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Size-exclusion Chromatography Coupled to Multi-angle Light Scattering (SEC-MALS)

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We use this protocol and it's working

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Abstract

Size exclusion chromatography coupled to light scattering (SEC-MALS) is a convenient method to determine the molecular weight of proteins and protein complexes in solution. It exploits the intensity of the isotropic scattering of proteins measured at several angles as a function of their concentration. Accurate measurements of concentration are determined in-line during the experiment by measuring the differential refractive index using an Optilab Refractometer. Here we describe a generic method for collecting data that can be exploited for diverse proteins in order to understand their size/stoichiometry in solution.

Materials

1. Consumables

- 3 or 10 kDa MWCO Amicon Ultra-15 concentrator tube, Merck

2. Reagents

- Purified proteins for analysis
- Gel filtration buffer (20 mM Tris pH 8.0 at 4ºC, 150 mM NaCl, 1 mM DTT)

3. Equipment

- FPLC system (i.e., AKTA basic or AKTA purifier, Cytiva)
- Size exclusion chromatography column: Superdex 75 10/300 GL (Cytiva)

Note

Depending on the size of a protein or protein complex a different type of column is recommended, ie. the superdex 200 10/300GL (Cytiva)

- MiniDAWN MALS spectrometer (Wyatt)
- Refractometer Optilab rEX (Wyatt)
- Wyatt Comet sonicator (cleaning device for the flow cell of the MiniDAWN spectrometer)
- PC with the AKTA control software (UNICORN 5.11) and the SEC-MALS control and analysis software (ASTRA 4.9)

Method

1 Connect the “miniDAWN” light scattering spectrometer and the refractometer to the FPLC System as indicated in Fig. 1.

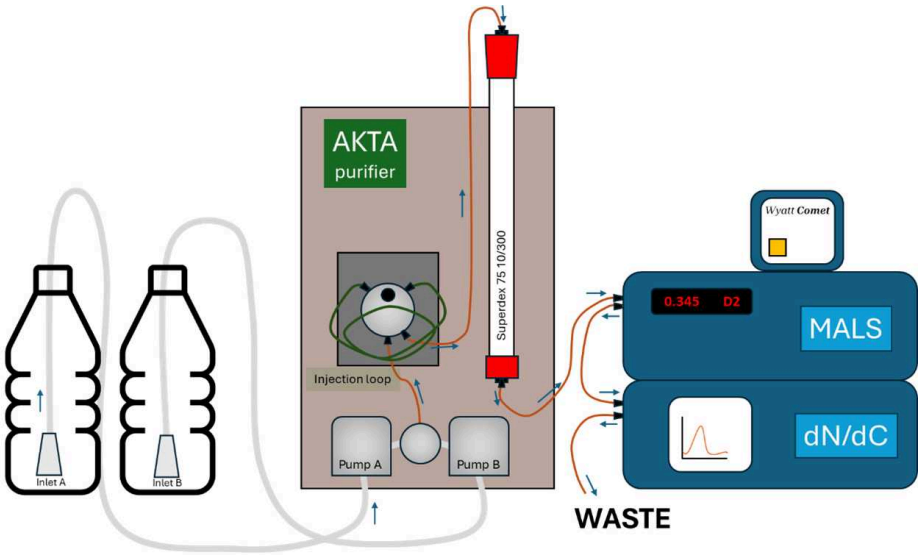

Figure 1: Setup SEC-MALS. The AKTA purifier is equipped with a 0.5 ml injection loop and superdex 75 10/300 GL size exclusion column. The column outlet is connected to the MiniDAWN light scattering spectrometer followed by a refractometer, which is used to collect information on the protein concentration.

2 Equilibrate column into Gel filtration buffer until signal on detector 2 (90° angle) of the miniDAWN is stable (signal between 0.3 and 0.4 with minimal fluctuations).

> Note
>
> This can take between 1 to 3 column volumes (1 CV = 24 ml)

3 During equilibration, purge cell of refractometer to minimize mismatch.

4 To improve signal stabilization, use the “Comet” sonicator. For conducting an experiment, it needs to be switched off.

5 Run the FPLC at a flow rate of 0.6 to 0.7 ml/min, maximum system pressure 1.8 MPa.

6 Concentrate/dilute protein to approximately 1 mg/ml.

Note

The smaller the protein is, the less it scatters. For example, our TMEM55B 80-166 2CysMUT construct has a MW of 9.8 kDa, which is at the lower end of what SLS can be used for. Smaller proteins might not scatter enough to produce a usable signal. For small proteins, higher concentrations help with the signal strength.

7 Attach a 0.5 ml injection loop to the FPLC System. Flush with a few ml of gel filtration buffer.

8 Load injection loop with the protein or protein complex.

9 Check settings of the ASTRA software:
- System setup: Flow Rate (i.e., 0.6 ml/min); leave the other parameters unchanged.
- Collection Setup: dn/dc (mL/g): 0.185; Collection Duration: 35 ml; Collection interval (sec) 1.

10 Start collecting light scattering data using the ASTRA software and set the FPLC software from 'load' sample to 'inject' directly after another.

11 Collect data for an appropriate time to allow proper baselining for analysis.

12 Analyze data using ASTRA.