

Practical course ESSON

Proteins and nanoparticle assemblies and interactions by AUC and SEC/MALS

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Analytical Ultracentrifugation (AUC) sedimentation velocity with absorbance, interference and fluorescence detections, and Size Exclusion Chromatography coupled to static and dynamic light scattering, absorbance and refractive index detections (SEC/MALS) are two techniques that combine separation and analysis, in an absolute manner, of the mass and size of macromolecules in solution. They allow: assessing sample homogeneity, determining the association state of complexes, providing indications on the general shape of the macromolecules, analysing complex multi-component systems, on the basis of their density and optical properties (absorbance, refractive index and fluorescence), and quantifying association constants and weak inter-molecular interactions (that determine protein solubility).

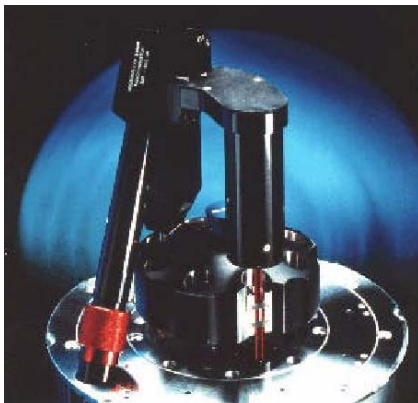
We will describe briefly the principles of the methods, what the detection systems used measures, some theoretical background and basic data analysis, with examples of applications. A sedimentation velocity experiment in AUC and a SEC-MALS experiment will be performed and analyzed with the participants.

The two methods are described on the web site of our platforms:

- <http://www.isbg.fr/caracterisations-biophysiques/>

References using the two techniques, for the characterisation

- of a membrane protein: Le Roy et al. (2015) *Methods Enzymol* **562** 257-86.
- of a monomer-dimer equilibrium: Echaliier et al. (2013) *PNAS* **110** 1273-8.



Rotor and optical arm of AUC



Picture of the SEC/MALS equipment