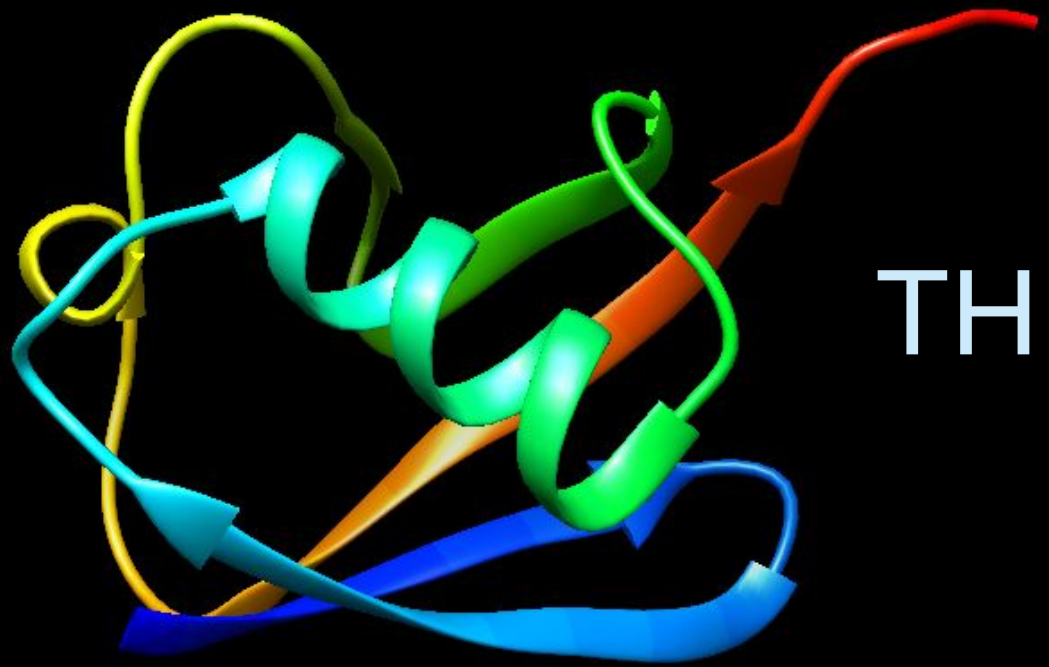

THE UBIQUITIN SYSTEM

Structural Biology 2023-2024

Maria Igual, Raoudha Somrani, David Roura and Laia Dalmacio

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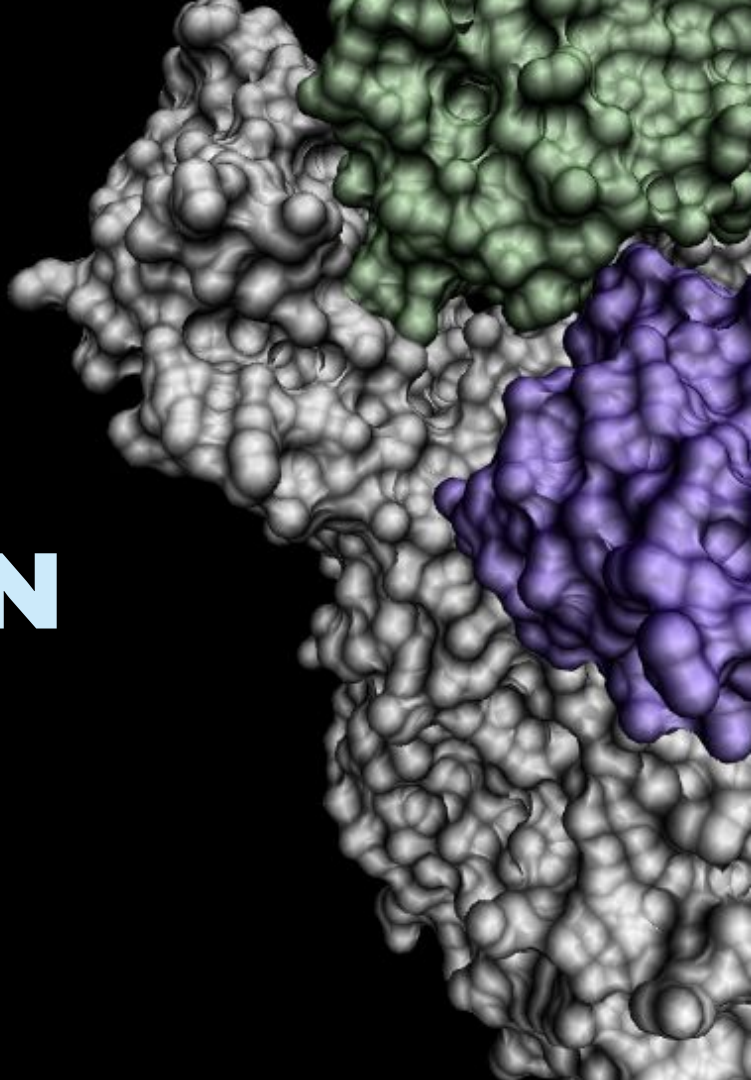
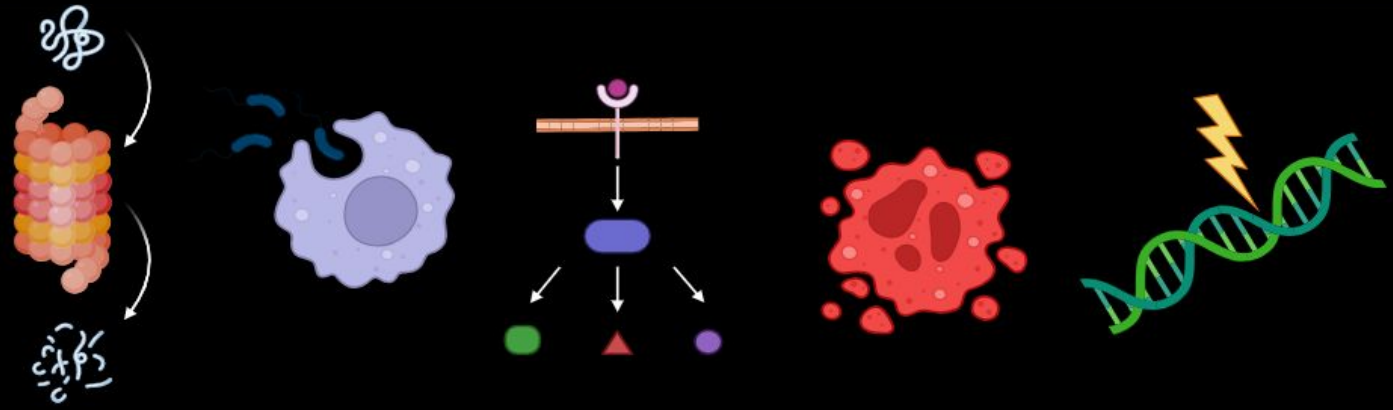
01.

UBIQUITINATION



INTRODUCTION



Normal ubiquitin signalling

Normal cellular function andhomeostasis

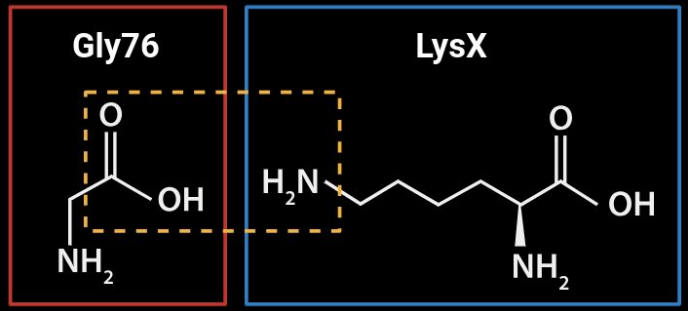
- proteostasis
- normal signalling
- cell survival and robustness
- adaption to stress

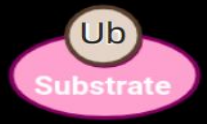
Dysfunctional ubiquitin signalling

Cellular dysfunction and disease

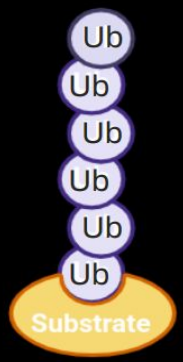
- developmental disease
- cancer
- immune disorders
- neurogenerative disease
- metabolic disorders
- ageing

UBIQUITIN FUNCTIONS

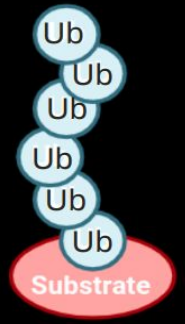




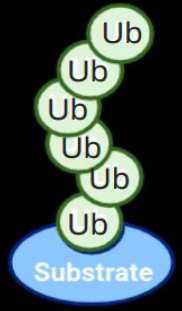
Mono-Ub
DNA repair



Poly-Ub (K48)
Proteasomal degradation

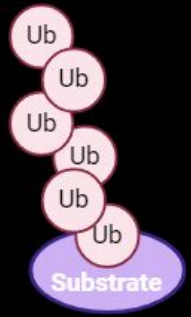


Poly-Ub (K27)
DNA damage
Antiviral immunity

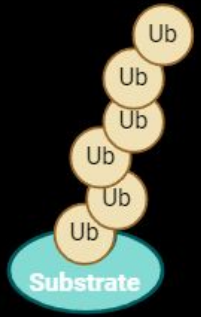


Poly-Ub (K33)
Interferon signaling
Autophagy

Poly-Ub (K63)
DNA damage
Anti-bacterial autophagy



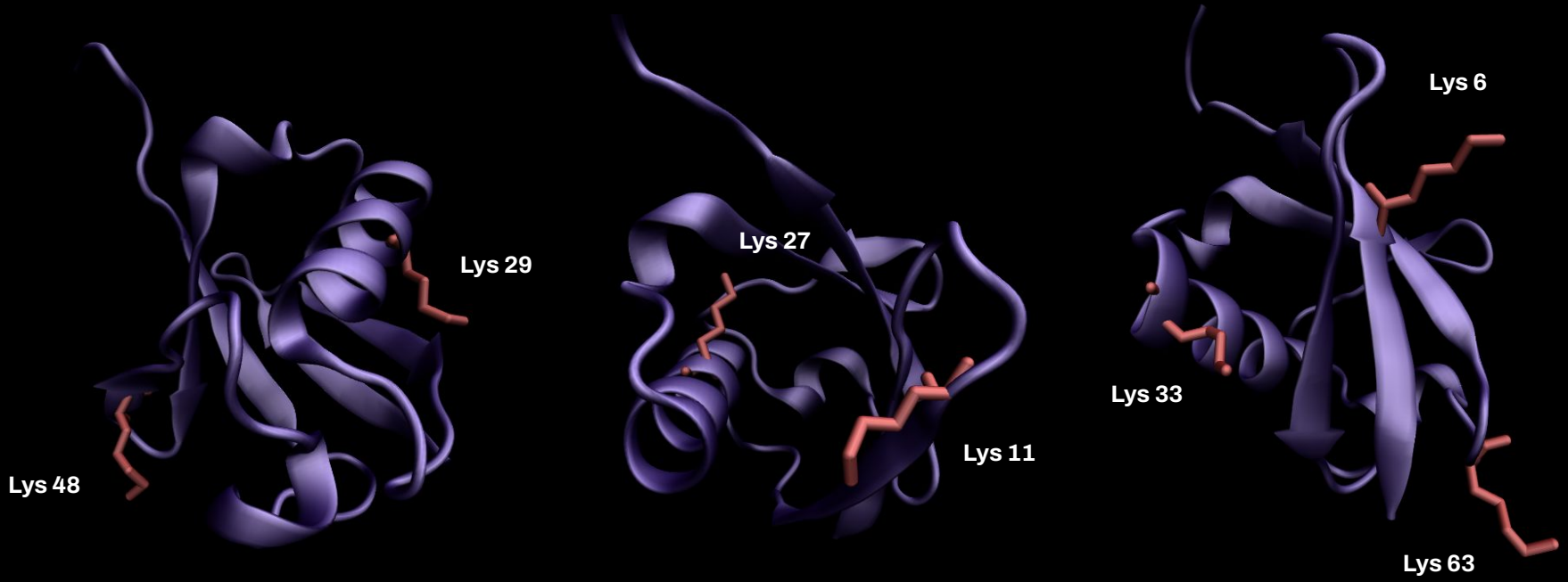
Poly-Ub (K6)
Protein stabilitazion and non-degradative process



Poly-Ub (K11)
Regulation cell-cycle proteasome-mediate degradation

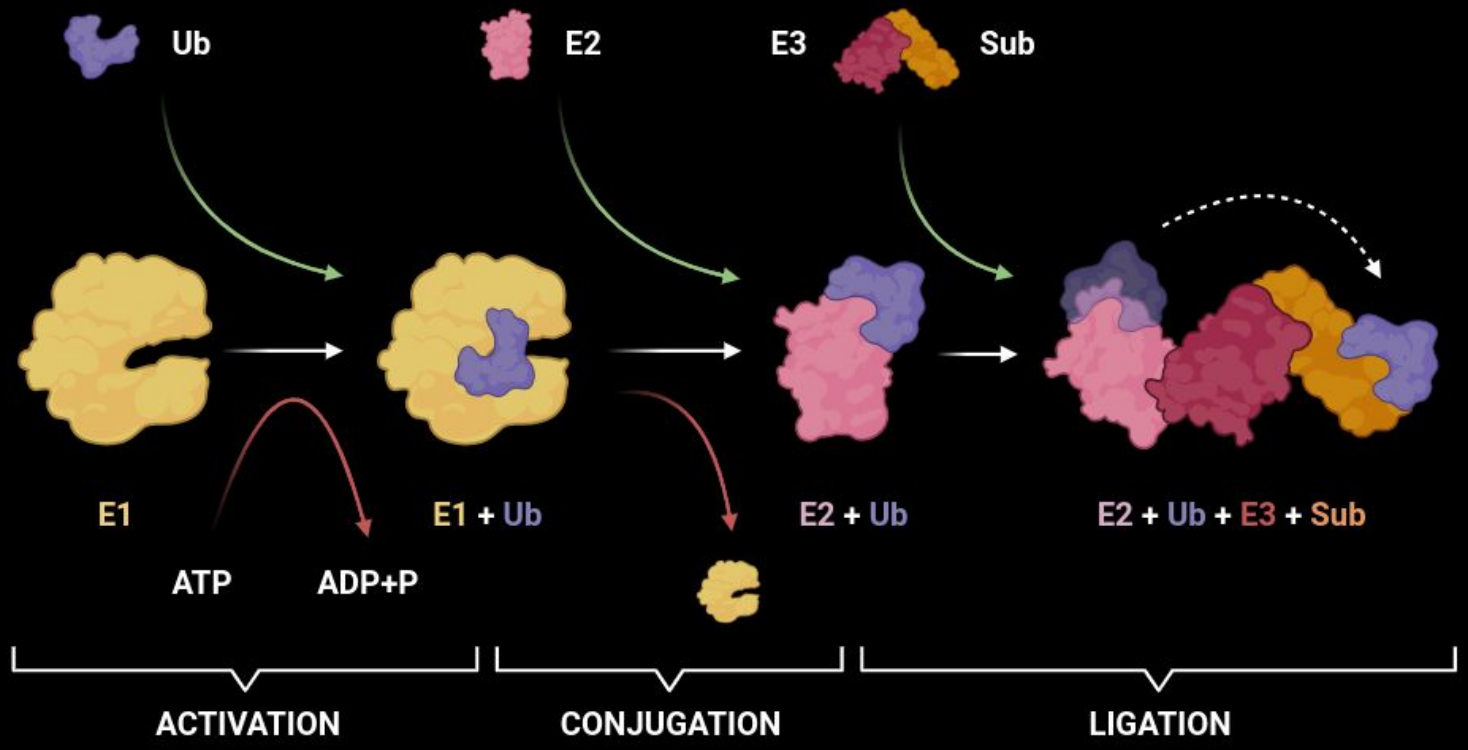
Poly-Ub (K29)
proteoxit stress response and cycle

