Serine Proteases and their Inhibitors

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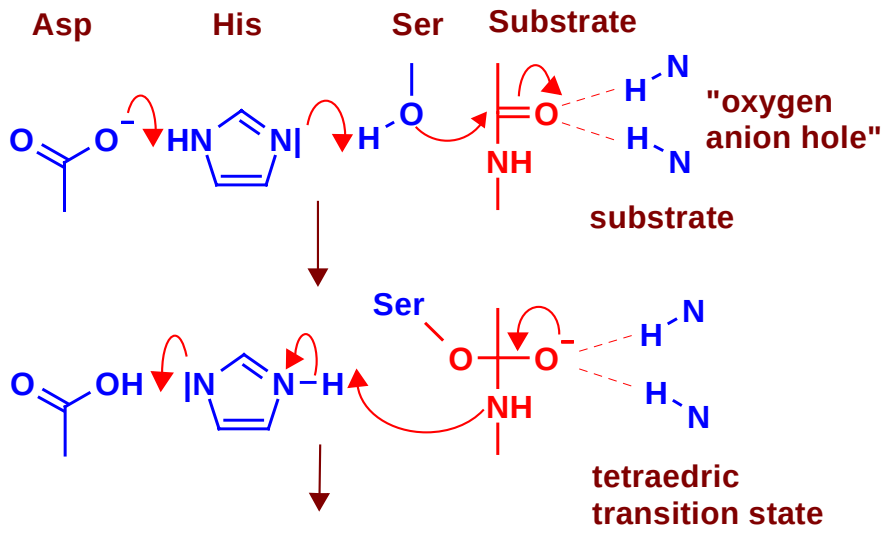
Serine Proteases of Physiological Importance

Protease	Cleavage Site	Function
Trypsin	Arg-X, Lys-X	Digestion
Chymotrypsin	Tyr-X, Phe-X, Trp-X	Digestion
Elastase	Val-X	Tissue degradation
Thrombin	Arg-Gly	Blood coagulation
Factor Xa	Arg-Ile, Arg-Gly	Blood coagulation

Other Important Serine Proteases:

Tryptase, Lipases, Phospholipases, Subtilisin

Catalytic Mechanism of Serine Proteases



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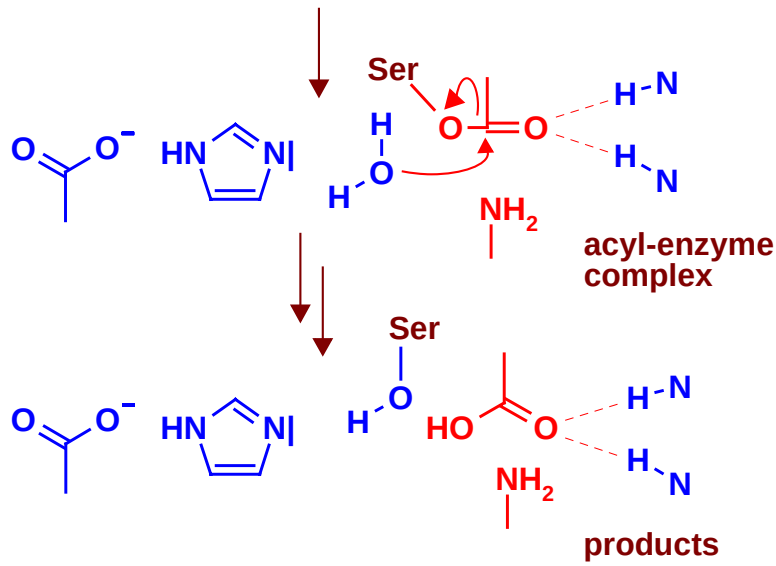
Catalytic Mechanism of Serine Proteases



Dissection of the Catalytic Triad of Subtilisin

(substrate: N-succinoyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide).
P. Carter and J. A. Wells, Nature 332, 564-568 (1988)

Subtilisin, wild type and mutants			K_m	k_{cat}/K_m
AA 32	AA 64	AA 221	(μM)	($s^{-1}mol^{-1}$)
Asp	His	Ser	220	250 000
Ala	His	Ser	480	5
Asp	Ala	Ser	390	0.1
Asp	His	Ala	420	0.1
Ala	Ala	Ala	330	0.1
uncatalysed reaction: k *vs.* $k_{Ala,Ala,Ala} \approx 0.0003$				

Why is the Ala-Ala-Ala mutant still enzymatically active ?

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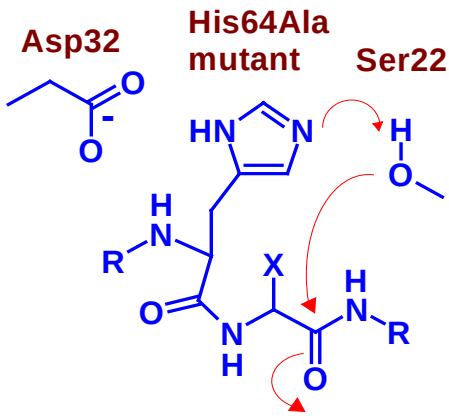
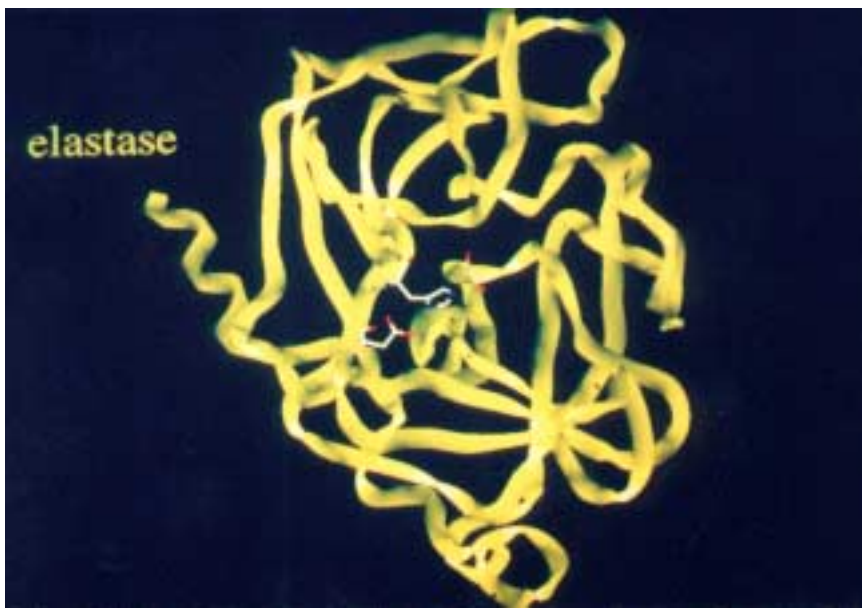
Substrate-Assisted Catalysis in a Subtilisin His64Ala Mutant

