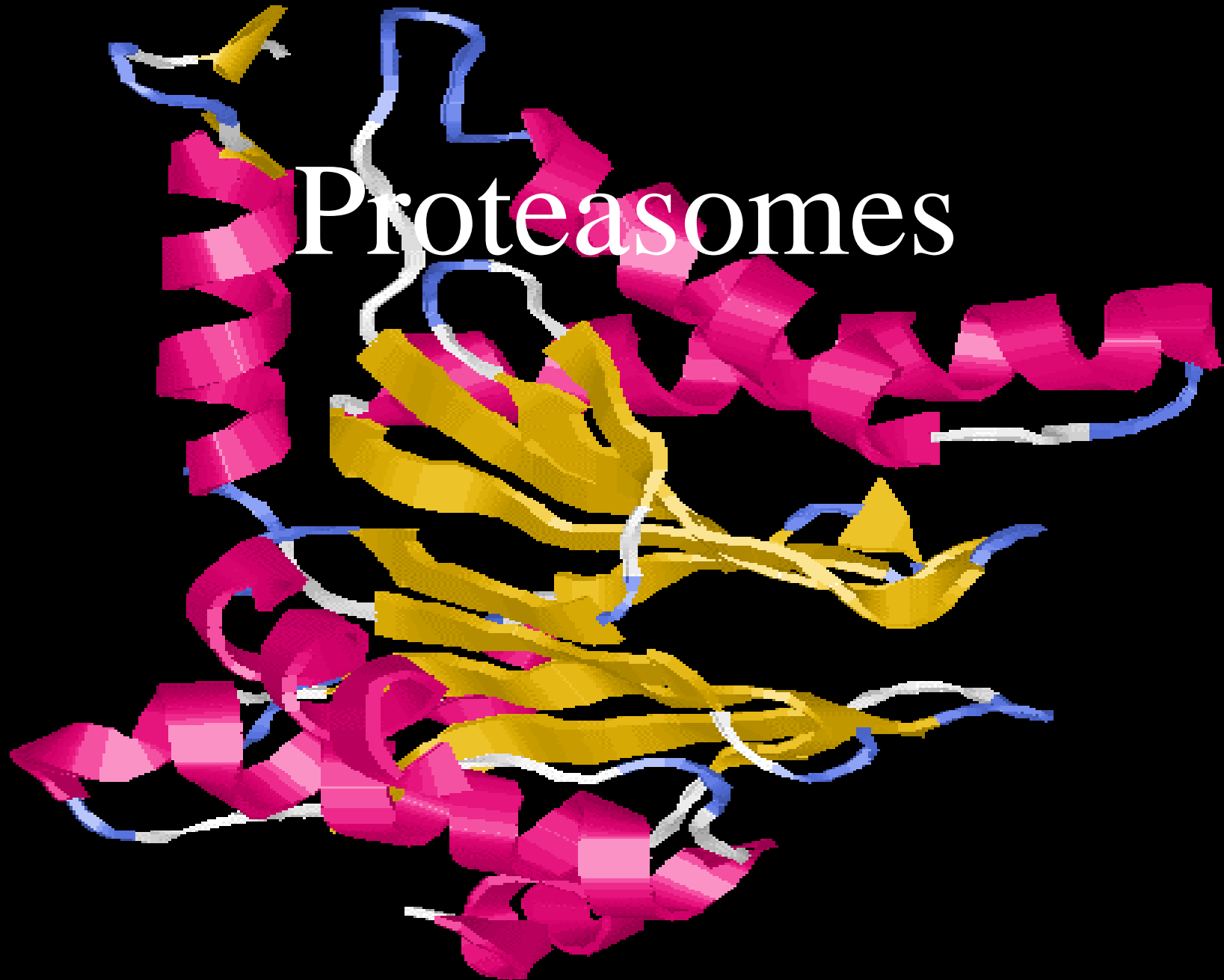


Proteasomes

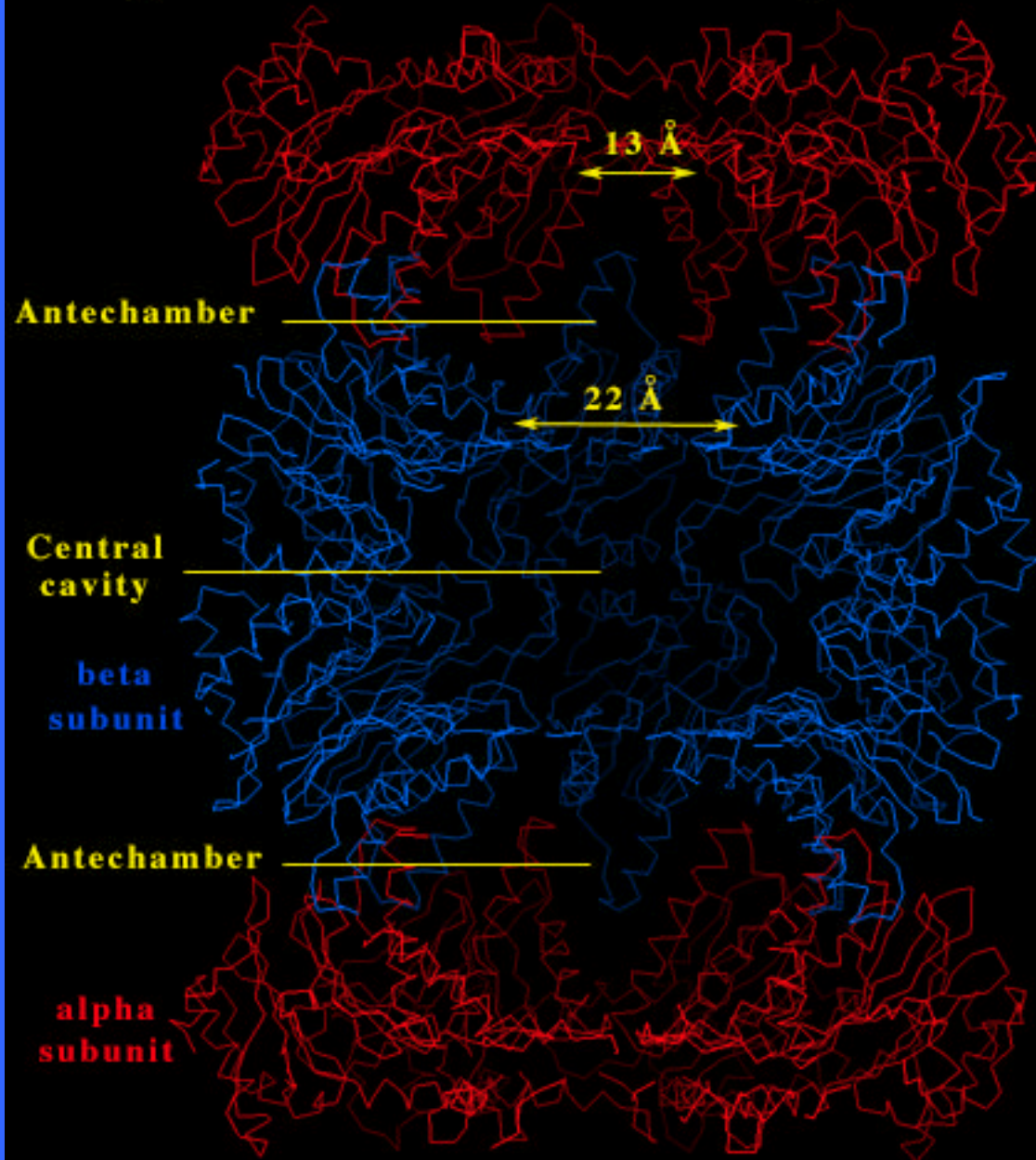


Proteasomes

- Proteasomes are responsible for degrading proteins that have been damaged, assembled improperly, or that are of no profitable use to the cell. The unwanted protein is literally chopped up into fragments that are further broken down into single amino acids by various enzymes.

- There are approximately 30,000 proteasomes in a typical human cell
- Each proteasome is approximately 700 kDa in size
- The proteasome is made up of 3 major components, a **20S unit** and two **19S subunits**, to compose the total **26S** proteasome.

