

SUPPLEMENTARY INFORMATION

Regulatory inter-domain interactions influence Hsp70 recruitment to the DnaJB8 chaperone

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Supplementary Data

Supplementary Data 1. Primary XL-MS data for DnaJB8 and its domains.

Supplementary Data 2. Primary DLS measurements for DnaJB8 oligomers.

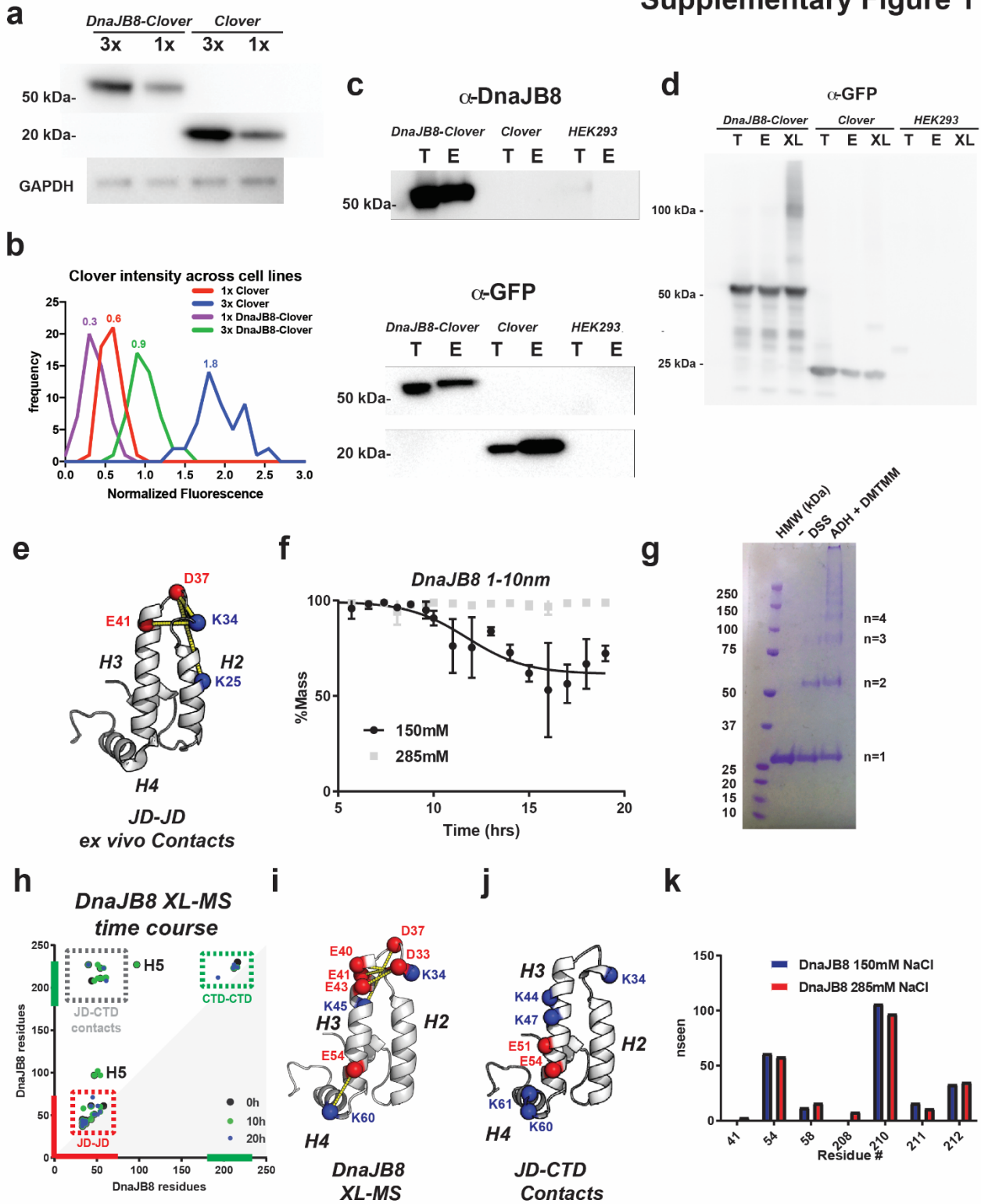
Supplementary Table 1. Residue distribution per domain with net charge for each domain.

