

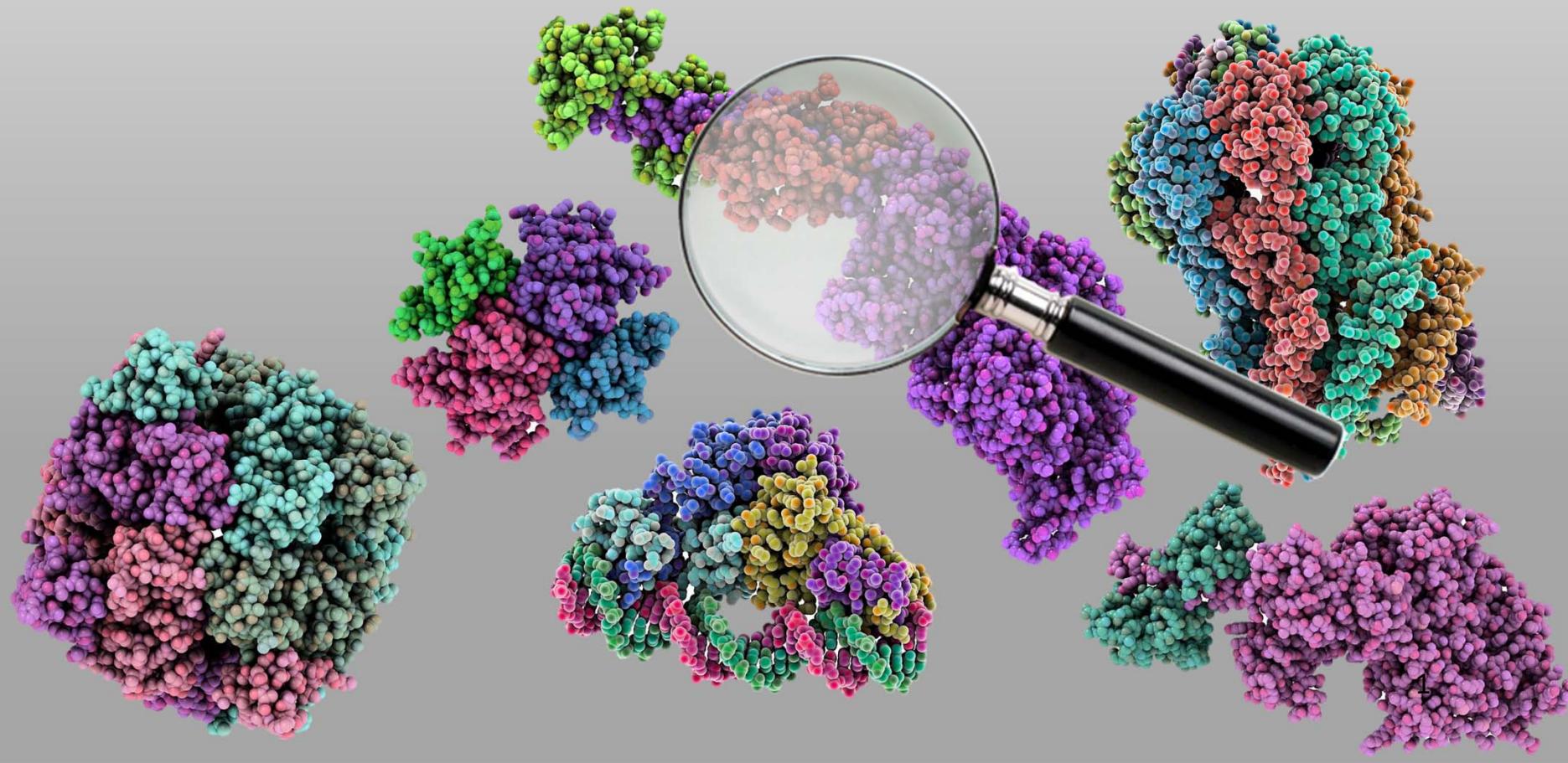
Molecular and Structural Biology 1 (Fall Semester 2019)

# Large Cellular Complexes in Protein Quality Control: Cellular Protein Degradation Part II

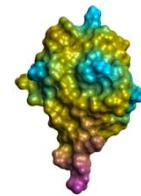
Prof. Eilika Weber-Ban

Institute for Molecular Biology & Biophysics

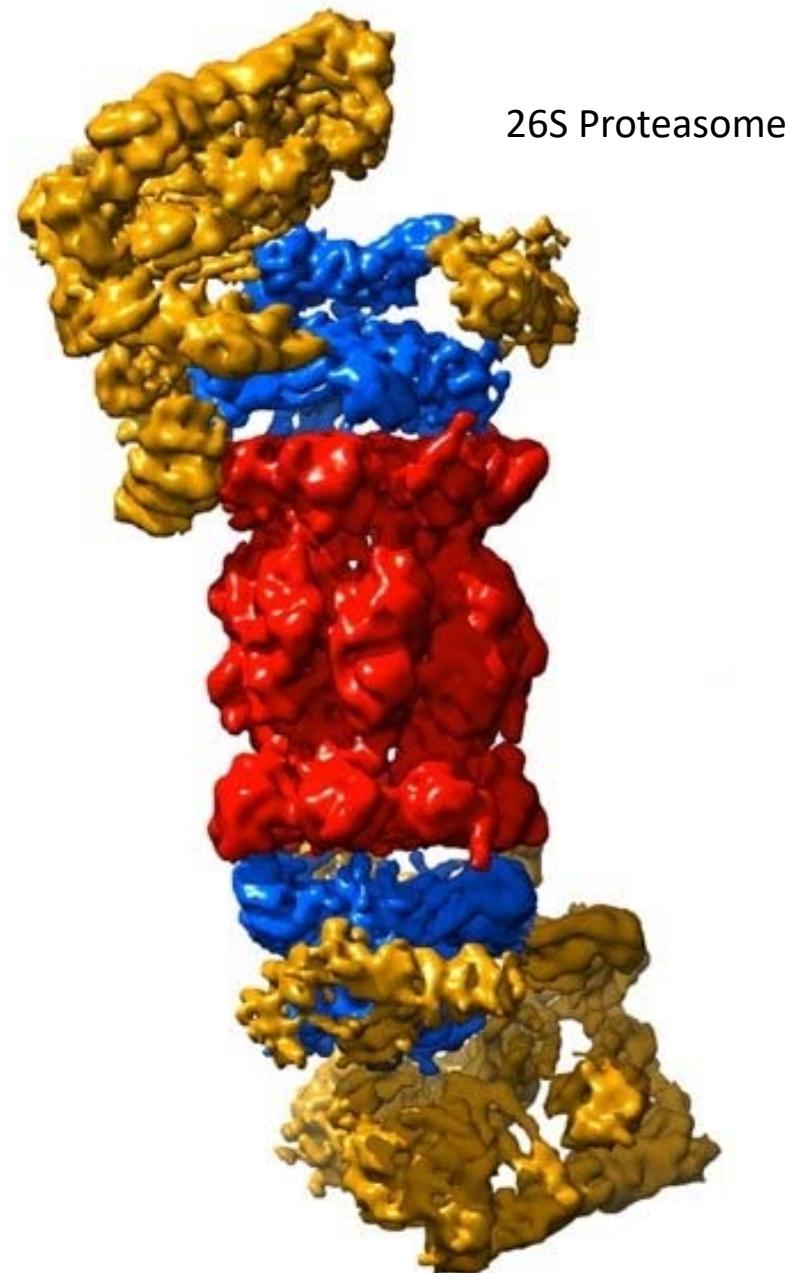
ETH Zurich



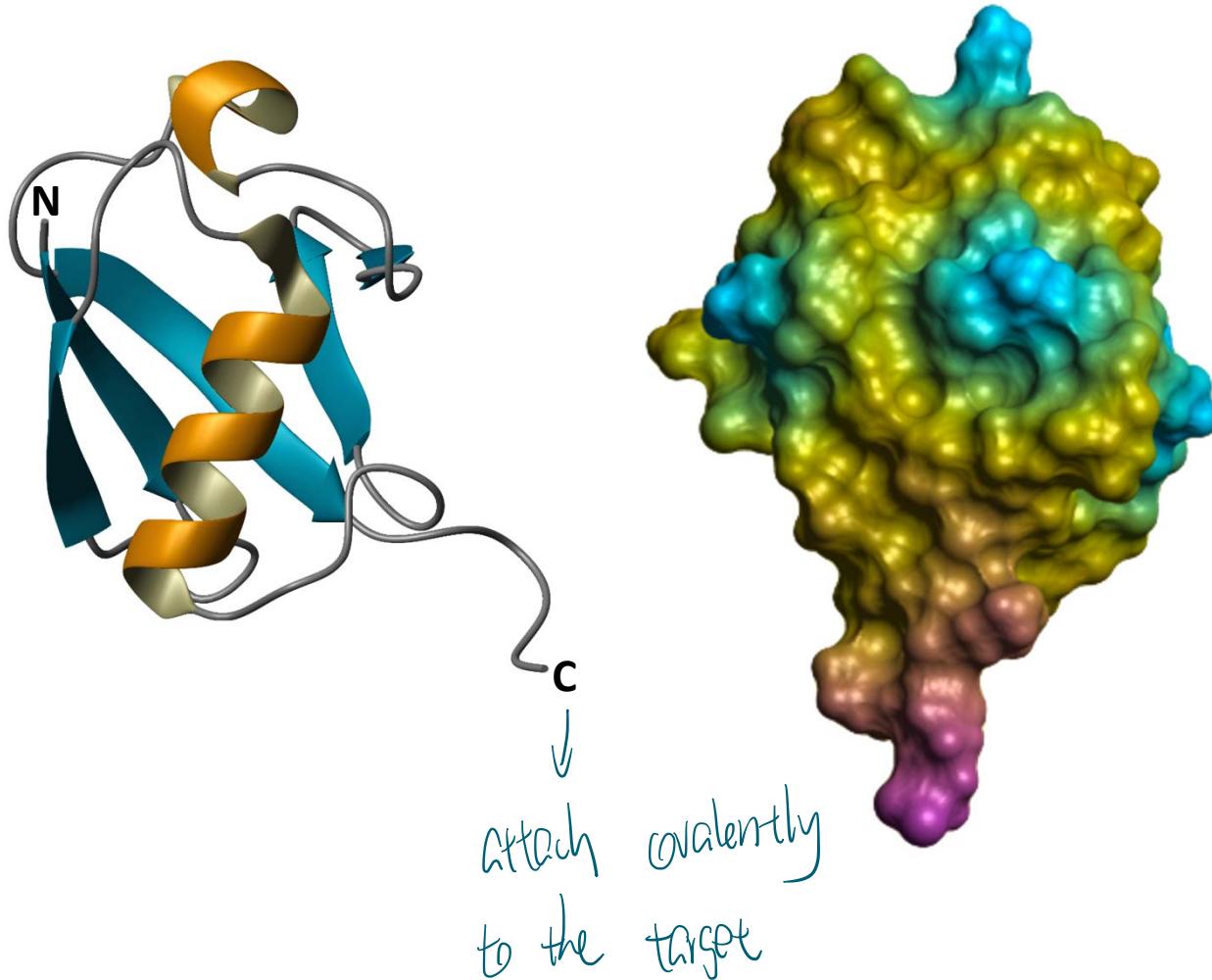
# The Ubiquitin-Proteasome System (UPS)



