**­­Supplementary Information for:**

**Conditional disorder in small heat-shock proteins**

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**Supplementary Figure 1. Histidine residues in the ACD and a pH titration followed by NMR.** (**A**) Crystal structure of the HSP27 ACD dimer (PDB: 4mjh) with the dotted line denoting separate monomers. Each ACD monomer contains four histidine residues that are located in the β2 strand (H90), the region between β3 and β4 (H103), the β5 strand (H124), and the loop between the β5 strand and the β6+7 strand (H131). Previous NMR data indicate that protonation of H124 at pH values below 7 is responsible for shifting the dimer-monomer equilibrium toward the monomer [1–3]. (**B**) 2D 1H-15N HSQC spectra of 15N-labeled cHSP27 (residues 84-171) at a total protein concentration of 250 µM in 30 mM sodium phosphate, 2 mM EDTA buffer as a function of pH. The C137S mutation was included to prevent disulfide formation. The pH values are indicated in the lower-right corner of each panel. The resonance from G116 near 105/8.8 ppm is boxed and the M and D refer to monomer and dimer, respectively, as determined previously [1]. Resonances from interfacial residues are circled and annotated in the pH 7 spectrum. The circles are present in the remaining spectra to highlight the location of the resonance frequencies at pH 7. Spectra were recorded on a 14.1 T spectrometer with the temperature unit set to 25 °C. Notably, only *ca.* 60 resonances are observed in the pH 5 spectrum compared to 75 resonances at pH 7.

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**Supplementary Figure 2. Inter-molecular association of the cHSP27 monomer at elevated concentrations.** 2D 1H-15N HSQC spectra of 15N-labeled cHSP27 monomer in 30 mM sodium phosphate, 2 mM EDTA buffer at pH 4.2 and 298 K. The protein concentration was 0.15 mM (**A**) or 0.45 mM (**B**). The spectra were acquired on a spectrometer operating at 14.1 T with identical parameters and processed in the same manner.

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**Supplementary Figure 3. Resonance assignments and secondary chemical shifts of the acid-unfolded form of cHSP27.** (**A**) Assigned spectrum of acid-unfolded cHSP27 at pH 3 at a static magnetic field of 14.1 T and 25 °C. (**B**-**D**) Secondary chemical shifts, Δδ, for 1HN, 13CO, and 13Cα. Neighbor-corrected random coil chemical shifts [4] were subtracted from the experimentally measured values. The uniformly small values indicate that the protein is disordered, but the small deviations from zero are suggestive of transient secondary structure. Slightly negative ΔδCα values are suggestive of low populations of extended conformations.

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**Supplementary Figure 4. Parameters obtained from the modelfree analysis of the cHSP27 monomer.** The 15N relaxation data (Figure 3) from the cHSP27 monomer were fit to Lipari-Szabo models that included a local τc value. (**A**) The locally fitted τc values are shown as a function of residue number. Excluding the disordered N-terminal region and the locally unfolded interfacial region, the average value of τc, <τc>, is 5.89 ± 0.8 ns, which closely agrees with the value of the global τc determined by 15N *R*2/*R*1 ratios (6.0 ± 0.8 ns). (**B**) τevalues describe rapid internal motion of N-H bond vectors on the picosecond timescale. The residues that required τe values are shown here. (**C**) Exchange contributions to *R*2 mainly cluster to the interfacial region, suggesting evidence of conformational exchange on the millisecond timescale that was not suppressed by the 1 kHz spin lock applied during the 15N *R*1ρ experiments.

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**Supplementary Figure 5. Analysis of 1*D*NH couplings for the cHSP27 dimer and monomer** (**A**) Correlation plot showing the measured values of 1*D*NH for the cHSP27 dimer (x-axis) or monomer (y-axis) in solutions of 4.2% PEG-hexanol (see Methods). The solid line indicates *y* = *x*. The graph on the left shows residues only in secondary structural elements (*n* = 36), and the graph on the right depicts all residues (*n* = 75). The values of Pearson’s correlation coefficient (*R*2) are indicated above each graph. (**B**) Comparison of experimentally measured (y-axis) and predicted (x-axis) values of 1*D*NH for the cHSP27 dimer or monomer (**C**). The solid lines depict *y* = *x*. The graphs on the left show residues located in regions of secondary structure and the graphs on the right depict all residues. The *Q* factors [5] are indicated above each graph.

