STRUCTURAL BIOLOGY

Universitat Pompeu Fabra Barcelona



THE UBIQUITIN SYSTEM

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2025/2026

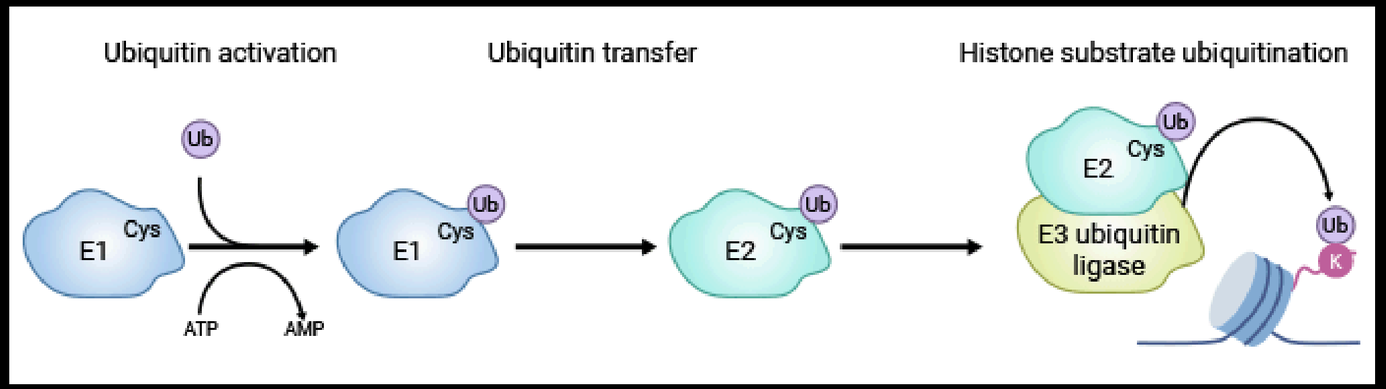
UBIQUITINATION PROCESS

Post-transational modification

Covalent attachment of the ubiquitin to substrate proteins

MAIN FUNCTIONS:

- Proteasomal degradation
- Cell signalling
- DNA damage response
- Cell cycle regulation



cusabio.com

FREE UBIQUITIN: THE START OF THE PROCESS

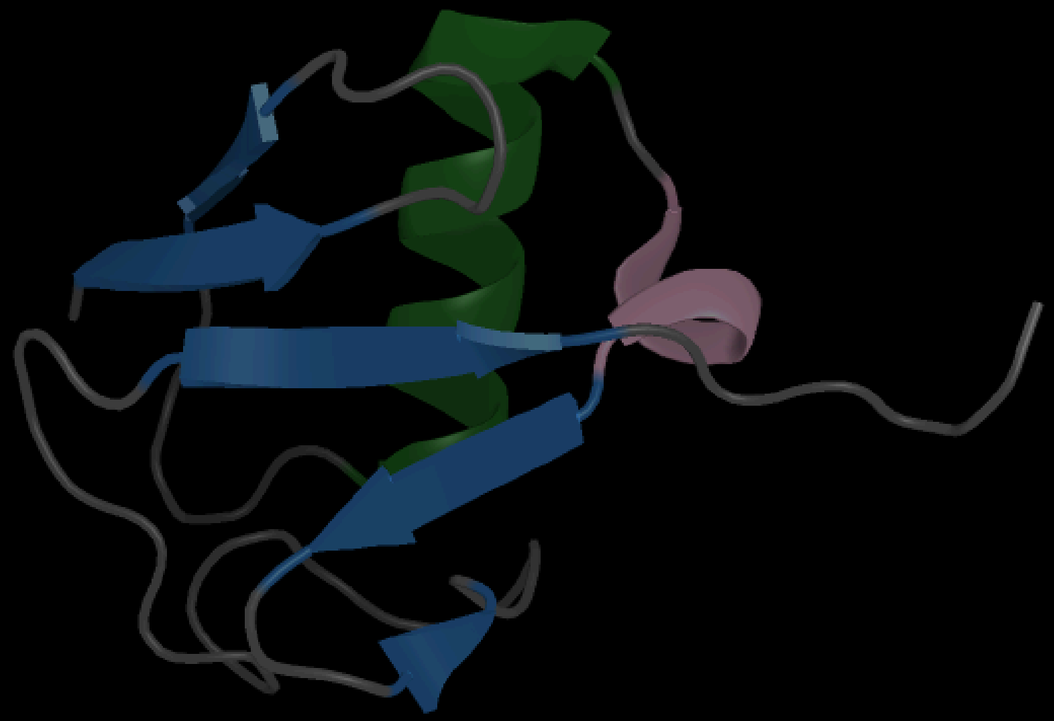
Ubiquitin:

- 76 amino acids
- β-grasp fold
- Stable hydrofobic core

In the surface:

- 7 LYS residues
- C-terminal GLY 76

Chemically inactive → It requieres activation

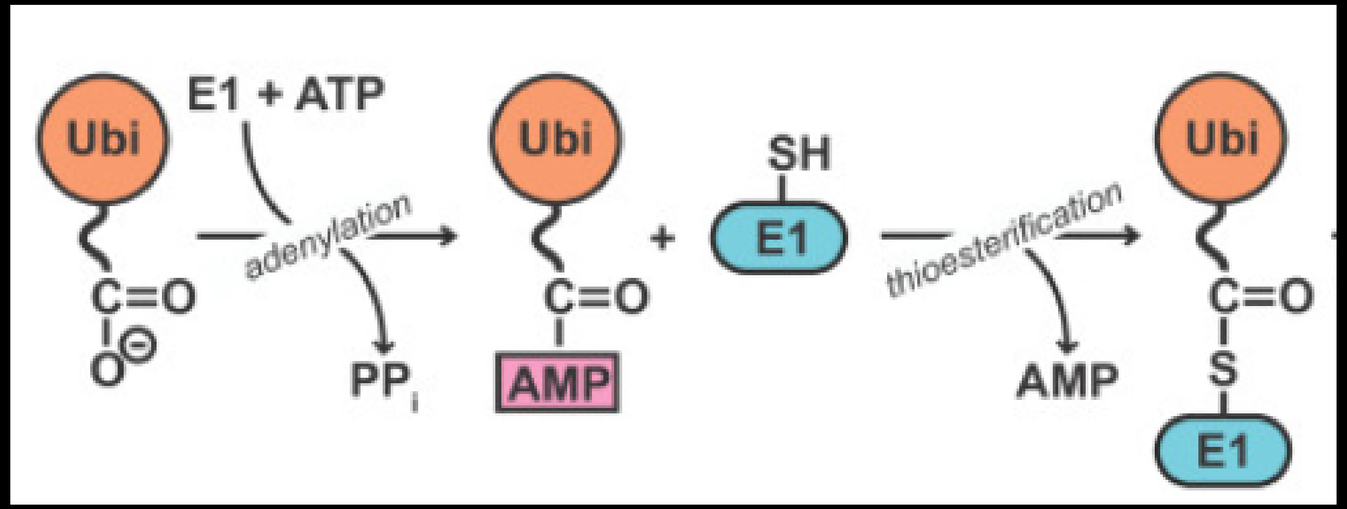


PDB ID: 3UGB
Resolution: 2.35 Å
Organism: Homo sapiens

ACTIVATION OF THE UBIQUITIN BY E1

1. UB + ATP → UB-AMP (adenylation)
2. Catalytic CYS attack

   E1-UB:
   - High-energy thioester bond
   - Energy stored for transfer



Lorenz et al. BMC Biology 2013, 11:65

TRANSFERENCE TO E2

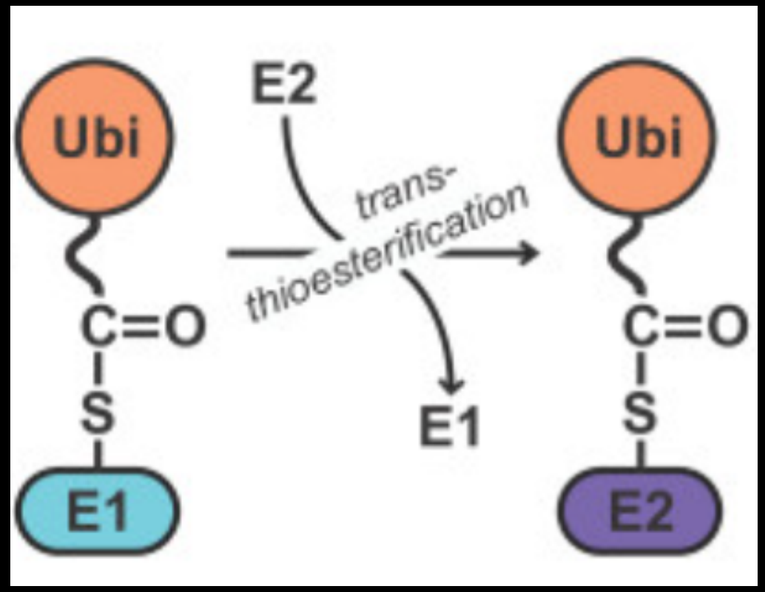
UBC domain

Catalytic CYS

TRANSTHIOLATION:

E1-UB → E2-UB

Activated ubiquitin remains preserved and E2 becomes the central catalytic component



Lorenz et al. BMC Biology 2013, 11:65

TYPES OF E2

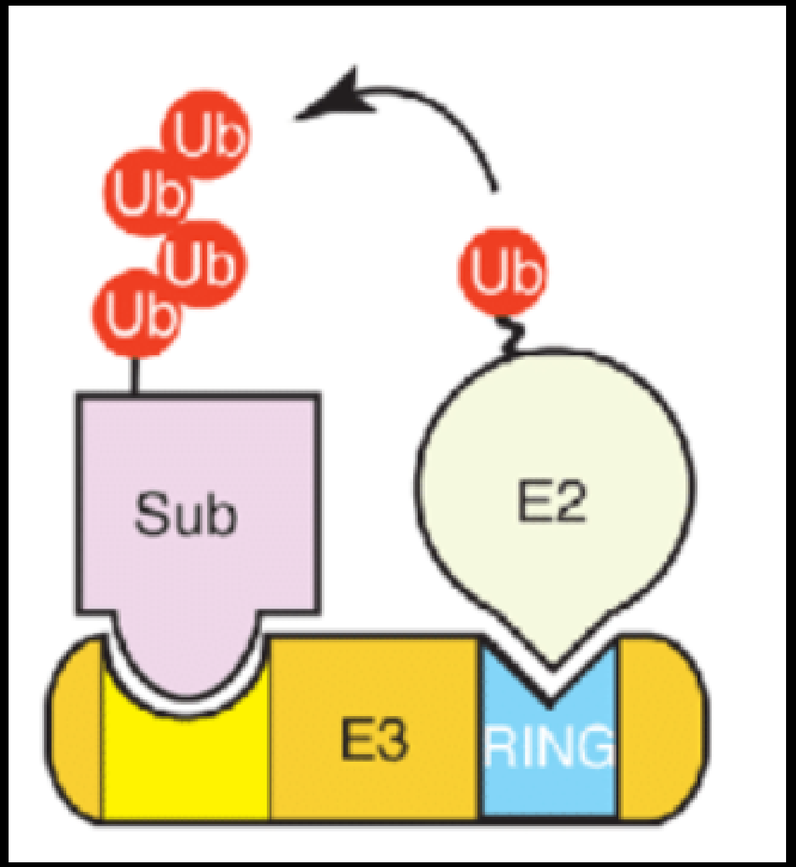
TYPES	STRUCTURE	EXAMPLES
CLASS I	ONLY UBC DOMAIN	UBE2D1
CLASS II	UBC DOMAIN + N-TERMINAL EXTENSIONS	UBE2E1
CLASS III	UBC DOMAIN + C-TERMINAL EXTENSIONS	UBE2R1
CLASS IV	UBS DOMAIN + BOTH N- AND C-TERMINAL EXTENSIONS	UBE2O

ROLE OF E3: SPECIFICITY

No UB intermediate

- Substrate recognition
- Positions the E2–ubiquitin complex close to a Lys

RING-type E3 ligase for catalytic orientation



F1000Research 2019, 8(F1000 Faculty Rev):725

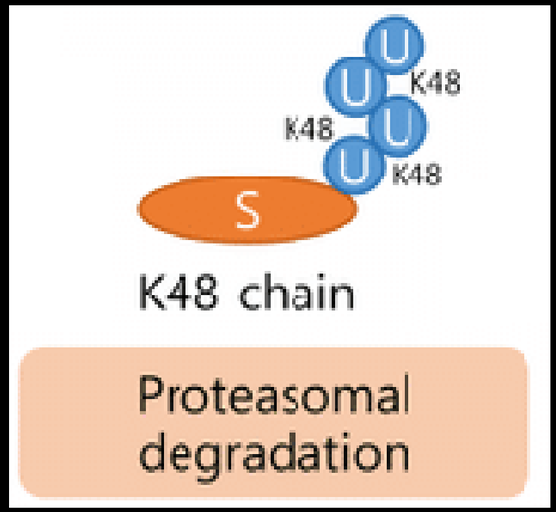
ISOPEPTIDE BOND FORMATION: FINAL EVENT

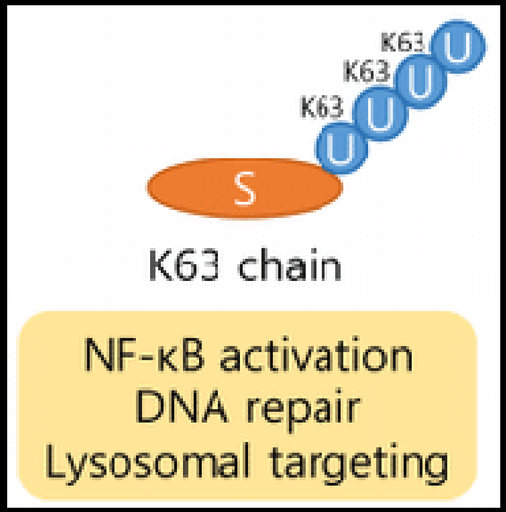
LYS of the substrate attacks THE E2~UB thioester bond → Formation of a stable isopeptide bond between ubiquitin and substrate protein

POLYUBIQUITIN CHAINS:

K48 → Degradation
K63 → Signalling

Reversible system → Deubiquitinases



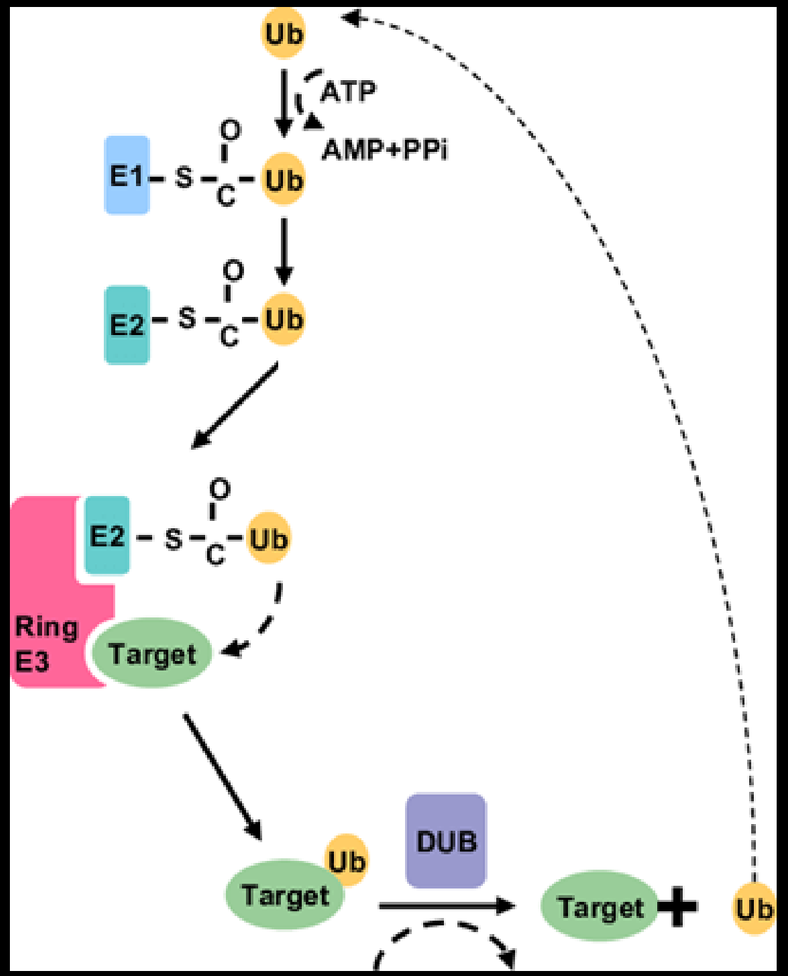


Park, C.-W., & Ryu, K.-Y. (2014). Cellular ubiquitin pool dynamics and homeostasis. BMB Reports, 47(9), 475–482.

SUMMARY

UBIQUITINATION CASCADE:

1. Activation (E1)
2. Transfer (E2)
3. Recognition (E3)
4. Isopeptide bond formation
5. Chain elongation
6. Deubiquitinases and recycling

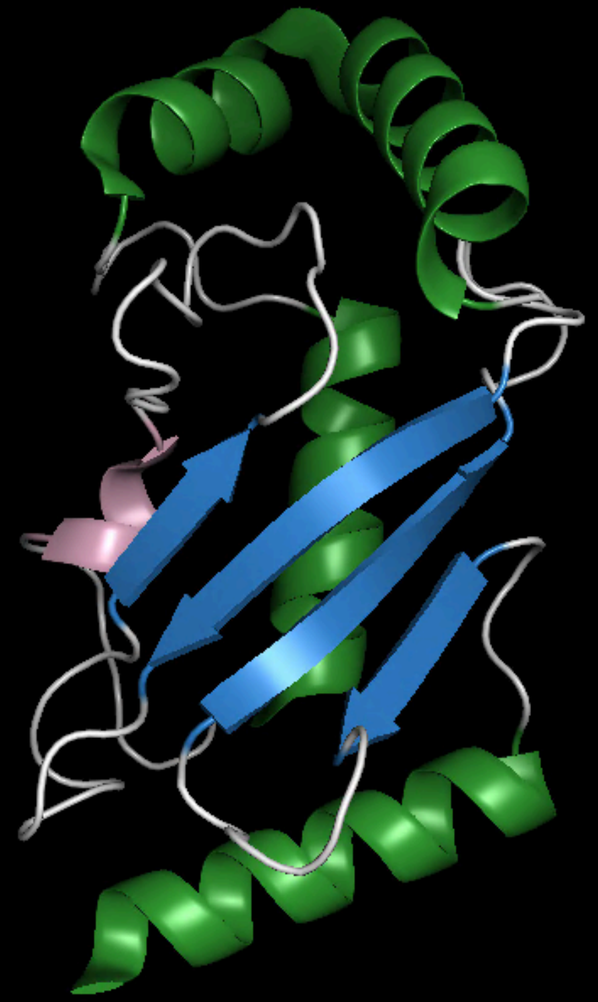


Yucer, N. et al. (2012). Protein ubiquitination in IR-induced DNA damage response. En M. Nenoi (Ed.), Current Topics in Ionizing Radiation Research. InTech.

