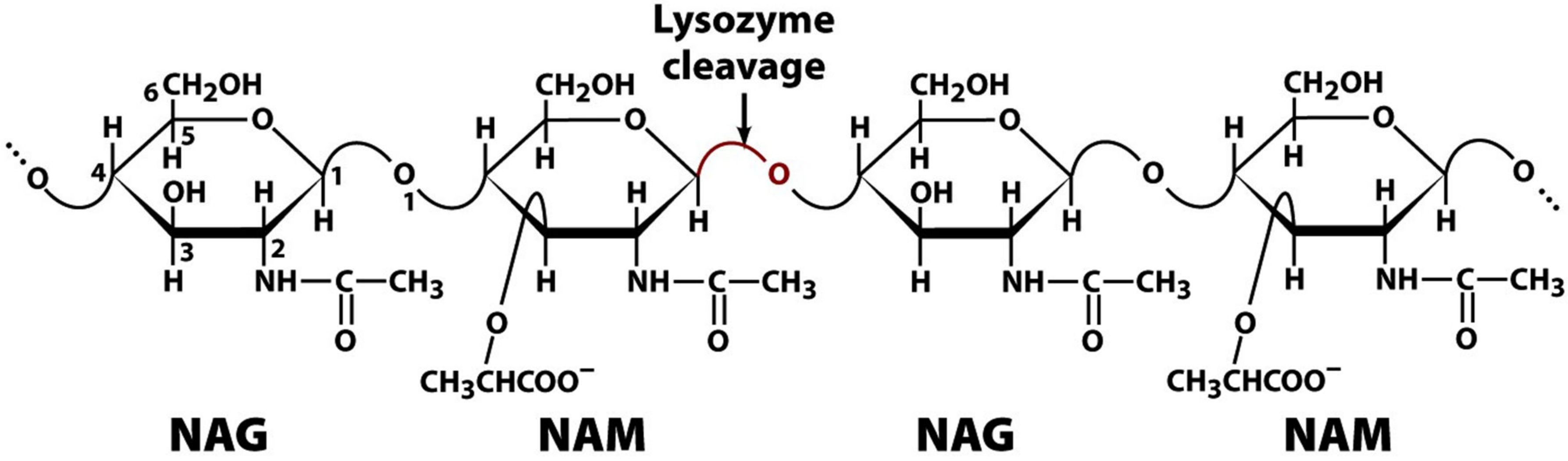
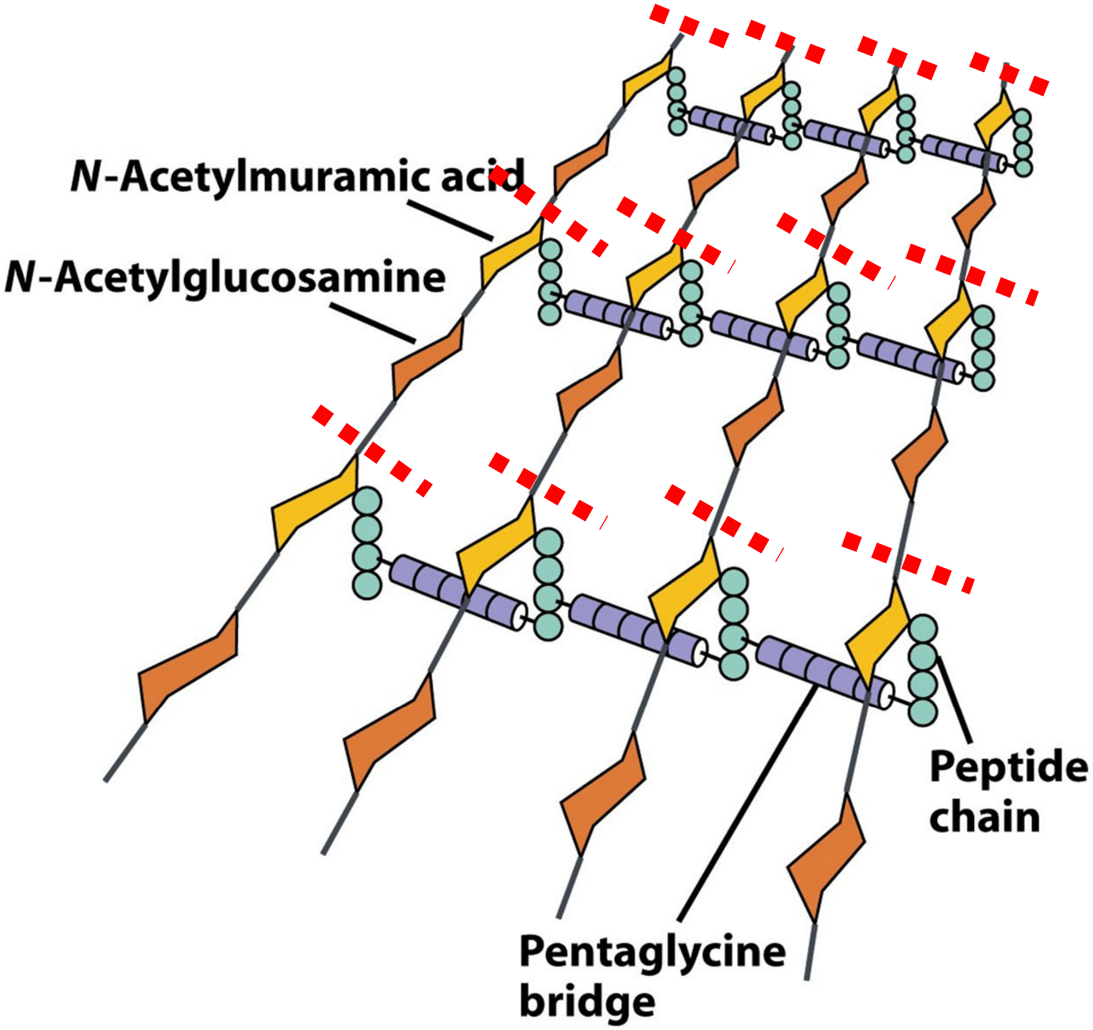
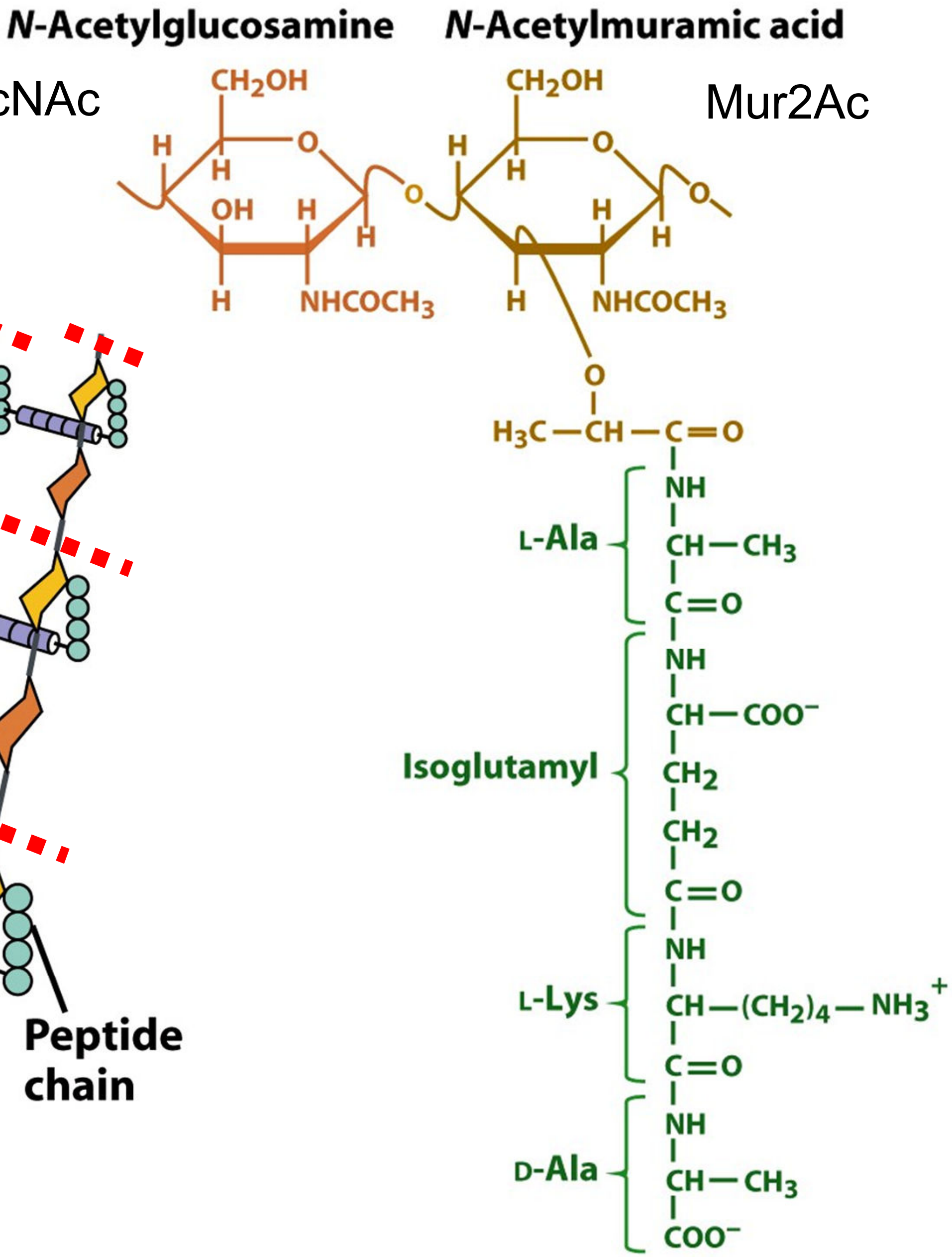
Lysozyme catalyzes the hydrolysis of a glycosidic bond of peptidoglycan



