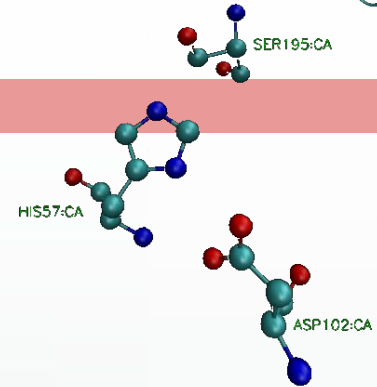
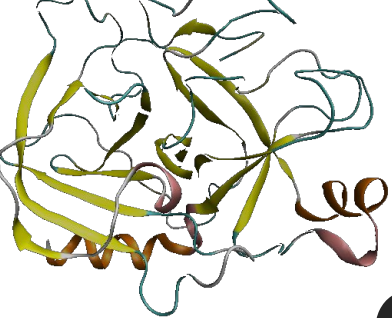


# SERINE PROTEASES

Maria Alós, Laura Carrillo, Alba  
Crespo, David Fernández





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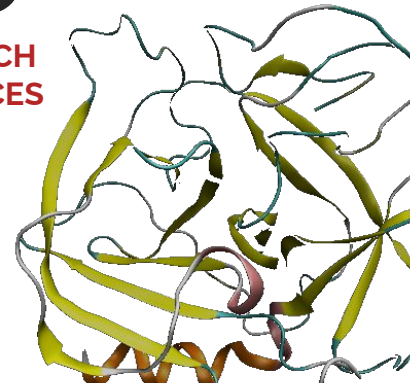
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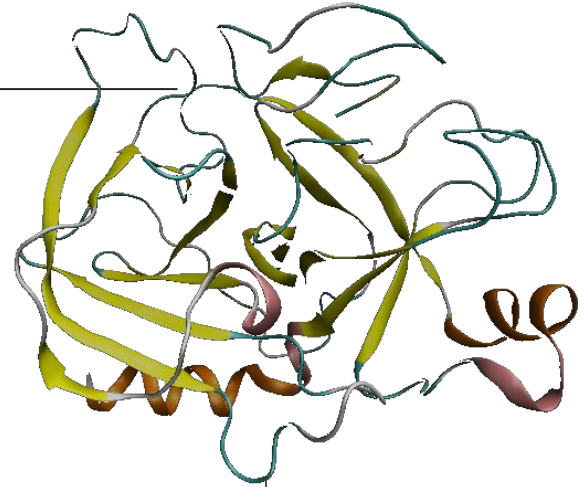
## **RESEARCH RESOURCES**



# 01

## INTRODUCTION

Classification and characteristic features of serine-proteases



# 1. PROTEASES

Enzymes that catalyse the breakdown of the peptide bonds

They are found in all the kingdoms of life and in viruses

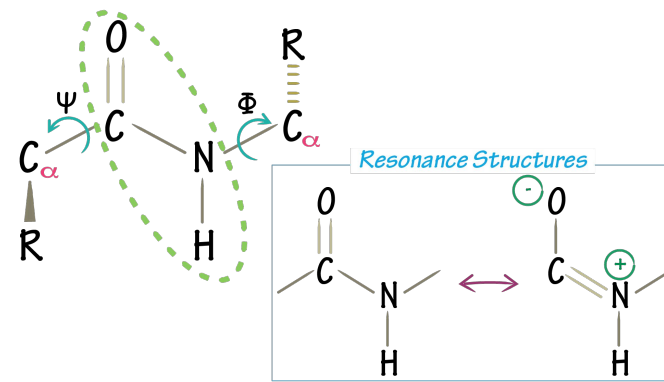


Figure 1. Peptide Bond

Independently evolved many times

Different catalytic mechanisms

Different structure

# PROTEASES: classification



MEROPS  
The Peptidase Database

Based on the residu that perform de catalysis

Clans of Serine Peptidases			
CLAN	FAMILY	TYPE PEPTIDASE	STRUCTURE
<a href="#">SB</a>	<a href="#">S8</a>	subtilisin Carlsberg ( <i>Bacillus licheniformis</i> )	Yes
	<a href="#">S53</a>	sedolisin ( <i>Pseudomonas sp. 101</i> )	Yes
<a href="#">SC</a>	<a href="#">S9</a>	prolyl oligopeptidase ( <i>Sus scrofa</i> )	Yes
	<a href="#">S10</a>	carboxypeptidase Y ( <i>Saccharomyces cerevisiae</i> )	Yes
	<a href="#">S15</a>	Xaa-Pro dipeptidyl-peptidase ( <i>Lactococcus lactis</i> )	Yes
	<a href="#">S28</a>	lysosomal Pro-Xaa carboxypeptidase ( <i>Homo sapiens</i> )	Yes
	<a href="#">S33</a>	prolyl aminopeptidase ( <i>Neisseria gonorrhoeae</i> )	Yes
	<a href="#">S37</a>	PS-10 peptidase ( <i>Streptomyces lividans</i> )	-
<a href="#">SE</a>	<a href="#">S82</a>	autocrine proliferation repressor protein A ( <i>Dictyostelium discoideum</i> )	-
	<a href="#">S11</a>	D-Ala-D-Ala carboxypeptidase A ( <i>Geobacillus stearothermophilus</i> )	Yes

## PROTEASES

Serine proteases

using a serine alcohol

Cysteine proteases

using a cysteine thiol

Threonine proteases

using a threonine secondary alcohol

Aspartic proteases

using a aspartate carboxylic acid

Glutamic proteases

using a glutamate carboxylic acid

Metalloproteases

using a metal, usually zinc

Asparagine peptide  
lyases

using an asparagine

# SERINE-PROTEASES: classification

## SERINE PROTEASES

Their catalytic mechanism depends upon the hydroxyl group of the **serine** residue acting as the **NUCLEOPHILE**

→ a donor of pair of electrons to form a chemical bond.

### Clans

Structure, mechanism and catalytic residue order

Trypsin-like  
peptidases

PA



23 families of **endopeptidases** that differ on its nucleophile (Ser or Cys)

Subtilisin-like  
peptidases

SB



2 families of **endopeptidases** and **exopeptidases** formed by a serine nucleophile.

### Families

Sequence similarity (homology)

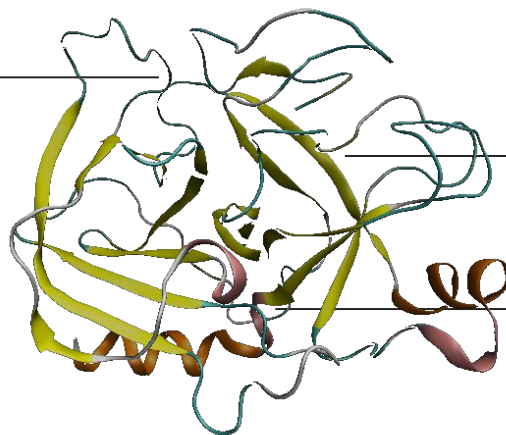
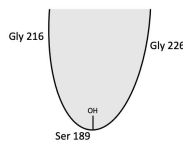
S1

S8

# SERINE-PROTEASES: characteristic features

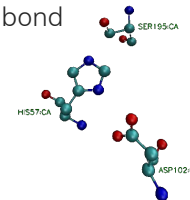
## SUBSTRATE SPECIFICITY POCKET

Determines which kind of residues interact with the protease



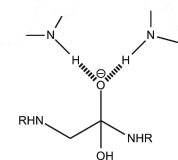
## CATALYTIC TRIAD

Breaks the peptide bond



## OXYANION HOLE

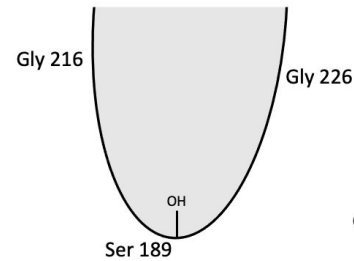
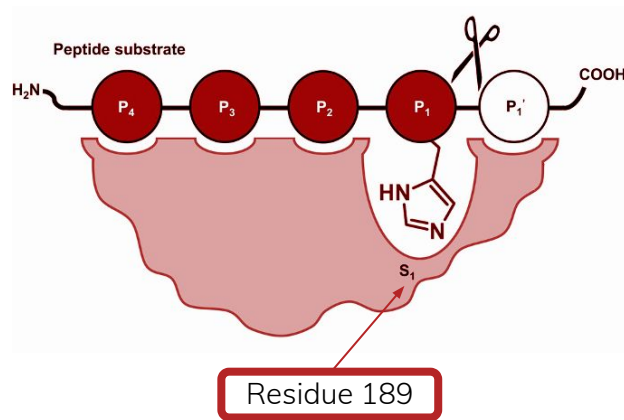
Stabilizes the tetrahedral intermediate



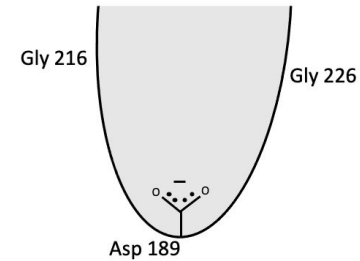


# Substrate specificity pocket

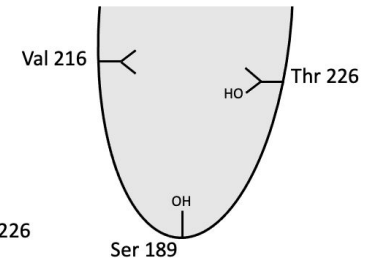
Function: to confer specificity to cut.



**Chymotrypsin**



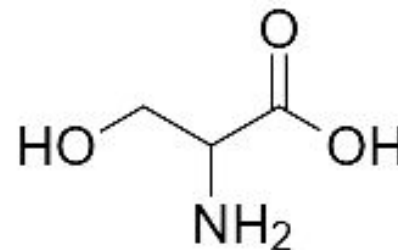
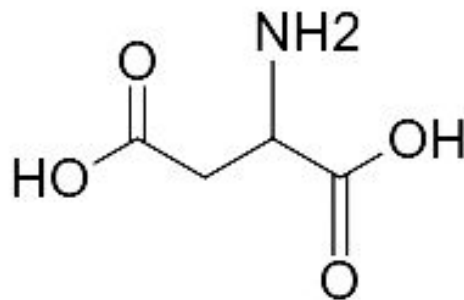
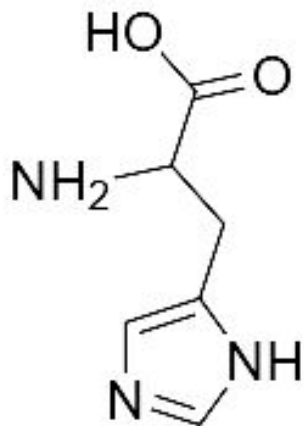
**Trypsin**



