

AN4007: Monitoring Protein Complexes in Solution

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Introduction

A non-aggregated sample is essential for small-angle neutron scattering (SANS). Studies to elucidate the low-resolution shape of a number of Type I bacterial restriction-modification systems have been greatly aided by the use of the DynaPro™ dynamic light scattering instrument. Although the protein samples looked fine by SDS-PAGE and UV-spec (as judged by the flat baseline and low minima at 260 nm), several SANS measurements showed that some of the protein samples were prone to aggregation and hence poor measurements were obtained. We then decided to use dynamic light scattering to access the monodispersity of the protein samples following purification.



Figure 1. The DynaPro™ NanoStar™ is a cuvette-based DLS instrument with a user-friendly touch interface. It allows for convenient, walk-up measurement of size, polydispersity, and particle concentration, requiring as little as 2 μ L of samples.

Experimental

Dynamic light scattering was performed at 10°C in 10 mM Tris.HCl pH 8.0, 100 mM NaCl, 1 mM Na₂EDTA, using a DynaPro cuvette-based dynamic light scattering instrument.

Results and Discussion

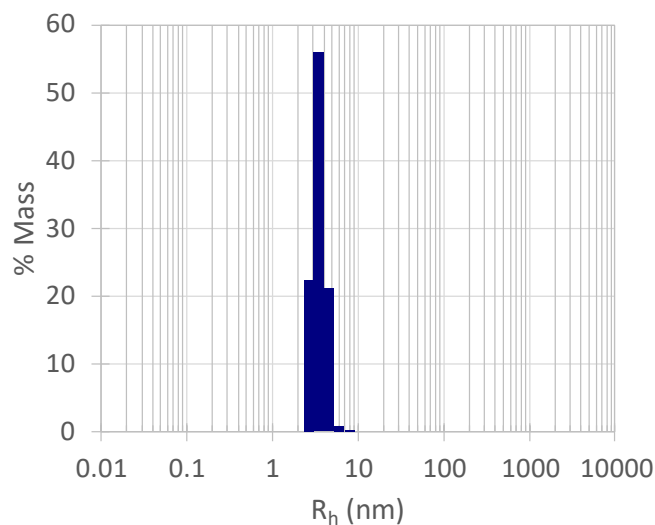


Figure 2. Histogram from the DYNAMICS software used to show the hydrodynamic radius and polydispersity of the R subunit of EcoR124I.

From thirty successful measurements, values for the hydrodynamic radius, R_h , and polydispersity were obtained using % mass calculations. The size-based molecular weight, M_r , was calculated using a volume shape hydration model and was compared to the theoretical M_r , which was found to be in very good agreement. Furthermore the sample was non-aggregated and thus suitable for SANS measurements.

This has enabled us to determine which of the proteins can be stored and which need to be purified “fresh” prior to complex formation. The maximum concentration of

the proteins prior to the formation of aggregates has thus been determined. This has enabled us to obtain the best quality SANS data, in the shortest measuring time on D22 (Institut Laue-Langevin, Grenoble), thereby allowing us to collect as much data possible in our beam-time allocation.

We now routinely use dynamic light scattering to monitor the behavior of the protein in solution.

To learn more about the DynaPro™ NanoStar™, please visit:

www.wyatt.com/NanoStar

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Acknowledgements

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Release update

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