THE 26S PROTEASOME

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THE 26S PROTEASOME

Protein degradation

↓

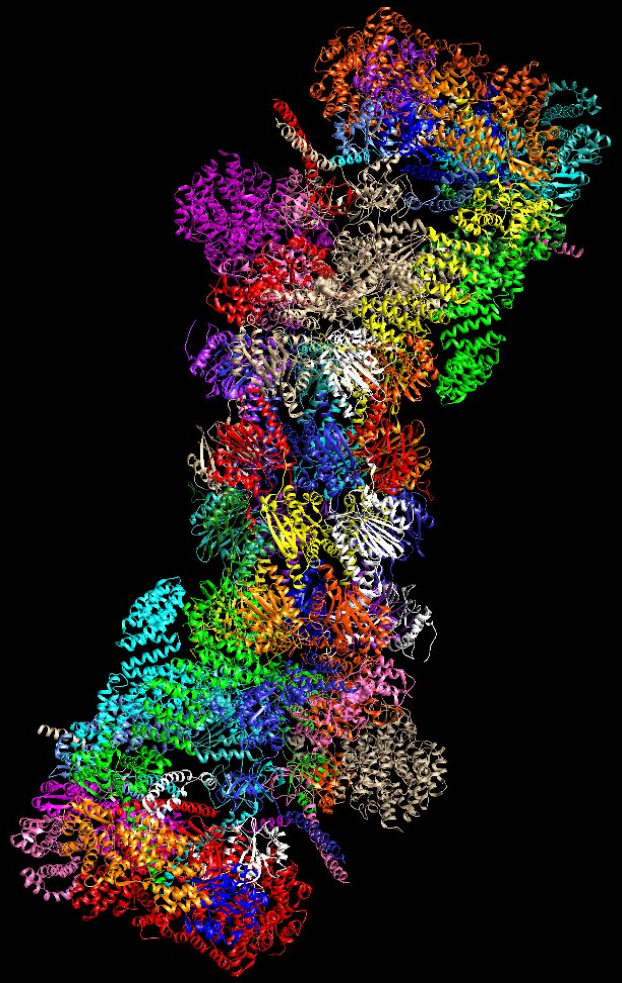
Ubiquitin - proteasome system

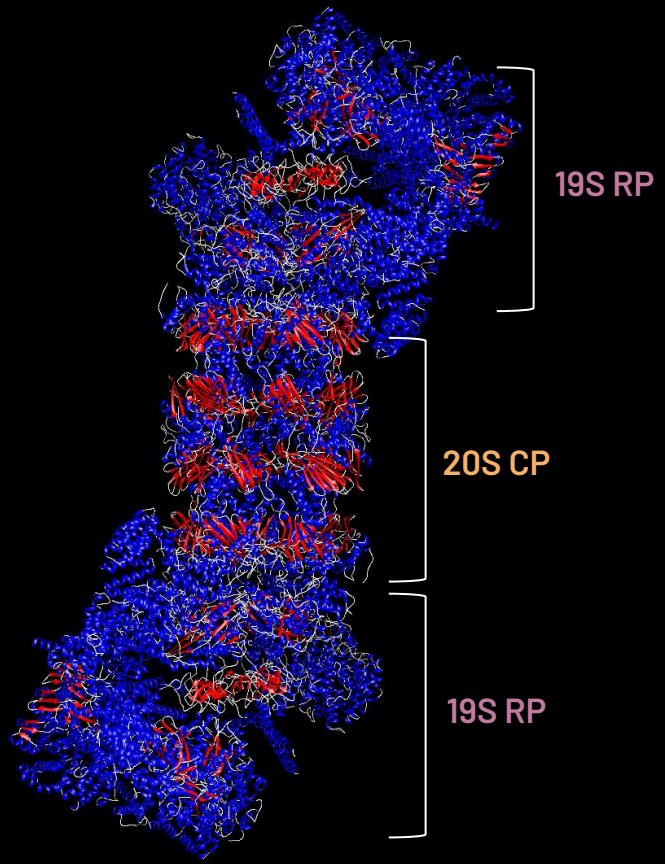
↓

Ubiquitin modifications target proteins to the

PROTEASOME

26S → 2.5 MDa





Homo Sapiens

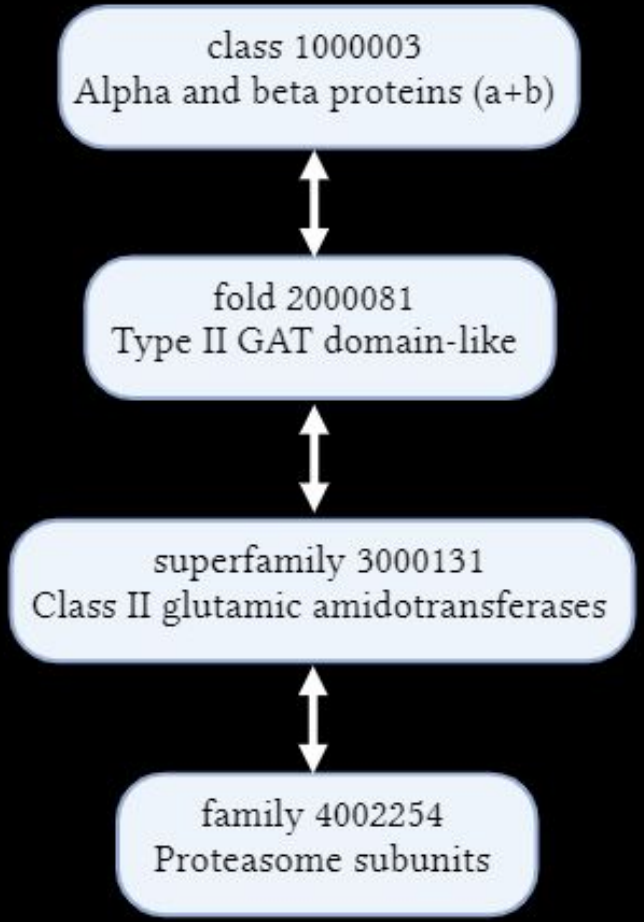
THE 26S PROTEASOME:

- **20S core particle (CP):**
  - **Peptides hydrolysis**
  - **28 α and β subunits**

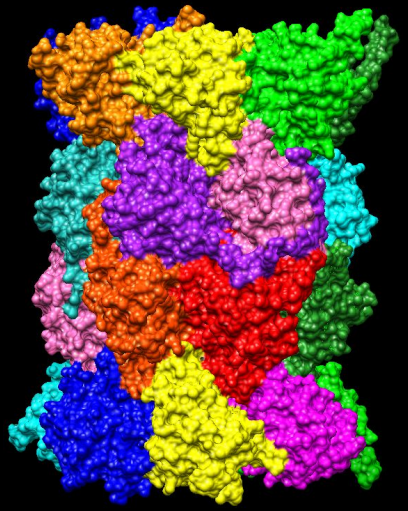
- **19S Regulatory particle (RP):**
  - **Ubiquitin recognition**
  - **17 subunits**

SCOP Classification

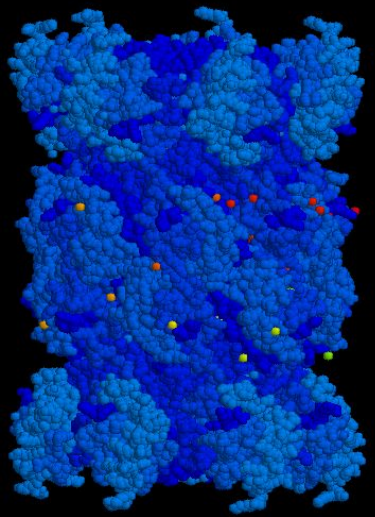
- **CLASS:** Alpha and beta proteins (a+b) class
- **FOLD:** Type II GAT domain-like
  Two antiparallel β-sheets, surrounded on either side by two α helix
- **SUPERFAMILY:** Class II glutamine amidotransferase
  Fold similar to i class I glutamine amidotransferase, which consists of an α/β/α sandwich.
- **FAMILY:** Proteasome subunits



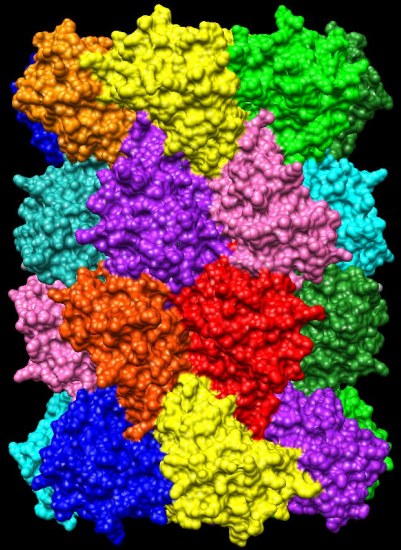
INTRODUCTION TO HOMOLOGUES



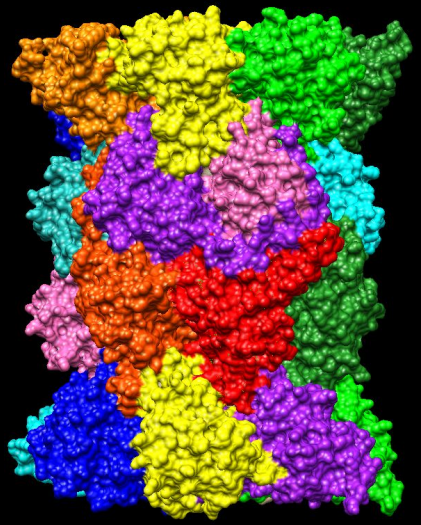
Eukaryota
Bos taurus



Archaea
Archaeoglobus fulgidus



Archaea
Thermoplasma acidophilum



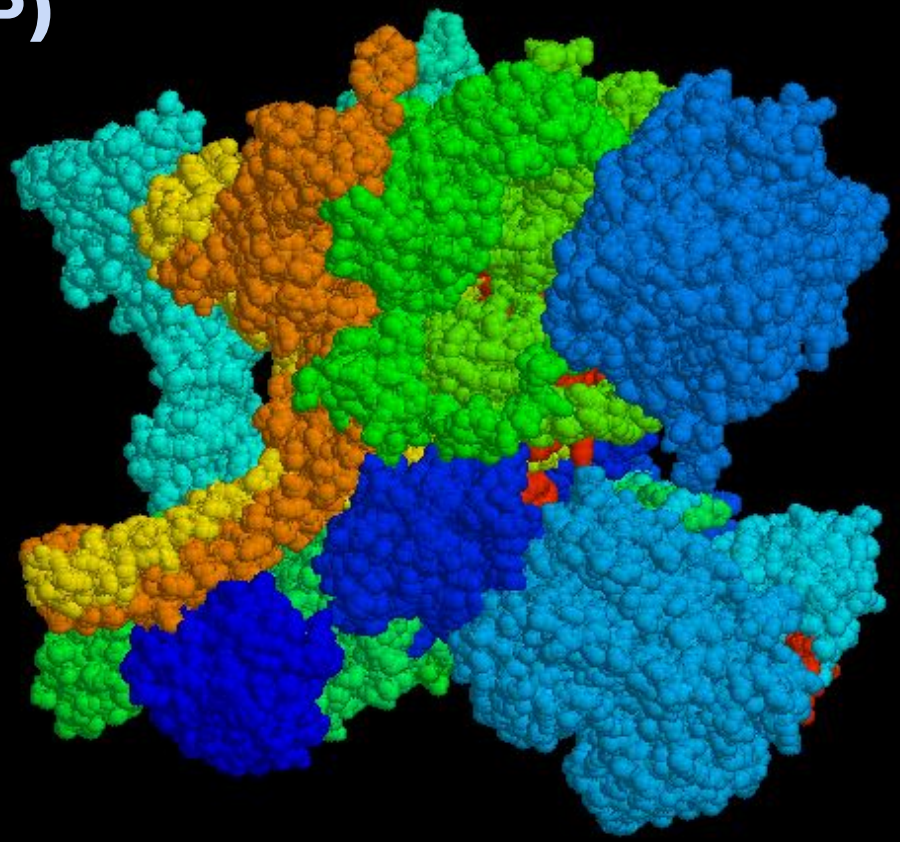
Fungi
Saccharomyces cerevisiae

STRUCTURE AND FUNCTION

19S - Regulatory Particle (RP)

17 SUBUNITS

- Captures ubiquitinated proteins
- Prometes substrate unfolding
- Opening of the gate to the α-ring



19S - Regulatory Particle (RP)

- **BASE**

Six → AAA + ATPases ring **Rpt 1 -6**

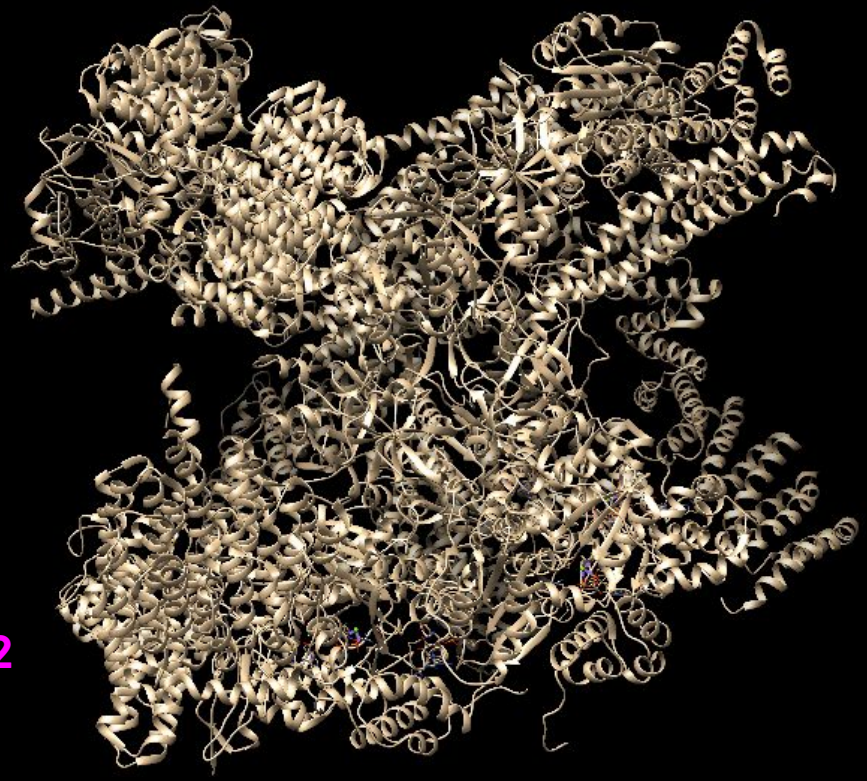
Responsible of the ATP hydrolysis that facilitates substrate translocation into the CP

Four → Non-ATPases **Rpn 1,2,10,13**

- **LIDS**

Ten → Non-ATPases **Rpn 3, 5, 6, 7, 8, 9, 11, 12**

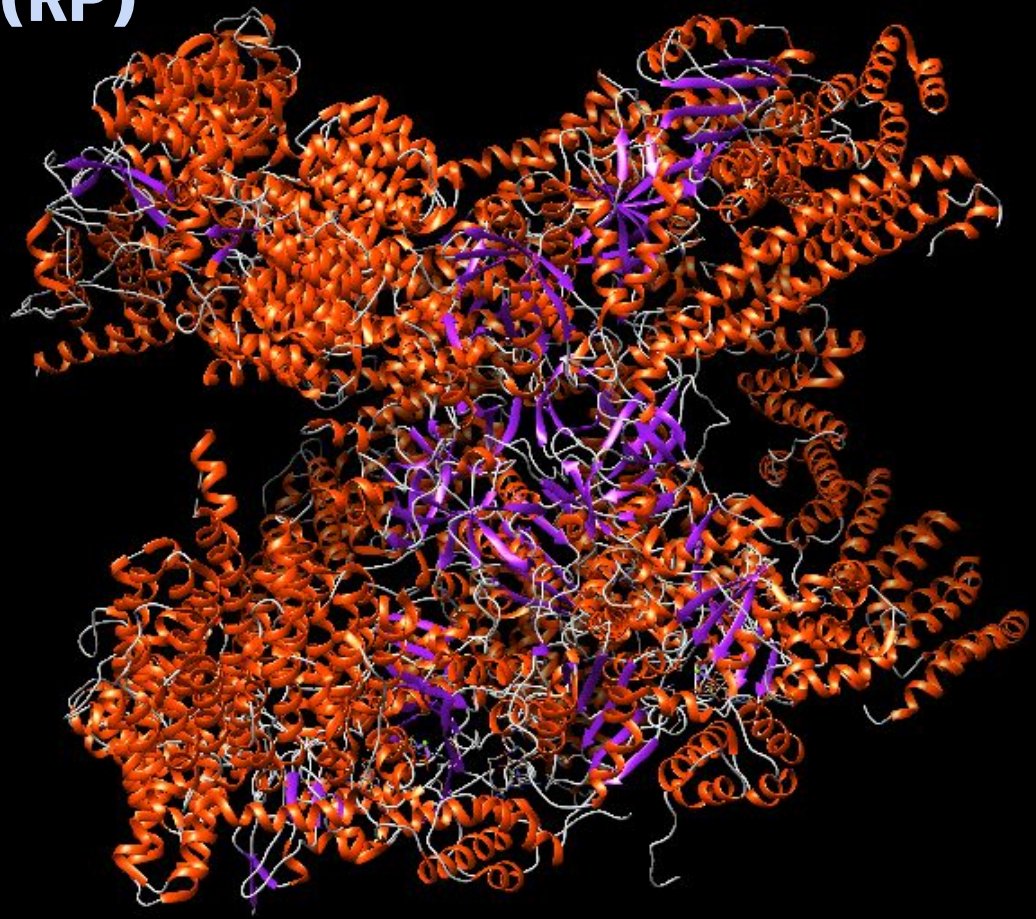
Recognises the ubiquitin complex



19S - Regulatory Particle (RP)