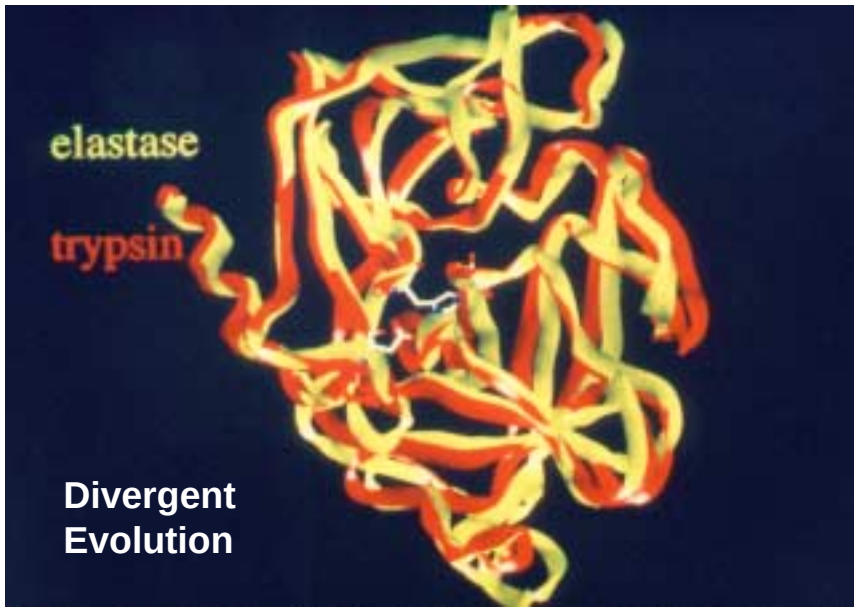


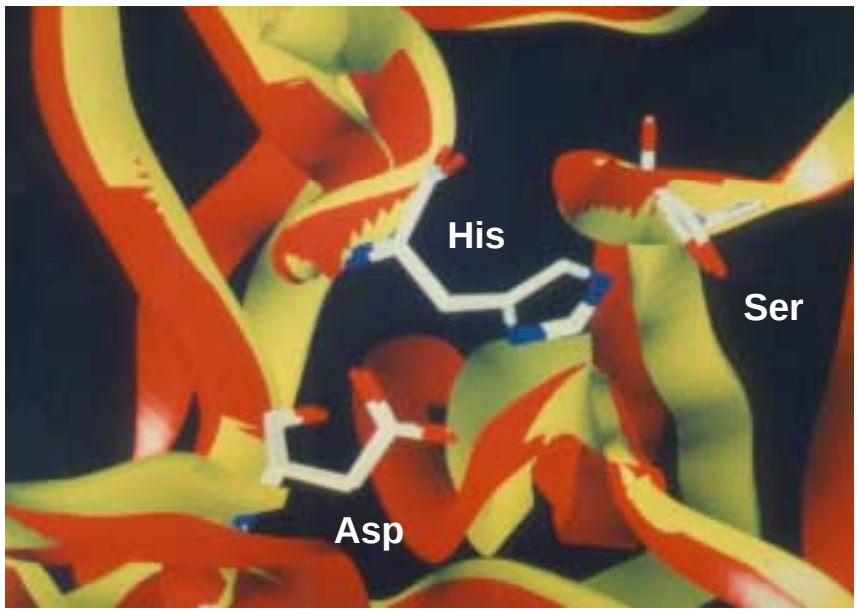
Subtilisin cleaves structurally different peptide substrates. The His64Ala mutant cleaves XXX-His-XXX peptides 4 orders of magnitude slower than wild-type subtilisin but about 200 times faster than substrates without His.

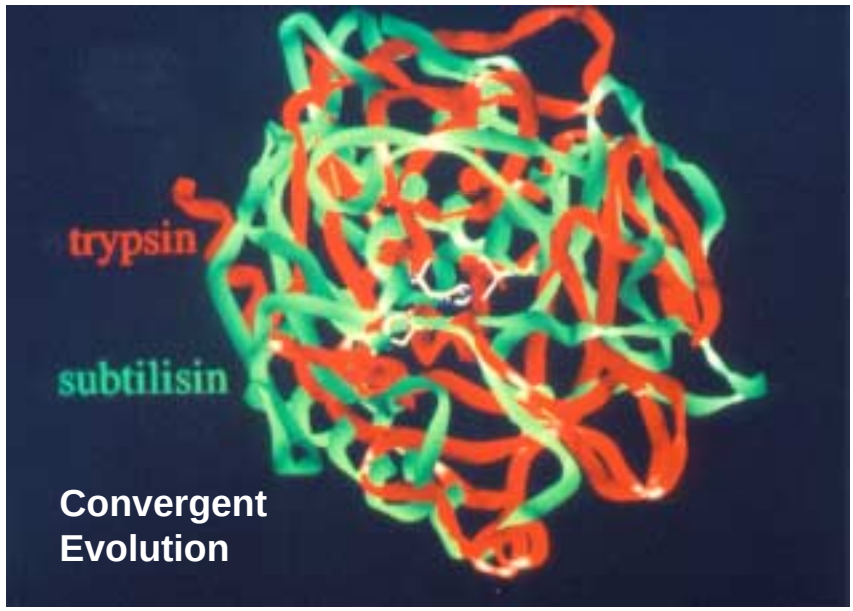
P. Carter and J. A. Wells, Science 237, 394-399 (1987)