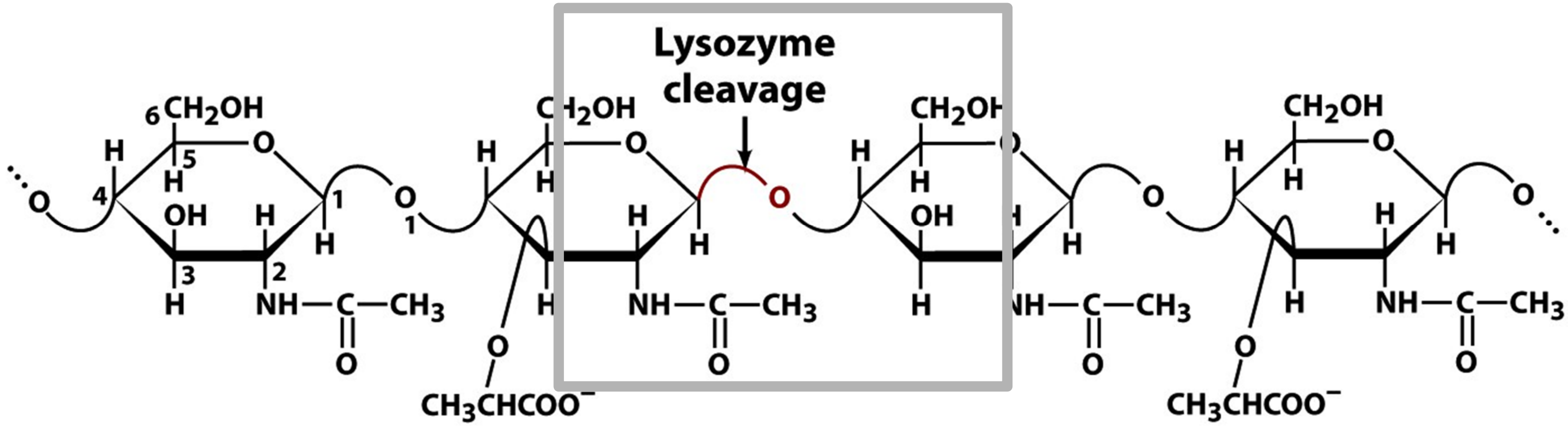
NAG = *N*-acetylglucosamine
NAM = *N*-acetylmuramic acid