Domain aa. length	Domain	Residue																				Net charge for the domain
		A	R	N	D	Q	E	G	H	I	L	K	M	F	P	S	T	Y	V	W	C	
1-75	JD	8	5	3	6	1	7	2	1	1	6	10	1	1	3	7	0	5	4	2	1	+2
76-148	G/F	6	5	2	5	0	5	13	2	1	2	0	1	14	6	6	3	2	0	1	0	-5
149-185	S/T	0	0	1	0	0	1	7	1	0	1	1	3	4	0	15	4	0	1	0	1	0
186-232	CTD	0	2	3	2	3	6	4	7	2	2	6	1	0	0	2	3	0	6	1	0	0

Supplementary Table 2. Primers used for cloning DnaJB8 variants.

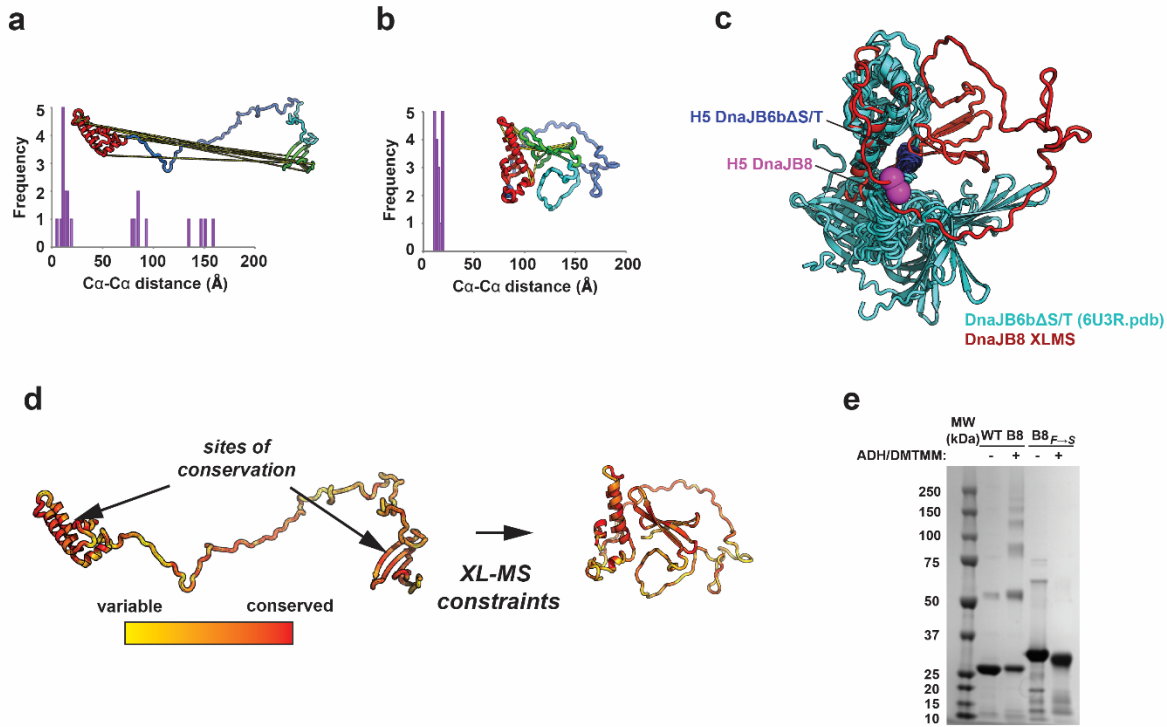
Primer Name	Sequence
DnaJB8_FM5_for	5'-GAACCGTCAGATCCGCTAGCGCCGCCACCATGGCTAACTACTACGAAGTGCTGGGCGTG-3'
DnaJB8_FM5_rev	5'-GCCGGATCCAGCAGCGGAGCCAGCAGAACCCTTGCTGTCCATCCATTTGAGCTGCTCC-3'
DnaJB8_pet29b_for	5'-ATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAACTACTACGAAGTGCTGG-3'
DnaJB8_pet29b_rev	5'-GCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGCTTGCTGTCCATCCATTTGAGC-3'
Jdomain_rev	5'-GATCTCAGTGGTGGTGGTGGTGGTGCTCGAGGGGCGTGCTGGCCCCGCCACCAGCCCCGCC-3'
CTD_for	5'-AATTTTGTTTAAACAAGAAGGAGATATACATATGGGCTCCAGTTCTGGCAGCTCGGGGTTTC-3'
CTDdel rev	5'-CCGGATCTCAGTGGTGGTGGTGGTGGTGCTCAGGAAATTCTCCAAAGCCTGCCGAG-3'