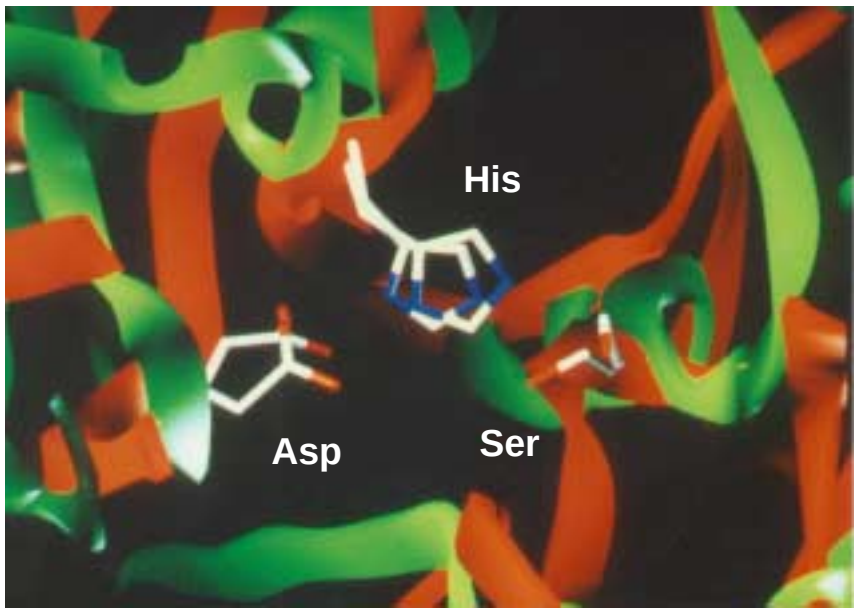

trypsin


elastase

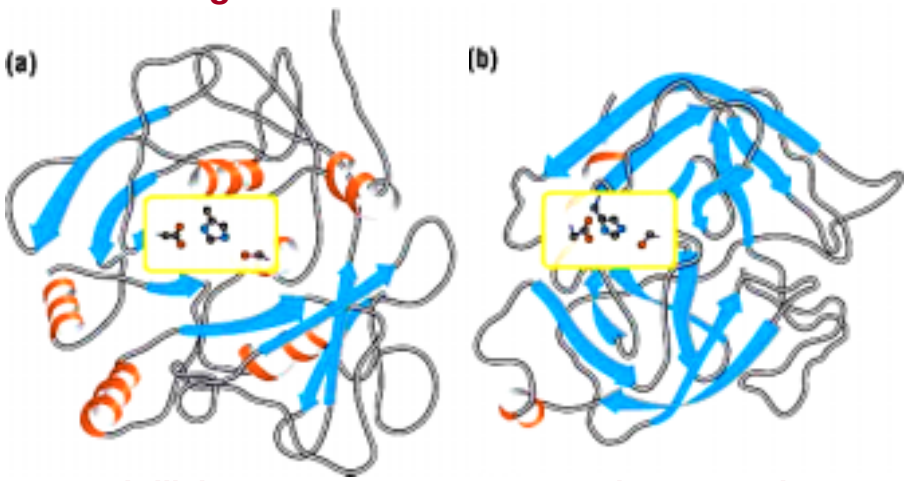

elastase
trypsin
Divergent
Evolution


His
Ser
Asp


trypsin
subtilisin
Convergent
Evolution


His
Asp
Ser

Convergent Evolution of Serine Proteases



subtilisin **chymotrypsin**

www.new-science-press.com/browse/protein/1/16

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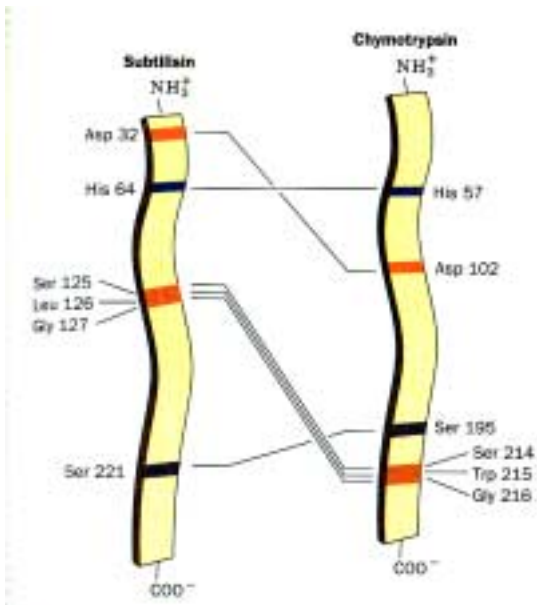
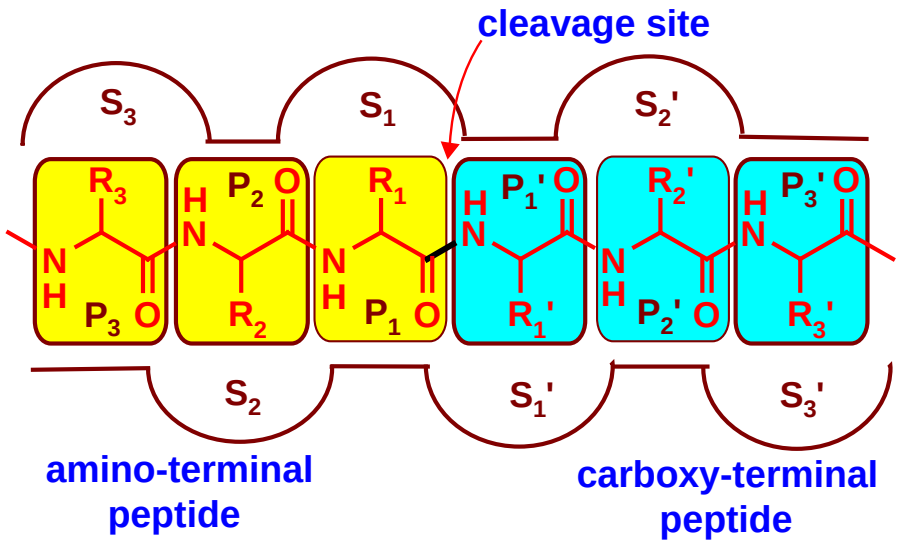


