Fast & reliable characterization of molecular weight, shape and polydispersity of biological particles by combined dynamic and static light scattering

Introduction

Light scattering in life science applications

Many questions in modern life sciences ask for precise characterization of particles. These particles range from protein, DNA, RNA to complex macromolecular complexes and mixtures. Their analysis often requires determination of the exact molecular weight (in Dalton), shape/size information (in nm) and polydispersity. These parameters in hand allow great assessment of the samples quality for basic research as well biotechnological and pharmaceutical applications (see below). Light scattering methods offer an elegant and fast way to characterize particles in molecular biology, biotechnology and medicine.

A very short survey into light scattering methods

When light hits a particle it scatters in all directions (Rayleigh scattering) as long as the particle size is small compared to the wavelength of the light. This principle underlies the static- and dynamic light scattering techniques (DLS and SLS, respectively).

In SLS measurements, the intensity of the scattered light (at one or more angles relative to the incident beam) is used to determine the absolute molecular weight of a particle (at a give concentration) in solution. SLS also allows determination of the root mean square radius (also radius of gyration) of solute particles and determination of the second virial coefficient (A_2).

DLS uses another phenomenon associated to light scattering by particle solutions. When a monochromatic and coherent light source (i.e., laser) is used the intensity of the scattered light fluctuates over time. This fluctuation is caused by the Brownian motion of the particles that constantly changes distance of the scattering particles over time. As a consequence, the scattered light undergoes destructive and constructive interference with the surrounding particles, which causes the fluctuations in scattering intensity. This phenomenon is used during Dynamic Light Scattering (DLS). Information on particles is derived from an autocorrelation from the intensity trace recorded during the experiment. Based on the Einstein-Stookes equation, DLS measurements then allow information on polydispersity and by assuming a particle shape (usually as sphere) also particle size and molecular weight. The later parameters become biased if the particle has a shape different from the assumed one. Also and as described below: precise determination of the particle concentration is essential to correct interpretation of light scattering experiments.

The Beckman Coulter DelsaMax Core combines dynamic and static light scattering (DLS and SLS, respectively) to obtain molecular weight, shape information and polydispersity of particle samples. Polydispersity is an important criterion to judge homo- and heterogeneity of the particle sizes (e.g., different oligomerization states of protein complexes), which is not easily accessed by other techniques. Taken together, parameters obtained by DelsaMax Core characterize samples for composition and quality to perform further experiments and applications.

Our test case: A hexameric protein

The protein FliI served as case to investigate the potential of DelsaMax Core. Our protein regulates the assembly of flagella which act as motors propelling bacteria through solutions. In nature this protein exists as a hexamer – which means: 6 FliI proteins arrange into a ring-like stable structure. In this study we assess the protein folding and quaternary structure of recombinant FliI was that was expressed and purified in *E. coli*. The question we asked was does the purified recombinant FliI form an hexamer as the native endogenously produced protein? The DLS and SLS techniques combined in DelsaMax Core provide an elegant and fast way to characterize the molecular weight, size and polydispersity of our protein sample.

Materials and Methods

Instrument Setup and Calibration

The instrument was calibrated one time according to the DelsaMax Core's operator's manual. Molecular weight of the sample was determined in kDa (kilo Dalton), the diameter in nanometer and the polydispersity index is a ratio. No blanking or calibration prior to the experiment was necessary.

Procedure

Protein purification

FliI was produced in E. coli BL21(DE3) and purified in a two-step protocol that consisted of a metal-affinity- and a size exclusion-chromatography step.

Sample & instrument preparation

For good measurement quality the sample was filtered through a 0.1 µm pore-sized filter to putative remaining dust particles and other high molecular weight compounds. The DelsaMax Core was switched-on 20 minutes before the measurements to ensure stability of laser and temperature during experiments.

Precise determination of protein concentration

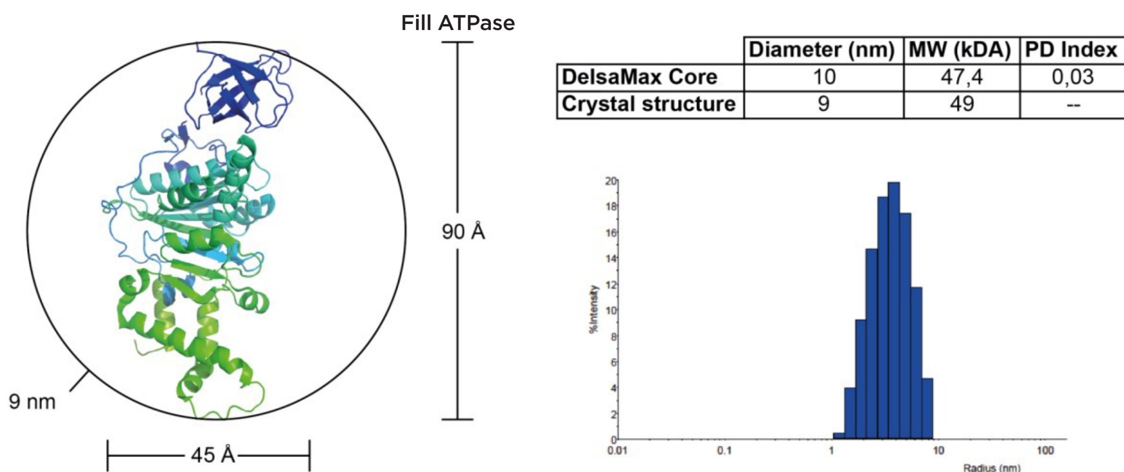
Most important to the correct interpretation of obtained data is a correctly determined sample concentration. A not correctly determined protein concentration will largely bias interpretation of results. Protein concentration was determined at a wavelength of 280 nm in spectrophotometer employing the Lambert-Beers Law with the proteins extinction coefficient calculated by the ProtParam tool at www.expasy.org. Protein determination by the Bradford assay was not suitable in our case because of high variance in the detection data.

Sample analysis

Prior to measurements the cuvette was carefully cleaned with filtrated, bi-distilled water and flushed with buffer. To obtain suitable results with DelsaMax Core, 50 – 100 µl of sample at concentrations between 5 – 20 mg/ml were employed. The measurement typically takes 2 – 5 minutes to achieve suitable results.

Results

The polydispersity index of the FliI sample was 0.03 indicating a highly homogeneous sample with only one particle size. The molecular weight was determined with 47.4 kDa, which corresponds well with the calculated molecular weight of 49 kDa of a monomer. The diameter of the sample was 10 nanometers, which matches well to the 9 nanometers from the crystal structure (Figure). Taken together, the measurements show that the FliI protein is a monomer and as such not in its biologically active hexameric form. We therefore will assess ways to produce the biologically active form of the protein. For these investigations, DelsaMax Core will be a great benefit to assess whether our material has the molecular weight and size of the biologically active form.



	Diameter (nm)	MW (kDA)	PD Index
DelsaMax Core	10	47,4	0,03
Crystal structure	9	49	--

Conclusions

DelsaMax Core is powerful stand-alone for the fast and reliable characterization of biological samples during daily labwork. The DelsaMax Core allows an easy and standardized way of lab work to assess the above named parameters. Taken together, DelsaMax Core allows simultaneous analysis of dynamic and static light scattering with two independent detection systems. It can measure molecular weight between 300 and 10E6 Da and particle size between 0.4 and 10.000nm at sample volumes as low as 1.25 µL.

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