Supplementary Figure 1

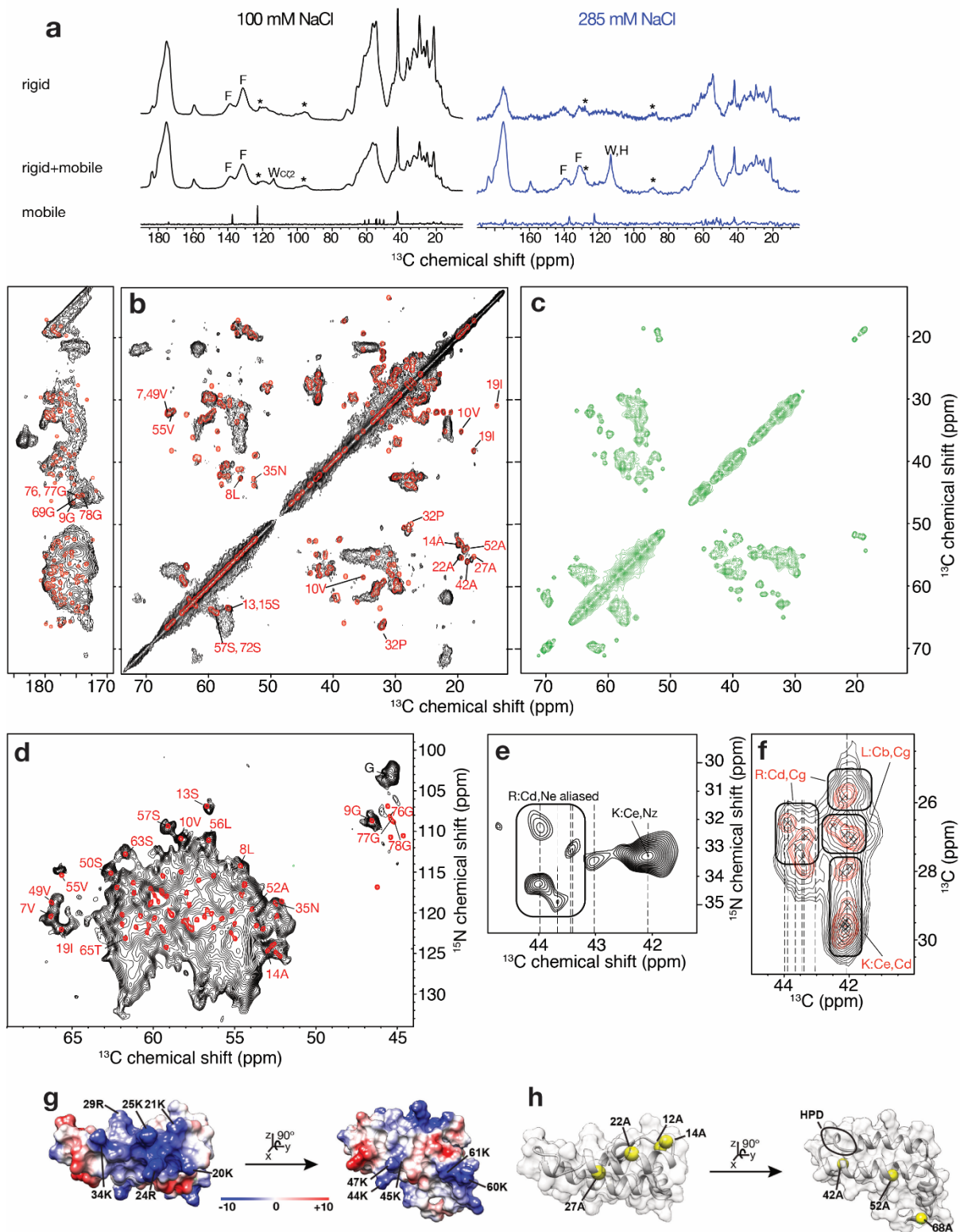


Supplementary Figure 1. Biochemical and mass spectrometry analysis of DnaJB8 in cells and *in vitro*. (a) Western blot analysis of 1x DnaJB8-Clover, 3x DnaJB8-Clover, 1x Clover and 3x Clover cell lines to quantify levels of expression in each cell line. GAPDH western blot is shown as a loading control. The experiment was performed 3 independent times. (b) Normalized FITC intensity analysis of 1x Clover (red), 3x Clover (blue), 1x DnaJB8-Clover (purple) and 3x DnaJB8-Clover (green) cell lines. Intensity measurements were calculated from 50 images for each cell line and the signal normalized to DAPI fluorescence intensity. (c) Western blot analysis of input and anti-GFP nanobody elutions DnaJB8-Clover, Clover and HEK293 cell lines as detected with anti-DnaJB8 and anti-GFP antibodies. Total (T) and elutions (E) for each condition are shown. This experiment was performed 3 independent times. (d) Western blot analysis of DnaJB8-Clover and Clover proteins isolated from mammalian cell lines. Total (T), elutions (E) and crosslinked elutions (XL) of each condition are shown. Western blot was probed with GFP antibodies. This experiment was performed 3 independent times. (e) JD intra-domain ADH/DMTMM crosslinks identified from DnaJB8-Clover isolated from cells mapped onto the JD structure. JD is shown in cartoon representation and is colored in white. Sites of crosslink are shown as spheres and are colored red or blue for aspartic/glutamic and lysines, respectively. Dashed yellow lines connect linked amino acid pairs. (f) DnaJB8 particles with a