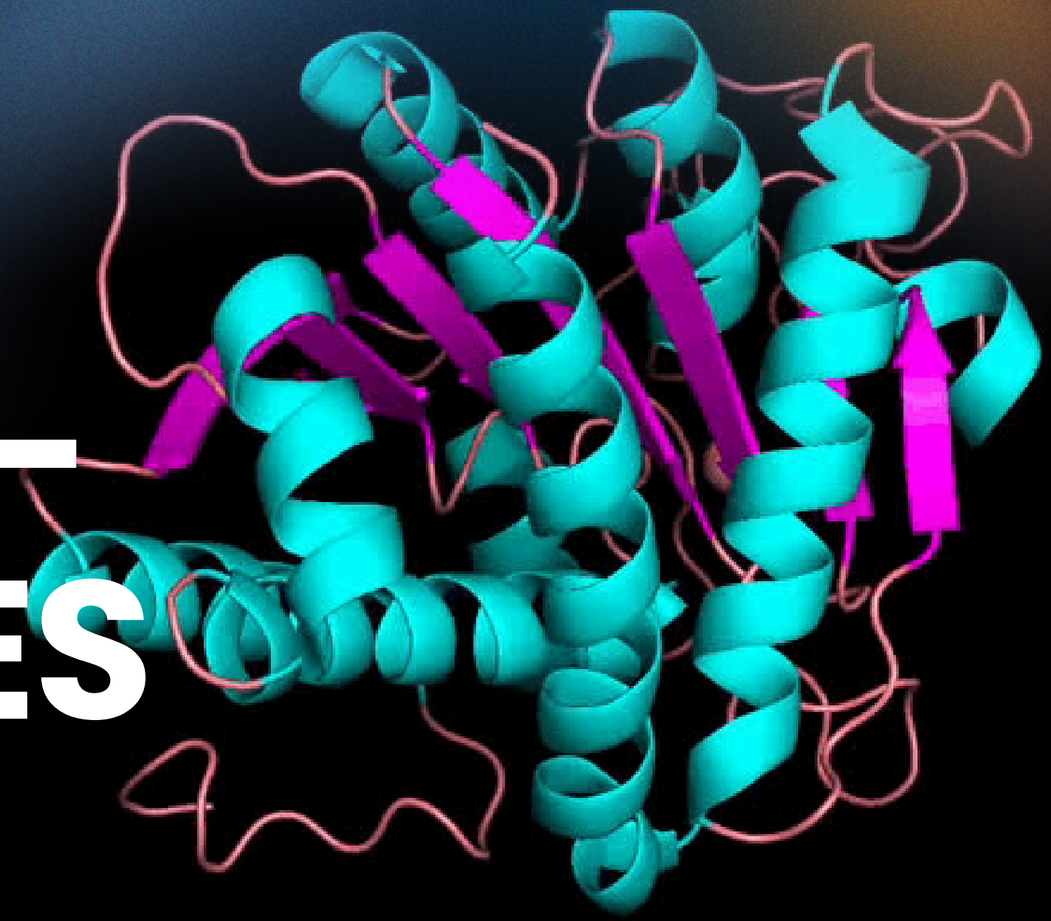
Universitat Pompeu Fabra Barcelona

CARBOXY-PEPTIDASES



MARTA ARJONA, MARTA CARRERAS,
JORGINA GARCIA I NATALIA GAVILAN

TABLE OF CONTENTS

01 Introduction

02 Classification

03 Scaffold

04 Active site

05 Catalysis

06 Specificity

07 Pro-carboxypeptidase

08 Inhibitors

09 Conservation and evolution

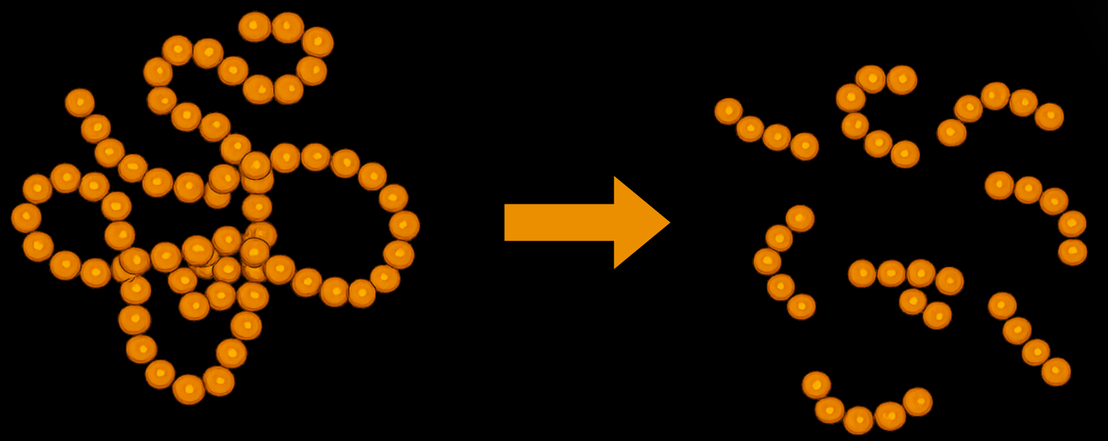
10 Conclusions

INTRODUCTION

PROTEASES

Hydrolytic **cleavage** of peptide bonds in proteins

- Large and diverse enzyme group
- Represented in all domains of life
- Essential for biological processes



- **ENDOPEPTIDASES**
- **EXOPEPTIDASES**
  - **AMINOPEPTIDASES**
  - **CARBOXYPEPTIDASES**

CLASSIFICATION

TYPE OF PROTEOLYTIC REACTION CATALYZED

Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)

1.OXIDOREDUCTASES
2.TRANSFERASES
3.HYDROLASES → 3.4. PROTEASES
4.LYASES
5.ISOMERASES
6.LIGASES

3.4.11	Aminopeptidases
3.4.13	Dipeptidases
3.1.14	Dipeptidyl-di-peptidases
3.4.15	Peptidyl-di peptidases
3.4.16	Serine-type carboxypeptidases
3.4.17	**Metallocarboxypeptidases**
3.4.18	Cysteine-type carboxypeptidases
3.4.19	Omega peptidases
3.4.21	Serine endopeptidases
3.4.22	Cysteine endopeptidases
3.4.23	Aspartic endopeptidases
3.4.24	Metalloendopeptidases
3.4.25	Threonine endopeptidases
3.4.99	Unknown type

TYPE OF PROTEOLYTIC REACTION CATALYZED

Nomenclature Committee of the Interantional Union of Biochemistry and Molecular Biology (NC-IUBMB)

1.OXIDOREDUCTASES
2.TRANSFERASES
3.HYDROLASES → 3.4. PROTEASES
4.LYASES
5.ISOMERASES
6.LIGASES

LIMITATION: Not able to account for the ample variability and evolutionary relationships

3.4.11	Aminopeptidases
3.4.13	Dipeptidases
3.1.14	Dipeptidyl-di-peptidases
3.4.15	Peptidyl-di peptidases
3.4.16	Serine-type carboxypeptidases
3.4.17	**Metallocarboxypeptidases**
3.4.18	Cysteine-type carboxypeptidases
3.4.19	Omega peptidases
3.4.21	Serine endopeptidases
3.4.22	Cysteine endopeptidases
3.4.23	Aspartic endopeptidases
3.4.24	Metalloendopeptidases
3.4.25	Threonine endopeptidases
3.4.99	Unknown type

MEROPS CLASSIFICATION: METALLOCARBOXYPEPTIDASES

FAMILY	SUBFAMILY	DESCRIPTION
M14	**A**	Pancreatic and pancreatic-like type
	B	Regulatory type
	C	Microbial enzymes that participate in sporulation
	D	Cytosolic carboxypeptidases

M14A METALLOCARBOXYPEPTIDASES

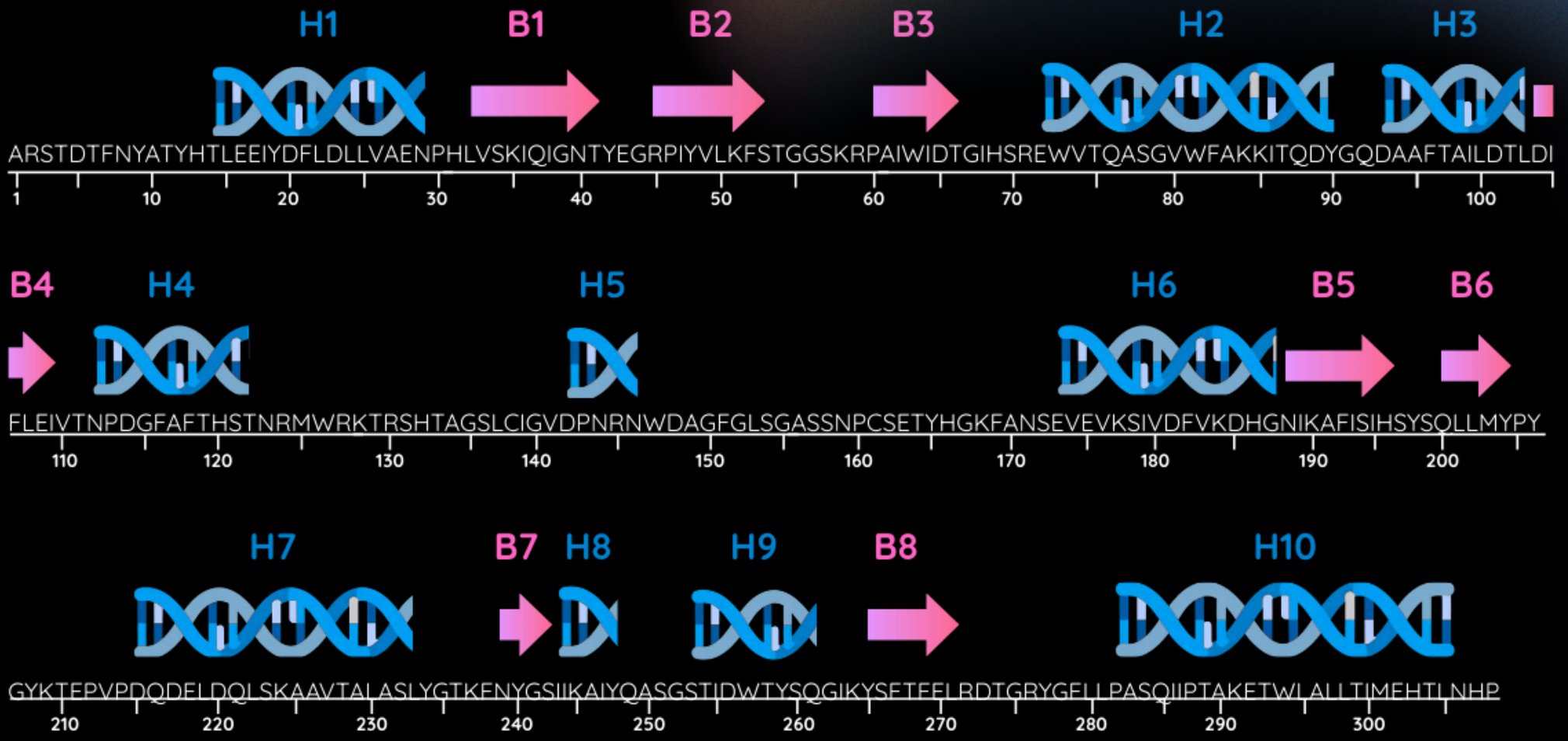
- 305-309 residues
- 34-36 kDa
- Structurally very uniform
- Pro-region in N-terminal

PANCREATIC	NON-PANCREATIC
CPA1 CPA2 (human) B	CPA3 CPA4 CPA5 CPA6 CPO CPU

SCOP

CLASS	FOLD	SUPERFAMILY	FAMILY
ALFA/BETA proteins (1000002)	Phosphorylase / hydrolase-like (2000408)	Zn-Dependent exopeptidases (3000662)	**Pancreatic carboxypeptidase-like (4000582)**
			Leucine aminopeptidase
			Bacterial dinuclear zinc exopeptidases
			FolH catalytic domain-like
			FGase-like
			AsteE/AspA-like
			Glutaminyl-peptide cyclotransferase-like
			CA_C2195 aminopeptidase-like domain-like
			Csd4 carboxypeptidase domain-like

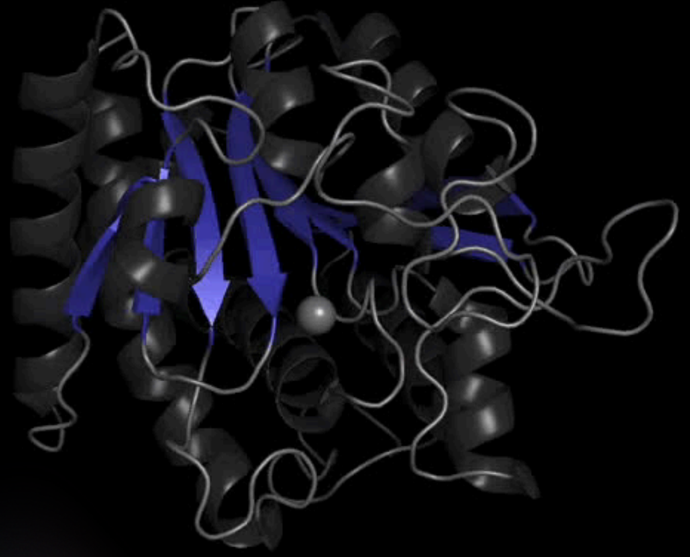
SCAFFOLD


H1
B1
B2
B3
H2
H3
ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGSKRPAIWIDTGIHSREWVTQASGVWFAKKITQDYGQDAAFTAILDTLDI
1
10
20
30
40
50
60
70
80
90
100
B4
H4
H5
H6
B5
B6
FLEIVTNPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVKDHGNIKAFISIHSYSQLLMYPY
110
120
130
140
150
160
170
180
190
200
H7
B7
H8
H9
B8
H10
GYKTEPVPDQDELDQLSKAAVTALASLYGTKENYGSIIKAIYQASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPASQIIPTAKETWLALLTIMEHTLNHP
210
220
230
240
250
260
270
280
290
300

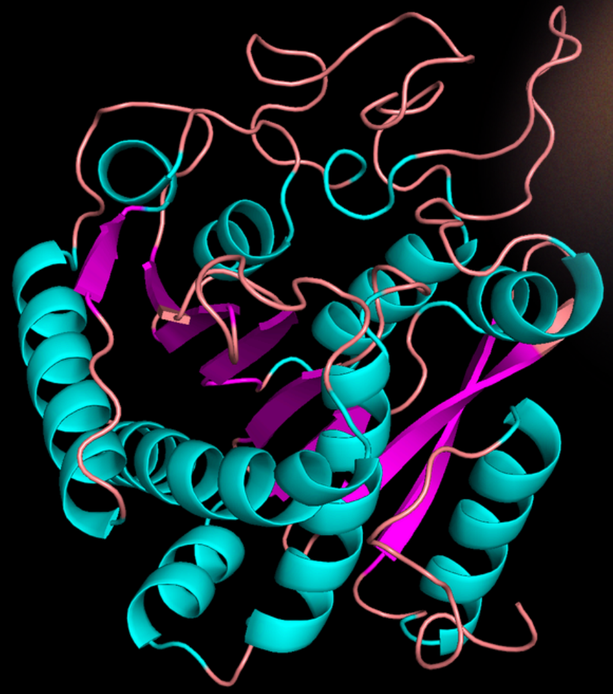
3-LAYER $\alpha\beta\alpha$ SANDWICH

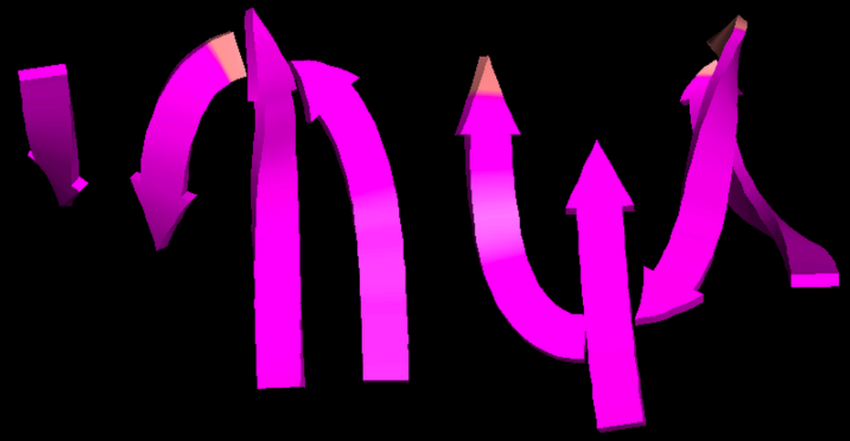


helix α

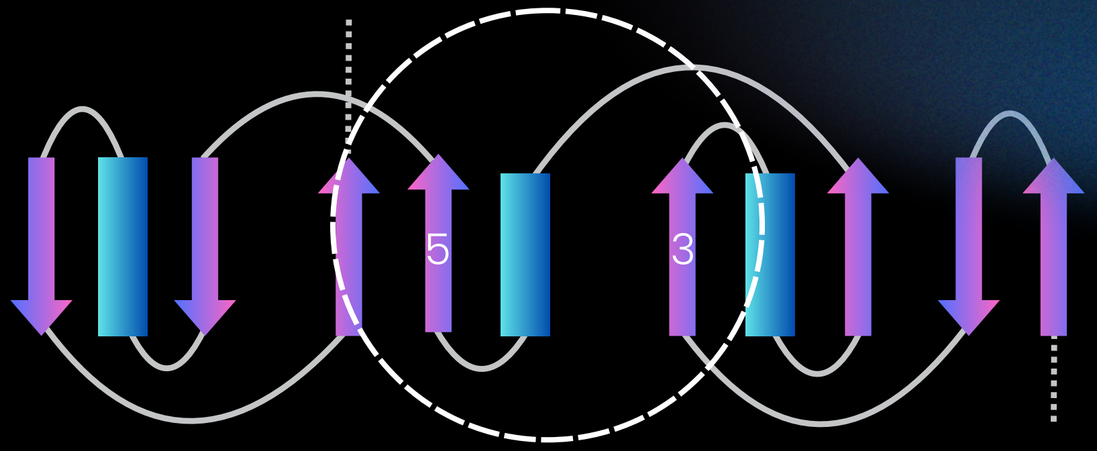


β sheet

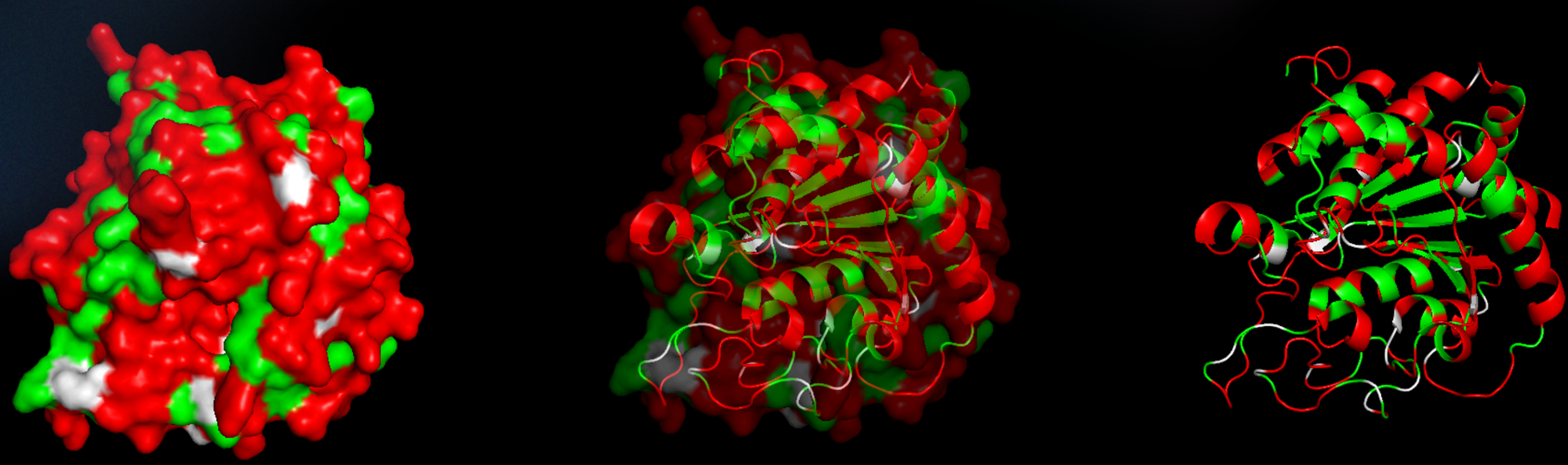




TOPOLOGICAL DIAGRAM

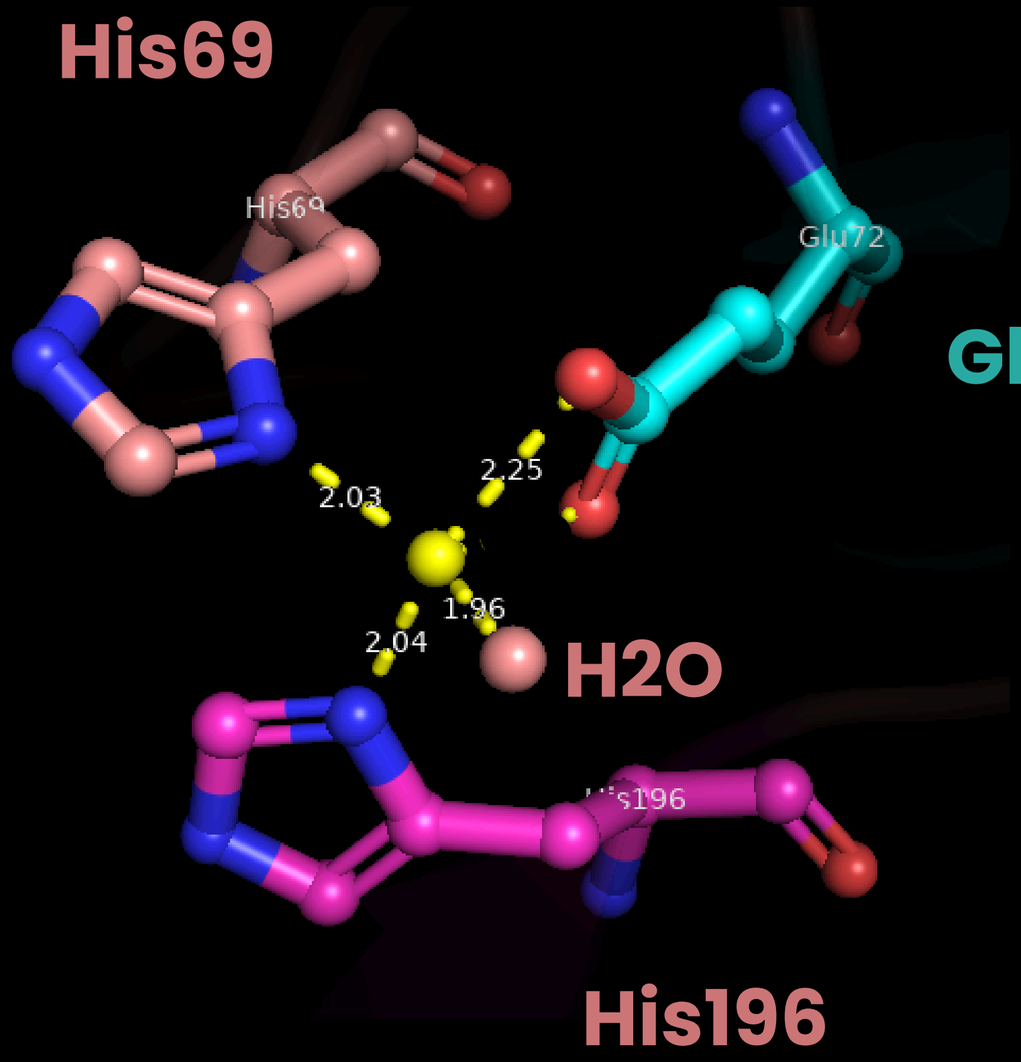


HIDROPHILIC HYDROPHOBIC



SOLUBLE AND GLOBULAR PROTEIN

ZINC COORDINATION



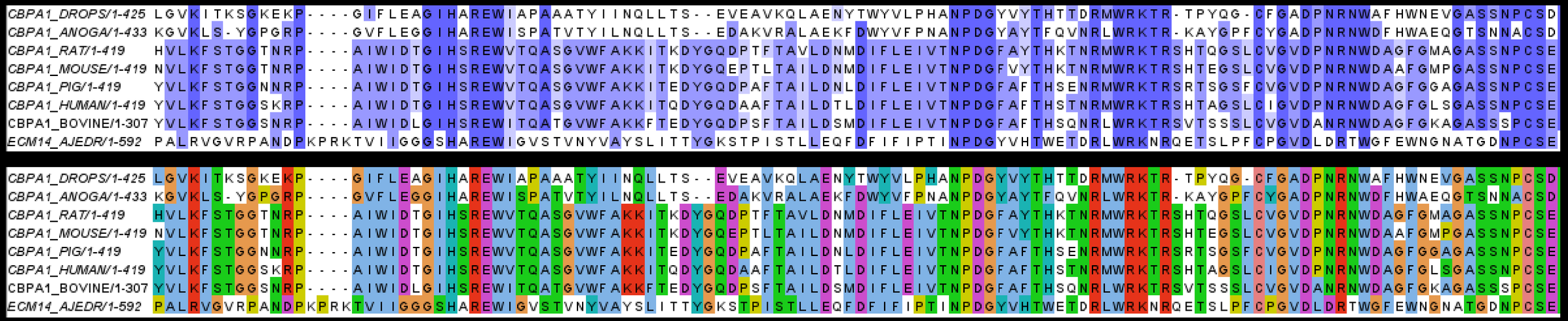
Tetrahedrally coordinated

HXXE + H motif

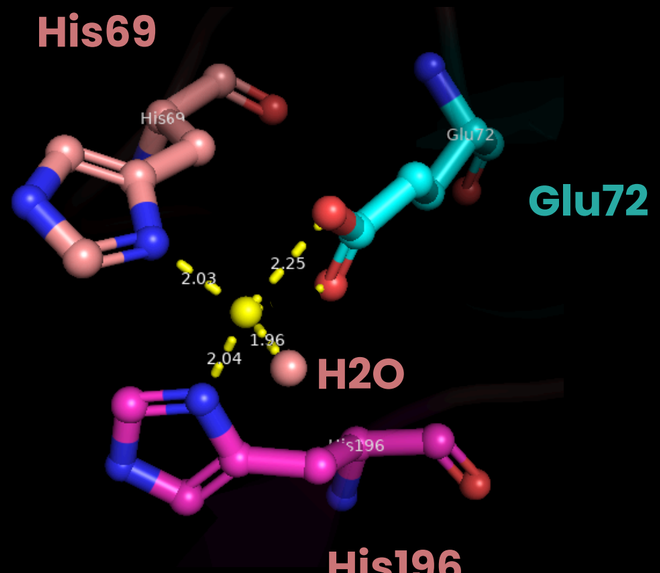
CARBOXYPEPTIDASE SEQ CONSERVATION

Sequences producing significant alignments:	Score (Bits)	E Value
sp|P00730|CBPA1_BOVIN Carboxypeptidase A1 OS=Bos taurus GN=CPA1 P...	637	0.0
sp|P09954|CBPA1_PIG Carboxypeptidase A1 OS=Sus scrofa GN=CPA1 PE=...	562	0.0
sp|P15085|CBPA1_HUMAN Carboxypeptidase A1 OS=Homo sapiens GN=CPA1...	544	0.0
sp|P00731|CBPA1_RAT Carboxypeptidase A1 OS=Rattus norvegicus GN=C...	533	0.0
sp|Q7TPZ8|CBPA1_MOUSE Carboxypeptidase A1 OS=Mus musculus GN=Cpa1...	521	0.0
sp|C5G6U8|ECM14_AJEDR Putative metallocarboxypeptidase ECM14 OS=A...	175	4e-49
sp|Q29NC4|CBPA1_DROPS Zinc carboxypeptidase A 1 OS=Drosophila pse...	218	7e-67
sp|O02350|CBPA1_ANOGA Zinc carboxypeptidase A 1 OS=Anopheles gamb...	258	2e-82

HYDROPHOBIC	A,C,I,L,M,F,W,V,C
POSITIVE CHARGE	K,R
NEGATIVE CHARGE	E,D
POLAR	N,Q,S,T
CYSTEINES	C
GLYCINES	G
PROLINES	P
AROMATIC	H,Y
UNCONSERVED	-



ZINC COORDINATION



His 196

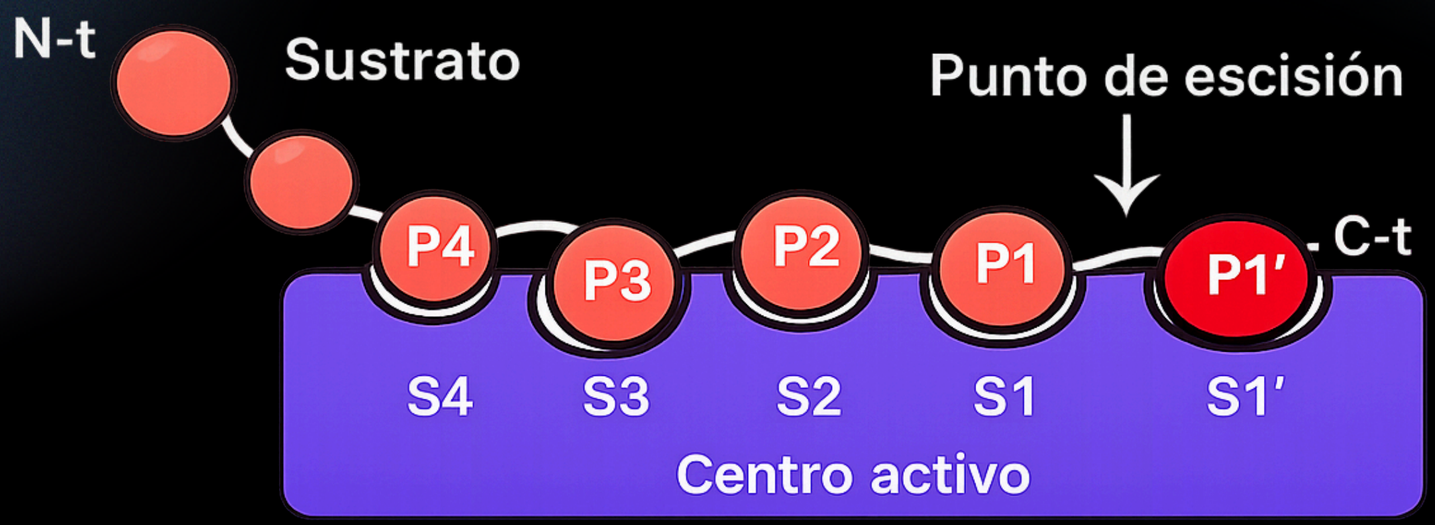
```
                                186H                 191A                 201L
CBPA1_PIG/1-419                 DHG-----NIKAFISIHSYSQLLLYPYG
CBPA1_BOVINE/1-307              DHG-----NFKAFLSIHSYSQLLLYPYG
CBPA1_HUMAN/1-419               DHG-----NIKAFISIHSYSQLLMYPYG
CBPA1_RAT/1-419                 SHG-----NIKAFISIHSYSQLLLYPYG
CBPA1_MOUSE/1-419               SHG-----NIKAFISIHSYSQLLLYPYG
CBPA1_ANOGA/1-433               KLRG----KLGAYIAFHSYSQLLLFPYG
CBPA1_DROPS/1-425               SLKG----KIQLYISLHAYSQYLLYPYG
ECM14_AJEDR/1-592               EQTEQHNVKFVAYLDLHSYSQQVLYPYS
```

Glu72

```
                10A                 20D                 30P                 40R                 50L                 60P                 66L                 76
CBPA1_PIG/1-419 ARTTSTFNYATYHTLEEIYDFMDILVAEHPQLVSKLQIGSSYEGRPIYVLKFSTGGNNRP----AIWIDTGIHSREWVTQA
CBPA1_BOVINE/1-307 ARSTNTFNYATYHTLDEIYDFMDLLVAEHPQLVSKLQIGRSYEGRPIYVLKFSTGGSNRP----AIWIDLGIHSREWITQA
CBPA1_HUMAN/1-419 ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGSKRP----AIWIDTGIHSREWVTQA
CBPA1_RAT/1-419 ALSTDSFNYATYHTLDEIYEFMDLLVAEHPQLVSKIQIGNTFEGRPIHVLKFSTGGTNRP----AIWIDTGIHSREWVTQA
CBPA1_MOUSE/1-419 ALSTDAFNYATYHTLDEIYEFMDLLVTEHPQLVSKIQIGSTFEGRPINVLKFSTGGTNRP----AIWIDTGIHSREWVTQA
CBPA1_ANOGA/1-433 -RAKGPFDWNDYHTLEEIHAWLDQLASEHPKEVELLDAGRSHQNRTMKGVKLS-YGPGRP----GVFLEGGIHAREWISPA
CBPA1_DROPS/1-425 GRAGEAYNWAQYYELDDTYAWLQTLAIENPGVVTLIEGGKTYQGRSILGVKITKSGKEKP----GIFLEAGIHAREWIAPA
ECM14_AJEDR/1-592 SDVTNIF-FEDYQPLSVIGPWMRLLASMFPSHVQLISIGSSFEGRDIPALRVGVRPANDPKPRKTVIIGGGSHAREWIGVS
```

