Supplemental Data

Structural Basis of Interdomain

Communication in the Hsc70 Chaperone

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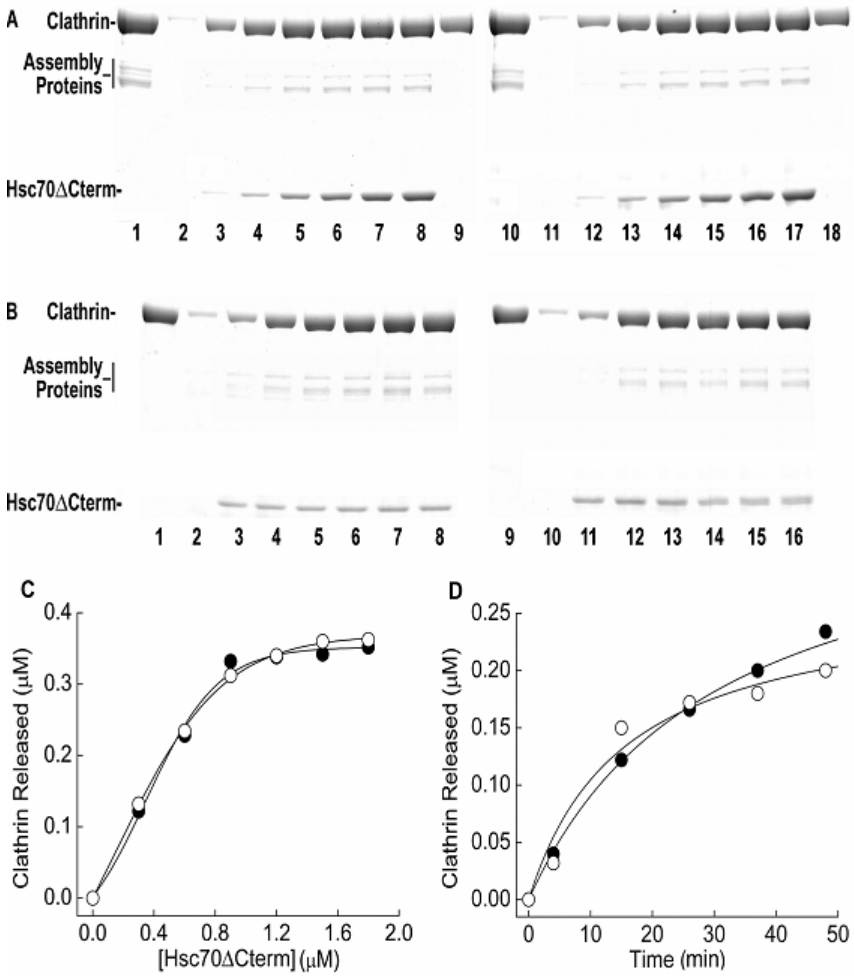

Figure S1. Stochiometry and Kinetics of Clathrin Cage Disassembly by Wt and E213A/D213A bHsc70ΔCterm Proteins

(A) SDS PAGE of cages prepared with clathrin and assembly proteins (lane 1), of ultracentrifugal supernatants from cages (intact cages are in the pellet; released clathrin is in the supernatant) incubated for 40 min. with ATP but no bHsc70ΔCterm (lane2), or incubated with ATP and 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 μM bHsc70ΔCterm in lanes 3, 4, 5, 6, 7, and 8, respectively (lane 9: pure clathrin). Lanes 10-18 repeat this experiment using E213A/D214A bHsc70ΔCterm.

(B) Cage disassembly as in (A) but with bHsc70ΔCterm at 0.6 μM and incubations of 4, 15, 26, 37, 48, and 59 min. in lanes 3, 4, 5, 6, 7, and 8, respectively (lanes 1 and 2 show, respectively, pure clathrin and supernatant from a disassembly assay incubated with ATP for 40 min. without bHsc70). Lanes 9-16 repeat this experiment with E213A/D214A bHsc70ΔCterm.

(C and D) Quantification of the experiments in (A) and (B), respectively. Empty circles: E213A/D214A; Filled circles: WT.