**Elastase**

Own source

# The catalytic triad



Own source

Trypsin-like

His57

Asp102

Ser195

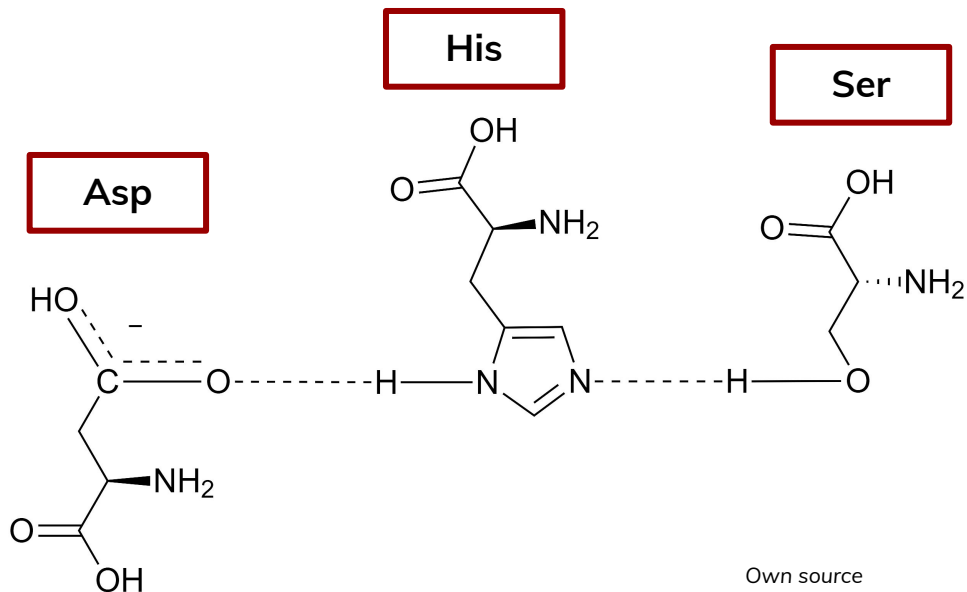
Subtilisins-like

His64

Asp32

Ser221

# The catalytic triad

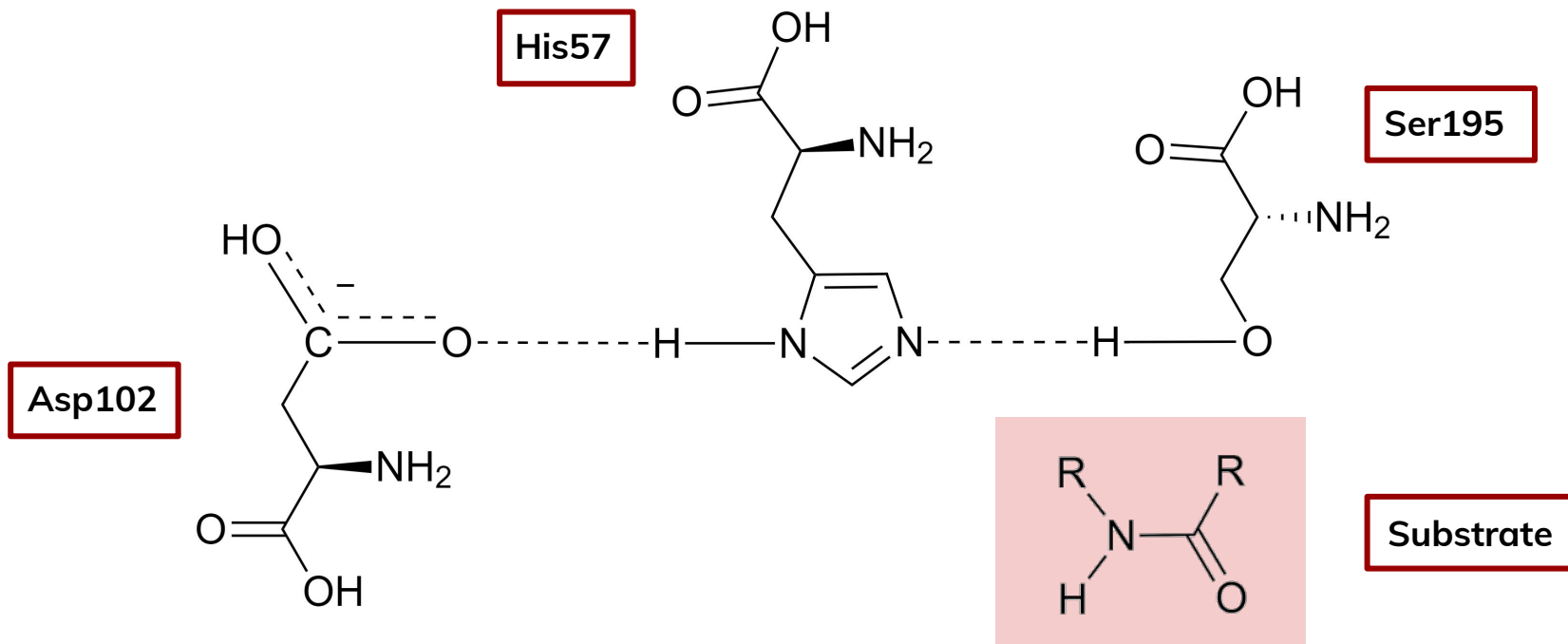


Differences in amino acids  
sequence

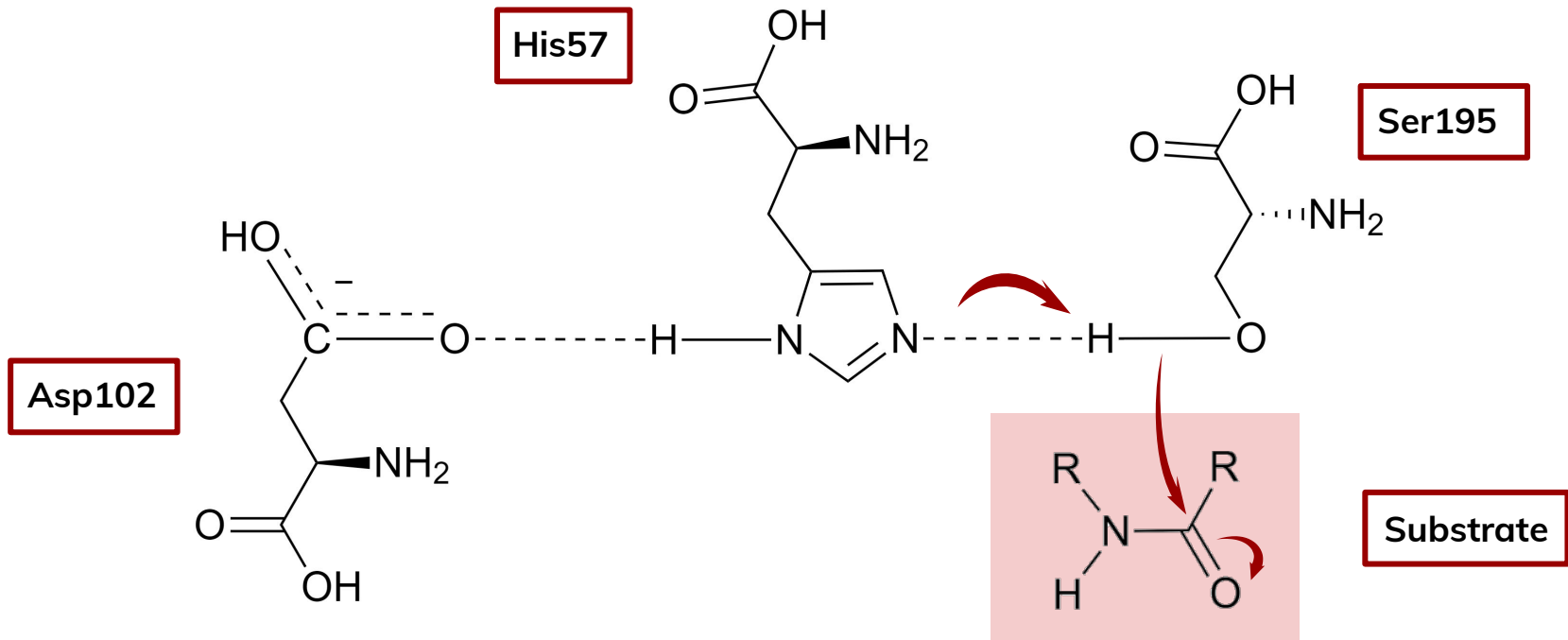


**CONVERGENT EVOLUTION**

# MECHANISM OF ACTION: The catalytic triad

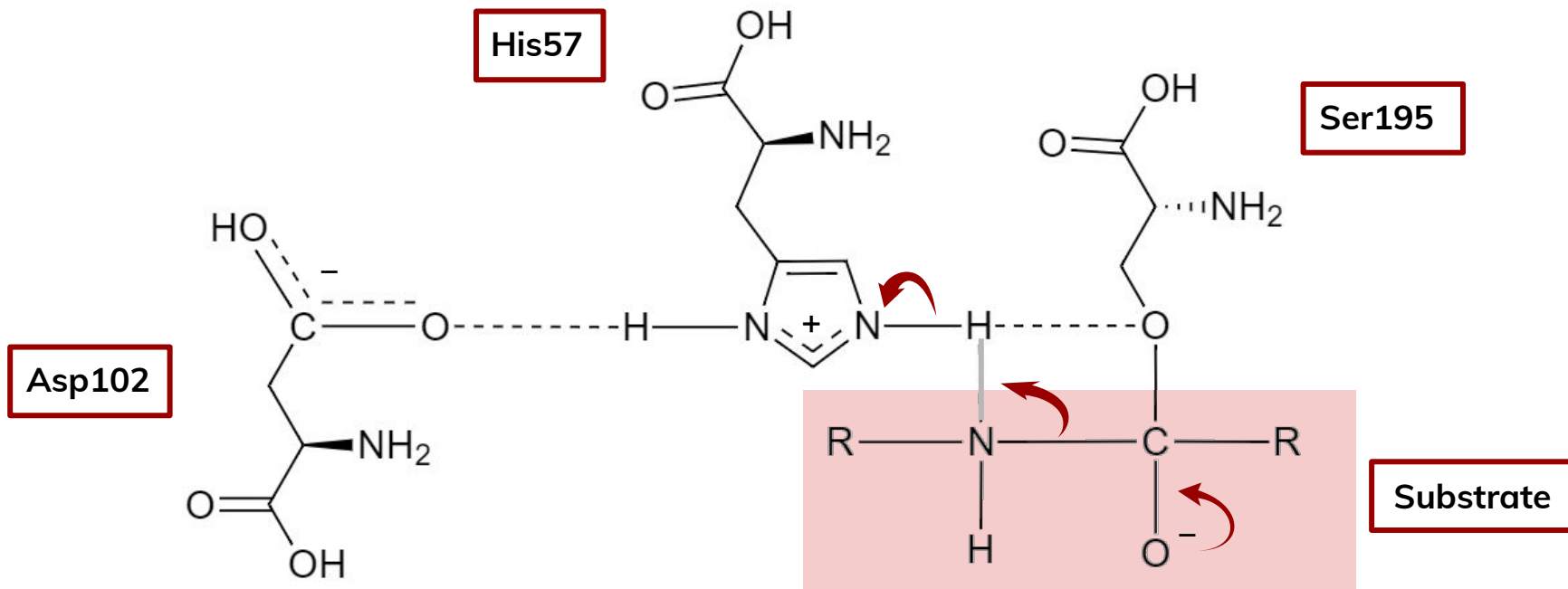


# Covalent bond formation

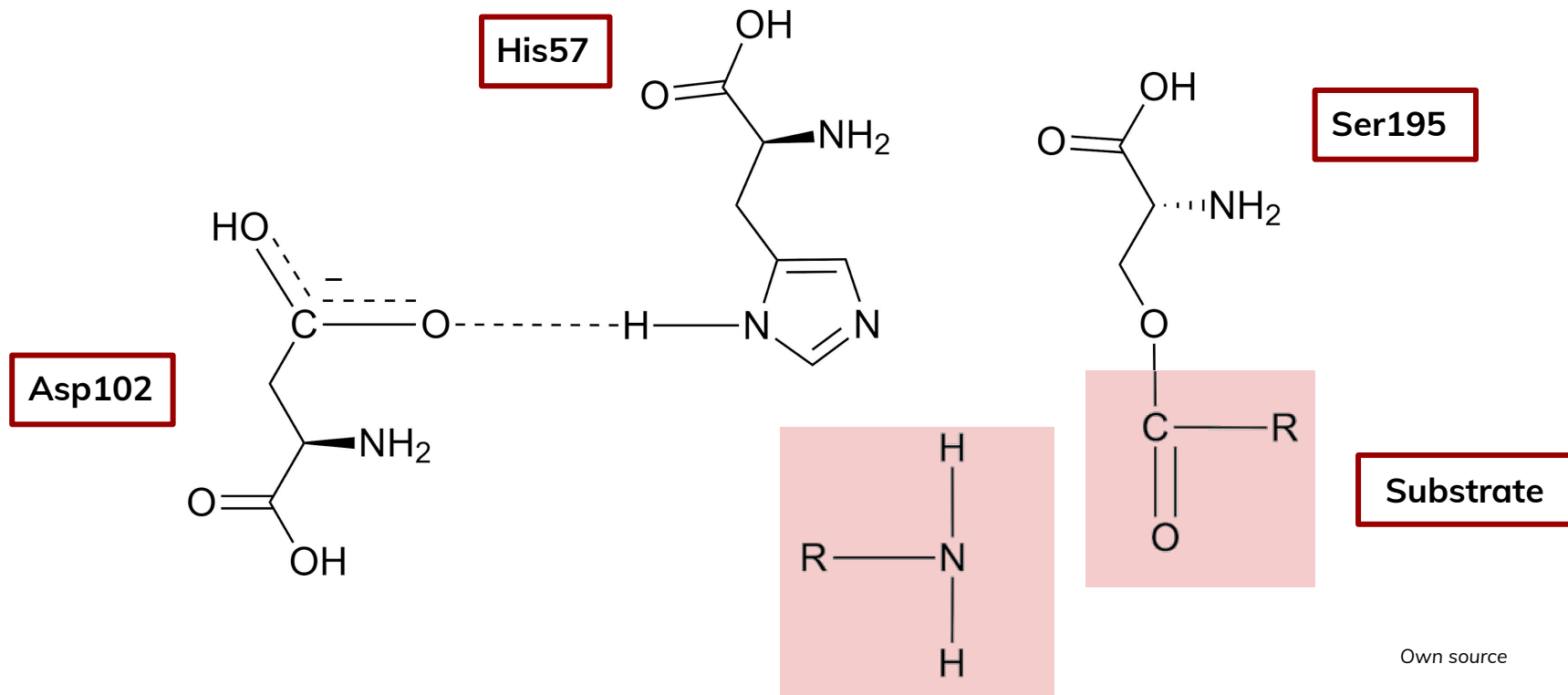




# First tetrahedral transition state

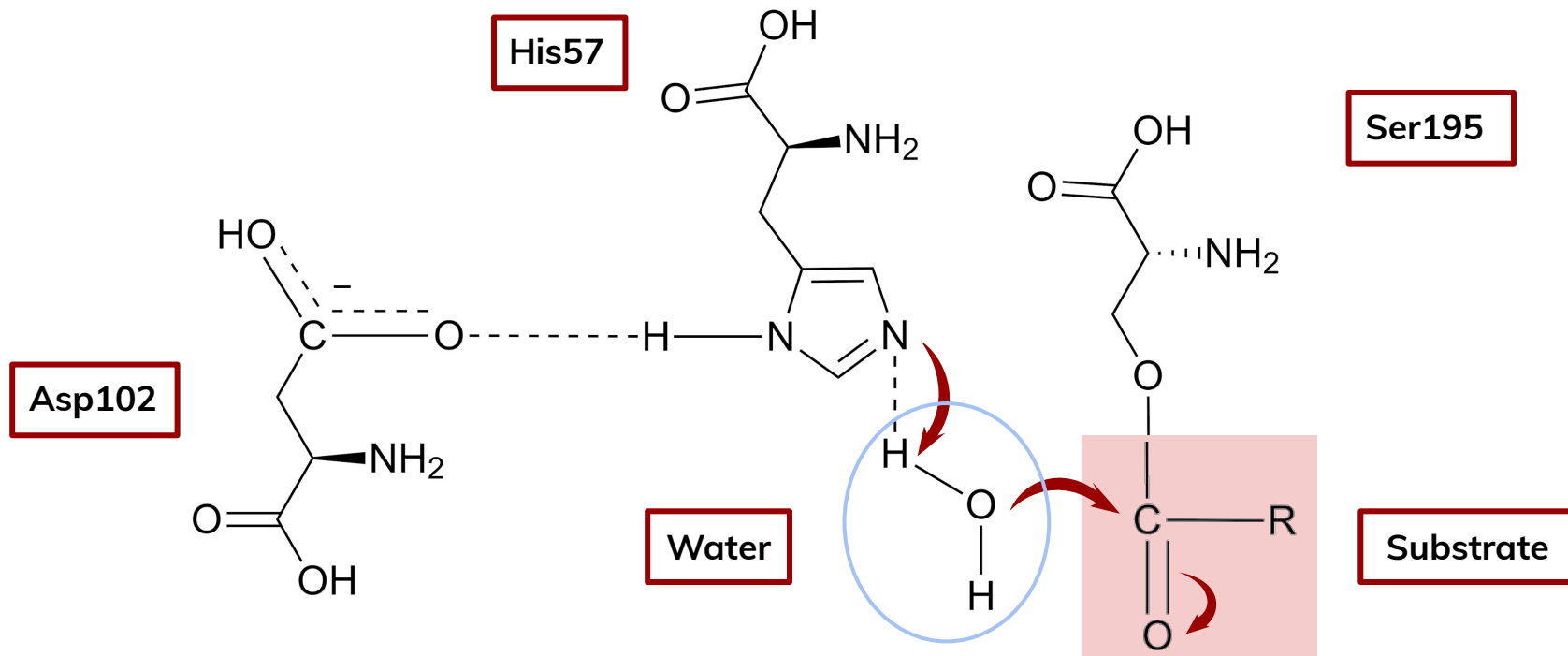


# Acyl-enzyme intermediate

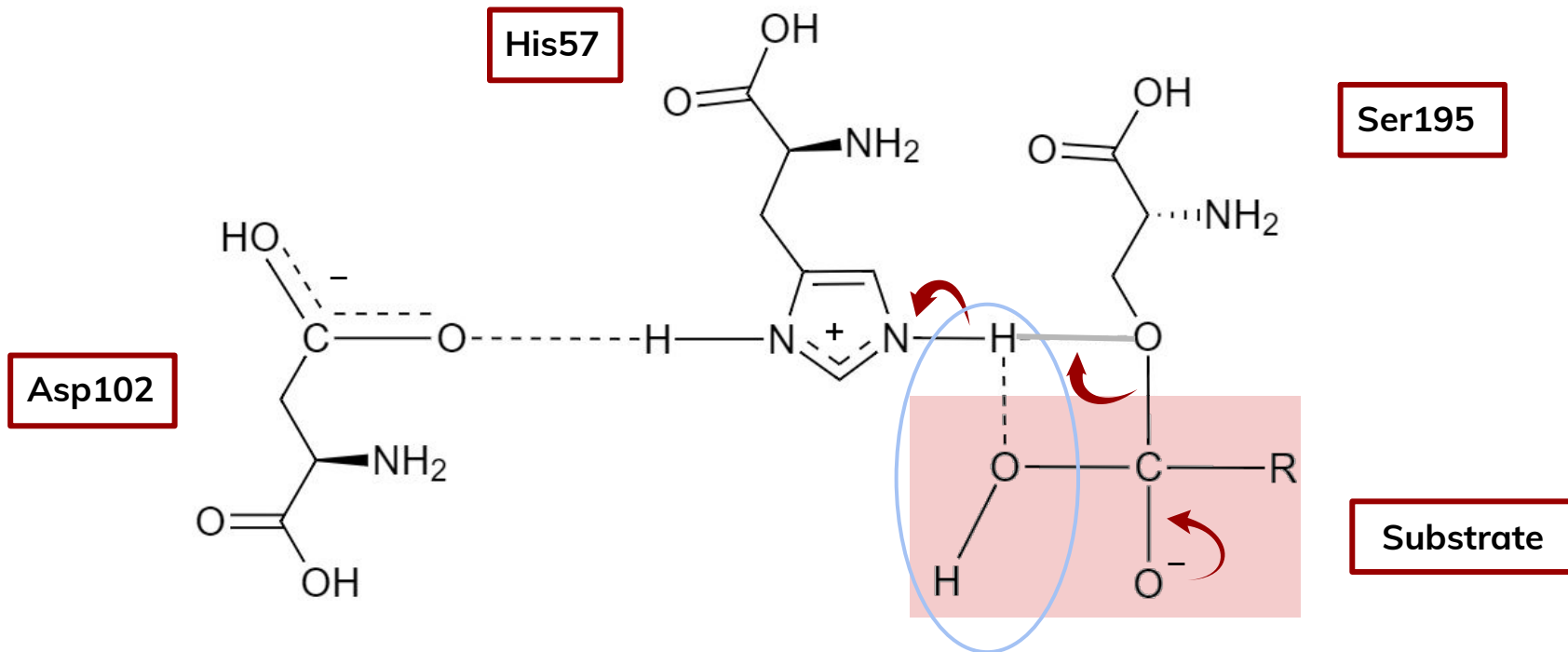




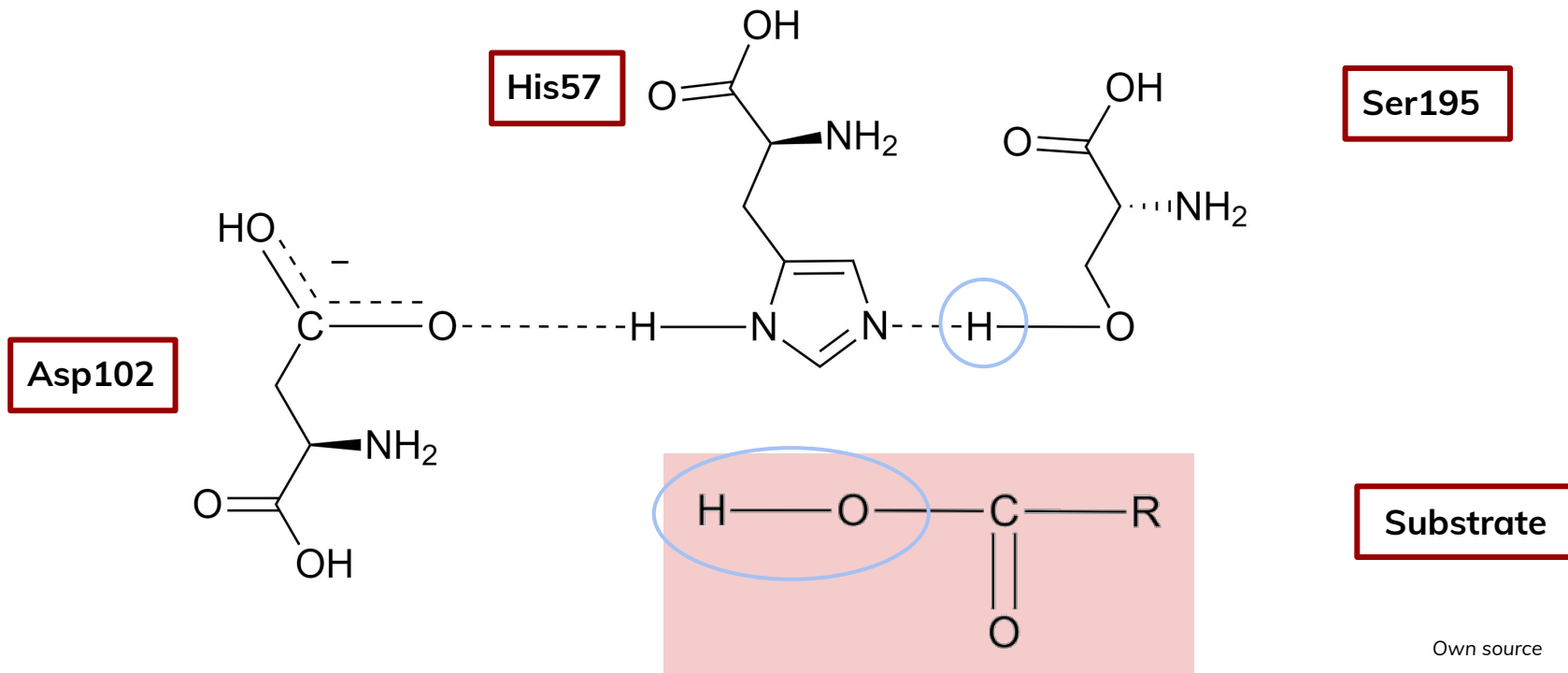
# Water activation



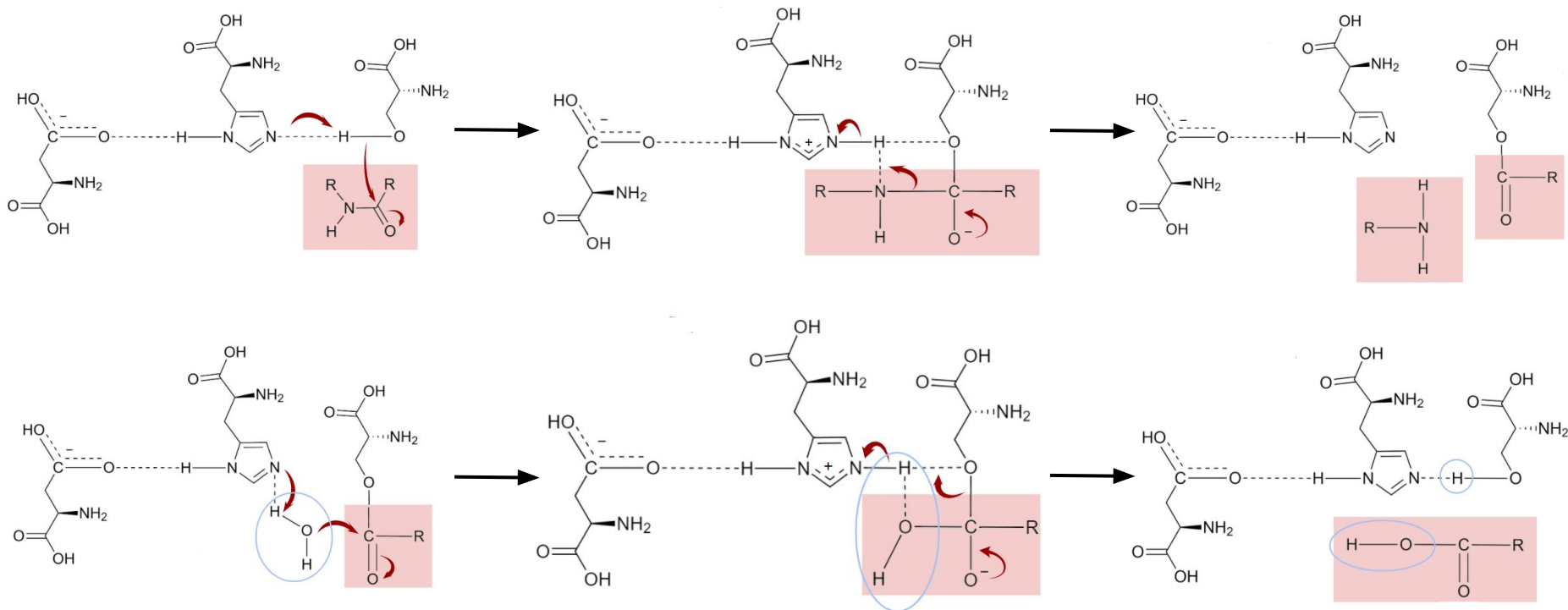
# Second tetrahedral transition state



# End of reaction: triad regeneration and product formation



# CATALYTIC MECHANISM: summary



# Oxyanion hole

Function: stabilisation of the tetrahedral intermediate.

## Trypsin-like

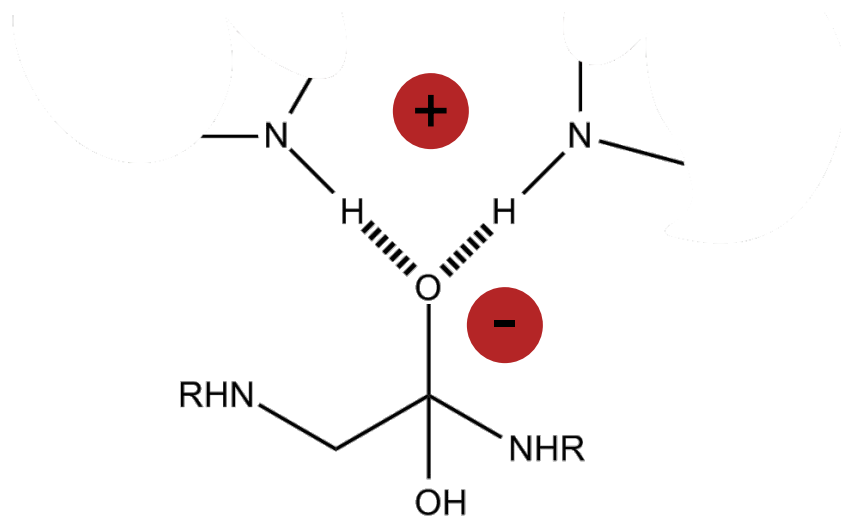
Gly193

Ser195

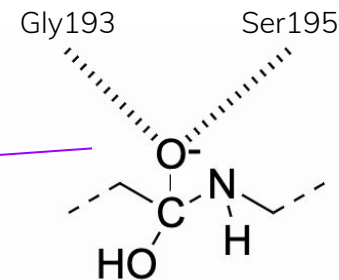
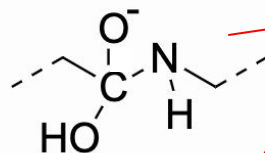
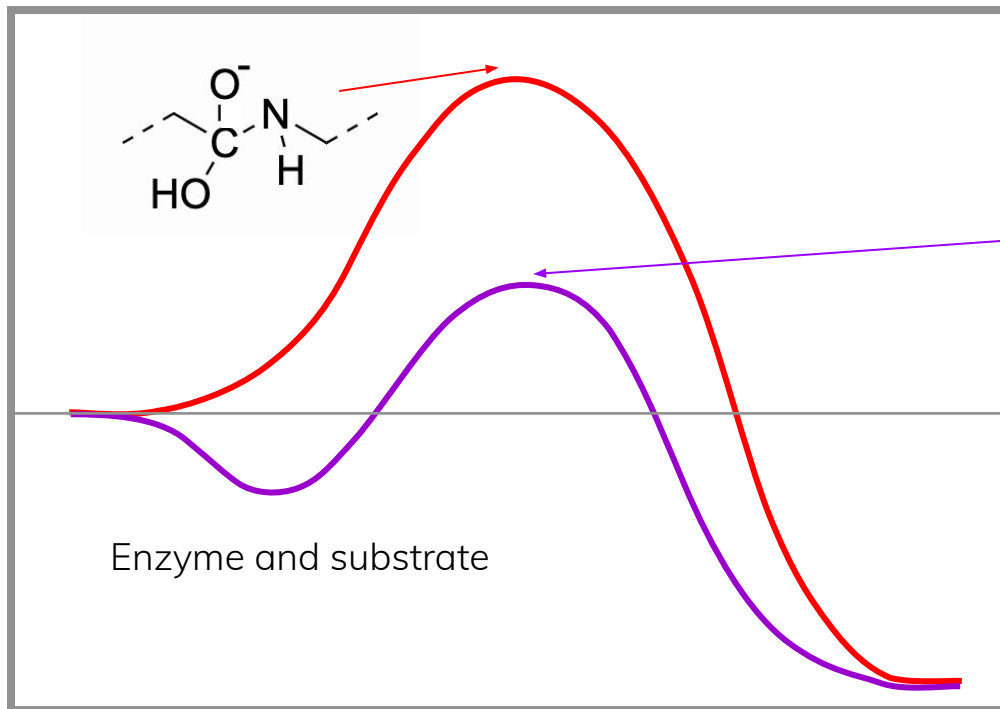
## Subtilisins-like

Asn155

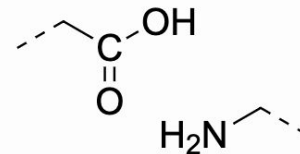
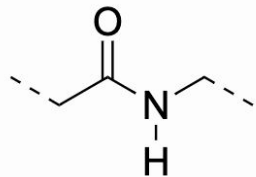
Ser221



# How do enzymes work?



Stabilization of the transition state brings down the activation energy.



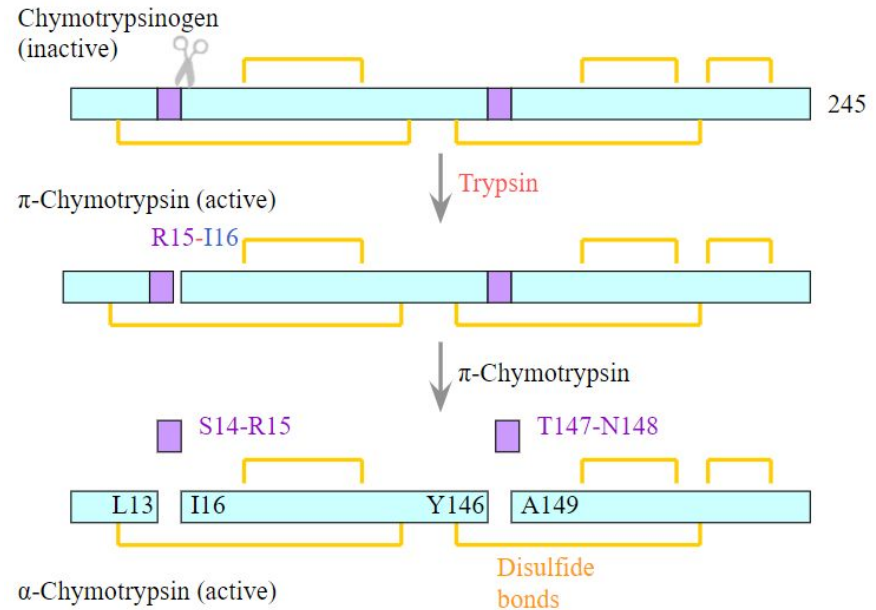
# Activation of Serine Proteases

**Trypsin-like proteases** are synthesized as inactive precursors: **ZYMOGEN**.

Proteolytic processing activates the zymogen.

1. Release of the **N-terminal Ile16** (depending on the enzyme).
2. Formation of salt bridge with **Asp194**.
3. Conformational change and creation of the active protease.

The mechanism of zymogen activation is conserved among mammalian trypsin-like serine proteases.



# Activation of serine proteases

**Elastase** consists of a single polypeptide chain of 240 amino acid.

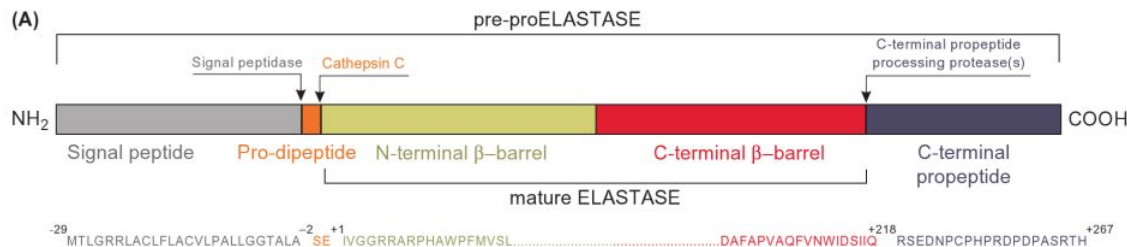
Synthesized as a **zymogen** (proelastase).  
Activation through limited trypsin proteolysis at its N-terminal.

Removal of an activation peptide from the N-terminal enables the enzyme to adopt its native conformation.

**Subtilisins** are synthesized as **zymogens**, with an approximately 77-long residue propeptide.

2 distinct autoproteolytic cleavages remove the propeptide, each with a different pH optimum.

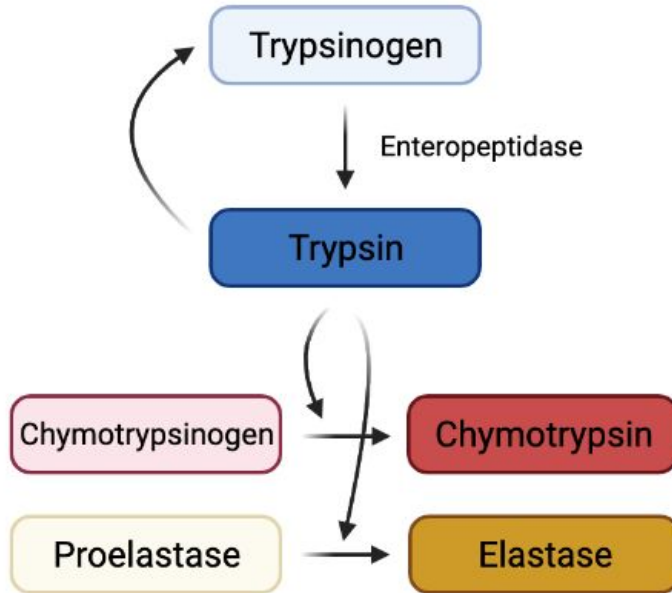
Maturation of the zymogen into enzymatically active subtilisin.



Handbook of Proteolytic Enzymes, Volume 1. Vol. 1. Elsevier, 2012.



# Integration of the activation of serine proteases



Trypsinogen activation is essential as it activates its own reaction, as well as the reaction of both chymotrypsin and elastase.

# Multiple Sequence Alignment Chymotrypsin + Chymotrypsinogen

Chymotrypsin A	-----	0
Chymotrypsin B	-----I VNGEEAVPGSWPWQVSLQDKTGFHFCCGSLINENWVVTAHCGV	45
Chymotrypsin C	CGVPAIQPVLSGL-----	13
Chymotrypsinogen	CGVPAIQPVLSGLSRI VNGEEAVPGSWPWQVSLQDKTGFHFCCGSLINENWVVTAHCGV	60

His 57

Ile 16

Chymotrypsin A	-----	0
Chymotrypsin B	TTSDVVVAGEFDQGSSEKIQLKIAKVFKNKYNSLTINNDITLLKLSTAASFSTVSA	105
Chymotrypsin C	-----	13
Chymotrypsinogen	TTSDVVVAGEFDQGSSEKIQLKIAKVFKNKYNSLTINNDITLLKLSTAASFSTVSA	120

Asp102

Chymotrypsin A	-----ANTPDRLQQASLPLLSNTNCKKYWGKIKDAM	32
Chymotrypsin B	VCLPSASDDFAAGTTCVTTGWGLTRY-----	131
Chymotrypsin C	-----	13
Chymotrypsinogen	VCLPSASDDFAAGTTCVTTGWGLTRYTNANTPDRLQQASLPLLSNTNCKKYWGKIKDAM	180

Chymotrypsin A	ICAGASGVSSCMGDSGGPLVCKKNGAWTLVGIVSWGSSSTCSTSTPGVYARVTALVNWVQQ	92
Chymotrypsin B	-----	131
Chymotrypsin C	-----	13
Chymotrypsinogen	ICAGASGVSSCMGDSGGPLVCKKNGAWTLVGIVSWGSSSTCSTSTPGVYARVTALVNWVQQ	240

Gly 193

Asp 194

Ser 195

Chymotrypsin A	TLAAN	97
Chymotrypsin B	-----	131
Chymotrypsin C	-----	13
Chymotrypsinogen	TLAAN	245

 Oxyanion hole

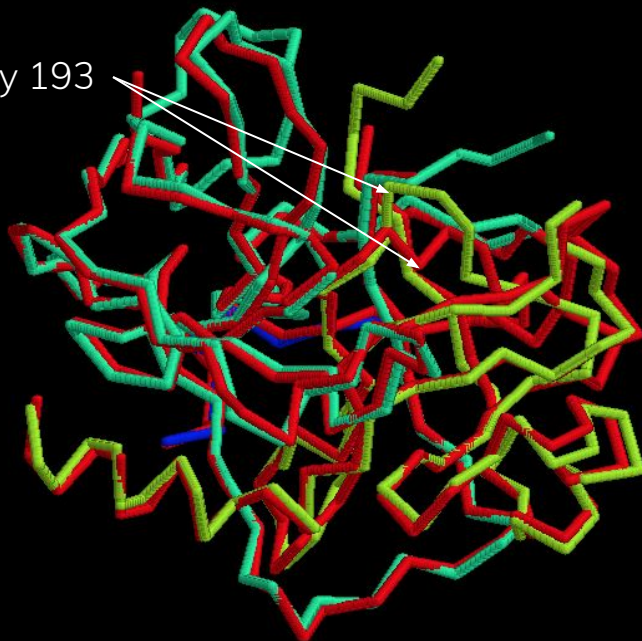
 Catalytic triad

 Activating bond

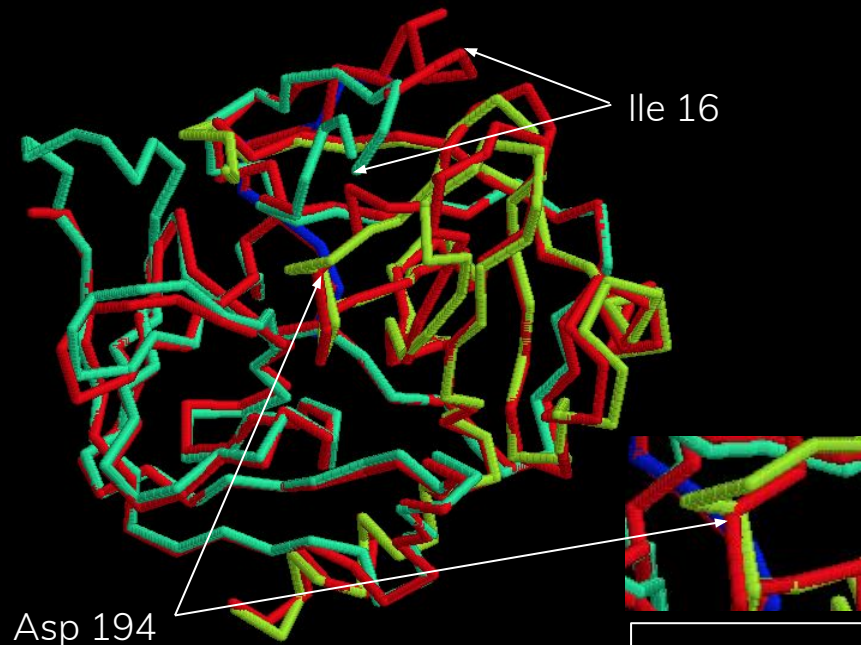
# Chymotrypsin + Chymotrypsinogen superimposition

Chymotrypsinogen  
Chymotrypsin Chain A  
Chymotrypsin Chain B  
Chymotrypsin Chain C

