

CLUTCH

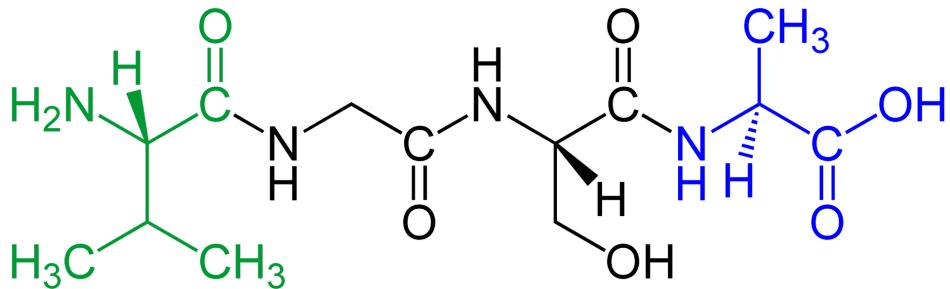
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CONCEPT: PROTEIN BASICS

Protein Structures

- Proteins are made by _____ amino acids
 - A **polypeptide chain** is formed by *peptide bonds* between amino acids
 - A **multimeric** protein is made up of multiple polypeptide chains, each called a **subunit**
 - A **monomeric** protein is made up of a single polypeptide.
 - The **polypeptide backbone** is formed with a repeating sequence of Nitrogen and Carbon atoms (-N-C-C-)
 - The **N-terminus** contains an amino group (NH_3^+) at its end
 - The **C-terminus** contains a carboxyl group (COOH) at its end

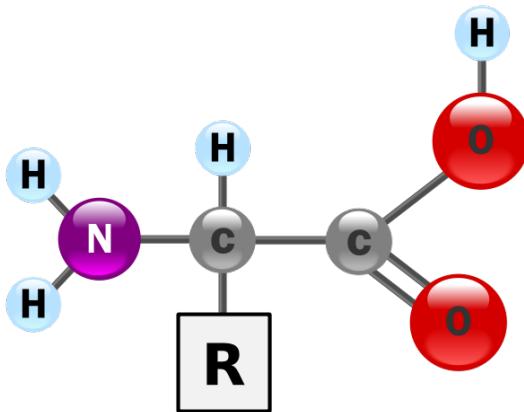
EXAMPLE: A three amino acid polypeptide chain with N and C termini



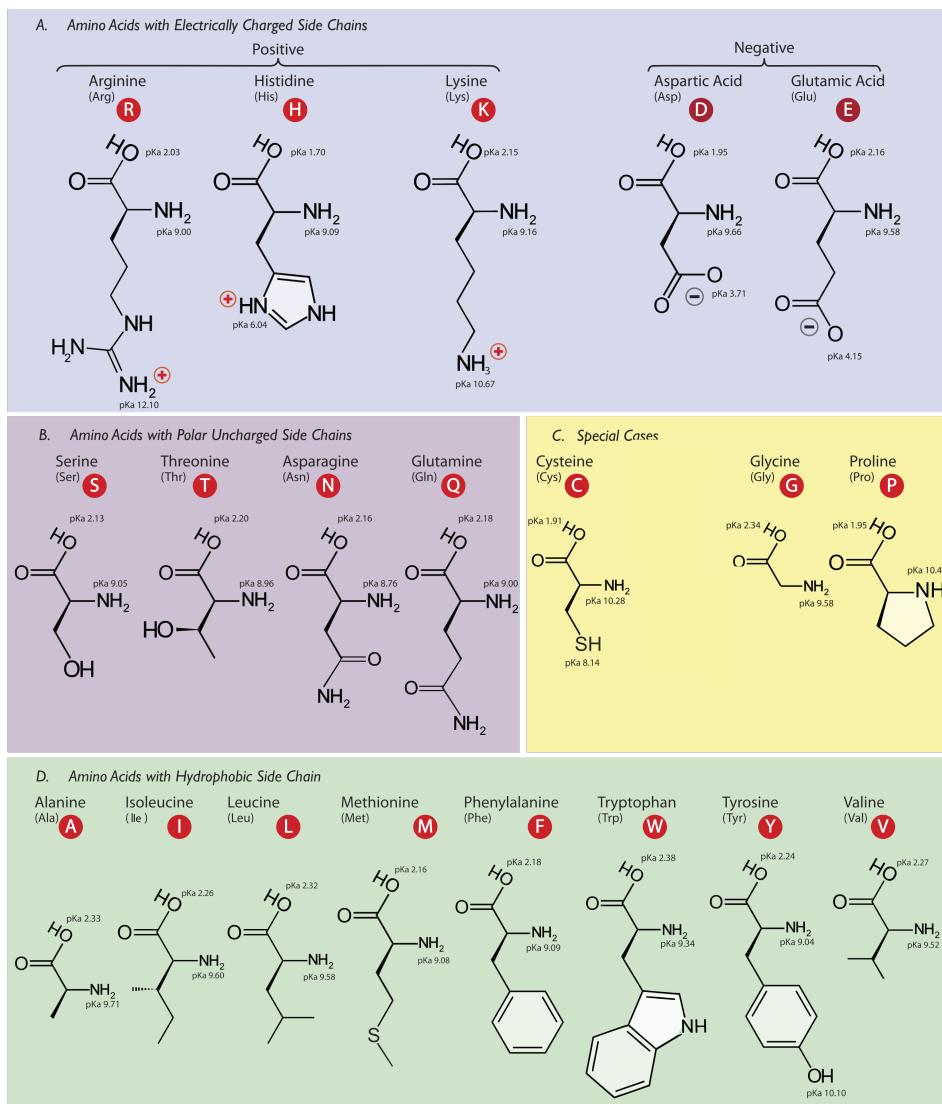
Amino Acids

- Each amino acid in a polypeptide chain has unique properties
 - All amino acids have the same carboxyl group, an amino group, and hydrogen
 - The **R group** is a side chain that differs between amino acids and gives them _____ properties
 - The *polar, charged* group can form ionic bonds with other charged molecules in the cell
 - The *polar, uncharged* group can form hydrogen bonds with other molecules including water
 - The *nonpolar* group cannot interact with water
 - The *other* group consists of three amino acids, each with unique properties that do not fit into other groups
 - Amino acids exist as **stereoisomers** because the four groups are asymmetrically arranged around the α -carbon
 - Two forms D- and L-; BUT the L forms is used in proteins