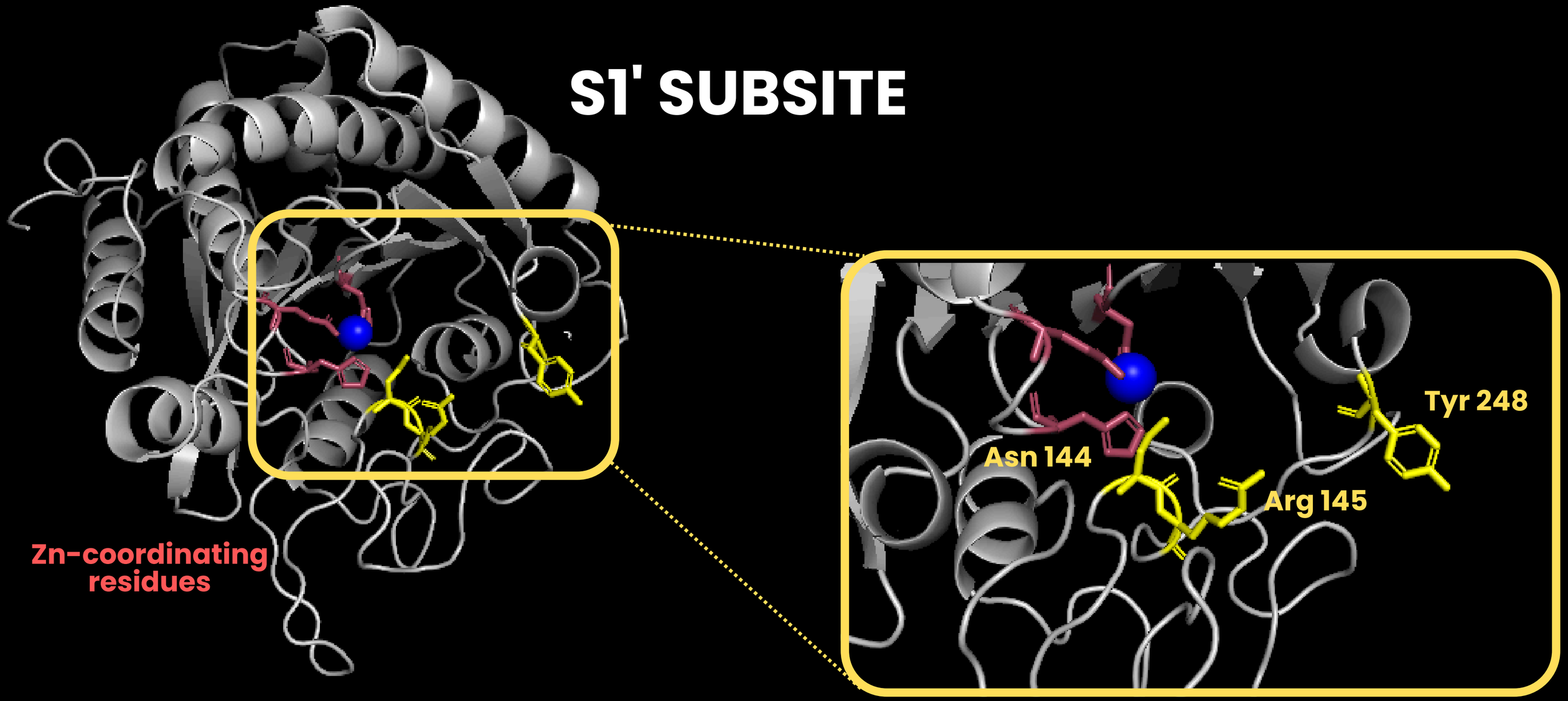
His 69

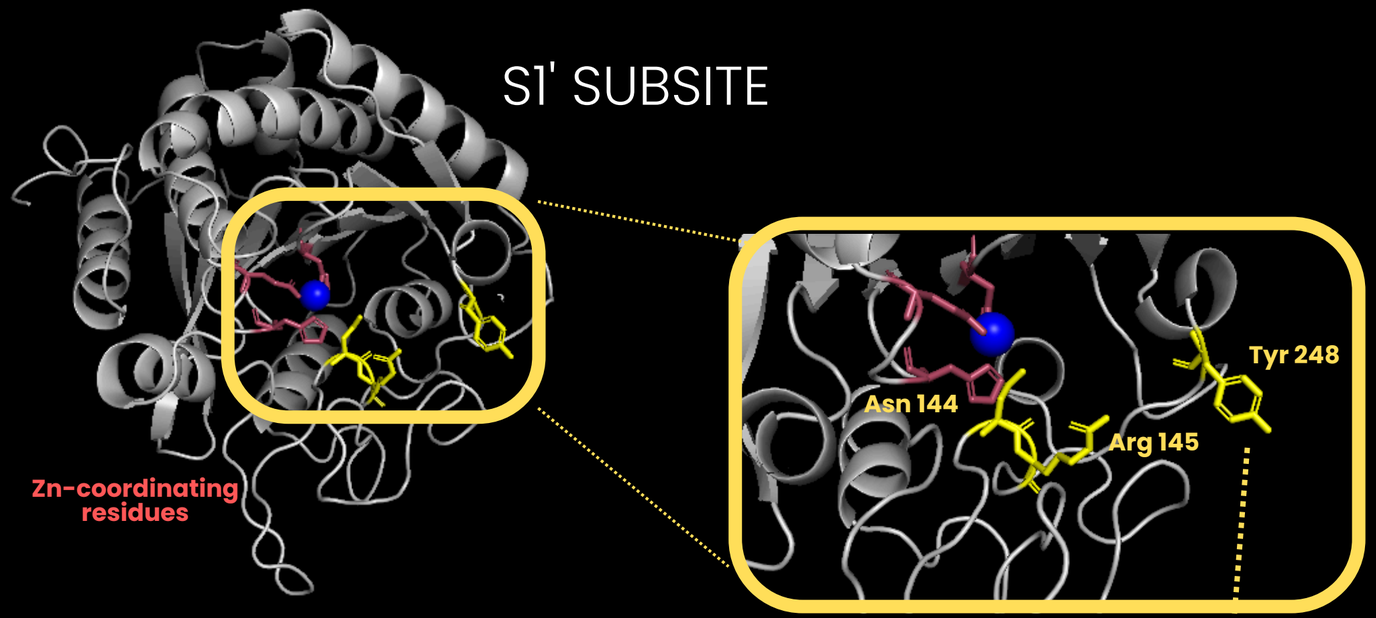
CATALYTIC SITE: SUBSITES



CPA ACTIVE SITE

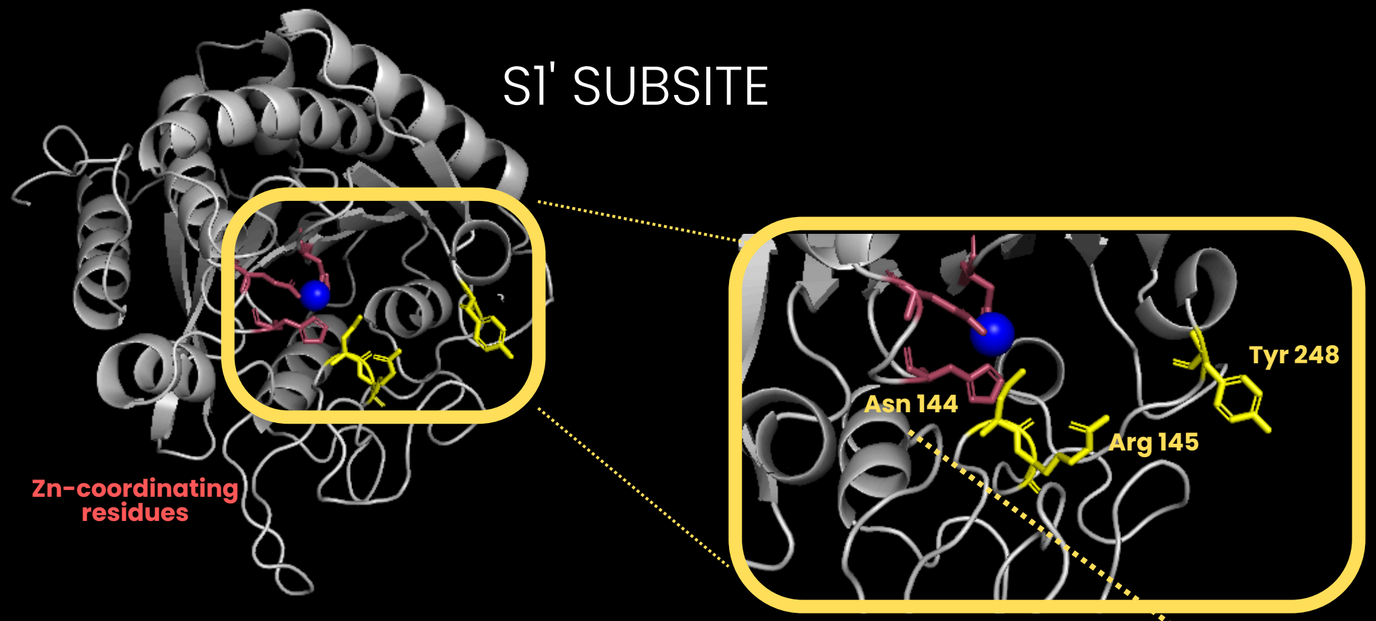
S1' SUBSITE





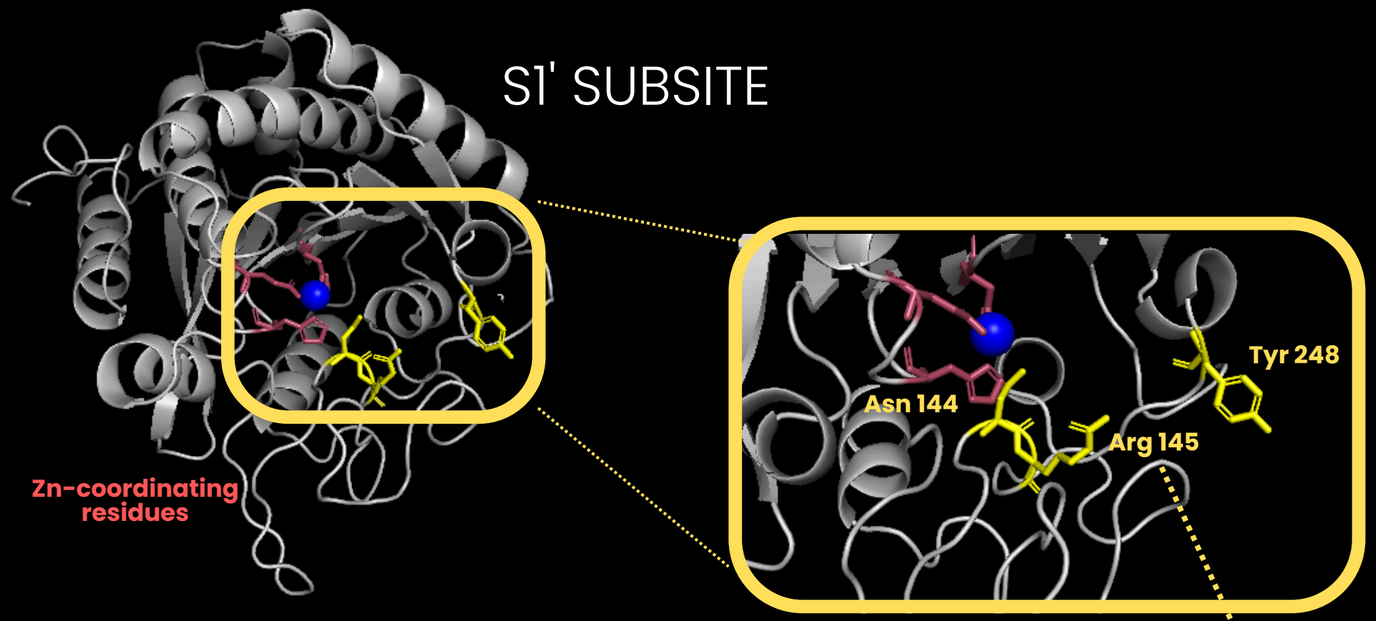
TYR248

```
                                240Y          242-          245T          255I          264K          274T
CBPA1_PIG/1-419          YGTKFQYG----------------SIITTIYQASGGTIDWTY-NQGIKYSFSFELRDTG---RYGFLL
CBPA1_BOVINE/1-307       YGTSYKYG----------------SIITTIYQASGGSIDWSY-NQGIKYSFTFELRDTG---RYGFLL
CBPA1_HUMAN/1-419        YGTKFNYG----------------SIIKAIYQASGSTIDWTY-SQGIKYSFTFELRDTG---RYGFLL
CBPA1_RAT/1-419          HGTKFKYG----------------SIIDTIYQASGSTIDWTY-SQGIKYSFTFELRDTG---LRGFLL
CBPA1_MOUSE/1-419        HGTKFKYG----------------SIIDTIYQASGSTIDWTY-SQGIKYSFTFELRDTG---LRGFLL
CBPA1_ANOGA/1-433        YGTQYKYG----------------NVYDAIYPASGSSVDWSYGAQDVKIAYTYELRPDGDAWN-GFVL
CBPA1_DROPS/1-425        YGTTYTGG----------------NIYDAIYPAAGASVDWAYGTQDVRMAFCYELRPSSNSFITGFKL
ECM14_AJEDR/1-592        NRQSYTVSSACQGFTASQKKVKLDTFPRMESTGGSALDWFYNDVGVKYSYQLKLRDKG---SYGFLL
```



ASN144

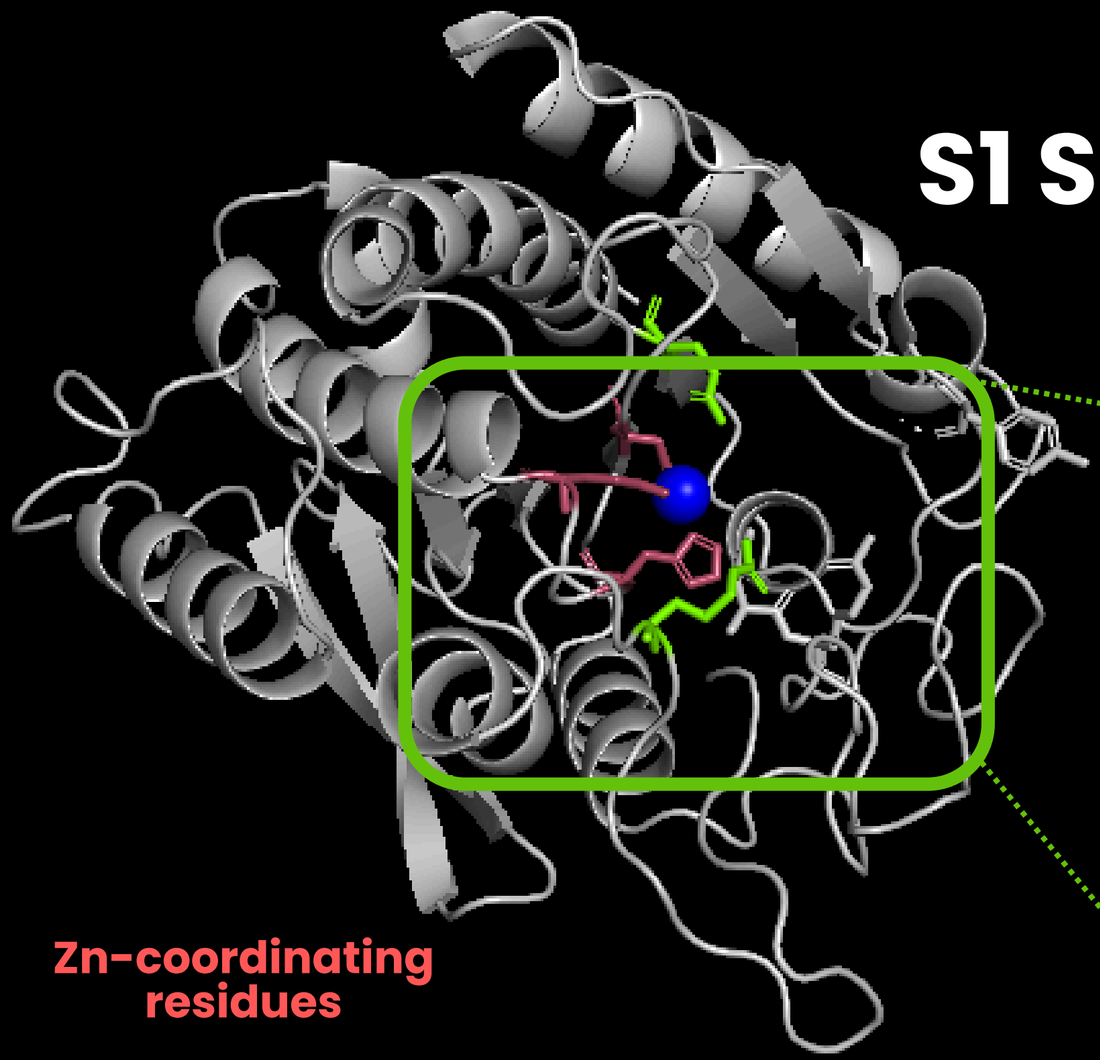
	116F	126W	136S	146N	156A	166H	176V
CBPA1_PIG/1-419	NPDGFAFTHSENRMWRKTRSRTSGSFCVGVDPNRNWDAGFGGAGASSNPCSETYHGKFPNSEVEVKS
CBPA1_BOVINE/1-307	NPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKS
CBPA1_HUMAN/1-419	NPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKS
CBPA1_RAT/1-419	NPDGFAYTHKTNRMWRKTRSHTQGSLCVGVDPNRNWDAGFGMAGASSNPCSETYRGKFPNSEVEVKS
CBPA1_MOUSE/1-419	NPDGFVYTHKTNRMWRKTRSHTEGSLCVGVDPNRNWDAAFGMPGASSNPCSETYRGKFPNSEVEVKS
CBPA1_ANOGA/1-433	NPDGYAYTFQVNRLWRKTR-KAYGPFCYGADPNRNWDFHWAEQGTSNNACSDTYHGSEAFSEVETRS
CBPA1_DROPS/1-425	NPDGYVYTHTTDRMWRKTR-TPYQG-CFGADPNRNWAFHWNEVGASSNPCSDTYAGPSAFSEIETLS
ECM14_AJEDR/1-592	NPDGYVHTWETDRLWRKNRQETSLPFCPGVDLDRTWGFEWNGNATGDNPCSESYGGDEPFAGTEARQ



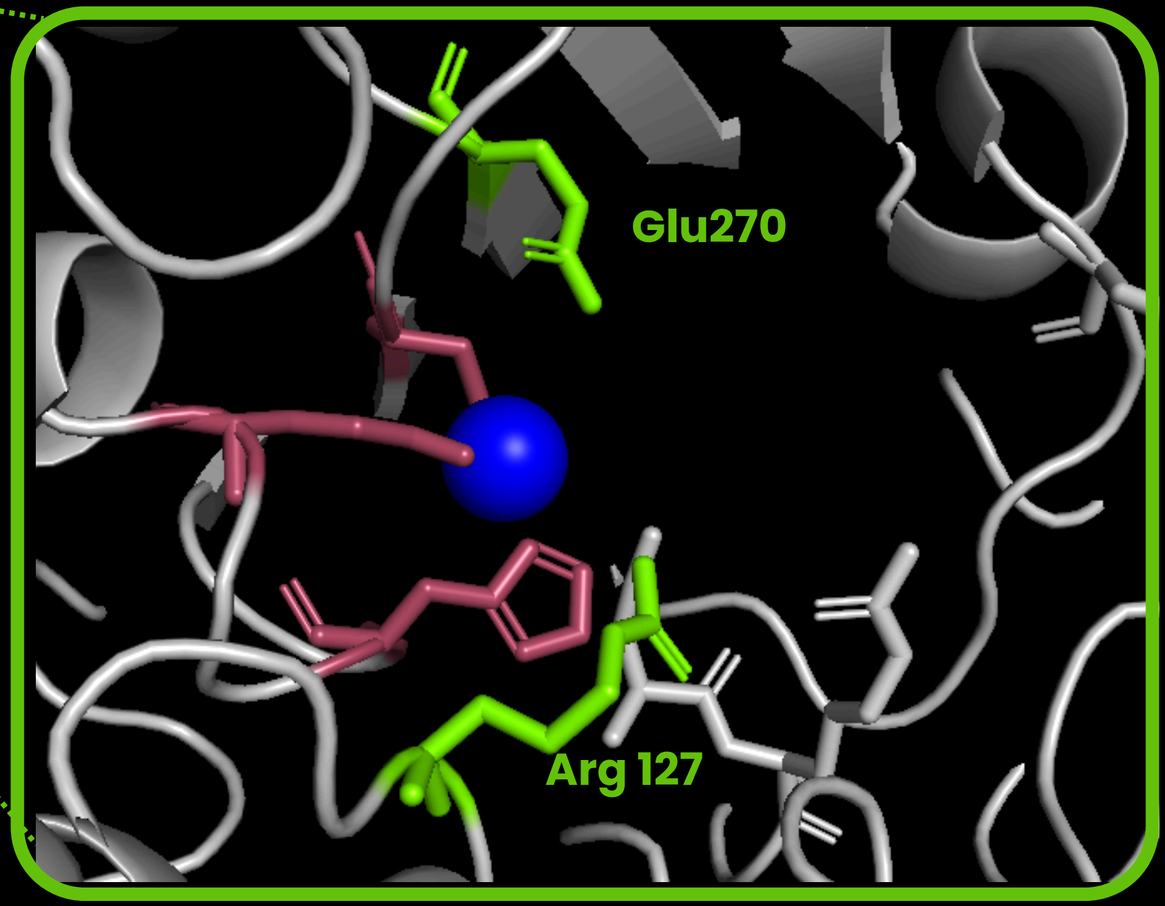
ARG145

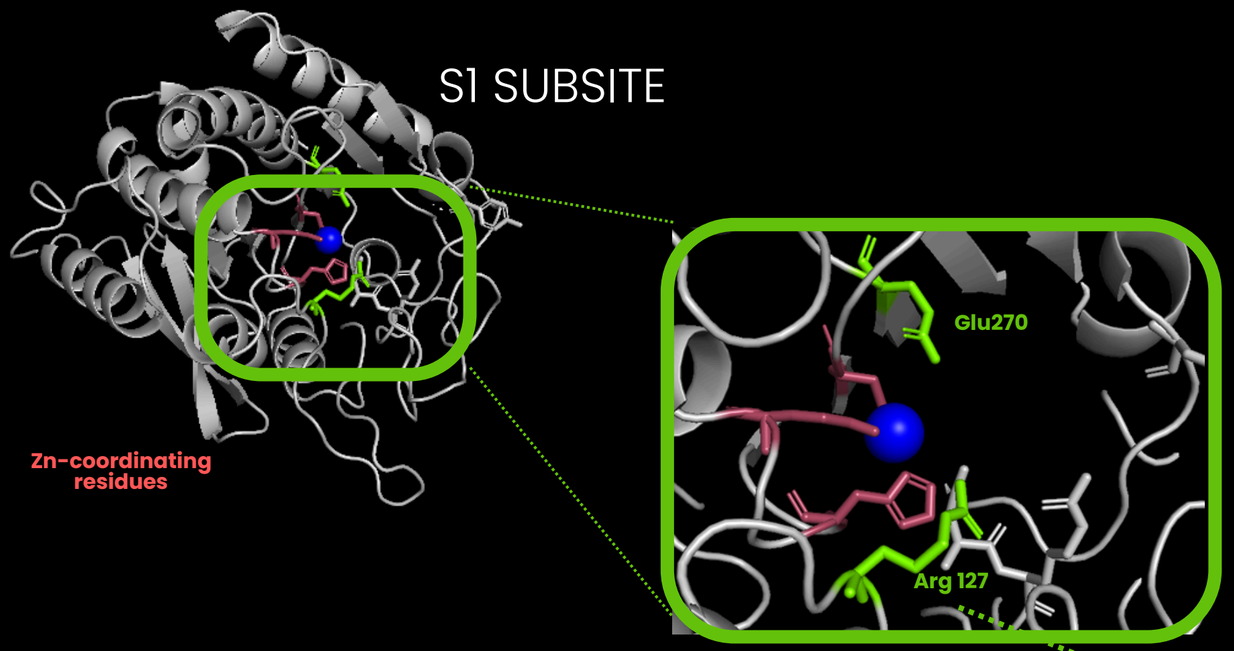
	116F	126W	136S	146N	156A	166H	176V
CBPA1_PIG/1-419	NPDGFAFTHSENRMWRKTRSRTSGSFCVGVDPNRNWDAGFGGAGASSNPCSETYHGKFPNSEVEVKS
CBPA1_BOVINE/1-307	NPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKS
CBPA1_HUMAN/1-419	NPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKS
CBPA1_RAT/1-419	NPDGFAYTHKTNRMWRKTRSHTQGSLCVGVDPNRNWDAGFGMAGASSNPCSETYRGKFPNSEVEVKS
CBPA1_MOUSE/1-419	NPDGFVYTHKTNRMWRKTRSHTEGSLCVGVDPNRNWDAAFGMPGASSNPCSETYRGKFPNSEVEVKS
CBPA1_ANOGA/1-433	NPDGYAYTFQVNRLWRKTR-KAYGPFCYGADPNRNWDFHWAEQGTSNNACSDTYHGSEAFSEVETRS
CBPA1_DROPS/1-425	NPDGYVYTHTTDRMWRKTR-TPYQG-CFGADPNRNWAFHWNEVGASSNPCSDTYAGPSAFSEIETLS
ECM14_AJEDR/1-592	NPDGYVHTWETDRLWRKNRQETSLPFCPGVDLDRTWGFEWNGNATGDNPCSESYGGDEPFAGTEARQ

S1 SUBSITE

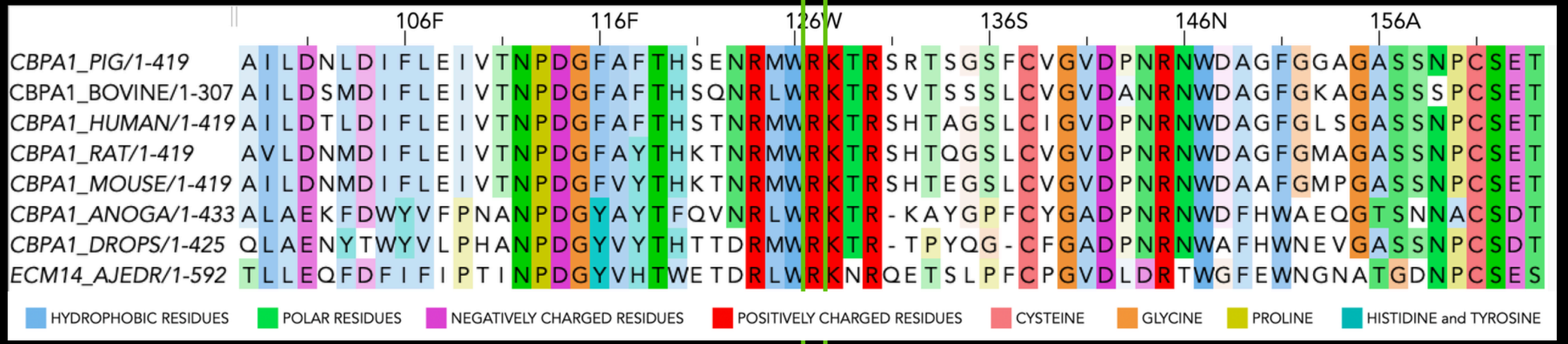


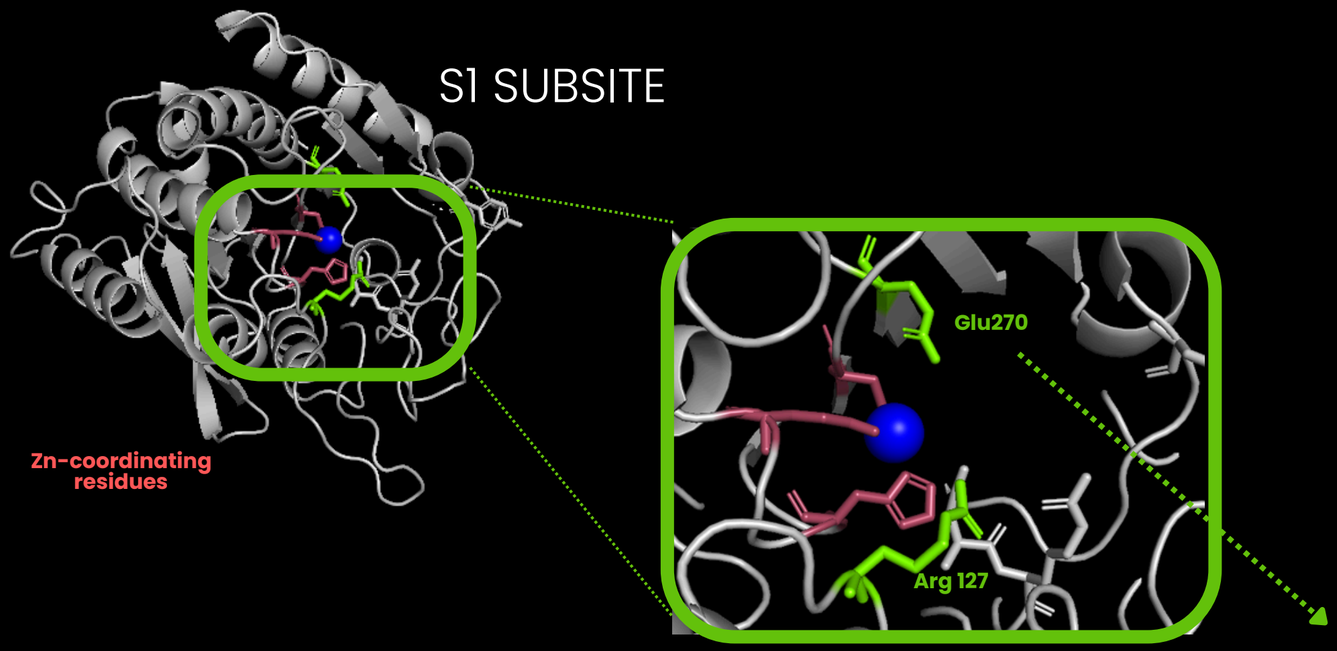
Zn-coordinating residues




S1 SUBSITE
Zn-coordinating residues
Glu270
Arg 127

ARG127

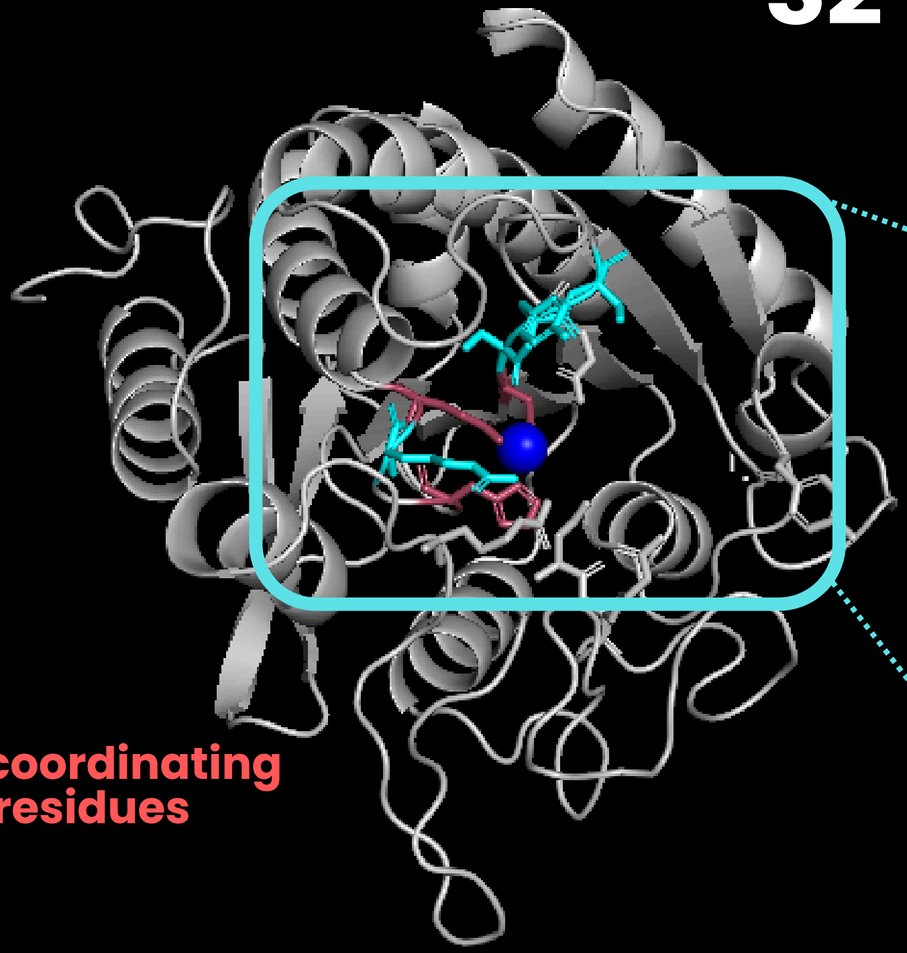

106F
116F
126W
136S
146N
156A
CBPA1_PIG/1-419 AILDNLDIFLEIVTNPDGFAFTHSENRMWRKTRSRTSGSFCVGVDPNRNWDAGFGGAGASSNPCSET
CBPA1_BOVINE/1-307 AILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGFGKAGASSSPCSET
CBPA1_HUMAN/1-419 AILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSET
CBPA1_RAT/1-419 AVLDNMDIFLEIVTNPDGFAYTHKTNRMWRKTRSHTQGSLCVGVDPNRNWDAGFGMAGASSNPCSET
CBPA1_MOUSE/1-419 AILDNMDIFLEIVTNPDGFVYTHKTNRMWRKTRSHTEGSLCVGVDPNRNWDAAFGMPGASSNPCSET
CBPA1_ANOGA/1-433 ALAEKFDWYVFPNANPDGYAYTFQVNRLWRKTR-KAYGPFCYGADPNRNWDFHWAEQGTSNNACSDT
CBPA1_DROPS/1-425 QLAENYTWYVLPHANPDGYVYTHTTDRMWRKTR-TPYQG-CFGADPNRNWAFHWNEVGASSNPCSDT
ECM14_AJEDR/1-592 TLLEQFDFIFIPTINPDGYVHTWETDRLWRKNRQETSLPFCPGVDLDRTWGFEWNGNATGDNPCSES
HYDROPHOBIC RESIDUES
POLAR RESIDUES
NEGATIVELY CHARGED RESIDUES
POSITIVELY CHARGED RESIDUES
CYSTEINE
GLYCINE
PROLINE
HISTIDINE and TYROSINE



