Mechanisms of Enzyme Catalysis

CHEM 420 – Principles of Biochemistry
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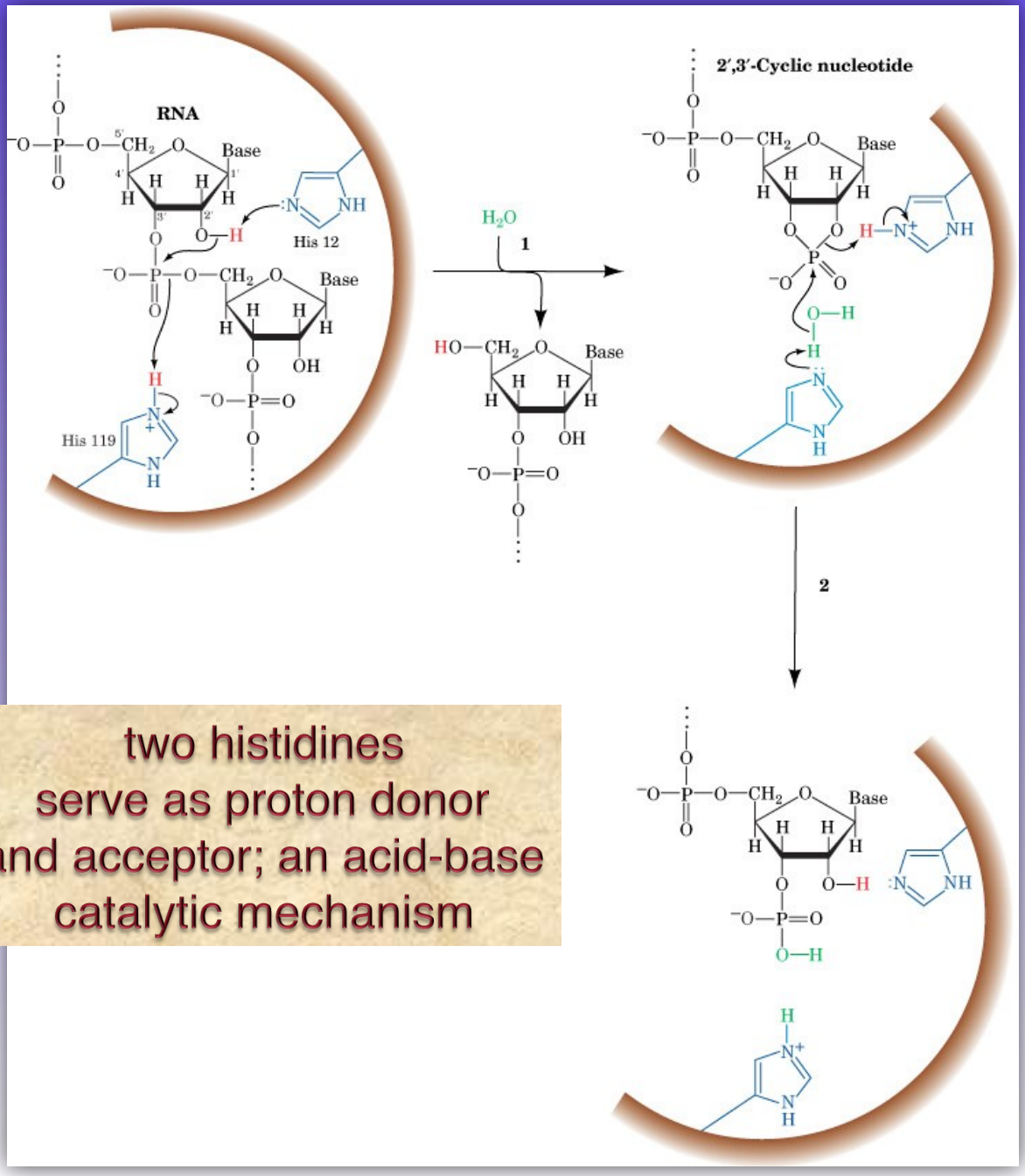
Chapter 15: Voet/Voet, *Biochemistry*, 2011
Fall 2015

October 9 & 12

Endonuclease mechanism
(acid-base catalysis)



two histidines serve as proton donor and acceptor; an acid-base catalytic mechanism

Mechanism of RNase A

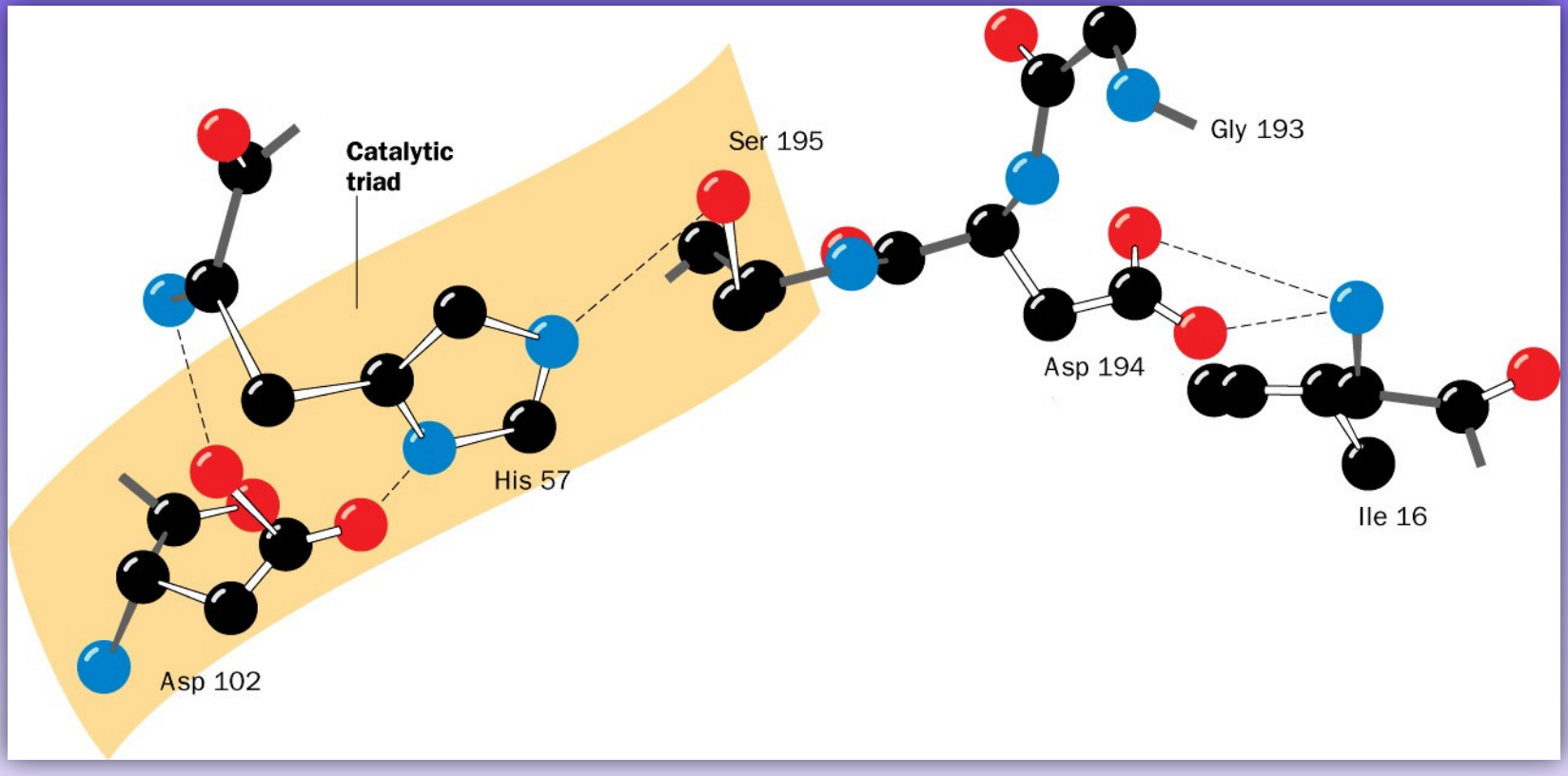
Bovine pancreatic RNase A catalyzed hydrolysis of RNA is a two-step process involving the formation of a 2′,3′-cyclic nucleotide intermediate.