EXAMPLE: A model of the structure of each amino acid



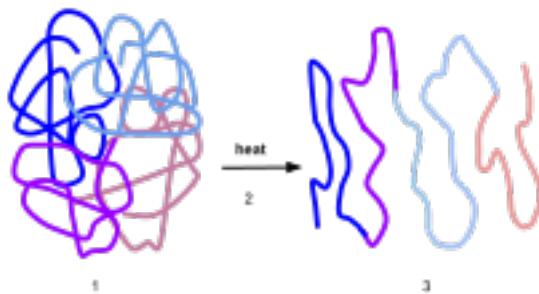
EXAMPLE: The 20 amino acid structures, classified by group



Self-Assembly and Protein Folding

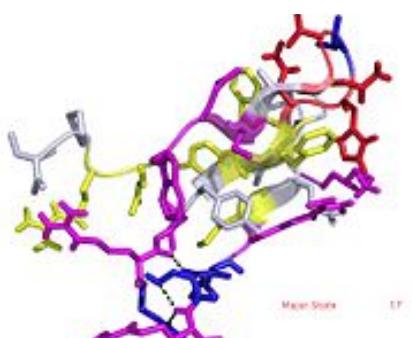
- Proteins form into _____ shapes
 - Most proteins are capable of **self-assembly**, meaning they can fold into their shape without assistance
 - Information required to specify the folding is inherent in the amino acid side chains (R group)
 - If the protein is **denatured** (unfolded) in one condition it will **renature** (reform) in proper conditions
 - The peptide bonds in the polypeptide backbone _____ movement
 - First limit on protein folding

EXAMPLE: Example of denatured and renatured forms of a protein



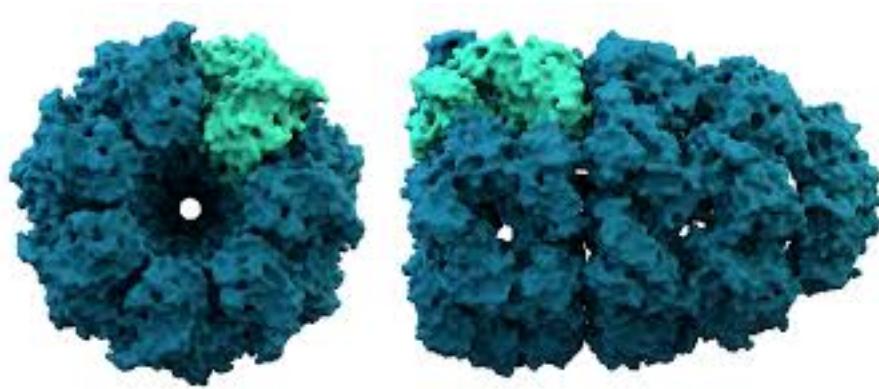
- The protein's **conformation** (folded shape) is determined through the properties of the amino acid R group
 - This forms with conformation with the _____ Gibbs free energy
 - The **native state** of a protein specifies a small number of conformations the protein will actually form
 - Out of 1000s of possibilities
- The protein's **conformation** (folded shape) is formed through noncovalent interactions
 - *Hydrogen bonds, Ionic bonds, Van der Waals interactions, and hydrophobic interactions*
- **Disulfide bonds** are stabilizing covalent bonds formed between sulfur atoms on two cysteine amino acids

EXAMPLE: Protein conformation is dictated by amino acid side chains



- **Chaperone** proteins are proteins that can _____ in protein folding
 - One group is the **molecular chaperones** that assist in stabilizing unfolded or partially folded proteins
 - Bind to short segments of the protein substrate
 - Prevent aggregation of unfolded, or misfolded proteins
 - Hsp70 is an example
 - The second group is the **chaperonins**, which form small folding chambers to sequester unfolded proteins
 - The sequestering allows the protein to refold without influence from molecules or water in cytosol
 - Contain a cylindrical folding core, and regulated by protein "lids" that allow proteins in and out
 - Hsp60 is an example
 - Chaperones _____ proteins from energy provided by ATP hydrolysis
 - Misfolded proteins results in diseases like Parkinson's, and Alzheimer's

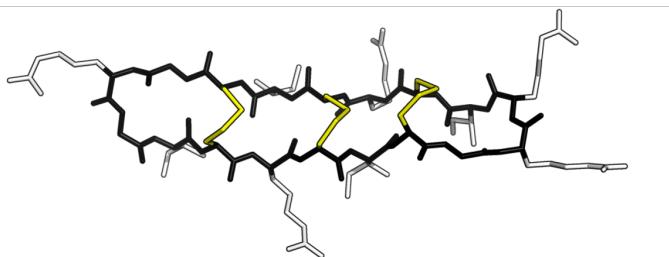
EXAMPLE: Structure of a chaperonin



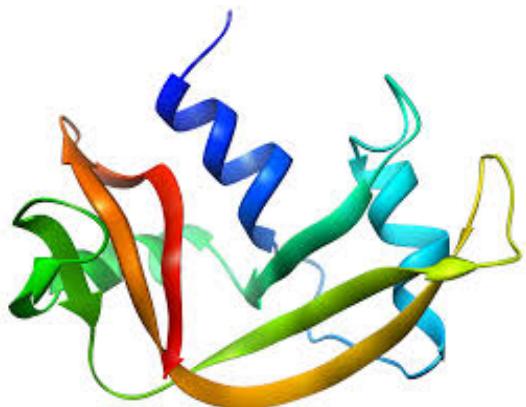
Four Protein Models

- There are four ways to present a protein's structure
 - The **backbone** model presents the overall organization of the polypeptide chain
 - The **ribbon** model shows the polypeptide backbone folding
 - The **wire** model shows the polypeptide backbone and the amino acid side chains
 - The **space-filling** model shows a contour map of the protein's surface

