

泛素结合酶E2的序列、结构及功能

Sequence, structure and function of ubiquitin-conjugating enzyme E2

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2019/01/13

目录

- 背景
- 序列搜寻
- GO,KEGG & 系统发育树
- 结构信息与活性位点
- 结构预测

Background

Background

泛素-蛋白酶体系统

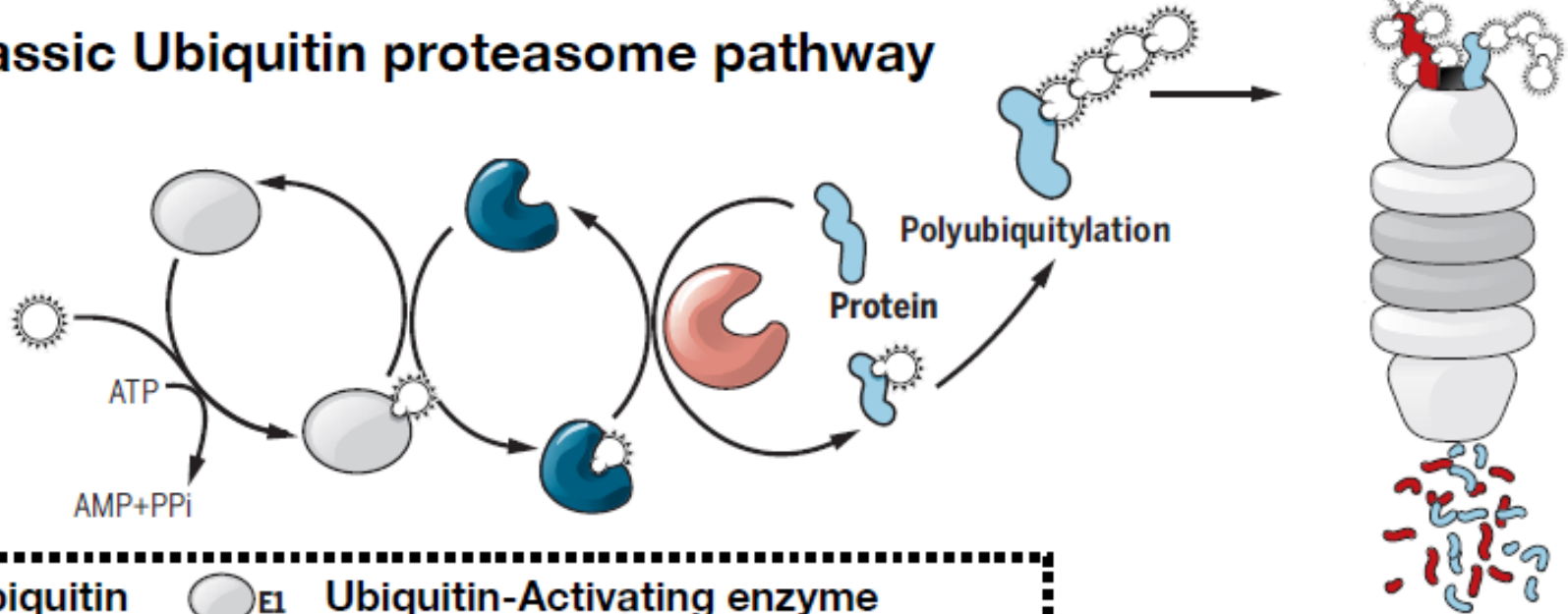
蛋白质


降解



泛素蛋白酶体蛋白质降解系统

溶酶体蛋白质降解系统

Classic Ubiquitin proteasome pathway



 Ubiquitin  E1 Ubiquitin-Activating enzyme

 E2 Ubiquitin-conjugating enzyme  E3 Ubiquitin-ligase

Degradation by
26S proteasome

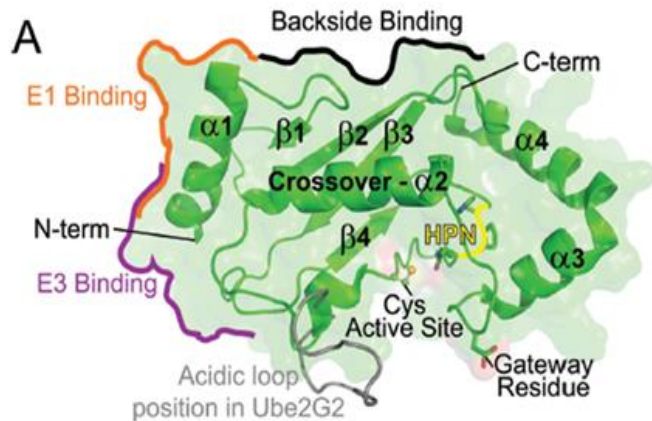
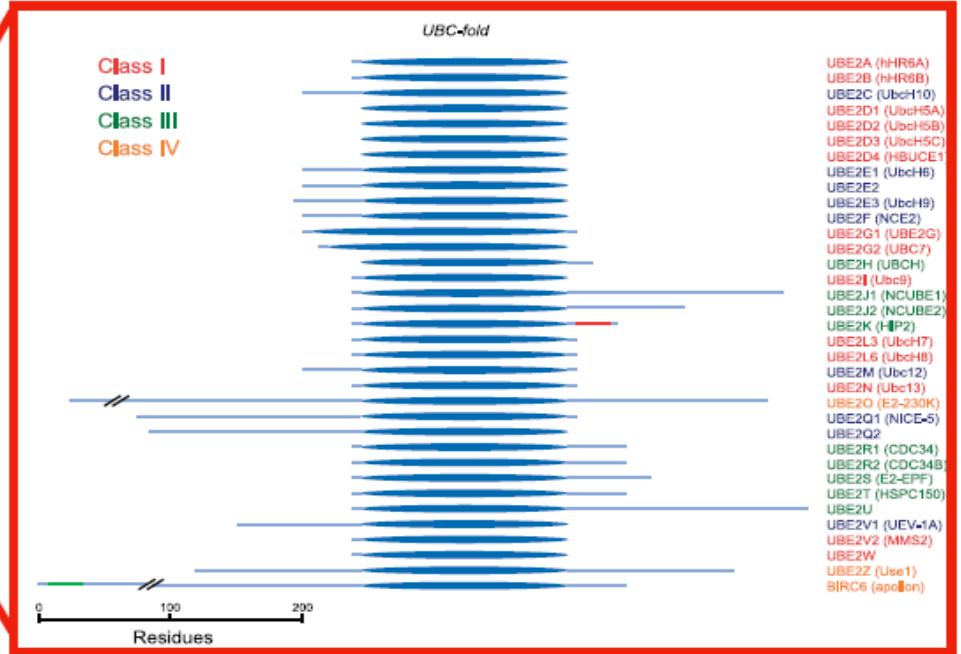
E1: only 2 was found

Each E1 could reaction with every E2

E2: ~40 in human

Each E2 could reaction with over 500 E3

E3: Unknown number...



-泛素结合酶 (E2s) 是负责将泛素 (Ub) 附着到细胞蛋白质的三重酶的中心参与者

-E2s主要参与两种类型的反应, 如下: (1) 转移 (从硫酯转移到巯基) 和 (2) 氨解 (从硫酯转移到氨基)

-E2s参与了细胞内很多细胞过程使得它成为药物靶点蛋白

-所有的E2都含有大约150个氨基酸的核心催化结构域, 称为UBC结构域。

Structure and function of UBE2S



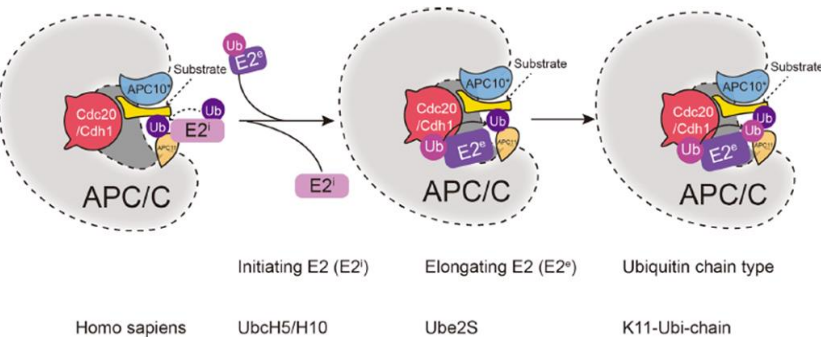
Feature key	Position(s)
Helix ⁱ	10 – 25
Beta strand ⁱ	31 – 35
Beta strand ⁱ	42 – 48
Turn ⁱ	54 – 57
Beta strand ⁱ	59 – 65
Turn ⁱ	68 – 72
Beta strand ⁱ	76 – 81
Beta strand ⁱ	92 – 94
Helix ⁱ	96 – 99
Turn ⁱ	100 – 102
Helix ⁱ	109 – 121
Helix ⁱ	125 – 127
Helix ⁱ	131 – 139
Helix ⁱ	141 – 155

PDB Entry	Method	Resolution	Chain	Positions	Links
1ZDN	X-ray	1.93 Å	A/B	1-156	PDBe RCSB PDB PDBj PDBsum
5BNB	X-ray	2.49 Å	A/B/C/D	1-156	PDBe RCSB PDB PDBj PDBsum
5L9T	Electron mi...	6.40 Å	T	1-222	PDBe RCSB PDB PDBj PDBsum

Secondary structure



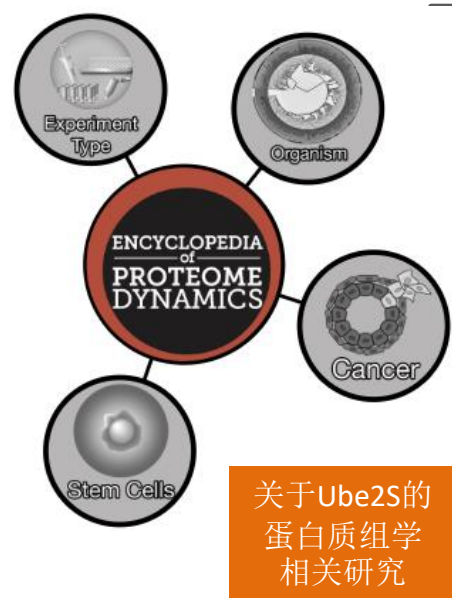
Legend: Helix Turn Beta strand PDB Structure known for this area



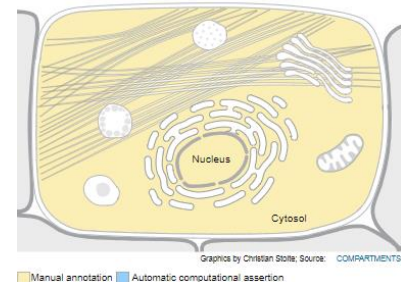
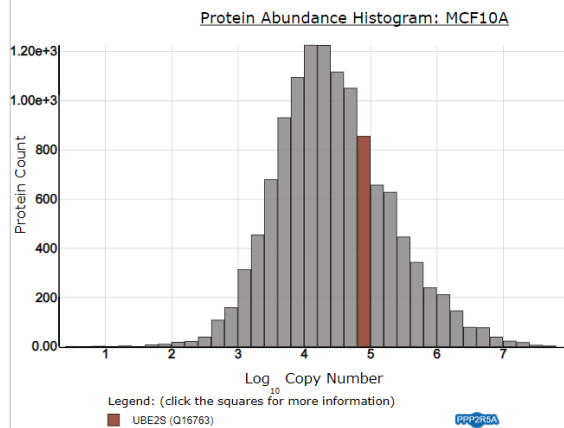
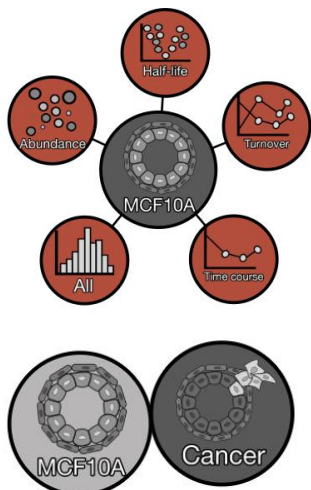
接受来自E1复合物的泛素蛋白并催化其与其他蛋白质的共价连接。催化‘Lys-11’连接的多泛素化。作为后期促进APC/C的必需因子，APC/C是细胞周期调节的泛素连接酶，其通过有丝分裂控制进展。通过在APC/C底物上特异性延长由E2酶UBE2C / UBCH10引发的‘Lys-11’连接的多聚泛素链，通过蛋白酶体增强APC/C底物的降解并促进有丝分裂退出。