Lysine residues

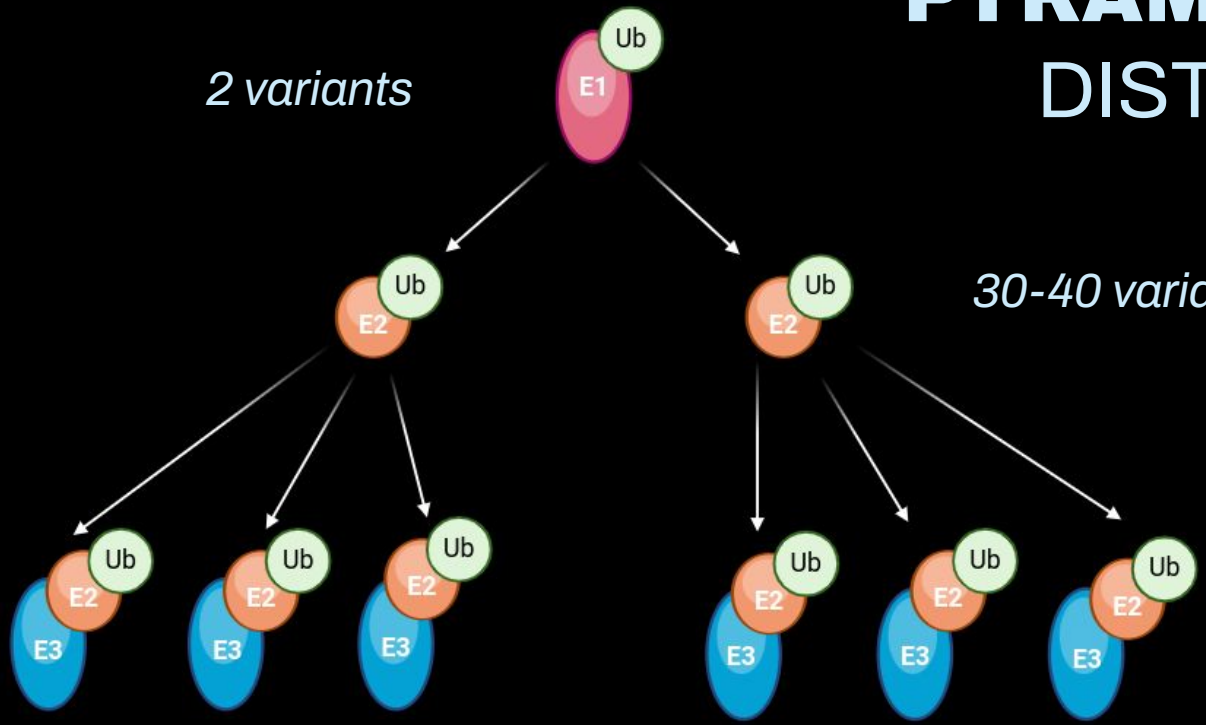


PDB: 1UBQ

PATHWAY


Ub
E2
E3
Sub
E1
E1 + Ub
E2 + Ub
E2 + Ub + E3 + Sub
ATP
ADP+P
ACTIVATION
CONJUGATION
LIGATION

PYRAMIDE-LIKE DISTRIBUTION



2 variants

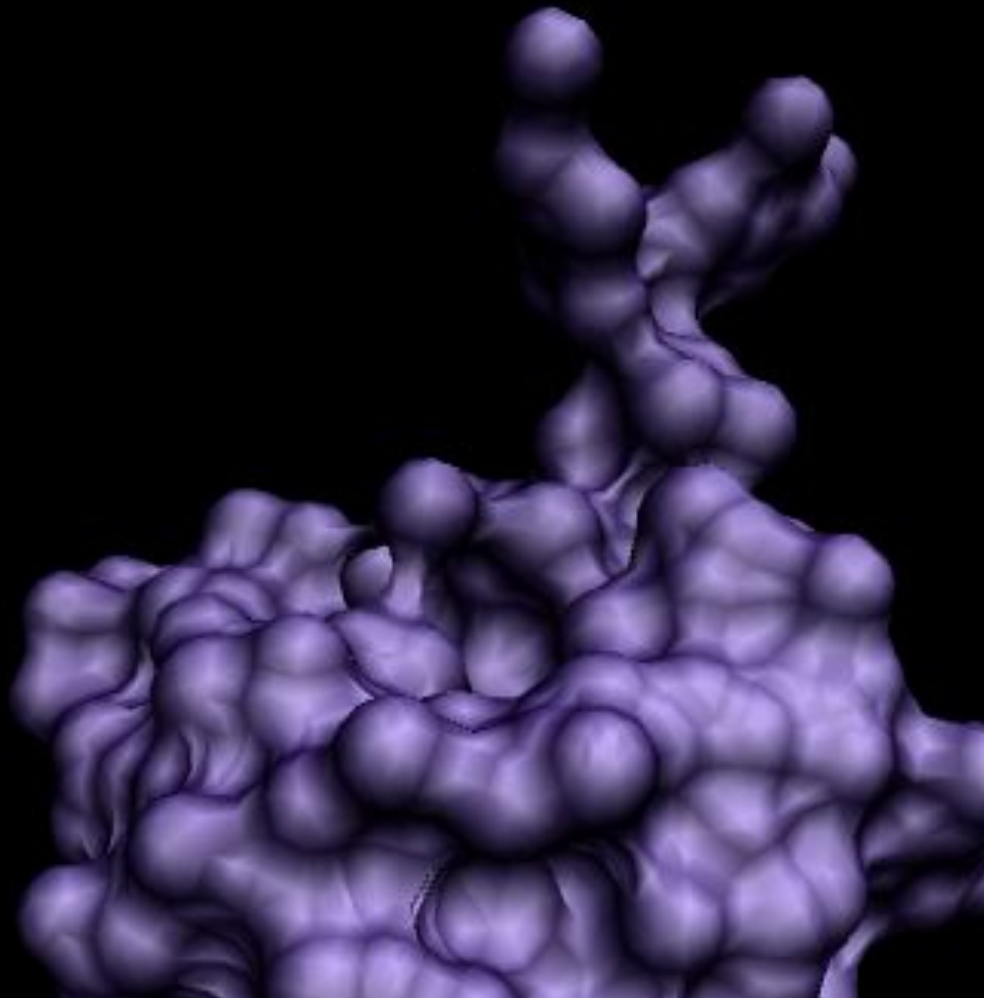
30-40 variants

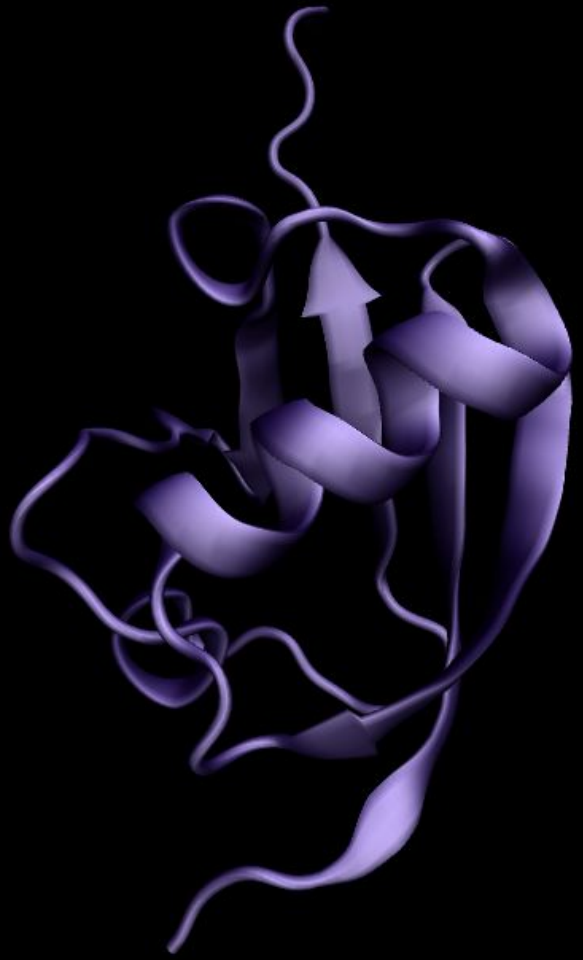
+500 variants

**data based on the human proteome*

02.

UBIQUITIN.





SCOP CLASSIFICATION

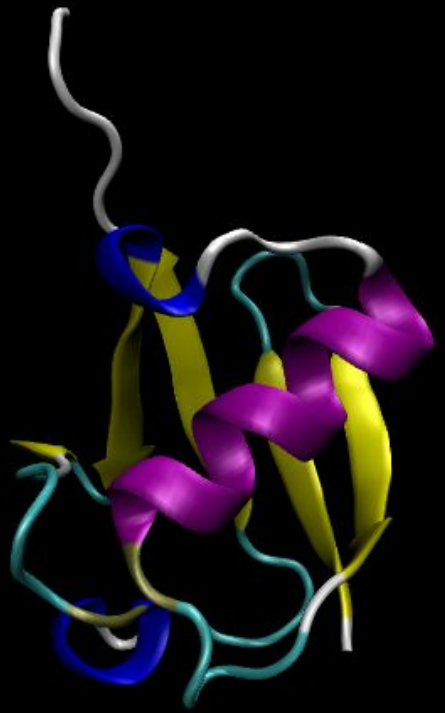
Class
Alpha and beta proteins (α + β)

Fold
beta-Grasp (ubiquitin-like)

Superfamily
Ubiquitin-like

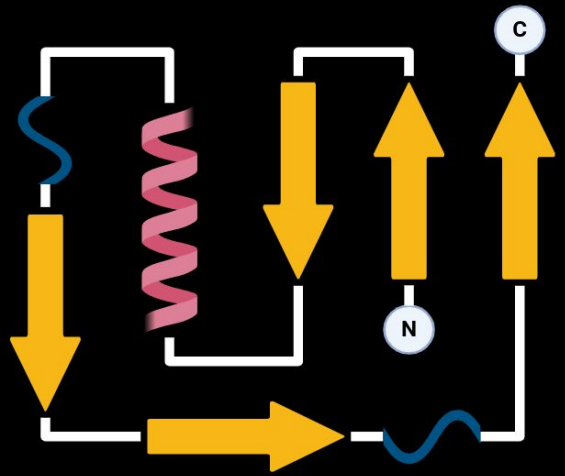
Family
Ubiquitin-related

TERTIARY STRUCTURE



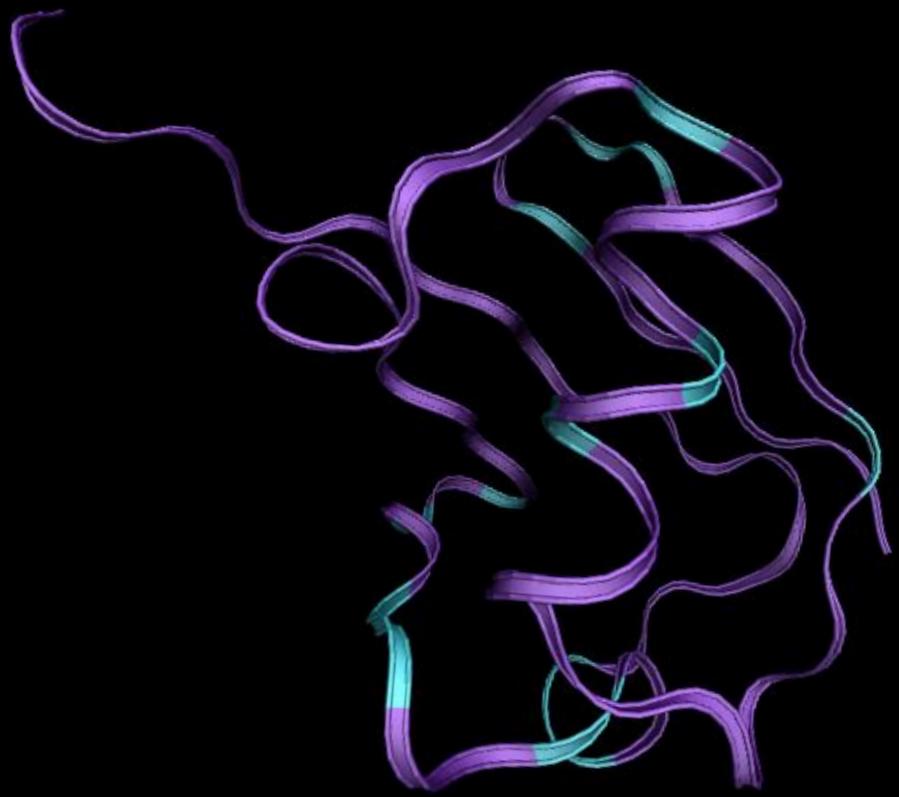
- β-strand
- α-helix
- 3_{10}-helix
- Loop

Ub Topological diagram



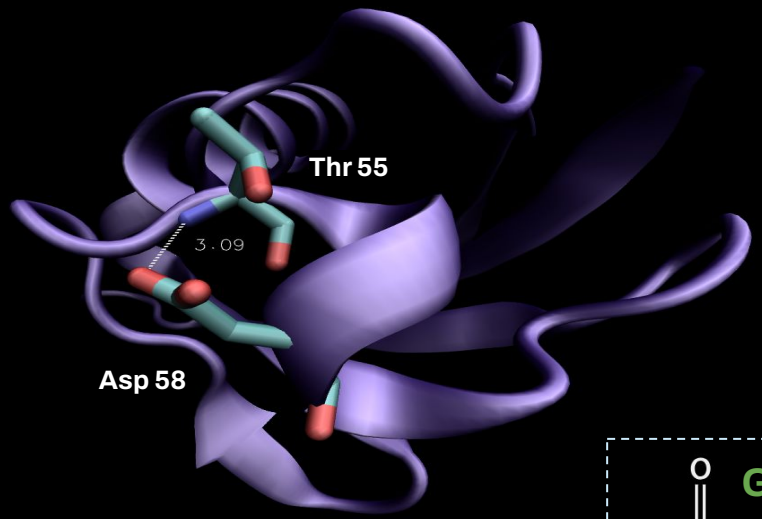
[1] MQIFVKTLTG KTITLEVEPS DTIENVKAKI QDKEGIPPDQ QRLIFAGKQL EDGRTLSDYN

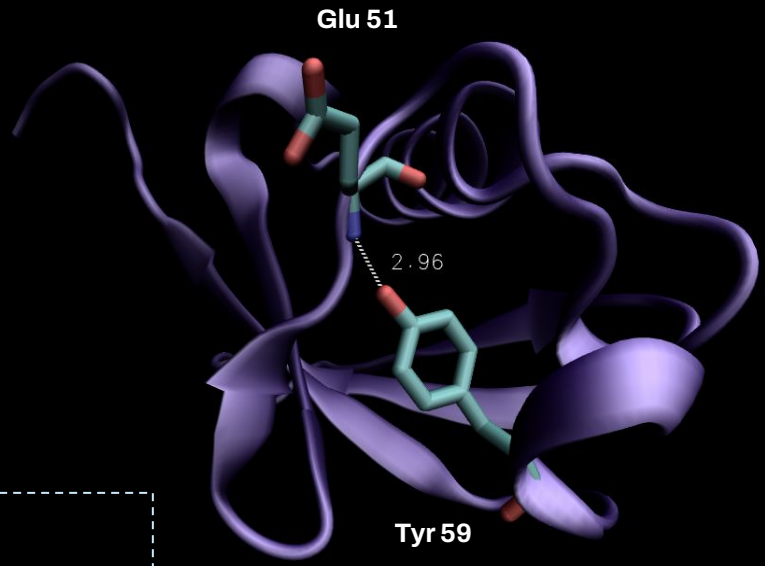
[61] IQKESTLHLV LRLRGG

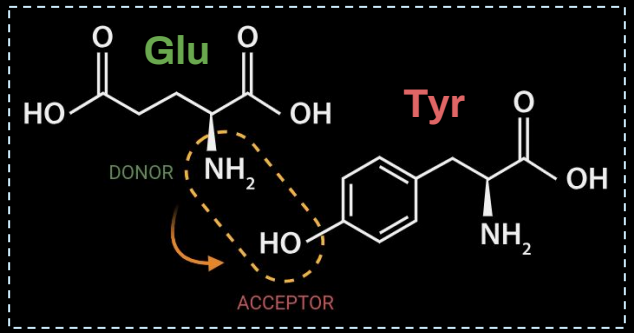


UBIQUITIN
INTERNAL INTERACTIONS

Hydrogen bonds

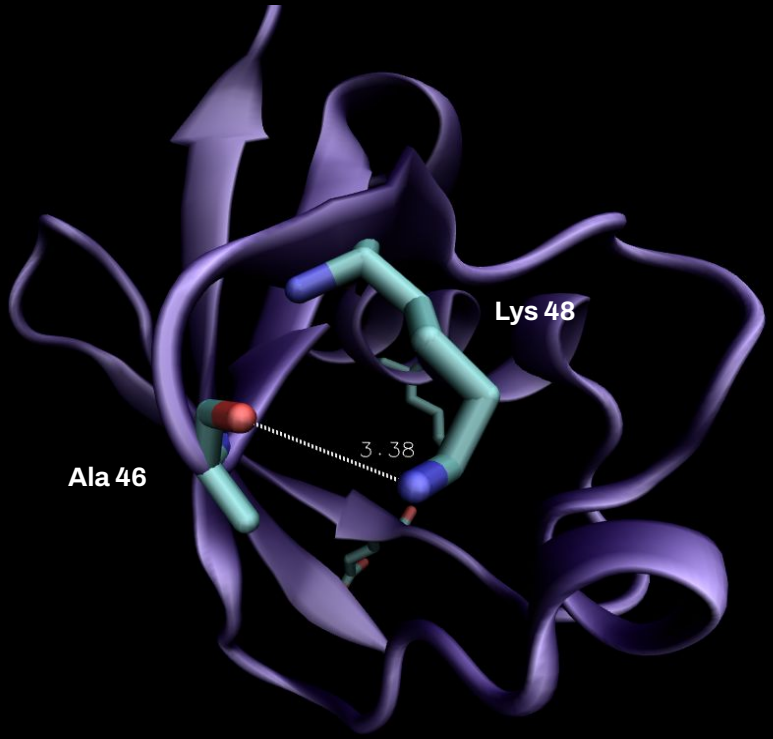


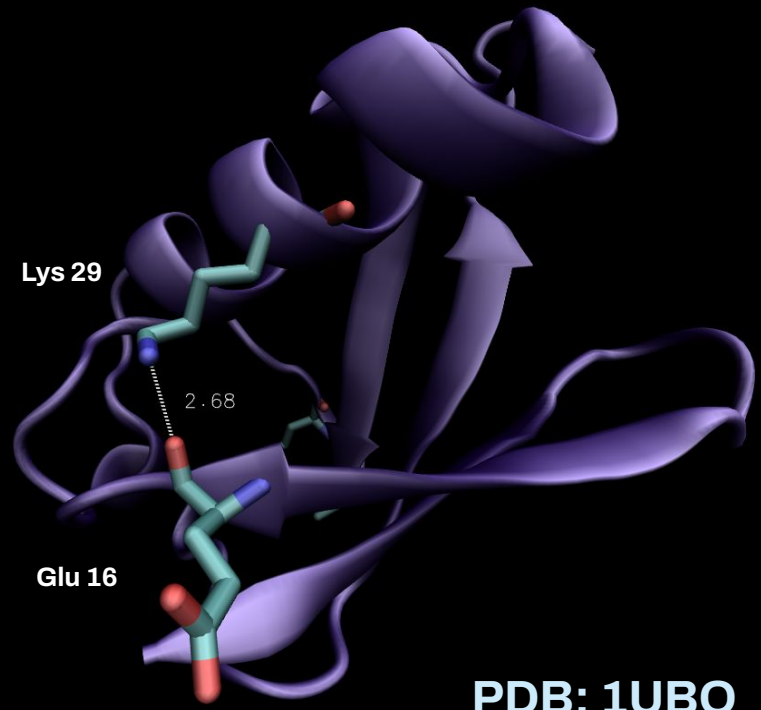




PDB: 1UBQ
(H.sapiens)