Convergent Evolution of Serine Proteases

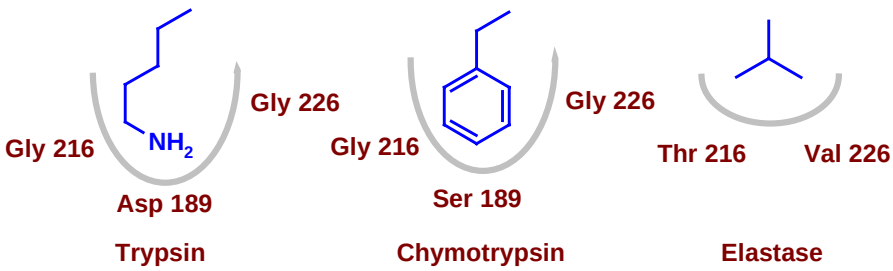
Asp, His and Ser are the amino acids of the catalytic triad.
Ser, Leu/Trp and Gly interact with the substrate.

J. D. Robertus et al., Biochemistry 11, 2439-2449 (1972)

Protease Binding Pockets for a Peptide Substrate



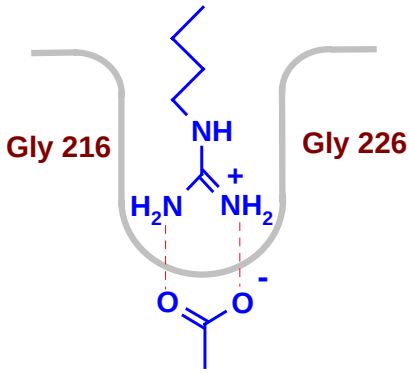
Comparison of the P_1 Pockets of Trypsin, Chymotrypsin and Elastase



The binding pockets of trypsin and thrombin accommodate positively charged amino acid side chains by the negatively charged Asp 189. The P_1 pocket of chymotrypsin is designed for large, lipophilic side chains. Elastase has a relatively small lipophilic P_1 pocket; it binds only small hydrophobic amino acids, like alanine and valine.

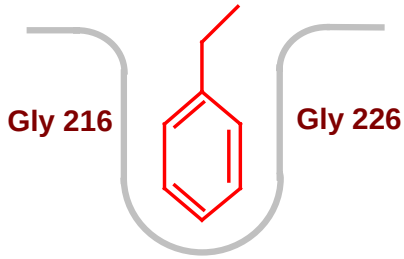
Specificity of a Trypsin Asp189Lys Mutant

Trypsin, wild-type enzyme



Asp 189

Trypsin Asp189Lys Mutant



???

Lys 189

What is the reason for this surprising selectivity?

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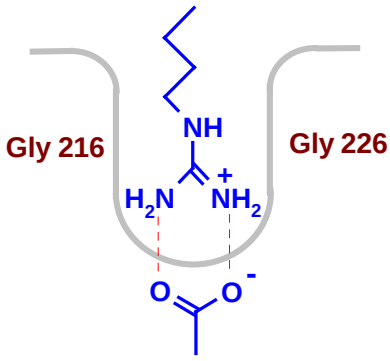
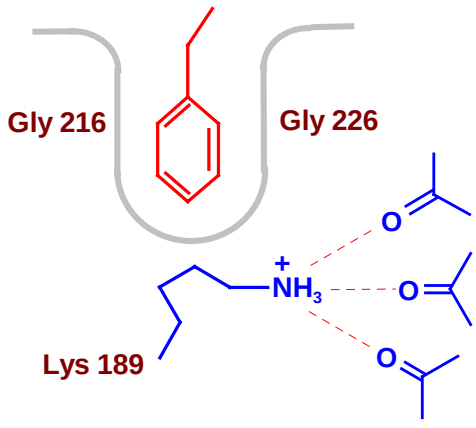
Specificity of a Trypsin Asp189Lys Mutant

