Module 1 : Protein Biochemistry

In this module, there are videos, animations and games to illustrate concepts on protein purification and protein structures.

After going through this module, you will gain understanding on different protein purification methods, gel filtration chromatography, protein separation procedures and determination of protein concentration. You will also learn different structures of proteins.

1a. What is Protein?

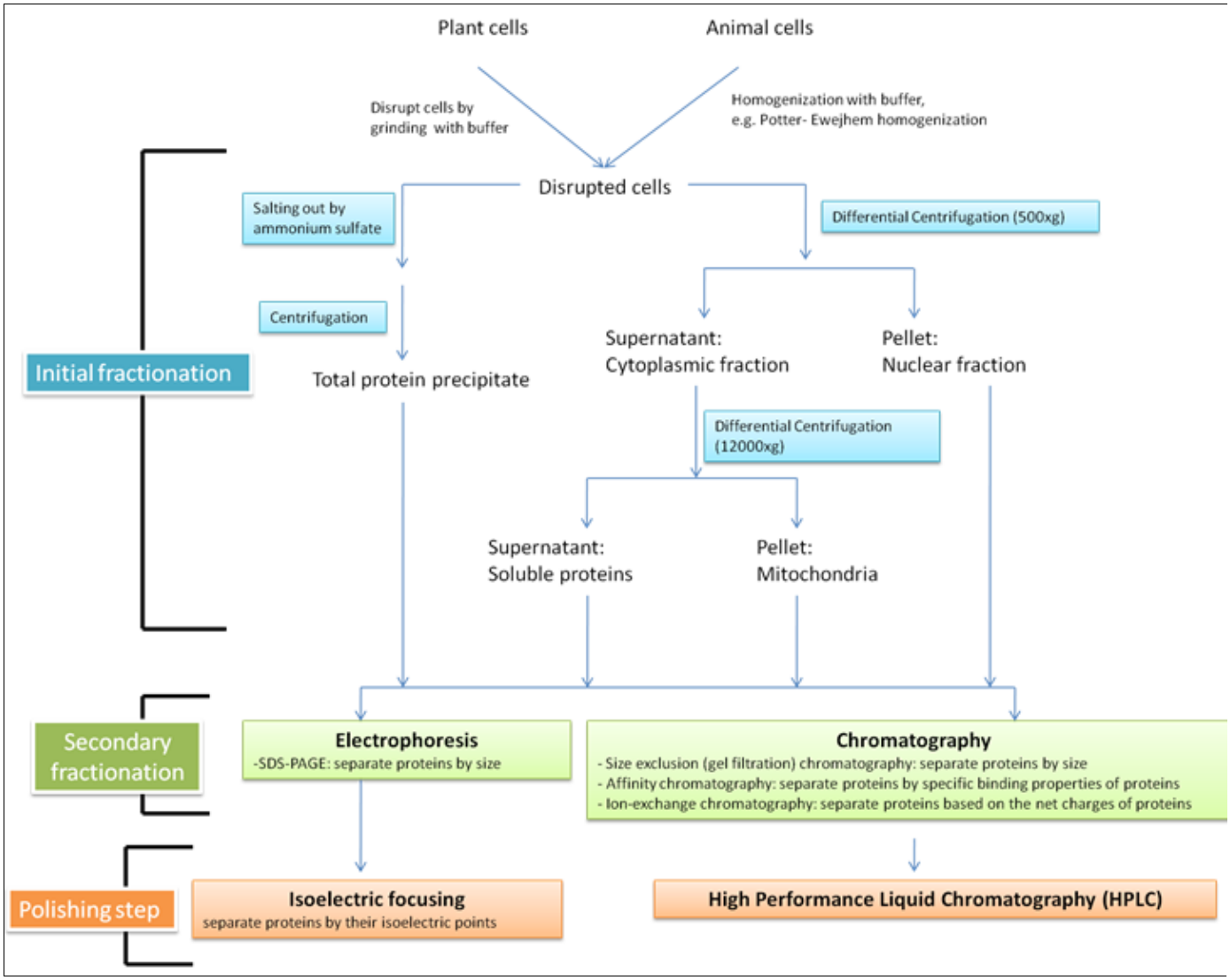
Proteins are macromolecules consisting of one or more polypeptides. Each polypeptide consists of a sequence of amino acids linked by peptide bonds. Polypeptide folds up to different structures that determines the unique three dimensional confirmation of a protein.