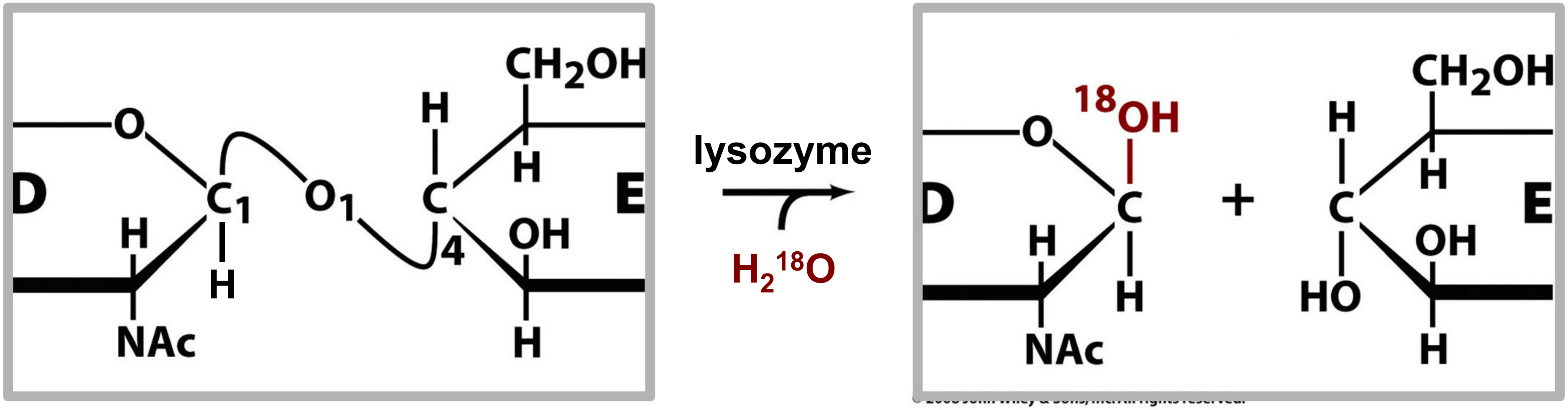
S. aureus peptidoglycan



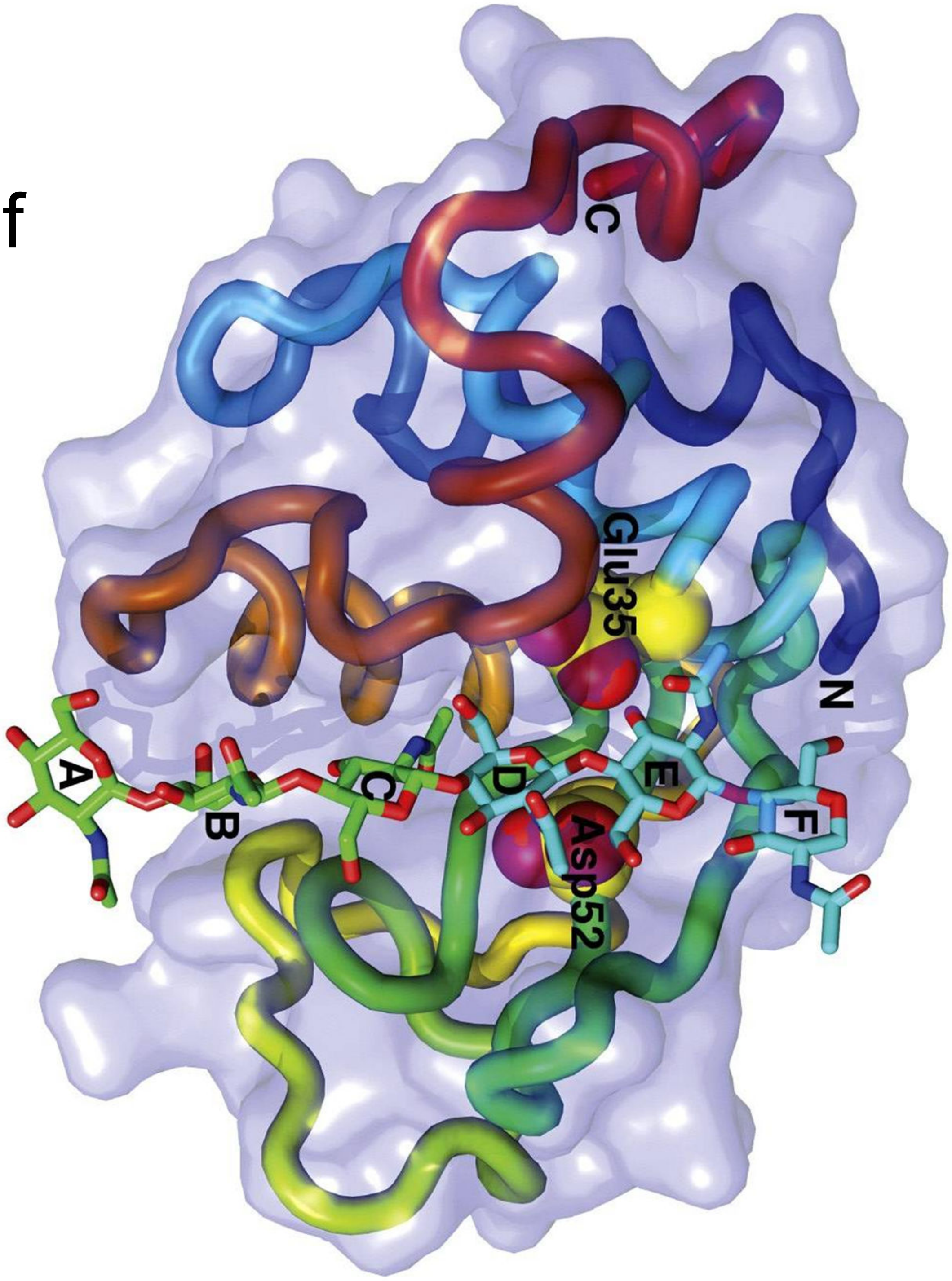


Using heavy water indicates which side of the glycosidic bond is cleaved



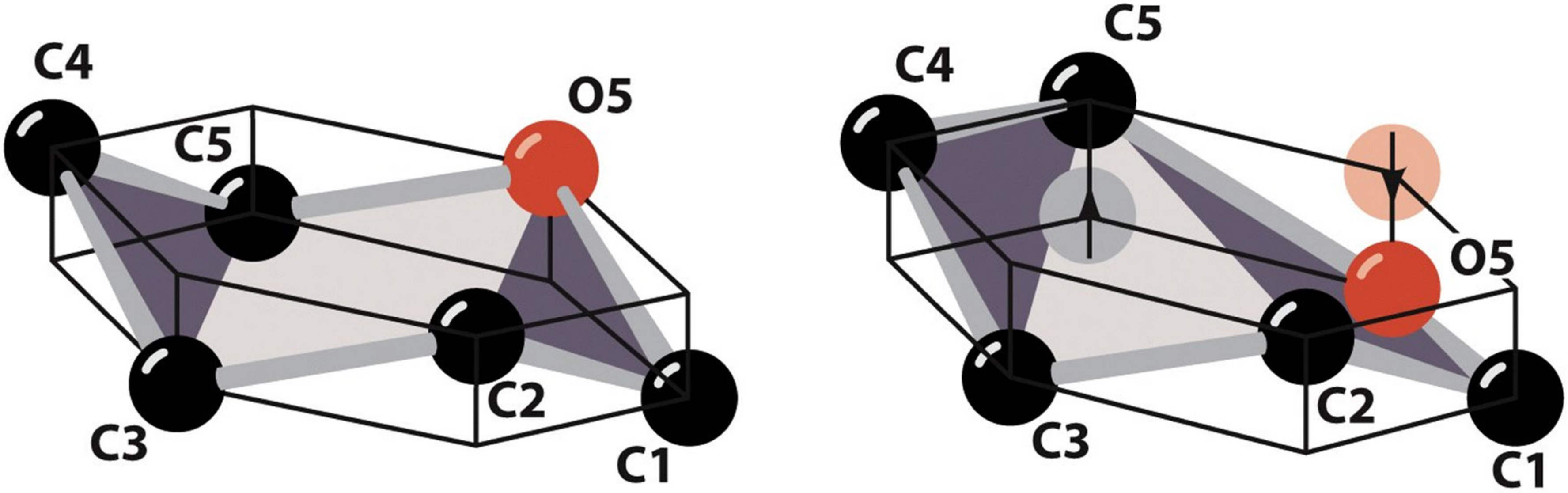


Lysozyme binds a 6-residue stretch of the peptidoglycan polysaccharide in its active site



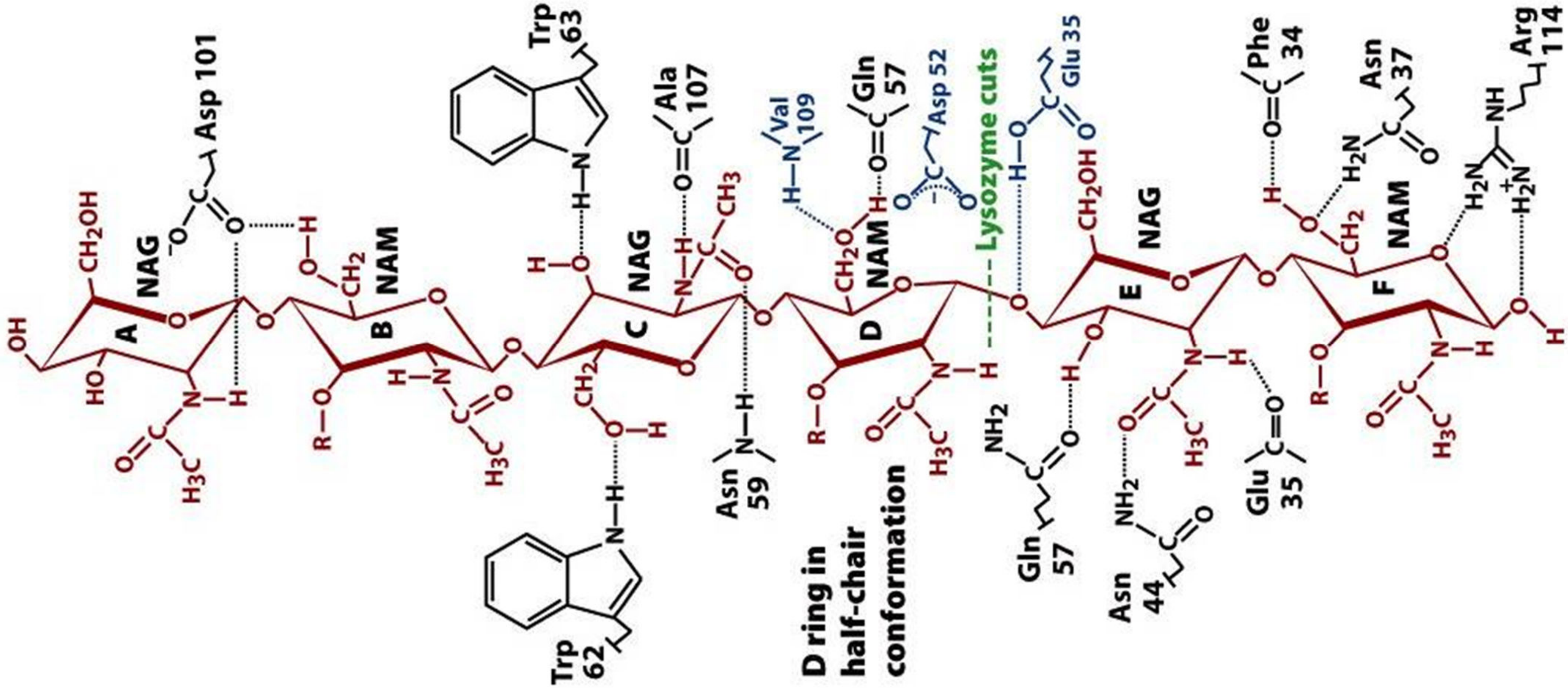
Binding sites for individual residues are labelled A-F