Gly 193



Ile 16

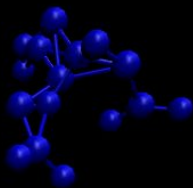


Asp 194

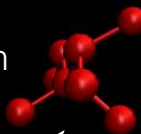
Sc 8.06  
RMS 0.81

# Conformational change

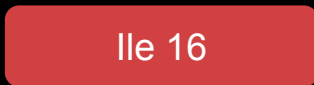
Asp 194



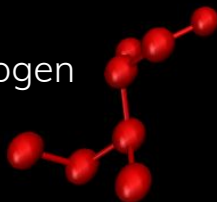
Chymotrypsin



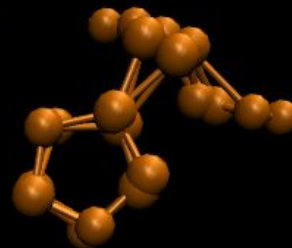
Ile 16



Chymotrypsinogen



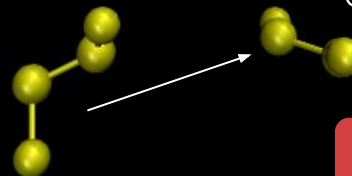
Catalytic triad



Chymotrypsin



Chymotrypsinogen



Gly 193

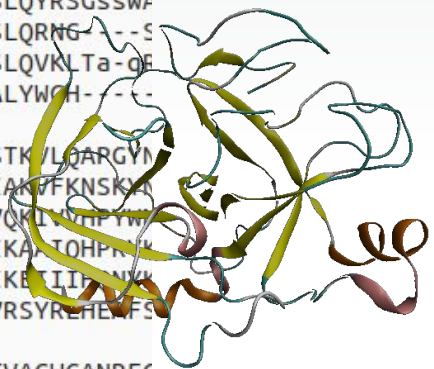
-----VVGGTTRAAQGEFFMVRRLSM-G-  
lsglsrIVNGEEAVPGSWPQVSLQDKTG---F  
-----VVGTEAQRNSWPSQISLQYRSGsswA  
-----IIGGREVIPHSRYPYMASLQRNG---S  
-----IVGGTESSWGEWPQVSLQVKLTa-qE  
---rsv-VGGLVALRGHAHPYIAALYWH---

NTSITATGGVVDLQSGAAVKVRSTK/LQARGYN  
DV-VVAGEFDQGSSEKIQKLIKIAKTFKNSKA  
TFRVVVGEHNLNQNDGTEQYVGVQKLVVTFYW  
QLRLVLGLHTLDSPG---LTFHIKATOHPRK  
VWRIYSGILELSDITKDTPFSSQIKETIIFNYK  
DLTVVLGQERRNHSCEPCQTLAVRSYREHELF

NQPTLKIATTTAYN---QG-TFTVAGWGANREC  
SQTVS AVCLPSASDDFAAGTTCVTTGWGLTRYI  
NSYVQLGVLPRA GTILANNSPCYITGWGLTRM  
SRTIRPLALPSKRQVVAAGTRCSMAGWGLTHQC  
TEFQKPI SLPSKGD TSTIYTNCWVTGWGFSKEK  
SPYVQPVS LPSGAARPSETTL CQVAGWGHQFEC

GNElVANEEICAGYPdtGGVDTCQGDSGGPMFF  
GTK- IKDAMICAGA---SGVSSCMGDSGGPLVC  
GST- VKNSMVCAGGD--GVRSGCQGDSGGPLHC  
NGS- LSPSMVCLAAD-sKDQAPCKGDSGGPLVC  
QDYkITQRMVCAGYK-eGGK DACKGDSGGPLVC  
GSS- ILPGMLCAGFL-eGGTDACQGDSGGPLVC

YPGVYTEVSTFASAIasaartl-----  
TPGVYARVTALVNwVqqtlaan-----  
KPTVFTRVSAYISWInnviasn-----  
KPPVATAVAPYVSWIrkvtgrsalehhhhh--  
QPGVYTKVAEYMDWIlektqssdgk-----  
KPGVYTDVAYYLAWIrehtvshhtgtrhhhhh



# 02

## SEQUENCE ANALYSIS

MSA based on sequence of trypsin-like serine proteases and subtilisin-like serine proteases



# Multiple Sequence Alignment TRYPsin-LIKE proteins

```

Trypsin          -----VVGGTAAQGEFFMVRISM-G-----CGGALYAQDIVLTAH
Chymotrypsinogen_A  cgvpalqpvlsgrIVNGEEAVPGSWPQVSLQDKTG---FHFCGGSLINENWVVTAAH
Elastase         -----VVGTEAQRNSWSPQISLQYRSGSswAHTCGGTLIRQNWVMTAAH
Granzyme_M       -----IIGGREVIPHRSRPMASLQRNG----SHLCGGVLVHPKRWLTAH
Kallikrein       -----IVGGTESSWGEWPWQVSLQVKLTa-qRHLCGGSLIGHQWVLTAAH
Coagulation_factorXII -----rsv-VGGLVALRGAHPYIAALYWGHI----SFCAGSLIAPCWVLTAAH
  
```

His 57

```

Trypsin          CVSG--sGNNTSITATGGVVDLQSGAAVKVRSTKVLQAPGYNGTG----FDNALIKLAQ
Chymotrypsinogen_A  CGVT---TSDV-VVAGEFDQGSSEKIQLKIAKVFKNISKYNSLT-I--NNDITLLKLST
Elastase         CVDR---ELTFRVVVGEHNLNQNDGTEQYVGVQKIVVHPYWNDD-VaaGVDIALLRLAQ
Granzyme_M       CLAQ--rMAQLRLVLGLHTLDSPG--LTFHIKAAIQHPRYKVPVpAL--ENDIALLQLDG
Kallikrein       CFDGLpLQDVWRIYSGILELSDITKDTPFSSQIKEIIIHQNYKVSE-G--NNDIALLKLQA
Coagulation_factorXII  CLQDrpaPEDLTVVVLGQERRNHSCEPCQTLAVRSYRLHEAFSPVS-Y--QNDIALLRLQE
  
```

Asp102

```

Trypsin          P-----INQPTLKIATTTAYN---QG-TFTVAGWGANREGG-sQQRYLKANVPFVSD
Chymotrypsinogen_A  A-----ASFSTQTVSAVCLPSASDDFAAGTTCVTTGWGLTRYTNanTPDRLQQASLPLLSN
Elastase         S-----VTLNSYVQLGVLPRAGTILANNSPCYITGWGLTRTNG-qLAQTLQQAYLPTVDY
Granzyme_M       K-----VKPSRTIRPLALPSKRQVVAAGTRCSMAGWGLTHQGG-rLSRVLRELDLQVLDY
Kallikrein       P-----LEYTEFQKPIISLPSKGDSTIYTNCWVTGWGFSKEKG-eIQNILQKVNIPVLTN
Coagulation_factorXII  DadgscALLSPYVQVPSLPSGAARPSETTLCQVAGWGHQFEGAeeYASFLQEAQVPFLSL
  
```

```

Trypsin          AACrSA--YGNELVANEELCAGYPdtGGVDTCGGSSGGPMFRKDNad--ewIQVGVISWG
Chymotrypsinogen_A  TNCCKY--WGTK-IKDAMI CAGA---SGVSSCMGGSSGGLVCKKNG---awTLVGVISWG
Elastase         AICSSSyWGST-VKNSMYCAGGD--GVRSSCCGGSSGGLHCLVNG---qyAVHGVTSFV
Granzyme_M       RNCINSrfWNGS-LSPSMYCLAAD-sKDCQACCKGSSGGLVCGK-----rVLAGVLSFS
Kallikrein       EECQKR--YQDYkITQRMYCAGYK-eGGKQACKGSSGGLVCKHNG---mwRLVGVTSWG
Coagulation_factorXII  EFCAPdvHGSS-ILPGMLCAGFL-eGGTDACGGSSGGLVCEdQaaerrlTLGVISWG
  
```

Residue 189

Gly 193

Ser 195



Catalytic triad



Oxyanion hole



Substrate specificity pocket



Example of disulfide bond

```

Trypsin          --YGCARPGYPGVYTEVSTFASAIasaartl-----
Chymotrypsinogen_A  --SSTCSTSTPGVYARVTALVNWVqqtlaan-----
Elastase         srLGCNVTRKPTVFRVSAYISWInnviasn-----
Granzyme_M       -sRVCTDIFKPPVATAVAPYVSWIrkvtgrsalehhhhhh--
Kallikrein       --EGCARREQPGVYTKVAEYMDWIlektqssdgk-----
Coagulation_factorXII --SGCGDRNKPGVYTDVAAYLAWIrehtshhtgrhhhhhh
  
```

# Multiple Sequence Alignment SUBTILISIN-LIKE proteins

Subtilisin_Carlsberg	aqtvpypgiplikadkvqaqgfkganvKVAVIDGIQASHPDLN---V-----VGGASFVA	Asp32
Bacillus_lentus	aqsvpwgisrvqapaahnrgltgsgvKVAVIDGI-STHPDLN---IR-----GGASFVP	
Subtilisin_BPN	aqsvpygvsqikapaahsqgytgsnvKVAVIDGIDSSHPDLK-----VAGGASMVP	
Subtilisin_NAT	aqsvpygisqikapaahsqgytgsnvKVAVIDGIDSSHPDLN-----VRGGASFVP	
Subtilisin_Savinase	aqsvpwgisrvqapaahnrgltgsgvKVAVIDGI-STHPDLN---IR-----GGASFVP	




Subtilisin_Carlsberg	GE---AYNTDGNHGHTHYAGTVAAL--DNTTGVLGVPVSVSLYAVKVLNSSGSGSYSGIV	His64
Bacillus_lentus	GE---PSTQDGNHGHTHYAGTIAAL--NNSIGV LGVAPSAELYAVKVLGASGSGSVSSIA	
Subtilisin_BPN	SE--TPNFQDDNSHGHTHYAGTVAAL--NNSIGV LGVAPSSALYAVKVLGDAGSGQYSWII	
Subtilisin_NAT	SE--TNPYQDGSSTHGHTHYAGTIAAL--NNSIGV LGVAPASLYAVKVL DSTGSGQYSWII	
Subtilisin_Savinase	GE---PSTQDGNHGHTHYAGTIAAL--NNSIGV LGVAPSAELYAVKVLGASGSGSVSSIA	

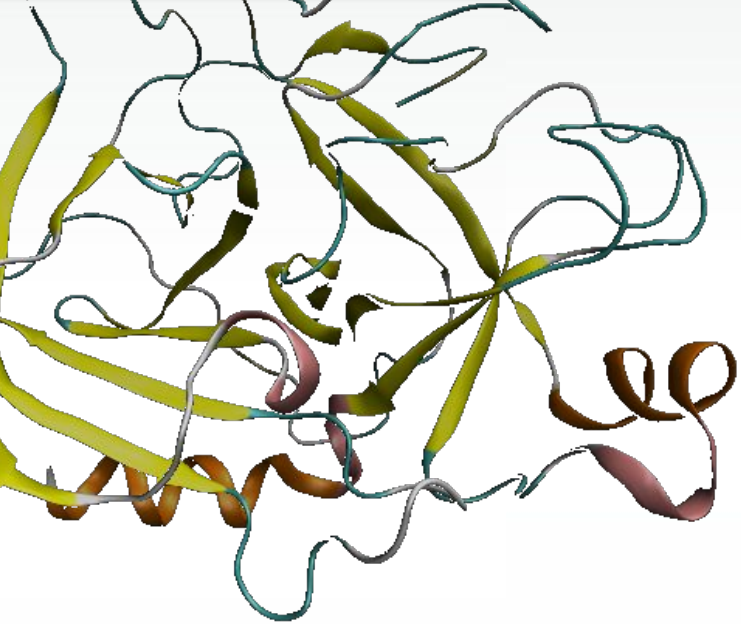
Subtilisin_Carlsberg	SGIEWAT-TNGMDVINMSLGG-----ASGSTAMKQAVDNAYARGVVVAAAQNSGSGS	Asn155
Bacillus_lentus	QGLEWAG-NNGMHVANLSLGS-----PSPSATLEQAVNSATSRGVLVAAAQNSGAGS-	
Subtilisin_BPN	NGIEWAI-ANNMDVINMSLGG-----PSGSAALKAAVDKAVASGVVVAAAQNECSTGS	
Subtilisin_NAT	NGIEWAI-SNNMDVINMSLGG-----PTGSTALKTVVDKAVSSGIVVAAAQNECSSLGS	
Subtilisin_Savinase	QGLEWAG-NNGMHVANLSLGS-----PSPSATLEQAVNSATSRGVLVAAAQNSGAGS-	

Subtilisin_Carlsberg	TNTGYPAKYDSVIAVGAVDNSNRRASFSSVGAEL-----EVMAPGAGVYSTYPTNT-
Bacillus_lentus	---SYPAFYANAMAVGATDQNNRRASFSSQYGAGL-----DIVAPGVNVQSTYPGST-
Subtilisin_BPN	SSTGYPGKYPYSVIAVGAVDSSNQRASFSSVGPPEL-----DVMAPGVSIQSTLPGNK-
Subtilisin_NAT	TSTGYPAKYDPTIIVGAVNSSNQRASFSSVGSSEL-----DVMAPGVSIQSTLPGGT-
Subtilisin_Savinase	---SYPAFYANAMAVGATDQNNRRASFSSQYGAGL-----DIVAPGVNVQSTYPGST-

Subtilisin_Carlsberg	--YATLNGSIATSPHVAGAAALILSKHPNLSASQVRNRLSSTATYLG-----SSFYYGK	Ser221
Bacillus_lentus	--YASLNGSIATPHVAGAAALVKQKNPSWSNVQIRNHLKNTATSLG-----STNLYGS	
Subtilisin_BPN	--YGAYNGSIATSPHVAGAAALILSKHPNWTNTQVRSLSLQNTTTKLG-----DSFYYGK	
Subtilisin_NAT	--YGAYNGSIATPHVAGAAALILSKHPTWTNAQVRDRLESTATYLG-----NSFYYGK	
Subtilisin_Savinase	--YASLNGSIATPHVAGAAALVKQKNPSWSNVQIRNHLKNTATSLG-----STNLYGS	

Subtilisin_Carlsberg	GLINVEAA--aq
Bacillus_lentus	GLVNAEAAT-r-
Subtilisin_BPN	GLINVQAA--aq
Subtilisin_NAT	GLINVQAA--aq
Subtilisin_Savinase	GLVNAEAAT-r-

-  Catalytic triad
-  Oxyanion hole
-  Substrate specificity pocket



**03**

## **STRUCTURE ANALYSIS**

Trypsin-like and subtilisin-like folding analysis



# SCOP classification

Class 1000001  
All beta proteins

Fold 2000083  
Trypsin-type beta(6)-barrel

Superfamily 3000114  
Trypsin-like serine proteases

Family 4000286  
Eukaryotic proteases

Clan PA

S1

Class 1000002  
Alpha and Beta (a/b)

Fold 2000207  
Subtilisin-like

Superfamily 3000226  
Subtilisin-like

Family 4000409  
Subtilases

Clan SB

S8

Trypsin

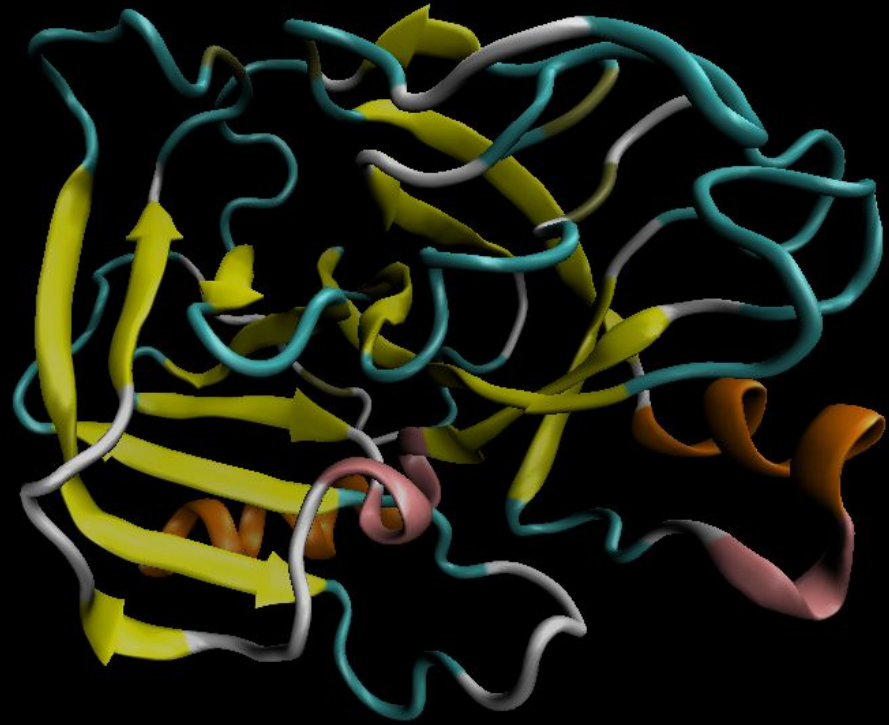
Chymotrypsin

Elastase

# TRYPSIN-LIKE

All beta structure

-  Alpha helix
-  Extended Beta
-  3 10 helix
-  Turn
-  Coil



# TRYPSIN-LIKE

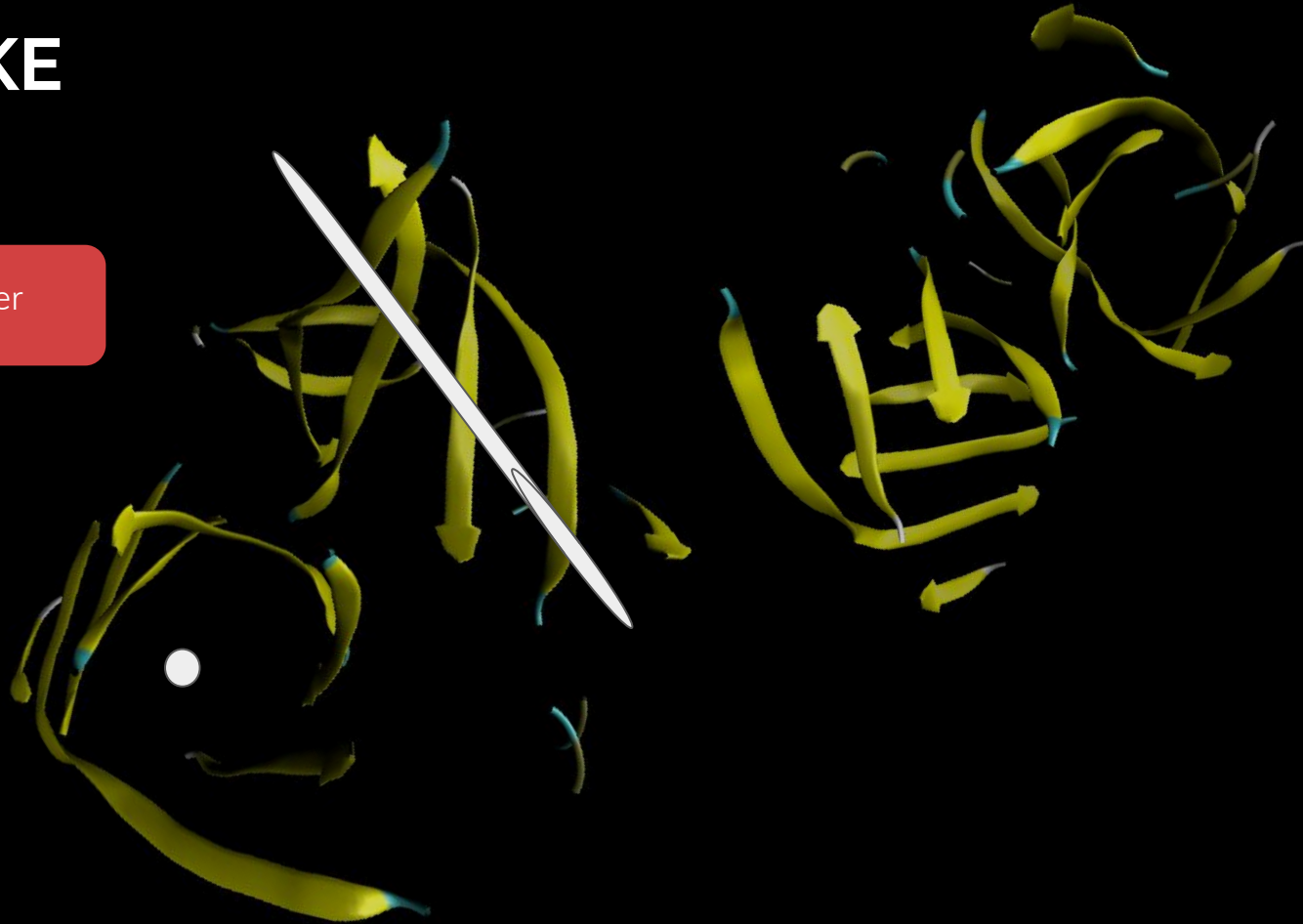
Two domains

- Alpha helix
- Extended Beta
- 3 10 helix
- Turn
- Coil



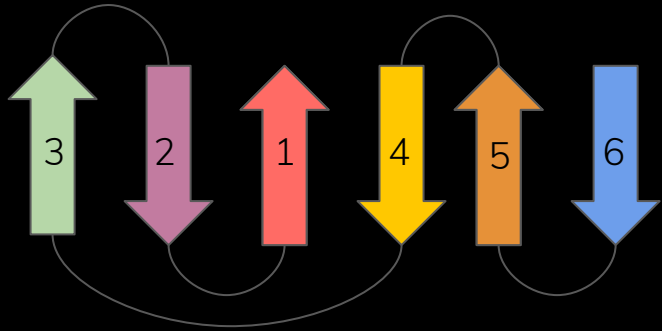
# TRYPSIN-LIKE

Perpendicular to each other

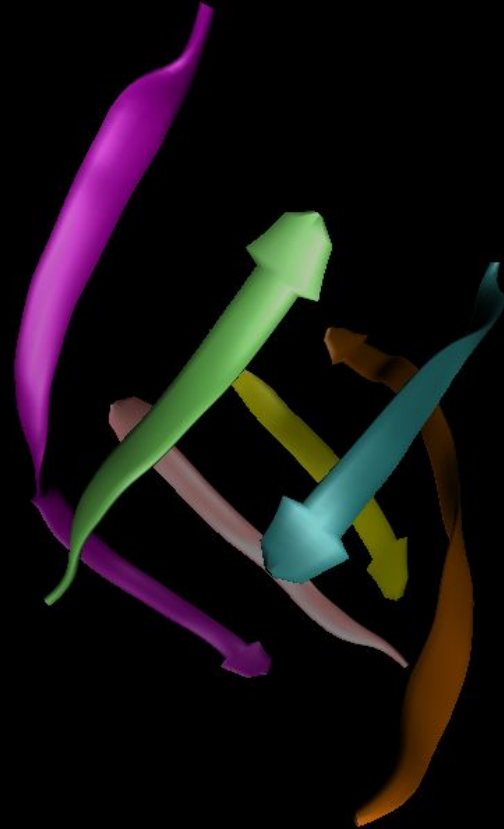


# TRYPSIN-LIKE

Beta-barrel 6 stranded Greek Key

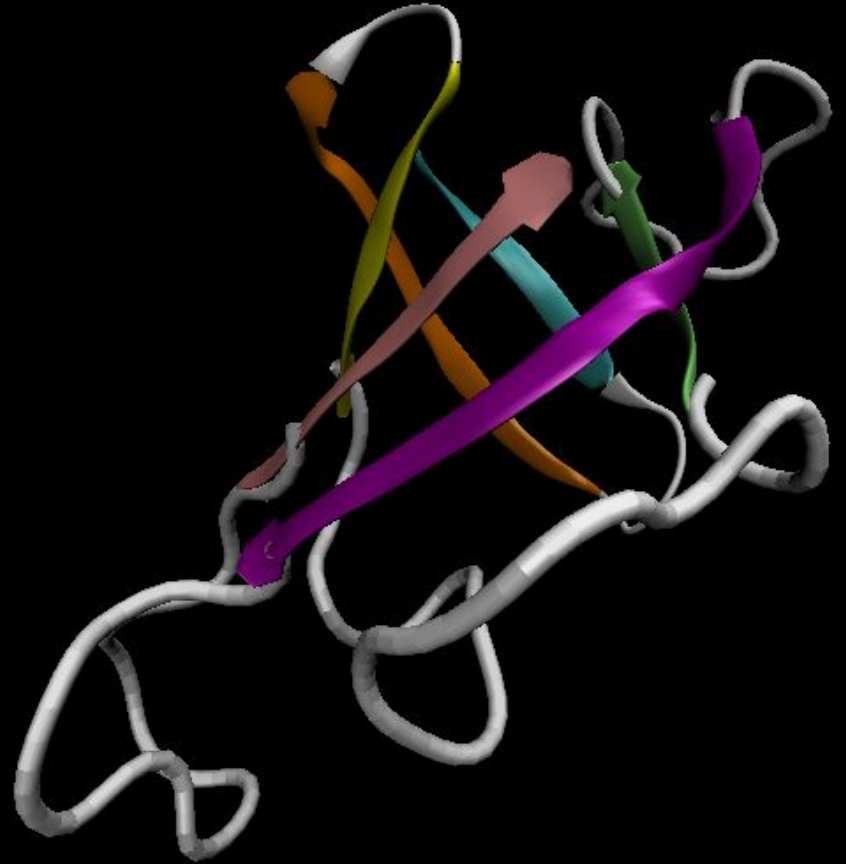
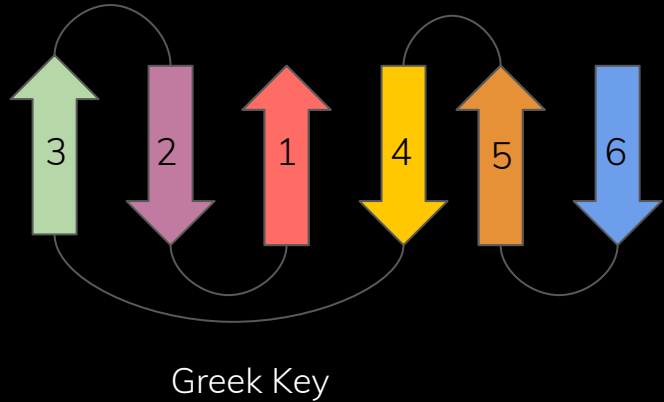


