Exam II

- **Thursday 10.30.08 in class**
- **Review Session Tuesday 10.28.08**

- Be able to draw a free energy diagram for an enzymatic reaction
- Know Michaelis-Menten Kinetics
- Understand the various types of inhibition (competitive, non-competitive, un-competitive)
- Know the mechanisms for two types of proteases (one with an acyl-enzyme intermediate, the other without)
- Lysozyme pick the mechanism you like best, compare and contrast evidence for both possible mechanisms to justify your choice

Lysozyme

- small enzyme in tears, mucus, cartilage, egg whites, etc. that attacks the protective cell walls of bacteria.
- breaks carbohydrate chains of petidoglycan, destroying the structural integrity of the cell wall - bacteria burst under their own internal pressure.
- **First Antibiotic:** Alexander Fleming discovered lysozyme1922 - it is the first enzyme crystal structure solved 1967.

ON THE MECHANISM OF LYSOZYME ACTION*

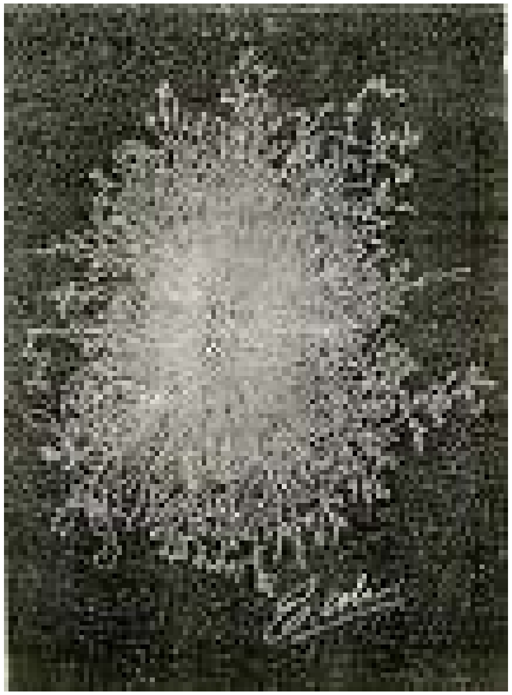
By KARL MEYER, JOHN W. PALMER, RICHARD THOMPSON, AND DEVORAH KHORAZO

(From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, and the Institute of Ophthalmology, Presbyterian Hospital, New York)

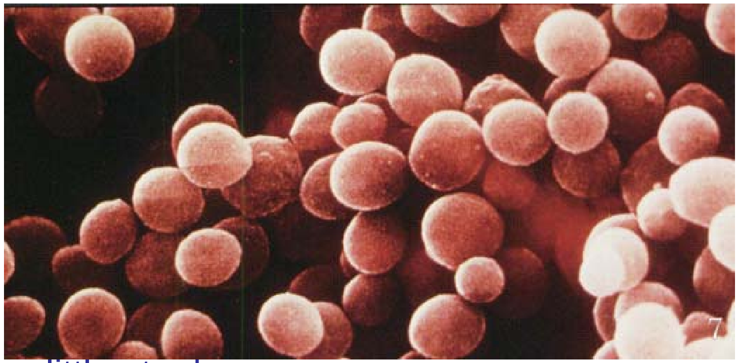
(Received for publication, November 19, 1935)

Lysozyme, the purification and chemical properties of which have recently been described (1), was considered by Fleming (2) to be an enzyme. Its heat stability rather suggested a physico-chemical action. Furthermore, some substances, such as rattle-

red blood cell lysis



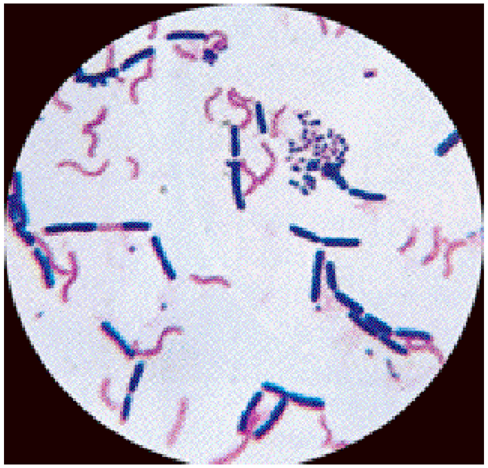
E. coli

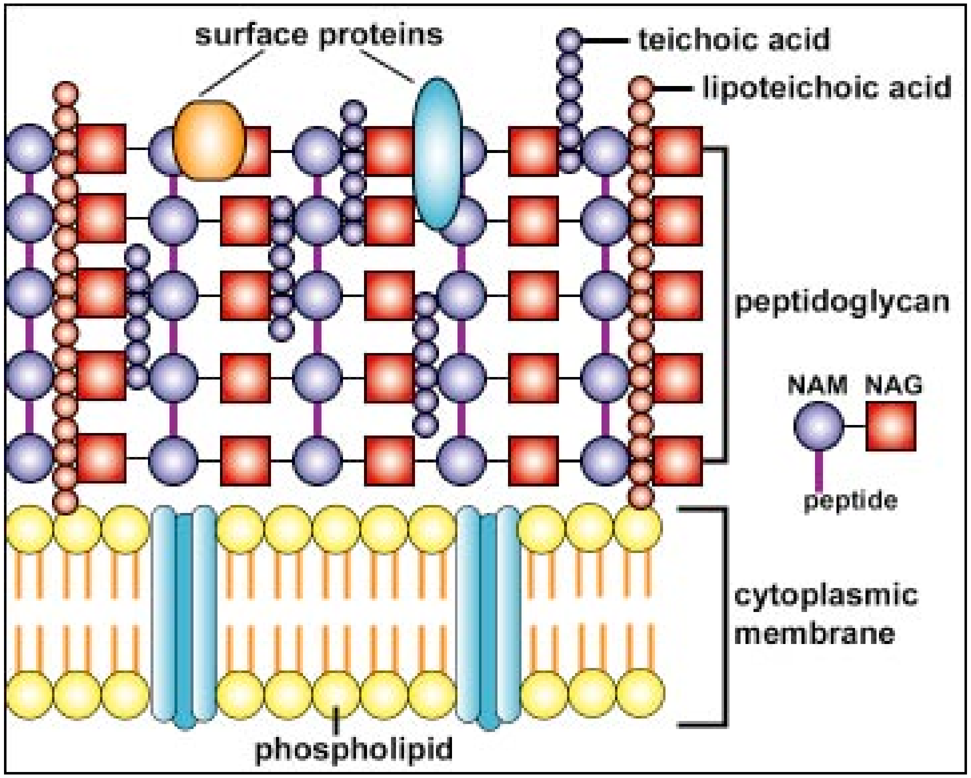


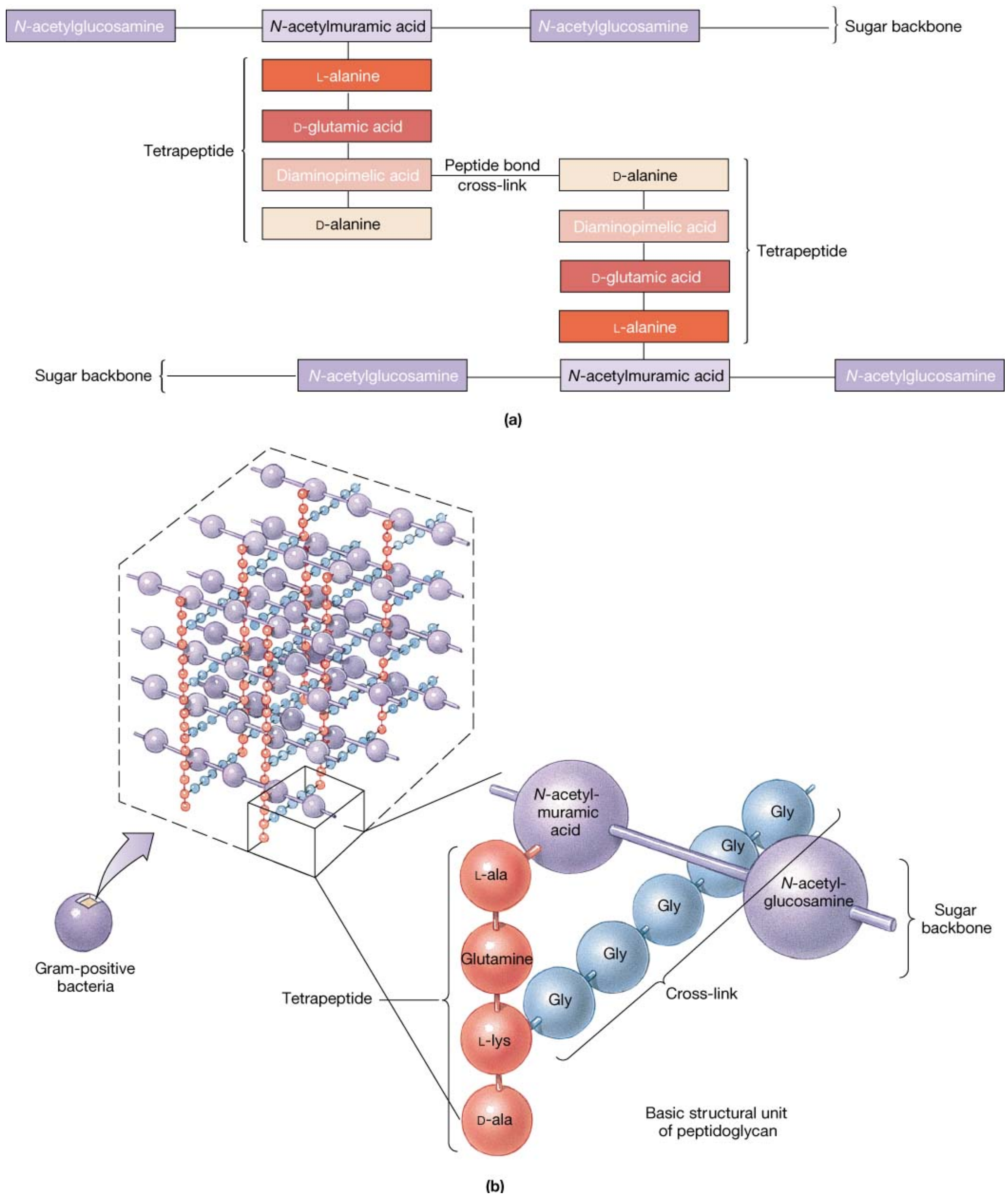
flee little staph...