Ubiquitin

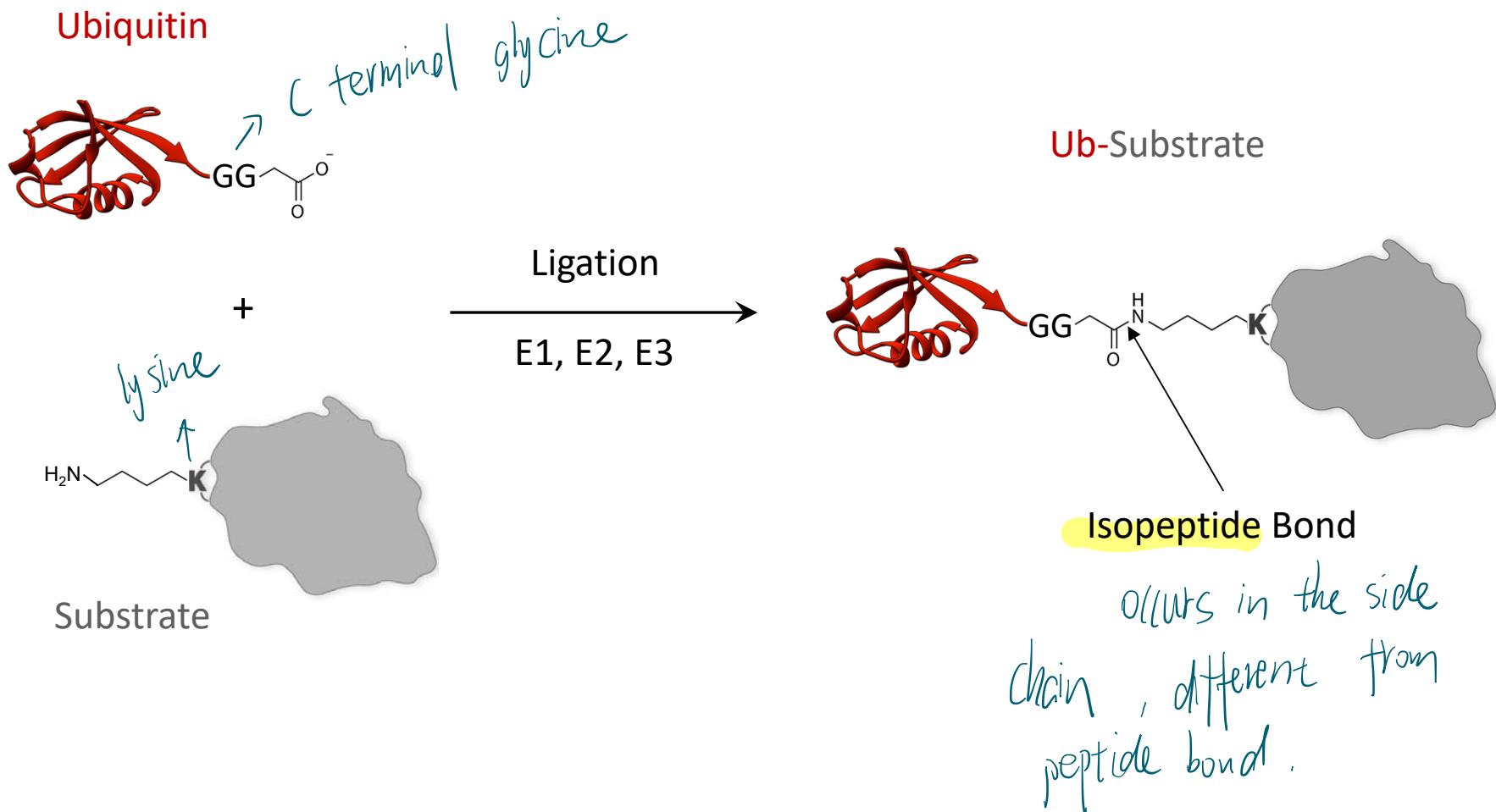


## Ubiquitin – Recruitment Tag for 26S Proteasome

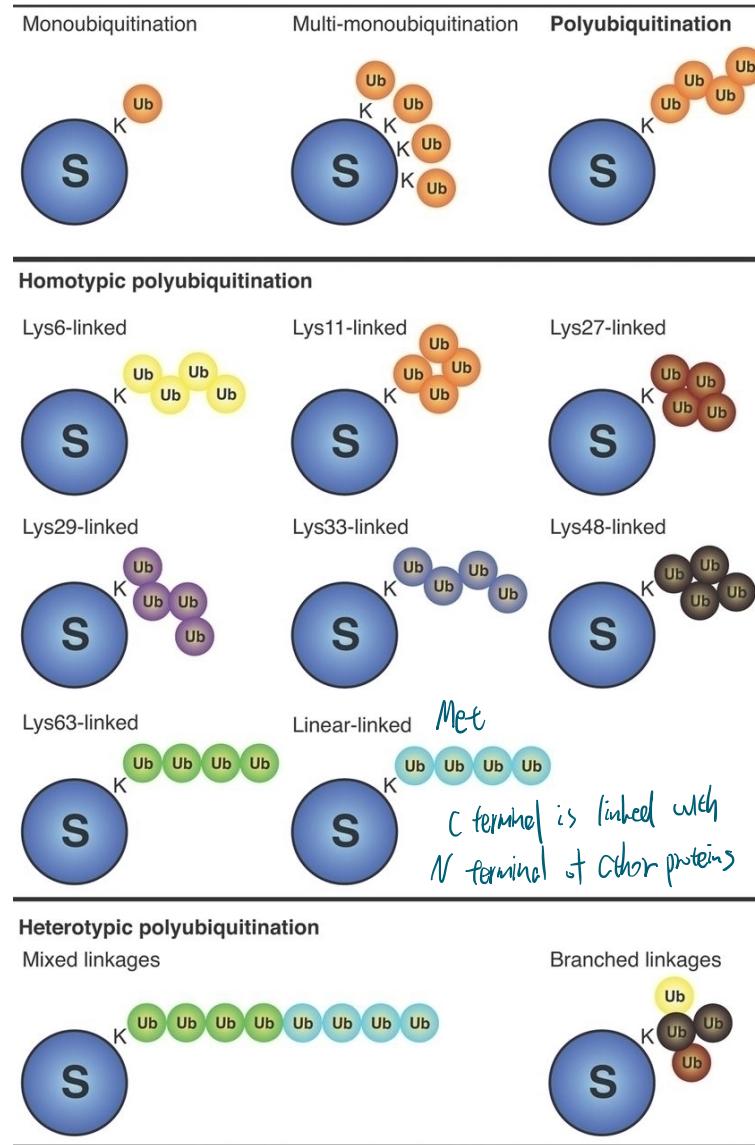
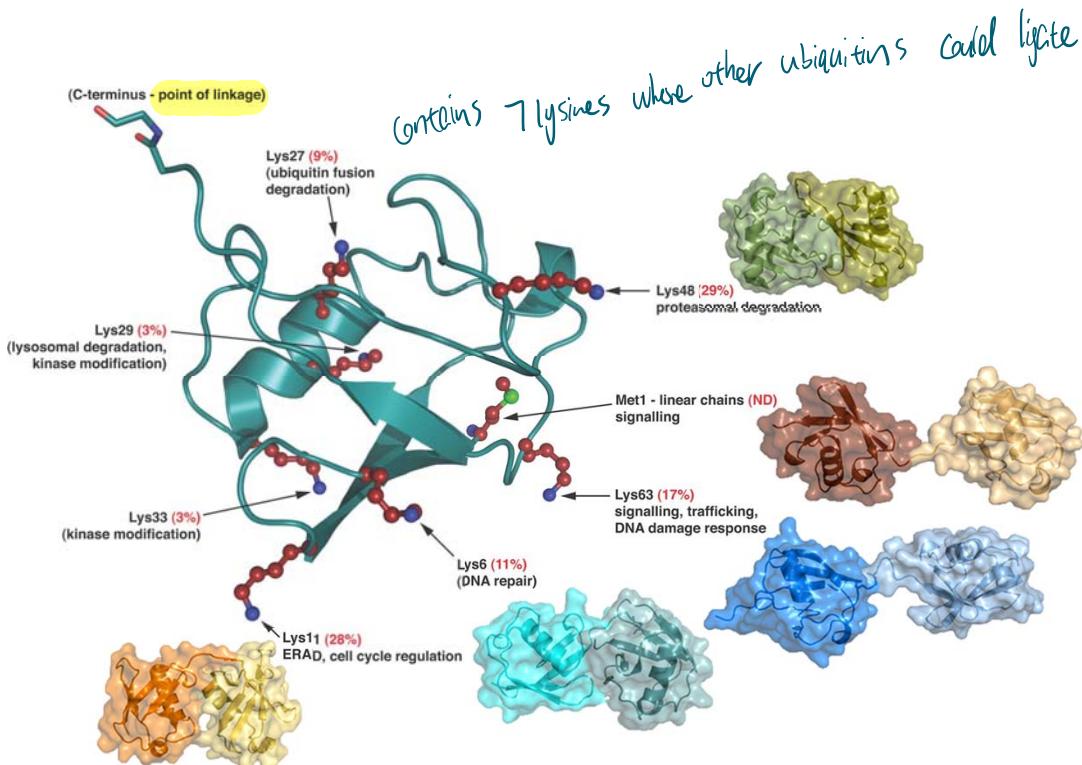


# Ubiquitination – Overall Reaction

post-translational

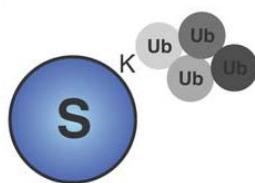


# Ubiquitination – A Tag with Complexity

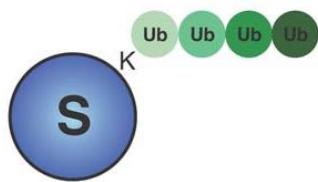


# Different Ub-chain types show different topology

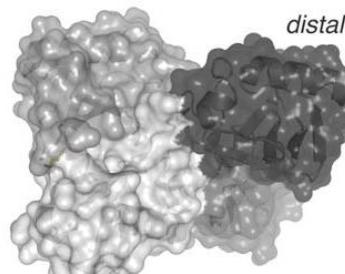
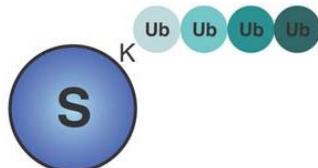
Lys48-linked



