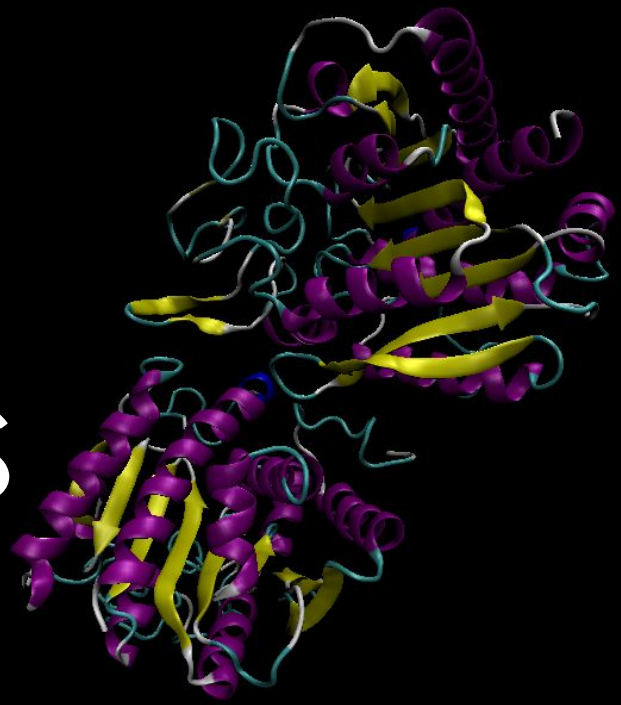
CARBOXYPEPTIDASES



Esther Colomé
Kristin Fichna
Carla Mateo
Maria Padial

upf.

INDEX

PEPTIDASES

- Proteolytic enzymes
- Broad spectrum of functions
- Medicine
- Biological processes

Dual classification depending on the cleavage they perform:

Endopeptidases

Exopeptidases →

Carboxypeptidases

Residue that is essential for catalysis:

- ➔ Serine proteases
- ➔ Cysteine proteases
- ➔ Aspartic acid proteases
- ➔ Threonine proteases
- ➔ Glutamic acid proteases
- ➔ Metalloproteases

CARBOXYPEPTIDASES CLASSIFICATION

By active site mechanism

- Metallocarboxypeptidases → Enzymes that use a metal in the active site
- Serine carboxypeptidases → Enzymes that use a serine in the active site
- Cysteine carboxypeptidase → Enzymes that use a cysteine in the active site

By substrate preference

- Carboxypeptidases A → Aromatic/aliphatic residues
- Carboxypeptidases B → Basic residues

MEROPS CLASSIFICATION

Clan	Family	Subfamily	Subfamily type peptidase
MC	M14	M14A	Carboxypeptidase A
		M14B	Carboxypeptidase E
		M14C	Gamma-D-glutamyl-(L)-meso-diaminopimelate peptidase I
		M14D	Cytosolic carboxypeptidase 6

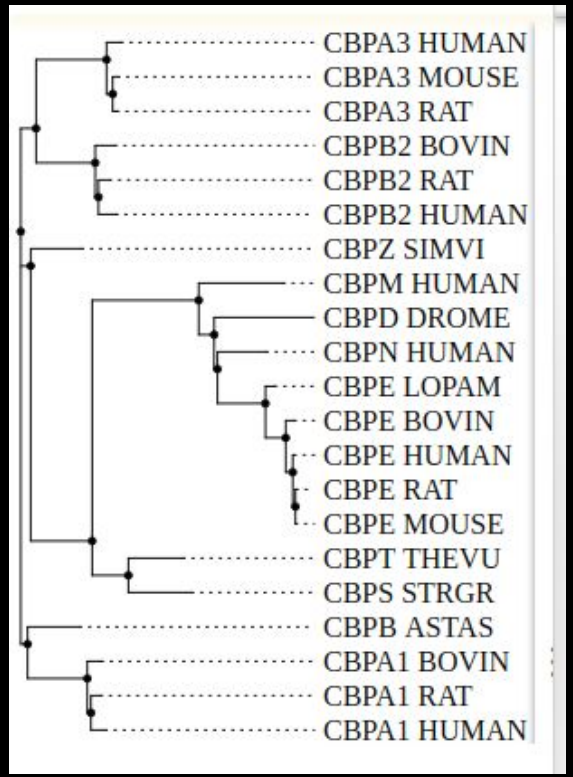
https://www.ebi.ac.uk/merops/cgi-bin/famsum?family=M14

SCOP CLASSIFICATION

Class	Fold	Superfamily	Family
Alpha and beta proteins (a/b)	Phosphorylase /hydrolase-like	Zn-dependent exopeptidases	Pancreatic carboxypeptidases
			Carboxypeptidase T
			Leucine aminopeptidase, C-terminal domain
			Bacterial dinuclear zin exopeptidases
			FolH catalytic domain-like
			N-acetylmuramoyl-L-alanine amidase-like
			AstE/AspA-like
			Glutaminyl-peptide cyclotransferase-like
			FGase-like

http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.d.hi.f.html

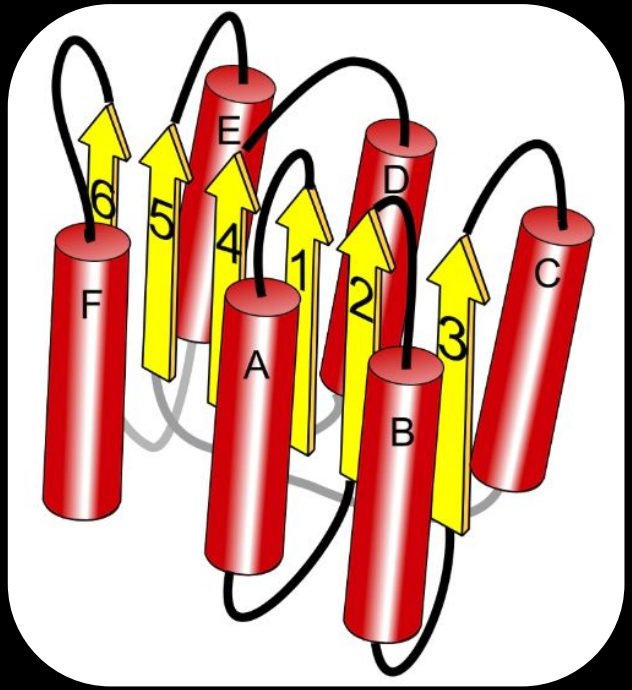
CARBOXYPEPTIDASES EVOLUTION



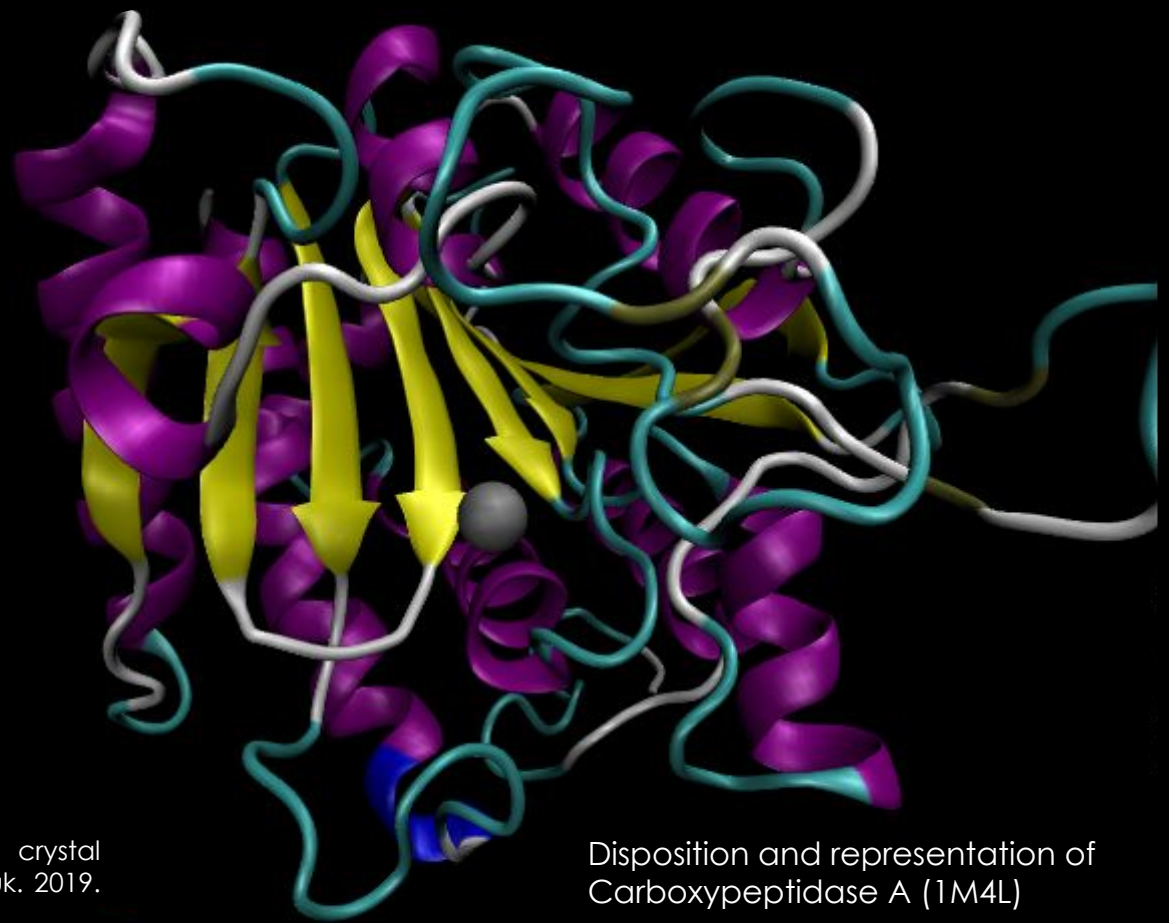
ConSurf Server

- Phylogenetic tree
- Carboxypeptidases A and B
- Mast cells
- Regulatory Carboxypeptidases

Scaffold

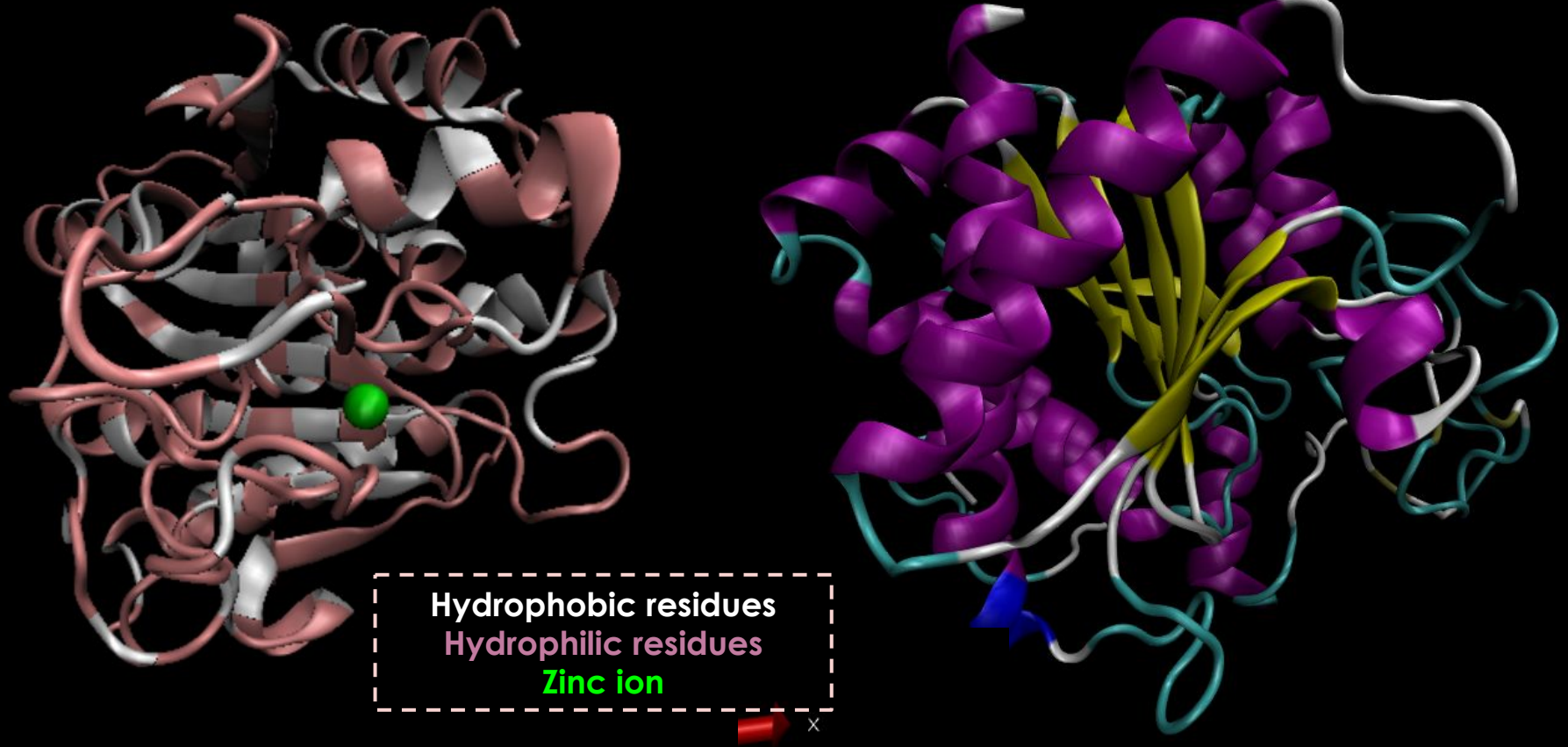


Europe P. Phaser - a "stunning" method for solving crystal structures ‹ Quips ‹ PDBe ‹ EMBL-EBI [Internet]. Ebi.ac.uk. 2019. Available from: http://www.ebi.ac.uk/pdbe/quips?story=Phaser



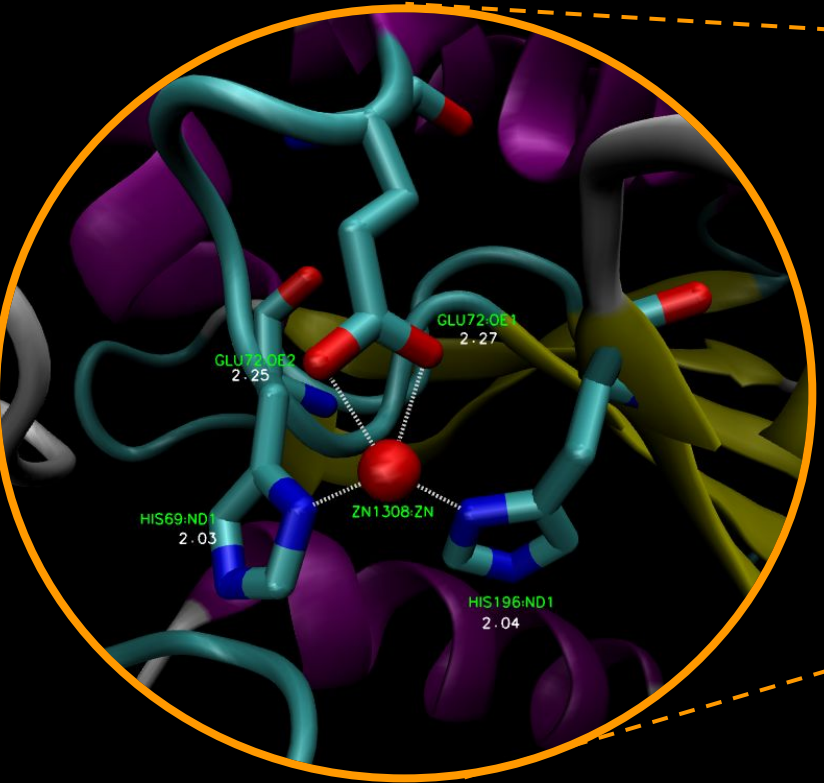
Disposition and representation of Carboxypeptidase A (1M4L)

