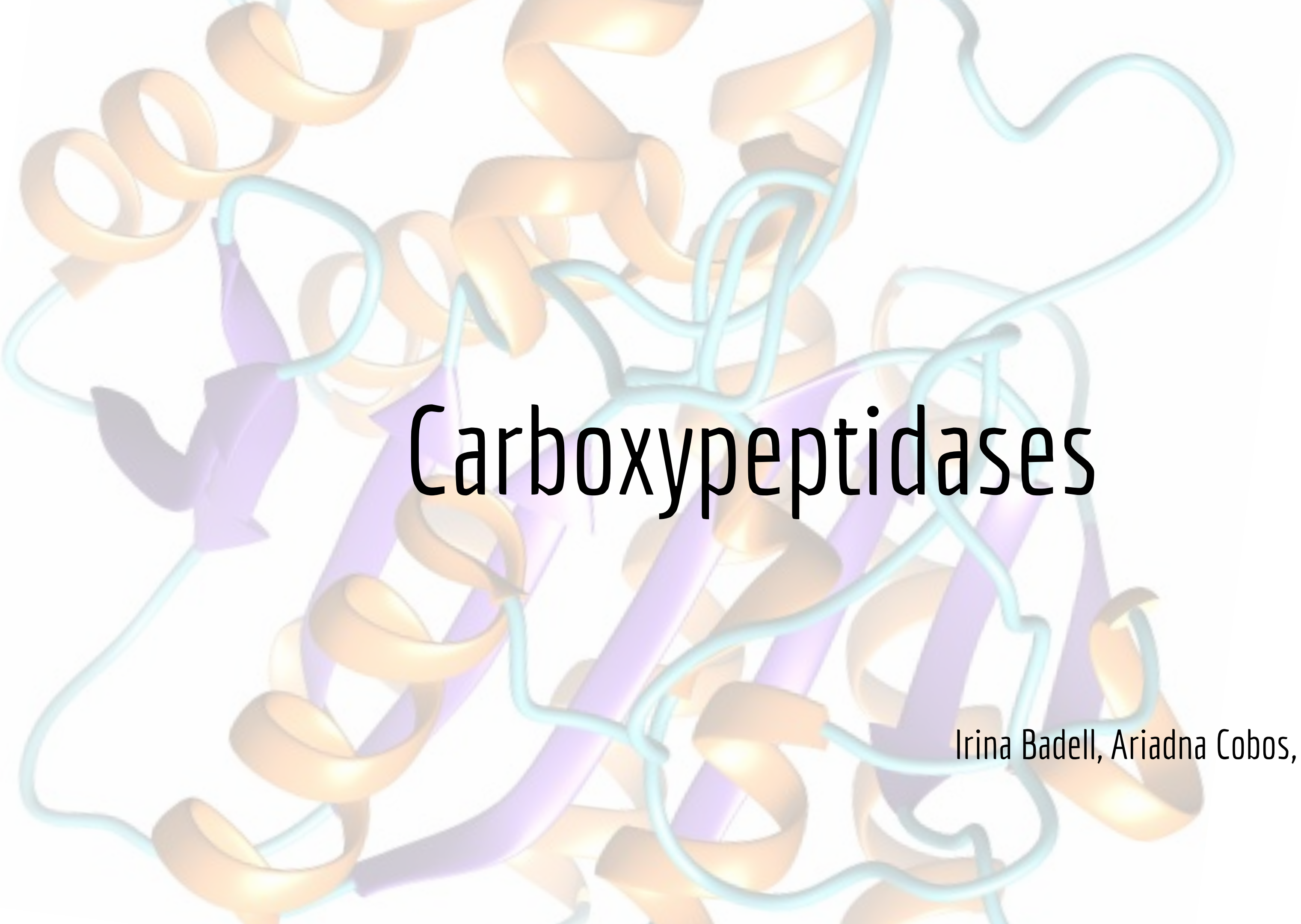
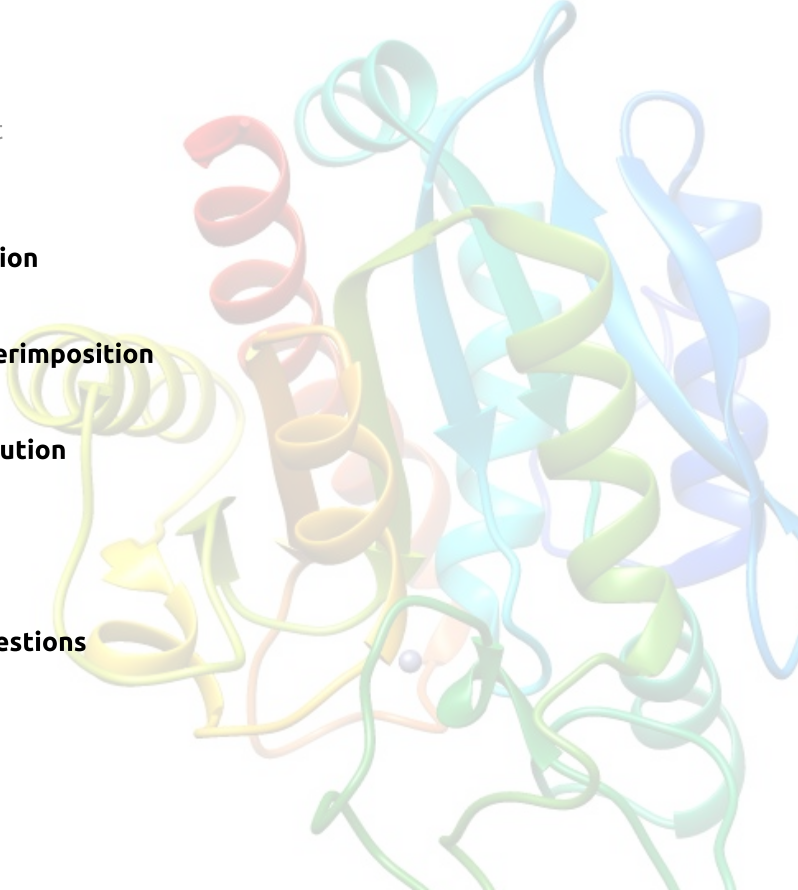


Carboxypeptidases

Irina Badell, Ariadna Cobos, Marta Dacosta

Index



Introduction

Peptidases

- ➢ Proteolytic enzymes
- ➢ Catalytic and regulatory functions
- ➢ Found in 237 species
- ➢ Essential for life

Action site:

- Endopeptidases
- Exopeptidases

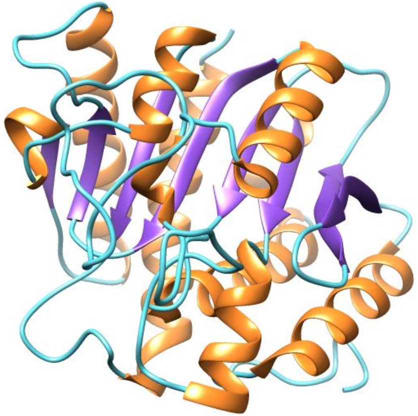
Catalytic site:

- Serine proteases
- Cysteine proteases
- Aspartic acid proteases
- Threonine proteases
- Glutamic proteases
- Metallo-peptidases

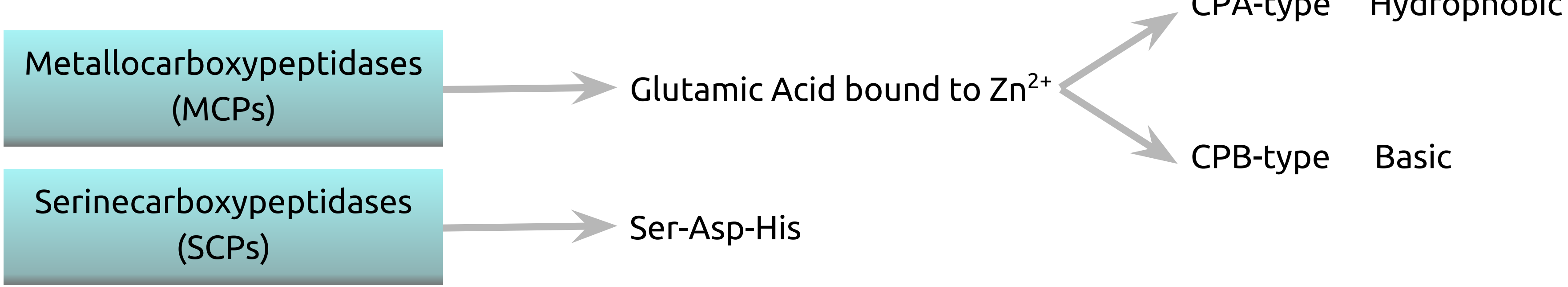
https://www.ebi.ac.uk/merops/cgi-bin/organism_famdist?family=M14;taxon=Animalia;id=peptidase

Introduction Carboxypeptidases

- ➢ Exopeptidases
- ➢ C-terminal
- ➢ Pancreatic secretions



Action site:



Metallocarboxypeptidases (MCPs) → Glutamic Acid bound to Zn^{2+}

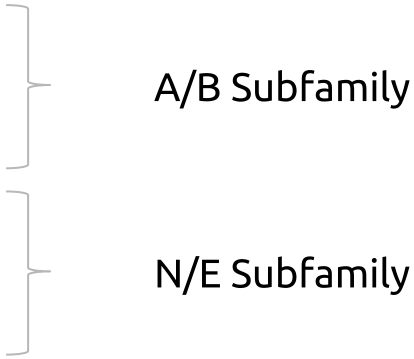
Glutamic Acid bound to Zn^{2+} → CPA-type Hydrophobic

Glutamic Acid bound to Zn^{2+} → CPB-type Basic

Serinecarboxypeptidases (SCPs) → Ser-Asp-His

Introduction MEROPS Classification

Clan	Family	Subfamily
MC	M14	M14A
		M14B
		M14C
		M14D



A/B Subfamily

N/E Subfamily

Rawlings, N.D., Barrett, A.J. & Finn, R.D. (2016) Twenty years of the *MEROPS* database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res* **44**, D343-D350

Introduction

A/B Subfamily

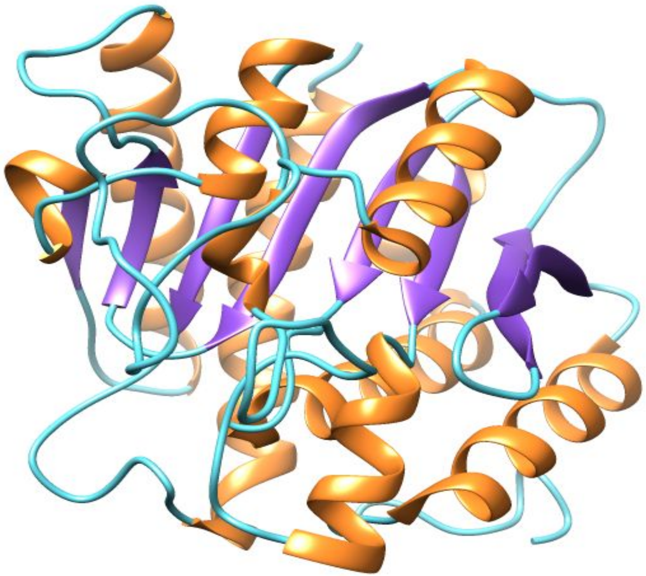
M14A (CPA)	M14B (CPH)
- Catalytic or digestive - Zymogens - Active site blocked	- Active enzymes - Transthyretin-like domain
CPA1 CPA2 CPA4 CPB	CPD CPE CPN CPM ...

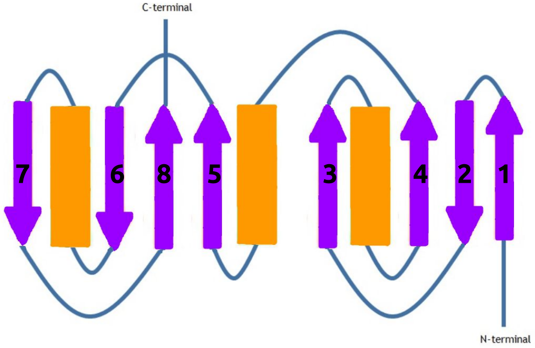
Introduction SCOP Classification

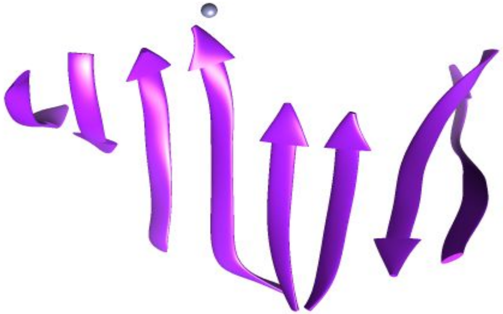
Class	Fold	Superfamily	Family
α/β proteins	Phosphorylase/hydrolase-like	Zn-dependent exopeptidases	**Pancreatic CPs**
			Carboxipeptidase T
			Leucine aminopeptidase, C-terminal domain
			Bacterial dinuclear Zn exopeptidases
			FolH catalytic domain-like
			N-acetylmuramoyl-L-alanine amidase-like
			AstE/AspA-like
			Glutaminyl-peptide cyclotransferase-like
			FGase-like

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

Introduction Rossmann-like Fold

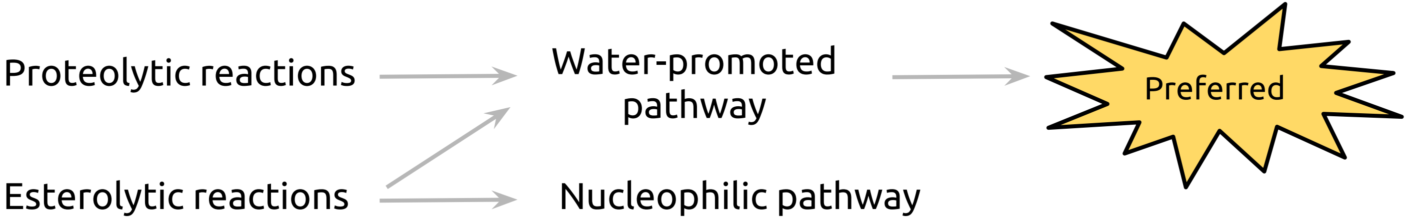






Introduction Catalysis

- Discrepancy with the catalytic mechanism of CPA
  - Different mechanisms
  - Different rate-limiting steps



Proteolytic reactions

Esterolytic reactions

Water-promoted pathway

Nucleophilic pathway

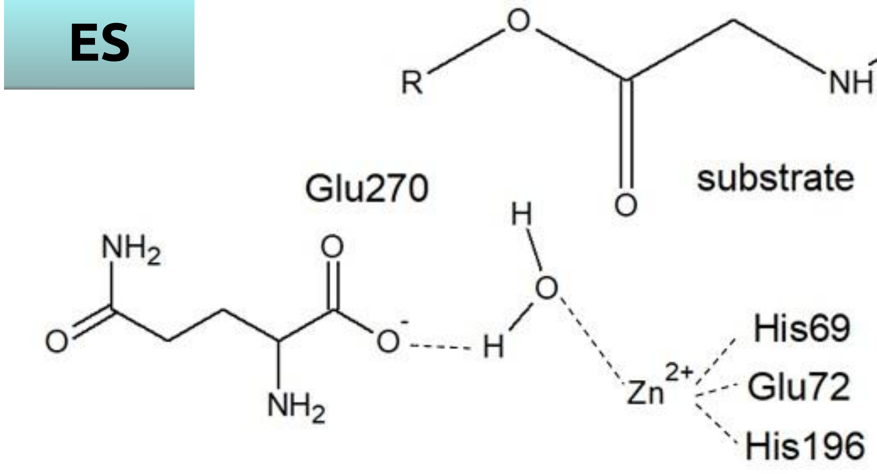
Preferred

Introduction

Water-promoted pathway

- Water acts as a proton donor for Glu270

ES



Arg127
Asn144
Arg145
Tyr127

C-terminal end

Arg127
Arg71

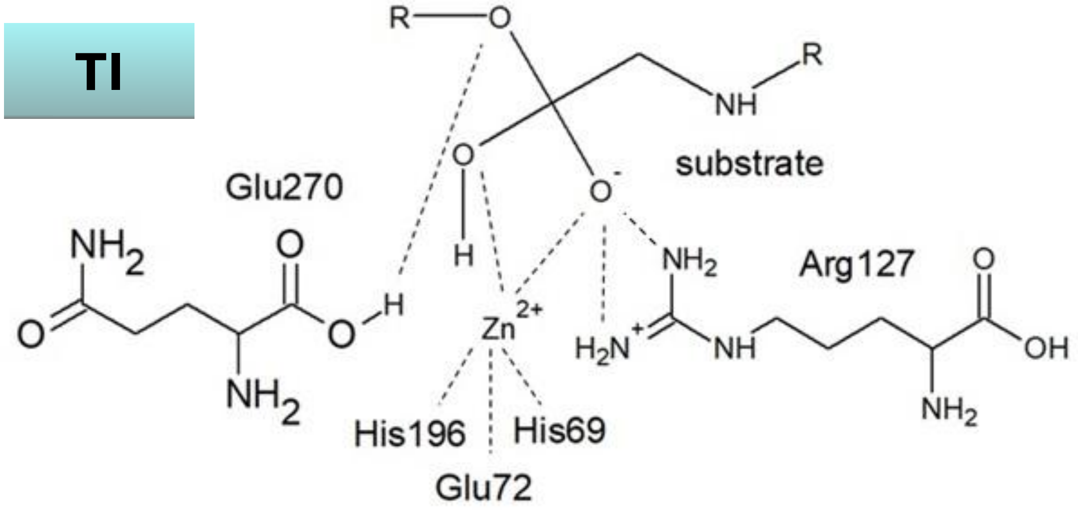
Benzoyl carbonyl oxygen

Ser197 → Stabilization

Introduction Water-promoted pathway

- STEP 1: nucleophilic addition

TI



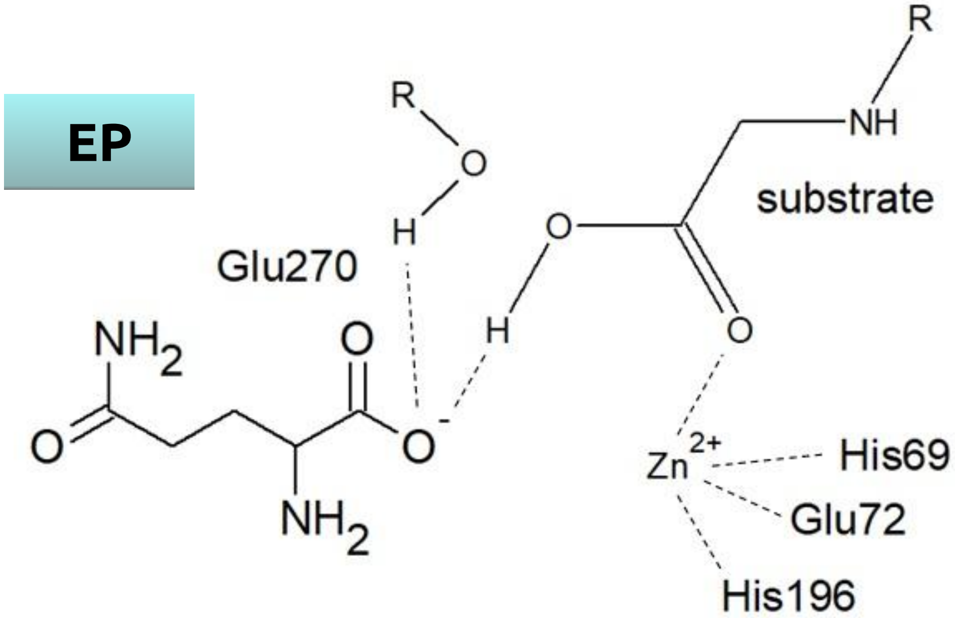
Glu270 and Zinc-bound water nucleophile attack the scissile carbonyl carbon resulting in the tetrahedral intermediate (TI)