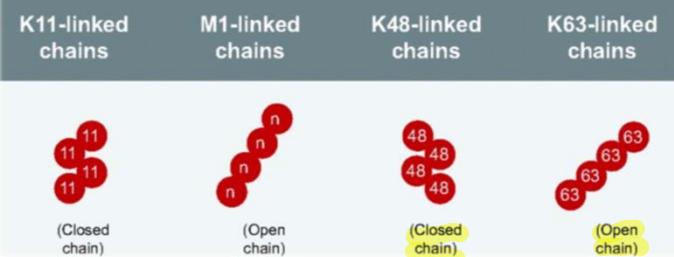
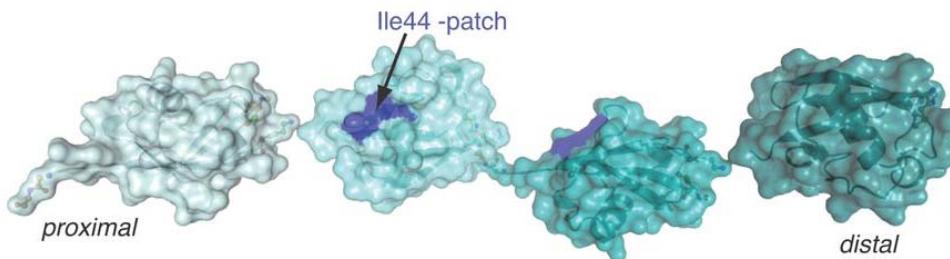
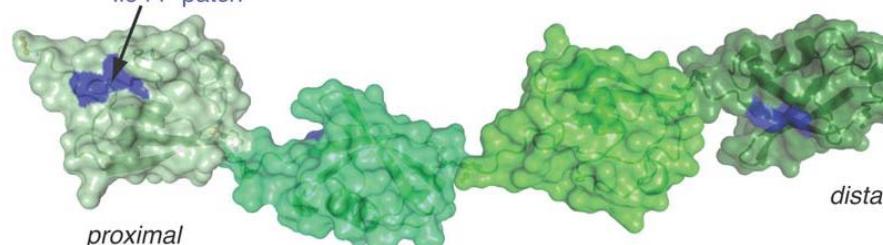
Lys63-linked



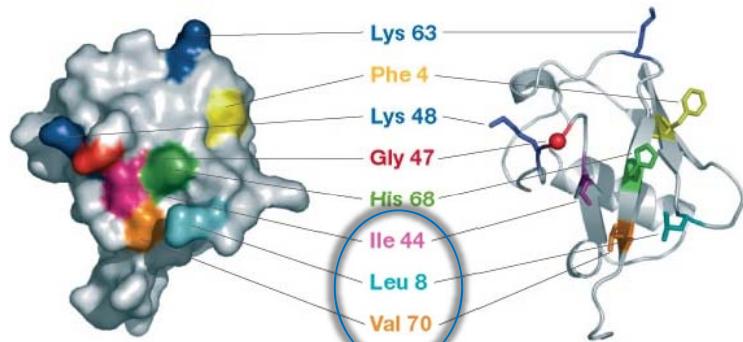
Linear



isoleucine, important region recognized by partners

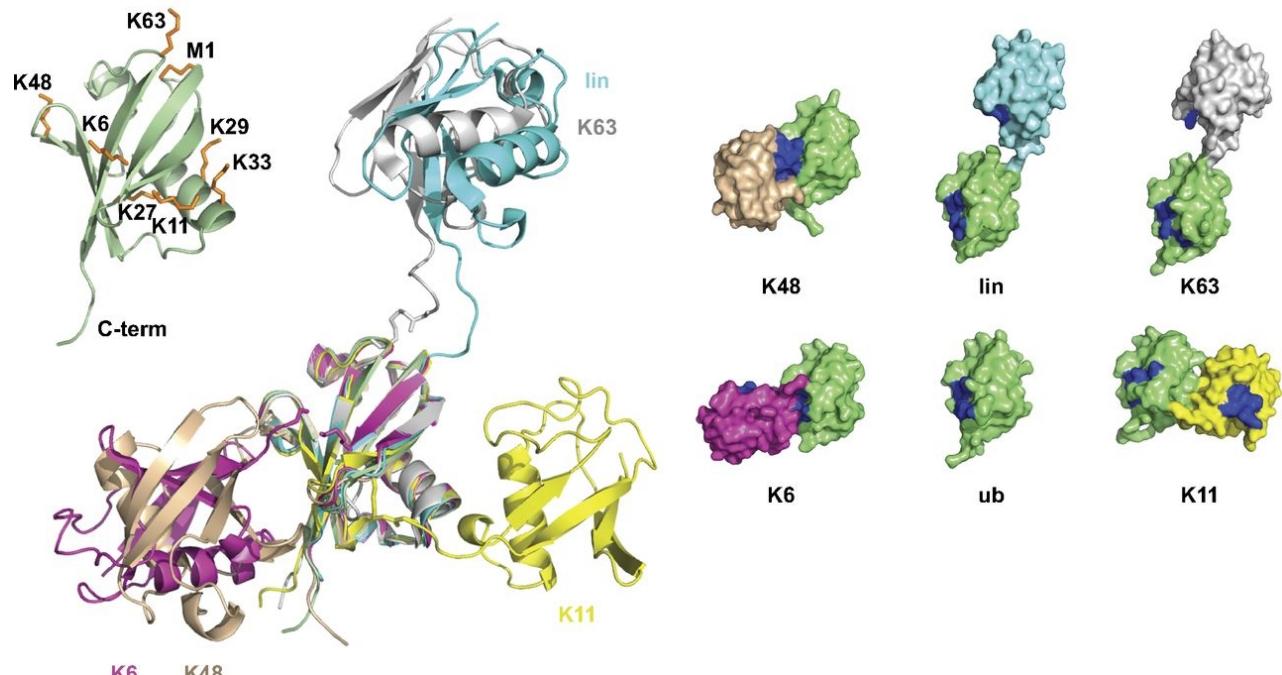


# Key Contact Surfaces on Ubiquitin

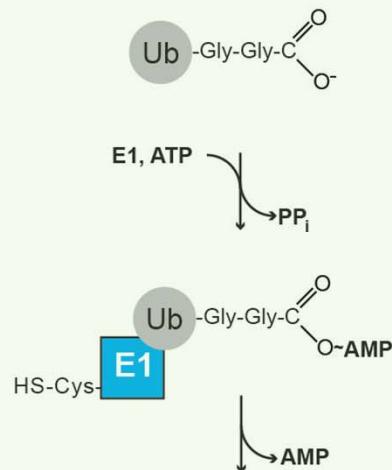


Leu8/Ile44/Val70 hydrophobic patch is required in context of a polyUb chain for proteasome binding and degradation.

this bind recognition could explain why some chains only bind to specific site of proteasome,  
( different chain shapes have different patch distribution )



# Ubiquitination – the chemistry

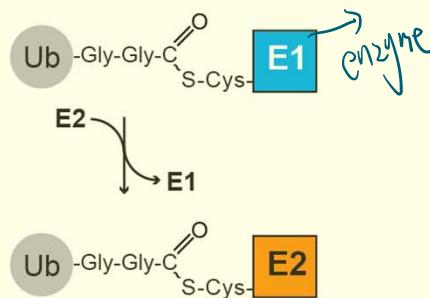


## Activation

The C-terminal carboxylate has to be activated. This occurs by **adenylylation**, where the AMP moiety of ATP is transferred to one of the oxygens.

Mechanistically that is the attack of the O<sup>-</sup> on the α-phosphate of ATP.

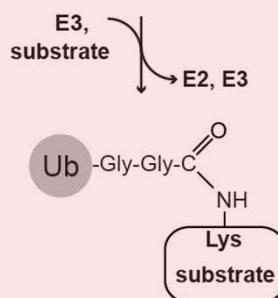
AMP is a good leaving group, so that the carbonyl carbon can now be attacked by a nucleophile, in this case a cysteine residue of the E1. The Ub is now bound to E1 as a thioester.



## Conjugation

The conjugation reaction is essentially a trans-thioesterification. The Ub is transferred from a cysteine on **E1** to a cysteine on **E2**.

Name: this is called conjugation, because the **E2** is able to bind to the **E3**, it makes the connection to the ligase in the next step.



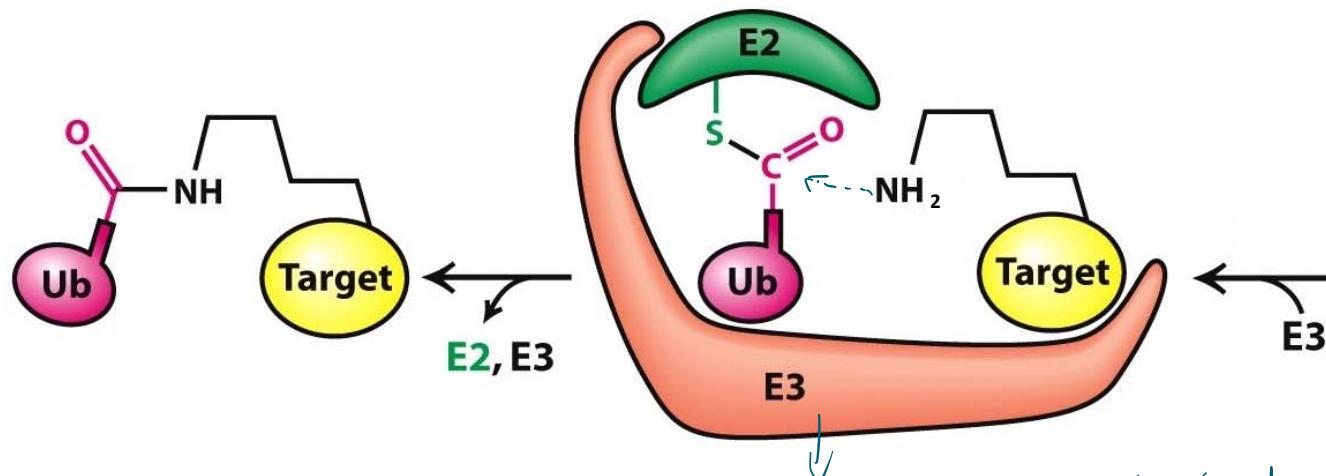
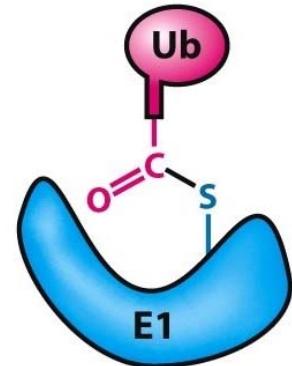
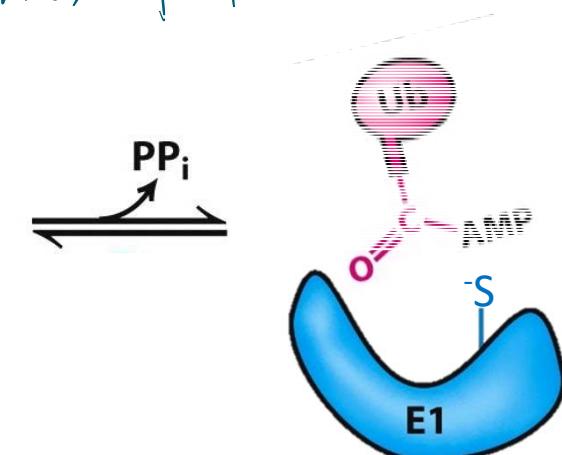
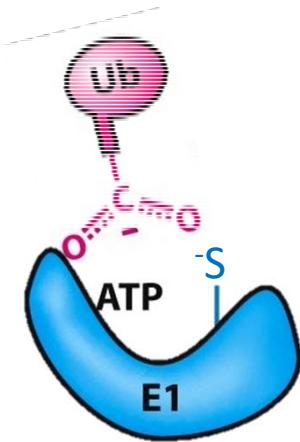
## Ligation