Steric hindrance at the ‘D’ site forces the ring into a strained, half-chair conformation

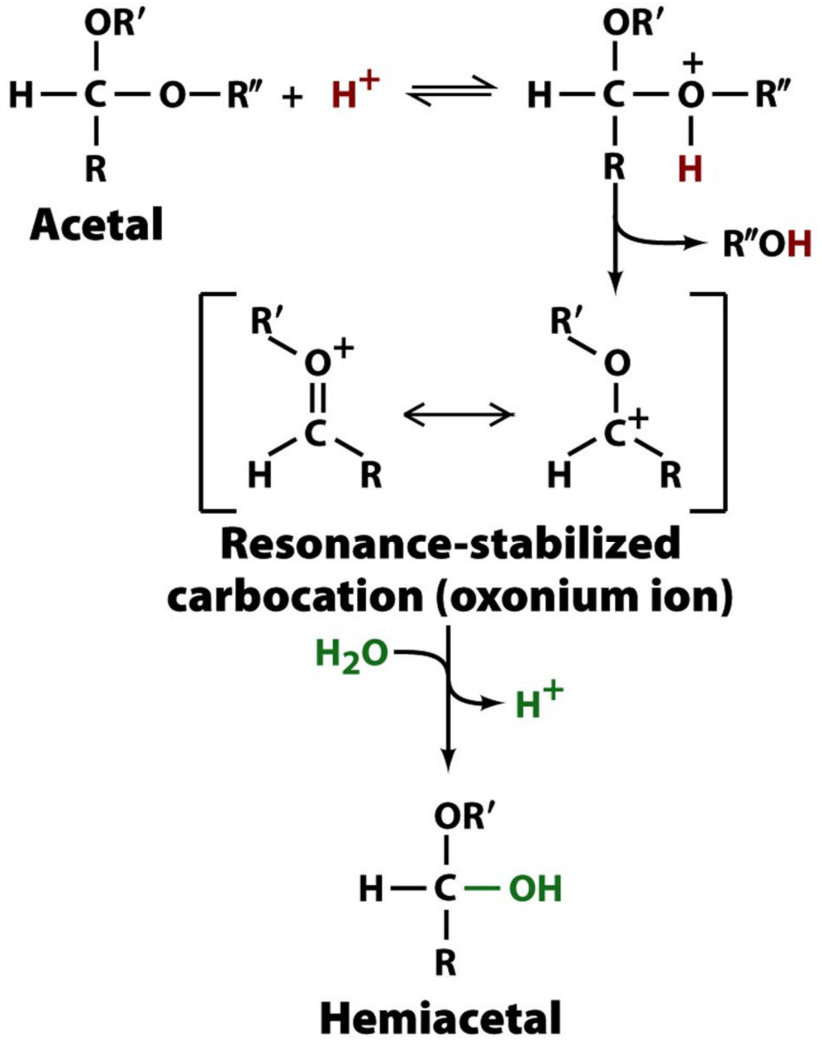


Chair conformation   **Half-chair conformation**

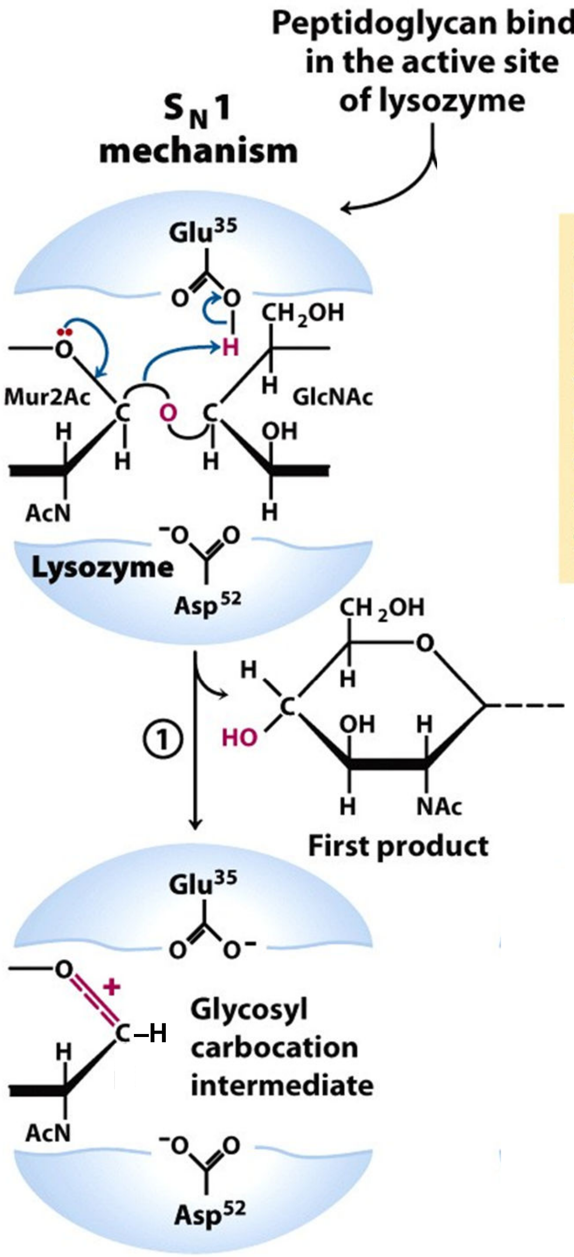
Binding interactions between enzyme and substrate offset strain at the ‘D’ site



Acid-catalyzed hydrolysis of an acetal produces a carbocation intermediate



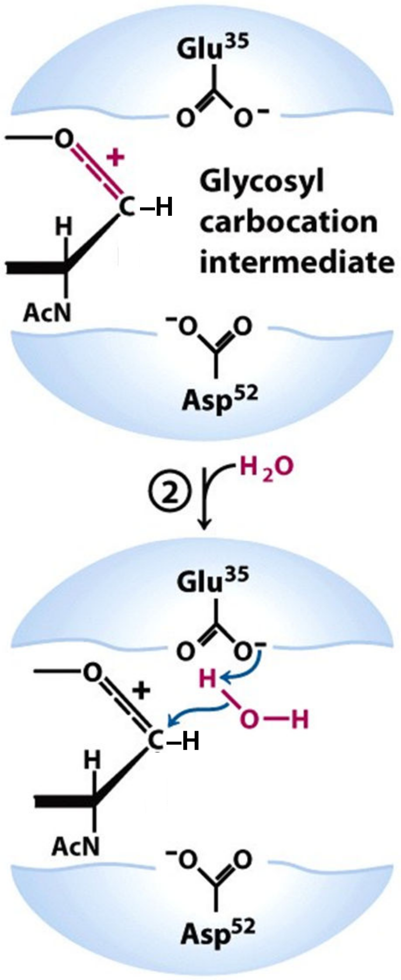
The S_N1 mechanism of lysozyme involves the formation of a carbocation intermediate



A rearrangement produces a glycosyl carbocation. General acid catalysis by Glu52 protonates the displaced GlcNAc oxygen and facilitates its departure.

