Supporting Information

Nucleotide-dependent interactions within a specialized Hsp70/Hsp40 complex involved in Fe-S cluster biogenesis

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Experimental Procedures

Sample Preparation

Unlabeled and uniformly-^{15}N-labeled ([U-^{15}N]) HscB[1,2] and unlabeled HscA and HscA(T212V)[3] were prepared as described previously.

HscA(NBD) and HscA(NBD;T212V) were prepared by using the QuikChange II (Agilent Technologies) site-directed mutagenesis kit to substitute the codon for M386 with the stop codon in the expression vectors for HscA and HscA(T212V), respectively.[3] Rosetta™ Blue DE3 competent cells (EMD Millipore) were transformed with the mutated vectors and selectively grown on media plates containing 100 μg/mL ampicillin. For expression of unlabeled protein, cultures were grown in 1 L of LB medium until the optical density reached 1.0, upon which IPTG was added to a final concentration of 0.4 mM. Protein expression continued for 4 hours, after which cells were harvested and frozen at -80 °C until use. For purification of HscA(NBD) and HscA(NBD;T212V), anion exchange chromatography was first performed using a DEAE Bio-Gel column (Bio-Rad) equilibrated with a pH 7.5 buffer (TED) containing 50 mM Tris-HCl, 0.5 mM EDTA, and 1 mM DTT. Elution of the desired product occurred over a 0–0.25 M NaCl gradient. Subsequent size-exclusion chromatography was performed with a HiLoad 16/60 Superdex 75 column (GE Healthcare), and protein was eluted in TED buffer supplemented with 150 mM NaCl.

NMR spectroscopy

All NMR spectra were obtained at 25°C with an 800 MHz Agilent NMRS spectrometer equipped with a *z*-gradient cryogenic probe. All NMR samples contained 7% D_2O, 0.7 mM 2,2-dimethyl-2-silapentane-5-sulfonate, and 0.02% sodium azide. NMRPipe[4] was used for processing raw NMR data, and SPARKY[5] was employed to analyze processed data and assign NMR signals.

Samples of [U-^{15}N]-HscB and [U-^{15}N]-HscB(H32A,P33A,D34A) used for NMR data collection contained 0.2 mM protein, 50 mM HEPES, 2 mM $MgCl_2$, and 1 mM DTT. The pH was adjusted to 7.0. As called for, the

samples also contained 10 mM ADP or 10 mM ATP. Note that HscA does not require a nucleotide exchange factor for its binding to ADP/ATP.[6] To form protein-protein complexes, aliquots of 0.2 mM unlabeled HscA(T212V) or 0.2 mM HscA(NBD;T212V) were added to achieve the specified protein ratios. Chemical shift perturbations were calculated by the following equation: $\Delta\delta_{NH}=[(\Delta\delta_N/5)^2+(\Delta\delta_H)^2]^{1/2}$.

ATPase activity assay

To quantify the rate of ATP hydrolysis catalyzed by HscA and HscA(NBD) in the absence and presence of HscB variants, steady-state assays were conducted at 25 °C in a pH 7.3 buffer containing 50 mM HEPES, 150 mM KCl, and 10 mM $MgCl_2$. The reaction mixture contained 1 μM HscA or HscA(NBD), and HscB or HscB(H32A,P33A,D34A) was added to 50 μM as previously reported.[1,2] Addition of 1 mM ATP initiated the reactions. ATPase rates were calculated by continuously monitoring the amount of phosphate released by using the enzyme-coupled EnzChek® Phosphate Assay Kit (Molecular Probes).