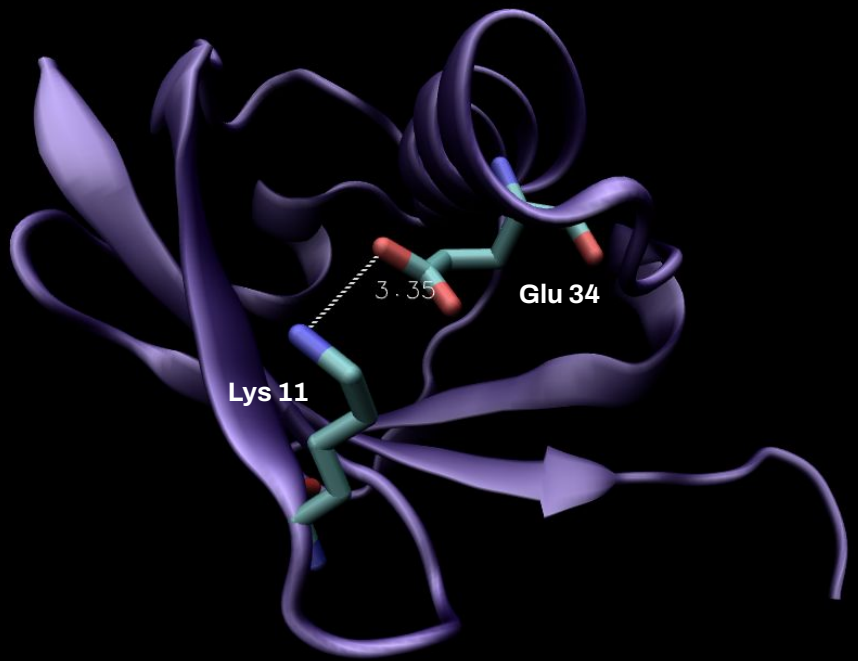
Hydrogen bonds





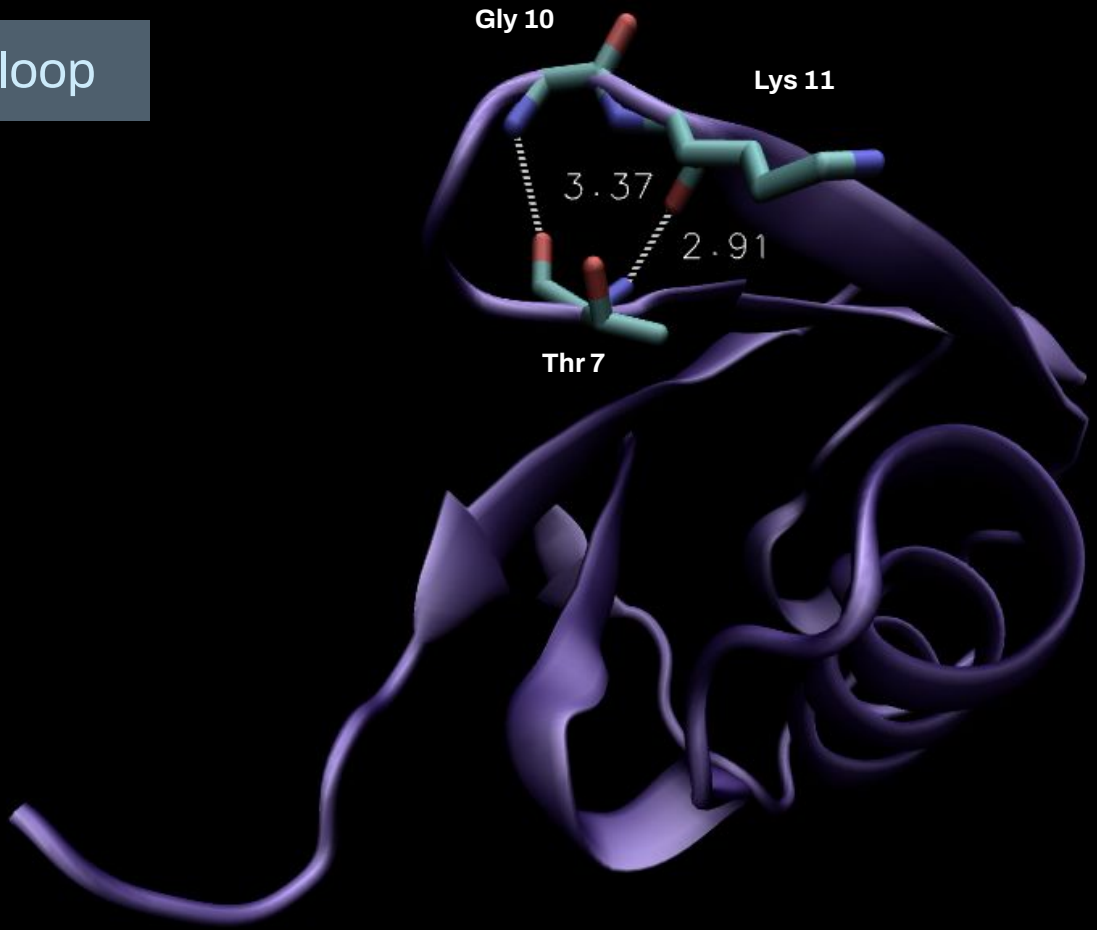
PDB: 1UBQ

Salt bridges



PDB: 1UBQ

Beta bulge loop

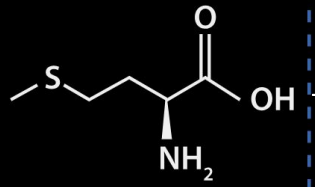


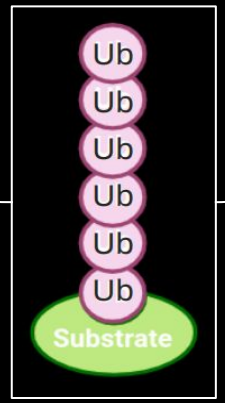
PDB: 1UBQ

N-terminal

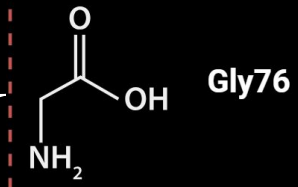
C-terminal

Met1

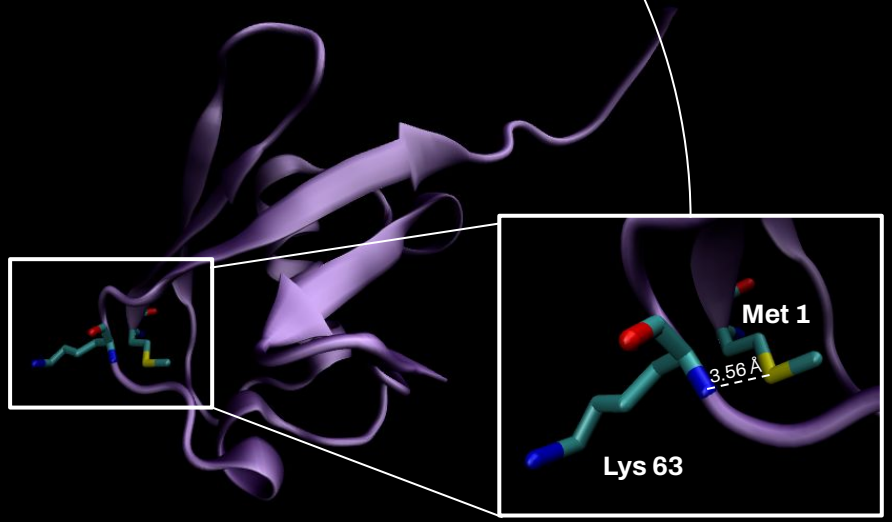




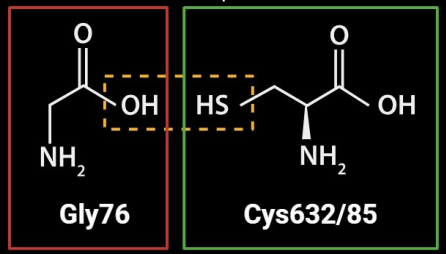
Isopeptide bond



Gly76

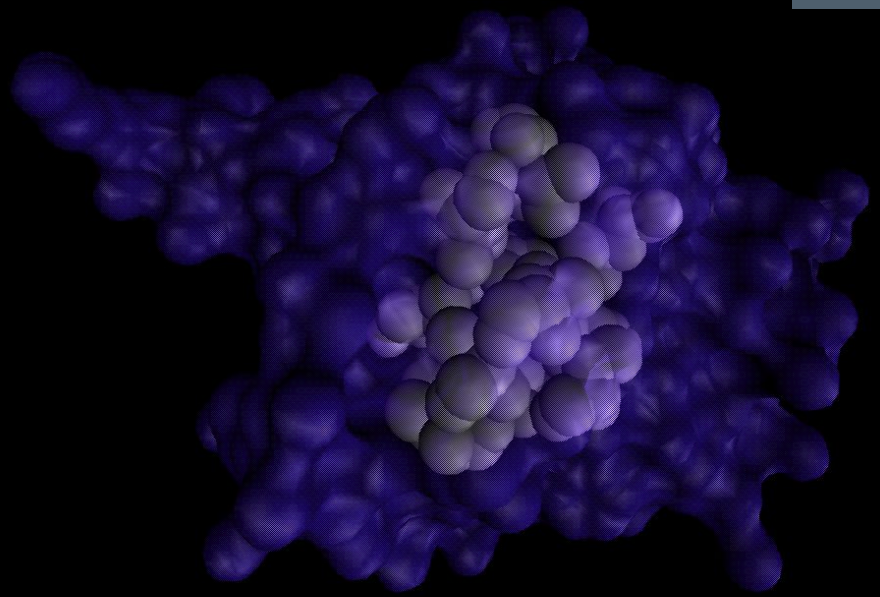


Hydrogen bond



Thioester bond

HYDROPHOBIC CORE

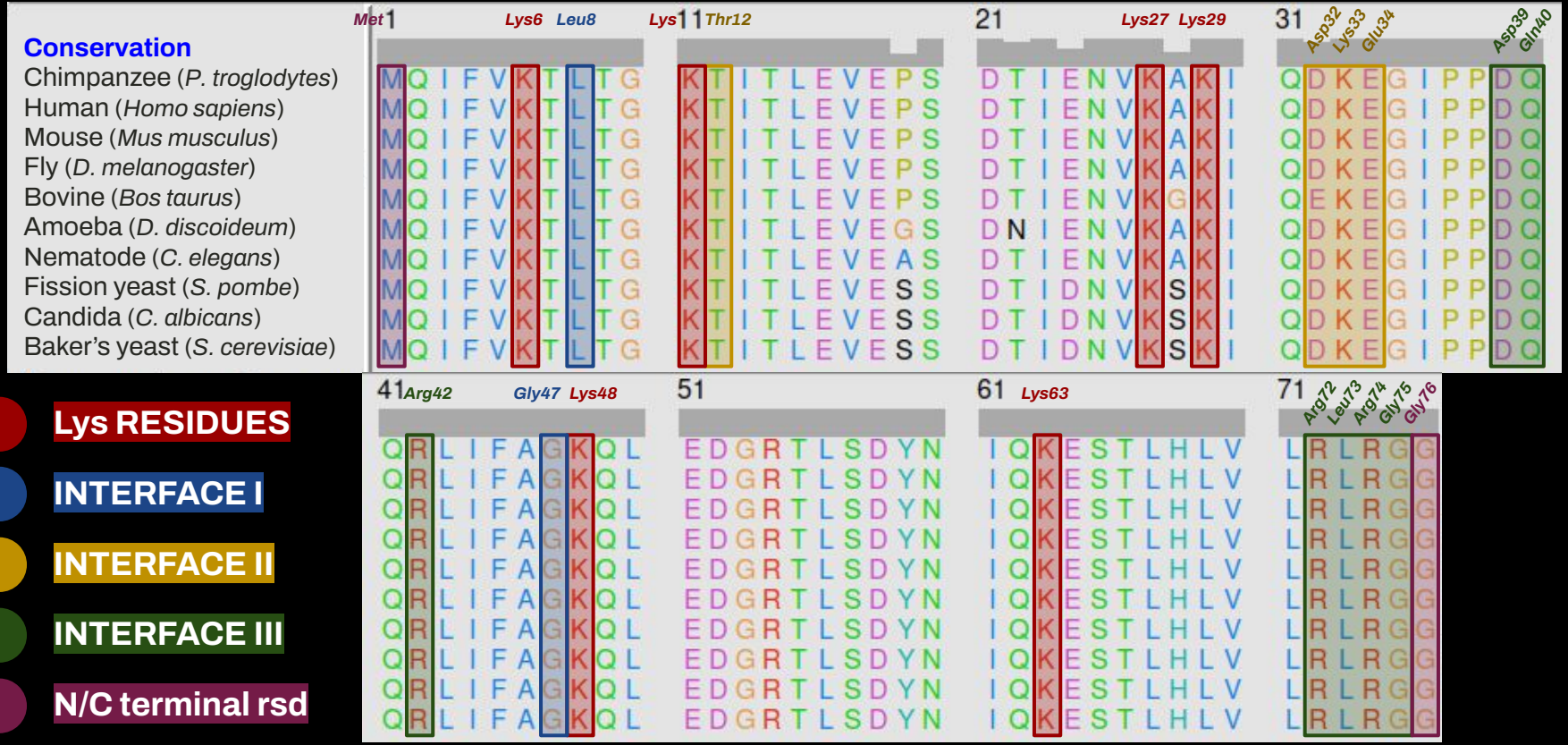


1 MQIFVKTLTG KTITLEVEPS DTIENVKAKI QDKEGIPPDQ QRLIFAGKQL EDGRTLSDYN

61 IQKESTLHLV LRLRGG

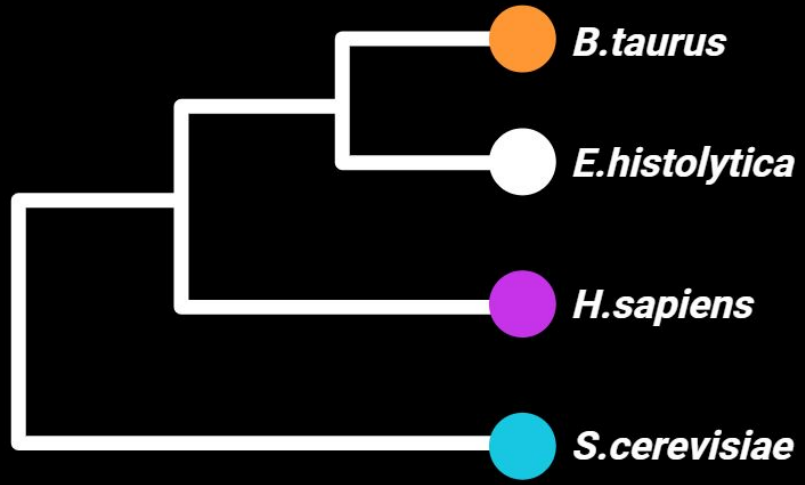
PDB: 1UBQ

MULTIPLE SEQUENCE ALIGNMENT



STRUCTURAL ALIGNMENT

RMSD: 0.45
SCORE: 8.95

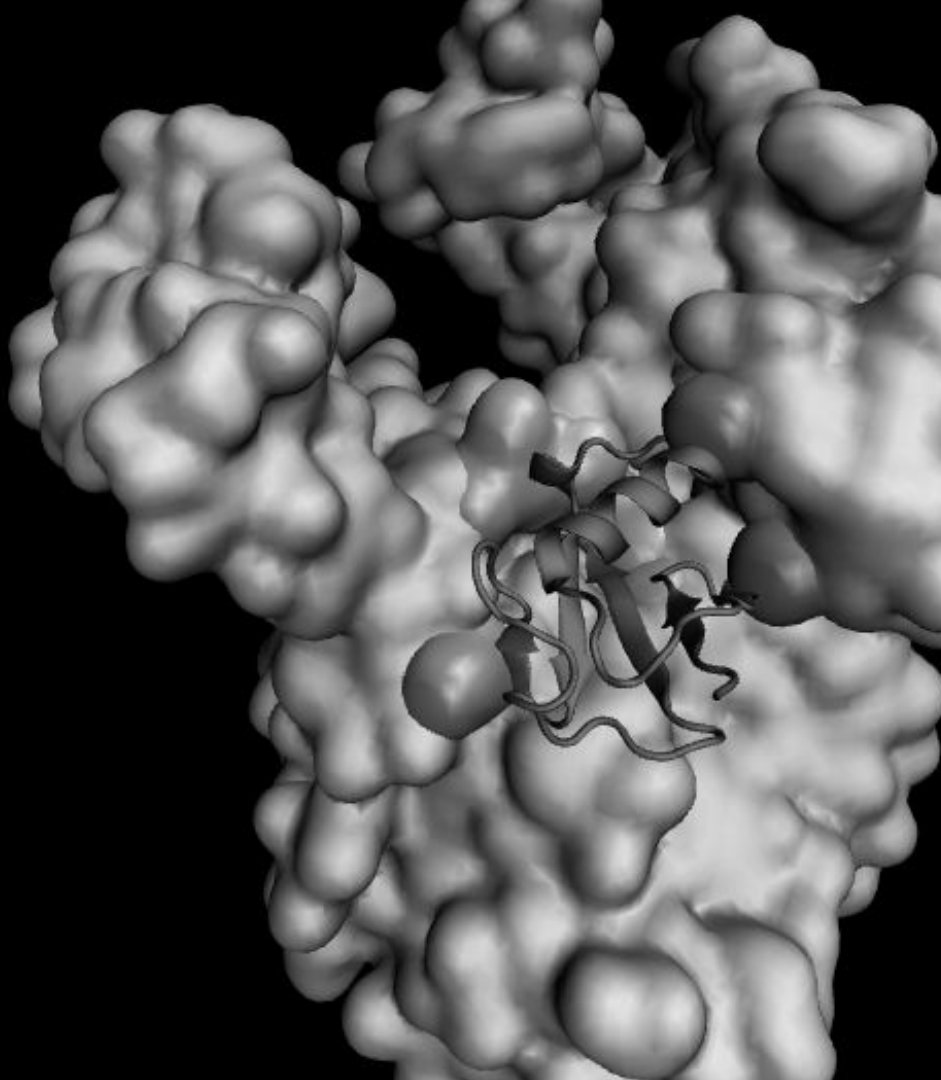


PDB ID: 6ZQH - *S.cerevisiae*
PDB ID: 1UBQ - *H.sapiens*
PDB ID: 2ZCC - *B.taurus*
PDB ID: 4GSW - *E.histolytica*

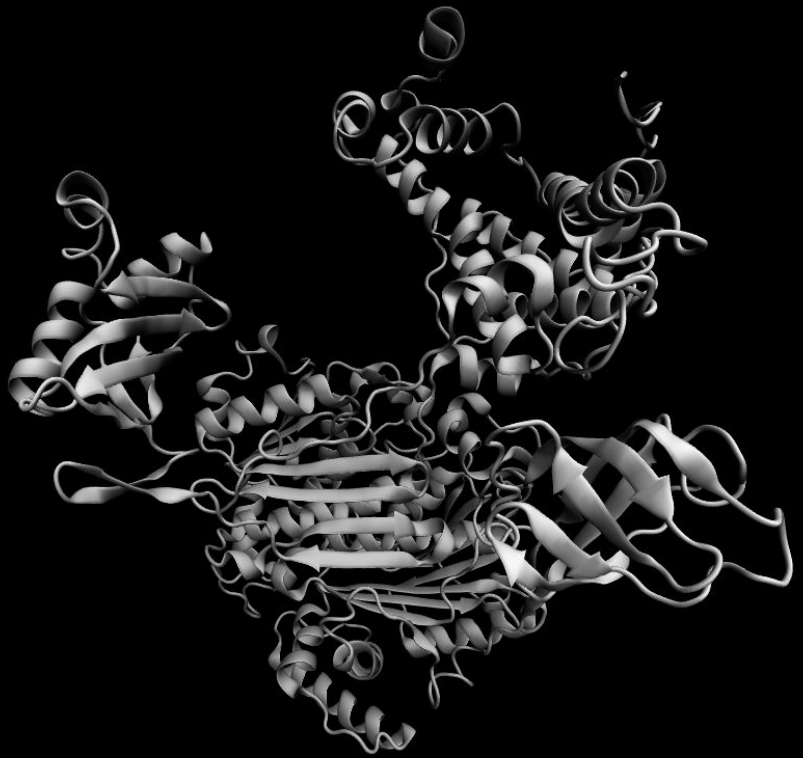
03.

E1.

Ub-activating enzyme



SCOP CLASSIFICATION



Class
Alpha and beta proteins (α/β)

Fold
Activating enzymes of the ubiquitin-like proteins

Superfamily
Activating enzymes of the ubiquitin-like proteins

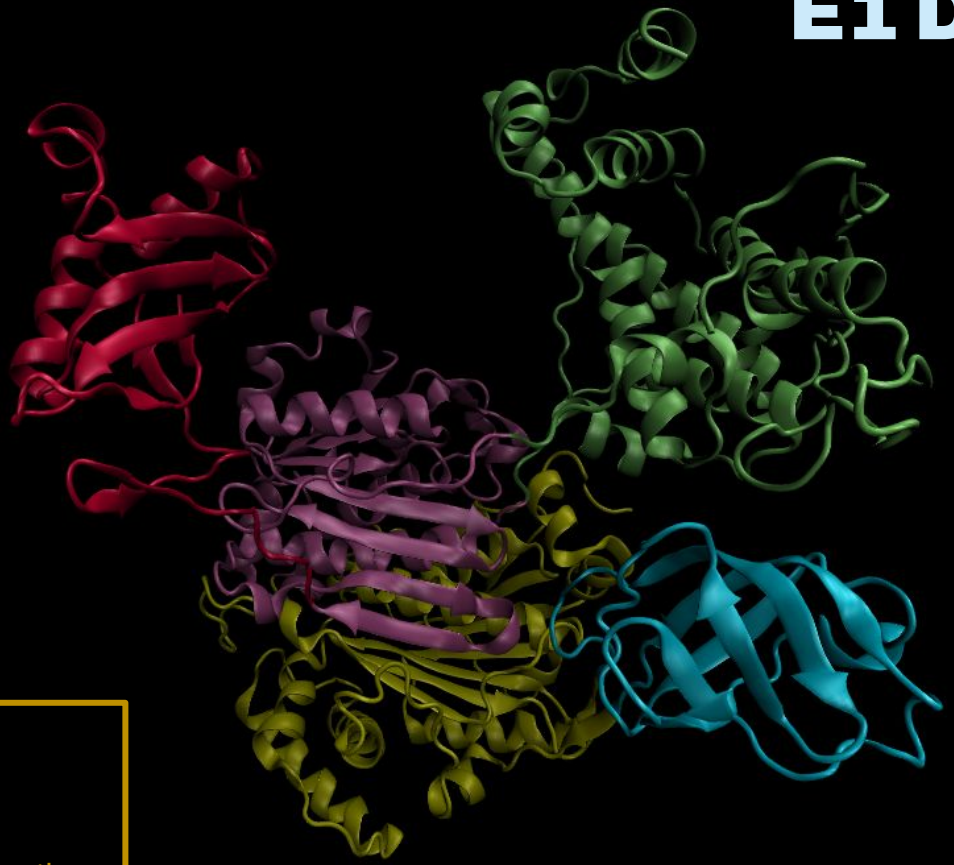
Family
Ubiquitin activation enzymes (UBA)

E1 DOMAINS

UFD
- Present in the C-terminus
- Recruitment of specific E2s

AAD
- Bound to ATP and Ub noncovalently
- Catalytically active in the adenylation reaction

IAD
- Present in the N-terminus
- Catalytically inactive

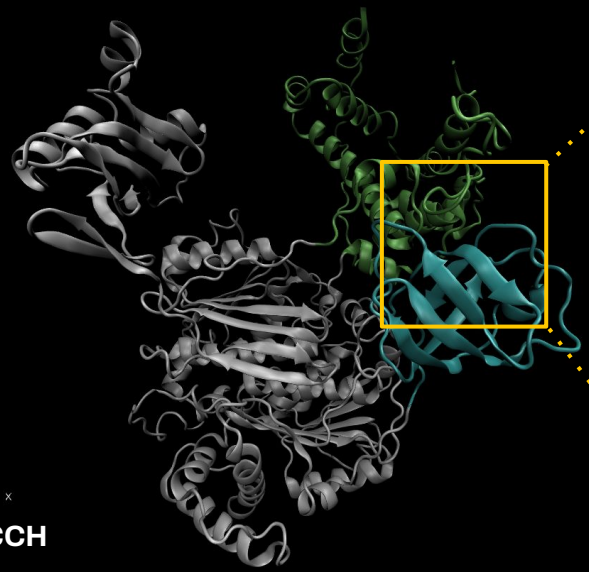


SCCH
- Carries the catalytic cysteine
- Forms a thioester bond with Ub

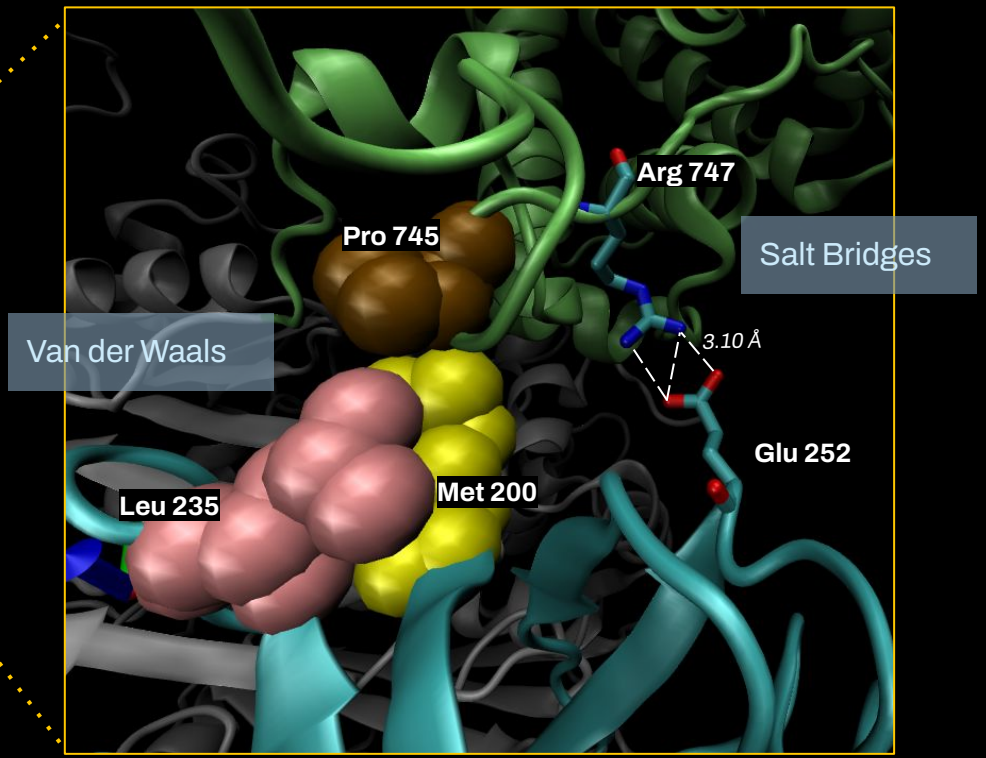
FCCH
- Associates with IAD
- Non-functional