R_h of 1-10nm in the DLS data were analyzed by proportion of the total sample (% mass) and binned by the size distribution of the constituent particles. The samples were run in triplicate with averages reported and error bars reporting the standard deviation. The time evolution of this mass fraction is shown for 150mM NaCl (black) and 285mM NaCl buffer conditions in 1xPBS. (g) SDS-PAGE coomassie gel showing DnaJB8 in the absence of crosslinker (left), with DSS (middle), and with DMTMM and ADH (right). (h) XL-MS contact map of DnaJB8 crosslinks identified using DMTMM and ADH from a time course: t=0hrs (large black dot), t=10hrs (medium green dot) and t=20hrs (small blue dot), small. The axes are colored in red and green for JD and CTD, respectively. Crosslink pairs between JD-CTD are shown in dashed box colored grey, red and green, respectively. Helix 5 crosslinks are denoted by H5. (i) JD intra-domain ADH/DMTMM *in vitro* crosslinks mapped onto the JD structure. JD is shown in cartoon representation and is colored in white. Sites of crosslink are shown as spheres and are colored red or blue for aspartic/glutamic and lysines, respectively. Dashed yellow lines connect linked amino acid pairs. (j) Seven cumulative crosslink sites identified from *in vitro* JD-CTD inter-domain ADH/DMTMM crosslinks mapped onto the JD structure. DnaJB8 JD is shown in cartoon representation and is colored in white. JD lysine sites that crosslink to CTD are shown as spheres and are colored blue and acids are colored red. (k) Histogram of frequency of ADH monolinks observed in normal and elevated ionic strength XLMS experiments.

