# Small-Angle X-ray Scattering from Mixtures of Eye Lens Crystallins

G. M. Thurston,[1] L. B. Lurio,[2] S. G. J. Mochrie[3]

[1] *Center for Ophthalmic Research, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, U.S.A.*

[2] *Center for Materials Science & Engineering and Dept. of Physics, Massachusetts Institute of Technology, Cambridge, MA, U.S.A.*

[3] *Department of Physics, Yale University, New Haven, CT, U.S.A.*

## Introduction

Cataract, the leading cause of blindness worldwide, is the end result of gradually increasing light scatter from the human ocular lens.[1] Much of the light scattering occurs within the lens cells, which contain proteins called crystallins, at concentrations up to 500 mg/ml. High-concentration solutions of crystallins can have a relatively uniform refractive index and scatter little light. However, aggregation of crystallins and liquid-liquid phase separation within the cytoplasm can both result in a nonuniform refractive index and can thereby increase light scatter in cataract.[1] We seek to understand the driving forces for aggregation and phase separation in solutions of lens crystallins.

Towards this end, we are studying high-concentration model mixtures of two key lens proteins, α- and γB-crystallin, using small-angle x-ray scattering, light scattering, phase boundary determinations, and Monte Carlo simulation. α-Crystallin (α) is a multisubunit protein of ~800,000 g/mol, which exhibits repulsive interactions in solution. γB-Crystallin (γB) is a globular protein of 21,000 g/mol, which exhibits attractive interactions. Solutions of γB alone show liquid-liquid phase separation below ~0°C. In contrast, we find that mixtures of α and γB can show phase separation well above body temperature. The rise in phase separation temperature is likely driven by the size disparity between α and γB, which enhances local fluctuations in protein species composition.

## Methods and Materials

Calf α and γB crystallins were isolated by chromatography and concentrated by ultrafiltration in phosphate buffer. X-ray scattering cross sections, Σ(q) vs. wavevector, q, were measured for $0.01 < q < 0.7$ Å$^{-1}$, using 8 keV photons, for concentrations from 2-400 mg/ml, for compositions from all α to all γB, and vs. temperature.

## Results

The Σ(q) for a agrees with previous findings of Tardieu and Delaye[2] and is consistent with largely temperature-independent packing of approximately spherical particles, modified by repulsive interactions. The Σ(q) for γB also is consistent with previous findings.[3] In contrast to α, γB solutions show a dramatic increase of scattering at low q as the temperature is lowered, consistent with incipient liquid-liquid phase separation. The low-q γB form factor agrees quantitatively with that expected from its crystal structure; the crystal structure of α has not been reported.

Using low-angle Σ(q) from dilute α-γB mixtures, we estimate the mixed second virial coefficient of α and γB to be about -80 times the volume of γB. This is close to the value expected from hard-core repulsions of α and γB. High-concentration mixtures show Σ(q) feaures that we believe give evidence for enhanced protein composition fluctuations. To test this, we are comparing Σ(q) with analytic models and Monte Carlo simulations.

## Discussion

We expect that the principal features of α-γB mixtures can be understood in terms of (i) size disparity between α and γB, leading to compositional phase separation; (ii) attractive interactions between γB-crystallins; and (iii) repulsive interactions between α-crystallins. An quantitative understanding of α-γB mixtures can be a basis for studying mixtures gradually more representative of eye lens cytoplasm, i.e., ones that include β-crystallins, cytoskeletal and membrane elements, and altered proteins associated with cataract.

## Acknowledgments

Supported by NIH EY11840. Use of the Advanced Photon Source was supported by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. Beamline 8-ID, Advanced Photon Source (APS) is supported by DOE (DE-FG02-96ER45593) and NSF (DMR 9312543).

## References

[1] G.B. Benedek, J. Pande, G.M. Thurston, and J.I. Clark, Progress in Retinal and Eye Research **18**, 391 (1999)

[2] A. Tardieu and M. Delaye, Ann. Rev. Biophys. Biophys. Chem. **17**, 47 (1988).

[3] P. Petitt, M.E. Edwards, and D. Forciniti, Eur. J. Biochem. **243**,415-421 (1997).