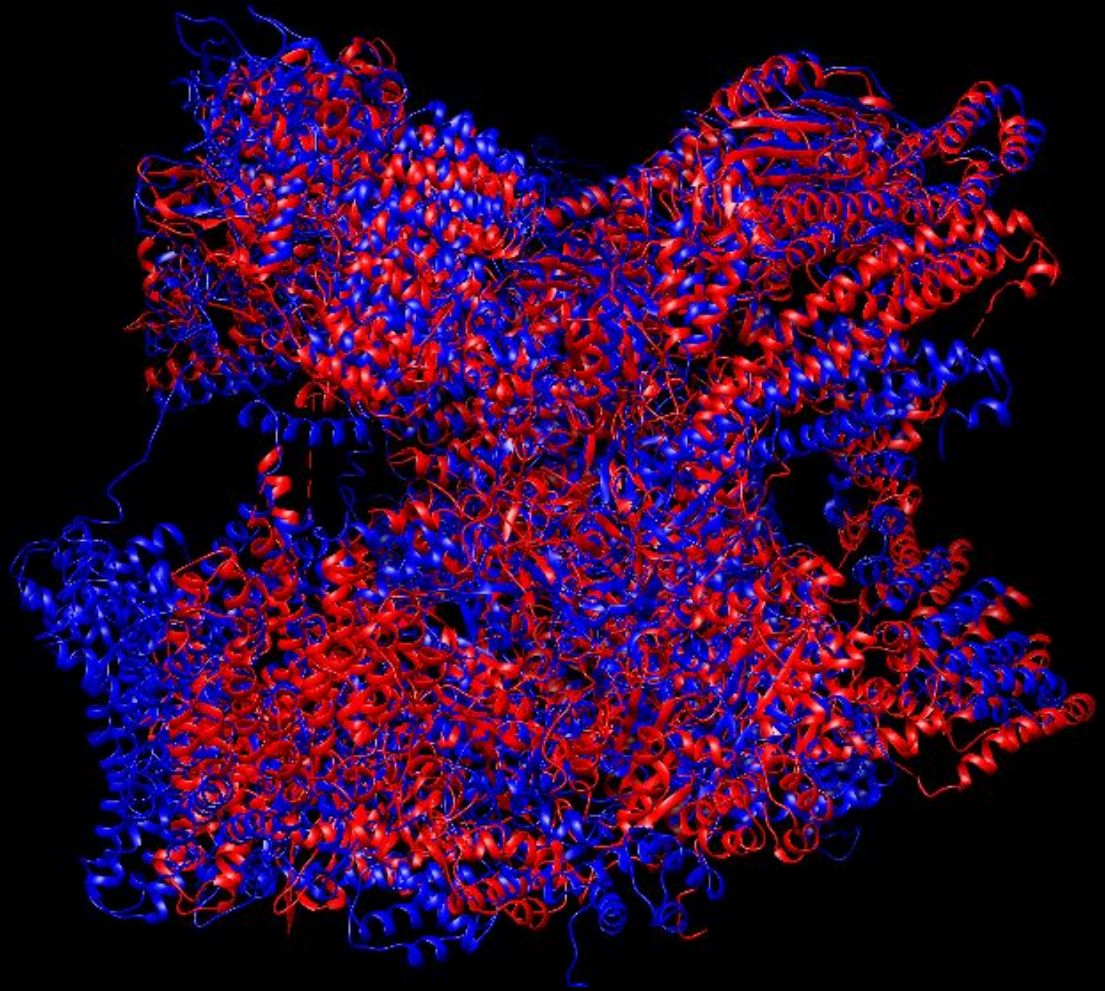
258 α - helix

111 β - strands



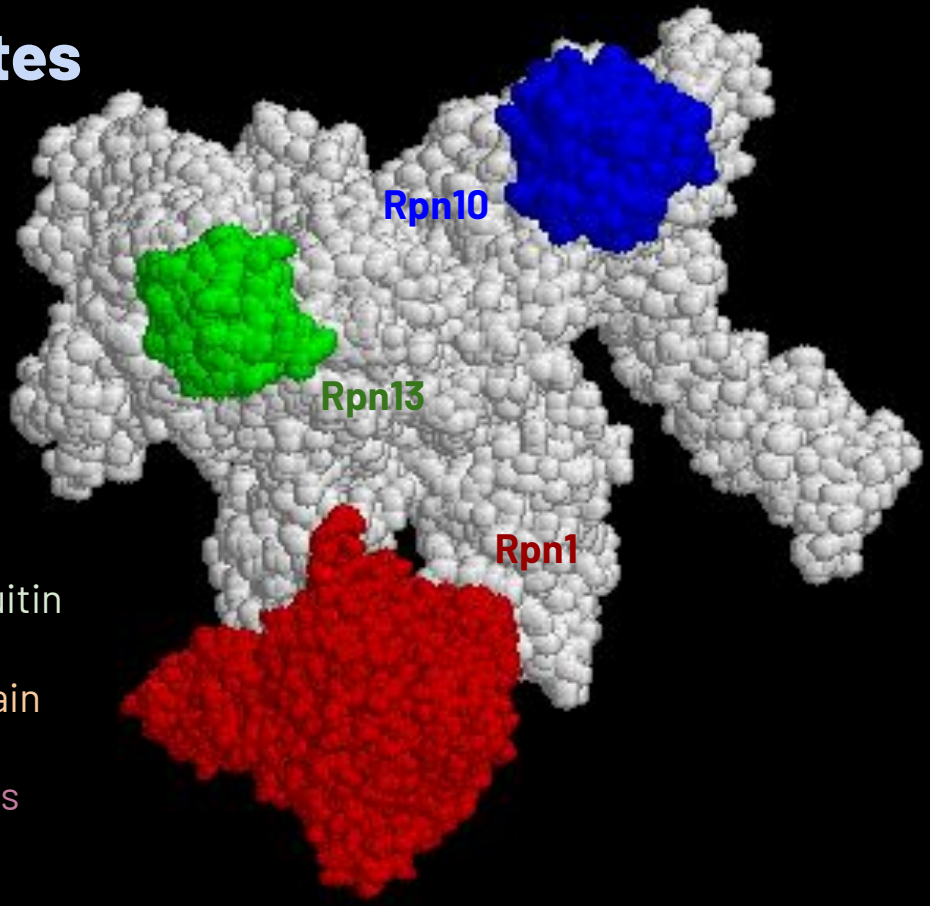
Homo Sapiens vs S. Cerevisiae

Alignment score	1.67
RMSD	2.8



19S - Ubiquitin interaction sites

Ubiquitinated protein → Ubiquitin receptors

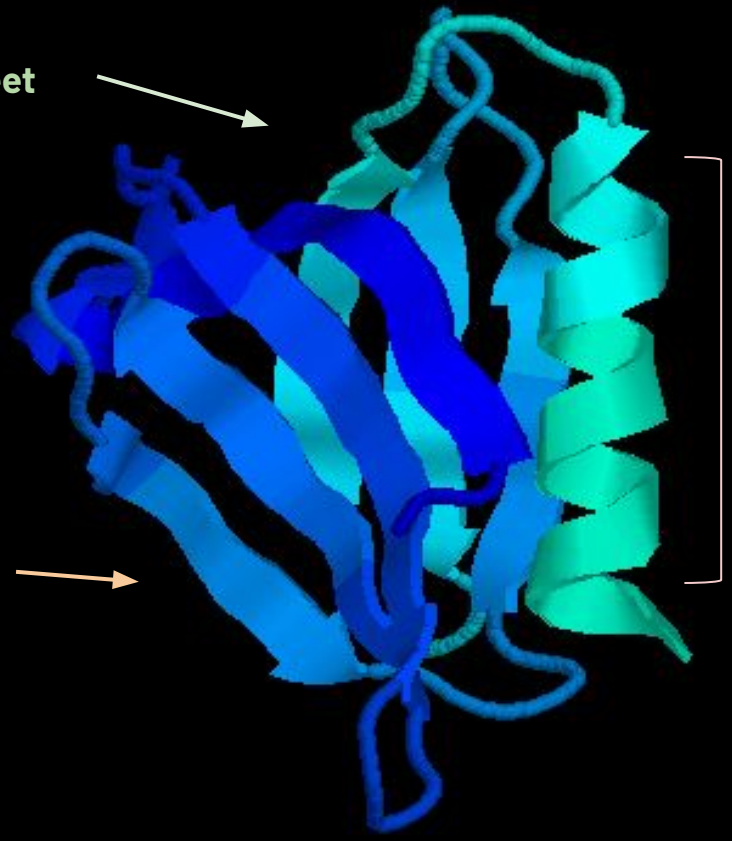


Rpn13 : PRU , Pleckstrin-like receptor for ubiquitin

Rpn10 : UIM, von Willebrand factor type A domain

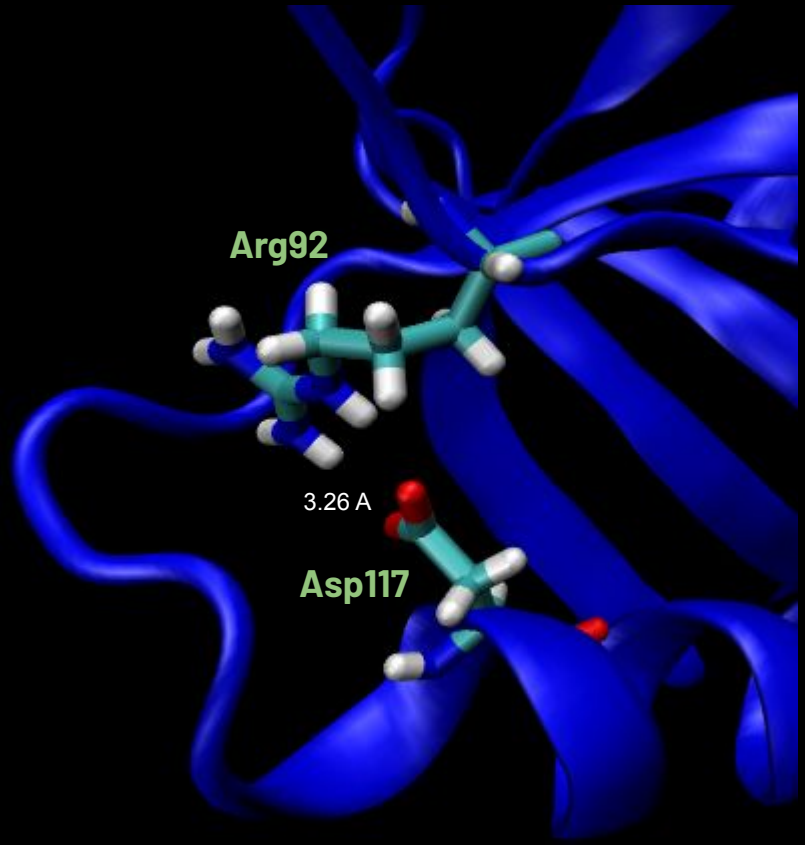
Rpn 1: UBL, ubiquitin and ubiquitin-like domains

RPN13: PRU Pleckstrin-like receptor for ubiquitin



RPN13

SALT BRIDGES
ASP117 - ARG92
ASP21 - LYS29
ASP41 - ARG43
ASP52 - LYS27
ASP54 - ARG42
ASP63 - ARG43
ASP71 - LYS30
ASP72 - HIS58
ASP79 - HIS68
GLU111 - ARG27
GLU70 - ARG43



RPN13

Interacting residues of Rpn13

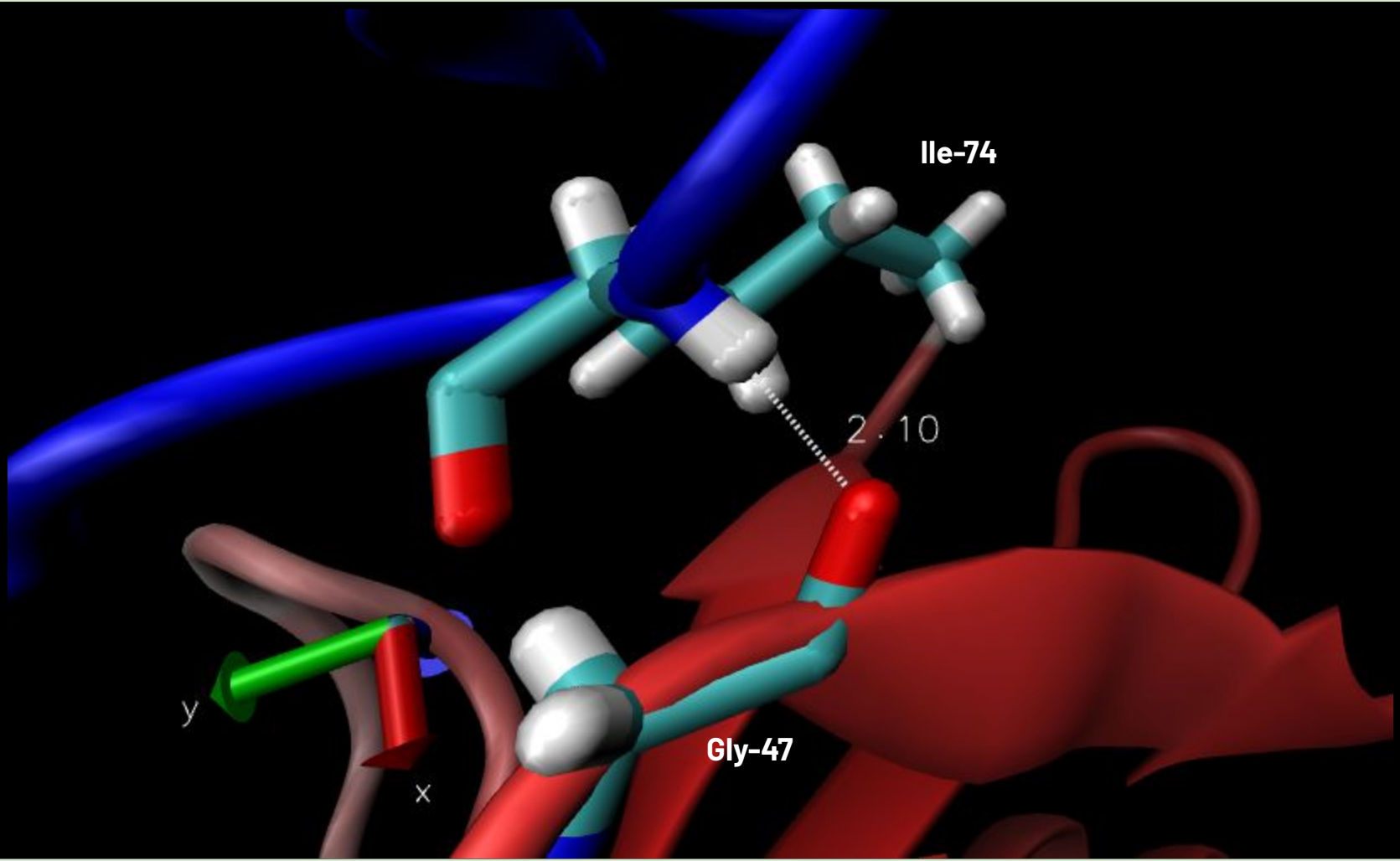
Leu-56

Leu-73

Ille-74

Phe-76

Phe-98


Ile-74
2.10
Gly-47
y
x

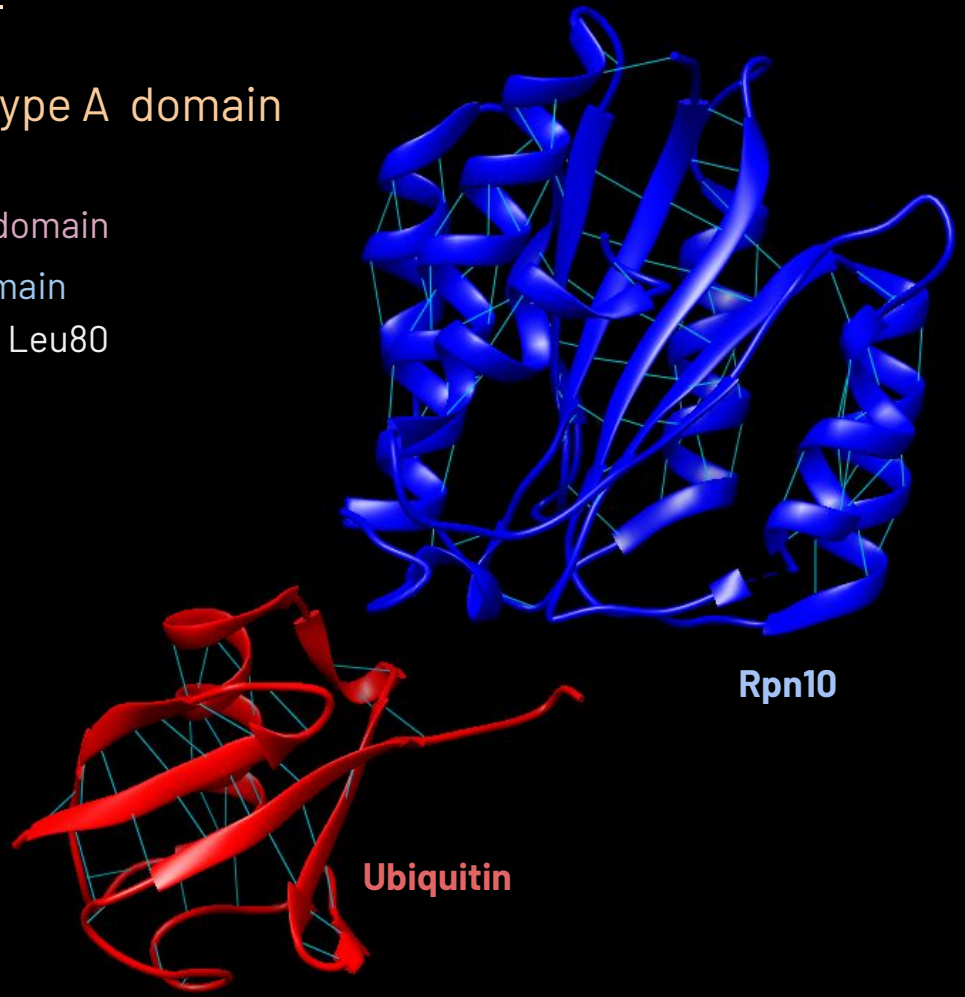
RPN10: UIM von Willebrand factor type A domain