http://www.biochemweb.org/fenteany/research/cell_migration/neutrophil.html

Lysozyme only works on Gram positive bacteria

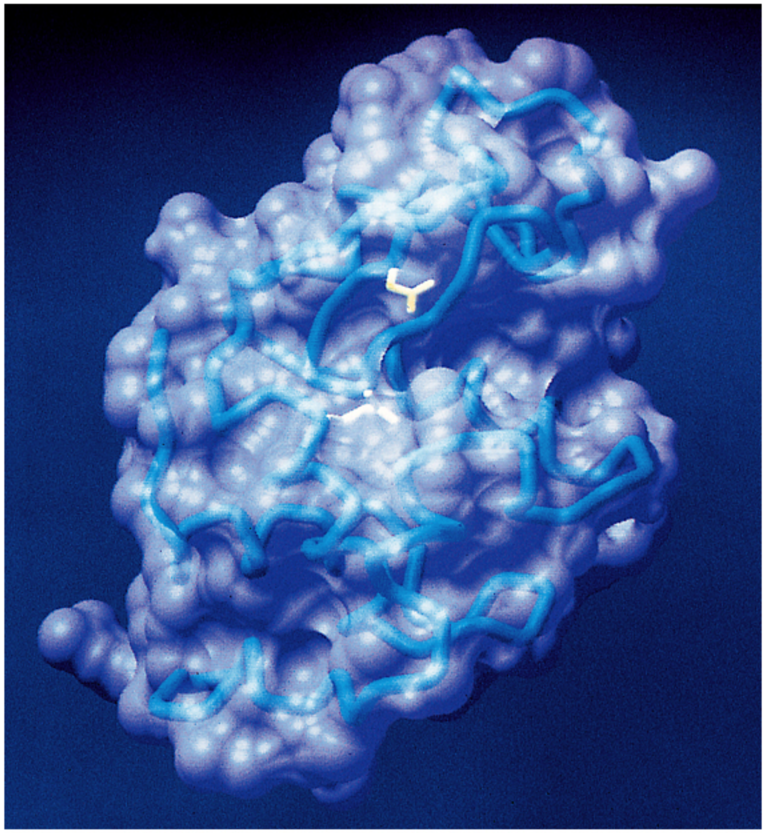






N-acetylglucosamine
N-acetylmuramic acid
N-acetylglucosamine
Sugar backbone
L-alanine
D-glutamic acid
Tetrapeptide
Diaminopimelic acid
Peptide bond cross-link
D-alanine
D-alanine
Diaminopimelic acid
Tetrapeptide
D-glutamic acid
L-alanine
Sugar backbone
N-acetylglucosamine
N-acetylmuramic acid
N-acetylglucosamine
(a)
N-acetyl-muramic acid
Gly
Gly
N-acetyl-glucosamine
Sugar backbone
L-ala
Gly
Gly
Glutamine
Gly
Gram-positive bacteria
Tetrapeptide
Cross-link
L-lys
D-ala
Basic structural unit of peptidoglycan
(b)

Lysozyme structure (1967)

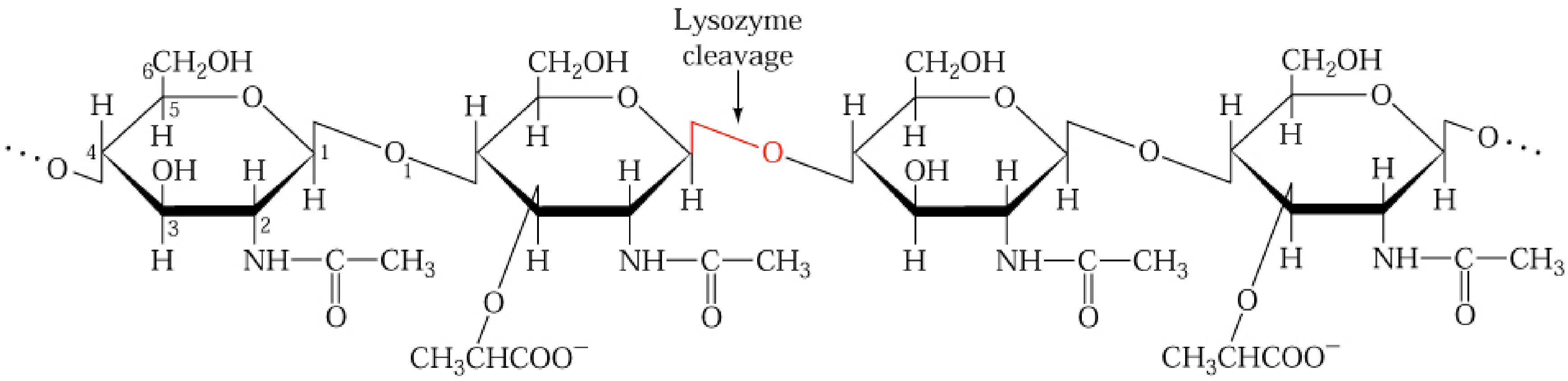


Courtesy of Arthur Olson, The Scripps Research Institute, La Jolla, California.

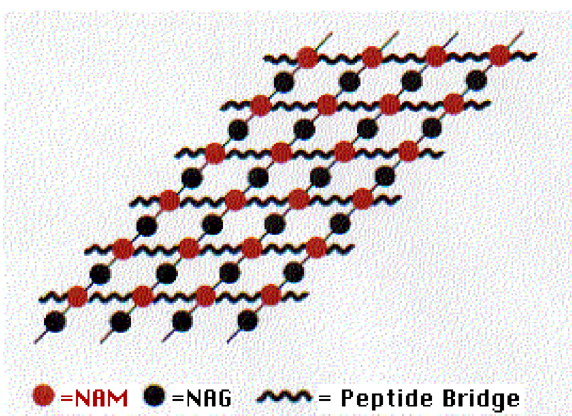
Gallus gallus lysozyme (1974)

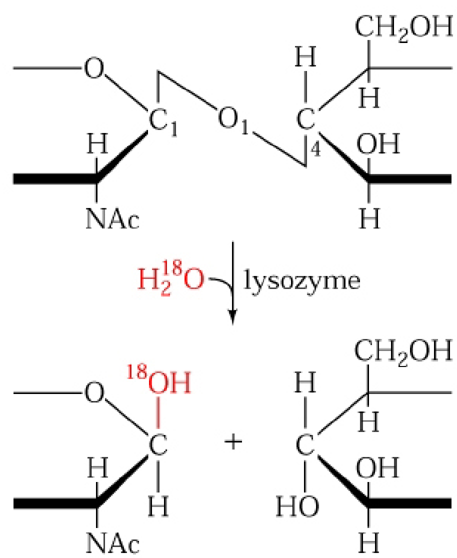
- **EC 3.2.1.17** mucopeptide *N*-acetylmuramoylhydrolase
- First cloned 1988 from human placenta
- 130 amino acids, 4 disulfide bonds
- 855 structures as recent as 7.05