Greek Key



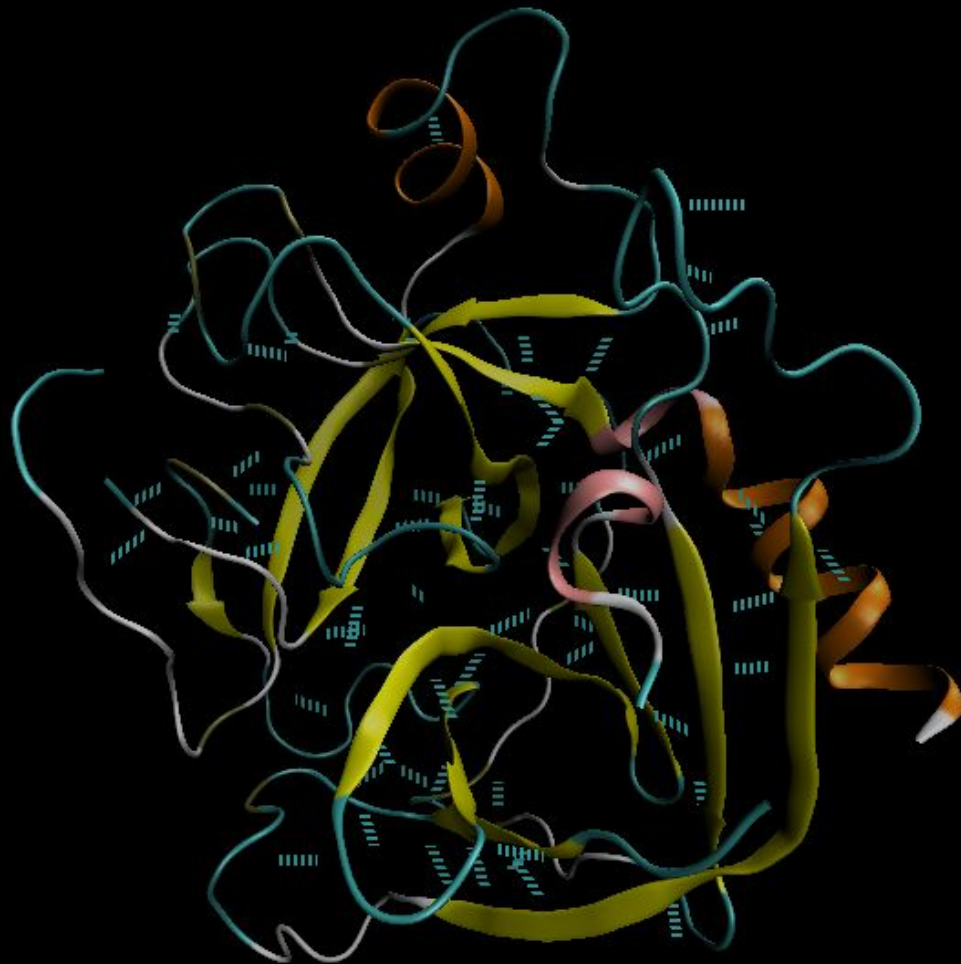
# TRYPSIN-LIKE

Beta-barrel 6 stranded Greek Key



# TRYPSIN-LIKE

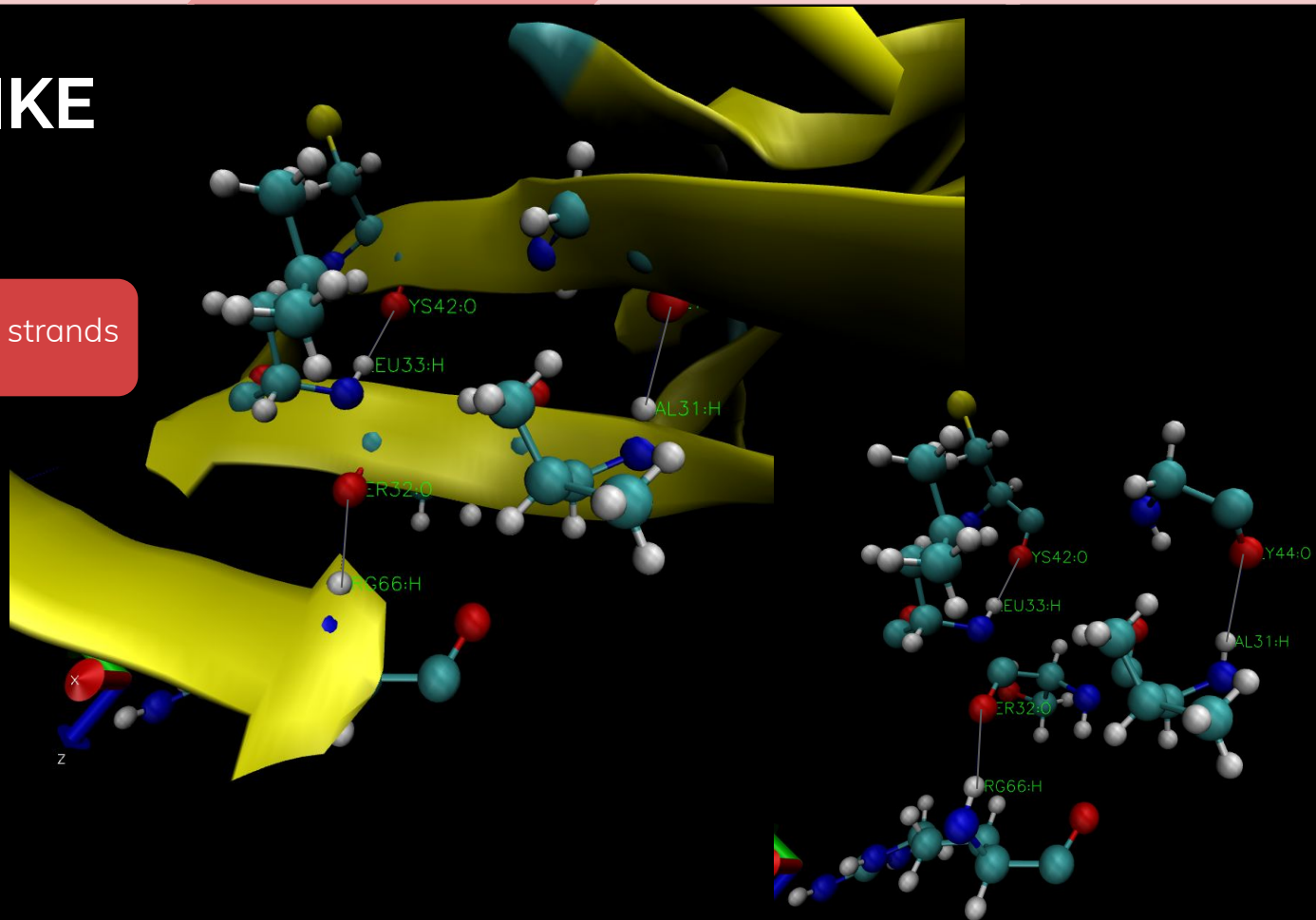
Hydrogen bonds between beta strands  
and alpha helix



# TRYPSIN-LIKE

Hydrogen bonds between beta strands

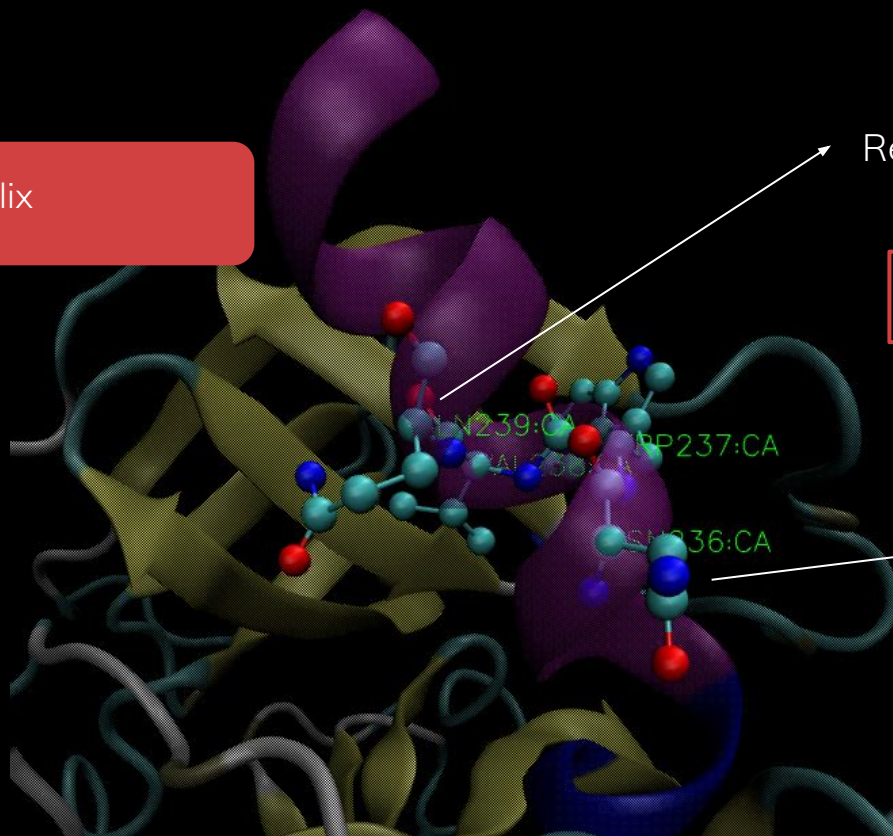
- Carbon
- Hydrogen
- Oxygen
- Nitrogen





# TRYPSIN-LIKE

Type of helix



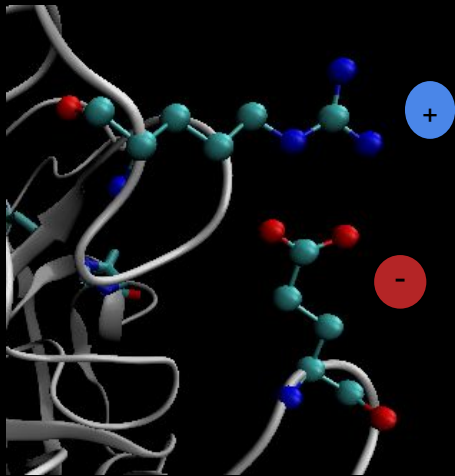
Residue 239



**4 residues → alpha helix**

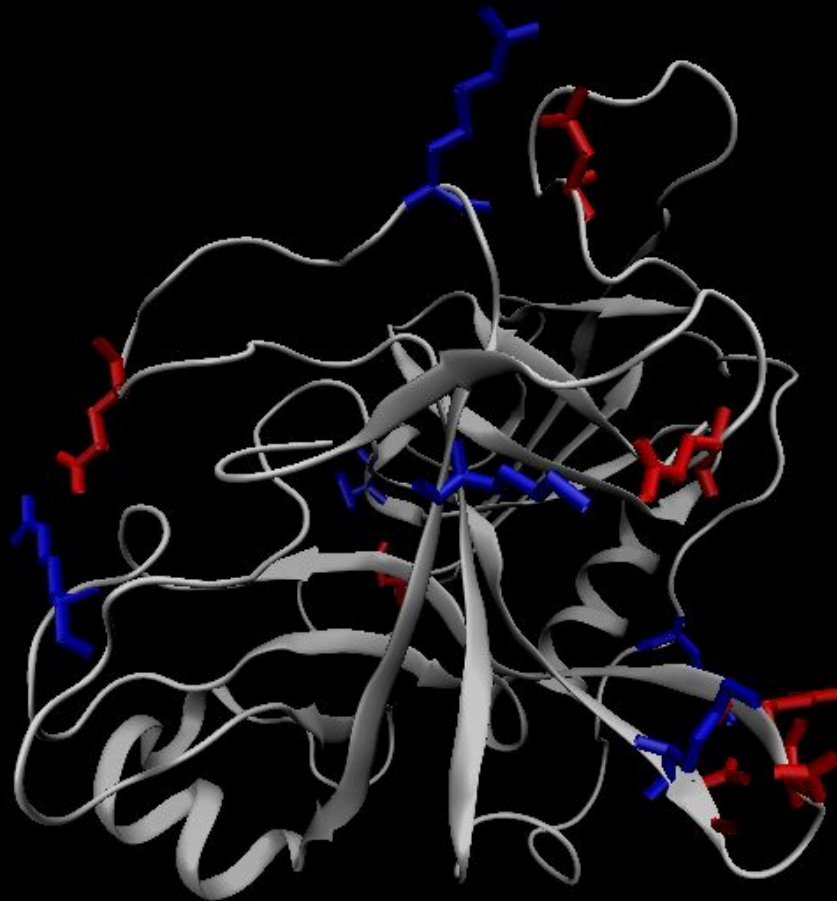
Residue 236

# TRYPSIN-LIKE

Salt bridges

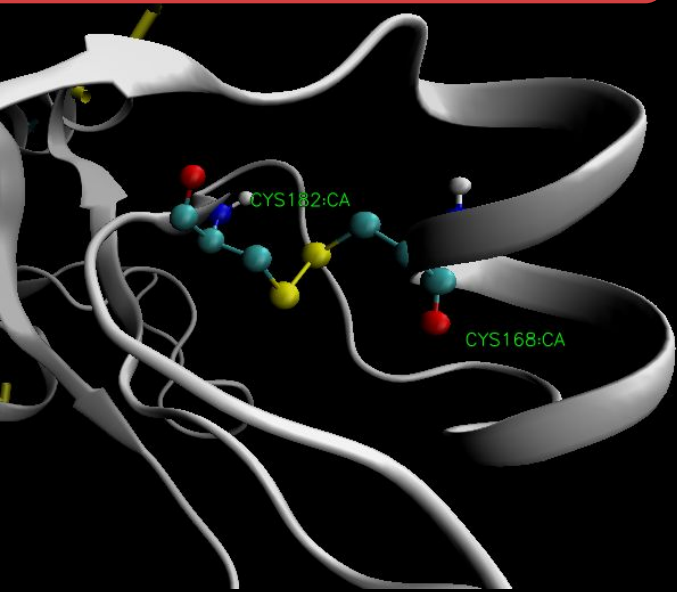


 Acid amino acids  
 Basic amino acids

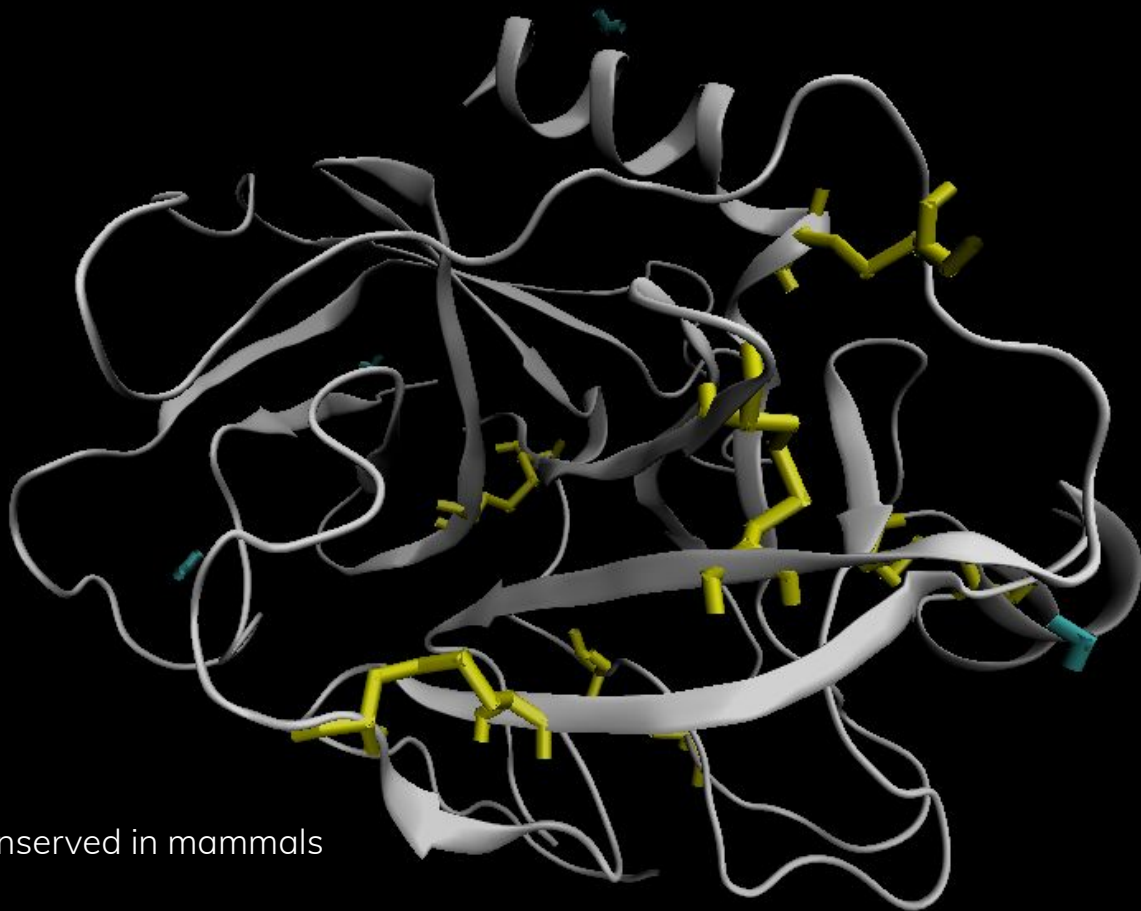


# TRYPSIN-LIKE

Disulfide bonds



6 conserved in mammals

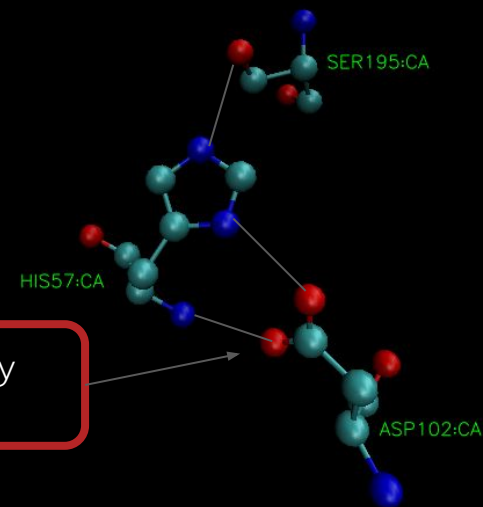
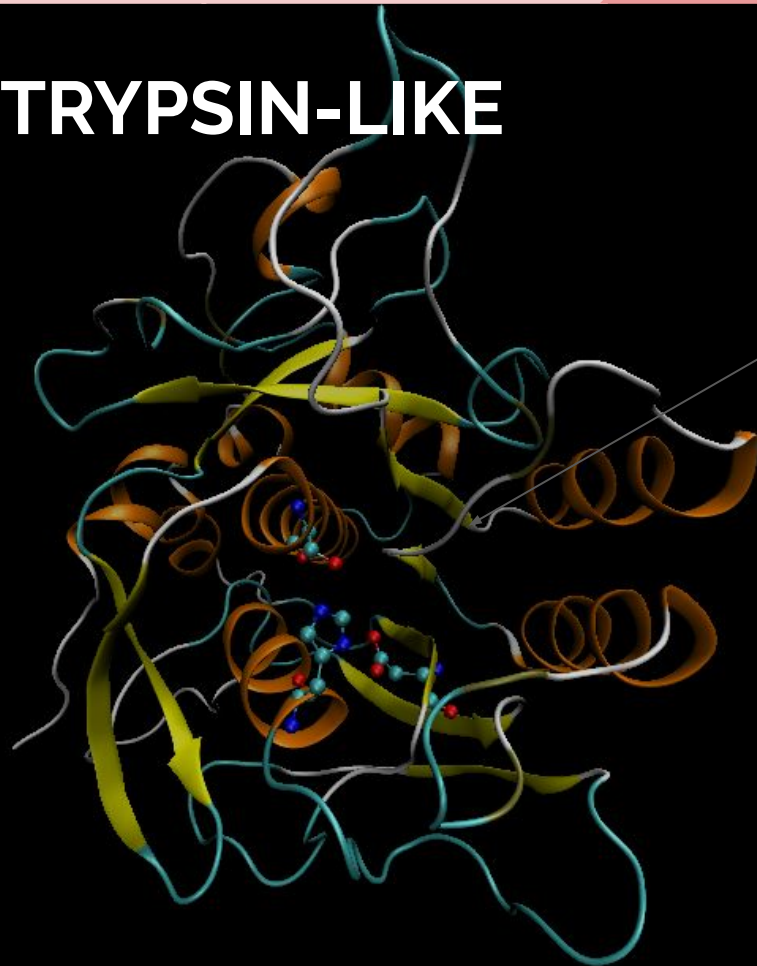


# TRYPSIN-LIKE

Catalytic Triad

Between the two domains

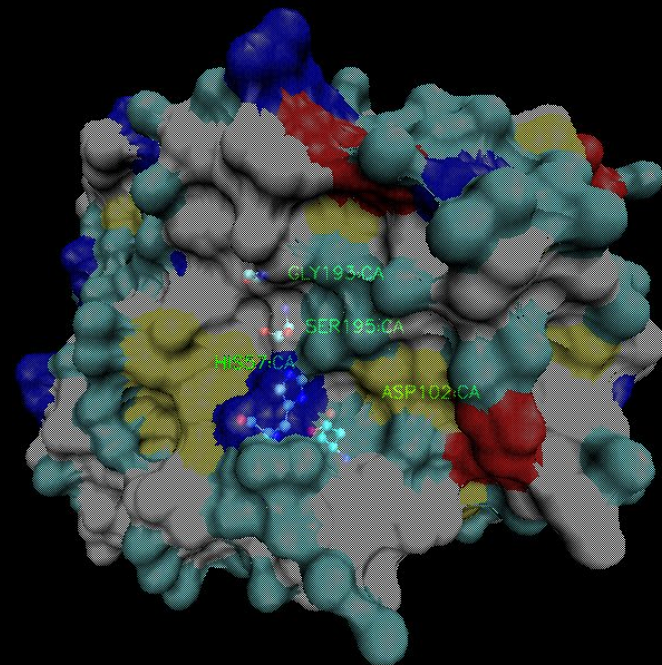
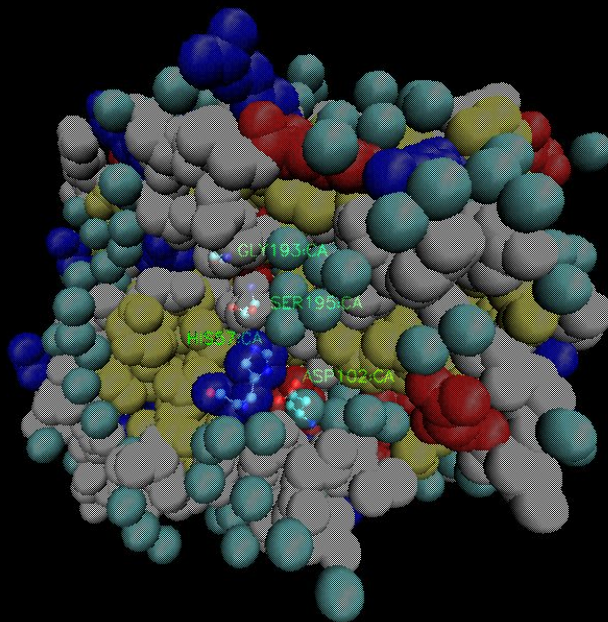
Conformation stabilized by  
Hydrogen Bonds



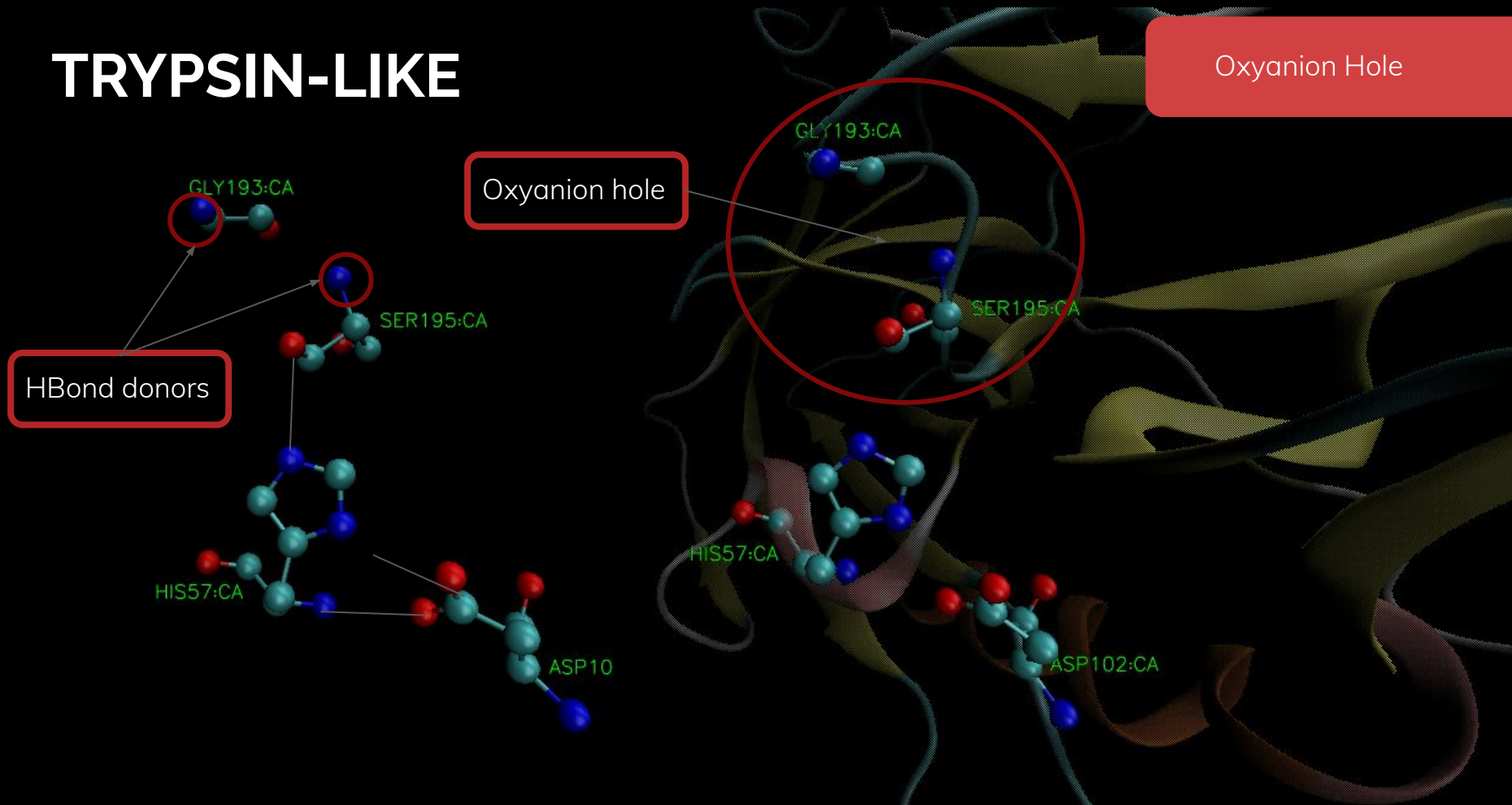


# TRYPSIN-LIKE

-  Non-polar amino acids
-  Polar amino acids
-  Acid amino acids
-  Basic amino acids
-  Unassigned

