

Ubiquitin *system*

Yichao Hong, Carla Sandi, Laia Pareras

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- Classification
- Structure
- Conservation

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- Classification
- Structure
- Conservation

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1. Ubiquitylation

The process of attaching ubiquitin to a substrate protein

Ubiquitin (Ub):

- Polypeptide of 76 amino acid (8,58 kDa)
- One of the most conserved of all eukaryotic protein

Three enzymes is required for ubiquitylation

- E1 **activating** enzyme
- E2 **conjugating** enzyme
- E3 **ligating** enzyme

Isopeptide bond

- ϵ -amino group of substrate **Lys residue**
- C-terminal **glycine residue**

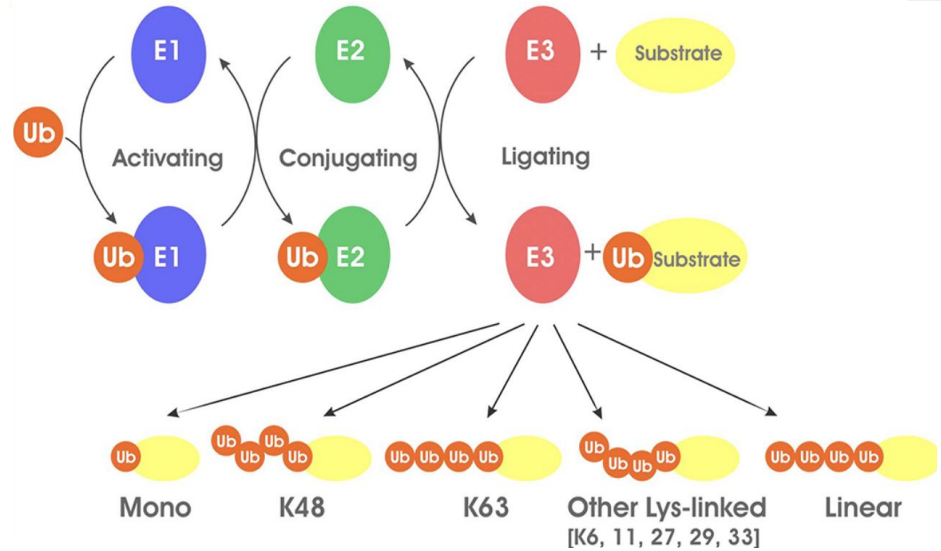


Figure 1. Dikic I, Wakatsuki S, Walters KJ. Ubiquitin binding domains - from structures to functions. Nat Rev Mol Cell Biol. 2009 Oct;10(10):659.

Ubiquitin (Ub):

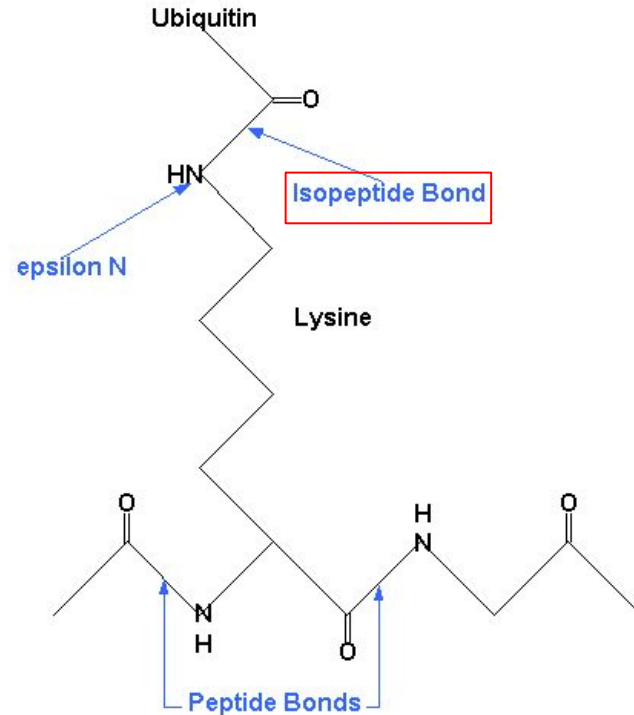
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- One of the most conserved of all eukaryotic protein

Three enzymes is required for ubiquitylation

- E1 **activating** enzyme
- E2 **conjugating** enzyme
- E3 **ligating** enzyme

Isopeptide bond

- ϵ -amino group of substrate **Lys** residue
- C-terminal **glycine** residue



Mono-ubiquitylation

VS

Poli-ubiquitylation

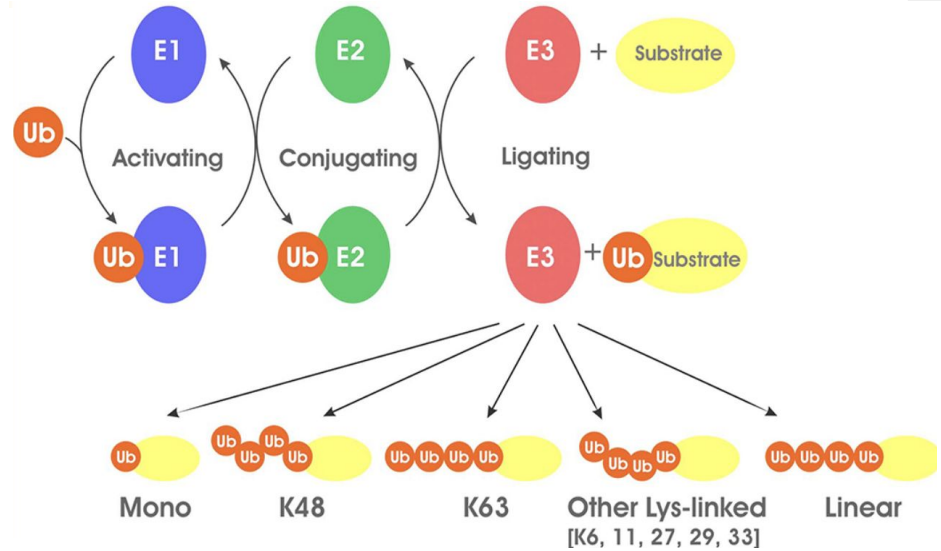


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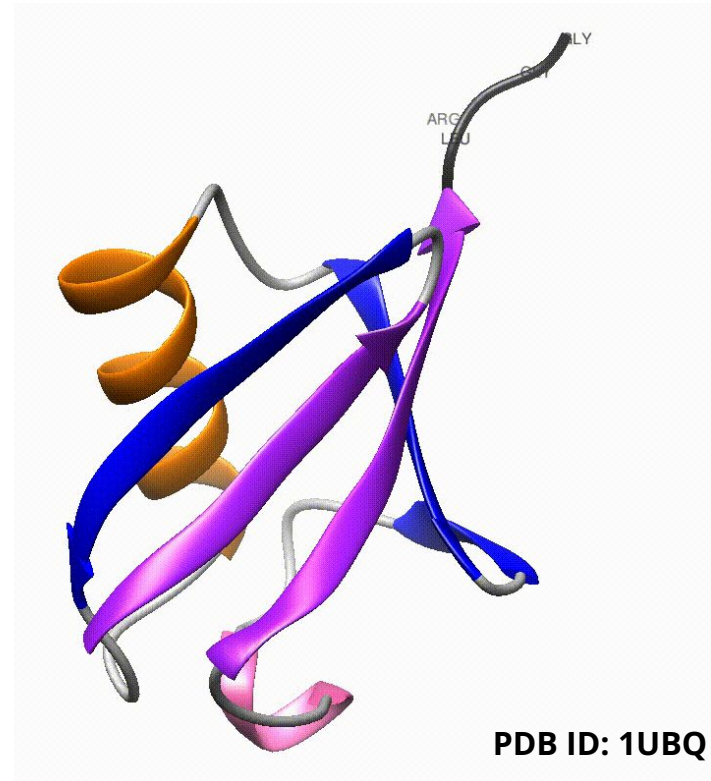


2. Ubiquitin



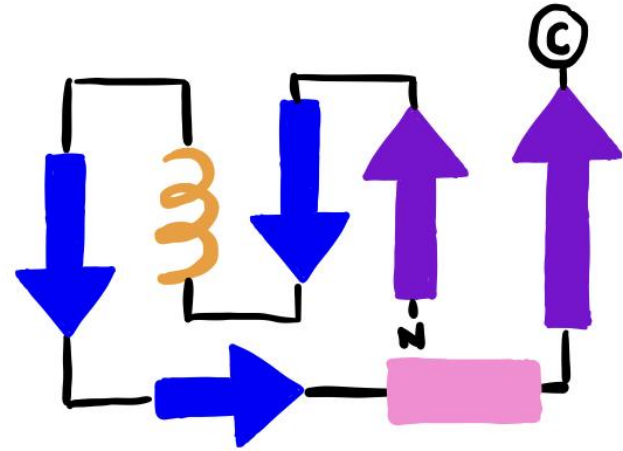
Ubiquitin SCOP Classification

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

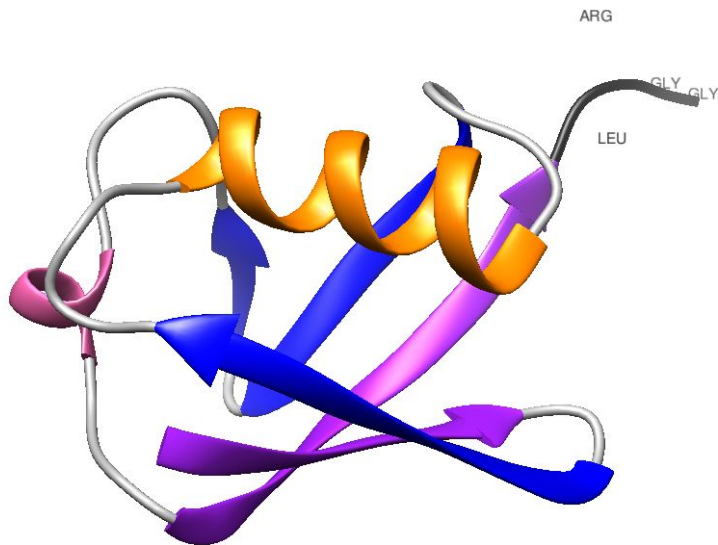


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Ubiquitin Secondary Structure



Mixed β -sheet

- Parallel
- Antiparallel

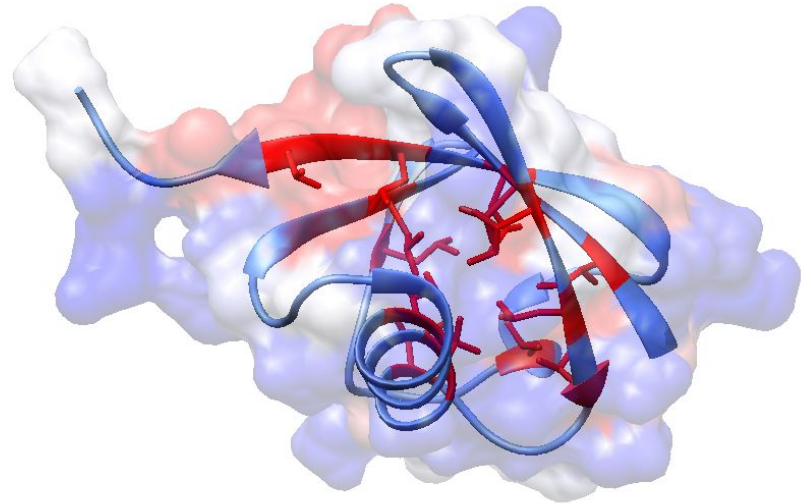
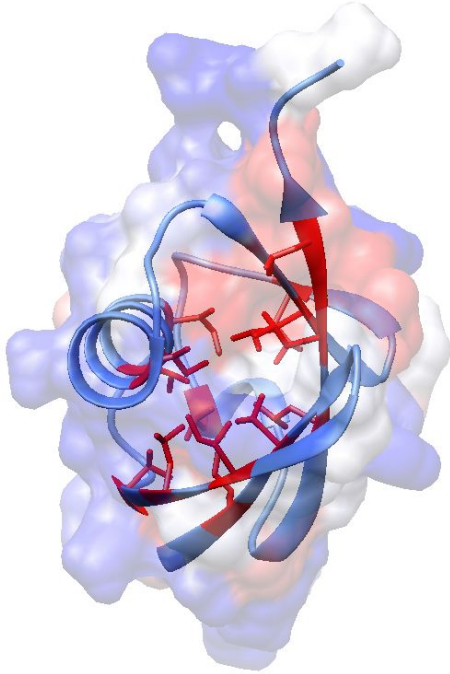
3.5 α -helix

3₁₀-helix

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRGG

C-terminal

Ubiquitin hydrophobic core

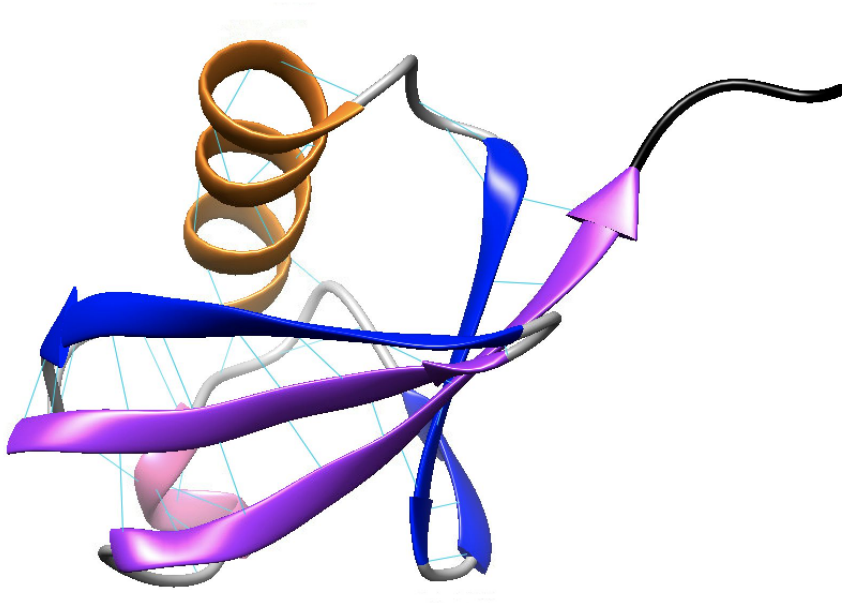


MQIFVKTLTGKTITLEVPSDTIENVKAKIQDKEGIPPDQQRLLFAGKQLEDGRTLSDYNIQKESTLHLVLLRGG

α -helix

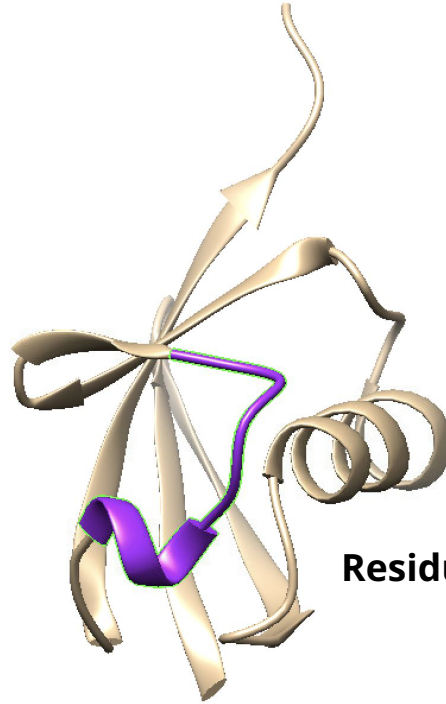
3_{10} -helix

Ubiquitin Hbonds



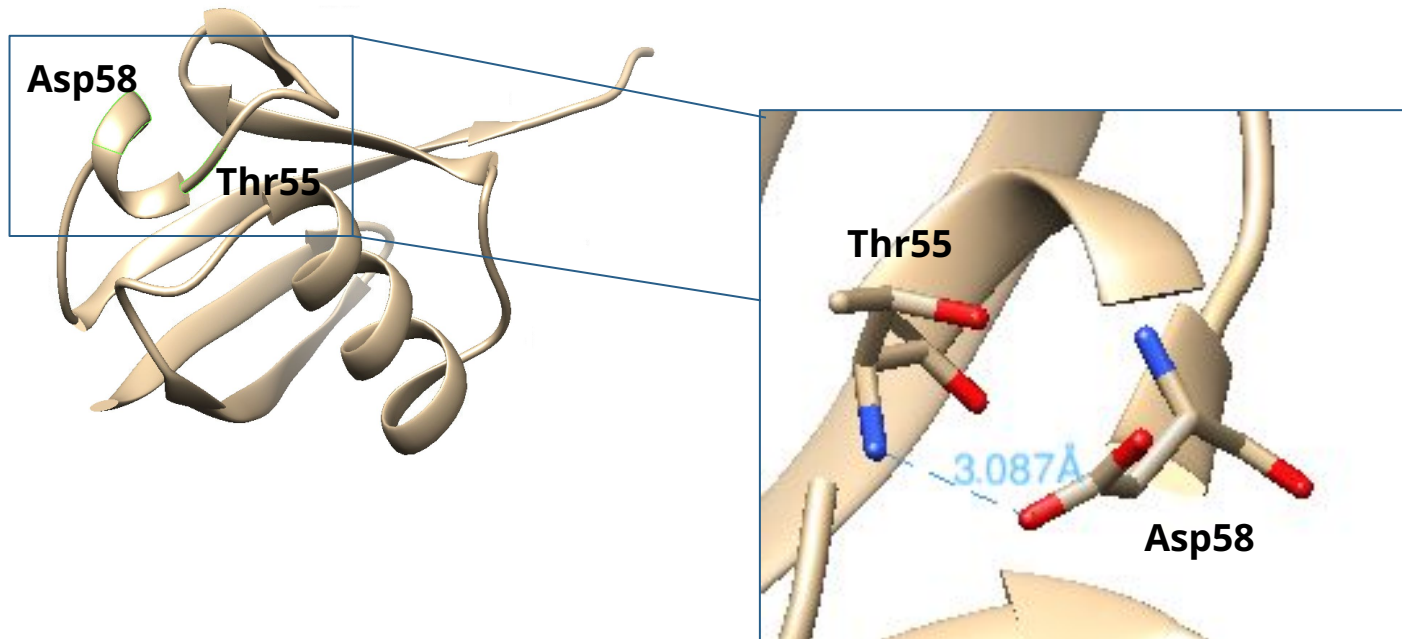
- **Rigid structure**
- **Thermal stability**
- **Resistance to a broad pH range**

