

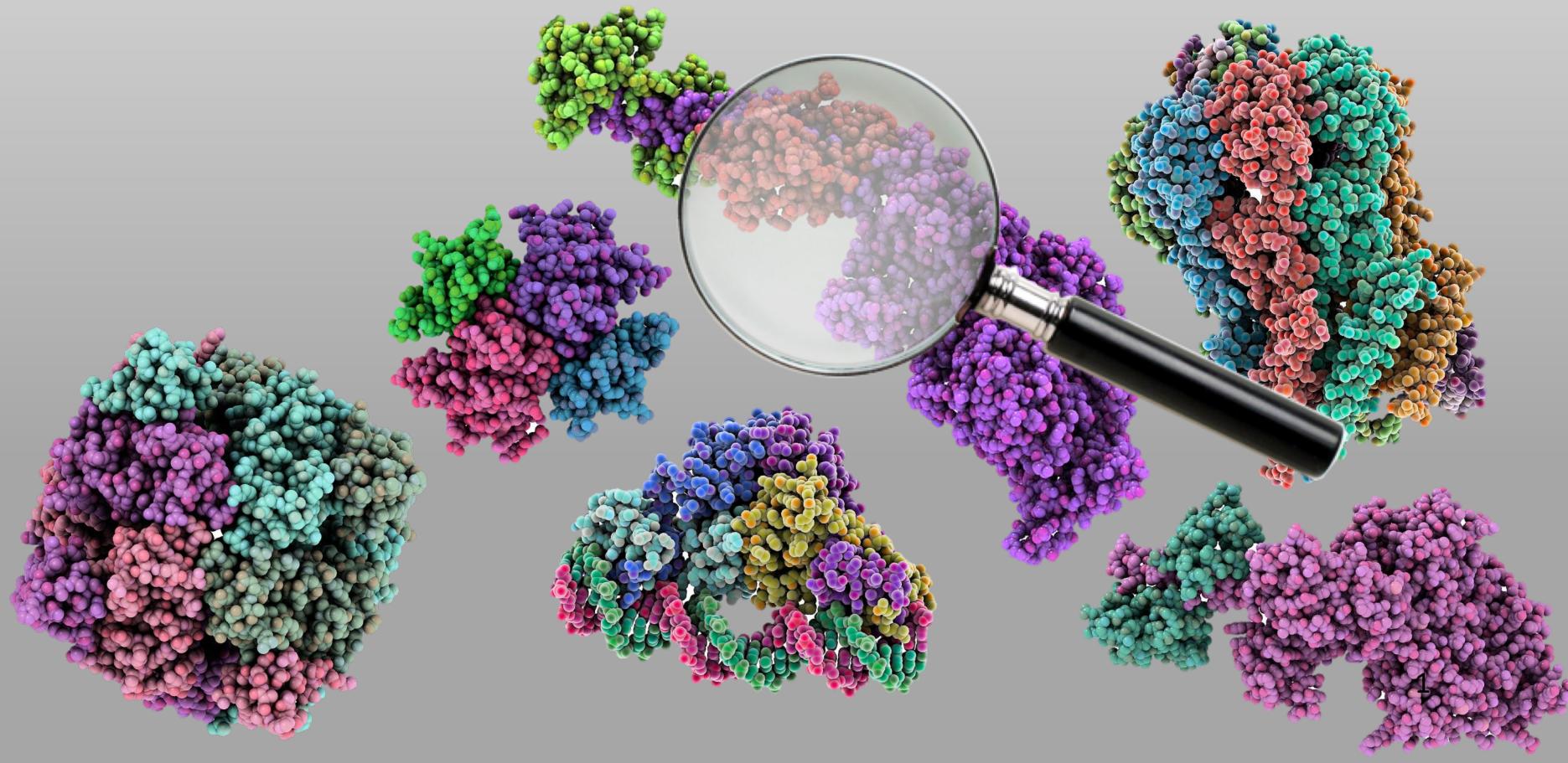
Molecular and Structural Biology 1 (Fall Semester 2019)

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

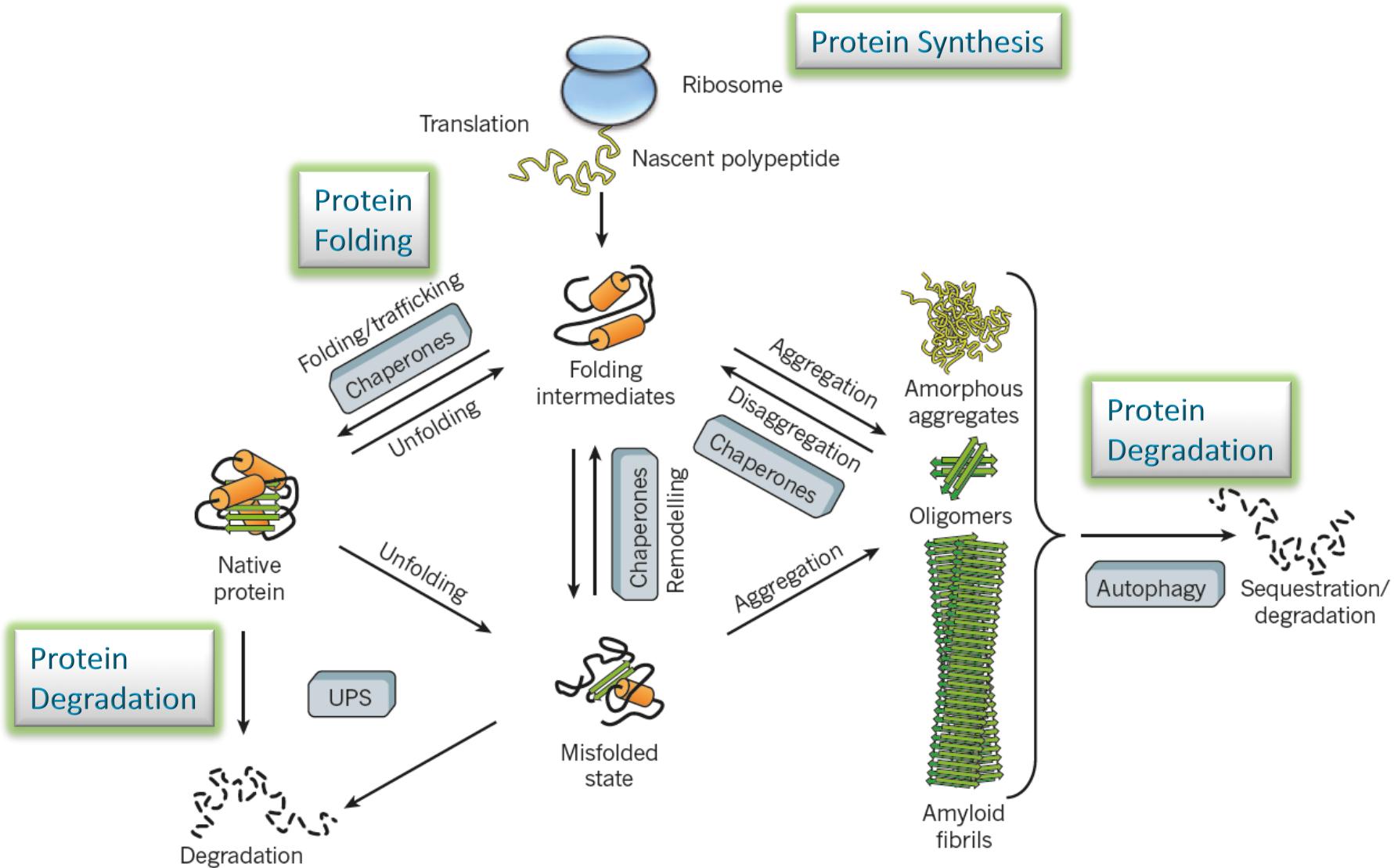
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Institute for Molecular Biology & Biophysics

ETH Zurich



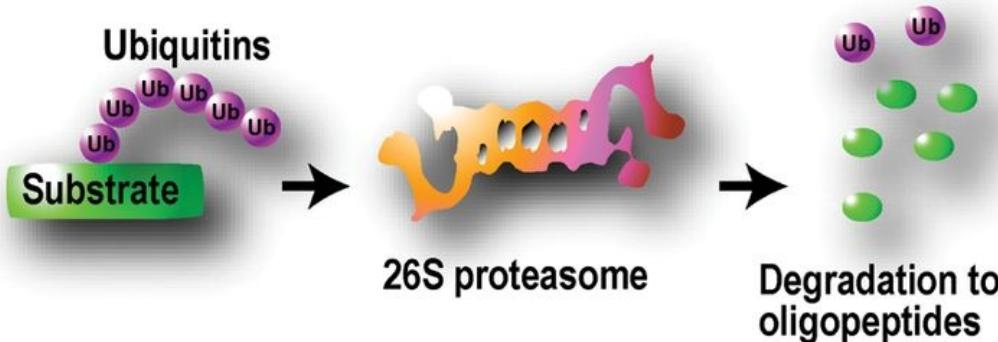
# Protein Turnover inside Cells



# Intracellular Protein Degradation

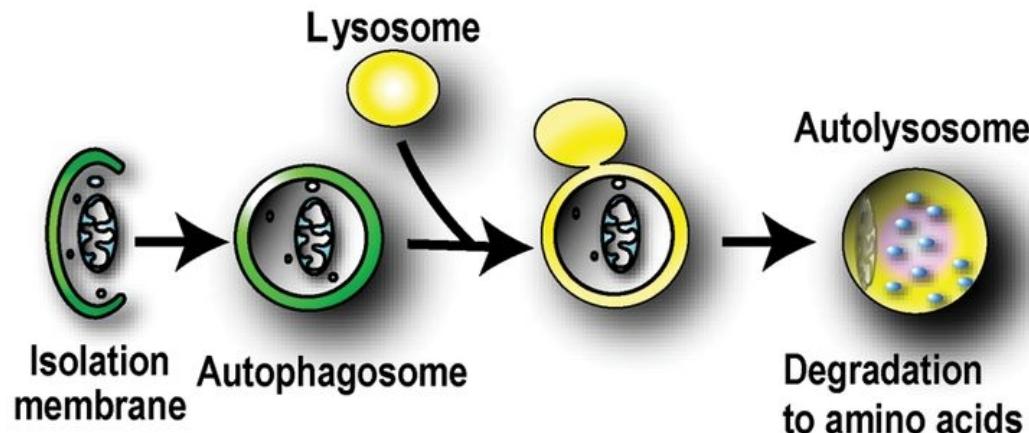
both are energy-dependent

## Ubiquitin–proteasome system



Compartmentalization inside a proteinaceous particle.