Oxyanion hole

Introduction Water-promoted pathway

- STEP 2: elimination

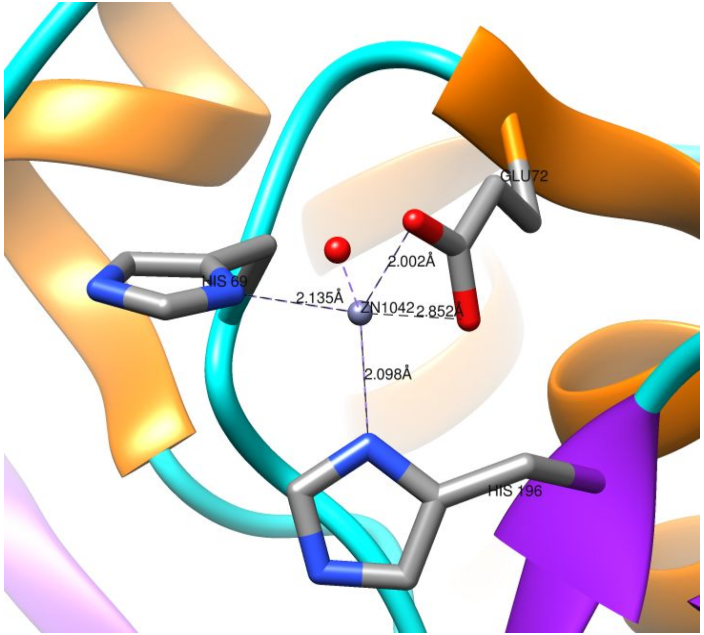
EP



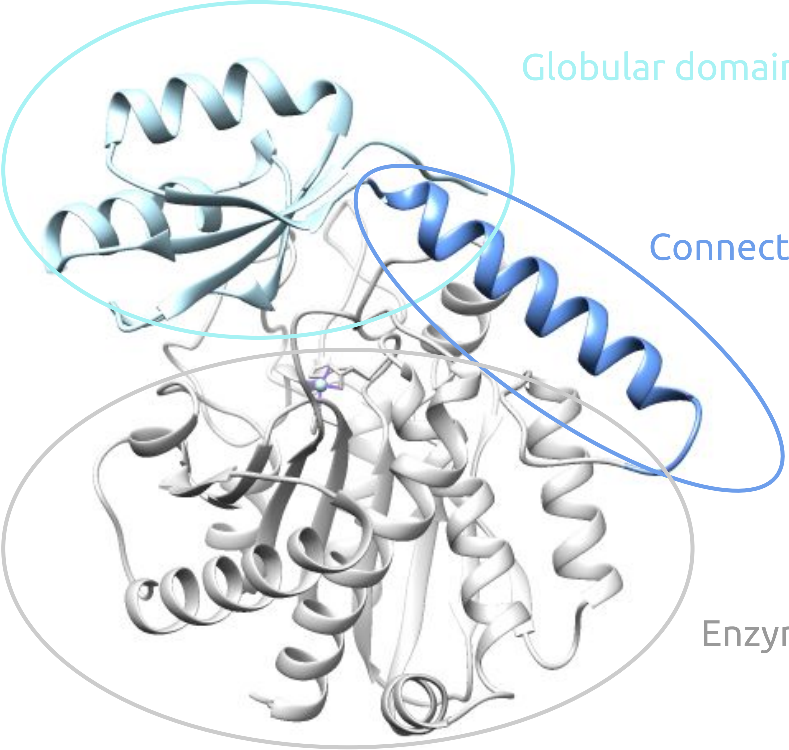
Cleavage of the esterolytic bond and obtaining the enzyme product (EP)

Introduction Nucleophilic pathway

- ➢ Anhydride mechanism
- ➢ Direct nucleophilic attack
- ➢ Acyl-enzyme intermediate (AI)

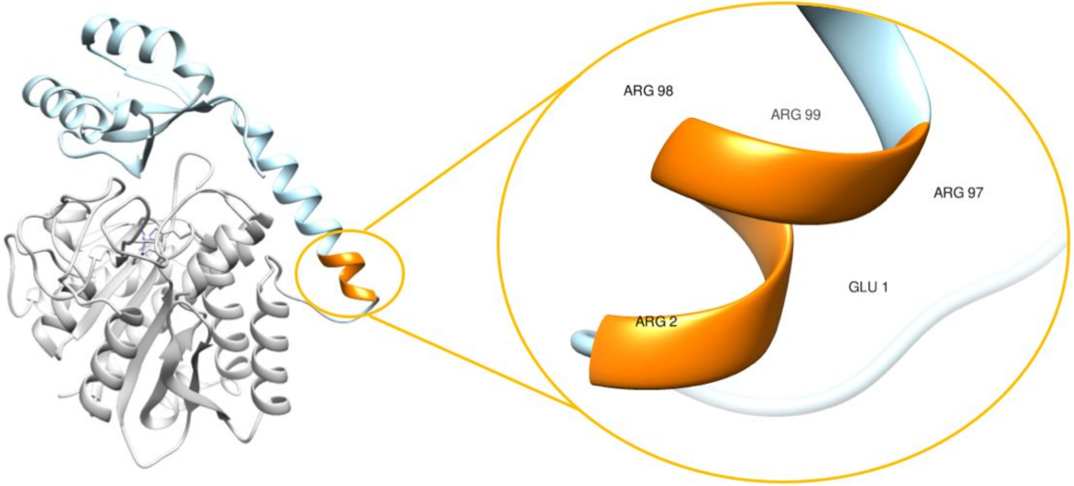


Pro-carboxypeptidase Structure



Pro-carboxypeptidase Alignment

```
hCPA2     -----------------------------------------FNFGAYHTLEEISQEMDNL
hPCPA2    KVFLESQGIAYSIMIEDVQVLLDKENEEMLFNRRRER-SGNFNFGAYHTLEEISQEMDNL
```



Pro-carboxypeptidase

Alignment

```
CBPA5_HUMAN         ESHGLAYSIMIKDIQVLLDEERQAMAKSRRLERSTNSFSYSSYHTLEEIY
CBPA5_MACFA         ESHGLAYSIMIKDIQVLLDEEREAMAKSRRLERSTSSFSYSSYHTLEEIS
CBPA2_MOUSE         ESQGITYSIMIEDVQVLLDQEREEMLFNQQRERGTN-FNFGAYHTLEEIY
CBPA2_RAT           ESQGIDYSIMIEDVQVLLDQEREEMLFNQQRERGGN-FNFEAYHTLEEIY
CBPA2_HUMAN         ESQGIAYSIMIEDVQVLLDKENEEMLFNRRRERSGN-FNFGAYHTLEEIS

CBPB2_RAT           NASRIPFNVLMNNVEDLIQQQTS--NDTVSPRAS--SSYYEQYHSLNEIY
CBPB2_MOUSE         NVSRIPFNVLMNNVEDLIEQQTF--NDTVSPRAS--ASYYEQYHSLNEIY
CBPB2_HUMAN         NVSGIPCSVLLADVEDLIQQQIS--NDTVSPRAS--ASYYEQYHSLNEIY
CBPB2 BOVIN         NASRIPFRVLVENVEDLIRQQTS--NDTISPRAS--SSYYEQYHSLNEIY
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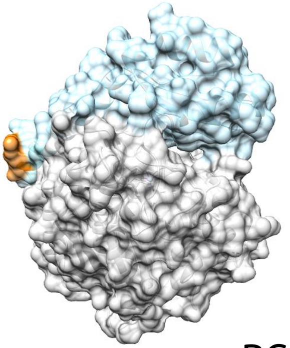
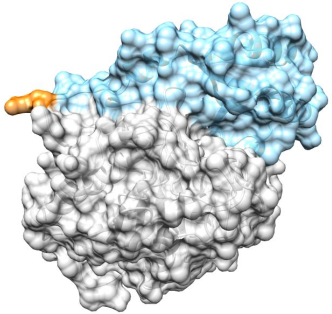


PCPA2 (PDBID: 1AYE)

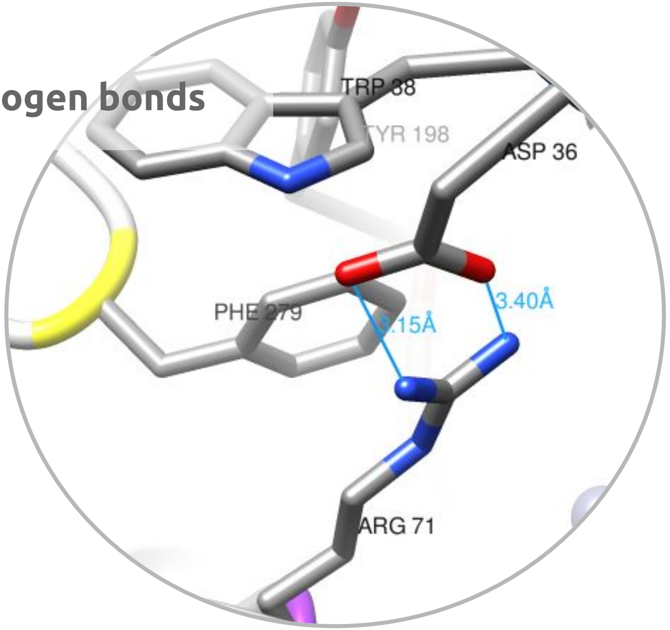


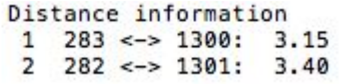
PCPB (PDBID: 1NSA)

Pro-carboxypeptidase

Interactions

Hydrogen bonds

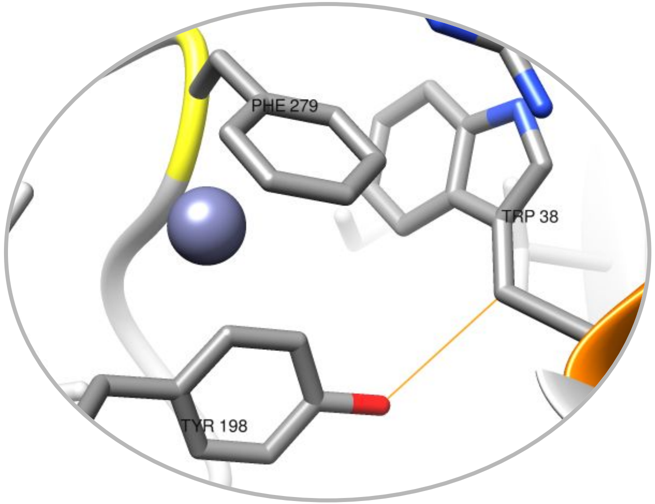


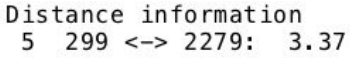


```
Distance information
 1  283 <-> 1300:  3.15
 2  282 <-> 1301:  3.40
```

Asp36A → Arg71

Other interactions





```
Distance information
 5  299 <-> 2279:  3.37
```

Tyr198 (π interaction)

↑

Trp38A

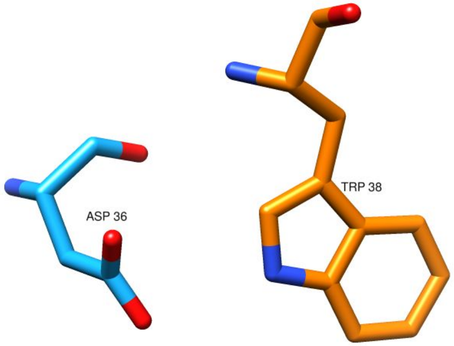
↓

Phe279 (CO···H)

Pro-carboxypeptidase Interactions

Asp 36A

Trp 38A



```
                                                          36A 38A
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sp|Q504N0|CBPA2_MOUSE   DEEQIKTLLQLEAEEHLEIDFWK----SPSVPRQTVHVRVPFASIQDVKV
sp|P48052|CBPA2_HUMAN   NEEQIKNLLQLEAQEHLQIDFWK----SPTTPGETAHVRVPFVNVQAVKV
sp|Q9UI42|CBPA4_HUMAN   NGDEISKLSQLVNSNNLKINFWK----SPSSFNRPVDVLVPSVSLQAFKS
sp|P09954|CBPA1_PIG     DEAQVQKVKELEDLEHLQIDFWR----GPARPGFPIDVRVPFPSIQAVKV
sp|P00730|CBPA1_BOVIN   DEAEVQTVKELEDLEHLQIDFWR----GPGQPGSPIDVRVPFPSLQAVKV
sp|P15085|CBPA1_HUMAN   DEAQVQKVKELEDLEHLQIDFWR----GPAHPGSPIDVRVPFPSIQAVKI
sp|P00731|CBPA1_RAT     DEAQVQKVKELEDLEHLQIDFWR----DAARAGIPIDVRVPFPSIQSVKA
sp|Q7TPZ8|CBPA1_MOUSE   DEAQVQKVRELEELEHLKIDFWR----DPARAGLPIDVRVPFPTIQSVKA
sp|Q8WXQ8|CBPA5_HUMAN   DEKQLSLLGDLEGLKPQKVDFWR----GPARPSLPVDMRVPFSELKDIKA
sp|Q4R7R2|CBPA5_MACFA   DEKQLSLLRDLEGLKPQKVDFWR----GPARPSLPVDMRVPFSELKYIKA
sp|Q8R4H4|CBPA5_MOUSE   NEKQLSLLRDLETQKPQKVDFWR----GPARPSLPVDMRVPFSELPSVKA
sp|P00732|CBPB1_BOVIN   DENHISLLHELASTR--QMDFWKPDSVTQVKPHSTVDFRVKAEDTVAVED
sp|P09955|CBPB1_PIG     DENDISLLHELASTR--QIDFWKPDSVTQIKPHSTVDFRVKAEDILAVED
sp|P55261|CBPB1_CANFA   DENHINLLHTLASTT--QIDFWKPDSVTQIKPHSTADFRVKAEDILTVED
sp|P15086|CBPB1_HUMAN   DENHINIIRELASTT--QIDFWKPDSVTQIKPHSTVDFRVKAEDTVTVEN
sp|P19223|CBPB1_RAT     GEDHVNLIQELANTK--EIDFWKPDSATQVKPLTTVDFHVKAEDVADVEN
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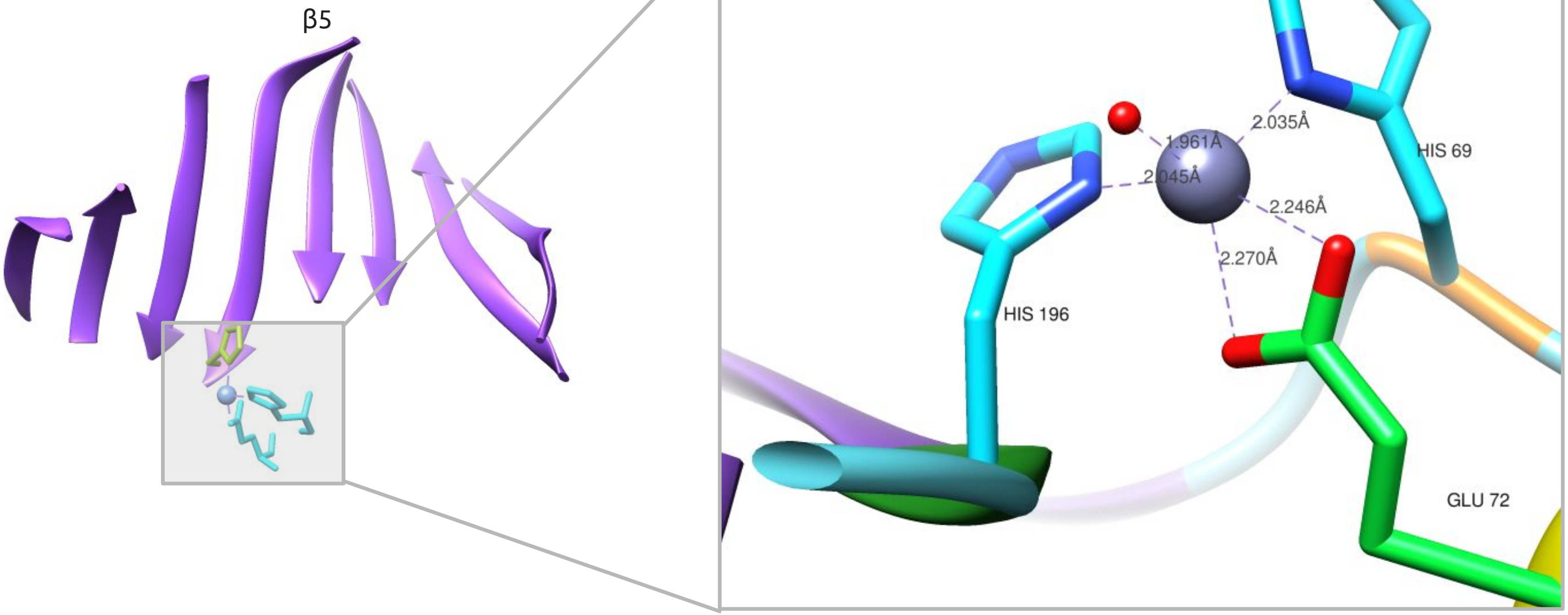
Active Site