A covalent bond is formed between the target and Ub. The **ε-amino group of a lysine** in the target protein makes a nucleophilic **attack on the carbonyl-carbon** of the thioester group. The thiol is the leaving group, thereby releasing the ubiquitin from the covalent bond with the enzyme. **The isopeptide bond with the substrate is formed.**

# Ubiquitination – Reaction Scheme with Enzymes

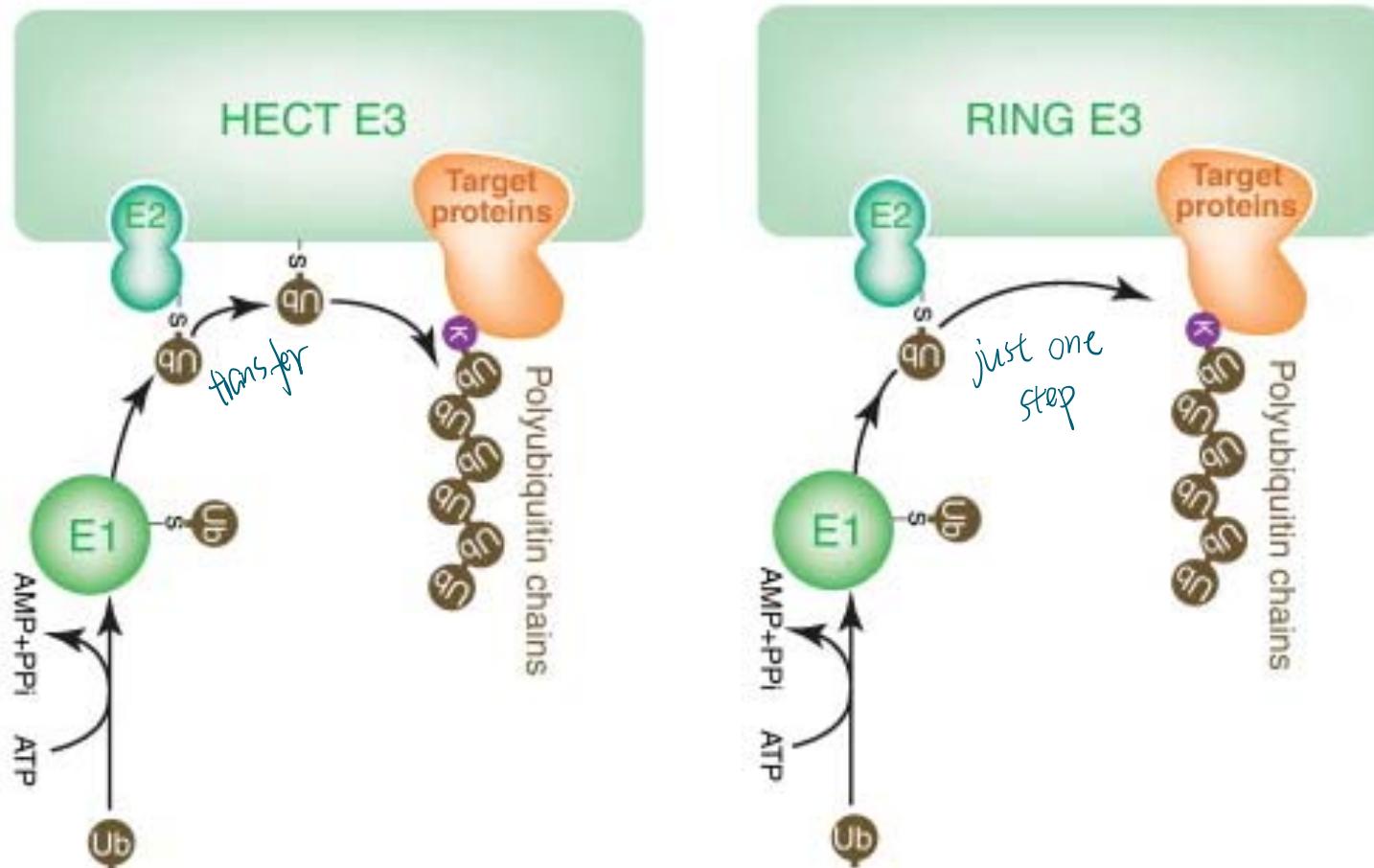
kinds of proteins:  $E_1 < E_2 < E_3$

↓  
most diverse where selectivity has  
shown



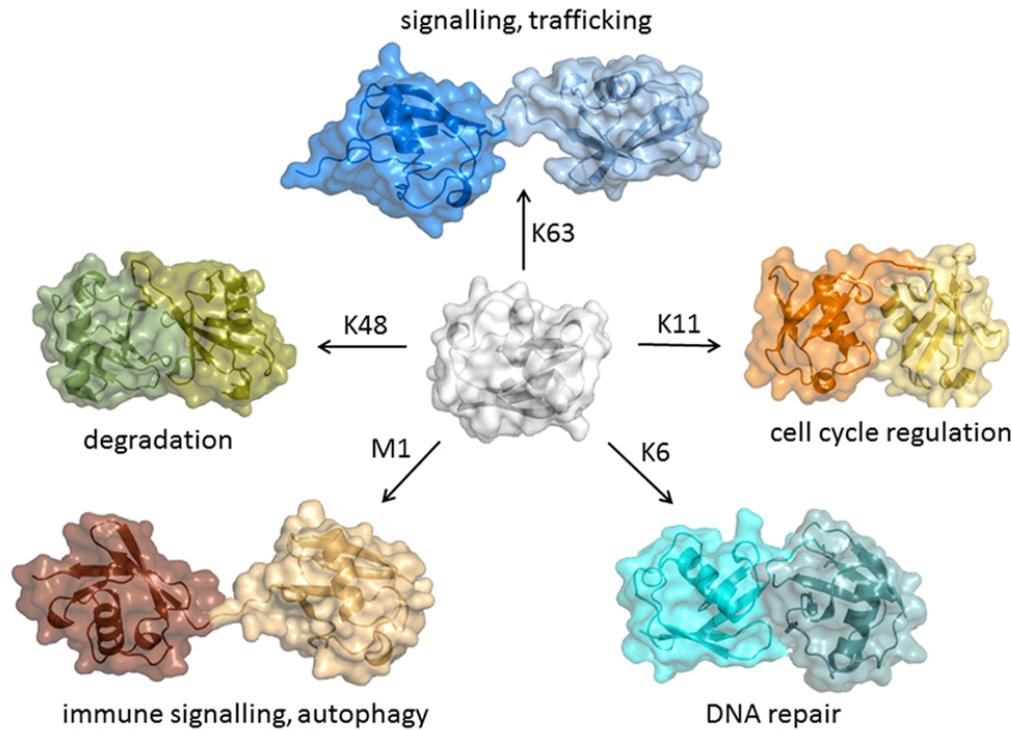
bring Ub and target together  
contain binding sites for Ub and target protein.

## Two main types of E3 ligases: HECT and RING



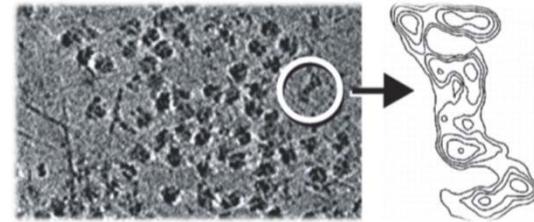
# Polyubiquitination leads to degradation (not always, but for certain linkages)

