

# Ubiquitination system



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# Summary

## **1. Introduction**

## **2. Ubiquitin**

- 2.1. Classification
- 2.2. Secondary structure
- 2.3. Residues

## **3. E3 enzymes**

- 3.1. RING
- 3.2. HECT

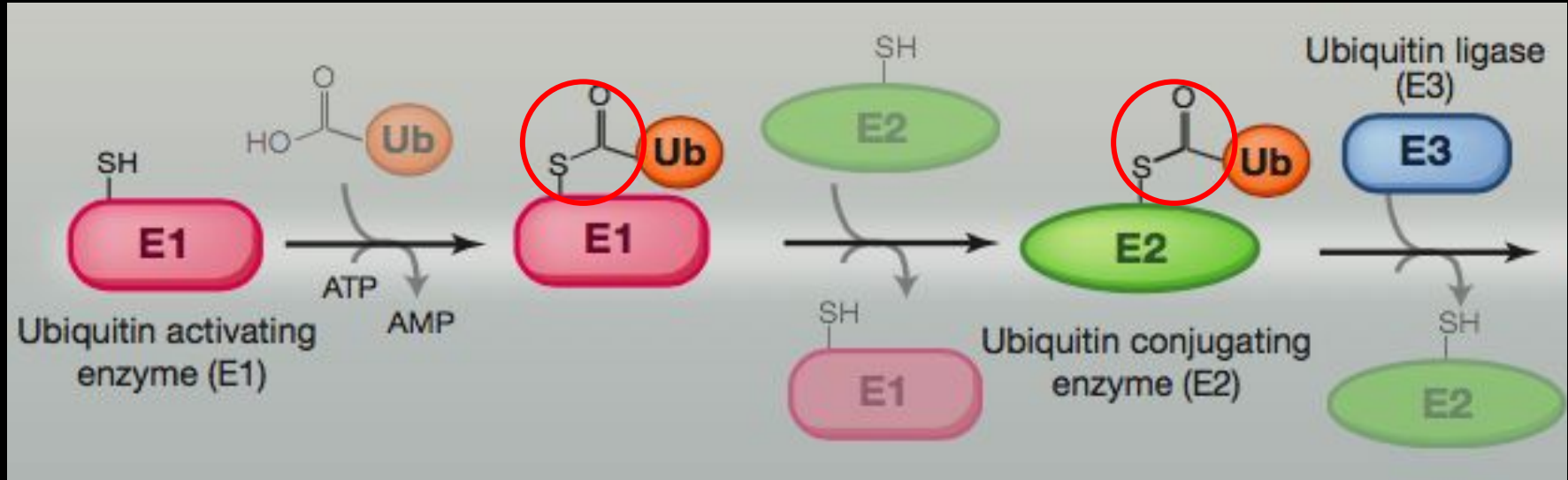
## **4. PARKIN**

- 4.1 Structure
- 4.2 Mutations
- 4.3 Parkinson's disease

## **5. CONCLUSIONS**

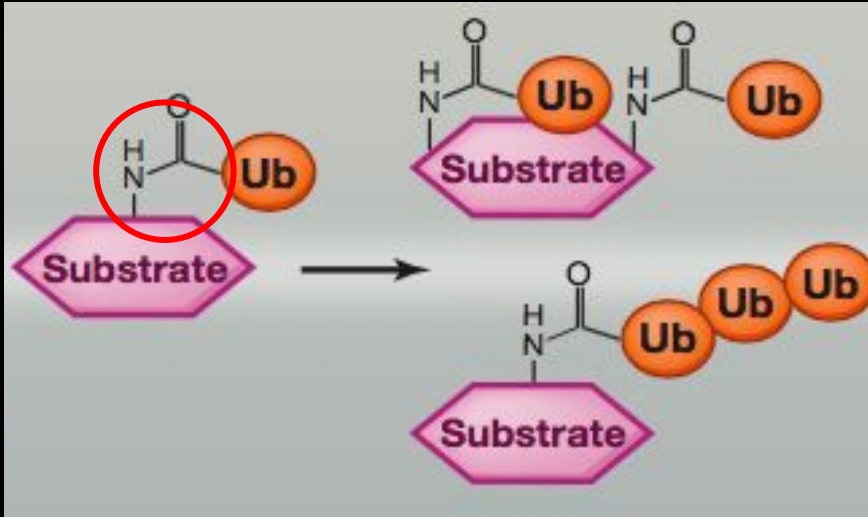
# 1. Introduction

# Ubiquitination process



Morreale F, Walden H. SnapShot: Types of ubiquitin ligases. 2016.

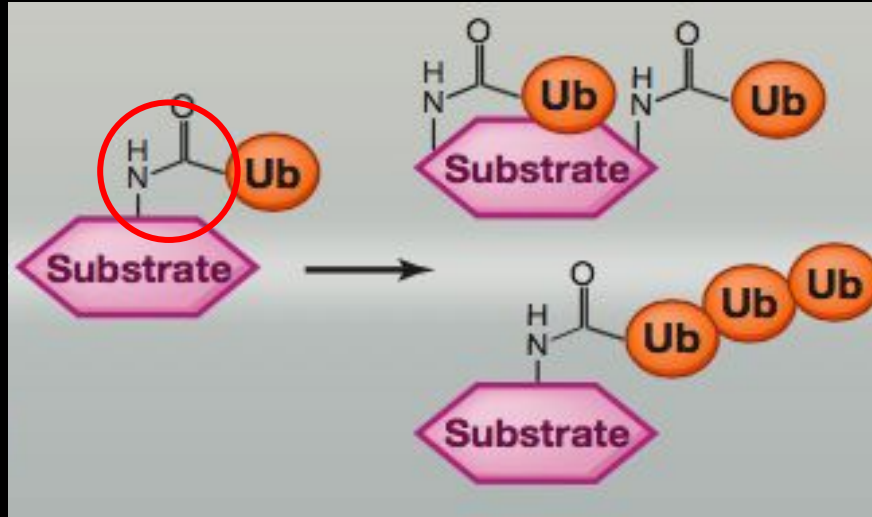
# Ubiquitination process



Degradation via the proteasome  
Cellular location and signalling  
DNA repair damage  
Intracellular trafficking  
Etc

Morreale F, Walden H. SnapShot: Types of ubiquitin ligases. 2016.

# Ubiquitination process



Degradation via the proteasome  
Cellular location and signalling  
DNA repair damage  
Intracellular trafficking  
Etc

Morreale F, Walden H. SnapShot: Types of ubiquitin ligases. 2016.

| E1 |
|----|
| 2  |

| E2 |
|----|
| 40 |

| E3  |
|-----|
| 600 |

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## 2. Ubiquitin

# Ubiquitin classification (SCOP)

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



PDB ID: 1UBQ

# Genes

**UBB**

**Poly-Ubq**  
**3 direct repeats**  
**17.p11.2**

**UBC**

**Poly-Ubq**  
**9 direct repeats**  
**12p24.31**

**UBA52**

**Ubiquitin fused**  
**19p13.11**

**RPS27A**

**Ubiquitin fused**  
**2p16.1**

**1**

**MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG**

**76**

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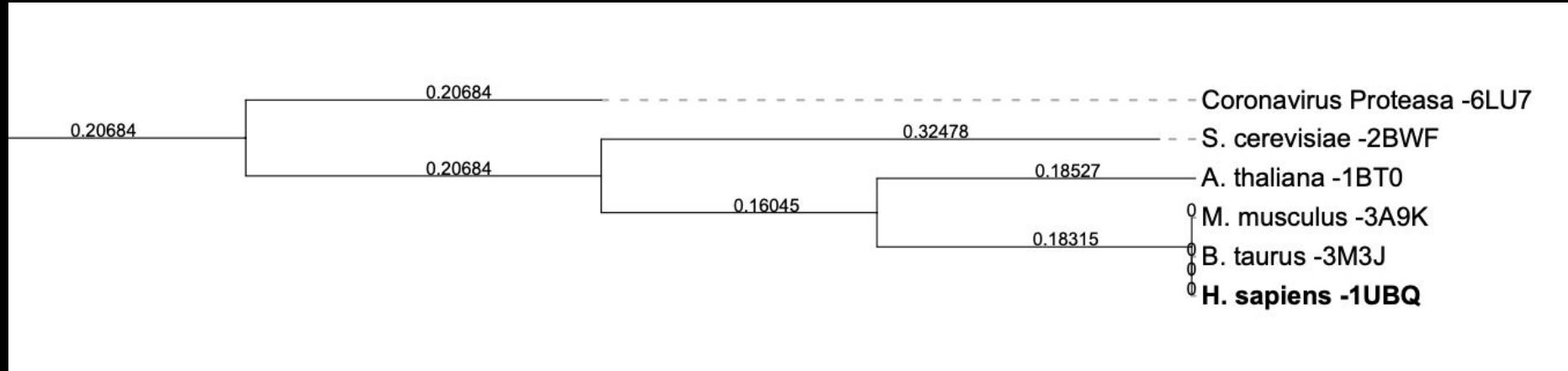
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# Evolution

CLUSTAL 2.1 multiple sequence alignment

```
M._musculus_-3A9K      --MQIFVKLTG-KTITLVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGGD
B._taurus_-3M3J       --MQIFVKLTG-KTITLVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG-
A._thaliana_-1BT0      --MLIKVKLTG-KEIEIDIEPTDTIDRIKERVEEKEGIPPVQQLIYAGKQLADDKTAKDYNIEGGSVLHLVLALRGG-
S._cerevisiae_-2BWF    LDMSLNIIHKSGQDKWEVNVAPESTVLQFKEAINKANGIPVANQRLIYSGKILKDDQTVESYHIQDGHSVHLVKSQP---
H._sapiens_-1UBQ       --MQIFVKLTG-KTITLVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG-
                        *  :  ::  :* .   ::: * .*: ..*   ::. :***  :*****:** * *.:* ..** :   :***
```

