Supplementary information for:

Probing dynamic conformations of the high molecular weight αB-crystallin heat shock protein ensemble by NMR spectroscopy

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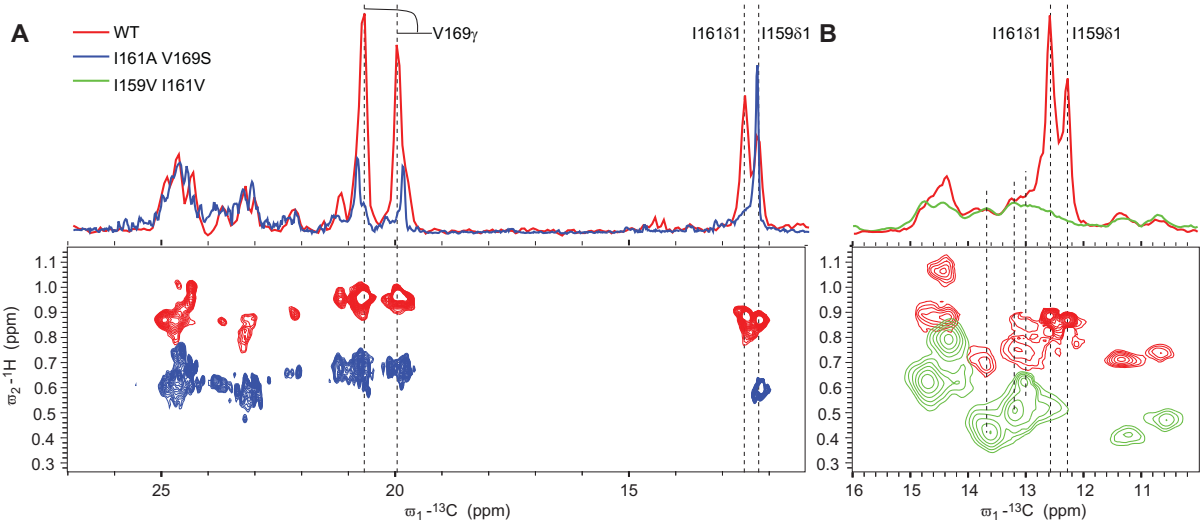

Figure S1: Assignment of αB-crystallin methyl groups I159δ1, I161δ1 and V169γ by mutagenesis. As described previously[1], the two double mutants used for assignment were (I159V I161V)-αB, (green) and (I161A V169S)-αB, (blue). The gene for each of the mutants was generated via mutagenesis of the wild-type sequence, with both mutant proteins expressed in *E. coli* and purified according to the protocol for the wild-type. Notably, their expression levels were comparable to that of the wild-type (red), and all three proteins eluted at the same volume when purified by size exclusion chromatography. The figure compares corresponding solution HMQC spectra, represented here both in 2D form and as 1D ^{13}C projections, obtained by directly summing over the proton dimension. The referencing in the proton dimension of the 2D spectra is correct for the data set recorded of the wild-type protein (red). Spectra of the mutants (I161A/V169S)-αB (blue) and (I159V/I161V)-αB (green) have been offset by 0.25 ppm (in ^{1}H) to enable a clear comparison. **A –**At high temperature (50^{o}C, 14 T) the relatively weak resonances that derived from residues tumbling with the bulk of the protein are maximally intense. Nevertheless, solution-state HMQC spectra of isoleucine, leucine and valine (ILV) methyl labelled wild-type αB-crystallin are still dominated by four sharp resonances, two in the isoleucine region (10-16ppm) and two in the leucine/valine region (18-30ppm). Comparing this spectrum (red) with that from (I161A/V169S)-αB (blue, 40^{o}C, 14 T) reveals that the two sharp resonances formerly in the leucine/valine region are absent in the data set recorded of the double mutant. The two remaining resonances in this region are significantly less intense and can be observed in wild-type spectra as 'shoulders' of the intense correlations. In addition to the loss of two leucine/valine resonances, one of the two sharp isoleucine resonances is also absent from the spectrum of the double mutant. By linking the missing resonances to the mutated residues they can be assigned to V169γ (γ1/γ2) and I161δ1. We note that the spectrum of (I161A/V169S)-αB was recorded at 40^{o}C, while that of the wild-type was obtained at 50^{o}C. That the remaining intense isoleucine resonance is relatively stronger for the (I161A/V169S)-αB data set, despite the lower temperature, is due to an exchange phenomenon that forms the basis of the present paper (see text). Note, however that the peak position is identical in the two spectra. **B –** The spectrum of the isoleucine labelled wild-type protein (red, 50^{o}C, 14 T) is compared to that of (I159V/I161V)-αB (green, 40^{o}C, 11.7 T). The two sharp resonances that dominate the Ile region of the wild-type spectrum (red) are not observed in the spectrum of the double mutant (green) and so can be assigned to I159δ1 and I161δ1. By contrast, the remaining peak positions are essentially unchanged.

