**Biophysical Journal, Volume *112***

## Supplemental Information

# A Combined NMR and SAXS Analysis of the Partially Folded Cataract-Associated V75D γD-Crystallin

**Matthew J. Whitley, Zhaoyong Xi, Jonathan C. Bartko, Malene Ringkjøbing Jensen, Martin Blackledge, and Angela M. Gronenborn**

# A Combined NMR and SAXS Structural Analysis of the Partially Folded Cataract-Associated V75D Mutant of γD-Crystallin

Matthew J. Whitley[1], Zhaoyong Xi[1], Jonathan C. Bartko[1], Malene Ringkjøbing Jensen[2], Martin Blackledge[2], and Angela M. Gronenborn[1]

[1] Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA
[2] Institut de Biologie Structurale, CEA, CNRS, Université Grenoble Alpes, 38044 Grenoble, France

## Supporting Material

Figure S1: Equilibrium denaturation curve for V75D human γD-crystallin recorded as the ratio of fluorescence intensity at 370 and 320 nm as a function of urea concentration.

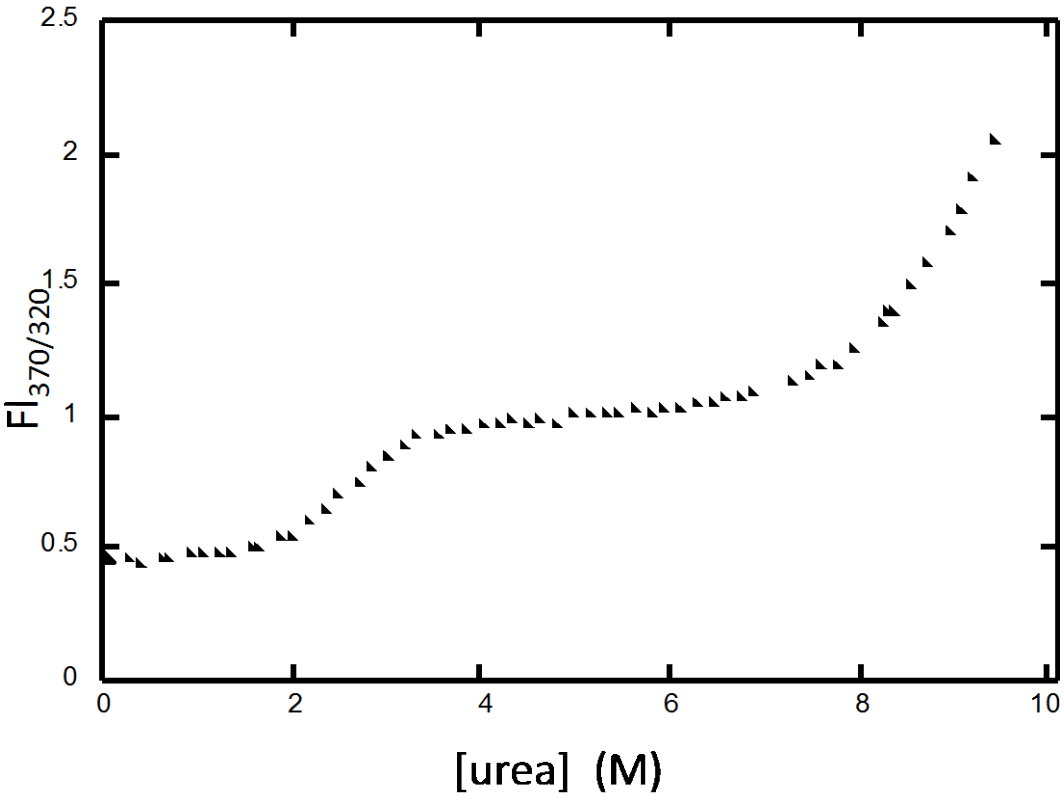

Figure S2: Superposition of the $^{1}H$-$^{15}N$ HSQC spectra of V75D human γD-crystallin in the absence (black) and presence (red) of 4.2 M urea. Several amide resonances associated with the folded N-terminal domain (black) are labeled by residue name and number. The corresponding resonances for the unfolded N-terminal domain reside in the central, crowded region of the red spectrum.

