IIT Guwahati

Lecture 26

Course BT 631

Protein Structure, Function and Crystallography

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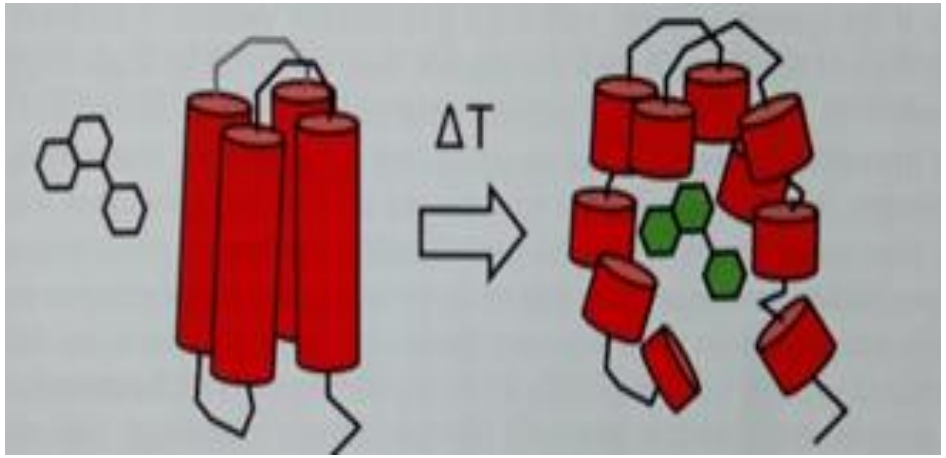
Protein stability and conformational state Analysis

- **Purity, stability and conformational homogeneity of a candidate protein for crystallization are important factors that influence the success of crystallization experiment.**
- **Molecular purity can be analyzed by using denaturing SDS-PAGE.**
- **Analysis of conformational homogeneity is comparatively harder, nevertheless it is important as differently shaped molecules do not arrange into ordered, periodic crystals. However, the conformational homogeneity is not an absolute measure to ensure crystallization.**

Stability Analysis by Thermal Shift Fluorescence Assays

Thermal Shift Fluorescence Assays for stability

- The ThermoFluor assay is a quick, temperature-based assay to assess the stability of proteins. It can be used to determine the melting temperature of proteins.
- This method is used to observe the effect of cofactors, buffers and other additives on protein stability and oligomerization.
- A hydrophobic fluoroprobe whose fluorescence is quenched in aqueous environment is added to protein solution.
- The fluorescence is enhanced when the probe attached to hydrophobic residues of proteins get exposed upon heating.

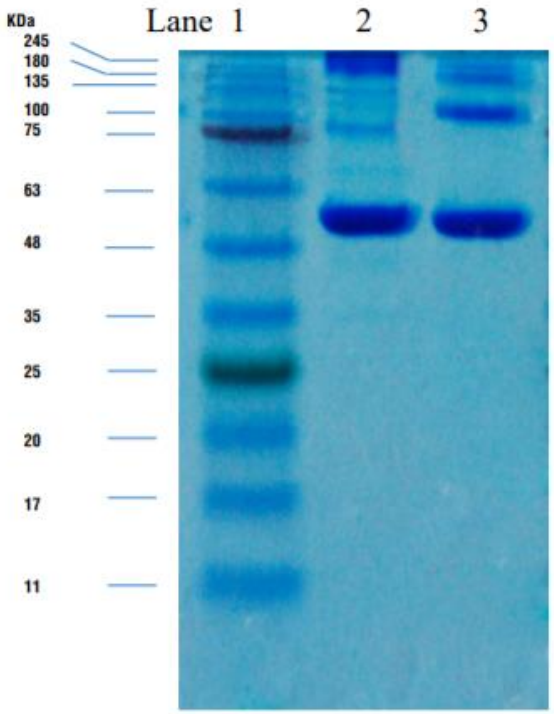


Representation of Thermofluor assay. Fluorescence of probe is quenched in aqueous environment (white), but enhanced when it binds to exposed hydrophobic residues on heating (green).