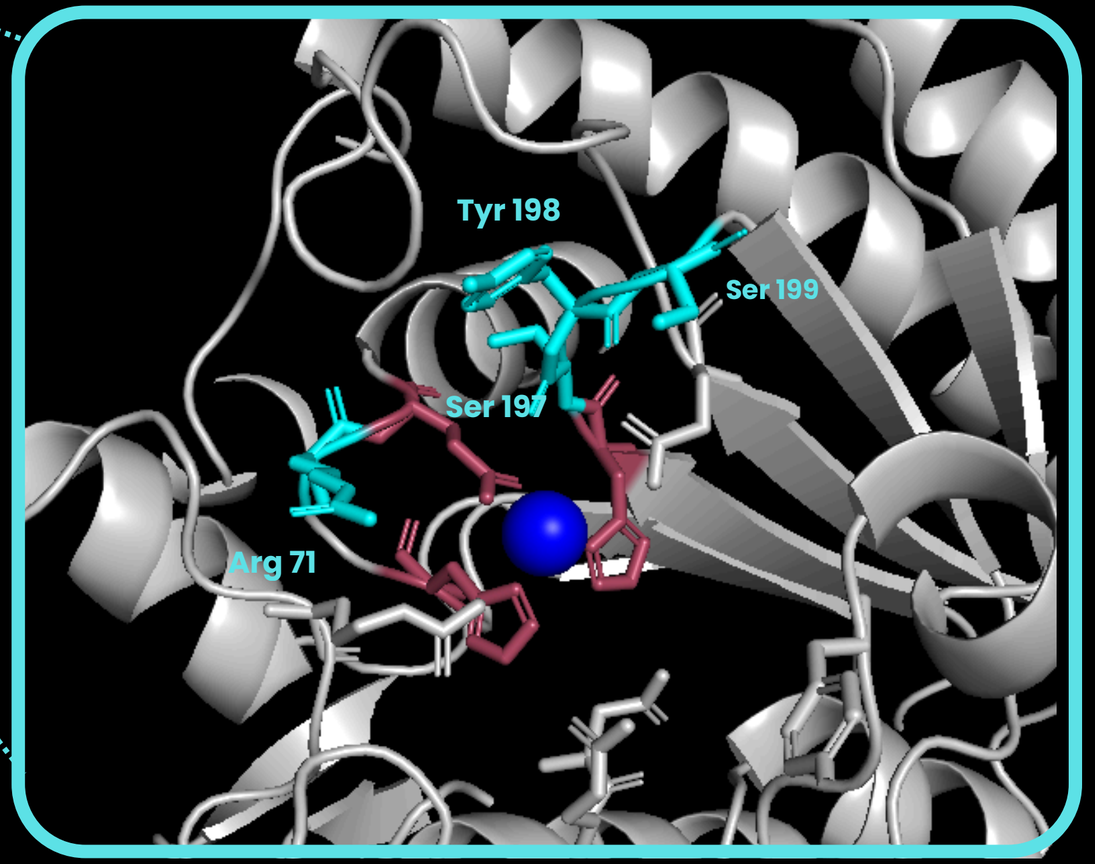
GLU270

```
                    245T          255I          264K          274T          281L          291Q          301M
CBPA1_PIG/1-419     --SIITTIYQASGGTIDWTY-NQGIKYSFSFELRDTG---RYGFLLPASQIIPTAQETWLALLTIME
CBPA1_BOVINE/1-307  --SIITTIYQASGGSIDWSY-NQGIKYSFTFELRDTG---RYGFLLPASQIIPTAQETWLGVLTIME
CBPA1_HUMAN/1-419   --SIIKAIYQASGSTIDWTY-SQGIKYSFTFELRDTG---RYGFLLPASQIIPTAKETWLALLTIME
CBPA1_RAT/1-419     --SIIDTIYQASGSTIDWTY-SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMD
CBPA1_MOUSE/1-419   --SIIDTIYQASGSTIDWTY-SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMD
CBPA1_ANOGA/1-433   --NVYDAIYPASGSSVDWSYGAQDVKIAYTYELRPDGDAWN-GFVLPPNEIVPTGEETLDSLITLLE
CBPA1_DROPS/1-425   --NIYDAIYPAAGASVDWAYGTQDVRMAFCYELRPSSNSFITGFKLPAAQIVPASEELLDSIVAMAA
ECM14_AJEDR/1-592   KLDTFPRMESTGGSALDWFYNDVGVKYSYQLKLRDKG---SYGFLLPRENIVPTGKEVFNAVMVLAK
```

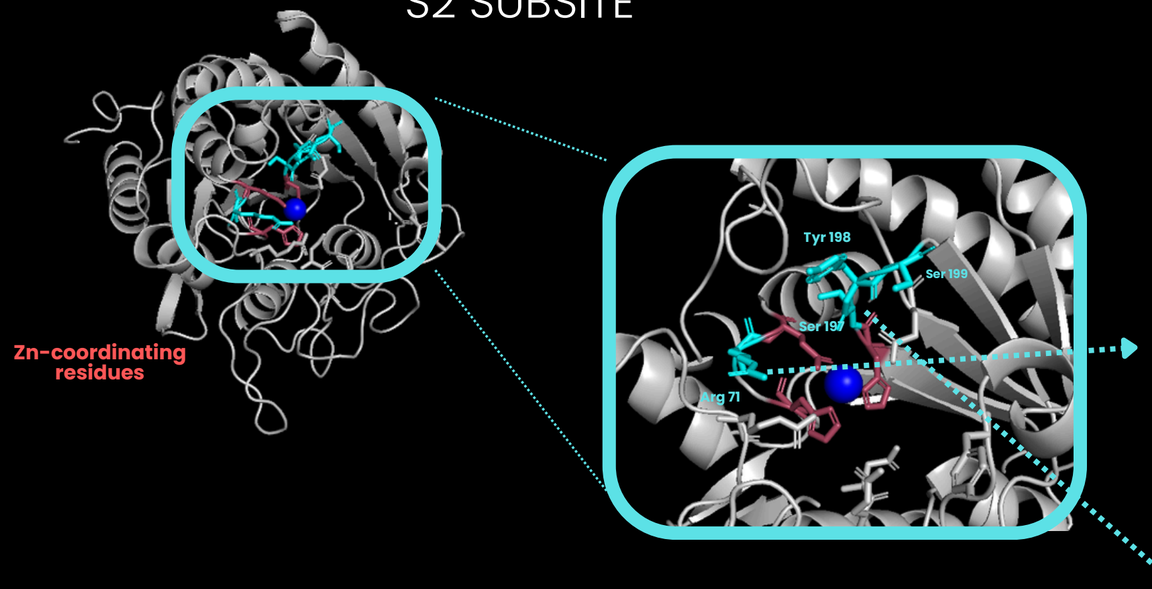
S2 SUBSITE



Zn-coordinating residues



S2 SUBSITE



ARG71

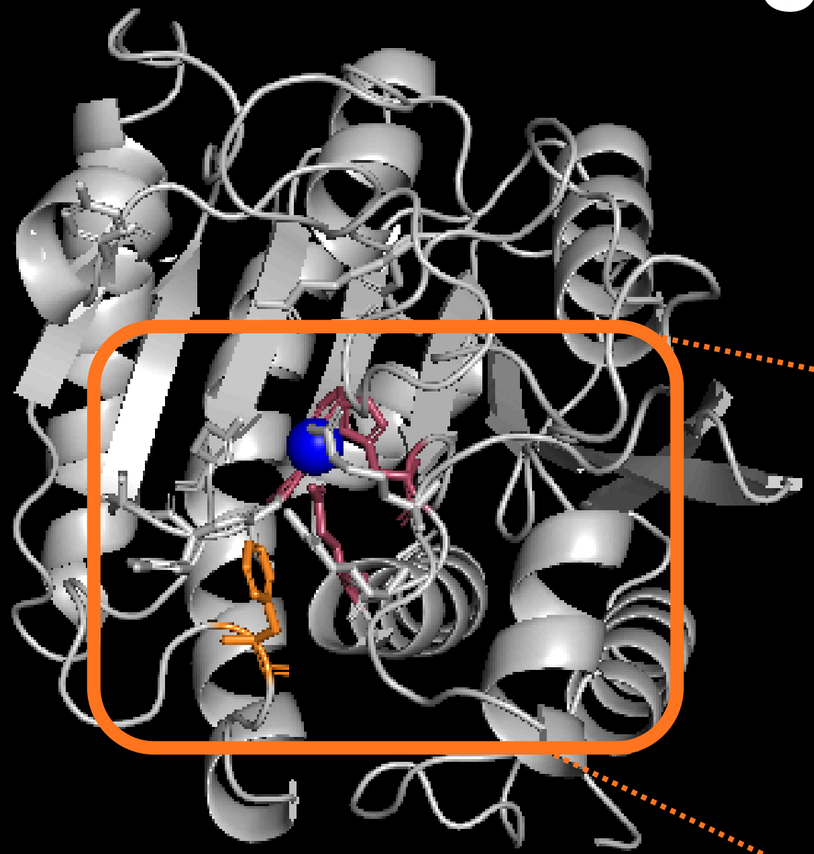
```
                    50L       60P       66L       76Q       86F       96F
CBPA1_PIG/1-419     GRPIYVLKFSTGGNNRP----AIWIDTGIHSREWVTQASGVWFAKKITEDYGQDPAFTAILDNLDIF
CBPA1_BOVINE/1-307  GRPIYVLKFSTGGSNRP----AIWIDLGIHSREWITQATGVWFAKKFTEDYGQDPSFTAILDSMDIF
CBPA1_HUMAN/1-419   GRPIYVLKFSTGGSKRP----AIWIDTGIHSREWVTQASGVWFAKKITQDYGQDAAFTAILDTLDIF
CBPA1_RAT/1-419     GRPIHVLKFSTGGTNRP----AIWIDTGIHSREWVTQASGVWFAKKITKDYGQDPTFTAVLDNMDIF
CBPA1_MOUSE/1-419   GRPINVLKFSTGGTNRP----AIWIDTGIHSREWVTQASGVWFAKKITKDYGQEPTLTAILDNMDIF
CBPA1_ANOGA/1-433   NRTMKGVKLS-YGPGRP----GVFLEGGIHAREWISPATVTYIINQLLTS--EDAKVRALAEKFDWY
CBPA1_DROPS/1-425   GRSILGVKITKSGKEKP----GIFLEAGIHAREWIAPAAATYIINQLLTS--EVEAVKQLAENYTWY
ECM14_AJEDR/1-592   GRDIPALRVGVRPANDPKPRKTVIIGGGSHAREWIGVSTVNYVAYSLITTYGKSTPISTLLEQFDFI
```

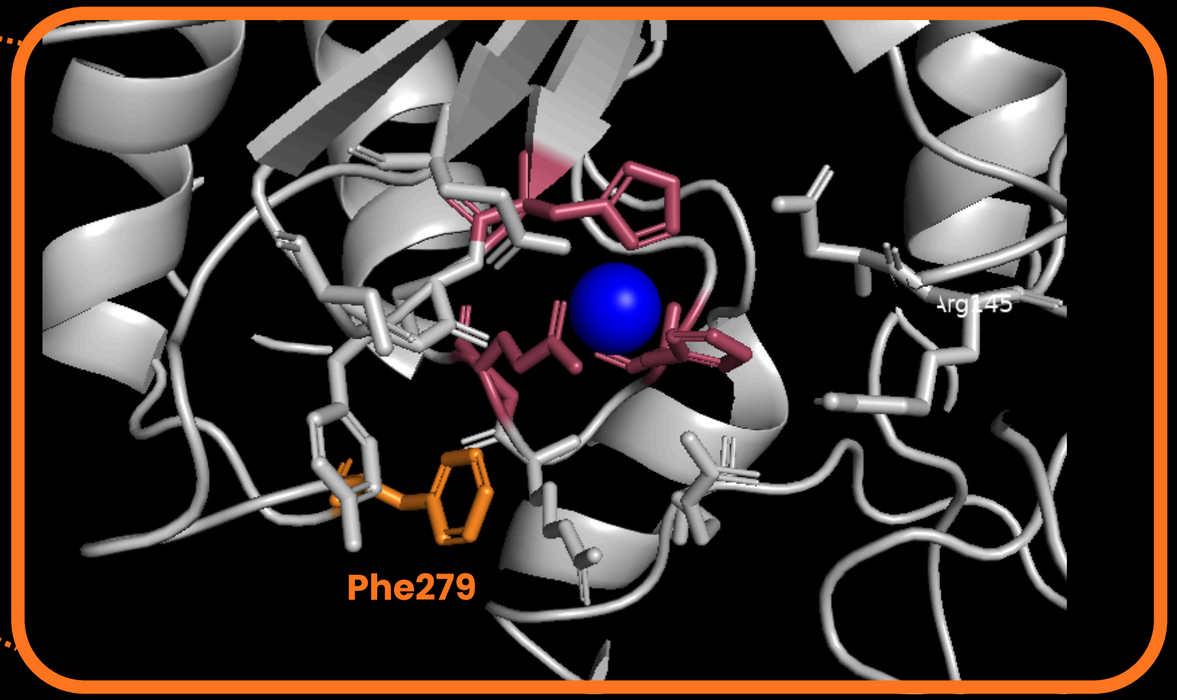
TYR198

SER197 SER199

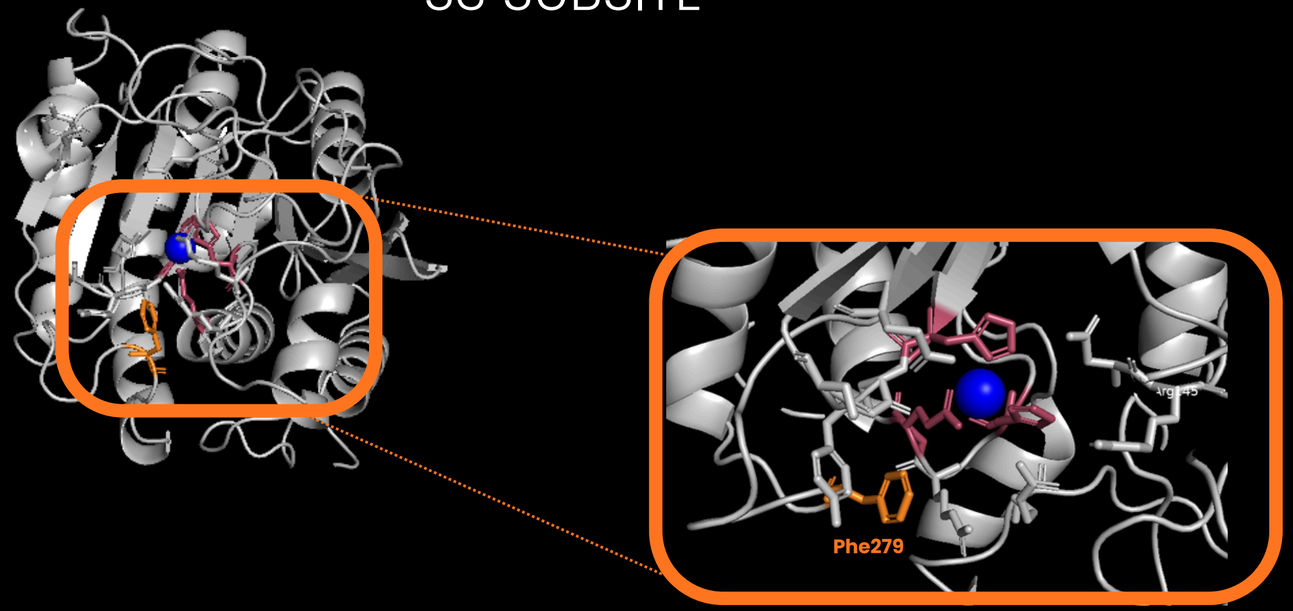
```
                    176V      186H      191A      201L      211Q      220N
CBPA1_PIG/1-419     PNSEVEVKSIVDFVNDHG-----NIKAFISIHSYSQLLLYPYGYKTEA-PADKDELDQISKSAVAAL
CBPA1_BOVINE/1-307  ANSEVEVKSIVDFVKDHG-----NFKAFLSIHSYSQLLLYPYGYTTQS-IPDKTELNQVAKSAVAAL
CBPA1_HUMAN/1-419   ANSEVEVKSIVDFVKDHG-----NIKAFISIHSYSQLLMYPYGYKTEP-VPDQDELDQLSKAAVTAL
CBPA1_RAT/1-419     PNSEVEVKSIVDFVTSHG-----NIKAFISIHSYSQLLLYPYGYTSEP-APDQAELDQLAKSAVTAL
CBPA1_MOUSE/1-419   PNSEVEVKSIVDFVTSHG-----NIKAFISIHSYSQLLLYPYGYTSEP-APDKEELDQLAKSAVTAL
CBPA1_ANOGA/1-433   AFSEVETRSLAAFVEKLRG----KLGAYIAFHSYSQLLLFPYGHTGEH-SPNHQDLNEIAEATVKSL
CBPA1_DROPS/1-425   AFSEIETLSLSNYLASLKG----KIQLYISLHAYSQYLLYPYGHTGDL-PDNVADFRKVYDVSIAAV
ECM14_AJEDR/1-592   PFAGTEARQLAGWVKEQTEQHNVKFVAYLDLHSYSQQVLYPYSYSCLPRPPNLENLEELAMGIAKAI
```

S3 SUBSITE





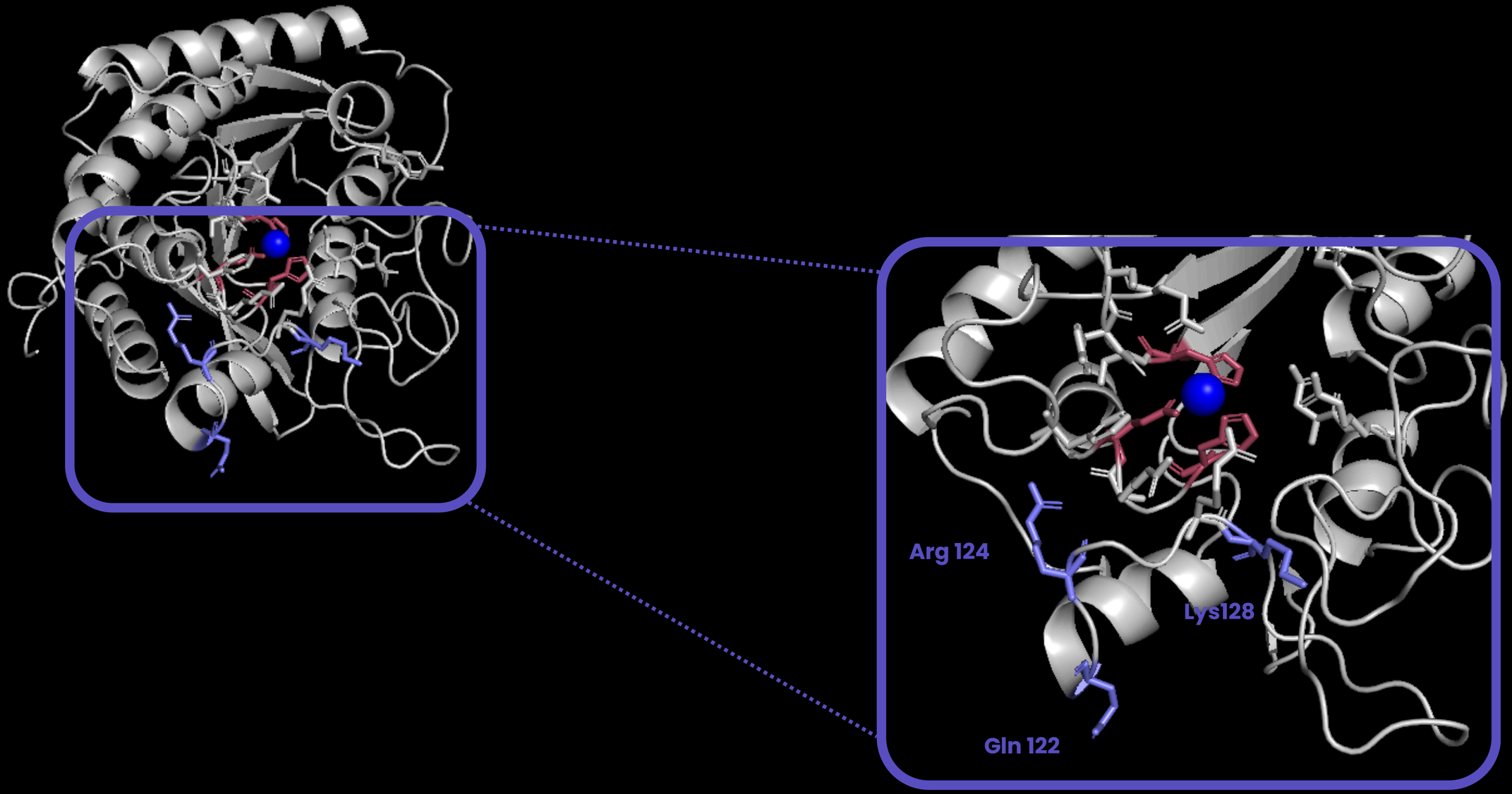
S3 SUBSITE



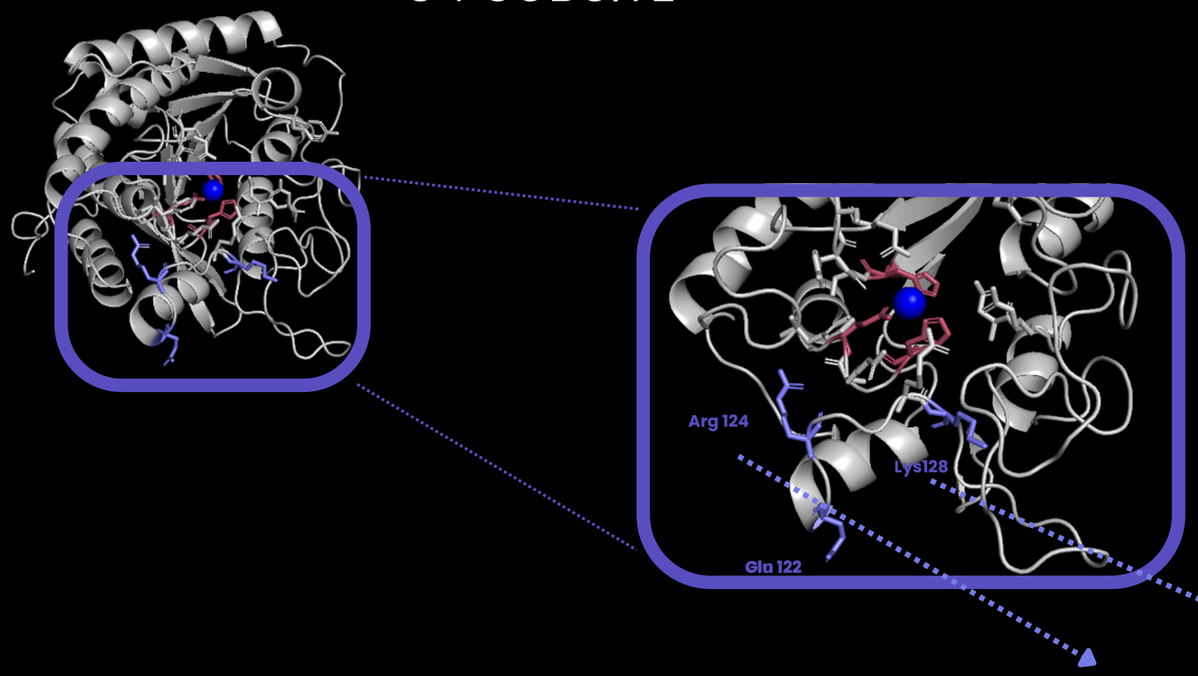
PHE279

```
                          255I          264K          274T          281L          291Q          301M
CBPA1_PIG/1-419           QASGGTIDWTY-NQGIKYSFSFELRDTG---RYGFLLPASQIIPTAQETWLALLTIMEHTLNHPY--
CBPA1_BOVINE/1-307        QASGGSIDWSY-NQGIKYSFTFELRDTG---RYGFLLPASQIIPTAQETWLGVLTIMEHTVNN----
CBPA1_HUMAN/1-419         QASGSTIDWTY-SQGIKYSFTFELRDTG---RYGFLLPASQIIPTAKETWLALLTIMEHTLNHPY--
CBPA1_RAT/1-419           QASGSTIDWTY-SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMDHTVKHPY--
CBPA1_MOUSE/1-419         QASGSTIDWTY-SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMDHTVKHPY--
CBPA1_ANOGA/1-433         PASGSSVDWSYGAQDVKIAYTYELRPDGDAWN-GFVLPPNEIVPTGEETLDSLITLLEESSARGYYD
CBPA1_DROPS/1-425         PAAGASVDWAYGTQDVRMAFCYELRPSSNSFITGFKLPAAQIVPASEELLDSIVAMAAEVNNLGYFN
ECM14_AJEDR/1-592         STGGSALDWFYNDVGVKYSYQLKLRDKG---SYGFLLPRENIVPTGKEVFNAVMVLAKFLLGSDGFE
```

S4 SUBSITE



S4 SUBSITE

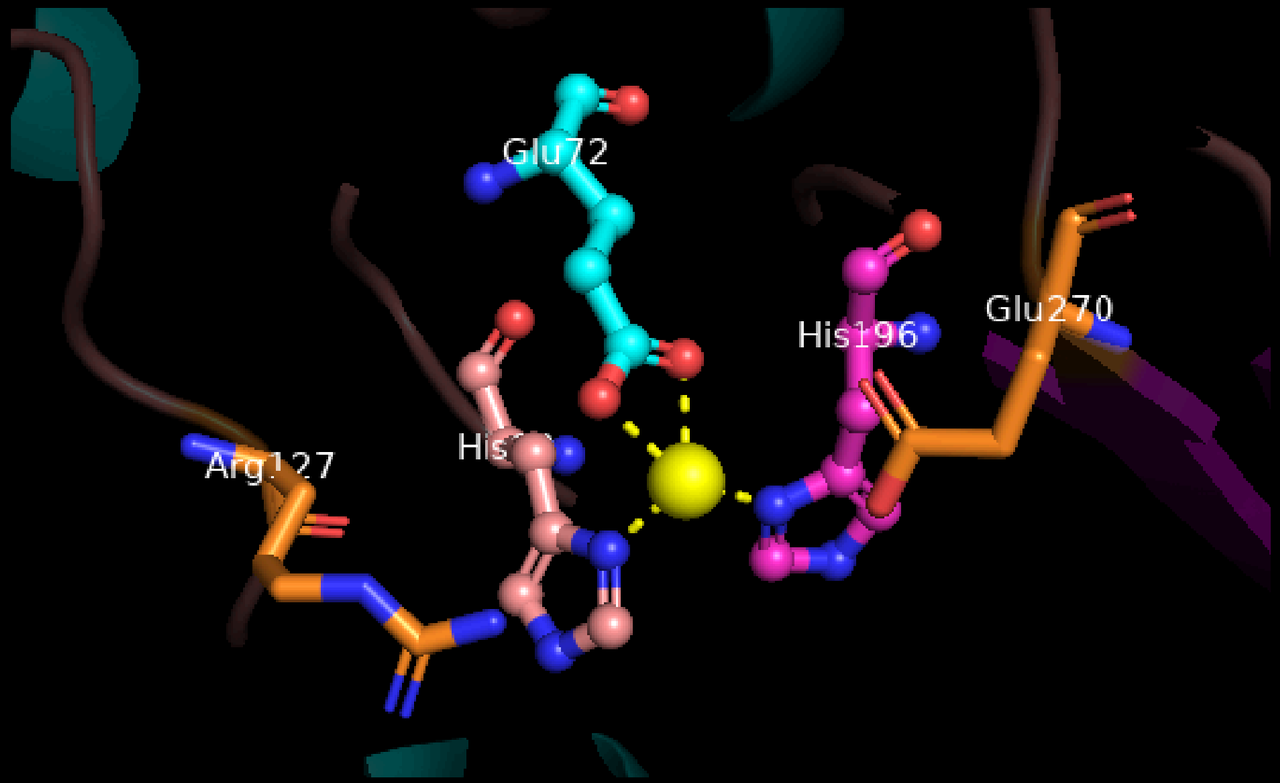


ARG124 **LYS128**

```
                                96F                 106F                116F                126W                136S                146N                156A
CBPA1_PIG/1-419          PAFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRKTRSRTSGSFCVGVDPNRNWDAGFGGAGASSNP
CBPA1_BOVINE/1-307       PSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGFGKAGASSSP
CBPA1_HUMAN/1-419        AAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNP
CBPA1_RAT/1-419          PTFTAVLDNMDIFLEIVTNPDGFAYTHKTNRMWRKTRSHTQGSLCVGVDPNRNWDAGFGMAGASSNP
CBPA1_MOUSE/1-419        PTLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRKTRSHTEGSLCVGVDPNRNWDAAFGMPGASSNP
CBPA1_ANOGA/1-433        AKVRALAEKFDWYVFPNANPDGYAYTFQVNRLWRKTR-KAYGPFCYGADPNRNWDFHWAEQGTSNNA
CBPA1_DROPS/1-425        EAVKQLAENYTWYVLPHANPDGYVYTHTTDRMWRKTR-TPYQG-CFGADPNRNWAFHWNEVGASSNP
ECM14_AJEDR/1-592        TPISTLLEQFDFIFIPTINPDGYVHTWETDRLWRKNRQETSLPFCPGVDLDRTWGFEWNGNATGDNP
```

CATALYSIS

CATALYTIC SITE



Zn2+ coordinating residues:

- His69
- His196
- Glu72

Other important residues:

- Glu270
- Arg127

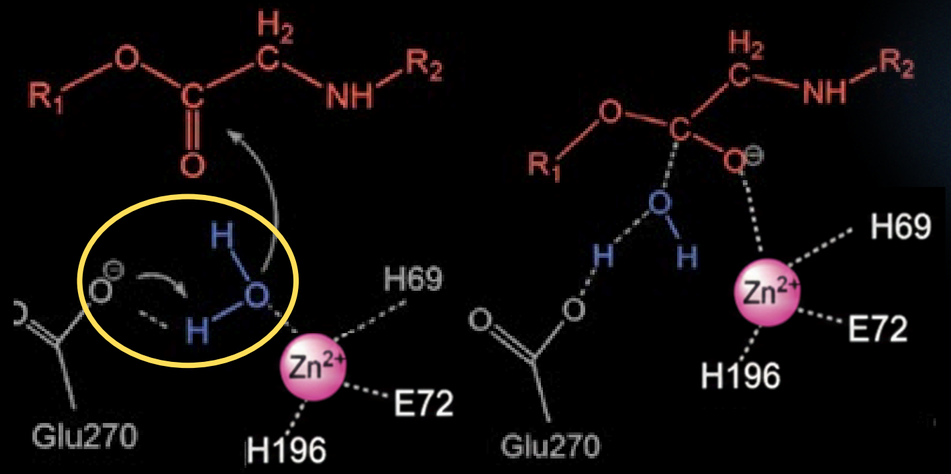
CATALYSIS

TWO PATHWAYS PROPOSED

PROMOTED-WATER
OR GENERAL BASE-GENERAL ACID

NUCLEOPHILIC
OR “ANHYDRIDE”

PROMOTED-WATER
OR GENERAL BASE-GENERAL ACID



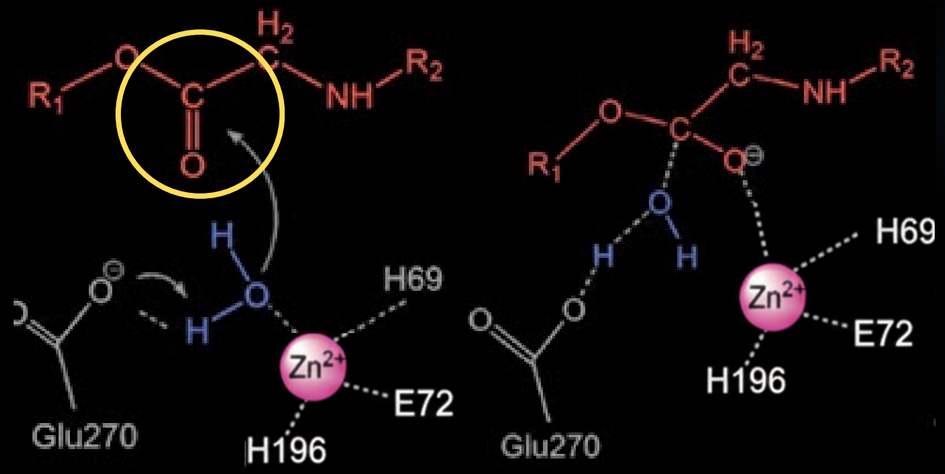
Adapted from Amangani et al. (1992)

Glu270 removes one H+ from H2O generating a nucleophilic OH-

STEP 1: Nucleophilic attack

- Glu270 → BASE

PROMOTED-WATER
OR GENERAL BASE-GENERAL ACID



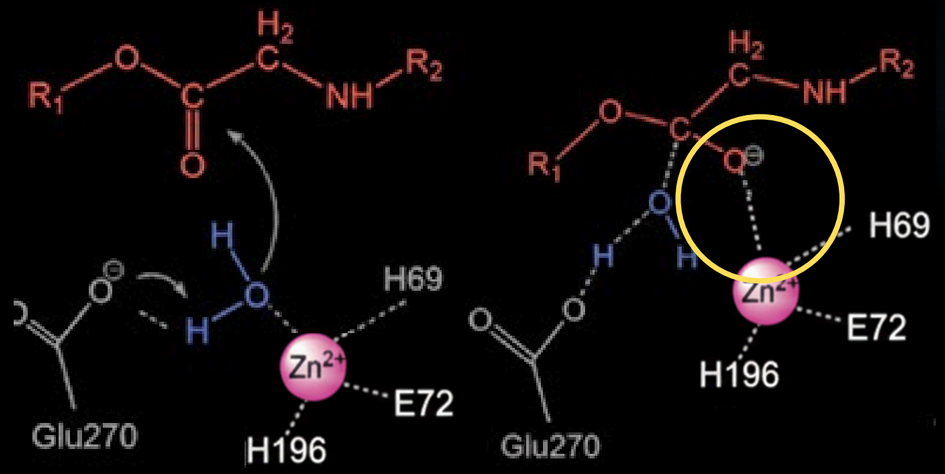
Adapted from Amangani et al. (1992)

The OH- attacks the cabonylic carbon forming a tetrahedral intermediate

STEP 1: Nucleophilic attack

- Glu270 → BASE

PROMOTED-WATER
OR GENERAL BASE-GENERAL ACID



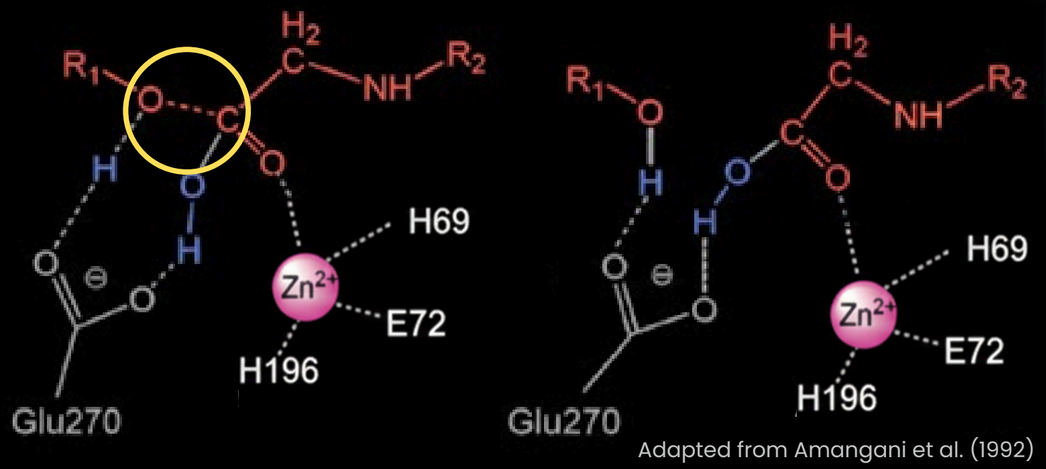
Adapted from Amangani et al. (1992)

