Supplemental data

Table S1

	DnaK (DnaJ, GrpE)		**Ssa1p** (Ydj1p)		**ClpB**		**Hsp104**	
GuHCl	-	+	-	+	-	+	-	+
ATPase activity [turn-over; min-1]	14,8	13,9	5,9	4,8	3,4	17,2	19,6	8,3

TABLE S1. **The influence of GuHCl on the ATPase activities of bacterial and yeast Hsp70 and Hsp100 chaperones.** The ATPase activities of particular chaperones were measured in the absence or presence of GuHCl (5 mM) in a coupled enzymatic assay. Chaperone concentrations: 1 μM DnaK (in the presence of 0.2 μM DnaJ and 0.1 μM GrpE); 3 μM Ssa1p (in the presence of 1 μM Yj1p); 4 μM ClpB; 1 μM Hsp104.

Figure S1

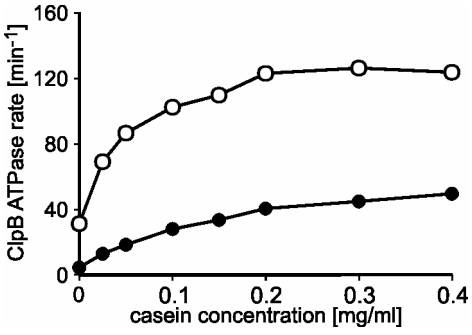

FIGURE S1. **GuHCl stimulation of the ATPase activity of ClpB is casein-independent.** In the presence of increasing concentrations of casein the ATPase activity of ClpB (1 μM) was measured in the absence (filled circles) or presence of 5 mM GuHCl (open circles).

Figure S2

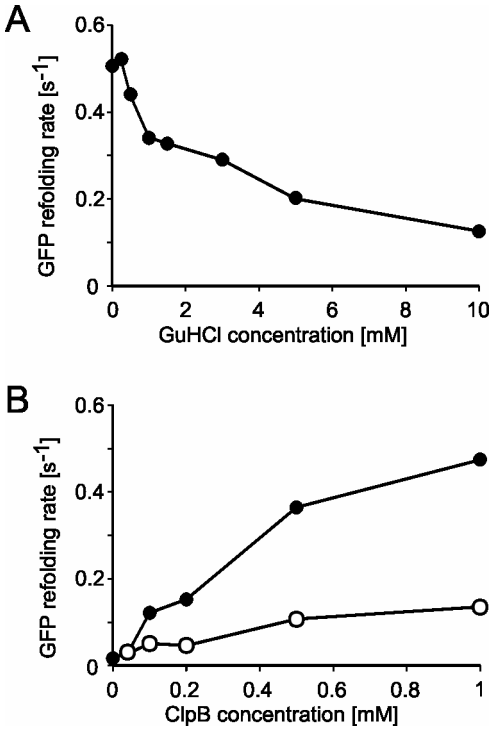

FIGURE S2. **GuHCl inhibits the disaggregation of heat aggregated GFP by the bacterial KJE-ClpB chaperone system *in vitro*.** *A*, heat-aggregated GFP (9 μg) was incubated with the KJE-ClpB chaperone system (0.5 μM ClpB, 1 μM DnaK, 0.2 μM DnaJ, 0.1 μM GrpE). GFP reactivation was monitored in real-time in a spectrofluorometer (excitation and emission wavelengths: 395 nm and 510 nm, respectively). GFP reactivation was measured in the presence of increasing concentrations of GuHCl. The rate of reaction (increase in GFP fluorescence/s) was calculated from the slope of the linear part of each curve and plotted as a function of GuHCl concentration. *B*, GFP refolding rate was measured as in *A*. In the absence (filled circles) or presence of 5 mM GuHCl (open circles) over a range of ClpB concentrations.