The resonances from the remaining isoleucine residues are slightly broader in the spectrum of the double mutant due to the lower temperature (these residues do not undergo exchange broadening).

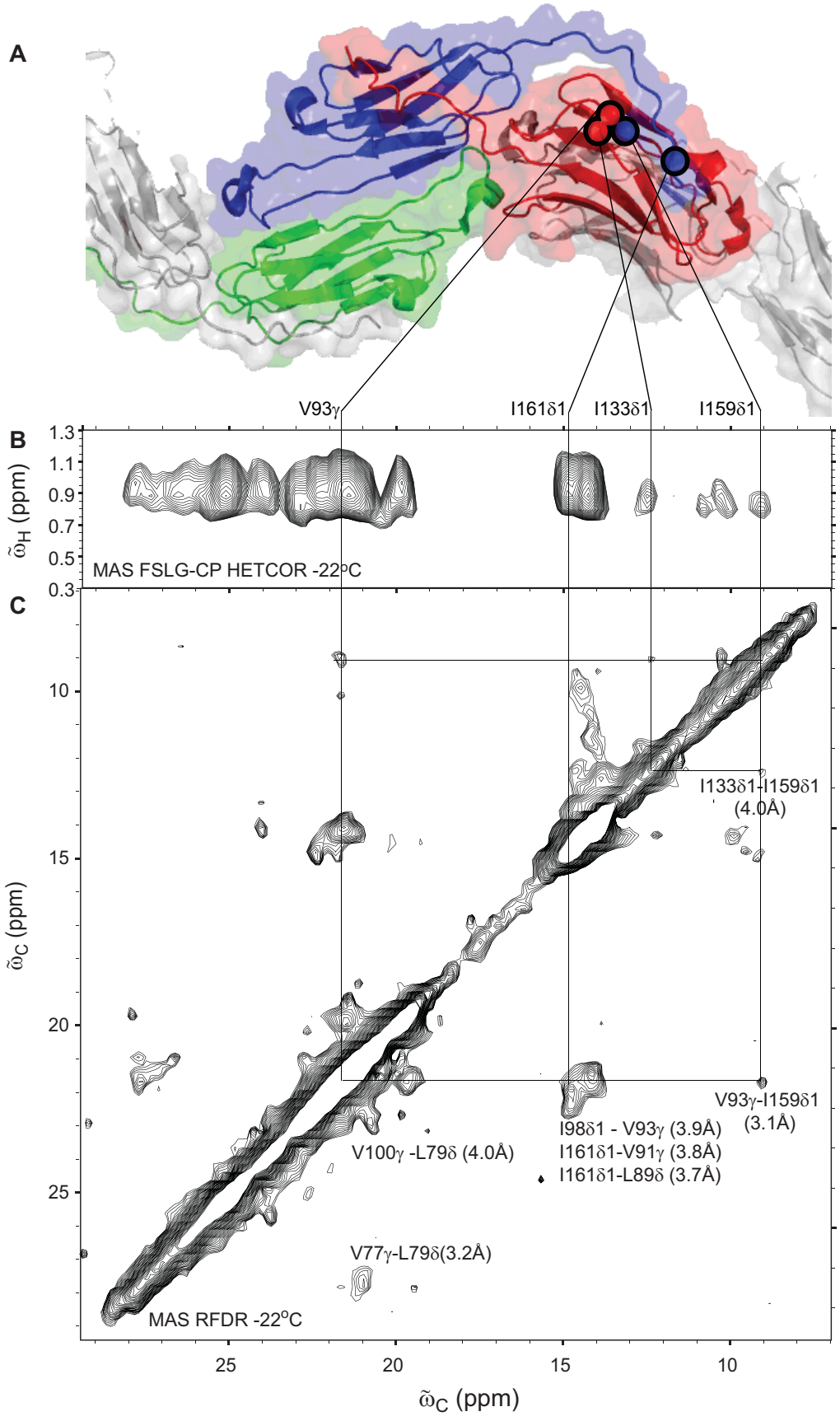

Figure S2: A) Residues 68-162 of αB-crystallin (PDB accession code 3L1G[2]) with a dimer building block highlighted in green/blue shading. The x-ray structure establishes that each dimer makes