**Table S1. 1*D*NH values for the cHSP27 monomer and dimer recorded at 900 MHz in samples that were aligned in 4.3% PEG-hexanol.** Columns that contain a superscript refer to the monomer and those with b refer to the dimer. The values under the column “Predicted (Hz)” refer to those back-calculated from the crystal structure using the fitted alignment tensor. See Methods for additional details.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Res** | **RDC (Hz)a** | **Error (Hz)a** | **Predicted (Hz)a** | **RDC (Hz)b** | **Error (Hz)b** | **Predicted (Hz)b** |
| 85 | 1.9 | 0.01 | N/A | N/A | N/A | N/A |
| 86 | 3.2 | 0.01 | 20.8 | 0.1 | 0.2 | 18.8 |
| 87 | 3.6 | 0.02 | 17.5 | 7.4 | 0.1 | 23.7 |
| 88 | 3.2 | 0.01 | 19.2 | 5.3 | 0.1 | 13.7 |
| 89 | 5.2 | 0.02 | 5.7 | 10.9 | 0.1 | 22.3 |
| 90 | 5.0 | 0.02 | 14.5 | N/A | N/A | 8.9 |
| 91 | 4.2 | 0.02 | 11.4 | 9.5 | 1.8 | 20.0 |
| 92 | 6.1 | 0.03 | 9.6 | 15.4 | 0.5 | 20.2 |
| 93 | 4.9 | 0.10 | 19.9 | 0.3 | 0.5 | 23.7 |
| 94 | 13.7 | 0.20 | 21.0 | 14.0 | 0.4 | 20.7 |
| 95 | 15.5 | 0.56 | 10.7 | 12.0 | 0.8 | -7.7 |
| 96 | 15.3 | 0.40 | 17.0 | N/A | N/A | 28.9 |
| 97 | 21.0 | 0.52 | 18.2 | 25.5 | 0.6 | 24.3 |
| 98 | 17.8 | 0.30 | 19.5 | 29.7 | 0.7 | 28.4 |
| 99 | 20.2 | 0.32 | 20.2 | 19.2 | 0.5 | 24.1 |
| 100 | 19.8 | 0.35 | 20.2 | 29.5 | 0.7 | 29.3 |
| 101 | 4.8 | 0.97 | -2.6 | N/A | N/A | 0.4 |
| 102 | 1.2 | 0.91 | 9.6 | 24.2 | 3.0 | 26.0 |
| 103 | 3.2 | 0.39 | 6.2 | -3.1 | 1.8 | 17.0 |
| 104 | 18.6 | 0.41 | -22.5 | -4.8 | 1.0 | -9.0 |
| 105 | 12.0 | 1.88 | -6.9 | -2.9 | 2.3 | 10.5 |
| 107 | 0.3 | 3.21 | -1.2 | -8.6 | 1.6 | -11.1 |
| 108 | 0.4 | 0.53 | 5.1 | 23.7 | 2.9 | 21.6 |
| 109 | 8.5 | 0.41 | 17.6 | 25.6 | 1.2 | 23.6 |
| 110 | 18.5 | 0.45 | 20.7 | 12.0 | 0.3 | 16.0 |
| 111 | 21.4 | 0.52 | 21.4 | 25.1 | 0.5 | 24.3 |
| 112 | 19.1 | 0.30 | 17.2 | 8.2 | 0.3 | 2.7 |
| 113 | 19.5 | 0.32 | 19.7 | 20.4 | 0.2 | 23.3 |
| 114 | 16.5 | 0.28 | 17.8 | 11.0 | 0.6 | 15.7 |
| 115 | 17.1 | 0.42 | 17.6 | 0.6 | 1.6 | 4.0 |
| 116 | 17.8 | 0.42 | 11.7 | 9.7 | 1.6 | 1.7 |
| 117 | 8.5 | 0.57 | 19.2 | 22.9 | 0.9 | 14.3 |
| 118 | 13.2 | 0.62 | 18.4 | 24.7 | 1.2 | 24.5 |
| 119 | 19.7 | 0.70 | 17.7 | 4.4 | 0.0 | 5.5 |
| 120 | 20.9 | 0.50 | 21.3 | 20.5 | 1.7 | 25.8 |