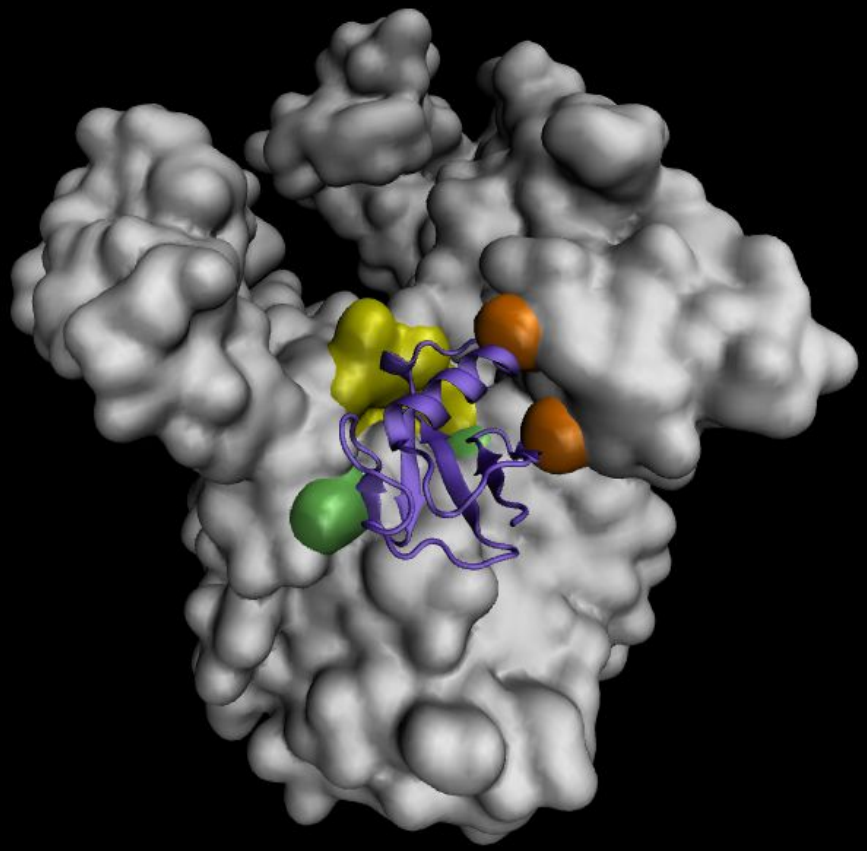
PDB: 6DC6
(H. sapiens)

FCCH-SCCH INTERACTIONS



PDB: 6DC6

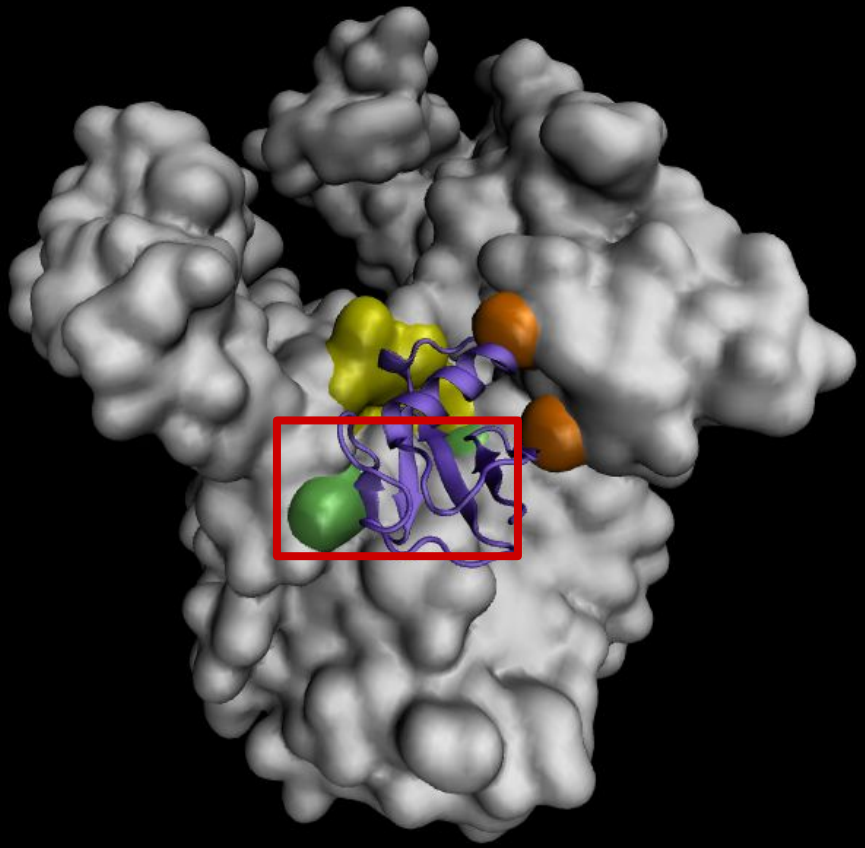




Ub-E1 INTERACTIONS

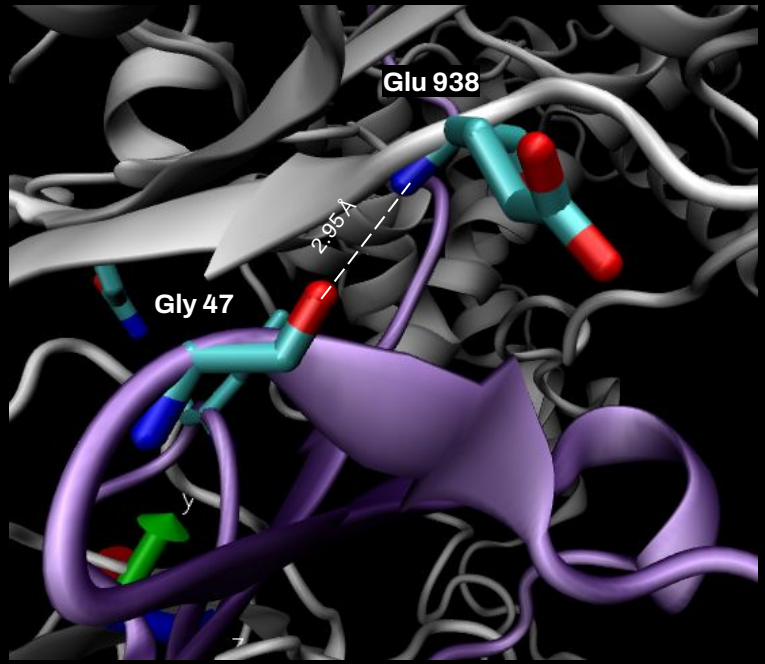
- Interface I
- Interface II
- Interface III