The substrate

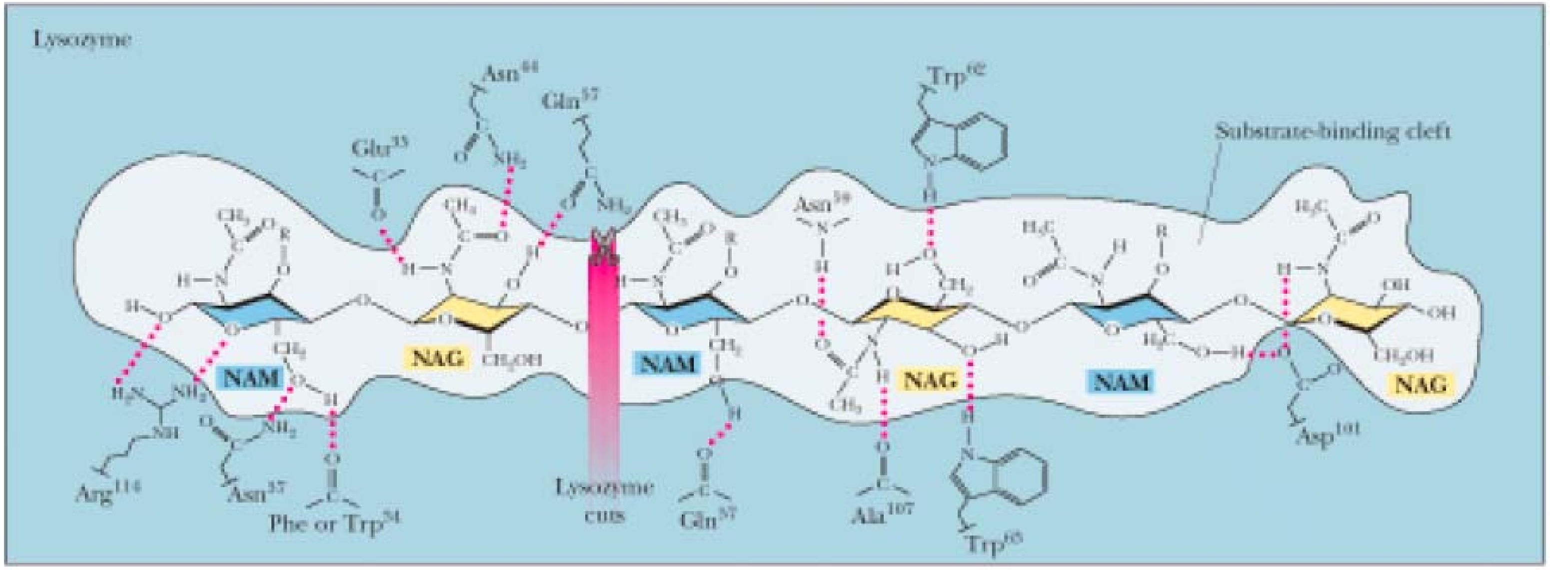


NAG **NAM** **NAG** **NAM**

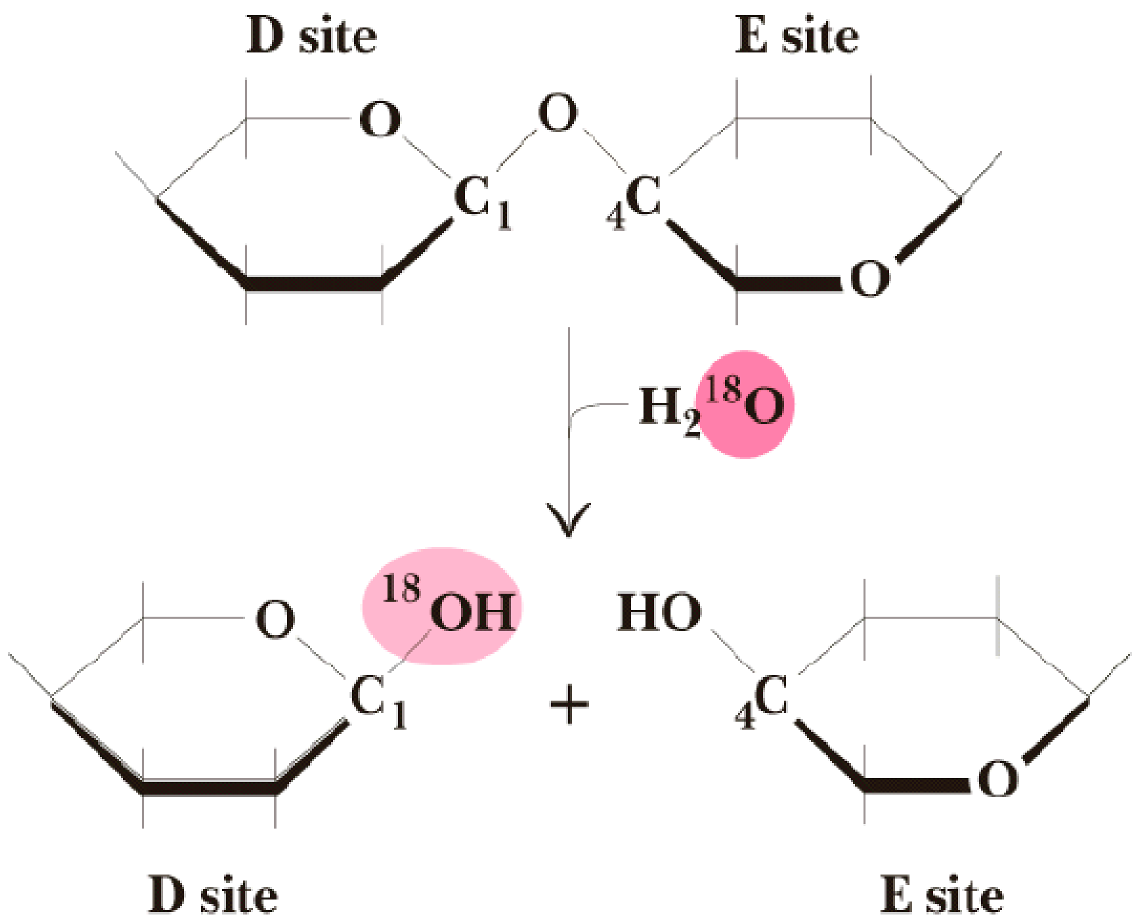




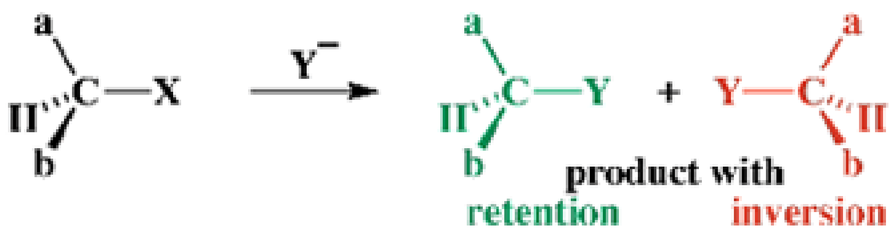
Substrate binding to lysozyme



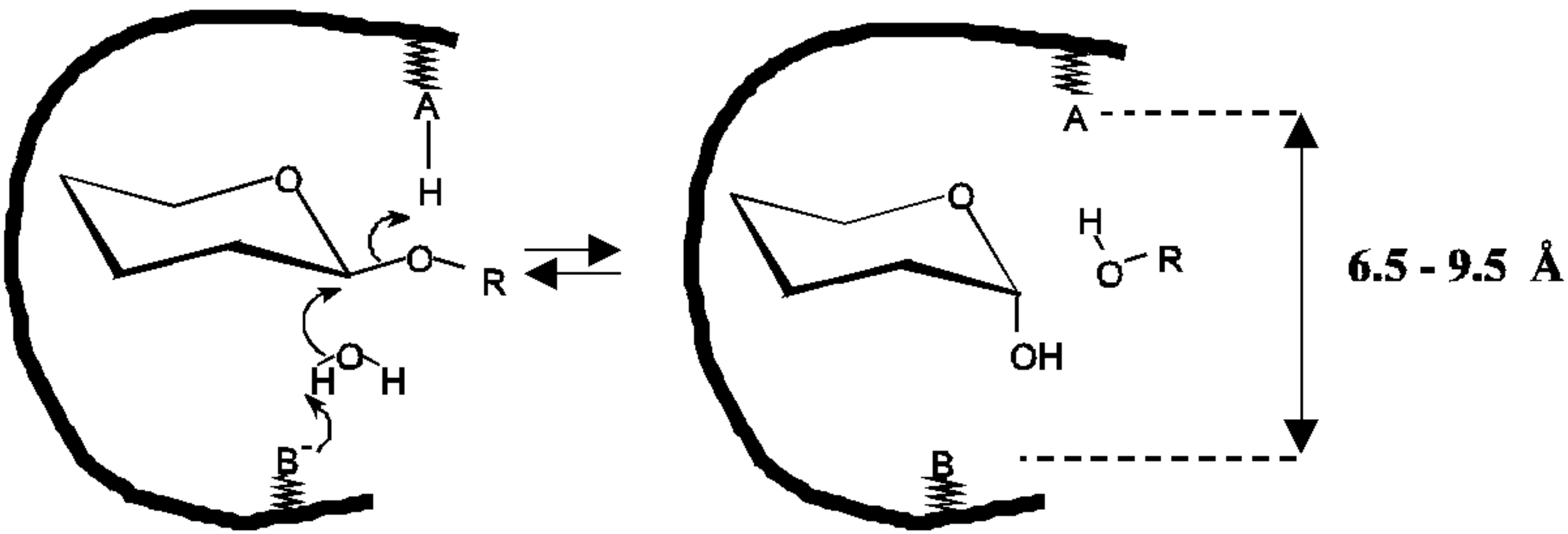
Lysozyme is a "retaining" hydrolase



Stereochemistry as a clue to mechanism

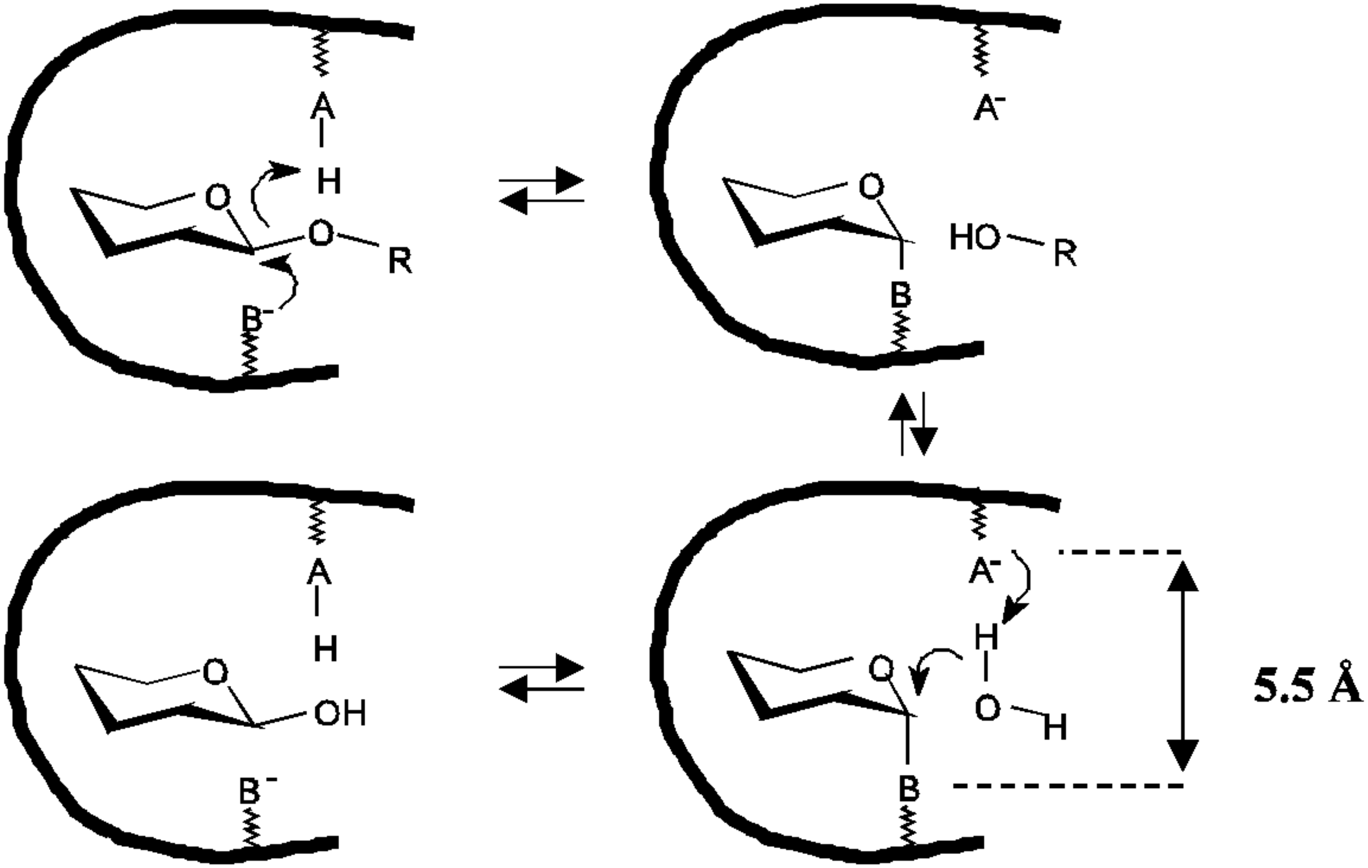


Glycosyl transferases
single nucleophilic replacement (inverting)



Glycosyl transferases

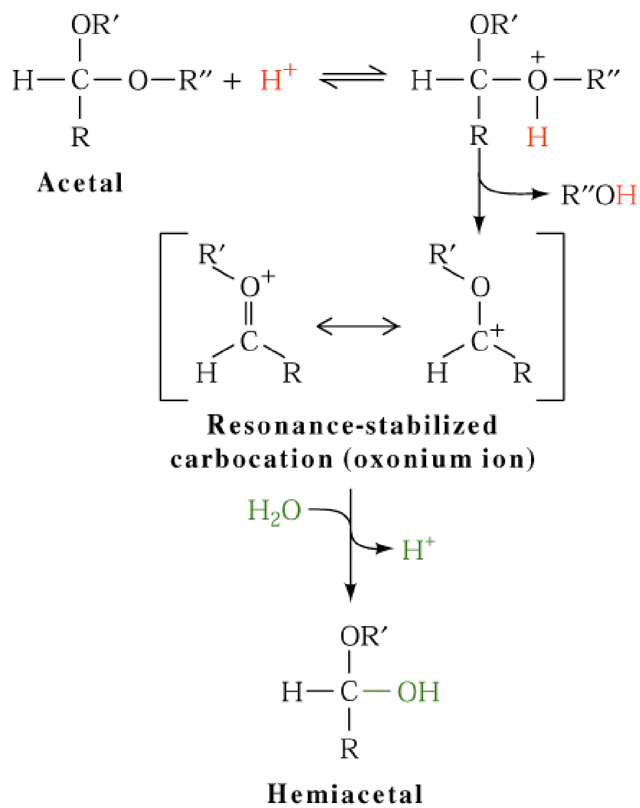
Double displacement (retaining)



