

Ubc12蛋白结构初步分析

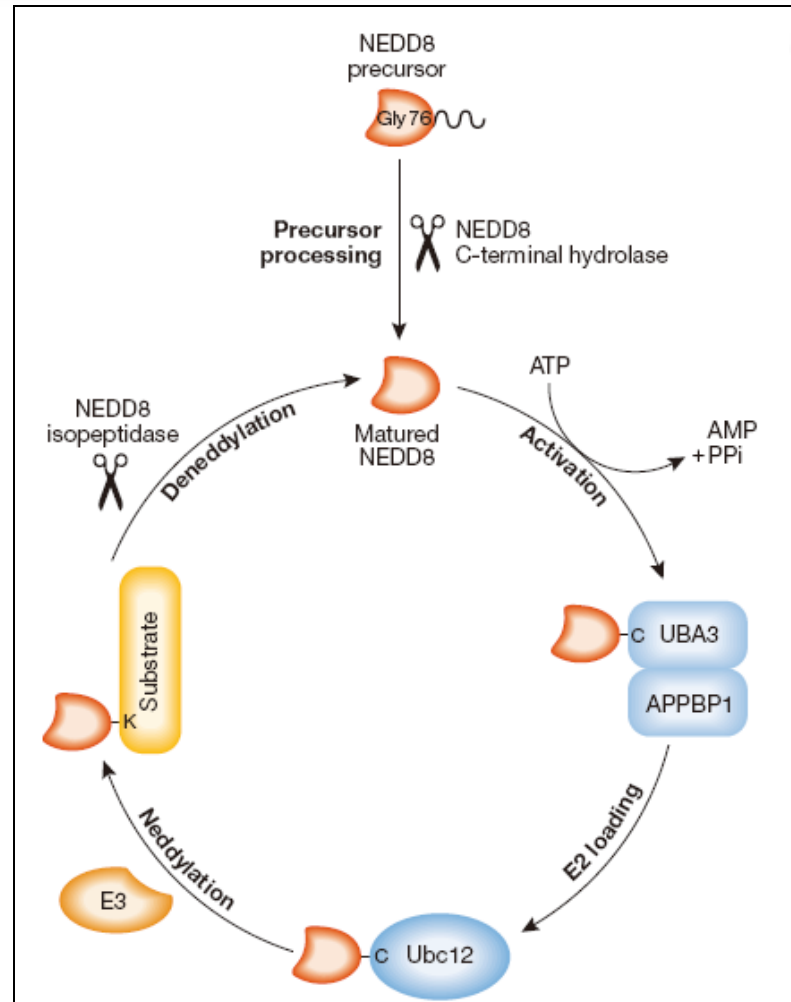
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Outline

- 1. Background on Neddylation and Ubc12
- 2. Goals of our project
- 3. Analysis by Bioinformatics tools
- 4. Conclusion

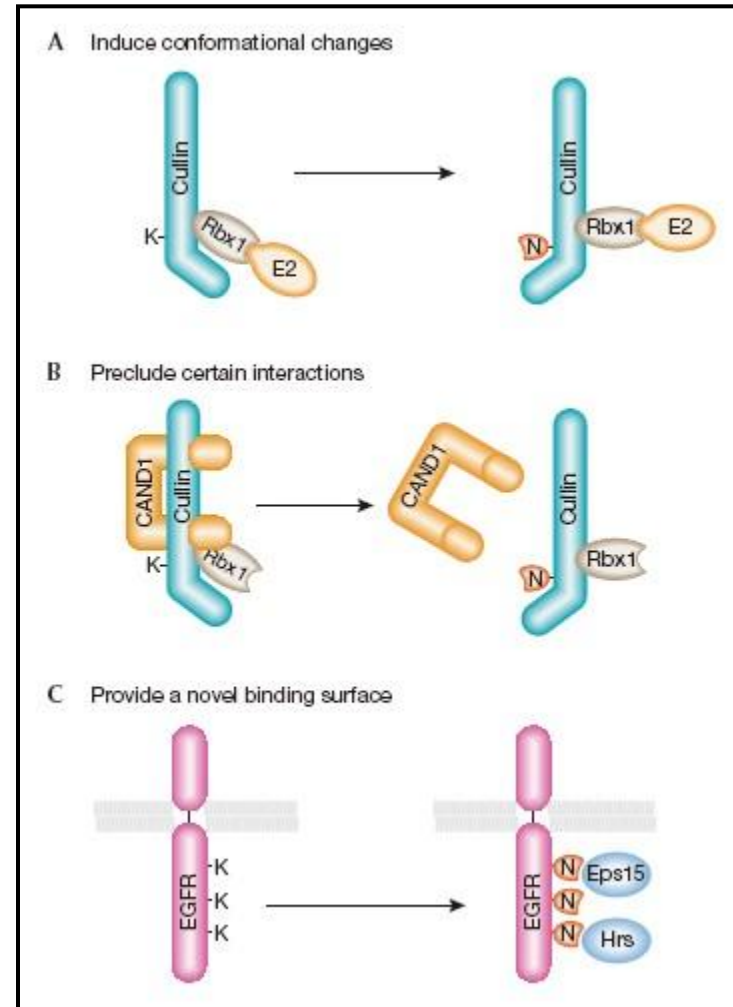
- NEDD8是一种类泛素化小分子，它在体内固有酶簇作用下被共价结合到底物蛋白上，参与蛋白质翻译后修饰，这一过程被称为Neddylation。



Neddylation过程 (Rabut G.et.al. 2008)

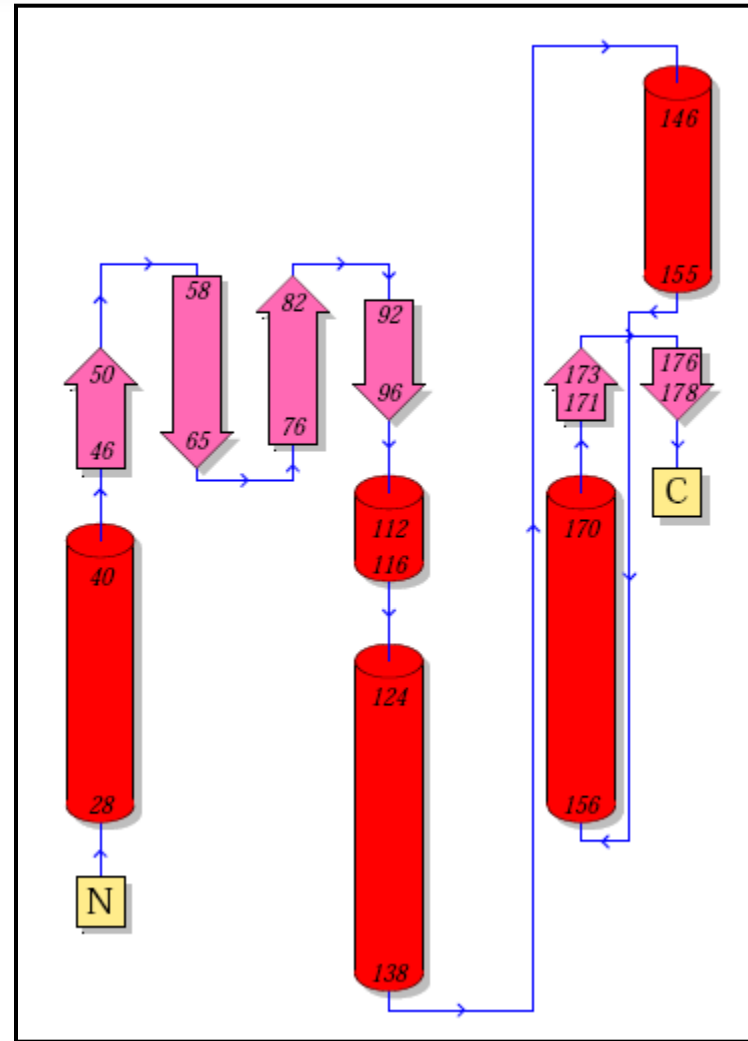
