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### **Contributed talk 5 - Rotational and translational diffusion of eye lens gamma crystallin at low and intermediate concentrations**

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We are conducting continuing studies of rotational diffusion, translational diffusion, and thermodynamic compressibility of the eye lens protein bovine gammaB crystallin at low and intermediate protein concentrations. For nuclear magnetic resonance (NMR) measurements, <sup>15</sup>N-labeled bovine gammaB crystallin was produced in transformed E Coli by recombinant means, and isolated using size-exclusion and cation-exchange chromatography. For light scattering measurements, protein was isolated from young bovine lenses and isolated in the same fashion. Protein was concentrated for measurements in 10% D<sub>2</sub>O 100mM sodium phosphate buffer, pH 7.1, with 20mM dithiothreitol to inhibit oxidation. NMR transverse and longitudinal relaxation profiles were used to study concentration- and temperature-dependent rotational self-diffusion. Translational collective diffusion was measured with use of quasielastic light scattering, and solution Rayleigh ratios were measured using static light scattering. Characteristic rotational diffusion times of gammaB crystallin slowed from 9 nanoseconds to 13 nanoseconds, as protein concentration was increased from 0.25 to 2.6 millimolar. This slowing-down is well above effects due to solution viscosity increases in this concentration range. Evidence for concentration-dependent, non single-exponential rotational relaxation emerged and is awaiting follow-up experiments. Collective translational diffusion coefficient slowed from  $9 \times 10^{-11} \text{ m}^2/\text{s}$  to  $6 \times 10^{-11} \text{ m}^2/\text{s}$  over the same concentration range. The dependence of the Rayleigh ratio on concentration is consistent with attractive interactions, as characterized previously. We compare these results with computational hydrodynamic and theoretical calculations. To fully interpret the combined data, models for rotational diffusion in the presence of orientation-dependent direct as well as hydrodynamic inter-protein interactions may need further development.

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