# Phylogenetic tree



# Phylogeny



 *B. taurus*  
 *M. musculus*  
 *S. cerevisiae*  
 *A. thaliana*  
 *H. sapiens*

Sc: 8.80 i RMS: 0.77

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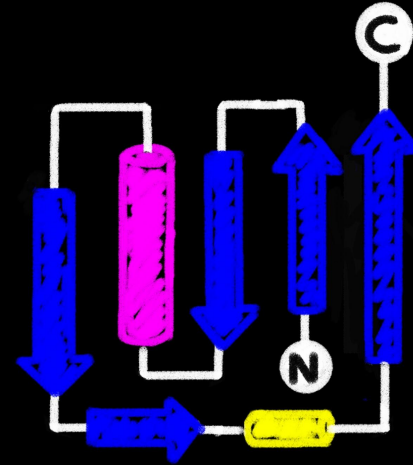
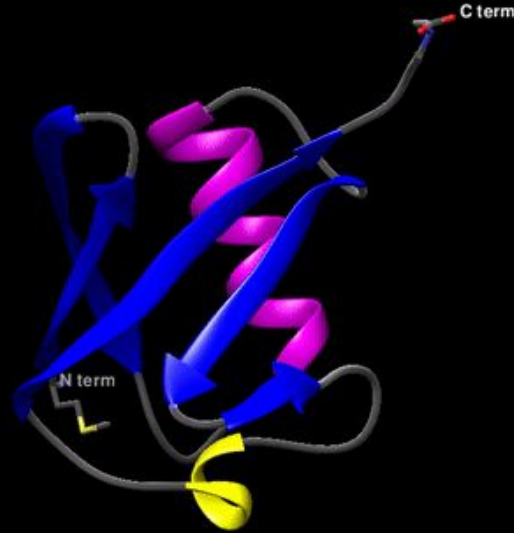
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# Secondary structure



PDB ID: 1UBQ

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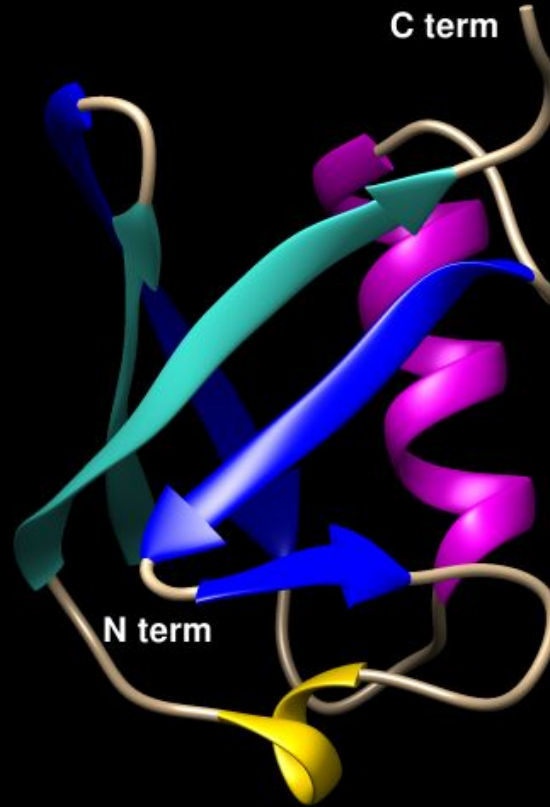
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# Secondary structure

Mixed  $\beta$ -sheet

$\alpha$ -helix

$3_{10}$  helix



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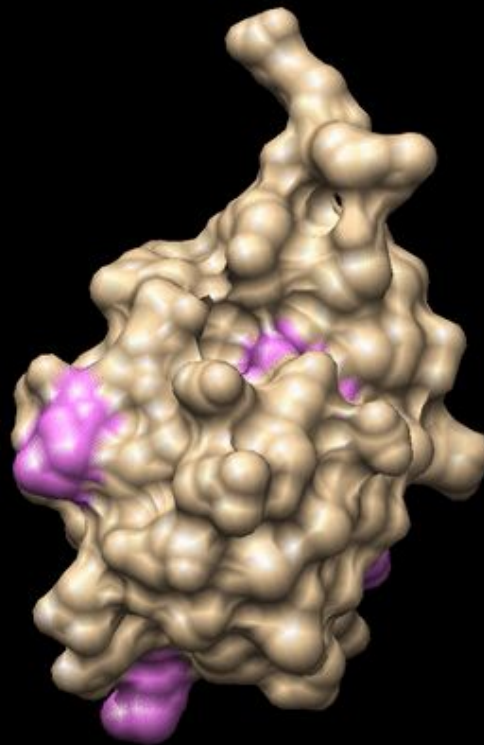
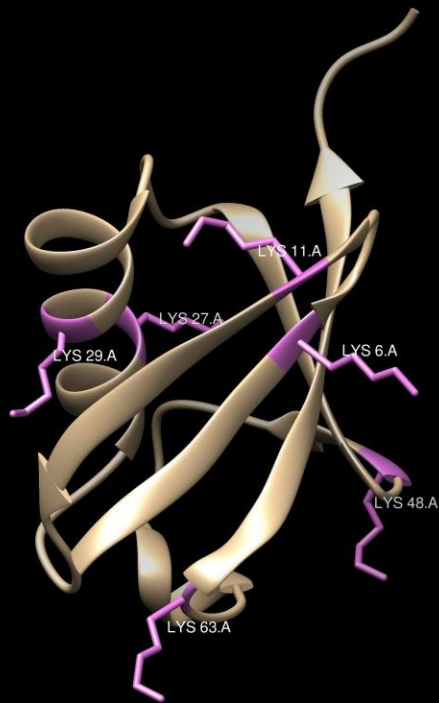
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# Lysines



MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**AKIQDKEGIPPDQQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRRLRGG

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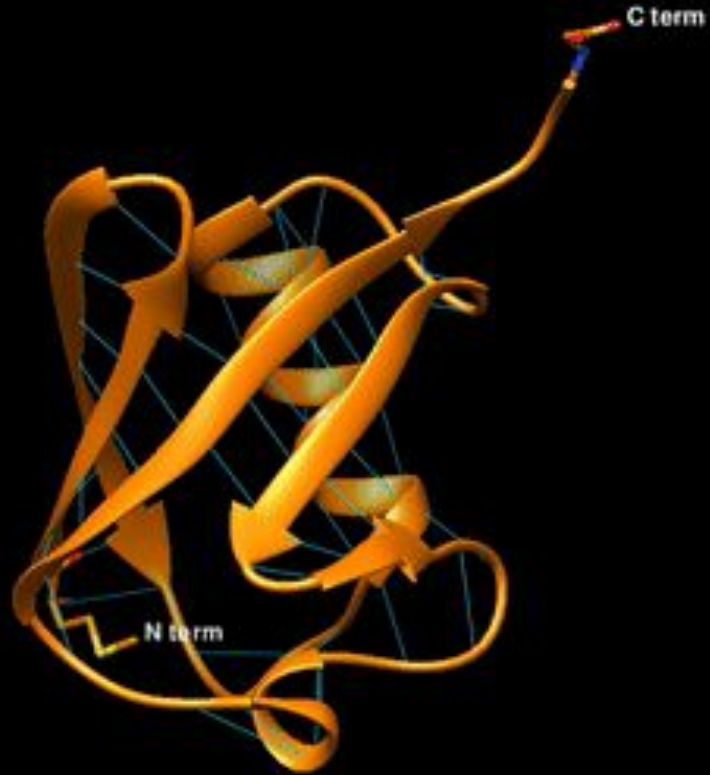
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# Hydrogen bonds



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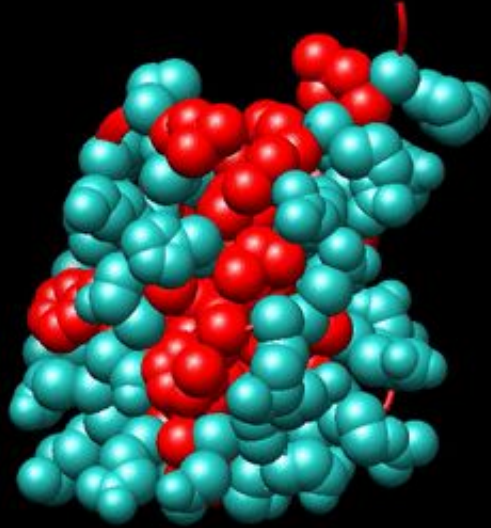
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# Hydrophobic core



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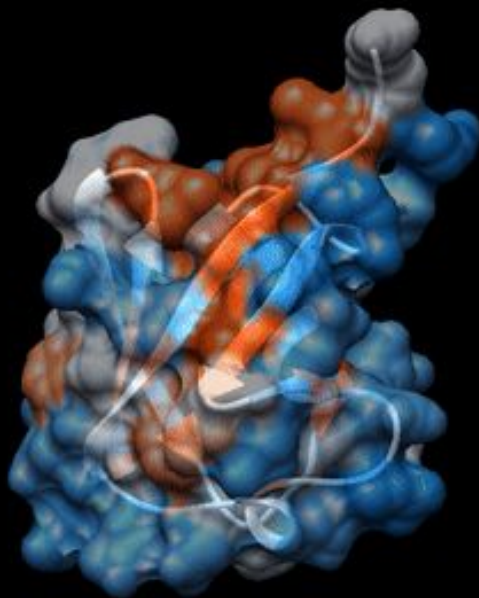
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# Hydrophobic surface



PDB ID: 1UBQ

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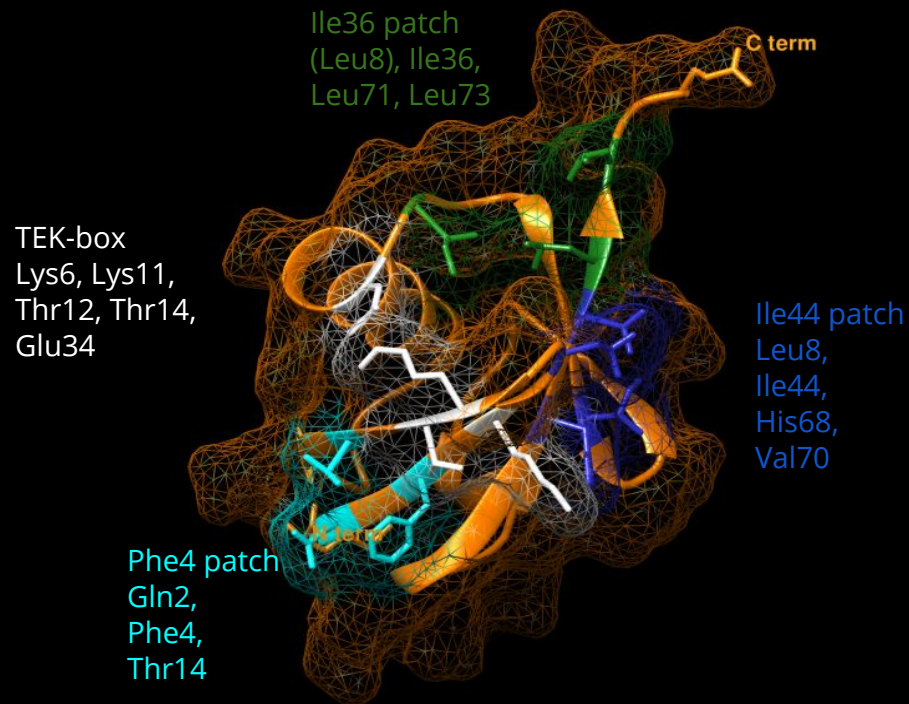
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# Patches



