Supporting Information for:

Structural and Functional Consequences of Age-Related Isomerization in α-Crystallins

Yana A. Lyon,[a] Dylan L. Riggs,[a] Miranda P. Collier,[b]

Matteo T. Degiacomi,[c] Justin L.P. Benesch,[b] Ryan R. Julian[a]

[a]Department of Chemistry, University of California, Riverside, 501 Big Springs Road, Riverside, CA 92521, USA; [b]Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ, UK; [c]Department of Chemistry, Durham University, South Road, Durham DH1 3LE, UK

Corresponding Authors: ryan.julian@ucr.edu, justin.benesch@chem.ox.ac.uk

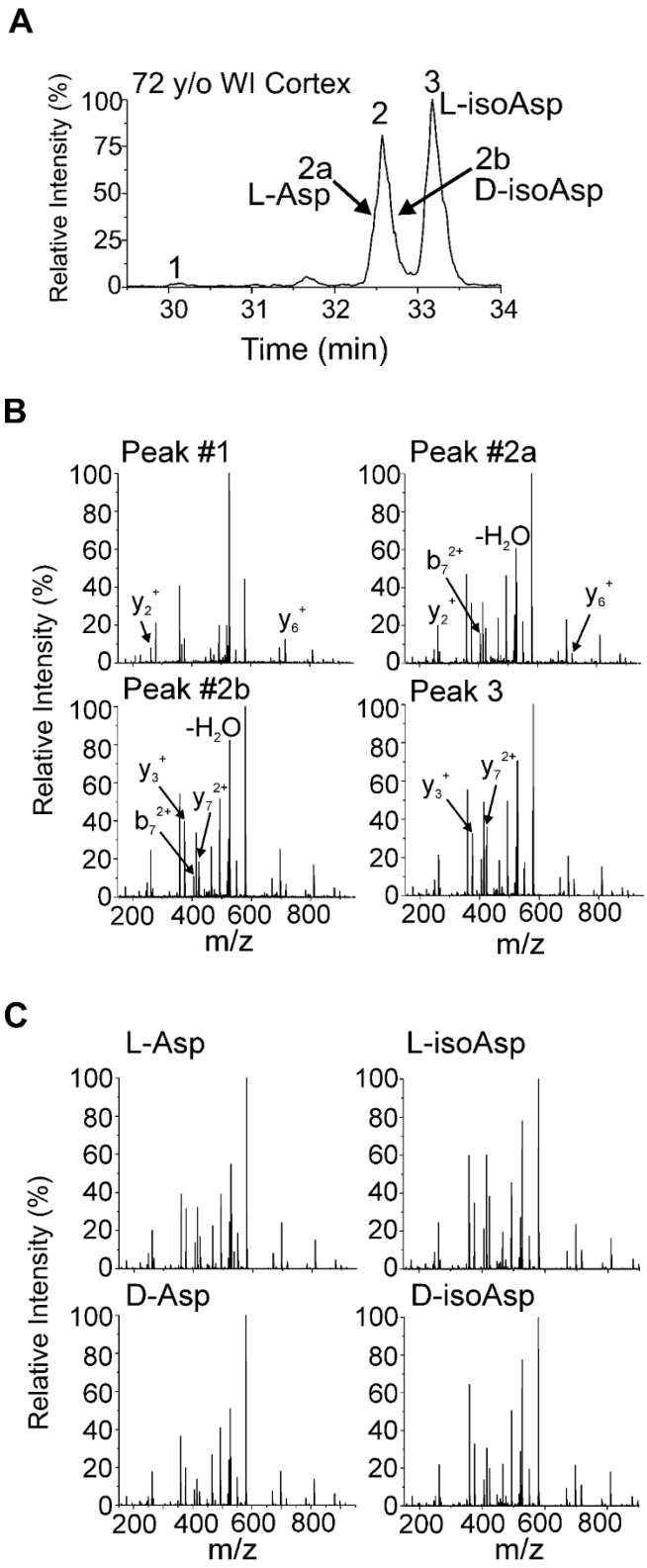

Fig. S1. (A) LC chromatogram of the ^{108}pQDEHGFISR^{116} isomers from a 72 y/o WI cortex digest.(B) Four spectra resulting from collision-induced dissociation from three isomer peaks in

the LC chromatogram. Peak 2 contains two co-eluting isomers and are labeled “2a and 2b”. Comparison of the fragmentation intensities in peak #1 and peak #2 show differences in the b_6^+ ion, and by also comparing the fragmentation intensities of y_2^+ an R_{isomer} score can be calculated. In this example:

$$R_{\text{isomer}} = \frac{R_{\text{Peak 1}}}{R_{\text{Peak 2a}}} = \frac{\frac{I_A}{I_B}}{\frac{I_A}{I_B}} = \frac{\frac{12.48}{5.13}}{\frac{7.95}{20.04}} = 6.1;$$

Where I_A = Relative intensity of y_6^+, I_B = Relative Intensity of y_2^+.

