

Supplementary Table 1. Binding affinities of PfHsp70-x/PfHsp70-xT for peptide substrates

Ligand	Analyte	k_a (1/ Ms)	k_a (1/s)	K_D (M)	χ^2
PfHsp70-x	-	NS	NS	NS	ND
	NRLLTG	3.86 (\pm 0.20)	9.97 (\pm 0.41) e ⁻⁶	2.50 (\pm 0.34) e ⁻⁷	0.98
	NRLLTG + ADP	1.62 (\pm 0. 22)	1.01 (\pm 0.28) e ⁻⁵	6.20 (\pm 0.12) e ⁻⁷	4.38
	NRLLTG + ATP	1.21 (\pm 0.23)	1.01 (\pm 0.13) e ⁻⁵	1.70 (\pm 0.30) e ⁻⁶	2.10
PfHsp70-xT	-	NS	NS	NS	ND
	NRLLTG	3.15 (\pm 0.31)	8.67 (\pm 0.42) e ⁻⁶	2.80 (\pm 0.14) e ⁻⁷	0.24
	NRLLTG + ADP	6.0 (\pm 0.51)	1.00 (\pm 0.21) e ⁻⁵	1.70 (\pm 0.34) e ⁻⁷	3.21
	NRLLTG + ATP	3.18 (\pm 0.42) e ¹	1.61(\pm 0.36) e ⁻⁴	1.80 (\pm 0.23) e ⁻⁶	2.37
PfHsp70-x	NRNNTG	2.43 (\pm 0.45)	9.49 (\pm 0.27) e ⁻⁷	6.91 (\pm 0.43) e ⁻⁹	1.31
	NRNNTG +ADP	2.55 (\pm 0.23)	1.03 (\pm 0.41) e ⁻⁷	4.31 (\pm 0.30) e ⁻⁹	3.98
	NRNNTG +ATP	7.33 (\pm 0.22)	1.01 (\pm 0.31) e ⁻⁶	1.37 (\pm 0.20) e ⁻⁷	4.10
PfHsp70-xT	NRNNTG	3.52 (\pm 0.18)	2.79 (\pm 0.19) e ⁻⁸	7.92 (\pm 0.50) e ⁻⁹	1.56
	NRNNTG +ADP	5.96 (\pm 0.1)	1.01 (\pm 0.31) e ⁻⁸	1.69 (\pm 0.30) e ⁻⁹	2.10
	NRNNTG +ATP	9.37 (\pm 0.17)	1.01 (\pm 0.21) e ⁻⁵	1.07 (\pm 0.20) e ⁻⁷	3.91
PfHsp70-x	GFTVVLMYRF	4.03 (\pm 0.19) e ²	1.03 (\pm 0.40) e ⁻⁵	1.09 (\pm 0.10) e ⁻⁷	0.812
	GFTVVLMYRF + ADP	3.53 (\pm 0.18) e ²	1.00 (\pm 0.14) e ⁻⁵	2.85 (\pm 0.20) e ⁻⁷	2.18
	GFTVVLMYRF + ATP	1.11 (\pm 0.20) e ²	1.17 (\pm 0.44) e ⁻⁴	1.90 (\pm 0.12) e ⁻⁶	4.12

PfHsp70-x _T	GFTVVLMYRF	2.48 (± 0.40) e ¹	2.52 (± 0.34) e ⁻⁶	2.99 (± 0.10) e ⁻⁷	0.68
	GFTVVLMYRF + ADP	1.14 (± 0.19) e ²	1.01 (± 0.22) e ⁻⁵	1.76 (±0.11) e ⁻⁷	2.36
	GFTVVLMYRF + ATP	1.02 (± 0.16) e ²	1.01 (± 0.23) e ⁻⁴	1.10 (± 0.20) e ⁻⁶	5.91
PfHsp70-x	GFTNNNMYRF	9.03 (± 0.34) e ²	1.28 (± 0.2) e ⁻⁷	2.40 (± 0.15) e ⁻⁹	0.89
	GFTNNNMYRF + ADP	4.60 (± 0.15) e ²	1.01(± 0.23) e ⁻⁷	6.25 (± 0.80) e ⁻⁹	4.21
	GFTNNNMYRF + ATP	2.54 (± 0.13) e ²	1.01 (± 0.11) e ⁻⁵	3.97 (±0.15) e ⁻⁷	2.58
PfHsp70-x _T	Peptide 2 GFTNNNMYRF	3.60 (± 0.34) e ²	1.01(± 0.14) e ⁻⁷	2.20 (±0.12) e ⁻⁹	1.37
	GFTNNNMYRF + ADP	5.32 (± 0.45) e ²	1.01 (± 0.40) e ⁻⁷	1.40 (± 0.15) e ⁻⁹	3.10
	GFTNNNMYRF + ATP	8.57 (± 0.14) e ²	1.00 (± 0.20) e ⁻⁵	5.17 (± 0.16) e ⁻⁷	1.87
PfHsp70-x	ALLMYRR	8.67 (±0.21) e ⁻¹	1.28 (±0.92) e ⁻⁶	1.47 (±0.30) e ⁻⁶	3.03
	ALLMYRR +ATP	2.65 (±0.11) e ⁻²	1.60 (±0.29) e ⁻⁶	6.06 (±0.41) e ⁻⁵	2.57
	ALLMYRR +ADP	1.04 (± 0.21) e ³	2.82 (±0.33) e ⁻³	2.82(±0.16) e ⁻⁶	1.50
PfHsp70-x _T	ALLMYRR	1.92 (±0.31) e ⁻²	1.04 (±0.45) e ⁻⁵	5.42 (0.33) e ⁻⁴	1.95
	ALLMYRR +ATP	ND	ND	ND	ND
	ALLMYRR +ADP	3.32 (±0.21) e ⁻²	1.04 (±0.25) e ⁻⁵	3.13(±0.42) e ⁻⁴	1.89
PfHsp70-x	ANNMYRR	3.31 (±0.31) e ⁵	7.32 (±0.43) e ⁻⁴	2.21 (±0.53) e ⁻⁹	4.59
	ANNMYRR +ATP	7.24 (±0.34) e ³	5.67 (±0.11) e ⁻³	7.83 (±0.33) e ⁻⁷	3.78

	ANNNMYRR +ADP	$1.74 (\pm 0.13) e^6$	$3.86 (\pm 0.24) e^{-3}$	$2.21 (\pm 0.31) e^{-9}$	1.12
PfHsp70-x _T	ANNNMYRR	$1.67 (\pm 0.34) e^{-2}$	$1.04 (\pm 0.51) e^{-5}$	$6.26 (\pm 0.32) e^{-4}$	3.24
	ANNNMYRR +ATP	ND	ND	ND	ND
	ANNNMYRR +ADP	$2.35 (\pm 0.37) e^{-2}$	$1.04 (\pm 0.21) e^{-5}$	$4.43 (\pm 0.43) e^{-4}$	2.66

ND, affinity too low to be determined; NS, No signal was detected; As a control a channel without immobilized protein was used as a background control for referencing; in bold are N residue insertions introduced in each peptide. The association rate constant is represented by (*ka*), dissociation rate constants (*kd*), and the equilibrium constant (denotes the affinity) is represented by (*K_D*). Standard deviations about the respective means are shown.