Different linkages have different functions for polyubiquitin



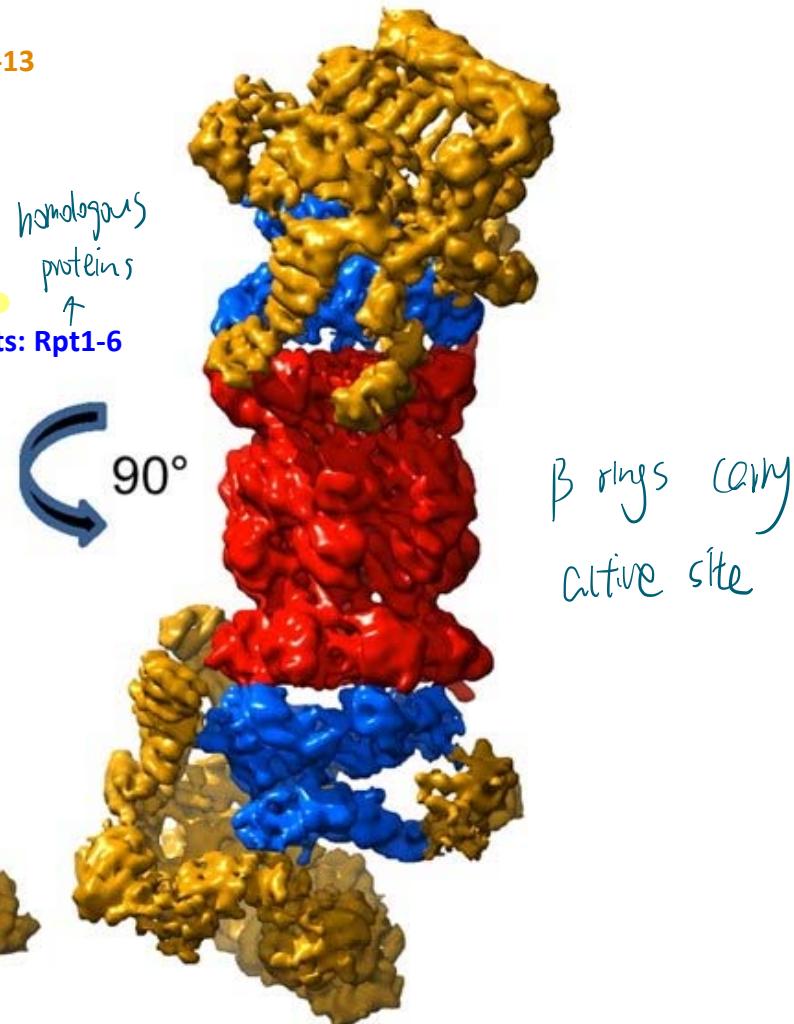
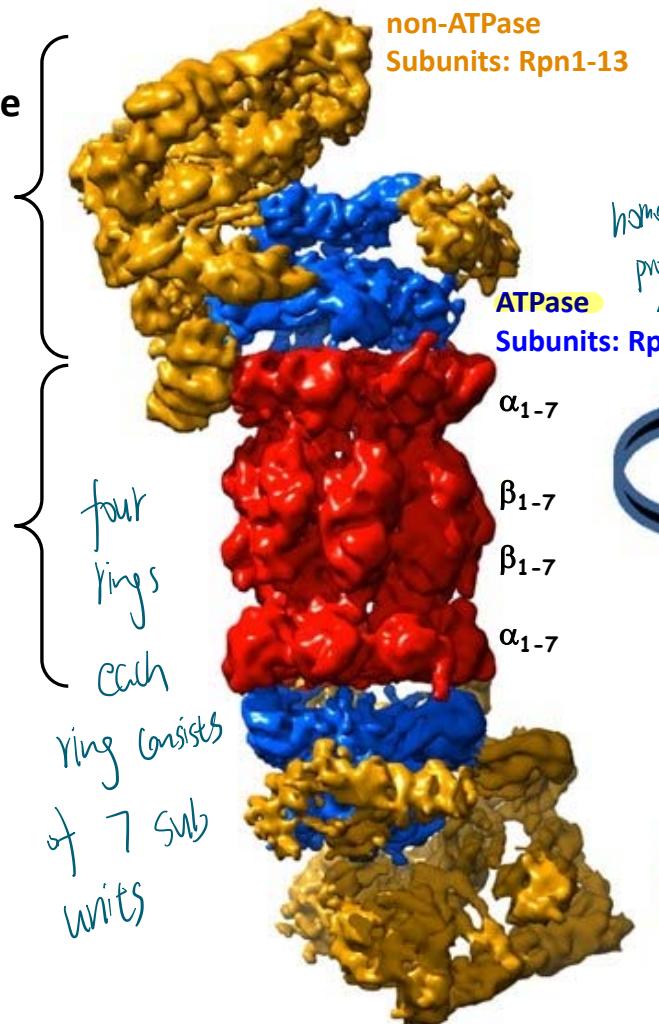
The main linkage type you should remember for degradation is via K48.

# The 26S Proteasome



recognize the tag  
and take off the tag  
↑

**19S Regulatory Particle**  
(~900 kD)  
two catalytic activities:  
**ATPase activity**  
Deubiquitination



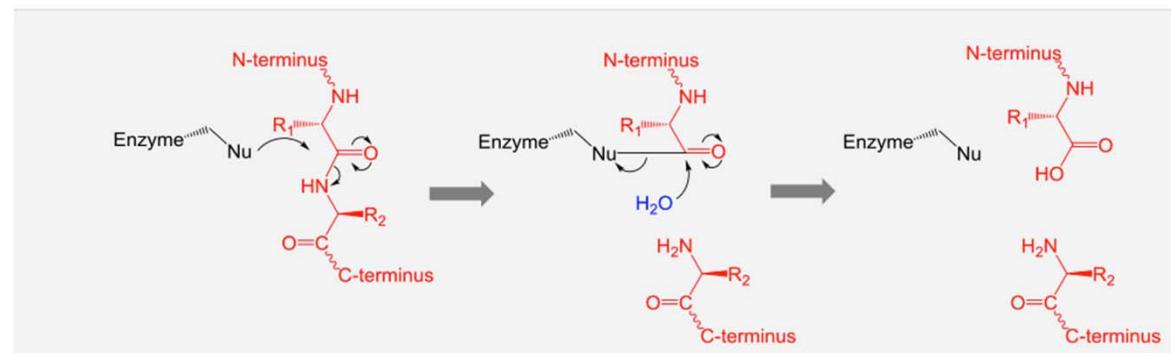
**20S Core Particle**  
(~700 kD)

↓  
where degradation  
occurs

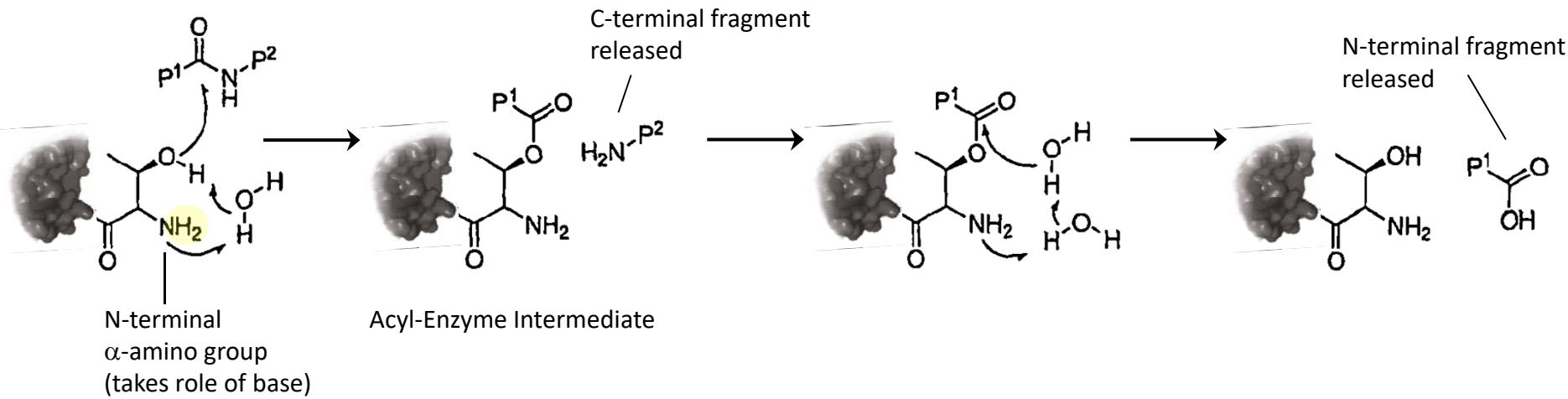
# 20S Proteasome: Protease Reaction

20S proteasome is an **N-terminal threonine protease** and functions according to the covalent type of protease catalysis

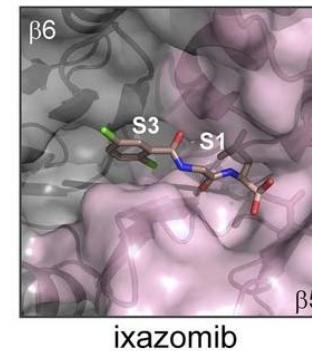
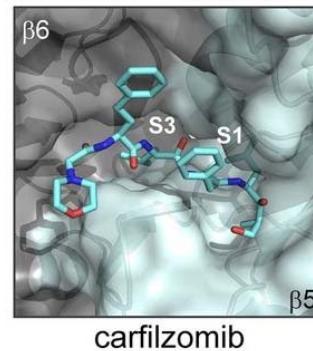
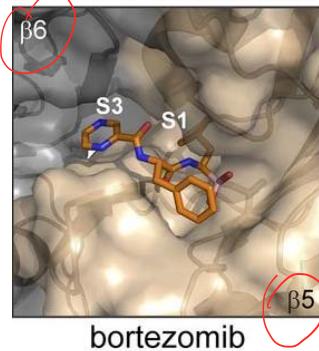
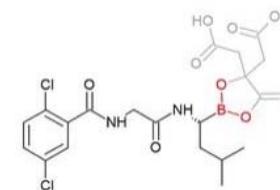
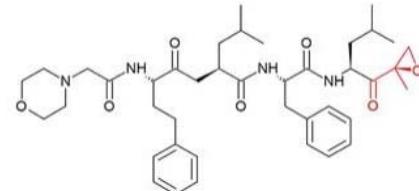
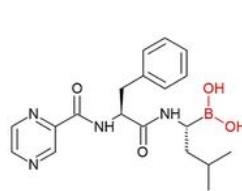
*catalytic residue*



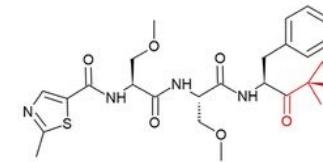
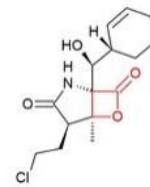
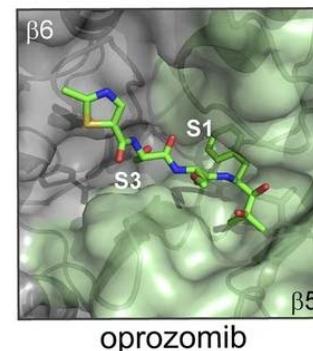
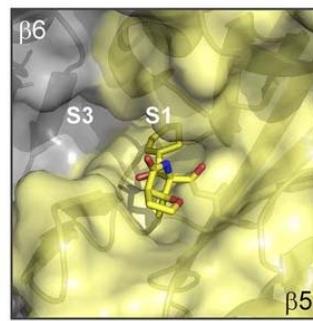
The Thr hydroxyl is not activated by a regular catalytic triade, but the N-terminal amino group takes the role of the base (via an H<sub>2</sub>O molecule that acts as relay).



