Introduction

The Wyatt DynaPro Plate Reader uses dynamic light scattering and static light scattering of analytes in solution to obtain physical properties of biomolecules like the second virial coefficient and diffusion interaction parameter. The DynaPro Plate Reader can also be used to study molecular interactions such as aggregation and dimerization.

The Dynamics software allows for the rigorous interpretation of data. Different fits (Cumulants vs Regularization) can be applied to the obtained autocorrelation functions to obtain results that best describe your sample.

Features of the DynaPro Plate Reader

Large experimental temperature ranges

Temperature controls ranging from 4°C - 85°C allows for studying protein stability. Various ramp rates available.

Hydrodynamic radius determination by DLS

Hydrodynamic radius determination ranging from 0.5-1000 nm as well as sample polydispersity (%Pd) determination.

Molecular weight determination via SLS

Protein molecular weight determination ranging from 1-1000 kDa.

Minimal sample required

Have sensitivity down to 0.125 mg/mL protein, with minimal sample volumes of 4 µL. All measurements carried out in 96, 384 and 1536-well plates allowing for the study of proteins without modifying the samples.

Detecting molecular interactions

Allows detection of multispecies processes like aggregation which can be used for applications like protein crystallization or buffer screening.

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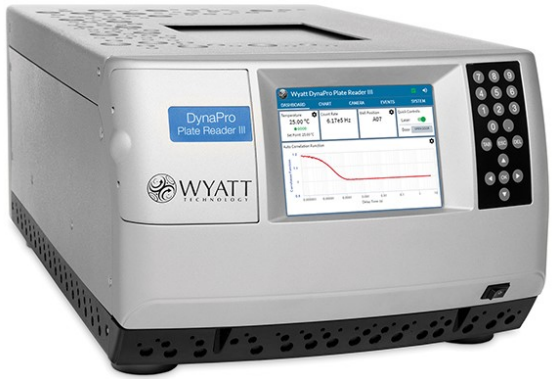


Wyatt DynaPro Plate Reader III for DLS/SLS

Application Note #2:

MW-S, A_2 and k_D Determination by SLS and DLS

- A_2 (B_{22}), the second virial coefficient is a measure of solute-solvent interaction.
- k_D, describes system-dependent self-interactions between molecules.
- MW-S is an average molecular weight that considers all species present in the sample.
- Determination of attractive/repulsive forces between sample molecules.

Wyatt DynaPro Plate Reader III— MW-S, A_2 and k_D of BSA

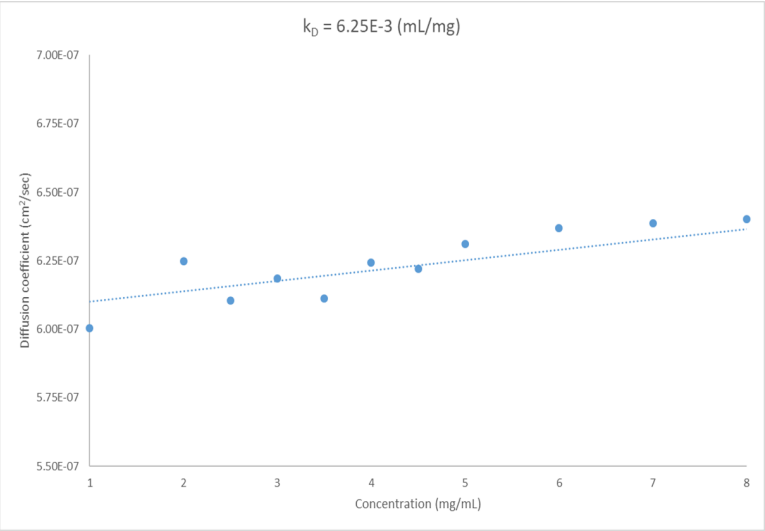
Determining MW-S, A_2 and k_D for BSA by DLS/SLS