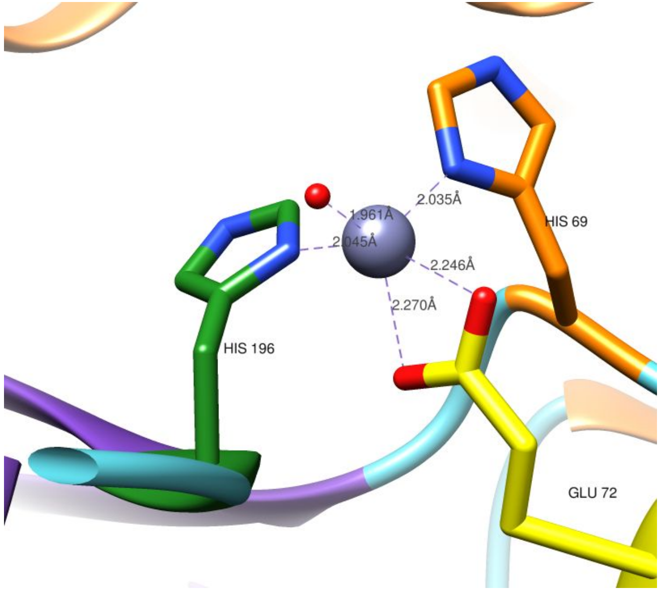
PDBID: 2V77

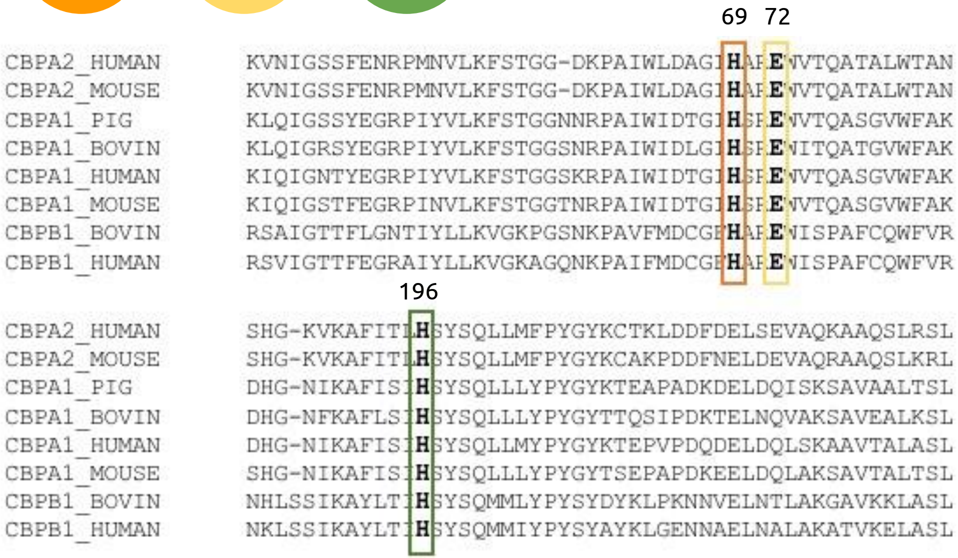
Active Site   Zn residues

His 69

Asp 72

His 196





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CBPA1_PIG      KLQIGSSYEGRPIYVLKFSTGGNNRPAIWIDTGIHSREWVTQASGVWFAK
CBPA1_BOVIN    KLQIGRSYEGRPIYVLKFSTGGSNRPAIWIDLGIHSREWITQATGVWFAK
CBPA1_HUMAN    KIQIGNTYEGRPIYVLKFSTGGSKRPAIWIDTGIHSREWVTQASGVWFAK
CBPA1_MOUSE    KIQIGSTFEGRPINVLKFSTGGTNRPAIWIDTGIHSREWVTQASGVWFAK
CBPB1_BOVIN    RSAIGTTFLGNTIYLLKVGKPGSNKPAVFMDCGFHAREWISPAFCQWFVR
CBPB1_HUMAN    RSVIGTTFEGRAIYLLKVGKAGQNKPAIFMDCGFHAREWISPAFCQWFVR

CBPA2_HUMAN    SHG-KVKAFITLHSYSQLLMFPYGYKCTKLDDFDELSEVAQKAAQSLRSL
CBPA2_MOUSE    SHG-KVKAFITLHSYSQLLMFPYGYKCAKPDDFNELDEVAQRAAQSLKRL
CBPA1_PIG      DHG-NIKAFISIHSYSQLLLYPYGYKTEAPADKDELDQISKSAVAALTSL
CBPA1_BOVIN    DHG-NFKAFLSIHSYSQLLLYPYGYTTQSIPDKTELNQVAKSAVEALKSL
CBPA1_HUMAN    DHG-NIKAFISIHSYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASL
CBPA1_MOUSE    SHG-NIKAFISIHSYSQLLLYPYGYTSEPAPDKEELDQLAKSAVTALTSL
CBPB1_BOVIN    NHLSSIKAYLTIHSYSQMMLYPYSYDYKLPKNNVELNTLAKGAVKKLASL
CBPB1_HUMAN    NKLSSIKAYLTIHSYSQMMIYPYSYAYKLGENNAELNALAKATVKELASL
```

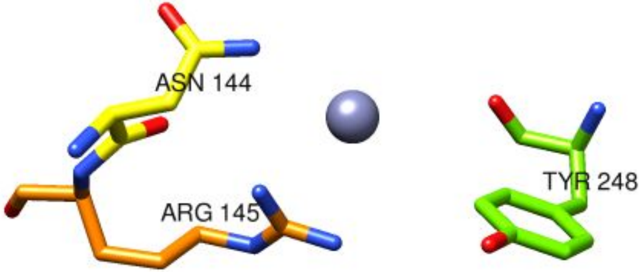
Active Site

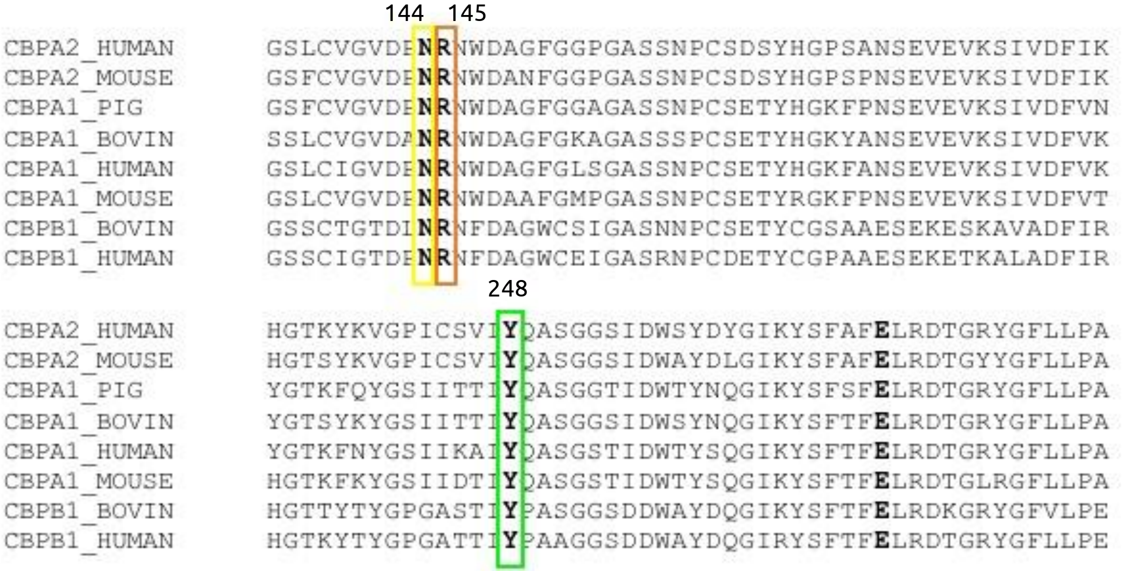
Subsite S1’

Asn 144

Arg 145

Tyr 248





```
                                144 145
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CBPA1_PIG       GSFCVGVDENRNWDAGFGGAGASSNPCSETYHGKFPNSEVEVKSIVDFVN
CBPA1_BOVIN     SSLCVGVDANRNWDAGFGKAGASSSPCSETYHGKYANSEVEVKSIVDFVK
CBPA1_HUMAN     GSLCIGVDENRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVK
CBPA1_MOUSE     GSLCVGVDENRNWDAAFGMPGASSNPCSETYRGKFPNSEVEVKSIVDFVT
CBPB1_BOVIN     GSSCTGTDINRNFDAGWCSIGASNNPCSETYCGSAAESEKESKAVADFIR
CBPB1_HUMAN     GSSCIGTDENRNFDAGWCEIGASRNPCDETYCGPAAESEKETKALADFIR
                                     248
CBPA2_HUMAN     HGTKYKVGPICSVIYQASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPA
CBPA2_MOUSE     HGTSYKVGPICSVIYQASGGSIDWAYDLGIKYSFAFELRDTGYYGFLLPA
CBPA1_PIG       YGTKFQYGSIITTIYQASGGTIDWTYNQGIKYSFSFELRDTGRYGFLLPA
CBPA1_BOVIN     YGTSYKYGSIITTIYQASGGSIDWSYNQGIKYSFTFELRDTGRYGFLLPA
CBPA1_HUMAN     YGTKFNYGSIIKAIYQASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPA
CBPA1_MOUSE     HGTKFKYGSIIDTIYQASGSTIDWTYSQGIKYSFTFELRDTGLRGFLLPA
CBPB1_BOVIN     HGTTYTYGPGASTIYPASGGSDDWAYDQGIKYSFTFELRDKGRYGFVLPE
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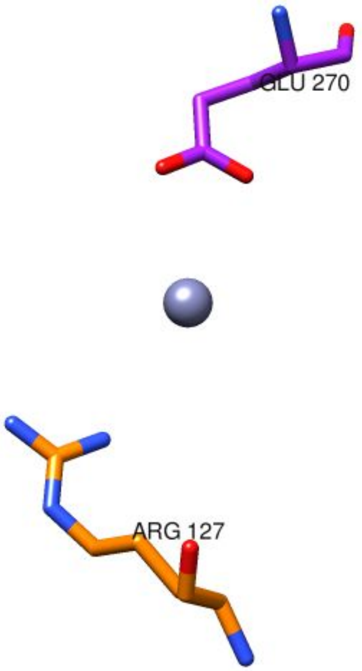
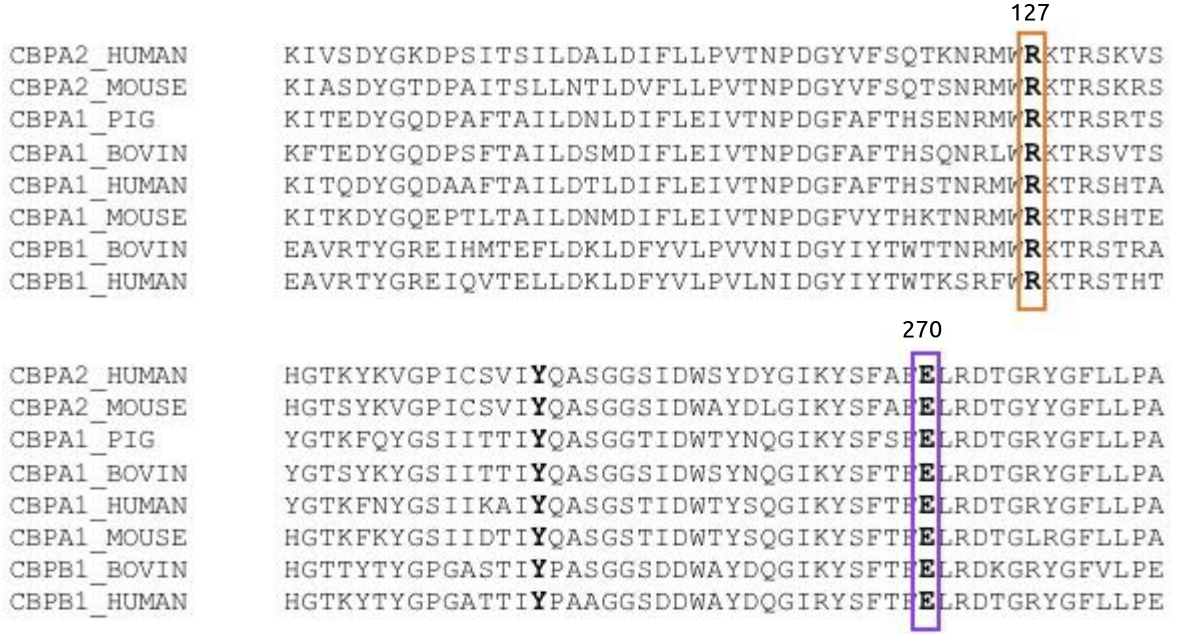
Active Site

Subsite S1

Arg 127

Glu 270





```
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CBPA2_MOUSE     KIASDYGTDPAITSLLNTLDVFLLPVTNPDGYVFSQTSNRMWRKTRSKRS
CBPA1_PIG       KITEDYGQDPAFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRKTRSRTS
CBPA1_BOVIN     KFTEDYGQDPSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTS
CBPA1_HUMAN     KITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTA
CBPA1_MOUSE     KITKDYGQEPTLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRKTRSHTE
CBPB1_BOVIN     EAVRTYGREIHMTEFLDKLDFYVLPVVNIDGYIYTWTTNRMWRKTRSTRA
CBPB1_HUMAN     EAVRTYGREIQVTELLDKLDFYVLPVLNIDGYIYTWTKSRFWRKTRSTHT

                                                            270
CBPA2_HUMAN     HGTKYKVGPICSVIYQASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPA
CBPA2_MOUSE     HGTSYKVGPICSVIYQASGGSIDWAYDLGIKYSFAFELRDTGYYGFLLPA
CBPA1_PIG       YGTKFQYGSIITTIYQASGGTIDWTYNQGIKYSFSFELRDTGRYGFLLPA
CBPA1_BOVIN     YGTSYKYGSIITTIYQASGGSIDWSYNQGIKYSFTFELRDTGRYGFLLPA
CBPA1_HUMAN     YGTKFNYGSIIKAIYQASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPA
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CBPB1_BOVIN     HGTTYTYGPGASTIYPASGGSDDWAYDQGIKYSFTFELRDKGRYGFVLPE
CBPB1_HUMAN     HGTKYTYGPGATTIYPAAGGSDDWAYDQGIRYSFTFELRDTGRYGFLLPE
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Active Site

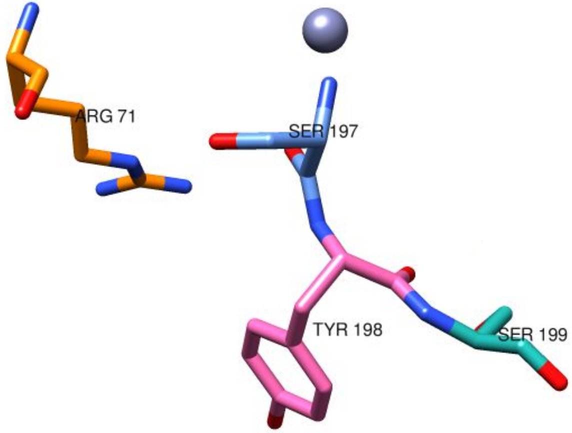
Subsite S2

Arg 71

Ser 197

Tyr 198

Ser 199



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                                                                            71
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CBPA1_PIG        KLQIGSSYEGRPIYVLKFSTGGNNRPAIWIDTGIHSREWVTQASGVWFAK
CBPA1_BOVIN      KLQIGRSYEGRPIYVLKFSTGGSNRPAIWIDLGIHSREWITQATGVWFAK
CBPA1_HUMAN      KIQIGNTYEGRPIYVLKFSTGGSKRPAIWIDTGIHSREWVTQASGVWFAK
CBPA1_MOUSE      KIQIGSTFEGRPINVLKFSTGGTNRPAIWIDTGIHSREWVTQASGVWFAK
CBPB1_BOVIN      RSAIGTTFLGNTIYLLKVGKPGSNKPAVFMDCGFHAREWISPAFCQWFVR
CBPB1_HUMAN      RSVIGTTFEGRAIYLLKVGKAGQNKPAIFMDCGFHAREWISPAFCQWFVR

                             197 198 199
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CBPA2_MOUSE      SHG-KVKAFITLHSYSQLLMFPYGYKCAKPDDFNELDEVAQRAAQSLKRL
CBPA1_PIG        DHG-NIKAFISIHSYSQLLLYPYGYKTEAPADKDELDQISKSAVAALTSL
CBPA1_BOVIN      DHG-NFKAFLSIHSYSQLLLYPYGYTTQSIPDKTELNQVAKSAVEALKSL
CBPA1_HUMAN      DHG-NIKAFISIHSYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASL
CBPA1_MOUSE      SHG-NIKAFISIHSYSQLLLYPYGYTSEPAPDKEELDQLAKSAVTALTSL
CBPB1_BOVIN      NHLSSIKAYLTIHSYSQMMLYPYSYDYKLPKNNVELNTLAKGAVKKLASL
CBPB1_HUMAN      NKLSSIKAYLTIHSYSQMMIYPYSYAYKLGENNAELNALAKATVKELASL
```