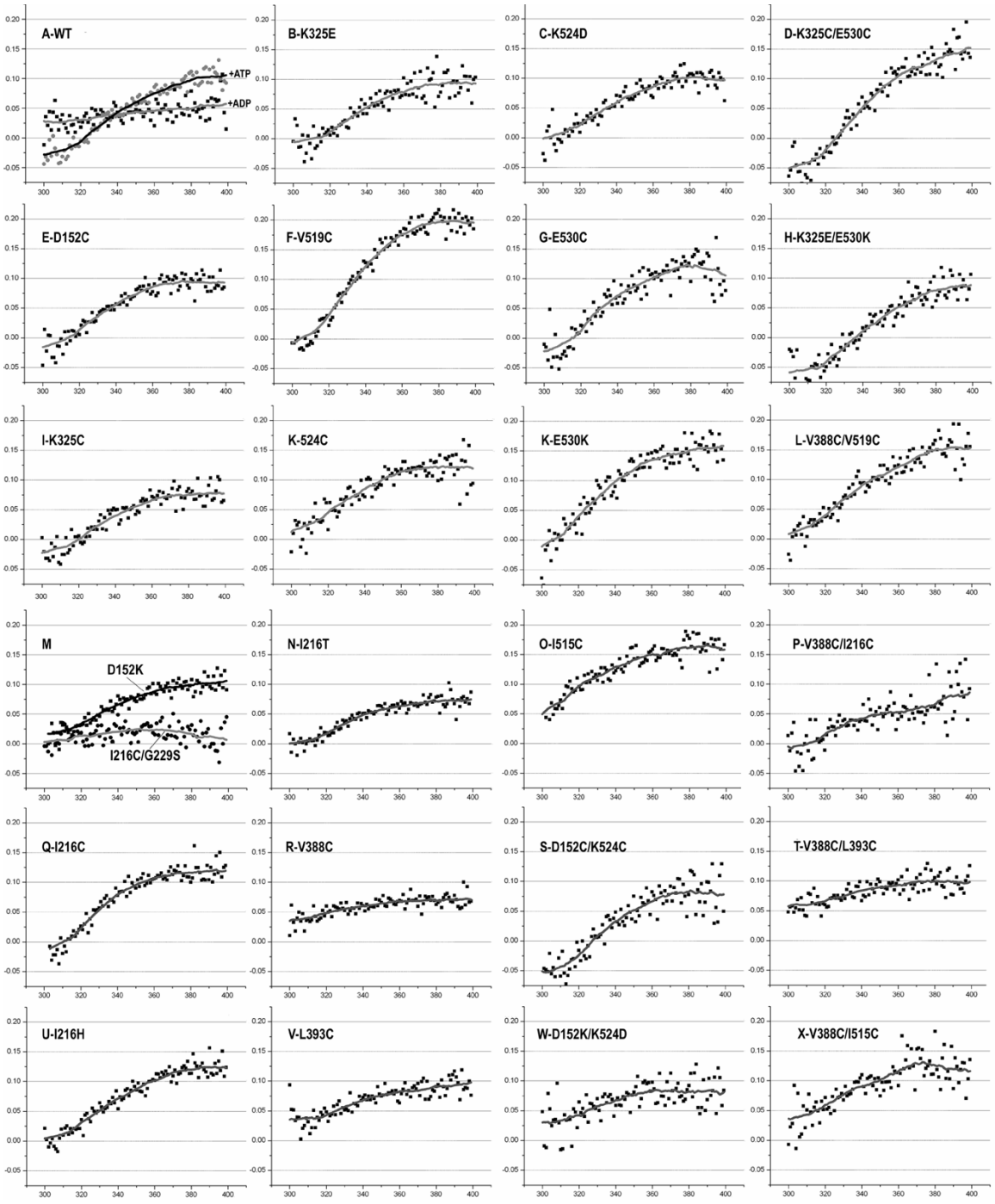

Figure S2. Effects of Interdomain Interface and Linker Mutations on ATP-Induced Fluorescence Changes

Fractional differences in W fluorescence spectra with and without ATP [(Fluorescence-ATP)-(Fluorescence+ATP))/(Fluorescence-ATP)] are plotted. In panel A spectral differences due to ADP are also plotted for comparison, and in panel C spectral differences -/+ ATP are also plotted for D152K and for a completely inactive mutant (I216CG229S).