Stabilization of the oxyanion by Zn2+ and Arg127

STEP 1: Nucleophilic attack

- Glu270 → BASE

PROMOTED-WATER
OR GENERAL BASE-GENERAL ACID



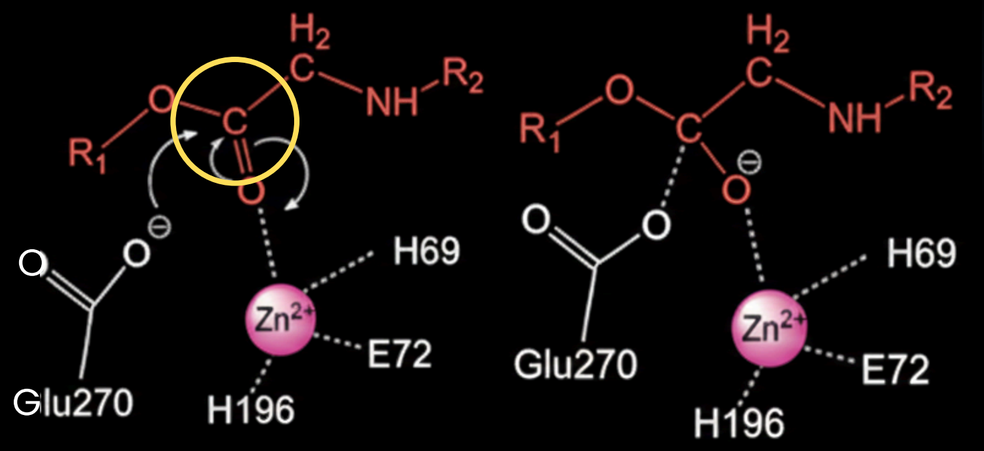
Adapted from Amangani et al. (1992)

Glu270 trasfers a H+ causing the break of the scissile bond

STEP 2: Elimination

- Glu270 → ACID

NUCLEOPHILIC OR “ANHYDRIDE”

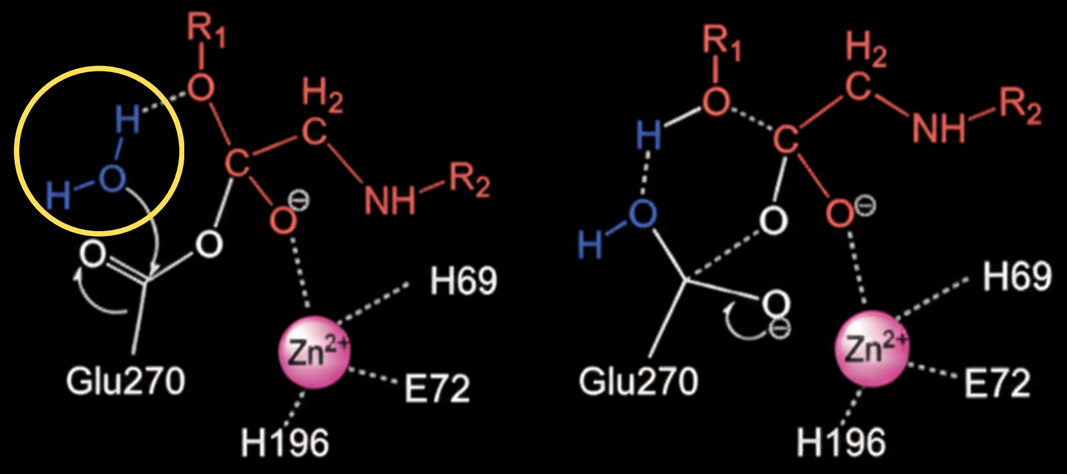


Adapted from Amangani et al. (1992)

Glu270 attacks the cabonylic carbon from the substrate

STEP 1: Acylation

NUCLEOPHILIC OR “ANHYDRIDE”

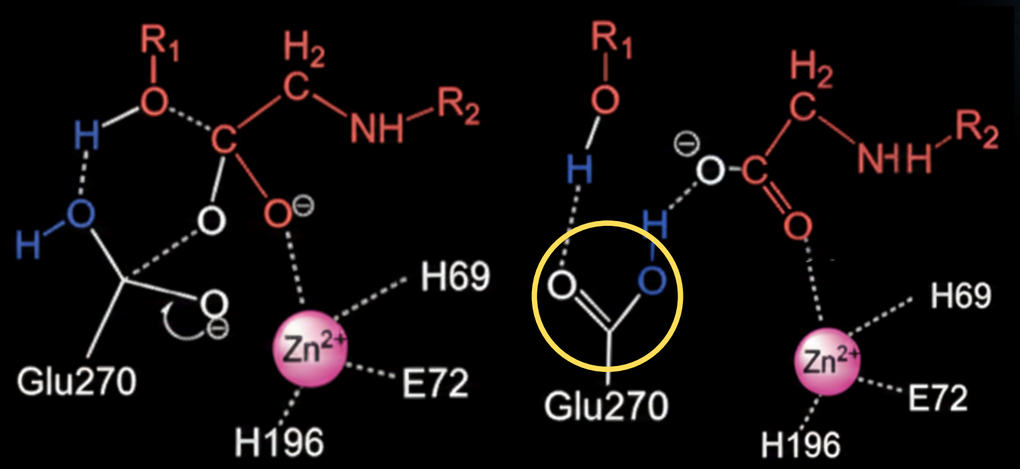


Adapted from Amangani et al. (1992)

H20 attacks the carboxylic carbon from Glu270

STEP 2: Desacylation

NUCLEOPHILIC OR “ANHYDRIDE”

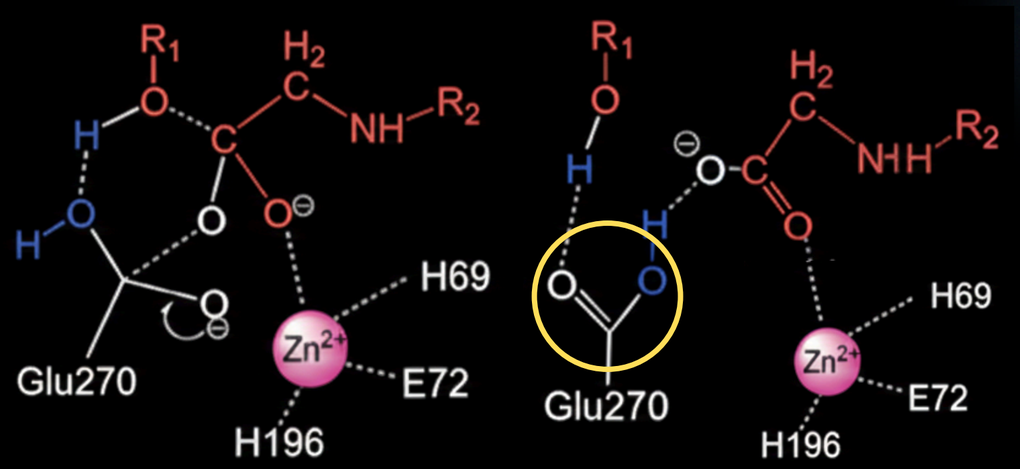


Adapted from Amangani et al. (1992)

Glu270 reforms and the substrate is liberated

STEP 2: Desacylation

NUCLEOPHILIC OR “ANHYDRIDE”



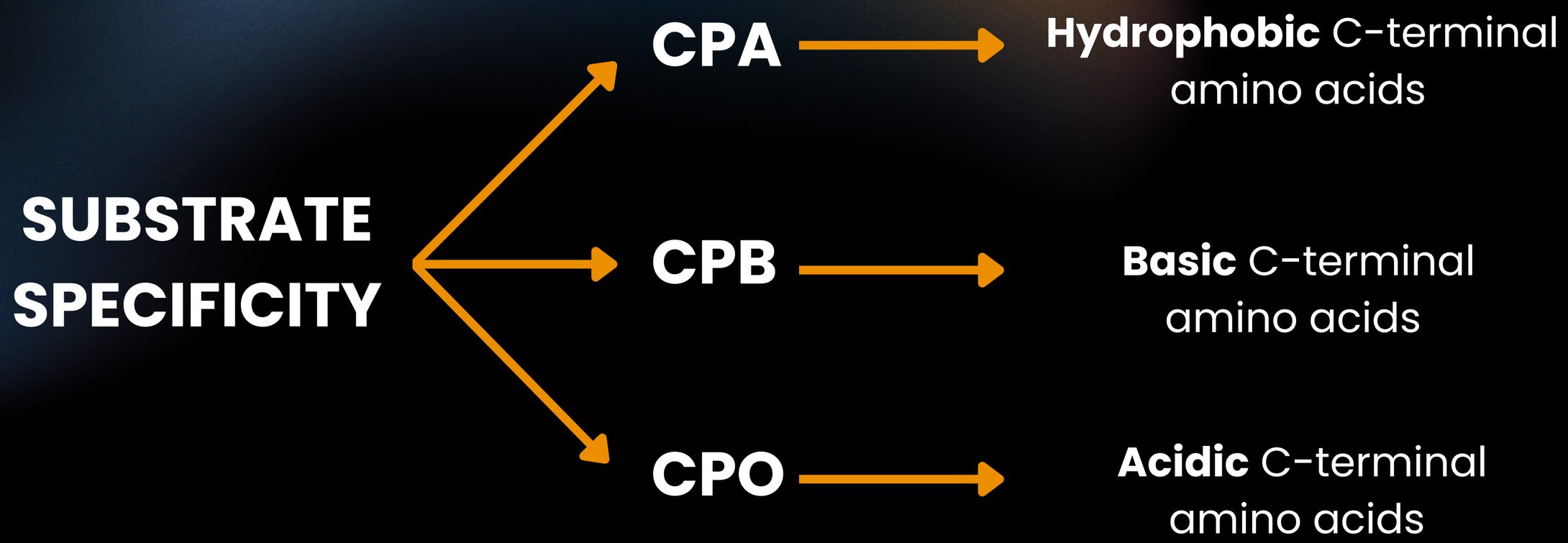
Adapted from Amangani et al. (1992)

STEP 2: Desacylation

Glu270 reforms and the substrate is liberated

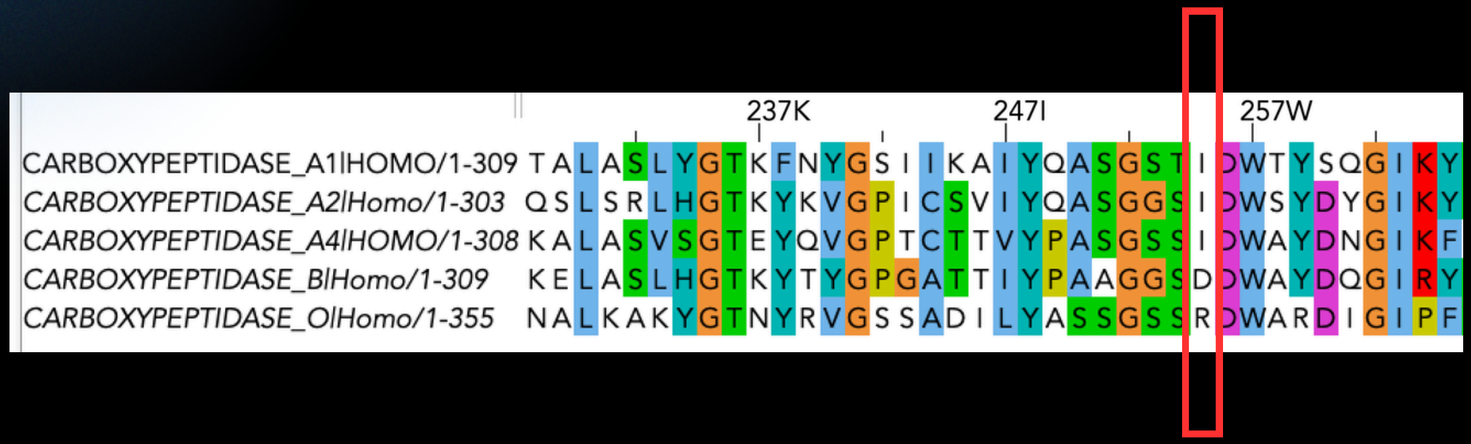
Less likely to occur with peptides

SPECIFICITY

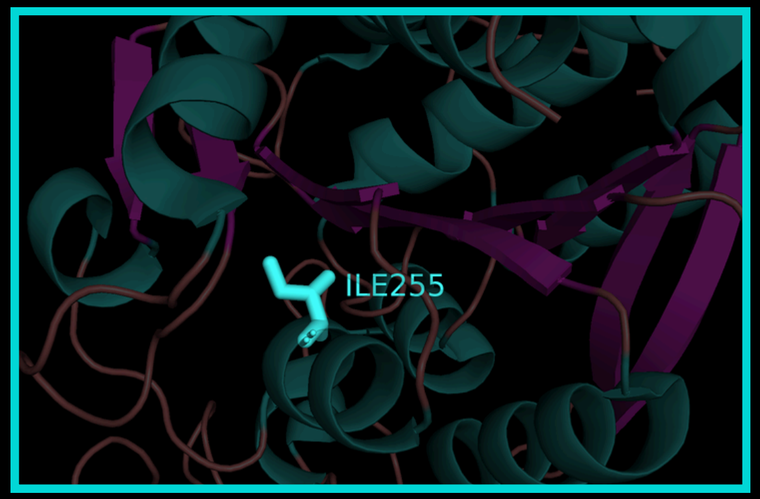

SUBSTRATE SPECIFICITY
CPA
Hydrophobic C-terminal amino acids
CPB
Basic C-terminal amino acids
CPO
Acidic C-terminal amino acids

SUBSTRATE SPECIFICITY

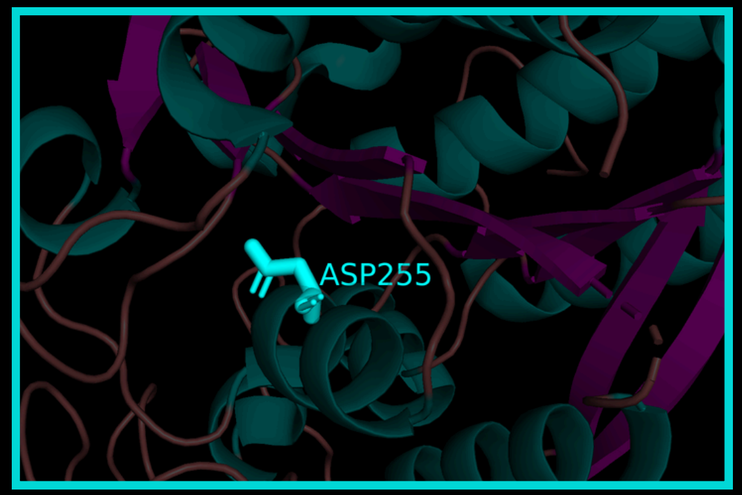
- **CPA** → Hydrophobic C-terminal amino acids
- **CPB** → Basic C-terminal amino acids
- **CPO** → Acidic C-terminal amino acids



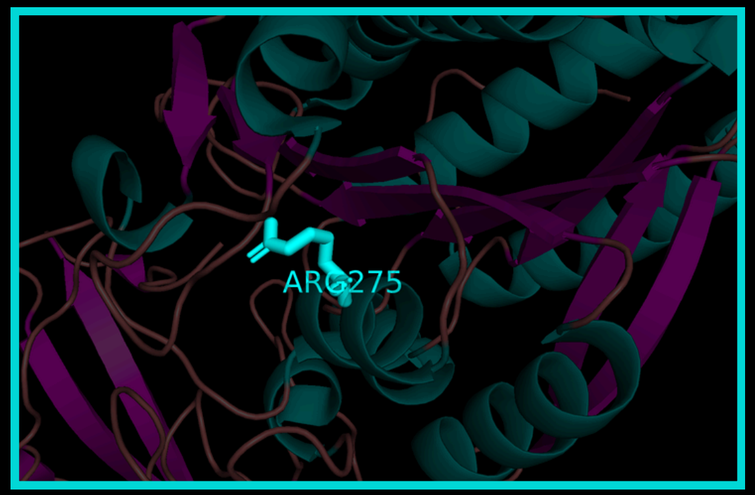
CARBOXYPEPTIDASE **A**

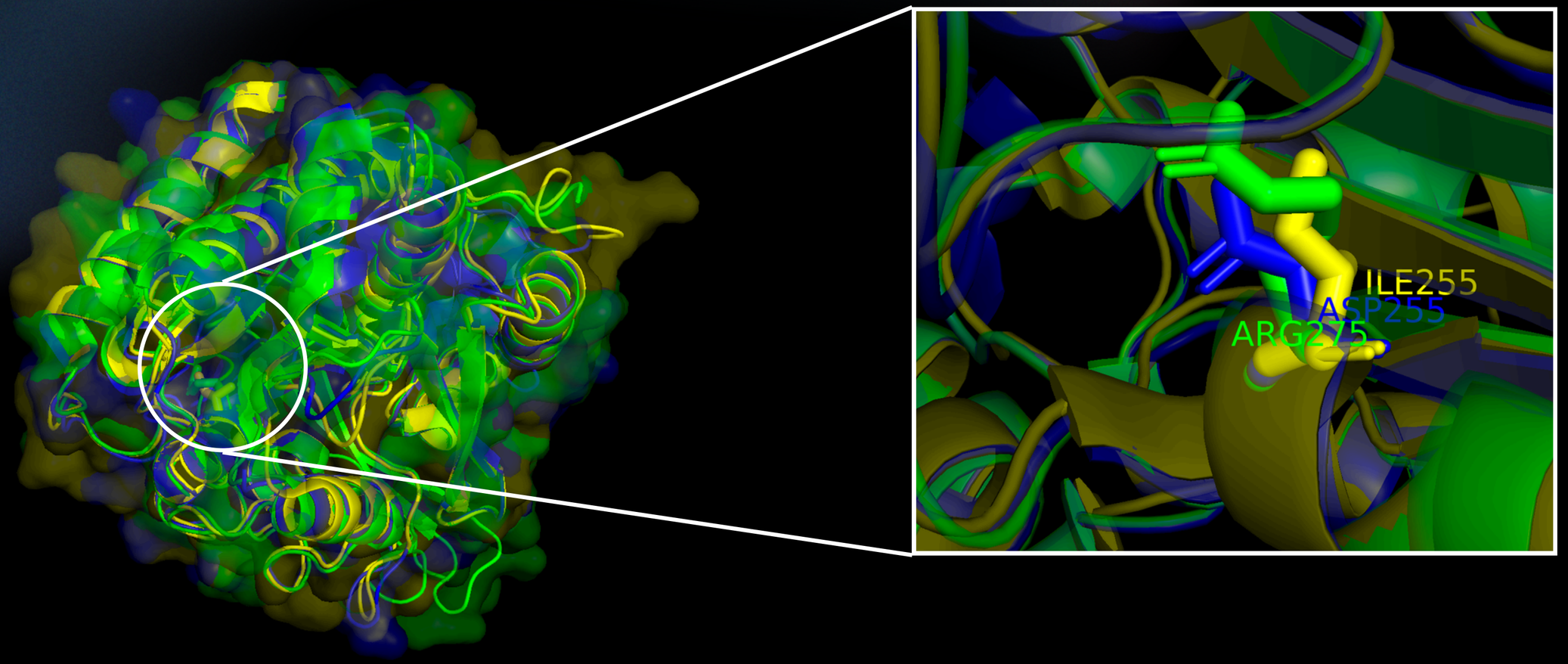


CARBOXYPEPTIDASE **B**



CARBOXYPEPTIDASE **O**





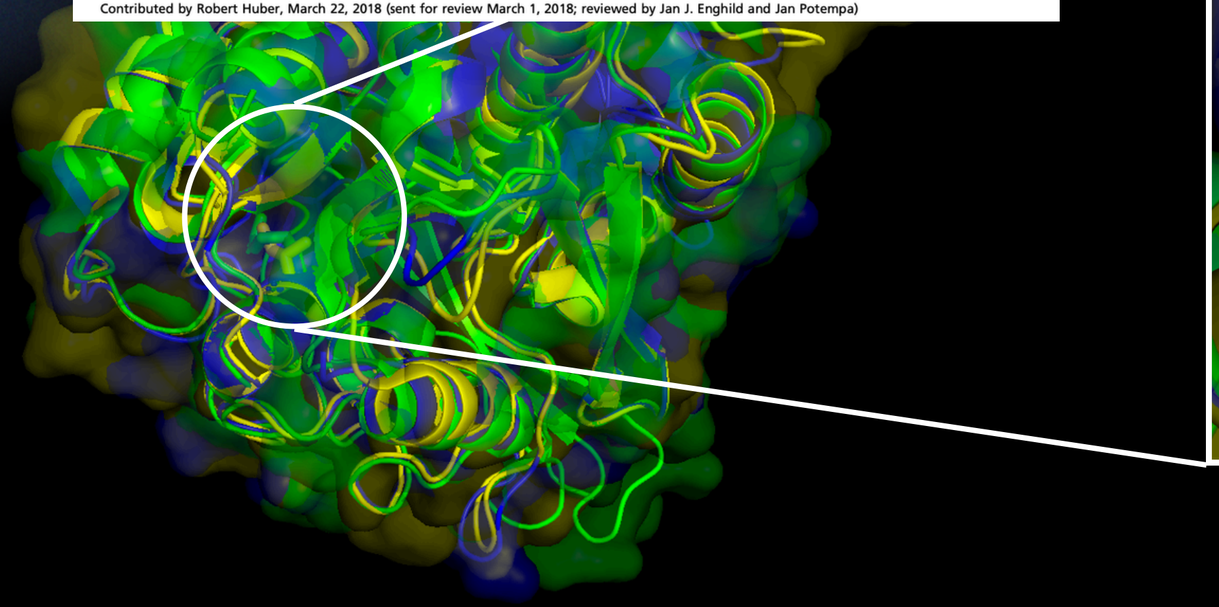
Specificity differences arise from localized side-chain variations

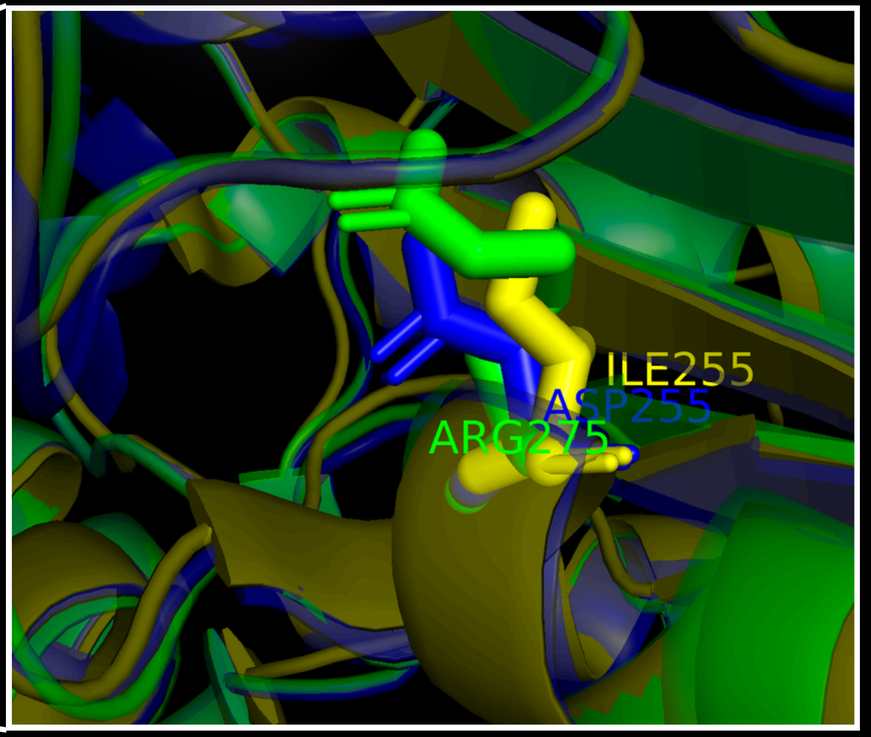
Crystal structure and mechanism of human carboxypeptidase O: Insights into its specific activity for acidic residues

Maria C. Garcia-Guerrero[a,b,1], Javier Garcia-Pardo[a,b,c,1], Esther Berenguer[a,b], Roberto Fernandez-Alvarez[a,b], Gifty B. Barfi[d], Peter J. Lyons[d], Francesc X. Aviles[a,b], Robert Huber[e,f,g,2], Julia Lorenzo[a,b,2], and David Reverter[a,b,2]

[a]Institute for Biotechnology and Biomedicine, Universitat Autonoma de Barcelona, 08193 Bellaterra, Barcelona, Spain; [b]Department of Biochemistry and Molecular Biology, Universitat Autonoma de Barcelona, 08193 Bellaterra, Barcelona, Spain; [c]Catalan Institute of Nanoscience and Nanotechnology, Consejo Superior de Investigaciones Cientificas, The Barcelona Institute of Science and Technology, 08193 Bellaterra, Barcelona, Spain; [d]Department of Biology, Andrews University, Berrien Springs, MI 49104; [e]Max Planck Institut für Biochemie, D-82152 Martinsried, Germany; [f]Zentrum für Medizinische Biotechnologie, Universität Duisburg-Essen, D-45117 Essen, Germany; and [g]Fakultät für Chemie, Technische Universität München, D-85747 Garching, Germany

Contributed by Robert Huber, March 22, 2018 (sent for review March 1, 2018; reviewed by Jan J. Enghild and Jan Potempa)





Specificity differences arise from localized side-chain variations

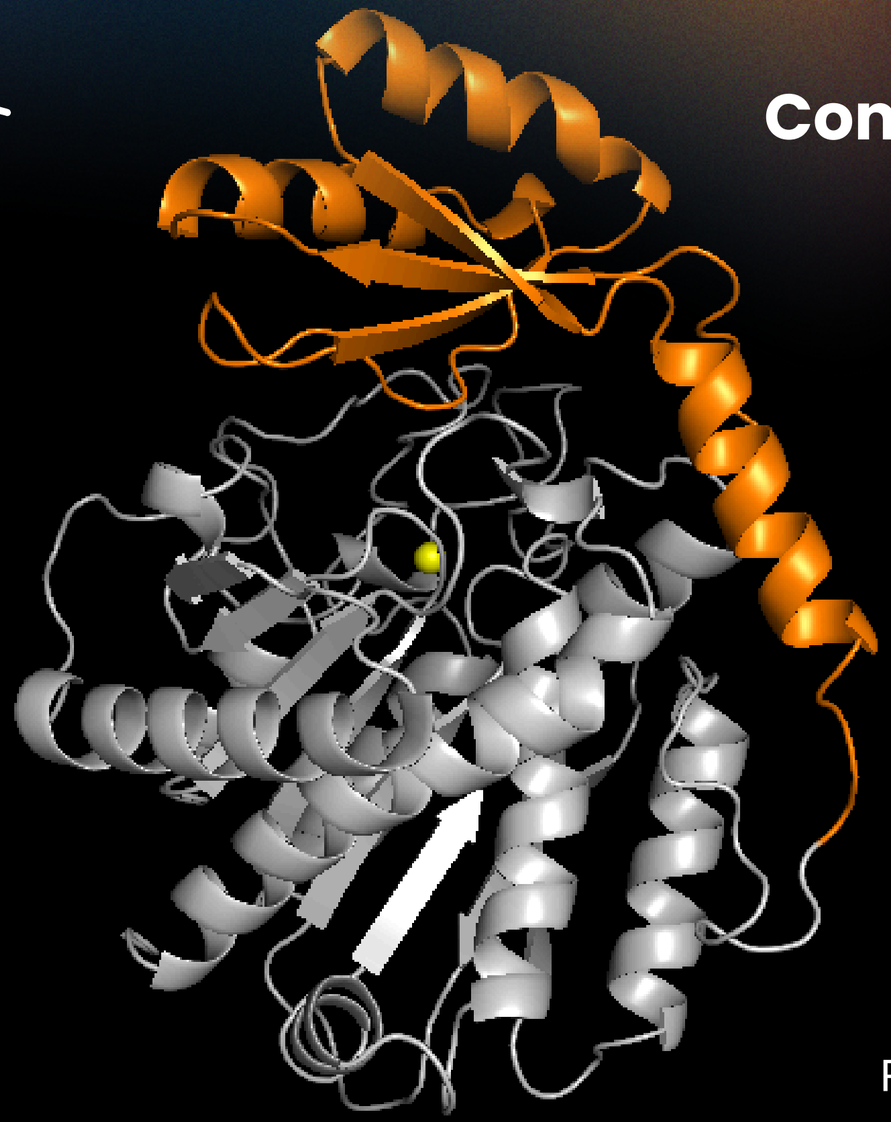
PRO-CARBOXYPEPTIDASE

GENERAL STRUCTURE

Activation segment

N-terminal
4 antiparallel B-sheets
2 a-helix
Globular structure

Enzime



Connecting segment

C-terminal
a-helix
Loop

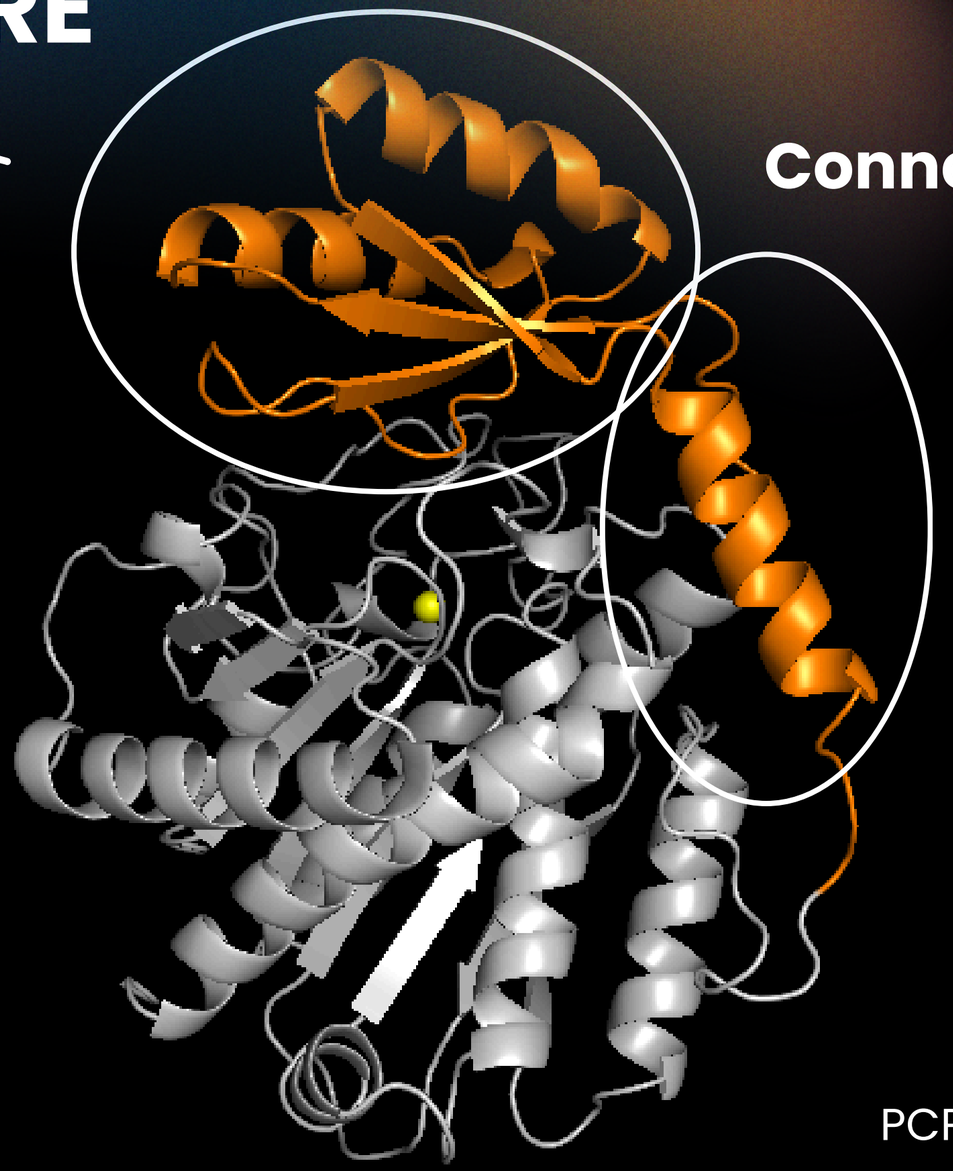
PCPA bovine

GENERAL STRUCTURE

Activation segment

N-terminal
4 antiparallel B-sheets
2 a-helix
Globular structure

Enzime

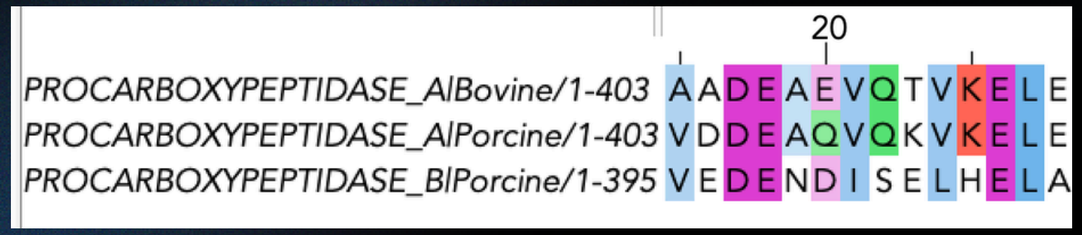


Connecting segment

C-terminal
a-helix
Loop

PCPA bovine

COMPARING PCPA AND PCPB



- Activation segments (94-96 residues) with equivalent folds
- Porcine and bovine PCPAs are homologues



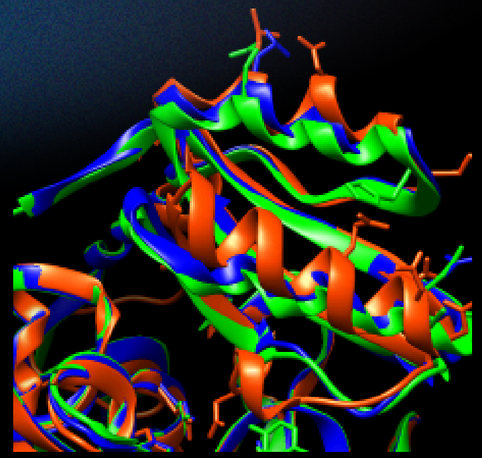
Porcine PCPB **Porcine PCPA** **Bovine PCPA**