Different proteins can be separated by different means of protein purification according to their nature: solubility; size; surface hydrophobicity and charge.

Proteins can be in different forms, possesses of different functions. They may act as hormones, enzymes, glycoproteins, antibody and signaling molecules.

1b. Protein Purification

Means of protein purification	
Method	Analytical v.s. Preparative
Analytical	Detect and identify a protein in a mixture
Preparative	Produce large quantities of the protein for other purposes
Means	a) Extraction b) Precipitation & differential solubilization c) Centrifugation d) Column Chromatography - By size: size-exclusion chromatography (gel filtration chromatography) - By charge/ hydrophobicity: ion exchange chromatography; affinity chromatography e) Electrophoresis



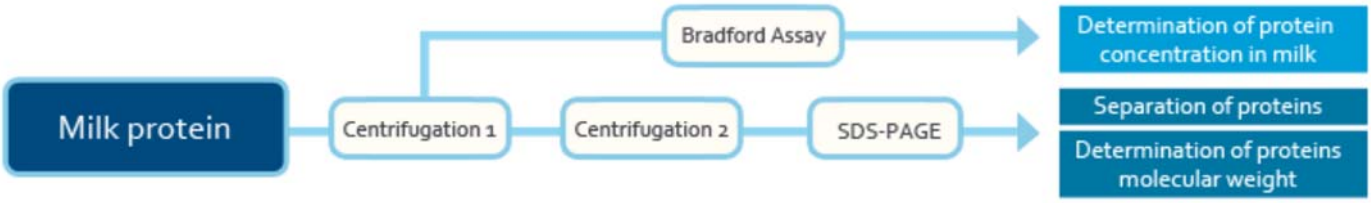
1b(i). Gel Filtration Chromatography

Gel filtration chromatography is also named as size-exclusion chromatography as it separates proteins based on their size. The column is packed with porous gel matrix in bead form. Sample is applied to the column for separation. Larger molecules that cannot enter the beads would elute out of the column first, while the smaller molecules which can enter the gel beads, would be retained longer within the gel structure and be eluted out later.

Animation : Gel filtration (see website)

Video : Gel Filtration Chromatography (see website)

1c. Case study: Milk protein separation



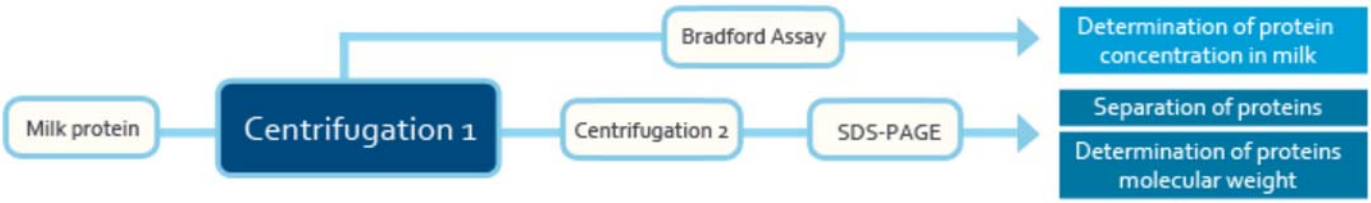
In this case study, we are trying to separate different kinds of milk protein out from the bovine milk sample.

Casein is the primary group of milk protein with total concentration around 25g/L in cow milk. It contributes about 80% to the total milk protein. There are 4 caseins in the milk of most species. They are αs1, αs2, β and κ-casein. Their molecular weight ranging from around 20-25 kDa. Centrifugation of skim milk results in pelleting of the casein with the supernatant whey proteins.

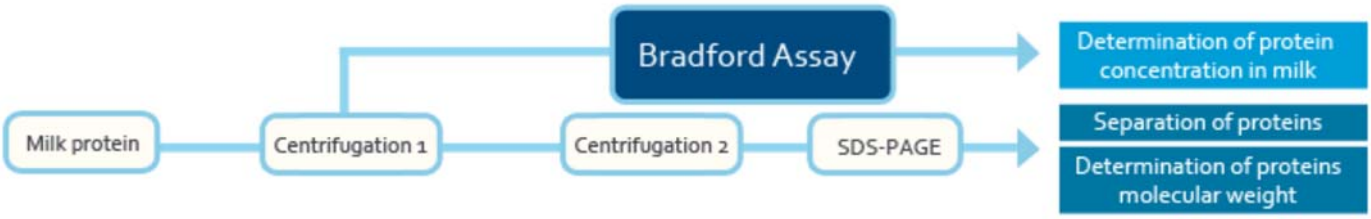
Whey proteins contribute approximately 20% to the total milk protein. Major whey proteins in bovine milk include β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins.