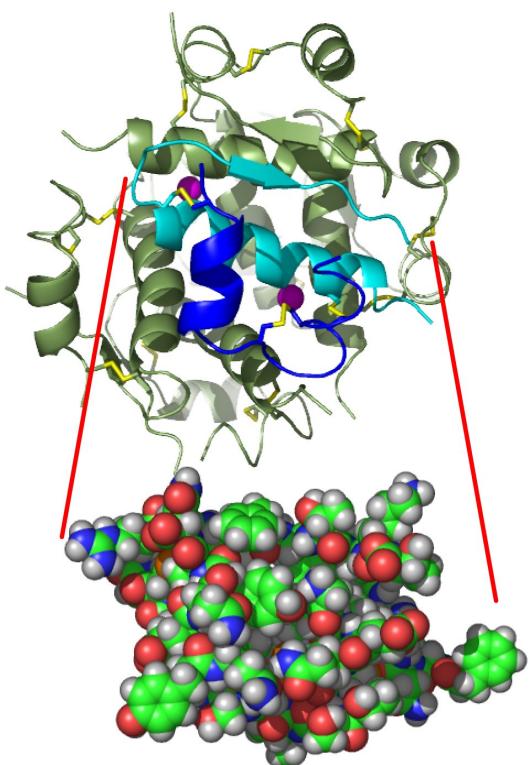
EXAMPLE: Four models of protein folding



Backbone model



Ribbon model



Wire model

Space Filling model

PRACTICE

1. Which of the following is false about the R group of amino acids?
 - a. They give polypeptide chains unique properties
 - b. They assist in forming complex protein structures
 - c. They control the stereoisomer form of the amino acid
 - d. They can be nonpolar

2. A protein C-terminus is named that way because it contains what molecule?
 - a. An extra carbon
 - b. A COOH group
 - c. A CH₃ group
 - d. A Chloride

3. A protein's conformation is formed through all but which of the following?

 - Hydrophobic Interactions
 - Disulfide bonds
 - Covalent bonds between carbons
 - Noncovalent bonds between R groups

4. Which of the following protein models would you use if you wanted to gain an idea of what the surface of a protein looked like?

 - Backbone
 - Ribbon
 - Wire
 - Space-Filing

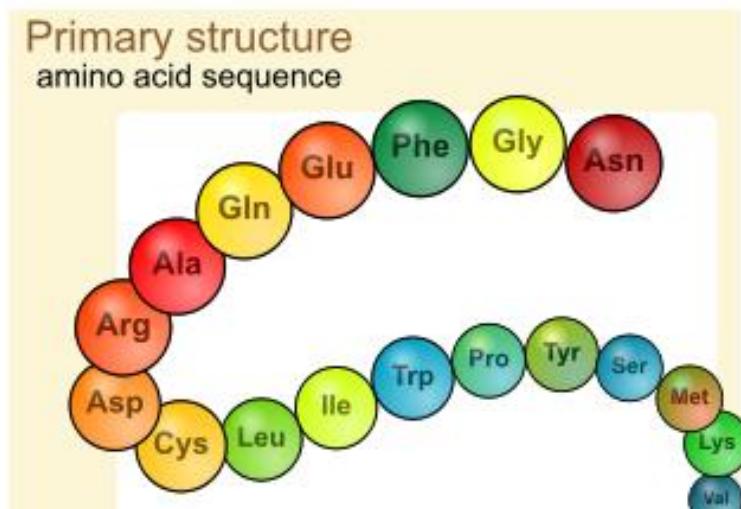
CONCEPT: PROTEIN FOLDING

Primary Structure

- Primary structure is the first of four protein folding levels

- Primary structure is the _____ sequence of amino acids in a polypeptide chain
- The sequence of amino acids, and their attached R groups provides information for folding the 3D conformation
- Covalent peptide bonds between amino acids hold together the primary structure

EXAMPLE: Primary structure of a protein



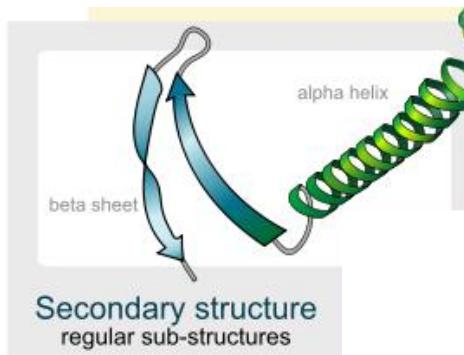
Secondary Structure

- The secondary structure refers to _____ structures formed by the polypeptide backbone.

- The **alpha helix** is one secondary folding pattern
 - Hydrogen bonds made between every fourth amino acid (carboxyl group bound to amino group)
 - Forms a rigid cylinder that can be right handed or left handed
 - Is abundant in skin protein
- A **beta sheet** is another main secondary folding pattern
 - Hydrogen bonds made between segments of the polypeptide chain that are arranged side by side
 - Can be **parallel** (two chains in the same direction) or **antiparallel** (two chains in opposite directions)
 - Is abundant in silk proteins

- **Disulfide bonds** between the side chains of cysteine can link proteins together and increase stability

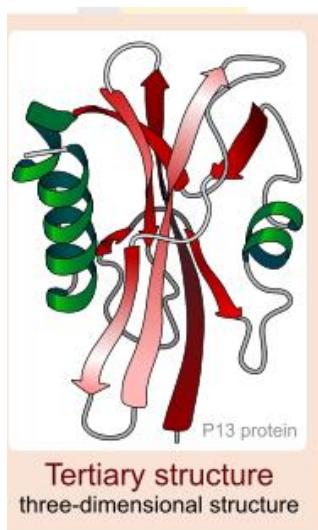
EXAMPLE: Secondary structure of a protein



Tertiary Structure

- The **tertiary structure** refers to ____ conformations formed by a single polypeptide chain. Can be functional or structural
 - **Structural motifs** are combinations of two or more secondary structures that form a 3D structure
 - A **coiled coil** is 2-3 helices that wrap around each other to form a very stable structure
 - The *helix-turn-helix* and *helix-loop-helix* are two common structural motifs named by their helical structure
 - Each structural motif usually has a specific function
 - Protein **domains** are segments of the polypeptide chain (40-350 aa) that fold into independent stable structures
 - Each domain usually has a specific _____
 - Certain domains are found in multiple proteins (SH2 domains is found in 120 polypeptide chains)
 - **Domain shuffling** is an evolutionary process that linked domains in new combinations
 - Two-thirds of proteins contain 2+ domains
 - Tertiary structure forms two main protein types: *Fibrous proteins* and *Globular proteins*
 - **Fibrous proteins** are proteins with an elongated shape
 - **Globular proteins** are proteins with a compact shape

