Multi-Angle Light Scattering and Flow-Field Fractionation (FFF/SEC-MALS)

In a nutshell: Molecular separations and measurements of molecular mass, size, and shape of macromolecules in solution

Services: We offer a limited sample processing service using standard SEC-MALS and FFF protocols. This service is intended for the occasional users of this system. Researchers who expect to use this instrument extensively can obtain training and gain access to the instrument reservation calendar. Trained user can perform their experiments independently.

Location: Building 50, room 3226

Description: The Multi Angle Light Scattering (MALS) detector measures the static intensity of laser light scattered at different angles by the macromolecules in a solution passing through a flow cell. It provides the same thermodynamically-rigorous molar mass value as the sedimentation equilibrium experiment in the analytical ultracentrifuge, but in a much shorter time. The MALS detector measures the weight-average molar mass (M_w). To obtain a meaningful information on heterogeneous samples MALS has to be coupled with a separation device. There are two options available for this purpose: gel filtration columns (SEC-MALS) or the FFF separation device (FFF-MALS).

The Eclipse flow controller can easily switch between the SEC and FFF separation modes. Size-exclusion chromatography is a familiar technique that is typically used for the analysis of proteins and similar samples. The advantage of SEC is its simplicity, since there are no run parameters to adjust. FFF can also be used for protein analysis, but this technique is ideal for separation of larger particles or mixtures with a very wide range of molecular sizes that cannot be separated using SEC columns. FFF can separate all types of analytes in the size range from 1 nm to 1000 nm with no stationary phase. This includes the separation of whole plasma, viruses, gene vectors, nanodrug carriers, exosomes, and synthetic nanoparticles. FFF is also a separation method of choice for the aggregation studies.

Smaller molecules, including most monomeric proteins, scatter light isotropically. In this case, multiple MALS detectors increase measurement accuracy by providing additional signals for averaging. Molecules larger than approximately one-tenth of the wavelength of the laser light display anisotropic scattering. For these particles the radius of gyration (r_g) can be calculated from the angular dependence of the scattered light. The rg value is rigorously defined and can be used to calculate dimensions of molecules of known shapes. DAWN can measure rg values of approximately 10 nm and larger.

The size of smaller molecules can be obtained from the dynamic light scattering (DLS) that operates in parallel with the MALS detectors. The DLS detector is installed on the flow cell and analyzes the fluctuations of the scattered light to obtain the translational diffusion coefficient (D_t) for the molecules in solution. The hydrodynamic radius (r_h) of the molecules is then calculated using the Stokes-Einstein equation. The DLS detector can measure hydrodynamic radii as small as 1 nm, extending the range of the molecular size measurements of the MALS system.

Molar mass calculations require concentration information and this instrument is equipped with two concentration detectors: UV absorption and differential refractive index (d*RI*). Combination of MALS, UV, and d*RI* signals can be used to analyze conjugation ratio or encapsulation efficiency. This is an ideal method for the analysis of glycoproteins, lipoproteins, PEGylated proteins, membrane proteins solubilized in detergent micelles, VLPs, LNPs, etc.

Typical applications:

- Measurements of the native molecular mass and size distributions of proteins and various macromolecules
- Size separations in the 1 nm to 1000 nm range with no stationary phase
- Oligomerization, interaction, and aggregation studies
- Analysis of protein conjugates, micelle-solubilized membrane proteins, protein-nucleic acid complexes, nanoconjugates
- Measurements of the second virial coefficient, conformation, and polymer branching

Basic instrument specifications: This system consists of multiple components:

- MALS detector – DAWN:
  - Molar mass range: 1 Da to 1 GDa
  - Molecular size range (rg): 10 to 500 nm
  - Flow cell: 18 angles, temperature controlled
- DLS - Dynapro Nanostar:
  - Molecular size range (r_h): 0.5 to 100 nm
- d*RI* detector – Optilab:
  - Noise: ±1 x 10^{-9} *RI*U
- HPLC: 1260 INFINITY II system:
  - Sample handling: Autosampler and fraction collector
  - UV detector: 190-950 nm WL range, 2 AU signal range, 10 mm pathlength
- FFF controller – ECLIPSE:
  - Options: FFF-SEC switching, Dilution Control Module (DCM)
  - FFF resolution size range: 1 – 1000 nm
  - Available FFF channels:
    - Standard channel with 350 µm and 250 µm channel height options and temperature control
    - Dispersion inlet channel – aggregation prone samples
    - Semi-preparative channel – up to 1 mg sample capacity
- Available SEC columns (all columns have 300 mm length):

column name	Diameter [mm]	Separation range [Da]	Particle size [µm]	Pore size [Å]
WTC-015S5	7.8	0.5k - 150k	7	150
WTC-050S5	7.8	15k - 5M	5	500
WTC-050N5	4.6	15k - 5M	5	500
TSKgel-G4000SWxL	7.8	up to7M	8	450
TSKgel-G3000SWxL	7.8	up to 500k	5	200
TSKgelSuper-SW2000W	4.6	up to 150k	4	125

Sample requirements and recommended buffers: The standard running buffer in our system is PBS pH 7.4. If the buffer will be used for several days, it should be substituted with 0.05% sodium azide. Samples for MALS analysis should be dialyzed in the running buffer. Unmatched buffer components injected with the sample, especially additional salts or glycerol, will result in large refractive index changes that affect the molar mass calculations. Different running buffers can be used for MALS analysis, but the buffer changing procedure is time consuming. It will require inline filters and membrane replacement followed by system equilibration. The users are responsible for changing the running buffer back to water after their experiments when a custom buffer was used. All buffers should be freshly filtered and degassed.

Sample preparation will slightly differ for SEC and FFF. SEC samples must be absolutely free of particles and aggregates. To this end, samples must be either filtered or ultracentrifuged directly before injection. The recommended filtering device is a Whatman Anotop-10 (0.02 µm small syringe filter). Alternatively, the Millipore Ultrafree-MC GV (0.22 µm centrifugal filter) allows filtration of smaller volumes. Samples that do not tolerate filtering can be ultracentrifuged at high speed. FFF can tolerate aggregates, but large particles that can clog the flow controllers should be removed.

The standard column used in the facility for protein analysis is the TSKgel-G3000SWxL (working pH range: 2.5–7.5). Other columns are available, and dedicated, clean users' columns can also be mounted if necessary. Please discuss column selection with us when you plan your experiments.

Minimum sample amount: The autosampler loop can hold up to 100 µL sample volume. Typical sample injection volume for SEC-MALS analysis is 50 µL, and 20 µL for FFF-MALS analysis with the standard channel.

The light scattering intensity is proportional to the product of the protein molar mass and concentration. As a reference, approximately 10-20 ng of BSA should be sufficient to obtain a good quality MALS data from a well-prepared sample. This corresponds to a 50 µL injection of 0.2-0.4 mg/mL of BSA. Smaller proteins require higher sample concentrations, with a practical analysis limit of approximately 10 kD.

In FFF runs with standard and semi-preparative channels during the focusing step the sample concentration will increase to values higher than the loading concentration. The dispersion inlet channel can be used to avoid this, for example in analysis of aggregation-prone samples.

Consumables: We provide channel membranes, filters, vials, and other consumables. Membranes and filters do not have to be changed after every run, and typically there are no consumable charges for standard analysis. If needed, consumables are provided at the manufacturer's prices.

SEC columns have a limited lifetime, especially when used for analysis of low-quality samples. If the sample recovery in a MALS run is lower than 75%, or if there is a system pressure increase, users will be charged a cleaning fee or column replacement fee, regardless of the subscription status.