NMR spectroscopy, mass spectrometry and electron microscopy elucidate the structure and dynamics of αB-crystallin oligomers

Iva Pritisanac[1], Hadi Lioe[1], Nik Sgourakis[2], Lindsay Baker[3], Simon Sharpe[3], John Rubinstein[3], David Baker[2], Justin Benesch[1], Lewis Kay[3], **Andrew Baldwin[1]**,

[1] Physical and Theoretical Chemistry, University of Oxford, UK

[2] University of Washington, Seattle, WA, USA

[3] Department of Medical Genetics, University of Toronto, ON, Canada

EMAIL andrew.baldwin@chem.ox.ac.uk

Small heat shock proteins (sHSPs) are a major class of cellular chaperone that inhibit protein aggregation and amyloid fibril formation *in vivo*. They form a front-line defence against aggregation in the brain whose failure can lead to conditions such as Alzheimer's and Parkinson's diseases. αB-crystallin is a human sHSP that naturally exists as a heterogeneous mixture of oligomers frequently comprising 10-50 monomers (average molecular weight ca. 600kDa), whose individual subunits freely exchange between the different oligomeric forms. The study of such heterogeneous mixtures has therefore proven highly challenging for conventional structural biology approaches. They are very tractable to NMR studies, however.

Here, we determine 'local' structural properties of this high molecular weight heterogeneous mixture using solution and solid NMR approaches. We combine this with 'global' information on shape and size from ion mobility mass spectrometry and tilted pair cryo-electron microscopy images. In doing so we are able to determine structures of the principally populated oligomers within the mileu. The models are refined using Rosetta and extensively cross-validated. This family of structures immediately reveal how the oligomers are able to inter-convert and explain why this protein exists as a poly-disperse ensemble.

To understand how these complexes function, we have developed and applied CPMG NMR relaxation dispersion experiments optimised for high molecular weight systems. Analysis and modelling of data allows us to determine specific conformational fluctuations that give rise to both oligomer inter-conversion, and function.

The methodology developed combines high-resolution structural information derived from NMR measurements with coarser information about shape size of individual complexes from electron microscopy and ion-mobility mass spectrometry. It is generally applicable to a wide range of heterogeneous systems that are otherwise intractable to biophysical analysis.