

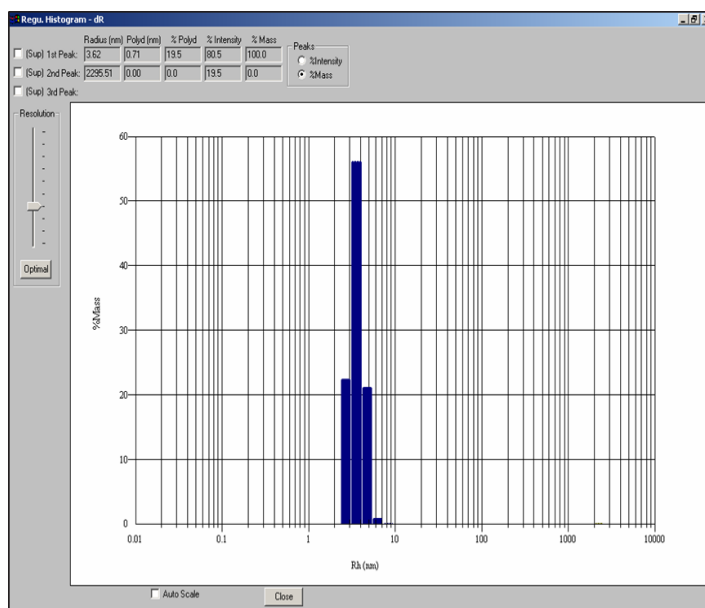
## Monitoring Protein Complex Formation in Solution

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.

Dynamic light scattering was performed at 10°C in 10 mM Tris.HCl pH 8.0, 100 mM NaCl, 1 mM Na<sub>2</sub>E-DTA, using a DynaPro MSTC800 light scattering instrument.

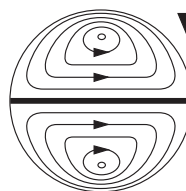
From thirty successful measurements, values for the hydrodynamic radius,  $R_h$ , and polydispersity were obtained using % mass calculations. The experimental molecular weight,  $M_r$ , was calculated using a volume shape hydration model and was compared to the theoretical  $M_r$ , which was found to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

We now routinely use dynamic light scattering to monitor the behavior of the protein in solution. 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, ILL, Grenoble, thereby allowing us to collect as much data possible in our beam-time allocation.



**Figure 1.** Histogram from the Dynamics software used to show the hydrodynamic radius and polydispersity of the R subunit of EcoR124I.

This note graciously submitted by Dr James Taylor, Biophysics Laboratories, School of Biological Sciences, University of Portsmouth, White Swan Road, Portsmouth, PO1 2DT, ENGLAND.



**Wyatt  
Technology**  
CORPORATION

6300 Hollister Avenue • Santa Barbara, CA 93117  
TEL (805) 681-9009 • FAX (805) 681-0123  
E-mail: [info@wyatt.com](mailto:info@wyatt.com) • URL <http://www.wyatt.com>