N-terminal vWA (Von Willebrand factor type A) domain

C-terminal Ubiquitin Interacting Motif (UIM) domain

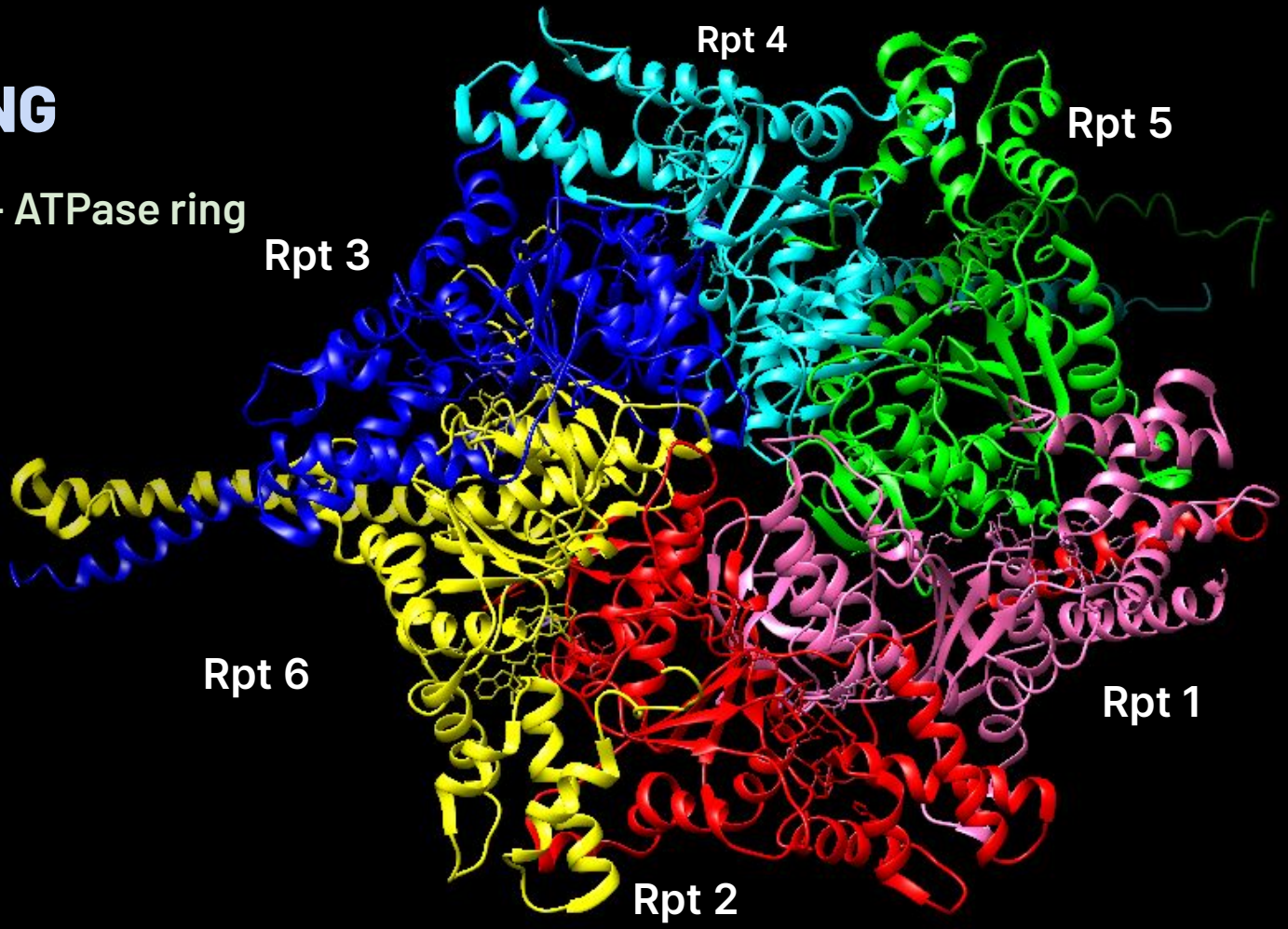
Ile 44, Val70, and Leu80

SALT BRIDGES
ASP140 - HIS170
ASP20 - ARG25
ASP21 - LYS29
GLU156 - ARG122
GLU27 - ARG23
GLU4 - LYS40



ATPase RING

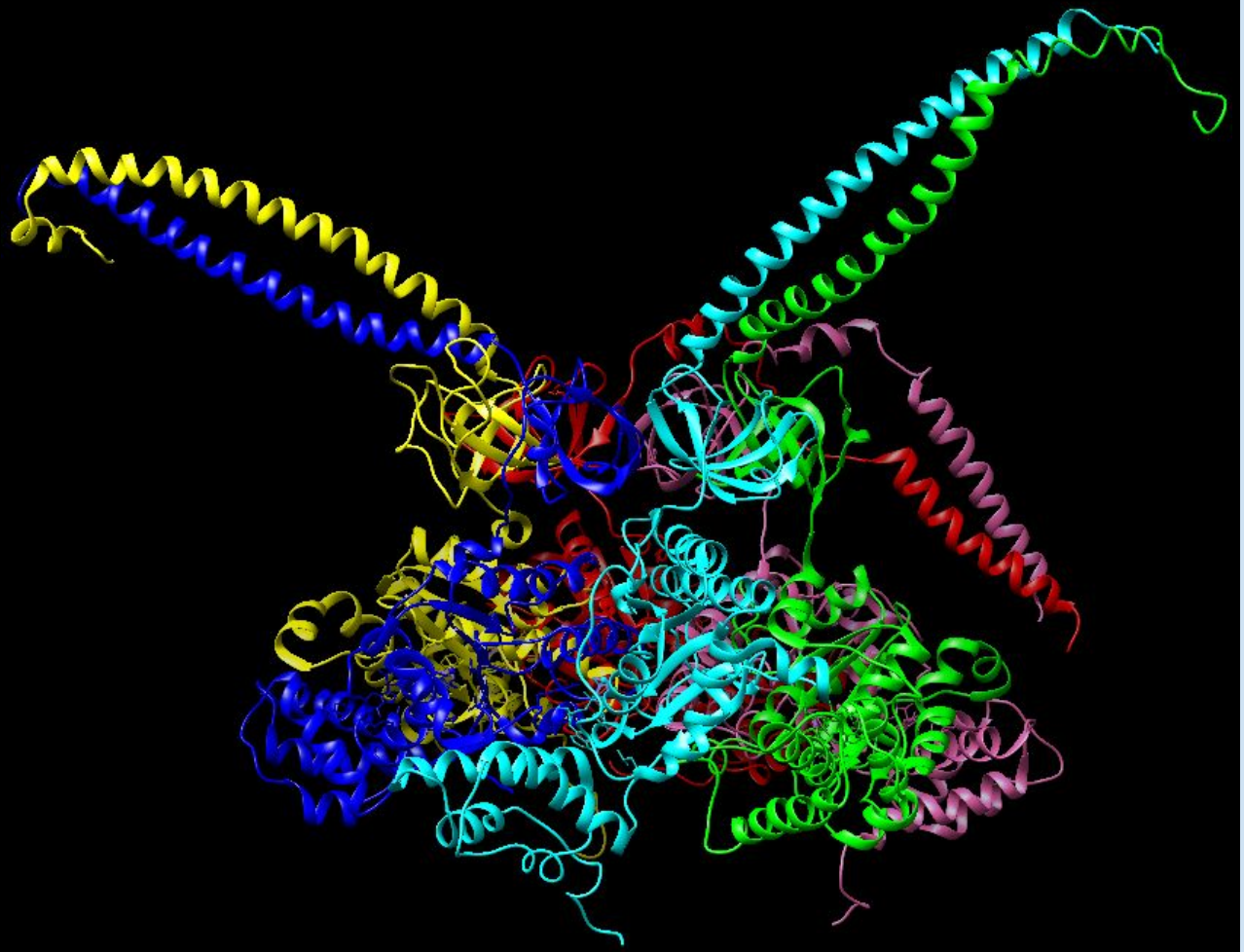
Six membered - ATPase ring



ATPase RING

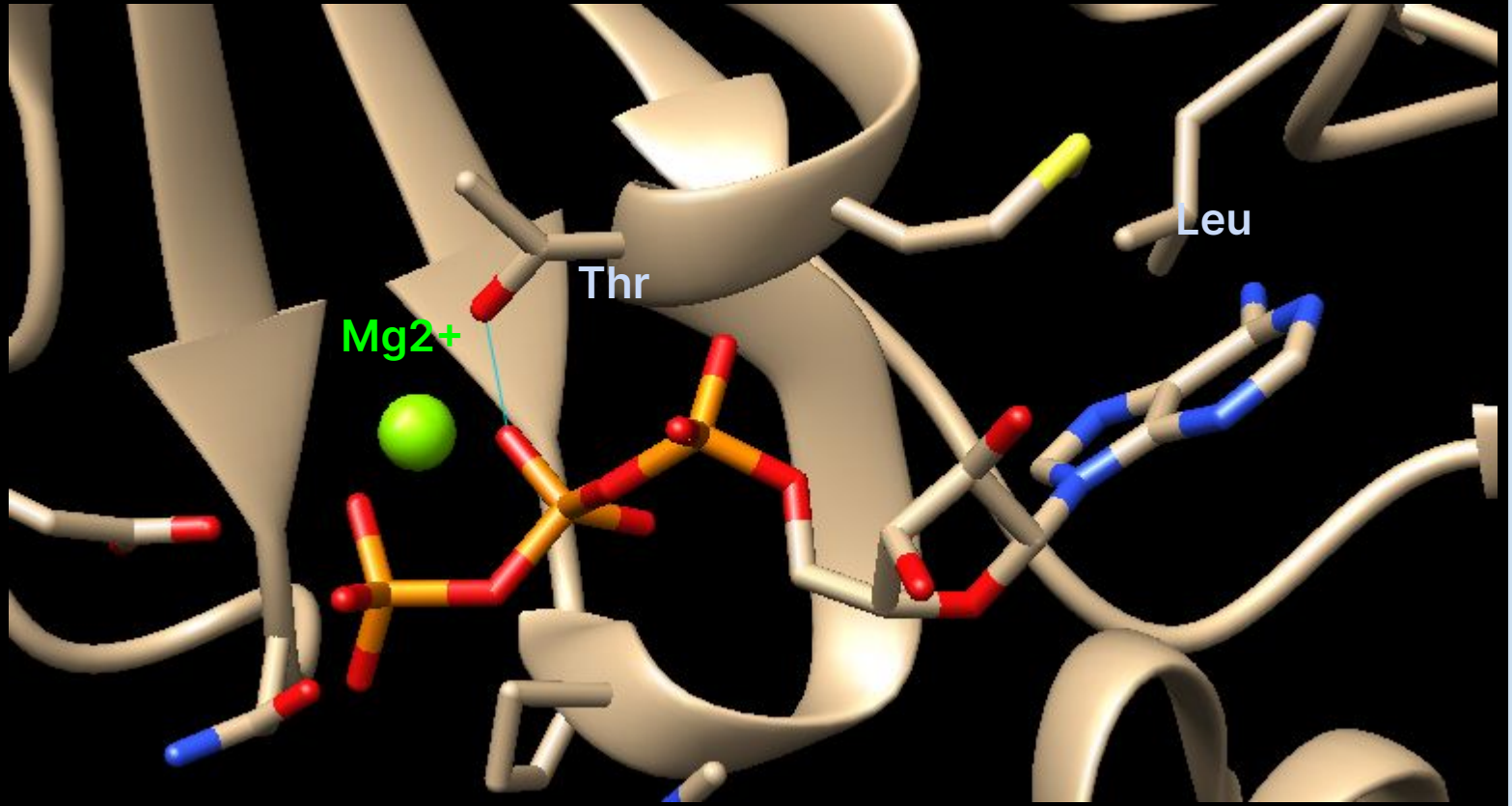
Pore 1 loop

Pore 2 loop



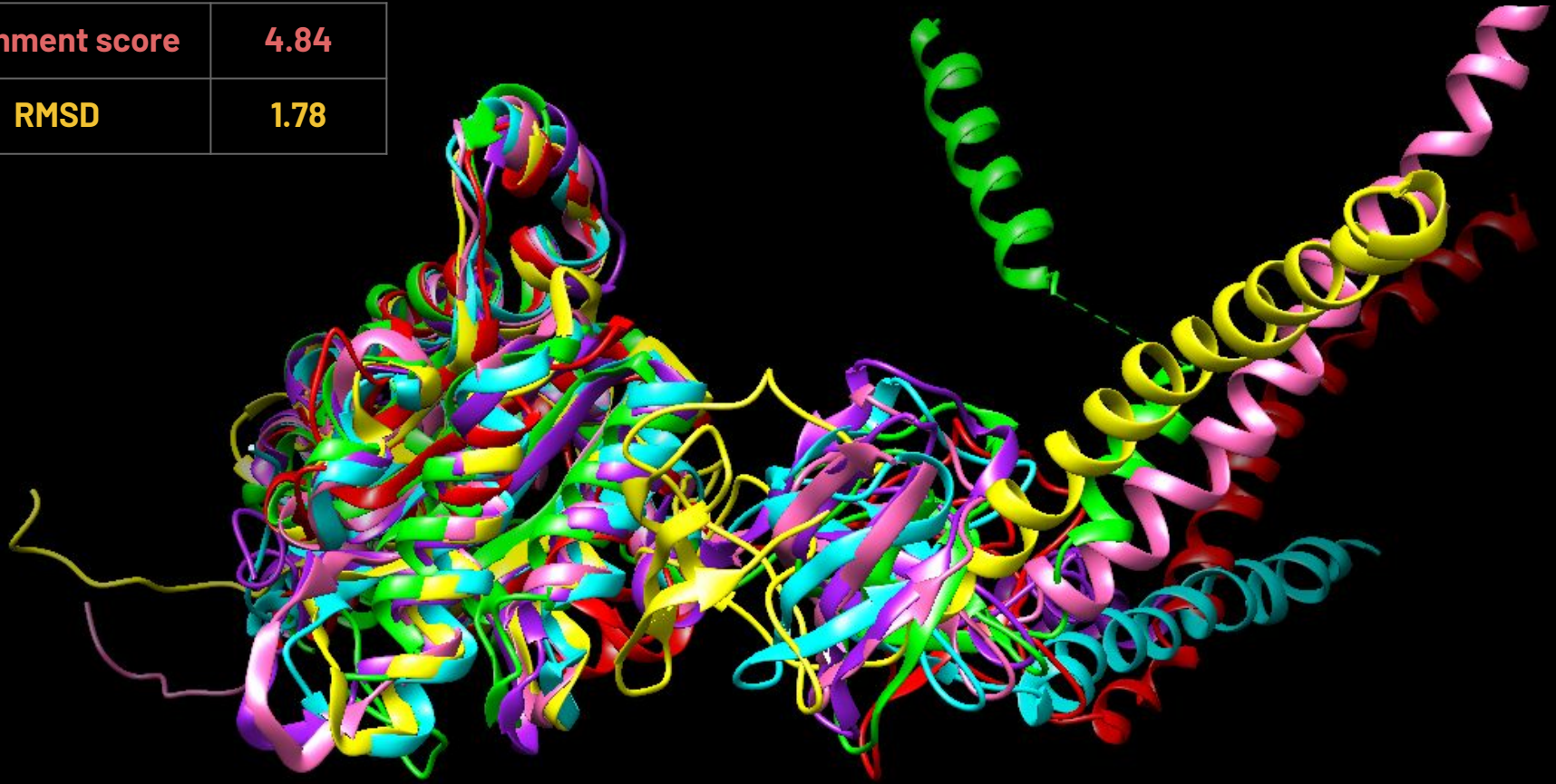
ADP - RPT3

2.1 - 2.8A



Homo Sapiens vs S. Cerevisiae vs T.Acidophilum

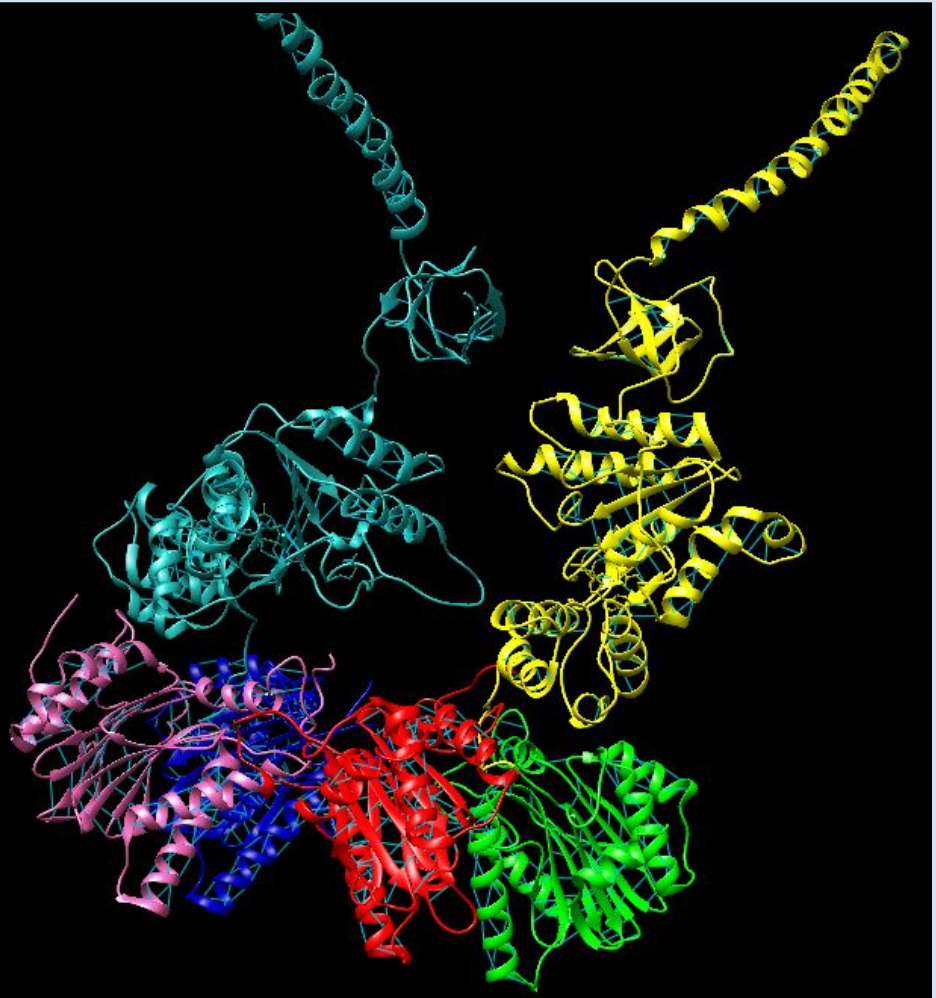
Alignment score	4.84
RMSD	1.78



Interaction 19S-20S

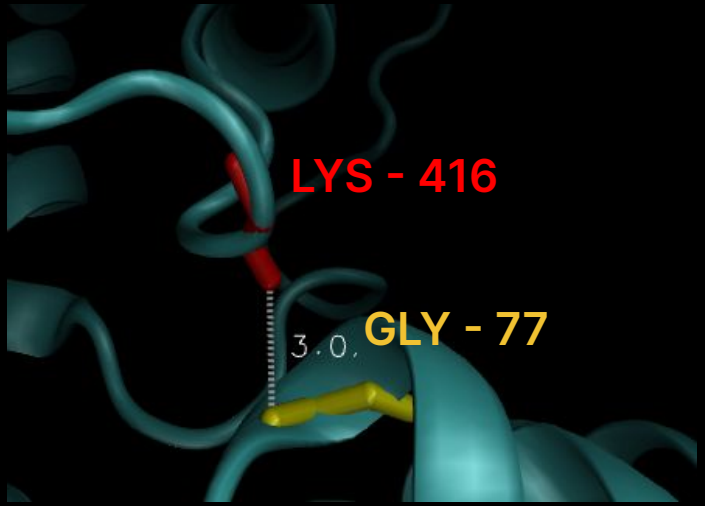
Rpt3 → α1, α2

Rpt5 → α5, α6

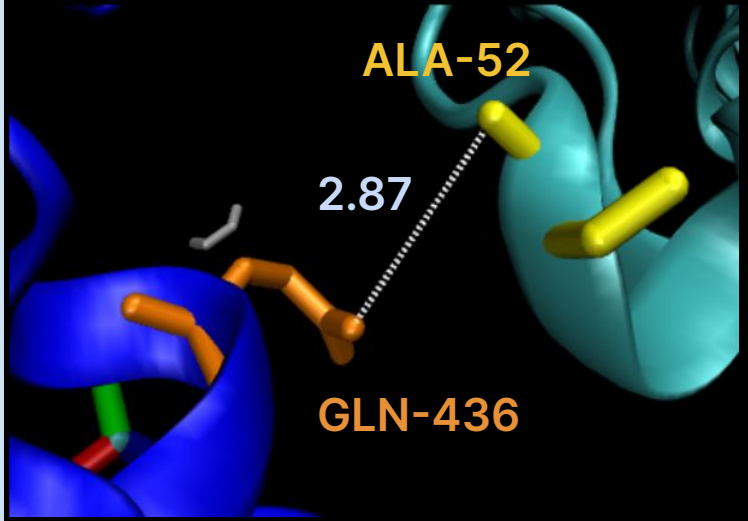


Rpt3: Hydrogen Bonds

Donor Amino Acid	Acceptor Amino Acid	Alpha Subunit
Lys418	Gly30	α 1
Lys418	Tyr75	α 1
Lys416	Gly77	α 2
Glu415	Lys52	α 2
Tyr417	Glu26	α 1
Tyr417	Leu22	α 1
Tyr417	Hydrophobic residues	α 1
Phe416	Hydrophobic residues	α 1

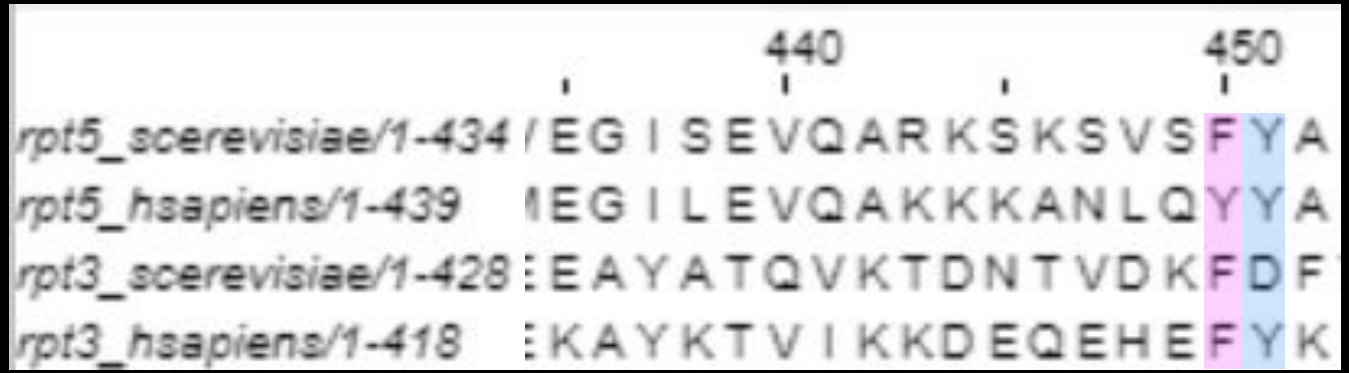


Rpt5: Hydrogen bonds



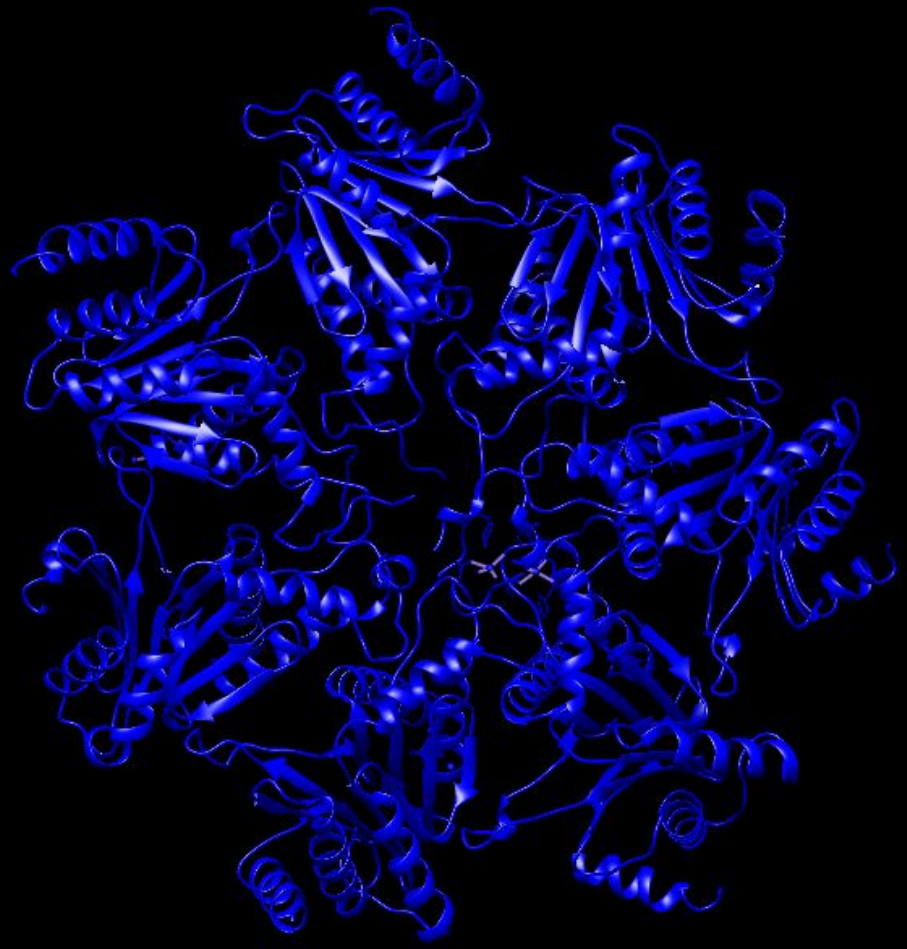
Donor Amino Acid	Acceptor Amino Acid	Alpha Subunit
Ala439	Gln60	α 6
Ala439	Lys62	α 6
Gln436	Ala52	α 5
Gln437	Gln53	α 5
Tyr437	*Nearby Residue*	α *5*
Tyr438	*Nearby Residue*	α *5*