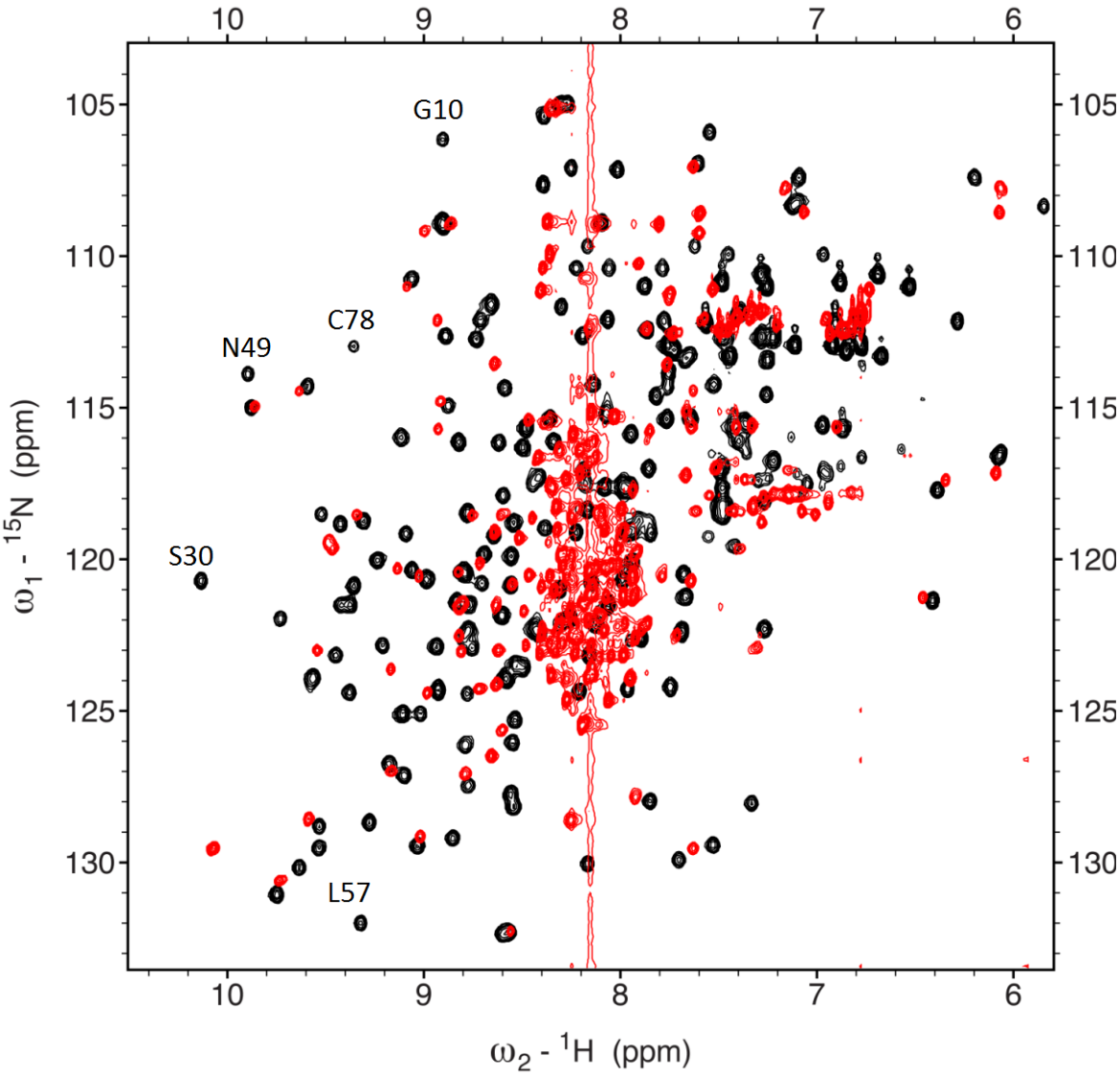

Figure S3: Experimental SAXS data (black), the calculated curve for the entire 25,000-structure starting ensemble (light blue), and the calculated curve from the 200-structure ASTEROIDS-ensemble optimized based on the SAXS data alone (red). The agreement between the experimental and ASTEROIDS-derived calculated data is only modestly improved compared to an ASTEROIDS-optimized ensemble in which all the experimental data were used (compare to Figure 7B). Note that all ASTEROIDS ensembles agree significantly better with the experimental data than does the starting pool ensemble.