Serine protease mechanism
(covalent catalysis)

Examples of serine proteases

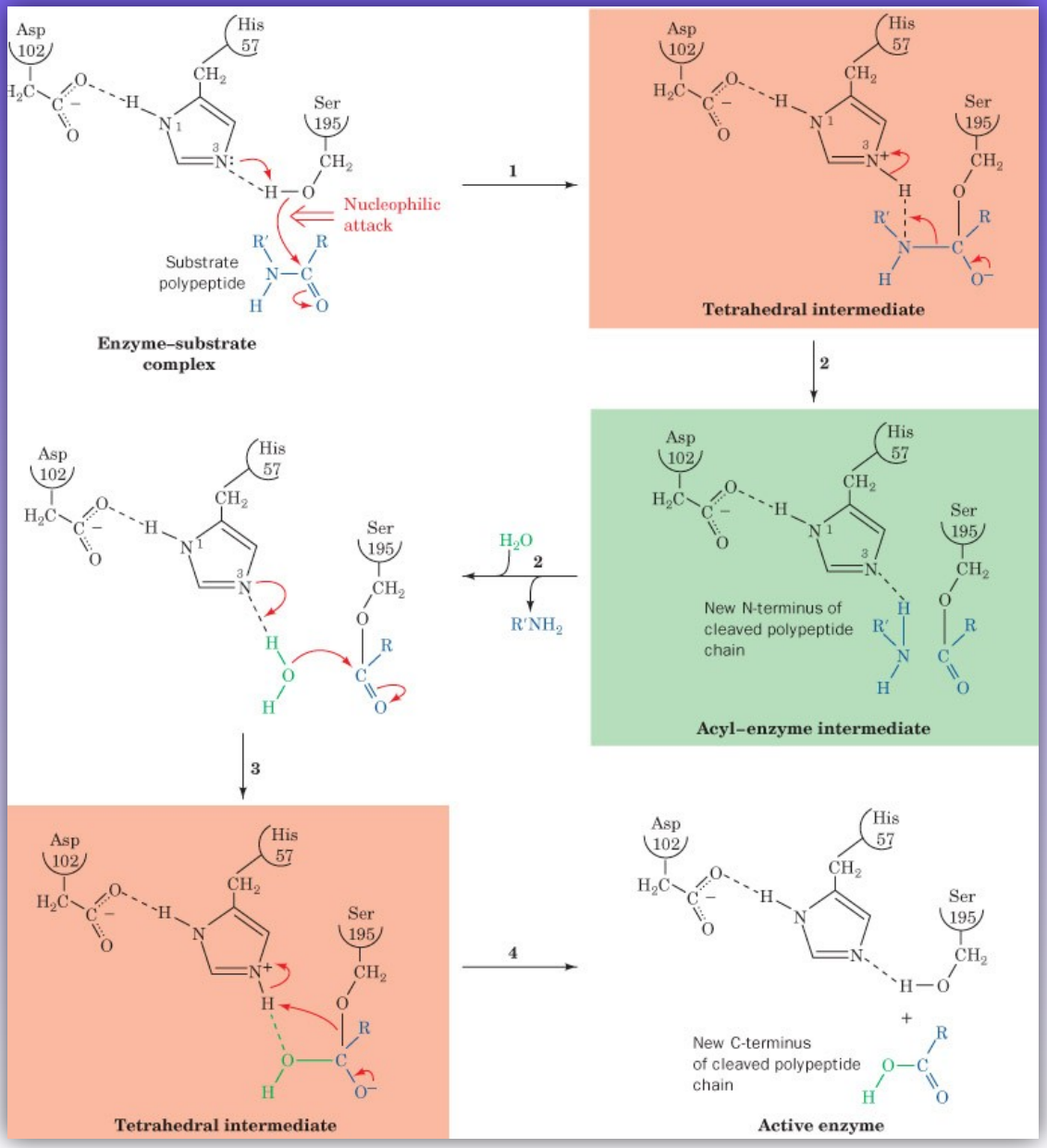
Enzyme	Source	Function
Trypsin	Pancreas	Digestion of proteins
Chymotrypsin	Pancreas	Digestion of proteins
Elastase	Pancreas	Digestion of proteins
Thrombin	Vertebrate serum	Blood clotting
Plasmin	Vertebrate serum	Dissolution of blood clots
Kallikrein	Blood and tissues	Control of blood flow
Complement C1	Serum	Cell lysis in the immune response
Acrosomal protease	Sperm acrosome	Penetration of ovum
Lysosomal protease	Animal cells	Cell protein turnover
Cocoonase	Moth larvae	Dissolution of cocoon after metamorphosis
α-Lytic protease	*Bacillus sorangium*	Possibly digestion
Proteases A and B	*Streptomyces griseus*	Possibly digestion
Subtilisin	*Bacillus subtilis*	Possibly digestion

Source: Stroud, R.M., *Sci. Am.* **231**(1), 86 (1974).



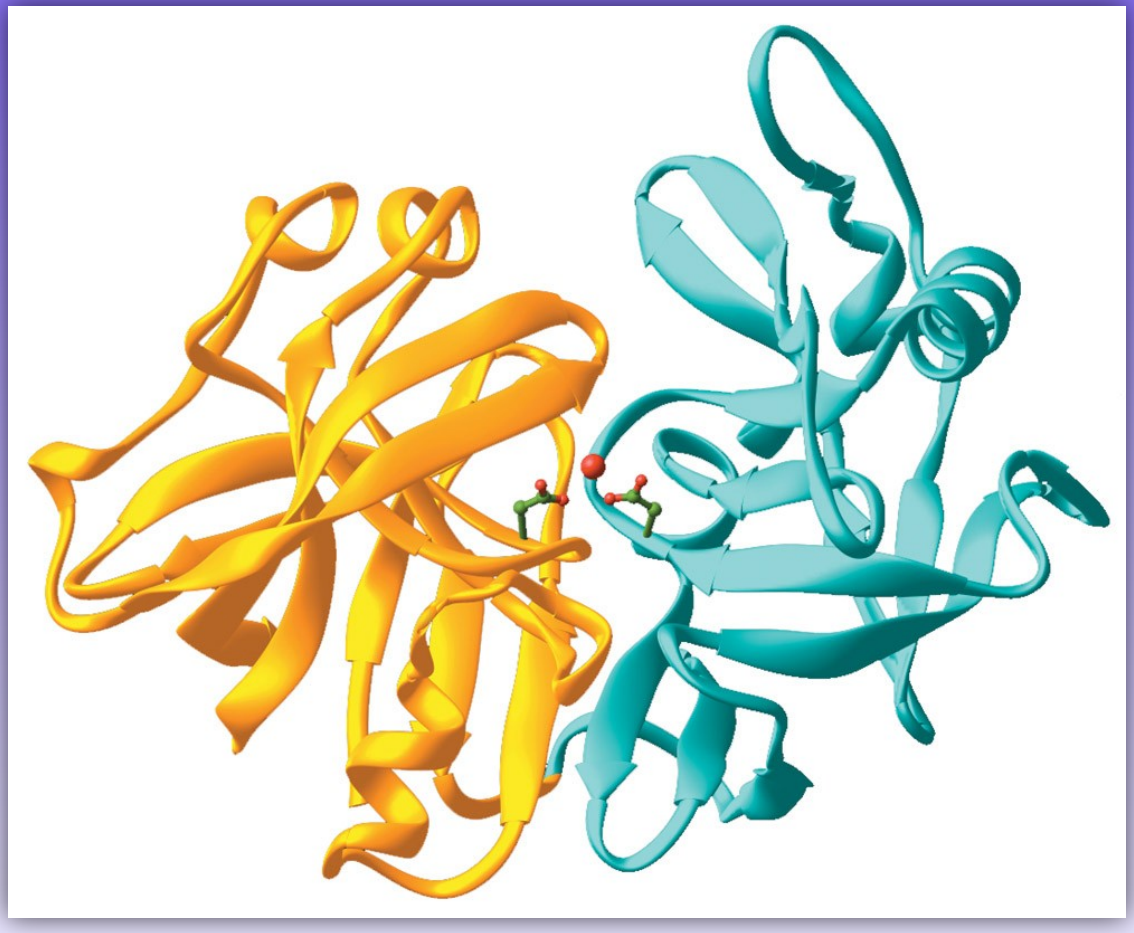
The active site residues of chymotrypsin
The catalytic triad is common to all serine proteases.