Trypsin, wild-type enzyme



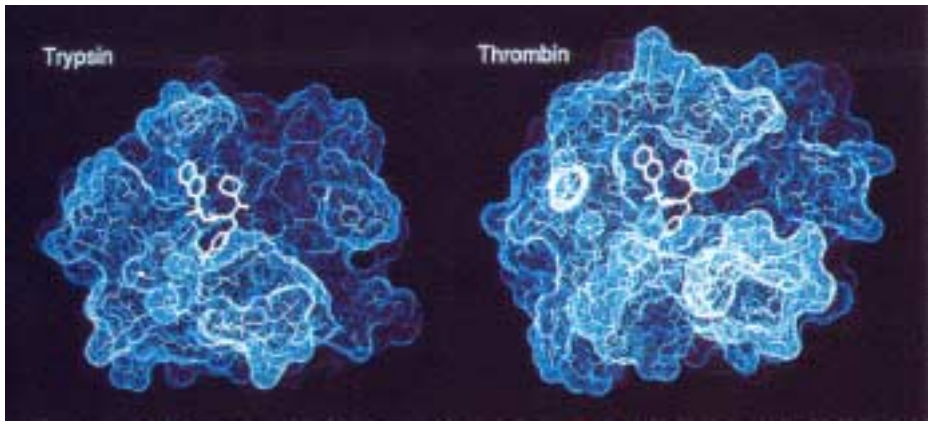
Asp 189

Trypsin Asp189Lys Mutant



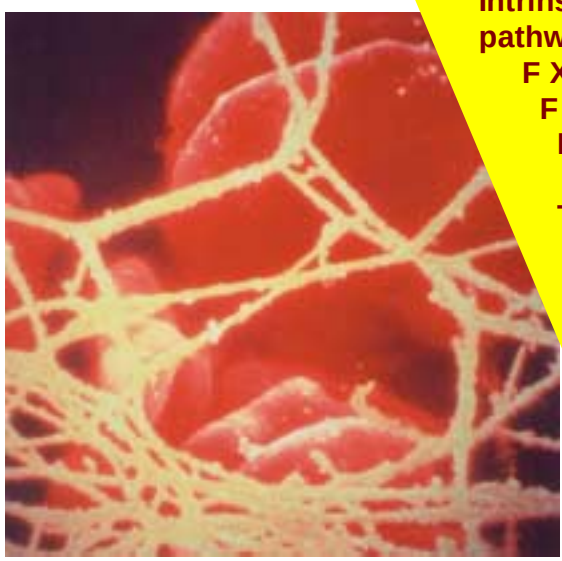
Lys 189

Trypsin and Thrombin Show Different Substrate Selectivity



open pocket, non-selective **closed pocket, selective**

Blood Coagulation

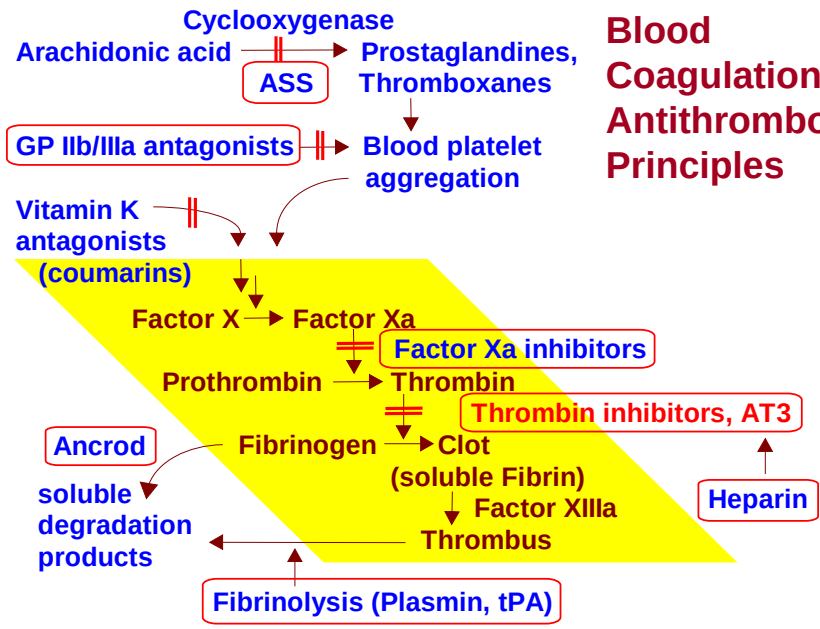


Intrinsic pathway
F XII
F XI
F IX
F X
Thrombin
↓
Fibrin
↓ **F XIII**
Thrombus

Extrinsic pathway
F VIII

Fibrinolysis
↑
Plasmin
Plasminogen

Blood Coagulation: Antithrombotic Principles



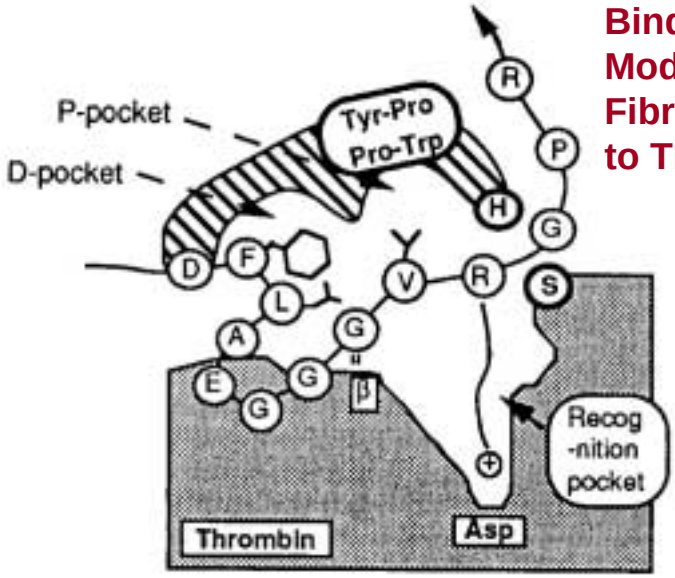
Relative Inhibitory Activities of Tripeptide Aldehydes vs. Thrombin

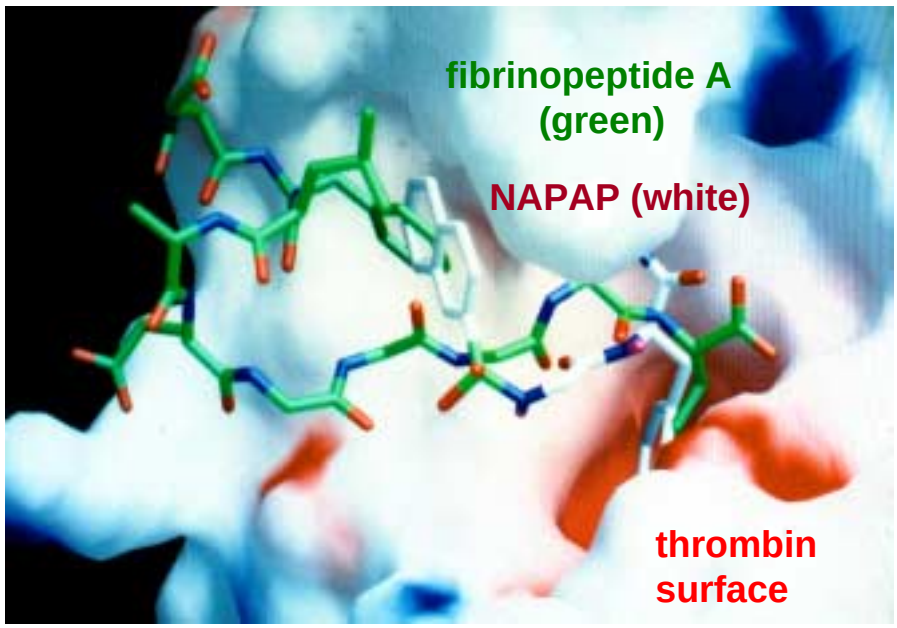
Peptide	Relative inhibitory activities
Gly-Val-Arg-H	1
Gly-Pro-Arg-H	9
Phe-Pro-Arg-H	57
D-Ala-Pro-Arg-H	469
D-Val-Pro-Arg-H	1 273
D-Phe-Pro-Arg-H	7 370

