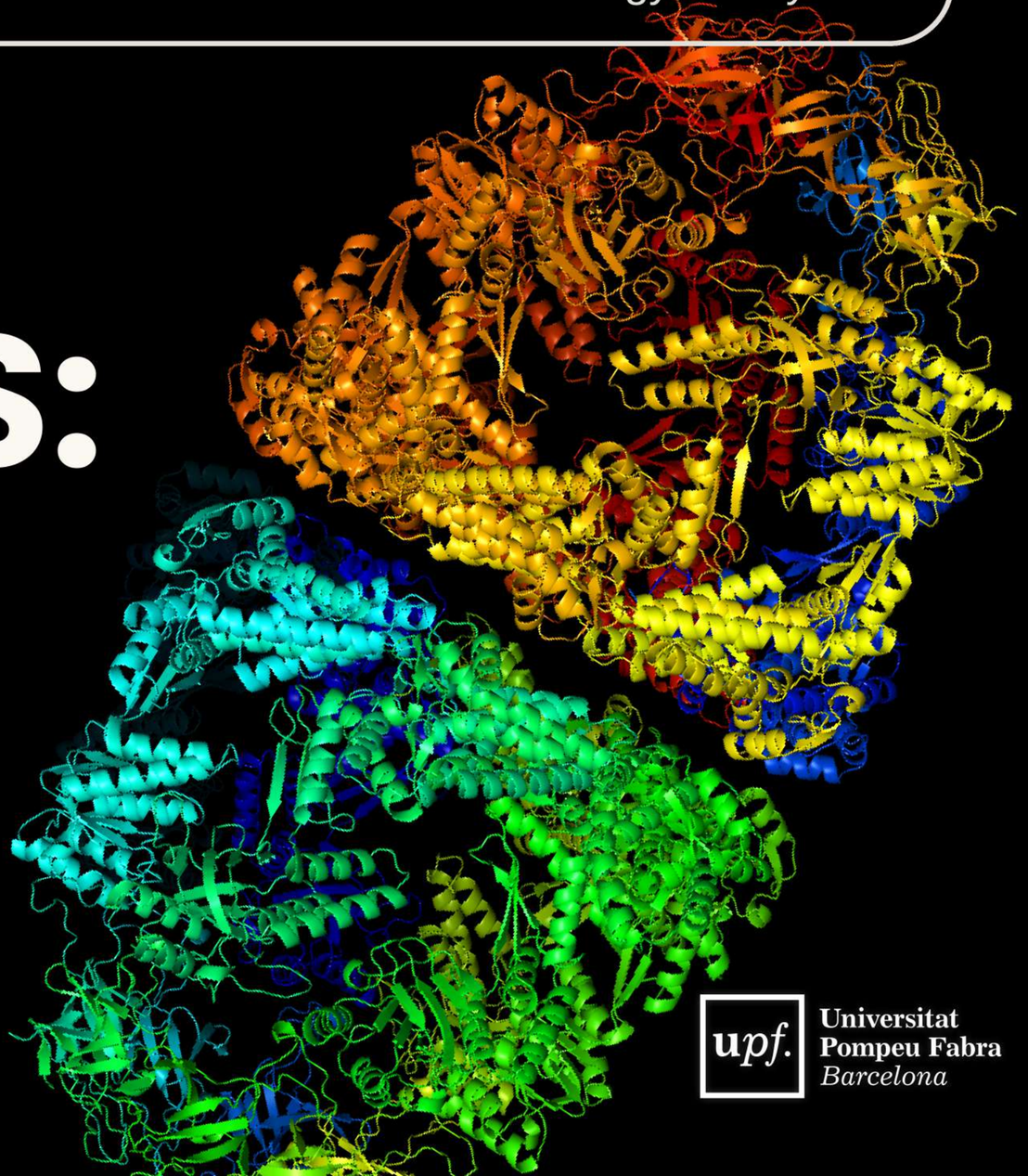


Hsp60, a protein folding container

Human Biology - 4th year

CHAPERONES: HSP60



Alba Fernández, Marie Gajardo, Aya Hacini, Joana Marí

upf. Universitat Pompeu Fabra Barcelona

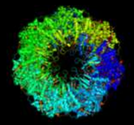


TABLE OF CONTENTS

1 Chaperones in protein folding

2 Heat Shock Proteins

3 Hsp60 family

4 Human Hsp60

5 Hsp60 protomer

6 GroEL vs mtHsp60

7 Dynamic Assembly
→ Intra/Inter ring interactions

8 apo/ATP-bound Superimposition

9 ATP binding

10 Hsp10 binding

11 Substrate binding

12 Evolution

CHAPERONES IN PROTEIN FOLDING

Protein function → Structure → ***CORRECT FOLDING***

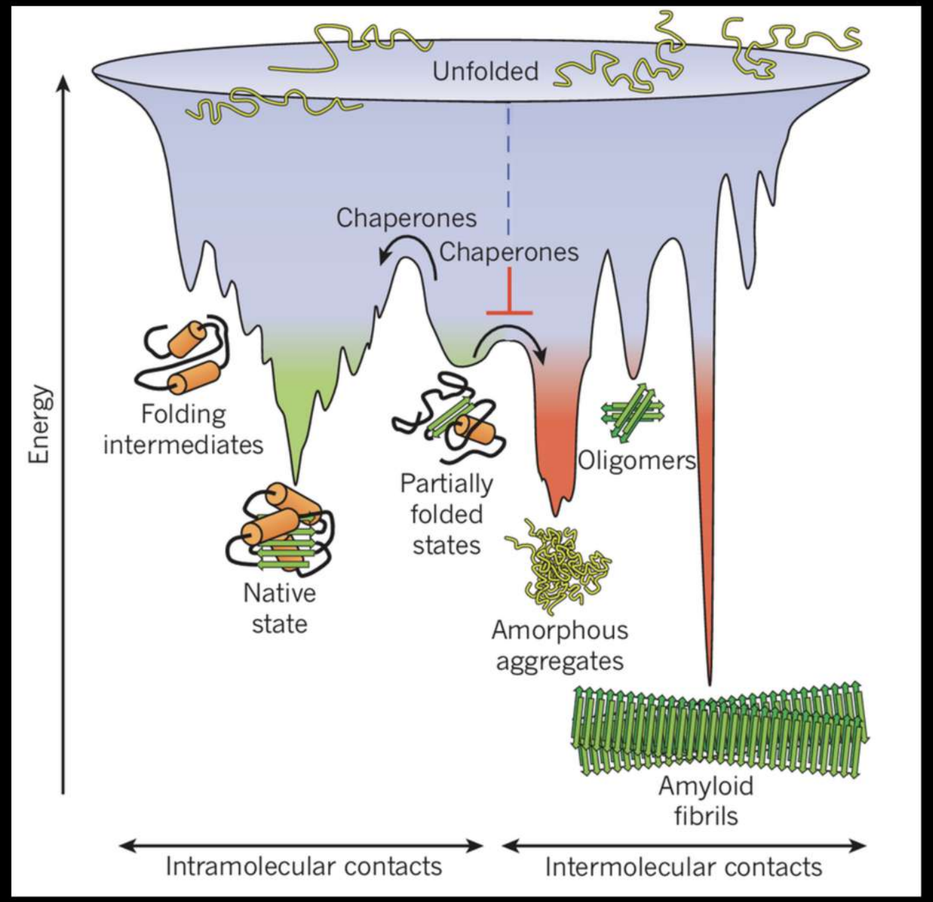
IF MISFOLDING:

exposure of hydrophobic or charged residues

↓

Protein homeostasis network

Degradation + **Chaperones**



Hartl, F., Bracher, A. & Hayer-Hartl, M. Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332 (2011).

CHAPERONES IN PROTEIN FOLDING

Protein function → Structure → ***CORRECT FOLDING***

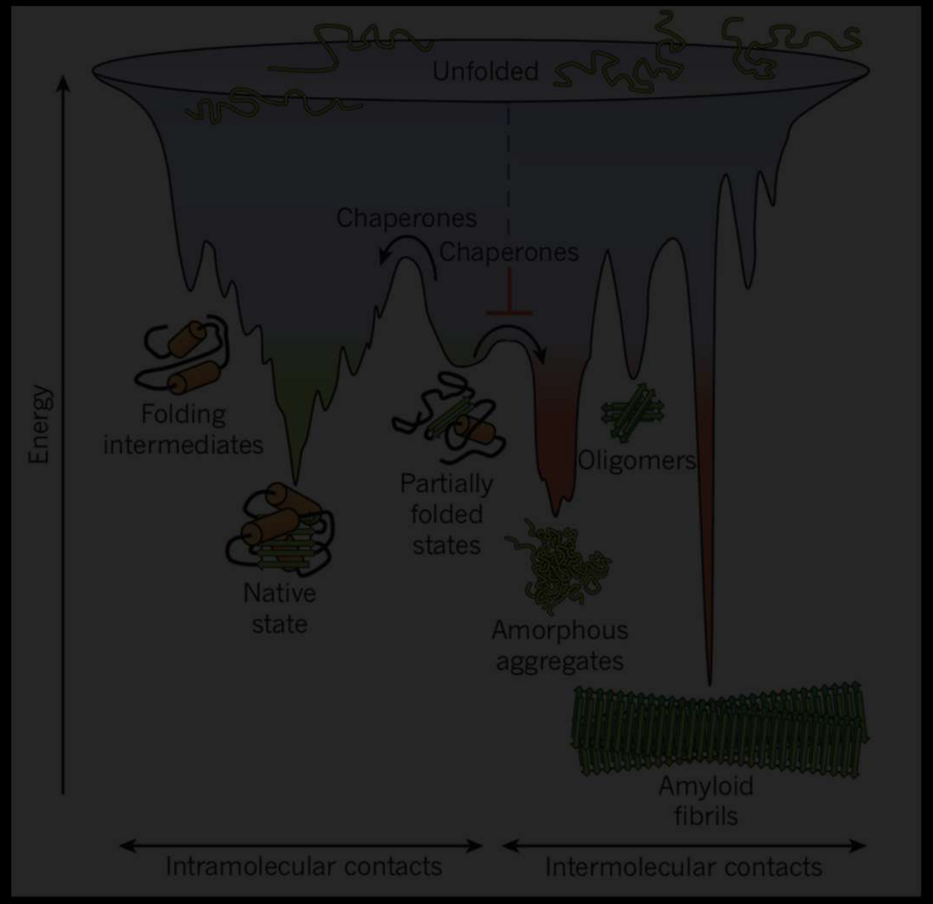
IF MISFOLDING:

exposure of hydrophobic or charged residues

Protein homeostasis network

Degradation + **Chaperones**

Correct folding: don't impose, bind transiently → Non native SPs are capture and trapped to fold safely

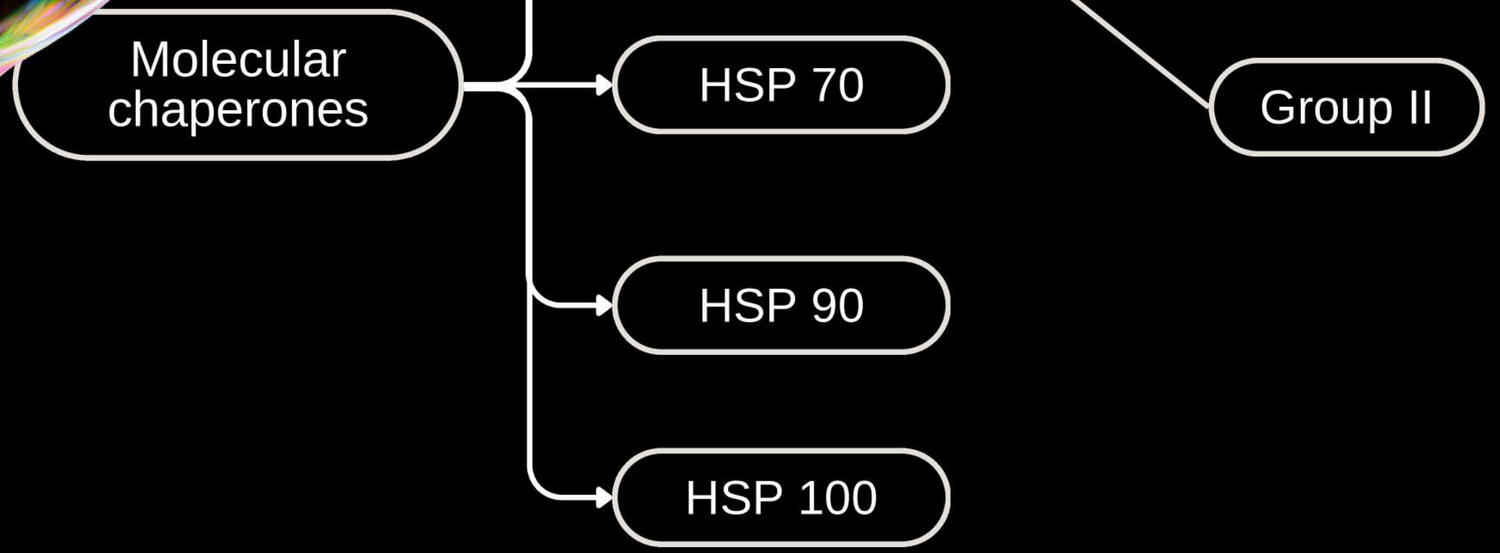


HEAT SHOCK PROTEINS
CLASSIFICATION





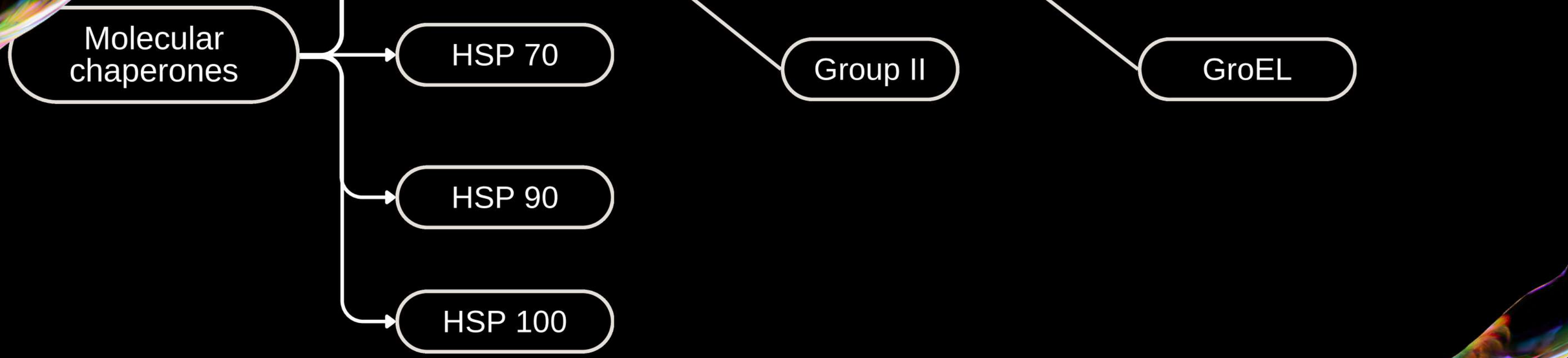
HEAT SHOCK PROTEINS
CLASSIFICATION

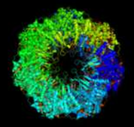


HEAT SHOCK PROTEINS
CLASSIFICATION

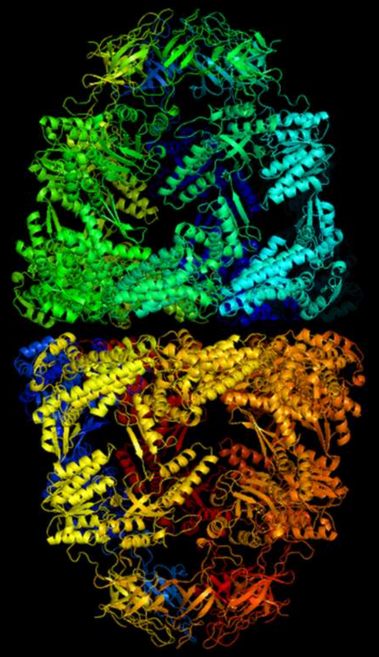


HEAT SHOCK PROTEINS
CLASSIFICATION





HEAT SHOCK PROTEINS



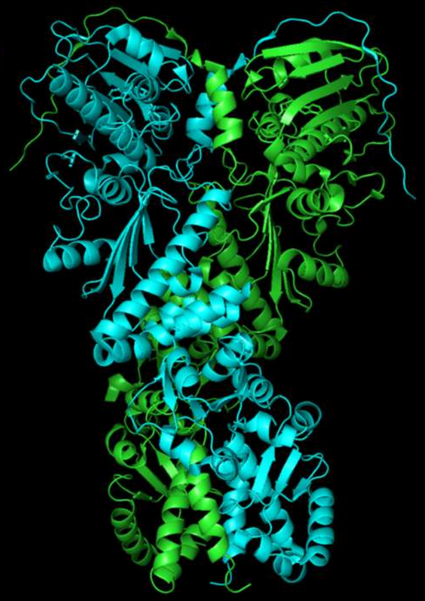
HSP 60

ID: 9SHG, 1.91 Å
Electron Microscopy



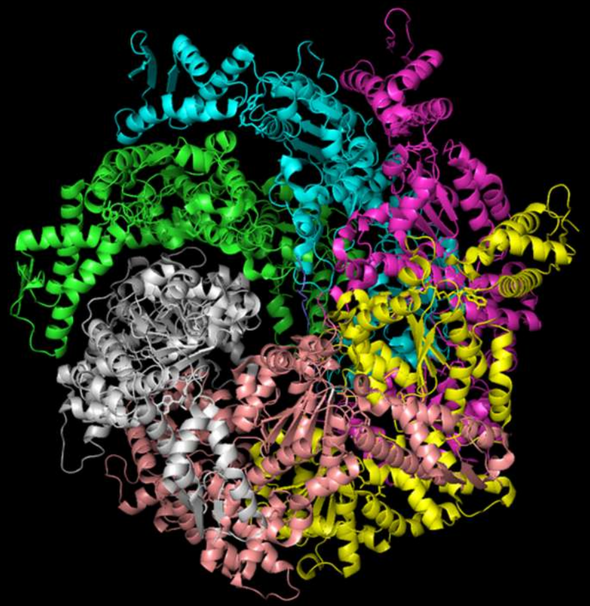
HSP 70

ID: 5E85, 2.99 Å
X-Ray Diffraction



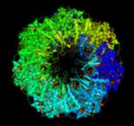
HSP 90

ID: 7KCK, 3.26 Å
Electron Microscopy

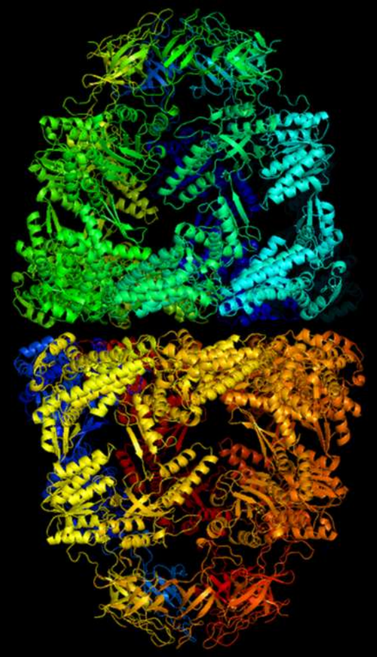


HSP 100

ID: 6W24, 3.4 Å
Electron microscopy

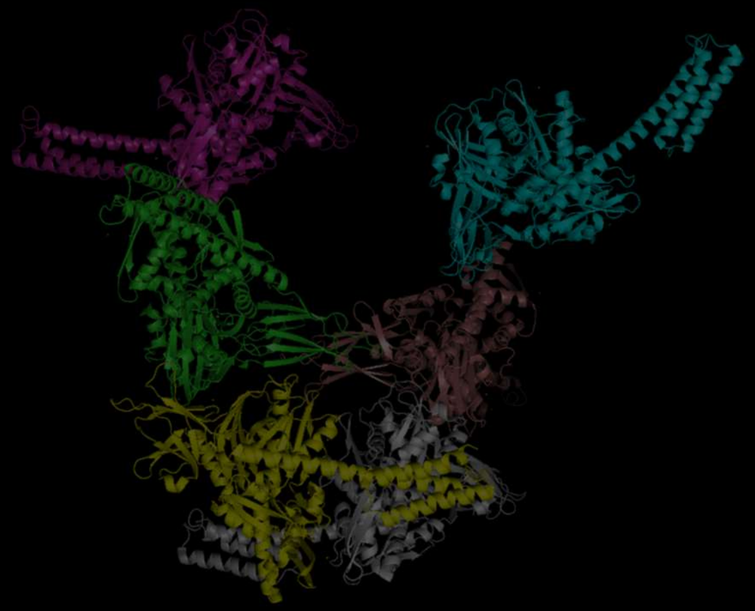


HEAT SHOCK PROTEINS



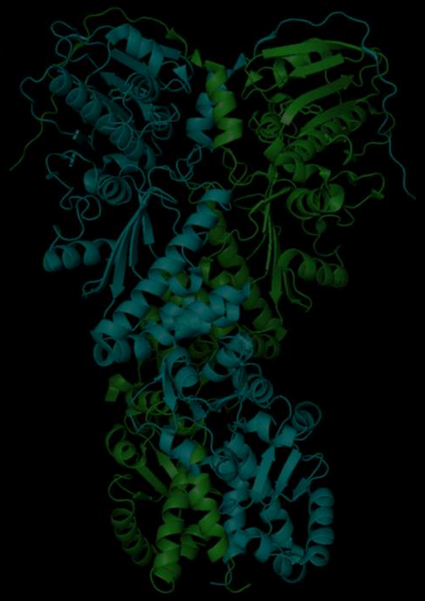
HSP 60

ID: 9SHG, 1.91 Å
Electron Microscopy



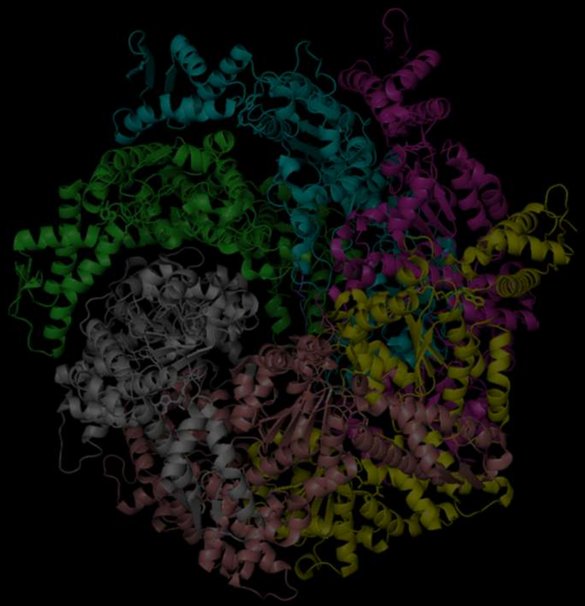
HSP 70

ID: 5E85, 2.99 Å
X-Ray Diffraction



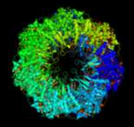
HSP 90

ID: 7KCK, 3.26 Å
Electron Microscopy

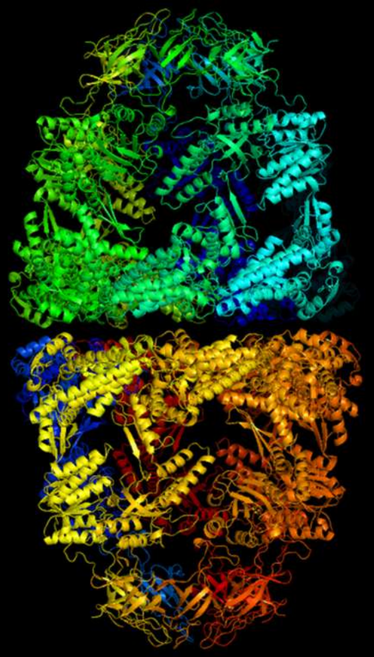


HSP 100

ID: 6W24, 3.4 Å
Electron microscopy



HEAT SHOCK PROTEINS



HSP 60

ID: 9SHG, 1.91 Å
Electron Microscopy

HSP60 = CHAPERONINES

- 60 kDa
- ATP dependent
- 2 groups

HSP60 FAMILY

→ ATP DEPENDENT

GROUP II

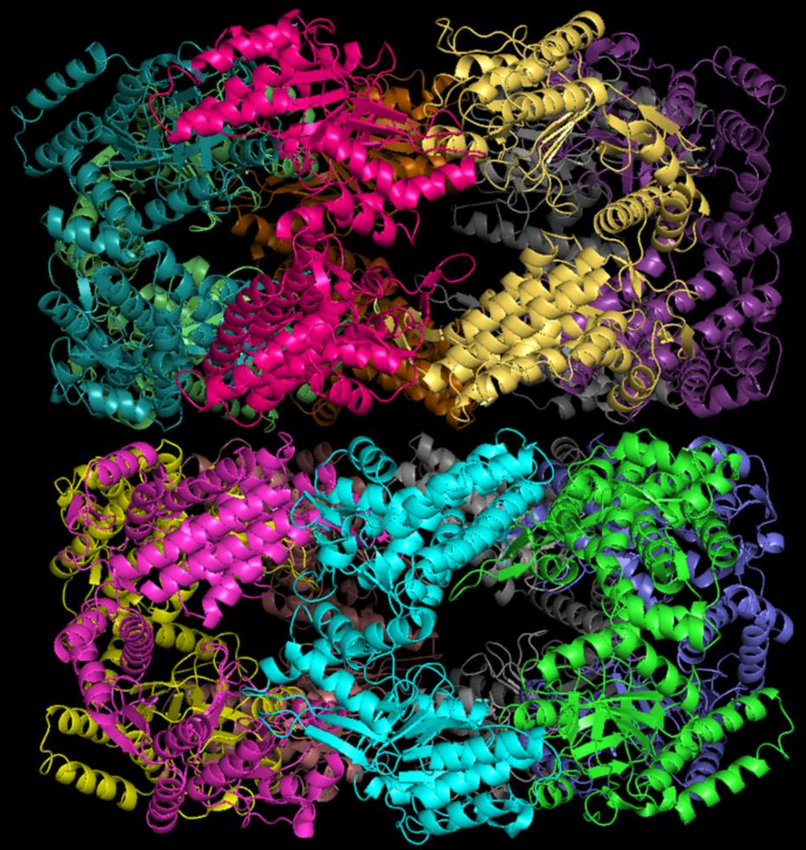
- Hetero oligomers
- Eightfold barrel-like structure
- Sixteen subunits
- Don’t need co-chaperones
- Build-in lid → enclosed chamber
- Archaea and eukaryotes
- Ex:
  - Chaperonin containing TCP1 complex (Eukaryotes)



ID: 6W24, 3.4 Å
Electron microscopy

HSP60 FAMILY

→ ATP DEPENDENT

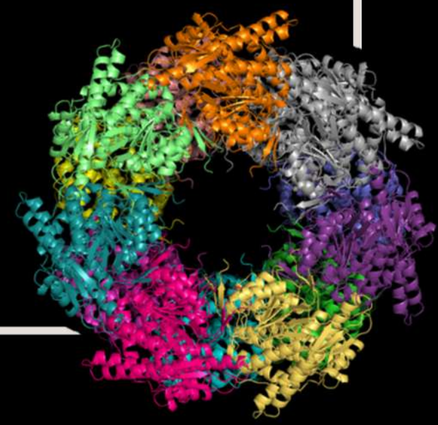


ID: 1KP8, 2 Å
X-ray diffraction

GROUP I

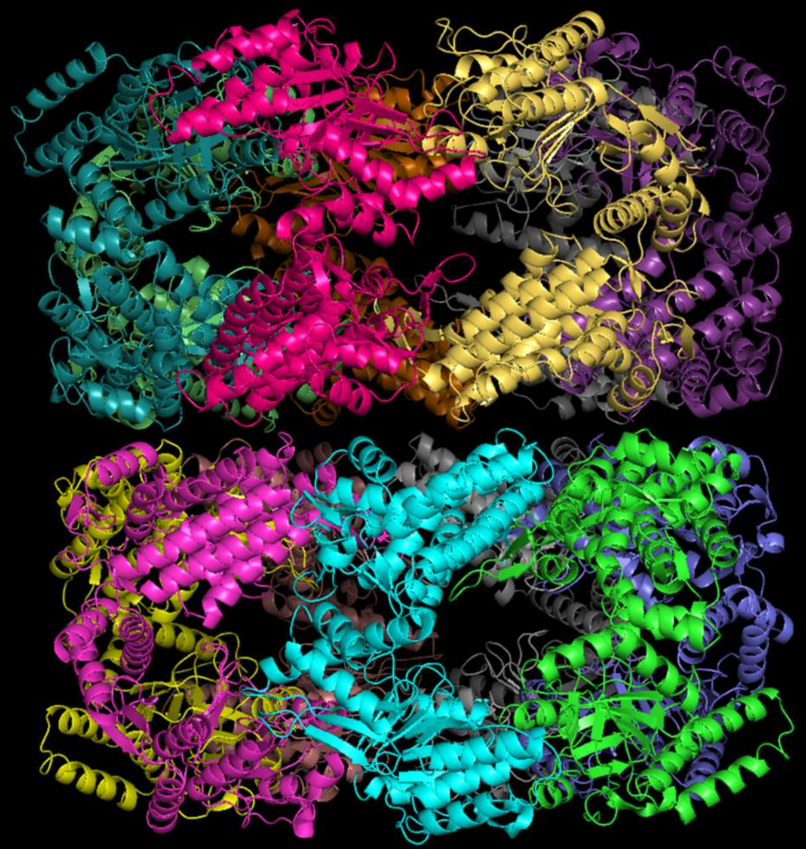
- Homo oligomers
- Sevenfold symmetric barrel-like structure
- Two rings
- Function with co-chaperones
- Eubacteria and end. organelles

- Ex:
  - GroEL/ES (Prokaryotes)
  - m**Hsp60/Hsp10 (human)**



HSP60 FAMILY

→ ATP DEPENDENT

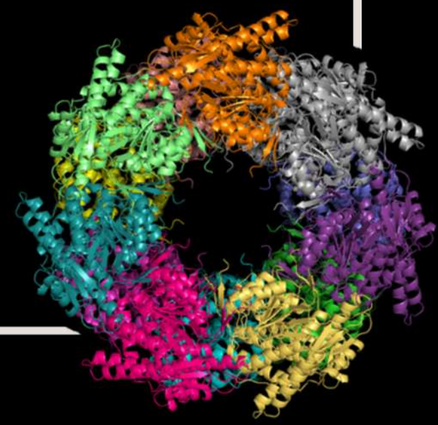


ID: 1KP8, 2 Å
X-ray diffraction

GROUP I

- Homo oligomers
- Sevenfold symmetric barrel-like structure
- Two rings
- Function with co-chaperones
- Eubacteria and end. organelles

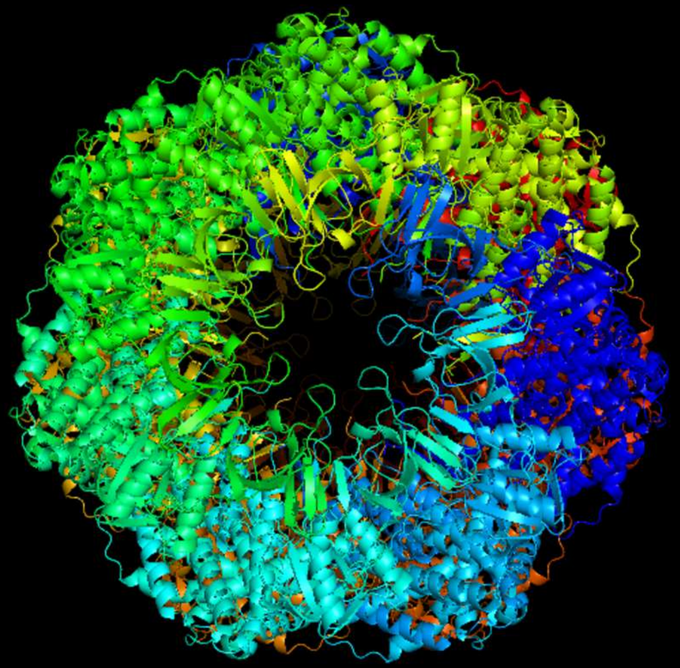
- Ex:
  - GroEL/ES (Prokaryotes)
  - m**Hsp60/Hsp10 (human)**

