

## Supplemental Table S1: Amino acid replacements causing defects in allosteric control in Hsp70s

Activities and conformational changes of amino acid replacement variants relative to the wild type protein.

Position in DnaK	replacement	protein analyzed <sup>b</sup>	Crystal structure contacts <sup>a</sup>		ATPase rel. $k_{cat}$ <sup>c</sup>	Effects on allostery					Reference	
			1HPM	4B9Q		Substrate stimulated ATPase <sup>d</sup>	DnaJ-stimulated ATPase <sup>e</sup>	DnaJ-Substrate stimulation <sup>f</sup>	ATP-stimulated substrate release <sup>g</sup>	ATP-induced conformational change <sup>h</sup>		
11	T37G	CgBiP	T13/OG1-Pi/O4	T13/OG1-ATP/O1G	0.19	-				-	- (proteolysis)	(1)
	T13G	BtHsc70	T13/ N-Pi/O3		0.002						no $\Delta$ Rg (SAXS) no blueshift	(2)
	T13S	BtHsc70			0.09						red. $\Delta$ Rg (SAXS)	(2)
	T13V	BtHsc70			0.02						no $\Delta$ Rg (SAXS) no blueshift	(2)
70	K71M	BtHsc70	K71/NZ-E175/OE2 K71/NZ-Pi/O3	K70/NZ-ATP/O1G K70/NZ-E171/OE2	0.00						no $\Delta$ Rg (SAXS)	(3)
	K70A	EcDnaK	K71/CE-P147/CG K71/CE-P147/CD	K70/CE-P143/CG K70/CE-P143/CD	0.04					-	no blueshift	(4)
143	P143S	EcDnaK	P147/CG-K71/CE P147/CD-K71/CE	P143/CG-K70/CE P143/CD-K70/CE	0.06	+	+					(5)
	P143A	EcDnaK	P147/CG-F150/CE1 P147/CD-F150/CE2	P143/CG-F146/CE1 P143/CD-F146/CE2	0.27	1.43	0.66	0.46	0.16		red. blueshift	(6)
	P143G	EcDnaK			0.12	0.49	0.39	0.24	0.67		red. blueshift	(6)
145,147,148	YND>AAA	EcDnaK			3.49	0.92	0.15	0.125	0.34		red. blueshift	(7)
145	Y145A	EcDnaK	Y149/OH-H2O-D206/OD2		16.10	0.85	0.30	0.03	0.27		red. blueshift	(8)
	Y145F	EcDnaK	Y149/OH-H2O-PiO1		2.20	1.27	2.88	0.60	0.61		wt blueshift	(8)
	Y145H	EcDnaK			2.40	3.13	7.34	1.00	0.58		wt blueshift	(8)
146	F146A	EcDnaK	F150/CZ-K70/CG F150/CE1-P143/CG F150/CE2-P143/CD	F146/CZ-K70/CG F146/CE1-P143/CG F146/CE2-P143/CD	5.17	0.66	1.33	0.34	0.06		red. blueshift	(8)
148	D148A	EcDnaK		D148/OD1-L484/N	3.41	0.55	1.05	0.07	2.4		wt blueshift	(8)
151	R151A	EcDnaK	R155/NH1-N170/OD1 R155/NH2-F146/O R155/NH2-A144/O	R151/NH1-N170/OD1 R151/NH2-F146/O R151/NH2-A144/O	4.5	0.12	0.37	0.03	0		no blueshift	(6)
	R151K	EcDnaK	R155/NE-F146/O	R151/NE-D481/O	17.6	0.26	0.29	0.02	0.06		red. blueshift	(6)
155	K155A	EcDnaK			0.86	0.82	1.79	0.16	0.03		red. blueshift	(9)
	K155D	EcDnaK			1.44	1.46	0.73	0.11	0.00		red. blueshift	(9)