Arg-H = arginine aldehyde

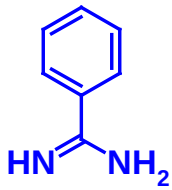
Why is the D-Phe analog more active than the L-Phe analog?

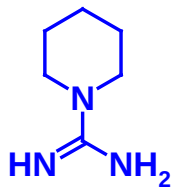
Binding Mode of Fibrinogen to Thrombin





Affinity of Inhibitors to Thrombin and Trypsin





K_i (thrombin) = 220 µM
K_i (trypsin) = 35 µM

K_i (thrombin) = 150 µM
K_i (trypsin) = 360 µM

Benzamidine binds specifically to trypsin, whereas N-amidino-piperidine has a slightly higher specificity for thrombin.

Thrombin 189-200: DACEGDSGGPFV
Trypsin 189-200: DSCQGDSGGPVV

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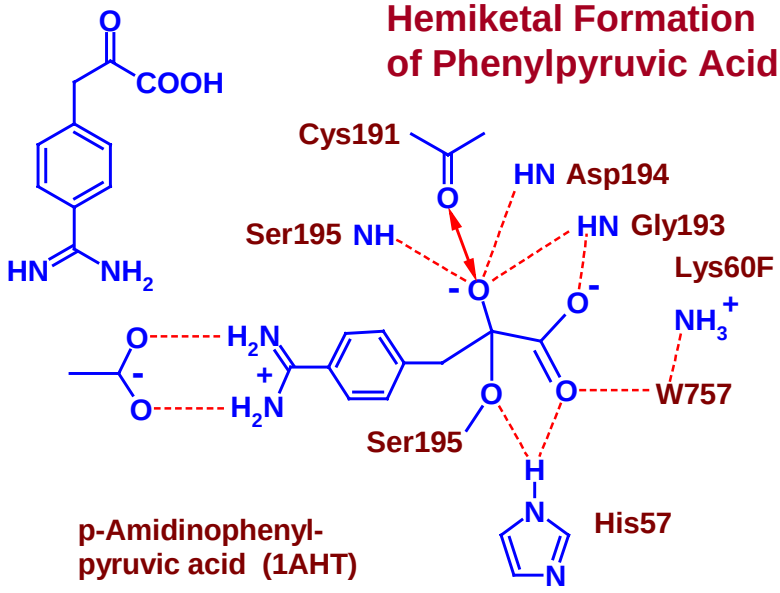
Hemiketal Formation of Phenylpyruvic Acid

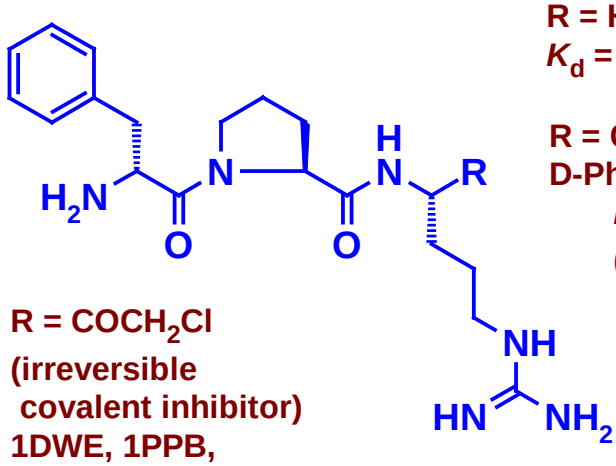


p-Amidinophenyl-pyruvic acid (1AHT)

Groups that Covalently Interact With the Catalytically Active Serine

Inhibitor Type	Functional Groups	
Irreversible Inhibitors	Chloromethylketones	-$COCH_2Cl$
	Sulfonylfluorides	-SO_2F
	Esters	-COOR
	Boronic Acids	-$B(OR)_2$
Reversible Inhibitors	Aldehydes	-CHO
	Arylketones	-CO-Aryl
	Trifluoromethylketones	-$COCF_3$
	Ketocarboxylic acids	-COCOOH

"Classical" Thrombin Inhibitors

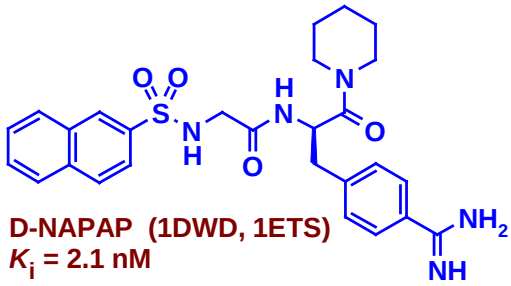


R = H
K_d = 180 nM

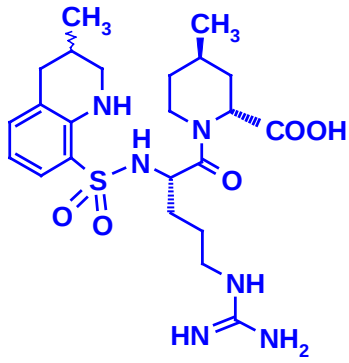
R = CHO
D-Phe-Pro-Arg-H
K_d = 1.8 nM
(reversible covalent inhibitor)

R = $COCH_2Cl$
(irreversible covalent inhibitor)
1DWE, 1PPB,
(cf. 1AI8, 1AIX, 1HAI)