Supplementary Figure 2

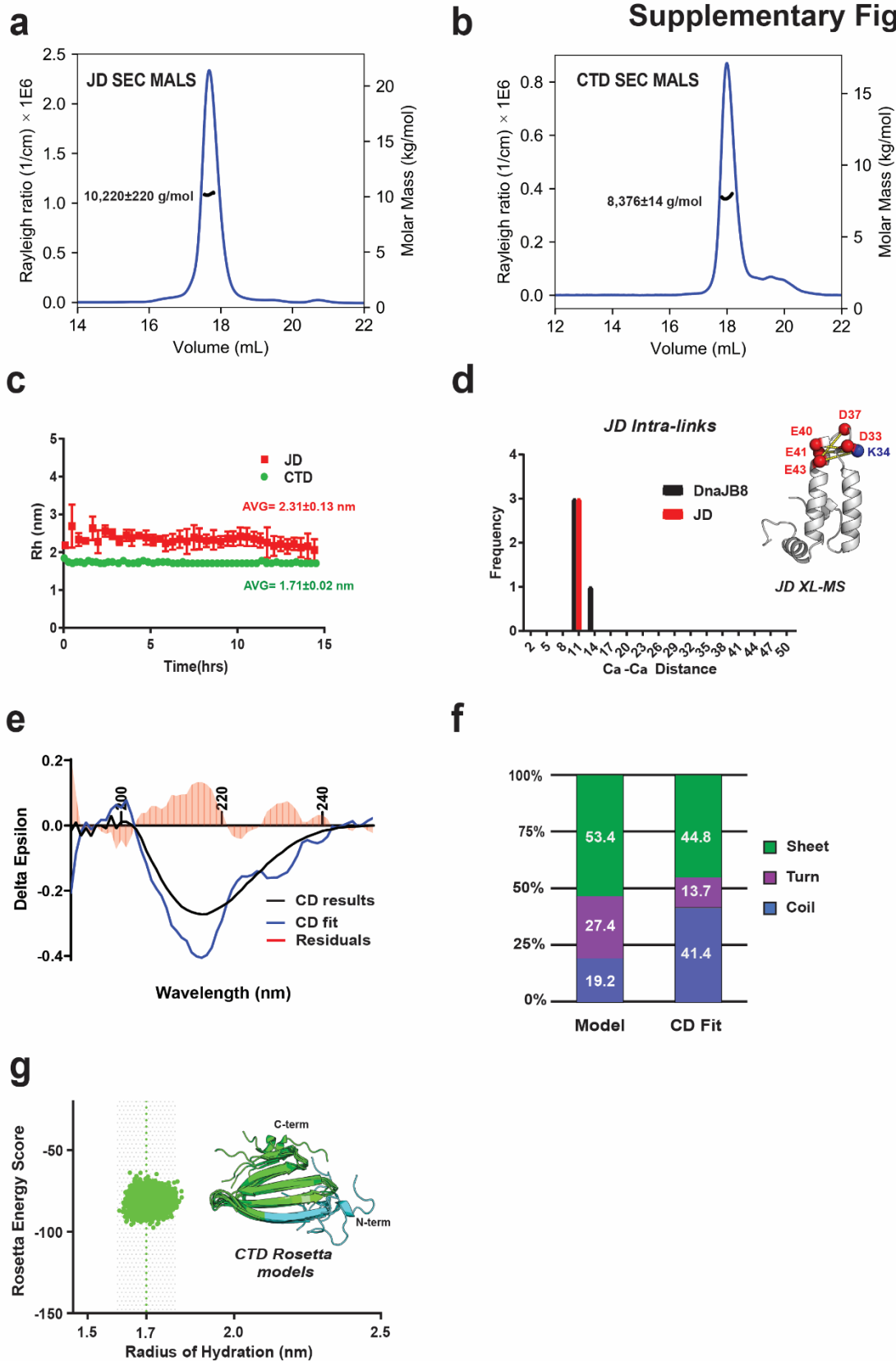


Supplementary Figure 2. Modeling the full length DnaJB8 monomer using crosslinks. **(a)** DnaJB8 ADH/DMTMM crosslinks mapped onto an expanded structure featuring the conserved JD and our predicted CTD model. The histogram of C α -C α distances shows 11 crosslinks that exceed the physical limitations for ADH/DMTMM. **(b)** Rosetta *ab initio* collapsed structure of DnaJB8 with ADH/DMTMM crosslinks mapped. The histogram of C α -C α distances shows that all crosslinks are satisfied when the JD and CTD are docked in this model. **(c)** Overlay of our Rosetta *ab initio* collapsed structure (red) with the solution NMR ensemble of the published DnaJB6bΔST deletion variant (cyan; PDBID: 6U3R). The proposed helix 5 (H5) is shown on the DnaJB8 (pink) and DnaJB6bΔST (blue) models. The position of H5 in our DnaJB8 XL-MS-constrained model is close to the position in the DnaJB6b model. The major difference is the position of the CTD, which is influenced by both the XL-MS constraints, and the inclusion of the S/T region that was deleted in the DnaJB6b construct. **(d)** Conserved surfaces on the JD and CTD in the full-length DnaJB8 model mediate the interaction. Highly conserved sites are colored in red (and highlighted by arrows) and variable positions are colored in yellow. Sequence-based conservation of DnaJB8 shows highly conserved faces along the JD, and some conserved faces in the CTD that overlap with the XL-MS identified surfaces. **(e)** SDS-PAGE coomassie gel of DnaJB8 and DnaJB8_{F→S} mutant crosslinked with ADH/DMTMM. This experiment was performed 3 independent times.