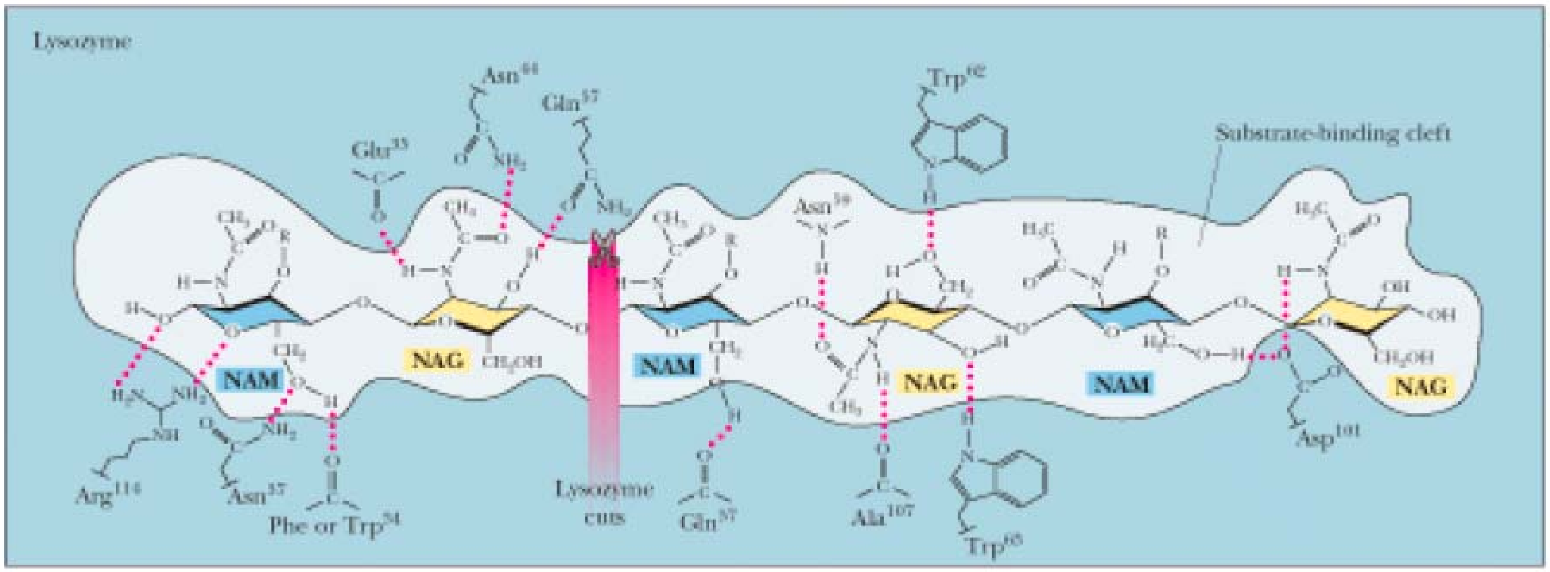
http://afmb.cnrs-mrs.fr/CAZY/acc.html

Glycosyl hydrolases

carbocation intermediates

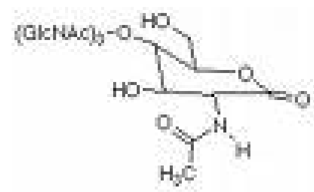


Substrate binding to lysozyme



Lactones inhibit lysozyme

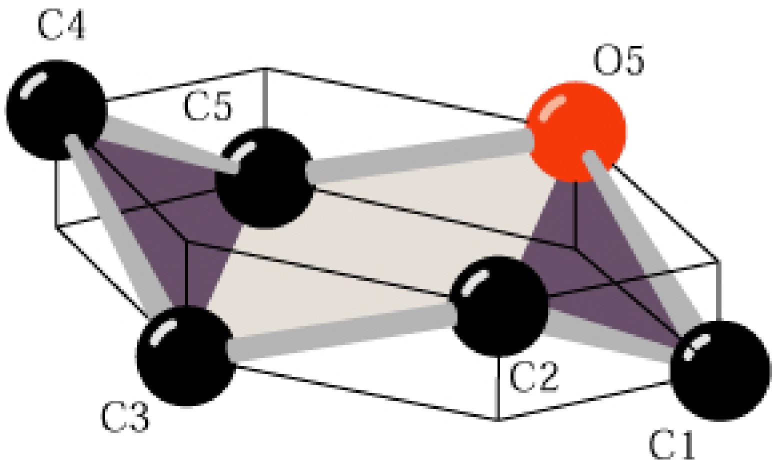
$K_i = 8 \times 10^{-8}$ M



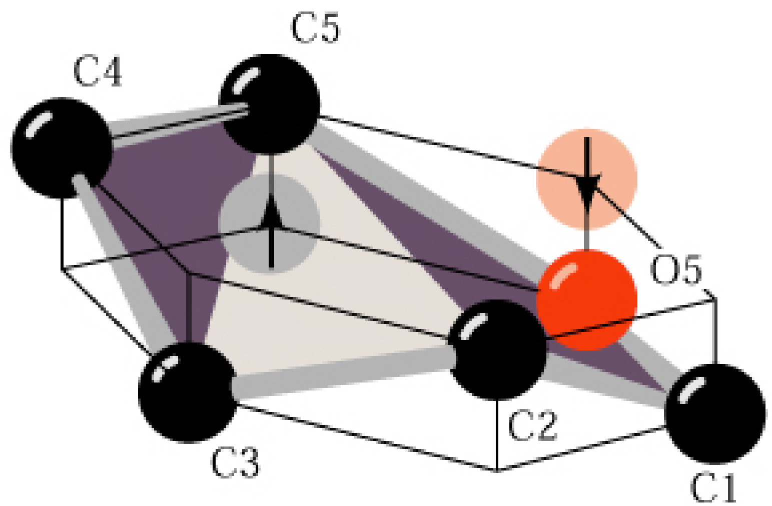
lactone

compared to $K_m = 1 \times 10^{-5}$ M for the substrate NAG_4

