Optima AUC

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.

Technical specifications

Features:

- maximum speed: 60,000 rpm
- temperature range: 4-40 °C
- absorbance optical system (ABS): 190-800 nm. possibility to run multi-wavelength experiments
  interference optical system (IF): 660 nm,
  CCD camera resolution 2048x1088 pixels

Accessories:

- rotors: An-60 Ti (4 holes), An-50 Ti (8 holes)
- quartz and sapphire windows
- double-sector cells for SV experiments, centerpieces made of epon, aluminum (Beckman Coulter) or titanium (Nanolytics Instruments)
- six-channel epon centerpiece cells for SE

Data analysis:

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

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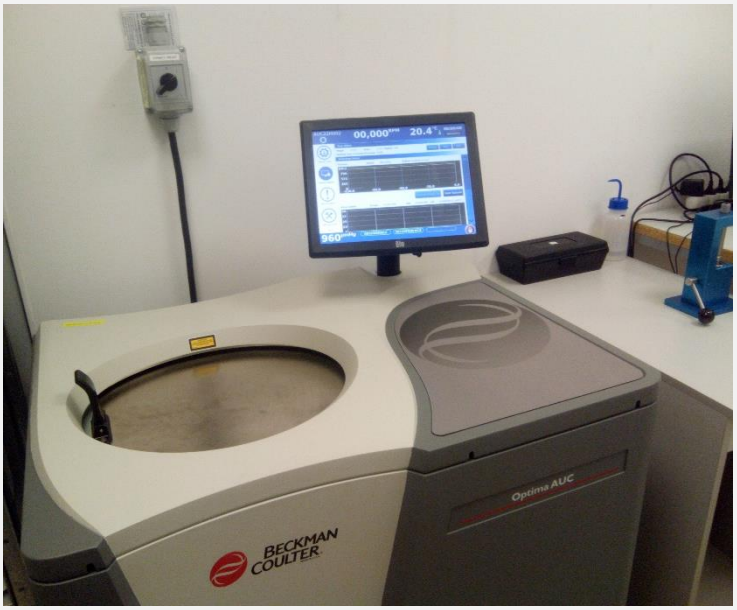
Instrument: **Optima AUC (Beckman Coulter)**

Optima AUC



rotor chamber with optical periscopes

cell accessories

An-50 Ti rotor

Instrument operation

Operational mode: Optima AUC is available in service mode only.

Provided services:

- Sedimentation velocity experiment (SV)
- Sedimentation equilibrium experiment (SE)
- Data analysis

Price of the service typically includes consultation about the experimental design, the experiment itself, and the data analysis. The customer receives written report with the results and other relevant information (usually within one week). Please note that assistance with the preparation of the publication or creating the figures is not a part of the service. Raw and processed data are available upon request.

Sample requirements

- **both sample and reference solution are required** - samples should be equilibrated into the experimental buffer by dialysis or size-exclusion/desalting chromatography (crucial especially for the use of IF system)
- **buffer** (usually 10-20 mM): buffers should not absorb at wavelength where the sample is measured (e.g. phosphate buffers work well for ABS 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 biomolecule
- 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 the aim of experiment, but usually no higher than 1-2 mg/ml
- **recommended volumes/concentrations for SV and SE experiments:**
  - for SV experiment: usually 450 µl of both the sample (optimal absorbance ~0.1-0.8 OD (ABS optics), concentration >0.1 mg/ml (IF optics) and the reference is required
  - for SE experiment: >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 measure at least 3 different concentrations to see eventual reversible interactions or sample non-ideality.

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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Instrument Location:

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