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Ubiquitin

Let's degrade something

Summary

1. Ubiquitination
2. Ubiquitin
 - a. Classification
 - b. Genes and sequence
 - c. Structure
 - d. Conservation
3. Ubiquitin Activating Enzymes (E1)
 - a. Classification
 - b. Genes and sequence
 - c. Structure
 - d. Conservation
4. Ubiquitin-E1 interaction
5. Ubiquitin conjugating enzymes (E2)
 - a. Classification
 - b. General features
 - c. Structure
 - d. Conservation
6. E1-E2 interaction
7. Ubiquitin-protein interaction



1. The Ubiquitination pathway

“Before creation comes destruction”

The Ubiquitin signal

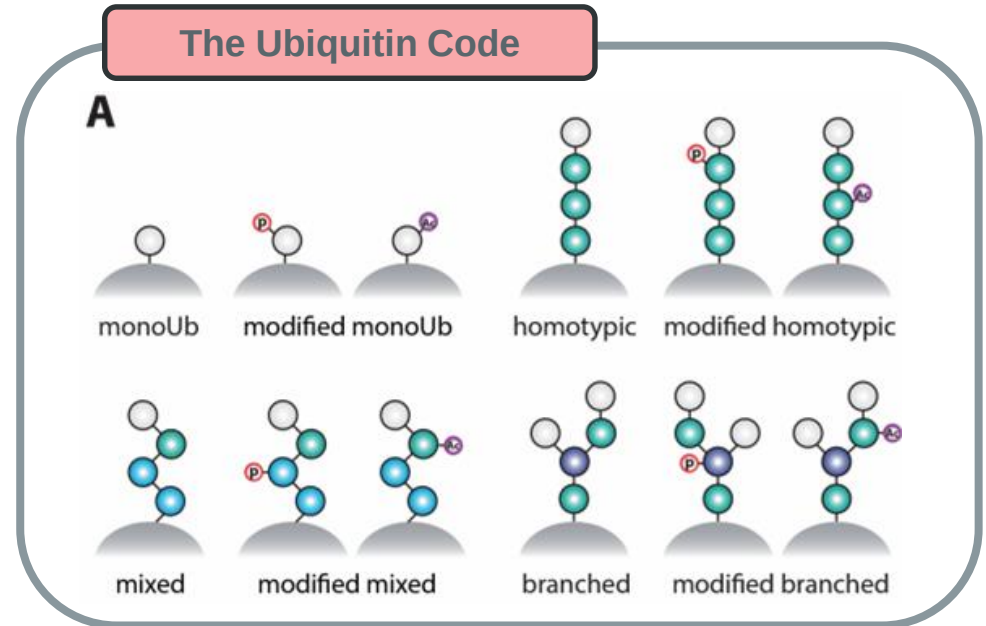
Ubiquitination: The addition of **ubiquitin** to a substrate protein

- **Ubiquitin domain**
- 76-amino-acid polypeptide of 8,5kDa
- Not present in prokaryotes
- Highly conserved among eukaryotes

Ubiquitination affects proteins **in many ways:**

- **Degradation**
- Cellular location
- Catalytic activity
- Cell cycle control
- Immune function

...Etcetera



Yau R, Rape M. The increasing complexity of the ubiquitin code. Nature Cell Biology. 2016;18(6):579-586.

The Ubiquitin Pathway

The conjugation of ubiquitin to its substrates requires of **3 steps**:

E1 Activation

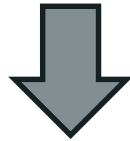
- Ubiquitin-activating enzymes

E2 Conjugation

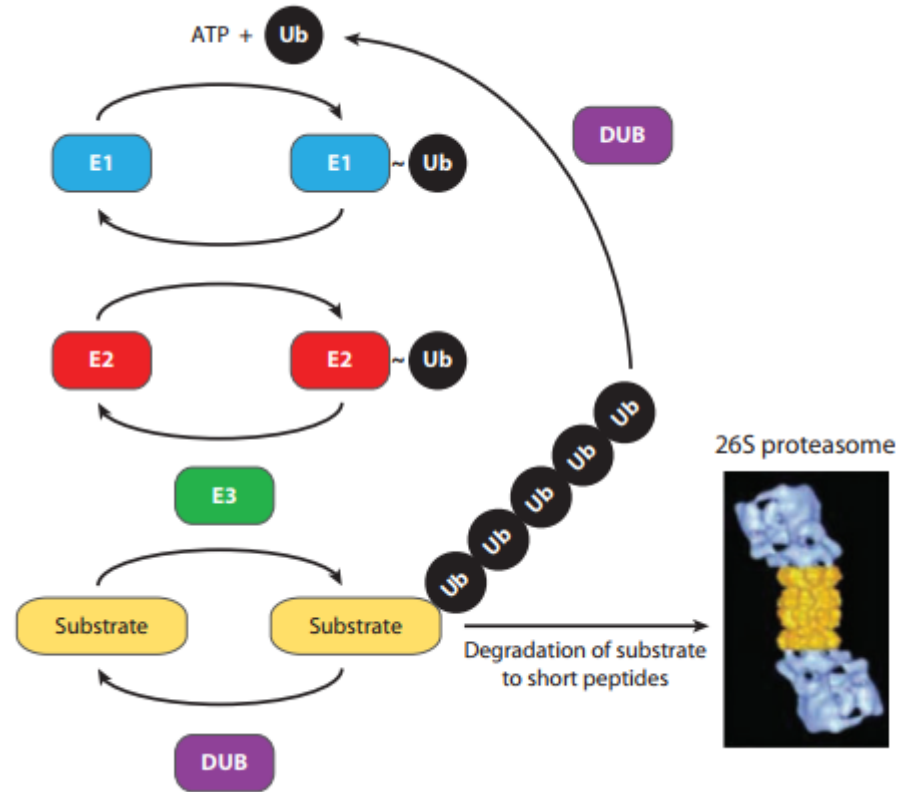
- Ubiquitin-conjugating enzymes

E3 | Ligation to target

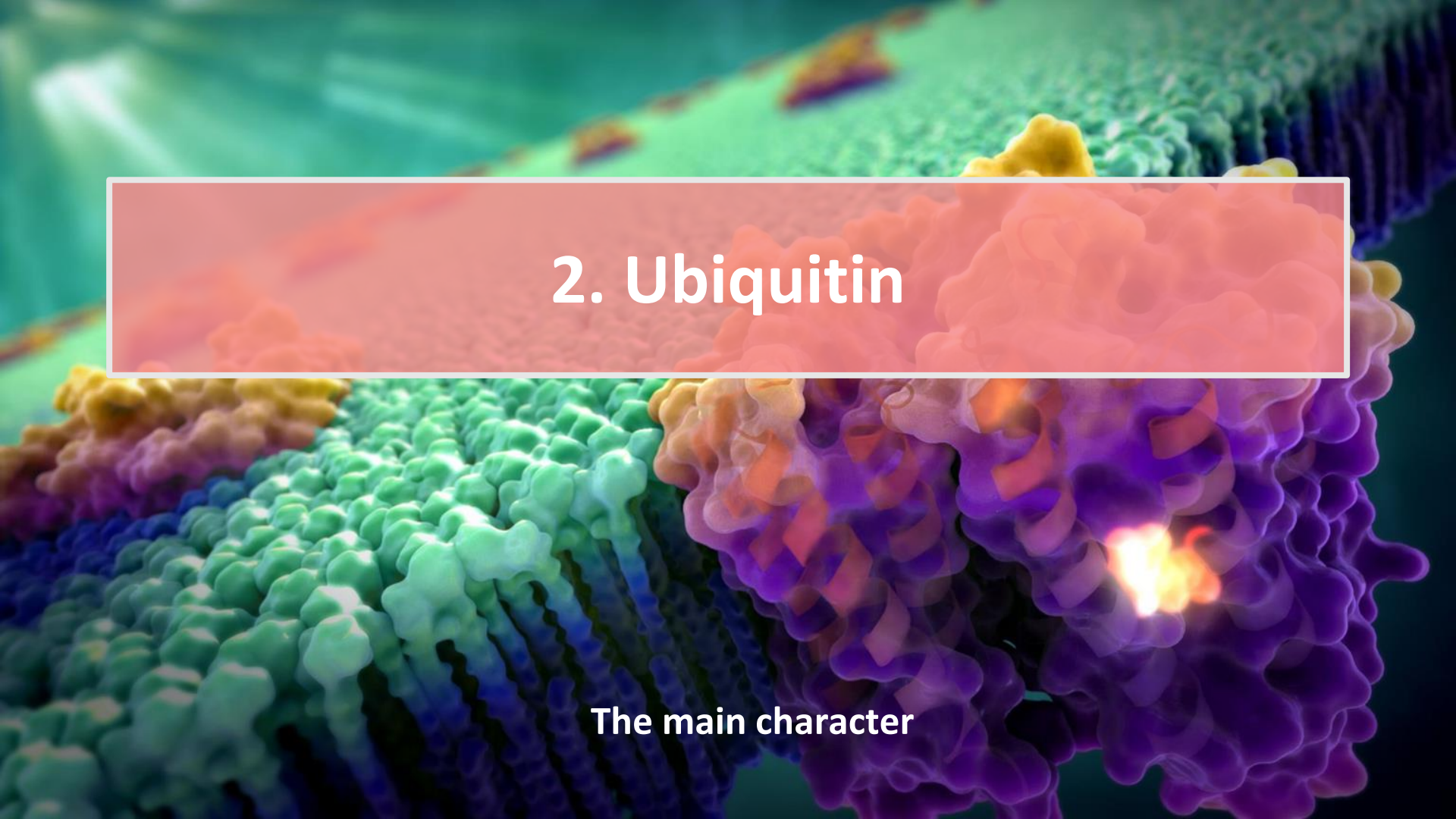
- Ubiquitin ligases



Pyramide-like distribution



Varshavsky A. The Ubiquitin System, an Immense Realm. *Annual Review of Biochemistry*. 2012;81(1):167-176.



2. Ubiquitin

The main character

Ubiquitin - Classification

SCOP Classification

- **Class:** Alpha and beta proteins ($\alpha+\beta$)
- **Fold:** beta-Grasp (Ubiquitin-like)
- **Superfamily:** Ubiquitin-like
- **Family:** Ubiquitin-related

CATH Classification

- **Class:** Alpha Beta (3)
- **Architecture:** Roll (3.10)
- **Topology:** Ubiquitin-like (UB roll) (3.10.20)
- **Homologous Superfamily:**
Phosphatidylinositol 3-kinase Catalytic Subunit; Chain A, domain 1 (3.10.20.90)

Ubiquitin - Genes

There are **4 genes** that encode for human **Ubiquitin**:

UBB

- Polyubiquitin precursor
- 3 direct repeats (229 aa)
- 17p11.2

UBC

- Polyubiquitin precursor
- 9 direct repeats (685 aa)
- 12p24.31

UBA52

- Ubiquitin fused to L40
- 19p13.11

RPS27A

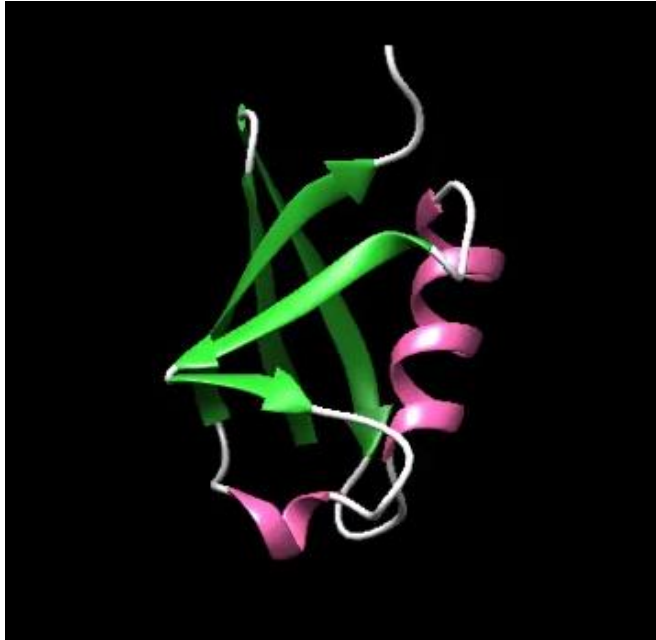
- Ubiquitin fused to S27a
- 2p16.1

The **Ubiquitin** sequence common in all these genes is the following:

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG

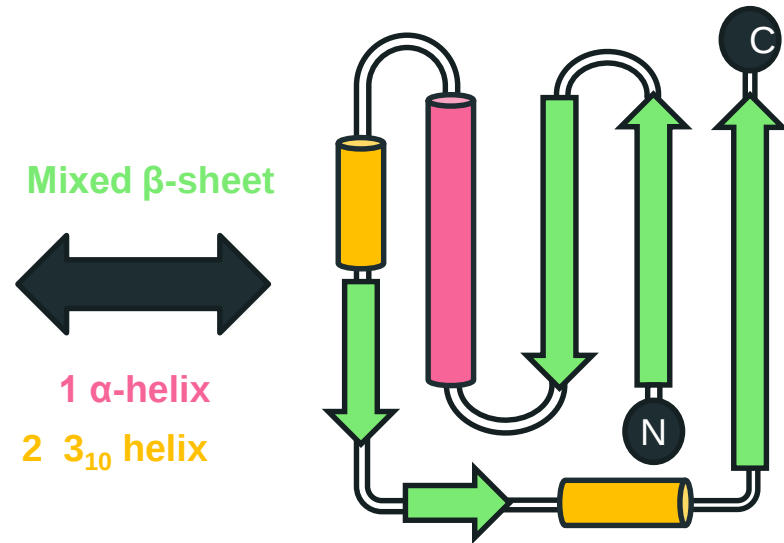
Ubiquitin - Structure

Ubiquitin 3D Structure



Human ubiquitin PDB ID: 1UBQ

Ubiquitin 2D Structure



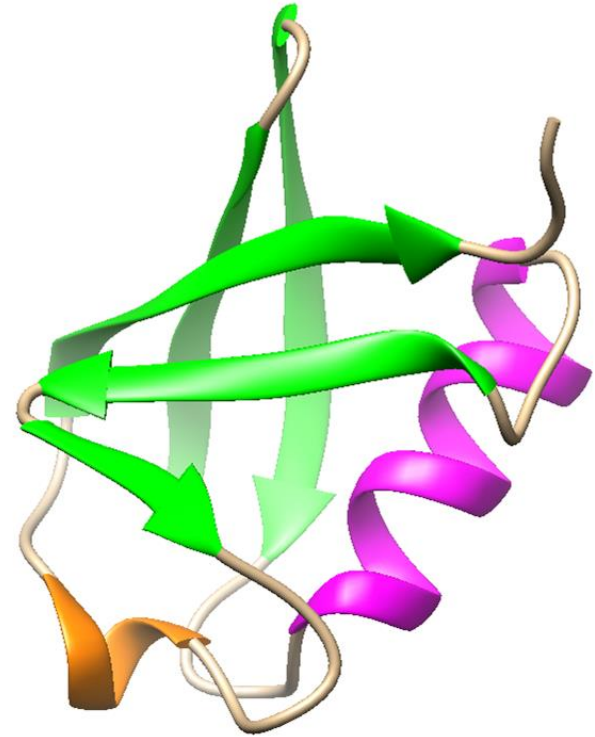
Ubiquitin - Structure

High chemical and thermal stability:

- **Hydrophobic core**
- High **hydrogen-bonding** interactions

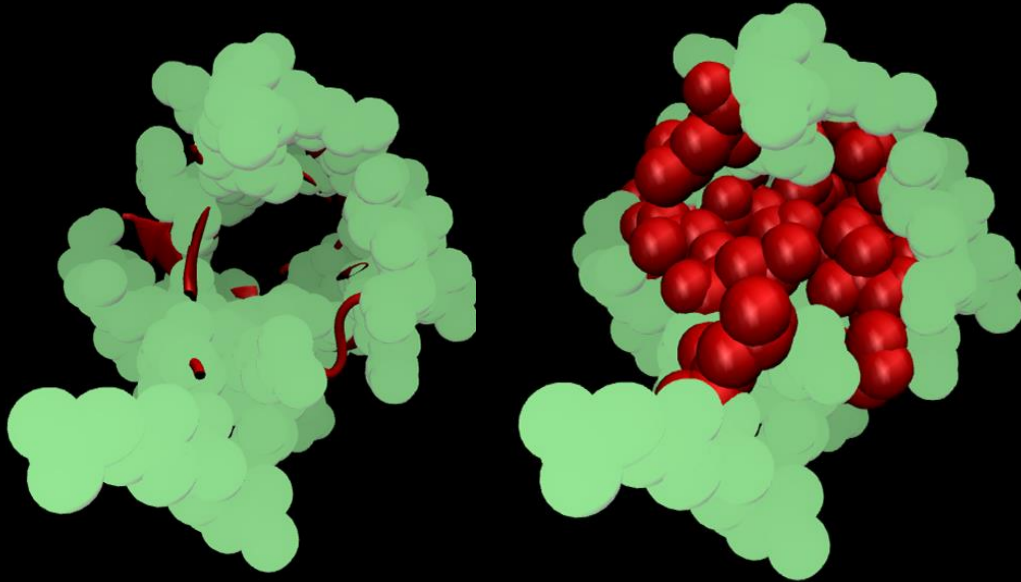
Surface side chains:

- **Acidic and basic**
- 5 Lys: Ubq-Ubq conjugation
- 3 hydrophobic residues: UBD interaction



PDB ID: 1UBQ

Ubiquitin Hydrophobic Core



Hydrophylic residues
Hydrophobic residues

High chemical and thermal stability:

- Hydrophobic core
- High **hydrogen-bonding** interactions

PDB ID: 1UBQ

Ubiquitin Hydrophobic Core



High chemical and thermal stability:

- Hydrophobic core
- High **hydrogen-bonding** interactions

PDB ID: 1UBQ

MQTIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQIEDGRITSDYNTQKESTHIVLRLGG

β1

β2

α

β3

β4

β5

Ubiquitin Hydrophobic Core



High chemical and thermal stability:

- Hydrophobic core
- High **hydrogen-bonding** interactions

PDB ID: 1UBQ

MQTIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQIEDGRTISDYNIQKESTHIVLRLRGG

β1

β2

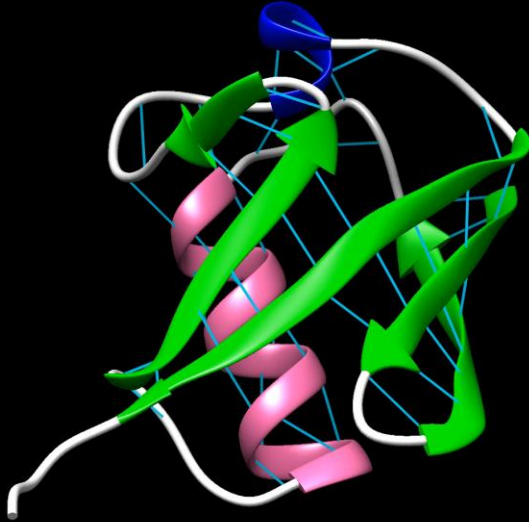
α

β3

β4

β5

Ubiquitin Hydrogen Bonds

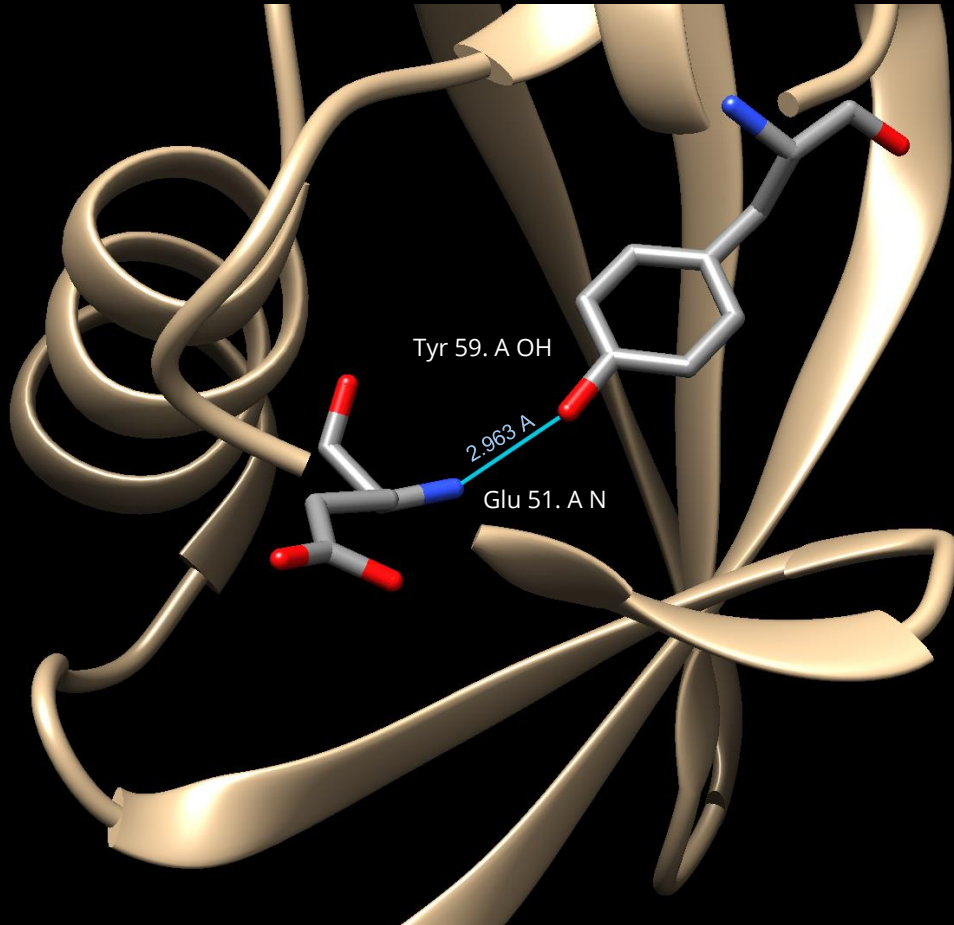


High chemical and thermal stability:

- Hydrophobic core
- High **hydrogen-bonding** interactions

PDB ID: 1UBQ

Ubiquitin - Hydrogen bonds (I)