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# Ubiquitin family

CLUSTAL 2.1 multiple sequence alignment

```
2bkr      -----SMLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQRLIYSGKQMDEKTAADYKILGGSVLHLVLALRGG
3dbl      GSRRASVGSGGSMLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQRLIYSGKQMDEKTAADYKILGGSVLHLVLQLRGG
1p3q      -----MQIFVKLTGTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLR--
1xd3      -----MQIFVKLTGTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-
1nbf      -----MQIFVKLTGTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-
1ubq      -----MQIFVKLTGTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG
1aar      -----MQIFVKLTGTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG
2zeq      -----MGMIVFVRFNSSYGFPVEVSDTSILQLKEVVAKRQGV PADQLRVIFAGKELPNH LTVQNC DLEQQSIVHIVQRPRRR
          * : * : . : : : : . : . : * : . : * : * : * : * : * : * : * : * : * : * : *
```

# Ubiquitin family



Sc: 8.37 Å RMS: 1.51 Å

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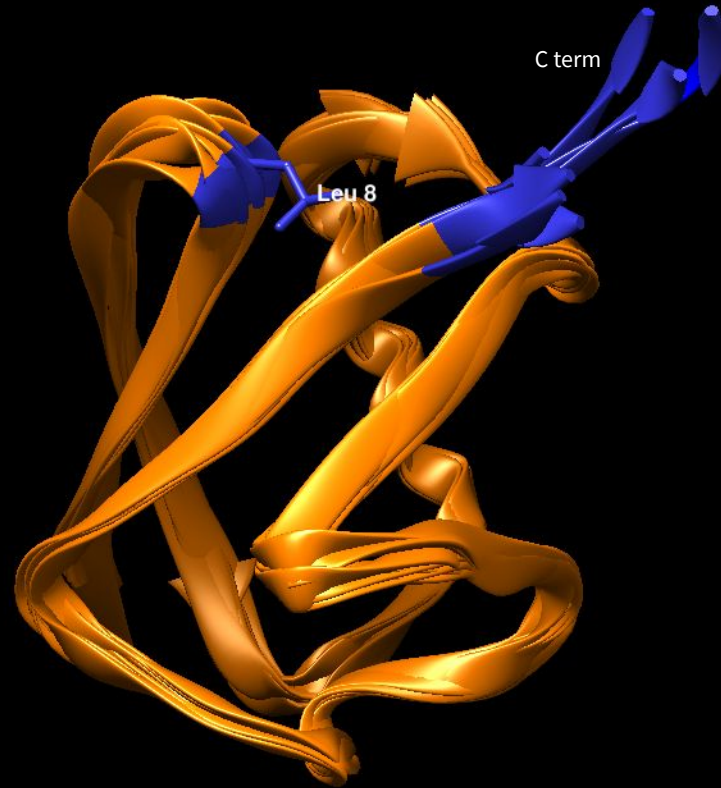
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# Ubiquitin family



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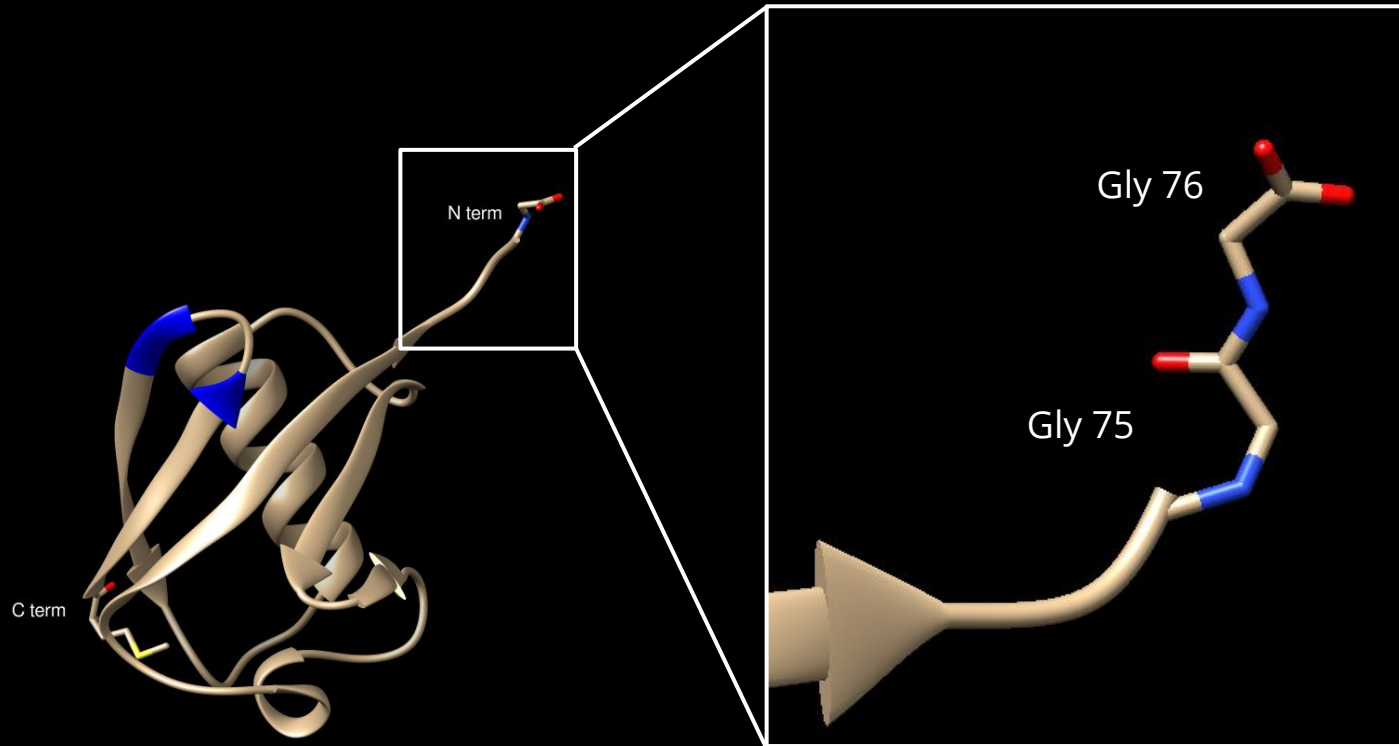
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# C-terminal



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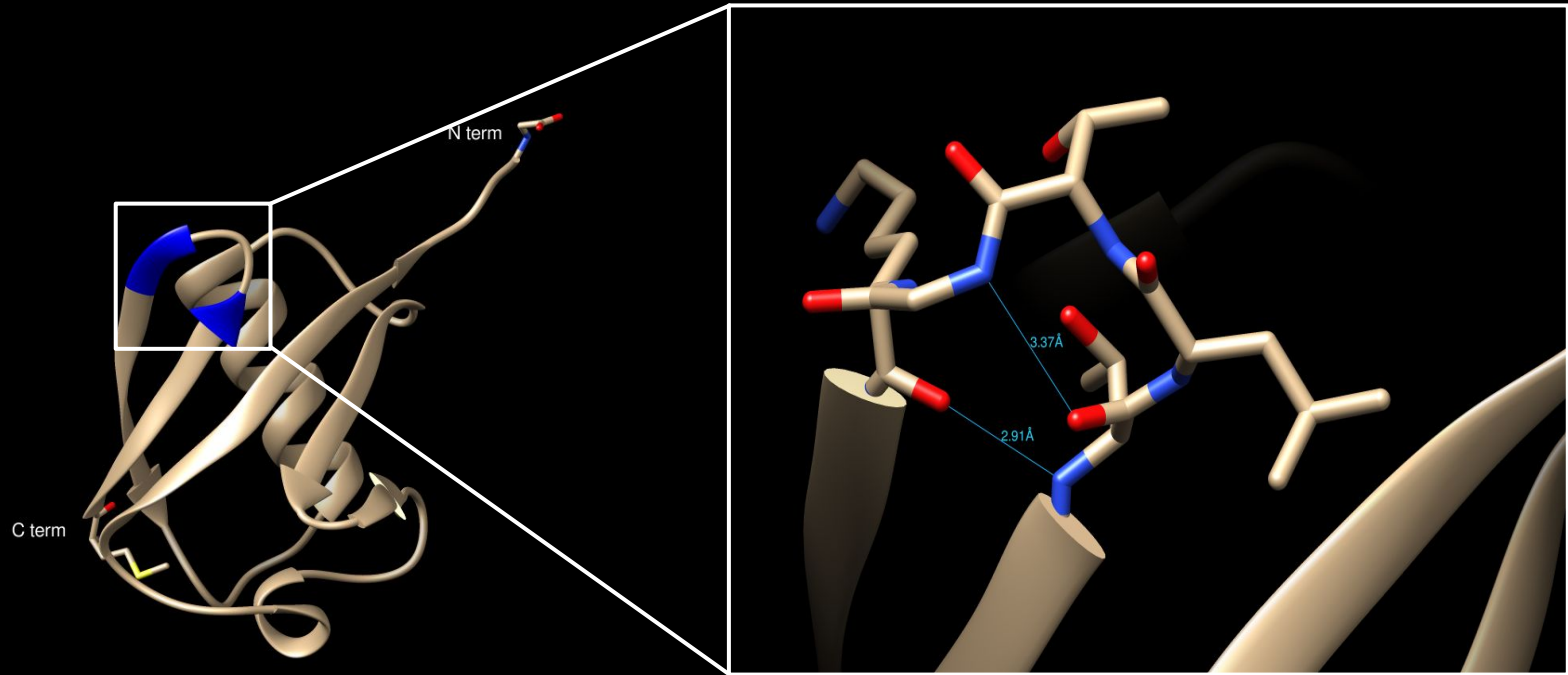
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# Beta bulge loop