Ubiquitin Hbonds

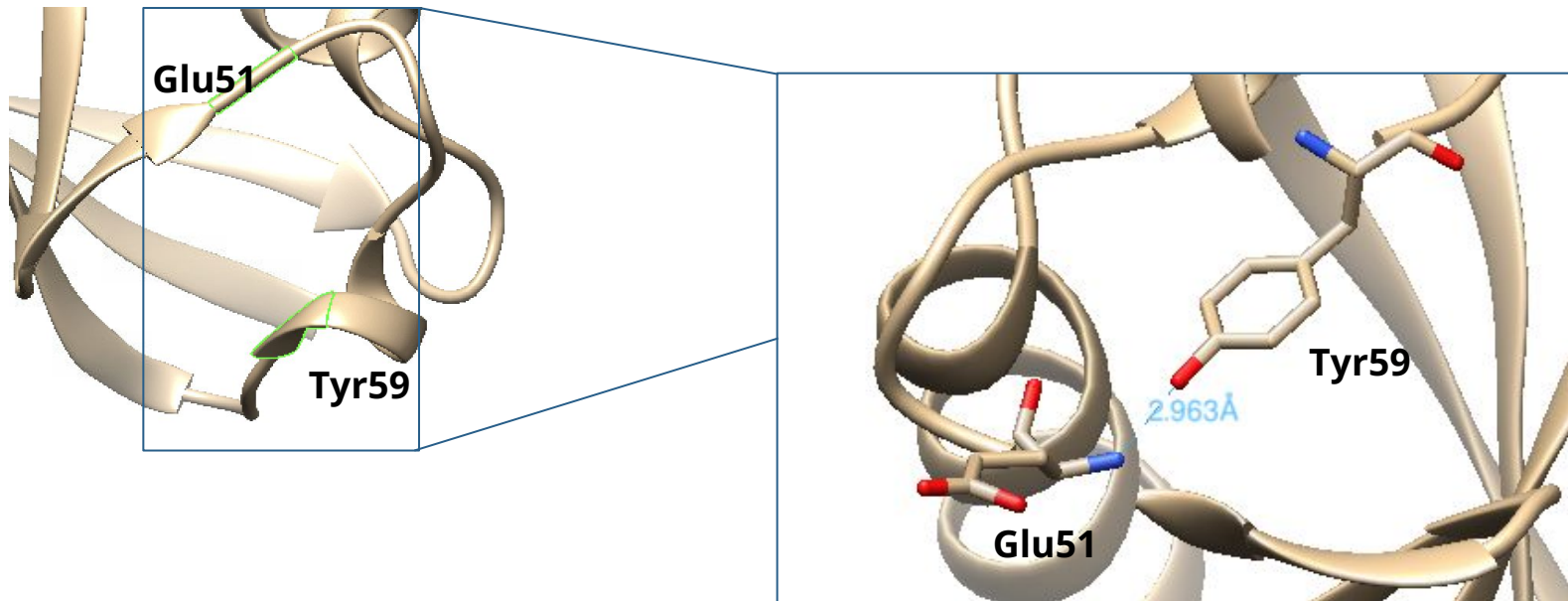


Residues 51-59

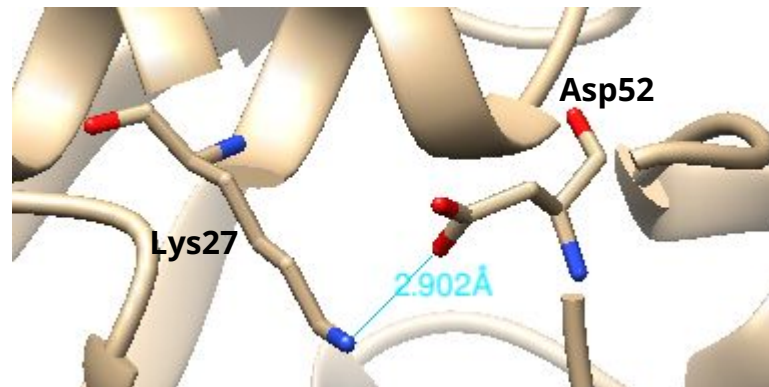
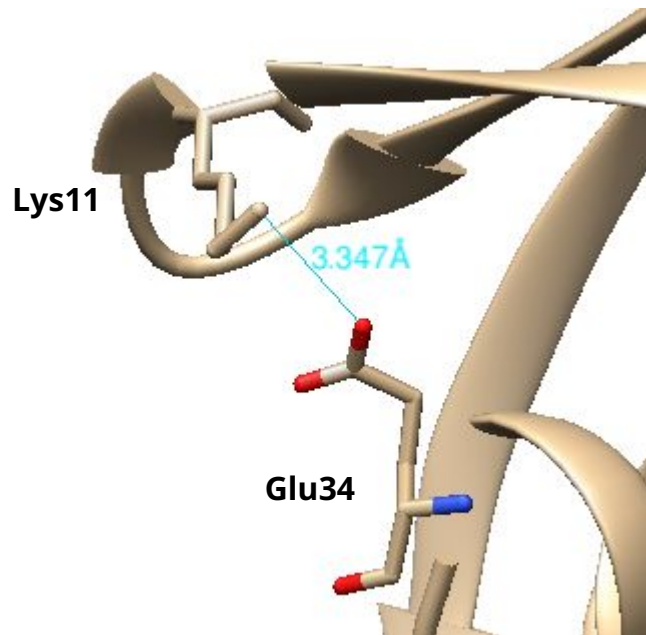
Ubiquitin Hbonds



Ubiquitin Hbonds



Ubiquitin Salt bridges



Ubiquitin Beta-bulges

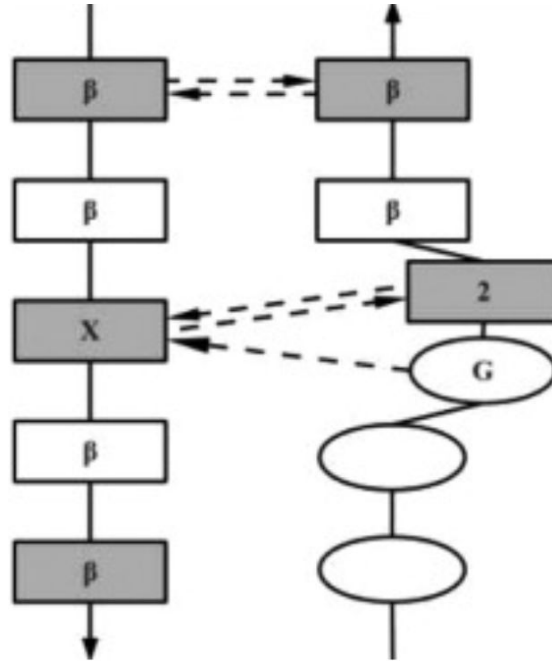
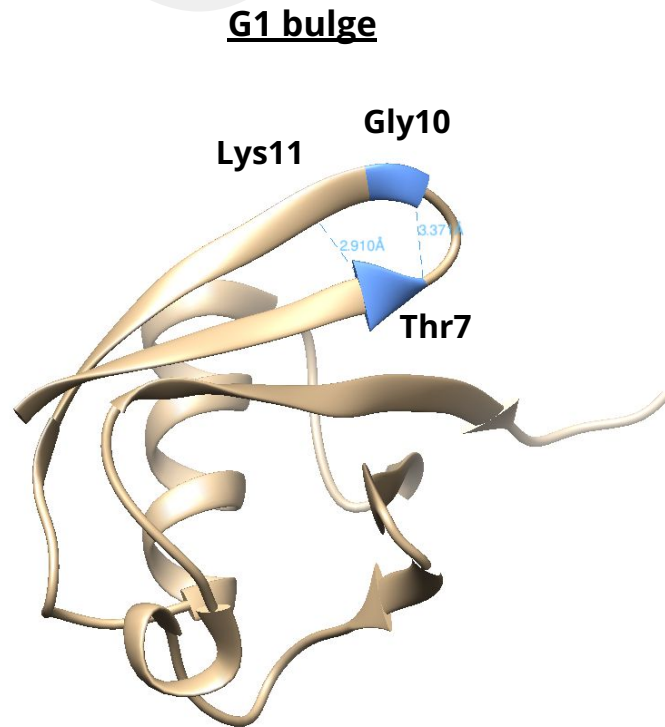
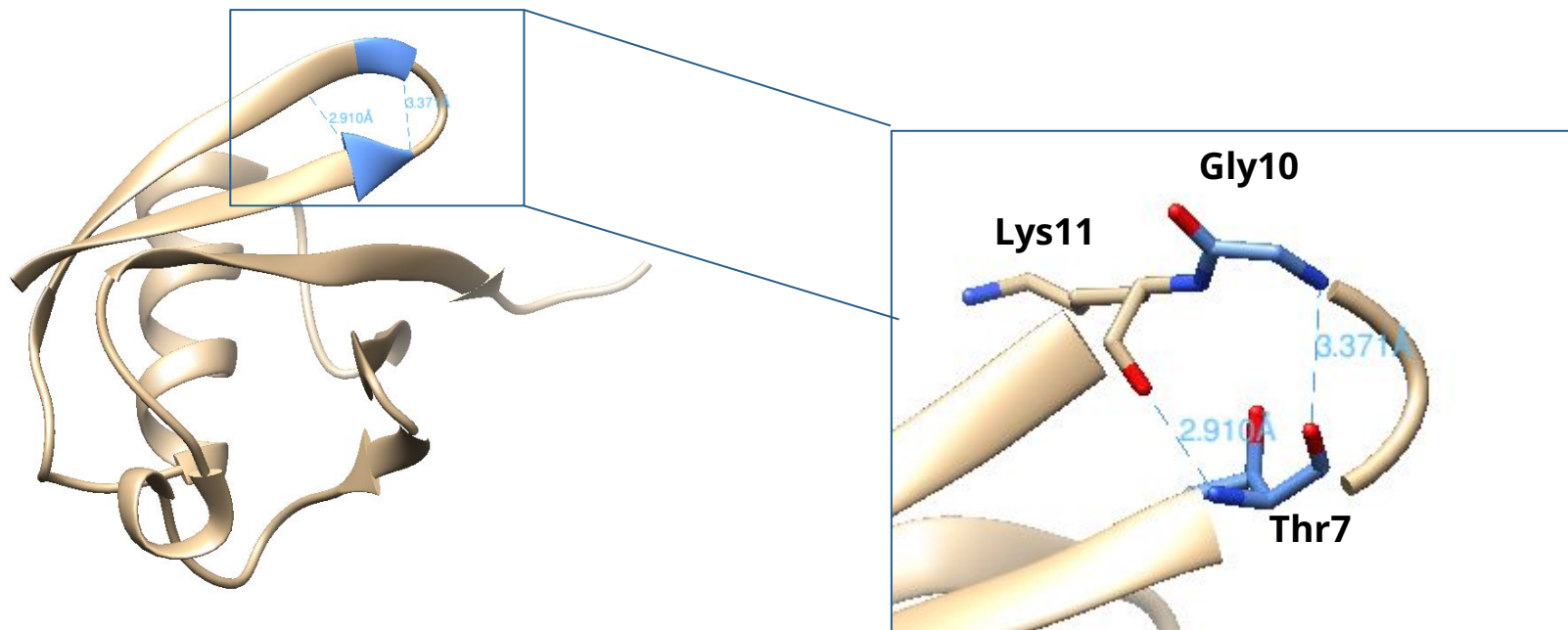


Figure 2. Craveur P, Joseph AP, Rebehmed J, De Brevern AG. β -Bulges: Extensive structural analyses of β -sheets irregularities. *Protein Sci.* 2013 Oct;22(10):1366.

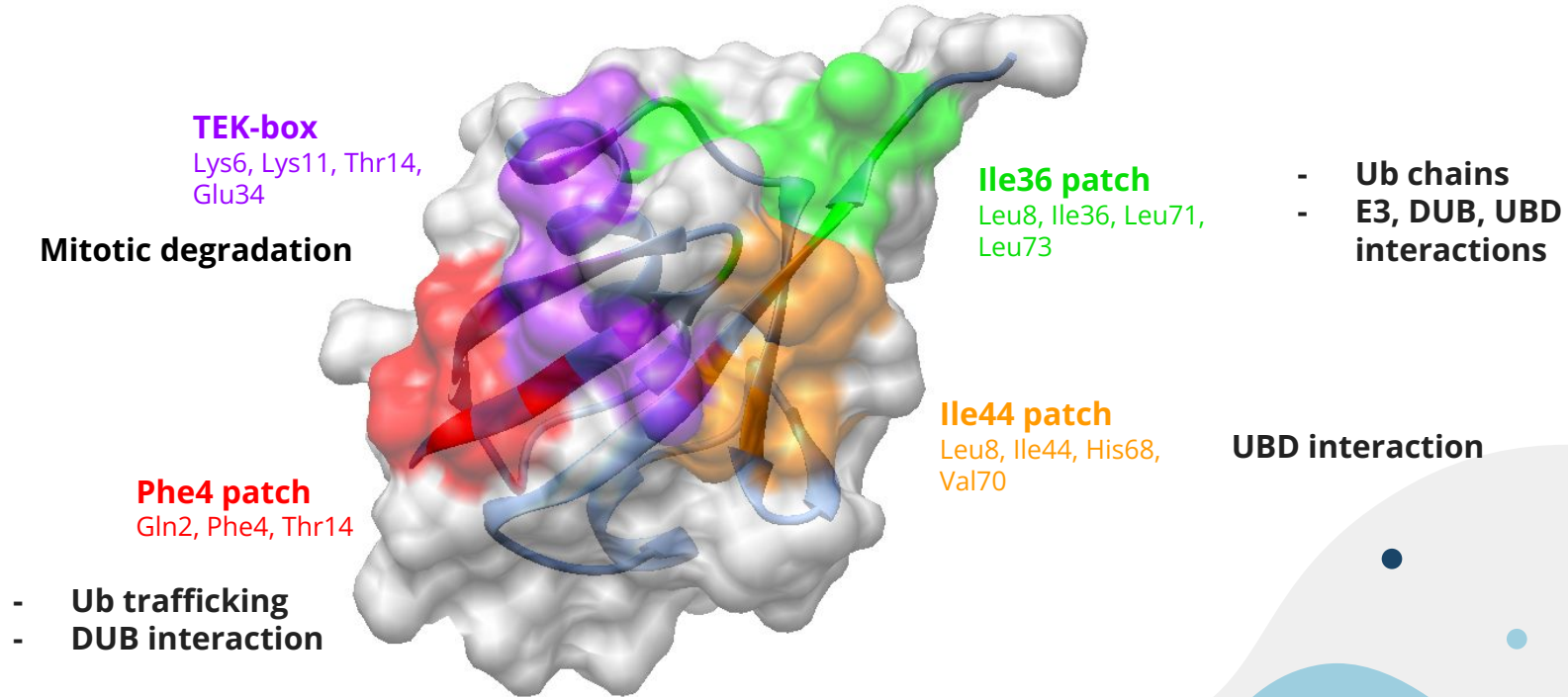
Ubiquitin Beta-bulges



Ubiquitin Beta-bulges

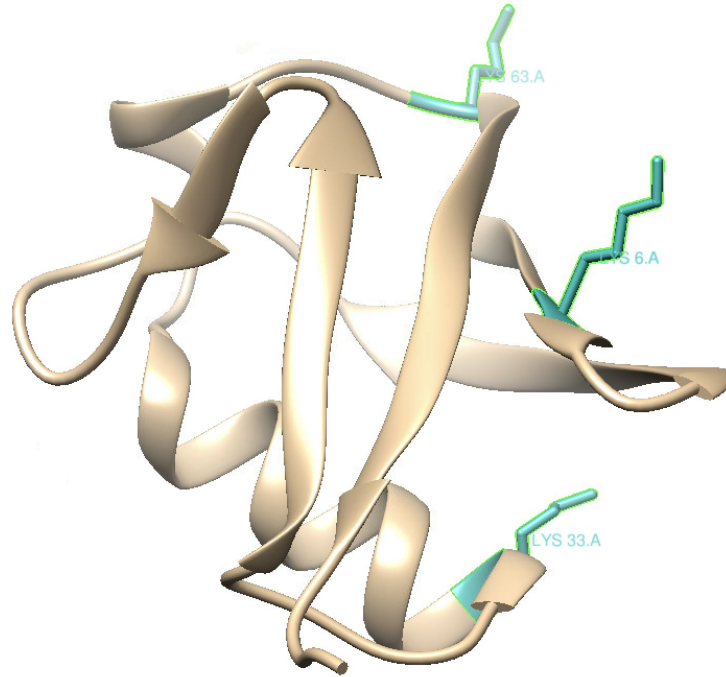


Ubiquitin Patches



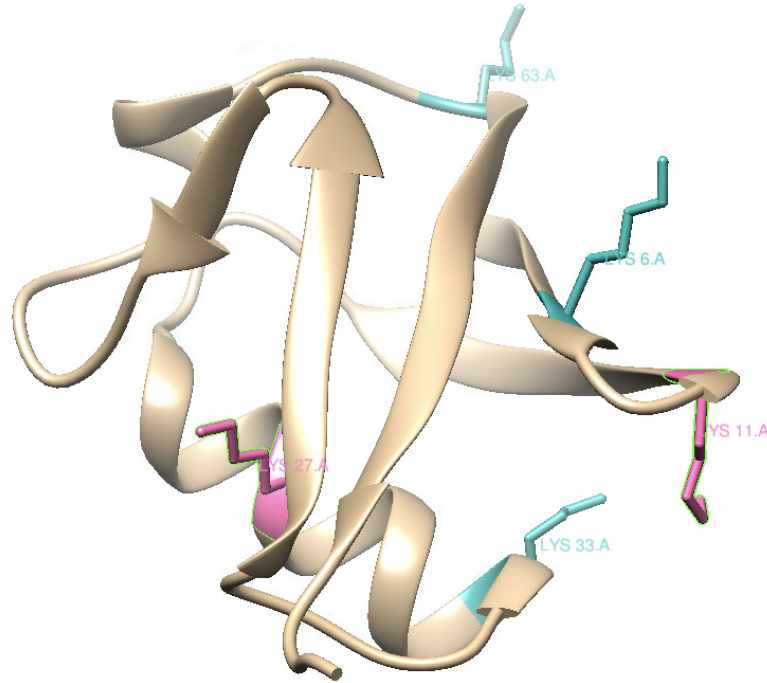
Ubiquitin Lysine residues

- **Lys6, 33, 63**
 - On the surface
 - No intramolecular contacts
- **Lys11, 27**
 - Salt bridge interactions
- **Lys29, 48**
 - Hydrogen bonds with Glu16 and Ala46



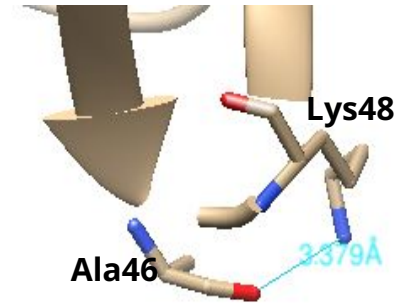
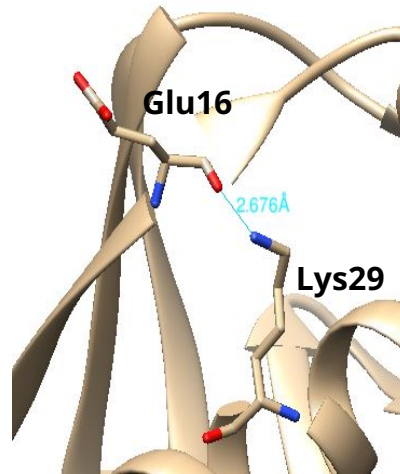
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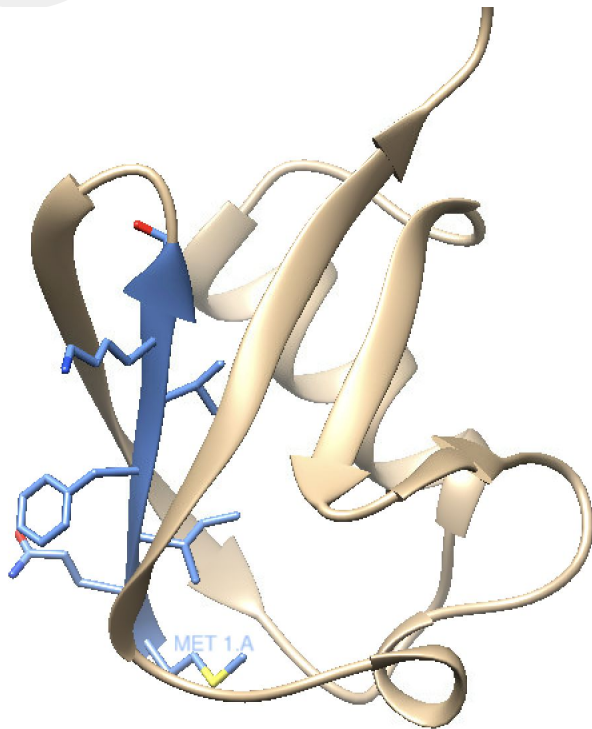


Ubiquitin Lysine residues

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Ubiquitin N-terminus



N-terminus virtually inaccessible



**Preventing the degradation
of ubiquitin**

Ubiquitin - Functions

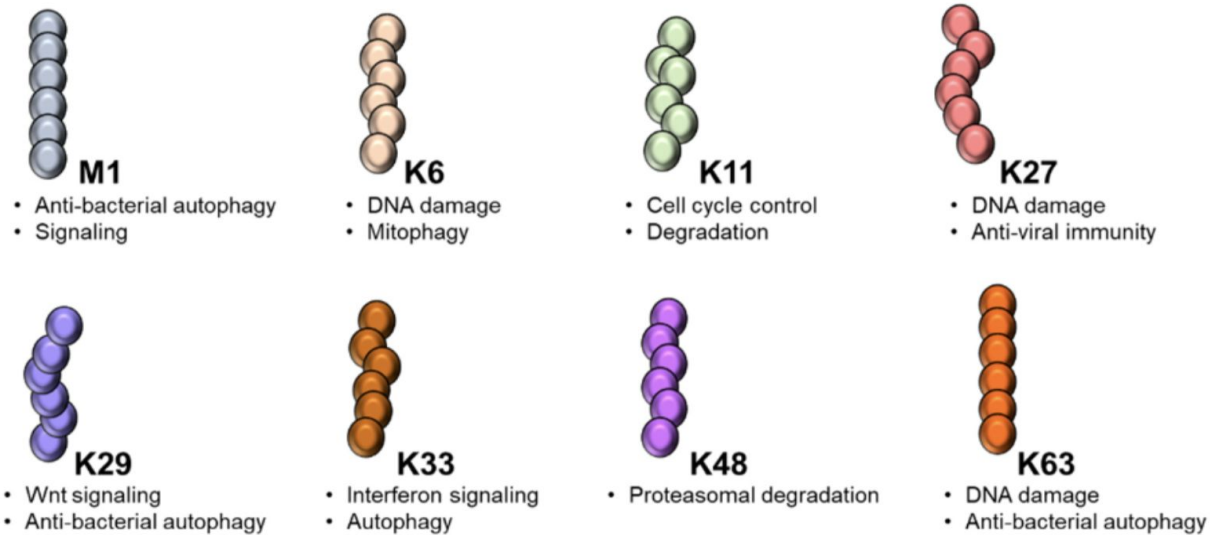
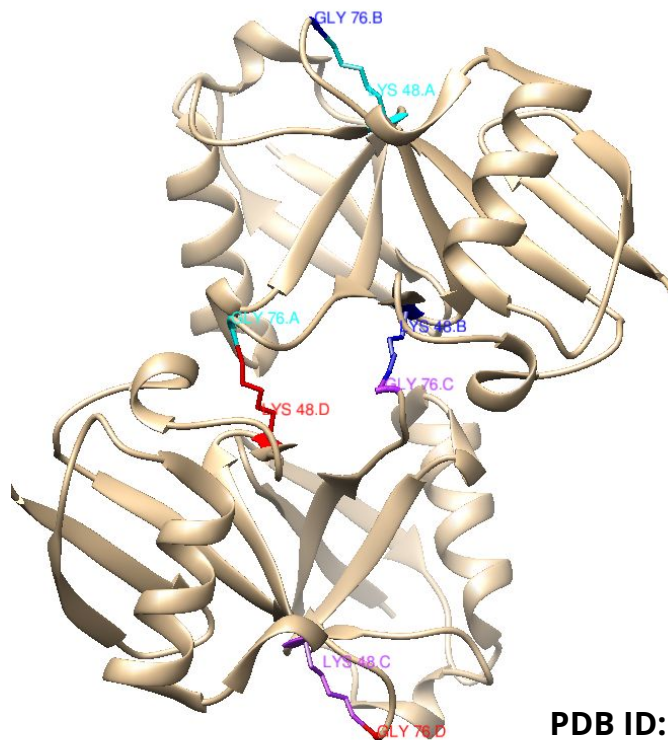


Figure 3. Kaushal K, Ramakrishna S. Deubiquitinating Enzyme-Mediated Signaling Networks in Cancer Stem Cells. *Cancers* 2020, Vol 12, Page 3253. 2020 Nov 4;12(11):3253.