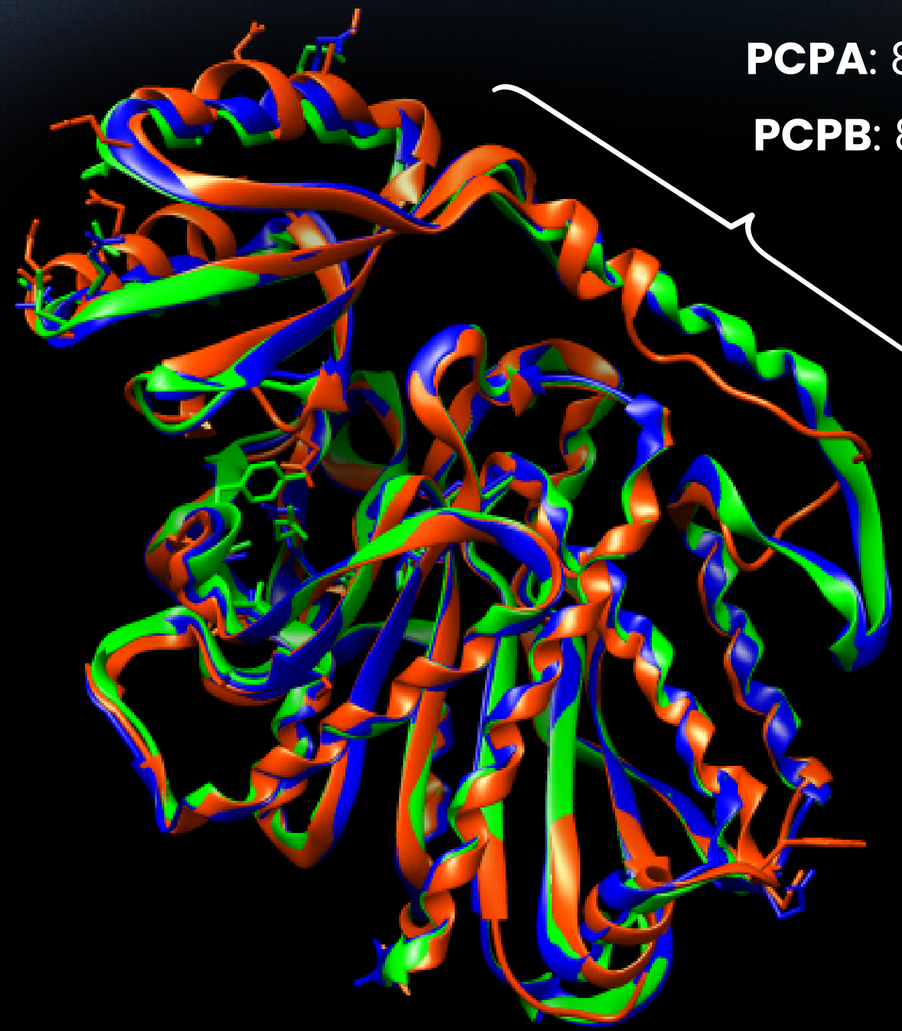
COMPARING PCPA AND PCPB

PCPA: 82-99

PCPB: 81-95

PCPB → helix 3_{10}





PCPAs → longer helix insertion (34-35)

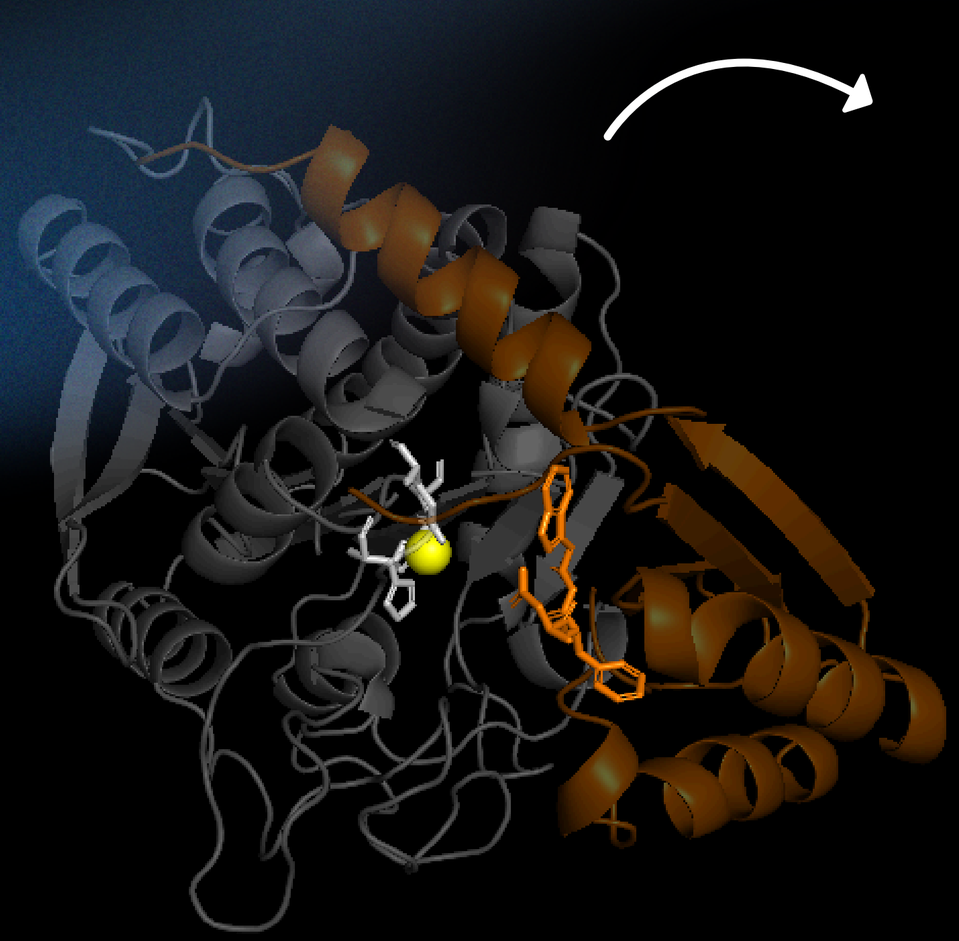
PCPAs → small intrinsic activity
vs
PCPB → completely inhibited

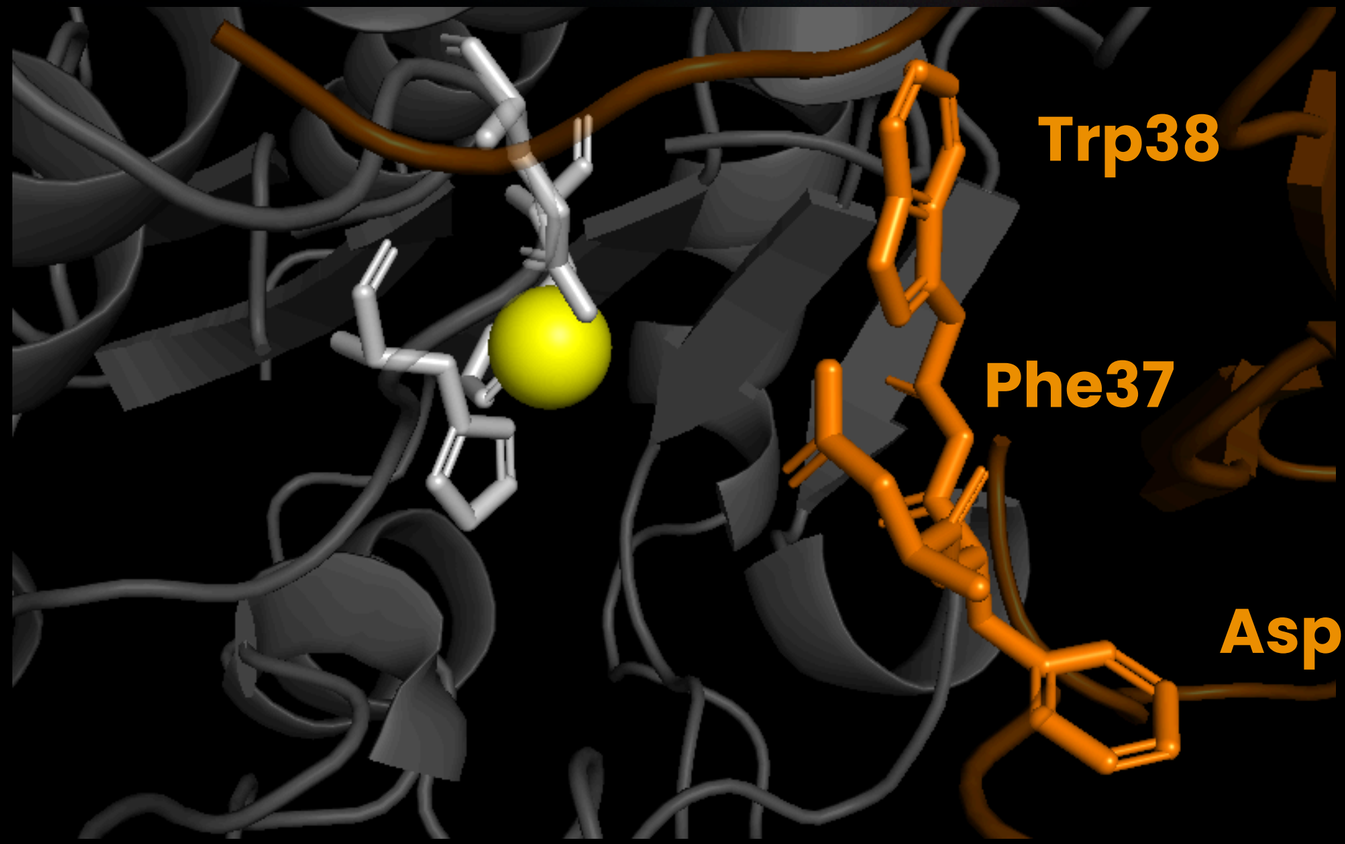
Porcine PCPB **Porcine PCPA** **Bovine PCPA**

INHIBITION MECHANISM

S1 AND S1' SUBSITES

- No direct blockage of the catalytic centre



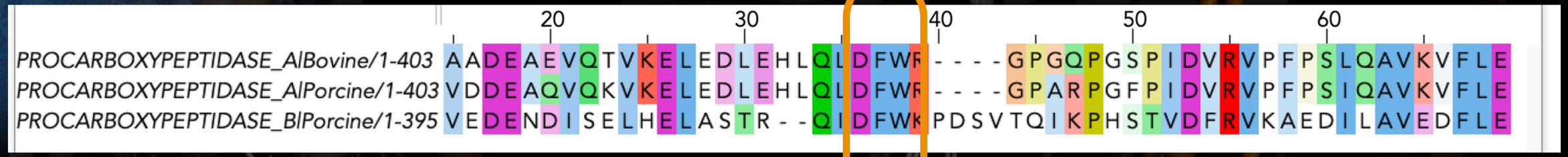


Zn-coordinating residues

INHIBITION MECHANISM

S1 AND S1' SUBSITES

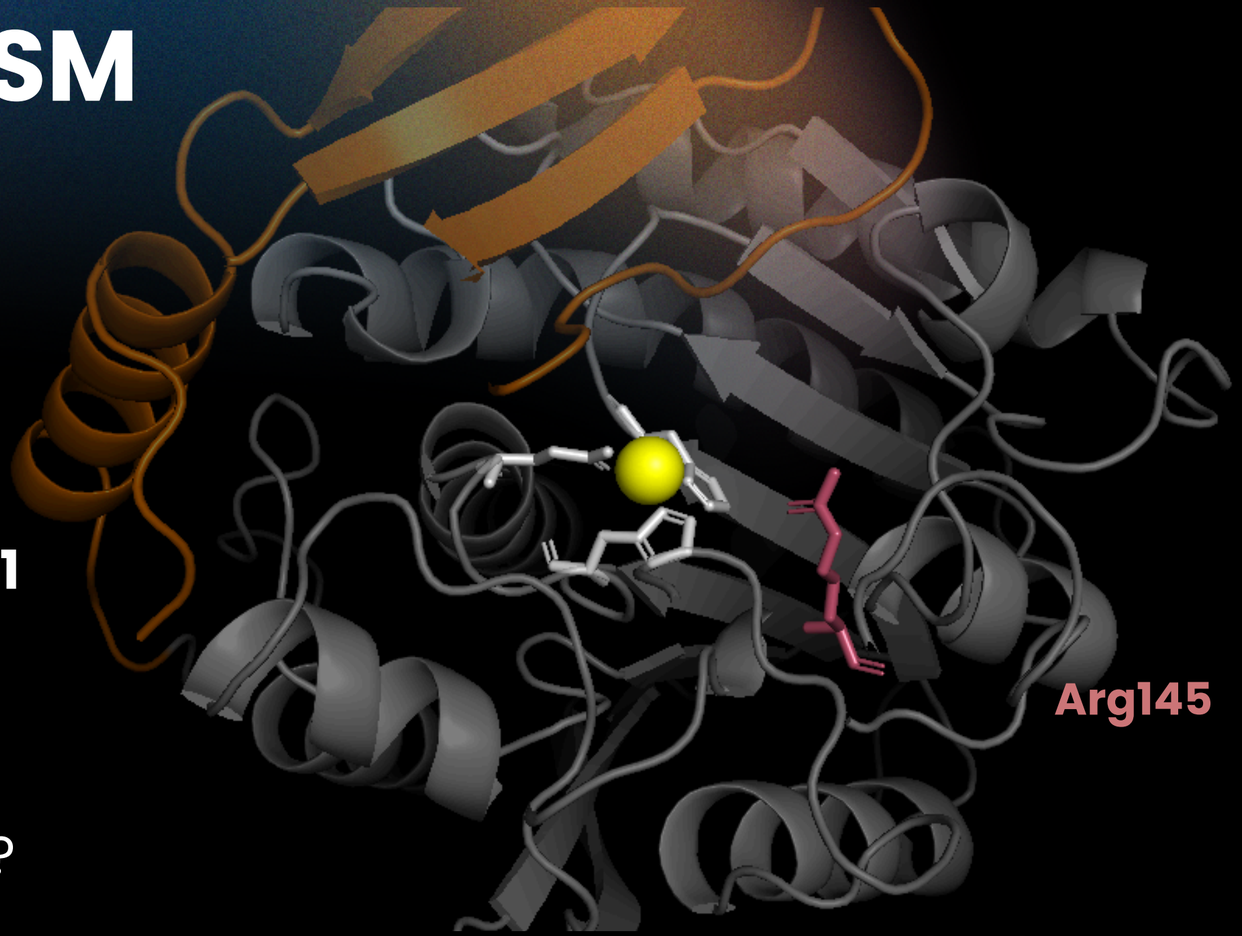
- No direct blockage of the catalytic centre



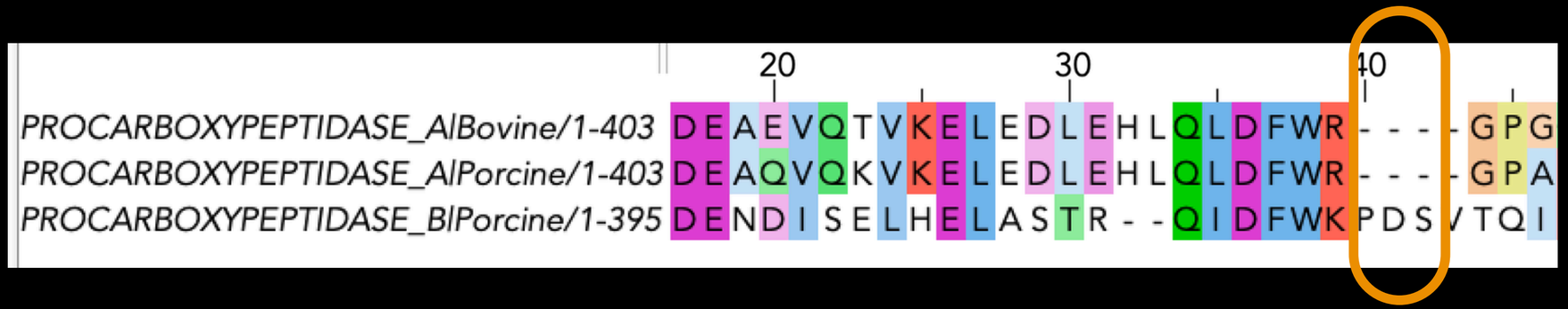
INHIBITION MECHANISM

S1 AND S1' SUBSITES

PCBs: Arg145 – Asp41 vs **PCPAs: No Asp41**

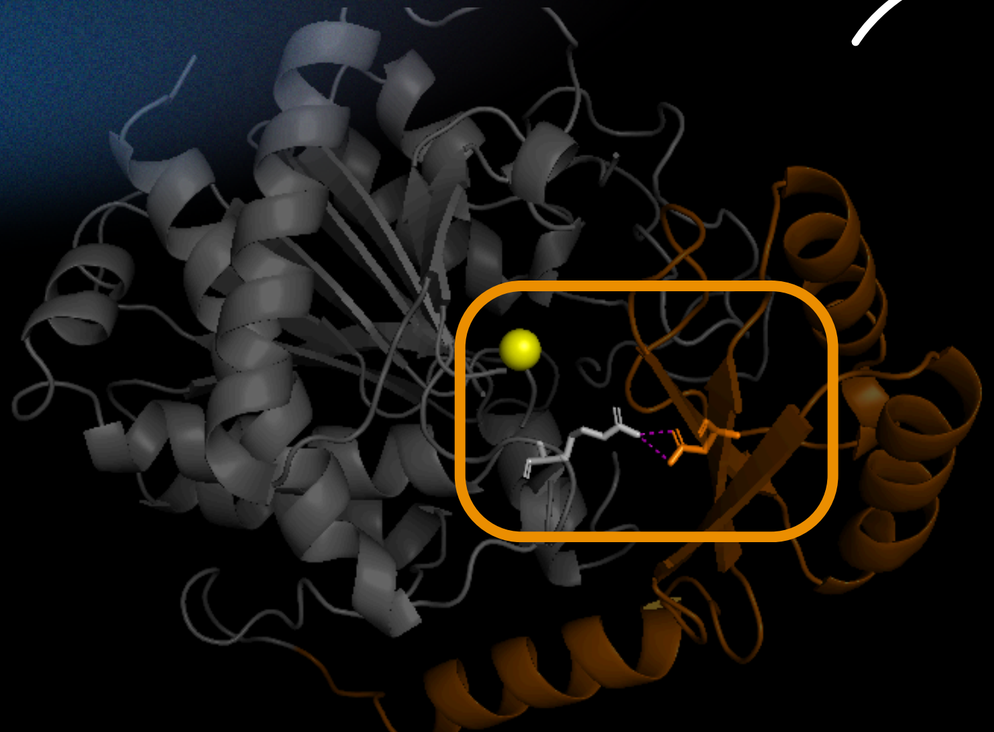


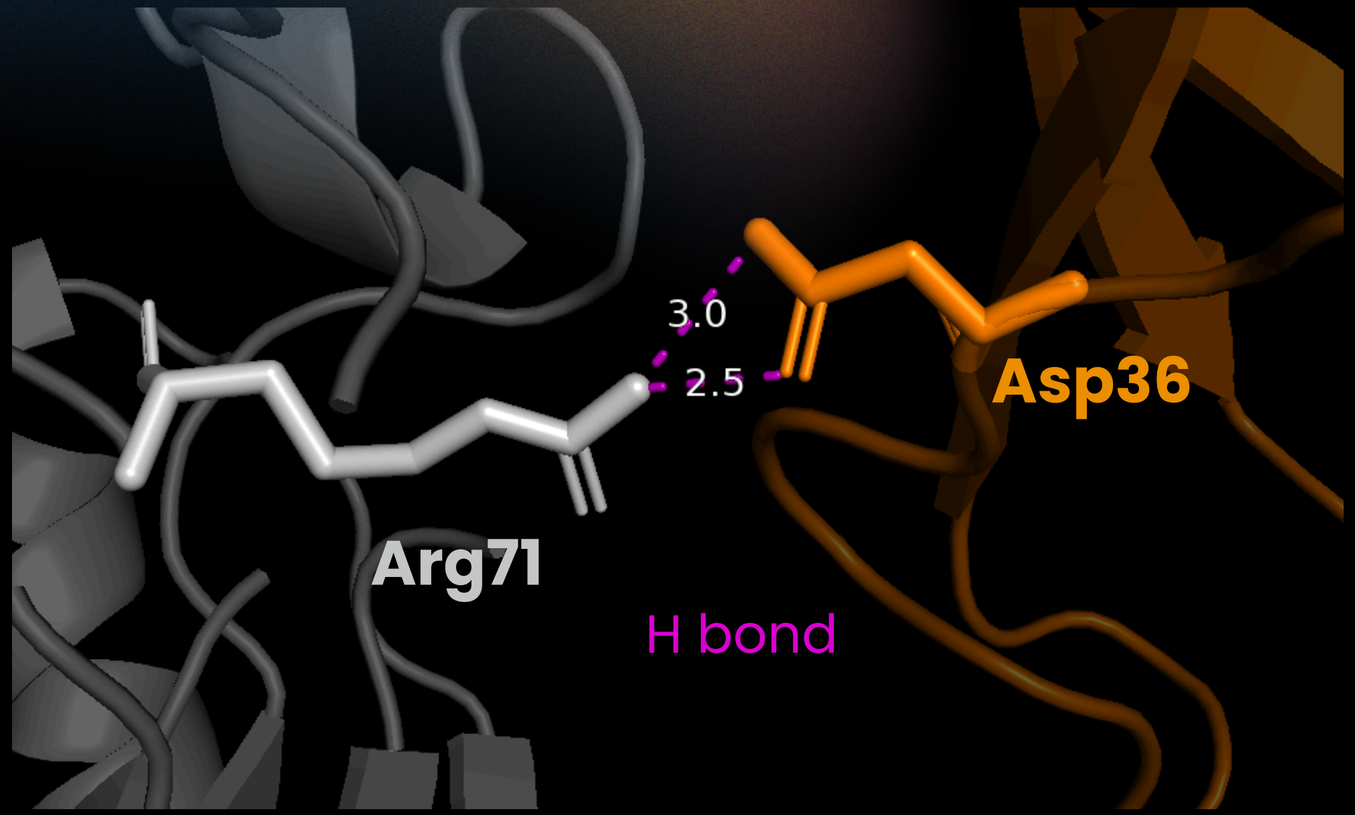
Responsible for PCPAs remaining activity?



INHIBITION MECHANISM

S2 SUBSITE



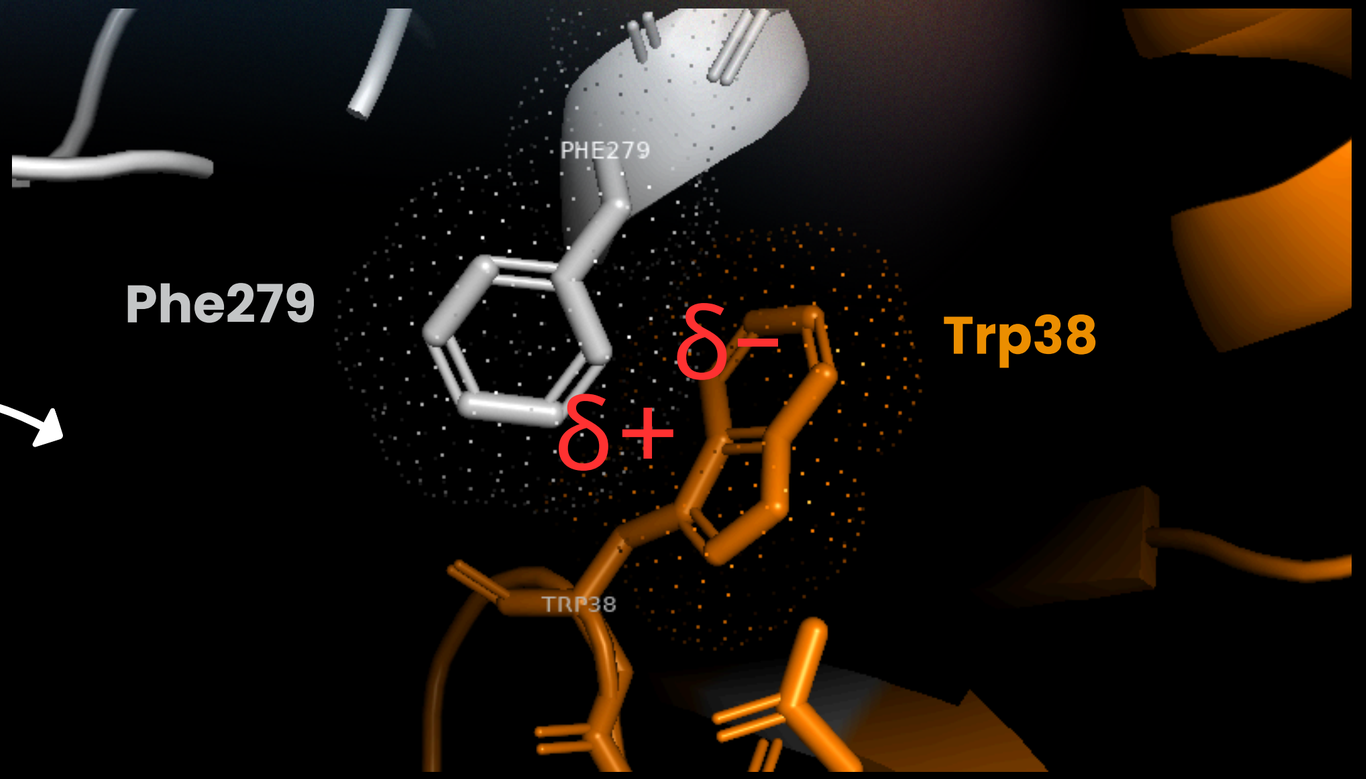


- Guides the Cterm of the substrate
- Stabilizes carbonyl group at P2

INHIBITION MECHANISM

S3 SUBSITE





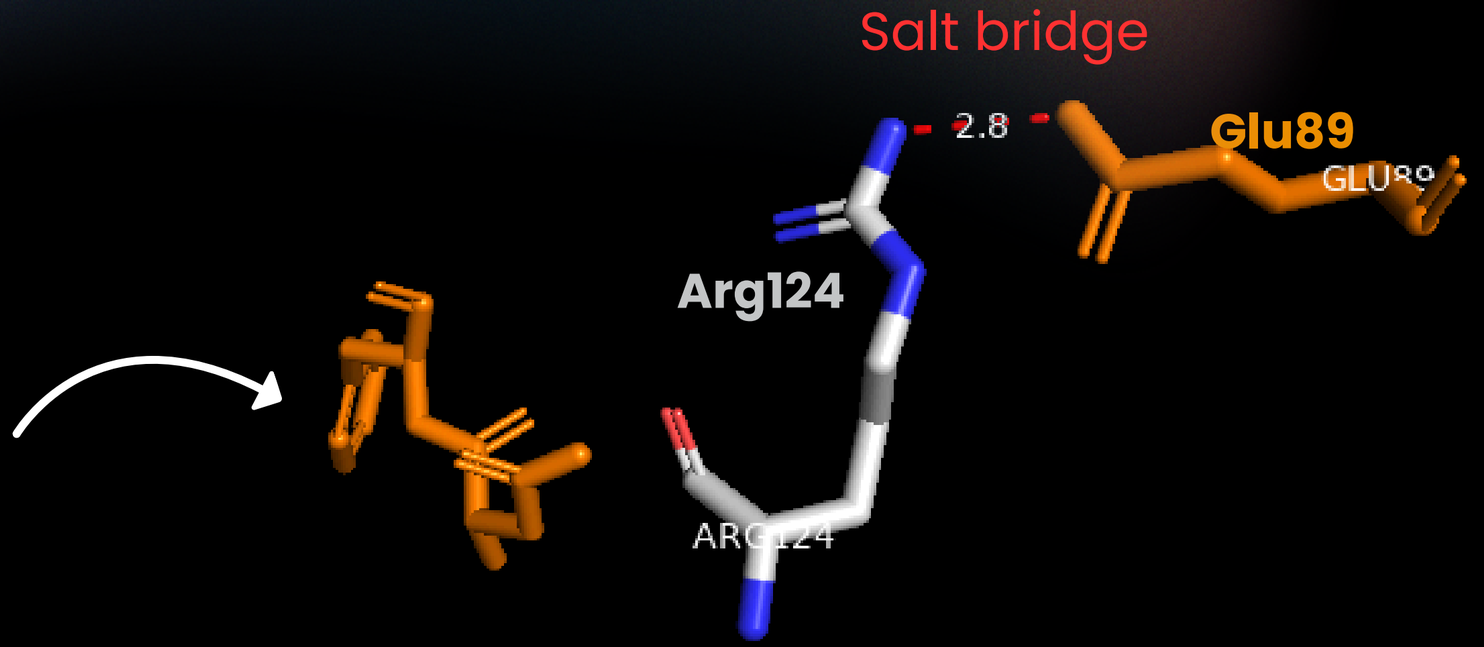
Edge-to-face interaction

- Electrostatic interaction that stabilizes and blocks the protein fold

INHIBITION MECHANISM

S4 SUBSITE



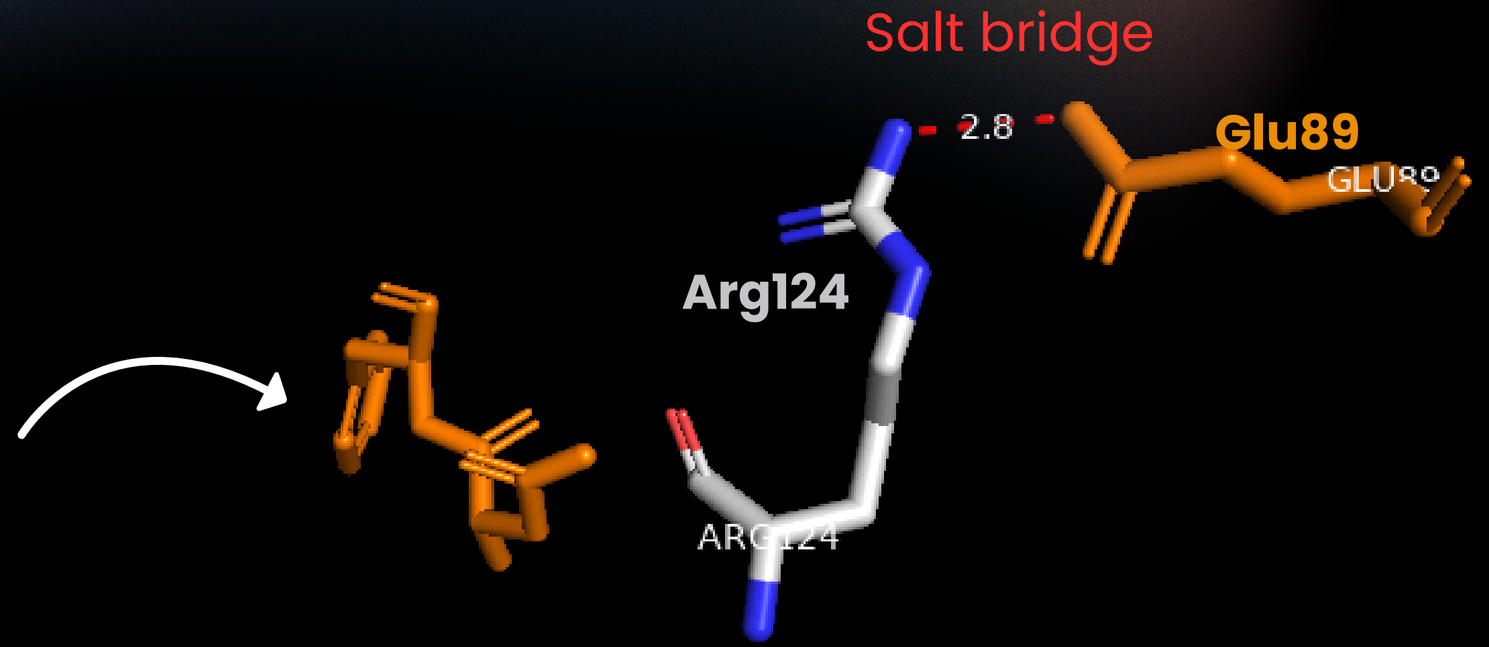


- H bond + electrostatic interaction, strong
- Acid proton donor → basic acceptor

