Structure-Function Studies of Proteins: β-Lactamase

A. Definition & Purpose

B. Steps in Structure-Function Studies

1. Structural analysis

2. Mutational design and mutagenesis
   i) Surface-exposed vs. core-buried residues

   ii) Conservative vs. non-conservative mutation

   E: $-CH_2CH_2COO-$ ⟶

   iii) Point, combinational, or scanning mutagenesis

   iv) Primer design, restriction sites, PCR strategy

3. Subcloning into an expression vector

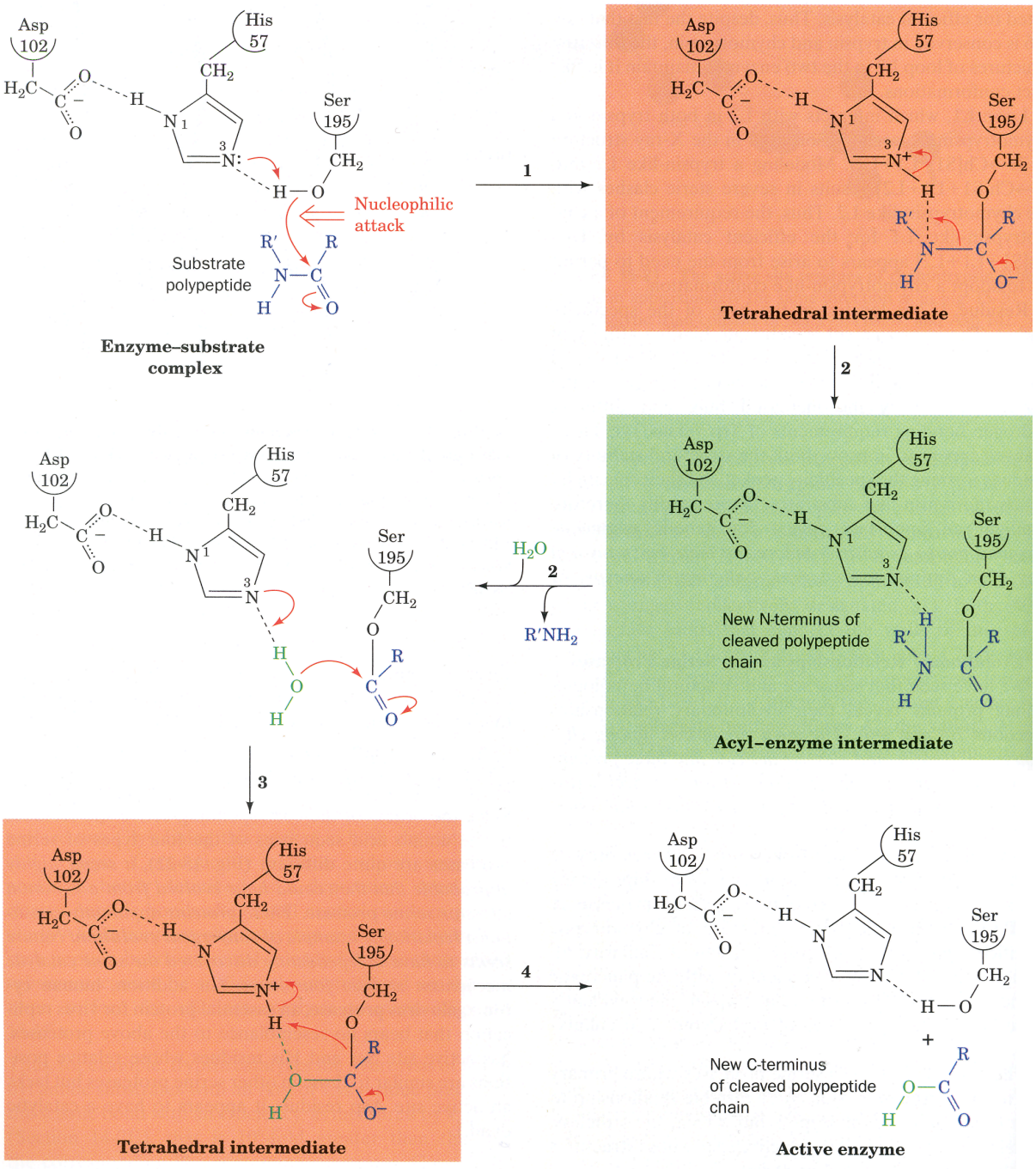
4. Transformation into expressing cells (*E. coli*)