Centrifugation will be first carried out to remove fat from the whole milk to yield skim milk. After that, the skim milk will be centrifuged to separate caseins and whey proteins. To examine the presence of different milk proteins and their molecular weights, SDS-PAGE technique will be employed. On another hand, the protein concentration of milk sample will to be determined by Bradford assay.

1c(i). Centrifugation 1



1c(ii). Determination of Protein using Bradford



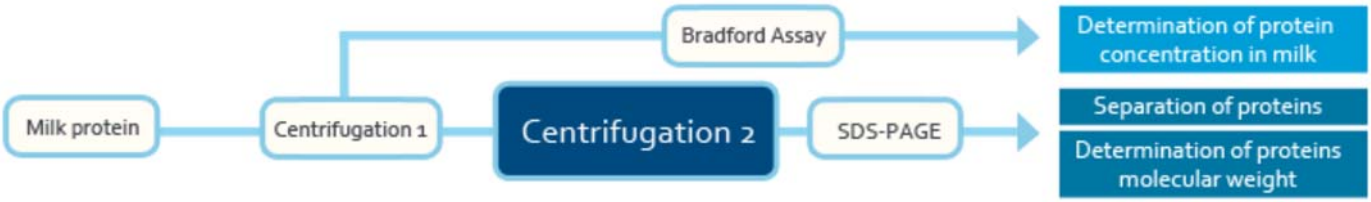
In this case, we are trying to determine the amount of protein in the sample of commercial brand milk.

The Bradford assay involves the addition of an acidic dye, Coomassie Brilliant blue, which change to blue in colour when binds to proteins. The absorption at 595nm is directly related to the protein concentration.

Before assay the commercial sample of milk, standard proteins sample mixed with Coomassie Brilliant blue should be prepared for plotting a standard curve of absorbance at 595nm against protein concentration. Bovine serum albumin (BSA) with known concentration is generally used as the standard protein.

Video : Bradford assay (see website)

1c(iii). Centrifugation 2

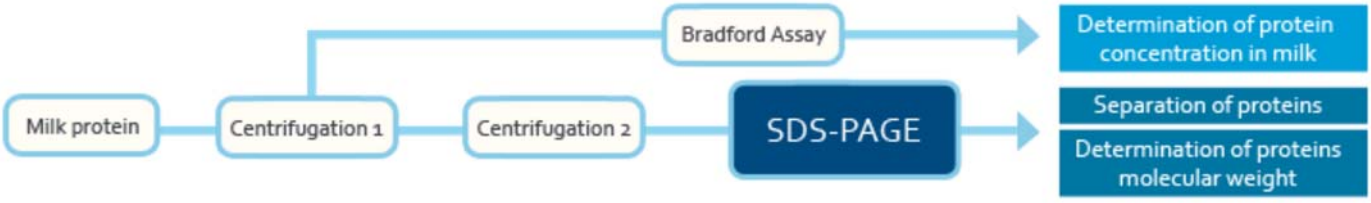


Centrifugation methods are based on the effects of force fields stronger than the gravitational force, to sediment different substances according to their density by different acceleration applied to the sample. In a centrifugation machine, there is a rotating unit, called rotor that has fixed holes drilled at an angle to the vertical. Samples in test tubes are placed in the rotor. The rotor rotates at a high speed in circular motion. Larger and heavier particles sediment to the bottom of the centrifuge tube at a relatively lower centrifugal force.

In addition to the size of the organelle, a number of other factors can also affect the time of sedimentation. They are the density and viscosity of the medium, the buoyant weight of the particle, the speed of rotation, the path length of the centrifuge tube and the temperature.

Video: Centrifugation (see website)

1c(iv). SDS-PAGE



To separate different milk proteins with different molecular mass, SDS-PAGE technique can be employed.