GATE OPENING

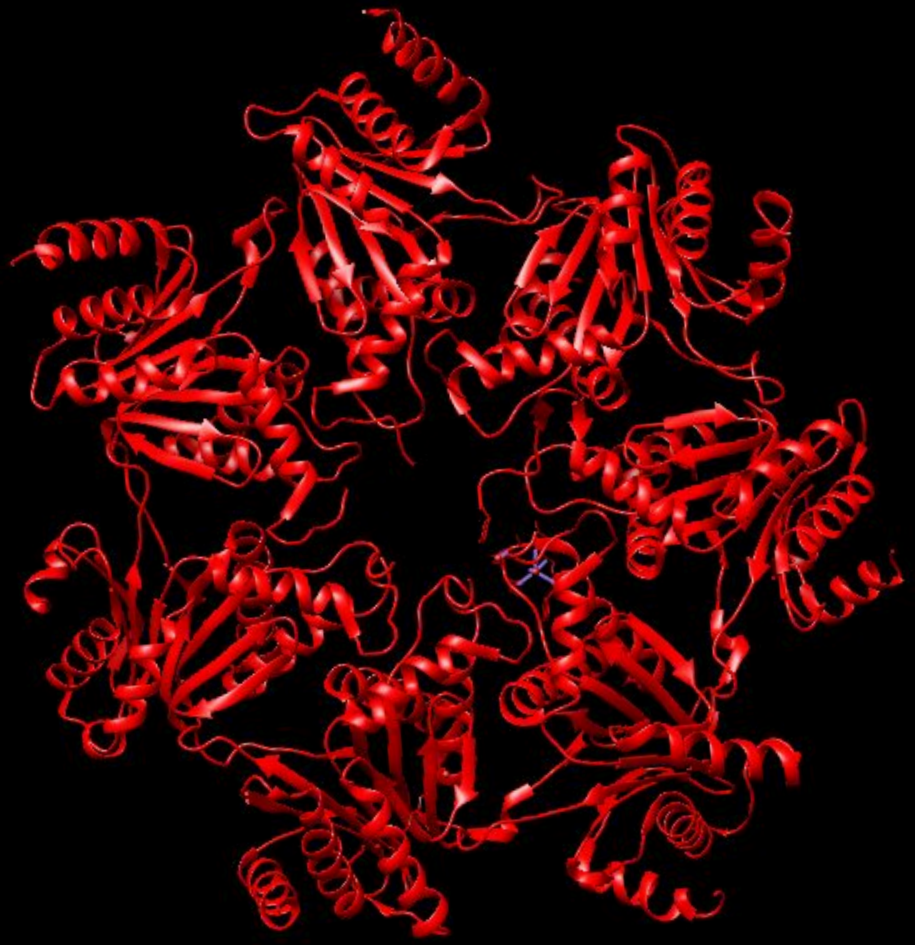


Hb-Y-X motifs

CLOSED GATE

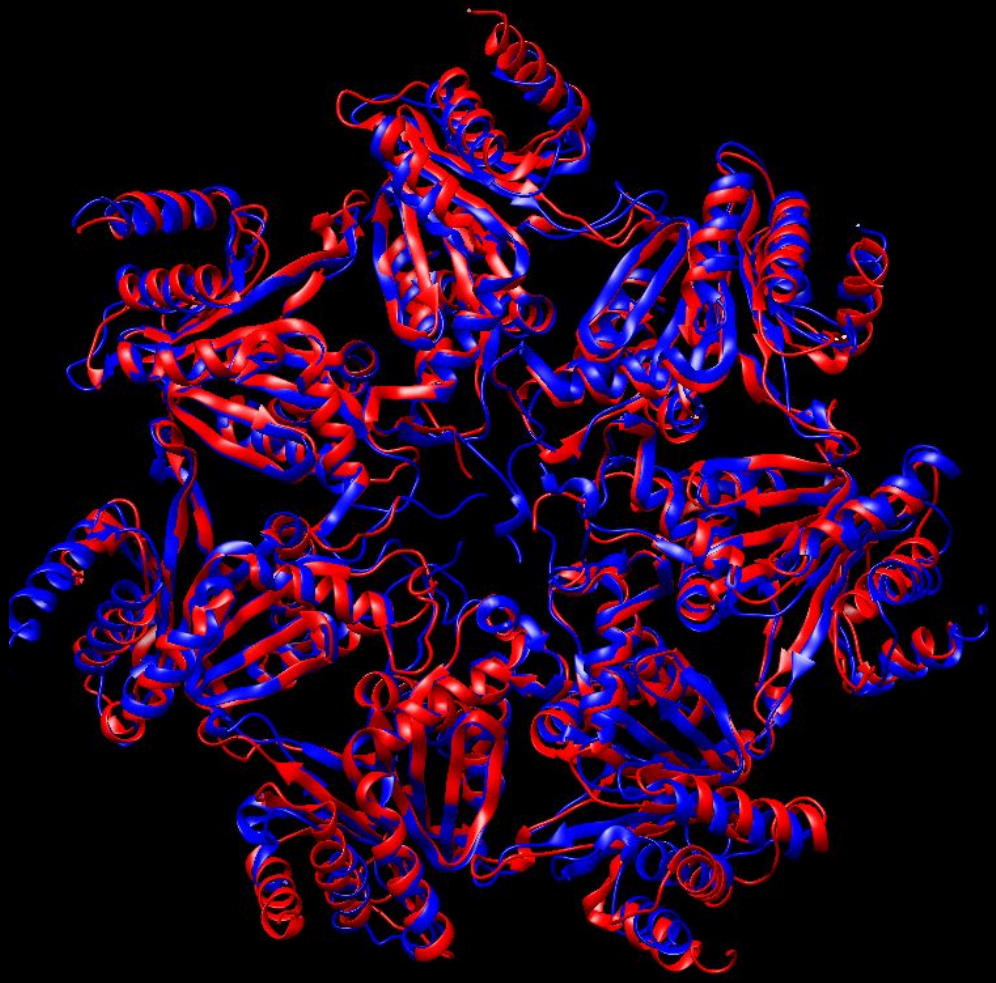


OPEN GATE



OPEN VS CLOSED GATE

Alignment score	7.04
RMSD	1.71

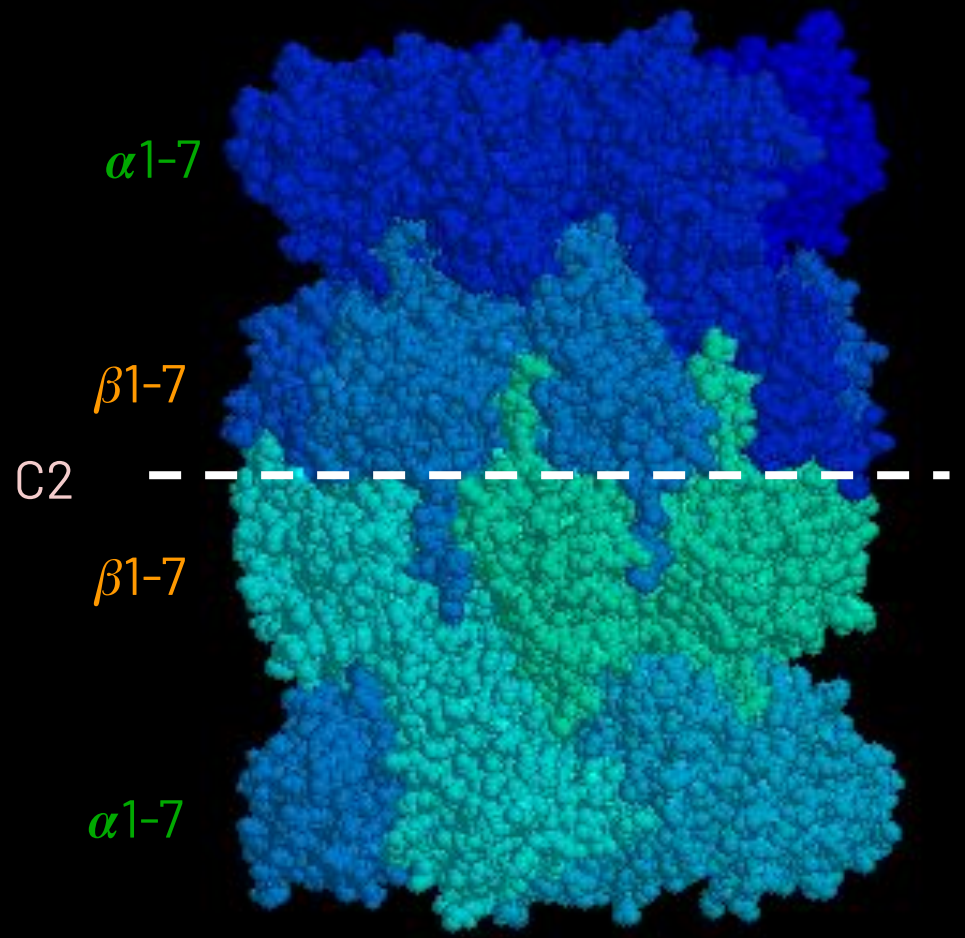


20S - Core Particle (CP)

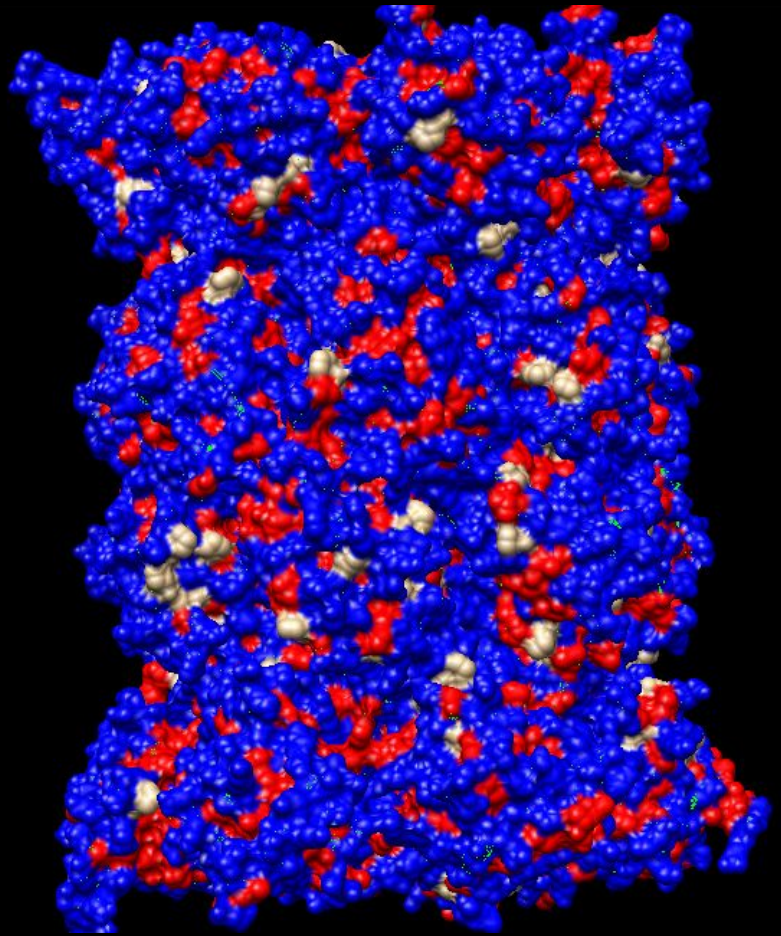
28 SUBUNITS

2 alpha rings

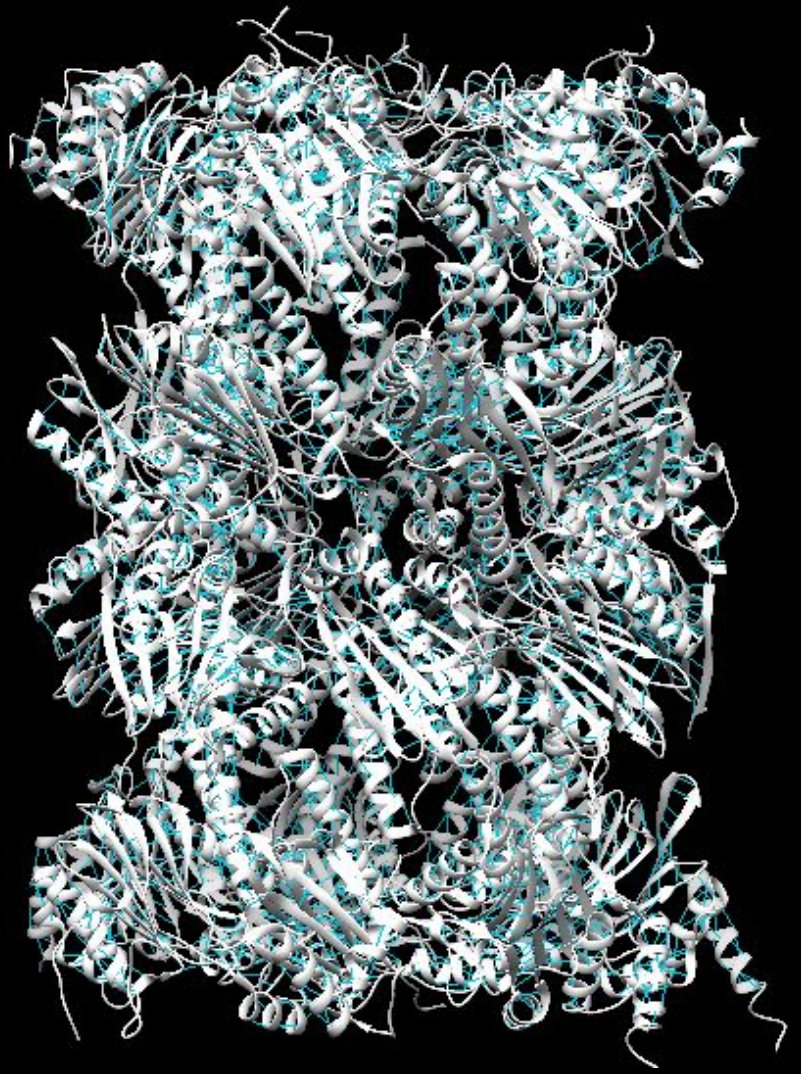
2 beta rings



HYDROPHOBICITY

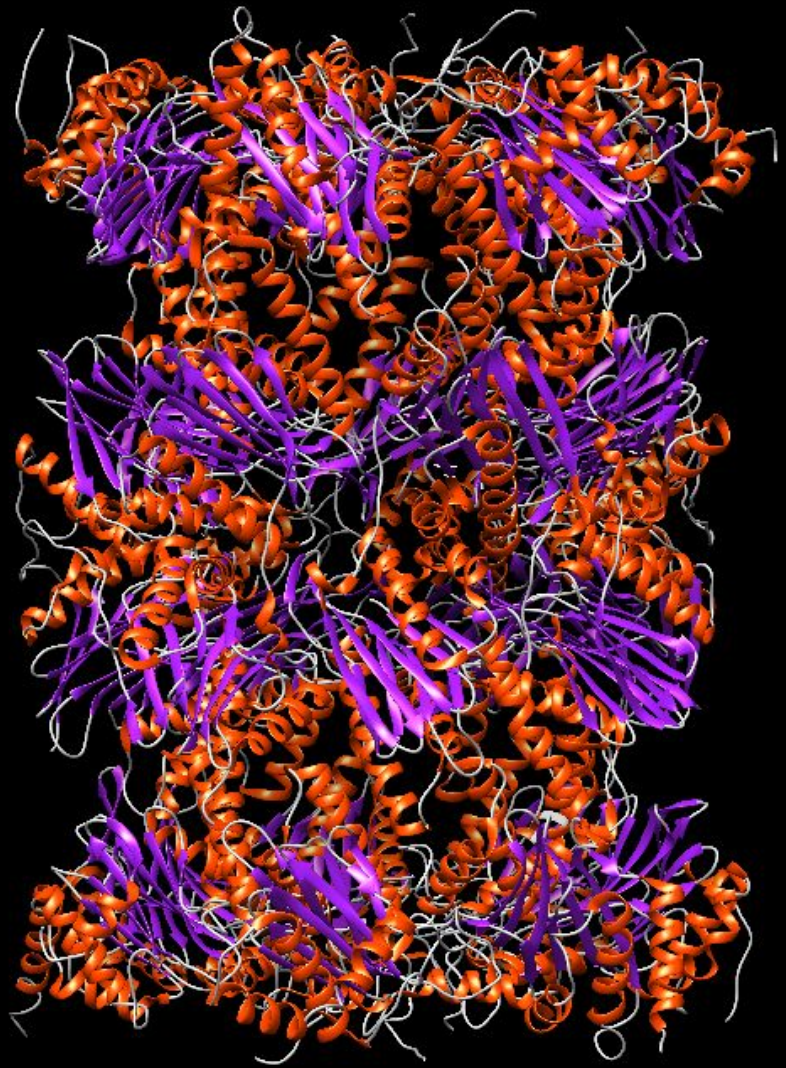


HYDROGEN BONDS



179 α helices

330 β strands

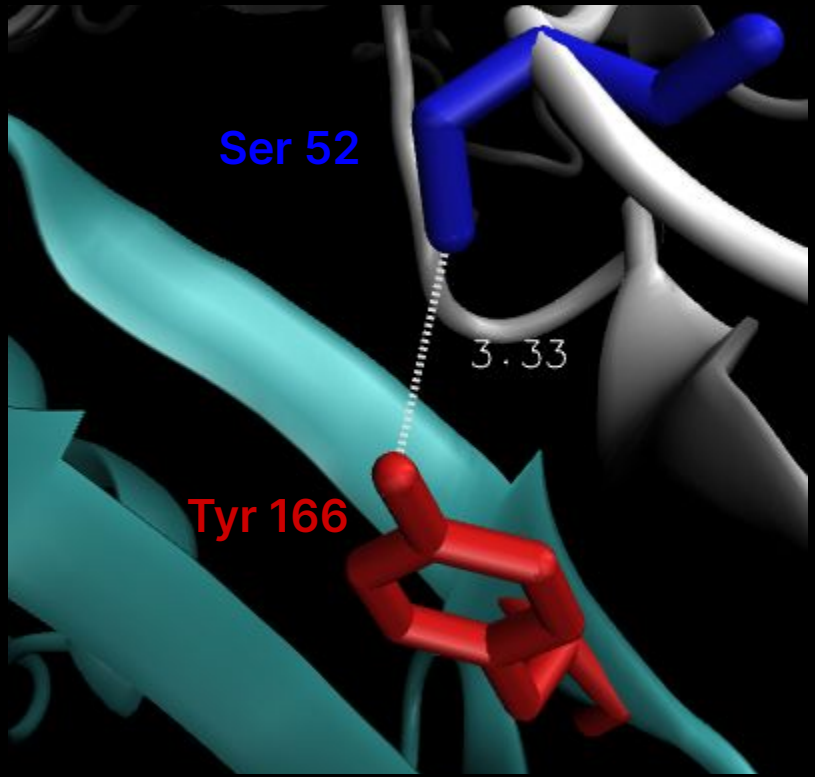


ALPHA RING

DONOR	ACCEPTOR
Asp166 (alpha5)	Leu56 (alpha1)
Ser 52 (alpha1)	Tyr 166 (alpha5)
Lys157(alpha7)	Leu58(alpha5)
Glu60(alpha5)	Ala155(alpha7)
Ser134(alpha5)	Gln120(alpha7)
Asn122(alpha7)	Ala132(alpha5)
Lys160(alpha4)	Leu53(alpha7)
Asp55(alpha7)	Gly158(alpha4)
Arg125(alpha7)	Gln123(alpha4)
Asp57(alpha4)	Ala156(alpha2)
Lys158(alpha2)	Leu55(alpha4)