Supplementary Figure 3. Additional ssNMR data and analysis for DnaJB8 oligomers in PBS. **(a)** ^{13}C 1D spectra at 100mM NaCl (black) and elevated ionic strength (blue) that show rigid residues (CP), rigid and mobile residues (SPE) and only mobile residues (INEPT). **(b)** Overlay of the experimental (black) 2D ^{13}C - ^{13}C CP-DARR spectrum of DnaJB8 oligomers uniformly labeled with ^{13}C and ^{15}N and synthetic (red) spectrum of the DnaJB8 JD in solution generated from BMRB chemical shifts (red assignments). **(c)** Simulated spectrum from three other domains (G/F, S/T and CTD), showing diagonal peaks and one-bond cross-peaks involving backbone $\text{C}\alpha$ - $\text{C}\beta$ and CO - $\text{C}\alpha$ carbons. **(d)** 2D ^{13}C - ^{15}N CP-based NCA experimental spectrum (black) of DnaJB8 oligomers uniformly labeled with ^{13}C and ^{15}N , overlaid with synthetic spectrum of the DnaJB8 JD in solution (red) from BMRB chemical shifts (red assignments). **(e)** 2D CP-based ^{13}C - ^{15}N spectrum with $\text{C}\epsilon$ - $\text{N}\zeta$ and (aliased) $\text{C}\delta$ - $\text{N}\epsilon$ correlations from immobilized Lysine and Arginine side chains, respectively. **(f)** Enlarged ^{13}C - ^{13}C spectral region from panel (A) demonstrating good alignment of Arg, Lys and Leu signals between experimental ssNMR (black) and solution chemical shifts of the JD (red). Dashed lines mark known ^{13}C positions used to confirm amino acid types of signals. **(g)** Electrostatic surface potential of the DnaJB8 JD, with selected residues indicated. Highly positive and negative potentials are colored blue and red, respectively. White color represents neutral charge. **(h)** The same view showing the location of Ala with narrow peaks at 100 mM NaCl which disappear at 285 mM NaCl (Fig. 3, panel “c”).

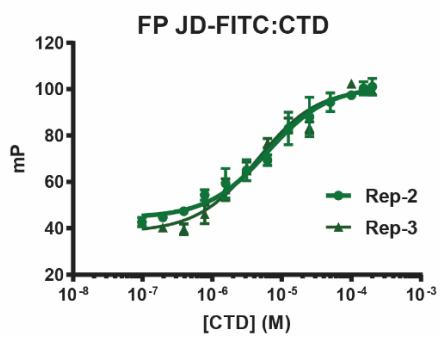
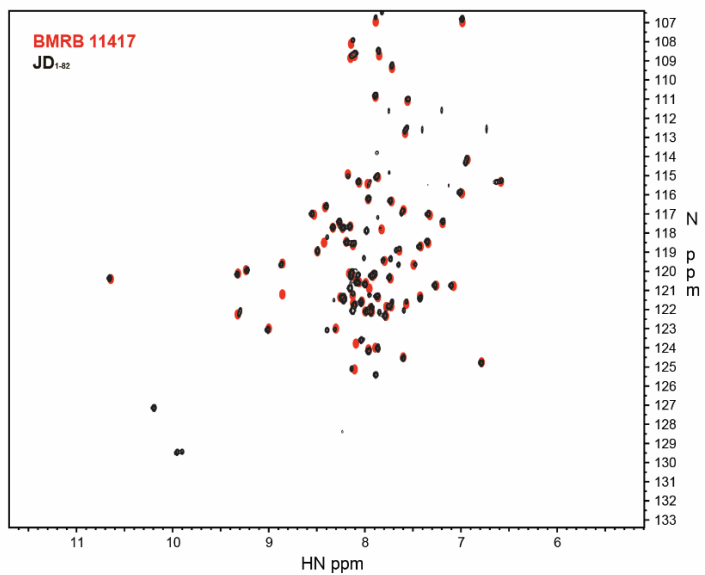
Supplementary Figure 4