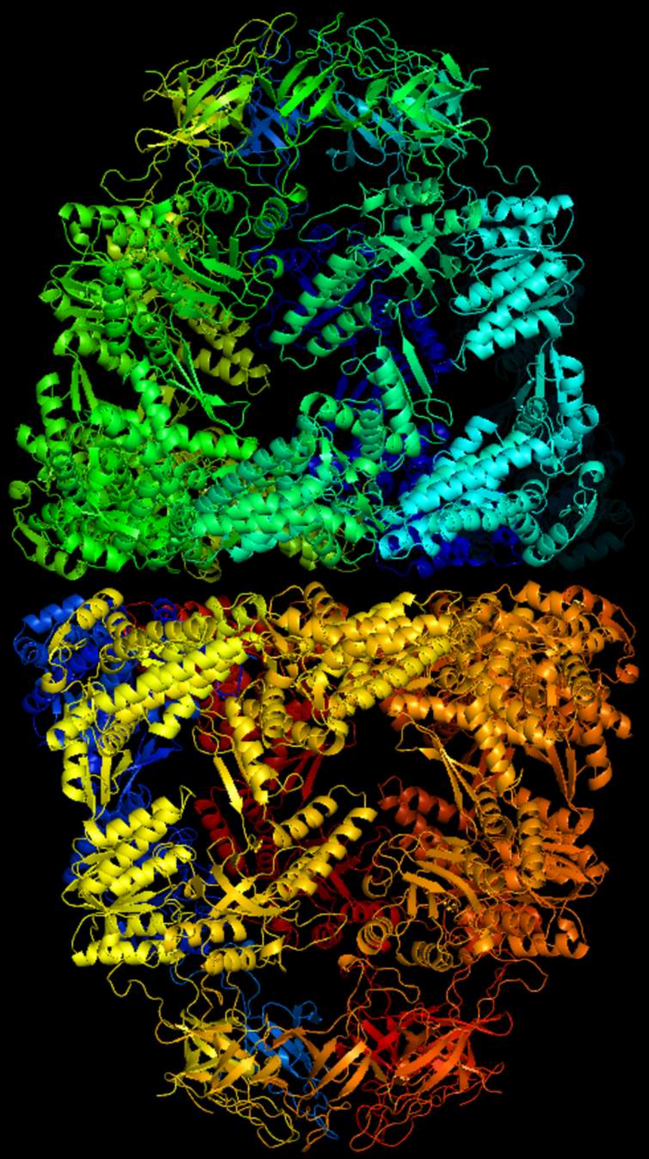


HUMAN mtHSP60

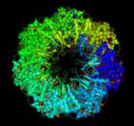
Mitochondria

Aid in protein folding
Regulation oxidative and free radical stress





PDB ID: 9SHG
Resolution: 1.91 Å
ELECTRON MICROSCOPY



GroEL vs mtHSP60

Query= Hsp60

Length=527

Sequences producing significant alignments:	Score (Bits)	E Value
sp|P10809|CH60_HUMAN 60 kDa heat shock protein, mitochondrial OS=...	1036	0.0
sp|Q5NVM5|CH60_PONAB 60 kDa heat shock protein, mitochondrial OS=...	1026	0.0
sp|P31081|CH60_BOVIN 60 kDa heat shock protein, mitochondrial OS=...	1020	0.0
sp|P63039|CH60_RAT 60 kDa heat shock protein, mitochondrial OS=Ra...	1015	0.0
sp|P63038|CH60_MOUSE 60 kDa heat shock protein, mitochondrial OS=...	1015	0.0
sp|P18687|CH60_CRIGR 60 kDa heat shock protein, mitochondrial OS=...	1011	0.0
sp|Q5ZL72|CH60_CHICK 60 kDa heat shock protein, mitochondrial OS=...	1000	0.0
sp|Q02649|CH60_DROME 60 kDa heat shock protein, mitochondrial OS=...	815	0.0
sp|Q19NJ4|CH602_ECOK1 60 kDa chaperonin 2 OS=Escherichia coli O1:...	554	0.0
sp|A9M0Q6|CH60_NEIM0 60 kDa chaperonin OS=Neisseria meningitidis ...	553	0.0
sp|Q2N5R9|CH602_ERYLH 60 kDa chaperonin 2 OS=Erythrobacter litora...	553	0.0
sp|Q8PPZ1|CH60_XANAC 60 kDa chaperonin OS=Xanthomonas axonopodis ...	553	0.0
sp|A1KW52|CH60_NEIMF 60 kDa chaperonin OS=Neisseria meningitidis ...	553	0.0
sp|P42385|CH60_NEIMB 60 kDa chaperonin OS=Neisseria meningitidis ...	553	0.0
sp|P57006|CH60_NEIMA 60 kDa chaperonin OS=Neisseria meningitidis ...	553	0.0
sp|A5EX17|CH60_DICNV 60 kDa chaperonin OS=Dichelobacter nodosus (...	553	0.0
sp|Q930Y0|CH603_RHIME 60 kDa chaperonin 3 OS=Rhizobium meliloti (...	553	0.0
sp|Q8RIT7|CH60_XANCH 60 kDa chaperonin OS=Xanthomonas campestris ...	553	0.0
sp|A4JPD1|CH602_BURVG 60 kDa chaperonin 2 OS=Burkholderia vietnam...	553	0.0
sp|Q1GVZ9|CH601_SPHAL 60 kDa chaperonin 1 OS=Sphingopyxis alasken...	553	0.0
sp|C0RKD5|CH60_BRUMB 60 kDa chaperonin OS=Brucella melitensis bio...	552	0.0

E-value: 0.0

GroEL vs mtHSP60

Sc

S = 100%

	No.	Domain1	Domain2	Sc	RMS	Len1	Len2	Align	NFit	Eq.	Secs	%I	%S	P(m)
Pair	1	hsp60	groel	3.69	1.35	527	525	706	282	272	0	47.43	100.00	7.33e-56

RMS < 2.0 A

I > 40%

GroEL vs mtHSP60



Apical domain different

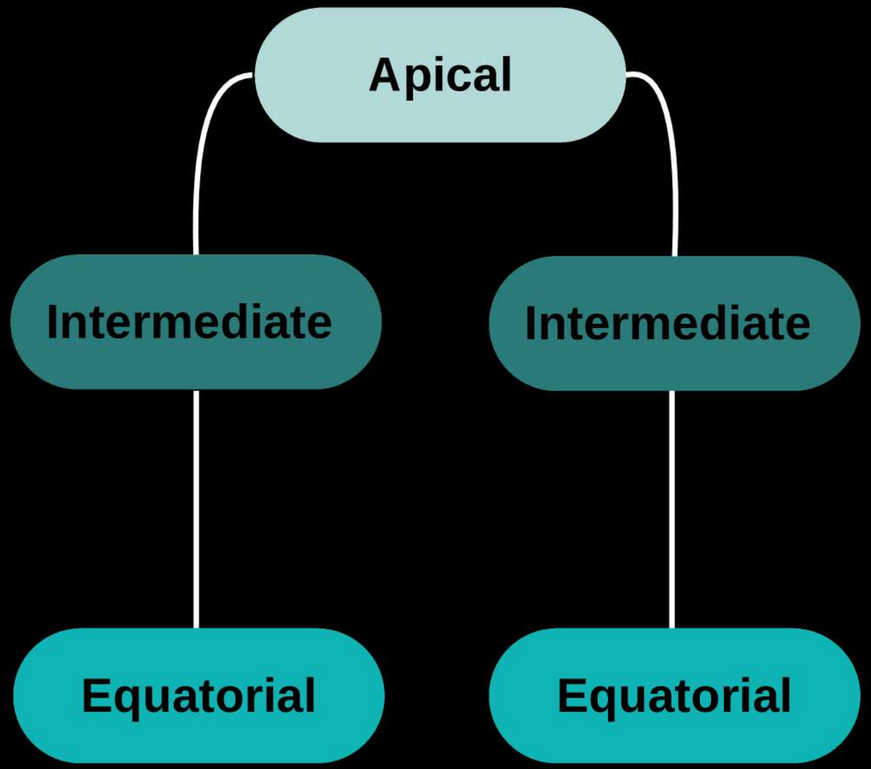
Substrate protein binding

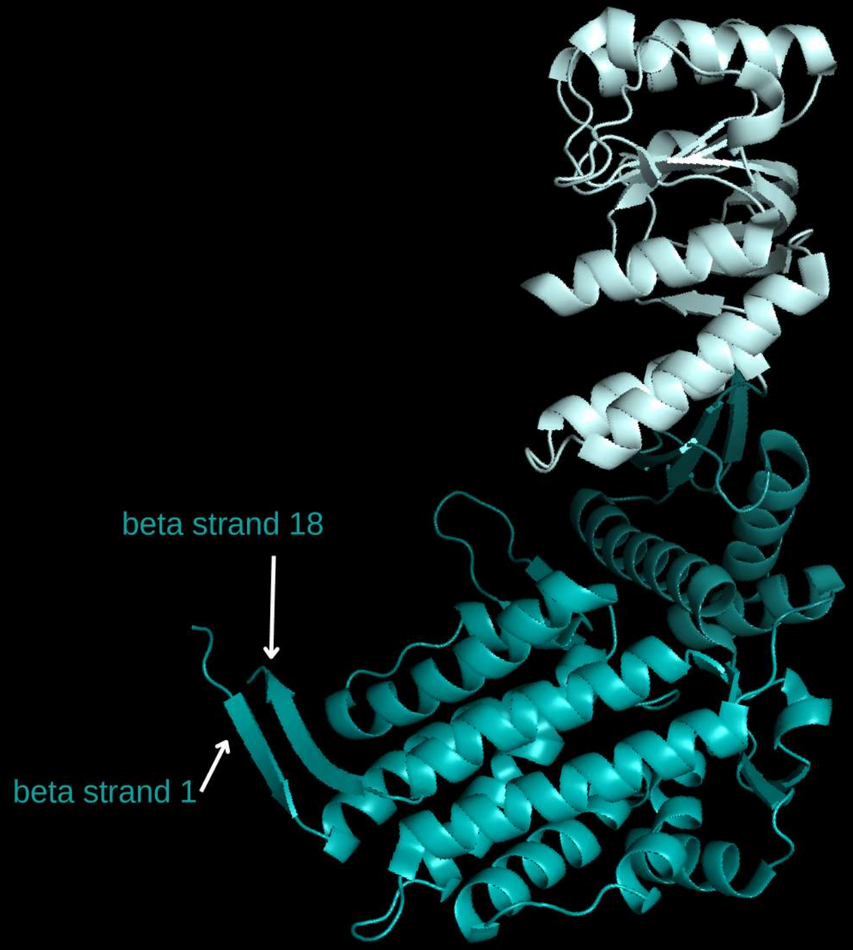
Intermediate and equatorial domains

mtHsp60 PDB ID: 9HGS
Resolution 1.91A

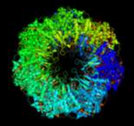
GroEL PDB ID: 1KP8
Resolution 2.0A

HSP60 PROTOMER





GSAKDVKFGADARALMLQGVDLLADAVAVTMGPKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDAVIA
ELKKQSKPVTTPEEIAQVATISANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVL
NRLKVGLQVVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKDDAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEK
KDRVTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIPK



HSP60 PROTOMER
SCOP

Equatorial Domain

Class: All alpha proteins

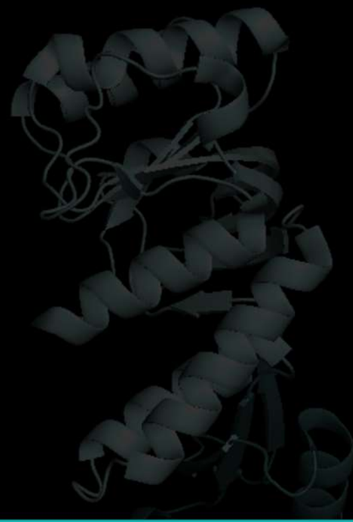
Fold: GroEL equatorial domain like

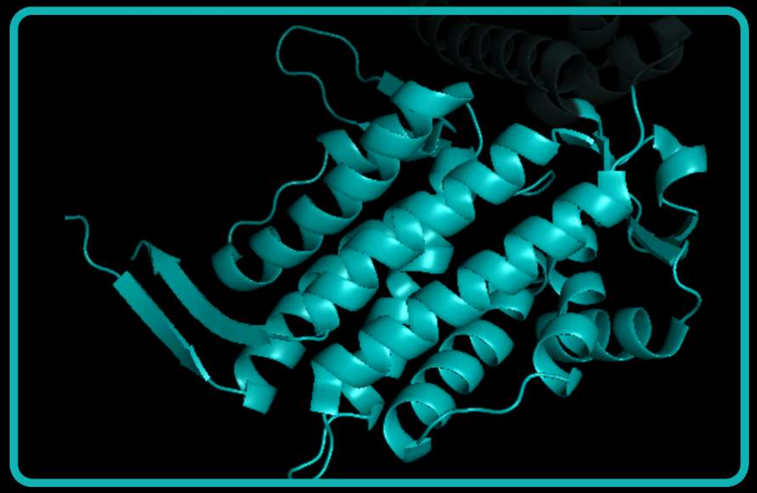
Superfamily: GroEL equatorial domain-like

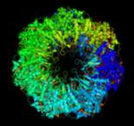
Family: GroEL chaperone, ATPase domain



2 subdomains
with 4 helices







HSP60 PROTOMER STRUCTURE

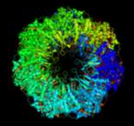
Equatorial Domain

1-137 and 411-526

- Most rigid
- ATP-binding pocket
- Stability of ring
- Inter and intra-ring contacts



GSAKDVKFGADARALMLQGVDLLADAVAVTMGPKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDAVIA
ELKKQSKPVTTPEEIAQVATISANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVL
NRLKVGLQVVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKDDAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEK
KDRVTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIPK



HSP60 PROTOMER
SCOP

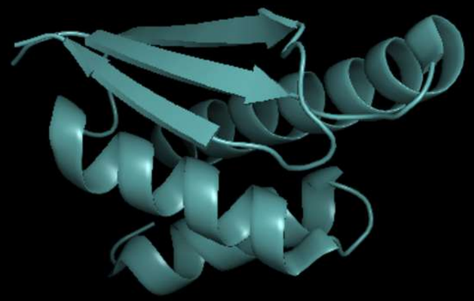
Intermediate Domain

Class: Alpha and beta proteins (a+b)

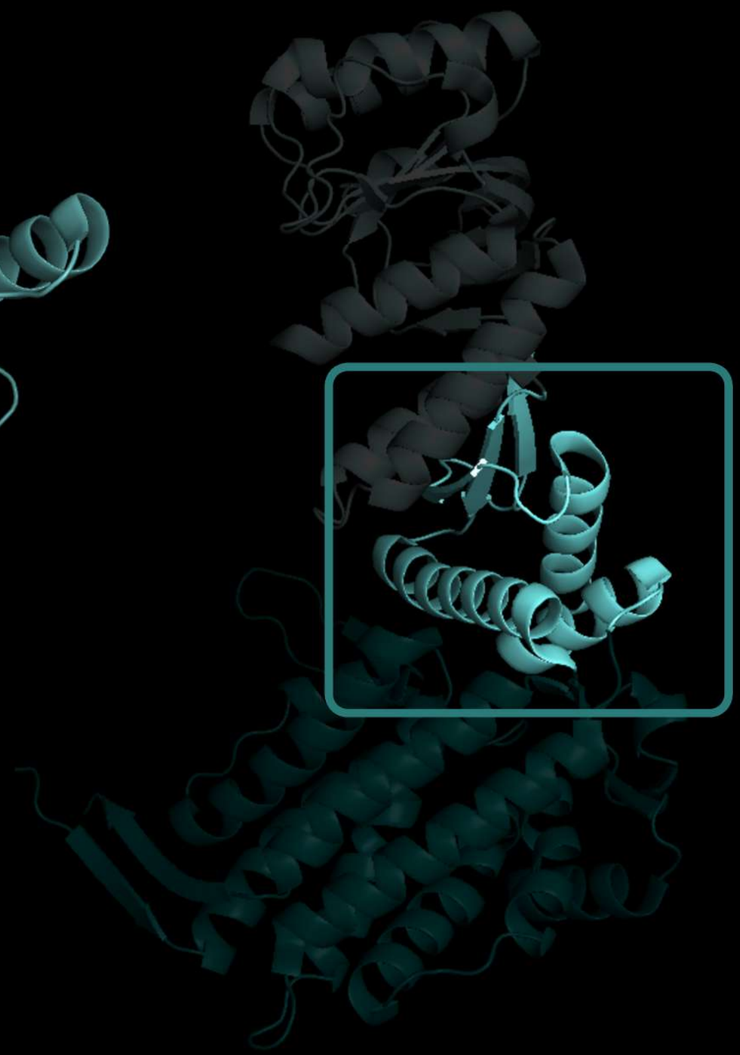
Fold: GroEL intermediate domain like

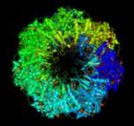
Superfamily: GroEL intermediate domain-like

Family: GroEL chaperones, intermediate domain



3 helices +
3 beta-strands





HSP60 PROTOMER STRUCTURE

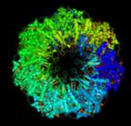
Intermediate Domain

138-191 and 375-411

- 2 flexible hinge regions
- Facilitate conformational change
- Flexible connector



GSAKDVKFGADARALMLQGVDLLADAVAVTMGPKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDAVIA
ELKKQSKPVTTPEEIAQVATISANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVL
NRLKVGLQVVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKDDAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEK
KDRVTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIPK



HSP60 PROTOMER
SCOP

Apical Domain

Class: Alpha and beta proteins (a/b)

Fold: The swivelling beta/beta/alpha domain

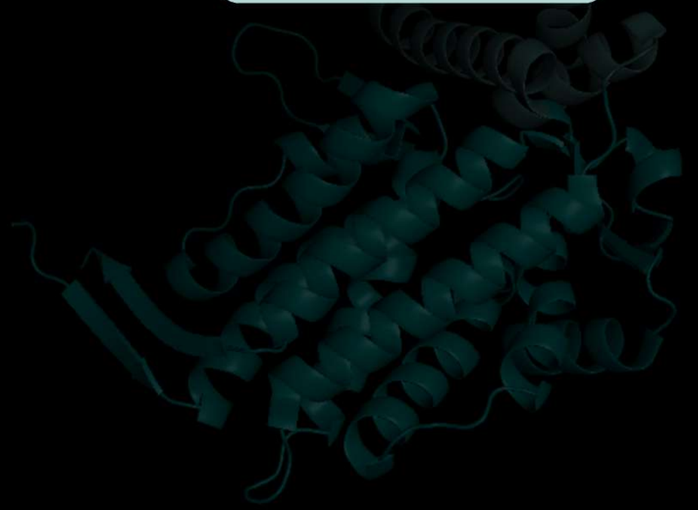
2 beta sheets ↑↑ ↓↑
and 1 helix layer

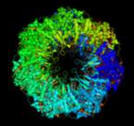
Superfamily: GroEL apical domain-like

Family: GroEL-like chaperone, apical domain









HSP60 PROTOMER STRUCTURE

Apical Domain

192-374

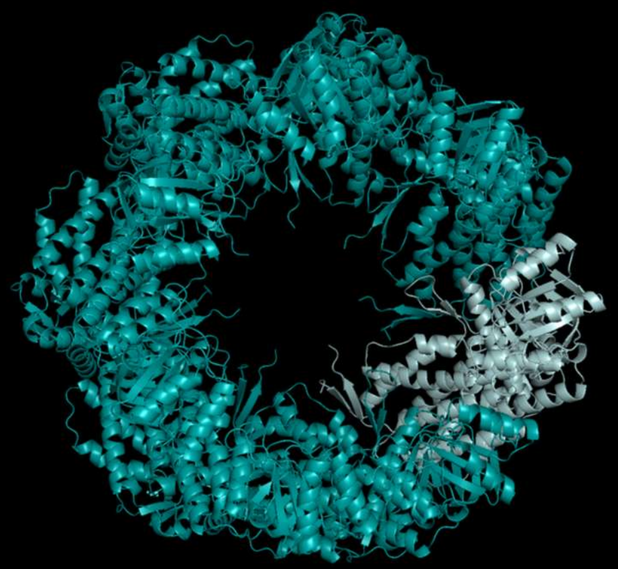
- Most flexible
- Contains binding sites



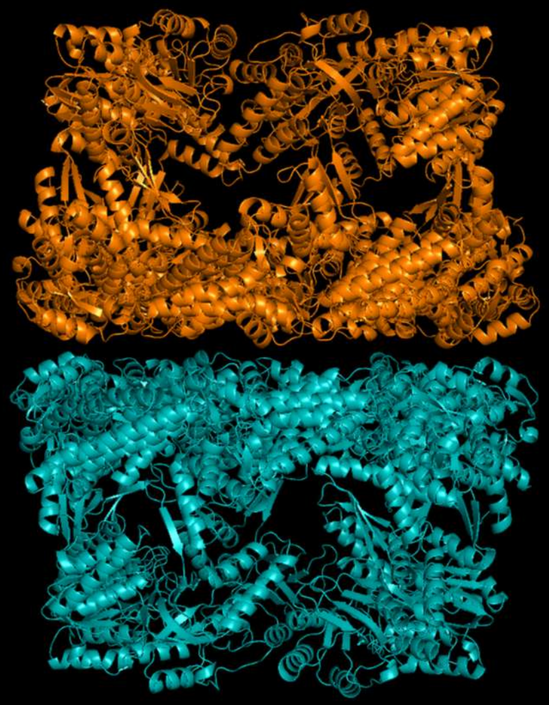


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ELKKQSKPVTTPEEIAQVATISANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKF**DRGYISPYFINTSKGQKCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVL**
NRLKVGLQVVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKDDAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEK
KDRVTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTEIPK

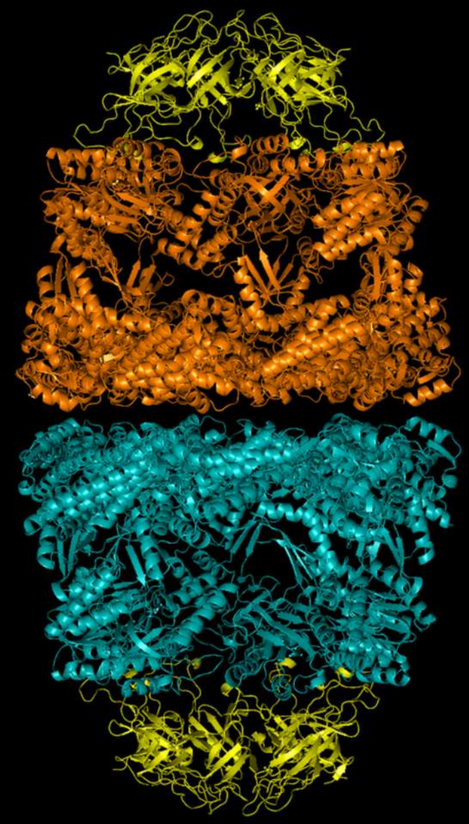
DYNAMIC ASSEMBLY



Heptamer
=
Single ring



Tetradecamer
=
Double ring

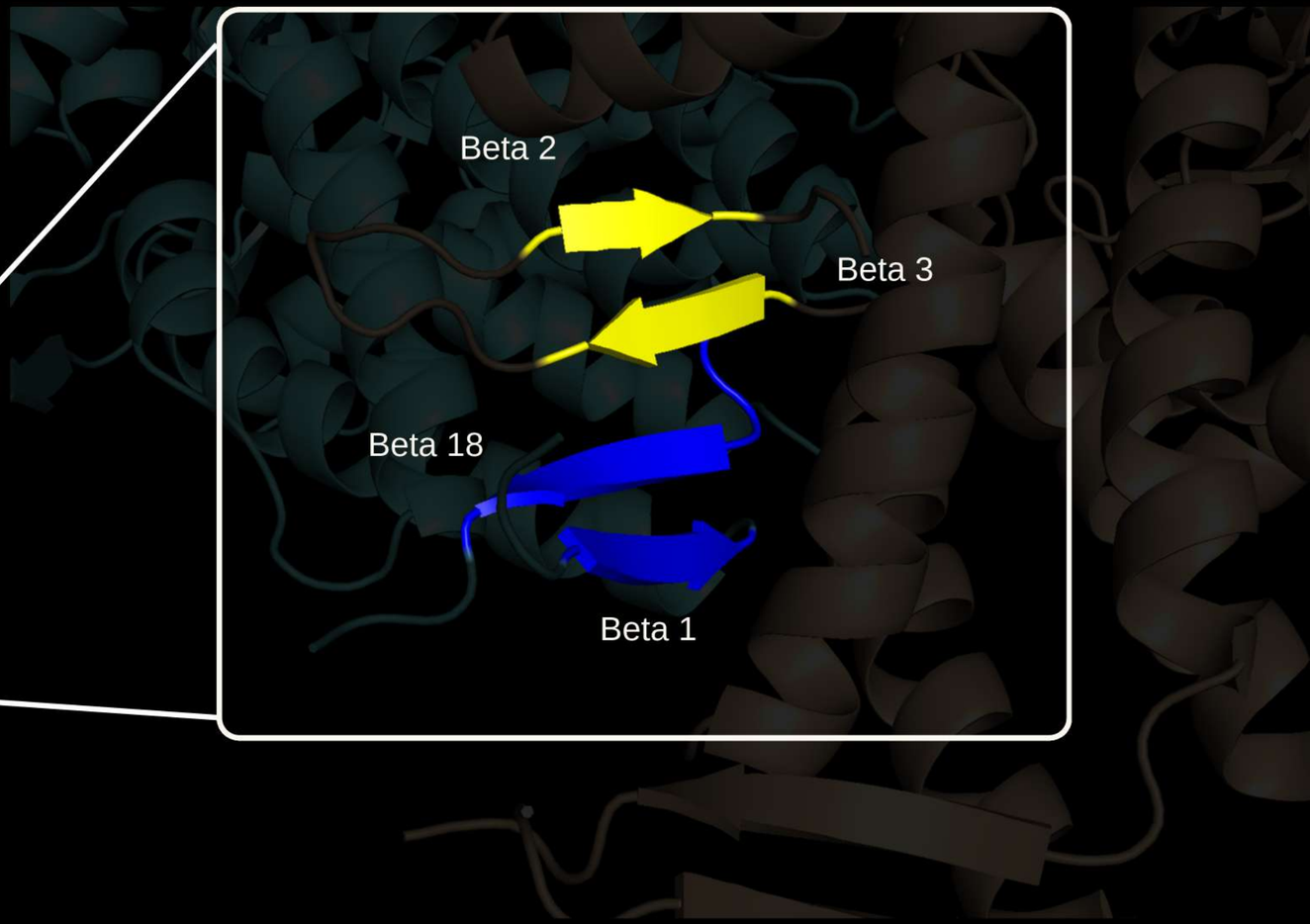


FOOTBALL COMPLEX

Hsp10

INTRA RING INTERACTIONS

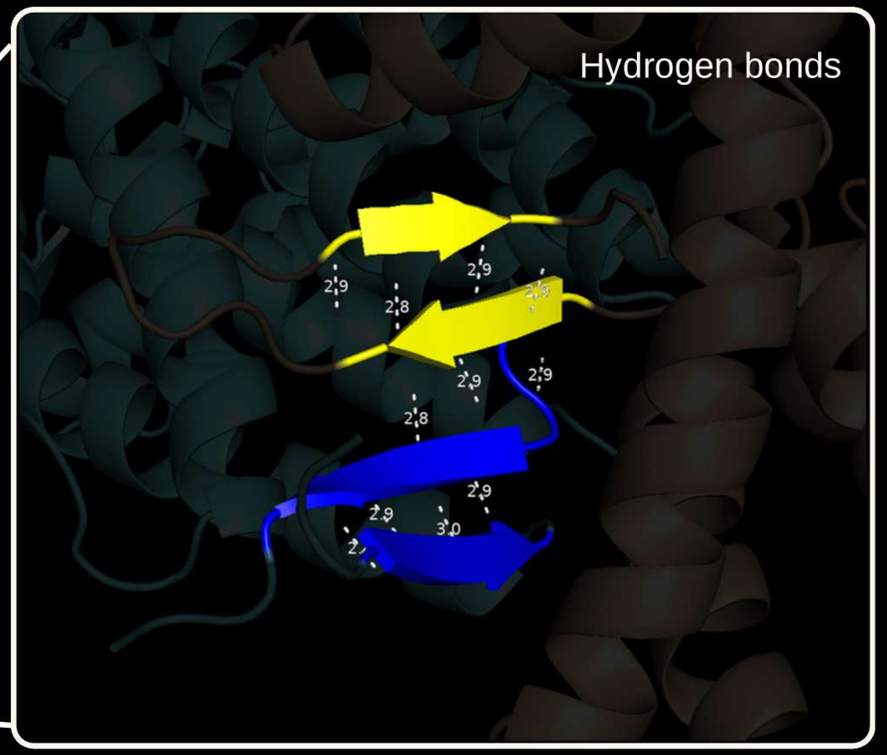




Equatorial

INTRA RING INTERACTIONS

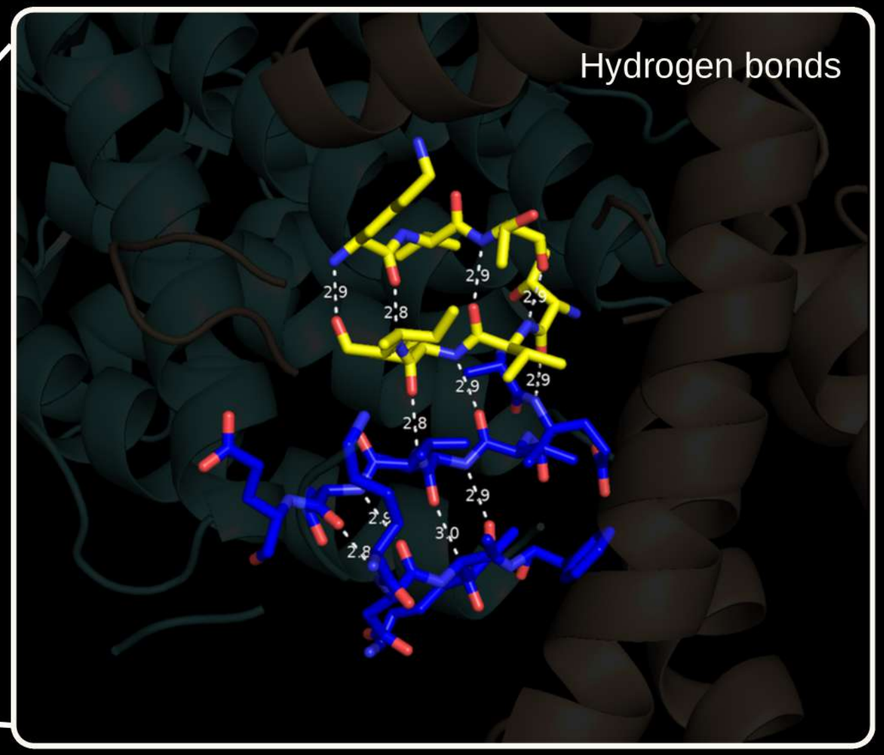




Equatorial

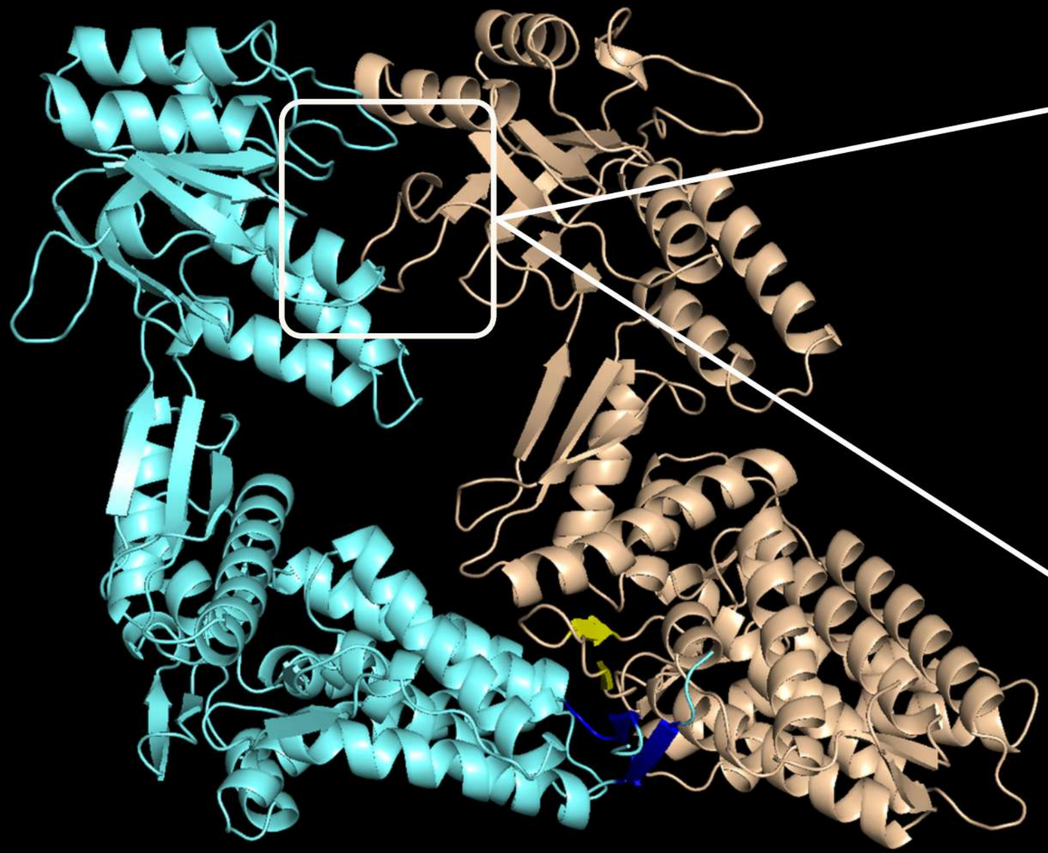
INTRA RING INTERACTIONS





Equatorial

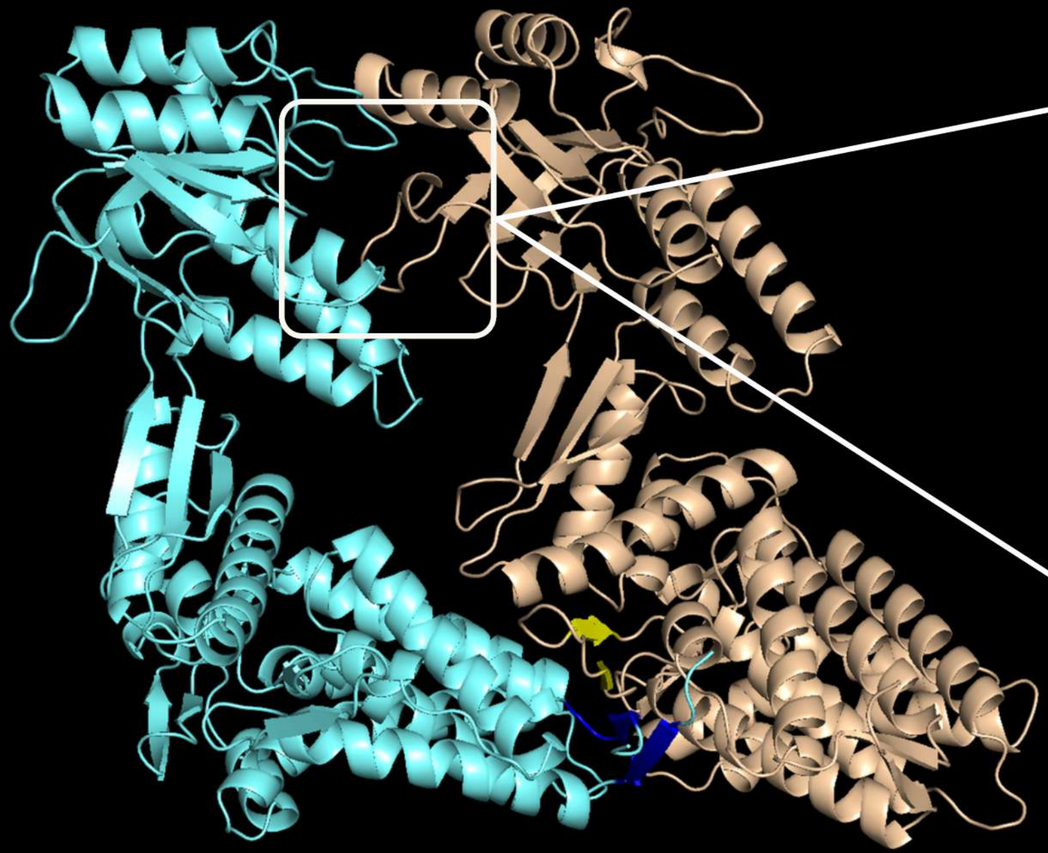
INTRA RING INTERACTIONS

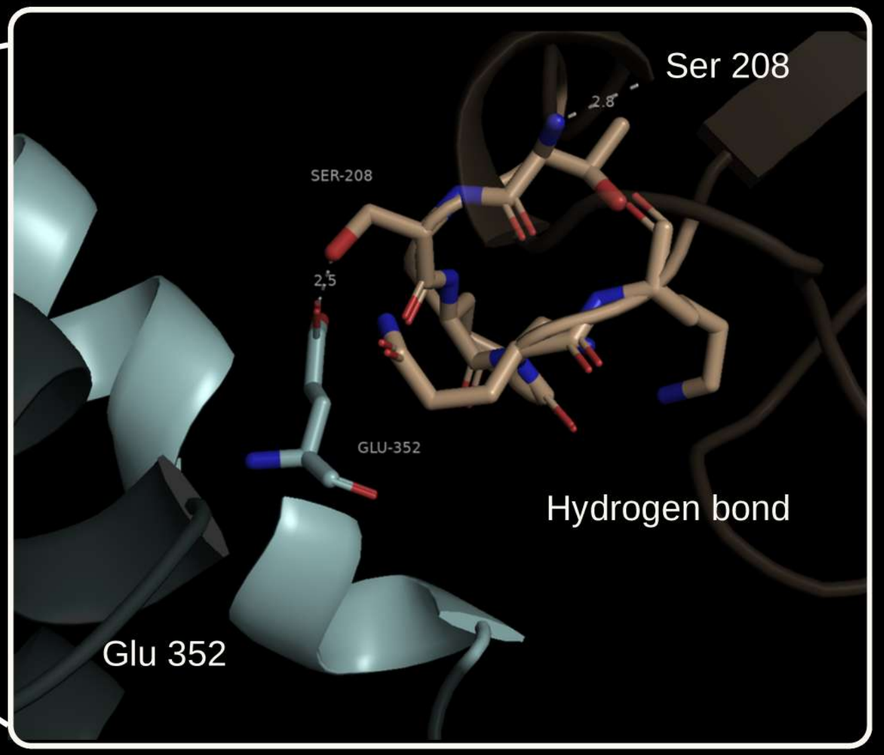




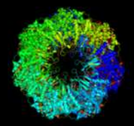
Apical

INTRA RING INTERACTIONS





Apical



EVOLUTION

```
Query= Hsp60

Length=527

                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

sp|P10809|CH60_HUMAN 60 kDa heat shock protein, mitochondrial OS=...  1036    0.0
sp|Q5NVM5|CH60_PONAB 60 kDa heat shock protein, mitochondrial OS=...  1026    0.0
sp|P31081|CH60_BOVIN 60 kDa heat shock protein, mitochondrial OS=...  1020    0.0
sp|P63039|CH60_RAT 60 kDa heat shock protein, mitochondrial OS=Ra...  1015    0.0
sp|P63038|CH60_MOUSE 60 kDa heat shock protein, mitochondrial OS=...  1015    0.0
sp|P18687|CH60_CRIGR 60 kDa heat shock protein, mitochondrial OS=...  1011    0.0
sp|Q5ZL72|CH60_CHICK 60 kDa heat shock protein, mitochondrial OS=...  1000    0.0
sp|O02649|CH60_DROME 60 kDa heat shock protein, mitochondrial OS=...  815     0.0
sp|Q9VMN5|CH60C_DROME 60 kDa heat shock protein homolog 2, mitoch...  793     0.0
sp|P50140|CH60_CAEEL Chaperonin homolog Hsp-60, mitochondrial OS=...  761     0.0
sp|P25420|CH63_HELVI 63 kDa chaperonin, mitochondrial OS=Heliothi...  712     0.0
sp|P29185|CH61_MAIZE Chaperonin CPN60-1, mitochondrial OS=Zea may...  642     0.0
sp|Q43298|CH62_MAIZE Chaperonin CPN60-2, mitochondrial OS=Zea may...  639     0.0
sp|Q05046|CH62_CUCMA Chaperonin CPN60-2, mitochondrial OS=Cucurbi...  634     0.0
sp|P29197|CH60A_ARATH Chaperonin CPN60, mitochondrial OS=Arabidop...  634     0.0
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sp|O60008|HSP60_PARBA Heat shock protein 60, mitochondrial OS=Par...  608     0.0
sp|Q5B041|HSP60_EMENI Heat shock protein 60 OS=Emericella nidulan...  605     0.0
sp|Q09864|HSP60_SCHPO Heat shock protein 60, mitochondrial OS=Sch...  603     0.0
sp|O74261|HSP60_CANAL Heat shock protein 60, mitochondrial OS=Can...  600     0.0
sp|Q3SQJ5|CH602_NITWN 60 kDa chaperonin 2 OS=Nitrobacter winograd...  600     0.0
sp|A5EM76|CH604_BRASB 60 kDa chaperonin 4 OS=Bradyrhizobium sp. (...  599     0.0
sp|A4YS25|CH602_BRASO 60 kDa chaperonin 2 OS=Bradyrhizobium sp. (...  598     0.0

sp|Q9XCA9|CH60_RHOMR 60 kDa chaperonin OS=Rhodothermus marinus GN...  554     0.0
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sp|Q0KDR7|CH60_CUPNH 60 kDa chaperonin OS=Cupriavidus necator (st...  554     0.0
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sp|Q2N5R9|CH602_ERYLH 60 kDa chaperonin 2 OS=Erythrobacter litora...  553     0.0
```

Blastp

Mammals

Bird

Arthropoda

Nematoda

Plants

Yeast

Fungi

Bacteria

Vertebrates

Invertebrates

Rattus norvegicus (Rat)

Gallus gallus (Chicken)

Drosophila melanogaster (Fruit fly)

Caenorhabditis elegans

Cucurbita maxima (Pumpkin)

Arabidopsis thaliana (Mouse-ear cress)