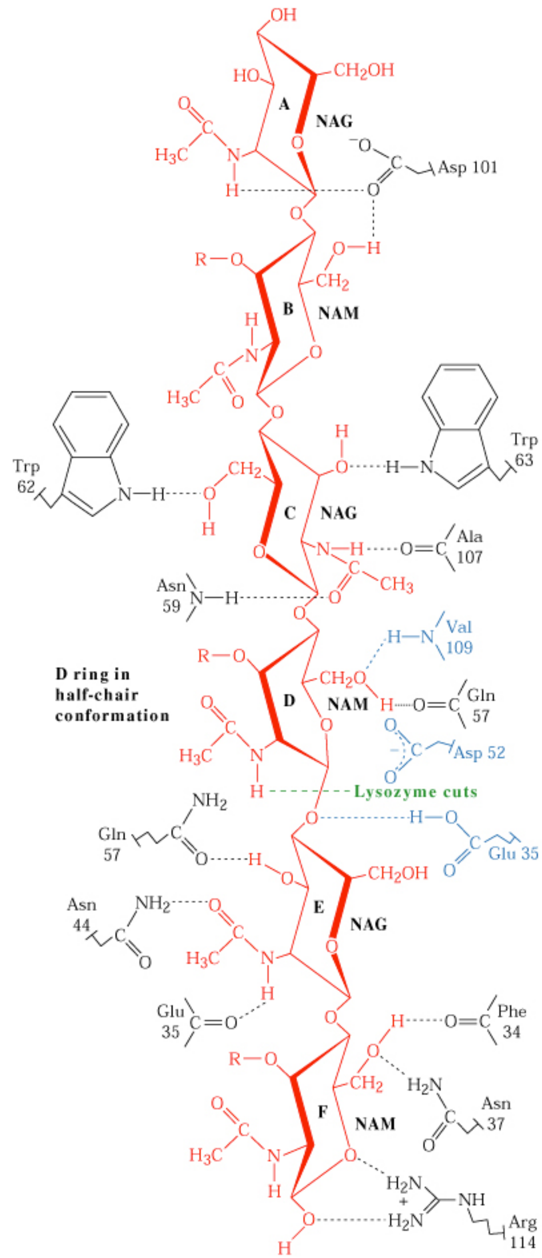
Ring distortion



Chair conformation



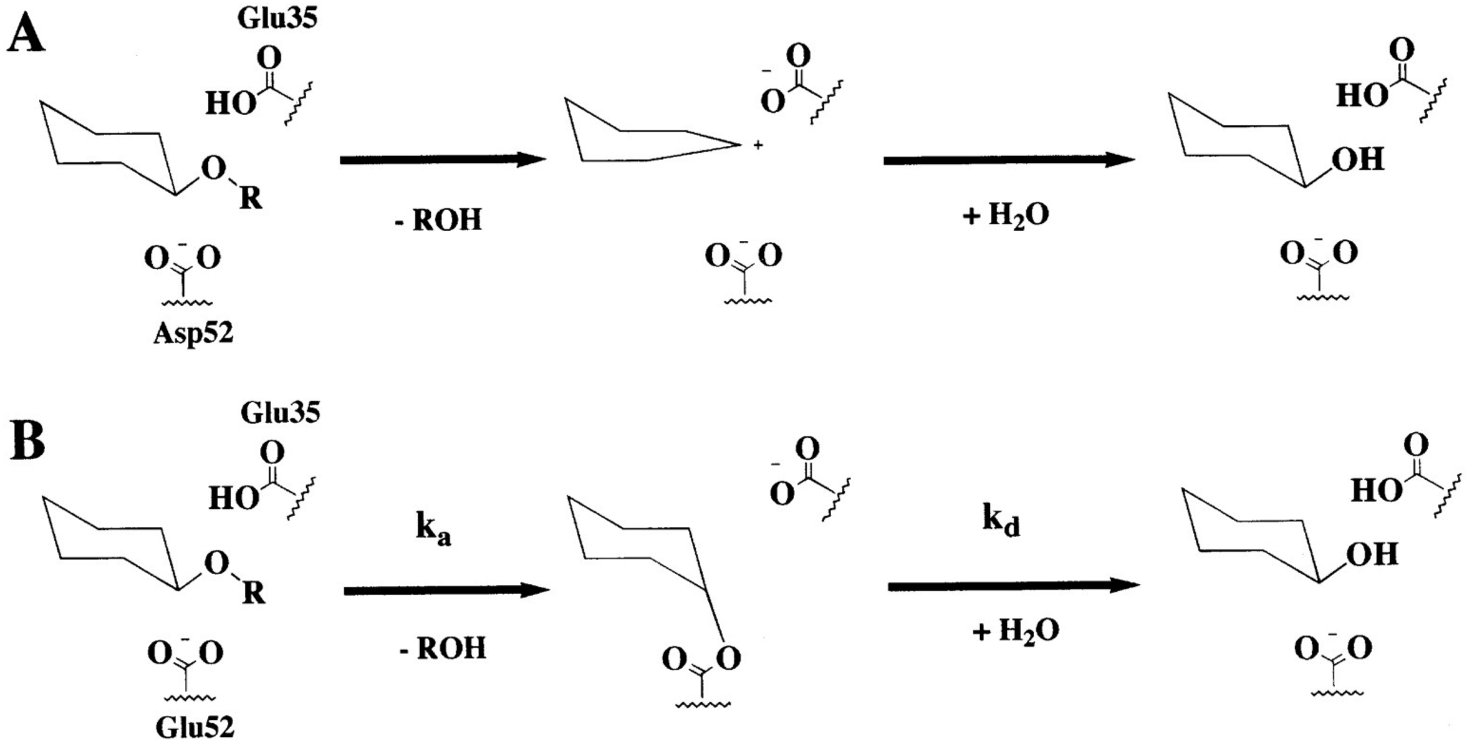
Half-chair conformation


A
NAG
Asp 101
B
NAM
Trp
62
Trp
63
C
NAG
Ala
107
Asn
59
D ring in
half-chair
conformation
D
NAM
Val
109
Gln
57
Asp 52
Lysozyme cuts
Gln
57
Glu 35
E
NAG
Asn
44
Glu
35
Phe
34
F
NAM
Asn
37
Arg
114

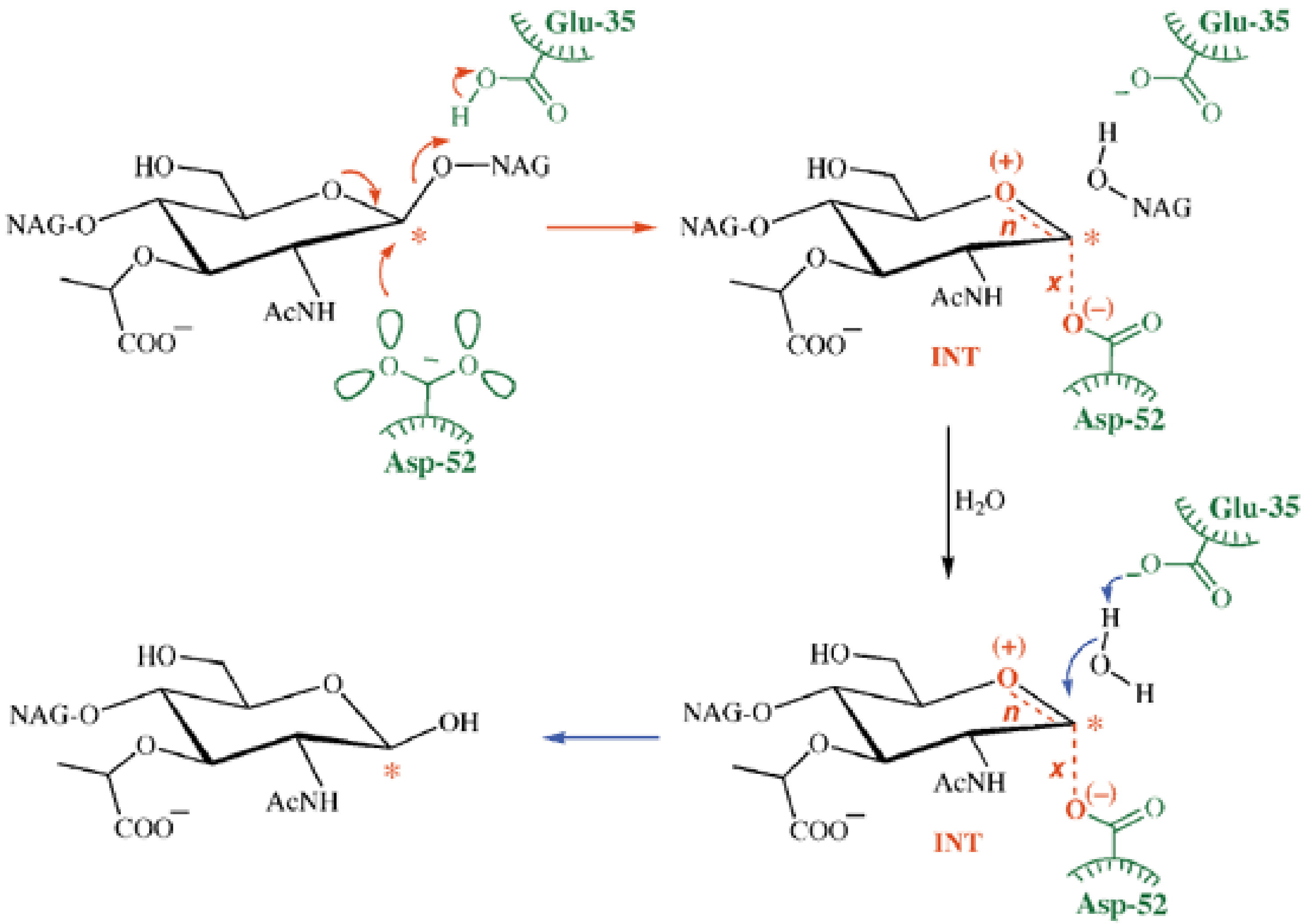
Is strain involved?