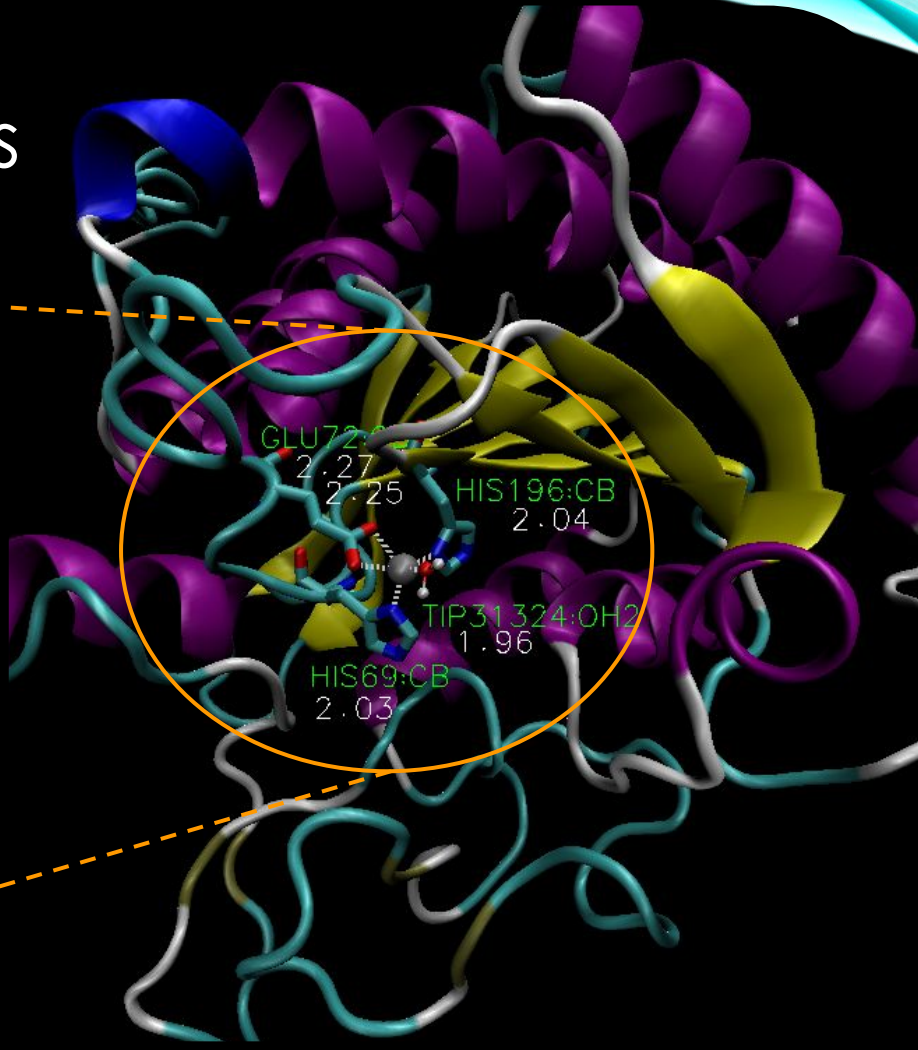
Bovine Carboxypeptidase A



CATALYTIC SITE

Zn coordination residues

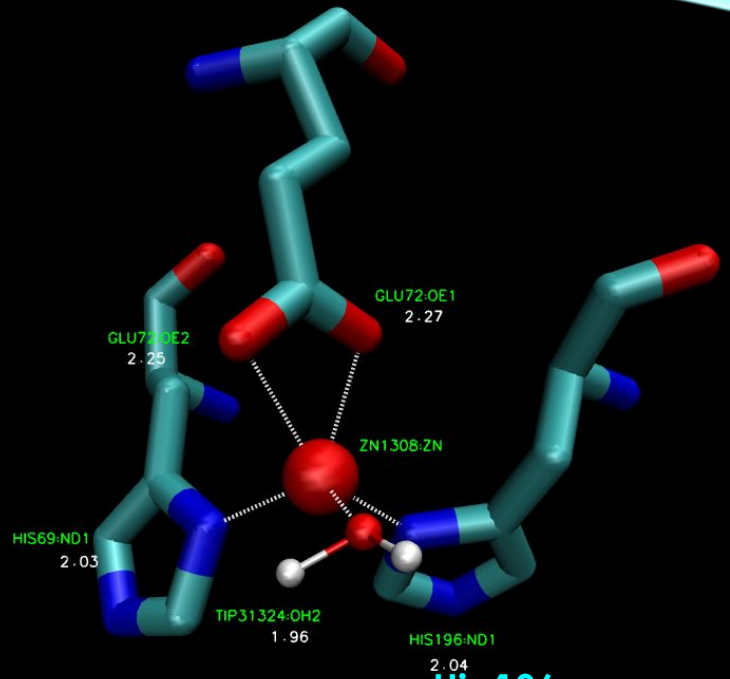




Zn coordination

His 60 Glu 72

```
CBPA2_HUMAN     HAREWVTQATALWTANKIVSDYGKDPS
CBPA1_RAT       HSREWVTQASGVWFAKKITKDYGQDPT
CBPA1_MOUSE     HSREWVTQASGVWFAKKITKDYGQEPT
CBPA1_BOVIN     HSREWITQATGVWFAKKFTEDYGQDPS
CBPA1_HUMAN     HSREWVTQASGVWFAKKITQDYGQDAA
CBPA1_PIG       HSREWVTQASGVWFAKKITEDYGQDPA
                *.***:***:.:* *:*:..***:: :
```

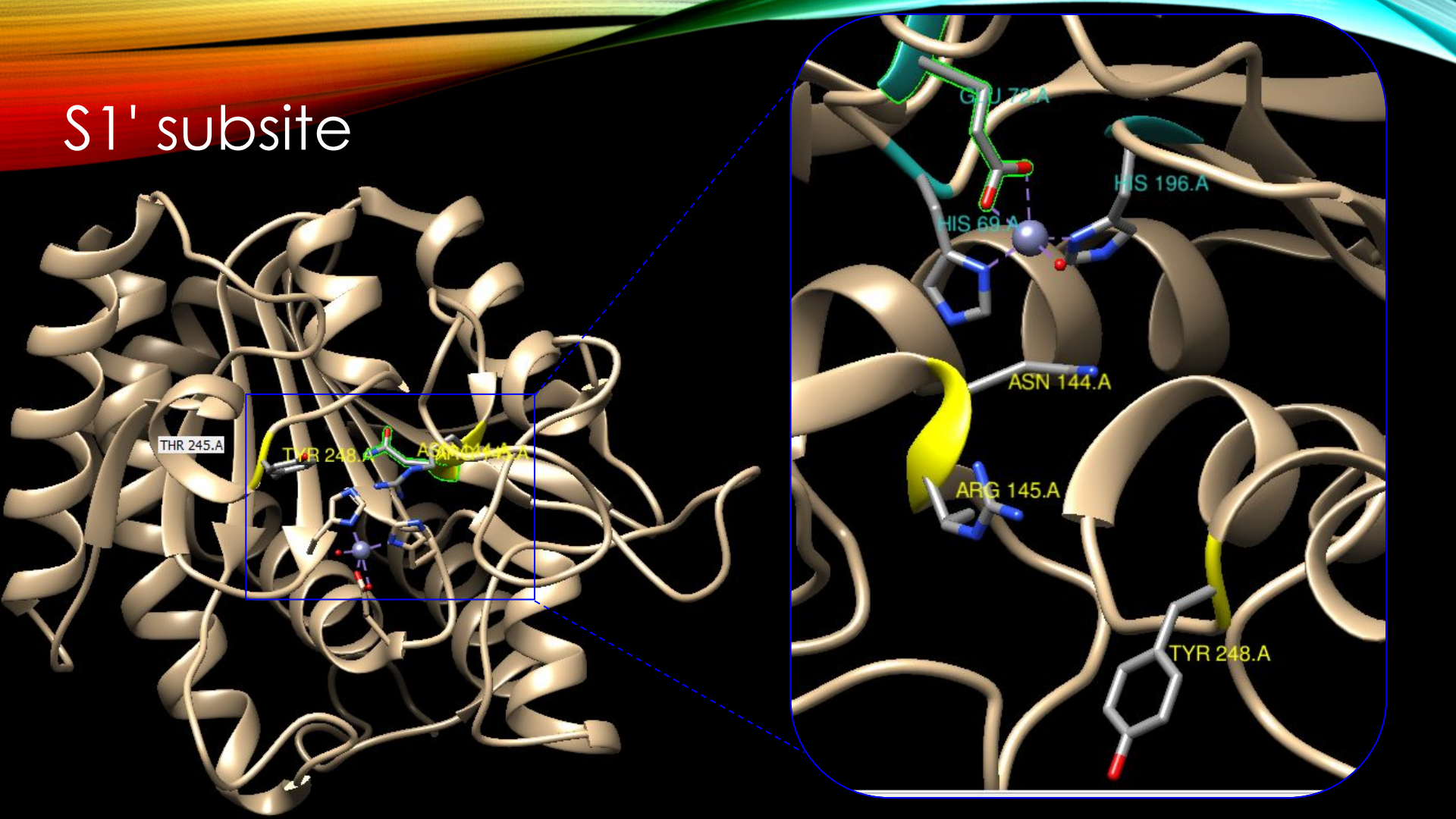


His 196

```
CBPA1_RAT       TRSHTQGSLCVGVDPNRNWDAGFGMAGASSNPCSETYRGKFPNSEVEVKSIVDFVTSHGN
CBPA1_MOUSE     TRSHTEGSLCVGVDPNRNWDAAFGMPGASSNPCSETYRGKFPNSEVEVKSIVDFVTSHGN
CBPA1_HUMAN     TRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVKDHGN
CBPA1_PIG       TRSRTSGSFCVGVDPNRNWDAGFGGAGASSNPCSETYHGKFPNSEVEVKSIVDFVNDHGN
CBPA1_BOVIN     TRSVTSSSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKSIVDFVKDHGN
CBPA2_HUMAN     TRSKVSGSLCVGVDPNRNWDAGFGGPGASSNPCSDSYHGPSANSEVEVKSIVDFIKSHGK
                ***    *.*.*** ****** **  **** ***.*.*.   ***********. **.
```

Residues His 69,His 196 and Glu 72 are conserved among species.

S1' subsite



S1' subsite

Asn 144-Arg 145

```
CBPA1_RAT       TRSHTQGSLCVGVDPNRNWDAGFGMAGASSNP
CBPA1_MOUSE     TRSHTEGSLCVGVDPNRNWDAAFGMPGASSNP
CBPA1_HUMAN     TRSHTAGSLCIGVDPNRNWDAGFGLSGASSNP
CBPA1_PIG       TRSRTSGSFCVGVDPNRNWDAGFGGAGASSNP
CBPA1_BOVIN     TRSVTSSSLCVGVDANRNWDAGFGKAGASSSP
CBPA2_HUMAN     TRSKVSGSLCVGVDPNRNWDAGFGGPGASSNP
                *** . .*:*:***.******.** .****.*
```

Tyr 248

```
LLMFPYGYKCTKLDDFDELSEVAQKAAQSLRSLHGTKYKVGPICSVIY
LLLYPYGYTSEPAPDQAELDQLAKSAVTALTSLHGTKFKYGSIIDTIY
LLLYPYGYTSEPAPDKEELDQLAKSAVTALTSLHGTKFKYGSIIDTIY
LLLYPYGYTTQSIPDKTELNQVAKSAVEALKSLYGTSYKYGSIITTIY
LLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIY
LLLYPYGYKTEAPADKDELDQISKSAVAALTSLYGTKFQYGSIITTIY
**::****.     *  **.:::: *. :* **:**.:: * *  .**
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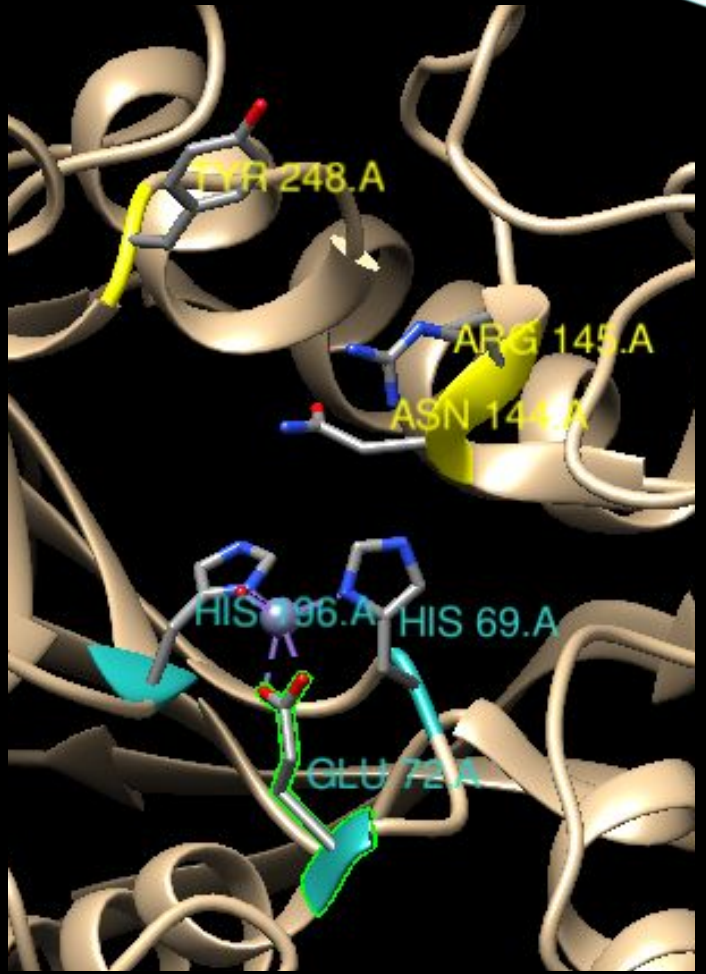
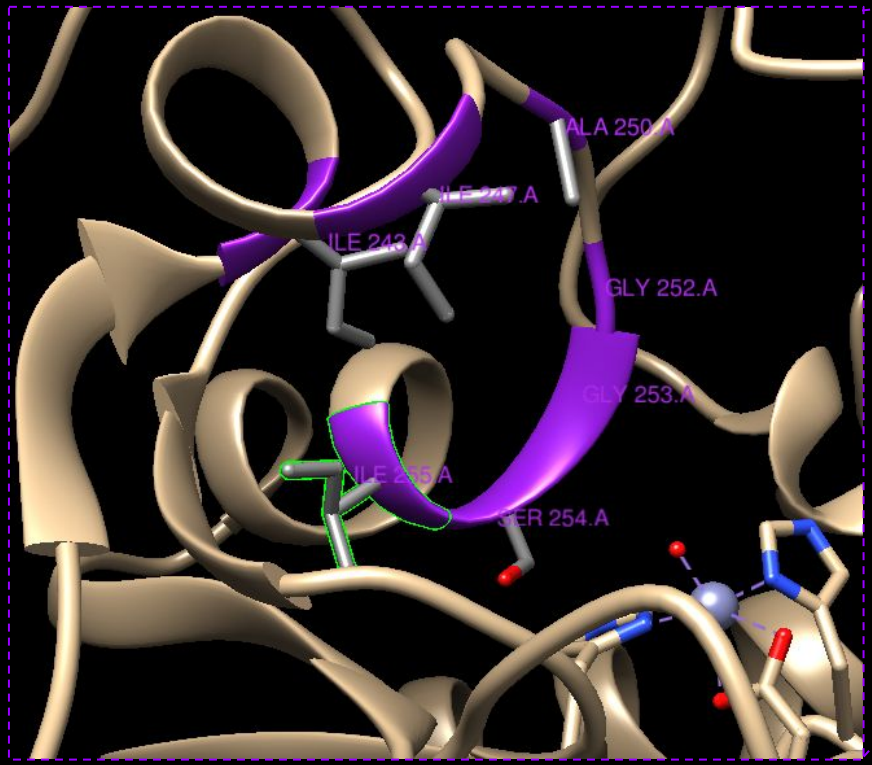
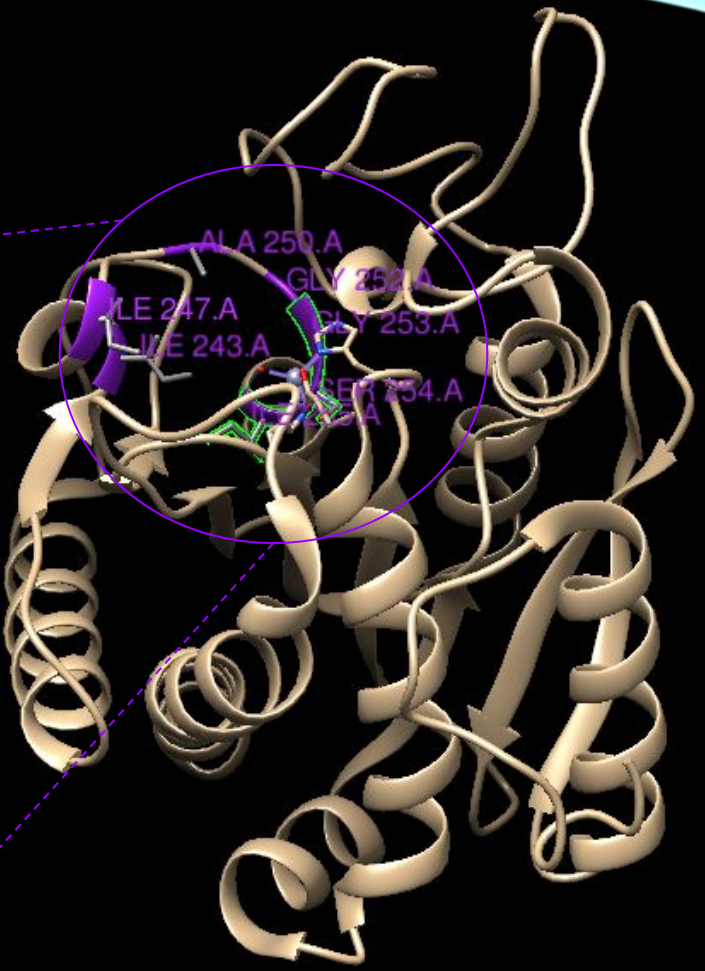
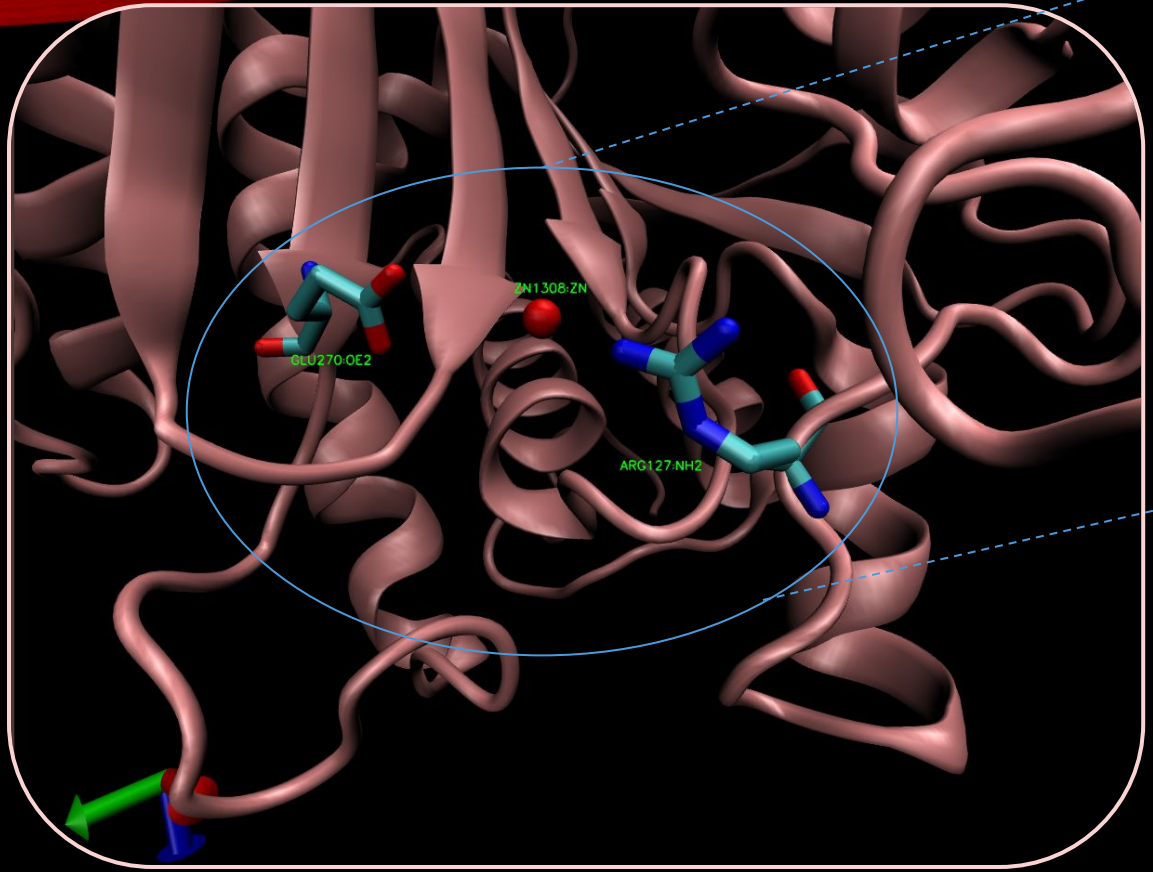


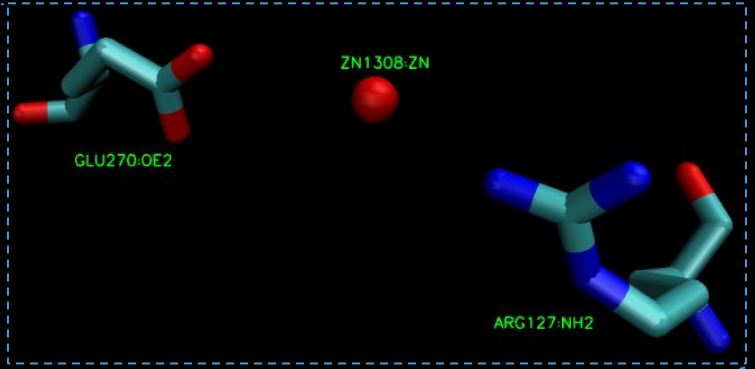
Hydrophobic Pocket





S1 subsite


ZN1308:ZN
GLU270:OE2
ARG127:NH2


ZN1308:ZN
GLU270:OE2
ARG127:NH2

S1 Subsite

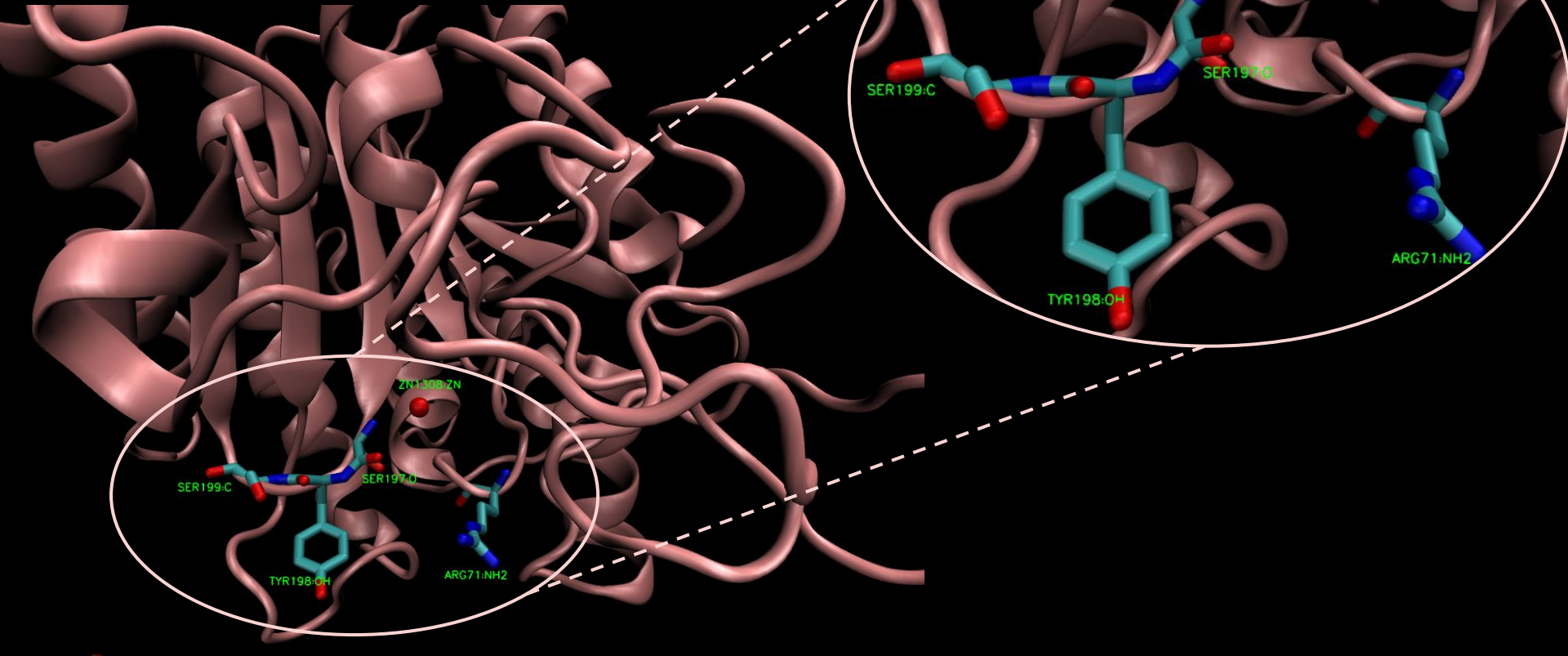
Arg 127