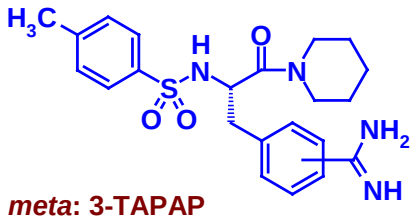
"Classical" Thrombin Inhibitors



D-NAPAP (1DWD, 1ETS)
K_i = 2.1 nM



Argatroban, MD-805
(1DWC, 1ETR)
K_i = 19 nM



meta: 3-TAPAP
para: 4-TAPAP (1ETT)

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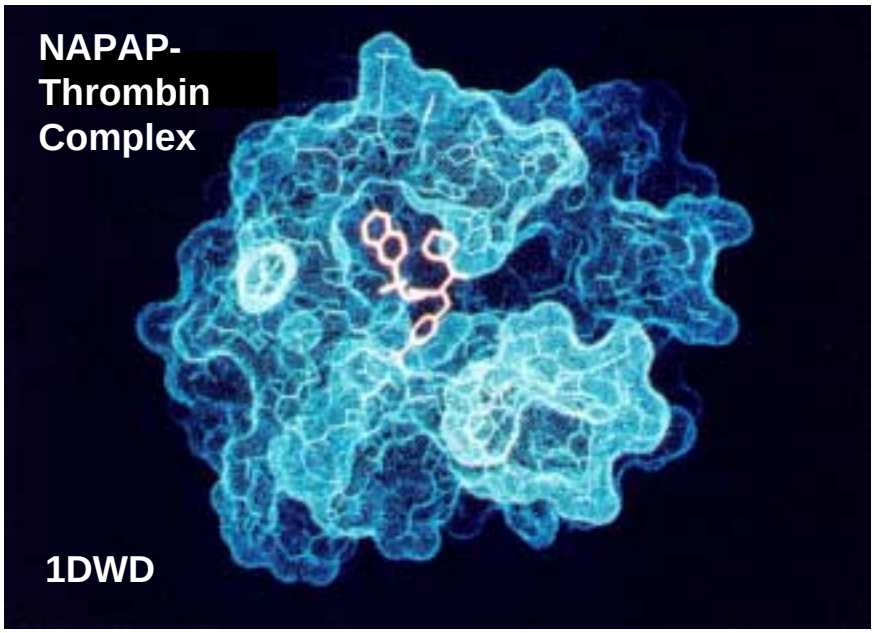
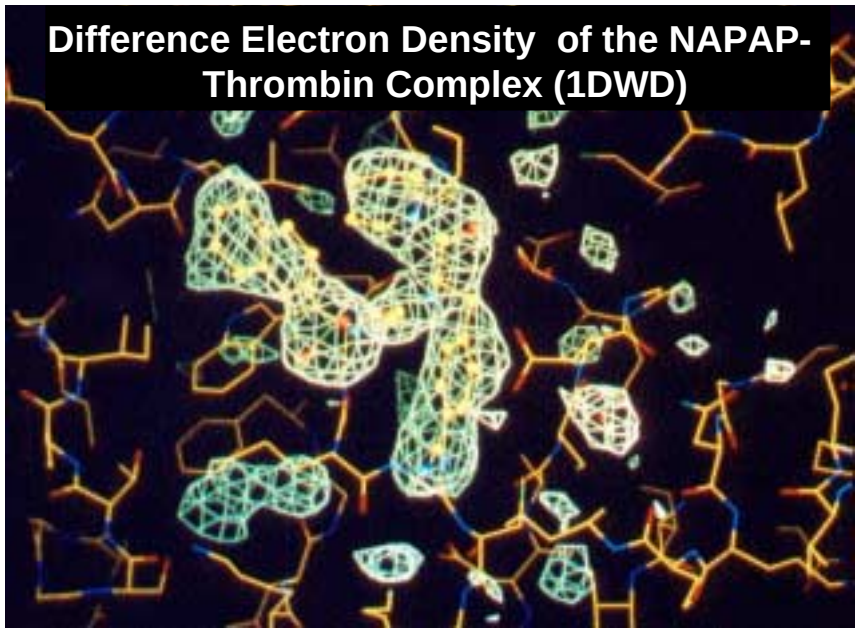
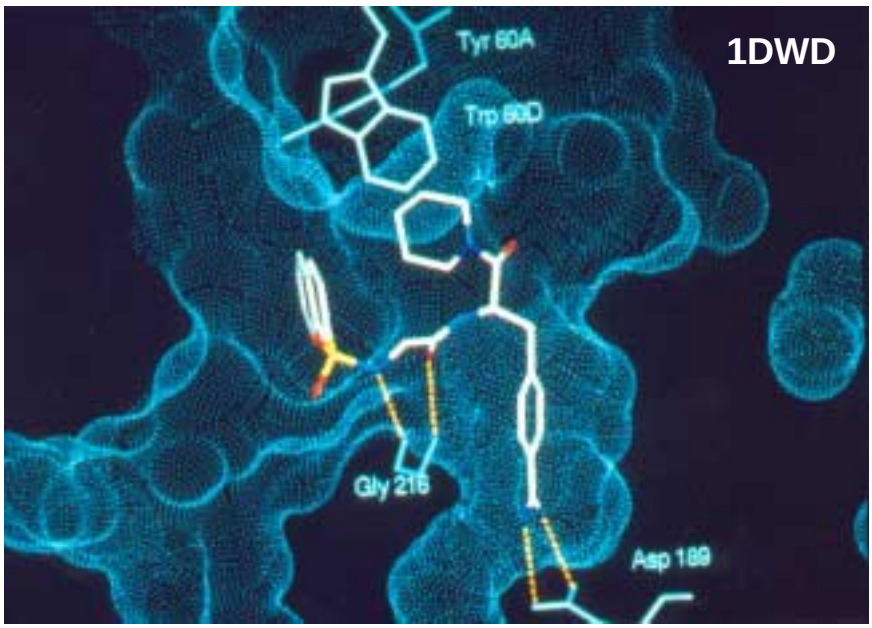
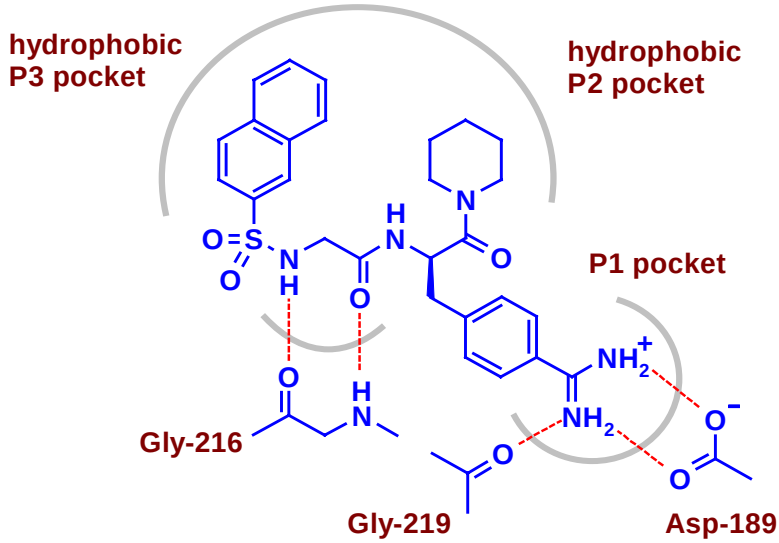