Lysozyme hydrolytic rate constants	
Oligosaccharide	rate constant (s^{-1})
$(NAG\text{-}NAM)_3$	0.5
$(NAG)_6$	0.25
$(NAG)_5$	0.033
$(NAG)_4$	7×10^{-5}
$(NAG)_3$	8×10^{-6}
$(NAG)_2$	2.5×10^{-8}

What is the mechanism?



What is the mechanism?



Lysozyme mechanism: carbocation or covalent intermediate?

- Nature Structural Biology 8, 737 - 739 (2001) Anthony J. Kirby “The lysozyme mechanism sorted — after 50 years”

Evidence for cation mechanism

- The pH dependence of lysozyme acid with pK ~6 and a base of pK ~ 4

Group	pK of E*	pK of ES	Titration
Glu-35	6.0	6.6	5.9
Asp-52	3.0	3.3	4.5

•Esterification of Asp-52 results in total loss of activity, as well as perturbation of the pK of Glu-35.

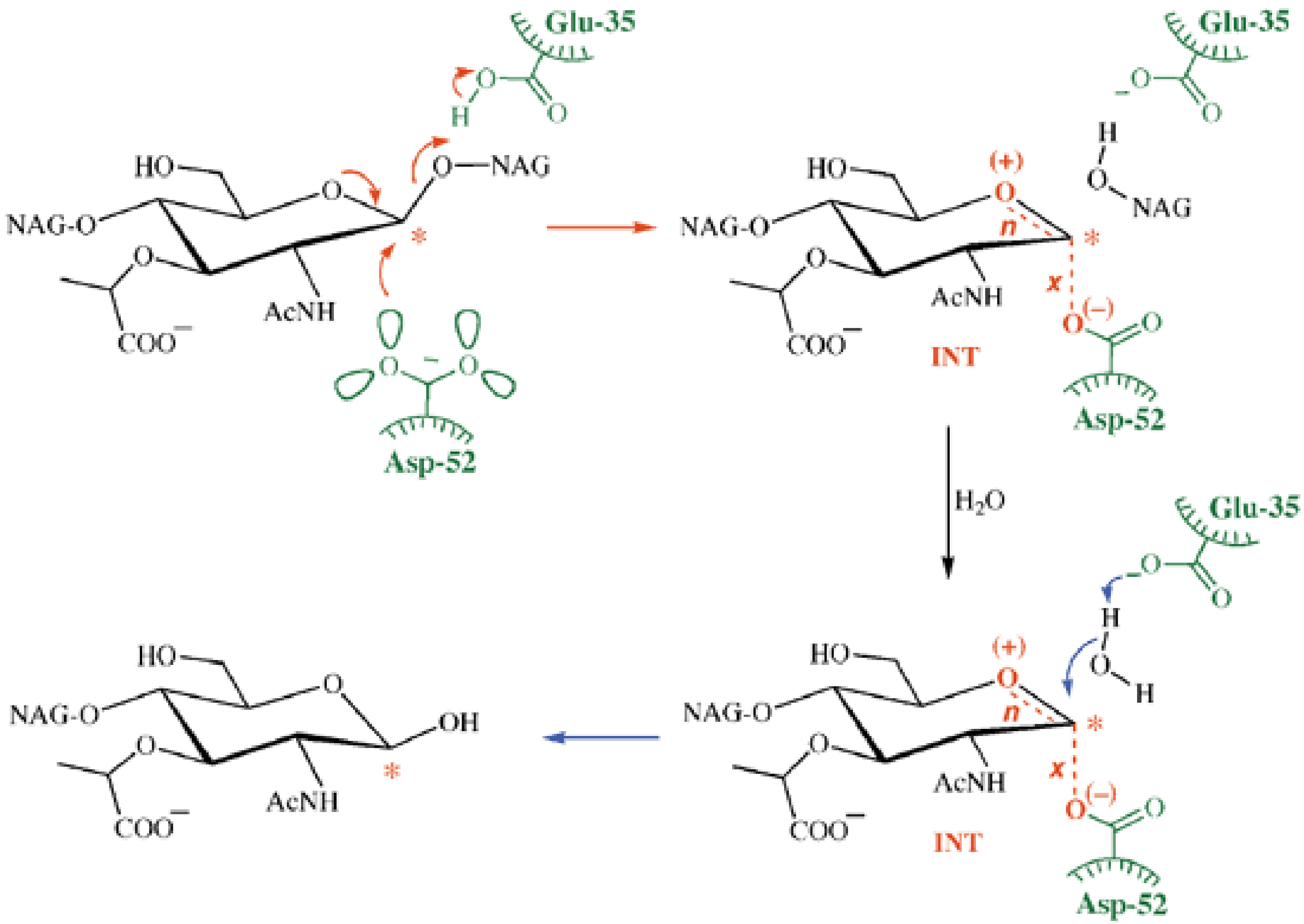
•*Site-directed mutagenesis:* Asp52Asn (5%), Glu35Gln (0%)

(T4 lysozyme no group corresponding to Glu-35 is found, suggesting that solvent water serves that purpose in this particular enzyme.)

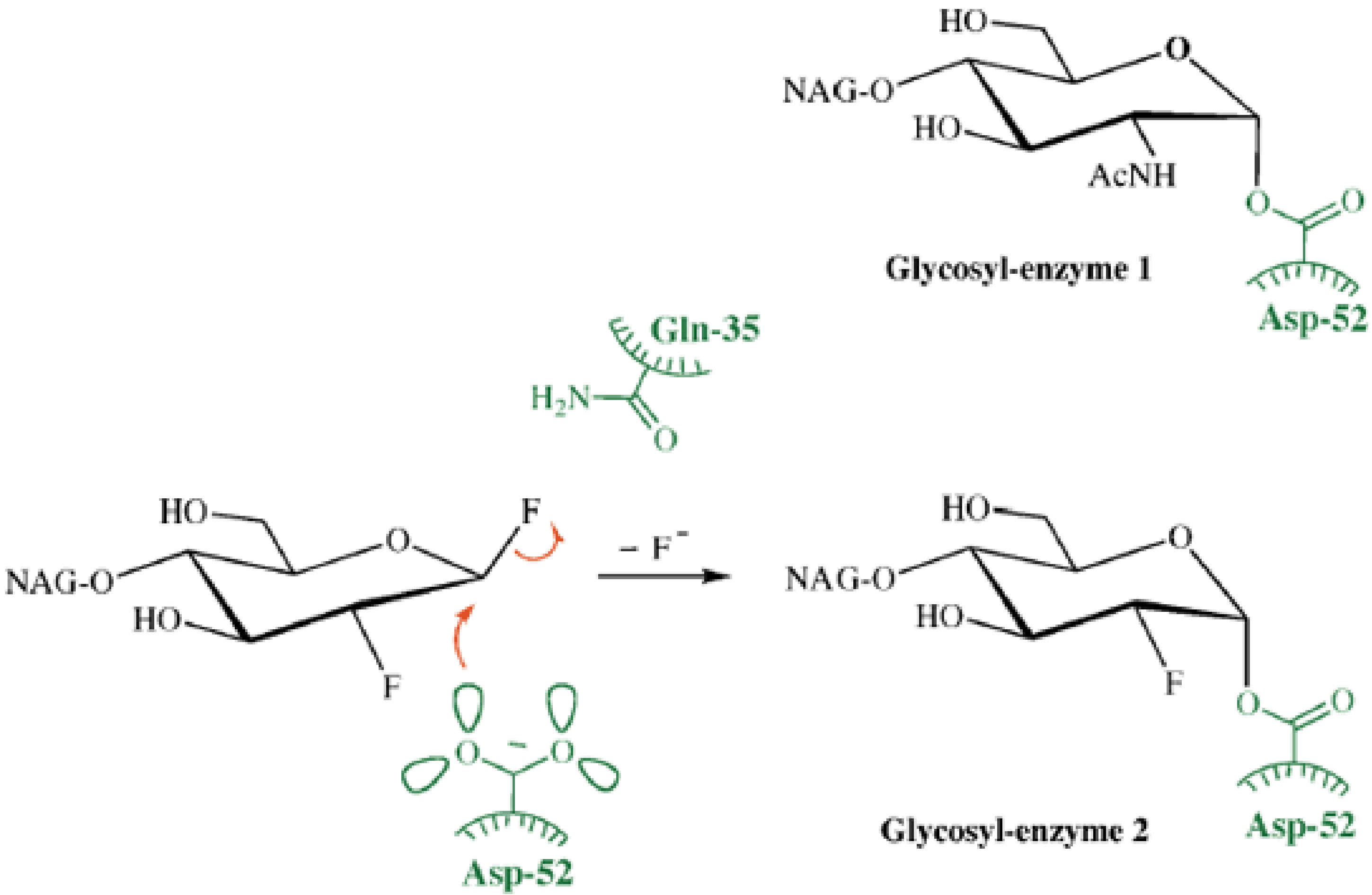
Animation of carbocation mechanism

- http://www.angelo.edu/faculty/nflynn/Biochemistry/Lysozyme%20Catalytic%20Mechanism.htm

What is the mechanism?