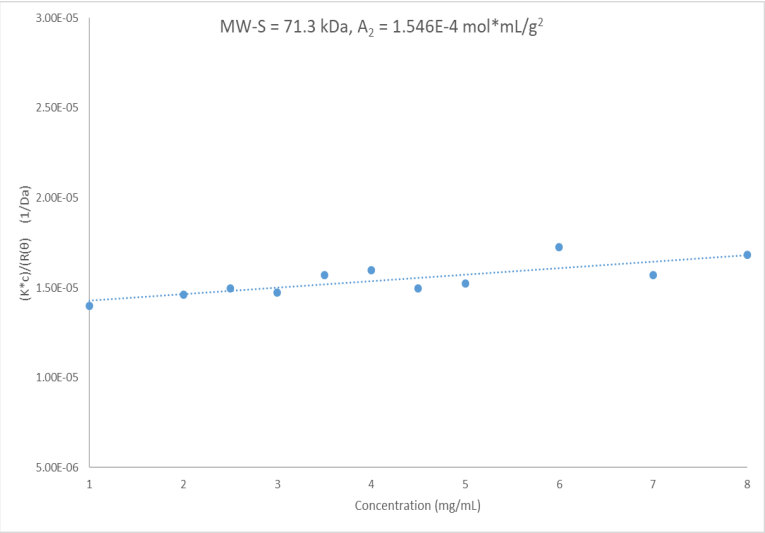
Protein sample: BSA (1-8 mg/mL)
Buffer: 1x PBS pH 7.4
Number of acquisitions: 15
Acquisition time: 5 seconds
Temperature: 25°C

To determine the MW-S, A_2 and k_D of BSA, a concentration series of BSA was prepared, ranging from 1 to 8 mg/mL and were read in four replicates using both DLS and SLS. The samples were centrifuged at 17,000 RPM for 15 minutes to remove aggregates and the A280 values for each sample was read to get accurate protein concentrations. Using DLS, the k_D was obtained, and by using SLS, the MW-S and the A_2 were obtained.

The diffusion interaction parameter (k_D) describes how the diffusion rate of the sample is impacted by concentration as a result of intermolecular forces. To get the k_D value, the diffusion coefficients of a samples are plotted against sample concentration as shown to the right. The slope of the plotted line is the k_D value of the sample set as obtained by re-arranging the equation below:

$$D_t = D_0 (1 + k_D c)$$





The obtained k_D and A_2 values were plotted as averages of scans done in triplicate and are in good agreement with values from the literature. Both the k_D and A_2 will change as a function of what the buffer is, as different buffer compositions will promote different interactions in the sample.

The MW of BSA taking into consideration the contributions of the monomer, dimer and the tetramer is ~70.5 kDa. The MW of our BSA samples as obtained from SLS is in good agreement with this value.

The k_D is an interaction parameter that is used to describe the system-dependent interactions between molecules. The k_D also relates concentration dependence to a particle's diffusion coefficient. For our sample, we find that the BSA tends to interact more with the solvent than with itself. This would mean that PBS is a good solvent for BSA.

The A_2 (B_{22}) value is the second virial coefficient and is a measurement of solute-solvent interaction. A positive A_2 value agrees with the k_D which suggests that the BSA interacts more with the PBS than with itself.

The MW-S of each sample was then determined by the DYNAMICS software after inputting the appropriate sample parameters. From here, A_2, the second virial coefficient which is a measure of the solvent-solute interaction was obtained using the equation below:

$$R(\theta) = K^* McP(\theta)[1 - 2\boldsymbol{A_2} McP(\theta)]$$

Positive k_D/ A_2 values are indicative of repulsive interactions in the sample (i.e. the sample associates more with the solvent than with itself). This results in an apparent decrease in the radius of the sample as the diffusion coefficient increases and would suggest good colloidal stability of the sample. This can be used for screening buffers for determining the optimal buffer for the sample.

Negative k_D/ A_2 values are indicative of attractive interactions in the sample. This results in an apparent increase in the sample radius as the diffusion coefficient decreases and suggest that the sample has bad colloidal stability. This can be used for screening buffers for protein crystallization in order to determine which buffers are more likely to cause the protein to crystallize.