Light Scattering

Light scattering is caused by the interaction of light with dispersed particles (typically in solution), while organized particles (typically in crystal) result in diffraction phenomenon. The intensity of the scattered light depends on the size and the shape of the interacting particles. The experiment using visible light may be performed in two different modes: dynamic and static light scattering. Dynamic light scattering (DLS) measurement analyses the time variance in the scattered light. Static light scattering (SLS) measurement depends on the analysis of light scattering at various angles with respect to the incident beam.

DLS/SLS can be used for:

- Determination of sample homogeneity and polydispersity
- Observation of protein oligomerization or aggregation in time or as a result of buffer composition
- Analyzing of thermal stability of biomacromolecules differentiating pure unfolding from aggregation
- Protein oligomeric state determination (applicability may be limited)
- Particle size / molecular weight estimation (0.5 – 5000 nm / 500 Da – 1 MDa) – applicable not only to biomacromolecules, but also viruses, colloids, liposomes, metallic nanoparticles, quantum dots

Technical equipment

Instrument: Delsa Max Core (Beckman Coulter)

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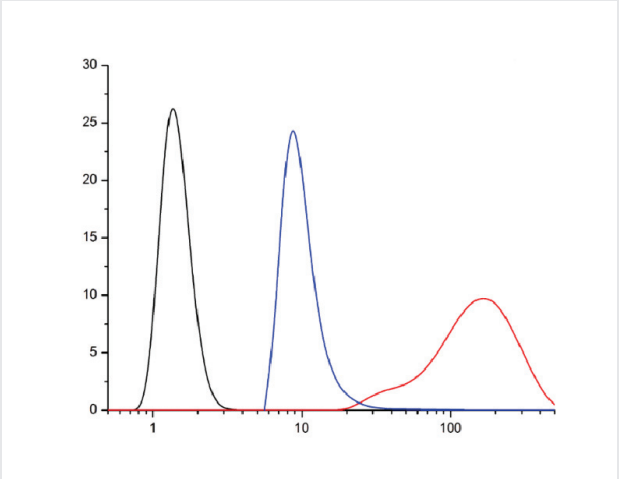
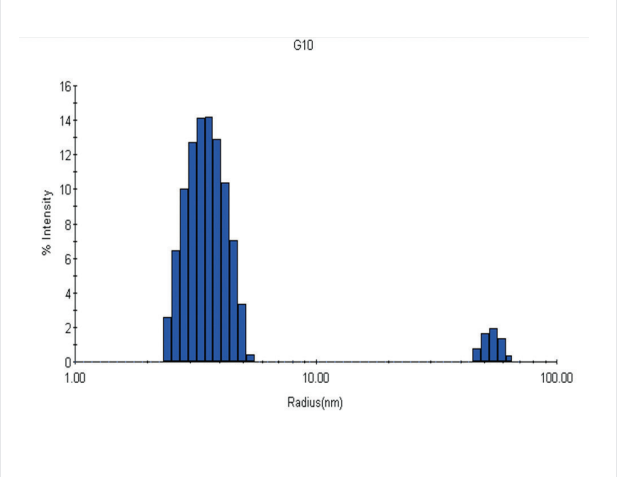


Size distribution curves



Size distribution curve and effect of sample polydispersity on its shape

Features:

- Dynamic and/or static light scattering measurement at 90°, with minimum measurement time of 1 sec and data acquisition time in range 1 -3 600 sec.
- Laser wavelength of 658 nm
- Fast data acquisition – less than 2 minutes per sample for typical DLS set-up
- Temperature range between 20°C and 80°C (lower temperatures down to 4°C on special demand)
- Possibility to programmably change the temperature during the experiment
- Plastic cuvettes for DLS with sample volume from 5 ul. Quartz cuvettes for SLS with sample volume from 45 ul.

Instrument: DynaPro Plate Reader (Wyatt)

Features:

- Accommodates 96, 384 and 1536 well plates
- Dynamic light scattering measurement at 158°.
- Laser wavelength of 830 nm
- Temperature range between 20°C and 70°C
- Possibility to programmably change the temperature during the experiment

Operational mode:

Light scattering measurements are performed by the users themselves. New users are obliged to attend special training that can be ordered as a service.

Operating software and data evaluation:

Both instruments utilize special software for data analysis. Data can be exported as images or tables for further processing.

Sample requirements - importance of sample preparation

- For DLS measurement at DelsaCore, at least 5 ul of the sample is required (20+ ul is recommended). DLS measurement in DynaPro Plate Reader requires at least 80 ul of each sample in 96well plate (20-50 ul in 384 well plate, 6-10 ul in 1536 well plate; 384 and 1536 plates currently not available on site).
- Sample concentration of 0.1 mg/ml and higher are generally suitable. Large particles at lower concentrations give strong enough signal as well.
- Detergents at higher concentrations should be avoided. Above MIC, the micelle formation interferes with the measurement of biomacromolecule size.
- For size analysis (especially for SLS), the sample should be filtrated or at least spin down prior to measurement. High purity and homogeneity is crucial for reliable result.
- For Static light scattering measurement, extra volume of buffer is necessary for cuvette calibration.

Contacts

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