Supplemental Material: Binding determinants of the small heat shock protein, αB-crystallin: recognition of the "IxI" motif

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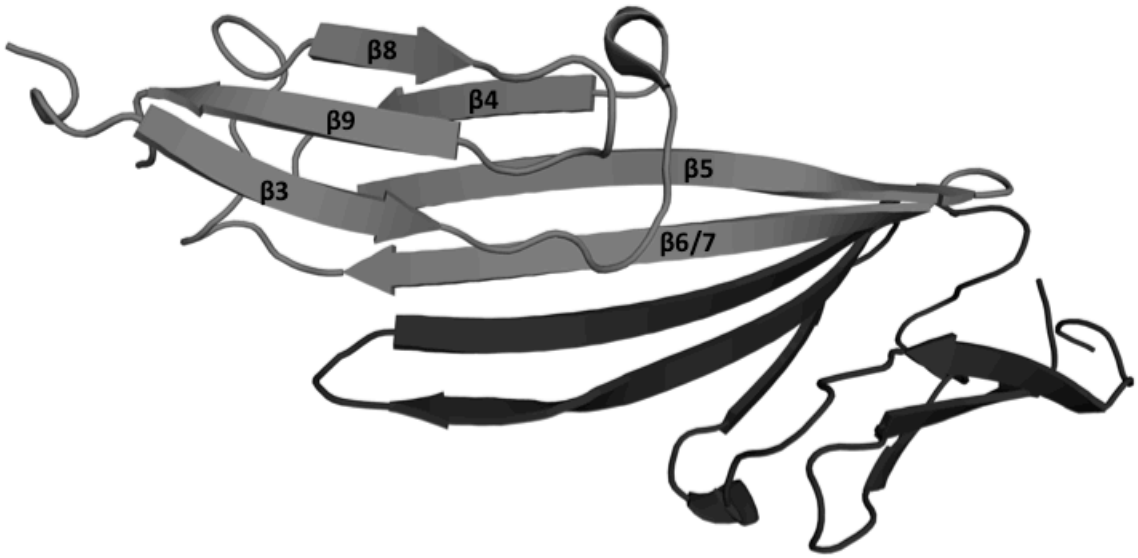
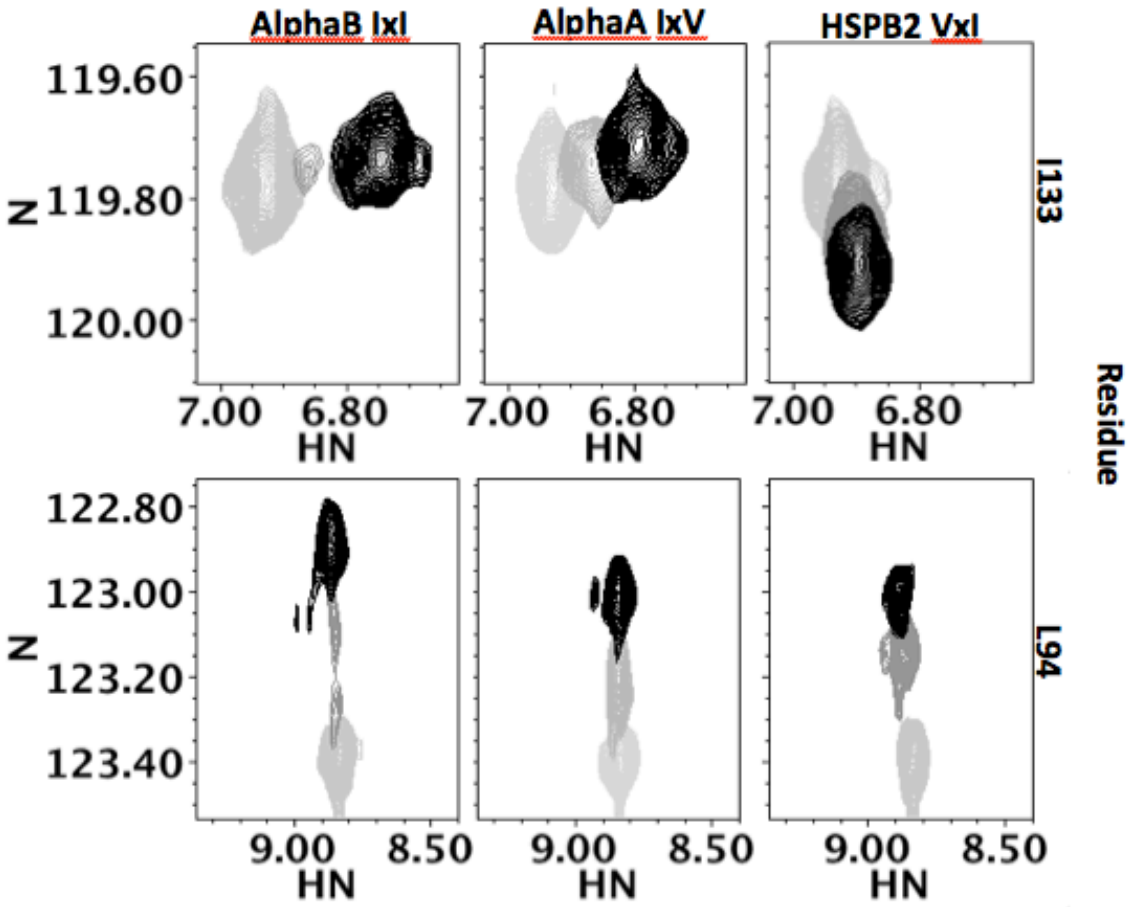
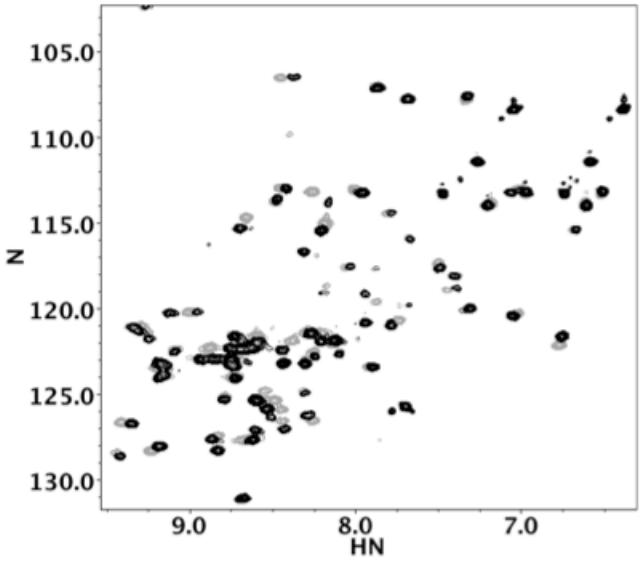