```
CBPA2_HUMAN      HAREWVTQATALWTANKIVSDYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRK
CBPA1_RAT        HSREWVTQASGVWFAKKITKDYGQDPTFTAVLDNMDIFLEIVTNPDGFAYTHKTNRMWRK
CBPA1_MOUSE      HSREWVTQASGVWFAKKITKDYGQEPTLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRK
CBPA1_BOVIN      HSREWITQATGVWFAKKFTEDYGQDPSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRK
CBPA1_HUMAN      HSREWVTQASGVWFAKKITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRK
CBPA1_PIG        HSREWVTQASGVWFAKKITEDYGQDPAFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRK
                 *:***:***:.:* *:*:..***:: ::*::** :****  ******:.:::. **:***
```

Glu 270

```
CBPA2_HUMAN      QASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPARQILPTAEETWLGLKAIMEHVRDHP
CBPA1_RAT        QASGSTIDWTYSQGIKYSFTFELRDTGLRGFLLPASQIIPTAEETWLALLTIMDHTVKHP
CBPA1_MOUSE      QASGSTIDWTYSQGIKYSFTFELRDTGLRGFLLPASQIIPTAEETWLALLTIMDHTVKHP
CBPA1_BOVIN      QASGGSIDWSYNQGIKYSFTFELRDTGRYGFLLPASQIIPTAQETWLGVLTIMEHTLNNL
CBPA1_HUMAN      QASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPASQIIPTAKETWLALLTIMEHTLNHP
CBPA1_PIG        QASGGTIDWTYNQGIKYSFSFELRDTGRYGFLLPASQIIPTAQETWLALLTIMEHTLNHP
                 ****.:***:*. ******:******  ****** **:***:****.: :**:*. .:
```

S2 Subsite


ZN1308:ZN
SER199:C
SER197:O
TYR198:OH
ARG71:NH2
ZN1308:ZN
SER199:C
SER197:O
TYR198:OH
ARG71:NH2

S2 Subsite

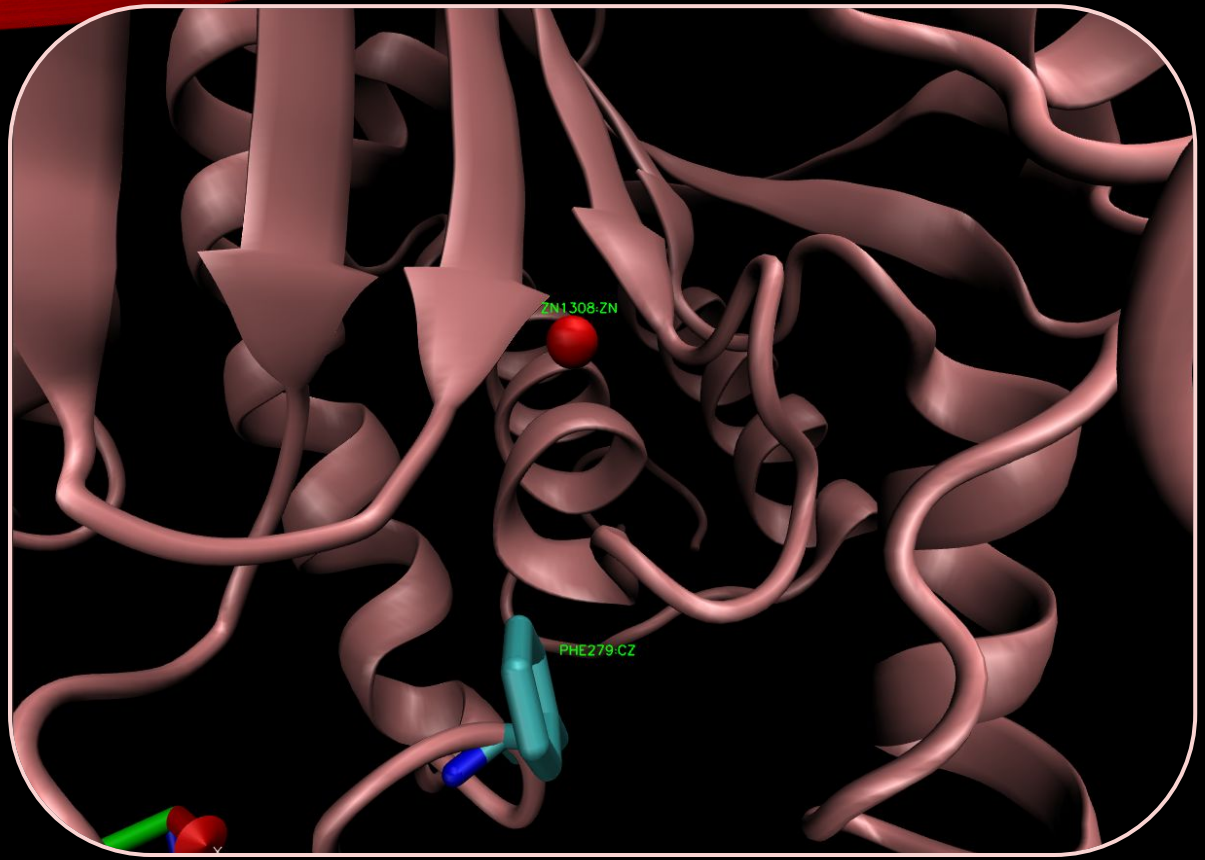
Arg 71

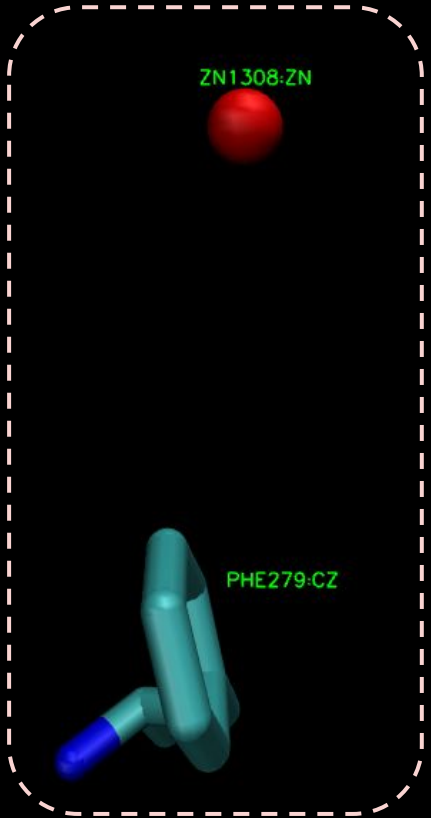
```
CBPA2_HUMAN     HAREWVTQATALWTANKIVSDYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRK
CBPA1_RAT       HSREWVTQASGVWFAKKITKDYGQDPTFTAVLDNMDIFLEIVTNPDGFAYTHKTNRMWRK
CBPA1_MOUSE     HSREWVTQASGVWFAKKITKDYGQEPTLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRK
CBPA1_BOVIN     HSREWITQATGVWFAKKFTEDYGQDPSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRK
CBPA1_HUMAN     HSREWVTQASGVWFAKKITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRK
CBPA1_PIG       HSREWVTQASGVWFAKKITEDYGQDPAFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRK
                *.***.***.:* *:*:.***:: ::*::** :****  ******.:::. **.***
```

Ser 197/Tyr 198/Ser 199

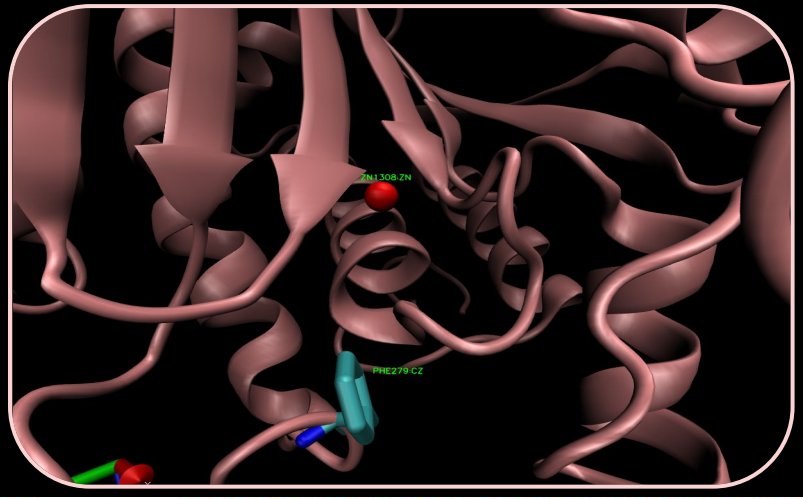
```
CBPA2_HUMAN     VKAFITLHSYSQLLMFPYGYKCTKLDDFDELSEVAQKAAQSLRSLHGTKYKVGPICSVIY
CBPA1_RAT       IKAFISIHSYSQLLLYPYGYTSEPAPDQAELDQLAKSAVTALTSLHGTKFKYGSIIDTIY
CBPA1_MOUSE     IKAFISIHSYSQLLLYPYGYTSEPAPDKEELDQLAKSAVTALTSLHGTKFKYGSIIDTIY
CBPA1_BOVIN     FKAFLSIHSYSQLLLYPYGYTTQSIPDKTELNQVAKSAVEALKSLYGTSYKYGSIITTIY
CBPA1_HUMAN     IKAFISIHSYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIY
CBPA1_PIG       IKAFISIHSYSQLLLYPYGYKTEAPADKDELDQISKSAVAALTSLYGTKFQYGSIITTIY
                .***:::*******:****.     *  **.:::: *. :* **:**.:: * *  .**
```

S3 subsite





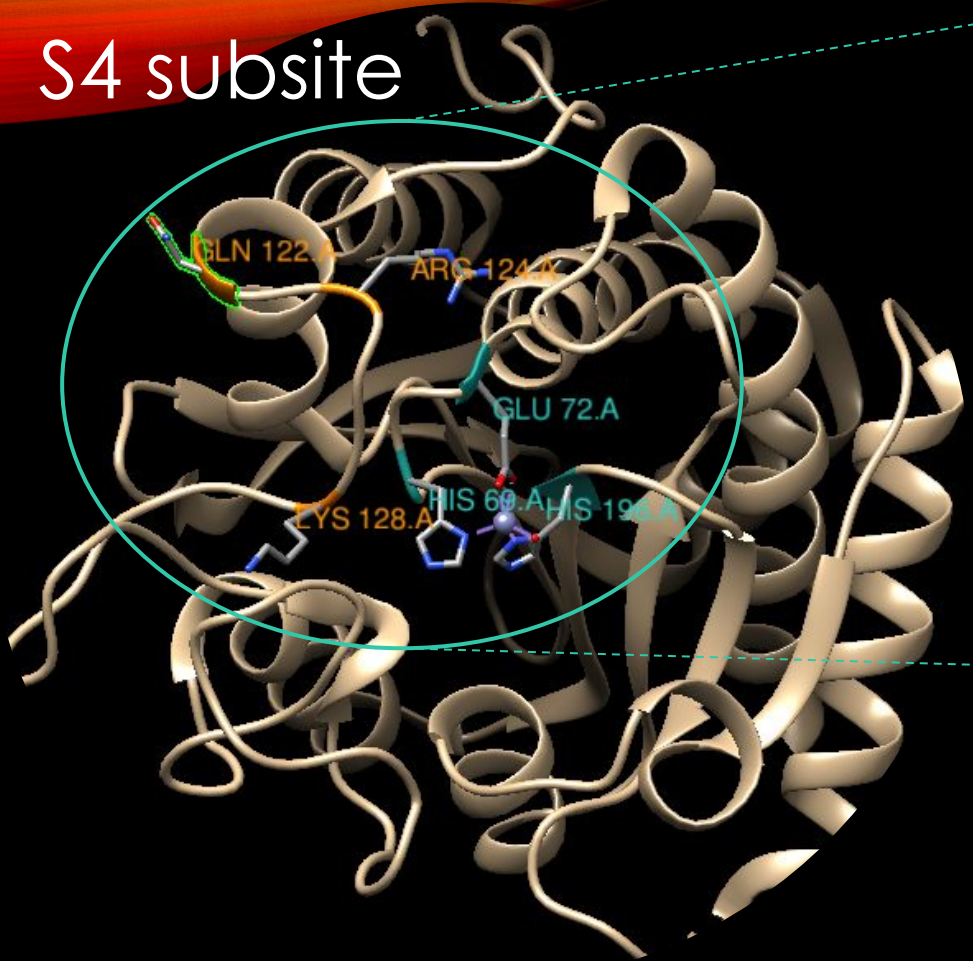
S3 subsite conservation

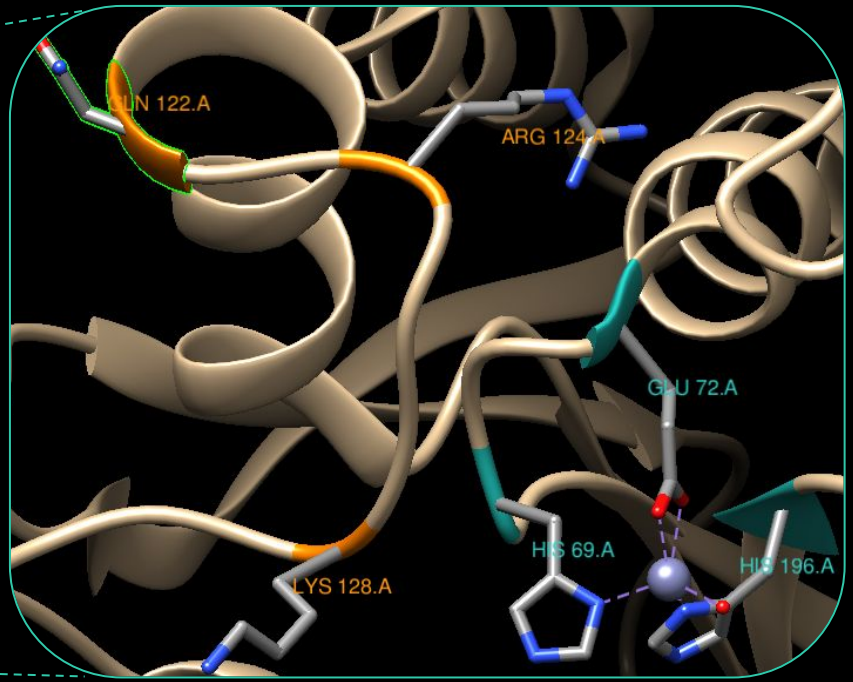


Phe 279