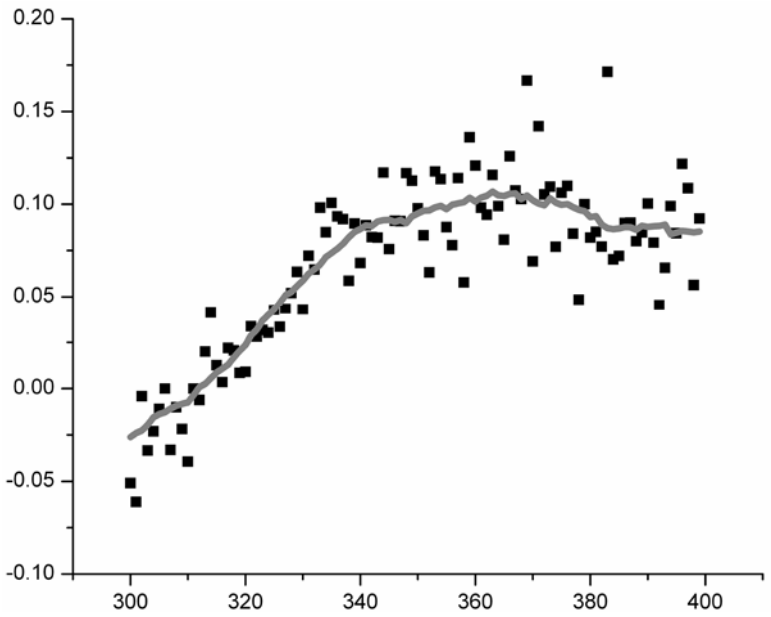

Figure S3. Fractional Difference between W Fluorescence Spectra with and Without ATP for Full-Length Hsc70 from Bovine Brain

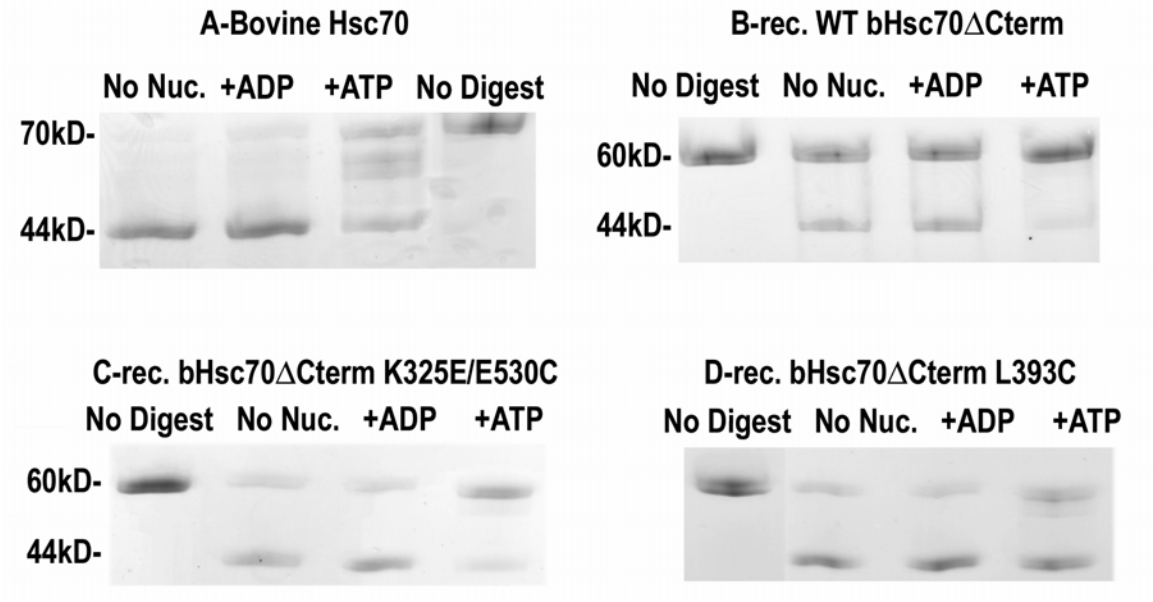

Figure S4. Effects of ATP and ADP on Interdomain Linker Protease Sensitivity in Bovine Hsc70 and Recombinant Wt and Mutant bHsc70ΔCterm Enzymes

(A) Products of proteinase K treatment of bovine brain Hsc70 in the absence of nucleotide ('No nuc.'), with ADP or ATP present ('No digest'=no protease control).