Supplemental Figures

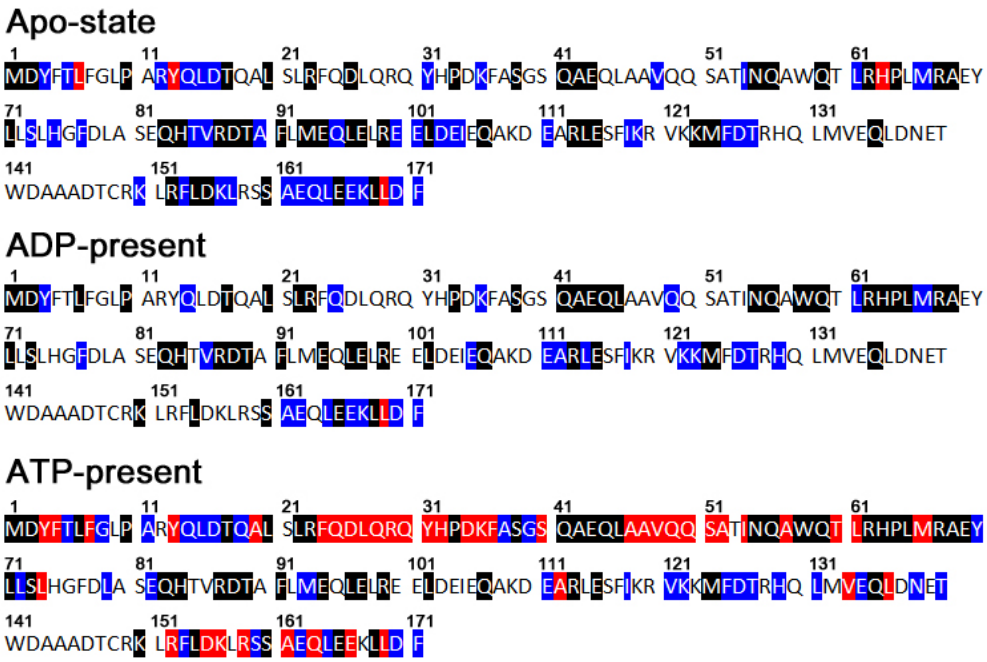

Figure S1. Summary of the perturbations of the 2D ^{1}H-^{15}N NMR signals of [U-^{15}N]-HscB upon the addition of 3-fold HscA(T212V): (*top*) in the absence of added nucleotide, (*middle*) in the presence of excess ADP, and (*bottom*) in the presence of excess ATP. The NMR data are shown in Figure 2, and the color coding is the same as that used in Figure 2: (*black*) residues with no signal (Pro), unassigned residues, or residues whose signals could not be followed upon addition of HscA(T212V); (*blue*) residues with $\Delta\delta_{NH}$ > 0.01 ppm; and (*red*) residues whose signals broadened beyond detection.

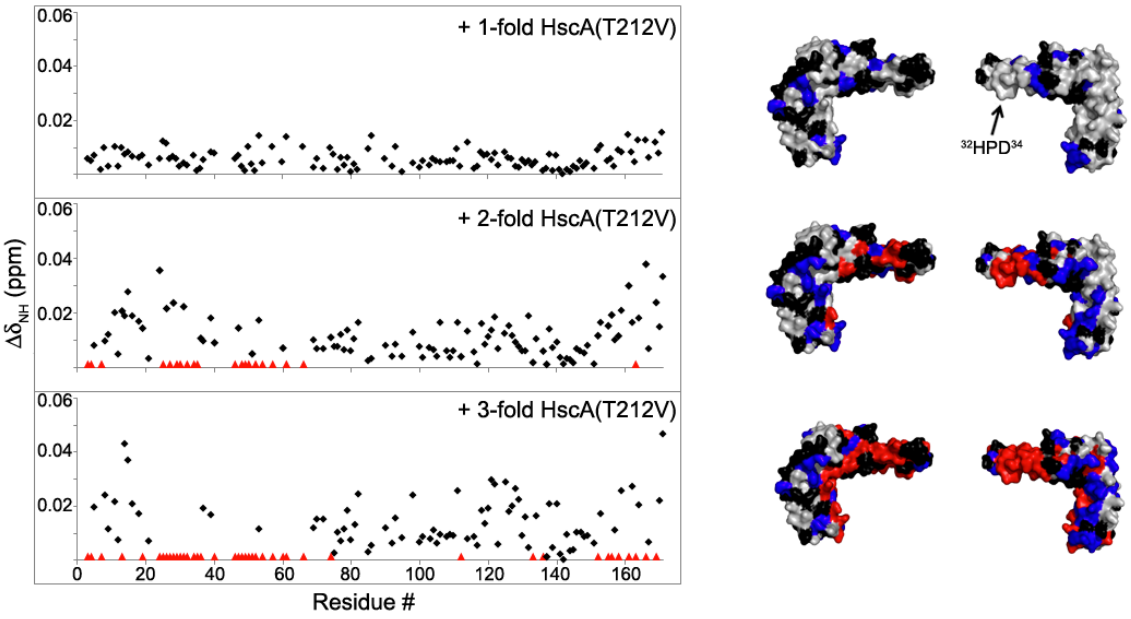

Figure S2. Data from 2D ^{1}H-^{15}N TROSY-HSQC NMR spectra of [U-^{15}N]-HscB in the presence of 10 mM ATP upon the addition of HscA(T212V) (spectra shown in Figure 2). The graphs on the left show the chemical shift perturbations ($\Delta\delta_{NH}$) of the backbone ^{1}H-^{15}N signals of [U-^{15}N]-HscB upon the addition of (*top*) one, (*middle*) two, and (*bottom*) three equivalent of HscA(T212V) as a function of residue number. Red triangles represent residues whose signals broadened beyond detection. On the right, these spectral changes are mapped onto the surface of the structural model for HscB (PDB 1FPO)[7] with the following color codes: (*black*) residues whose signals could not be assigned unambiguously; (*gray*) residues whose signals were perturbed minimally ($\Delta\delta_{NH} \leq 0.01$ ppm); (*blue*) residues with $\Delta\delta_{NH} > 0.01$ ppm; (*red*) residues whose signals broadened beyond detection.