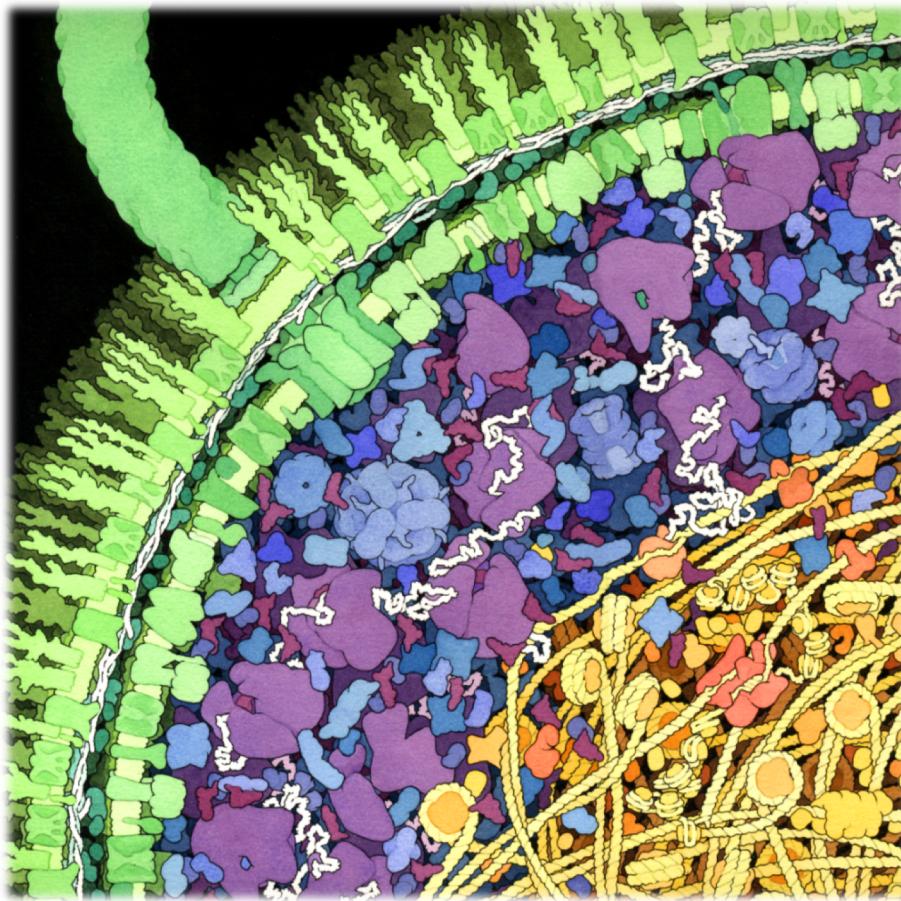
## Autophagy–lysosome system



Compartmentalization inside an organelle, separated from cytosol by membrane.

# Degradation is an important Element of Protein Homeostasis

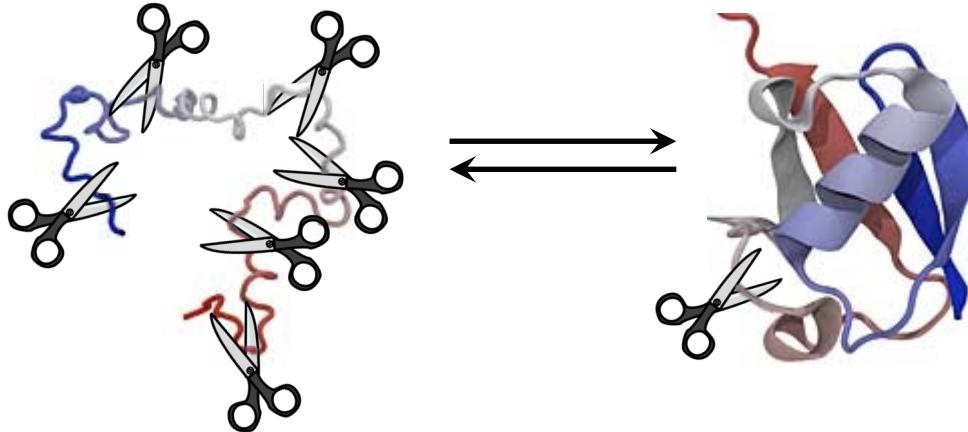
Proteins have to be synthesized and degraded constantly in the cell.



reasons:

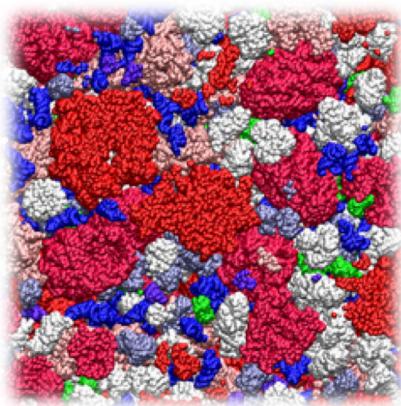
- old, damaged proteins must be removed
- some proteins should be present only a short while (regulation)
- growth and development

# Challenges of Protein Degradation in the Cytosol



Folded proteins have evolved to withstand the repertoire of small single-hit proteases in the cell.

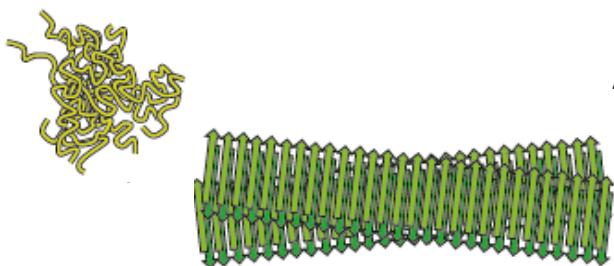
→ To degrade them, their 3D structure has to be unraveled.



Degradate just the little green guy?

Damage to other cellular proteins has to be avoided when degrading a subset of proteins.

→ Compartmentalization necessary.



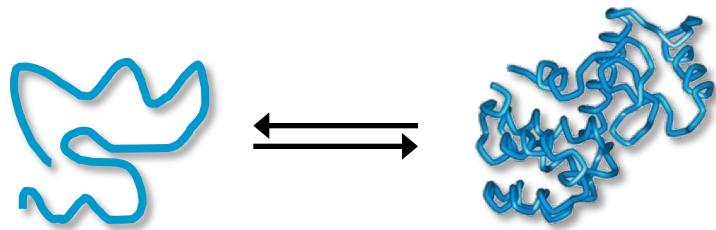
Aggregates/Amyloids

Proteins have to be cleaved processively to avoid truncated forms that might be aggregation prone and otherwise damaging.

# Energy-dependent proteases: Chaperone-Proteases

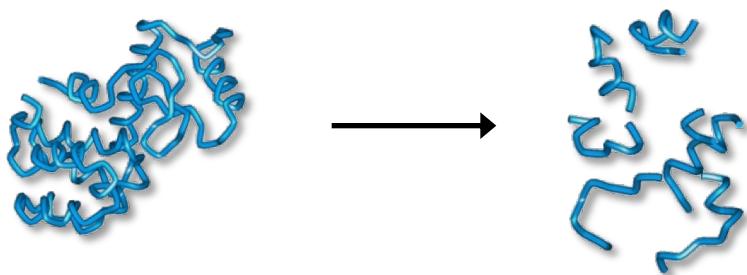
## Chaperones:

Proteins that modulate the conformational or assembly state of their protein substrates (i.e. they assist folding/unfolding or assembly/disassembly).



## Proteases:

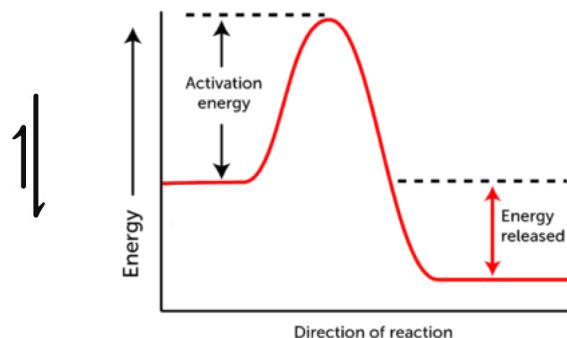
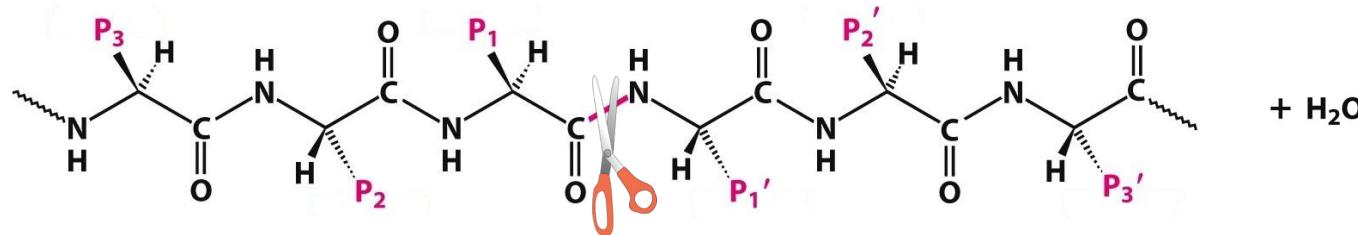
Enzymes that catalyze hydrolysis of peptide bonds.



## Chaperone-Proteases:

Complexes that combine these two activities to achieve complete and processive degradation of their protein substrates into small peptides.