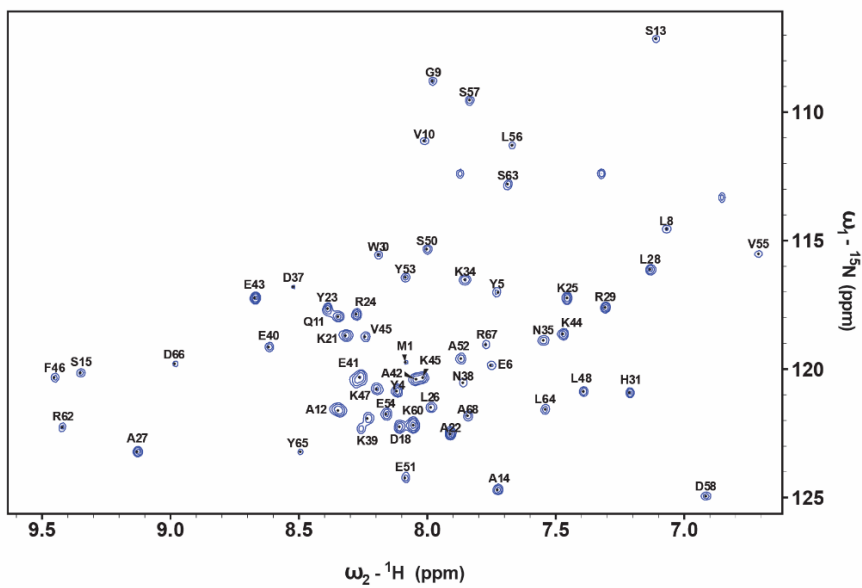
Supplementary Figure 4. JD and CTD are stable monomers. (a) SEC-MALS of JD₁₋₈₂ shows a single peak with a calculated molar mass of $10,220 \pm 220$ g/mol consistent with a monomer. (b) SEC-MALS of CTD₁₇₀₋₂₃₂ shows a single peak with a calculated molar mass of $8,376 \pm 14$ g/mol consistent with a monomer. (c) DLS time course of JD₁₋₈₂ and CTD₁₇₀₋₂₃₂ constructs. Samples were run in triplicates with averages reported and error bars reporting the standard deviation. The average R_h of JD₁₋₈₂ and CTD₁₇₀₋₂₃₂ was calculated to be 2.30 ± 0.12 nm and 1.71 ± 0.02 nm, respectively. (d) Histogram of JD intra-domain crosslinks across XLMS experiments for DnaJB8 (black), JD₁₋₈₂ (red), and JD₁₋₈₂ mixed with CTD₁₇₀₋₂₃₂ (grey). All intra-domain crosslinks satisfy the physical constraint of < 20 nm for the ADH/DMTMM crosslinkers. (e,f) CD spectra of CTD was analyzed using the Bestsel server. Secondary structure analysis of the data shows high β -sheet character (yellow) with some helix character (purple) and no random coil (blue). The results are compared to the CTD model generated from *ab initio*. (g) 5,000 Rosetta *ab initio* generated models of CTD. The structural models are consistent with R_h values derived from DLS (dashed line). Structural overlay of low energy scoring models reveals consistent pleated beta-sheet fold. Models are shown in cartoon representation and are colored in green.

