

Supplemental materials

TABLE S1: Backbone Resonance Assignments for Hsc70									
res	Nr	N	H	CA(i-1)	CA(i)	CB(i-1)	CB(i)	CO(i-1)	CO(i)
		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
~	~								
G	TAG10								
V	TAG11	119.25	7.789	44.97	61.63		32.05	173.99	175.58
D	TAG12	124.16	8.296	61.59	53.73	32.03	40.55	175.60	176.19
L	TAG13	124.05	8.237	53.71	55.02	40.67	41.20	176.18	178.11
G	TAG14	109.54	8.423	55.03	45.20	41.20		178.13	174.74
T	TAG15	113.39	7.887	45.20	61.75		69.09	174.80	174.93
E	TAG16	122.97	8.421	61.80	56.70	69.16	29.17	174.95	176.27
N	TAG17	119.35	8.277	56.70	53.07	29.11	38.23	176.29	175.12
L	TAG18	122.60	7.996	53.10	55.27	38.27	41.14	175.11	177.13
Y	TAG19	119.92	7.935	55.33	57.65		37.84	177.20	175.66
F	TAG20	121.16	7.851	57.81	57.47		38.81	175.69	175.52
Q	TAG21	121.77	8.056	57.49	55.59		28.46	175.53	175.80
S	TAG22								
N	TAG23								
A	TAG24	118.28	8.031	52.91	55.49			177.99	
M	TAG25	122.39	7.861	55.47	55.04			176.32	
L	391	122.38	7.896	54.95	54.62		41.24	176.95	
L	392	122.95	7.958	54.68	54.44		41.06	176.63	
L	393	122.66	7.914	54.36	54.36	41.13	41.67	176.62	
L	394								
D	395	122.43	7.964	53.11	55.27		41.12	175.13	177.15
V	396	119.68	7.927	55.33	57.76		37.77	177.21	
T	397								
P	398								
~	~								
G	616								
M	617	121.23	8.173	44.68	52.95		31.69	173.78	174.46
P	618								
G	619	110.04	8.432	63.17	44.85			177.55	174.58
G	620	108.71	8.112	44.83	44.62			174.66	173.70
M	621	121.05	8.111	44.67	52.91		31.74	173.71	174.44
M(2nd)	621	120.74	8.046	44.63	52.86		31.79	173.63	174.40

P	622								
G	623	109.91	8.399	63.12	44.88			177.47	174.56
G	624								
F	625	121.23	8.056	44.56	55.34		38.64	173.35	174.35
P	626								
G	627								
G	628	109.17	8.186	45.01	44.94			174.83	174.57
G	629	108.68	8.050	44.87	44.60			174.55	173.32
A	630	121.27	8.021	44.57	55.44			173.32	174.36
P	631								
P	632								
S	633	116.38	8.317	62.66	58.03			177.18	
G	634								
G	635	109.16	8.161	44.92	44.52			174.59	173.26
A	636	125.06	8.017	44.41	49.88		17.46	173.27	175.11
S	637								
S	638								
G	639	111.02	8.116	57.94	44.06			174.46	171.57
P	640								
T	641	115.88	8.244	62.66	61.55	31.37	69.34	177.22	174.46
I	642	124.00	8.112	61.57	60.50	69.30	37.88	174.48	175.91
I(2nd)	642	124.48	8.252	61.76	60.44		37.92	174.27	
E	643	125.35	8.329	60.52	55.88	37.90	29.69	175.92	176.06
E	644	123.22	8.314	55.96	55.85		29.57	176.09	176.07
V	645	121.20	8.067	55.89	61.44	29.52	32.42	176.12	175.00
D	646	129.16	7.882	61.43	55.60	32.38	41.69	175.00	180.82