# Peptide bonds are kinetically stable



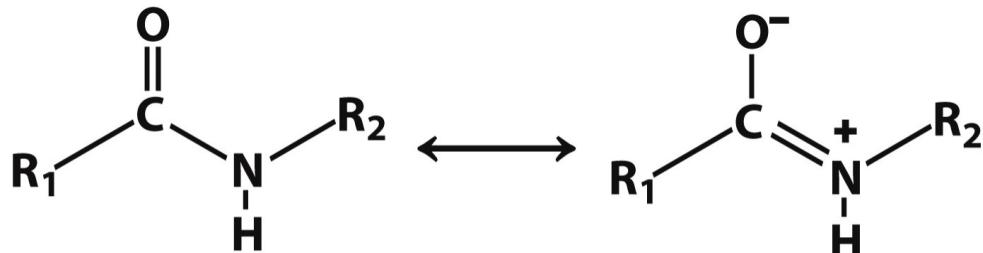
$\Delta G^{0'} = -20 \text{ kJ/mol}$  bonds cleaved  
 $t_{1/2} \text{ uncat} = 10-1000 \text{ years}$



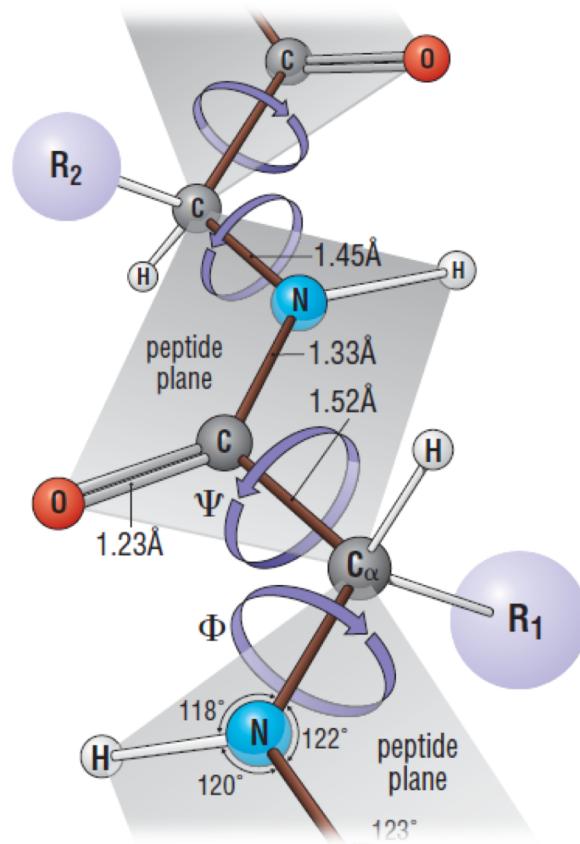
N-terminal fragment

C-terminal fragment

# Peptide Bond – Kinetic Stability



The **resonance structure** of the peptide bond shows that it has partial double-bond character. The electron lone pair of the nitrogen delocalizes such that a higher electron density is found between the C and N-Atom than in a regular single bond. This on the other hand makes the carbonyl carbon less electrophilic and thus less susceptible to a nucleophilic attack.

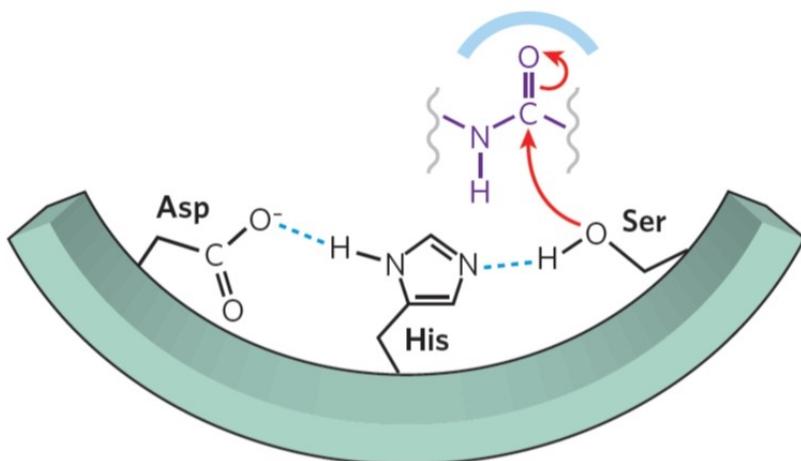
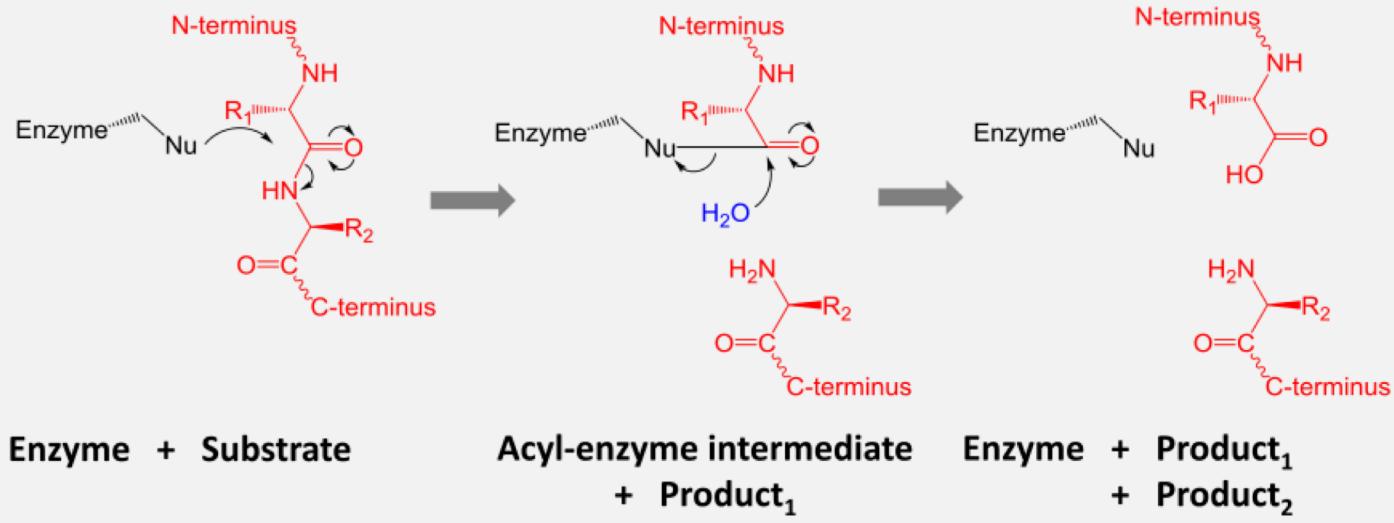


# Proteases – Catalysis Principles

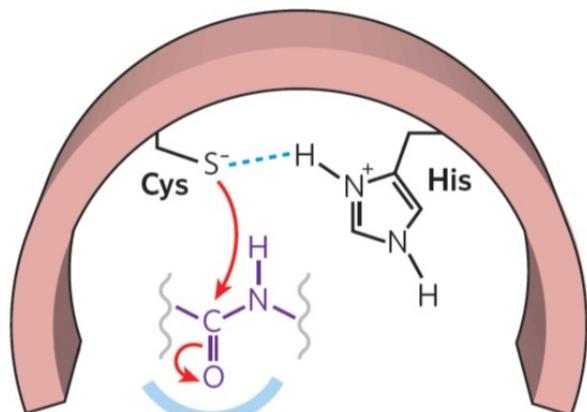
## covalent catalysis

attack by enzyme residue as nucleophile

Nu =  
Cys S-  
Ser O-  
Thr O-  
(activated by a  
catalytic triade or  
diade)



examples: chymotrypsin, trypsin (digestive enzymes excreted from pancreas)



examples: papain (Cys protease from Papaya fruit), Tev protease

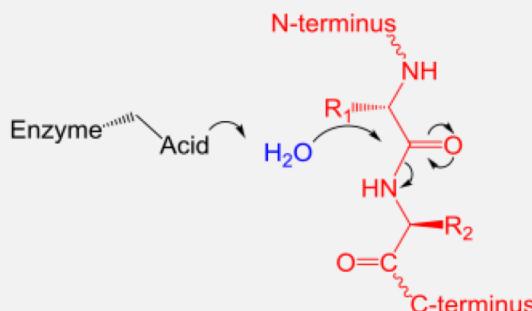
# Proteases – Catalysis Principles

non-covalent  
catalysis

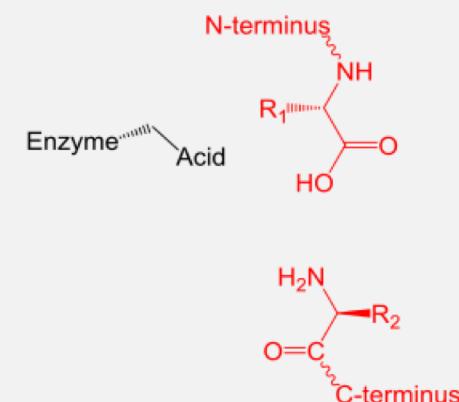
attack by water  
(activated by  
acidic residue)

Acid =  
Asp  
Glu  
Metal ion

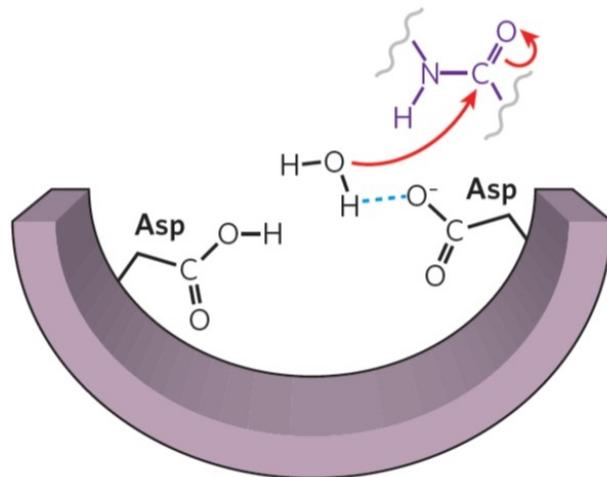
Enzyme + Substrate



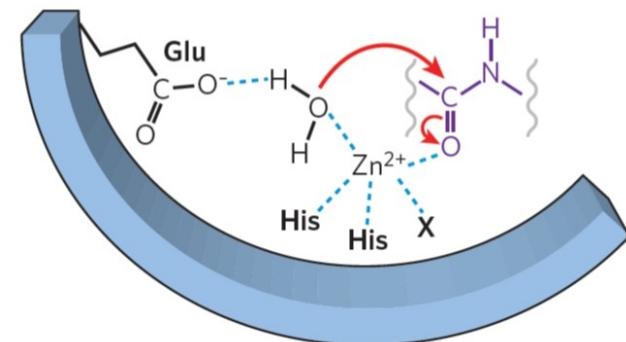
Enzyme + Product<sub>1</sub>  
+ Product<sub>2</sub>