INHIBITION MECHANISM

S4 SUBSITE

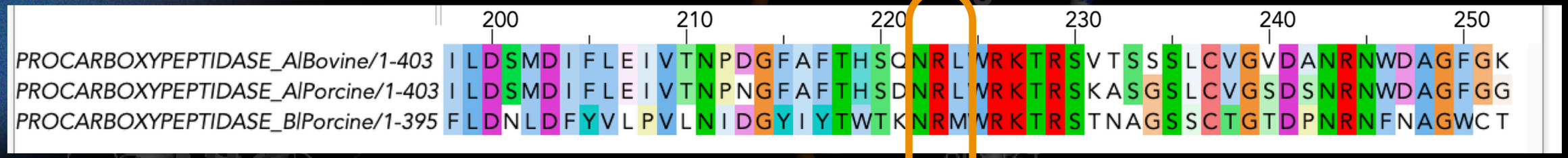


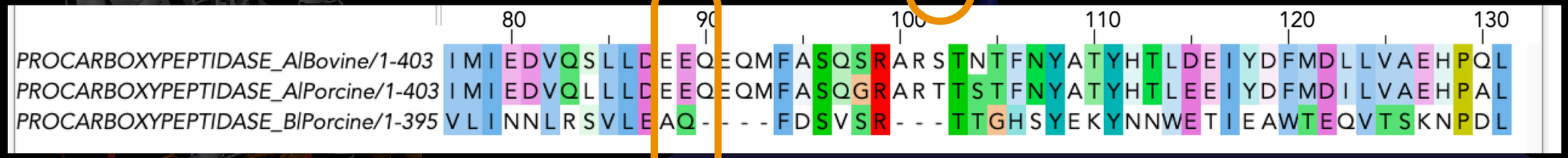


- Essential to position the globular domain on the active site of the enzyme

INHIBITION MECHANISM

S4 SUBSITE





- Essential to position the globular domain on the active site of the enzyme

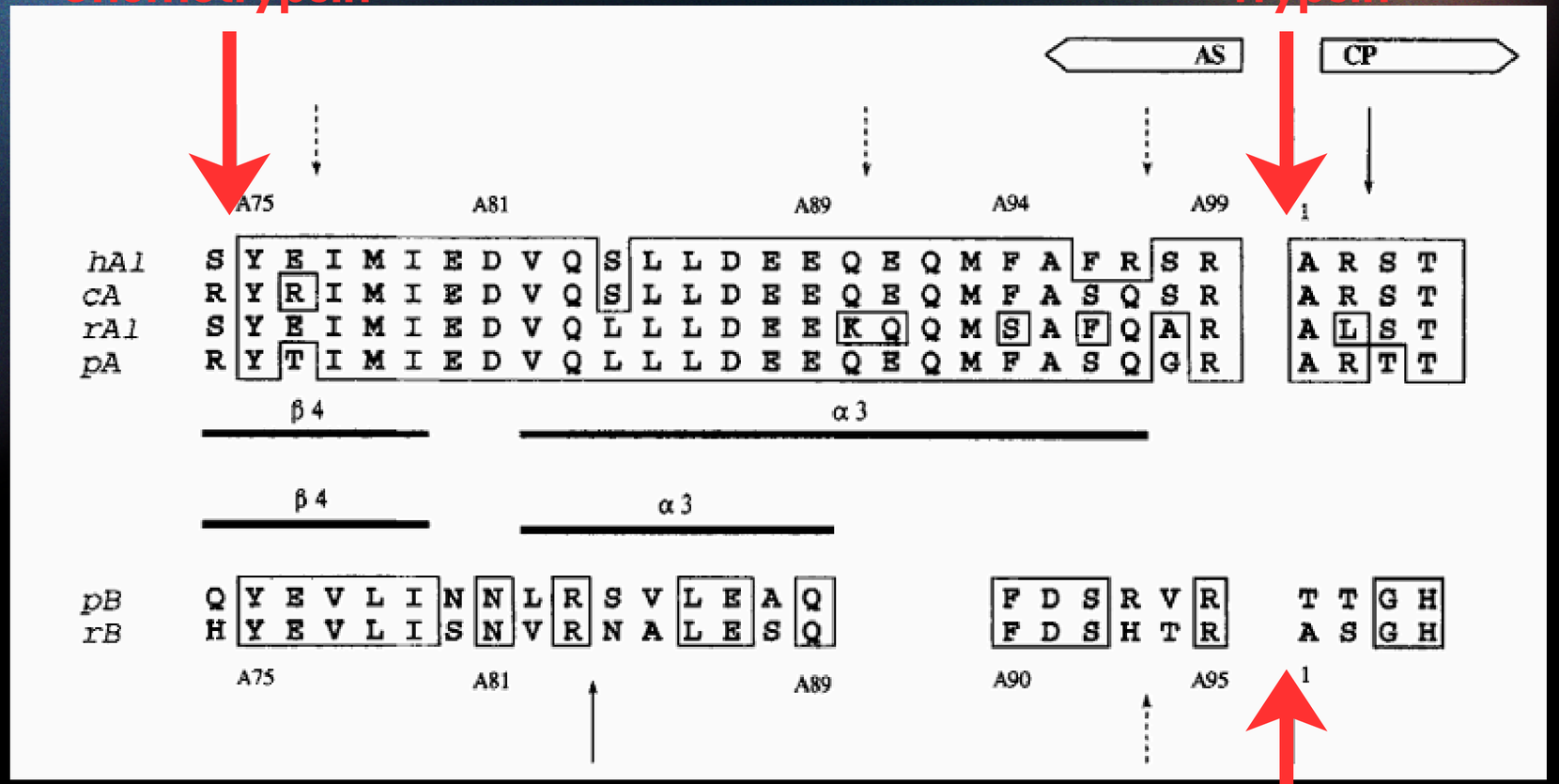
ACTIVATION MECHANISM

Chemotrypsin

Trypsin

PCPA

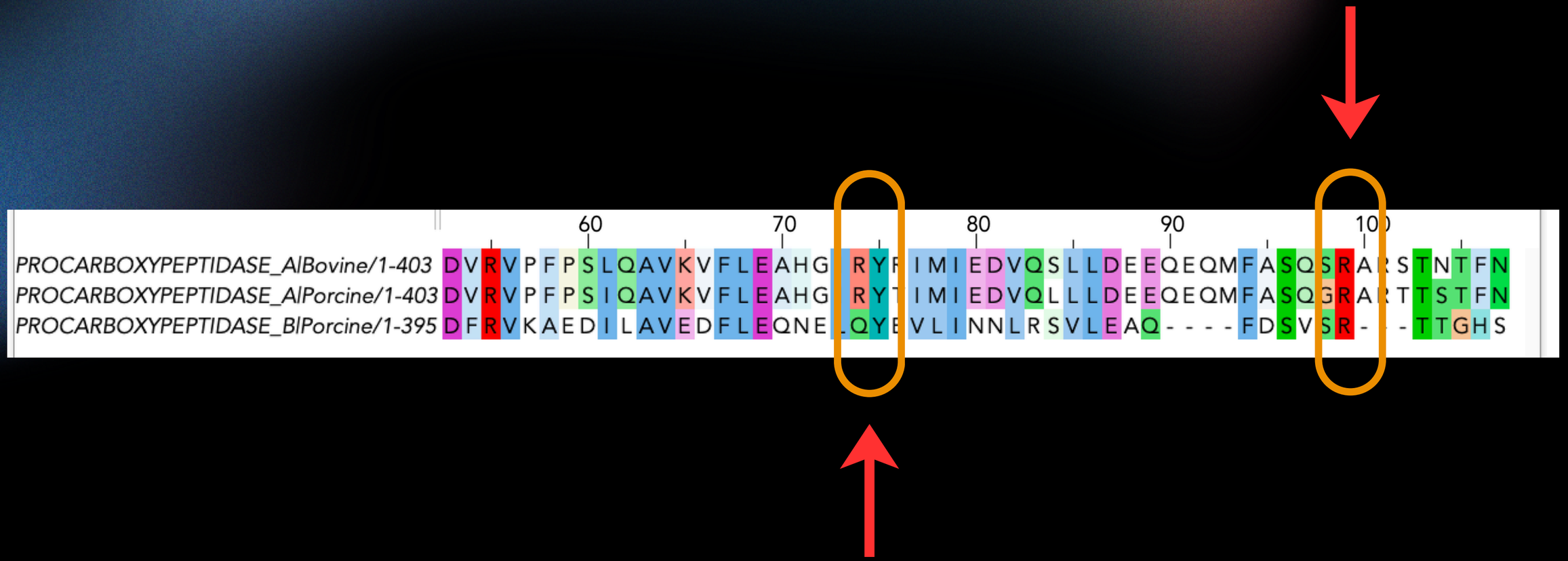
PCPB



https://febs.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1432-1033.1993.tb17561.x

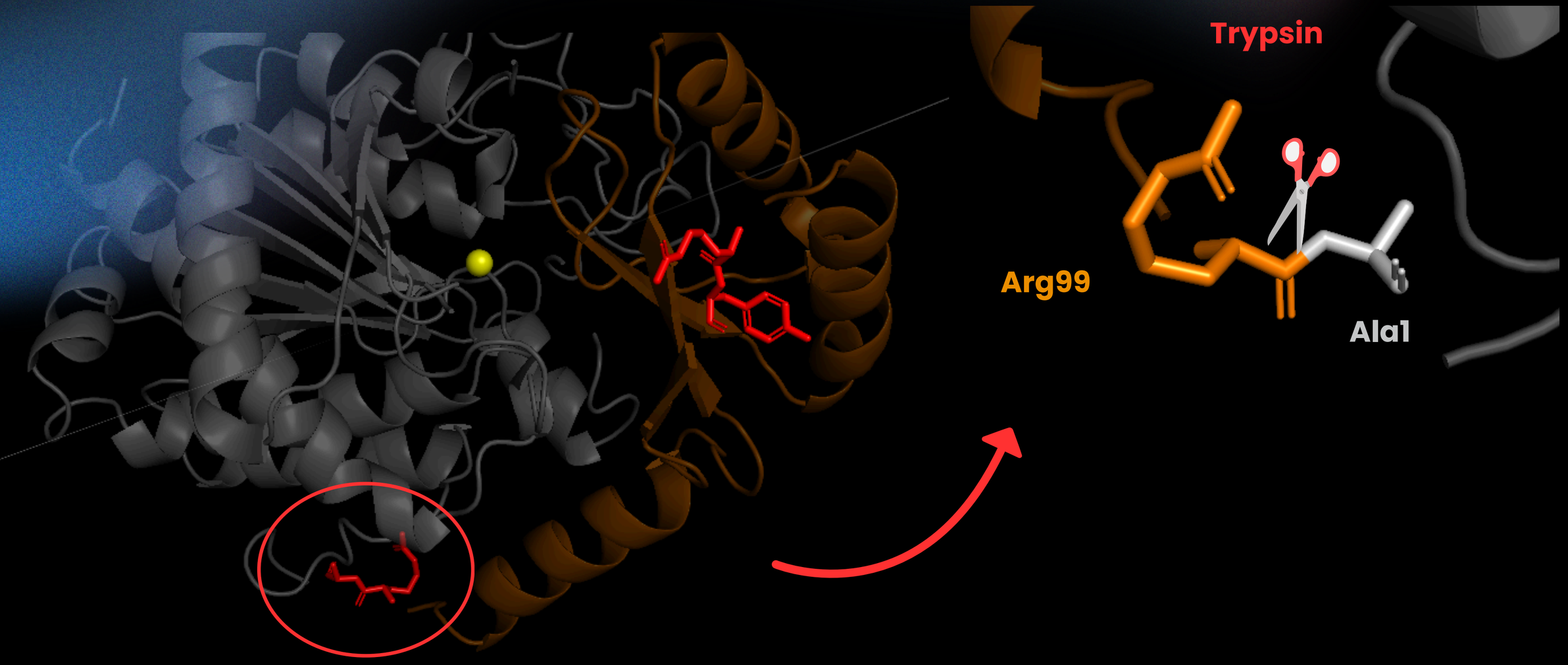
Trypsin

ACTIVATION MECHANISM



ACTIVATION MECHANISM

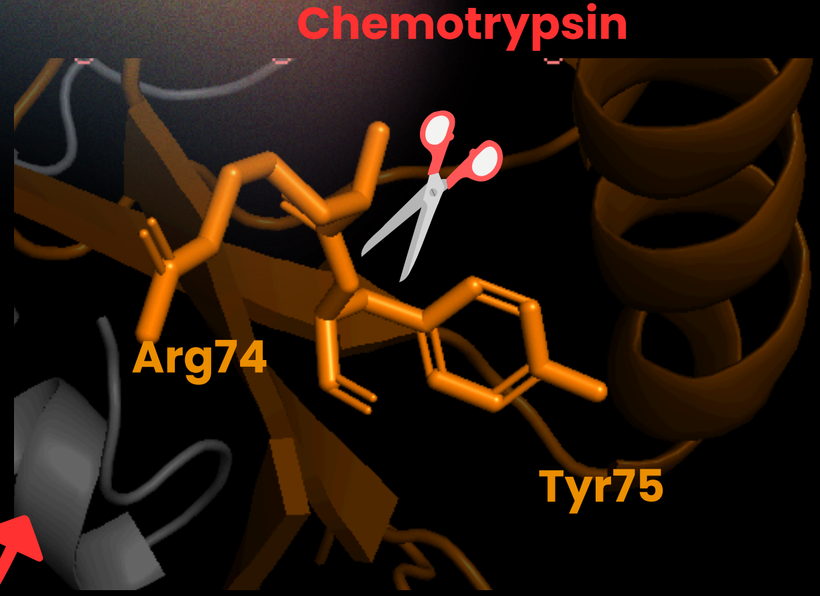
FIRST CUT



ACTIVATION MECHANISM

SECOND CUT





INHIBITION

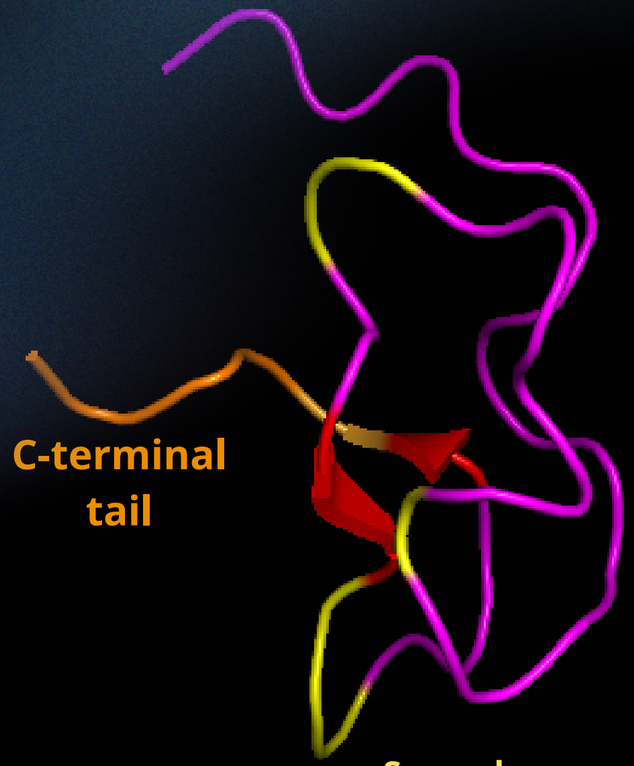
INHIBITION MECHANISMS

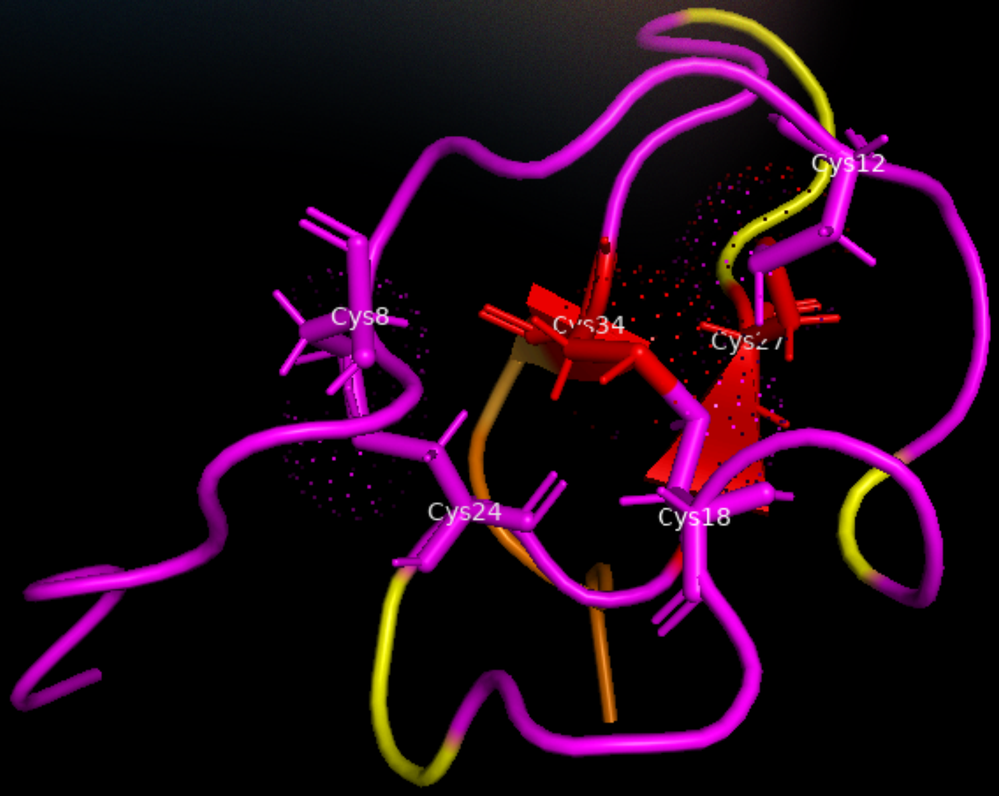
1. Autologous Inhibitors: Pro - segment

2. Heterologous Inhibitors
    - Potato Carboxypeptidase Inhibitor (PCI)
    - Transition State Mimetic: L-benzylsuccinate acid (BZS)

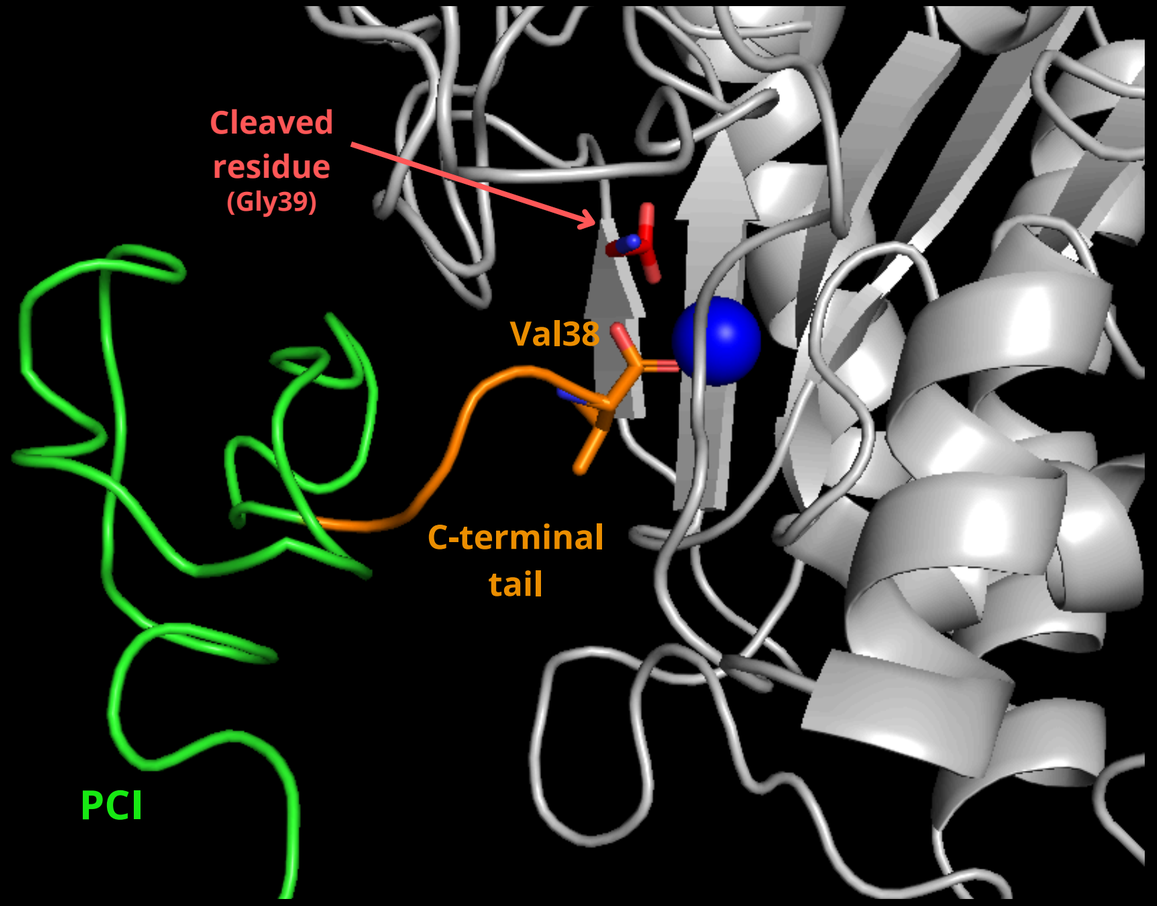
3. Inhibition by Excess of Zinc

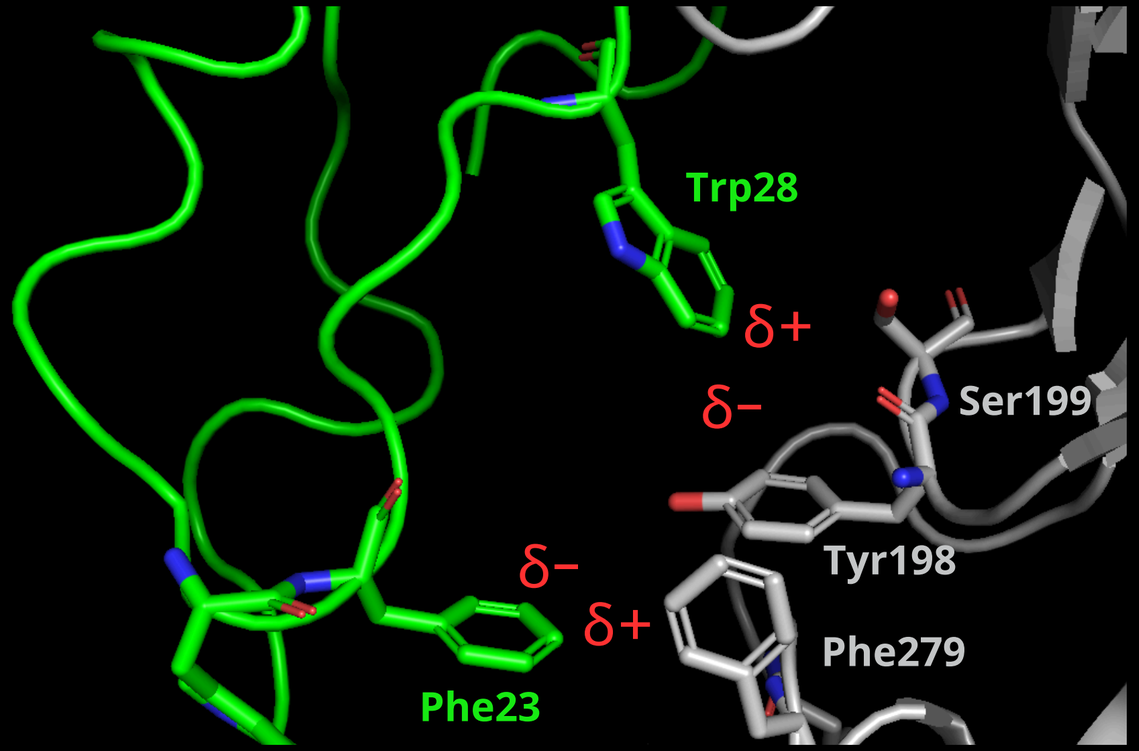
POTATO CARBOXYPEPTIDASE INHIBITOR (PCI)





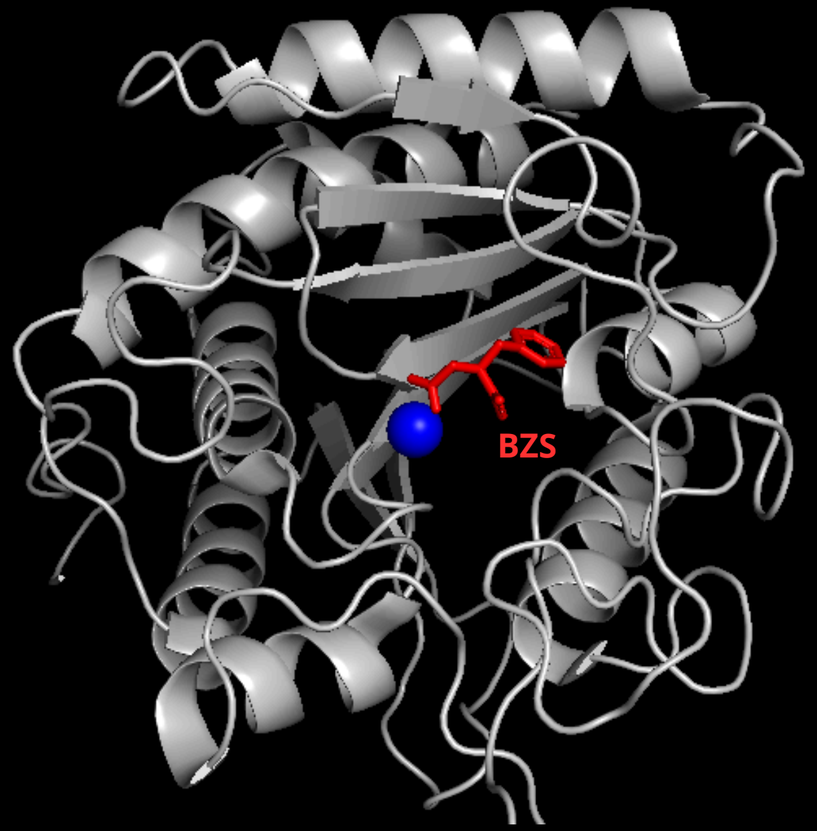
Disulphide bridges

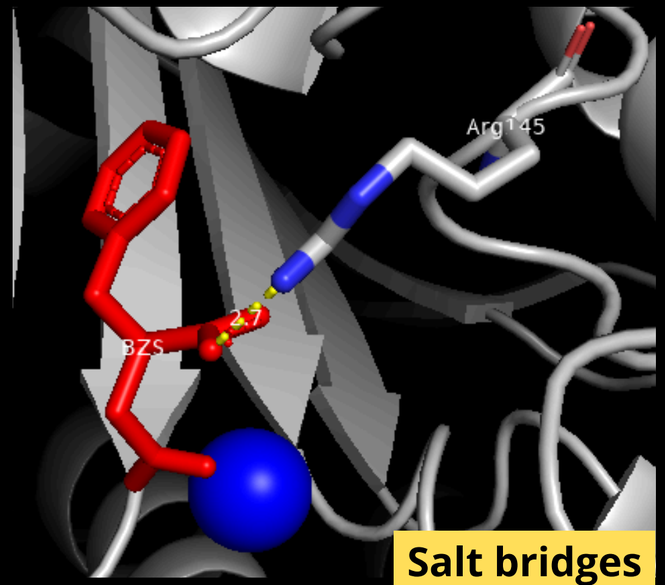

Cleaved
residue
(Gly39)
Val38
C-terminal
tail
PCI


Trp28
δ+
δ−
Ser199
Tyr198
Phe279
δ−
δ+
Phe23

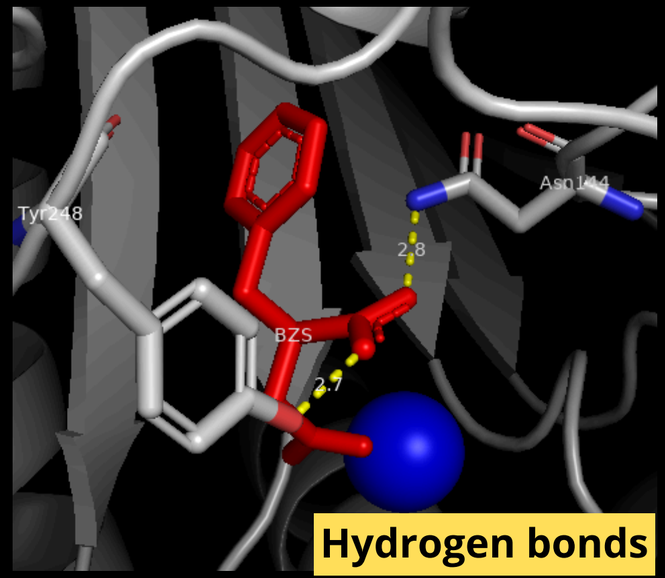
TRANSITION STATE MIMETIC

L-benzylsuccinate acid (BZS)



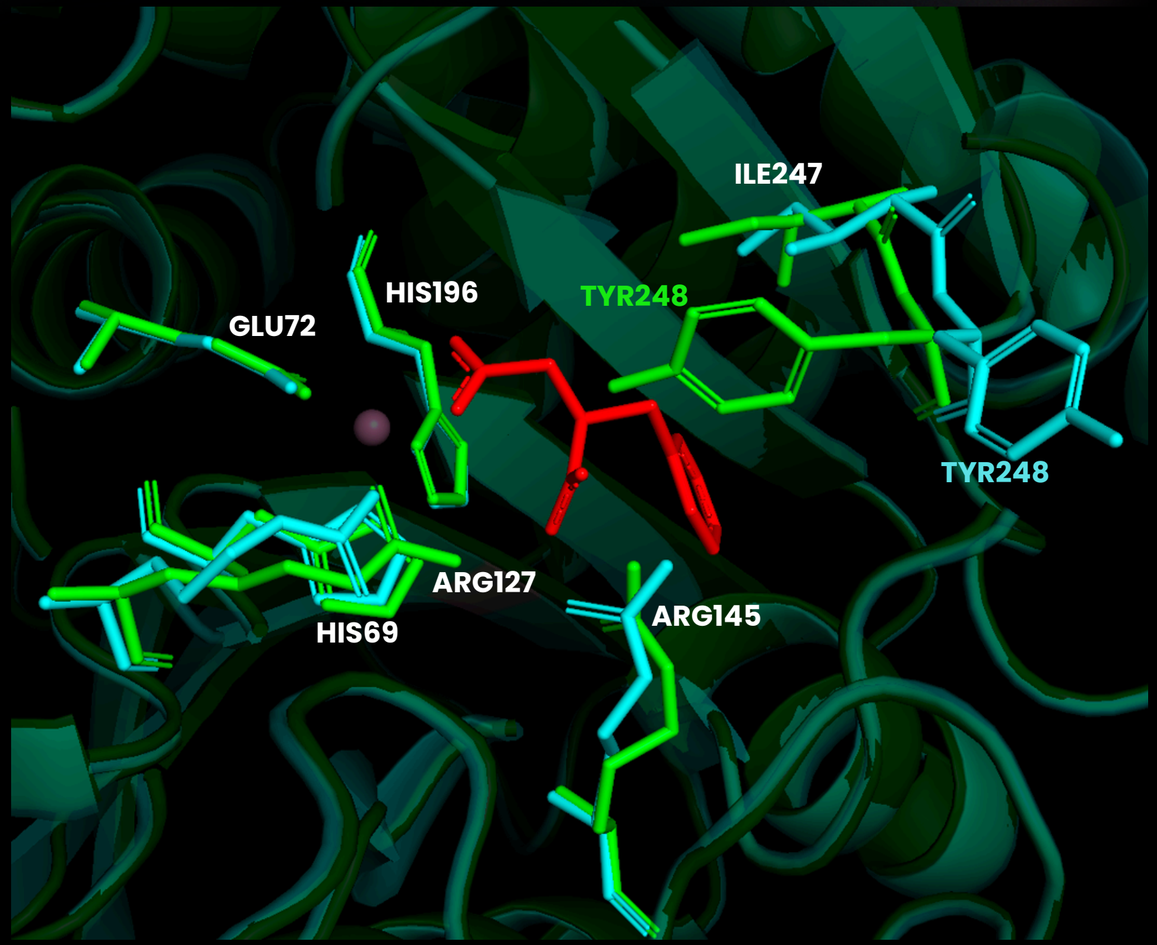


Salt bridges

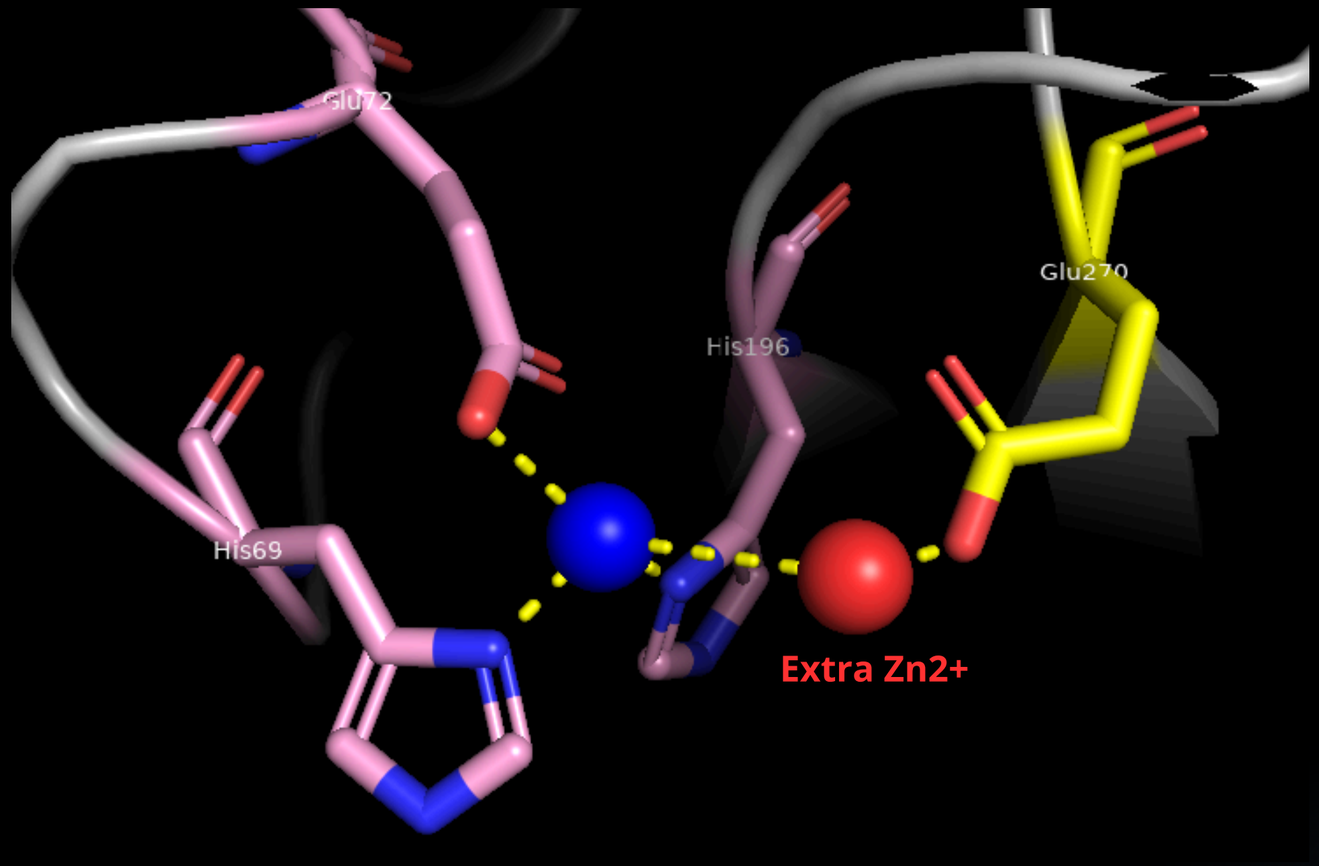


Hydrogen bonds

DIFFERENCES IN 3-D STRUCTURE OF CARBOXYPEPTIDASE A AT ITS ACTIVE CENTRE WHEN UNLIGANDED OR LIGANDED WITH BENZYLSUCCINIC ACID