Protein information of Ube2S

亚细胞定位：在细胞质及核中均有出现



关于Ube2S的蛋白质组学相关研究



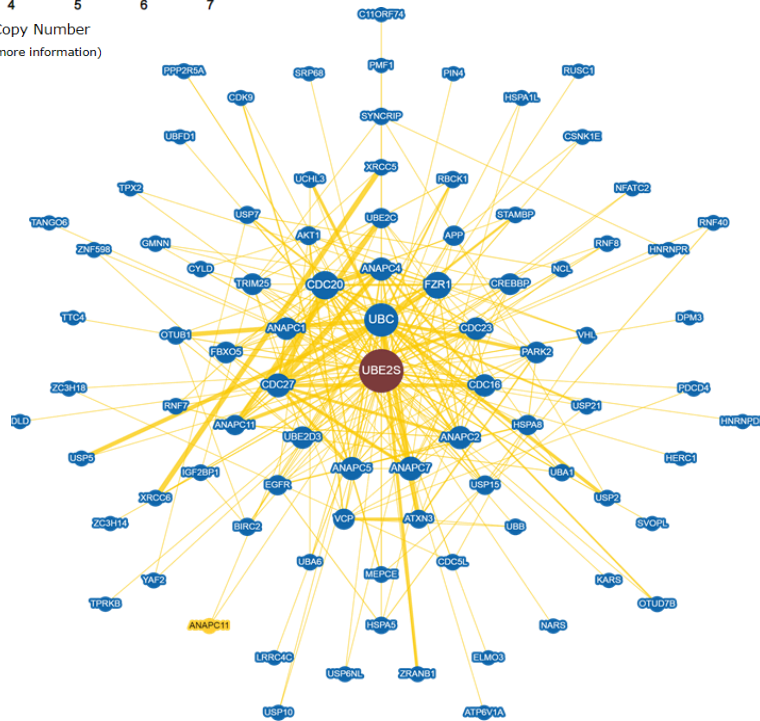
Interactor (Node) Distribution:

Query Node: 1	Same Organism Nodes: 86
Different Organism Node: 1	Chemical Node: 0

Interaction (Edge) Distribution:

Physical Edges: 251	Genetic Edge: 0
Physical/Genetic Edge: 0	Chemical Edge: 0

根据Switch View相关信息可以得到与Ube2S发生互作的蛋白有88个其自身的翻译后修饰位点有十六个其互作网络:



APC / C复合物的组分，由至少14个不同的亚基组成，这些亚基组装成至少19个链的复合物，其组合分子量约为1.2MDa。在这个复合体内，直接与ANAPC2和ANAPC4互作

Annotation information of Ube2S

UniProt还提供了多种多样的相关Ube2S的数据库：3D结构，蛋白-蛋白互作；PTM多态性和突变；蛋白质组学；基因组注释；组织特异性；系统发生学；酶和通路数据库；基因表达及家族和结构域数据库。

MENU ▾

nature
cell biology

Letter | Published: 11 October 2009

UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit

Mathew J. Garnett, Jörg Mansfeld, Colin Godwin, Takahiro Matsusaka, Jiahua Wu, Paul Russell, Jonathon Pines & Ashok R. Venkitaraman

Nature Cell Biology **11**, 1363–1369 (2009) | [Download Citation](#) ↓

包括了UBE2S蛋白的序列，通路，蛋白相互作用，功能，表达，结构，蛋白家族和结构域，蛋白质组学，表征，作用机制等等方面。如：“UBE2S可延长APC / C底物上的泛素链，促进有丝分裂的结束。

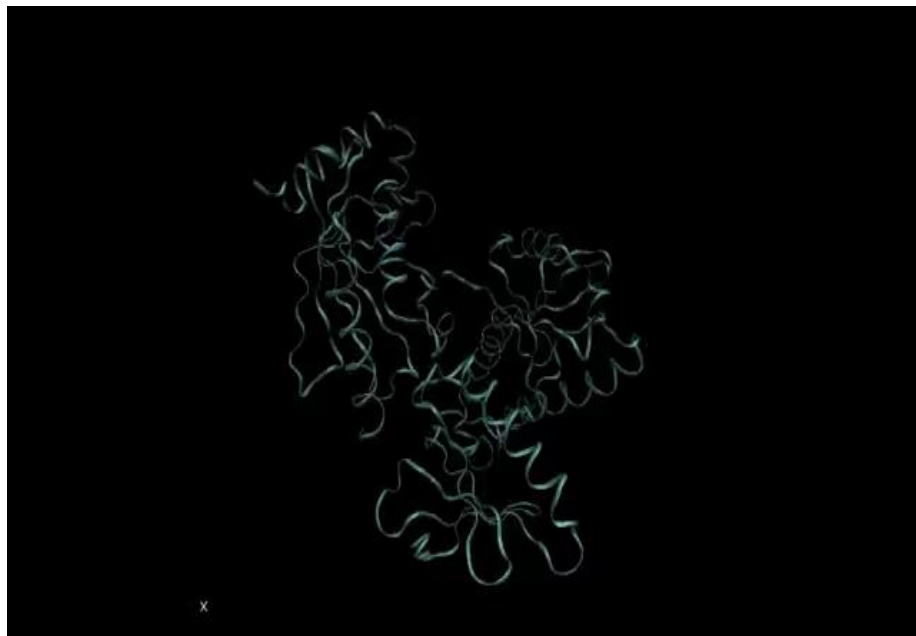
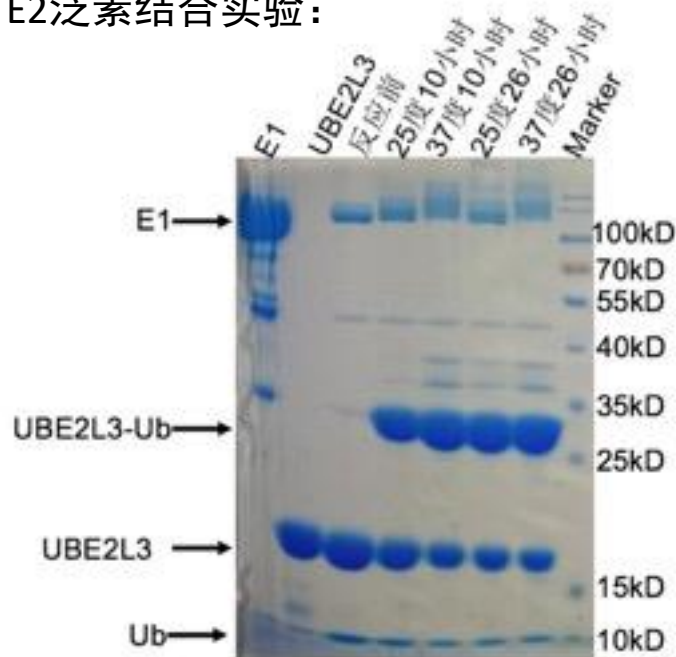
Experimental information

上游引物设计从第23位碱基开始的20个碱基，下游引物设计用反向配对序列第467至487位。

UBE2L3引物设计：上游 5' taagaaggagatatATGGCGGCCAGCAGGAGGCT 3'

下游 5' gtggtggtggtggtgGTCCACAGGTCGCTTTTCCCAT 3'

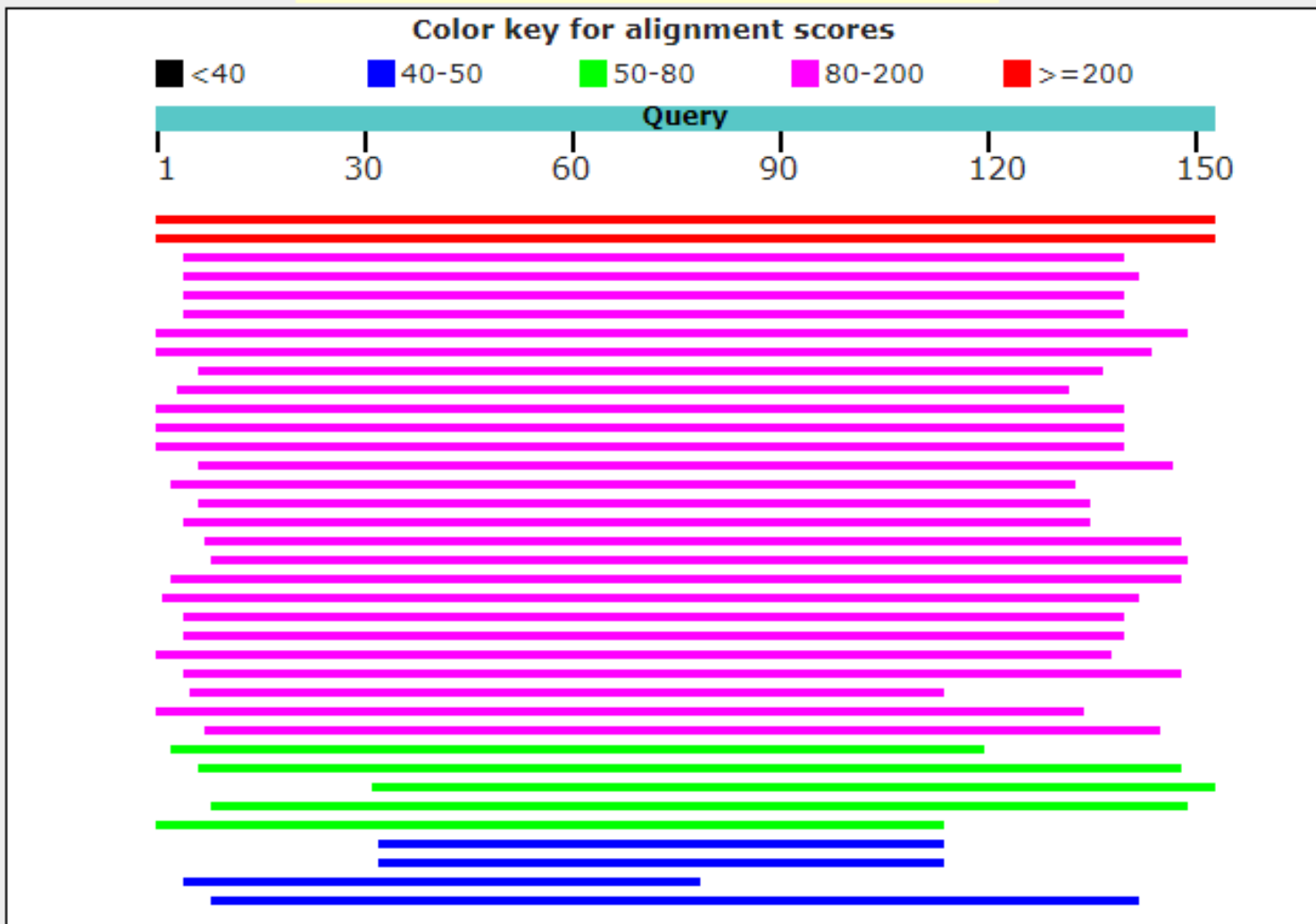
E2泛素结合实验：



Sequence search

Distribution of the top 37 Blast Hits on 37 subject sequences

Mouse over to see the title, click to show alignments



在N
选择
(C
seq
(A
行E

据库

点击运

Enter Quer

Enter accessio
>sp |P63146 |UBE2
GN=UBE2B PE=1 S
MSTPARRRLGRDFYK
EEYFNKFPPTVRFLSI
PANSQAAQLYQENED

Or, upload file

Job Title

Align two or

Choose Se

Database

Organism
Optional

Exclude
Optional

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PSI-BLAST (P

differ from the

ces

Database advanced search of E2s

点击 UniProt 数据库主页 Advanced，打开高级检索窗口，选择 Protein Name [DE]，输入 E2 酶的英文 ubiquitin-conjugating enzymes，选择基因名 Gene Name [GN]，输入 E2 酶的基因名缩写 UBE2*，选择物种名 Organism [OS]，输入 Human，选择 Human [9606]，选择 Reviewed，提交检索策略，页面显示 UniProt 数据库中收录的 E2 酶序列条目，共 39 条。