Difference Electron Density of the NAPAP-Thrombin Complex (1DWD)





Binding Mode of NAPAP (1DWD)

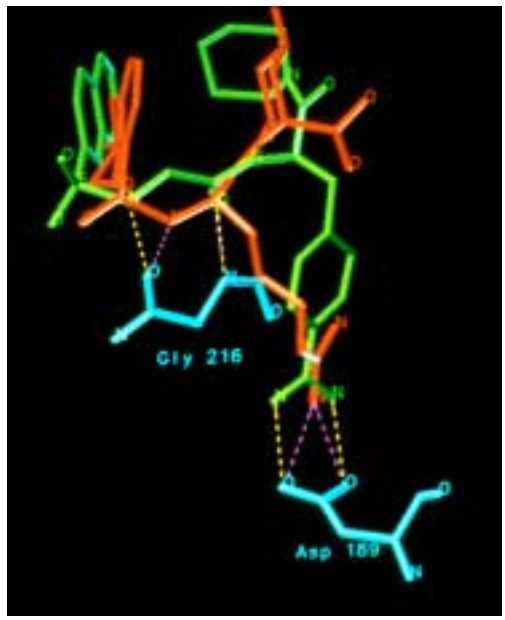


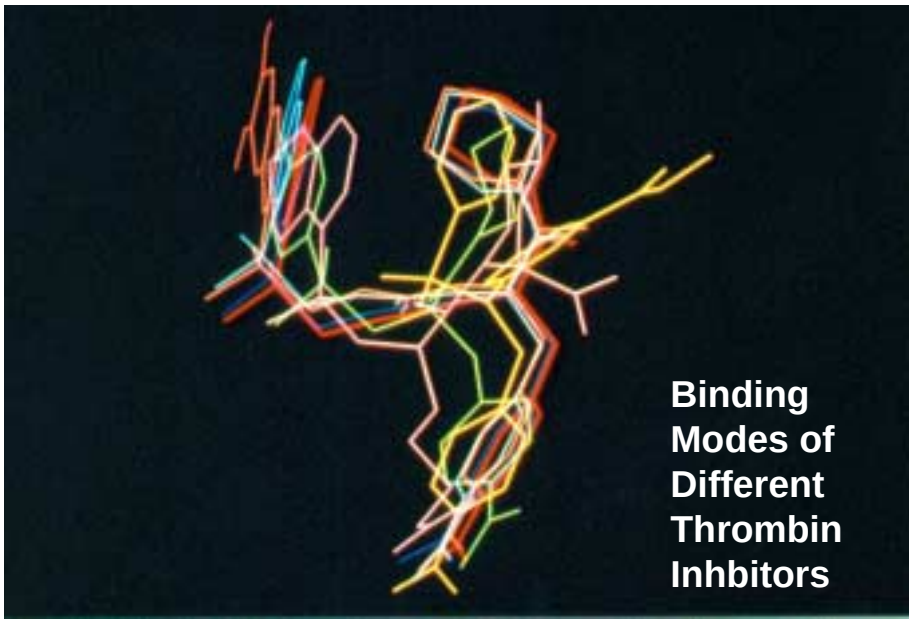
Binding Modes of Thrombin Inhibitors

(„hydrophobic collapse“)

NAPAP
(1DWD)

Argatroban
(1DWC)





Binding Modes of Different Thrombin Inhbitors

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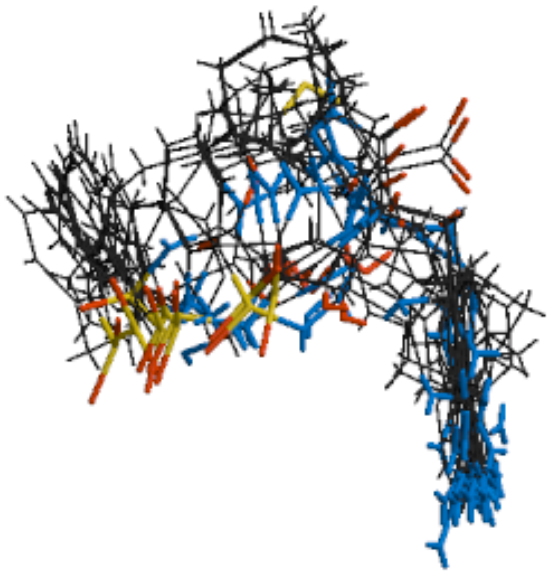

Binding Modes of Different Thrombin Inhbitors

(coordinates from X-ray structure analyses and from docking)