Vocadlo's mutant



Evidence for covalent mechanism?

- 20 August 2001 issue of **Nature**, Vocadlo, D. J., et al., report evidence that Asp-52 stabilizes ring 4 by forming a transient covalent bond rather than through ionic interactions.
- BUT....enzyme altered and substrates are modified disaccharides

- What about Protein Engineering, Vol. 12, No. 4, 327-331, April 1999? D52E....inactive
- D52S lysozyme with no negative charge at the 52 site (Hashimoto et al., 1996) retained more detectable activity (as much as 2% of the wild-type enzyme) than D52E lysozyme (0.7%). Xray structure of D52S: no adduct

Can you reconcile the mechanisms?

Classification of enzymes

IUPAC (International Union of Pure and Applied Chemistry)
IUBMB (International Union of Biochemistry and Molecular Biology)

1. Oxidoreductases (electron transfer)
   - donor (e.g. 1.1 CH-OH)
     - acceptor (e.g. 1.1.1 NAD+)
2. Transferases (group transfer)
   - group (e.g. 2.4 glyco-)
3. Hydrolases (transfer to water)
4. Lyases (double bonds - addition or elimination)
5. Isomerases (transfer within molecule)
6. Ligases (condensation coupled to ATP hydrolysis)

Example

http://www.chem.qmul.ac.uk/iubmb/enzyme/

- common name: trypsin
- IUPAC/IUBMB designation: **EC 3.4.21.4**

  EC 3 (hydrolases)

  EC 3.4 (peptide hydrolases - peptidases)

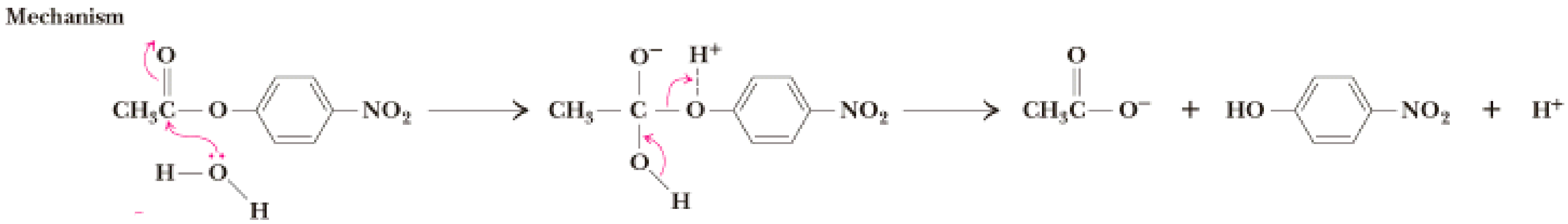
  EC 3.4.21 (serine endopeptidases)

- EC 2.4.1.40 glycoprotein-fucosylgalactoside α-N-acetylgalactosaminyltransferase

Group transfer by displacement reactions

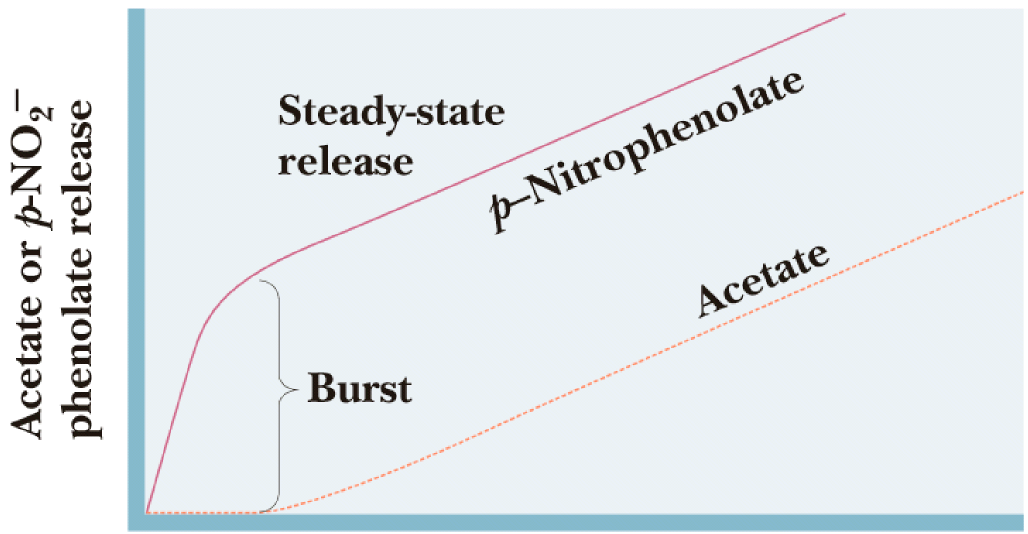
- Factors affecting displacement
  - equilibrium (e.g. hydrolysis)
  - reactivity of nucleophile (basicity, polarizability)
  - leaving group displaced (must accomodate a pair of electrons)
  - other (enzyme substrate interactions lowering energy of transition state)

Example: Hydrolysis

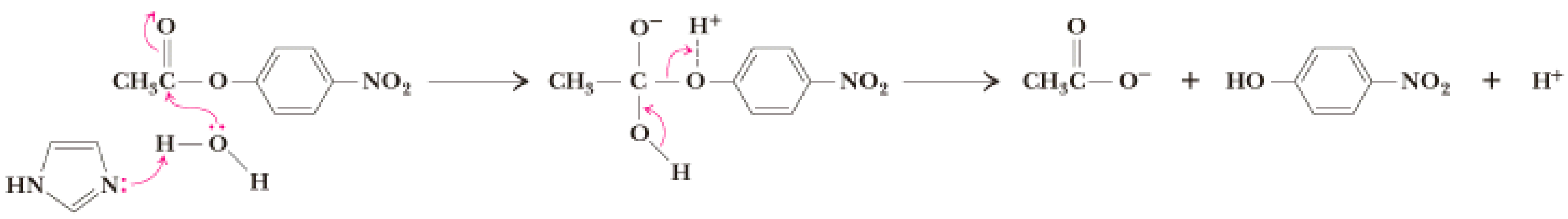


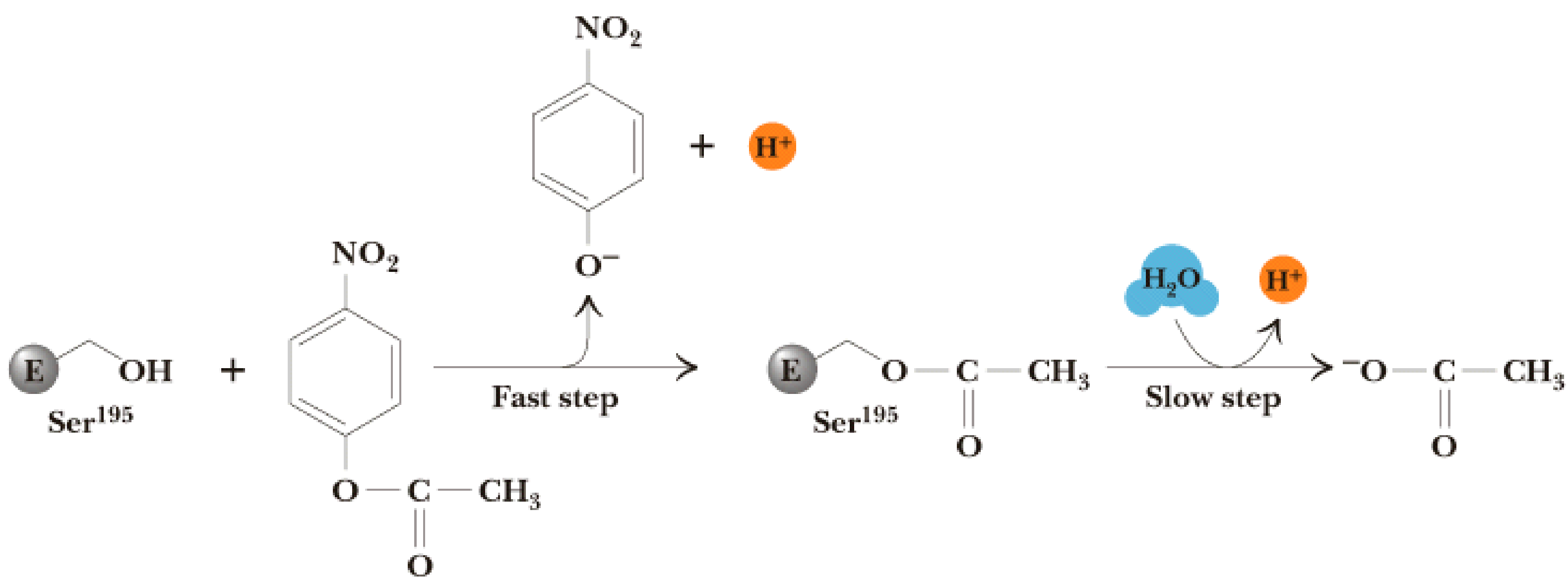
How could we lower the transition state energy?

What does kinetics tell us about the mechanism?