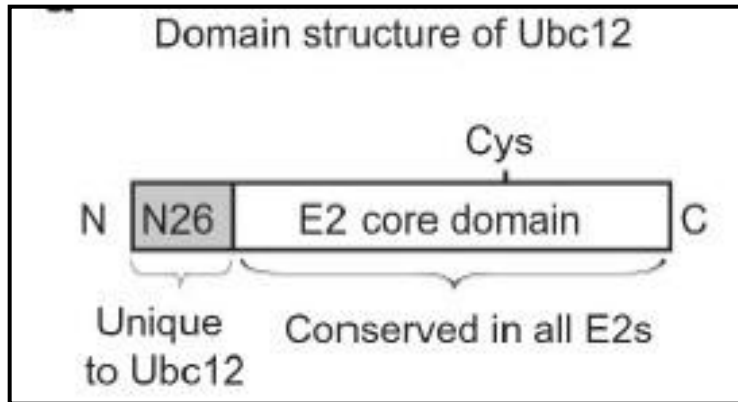
- Neddylation可以参与到细胞发育、细胞周期、信号转导等许多细胞调控。
- 目前发现的Neddylation的底物蛋白有：
Cullin家族、p53、Mdm2、p73、表皮生长因子受体EGFR、核糖体蛋白家族等。
- E1：APPBP1-UBA3复合物
- E2：Ubc12 (UBE2M)、UBE2F
- E3：Mdm2、Rbx1、Dcn1、SCF、c-Cb1等

- 改变底物蛋白的构象
- 和其它相互作用蛋白竞争性结合
- 招募NEDD8相互作用的蛋白。



Direct effects of neddylation (Rabut G.et.al. 2008)

- Ubc12由183个氨基酸残基组成，具有5个helix和6个strand。



- 1. How does Ubc12 interact with E1?