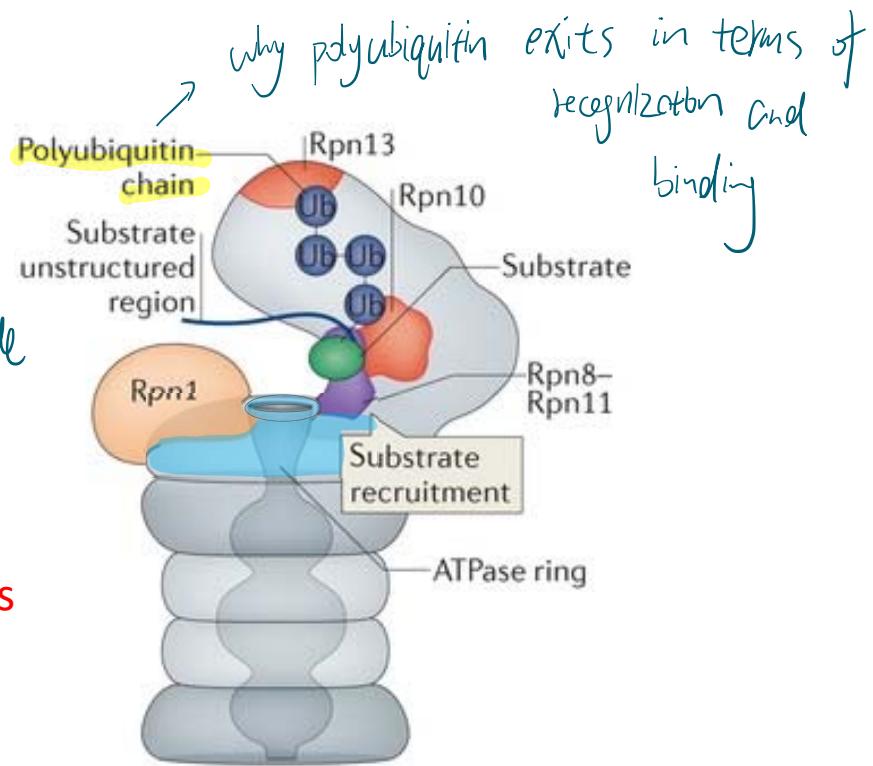
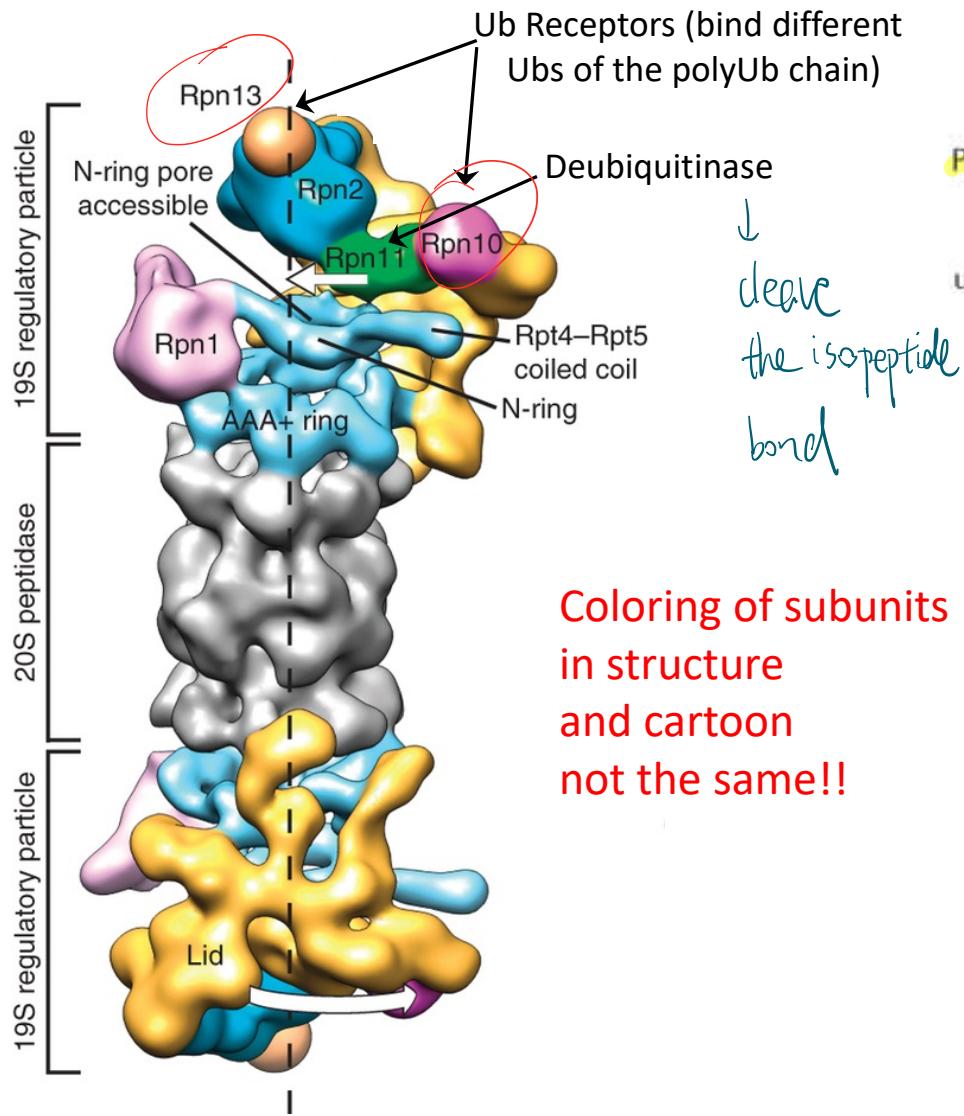
# Proteasome Inhibitors in Pharmacology



binds to  
active site

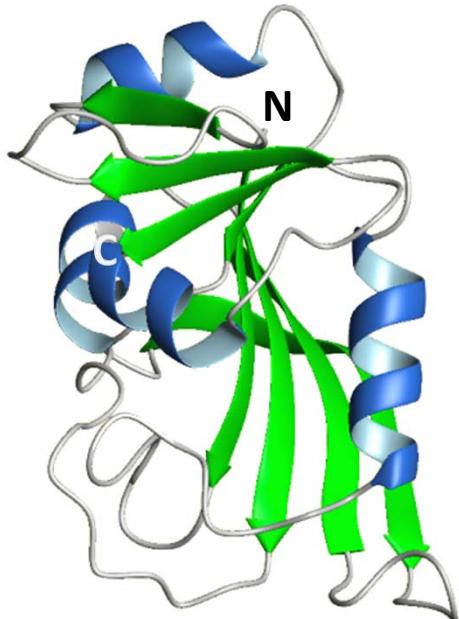


# Substrate Recognition and Deubiquitination



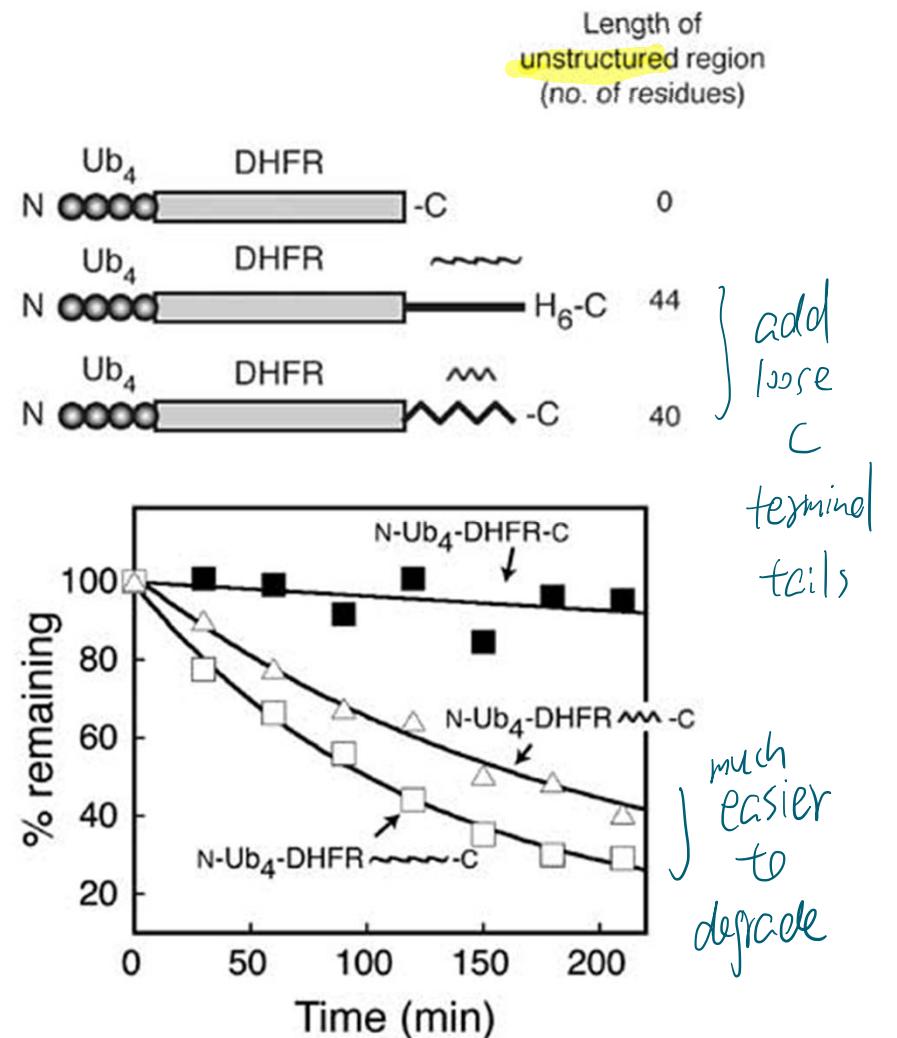
# Threading of the Substrate?

Dihydrofolate Reductase (DHFR)