Schizosaccharomyces pombe

Candida albicans

Escherichia coli

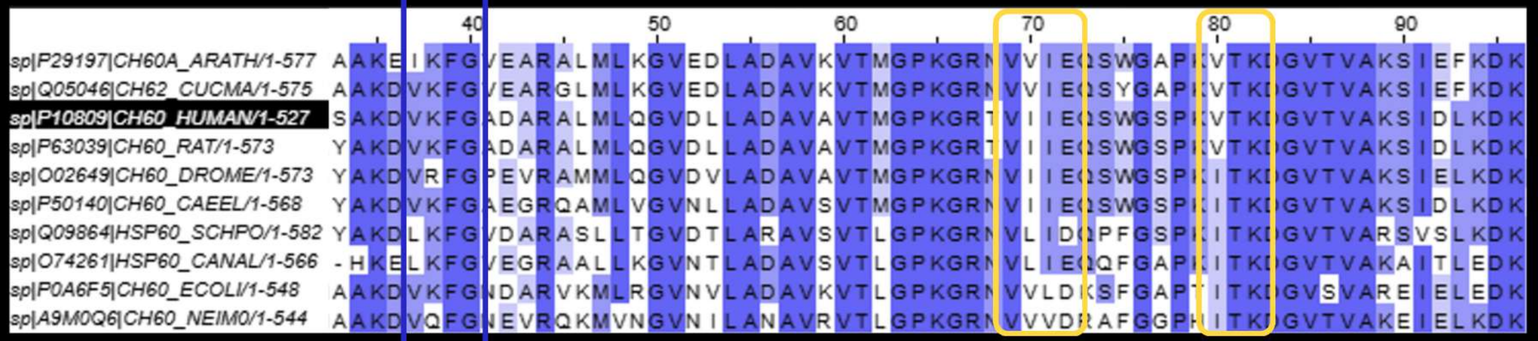
Neisseria meningitidis

INTRA RING INTERACTIONS

β 1

β 2

β 3

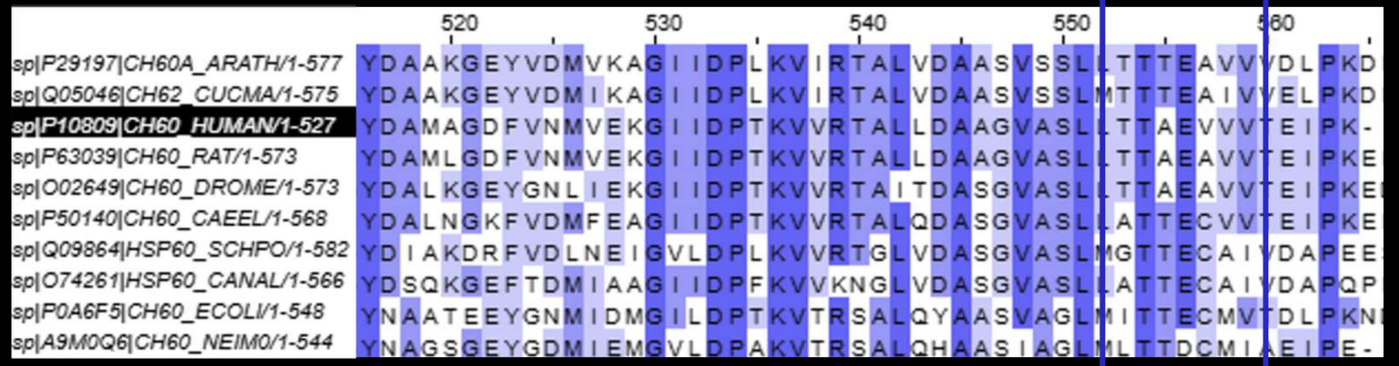


4 - 8

37 - 40

48 - 50

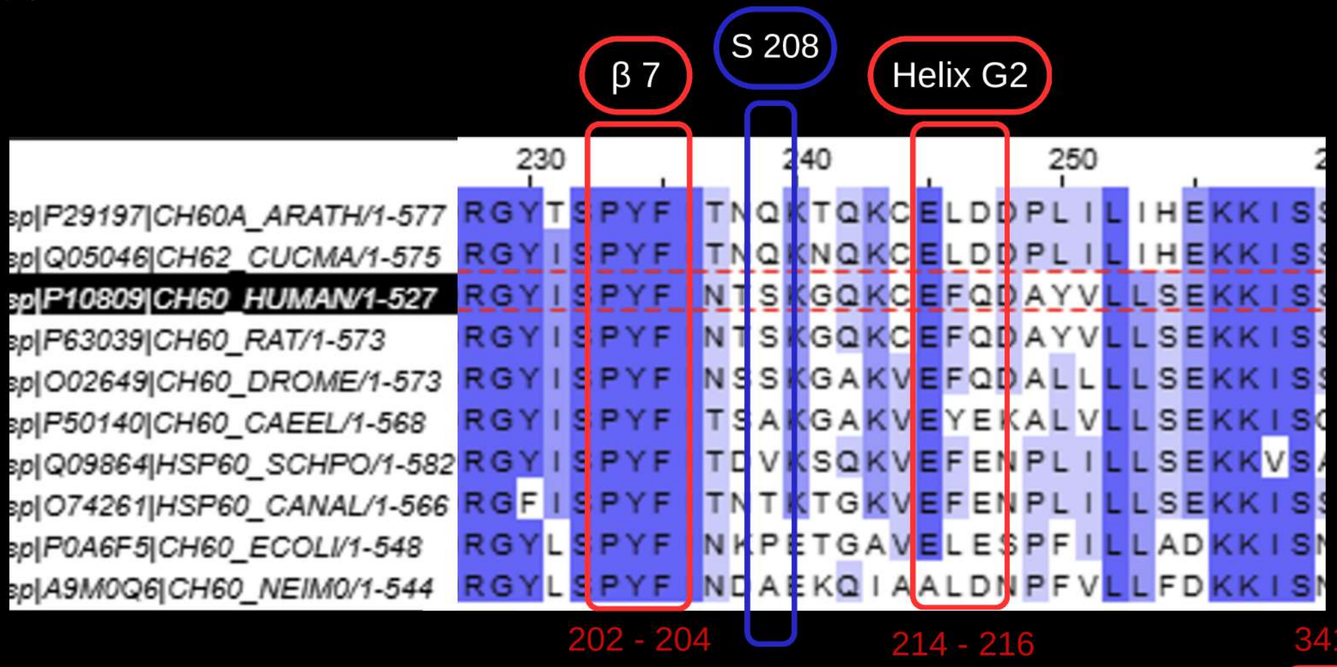
β 18



518 - 524

Equatorial

INTRA RING INTERACTIONS





Apical

STAMP SUPERIMPOSITION
ATP IMPORTANCE

apo mHsp60

ATP-bound mHsp60

RMSD: 1.62 Å



7AZP
3.50 Å
ELECTRON MICROSCOPY

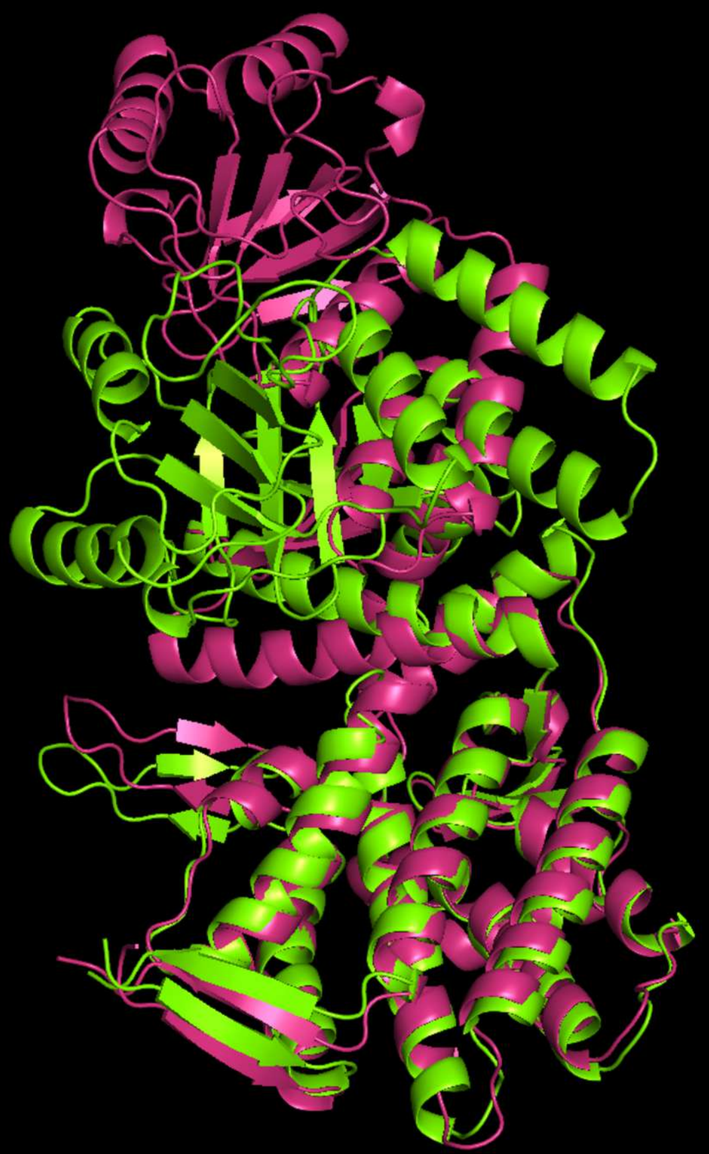
9SHG
1.91 Å
ELECTRON MICROSCOPY

- ATP bound
- APO

STAMP SUPERIMPOSITION

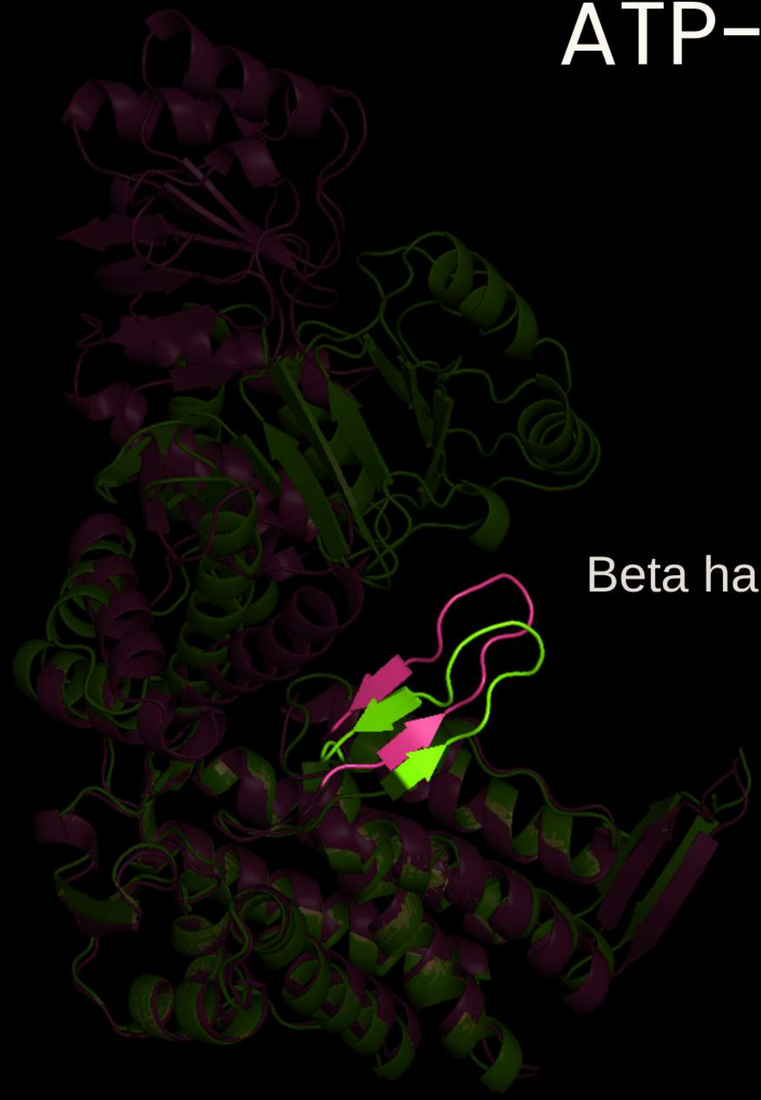
ATP-BOUND/APO



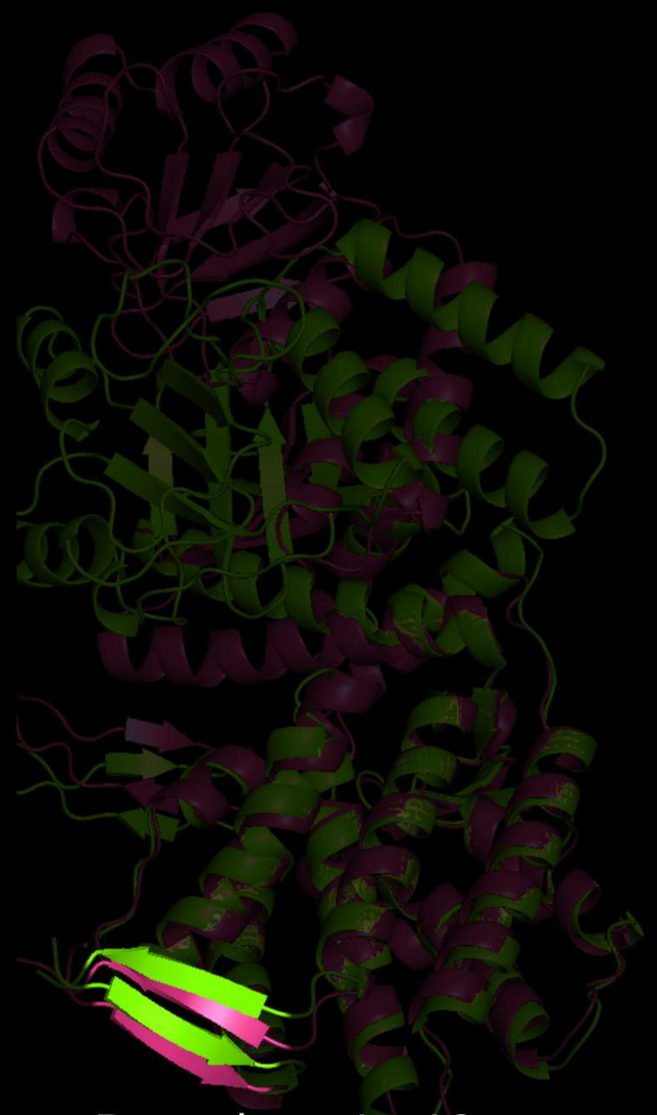


- ATP bound
- APO

STAMP SUPERIMPOSITION
ATP-BOUND/APO



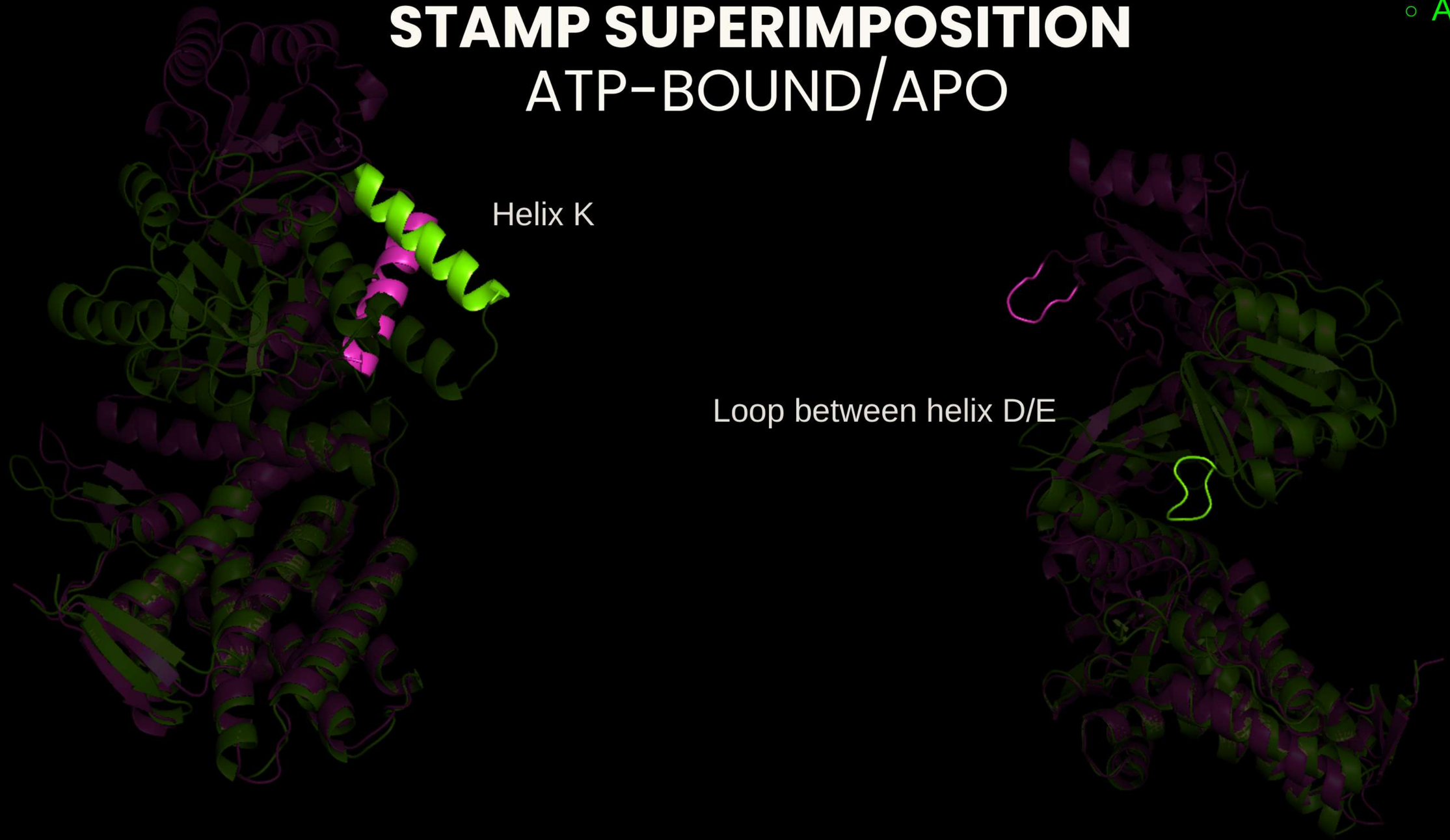
Beta hairpin



Beta sheet 1 - 18

- ATP bound
- APO

STAMP SUPERIMPOSITION
ATP-BOUND/APO

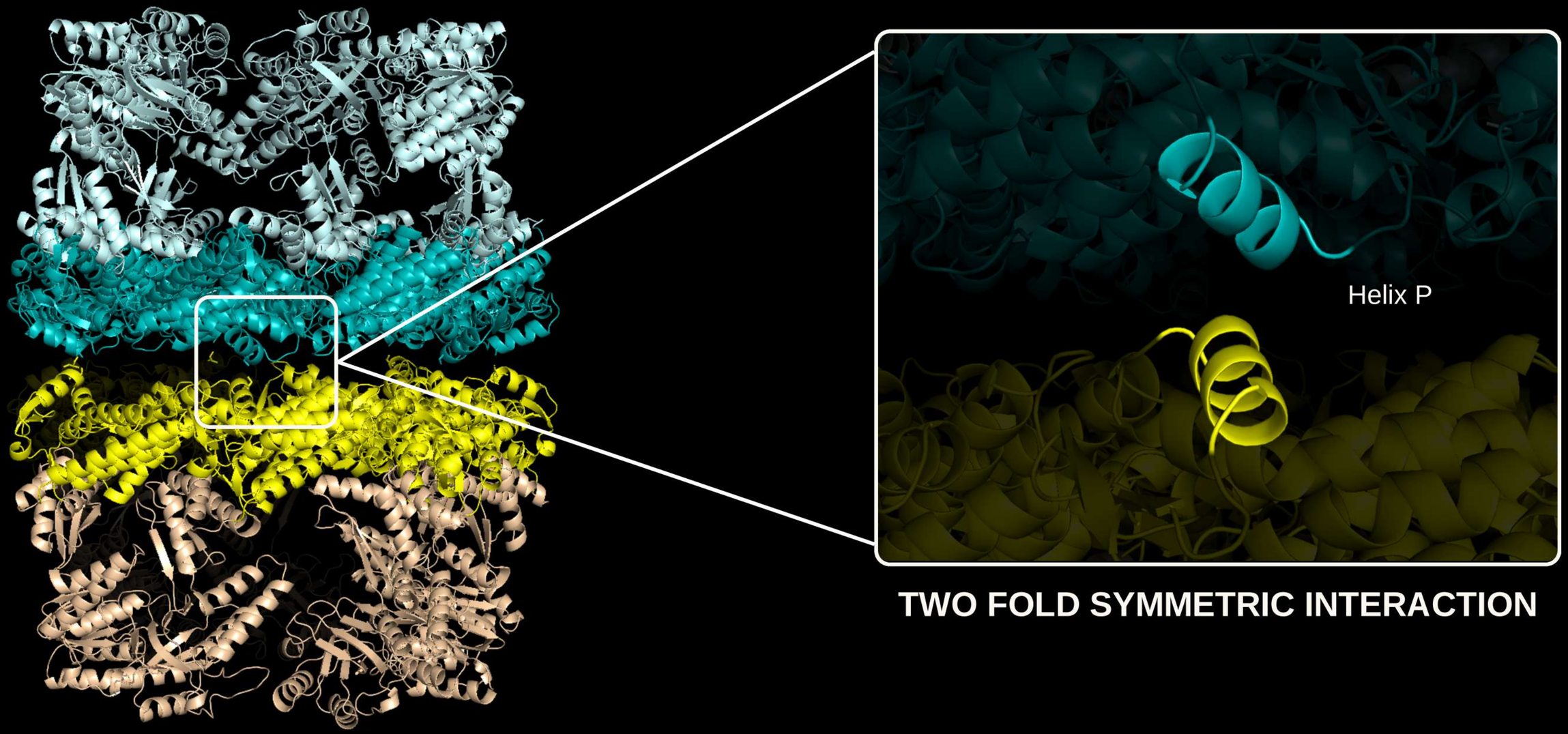


INTER RING INTERACTIONS



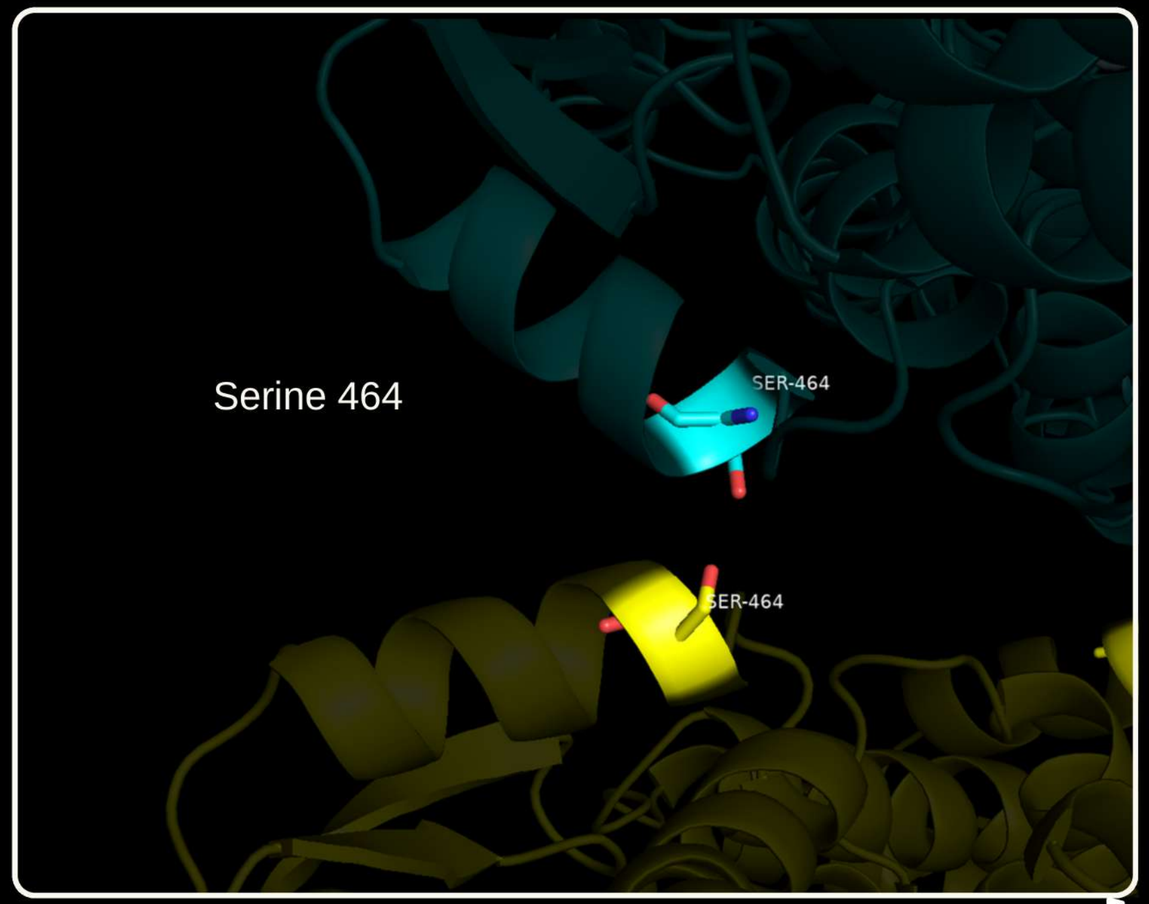
TWO FOLD SYMMETRIC INTERACTION

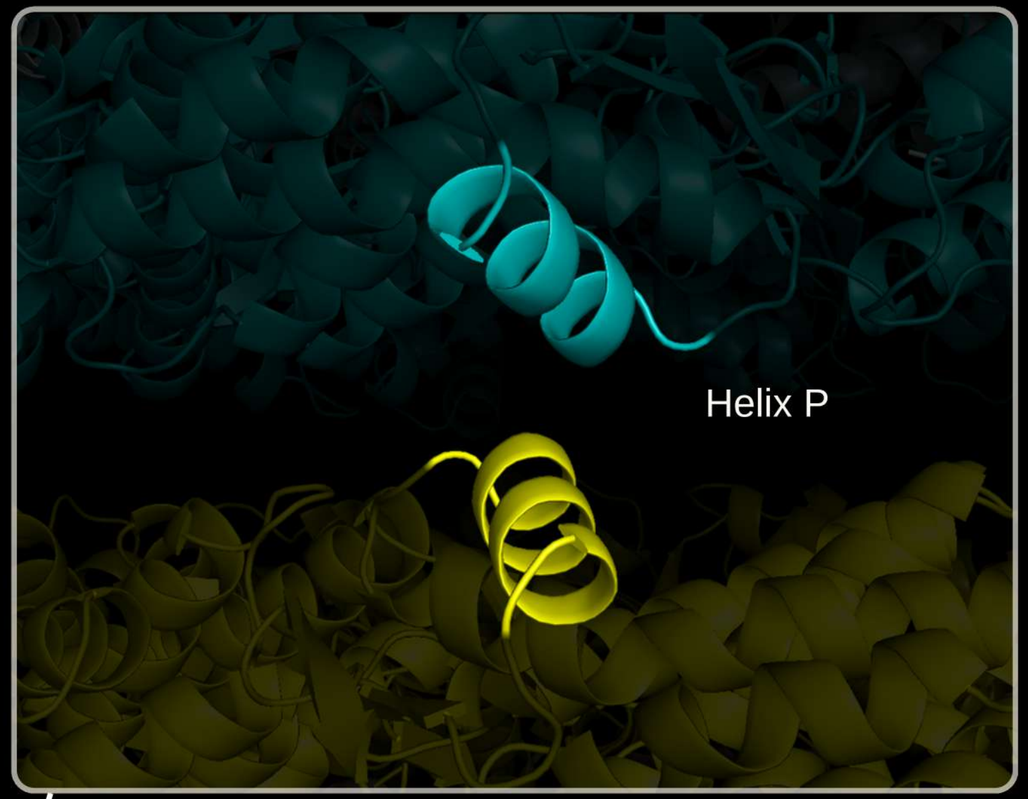
INTER RING INTERACTIONS



TWO FOLD SYMMETRIC INTERACTION

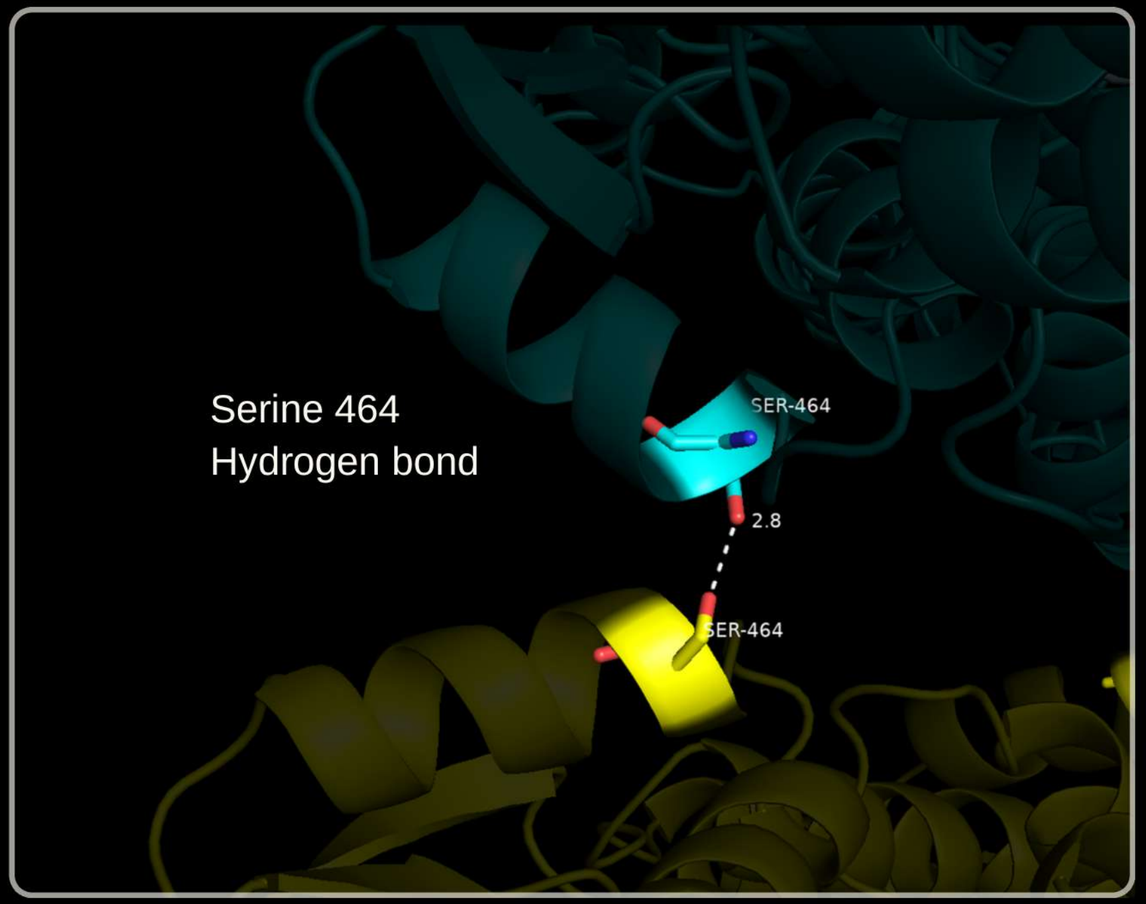
INTER RING INTERACTIONS

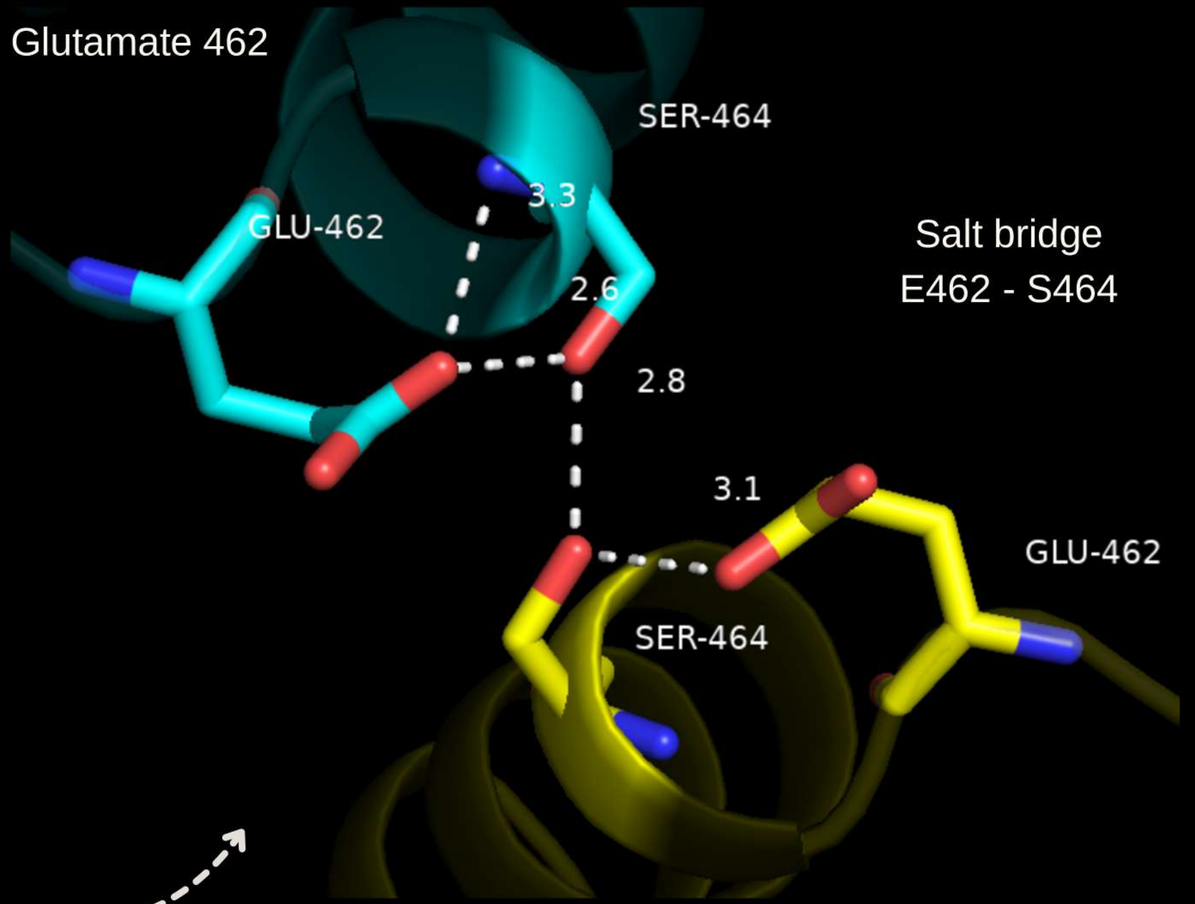




ROTATION

INTER RING INTERACTIONS

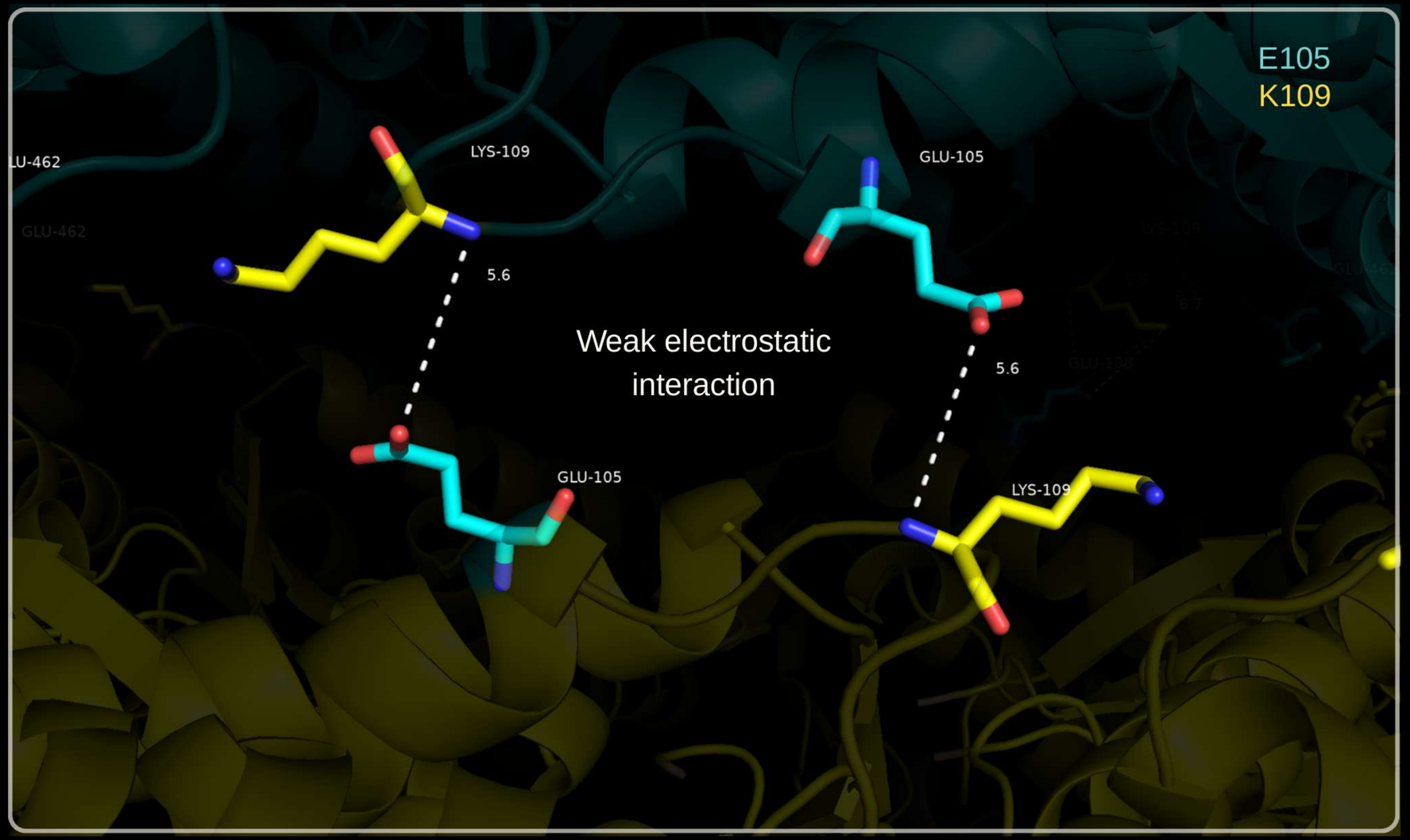


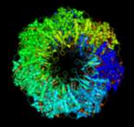


Salt bridge
E462 - S464

INTER RING INTERACTIONS

Loop between helix D/E

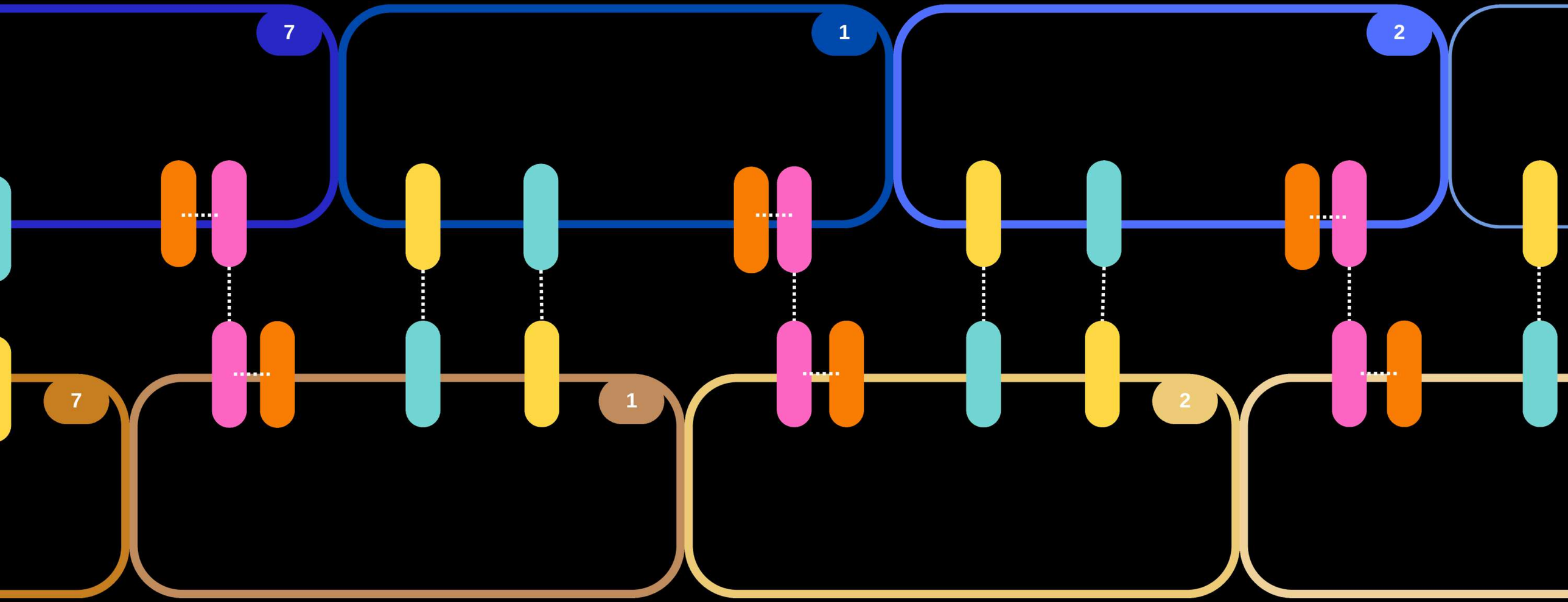




INTER RING INTERACTIONS

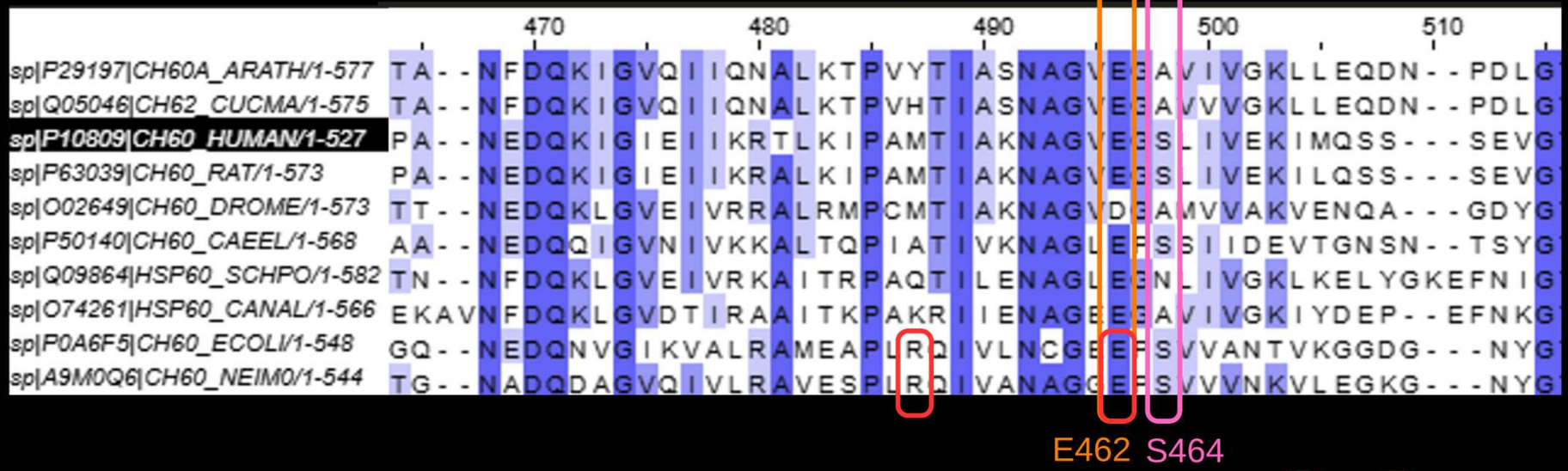
E462 E105 S464 K109

1ST RING

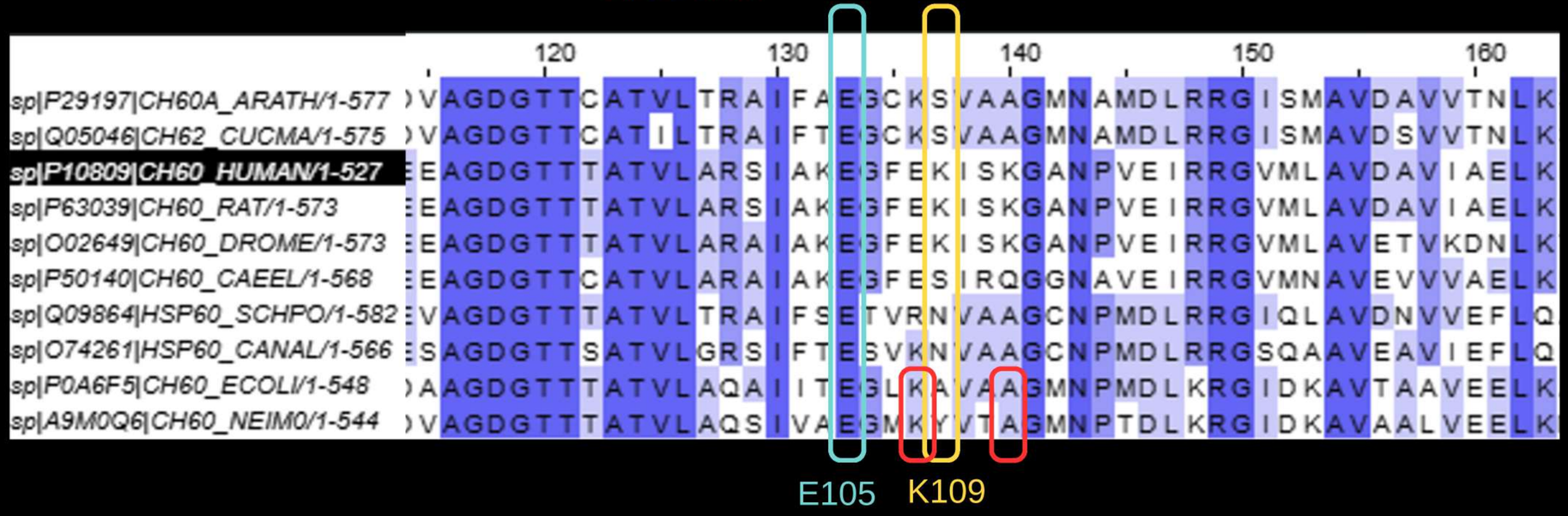


2ND RING

INTER RING INTERACTIONS



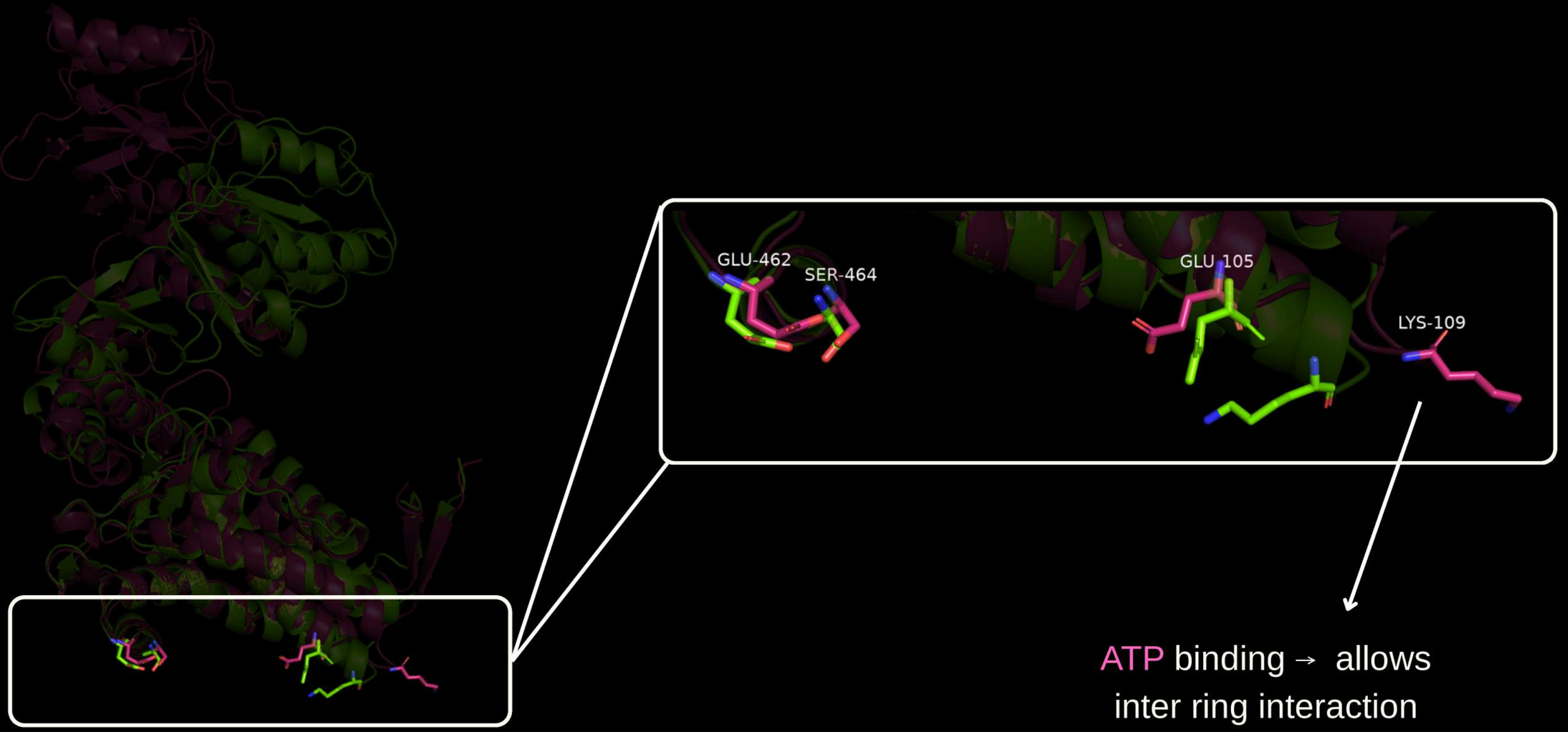
GroEL interactions



Equatorial

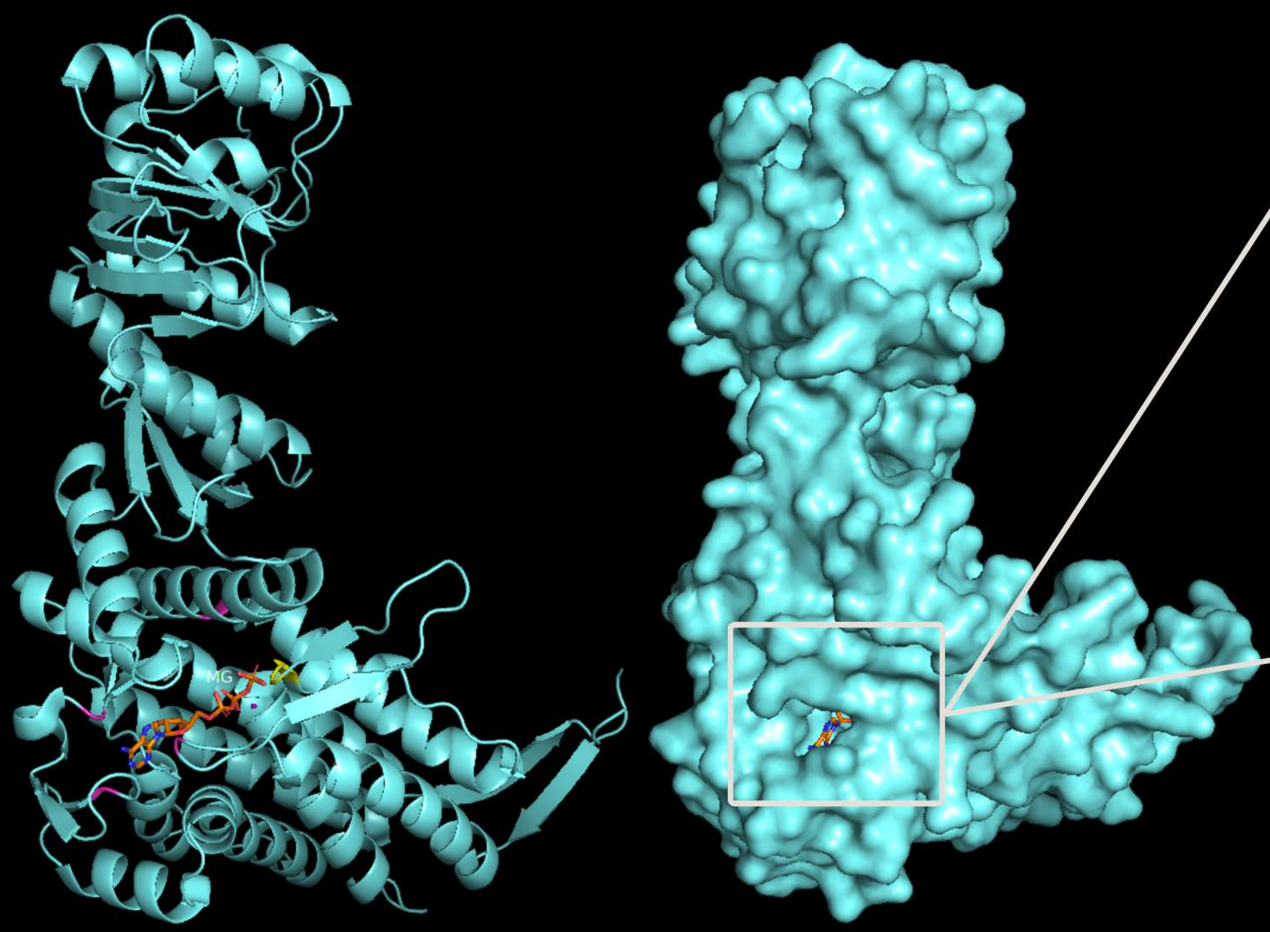