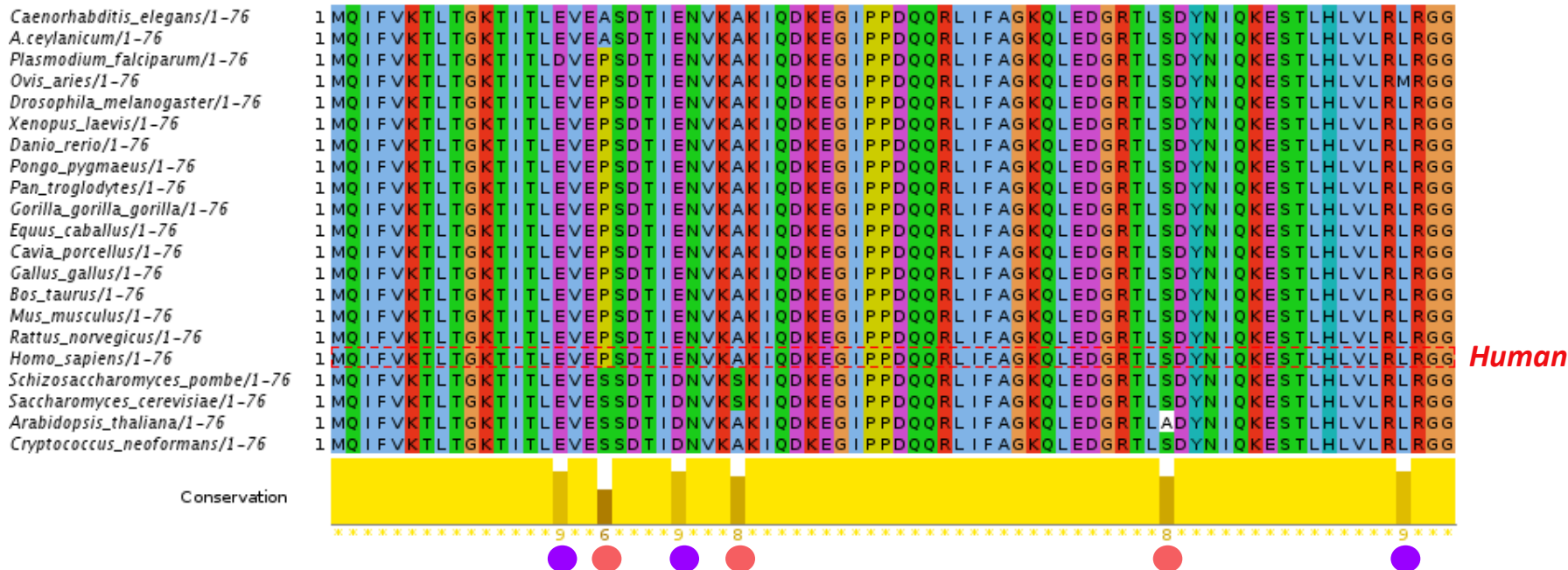


High chemical and thermal stability:

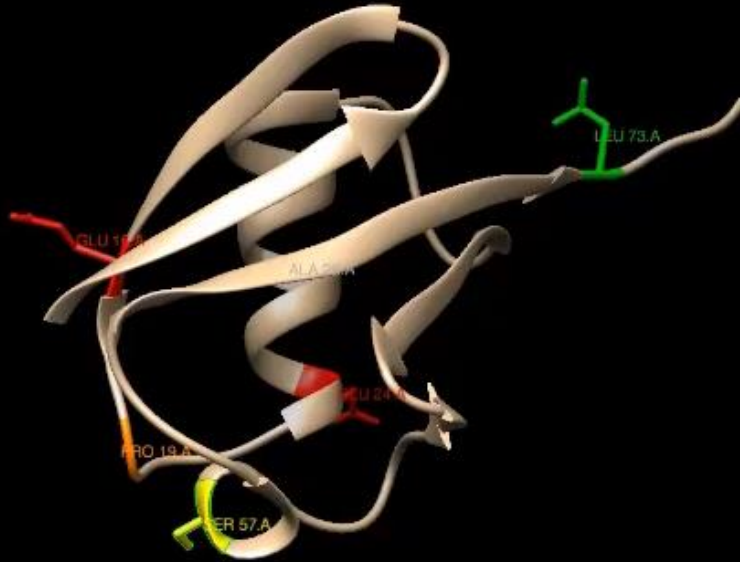
- Hydrophobic core
- High **hydrogen-bonding** interactions

PDB ID: 1UBQ

Ubiquitin Evolution



Ubiquitin non-conserved residues



Glutamate 16 ●

Proline 19 ●

Glutamate 24 ●

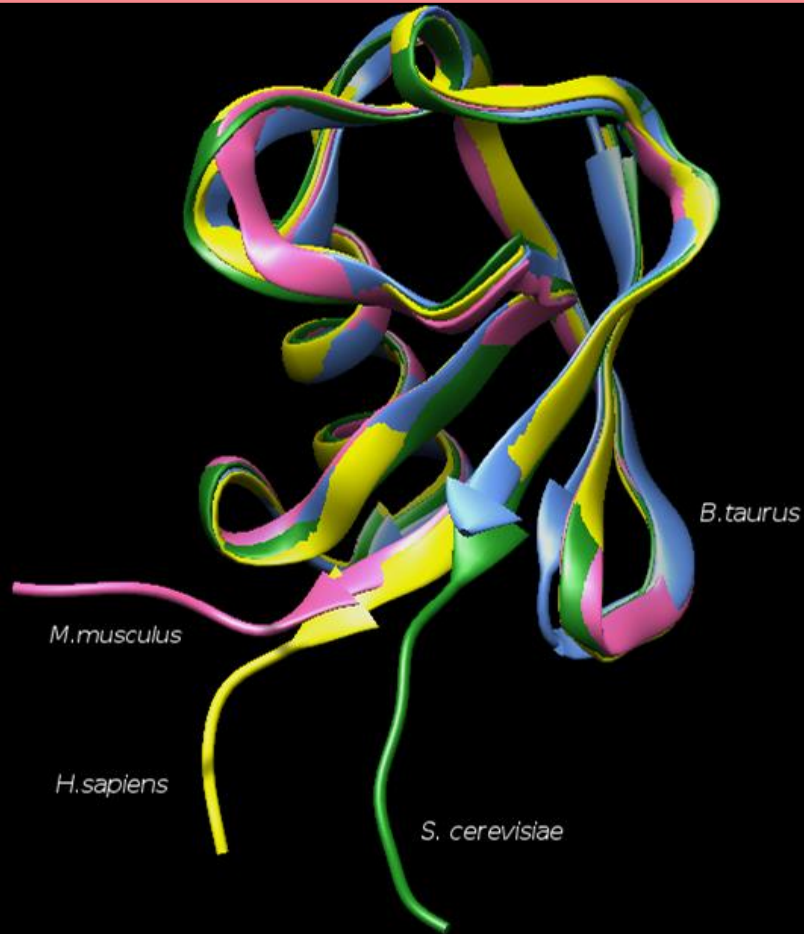
Alanine 28 ●

Serine 57 ●

Leucine 73 ●

PDB ID: 1UBQ

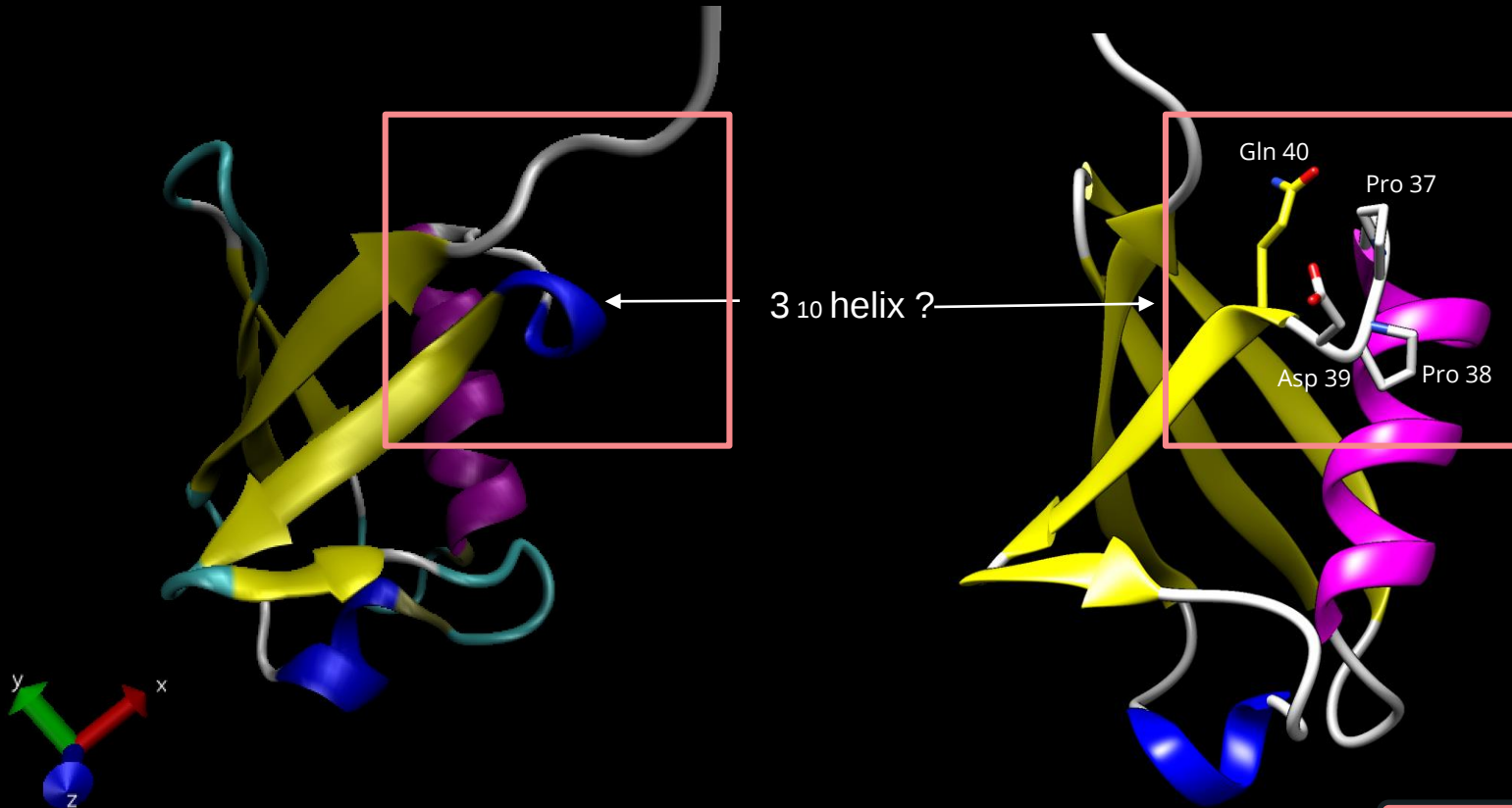
Ubiquitin Conservation



$Sc > 8$ $RMSD < 0,65$

-  Ubiquitin (*H. sapiens*)
-  Ubiquitin (*M. musculus*)
-  Ubiquitin (*B. taurus*)
-  Ubiquitin (*S. cerevisiae*)

Ubiquitin - Helix 3₁₀



PDB ID: 1UBQ

Ubiquitin: 3-turn helix

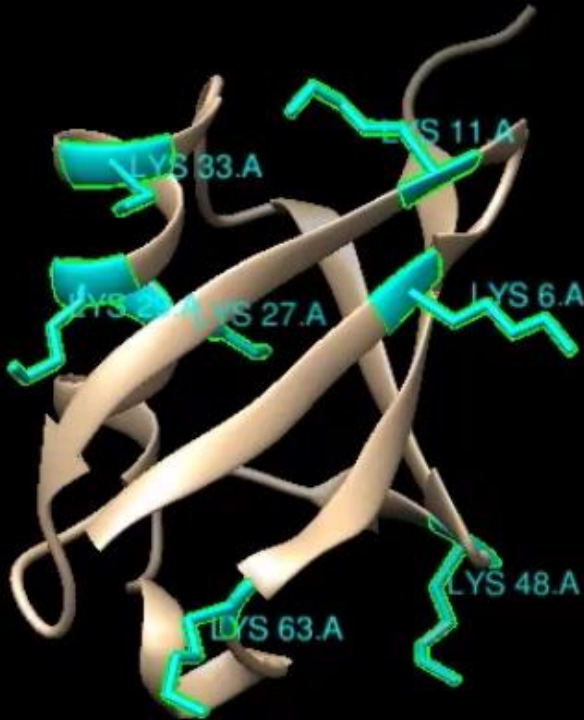
PDBid

3cmm	S.cerevisiae	MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLL--RGG	
2zcc	B.taurus	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLV-----	
2a9k	M.musculus	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPDQQRLLIFAGKQLEDGRTLSDYNIQRESTLHLVLR-LRGG--	
1ubq	H.sapiens	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLR-LRGG--	
	S.cerevisiae	-EEEEETT--EEEE--TT-BHHHHHHHHHHHH--GGG-EEEETTEE--TTSBTGGGT--TT-EEEE-----	
	B.taurus	-EEEE-TTS-EEEE--TT-BHHHHHHHHHHHH--GGG--EETTEE--TTSBTGGGT--TT-EEEE-----	
	M.musculus	-EEEEETTS-EEEE--TT-BHHHHHHHHHHHH--GGGEEEETTEE--TT-BTGGGT--TT-EEEEEE-----	
	H.sapiens	-EEEEETTS-EEEE--TTSBHHHHHHHHHHHH--GGGEEEETTEE--TTSBTGGGT--TT-EEEEEE---S----	
			3₁₀ helix
			3₁₀ helix

Sc > 8 RMSD < 0,65

- H = α -helix
- B = residue in isolated β -bridge
- E = extended strand (β ladder)
- G = 3-helix (3₁₀ helix)
- T = hydrogen bonded turn
- S = bend

Ubiquitin Lysine residues (I)

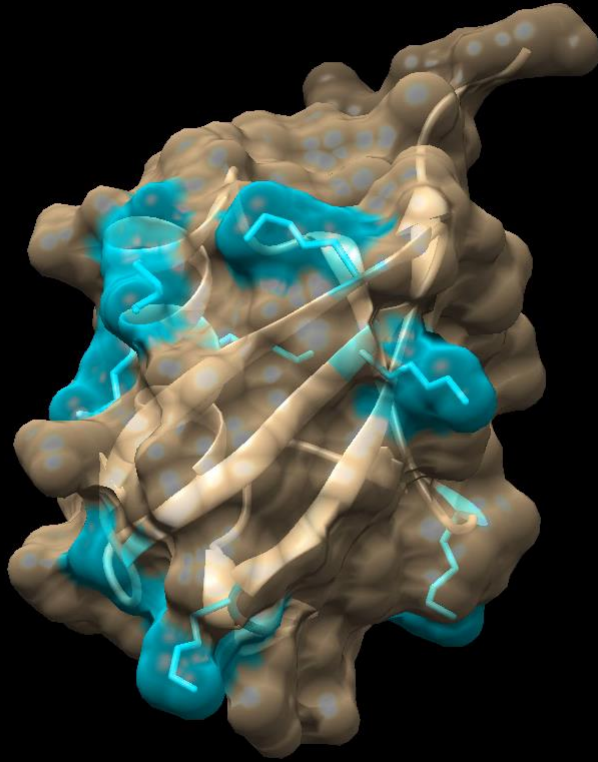


All surfaces covered by lysines:

- **Lys 29, 48** - Proteasome degradation
- **Lys 6, 11** and **63** - DNA repair and signalling transduction
- **Lys 27** - hidden → Requires conformational changes in order to be functional

PDB ID: 1UBQ

Ubiquitin Lysine residues (I)



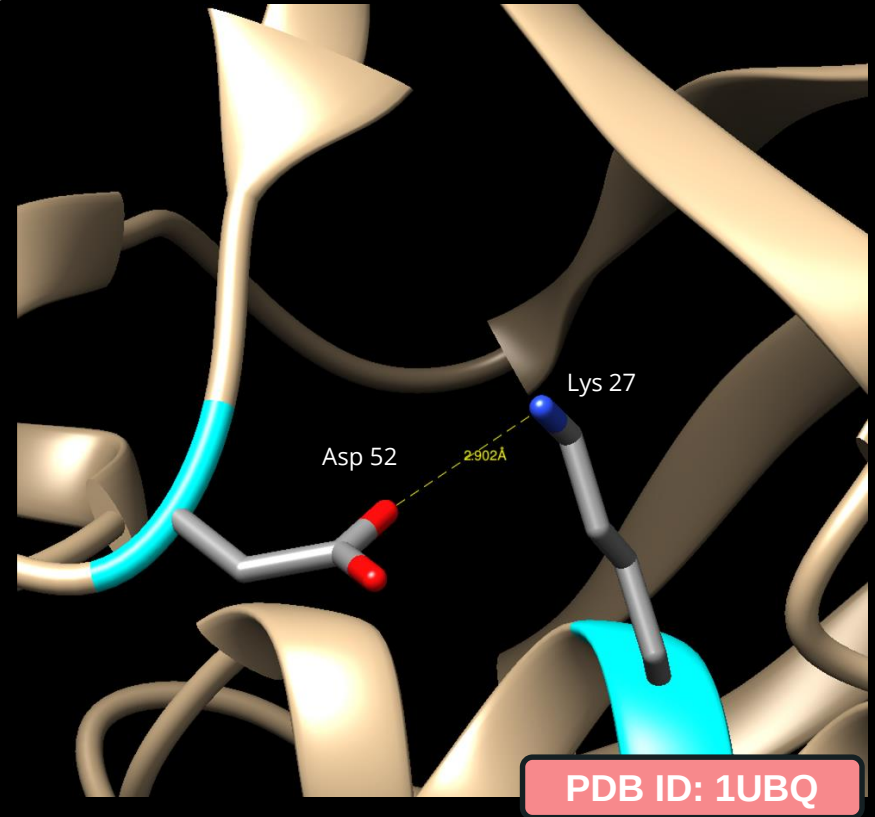
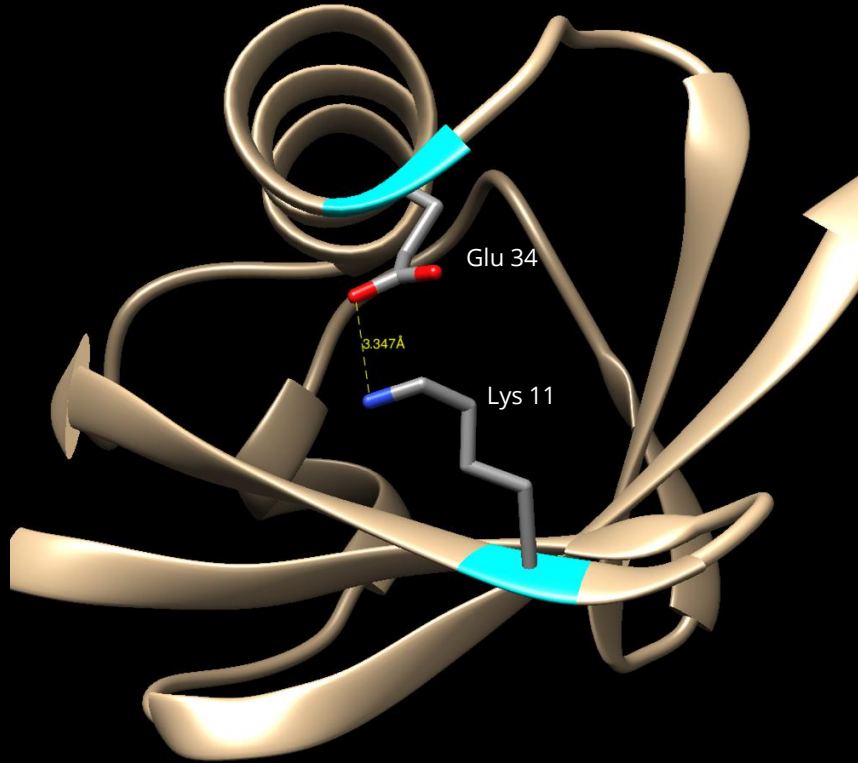
All surfaces covered by lysines:

- **Lys 29, 48** - Proteasome degradation
- **Lys 6, 11** and **63** - DNA repair and signalling transduction
- **Lys 27** - hidden → Requires conformational changes in order to be functional

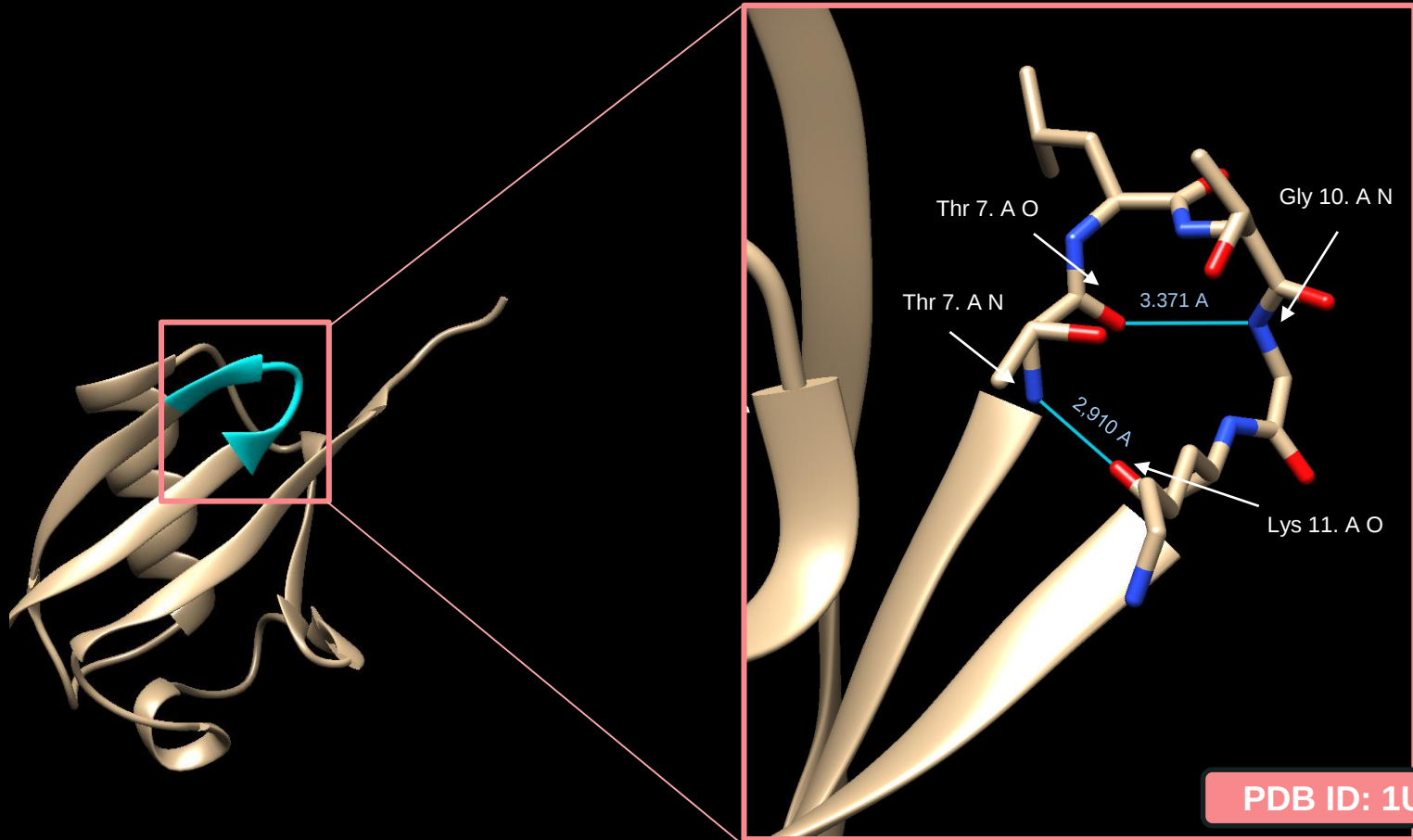
PDB ID: 1UBQ

Ubiquitin Lysine residues (II)