UBIQUITIN-CONJUGATING ENZYMES (E2)

E2 SCOP CLASSIFICATION

- **Class:** Alpha and beta proteins
- **Fold:** UBC-like
- **Superfamily:** UBC-like
- **Family:** UBC-related

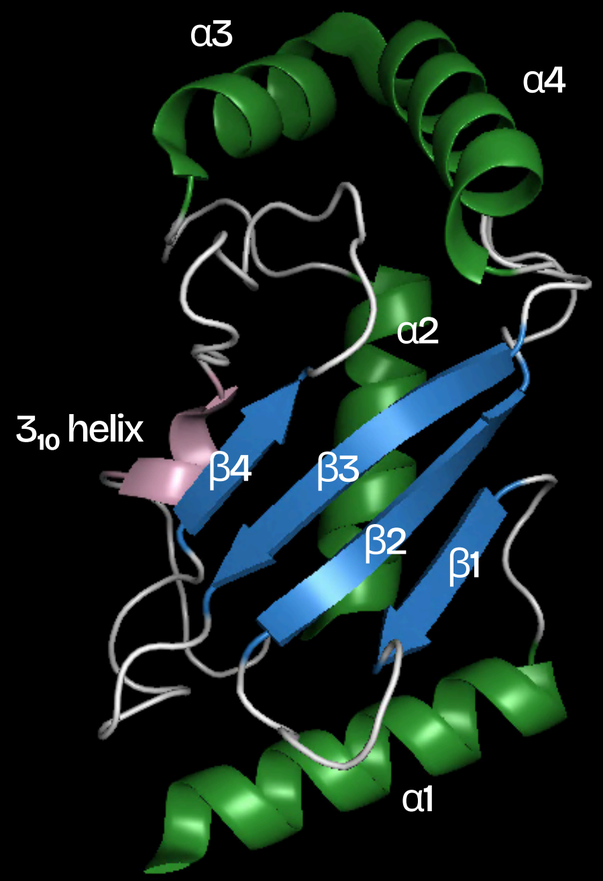


PDB ID: 7BOL
Resolution: 1.80 Å
Organism: Homo sapiens

UBC: Ubiquitin-Conjugating enzyme

E2 SECONDARY STRUCTURE

UBE2D2
(UBCH5B)

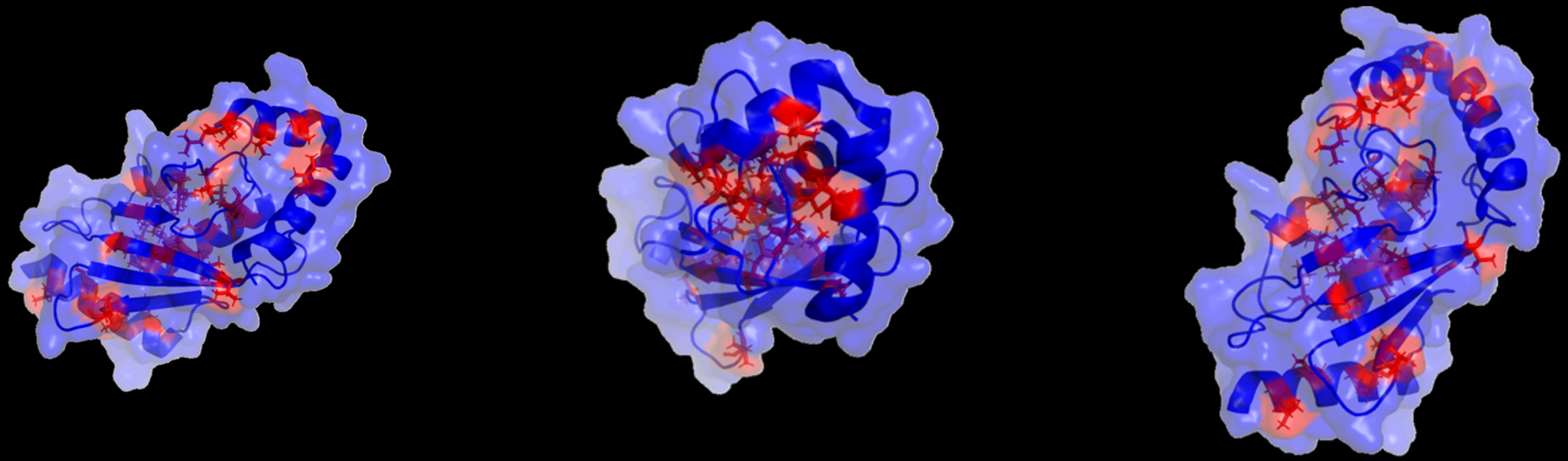


α-helix
β-strands
3_{10} helix

α/β fold

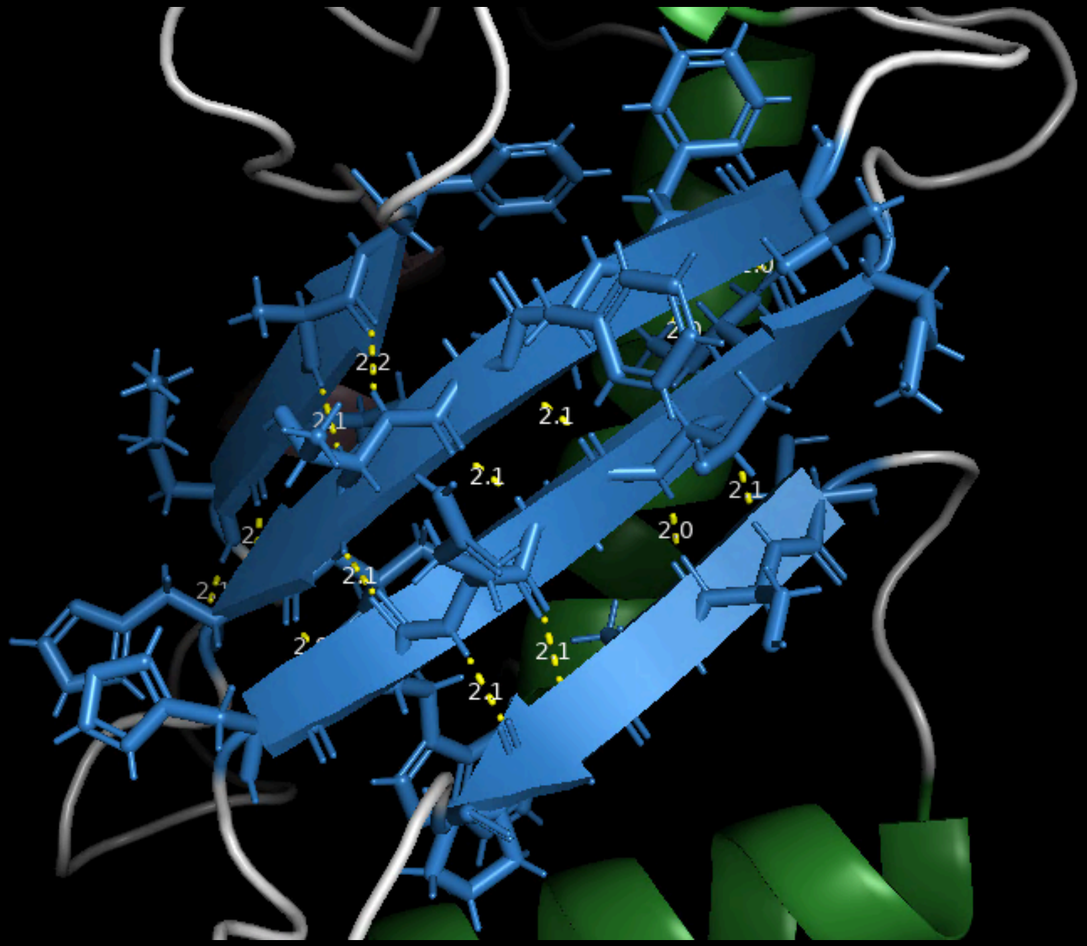
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YNRIAREWTQKYAM

HYDROPHOBIC CORE

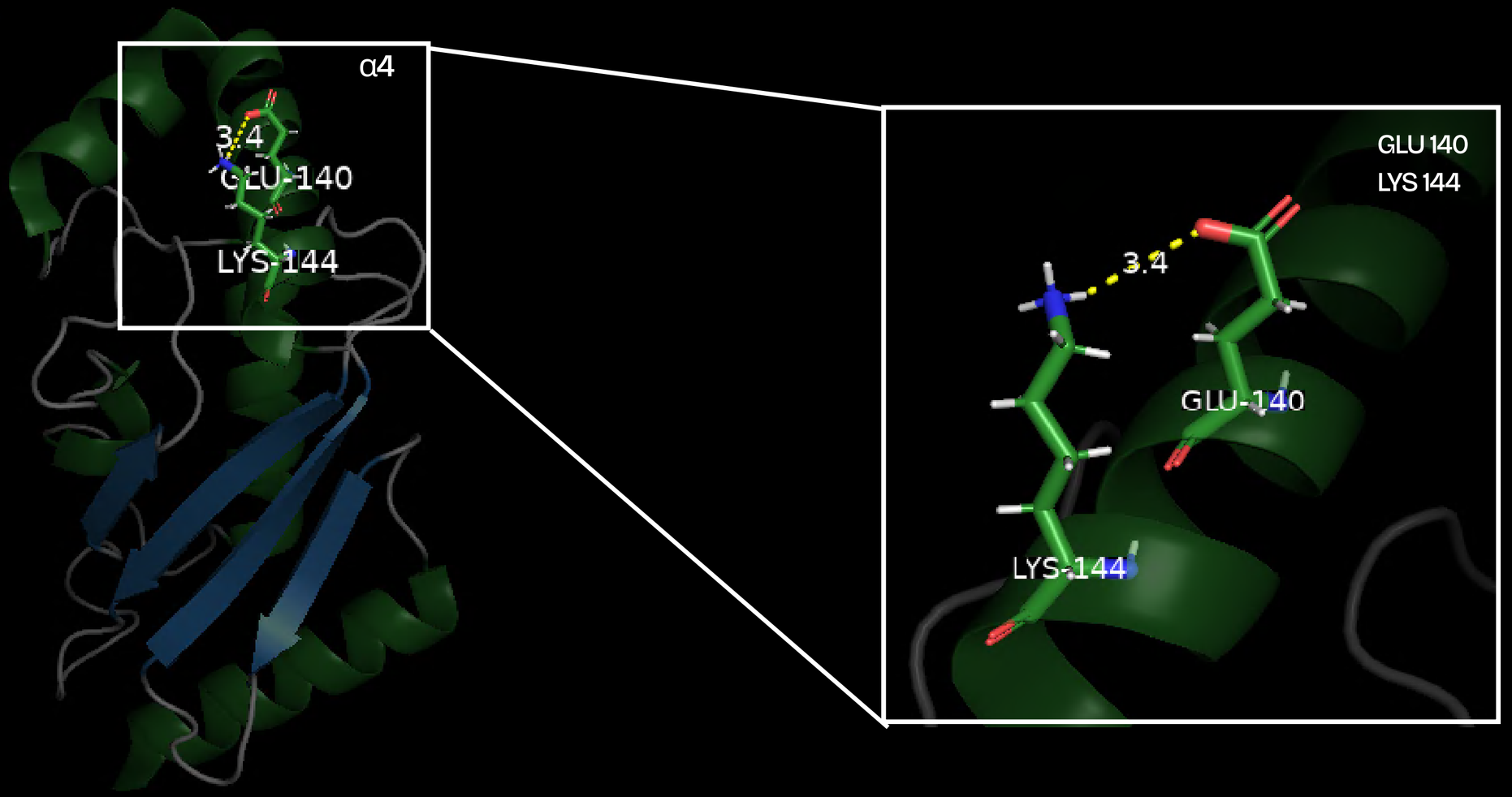


MALKRIHKELNDLARDPPAQCSAGPVGDDMFHWQATIMGPNDSPYQGGVFFLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDDPLVPEIARIYKTDREK
YNRIAREWTQKYAM