Searching in UniProtKB [? Help](#)

Term
Protein name [DE]

Term
AND Gene name [GN]

AND Reviewed > Reviewed

Term
AND Organism [OS]

Entry	Entry name	Protein names	Gene names	Organism	Length
P62253	UB2G1_HUMAN	Ubiquitin-conjugating enzyme E2 G1	UBE2G1 UBE2G	Homo sapiens (Human)	170
P61086	UBE2K_HUMAN	Ubiquitin-conjugating enzyme E2 K	UBE2K HIP2, LIG	Homo sapiens (Human)	200
P51668	UB2D1_HUMAN	Ubiquitin-conjugating enzyme E2 D1	UBE2D1 SFT, UBC5A, UBCH5, UBCH5A	Homo sapiens (Human)	147
Q9C0C9	UBE2O_HUMAN	(E3-independent) E2 ubiquitin-conju...	UBE2O KIAA1734	Homo sapiens (Human)	1,292
P61077	UB2D3_HUMAN	Ubiquitin-conjugating enzyme E2 D3	UBE2D3 UBC5C, UBCH5C	Homo sapiens (Human)	147
Q16763	UBE2S_HUMAN	Ubiquitin-conjugating enzyme E2 S	UBE2S E2EPF, OK/SW-cl.73	Homo sapiens (Human)	222
P62837	UB2D2_HUMAN	Ubiquitin-conjugating enzyme E2 D2	UBE2D2 PUBC1, UBC4, UBC5B, UBCH4, UBCH5B	Homo sapiens (Human)	147

GO,KEGG & Phylogenetic trees

Gene ontology (UBE2B)

BP

(Biological Process)

- ubiquitin-dependent protein catabolic process
- protein polyubiquitination
- protein K63-linked ubiquitination

MF

(Molecular Function)

- ubiquitin-protein transferase activity
- ATP binding
- ubiquitin conjugating enzyme activity

CC

(Cellular component)

- replication fork
- nuclear chromatin
- cytoplasm

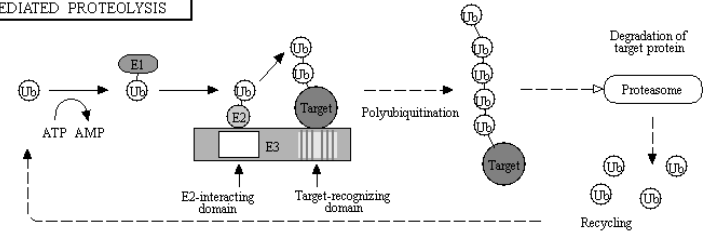
KEGG (UBE2B)

Catalytic activity: ATP + ubiquitin + protein lysine = AMP + diphosphate + protein N-ubiquityllysine.

Function: Catalyzes the covalent attachment of ubiquitin to other proteins. Required for postreplication repair of UV-damaged DNA. Associates to the E3 ligase RAD18 to form the UBE2B-RAD18 ubiquitin ligase complex involved in mono-ubiquitination of DNA-associated PCNA on 'Lys-164'.

Disease: Male infertility

UBIQUITIN MEDIATED PROTEOLYSIS



E1
(Ubiquitin-activating enzyme)

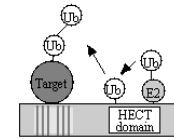
- UBE1
- UBLE1A
- UBLE1B
- UBE1C

E2
(Ubiquitin-conjugating enzyme)

- UBE2A
- UBE2B
- UBE2C
- UBE2D
- UBE2E
- UBE2F
- UBE2G
- UBE2H
- UBE2I
- UBE2J
- UBE2K
- UBE2L
- UBE2M
- UBE2N
- UBE2O
- UBE2P
- UBE2Q
- UBE2R
- UBE2S
- UBE2U
- UBE2V
- UBE2W
- UBE2X
- UBE2Y
- UBE2Z
- HIP2
- AF1CN1

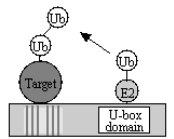
E3
(Ubiquitin ligase)

HECT type E3



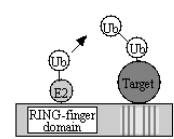
- E6AP
- UBE3B
- UBE3C
- Smurf1
- Itch
- WWP1
- WWP2
- TRIP12
- NEDD4
- ARF-BP1
- EDD1
- HERC1
- HERC2
- HERC3
- HERC4

U-box type E3



- UBE4A
- UBE4B
- CHIP
- CYC4
- PRP19
- UIP5

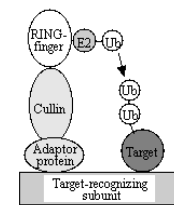
single RING-finger type E3



- Mdm2
- CEB1
- Parkin
- SIAH-1
- PML
- TRAF6
- MEK1
- COP1
- PIRHR2
- cIAPs
- PIAS
- SYVN
- NHLRC1
- AIRE
- MGRN1
- BRCA1
- PANCL
- MID1
- Trm32
- Trm37

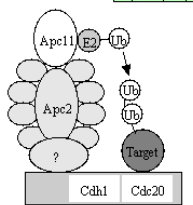
multi subunit RING-finger type E3

Cullin-Rbx E3



- | | RING finger | Cullin | Adaptor protein | Target recognizing subunit |
|--------------|-------------|--------|-----------------|----------------------------|
| SCF complex | RBX1 | Cul1 | Skp1 | F-box |
| ECV complex | RBX1 | Cul2 | EloB
EloC | VHLbox |
| Cul3 complex | RBX1 | Cul3 | | BTB |
| Cul4 complex | RBX1 | Cul4 | DDB1 | DCAF |
| ECS complex | RBX2 | Cul5 | EloB
EloC | SOC3box |
| Cul7 complex | RBX1 | Cul7 | Skp1 | F-box2 |

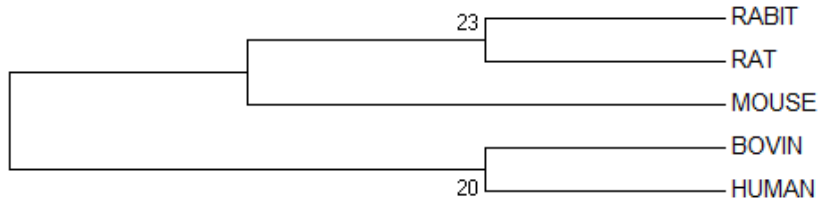
APC/C



- | RING finger | Cullin | Adaptor protein | Target recognizing subunit | Other subunits |
|-------------|--------|-----------------|----------------------------|---|
| Apc11 | Apc2 | ? | Cdc20 | Apc1
Apc2
Apc3
Apc4
Apc5
Apc6
Apc7
Apc8
Apc9
Apc10
Apc12
Apc13 |
| | | | Cdh1 | |

phylogenetic tree (UBE2B,UBE2C)

UBE2B



Method:Neighbor-Joining
 Bootstrap:1000
 Model:p-distance

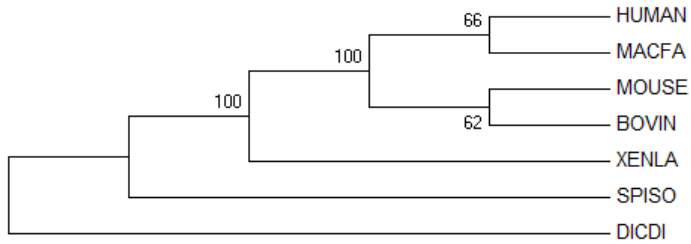
P63146	UBE2B_HUMAN	1	MSTPARRRLMRDFKRLQEDPPVG VSGAPSENNIMQWNAVIFGPEG TPFEDG TFKL VIEF S	60
P63147	UBE2B_MOUSE	1	MSTPARRRLMRDFKRLQEDPPVG VSGAPSENNIMQWNAVIFGPEG TPFEDG TFKL VIEF S	60
Q32P99	UBE2B_BOVIN	1	MSTPARRRLMRDFKRLQEDPPVG VSGAPSENNIMQWNAVIFGPEG TPFEDG TFKL VIEF S	60
P63148	UBE2B_RABIT	1	MSTPARRRLMRDFKRLQEDPPVG VSGAPSENNIMQWNAVIFGPEG TPFEDG TFKL VIEF S	60
P63149	UBE2B_RAT	1	MSTPARRRLMRDFKRLQEDPPVG VSGAPSENNIMQWNAVIFGPEG TPFEDG TFKL VIEF S	60

P63146	UBE2B_HUMAN	61	EEYPNKPPTVRFL SKMFHPNVYADG SICLDILQNRWSPTYDVS SILT SIQSLLDEPNPNS	120
P63147	UBE2B_MOUSE	61	EEYPNKPPTVRFL SKMFHPNVYADG SICLDILQNRWSPTYDVS SILT SIQSLLDEPNPNS	120
Q32P99	UBE2B_BOVIN	61	EEYPNKPPTVRFL SKMFHPNVYADG SICLDILQNRWSPTYDVS SILT SIQSLLDEPNPNS	120
P63148	UBE2B_RABIT	61	EEYPNKPPTVRFL SKMFHPNVYADG SICLDILQNRWSPTYDVS SILT SIQSLLDEPNPNS	120
P63149	UBE2B_RAT	61	EEYPNKPPTVRFL SKMFHPNVYADG SICLDILQNRWSPTYDVS SILT SIQSLLDEPNPNS	120

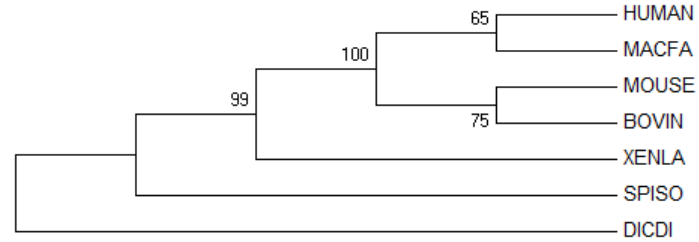
P63146	UBE2B_HUMAN	121	PANSQAAQLYQENKREYERKRVSA IVEQSWND S	152
P63147	UBE2B_MOUSE	121	PANSQAAQLYQENKREYERKRVSA IVEQSWND S	152
Q32P99	UBE2B_BOVIN	121	PANSQAAQLYQENKREYERKRVSA IVEQSWND S	152
P63148	UBE2B_RABIT	121	PANSQAAQLYQENKREYERKRVSA IVEQSWND S	152
P63149	UBE2B_RAT	121	PANSQAAQLYQENKREYERKRVSA IVEQSWND S	152

UBE2C

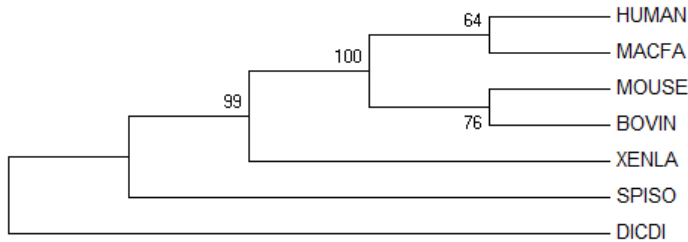
phylogenetic tree (UBE2B,UBE2C)