Lys48-linked polyubiquitin chain



PDB ID: 3ALB

- Ub 1
- Ub 2
- Ub 3
- Ub 4

Ubiquitin - Functions

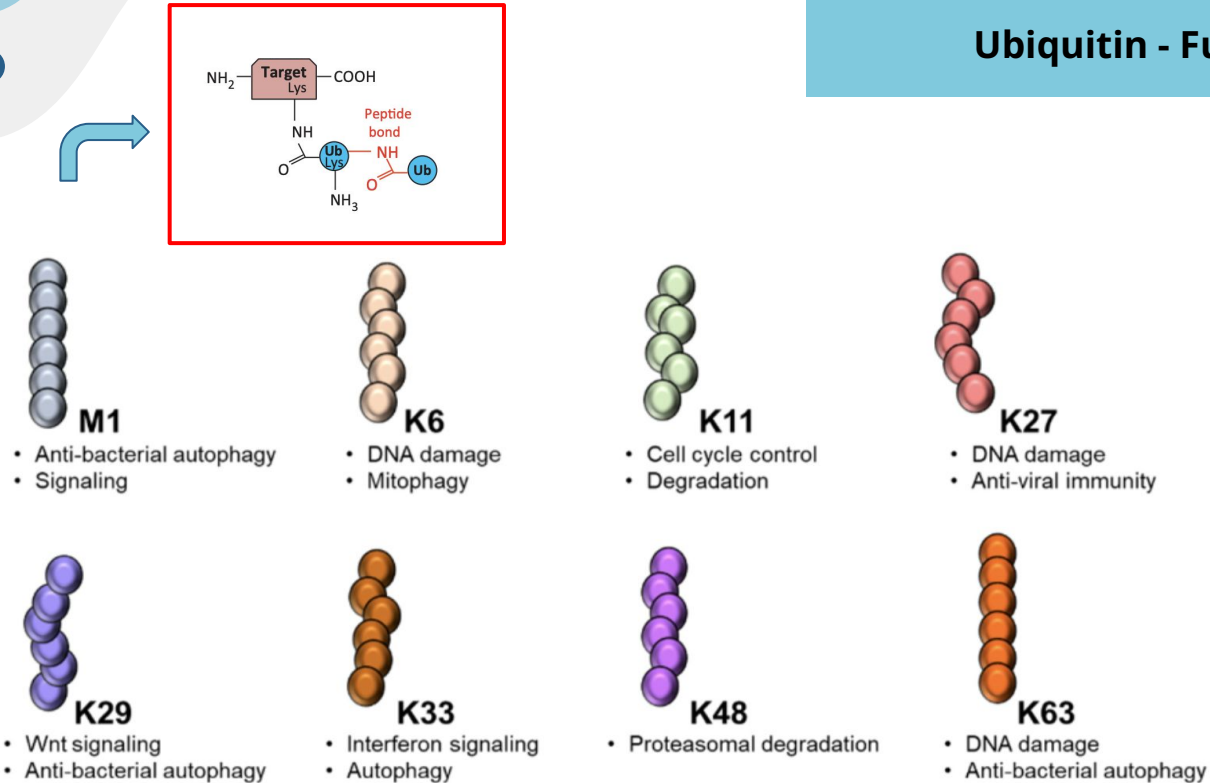


Figure 3. Kaushal K, Ramakrishna S. Deubiquitinating Enzyme-Mediated Signaling Networks in Cancer Stem Cells. Cancers 2020, Vol 12, Page 3253. 2020 Nov 4;12(11):3253.

Ubiquitin Genes

UBB

UBC

Uba52

Rps27a

Polyubiquitin precursor

n° of repeats differ between species

Ubiquitin fused to L40

Ubiquitin fused to S27a

**Almost the same
sequence in all
eukaryotes**

Ubiquitin MSA

H.sapiens
X.tropicalis
D.melanogaster
M.musculus
B.taurus
S.cerevisiae
A.thaliana

MQIFVKLTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG
 MQIFVKLTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG
 MQIFVKLTLTGKTITLEVEPSDTXENVKAKIQDKEGIPPDQQRLLIFAGKQLXXGRTLSDYNIQKESTLHLVLRRLRG
 MQIFVKLTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQRESTLHLVLRRLRG
 MQIFVKLTLTGKTITLEVEPSDTIENVKAKIQEKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG
 MQIFVKLTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG
 MQIFVKLTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLADYNIQKESTLHLVLRRLRG
 ***** .*** .***.***.***** ***** .*****.*****



Ubiquitin MSA

H.sapiens
X.tropicalis
D.melanogaster
M.musculus
B.taurus
S.cerevisiae
A.thaliana

```
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG
MQIFVKTLTGKTITLEVEPSDTXENVKAKIQDKEGIPPDQQRLIFAGKQLXXGRTLSDYNIQKESTLHLVLRRLGG
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQEKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG
MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG
MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRRLGG
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****
```



$P \rightarrow S$
 $E \rightarrow D$

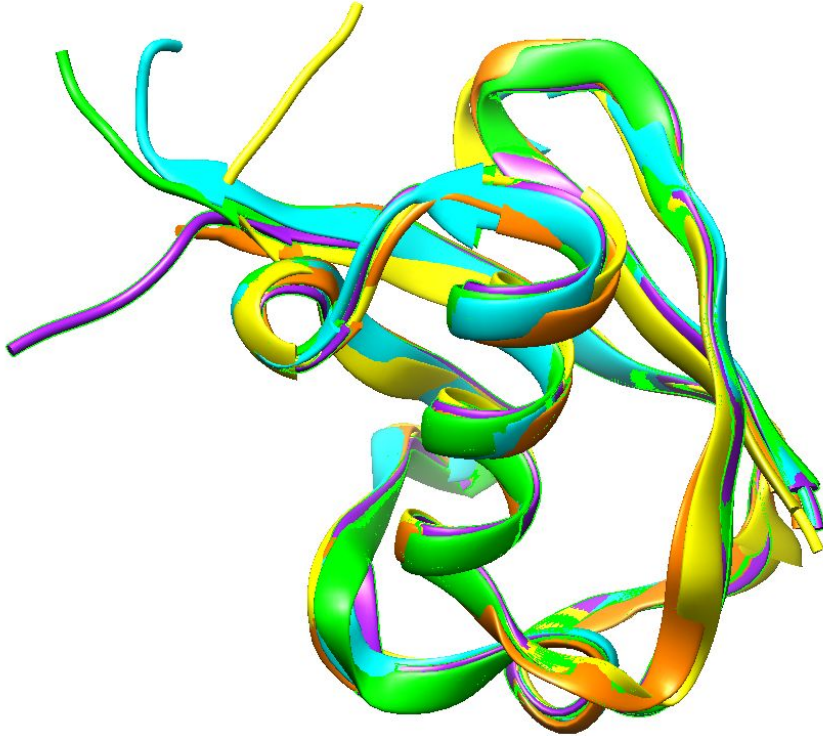


$S \leftrightarrow A$

Residues with the **similar chemical properties**

Residues with the **similar size**

Ubiquitin structural conservation



 **PDB ID: 1WR1** - *S. cerevisiae*

 **PDB ID: 1UBQ** - *H. sapiens*

 **PDB ID: 3M3J** - *B. taurus*

 **PDB ID: 1BT0** - *A. thaliana*

 **PDB ID: 3A9K** - *M. musculus*

SC > 8,57 RMS < 1,01



3. E1 activating enzyme

E1 - Function

E1-Ubiquitin Activating Enzyme (Uba1)

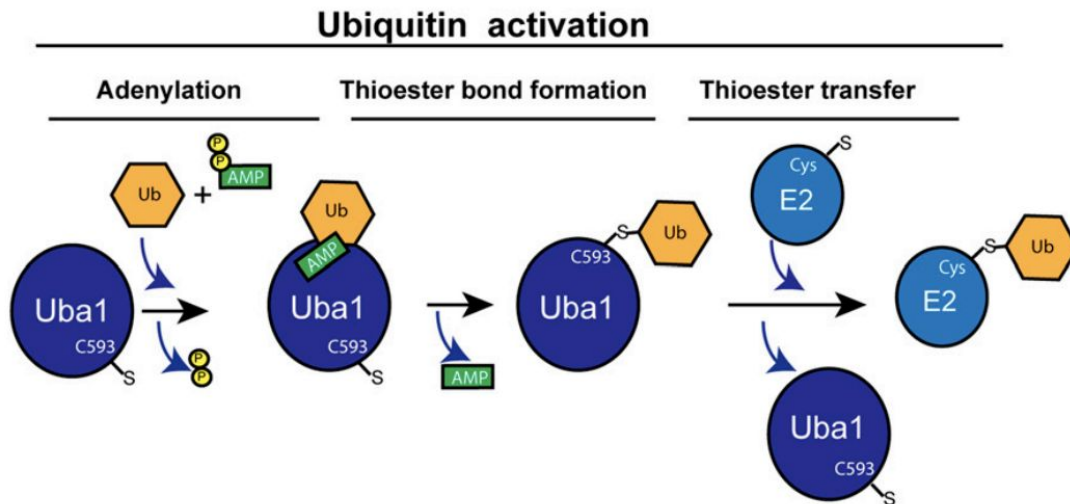


Figure 4. Kumar M, Wiener R. Closing the gap of ubiquitin activation. Proc Natl Acad Sci USA. 2019 Jul 30;116(31):15319-21.

PDB ID: 6DC6

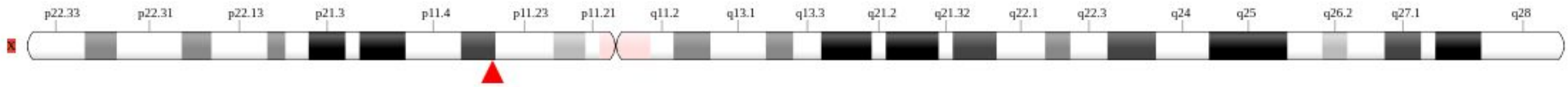
E1 - Classification

SCOP Classification

- **Class:** Alpha and beta proteins (α/β)
- **Superfamily:** Activating enzymes of the ubiquitin-like proteins
- **Family:** Ubiquitin activation enzymes (UBA)
- **Species:** Human (*Homo sapiens*)

E1 - Genome and sequence

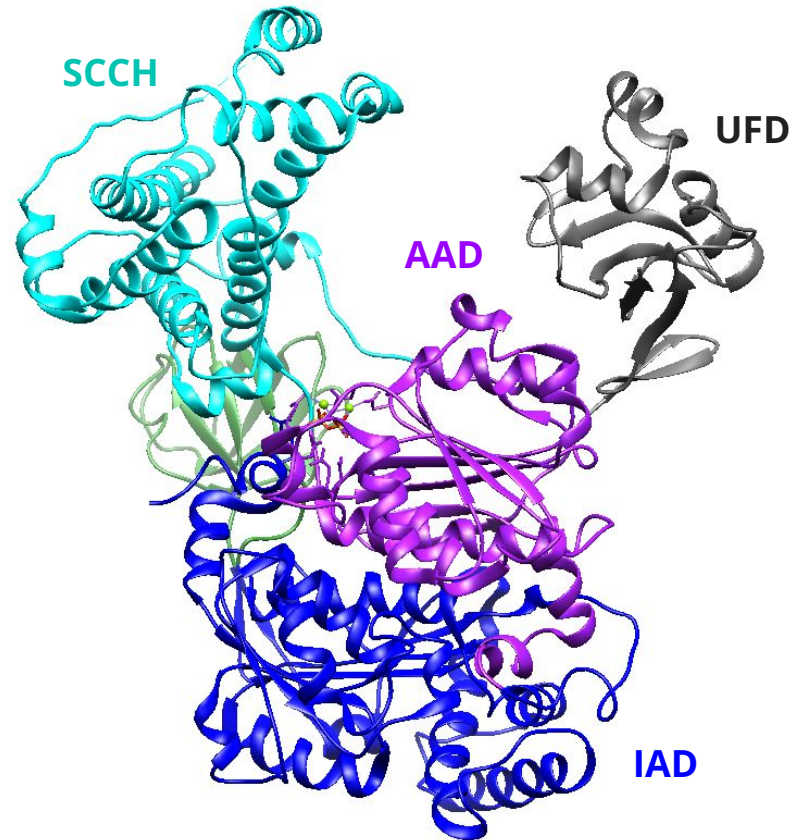
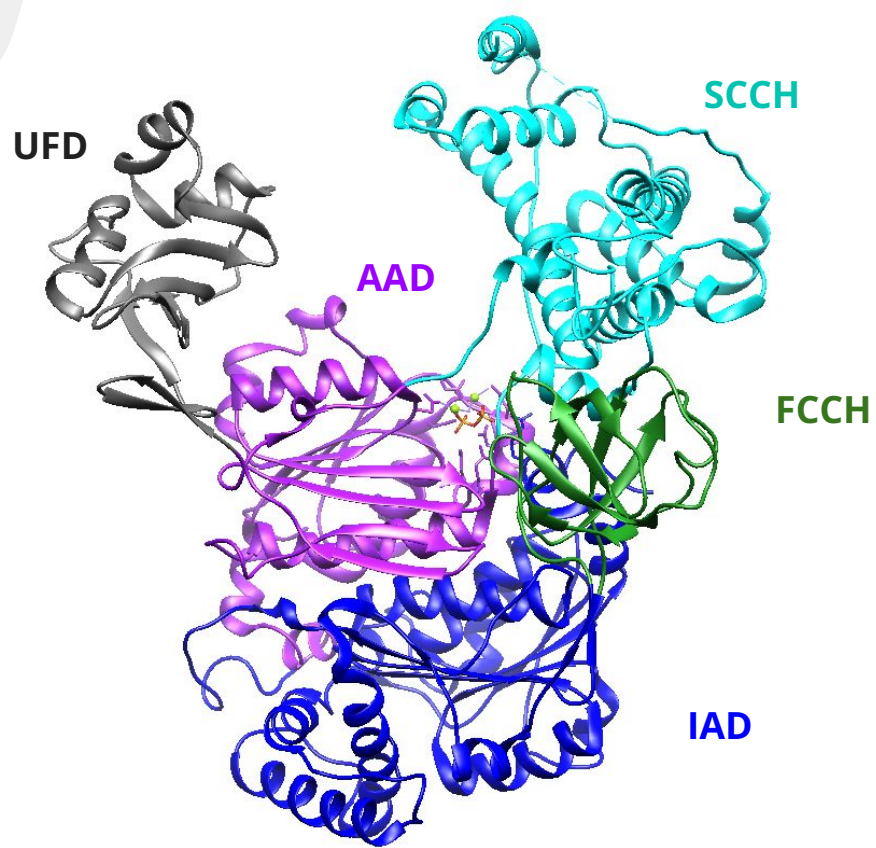
Gene: *uba1* → **Chromosome X:** 27 exons (alternative 1a exon)



Gene encodes a 1.058 amino acid

```
MSSSPLSKKR RVSGPDPKPG SNCSPAQSVL SEVPSVPTNG MAKNGSEADI DEGLYSRQLY VLGHEAMKRL QTSSVLVSGL RGLGVEIAKN
IILGGVKAVT LHDQGTAWA DLSSQFYLR EDIGKNRAEV SQPRLAELNS YVPVTAYTGP LVEDFLSGFQ VVVLNTNPLE DQLRVGEFCH
NRGIKLVVAD TRGLFGQLFC DFGEEMILTD SNGEQPLSAM VSMVTKDNPG VVTCLDEARH GFESGDFVSF SEVQGMVELN GNQPMIEIKVL
GPYTFSIDCT SNFSDYIRGG IVSQVKVPPK ISFKSLVASL AEPDFVVTDF AKFSRPAQLH IGFQALHQFC AQHGRPPRPR NEEDAAELVA
LAQAVNARAL PAVQQNNLDE DLIRKLAYVA AGDLAPINAF IGLLAAQEVN KACSGKFMPI MQWLYFDALE CLPEDKEVLT EDKCLQRQNR
YDGQVAVFGS DLQEKLGKQK YFLVGAGAIG CELLKNFAMI GLGCGEGGEI IVTDMDTIEK SNLNRQFLFR PWDVTKLKSD TAAAVRQMN
PHIRVTSHQN RVGPDTERIY DDDFFQNLGD VANALDNVDA RMYMDRRVCY YRKPLLESgt LGTKGNVQVV IPFLTESYSS SQDPPEKSIP
ICTLKNFPNA IEHTLQWARD EFEGLFKQPA ENVNQYLTDP KFVERTLRLA GTQPLEVLEA VQRSLVLQRP QTWADCVTWA CHHWHTQYSN
NIRQLLNHNP PDQLTSSGAP FWSGPKRCPH PLTFDVNNPL HLDYVMAAAN LFAQTYGLTG SQDRAAVATF LQSVQVPEFT PKSGVKIHVS
DQELQSANAS VDDSRLEELK ATLPSPDKLP GFKMYPIDFE KDDDSNFHMD FIVAASNLR ENYDIPSADR HSKSLIAGKI IPAIATTTAA
VVGVLVCELY KVVQGHRQLD SYKNGFLNLA LPFFGFSEPL AAPRHQYYNQ EWTLWDRFEV QGLQPNGEEM TLKQFLDYFK TEHKLEITML
SQGVSMYLSF FMPPAAKLKER LDQPMTEIVS RVSKRKLGRH VRALVLELCC NDESGEDVEV PYVRYTIR
```