Mechanism





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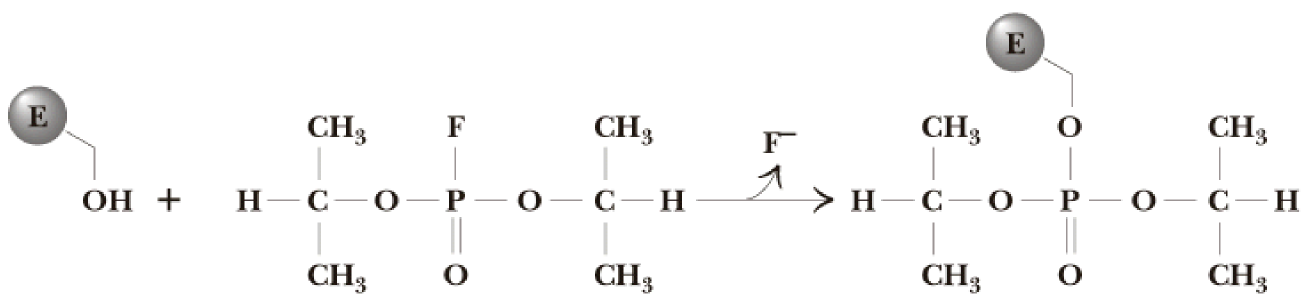
Catalytic serine identified by pseudosubstrate DFP



Diisopropylfluorophosphate

Diisopropylphosphoryl derivative of chymotrypsin

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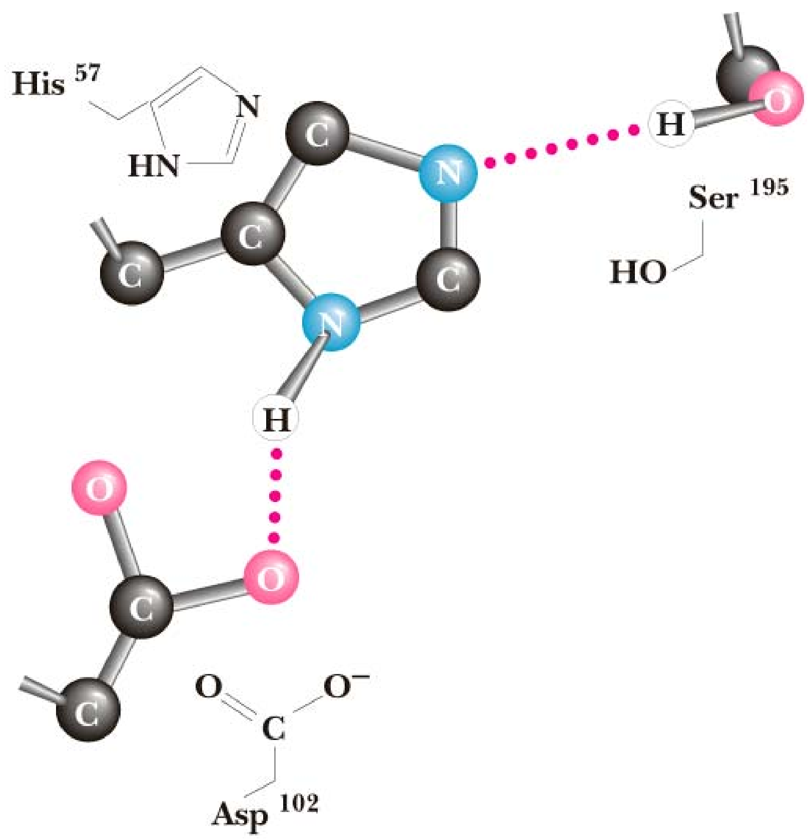
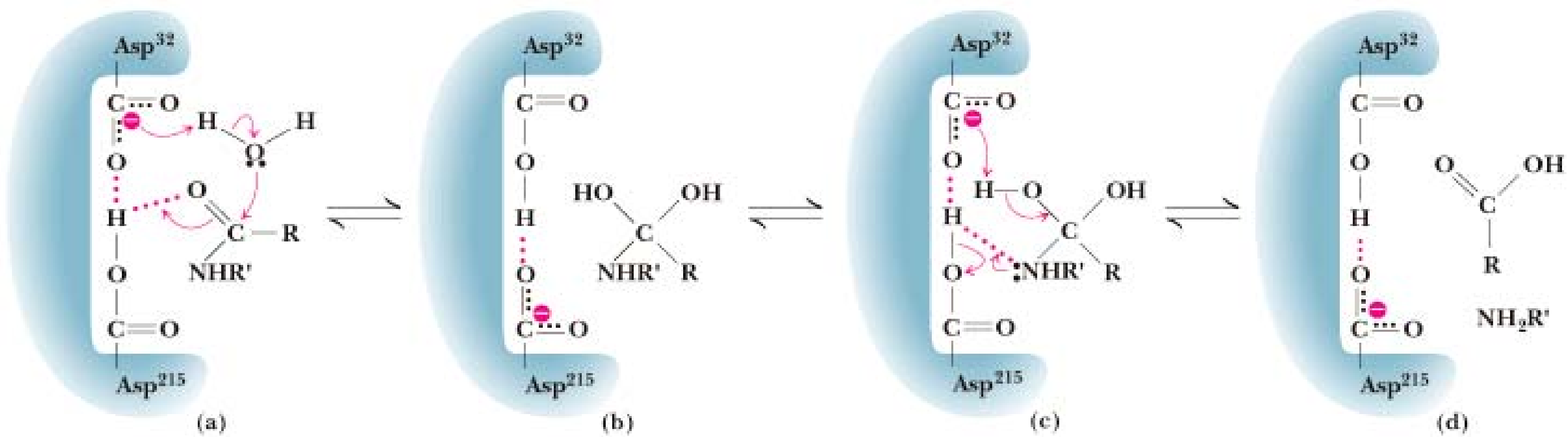
“Catalytic triad”



Aspartyl proteases



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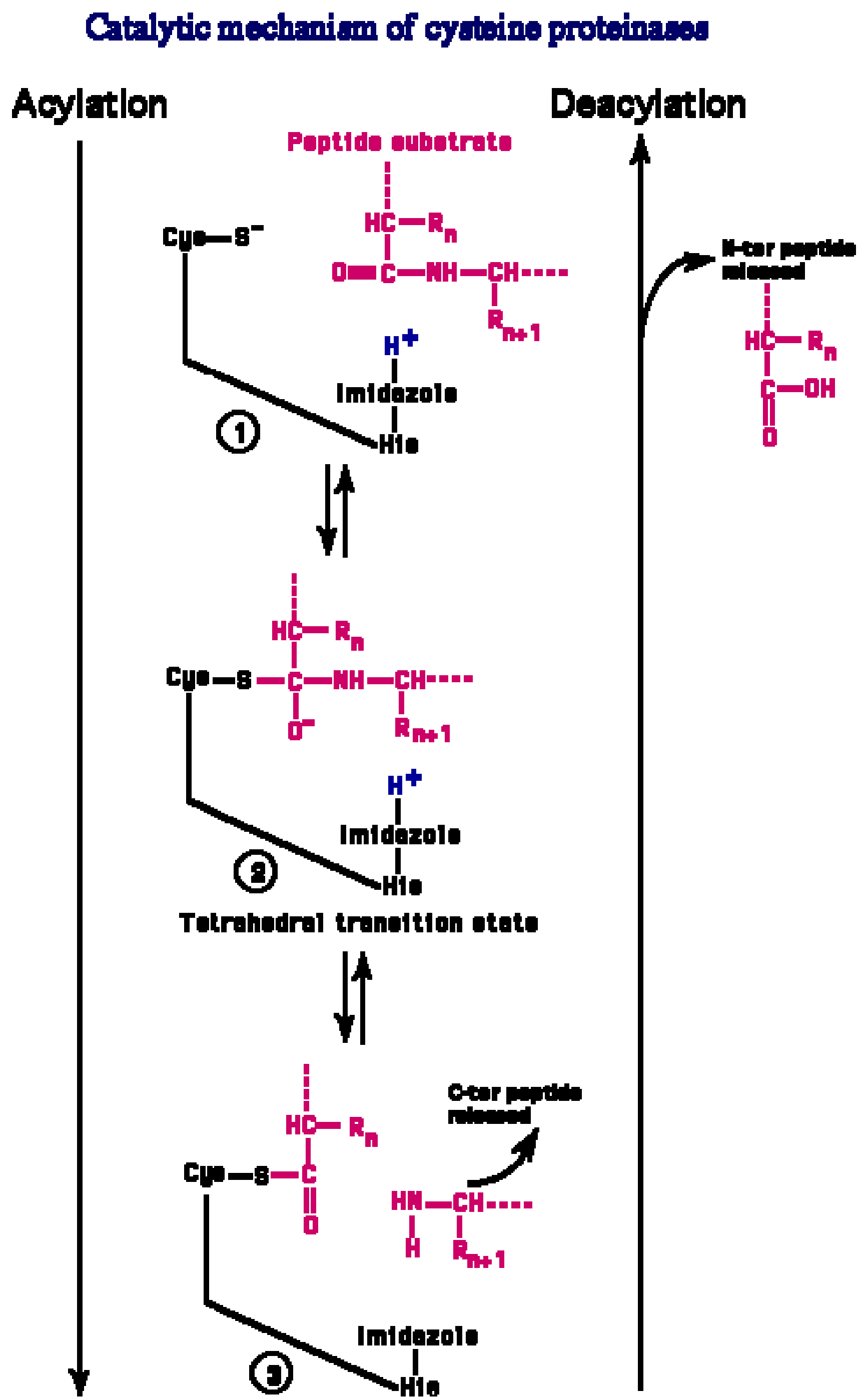
Cysteine proteases

- mammalian lysosomal cathepsins, plant papain
- nucleophile is a thiolate ion
- covalent intermediate



Metalloproteases

Catalytic mechanism of metallo proteinases