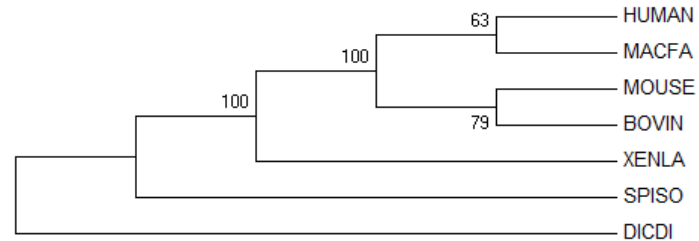
Method:Neighbor-Joining
Bootstrap:1000
Model:p-distance



Method:Neighbor-Joining
Bootstrap:1000
Model:Poisson



Method:Neighbor-Joining
Bootstrap:1000
Model:Equal Input

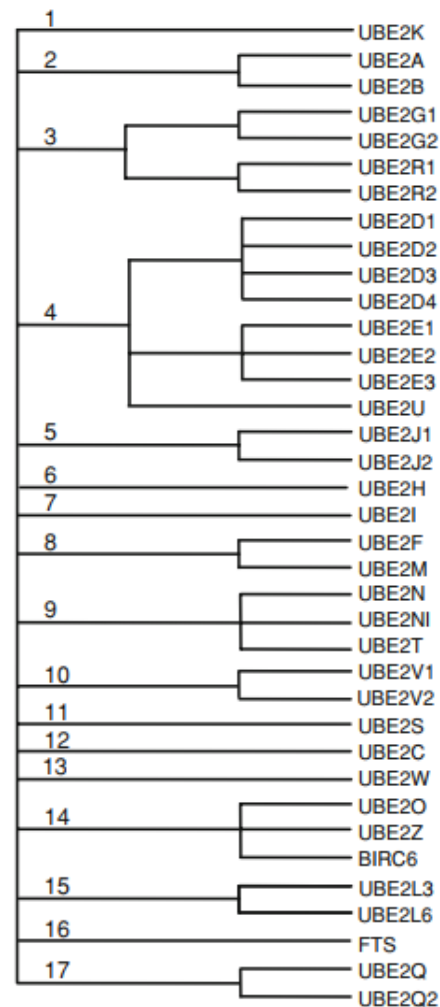
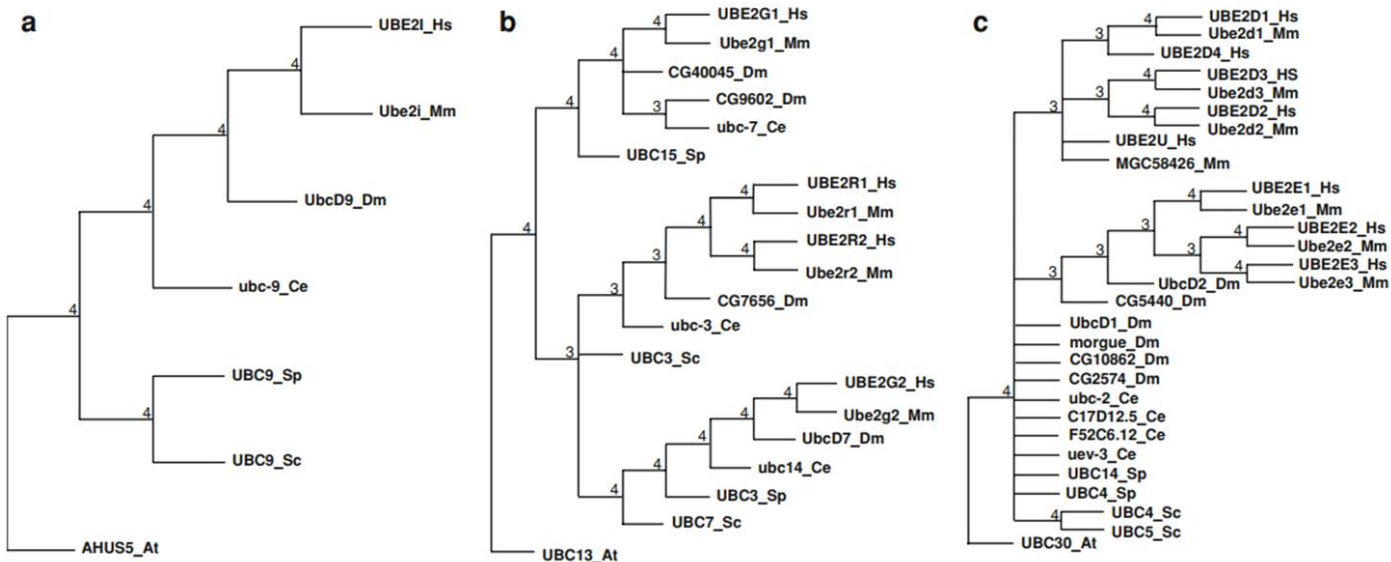


Method:Neighbor-Joining
Bootstrap:1000
Model:JTT matrix-based
method

phylogenetic tree

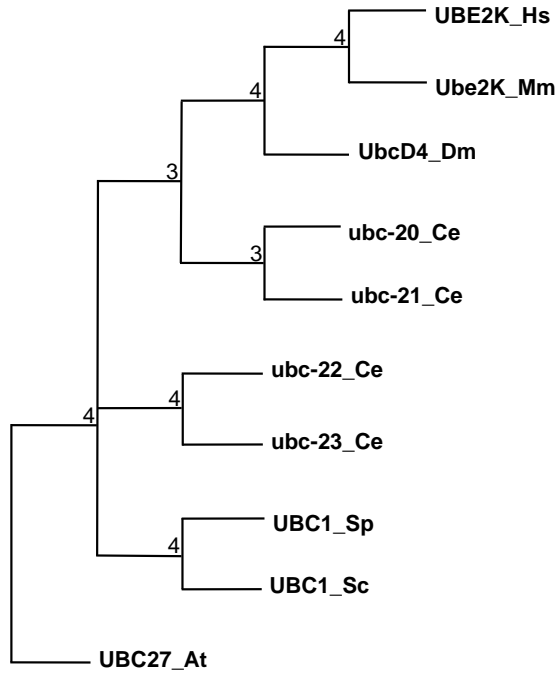
将每个物种的每个家族中选择一个直系同源基因。将蛋白质序列以相同的顺序连接以获得每个物种一个序列。这种连接用于构建所研究物种的系统发育树。使用了四种算法（NJ，ML，MP和BI），绘制了共识树。

计算分析后绘制的37种人E2酶的简化系统发育树。每个分支代表一个不同的系列，其数量位于根部附近

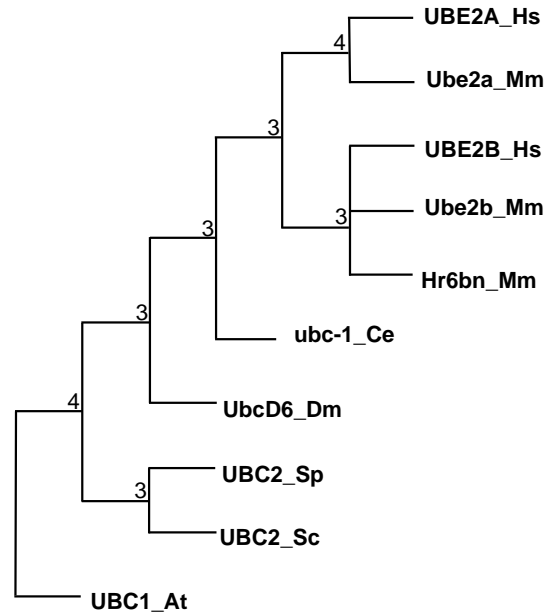


家族的系统发育树的例子，如家庭7 (a)，和复杂家庭，如家庭3 (b) 和4 (c)。每棵树代表四种算法 (NJ, ML, MP和BI) 的共识。仅显示至少三种算法中存在的分支，而其他分支则隐藏。数字表示支持节点存在的算法的数量

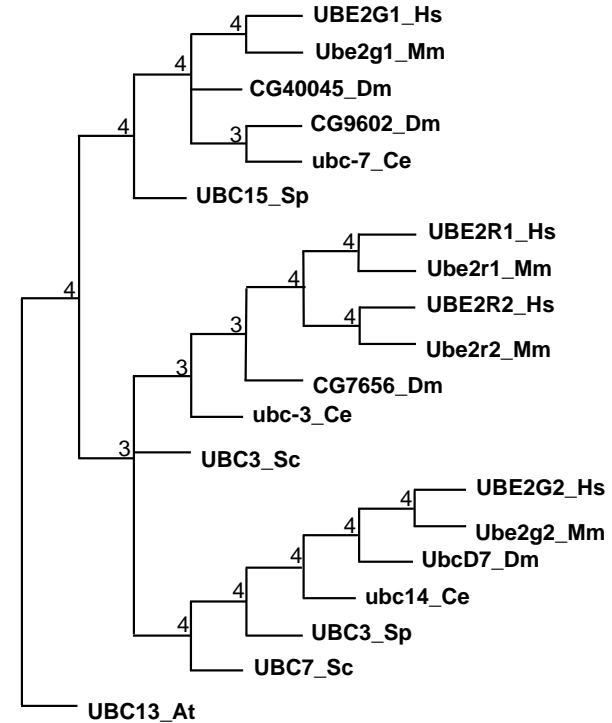
phylogenetic tree



Family 1



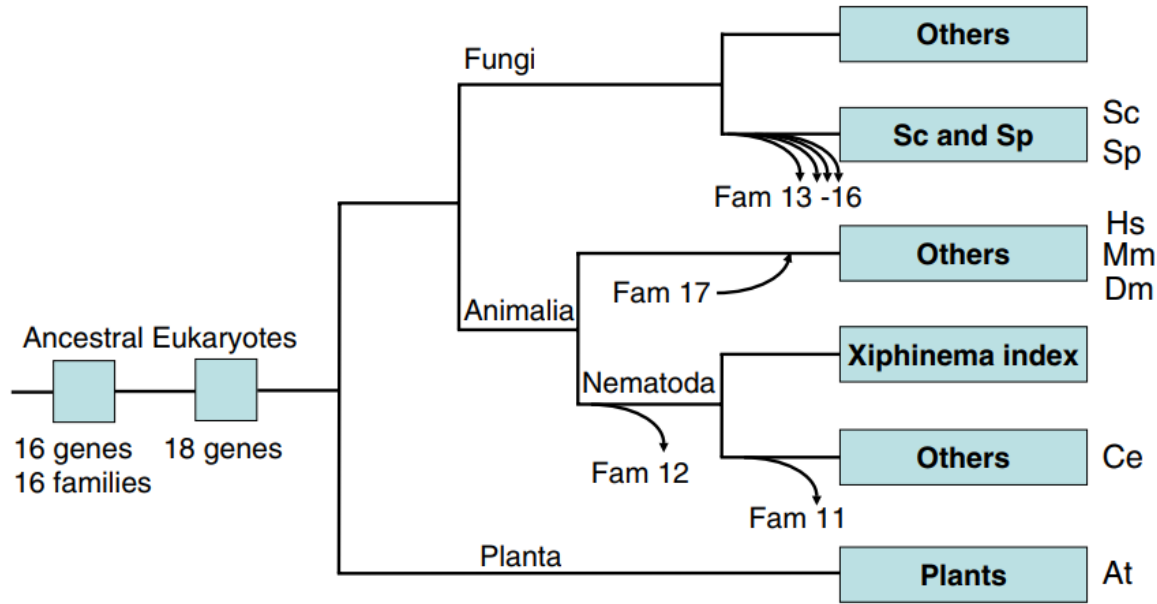
Family 2



Family 3

作者发现在所有7种物种中仅存在10种直向同源基因。将这10个明确定义的序列连接起来并用于构建系统发育树。五个家族的*D. melanogaster*基因比*C. elegans*成员更接近哺乳动物的基因，而只有3个家族的*C. elegans*基因更接近哺乳动物的基因。采用简单多数规则，这与最近将昆虫放在最接近哺乳动物的结果一致。研究结果也证实，大多数秀丽隐杆线虫（Ce）基因的进化速度比果蝇（Dm）基因的速度更快。