In SDS-PAGE, protein sample is first treated with detergent sodium dodecyl sulfate (SDS), $CH_3(CH_2)10CH_2OSO_3^-Na^+$,before applied to the gel. The anions bind to proteins via non-specific adsorption. Larger the protein, more anions adsorb. All proteins are negatively charged as a result of adsorption of anionic SO_3^-. SDS completely denatures the proteins by disrupting the tertiary and quaternary structures.

Therefore, the shape and charge are approximately the same for all proteins in the sample, size of proteins become the determining factor in the separation. The gel is more resistant to large molecules than the small ones. Smaller proteins migrate more rapidly to the positive pole of the gel. Molecular weight of proteins can be determined by comparison to protein ladder/ molecular weight ladder ran on the same gel with the sample.

Limitation of molecular weight determination of protein sample by SDS-PAGE

1. Proteins are fully denatured.
2. Gel % matches the molecular weight range of the sample.

A plot of log (molecular weight) of protein against the electrophoretic mobility (distance migrated) of proteins can be plotted to find the molecular weight of proteins separated.

Video : SDS-PAGE (see website)

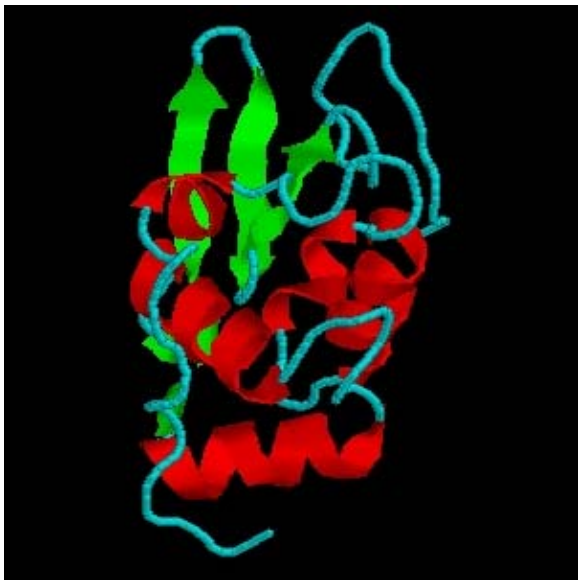
1d. Protein Structure

Lysozyme acts as a model of protein structure.

Lysozyme is a component of mucous and tears and acts as a protective role in defending the body against bacterial infection. Lysozyme hydrolyzes the mucopolysaccharide portion of bacterial cell wall.

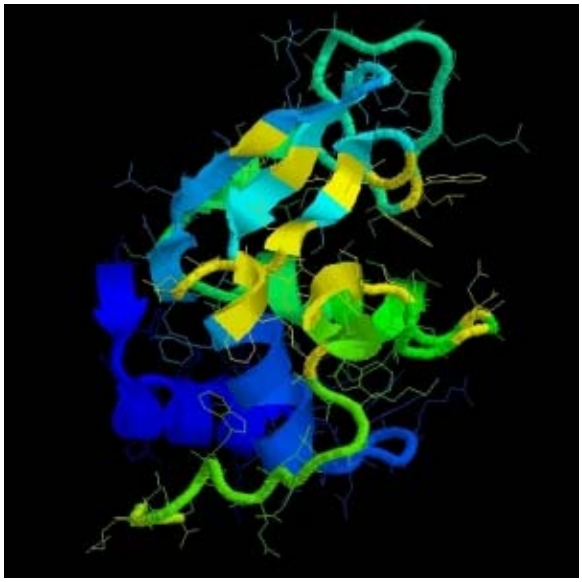
Specifically, the enzymehydrolyzes the 1-4 glycosidic linkage between alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues.