Catalytic mechanism of serine proteases

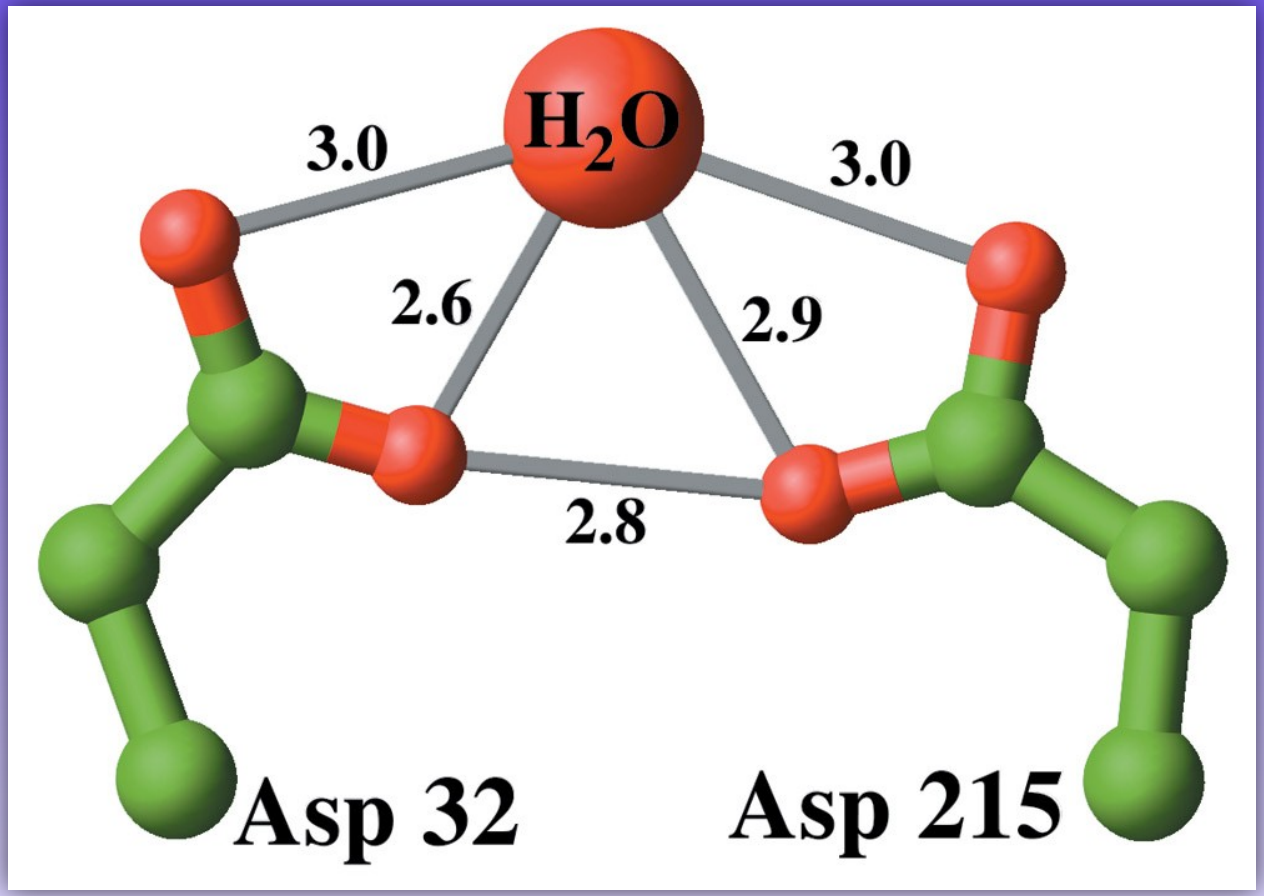


Aspartic protease mechanism
(acid-base catalysis)

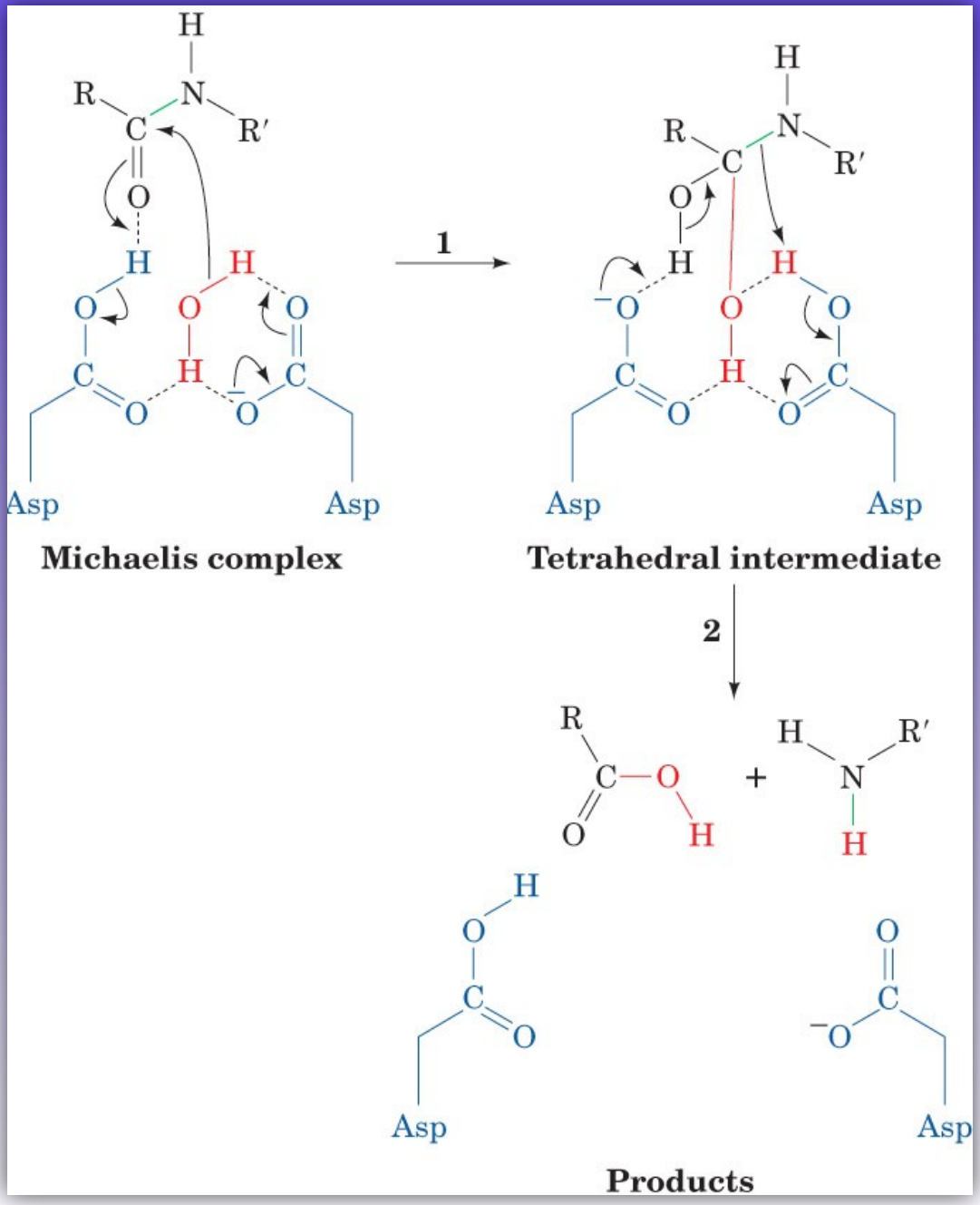
Pepsin - an aspartic protease



X-ray structure of pepsin (two domains)



The active site Asp residues in pepsin and the bound water molecule indicating the lengths (in Å) of possible hydrogen bonds (*gray*).



Michaelis complex

Tetrahedral intermediate

Products

Catalytic mechanism of aspartic proteases

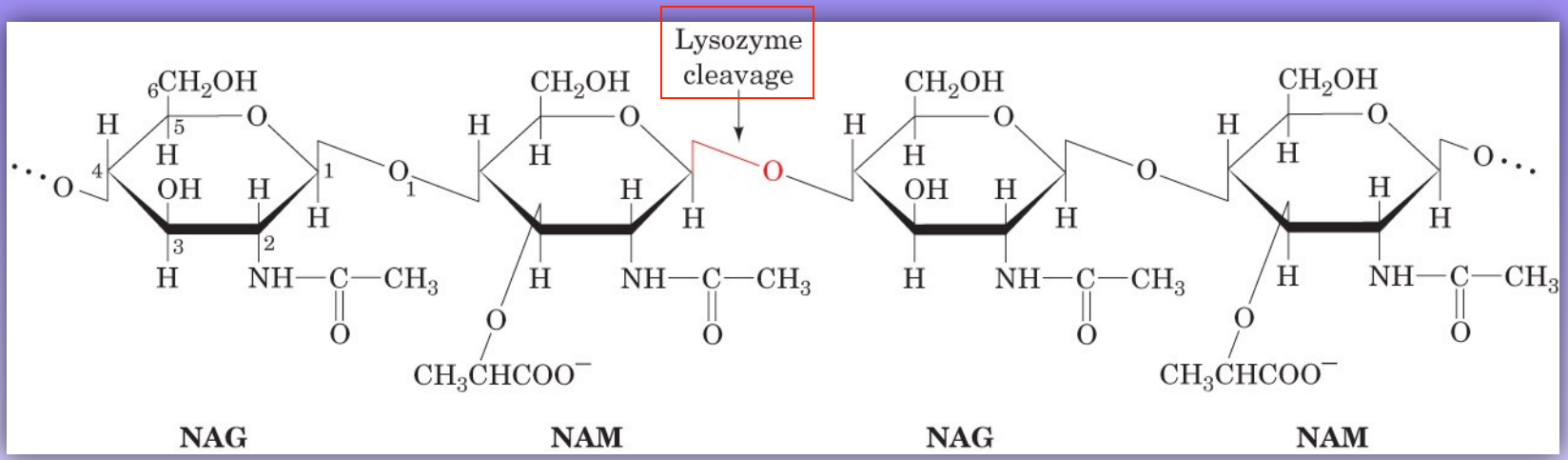
HIV-1 protease is an aspartic protease.