This calculation is then used to compare the front end of peak 2 (2a) to the back end of peak 2 (2b), and then peak 2b to peak 3. The R_{isomer} scores for each of these subsequent calculations are all above the threshold of 1.9, indicating that they are different isomers. (C) Synthetic standards of the pQDEHGFISR are then used to identify the specific isomer in each of the peaks. This is done by comparing the fragmentation spectrum in each of the peaks to the fragmentation spectra from the authentic L-Asp, L-isoAsp, D-Asp and D-isoAsp synthetic versions. An R_{isomer} score below the 1.9 threshold allows for confident confirmation of the isomer.

Table S1. Idenfication of Asp isomers from ^{108}pQDEHGFISR^{116} from αB in WI Cortex of 72 y/o Lens using R_{isomer} scores

Peak Observed from Lens	Synthetic standard	R_{isomer}
Peak 1	L-Asp	5.4
Peak 1	L-isoAsp	3.9
Peak 1	D-Asp	5.9
Peak 1	D-isoAsp	4.1

Peak Observed from Lens	Synthetic standard	R_{isomer}
Peak 2a	**L-Asp**	**1.2**
Peak 2a	L-isoAsp	2.6
Peak 2a	D-Asp	2.3
Peak 2a	D-isoAsp	2.4

Peak Observed from Lens	Synthetic standard	R_{isomer}
Peak 2b	L-Asp	2.7
Peak 2b	L-isoAsp	3.8
Peak 2b	D-Asp	2.4
Peak 2b	**D-isoAsp**	**1.5**

Peak Observed from Lens	Synthetic standard	R_{isomer}
Peak 3	L-Asp	2.8
Peak 3	**L-isoAsp**	**1.2**
Peak 3	D-Asp	5.1
Peak 3	D-isoAsp	2.3

Table S1 lists the results of the isomer identification of the pQDEHGFISR peaks from the WI cortex of the 72 y/o lens, and the best matching isomer is indicated in red. When matching observed peptides to synthetic standards, low R_{isomer} values are desired to indicate similarity (the opposite of what is done to distinguish isomers from each other, where high R_{isomer} values are meaningful). Peak 1 (Figure S1a) centered at 30 min is an isomer of this peptide, but does not match any of the Asp synthetics suggesting that it contains a different site of isomeriation.

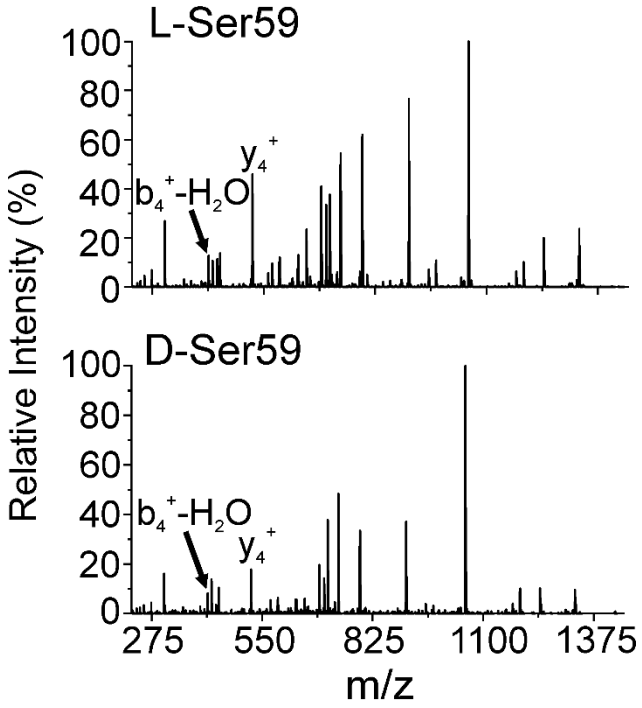

Fig. S2. Resulting CID spectra of the APSWFDTGLSEMR L-Ser59 and D-Ser59 synthetic standards. Comparison of the b_4^+ -H2O and y_4^+ ions yields an R_{isomer} score of 4.1 indicating that CID can be used to detect D-Ser59 in this peptide. The fragmentation patterns of these two synthetics are then checked against each of the 9 peaks, allowing for identification of both L-Ser59 and D-Ser59.