- 2. How does Nedd8 transfer from E1 to E2?
- 3. How does Nedd8 transfer from E2 to substrate?



- 1.Sequence analysis

```
1 MIKLFSLKQQKKEEESAGGTKGSSKKA SAAQLRIQKDI NELNLPKTCETEFSDHDDLIN 60 Q6P8D9 UBC12_XENTR
1 MIKLFSLKQQKKEEESAGGTKGSSKKA SAAQLRIQKDI TELNLPKTCETEFSDHDDLIN 60 Q6DCZ9 UBC12_XENLA
1 MIKLFSLKQQKKEEESAGGTKGSSKKA SAAQLRIQKDI NELNLPKTCDISFSDEDDLIN 60 A3KN22 UBC12_BOVIN
1 MIKLFSLKQQKKEEESAGGTKGSSKKA SAAQLRIQKDI NELNLPKTCDISFSDEDDLIN 60 P61082 UBC12_MOUSE
1 MIKLFSLKQQKKEEESAGGTKGSSKKA SAAQLRIQKDI NELNLPKTCDISFSDEDDLIN 60 P61081 UBC12_HUMAN
1 MIKLFSLKQQKKEEESAGGTKGSSKKA SAAQLRIQKDI NELNLPNT CATDFPDDNDLIN 57 Q9VSF3 UBC12_DROME