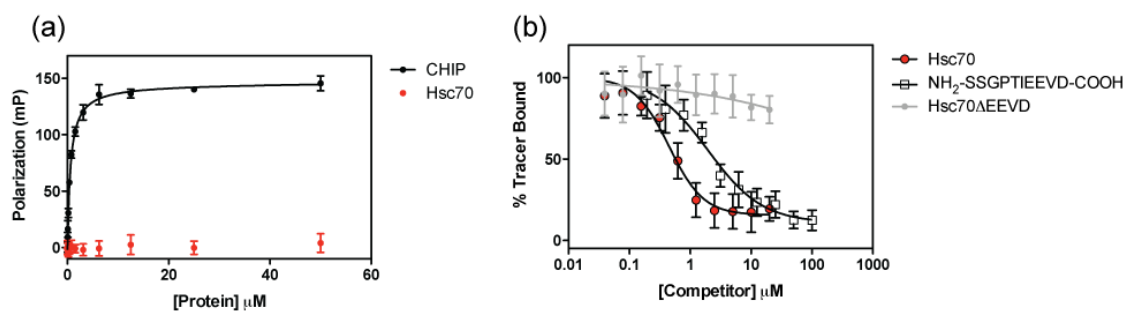


Fig. S1. Fluorescence polarization assay for Hsc70 – CHIP binding. (a) The C-terminal peptide tracer (5-FAM-SSGPTIEEVD) binds CHIP with a K_d of $0.61 \pm 0.04 \mu\text{M}$. Free Hsc70 does not bind the tracer to any appreciable extent. (b) At 20 nM tracer, full length Hsc70 out-competes both the C-terminal peptide from which the tracer was derived and Hsc70 without the EEVD motif for CHIP binding. IC_{50} values are $0.45 \mu\text{M}$, $2 \mu\text{M}$, and non-determinable, respectively.