(B) As in (A), but with recombinant WT bHsc70ΔCterm.

(C) As in (A), but with an active mutant bHsc70ΔCterm (K325E/E530C).

(D) As in (A), but with a poorly active mutant bHsc70ΔCterm (L393C).

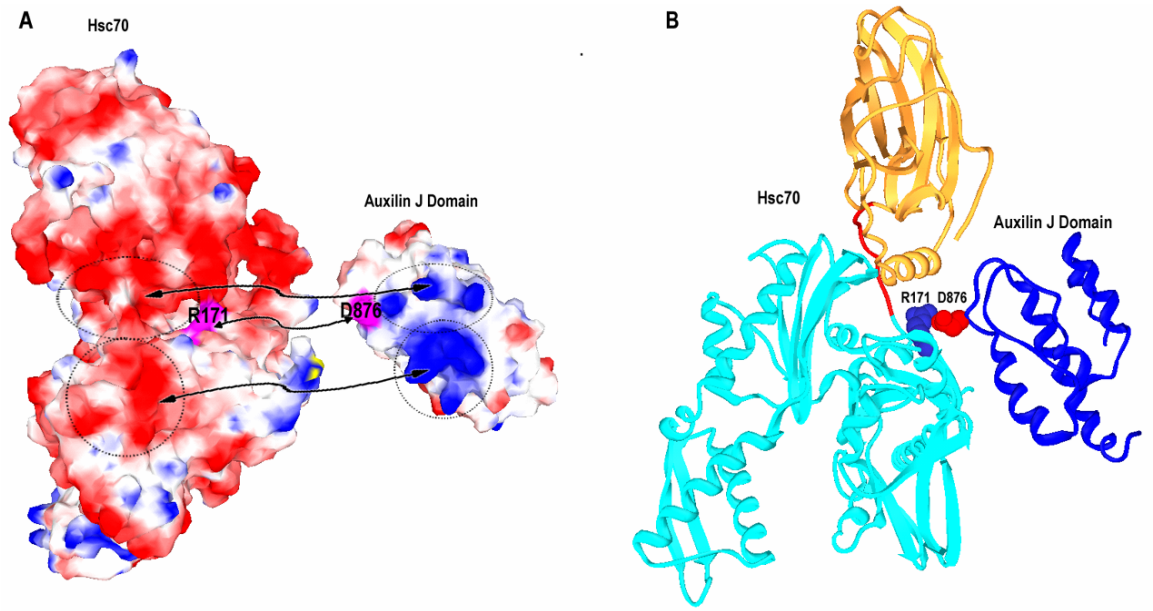

Figure S5. Modeling of Auxilin J Domain:bHsc70 Interaction

(A) Electrostatic surface representations of bHsc70ΔCterm and the auxilin J domain. Intergenic suppression analysis (Suh et al., 1998) and disulfide cross-linking of R171C and D876C mutants (unpublished) suggest that R171 and D876 interact in the complex. Positively charged residues on the auxilin J domain surface known to be important for Hsc70 binding (Jiang et al., 2003 and references therein) and negatively charged regions on Hsc70 which may be positioned to interact with these positively charged regions are indicated.

(B) Modeling of the auxilin J domain:bHsc70 complex on the basis of the considerations outlined in A places the J domain near the interdomain interface where it could simultaneously interact with the NBD and SBD.

Table S1. Data Collection and Refinement Statistics

Space Group	$P2_1$
Unit cell (Å)	a=65.7, b=50.1, c=87.2, β=99.9^o
Solvent content	47% (1 mol per A.U.)
Resolution (Å)	50-2.6 (2.69-2.60)
Wavelength (Å)	1.54
R_{merge} (%)	7.9 (45.9)
I/σ	11.6 (2.4)
Unique reflections	16,521
Completeness (%)	98.7
R factor	22.0
R_{free}	29.7
RMSD bonds (Å)	0.011
RMSD angles (^{o})	1.57
non-gly Ramachandran allowed (%)	99.2