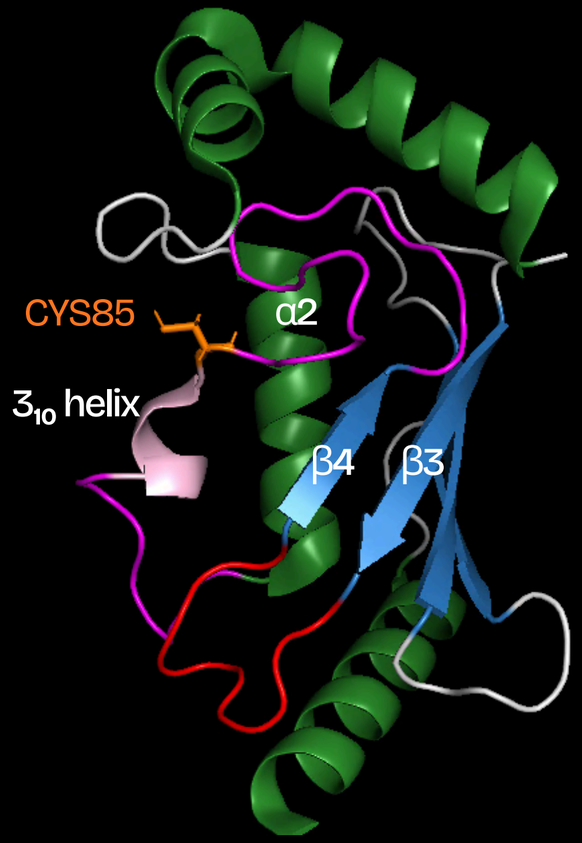
SECONDARY STRUCTURE STABILITY



SECONDARY STRUCTURE STABILITY



LOOPS L4 AND L7



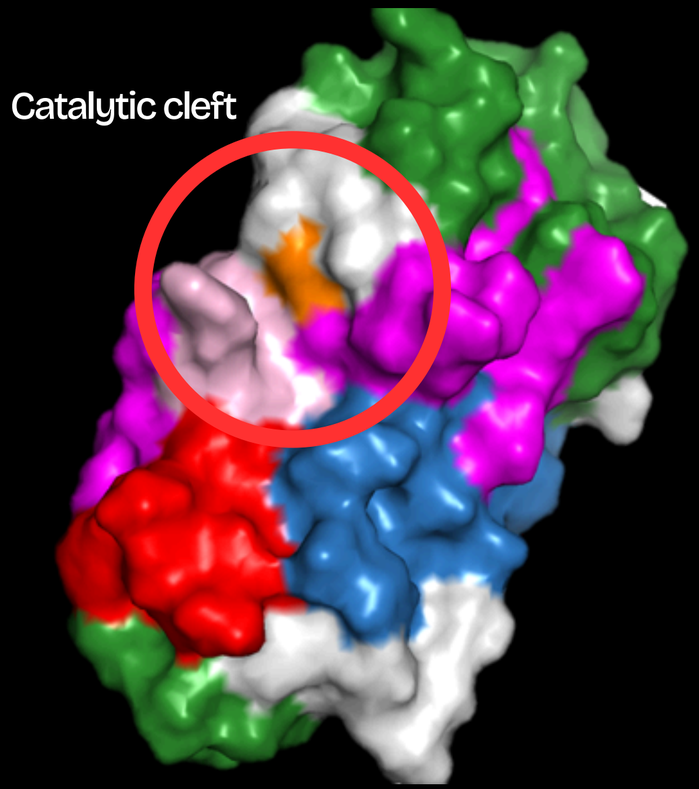
α-helix

β-strands

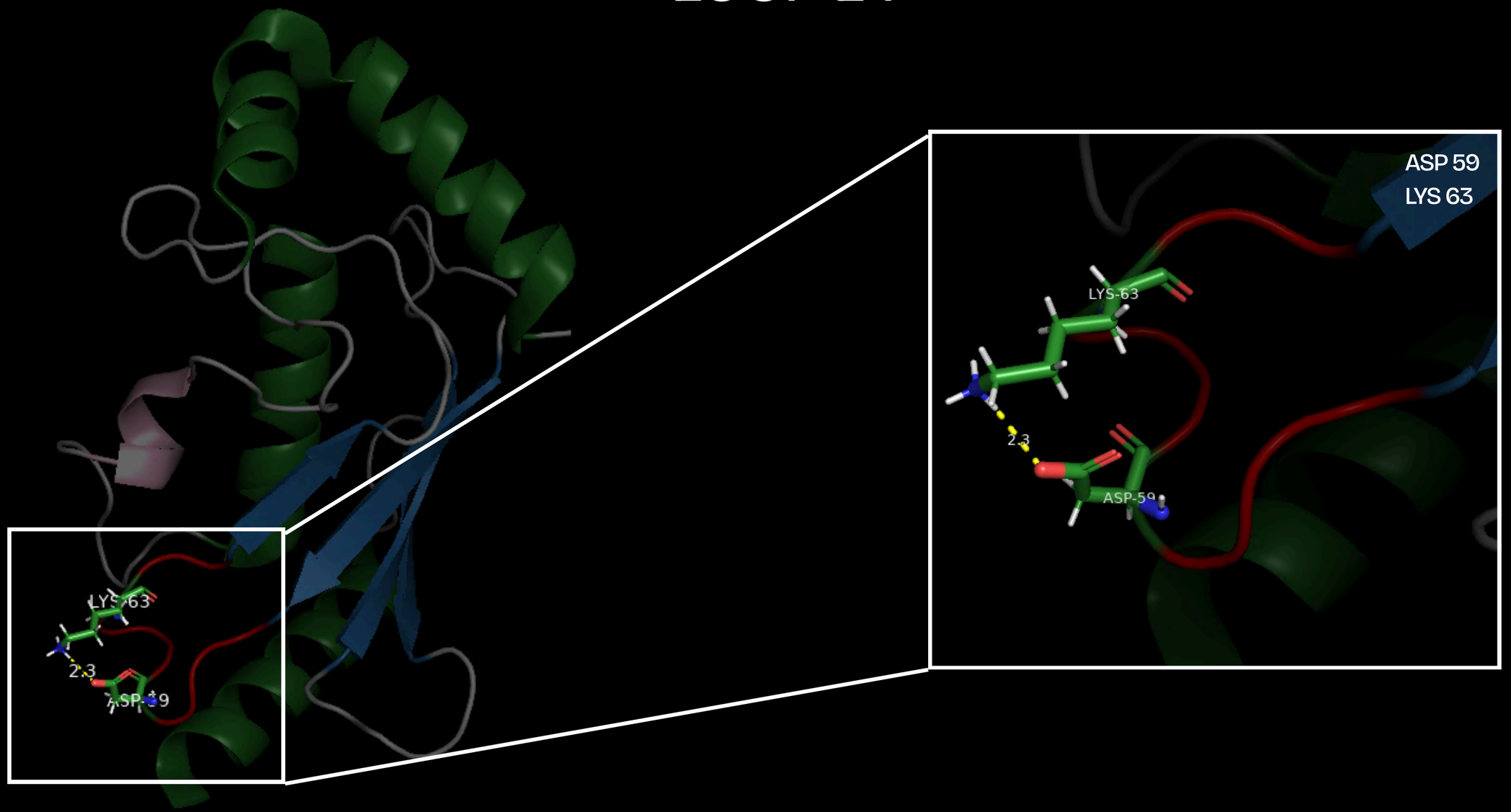
3_{10} helix

L4

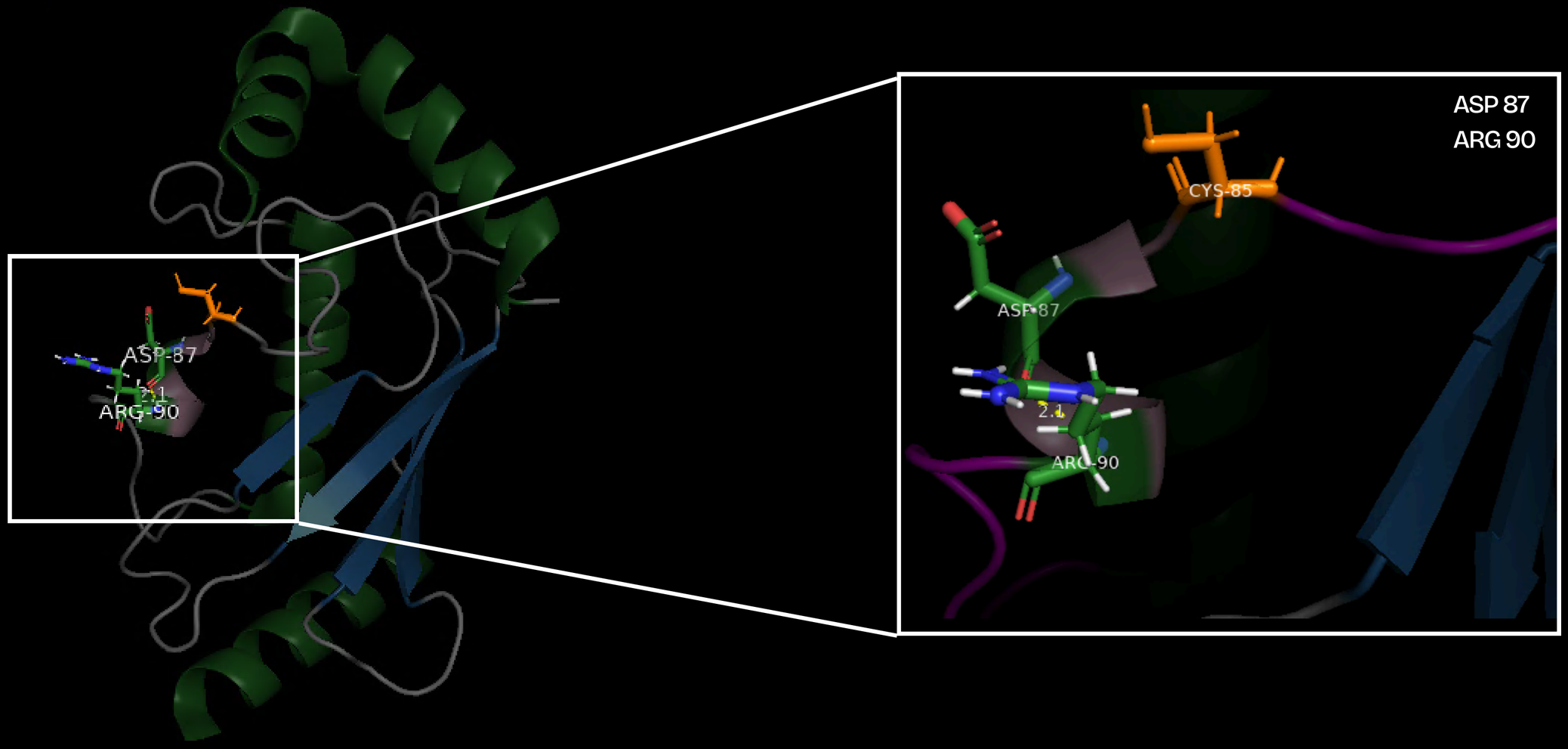
L7



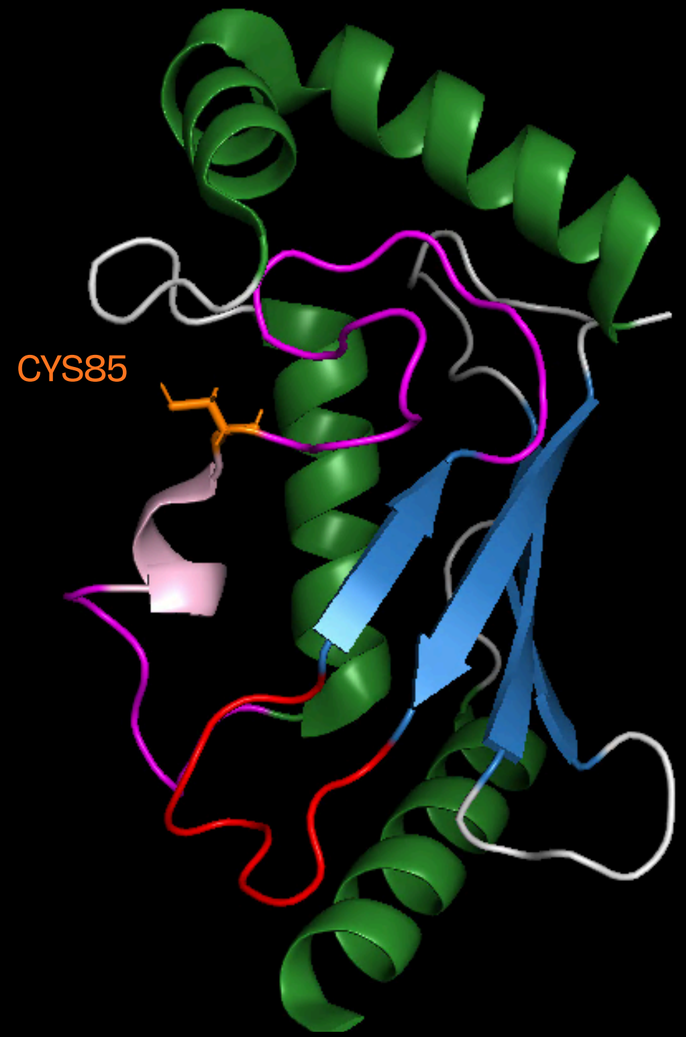
LOOP L4

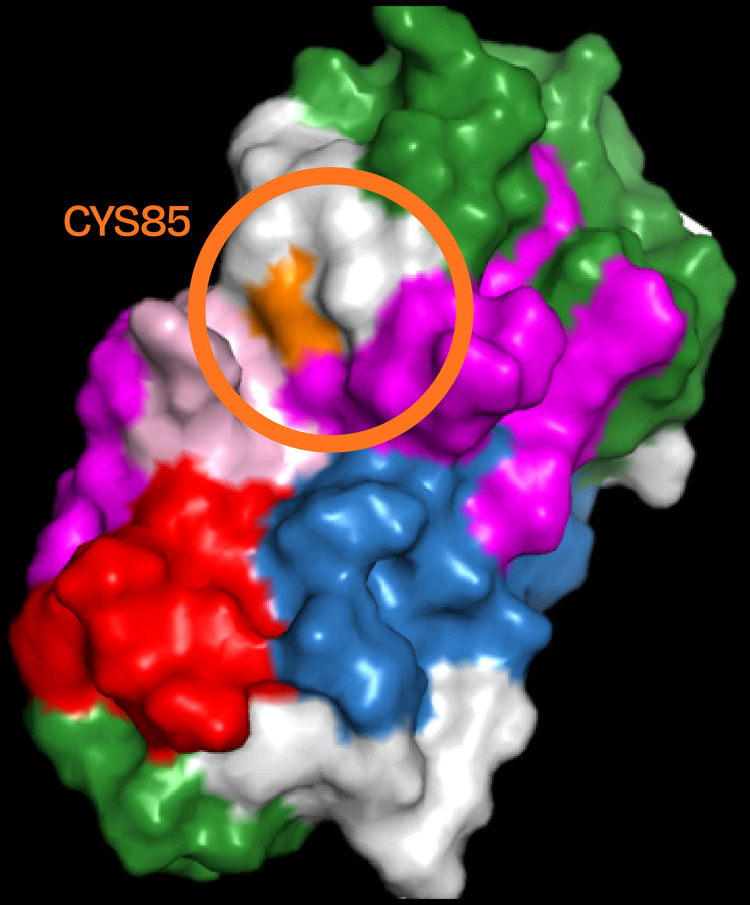


LOOP L7

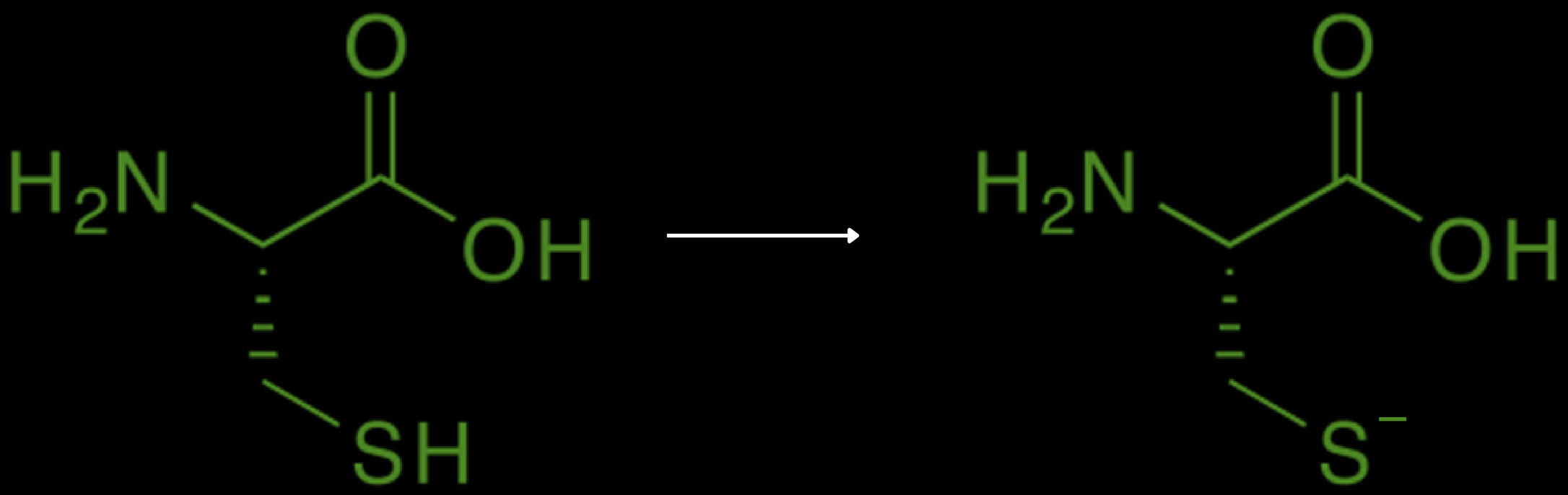


CYS85



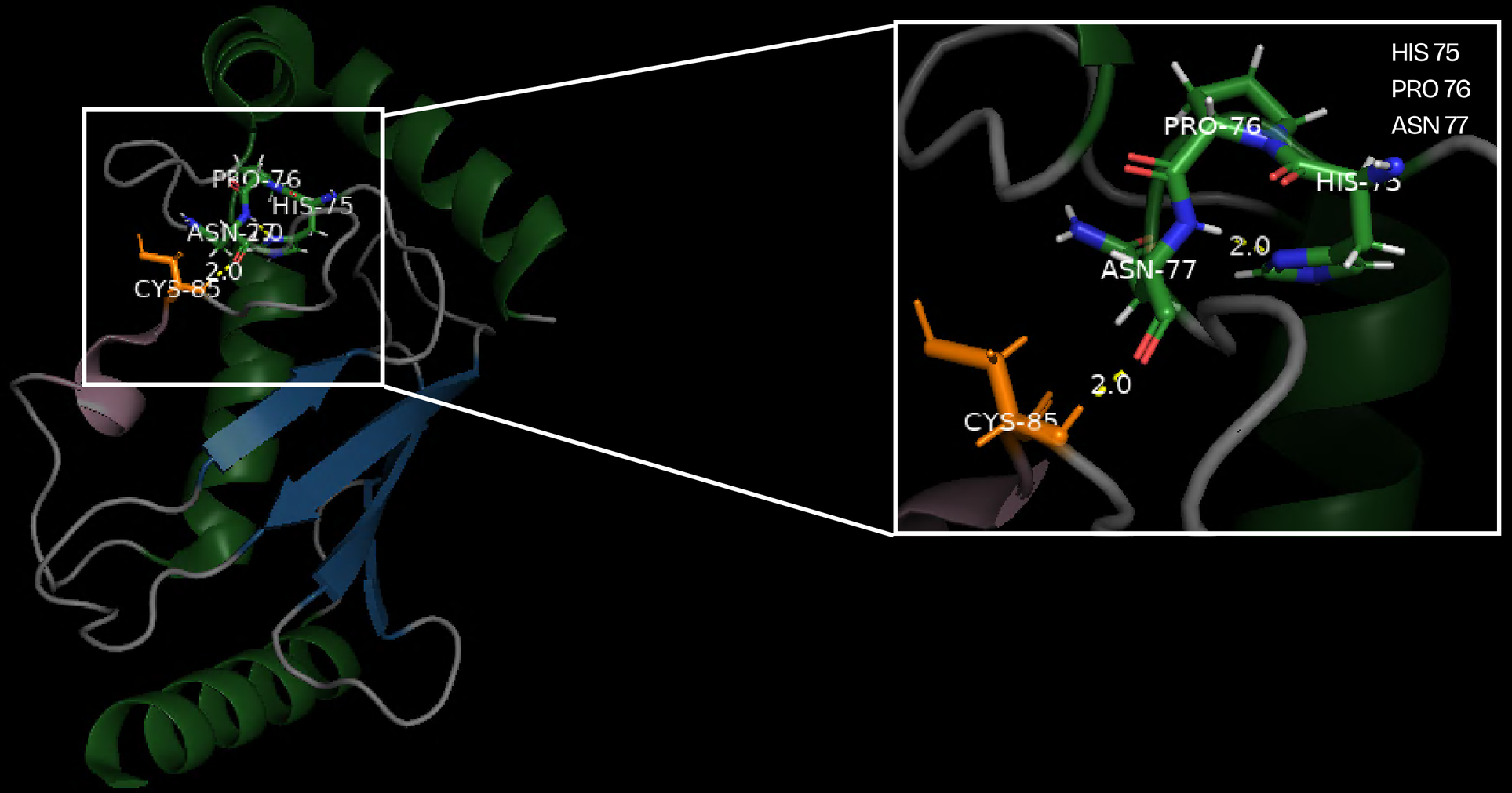


CYS85

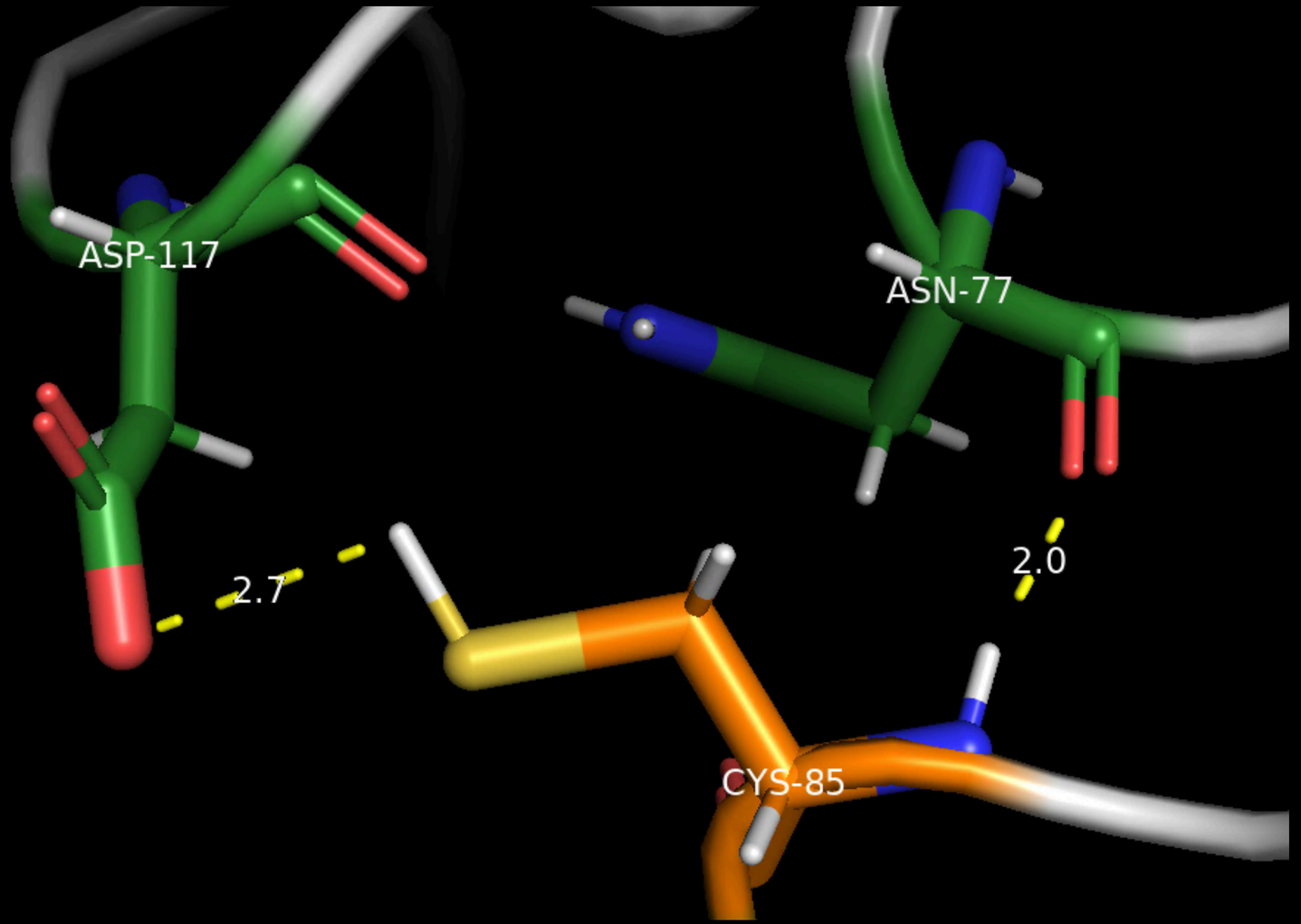


thiolate ion
nucleophile

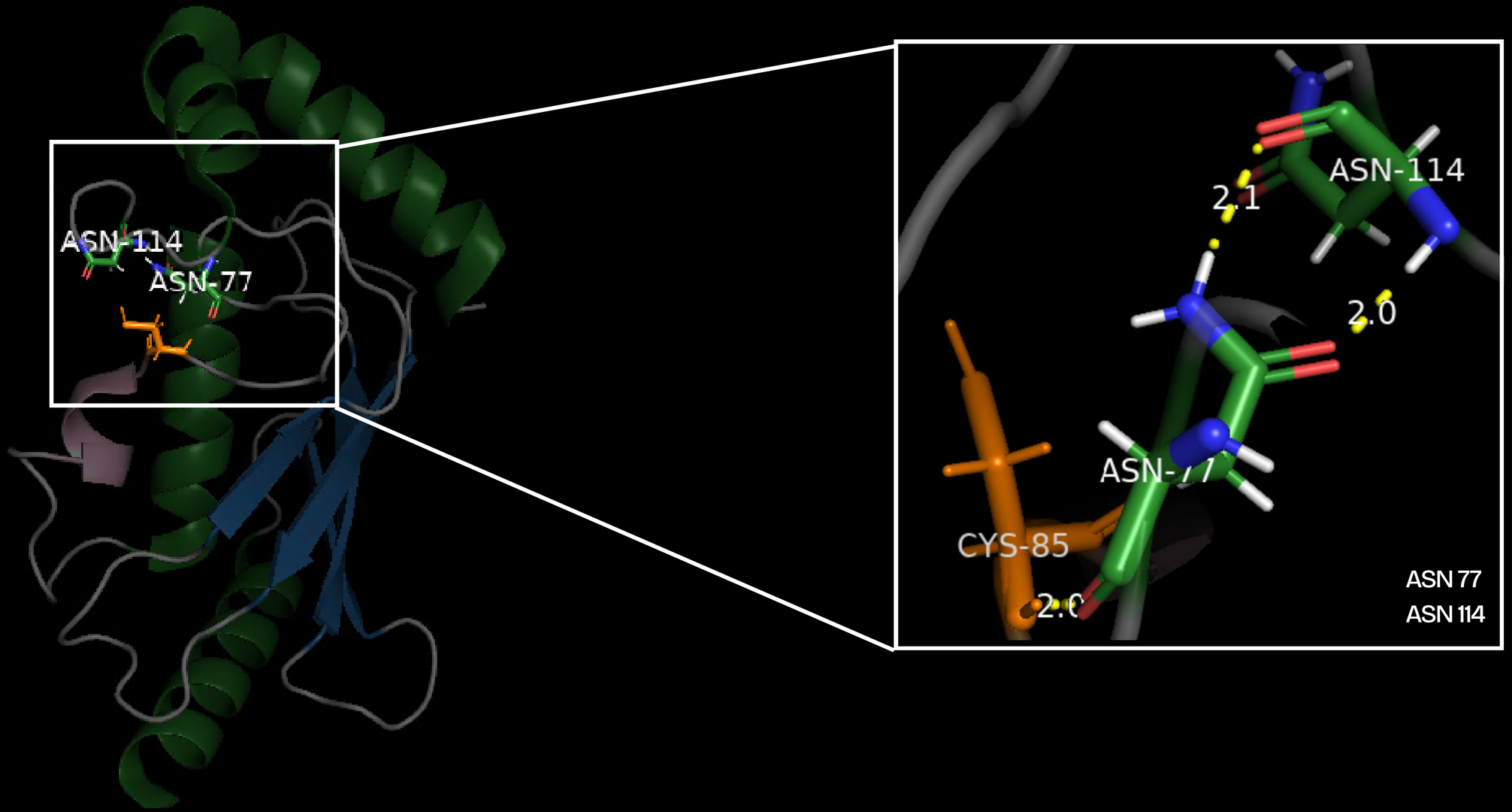
HPN MOTIF


PRO-76
HIS-75
ASN-77
2.0
CYS-85
HIS 75
PRO 76
ASN 77
PRO-76
HIS-75
2.0
ASN-77
2.0
CYS-85

HPN MOTIF



HYDROGEN BONDS



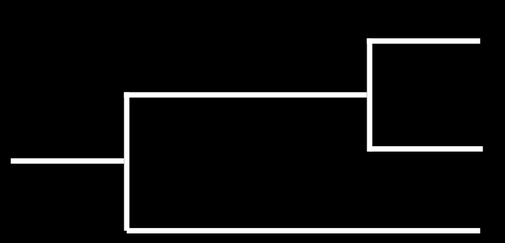
ALIGNMENT



- cys85
- HPN motif
- 3_{10} helix
- Asp59-Lys63
- Asn114
- Asp117

STRUCTURAL ALIGNMENT

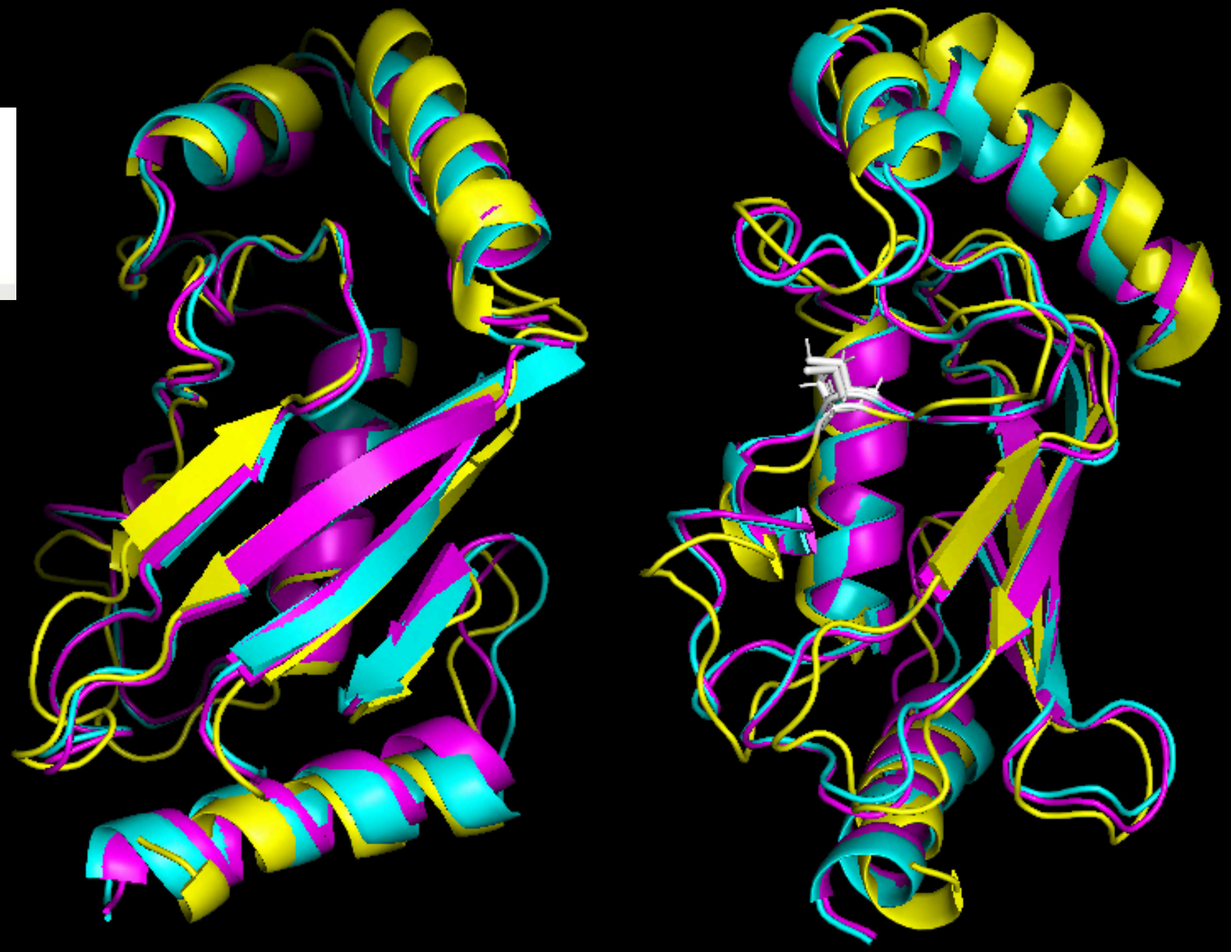
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22 Doing cluster analysis...
23 Cluster:  1 (    1qc1  &     7bol ) Sc  9.39 RMS   0.81 Len 148 nfit 147
24  See file ube2d2.1 for the alignment and transformations
25 Cluster:  2 (    2fuh  &     1qc1     7bol ) Sc  9.17 RMS   1.88 Len 146 nfit 144
26  See file ube2d2.2 for the alignment and transformations
```



Saccharomyces cerevisiae

Homo sapiens

Rattus norvegicus



E1 - UBIQUITIN - E2

E1: Ubiquitin-activating enzymes

E2: Ubiquitin-conjugating enzymes

COMPLEX E1 - UBIQUITIN - E2

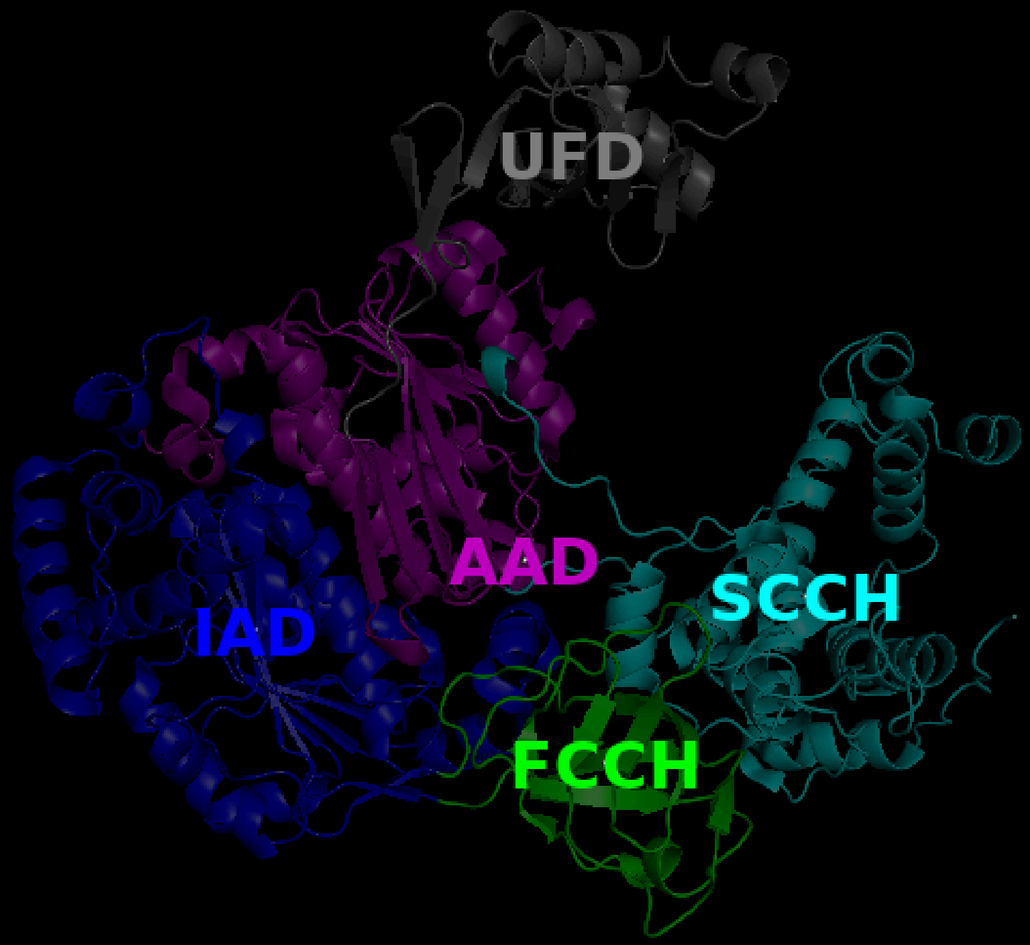


PDB ID: 4II2

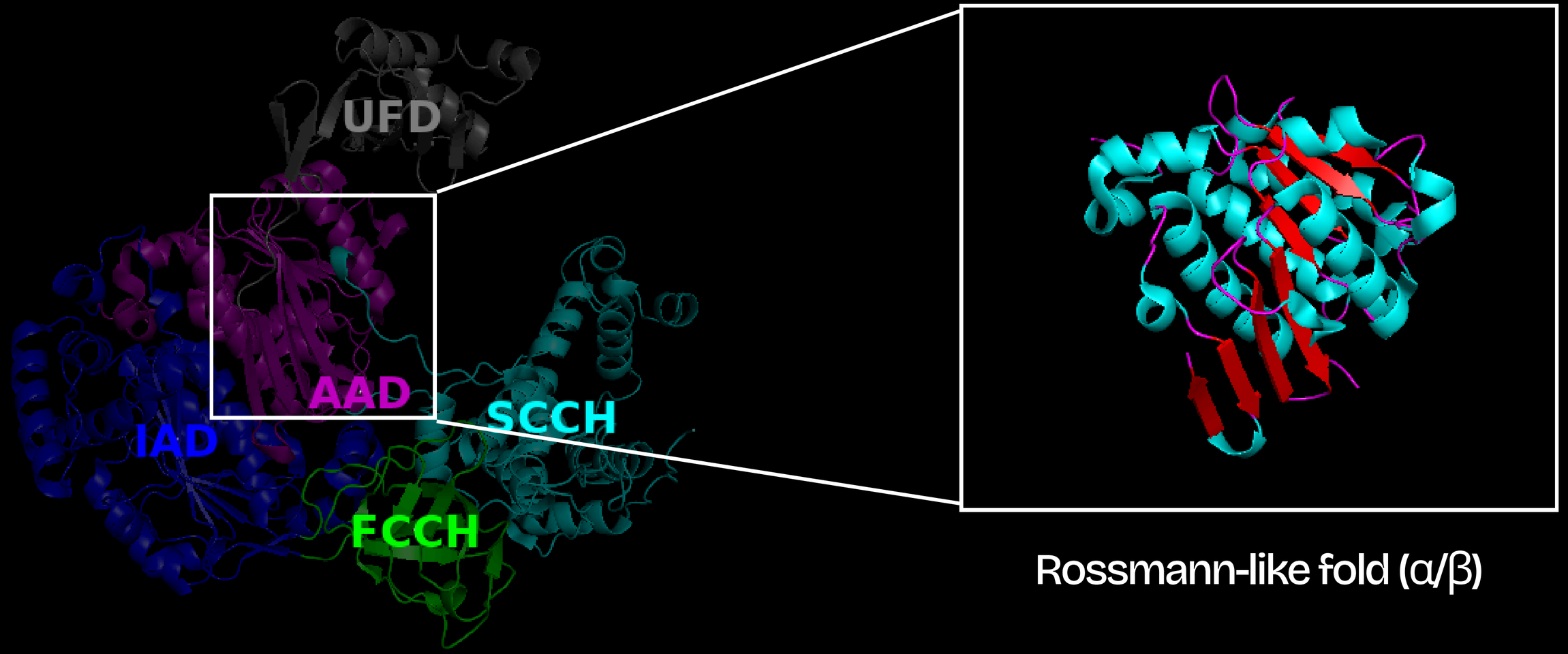
Resolution: 2.20 Å

Organism: Schizosaccharomyces pombe

E1 DOMAINS

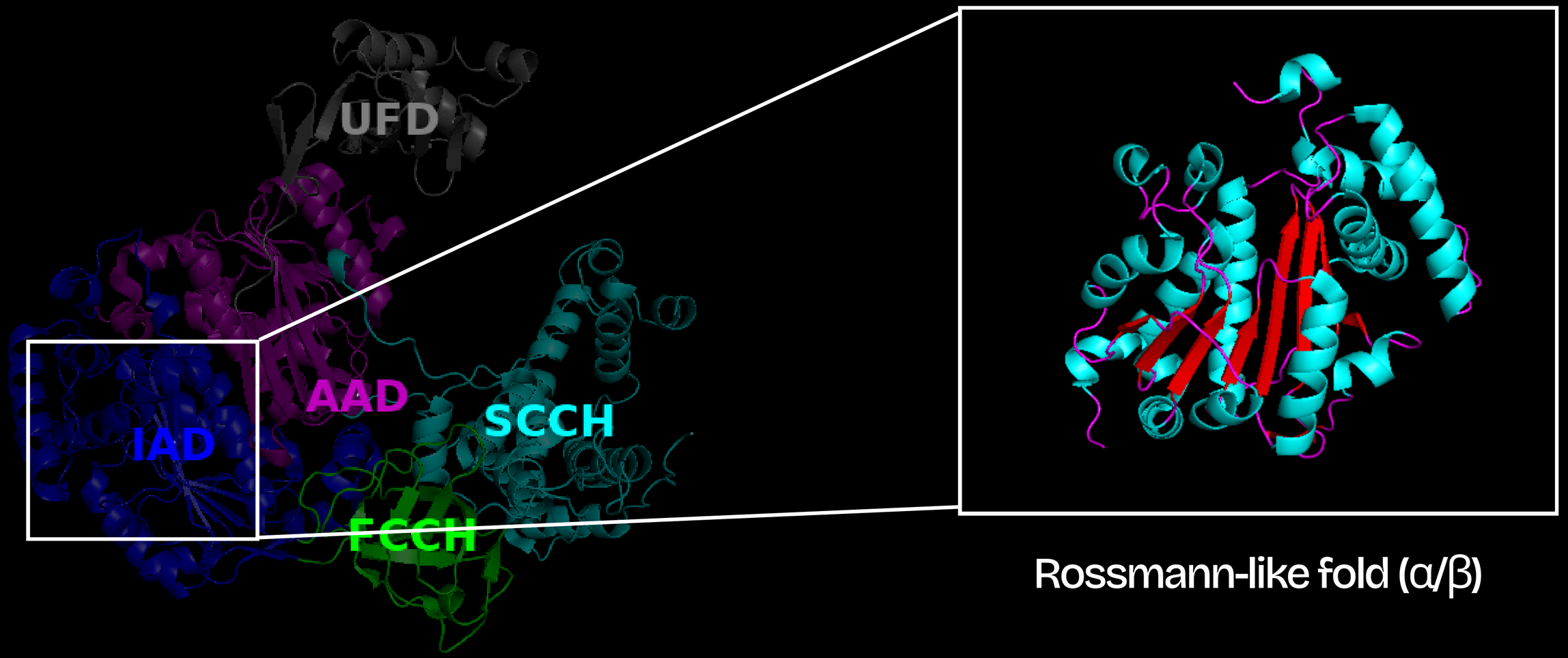


E1 DOMAINS: AAD