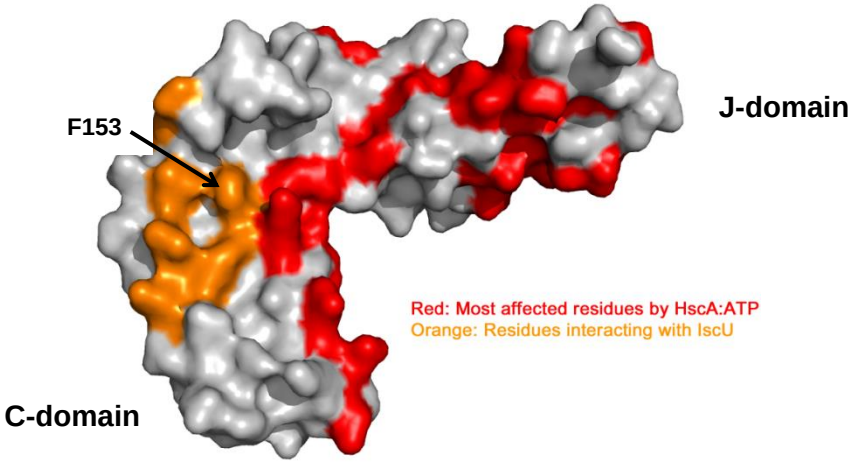

Figure S3. Structural model for HscB (PDB 1FPO).[7] Colored in orange are residues found in a previous NMR investigation[8] to interact with IscU (R87, L92, L96, R99, E100, and F153). Colored in red are residues found

in the current study to interact with HscA:ATP and broaden beyond detection (e.g. R152, D155, K156, R158, and Q163).

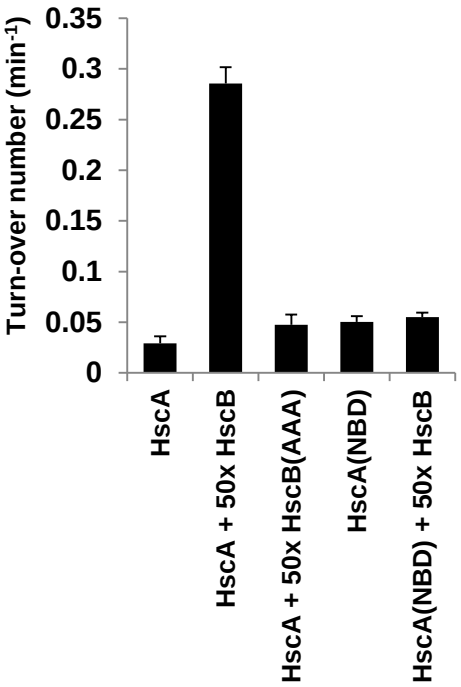

Figure S4. Results from ATPase activity measurements of full-length HscA and HscA(NBD) in the absence/presence of HscB or HscB(H32A,P33A,D34A) [denoted as HscB(AAA)]. The *y*-axis indicates the turnover number per minute (moles of ATP hydrolyzed per mole of HscA per minute) at 25 °C. The pH 7.3 reaction buffer contained 50 mM HEPES, 150 mM KCl, and 10 mM $MgCl_2$. The concentrations of HscA and HscA(NBD) were each 1 μM, and HscB or HscB(AAA) was added to 50 μM. Reactions were initiated by adding 1 mM ATP. The error bars were calculated from the standard deviations of three measurements obtained under each reaction condition. Whereas the ATPase activity of full-length HscA was stimulated up to 10-fold by HscB, the ATPase activity of HscA(NBD) showed negligible increase upon adding HscB.