Glycosidase mechanism

(strain; acid-base catalysis; covalent catalysis)

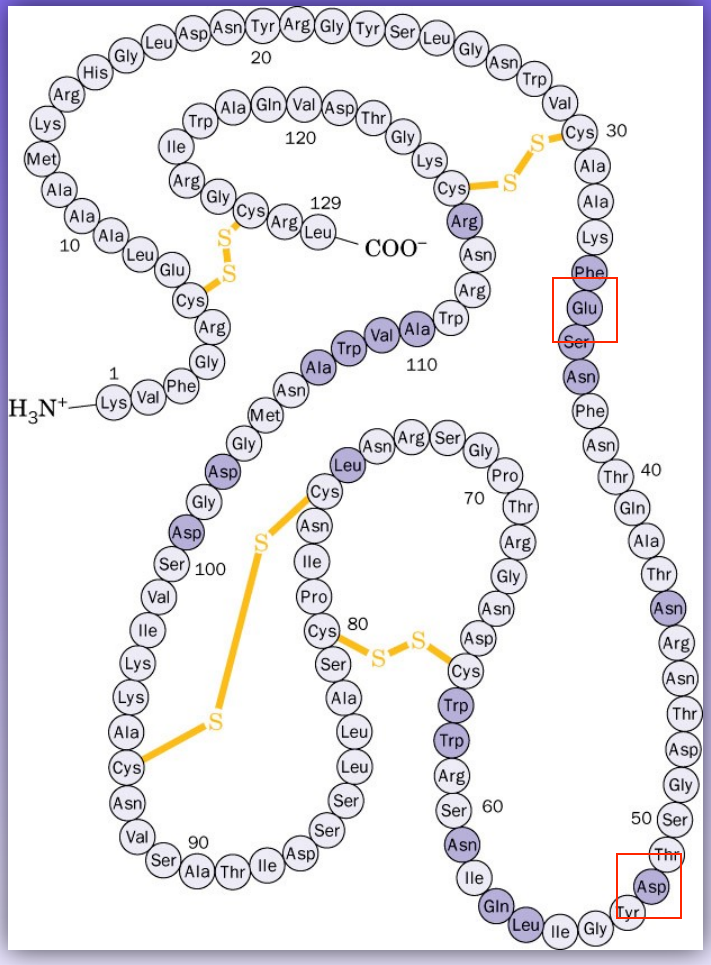
Lysozyme – a glycosidase

The substrate: The NAG-NAM polysaccharide of bacterial cell peptidoglycans (also hydrolyzes chitin in fungal cell walls)

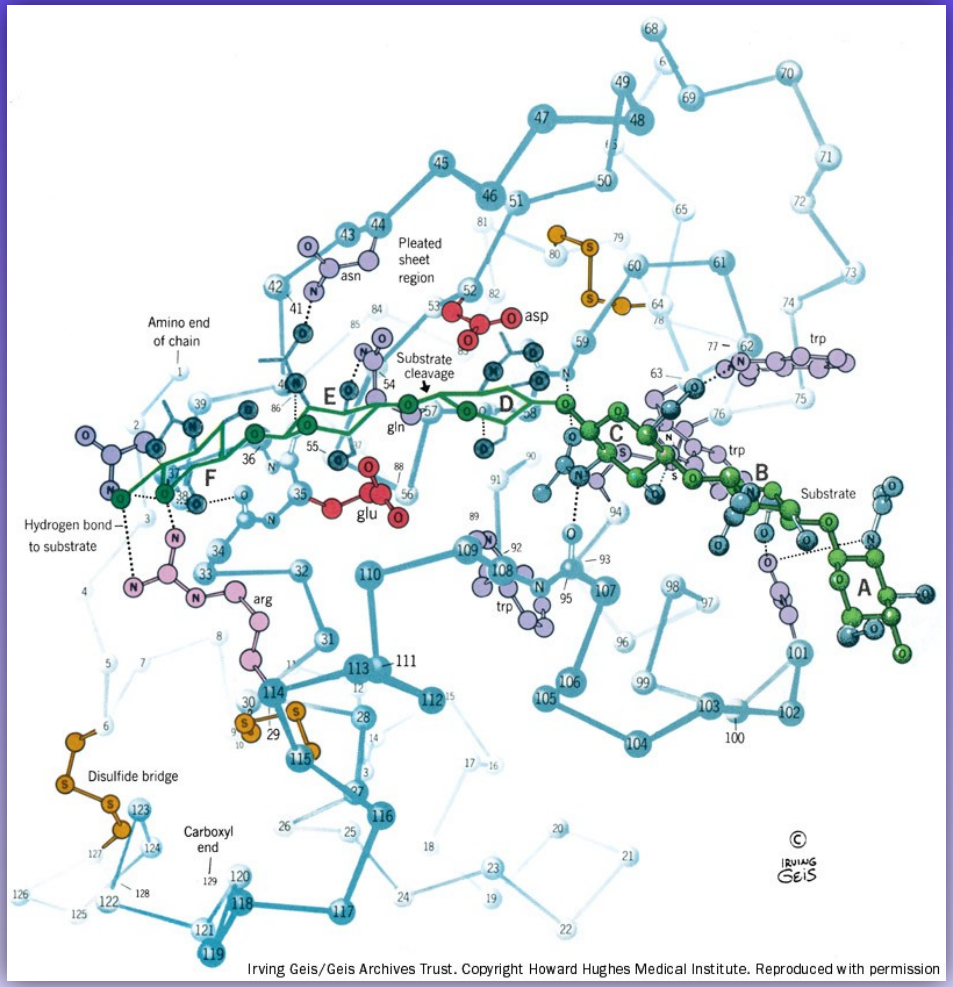


NAM = *N*-acetylmuramic acid (a GlcNAc residue to which has been attached L-lactic acid in ether linkage at O3)

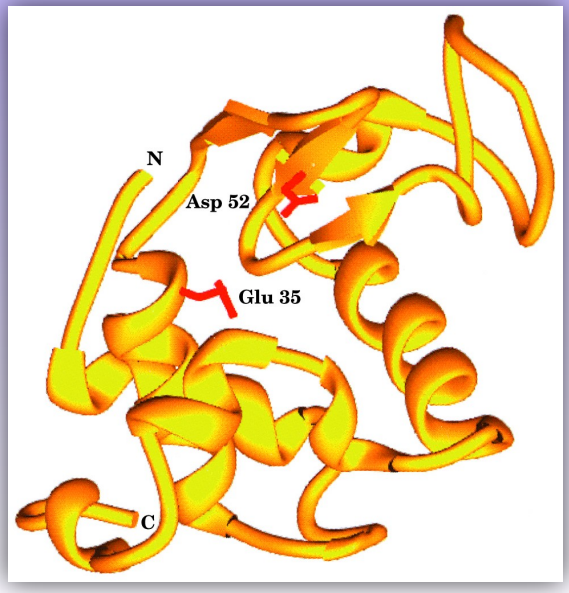
Lysozyme hydrolyzes the βNAM (1 → 4)-βNAG glycosidic linkage.