Active Site

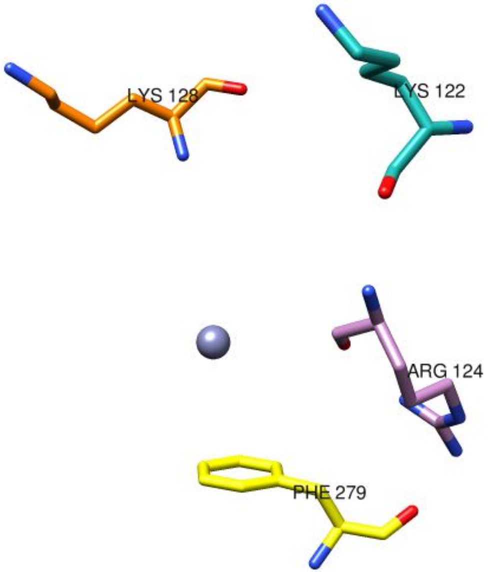
Subsites S3 and S4

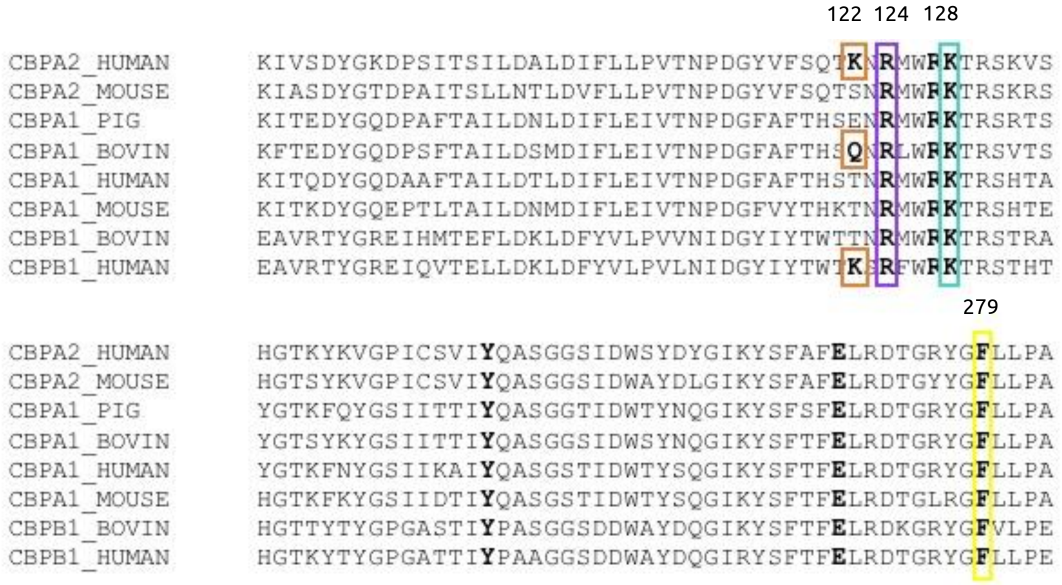
Lys 122

Arg 124

Lys 128

Phe 279





```
                                                    122 124 128
CBPA2_HUMAN     KIVSDYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKVS
CBPA2_MOUSE     KIASDYGTDPAITSLLNTLDVFLLPVTNPDGYVFSQTSNRMWRKTRSKRS
CBPA1_PIG       KITEDYGQDPAFTAILDNLDIFLEIVTNPDGFAFTHSENRMWRKTRSRTS
CBPA1_BOVIN     KFTEDYGQDPSFTAILDSMDIFLEIVTNPDGFAFTHSQNRLWRKTRSVTS
CBPA1_HUMAN     KITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTA
CBPA1_MOUSE     KITKDYGQEPTLTAILDNMDIFLEIVTNPDGFVYTHKTNRMWRKTRSHTE
CBPB1_BOVIN     EAVRTYGREIHMTEFLDKLDFYVLPVVNIDGYIYTWTTNRMWRKTRSTRA
CBPB1_HUMAN     EAVRTYGREIQVTELLDKLDFYVLPVLNIDGYIYTWTKSRFWRKTRSTHT

                                                            279
CBPA2_HUMAN     HGTKYKVGPICSVIYQASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPA
CBPA2_MOUSE     HGTSYKVGPICSVIYQASGGSIDWAYDLGIKYSFAFELRDTGYYGFLLPA
CBPA1_PIG       YGTKFQYGSIITTIYQASGGTIDWTYNQGIKYSFSFELRDTGRYGFLLPA
CBPA1_BOVIN     YGTSYKYGSIITTIYQASGGSIDWSYNQGIKYSFTFELRDTGRYGFLLPA
CBPA1_HUMAN     YGTKFNYGSIIKAIYQASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPA
CBPA1_MOUSE     HGTKFKYGSIIDTIYQASGSTIDWTYSQGIKYSFTFELRDTGLRGFLLPA
CBPB1_BOVIN     HGTTYTYGPGASTIYPASGGSDDWAYDQGIKYSFTFELRDKGRYGFVLPE
CBPB1_HUMAN     HGTKYTYGPGATTIYPAAGGSDDWAYDQGIRYSFTFELRDTGRYGFLLPE
```

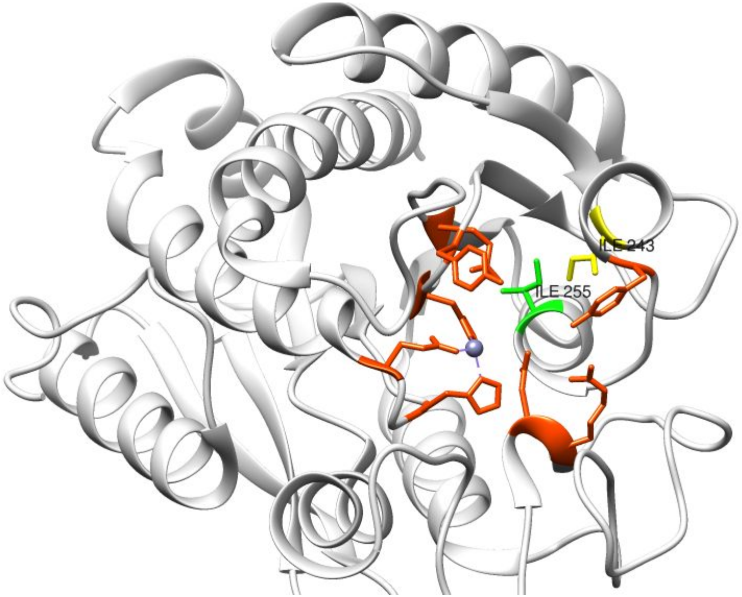
Substrate Specificity CPA vs CPB

```
hCPA1   ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVL
hCPA2   ------FNFGAYHTLEEISQEMDNLVAEHPGLVSKVNIGSSFENRPMNVL
hCPB    -VRATGHSYEKYNNWETIEAWTQQVATENPALISRSVIGTTFEGRAIYLL

                          *  *
hCPA1   KFSTGGSKRPAIWIDTGIHSREWVTQASGVWFAKKITQDYGQDAAFTAIL
hCPA2   KFSTGGDK-PAIWLDAGIHAREWVTQATALWTANKIVSDYGKDPSITSIL
hCPB    KVGKAGQNKPAIFMDCGFHAREWISPAFCQWFVREAVRTYGREIQVTELL

                                                     *
hCPA1   DTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTAG-SLCIGVDPNRNWDA
hCPA2   DALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKVSAGSLCVGVDPNRNWDA
hCPB    NKLDFYVLPVLNIDGYIYTWTKSRFWRKTRSTHTG-SSCIGTDPNRNFDA

                                                       * *
hCPA1   GFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVKDHG-NIKAFISIHSY
hCPA2   GFGGPGASSNPCSDSYHGPSANSEVEVKSIVDFIKSHG-KVKAFIILHSY
hCPB    GWCEIGASRNPCDETYCGPAAESEKETKALADFIRNKLSSIKAYLTIHSY

                                                    243
                                                         *
hCPA1   SQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIY
hCPA2   SQLLMFPYGYKCTKLDDFDELSEVAQKAAQSLSRLHGTKYKVGPICSVIY
hCPB    SQMMIYPYSYAYKLGENNAELNALAKATVKELASLHGTKYTYGPGATTIY

              255
                             *
hCPA1   QASGSTIDWTYSQGIKYSFTFELRDTGRYGFLLPASQIIPTAKETWLALL
hCPA2   QASGGSIDWSYDYGIKYSFAFELRDTGRYGFLLPARQILPTAEETWLGLK
hCPB    PAAGGSDDWAYDQGIRYSFTFELRDTGRYGFLLPESQIRATCEETFLAIK

hCPA1   TIMEHTLNHPY
hCPA2   AIMEHVRDHPY
hCPB    YVASYVLEHLY
```

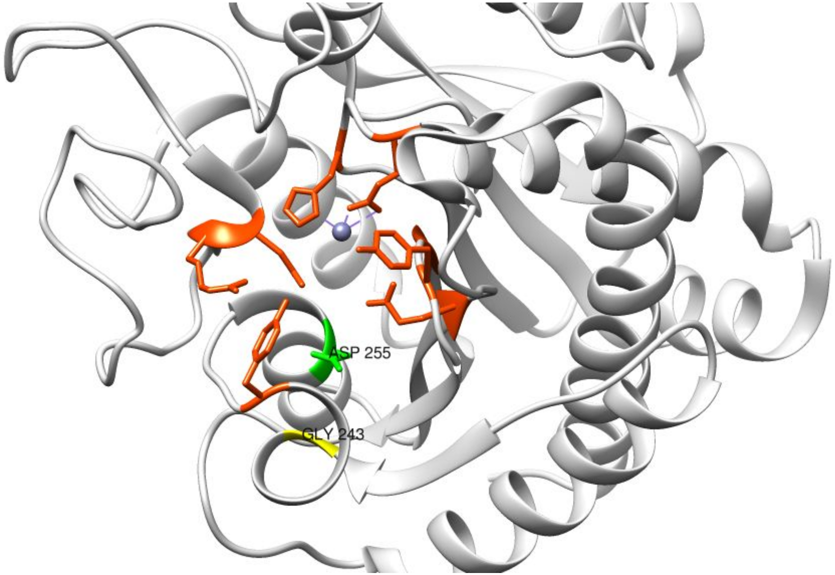
	243		255
	Ile	CPA	Ile
	Gly	CPB	Asp

PDBID: 1UEE, 1DTD, 1ZLI

Substrate Specificity CPA vs CPB

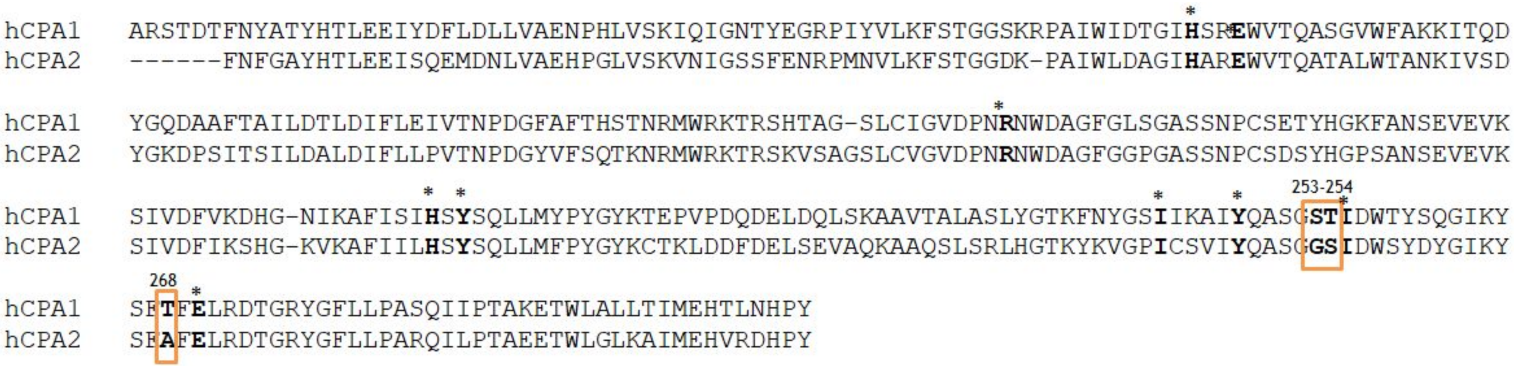


CPA (PDBID: 1UEE)



CPB (PDBID: 1ZLI)

Substrate Specificity CPA1 vs CPA2

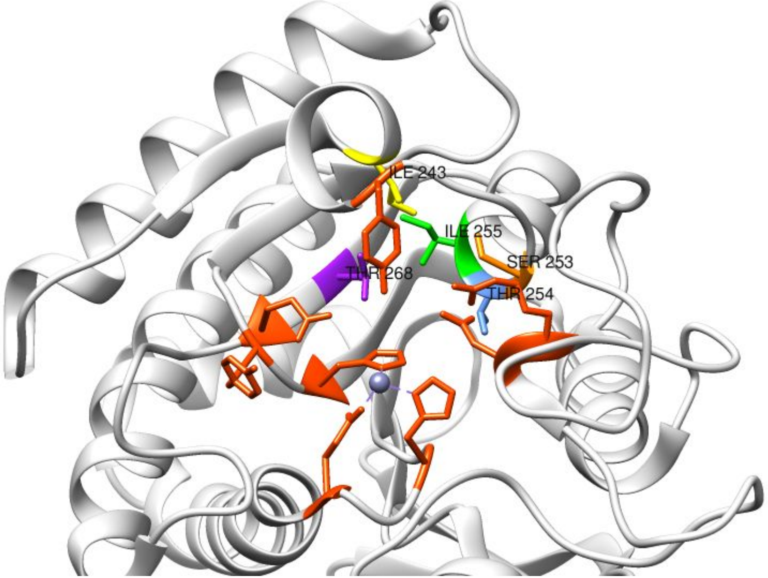


```
                                                                              *  *
hCPA1     ARSTDTFNYATYHTLEEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTGGSKRPAIWIDTGIHSREWVTQASGVWFAKKITQD
hCPA2     ------FNFGAYHTLEEISQEMDNLVAEHPGLVSKVNIGSSFENRPMNVLKFSTGGDK-PAIWLDAGIHAREWVTQATALWTANKIVSD

                                                                  *
hCPA1     YGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHTAG-SLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVK
hCPA2     YGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKVSAGSLCVGVDPNRNWDAGFGGPGASSNPCSDSYHGPSANSEVEVK

                                                                                   253-254
                             * *                                            *    *      *
hCPA1     SIVDFVKDHG-NIKAFISIHSYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIYQASGSTIDWTYSQGIKY
hCPA2     SIVDFIKSHG-KVKAFIILHSYSQLLMFPYGYKCTKLDDFDELSEVAQKAAQSLSRLHGTKYKVGPICSVIYQASGGSIDWSYDYGIKY

           268
              *
hCPA1     SFTFELRDTGRYGFLLPASQIIPTAKETWLALLTIMEHTLNHPY
hCPA2     SFAFELRDTGRYGFLLPARQILPTAEETWLGLKAIMEHVRDHPY
```

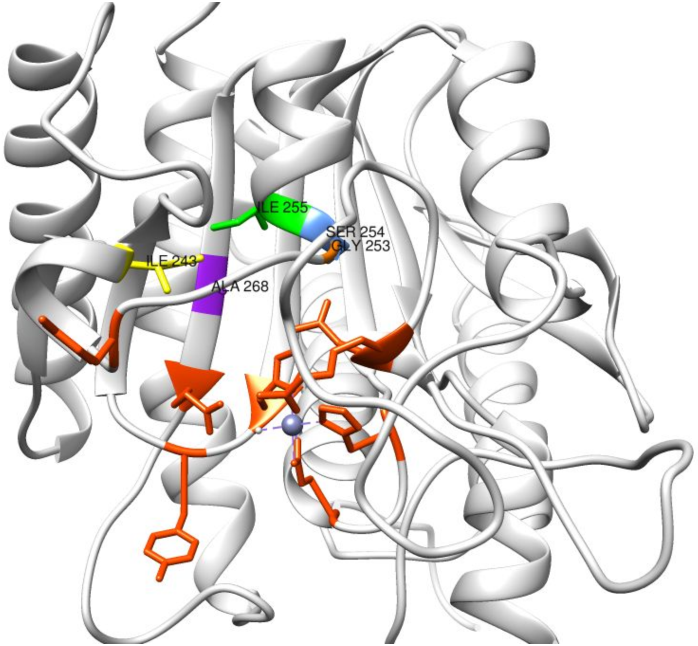
	253	254	268
CPA1	Ser	Thr	Thr
CPA2	Gly	Ser	Ala