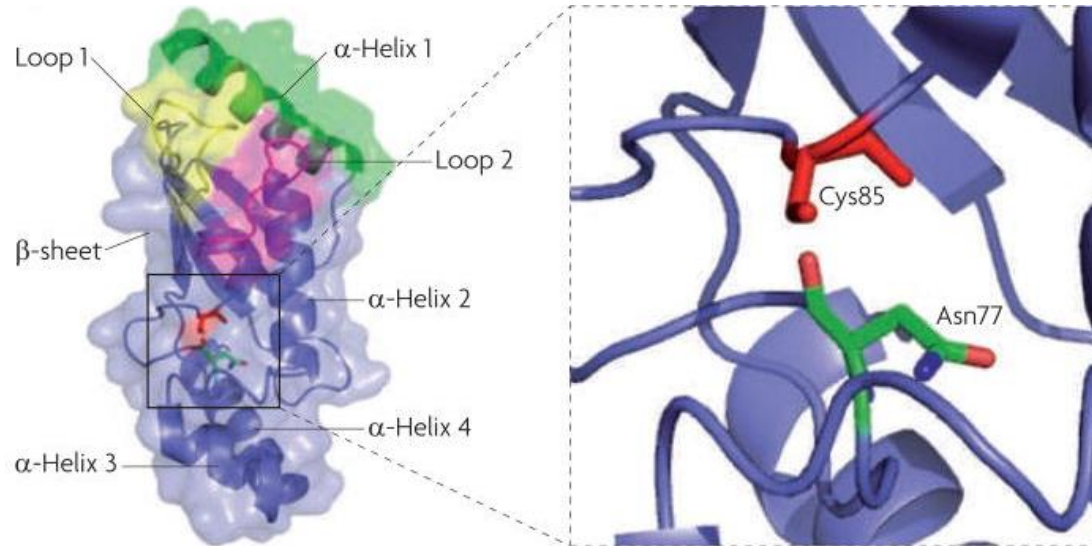
phylogenetic tree



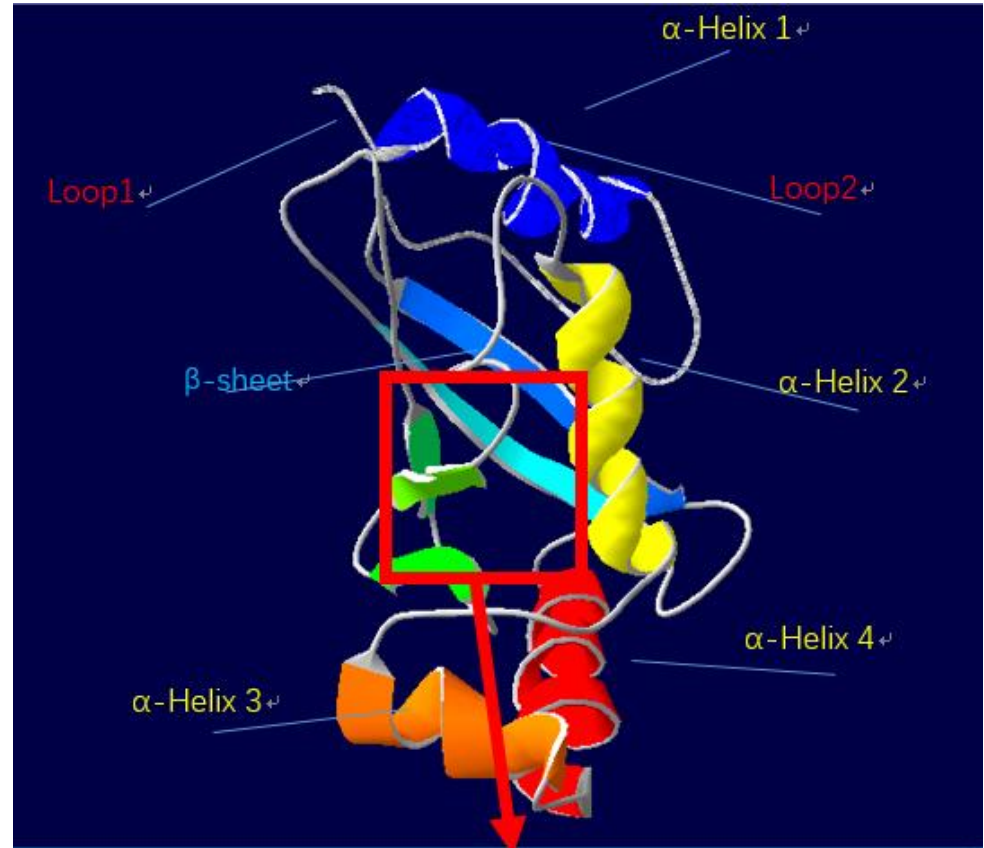
10个家庭在所有物种可能对应于最小数量的初始基因在真核生物的祖先。然而,更可能的是,所有人的共同祖先3门已经拥有一组18祖先基因在16个家庭,考虑到拟南芥拥有16个家庭的基因。秀丽隐杆线虫失去2家庭(家庭11和12),和酵母失去4家庭(家庭13到16)。拟南芥是最富有的基因组酶E2基因,表明这个途径在植物的重要性。基因组重复的几个事件是拟南芥谱系中这组丰富的UBC基因的起源 (Adams和Wendel, 2005)。

structure and active sites of ubiquitin- conjugating enzymes E2

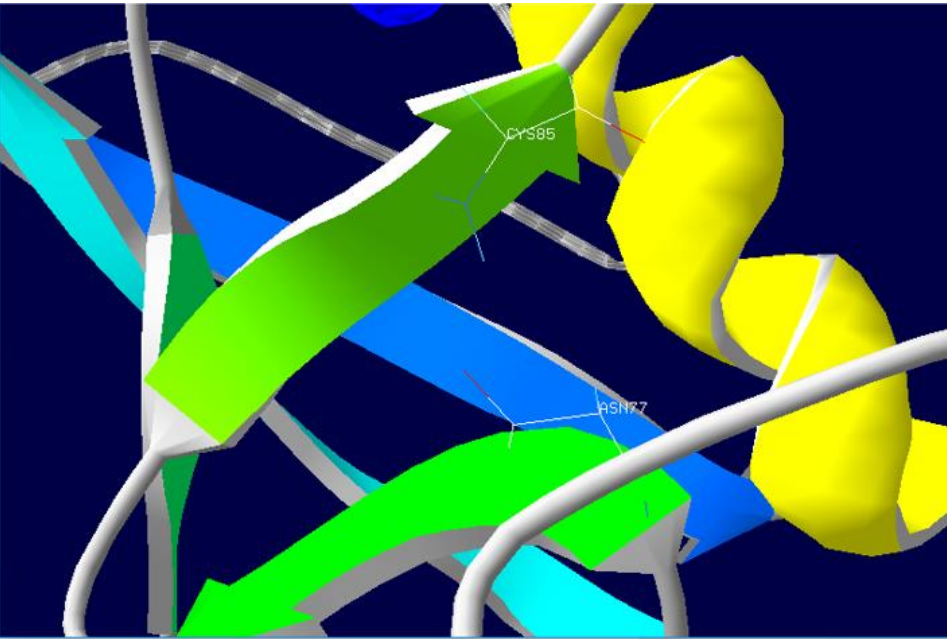
活性E2具有核心蛋白缀合（UBC）结构域，其含有催化性Cys残基并与E1相互作用。在蛋白E2变体（UEV）蛋白质也具有UBC结构域但缺乏活性位点Cys残基。不同的E2有同样UBC结构域，具有高度的序列同源性，并且采用由四个 α 螺旋，由四股形成反平行的 β 折叠，和一个短的3类似的结构螺旋。高度保守的活性位点Cys位于由连接 α -螺旋2与 α -螺旋3的短环形成的浅沟槽和靠近活性位点的长环。



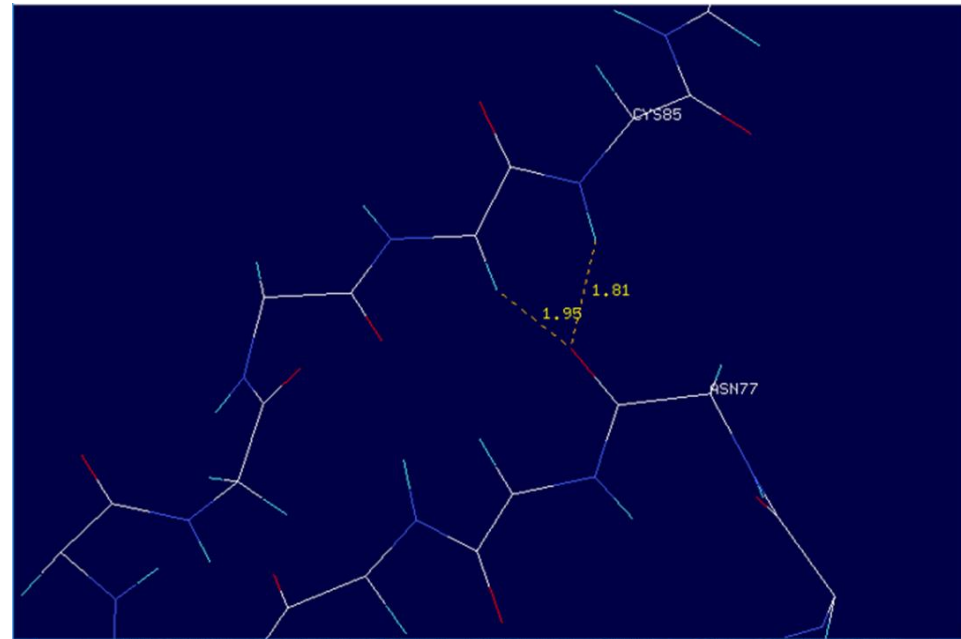
E2 UBE2D2的核心为蛋白缀合
(UBC) 结构域。UBC核心由四
个 α -螺旋和由四个 β -链形成的反
平行 β -折叠组成。E1相互作用的
 α -螺旋1以深蓝色显示，两个E3
相互作用的环以环1和环2显示。



structure and active sites of ubiquitin-conjugating enzymes E2

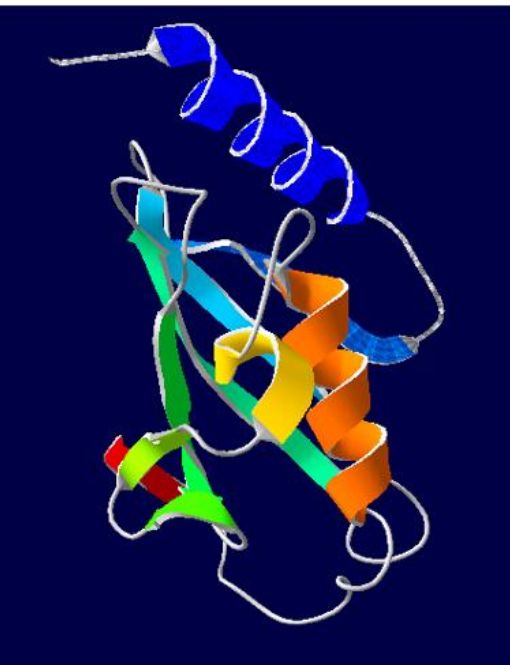


特写视图显示了活性位点Cys85和Asn77的侧链的相对空间位置。

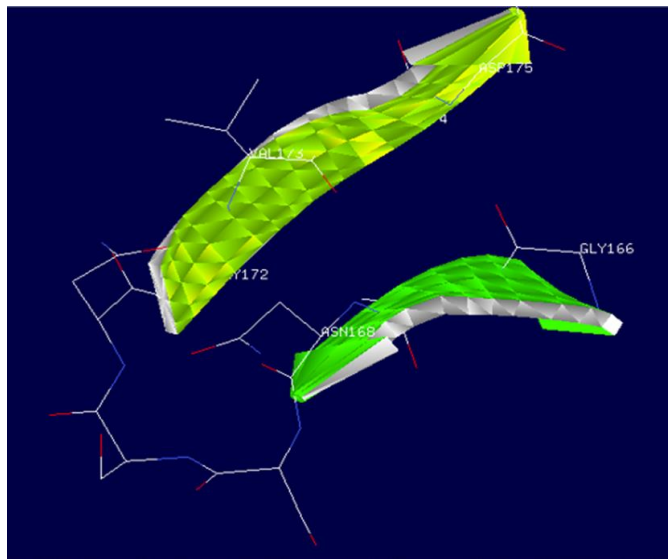


测量原子间距离如图

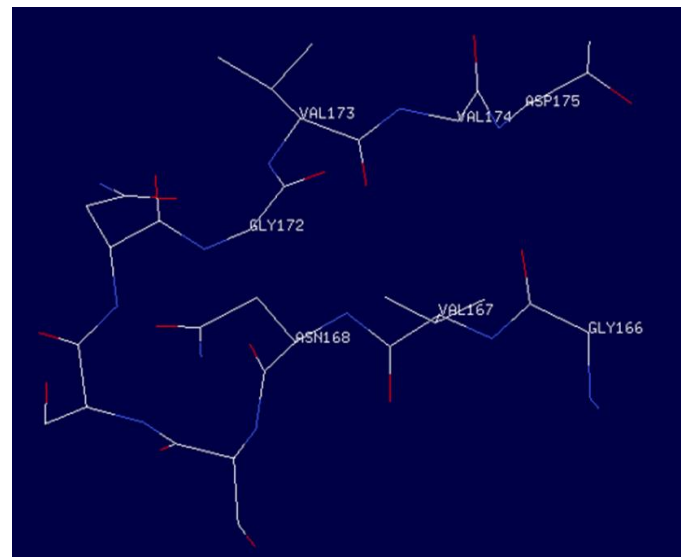
structure and active sites of ubiquitin-conjugating enzymes E2