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**UBIQUITIN**

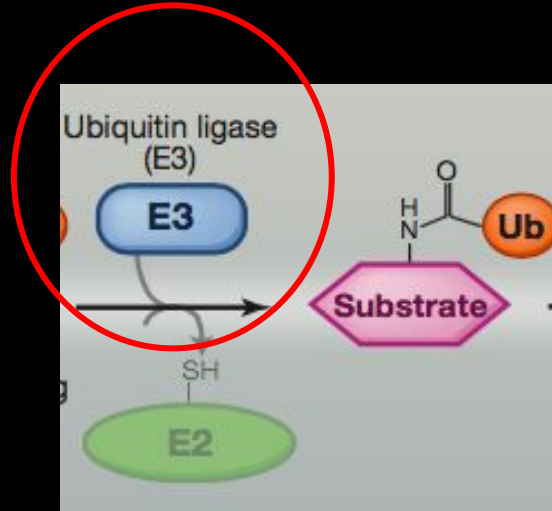
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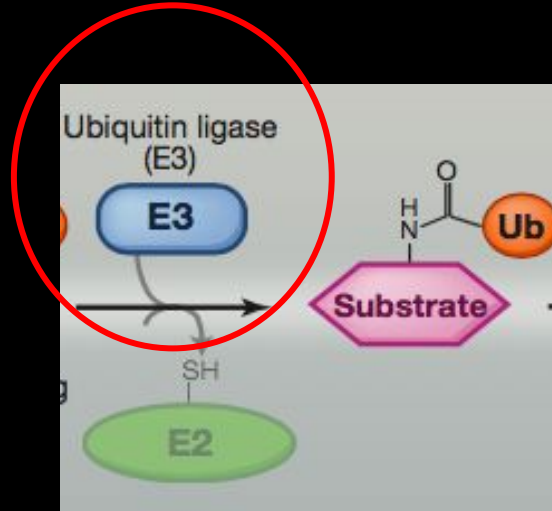
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## 3. E3 ENZYMES





**HECT**

**RING**

**RBR**

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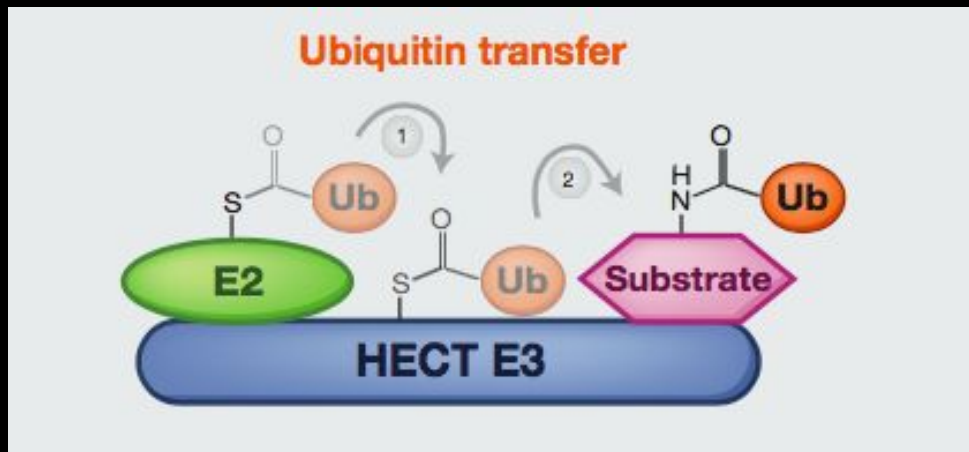
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# HECT characteristics



Morreale F, Walden H. SnapShot: Types of ubiquitin ligases. 2016.

|                   |                            |                            |
|-------------------|----------------------------|----------------------------|
| NEDD4             | NEDD4<br>ITCH<br>WWP       | SMURF<br>HECTW             |
| WW motifs         |                            |                            |
| HERC              | HERC 1<br>HERC 2<br>HERC 3 | HERC 4<br>HERC 5<br>HERC 6 |
| RCC-1 like domain |                            |                            |
| Other HECTs       | HUWE 1<br>UBE3<br>HECTD    | AREL1<br>G2E3<br>1BR5      |
| Varied domains    |                            |                            |

# E6AP - Structure

C-lobe

Hinge region

Cys280

N-lobe

PDB ID: 1D5F

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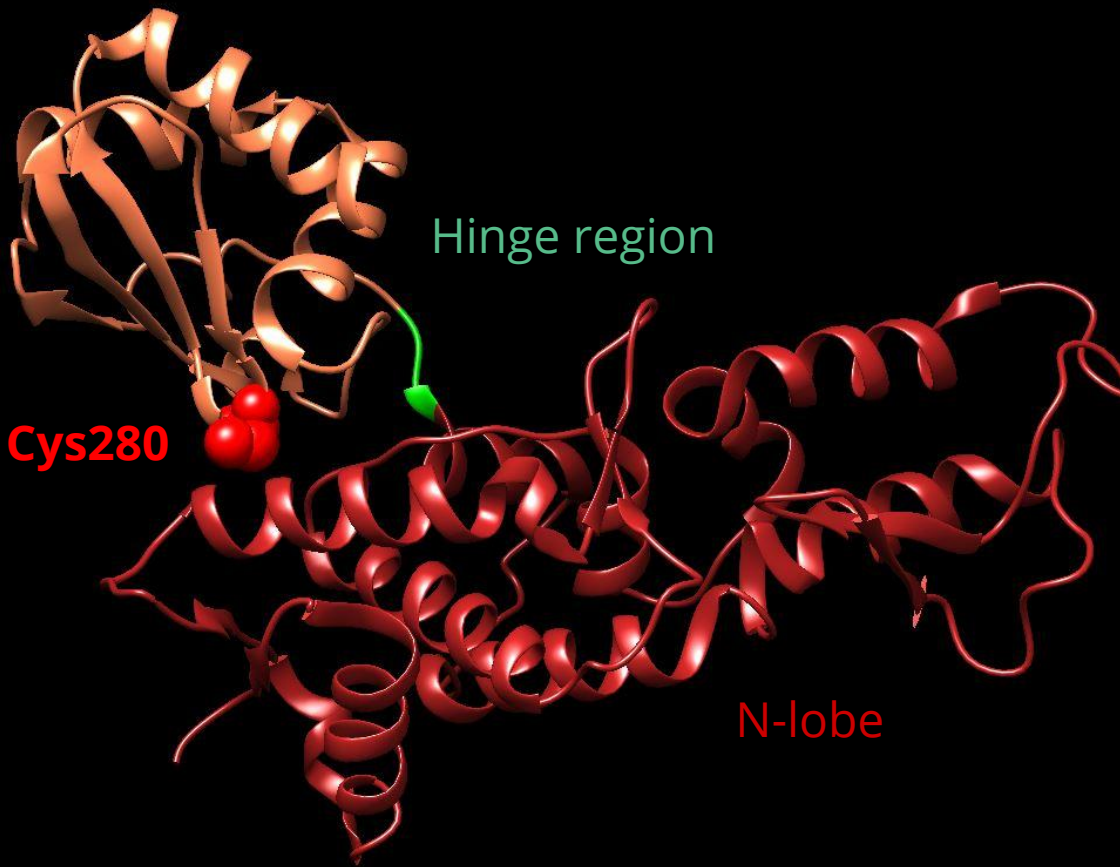
UBIQUITIN

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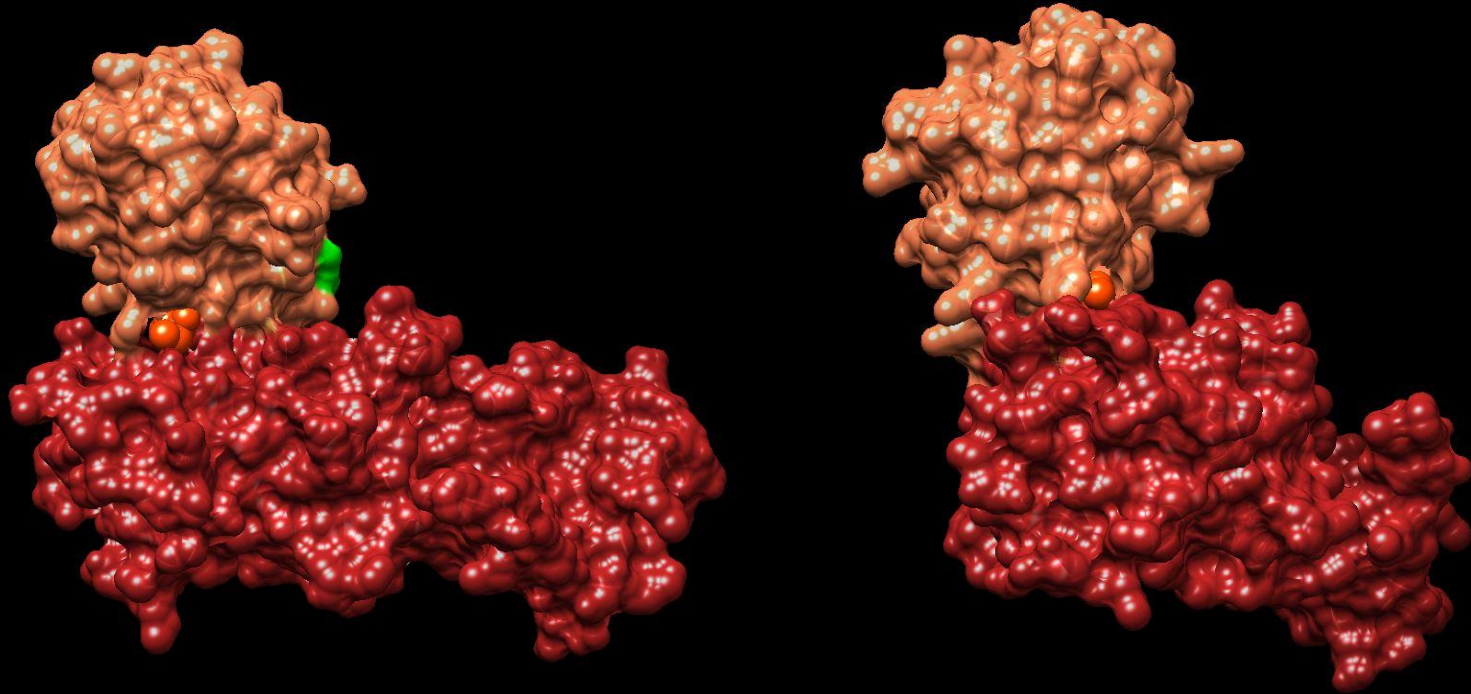
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# E6AP - Structure