contacts to adjacent dimers via the C-terminus of a monomer (eg, blue with red). This inter-dimer interaction places I159 of the blue monomer proximal to I133 and V93 of the red monomer. An identical interaction was also observed in solid-state NMR experiments[3]. **B)** FROSTY MAS ^{1}H-^{13}C FSLG-CP HETCOR[4,5] spectrum of U-[^{2}H], Ile-[$^{13}CH_3$ δ1], Leu,Val-[$^{13}CH_3$,$^{12}CD_3$] αB-crystallin, -22^{o}C, 11.7T. Assignments are based on the tabulated chemical shift values from Oschkinat and coworkers[3]. The isolated downfield resonance from I159δ1 is readily distinguished; note that its position is slightly different from that reported previously (11 ppm) based on measurements on lyophilized samples, but agrees very well with FROSTY spectra kindly provided by B. Reif. The assignment has been confirmed by an RFDR experiment (see below). The resonance from I161δ1 at 15ppm is overlapped with a large number of additional peaks and cannot be directly discerned. **C)** A 2D RFDR[6] spectrum of U-[^{2}H], Ile-[$^{13}CH_3$ δ1], Leu,Val-[$^{13}CH_3$,$^{12}CD_3$] αB-crystallin, -22^{o}C, 11.7T. Seven out of eight (88%) inter-methyl contacts with a carbon-carbon distance of 4 Å or less were observed. Notably, contacts are obtained from I159δ1 to both V93γ and I133δ1, consistent with both X-ray and the solid-state NMR structures[2,3]. While they cannot be individually assigned, cross peaks are observed consistent with interactions between I161δ1 - L89δ, V91γ - L69δ, as well as between V77γ, L79δ and V100γ. Finally, because of the nature of the samples we use (only one of the two prochiral methyl groups of Leu/Val is $^{13}CH_3$) intra-residue methyl NOEs are not observed.

