## Introduction

The Wyatt DynaPro Plate Reader uses dynamic light scattering of analytes in solution to obtain key physical properties of biomolecules like hydrodynamic radii and melting temperatures. 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 the sample. The software is able to determine transition points for various events like aggregation, complex formation and is able to carry out statistical analysis of the data.

## 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.

**Structural & Biophysical Core Facility**


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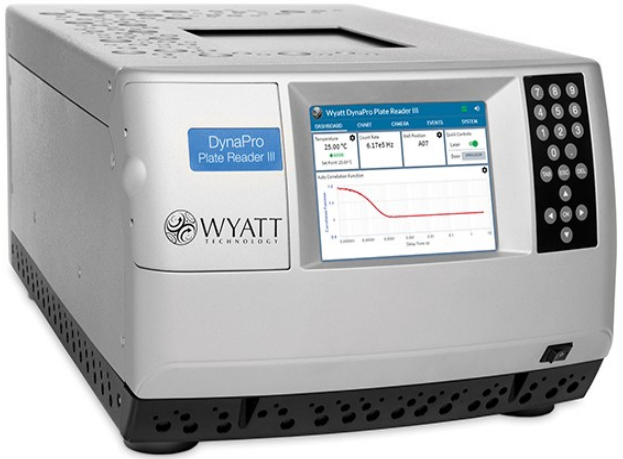
# Structural and Biophysical Core Facility




# Wyatt DynaPro Plate Reader III for DLS/SLS

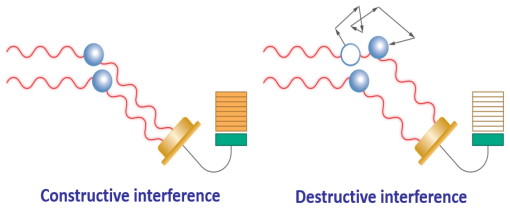
## Dynamic Light Scattering Analysis of Proteins

- Measure the size, polydispersity and size distribution of proteins or other particles directly in 96, 384 or 1536-well plates.
- Monitor the oligomerization, aggregation or conformational changes of proteins.
- Determine the thermal stability of protein via DLS temperature ramp experiments.
- Investigate biomoleular interactions by looking for size changes from complex formation.

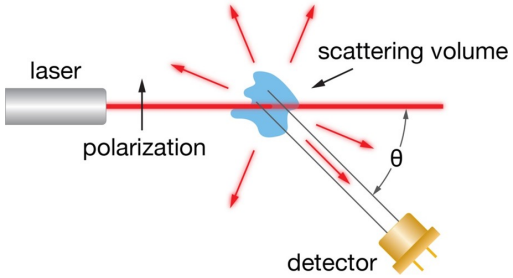
# Wyatt DynaPro Plate Reader III—Determining $R_h$ by DLS

## Theoretical Background

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




Static Light Scattering (SLS) monitors the intensity of scattered light to determine the average molecular weight of the sample, and the radius of gyration ($R_g$). In order to calculate the amount of light scattered at each angle, it is necessary to integrate over the contributions of each scattering sample and to probe the target from many angles.



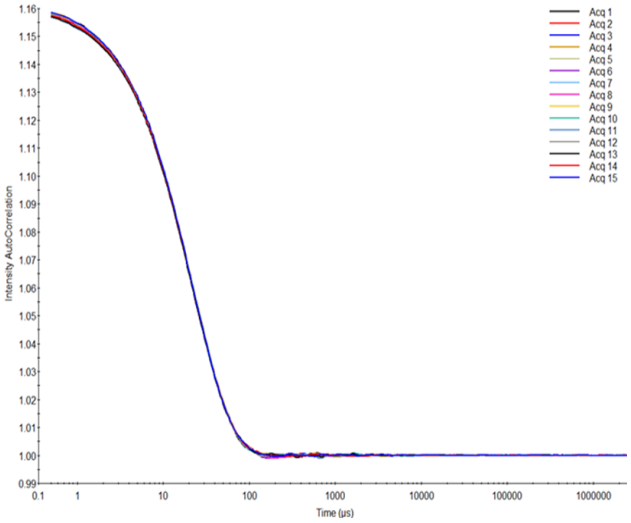

## Sample Types

Samples can be described as monomodal and monodisperse (one particle type with a narrow size distribution), monomodal and polydisperse (one particle type with a wide size distribution) or multimodal (several different particle types with wide size distributions). The type of sample will determine the appropriate analysis to be done.

## Hydrodynamic radius of BSA using DLS

**Protein sample:** BSA (2 mg/mL)
**Buffer:** 0.9% saline (w/v)
**Number of acquisitions:** 15
**Read time:** 5 seconds

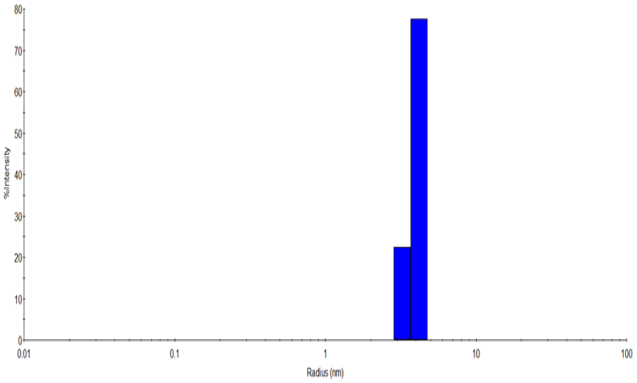
Below is a representative autocorrelation function (ACF) which is the raw data output from a DLS experiment. This function plots the average change in scattered light intensity versus time for a given time interval. Smaller particles diffuse quicker and will have faster fluctuations in their light intensity and shorter decay times in the function whereas the opposite is true for larger particles. From here, a hydrodynamic radius can be determined.




Depending on the sample type, either a cumulants fit or a regularization fit will be applied to the data. The cumulants fit assumes a distribution of diffusion rates and determines and determines an average $R_h$ which is valid for monodisperse samples. The regularization method assumes that the $R_h$ distribution is smooth, is able to resolve multiple peaks and is suitable for polydisperse samples.

## Hydrodynamic radius of BSA using DLS contd.

Shown below is the regularization fit applied to the previously shown autocorrelation function.




| Peak | Hydrodynamic Radius (nm) | % Pd | % Intensity | % Mass | % Number |
|---|---|---|---|---|---|
| 1 | 4 | 10 | 100 | 100 | 100 |

After applying a regularization fit to the auto-correlation function (left figure), we find that our BSA sample has an $R_h$ of 4.0 nm, which compares well with previously reported data for BSA. The sample also has a %Pd of 10.3%, indicating that it is monomodal. %Pd refers to polydispersity which is an indication of the homogeneity of the sample. BSA in a given sample exists as a monomer, dimer or as a tetramer. DLS has issues with resolving the small size differences between the BSA monomer, dimer and tetramer, and therefore the regularization fit that is applied has some contribution from all three samples which is seen in the %Pd value.

| Instrument specifications | |
|---|---|
| Sample Volume | 4 to 20 µL |
| Temperature range | 4°C to 85 °C |
| Protein concentration | Down to 0.125 mg/mL |
| Min. read time per well | 5 seconds |