```
CBPA2_HUMAN      QASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPARQILPTAEETWLGLKAIMEHVRDHP
CBPA1_RAT        QASGSTIDWTYSQGIKYSFTFELRDTGLRGFLLPASQIIPTAEETWLALLTIMDHTVKHP
CBPA1_MOUSE      QASGSTIDWTYSQGIKYSFTFELRDTGLRGFLLPASQIIPTAEETWLALLTIMDHTVKHP
CBPA1_BOVIN      QASGGSIDWSYNQGIKYSFTFELRDTGRYGFLLPASQIIPTAQETWLGVLTIMEHTLNNL
CBPA1_HUMAN      QASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPASQIIPTAKETWLALLTIMEHTLNHP
CBPA1_PIG        QASGGTIDWTYNQGIKYSFSFELRDTGRYGFLLPASQIIPTAQETWLALLTIMEHTLNHP
                 ****.:***:*. ******:******* ****** **:***:****.: :**:*. .:
```

S4 subsite

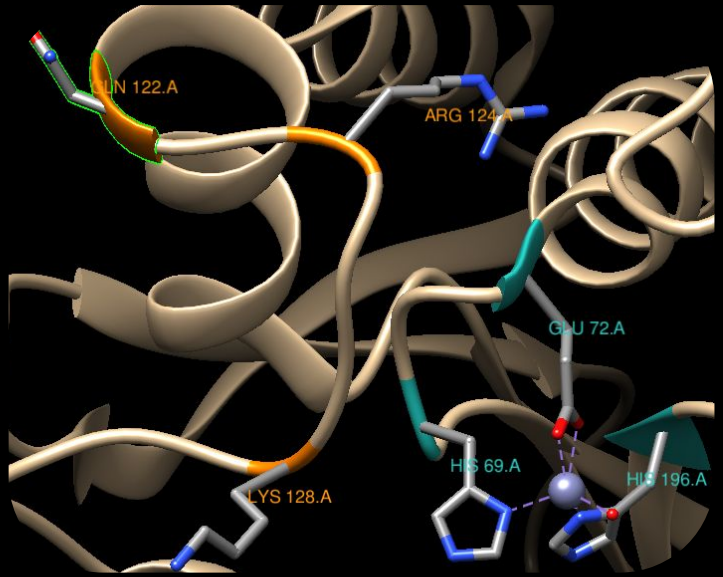

GLN 122.A
ARG 124.A
GLU 72.A
LYS 128.A
HIS 69.A
HIS 196.A


GLN 122.A
ARG 124.A
GLU 72.A
LYS 128.A
HIS 69.A
HIS 196.A

S4 subsite alignment

Residue 122 is variable between species and between carboxypeptidases.

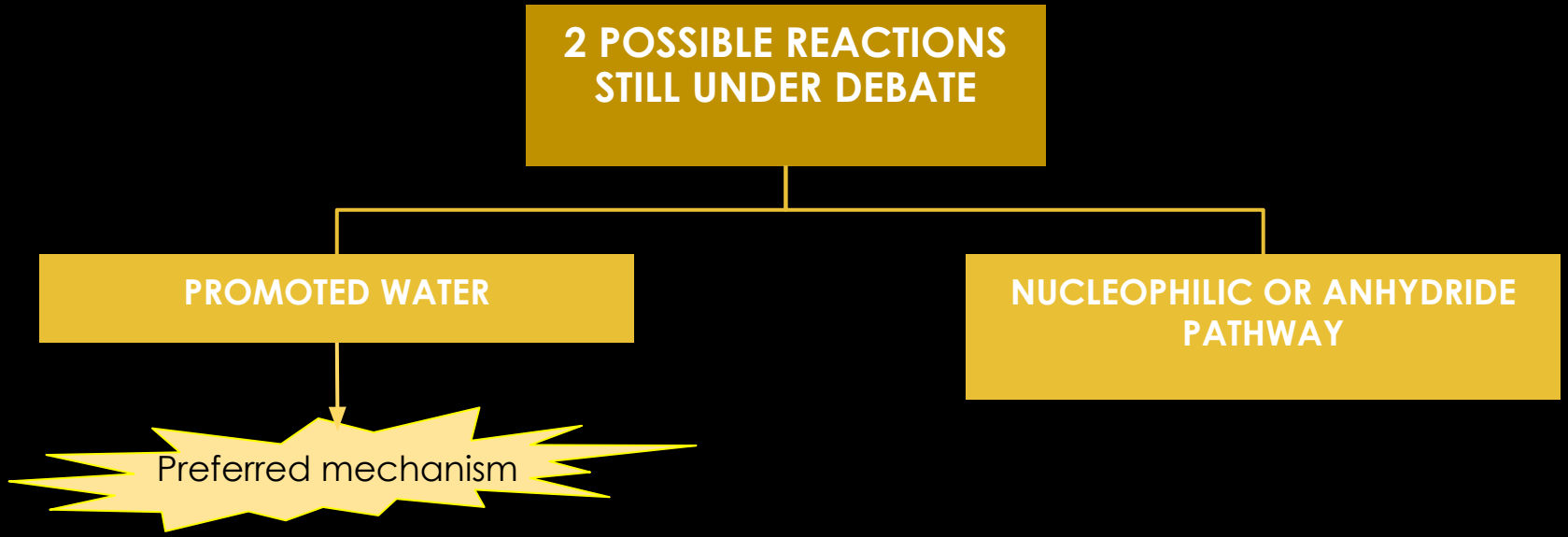
Despite this, the amino acid in this position conserves the polarity feature.



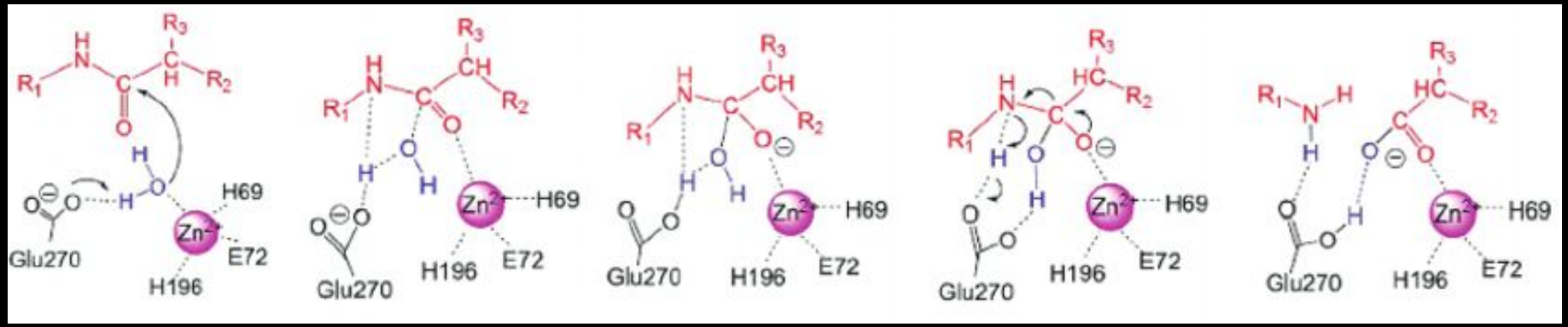
Gln 122 Arg 124 Lys 128