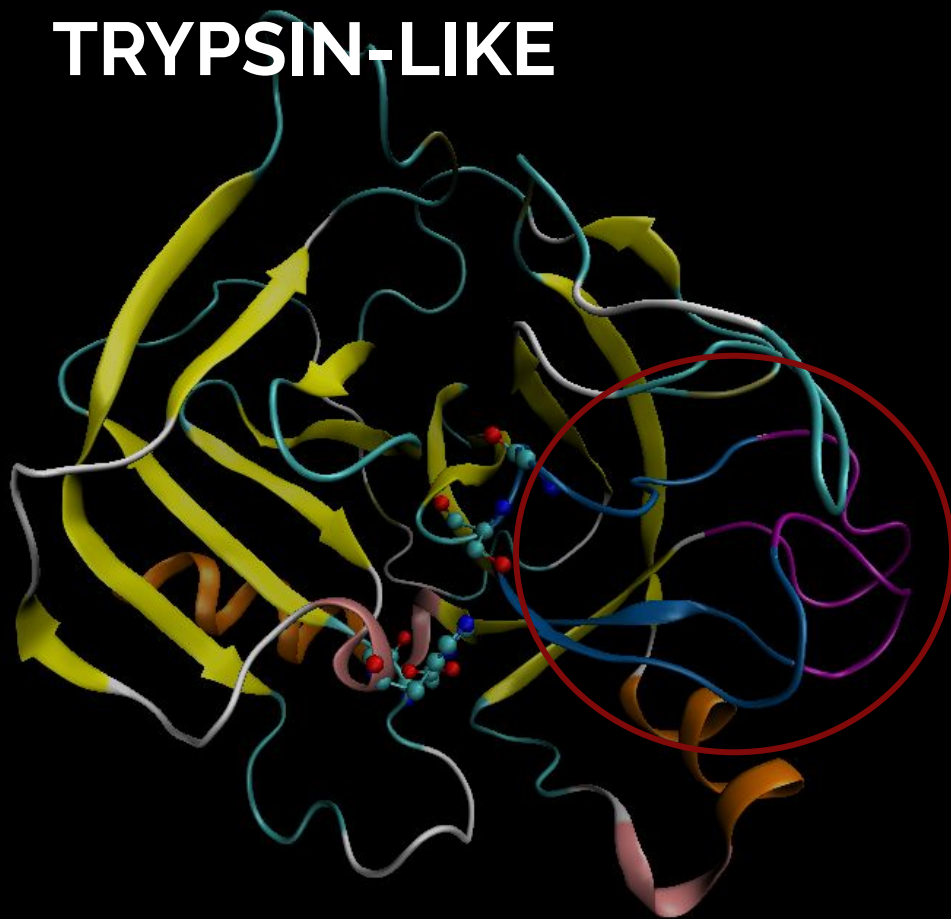


# TRYPSIN-LIKE



# TRYPSIN-LIKE

Substrate pocket



Substrate Specificity pocket



S1 pocket







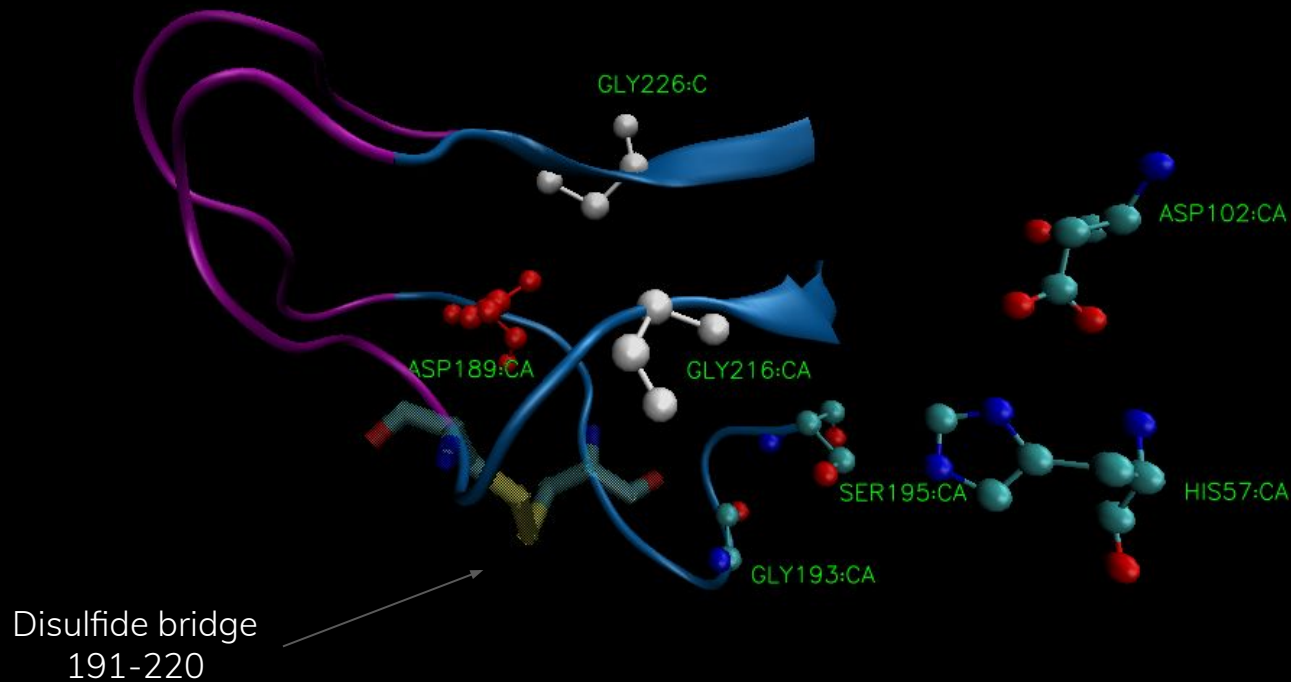
L1 and L2 loops

# TRYPSIN-LIKE

Substrate pocket

Trypsin





-  S1 pocket
-  L1 and L2 loops
-  Polar amino acids
-  Acid amino acids

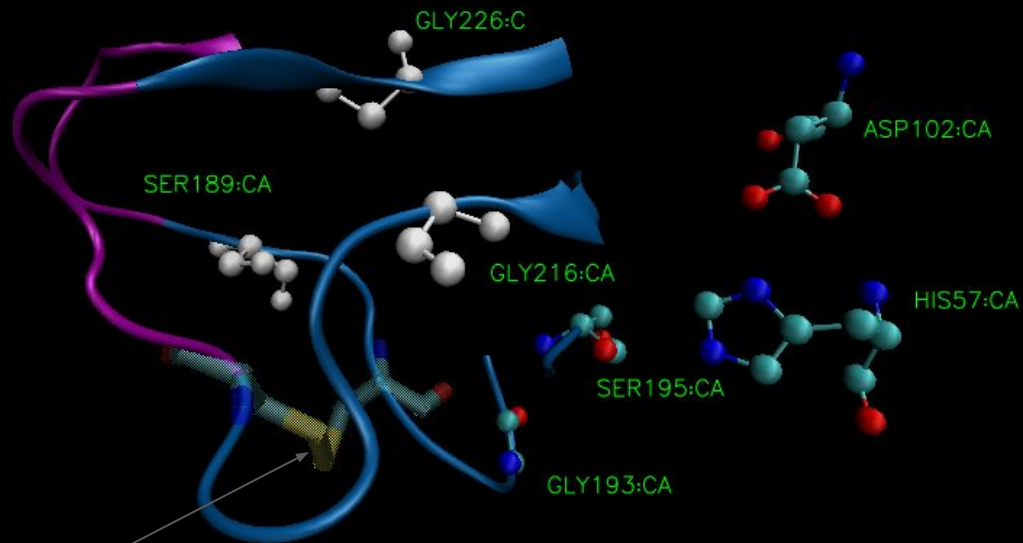




# TRYPSIN-LIKE

## Chymotrypsin

-  S1 pocket
-  L1 and L2 loops
-  Polar amino acids
-  Acid amino acids







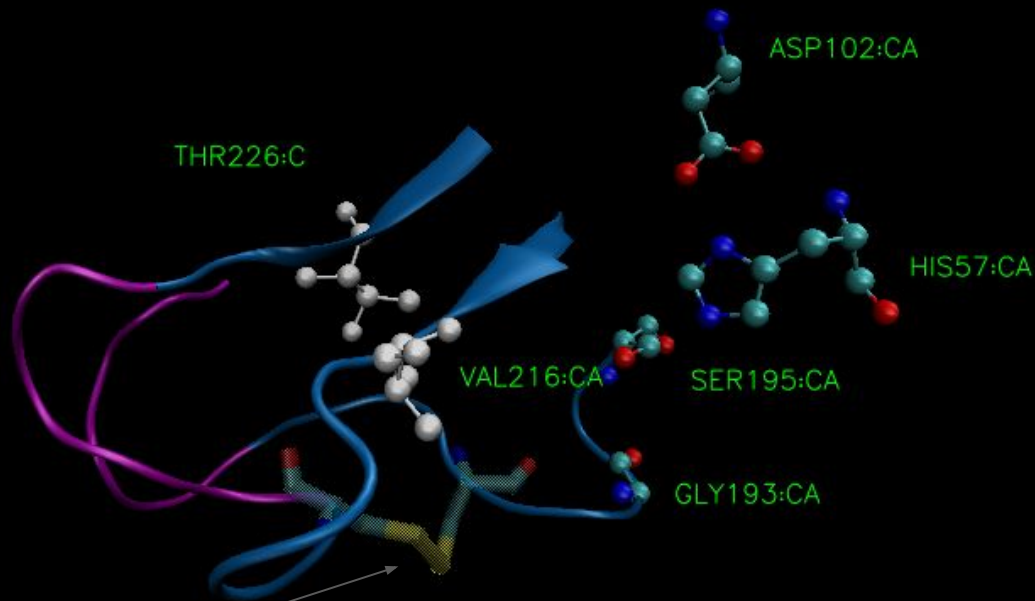
Disulfide bridge  
191-220

Substrate pocket

# TRYPSIN-LIKE

Elastase

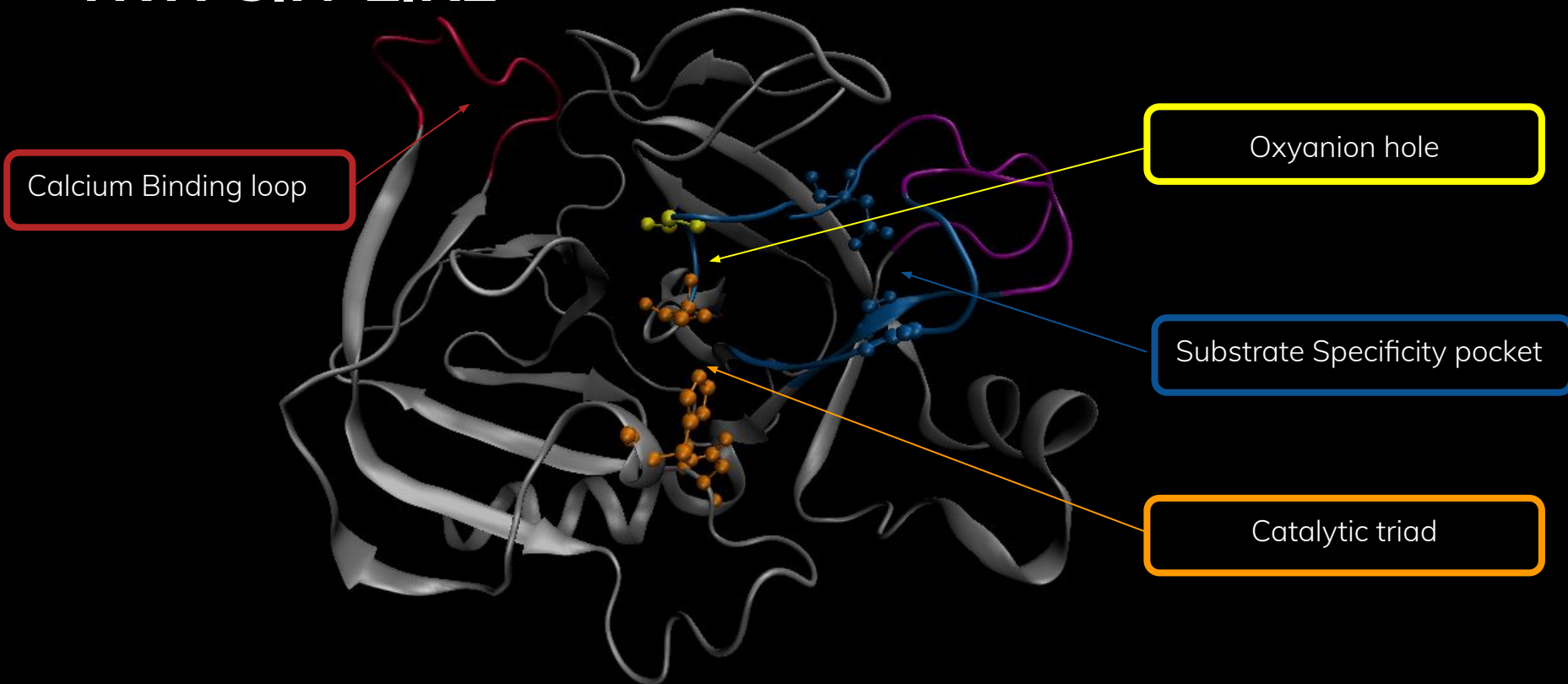
-  S1 pocket
-  L1 and L2 loops
-  Polar amino acids
-  Acid amino acids



Disulfide bridge  
191-220

Substrate pocket

# TRYPSIN-LIKE



# TRYPSIN-LIKE proteins superimposition



- Coagulation factor XII
- Trypsin
- Kallikrein
- Granzyme M
- Elastase
- Chymotrypsinogen A

Sc 7.96  
RMS 1.26

# Multiple Sequence Alignment TRYPSIN-LIKE proteins by structure

```

Coagulation_factorXII  -----VALRGAHPYIAALYWG-----HSF-CAGSLIAPCWVLTAAHCLQDRP---APEDL
Trypsin                VVGGTRAAQGEFFPMVRLS-----M-G-CGGALYAQDIVLTAACHV-S-GSGNN-TSI
Kallikrein             IVGGWECEQHSQPWQAALYHF----ST-FQCGGILVHRQWVLTAAHCSI-S-----D-N-Y
Granzyme_M             IIGGREVIPHRSRPYMASLQRN----GS-HLCGGVLVHPKWVLTAAHCLAQ-R----M-AQL
Elastase               VVGGTEAQRNSWSPQISLQYRSGSSWA-HTCGGTLIRQNWVMTAAHCVDR-E---L-T-F
Chymotrypsinogen_A    IVGGTESSWGEWPWQVSLQVKLT-AQR-HLCGGSLIGHQWVLTAAHCFDGLP---LQDVW
  
```

His 57

```

Coagulation_factorXII  TVVLGQERRNHSC--EPCQTLAVRSYRLHEAFSP-V-----SY--QIDIALLRLQE
Trypsin                TATGGVVDLQSG---A-AVKVRSTKVLQAPGYN-----GT--GKDIALIKLAQ
Kallikrein             QLWLGRHNLFDDE--NTAQFVHVSEFPHPGFNM-SLLENRQADEDY--SIDIMLLRLTE
Granzyme_M             RLVLGLHTLDSPL----GLTFHIKAAIQHPRYPKVP-----AL--EIDIALQLDQ
Elastase               RVVVGGEHNLNQND--GTEQYVGQKIVVHPYWNT-D-----DVAAGTDIALLRLAQ
Chymotrypsinogen_A    RIYSGILELSDI-TKD-TPFSQIKEIIHQNYKV-S-----EG--NIDIALIKLQA
  
```

Asp102

```

Coagulation_factorXII  DADGSCALL-SPYVQPVSLPSGAARPS-ETTLQVAGWGH----QFEGAEYASFLQEA
Trypsin                -----PIN--Q---PTLKIAT-TTAYN-Q-GTFTVAGWGANR-EGGSQ-----QRYLLKA
Kallikrein             -----PADTITDAVKVVELPT--EEPE-VGSTCLASGWGSIEPENFSF----PDDLQCV
Granzyme_M             -----KVKP-SRTIRPLALPSKRQVVA-AGTRCSMAGWGLTH-QGGRL-----SRVLREL
Elastase               -----SVTL-NSYVQLGVLPRAGTILA-NNSPCYITGWGLTR-TNGQL-----AQTLQQA
Chymotrypsinogen_A    -----PLEY-TEFQKPIISLPSKGDSTIY-TNCWVTGWGFSK-EKGEI-----QNILQKV
  
```

Residue 189

```

Coagulation_factorXII  QVPFLSLERCSAPDVH-GSSIL-PGMLCAG-FL-EGGDDCCGDSCGPLVCEQAERR
Trypsin                NVPFVSDAACR-S-AY-GNELVANEIICAG-YPDTGGDDCCDGGSCGPMFRKDN--ADE
Kallikrein             DLKILPNDECK-K-AH-VQKVT-DFMLCVG-HL-EGGDDCCVGGSCGPLMCD-----G
Granzyme_M             DLQVLDTRMCNNSRFW-NGSL-S-PSMVCLAAD--SKDDAACCGKSCGPLVCGK-----G
Elastase               YLPTVDYAIICSSSYW-GSTVK-NSMVCAG--G-DGVRSCDGGSCGPLHCLVN---GQ
Chymotrypsinogen_A    NIPLVTNEEQ-K-RYQDYKIT-QRMVCAG-YK-EGGDDCCCGSCGPLVCKHN---GM
  
```

Gly 193

Ser 195

```

Coagulation_factorXII  LTLGGIISWGS--CCDRNK-PGVYTDVAYYLAWIREHTVSHHT
Trypsin                WIQGGIVSWGY--CC-ARPGVPGVYEVSTFASAIASAAR-TL-
Kallikrein             GVLGGVTSWGYV-CC-GTPNPKPSVAVRVLVSYVKWIEDTIA-ENS
Granzyme_M             RVLGGVLSFSSR-VC-TDIFKPPVATAVAPYVSWIRKVTGRS--
Elastase               YAVHGVTSFVSRLLCC-NVTRKPTVFRVSAIYISWINNVIA-SN-
Chymotrypsinogen_A    WRLVGGITSWGE--CC-ARREQPGVYTKVAEYMDWILEKTQSS--
  
```

Cys 191 -  
Cys220

Catalytic triad



Oxyanion hole

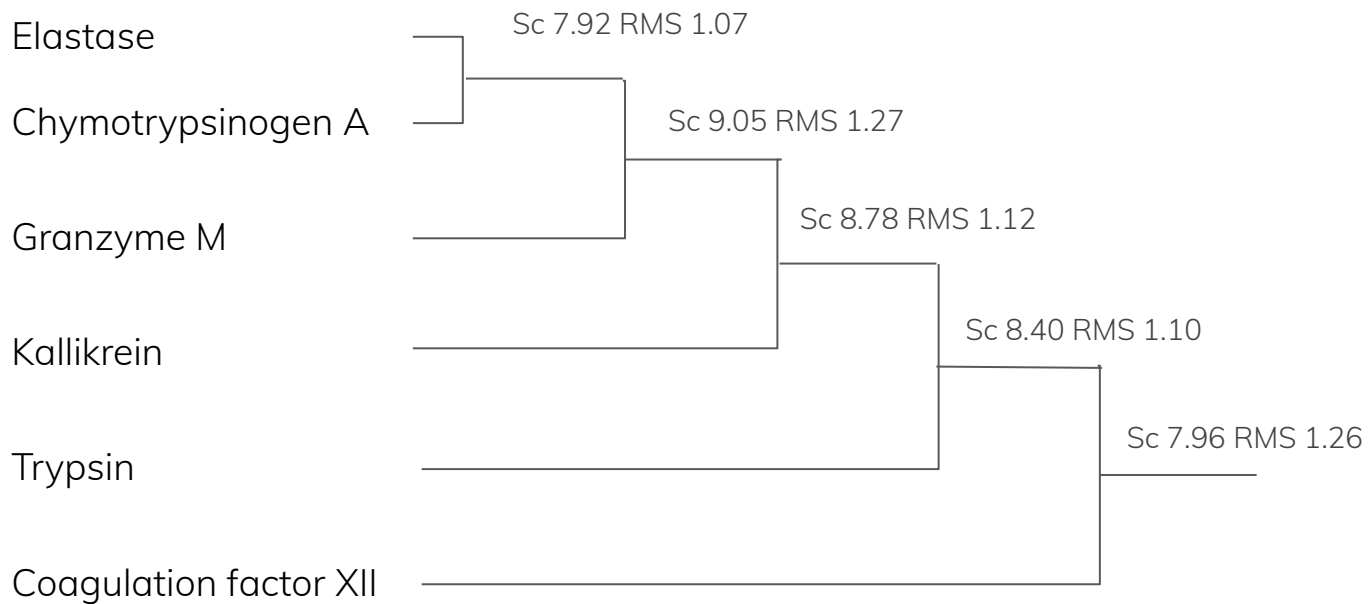


Substrate specificity pocket



Example of disulfide bond

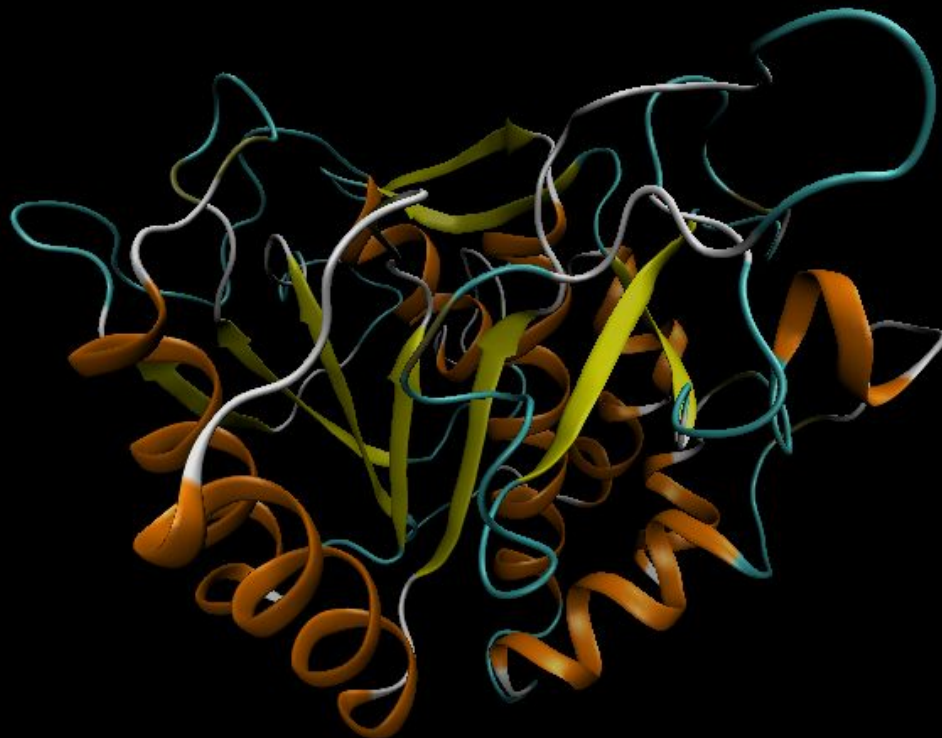
## TRYPSIN-LIKE proteins Clusters



# SUBTILISIN-LIKE

Alpha / beta / alpha

-  Alpha helix
-  Extended Beta
-  3 10 helix
-  Turn
-  Coil



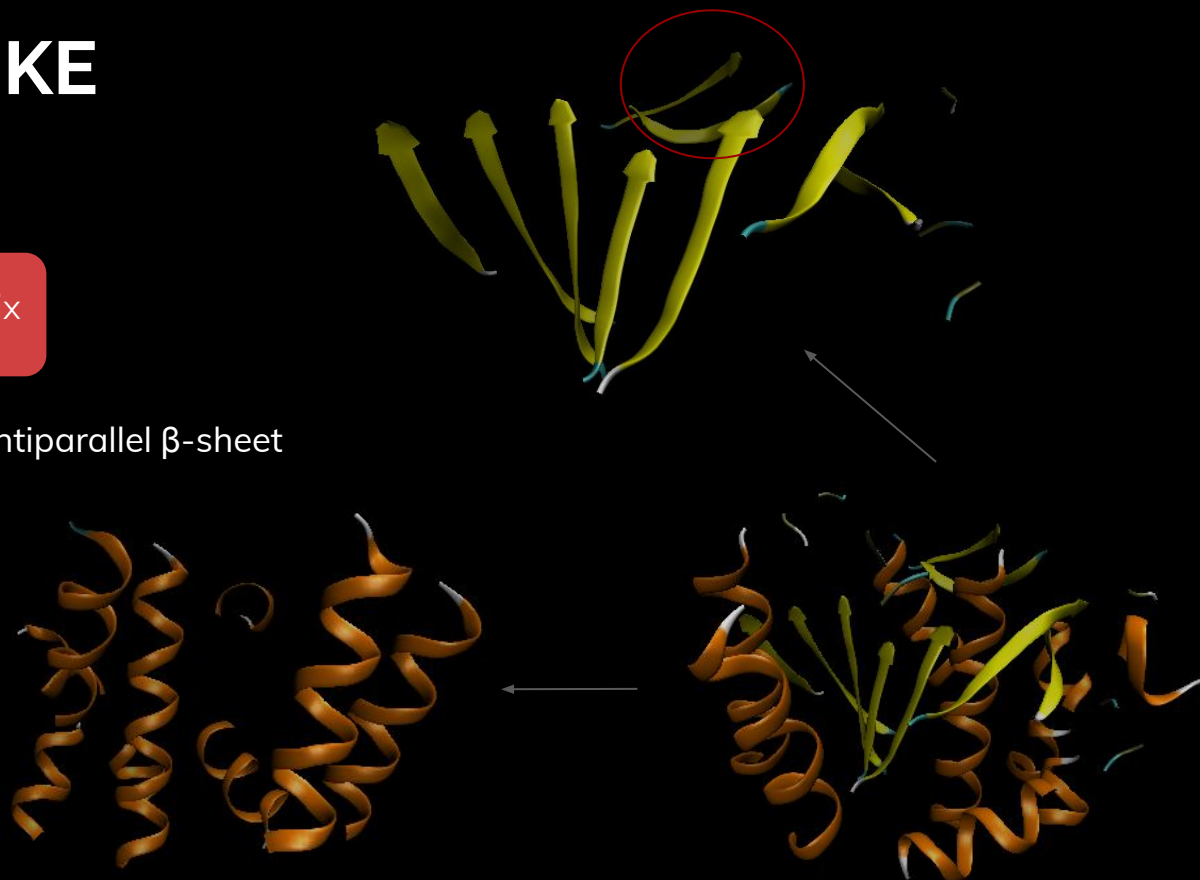


# SUBTILISIN-LIKE

7 parallel beta-strand + 9 alpha-helix

Two additional strands of antiparallel  $\beta$ -sheet

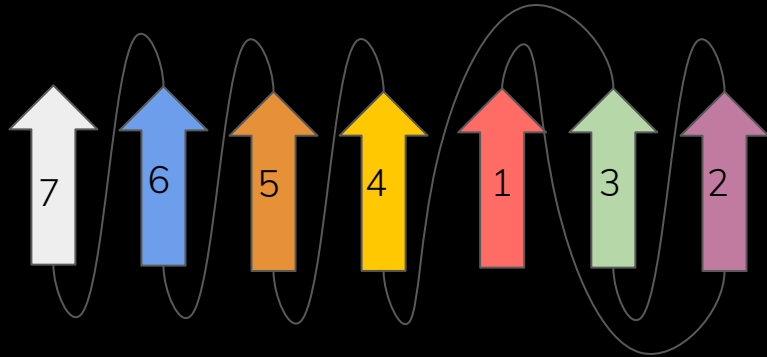
- Alpha helix
- Extended Beta
- 3 10 helix
- Turn
- Coil