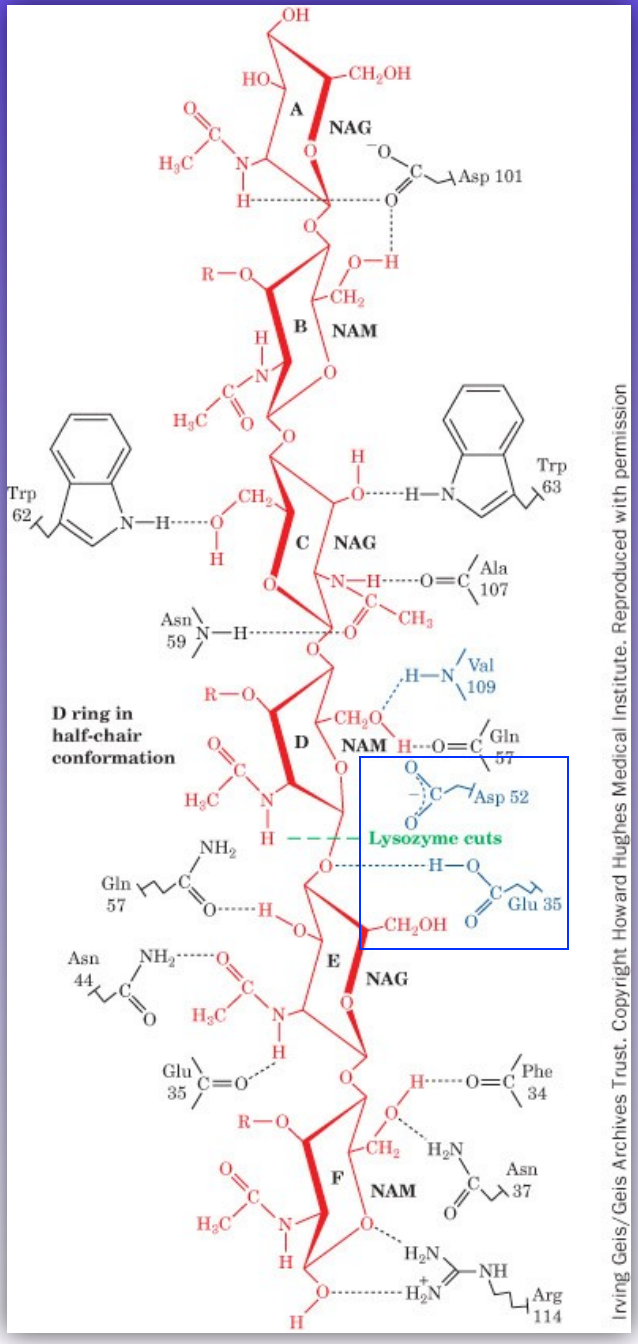
Primary structure of hen egg white (HEW) lysozyme (129 residues). Residues that comprise the substrate binding site are shown in dark purple. Protein is stabilized by four disulfide bonds (common for secreted proteins). Note the location of the two catalytic residues, Glu 35 and Asp 52 (surrounded by red boxes).



$(NAG)_6$ binding by HEW lysozyme. The enzyme contains a prominent cleft that accommodates binding of the hexasaccharide. Hexasaccharide residues are denoted A-F, with cleavage occurring between residues D and E.

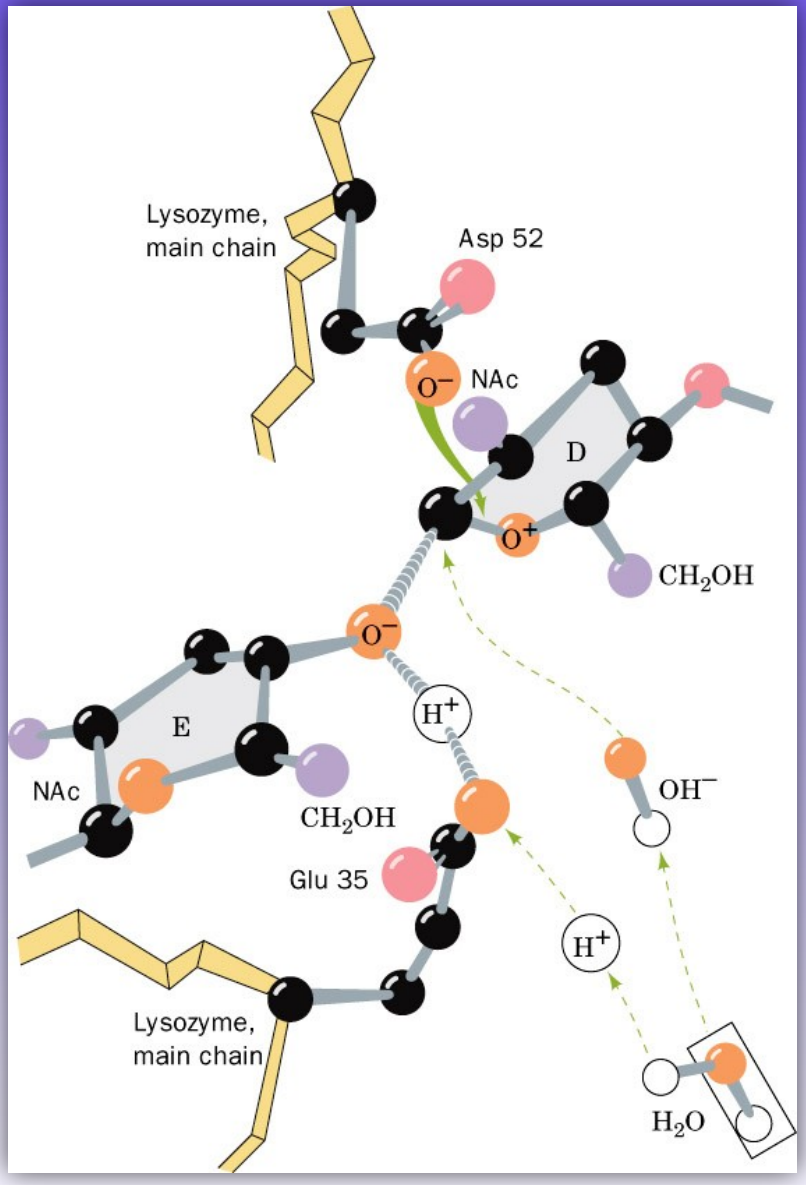


Ribbon diagram of HEW lysozyme



Lysozyme interactions with substrate.

Multiple H-bonds secure the (NAG-NAM)$_3$ hexasaccharide to the catalytic site, positioning the NAM-NAG glycosidic linkage joining residues D and E in proximity to the acidic side-chains of Glu 35 and Asp 52. The enzyme uses these acidic functionalities to hydrolyze the intrinsically acid-labile acetal.



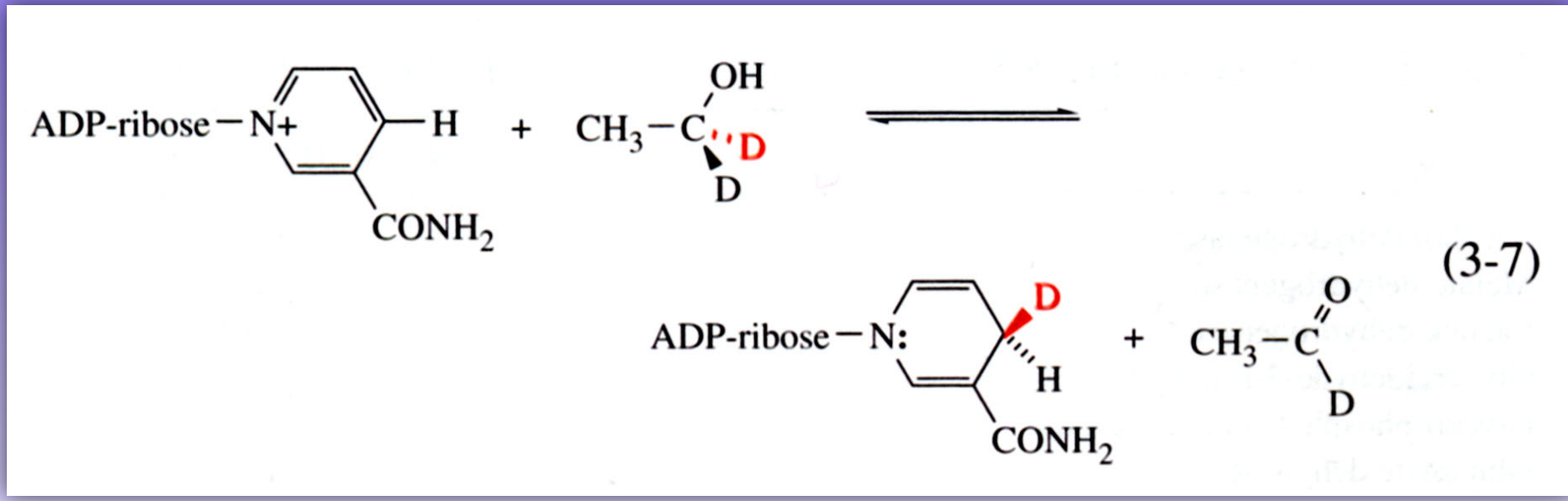
The Phillips mechanism of glycoside hydrolysis by lysozyme. Glu 35 protonates the linkage oxygen. The resulting D-ring oxonium cation is stabilized by proximity to the negatively-charged Glu 52 side-chain and by enzyme-induced distortion of the D-ring. Upon release of the E-ring, solvent H_2O provides OH^- and H^+ that react with the oxonium ion and re-protonate Glu 35, respectively. Note that in this mechanism, the Glu 35 side-chain is protonated, and the Glu 52 side-chain is deprotonated in the active form of the enzyme.