Longitudinal section of the 20S proteasome



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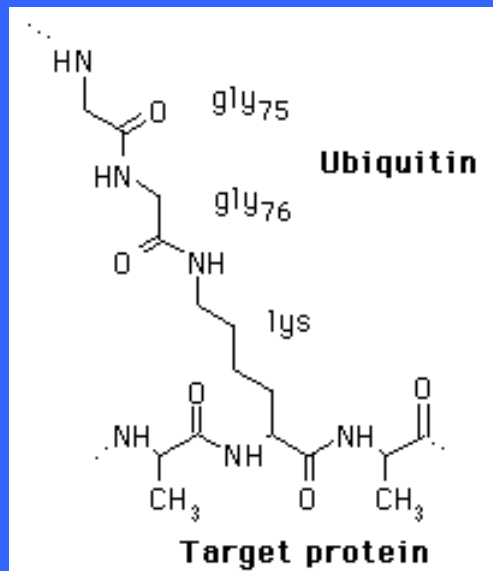
- The **20S** core unit is composed of 28 subunits that are arranged as stacks of 4 disks
 - 2 Beta disks and 2 Alpha disks
 - Each disk is composed of 7 subunits

This constitutes the degradation chamber of the proteasome, giving it its multicatalytic, multisubunit characteristics.

- The two **19S** subunits are responsible for modulating the activity of the central **20S** unit.
- The **19S** subunits can include ATPase, isopeptidase and proteins that are thought to be responsible for unfolding the protein substrates. These also serve to act as “caps,” keeping unwanted proteins from entering the **20S** complex.
- The protein is then fed into the **20S** unit to be degraded.

Which proteins get degraded?

- Proteins that are degraded are first labeled with the protein **ubiquitin**.



- **Ubiquitin** consists of only 77 amino acids and is found universally in all eukaryotic cells.
- It is linked to the target protein, in an ATP dependent process, through several steps using the enzymes **E1, E2, and E3**.

Protein labeling

- **E1** binds free ubiquitin and activates it
- It then transfers it to **E2**
- **E3** then binds the activated ubiquitin to the target protein via formation of an amide bond with its carboxy-terminal glycine residue linked to the lysine side chain of the target protein.

E3-Ubiquitin binding

- There are hundreds of different and distinct **E3** complexes that recognize specific amino acid sequences of target proteins.
- This is accomplished by various **F-box proteins** that bind to the **E3** enzyme, enabling it to facilitate ubiquitin binding to the target protein.

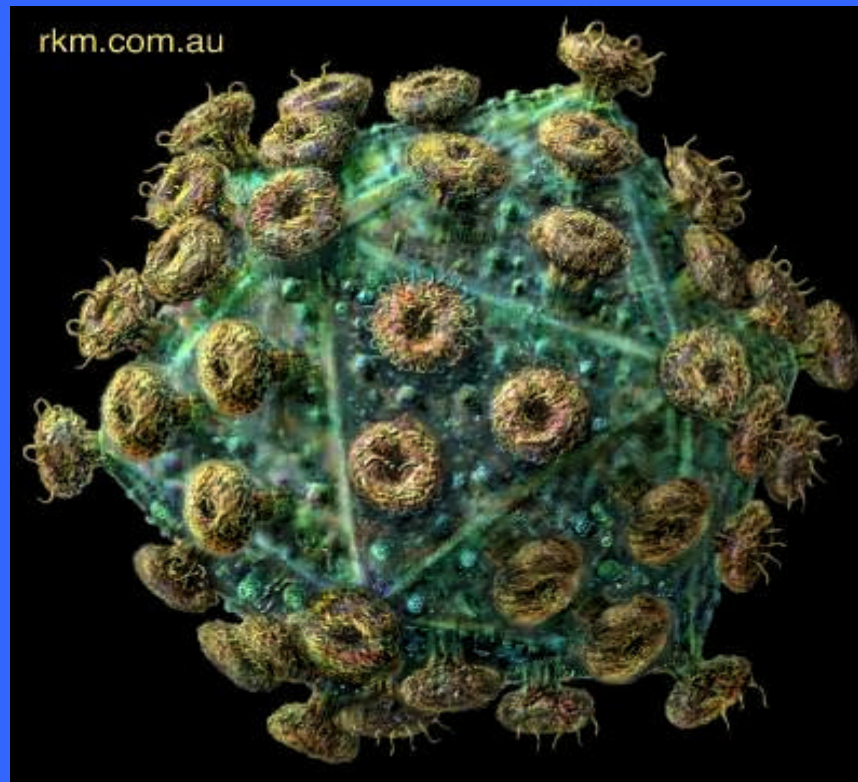
- The **E3** enzyme acts as the “socket wrench” and the **F-box proteins** are the “sockets.”
- This enables the **E3** enzyme to exhibit the degree of specificity and diversity needed to identify and label hundreds of proteins for degradation.
- The process of ubiquitination of the target protein is repeated until it’s bound to a chain of ubiquitin labeling proteins.
- If only one ubiquitin molecule is added, it will only serve to regulate that protein’s function.