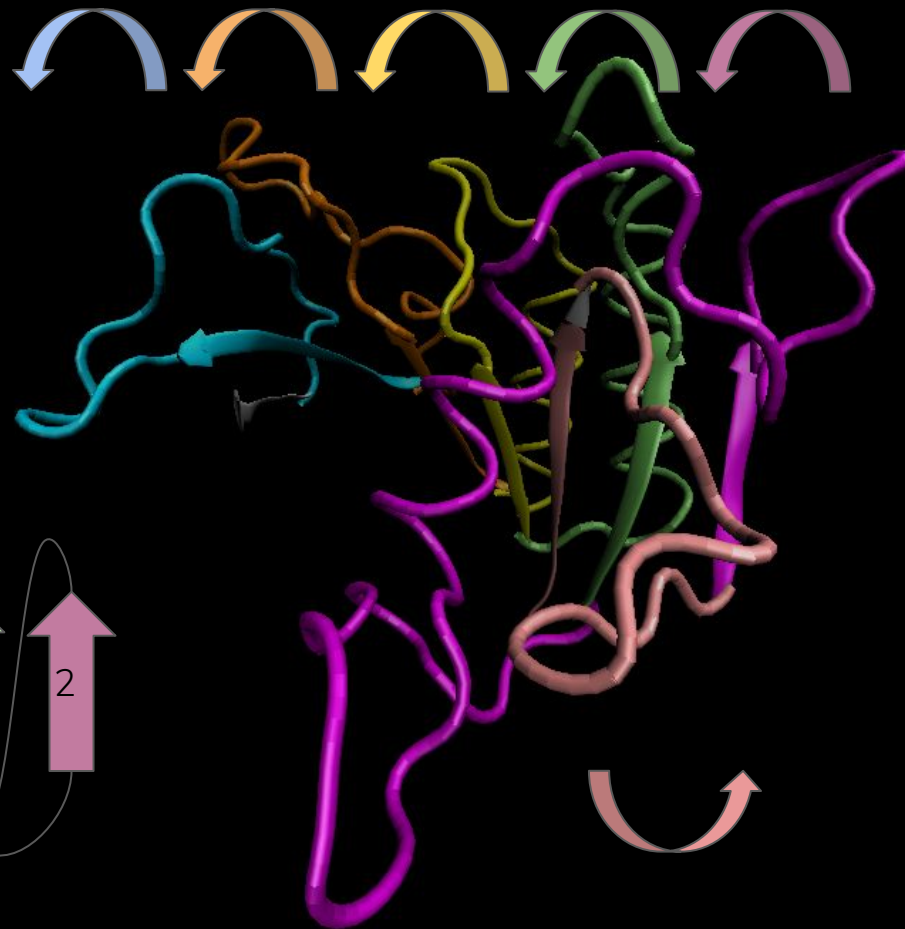
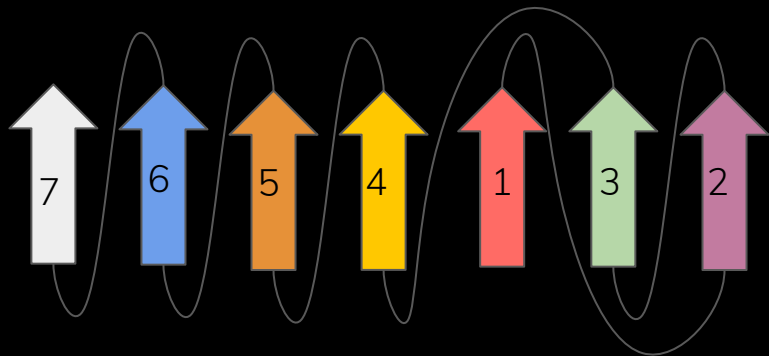
# SUBTILISIN-LIKE

Left-handed connection between strand  
2 - 3



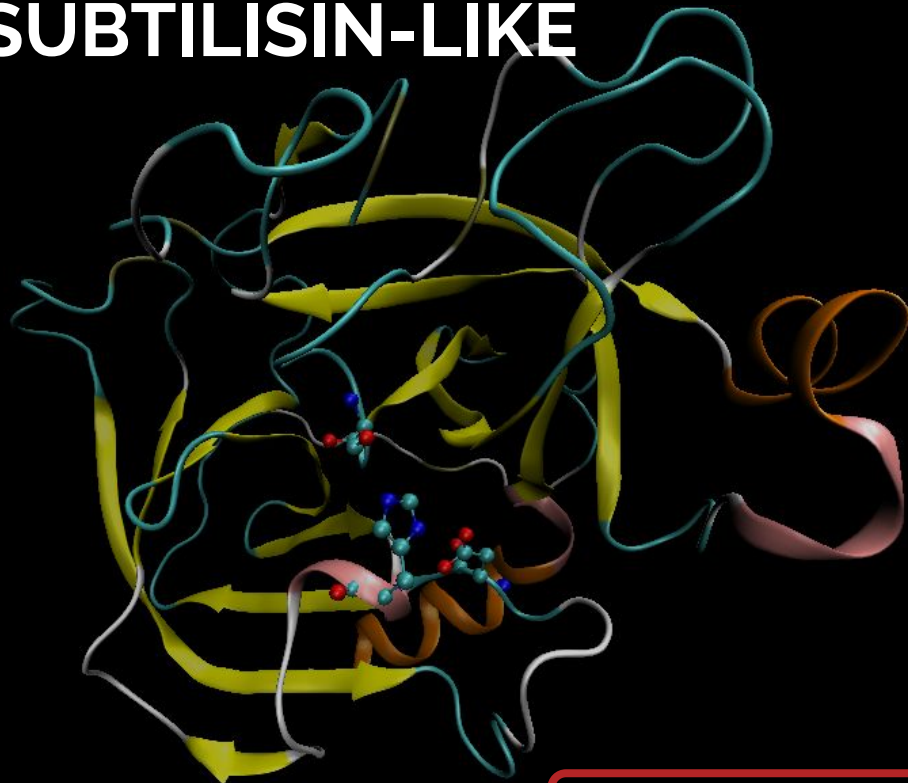
# SUBTILISIN-LIKE

Left-handed connection between strand  
2 - 3

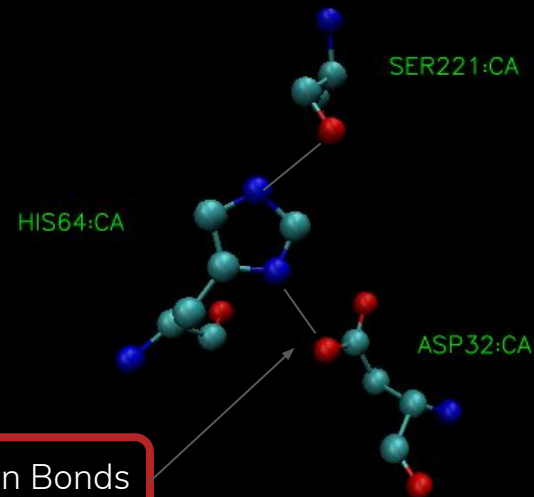


# SUBTILISIN-LIKE

Catalytic Triad

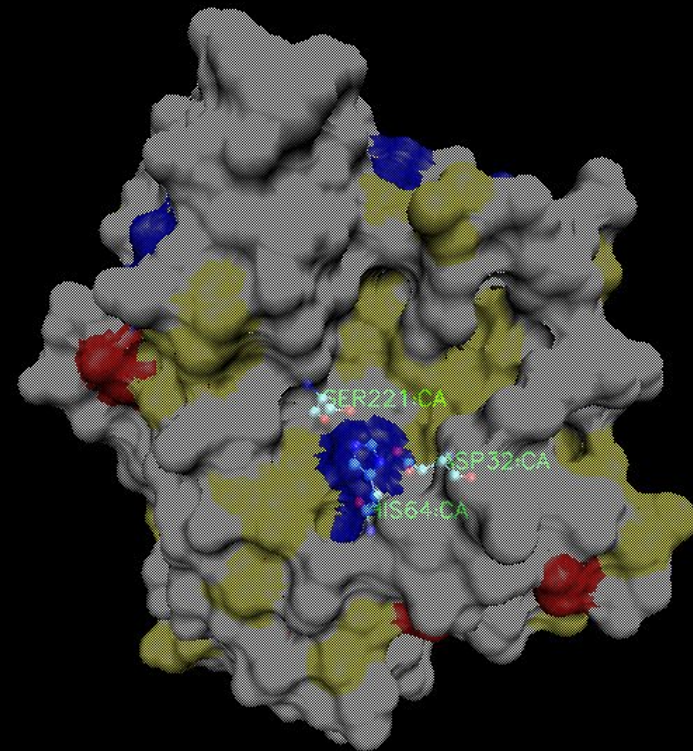
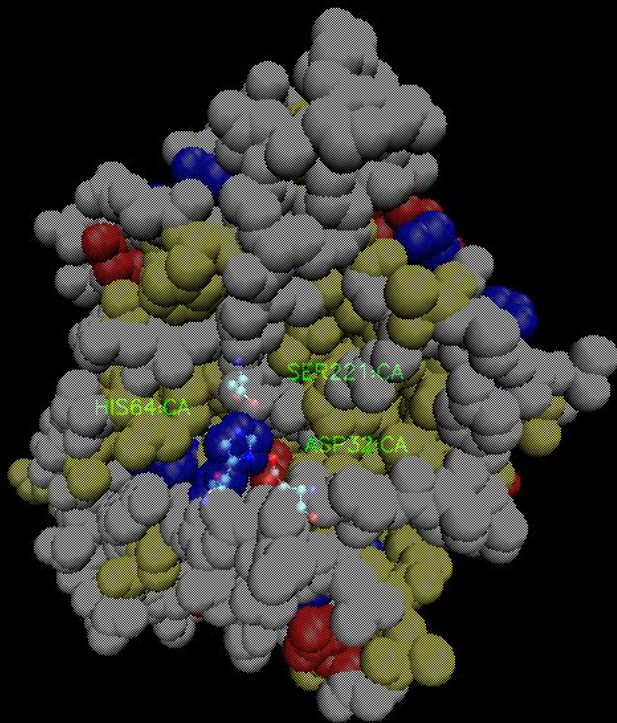


Conformation stabilized by Hydrogen Bonds



# SUBTILISIN-LIKE

-  Non-polar amino acids
-  Polar amino acids
-  Acid amino acids
-  Basic amino acids
-  Unassigned

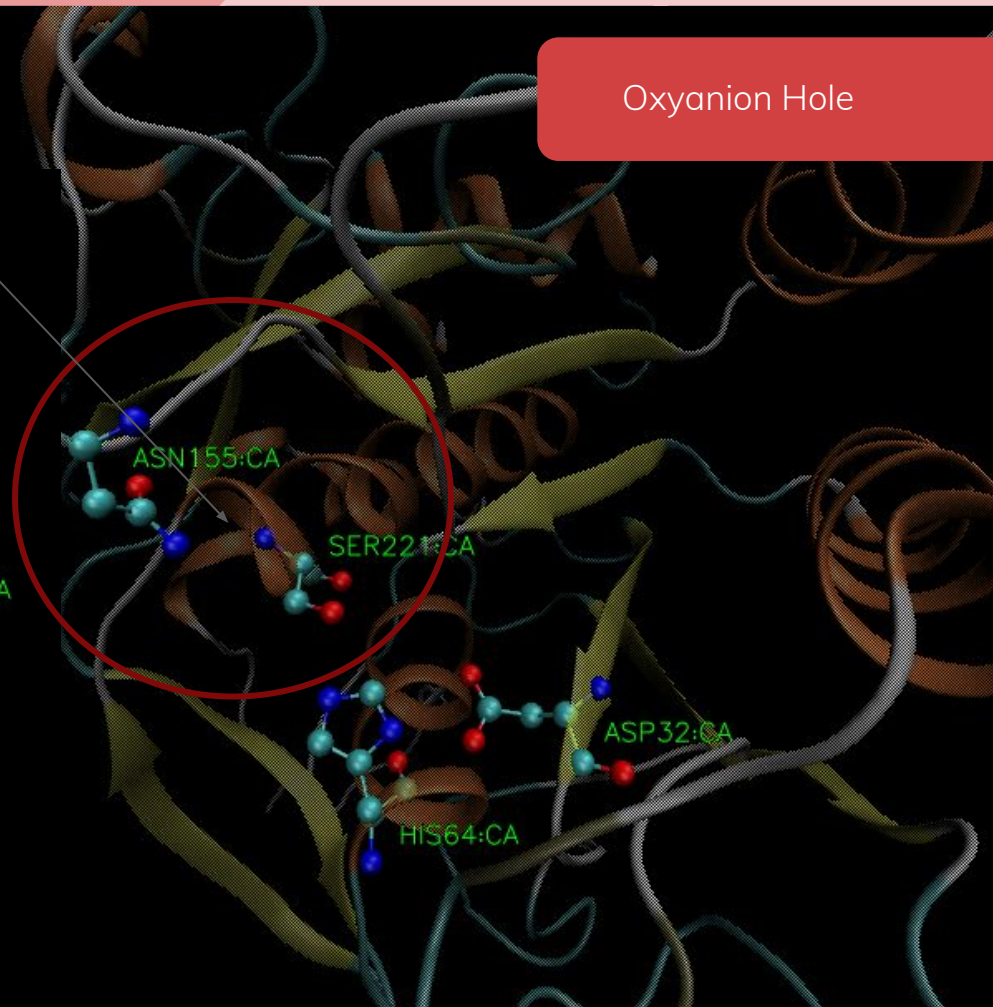
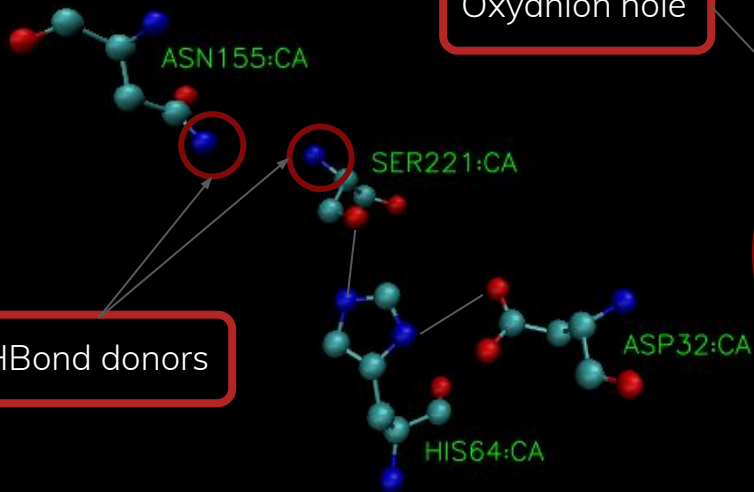
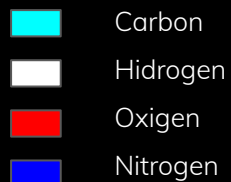


# SUBTILISIN-LIKE

Oxyanion Hole

Oxyanion hole

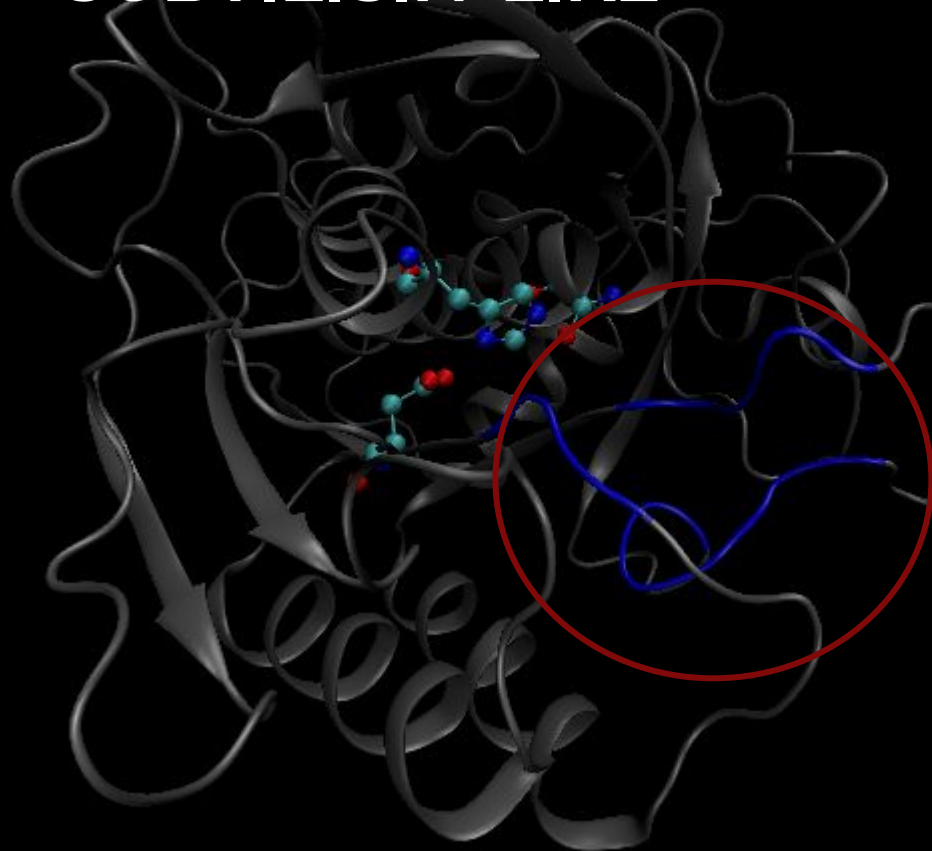
HBond donors





# SUBTILISIN-LIKE

Substrate pocket




Substrate Specificity pocket




S1 pocket


# SUBTILISIN-LIKE


Substrate pocket

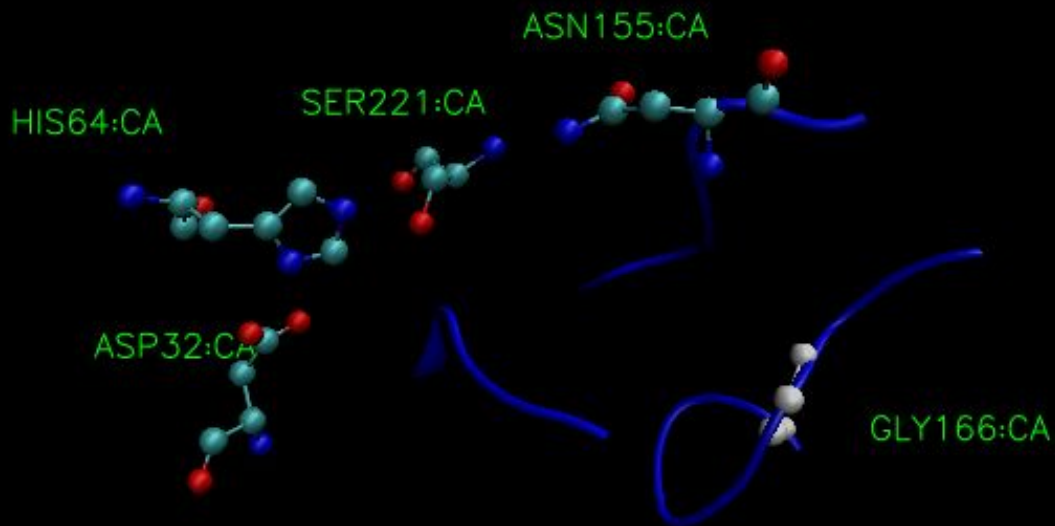
 S1 pocket

 Non-polar amino acids

 Polar amino acids

 Acid amino acids

 Basic amino acids



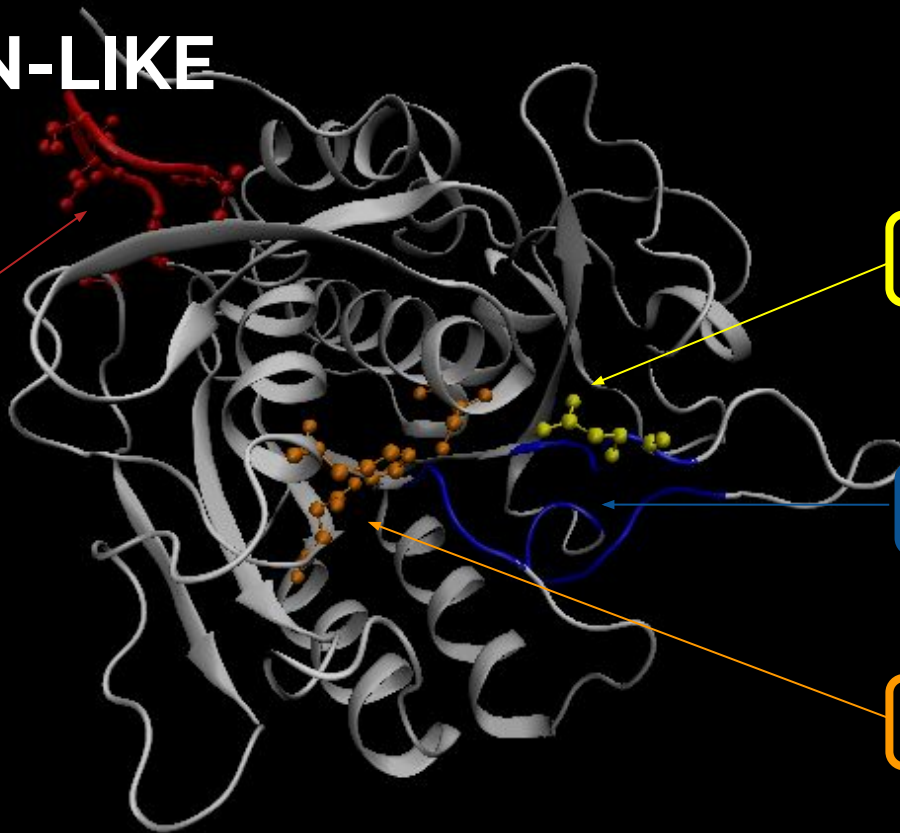
# SUBTILISIN-LIKE

Calcium Binding loop

Oxyanion hole

Substrate Specificity pocket






Catalytic triad





# SUBTILISIN-LIKE proteins superimposition



-  Subtilisin BPN
-  Bacillus lentus subtilisin
-  Subtilisin Savinase
-  Subtilisin Carlsberg
-  Subtilisin NAT

Sc 9.26  
RMS 0.54

# Multiple Sequence Alignment SUBTILISIN-LIKE proteins by structure

```

Subtilisin_BPN      ---VPYGVSQIKAPALHSQGYTGSNVKVAVLDISGIDSSHPDLNVAGGASFVPSETPNFQD
Bacillus_lentus    AQSVPWGISRVQAPAAHNRGLTGSQVKVAVLDITGIST-HPDLNIRGGASFVPGEPST-QD
Subtilisin_Savinase AQSVPWGISRVQAPAAHNRGLTGSQVKVAVLDITGIST-HPDLNIRGGASFVPGEPST-QD
Subtilisin_Carlsberg AQTVPYGIPLIKADKQVAQGFKGANVKVAVLDITGIQASHPDLNVVGGASFVAGE-AYNTD
Subtilisin_NAT     AQSVPYGVSQIKAPALHSQGYTGSNVKVAVLDISGIDSSHPDLKVAGGASMVPSETPNFQD
  
```

Asp 32

```

Subtilisin_BPN      NNSHGTHVAGTVL-----AVAPSASLYAVKVLGADGSGQYSWIINGIEWAIANMMD
Bacillus_lentus    GNGHGHVAGTIAALNNSIGVGLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
Subtilisin_Savinase GNGHGHVAGTIAALNNSIGVGLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
Subtilisin_Carlsberg GNGHGHVAGTVAALDNTTGVGLGVAPSVSLYAVKVLNSSGSGSYSGIVSGIEWATTNGMD
Subtilisin_NAT     DNSHGHVAGTVAALNNSIGVGLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANMMD
  
```

His 64

```

Subtilisin_BPN      VINMSLGGPSGSAALKAAVDKAVASGVVVAAAGNIGTSGSSSTVGYPGHYPSVIAVGAV
Bacillus_lentus    VANLSLGSPPSATLEQAVNSATSRGVLVVAASGNLGA--G--SISYPARYANAMAVGAT
Subtilisin_Savinase VANLSLGSPPSATLEQAVNSATSRGVLVVAASGNLGA--G--SISYPARYANAMAVGAT
Subtilisin_Carlsberg VINMSLGGASGSTAMKQAVDNAYARGVVVAAAGNIGNSGSTNTIGYPAHYDSVIAVGAV
Subtilisin_NAT     VINMSLGGPSGSAALKAAVDKAVASGVVVAAAGNIGTSGSSSTVGYPGHYPSVIAVGAV
  
```

Asn 155




```

Subtilisin_BPN      DSSNQRAFSSVGPELDVMAPGVSIIVSTLPGNKYGAKSGTAMASPHVAGAAALILSKHPN
Bacillus_lentus    DQNNNRASFQYAGGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
Subtilisin_Savinase DQNNNRASFQYAGGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
Subtilisin_Carlsberg DSNSNRASFSSVGAEEVMAPGAGVYSTYPTNTYATLNGTSMASPHVAGAAALILSKHPN
Subtilisin_NAT     DSSNQRAFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
  
```

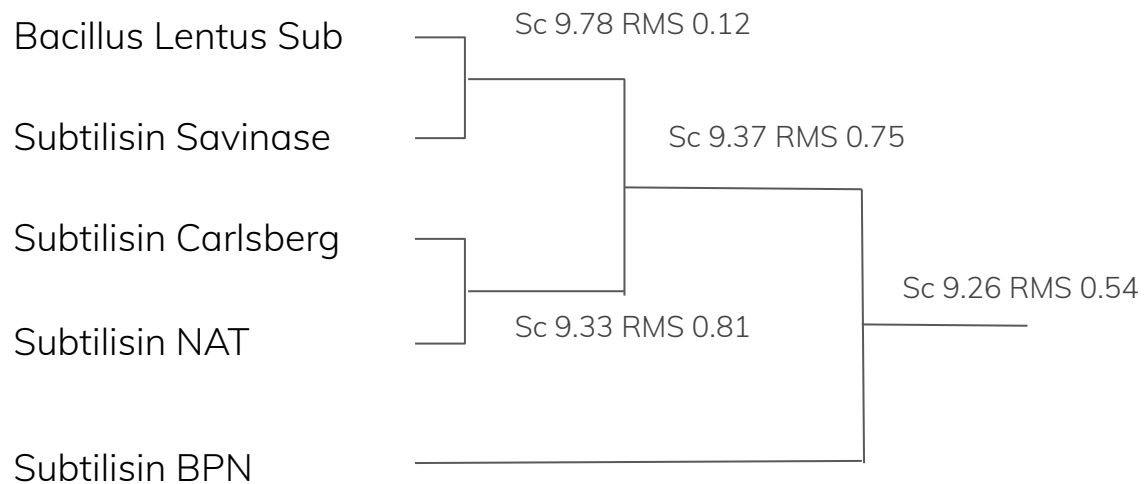
Ser 221

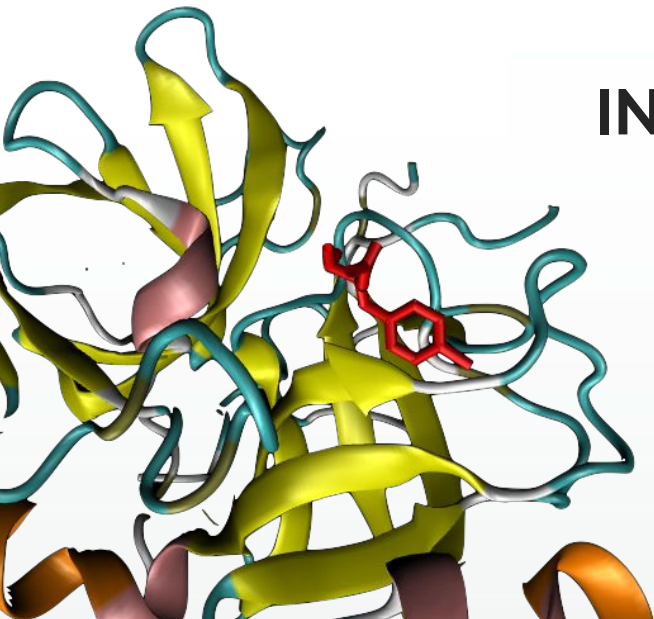
```

Subtilisin_BPN      WTNTQVRSSLENTTTKLGDSFYYGKGLINVEAAAQALAL
Bacillus_lentus    WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR----
Subtilisin_Savinase WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR----
Subtilisin_Carlsberg LSASQVRNRLSSTATYLGSSFYYGKGLINVEAAAQ----
Subtilisin_NAT     WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ----
  