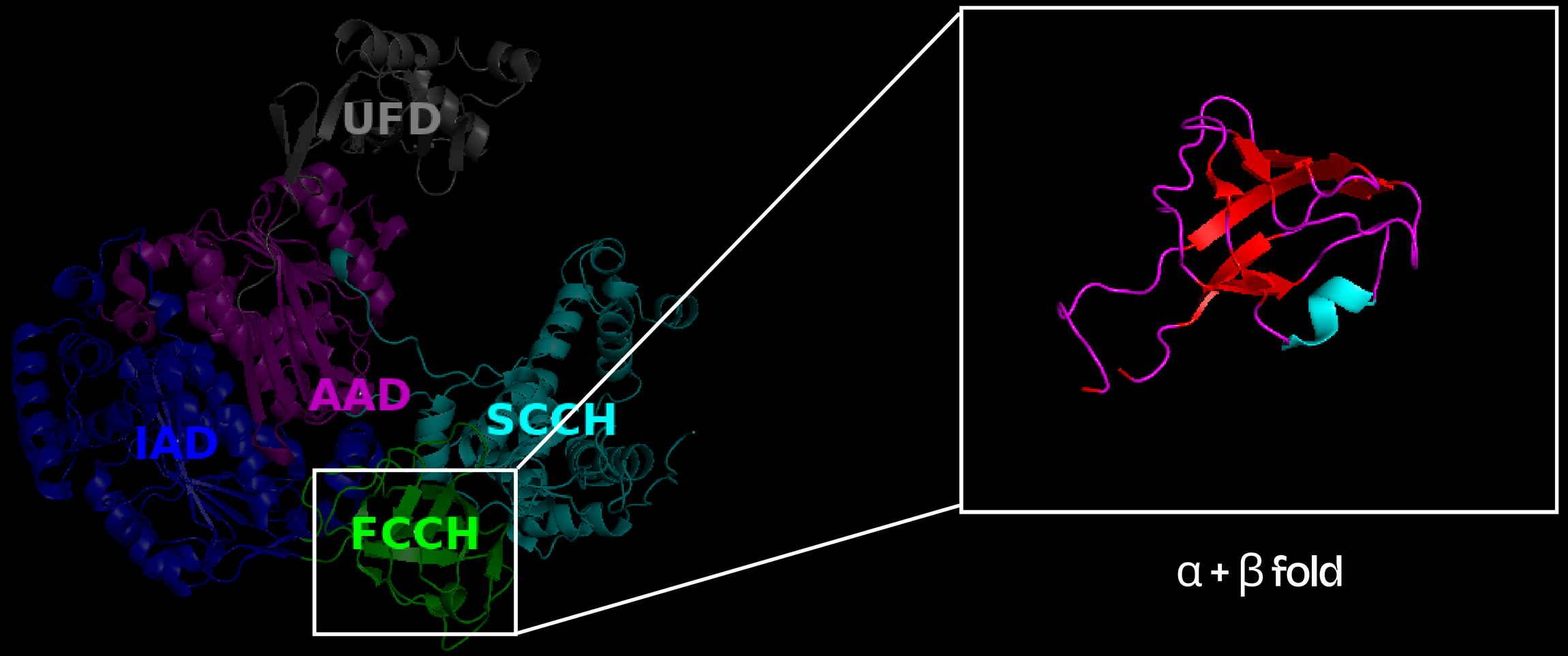
Rossmann-like fold (α/β)

E1 DOMAINS: IAD

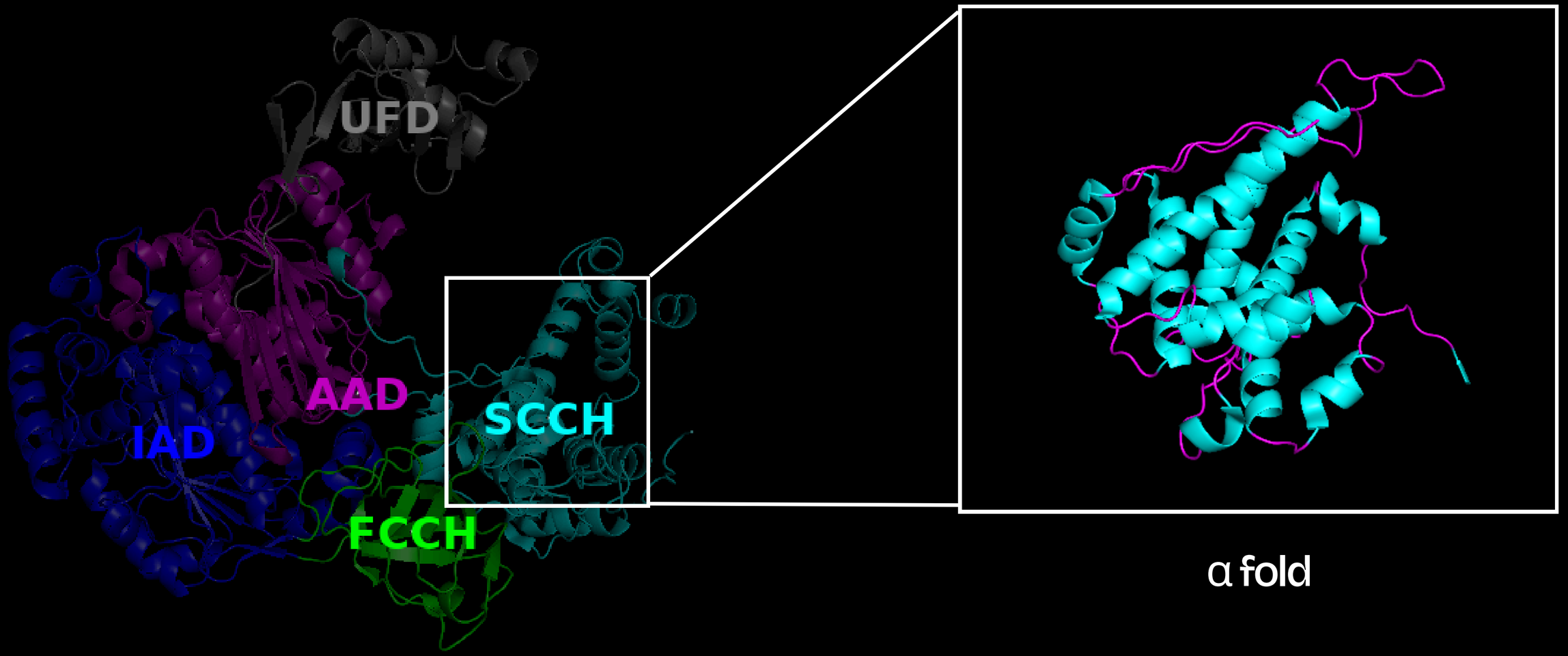


Rossmann-like fold (α/β)

E1 DOMAINS: FCCH

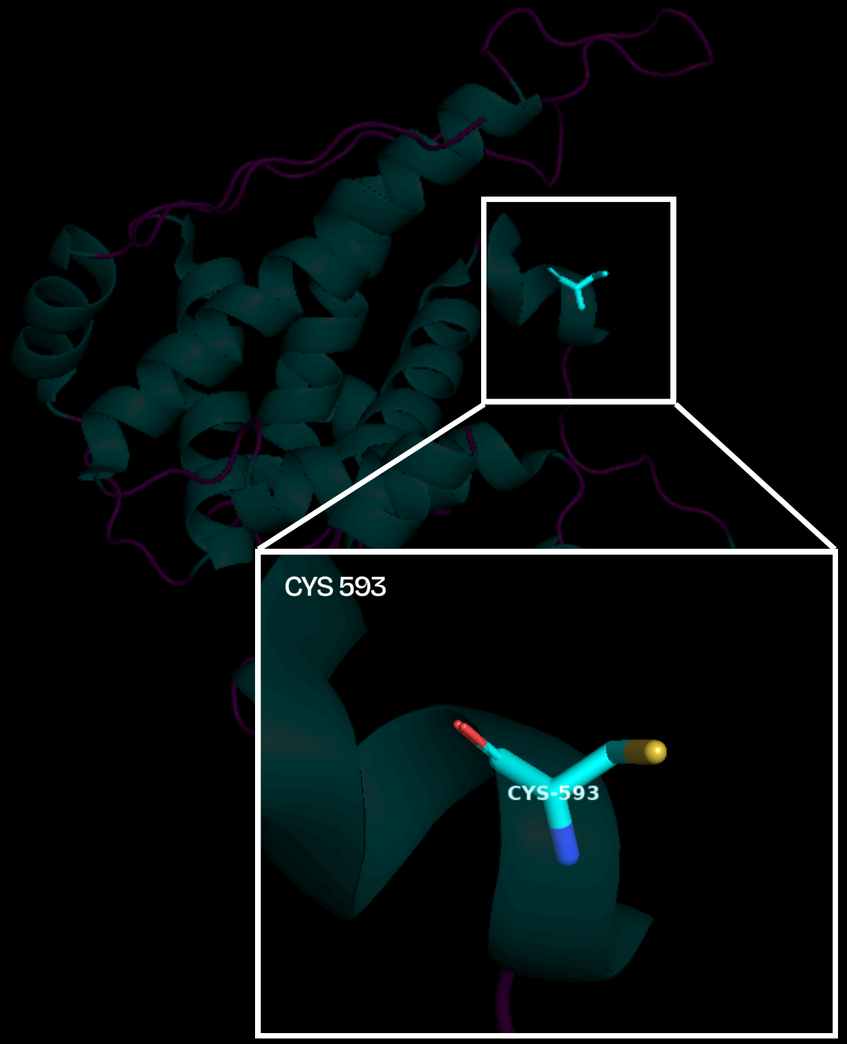


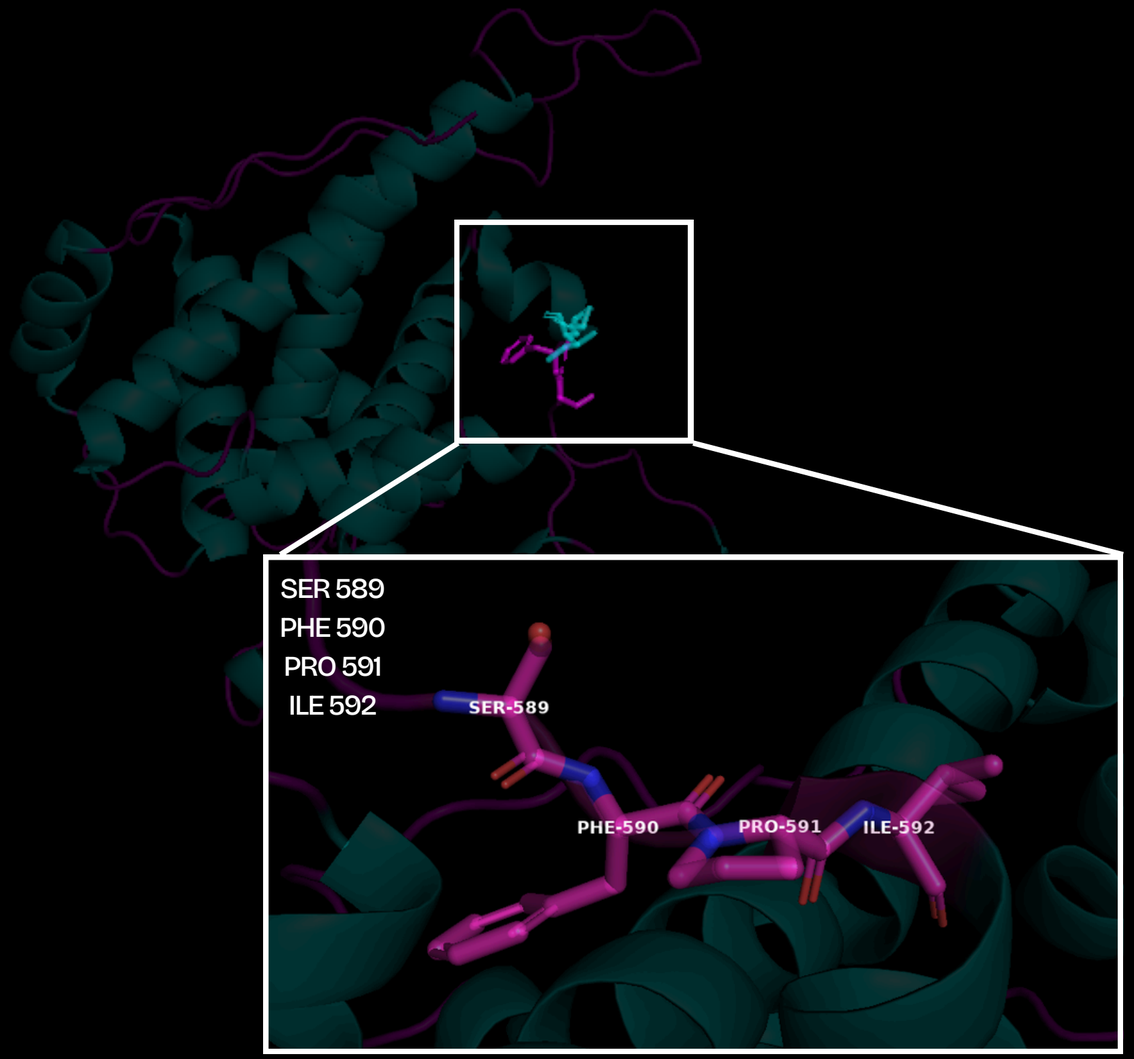
E1 DOMAINS: SCCH



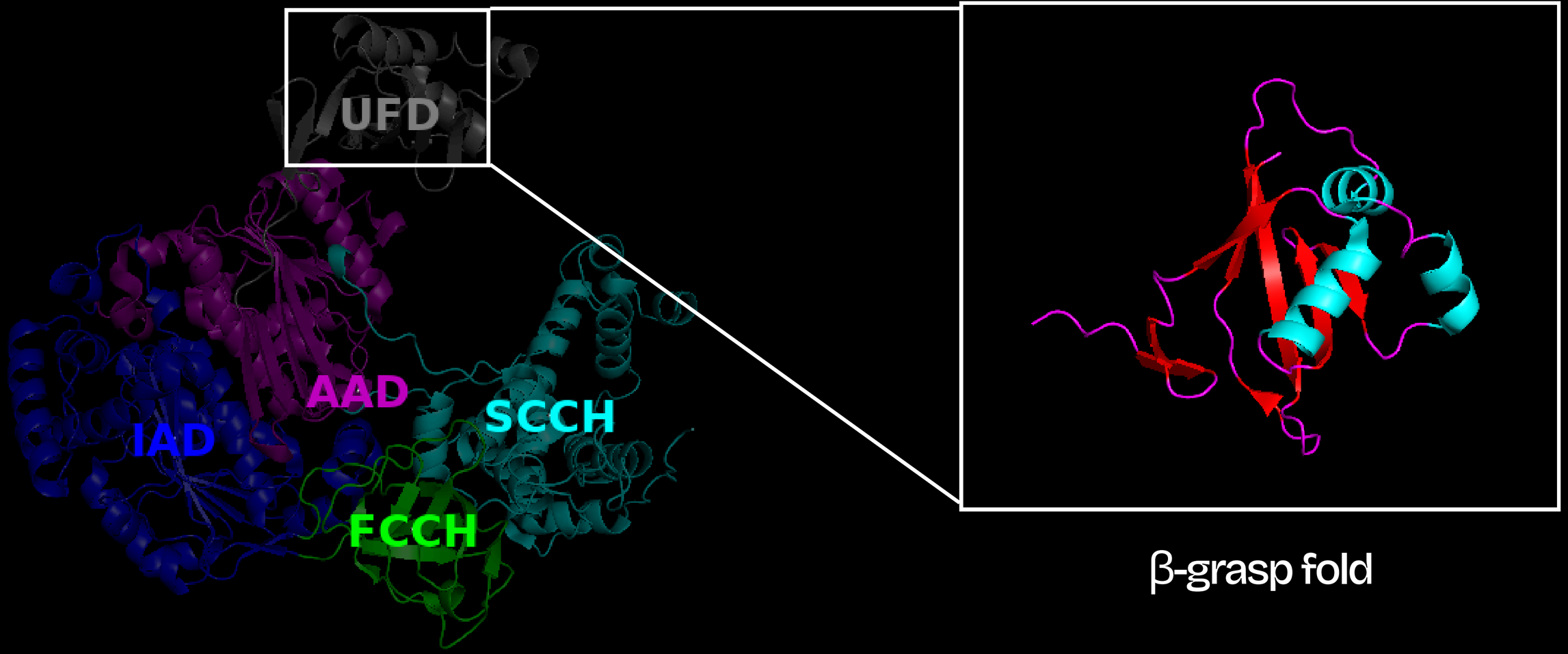
α fold

E1 DOMAINS: SCCH



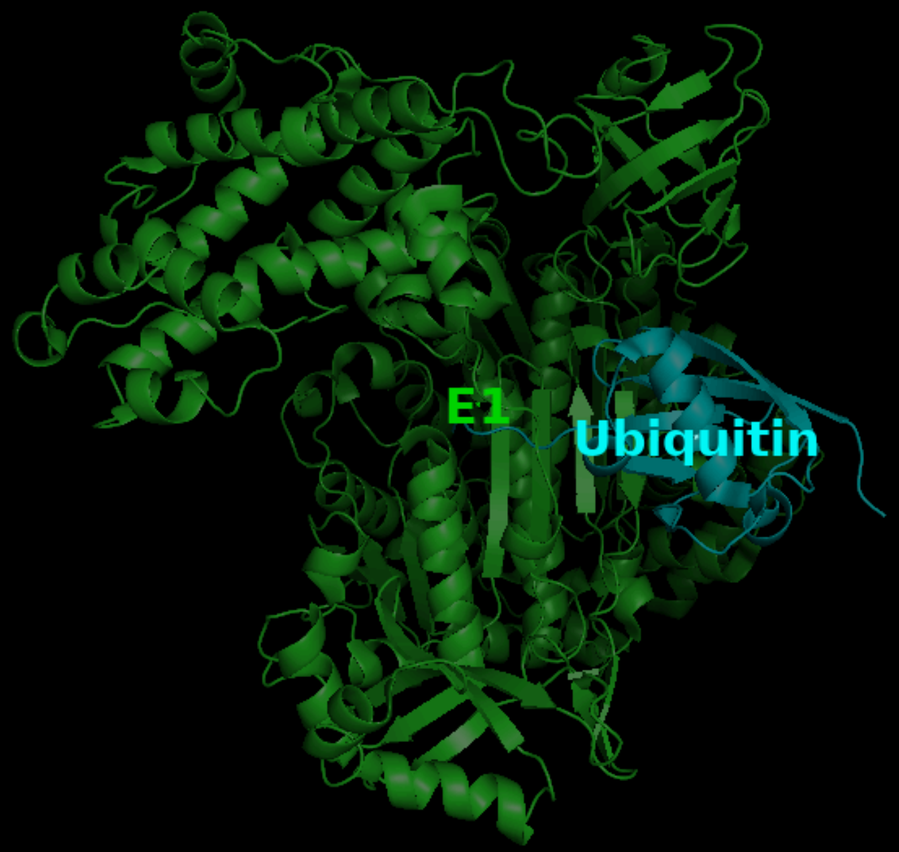


E1 DOMAINS: UFD

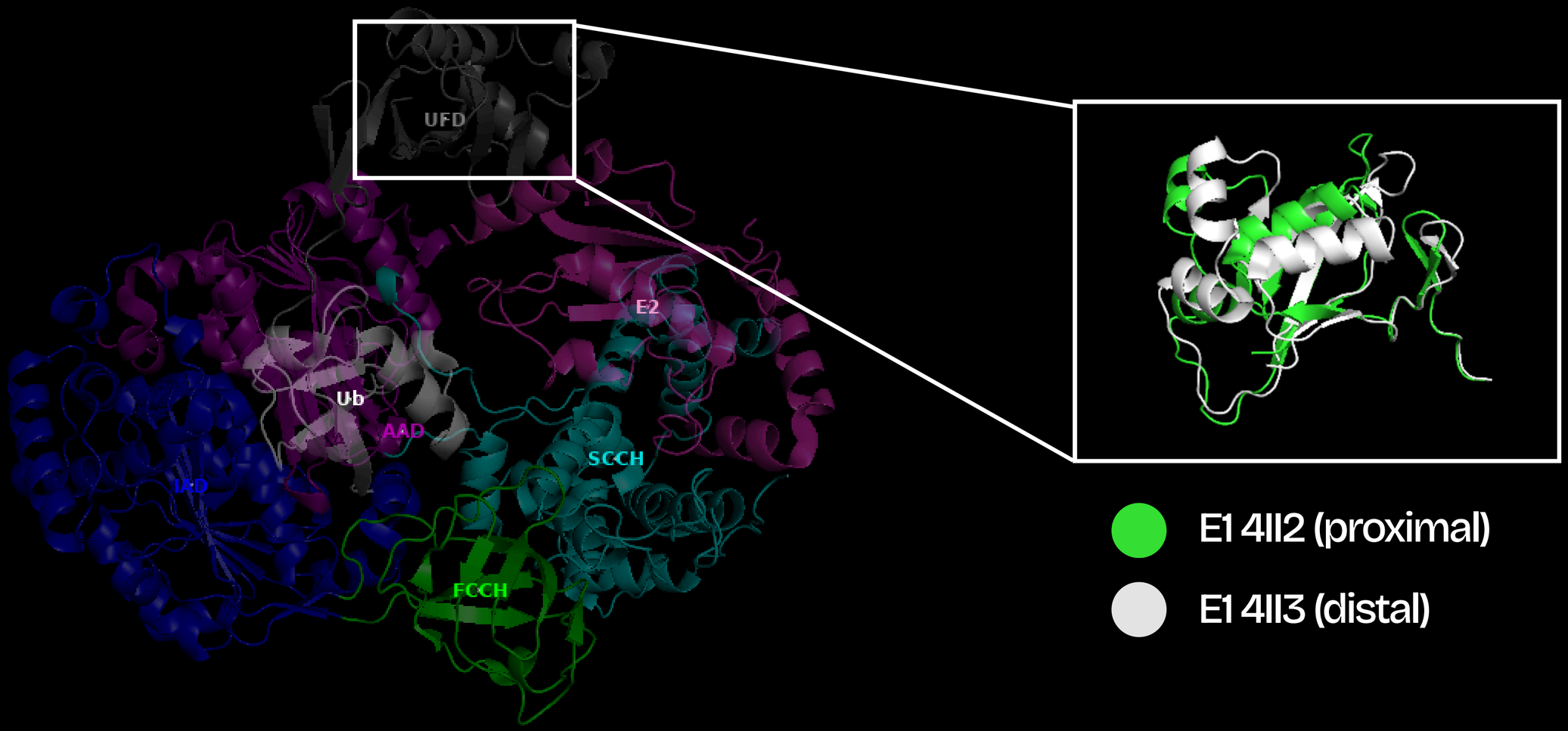


β-grasp fold

E1 PRIMING



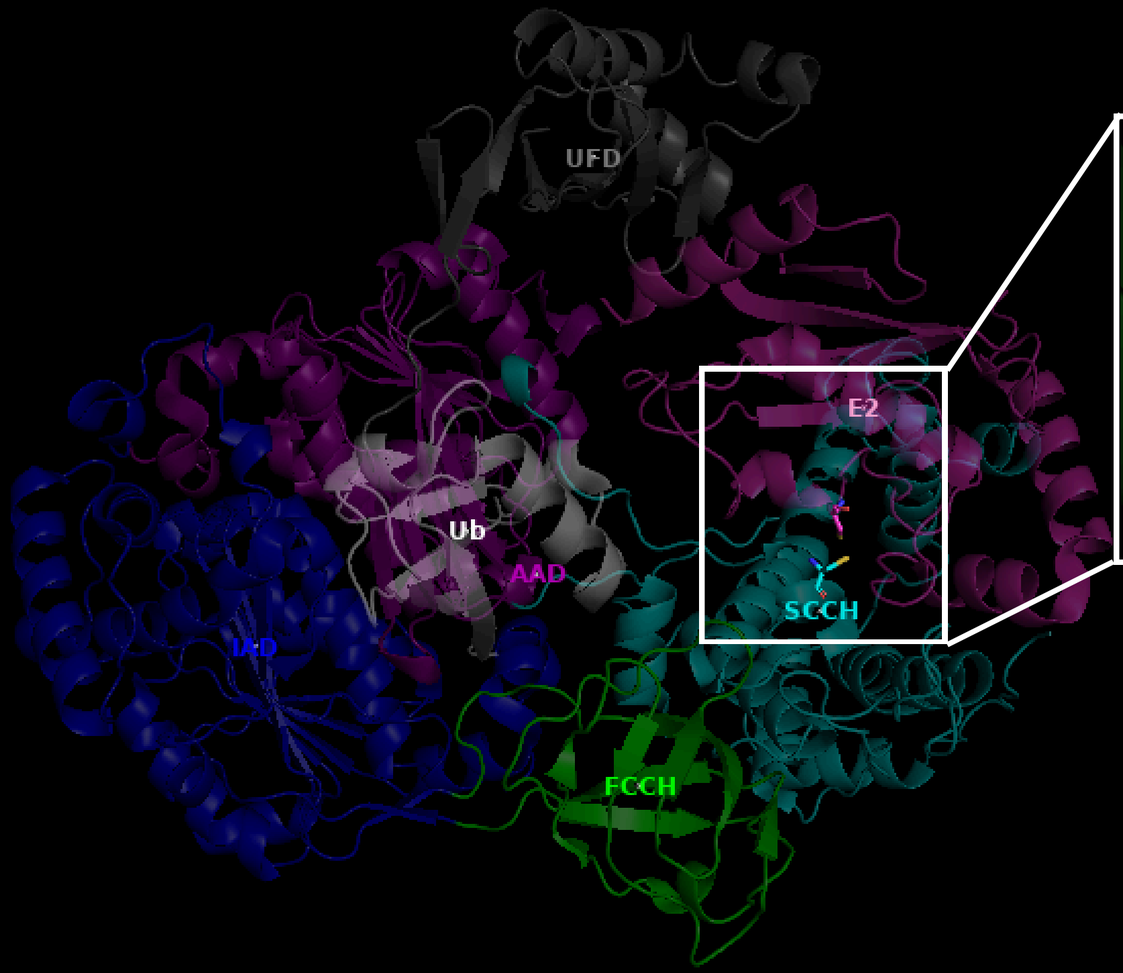
E2 RECRUITMENT

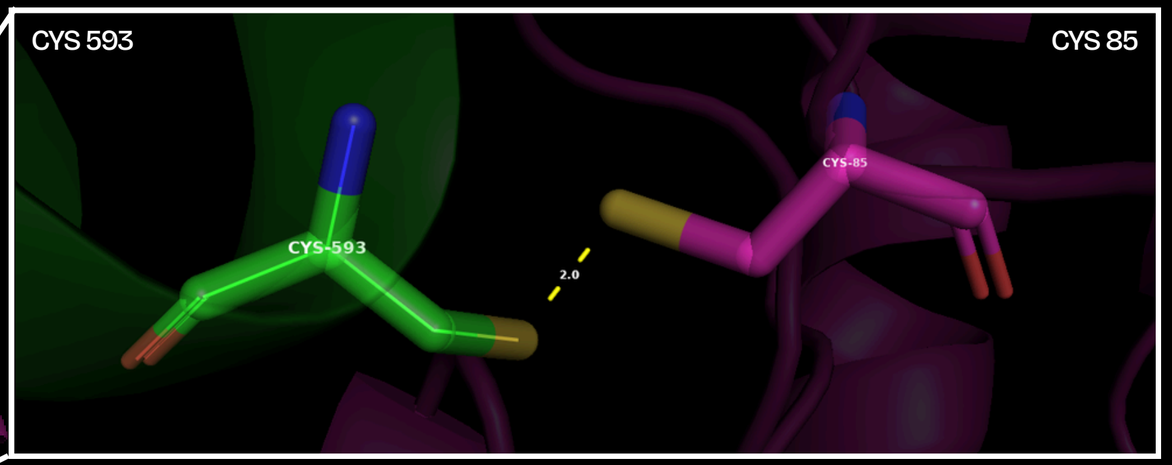


E1 4II2 (proximal)

E1 4II3 (distal)

E2 RECRUITMENT

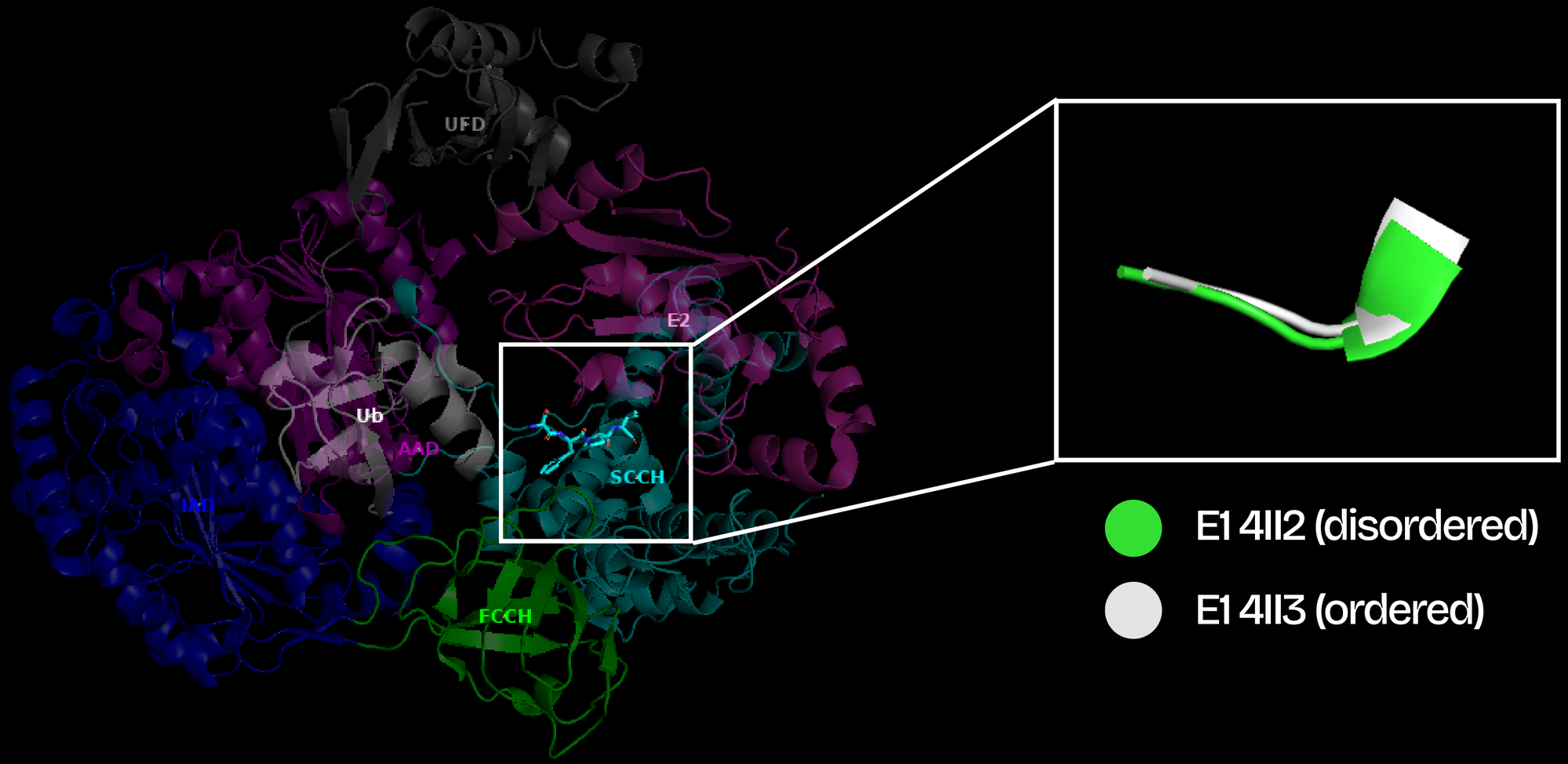




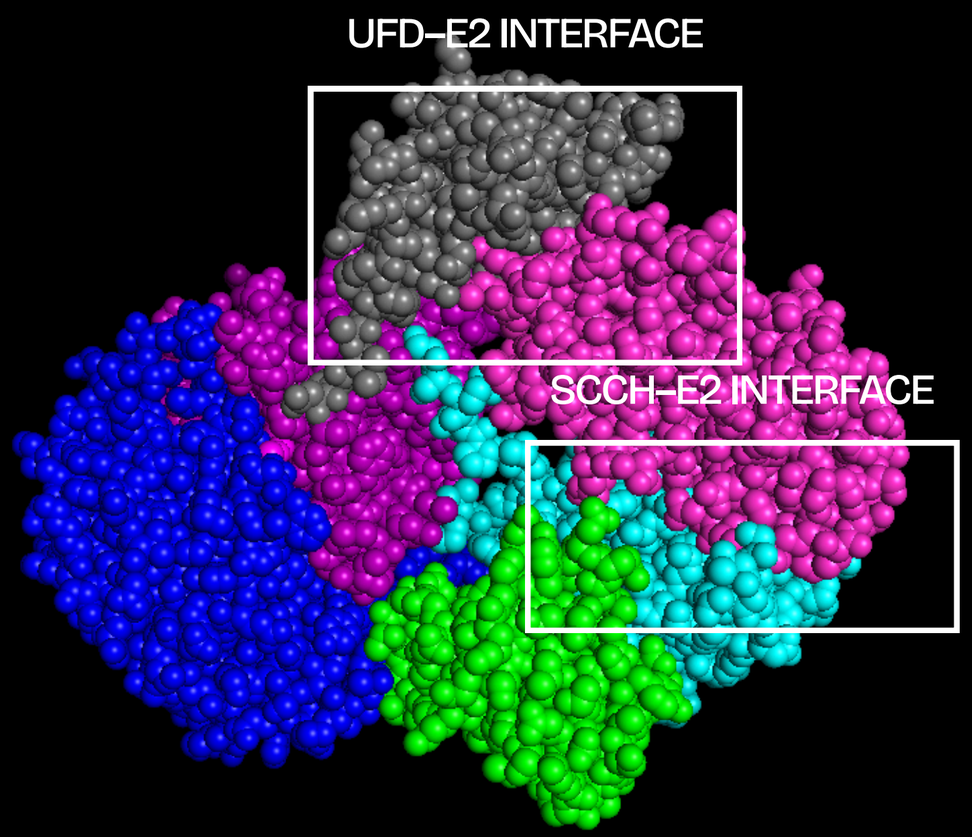
E1 4II2

E2 4II2

E2 RECRUITMENT



E1 - E2 INTERACTION



E2

UFD

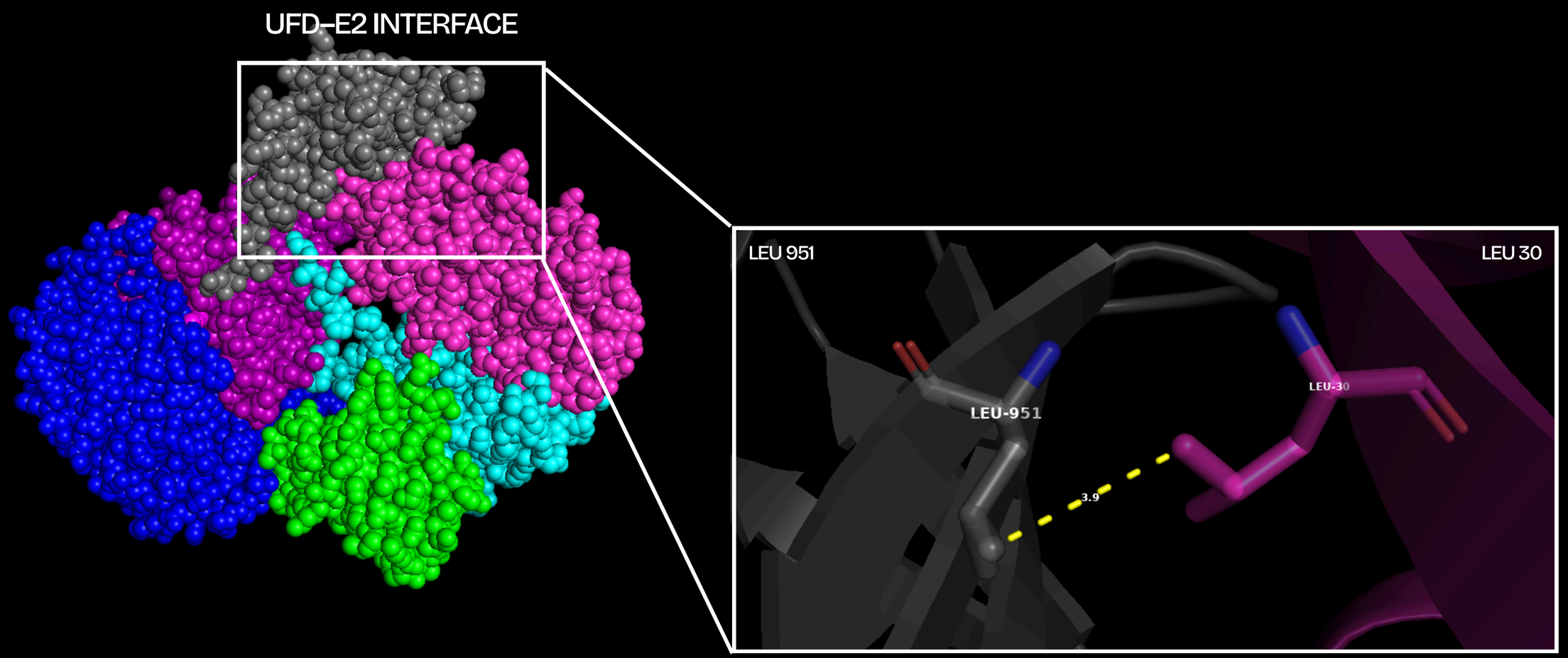
SCCH

AAD

IAD

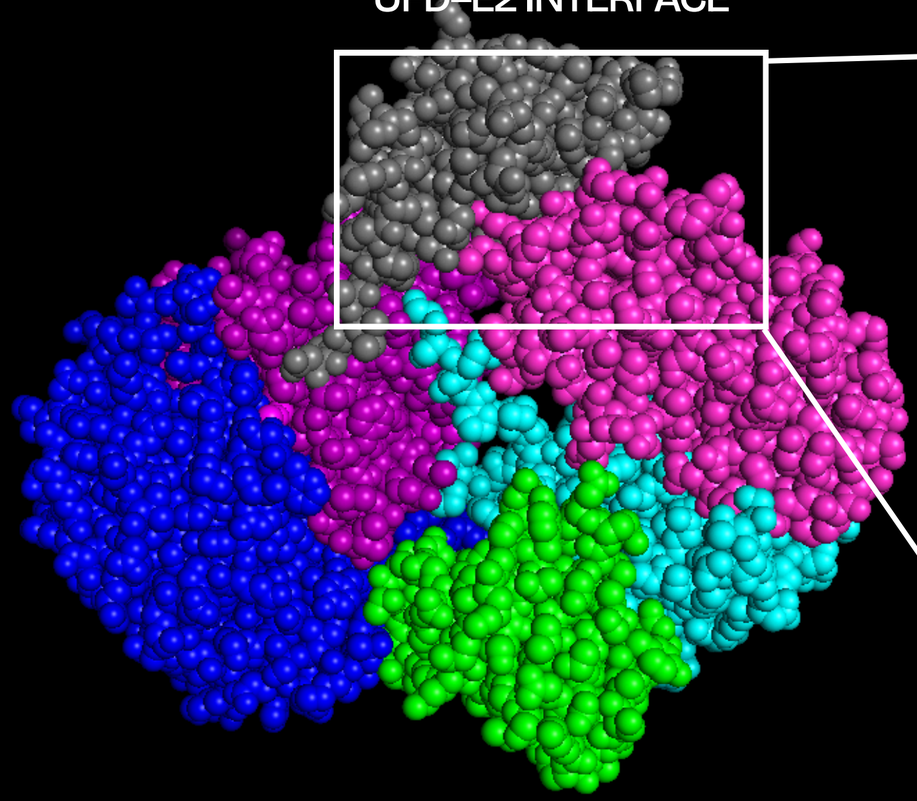
FCCH

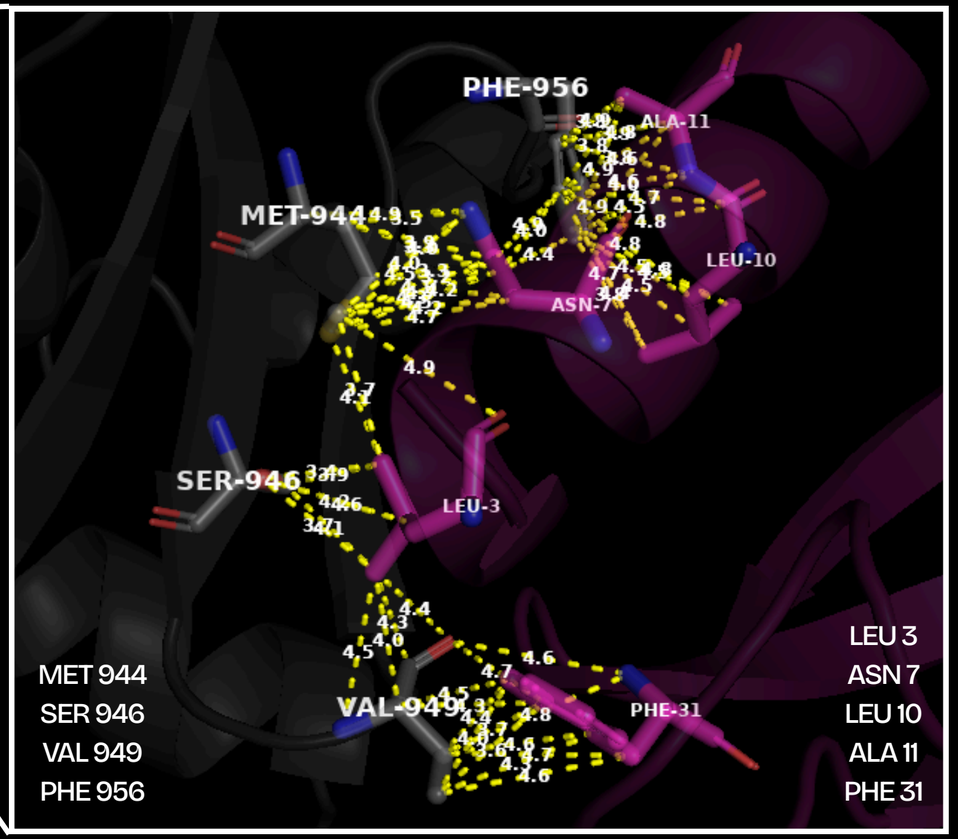
E1 - E2 INTERACTION



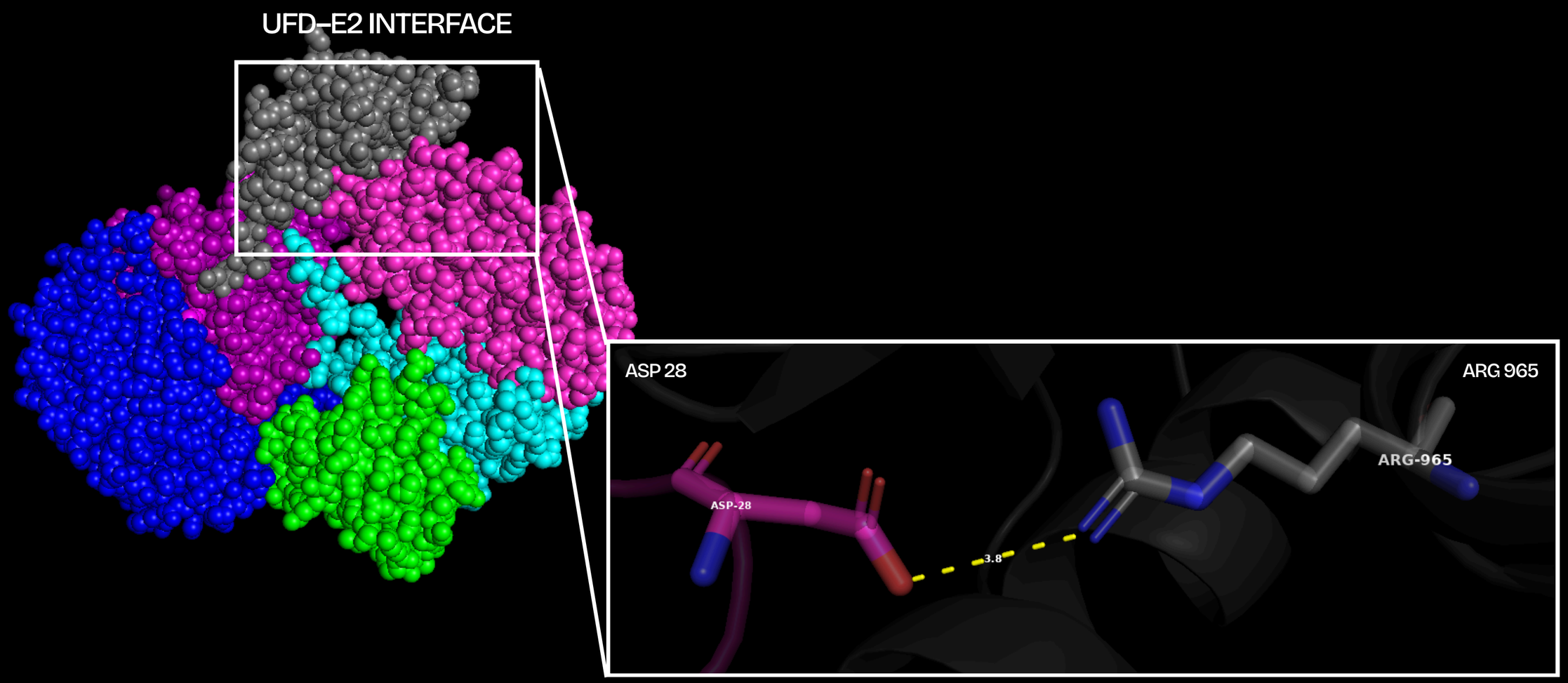
E1 - E2 INTERACTION

UFD–E2 INTERFACE

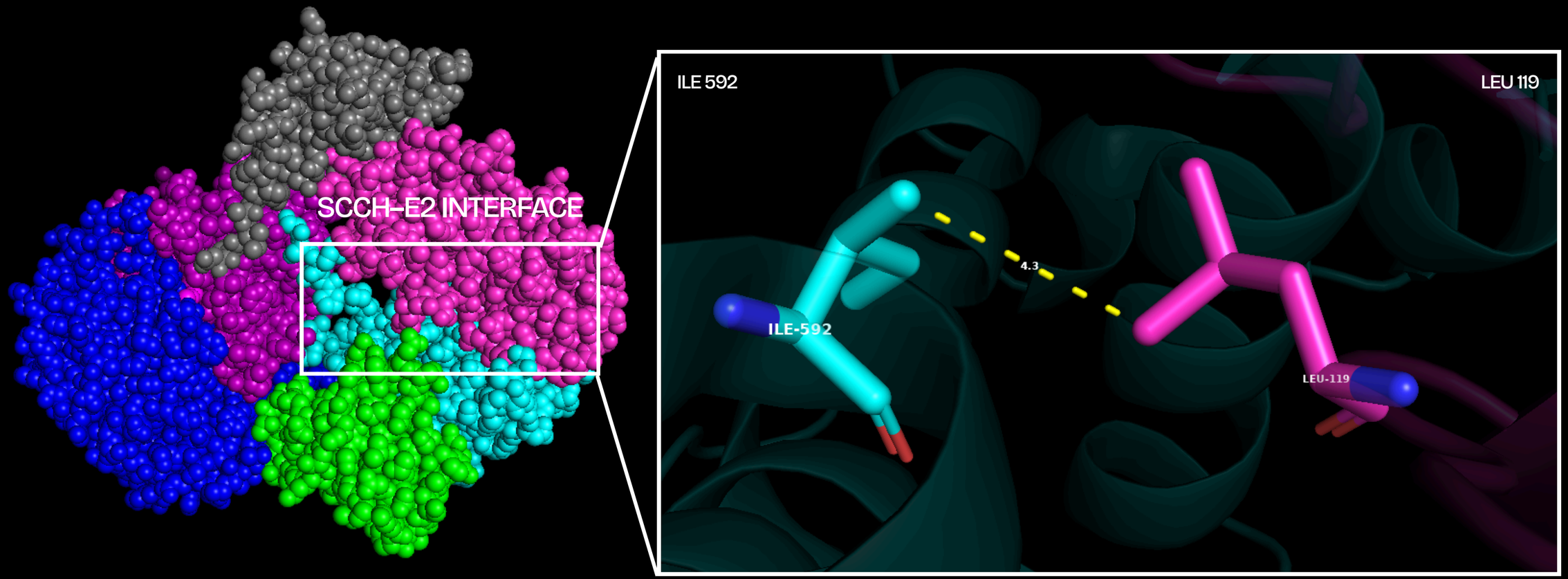




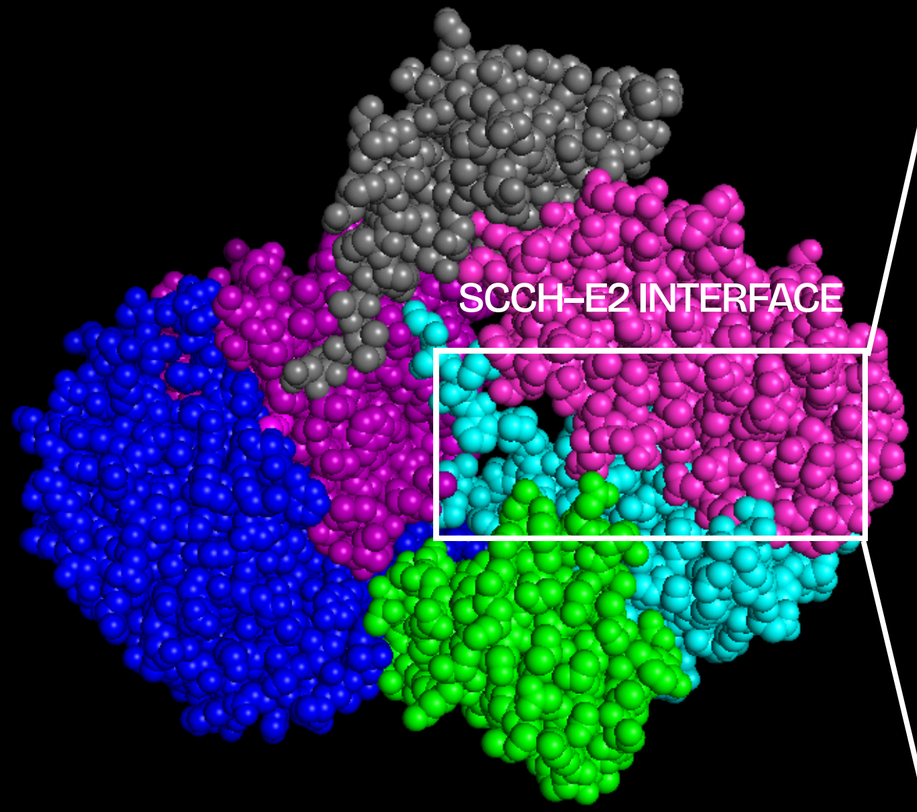
E1 - E2 INTERACTION

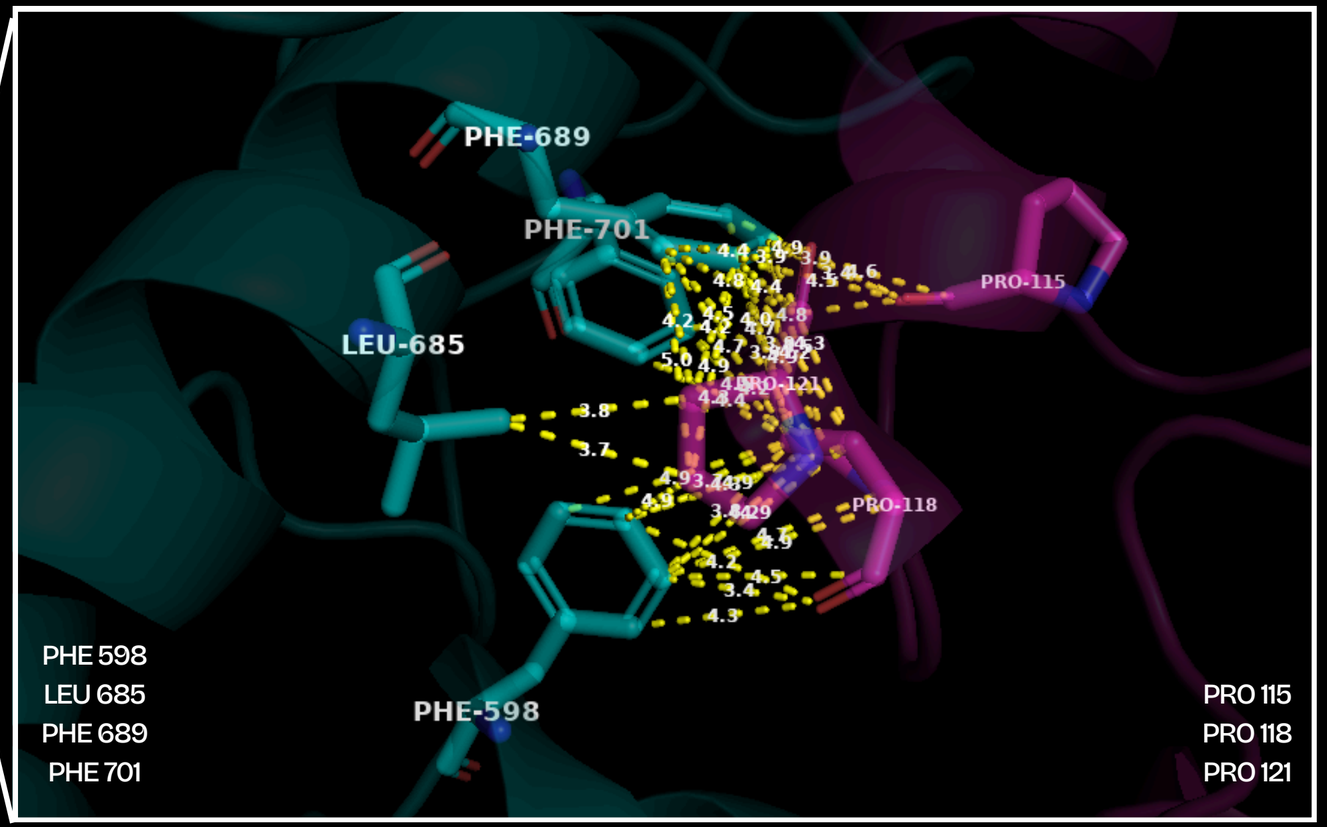


E1 - E2 INTERACTION



E1 - E2 INTERACTION





PHE 598
LEU 685
PHE 689
PHE 701

PRO 115
PRO 118
PRO 121

E2 - UBIQUITIN

E2: Ubiquitin-conjugating enzymes

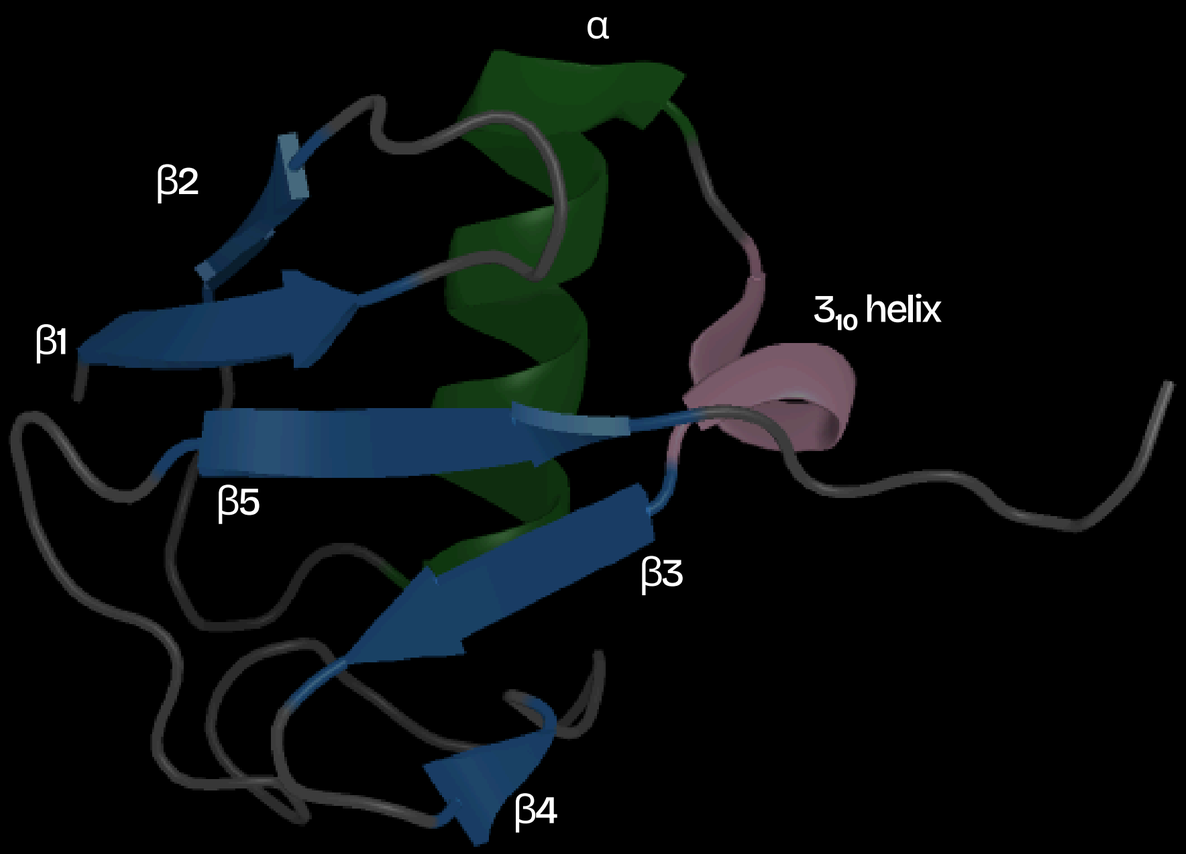
UBIQUITIN SCOP CLASSIFICATION

- **Class:** Alpha and beta proteins
- **Fold:** beta-Grasp
- **Superfamily:** Ubiquitin-like
- **Family:** Ubiquitin-related



PDB ID: 3UGB
Resolution: 2.35 Å
Organism: Homo sapiens

UBIQUITIN SECONDARY STRUCTURE



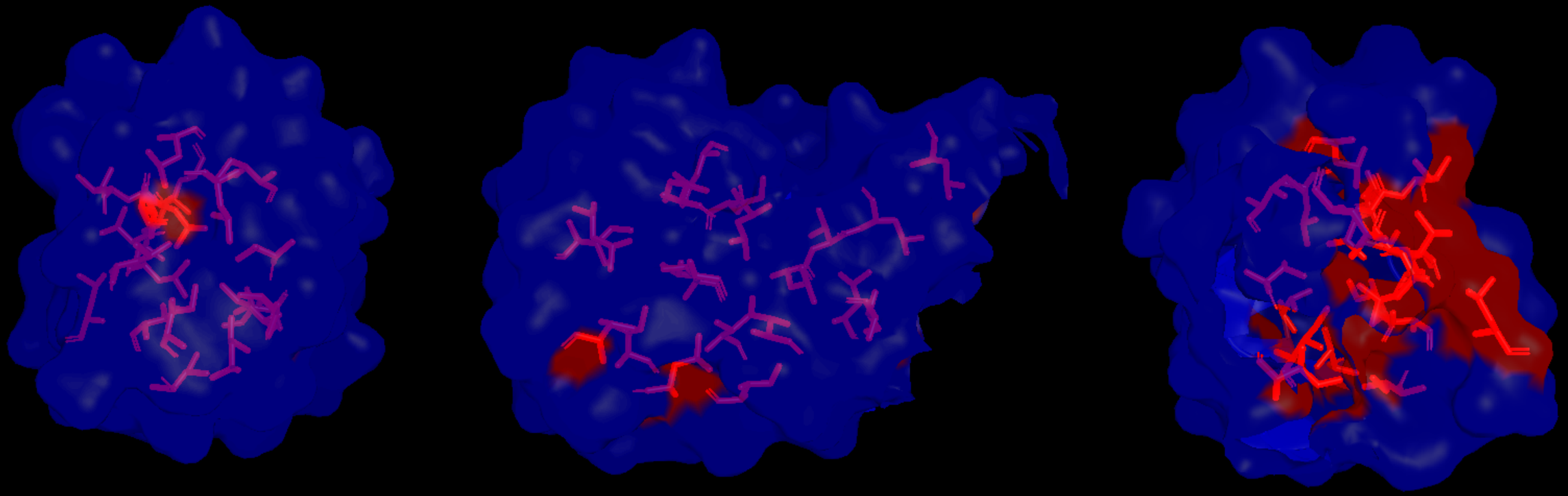
α-helix

β-strands