PDB ID: 1D5F

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# E6AP - Catalytic Cysteine

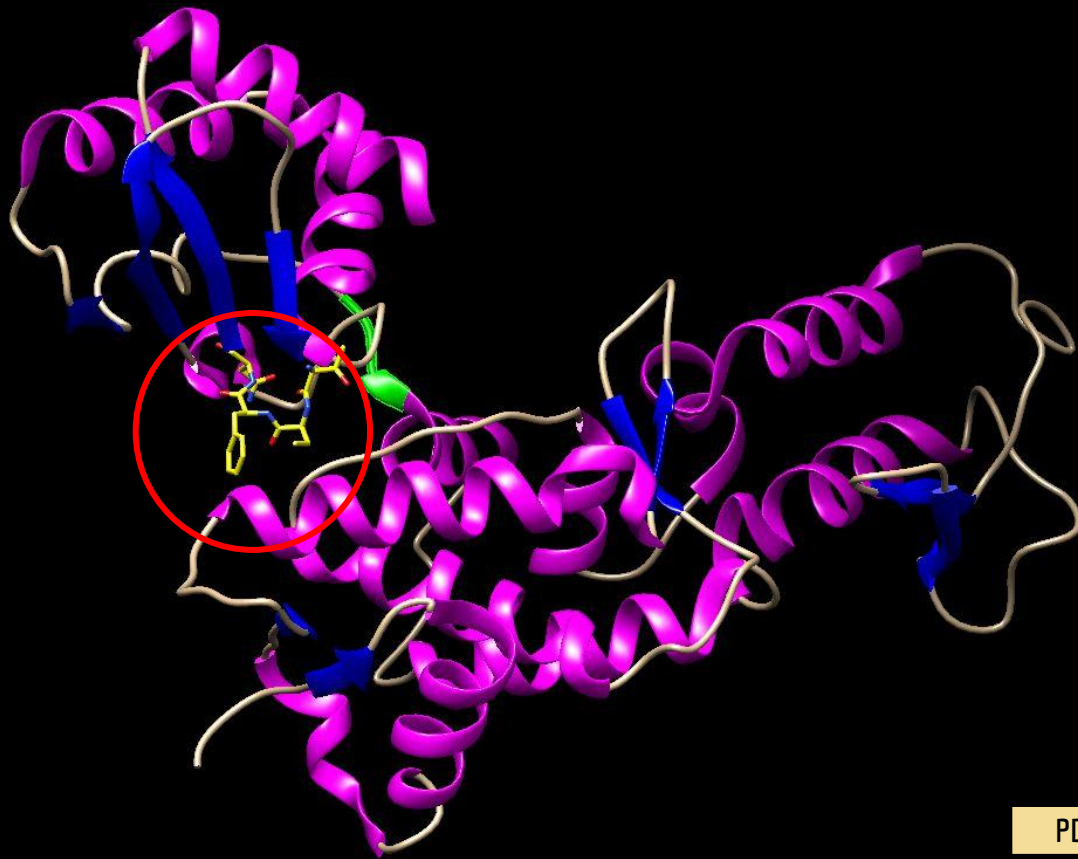
Active loop

Thr819

**Cys280**

Phe821

Asn822



PDB ID: 1D5F

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## E6AP - Catalytic Cysteine

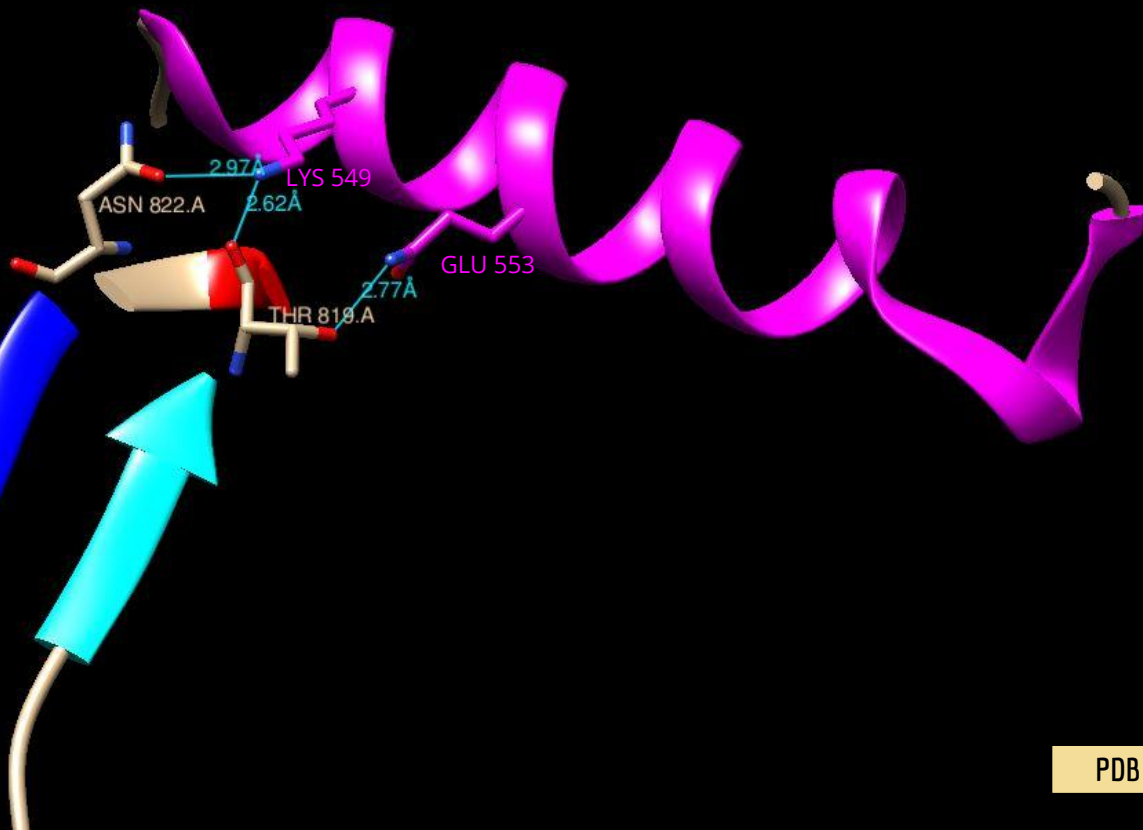
## Active loop

Thr819

**Cys280**

Phe821

Asn822



**PDB ID: 1D5F**

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# PARKIN

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# E6AP - Sequence alignment

CLUSTAL 2.1 multiple sequence alignment

```
WWP1-1ND7      -----HMG---FRWKLAFHR-YLCQSNA--LPSHVKINVSQRQTLFED
NEDD4-2XBF      -----GPLGSRDYKRKYEFFRRKLKKQND--IPNKFEMKLRRATVLED
Smurf2-1ZVD      -----KRDLVQKLKILRQELSQQQP--QAGHCRIEVSREEIFEE
HuWE1-3H1D      MGSSHHHHHHSSGLEVLFGQPHMDFDVKRKYFRQELERLDEGLRKEDMAVHVRRDHVFED
E6AP-1D5F      -----QLNP-----YLRLKVRRDHIIDD
                                   : :                :.: *      :.:.:
```

[...]

```
WWP1-1ND7      KFCIEKVGKDTW-LPRSHTCFNRLDLPPYKSYEQLKEKLLFAIEET-E-----
NEDD4-2XBF      SFTVEQWGTPEK-LPRAHTCFNRLDLPPYESFEELWDKLQMAIENT-QGFDGVD
Smurf2-1ZVD      LFTIHQIDACTNNLPKAHTCFNRIDIPPYESYEKLYEKLLTAIEET-CGFAVE-
HuWE1-3H1D      KFQIHRDDRSTDRLPSTAHTCFNQDLPAYESFEKLRHMLLLAIQEASEGFGLA-
EGAP-1D5F      -MIIAKNGPDTERLPTSHTCFNVLLLPEYSSKEKLKERLLKAITYA-KGFGML-
      : : : .      ** :***** : : * *. * *: * . * ** :
```

# E6AP - Sequence alignment

CLUSTAL 2.1 multiple sequence alignment

```
WWP1-1ND7      -----HMG---FRWKLAHFR-YLCQSNA--LPSHVKINVSQRQTLFED
NEDD4-2XBF      -----GPLGSRDYKRKYEFFRRKLKKQND--IPNKFEMKLRRATVLED
Smurf2-1ZVD      -----KRDLVQKLKILRQELSQQQP--QAGHCRIEVSREEIFEE
HuWE1-3H1D      MGSSHHHHHHSSGLEVLFGQPHMDFDVKRKYFRQELERLDEGLRKEDMAVHVRRDHVFED
E6AP-1D5F       -----QLNP-----YLRLKVRRDHIIDD
                                   : :                :. : *   :. : :
```

[...]

```
WWP1-1ND7      KFCIEKVGKDTW-LPRSHTCFNRRLDLPPYKSYEQLKEKLLFAIEET-E-----
NEDD4-2XBF      SFTVEQWGTPEK-LPRAHTCFNRRLDLPPYESFEELWDKLQMAIENT-QGFDGVD
Smurf2-1ZVD      LFTIHQIDACTNNLPKAHTCFNRIDIPPYESYEKLYEKLLTAIEET-CGFAVE-
HuWE1-3H1D      KFQIHRDDRSTDRLPSTAHTCFNQDLDPAYESFEKLRHMLLLAIQEASEGFGLA-
EGAP-1D5F       -MIIAKNGPDTERLPTSHTCFNVLLLPEYSSKEKLKERLLKAITYA-KGFGML-
               : : : .      ** :***** : : * * . * : * . * ** :
```



PDB ID: 2Y1M

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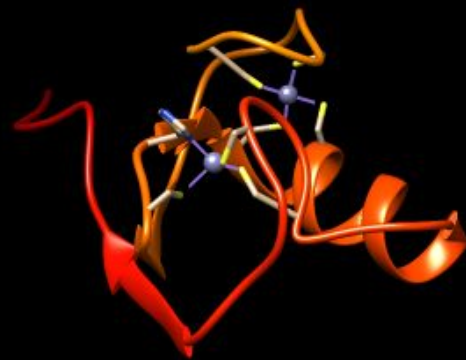
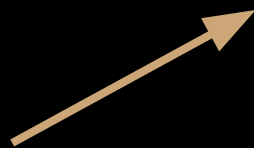
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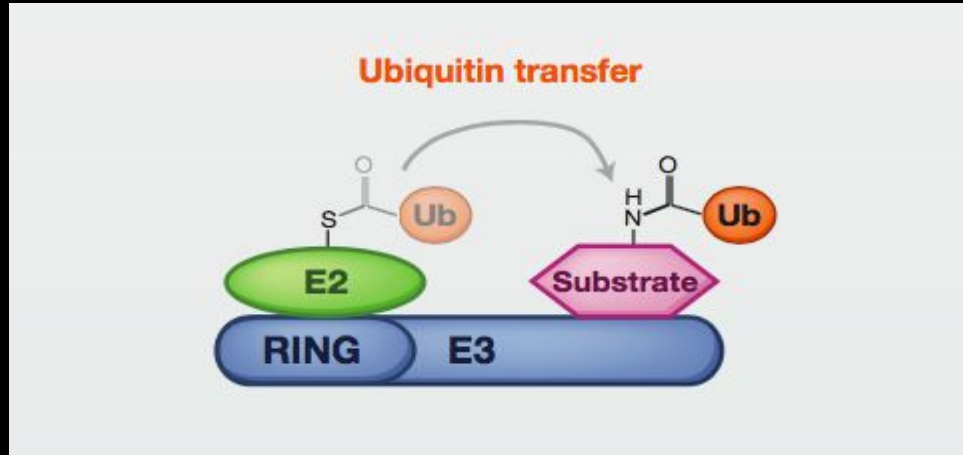
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# RING characteristics



Morreale F, Walden H. SnapShot: Types of ubiquitin ligases. 2016.