Supplementary Figure 5

a

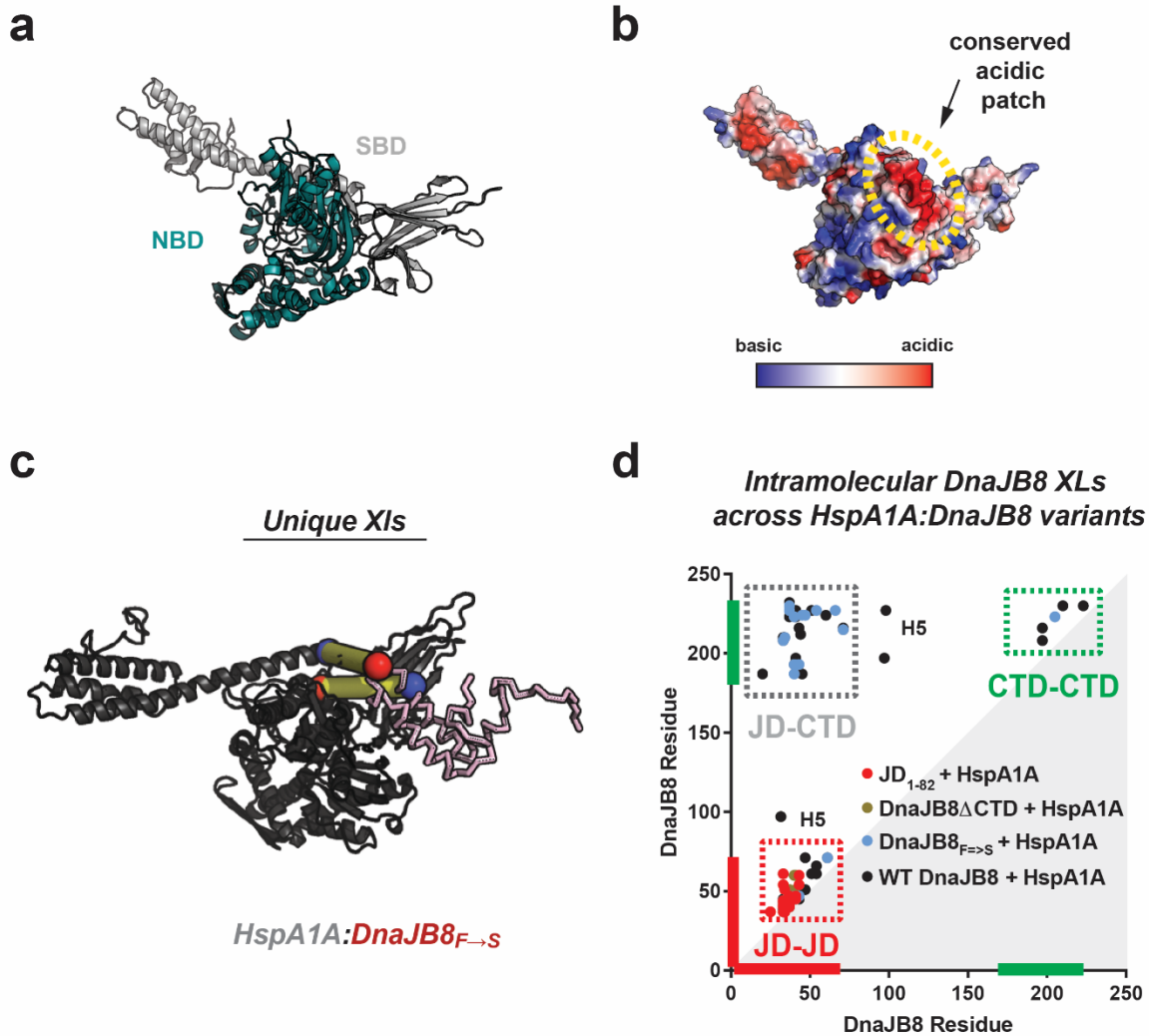
**b**

C



Supplementary Figure 5. JD₁₋₈₂ construct spectra consistent with deposited chemical shifts. **(a)** Two technical replicates of the JD-FITC fluorescence polarization binding assay onto CTD as shown in Fig. 5a. Each replicate was performed in triplicate and shown as averages with standard deviations, yielding affinities of 6.21 ± 0.94 μ M and 4.43 ± 0.55 μ M, respectively. **(b)** Overlay of our ^{15}N - ^1H HSQC spectra for JD₁₋₈₂(black) onto the submitted spectra for DnaJB8 JD (red) (BMRB:11417). 97% of assigned peaks in the published spectra were matched to our data. **(c)** Amino acid assignments mapped onto the HSQC spectra for the JD₁₋₈₂ protein.

Supplementary Figure 6



Supplementary Figure 6. Hsp70 proteins present a conserved acidic surface. (a) Structure of HspA1A colored by domain with the nucleotide binding domain (NBD) in turquoise and substrate binding domain (SBD) in grey. (b) Electrostatic surface mapped onto the HspA1A structure with the negatively charged Hsp40-JD-binding site circled in yellow. (c) The two intermolecular crosslinks identified across three XL-MS datasets between JD and HspA1A in the DnaJB8:HspA1A complex mapped onto JD-HspA1A model. JD is shown in ribbon representation and HspA1A in cartoon representation, colored pink and black respectively. Sites of crosslink are shown as red or blue spheres for aspartic/glutamic acid and lysines, respectively. Yellow lines connect linked amino acid pairs. (d) XL-MS contact map of changes in intramolecular DnaJB8 crosslinks identified using DMTMM and ADH in co-mixtures of different variants of DnaJB8 with HspA1A: WT DnaJB8:HspA1A (black), DnaJB8 Δ CTD:HspA1A (brown), DnaJB8_{F→S}:HspA1A (blue) and JD₁₋₈₂:HspA1A (red). The axes are colored in red and green for JD and CTD, respectively. Crosslink pairs between JD-CTD are shown in dashed box colored grey, red and green, respectively. Contacts to helix 5 in WT DnaJB8 are denoted with H5.