Importance of E3

- **E3** also plays a key role in the body's immune response and cellular division
- Viral proteins that enter the cell are labeled and then degraded by immunoproteasomes. Portions of the virus, 8-10 amino acids long, are joined with MHC class I molecules and fuse with the cell membrane. This assists in the activation of cytotoxic T-cells to destroy the virally infected cell.

- Cancers may also arise when **E3** prematurely labels inhibitory growth proteins, causing cellular replication to proceed at too rapid a rate.

HIV



HIV

- The HIV virus has 3 polyprotein complexes that produce its various components:
 - Gag** – the inner virion core
 - Pol** – the viral enzymes
 - Env** – the glycoproteins of the virion envelope

- HIV needs the proteasome to become active
- One translated in the cell, the newly formed HIV **Gag** polyprotein must undergo cleavage by the proteasome.
- The **Gag** complex is cleaved into the **matrix**, **capsid**, **nucleocapsid**, and **p6^{gag}** proteins.
- This is a necessary step in order to produce mature, infectious viral particles.

- The virus also uses the proteasome for another function....
- The HIV-1 protein **Vpu** utilizes the proteasome-ubiquitin system by labeling CD4 molecules with ubiquitin, causing them to undergo proteolytic degradation.
- This decreases the ability of T-cells to identify virally infected cells as containing foreign antigen, and thus eliminate them.

Proteasome Inhibition

- The three cleaving activities that could be selectively repressed with different proteasome inhibitors are referred to as:
 - Chymotrypsin-like
 - Trypsin-like
 - Peptidylglutamyl peptide-hydrolyzing (PGPH)

- The drug **Ritonavir**, a drug used to inhibit HIV-1 protease, has been shown to inhibit the **chymotrypsin-like** activity, AND to simultaneously enhance the **trypsin-like** activity.



- The reasons for this selectivity are still unknown.

- This up-regulation of the **trypsin-like** activity is not, however, affected by specific active site inhibitors of **chymotrypsin** or **PGPH** activity.
- This suggests that the **chymotrypsin-like** activity and the **trypsin-like** activity of the proteasome do not allosterically interact with each other.

- It has also been found that selectively inhibiting the **chymotrypsin-like** activity and **PGPH** activity does not affect the enhancement of the **trypsin-like** activity by Ritonavir.
- It has been suggested that Ritonavir accomplishes this by binding to an “unidentified modifier site” that is not identical with any of the known active centers on the proteasome.

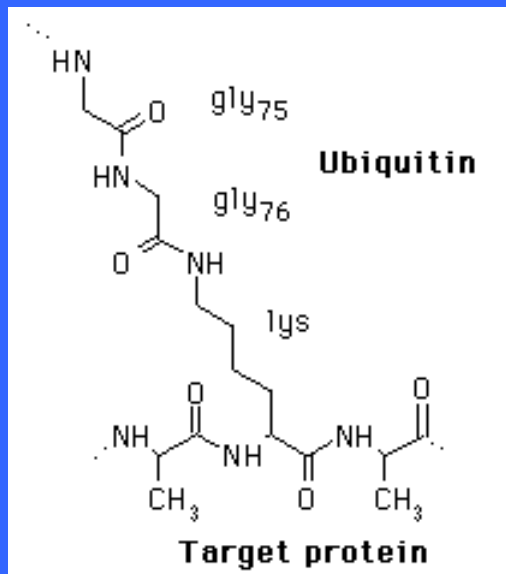
- The theory of there being two binding sites for **Ritonavir**, one at the active center of the proteasome and another “modifier site” elsewhere, suggests that there may possibly be better and more efficient ways to regulate proteasomal activity in the fight against HIV and AIDS.

The End

Possible final exam questions:



name this structure...



True or false?

The major 3 polyprotein complexes of HIV are Gag, Pol, and Vpu, or are they Gag, Pol, and Env?