Software applied: raswin/rasmol http://rasmol.org/OpenRasMol.html
PDB download site: http://www.rcsb.org/pdb/explore/explore.do?structureId=1HEW

Lysozyme molecule without substrate(1)	**Lysozyme molecule without substrate(2)**
 Helices --- Red color Sheets --- Green color Notice the three larger helices (and two smaller helices) in red and the three main beta sheets (and two smaller beta sheets) in green. The figure also shows two closer antiparallel pleated sheets.	 Lysozyme is displayed in cartoon ribbons, with a group color scheme. The C terminus is shown in red , and N terminus is shown in purple
Lysozyme molecule shows disulfide bonds **(See next page)**	**Lysozyme molecule shown cleft** **(See next page)**

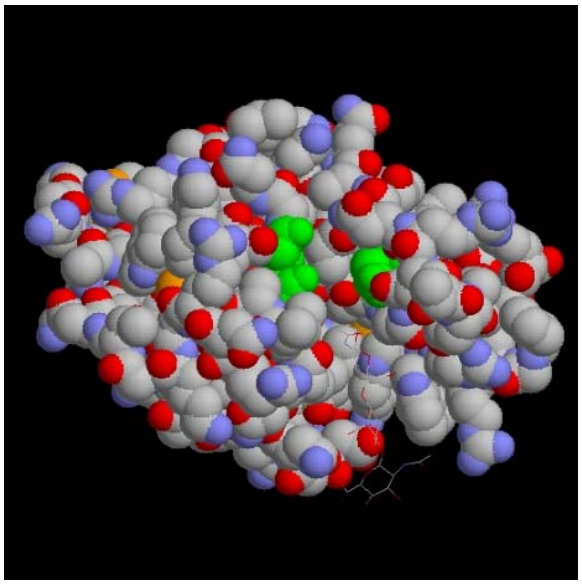
Cysteine residues shown in cyan, and four disulfide bonds are illustrated.

The cleft in lysozyme, with function groups shown

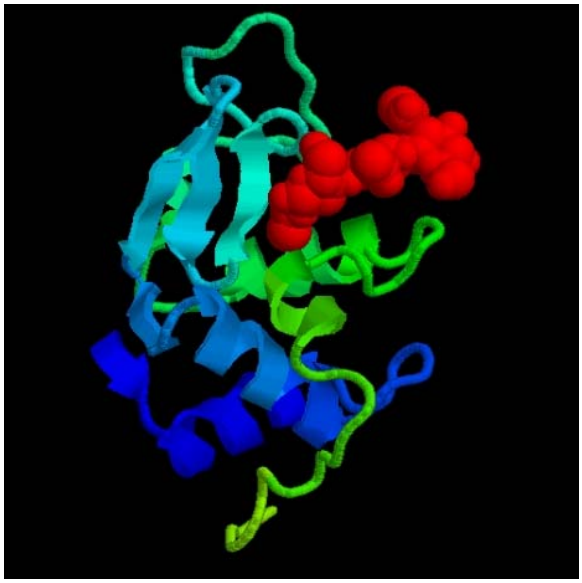


The cleft is shown in yellow, it is overlaying the helices and sheets. The cleft actually traverses all three beta sheets.

Lysozyme molecule with N-acetylglucosamine (NAG) substrate(1)



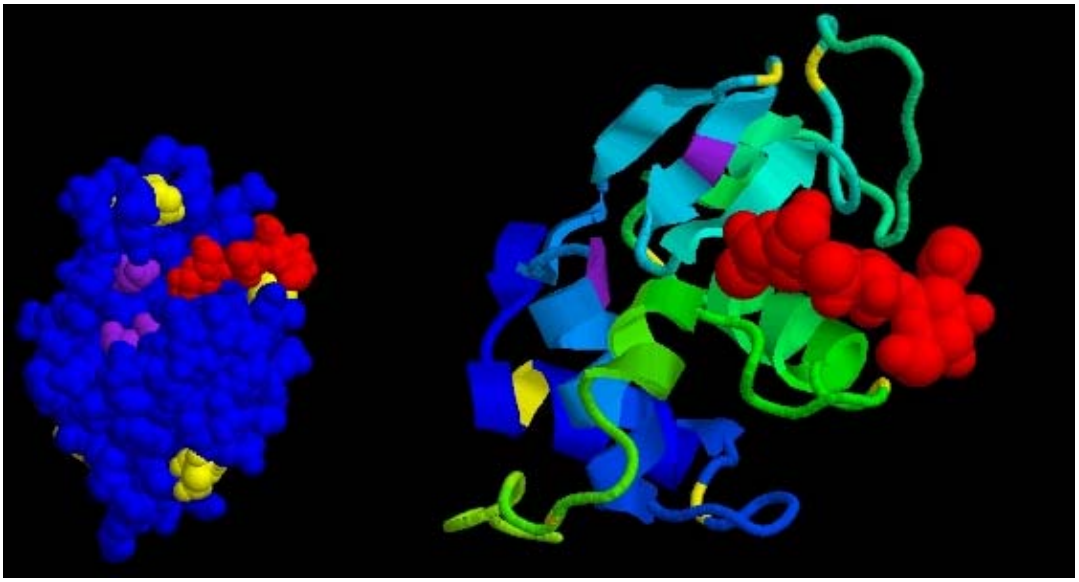
The figure is in spacefill view. The two functional groups are shown in green, they are at Glu 35 and Asp 52. It is the reactive center's catalytic groups