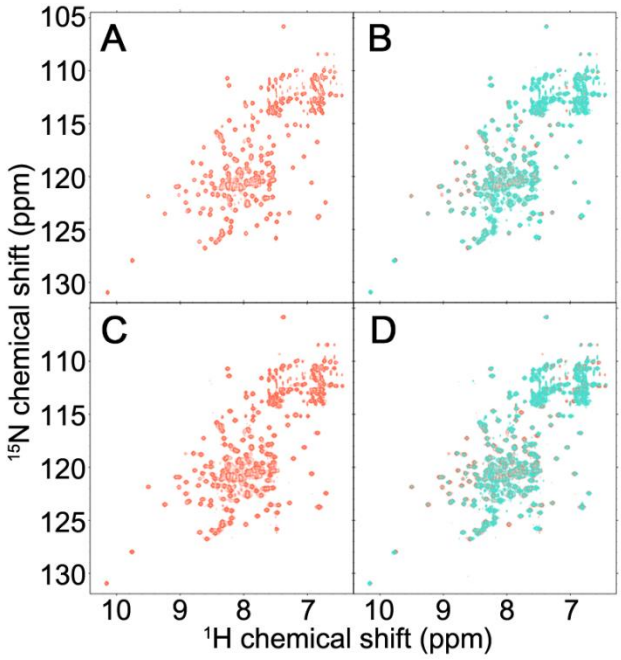

Figure S5. 2D ^{1}H-^{15}N TROSY-HSQC NMR spectra of HscB(H32A,P33A,D34A) under various conditions: (A) in the presence of 10 mM ADP; (B) in the presence of 10 mM ADP and 0.6 mM HscA(T212V), cyan spectrum overlaid with the spectrum from A; (C) in the presence of 10 mM ATP; (D) in the presence of 10 mM ATP and 0.6 mM HscA(T212V), cyan spectrum overlaid with the spectrum from C.

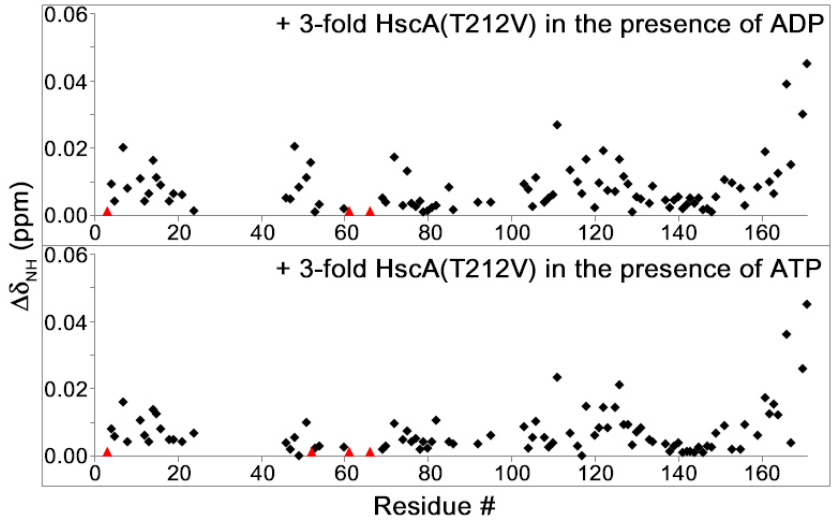

Figure S6. Plot of residue-specific spectral perturbations abstracted from the 2D ^{1}H-^{15}N TROSY-HSQC NMR spectra (shown in Figure S5) of HscB(H32A,P33A,D34A) under various conditions. (*Upper panel*) black diamonds represent chemical shift changes in 2D ^{1}H-^{15}N peaks from HscB(H32A,P33A,D34A) in the presence of ADP upon addition of a 3-fold excess of HscA(T212V); red triangles represent residues whose peaks broadened beyond detection (*Lower panel*) black diamonds represent chemical shift changes in 2D ^{1}H-^{15}N peaks from HscB(H32A,P33A,D34A) in the presence of ATP upon addition of a 3-fold excess of HscA(T212V); red triangles represent residues whose peaks broadened beyond detection. NMR signals from residues in and near the mutated site (^{32}HPD^{34}) were not assigned because the amino acid substitutions led to large chemical shift changes that could not be tracked between the two variants.

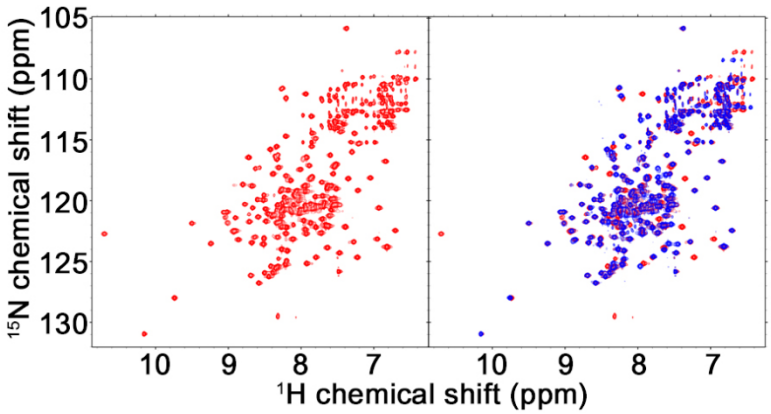

Figure S7. Comparison of 2D ^{1}H-^{15}N TROSY-HSQC NMR spectra of (*red*) wild-type [U-^{15}N]-HscB and (*blue*) [U-^{15}N]-HscB(H32A,P33A,D34A). The spectra indicate overall structural similarity for the two variants; the few signals exhibiting large chemical shift differences come from residues at or near the mutated site.

References

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