BBCF Facilities and Equipment

Circular Dichroism (CD)

Chiral molecules absorb different forms of polarised light to different degrees. If the light is circularly polarised the effect is known as circular dichroism (CD). Measurement of these differences for an unknown compound allows us to determine which chiral moieties are present. Circular dichroism is measured using a spectropolarimeter (a highly sensitive spectrophotometer modified to produce circular polarised light).

Our CD Facility is based around a JASCO J-810 Spectropolarimeter. We have both cylindrical cell holders as well as a 1cm temperature controlled holder (-4°C 10 110°C). Titrations to examine protein-protein, protein-DNA and protein-ligand interactions can be carried out using a dedicated dual syringe JASCO auto-titrator.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR like CD provides information on the structure of biomolecules. In the case of proteins, FTIR provides high quality information on the amount and type of β-sheet structures. This is of particular interest in the study of protein aggregation and the formation of amyloids. FTIR can also provide estimations of the α-helical content within a protein. FTIR has also been applied to the study of reaction chemistries in enzyme catalysed reactions. Unlike CD, FTIR is insensitive to scattering and is therefore the technique of choice for the study of large molecular assemblies (membrane vesicles).

Applications of CD and FTIR

Proteins are made up of chiral amino acids which themselves are organised into chiral structures (e.g. α-helix and β-sheet). Circular dichroism and FTIR allow the quantities of components in a protein to be measured. This is particularly important in a number of situations:

- Structure determination of an unknown protein
- Determination of solution conditions to maximise protein folding
- Determination of solution conditions to minimise protein folding and aggregation
- To determine whether mutations have altered protein folding or stability

Analytical Ultracentrifugation (AUC)

Analytical ultracentrifugation (AUC) provides a tool to probe the masses of molecules in solution. The AUC utilises the centrifugal field developed in a centrifuge to make particles move with respect to a solvent. The AUC can measure this movement and these data can then be used to calculate particle mass.

Applications of AUC

Unlike mass spectrometer measurements, AUC provides a mass where non-covalent interactions are maintained. This allows the interactions between molecules to be analysed, providing both information on the size of molecular complexes and the strength of the interactions that hold the complex together.

The Beckman XLI instrument offers two types of experiment:

1. **Sedimentation Velocity (SV).** SV data provides information on the size distribution of particles in solution in terms of their sedimentation coefficient (s). In some cases an accurate molecular mass can be determined. SV experiments can also be designed to provide information on the strengths of molecular interactions.
2. **Sedimentation Equilibrium (SE).** SE data provides very accurate estimations of masses of particles in solution. SE is particularly suited to solutions containing a single species. SE is also the technique of choice for the study of molecular homo-associations (e.g. polymerisations), and in some cases can be used to study hetero-associations.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) monitors the fluctuation in scattered light in a solution caused by the Brownian motion induced movement of molecules. These data can be used to determine the molecular weight of the molecule.

Applications of AUC and DLS

- Assessment of purity
- Detection of aggregation
- Measurement of particle size
- Measurement of binding affinities

A typical application of DLS is the investigation of protein aggregation as a function of buffer composition. DLS reacts sensitively to protein aggregation in solution long before precipitation may become apparent. The technique is also suitable to examine protein self-association in relation to buffer pH, ionic strenght, etc, for instance ahead of NMR experiments or protein crystallisation efforts.

For more information on sample requirements please visit the **Birmingham Structural Biology Forum. (http://www.bsbf.bham.ac.uk/facilities/)**