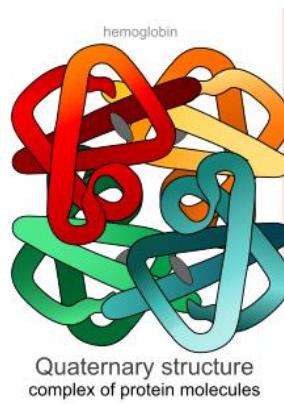
EXAMPLE: Tertiary structure of a protein



Quaternary Structure

- The **quaternary structure** refers to a protein complex with _____ one polypeptide chain
 - Only found in **multimeric proteins** which are composed of 2+ polypeptide chains
 - A **subunit** is a single polypeptide chain which is a part of a larger protein complex
 - Each subunit can be identical or non-identical to other subunits
 - A **homomeric proteins**: composed of identical subunits
 - A **heteromeric proteins**: composed of non-identical subunits
 - Quaternary structure is stabilized by the same non-covalent interactions and *disulfide bonds* as tertiary structure
 - *Hydrogen bonds, van der Waals forces, hydrophobic interactions, ionic bonds*

EXAMPLE: Quaternary structure of a protein

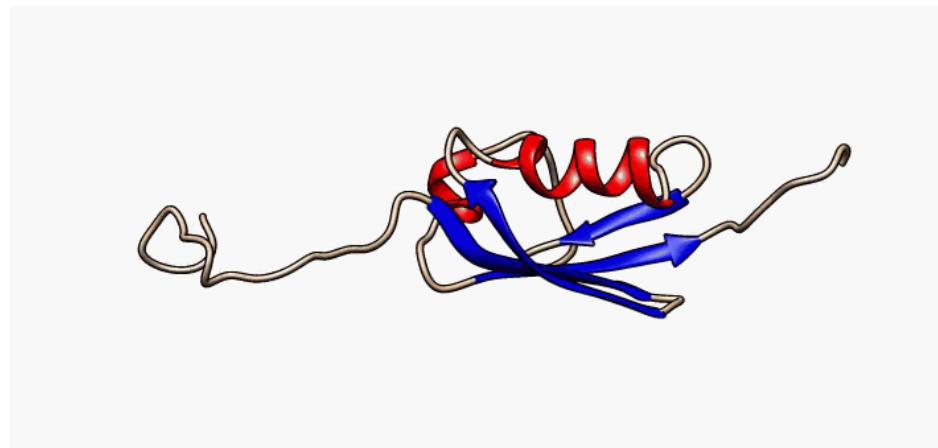


Unstructured Regions of a Protein

- **Unstructured regions** (disordered regions) exist between _____ protein structures or domains

- Provide flexibility to protein structure and folding
 - Wrap around target proteins with high specificity but low affinity
 - Can help scaffold proteins together
- Nearly one-third of eukaryotic proteins have unstructured regions in at least one polypeptide chain
 - Some can be found as the _____ polypeptide chain – usually form aggregates in cytosol
- Disulfide bonds** between the side chains of cysteine can link proteins together and increase stability

EXAMPLE: Unstructured regions of a protein (grey) surround structured regions (red/blue)



PRACTICE

1. Match the following protein structures with their appropriate definitions
 - i. Primary Structure _____
 - ii. Secondary Structure _____

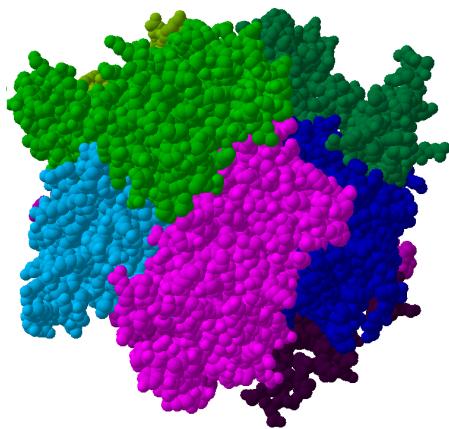
iii. Tertiary Structure _____
iv. Quaternary Structure _____

- A. 3D conformation of a single polypeptide chain
- B. Linear sequence of amino acids
- C. Folding of multiple polypeptide chains together
- D. Local structures of a single polypeptide chain

CONCEPT: COMPLEX PROTEIN STRUCTURES

- Proteins are often made up of more than one polypeptide chain
 - **Binding sites** on polypeptide chains allow for them to interact with other polypeptide chains and complexes
 - **Multi-protein complexes** are made up of multiple polypeptide chains with _____ functions
 - These are dynamic structures, and can assemble and disassemble in response to cellular signals
 - Examples include protein machines that drive DNA synthesis, RNA processing, and ATP creation
 - Stabilization of large protein complexes occurs through covalent _____ bonds
 - Especially common in the extracellular matrix
 - Mostly, noncovalent bonds connect polypeptide chains together