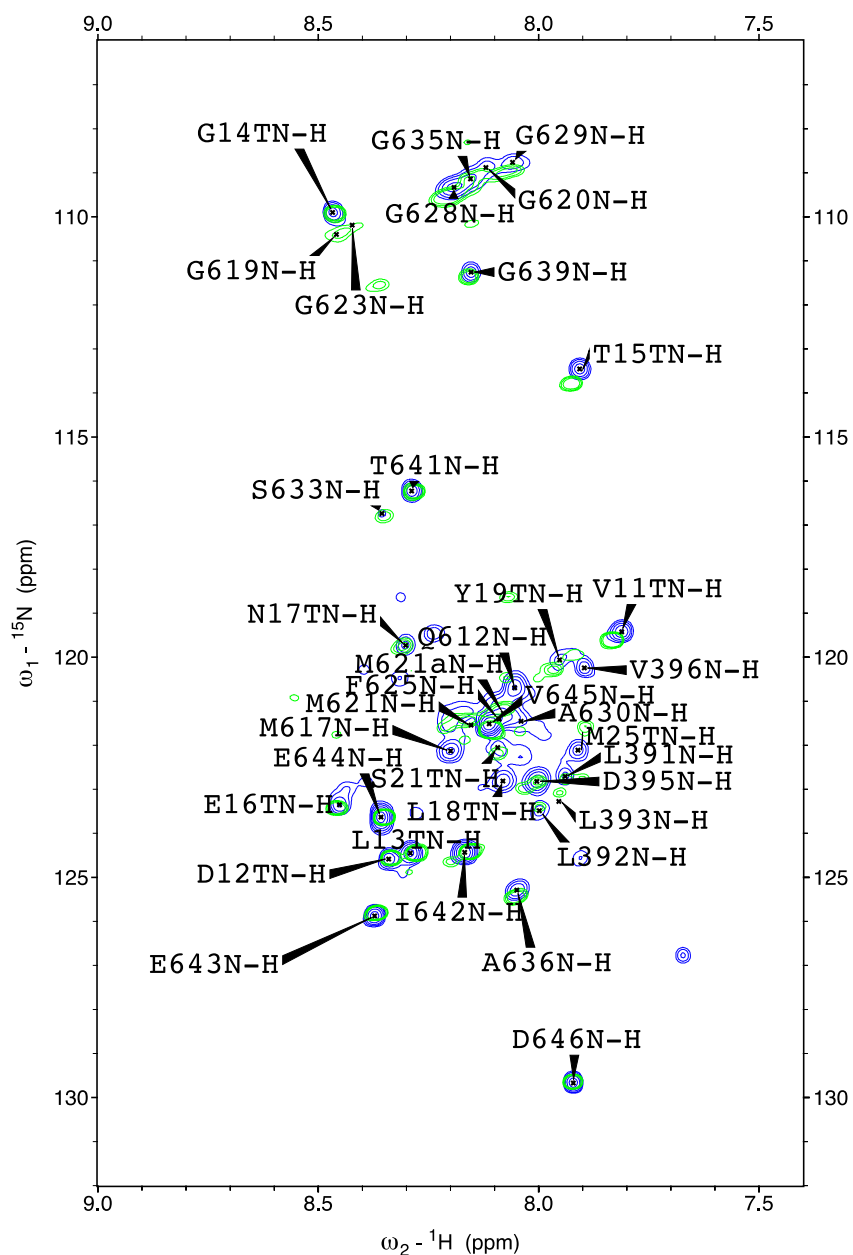


Fig. S2. Overlay of the 800 MHz TROSY spectrum of ^{15}N -labeled full length Hsc70 (646 residues, 72 kDa) in blue, with that of the isolated SBD (391-646) (Green). ADP and peptide NR (NRLLLTG) were present in the samples. This figure is a high-level enlargement of Figure 3 in the main text.

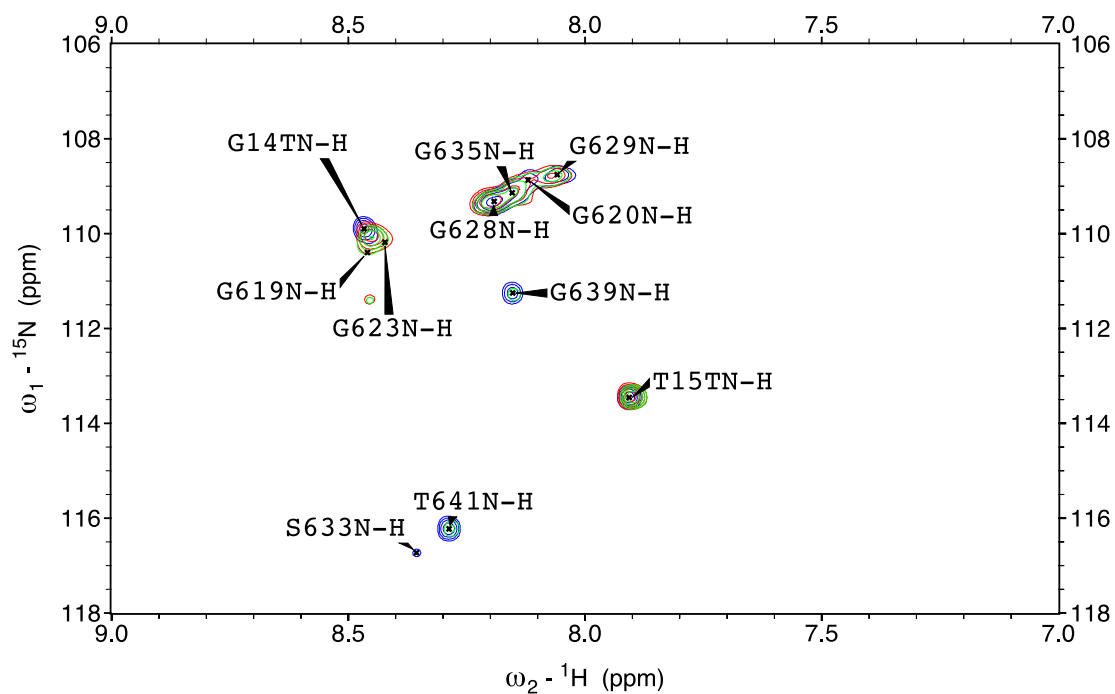


Fig. S3. Enlargement of the 800 MHz TROSY spectrum of ^{15}N -labeled full length Hsc70 (646 residues, 72 kDa). In blue, Hsc70; in green Hsc70+CHIP (1:0.5), in red, Hsc70+CHIP (1:1);

