

## Protein Folding

Protein folding and unfolding is an extremely active field of research. The main rules of protein folding/unfolding have been established from *in vitro* experiments and the obtained information can be related to the function and behavior of proteins *in vivo*. Unfolding of proteins *in vitro* can be induced chemically by using chaotropic agents, like guanidine hydrochloride or urea.

Protein Y is a 16 kDa, almost fully helical human protein. At pH 5.0 it forms oligomers of different sizes. Studying the unfolding of protein Y by circular dichroism, the main transition in the secondary structure can be observed. Our aim was to follow the unfolding process by another independent method.

Far UV CD spectra were taken at 25°C on an AVIV 60DS circular dichroism spectrophotometer using a 0.01 cm cell. 5 spectra were recorded with a step size of 1.0 nm, a bandwidth of 1.5 nm and an averaging time of 5 seconds.

Dynamic light scattering measurements were carried out using DynaPro-MSXTC using a 1.5-mm path length 12µl quartz cuvette. Samples were filtered with a 0.01µm Whatman Anodisc-13 filter. Data were analyzed with the Dynamics software and 25-30 acquisitions were averaged.

The main transition in secondary structure can be observed by estimating the Stokes radius of the protein under different concentrations of the chaotropic agent (Fig. 1.). The mid-point of the transition agrees exceptionally well with the CD data (2.6M GnHCl). However, it is possible to observe *additional* molecular events applying DLS measurements: the dissociation of oligomers in the 0-2 M GnHCl concentration range.

Dynamic light scattering measurements are capable of monitoring the dissociation of an oligomeric protein and unfolding of monomers simultaneously using chaotropic agents.

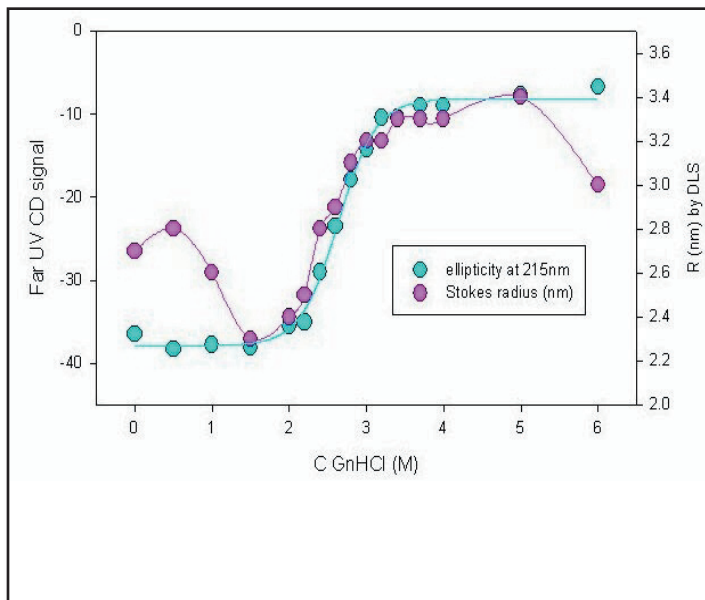


Figure 1. Folding transition in the presence of varying concentration of GnHCl at pH 5.0 at 25°C. Samples were prepared 3 hr before measurements to allow time for the unfolding. Protein concentration was 2 mg/ml. The inflexion point of the unfolding curve is at 2.6 M GnHCl by both methods.

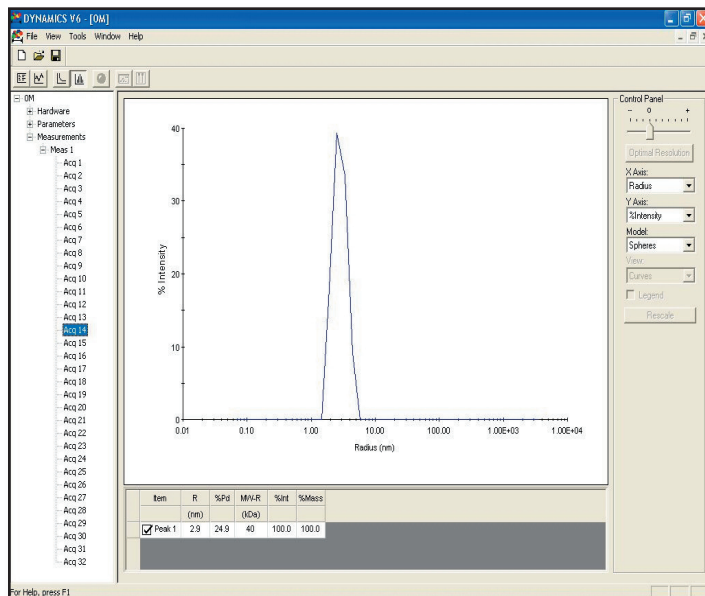


Figure 2. Snapshot of a size distribution view of a single DLS acquisition in the presence of 0M GnHCl at pH 5.0, 2mg/ml protein concentration at 25°C.

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