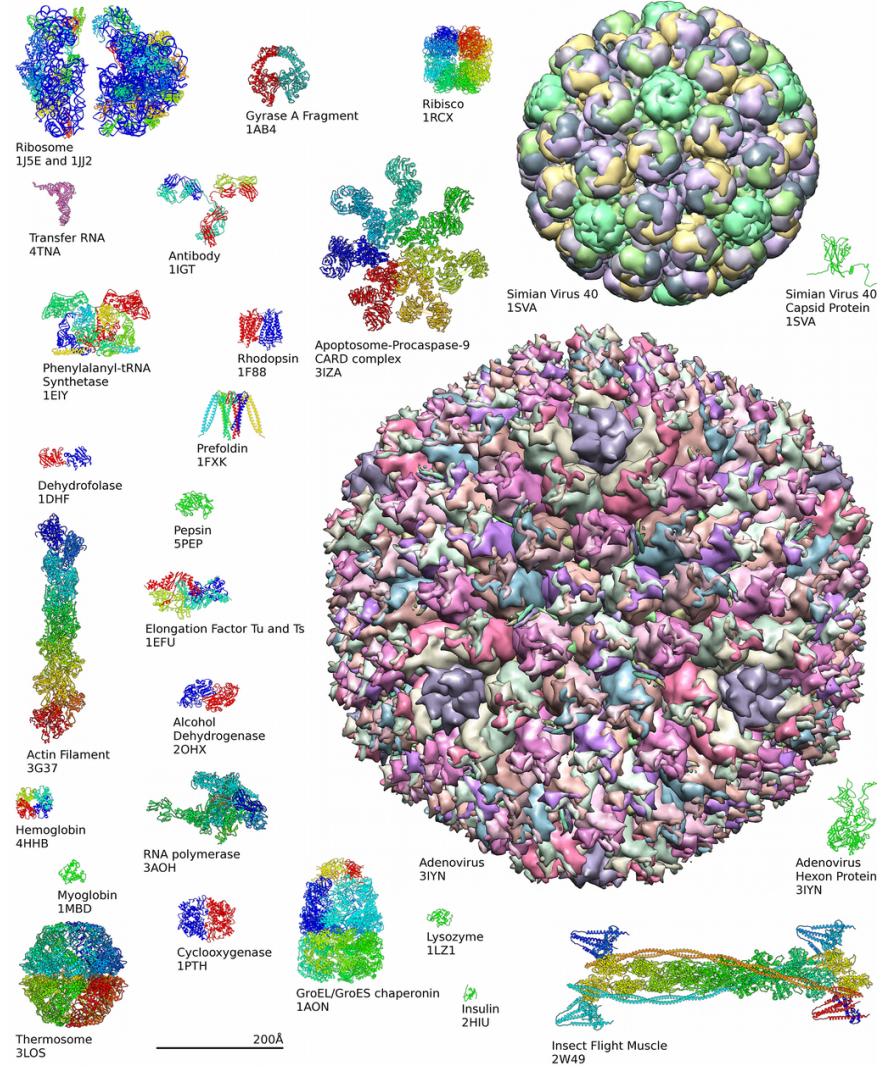
EXAMPLE: The exosome multi-protein complex is made up of 6 proteins represented by a different color



- Proteins and multi-protein complexes can form _____ shapes
 - *Helices* are a common shape of proteins
 - Helix is most energetically favorable way to link similar subunits in a long repetitive chain
 - Elongated fibrous shapes are another common shape of proteins
 - Fibrous proteins found in skin or the extracellular matrix
 - Compact globular shapes are a common shape of proteins
 - Unstructured polypeptide chains can provide flexibility to large protein structures

- Can be covalently cross-linked to create an elastic meshwork (Ex. Elastin)

EXAMPLE: Examples of diverse protein shapes



PRACTICE

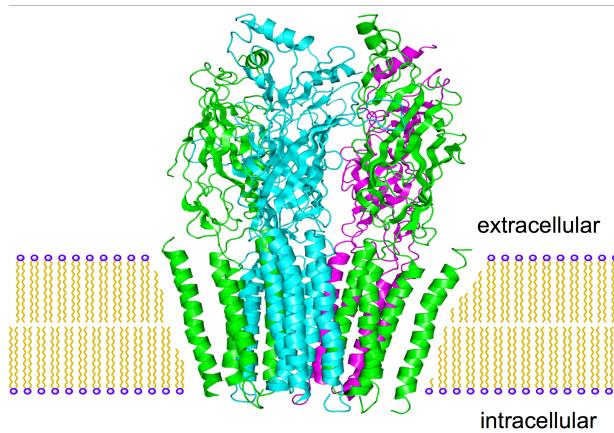
1. What type of bonds hold polypeptide chains together to form complex proteins?
 - a. Covalent bonds
 - b. Phosphodiester bonds
 - c. Ester bonds
 - d. Noncovalent bonds
 2. True or False: Due to the number of polypeptide chains, multiple protein complexes can only have a singular, globular shape.
 - a. True
 - b. False

CONCEPT: PROTEIN BINDING

Protein Binding

- All proteins bind to other _____
 - The *binding site* is the region where proteins bind
 - It is highly **specific** and can usually only bind to one or a few particles
 - The **ligand** is any substance bound to a protein
 - **Affinity** measures the strength of protein binding
 - Noncovalent bonds between amino acids in the binding site allow for the ligand to bind to the protein
 - Also called *molecular complementarity* when binding site and ligand properties are complementary
 - Surface conformation of the protein binding site provides it a certain _____
 - Can restrict the ligand's access to water and prevent the formation of hydrogen bonds
 - Allow for reactions between ligands and polar amino acid side chains

EXAMPLE: Extracellular ligand binding to a protein in the membrane bilayer



- There are _____ forms of protein binding
 - *Surface-String*: One large protein surface interacts with polypeptide chain of another protein (string)
 - *Helix-Helix*: Two polypeptide helices interact
 - *Surface-Surface*: Two protein surfaces interact – most common form of protein interaction
 - *Molecular channels*: One protein forms a channel containing an active site on the inside
 - Allows for processing of an unstable intermediate protein quickly without it diffusing into cytosol

- Small Molecule:* Nonprotein binding of a small molecule that gives the protein a specific function

EXAMPLE: The small molecule retinol (grey) bound to the human rhodopsin protein

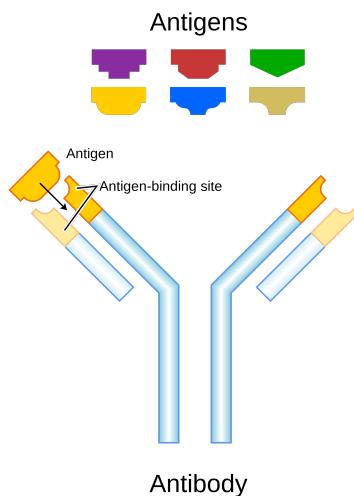


Antibodies

- **Antibodies** are proteins (also called immunoglobulin proteins) produced by the immune system