Acid in its basic form, eg  $\text{COO}^-$ , takes up proton  
from water to activate it for nucleophilic attack



examples: aspartyl proteases contain two  
Asp; eg pepsin (digestive enzyme in stomach)



examples: thermolysine (thermostable  
 $\text{Zn}^{2+}$ -protease from *B. subtilis*)

# Classification of Proteases

1. nature of substrate: protein or peptide

3. catalytic residue: nucleophile attacking peptide bond

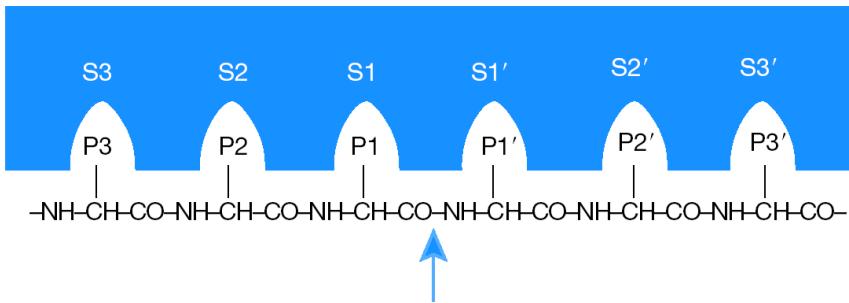
aspartic proteases: Asp activates H<sub>2</sub>O (HIV protease)

metalloproteases: Zn<sup>2+</sup> activates H<sub>2</sub>O (FtsH)

cysteine proteases: SH acts as nucleophile (caspases)

serine or threonine proteases: OH acts as nucleophile  
(trypsin, proteasome)

4. residue-specificity of bonds cleaved



6. cell compartment they are found in  
for bacteria (cytoplasmic, periplasmic, secreted)

7. energy-dependence

energy-independent:

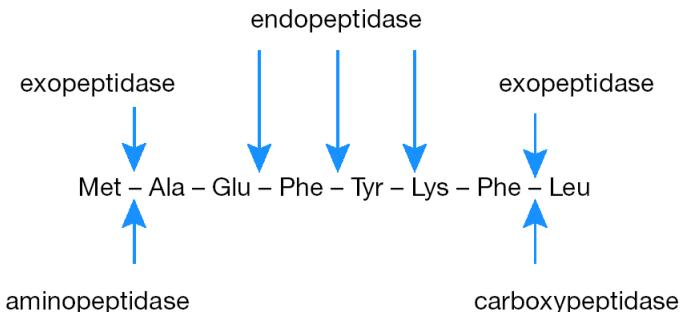
small single-hit proteases or peptidases

large complexes that work together with proteasome or other proteases

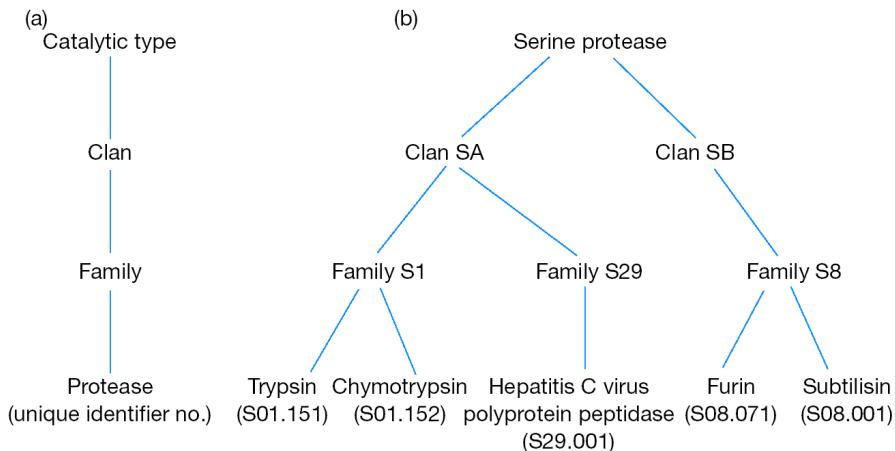
energy-dependent:

chaperone-proteases, usually the energy-dependence resides  
on the chaperone portion

2. location of peptide bond within protein



5. structural and sequence similarities



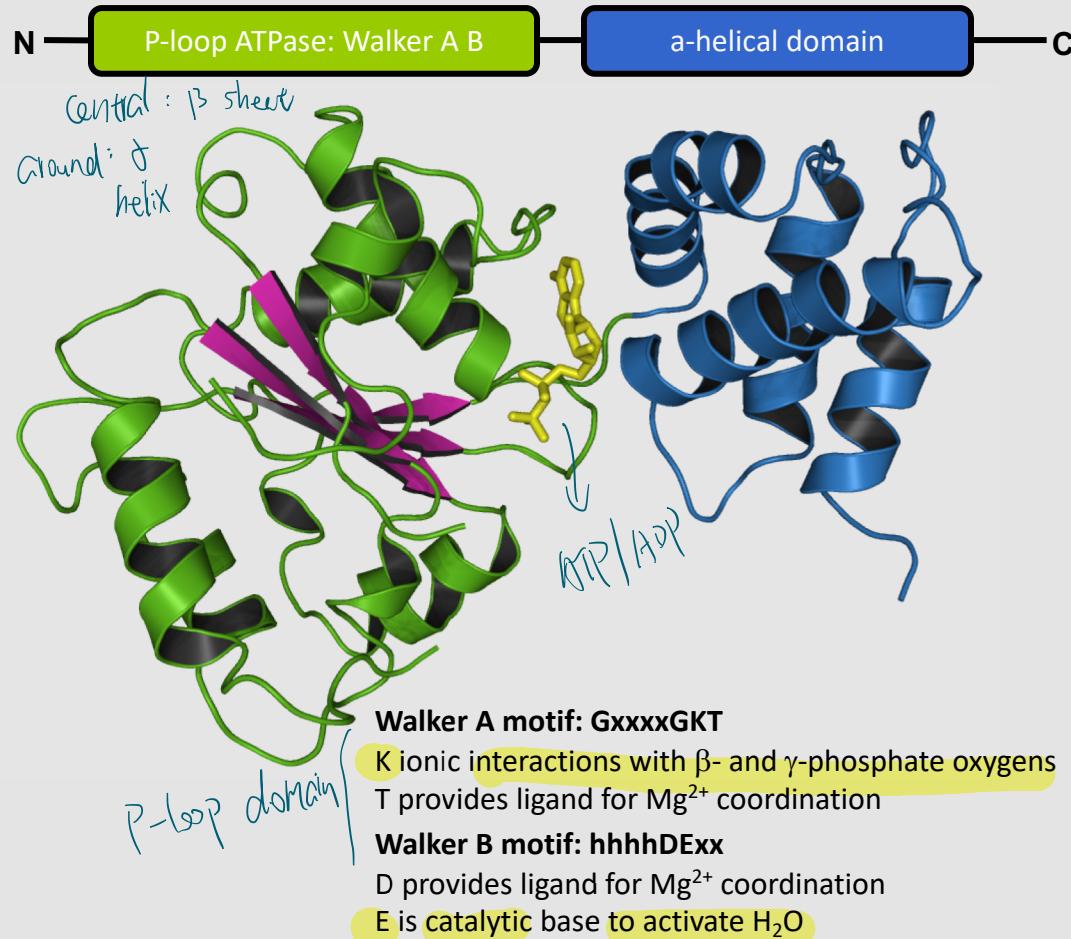
# AAA proteins: ATPases Associated with various cellular Activities

Function:

mediators of conformation or assembly state  
of proteins or nucleic acids

consists of two domains

**AAA module:** 230-250 aa sequence containing  
**P-loop ATPase fold** and  **$\alpha$ -helical domain**

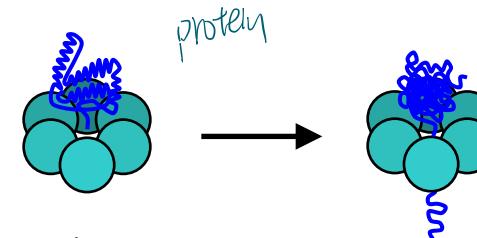


Mechano-chemical coupling of ATP hydrolysis and conformational changes to thread DNA or protein substrates through central pore:

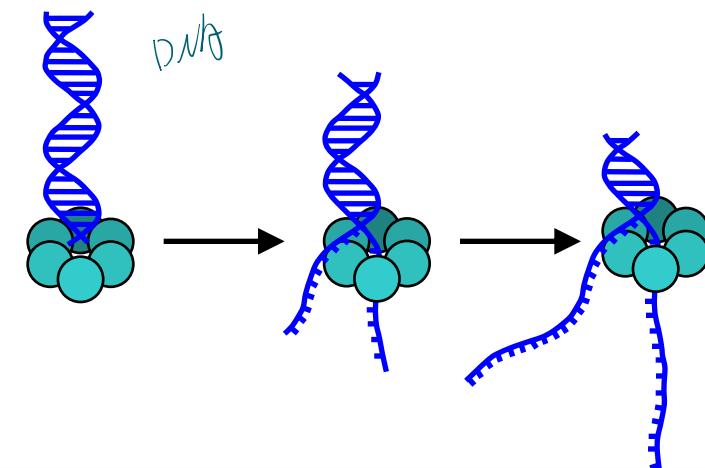
Disassembly



Translocation



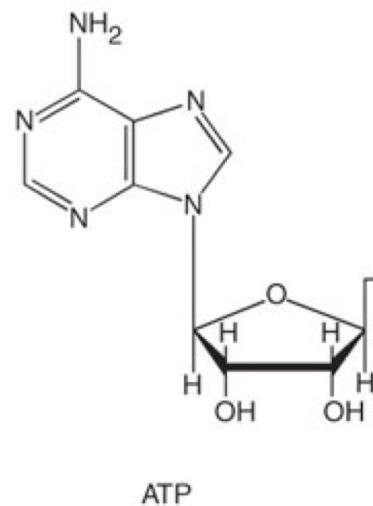
Unwinding



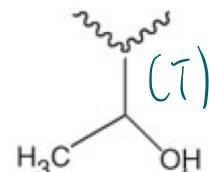
# Mechanism of ATP Hydrolysis

## Walker A motif: GxxxxGKT

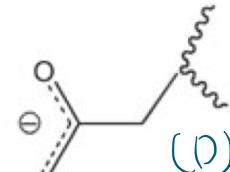
K (not shown) ionic interactions with  
 $\beta$ -/ $\gamma$ -phosphate oxygens  
T provides ligand for  $Mg^{2+}$  coordination



