APPLICATION NOTE

AN1608: Transient protein self-association determined by SEC-MALS

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Introduction

Domain antibodies (dAbs) are the smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy (VH) or light (VL) chains of human antibodies. Different dAbs can show different degrees of self-association which drives the need for a cheap, quick—yet robust—technique to study self-association behavior in drug discovery. This study outlines the use of SEC-MALS to determine self-association properties and validates the work by comparison with Analytical Ultracentrifugation (AUC).

Materials and Methods

A Shimazdu LC-20AD Prominence HPLC was used in series with miniDAWN® and Optilab® for this study. The columns used were TSK-GEL3000SW$_{XL}$ and Superdex S200. Data were collected and analyzed in ASTRA® software to determine weight-average molar mass at each concentration.

dAb_1 was expressed, purified and dialyzed into PBS, pH 7.4 and submitted to SEC-MALS and AUC. For MALS, the sample was concentrated to 2.4, 7.0, 75 and 130 mg/mL and followed by appropriate dilution to obtain 9 different concentrations which were run on SEC-MALS (Table 1).

The equilibrium dissociation constant for dimerization $K_{2,1}$ was determined by plotting a graph of M_w versus log-concentration obtained from SEC-MALS mid peak.

Results and Discussion

Figure 1 shows the overlay of dRI and light scattering signals for five injections corresponding to stock concentrations from 5 mg/mL to 120 mg/mL (additional injections at lower concentration were also performed, per Table 1). Dilution on the size-exclusion columns decreases the peak concentration by approximately 7-fold and other areas of the peak have respectively lower concentrations.

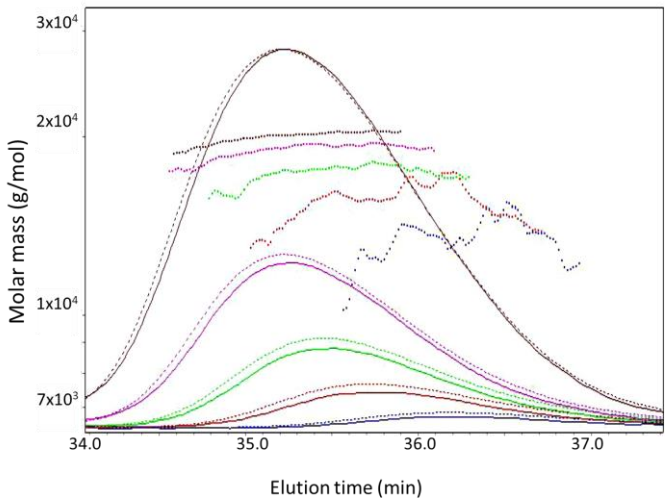


Figure 1. Overlay of the five different sample results from SEC-MALS highlighting the 'Determined Mw'. Samples with stock concentrations 5mg/mL, 15mg/mL, 30mg/mL, 60mg/mL and 120mg/mL, representing the smallest to biggest peaks respectively.

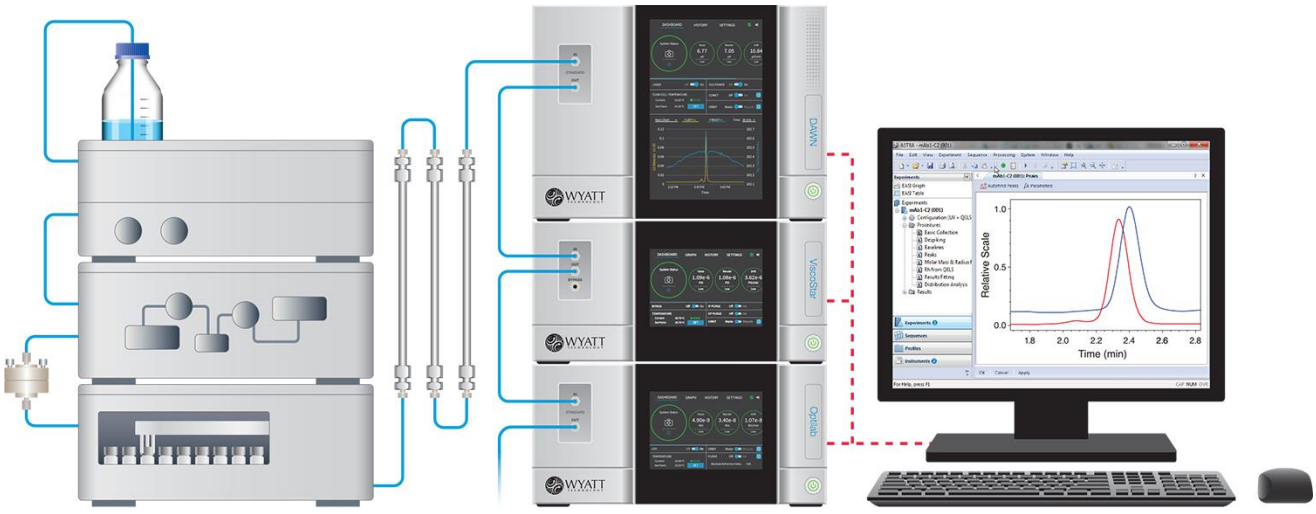
Due to dynamic monomer-dimer equilibrium, the molar mass determined by MALS is not uniform across the peak. For best accuracy, the calculations are performed using the concentration and molar mass values determined at the maximum of each peak. The $K_{2,1}$ values for dAb_1 from SEC-MALS was calculated as 300 µM, comparing well to 391 µM from the AUC experiment.

While AUC is often considered the gold standard for the determination of self-association in solution, scientists are looking for alternatives due to AUC's high costs, low throughput and expertise requirements. Here, SEC-MALS has shown excellent agreement with AUC results and can be used as a cheaper, easier and quicker alternative.

Another light-scattering method that is suitable for characterization of transient self-association beyond monomer-dimer equilibrium is CG-MALS. With similar low cost and ease of use, CG-MALS provides extended analysis of self- and hetero-association, similar to equilibrium sedimentation by AUC.

Stock Conc. (mg/mL)	Sequence Monomer Mw (kDa)	Sequence Monomer Mw (kDa)	
		Concentration mid-peak (mg/mL)	Determined Mw
0.1	11.634	0.015	11.5
0.5		0.048	11.26
1		0.092	11.9
2		0.185	12.85
5		0.369	13.54
15		1.03	15.59
30		2.11	17.35
60		4.08	18.92
120		9.05	19.92

Table 1. List of the samples injected, M_w and concentration as determined by SEC-MALS. A plot of M_w against log(concentration) is used to determine K_d



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