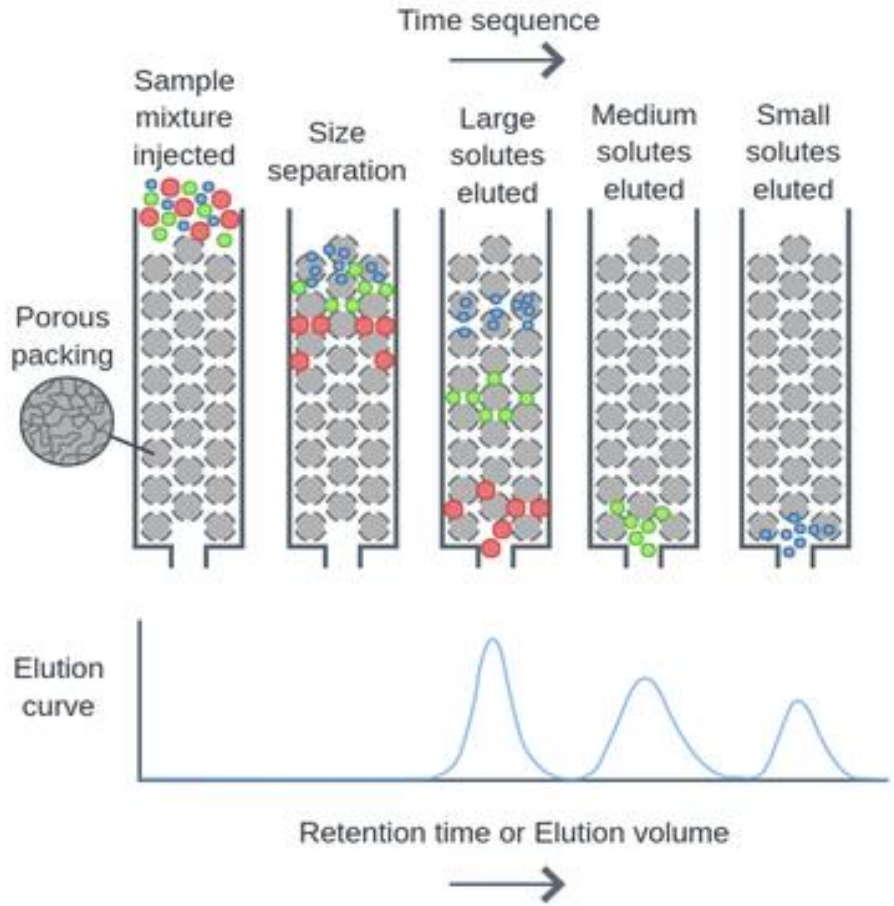
Stability and oligomerization state analysis

- Oligomerization state of a ptotein can be also studied by Native-PAGE and Size-exclusion chromatography (SEC). In case of stable oligomer a single band is observed on the Native gel and a single peak in the SEC chromatogram.
- When oligomers are unstable, the chemical environment during purification, especially the elution buffer composition influences the equilibrium between monomers and oligomers.
- The combination of SEC and Dynamic Light Scattering (DLS) methods is used to estimate the molecular mass and assess the size distribution of protein.