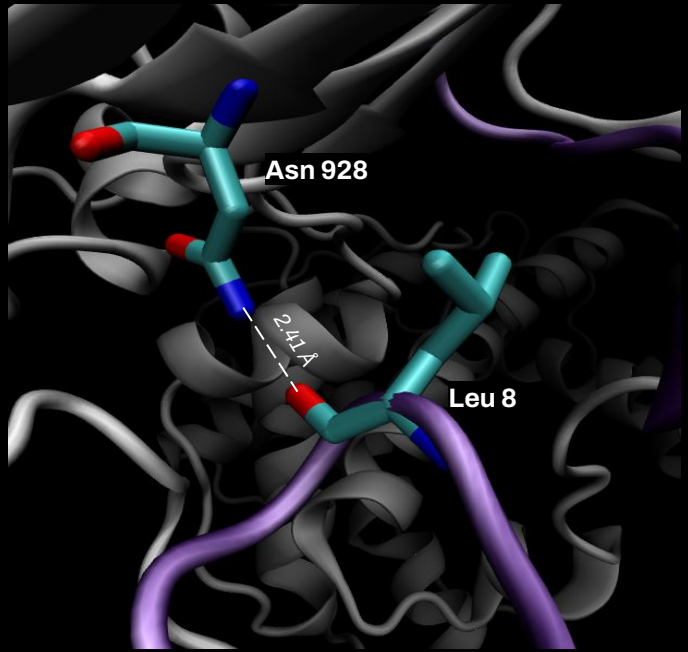
PDB: 6DC6



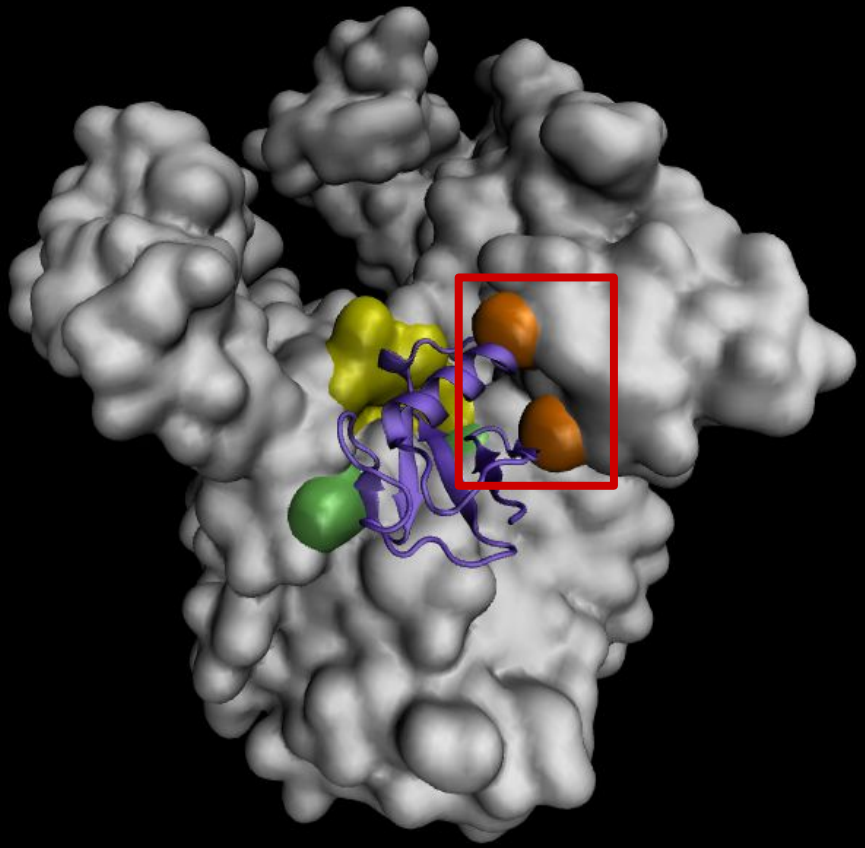
INTERFACE I

Hydrogen bonds



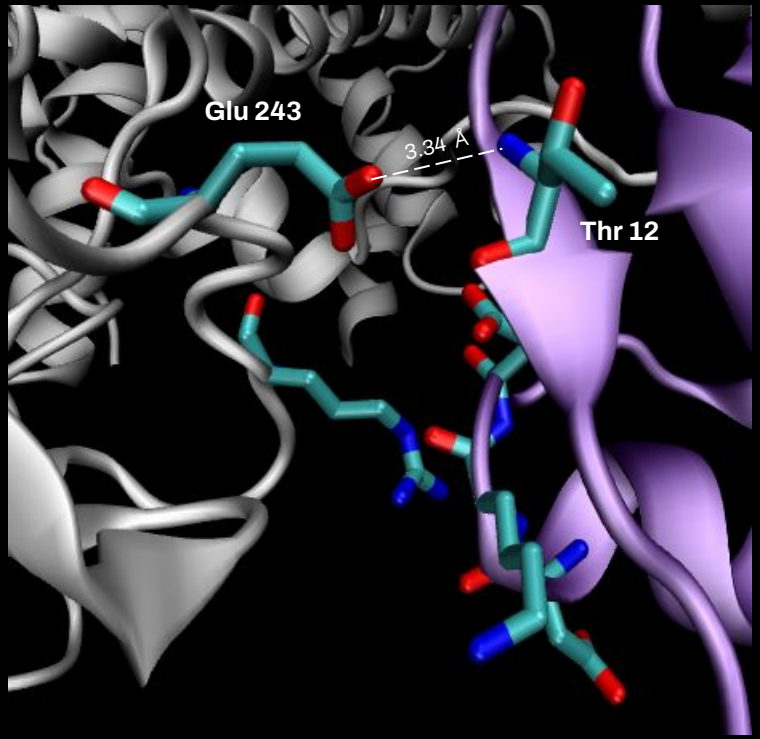


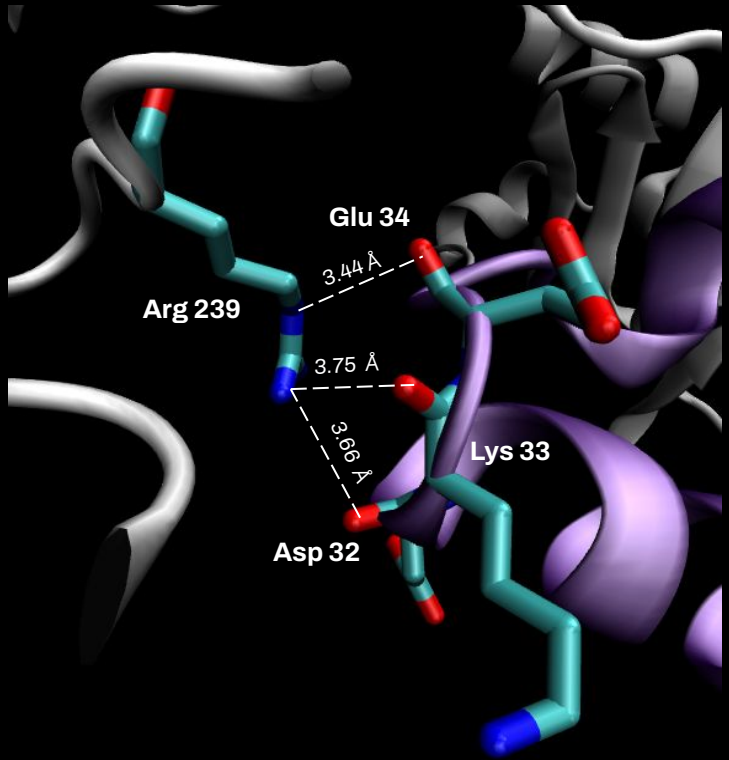
PDB: 6DC6



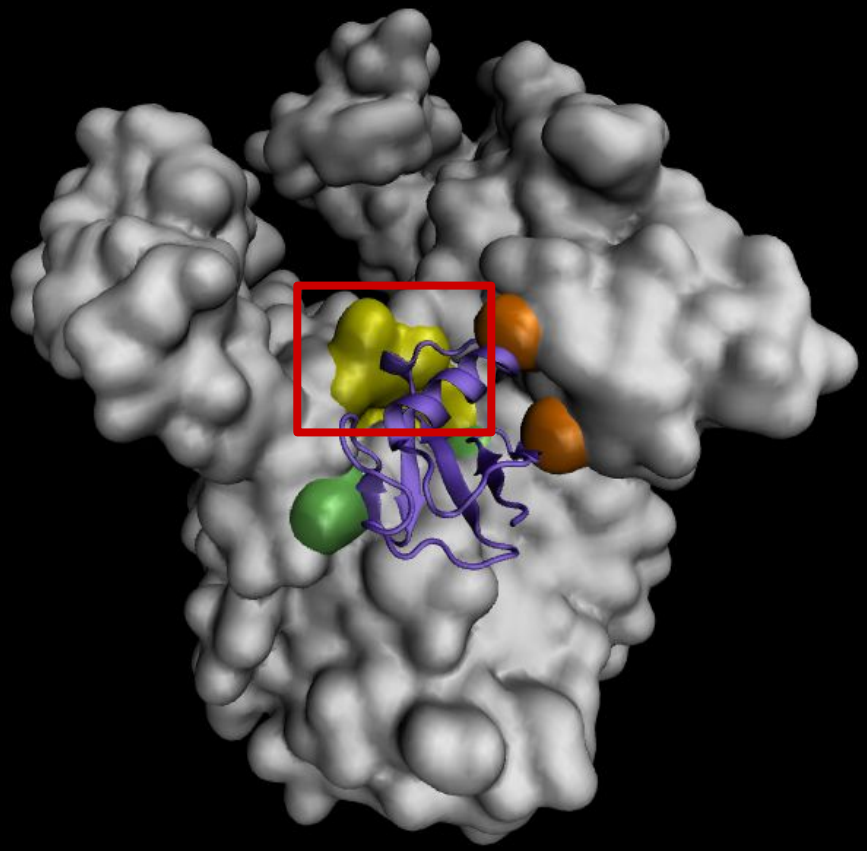
INTERFACE II

Hydrogen bonds



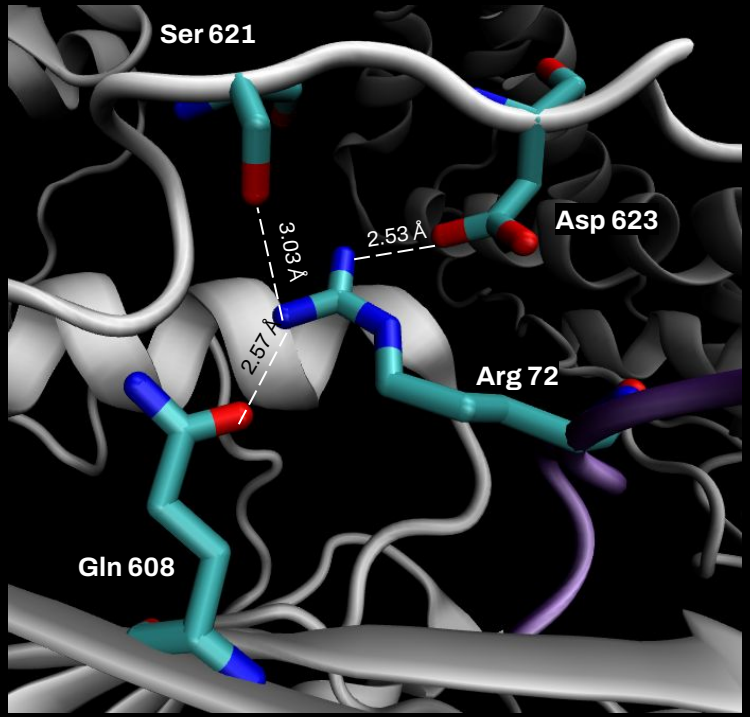


PDB: 6DC6



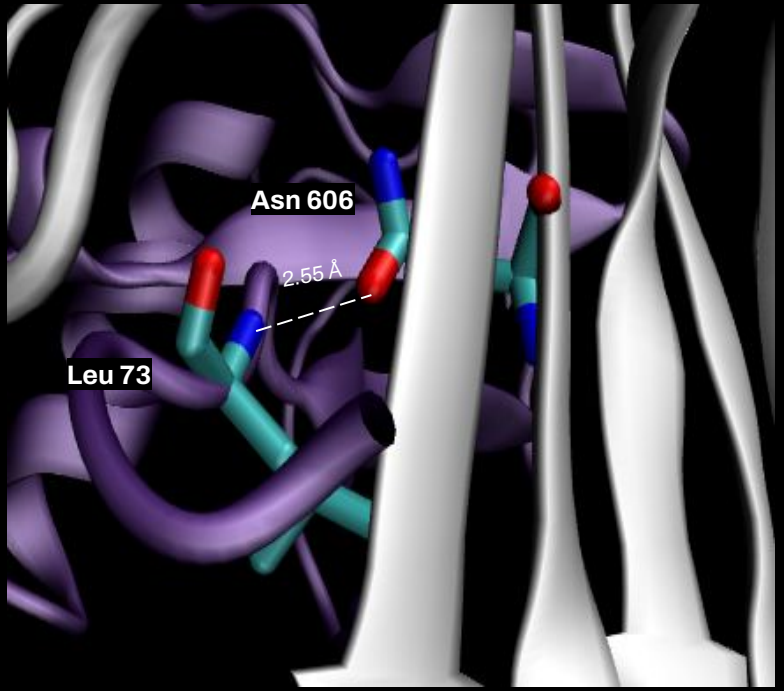
INTERFACE III

Crossover loop



AAD

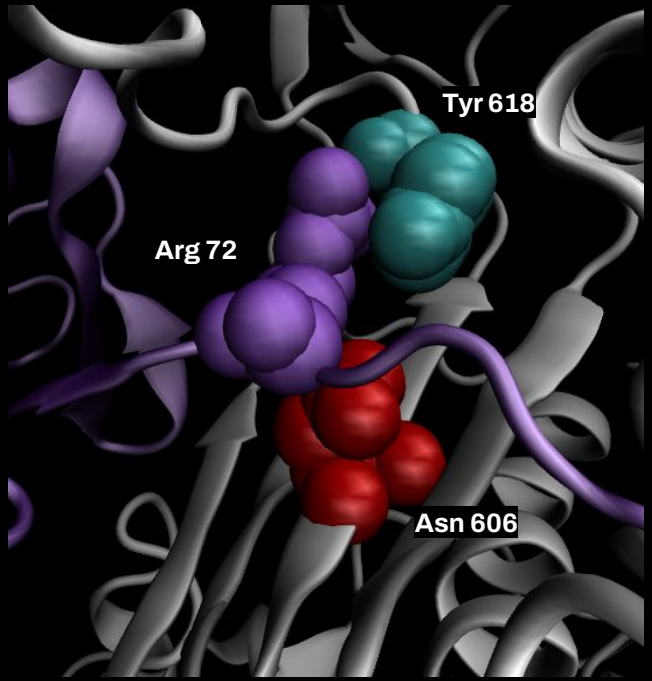
Hydrogen bonds



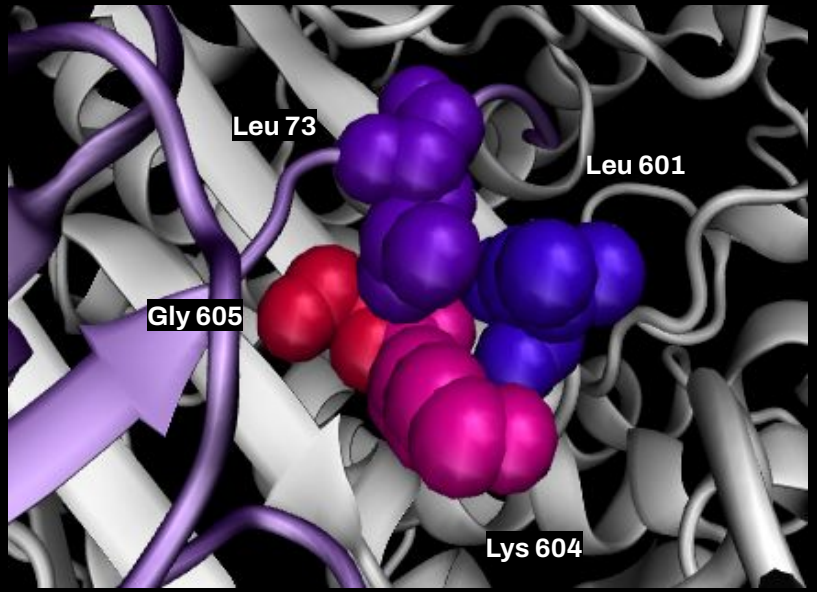
PDB: 6DC6

PDB: 6DC6

Van der Waals

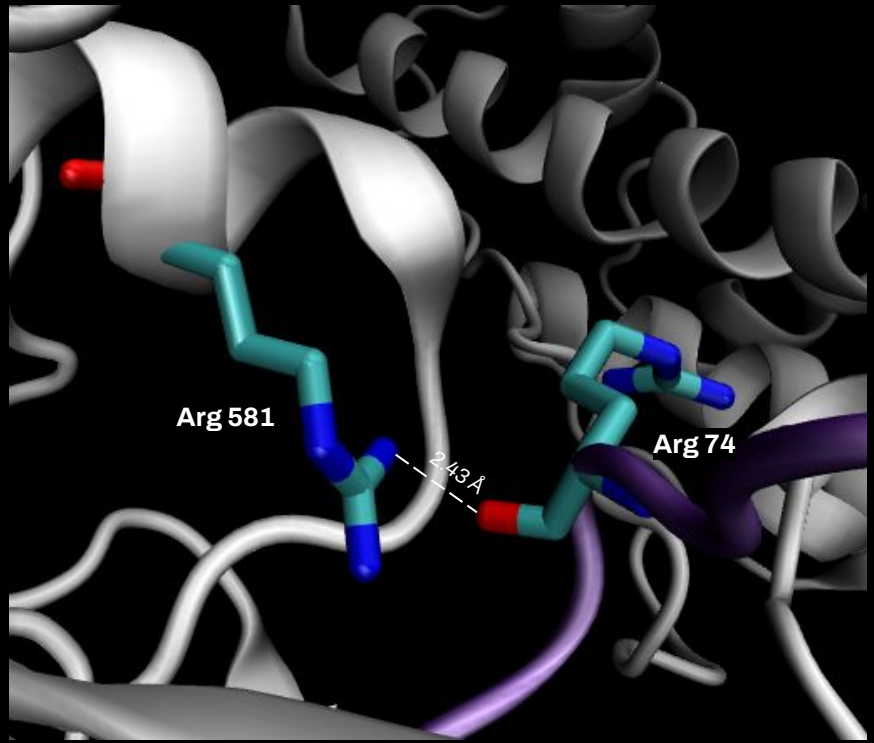


- AAD residues (E1)
- Crossover loop residues (E1)
- Ubiquitin

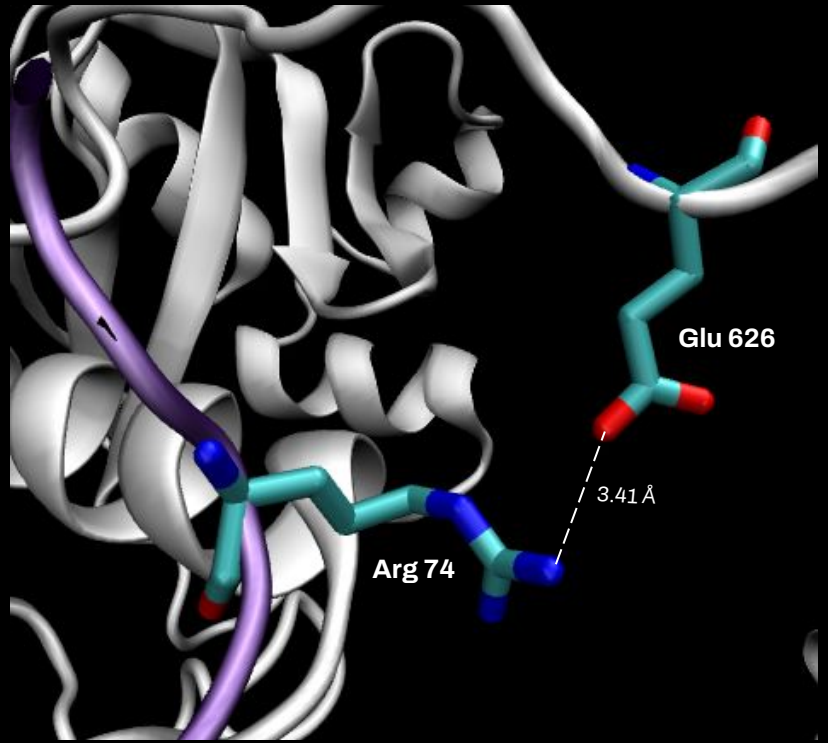


- AAD domain residues (E1)
- Ubiquitin

Hydrogen Bonds



Salt Bridges

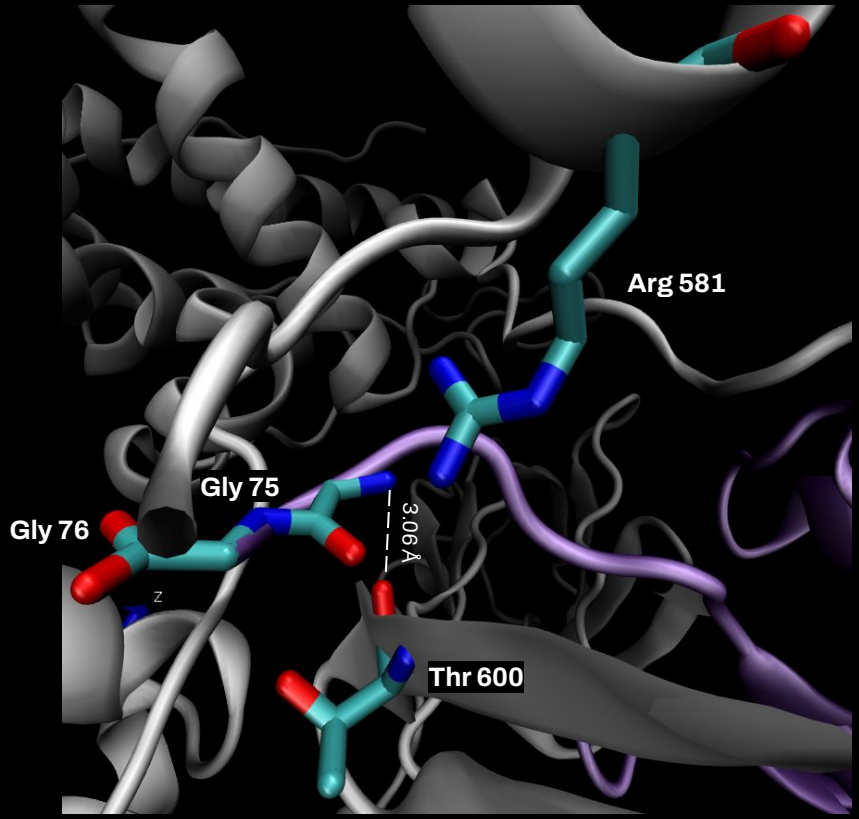


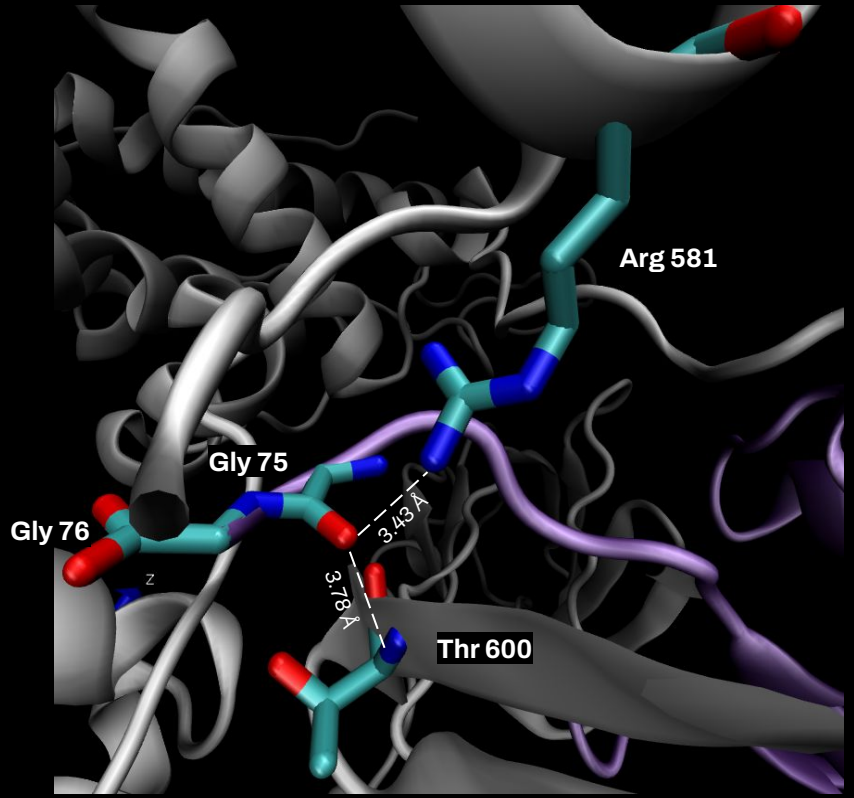
AAD(E1)

Crossover loop(E1)

PDB: 6DC6

Hydrogen bonds



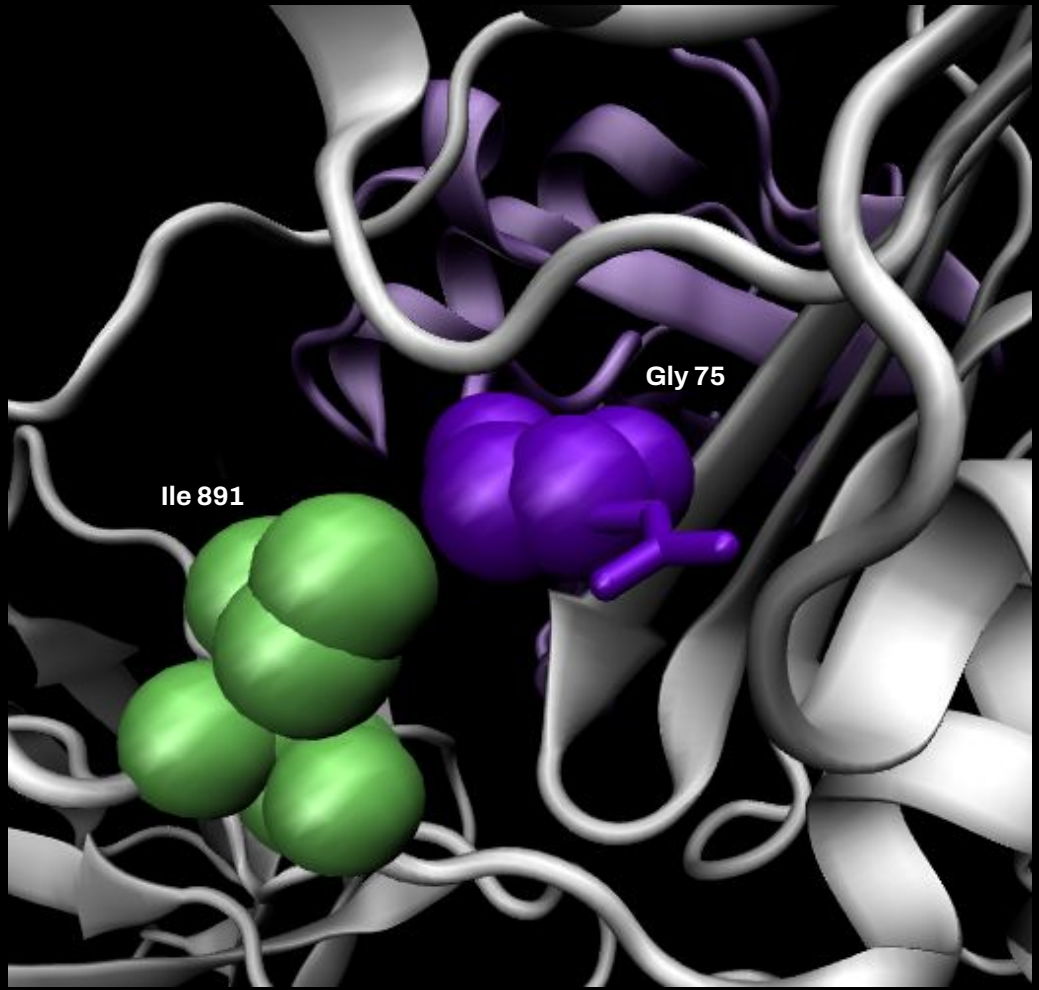


AAD(E1)

Ubiquitin

PDB: 6DC6

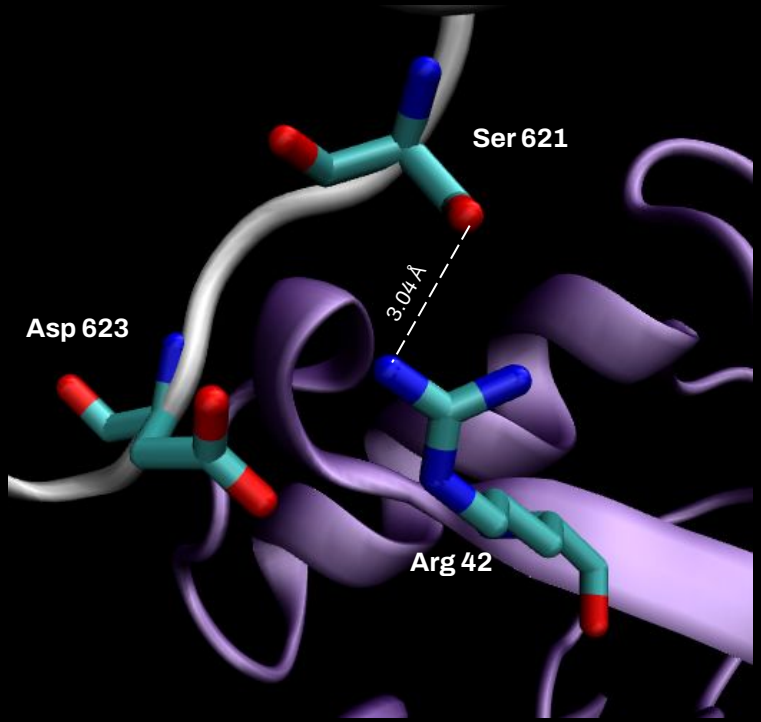
Van der Waals



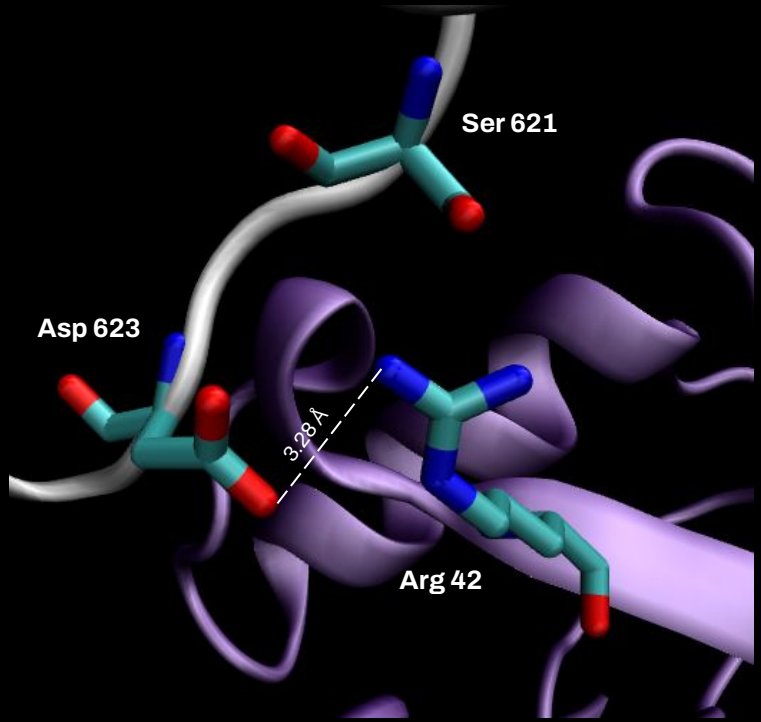
- SCCH domain residues (E1)
- Ubiquitin

PDB: 6DC6

Hydrogen Bonds

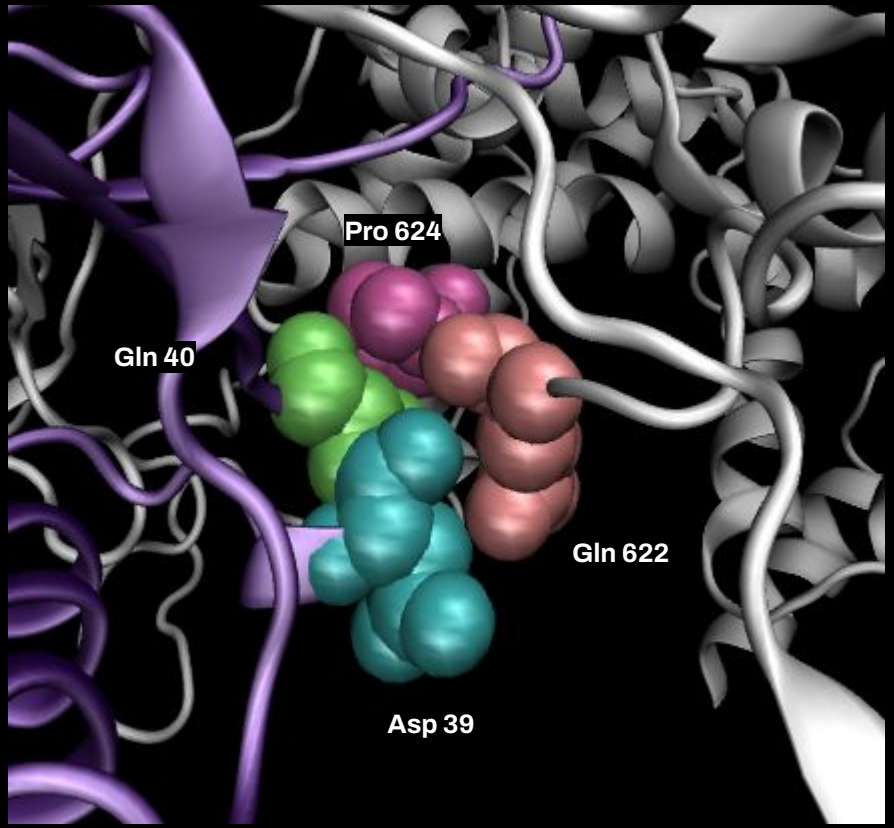


Salt Bridges



PDB: 6DC6

Van der Waals

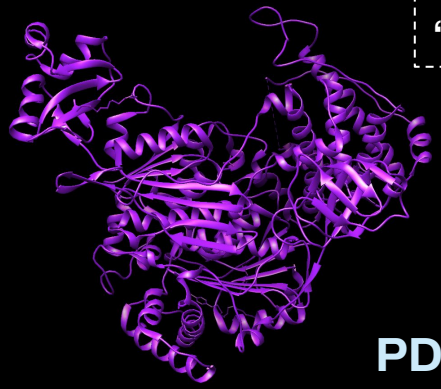


Crossover loop residues (E1)

g1 helix residues (Ub)

