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Technical Note

Observing Real Time Protein Aggregation Using the pUNk

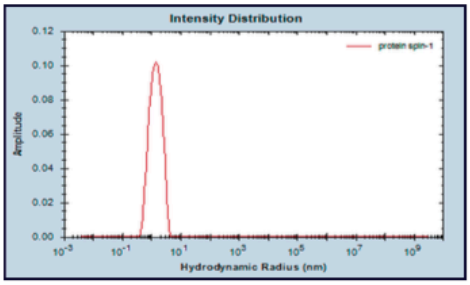

Fig 1 : 0 min - after centrifuge

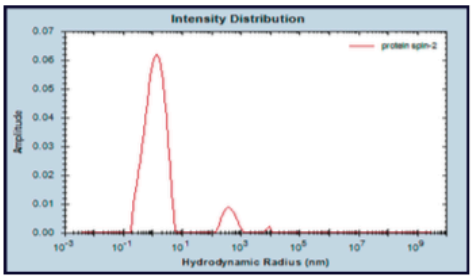

Fig 2 : 4 min - 2nd peak visible

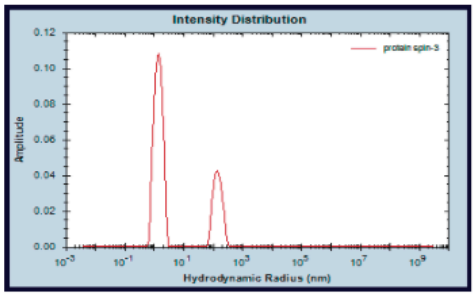

Fig 3 : 20 min - aggregates forming

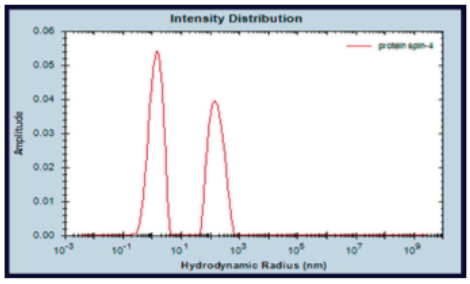

Fig 4 : 45 min - equilibrium restored

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 a recent case study the speed and power of DLS was demonstrated by revealing the spontaneous self-association of a molecule in solution. Dynamic processes can be easily observed in real time by DLS due to the short acquisition times and 'ensemble' measurement method.

Experiment & Results

In our study, a peptide compound in solution was initially shown to contain a mixture of monomer and aggregates when measured on the pUNk DLS system. To reduce the aggregate content the sample was centrifuged at 10,000 rpm for 10 minutes.

An aliquot was re-analysed to reveal a single peak of narrow size distribution and no aggregate peak (Fig 1). A second measurement was taken four minutes later (Fig 2) and on this occasion a small secondary peak was detected. Subsequent measurements at later time intervals indicated an increasing light scattering signal from the aggregate content (Fig 3) whilst the monomer peak remained constant in size. The process continued for 45 minutes until the original monomer/ aggregate equilibrium was restored (Fig 4).

Conclusion

DLS measurements are performed quickly and non-invasively, allowing dynamic processes to be observed very easily and with great sensitivity.

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