5. Protein Expression

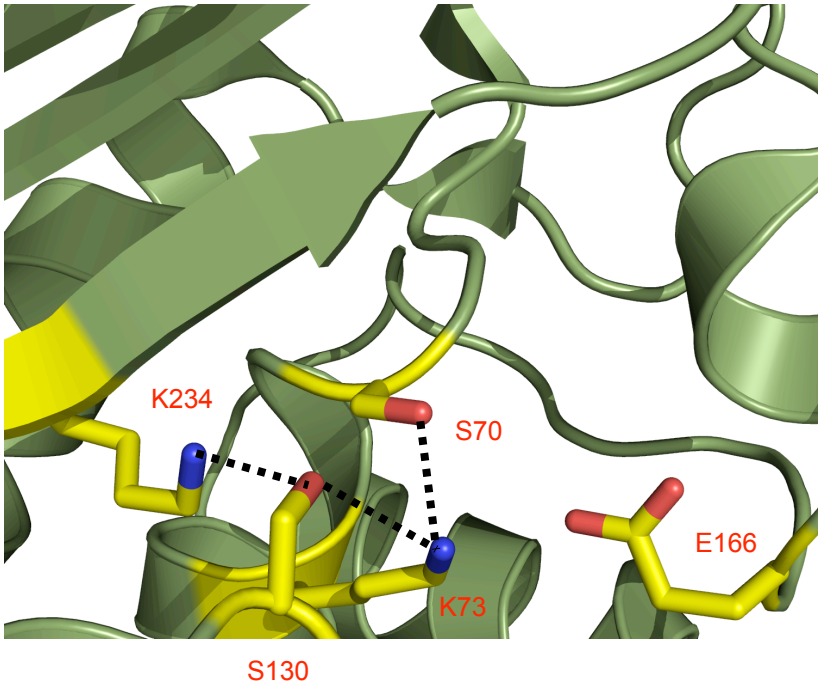
6. Protein Purification

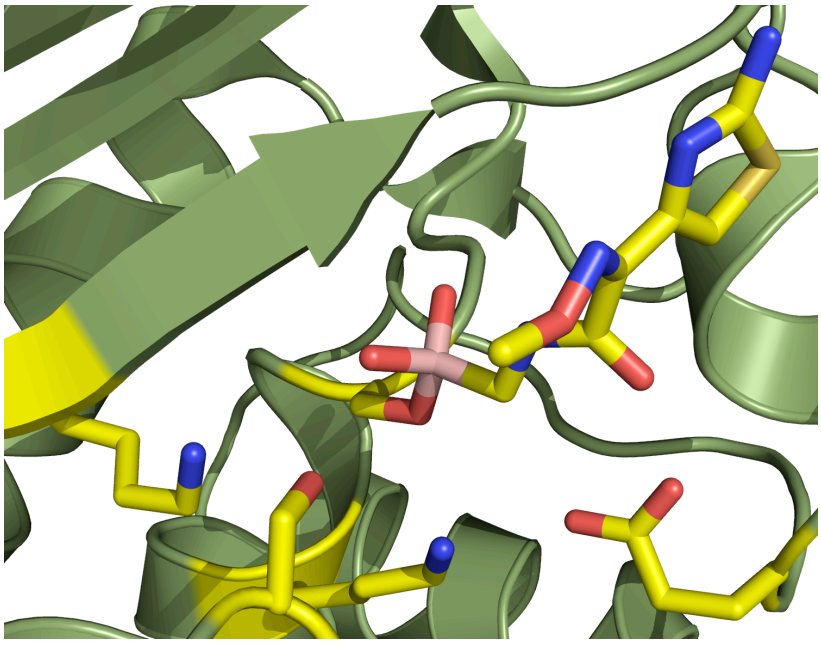
7. Characterization
   i) Structural analysis
   ii) Protein stability
   iii) Functional analysis: activity, binding, etc.