PDBID: 1UEE, 1DTD

Substrate Specificity CPA1 vs CPA2

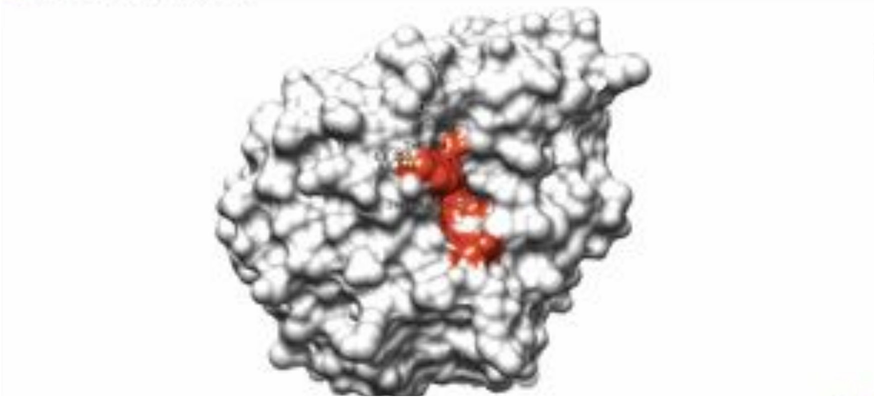


CPA1 (PDBID: 4UEE)

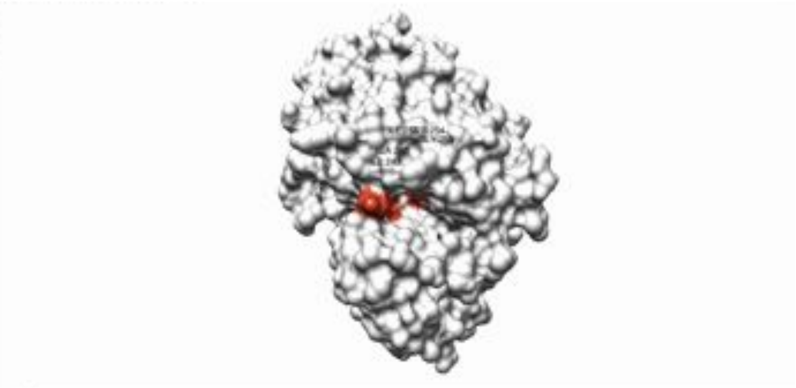


CPA2 (PDBID: 1DTD)

Substrate Specificity CPA1 vs CPA2



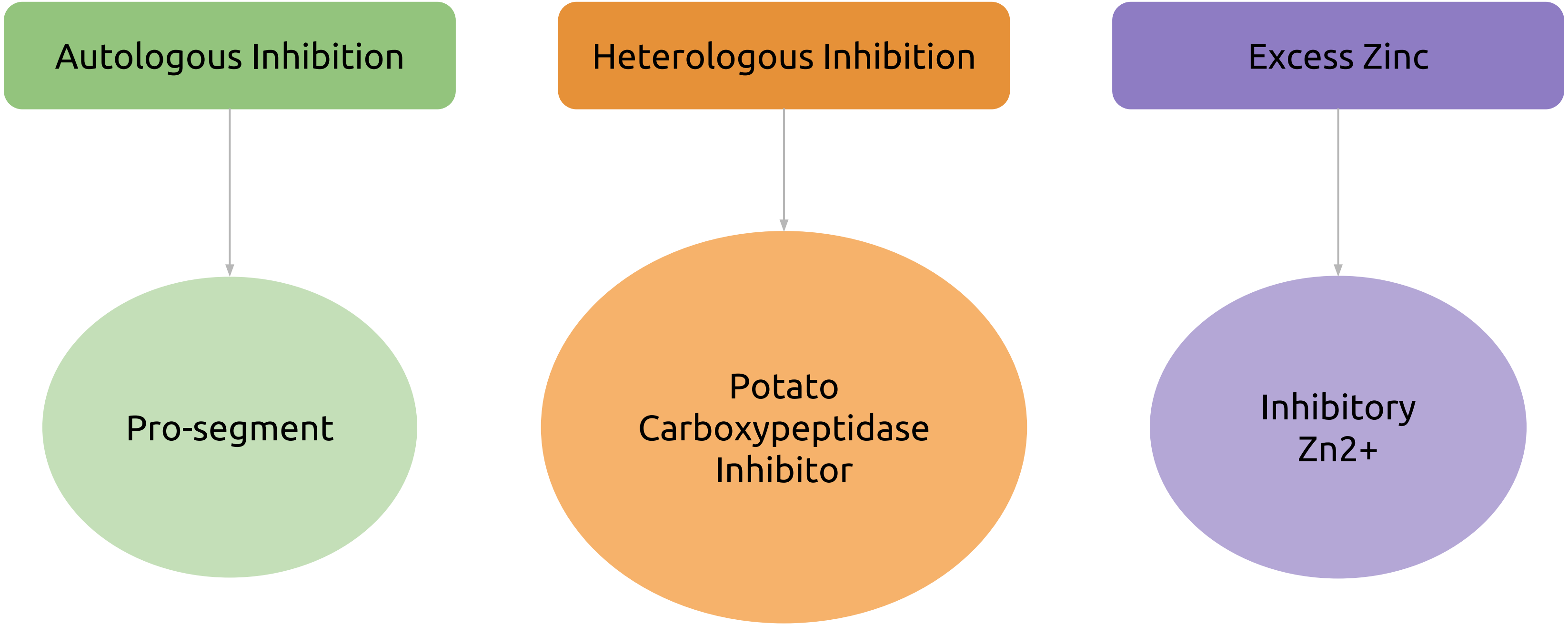
CPA1 (PDBID: 4UEE)



CPA2 (PDBID: 1DTD)

Inhibitors

Types



Autologous Inhibition

Pro-segment

Heterologous Inhibition

Potato Carboxypeptidase Inhibitor

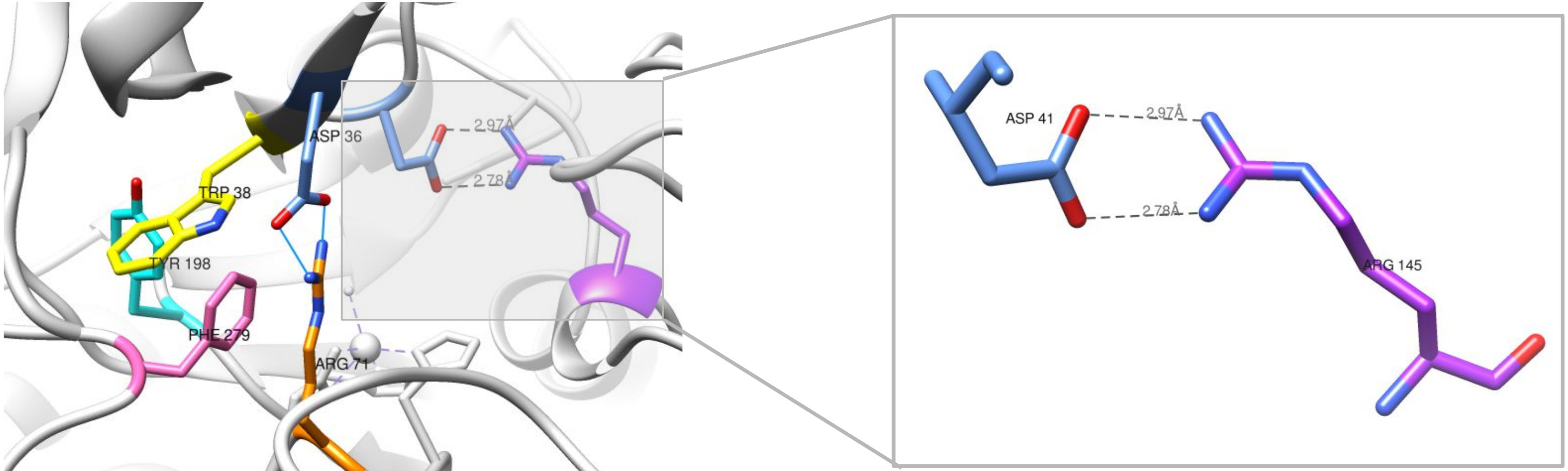
Excess Zinc

Inhibitory Zn2+

Inhibitors

Autologous Inhibitors

Pro-carboxypeptidase

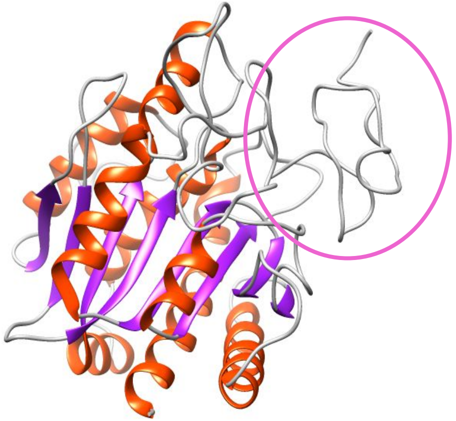


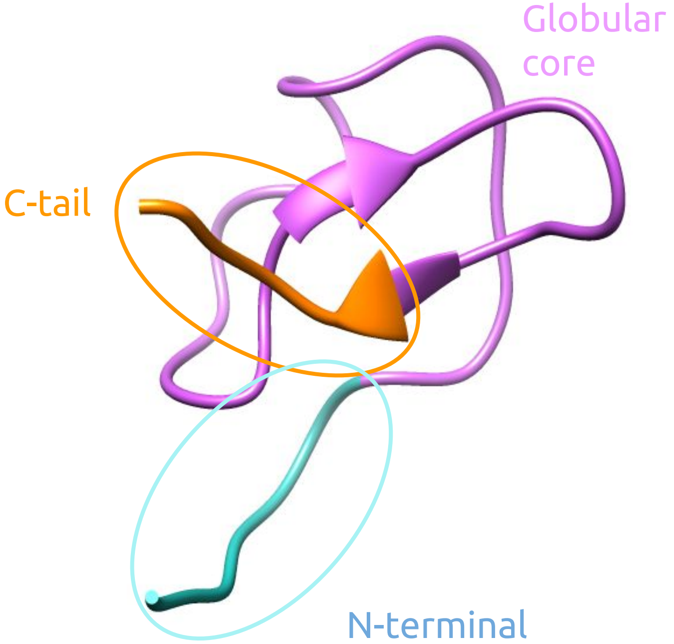
PDBID: 1NSA

Inhibitors

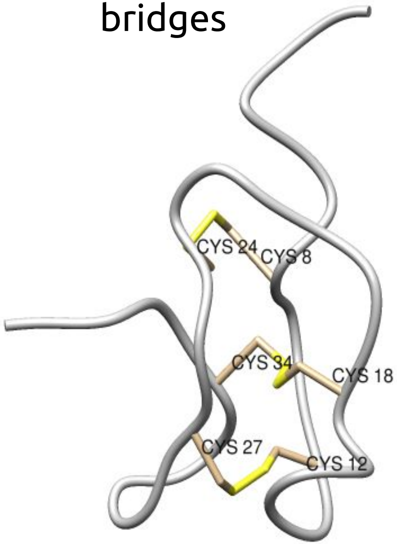
Heterologous Inhibitors

Potato Carboxypeptidase Inhibitor (PCI)