```
CBPA2_HUMAN     HAREWVTQATALWTANKIVSDYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRK
CBPA1_RAT       HSREWVTQASGVWFAKKITKDYGQDPTFTAVLDNMDIFLEIVTNPDGFAYTHKTNRMWRK
CBPA1_MOUSE     HSREWVTQASGVWFAKKITKDYGQEPTLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRK
CBPA1_BOVIN     HSREWITQATGVWFAKKFTEDYGQDPSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRK
CBPA1_HUMAN     HSREWVTQASGVWFAKKITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRK
CBPA1_PIG       HSREWVTQASGVWFAKKITEDYGQDPAFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRK
                *:***:***:.:* *:*:..***:: ::*:** :**** ******:::::. **:***
```

Catalytic reaction



Promoted Water (general base-general acid)

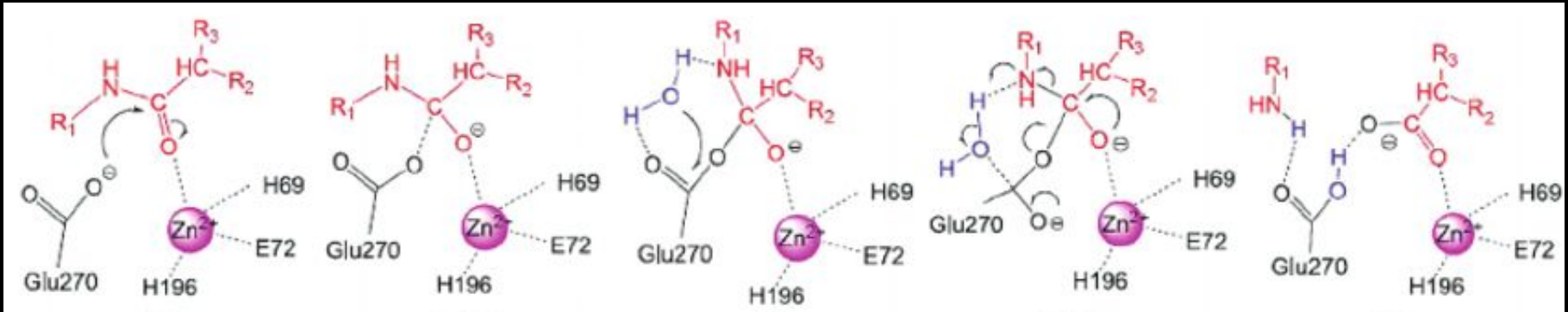


Wu S, Zhang C, Xu D, Guo H. Catalysis of Carboxypeptidase A: Promoted-Water versus Nucleophilic Pathways. The Journal of Physical Chemistry B. 2010;114(28):9259-9267.

STEP 1 Nucleophilic addition: Glu 270 acts as a general base facilitating the attack of the zinc-bound water at the scissile carbonyl carbon by transferring a water H^+ to a carboxylate oxygen

STEP 2 Elimination: Glu 270 acts as a general acid where gives the H^+ to the leaving nitrogen group

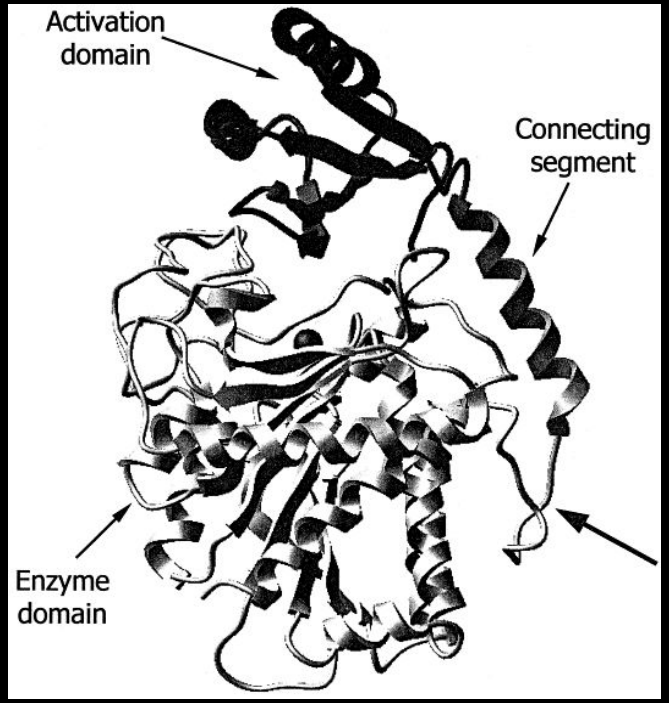
Anhydride or nucleophilic mechanism



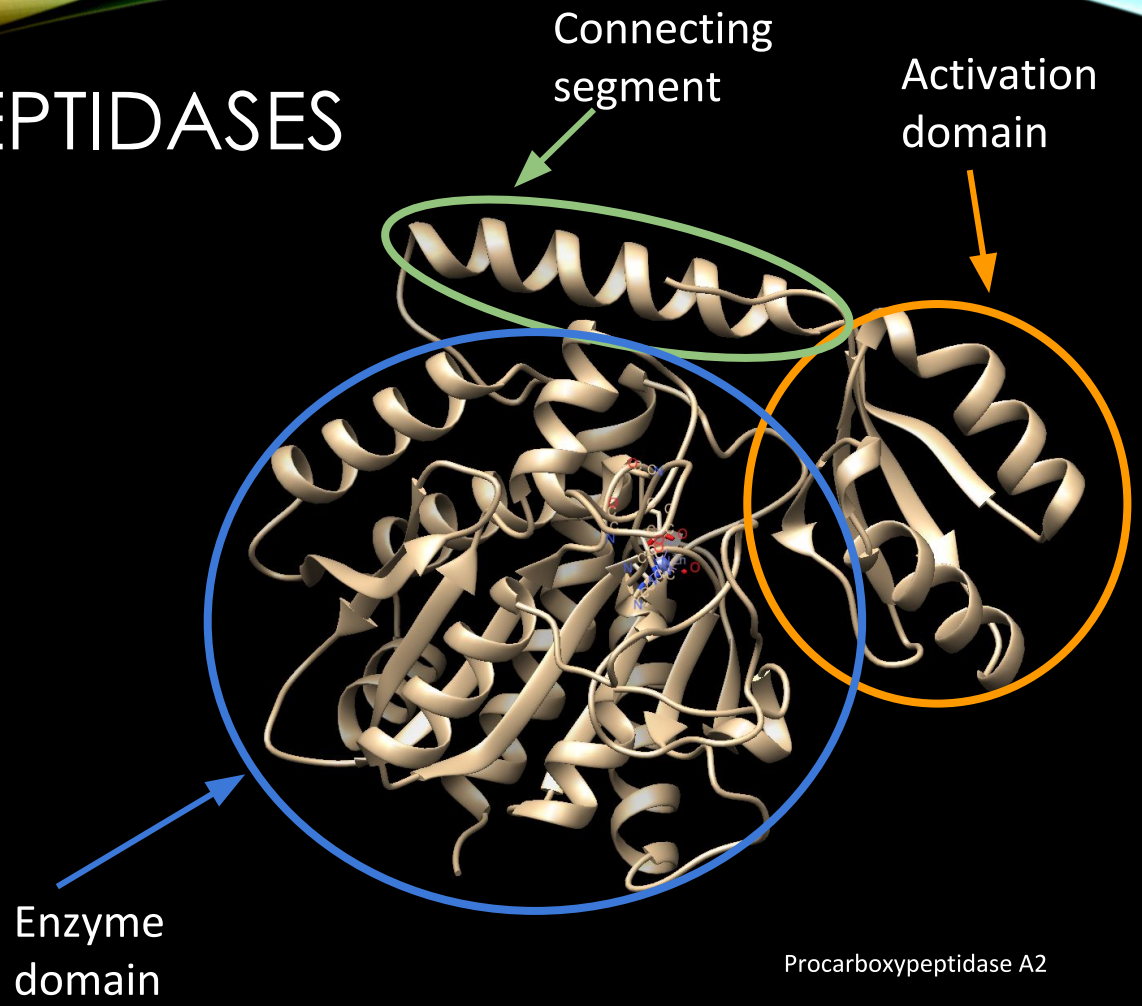
Wu S, Zhang C, Xu D, Guo H. Catalysis of Carboxypeptidase A: Promoted-Water versus Nucleophilic Pathways. The Journal of Physical Chemistry B. 2010;114(28):9259-9267.

Glu 270 performs a nucleophilic attack at the scissile carbonyl carbon, this needs an acyl-enzyme intermediate which can be posteriously hydrolysed by water.

PROCARBOXYPEPTIDASES



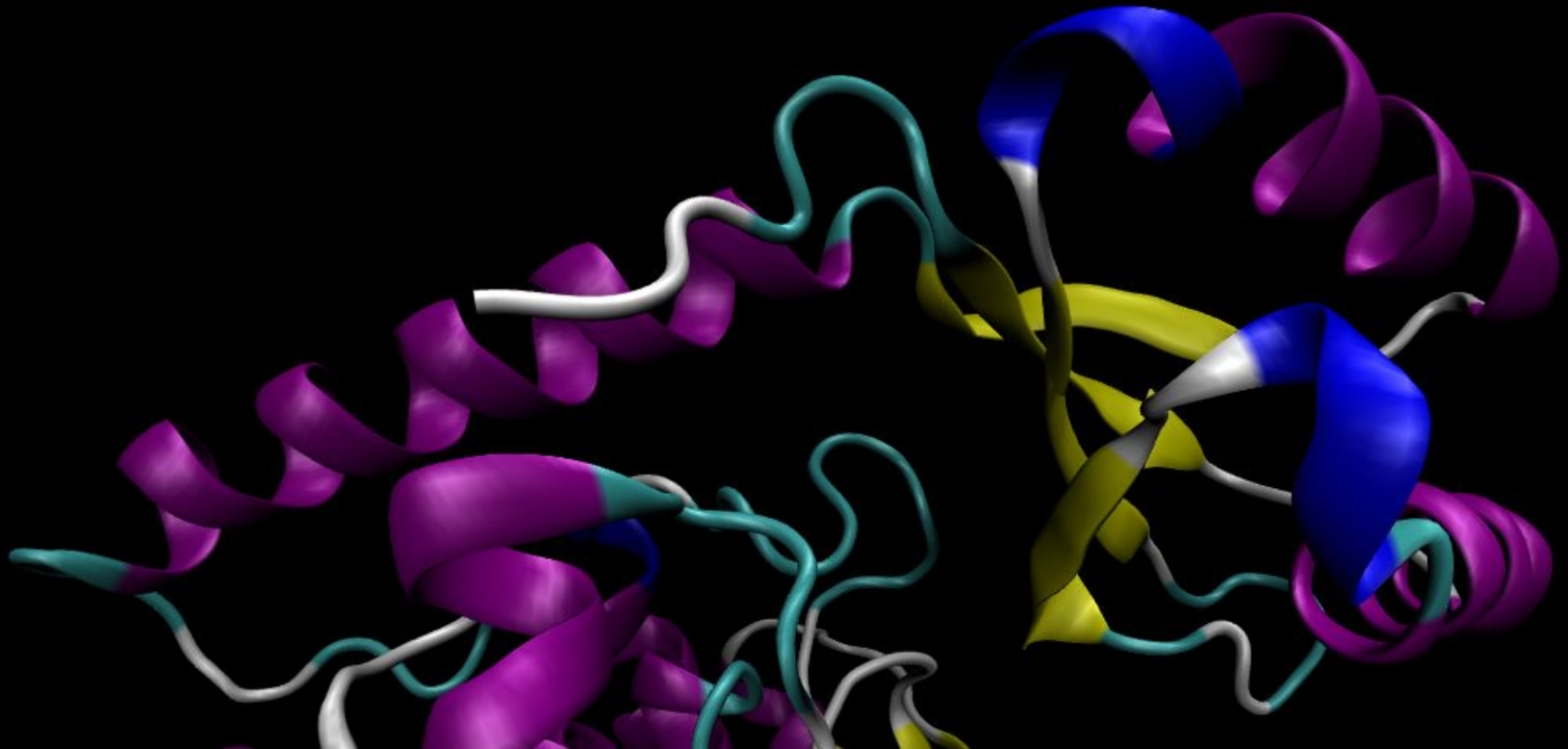
Vendrell J, Querol E, Avilés F. Metallocarboxypeptidases and their protein inhibitors. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology. 2000;1477(1-2):284-298.



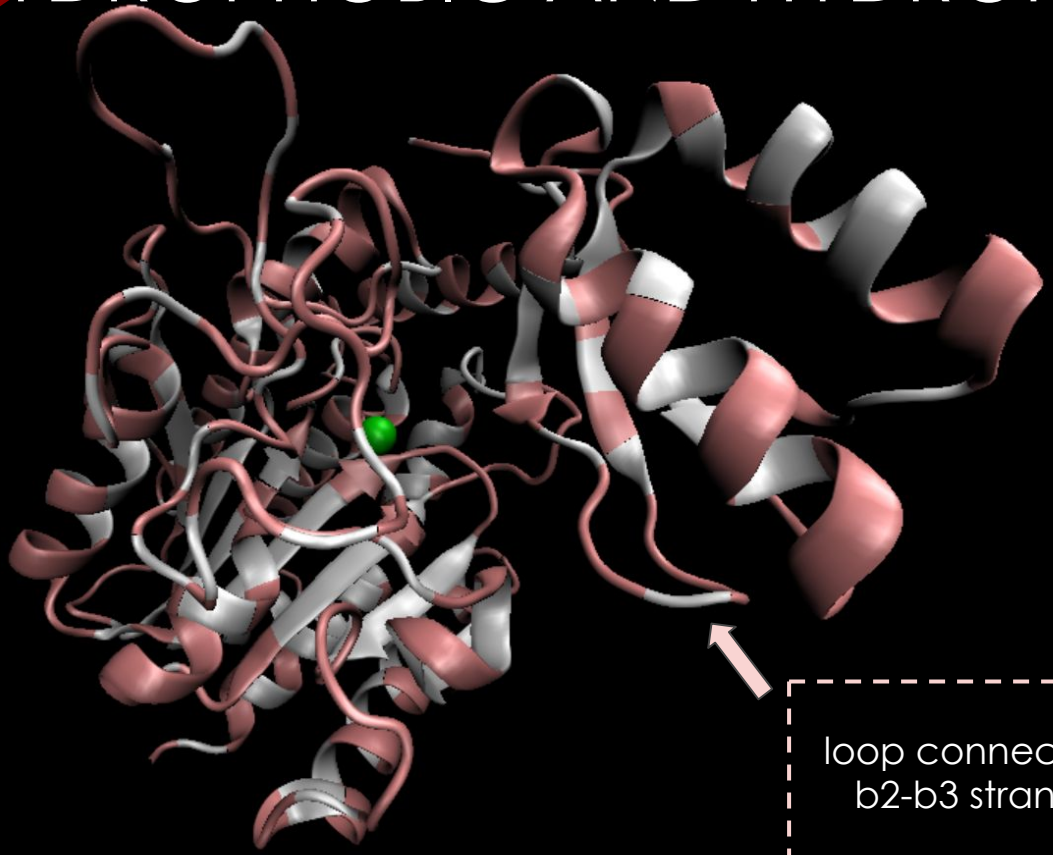
Procarboxypeptidase A2

ACTIVATION DOMAIN

4 antiparallel b-strands
3 alpha helix
2 short 3_{10} helix
No disulfide bridges



HYDROPHOBIC AND HYDROPHILIC REGIONS



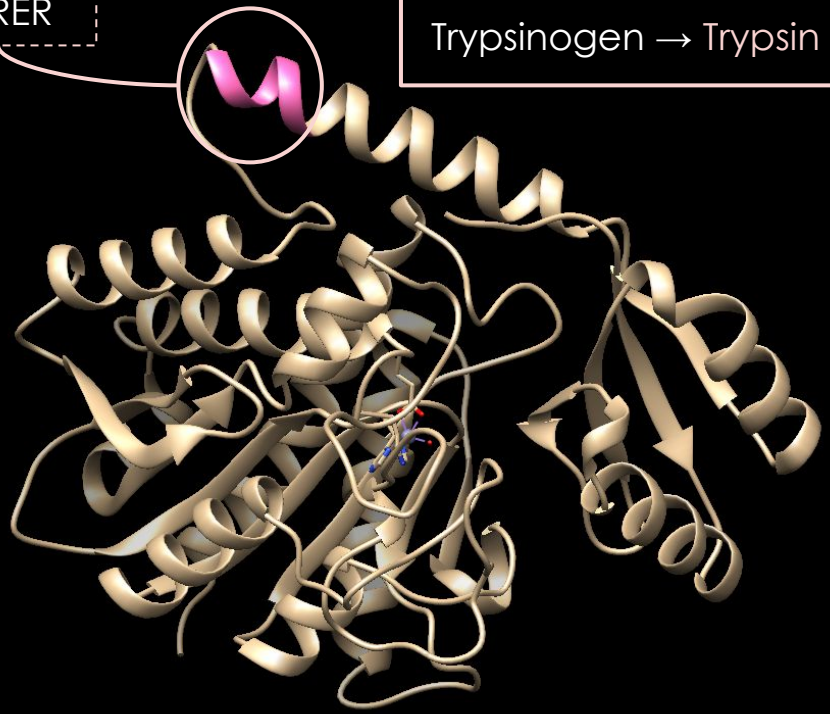
Hydrophobic residues
Hydrophilic residues
Zinc ion

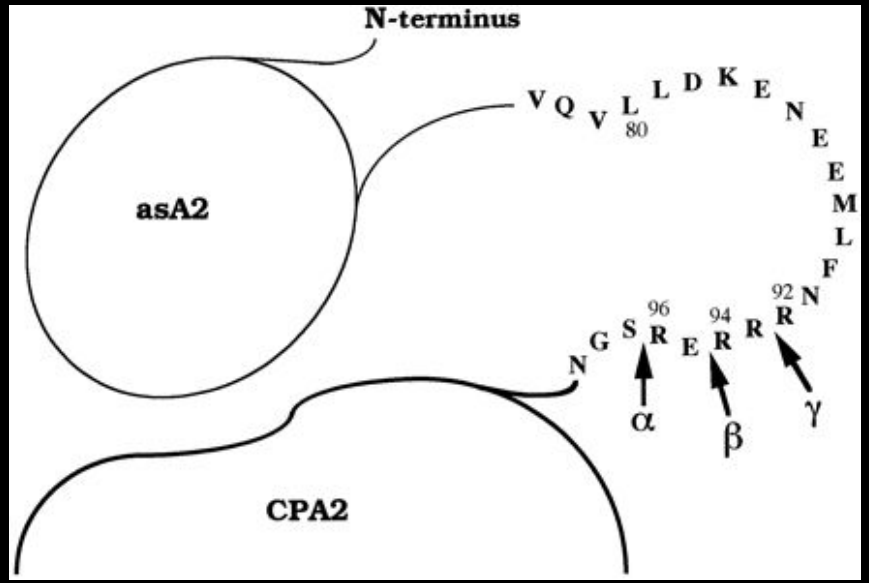
loop connecting
b2-b3 strands

TRYPSIN TARGET

RRRER

Trypsinogen → Trypsin





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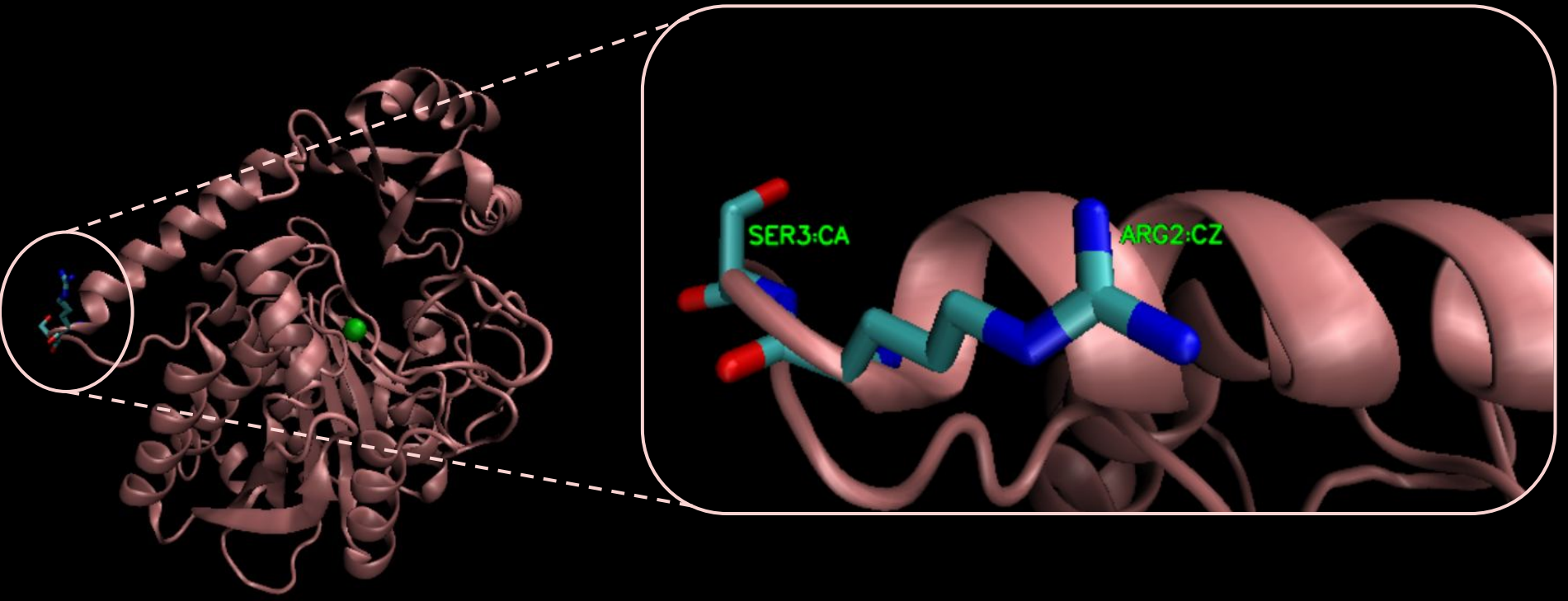
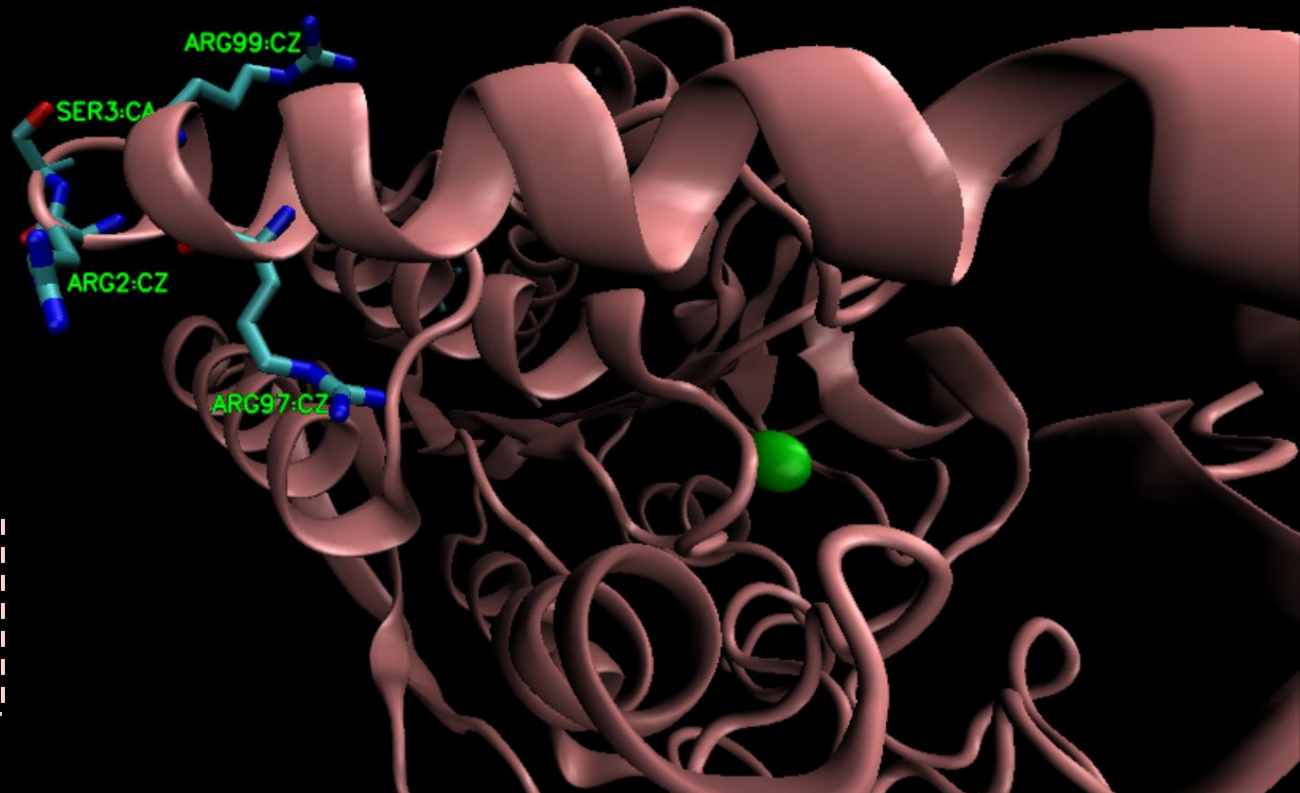
TRYPSIN CLEAVAGE 1



TRYPSIN CLEAVAGE

1. **Arg2-Ser3**
2. Arg97-Arg99



- Porcine PCPA1 → 4 helix with Arg99
- Porcine PCPB → 2 helix

ALIGNMENT PCPA2-CPA2

PCPA2→ 1AYE
CPA2 → 1DTD

```
CLUSTAL O(1.2.4) multiple sequence alignment


1AYE      LETFVGDQVLEIVPSNEEQIKNLLQLEAQEHLQLDFWKSPTTPGETAHVRVPFVNVQAVK  60
1DTD      ------------------------------------------------------------  0


1AYE      VFLESQGIAYSIMIEDVQVLLDKENEEMLFNRRRERSGNFNFGAYHTLEEISQEMDNLVA  120
1DTD      -----------------------------------FNFGAYHTLEEISQEMDNLVA  21
                                              **********************


1AYE      EHPGLVSKVNIGSSFENRPMNVLKFSTGGDKPAIWLDAGIHAREWVTQATALWTANKIVS  180
1DTD      EHPGLVSKVNIGSSFENRPMNVLKFSTGGDKPAIWLDAGIHAREWVTQATALWTANKIVS  81
          ************************************************************


1AYE      DYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKVS-GSLCVGVDPNRNW  239
1DTD      DYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKVSAGSLCVGVDPNRNW  141
          ********************************************* ************


1AYE      DAGFGGPGASSNPCSDSYHGPSANSEVEVKSIVDFIKSHGKVKAFIILHSYSQLLMFPYG  299
1DTD      DAGFGGPGASSNPCSDSYHGPSANSEVEVKSIVDFIKSHGKVKAFIILHSYSQLLMFPYG  201
          ************************************************************


1AYE      YKCTKLDDFDELSEVAQKAAQSLRSLHGTKYKVGPICSVIYQASGGSIDWSYDYGIKYSF  359
1DTD      YKCTKLDDFDELSEVAQKAAQSLSRLHGTKYKVGPICSVIYQASGGSIDWSYDYGIKYSF  261
          **********************  ***********************************