Table S2. Identification of L-Ser59 of ^{57}APSWFDTGLSEMR^{69} from αB in WI Cortex

Synthetic standard	Peak Observed from Lens	R_{isomer}
L-Ser59	Peak 1	5.7
L-Ser59	Peak 2	3.4
L-Ser59	Peak 3	3.8
L-Ser59	Peak 4	4.2
L-Ser59	Peak 5	2.5
L-Ser59	Peak 6	8.3
L-Ser59	**Peak 7**	**1.3**
L-Ser59	Peak 8	2.9
L-Ser59	Peak 9	3.5

Table S3. Identification of D-Ser59 of ^{57}APSWFDTGLSEMR^{69} from αB in WI Cortex

Synthetic standard	Peak Observed from Lens	R_{isomer}
D-Ser59	Peak 1	5.4
D-Ser59	Peak 2	4.8
D-Ser59	Peak 3	4.9
D-Ser59	**Peak 4**	**1.4**
D-Ser59	Peak 5	3.3
D-Ser59	Peak 6	7.6
D-Ser59	Peak 7	4.9
D-Ser59	Peak 8	4.7
D-Ser59	Peak 9	4.5

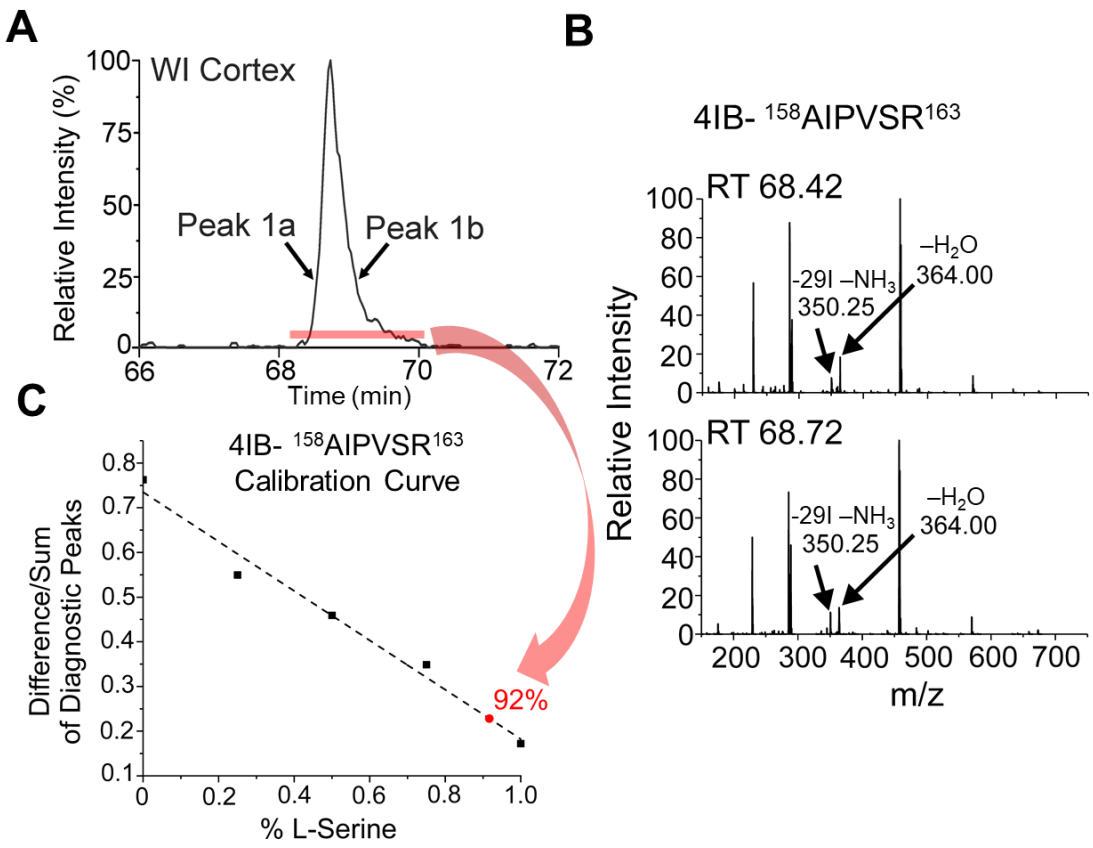

Fig. S3 (A) LC chromatogram of 4IB-AIPVSR in the WI cortex digest of the 72 y/o lens. (B) RDD spectra from the front-end (peak 1a) and back-end (peak 1b) of the corresponding LC peak. (C) A calibration curve is then used to quantify the amount of D-Ser that co-elutes in the LC chromatogram. The curve is generated by making standard solutions that contain known amounts of both isomers and taking the difference over the sum of the