3 disulfide bridges

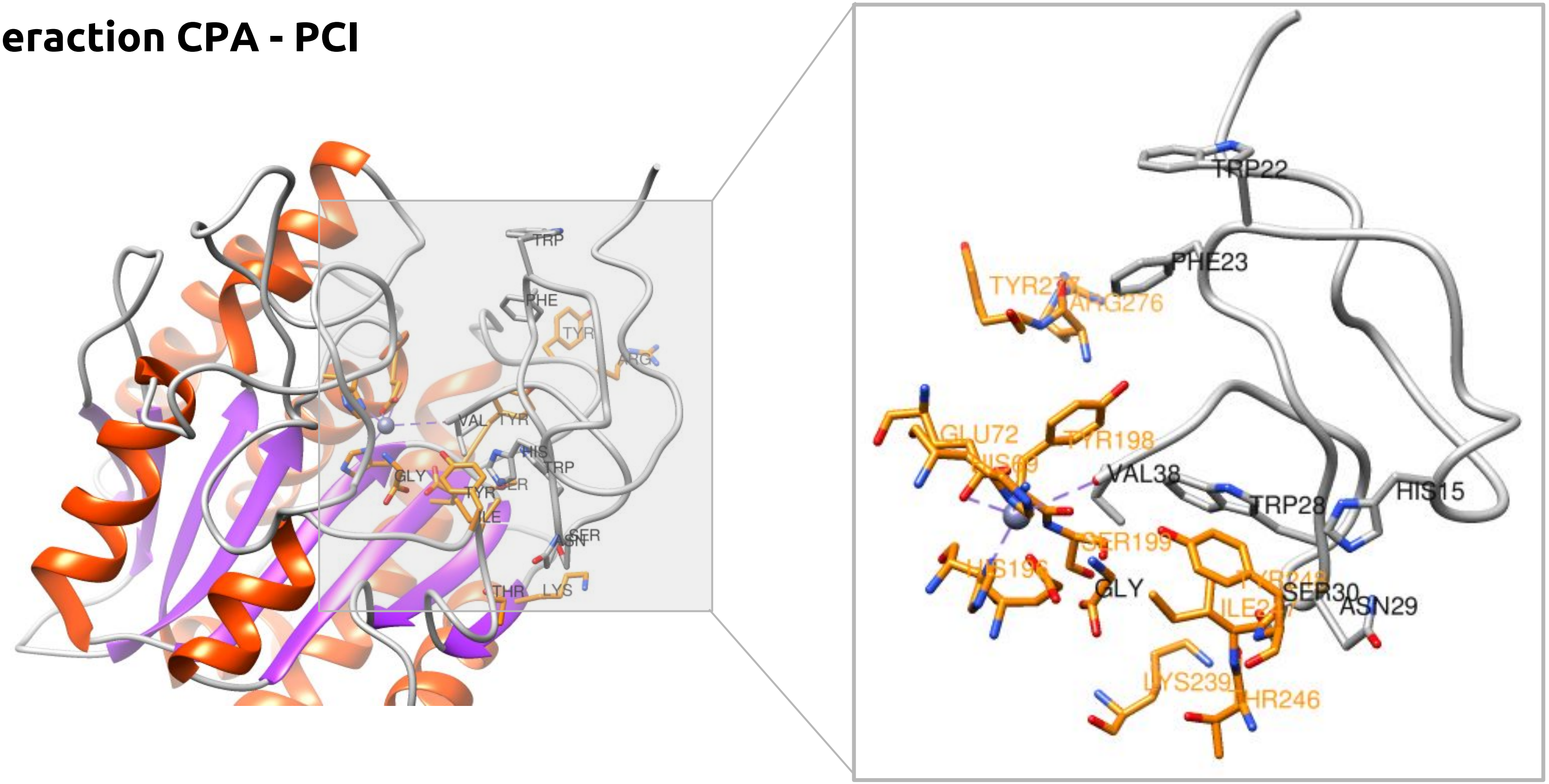


PDBID: 1H20

Inhibitors

Heterologous Inhibitors

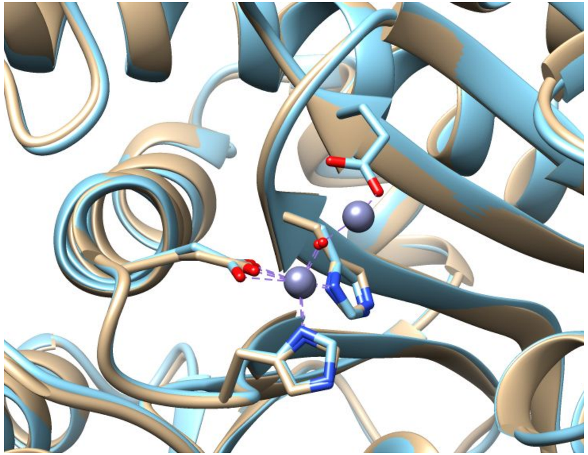
Interaction CPA - PCI



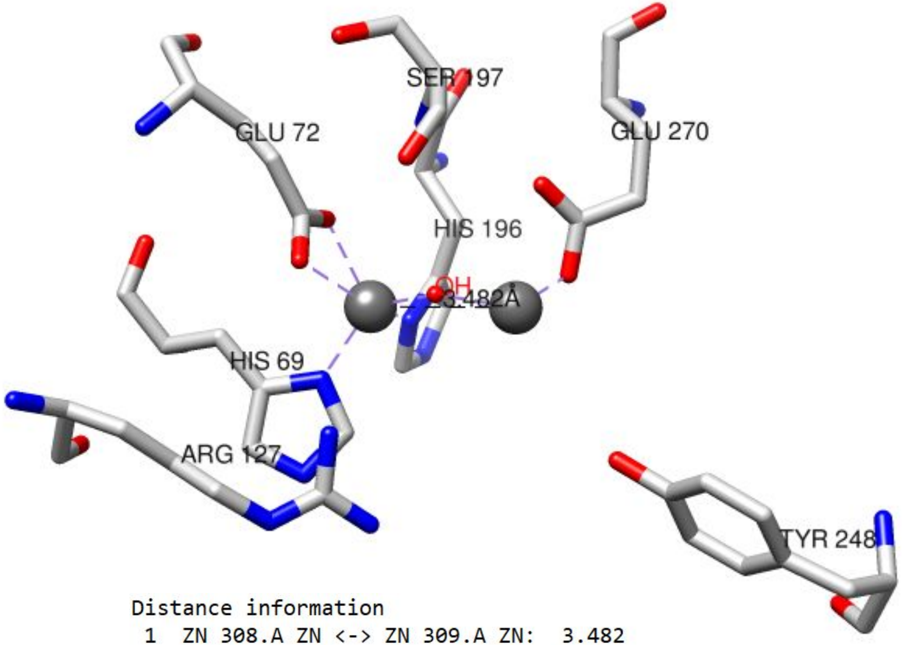
PDBID: 4CPA

Inhibitors

Excess Zinc



Superimposition of CPA and CPA+Zinc

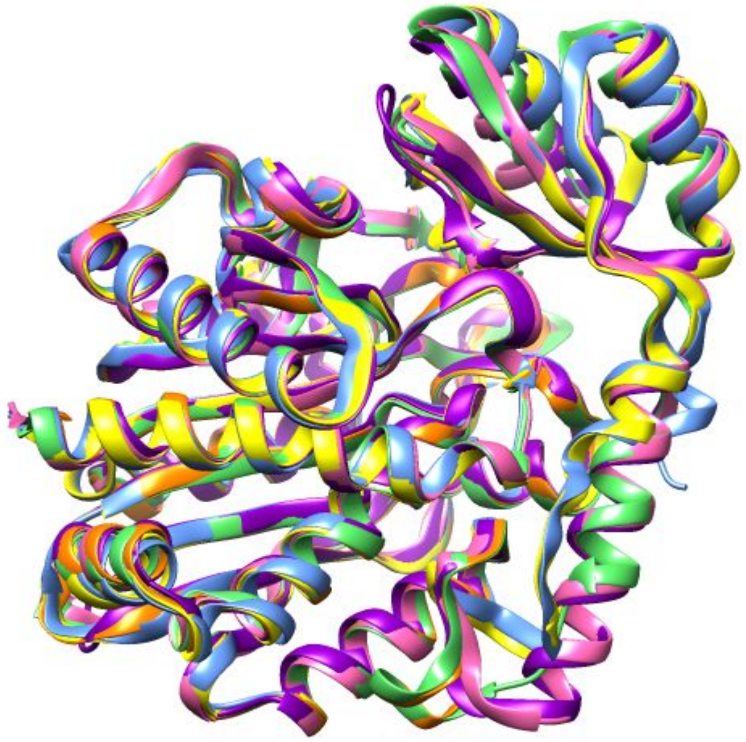


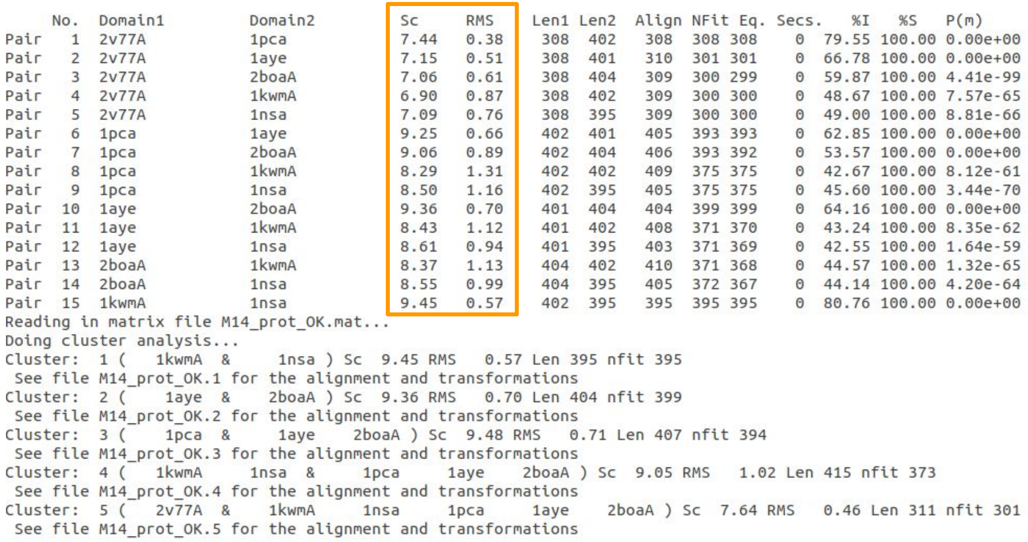
```
Distance information
 1  ZN 308.A ZN <-> ZN 309.A ZN:   3.482
 2  ZN 308.A ZN <-> OH 541.A O:    1.752
 3  ZN 309.A ZN <-> OH 541.A O:    1.846

Angles/Torsions
 ZN 308.A ZN -> OH 541.A O -> ZN 309.A ZN:  150.887
```

PDBID: 1CPX

M14A Carboxypeptidases

Superimposition





```
     No.  Domain1          Domain2          Sc     RMS    Len1 Len2  Align NFit Eq. Secs.    %I    %S    P(m)
Pair   1  2v77A            1pca             7.44    0.38   308  402   308  308 308     0  79.55 100.00 0.00e+00
Pair   2  2v77A            1aye             7.15    0.51   308  401   310  301 301     0  66.78 100.00 0.00e+00
Pair   3  2v77A            2boaA            7.06    0.61   308  404   309  300 299     0  59.87 100.00 4.41e-99
Pair   4  2v77A            1kwmA            6.90    0.87   308  402   309  300 300     0  48.67 100.00 7.57e-65
Pair   5  2v77A            1nsa             7.09    0.76   308  395   309  300 300     0  49.00 100.00 8.81e-66
Pair   6  1pca             1aye             9.25    0.66   402  401   405  393 393     0  62.85 100.00 0.00e+00
Pair   7  1pca             2boaA            9.06    0.89   402  404   406  393 392     0  53.57 100.00 0.00e+00
Pair   8  1pca             1kwmA            8.29    1.31   402  402   409  375 375     0  42.67 100.00 8.12e-61
Pair   9  1pca             1nsa             8.50    1.16   402  395   405  375 375     0  45.60 100.00 3.44e-70
Pair  10  1aye             2boaA            9.36    0.70   401  404   404  399 399     0  64.16 100.00 0.00e+00
Pair  11  1aye             1kwmA            8.43    1.12   401  402   408  371 370     0  43.24 100.00 8.35e-62
Pair  12  1aye             1nsa             8.61    0.94   401  395   403  371 369     0  42.55 100.00 1.64e-59
Pair  13  2boaA            1kwmA            8.37    1.13   404  402   410  371 368     0  44.57 100.00 1.32e-65
Pair  14  2boaA            1nsa             8.55    0.99   404  395   405  372 367     0  44.14 100.00 4.20e-64
Pair  15  1kwmA            1nsa             9.45    0.57   402  395   395  395 395     0  80.76 100.00 0.00e+00
Reading in matrix file M14_prot_OK.mat...
Doing cluster analysis...
Cluster:  1 (    1kwmA  &     1nsa ) Sc  9.45 RMS   0.57 Len 395 nfit 395
 See file M14_prot_OK.1 for the alignment and transformations
Cluster:  2 (     1aye  &    2boaA ) Sc  9.36 RMS   0.70 Len 404 nfit 399
 See file M14_prot_OK.2 for the alignment and transformations
Cluster:  3 (     1pca  &     1aye    2boaA ) Sc  9.48 RMS   0.71 Len 407 nfit 394
 See file M14_prot_OK.3 for the alignment and transformations
Cluster:  4 (    1kwmA     1nsa  &     1pca     1aye    2boaA ) Sc  9.05 RMS   1.02 Len 415 nfit 373
 See file M14_prot_OK.4 for the alignment and transformations
Cluster:  5 (    2v77A  &    1kwmA     1nsa     1pca     1aye    2boaA ) Sc  7.64 RMS   0.46 Len 311 nfit 301
 See file M14_prot_OK.5 for the alignment and transformations
```

2v77A → hCPA1
1PCA → pPCPA1
1AYE → hPCPA2
2BOA → hPCPA4
1KWM → hPCPB
1NSA → pPCPB

M14A Carboxypeptidases

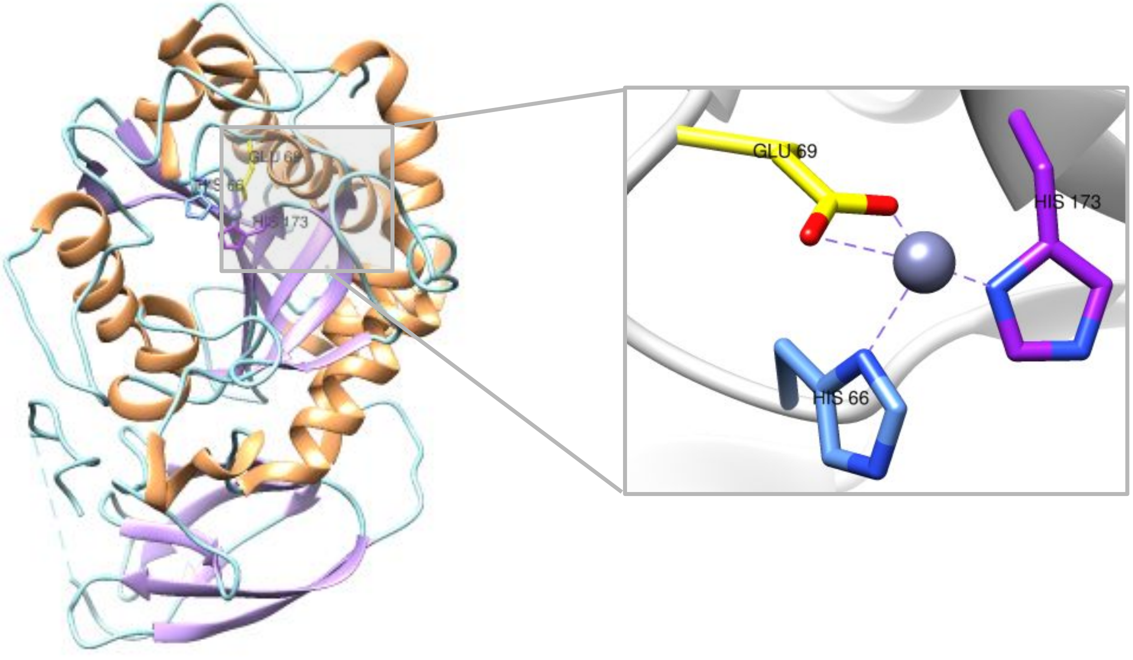
Structural Alignment

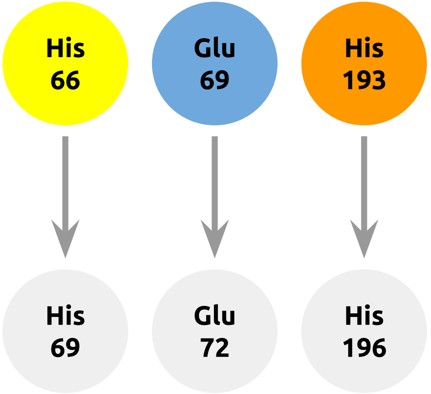
```
hCPA1     ---------------------------------------------------------------------------------------------------------ARSTDTFNYATYHTL
hPCPB     HHGGE-HFEGEKVFRVNVEDENHINIIRELAS--TTQIDFWKPDSVTQIKPHSTVDFRVKAEDTVTVENVLKQNELQYKVLISNLRNVVEAQF-DS-RV-RAT-G------HSYEKYNKW
pPCPB     -------FEGEKVFRVNVEDENDISELHELAS--TRQIDFWKPDSVTQIKPHSTVDFRVKAEDILAVEDFLEQNELQYEVLINNLRSVLEAQF-DS-VS-RTT-G------HSYEKYNNW
pPCPA1    --K--EDFVGHQVLRISVDDEAQVQKVKELEDLEHLQLDFWR-G-P--ARPGFPIDVRVPFPSIQAVKVFLEAHGIRYTIMIEDVQLLLDEEQEQMFASQ--G-RARTTSTFNYATYHTL
hPCPA2    --L--ETFVGDQVLEIVPSNEEQIKNLLQLEAQEHLQLDFWK-S-P--TTPGETAHVRVPFVNVQAVKVFLESQGIAYSIMIEDVQVLLDKENEEMLFNRRRERSG-N---FNFGAYHTL
hPCPA4    --R--EKFFGDQVLRINVRNGDEISKLSQLVNSNNLKLNFWK-S-P--SSFNRPVDVLVPSVSLQAFKSFLRSQGLEYAVTIEDLQALLDNEDDEMQHNEGQERSSNN---FNYGAYHSL

hCPA1     EEIYDFLDLLVAENPHLVSKIQIGNTYEGRPIYVLKFSTG--GSKRPAIWIDTGIHSREWVTQASGVWFAKKITQDYGQDAAFTAILDTLDIFLEIVTNPDGFAFTHSTNRMWRKTRSHT
hPCPB     ETIEAWTQQVATENPALISRSVIGTTFEGRAIYLLKVGKAG-Q-NKPAIFMDCGFHAREWISPAFCQWFVREAVRTYGREIQVTELLNKLDFYVLPVLNIDGYIYTWTKSRFWRKTRSTH
pPCPB     ETIEAWTEQVTSKNPDLISRSAIGTTFDGDNIYLLKVGKPG-S-NKPAIFMDCGFHAREWISQAFCQWFVRDAVRTYGYEAHMTEFLDNLDFYVLPVLNIDGYIYTWTKNRMWRKTRSTN
pPCPA1    EEIYDFMDILVAEHPALVSKLQIGRSYEGRPIYVLKFSTG--GSNRPAIWIDSGIHSREWITQASGVWFAKKITENYGQNSSFTAILDSMDIFLEIVTNPNGFAFTHSDNRLWRKTRSKA
hPCPA2    EEISQEMDNLVAEHPGLVSKVNIGSSFENRPMNVLKFSTG--G-DKPAIWLDAGIHAREWVTQATALWTANKIVSDYGKDPSITSILDALDIFLLPVTNPDGYVFSQTKNRMWRKTRSKV
hPCPA4    EAIYHEMDNIAADFPDLARRVKIGHSFENRPMYVLKFSTGKGV-RRPAVWLNAGIHSREWISQATAIWTARKIVSDYQRDPAITSILEKMDIFLLPVANPDGYVYTQTQNRLWRKTRSRN

hCPA1     AGSLCIGVDPNRNWDAGFGLSGASSNPCSETYHGKFANSEVEVKSIVDFVKDHG-NIKAFISIHSYSQLLMYPYGYKTEPVPDQDELDQLSKAAVTALASLYGTKFNYGSIIKAIYQASG
hPCPB     TGSSCIGTDPNRNFDAGWCEIGASRNPCDETYCGPAAESEKETKALADFIRNKLSSIKAYLTIHSYSQMMIYPYSYAYKLGENNAELNALAKATVKELASLHGTKYTYGPGATTIYPAAG
pPCPB     AGSSCTGTDPNRNFNAGWCTVGASVNPCNETYCGSAAESEKETKALADFIRNNLSSIKAYLTIHSYSQMILYPYSYDYKLPENDAELNSLAKGAVKELASLYGTSYSYGPGSTTIYPAAG
pPCPA1    SGSLCVGSDSNRNWDAGFGGAGASSSPCAETYHGKYPNSEVEVKSITDFVKNNG-NIKAFISIHSYSQLLLYPYGYKTQSPADKSELNQIAKSAVAALKSLYGTSYKYGSIITVIYQASG
hPCPA2    SGSLCVGVDPNRNWDAGFGGPGASSNPCSDSYHGPSANSEVEVKSIVDFIKSHG-KVKAFIILHSYSQLLMFPYGYKCTKLDDFDELSEVAQKAAQSLRSLHGTKYKVGPICSVIYQASG
hPCPA4    PGSSCIGADPNRNWNASFAGKGASDNPCSEVYHGPHANSEVEVKSVVDFIQKHG-NFKGFIDLHSYSQLLMYPYGYSVKKAPDAEELDKVARLAAKALASVSGTEYQVGPTCTTVYPASG

hCPA1     STIDWTYSQGIKYSFTFELRDTGRYGFLLPASQIIPTAKETWLALLTIMEHTLNHP-
hPCPB     GSDDWAYDQGIRYSFTFELRDTGRYGFLLPESQIRATCEETFLAIKYVASYVLEHLY
pPCPB     GSDDWAYNQGIKYSFTFELRDKGRFGFVLPESQIQATCQETMLAVKYVTNYTLEHL-
pPCPA1    GVIDWTYNQGIKYSFSFELRDTGRRGFLLPASQIIPTAQETWLALLTIMEHTLNNS-|
hPCPA2    GSIDWSYDYGIKYSFAFELRDTGRYGFLLPARQILPTAEETWLGLKAIMEHVRDHPY
hPCPA4    SSIDWAYDNGIKFAFTFELRDTGTYGFLLPANQIIPTAEETWLGLKTIMEHVRDNLY
```

M14B Carboxypeptidases

CPE	**Neuroendocrine enzyme** implicated in the **biosynthesis** of numerous **peptide hormones** and **neurotransmitters** by removing the C-terminal from peptide processing **intermediates** that are formed by the action of **prohormone convertases** on the peptide precursor.
CPD	Broadly distributed throughout the body. Primarily functions in the **trans-Golgi network** and **constitutive secretory pathway**. Main substrates are **growth factors** and **receptors** that are produced from larger precursors.
CPM	**Attached** to a wide variety of cells via a **glycosylphosphatidylinositol linkage** and it's involved in the **extracellular processing** of peptides and proteins.
CPN	The **largest regulatory carboxypeptidase described**, it circulates in the plasma forming a 280kDa protein complex. It's the major **blood inactivator** of potent peptides such as kinins and anaphylotoxins.
CPZ	Present in the **extracellular matrix**, with a broad distribution during embryogenesis and a more restricted pattern in adult tissues. **Precise function is not known**, it is likely that this enzyme cleaves intermediates generated by the various matrix endopeptidases