Monomers

Single polypeptide

Homodimers

c-Cbl

E3 complex

Heterodimers

APC

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## RING domain. c-Cbl

Canonical sequence

**Cys**-X<sub>2</sub>-**Cys**-X<sub>(9-39)</sub>-**Cys**-X<sub>(1-3)</sub>-**His**-X<sub>(2-3)</sub>-**Cys**-X<sub>2</sub>-**Cys**-X<sub>(4-48)</sub>-**Cys**-X<sub>2</sub>-**Cys**

*Homo sapiens* c-Cbl

**Cys**<sub>381</sub>-X<sub>2</sub>-**Cys**<sub>384</sub>-X<sub>11</sub>-**Cys**<sub>396</sub>-X-**His**<sub>398</sub>-X<sub>2</sub>-**Cys**<sub>401</sub>-X<sub>2</sub>-**Cys**<sub>404</sub>-X<sub>11</sub>-**Cys**<sub>416</sub>-X<sub>2</sub>-**Cys**<sub>419</sub>

# RING domain. c-Cbl

Canonical sequence

**Cys**-X<sub>2</sub>-**Cys**-X<sub>(9-39)</sub>-**Cys**-X<sub>(1-3)</sub>-**His**-X<sub>(2-3)</sub>-**Cys**-X<sub>2</sub>-**Cys**-X<sub>(4-48)</sub>-**Cys**-X<sub>2</sub>-**Cys**

*Homo sapiens* c-Cbl

**Cys**<sub>381</sub>-X<sub>2</sub>-**Cys**<sub>384</sub>-X<sub>11</sub>-**Cys**<sub>396</sub>-X-**His**<sub>398</sub>-X<sub>2</sub>-**Cys**<sub>401</sub>-X<sub>2</sub>-**Cys**<sub>404</sub>-X<sub>11</sub>-**Cys**<sub>416</sub>-X<sub>2</sub>-**Cys**<sub>419</sub>



PDB ID: 2Y1M

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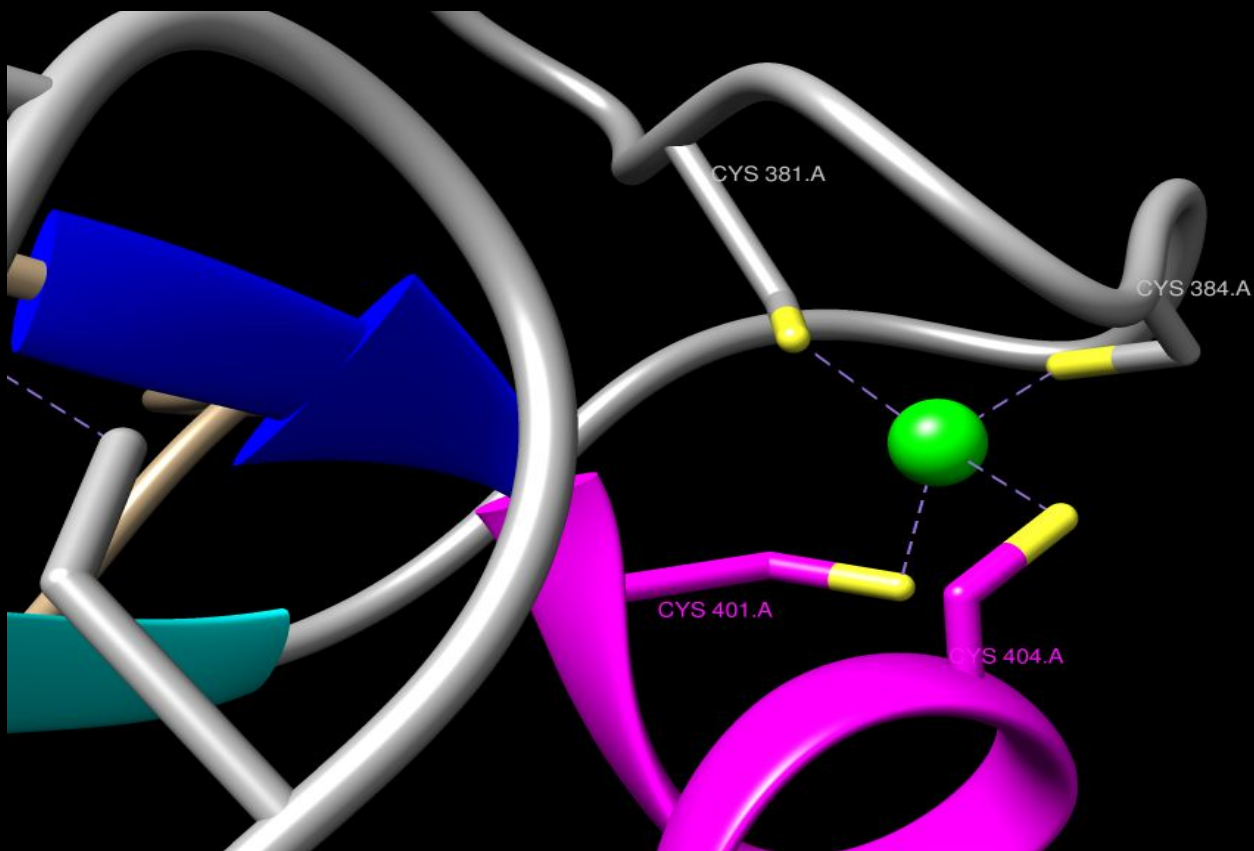
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## Zn atoms



PDB ID: 2Y1M

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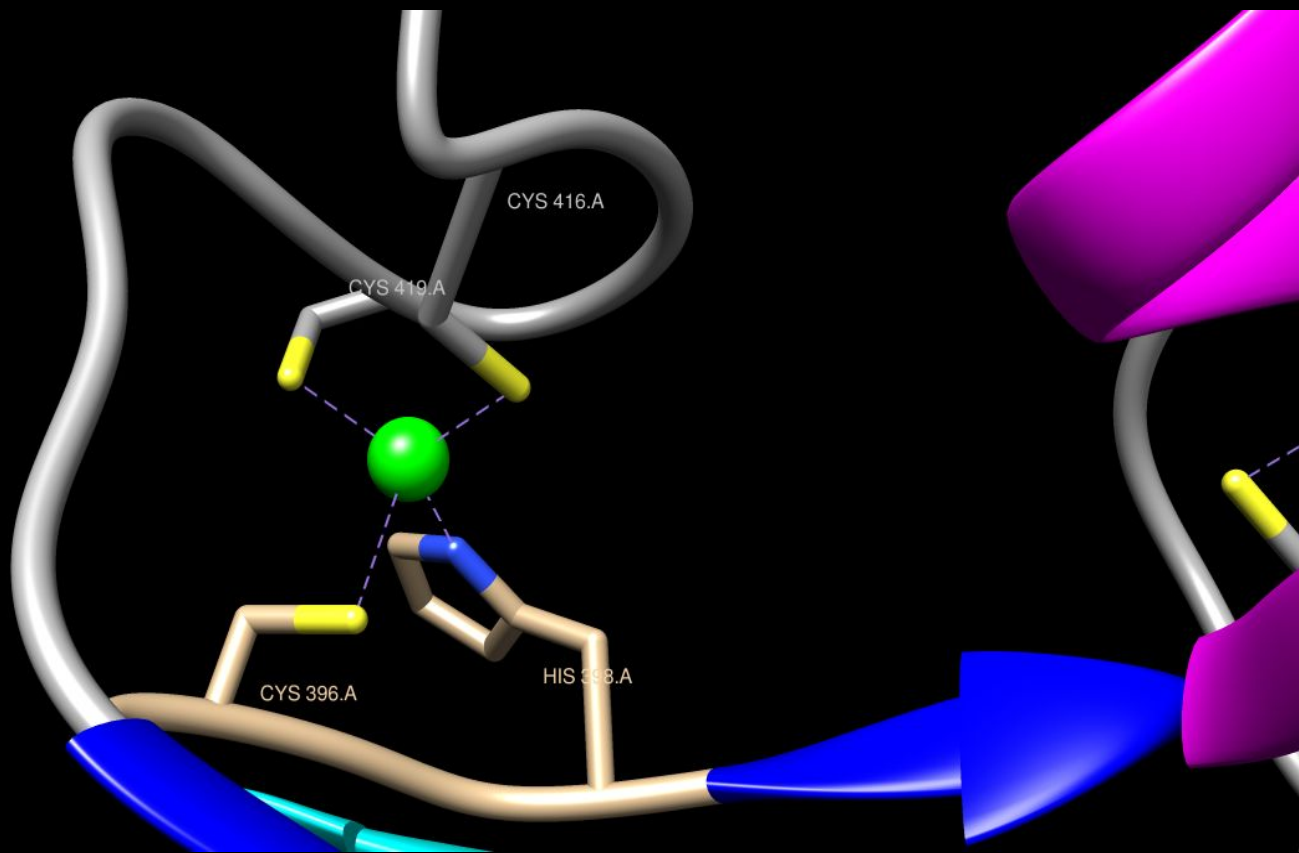
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## Zn atoms