UEV变体的结构，可以看到依然有UBC结构域， α -Helix， β -sheet和两个loop

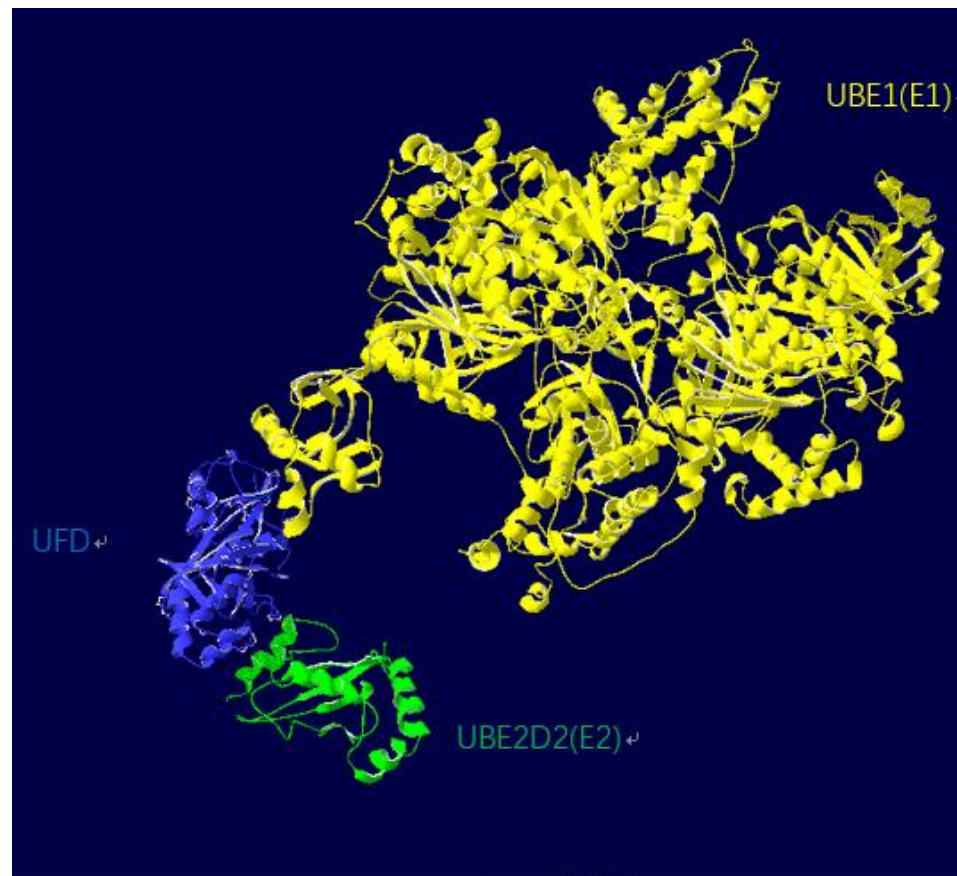


选取同样的位置

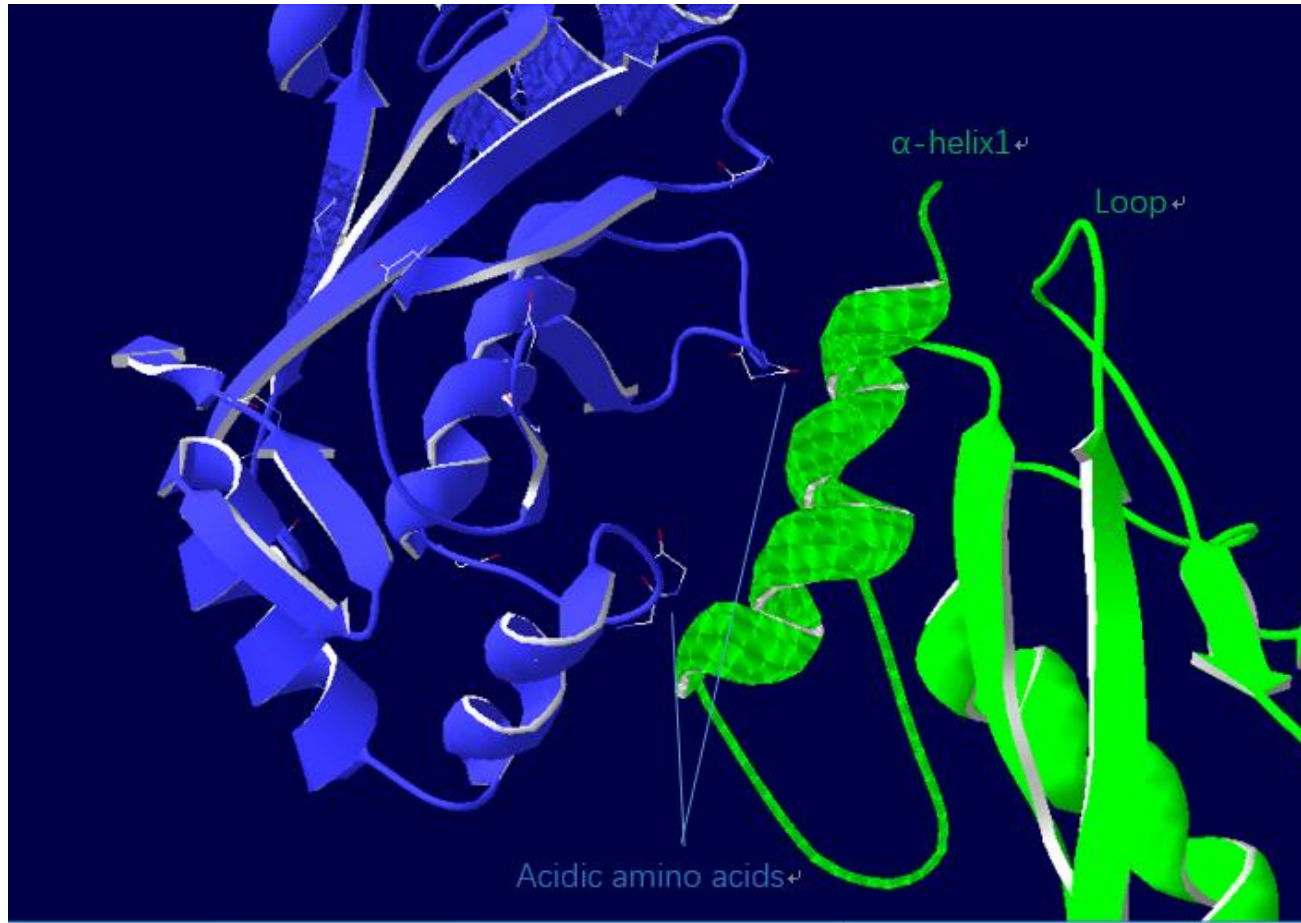


可以看到，没有了活性位点Cys残基

泛素缀合酶（E2）- 泛素激活酶（E1）相互作用的结构模型。显示的例子是E2 UBE2D2与E1 UBE1（也称为UBA1）的复合物。与相应的E1在蛋白折叠结构域（UFD）复合的UBE2M（神经元前体细胞表达的发育上调下蛋白8（NEDD8）E2）的结构用作参照



