Dynamic Light Scattering (DLS)- DynaPro NanoStar II & Mobius

In a nutshell: Fast measurements of the molecular sizes of proteins, micelles, liposomes, and nanoparticles. Detecting aggregation, measuring molecular mass and nanoparticle concentration.

Services: Biophysics Facility offers DLS as an open-access instrument. First-time users must complete a short training session before gaining access to the instrument reservation calendar. Training includes DLS analysis of small- and large-molecular size standards.

Location: Building 50, room 3123

Description: DLS is a light-scattering technique that allows the estimation of the hydrodynamic radius (molecular size) of particles. Solutions of small molecules undergo rapid Brownian motion and generate fast fluctuations of the scattered light. Conversely, large macromolecules diffuse slowly and generate slower light fluctuations. DLS analyses these fluctuations to find the autocorrelation function. From the analysis of the autocorrelation function, we obtain the diffusion coefficients of the scattering molecules. The diffusion coefficient of a spherical particle is directly related to its radius as described by the Stokes-Einstein relationship: $D=kBT/6\pi\eta r$. Thus, an equivalent radius of a spherical particle (the so-called hydrodynamic radius, R_h) may be associated with the diffusion coefficient measured for the molecule, and this is the value reported by the DLS instrument.

Different types of the autocorrelation function decay analysis are used for the monomodal and multimodal particle distributions. The most robust and numerically stable DLS result is the z-average radius. This is the intensity-weighted mean R_h obtained from the cumulants analysis, and it is used for monomodal size distributions. Samples are usually considered monomodal when the polydispersity index *PD* obtained from the cumulant analysis is smaller than 15%. For multimodal samples with larger *PD* values, a distribution of particle sizes can be obtained from the autocorrelation function by applying the regularization analysis. In practice, regularization analysis can resolve species with four- to five-times molecular weight difference.

For multimodal samples, the primary size distribution reported from DLS is the intensity-based regularized distribution. The DLS software has options to transform this distribution to mass- or number-distributions by applying certain assumption about the scattering macromolecules. These transformations are not always reliable and their results can be easy overinterpreted. The volume and number size distributions should only be used to roughly estimate the relative amounts of material in the distribution peaks.

Mobius, our second DLS instrument, is a specialized DLS system with phase analysis light scattering (PALS) capability. This method is used for electrophoretic mobility measurements to analyze the zeta potential of particles in a wide molecular size range. The Atlas module can pressurize the Mobius flow cell for measurements in solvents with high conductivity, such as the physiological saline.

DLS makes "bulk" type-measurements analyzing light fluctuations from a small scattering volume in the sample. Biophysics core also has the NTA (nanoparticle tracking analysis) instrument that measures particle diffusion (and in consequence, hydrodynamic radius distributions) by following Brownian motion of the individual particles.

Typical applications:

- Measurements of the hydrodynamic radius of proteins, liposomes, viruses, nanoparticles, etc.
- Assessing the aggregation state of a sample
- Measurements of macromolecular interactions (detection of macromolecular complexes)
- Molecular mass measurements with the static light scattering capability
- Measurements of the thermal stability of molecules
- Concentration measurements of particle solutions (viruses, lipid nanoparticle, etc.)
- Electrophoretic mobility, net charge, and zeta potential analysis (Mobius)

Basic instrument specifications:

- Size range (DLS hydrodynamic radius, Rh): 0.5 – 1,000 nm
- Molar Mass range (SLS): 300 Da to 10 MDa
- Temperature range: 4°C – 120°C, up to 15°C/min ramp rate
- Particle concentration range: 10^5 – 10^{15} particles per milliliter
- Sample volume:
    - 2 µL (quartz cuvette)
    - 4 µL (disposable cuvette)
    - 65 µL (Mobius dip cell for zeta potential measurements)
    - 170 µL (Mobius flow cell for zeta potential measurements)
    - 50 µL disposable cuvettes and large volume generic glass cuvettes can also be used
- Non-destructive, fast measurements: less than 2 min per experiment

Sample requirements and recommended buffers: DLS can use a wide range of buffers and solvents. It is very important to use only freshly filtered buffers and dust-free labware for DLS sample preparation. The standard DLS buffer is the PBS. For custom buffers their viscosity (for DLS) and refractive index (for SLS) will need to be provided. Unless DLS is used for the aggregation analysis, all aggregates and foreign particles (dust) must be removed from samples. We recommend filtering samples and buffers with the Whatman Anotop-10 filters (0.02 µm pore size) or the high-speed ultracentrifugation just prior to the DLS measurements.

Minimum sample amount: The minimum sample concentration is particle size dependent; large particles scatter strongly and require much lower concentration than small molecules. For orientation, the minimum measurable lysozyme (14 kDa) concentration is 0.1 mg/mL. Analysis of multimodal distributions requires higher sample concentrations.

Consumables: We stock disposable DLS cuvettes and the small-pore filters. Consumables are provided at the manufacturer's prices.