PDB: 6DC6

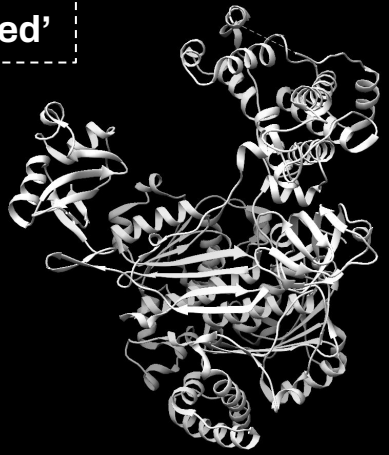
SCCH ROTATION



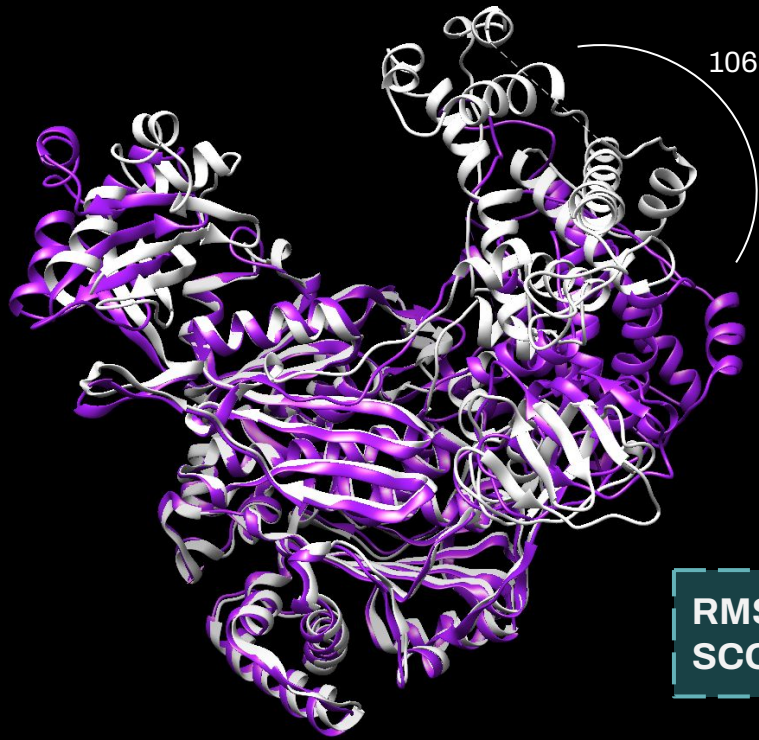
‘Closed’

PDB: 5UM6
(S. pombe)

‘Opened’



PDB: 4II3
(S. pombe)



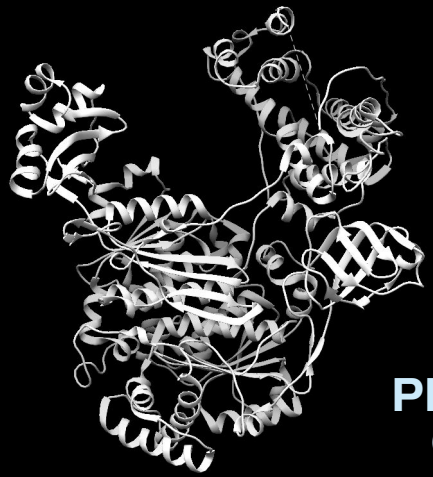
RMSD: 1.53
SCORE: 4.46

UFD ROTATION

Without E2

PDB: 4II3
(S.pombe)

With E2

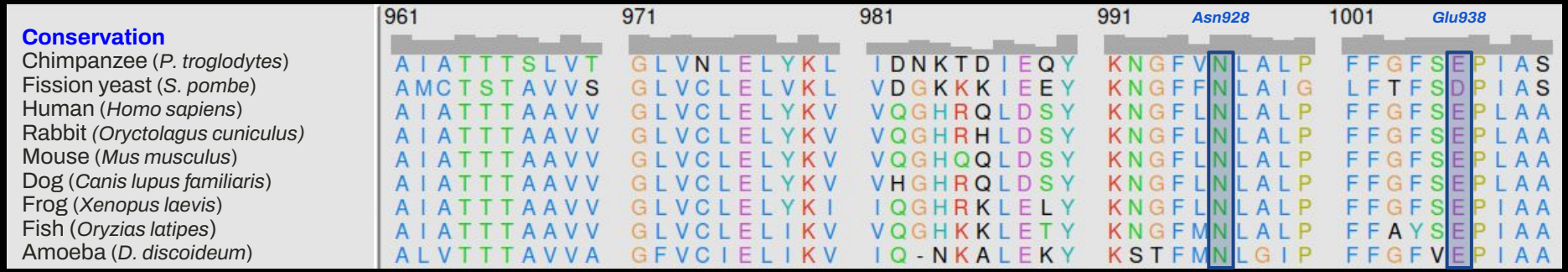


PDB: 4II2
(S. pombe)

RMSD: 1.22
SCORE: 8.34

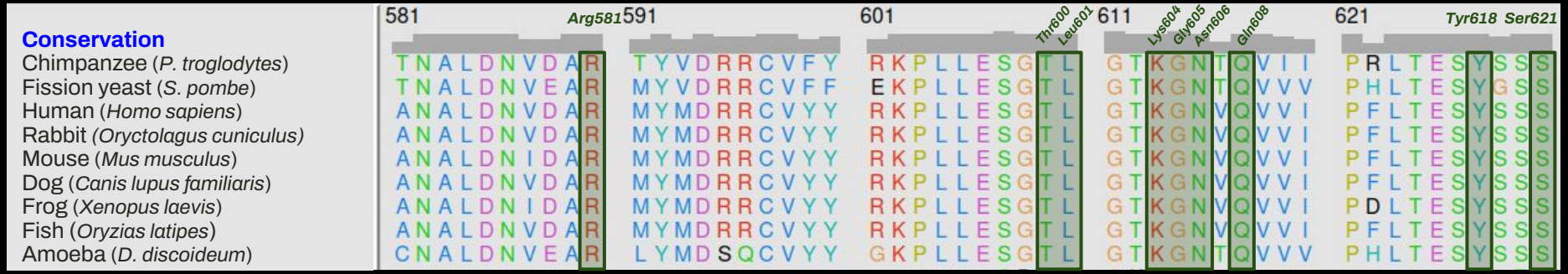
MULTIPLE SEQUENCE ALIGNMENT

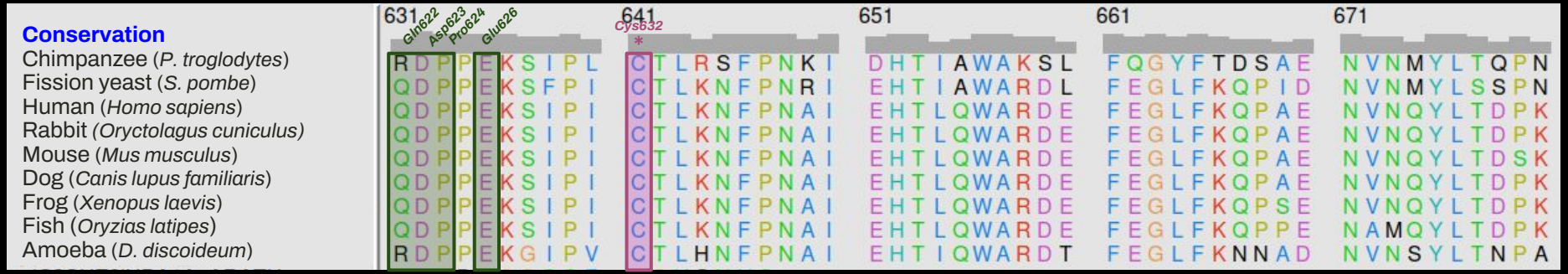
INTERFACE I



INTERFACE II

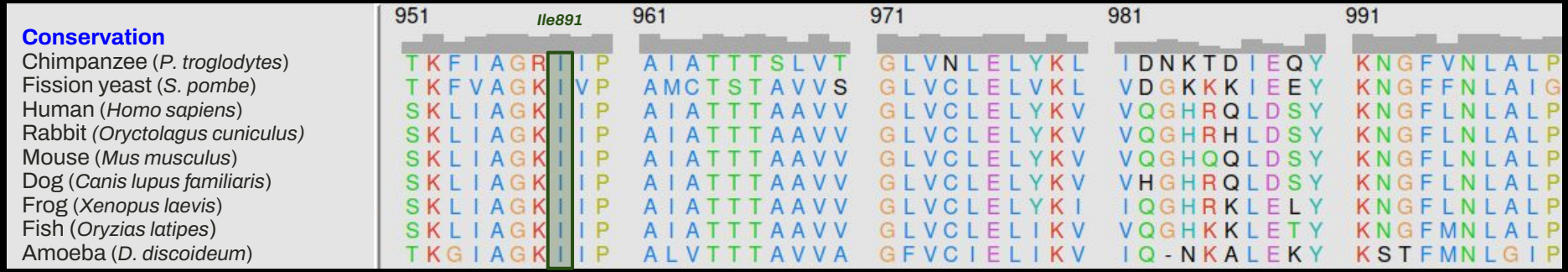
INTERFACE III





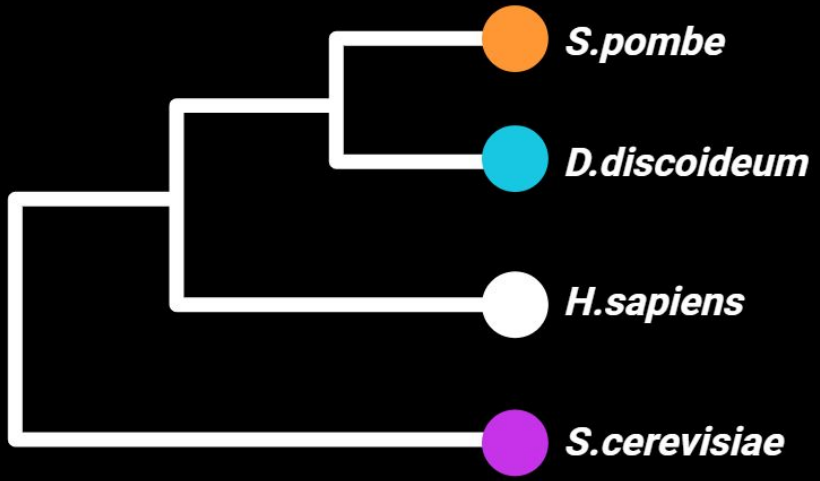
*Catalytic cysteine

INTERFACE III



STRUCTURAL ALIGNMENT

RMSD: 1.47
SCORE: 9.36

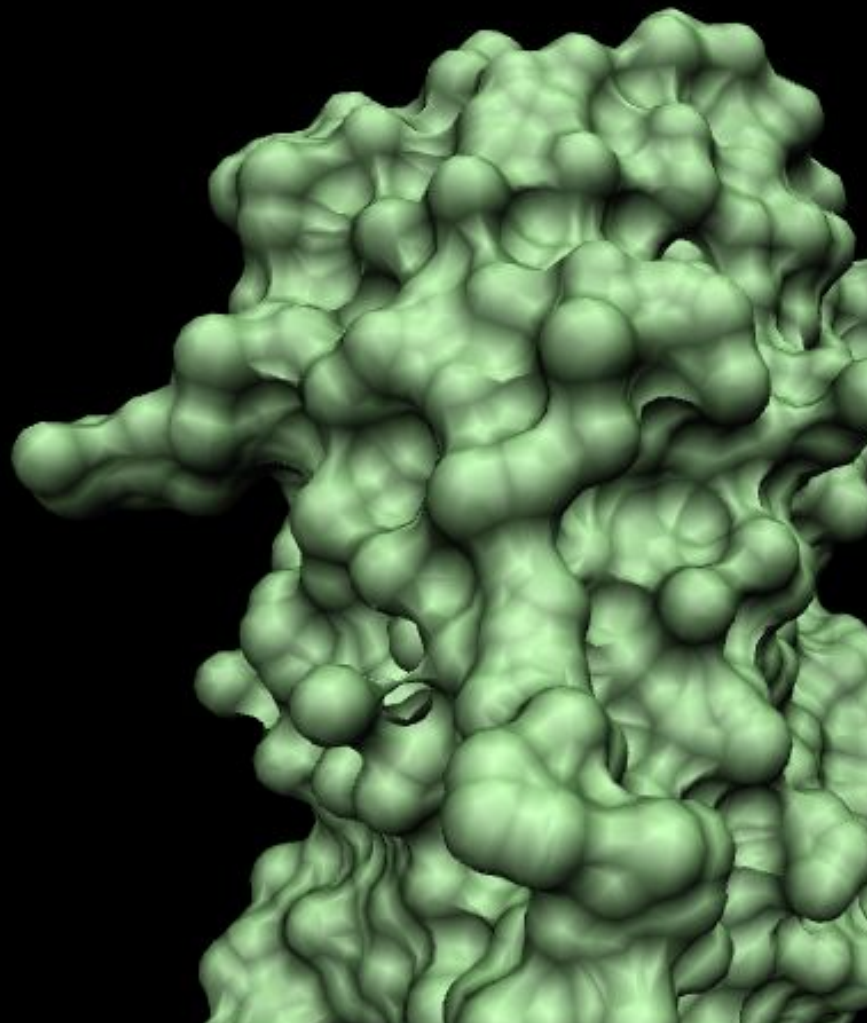


Predicted: Q55C16 - *D.discoideum*
PDB ID: 3CMM - *S.cerevisiae*
PDB ID: 4II3 - *S.pombe*
PDB ID: 6DC6 - *H.sapiens*

04.

E2.

Ub-conjugating enzyme



SCOP CLASSIFICATION

Class
Alpha and beta proteins (α + β)