*****:*****:*.*. : *****:*****:***.***:*****
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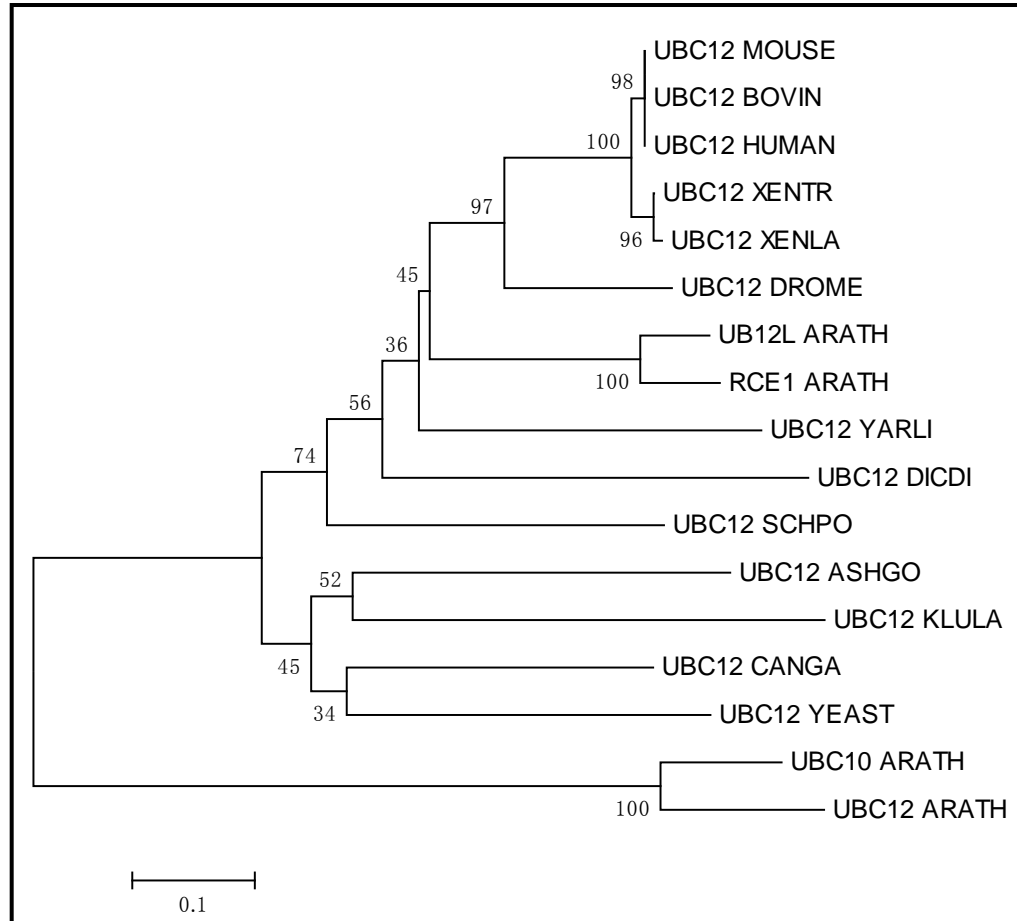
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61 KLVICPDEGFYKGGKFVFSFKVGGQGYPHDPPKV K CETMVYHPNIDL EGNVCLN I LREDWK 120 Q6P8D9 UBC12_XENTR
61 KLVICPDEGFYKGGKFVFSFKVGGQGYPHDPPKV K CETMVYHPNIDL EGNVCLN I LREDWK 120 Q6DCZ9 UBC12_XENLA
61 KLVICPDEGFYKSGKFVFSFKVGGQGYPHDPPKV K CETMVYHPNIDL EGNVCLN I LREDWK 120 A3KN22 UBC12_BOVIN
61 KLVICPDEGFYKSGKFVFSFKVGGQGYPHDPPKV K CETMVYHPNIDL EGNVCLN I LREDWK 120 P61082 UBC12_MOUSE
61 KLVICPDEGFYKSGKFVFSFKVGGQGYPHDPPKV K CETMVYHPNIDL EGNVCLN I LREDWK 120 P61081 UBC12_HUMAN