two peaks that have the largest differences in the fragmentation spectra. For this peptide, the -29I-NH_3 losses from the precursor ion and the -H_2O loss from the precursor ion were chosen as the diagnostic peaks. The percent D-Ser/L-Ser in the digest is then determined by averaging the RDD spectra for the entire peak in part A (indicated by the red bar). This value maps to the red point in part (C), 92% L-Ser and 8% D-Ser.

Table S4. Idenfication of Ser epimers from 4IB-^{158}AIPVSR^{163} from αA in WI Cortex of 72 y/o Lens using R_{isomer} scores

Peak Observed from Lens	Synthetic standard	R_{isomer}
RT 68.42	L-Ser	4.6
RT 68.42	**D-Ser**	**1.7**

Peak Observed from Lens	Synthetic standard	R_{isomer}
RT 68.42	**L-Ser**	**1.3**
RT 68.42	D-Ser	6.0

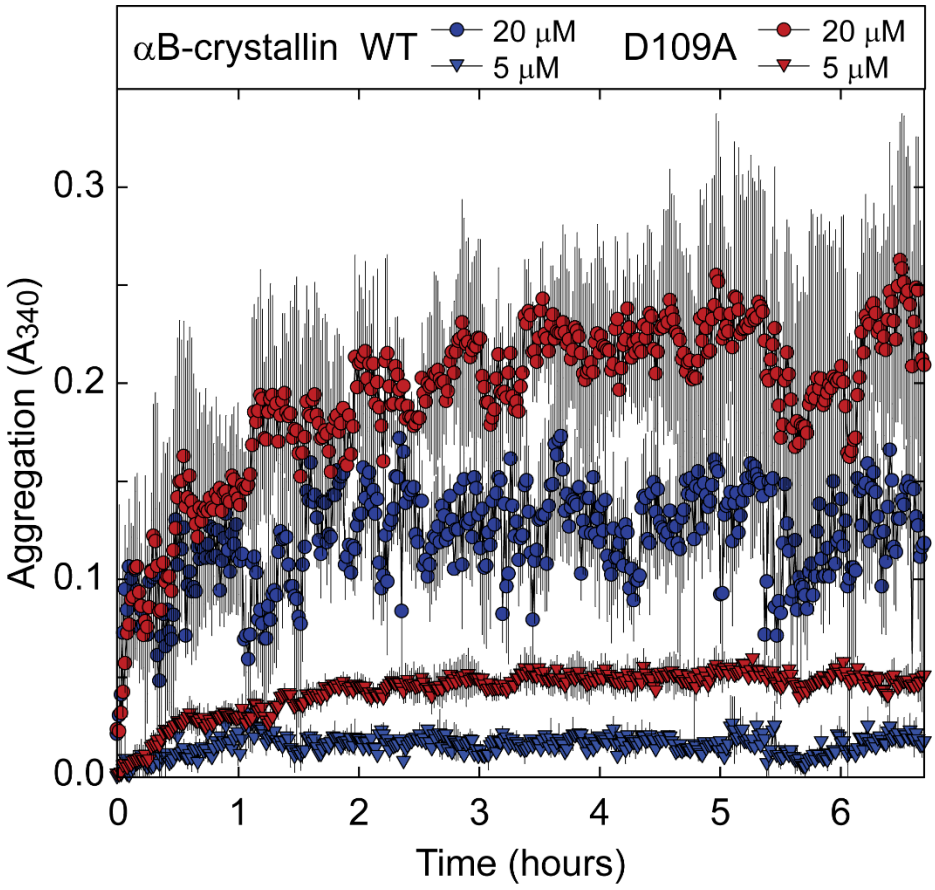

Fig. S4. Aggregation of αB-crystallin (WT or D109A) over time at 42°C monitored by light scattering at 340 nm. Molar concentrations correspond to monomers. Error bars represent one s.d. (n = 3). The D109A substitution predisposes αB-crystallin to form large aggregates, in agreement with the native-MS results.