M14B Carboxypeptidases



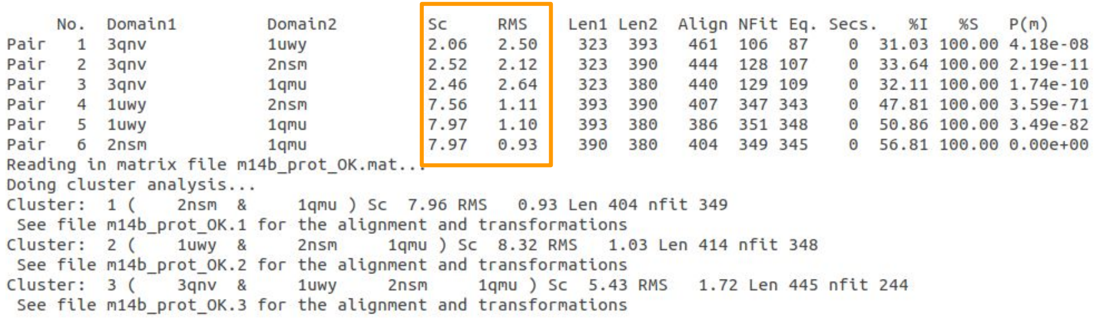

His 66 → His 69

Glu 69 → Glu 72

His 193 → His 196

M14B Carboxypeptidases

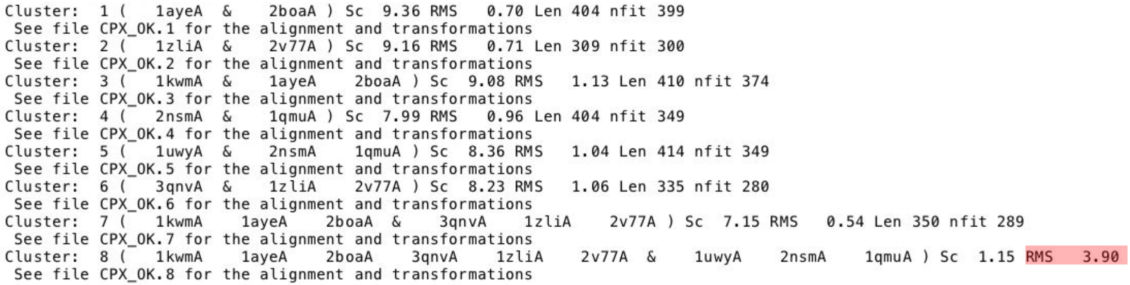
Superimposition



```
     No.  Domain1          Domain2          Sc      RMS     Len1 Len2  Align NFit Eq. Secs.   %I    %S    P(m)
Pair   1  3qnv             1uwy             2.06    2.50     323  393   461  106  87    0  31.03 100.00 4.18e-08
Pair   2  3qnv             2nsm             2.52    2.12     323  390   444  128 107    0  33.64 100.00 2.19e-11
Pair   3  3qnv             1qmu             2.46    2.64     323  380   440  129 109    0  32.11 100.00 1.74e-10
Pair   4  1uwy             2nsm             7.56    1.11     393  390   407  347 343    0  47.81 100.00 3.59e-71
Pair   5  1uwy             1qmu             7.97    1.10     393  380   386  351 348    0  50.86 100.00 3.49e-82
Pair   6  2nsm             1qmu             7.97    0.93     390  380   404  349 345    0  56.81 100.00 0.00e+00
Reading in matrix file m14b_prot_OK.mat...
Doing cluster analysis...
Cluster:  1 (    2nsm  &     1qmu ) Sc  7.96 RMS   0.93 Len 404 nfit 349
 See file m14b_prot_OK.1 for the alignment and transformations
Cluster:  2 (    1uwy  &     2nsm     1qmu ) Sc  8.32 RMS   1.03 Len 414 nfit 348
 See file m14b_prot_OK.2 for the alignment and transformations
Cluster:  3 (    3qnv  &     1uwy     2nsm     1qmu ) Sc  5.43 RMS   1.72 Len 445 nfit 244
 See file m14b_prot_OK.3 for the alignment and transformations
```

3QNV → CPT
1UWY → CPM
2NSM → CPN
1QMU → CPD

M14A vs M14B: Superimposition



```
Cluster:  1 (   1ayeA  &    2boaA ) Sc  9.36 RMS   0.70 Len 404 nfit 399
 See file CPX_OK.1 for the alignment and transformations
Cluster:  2 (   1zliA  &    2v77A ) Sc  9.16 RMS   0.71 Len 309 nfit 300
 See file CPX_OK.2 for the alignment and transformations
Cluster:  3 (   1kwmA  &    1ayeA    2boaA ) Sc  9.08 RMS   1.13 Len 410 nfit 374
 See file CPX_OK.3 for the alignment and transformations
Cluster:  4 (   2nsmA  &    1qmuA ) Sc  7.99 RMS   0.96 Len 404 nfit 349
 See file CPX_OK.4 for the alignment and transformations
Cluster:  5 (   1uwyA  &    2nsmA    1qmuA ) Sc  8.36 RMS   1.04 Len 414 nfit 349
 See file CPX_OK.5 for the alignment and transformations
Cluster:  6 (   3qnvA  &    1zliA    2v77A ) Sc  8.23 RMS   1.06 Len 335 nfit 280
 See file CPX_OK.6 for the alignment and transformations
Cluster:  7 (   1kwmA    1ayeA    2boaA  &    3qnvA    1zliA    2v77A ) Sc  7.15 RMS   0.54 Len 350 nfit 289
 See file CPX_OK.7 for the alignment and transformations
Cluster:  8 (   1kwmA    1ayeA    2boaA    3qnvA    1zliA    2v77A  &    1uwyA    2nsmA    1qmuA ) Sc  1.15 RMS   3.90
 See file CPX_OK.8 for the alignment and transformations
```

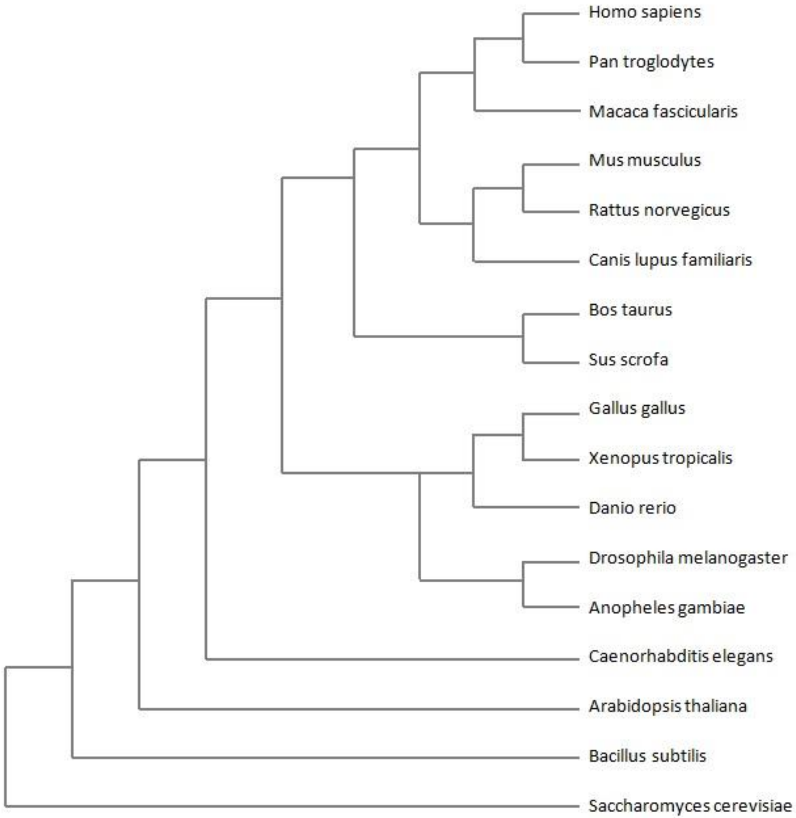
```
       No.  Domain1           Domain2           Sc      RMS
Pair    1   1ayeA             1zliA             7.06    0.77
Pair    2   1ayeA             2v77A             7.15    0.51
Pair    3   1ayeA             2boaA             9.36    0.70
Pair    4   1ayeA             1uwyA             0.35    2.34
```

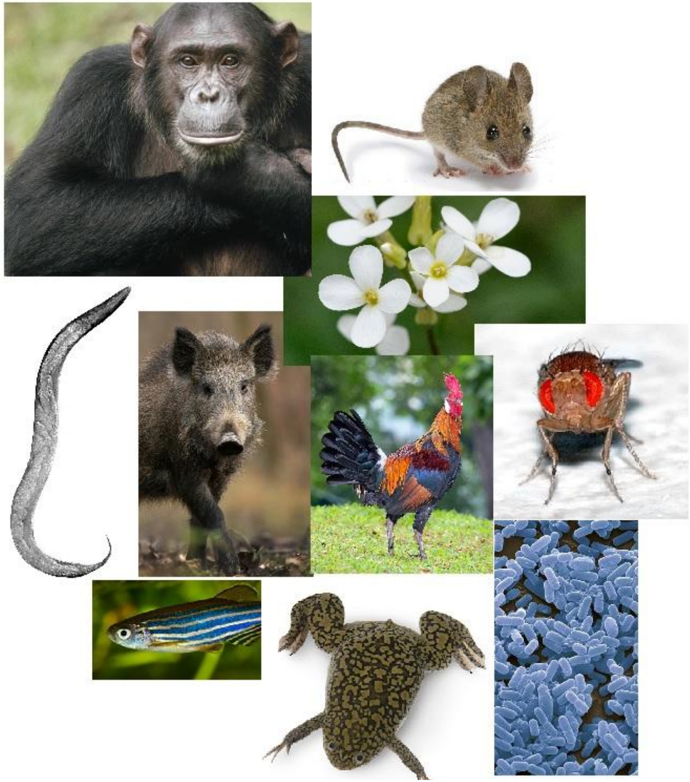
2NSM → CPN
1QMU → CPD
1KWM → PCPB
1AYE → PCPA2
2BOA → PCPA4

3QNV → CPT
1ZLI → CPB
2V77 → CPA1
1UWY → CPM

Phylogeny and Evolution

Phylogenetic tree

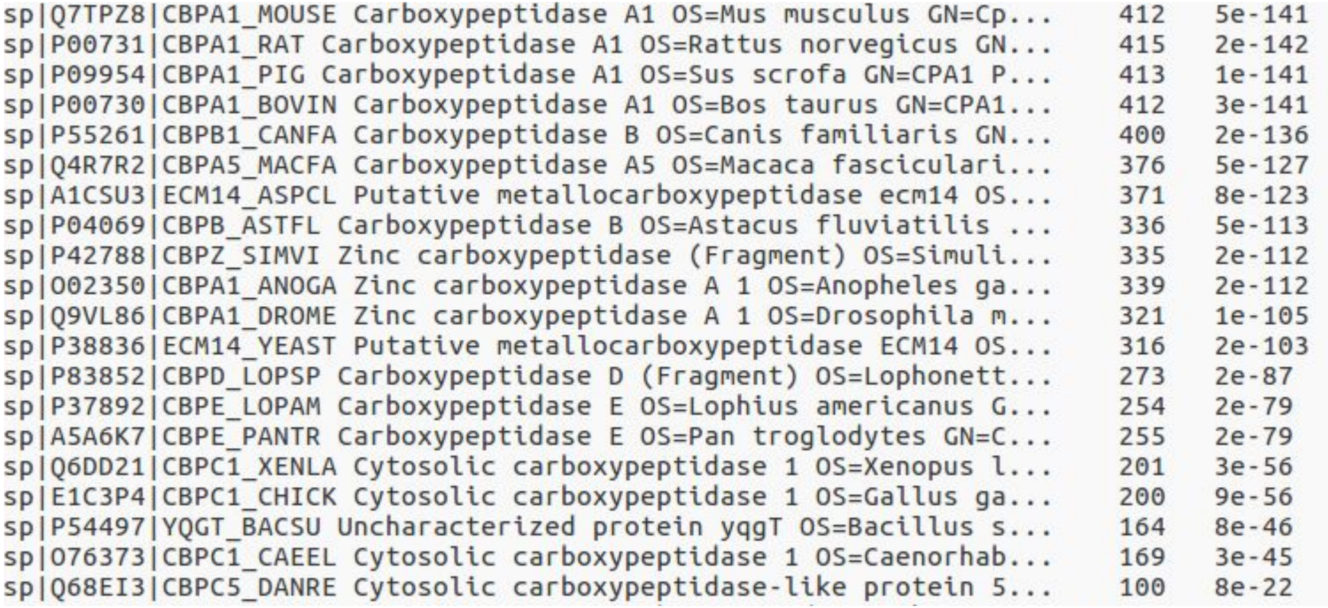




"InParanoid 8: orthology analysis between 273 proteomes, mostly eukaryotic" Erik L.L.Sonnhammer and Gabriel Östlund
Nucleic Acids Res. 43:D234-D239 (2015)

Phylogeny and Evolution

Multiple Sequence Alignment



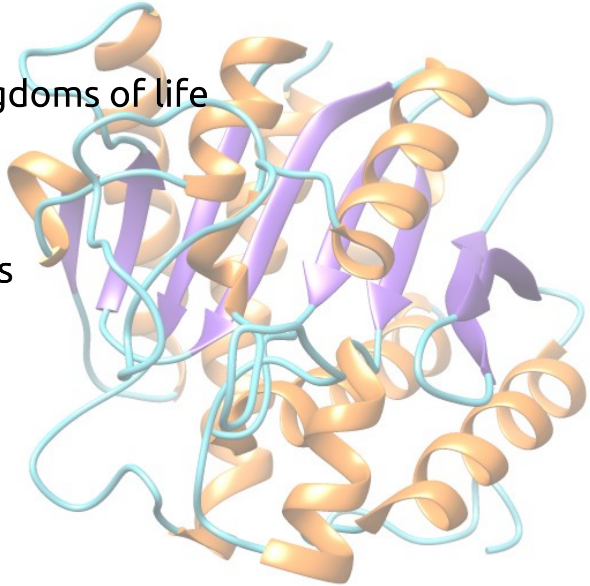
```
sp|Q7TPZ8|CBPA1_MOUSE Carboxypeptidase A1 OS=Mus musculus GN=Cp...     412   5e-141
sp|P00731|CBPA1_RAT Carboxypeptidase A1 OS=Rattus norvegicus GN...     415   2e-142
sp|P09954|CBPA1_PIG Carboxypeptidase A1 OS=Sus scrofa GN=CPA1 P...     413   1e-141
sp|P00730|CBPA1_BOVIN Carboxypeptidase A1 OS=Bos taurus GN=CPA1...     412   3e-141
sp|P55261|CBPB1_CANFA Carboxypeptidase B OS=Canis familiaris GN...     400   2e-136
sp|Q4R7R2|CBPA5_MACFA Carboxypeptidase A5 OS=Macaca fasciculari...     376   5e-127
sp|A1CSU3|ECM14_ASPCL Putative metallocarboxypeptidase ecm14 OS...     371   8e-123
sp|P04069|CBPB_ASTFL Carboxypeptidase B OS=Astacus fluviatilis ...     336   5e-113
sp|P42788|CBPZ_SIMVI Zinc carboxypeptidase (Fragment) OS=Simuli...     335   2e-112
sp|O02350|CBPA1_ANOGA Zinc carboxypeptidase A 1 OS=Anopheles ga...     339   2e-112
sp|Q9VL86|CBPA1_DROME Zinc carboxypeptidase A 1 OS=Drosophila m...     321   1e-105
sp|P38836|ECM14_YEAST Putative metallocarboxypeptidase ECM14 OS...     316   2e-103
sp|P83852|CBPD_LOPSP Carboxypeptidase D (Fragment) OS=Lophonett...     273   2e-87
sp|P37892|CBPE_LOPAM Carboxypeptidase E OS=Lophius americanus G...     254   2e-79
sp|A5A6K7|CBPE_PANTR Carboxypeptidase E OS=Pan troglodytes GN=C...     255   2e-79
sp|Q6DD21|CBPC1_XENLA Cytosolic carboxypeptidase 1 OS=Xenopus l...     201   3e-56
sp|E1C3P4|CBPC1_CHICK Cytosolic carboxypeptidase 1 OS=Gallus ga...     200   9e-56
sp|P54497|YQGT_BACSU Uncharacterized protein yqgT OS=Bacillus s...     164   8e-46
sp|O76373|CBPC1_CAEEL Cytosolic carboxypeptidase 1 OS=Caenorhab...     169   3e-45
sp|Q68EI3|CBPC5_DANRE Cytosolic carboxypeptidase-like protein 5...     100   8e-22
```

48% identity

30% identity

Phylogeny and Evolution

Multiple Sequence Alignment



- Carboxypeptidases are present in all kingdoms of life
- Highly conserved residues
- Observed similarity fits the classifications
- Further research on structure is needed

Conclusions

- ➔ CPs have a dual classification in different families
- ➔ Structural similarity in the catalytic domain
- ➔ Highly conserved residues in the active site
- ➔ Discrepancies in catalysis
- ➔ Structural differences between M14A and M14B
- ➔ Present in all kingdoms of life

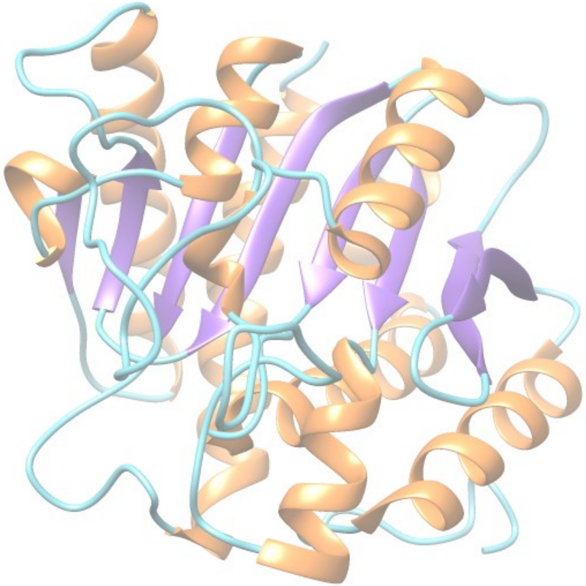
Materials and Methods

Programs used:

- Psi-blast
- Clustalw
- STAMP (Alignfit)
- Chimera

Databases used:

- PDB
- UniProt
- MEROPS
- InParanoid8



Bibliography

Aloy P, Catasus L, Villegas V, Reverter D, Vendrell J, Aviles FX. Comparative Analysis of the Sequences and Three-Dimensional Models of Human Procarboxypeptidases A1, 2 and B. 1998;379(February):149–55.