E1 - Domains

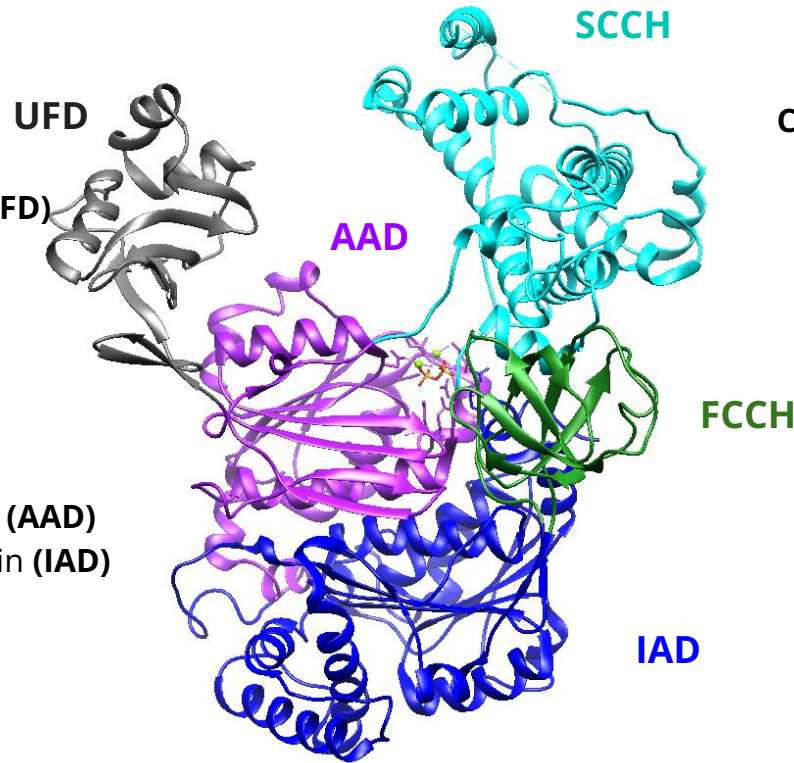


E1 - Domains' functions

Ubiquitin Fold Domain (UFD)

Interacts with the E2

Active Adenylation Domain (**AAD**)
Inactive Adenylation Domain (**IAD**)



Catalytic cysteine domain:

- First catalytic cysteine half-domain (**FCCH**)
- Second catalytic cysteine half-domain (**SCCH**)

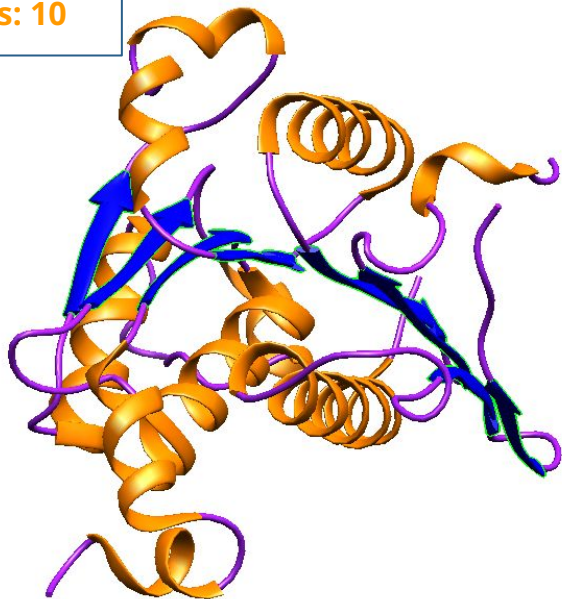
PDB ID: 6DC6

E1 - Relevant domain folds

β-sheet: 8

Helices: 10

AAD

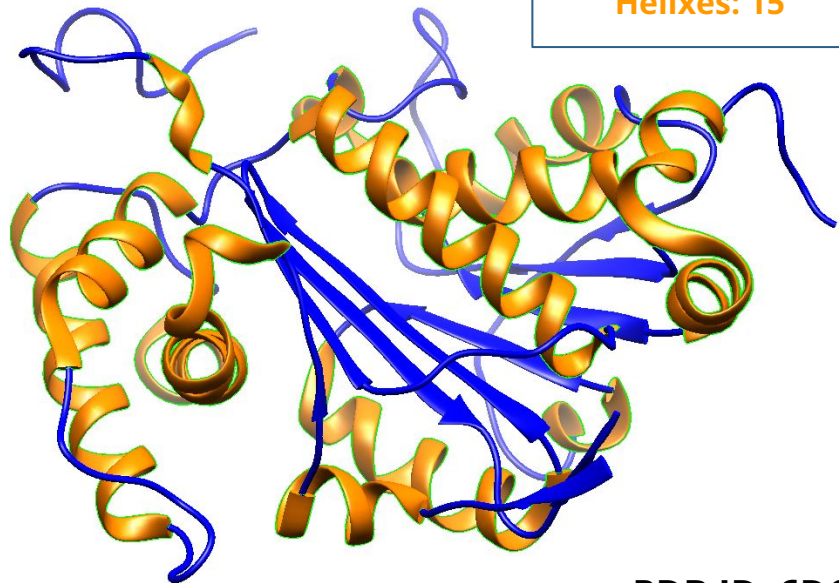


Rossman-like fold

IAD

β-sheet: 9

Helices: 15



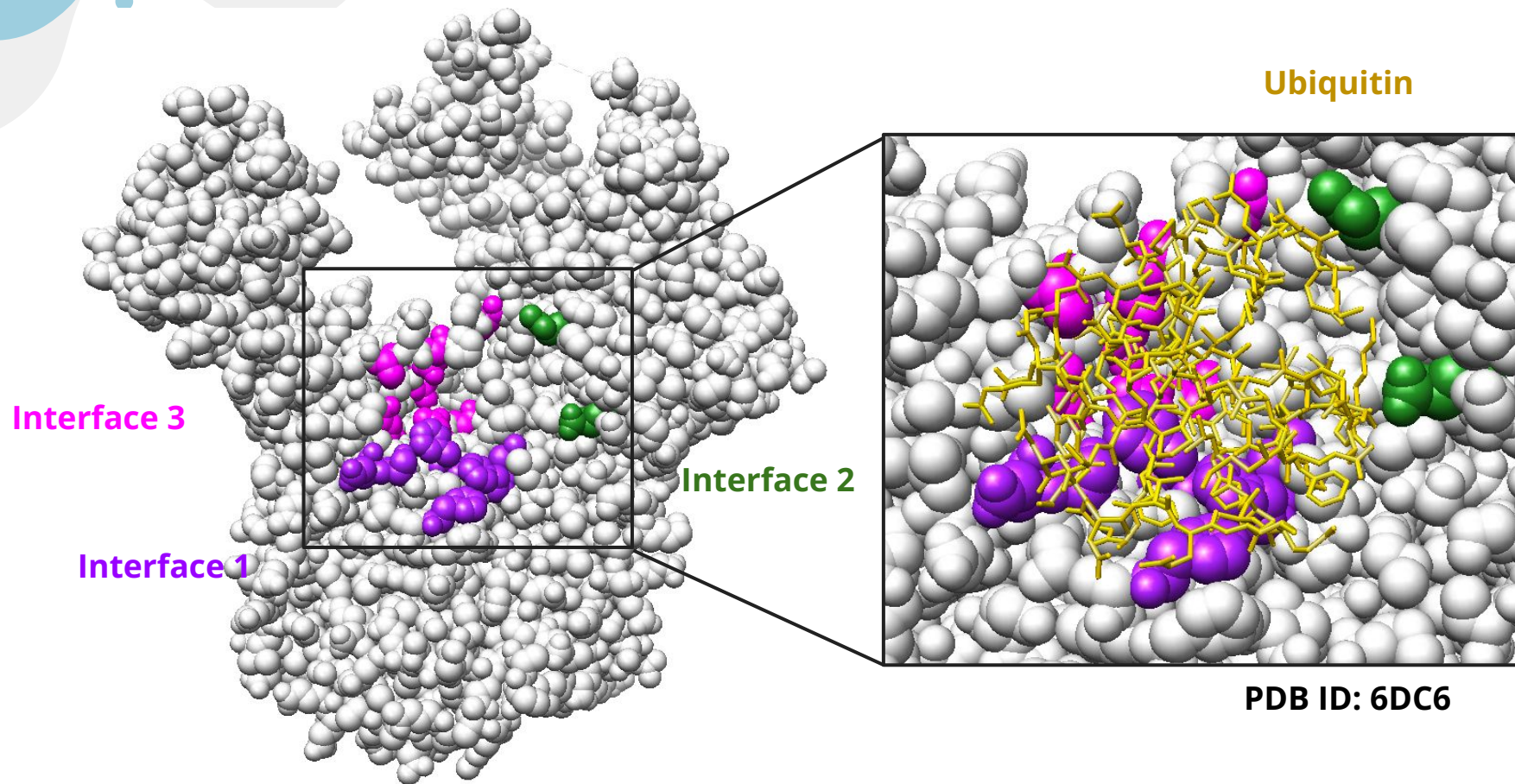
PDB ID: 6DC6



4. Ubiquitin-E1 interaction

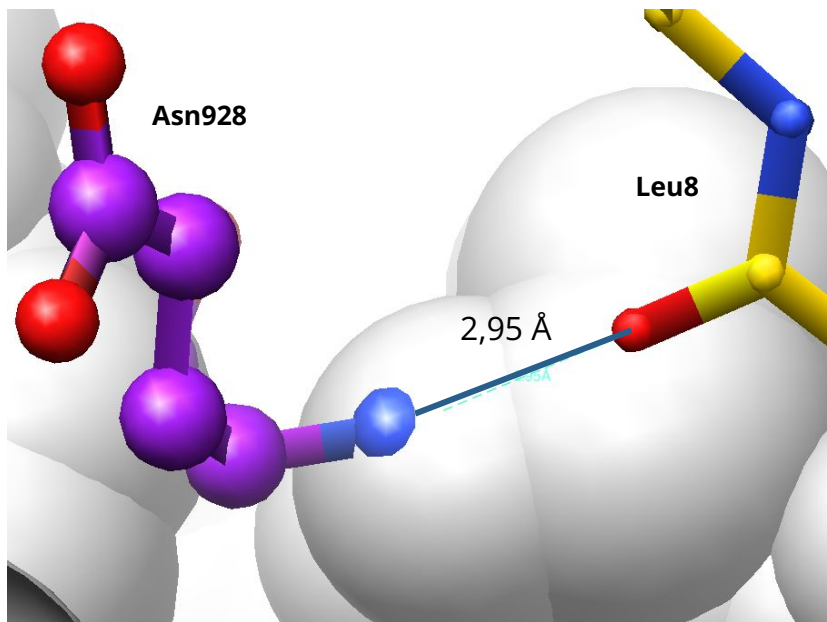


E1- Interfaces

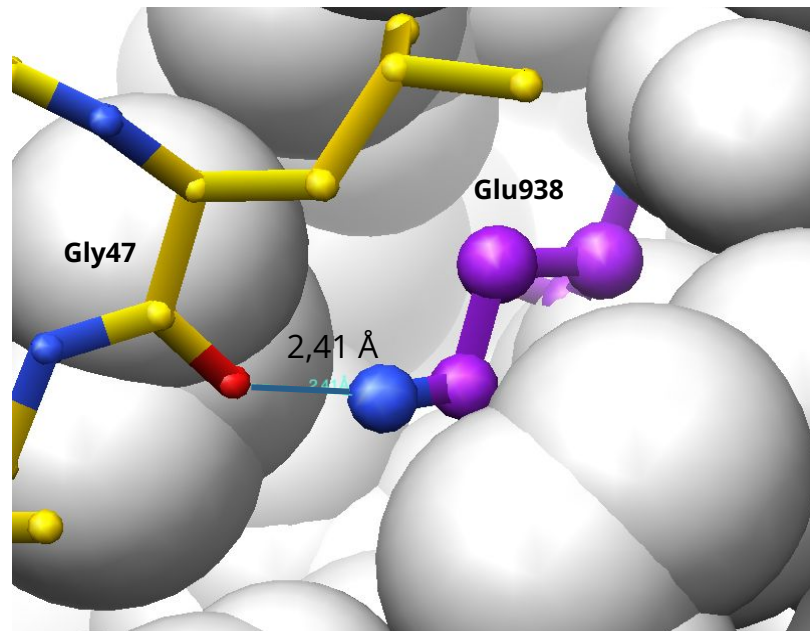


● E1-Ub Interface 1 interactions

Hydrogen bond

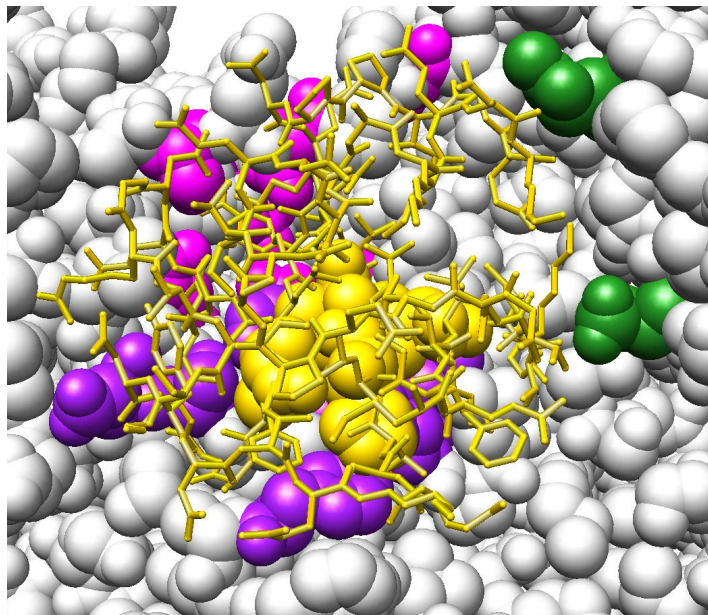
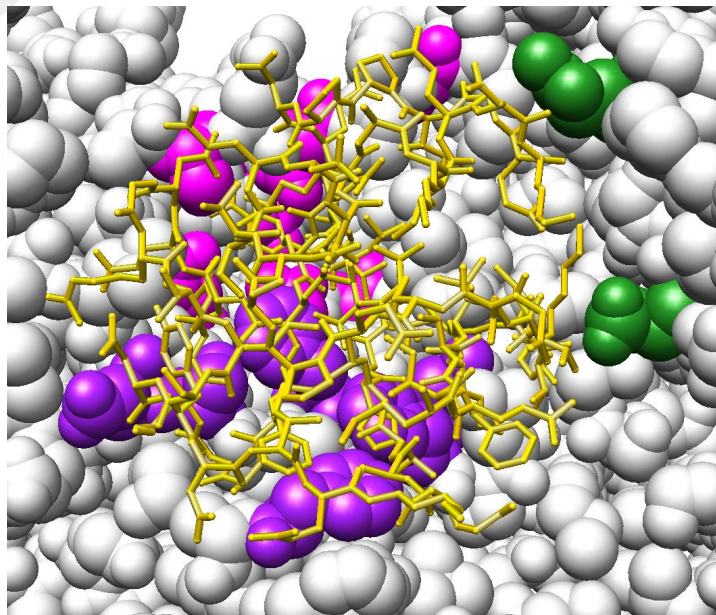


Hydrogen bond



PDB ID: 6DC6

- **E1-Ub Interface 1 hydrophobic interactions**

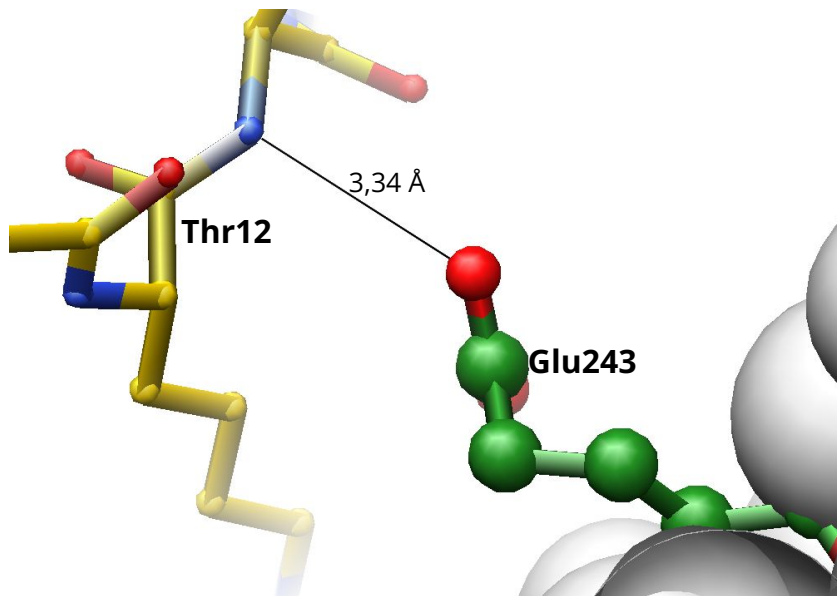


Ub	Leu-8 Ile-44 His-68 Val-70
E1	Phe-320 Phe-926 Phe-933 Ser-937

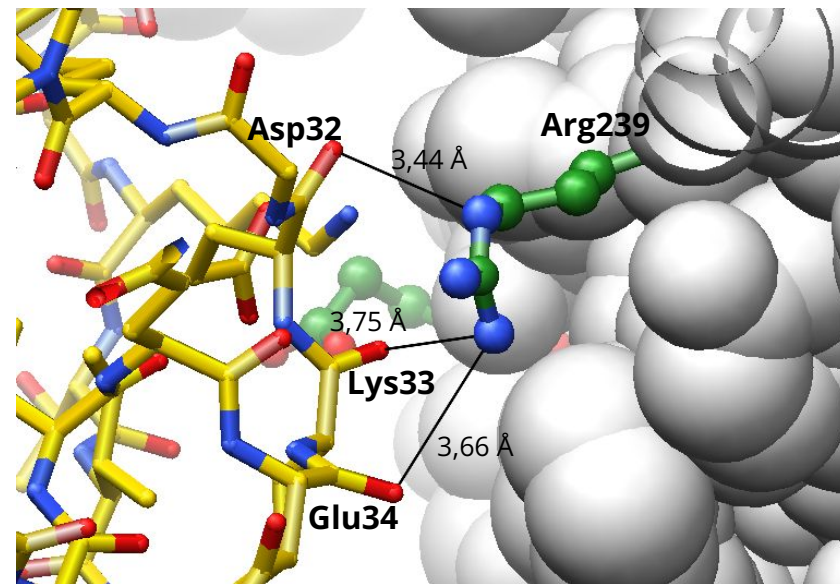
PDB ID: 6DC6

● E1-Ub Interface 2 interactions

Hydrogen bonds



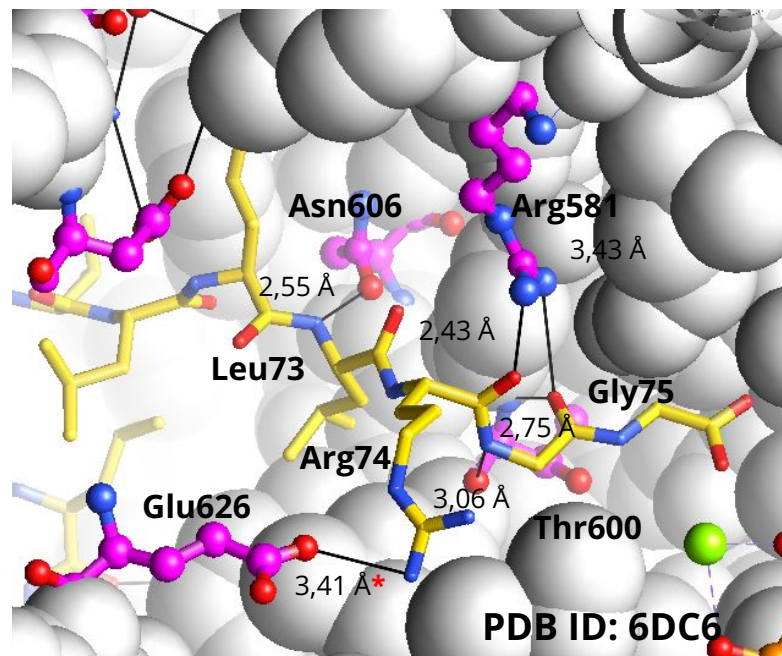
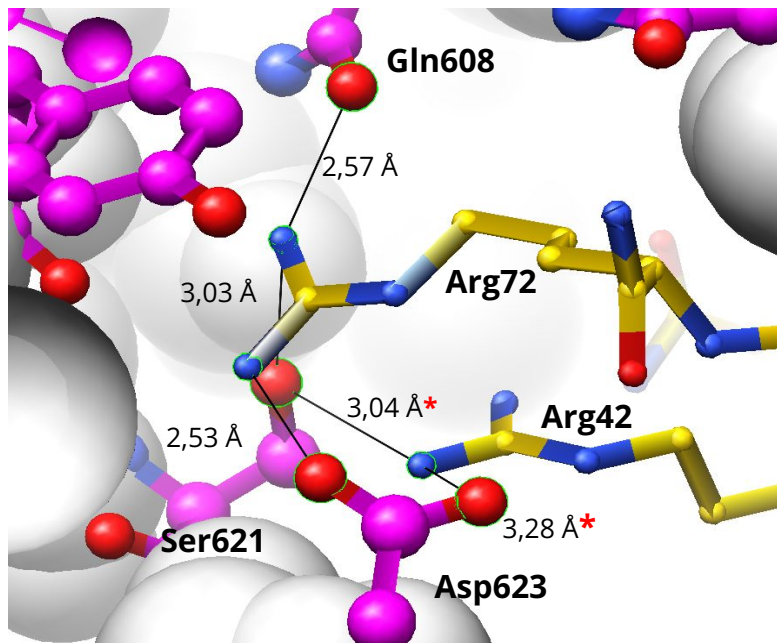
Hydrogen bonds



