

# THE <u>UBIQUITIN</u> System

#### Structural Biology 2023-2024

Maria Igual, Raoudha Somrani, David Roura and Laia Dalmacio

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# UBIQUITINATION

### INTRODUCTION





#### Lysine residues





### PATHWAY





#### \*data based on the human proteome



# **UBIQUITIN.**



### **SCOP** CLASSIFICATION

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



<sup>1</sup> MQIFVKTLTG KTITLEVEPS DTIENVKAKI QDKEGIPPDQ QRLIFAGKQL EDGRTLSDYN <sup>61</sup> IQKESTLHLV LRLRGG



### UBIQUITIN INTERNAL INTERACTIONS









#### PDB: 1UBQ

#### Beta bulge loop





#### HYDROPHOBIC CORE



<sup>1</sup> MQIFVKTLTG KTITLEVEPS DTIENVKAKI QDKEGIPPDQ QRLIFAGKQL EDGRTLSDYN

<sup>61</sup> **I**QKEST**L**H**L**V LRLRGG



### **MULTIPLE SEQUENCE** ALIGNMENT









E1. **Ub-activating** enzyme



### **SCOP CLASSIFICATION**

**Class** Alpha and beta proteins  $(\alpha/\beta)$ 

**Fold** Activating enzymes of the ubiquitin-like proteins

Superfamily Activating enzymes of the ubiquitin-like proteins

**Family** Ubiquitin activation enzymes (UBA)



#### UFD

- Present in the C-terminus

- Recruitment of
- specific E2s

#### AAD

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

#### IAD

Present in theN-terminus Catalytically inactive



### **E1 DOMAINS**

#### SCCH

Carries the catalytic cysteine
Forms a thioester bond with Ub

#### FCCH

- Associates with IAD
- Non-functional

PDB: 6DC6 (H. sapiens)

### FCCH-SCCH INTERACTIONS





### Ub-E1 INTERACTIONS







### INTERFACEI





PDB: 6DC6



### INTERFACE II







### INTERFACE III













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



Lys 604



AAD domain residues (E1)

Ubiquitin







#### Van der Waals



Ubiquitin





PDB: 6DC6



#### PDB: 6DC6





### **MULTIPLE SEQUENCE** ALIGNMENT

#### **INTERFACE** I

	961	971	981	991 Asn928	1001	Glu938
Conservation	Manual Statistics - Manual Street	Manufacture and American Street	and the second s	Report of the local division of the		
Chimpanzee (P. troglodytes)	AIATTTSLVT	GLVNLELYKL	IDNKTDIEQY	KNGFVNLALP	FFG	FSEPIAS
Fission yeast (S. pombe)	AMCTSTAVVS	GLVCLELVKL	VDGKKKIEEY	KNGFFNLAIG	LFT	FSDPIAS
Human (Homo sapiens)	AIATTTAAVV	GLVCLELYKV	VQGHRQLDSY	KNGFLNLALP	FFG	FSEPLAA
Rabbit (Oryctolagus cuniculus)	AIATTTAAVV	GLVCLELYKV	VQGHRHLDSY	KNGFLNLALP	FFG	FSEPLAA
Mouse (Mus musculus)	AIATTTAAVV	GLVCLELYKV	VQGHQQLDSY	KNGFLNLALP	FFG	FSEPLAA
Dog (Canis lupus familiaris)	AIATTTAAVV	GLVCLELYKV	VHGHRQLDSY	KNGFLNLALP	FFG	FSEPLAA
Frog (Xenopus laevis)	AIATTTAAVV	GLVCLELYKI	IQGHRKLELY	KNGFLNLALP	FFG	FSEPIAA
Fish (Oryzias latipes)	AIATTTAAVV	GLVCLELIKV	VQGHKKLETY	KNGFMNLALP	FFA	YSEPIAA
Amoeba (D. discoideum)	ALVTTTAVVA	GFVCIELIKV	IQ-NKALEKY	KSTFMNLGIP	FFG	FVEPIAA