58 KLIISPEDEGFYRDRFVFNFRVGSNYEHE PPKVKATQVYHPNIDL DGNVCLN I LREDWN 117 Q9VSF3 UBC12_DROME
**:*.******:.*:***.*:***.***:***** * *****:*****:*****:
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121 PVLTIINSIIYGLQYLFLEPNPEDPLNKEAAEVL QNNRR LFEQNVQRS MRGGYIGSTYFER 180 Q6P8D9 UBC12_XENTR
121 PVLTIINSIIYGLQYLFLEPNPEDPLNKEAAEVL QNNRR LFEQNVQRS MRGGYIGSTYFER 180 Q6DCZ9 UBC12_XENLA
121 PVLTIINSIIYGLQYLFLEPNPEDPLNKEAAEVL QNNRR LFEQNVQRS MRGGYIGSTYFER 180 A3KN22 UBC12_BOVIN
121 PVLTIINSIIYGLQYLFLEPNPEDPLNKEAAEVL QNNRR LFEQNVQRS MRGGYIGSTYFER 180 P61082 UBC12_MOUSE
121 PVLTIINSIIYGLQYLFLEPNPEDPLNKEAAEVL QNNRR LFEQNVQRS MRGGYIGSTYFER 180 P61081 UBC12_HUMAN