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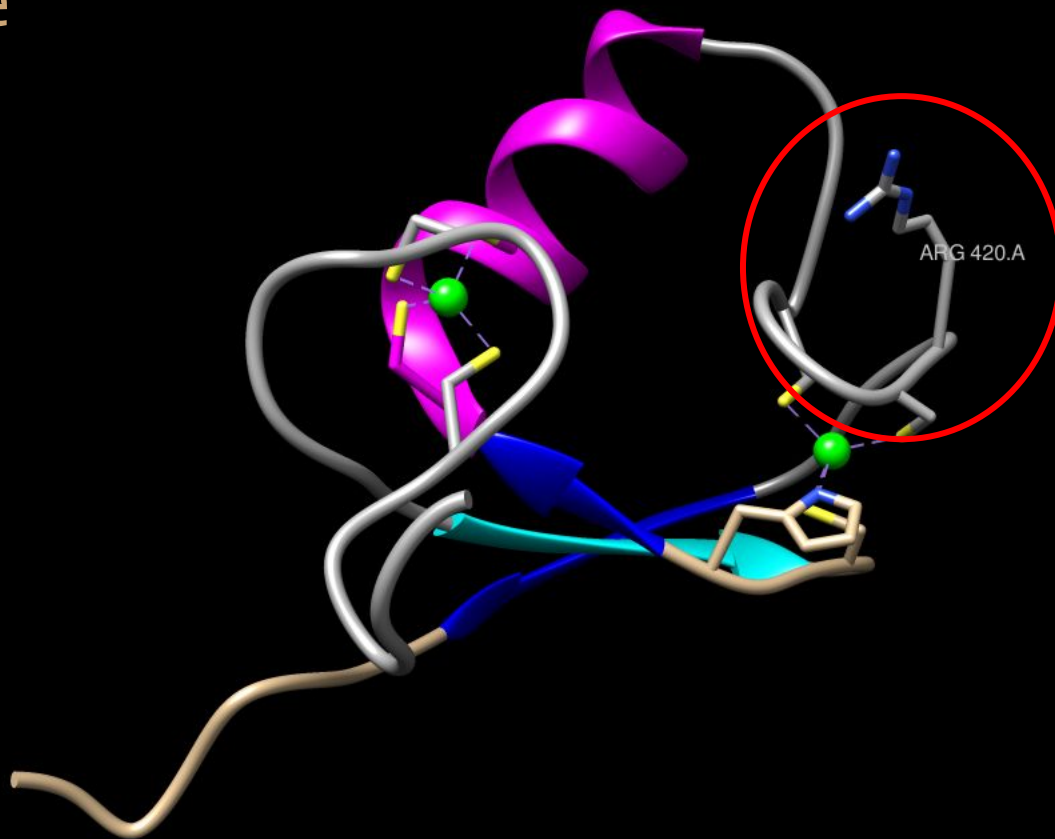
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# Arg residue



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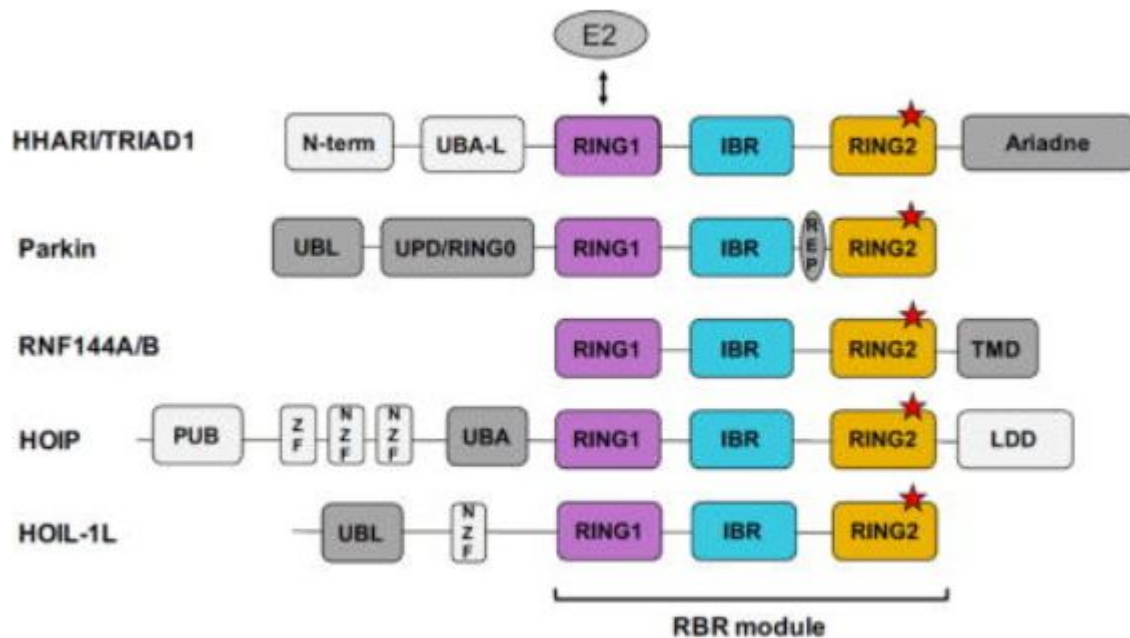
RING

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## 4. RBR-PARKIN

# RBR



Dove K, Klevit RE. RING-Between-RING E3s ligases: Emerging themes amid the variations. 2017.



PDB ID: 411F

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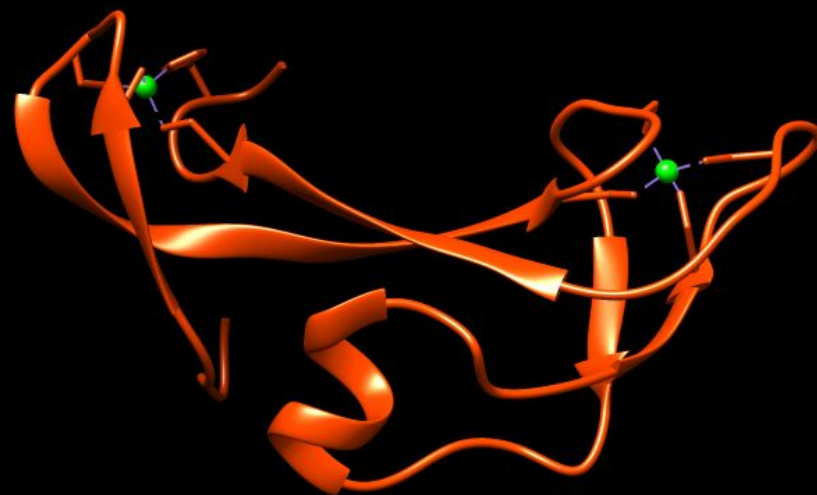
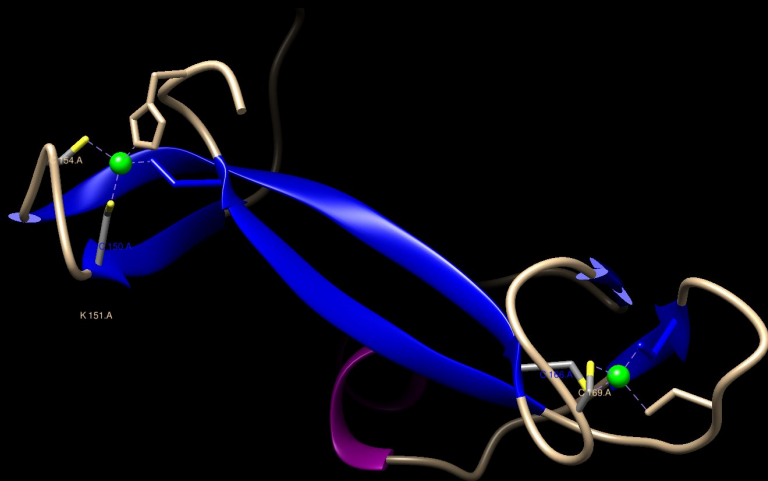
**PARKIN**

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# R0



CYS-rich clusters

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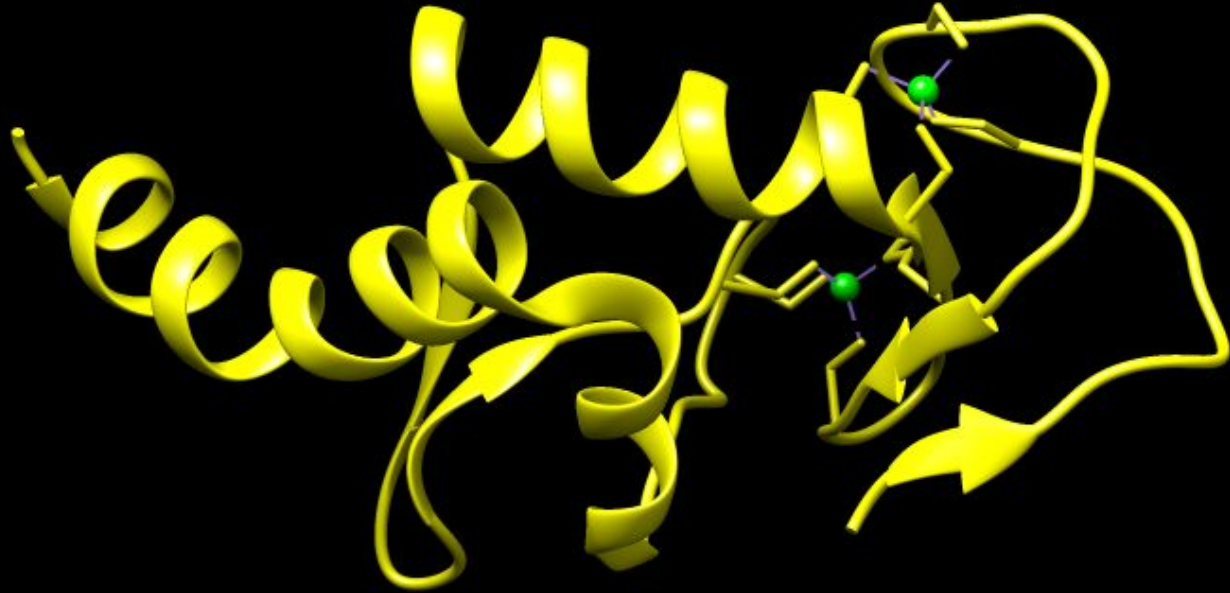
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R1



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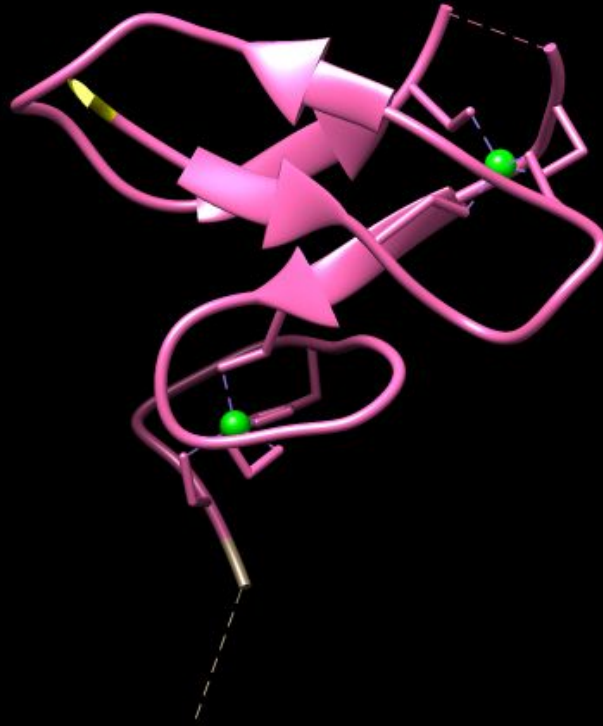
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# IBR