PDB ID: 6DC6

● E1-Ub Interface 3 interactions

Hydrogen bonds and salt bridges



• E1-Ub Interfaces amino acid conservation

E1 Activating enzyme

S. pombe --DGVVTMLEETRHGLENGDFVKFTEVKGMPLNDGTPRKVEVKGPYTFSIGSVKDLGSA
H. sapiens 227 DNPGVVTCLEEARHGFESGDFVSFSEVQGMVELNGNQPMKIKVLGPYTFISICDTSNFSKY
B. taurus --DGTVTMLDDNRHGLEDGNFVRFSEVEGLDKLNDGTLFKVEVLGPFAFRIGSVKEYGEY
S. cerevisiae --DGTVTMLDDNRHGLEDGNFVRFSEVEGLDKLNDGTLFKVEVLGPFAFRIGSVKEYGEY

S. pombe GYNGVFTQVKVPTKISFKSLRESLKDPEYVYPDFGKMMRPPQYHIAFQALSAFADAHEGS
H. sapiens 287 IRGGIVSQVKVPKISFKSLVASLAEPDFVVDFAKFSRPAQLHIGFQALHQFCAHQH-R
B. taurus KKGGFTEVKVPRKISFKSLKQQLSNPEFVFSDFAKFDRAAQLHLGFQALHQFAVRHNGE
S. cerevisiae KKGGFTEVKVPRKISFKSLKQQLSNPEFVFSDFAKFDRAAQLHLGFQALHQFAVRHNGE

S. pombe ARMYVDRRCVFFEKPLLESQTLGTKCNTQVVPHLTESYSSQDPPEKSFPICTLKNFPN
H. sapiens 580 ARMYMDRRCVYRKPLLESQTLGTKCNVQVVPFLTESYSSQDPPEKSIPIATLKNFPN
B. taurus ARYVDRRCVYRKPLLESQTLGTKCNTQVIIPRLTESYSSSRDPPEKSIPLCTLRSPN
S. cerevisiae ARYVDRRCVYRKPLLESQTLGTKCNTQVIIPRLTESYSSSRDPPEKSIPLCTLRSPN

S. pombe ITPADRFKTKFVAGKIVPAMCTSTAVVSGLVCLLVKLVGDGKKKIEEYKNGFFNLAIQLF
H. sapiens 875 IPSADRHKSKLIAGKIIIPAIATTTAAVVGVLVCLLYKVVQGHRQLDSYKNGFFNLALPFF
B. taurus IETADRQKTKFIAGRIIPAIATTTSLVTGLVNLELYKLIDNKTDIEQYKNGFFNLALPFF
S. cerevisiae IETADRQKTKFIAGRIIPAIATTTSLVTGLVNLELYKLIDNKTDIEQYKNGFFNLALPFF

S. pombe TFSDFIASPKMKVNGKEIDKIWDRYNLPD-----CTLQELIDYFQKEEGLEVMT--LS
H. sapiens 935 GFSEPLAAPRHQYYNQEWL-LWDRFEVQGLQPNGEEMTLKQFLDYFKTE--HKLEITMLS
B. taurus GFSEPIASPKGEYNNKKYDKIWDRFDIKGD-----IKLSDLIEHFED--EGLEITMLS
S. cerevisiae GFSEPIASPKGEYNNKKYDKIWDRFDIKGD-----IKLSDLIEHFED--EGLEITMLS

Interface 1
Interface 2
Interface 3

• E1-Ub Interfaces amino acid conservation

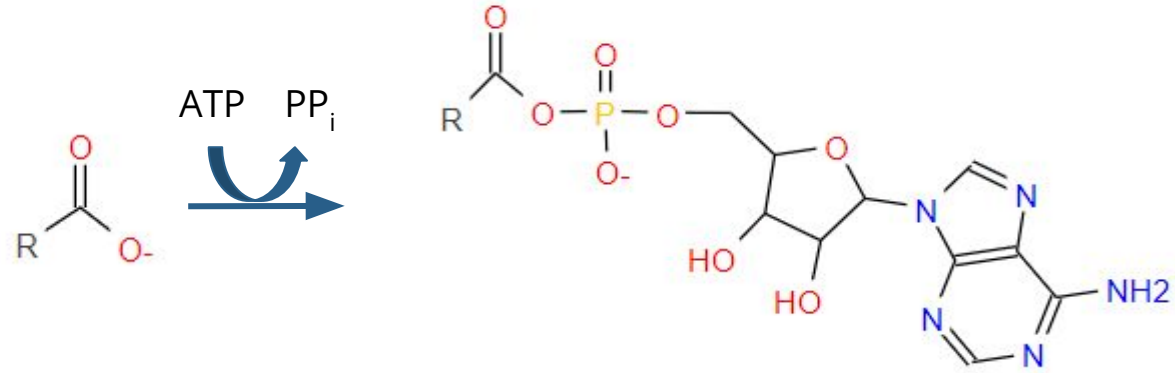
Ubiquitin

	Leu-8	Thr-12	32-34	Arg-42	Gly-47	72-75	
<i>S.cerevisiae</i>	MQIFVKT	LTGKT	TITLEVESSDTIDNVKSKI	QDK	EGIPPDQQR	LIFAGKQLEDGRTLSDYNIQKESTLHLVLR	LRGG
<i>H.sapiens</i>	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKI	QDK	EGIPPDQQR	LIFAGKQLEDGRTLSDYNIQKESTLHLVLR	LRGG
<i>B.taurus</i>	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKI	QDK	EGIPPDQQR	LIFAGKQLEDGRTLSDYNIQKESTLHLVLR	LRGG
<i>A.thaliana</i>	MQIFVKT	LTGKT	TITLEVESSDTIDNVKAKI	QDK	EGIPPDQQR	LIFAGKQLEDGRTLADYNIQKESTLHLVLR	LRGG
<i>M.musculus</i>	MQIFVKT	LTGKT	TITLEVEPSDTIENVKAKI	QDK	EGIPPDQQR	LIFAGKQLEDGRTLSDYNIQRESTLHLVLR	LRGG

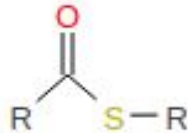
- Interface 1
- Interface 2
- Interface 3

● E1 - Adenylation site & interactions

Adenylation



Thioester bond



Glycine-76: the Ubiquitin C-terminal amino acid

ATP molecule

Magnesium ions: stabilize the ATP phosphate groups

Catalytic cysteine (Cys-632) from the E1 enzyme

Several **E1 amino acids**

● E1 - Adenylation site & interactions

Step 1:

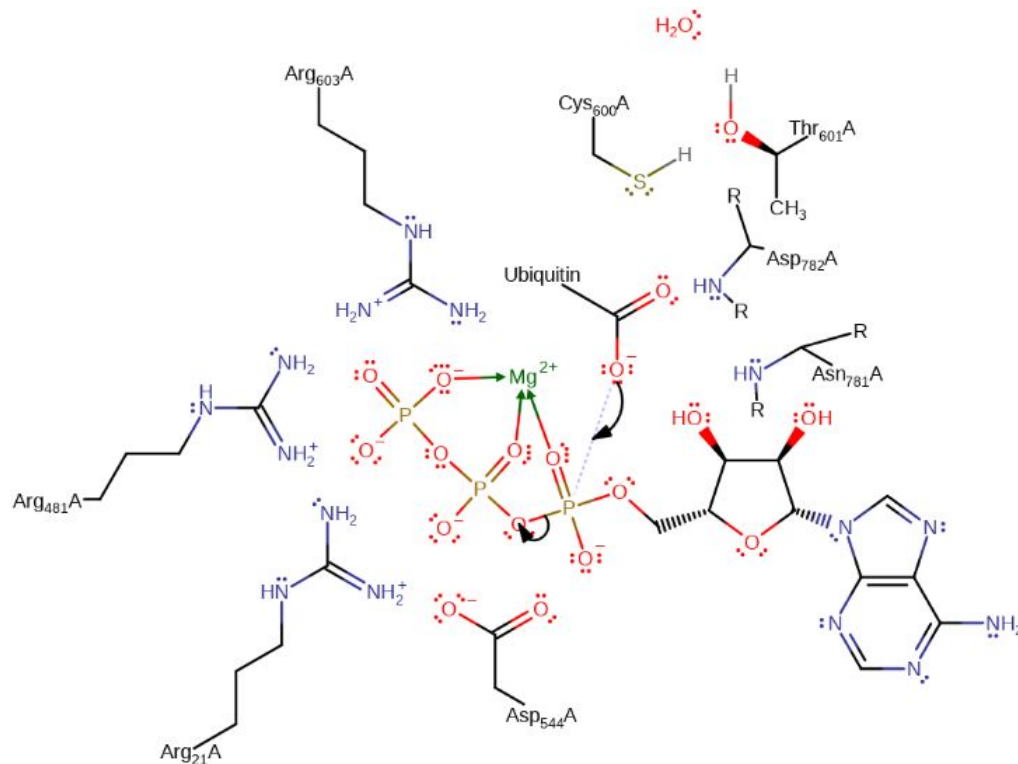


Figure 5. E1 ubiquitin-activating enzyme. M-CSA Mechanism and Catalytic Site Atlas. Available from: <https://www.ebi.ac.uk/thornton-srv/m-csa/entry/307/>

● E1 - Adenylation site & interactions

Step 2:

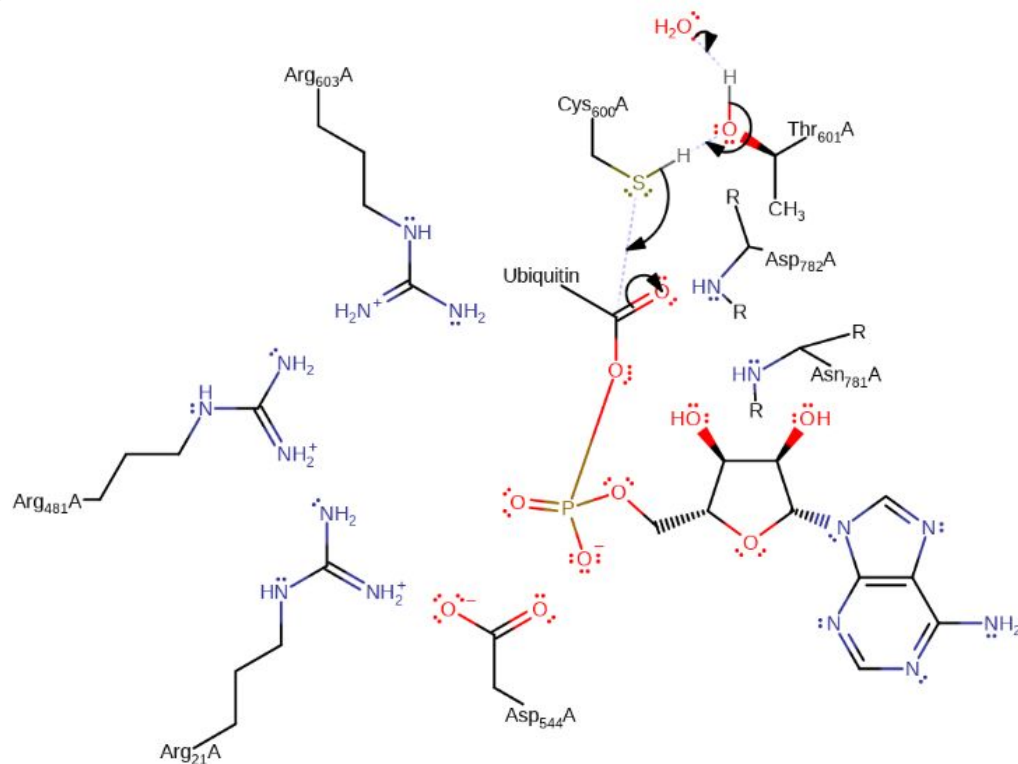


Figure 6. E1 ubiquitin-activating enzyme. M-CSA Mechanism and Catalytic Site Atlas. Available from: <https://www.ebi.ac.uk/thornton-srv/m-csa/entry/307/>

● E1 - Adenylation site & interactions

Step 3:

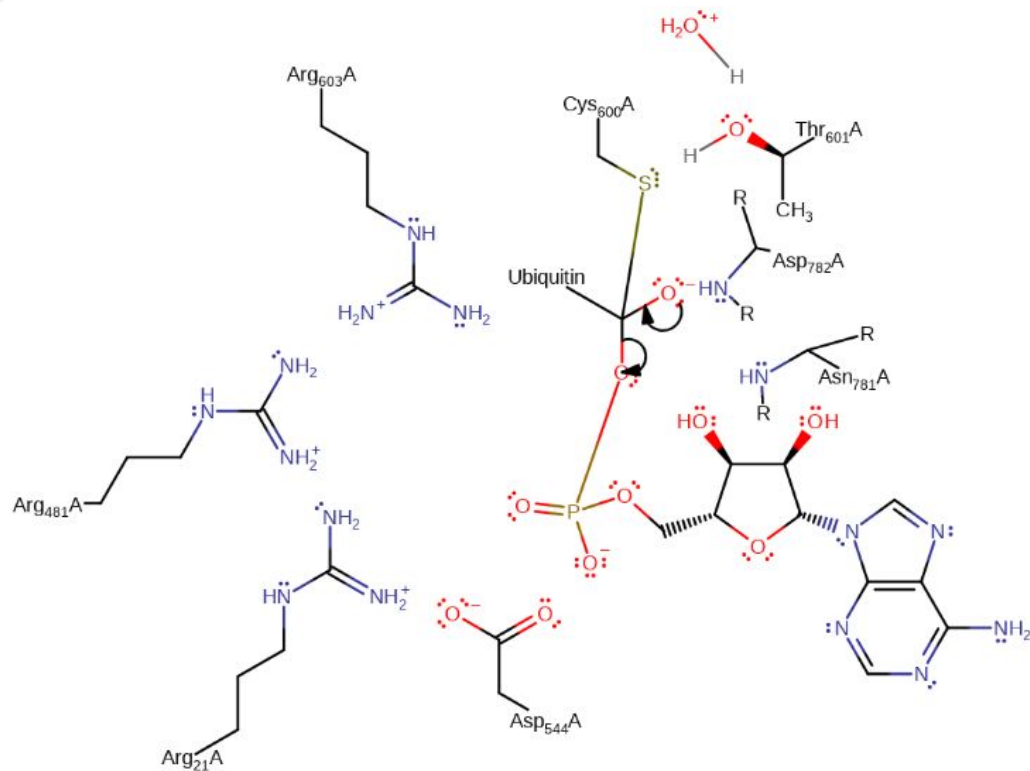


Figure 7. E1 ubiquitin-activating enzyme. M-CSA Mechanism and Catalytic Site Atlas. Available from: <https://www.ebi.ac.uk/thornton-srv/m-csa/entry/307/>

● E1 - Adenylation site & interactions

Step 4:

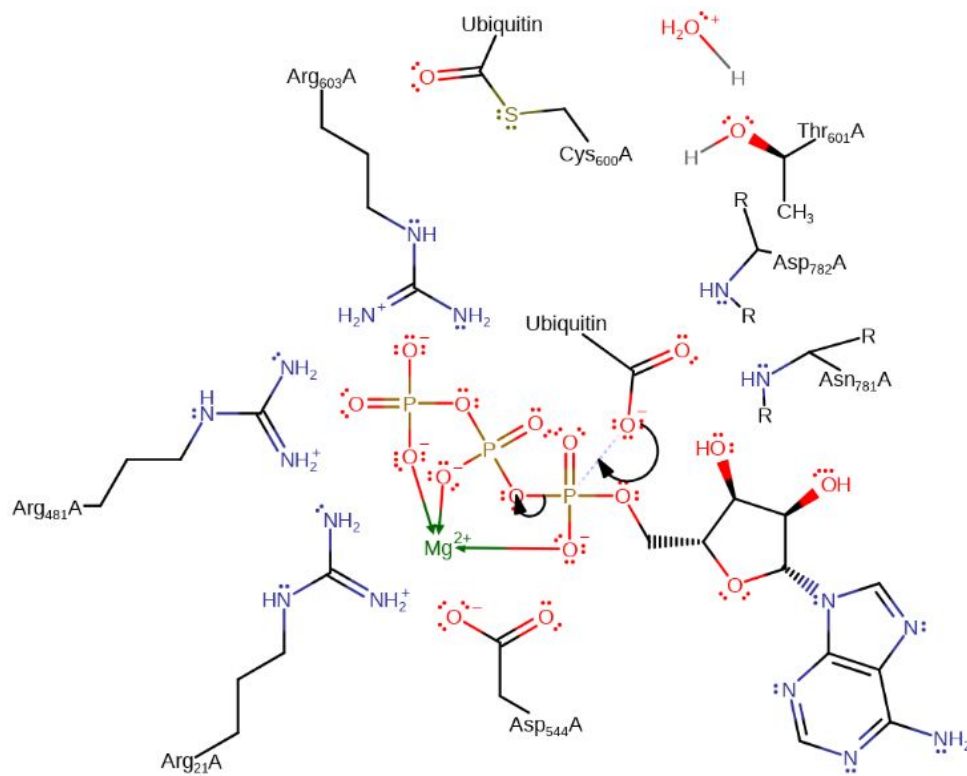
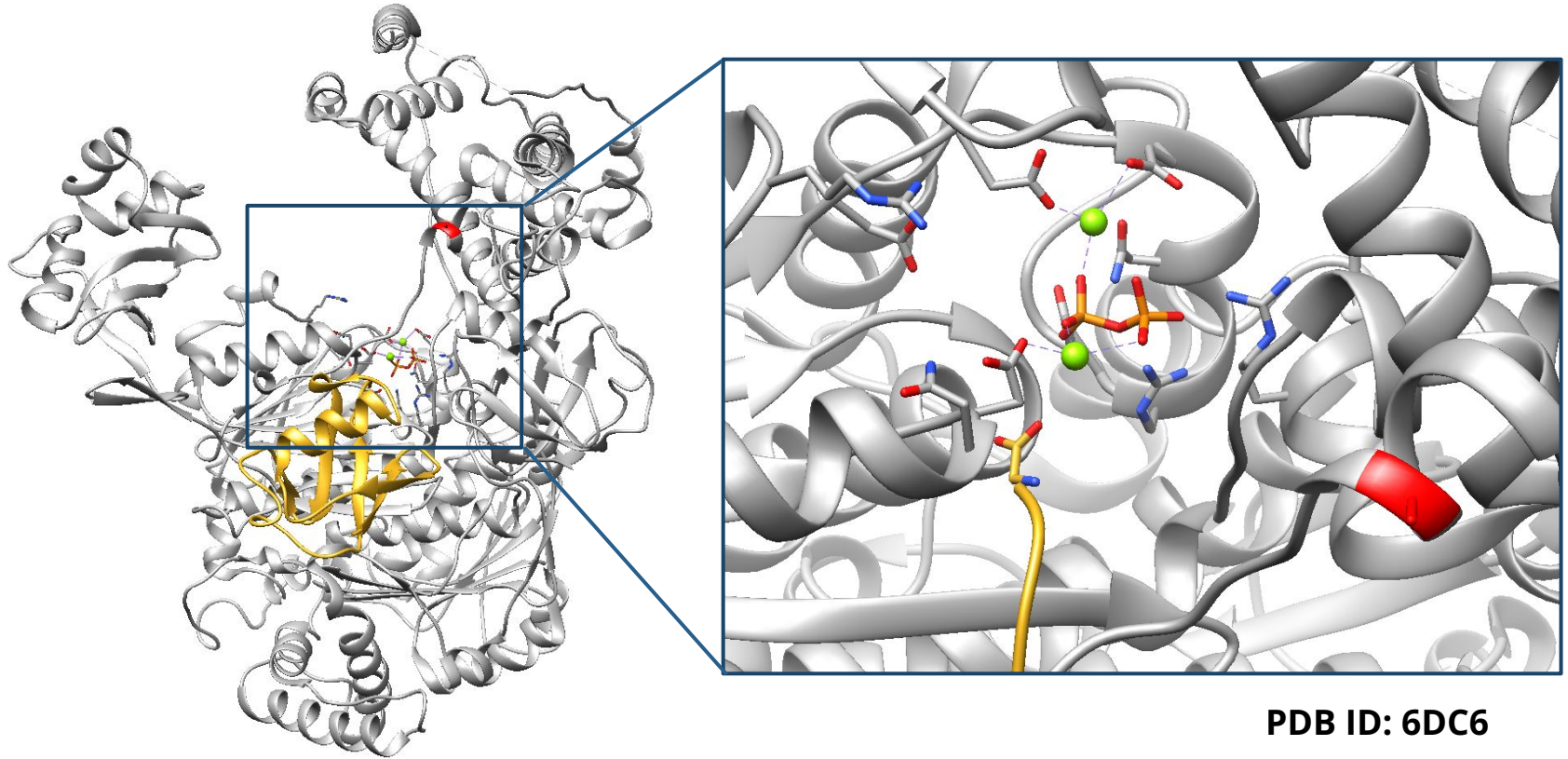
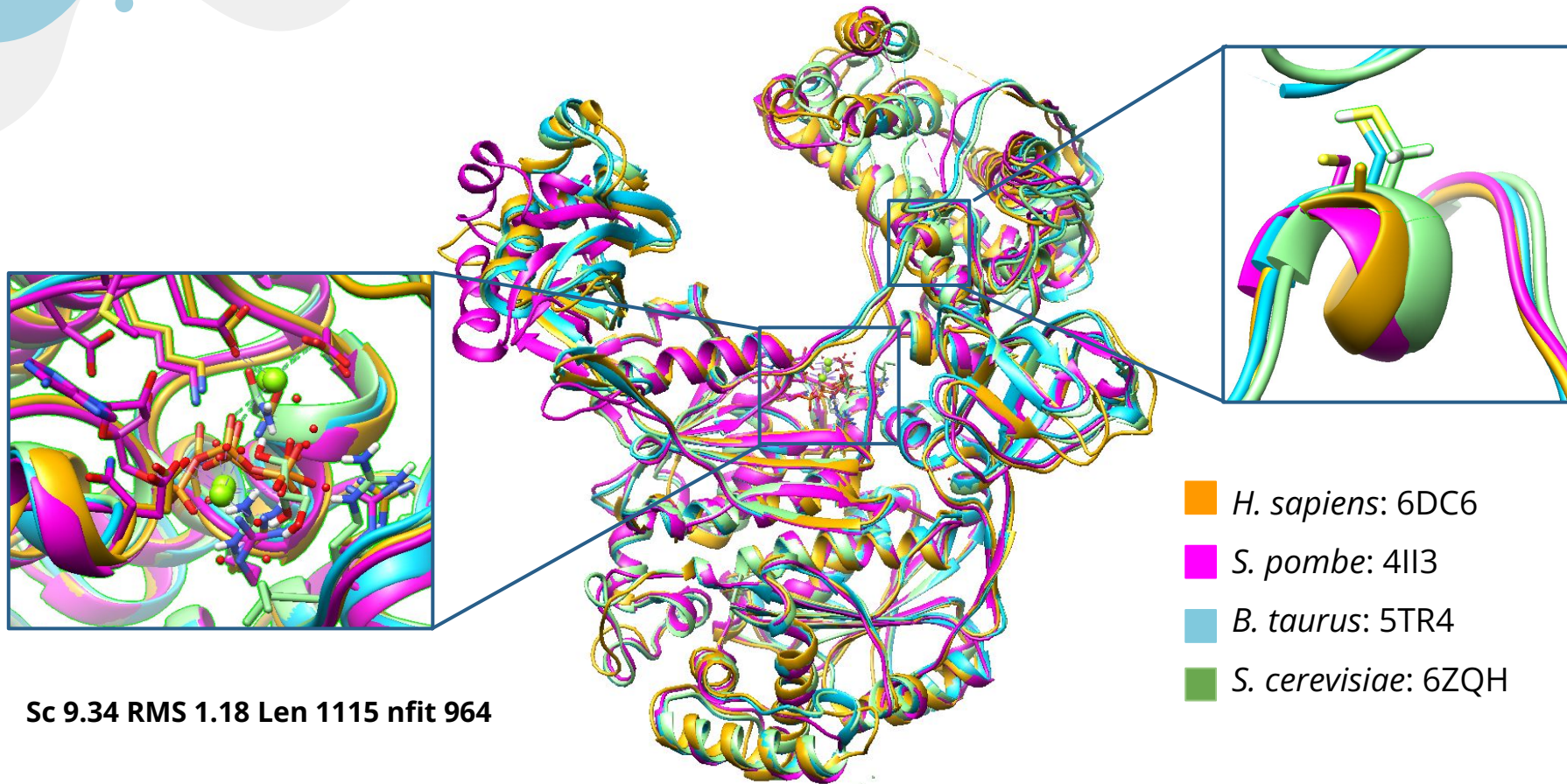