3_{10} helix

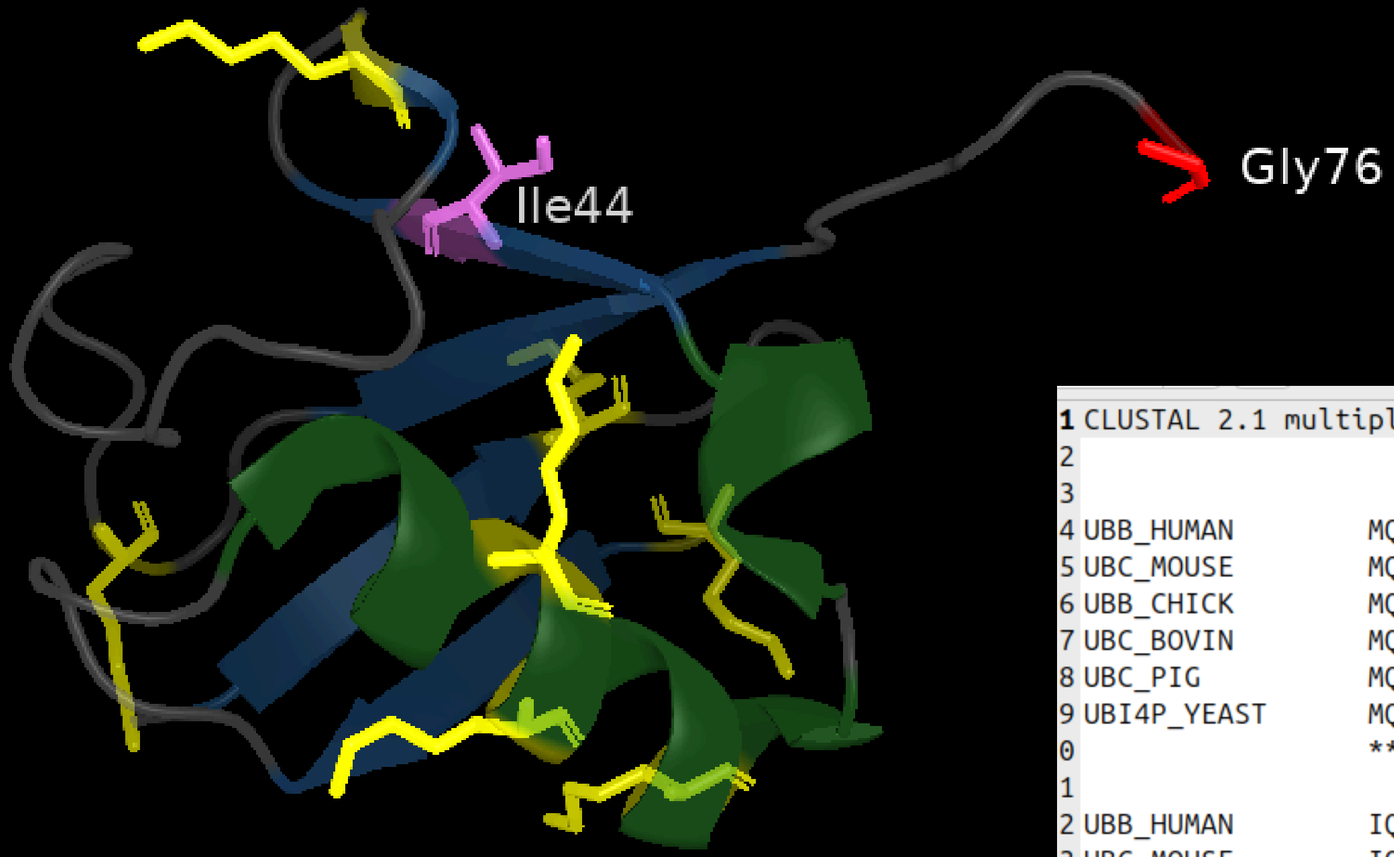
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

HYDROPHOBIC CORE



MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

KEY UBIQUITIN RESIDUES AND CONSERVATION



K6, K11, K27, K29, K33, K48, K63

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CLUSTAL 2.1 multiple sequence alignment

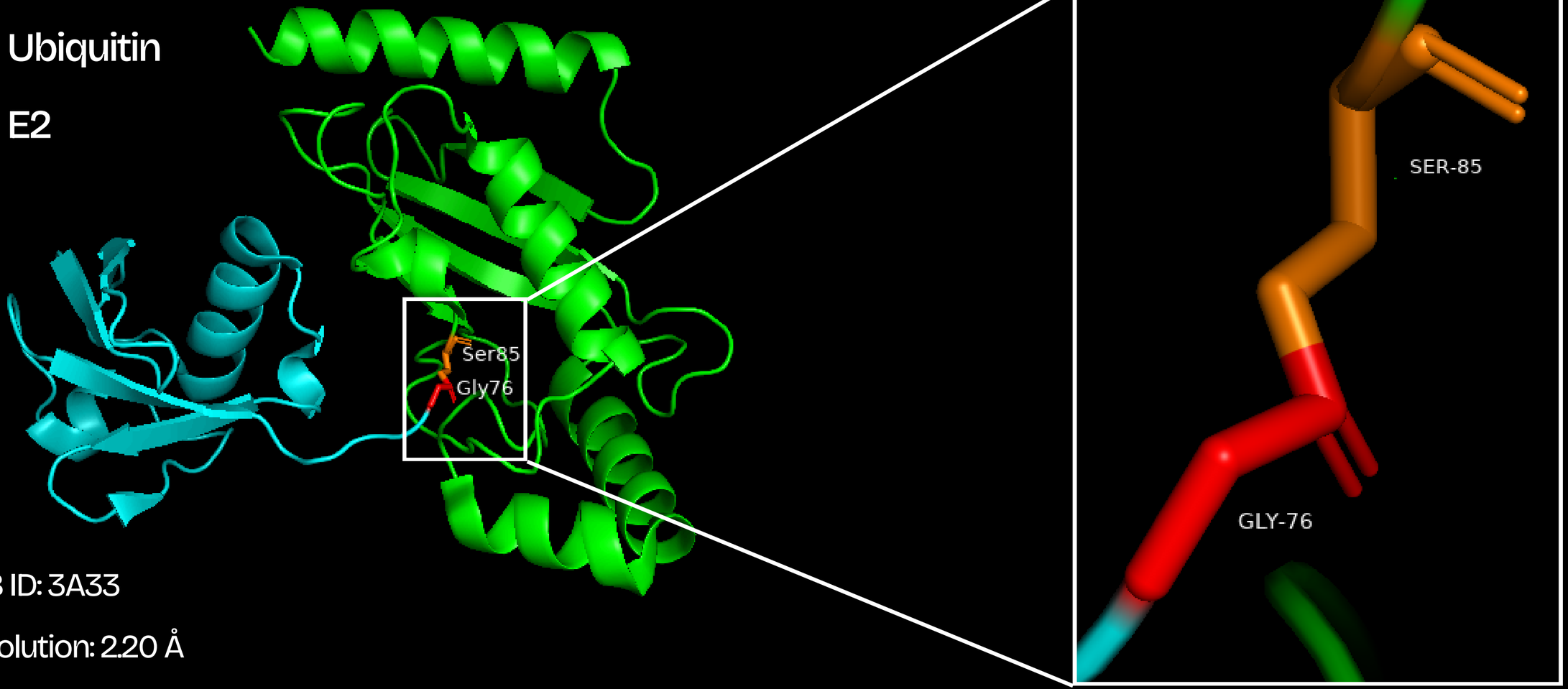

UBB_HUMAN       MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN
UBC_MOUSE       MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN
UBB_CHICK       MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN
UBC_BOVIN       MQIFVKTLTGKTITLEVEPSDTIENVKCKIQEKEGIPPDQQRLIFAGKQLEDGRTLSDYN
UBC_PIG         MQIFVKTLTGKTITLEVEPSDTIENVKCKIQEKEGIPPDQQRLIFAGKQLEDGRTLSDYN
UBI4P_YEAST     MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN
                ********************.****:***.***:**********.*****************

UBB_HUMAN       IQKESTLHLVLRLRGG
UBC_MOUSE       IQKESTLHLVLRLRGG
UBB_CHICK       IQKESTLHLVLRLRGG
UBC_BOVIN       IQKESTLHLVLRLRGG
UBC_PIG         IQKESTLHLVLRLRGG
UBI4P_YEAST     IQKESTLHLVLRLRGG
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THIOESTERS ARE DIFFICULT TO CRYSTALLIZE

Ubiquitin

E2



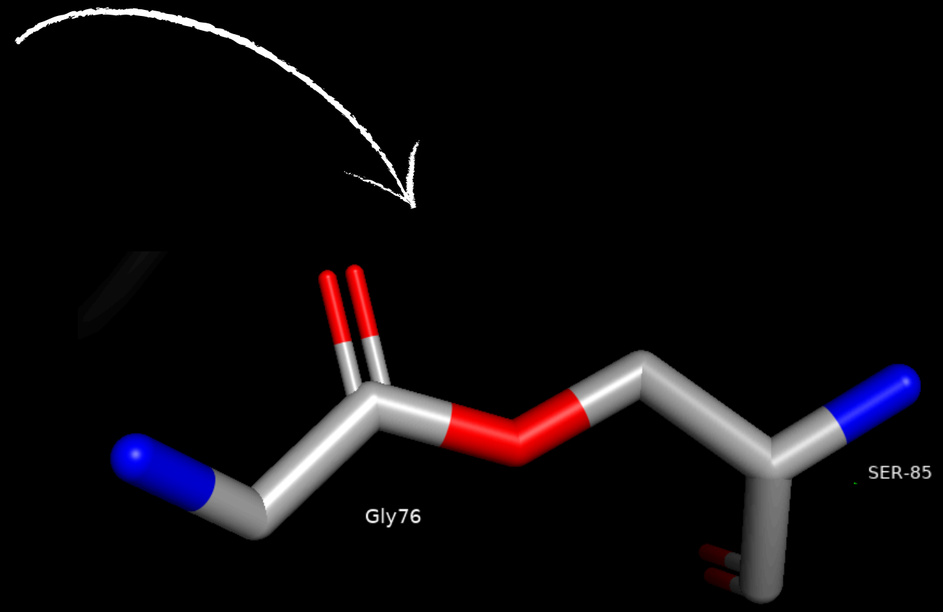
PDB ID: 3A33

Resolution: 2.20 Å

Organism: Homo sapiens

THIOESTERS ARE DIFFICULT TO CRYSTALLIZE





Oxyester bond

NUCLEOPHILIC ATTACK DURING THE E1-E2 TRANSFER: TRANSTHIOESTERIFICATION



Ubiquitin-charged E1 (E1~Ub)

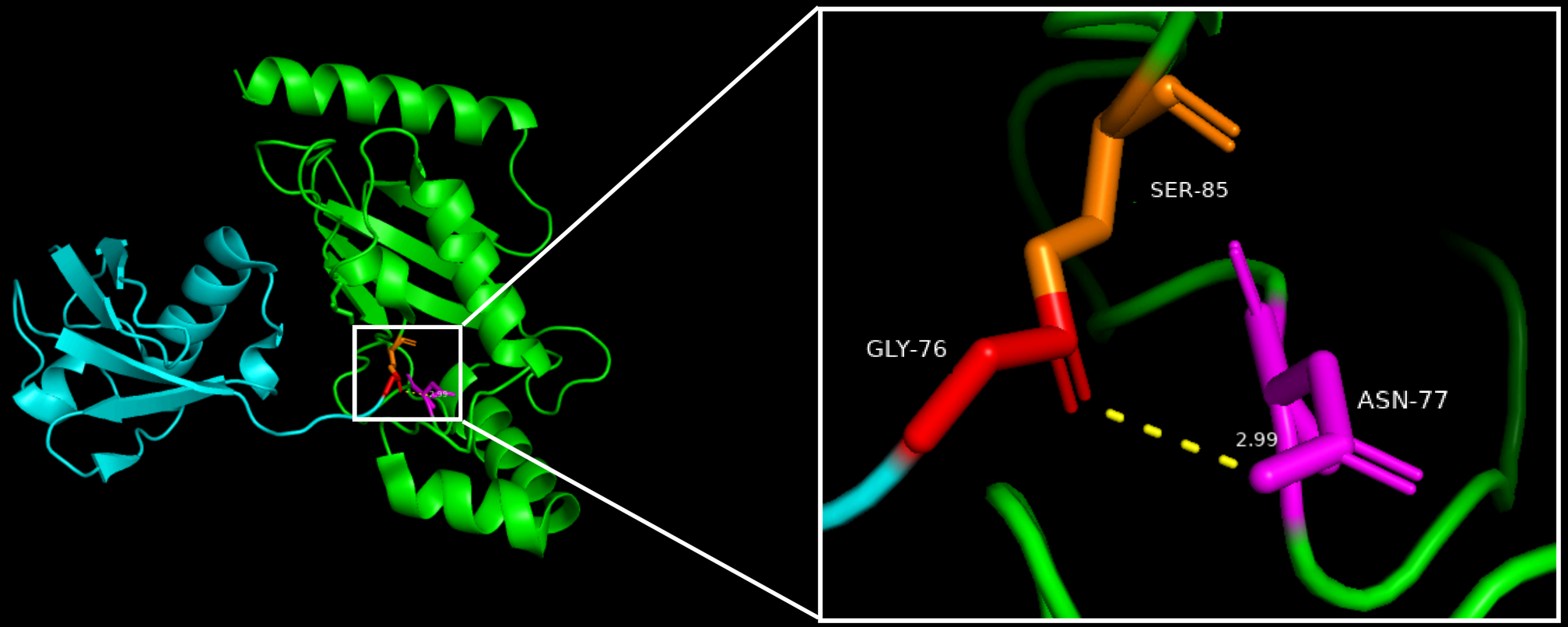
Apo-E2

Apo-E1

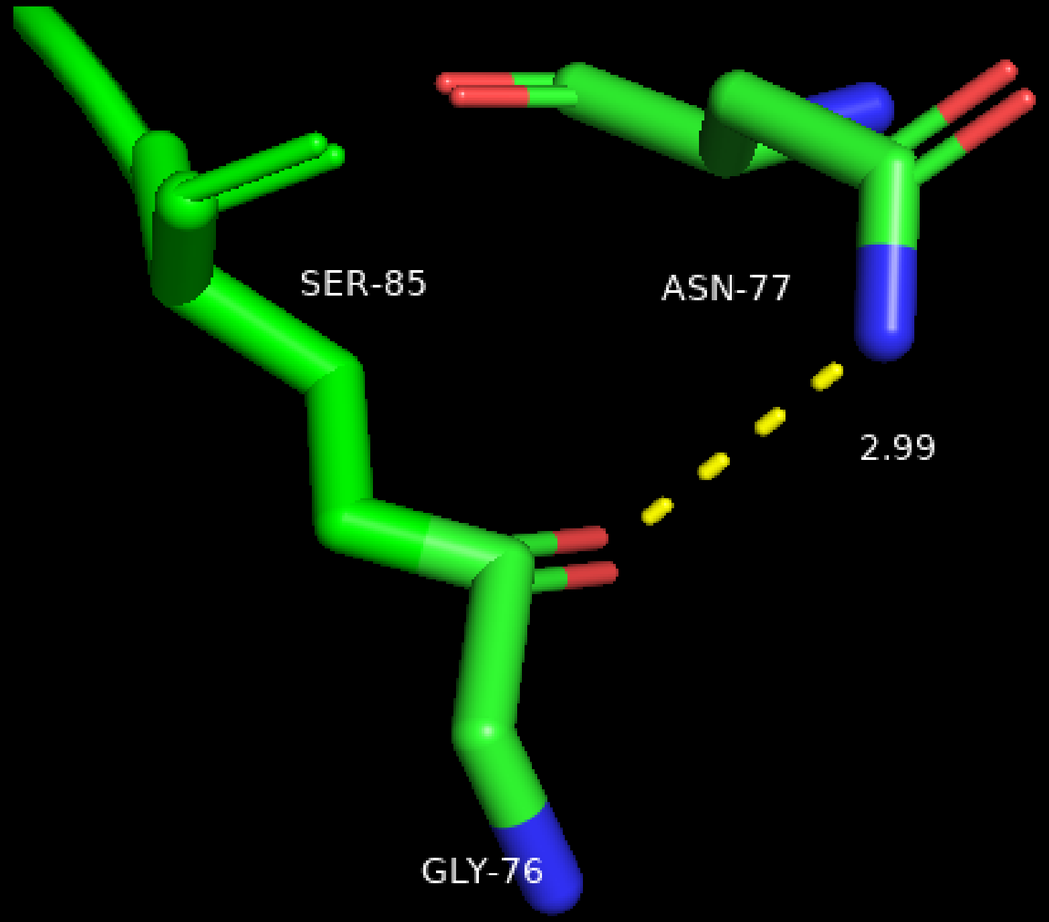
Ubiquitin-charged E2 (E2~Ub)

Ye, Y., & Rape, M. (2009). Building ubiquitin chains: E2 enzymes at work. *Nature Reviews Molecular Cell Biology*, 10, 755–764