## Walker A

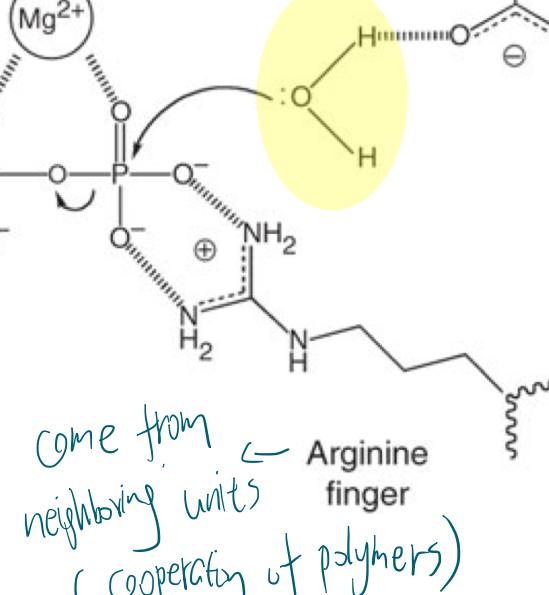


## Walker B



## Walker B motif: hhhhDExx

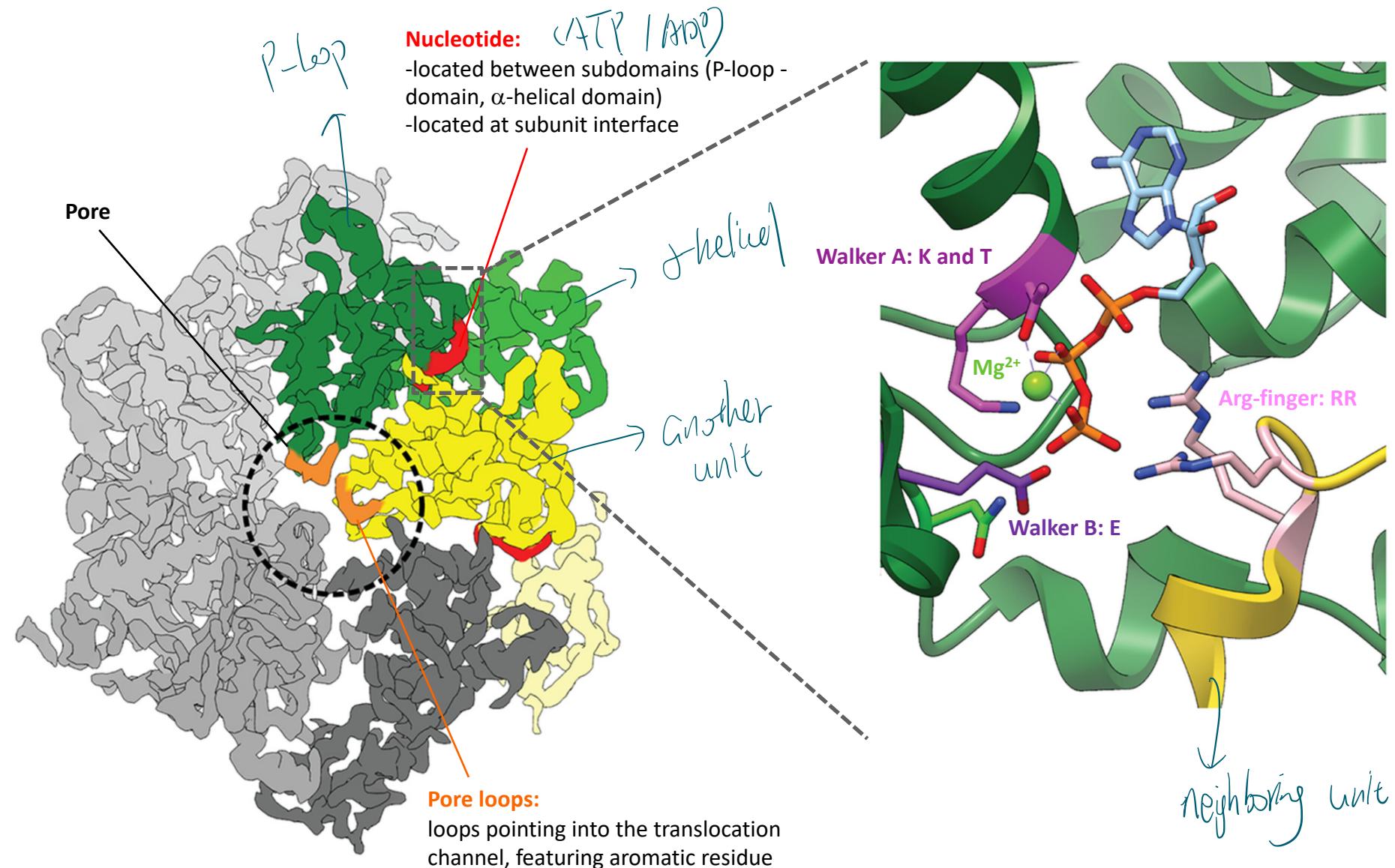
D provides ligand for  $Mg^{2+}$  coordination  
E is catalytic base to activate  $H_2O$



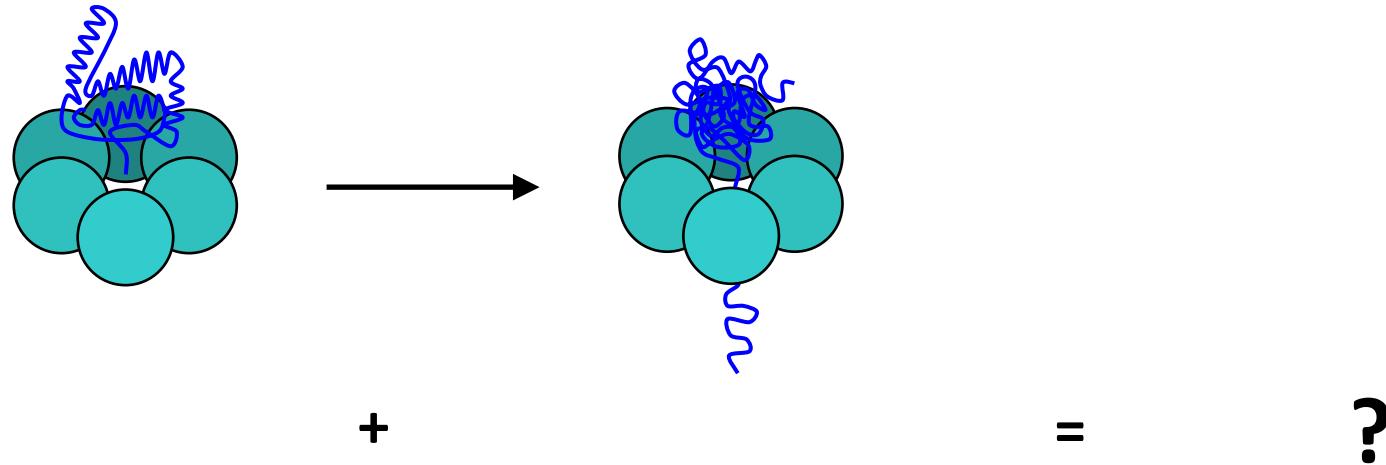
## Mechanism of ATP hydrolysis:

Nucleophilic attack of activated  $H_2O$  at the  $\gamma$ -phosphorus of ATP and formation of 5-coordinate transition state, the negative charge accumulating at the  $\gamma$ -phosphate will be stabilized by  $Mg^{2+}$  and surrounding positively charged groups or H-bond donors.