The Phillips (strain) mechanism has been recently challenged by a covalent mechanism supported by studies of fluorinated substrates (Stephen Withers; see Voet/Voet, 4th edition, 2011, pages 523-525).

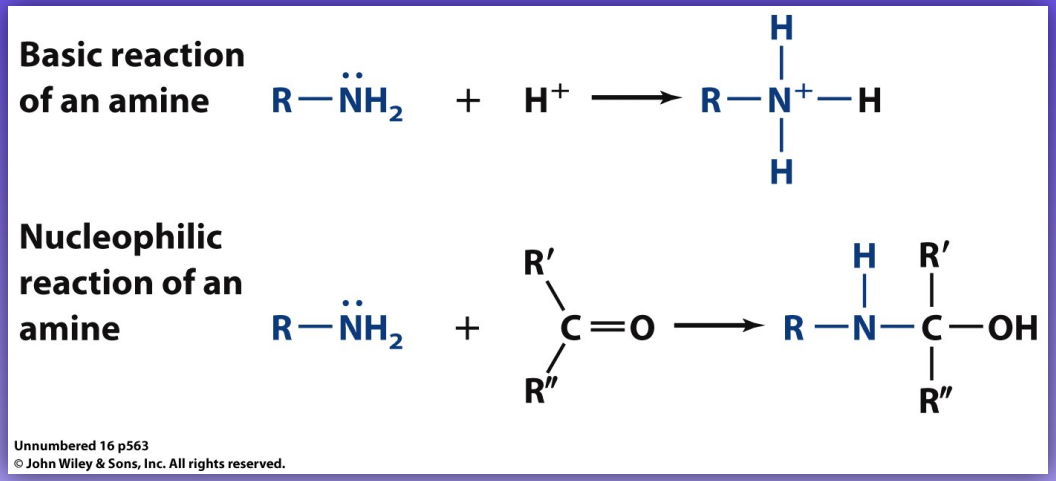
Prochirality

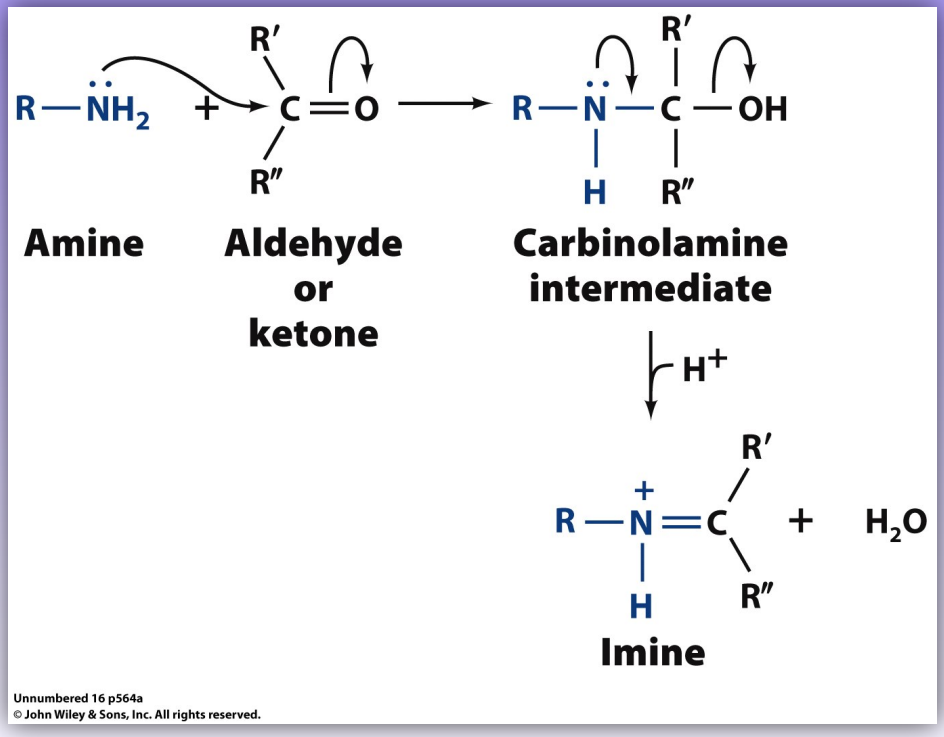
Stereospecific reduction of NAD^+

Prochiral recognition by alcohol dehydrogenase (ADH; EC 1.1.1.1)



The reduction of NAD^+ with 1,1-dideuteroethanol as the substrate gives 4*R*-[nicotinamide-4-$^2H_2$]NADH as product; the ^2H is transferred specifically to the 4-pro-*R* site of NAD^+.

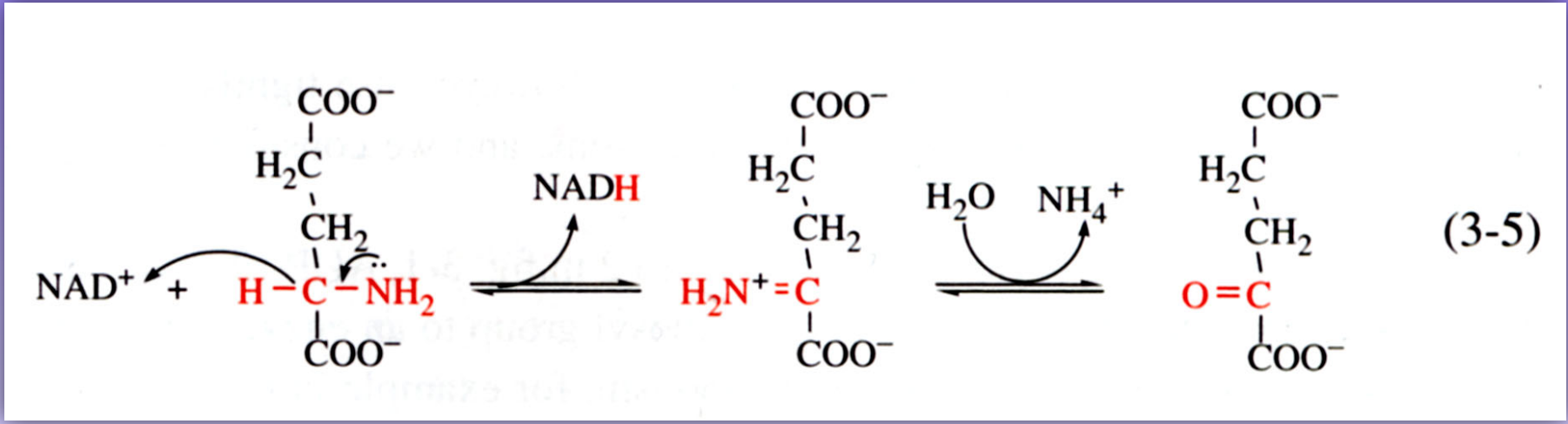




Involvement of iminium cations in enzyme catalysis

Protonated imines = activated carbonyls

L-glutamate dehydrogenase (EC 1.4.1.3): Conversion of an α-amino acid into an α-ketoacid (α-ketoglutarate)



Note the formation of an iminium cation (protonated imine) intermediate that subsequently undergoes hydrolysis to form the α-ketoacid.

This reaction is an example of oxidative deamination.