1AYE      AFELRDTGRYGFLLPARQILPTAEETWLGLKAIMEHVRDHPY    401
1DTD      AFELRDTGRYGFLLPARQILPTAEETWLGLKAIMEHVRDHPY    303
          *****************************************
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INTERACTIONS

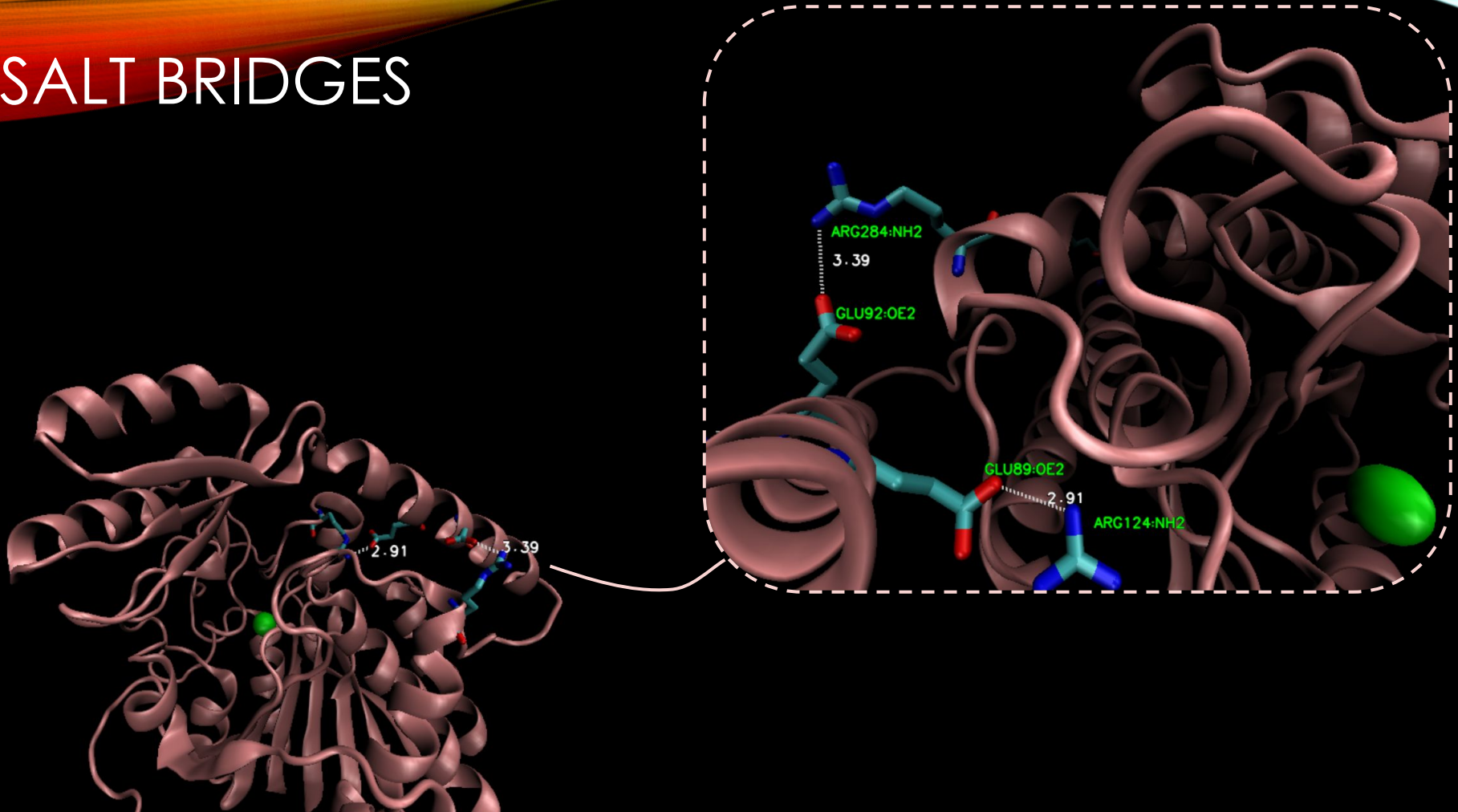
Garcia-Saez I, Reverter D, Vendrell J, Avilés F, Coll M. The three-dimensional structure of human procarboxypeptidase A2. Deciphering the basis of the inhibition, activation and intrinsic activity of the zymogen. The EMBO Journal. 1997;16(23):6906-6913.

Table I. Interactions[a] between the pro-segment and the CPA2 moiety in human pro-CPA2

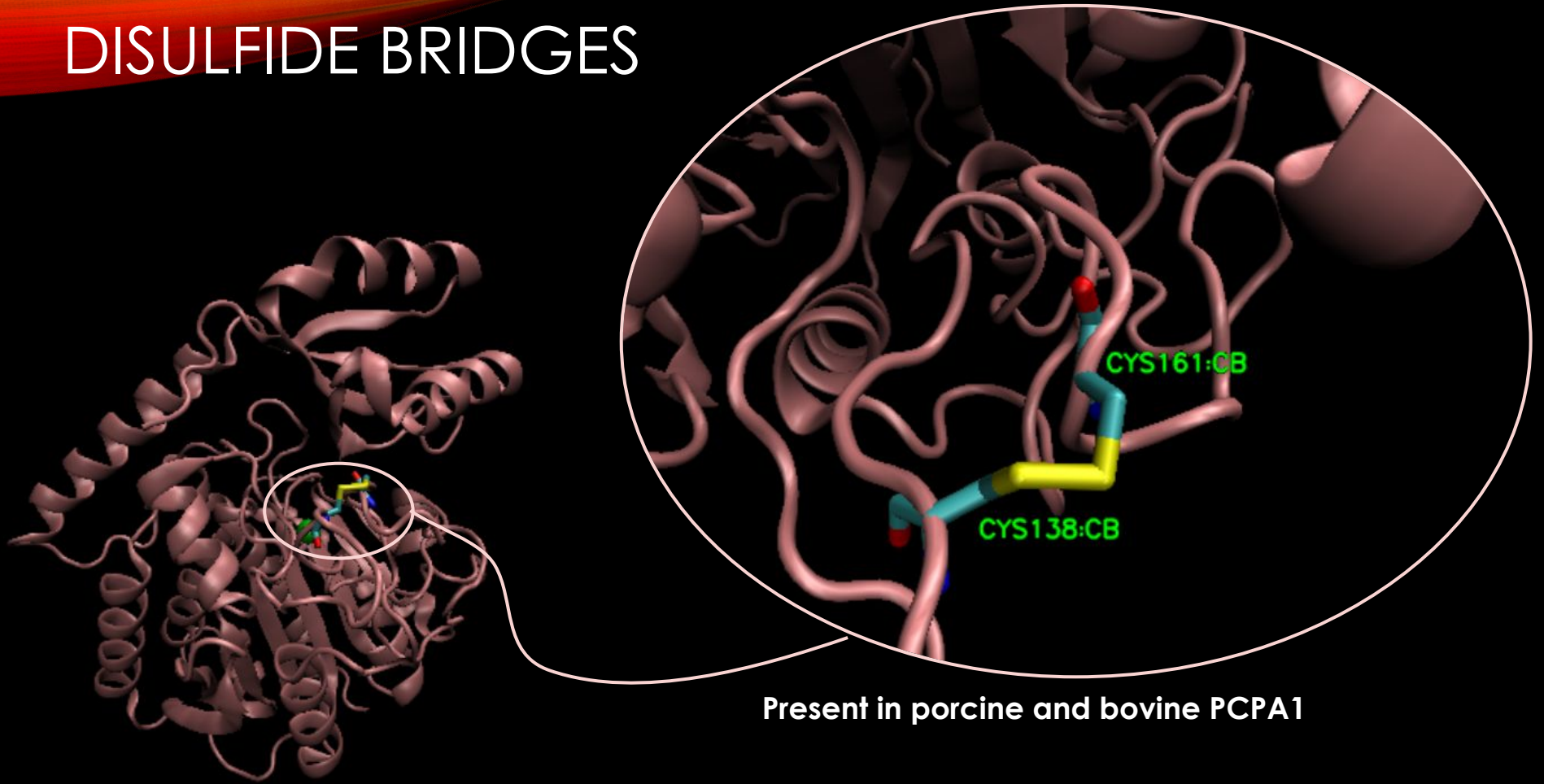
Globular domain/CPA2	Distance (Å)	Connecting segment/CPA2	Distance (Å)
N(Glu5A)--O(Lys122)	3.0	$C^{\gamma 1}$(Val82A)...$C^{\delta 1}$(Leu280)	4.0
O(Glu5A)--W2--N(Lys124)	3.0/2.9	C^{β}(Leu85A)...$C^{\delta 1}$(Leu280)	3.9
O(Glu5A)--W2--O(Lys124)	3.0/3.5	O(Leu85A)--W64--O (Leu280)	3.4/2.9
$C^{\varepsilon 1}$(Phe7A)...C^{γ}(Met125)	3.7	$C^{\delta 1}$(Leu86A)...C^{γ}(Arg124)	3.8
C^{ζ}(Phe7A)...C^{γ}(Met125)	3.7	$O^{\varepsilon 1}$(Glu89A)--$N^{\eta 2}$(Arg124)	2.9
$O^{\varepsilon 2}$(Glu33A)--$N^{\delta 2}$(Asn159)	3.3	$O^{\varepsilon 2}$(Glu89A)--N^{ε}(Arg124)	2.8
$O^{\delta 1}$(Asp36A)--$N^{\eta 2}$(Arg71)	2.8	C^{δ}(Glu89A)--$C^{\zeta 3}$(Trp73)	3.7
$O^{\delta 1}$(Asp36A)--$N^{\eta 1}$(Arg71)	3.4	$O^{\varepsilon 1}$(Glu89A)--W65--O(Leu281)	2.8/2.9
$O^{\delta 2}$(Arg36A)--$N^{\eta 2}$(Arg71)	3.0	$O^{\varepsilon 2}$(Glu92A)--$N^{\eta 2}$(Arg284)	3.3
$O^{\delta 1}$(Asp36A)--W55--$O^{\delta 2}$(Asp163)	2.7/2.8	$O^{\varepsilon 1}$(Glu92A)--W132--N^{ε}(Ala283)	2.9/3.0
O(Trp38A)--W120--$N^{\eta 1}$(Arg71)	3.1/3.1	$O^{\varepsilon 1}$(Glu92A)--W100--N(Arg284)	2.8/2.9
C^{γ}(Trp38A)...$C^{\varepsilon 2}$(Phe279)[b]	3.5	$O^{\varepsilon 1}$(Glu92A)--W00--N^{ε}(Arg284)	2.8/2.9
$C^{\zeta 3}$(Trp38A)...C^{α}(Gly278)	4.0	O(Met93A)--W87--O(Tyr12)	2.9/2.9
N^{ζ}(Lys39A)--W256--O(Val246)	2.8/2.7	O(Asn96A)--N(Glu1)[e]	2.8
N(Ser40A)--W13--O(Tyr248)[c]	3.3/3.2	$N^{\delta 2}$(Asn96A)--$O^{\varepsilon 2}$(Glu1)	2.9
O(Ser40A)--W13--O(Tyr248)[c]	3.2/3.2	$O^{\delta 1}$(Asn96A)--$N^{\delta 2}$(Asn8)	3.0
$C^{\gamma 2}$(Thr42A)...C(Ile247)	3.9	C^{γ}(Asn96A)--C^{β}(Ala11)	3.6
$C^{\gamma 2}$(Thr42A)...$C^{\varepsilon 2}$(Tyr248)[d]	3.5	O(Arg97A)--N(Arg2)[e]	3.5
$C^{\gamma 2}$(Thr42A)...C^{β}(Tyr248)[c]	3.8	O(Arg97A)--N(Glu1)[e]	3.3
$C^{\gamma 2}$(Thr47A)...$C^{\varepsilon 2}$(Tyr248)[d]	4.0	$N^{\eta 2}$(Arg97A)--W36--O(Tyr12)	2.7/3.5
$N^{\delta 1}$(His53A)--O^{η}(Tyr198)	2.7	Nε(Arg97A)--W36--O(Tyr12)	2.9
S^{δ}(Met78A)...O(Gly275)	3.7	O(Arg98A)--N(Glu1)[e]	3.3
S^{δ}(Met78A)...C(Gly275)	4.0	O(Arg98A)--N(Arg2)[e]	3.9
		O(Arg99A)--N(Gly4)[e]	3.1
		O(Arg99A)--N(Gly3)[e]	3.1
		O(Met93A)--W87--O(Tyr12)	2.9/2.9
		$N^{\delta 2}$(Asn96A)--W87--O(Tyr12)	2.7/2.9
		N^{ε}(Arg97A)--W36--O(Tyr12)	2.9/3.5
		$N^{\eta 2}$(Arg97A)--W36--O(Tyr12)	2.7/3.5

[a](...):van der Waal's contacts ≤4.0 Å; (--): H-bonds ≤3.5 Å.
[b]Aromatic–aromatic interaction; only the shortest distance between the two rings is listed.
[c]In the benzylsuccinate complexed structure.
[d]In the uncomplexed structure.
[e]Intrahelical H-bond.