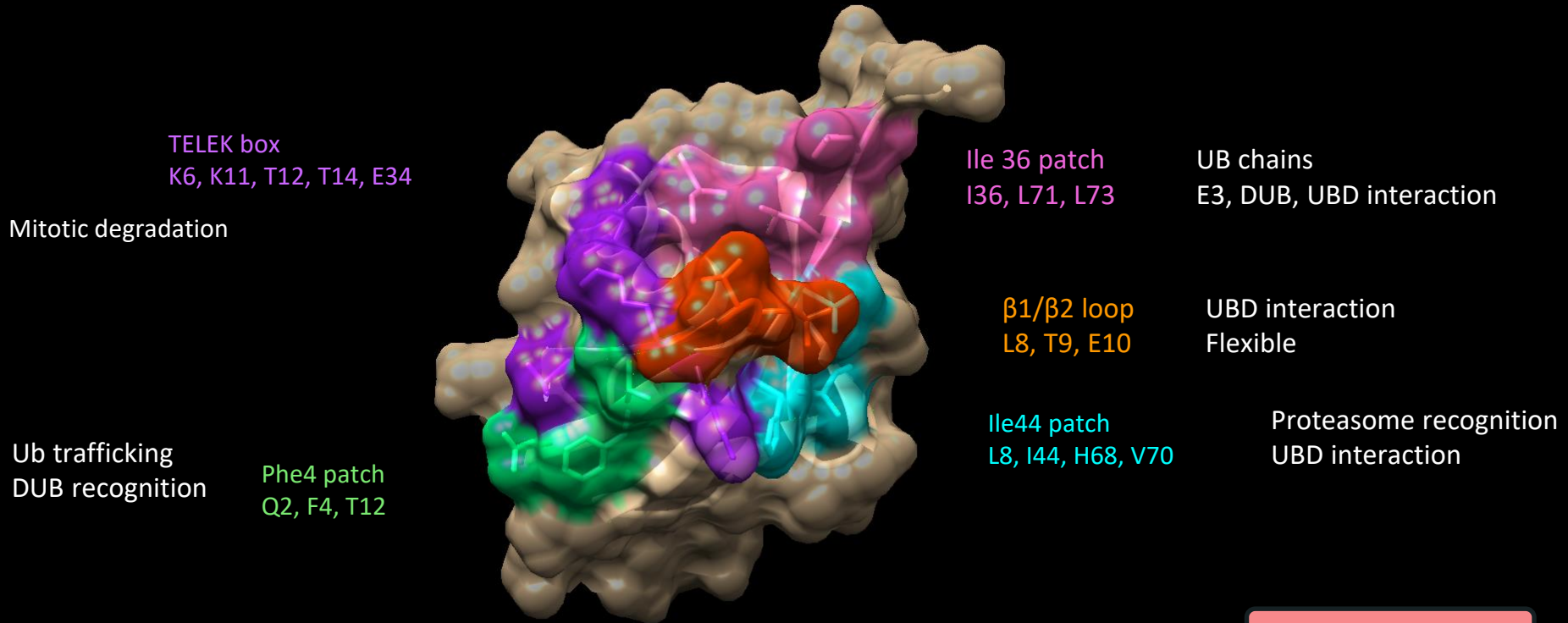
Salt bridges



Ubiquitin - Beta bulge loop

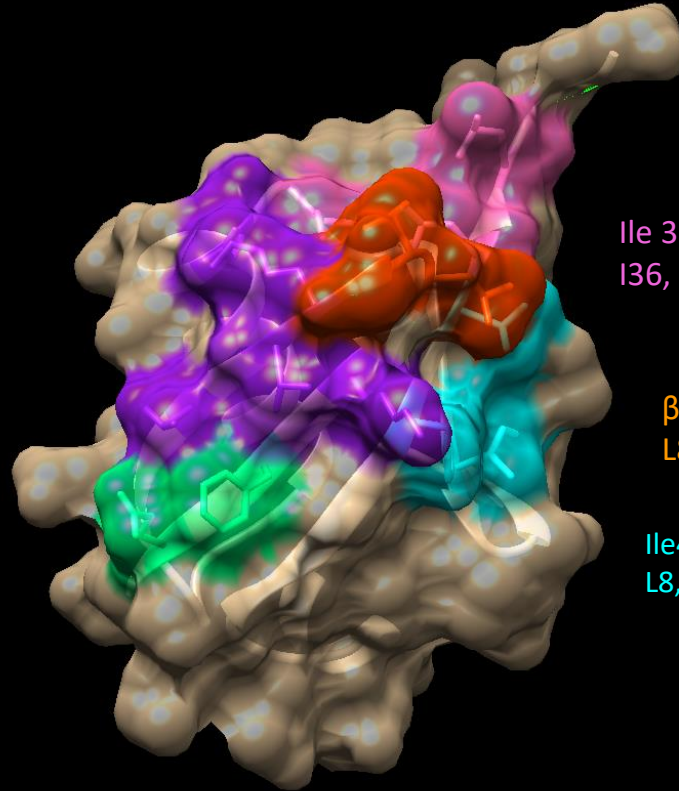


Ubiquitin - Important regions



PDB ID: 1UBQ

Ubiquitin - Important regions



TELEK box
K6, K11, T12, T14, E34

Mitotic degradation

Ile 36 patch
I36, L71, L73

UB chains
E3, DUB, UBD interaction

β1/β2 loop
L8, T9, E10

UBD interaction
Flexible

Ile44 patch
L8, I44, H68, V70

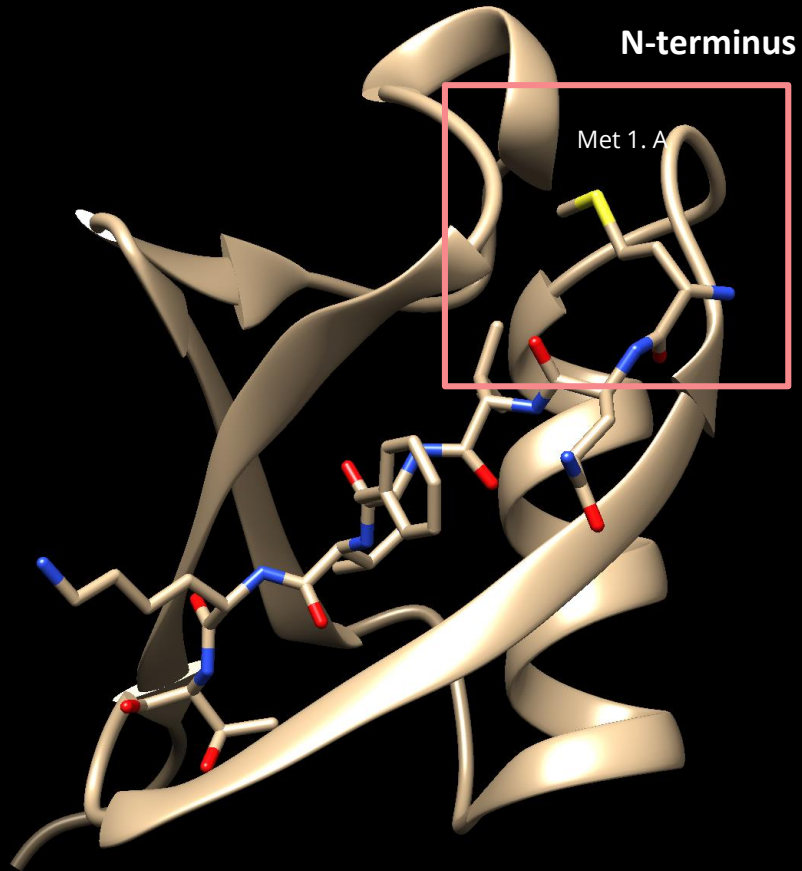
Proteasome recognition
UBD interaction

Ub trafficking
DUB recognition

Phe4 patch
Q2, P4, T12

PDB ID: 1UBQ

Ubiquitin - N-terminus



Hidden N-terminus

7 residues tightly conserved



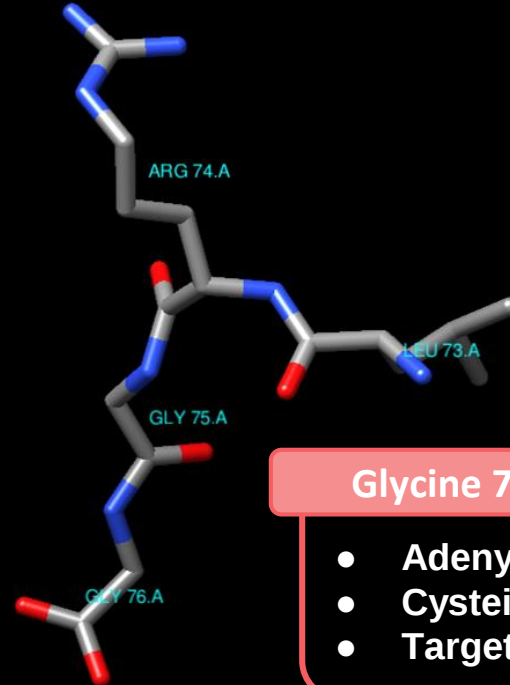
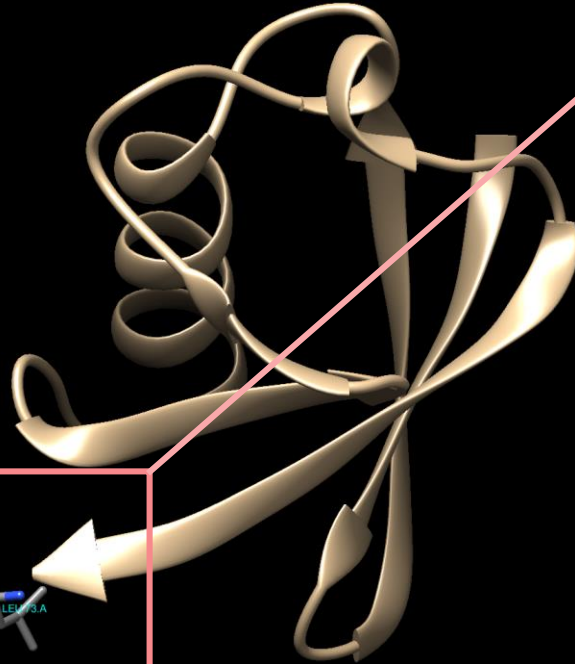
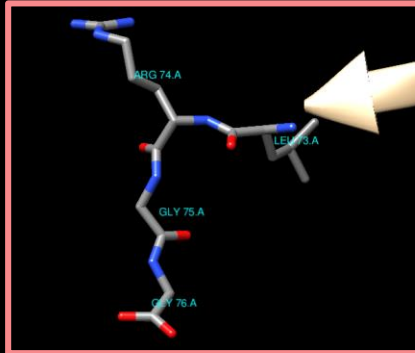
Prevent degradation

PDB ID: 1UBQ

Ubiquitin - C-terminus and Flexibility

PDB ID: 1UBQ

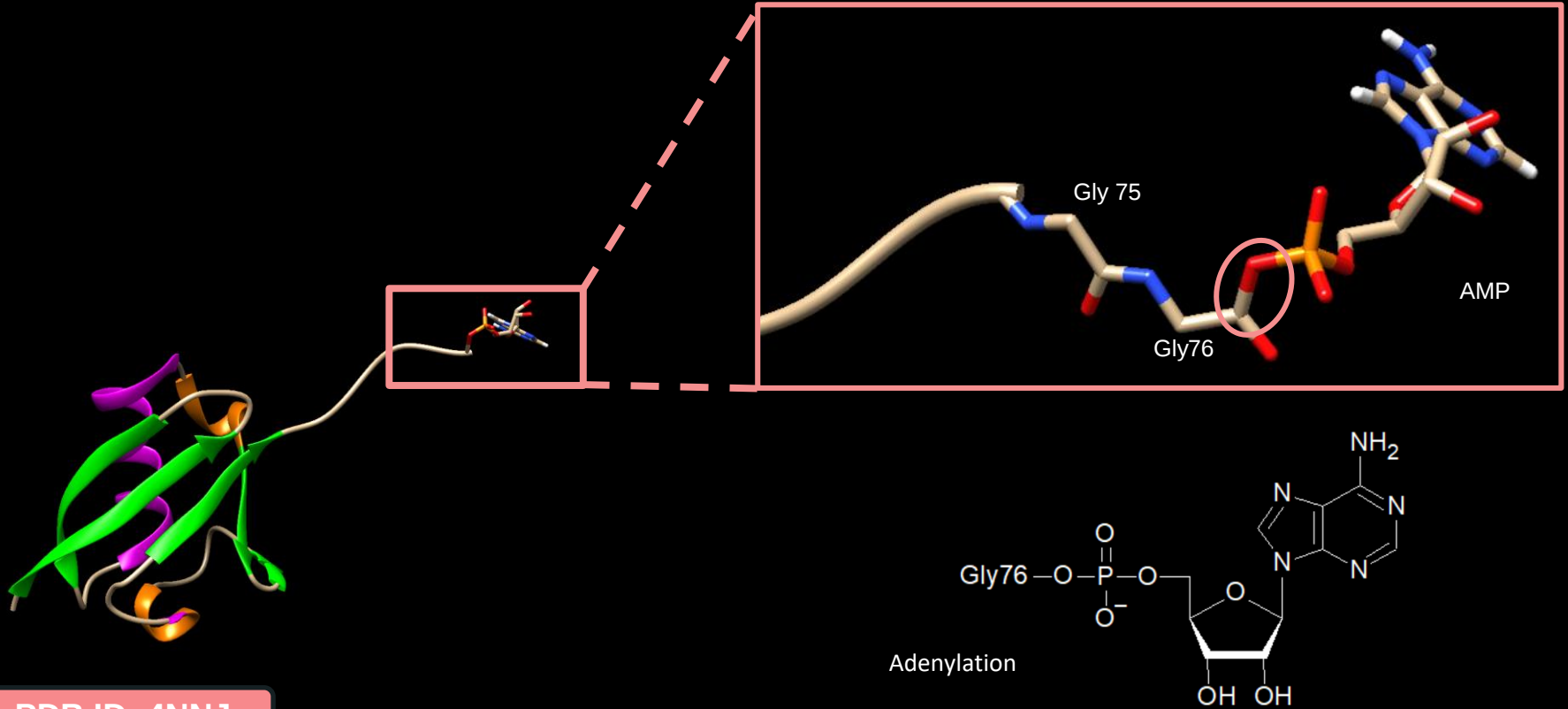
C-terminus



Glycine 76

- Adenylation reaction
- Cystein bonding
- Target unions

Ubiquitin - Adenylation



Ubiquitin - Adenylation



Structural alignment

RMSD 1,11

Sc = 8,69

PDB ID: 4NNJ

A detailed 3D molecular model of a protein complex, likely a ubiquitin-activating enzyme. The protein is shown in a multi-colored representation (green, blue, purple, orange, yellow) to highlight different structural domains. A bright, glowing orange-yellow spot is visible within the purple domain, indicating the active site. The background is a soft, out-of-focus green and blue gradient.

3. Ubiquitin-activating enzymes

E1 - Classification

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 activating enzymes (UBA)

CATH Classification

- **Class:** Alpha Beta (3)
- **Architecture:** 3-Layer(aba) Sandwich (3.40)
- **Topology:** Rossmann fold (3.40.50)
- **Homologous Superfamily:** NAD(P)-binding Rossmann-like Domain (3.40.50.720)

E1 – General features

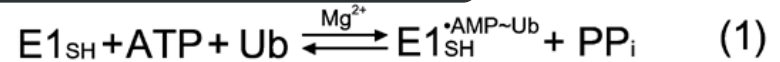
Functions

- Selection of ubiquitin for the pathway.
- Activation of ubiquitin C-terminus for further reactions.
- Coordination of the ubiquitin with correct downstream E2.

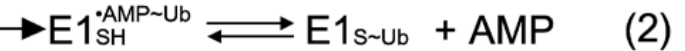
Reactions

- Ubiquitin activation by adenylation.
- E1 forms a thioester between a conserved catalytic cysteine and the Ubiquitin.
- Adenylation of another ubiquitin

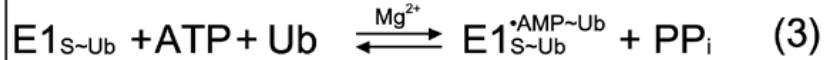
Ubiquitin activation



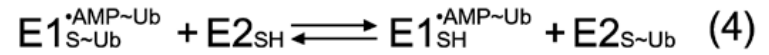
- Activates ubl C-terminus for the remaining reactions in the pathway
- Selects correct ubl for pathway



- E1-ubl thioester intermediate required for further chemical reactions



- Loaded E1 is primed for rapid turnover
- Nucleotide binding promote ubiquitin transfer from E1 to E2



- Coordinates the correct ubl with its cognate E2

Huang D, Walden H, Duda D, Schulman B. Ubiquitin-like protein activation. *Oncogene*. 2004;23(11):1958-1971.

E1 – Genes and Sequence

There are **8 genes** that encode for human **Ubiquitin-activating enzymes**:

UBA1

UBA3

UBA5

UBA6

UBA7

UBA1

SAE1

SAE2

Uba1 gene encodes for a 1.058 aa protein:

MSSNNSGLSAAGEIDESLYSRQLYVLGKEAMLKMQTSNVLILGLKGLGVEIAKNVVLAVGKSMVTFDPEPVQLADLSTQFFLTEKDIGQKRGDVTRAKLAELNAYVPVNVLDLDDVTQLSQFQV
VVATDTVSLEDKVKINEFCHSSGIRFISSETRGLFGNTFVDLGEFTVLDPTGEEPRGTGMVSDIEPDGTVTMLDDNRHGLEDGNFVRFSEVEGLDKLNDGTLFKVEVLGPFAFRIGSVKEYGEYKKG
GIFTEVKVPRKISFKSLKQQLSNPEFVFSDFAKFDRAAQLHLGFQALHQFAVRHNGELPRTMNDEDEDANELIKLVTDLVQQPEVLGEGVDVNEGLIKELSYQARGDIPGVVAFFGGLVAQEVKAC
SGKFTPLKQFMYFDSLESPLDPKNFPRNEKTTQPVNSRYDNQIAVFGLDQKKIANSKVFLVGSGAIGCEMLKNWALLGLGSGSDGYIVVTDNDSIEKSNLNRQFLFRPKDVGKNKSEVAAEAVC
AMNPLKGKINAKIDKVGPETEEIFNDSFWESLDFVTNALDNVDARTYVDRRCVYRKPPLSEGLTGKNTQVIIPRLTESYSSSRDPPEKSIPLCTLRSPNKIDHTIAWAKSLFQGYFTDSAENVN
MYLTQPNFVEQTLKQSGDVKGVLSEISDSLSSKPHNFEDCIKWARLEFEKKFNHDIKQLLFNFPKDAKTSNGEPFWGAKRAPTPLEFDIYNNDHFHVVAGASLRAYNYGIKSDDSNSKPNVDEY
KSVIDHMIIEFTPNANLKIQVNDLDDPDPNANAANGSDEIDQLVSSLPDPSTLAGFKLEPVDFEKDDDTNHHIEFITACSNCRANQNYFIETADRQTKFIAGRIIPAIATTTSLVTGLVNLELYKLIDNK
TDIEQYKNGFVNALLPFGFSEPIASPKGEYNNKKYDKIWDREFDIKGDIKLSDLIEHFEKDEGLEITMLSYGVSLLYASFFPPKKLKERLNLPIQLVVKLVTKKDIPAHVSTMLEICADDKEGEDVEVPFI
TIHL

E1 - Structure

Consists of 4 building blocks:

Adenylation domains:

IAD: Inactive Adenylation Domain

AAD: Active Adenylation Domain

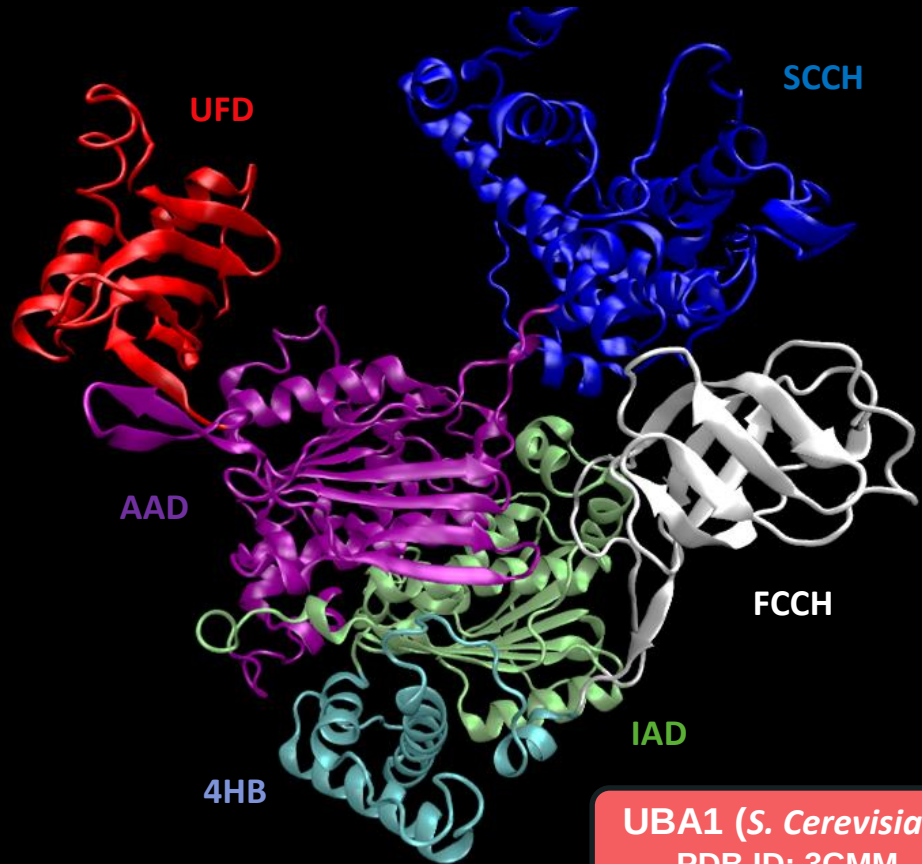
Cysteine catalytic half-domains (CCHs)

FCCH: First CCH

SCCH: Second CCH

Ubiquitin-fold domain (UFD)

Four-helix bundle (4HB)



UBA1 (*S. Cerevisiae*)
PDB ID: 3CMM

UBA1 Evolution

Second Cysteine Catalytic Half-Domain (partially)

	620	630	640	650	660	670	680		
<i>Arabidopsis_thaliana</i> /1-1080	VNALD	NVNARLYVDS	SRCLYFQKPLLES	SGTLG	TKCNTQSVIPHLT	ENYGASRD	PPEKQAPMCTVHSFPH		
<i>Triticum_aestivum</i> /1-1051	VNALD	NVTARMYIDS	SRCVYFQKPLLES	SGTLG	AKCNTQMVIPHLT	ENYGASRD	PPEKQAPMCTVHSFPH		
<i>Saccharomyces_cerevisiae</i> /1-1024	TNALD	NVDARTYVDRRC	VYFQKPLLES	SGTLG	TKGNTQVVIIPRLT	ESYSSSRD	PPEKSIPLCTLR	SFPN	
<i>Schizosaccharomyces_pombe</i> /1-1012	TNALD	NVEARMYVDRRC	VYFQKPLLES	SGTLG	TKGNTQVVVPHLT	ESYSSSQD	PPEKSFPICTLR	KNFPN	
<i>Mus_musculus</i> /1-1058	ANALD	NIDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Rattus_norvegicus</i> /1-1058	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Cricetulus_griseus</i> /1-1058	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Homo_sapiens</i> /1-1058	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Pan_troglodytes</i> /1-1058	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Pongo_abelii</i> /1-1056	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Papio_anubis</i> /1-1058	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Chlorocebus_sabaeus</i> /1-1058	ANALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN
<i>Bos_taurus</i> /1-1058	TNALD	NVDARMYMDRR	CVYRKPLLES	SGTLG	TKGNVQVVI	PFLT	ESYSSSQD	PPEKSIPICTLR	KNFPN



Catalytic Cysteine

UBA1: Catalytic Cysteine

100% conserved!