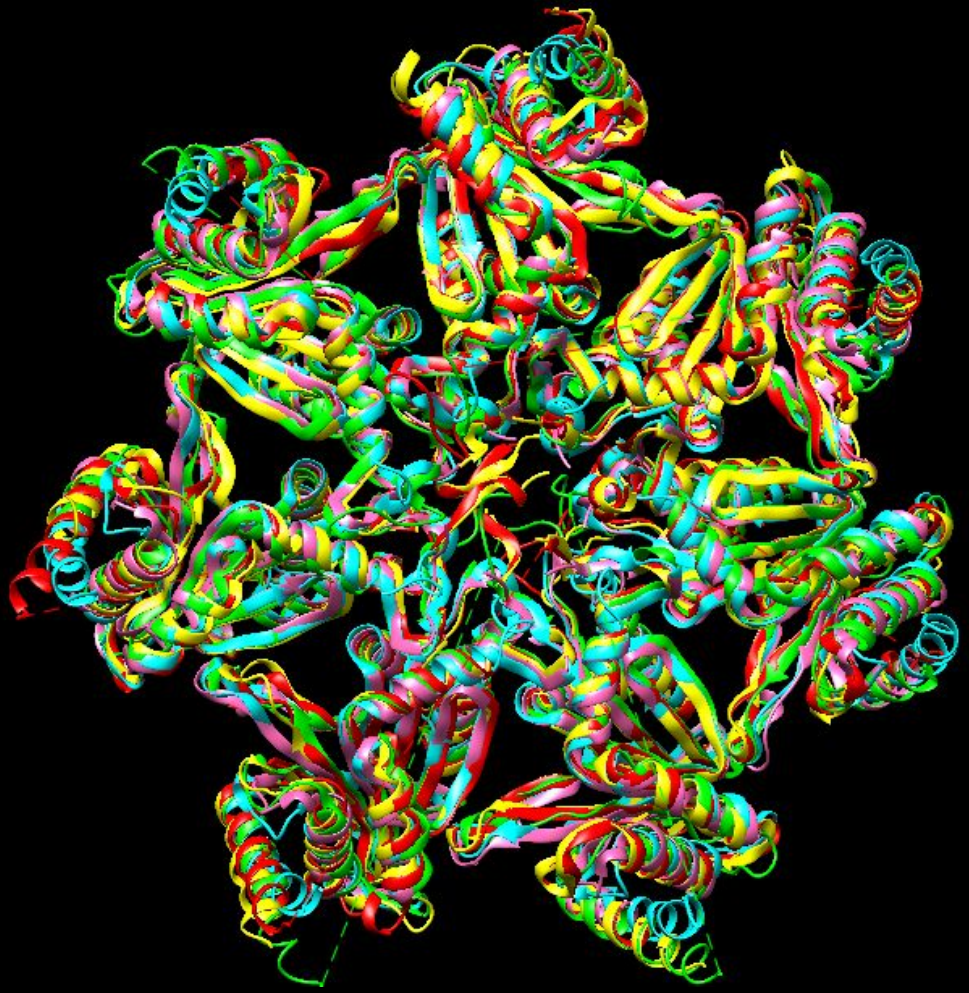
DONOR	ACCEPTOR
Arg127(alpha2)	Gln127(alpha6)
Ala129(alpha6)	Gly125(alpha2)
Lys164(alpha6)	Leu55(alpha2)
Asp62(alpha6)	Gly159(alpha3)
Trp161(alpha3)	Leu60(alpha6)
Gly162(alpha3)	Leu60(alpha6)
Arg132(alpha6)	Leu124(alpha3)
Tyr123(alpha1)	Ala127(alpha3)
Arg129(alpha3)	Gln121(alpha1)
Glu59(alpha3)	Asp155(alpha1)
Arg157(alpha1)	Leu57(alpha3)