Alvarez-Santos S, Gonzalez-Lafont A, Lluch JM. On the water-prompted mechanism of peptide cleavage by carboxypeptidase A. A theoretical study. Can J Chem. 1994;72(25):2077.

Arolas JL, Lorenzo J, Rovira A, Vendrell J, Aviles FX, Ventura S. Secondary Binding Site of the Potato Carboxypeptidase Inhibitor . Contribution to Its Structure, Folding , and Biological Properties. 2004;7973–82.

Arolas JL, Vendrell J, Aviles FX, Fricker LD. Metallocarboxypeptidases: emerging drug targets in biomedicine. Curr Pharm Des [Internet]. 2007;13(4):349–66. Available from: http://cat.inist.fr/?aModele=afficheN&cpsidt=18654429

Banci L, Bertini I, Penna G La. The Enzymatic Mechanism of Carboxypeptidase : A Molecular Dynamics Study. 1994;197:186–97.

Bukrinsky JT, Bjerrum MJ, Kadziola A. Native Carboxypeptidase A in a New Crystal Environment Reveals a Different Conformation of the Important Tyrosine 248. 1998;16555–64.

Bukrinsky JT, Bjerrum MJ, Kadziola A. Native carboxypeptidase A in a new crystal environment reveals a different conformation of the important tyrosine 248. Biochemistry. 1998;37(47):16555–64.

Deiteren K, Surpateanu G, Gilany K, Willemse JL, Hendriks DF, Augustyns K, et al. The role of the S1 binding site of carboxypeptidase M in substrate specificity and turn-over. Biochim Biophys Acta - Proteins Proteomics. 2007;1774(2):267–77.

Fernández D, Boix E, Pallarès I, Avilés FX, Vendrell J. Analysis of a new crystal form of procarboxypeptidase B: Further insights into the catalytic mechanism. Biopolymers. 2010;93(2):178–85.

Alvarez-Santos S, González-Lafont A, Lluch JM, Oliva B, Avilés FX. On the water-promoted mechanism of peptide cleavage by carboxypeptidase A. A theoretical study. Can J Chem 1994 march 21; 72: 2077-2083.

Carbalho HF, Roque ACA, Iranzo O, Branco RJF. Comparison of the Internal Dynamics of Metalloproteases Provides New Insights on Their Function and Evolution. PLoS ONE 2015 september 23; 10 (9): 1-22.

Bibliography

Fernández D, Pallarès I, Vendrell J, Avilés FX. Biochimie Progress in metallocarboxypeptidases and their small molecular weight inhibitors. Biochimie [Internet]. Elsevier Masson SAS; 2010;92(11):1484–500. Available from: http://dx.doi.org/10.1016/j.biochi.2010.05.002

Gallivan JP, Dougherty DA. Cation-pi interactions in structural biology. Proc Natl Acad Sci [Internet]. 1999;96(17):9459–64. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.96.17.9459

García-castellanos R, Bonet-figueredo R, Pallarés I, Ventura S, Avilés FX, Vendrell J. Detailed molecular comparison between the inhibition mode of A / B-type carboxypeptidases in the zymogen state and by the endogenous inhibitor latexin. 2014;62(2005):1996–2014.

García-Sáez I, Reverter D, Vendrell J, Avilés FX, Coll M. The three-dimensional structure of human procarboxypeptidase A2. Deciphering the basis of the inhibition, activation and intrinsic activity of the zymogen. EMBO J. 1997;16(23):6906–13.

Gardell SJ, Craik S, Clauserll E, Goldsmith J, Stewart C, Graf M, et al. A Novel Rat Carboxypeptidase, CPAB : Characterization, Molecular Cloning, and Evolutionary Implications on Substrate Specificity in the Carboxypeptidase Gene Family. 1988;263(33):17828–36.

Gomez-ortiza M, Gomis-riithb FX, Huber R, Aviles FX. Inhibition of carboxypeptidase A by excess zinc : analysis of the structural determinants by X-ray crystallography. 1997;400:336–40.

Gomis-r FX. Structure and Mechanism of Metallocarboxypeptidases. *Critical Reviews in Biochemistry and Molecular Biology*, 43:319–345, 2008.

Ibarz A, Garvín A, Garza S, Pagán J. Inactivation of carboxypeptidase A and trypsin by UV-visible light. Innov Food Sci Emerg Technol [Internet]. 2009;10(4):517–21. Available from: http://dx.doi.org/10.1016/j.ifset.2009.03.006

Jiménez M, Villegas V, Santoro J. NMR solution structure of the activation domain of human procarboxypeptidase A2. Protein … [Internet]. 2003;296–305. Available from: http://onlinelibrary.wiley.com/doi/10.1110/ps.0227303/full

Kilshtain AV, Warshel A. On the origin of the catalytic power of carboxypeptidase a and other metalloenzymes. Proteins Struct Funct Bioinforma. 2009;77(3):536–50.

Bibliography

Kilshtain-vardi A, Greenblatt HM. research papers Refined structure of bovine carboxypeptidase A at Ê resolution research papers. 2003;323–33.

Lee KJ, Kim DH. Design of Mechanism-Based Carboxypeptidase A Inactivators on the Basis of the X-ray Crystal Structure and Catalytic Reaction Pathway 1. 1998;6:1613–22.

Manuscript A, Pathways N. NIH Public Access. 2011;114(28):9259–67.

Petrera A, Lai ZW, Schilling O. Carboxyterminal protein processing in health and disease: Key actors and emerging technologies. J Proteome Res. 2014;13(11):4497–504.

Pinto GA, Tardioli PW, Cabrera-Padilla RY, Galvão CMA, Giordano RC, Giordano RLC. Amino acids yields during proteolysis catalyzed by carboxypeptidase A are strongly dependent on substrate pre-hydrolysis. Biochem Eng J. 2008;39(2):328–37.

Puigserver a, Desnuelle P. Dissociation of bovine 6S procarboxypeptidase A by reversible condensation with 2,3-dimethyl maleic anhydride: application to the partial characterization of subunit III. Proc Natl Acad Sci U S A. 1975;72(6):2442–5.

Rawlings ND, Barrett AJ & Finn RD (2016) Twenty years of the *MEROPS* database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res* 44, D343-D350

Shushanyan M, Khoshtariya DE, Tretyakova T, Makharadze M, Van Eldik R. Diverse role of conformational dynamics in carboxypeptidase A-driven peptide and ester hydrolyses: Disclosing the “perfect Induced Fit” and “protein Local Unfolding” pathways by altering protein stability. Biopolymers. 2011;95(12):852–70.

Szeto MWY, Mujika JI, Zurek J, Mulholland AJ, Harvey JN. Journal of Molecular Structure : THEOCHEM QM / MM study on the mechanism of peptide hydrolysis by carboxypeptidase A. J Mol Struct THEOCHEM [Internet]. Elsevier B.V.; 2009;898(1–3):106–14.

Szeto MWY, Mujika JI, Zurek J, Mulholland AJ, Harvey JN. QM/MM study on the mechanism of peptide hydrolysis by carboxypeptidase A. J Mol Struct THEOCHEM [Internet]. 2009;898(1–3):106–14. Available from: http://dx.doi.org/10.1016/j.theochem.2008.06.033

Shanshan W, Chunchun Z, Dingguo X, Hua G. Catalysis of Carboxypeptidase A: Promoted-water vs Nucleophilic Pathways. J Phys Chem B 2010 July 22; 114 (28): 9259-9267.

Bibliography

Szmola R, Bence M, Carpentieri A, Szab?? A, Costello CE, Samuelson J, et al. Chymotrypsin C is a co-activator of human pancreatic procarboxypeptidases A1 and A2. J Biol Chem. 2011;286(3):1819–27.

Testero S. The X-Ray Structure of Carboxypeptidase A Inhibited by a Thiirane Mechanism-Based Inhibitor. 2010;29–34.

Testero SA, Granados C, Fernandez D, Gallego P, Covaleda G, Reverter D, et al. Discovery of mechanism-based inactivators for human pancreatic carboxypeptidase A from a focused synthetic library Discovery of mechanism-based inactivators for human pancreatic carboxypeptidase A from a focused synthetic library. 2017.

Vallee BL. Metallocarboxypeptidases : Mechanism. 1962;1(5).

Vendrell J, Aviles FX, Fricker LD. Josep Vendrell , Francesc X Aviles and Lloyd D Fricker. 2011;1–15.

Vendrell J, Querol E, Avile FX. Metallocarboxypeptidases and their protein inhibitors Structure , function and biomedical properties. 2000;1477:284–98.

Wang SF, Jin JY, Zeng ZH, Tian GR. Optical 2-benzyl-5-hydroxy-4-oxopentanoic acids against carboxypeptidase A: Synthesis, kinetic evaluation and X-ray crystallographic study. Chinese Chem Lett. 2010;21(2):159–62.

Wang SH, Wang SF, Xuan W, Zeng ZH, Jin JY, Ma J, et al. Nitro as a novel zinc-binding group in the inhibition of carboxypeptidase A. Bioorganic Med Chem. 2008;16(7):3596–601.

Web sites:

EMBL-EBI I. Peptidase M14, carboxypeptidase A (IPR000834) < InterPro < EMBL-EBI [Internet]. Ebi.ac.uk. 2017 [cited 15 February 2018]. Available from: http://www.ebi.ac.uk/interpro/entry/IPR000834
MEROPS - the Peptidase Database [Internet]. Merops.sanger.ac.uk. 2017 [cited 15 February 2018]. Available from: http://merops.sanger.ac.uk/cgi-bin/famsum?family=m14
SCOP: Family: Pancreatic carboxypeptidases [Internet]. Scop.mrc-lmb.cam.ac.uk. 2017 [cited 15 February 2018]. Available from: http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.d.hi.f.b.html
InParanoid 8: orthology analysis between 273 proteomes, mostly eukaryotic" Erik L.L.Sonnhammer and Gabriel Östlund. Nucleic Acids Res. 43:D234-D239 (2015) http://inparanoid.sbc.su.se/cgi-bin/index.cgi

Multiple Choice Questions

1. Regarding Carboxypeptidases:

a) Endopeptidases that cut the C-terminal end of peptides
b) Endopeptidases that cut the N-terminal end of peptides
c) Exopeptidases that cut the C-terminal end of proteins
d) Exopeptidases that cut the N-terminal end of proteins
e) None of the above

2. Select the correct statements about Carboxypeptidases M14A:

1. Catalytic proteins
2. Secreted as zymogens
3. Found in pancreatic secretions
4. Have a transthyretin-like domain

a)1, 2, 3
b)2, 4
c)1, 3
d) 4
e)1, 2, 3, 4

3. Related to the water-promoted pathway in Carboxypeptidases:

a) It's divided in nucleophilic addition and elimination steps
b) Water acts as a proton donor for Glu270
c) An oxyanion hole is formed
d) There's a tetrahedral intermediate
e) All of the above

Multiple Choice Questions

4. Regarding the catalytic mechanism of Carboxypeptidases:

a) There are discrepancies despite the experimental data
b) Water-promoted pathway is the sole mechanism for proteolytic reactions
c) Nucleophilic pathway is not the preferred mechanism
d) Both pathways are viable for esterolytic reactions
e) All of the above

5. Procarboxypeptidases…

a) Are secreted in the liver
b) Their pro-peptide is 50 residues long
c) Need activation by trypsin
d) Release the pro-peptide when cut in a cysteine residue
e) Contain only globular domains

6. Why are residues 69, 72 and 196 highly conserved?

a) Because they are in the pro-segment
b) Because they are crucial for catalysis
c) Because they coordinate Zinc atom
d) Because they have positive charge
e) Because they are aromatic amino acids

7. Regarding the substrate specificity of Carboxypeptidases:

a) CPA1 has preference for aliphatic residues
b) CPA's have an Isoleucine in position 255 and 243.
c) Both a and b are correct
d) CPB cleaves off basic residues
e) All of them are correct

Multiple Choice Questions

8. Which of the following are CPA inhibitors?

- a) PCI (Potato Carboxypeptidase Inhibitor)
- b) Presence of a second zinc ion
- c) Both a and b are correct
- d) Autologous inhibition by the pro-segment
- e) All of them are correct

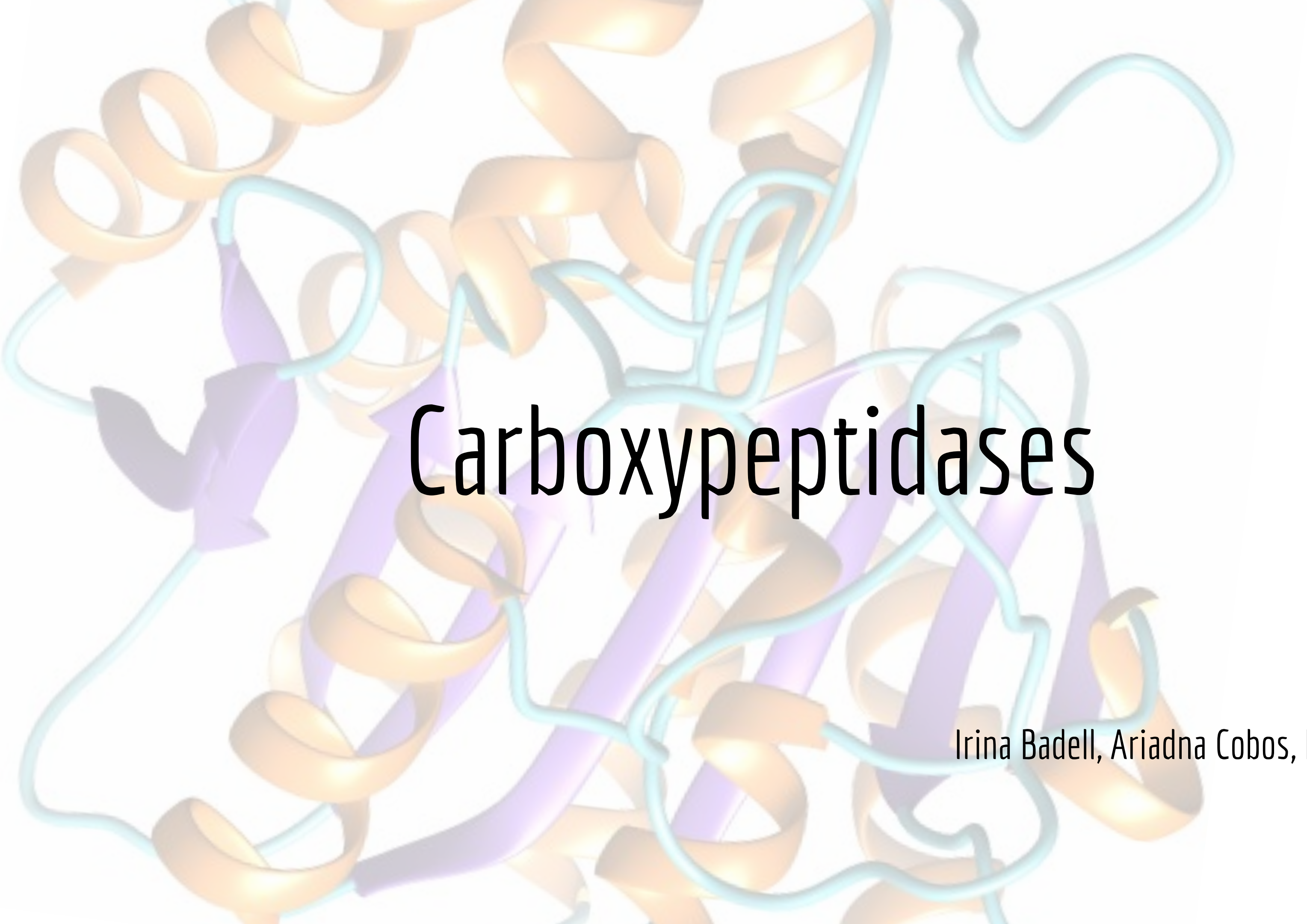
9. About M14 subfamilies superimposition:

1. M14A carboxypeptidases are more similar among them that when compared to the M14B
2. Sc values of 9 and RMS values of <2.0 suggest that proteins have a common ancestor
3. M14B superimposition has a higher RMS value than the M14A subfamily superimposition
4. Sequence is more similar between the two carboxypeptidase subfamilies than structure

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

10. About evolution of Carboxypeptidases:

- a) They are present only in eukaryotes
- b) Low number of residues are conserved along different species.
- c) Are found in all the kingdoms of life
- d) Catalytic CPs are only found in humans
- e) All of them are false



Carboxypeptidases

Irina Badell, Ariadna Cobos, Marta Dacosta