Catalytic Mechanism of Serine Proteases: Chymotrypsin



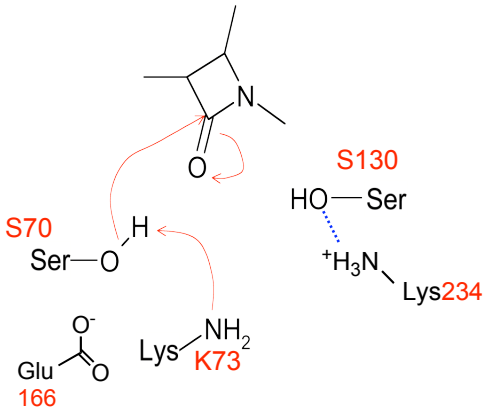
Active Site Residues of β-Lactamase



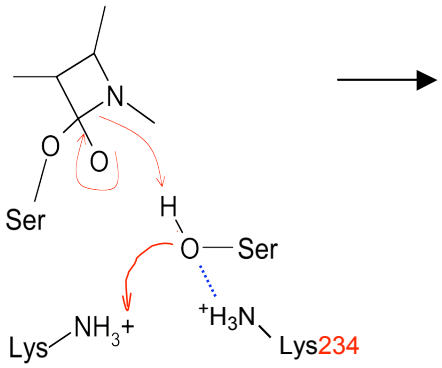


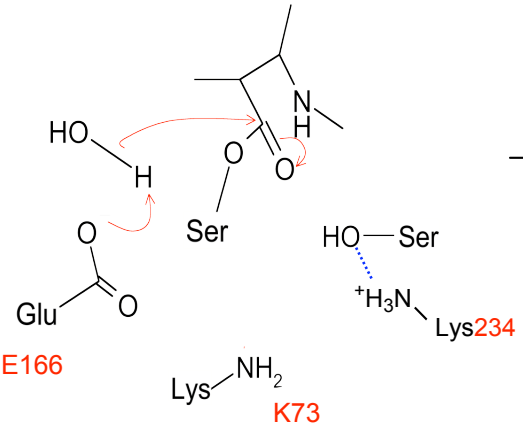
Proposed Catalytic Mechanism of β-Lactamase

Michaelis complex (E•S)

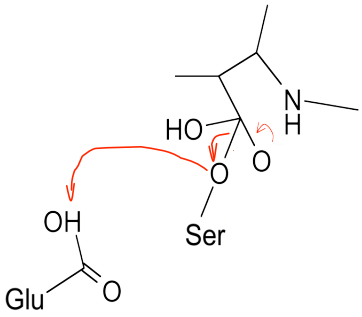


Tetrahedral complex I

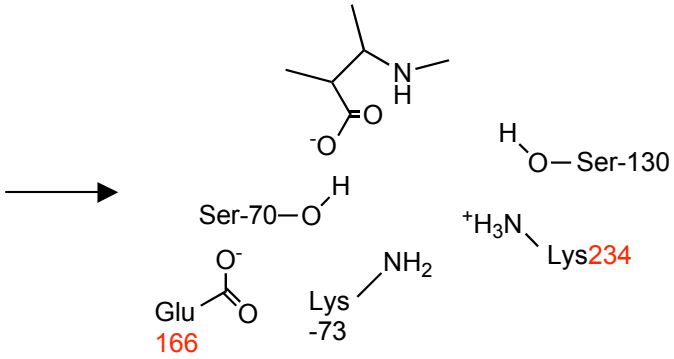




Acyl-enzyme Intermediate



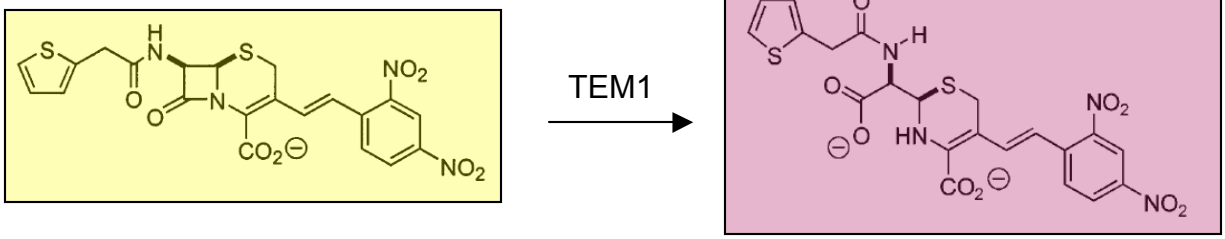
Tetrahedral complex II



Enzyme-Product complex (E•P)

Spectrophotometric (Colorimetric) β-Lactamase Assay

Nitrocefin



λ_{max} = 390 nm at pH 7.0

λ_{max} = 486 nm at pH 7.0

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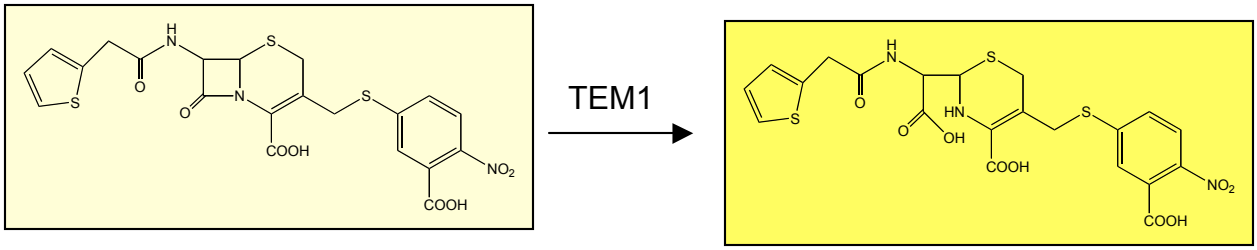

λ_{max} = 340 nm

λ_{max} = 405 nm