Analytical Ultracentrifugation

Analytical ultracentrifugation (AUC) has a broad applicability in life sciences and can be used to analyze a variety of molecules in a broad range of solvents. In AUC, molecules are characterized directly in solution, often under biologically relevant conditions. In contrast to many other methods, there are no complications caused by interactions with matrices or surfaces. Also, no immobilization or labeling is necessary for the analysis. Analytical ultracentrifugation is considered to be one of the most accurate methods for determination of molar mass of the molecule. Since it is a first-principle method, no calibration is required to determine the mass. Analytical ultracentrifugation is a non-destructive technique which is applicable to particles with molar masses ranging from several hundreds of Da (small peptides) to hundreds of MDa (viruses).

Two different but complementary methods are possible using analytical ultracentrifuge. Sedimentation velocity technique (SV) provides hydrodynamic information about the size and shape of a molecule, while sedimentation equilibrium (SE) is a thermodynamic technique which provides the information about the molecular weight.

AUC can be used for the study of:

- proteins and glycoproteins
- nucleic acids (DNA, RNA)
- polysaccharides
- viruses
- lipids and lipoproteins
- nanoparticles
- polymers, dyes (material chemistry)

Applications of AUC:

- sample dispersity (SV)
- oligomeric state/molar mass (SV, SE)
- size and shape of the particle, conformational changes (SV)
- analysis of aggregates (SV)
- study of biomolecular interactions - determination of stoichiometry, K_D (in the range of 10^4-10^8 M^{-1}) and molar mass of the complex (SV, SE)

Technical Specifications

Instrument: ProteomeLab XL-I (Beckman Coulter)

Features:

- maximum speed: 60,000 rpm
- temperature range: 4-40 °C
- absorbance optical system (ABS): wavelength range 190-800 nm
- interference optical system (IF): laser wavelength 660 nm, CCD camera resolution 2048x96 pixels

Accessories:

- four hole An-60 Ti rotor
- quartz and sapphire windows
- flow-through double-sector centerpiece cells for SV experiments
- six-channel centerpiece cells for SE experiments
- additional cells for special purposes

Data analysis:

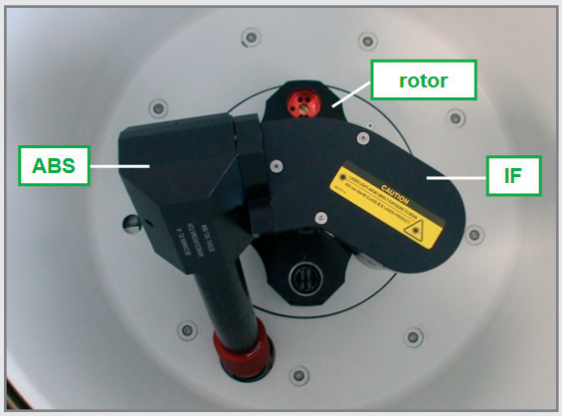
- Sednterp (prediction of partial specific volume, density and viscosity)
- SEDFIT, SEDPHAT, Ultrascan (packages for analysis of SV and SE experiments)
- GUSSI (graphical output)
- HydroPro, SOMO (hydrodynamic modelling)

Operational mode:

Analytical ultracentrifugation experiments are performed by Core Facility staff only.

analytical ultracentrifuge

optical systems in the rotor chamber

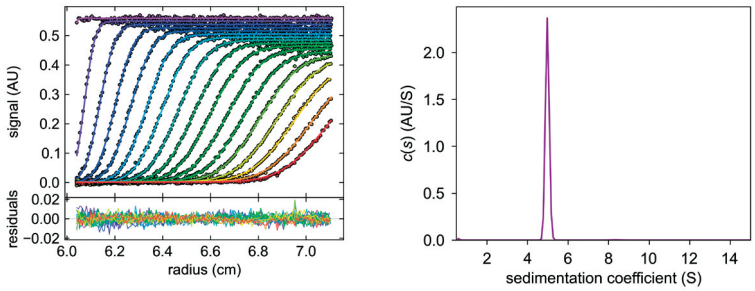
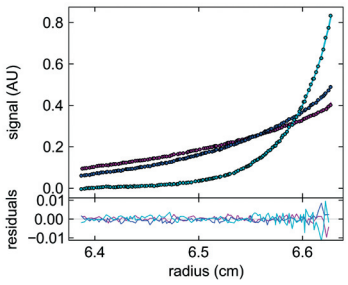


AUC accessories

An-60 Ti rotor

Established Methodologies and Provided Services

- Sedimentation velocity experiment (SV)
- Sedimentation equilibrium experiment (SE)
- Data analysis
- Training in data analysis

SV experiment (determination of sample dispersity, oligomeric form of protein)	**SE experiment** (molecular weight determination)
	

Sample Requirements

- both sample and reference buffer are required – samples should be equilibrated into the experimental buffer by dialysis or size-exclusion/desalting chromatography (crucial especially for the use of interference optical system)
- buffer (usually 10-20 mM): buffers should not absorb at wavelength where the sample is measured (e.g. phosphate buffers work well for absorbance optics, Tris and Hepes are tolerable at low concentrations for 280 nm)
- ionic strength (at least 100-200 mM NaCl, or even higher for highly charged proteins): sufficient ionic strength is needed to prevent electrostatic interactions that would affect sedimentation of proteins
- if possible substances generating density gradients (glycerol, sucrose, cesium chloride) should be avoided
- if the use of reductants (DTT, β-mercaptoethanol) is necessary, they should be used at low concentrations
- concentrations: dependent on absorbtivity, but usually no higher than 1 mg/ml

- volumes:
  - for SV experiment usually 450 µl of both the sample (optimal loading absorbance 0.5-1.0 OD for absorbance optics, optimal loading concentration >0.1 mg/ml for interference optics) and the reference is required
  - for SE experiment: at least 95% purity of a sample, usually 150 µl of both the sample (optimal loading absorbance 0.2-0.5 OD) and the reference

It is recommended to discuss the project and the details of the experiment (sample requirements, choice of method and optical system) with the Core Facility staff in advance.

Contacts

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