- ATP bound
- APO

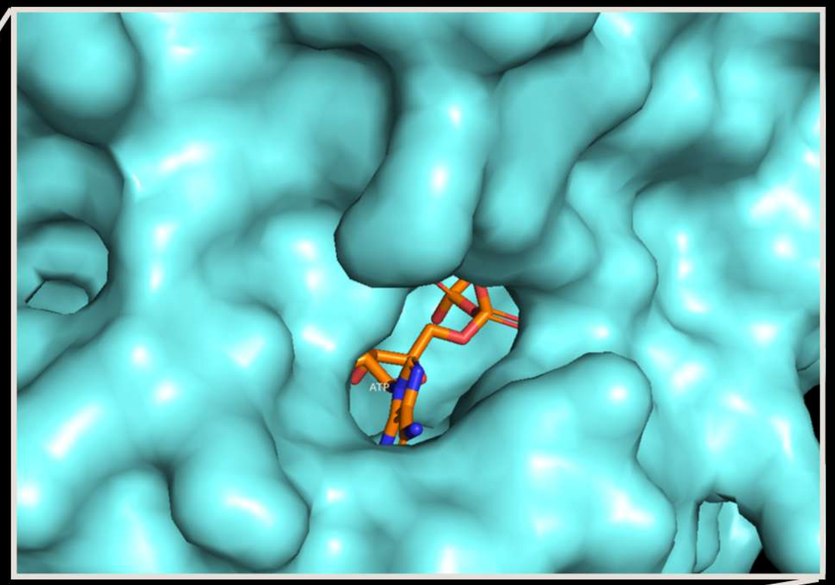
INTER RING INTERACTIONS



ATP binding → allows inter ring interaction

ATP BINDING





ATP BINDING POCKET

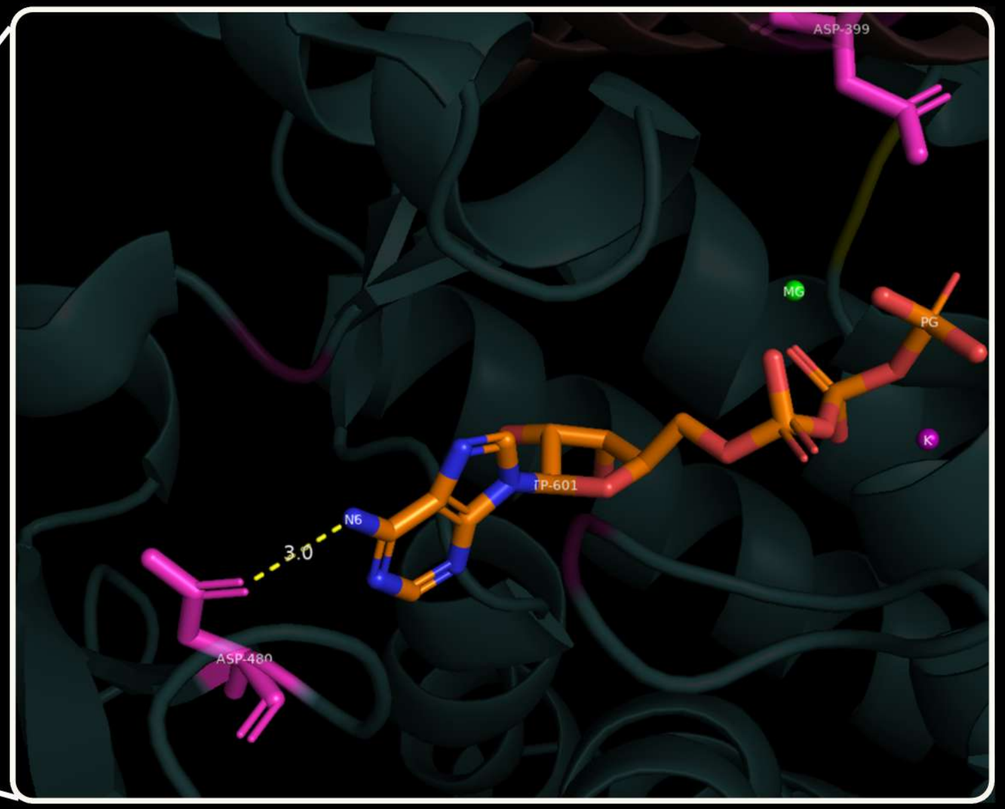
Equatorial

ATP BINDING

ATP STABILISATION

ATP adenine half N6 atom forms a hydrogen bond with **D480**

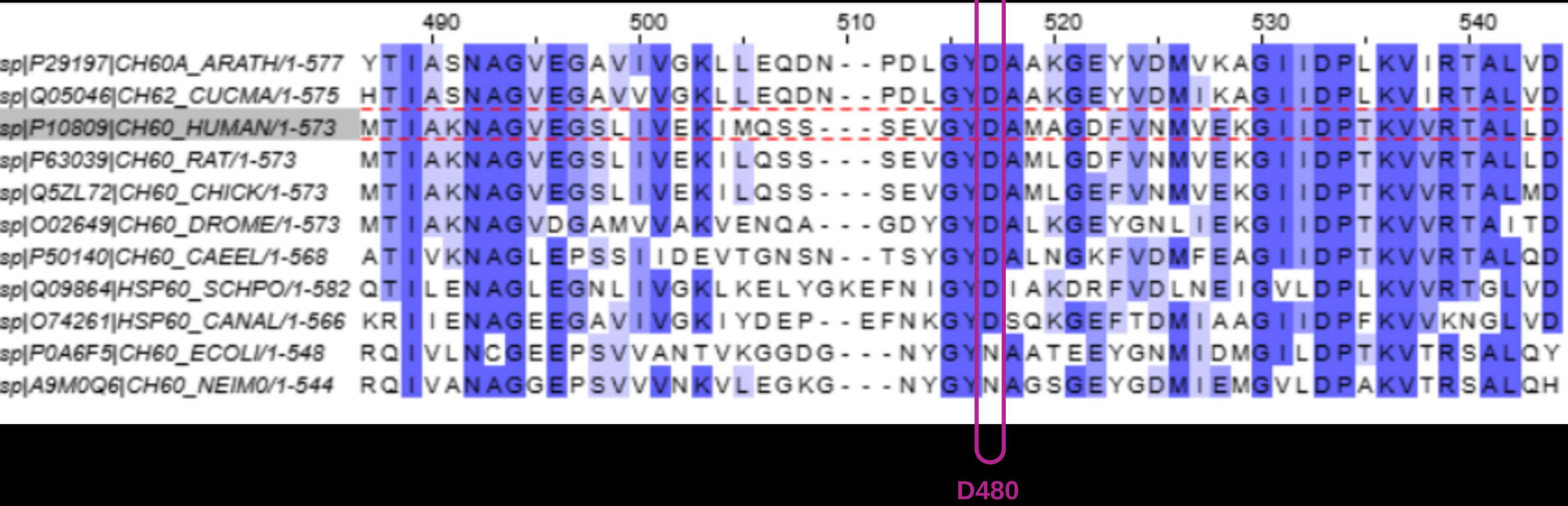




ATP BINDING

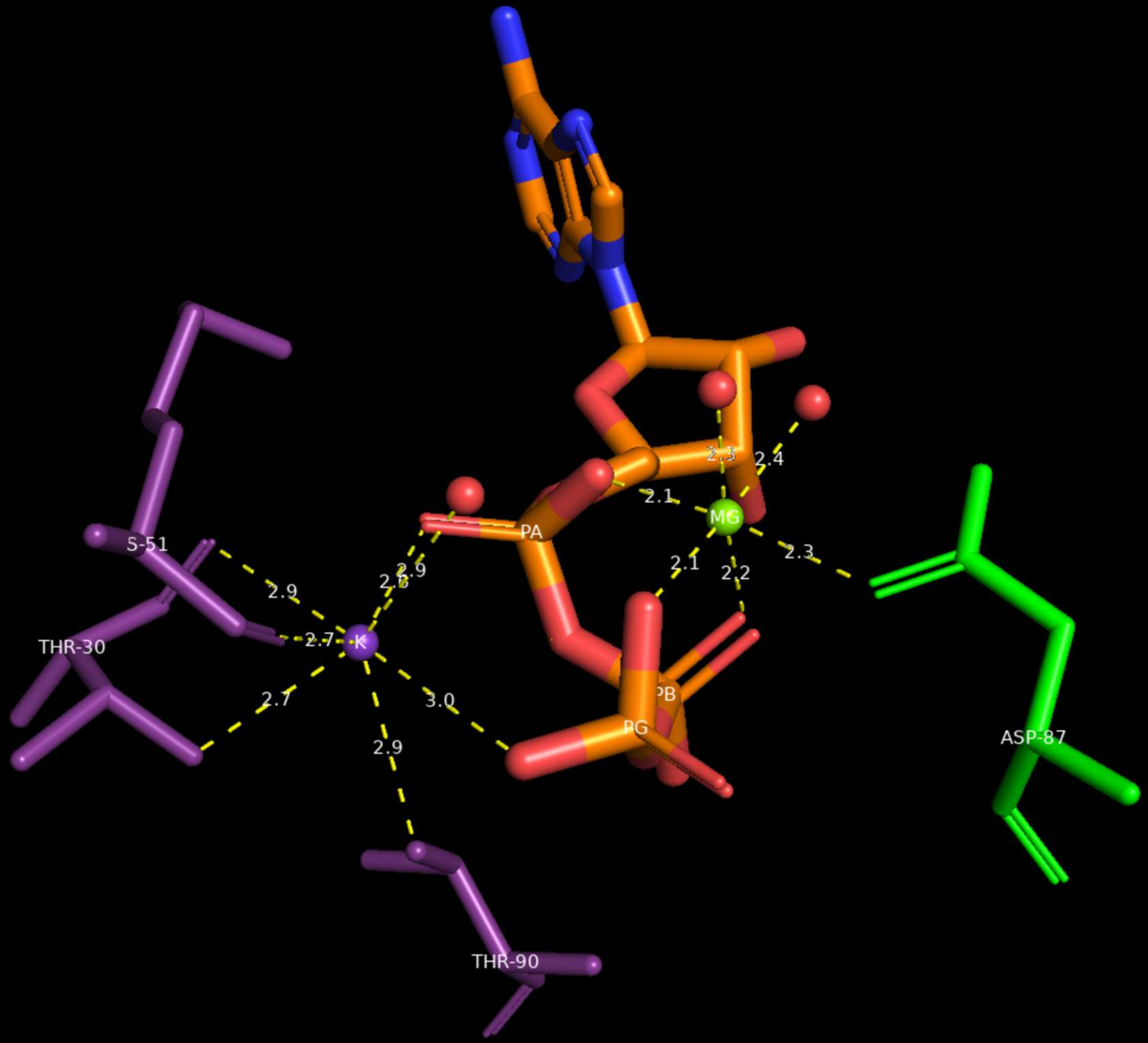
ATP STABILISATION

- Residues **conservation** involved in ATP stabilisation:



ATP BINDING

METAL IONS COORDINATION

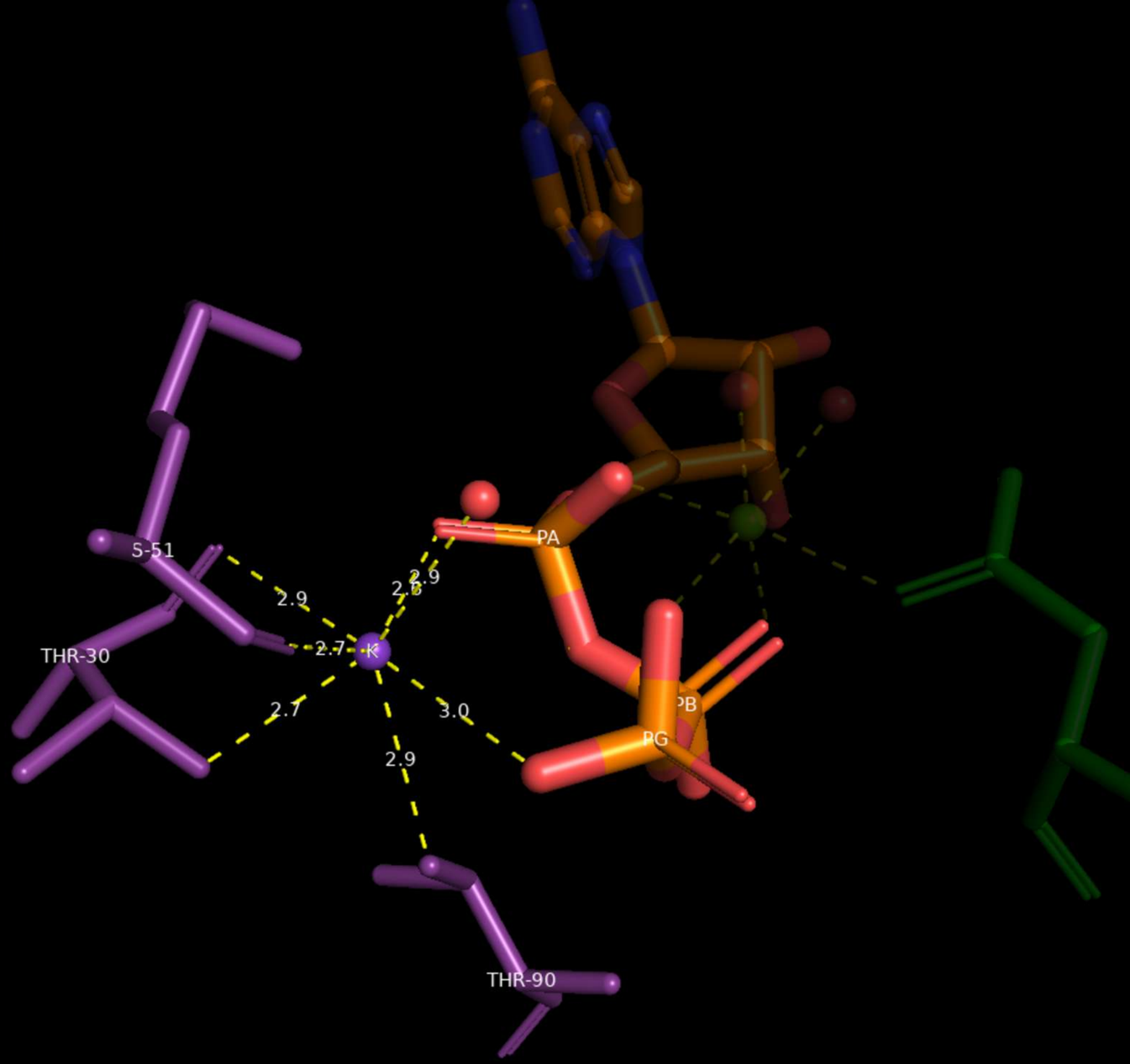


ATP BINDING

METAL IONS COORDINATION

K+

- **distorted polyhedral coordination**
- **K51** brought within coordination distance
- T30, T90, HOH

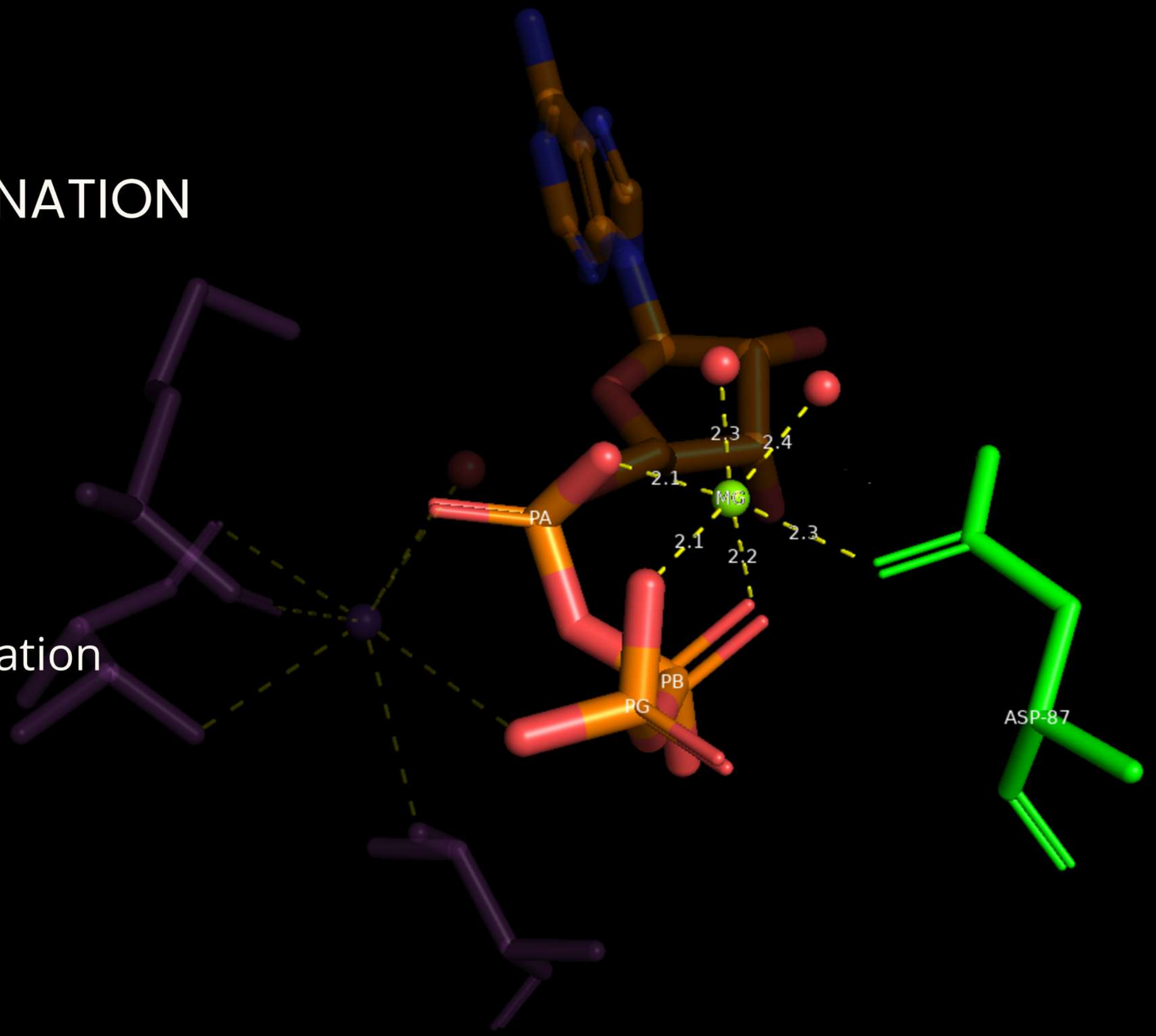


ATP BINDING

METAL IONS COORDINATION

Mg2+

- **octahedral coordination**
- **D87** brought within coordination distance
- 2 HOH

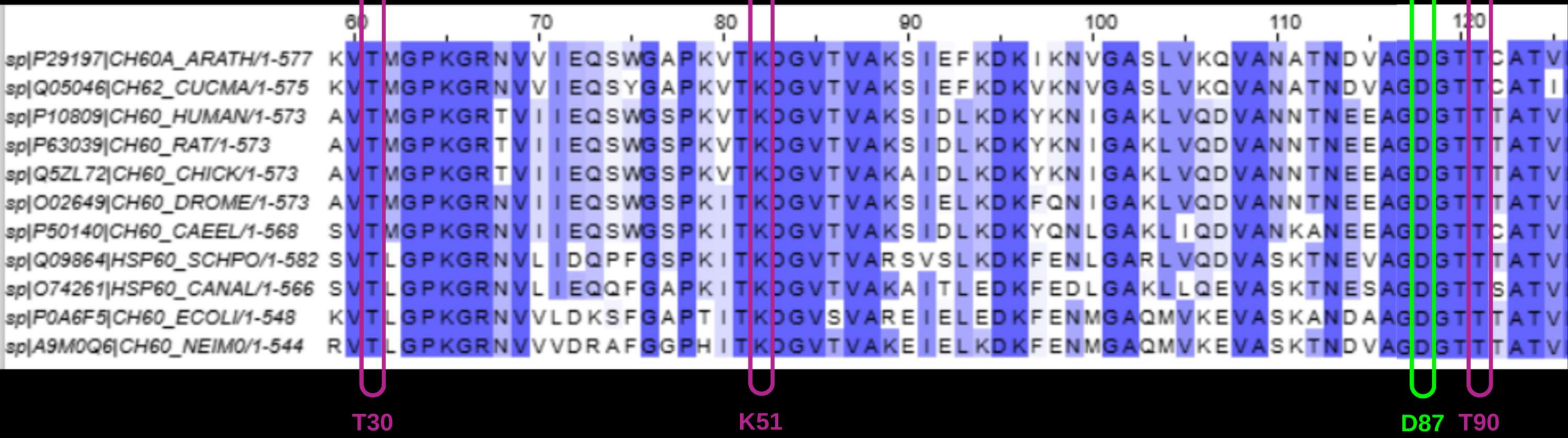


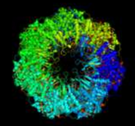
ATP BINDING

METAL IONS COORDINATION

Residues **conservation** involved in metal coordination:

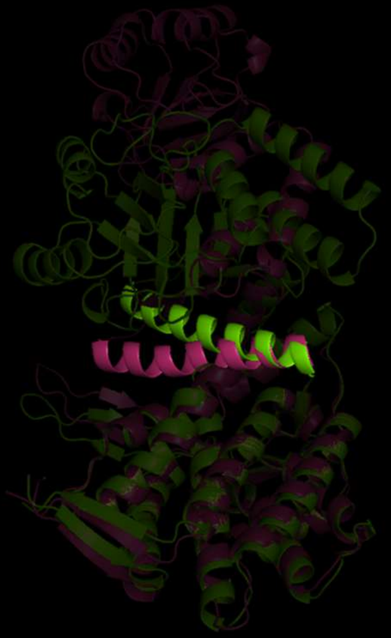
- K51, T30, T90 for **K+**
- D87 for **Mg2+**





ATP BINDING

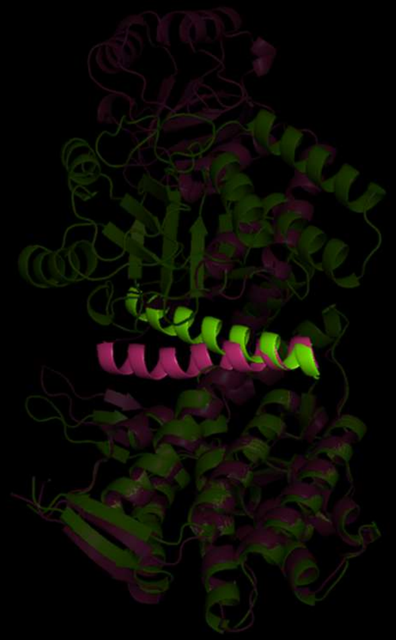
CATALYTIC ACTIVITY



ATP–APO
SUPERIMPOSITION

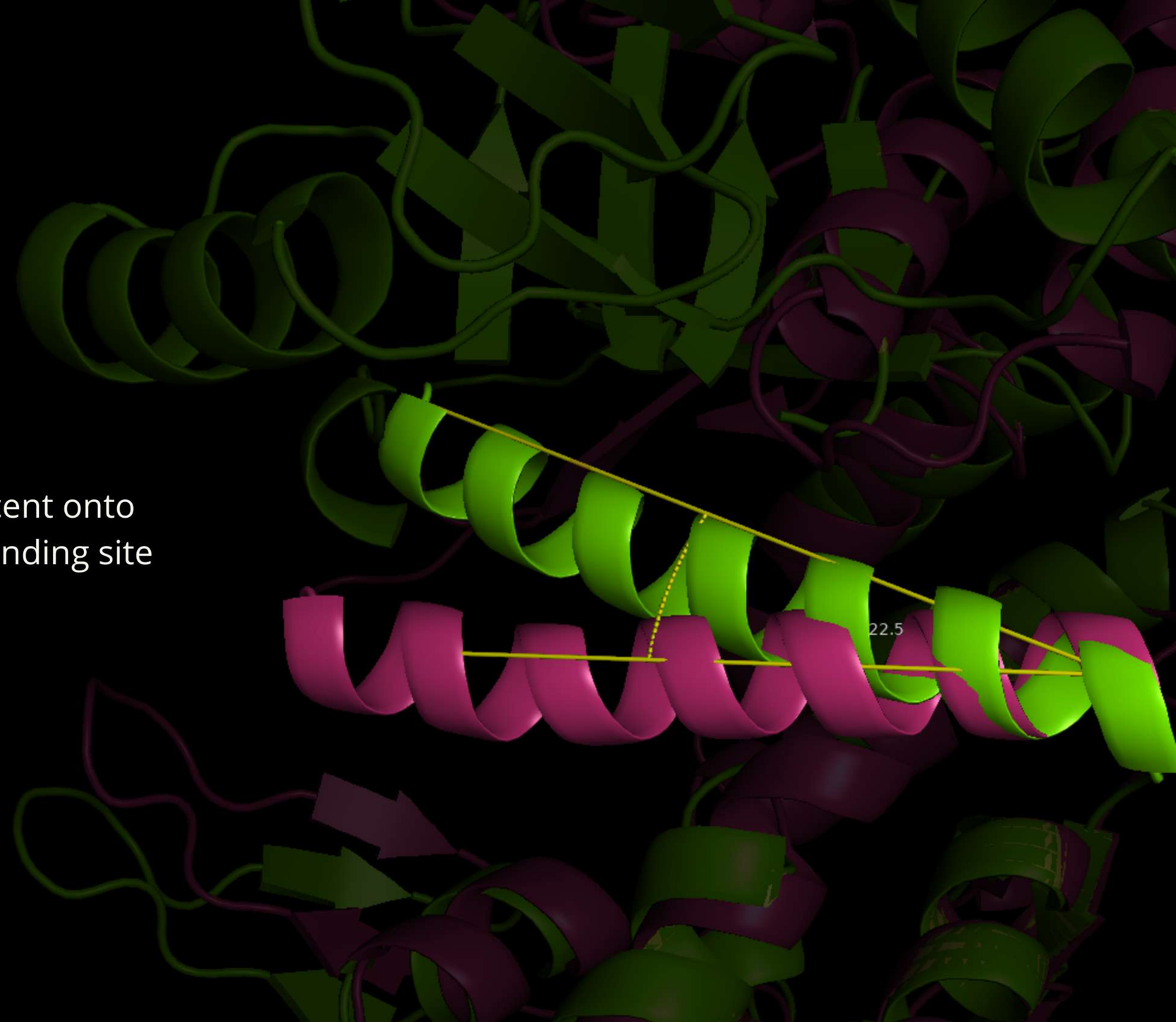
- **Helix M descent** onto nucleotide-binding site upon **ATP** binding

ATP BINDING
CATALYTIC ACTIVITY



ATP–APO
SUPERIMPOSITION

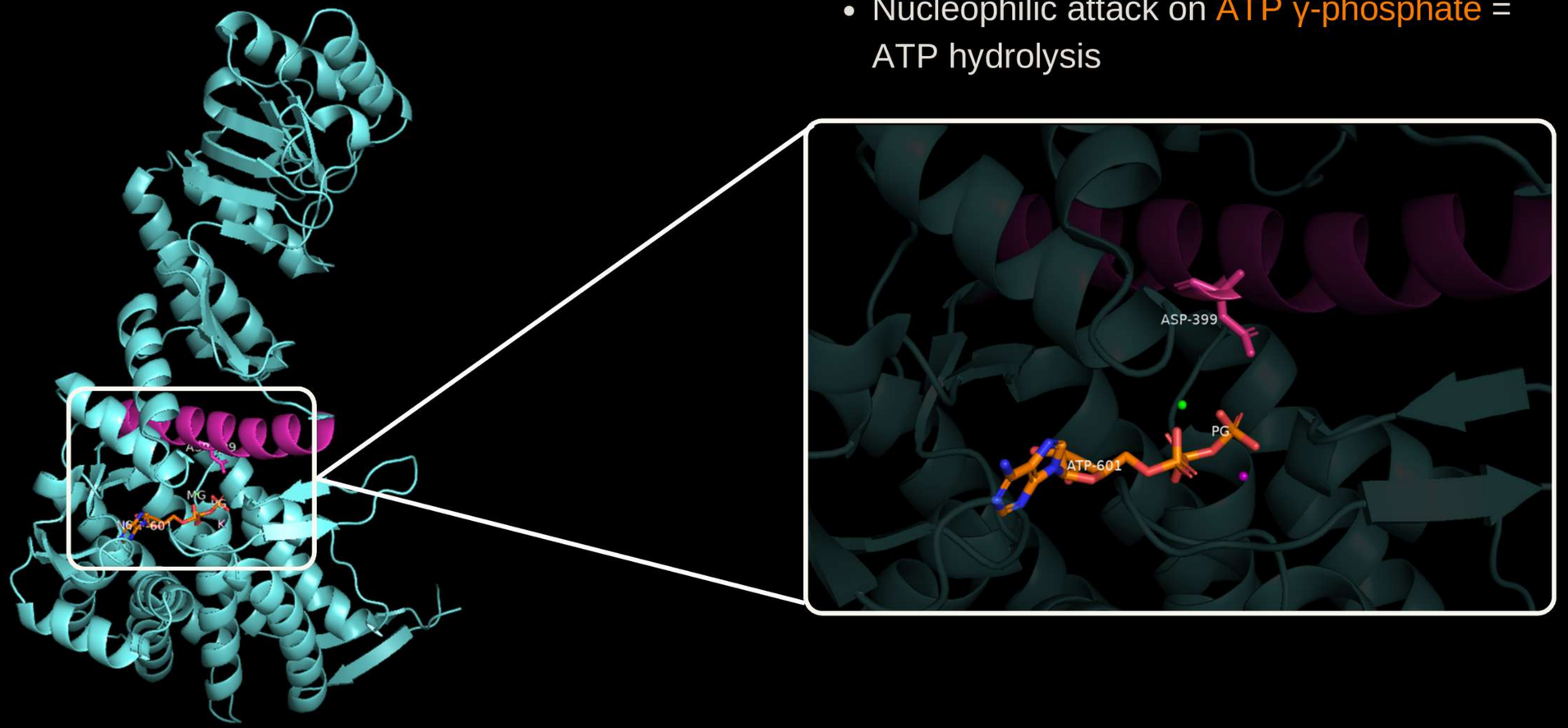
- **Helix M** descent onto nucleotide-binding site
  - 22.5º



ATP BINDING

CATALYTIC ACTIVITY

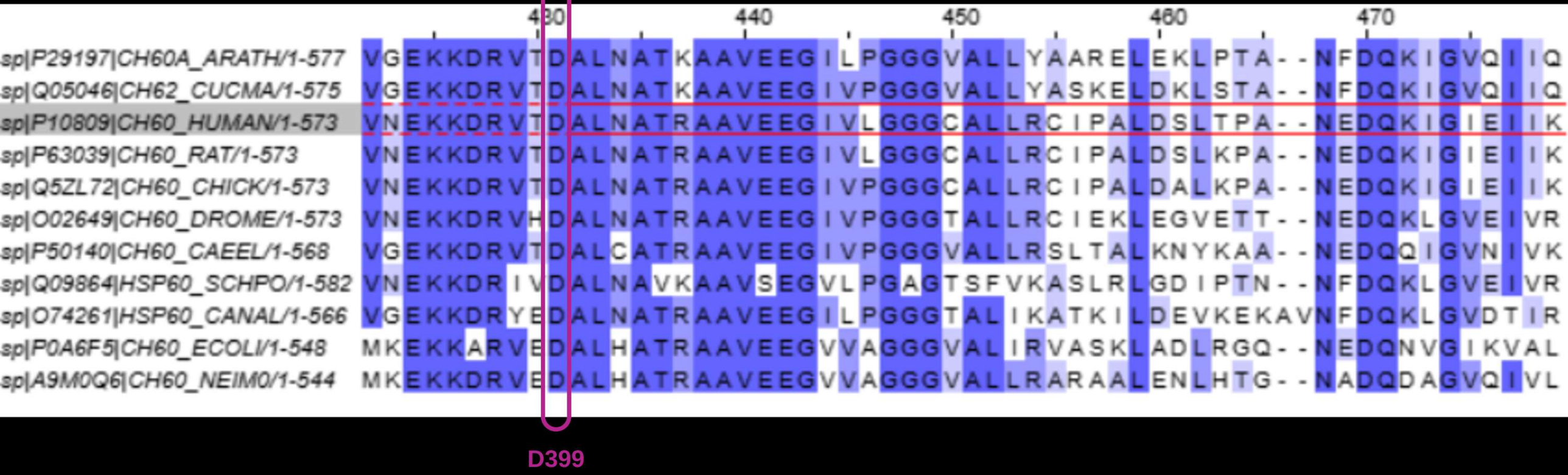
- Catalytic **D399** in Helix M brought closer towards the ATP-binding site
- Nucleophilic attack on ATP γ-phosphate = ATP hydrolysis