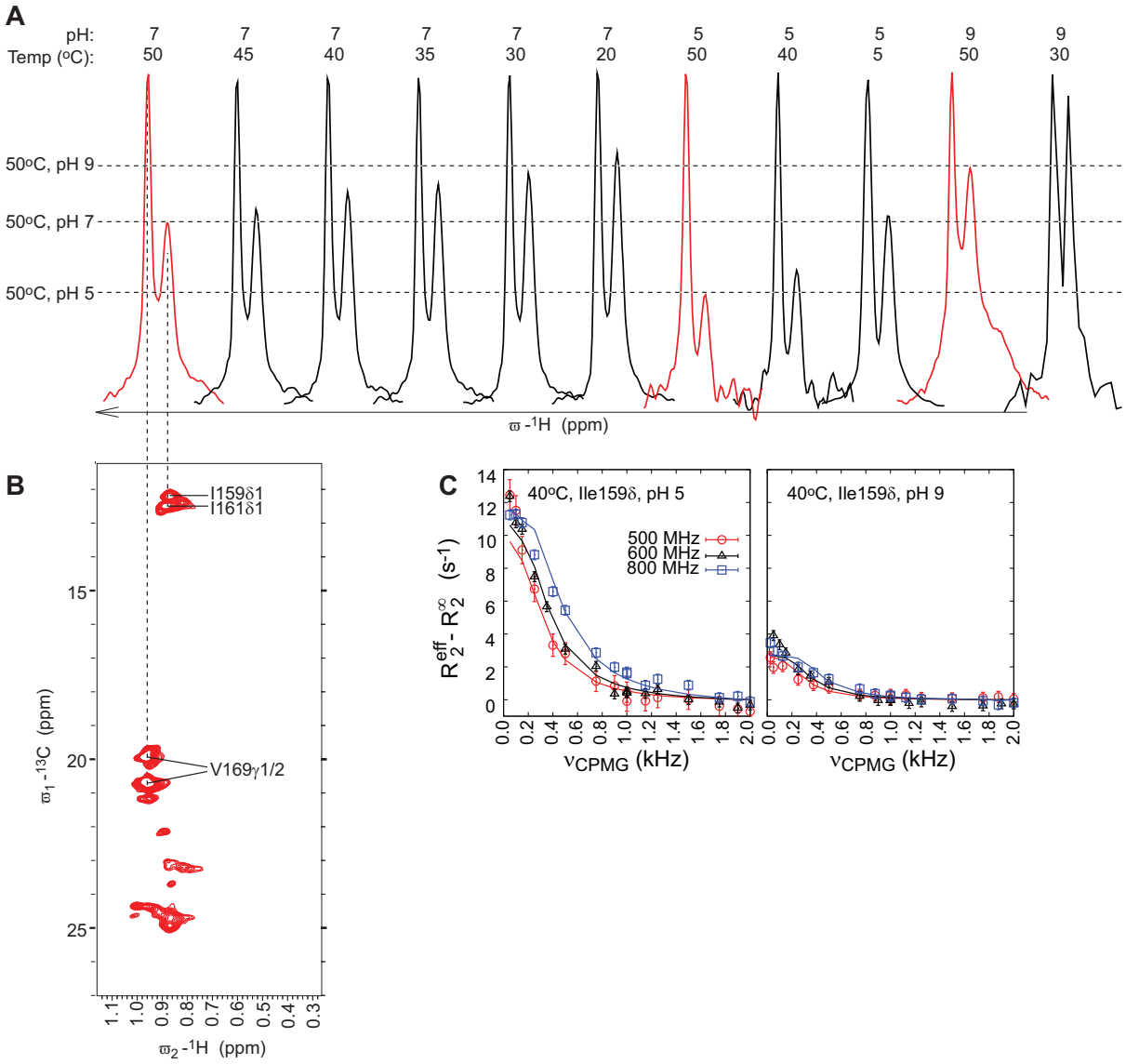

Figure S3: The isoleucine, leucine and valine methyl-labelled spectrum of wild-type αB-crystallin is dominated by four resonances as described elsewhere[1], in the text and in Figure S1. In a one-dimensional proton spectrum resonances from I159δ1 and I161δ1 overlap as do those from V169γ1 and 2 (**A**). The upfield resonance is dominated by the superposition of I159δ1 and I161δ1, and the downfield resonance arises due to the superposition of signals from V169γ1 and 2. (**B**). The two resonances dominate the spectrum over an exceedingly wide range of solution conditions. Selected spectra are shown in A that span pH 5 to 9 and 5°C to 50°C. Individual spectra are normalized so that the intensity of the downfield resonance formed from the combination of V169γ 1 and 2 is the same in all spectra. Taken together, residues I161δ1 and I159δ1 populate predominantly the disordered state over the range of solution conditions shown. The relative intensity of the two observed resonances vary significantly with changing solution conditions. Specifically, the relative intensity of the isoleucines decreases with increasing temperature and decreasing pH. CPMG experiments reveal that the variance in signal intensity is due to chemical exchange. Under conditions where the two isoleucine resonances are relatively weak (pH 5, 50°C) the effects of chemical exchange are at a maximum and large relaxation dispersion curves are observed (**C**). Under conditions where the relative isoleucine intensity is high (pH 9, 20°C), the effects of chemical exchange are significantly

reduced. From a detailed analysis of CPMG relaxation dispersion curves the contribution of chemical exchange to the measured effective relaxation rates can be quantified. The CPMG data can be well explained in terms of the inter-conversion between a principally populated flexible 'ground' state (populated >95% under all conditions surveyed) and a sparsely populated 'excited' state (populated <5% under all conditions surveyed)[1]. The chemical shifts of the excited state are found to be similar under all conditions analyzed, and similar to that of the bound state observed by solid-state NMR experiments at low temperature (<0°C), as described in the text. Both the forward and backward rates vary smoothly between pH 5 and 9, in the temperature range shown, as described previously[1]. Moreover, a comparison of the forward and backward rates isolated from the analysis of the dispersion data with global monomer exchange rates determined by mass spectrometry establish that the formation of the excited state is the rate-limiting step for subunit-exchange [1].