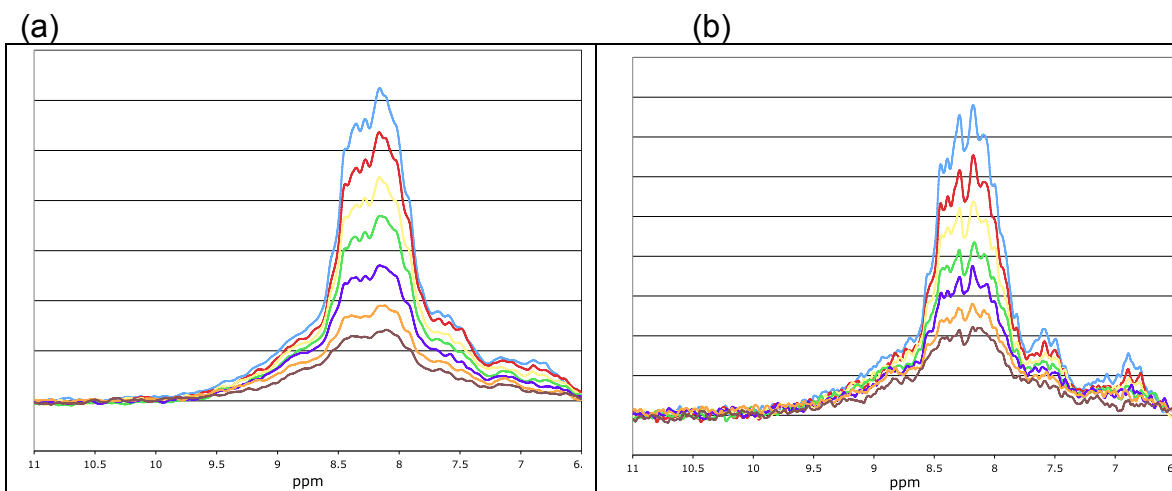


Fig. S4. 1D ^1H NMR spectra of the amide proton region of ^{15}N -labeled Hsc70, acquired with a HSQC-style ^{15}N -R1 relaxation experiment. (a) 40 μM Hsc70 without CHIP with T1 relaxation times of 50, 200, 400, 600, 1000, 1500, 2000ms (top to bottom). (b) 40 μM Hsc70 with 30 μM CHIP with T1 relaxation times of 50, 200, 400, 600, 1250, 1500 and 2000ms (top to bottom)