INHIBITION BY EXCESS OF ZINC

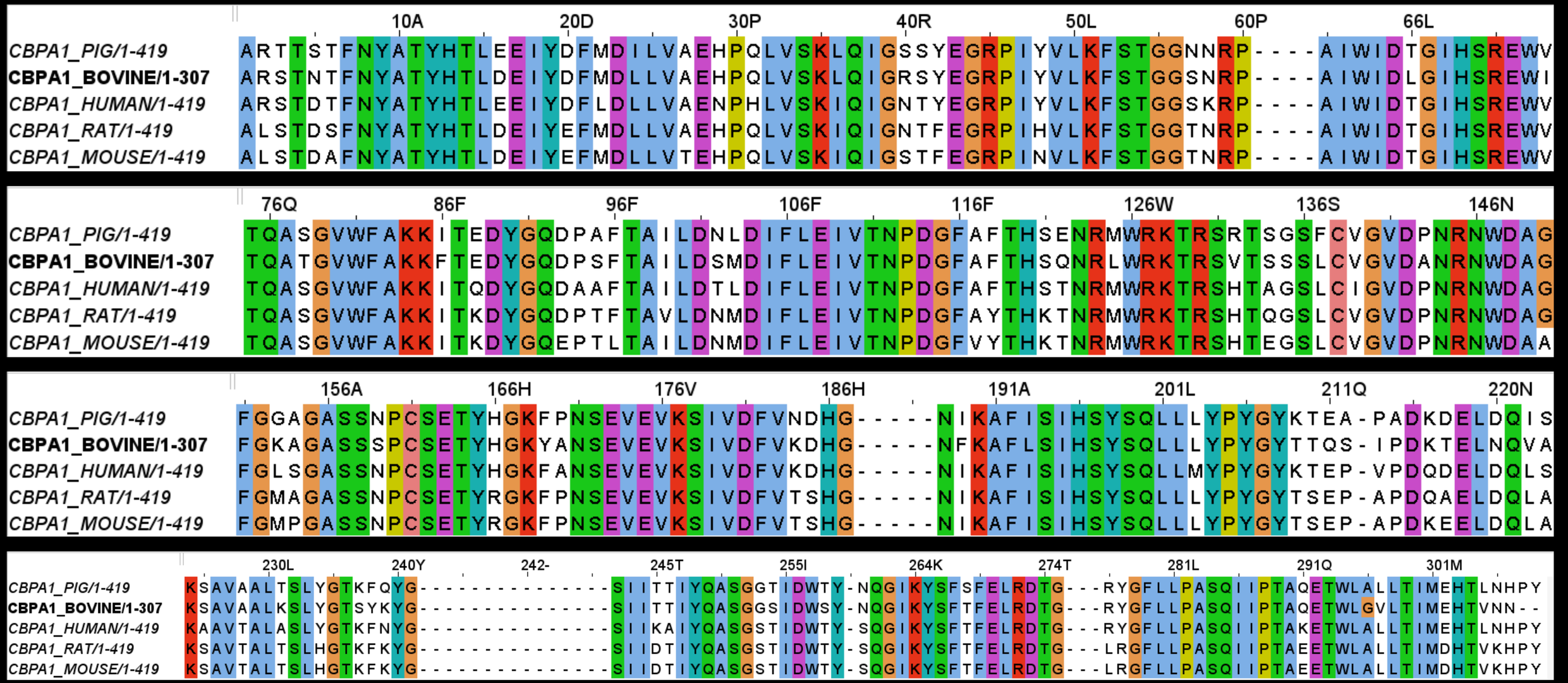


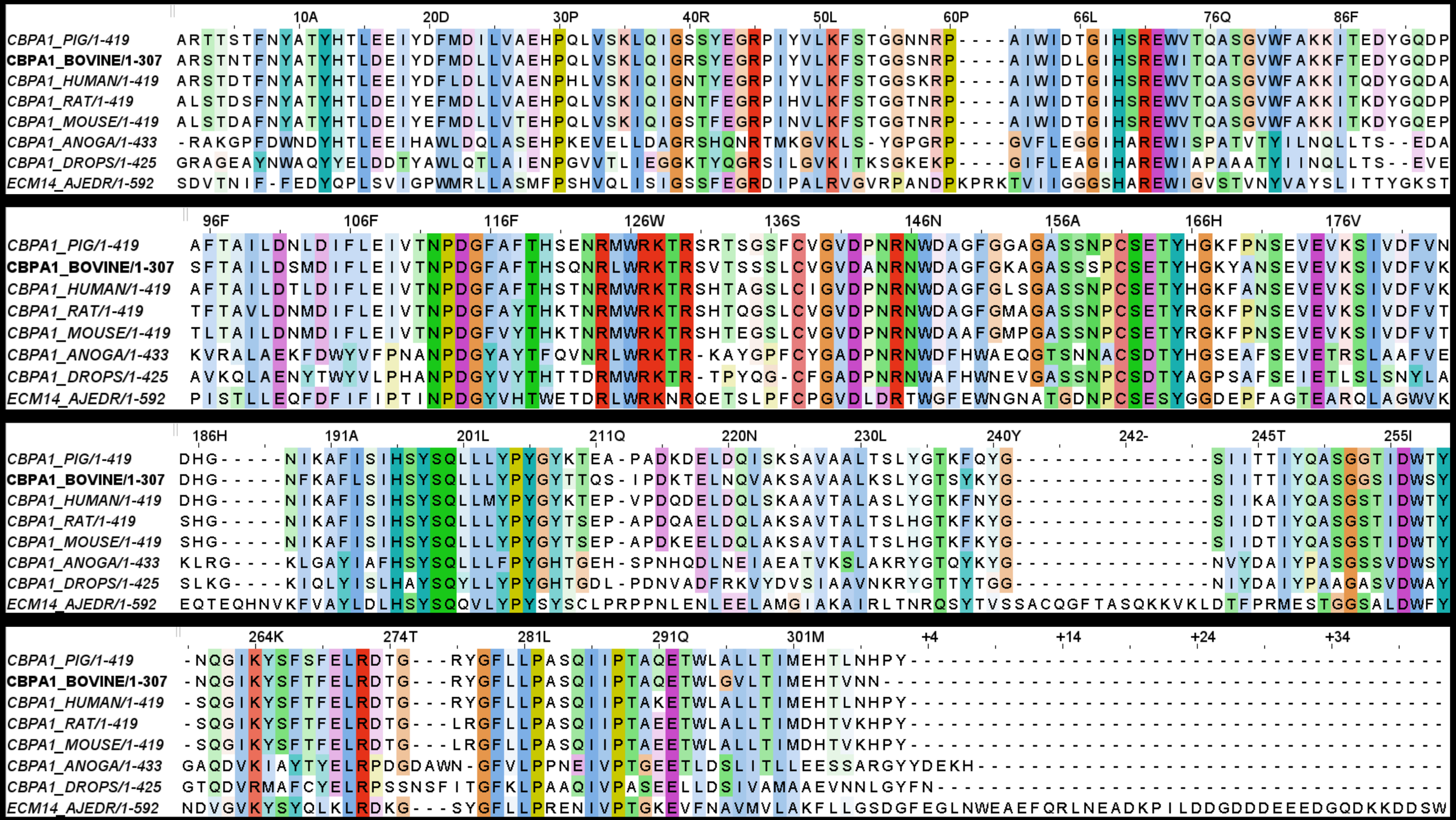
Binuclear Zn2+ center:

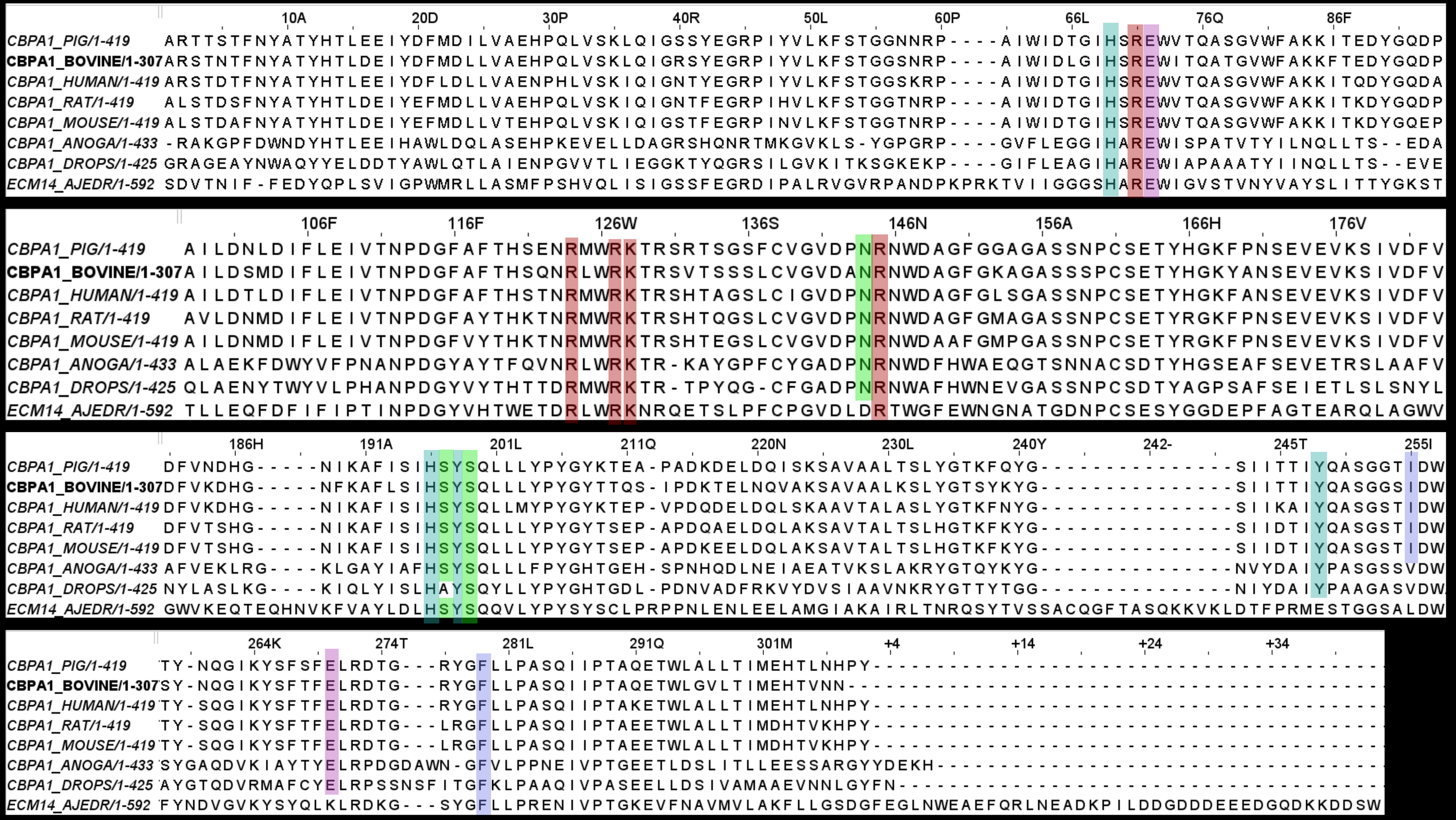
- Disorted coordination geometry
- Dysfunctional catalytic activity

CONSERVATION AND EVOLUTION

MSA VERTEBRATES



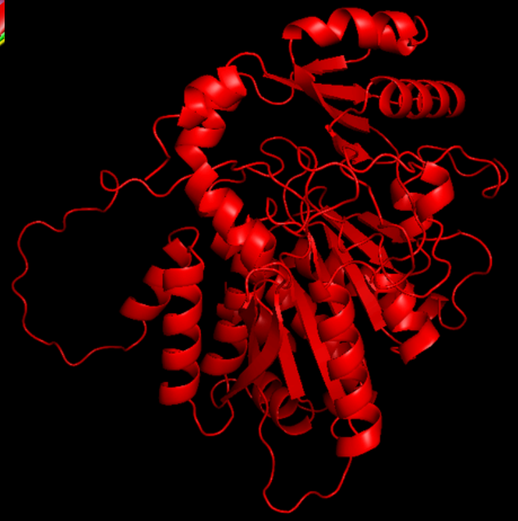

10A 20D 30P 40R 50L 60P 66L 76Q 86F
CBPA1_PIG/1-419 ARTTSTFNYATYHTLEEIYDFMDILVAEHPQLVSKLQIGSSYEGRPIYVLKFSTGGNNRP----AIWIDTGIHSREWVTQASGVWFAKKITEDYGQDP
CBPA1_BOVINE/1-307 ARSTNTFNYATYHTLDEIYDFMDLLVAEHPQLVSKLQIGRSYEGRPIYVLKFSTGGSNRP----AIWIDLGIHSREWITQATGVWFAKKFTEDYGQDP
CBPA1_HUMAN/1-419 ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGSKRP----AIWIDTGIHSREWVTQASGVWFAKKITQDYGQDA
CBPA1_RAT/1-419 ALSTDSFNYATYHTLDEIYEFMDLLVAEHPQLVSKIQIGNTFEGRPIHVLKFSTGGTNRP----AIWIDTGIHSREWVTQASGVWFAKKITKDYGQDP
CBPA1_MOUSE/1-419 ALSTDAFNYATYHTLDEIYEFMDLLVTEHPQLVSKIQIGSTFEGRPINVLKFSTGGTNRP----AIWIDTGIHSREWVTQASGVWFAKKITKDYGQEP
CBPA1_ANOGA/1-433 -RAKGPFDWNDYHTLEEIHAWLDQLASEHPKEVELLDAGRSHQNRTMKGVKLS-YGPGRP----GVFLEGGIHAREWISPATVTYILNQLLTS--EDA
CBPA1_DROPS/1-425 GRAGEAYNWAQYYELDDTYAWLQTLAIENPGVVTLIEGGKTYQGRSILGVKITKSGKEKP----GIFLEAGIHAREWIAPAAATYIINQLLTS--EVE
ECM14_AJEDR/1-592 SDVTNIF-FEDYQPLSVIGPWMRLLASMFPSHVQLISIGSSFEGRDIPALRVGVRPANDPKPRKTVIIGGGSHAREWIGVSTVNYVAYSLITTYGKST
96F 106F 116F 126W 136S 146N 156A 166H 176V
CBPA1_PIG/1-419 AFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRKTRSRTSGSFCVGVDPNRNWDAGFGGAGASSNPCSETYHGKFPNSEVEVKSIVDFVN
CBPA1_BOVINE/1-307 SFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKSIVDFVK
CBPA1_HUMAN/1-419 AFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVK
CBPA1_RAT/1-419 TFTAVLDNMDIFLEIVTNPDGFAYTHKTNRMWRKTRSHTQGSLCVGVDPNRNWDAGFGMAGASSNPCSETYRGKFPNSEVEVKSIVDFVT
CBPA1_MOUSE/1-419 TLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRKTRSHTEGSLCVGVDPNRNWDAAFGMPGASSNPCSETYRGKFPNSEVEVKSIVDFVT
CBPA1_ANOGA/1-433 KVRALAEKFDWYVFPNANPDGYAYTFQVNRLWRKTR-KAYGPFCYGADPNRNWDFHWAEQGTSNNACSDTYHGSEAFSEVETRSLAAFVE
CBPA1_DROPS/1-425 AVKQLAENYTWYVLPHANPDGYVYTHTTDRMWRKTR-TPYQG-CFGADPNRNWAFHWNEVGASSNPCSDTYAGPSAFSEIETLSLSNYLA
ECM14_AJEDR/1-592 PISTLLEQFDFIFIPTINPDGYVHTWETDRLWRKNRQETSLPFCPGVDLDRTWGFEWNGNATGDNPCSESYGGDEPFAGTEARQLAGWVK
186H 191A 201L 211Q 220N 230L 240Y 242- 245T 255I
CBPA1_PIG/1-419 DHG-----NIKAFISIHSYSQLLLYPYGYKTEA-PADKDELDQISKSAVAALTSLYGTKFQYG----------------SIITTIYQASGGTIDWTY
CBPA1_BOVINE/1-307 DHG-----NFKAFLSIHSYSQLLLYPYGYTTQS-IPDKTELNQVAKSAVAALKSLYGTSYKYG----------------SIITTIYQASGGSIDWSY
CBPA1_HUMAN/1-419 DHG-----NIKAFISIHSYSQLLMYPYGYKTEP-VPDQDELDQLSKAAVTALASLYGTKFNYG----------------SIIKAIYQASGSTIDWTY
CBPA1_RAT/1-419 SHG-----NIKAFISIHSYSQLLLYPYGYTSEP-APDQAELDQLAKSAVTALTSLHGTKFKYG----------------SIIDTIYQASGSTIDWTY
CBPA1_MOUSE/1-419 SHG-----NIKAFISIHSYSQLLLYPYGYTSEP-APDKEELDQLAKSAVTALTSLHGTKFKYG----------------SIIDTIYQASGSTIDWTY
CBPA1_ANOGA/1-433 KLRG----KLGAYIAFHSYSQLLLFPYGHTGEH-SPNHQDLNEIAEATVKSLAKRYGTQYKYG----------------NVYDAIYPASGSSVDWSY
CBPA1_DROPS/1-425 SLKG----KIQLYISLHAYSQYLLYPYGHTGDL-PDNVADFRKVYDVSIAAVNKRYGTTYTGG----------------NIYDAIYPAAGASVDWAY
ECM14_AJEDR/1-592 EQTEQHNVKFVAYLDLHSYSQQVLYPYSYSCLPRPPNLENLEELAMGIAKAIRLTNRQSYTVSSACQGFTASQKKVKLDTFPRMESTGGSALDWFY
264K 274T 281L 291Q 301M +4 +14 +24 +34
CBPA1_PIG/1-419 -NQGIKYSFSFELRDTG---RYGFLLPASQIIPTAQETWLALLTIMEHTLNHPY----------
CBPA1_BOVINE/1-307 -NQGIKYSFTFELRDTG---RYGFLLPASQIIPTAQETWLGVLTIMEHTVNN----------
CBPA1_HUMAN/1-419 -SQGIKYSFTFELRDTG---RYGFLLPASQIIPTAKETWLALLTIMEHTLNHPY----------
CBPA1_RAT/1-419 -SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMDHTVKHPY----------
CBPA1_MOUSE/1-419 -SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMDHTVKHPY----------
CBPA1_ANOGA/1-433 GAQDVKIAYTYELRPDGDAWN-GFVLPPNEIVPTGEETLDSLITLLEESSARGYYDEKH----------
CBPA1_DROPS/1-425 GTQDVRMAFCYELRPSSNSFITGFKLPAAQIVPASEELLDSIVAMAAEVNNLGYFN----------
ECM14_AJEDR/1-592 NDVGVKYSYQLKLRDKG---SYGFLLPRENIVPTGKEVFNAVMVLAKFLLGSDGFEGLNWEAEFQRLNEADKPILDDGDDDEEEDGQDKKDDSW


10A 20D 30P 40R 50L 60P 66L 76Q 86F
CBPA1_PIG/1-419 ARTTSTFNYATYHTLEEIYDFMDILVAEHPQLVSKLQIGSSYEGRPIYVLKFSTGGNNRP----AIWIDTGIHSREWVTQASGVWFAKKITEDYGQDP
CBPA1_BOVINE/1-307 ARSTNTFNYATYHTLDEIYDFMDLLVAEHPQLVSKLQIGRSYEGRPIYVLKFSTGGSNRP----AIWIDLGIHSREWITQATGVWFAKKFTEDYGQDP
CBPA1_HUMAN/1-419 ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGSKRP----AIWIDTGIHSREWVTQASGVWFAKKITQDYGQDA
CBPA1_RAT/1-419 ALSTDSFNYATYHTLDEIYEFMDLLVAEHPQLVSKIQIGNTFEGRPIHVLKFSTGGTNRP----AIWIDTGIHSREWVTQASGVWFAKKITKDYGQDP
CBPA1_MOUSE/1-419 ALSTDAFNYATYHTLDEIYEFMDLLVTEHPQLVSKIQIGSTFEGRPINVLKFSTGGTNRP----AIWIDTGIHSREWVTQASGVWFAKKITKDYGQEP
CBPA1_ANOGA/1-433 -RAKGPFDWNDYHTLEEIHAWLDQLASEHPKEVELLDAGRSHQNRTMKGVKLS-YGPGRP----GVFLEGGIHAREWISPATVTYILNQLLTS--EDA
CBPA1_DROPS/1-425 GRAGEAYNWAQYYELDDTYAWLQTLAIENPGVVTLIEGGKTYQGRSILGVKITKSGKEKP----GIFLEAGIHAREWIAPAAATYIINQLLTS--EVE
ECM14_AJEDR/1-592 SDVTNIF-FEDYQPLSVIGPWMRLLASMFPSHVQLISIGSSFEGRDIPALRVGVRPANDPKPRKTVIIGGGSHAREWIGVSTVNYVAYSLITTYGKST
106F 116F 126W 136S 146N 156A 166H 176V
CBPA1_PIG/1-419 AILDNLDIFLEIVTNPDGFAFTHSENRMWRKTRSRTSGSFCVGVDPNRNWDAGFGGAGASSNPCSETYHGKFPNSEVEVKSIVDFV
CBPA1_BOVINE/1-307 AILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTSSSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKSIVDFV
CBPA1_HUMAN/1-419 AILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTAGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFV
CBPA1_RAT/1-419 AVLDNMDIFLEIVTNPDGFAYTHKTNRMWRKTRSHTQGSLCVGVDPNRNWDAGFGMAGASSNPCSETYRGKFPNSEVEVKSIVDFV
CBPA1_MOUSE/1-419 AILDNMDIFLEIVTNPDGFVYTHKTNRMWRKTRSHTEGSLCVGVDPNRNWDAAFGMPGASSNPCSETYRGKFPNSEVEVKSIVDFV
CBPA1_ANOGA/1-433 ALAEKFDWYVFPNANPDGYAYTFQVNRLWRKTR-KAYGPFCYGADPNRNWDFHWAEQGTSNNACSDTYHGSEAFSEVETRSLAAFV
CBPA1_DROPS/1-425 QLAENYTWYVLPHANPDGYVYTHTTDRMWRKTR-TPYQG-CFGADPNRNWAFHWNEVGASSNPCSDTYAGPSAFSEIETLSLSNYL
ECM14_AJEDR/1-592 TLLEQFDFIFIPTINPDGYVHTWETDRLWRKNRQETSLPFCPGVDLDRTWGFEWNGNATGDNPCSESYGGDEPFAGTEARQLAGWV
186H 191A 201L 211Q 220N 230L 240Y 242- 245T 255I
CBPA1_PIG/1-419 DFVNDHG----NIKAFISIHSYSQLLLYPYGYKTEA-PADKDELDQISKSAVAALTSLYGTKFQYG----------SIITTIYQASGGTIDW
CBPA1_BOVINE/1-307 DFVKDHG----NFKAFLSIHSYSQLLLYPYGYTTQS-IPDKTELNQVAKSAVAALKSLYGTSYKYG----------SIITTIYQASGGSIDW
CBPA1_HUMAN/1-419 DFVKDHG----NIKAFISIHSYSQLLMYPYGYKTEP-VPDQDELDQLSKAAVTALASLYGTKFNYG----------SIIKAIYQASGSTIDW
CBPA1_RAT/1-419 DFVTSHG----NIKAFISIHSYSQLLLYPYGYTSEP-APDQAELDQLAKSAVTALTSLHGTKFKYG----------SIIDTIYQASGSTIDW
CBPA1_MOUSE/1-419 DFVTSHG----NIKAFISIHSYSQLLLYPYGYTSEP-APDKEELDQLAKSAVTALTSLHGTKFKYG----------SIIDTIYQASGSTIDW
CBPA1_ANOGA/1-433 AFVEKLRG----KLGAYIAFHSYSQLLLFPYGHTGEH-SPNHQDLNEIAEATVKSLAKRYGTQYKYG----------NVYDAIYPASGSSVDW
CBPA1_DROPS/1-425 NYLASLKG----KIQLYISLHAYSQYLLYPYGHTGDL-PDNVADFRKVYDVSIAAVNKRYGTTYTGG----------NIYDAIYPAAGASVDW
ECM14_AJEDR/1-592 GWVKEQTEQHNVKFVAYLDLHSYSQQVLYPYSYSCLPRPPNLENLEELAMGIAKAIRLTNRQSYTVSSACQGFTASQKKVKLDTFPRMESTGGSALDW
264K 274T 281L 291Q 301M +4 +14 +24 +34
CBPA1_PIG/1-419 TY-NQGIKYSFSFELRDTG---RYGFLLPASQIIPTAQETWLALLTIMEHTLNHPY----------
CBPA1_BOVINE/1-307 SY-NQGIKYSFTFELRDTG---RYGFLLPASQIIPTAQETWLGVLTIMEHTVNN----------
CBPA1_HUMAN/1-419 TY-SQGIKYSFTFELRDTG---RYGFLLPASQIIPTAKETWLALLTIMEHTLNHPY----------
CBPA1_RAT/1-419 TY-SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMDHTVKHPY----------
CBPA1_MOUSE/1-419 TY-SQGIKYSFTFELRDTG---LRGFLLPASQIIPTAEETWLALLTIMDHTVKHPY----------
CBPA1_ANOGA/1-433 SYGAQDVKIAYTYELRPDGDAWN-GFVLPPNEIVPTGEETLDSLITLLEESSARGYYDEKH----------
CBPA1_DROPS/1-425 AYGTQDVRMAFCYELRPSSNSFITGFKLPAAQIVPASEELLDSIVAMAAEVNNLGYFN----------
ECM14_AJEDR/1-592 FYNDVGVKYSYQLKLRDKG---SYGFLLPRENIVPTGKEVFNAVMVLAKFLLGSDGFEGLNWEAEFQRLNEADKPILDDGDDDEEEDGQDKKDDSW

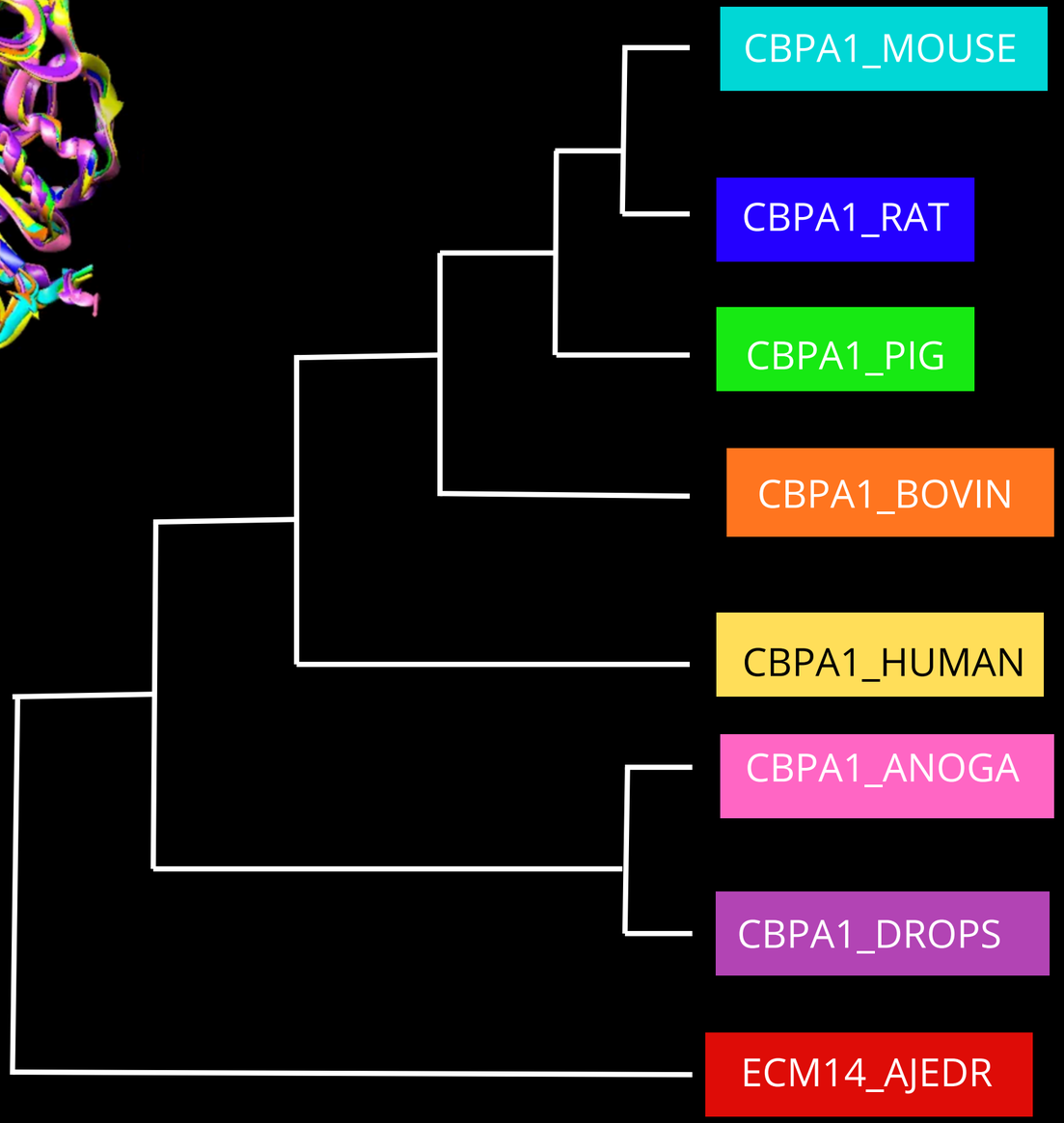
SUPERIMPOSITION





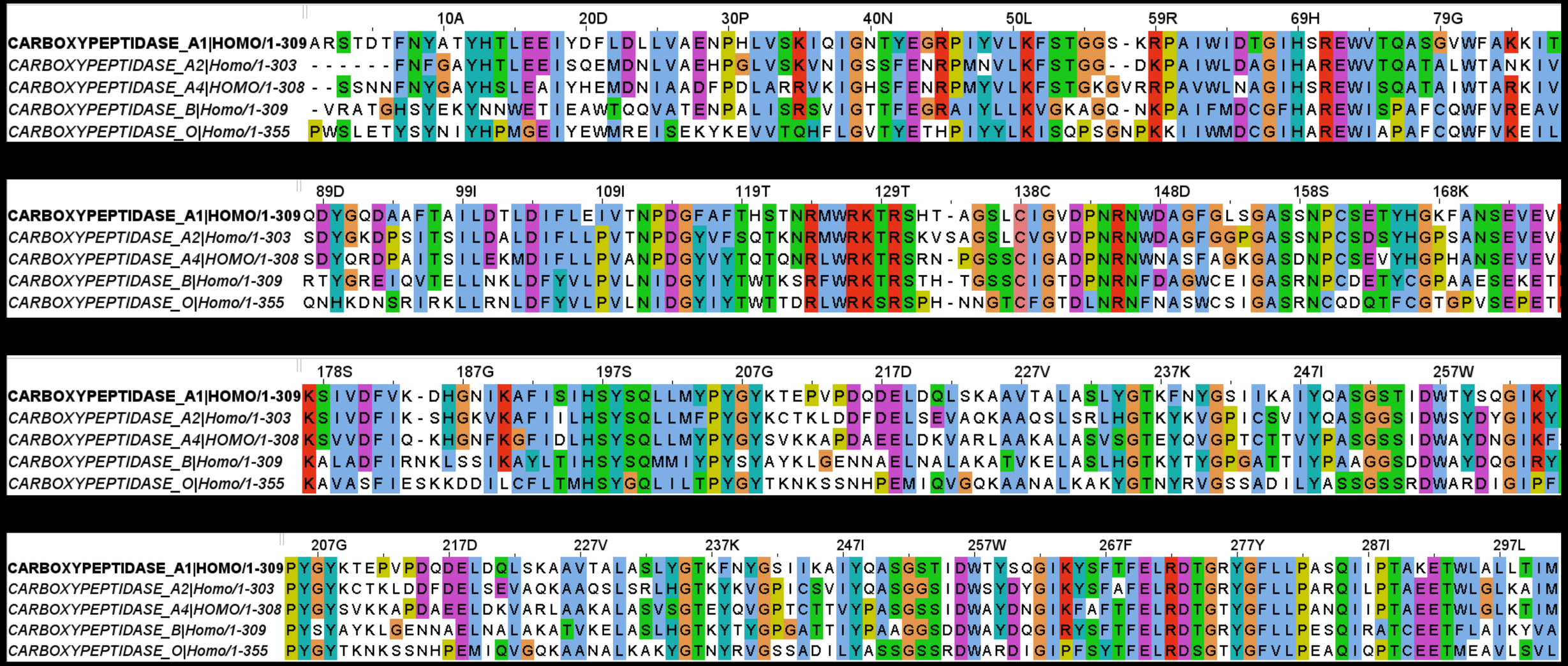


CLUSTERS:



	No.	Domain1	Domain2	Sc	RMS	Len1	Len2	Align	NFit	Eq.	Secs.	%I	%S	P(m)
Pair	1	CBPA1_ANOGA_	CBPA1_BOVIN_	8.30	1.32	405	403	411	382	378	0	38.36	100.00	1.02e-47
Pair	2	CBPA1_ANOGA_	CBPA1_DROPS_	8.73	1.01	405	400	405	384	381	0	50.39	100.00	4.47e-88
Pair	3	CBPA1_ANOGA_	CBPA1_HUMAN_	8.07	1.47	405	396	405	373	373	0	38.34	100.00	8.72e-48
Pair	4	CBPA1_ANOGA_	CBPA1_MOUSE_	8.34	1.29	405	403	409	382	379	0	36.41	100.00	3.44e-43
Pair	5	CBPA1_ANOGA_	CBPA1_PIG_	8.35	1.30	405	403	409	383	379	0	36.15	100.00	1.76e-42
Pair	6	CBPA1_ANOGA_	CBPA1_RAT_	8.35	1.28	405	403	409	382	379	0	37.20	100.00	2.38e-45
Pair	7	CBPA1_ANOGA_	ECM14_AJEDR_	6.44	1.48	405	456	454	342	335	0	33.13	100.00	6.59e-31
Pair	8	CBPA1_BOVIN_	CBPA1_DROPS_	7.77	1.61	403	400	412	366	357	0	33.33	100.00	2.80e-33
Pair	9	CBPA1_BOVIN_	CBPA1_HUMAN_	9.21	0.88	403	396	402	393	393	0	82.44	100.00	0.00e+00
Pair	10	CBPA1_BOVIN_	CBPA1_MOUSE_	9.63	0.53	403	403	403	403	403	0	76.67	100.00	0.00e+00
Pair	11	CBPA1_BOVIN_	CBPA1_PIG_	9.64	0.52	403	403	403	403	403	0	85.36	100.00	0.00e+00
Pair	12	CBPA1_BOVIN_	CBPA1_RAT_	9.63	0.54	403	403	403	403	403	0	78.66	100.00	0.00e+00
Pair	13	CBPA1_BOVIN_	ECM14_AJEDR_	6.63	1.35	403	456	451	350	337	0	34.72	100.00	1.24e-34
Pair	14	CBPA1_DROPS_	CBPA1_HUMAN_	7.66	1.68	400	396	409	365	361	0	34.35	100.00	4.58e-36
Pair	15	CBPA1_DROPS_	CBPA1_MOUSE_	7.91	1.55	400	403	412	373	370	0	33.78	100.00	1.61e-35
Pair	16	CBPA1_DROPS_	CBPA1_PIG_	7.91	1.54	400	403	412	372	367	0	32.97	100.00	2.72e-33
Pair	17	CBPA1_DROPS_	CBPA1_RAT_	7.92	1.54	400	403	412	373	368	0	33.97	100.00	8.69e-36
Pair	18	CBPA1_DROPS_	ECM14_AJEDR_	6.35	1.50	400	456	453	340	330	0	30.30	100.00	1.17e-24
Pair	19	CBPA1_HUMAN_	CBPA1_MOUSE_	9.34	0.67	396	403	402	393	393	0	82.70	100.00	0.00e+00
Pair	20	CBPA1_HUMAN_	CBPA1_PIG_	9.35	0.69	396	403	402	393	393	0	88.04	100.00	0.00e+00
Pair	21	CBPA1_HUMAN_	CBPA1_RAT_	9.35	0.66	396	403	402	393	393	0	85.24	100.00	0.00e+00
Pair	22	CBPA1_HUMAN_	ECM14_AJEDR_	6.58	1.39	396	456	449	342	332	0	32.23	100.00	1.02e-28
Pair	23	CBPA1_MOUSE_	CBPA1_PIG_	9.77	0.19	403	403	403	403	403	0	81.39	100.00	0.00e+00
Pair	24	CBPA1_MOUSE_	CBPA1_RAT_	9.79	0.12	403	403	403	403	403	0	94.54	100.00	0.00e+00
Pair	25	CBPA1_MOUSE_	ECM14_AJEDR_	6.66	1.32	403	456	452	351	340	0	33.53	100.00	3.21e-32
Pair	26	CBPA1_PIG_	CBPA1_RAT_	9.78	0.16	403	403	403	403	403	0	83.37	100.00	0.00e+00
Pair	27	CBPA1_PIG_	ECM14_AJEDR_	6.72	1.27	403	456	450	350	337	0	32.34	100.00	2.27e-29
Pair	28	CBPA1_RAT_	ECM14_AJEDR_	6.68	1.34	403	456	451	352	339	0	32.74	100.00	2.13e-30