ALPHA RING INTERACTION

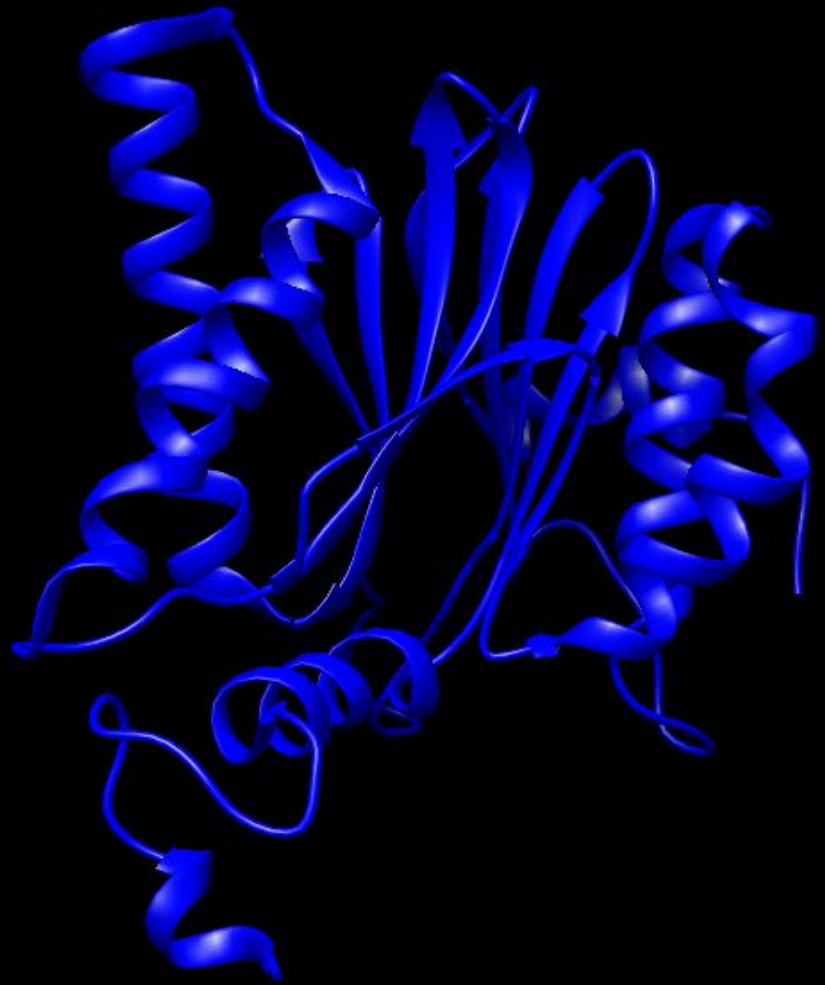


ALPHA RING AMONG SPECIES

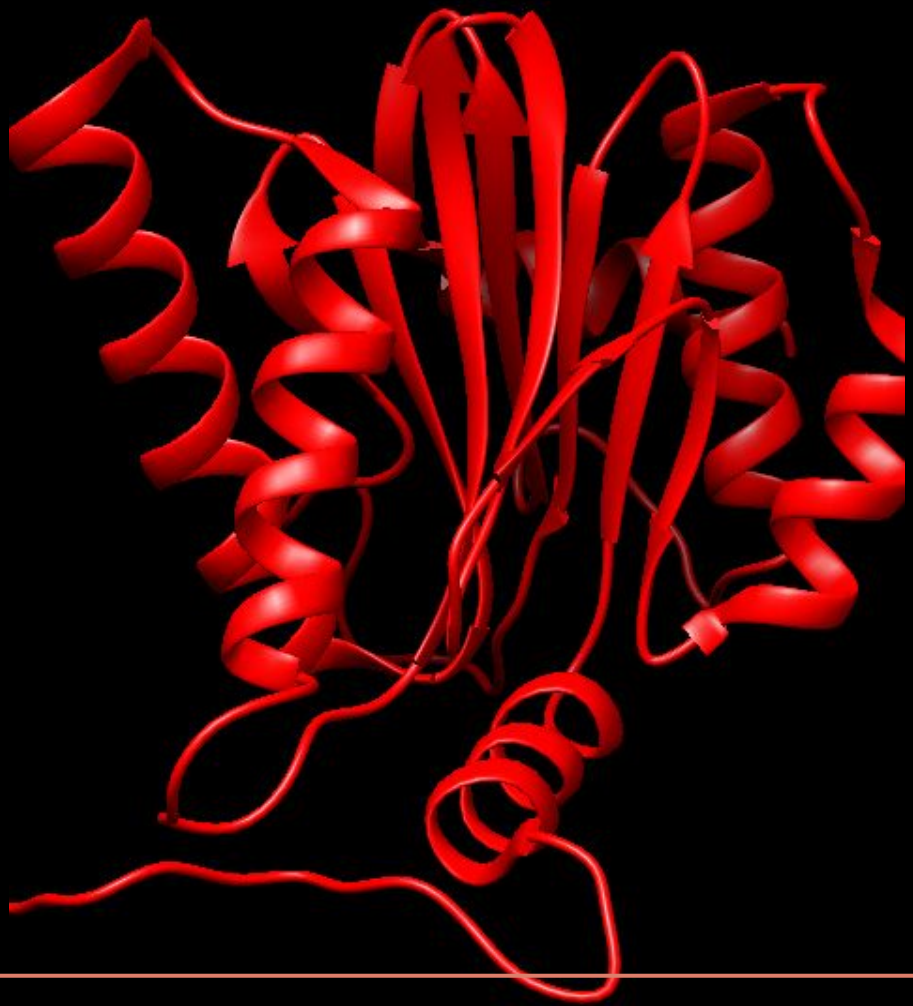
Alignment score	8.98
RMSD	1.48



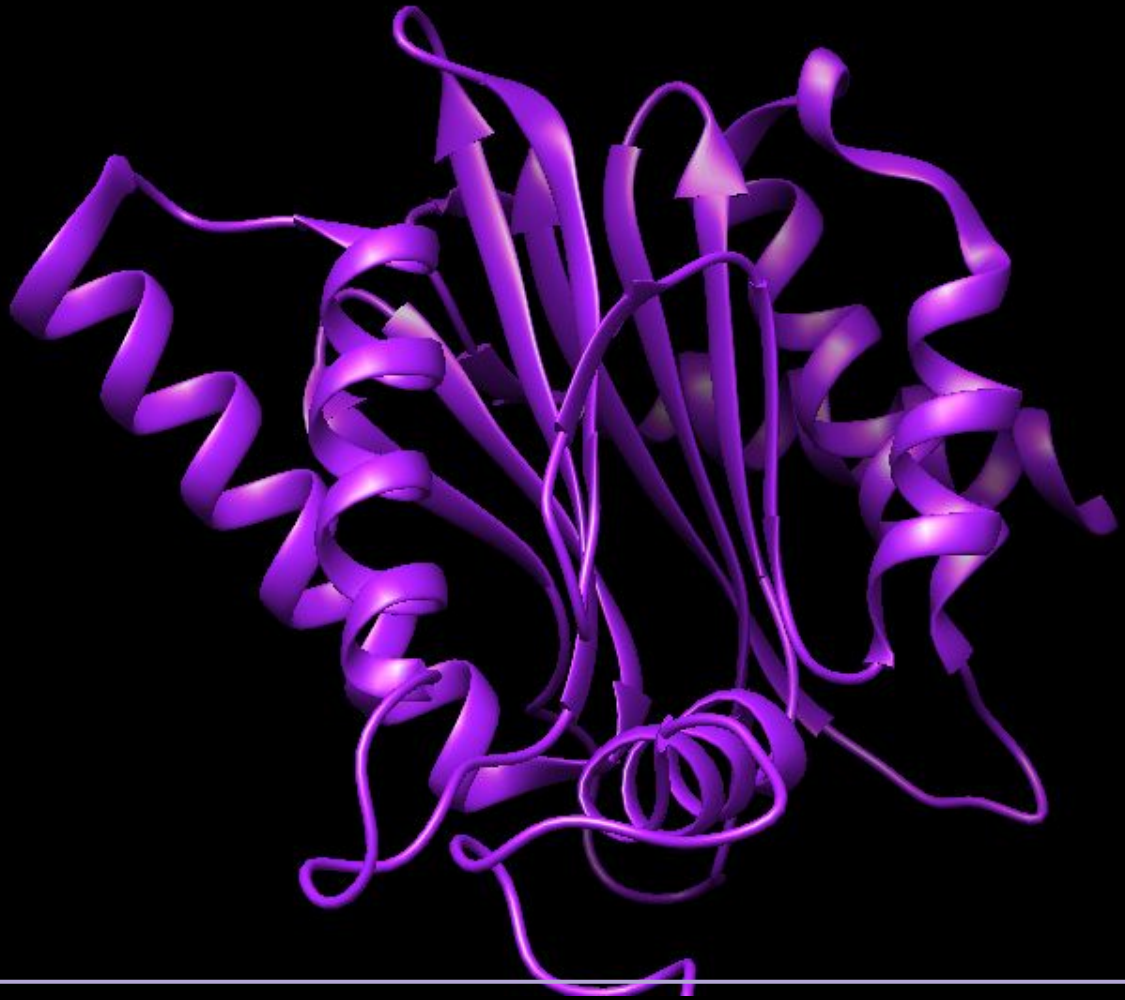
ALPHA 1



ALPHA 2



ALPHA 3



ALPHA 4

ALPHA 5

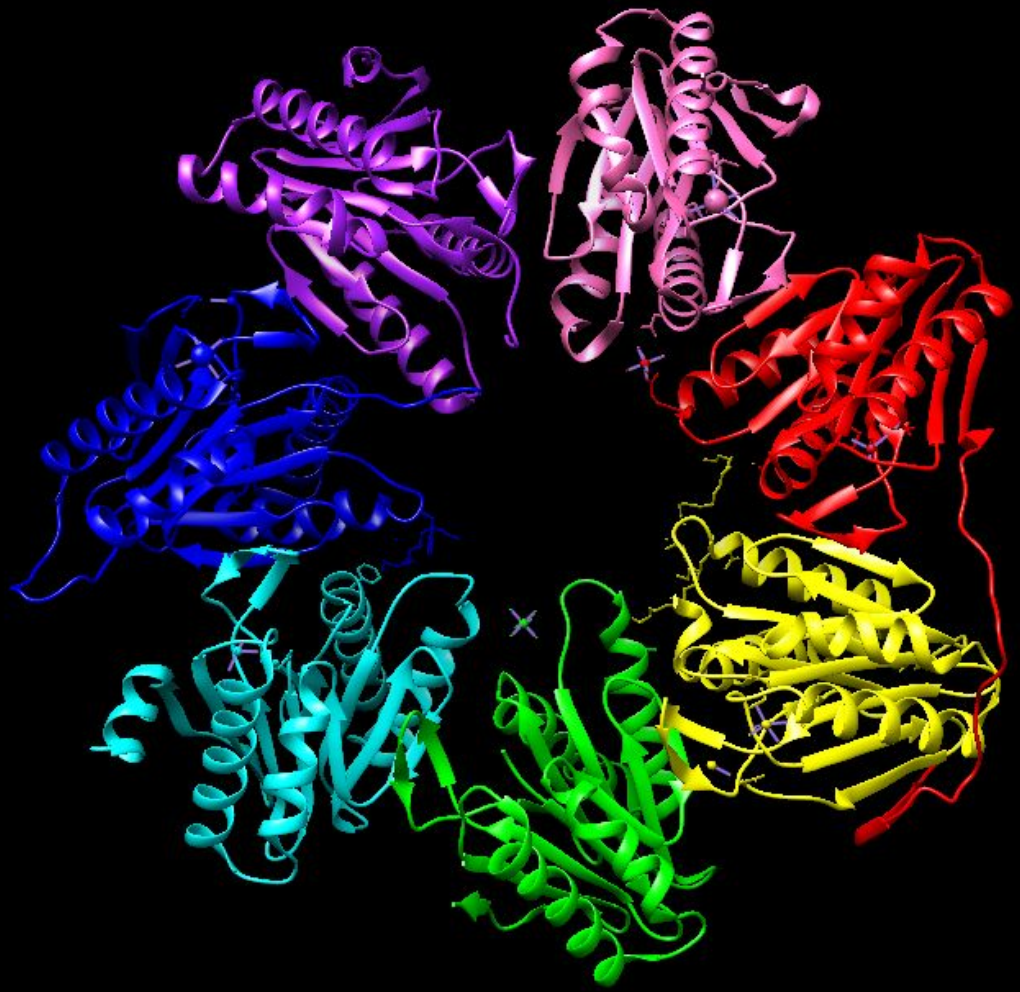
ALPHA 6

ALPHA 7

SUPERIMPOSITION ALPHA RING

Alignment score	8.47
RMSD	1.21

BETA RING



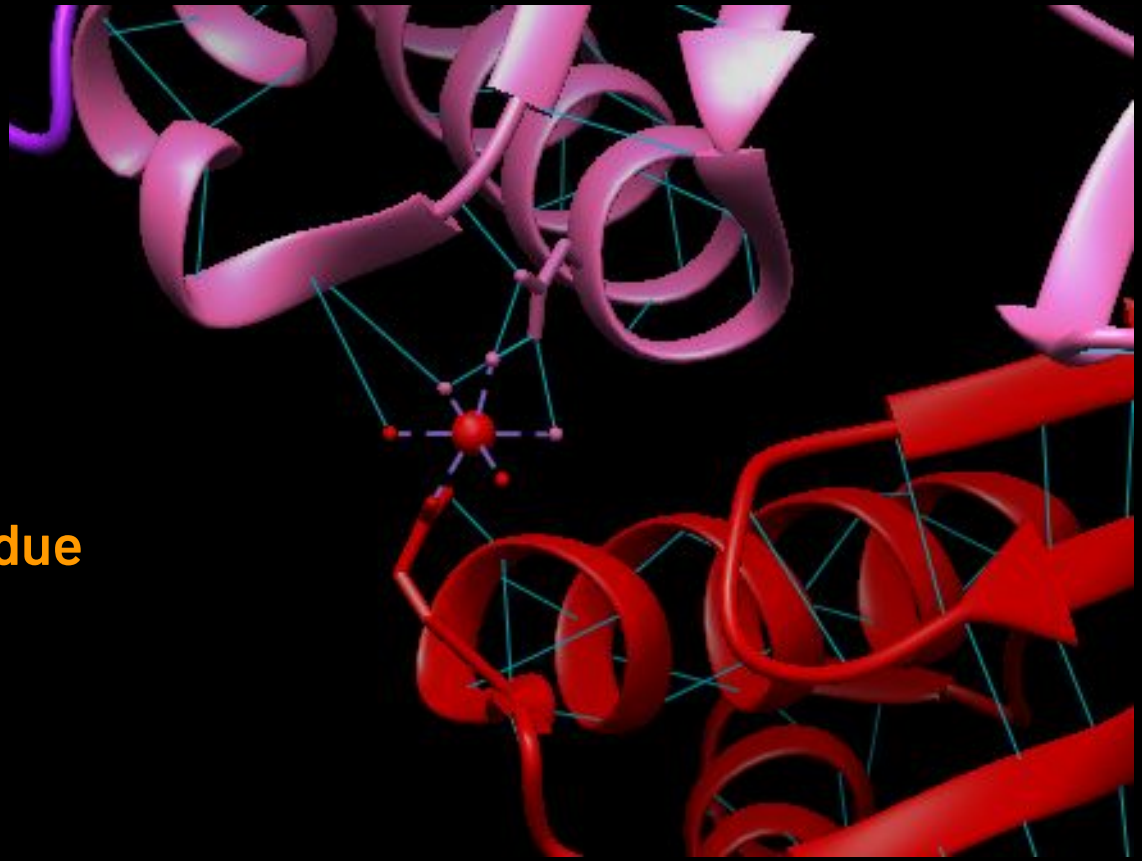
Mg^{2+} interaction

Positively charged

⬇

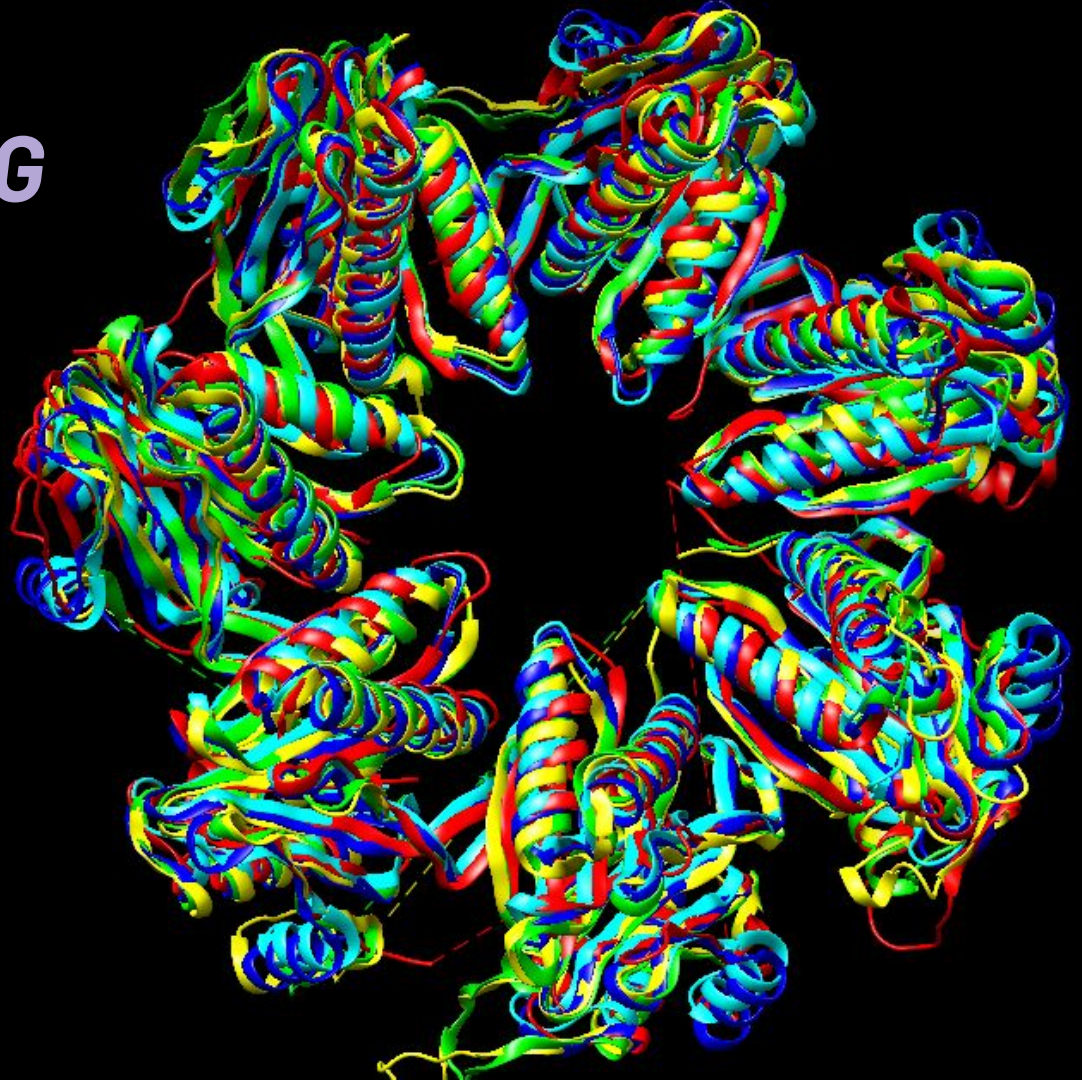
Negatively charged residue

Asp or Glu



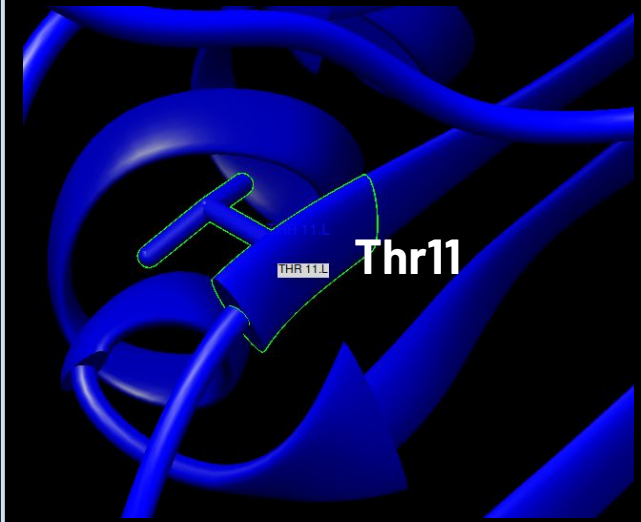
BETA RING AMONG SPECIES

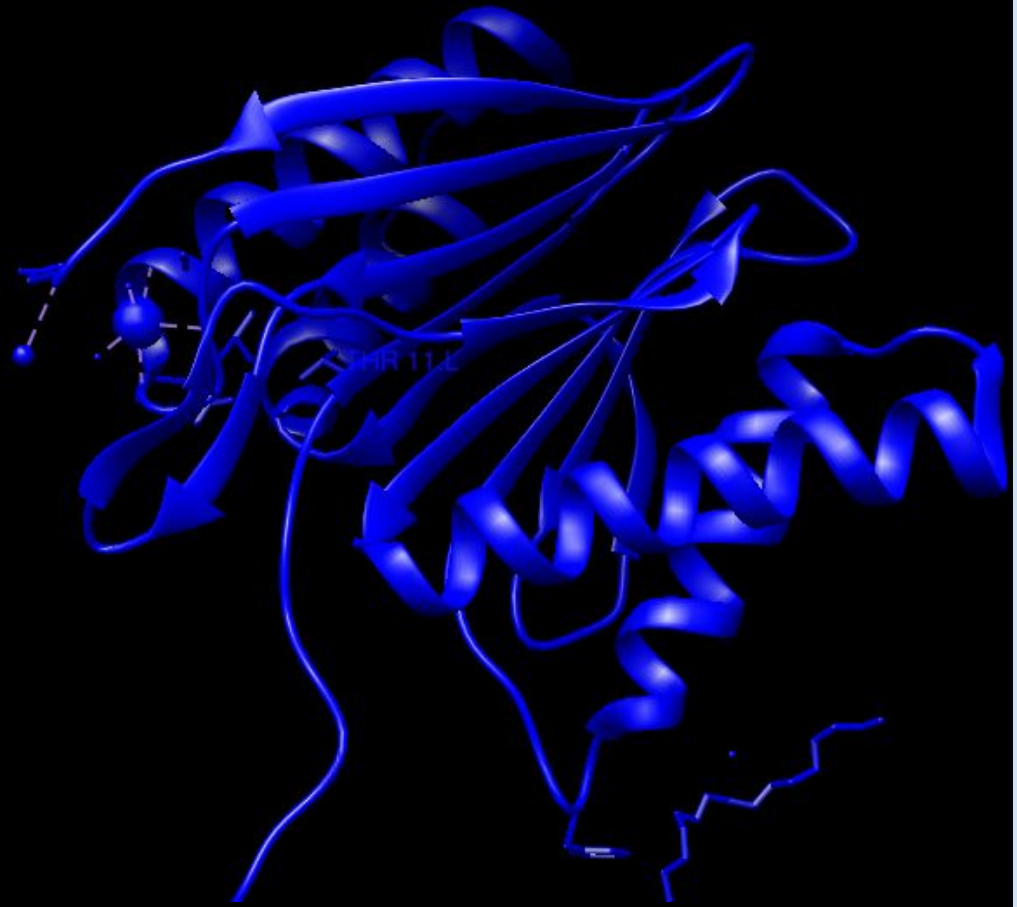
Alignment score	8.73
RMSD	1.70



BETA 1

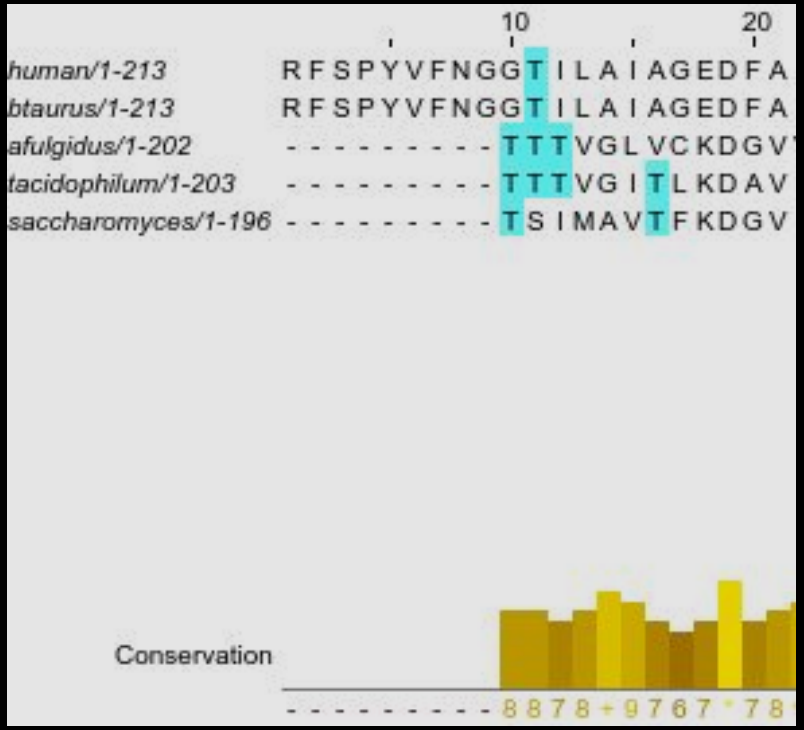
Caspase-like
catalytic activity





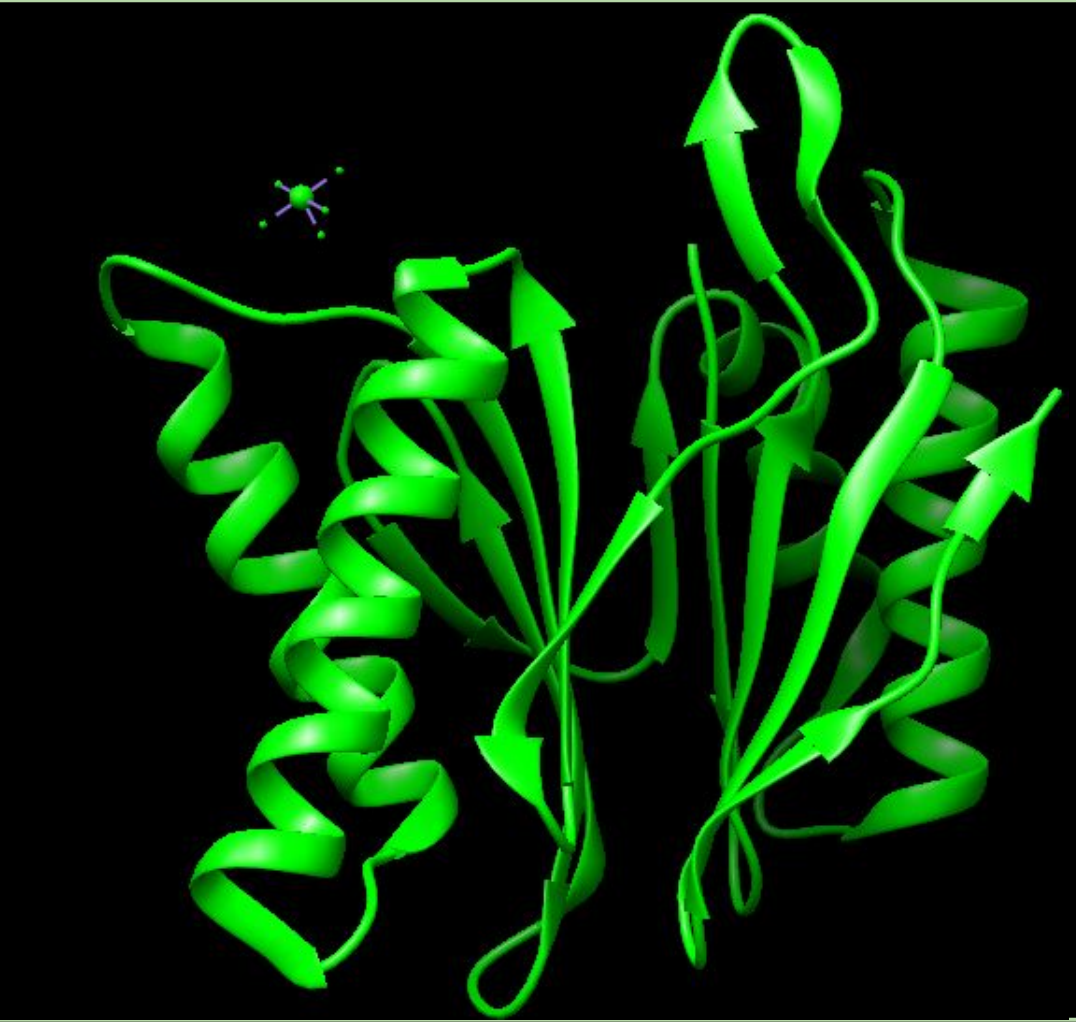
BETA 1

Threonine residues are conserved in all the species



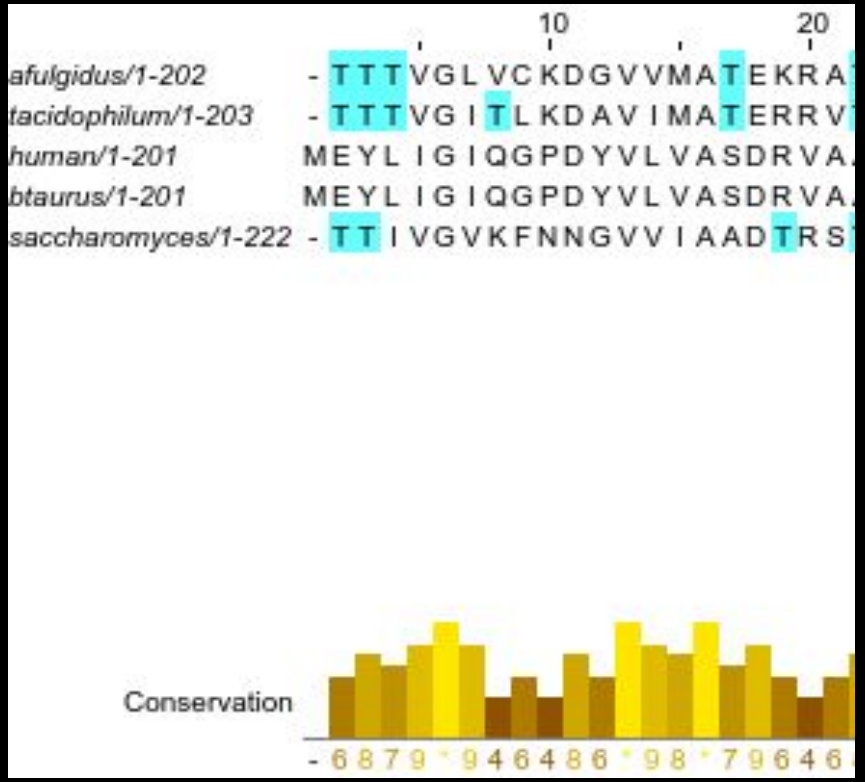
BETA 2

Trypsin-like
catalytic activity

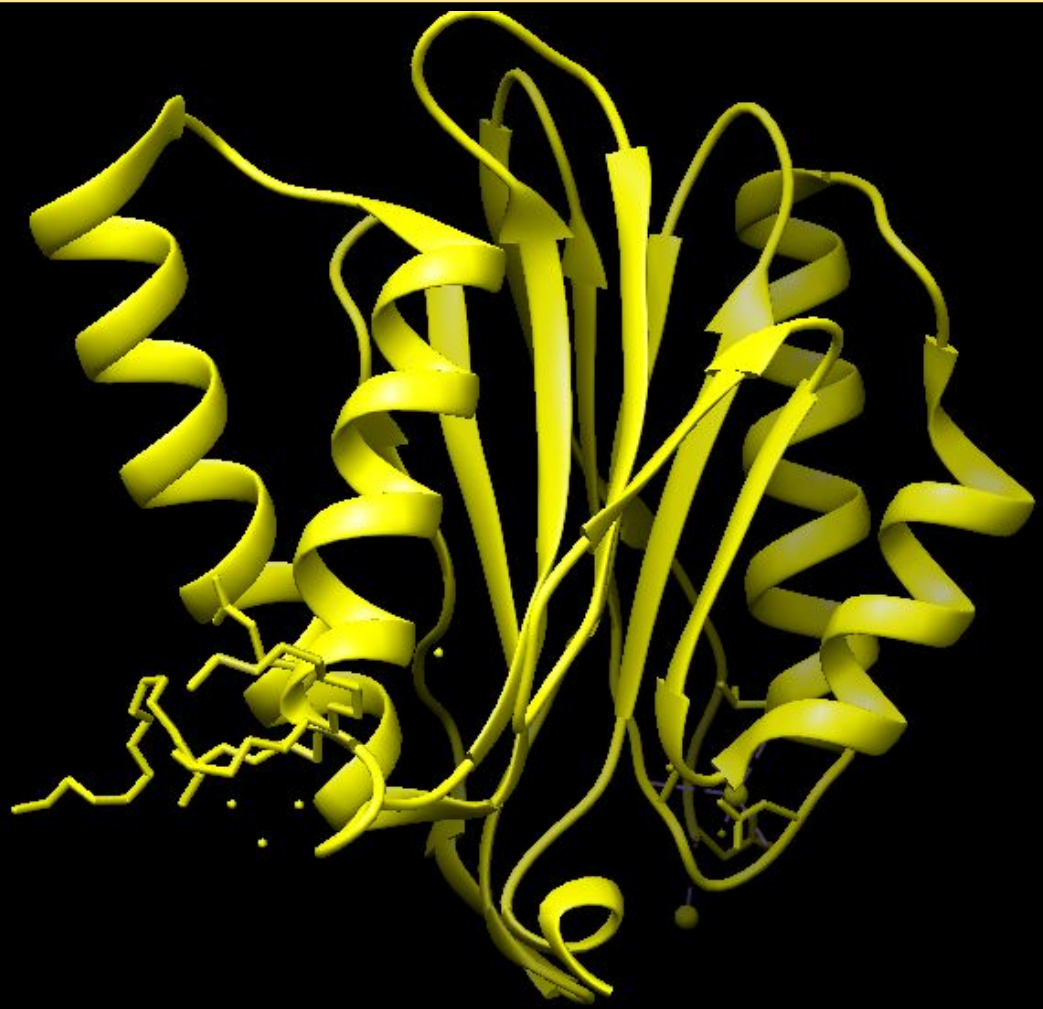


BETA 2

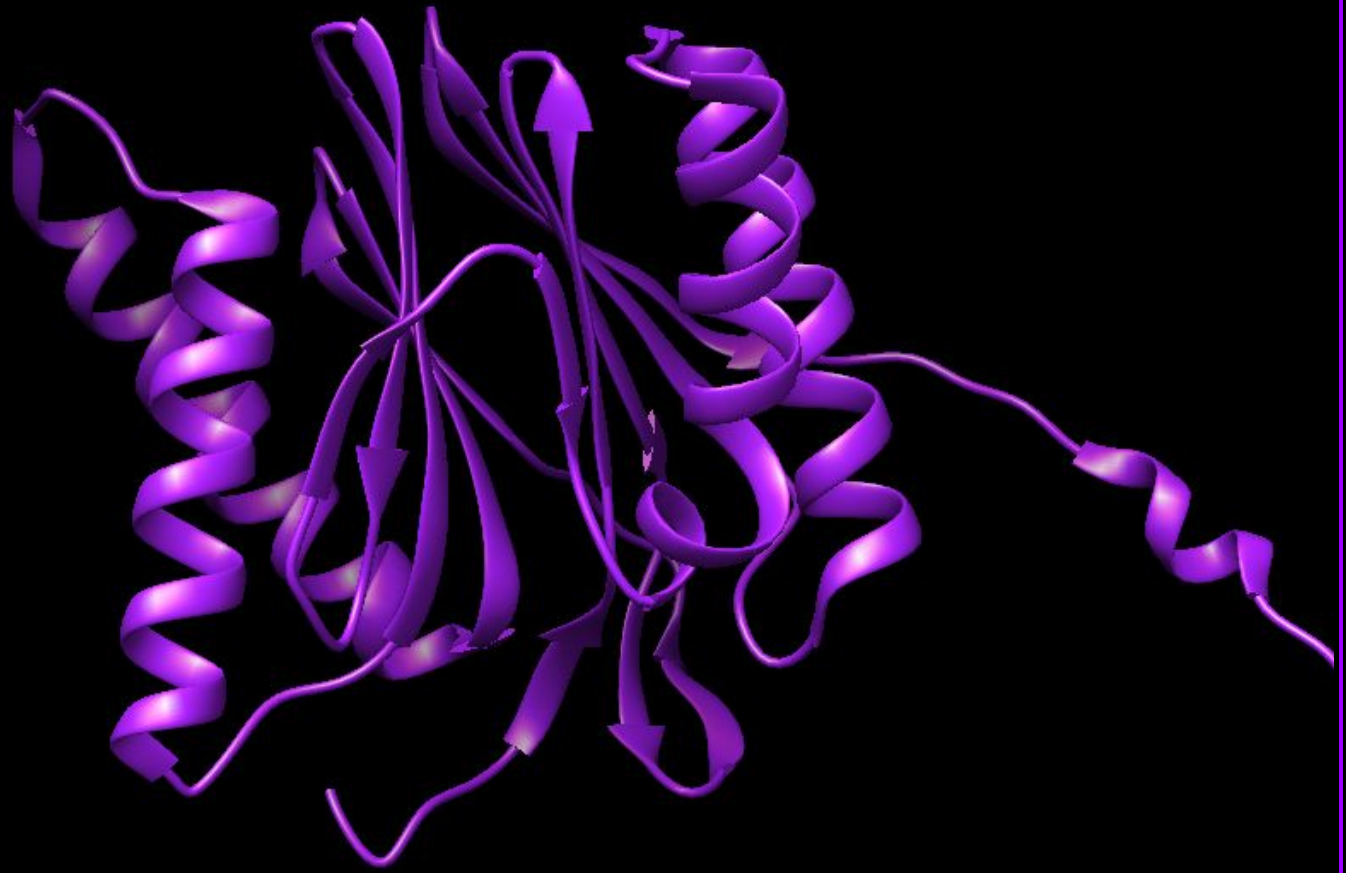
Threonine residues are not conserved in eukaryotes