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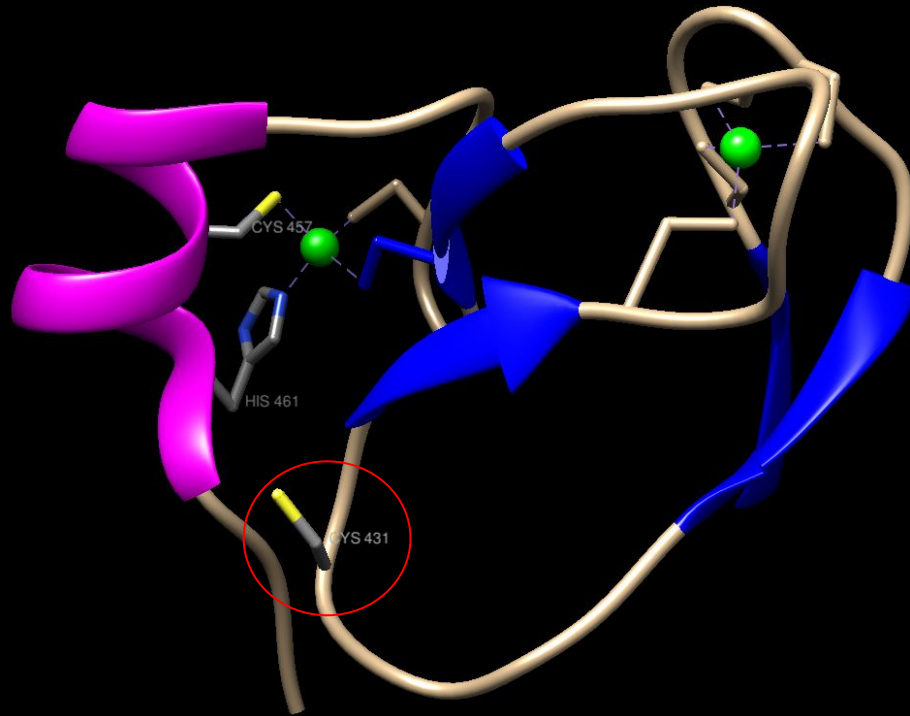
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R2



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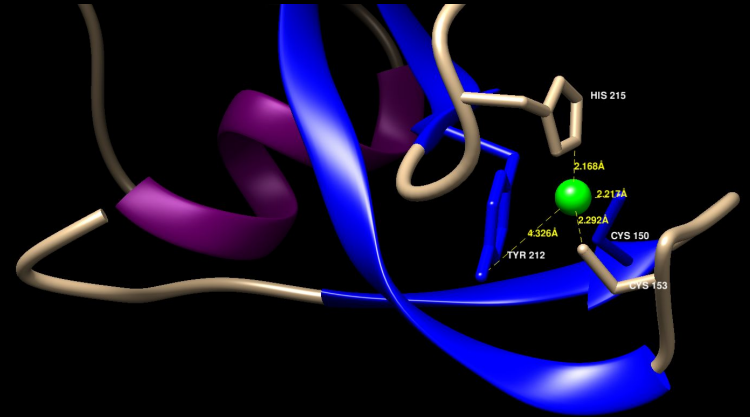
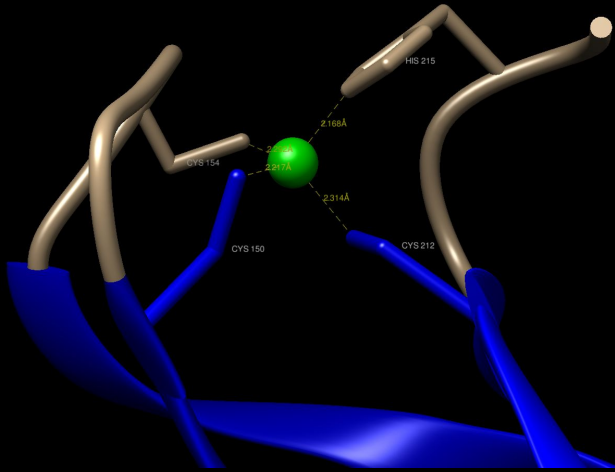
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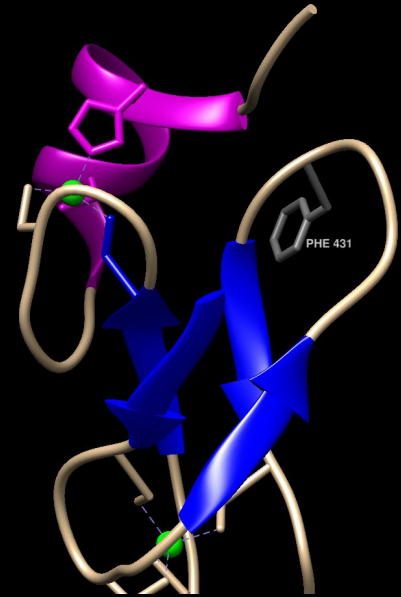
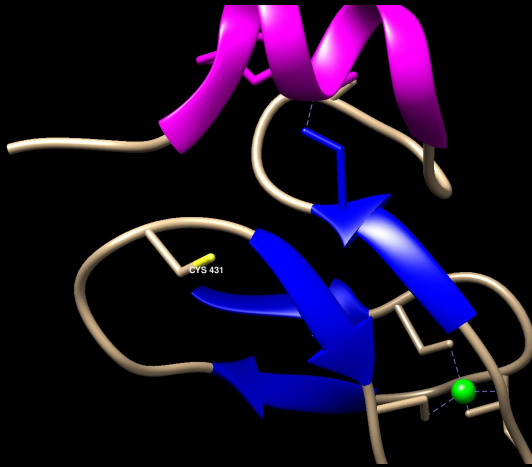
# Mutations that disrupt Zn coordination or protein folding

- CYS 212 → TYR



# Mutations that affect the catalytic site

- CYS 431 → PHE



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# Mutations that affect protein contacts

- GLY 328 → GLU

# Conclusions

- Ubiquitin is a highly conserved protein among Eukaryotes
- The  $\beta$ -grasp fold is only present in the ubiquitin-like family
- E3 - Ubiquitin protein-ligases determine substrate specificity
- There are currently characterized three types of E3, which transfer ubiquitin by different mechanisms
- Different mutations in PARKIN E3 cause a loss of function that can lead to Parkinson's disease.

# PEM questions

1. Choose the correct answer about ubiquitin:

- a) It is encoded by four genes
- b) It is not present in prokaryotes
- c) It's a small protein, only 8.5 kDa
- d) It participates in protein degradation, endocytosis signalling and DNA repair, among others.

**e) All the above are correct**

2. Ubiquitin is bound to the substrate proteins by its:

- a) Surface Lysine
- b) Surface Cysteine
- c) N-terminal Glycine
- d) C-terminal Glycine**
- e) Ile144 patch

3. Choose the correct answers about E3 enzymes:

- 1- They are also called ubiquitin-protein ligases
- 2- There are three categorized families
- 3- They determine substrate specificity
- 4- There are about fifty different E3

**a) 1, 2, 3**

b) 1, 3

c) 2, 4

d) 4

e) 1, 2, 3, 4

4. About HECT E3:

- a) Human HECTs can be classified in three subfamilies
- b) They have a catalytic Cysteine to which ubiquitin is transferred
- c) a and b are correct**
- d) They coordinate with four zinc ions, which directly bind to the substrate
- e) All the above are correct

5. The overall structure of E6AP HECT domain consists of:

- a) A big N-lobe and a smaller C-lobe connected by a short linker**
- b) One domain with a three-stranded beta-sheet, one alpha-helix and two bulges
- c) Two identical lobes that coordinate with a Zn ion each
- d) A big adenylation lobe and a smaller E2 binding lobe
- e) All the above are incorrect

6. About RING domain ligases:

- a) Only one Zn atom is needed
- b) They form a link with Ub in order to get it transferred to the substrate
- c) Cysteines are essential to stabilize the overall structure**
- d) No alpha-helix are present in the structure
- e) All of the above are correct

# PEM questions

7. About c-Cbl, it is true that:

- a) Its sequence does not fit the canonical sequence
- b) It presents a His residue which helps coordinating one of the Zn atoms**
- c) It is a multicomponent E3 complex
- d) It is not present in humans
- e) None of the above is correct

8. Which are the typical domains of E3 RBR?

- a) UbL
- b) RING1-IRB-RING2**
- c) RING1
- d) 2 random RING and IRB
- e) None of the above is correct

9. In which RING domain of Parkin protein is there a typical E2 binding motif ( $\alpha$ -helix and two loops)?

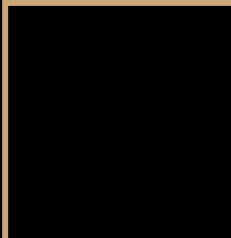
- a) R2
- b) R1**
- c) IRB
- d) a and b are correct
- e) None of the above is correct

10. How are Parkin mutations associated to Parkinson's Disease classified?

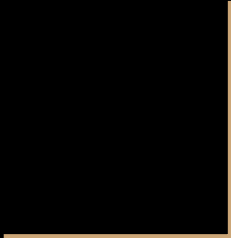
- a) Mutations that disrupt Zn coordination or protein folding
- b) Mutations that affect the catalytic site
- c) Mutations that affect proteins contacts
- d) All of the above are correct**
- e) None of the above is correct

# Bibliography

- Chaugule VK, Walden H. Specificity and disease in the ubiquitin system. *Biochem Soc Trans.* 2016; 44 (1): 212-227.
- Dove KK, Klevit RE. RING-Between-RING E3s ligases: emerging themes amid the variation. *J Mol Biol.* 2017; 429 (22): 3363-3375.
- Huang L. Structure of an E6AP-UbcH7 complex: insights into ubiquitination by the E2-E3 enzyme cascade. *Science.* 1999; 286 (5443): 1321-1326.
- Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem.* 2012; 81: 203-229.
- Lorenz S. Structural mechanisms of HECT-type ubiquitin ligases. *Biol Chem.* 2018; 399 (2): 127-145.
- Morreale FE, Walden H. SnapShot: Types of ubiquitin ligases. *Cell.* 2016; 165: 248.
- Riley BE, Loughheed JC, Callaway K, Velasquez M, Brecht E, Nguyen L, *et al.* Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. *Nat Commun.* 2013; 4: 1982.
- Trempe JF, Sauvé V, Grenier K, Seirafi M, Yang MY, Ménade M, *et al.* Structure of Parkin reveals mechanisms for ubiquitin ligase activation. *Science.* 2013; 340 (6139): 1451-1455.
- Vijay-Kumar S, Bugg CE, Cook WJ. Structure of ubiquitin refined at 1.8 Å resolution. *J Mol Biol.* 1987; 194: 531-544.
- Zheng N, Shabek N. Ubiquitin ligases: structure, function and regulation. *Annu Rev Biochem.* 2017; 86: 129-157.



Thank you for  
your attention



# Ubiquitination system



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