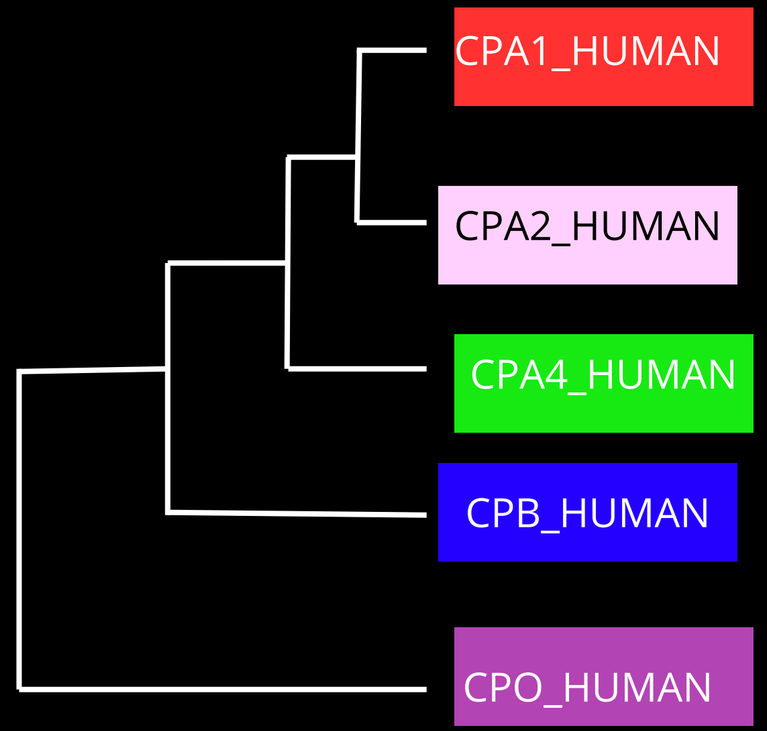
MSA


10A 20D 30P 40N 50L 59R 69H 79G
CARBOXYPEPTIDASE_A1|HOMO/1-309 ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGS-KRPAIWIDTGIHSREWVTQASGVWFAKKIT
CARBOXYPEPTIDASE_A2|Homo/1-303 ------FNFGAYHTLEEISQEMDNLVAEHPGLVSKVNIGSSFENRPMNVLKFSTGG--DKPAIWLDAGIHAREWVTQATALWTANKIV
CARBOXYPEPTIDASE_A4|HOMO/1-308 --SSNNFNYGAYHSLEAIYHEMDNIAADFPDLARRVKIGHSFENRPMYVLKFSTGKGVRRPAVWLNAGIHSREWISQATAIWTARKIV
CARBOXYPEPTIDASE_B|Homo/1-309 -VRATGHSYEKYNNWETIEAWTQQVATENPALISRSVIGTTFEGRAIYLLKVGKAGQ-NKPAIFMDCGFHAREWISPAFCQWFVREAV
CARBOXYPEPTIDASE_O|Homo/1-355 PWSLETYSYNIYHPMGEIYEWMREISEKYKEVVTQHFLGVTYETHPIYYLKISQPSGNPKKIIWMDCGIHAREWIAPAFCQWFVKEIL
89D 99I 109I 119T 129T 138C 148D 158S 168K
CARBOXYPEPTIDASE_A1|HOMO/1-309 QDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHT-AGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEV
CARBOXYPEPTIDASE_A2|Homo/1-303 SDYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKVSAGSLCVGVDPNRNWDAGFGGPGASSNPCSDSYHGPSANSEVEV
CARBOXYPEPTIDASE_A4|HOMO/1-308 SDYQRDPAITSILEKMDIFLLPVANPDGYVYTQTQNRLWRKTRSRN-PGSSCIGADPNRNWNASFAGKGASDNPCSEVYHGPHANSEVEV
CARBOXYPEPTIDASE_B|Homo/1-309 RTYGREIQVTELLNKLDFYVLPVLNIDGYIYTWTKSRFWRKTRSTH-TGSSCIGTDPNRNFDAGWCEIGASRNPCDETYCGPAAESEKET
CARBOXYPEPTIDASE_O|Homo/1-355 QNHKDNSRIRKLLRNLDFYVLPVLNIDGYIYTWTTDRLWRKSRSPH-NNGTCFGTDLNRNFNASWCSIGASRNCQDQTFCGTGPVSEPET
178S 187G 197S 207G 217D 227V 237K 247I 257W
CARBOXYPEPTIDASE_A1|HOMO/1-309 KSIVDFVK-DHGNIKAFISIHSYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIYQASGSTIDWTYSQGIKY
CARBOXYPEPTIDASE_A2|Homo/1-303 KSIVDFIK-SHGKVKAFIILHSYSQLLMFPYGYKCTKLDDFDELSEVAQKAAQSLSRLHGTKYKVGPICSVIYQASGGSIDWSYDYGIKY
CARBOXYPEPTIDASE_A4|HOMO/1-308 KSVVDFIQ-KHGNFKGFIDLHSYSQLLMYPYGYSVKKAPDAEELDKVARLAAKALASVSGTEYQVGPTCTTVYPASGSSIDWAYDNGIKF
CARBOXYPEPTIDASE_B|Homo/1-309 KALADFIRNKLSSIKAYLTIHSYSQMMIYPYSYAYKLGENNAELNALAKATVKELASLHGTKYTYGPGATTIYPAAGGSDDWAYDQGIRY
CARBOXYPEPTIDASE_O|Homo/1-355 KAVASFIESKKDDILCFLTMHSYGQLILTPYGYTKNKSSNHPEMIQVGQKAANALKAKYGTNYRVGSSADILYASSGSSRDWARDIGIPF
207G 217D 227V 237K 247I 257W 267F 277Y 287I 297L
CARBOXYPEPTIDASE_A1|HOMO/1-309 PYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIYQASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPASQIIPTAKETWLALLTIM
CARBOXYPEPTIDASE_A2|Homo/1-303 PYGYKCTKLDDFDELSEVAQKAAQSLSRLHGTKYKVGPICSVIYQASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPARQILPTAEETWLGLKAIM
CARBOXYPEPTIDASE_A4|HOMO/1-308 PYGYSVKKAPDAEELDKVARLAAKALASVSGTEYQVGPTCTTVYPASGSSIDWAYDNGIKFAFTFELRDTGTYGFLLPANQIIPTAEETWLGLKTIM
CARBOXYPEPTIDASE_B|Homo/1-309 PYSYAYKLGENNAELNALAKATVKELASLHGTKYTYGPGATTIYPAAGGSDDWAYDQGIRYSFTFELRDTGRYGFLLPESQIRATCEETFLAIKYVA
CARBOXYPEPTIDASE_O|Homo/1-355 PYGYTKNKSSNHPEMIQVGQKAANALKAKYGTNYRVGSSADILYASSGSSRDWARDIGIPFSYTFELRDSGTYGFVLPEAQIQPTCEETMEAVLSVL

HUMAN M14A SUPERIMPOSITION



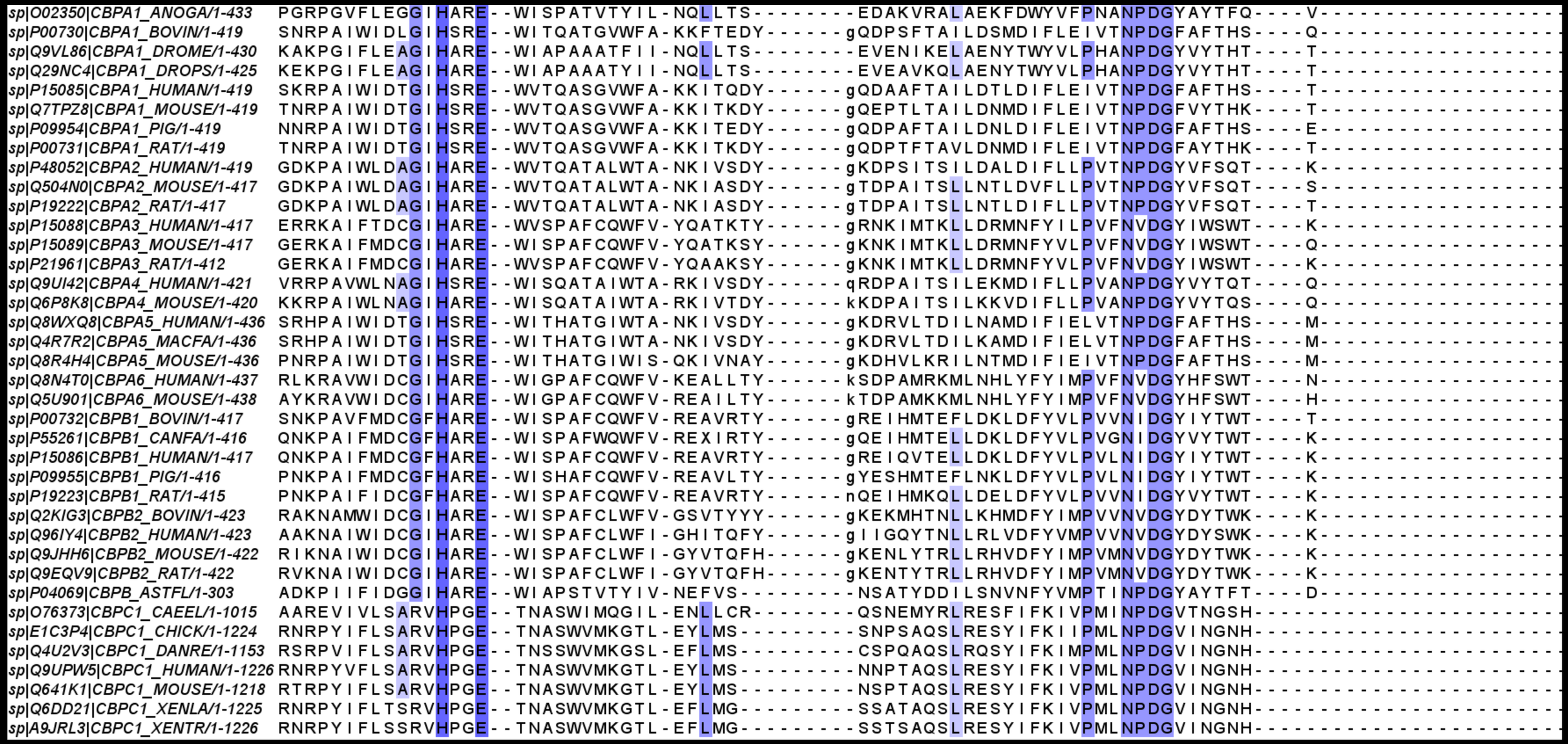
CLUSTERS:



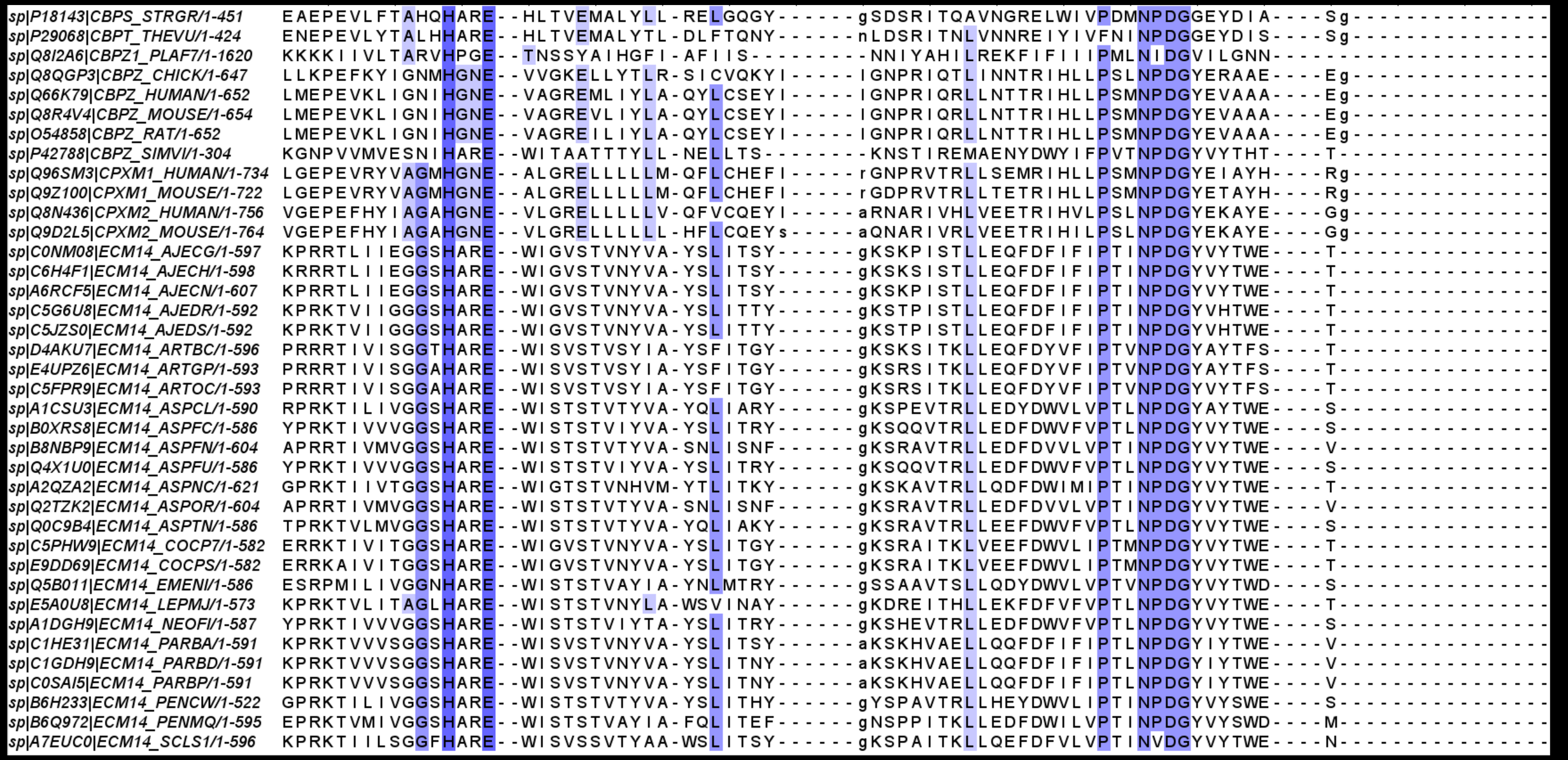
M14A SUPERIMPOSITION

	No.	Domain1	Domain2	Sc	RMS	Len1	Len2	Align	NFit	Eq.	Secs.	%I	%S	P(m)
Pair	1	CPA2	CPB	9.26	0.78	303	306	305	301	301	0	46.51	100.00	3.87e-59
Pair	2	CPA2	CPA4	9.33	0.58	303	307	304	301	301	0	68.11	100.00	0.00e+00
Pair	3	CPA2	CPA1	9.36	0.50	303	308	303	301	301	0	66.11	100.00	0.00e+00
Pair	4	CPA2	CPO	9.04	0.75	303	307	306	293	293	0	39.25	100.00	5.90e-40
Pair	5	CPB	CPA4	9.13	0.80	306	307	306	299	299	0	48.16	100.00	3.10e-63
Pair	6	CPB	CPA1	9.16	0.74	306	308	309	301	301	0	48.50	100.00	1.32e-64
Pair	7	CPB	CPO	9.12	0.79	306	307	307	297	297	0	48.15	100.00	8.63e-63
Pair	8	CPA4	CPA1	9.35	0.57	307	308	308	301	300	0	60.00	100.00	7.35e-100
Pair	9	CPA4	CPO	8.94	0.92	307	307	308	295	293	0	41.30	100.00	1.17e-44
Pair	10	CPA1	CPO	9.00	0.80	308	307	307	296	296	0	39.19	100.00	3.28e-40

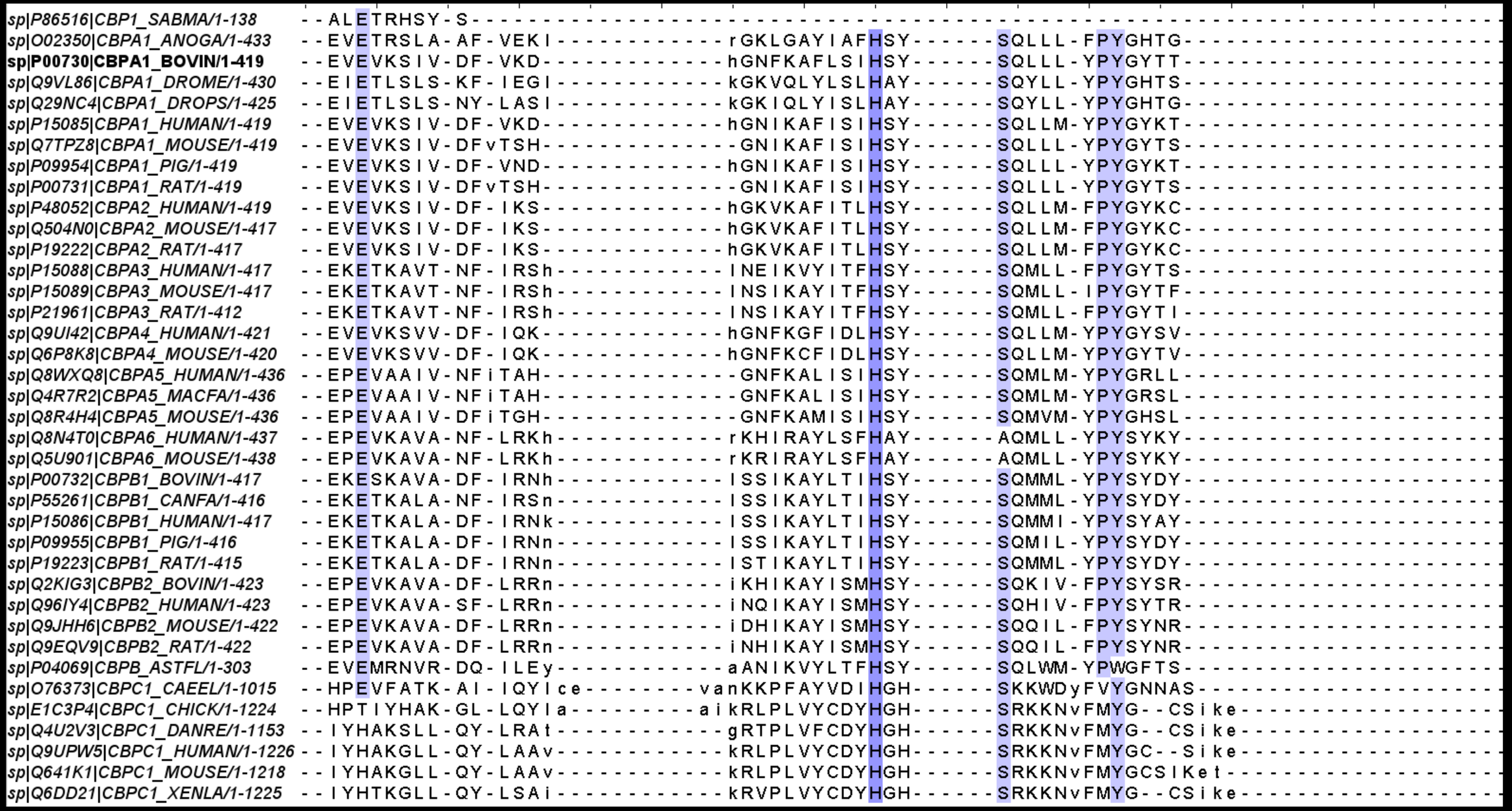
HMMERALIGN HIS69 I GLU72



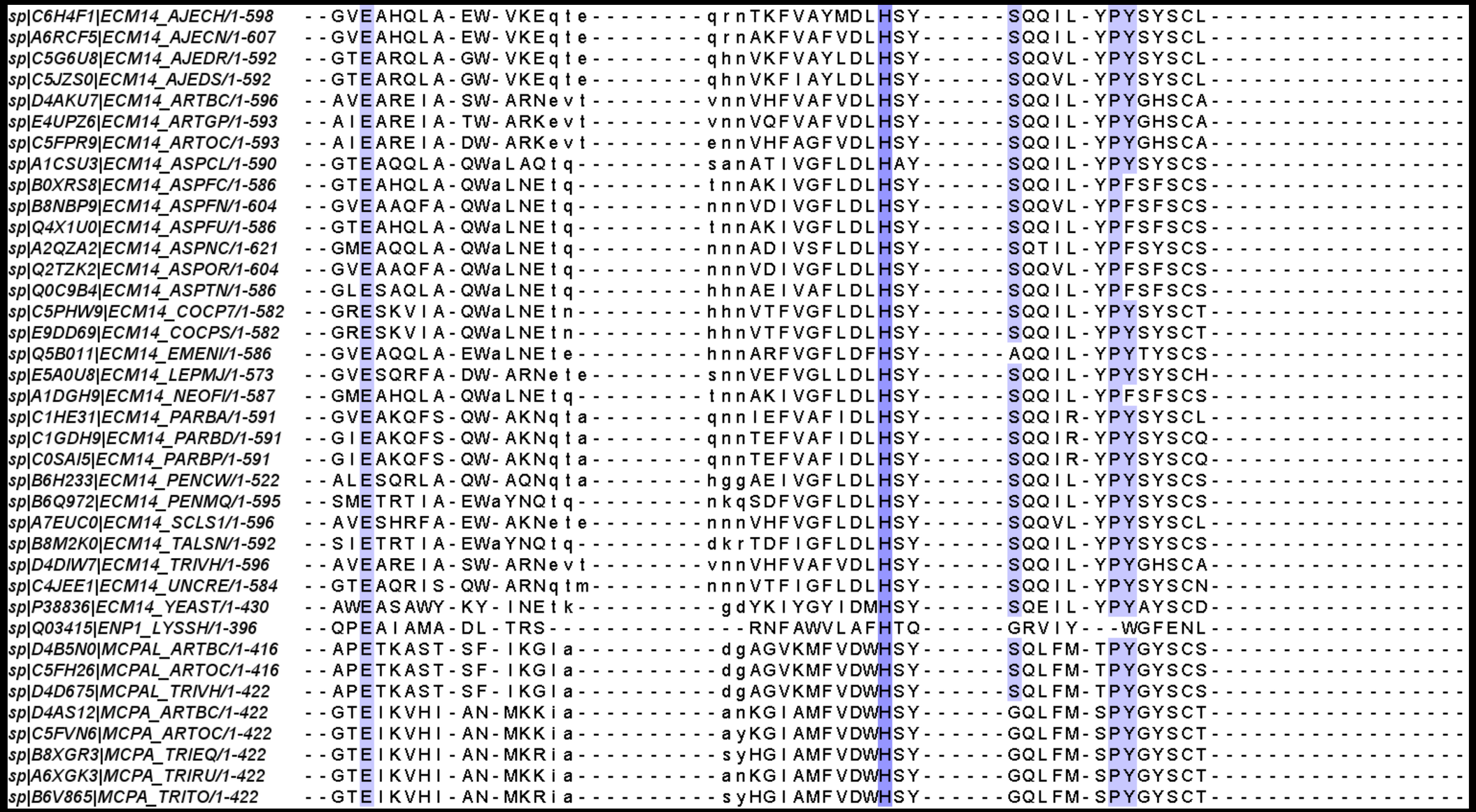
HMMERALIGN HIS69 I GLU72



HMMALIGN HIS196



HMMERALIGN HIS196



FROM SEQUENCE TO DISEASE:
HOW A SINGLE MUTATION DISRUPTS CPA1 STRUCTURE AND FUNCTION

PATHOGENIC MUTATION **N256K**

LOCAL DESTABILIZATION AND OVERALL PROTEIN **MISFOLDING**

INSOLUBLE AGGREGATES

ENDOPLASMIC RETICULUM STRESS

PROGRESSIVE CHRONIC PANCREATITIS

CONCLUSIONS

- The active site is strictly mantained
- Autologous inhibition by the pro-segment
- Heterologous inhibitors: PCI (stopper-like mechanism) vs BZS (transition state mimetic)
- Minimal changes determine specificity
- In the alignments we do not only see conservation, we find identity


THANK YOU

BIBLIOGRAPHY

Alvarez-Santos, S., González-Lafont, A., Lluch, J. M., Oliva, B., & Avilés, F. X. (1994). On the water-promoted mechanism of peptide cleavage by carboxypeptidase A. A theoretical study. *Canadian Journal Of Chemistry*, *72*(10), 2077-2083. https://doi.org/10.1139/v94-264

Arolas, J. L., Lorenzo, J., Rovira, A., Vendrell, J., Aviles, F. X., & Ventura, S. (2004). Secondary Binding Site of the Potato Carboxypeptidase Inhibitor. Contribution to Its Structure, Folding, and Biological Properties. *Biochemistry*, *43*(24), 7973-7982. https://doi.org/10.1021/bi049596j

Avilés, F. X., Vendrell, J., Guasch, A., Coll, M., & Huber, R. (1993). Advances in metallo-procarboxypeptidases. *European Journal Of Biochemistry*, *211*(3), 381-389. https://doi.org/10.1111/j.1432-1033.1993.tb17561.x

Cerdà-Costa, N., & Gomis-Rüth, F. X. (2013). Architecture and function of metallopeptidase catalytic domains. *Protein Science*, *23*(2), 123-144. https://doi.org/10.1002/pro.2400

Enzyme Active Sites and their Reaction Mechanisms. (2021). En *Elsevier eBooks*. https://doi.org/10.1016/c2019-0-03090-7

Fajardo, D., Jean, R. S., & Lyons, P. J. (2023). Acquisition of new function through gene duplication in the metallocarboxypeptidase family. *Scientific Reports*, *13*(1), 2512. https://doi.org/10.1038/s41598-023-29800-9

Garcia-Guerrero, M. C., Garcia-Pardo, J., Berenguer, E., Fernandez-Alvarez, R., Barfi, G. B., Lyons, P. J., Aviles, F. X., Huber, R., Lorenzo, J., & Reverter, D. (2018). Crystal structure and mechanism of human carboxypeptidase O: Insights into its specific activity for acidic residues. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(17), E3932–E3939. https://doi.org/10.1073/pnas.1803685115

Guasch, A., Coll, M., Avilés, F. X., & Huber, R. (1992). Three-dimensional structure of porcine pancreatic procarboxypeptidase A. *Journal Of Molecular Biology*, *224*(1), 141-157. https://doi.org/10.1016/0022-2836(92)90581-4

Kilshtain-Vardi, A., Glick, M., Greenblatt, H. M., Goldblum, A., & Shoham, G. (2003). Refined structure of bovine carboxypeptidase A at 1.25 Å resolution. *Acta Crystallographica Section D Biological Crystallography*, *59*(2), 323-333. https://doi.org/10.1107/s0907444902015706

Ma, W., Tang, C., & Lai, L. (2005). Specificity of Trypsin and Chymotrypsin: Loop-Motion-Controlled Dynamic Correlation as a Determinant. *Biophysical Journal*, *89*(2), 1183-1193. https://doi.org/10.1529/biophysj.104.057158

BIBLIOGRAPHY

Mangani, S., Carloni, P., & Orioli, P. (1992). Crystal structure of the complex between carboxypeptidase A and the biproduct analog inhibitor l-benzylsuccinate at 2·0 Å resolution. *Journal Of Molecular Biology*, *223*(2), 573-578. https://doi.org/10.1016/0022-2836(92)90671-6

Németh, B. C., Demcsák, A., Geisz, A., & Sahin-Tóth, M. (2022). Misfolding-induced chronic pancreatitis in CPA1 N256K mutant mice is unaffected by global deletion of Ddit3/Chop. Scientific reports, 12(1), 6357. Alvarez-Santos, S., González-Lafont, A., Lluch, J. M., Oliva, B., & Avilés, F. X. (1994). On the water-promoted mechanism of peptide cleavage by carboxypeptidase A. A theoretical study. Canadian Journal Of Chemistry, 72*(10), 2077-2083.* https://doi.org/10.1139/v94-264

Oliva, B., Marino, C., Daura, X., Molina, M. A., Canals, F., Aviles, F. X., & Querol, E. (1995). On the Entropic and Hydrophobic Properties Involved in the Inhibitory Mechanism of Carboxypeptidase A by its Natural Inhibitor from Potato. *Journal Of Molecular Modeling*, *1*(2), 54-67. https://doi.org/10.1007/s008940050007

Sándor, M., Thiel, F. G., Schmid, M., Demcsák, A., Morales Granda, N. C., Németh, B. C., Vajda, S., Hoerning, A., & Sahin-Tóth, M. (2022). Novel p.G250A Mutation Associated with Chronic Pancreatitis Highlights Misfolding-Prone Region in Carboxypeptidase A1 (CPA1). *International journal of molecular sciences*, *23*(24), 15463. https://doi.org/10.3390/ijms232415463

Tanco, S., Lorenzo, J., Garcia-Pardo, J., Degroeve, S., Martens, L., Aviles, F. X., Gevaert, K., & Van Damme, P. (2013). Proteome-derived peptide libraries to study the substrate specificity profiles of carboxypeptidases. *Molecular & cellular proteomics : MCP*, *12*(8), 2096–2110. https://doi.org/10.1074/mcp.M112.023234

Vendrell, J., Querol, E., & Avilés, F. X. (2000). Metallocarboxypeptidases and their protein inhibitors. Biochimica Et Biophysica Acta (BBA) - Protein Structure And Molecular Enzymology, 1477(1-2), 284-298. https://doi.org/10.1016/s0167-4838(99)00280-0 ytic domains. Protein Science, 23(2), 123-144. https://doi.org/10.1002/pro.2400

PEM QUESTIONS

1. Which classification system was created in 1983 to classify proteases based on structural features and evolutionary relationships?
A) NC-IUBMB
B) MEROPS
C) SCOP
D) EC Indexing
E) CATH

2. What is the characteristic architecture of the Carboxypeptidase A (CPA) scaffold?
A) All-alpha helix bundle
B) Single-layer beta-barrel
C) Three-layer αβα sandwich
D) Disordered fibrous mesh
E) Purely antiparallel beta-propeller

3. Which metal ion is essential for the catalytic activity of CPA and how is it coordinated?
A) Iron, coordinated octahedrally
B) Magnesium, coordinated by three glutamates
C) Zinc, coordinated tetrahedrally
D) Copper, coordinated by four histidines
E) Calcium, coordinated by the pro-peptide

4. Which residue is considered "key for the catalytic function" and acts as a general acid/base in the promoted-water pathway?
A) Arg127
B) Glu270
C) Ile255
D) Asp41
E) His196

PEM QUESTIONS

5. How does the S1' pocket of Human Carboxypeptidase O (hCPO) differ from CPA and CPB?
A) It contains a hydrophobic residue for aliphatic chains.
B) It contains a negatively charged residue for basic substrates.
C) It contains a positively charged Arg275 for acidic substrates.
D) It lacks an S1' pocket entirely.
E) It uses a zinc-bridged water molecule for specificity.

6. Which enzyme is responsible for the first proteolytic cleavage of PCPA between Arg99 and Ala1?
A) Chymotrypsin
B) Pepsin
C) Trypsin
D) Elastase
E) CPA itself (autocatalysis)

7. What is the "stopper-like" mechanism used by the Potato Carboxypeptidase Inhibitor (PCI)?
A) It removes the zinc ion from the active site.
B) Its C-terminal tail docks into the active site, blocking substrate access.
C) It denatures the αβα sandwich.
D) It binds to the N-terminus and prevents folding.
E) It induces a permanent salt bridge with Arg127.

8. What happens to the active site of CPA when there is an "excess of zinc"?
A) Catalytic efficiency increases by 200%.
B) The protein becomes more soluble and stable.
C) A second metal-binding site forms, creating an inhibitory binuclear zinc center.
D) The zinc ion is replaced by copper.
E) The pro-peptide is unable to be cleaved by trypsin.

PEM QUESTIONS

9. Analysis of orthologues shows that the zinc-binding ligands (His69, Glu72, His196) are:
A) Highly variable among mammals.
B) Strictly invariant across species.
C) Replaced by cysteines in fungi.
D) Only conserved in the pro-peptide form.
E) Frequently mutated to increase catalytic speed.

10. What structural difference explains why PCPA activation is "gradual and progressive" compared to the "rapid" activation of PCPB?
A) PCPA requires two proteolytic cleavages (by trypsin and chymotrypsin)
B) PCPA lacks a zinc ion during its initial maturation
C) PCPA contains a positively charged Arg275 in its active site
D) PCPA is inhibited by an excess of zinc in the pancreatic duct
E) PCPA has a simpler one-step cleavage by trypsin only