BETA 3

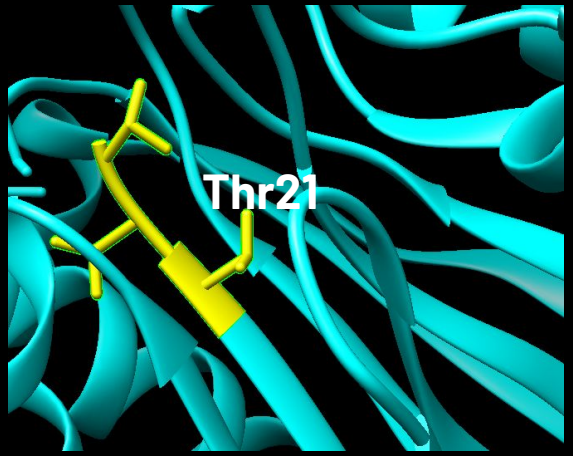


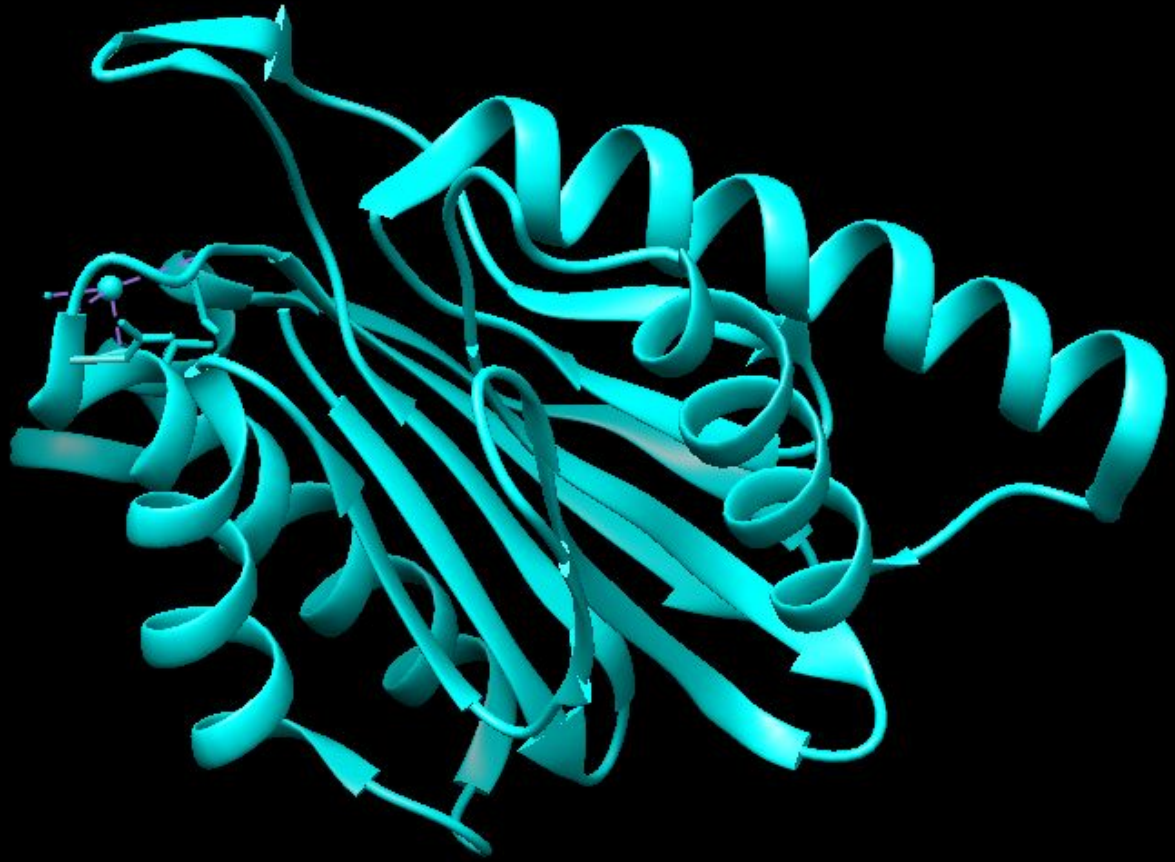
BETA 4



BETA 5

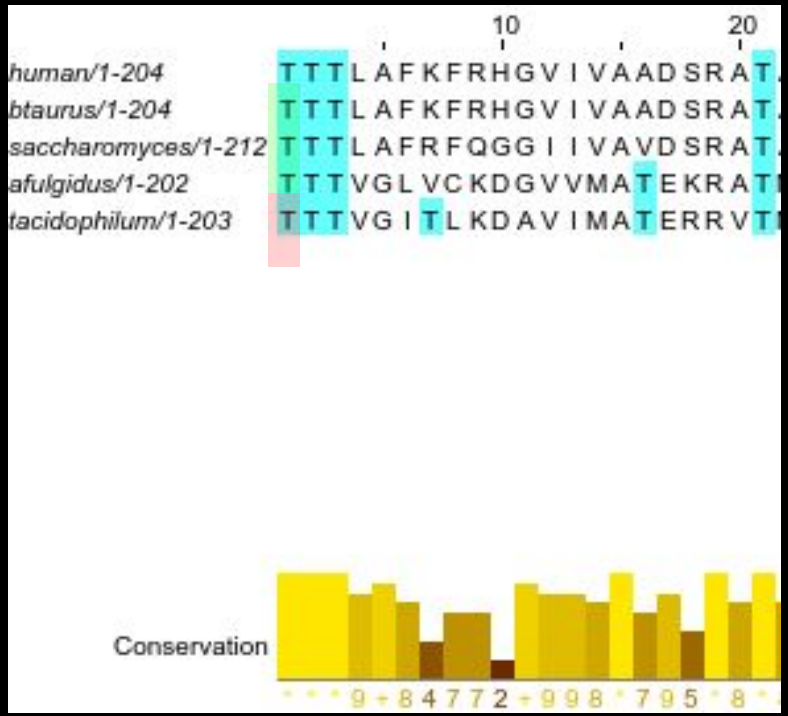
Chymotrypsin-like
catalytic activity



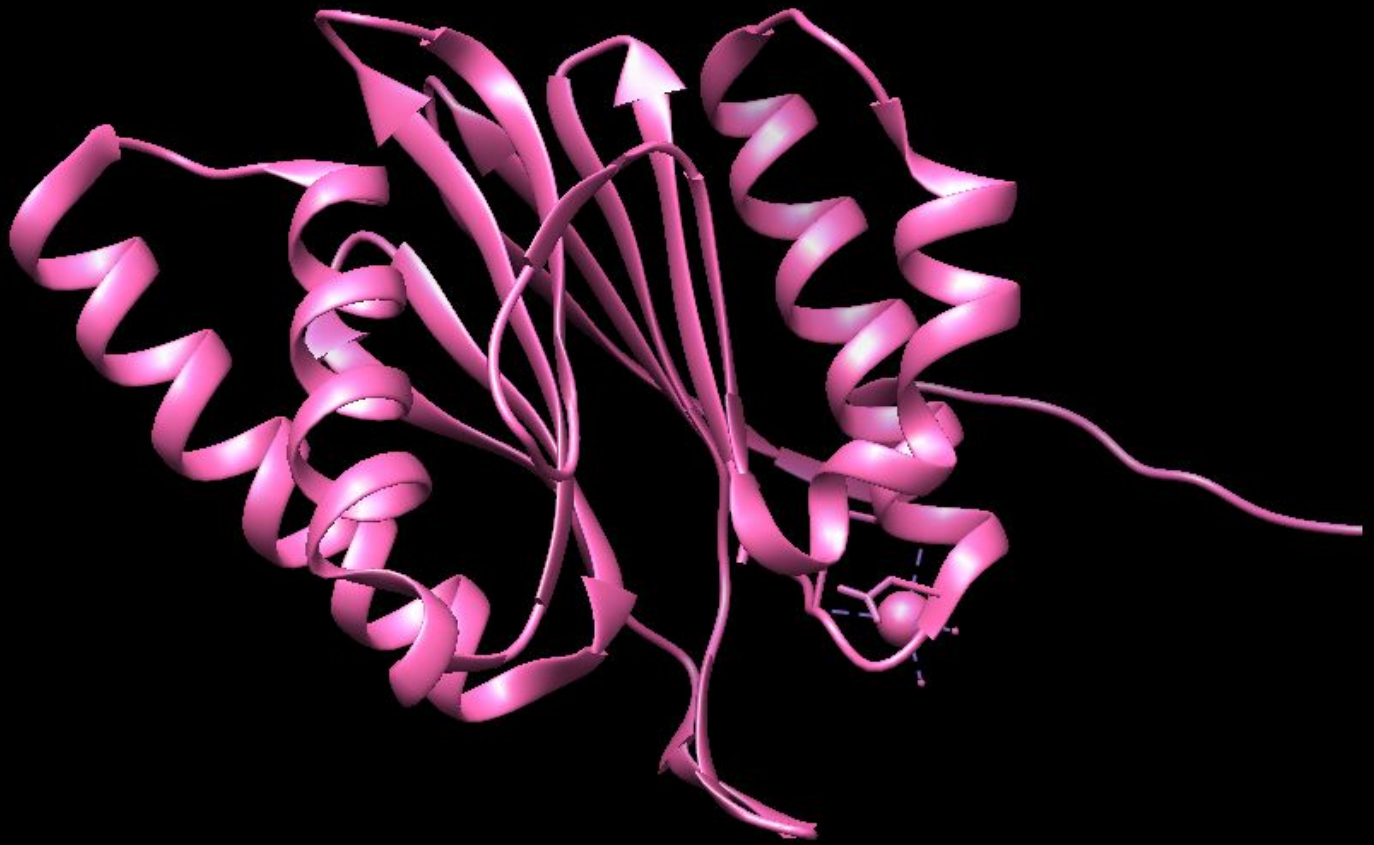


BETA 5

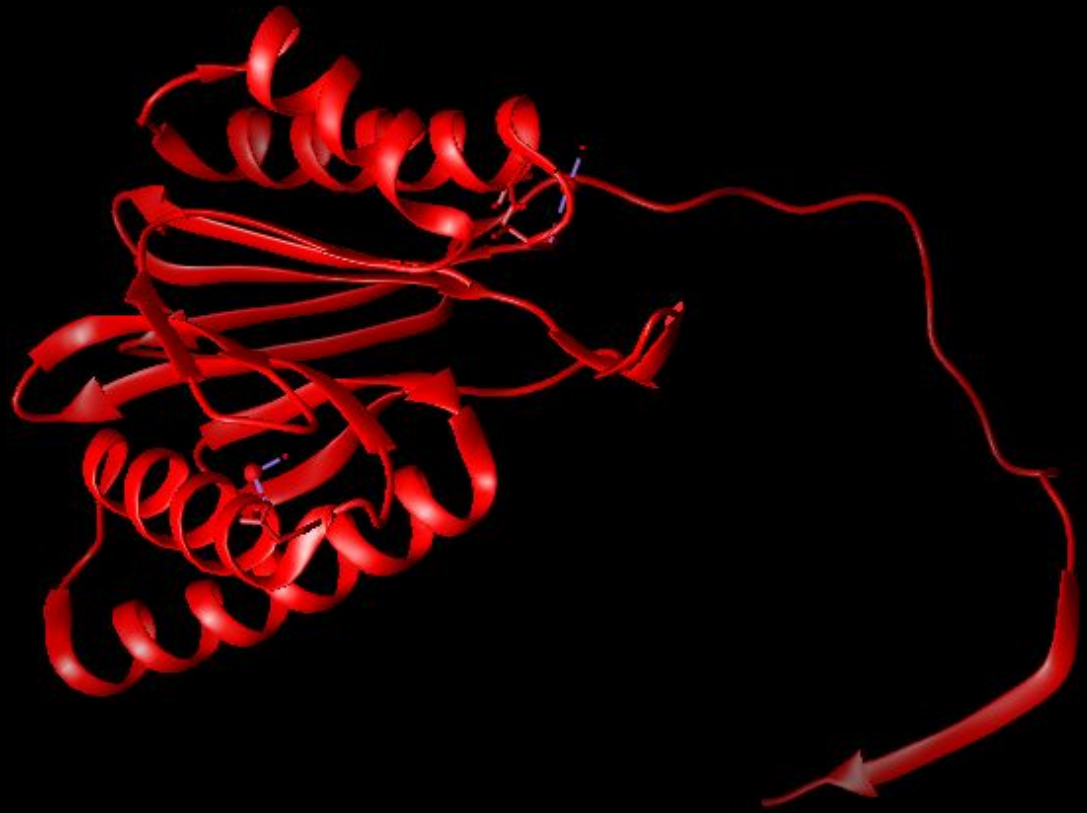
Threonine residues are conserved in all the species



BETA 6



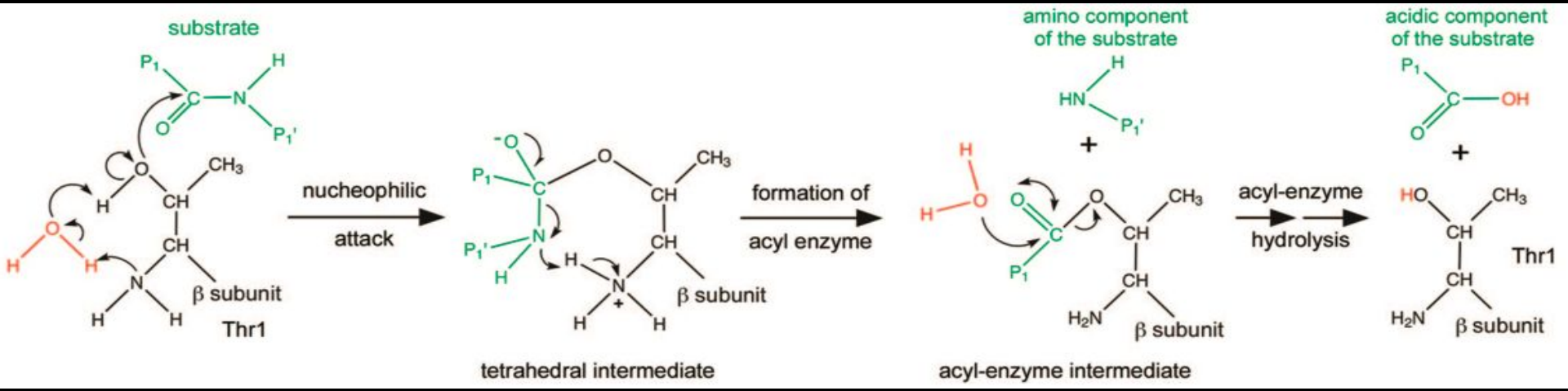
BETA 7

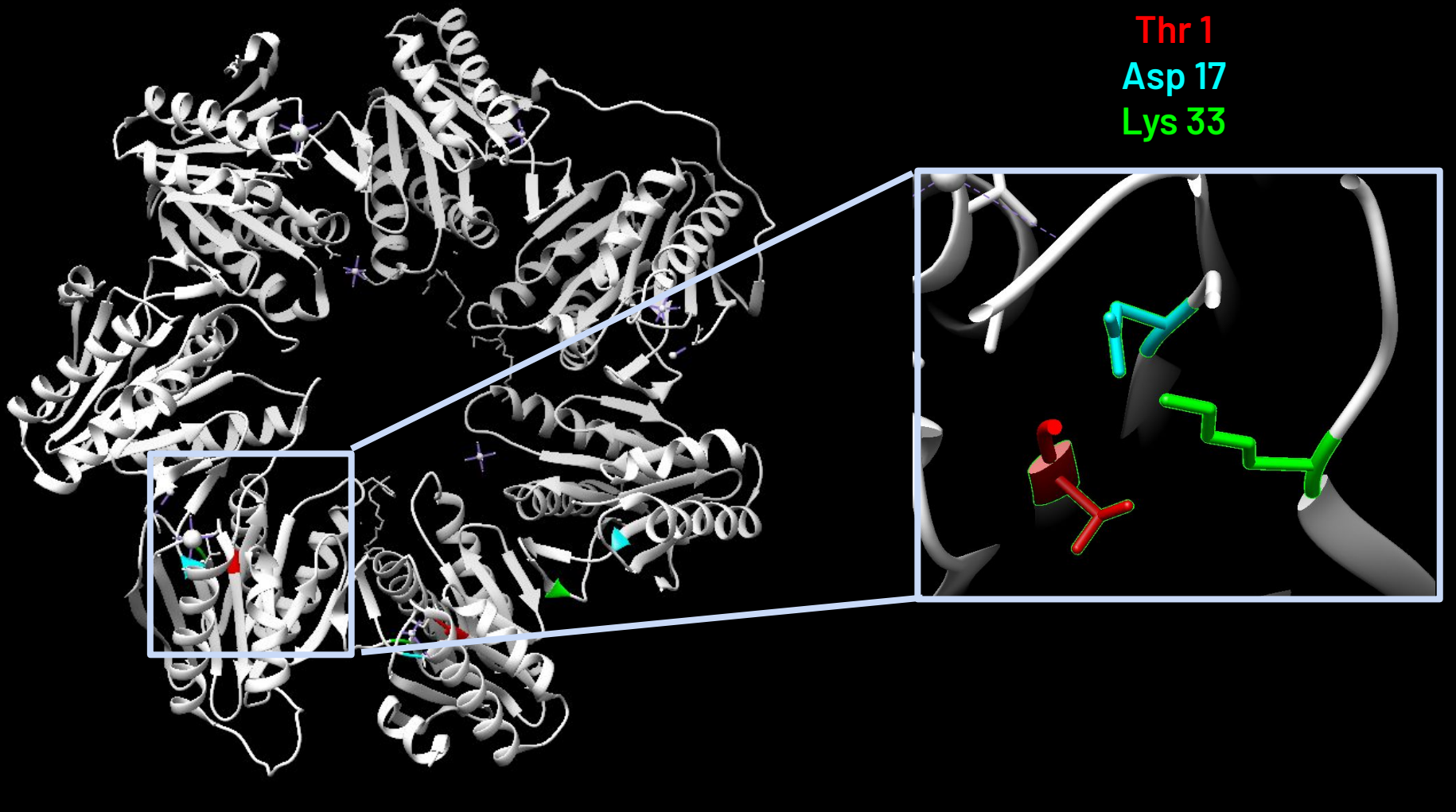


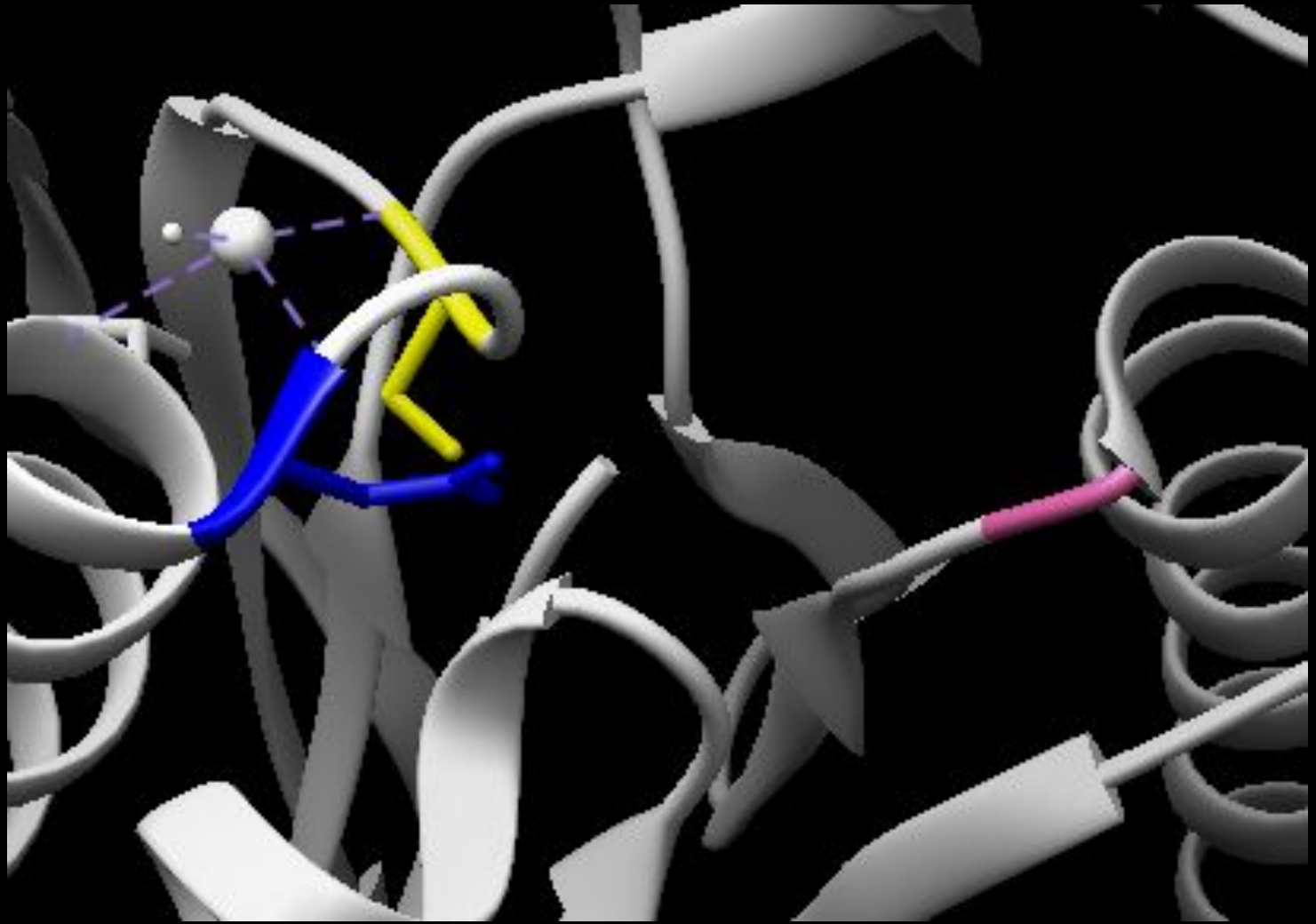
SUPERIMPOSITION BETA RING

Alignment score	8.11
RMSD	1.40

CATALYTIC MECHANISM


substrate
nucheophilic
attack
tetrahedral intermediate
formation of
acyl enzyme
amino component
of the substrate
acyl-enzyme intermediate
acyl-enzyme
hydrolysis
acidic component
of the substrate
β subunit
Thr1