167	R167A	EcDnaK		R167/NE-D481/OD1	2.8	1.04	0.56	0.11	0.03	red. blueshift	(9,10)
	R167D	EcDnaK			7.3	0.42	0.51	0.03	0	no blueshift	(9)
	R167H	EcDnaK						reduced			(10)
170	N170A	EcDnaK	N174/OD1-R155/NH1	N170/ND2-D393/OD2				reduced			(10)
171	E175S	BtHsc70	E175/OE1-Pi/O1	E171/OE2-K70/NZ	0.01					red ΔRg (SAXS)	(3)
	E171D	EcDnaK	E175/OE2-K71/NZ	E171/OE2-H2O-Mg	0.02	1.15	0.5	0.25	0.18	red. blueshift	(6)
	E171Q	EcDnaK	E175/OE2-H2O-Mg	E171/OE1-H2O-D201/OD2	0.01	1.83	0.4	0.18	0.11	red. blueshift	(6)
173	T173A	EcDnaK		T173/OG1-D393/OD2 T173/CG2-L391/CD2				reduced			(10)
194	D199S	BtHsc70	D199/OD1-H2O-Mg D199/OD2-H2O-Mg D199/N-D206/O D199/O-D206/N	D194/OD1-H2O-Mg D194/OD2-H2O-Mg D194/N-D201/O D194/O-D201/N	0.32					no ΔRg (SAXS)	(3)
196	G226D	CgBiP			0.33				-	- (proteolysis)	(1)
197	G227D	CgBiP	G202/N-ADP/O1B	G197/N-ATP/O3B	0.29				-	- (proteolysis)	(1)
199	T199A	EcDnaK	T204/OG1-Pi/O4	-	0.030				+	wt blueshift	(4)
	T204E	BtHsc70			7.500						(11)
	T204V	BtHsc70			0.350						(11)
	T229G	CgBiP			-				+	yes	(1)
201	D201N	EcDnaK	D206/OD2-H2O-Y149/OH	D201/OD2-H2O-E171/OE1	10.3	0.1-0.2	-			red. (proteolysis)	(12)
	D206S	BtHsc70	D206/OD2-H2O-Pi/O1		0.28					red ΔRg (SAXS)	(3)
217,218	EV>AA	EcDnaK			4.29	0.85	0.11	0.07	0.10	red. blueshift	(7)
326	D326V	EcDnaK		D326/OD2-K414/NZ D326/OD2-N415/ND2	ca. 2	0.43				red. blueshift	(13)
388	D388R	EcDnaK			1.33	0.81	2.3	0.35	0.96	wt blueshift	(9)
389-392	VLLL>AAAA	EcDnaK				-	-	-	-		(14,15)
389-392	VLLL>VDDL	EcDnaK				-	-	-	-		(14,15)
389	V389A	EcDnaK		V389/CG1-L177/CD2 V389/CG1-V281/CG2 V389/CG1-F216/CD1		-	+	+	reduced		(16)
	V389D	EcDnaK				-	-	reduced	-		(16)
390	L390A	EcDnaK				reduced	reduced	reduced			(16)
	L390D	EcDnaK				reduced	-	-			(16)
391	L391A	EcDnaK		L391/CD1-L177/CD2 L391/CD1-F216/CB L391/CD2-T173/CG2		-	-	reduced	reduced		(16)
	L391D	EcDnaK				-	-	-	-		(16)

392	L392A	EcDnaK			reduced	-	-				(16)
	L392D	EcDnaK			reduced	-	-	reduced			(16)
393	D393A	EcDnaK	D393/OE2-N170/ND2	2.40	0.44	0.51	0.02	0.01	no blueshift		(9)
	D393R	EcDnaK	D393/OE2-T173/OG1	1.36	0.36	0.51	0.02	0.00	no blueshift		(9)
414	K414I	EcDnaK	K414/NZ-D326/OD2	25.9				0.002	no blueshift		(8,17)
415	N415G	EcDnaK	N415/ND2-D326/OD2 N415/OD1-T221/N	ca. 1.6	0.5				red. blueshift		(13)
440	V440A	EcDnaK	V440/CG1-L484/CD2	1.97	0.42	1.43	0.06	2.4	red. blueshift		(8)
481	D481A	EcDnaK	D481/OD1-I168/N D481/OD1-R167/NE	84	0.56	0.89	0.03	0.002	no blueshift		(8)
	D481K	EcDnaK	D481/O-R151/NE	70	0.64	0.57	0.02	0.001	no blueshift		(8)
484	L484A	EcDnaK	L484/CD2-V440/CG1	1.72	0.5	0.98	0.06	2.9	red. blueshift		(8)

a: Crystal structure contacts in the ADP·Pi bound NBD (1HPM, DnaK numbering) and the ATP bound DnaK (4B9Q) in pdb nomenclature. Distances between H-bond donor and acceptor <3.5 Å; hydrophobic contacts <4.2 Å; b: Cg, *Cricetulus griseus*; Bt, *Bos taurus*; Ec, *Escherichia coli*; c:  $V_{max}(mut)/V_{max}(wt)$  or  $k_{cat}(mut)/k_{cat}(wt)$ ; d: Substrate-stimulated ATPase rate; -, not detectable; +, proficient; numbers=  $[k_{cat}(mut+substrate)/k_{cat}(mut)]/[k_{cat}(wt+substrate)/k_{cat}(wt)]$ ; e: DnaJ-stimulated ATPase rate; -, not detectable; +, proficient; numbers=  $[k_{cat}(mut+DnaJ)/k_{cat}(mut)]/[k_{cat}(wt+DnaJ)/k_{cat}(wt)]$ ; f: synergistic stimulation by DnaJ+substrate; -, not detectable; +, proficient; numbers=  $[k_{cat}(mut+DnaJ+substrate)/k_{cat}(mut)]/[k_{cat}(wt+DnaJ+substrate)/k_{cat}(wt)]$ ; g: ATP-stimulated substrate release; -, not detectable; +, proficient; numbers=  $[k_{off}(mut+ATP)/k_{off}(mut)]/[k_{off}(wt+ATP)/k_{off}(wt)]$ ; h: ATP-induced conformational changes as determined by proteolysis, tryptophane fluorescence (blueshift), or small angle X-ray scattering (SAXS); red., reduced; wt, wild type-like;  $\Delta Rg$ , change in radius of gyration.

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