- Antibodies help the body _____ and respond to foreign molecules
 - Recognize an **antigen**, which is any foreign molecule – VERY specific
 - Antibodies recognize an antigen's **epitope** or small specific region that the antibody binds
- Antibodies are shaped like a "Y" with a heavy and light chain segment
 - There are variable regions that recognize specific antigens, and constant regions specific to antibodies
- Antibodies are extremely _____. They can tell the difference between a single amino acid

EXAMPLE: An antibody structure is specific for one antigen

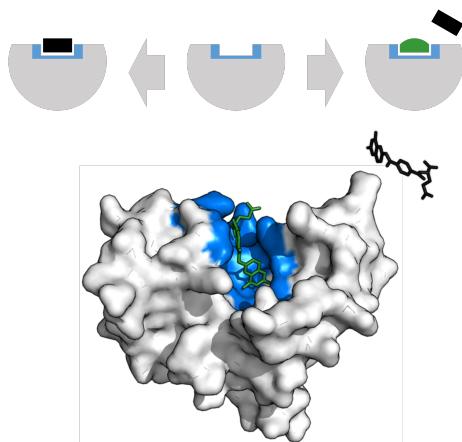


Enzymes

- **Enzymes** are proteins that help to _____ (speed up) reactions

- Enzymes bind to **substrates**, which are ligands that bind to enzymes
 - Binds to the active site portion of the enzyme
 - Once bound the enzyme stabilizes the *transition state* to lower activation energy
 - Chemical reaction or modification proceeds
 - Many pharmaceutical drugs inhibit enzymes

EXAMPLE: Enzymes bind to substrates to catalyze chemical reactions



PRACTICE

1. Which of the following is not a form of protein binding?
 - a. Helix-helix
 - b. Surface-string
 - c. Surface-surface
 - d. Surface-helix

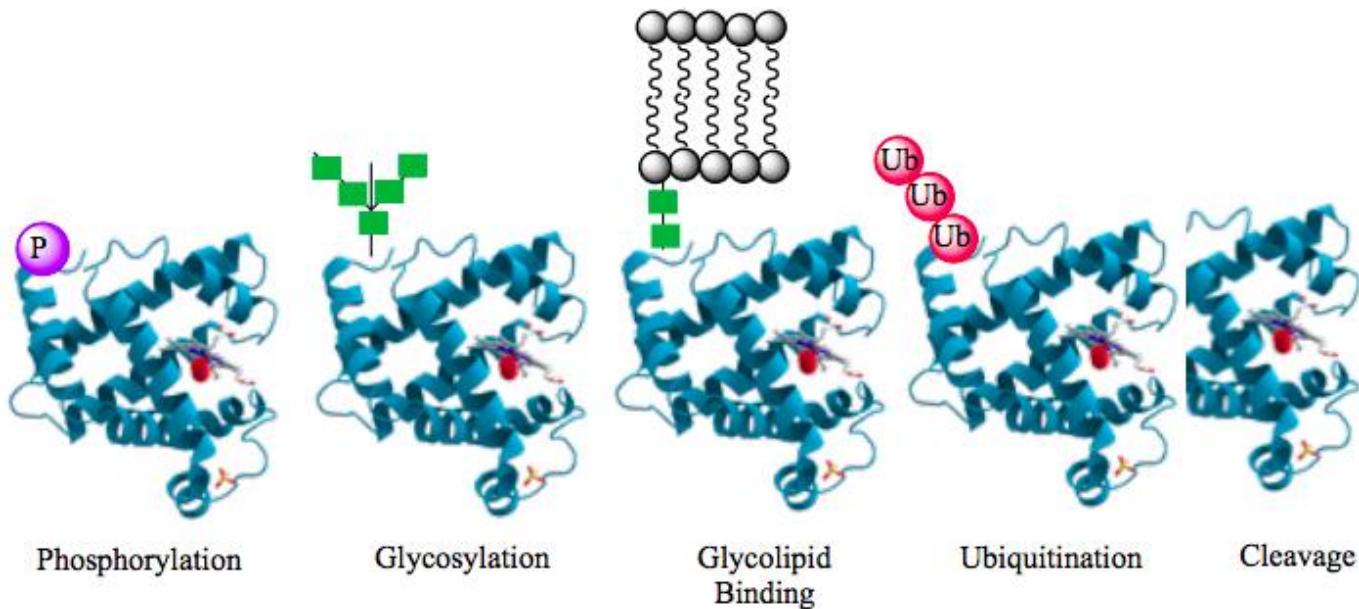
2. The region of a molecule that an antibody binds is called what?
 - a. Antigen
 - b. Epitope
 - c. Ligand
 - d. Substrate

3. True or False: The binding site of a protein is highly specific for one or a few ligands
- a. True
 - b. False

CONCEPT: PROTEIN REGULATION

Covalent Modifications

- Modifications to proteins can affect their _____.
 - **Phosphorylation** is the reversible addition of a phosphate group to one or more amino acid side chains
 - Carries two negative charges and can cause conformational change
 - Kinases catalyze addition of the phosphate group; Phosphatases causes removal (dephosphorylation)
 - **Glycosylation** is the reversible addition of carbohydrates
 - N-linked if attached to nitrogen atom; O-linked if attached to oxygen atom
 - Modifications also occur by the _____ addition of lipids
 - **Glycolipids** are lipids linked to oligosaccharides (sugars) which can be added to proteins to anchor them
 - Other types include *prenylation*, *palmitoylation* and *N-myristoylation* (each named based on the lipid type)
 - **Ubiquitination** is the addition of ubiquitin proteins which target the protein for degradation
 - **Cleavage** can occur to irreversibly remove a section of the protein
 - Cleavage of targeting signal sequences allows release of proteins sequestered in one area