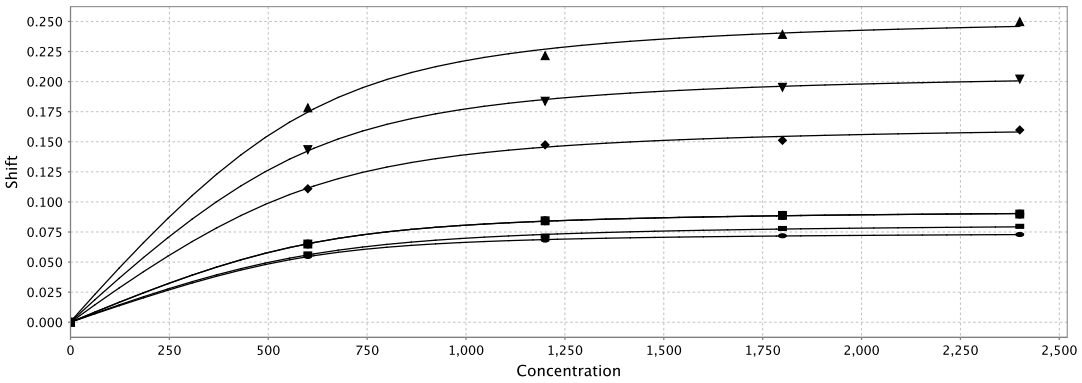
Supplemental Figure S1. Secondary structure representation of the αB-ACD determined by ssNMR (2KLR). The two monomers are shown in different shades of gray and β strands are labeled.



Supplemental figure S2. αB-IxI peptide, αA-IxV and HSPB2-VxI peptides show different affinities and residence times. The resonances of residue I133 (Top) from the β8 strand and L94 (Bottom) from the β4 strand of the αB ACD are plotted in the absence of peptide and in the presence of 1-fold and 4-fold αB-IxI-peptide (Left) and αA-IxV peptide (Middle) and HSPB2 peptide (Right). Overlay of spectra measured from αB-ACD samples containing no peptide, 1-fold, 2-fold and 4-fold excess peptide are shown in progressively darker shades of gray. Broadening in the spectrum collected with 1-fold αB-IxI reflects the longer lifetime (Left Bottom) of the bound state relative to the other peptides.



Supplemental Figure S3. ^{1}H-^{15}N HSQC Spectra of wt αB-ACD (Gray) and the β8 mutant S135Q αB-ACD indicate the mutant does not disrupt the ACD structure (Black). Peaks that show a chemical shift greater than .1 as a result of the mutation are V77, V91, L131, I133, T134, S136 and V145.



Supplemental Figure S4. Chemical shift perturbations fit to quadratic binding curves for the HSPB2 peptide. 6 peaks were fit for the HSPB2 peptide (92, 94, 136, 95, 78, 127 and 97) yielding an average K_d of 87 +/- 12 μM. Chemical shift perturbation is plotted on the y-axis and total peptide concentration(μM) is plotted on the x-axis.