#### INTERFACE II

	221	231	. 241. Glu243	251	261
Conservation	- III. III. III.		Arg239	and the second second	Internet and the second
Chimpanzee (P. troglodytes)	GMVSDIEPD -	- GTVTMLDDN	RHGLEDGNEV	RESEVEGLDK	LNDGTLFKVE
Fission yeast (S. pombe)	GMIASITDD -	- GVVTMLEET	RHGLENGDFV	KFTEVKGMPG	LNDGTPRKVE
Human ( <i>Homo sapiens</i> )	AMVSMVTKDN	PGVVTCLDEA	RHGFESGDFV	SFSEVQGMVE	LNGNQPMEIK
Rabbit (Oryctolagus cuniculus)	TMVSMVTKDN	PGVVTCLDEA	RHGFESGDFV	SFSEVQGMTE	LNGNOPIEIK
Mouse (Mus musculus)	AMVSMVTKDN	PGVVTCLDEA	RHGFETGDFV	SFSEVQGMIQ	LNGCQPMEIK
Dog (Canis lupus familiaris)	AMVSMVTKDS	PGVVTCLDEA	RHGFESGDYV	SFSEVQGMVE	LNGSQPMEIK
Frog (Xenopus laevis)	AMISMITKDN	PGVVTCLDEA	RHGFETGDFV	TFTEVRGMTE	LNGCEPVEIK
Fish ( <i>Oryzias latipes</i> )	AMISMITKDT	AGVVTCLDEA	RHGFESGDYV	TFTEVQGMVE	LNGCQPVEIK
Amoeba (D. discoideum)	YMISSISQDK	EGIVTVVEEQ	KLQLLDGDLV	TFKEVNGMSA	LNDLPPQKIK

#### INTERFACE III

	581 Arg58	31591	601	60° 611	60 <sup>4</sup> 60 <sup>5</sup> 60 <sup>6</sup> 60 <sup>8</sup>	621	Tyr618 Ser621
Conservation			11	Lev.	My CHAST. OIL		
Chimpanzee (P. troglodytes)	TNALDNVDAR	TYVDRRCVFY	RKPLLESGT	L GI	KGNTQVII	PRL	TESYSSS
Fission yeast (S. pombe)	TNALDNVEAR	MYVDRRCVFF	EKPLLESGT	L G1	KGNTQVVV	PHL	TESYGSS
Human (Homo sapiens)	ANALDNVDAR	MYMDRRCVYY	RKPLLESGT	L G1	KGNVQVVI	PFL	TESYSSS
Rabbit (Oryctolagus cuniculus)	ANALDNVDAR	MYMDRRCVYY	RKPLLESGT	L G1	KGNVQVVI	PFL	TESYSSS
Mouse (Mus musculus)	ANALDNIDAR	MYMDRRCVYY	RKPLLESGT	L G1	KGNVQVVI	PFL	TESYSSS
Dog (Canis lupus familiaris)	ANALDNVDAR	MYMDRRCVYY	RKPLLESGT	L G1	KGNVQVVI	PFL	TESYSSS
Frog (Xenopus laevis)	ANALDNIDAR	MYMDRRCVYY	RKPLLESGT	L G1	KGNVQVVI	PDL	TESYSSS
Fish (Oryzias latipes)	ANALDNVDAR	MYMDRRCVYY	RKPLLESGT	L G1	KGNVQVVI	PFL	TESYSSS
Amoeba (D. discoideum)	CNALDNVEAR	LYMDSQCVYY	GKPLLESGT	L G1	KGNTQVVV	PHL	TESYSSS