| 121 | 18.3 | 1.03 | 14.0 | 3.5 | 4.0 | 3.3 |
| 122 | 20.2 | 0.50 | 18.3 | 20.7 | 0.7 | 21.3 |
| 123 | 15.1 | 0.90 | 11.6 | 14.4 | 4.0 | 12.7 |
| 124 | 17.8 | 0.90 | 17.4 | 13.1 | 5.3 | 12.3 |
| 125 | 10.9 | 0.35 | 16.5 | 17.9 | 4.0 | 12.1 |
| 126 | 4.3 | 0.08 | 5.7 | 23.6 | 1.0 | 22.0 |
| 127 | 10.0 | 0.20 | 17.1 | 22.0 | 1.2 | 27.9 |
| 128 | 7.6 | 0.25 | 18.8 | 16.7 | 3.5 | 27.6 |
| 129 | 2.7 | 0.30 | -16.9 | -7.8 | 4.0 | -36.0 |
| 130 | 0.5 | 0.46 | -31.0 | -29.1 | 2.7 | -31.9 |
| 131 | 3.4 | 0.28 | -26.5 | -37.3 | 3.2 | -44.5 |
| 132 | 8.1 | 0.25 | -9.8 | -11.9 | 3.2 | -19.6 |
| 133 | 6.3 | 0.32 | -10.4 | 2.8 | 3.6 | -25.5 |
| 134 | 8.6 | 0.67 | 17.8 | 25.4 | 1.6 | 22.9 |
| 135 | 8.5 | 0.60 | 20.3 | 12.5 | 1.6 | 17.1 |
| 136 | 6.8 | 0.36 | 20.6 | N/A | N/A | 23.3 |
| 137 | 11.6 | 0.36 | 19.7 | 14.1 | 3.9 | 13.8 |
| 138 | 15.3 | 0.22 | 19.9 | 20.8 | 2.7 | 12.2 |
| 139 | 18.2 | 0.42 | 18.8 | 23.6 | 1.2 | 11.4 |
| 140 | 21.9 | 1.10 | 21.1 | 26.6 | 3.4 | 26.0 |
| 141 | 18.8 | 0.49 | 12.9 | 20.3 | 2.5 | -1.3 |
| 142 | 18.6 | 0.66 | 20.6 | 23.4 | 1.3 | 23.5 |
| 143 | 18.3 | 0.34 | 17.7 | 8.9 | 0.3 | 11.8 |
| 144 | 5.0 | 0.28 | 0.4 | 23.2 | 0.4 | 19.7 |
| 147 | 0.2 | 0.20 | -6.1 | 13.8 | 0.2 | 14.5 |
| 148 | 11.6 | 0.25 | 18.8 | -1.7 | 0.3 | 11.4 |
| 149 | 3.3 | 0.28 | -4.4 | 14.0 | 0.2 | 11.9 |
| 151 | 17.9 | 0.36 | -15.3 | -27.4 | 1.5 | -19.0 |
| 152 | 13.5 | 0.30 | 12.1 | 23.8 | 2.4 | 23.2 |
| 153 | 8.1 | 0.21 | 9.3 | 22.4 | 1.1 | 20.8 |
| 154 | 6.4 | 0.31 | 6.4 | 24.5 | 0.4 | 24.2 |
| 155 | 18.3 | 0.22 | 20.9 | 25.8 | 0.3 | 24.5 |
| 156 | 19.5 | 0.26 | 18.1 | 26.7 | 0.4 | 14.3 |
| 157 | 19.9 | 0.28 | 21.4 | 22.8 | 0.3 | 23.9 |
| 158 | 20.5 | 0.26 | 18.0 | 23.2 | 0.4 | 24.4 |
| 160 | -26.8 | 0.44 | -31.6 | -37.3 | 1.8 | -41.8 |
| 161 | 12.0 | 0.24 | 20.6 | -6.1 | 0.7 | 19.9 |
| 162 | 12.6 | 0.21 | 13.7 | 18.7 | 0.6 | 17.2 |
| 163 | 15.8 | 0.50 | 6.1 | 6.1 | 1.0 | -5.3 |
| 164 | 22.0 | 0.41 | 19.2 | 32.5 | 0.6 | 29.4 |
| 165 | 17.8 | 0.44 | 18.1 | 24.4 | 0.5 | 25.5 |
| 166 | 12.6 | 0.40 | 15.0 | 28.3 | 0.7 | 28.7 |
| 167 | 11.4 | 0.54 | 9.2 | 25.9 | 0.7 | 23.9 |
| 169 | 6.5 | 0.01 | 0.0 | 21.5 | 0.3 | 18.9 |
| 171 | 6.6 | 0.01 | N/A | 13.1 | 0.1 | N/A |

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