Figure 6-25a part 1
Lehninger Principles of Biochemistry, Fifth Edition

The S_N1 mechanism of lysozyme involves the formation of a carbocation intermediate



General base catalysis by Glu35 facilitates the attack of water on the glycosyl carbocation to form product.

Figure 6-25a part 2
Lehninger Principles of Biochemistry, Fifth Edition

The S_N1 mechanism of lysozyme involves the formation of a carbocation intermediate

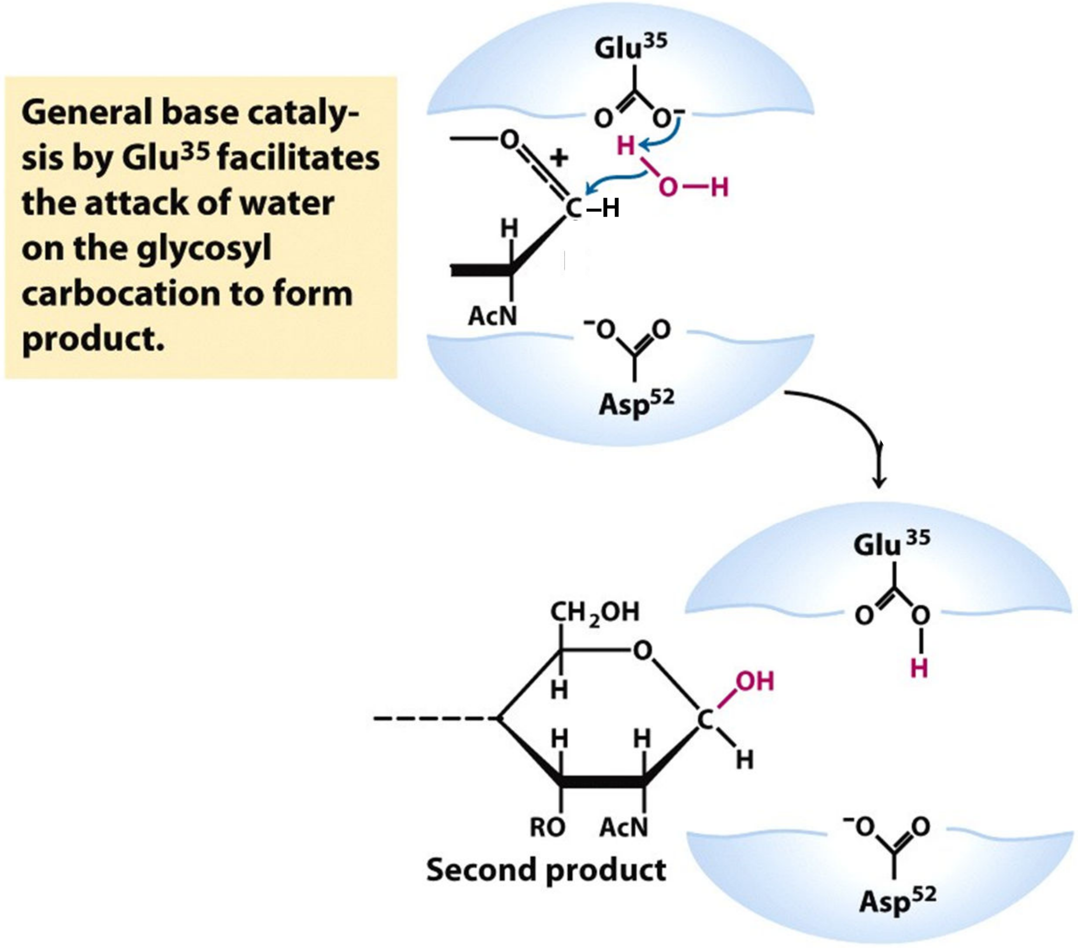

General base catalysis by Glu^{35} facilitates the attack of water on the glycosyl carbocation to form product.

Figure 6-25a part 3
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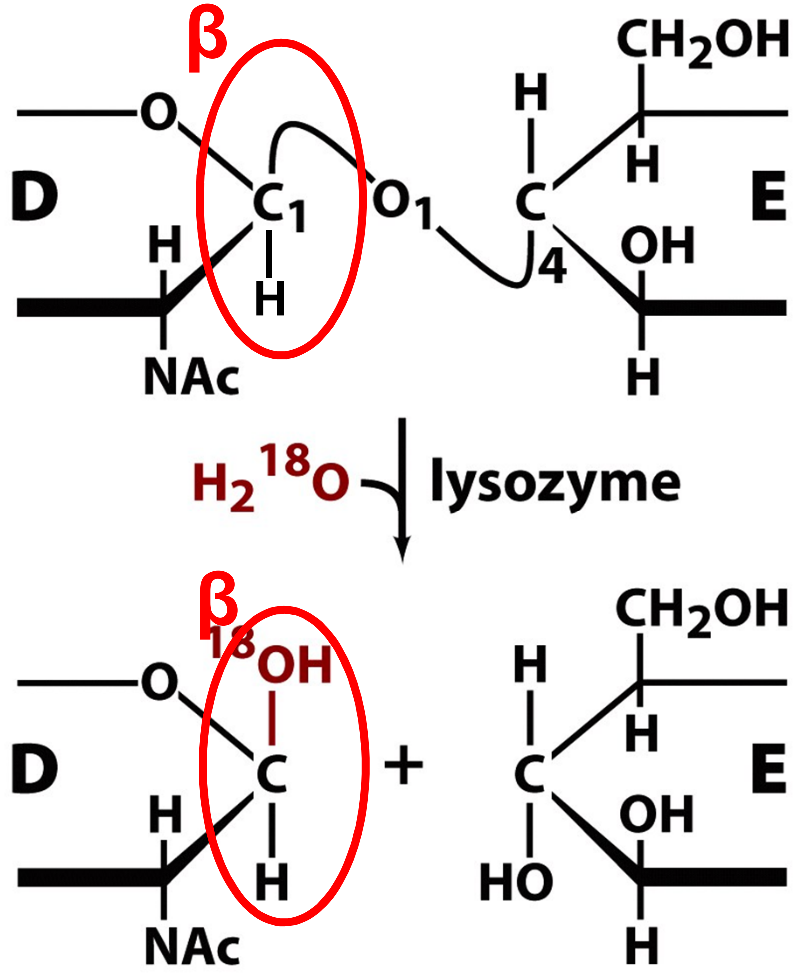
Lysozyme is a retaining glycosidase; products retain the anomeric configuration