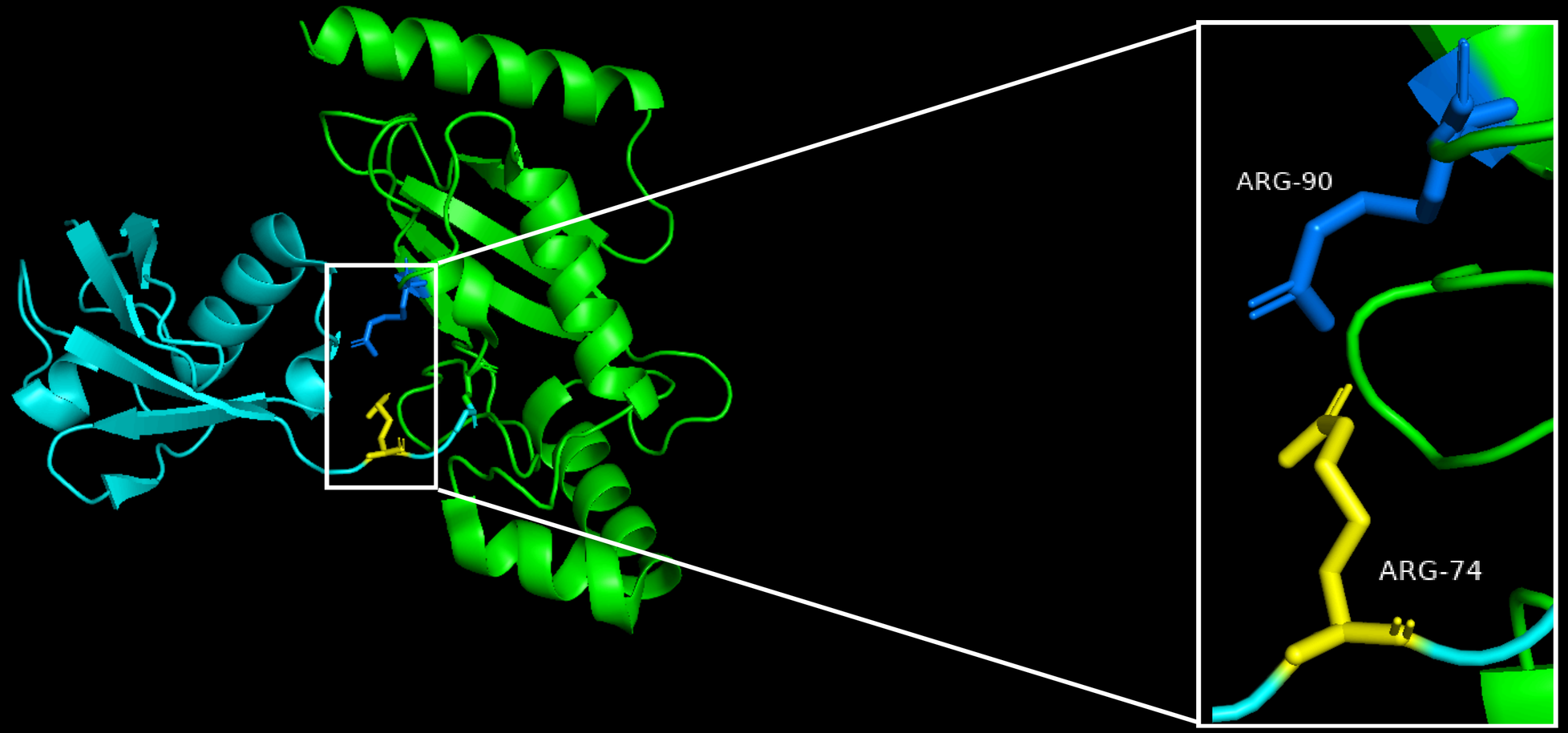
ACTIVE SITE AND STABILIZATION



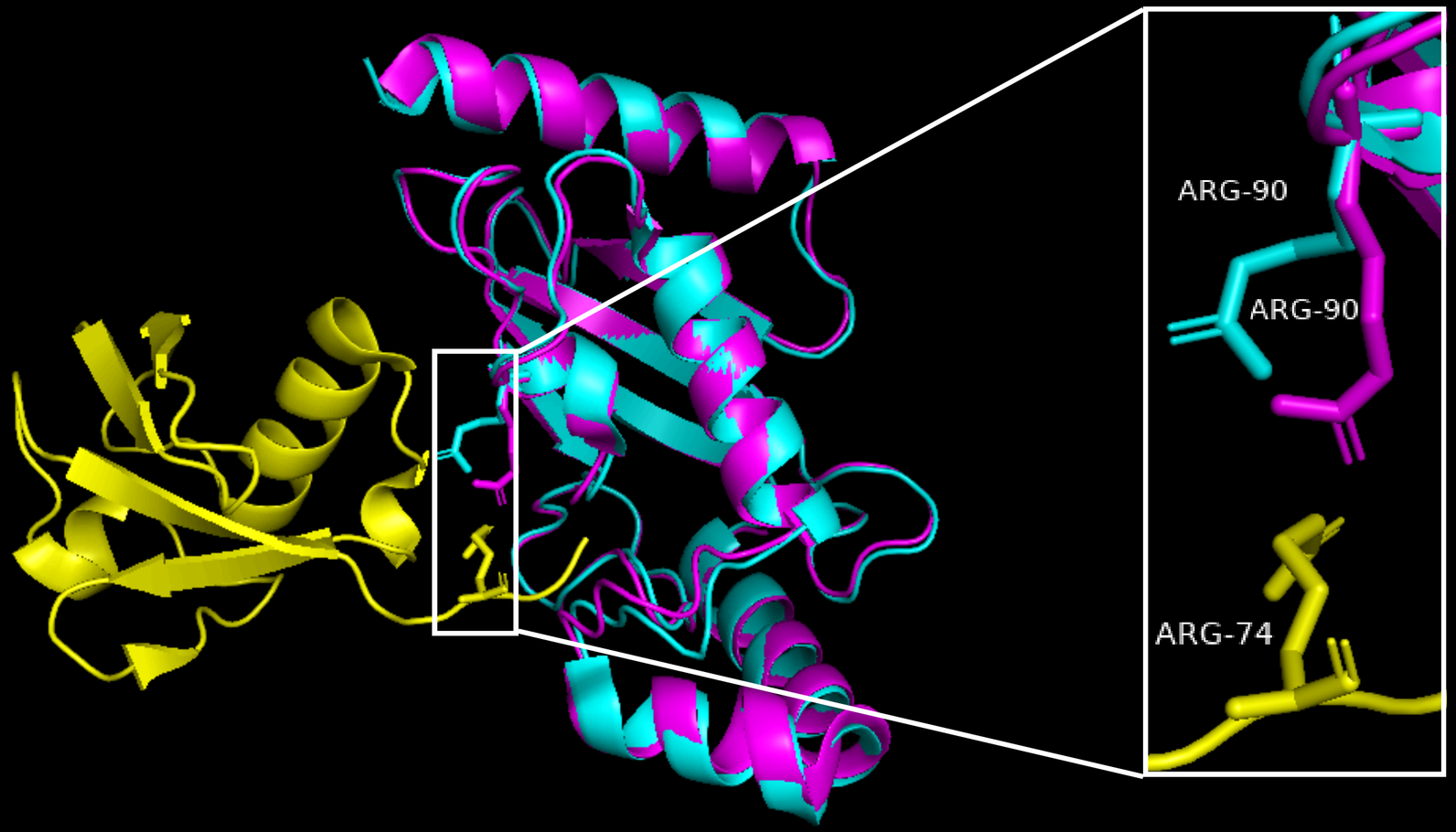
ACTIVE SITE AND STABILIZATION



ARG90 (E2) AND ARG74 (UB): MANAGING CHARGE REPULSION



ARG90 SHIFT



PDB ID: 2ESK

Resolution: 1.36 Å

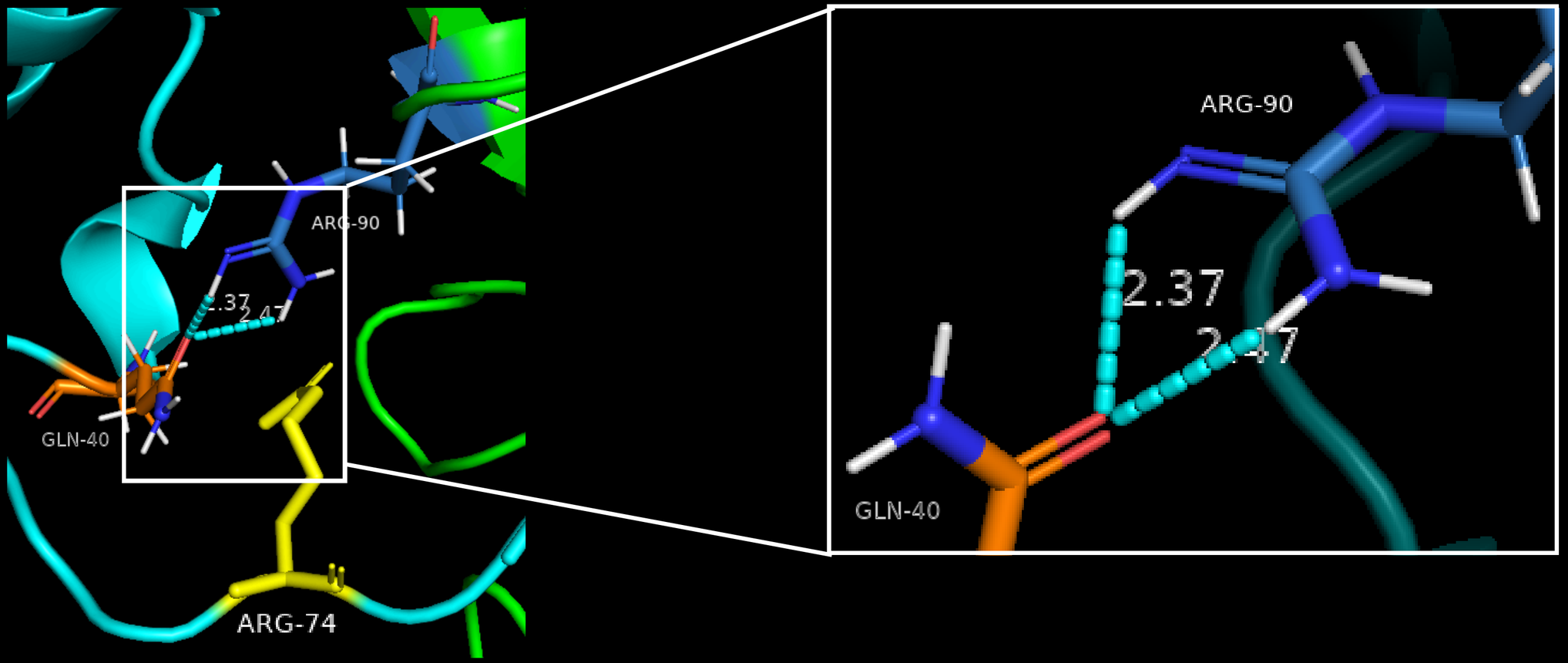
Organism: Homo sapiens

E2: free state

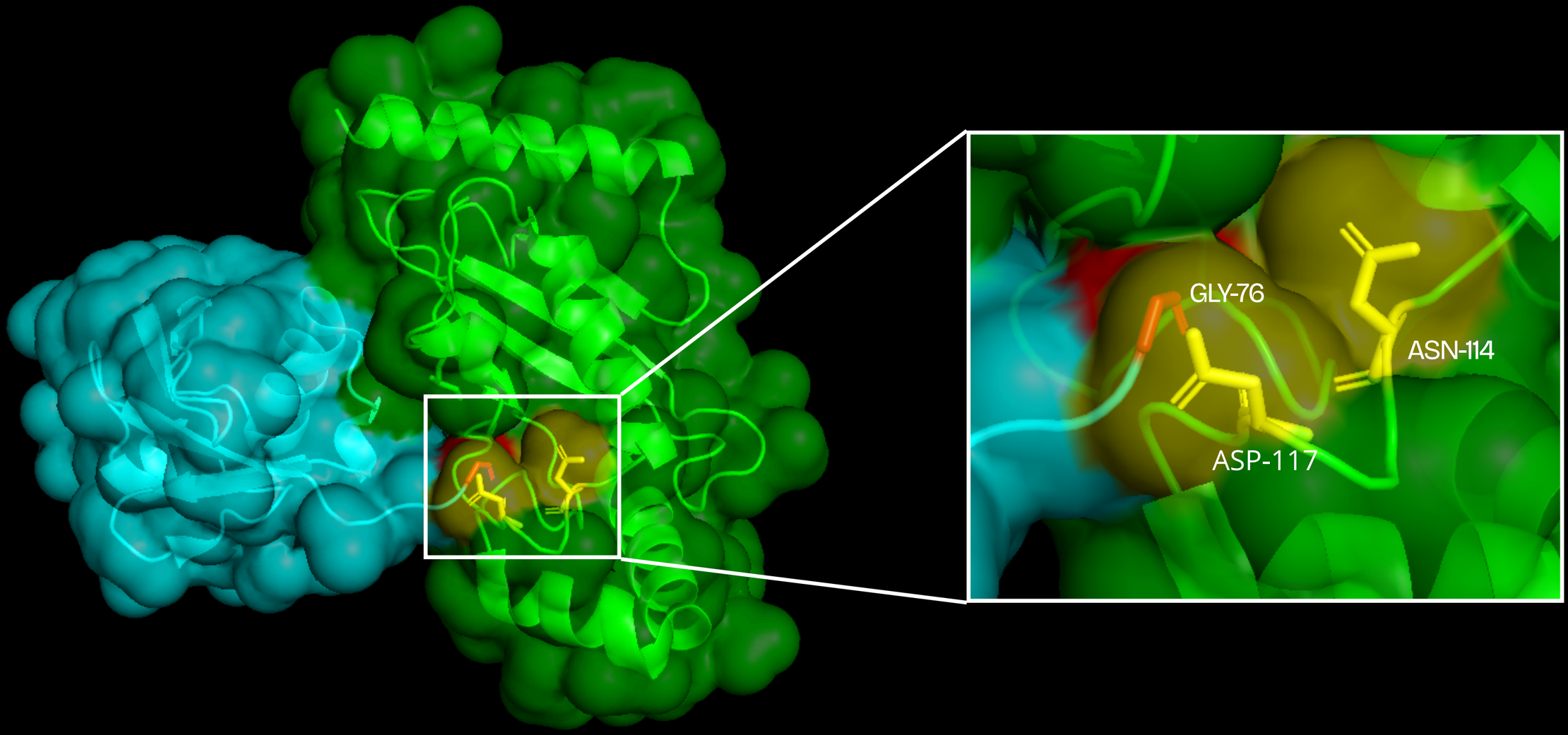
E2: conjugated state

Ubiquitin

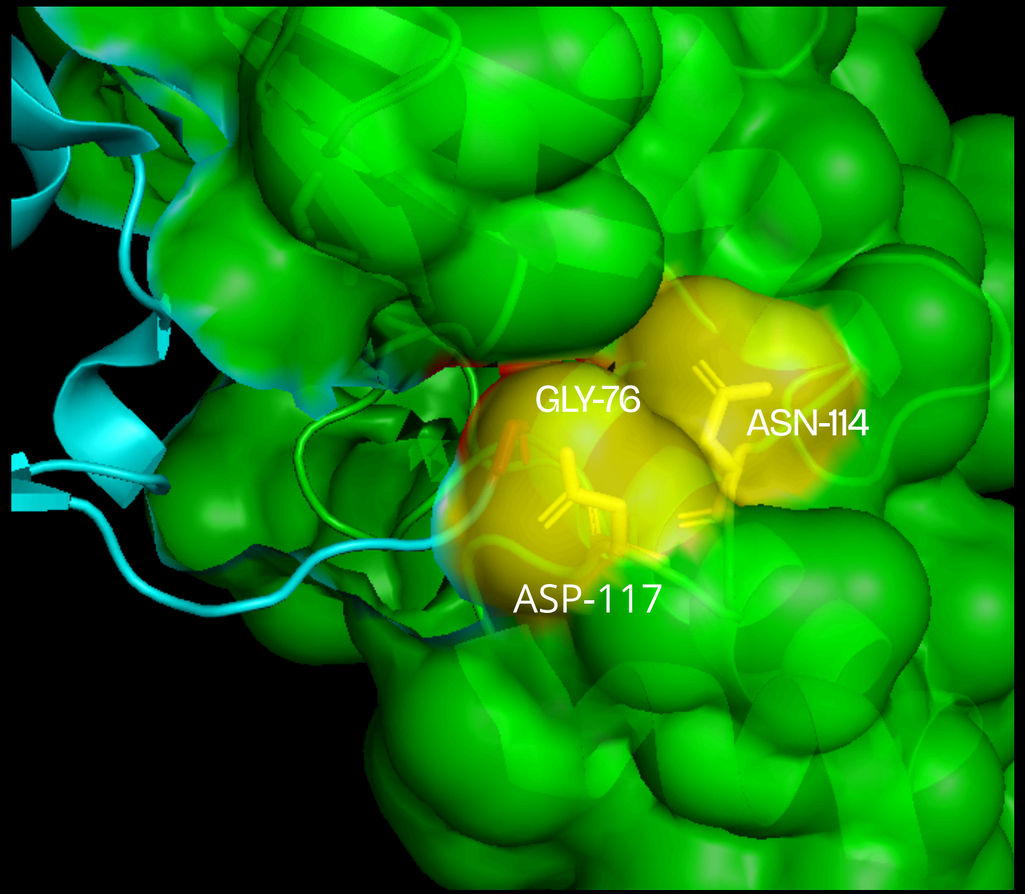
ARG90 (E2) AND ARG74 (UB): MANAGING CHARGE REPULSION

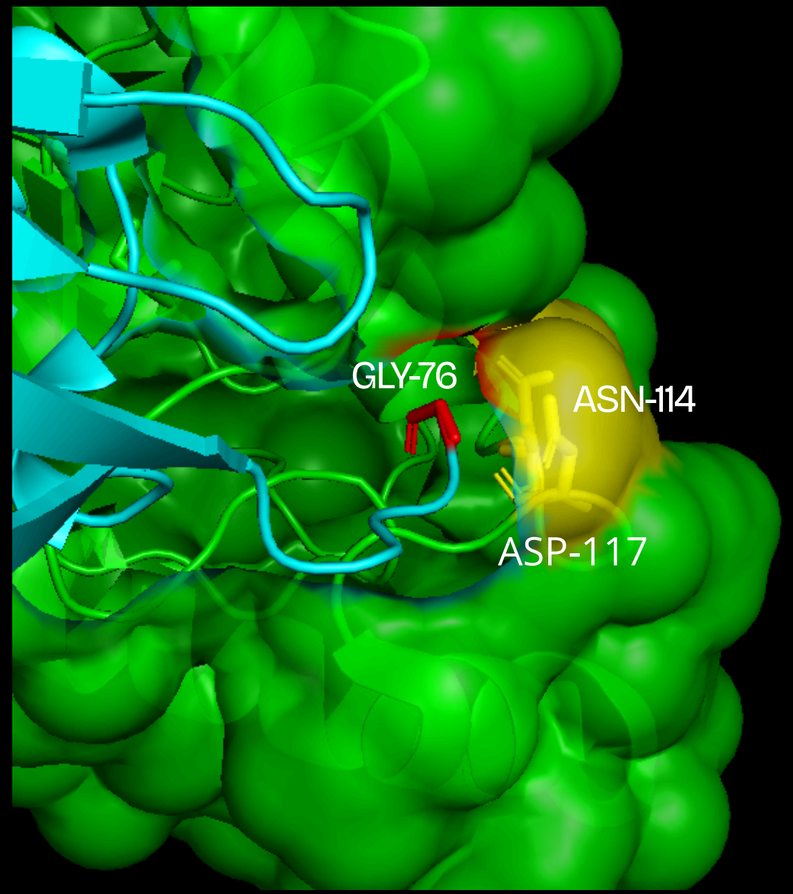


EXPOSED GROOVE



EXPOSED GROOVE





CONCLUSIONS

- Ubiquitination is a highly coordinated and structurally conserved enzymatic process essential for protein regulation.
- E1 enzymes initiate the cascade by activating ubiquitin in an ATP-dependent manner and transferring it to E2 through a thioester bond.
- UBE2D2, as an E2 enzyme, adopts a conserved UBC fold that provides the structural framework necessary for catalysis. Its activity depends on the precise positioning of Cys85, which forms the transient thioester bond with ubiquitin at Gly76, supported by Asp117 and stabilized by the conserved HPN motif.
- The strong evolutionary conservation of both sequence and structure highlights the fundamental importance of this catalytic mechanism across species, emphasizing the central role of E2 enzymes in the ubiquitination pathway.

THANK YOU!

ANY QUESTION?

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PEM QUESTIONS

1. Which of the following statements correctly describes the ATP-dependent activation step of ubiquitination?

a) The catalytic cysteine of E2 directly binds free ubiquitin in an ATP-independent manner.