UBA1 (*S. Cerevisiae*)
PDB ID: 3CMM

The Ubiquitin Folded Domain



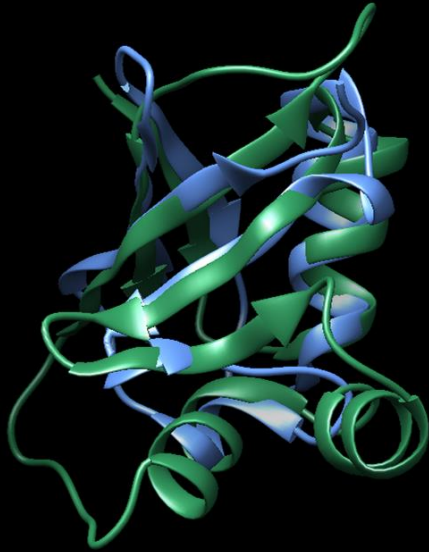
Ubiquitin (*S. cerevisiae*)

PDB: 1UBQ



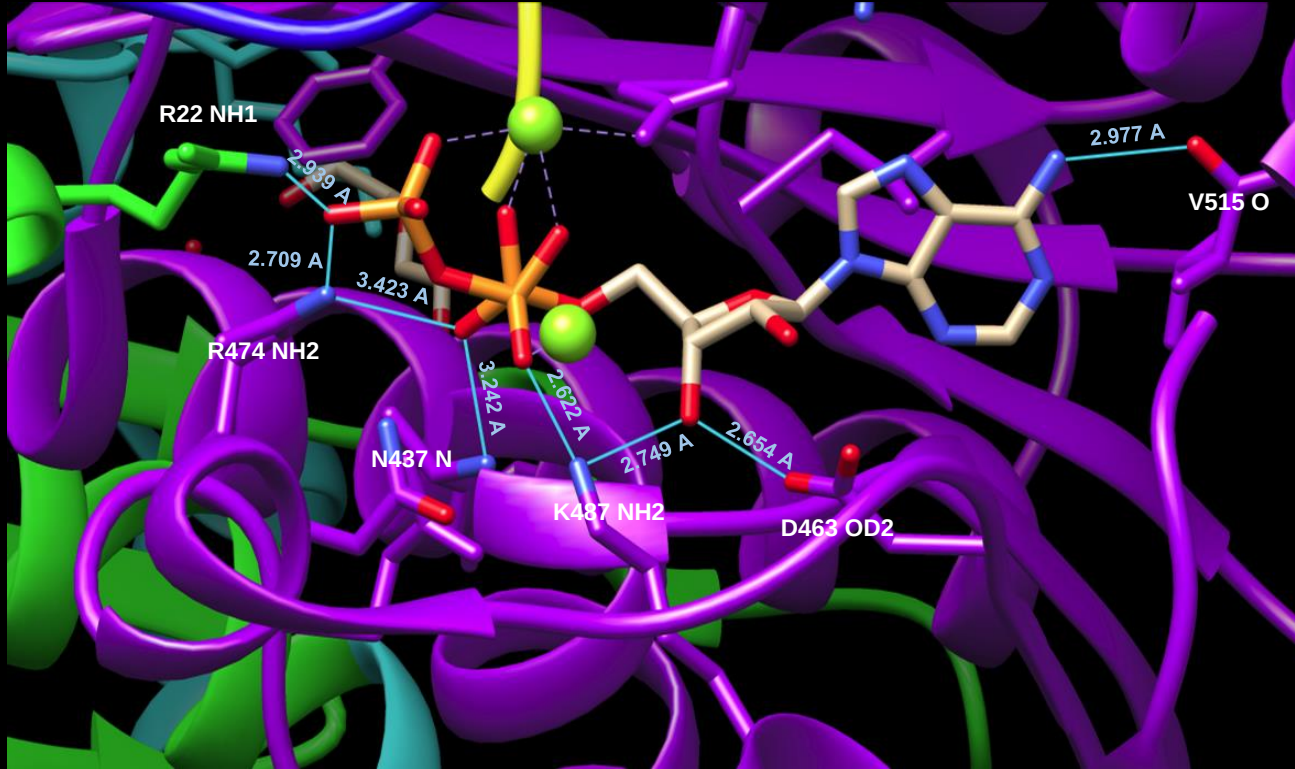
UFD from UBA1 (*S. cerevisiae*)

PDB: 3CMM



SC: 3.48
RMSD: 1.78

ATP - binding



8 Hydrogen Bonds

UBA1 involved residues:

IAD: Arg22

AAD: Val515, Lys487,
Ala437, Arg474 and
Asp463.

Uba1 - Ubc4
(*S. pombe*)
PDB ID: 4II2C

A 3D molecular model showing the interaction between Ubiquitin (a yellow/orange structure) and E1 (a purple structure). The Ubiquitin molecule is shown as a yellow/orange structure, and the E1 molecule is shown as a purple structure. They are interacting at a site highlighted by a bright orange/yellow glow. The background is a blurred green and blue surface.

4. Ubiquitin-E1 interaction

Ubiquitin - E1 interactions

UBA1 (*S. Cerevisiae*)
PDB ID: 3CMM

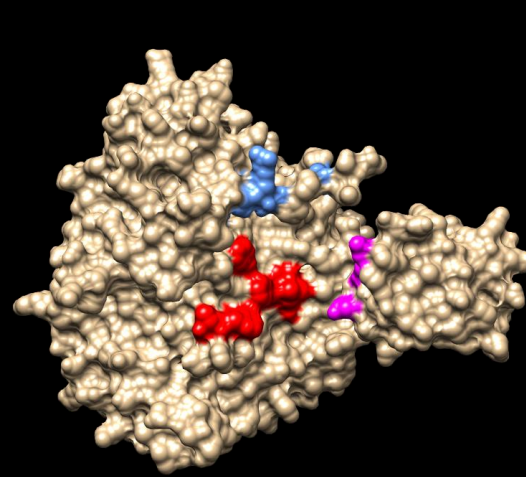
E1 enzymes can interact covalently and non covalently with Ub.

Non-covalently (3 interfaces):

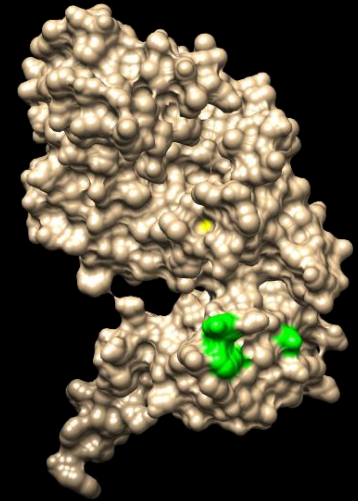
- Interface 1
- Interface 2
- Interface 3

Covalently linked to **Cys600**:

- Interface 1

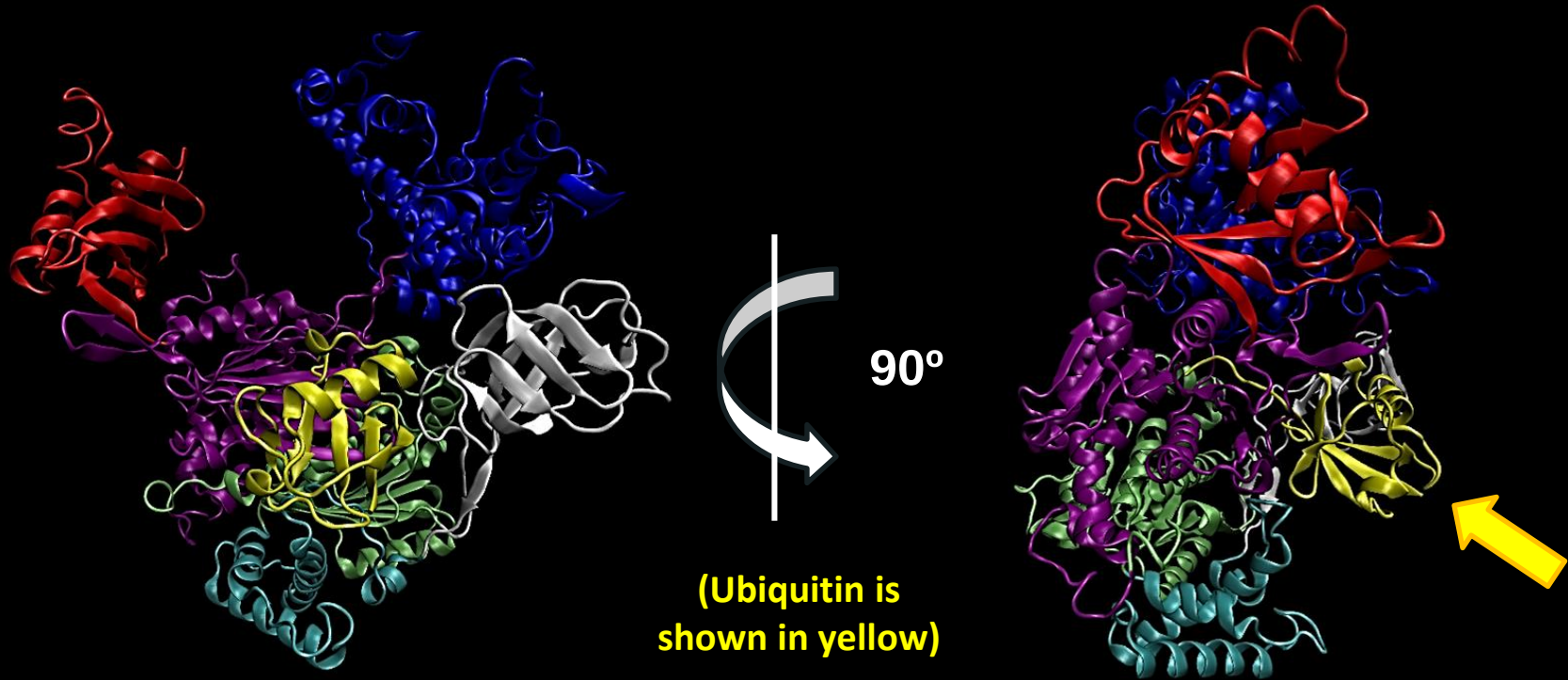


UFD and SCCH are not shown



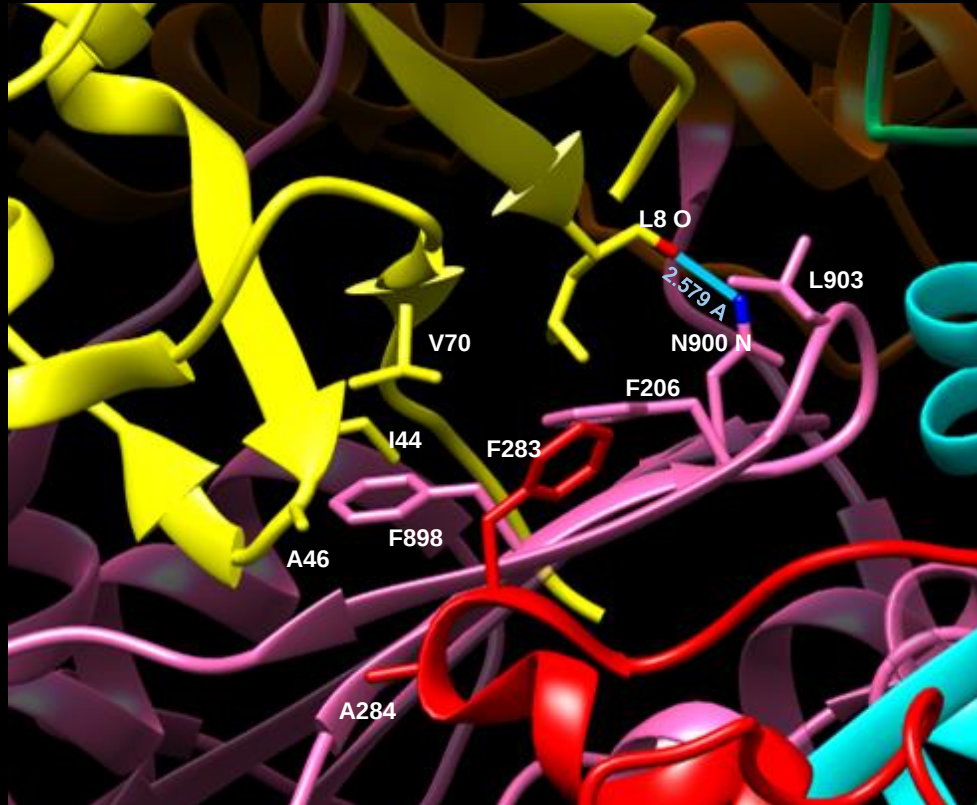
Only FCCH and SCCH are shown

E1 + Ubiquitin



PDB ID: 3CMM

INTERFACE 1



Hydrophobic interaction

+

Hydrogen Bond

UBA1 involved residues:

AAD: Phe 898, Asn 900, Leu903 and Phe905

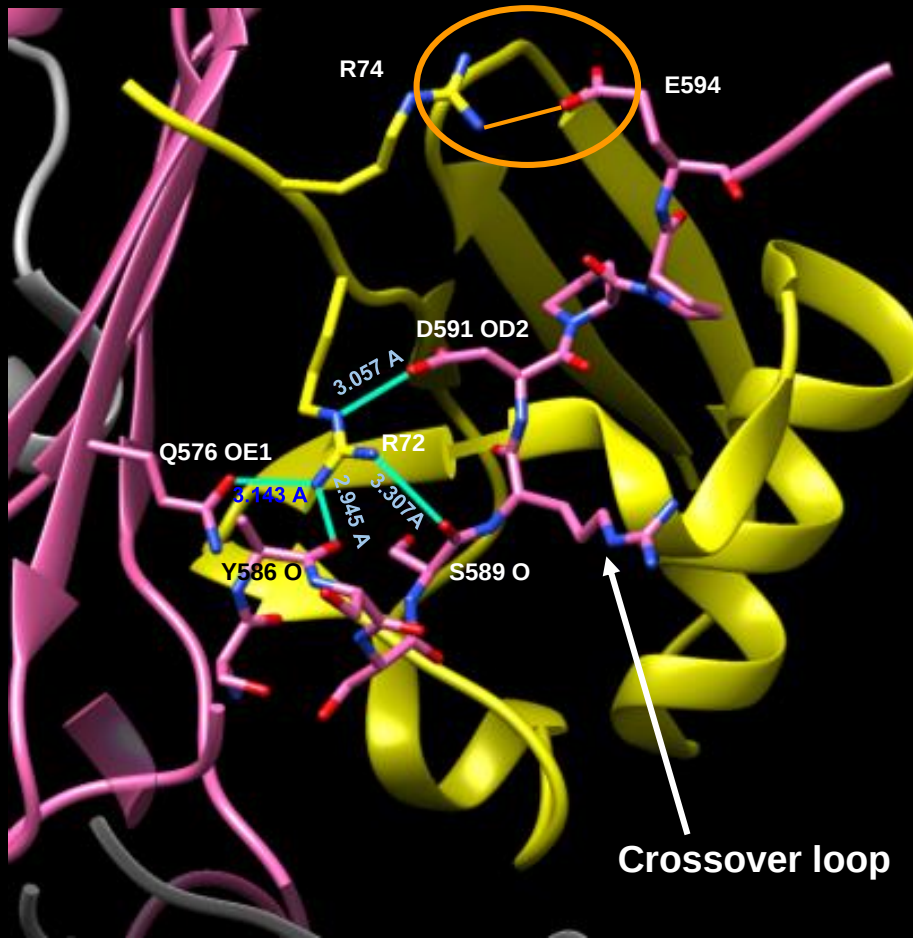
4HB: Phe 283 and Ala 284

Ubiquitin involved residues:

- Leu 8, Ile 44, Ala 46 and Val 70

PDB ID: 3CMM

INTERFACE 2



Salt Bridge
+
4 Hydrogen Bond

UBA1 involved residues:

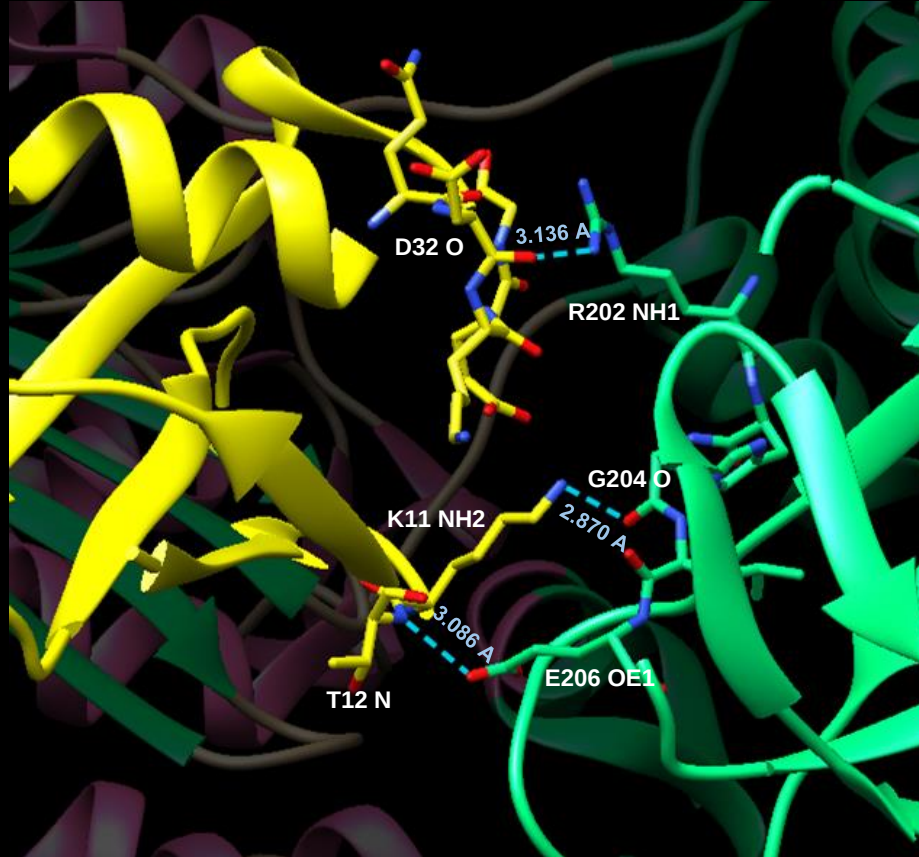
AAD: Gln 576, Tyr 586, Ser 589, Asp 591 and Glu 594

Ubiquitin involved residues:

Arg 74 and Arg 72

PDB ID: 3CMM

INTERFACE 3



3 Hydrogen Bonds

UBA1 - FCCH involved residues:

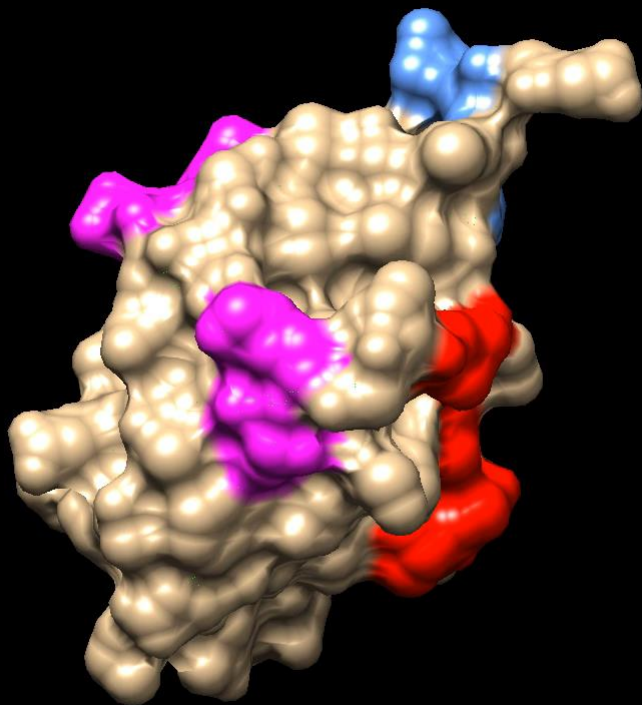
- Arg202, Gly204, and Glu206

Ubiquitin involved residues:

- Lys11, Thr12, and Asp32

PDB ID: 3CMM

Ubiquitin Surface



Interface 1

Interface 2

Interface 3

PDB ID: 1UBQ

Ubiquitin - Involved residues

	L8	K1	T12	D32	K42	I44	A46	V70	R72	R74
Caenorhabditis_elegans	MQIFVKT	LTGKT	TITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
A.ceylanicum	MQIFVKT	LTGKT	TITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Plasmodium_falciparum	MQIFVKT	LTGKT	TITLDVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Ovis_aries	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	MR	RG				
Drosophila_melanogaster	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Xenopus_laevis	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Danio_rerio	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Pongo_pygmaeus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Pan_troglodytes	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Gorilla_gorilla_gorilla	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Equus_caballus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Cavia_porcellus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Gallus_gallus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Bos_taurus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Mus_musculus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Rattus_norvegicus	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Homo_sapiens	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Schizosaccharomyces_pombe	MQIFVKT	LTGKT	TITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Saccharomyces_cerevisiae	MQIFVKT	LTGKT	TITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
Arabidopsis_thaliana	MQIFVKT	LTGKT	TITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHL	VLR	LR	RG				
Cryptococcus_neoformans	MQIFVKT	LTGKT	TITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHL	VLR	LR	RG				
*****	***	***	***:***:***:***:*****	*****	***	***	*****:*****	***	***	***

■ Interface 1
■ Interface 2
■ Interface 3

Interface 1 - Residues involved in interaction

Arabidopsis_thaliana
Triticum_aestivum
Saccharomyces_cerevisiae
Schizosaccharomyces_pombe
Mus_musculus
Rattus_norvegicus
Cricetulus_griseus
Homo_sapiens
Pan_troglodytes
Pongo_abelii
Papio_anubis
Chlorocebus_sabaeus
Bos_taurus
Ovis_aries
Canis_lupus
Equus_caballus
Cavia_porcellus
Ictidomys_tridecemlineatus
Oryctolagus_cuniculus
Monodelphis_domestica
Anolis_carolinensis
Drosophila_melanogaster
Brugia_malayi

F283
A284
FSDFSKFDRPPLLHLAFQALDHFKA EA-GRFPVAGSEEDAQKLISIATAI
MSDFSKFERPPLLHLAFQALDKFRTEL-SRFPVAGSTDDVQRVIEYAI SI
FSDFAKFDRAAQLHLGFQALHQFAVRHNGELPRTMNDEDANELIKLVTDL
YPD FGKMMRPPQYHIAFQALSAFADAHEGSLPRPRNDIDAAEFFEFCKKI
MTDFAKYSRPAQLHIGFQALHQFCALH-NQPPRPRNEEDATELVGLAQAV
MTDFAKYSRPAQLHIGFQALHQFCAQH-NRPPRPRNEEDATELVTLAQAV
MTDFAKFSRPGQLHIGFQALHQFCAQH-NRPPRPRNEEDATELVALAQAM
VTDFAKFSRPAQLHIGFQALHQFCAQH-GRPPRPRNEEDAAELVALAQAV
MTDFAKFSRPAQLHIGFQALHQFCAQH-GRPPRPRNEEDAAELVALAQAV
MTDFAKFSRPAQLHIGFQALHQFCAQH-GRPPRPRNEEDAAELVALAQAV
MTDFAKFSHPAQLHIGFQALHHFCAQH-GRPPRPRNEEDATELVALAQAV
MTDFAKFSHPAQLHIGFQALHHFCAQH-GRPPRPRNEEDATELVALAQAV
MTDFAKFSHPAQLHIGFQALHHFCAQH-GRPPRPRNEEDATELVALAQAV
MTDFAKYSRPAQLHIGFQALHHFCAQH-GRSPRPHNEEDAAELVTIAQAV
MTDFAKYSRPAQLHIGFQALHHFCAQH-GRSPRPHNEEDAAELVTIAQAV
MTDFAKYSRPAQLHLGFQALHQFCAQH-GRPPRPRNEEDATELVALARAV
MTDFAKYSRPAQLHIGFQALHQFCAQH-GRPPRPRNEEDAAELVTIAQAV
MTDFAKYSRPAQLHIGFQALHQFCAQH-NRPPRPRNEEDATKLVALAQAV
MTDFAKFSRPAQLHIGFQALHQFCAQH-SRPPRPRNEEDATELVALAQAV
MTDFAKFSRPAQLHIGFQALHKFCAQH-SRPPRPRNEEDAAELVTLARAV
MTDFAKFSRPAHLHVAFQALHQFYSSQR-GR LPHPQNQLDAAEMVSLAQGI
ITD FGKFD RPAQLHLGFQGLQEFHKKH-ERFPKPRNQADASEVLT LVKEL
ISDFAKLDSPATLHVAFNALSSYRKAHNGALPRPWNEEDANSFLEVVRAS
MWDFAKFENPSQLHALWQALHSFEAKH-KRSPMPRSNEDVGLLKVELP--

Interface 1
Interface 2
Interface 3

Interface 1 - Residues involved in interaction

Arabidopsis_thaliana	IPAIATSTAMATGLVCELYKVL DGGHK-----VEAYRNTFANLALPLFS	F398	N900	L903	L905
Triticum_aestivum	IPAIATSTAMATGLVCELYKALAGGHK-----VEDYRNTFANLAIP LFS				
Saccharomyces_cerevisiae	IPAIATTTSLVTGLVNL ELYKLI-DNKT----DIEQYKNGFVNLALPFFG				
Schizosaccharomyces_pombe	VPAMCTSTAVVSGLVCELVKLV-DGKK---KIEEYKNGFFNLALGLFT				
Mus_musculus	IPAIATTTAAVVGLVCELYKVV-QGHQ----QLDSYKNGFLNLALPFFG				
Rattus_norvegicus	IPAIATTTAAVVGLVCELYKVV-QGHQ----QLDSYKNGFLNLALPFFG				
Cricetulus_griseus	IPAIATTTAAVVGLVCELYKVV-QGHQ----QLDSYKNGFLNLALPFFG				
Homo_sapiens	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Pan_troglodytes	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Pongo_abelii	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Papio_anubis	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Chlorocebus_sabaeus	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Bos_taurus	IPAIATTTAAVVGLVCELYKVV-QGHR----QLNSYKNGFLNLALPFFG				
Ovis_aries	IPAIATTTAAVVGLVCELYKVV-QGHR----QLNSYKNGFLNLALPFFG				
Canis_lupus	IPAIATTTAAVVGLVCELYKVV-HGHR----QLDSYKNGFLNLALPFFG				
Equus_caballus	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Cavia_porcellus	IPAIATTTAAVVGLVCELYKVV-QGHR----KLDSYKNGFLNLALPFFG				
Ictidomys_tridecemlineatus	IPAIATTTAAVVGLVCELYKVV-QGHR----QLDSYKNGFLNLALPFFG				
Oryctolagus_cuniculus	IPAIATTTAAVVGLVCELYKVV-QGHR----HLDSYKNGFLNLALPFFG				
Monodelphis_domestica	IPAIATTTAAVVGLVCELYKVV-QGHQ----RLEAYKNGFLNLALPFFG				
Anolis_carolinensis	IPAIATTTAAVVGLVCELYKII-QGHK----RLESFKNGFLNLALPFFG				
Drosophila_melanogaster	IPAIATTTSVLSGLACLEVIKLI-VGHR----DLVKFKKPCANLALPFMA				
Brugia_malay	IPALATTTAAVAGLVCI ELYKMIGDGHQPPNVPLKVFKNGFLNLALPFFG				

- Interface 1
- Interface 2
- Interface 3

Interface 2 - Residues involved in interaction

	Q576	Y586	S589	R590	D591	E594
Arabidopsis_thaliana	CNTQSVIPHLTEN	YGASRD	PPEKQAPMCTVHSFPHNIDHCLTWARSEFEG			
Triticum_aestivum	CNTQMVIPHLTEN	YGASRD	PPEKQAPMCTVHSFPHNIDHCLTWARSEFEG			
Saccharomyces_cerevisiae	GNTQVVIIPRLTES	YSSSRD	PPEKSIPLCTLRSPNIDHTIAWAKSLFQG			
Schizosaccharomyces_pombe	GNTQVVVPHLTES	YGSSQD	PPEKSFPICTLKNFNPRIEHTIAWARDLFEG			
Mus_musculus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Rattus_norvegicus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Cricetulus_griseus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Homo_sapiens	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Pan_troglodytes	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Pongo_abelii	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Papio_anubis	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Chlorocebus_sabaeus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Bos_taurus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Ovis_aries	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Canis_lupus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Equus_caballus	GNVQVVIPFLTES	YNSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Cavia_porcellus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Ictidomys_tridecemlineatus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Oryctolagus_cuniculus	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Monodelphis_domestica	GNVQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Anolis_carolinensis	GNIQVVIPFLTES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDEFEG			
Drosophila_melanogaster	GNVQVIVPFATES	YSSSQD	PPEKSIPICTLKNFNPNAIEHTLQWARDAFEG			
Brugia_malay	GNTQVVYPHLTES	YGSSVD	PPEKDIPICTLKNFNPNEIQHTIQWARDLFEG			

■ Interface 1
■ Interface 2
■ Interface 3

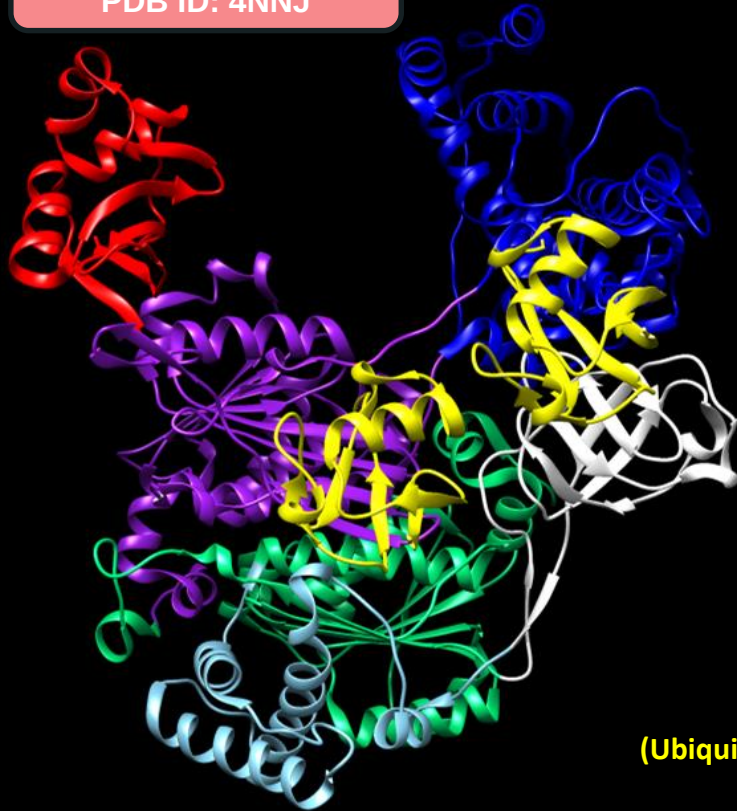
Interface 3 - Residues involved in interaction

		R202	E204	E206	
Arabidopsis_thaliana	GIIASISNENQAFISCVDDER	RL	EF		EDGDLVVFSEVEGMTELNDGKPRKIK
Triticum_aestivum	GIVASISNDNPALVSCVDDER	RL	FQ		DGDLVVFSEVHGMTELNDGKPRKVK
Saccharomyces_cerevisiae	GMVSDIEPDG - - TVTMLDDN	RH	GL		EDGNFVRFSEVEGLDKLNDGTLFKVE
Schizosaccharomyces_pombe	GMIASITDDG - - VVTMLEET	RH	GL		ENGDFVKFTEVKGMPGLNDGTPRKVE
Mus_musculus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		TGDFVSFSEVQGMISQLNGCQPMIEIK
Rattus_norvegicus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		TGDFVSFSEVQGMVQLNGCQPIEIK
Cricetulus_griseus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMISQLNGCQPIEIK
Homo_sapiens	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMVELNGNQPMIEIK
Pan_troglodytes	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMVELNGNQPMIEIK
Pongo_abelii	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMVELNGNQPMIEIK
Papio_anubis	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMVELNGNQPMIEIK
Chlorocebus_sabaeus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMVELNRNQPMIEIK
Bos_taurus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMIELNGSQPMIEIK
Ovis_aries	AMVSMVTKDNPGVVTCLDEAR	HG	FE		TGDFVSFSEVQGMIELNGSQPMIEIK
Canis_lupus	AMVSMVTKDSPGVVTCLDEAR	HG	FE		SGDYVSFSEVQGMVELNGSQPMIEIK
Equus_caballus	AMVSMVTKDSPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMIELNGSQPMIEIK
Cavia_porcellus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMNELNGNQPIEIK
Ictidomys_tridecemlineatus	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMIELNGNQPIEIK
Oryctolagus_cuniculus	TMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVSFSEVQGMTELNGNQPIEIK
Monodelphis_domestica	AMVSMVTKDNPGVVTCLDEAR	HG	FE		SGDFVTFTEVQGMSELNGISPMEIK
Anolis_carolinensis	AMVSMVTKGIPGEVTCLEAR	HG	FE		SGDFVSFTEVEGMSELNSCEPMEIK
Drosophila_melanogaster	TMIASITHDAQGVVTCLEDET	RH	GN		DGDLTLFSEVQGMQELNGCQPLKIT
Brugia_malay	LLIEHVDAET - GDVTTLDNV	MH	GL		DGDYVTFSEVKGMTLNGIEPLKIT

■ Interface 1
■ Interface 2
■ Interface 3

E1 + 2 Ubiquitins - Ternary Complex

UBA1 (*S. cerevisiae*)
PDB ID: 4NNJ

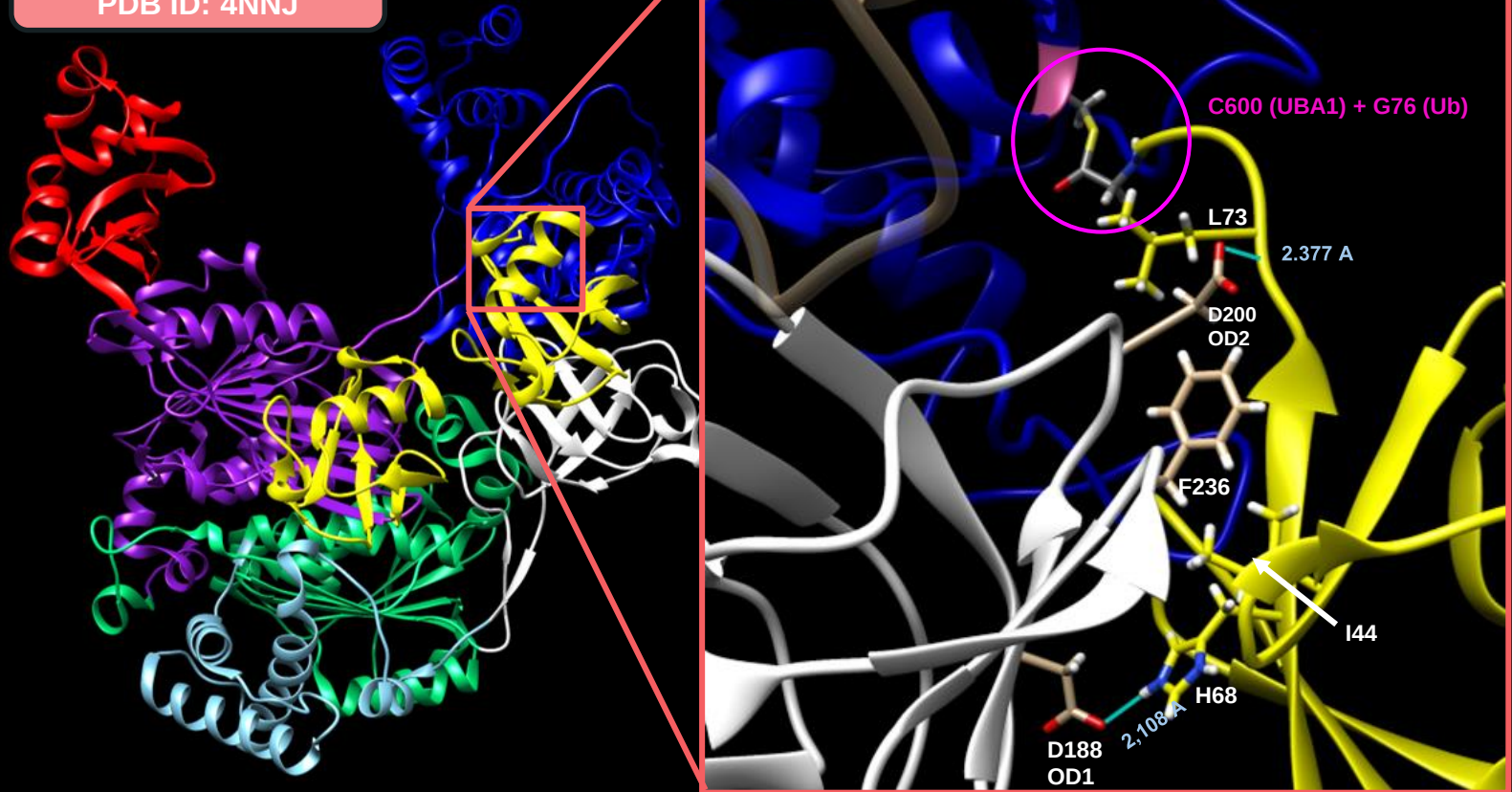


(Ubiquitins are shown in
yellow)

E1 + 2 Ubiquitins - Ternary Complex

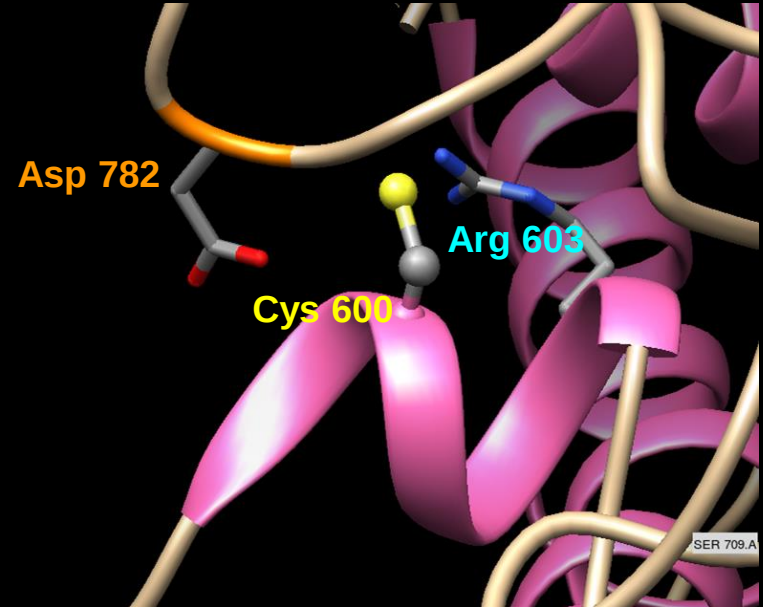
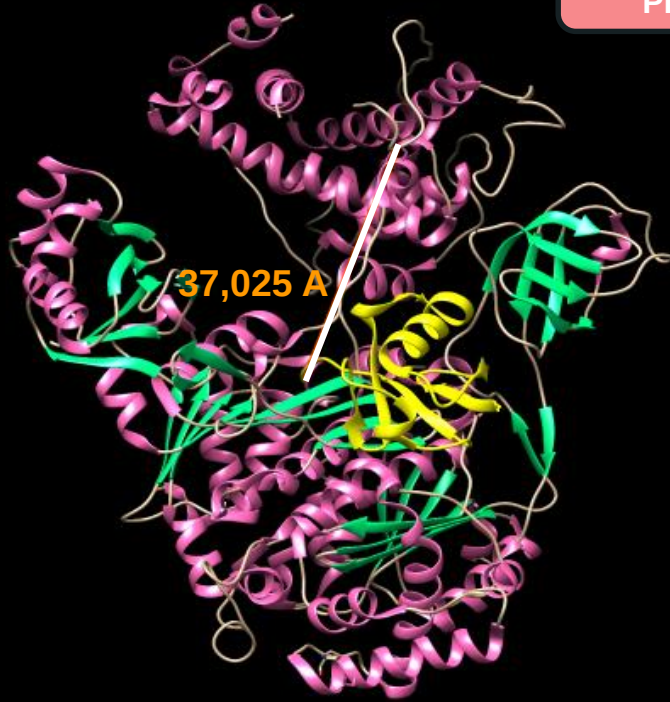
UBA1 (*S. cerevisiae*)

PDB ID: 4NNJ

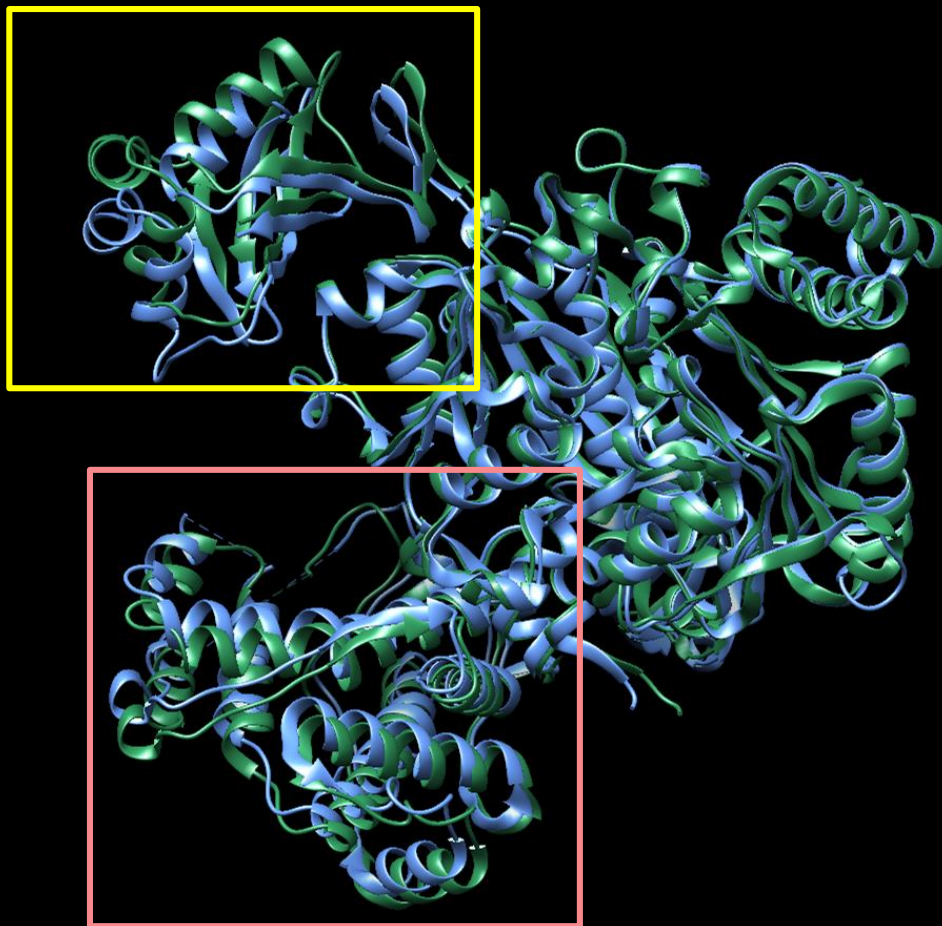


SCCH restrictions

UBA1 (*S. cerevisiae*)
PDB ID: 3CMM



Some movements



UBA1 with 1 Ub (ID: 3CMM)

UBA1 with 2 Ub (ID: 4NNJ)

***Both structures are from S. cerevisiae*

Sc: 8,58

RMS: 1,55

A detailed 3D molecular model of a protein complex, likely a ubiquitin-conjugating enzyme (E2). The protein is shown in a multi-colored representation, with different domains or regions highlighted in green, yellow, orange, and purple. The surface is highly textured, showing various loops and folds. A bright, glowing yellow-orange spot is visible within the purple region, indicating a specific active site or binding pocket. The background is a soft, out-of-focus gradient of green and blue.