ATP BINDING

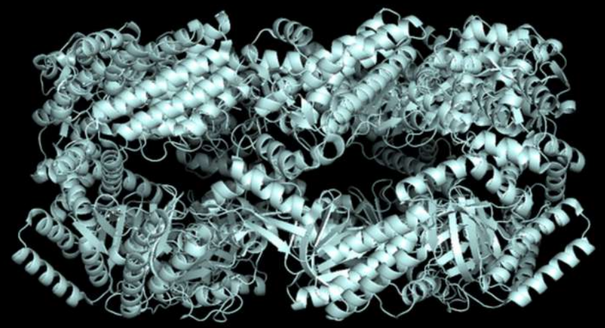
CATALYTIC ACTIVITY

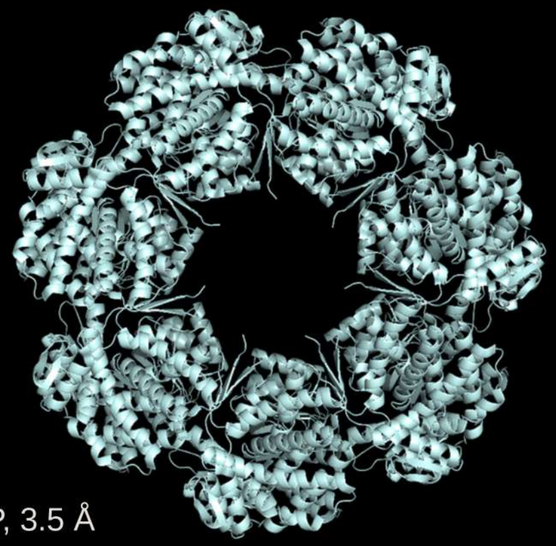
- Conservation of catalytic residue **D399**



HSP60 RINGS COMPARISONS

APO

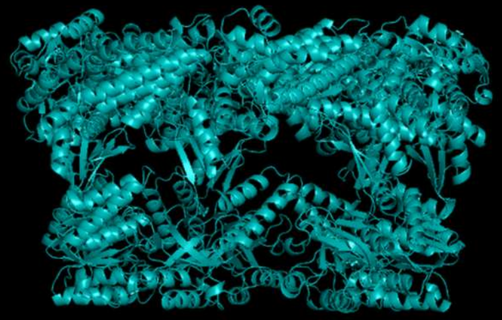


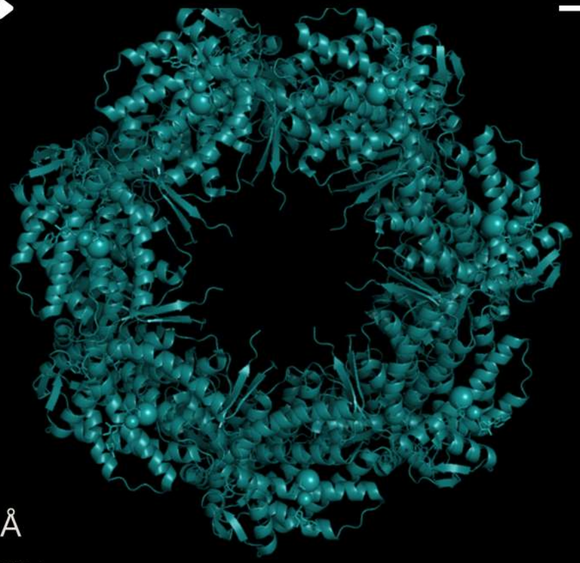


ID: 7AZP, 3.5 Å
Electron Microscopy

+ ATP

ATP bound

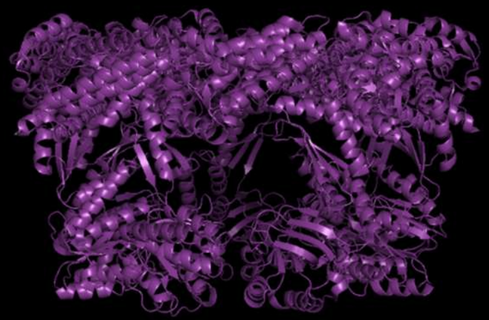


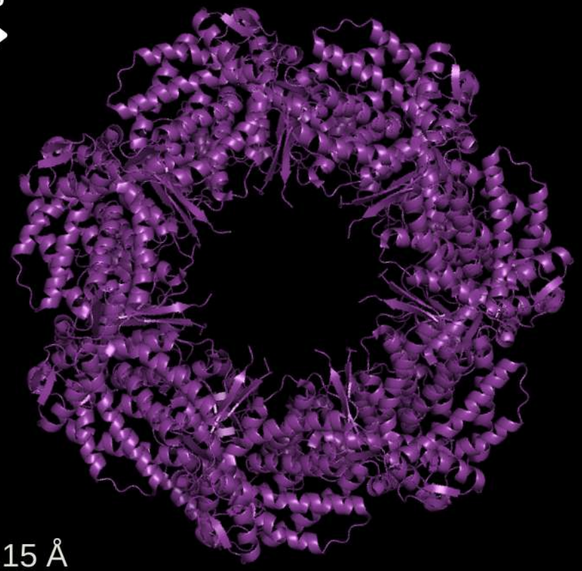


ID: 9SHG, 1.91 Å
Electron Microscopy

ATP hydrolysis

ADP bound

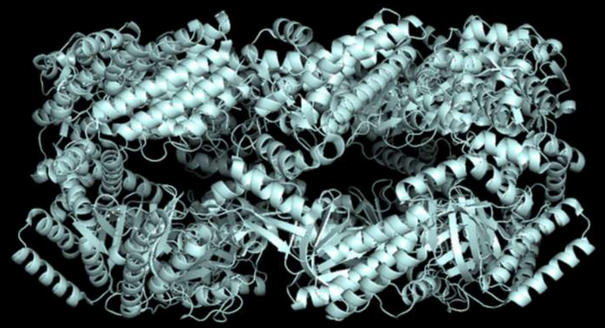


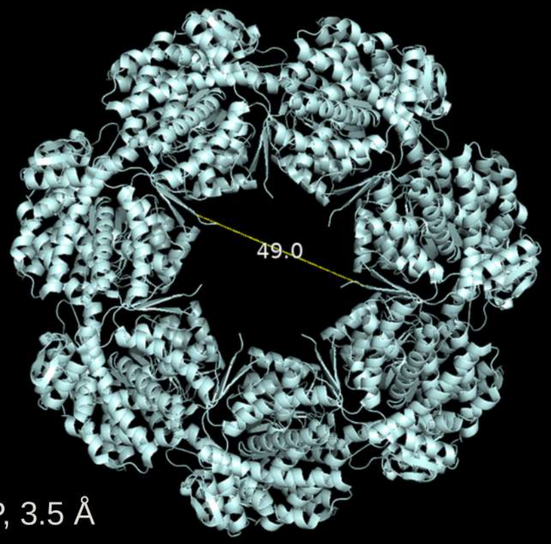


ID: 4PJ1, 3.15 Å
X-Ray Diffraction

HSP60 RINGS COMPARISONS

APO

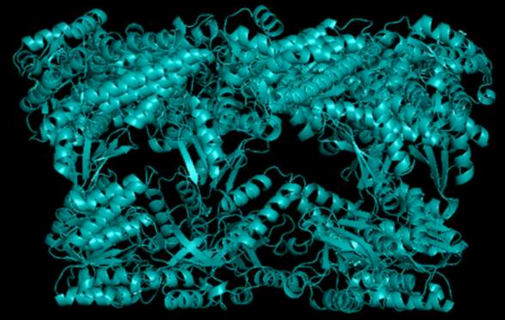


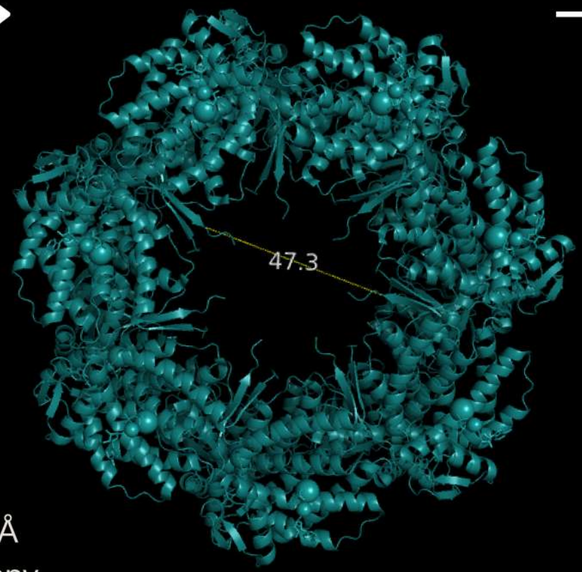


ID: 7AZP, 3.5 Å
Electron Microscopy

+ ATP

ATP bound

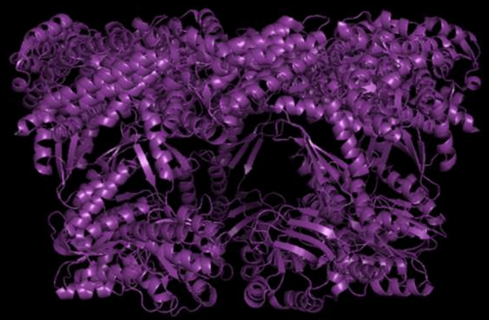


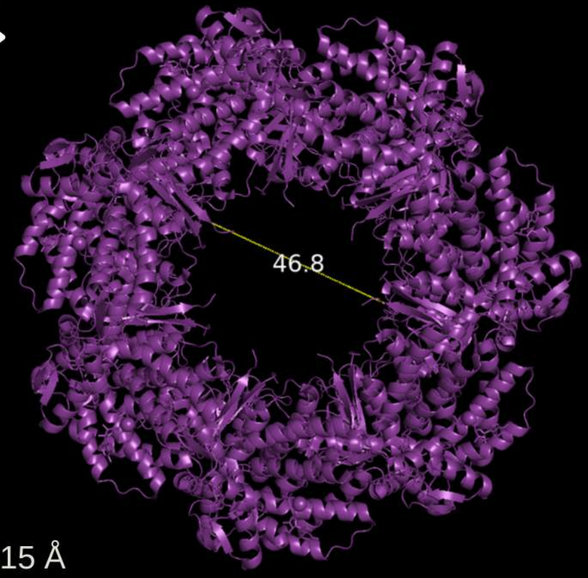


ID: 9SHG, 1.91 Å
Electron Microscopy

ATP hydrolysis

ADP bound

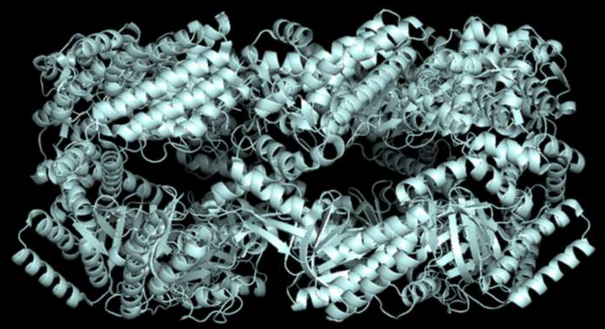


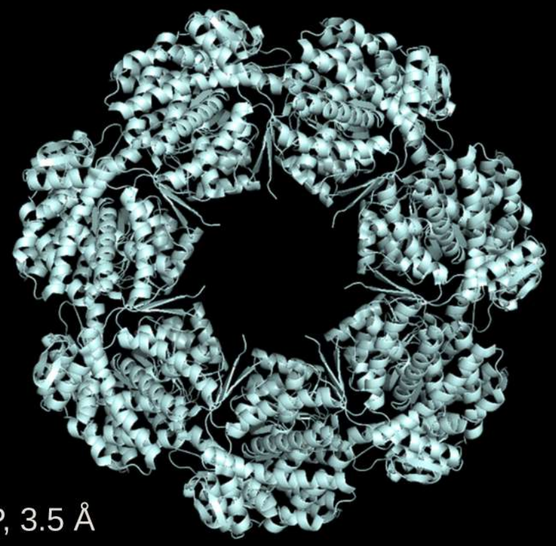


ID: 4PJ1, 3.15 Å
X-Ray Diffraction

HSP60 RINGS COMPARISONS

APO

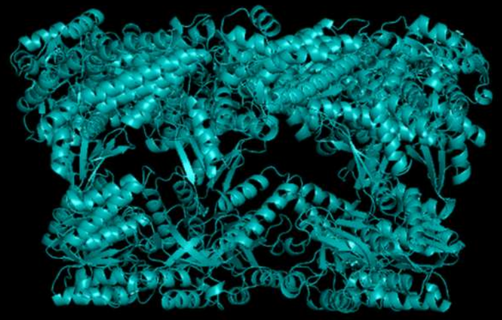


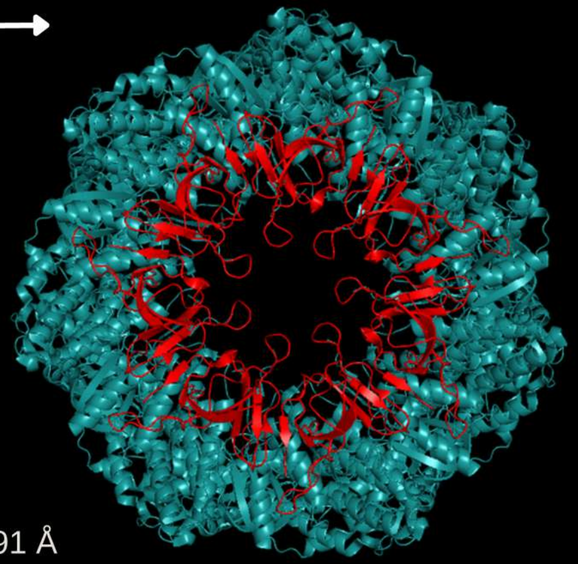


ID: 7AZP, 3.5 Å
Electron Microscopy

+ ATP

ATP bound

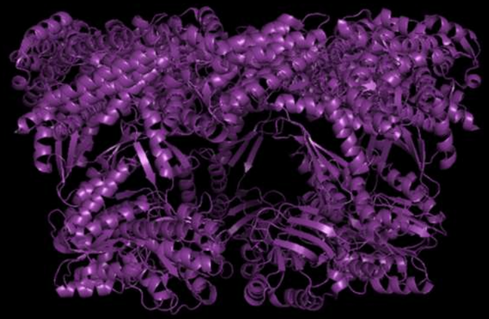


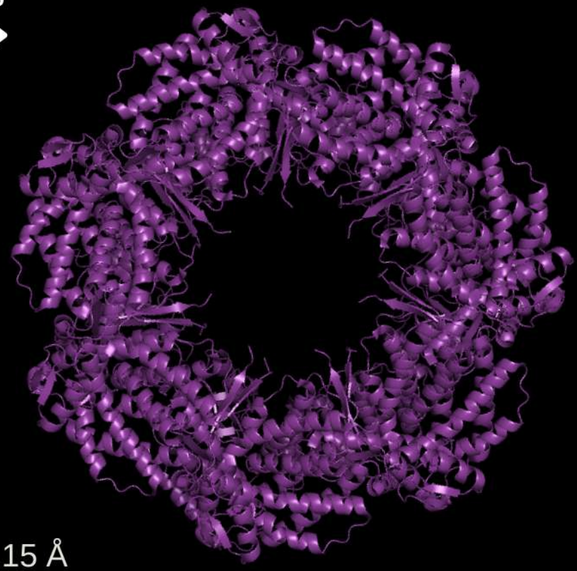


ID: 9SHG, 1.91 Å
Electron Microscopy

ATP hydrolysis

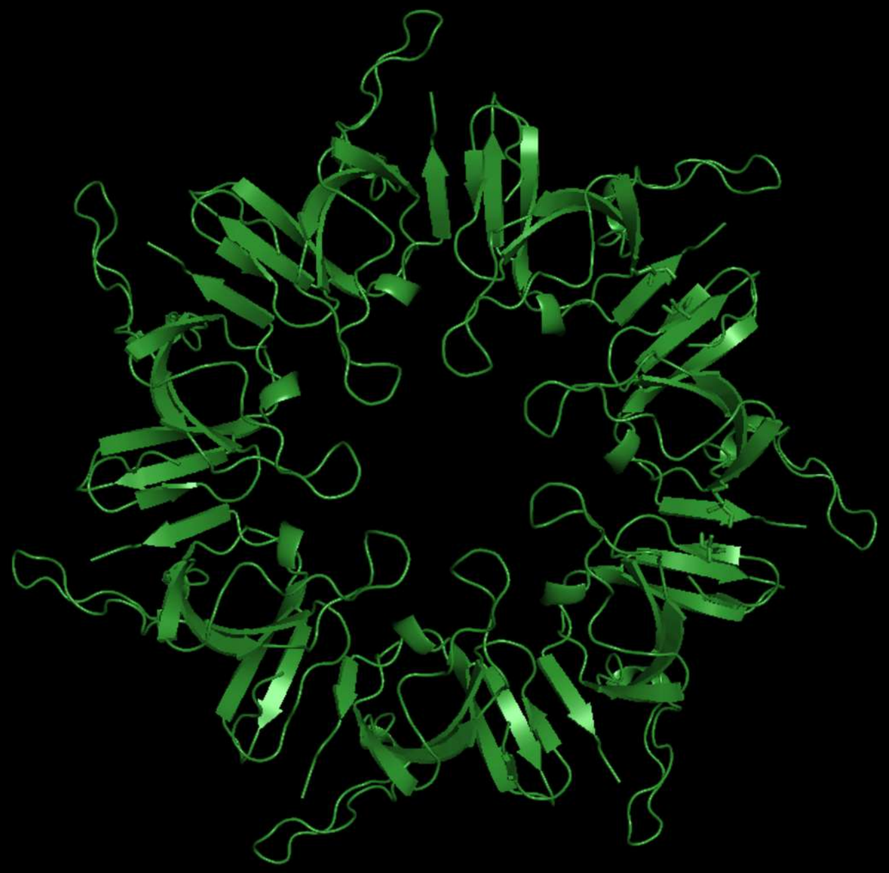
ADP bound





ID: 4PJ1, 3.15 Å
X-Ray Diffraction

HSP10 BINDING



Co - chaperone

10 kDa

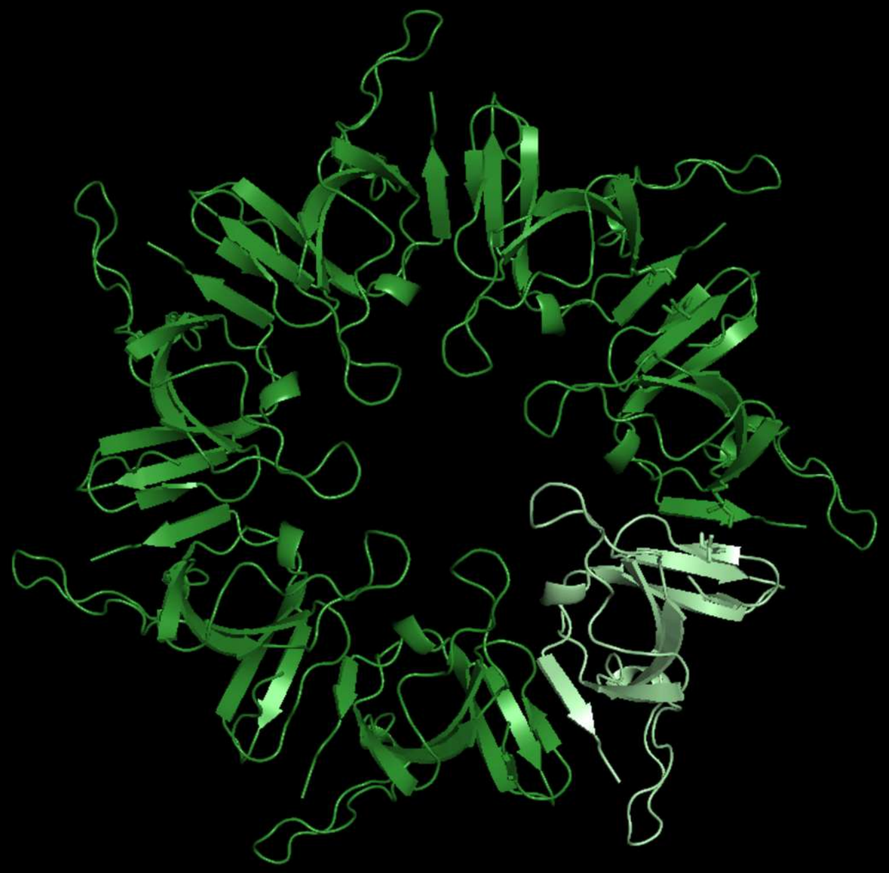
Heptamer

HSP10 BINDING

Co - chaperone

10 kDa

Heptamer



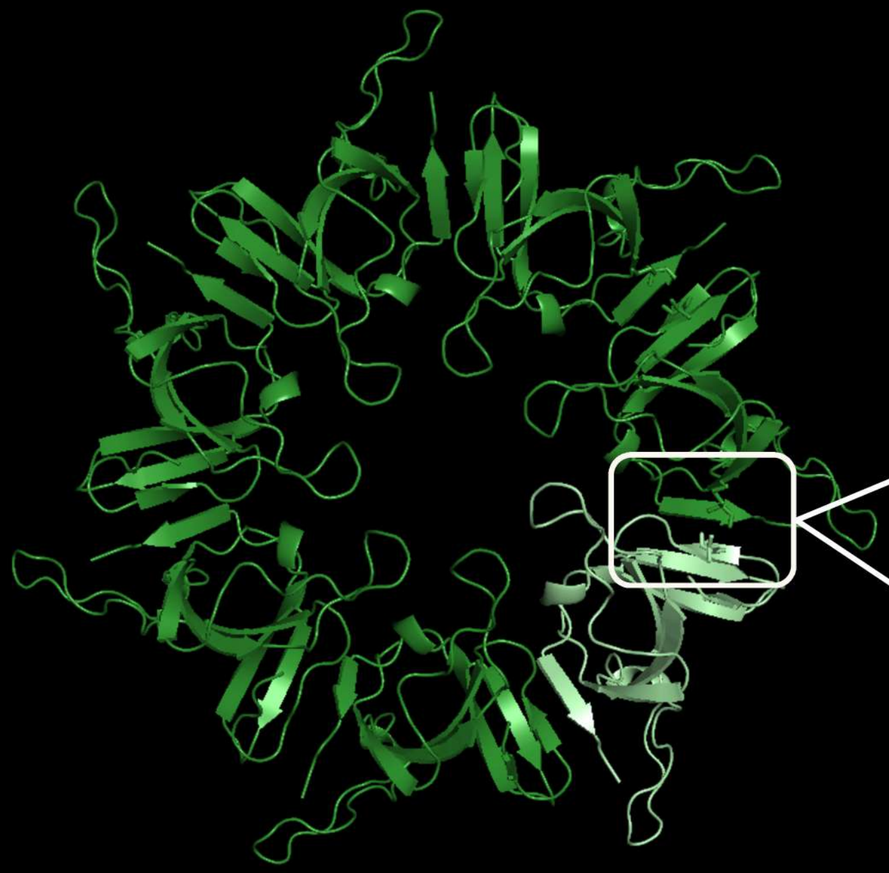
- Hsp10 Cap
- Class: all beta
- Fold: GroES like