Fold
UBC-like

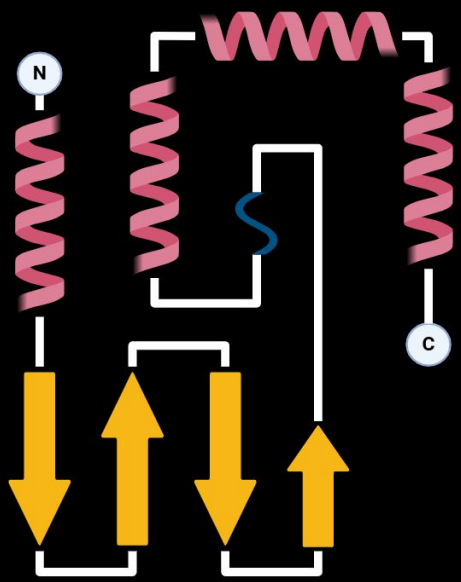
Superfamily
UBC-like

Family
UBC-related

TERTIARY STRUCTURE

- β-strand
- α-helix
- 3_{10}-helix
- Loop

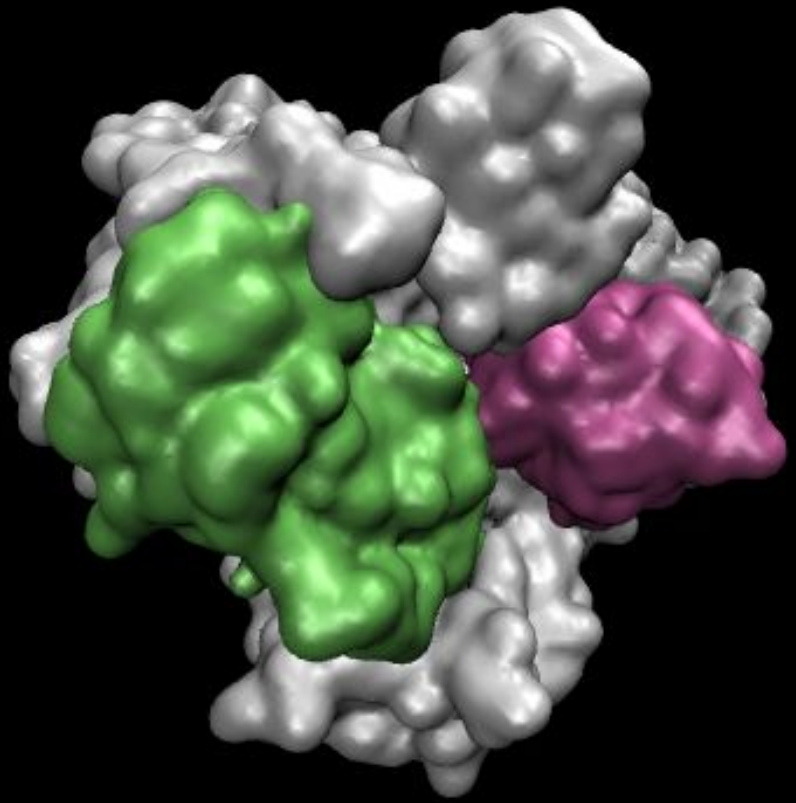
E2 Topological diagram



[1] MSSSKRIAKE LSDLERDPPT SCSAGPVGDD LYHWQASIMG PADSPYAGGV FFLSIHFPTD

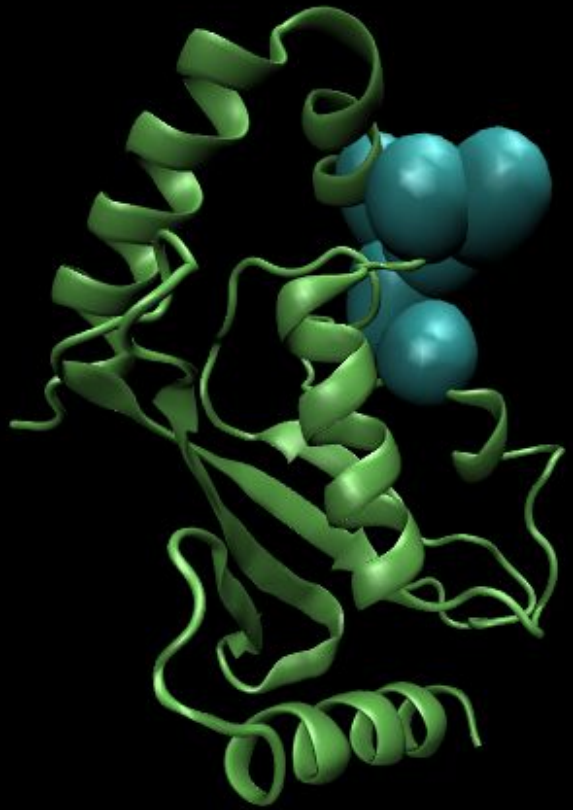
[61] YPFKPPKISF TTKIYHPNIN ANGNICLDIL KDQWSPALTL SKVLLSICSL LTDANPDDPL

[91] VPEIAHIYKT DRPKYEATAR EWTKKYAV



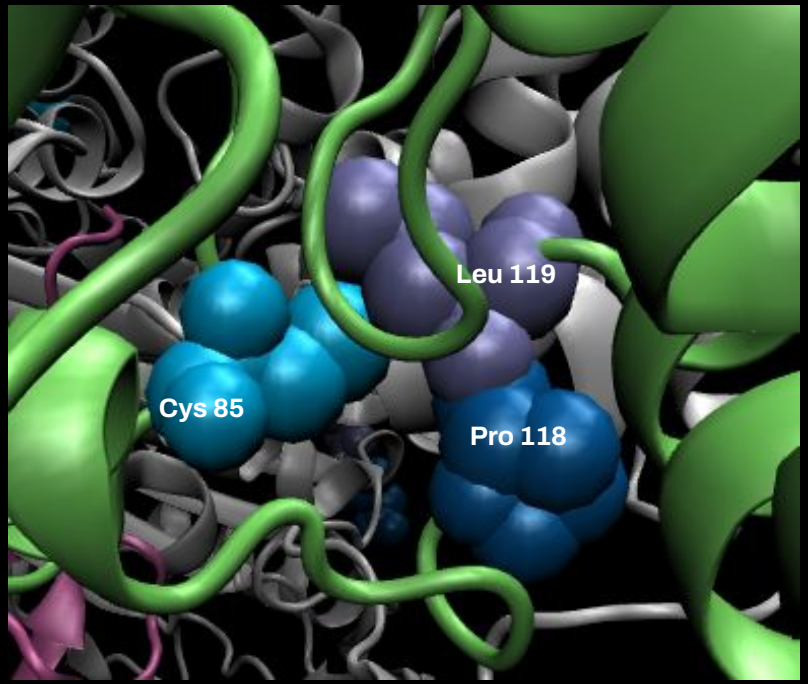
INTERACTIONS

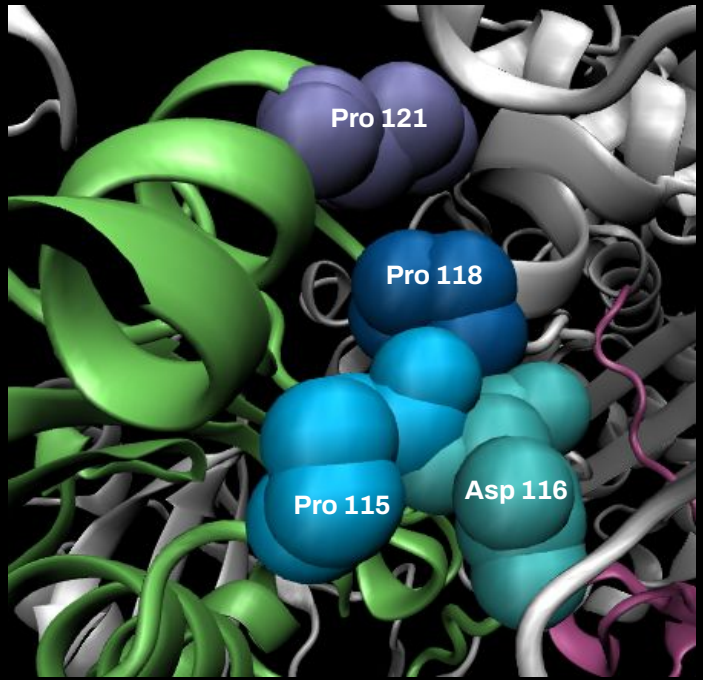
- E2
- E1
- Ubiquitin



Intramolecular interactions

Van der Waals



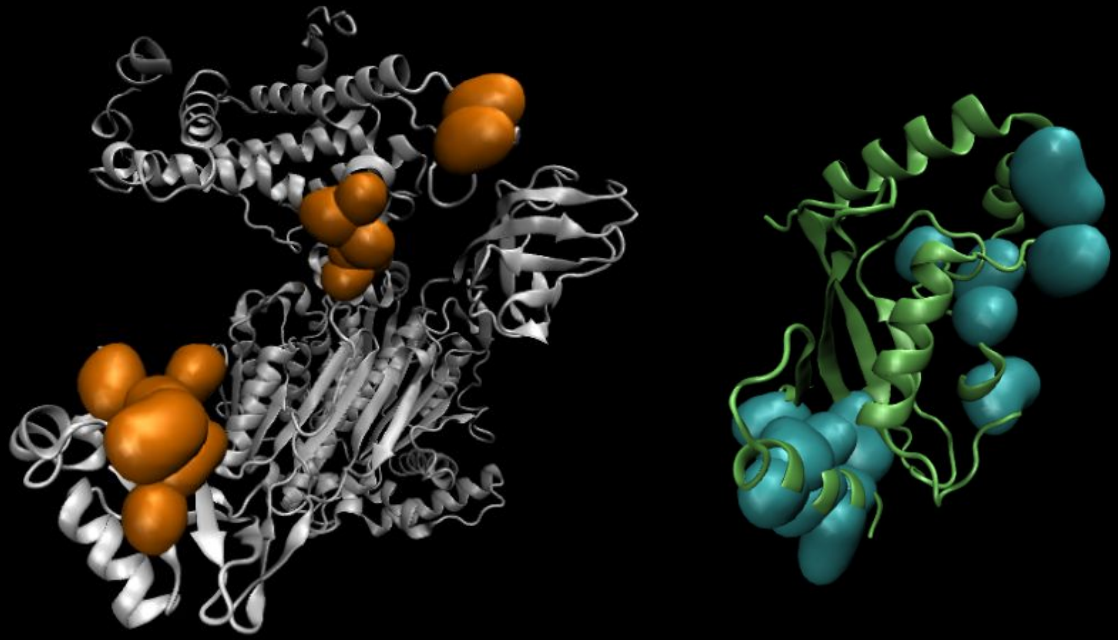


E2
E1

E2 residues

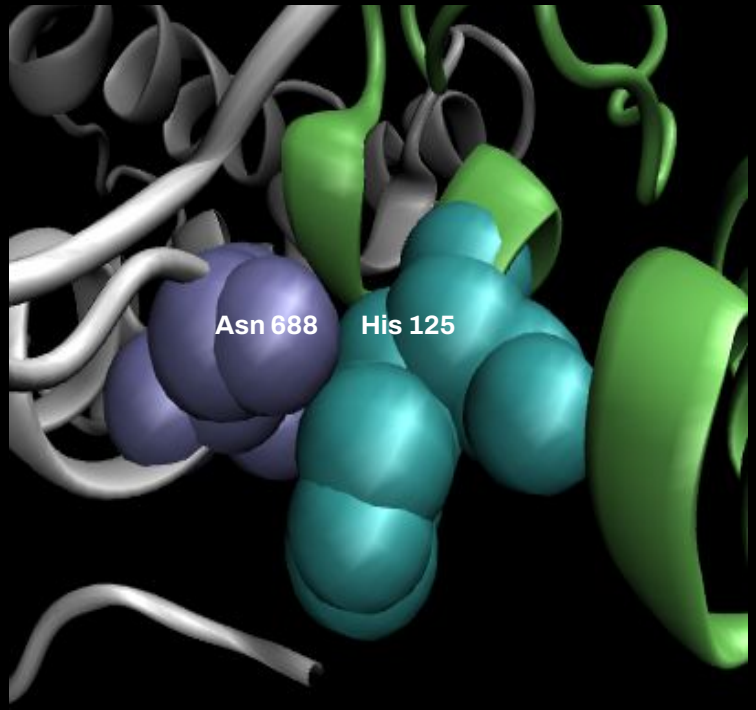
E2 residues

PDB: 4II2
(S. pombe)