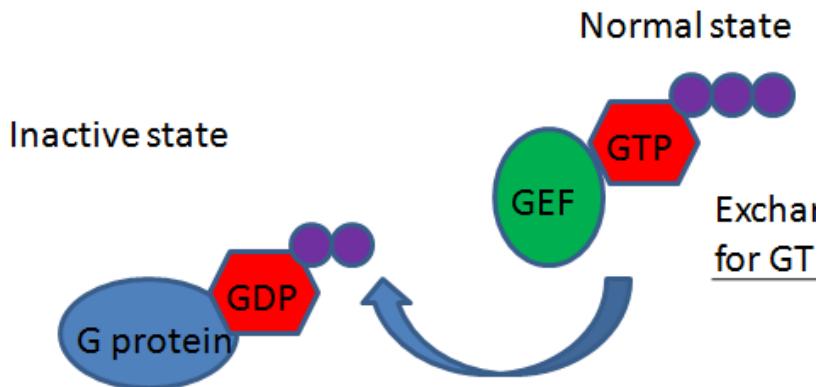


EXAMPLE: Types of protein modifications

GTP and Calcium Binding

- Binding and hydrolysis of **GTP** acts as a major source of protein _____
 - Proteins bind GTP in a special GTP-binding domain
 - GTP hydrolysis can control itself OR the function of other proteins to which the GTP-protein is bound
 - GTP hydrolysis to GDP results in conformational changes that inactivates the GTP- protein
 - The **Ras** protein is a major GTP binding cellular regulator
 - Misregulation of this protein leads to a variety of cancers
- Binding of Calcium _____ a variety of proteins
 - Calcium concentration is low in the cytosol
 - Changes in concentration can cause activation/inactivation of calcium binding proteins

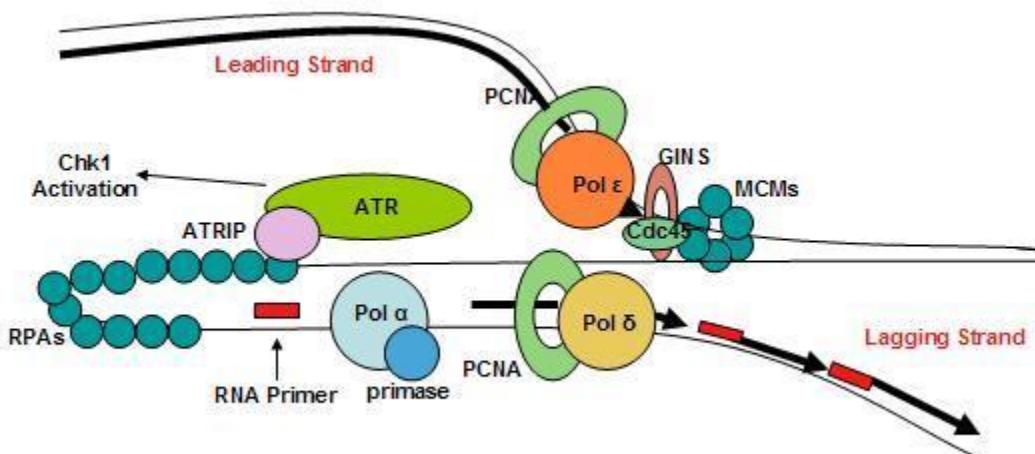
EXAMPLE: G proteins are inactive when bound to GDP and active when bound to GTP



Protein Machines

- **Protein Machines** are protein complexes made up of 10+ proteins
 - These machines have _____ and dynamic parts
 - Each part has to be positioned in a specific way to work properly
 - Control of these machines depends on control of each individual part
 - Many layers of regulation

EXAMPLE: A protein machine consists of many parts that are each independently regulated



PRACTICE

1. Which of the following is not a protein modification that allows for protein regulation?
 - a. Ubiquitination
 - b. Phosphorylation
 - c. Glycosylation
 - d. Noncovalent interactions in the binding site

2. Hydrolysis of GTP to GDP causes what to occur to a GTP-Binding protein

- a. It marks it for degradation
- b. It cleaves it
- c. It activates it
- d. It inactivates it

3. True or False: To regulate large protein complexes there is a single, powerful regulator of the entire complex.

- a. True
- b. False

CONCEPT: PROTEIN DEGRADATION

Ubiquitin-Proteasome Pathways

- The **proteasome** is a multisubunit protein complex that is able to _____ proteins

□ Proteins labeled with **ubiquitin** protein are targeted to the proteasome

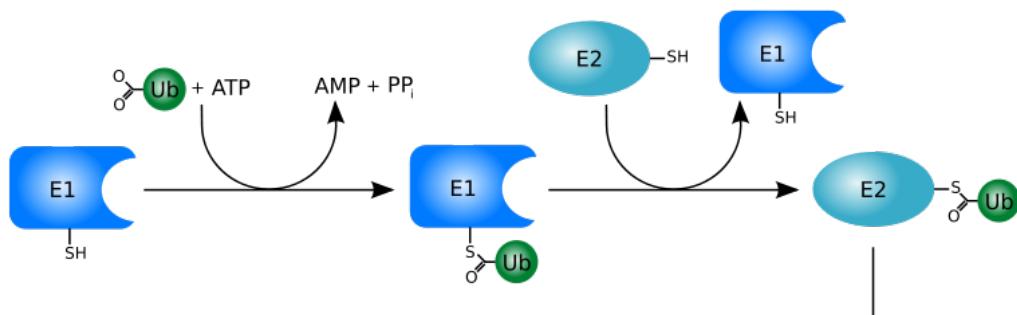
- Ubiquitin is a 76 amino acid protein conserved across all eukaryotic organisms

- Proteins can be labeled with one (mono) or more than one (poly) ubiquitin proteins

□ This pathway occurs in steps and requires energy from ATP

1. Ubiquitin is activated by the E1 ubiquitin activation enzyme

2. Activated ubiquitin then binds to the E2 ubiquitin conjugating enzyme



3. Then this complex is attached onto the target protein via E3 ubiquitin ligase

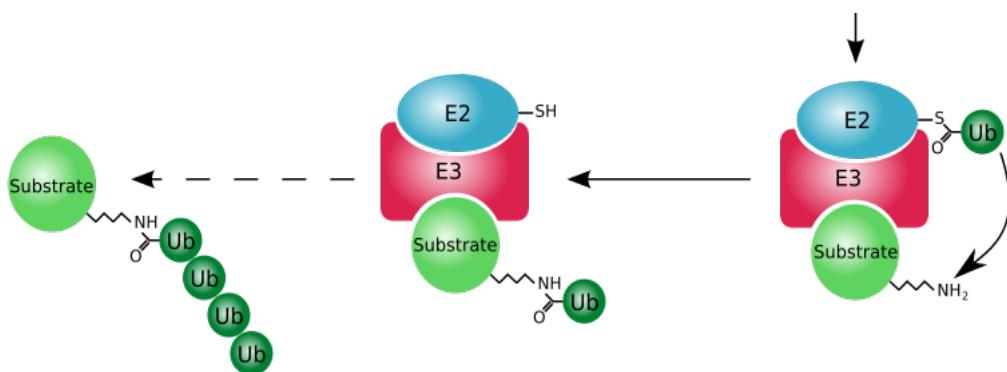
- Each E3 recognizes a different substrate protein – it “selects” the correct protein

