

BL/CH401 Lecture 15B Add-on

Enzymatic Mechanism of Catalysis

How do enzymes catalyze a reaction? Enzyme's amino acid side chains at the active site *interact* with the substrate's chemical bonds to facilitate *bond rearrangement* leading to products.

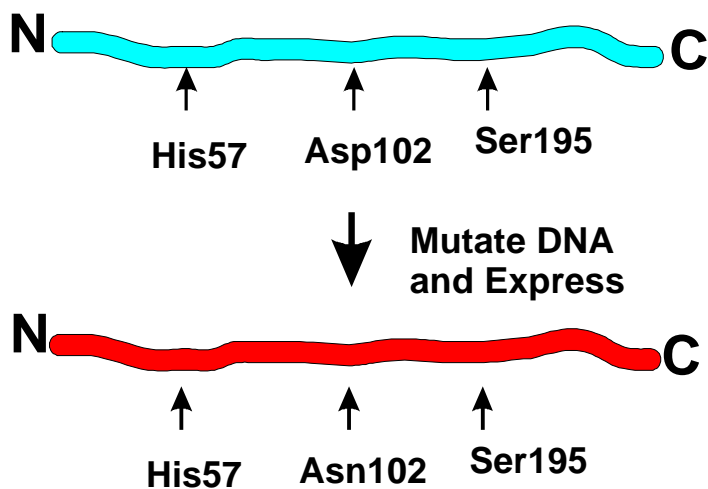
To understand an Enzyme's Catalytic Mechanism, we must:

1. Identify AA's involved in catalysis at the active site
2. Make a hypothesis for how the AA side chains assist bond rearrangement
3. Test the hypothesis by appropriate experiments.

Identification of Asp-102 of Trypsin

After the gene for trypsin was cloned from rat and the enzyme expressed in *E. coli*, the protein was modified by site-direct mutagenesis. Asp-102 was shown to be required for enzyme activity by site-directed mutagenesis and mutant protein expression:

Site-Directed Mutagenesis of Trypsin

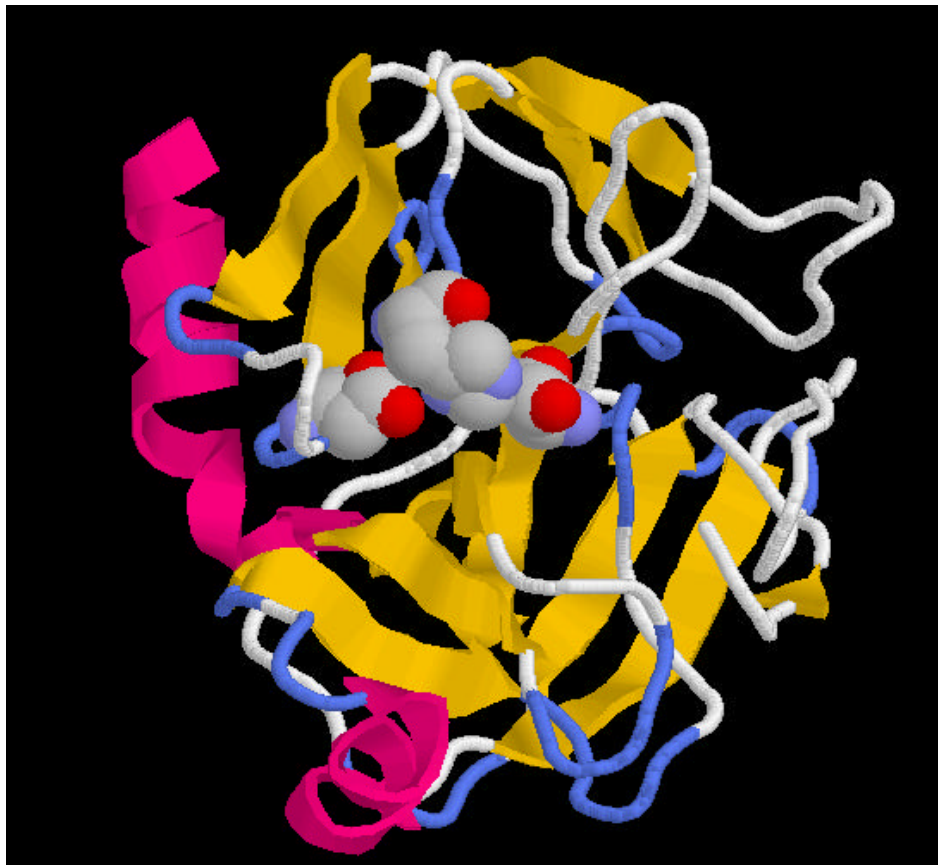


When Asn was substituted for Asp102, the following results were obtained:

Trypsin Form	$k_{\text{cat}} \text{ min}^{-1}$	Relative (Mutant k_{cat} / WT k_{cat})
Wild-type	63,100	1
Asn102 Mutant	16	~0.00025

Asn102 Mutant of Trypsin lost most of its activity. In addition, the mutant was crystallized and shown to have same conformation as the wild-type trypsin.

In addition, Ser195 in the mutant Trypsin no longer reacted with DFP and was not inhibited. **These results show that Asp102 is essential for trypsin catalytic activity.**



3-D Model of Trypsin showing Asp102, His57 and Ser195.

© Wilbur H. (Bill) Campbell 1997; wcampbel@mtu.edu