gla

1) Vitamin K extracts a γ proton from Glu, yielding a carbanion

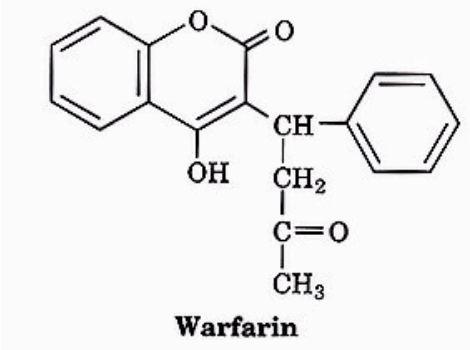
2) The carbanion then reacts with CO_2 to yield Gla

3 & 4) Vitamin K is regenerated



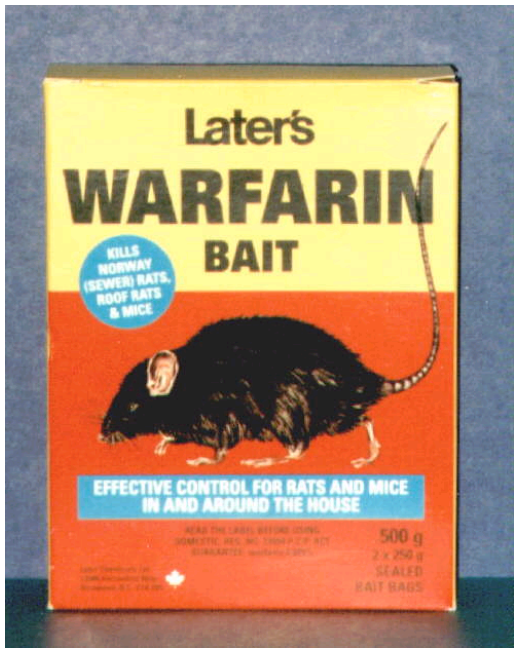
Inhibitors of Vitamin K Regeneration

warfarin (rat poison)

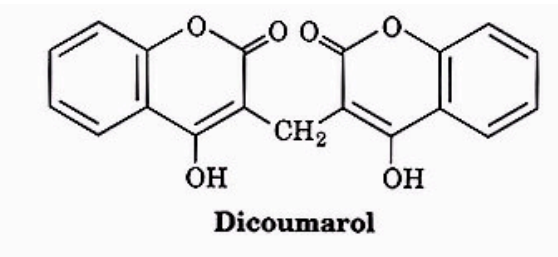


Warfarin

- Take awhile to take effect because turnover of coagulation proteins is relatively slow (~5-7 days).
- Wont affect prots synthesized before ingestion



dicoumarol (spoiled sweet clover)



Dicoumarol

Isozymes of Lactate Dehydrogenase

Garrett/Grisham, Biochemistry with a Human Focus
Figure 10.26