Native-PAGE and Size-Exclusion Chromatography



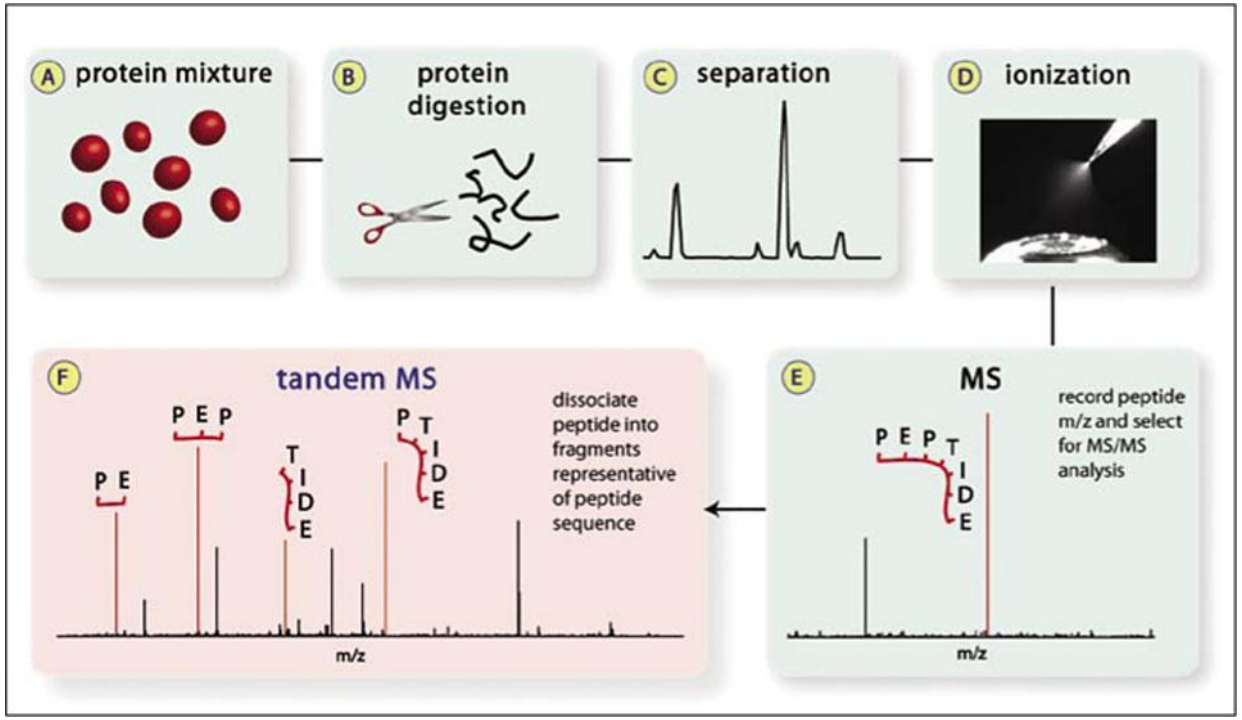
Native-PAGE Gel of Protein samples.
Lane 1: Protein marker, Lanes 2 and 3: Protein Samples



Size-Exclusion Chromatography for separation of proteins based on their size.
Elution of protein in order Large>medium>small

Molecular mass analysis by Mass Spectrometry

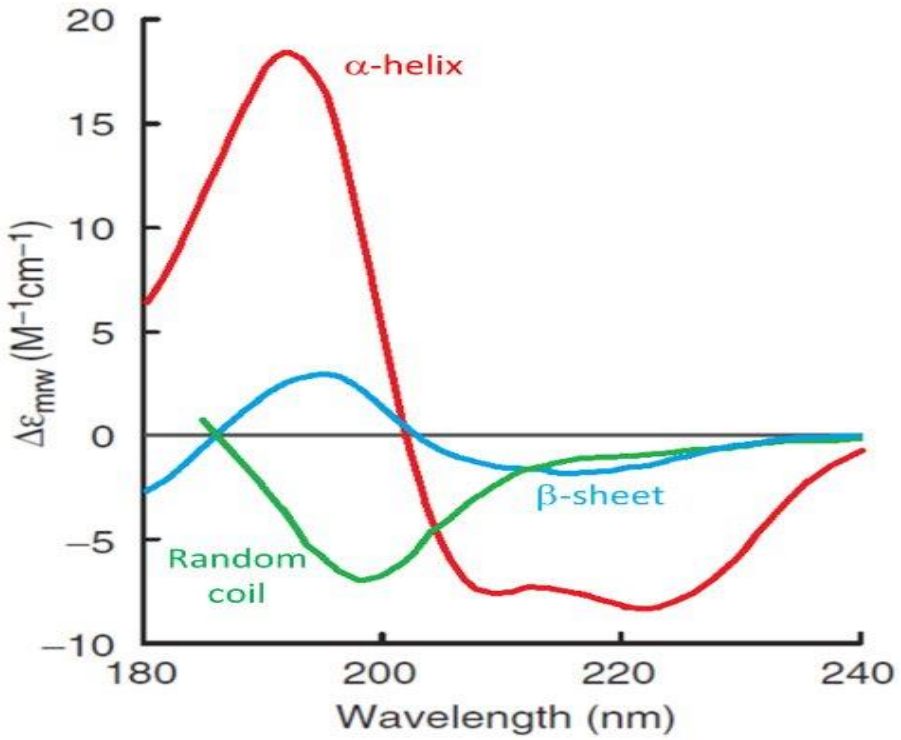
- Any modification in the protein can be detected as change in molecular weight that can be quantified by Mass Spectrometry.
- Thus, this technique supports crystallographic studies in a number of ways such as determination of surface lysine methylation, heavy metal incorporation for phasing studies and glycosylation patterns.
- Deuterium exchange mass spectroscopy can be used to detect unstructured regions of proteins.