Potentiation

Ornithine cyclodeaminase – biosynthesis of L-proline

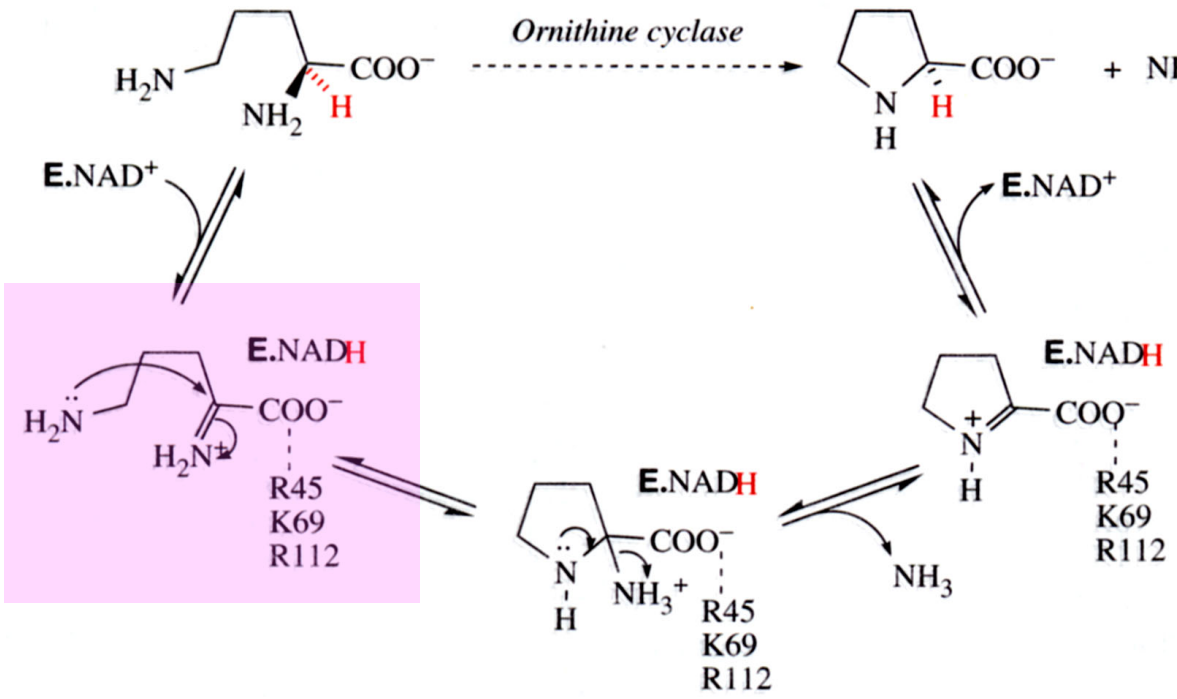


Fig. 3-7. Hypothetical mechanism for NAD⁺-dependent cyclization of ornithine into proline.

The first step involves the formation of a protonated imine (iminium cation) intermediate.