b) The C-terminal Gly76 of ubiquitin reacts with ATP to form a ubiquitin–adenylate intermediate, releasing pyrophosphate (PPi).

c) Ubiquitin is activated when E3 ligase forms a covalent intermediate with Gly76.

d) ATP is hydrolysed only after ubiquitin has been transferred to E2.

e) The lysine residues of ubiquitin are adenylated before substrate recognition.

2. During the final step of ubiquitination, which of the following molecular events occurs?

a) The catalytic cysteine of E1 forms an isopeptide bond with the substrate lysine.

b) The ε-amino group of a substrate lysine attacks the E2~Ub thioester bond, forming a stable isopeptide bond.

c) The E3 ligase forms a permanent covalent bond with ubiquitin before transfer to the substrate.

d) ATP is required again to drive isopeptide bond formation.

e) A disulfide bond is formed between ubiquitin and the substrate protein.

PEM QUESTIONS

3. According to the SCOP database, how is UBE2D2 structurally organized?

a) It has a C-terminal extension that stabilizes the main domain.

b) It is mainly composed of multiple beta sheets forming the protein core.

c) It contains only the conserved UBC domain of about 150 amino acids.

d) Its stability depends only on backbone hydrogen bonds.

e) Its hydrophobic core is completely surrounded by alpha helices.

4. How is the catalytic cysteine in UBE2D2 activated to become reactive?

a) The cysteine is naturally reactive at any pH.

b) An asparagine residue directly removes the proton from the thiol group.

c) A nearby aspartate residue weakens the S–H bond, making the sulfur more reactive.

d) A structural movement pushes the sulfur toward ubiquitin.