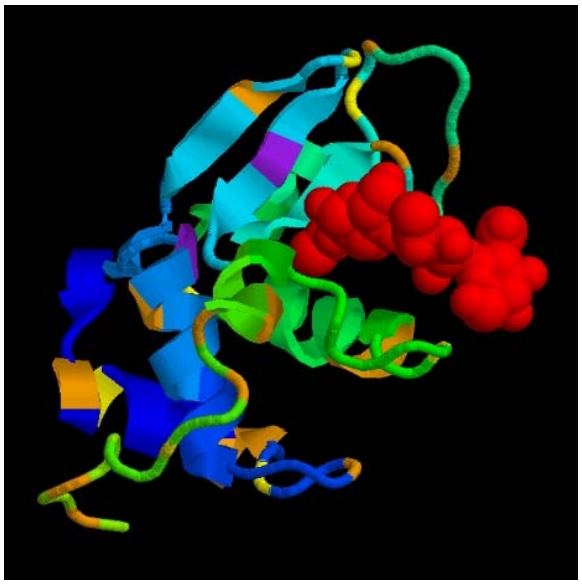
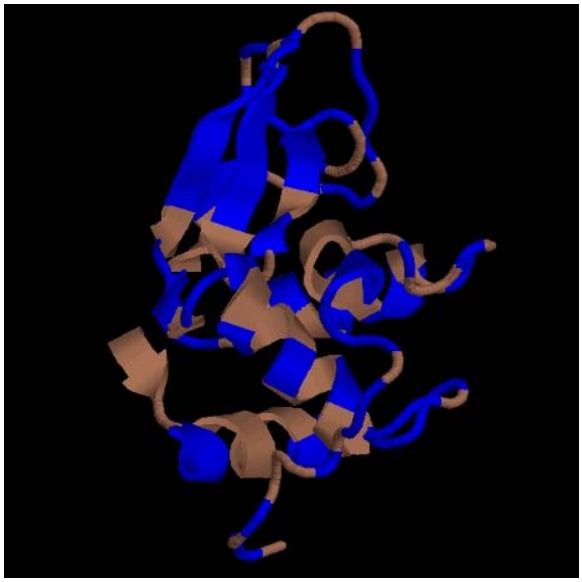
The substrate NAG is shown in Red.

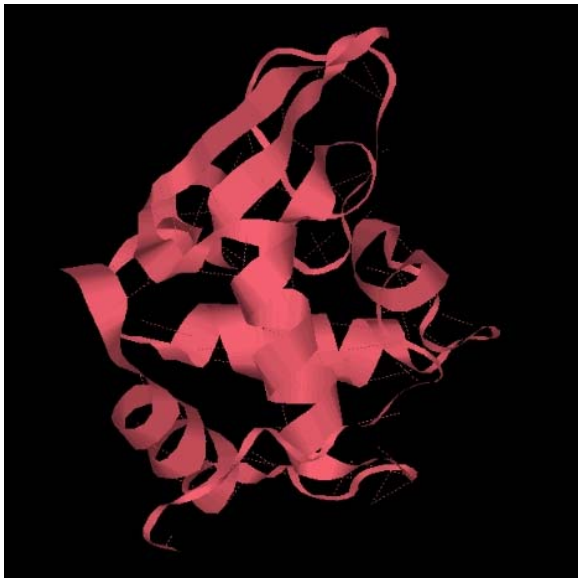
Lysozyme molecule with N-acetylglucosamine (NAG) substrate(2)

(See next page)