Binding sites of E2 and E1

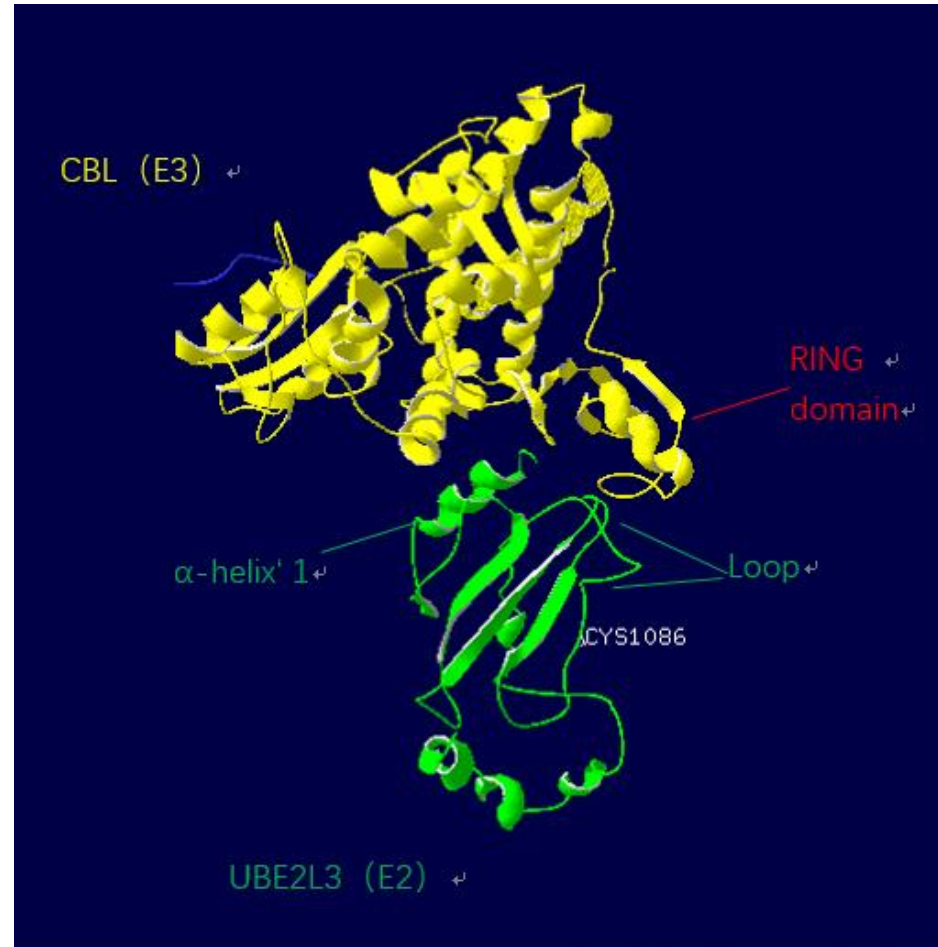


放大视图显示UFD包含由酸性氨基酸形成的凹槽

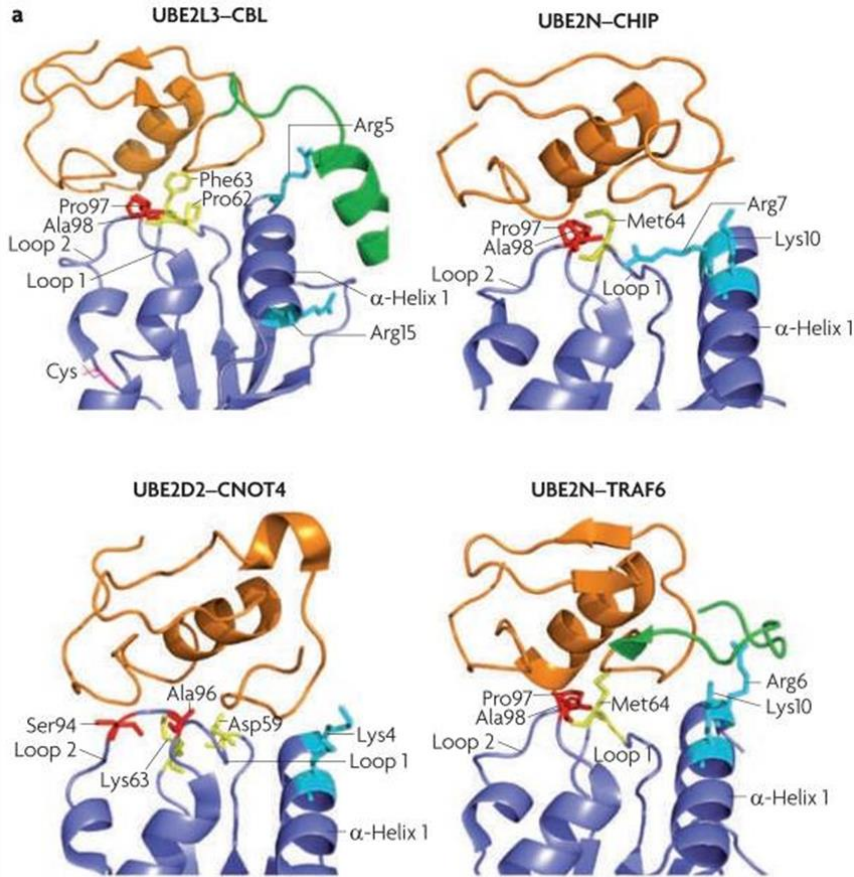
Binding sites of E2 and E3

显示的实例是E2 UBE2L3与RING E3 casitas B系淋巴瘤（CBL）的复合物。对于E1和E3，E2在 α -螺旋1上具有重叠的结合位点。还显示了与RING结构域和催化Cys86接触的两个环。

所有E2都通过L1和L2环以及E2表面上的N-末端 α -螺旋1识别E3。这些基序中的轻微序列变异有助于E3结合的特异性。例如，E2 UBE2L3在L1环中使用Pro62和Phe63，在L2环中使用Pro97和Ala98，在 α -螺旋1中使用Arg5和Arg15与E3 casitas B系淋巴瘤（CBL）相互作用，这是一种原癌基因负调节受体Tyr激酶信号通路。



Binding sites of ubiquitin-conjugating enzymes E2



这里列出了文献中不同的结合方式。

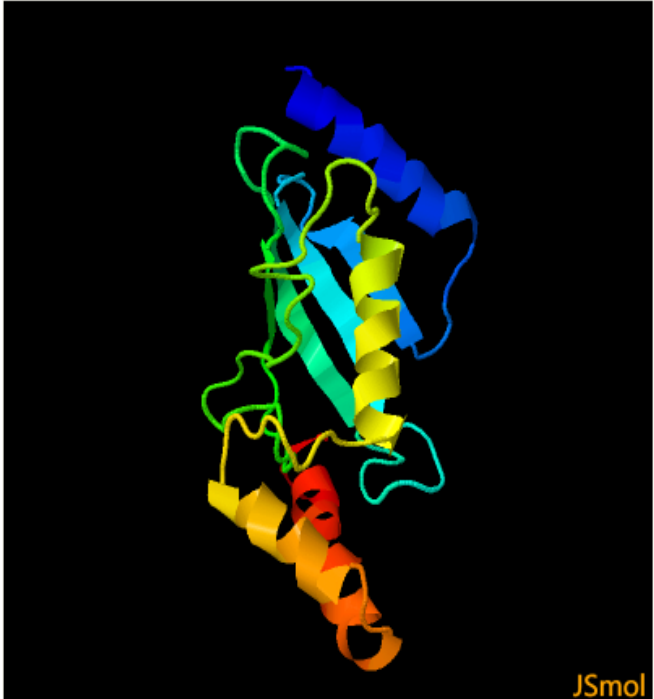
所有E2使用环1，环2和 α -螺旋1与E3相互作用。参与E3识别的残基的侧链是颜色编码的（环1中的残基是黄色，环2中的残基是红色， α -螺旋1中的残基是青色的）。对于CBL和TRAF6，RING结构域外的E3元件（以绿色显示）参与E2结合。RING结构域（或用于CHIP的U-Box）为橙色，同源E2为蓝色。

structure prediction of UBE2 E2

structure prediction of UBE2 E2

UBE2 E2接受来自E1复合物的泛素并催化其与其他蛋白质的共价连接。体外催化单泛素化和'Lys-48'连接的多泛素化。可能参与了katenin的降解。其蛋白结构还没有实验室解析。

Top model



Model (left) based on template [c4ddiB](#)

Top template information

PDB header:hydrolase/ligase
Chain: B: **PDB Molecule:**ubiquitin-conjugating enzyme e2 d2, ubiquitin thioesterase
PDBTitle: crystal structure of human otub1/ubch5b~ub/ub

Confidence and coverage

Confidence: **100.0%** Coverage: **62%**

147 residues (62% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

[3D viewing](#)

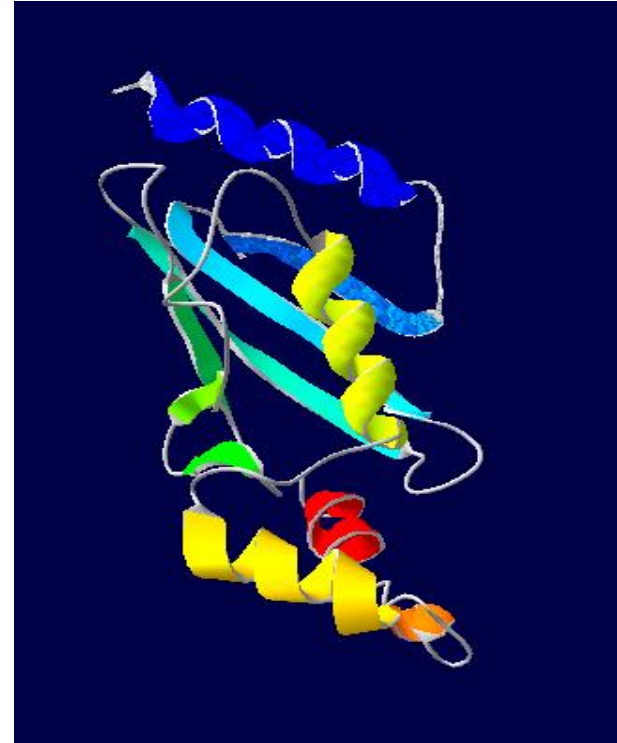
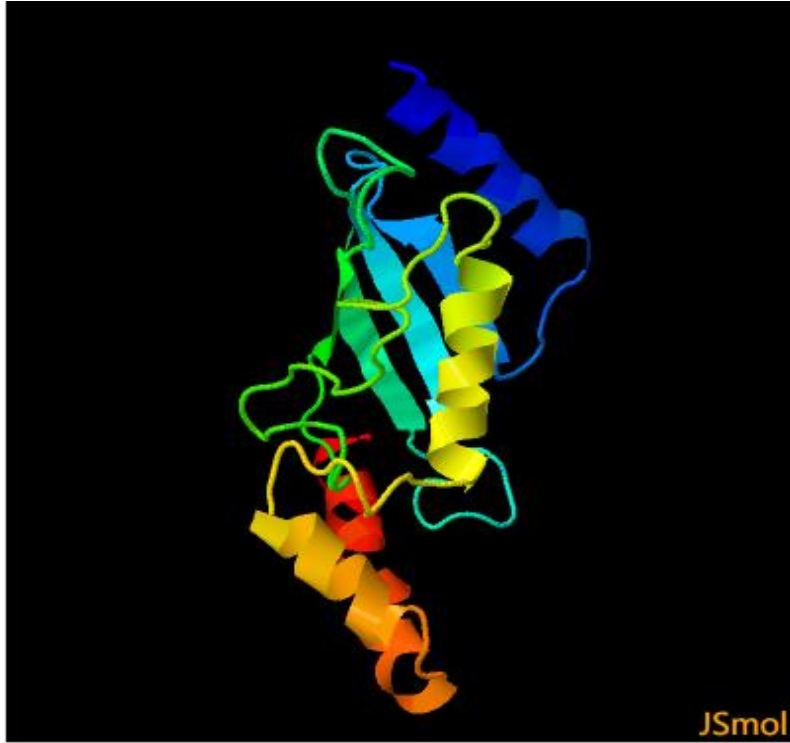
[Interactive 3D view in JSmol](#)

For other options to view your downloaded structure offline see the [FAQ](#)

JSmol

在Uniprot中获取UBE2 E2的序列，提取该蛋白恒定结构域7-183位序列，在蛋白质结构预测网站Phyre2中预测上述结构域空间结构


structure prediction of UBE2 E2



可以看到有E2酶的结构域，4个 α 螺旋，4个 β 折叠和2个loop

structure prediction of UBE2 E2

1 c4ddiB Alignment



100.0 35

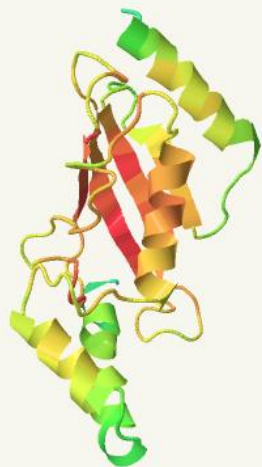
PDB header:hydrolase/ligase
Chain: B: **PDB Molecule:**ubiquitin-conjugating enzyme e2 d2, ubiquitin thioesterase
PDBTitle: crystal structure of human otub1/ubch5b~ub/ub

✓ [View investigator results](#)