```

-  Catalytic triad
-  Oxyanion hole
-  Substrate specificity pocket

## SUBTILISIN-LIKE proteins Clusters





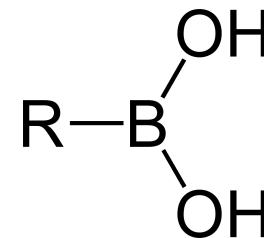
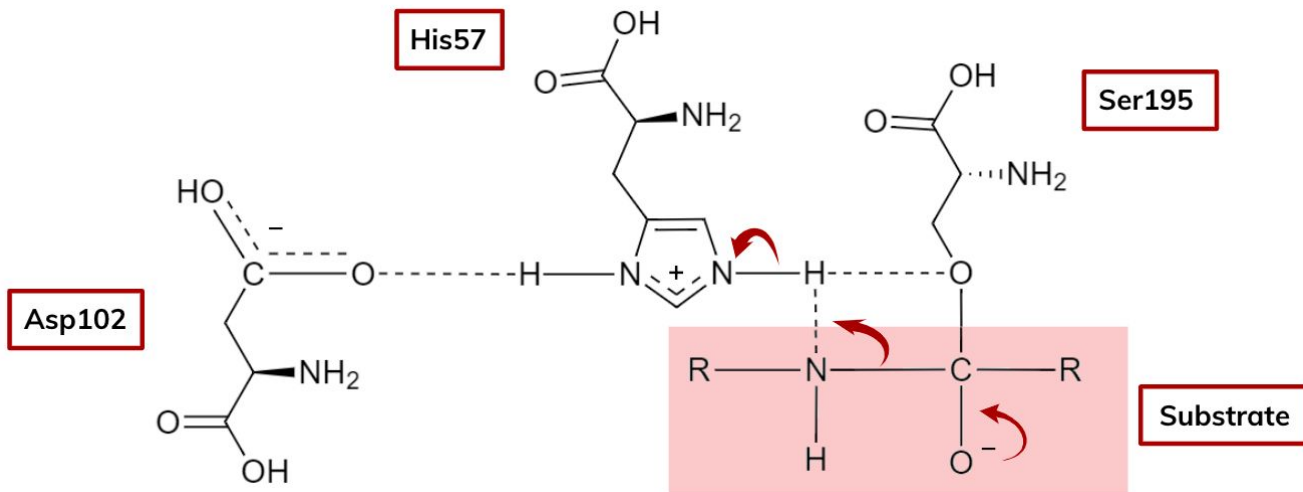
# 04

## INTERACTION WITH AN INHIBITOR

Interaction of a trypsin-like protease with an inhibitor

# Chymotrypsin: interaction with an inhibitor

**Boronic acids inhibitors** → transition state analogs

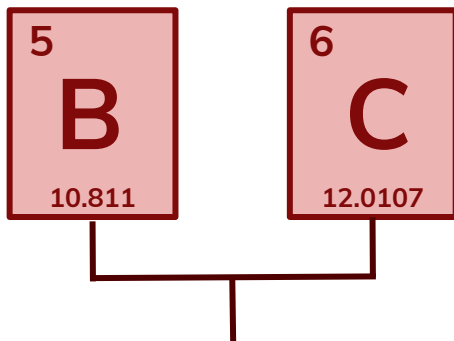


From Wikipedia, Boronic Acid

Own source

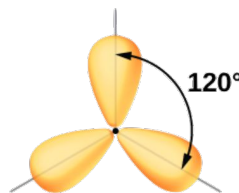
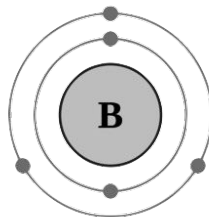
# Chymotrypsin: interaction with an inhibitor

## Boronic acids inhibitors



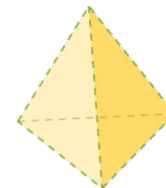
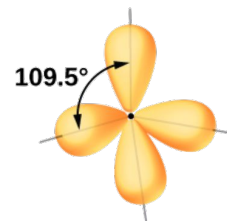
Similarities in structural features

*Analogues in the binding process, but not in reactions.*



$sp^2$

Trigonal planar



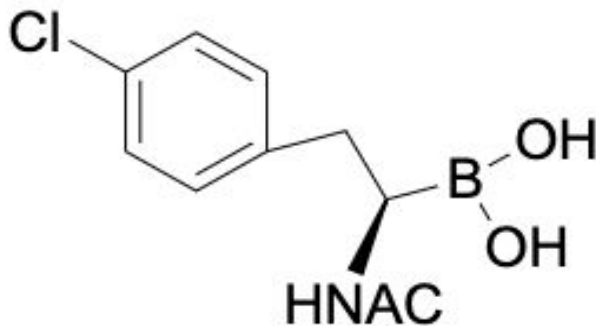
$sp^3$

Tetrahedral

# Chymotrypsin: interaction with an inhibitor

*gamma-chymotrypsin L-para-chloro-1-acetamido  
boronic acid inhibitor complex*

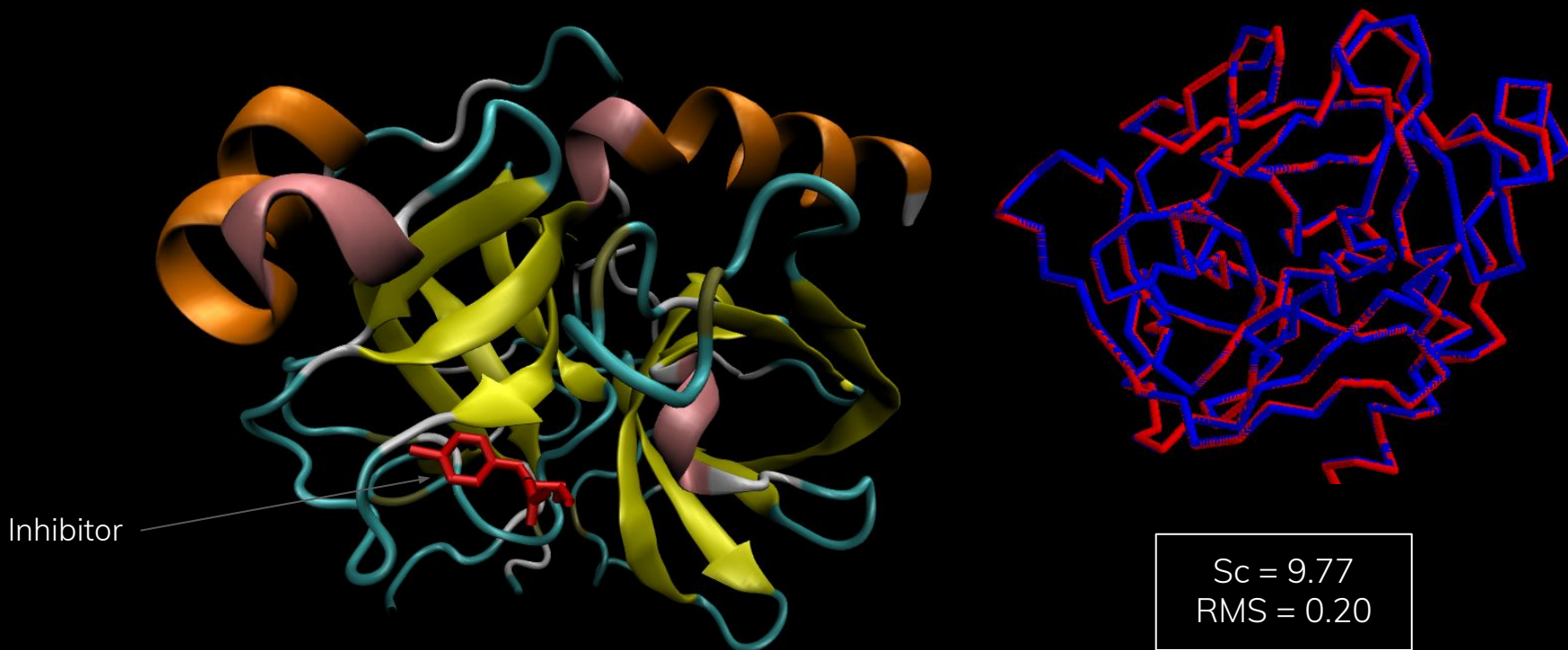
PDB: 1VGC



Own source

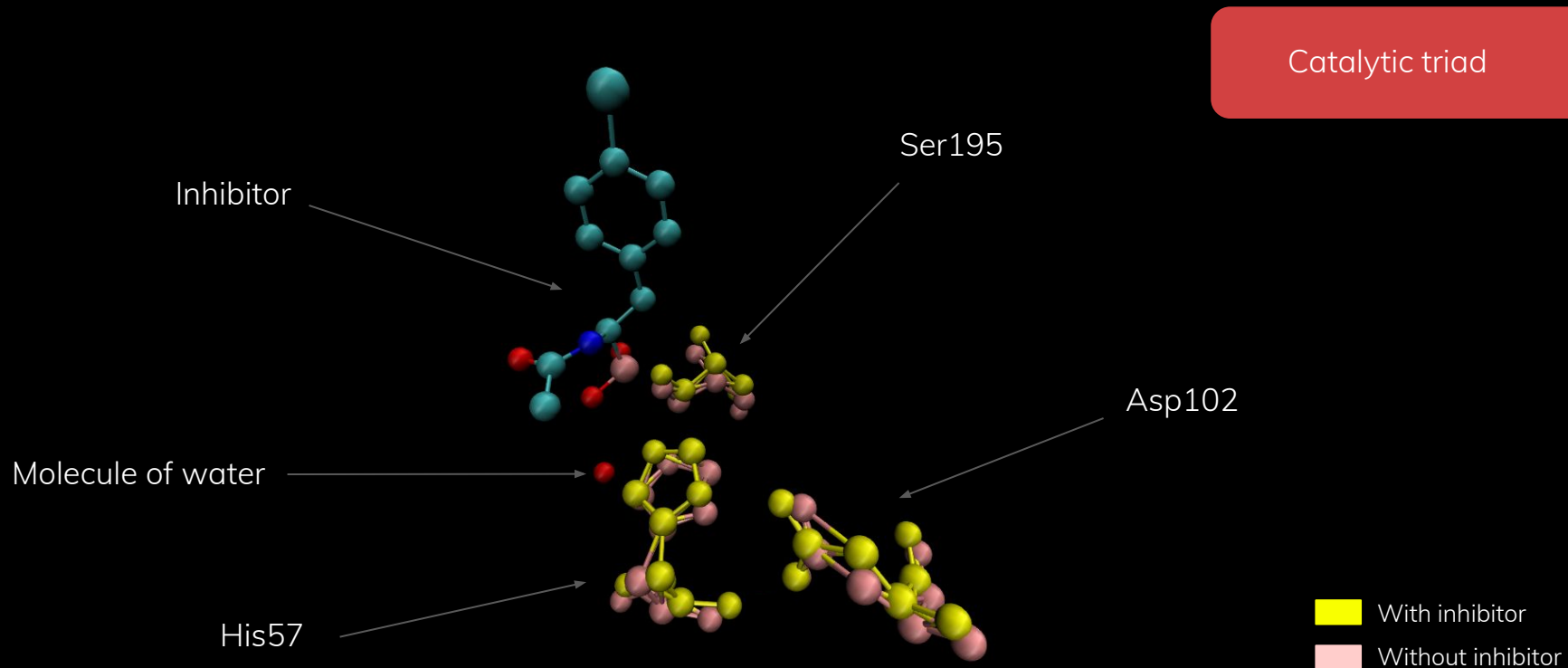
$K_i = 1.20 \pm 0.05 \mu\text{M}$

# Chymotrypsin: interaction with an inhibitor

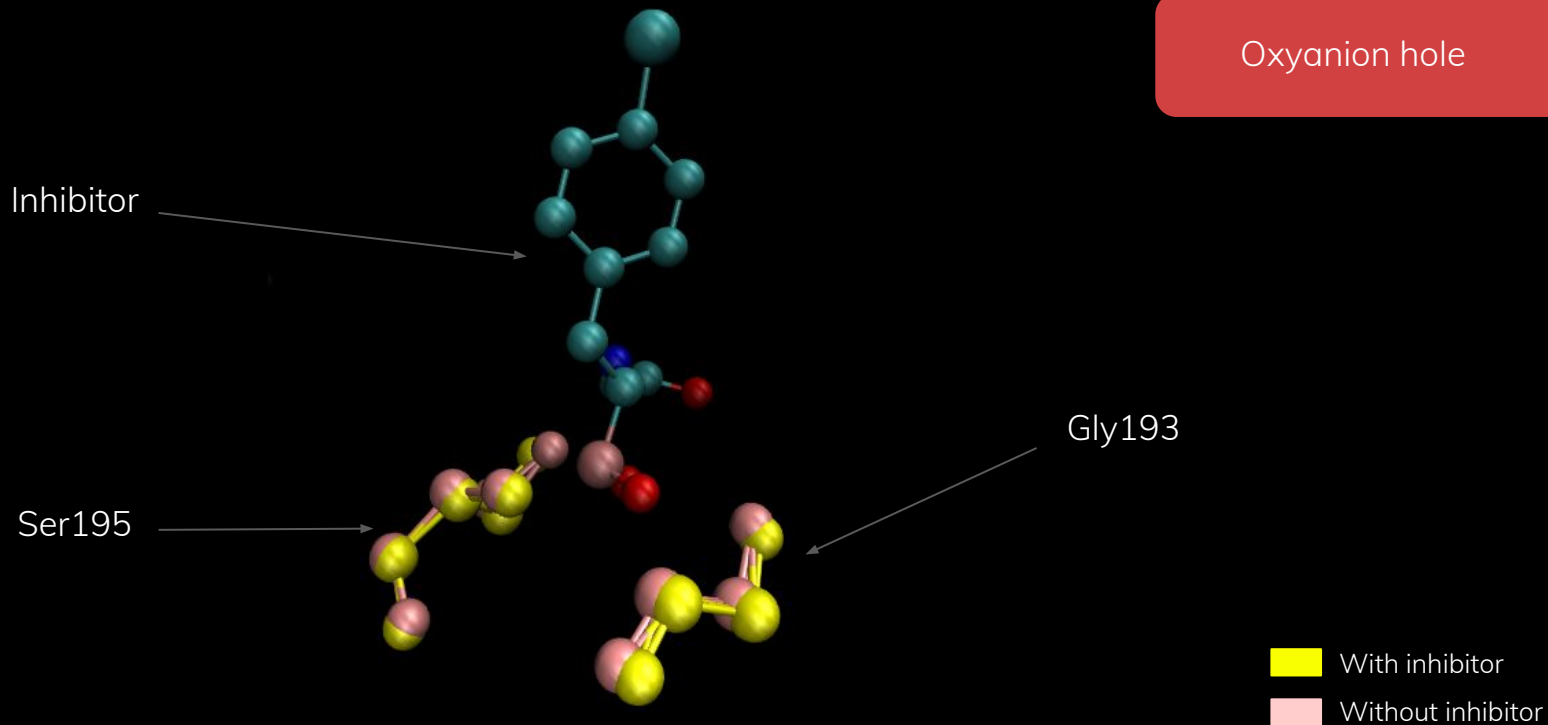




# Chymotrypsin: interaction with an inhibitor

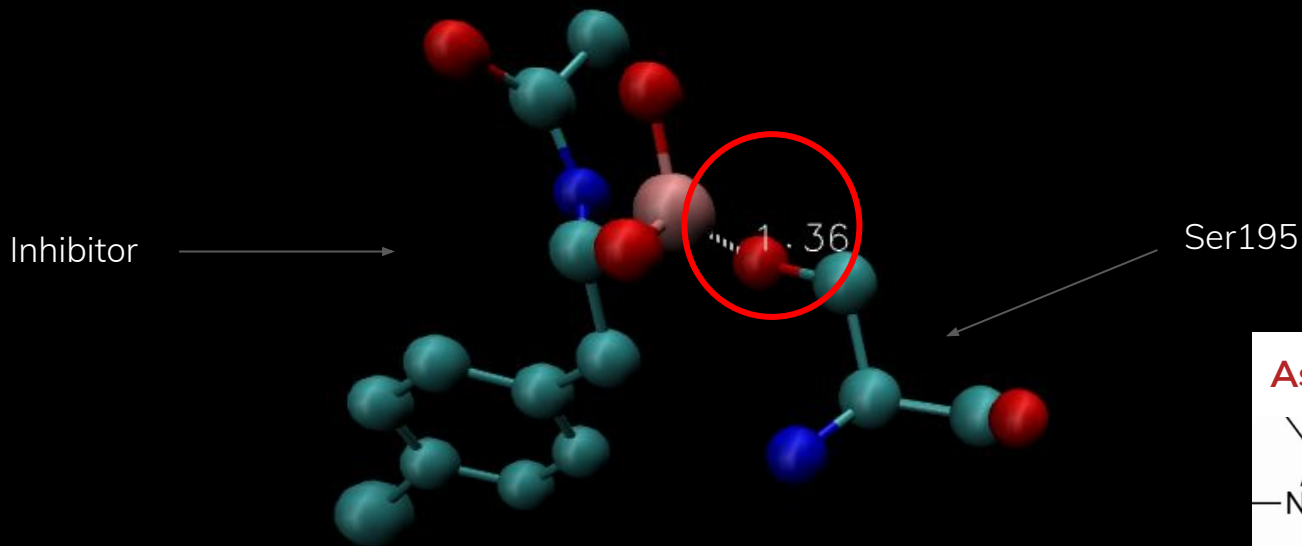


# Chymotrypsin: interaction with an inhibitor

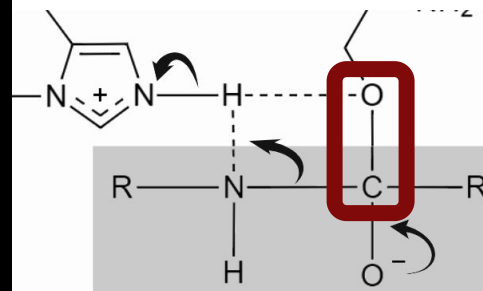


# Chymotrypsin: interaction with an inhibitor

Transition state

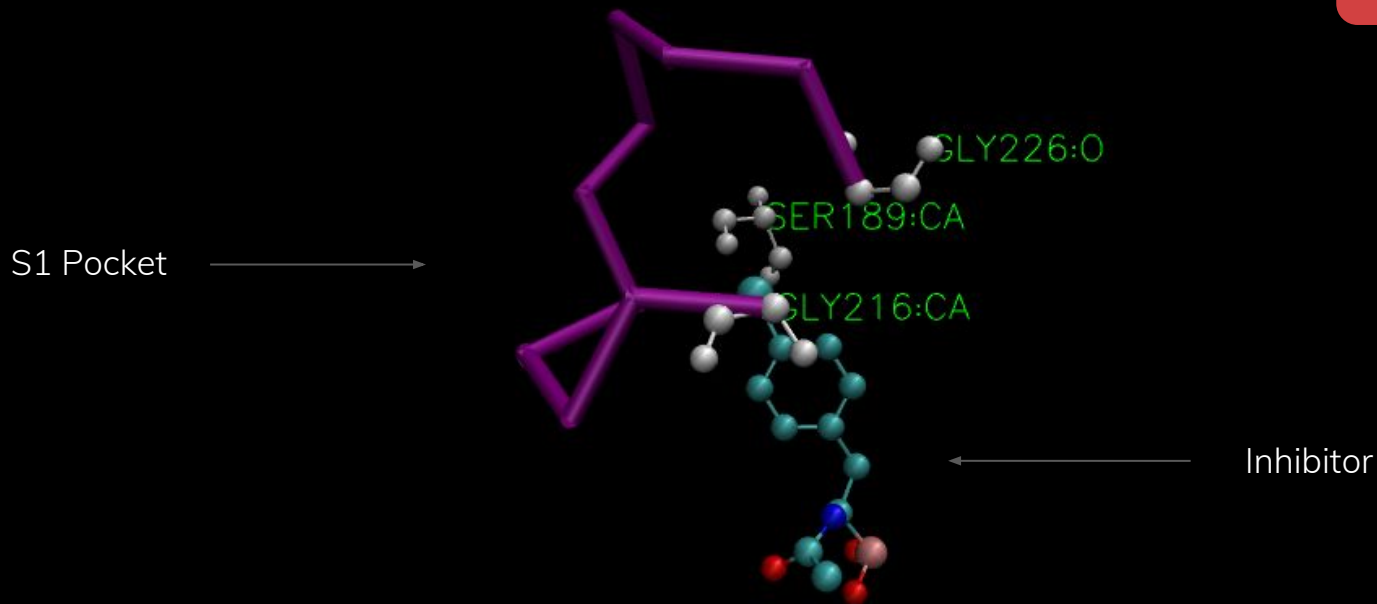


As we saw before...

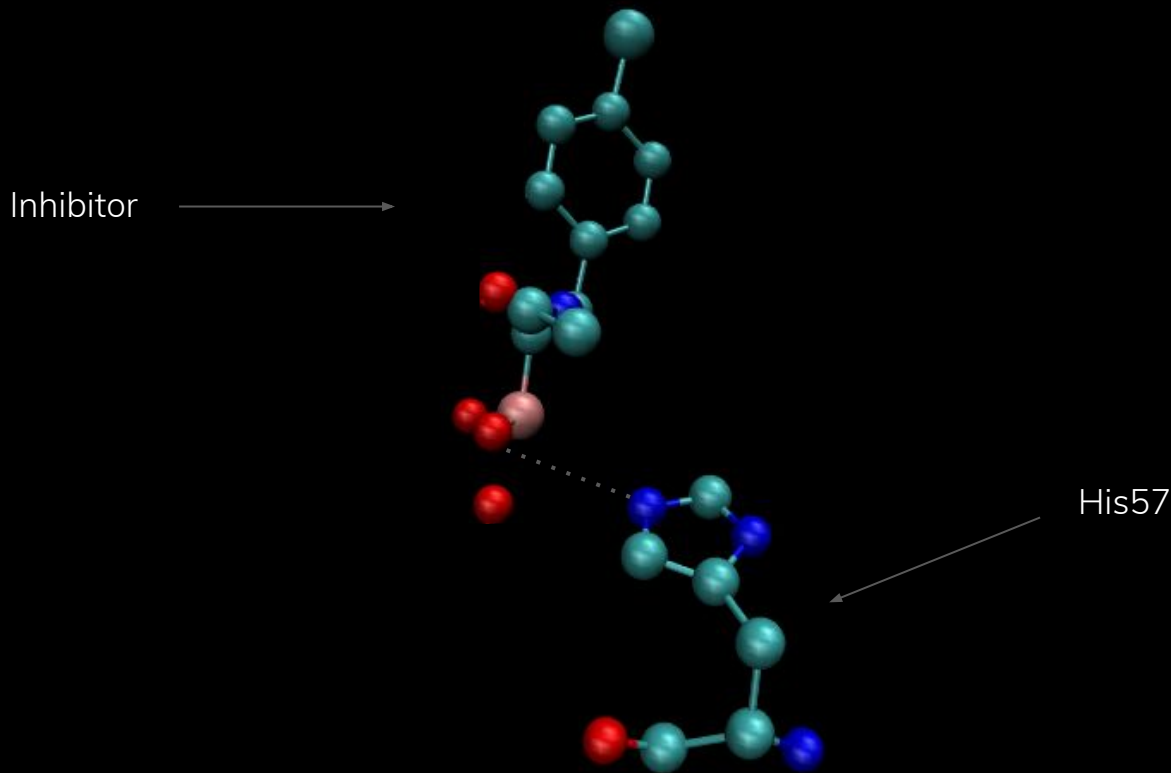


# Chymotrypsin: interaction with an inhibitor

Substrate specificity pocket

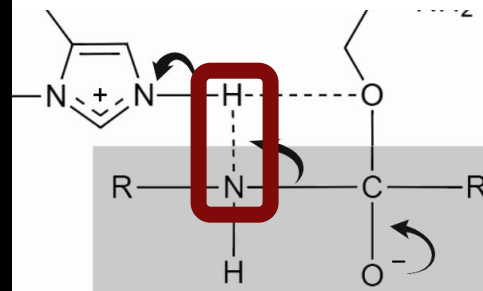


# Chymotrypsin: interaction with an inhibitor

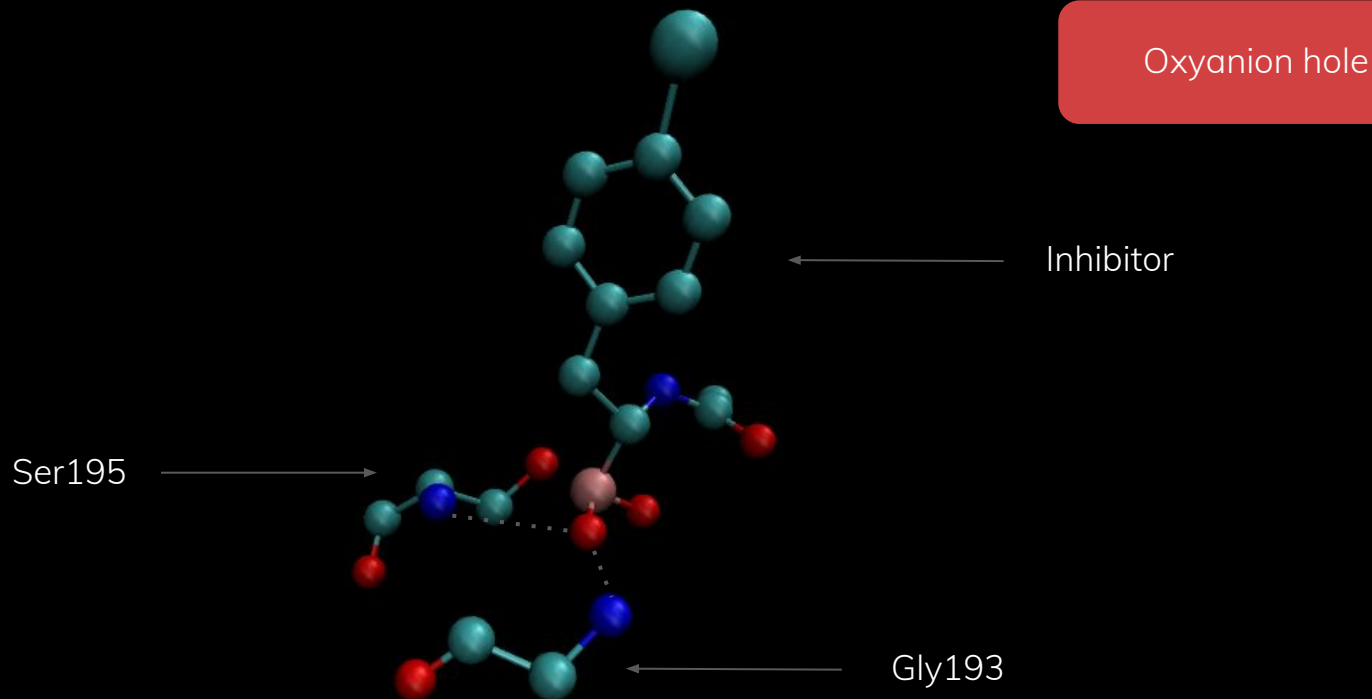


Transition state

As we saw before...