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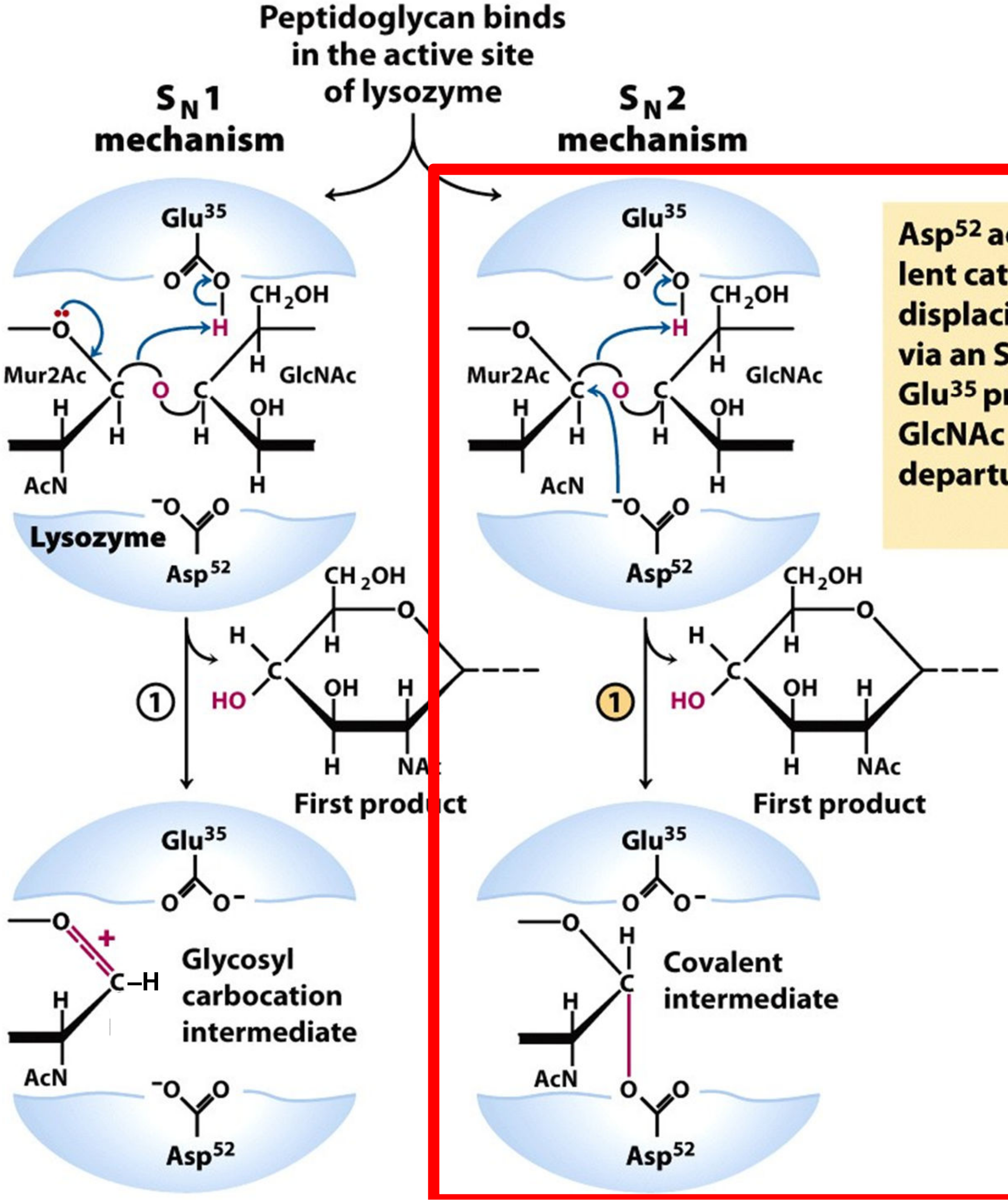
Other retaining glycosidases have S_N2 mechanism

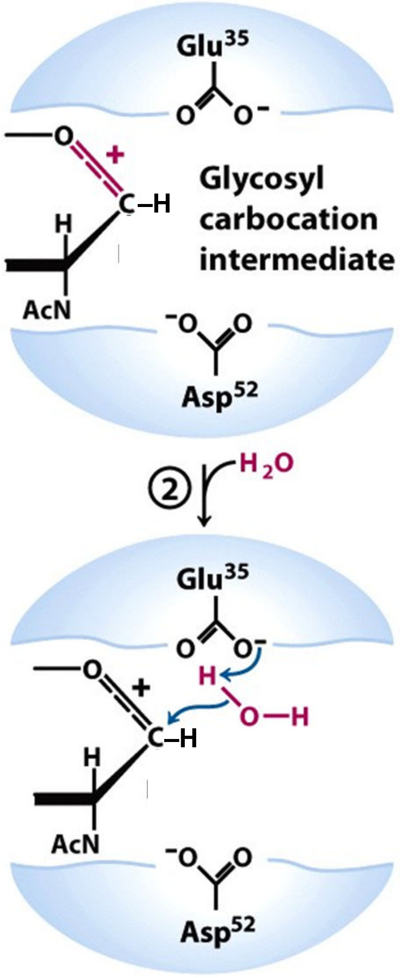
S_N2 mechanism has a covalent intermediate

A rearrangement produces a glycosyl carbocation. General acid catalysis by Glu^{52} protonates the displaced GlcNAc oxygen and facilitates its departure.

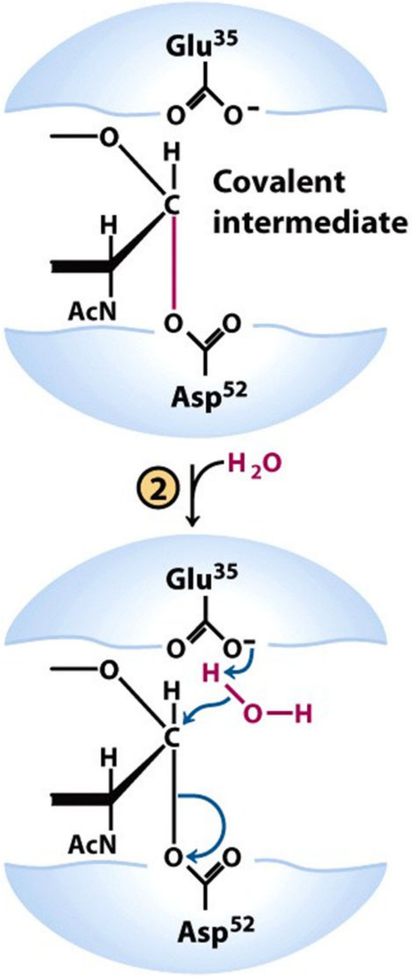


Asp^{52} acts as a covalent catalyst, directly displacing the GlcNAc via an S_N2 mechanism. Glu^{35} protonates the GlcNAc to facilitate its departure.

S_N2 mechanism has a covalent intermediate



General base catalysis by Glu^{35} facilitates the attack of water on the glycosyl carbocation to form product.



Glu^{35} acts as a general base catalyst to facilitate the S_N2 attack of water, displacing Asp^{52} and generating product.

Figure 6-25a part 2
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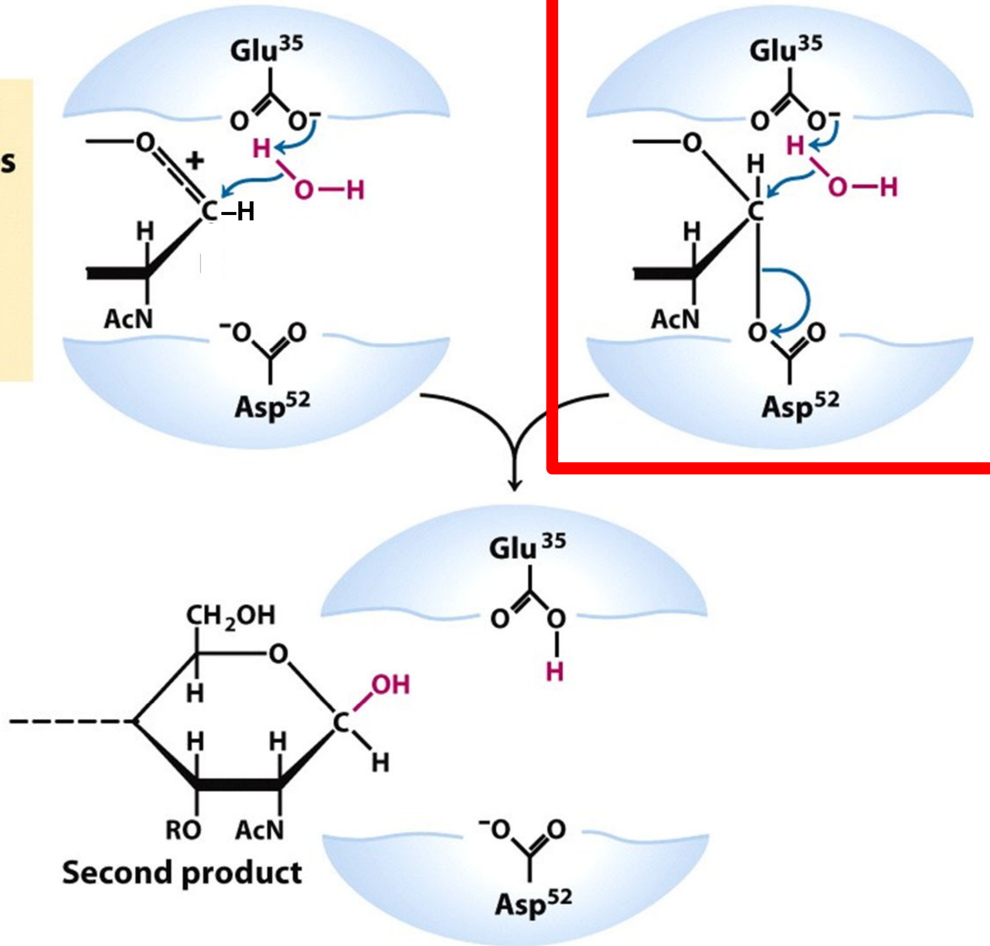
S_N2 mechanism has a covalent intermediate

General base catalysis by Glu^{35} facilitates the attack of water on the glycosyl carbocation to form product.