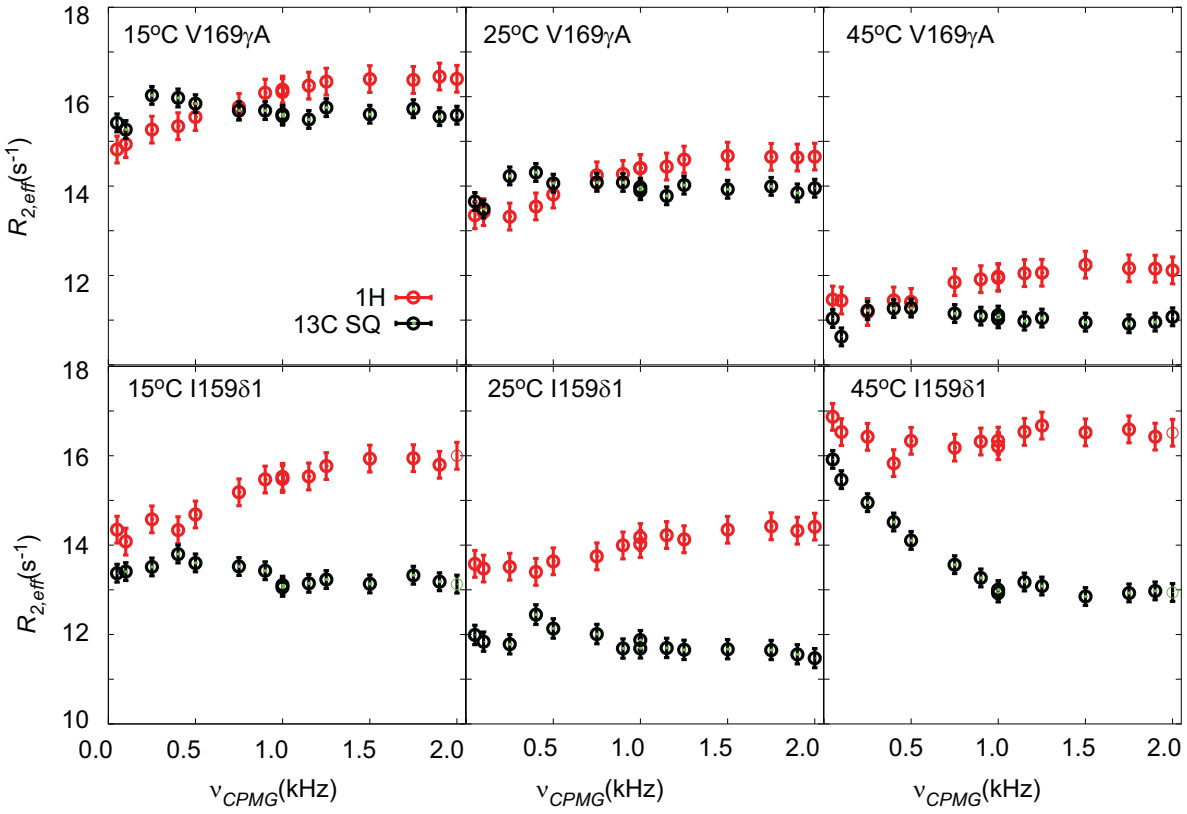

Figure S4: ^{13}C (black) and ^{1}H (red) CPMG relaxation dispersion profiles for V169γA (stereospecific assignments for Val residues not available) and I159δ1 recorded at 15°C, 25°C and 45°C. The curves for V169 show essentially no dependence on ν_{CPMG}; average $R_{2,eff}$ values decrease with increasing temperature, consistent with increased tumbling rates at the higher temperatures. In contrast, above 25°C, I159δ1 ^{13}C (black) dispersion profiles show a clear contribution from chemical exchange (the profile recorded at 45°C is illustrate here). ^{1}H dispersions show no obvious signs of exchange; the small increase in $R_{2,eff}$ with ν_{CPMG} is the result of mixing slow and fast relaxing proton transitions due to imperfections in ^{1}H 180° refocusing pulses of the CPMG element, as described previously[7]. The profiles establish that the difference in ^{1}H chemical shifts between ground and excited states for I159δ1 is small (same for I169δ1) so that exchange is fast (exchange rates on the order of 2000 s^{-1} [1]) and the ^{1}H transverse relaxation rate is well approximated by a population weighted average of rates for the ground and excited states.

Supplementary Material References

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