5. Ubiquitin conjugating enzymes (E2)

E2 - Classification

SCOP Classification

- **Class:** Alpha and beta proteins ($\alpha + \beta$)
- **Fold:** UBC-like
- **Superfamily:** UBC-like
- **Family:** UBC-like

CATH Classification

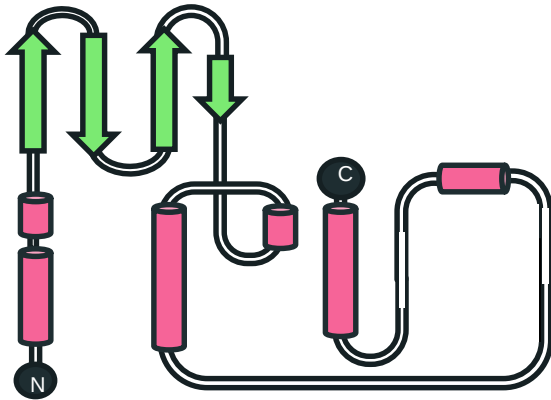
- **Class:** Alpha Beta (3)
- **Architecture:** Roll (3.10)
- **Topology:** Ubiquitin conjugating enzyme (3.10.110)
- **Homologous Superfamily:** Ubiquitin conjugating enzyme (3.10.110.10)

E2 - General features

Function

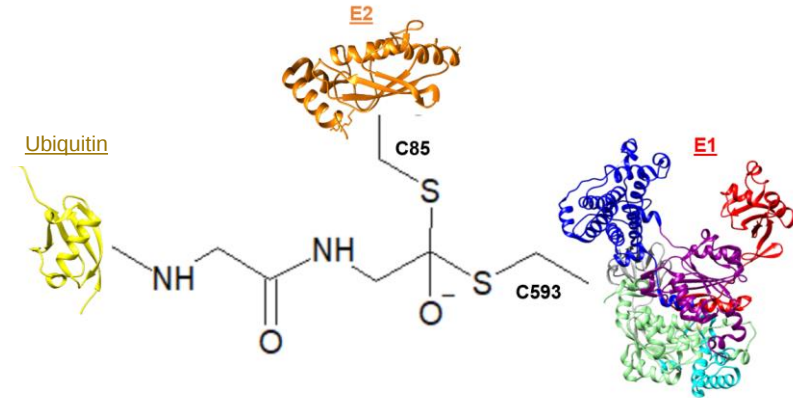
- Covalently attaches to ubiquitin
- Binds to ubiquitin ligase (E3)

Topological diagram



Process

$E1 \sim E2 \sim Ubl \text{ intermediate} \rightarrow E2 \sim Ubl$



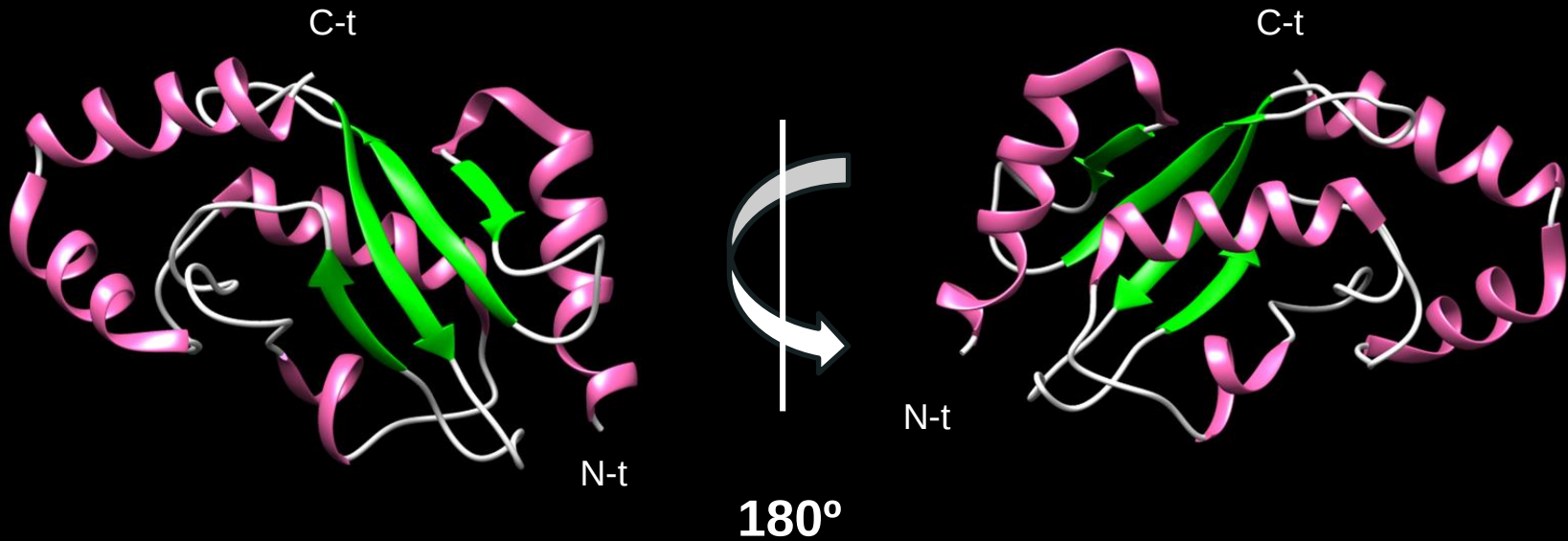
$E2 \sim Ubl \rightarrow \text{Target protein}$

$E2 \sim Ubl \rightarrow E3 \rightarrow \text{Target protein}$

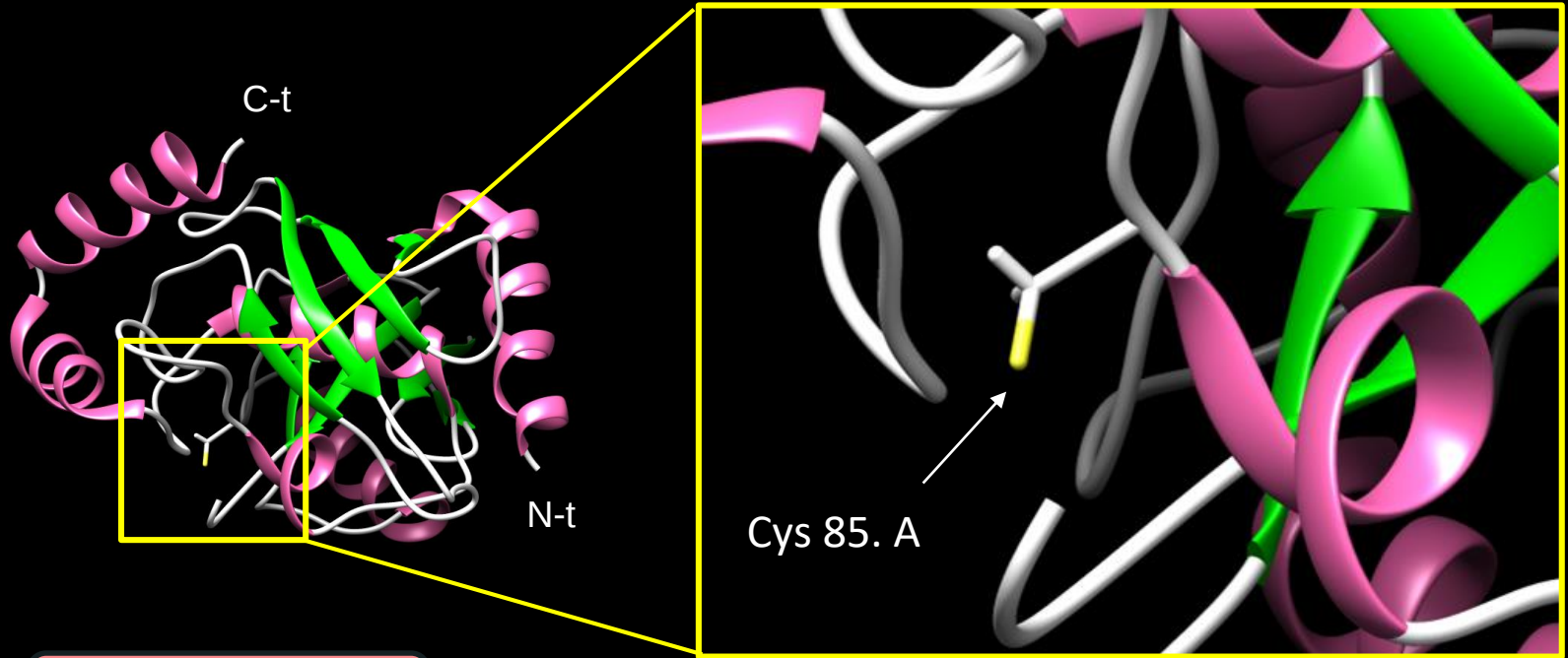
(Two mechanisms)

E2 - Structure

Ubc1 (*S. Cerevisiae*)
PDB ID: 1FXT



E2 - Structure



Ubc1 (*S. cerevisiae*)
PDB ID: 1FXT

E2 - Conservation

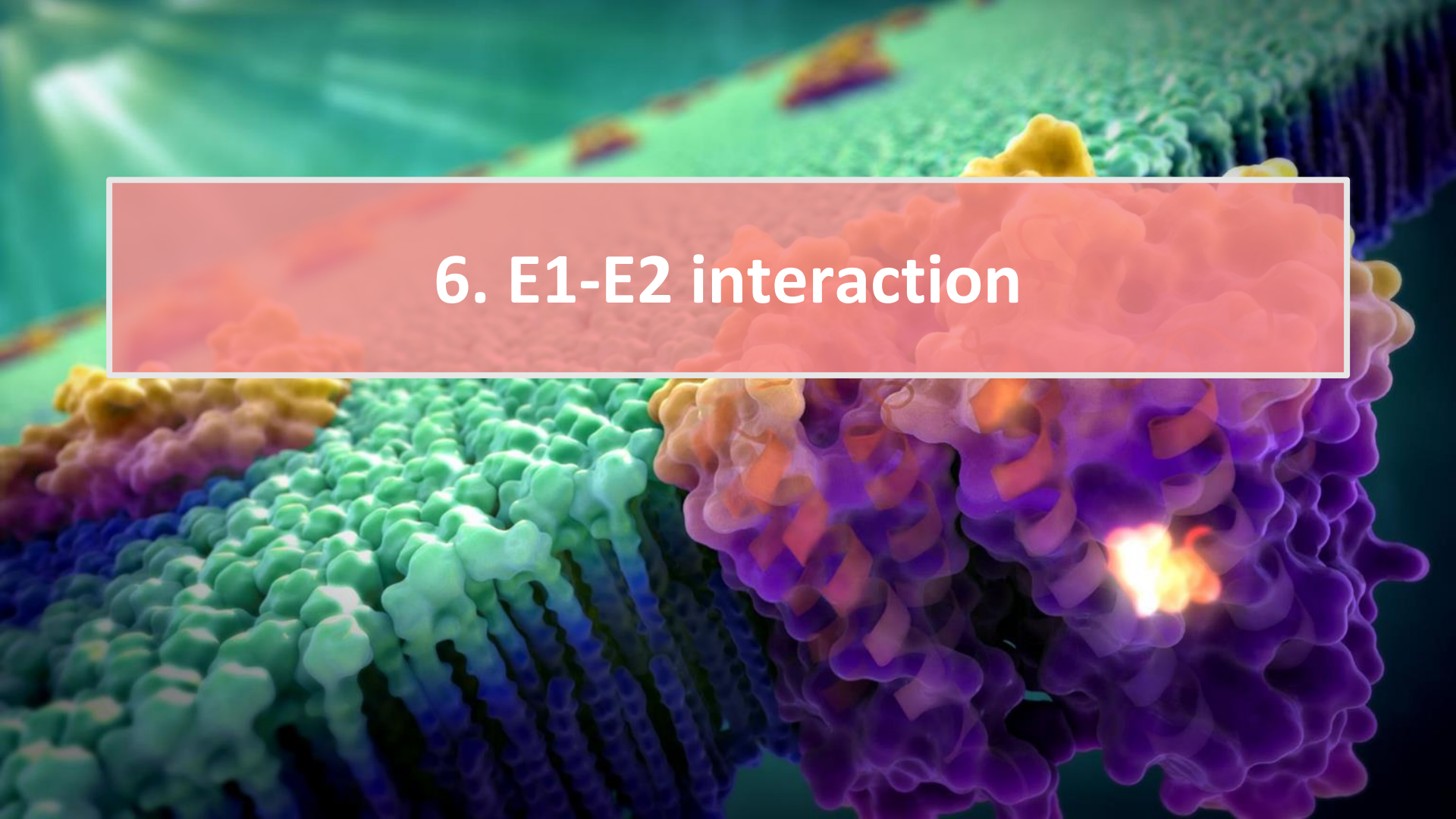
Homo_sapiens (Ubc5)
Mus_musculus (Ube2d3)
Xenopus_laevis (Ube2d3)
Arabidopsis_thaliana (Ubc8)
Saccharomyces_cerevisiae (Ubc7)
Bos_taurus (Ube2k)
Schizosaccharomyces_pombe (Ubc2)
Drosophila_melanogaster (Ubch10)

FTTRIIYHPNIN-SNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDD
FTTRIIYHPNIN-SNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDD
FTTKIYHPNIN-SNGSICLDILRSQWSPALTVSKVLLSICSLLCDPNPDD
FRTKVFHPNIN-SNGSICLDILKEQWSPALTISKVLLSICSLLTDPNPDD
FDTKVYHPNISSVTGAICLDILKNAWSPVITLKSALISLQALLQSPEPND
FITKIWHPNISSVTGAICLDILKDQWAAAMTLRTVLLSLQALLAAEPDD
FLTkiYHPNVD-KLGRICLSTLKKDWSPALQIRTVLLSIQALMGAPNPDD
FLTSCFHPNVD-LQGAICLDILKDKWSALYDVRTILLSIQSLLGEPNNE
* * :***:. * ** . *: . *: . : . *:*: **:

C85

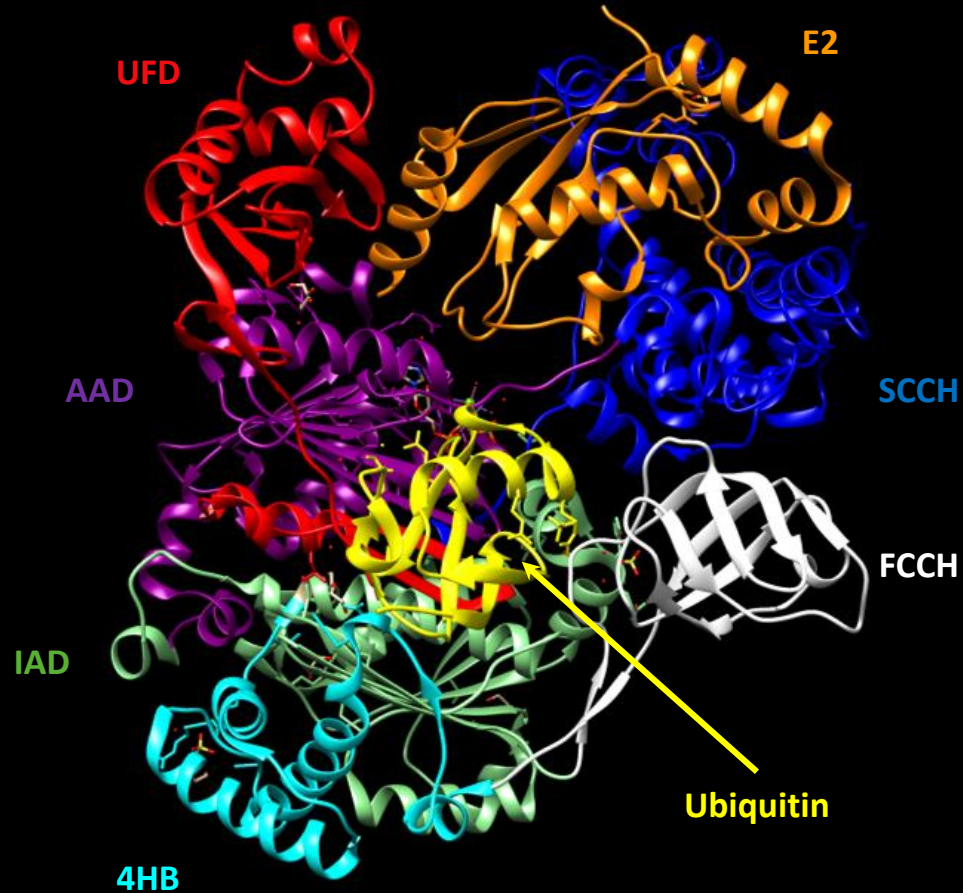


Catalytic Cysteine

A 3D molecular model of a protein complex, likely a photosynthetic reaction center. The structure is composed of several subunits, each represented by a different color: green, blue, yellow, and purple. The surface of the protein is highly textured, showing various loops and helices. A bright, glowing orange and yellow spot is visible on the purple subunit, indicating a site of high energy or interaction. The background is a soft, out-of-focus green and blue gradient.