Glu^{35} acts as a general base catalyst to facilitate the S_N2 attack of water, displacing Asp^{52} and generating product.

Figure 6-25a part 3
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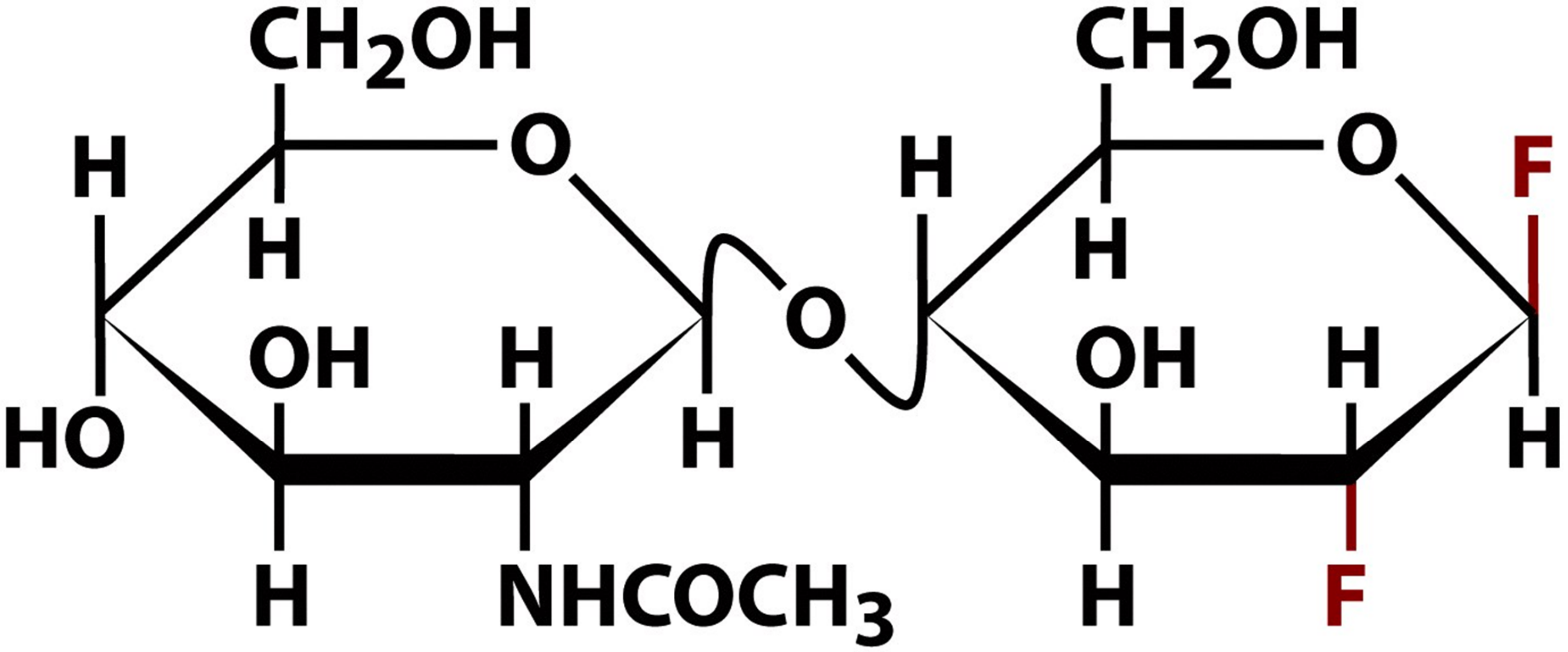
A substrate analog was used to trap the covalent intermediate of lysozyme



