

Characterization of High Concentration Protein Solutions with the pUNk

Background

Dynamic light scattering (DLS) is widely used to detect the size and distribution of molecules, particles and their aggregates under a variety of solvent conditions. In the field of protein crystallography, the process of increasing the concentration of protein solutions required for crystallization often encourages unwanted aggregation, however some DLS systems are incapable of detecting these aggregates due to excessive light scattering from the concentrated sample. The pUNk DLS system from Unchained Labs incorporates an automatic, wide range optical attenuator, which permits measurement of protein samples at concentrations far in excess of what is possible with other DLS systems.

Experiment & Results

Concentrations of 50, 75 & 100mg/ml BSA protein (66kDa MW) was prepared in a phosphate buffer and analyzed using the pUNk, i-Size software and Unchained Labs' unique 5µl BladeCell sample cuvettes. All samples produced distributions with small secondary aggregate peaks, which is common for very high concentrations but in the case of BSA is not severe enough to inhibit crystal nucleation and growth. At 50mg/ml, the measured size and MW estimate are very close to the true values for BSA. At 75 and 100mg/ml there is a noticeable incremental decrease in reported sizes and corresponding MWs.

Conclusion

The raw DLS data was of high quality and the preparations optically transparent so the apparent size change is unlikely to be caused by multiple scattering phenomena and more likely due to molecule-molecule interaction. The pUNk DLS system automatically compensates for highly scattering samples making it possible to measure the size and distribution of proteins at a wide range of concentrations, however at very high concentrations the measured size may differ from the true size due to molecule-molecule interaction.

Experiment	Rad (nm)	Est. MW (kDa)	Intensity	Mass
50mg/ml BSA	3.66	69.6	91.2%	100.0%
	102.76	---	8.8%	
75mg/ml BSA	3.39	57.4	88.1%	100.0%
	55.26	---	11.9%	
100mg/ml BSA	3.02	43.2	85.0%	100.0%
	42.71	---	15.0%	