Sequence of events defining a contemporary Mass Spectrometry (MS)-based proteomics experiment.

Circular Dichroism Spectroscopy

- CD spectroscopy provides information about the secondary structure of proteins in solution.
- It can determine the thermal and chemical denaturation of enzymes.
- CD is also employed in crystallization studies to detect any unfolding, large stretches of random coils or to get an idea about the secondary structure composition.



Typical CD spectra of proteins. Signal indicating helical (red), random coil (green) and sheet (light blue) structures.

Dynamic Light Scattering (DLS)

- Conformational heterogeneity results usually due to partial folding and nonspecific association and aggregation of protein molecules. This can be detected by analysing the size distribution and estimating the molecular weight from the hydrodynamic radius obtained from DLS experiments.

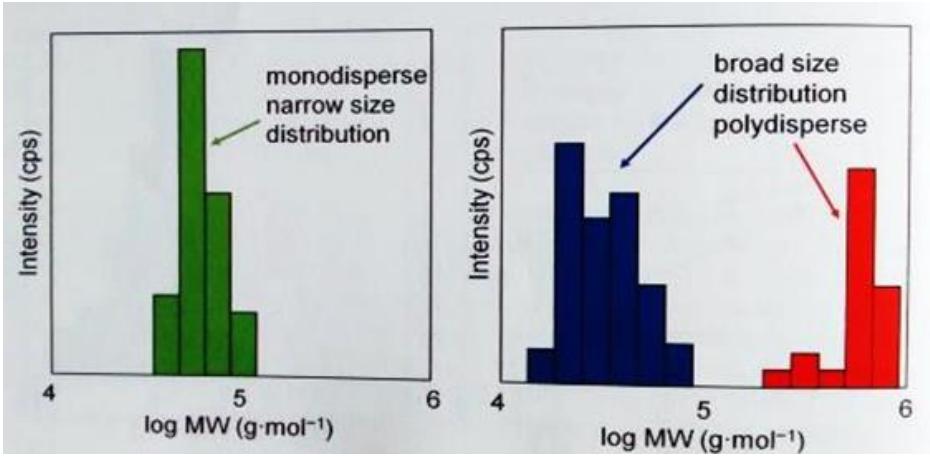
- Protein molecules undergoing random thermal motion move slow when they are large, this changes the Diffusion coefficient (D) which is used to estimate the Hydrodynamic radius (r_h) using the Stokes- Einstein relation:

$$r_h = k_B T / 6\pi\eta D$$

Where, η (eta) is the viscosity of the medium.

k_B =Boltzmann Constant, T =Absolute Temperature

- Some of the DLS instruments are used in-line with size exclusion chromatography to detect any multiple oligomer population in elution peaks.