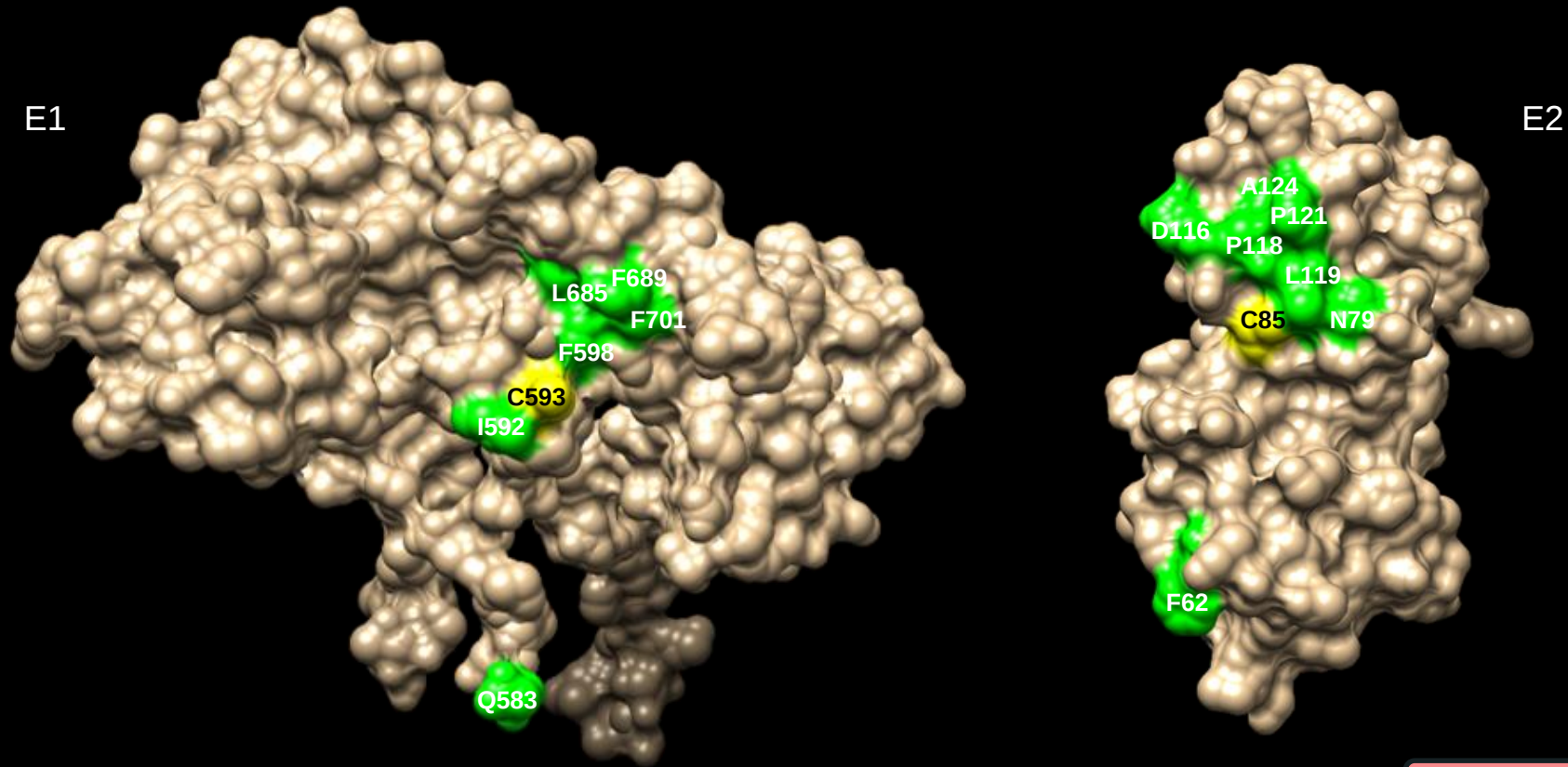
6. E1-E2 interaction

E1-E2 Complex: a general view



Uba1 - Ubc4
(*S. pombe*)
PDB ID: 4II2C

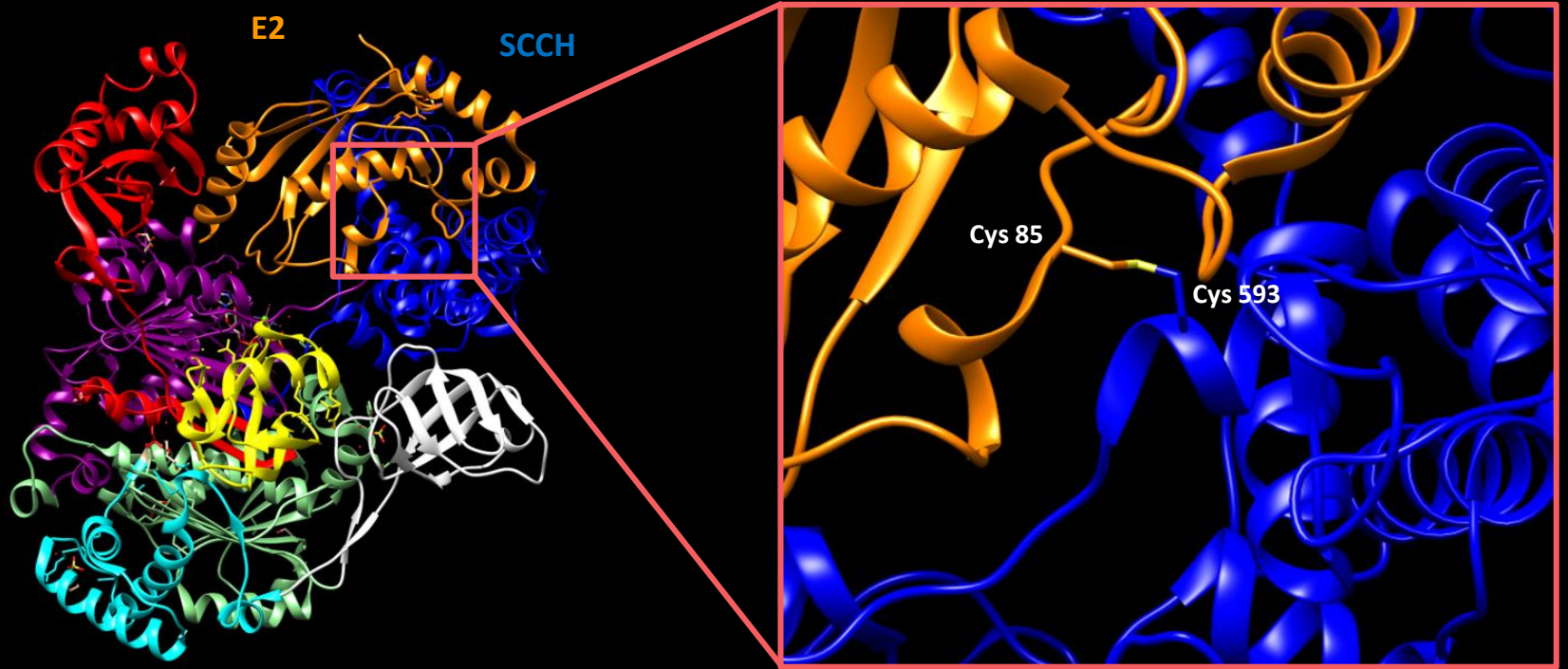
E1-E2 interaction surface



(Only SCCH domain is shown)

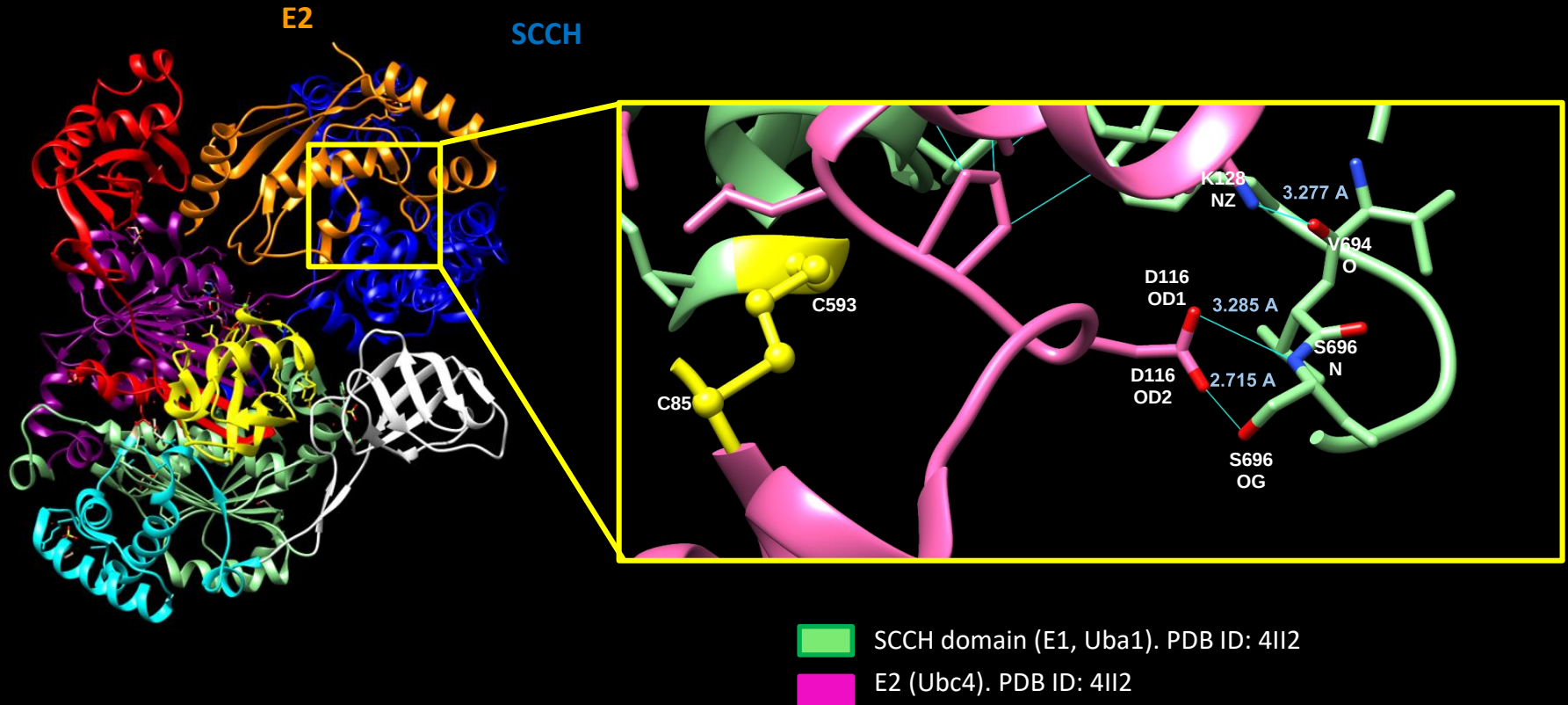
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Catalytic cysteines

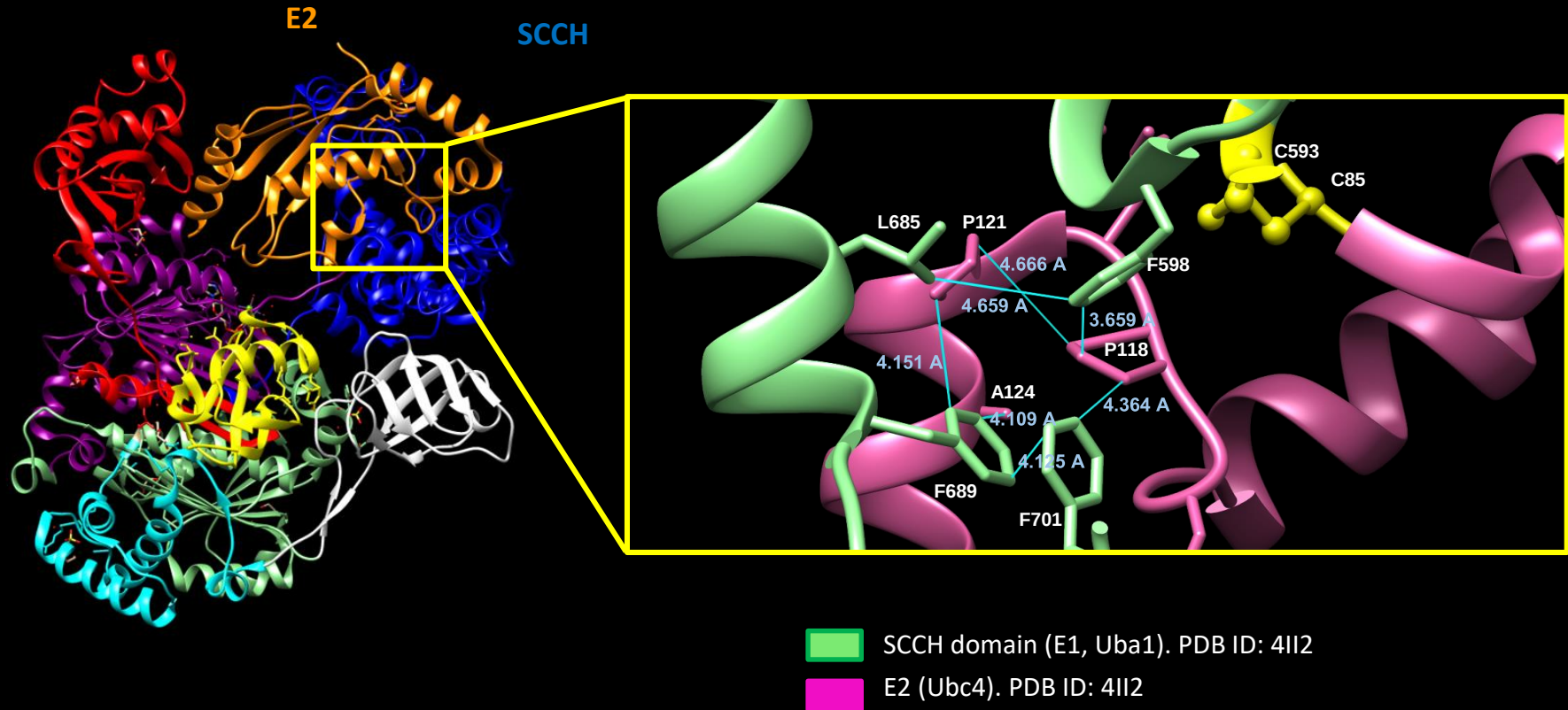


PDB ID: 4II2

E1-E2 interacting residues (I): Hydrogen bonds



E1-E2 interacting residues (II): Hydrophobic contacts



E1-E2 interacting residues (III): Conserved E1 residues

	Q583	I592 C593	F598
Arabidopsis_thaliana	CNTQSVIPHLTENYGASRDPPEKQAP	MCTVHS	FPHNIDHCLTWARSEFEG
Triticum_aestivum	CNTQMVIPHLTENYGASRDPPEKQAP	MCTVHS	FPHNIDHCLTWARSEFEG
Saccharomyces_cerevisiae	GNTQVIIIPRLTESYSSSRDPPEKSIP	LCTLR	SFPNKIDHTIAWAKSLFQG
Schizosaccharomyces_pombe	GNTQVVVPHLTESYGSSQDPPEKSFP	ICTLKN	FNPRIEHTIAWARDLFEG
Mus_musculus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Rattus_norvegicus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Cricetulus_griseus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Homo_sapiens	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Pan_troglodytes	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Pongo_abelii	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Papio_anubis	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Chlorocebus_sabaeus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Bos_taurus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Ovis_aries	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Canis_lupus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Equus_caballus	GNVQVVIPFLTESYNSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Cavia_porcellus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Ictidomys_tridecemlineatus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Oryctolagus_cuniculus	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Monodelphis_domestica	GNVQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Anolis_carolinensis	GNIQVVIPFLTESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDEFEG
Drosophila_melanogaster	GNVQVIVPFATESYSSSQDPPEKSIP	ICTLKN	FNPNAIEHTLQWARDAFEG
Brugia_malay	GNTQVVYPHLTESYGSSVDPPEKDIP	ICTLKN	FNPNEIQHTIQWARDLFEG

E1-E2 interacting residues (IV): Conserved E1 residues

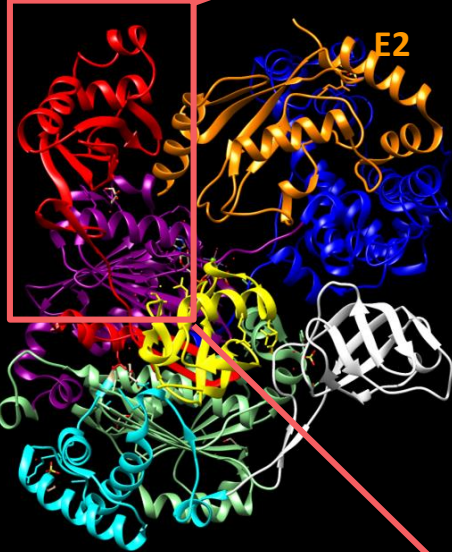
Arabidopsis_thaliana	QDCLTWARLRFEDYFVNRVKQ	LIYT	F	PEDAATSTGAP	FWSAPKRFPRPLQ
Triticum_aestivum	QDSITWARLKFEDYFSNRVKQ	LTFT	F	PEDSMTSSGAP	FWSAPKRFPRPVE
Saccharomyces_cerevisiae	EDCIKWARLEFEKKFNHDIKQ	LLFN	F	PKDAKTSNGEP	FWSGAKRAPTPLE
Schizosaccharomyces_pombe	EECIMWARLQFDKFFNNNIQQ	LLFN	F	PKDSVTSTGQP	FWSGPKRAPTPLS
Mus_musculus	GDCVTWACHHWHTQYCNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Rattus_norvegicus	GDCVTWACHHWHTQYCNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Cricetulus_griseus	GDCVTWACHHWHTQYCNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Homo_sapiens	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Pan_troglodytes	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Pongo_abelii	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Papio_anubis	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Chlorocebus_sabaeus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Bos_taurus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Ovis_aries	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Canis_lupus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Equus_caballus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Cavia_porcellus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Ictidomys_tridecemlineatus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Oryctolagus_cuniculus	ADCVTWACHHWHTQYSNNIRQ	LLHN	F	PPDQLTSSGAP	FWSGPKRCPHPLT
Monodelphis_domestica	ADCVAWACLHWHAQYANNIRQ	LLHN	F	PPPEQLTSSGAP	FWSGPKRCPHPLT
Anolis_carolinensis	ADCVTWACNHWHTQYSNNIRQ	LLHN	F	PPNQKTNSGTL	FWSGPKRCPHPLT
Drosophila_melanogaster	AHCVEWARLYWEDQYVNQIKQ	LLFN	F	PPDQITSSGQP	FWSGPKQCPDPLV
Brugia_malayi	EDCIKWARMNFQEYFHNMIQ	LLHM	F	PPDQVTEQGIK	FWSGSKRCPHVLD

E1-E2 interacting residues (V): Conserved E2 residues

	P118	L119	P121	A124	
Homo_sapiens (Ubc5)	PLVPEI	ARIYKTDREKYNRIAREWTQKYAM	-----		
Mus_musculus (Ube2d3)	PLVPEI	ARIYKTDREKYNRIAREWTQKYAM	-----		
Xenopus_laevis (Ube2d3)	PLVPEI	AHTYKADREKYNRLAREWTQKYAM	-----		
Arabidopsis_thaliana (Ubc8)	PLVPEI	AHMYKTDRAKYEATARNWTQKYAMG	-----		
Saccharomyces_cerevisiae (Ubc7)	PQDAEVA	QHLYLRDRESFNKTAALWTRLYASETSNGQKGNVEESDLYGIDH			
Bos_taurus (Ube2k)	PQDAVVA	NQYKQNPENFKQTARLWAHVYAGAPVSSPEYTKKIENLCAMGF			
Schizosaccharomyces_pombe (Ubc2)	PLDNDVA	KIWKENEPQAIANAREWTKKYAV	-----		
Drosophila_melanogaster (Ubch10)	PLNAQA	MMWNDQKEYKKYLDAFYEKHKDT	-----		
	*		*	:	:

UFD displacement

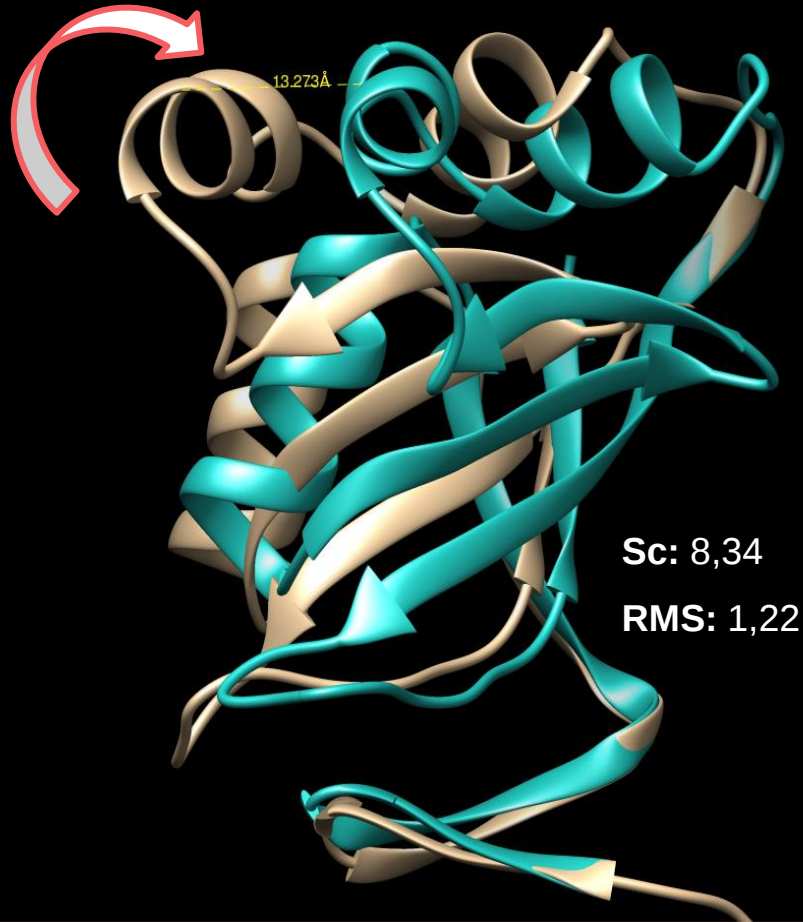
UFD



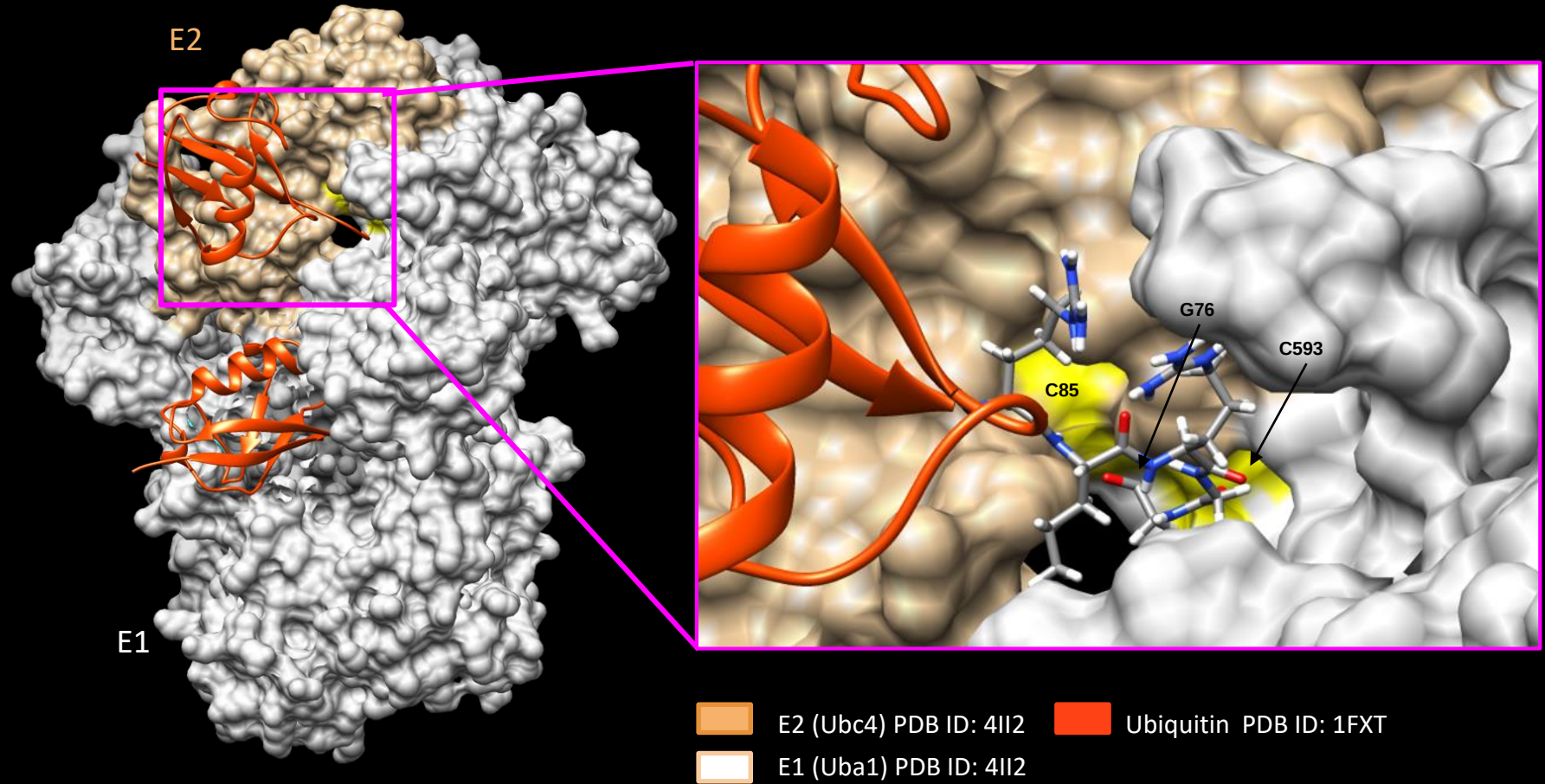
E2

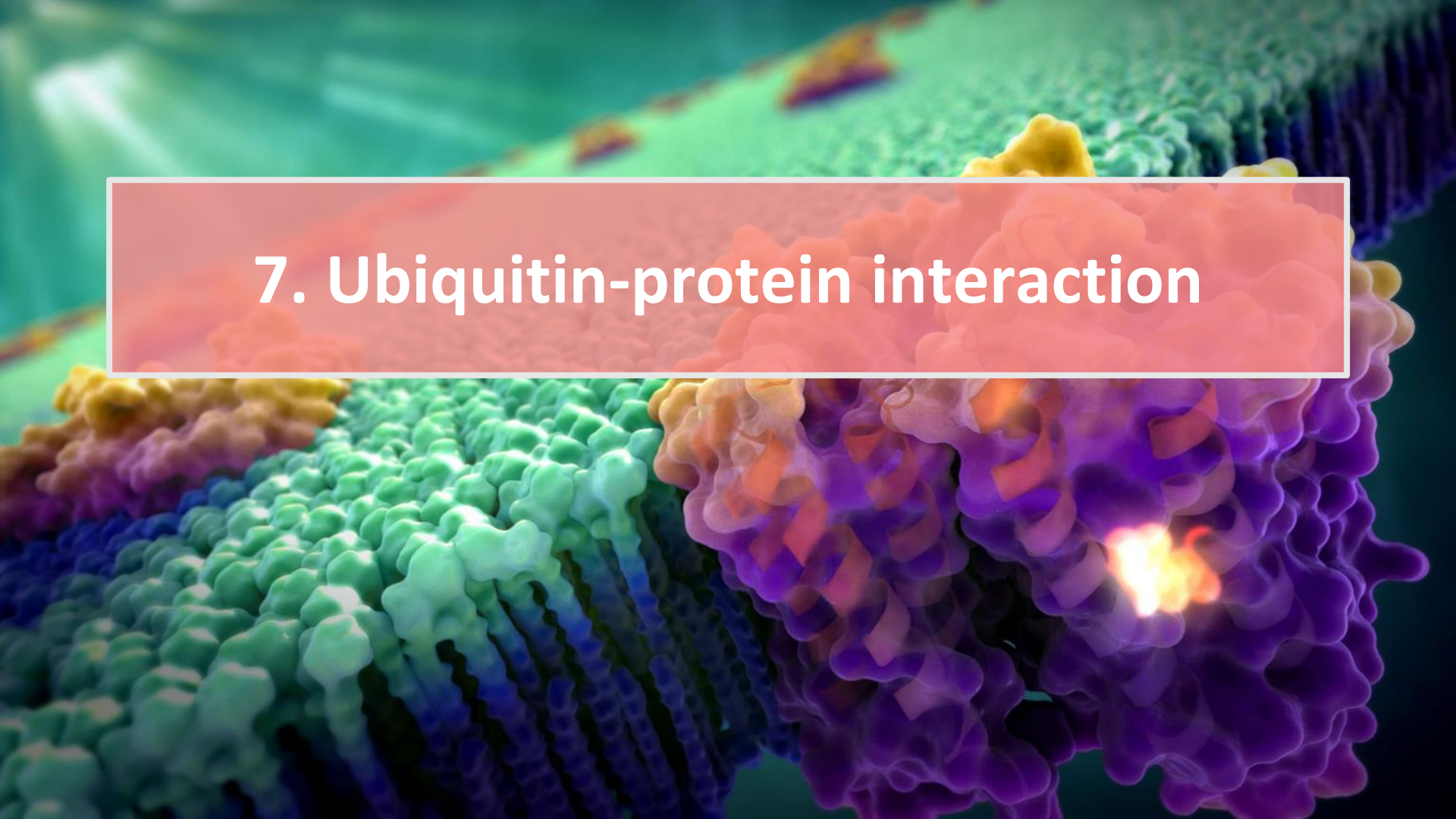
E1 (Uba1) interacting with E2 (Ubc4) PDB ID: 4II2

E1 (Uba1) PDB ID: 4II3



Doubly Loaded Uba1-Ubc4



A 3D molecular model of a protein surface, rendered in a multi-colored, textured style. The surface is composed of various colored regions: green, yellow, orange, and purple. A specific area on the right side, colored purple, is highlighted with a bright, glowing orange and yellow light, indicating a site of interest or interaction. A semi-transparent red rectangular box is overlaid on the image, containing the section title.

7. Ubiquitin-protein interaction

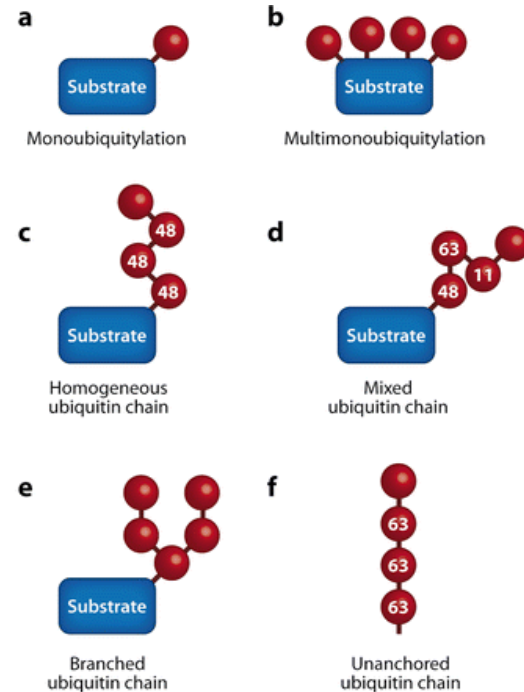
Protein ubiquitination

Residues:

- **Lys** → isopeptide bond
- **Cys** → thioester bond
- **Ser/Thr** → ester bond
- **N-terminal** → peptide bond

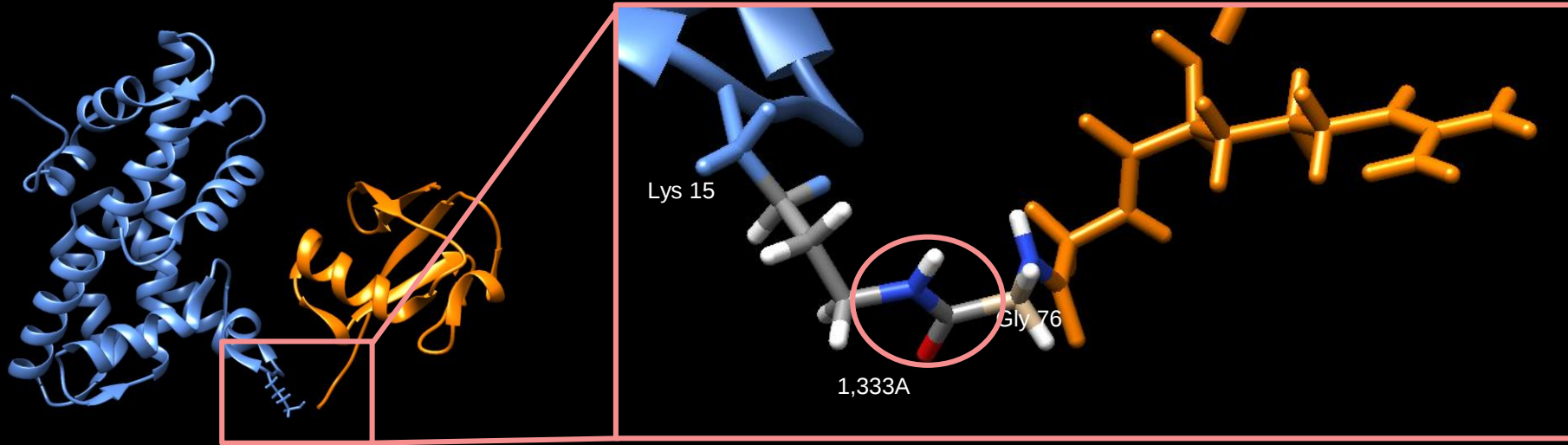
Outcome:

- **N-terminal:** proteasome degradation
- Depends on Ubiquitin chain



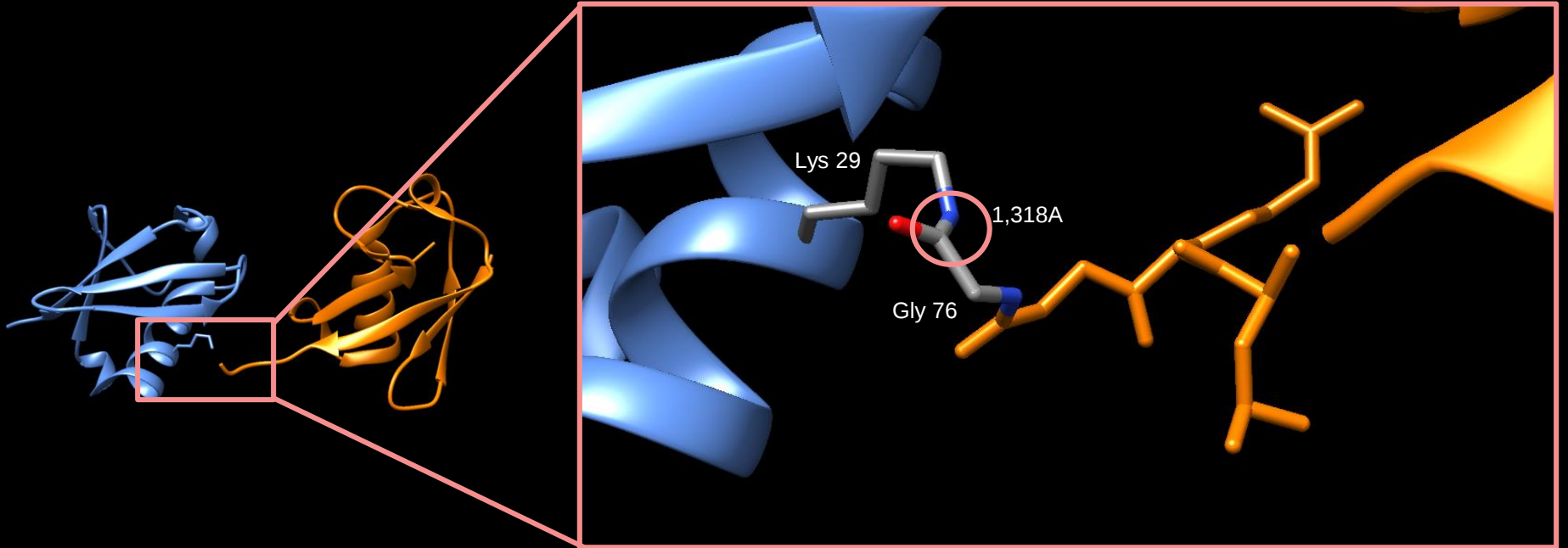
Komander D, Rape M. The Ubiquitin Code. Annual Review of Biochemistry. 2012;81(1):203-229.

H2A Lys15 ubiquitylation



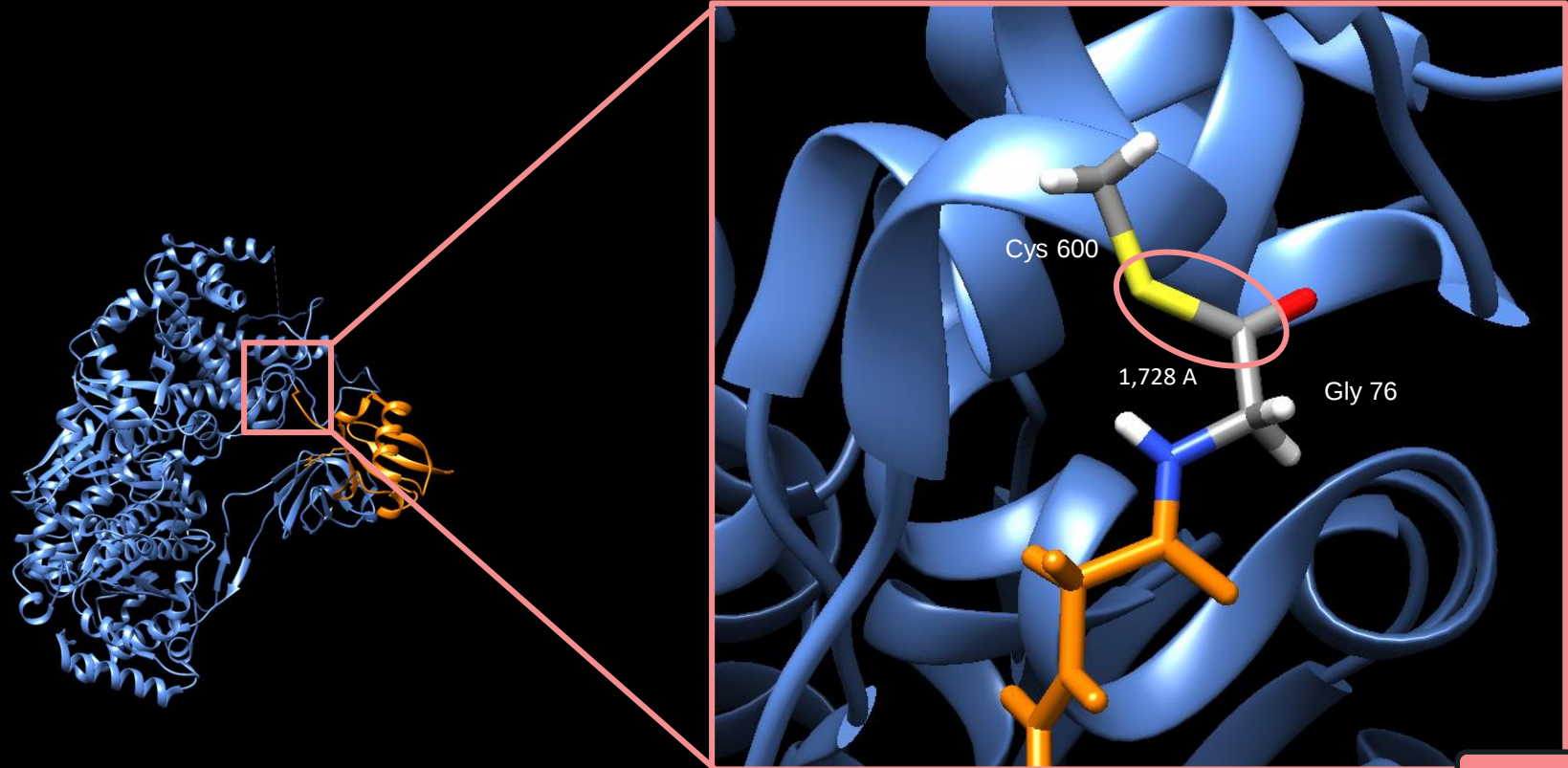
PDB ID: 5VEY

Lys29 ubiquitin chain



PDB ID: 4S22

UBA1 Cys600 ubiquitination



PDB ID: 4NNJ

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8. Schäfer A, Kuhn M, Schindelin H. Structure of the ubiquitin-activating enzyme loaded with two ubiquitin molecules. *Acta Crystallographica Section D Biological Crystallography*. 2014;70(5):1311-1320.
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10. Varshavsky A. The Ubiquitin System, an Immense Realm. *Annual Review of Biochemistry*. 2012;81(1):167-176.
11. Vijay-kumar S, Bugg C, Cook W. Structure of ubiquitin refined at 1.8 Å resolution. *Journal of Molecular Biology*. 1987;194(3):531-544.

PEM questions

1) Choose the correct answer:

- a. **Ubiquitin is not present in prokaryotes.**
- b. Ubiquitination only participates in protein degradation.
- c. There is a larger number of E1s than E2s.
- d. Ubiquitin is encoded for only one gene.
- e. Ubiquitin is bonded to target proteins through its catalytic cysteine.

2) Choose the incorrect answer:

- a. E1 catalytic cysteine is highly conserved.
- b. **Human E1 is encoded for more than 20 genes.**
- c. E1 is formed by 6 domains.
- d. UFD domain has a similar structure to Ubiquitin.
- e. All of them.

3) Which is not a function of Ubiquitin Activating Enzyme E1?

- a. Binds Ubiquitin.
- b. Activates Ubiquitin.
- c. Interacts with E2.
- d. Degrades Ubiquitin.**
- e. All of them.

4) Which is a domain of the Ubiquitin Activating Enzyme E1?

- a. IAD
- b. AAD
- c. UFD
- d. FCCH
- e. All of them**

5) Mark the correct answer:

- a. Ubiquitin's N-terminal is the most flexible part of the protein.
- b. Ubiquitin has thermic but not chemical stability.
- c. The human ubiquitin's Gly76 is hidden and needs a phosphorylation to be activated.
- d. Ubiquitin B1/B2 loop contains a G1 B-bulge.**
- e. None of the above is correct.

6) Mark the incorrect one:

- a. Ubiquitin is a really stable protein due to hydrophobic interactions.**
- b. Two of the lysines of ubiquitin form intramolecular salt bridges.
- c. Human ubiquitin is encoded in 4 different genes present in different chromosomes.
- d. The ubiquitin's N-terminal Met is hidden to protect the protein of degradation.
- e. E1's UFD suffers a conformational change in order to facilitate the transesterification reaction.

7) Select the correct answer

- a) Ubiquitin activating enzymes must suffer conformational changes for doing their functions
- b) The non-covalent interactions between UBA1(E1 enzyme) and Ubiquitin are related to the adenylation process
- c) **Both of them are correct**
- d) The catalytic cysteine of UBA (E1 enzyme) is not conserved among plants, because of its different function.
- e) all of them are correct

8) Choose the incorrect sentence about Ubiquitin and Ub activating enzymes:

- a) Ubiquitin sequence is conserved among eukarya
- b) Ubiquitin structure is conserved among eukarya
- c) **There is a catalytic cysteine in each cysteine catalytic half domain (in UBA1 enzyme)**
- d) UBA1 can load 2 ubiquitin at the same time
- e) All of them all correct

9) Which is the most common functional residue ubiquitinated?

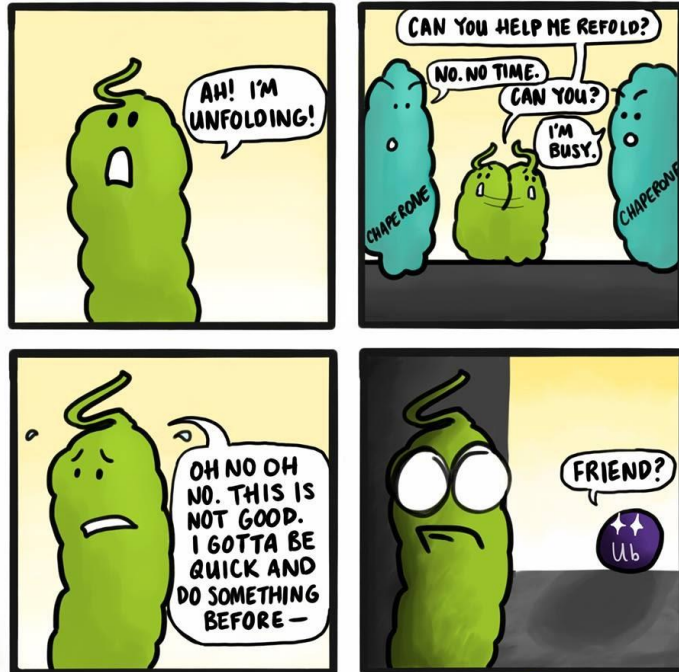
- a. Met
- b. Ser
- c. Glu
- d. Lys**
- e. Pro

10) Which is/are the most flexible regions of ubiquitin?

- a. C-terminal tail**
- b. N-terminal end
- c. Both
- d. Telek box
- e. All of them

THE END

"unfolded protein response"



Cellular Scribbles by Vicky Chou

cellularscribbles

"ubiquitin"



Cellular Scribbles by Vicky Chou

cellularscribbles

Iván Company, Albert Edo, Marc
González, Guillem Murciano and
Alejandra Rodríguez

Ubiquitin

Let's degrade something

Addendum : UBA1 domains

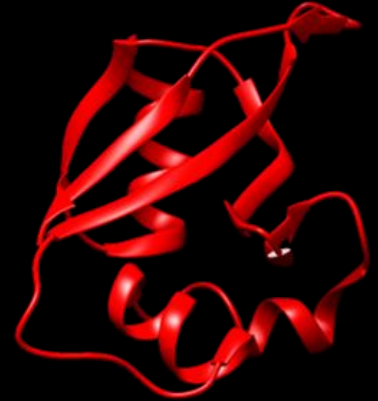
IAD



AAD



UFD



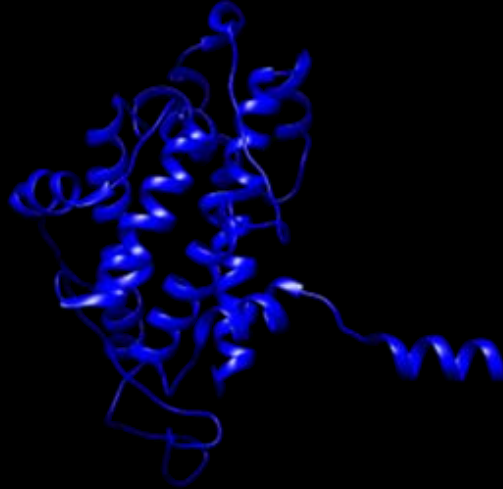
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Addendum : UBA1 domains

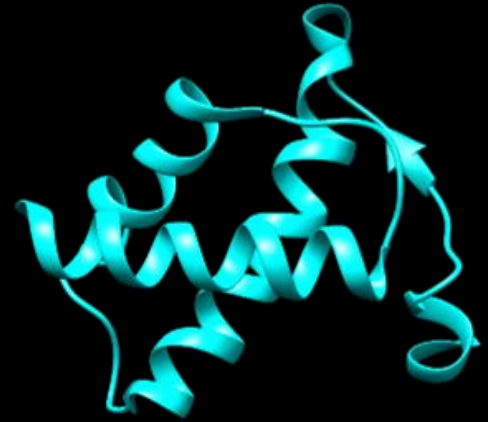
FCCH



SCCH



4HB



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