	631 a a a a	641	651	661	671
Conservation	GIND ASP Prob GIUD	Cys632 *			
Chimpanzee (P. troglodytes)	RDPPEKSIPL	CITLRSEPNKI	DHTLAWAKSL	FOGYETDSAE	NVNMYLTOPN
Fission yeast (S. pombe)	<b>ODPPEKSFPI</b>	CTLKNEPNBL	EHTIAWARDL	FEGLFKOPID	NVNMYLSSPN
Human (Homo sapiens)	<b>ODPPEKSIPI</b>	CTLKNEPNAL	EHTLQWARDE	FEGLFKQPAE	NVNQYLTDPK
Rabbit (Oryctolagus cuniculus)	<b>QDPPEKSIPI</b>	CTLKNFPNAI	EHTLQWARDE	FEGLFKQPAE	NVNQYLTDPK
Mouse (Mus musculus)	QDPPEKSIPI	CTLKNFPNAI	EHTLQWARDE	FEGLFKQPAE	NVNQYLTDSK
Dog (Canis lupus familiaris)	QDPPEKSIPI	CTLKNFPNAI	EHTLQWARDE	FEGLFKQPAE	NVNQYLTDPK
Frog (Xenopus laevis)	QDPPEKSIP1	CTLKNFPNAI	EHTLQWARDE	FEGLFKQPSE	NVNQYLTDPK
Fish (Oryzias latipes)	QDPPEKSIPI	CTLKNFPNAI	EHTLQWARDE	FEGLFKQPPE	NAMQYLTDPK
Amoeba (D. discoideum)	RDPPEKGIPV	CTLHNFPNAI	EHTIQWARDT	FEGLFKNNAD	NVNSYLTNPA
					<ul> <li>A set of the set of</li></ul>

\*Catalytic cysteine

#### INTERFACE III

	951	lle891	961	971	981	991
Conservation	and the second second		Manual Statistics and the	Manufacture and States and Street	and the second s	Manual Local Distances in the
Chimpanzee (P. troglodytes)	TKFIAG	RIP	AIATTTSLVT	GLVNLELYKL	IDNKTDIEQY	KNGFVNLALP
Fission yeast (S. pombe)	TKFVAG	KIVP	AMCTSTAVVS	GLVCLELVKL	VDGKKKIEEY	KNGFFNLAIG
Human (Homo sapiens)	SKLIAG	KIIP	AIATTTAAVV	GLVCLELYKV	VQGHRQLDSY	KNGFLNLALP
Rabbit (Oryctolagus cuniculus)	SKLIAG	KIP	AIATTTAAVV	GLVCLELYKV	VQGHRHLDSY	KNGFLNLALP
Mouse (Mus musculus)	SKLIAG	KIIP	AIATTTAAVV	GLVCLELYKV	VQGHQQLDSY	KNGFLNLALP
Dog (Canis lupus familiaris)	SKLIAG	KIIP	AIATTTAAVV	GLVCLELYKV	VHGHRQLDSY	KNGFLNLALP
Frog (Xenopus laevis)	SKLIAG	KIIP	AIATTTAAVV	GLVCLELYKI	IQGHRKLELY	KNGFLNLALP
Fish ( <i>Oryzias latipes</i> )	SKLIAG	KIP	AIATTTAAVV	GLVCLELIKV	VQGHKKLETY	KNGFMNLALP
Amoeba (D. discoideum)	TKGIAG	KIIP	ALVTTTAVVA	GFVCIELIKV	IQ-NKALEKY	KSTFMNLGIP



**E2.** Ub-conjugating enzyme

 $\mathbf{0}\mathbf{4}$ 





### **SCOP CLASSIFICATION**





### **TERTIARY** STRUCTURE



 $\alpha$ -helix

3<sub>10</sub>-helix

🛑 Loop

E2 Topological diagram







## INTERACTIONS

E2E1Ubiquitin



# Intramolecular interactions

#### Van der Waals





E2 residues

PDB: 4112 (S. pombe)