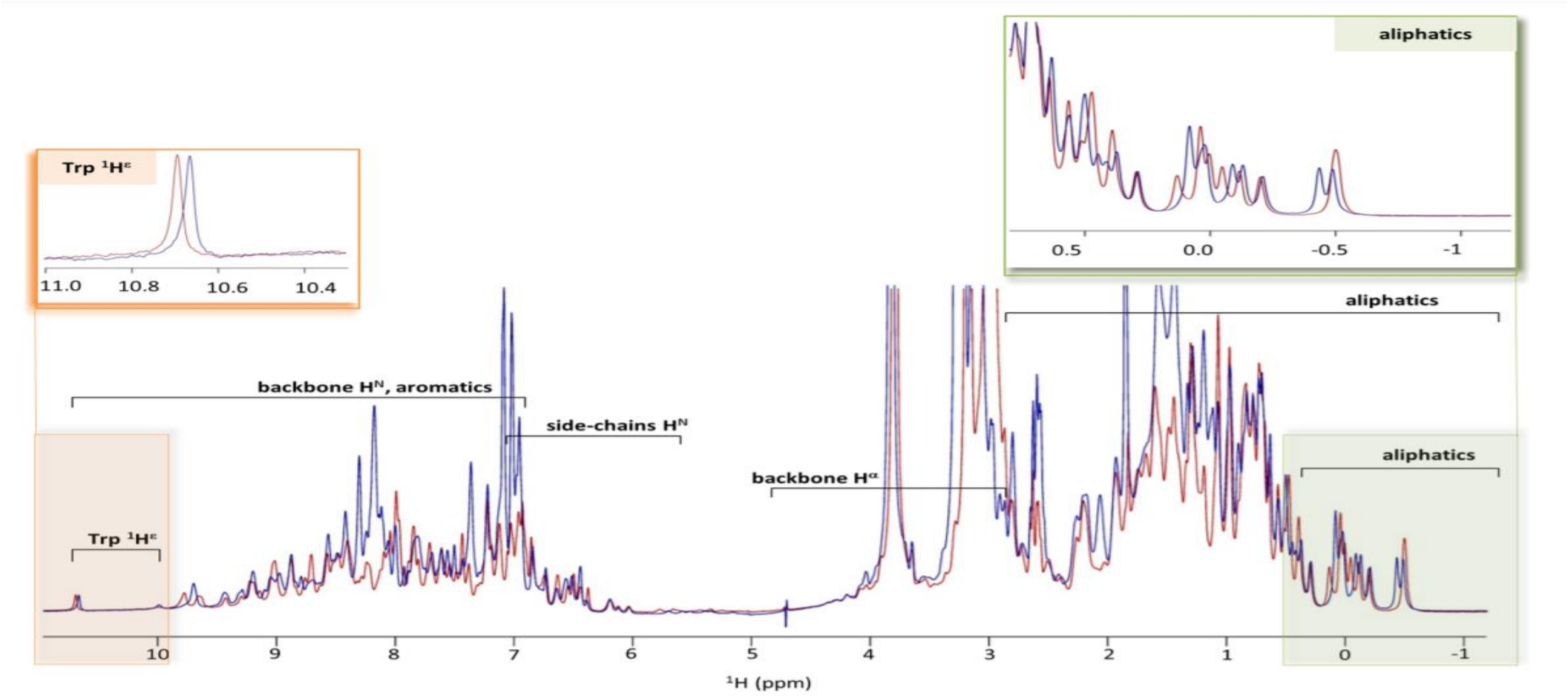
The left panel shows mono-disperse sample as size distribution is narrow,

The right panel shows the sample in poly dispersed state with two species having broad size distribution.

Nuclear Magnetic Resonance (NMR) Spectroscopy

- **By using NMR technique it is possible to detect folded proteins and unstructured regions or aggregates.**
- **A 1-Dimensional ^{1}H NMR spectrum can provide information about conformational state of the protein molecules, as the large soluble aggregates do not yield an interpretable high resolution spectrum with sharp resonance.**

Nuclear Magnetic Resonance (NMR) Spectroscopy



1D ^{1}H NMR spectra of a protein collected in the absence (red) and presence (blue) of a ligand.

Nuclear Magnetic Resonance (NMR) Spectroscopy

- **In 2-Dimensional hetero-nuclear single quantum coherence NMR spectra, the difference between unstructured and structured regions is very distinct.**
- **In 2D spectrum, the amide backbone is mapped according to the ^{1}H and ^{15}N resonance frequencies. These experiments require production of labelled proteins and large sample amount.**