Supplementary Experimental Procedures

Isotopic labeling

Uniformly ^{15}N-labeled protein was grown in M9 minimal medium containing [^{15}N]-ammonium chloride. Proteins selectively labeled with [^{15}N]-leucine, [^{15}N]-valine, [^{15}N]-isoleucine, [^{15}N]- or [^{13}C]-alanine and [^{13}C]-proline were expressed in *E. coli* strain DL39 *avtA* (LeMaster and Richards, 1988) in minimal medium containing 2X M9 salts and supplemented with 200 µg/ml tyrosine, phenylalanine, alanine, and aspartate, 300 µg/ml isoleucine and leucine, and 400 µg/ml valine. Labeled amino acids were substituted for their unlabeled counterparts at half the concentration, as a cost-saving measure. Labeled proline was added to 100 µg/ml.

Preparation of NMR samples

Proteins were concentrated to 0.3-0.7 mM and buffer-exchanged in a Centricon-10 device (Millipore, Billerica, MA) into 10 mM potassium phosphate, 10 mM potassium chloride, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol, 10% D_2O, 1 mM AEBSF (Pefabloc), and 0.02% sodium azide. Buffers were pH 7 and contained 0.5 mM 3-(trimethylsilyl)propane sulfonic acid as an internal standard.

Assignment of linker binding site

As an example of the strategy utilized, a sample of DnaK(1-392) labeled with ^{13}C'-Ala and ^{15}N-Leu should display two HNCO peaks, corresponding to AL^{177}, located in an exposed hydrophobic cleft between subdomains IA and IIA (Mayer and Bukau, 2005), and AL^{324}, on the opposite site of subdomain IIA beyond the reach of the linker. In fact, three peaks were observed (Fig. 3C), due to partial

proteolysis of the linker during sample preparation (verified by mass spectrometry, data not shown). One of these peaks is insensitive to linker binding, and was therefore assigned to L324. The other two resonances correspond to a single leucine that undergoes a large linker-dependent shift ($\Delta\delta_{av}$ = 0.13 ppm); these peaks were assigned to L177. Two PA and three AI pairs were assigned using a similar strategy.

Supplementary References

LeMaster, D. M., and Richards, F. M. (1988). NMR sequential assignment of Escherichia coli thioredoxin utilizing random fractional deuteriation. Biochemistry *27*, 142-150.

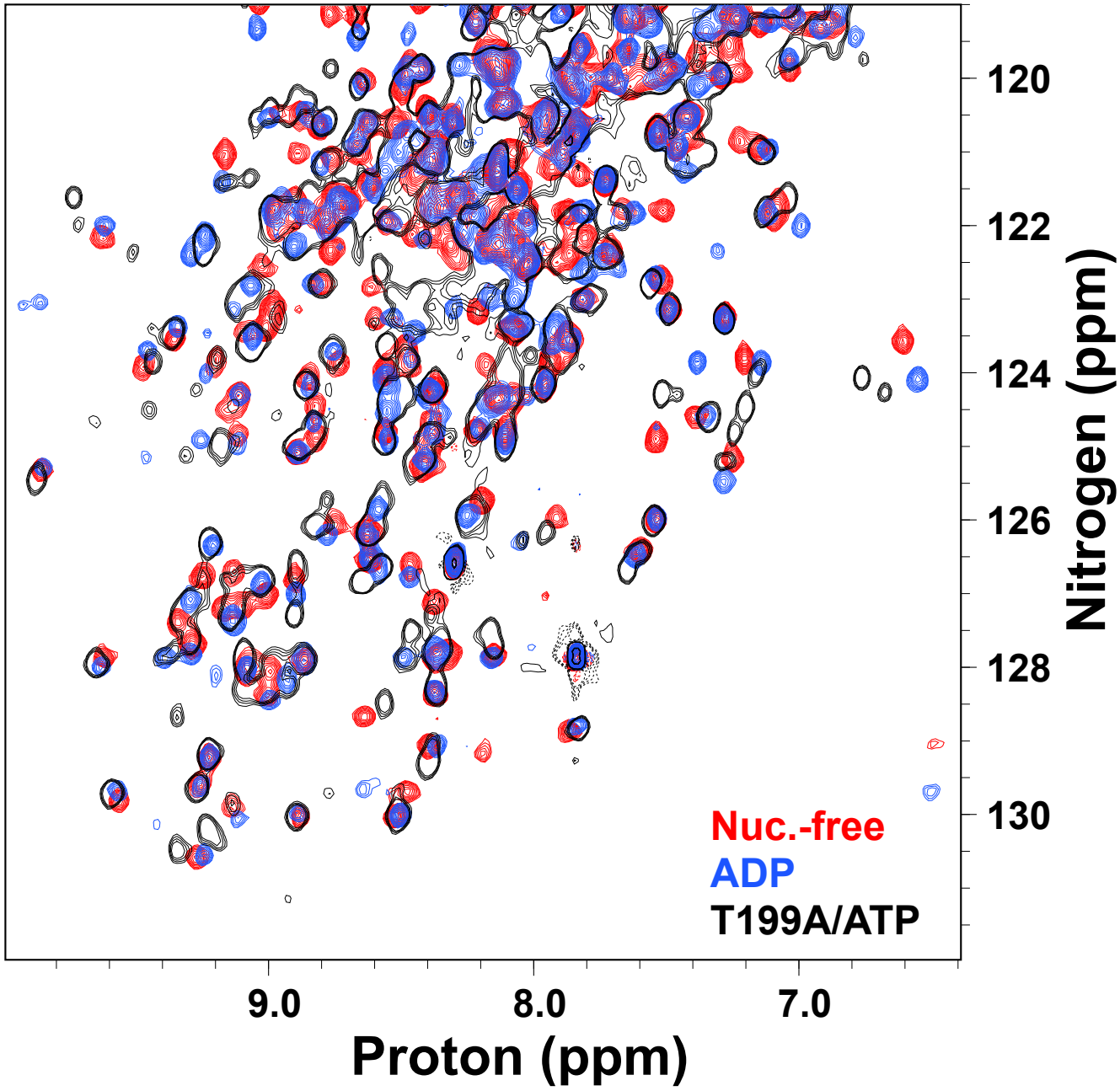
Mayer, M. P., and Bukau, B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci *62*, 670-684.

Table S1: HDX peak intensity ratios for DnaK(1-552)ye in ATP versus ADP

Residue Number	I_{ATP}/I_{ADP}
397	1.31
400	1.63
401	0.87
403	<0.44
409	0.91
420	0.72
422	1.13
436	<0.32
441	1.15
443	0.56
450	<0.42
451	<0.24
457	0.79
459	0.67
472	0.91
474	0.96
476	1.12
477	1.09
478	0.78
479	1.01
484	1.02
485	0.97
490	<0.48

Supplementary Figure Legend

Figure S1. Nucleotide binding causes widespread chemical shift changes in the isolated ATPase domain. TROSY NMR spectra of 0.3 mM DnaK(1-388) are displayed in the nucleotide-free state (red), bound to ADP (0.5 mM, blue), and bound to ATP with the T199A hydrolysis-defective mutation (5 mM ATP, black outlines).



Swain et al., Supplementary Figure 1