e) The sulfur is stabilized by interaction with a lysine residue.

PEM QUESTIONS

5. How do loops L4 and L7 contribute structurally and evolutionarily to E2 function?

a) Loop L4 and L7 are highly flexible regions with low sequence conservation, allowing E2 to bind to any protein.

b) The stability of Loop L7 is reinforced by a 3_{10} helix where a strong hydrogen bond between Asn87 and Arg90 maintains a rigid conformation.

c) In yeast, the essential Arg90 is lost and replaced by a hydrophobic leucine, significantly changing the loop's architecture.

d) Loop L4 contributes to the core stability through a covalent disulfide bridge between Asp59 and Lys63.

e) Evolutionary alignment shows that while the UBC core is conserved, the HPN motif and Loop L7 vary greatly between fish and mammals.

6. The main interactions between E1 and E2 are:

a) Hydrophobic

b) Electrostatic

c) H bonds

d) Covalent

e) They do not interact with each other

PEM QUESTIONS

7. E2 active site Cys85 interacts with which one of the following E2 residues?

a) Met944

b) Ser589

c) Cys593

d) Phe701

e) Arg965

8. E2 interacts mainly with E1 through:

a) AAD domain

b) UFD domain

c) SCCH domain

d) UFD + SCCH domains

e) All the domains

PEM QUESTIONS

9. What is the role of Gly76 in ubiquitin?
a) It stabilizes the Ile44 hydrophobic patch
b) It forms the covalent linkage to E1/E2 and substrates
c) It recognizes the E3 ligase
d) It determines the type of ubiquitin chain formed
e) It mutates to Serine

10. In what order do the proteins interact during the ubiquitination cascade?
a) E1 → E2 → E3 → Ub
b) E3 → E2 → E1
c) E1 + Ub → E2 + Ub
d) Ub → E3 → E2 + Ub → E1
e) E2 → E1 → E3 → Ub