

# *Measuring and Modelling the Structure and Dynamics of Heterogeneous Protein Assemblies*

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The function of protein assemblies is governed by the interplay between their structure and dynamics, however many of the most important targets in structural biology are complicated by heterogeneity. This inherent tendency for proteins to often populate a number of states simultaneously presents a considerable challenge as most techniques provide an ‘average view’ of the protein in solution. We have developed a combined X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and ion mobility (IM) mass spectrometry (MS) approach to allow us to characterise the heterogeneous molecular chaperone  $\alpha$ B-crystallin.

We use MS to measure oligomeric distributions and to assay quaternary dynamics, NMR to measure secondary/tertiary dynamics, and crystallography to obtain the structure of the protein core. We find that our results are described well by very simple mathematical models which reveal not only the underlying rate constants and free energies, but also the remarkable fact that the protein oligomers have a Poisson distribution. This tells us that all subunits within the heterogeneous ensemble are essentially equivalent, and allows us to build oligomeric models. We construct our models on geometrical principles derived from regular convex polyhedra, and filter them according to correspondence with IM data. Ultimately we obtain pseudo-atomic models for  $\alpha$ B-crystallin which provide a rationale for its heterogeneity and dynamic quaternary structure.