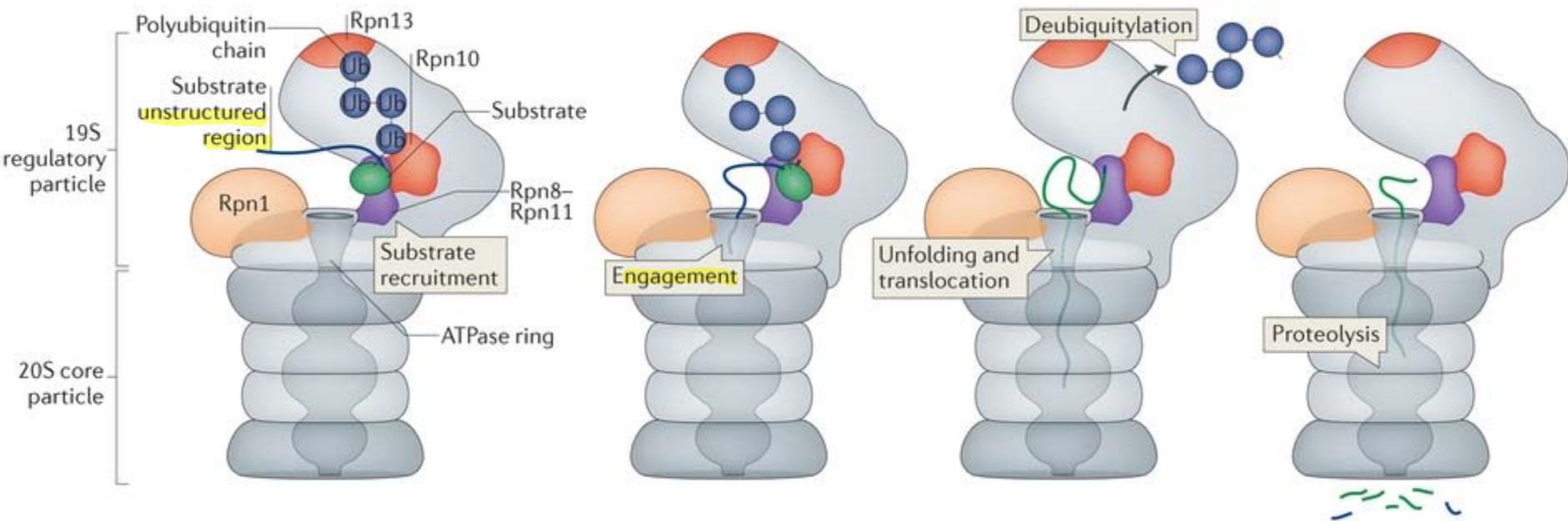


just to show  
the structure

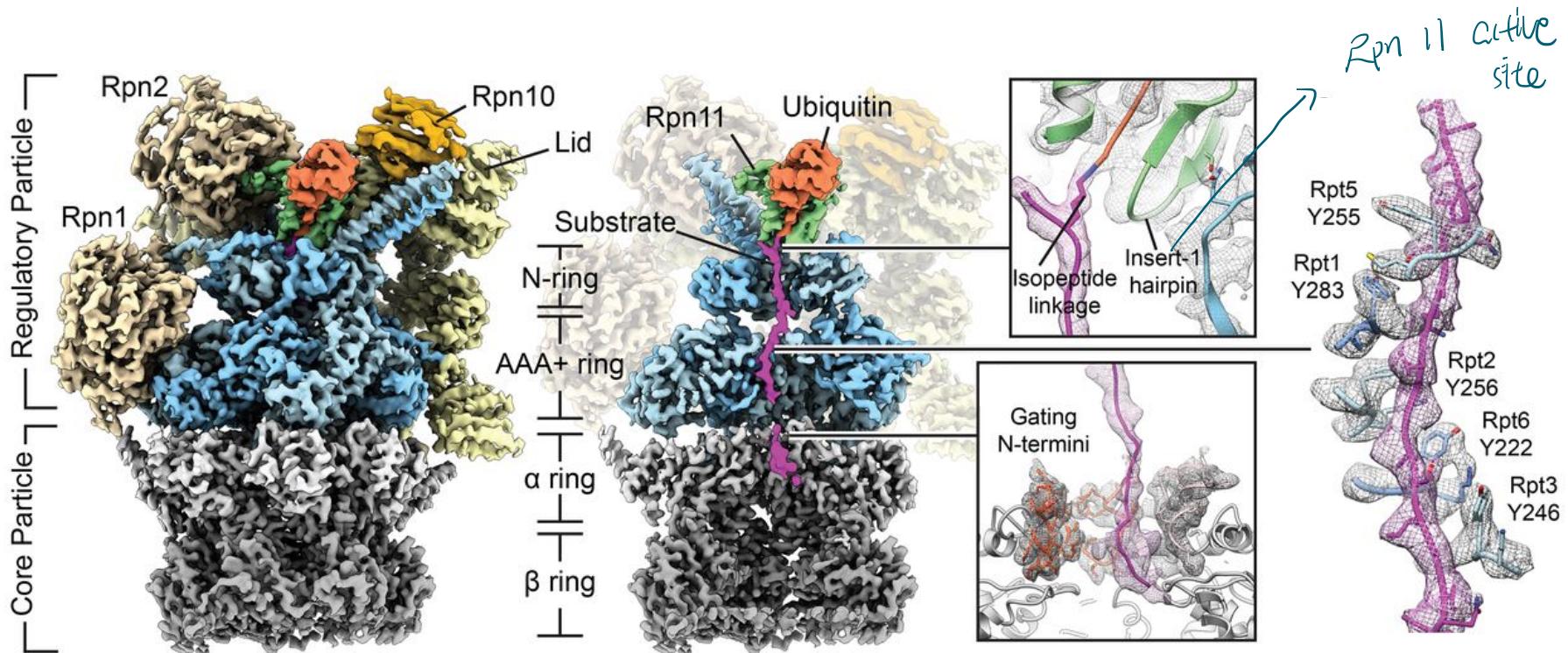
stable and tightly folded protein  
no loose C- or N-terminal tails



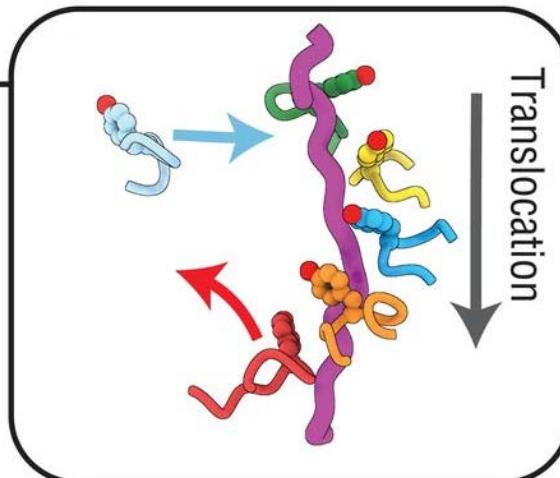
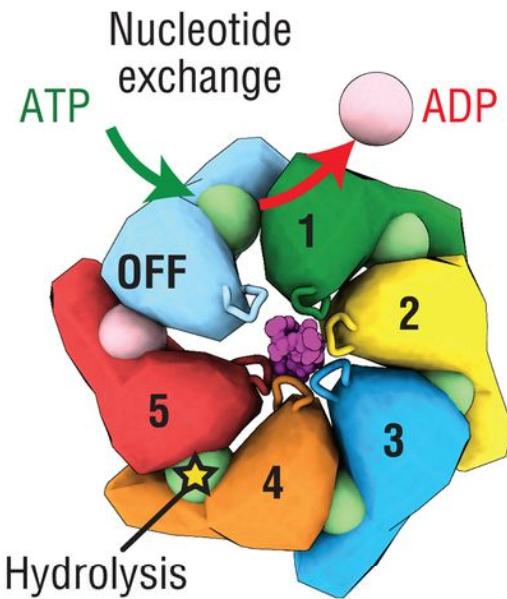
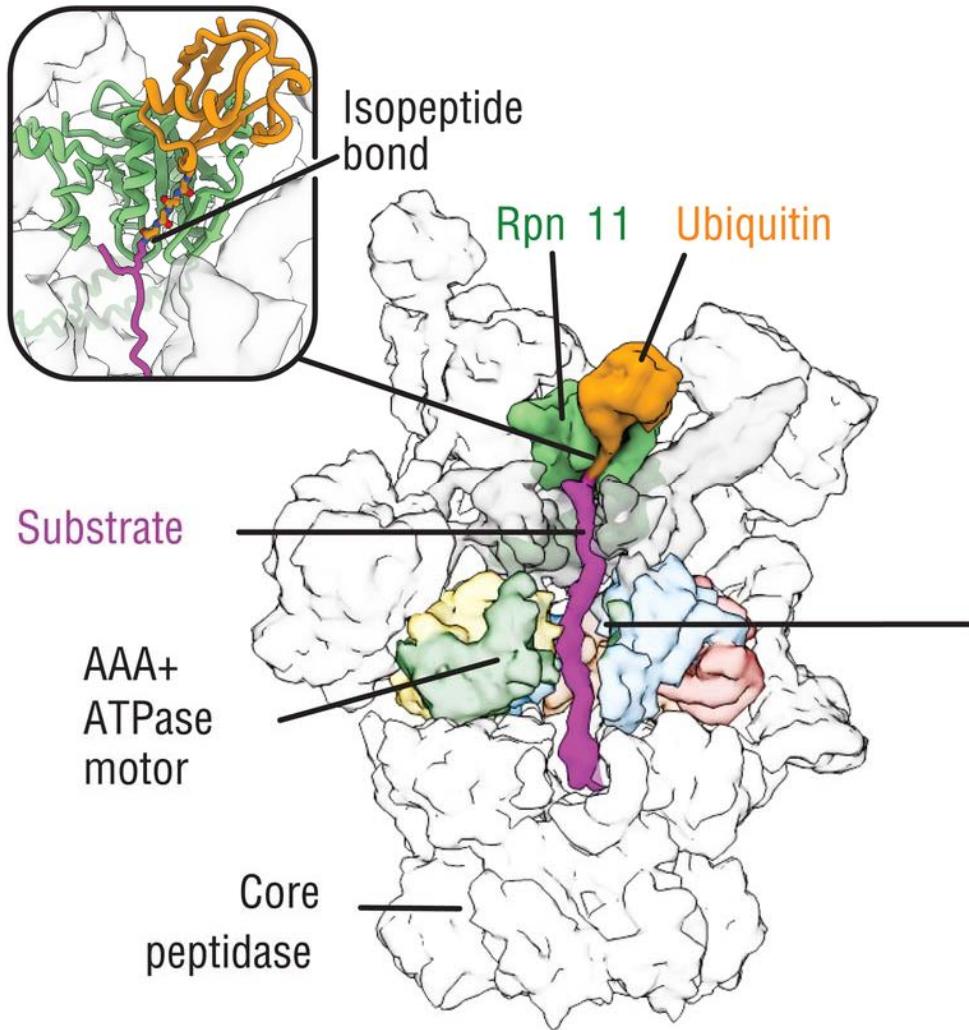
# Degradation of a polyubiquitinated substrat