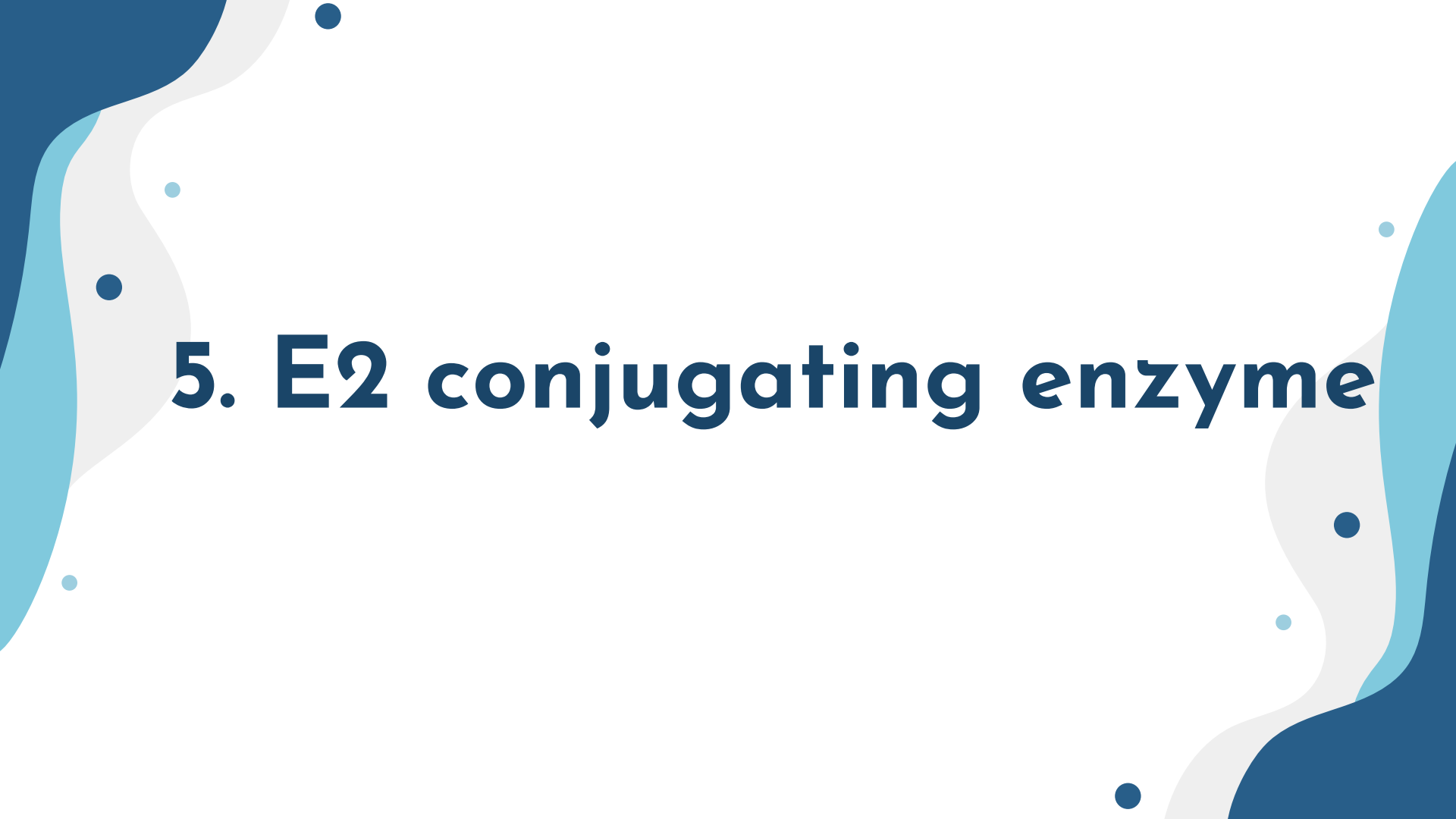
Figure 8. E1 ubiquitin-activating enzyme. M-CSA Mechanism and Catalytic Site Atlas. Available from: <https://www.ebi.ac.uk/thornton-srv/m-csa/entry/307/>

E1 - PDB's Adenylation site



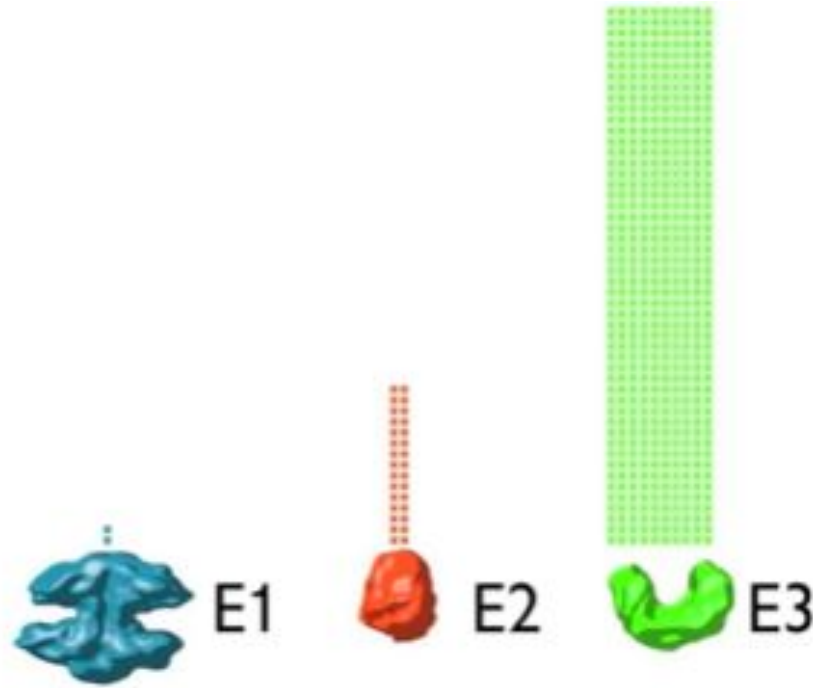
E1 - Structural alignment





5. E2 conjugating enzyme

E2 conjugating enzymes

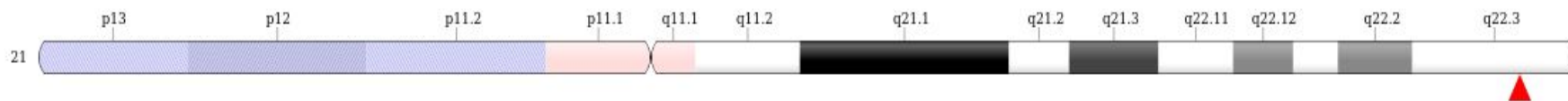


E2- Sequence

Ubiquitin-conjugating enzyme E2 G2 - *Homo sapiens*

Gene Name: *ube2g2*

Homo sapiens Chromosome 21, GRCh38.p13



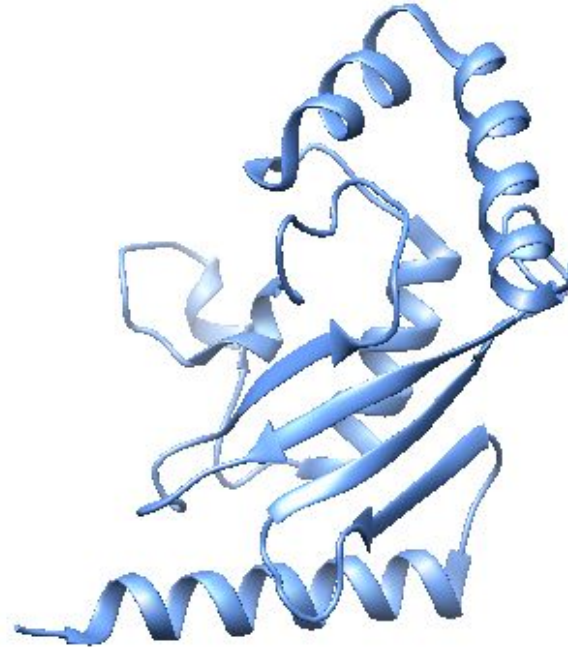
Sequence Length → 170 aa

GGSEFMAGTALKRLMAEYKQLTLN PPEGI VAGPMNEENFF EWEALIMGPEDTCFEFG VFPAILS FPLDY
PLSPP KMRF TCEMFHPNIYPDGRV C I S I L H A P G D D P M G Y E S S A E R W S P V Q S V E K I L L S V V S M L A E P N D E
SGANVDASKMWRDDREQFYKIAKQIVQKSL GL

E2 - Classification

SCOP Classification

- **Class:** Alpha and beta proteins ($\alpha + \beta$)
- **Fold:** UBC-like
- **Superfamily:** UBC-like
- **Family:** UBC-related
- **Domain:** Ubiquitin conjugating enzyme, UBC
- **Species:** Human (*Homo sapiens*)

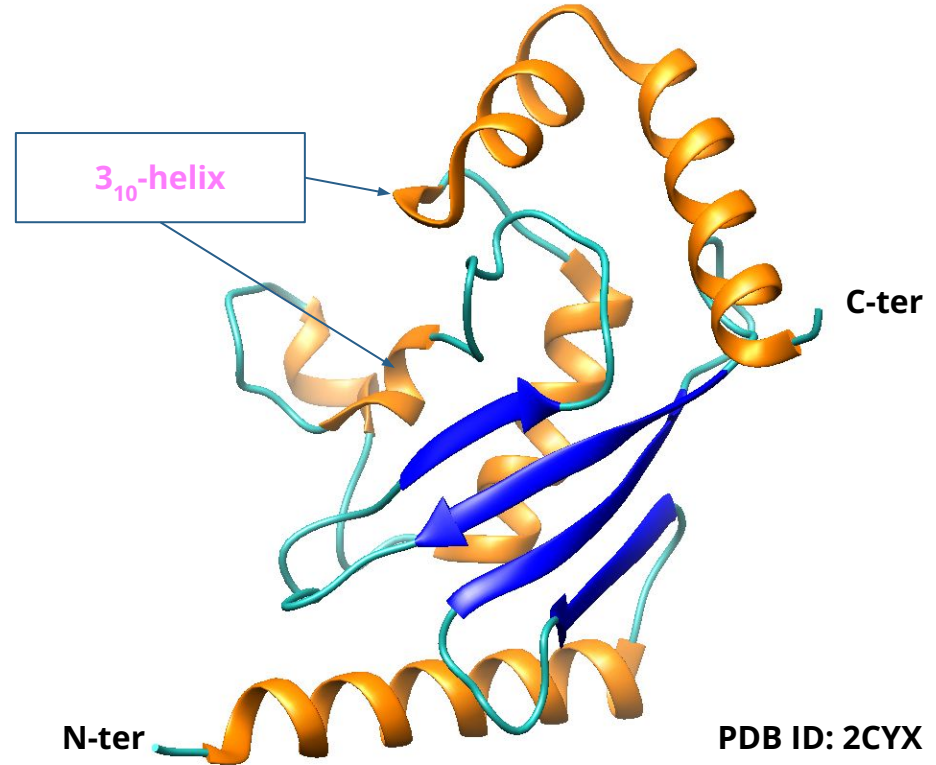
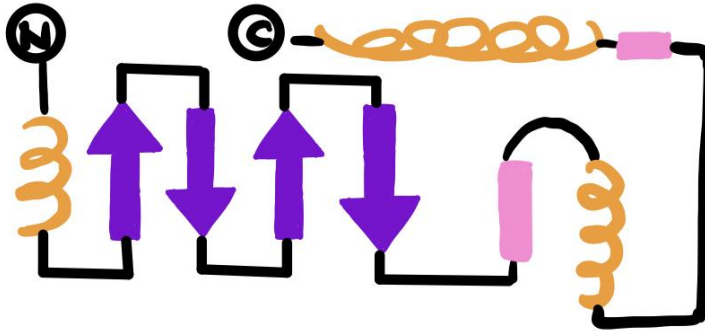