SALT BRIDGES

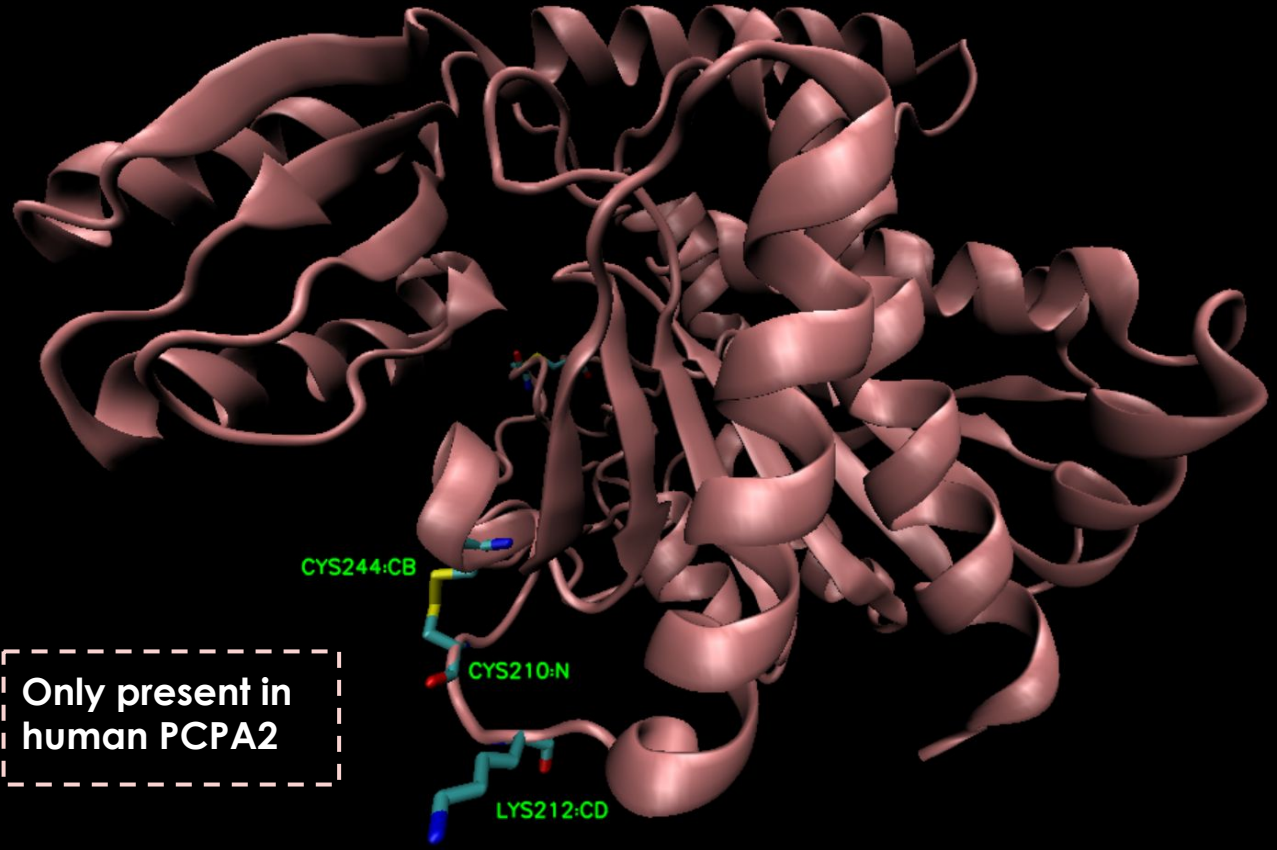


DISULFIDE BRIDGES



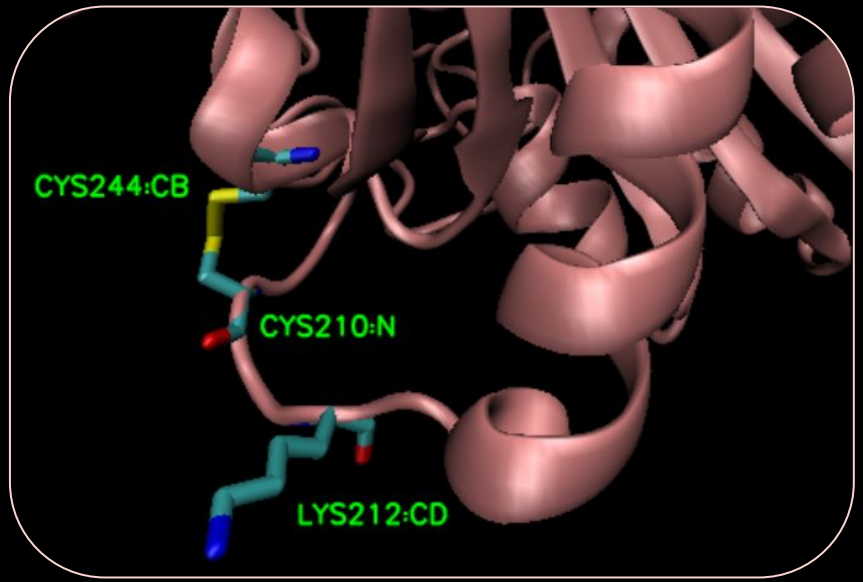
Present in porcine and bovine PCPA1

DISULFIDE BRIDGES

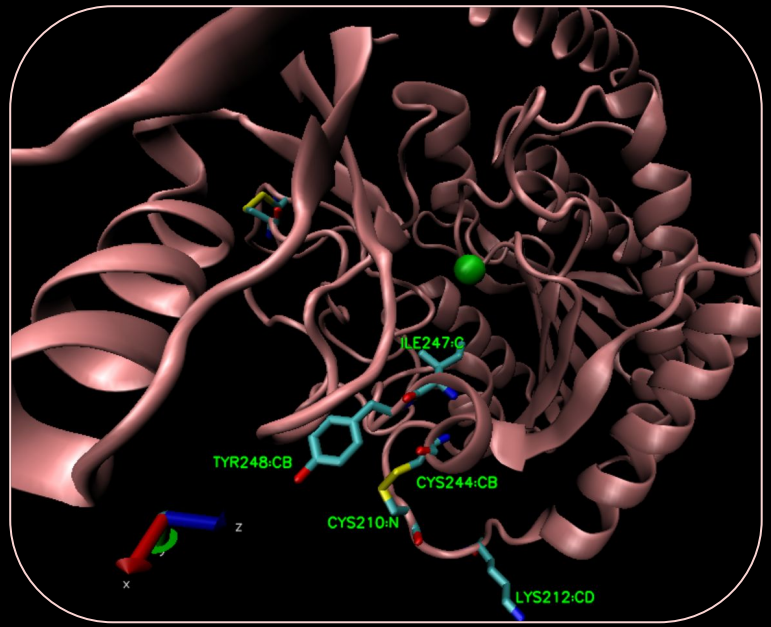


Only present in human PCPA2

DISULFIDE BRIDGES

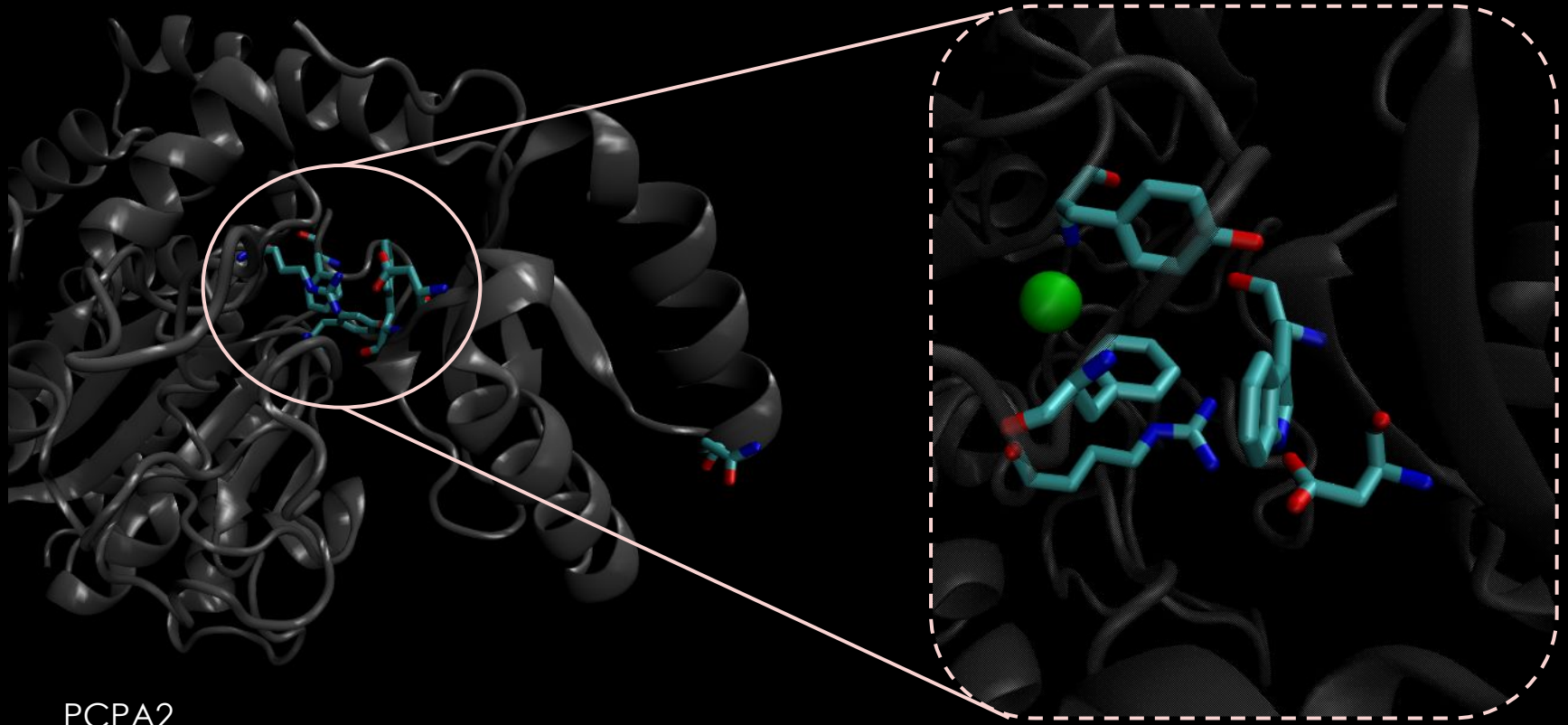


Displaces Lys 1.8A



Displaces residues 247-248

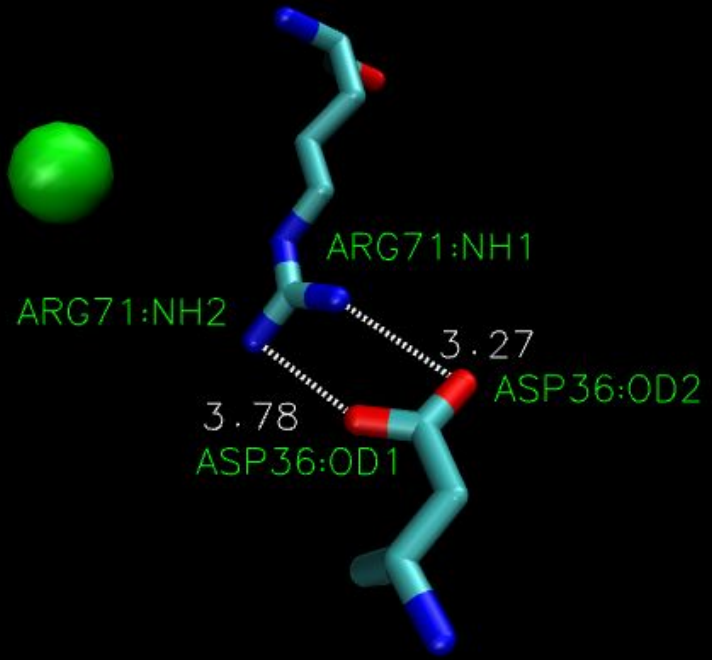
HYDROGEN BONDS

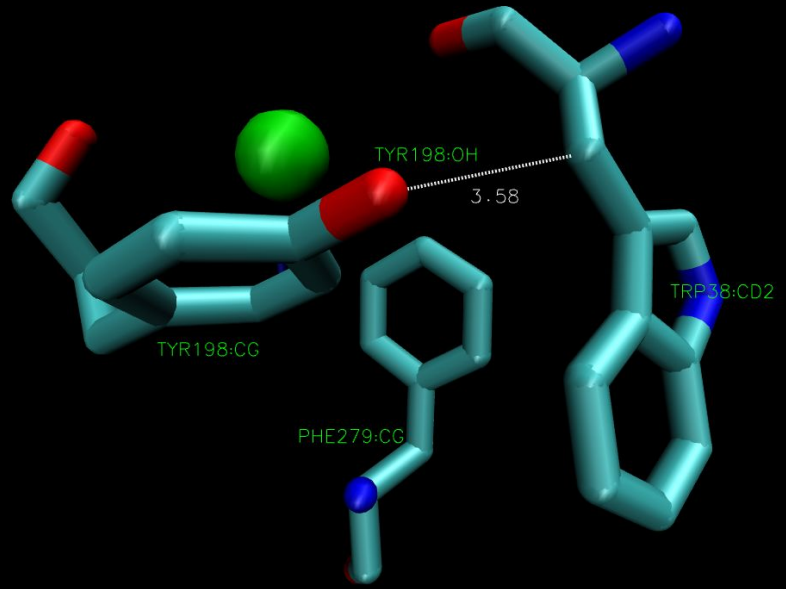


PCPA2

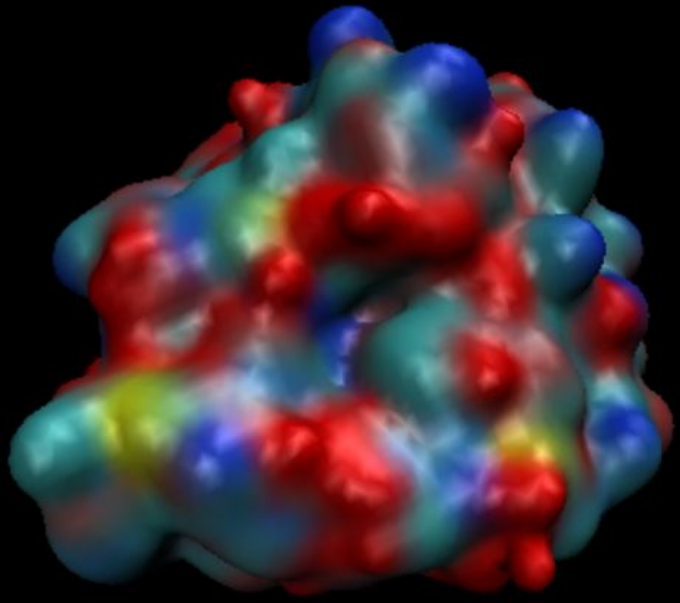
HYDROGEN BONDS

Pi-type interaction (Phe279-Trp38) -- 3.5

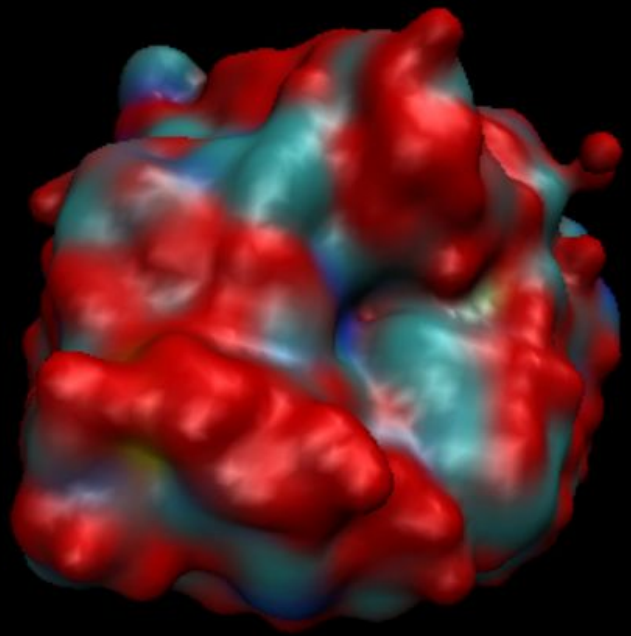




CARBOXYPEPTIDASE A VS B

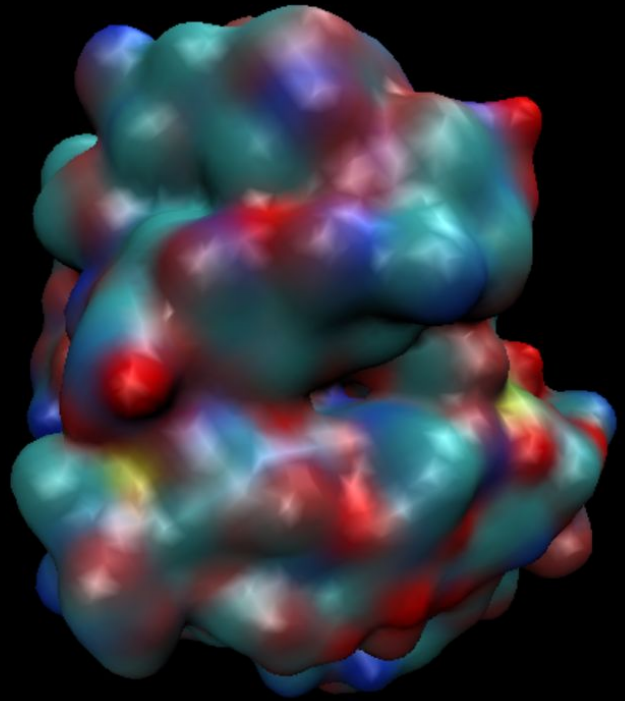


CPA2

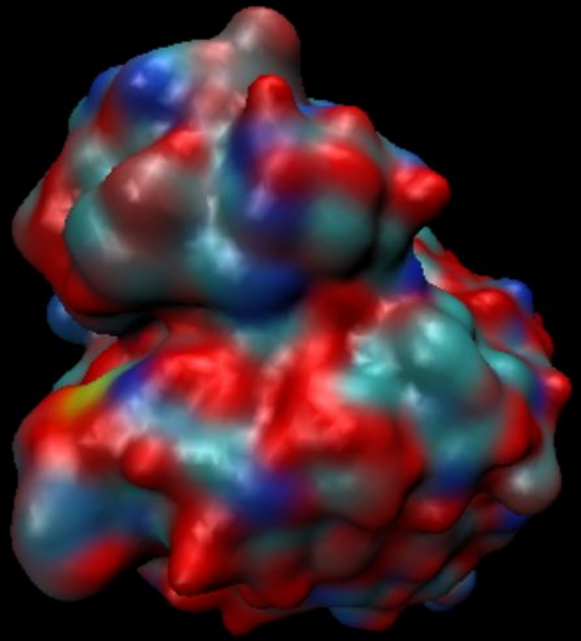


CPB

PROCARBOXYPEPTIDASE A VS B



PCPA2
Little activity



PCPB
No activity

TYPES OF CARBOXYPEPTIDASE INHIBITION

Autologous Inhibition

by Pro-Segment

Heterologous Inhibition

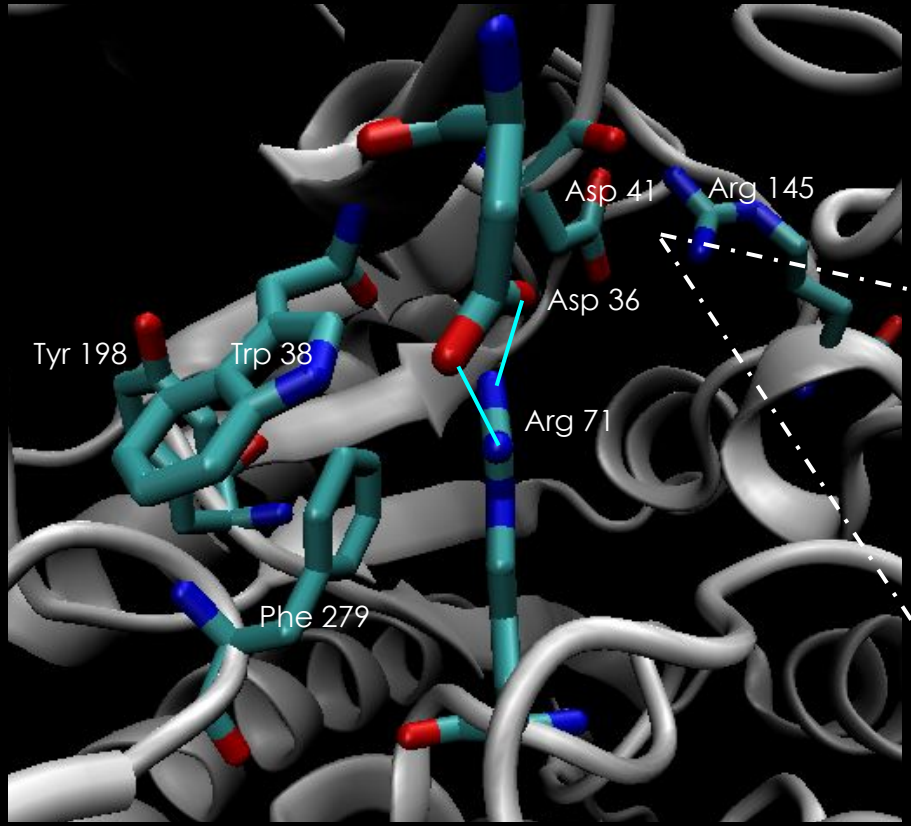
by Potato Carboxypeptidase Inhibitor

= PCI

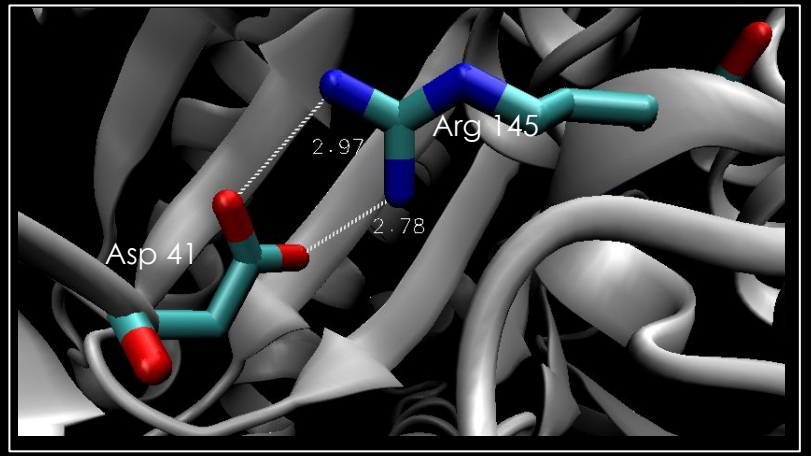
Excess of Zinc

Inhibition by a second eq. of Zn^{2+}

AUTOLOGOUS INHIBITORS: PRO-SEGMENT



salt bridge: Asp 41 - Arg 145



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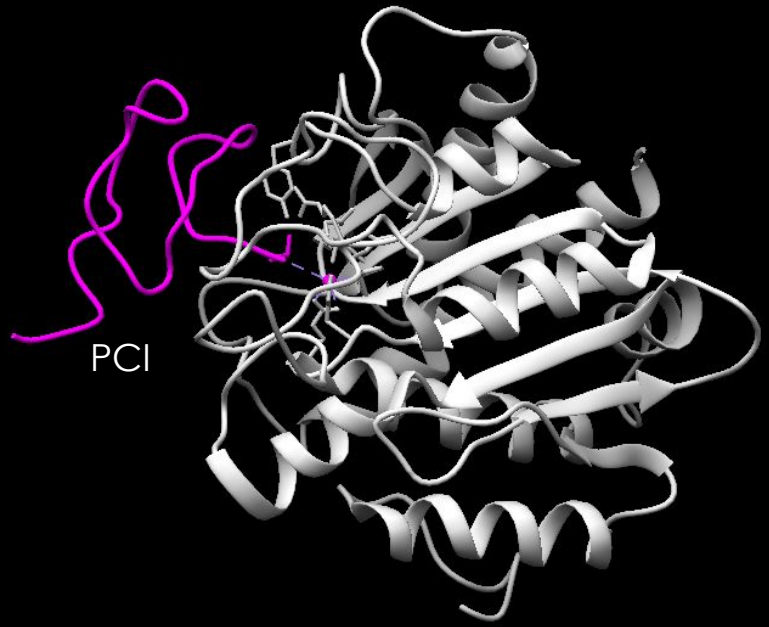
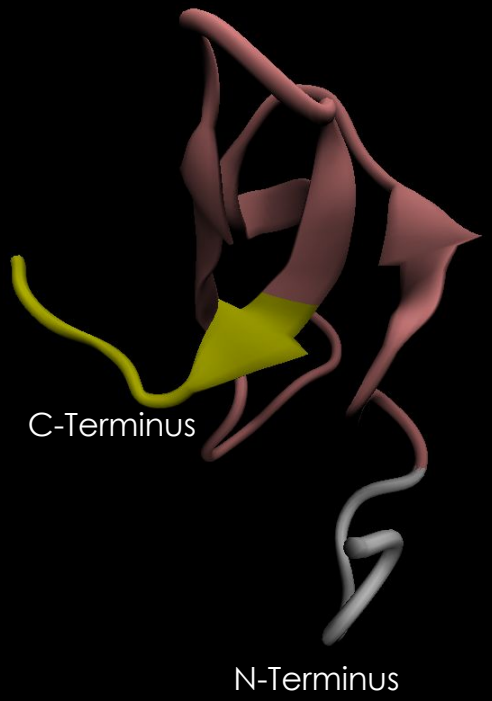
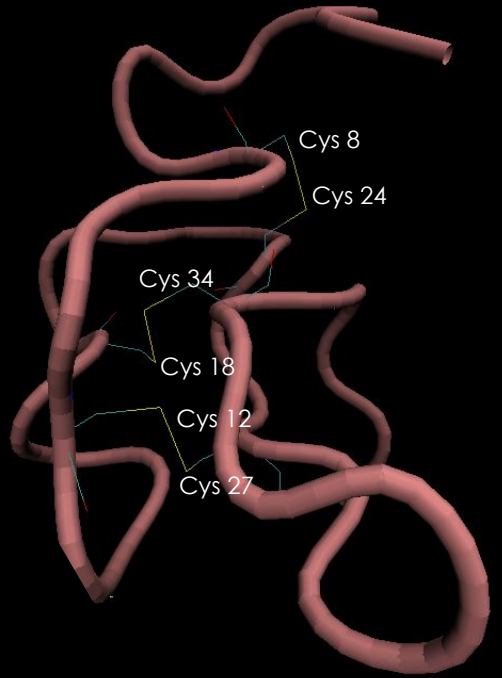
HETEROLOGOUS INHIBITORS: PCI



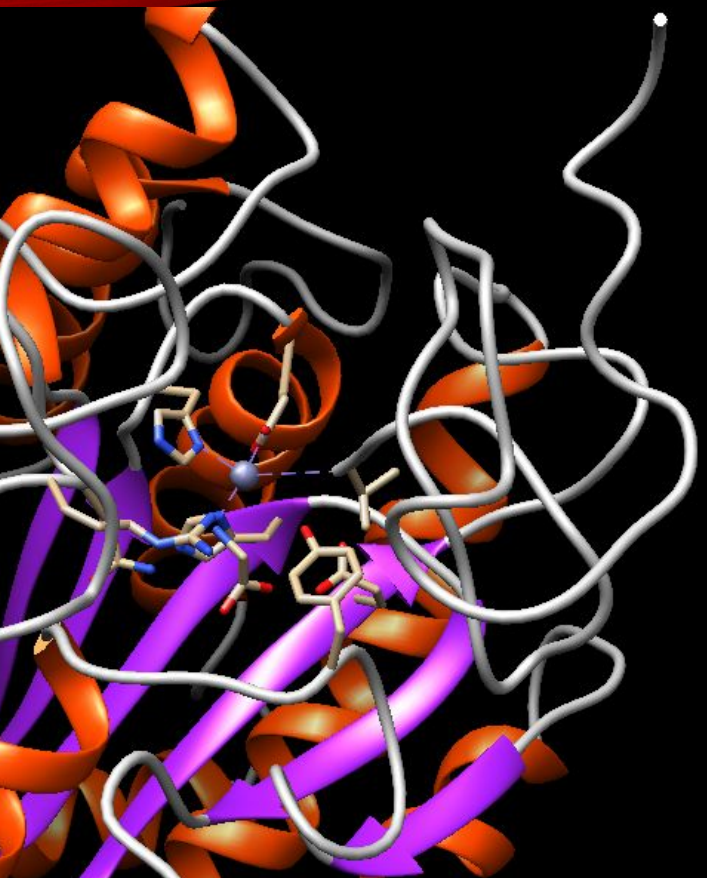


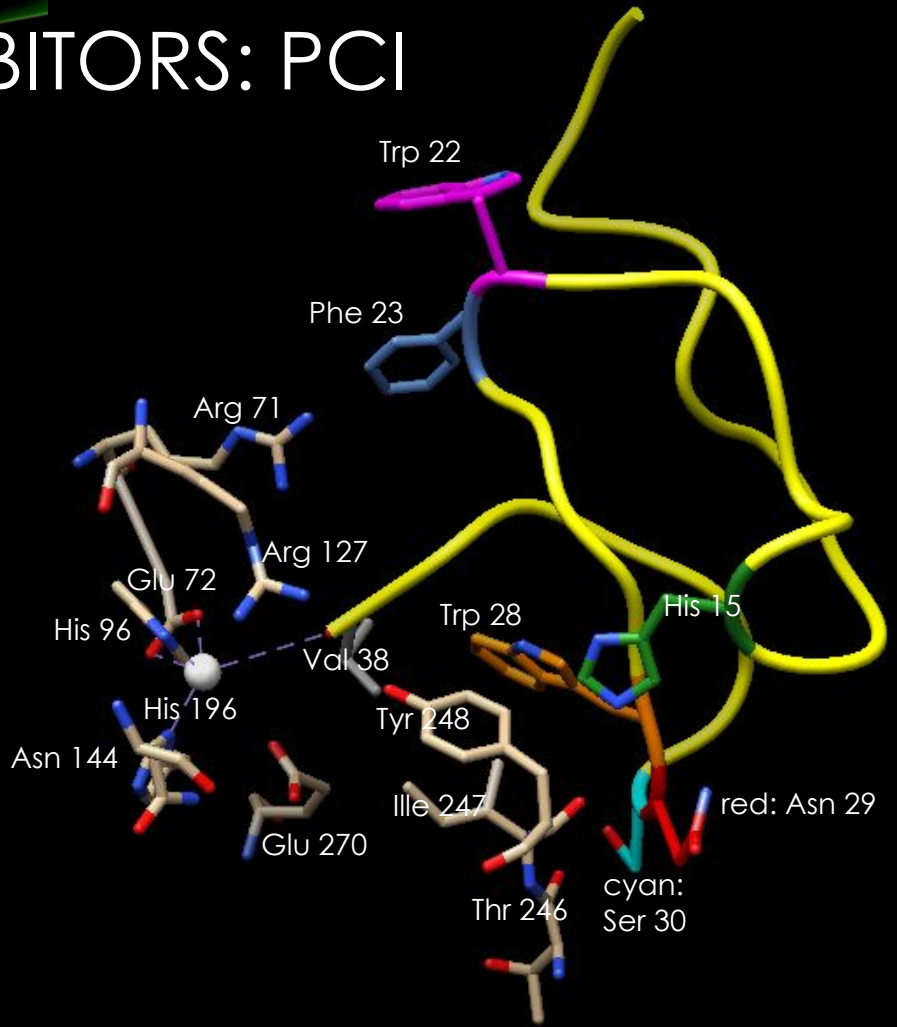


PDBID: 4cpa, 1h20

HETEROLOGOUS INHIBITORS: PCI

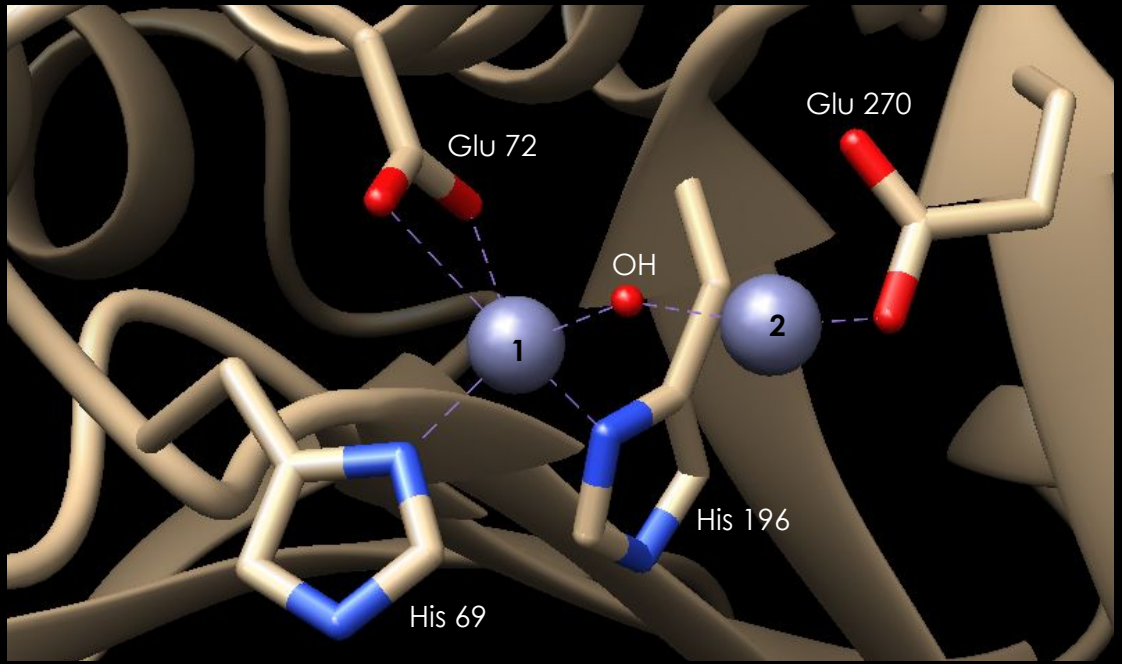
Interaction CPA-PCI:

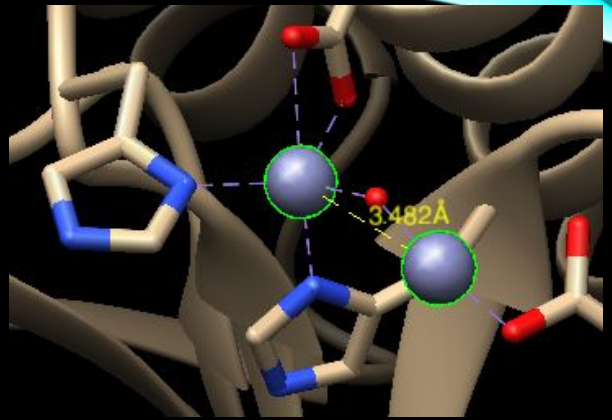




PDBID:4cpa

EXCESS OF ZINC





atoms	distance in Å
Zn 1 - Zn 2	3.482
Zn 1 - OH	1.752
OH - Zn 2	1.846

- Larsen and Auld: Inhibition *via* Zincmonohydroxide ($ZnOH^+$)
- Zn^{2+} 1: catalytic Zn^{2+} 2: excess

<u>torsion angle:</u>

Zn 1 - OH - Zn 2: 150.887°

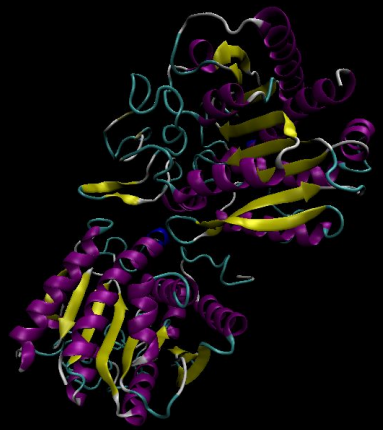
PDBID: 1cpx

FURTHER INHIBITORS OF CARBOXYPEPTIDASES

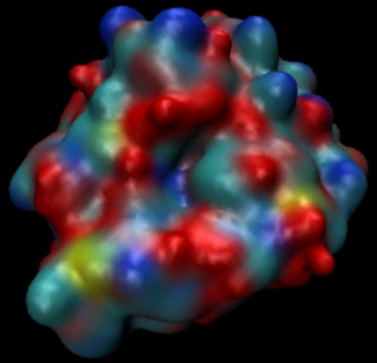
- Tomato Carboxypeptidase Inhibitor
- Ascaris suum Carboxypeptidase Inhibitor (parasit)
- Medical leech Carboxypeptidase Inhibitor (Hirudo medicinalis)
- Rat brain Carboxypeptidase Inhibitor or tissue Carboxypeptidase Inhibitor (TCI)

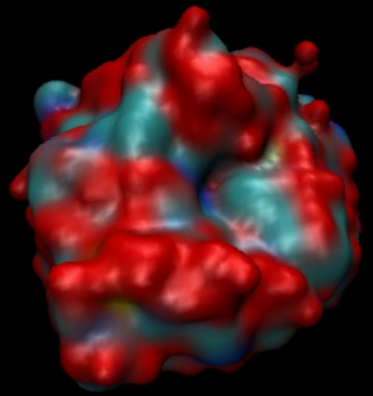
CONCLUSIONS

1. There are several kinds of CP's classifications
2. Carboxypeptidases belong to the exopeptidases
3. CPs act at C-terminals of the polypeptide chain of proteins and liberate a single amino acid or a dipeptide there
4. All of them show structural similarity in the catalytic domain
5. Pro-carboxypeptidases are the inactive form of carboxypeptidases and get activated by a trypsin cleavage of the pro-segment (96 aa).
6. There exist different ways of possible inhibitions
   → e.g. the autologous form occurs constantly in our organism
7. Play an important role in many processes such as digestion.



THANKS FOR YOUR ATTENTION!