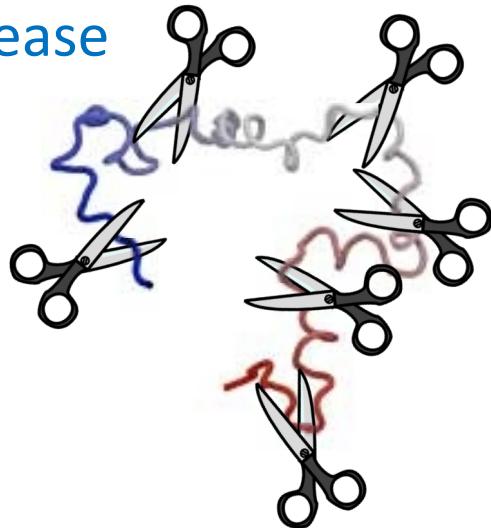
# AAA proteins: Hexameric Ring, Pore, ATP-binding/hydrolysis



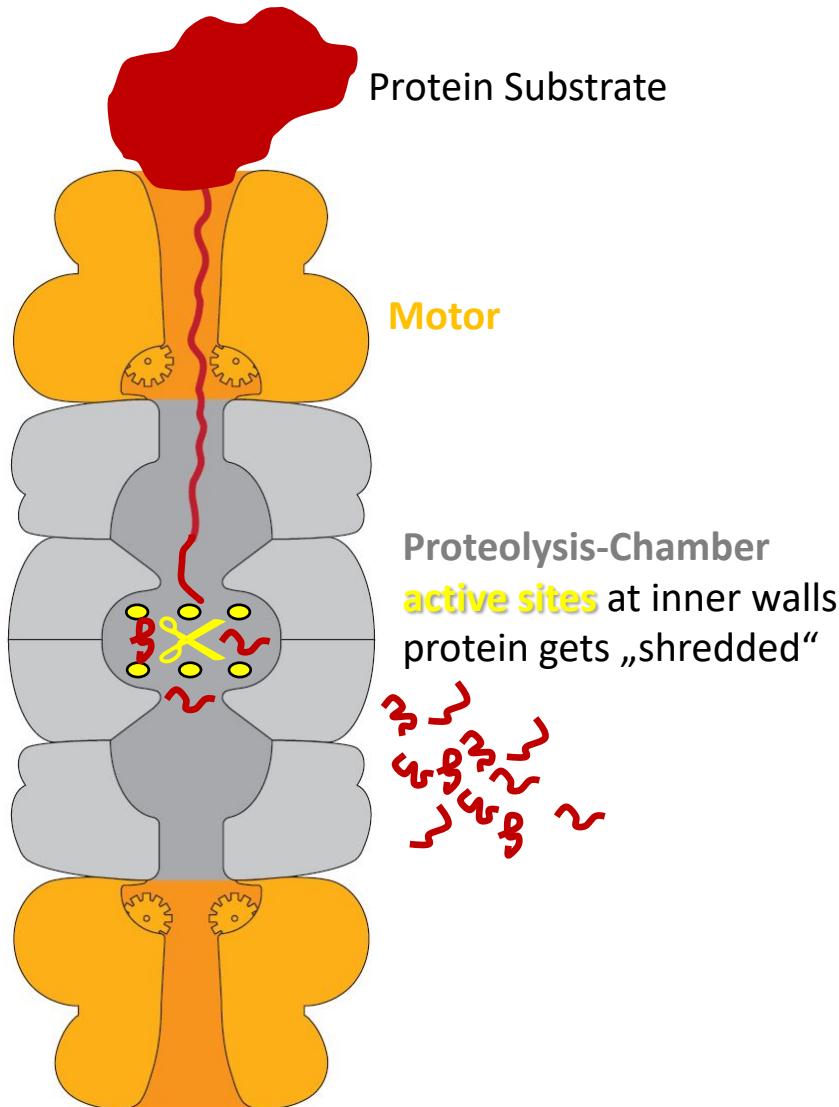
## Chaperone



## Protease

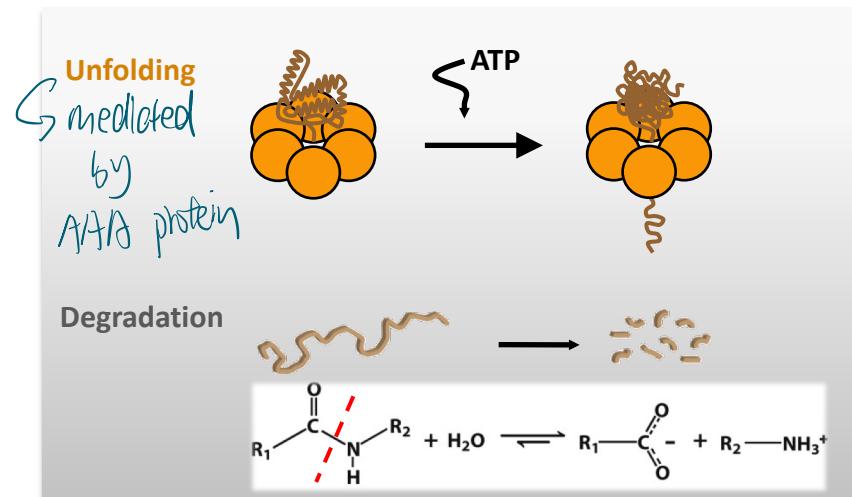


# Compartmentalizing Architecture



Compartmentalization provides the following features:

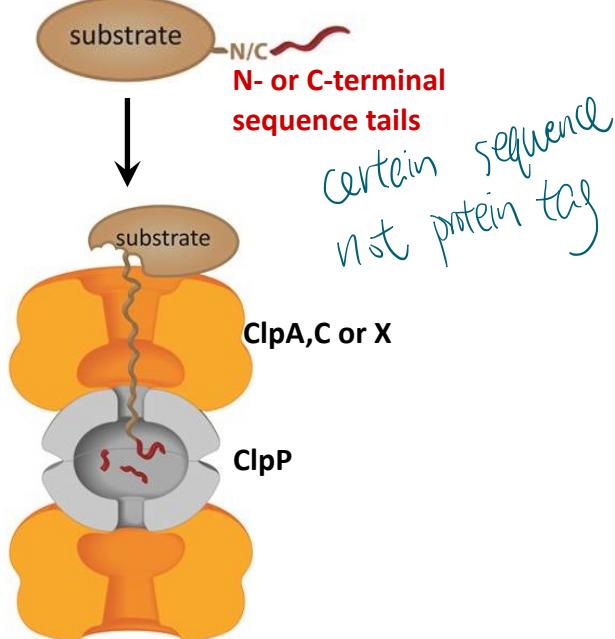
- Separation of substrate from bulk cytosol
- Selection via folding-state → narrow entrance pores
- Processivity by encapsulation of the protein → substrate is cleaved down to peptide size
- ring-stacking principle allows association with ring-shaped partners lining up the pores as a conduit into the proteolytic core