NAG - Red
Acidic function group - yellow
Two acidic catalytic residues - purple

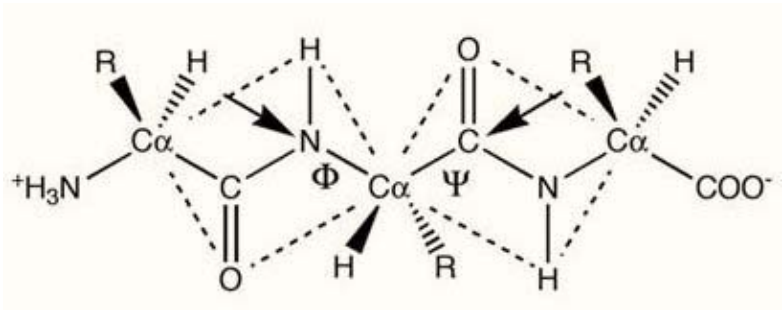
Lysozyme molecule with N-acetylglucosamine (NAG) substrate(3)	**Hydrophobic residues in lysozyme molecule**
 NAG -- Red The basic residues are in orange color. Please note that there are more basic residues in the structure. And they stick predominantly to the outside of the structure	 The hydrophobic residues are shown in brown color. These residues stick predominantly to the inside of the structure.
Hydrogen bonds in lysozyme molecule **(See next page)**	

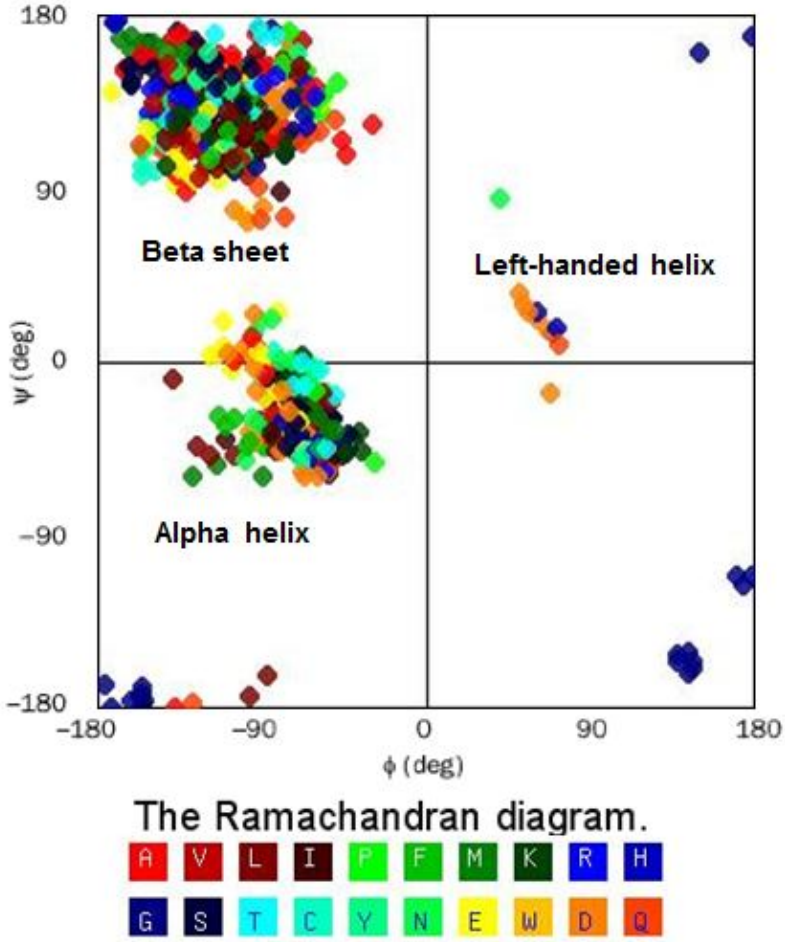


Hydrogen bonds in lysozyme molecule.

1d(i). Ramachandran Plot

A plot that displays the pairs of angles (psiΨ , phiΦ) that govern the twists in the chain of amino-acids forming a protein. Each pair of angles is represented by a point in a square whose limits, on each axis, correspond to (-180°, 180°). The Ramachandran plot shows the permitted range within a plot of Ψ against Φ.





A Ramachandran plot generated from human Proliferating Cell Nuclear Antigen (PCNA) that is composed of both beta sheets and alpha helices (PDB ID: 1AXC). Points that lie on the axes indicate N- and C-terminal residues for each subunit. The green regions show possible angle formations that include glycine, while the blue areas are for formations that don't include glycine.