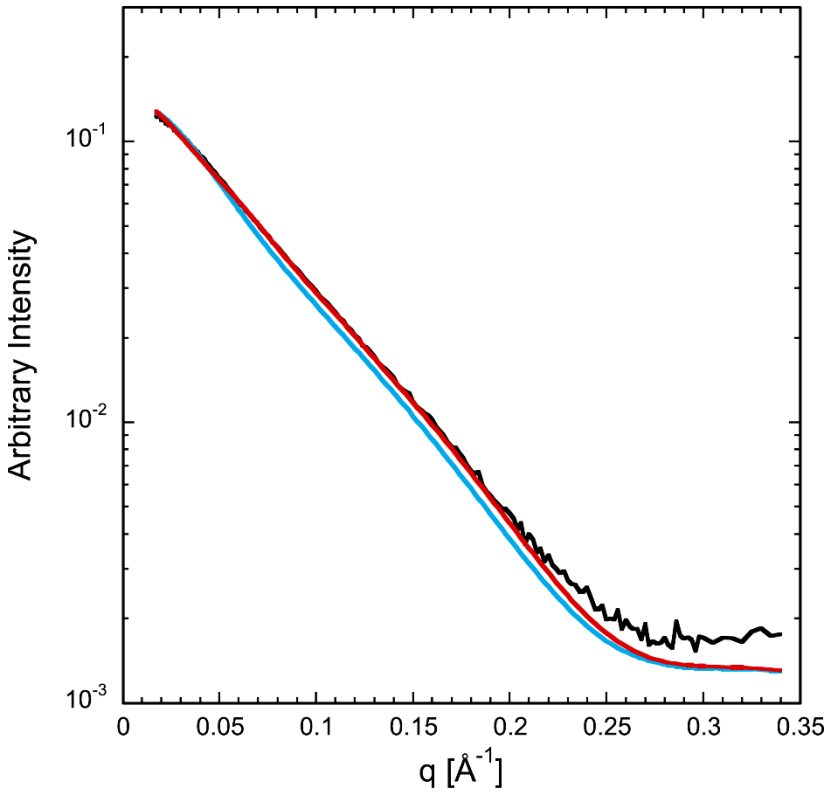

Figure S4: Validation of the ASTEROIDS-optimized ensembles using chemical shifts. Black data points were used in ASTEROIDS, whereas the cyan data points were withheld from the ASTEROIDS selection process. The ASTEROIDS ensembles reproduce the withheld data points with high accuracy.

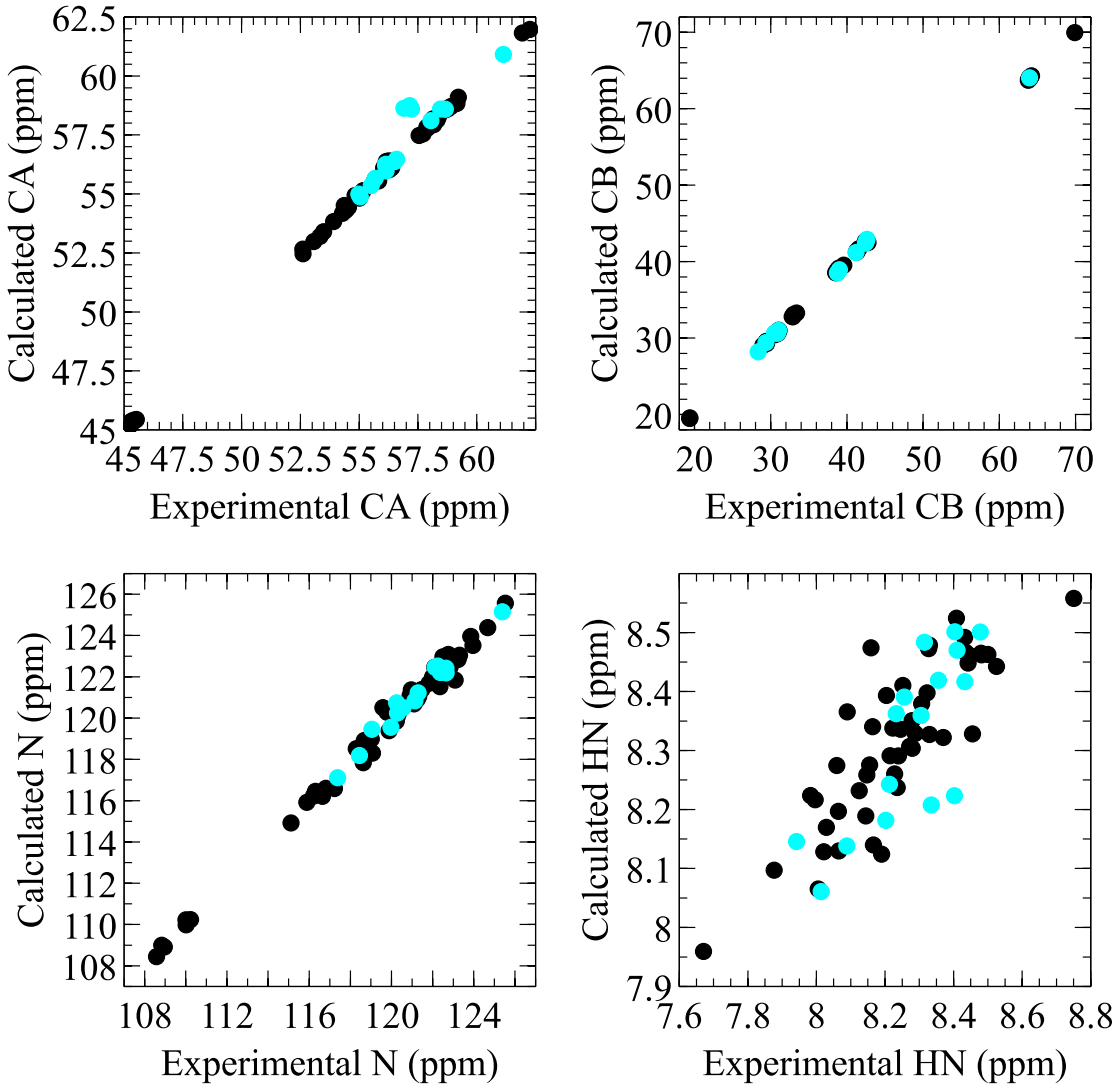

Figure S5: Validation of the ASTEROIDS-optimized ensembles using RDCs. Black data points were used in ASTEROIDS, whereas the cyan data points were withheld from the ASTEROIDS selection process. The panels labeled "Xval 20s," "Xval 30s," and so on, show the correlation between calculated and experimental RDCs when, in the calculation, the experimental RDCs of residues 20-29, 30-39, and so on (cyan) were omitted.