# Substrate Recruitment to Compartmentalizing Proteases

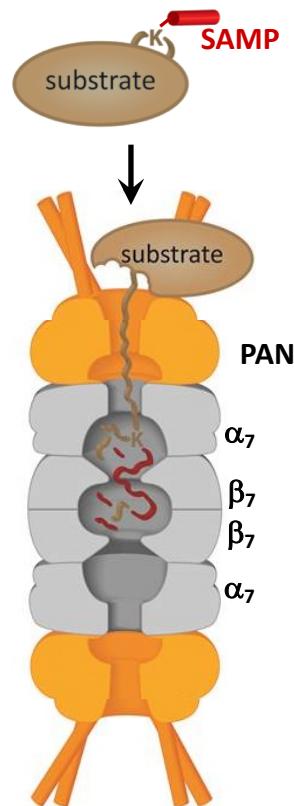
## Bacteria

### Clp Protease



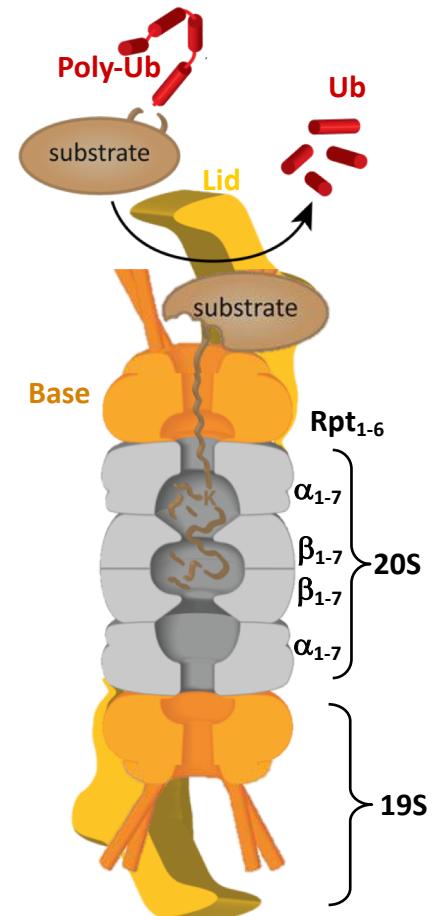
## Archaea

### Proteasome

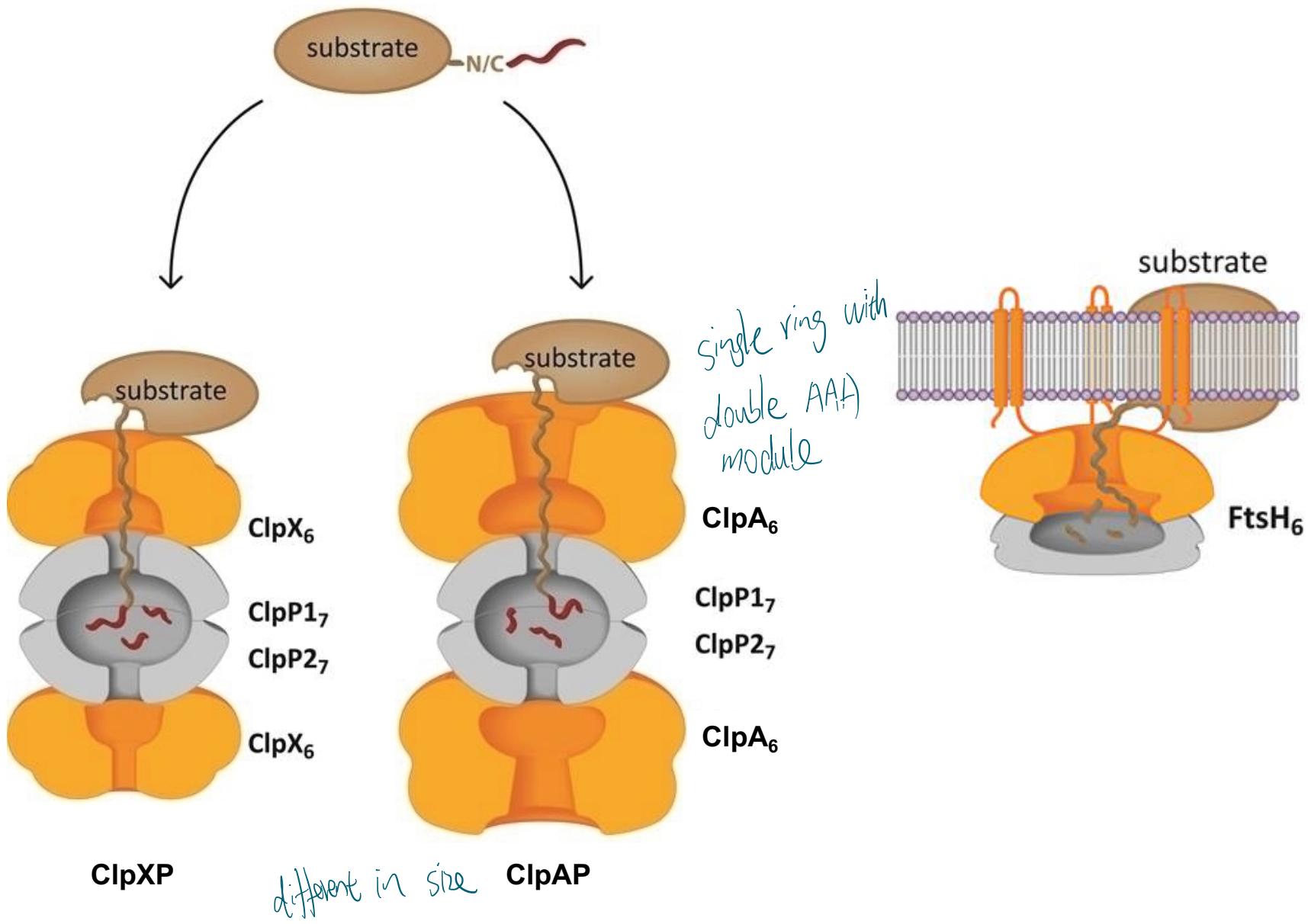


## Eukaryotes

### Proteasome

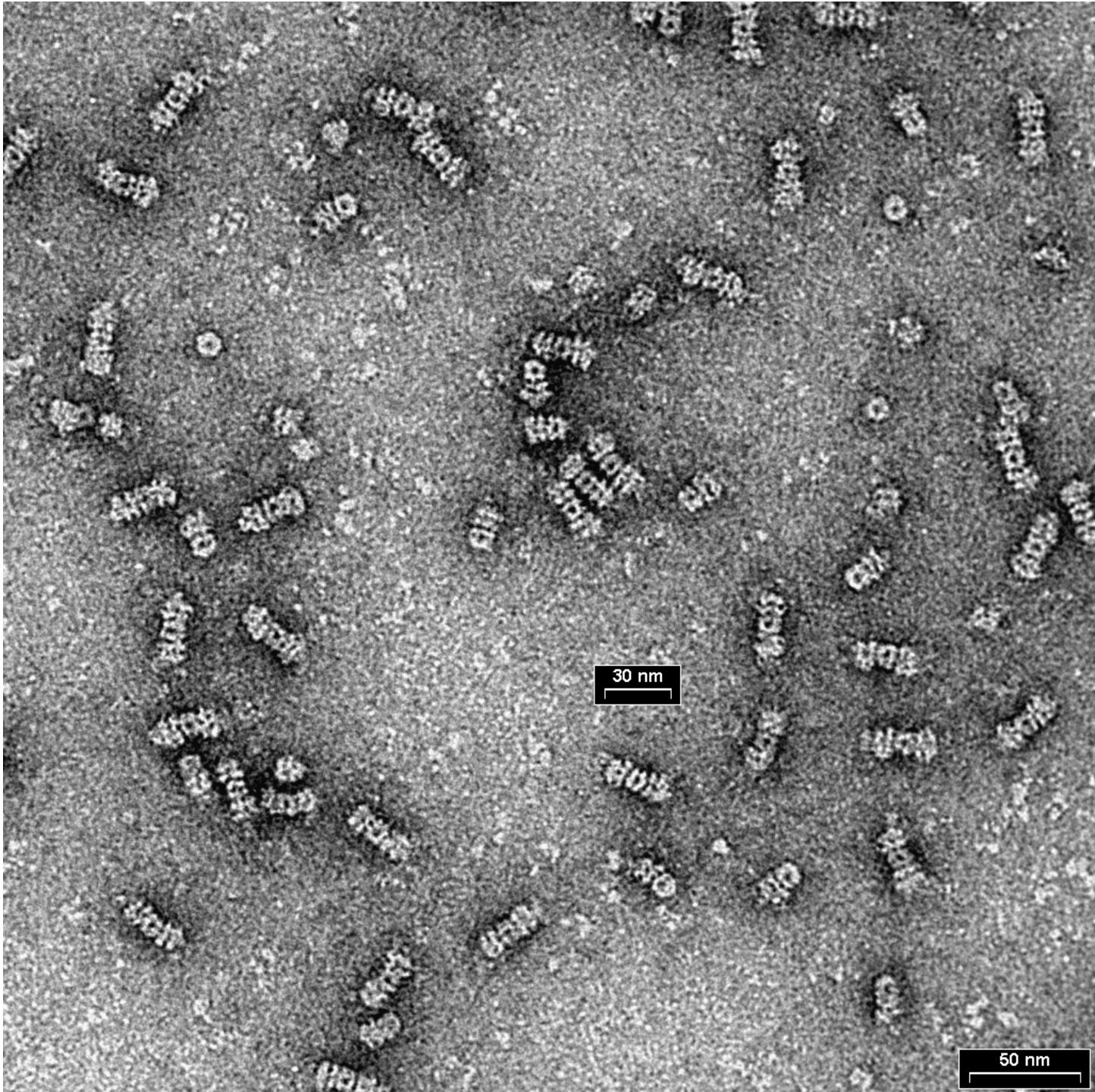


# The Bacterial Caseinolytic Protease Clp

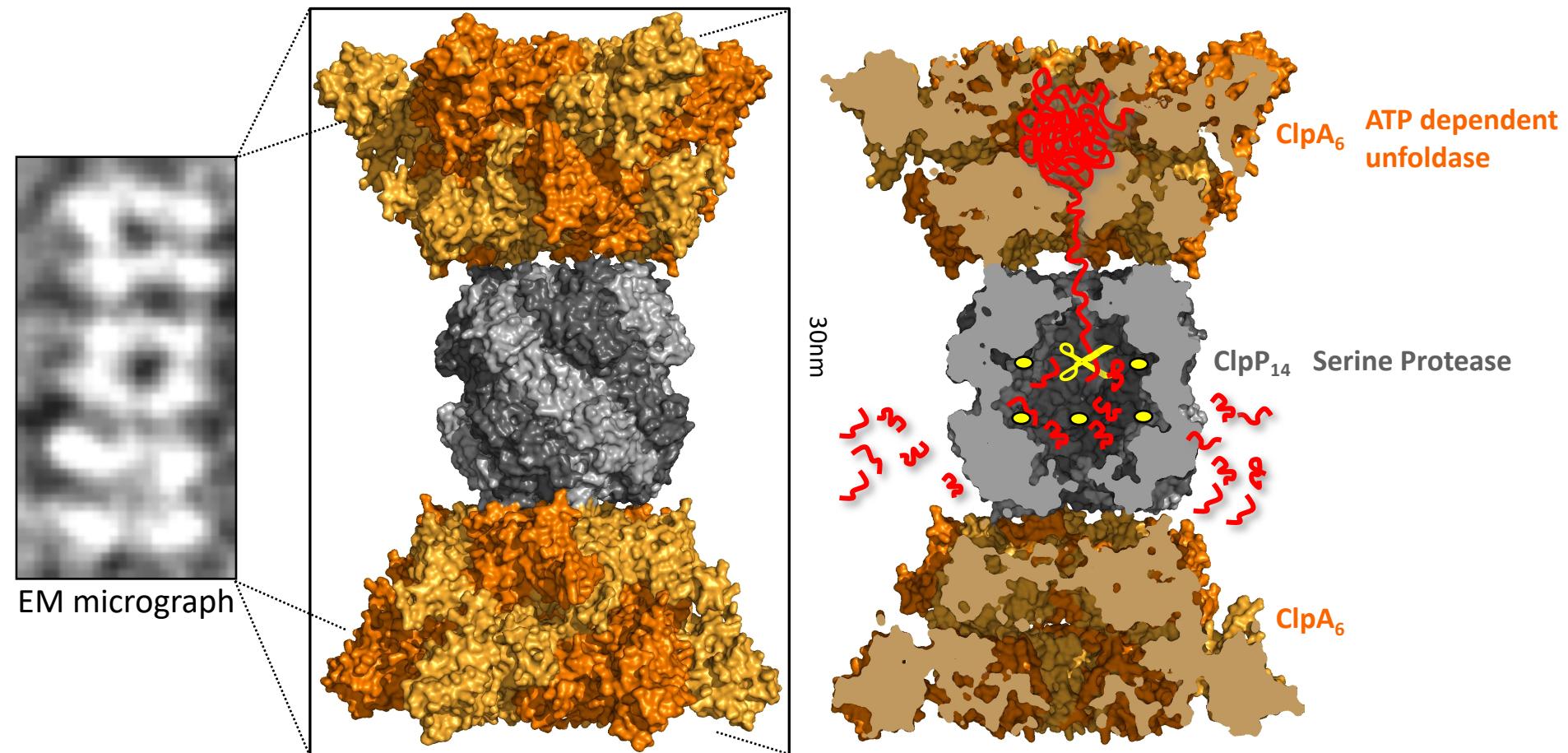


## Electron Microscopy

negative stain  
ClpA/ClpP 2:1



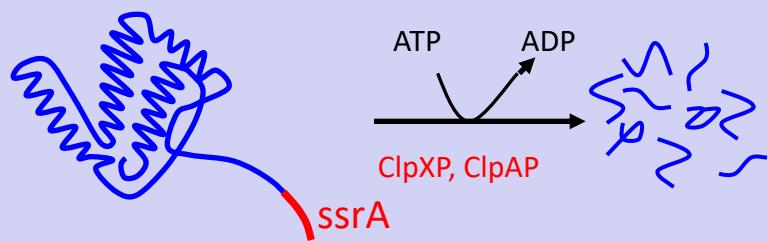
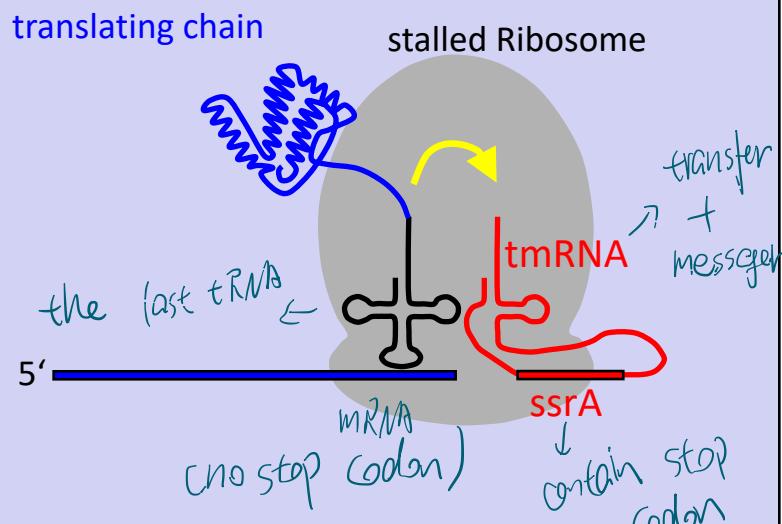
# ClpAP: Caseino-lytic protease with ATPase and Protease Rings