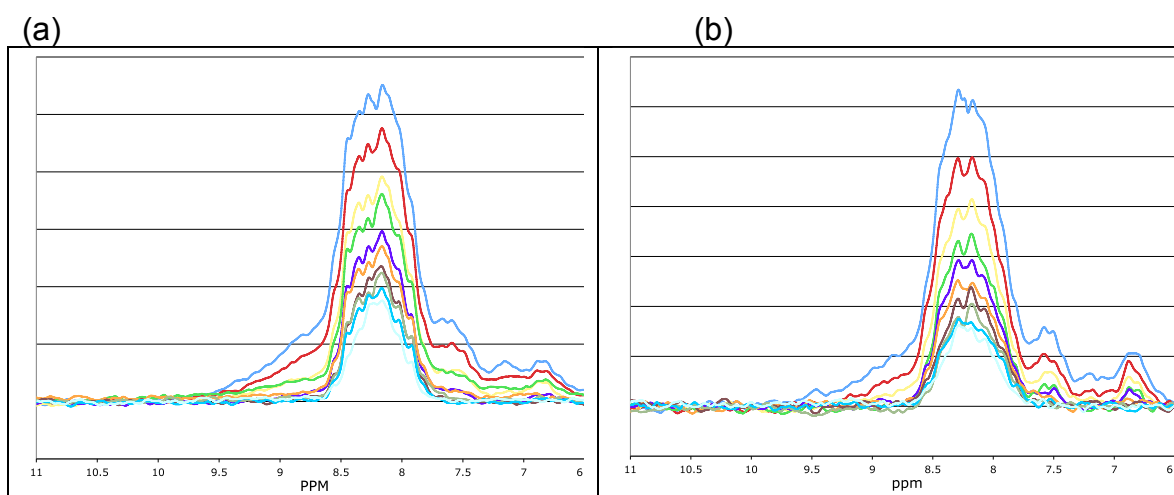


Fig. S5. 1D ^1H NMR spectra of the amide proton region of ^{15}N -labeled Hsc70, acquired with a HSQC-style ^{15}N - R_2 relaxation experiment. (a) 40 μM Hsc70 without CHIP with T_2 relaxation times of 2, 4, 6, 10, 14, 20, 30, 40, 60, 800 and 100 ms (top to bottom). (b) 40 μM Hsc70 with 30 μM CHIP with T_2 relaxation times of 2, 10, 20, 30, 40, 60, 70, 80 and 90 ms (top to bottom)

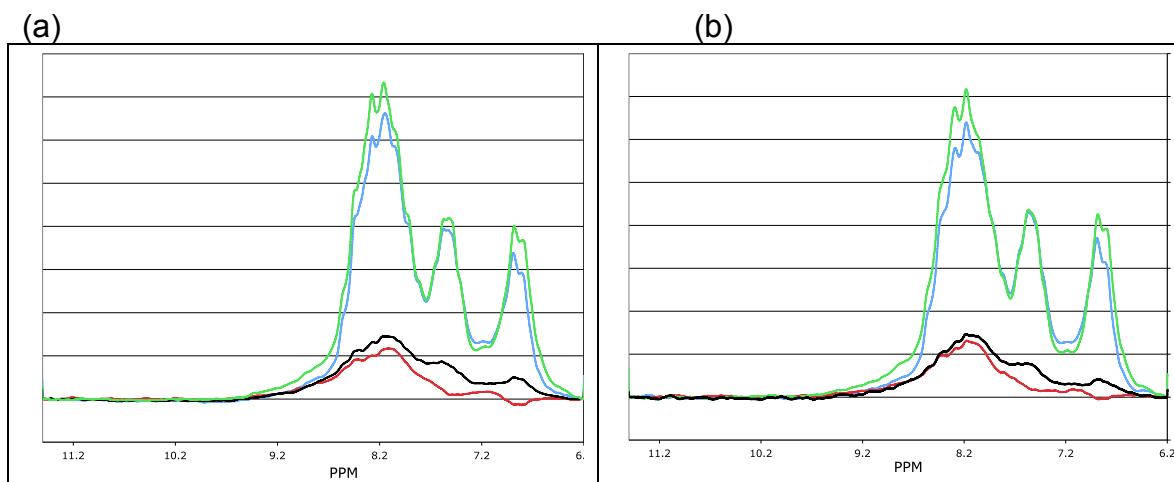


Fig. S6. 1D symmetrical reconversion η_{xy} spectra ¹ of ¹⁵N-labeled wt-Hsc70 (a) No CHIP (b) with 1:1 CHIP. Blue: $N_{xy} \rightarrow N_{xy}$; red: $N_{xy} \rightarrow 2N_{xy}H_z$; black: $2N_{xy}H_z \rightarrow N_{xy}$; green: $2N_{xy}H_z \rightarrow 2N_{xy}H_z$

1. Pelupessy, P., Espallargas, G. M. & Bodenhausen, G. (2003). Symmetrical reconversion: measuring cross-correlation rates with enhanced accuracy. *J Magn Reson* **161**, 258-64.