选择其中一个结果，以c4ddiB为模板 查看investigator结果

structure prediction of UBE2 E2

Good
Bad



JSmol

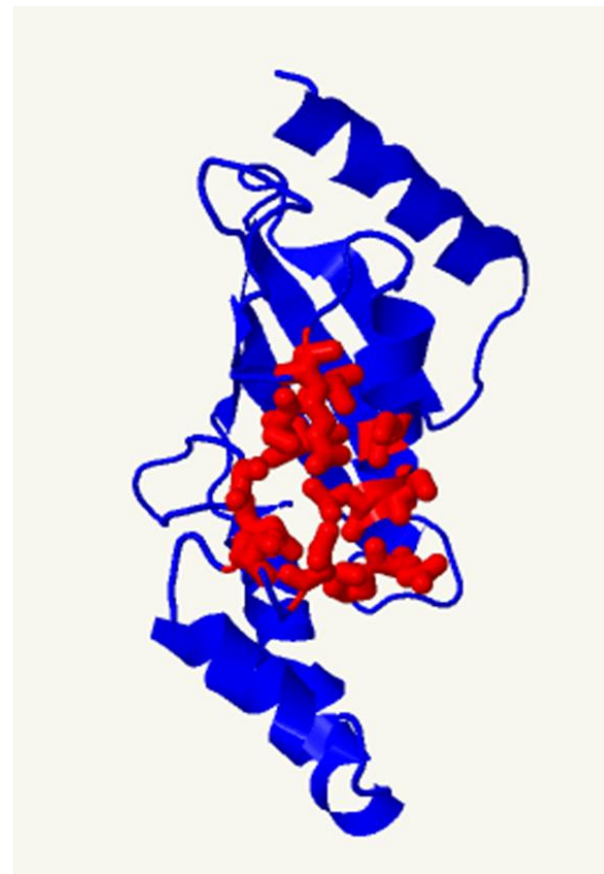
质量
评估

Relative
Conservation
High
Low



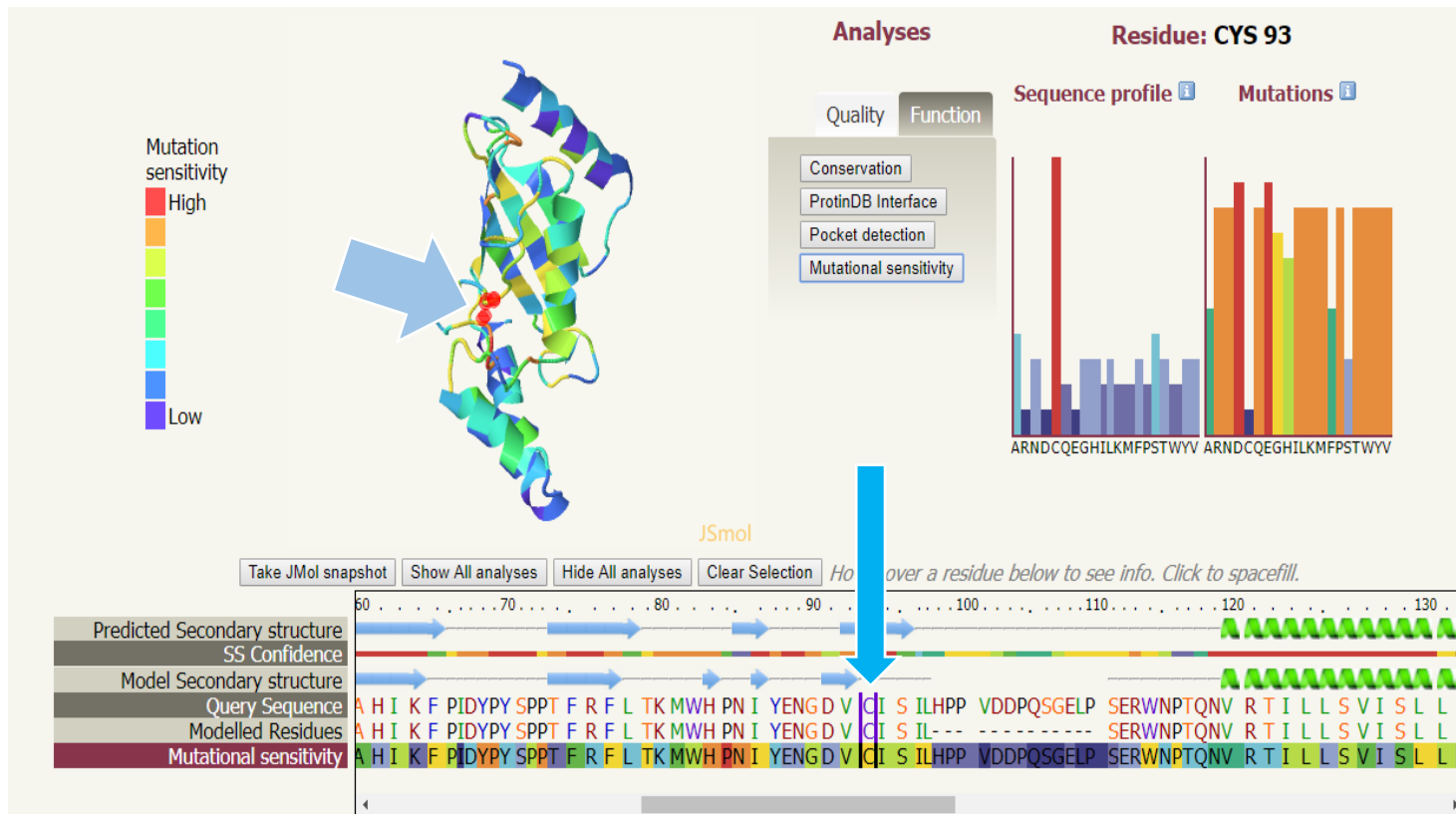
JSmol

保守
性分
析



口袋

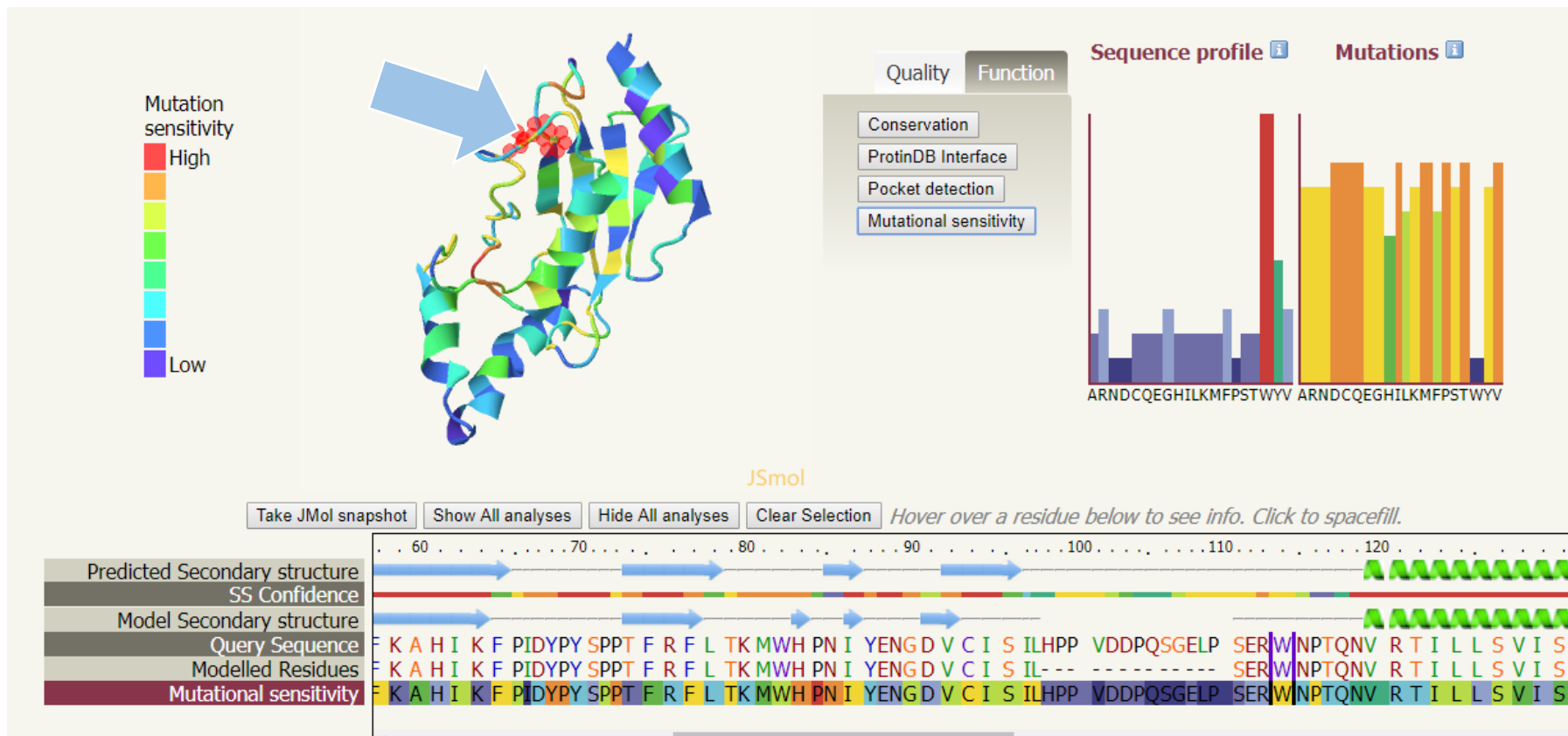
突变敏感性分析



观察93位活性位点的半胱氨酸保守性和突变敏感性，可以看到氨基酸高度保守，突变敏感性也比其他位置高很多。

structure prediction of UBE2 E2

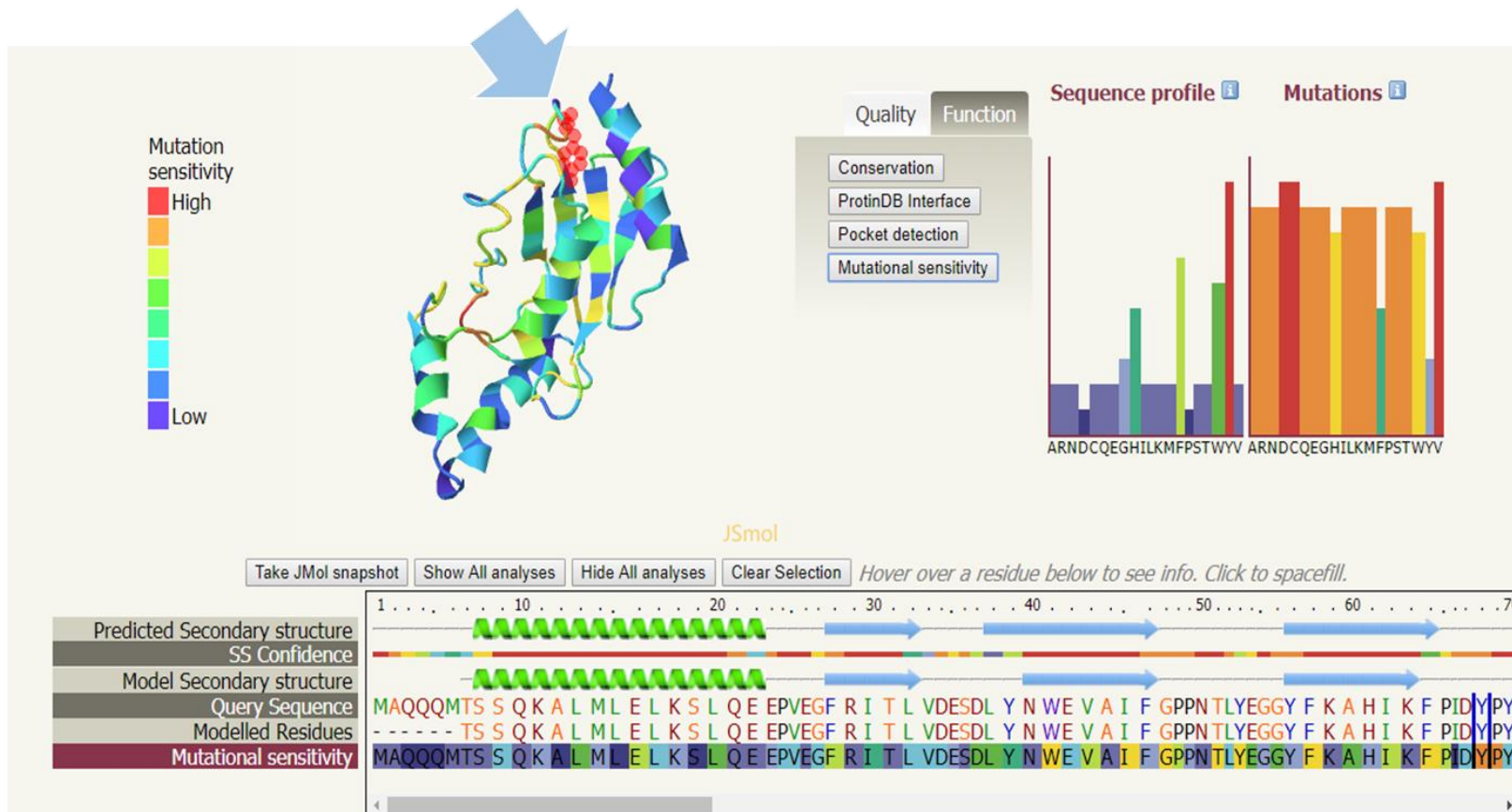
突变敏感性分析



在两个loop区也可以看到相似的结果

structure prediction of UBE2 E2

突变敏感性分析



在两个loop区也可以看到相似的结果

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Thanks For Ur Attention