# Substrate recruitment to ClpAP: two classes of substrates

## SsrA-tagged Substrates

NH<sub>2</sub> — blue bar — AANDENYALAA — C terminus



## N-end rule Substrates

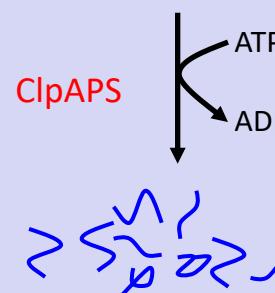
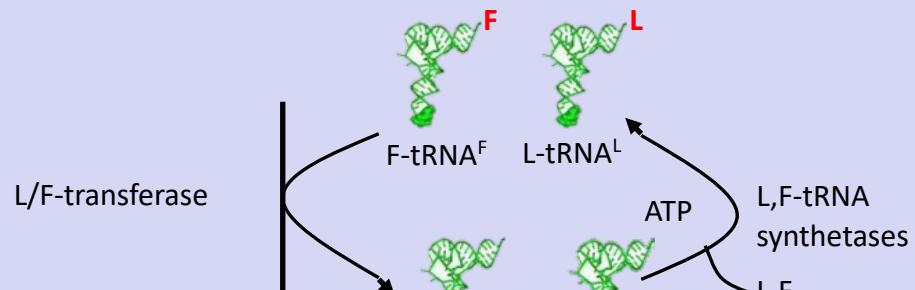
F  
L  
W  
Y

one of these residue

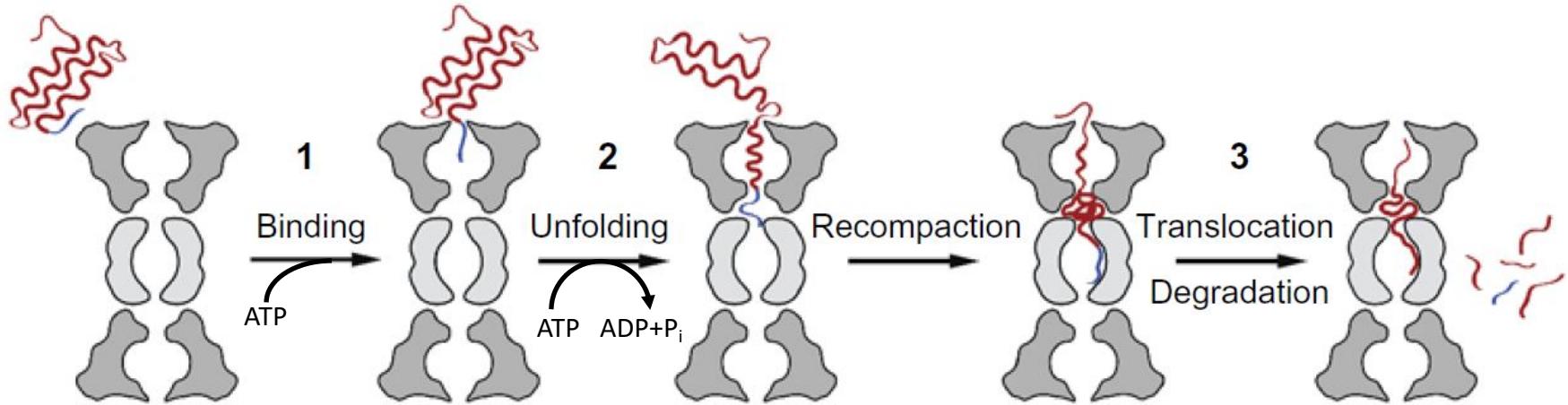
primary destabilizing residues

secondary destabilizing residues

R  
K



## ClpAP Reaction Stages



ClpAP has to accomplish three major tasks  
after first capture of substrate:

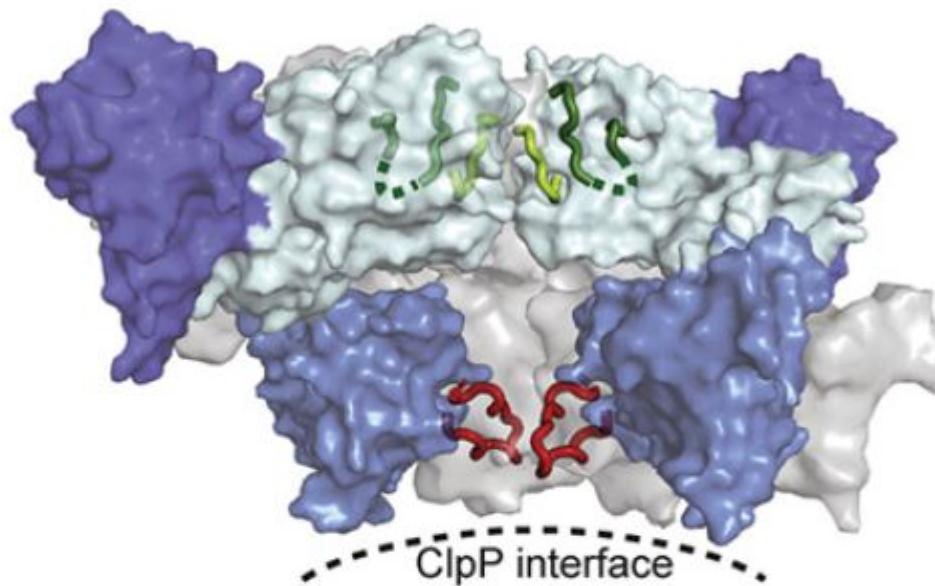
1. Unfolding
2. Translocation
3. Degradation

# ClpAP: Unfoldase/Translocase Reaction

ClpA unfolds and translocates by driving the movement of pore loops.

Together with the **ATPase cycle** comes a **cycle of directional movement**:

- loops attach to the polypeptide chain
- loops go from an «up» to a «down» position, along with it they drag the polypeptide
- loops release the chain
- loops move back to the «up» position



D1 loop 1  
SLDIGSLLAGTKYRGD (247-262)

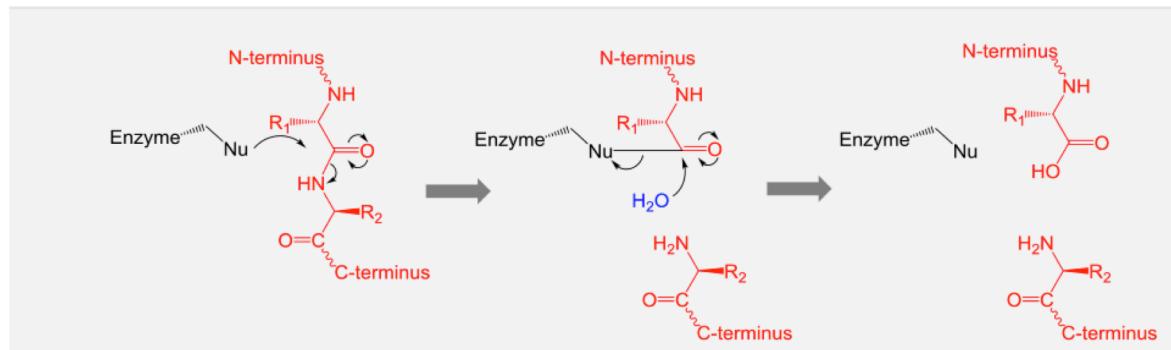
D1 loop 2  
IGAGAAASGG (291-299)

GYVG-loop (D2 loop)  
YMERHTVSRLIGAPPGYVG (524-542)

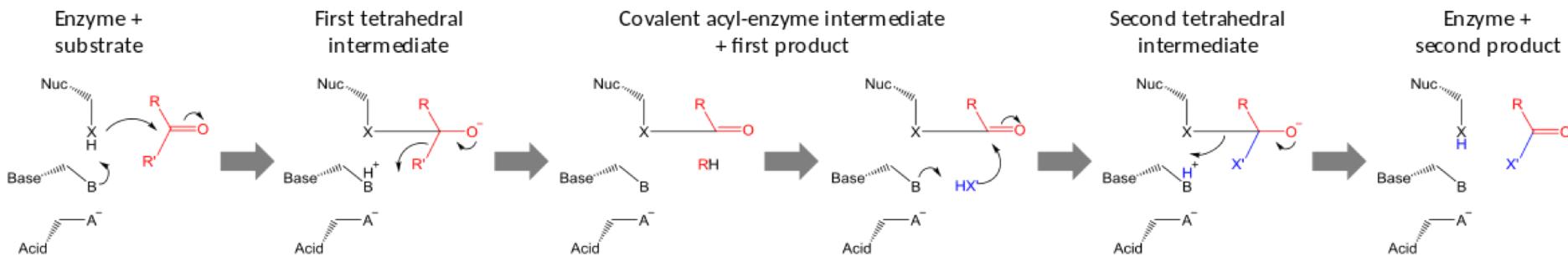
# ClpAP: Protease Reaction

ClpP is a **serine protease**

And functions according to the covalent type of protease catalysis



Covalent catalysis occurs usually with the help of so-called **catalytic triades**



ClpP triade:  
Asp-His-Ser

