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 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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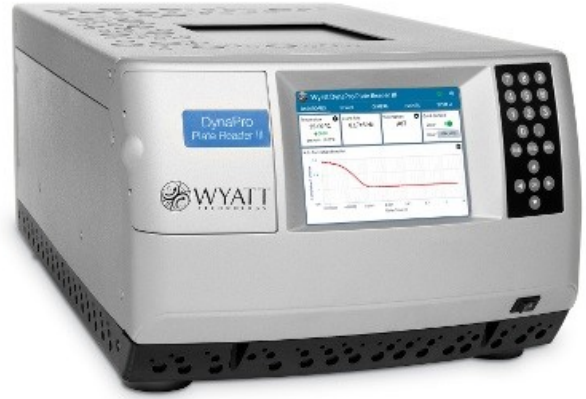


DynaPro Plate Reader III

Dynamic & Static Light Scattering

Application Note:

Characterization of protein-protein interaction and small molecule inhibition

- Quantification of protein-protein interactions using the Method of Continuous Variation
- Measure protein molecular weight and size distribution
- Modelling of average hydrodynamic radii of monomers and complex formation in solution
- Label-free, in-solution characterization
- High-throughput using micro-well plate format

Wyatt DynaPro Plate Reader III—Dynamic & Static Light Scattering

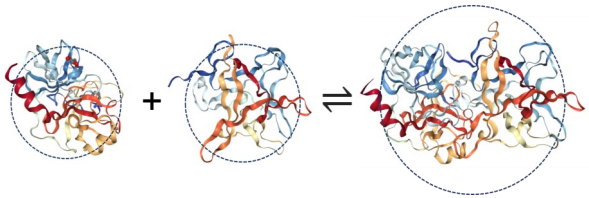
Theoretical background

Dynamic light scattering (DLS) uses the Brownian motion of particles in solution to measure the hydrodynamic radius (R_h). A laser illuminates the sample, and any particle in the beam scatters light in all directions. Any movement of a particle will cause fluctuations in the scattered light intensity, and since Brownian motion is random, the scattered light that the detector measures appears as random noise.

However, this noise is what results from changes in constructive and destructive interference as particles move around in relation to each other. The raw data undergoes autocorrelation analysis to give the decay rate and the translational diffusion coefficient. The translational diffusion coefficient and the Stokes-Einstein equation are then used to calculate the R_h of the sample.

Measurement of the hydrodynamic radius can be used to characterize the interaction between two proteins. The underlying principle is that when two proteins interact, they bind each other and form a complex with a larger hydrodynamic radius than either of the individual components. Scattered light from a dimer has a higher intensity than the total light scattered from two independent monomers. If the two proteins are roughly equal in size, this change is detectable and suggests an interaction.

E.g. Complex of trypsin and trypsin inhibitor:



R_h ~ 2.0 nm R_h ~ 2.2 nm R_h ~ 2.9 nm

If an interaction is 1:1, we would expect to observe the maximum R_h when both proteins are present in an equal molar ratio.

Characterization of protein-protein interactions

Experimental setup — Method of Continuous Variation (AKA Job Plot)

Trypsin and trypsin inhibitor (TI) protein solutions were mixed according to the table below following a reciprocal titration (i.e. as trypsin concentration is decreased, TI concentration is increased and vice versa). The mole fraction of TI is defined as $([TI]_{tot} / ([Trypsin]_{tot} + [TI]_{tot})$. The proteins are at equal molar ratio when the mole fraction is 0.5.

Trypsin (μM)	200	175	150	125	100	75	50	25	0
TI (μM)	0	25	50	75	100	125	150	175	200
Mole Fraction (TI)	0	0.125	0.25	0.375	0.5	0.625	0.75	0.875	1.0

20μL of each mixture was transferred to a 384-well plate and DLS analysis was conducted in triplicate at 25°C.

Graph (A) shows that the R_h of each individual protein stays relatively constant as concentration is varied.

In Graph (B), the average R_h (■) of the Trypsin-TI complex is observed to be at its maximum value when the mole fraction is equal to 0.5 (equal molar ratio, see arrow on graphs). This is indicative of a 1:1 interaction. A subsequent experiment was conducted, now with a pre-incubation with 2 mM AEBSF (a small molecule competitive inhibitor of trypsin). AEBSF prevents formation of the trypsin-TI complex, so the observed R_h should reflect this inhibition. In the presence of AEBSF (▲), the maximum R_h of the complex is no longer observed when Trypsin & TI are at an equal molar ratio. The plotted R_h is now a linear average of the non-complexed proteins.

Graph (C) has constant trypsin-TI concentration as AEBSF is titrated. When AEBSF is absent, the R_h is equal to uninhibited trypsin-TI complex. As AEBSF concentration increases, the R_h is observed to decrease as the complex is inhibited.