NAG2FGlcF

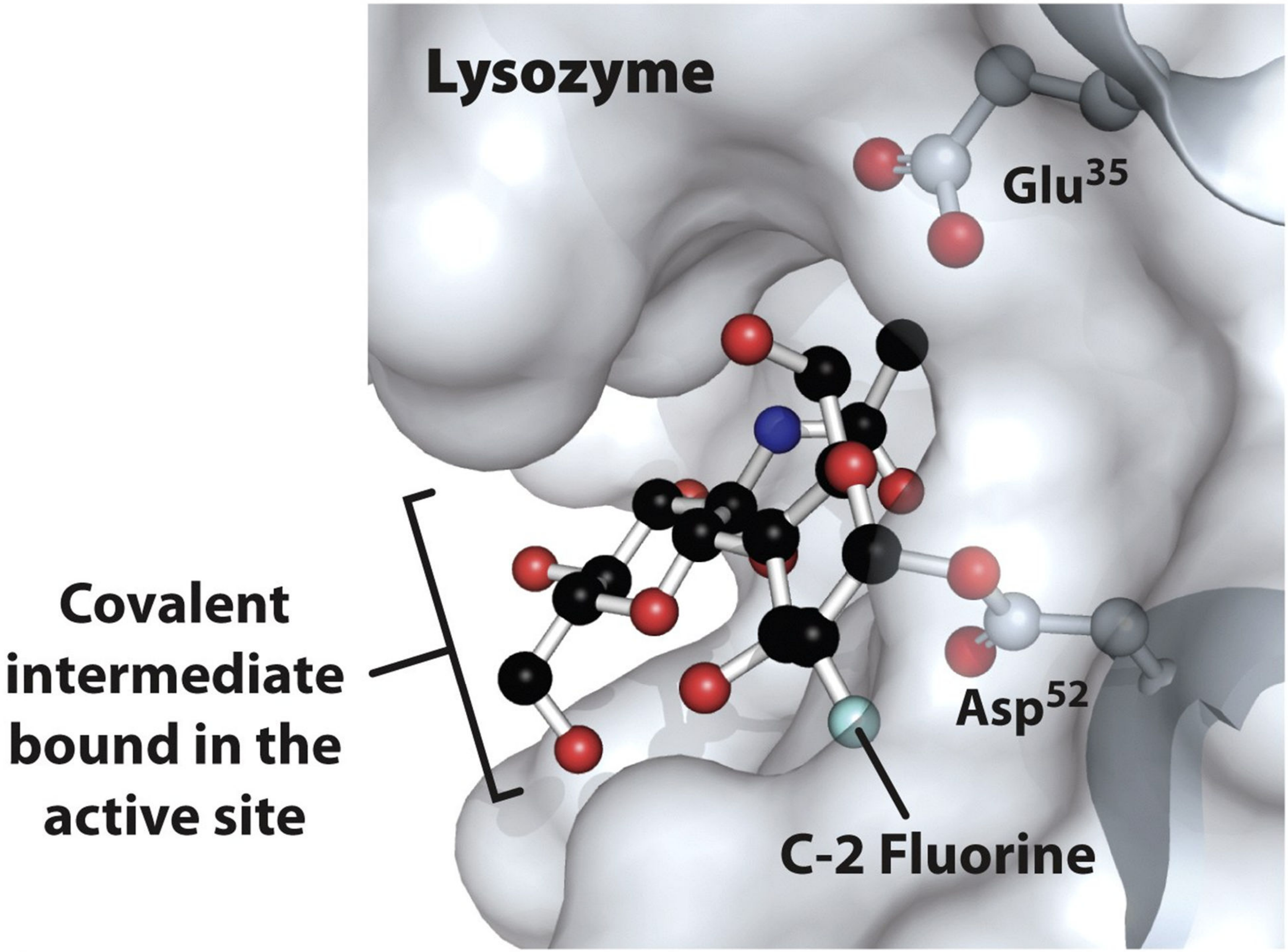


Figure 6-25b
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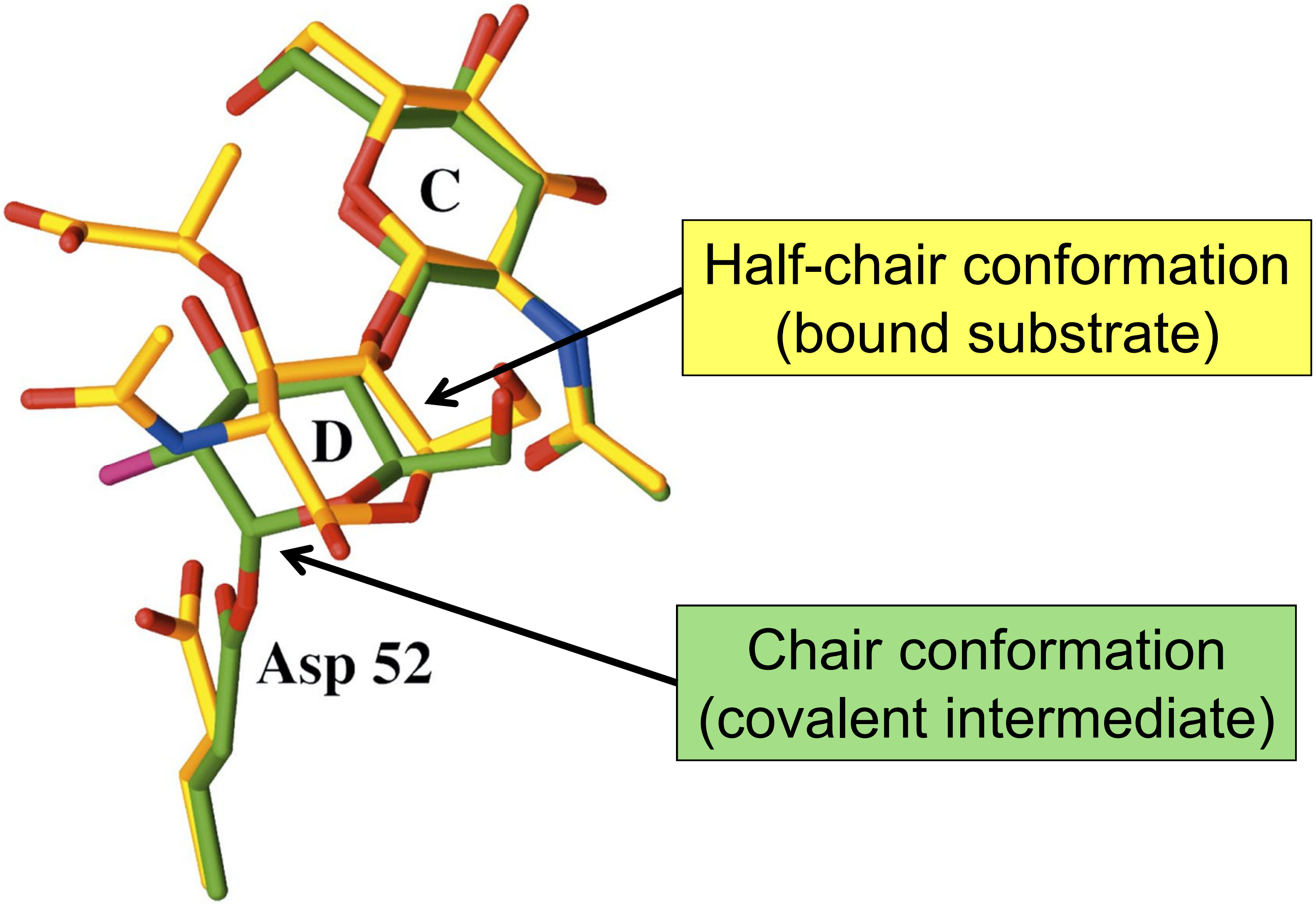
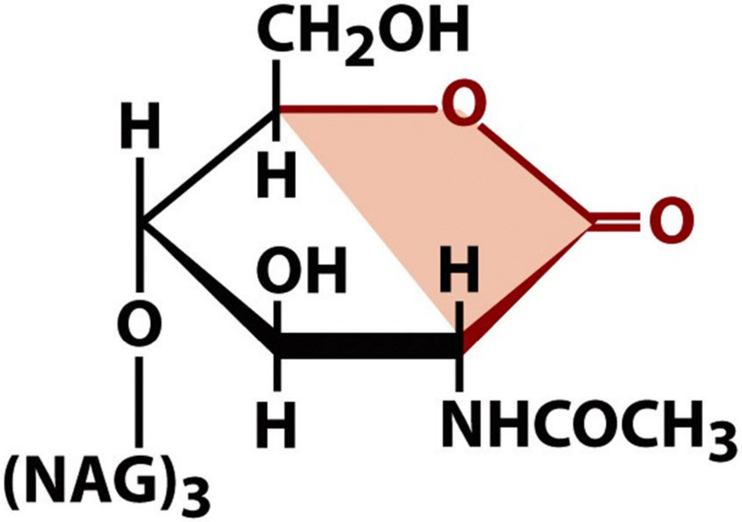
Formation of the covalent intermediate releases strain of the D-site residue



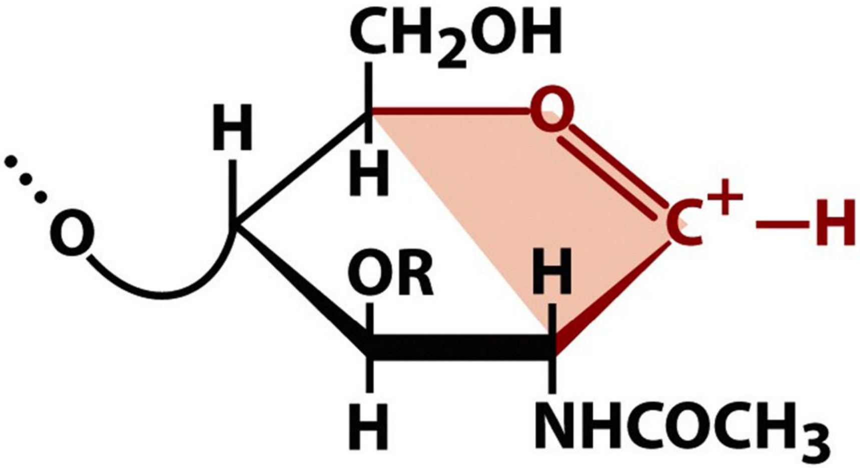
Half-chair conformation
(bound substrate)

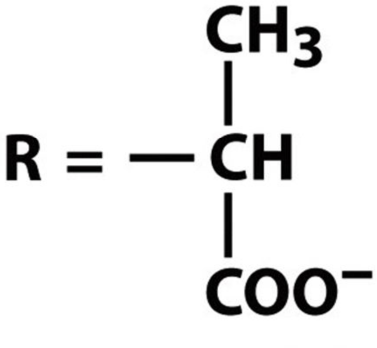
Chair conformation
(covalent intermediate)

Transition state analogs mimic a transition state and bind more tightly than substrate



δ-Lactone analog of (NAG)$_4$





$$R = -\underset{\displaystyle COO^-}{\overset{\displaystyle CH_3}{CH}}$$

Lysozyme transition state