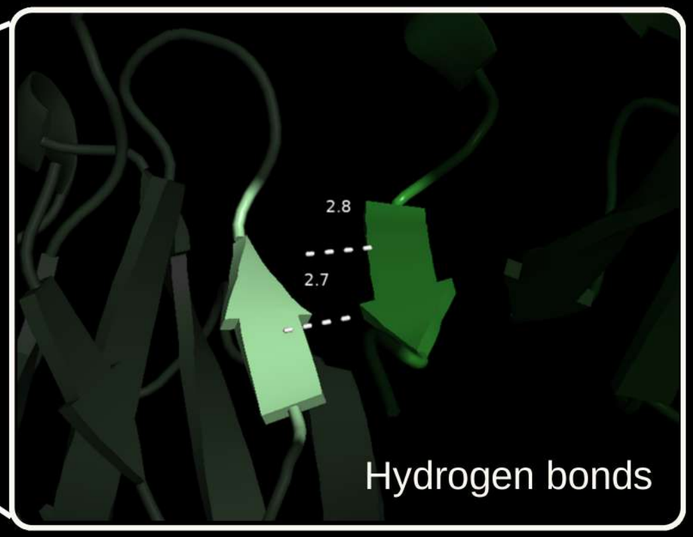
HSP10 BINDING

Co - chaperone

10 kDa

Heptamer

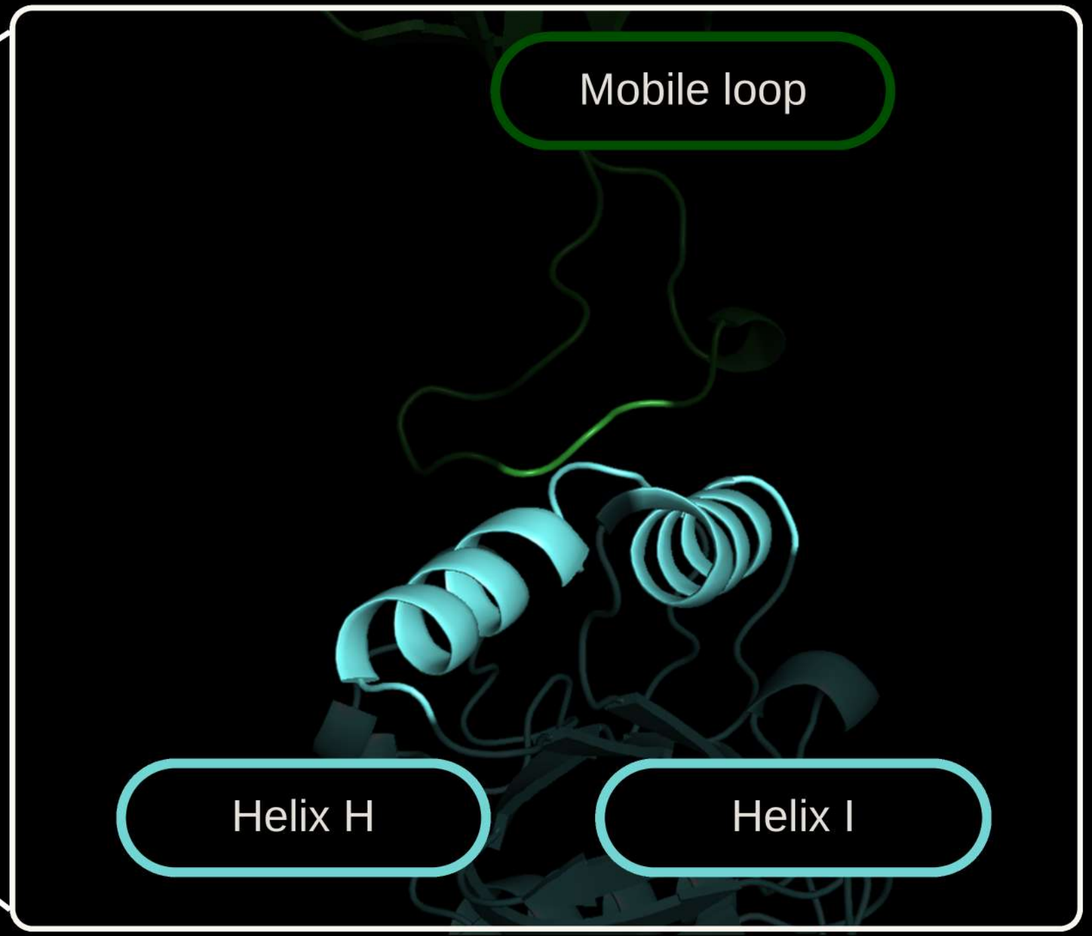




HSP10 BINDING







Apical

HSP10 BINDING



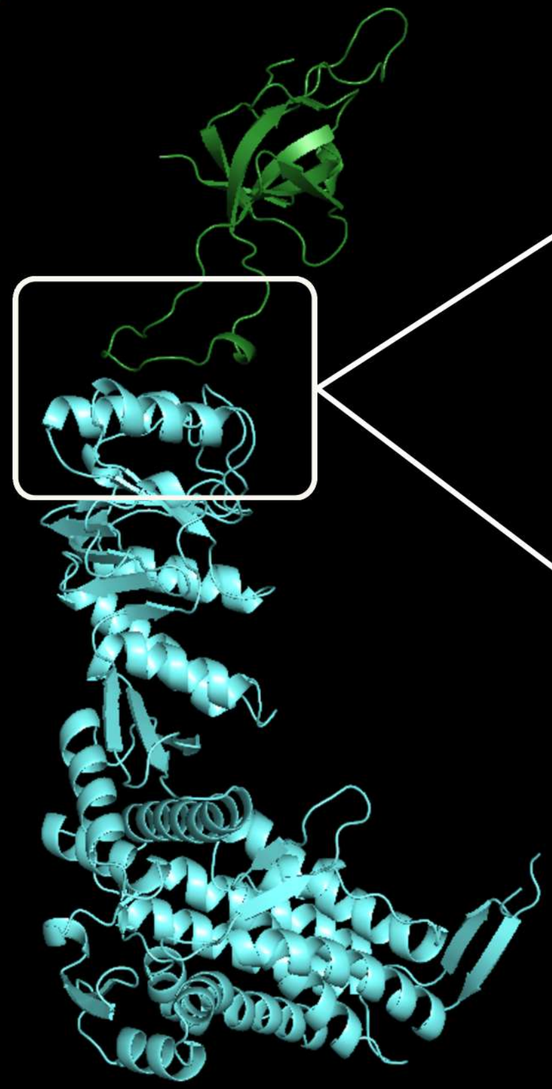


HYDROPHOBIC HYDROPHILIC



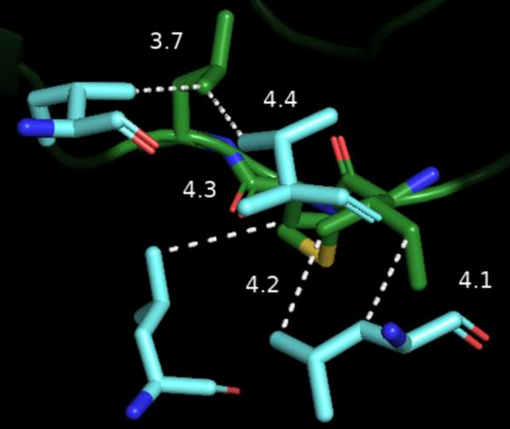
Apical

HSP10 BINDING



Hydrophobic interactions

I 31, M 32, L 33



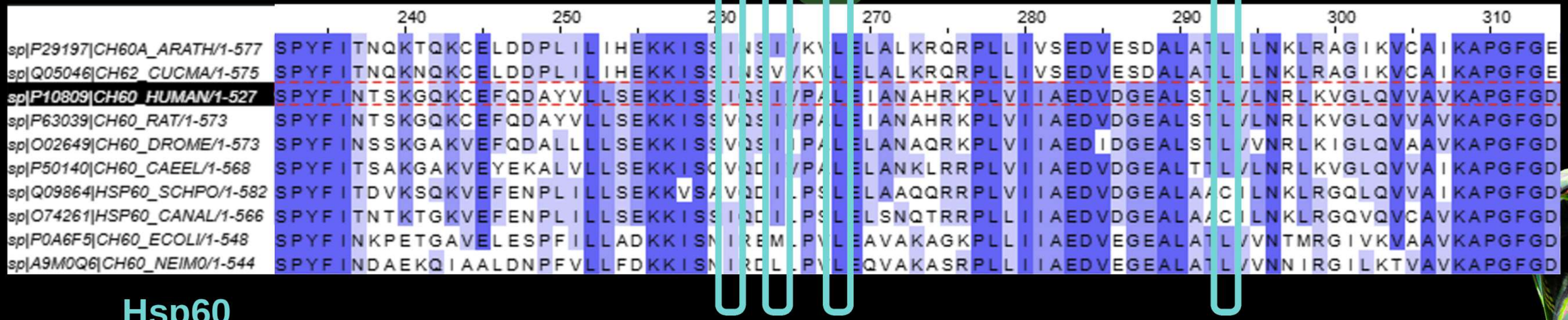
I 230
V 234
L 237
L 264

HSP10 BINDING

I31
M32
I33

Hsp10





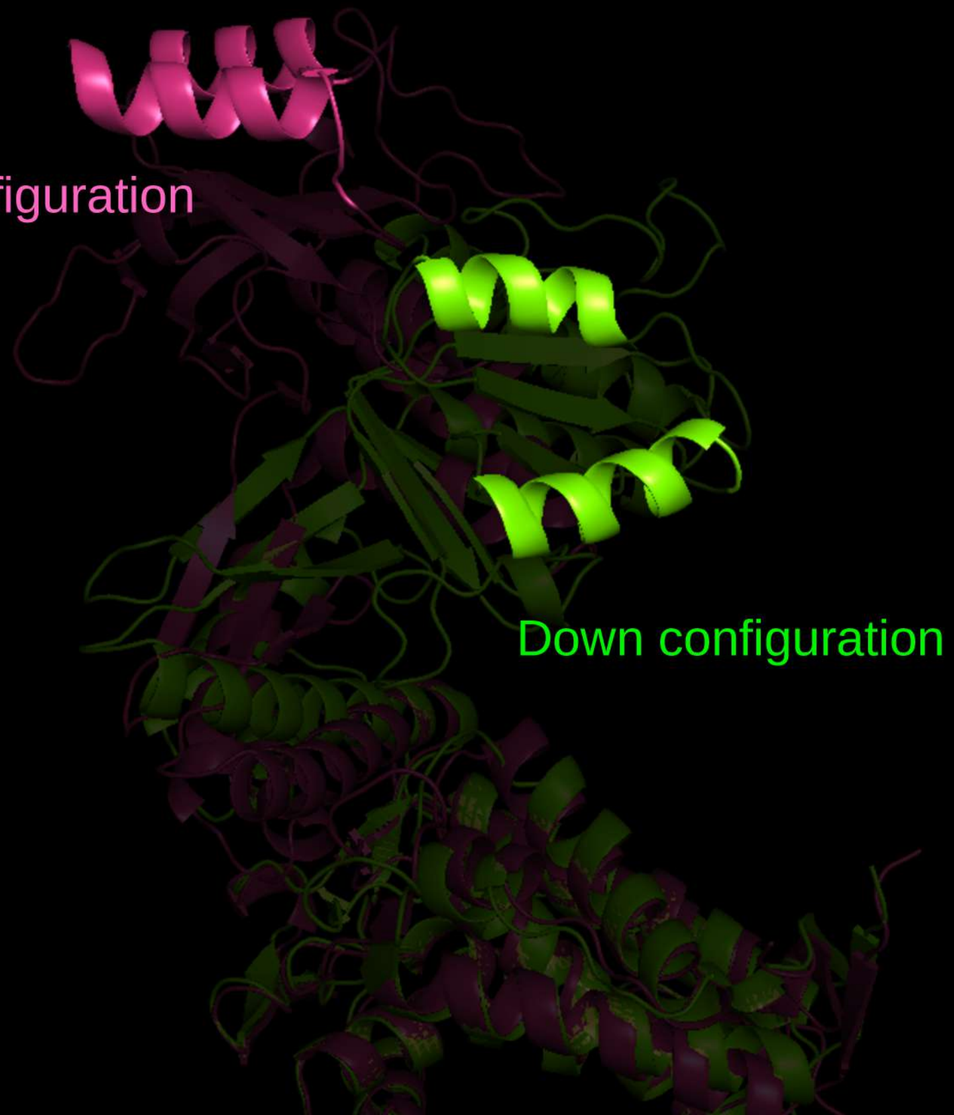
Hsp60

I 233 V 234 L 237

L 264

- ATP bound
- APO

HSP10 BINDING

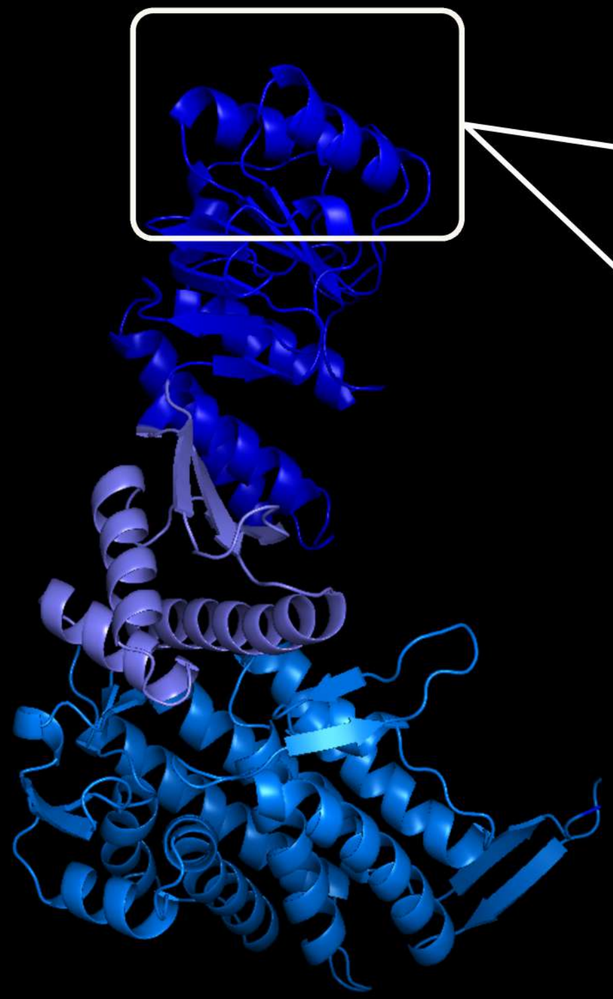


ATP binding → recruits Hsp10

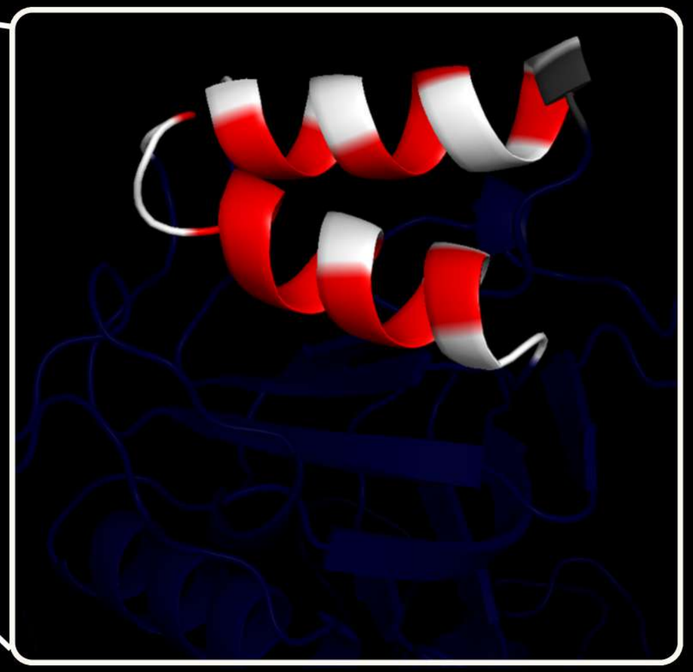
SUBSTRATE BINDING

HYDROPHOBIC

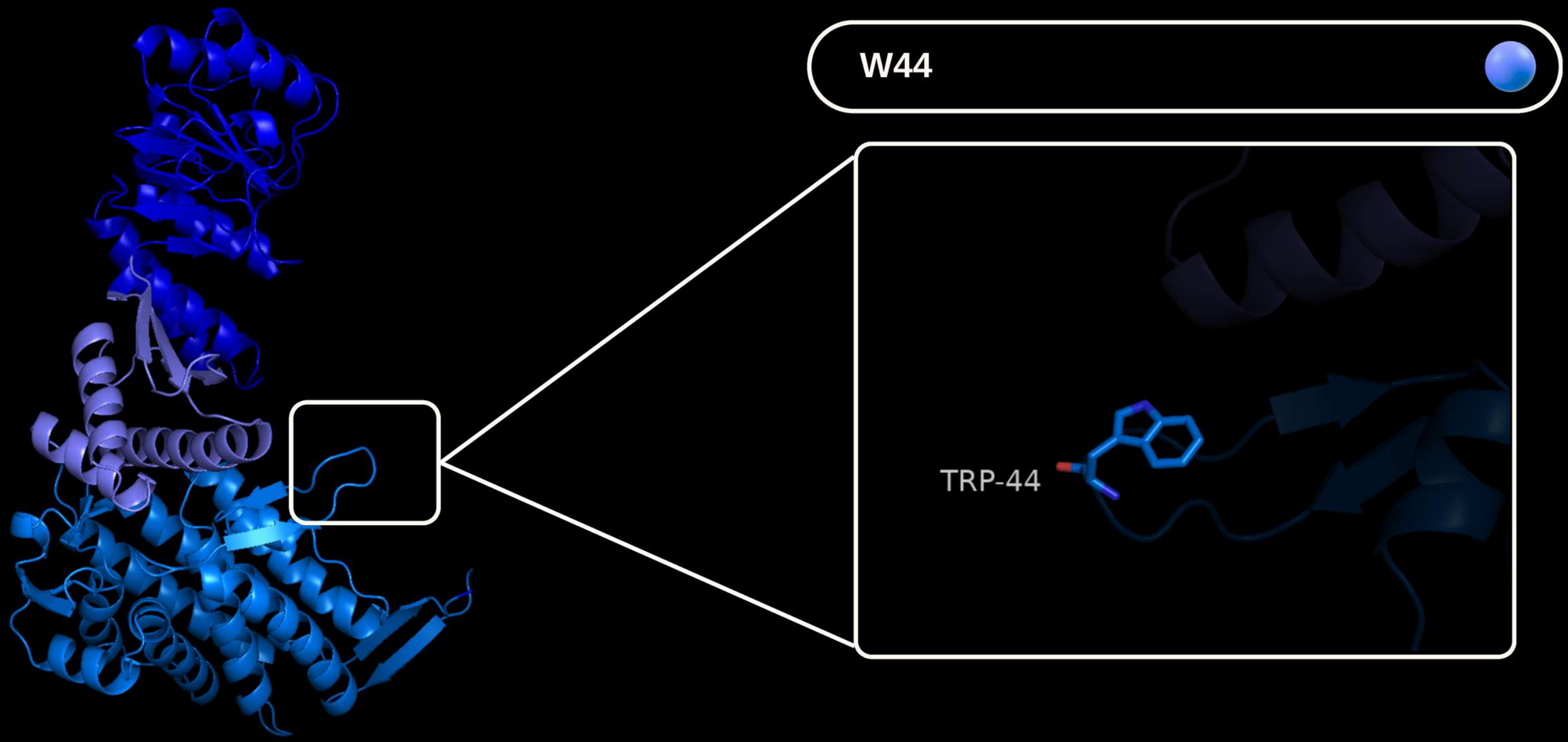
HYDROPHILIC



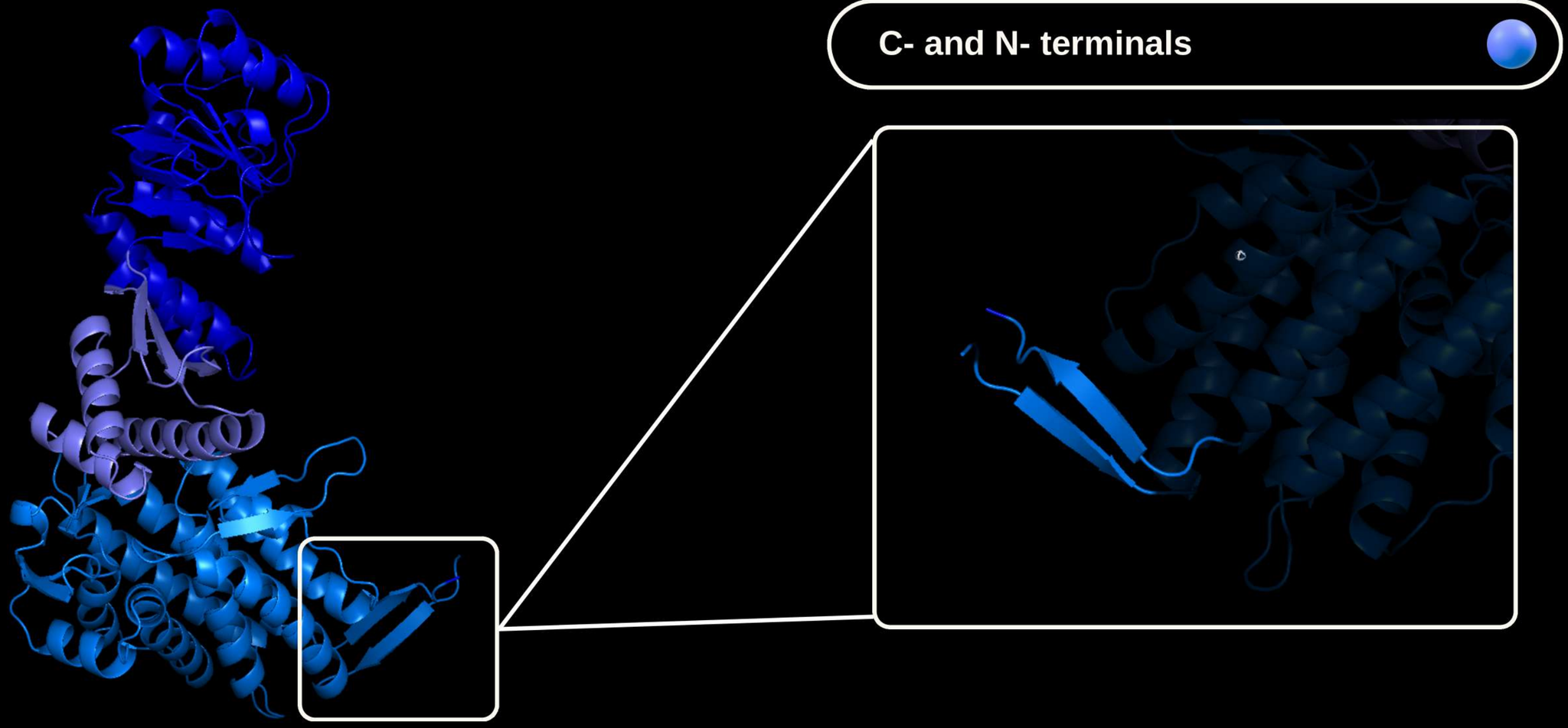
HELIX H AND I



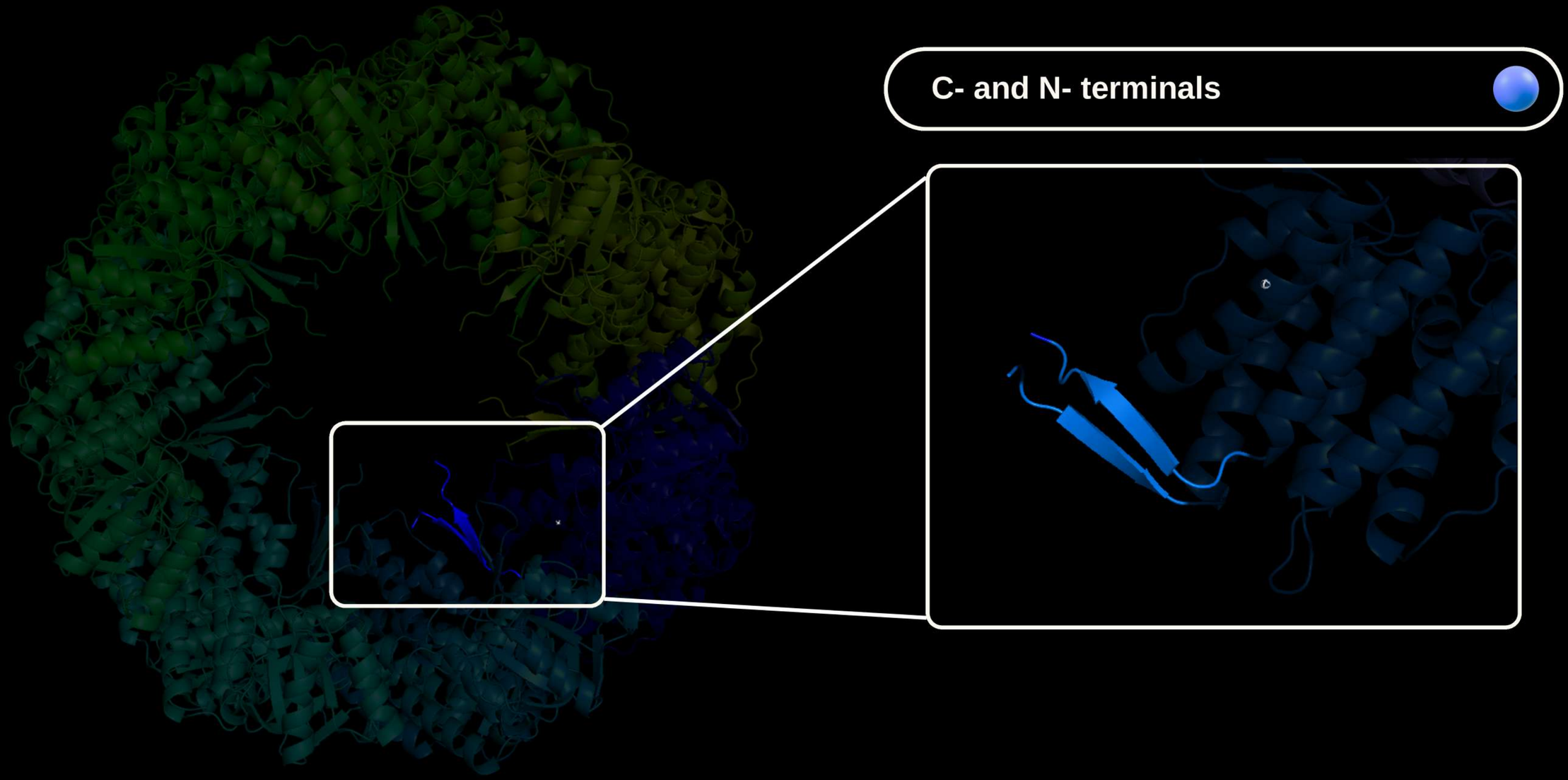
SUBSTRATE BINDING

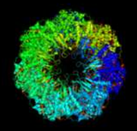


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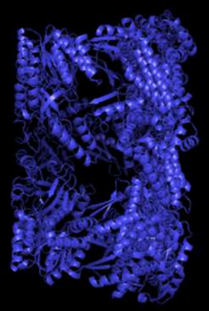


SUBSTRATE BINDING

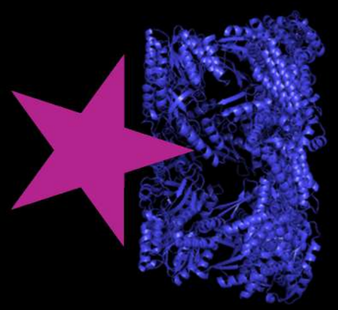




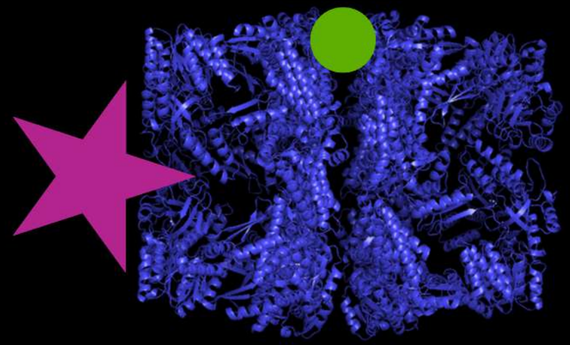
FOLDING CYCLE



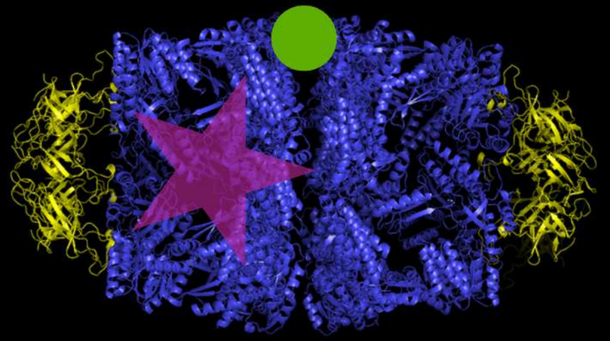
Substrate protein



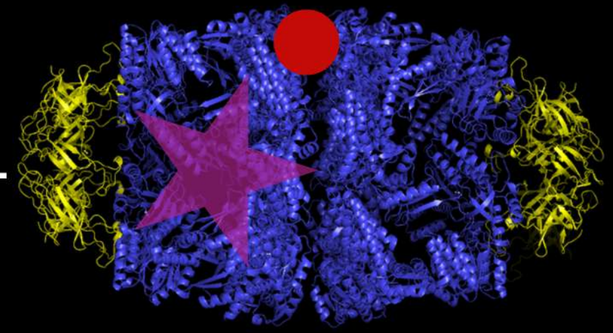
+ATP



+ Hsp10

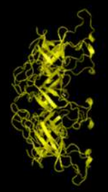


ATP hydrolysis



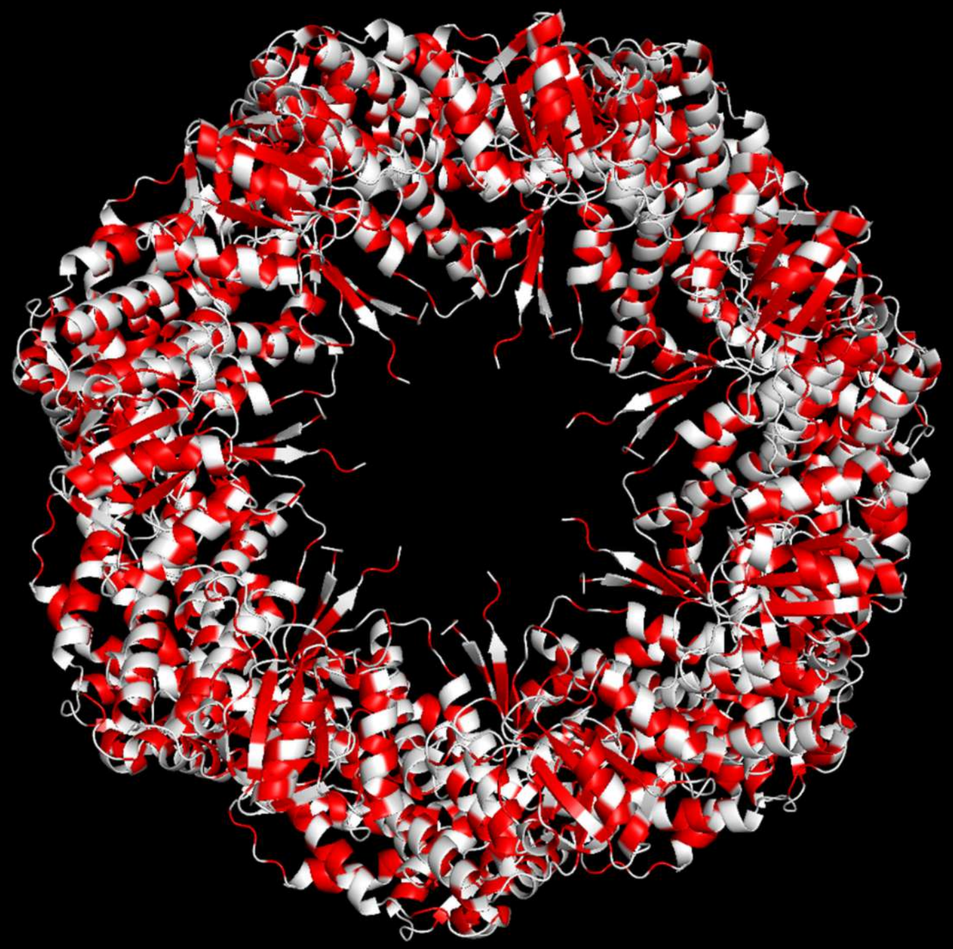
ADP





FOLDING CYCLE

HYDROPHOBIC HYDROPHILIC



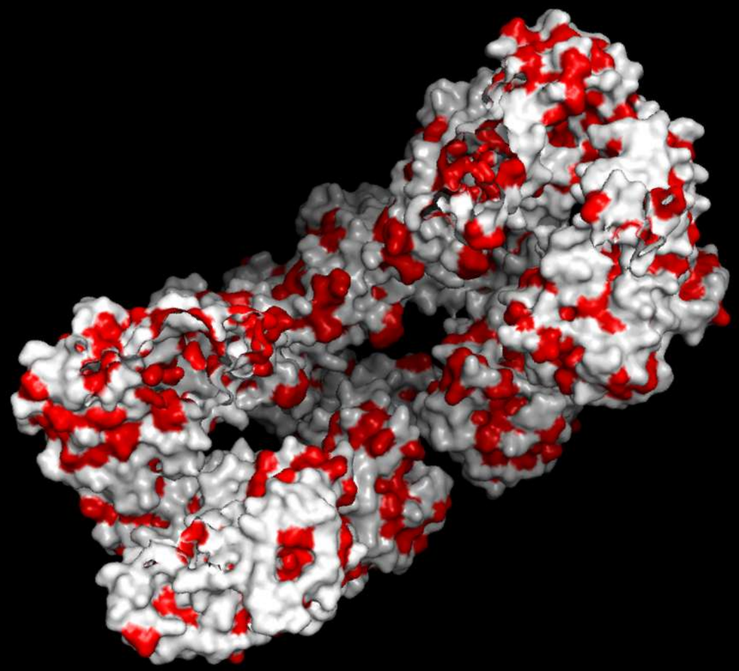
Anfinsen cage

- Proteins fold spontaneously
- Chaperones provide the right environment

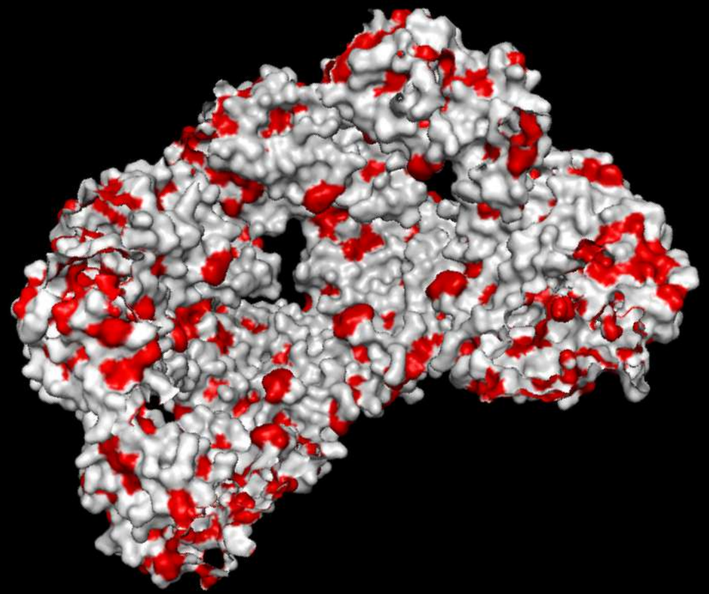
SUBSTRATE BINDING

HYDROPHOBIC HYDROPHILIC

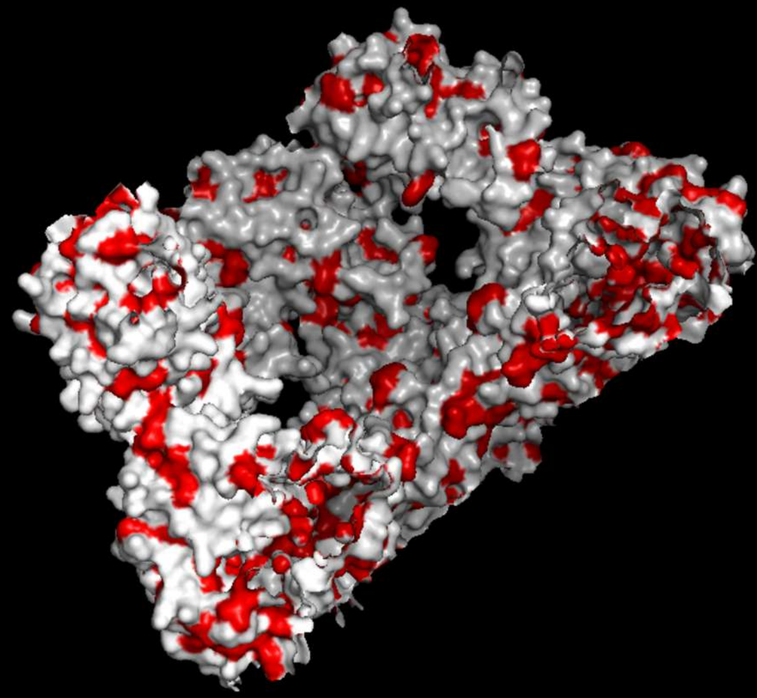
INNER CAVITY



APO (nucleotide free)

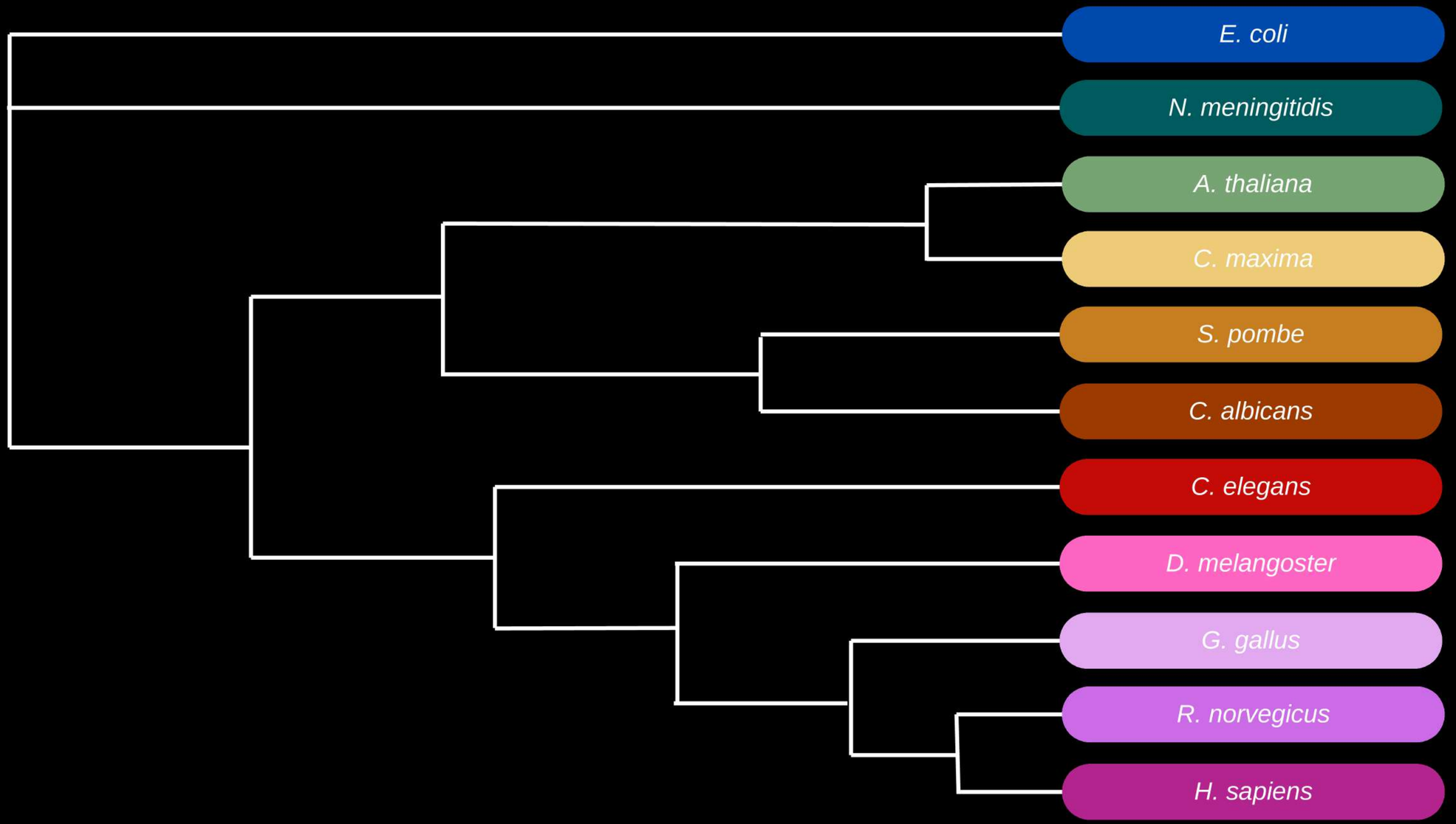


ATP bound



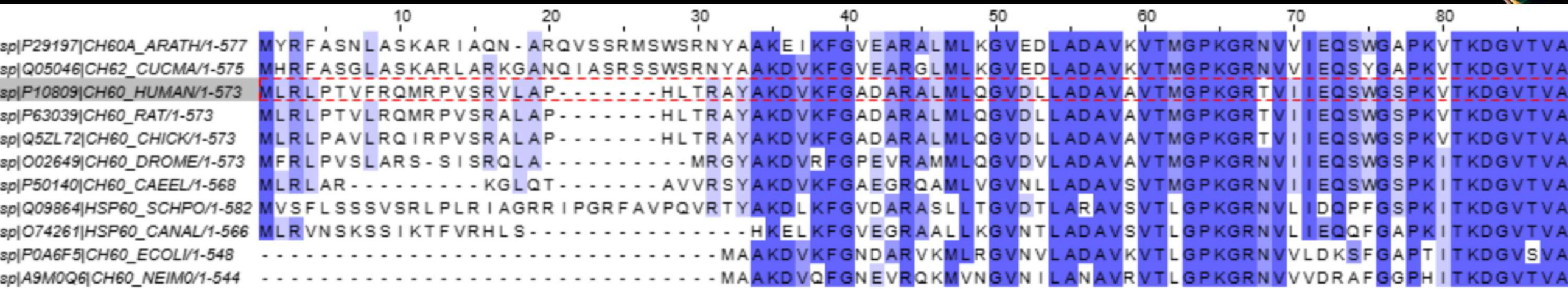
ADP bound

EVOLUTION

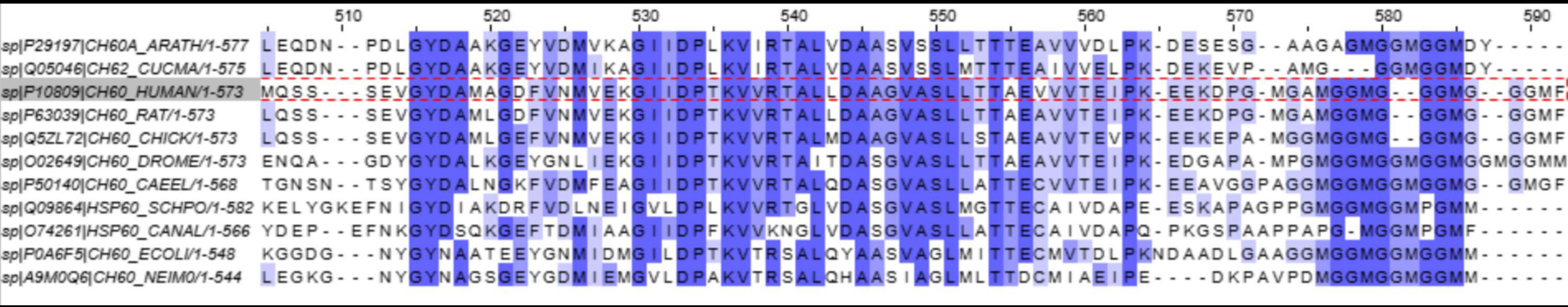

E. coli
N. meningitidis
A. thaliana
C. maxima
S. pombe
C. albicans
C. elegans
D. melangoster
G. gallus
R. norvegicus
H. sapiens

EVOLUTION

N-TERMINUS REGION



C-TERMINUS REGION



CONCLUSIONS

CONCLUSIONS

- Chaperones are a highly diverse group of proteins that aid in protein folding.
- The hsp60 family is a highly conserved family across evolution.
- The single ring complex is formed from a repeated subunit with three domains. These are bound together through beta sheets and hydrogen bonds between a helix and a loop.
- The double ring is bound through the equatorial domain and is assembled when ATP binds.
- ATP binding is stabilised in the nucleotide-binding pocket to be hydrolysed. Its union induces important conformational changes in the protein.
- ATP binding moves the folding cicle forward, allowing hsp10 and substrate protein binding.
- The main differences between species is the addition of the mitochondrial targeting sequence in eukaryotic organisms.



