Supplemental Data

INSIGHTS INTO THE CLP/HSP100 CHAPERONE SYSTEM FROM CHLOROPLASTS OF *ARABIDOPSIS THALIANA*

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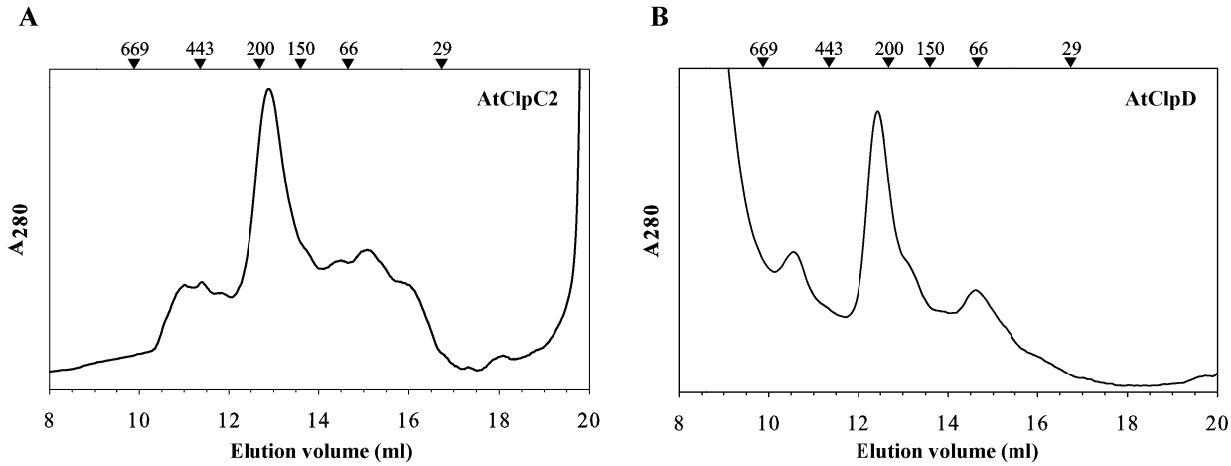

Fig. S1. Gel filtration analysis of the purified proteins. Panel A and B show typical chromatograms for AtClpC2 and AtClpD, respectively, in the absence of ATP. Arrows above the chromatograms indicate the migration of molecular weight standards.

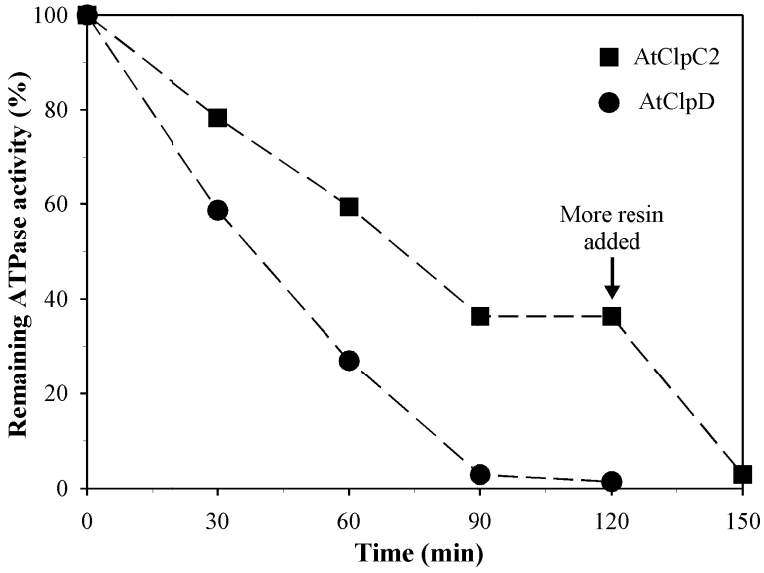

Fig. S2. ATPase activity after chaperone removal. 20 µl of polyclonal antibodies raised against each chaperone were bound overnight to 20 µl of protein A-Sepharose beads (pre-swollen in P buffer) at 4 °C under constant agitation. The antibody-decorated beads were washed three times with 1 ml of P buffer and then added to 0.1 mg/ml AtClpC2 or AtClpD in P buffer (final reaction mixture volume: 200 µl). The mixtures were incubated at 25 °C under constant agitation. Every 30 min, the resin was mildly centrifuged and ATPase activity was analyzed in 10-µl aliquots of the supernatant (reaction conditions:

37 °C, pH: 7.5, 50-µl reaction mixture containing 5 mM ATP, 30 min incubation). The results are expressed as a percent of ATPase activity with respect to a control mixture containing either chaperone incubated with protein A-Sepharose beads with no antibodies added. AtClpC2 (■), AtClpD (●). If at two time points ATPase activity remained unchanged, more antibody-decorated beads (20 µl) were added.

Table S1. Kinetic parameters of various members of the HSP100 family. Adapted from Schirmer *et al*. (29 and references therein).

Hsp	Organism	K_m (mM)	V_{max} (nmol min^{-1} μg^{-1})
Class 1			
Hsp104	*S. cerevisiae*	5	2
ClpA	*E. coli*	0.2	0.7 - 0.9
ClpB	*E. coli*	1,1	0.8
ClpC	*B. subtilis*	-	0.4
ClpC	*S. elongatus*	2.05	-
ClpC[a]	*M. tuberculosis*	5.6	-
ClpC2[b]	*A. thaliana*	1.42	0.62
ClpD[b]	*A. thaliana*	19.8	0.19
Class 2			
AmiB	*Pseudomonas aeruginosa*	0.174	2.4
ClpX	*E. coli*	0.5	0.5

[a] Kar, N. P., Sikriwal, D., Rath, P., Choudhary, R. K., and Batra, J. K. (2008) *FEBS J.* **275,** 6149-6158
[b] This work