Mechanism of post-translational modification of proteins: γ-carboxyglutamylation

A blood-clotting cascade

Carboxylation of prothrombin: vitamin K-dependent

↓

Ca^{2+}-prothrombin + factors Va and Xa:
bind to membrane surface

↓

Cleavage of prothrombin by factor Xa gives thrombin

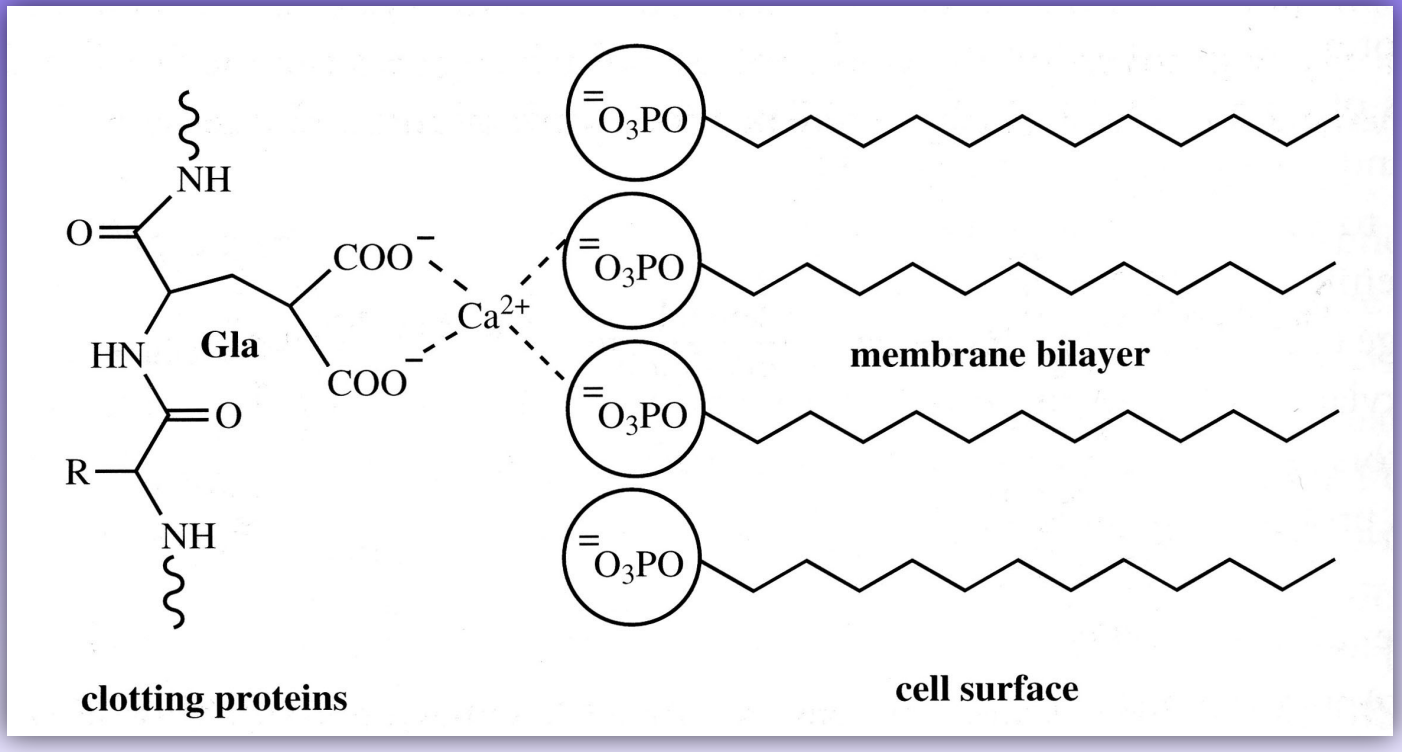
↓

Thrombin cleaves fibrinogen to fibrin

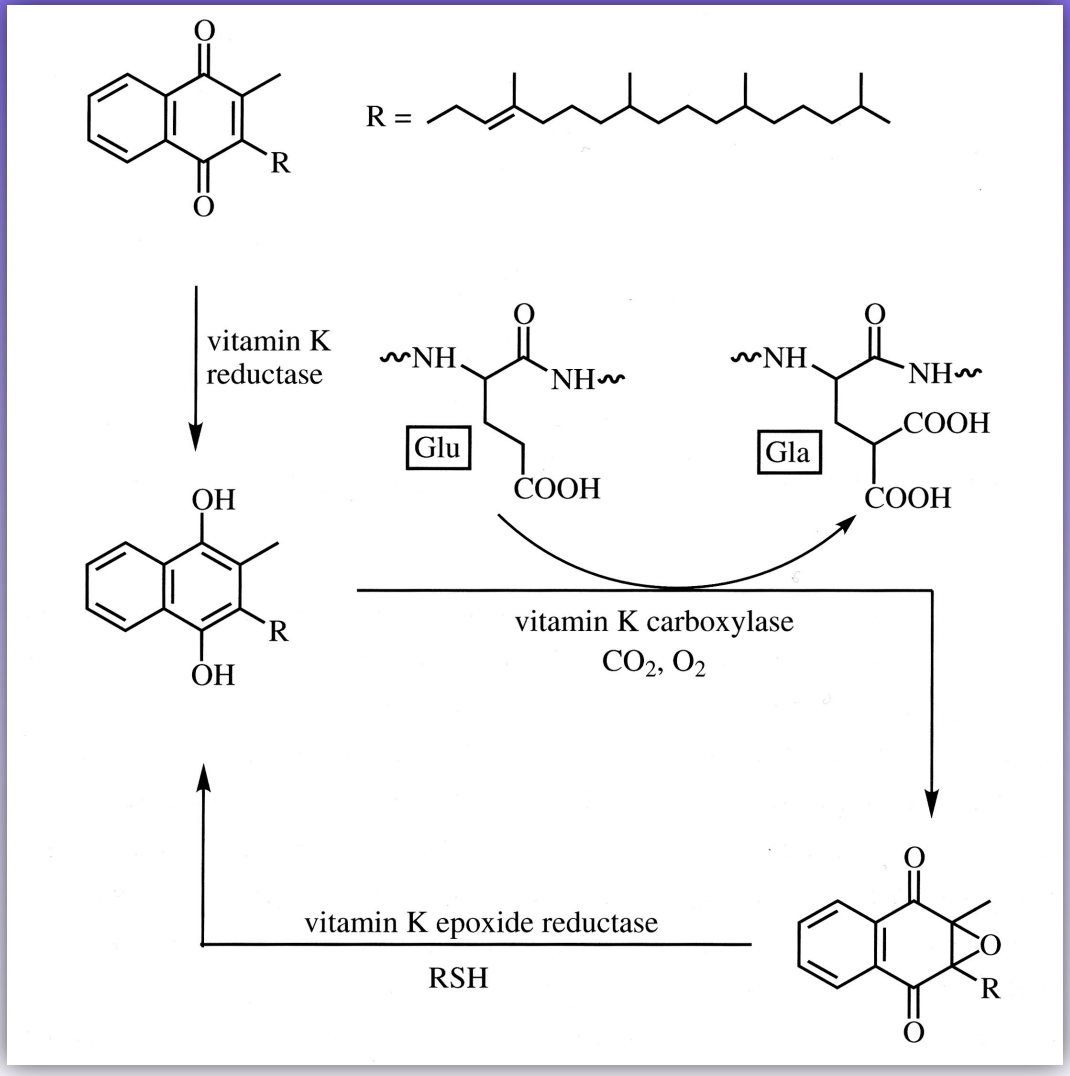
↓

Fibrin induces blood-clotting

Calcium-dependent binding of clotting proteins to cell surfaces

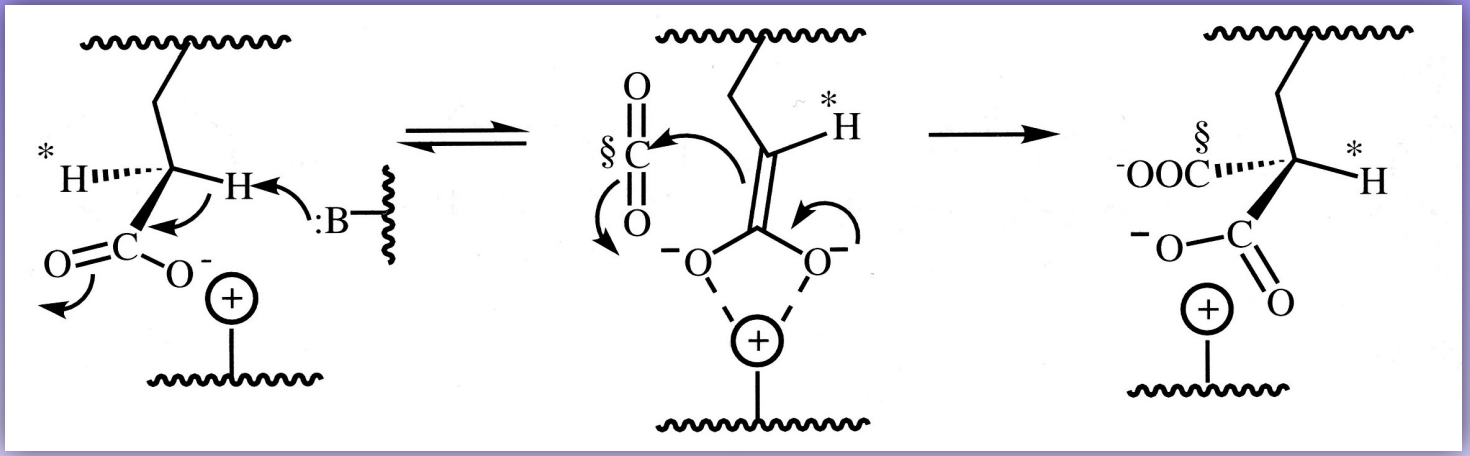


Vitamin K-dependent γ-carboxyglutamylation of blood-clotting proteins



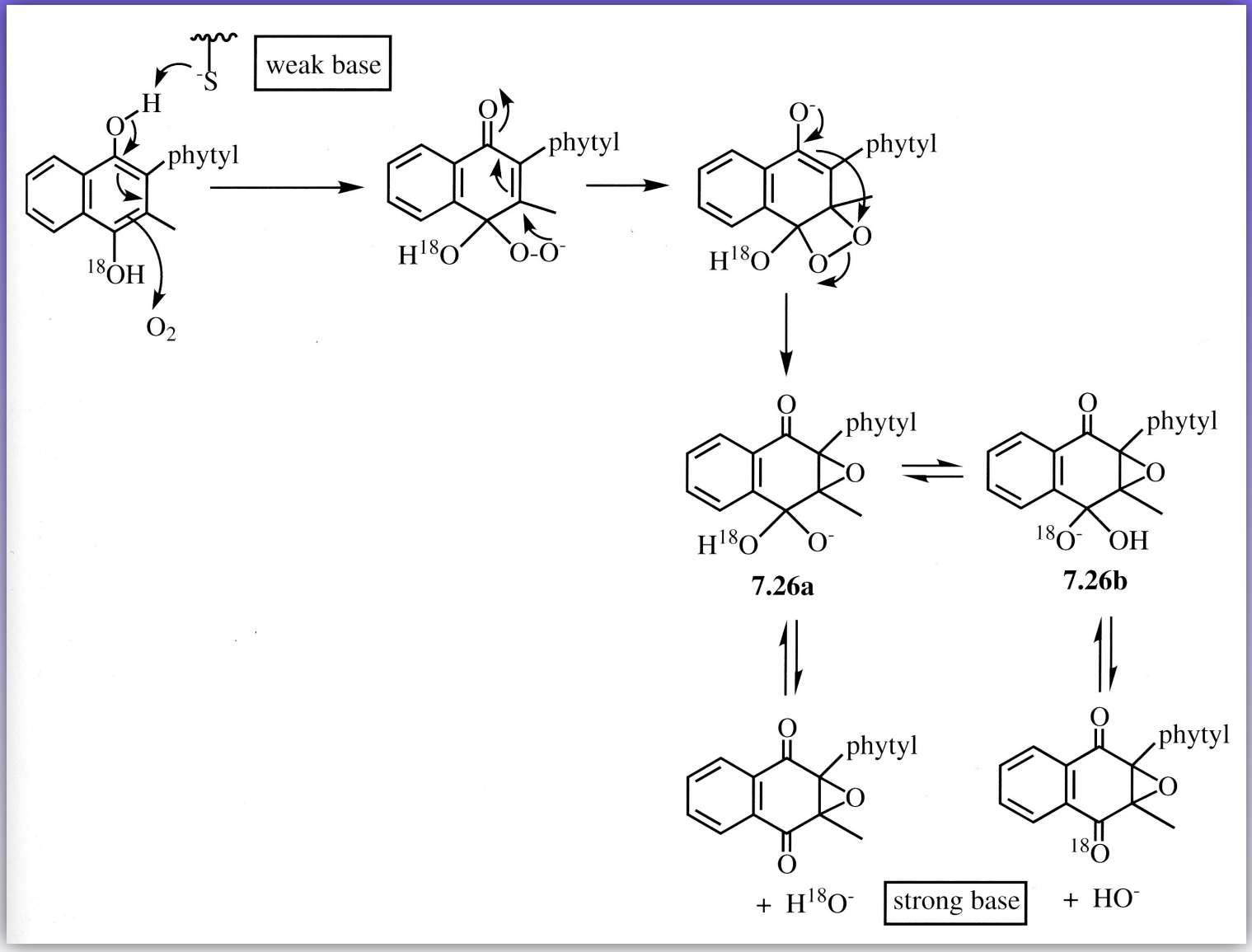
Reduced vitamin K is an obligatory substrate for a carboxylase that activates proteins in the blood-clotting cascade.

Proposed mechanism of Vitamin K carboxylase: Requires CO_2 as substrate and involves a carbanion intermediate



What is the role of reduced vitamin K?

The base strength amplification mechanism of Vitamin K carboxylase



Explanation of the base strength amplification mechanism

A weak base (active site cysteine) removes the hydroquinone proton from reduced vitamin K, which then reacts with O_2, leading to the strong base, the ketal anion. This anion does not remove the glutamate proton directly, but rather, the elimination product, *hydroxide ion*, is proposed to be the strong base involved in this abstraction.

The function of vitamin K appears to be to convert O_2 into hydroxide anion in a hydrophobic environment where it can deprotonate Glu residues. This is more effective than aqueous hydroxide because bases are known to be stronger in hydrophobic solvents than in aqueous media.