Introduction

The Wyatt DynaPro Plate Reader lll uses dynamic 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 III 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 produce results that best describe your sample.

Features of the DynaPro Plate Reader

Wide 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 20 µL. All measurements carried out in 384 and 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 such as protein crystallization or buffer screening.

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Wyatt DynaPro Plate Reader III for DLS/SLS

Application Note #4:

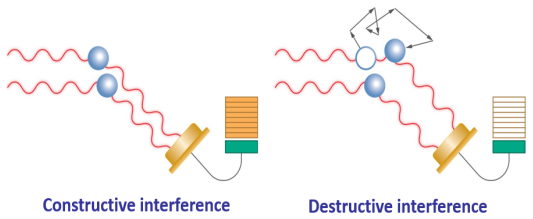
Thermal & Colloidal Stability Characterization by DLS/SLS.

- k_D, describes system-dependent self-interactions between molecules.
- D_t, describes the translational diffusion speed of particles.
- R_h, describes the size of particles in solution as if perfect spheres relating to stokes radius.
- Monitor the unfolding (T_{onset}), and the aggregation (T_{agg}) of samples as a function of temperature.

Wyatt DynaPro Plate Reader III - Thermal & Colloidal Stability by DLS

Theoretical Background

Dynamic Light Scattering (DLS) monitors rapid fluctuations in light intensity due to scattering of light by a particle undergoing Brownian motion. As the particles diffuse randomly, the amount of light scattered will change. The scattered light is converted by a detector to give a signal that is



converted into a hydrodynamic radius (R_h). The thermal and colloidal stability of proteins is an important property to know when developing formulation studies of potential therapeutic biomolecules. The key parameters that can be used to monitor the stability of the biomolecule are: the hydrodynamic radius (R_h), the diffusion coefficient (D_t), and the first order diffusion interaction parameter (K_D). These parameters are described by the following equations. Where K_B is the Boltzmann constant, T is the solvent temperature, and η is the solvent viscosity.

$$D_t = D_0 (1 + k_D c) = \frac{K_B T}{6\pi\eta R_h}$$

The hydrodynamic radius, also termed the Stokes radius, is inversely proportional to the diffusion coefficient. In simple terms, larger particles move slower than smaller particles. It then follows reason to observe the D_t decrease with increasing temperature as the protein begins to aggregate increasing the measured R_h.

Thermal & Colloidal Stability of Xylanase by DLS Analysis.

Sample: Xylanase

Concentration: 0.4 mg/mL

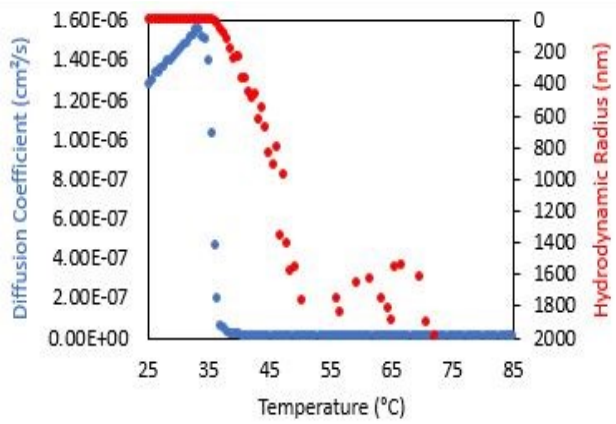
Buffer: PBS

Temperature Range: 25 °C - 85 °C

Temperature Ramp: 0.1 °C/min

Data Interval: 0.5 °C/data point

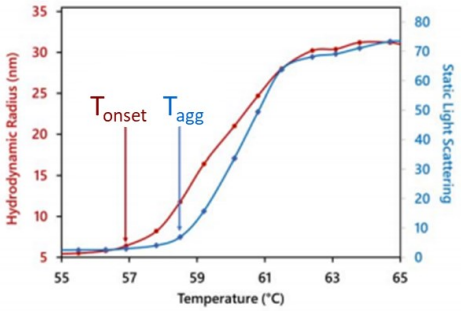
A 0.4 mg/mL aqueous sample of Xylanase in PBS was studied to determine the temperature at which the protein begins to denature and aggregate by DLS. The hydrodynamic radius (R_h) as well as the diffusion coefficient (D_t) were measured over the



temperature range 25 °C - 85 °C. The hydrodynamic radius (red) plotted in this figure gives a physical explanation to the behavior observed from the diffusion coefficient. Before the thermal transition the radius is seen to be relatively stable with respect to temperature, until about 36 °C. After which the radius is seen to quickly increase with increasing temperature. This behavior suggests that the particles are beginning to aggregate at this temperature a marker for determining that the protein is becoming unstable due to thermo-denaturation.

Monitoring for T_{onset} and T_{agg} of IgG by DLS & SLS Analysis

The DynaPro Plate reader can perform DLS and SLS experiments simultaneously in order to obtain the T_{onset}, and T_{agg} of your sample. T_{onset} is the onset temperature for protein unfolding, and T_{agg} is the temperature of the onset of aggregation of a protein. As the protein begins to aggregate, the intensity of scattered light will increase as large aggregates scatter more light than individual proteins. The point where the SLS signal begins to increase is defined as T_{agg}.



The plate reader can also be used to assess how changes in buffer composition and the addition of small molecules/other binding partners impact the stability of the protein. In the instance shown below, increasing the protein concentration results in a decrease in the observed T_{onset} of the protein.