

S005 Insight into protein:protein interactions from analytical ultracentrifugation

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The analytical ultracentrifuge – a high speed centrifuge with optical system(s) recording macromolecular concentration as a function of radial displacement - is a versatile tool permitting the analysis of a wide range of interacting systems. It is a free-resolution technique, not requiring immobilisation onto a surface nor a separation medium such as a column or membrane, with concomitant assumptions of inertness.

This talk outlines the basics of the two principal (and complementary) types of analytical ultracentrifuge measurement. Sedimentation velocity – which follows the evolution of the sedimentation concentration distribution with time and (at lower rotor speed) sedimentation equilibrium which involves the final steady-state concentration distribution following equilibration of sedimentation and diffusive forces. Complications through thermodynamic non-ideality effects can be dealt with both experimentally and computationally.

We will consider how the method – by itself or in conjunction with other methods - is suitable for the study of self-association phenomena, protein-protein interaction phenomena and protein interactions with other types of ligand – polysaccharides and glycoconjugates, DNA, RNA and small ligands, in terms of reversibility, stoichiometry and strengths ranging from very weakly interacting systems (interaction strengths or “ K_d ” values $>100\mu\text{M}$) to strong interactions $K_d < 1\mu\text{M}$) and the affects of external factors such as osmolytes and stresses caused by bioprocessing.