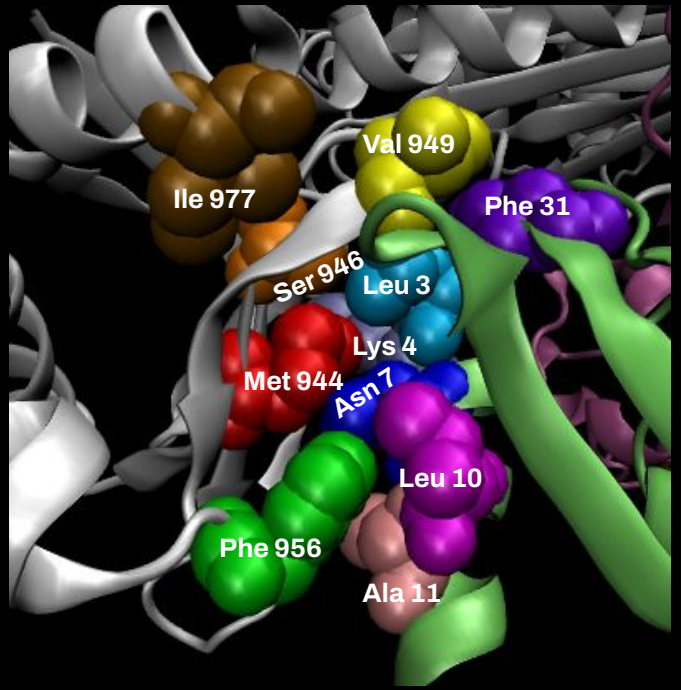


E2 - E1 Interactions

Van der Waals



E1 residues E2 residues



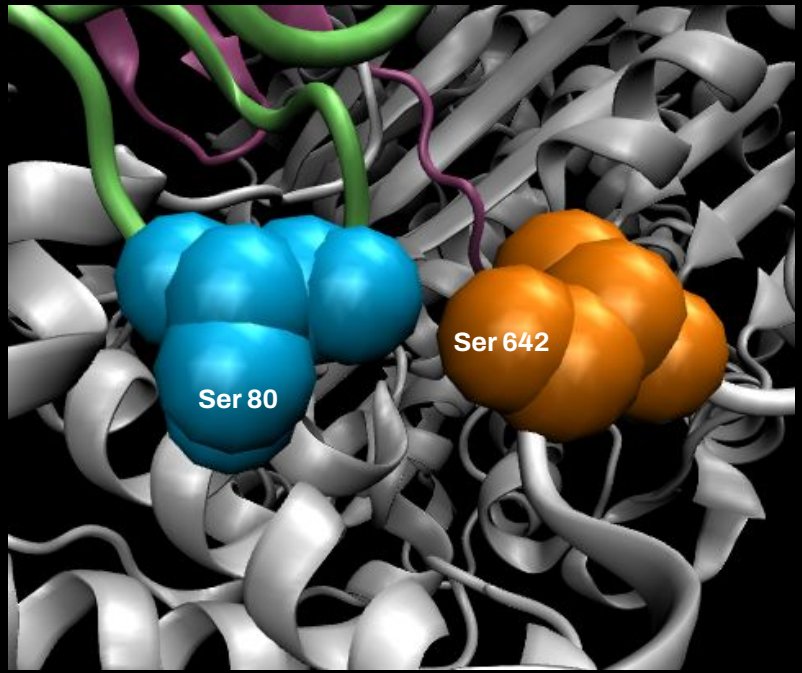
E2
E1

E1 residues

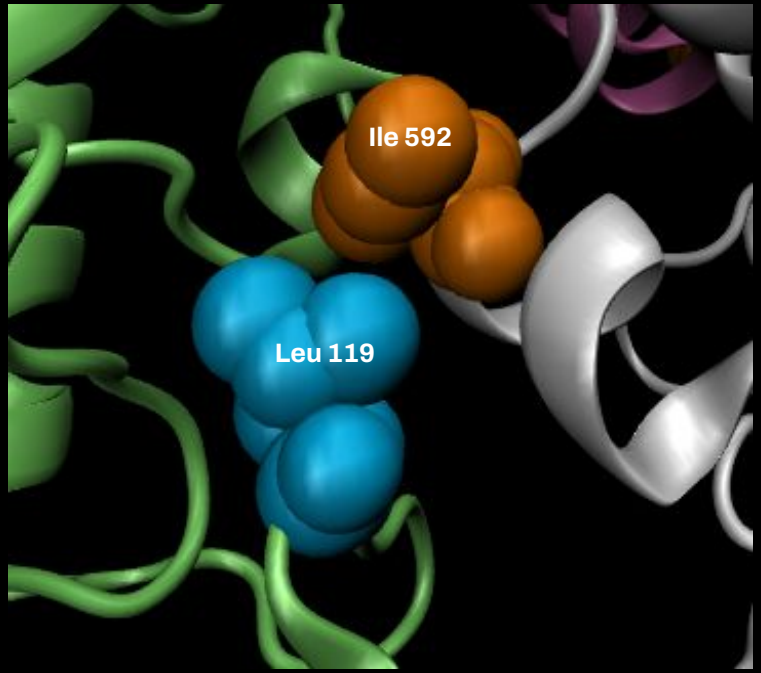
E2 residues

PDB: 4II2

Van der Waals



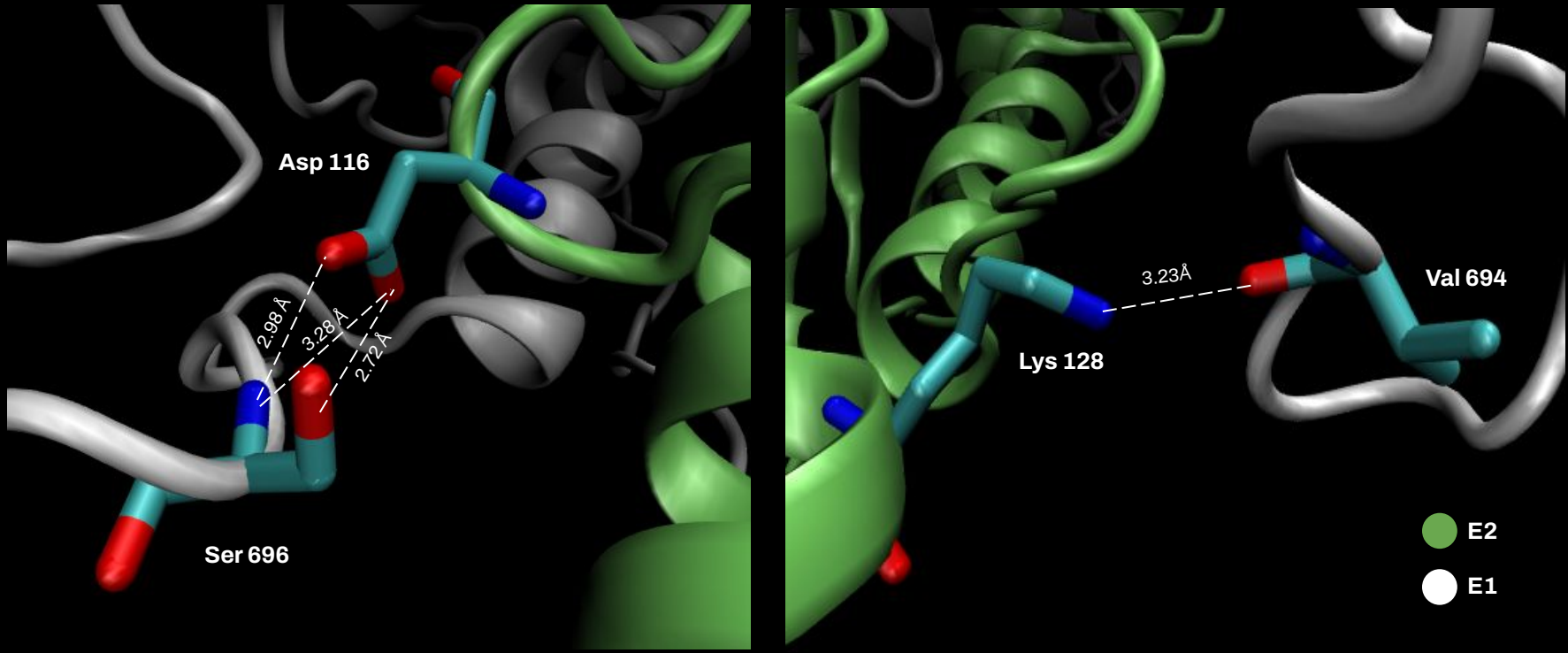
E2 residues E1 residues



E2 residues E1 residues

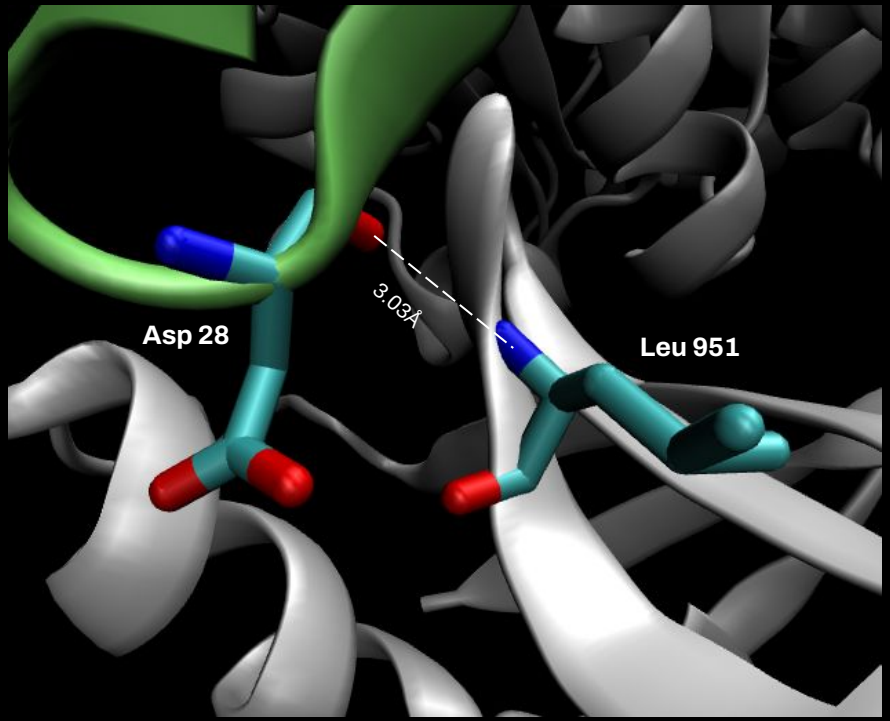
PDB: 4II2

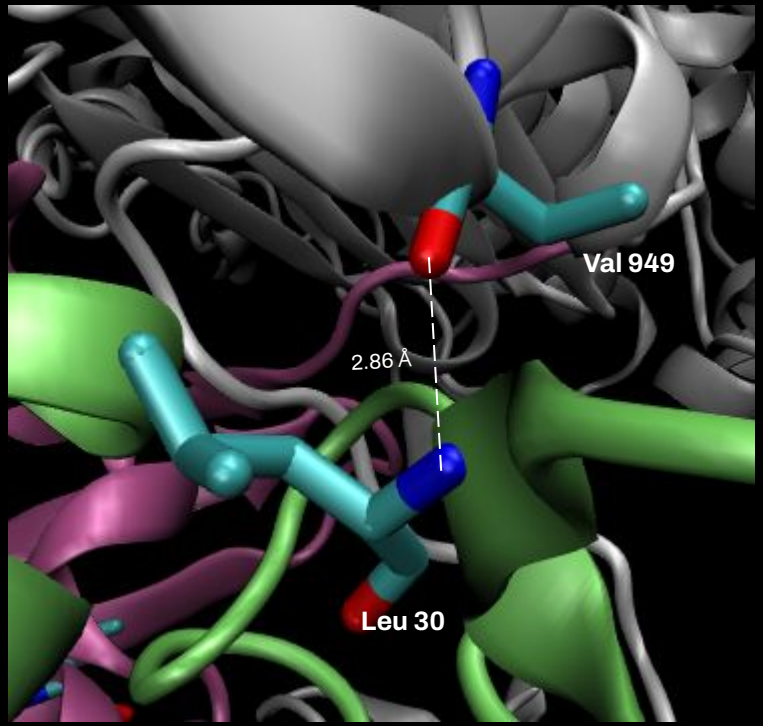
Hydrogen bonds



PDB: 4II2

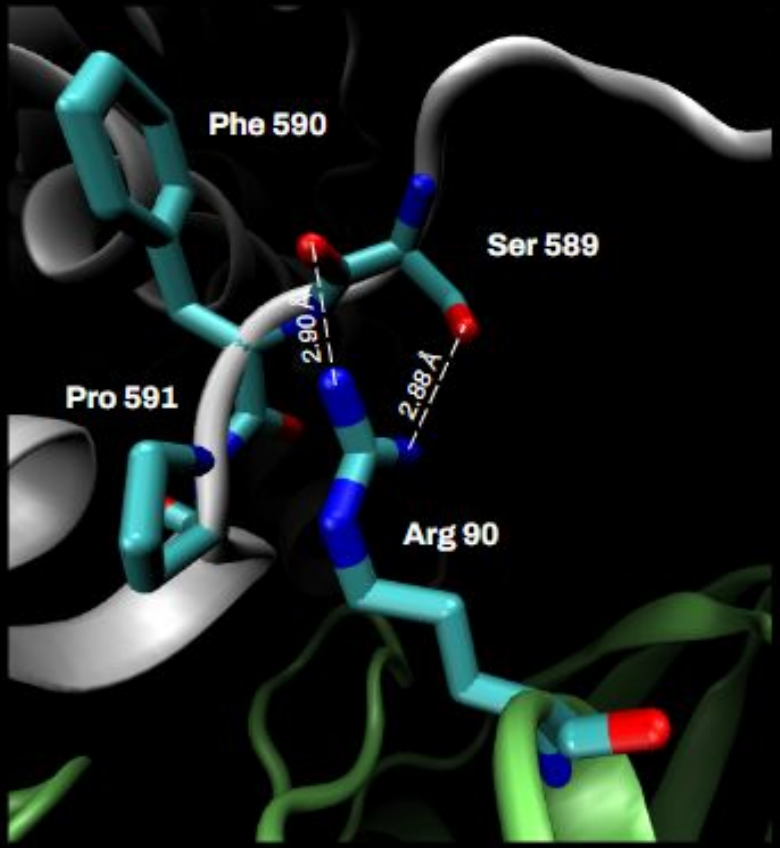
Hydrogen bonds





PDB: 4II2

Hydrogen bonds

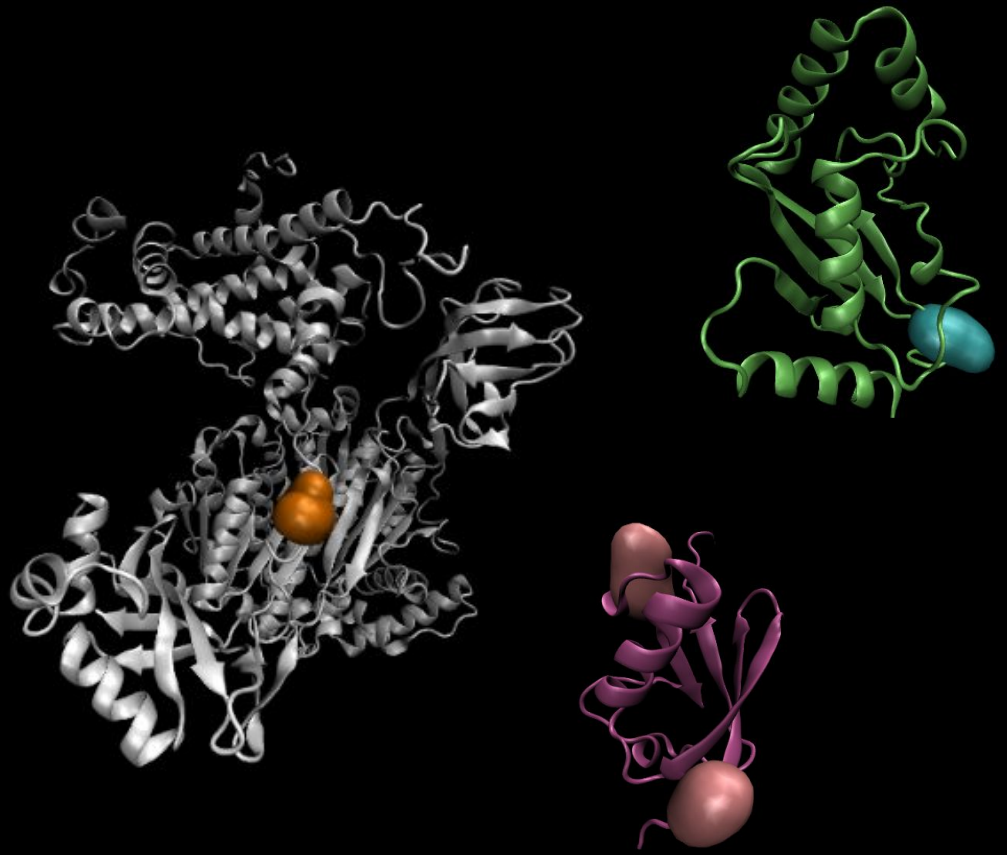


E2

E1

Crossover loop

PDB: 4II2



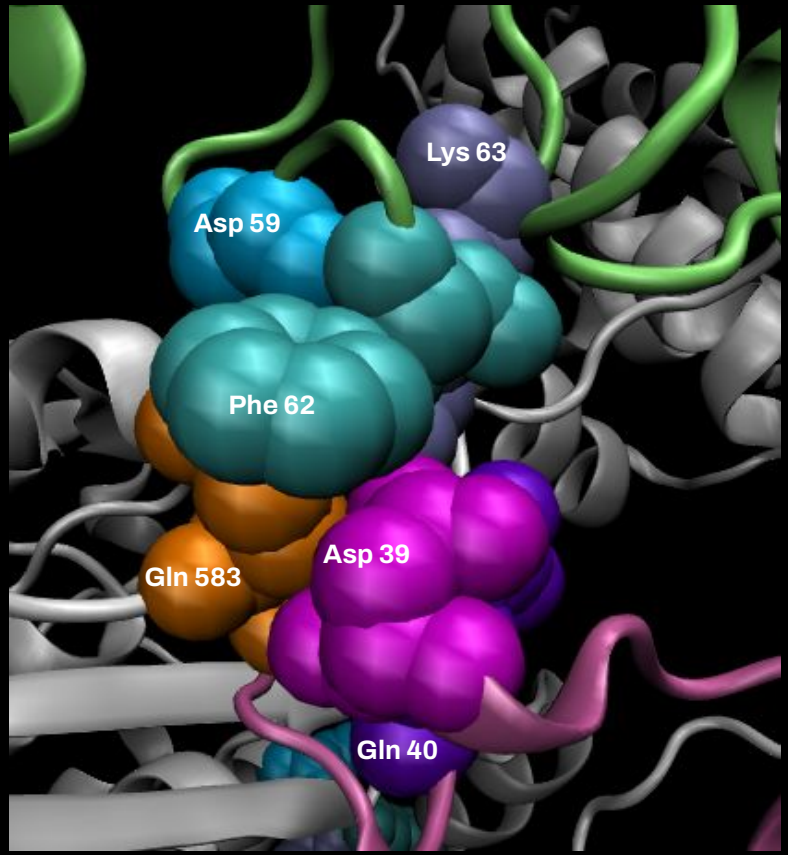
E2 - E1 - Ub Interactions

Van der Waals

E2 residues

Ubiquitin residues

E1 residues



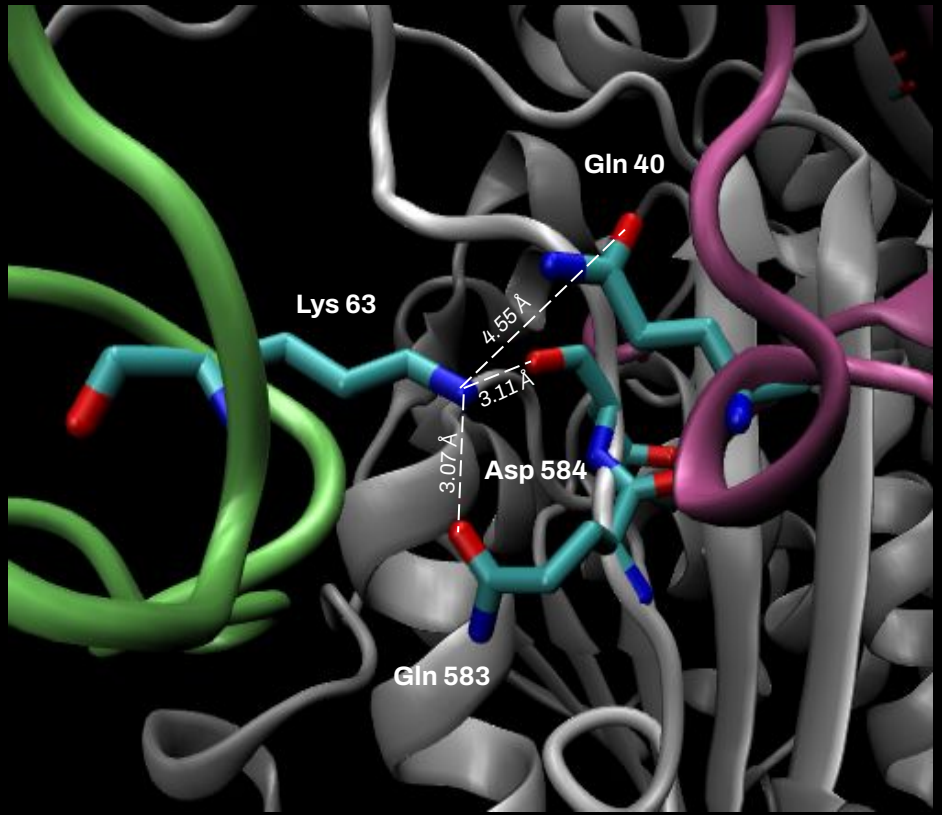
E2

E1

Ubiquitin

PDB: 4II2

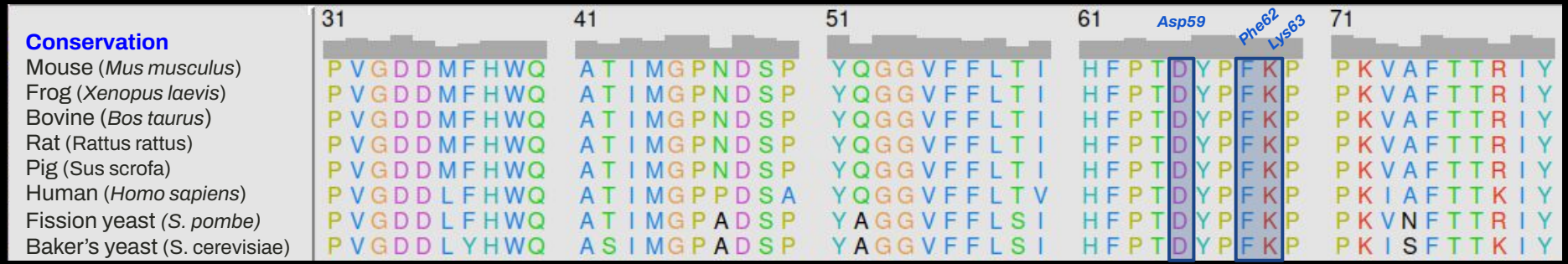
Hydrogen bonds

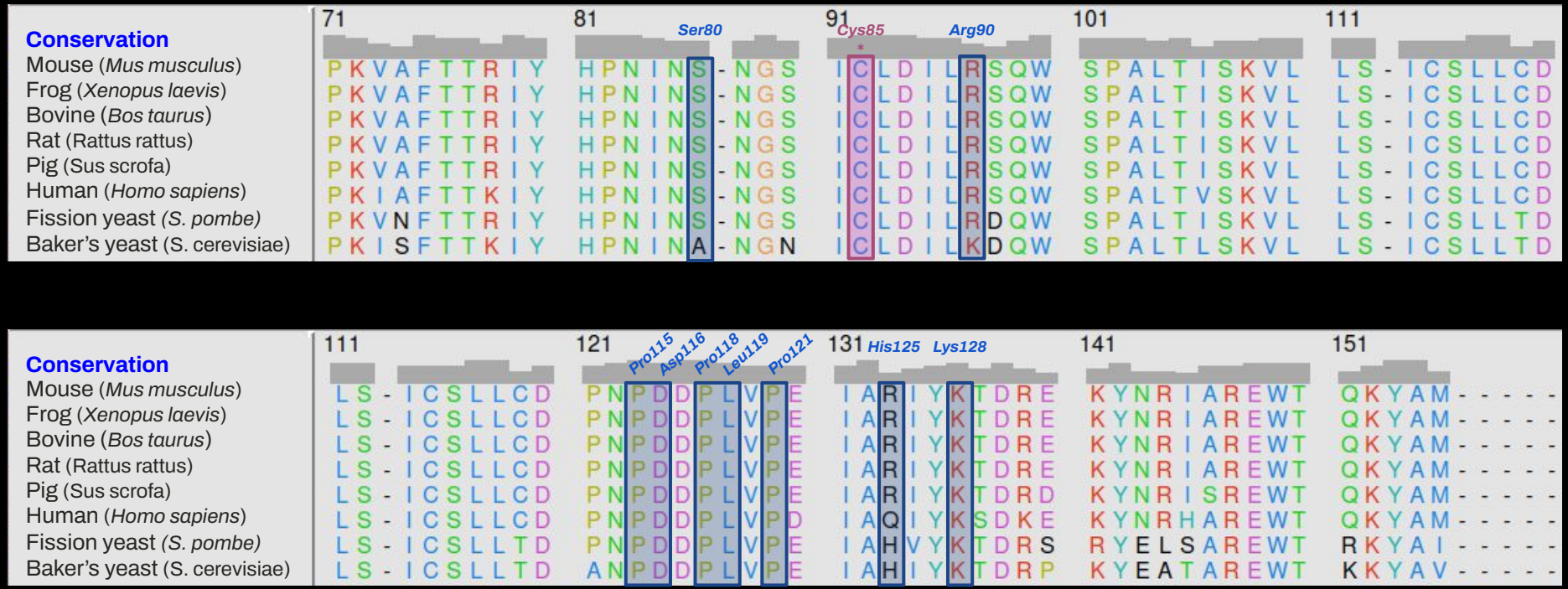


E2 E1 Ubiquitin

PDB: 4II2

MULTIPLE SEQUENCE ALIGNMENT

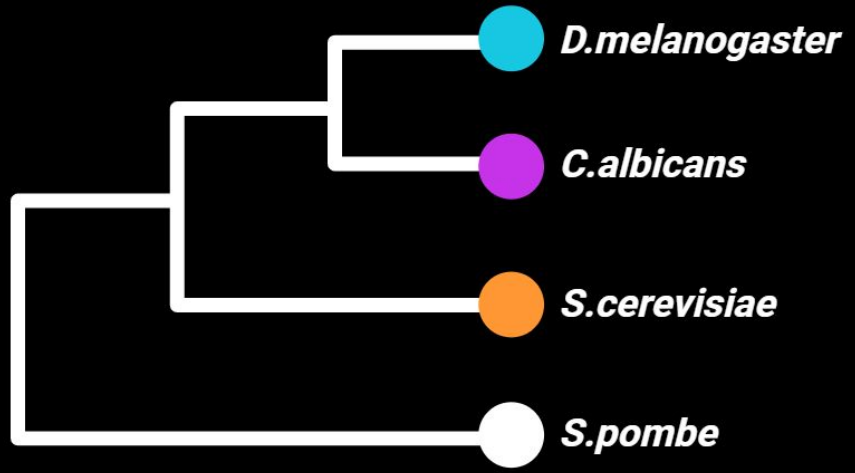




*Catalytic cysteine

STRUCTURAL ALIGNMENT

RMSD: 0.48
SCORE: 9.64



Predicted: P25867 - *D.melanogaster*

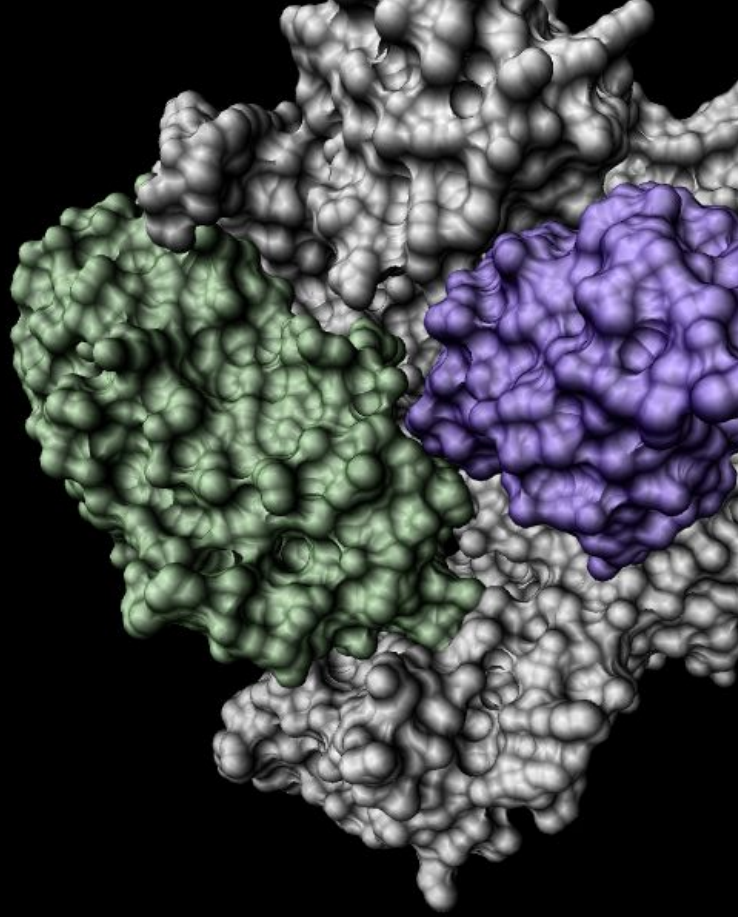
Predicted: P43102 - *C.albicans*

PDB ID: 1QCQ - *S.cerevisiae*

PDB ID: 4II2 - *S.pombe*

06.

CONCLUSIONS



- The **ubiquitination process** implies the ubiquitin and three enzymes
  - **E1** → activates the ubiquitin
  - **E2** → transfer the ubiquitin from the E1 enzyme to the substrate
  - **E3** → selects the substrate and assists in the transfer of ubiquitin from E2 to the substrate

- **Ubiquitin**: highly structurally and sequentially conserved multifunctional protein.
  - Presents a **hydrophobic core** and **hydrogen bonds** that are involved in its stability.

- **E1 Ub-activating enzyme** is a multidomain protein that catalyses the ubiquitin adenylation and the formation of a thioester bond during the first step of ubiquitination. Due to its relevance, both its structure and sequence are preserved among species.

- **E2 Ub-conjugating enzyme** is involved in the transthiolation step through its catalytic cysteine, and is the intermediate step before the substrate ubiquitination.

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PEM QUESTIONS

1. **Which are the ubiquitin residues that allows to do a polibuquination?**
   a) Lys
   b) Met
   c) a) and b) are correct
   d) Ser
   e) All of them are correct

2. **E1 enzyme is involved in:**
   i. The activation of the ubiquitin
   ii. E2 recruitment
   iii. The thioester bond transfer
   iv. The union of the ubiquitin to the target protein
   a) 1,2 and 3
   b) 1 and 3
   c) 2 and 4
   d) 4
   e) 1,2,3 and 4

3. The catalític Cys in the E1 enzyme is located at…

a) AAD domain
b) C-terminal
c) SCCH domain
d) FCCH domain
e) UFD domain

4. Which is the E1 domain that recruits E2?

a) SCCH
b) FCCH
c) UFD
d) IAD
e) IAD

5. Mark the correct answer about E2:

a) Its active site is located in Cysteine 85
b) It's a quite structurally conserved protein
c) Aspartic acid 28 is located proximal to the e1 UFD domain and it plays an important role during the E1-E2 thioester transfer.
d) It presents both intra and intermolecular interactions
e) All of them are correct

6. Mark the correct answer about ubiquitin residues conservation.

a) Ubiquitin residues involved in the E1 interfaces are not conserved in Drosophila
b) The Lys residues are not conserved in bacteria
c) The C-terminal diglycine motif only appears in H. sapiens
d) a) and c) are correct
e) All options are incorrect

7. About the ubiquitination mark the correct answer.

i. In the ubiquitination are involved two enzymes.
ii. ATP is only necessary to do the ubiquitin conjugation
iii. E3 is involved in ubiquitin activation
iv. E2 is involved in ubiquitination conjugation

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

8. About E1 enzyme conformational changes the incorrect answer is…

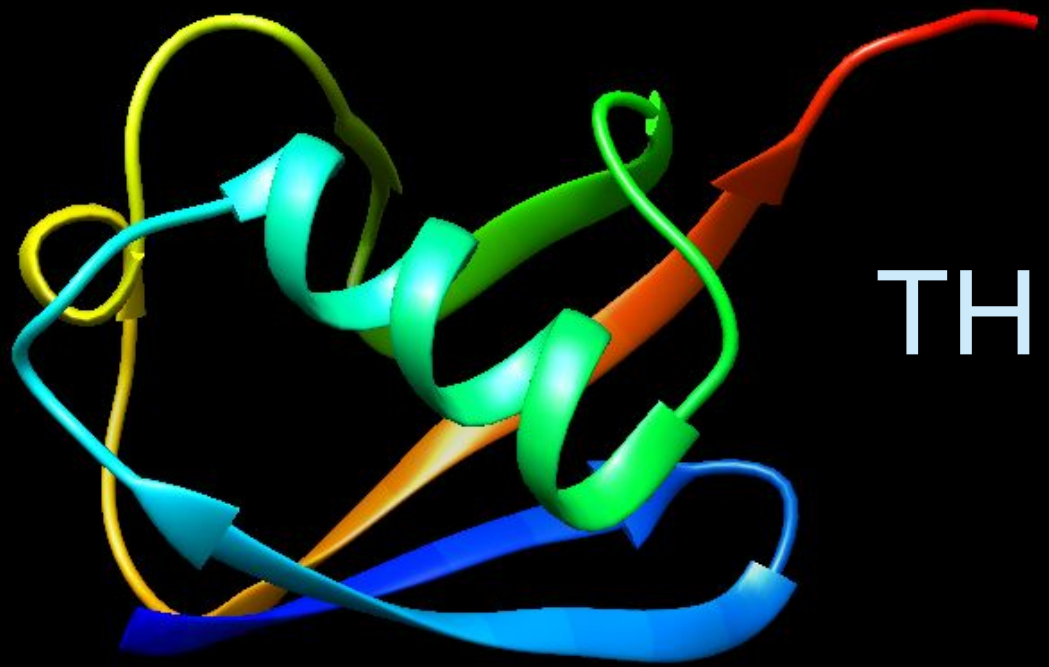
a) SCCH rotation facilitates the thioester bond transfer.
b) There are two major conformational changes related to E1
c) SCCH domain undergoes a rotation to switch from the ‘opened’ to the ‘closed’ conformation.
d) UFD rotation implies E2 interactions.
e) All answers are incorrect

9. How many Lysine residues has ubiquitin?

a) 3
b) 5
c) 7
d) 9
e) 11

10. Which of the following options is not an ubiquitin function?

a) Proteasomal degradation
b) DNA damage response
c) Selective autophagy
d) Cell cycle regulation
e) All options are correct



THE UBIQUITIN SYSTEM

Structural Biology 2023-2024

Maria Igual, Raoudha Somrani, David Roura and Laia Dalmacio