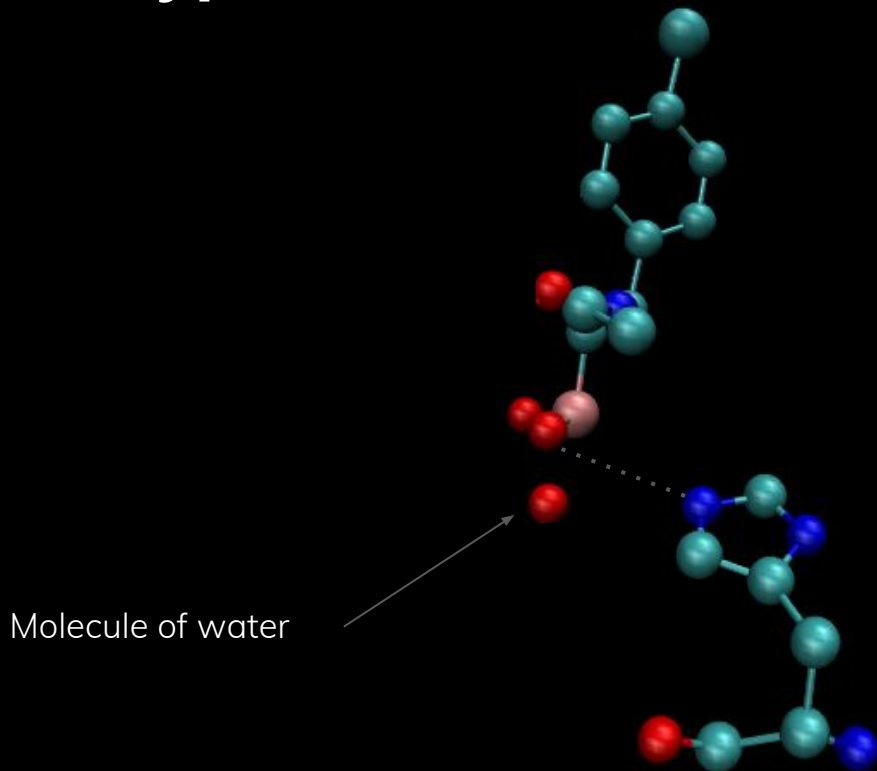


# Chymotrypsin: interaction with an inhibitor

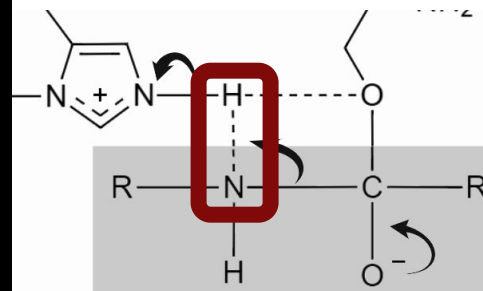


# Chymotrypsin: interaction with an inhibitor

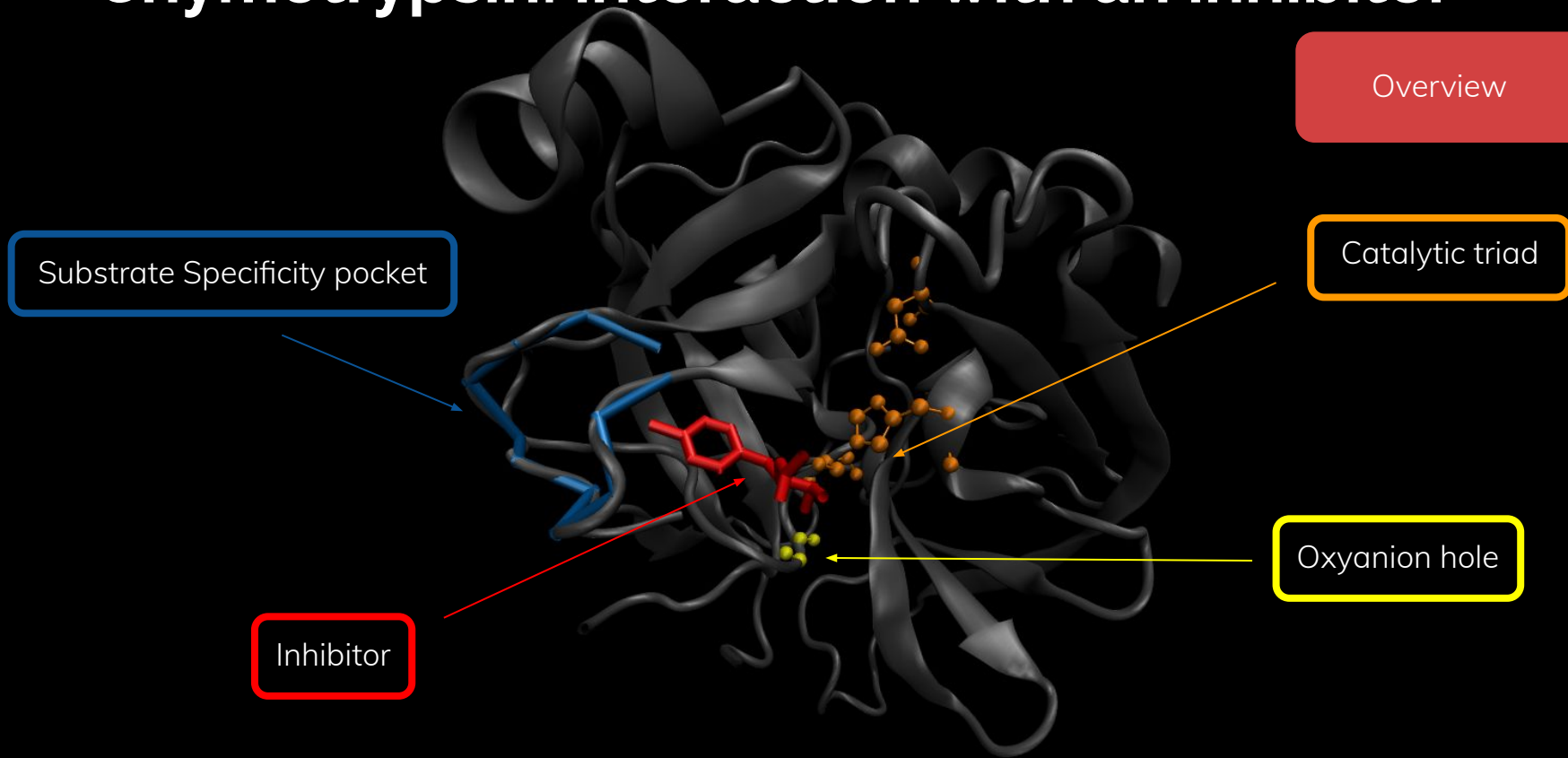
Catalytic mechanism



As we saw before...



# Chymotrypsin: interaction with an inhibitor



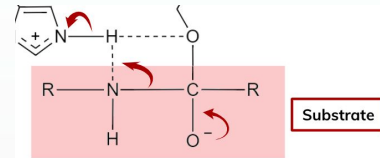
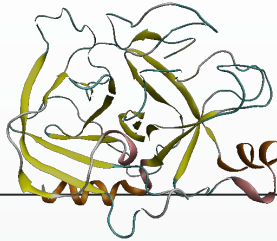


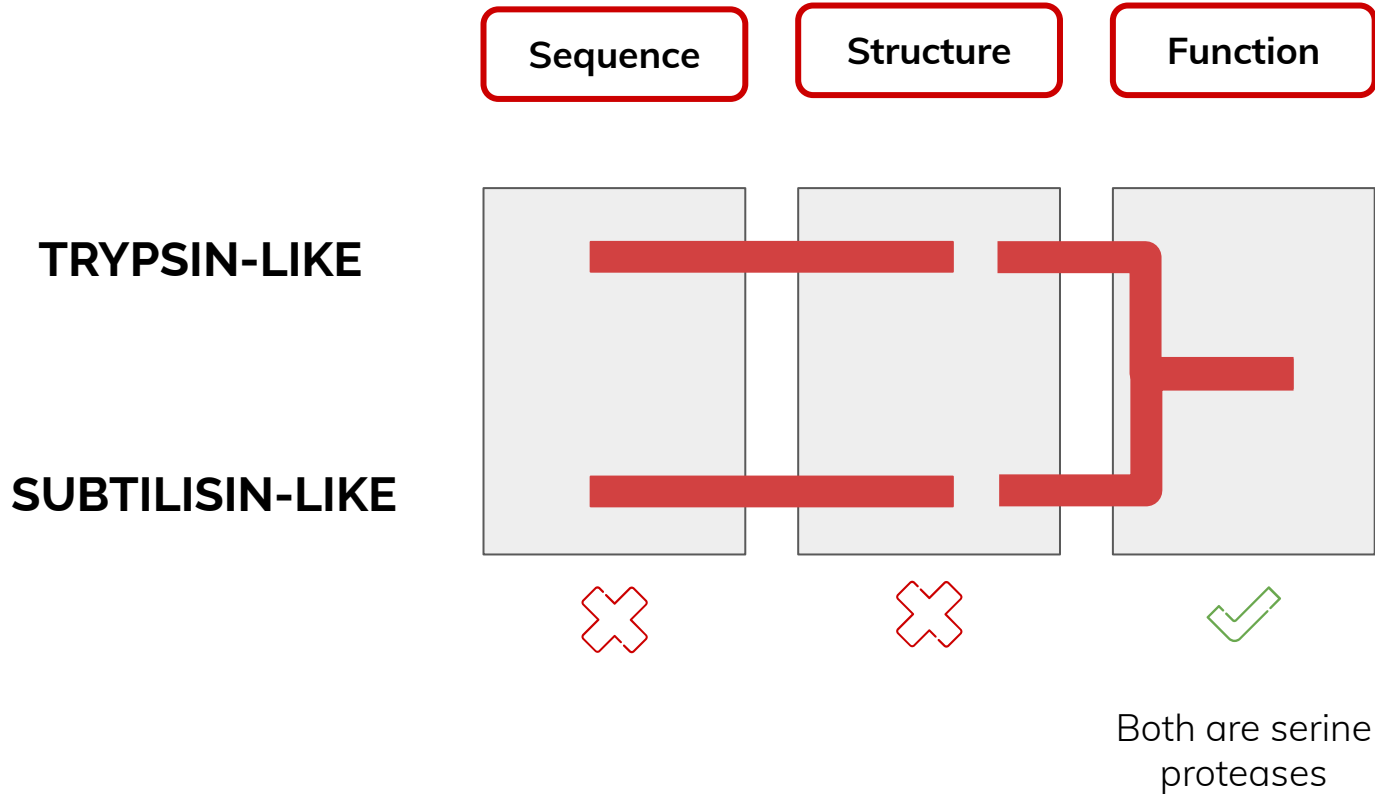
05

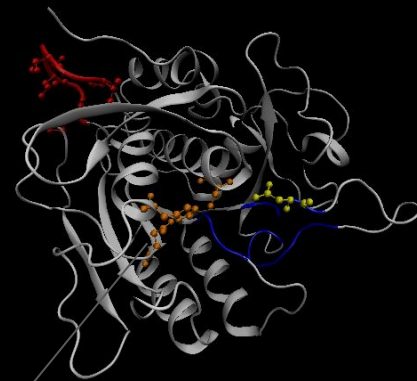
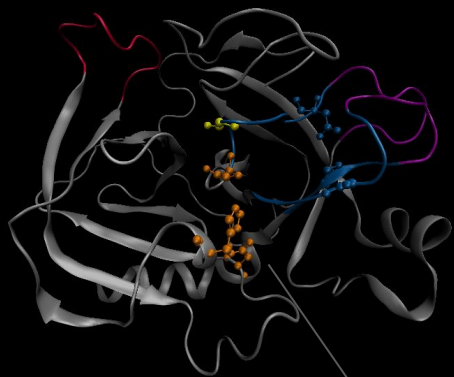
# CONCLUSIONS

## SEQUENCE - STRUCTURE - FUNCTION RELATIONSHIP

'TAYN- - -QG- TFT  
'SASDDFAAGTTCV  
'RAGTILANNSPCY  
'SKRQVVAAGTRCS  
'SKGDTSTIYTNCW  
'SGAARPSETTLCQ

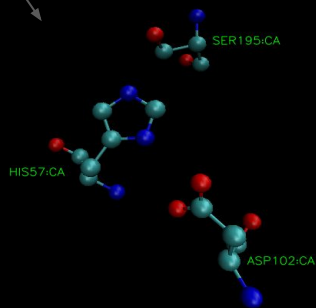




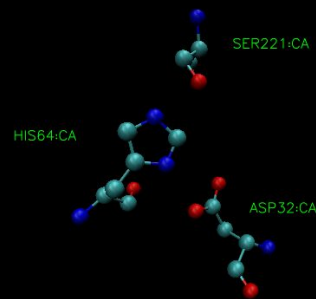


## Convergent evolution

TRYPSIN-LIKE



SUBTILISIN-LIKE



## Substrate Specificity

Sequence

Structure

Function

Trypsin

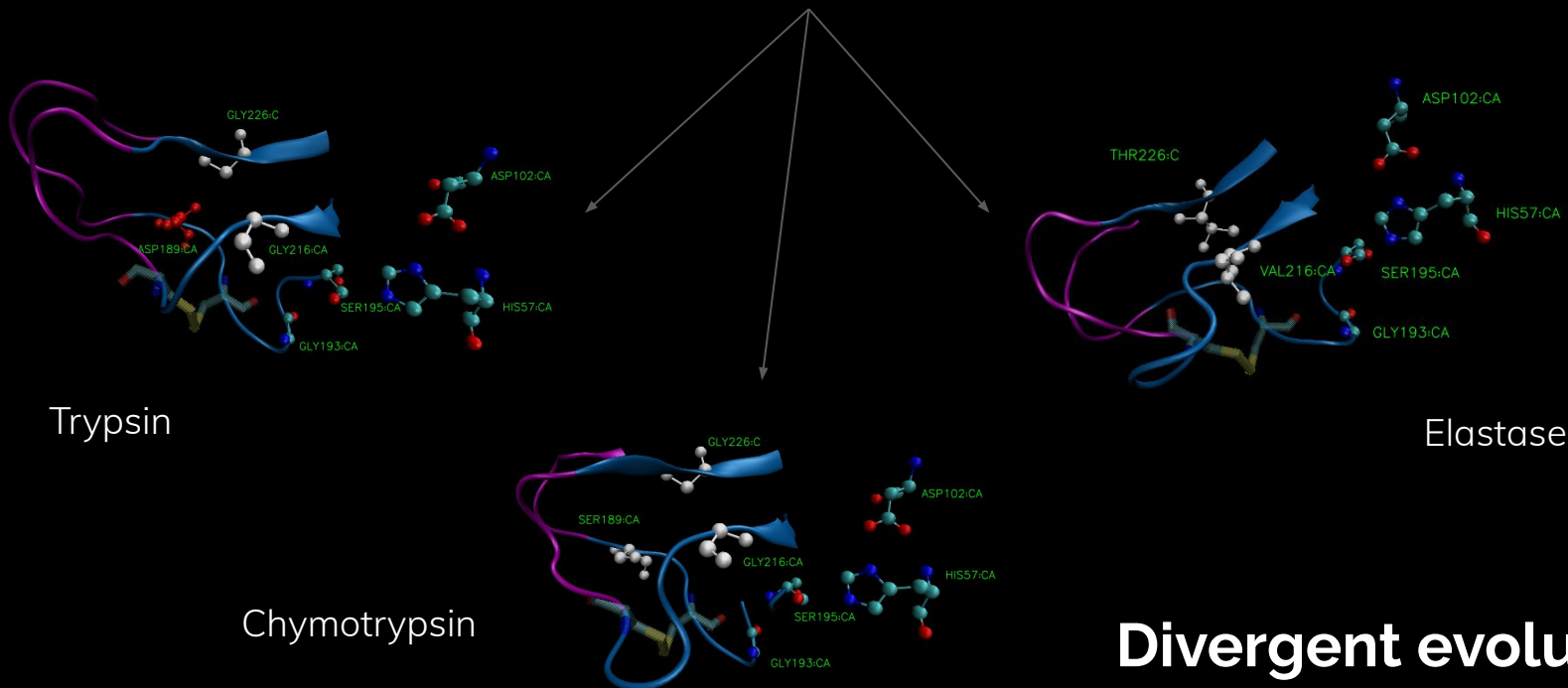
Chymotrypsin

Elastase

Divergence from a  
common ancestor

The diagram illustrates a phylogenetic tree where three enzymes—Trypsin, Chymotrypsin, and Elastase—diverge from a common ancestor. The tree is represented by red lines. A vertical line on the left indicates the common ancestor. Three horizontal lines branch out to the right, representing the divergence of each enzyme. These lines pass through three vertical grey boxes labeled 'Sequence', 'Structure', and 'Function'. Below each of these boxes is a red 'X' mark, indicating a point of divergence or analysis. A line from the text 'Divergence from a common ancestor' points to the branching point of the tree.

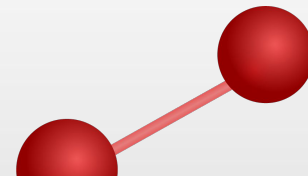
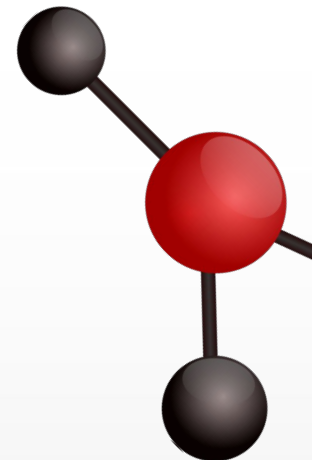
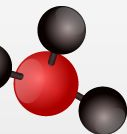
## Common ancestor



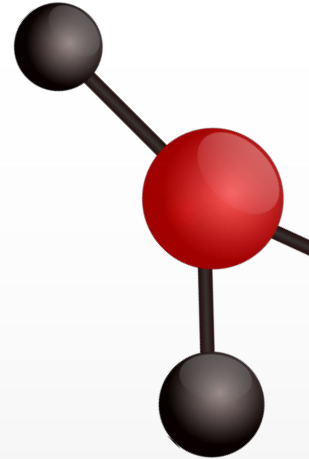
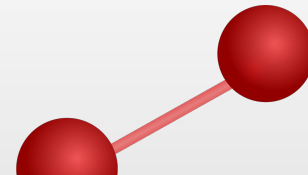
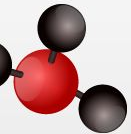
**Divergent evolution**  
(Diversification)

# 06 RESEARCH RESOURCES

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- Perona, John J., and Charles S. Craik. "Structural basis of substrate specificity in the serine proteases." Protein Science 4.3 (1995): 337-360.
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- Polgár, László. "The catalytic triad of serine peptidases." Cellular and molecular life sciences CMLS 62.19 (2005): 2161-2172.
- Pulido, Marian, et al. "Ca<sup>2+</sup>-dependent maturation of subtilisin from a hyperthermophilic archaeon, Thermococcus kodakaraensis: the propeptide is a potent inhibitor of the mature domain but is not required for its folding." Applied and environmental microbiology 72.6 (2006): 4154-4162.
- Stoll, Vincent S., et al. "Differences in binding modes of enantiomers of 1-acetamido boronic acid based protease inhibitors: crystal structures of  $\gamma$ -chymotrypsin and subtilisin Carlsberg complexes." Biochemistry 37.2 (1998): 451-462.
- Yang, Wenqian, Xingming Gao, and Binghe Wang. "Boronic acid compounds as potential pharmaceutical agents." Medicinal research reviews 23.3 (2003): 346-368.
- SCOP
- MEROPS



# PDBs

Approach	PDBs
<b>Chymotrypsin activation:</b> MSA and superimposition	1ab9 - Chymotrypsin (bovine) 1chg - Chymotrypsinogen (bovine)
<b>Sequence and structure analysis:</b> MSA by sequence trypsin-like proteins	1c1m - Elastase (porcine) 1sgt - Trypsin ( <i>S. griseus</i> ) 1spj - Kallikrein (human) 2any - Chymotrypsinogen A (human) 2zgc - Granzyme M (human) 4xde - Coagulation factor XII (human)
<b>Sequence and structure analysis:</b> MSA by sequence subtilisin-like proteins	1af4 - Subtilisin Carlsberg ( <i>B. licheniformis</i> ) 1ndq - Bacillus lentus subtilisin ( <i>L. lentus</i> ) 1sbt - Subtilisin NAT ( <i>B. amyloliquefaciens</i> ) 1sua - Subtilisin BPN ( <i>B. amyloliquefaciens</i> ) 6y5t - Subtilisin Savinase ( <i>L. lentus</i> )



# PDBs

Approach	PDBs
<b>Structure analysis:</b> trypsin-like folding	1sgt - Trypsin ( <i>S. griseus</i> ) 1ab9 - Chymotrypsin (bovine) 1c1m - Elastase (porcine)
<b>Structure analysis:</b> subtilisin-like folding	1sbt - Subtilisin NAT ( <i>B. amyloliquefaciens</i> )
<b>Interaction with inhibitor:</b> superimposition	1vgc - Chymotrypsin with inhibitor (bovine) 2gch - Chymotrypsin (bovine)

The slide features several decorative molecular models. In the top-left, a large water molecule is shown with a red oxygen atom and two black hydrogen atoms. In the top-right, a smaller water molecule is visible. On the right side, another large water molecule is shown. In the bottom-left, a small water molecule is present. At the bottom, two red spheres are connected by a red line, possibly representing a diatomic molecule or a specific bond type. The background is a light gray gradient.

**THANKS**

Does anyone have any question?

# PEM QUESTIONS

## 1. Which of the following statements about serine proteases classification according to MEROPS are true:

1. The words “clan” and “superfamily” can be used as synonyms.
2. The serine proteases are characterized by using a serine alcohol for their catalytic function.
3. S8 is a name of a family from the SB clan, which corresponds to serine proteases.
4. The MEROPS database distinguishes different types of proteases based on the structure of the proteases.

**a) 1, 2 and 3**

b) 1 and 3

c) 2 and 4

d) 4

e) 1, 2, 3 and 4

## 2. Which of the following statements are false?

- a) Serine proteases break peptide bonds thanks to the presence of a serine residue in the active site
- b) Serine proteases work by stabilizing the transition state which in turn brings down the activation energy.
- c) a) and b) are false.

**d) Serine proteases are only present in prokaryotes.**

e) All the above are false.

**3. Which of the followings statements about serine proteases is true:**

1. The catalytic triads in trypsin-like and subtilisin-like proteases are conformed by the same three residues (histidine, aspartic acid and serine), although they present differences in the amino acid sequence.
2. The oxyanion hole is a region implicated in the stabilisation of the tetrahedral intermediate.
3. The negatively charged oxygen atom from the scissile bond forms two hydrogen bonds with the amides of the two residues conforming the oxyanion hole.
4. Differences in the amino acid conservation of the catalytic triad between trypsin-like and subtilise-like are an example of homology.

- a) 1, 2 and 3**  
b) 1 and, 3  
c) 2 and 4  
d) 4  
e) 1, 2, 3 and 4

**4. Which of these statements about the activation of serine proteases is false:**

- a) All trypsin-like proteases are synthesized as proteases.  
**b) Chymotrypsin activates proelastase.**  
c) The mechanism is preserved among the mammalian trypsin-like proteases.  
d) A formation of a salt bridge is required.  
e) All the above are false.

5. Which of the following statements about the activation of serine proteases is true:

- a) Glycine 193 is not part of the oxyanion hole of chymotrypsin.
- b) Elastase is not secreted as a zymogen.
- c)  $\alpha$ -Chymotrypsin is inactive.
- d) The processing of trypsinogen into trypsin changes the conformation of the oxyanion hole.**
- e) All the above are true.

6. Which of the following statements about the trypsin-like folding is true:

- 1. Trypsin-like serine proteases are all-alpha proteins.
  - 2. Trypsin-like serine proteases do NOT have beta barrels.
  - 3. Trypsin-like serine proteases only have one domain.
  - 4. The catalytic triad in trypsin-like proteases is located between the two domains that these proteins have.
- 
- a) 1, 2 and 3
  - b) 1 and, 3
  - c) 2 and 4
  - d) 4**
  - e) 1, 2, 3 and 4

**7. Which option is true:**

- a) Disulfide bonds are important to maintain the structure of trypsin-like serine proteases, for this reason there are some conserved disulfide bonds.
- b) Some residues into the S1 pocket are different in chymotrypsin and trypsin and it confers specificity of substrate to each serine protease.
- c) There is a conserved disulfide bond that contributes to the structure of the S1 pocket
- d) S1 pocket is located near the catalytic triad.
- e) **All of them are true.**

**8. Which of the following statements about the subtilisin-like proteins is true:**

- 1. Subtilisins are considered alpha/beta/alpha proteins.
  - 2. The amino acids that form the oxyanion hole in trypsin-like proteins and subtilisin-like proteins are the same (Gly193 and Ser195).
  - 3. Subtilisin-like proteins and trypsin-like proteins have a calcium-binding loop.
  - 4. Subtilisins are classified as glutamic proteases.
- 
- a) 1, 2 and 3
  - b) 1 and, 3
  - c) 2 and 4
  - d) **4**
  - e) 1, 2, 3 and 4

9. According to boronic acids and serine proteases complexes, choose which of the followings statements is false:

- a) **The complex of a chymotrypsin with a boronic acid showed big changes in the structure conformation of the oxyanion hole.**
- b) Boronic acids can form tetrahedral boronate complexes.
- c) Boronic acids are transition-state analogues of serine proteases.
- d) One of the hydroxyl groups of the boron interacts with the oxyanion hole, while the other is involved in the formation of the transition state conformation.
- e) None all the above.

10. A superimposition between different serine-proteases from the same family and different species:

- a) Will have a score lower than 5.5 and a RMSD value higher than 2
- b) Will have a score between 5.5 and 9.8 and a RMSD value higher than 2
- c) **Will have a score between 5.5 and 9.8 and a RMSD value lower than 2**
- d) Will have a score higher than 9.8 and a RMSD value lower than 2
- e) Will have a score lower than 5.5 and a RMSD value higher than 9.8