THANK YOU

upf. Universitat Pompeu Fabra Barcelona

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QUESTIONS:

1. Why was the structure of E. coli GroEL used to infer the structural classification of human mitochondrial Hsp60?

A. Because GroEL and mtHsp60 share extremely high sequence identity and identical gene regulation.

B. Because structural alignment showed a low RMSD and conserved secondary structure between the two proteins.

C. Because GroEL is the only experimentally solved structure among chaperonins.

D. Because mitochondrial proteins cannot be analyzed using structural comparison methods.

E. Because SCOP classification is determined solely by evolutionary lineage rather than structural features.

2. What is the primary function of the equatorial domain in the Hsp60 protomer?

A. It forms the substrate-binding surface responsible for recognizing unfolded proteins.

B. It contains the ATP-binding pocket and stabilizes both intra-ring and inter-ring interactions.

C. It interacts exclusively with the co-chaperonin Hsp10 during the folding cycle.

D. It forms the most flexible region of the protein responsible for conformational rearrangements.

E. It contains the mitochondrial targeting signal that directs the protein to mitochondria.

3. Which of the following statements is true about the inter ring interaction in mtHsp60

A. GroEL forms these interactions with the same residues.

B. The electrostatic interaction between the glutamate and lysine is the most essential.

C. The hydrogen bond between serines of opposing rings is a two fold symmetric interaction.

D. The salt bridge between glutamate and serine of the same ring dissasembles the double ring structure.

E. A monomer interacts with all its residues only with another monomer.

QUESTIONS:

4. What structural change allows the double-ring Hsp60 complex to form upon ATP binding?

A. ATP binding causes the apical domains to detach completely from the rest of the protomer.
B. ATP binding flattens the equatorial domain surfaces, allowing interactions between opposing rings.
C. ATP binding removes the intermediate domain from the protein structure.
D. ATP binding destabilizes the equatorial interface between protomers.
E. ATP binding converts the heptameric ring into a hexameric ring.

5. Why is the catalytic aspartatic acid residue involved in ATP hydrolysis strictly conserved across species?

A. Because it positions and activates a water molecule for nucleophilic attack on the ATP γ-phosphate.
B. Because it directly binds substrate proteins within the folding chamber.
C. Because it stabilizes the mitochondrial targeting sequence.
D. Because it forms part of the inter-ring salt bridge interface.
E. Because it interacts with Hsp10 during substrate encapsulation.

6. What is the functional significance of the conformational change in helix M after ATP binding?

A. It disrupts the interaction between Hsp60 and its substrate protein.
B. It disassembles the heptameric ring structure.
C. It prevents the association of the co-chaperonin Hsp10.
D. It removes metal ions from the ATP-binding pocket.
E. It repositions catalytic residues necessary for ATP hydrolysis within the nucleotide-binding pocket.

QUESTIONS:

7. In the single ring assembly, how could the interactions involved be described?

A. Salt bridges cause monomer association.
B. The helices beta sheets form hydrogen bonds along all domains of the monomer.
C. The intermediate domains form a beta sheet that unite the monomers.
D. A beta sheet forms in the equatorial domain, while a helix interacts with the loop of the adjacent monomer.
E. Two helices interact in the equatorial domain, while a loop interacts with a beta strand in the apical domain.

8. What is false about the folding cycle of misfolded proteins in mtHsp60 chaperonin?

A. The substrate binds to the single ring complex before ATP binding.
B. Hsp10 binding causes the substrate protein to be released in the mitochondrial matrix.
C. ATP hydrolysis aids substrate protein release.
D. The double ring complex forms after ATP binding.
E. Once a protein was folded, the chaperonin single ring is ready for another folding cycle.

9. About substrate protein binding

A. The chaperonin imposes a specific fold on the protein.
B. The inner cavity is more hydrophilic in the APO (nucleotide free) state.
C. The C and N terminals are important for substrate protein release.
D. The two helices I and H have a hydrophilic surface to capture the substrate protein.
E. The C and N terminals, with the tryptophan in the inner cavity, anchor the substrate protein.

QUESTIONS:

10. Regarding the evolution of this hsp 60 group I family:

A. The N terminal aminoacids are part of the mitochondrial targeting sequence, being only present in eukaryoteic species.

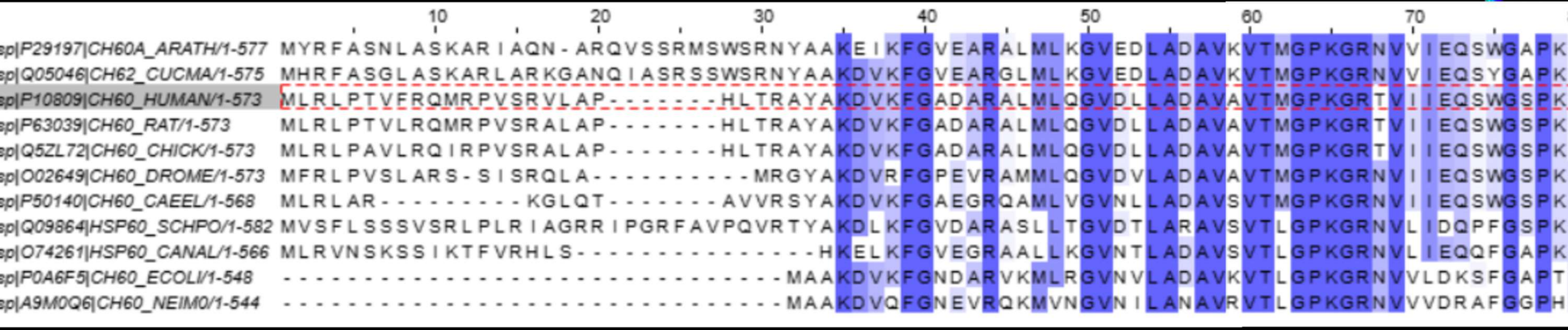
B. The C terminal contains the mitochondrial targeting sequence, being only present in eukaryotic species.

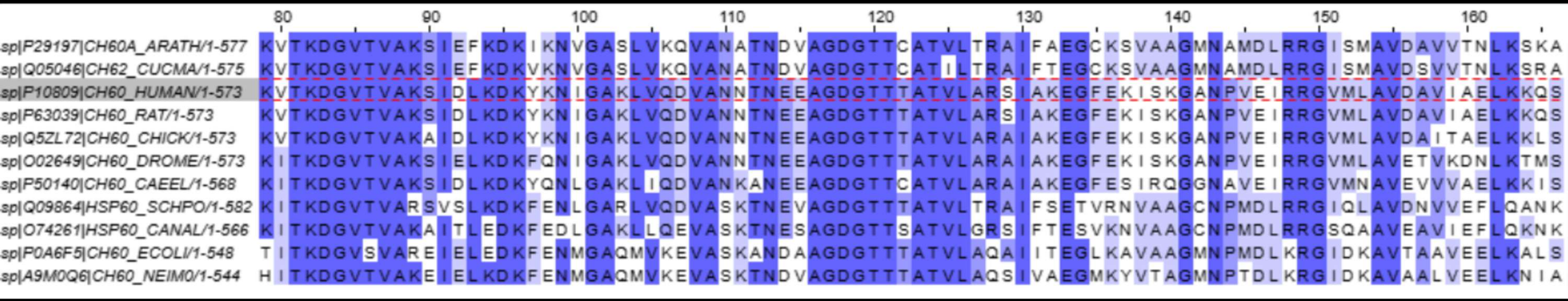
C. Both the N and C terminal are conserved in all species, both prokaryotic and eukaryotic.

D. The overall conservation seen in the sequence alignment is inconsistent with the relative importance of chaperone function.

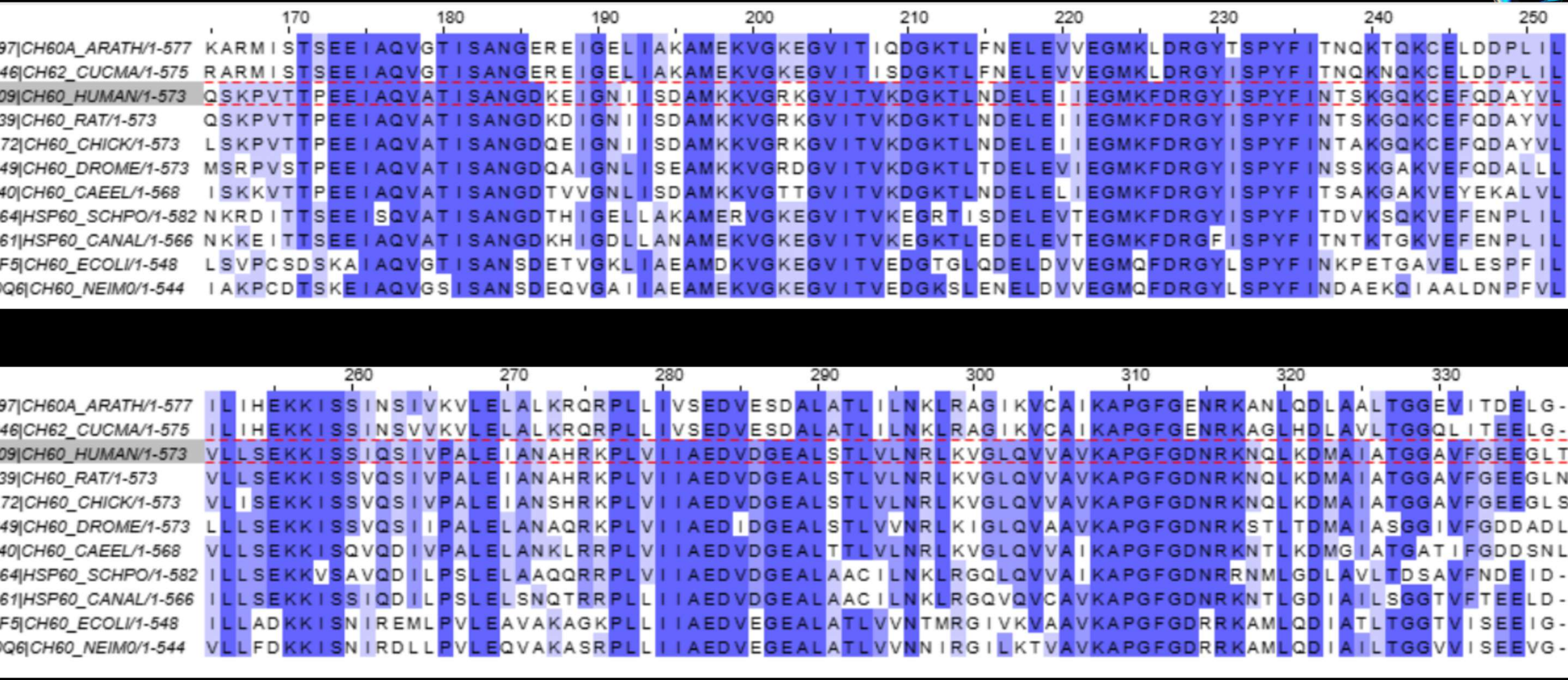
E. Most residues mentioned in the interactions are not conserved.

EXTRA MATERIAL: EVOLUTION

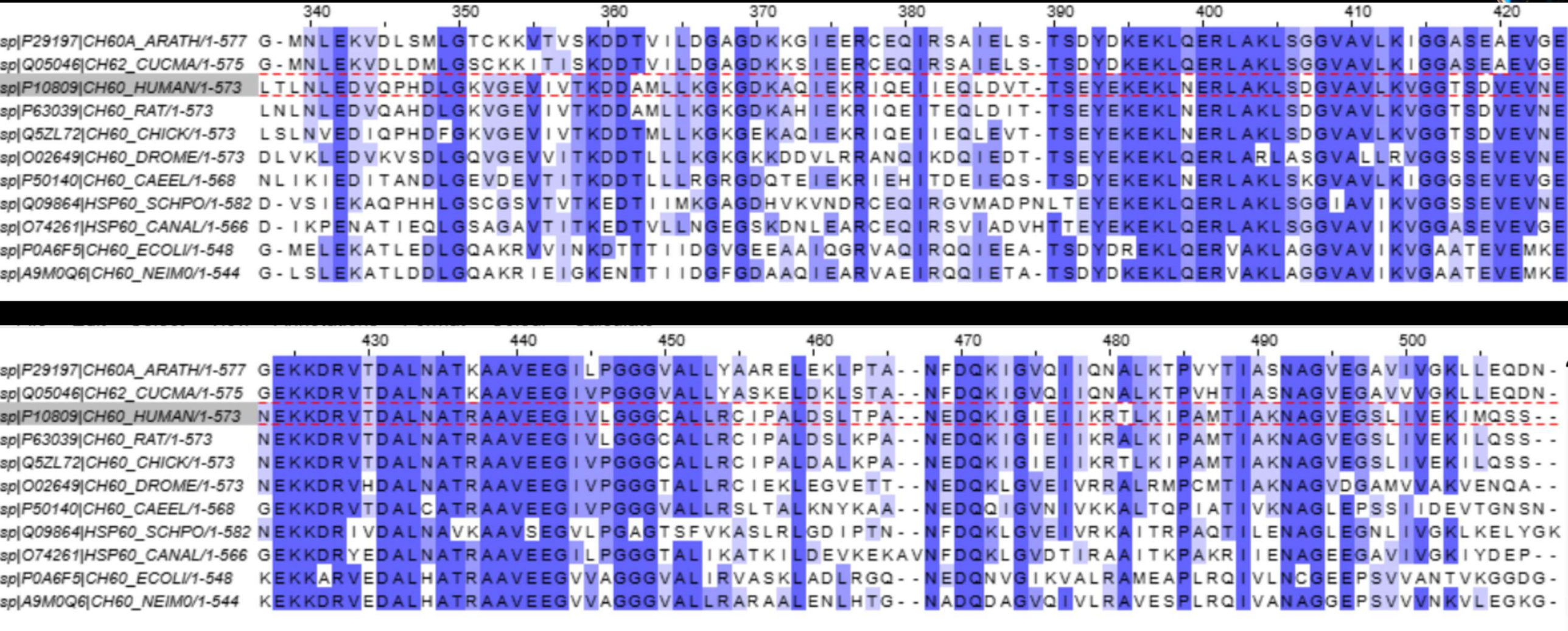


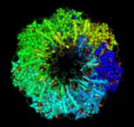


EXTRA MATERIAL: EVOLUTION

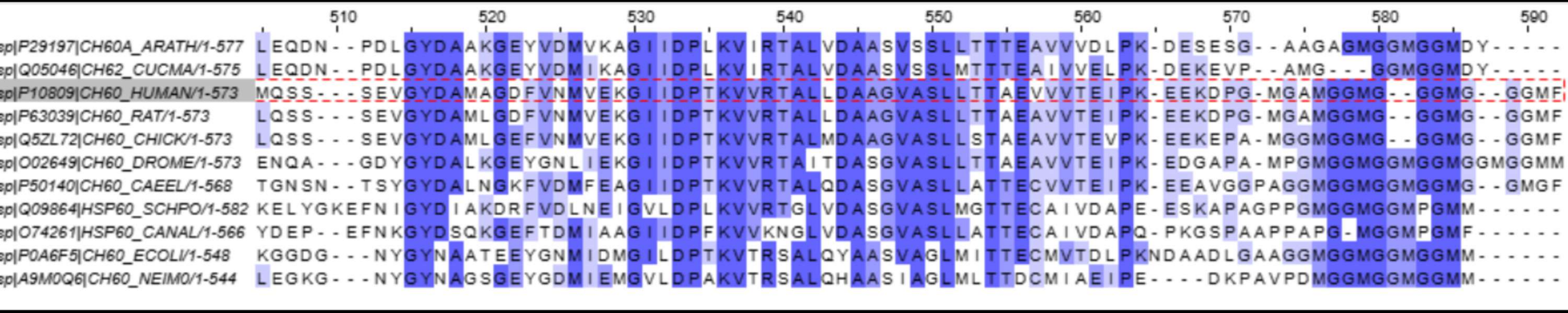


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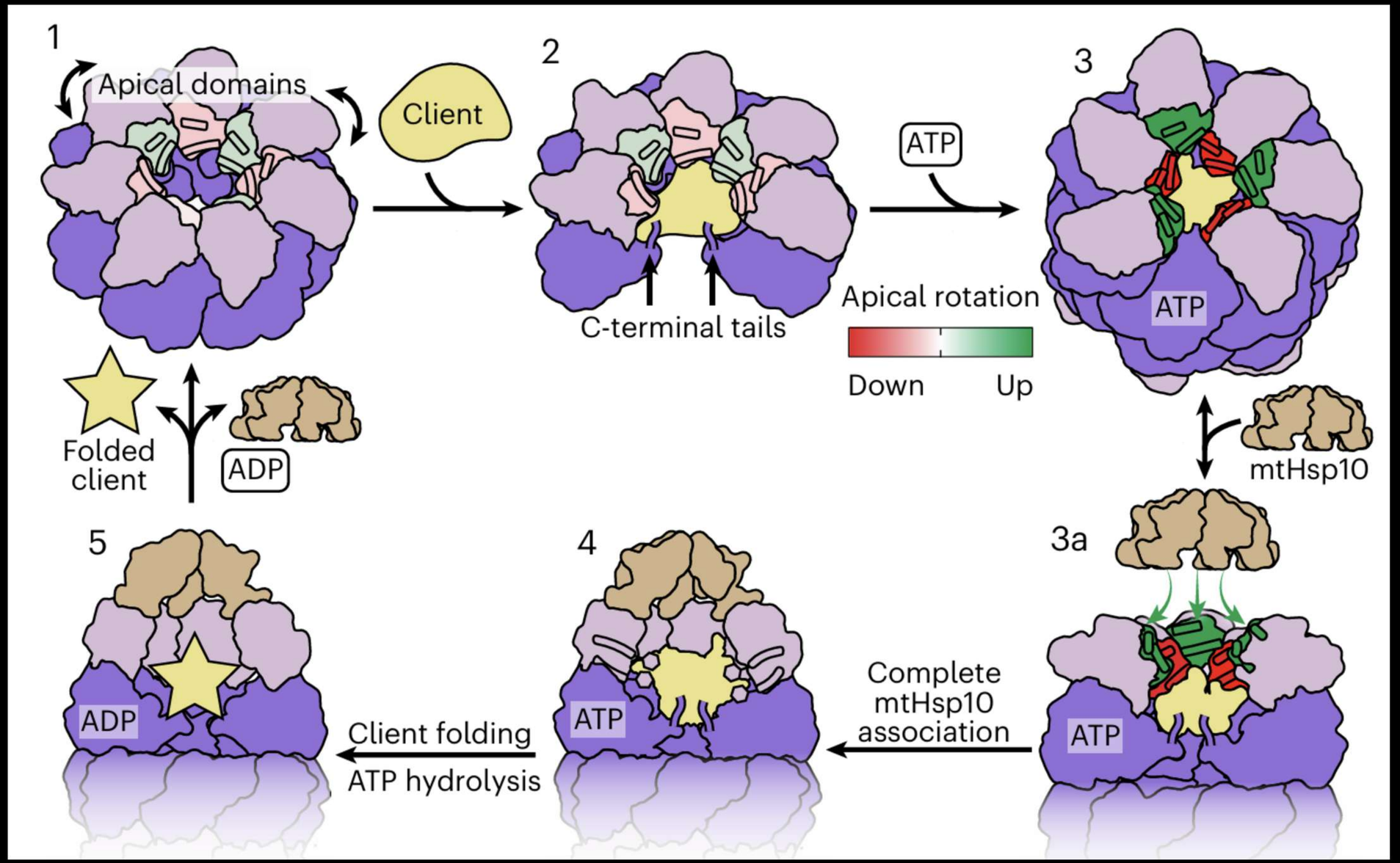




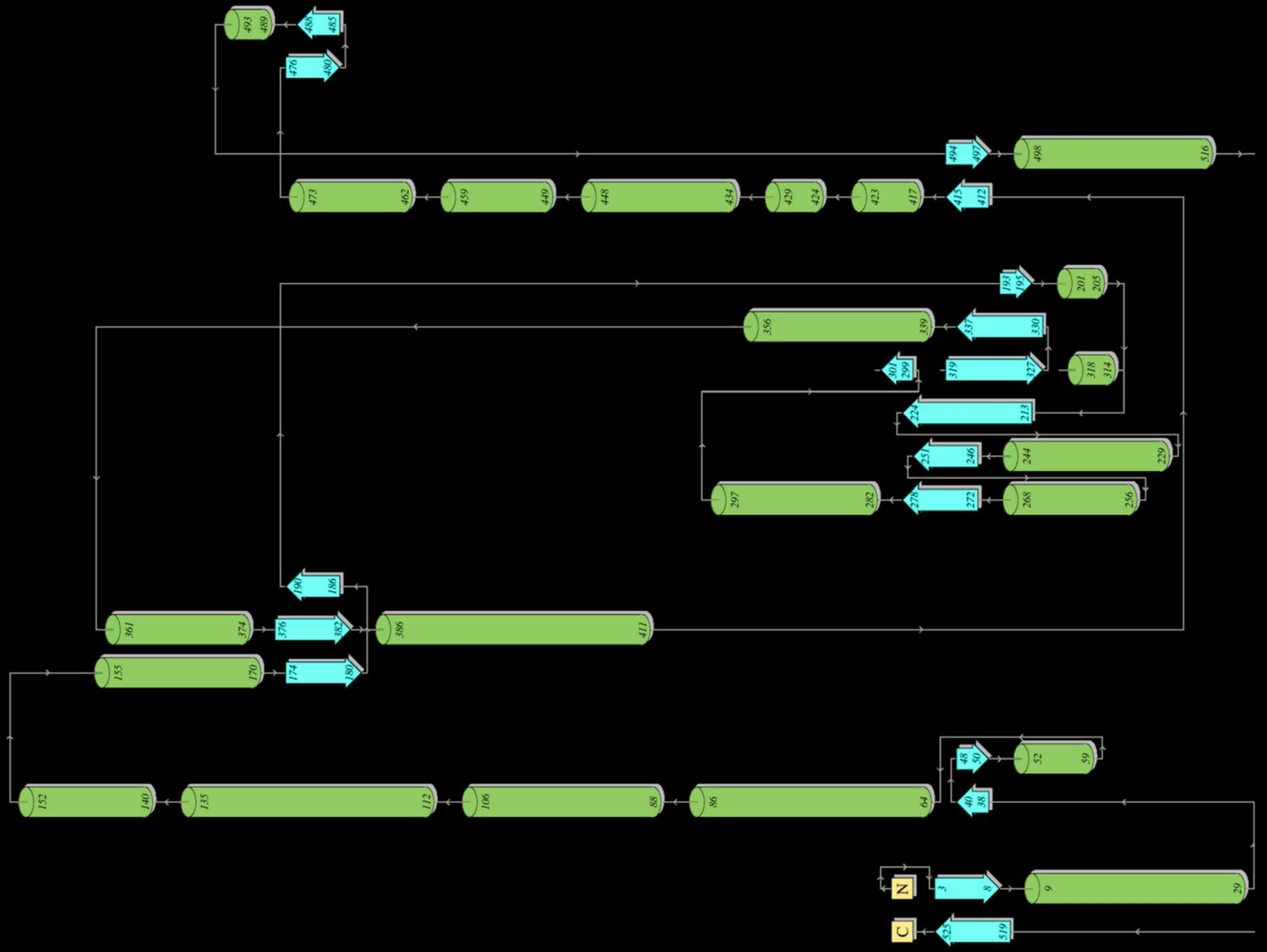
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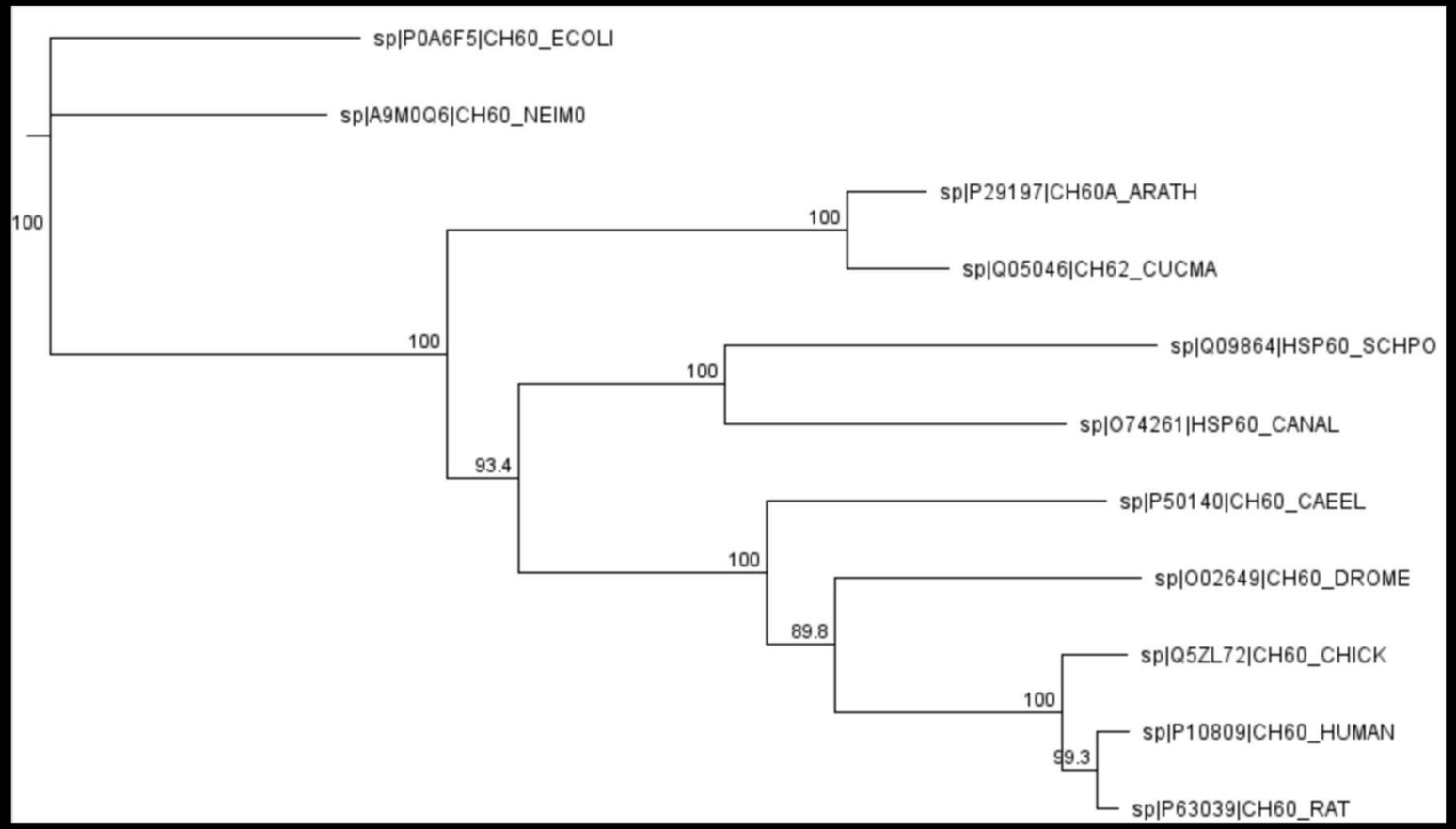
EXTRA MATERIAL



EXTRA MATERIAL



EXTRA MATERIAL: EVOLUTION



Genus Prime used to do the phylogenetic tree from the sequence alingment

SUPERIMPOSITOIN

Superimposition between a fungic group Ichaperonin glomalin, from *Rhizophagus irregularis,* and GroEL of *E. coli*



GroEL PDB ID: 1KP8
Resolution 2.0A

Glomalin PDB ID:)K47
Resolution 2.85A