E1 residues

PDB: 4112

E1 residues



#### Van der Waals















PDB: 4112



PDB: 4112



PDB: 4112

E2

E1



### E2 - E1 - Ub Interactions

Van der Waals

E2 residues
 Ubiquitin residues
 E1 residues









PDB: 4112

### **MULTIPLE SEQUENCE** ALIGNMENT

	1 .0	3 JSH Asn7	11 w10 at 1	21	31 02 <sup>8</sup> 11 <sup>30</sup> 23 <sup>1</sup>	41
Conservation			Let Ale		AST Let Ph	Statistics Statistics
Mouse (Mus musculus)	MAL	KRIH	KELNDLAR - D	PPAQCSAG	PVGDDMFHWQ	ATIMGPNDSP
Frog (Xenopus laevis)	MAL	KRIH	KELNDLAR-D	PPAQCSAG	PVGDDMFHWQ	ATIMGPNDSP
Bovine ( <i>Bos taurus</i> )	MAL	KRIH	KELNDLAR-D	PPAQCSAG	PVGDDMFHWQ	ATIMGPNDSP
Rat (Rattus rattus)	MAL	KRIH	KELNDLAR-D	PPAQCSAG	PVGDDMFHWQ	ATIMGPNDSP
Pig (Sus scrofa)	MAL	KRIH	KELNDLAR-D	PPAQCSAG	PVGDDMFHWQ	ATIMGPNDSP
Human (Homo sapiens)	MAL	KRIQ	KELSDLQR-D	PPAHCSAG	PVGDDLFHWQ	ATIMGPPDSA
Fission yeast (S. pombe)	MAL	KRIN	RELADLGK-D	PPSSCSAG	PVGDDLFHWQ	ATIMGPADSP
Baker's yeast (S. cerevisiae)	MSSS	KRIA	KELSDLER - D	PPTSCSAG	PVGDDLYHWQ	ASIMGPADSP

R	31	41	51	61 Asp59 62 63	71
Conservation	and the second division of the second divisio	Statistics. Shirts	Manufacture and American Street of S	Phr Lyst	States - States - States
Mouse (Mus musculus)	PVGDDMFHWQ	ATIMGPNDSP	YQGGVFFLTI	HFPTDYPFKP	PKVAFTTRIY
Frog (Xenopus laevis)	PVGDDMFHWQ	ATIMGPNDSP	YQGGVFFLTI	HFPTDYPFKP	PKVAFTTRIY
Bovine ( <i>Bos taurus</i> )	<b>PVGDDMFHWQ</b>	ATIMGPNDSP	YQGGVFFLTI	HFPTDYPFKP	PKVAFTTRIY
Rat (Rattus rattus)	PVGDDMFHWQ	ATIMGPNDSP	YQGGVFFLTI	HFPTDYPFKP	PKVAFTTRIY
Pig (Sus scrofa)	PVGDDMFHWQ	ATIMGPNDSP	YQGGVFFLTI	HFPTDYPFKP	PKVAFTTRIY
Human ( <i>Homo sαpiens</i> )	PVGDDLFHWQ	ATIMGPPDSA	YQGGVFFLTV	HFPTDYPFKP	PKIAFTTKIY
Fission yeast (S. pombe)	PVGDDLFHWQ	ATIMGPADSP	YAGGVFFLSI	HFPTDYPFKP	PKVNFTTRIY
Baker's yeast (S. cerevisiae)	PVGDDLYHWQ	ASIMGPADSP	YAGGVFFLSI	HFPTDYPFKP	PKISFTTKIY

	71	81 50590	91 4000	101	111
Conservation	Manager Manager Manager	Seroo	* Arg90	Contraction of Contra	and the second s
Mouse (Mus musculus)	PKVAFTTRIY	HPNINS-NGS	ICLDILRSQW	SPALTISKVL	LS-ICSLLCD
Frog (Xenopus laevis)	PKVAFTTRIY	HPNINS-NGS	ICLDILRSQW	SPALTISKVL	LS-ICSLLCD
Bovine ( <i>Bos taurus</i> )	PKVAFTTRIY	HPNINS-NGS	ICLDILRSQW	SPALTISKVL	LS-ICSLLCD
Rat (Rattus rattus)	PKVAFTTRIY	HPNINS-NGS	ICLDILRSQW	SPALTISKVL	LS-ICSLLCD
Pig (Sus scrofa)	PKVAFTTRIY	HPNINS-NGS	ICLDILRSQW	SPALTISKVL	LS-ICSLLCD
Human (Homo sapiens)	PKIAFTTKIY	HPNINS-NGS	ICLDILRSQW	SPALTVSKVL	LS-ICSLLCD
Fission yeast (S. pombe)	PKVNFTTRIY	HPNINS-NGS	ICLDILRDQW	SPALTISKVL	LS-ICSLLTD
Baker's yeast (S. cerevisiae)	PKISFTTKIY	HPNINA-NGN	ICLDILKDQW	SPALTLSKVL	LS-ICSLLTD