PDB ID: 2CYX

E2 - Secondary Structure

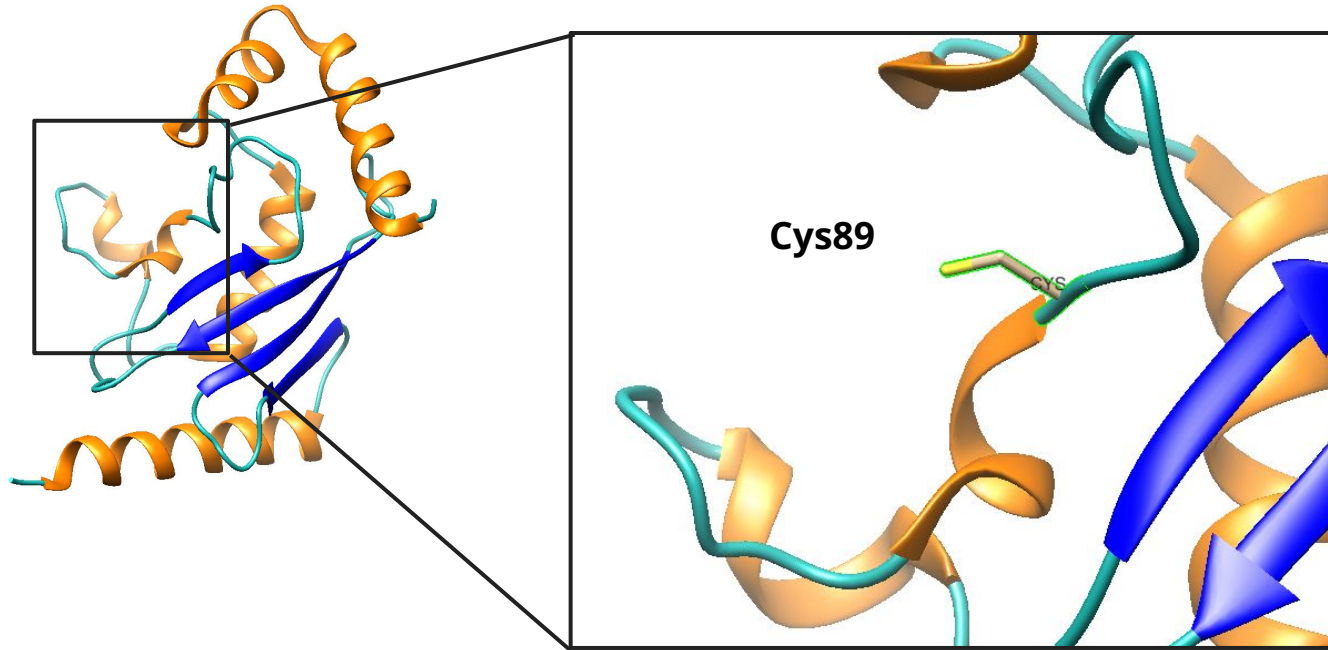
Antiparallel Beta-sheet

3.5 α -helix



PDB ID: 2CYX

E2 - Active Site



E2 - Active Site

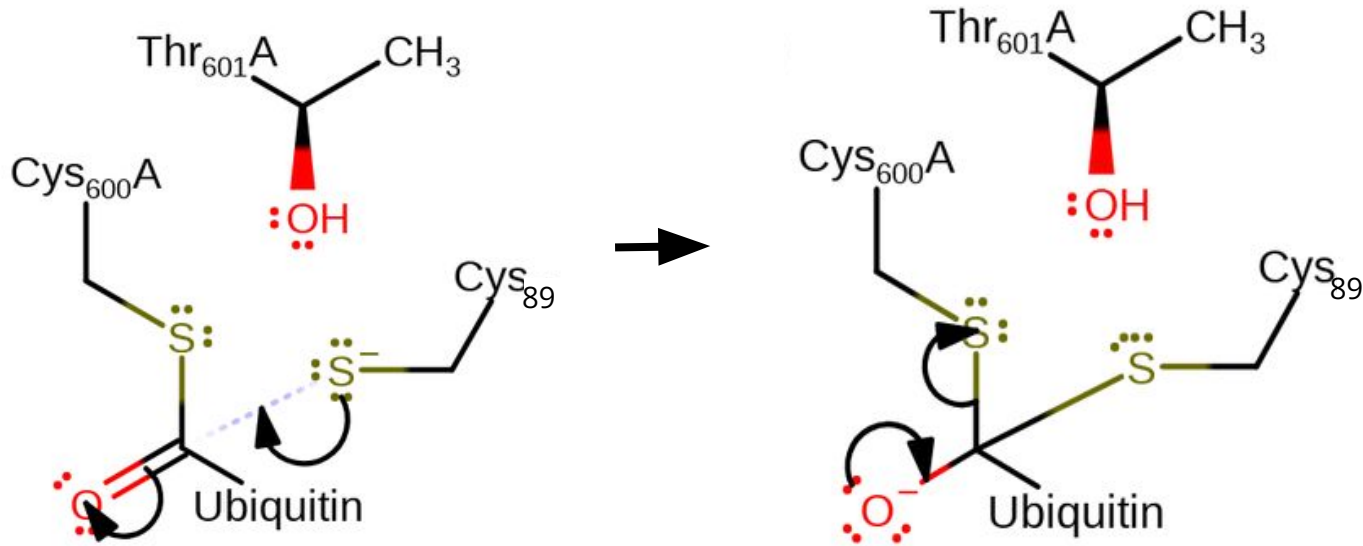
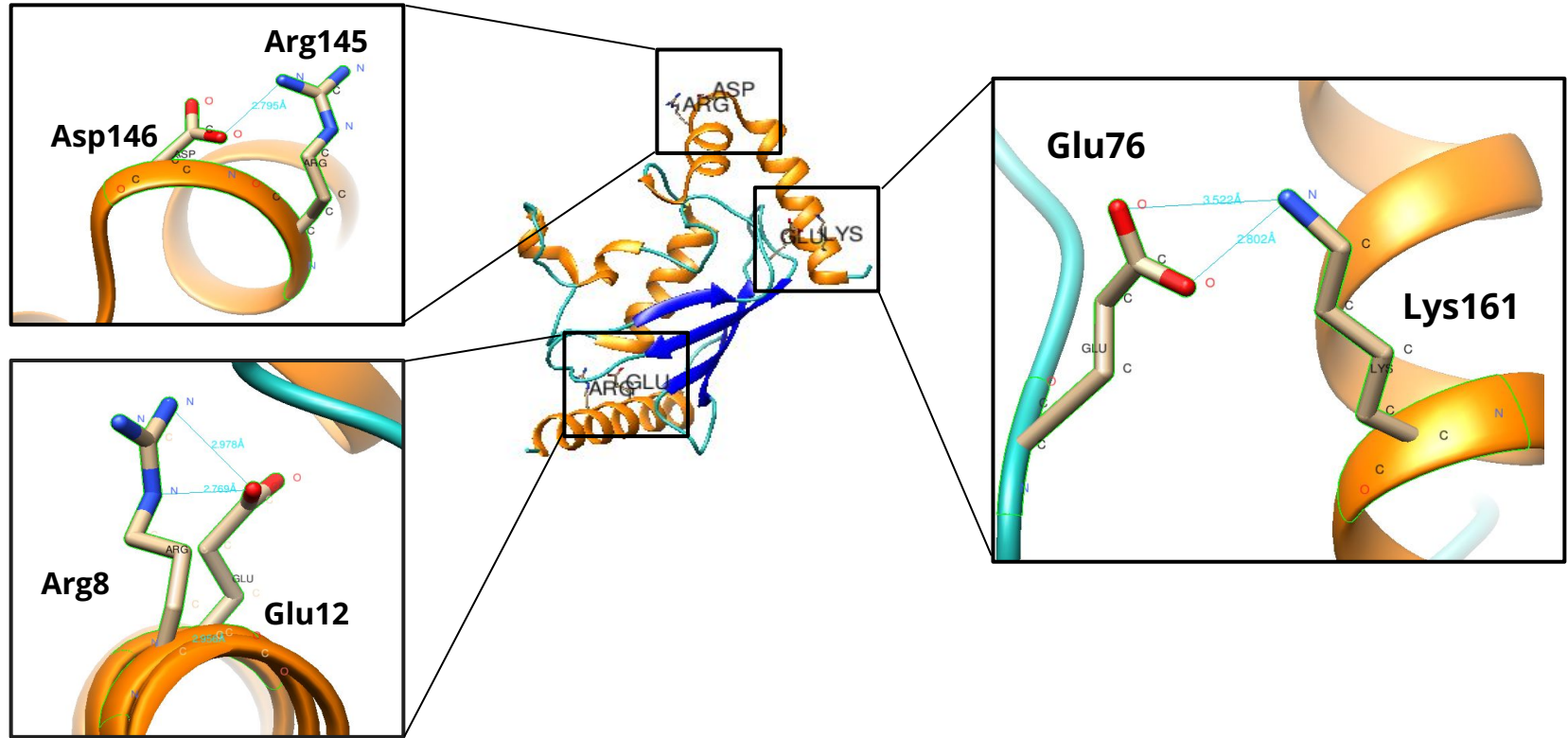
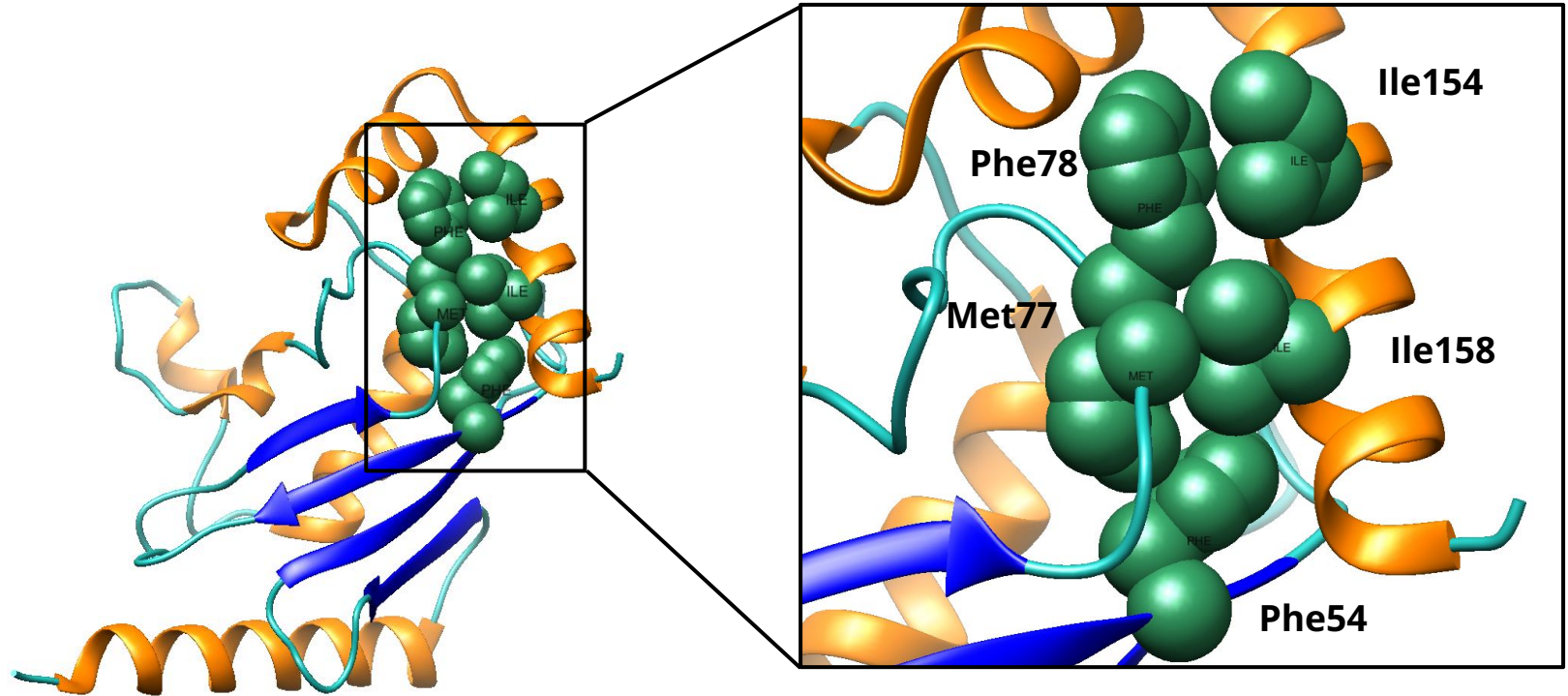


Figure 9. E2 ubiquitin-conjugating enzyme. M-CSA Mechanism and Catalytic Site Atlas. Available from: <https://www.ebi.ac.uk/thornton-srv/m-csa/entry/939/>

E2 - Salt Bridges



E2 - Hydrophobic Bonds



E2 - Sequence Conservation

Between different species of E2

M.musculus	MSTEAQRVDDSPSTSGG--SSDGDQRESVPKGDNIYEWRS	ILGPPGSVYEGGVFFLDITFSPDYPFKPPKVTFRTRIYHCNINSQ-GV
X.tropicalis	MSTEGQRVDDSPSTSGG--SSDGDQRDGVKGDNIYEWRS	ILGPPGSVYEGGVFFLDIAFSPDYPFKPPKVTFRTRIYHCNINSQ-GV
R.norvegicus	RSSQTQREGPFAGWGGGFAMSDDDSRASTPKGDNIYEWRS	ILGPPGSVYEGGVFFLDITFTPEYPFKPPKVTFRTRIYHCNINSQ-GV
D.melanogaster	-----	LVNDSWTELARGEIAGPPDTPYEGGKFVLEIKVPETYPFNPPKVRFITRIWHPNISSVTGA
C.elegans	-----	IKENNLMDIKGFIKGPEGTPYAGGTFEIKVDIPEHYPFEPKAKFVTRIWHPNISSQTGT
H.sapiens	-----	ILG-GLNEFVVKFYGPQGTPEYEGGVWVKVRVDLPDKYPFKSPSIGFMNKIFHPNIDEASGT

. : : ** .: * ** : : : .. ***:.* * .:.* **.. *

M.musculus	ICLDILKDNWSPALTISKVLLS-ICSLLTDCNPADPLVGS	IATQYMTNRAEHDRMARQWTKRYAT-----
X.tropicalis	ICLDILKDNWSPALTISKVLLS-ICSLLTDCNPADPLVGS	IATQYMTNRAEHDRMARQWTKRYAT-----
R.norvegicus	ICLDILKDNWSPALTISKVLLS-ICSLLTDCNPADPLVGS	IATQYMTNRAEHDRMARQWTKRYAT-----
D.melanogaster	ICLDILKDNWAAAMTLRTVLLS-LQALLAAAEPPDQDA	VAVAYQFKDKYDLFLLTAKHWTNAYAGG-PHTFPDCD
C.elegans	ICLDILKDKWTASLTLRTVLLS-LQAMLCSPESDPQDA	VAVAKQFINNYPMFTATAVYWTSYFANSKKDVEPDFN
H.sapiens	VCLDVINQWTALYDLTNIFESFLPQLLAYPNPIDPLN	GDAAMYLRPEEYKQKIKEYIQKYATEEALKEQEEG

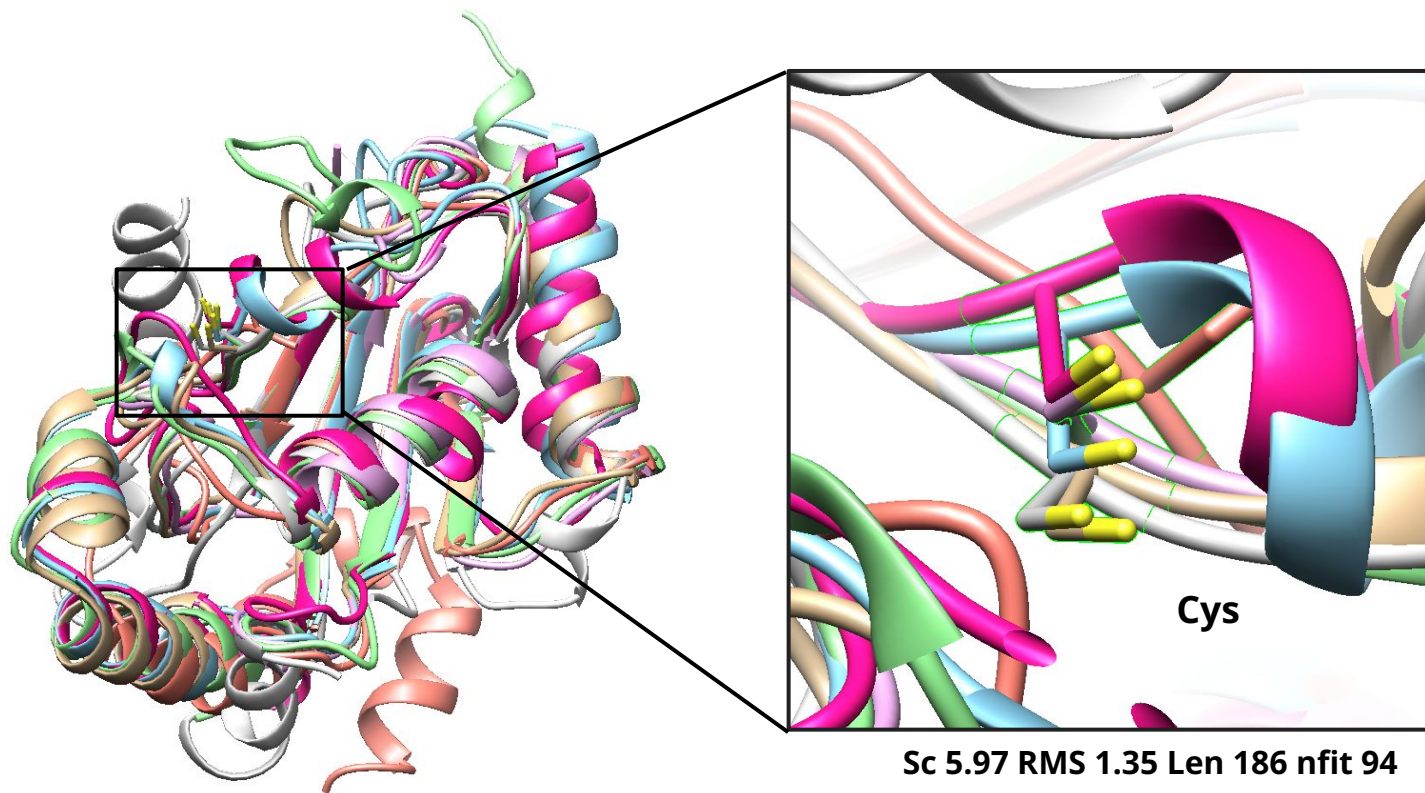
:***:::.*.: : .:: * : :* :* ** . * : . . ::.:*

E2 - Structural Conservation

Between different proteins of E2 paralogs

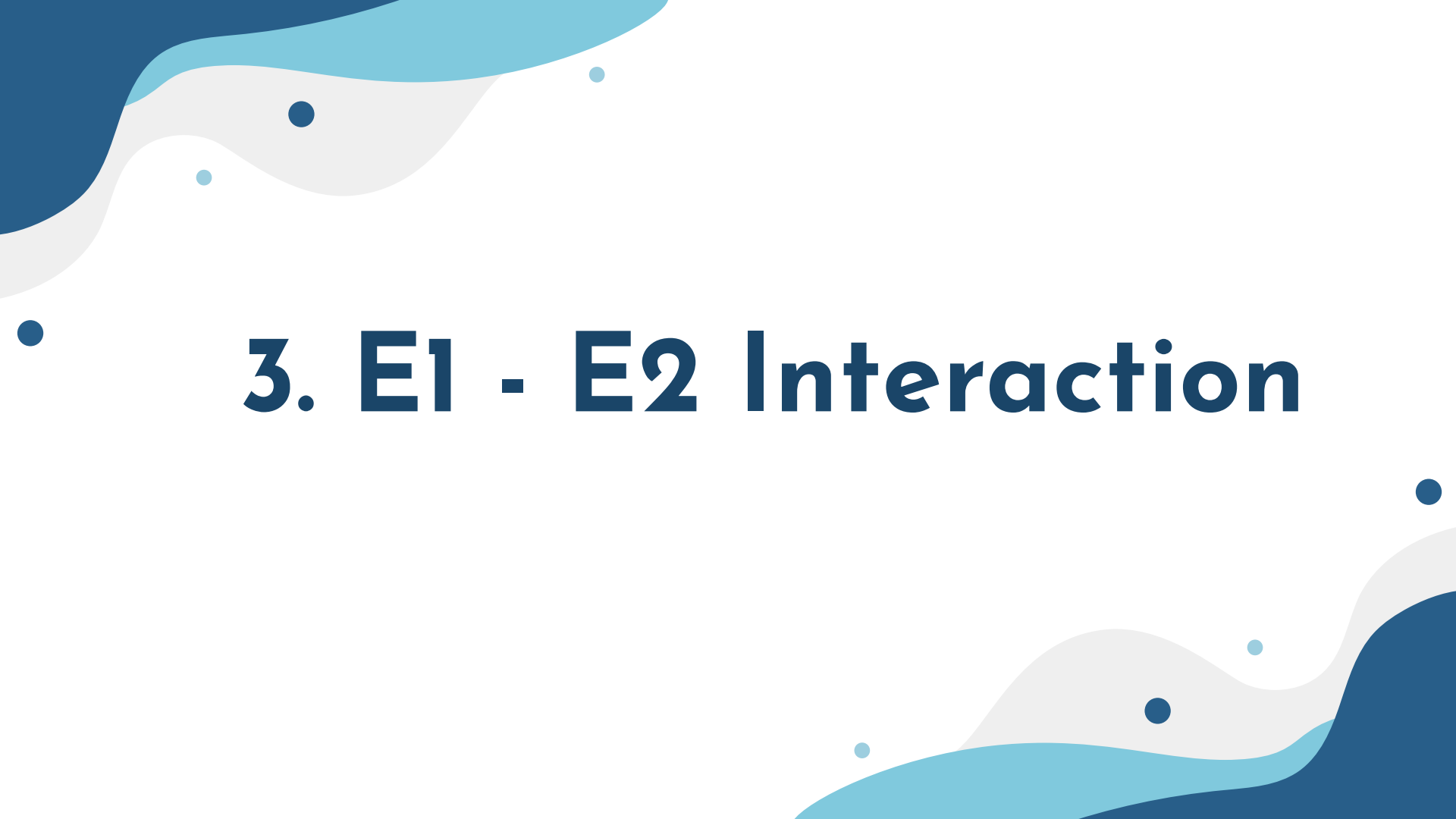
1zdn	--ENLPPIIIRLVYKEVTTLTADPPDGIKVPNEE-DLTDLQVTIEGPEGTPY-----	--RDWTAELGIRHVLLTIK-CLLIHPNPESALNEE--AGRLLLENYEEYAARARLLTEIH
1y6l	-----STSAKRIQKELAEITLDPNPNCISAGPKGD-NIYEWSTILGPPGSVY-----	--DNWSPALTISKVLLSIC-SLLTDCNPADPLVGS--IATQYMTNRAEHRMARQWTKRY
2awf	GLVPRGSLLLRRQLAELNK---NPVEGFSAGLIDDNDLYRWEVLIGPPDTLY-----	--ERWLPiHTVETIMISVI-SMLADP-----
2cyx	GSEFMAGTALKRLMAEYKQLTLNPPPEGIVAGPMNEENFFEWELIMGPEDTCF-----	SAERWSPVQSVEKILLSVV-SMLAEPNDESGANVD--ASKMWRDDREQFYKIAKQIVQKS
2z5d	-----PGKRMDTDVIKLIESKHEVTILG-----GLNEFVVKFYGPQGTPY-----	--QTWTALYDLTNIFESFLPQLLAYPNPIDPLNGD--AAAMYLHRPEEYKQKIKEYIQKY
2f4w	-----TTATQRLKQDYLRICKDPVPYICAEPLPS-NILEWHYVVRGPEMPY-----	----WNPASVSTILTGLLSFMVEKGPTLGSIIETSDFTKRQLAVQSLAFNLKDKVFCFL
2qgx	--SVQATDRLMKELRDIYRSQSFKGGNYAVELVND-SLYDWNVKKLVDSALHNDLQILK	-KQGWSSAYSIESVIMQISATLVKKG-----ARVQFGANKSQYSLTRAQQSYKS
	: .: . : .	* . : :: . ::
1zdn	---AGGLFRMKLLLGKDFPASPPKGYFLTIFHPNVGAN-GEICVNVLK-----	G-----
1y6l	---EGGVFFLDITFSPDYPFKPPKVTFRTRIYHCNINSQ-GVICDILK-----	AT-----
2awf	---EGGVFKAHLTFPKDYPLRPPKMKFITEIWHPNVDKN-GDVCSILHEPPE-----	-----
2cyx	---EFGVFPAILSFPLDYPLSPPKMRFTCEMFHPNIYPD-GRVCISILHAPGDDPMGYES	LGL-----
2z5d	---EGGVWVRVDLPDKYPFKSPSIGFMNKIFHPNIDEASGTVCLDVIN-----	ATEEALKE---
2f4w	---EGGYHGLIFPREFPFKPPSIYMITPNGRFKCNTR---LCL-----	PEVVEEIKQKQ
2qgx	EKEGADFILLNFSFKDNFPDPPFVRVSPVLGGYVLGGGATCMELLT-----	LV-----
	. . : .:* .*	:*:

E2 - Conservation

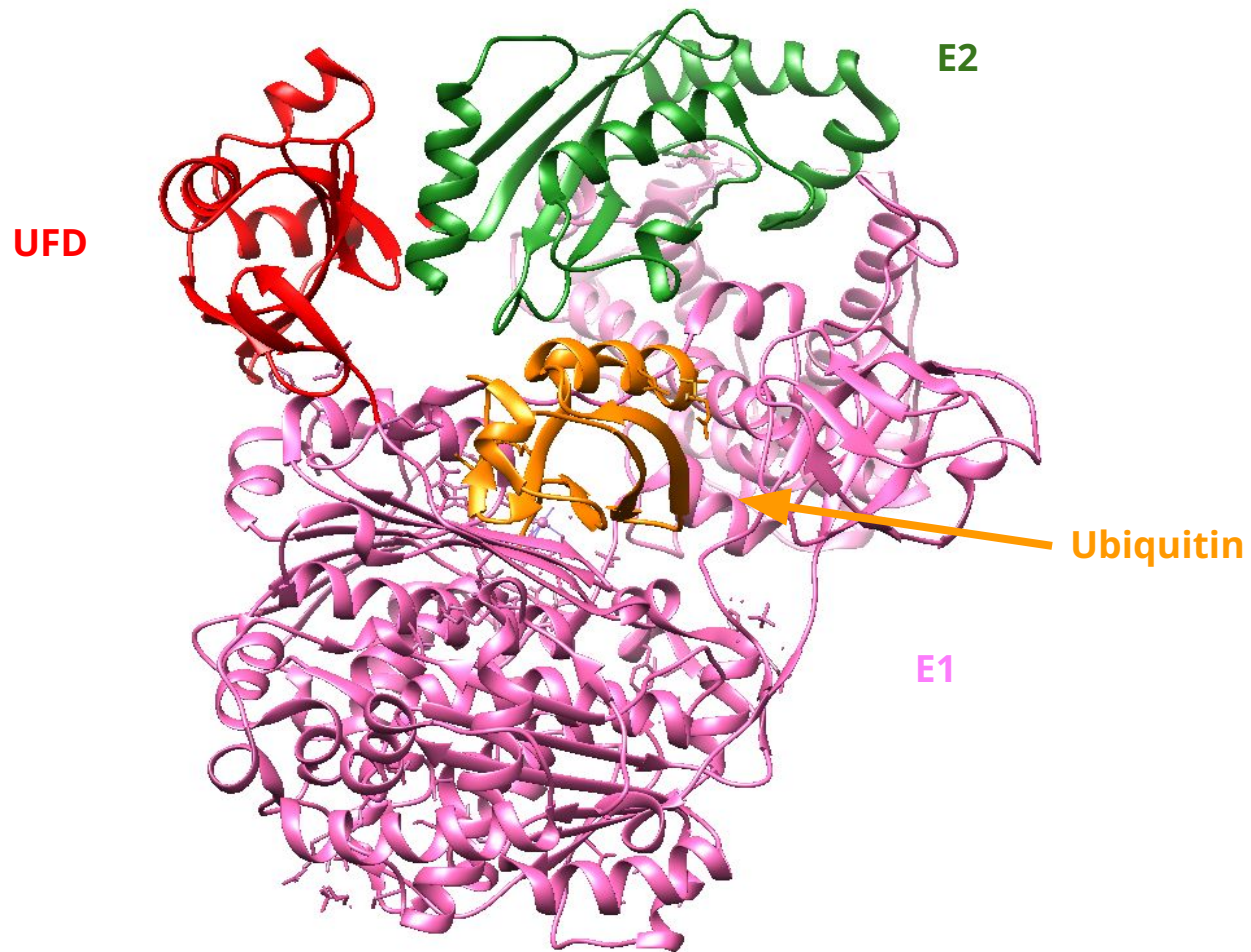


- 2qgx E2 Q
- 2f4w E2 J2
- 2awf E2 G1
- 2z5d E2 H
- 2cyx E2 G2
- 1y6l E2 E2
- 1zdn E2 S

Sc 5.97 RMS 1.35 Len 186 nfit 94

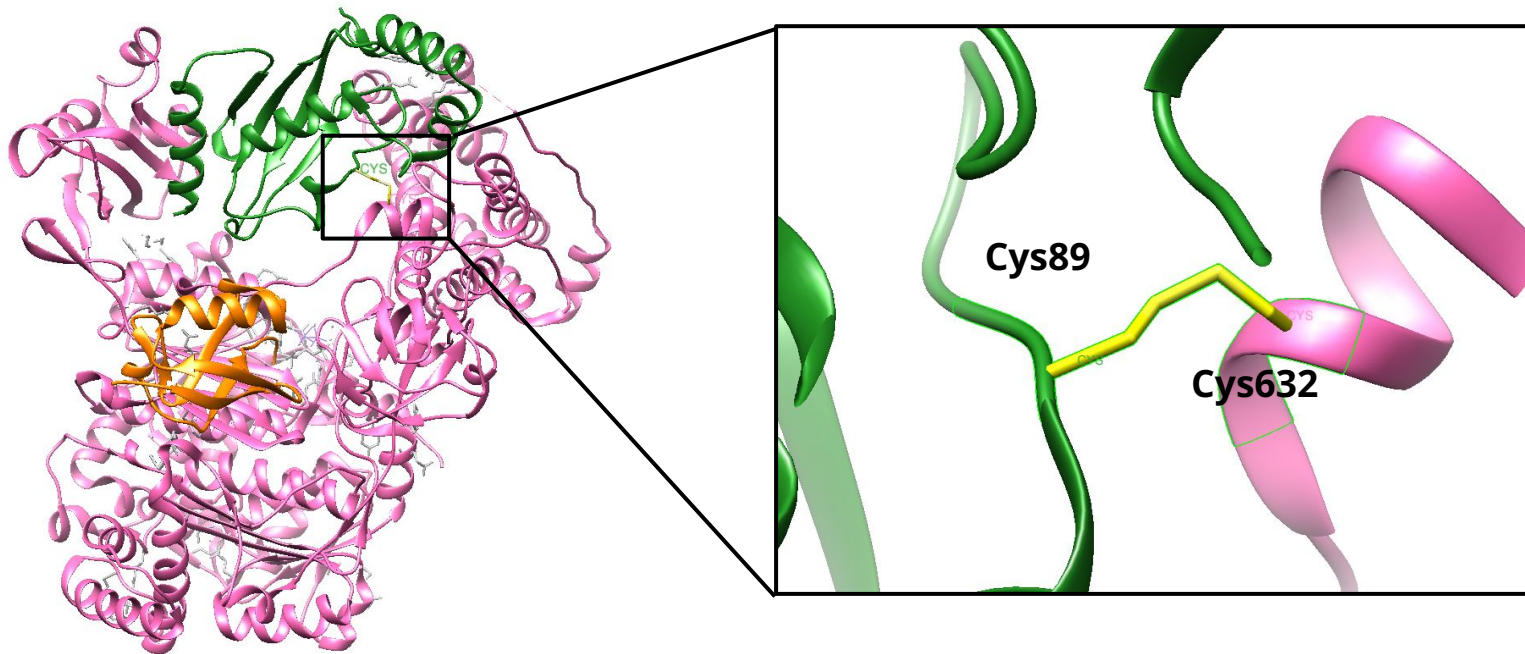


3. E1 - E2 Interaction

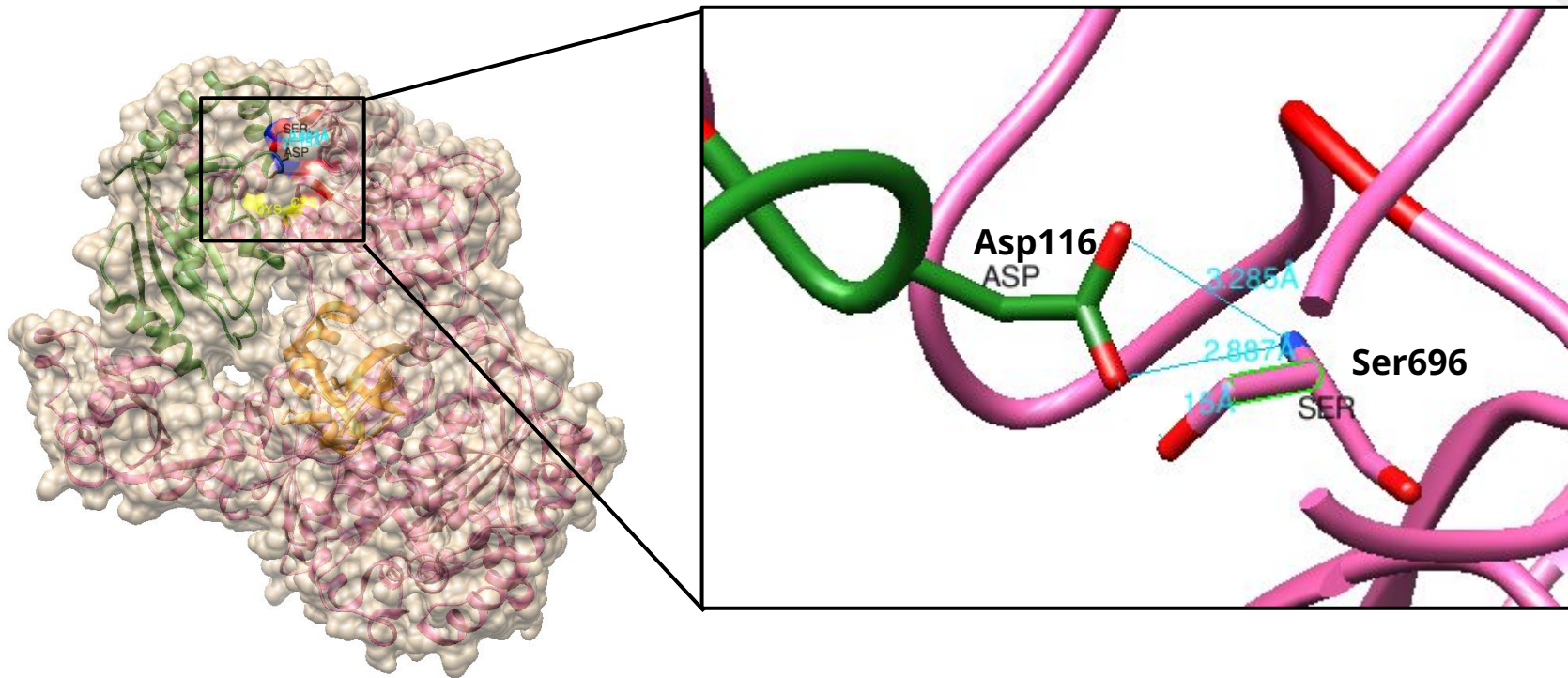


PDB ID: 4II2

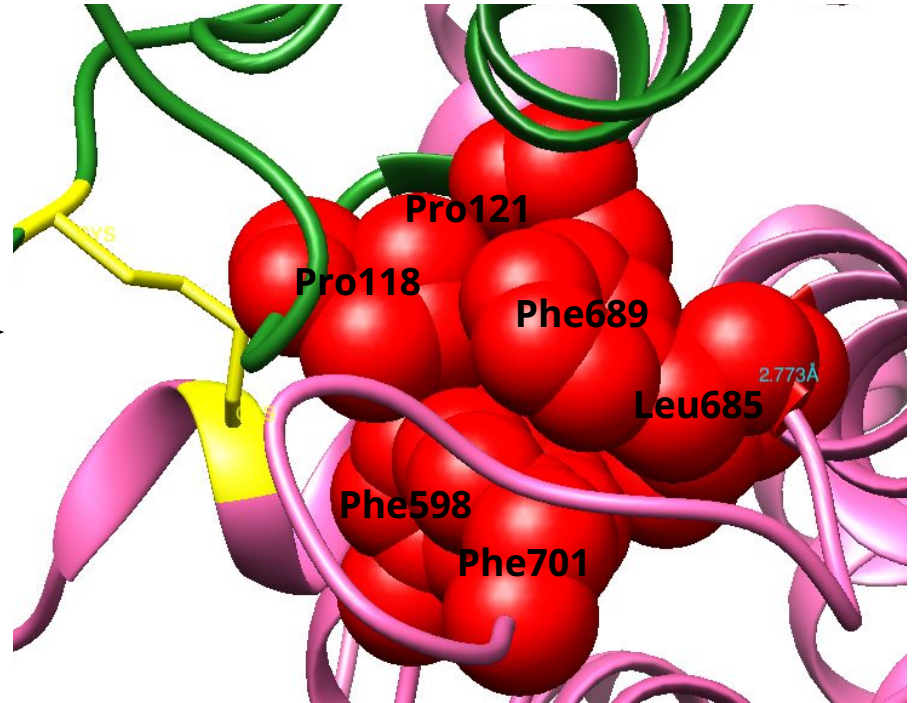
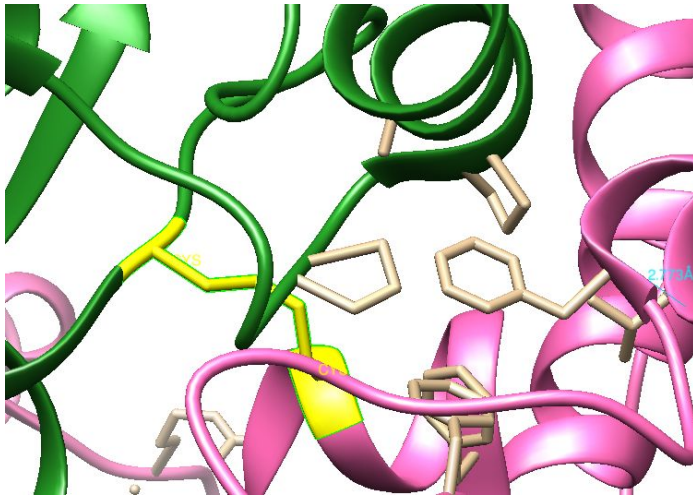
E1 - E2 Cys-Cys Bond



E1 - E2 Hydrogen Bonds



E1 -E2 Hydrophobic interactions



Conclusions

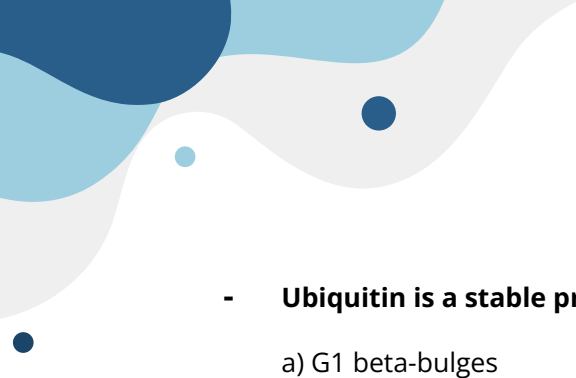
- **Ubiquitin** is a highly conserved protein in eukaryotes. Its stability is enhanced by the pronounced hydrophobic core and the extensive hydrogen-bonding interactions.
- **E1 activating enzyme** is the key and initial step in activating the ubiquitin, and is a highly conserved protein, due to its important role in ubiquitination.
- **E2 conjugating enzyme** plays an important role in the chain of reactions that enable the ubiquitin to bind the variety of proteins that have to be degraded. The amino acid that plays a key role in this enzyme is the Cys that binds the E1 enzyme.

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Questions

- **Ubiquitin is bound to the substrate protein by its:**
 - a) N-terminal methionine
 - b) Lys 48
 - c) **C-terminal glycine residue**
 - d) Phe4 patch
 - e) C-terminal lysine residue
- **Choose the correct answer about ubiquitin:**
 - a) Ubiquitin is one of the most conserved protein in prokaryotes
 - b) Ubiquitin is encoded only in the form of a monomer
 - c) Ubiquitin is bigger than E1 and E2 enzymes
 - d) Its methionine residue is found in the C-terminal
 - e) **Ubiquitin has a beta-Grasp fold**



Questions

- **Ubiquitin is a stable protein due to its:**
 - a) G1 beta-bulges
 - b) seven lysine residues
 - c) small molecular weight
 - d) **hydrophobic core and extensive hydrogen bonds**
 - e) interaction with a large number of enzymes

- **Which are the functional residues of ubiquitin:**
 - a) Lys
 - b) Met
 - c) Ser
 - d) **a and b**
 - e) b and c

Questions

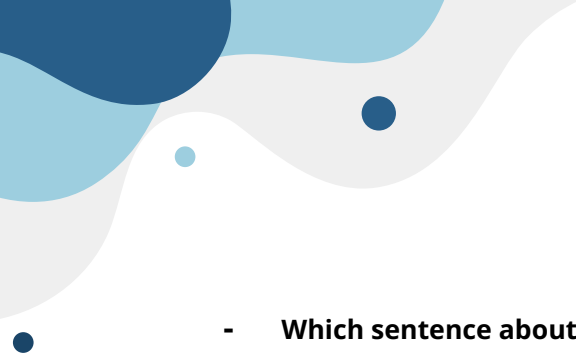
- **E1 enzyme is directly involved in:**

- a) The first step of the ubiquitylation process.
- b) The activation of the Ubiquitin
- c) A and B**
- d) The union of the ubiquitin to the target protein.
- e) None of the above

- **The adenylation that occurs in the adenylation site is:**

- 1) Only happens one time during the lifetime of each E1 activating enzyme.
- 2) One of the reagents is the catalytic cysteine of Ubiquitin.
- 3) Ions of calcium stabilize the charges of the substrates.
- 4) A biochemical reaction dependent of ATP.

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



Questions

- **Which sentence about the interfaces of E1 activating enzyme is FALSE?**
 - a) Interfaces are surfaces of interaction with the ubiquitin protein.
 - b) There are three different interfaces.
 - c) They are really conserved structurally among different species.
 - d) The only interaction between Ubiquitin and E1 amino acids are hydrogen bonds.**
 - e) None are false.

- **Which of the following is the less conserved protein:**
 - a) E1
 - b) E2**
 - c) Ubiquitin
 - d) All are conserved
 - e) None are conserved



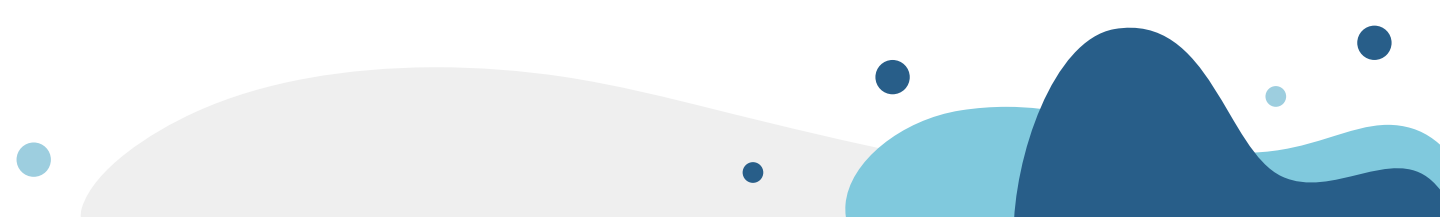
Questions

- **How many human E2 conjugating enzymes exist:**
 - a) One
 - b) Three
 - c) Four
 - d) Seven
 - e) None of the above**

- **Which is the most important bond between E1 and E2 :**
 - a) The hydrophobic bonds
 - b) The hydrogens bonds
 - c) The Cys-Cys tetramer**
 - d) There is no interaction between both
 - e) The salt bridges



**Thank you for
your attention**



Ubiquitin *system*

Yichao Hong, Carla Sandi, Laia Pareras