118 PVLNINSIVYGLQFLFLEPNPEDPLNKEAADVL QTNRRQ FENN VKKAMRGGCVGETYFEC 177 Q9VSF3 UBC12_DROME
***.*****:*****:*****:*****:*****:***.*** **:*.*:*****:*****:***.***
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```
181 CLK- 183 Q6P8D9 UBC12_XENTR
181 CLK- 183 Q6DCZ9 UBC12_XENLA
181 CLK- 183 A3KN22 UBC12_BOVIN
181 CLK- 183 P61082 UBC12_MOUSE
181 CLK- 183 P61081 UBC12_HUMAN
178 CLK 181 Q9VSF3 UBC12_DROME
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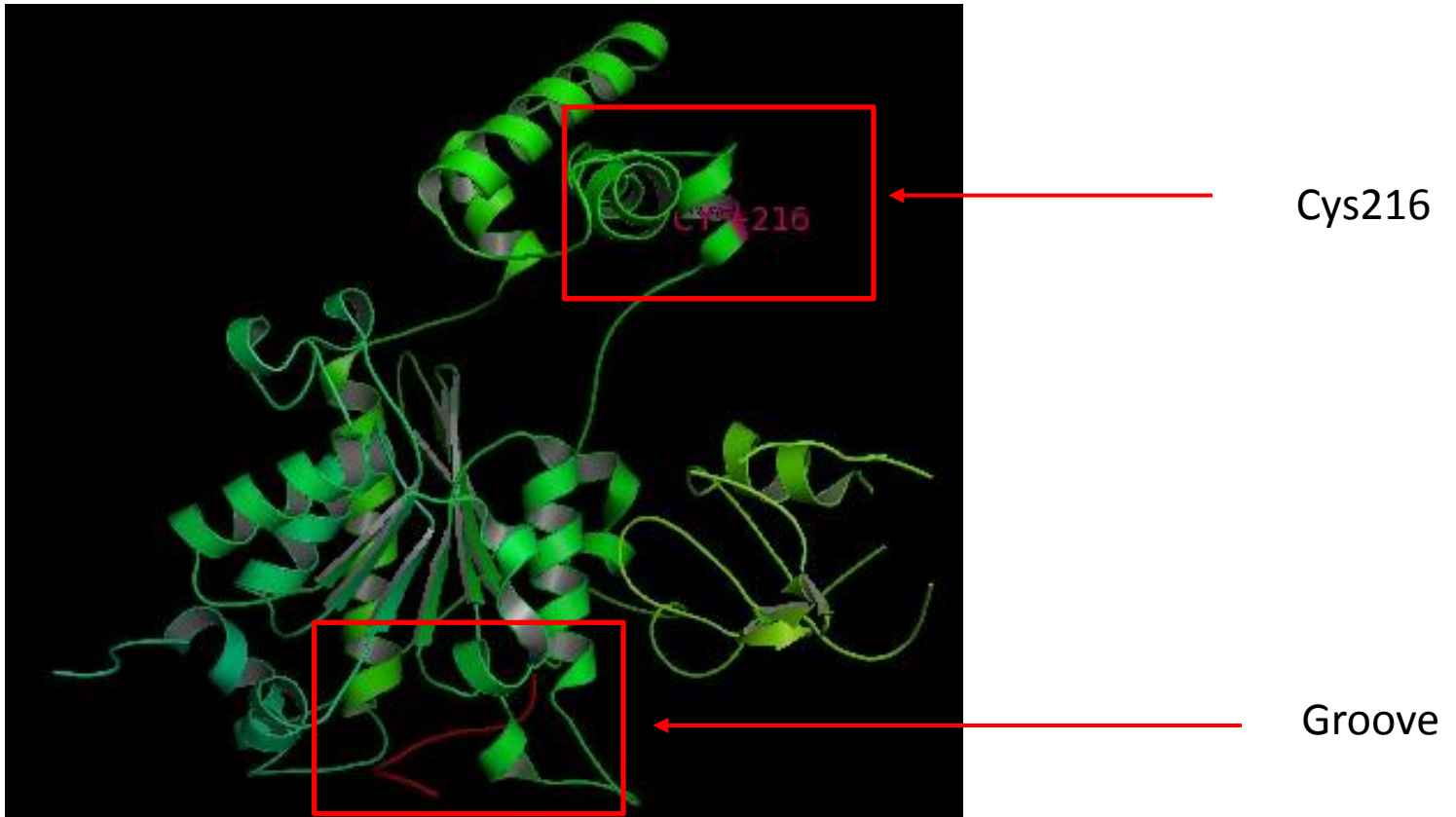
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- 1. Sequence analysis

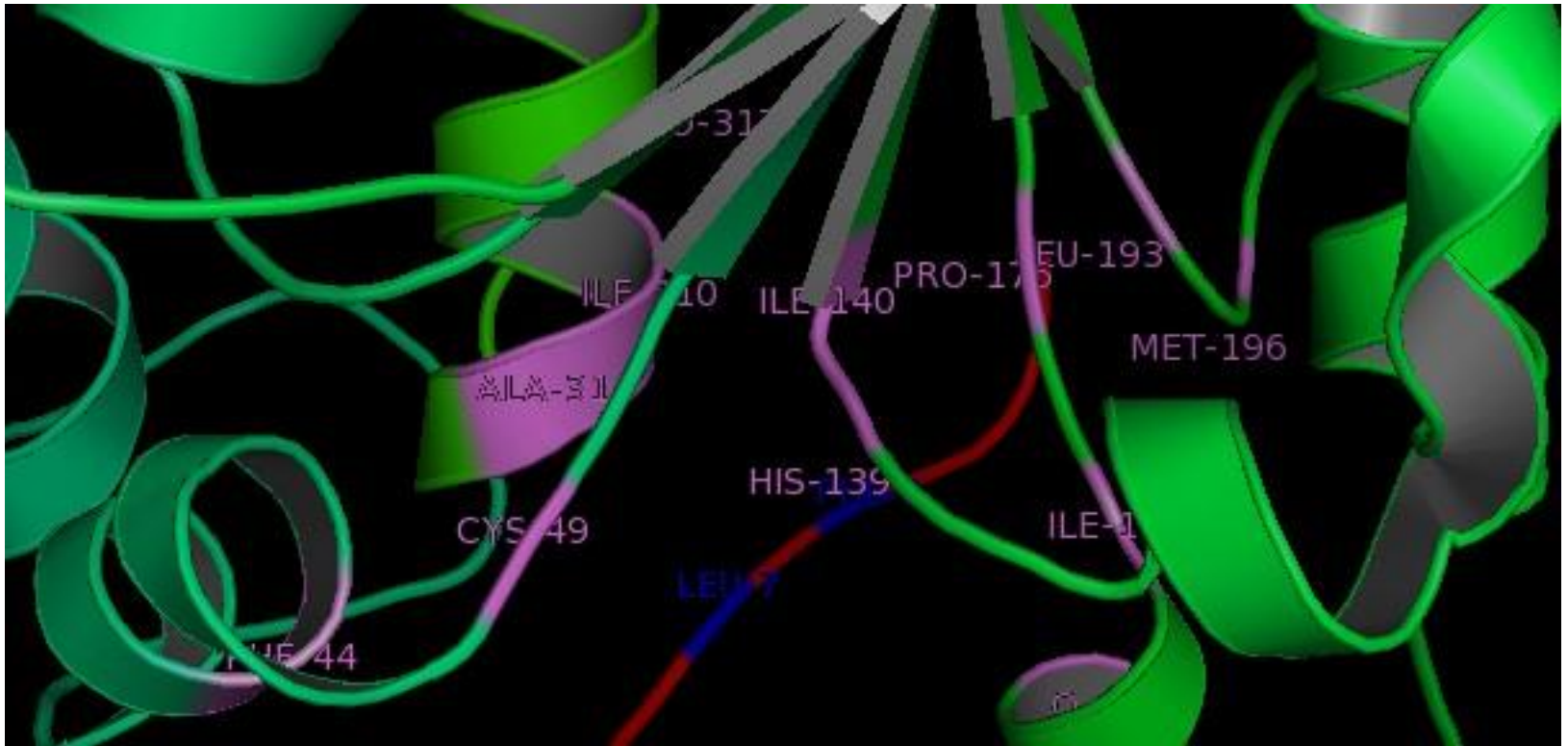


Ubc12直系同源系统发育树

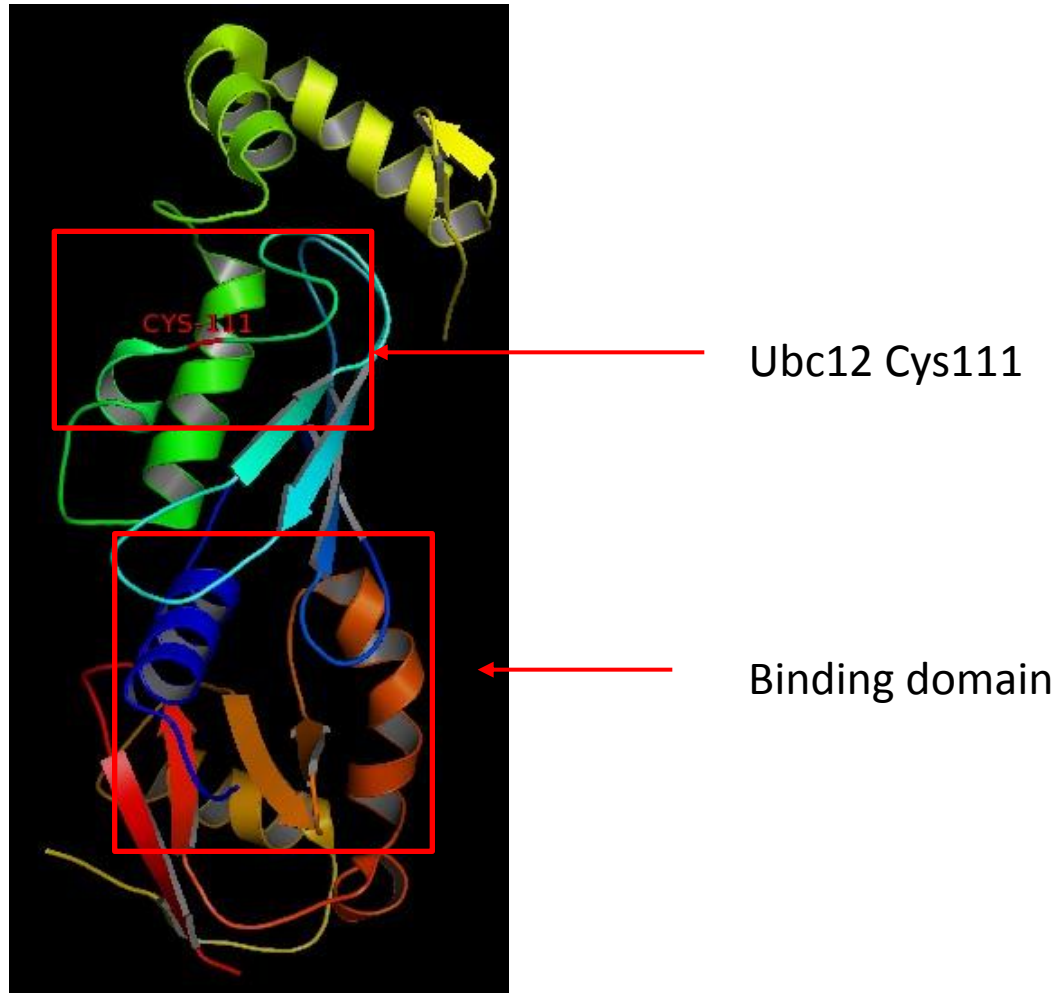