4. The ubiquitinated protein is recognized by the outside of the proteasome

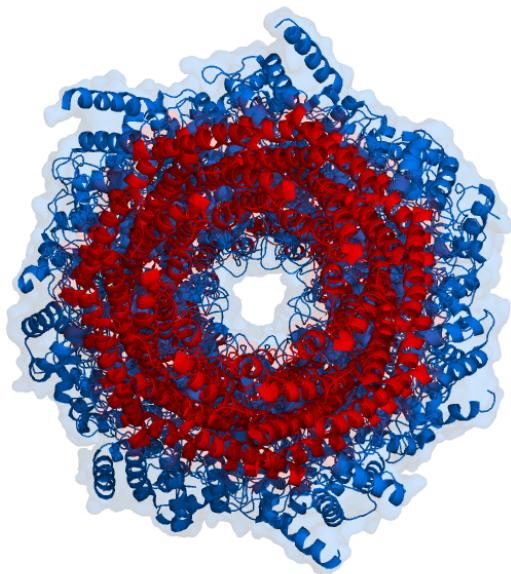
5. The protein is unfolded and fed through the inside of the cylindrical proteasome

- Contains ATP-dependent **proteases** which chop up the protein into short peptides

- The entire protein is bound until it is entirely cut



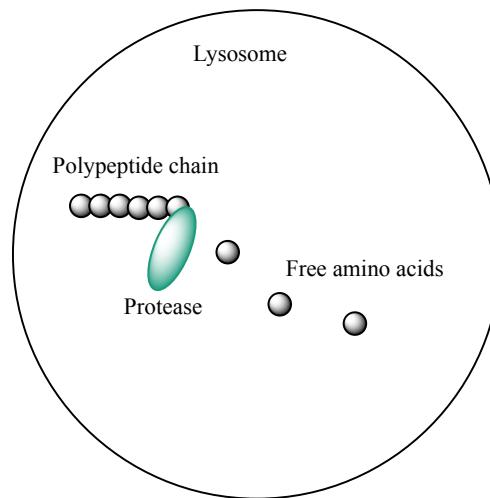
EXAMPLE: Proteasome structure



Lysosomal Pathway

- The **lysosome** breaks down proteins
 - The lumen of the lysosome contains _____ that chop up proteins
 - *Autophagy* is the process of cell death, and involves a lot of protein destruction via lysosomes
 - Can rapidly respond to nutrients and external signals

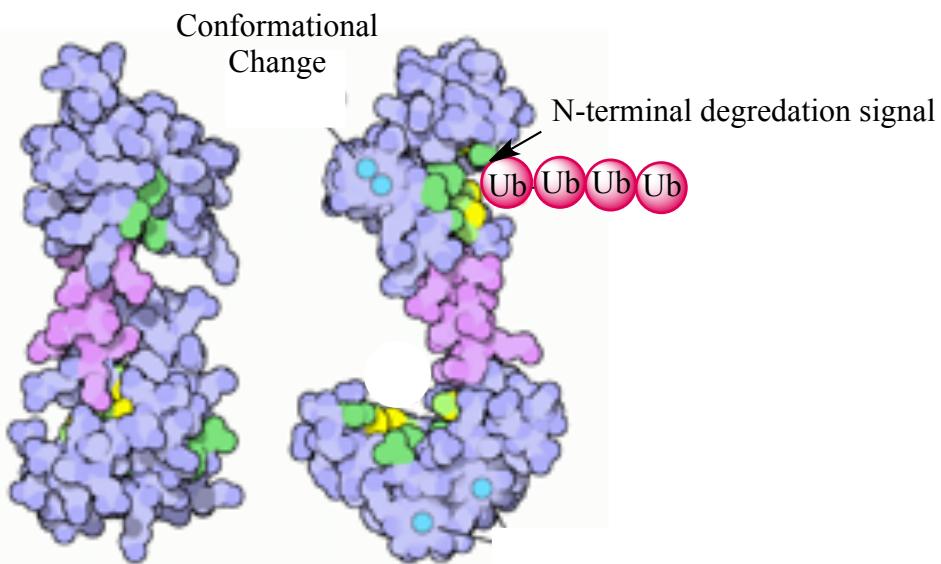
EXAMPLE: Lysosomal protein degradation



Degradation Regulation

- Protein degradation _____ the amount of protein in the cell at a certain time
 - One reason for regulation is that a protein's lifespan can vary from seconds to decades
 - Proper protein levels are crucial for certain chemical reactions
 - An **N-terminal degradation signal** is a hidden signal released when its time for degradation
 - Ubiquitin binds this region
 - A second reason for regulation is proteins occasionally _____
 - Abnormally folded proteins can form aggregates in the cell and cause disease

EXAMPLE: A conformational change releases an N-terminal degradation signal which becomes ubiquitinated



PRACTICE

1. Which of the following is not associated with protein degradation?
 - a. Ubiquitin-proteasome pathway
 - b. Lysosomal pathway
 - c. 5' Cap
 - d. N-terminal degradation signal

2. Which protein is responsible for attaching a ubiquitin molecule onto a protein to target it for degradation?
 - a. E1 ubiquitin activation enzyme
 - b. E2 ubiquitin conjugating enzyme
 - c. E3 ubiquitin ligase
 - d. E4 ubiquitin attachase

3. True or False: Before entering the proteasome the protein marked for degradation is unfolded.
- True
 - False
4. What is the name of the signal released by some proteins when they can be degraded?
- Ubiquitin binding signal
 - N-terminal degradation signal
 - C-terminal degradation signal
 - Proteosome signal