	111	121 15 16 18 19 12	131 His125 Lys128	141	151
Conservation		pro Asp pro Leu pro	and the second second second	and the second s	
Mouse (Mus musculus)	LS-ICSLLCD	PNPDDPLVPE	IARIYKTDRE	KYNRLAREWT	QKYAM
Frog (Xenopus laevis)	LS-ICSLLCD	PNPDDPLVPE	IARIYKTDRE	KYNRIAREWT	QKYAM
Bovine ( <i>Bos taurus</i> )	LS-ICSLLCD	PNPDDPLVPE	IARIYKTDRE	KYNRIAREWT	QKYAM
Rat (Rattus rattus)	LS-ICSLLCD	PNPDDPLVPE	IARIYKTDRE	KYNRIAREWT	QKYAM
Pig (Sus scrofa)	LS-ICSLLCD	PNPDDPLVPE	IARIYKTDRD	KYNRISREWT	QKYAM
Human (Homo sapiens)	LS-ICSLLCD	PNPDDPLVPD	IAQIYKSDKE	KYNRHAREWT	QKYAM
Fission yeast (S. pombe)	LS-ICSLLTD	PNPDDPLVPE	IAHVYKTDRS	RYELSAREWT	RKYAI
Baker's yeast (S. cerevisiae)	LS-ICSLLTD	ANPDDPLVPE	IAHIYKTDRP	KYEATAREWT	K K Y A V

#### \*Catalytic cysteine



Predicted: P25867 - D.melanogaster

Predicted: P43102 - C.albicans

PDB ID: 1QCQ - S.cerevisiae

PDB ID: 4112 - S.pombe

# 06. CONCLUSIONS



- The **ubiquitination process** implies the ubiquitin and three enzymes
  - $E1 \rightarrow activates the ubiquitin$
  - **E2**  $\rightarrow$  transfer the ubiquitin from the E1 enzyme to the substrate
  - **E3**  $\rightarrow$  selects the substrate and assists in the transfer of ubiquitin from E2 to the substrate
- **Ubiquitin**: highly structurally and sequentially conserved multifunctional protein.
  - Presents a hydrophobic core and hydrogen bonds that are involved in its stability.
- **E1 Ub-activating enzyme** is a multidomain protein that catalyses the ubiquitin adenylation and the formation of a thioester bond during the first step of ubiquitination. Due to its relevance, both its structure and sequence are preserved among species.
- **E2 Ub-conjugating enzyme** is involved in the transthiolation step through its catalytic cysteine, and is the intermediate step before the substrate ubiquitination.

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### **PEM QUESTIONS**

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

#### 2. E1 enzyme is involved in:

- i. The activation of the ubiquitin
- ii. E2 recruitment
- iii. The thioester bond transfer
- iv. The union of the ubiquitin to the target protein
- a) 1,2 and 3
- b) 1 and 3
- c) 2 and 4
- d) 4
- e) 1,2,3 and 4

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

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

#### 4. Which is the E1 domain that recruits E2?

- a) SCCHb) FCCHc) UFDd) IADa) IAD
- e) IAD

#### 5. Mark the correct answer about E2:

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

#### 6. Mark the correct answer about ubiquitin residues conservation.

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

#### e) All options are incorrect

#### 7. About the ubiquitination mark the correct answer.

- i. In the ubiquitination are involved two enzymes.
- ii. ATP is only necessary to do the ubiquitin conjugation
- iii. E3 is involved in ubiquitin activation
- iv. E2 is involved in ubiquitination conjugation
- a) 1,2 and 3
- b) 1 and 3
- c) 2 and 4
- d) 4 e) 1,2,3 and 4

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

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

#### 9. How many Lysine residues has ubiquitin?



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

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



# THE <u>UBIQUITIN</u> System

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Maria Igual, Raoudha Somrani, David Roura and Laia Dalmacio