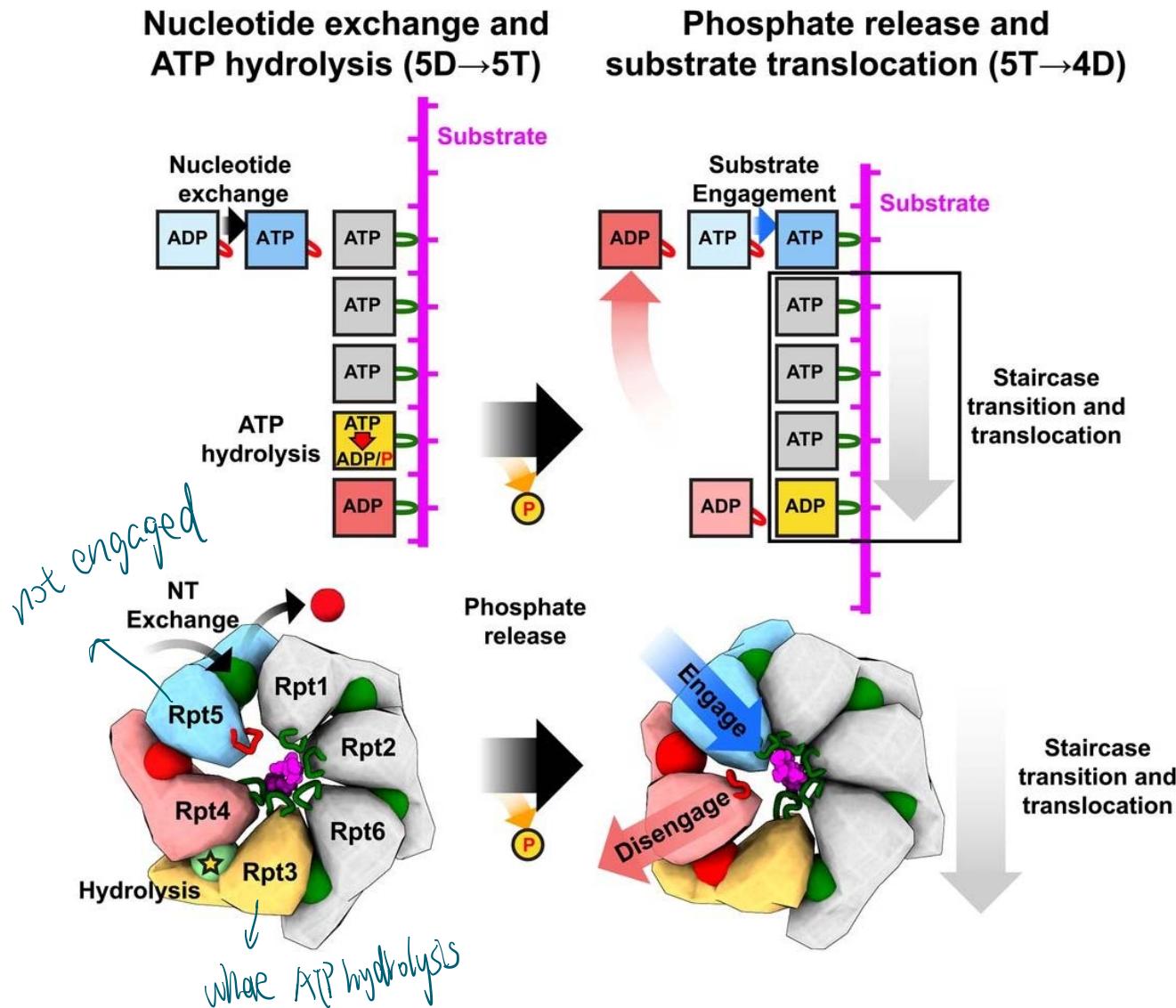
# High-resolution structure of substrate-engaged 26S proteasome



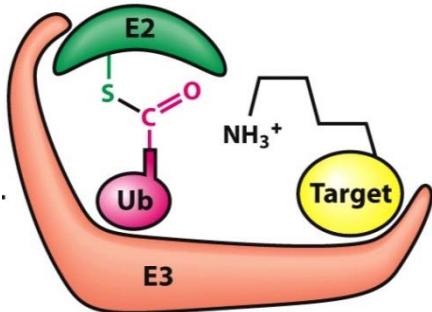
# Substrate Path Through the Proteasome



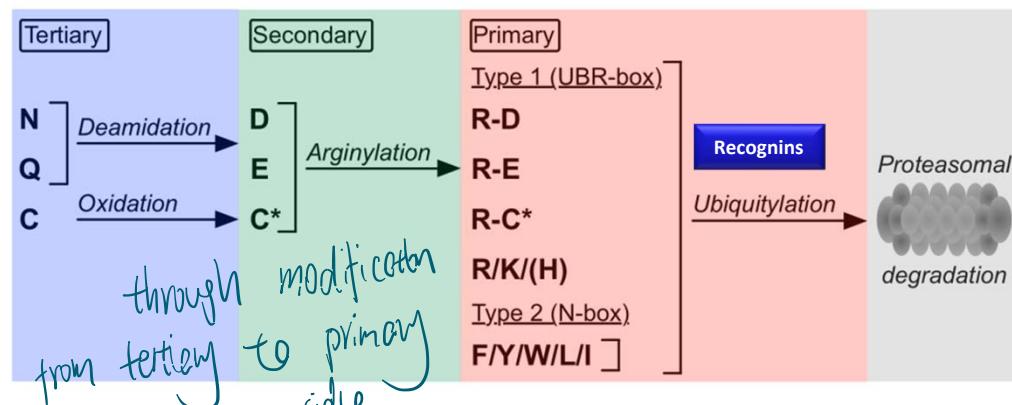
# Coordinated ATP-Hydrolysis and Substrate-Translocation Cycles



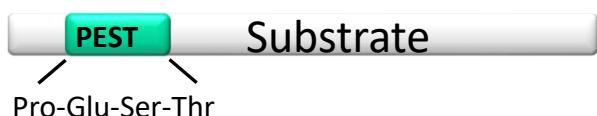
# What renders a protein a target for ubiquitination?



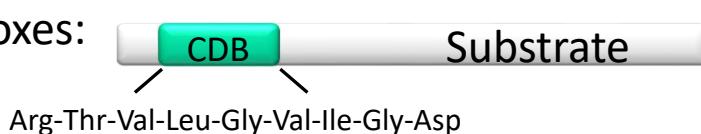
N-end rule: in bacteria



PEST Sequences:



Cyclin Destruction Boxes:



E3 ligases make a large contribution to the selection process, influencing which proteins are marked with Ub and which are not. The reason is that they possess a specific binding site for a certain protein substrate or a certain class of protein substrates.

allow the binds to E3 and poly-ubiquitinated

Highly stabilizing residues  
 $t_{1/2} > 20$  hours

Ala	Cys	Gly	Met
Pro	Ser	Thr	Val

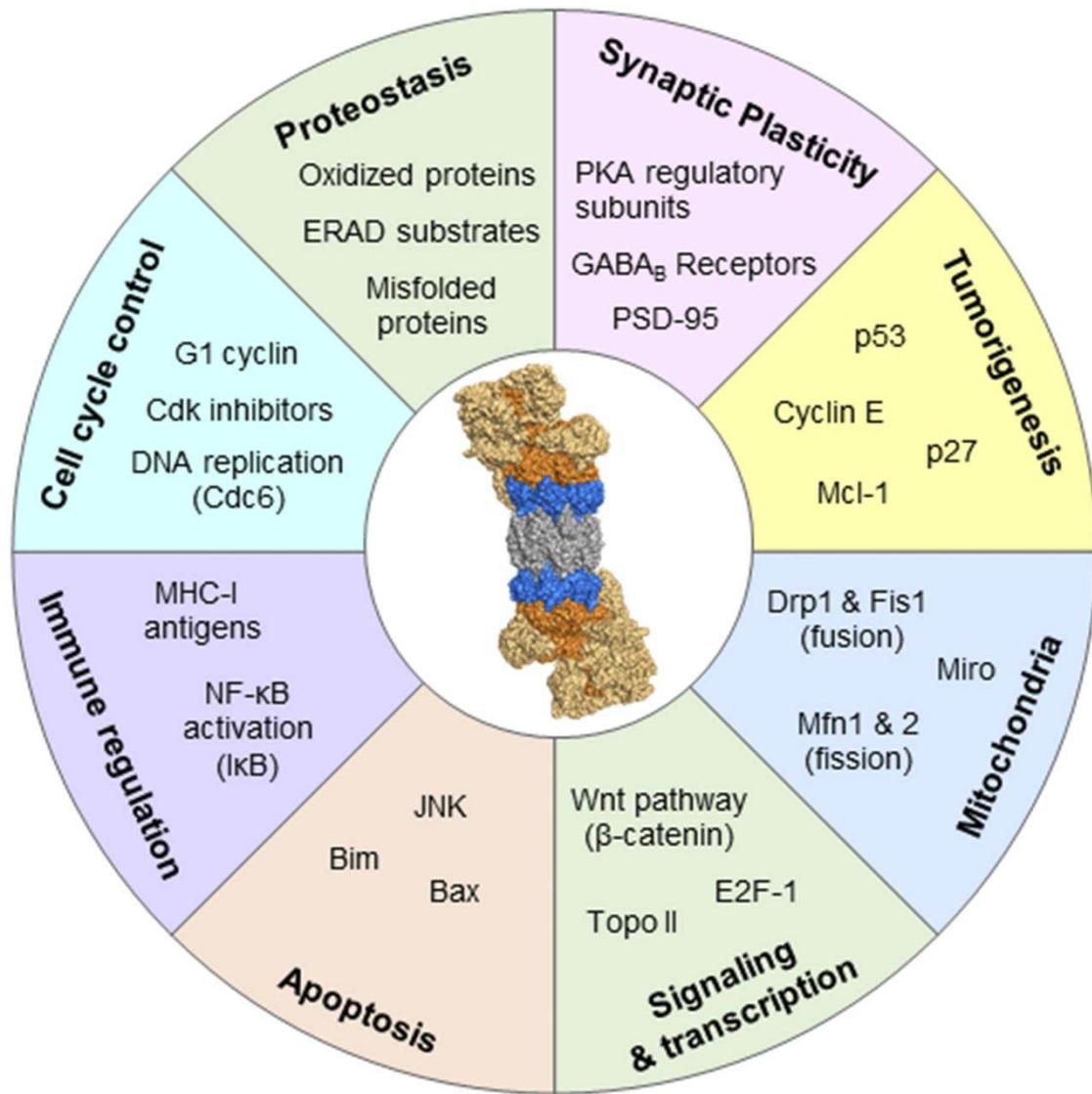
Intrinsically destabilizing residues  
 $t_{1/2} = 2$  to 30 minutes

Arg	His	Ile	Leu
Lys	Phe	Trp	Tyr

Destabilizing residues after chemical modification  
 $t_{1/2} = 3$  to 30 minutes

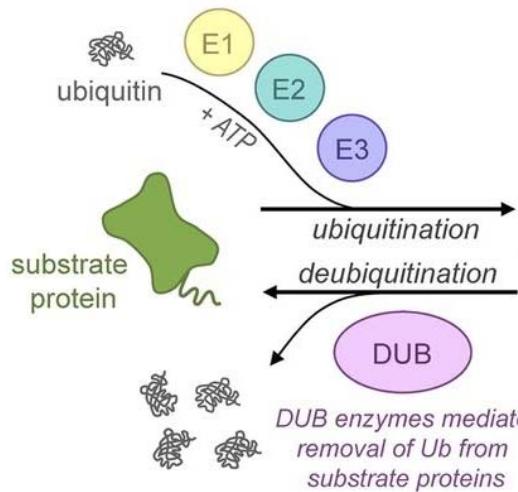
Asn	Asp	Gln	Glu
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# Cellular Functions Dependent on Proteasomal Degradation



# Summary of the Ubiquitin-Proteasome System

## Substrate ubiquitination



## Substrate processing by the 26S proteasome

