Analytical Ultracentrifugation (AUC) – Beckman Proteomelab XLI

In a nutshell: A gold-standard biomolecular analysis technique. A wide range of AUC-based methods are available for the analysis of interactions, oligomerization, composition, aggregation, membrane proteins, conformational changes, etc.

Services: This instrument is not user-accessible. We provide both data collection and data analysis services.

Location: Building 50, room 3331

Description: An analytical ultracentrifuge is equipped with absorption and interference optical systems that monitor the evolution of the sample concentration gradient during the course of sedimentation. To allow these measurements, samples are loaded into specialized optical cells and rotors. Sedimentation Velocity (SV-AUC) experiments are conducted at high rotor speeds to achieve a complete sedimentation of all sample components during the course of the experiment. Sedimentation equilibrium (SE-AUC) uses lower rotor speeds to form stable sample concentration gradients in the cell. SV-AUC has a high resolving power useful for the analysis of multiple sample components. SE-AUC is typically used when the number of sample species is limited, but it is a convenient method for obtaining affinity and oligomerization constants.

An important advantage of AUC is that in these experiments samples are analyzed in solution in their native state, exposed only to a centrifugal force. This way AUC analysis avoids the artefacts that may occur in the presence of a SEC stationary phase, immobilization, etc. AUC samples are typically not labeled with fluorescent probes, with the exception of specialized FD-AUC protocols.

Many analytical methods have been developed to obtain size and mass distributions, as well as interaction parameters from the AUC data files. The SV-AUC data also provides information on shape of the sample molecular components. AUC is a method based on first principles and does not use external standards. For this reason, AUC is often considered a gold-standard method of molecular characterization.

Typical applications:

- SV-AUC:
    - Oligomerization and interaction studies
    - Analysis of sample composition, aggregation, presence of trace components
    - Analysis of conformational changes
    - Analysis of viruses and nanoparticles

- SE-AUC:
    - Measurements of interaction and oligomerization affinities
    - Analysis of membrane proteins

Basic instrument specifications:

- Rotors: Analytical titanium 4- and 8-hole rotors.
- Temperature range: 4°C to 20°C
- Molecular size range: kDa to MDa
- Analytical AUC cells:
    - 1 mm, 3 mm, and 12 mm pathlength
    - 2-channel and 6-channel (SE only)
    - Sapphire and Quartz optical windows
    - Specialized centerpieces (gradient forming, meniscus balancing, titanium, etc.)
- Absorbance detection system:
    - Range: 190 nm to 800 nm
    - Typical noise: 0.005 OD
    - Radial resolution: 10 µm
- Interference detection system:
    - Laser wavelength: 675 nm
    - Typical noise: 0.003 fringe
    - Scan time: 5 s per scan

Sample requirements and recommended buffers: Buffers should be transparent at the wavelength of detection and should not contain readily sedimenting components. Consider using buffers with tabularized values of densities and viscosities of all components unless you plan to measure these values. Use salt concentration high enough to screen long-range interactions. PBS is a good buffer choice for most experiments.

For a good signal-to-noise ratio in a SV run, sample should be at a concentration sufficient to yield at least 0.1 OD or fringe value.

Minimum sample amount: SV experiments: 410 µL of sample and reference buffer are required to load the 12 mm pathlength 2-channel cell. SE experiments: We recommend using 2-channel cells loaded with approximately 120 µL sample volume (optimum volume depends on the sample molecular size). Required sample concentration depends on the intended protocol. As a general guideline, SV samples for absorption detection should have 0.1 to 1.2 OD in range.

Consumables: AUC does not require consumables. Cells and rotors are provided by the core facility.