- 2. A Common Mechanism for E1-E2 Interactions



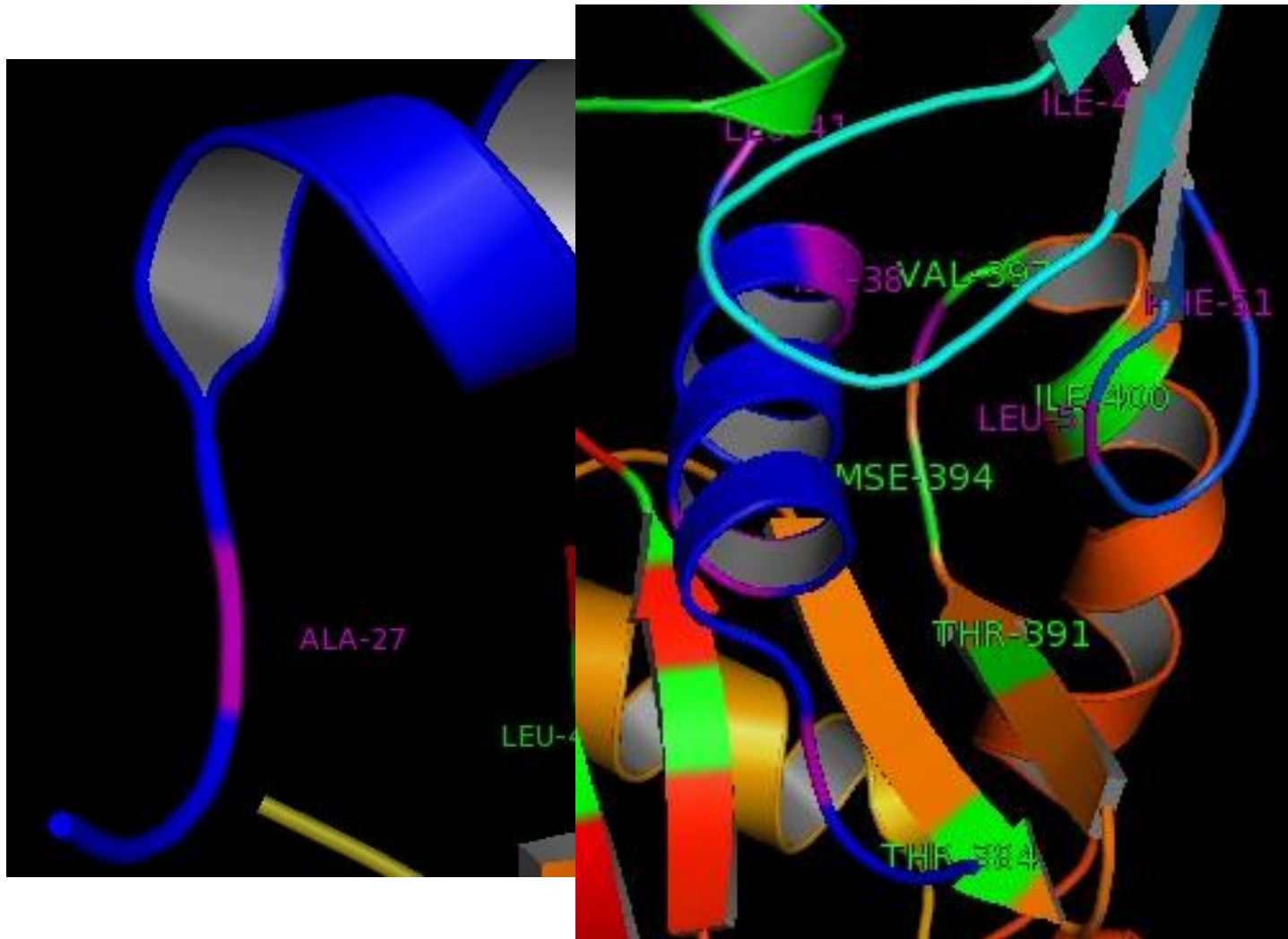
Overall structure of the Ubc12N26 complex with UBA3



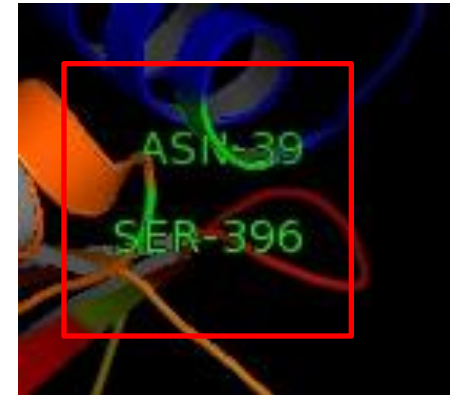
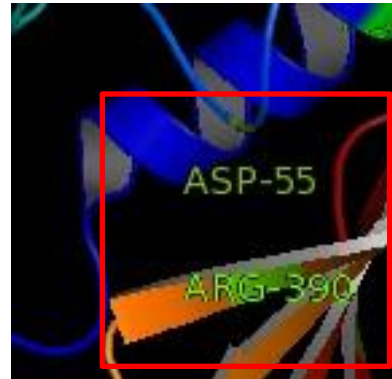
Contributions of individual residues from the Ubc12N26 to UBA3 binding



Overall Structure of the NE1^{ufd}-Ubc12^{core} Complex

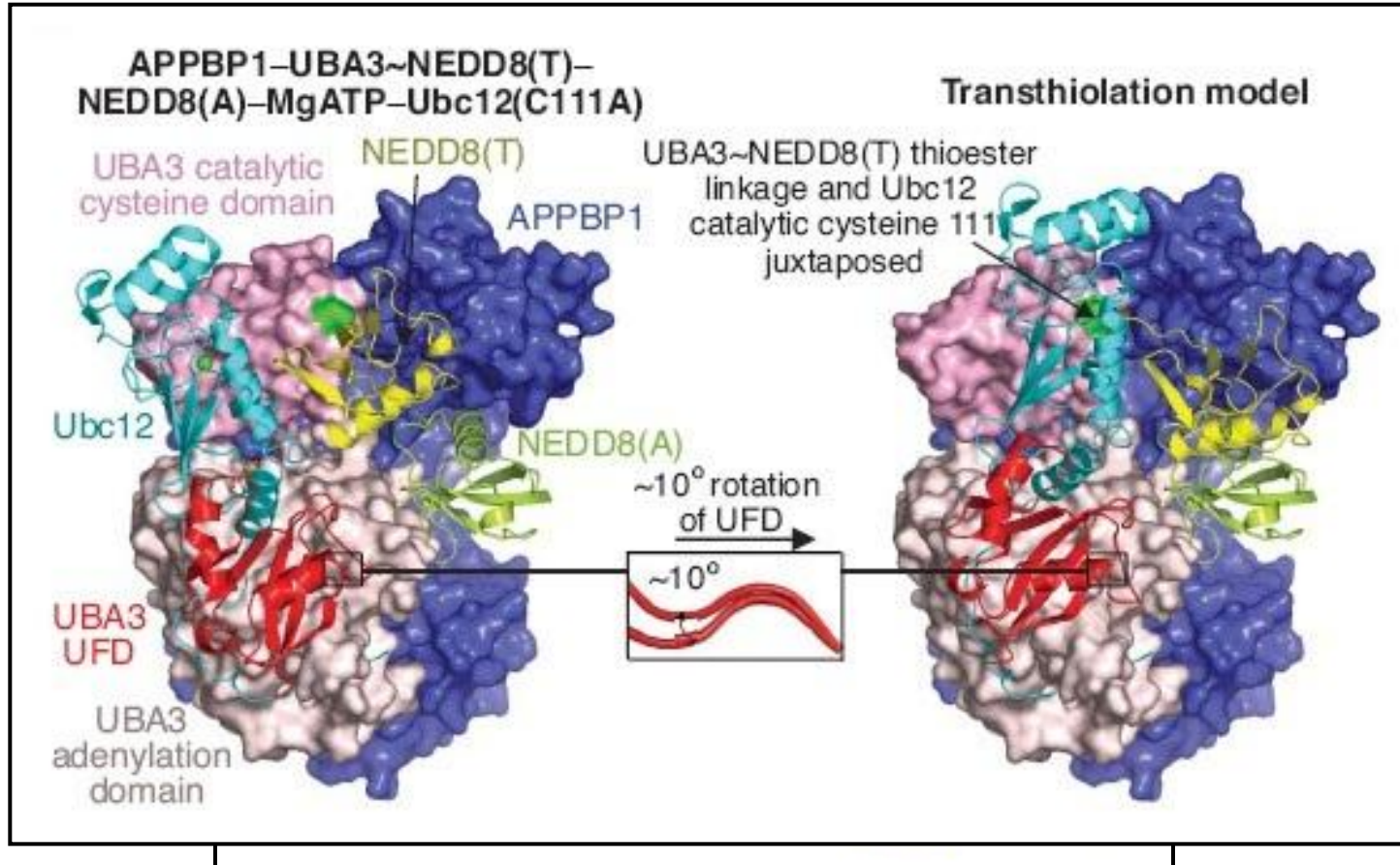


Contributions of individual residues from the Ubc12 core domain to UBA3 binding



**Contributions of individual residues from the Ubc12
core domain to UBA3 binding**

- 3. How does Nedd8 transfer from E1 to E2



**Evidence for Rotation of the E1's Ubiquitin-Fold Domain in Ubc12
 NEDD8 Thioester Formation(Danny T. Huang.et.al. 2005)**

- 4. How does Nedd8 transfer from E2 to substrate
 - No Structure of Ubc12 and E3, maybe domain of E2/E1 and E2/E3 overlaps.
 - Suppose: Ubc12-Nedd8 but not free Ubc12 can interact with E3, and E3 can change its formation and then Nedd8 transfer from E2 to substrate.

- 1. Ubc12 is very conservative.
- 2. Both the N-terminal domain and the core domain of Ubc12 can interact with E1, this stabilize the complex between Ubc12/E1.
- 3. Transferring of Nedd8 from E1 to Ubc12 needs conformation change.
- 4. It needs more study of how Nedd8 transfers from E2 to substrate.

Thank you