Figure S3

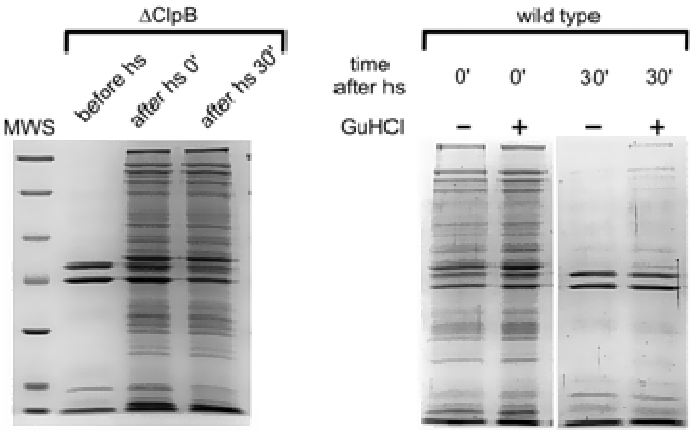

FIGURE S3. **GuHCl inhibits the removal of protein aggregates following heat stress.** *E. coli* MC1000 (parent) and *ΔclpB* strains were grown at 37ºC to OD_{600} = 0.3; GuHCl was then added to the final concentration of 20 mM and cultures were shifted to 50ºC for 8 min. After heat-shock bacteria were returned to 37ºC to allow protein disaggregation. At the indicated timepoints samples were taken (corresponding to 12 ml of culture of OD_{600} = 1), rapidly cooled in an ice-water bath and cells were harvested by centrifugation for 10 min at 5000×g at 4ºC. Pellets were resuspended in 2.5 ml of buffer K (10 mM potassium phosphate buffer, pH 6.8; 1 mM EDTA) and cell lysis was performed in an SLM Aminco French pressure cell. Cell lysates were centrifuged for 15 min at 2000×g, 4ºC to eliminate intact cells. The insoluble cell fraction (containing membranes and protein aggregates) was isolated by subsequent centrifugation at 15000×g, 4ºC for 20 min. Pellet fractions were resuspended in 400 µl of buffer K and centrifuged once more (20 min, 15 000×g, 4ºC). After centrifugation the pellets were resuspended in 320 µl of buffer K, followed by addition of 80 µl of 10% (v/v) NP-40; protein aggregates were isolated by centrifugation (30 min, 15000×g, 4ºC). The washing procedure was repeated to completely dissolve membranes. The detergent-insoluble fraction was washed with 400 µl of buffer K and centrifuged (30 min, 15000×g, 4ºC). Pellets were resuspended in 65 µl of buffer K and then analyzed by SDS-PAGE (12.5%) followed by Coomassie Blue staining.

Figure S4

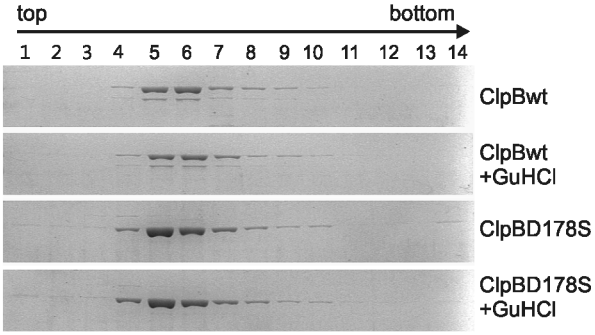

FIGURE S4. **Mutation D178S in ClpB does not influence the oligomeric state of the protein.** Sedimentation analysis of ClpBwt, ClpBD178S oligomerization. ClpBwt (*upper panels*) and ClpBD178S (*lower panels*) diluted in buffer U were loaded onto a glycerol gradient in buffer C supplemented with 5 mM ATP and (where indicated) with 5mM GuHCl. Sedimentation analysis of the proteins was performed (Beckman SW60 rotor; 15-45% (v/v) glycerol gradient; 46 000 rpm; 16 h; 4ºC). The position of ClpBw and ClpBD178S in the gradients was visualized by SDS-PAGE and Coomassie Blue staining.

Figure S5

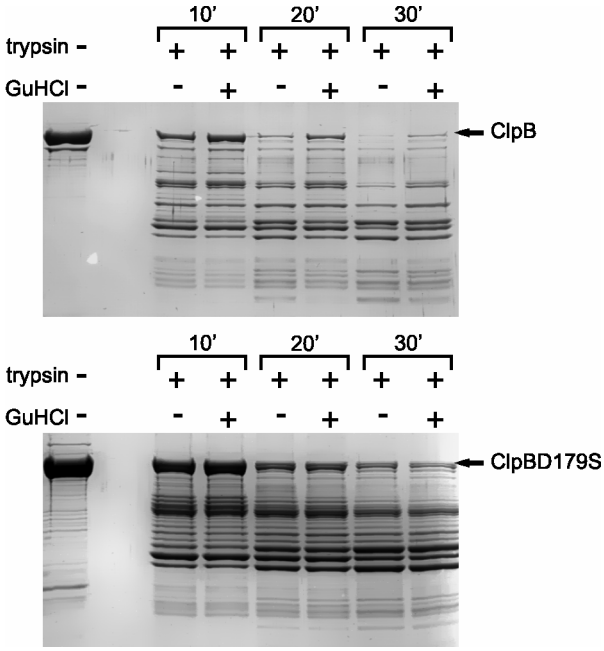

FIGURE S5. **Mutation D179S in ClpB influences both the trypsin digestion pattern and the rate of digestion.** Kinetics of the partial trypsin digestion of 4 μg of ClpB (*upper panel*) and ClpB (D179S) (*lower panel*) in the presence of 2 mM ATP and the presence or absence of 5 mM GuHCl (as indicated). Reactions (10 μl) were assembled and incubated at 30°C with 0.07 μg of trypsin. At the indicated time points reactions were stopped by the addition of Laemmli buffer. Proteolysis products were analyzed by SDS-PAGE and Coomassie Blue staining.

Figure S6

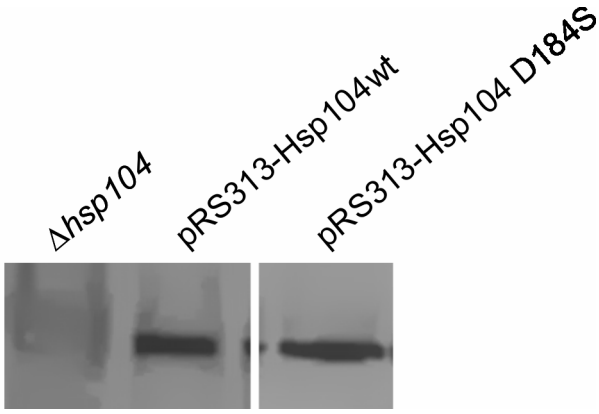

FIGURE S6. **Hsp104 and Hsp104 (D184S) are present in yeast cells in similar amounts.** Equal amounts of yeast strain *Δhsp104* carrying the plasmids pRS313-Hsp104 wt or pRS313-Hsp104(D184S) were collected and lysed. The samples were resolved by SDS-PAGE, followed by western blot analysis using anti-Hsp104 antibodies.