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PEM QUESTIONS

1. Which is correct about carboxypeptidases:
   a. Procarboxypeptidase's prosegment is usually short, about 40aa.
   b. Procarboxypeptidases have the same conformation as carboxypeptidases but with an activation domain or prosegment
   c. Carboxypeptidases and procarboxypeptidases have a different folding on the catalytic center.
   d. Procarboxypeptidases are all beta proteins.
   e. Carboxypeptidases have a selenium ion on its catalytic center

2. Which is the correct?
   a. All procarboxypeptidases are fully inactive.
   b. Procarboxypeptidases A1 and B have little activity.
   c. Procarboxypeptidase A2 is fully inactive.
   d. The trypsin target has lots of Asp.
   e. All the answers are incorrect.

PEM QUESTIONS

3. Which of the following examples can act as Inhibitors of CPs:
   a. Potato Carboxypeptidase inhibitor (PCI)
   b. Pro-segments
   c. Tomato carboxypeptidase inhibitor
   d. Only a and b are correct
   e. **All the answers are correct**

4. The pro-segments acts as inhibitors until … :
   a. A second zinc-ion binds to the catalytic zinc-ion
   b. **Trypsin activation occurs**
   c. They can't act as inhibitors
   d. Tyrosine activation occurs
   e. None of this answers is correct

5. Why are His 96, His196 and Glu 72 so important residues?
   a. **They are zinc-coordinated**
   b. They don't have an important role
   c. Because they have positive charge
   d. Because they are aromatic residues
   e. They are residues in the sidechains which are responsible for the correct protein conformation

PEM QUESTIONS

6. Carboxypeptidases are ...?
   a. Endopeptidases which cleave one amino acid from the C-terminal end of the polypeptide substrates
   b. Endopeptidases which cleave one amino acid from the N-terminal end of the polypeptide substrates
   c. Exopeptidases which cleave one amino acid from the N-terminal end of the polypeptide substrates
   d. Exopeptidases which cleave one amino acid from the C-terminal end of the polypeptide substrates
   e. No answer of above is correct

7. Which metal ion uses the metalloenzyme CPA?
   a. Fe^{2+}
   b. Mn^{2+}
   c. Zn^{2+}
   d. Mg^{2+}
   e. Cu^{2+}

PEM QUESTIONS

8. Which sentence about procarboxypeptidases is correct?
    a. They are secreted in the liver
    b. They need activation by trypsin
    c. The pro-peptide is about 60 residues long
    d. They release the pro-peptide when they were cut by histidine
    e. All answers are correct

9. Which of the following answers is correct? (CP=carboxypeptidase)

    a. Only CP A is secreted as a zymogen
    b. Only CP B is secreted as a zymogen
    c. Both are secreted as zymogens
    d. CP B doesn't exist
    e. None of the answers is correct

PEM QUESTIONS

10. How many subsites has Carboxypeptidase A?
    a. 0
    b. 1
    c. 5
    d. 3
    e. 2

MATERIALS AND METHODS

Programs:

Chimera
VMD
Clustalw
STAMP

Databases:

PDB
Uniprot
PFAM