Thr 1
Asp 17
Lys 33



SUBSTRATE PROTEOLYSIS

19S

↓

20S

Alpha

Beta

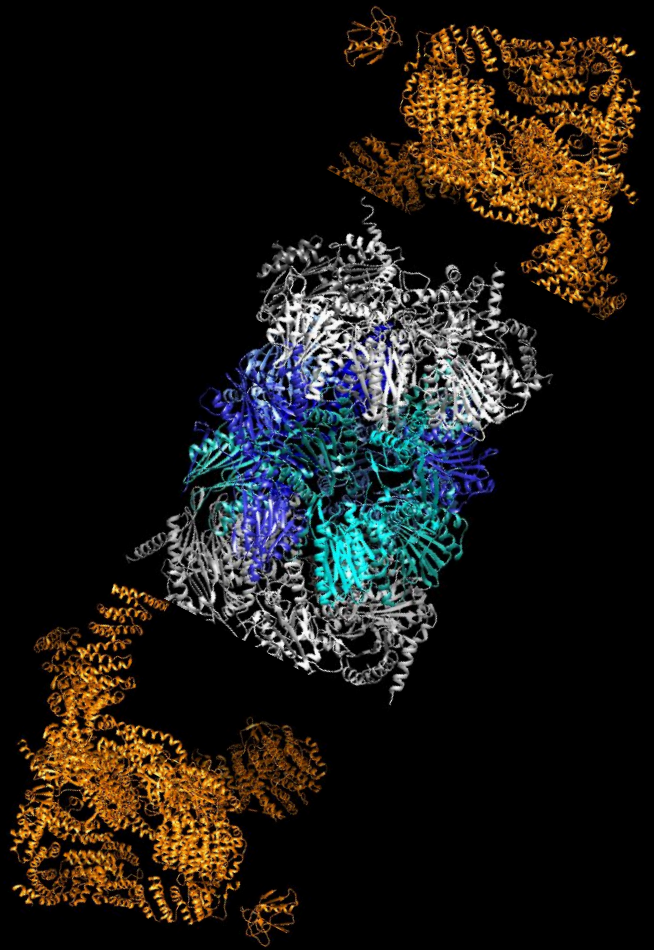
Recognition

↓

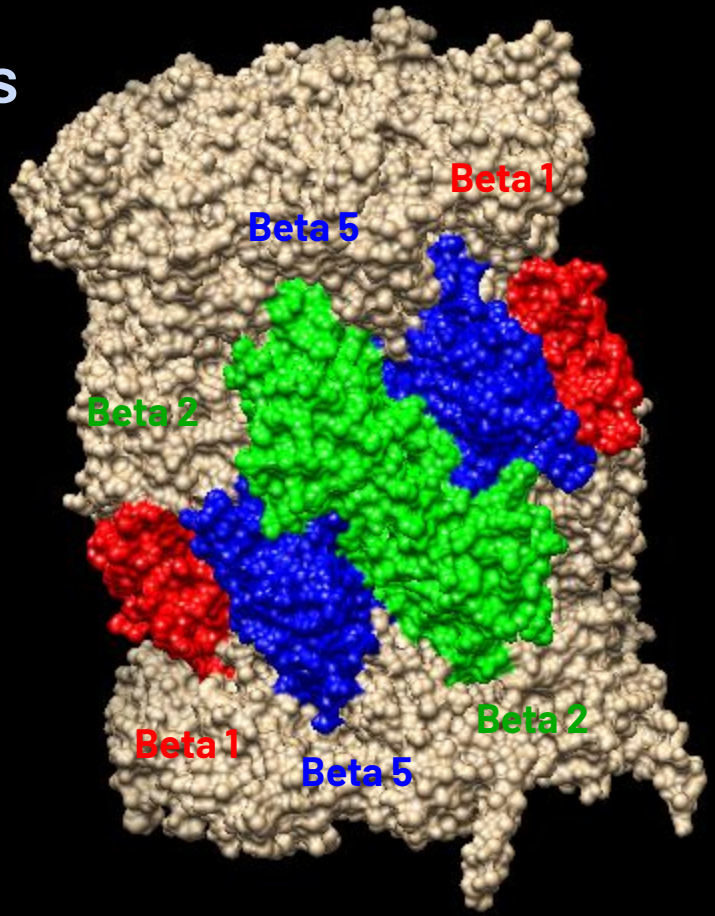
Translocation

↓

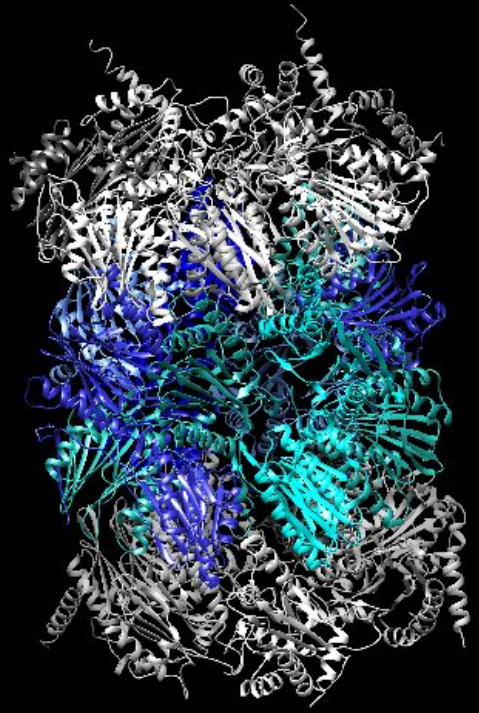
Degradation



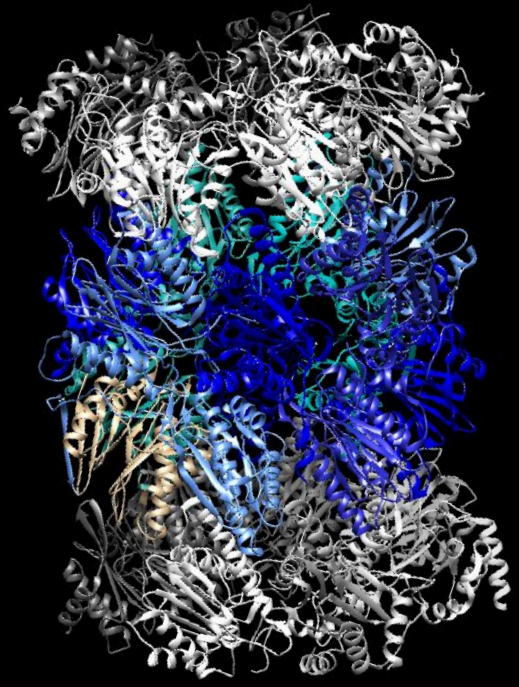
Hydrolisation sites



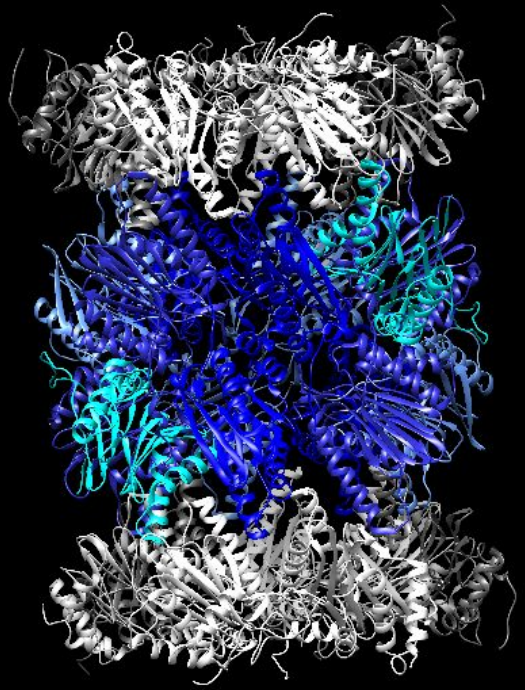
TYPES OF PROTEASOME



CONSTITUTIVE



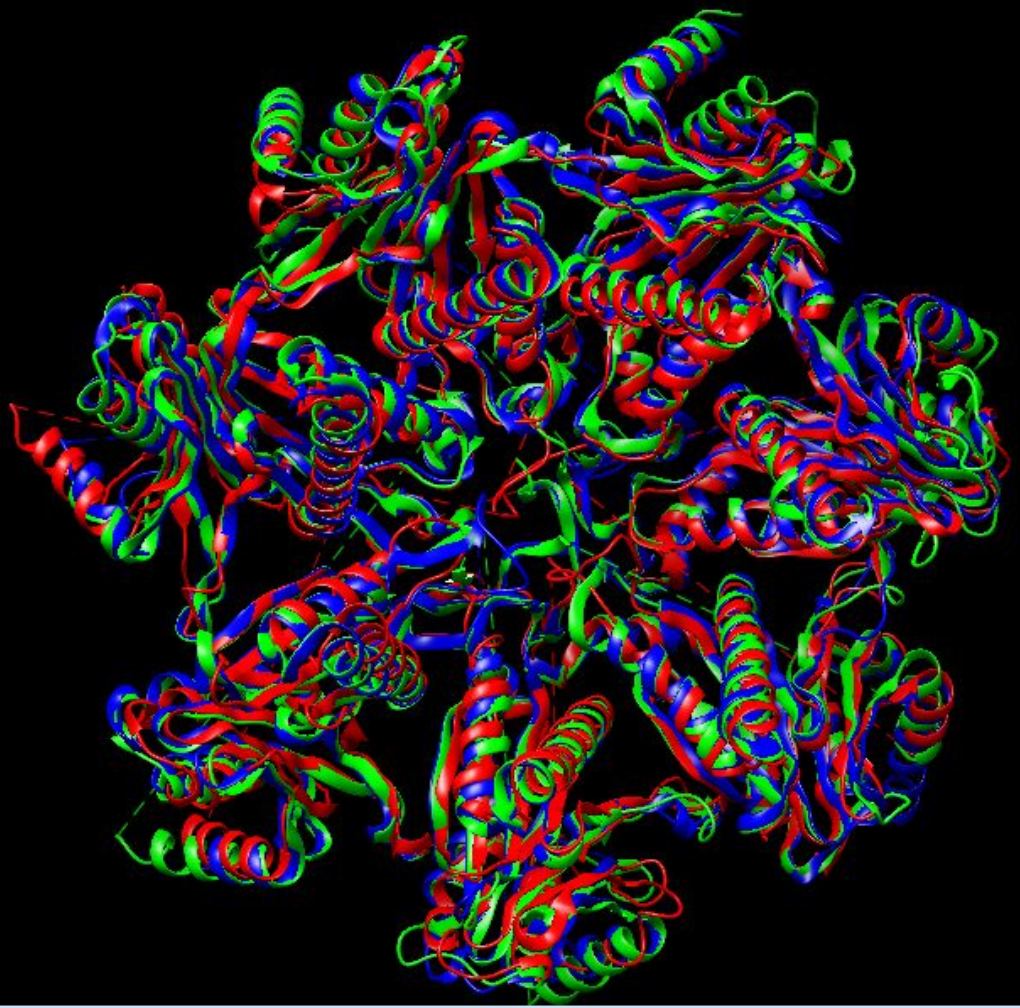
IMMUNOPROTEASOME



THYMOPROTEASOME

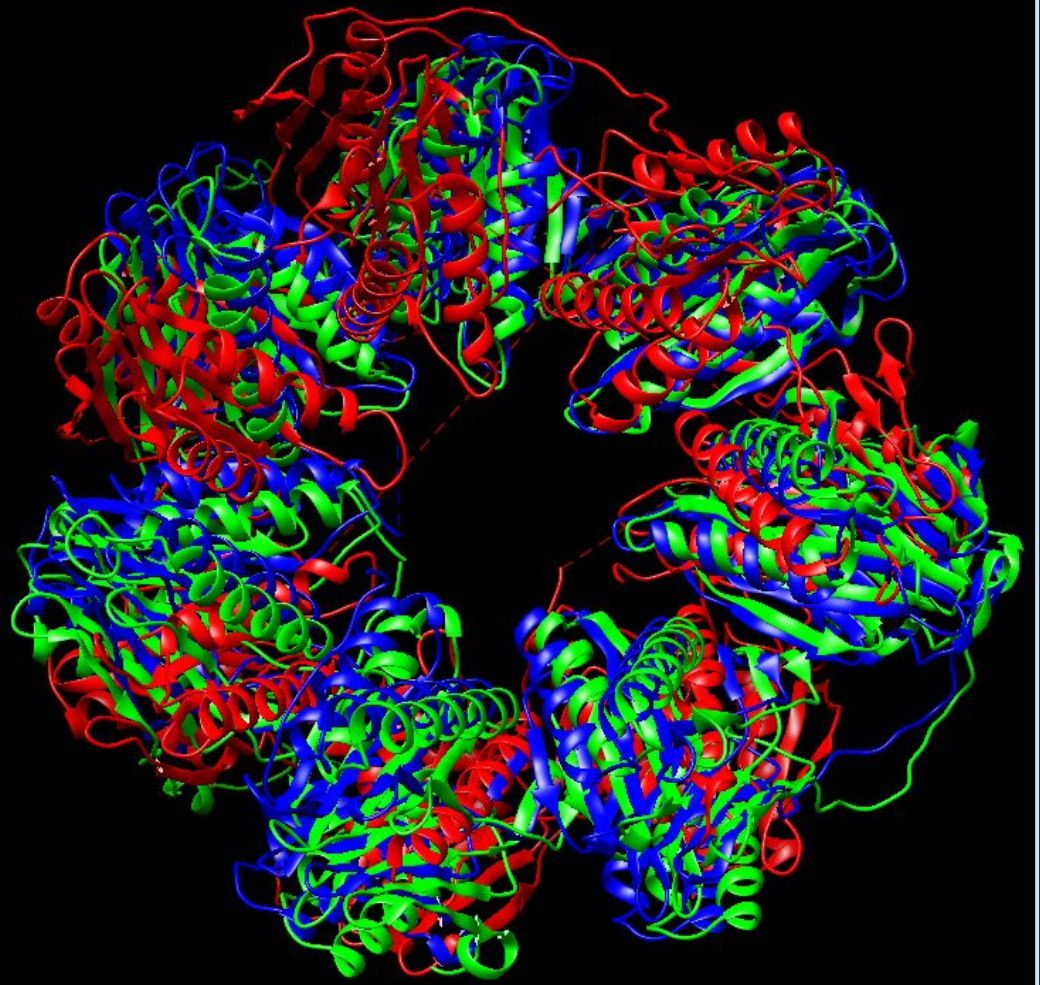
ALPHA RING

ALIGNMENT	SCORE	RMSD
IMMUNO VS THYMUS	8.32	1.14
VS CONSTITUTIVE	3.89	1.78



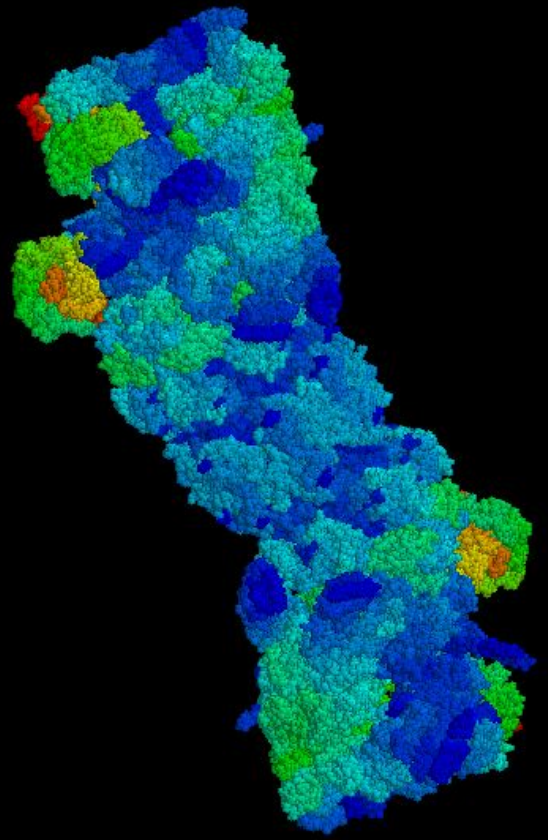
BETA RING

ALIGNMENT	SCORE	RMSD
IMMUNO VS THYMUS	8.78	0.97
VS CONSTITUTIVE	1.98	4.03



CONCLUSIONS

1. Function and importance
2. Structure
3. Gate opening
4. Evolution
5. Complexity



QUESTIONS

Which beta subunits of the proteasome are responsible for substrate proteolysis?

a. beta 1
b. beta 2
c. a and b are correct
d. beta 5
e. all of the above are correct

Which of the following is not a role of the 19S (RP) proteasome?

a. ATP hydrolysis
b. Ubiquitin recognition
c. Unfolding of the substrate
d. Peptide proteolysis
e. Aperture of the gate

About the structure of the proteasome, select the CORRECT one:

a. The 20S (CP) is organized forming a C2 symmetry, and divided in 4 subunits (A,b,b,a)
b. The 26S is the regulatory part of the proteasome
c. The 20S (CP) does not have symmetry and it is divided in 4 alpha rings
d. The proteasome is divided in two different parts, the 26S regulatory and the 19S core particles
e. All the answers are incorrect

Which statement about the catalytic activity of β5 in the proteasome complex is true?

a. β5 cleaves bonds on the amino side of hydrophobic amino acids.
b. β5 cleaves bonds on the carboxyl side of acidic amino acids.
c. β5 cleaves bonds on the carboxyl side of large hydrophobic amino acids.
d. β5 cleaves bonds on the amino side of basic amino acids.
e. β5 does not present catalytic activity

Which protein domain in Rpn13 interacts with ubiquitin by forming a specific groove to accommodate the hydrophobic patch and C-terminal tail of ubiquitin?

a. PRU domain
b. UIM domain
c. vWA domain
d. ATPase domain
e. Non-ATPase domain

What is the main function of the interaction between the C-terminal domains of the ATPases Rpt2, Rpt3, and Rpt5 and the hydrophobic pockets on the axial face of the core particle in the 26S proteasome?

a. Binding to the ubiquitinated substrates
b. Inducing gate opening to allow substrate passage into the proteolytic chamber
c. Facilitating ATP hydrolysis to provide the energy required for proteolytic activity
d. Establishing asymmetric interactions with the hexameric Rpt ring and the heptameric α ring of the proteasome
e. Hydrolysis of peptides

What is the function of the HbYX motif found in the C-terminal tails of ATPases Rpt3, Rpt2, and Rpt5?

a. Substrate recognition
b. Unfolding of the substrate
c. Inducing gate opening in the 20S core particle
d. Catalyzing the hydrolysis of ATP
e. Anchoring the ATPases to the proteasomal core

What type of catalytic activity does subunit beta 2 (β2) of the proteasome exhibit?

a. Caspase-like activity
b. Trypsin-like activity
c. Chymotrypsin-like activity
d. Metalloprotease activity
e. Subunit beta 2 does not present catalytic activity

Which statement regarding the proteasome is incorrect?

a. The proteasome is primarily responsible for protein degradation in eukaryotic cells.
b. The N-terminal threonine residues of the beta-subunits act as nucleophiles in peptide bond hydrolysis.
c. The outer part of the proteasome is composed of hydrophilic residues, while the inside part is composed of hydrophobic residues.
d. The 19S regulatory particle directly interacts with the catalytic sites of the proteasome to facilitate substrate degradation.
e. The proteasome contains only one type of catalytic site, located on the β1 subunit.

Which of the following statements about the catalytic mechanism of the proteasome is incorrect?

a. The proteasome belongs to the family of N-terminal nucleophilic (Ntn) hydrolases.
b. The catalytic mechanism involves a conserved threonine residue on the N-termini of all active β-subunits.
c. The free N-terminal Thr1 deprotonates the Thr1 hydroxyl group to generate a nucleophilic Thr10 for peptide-bond cleavage.
d. Proteasomes cleave peptide bonds by a mechanism in which a hydroxyl group of the N-terminal threonine serves as the catalytic nucleophile.
e. The catalytic mechanism of the proteasome does not involve interactions with aspartic acid and lysine residues for catalytic activity and structural integrity.

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