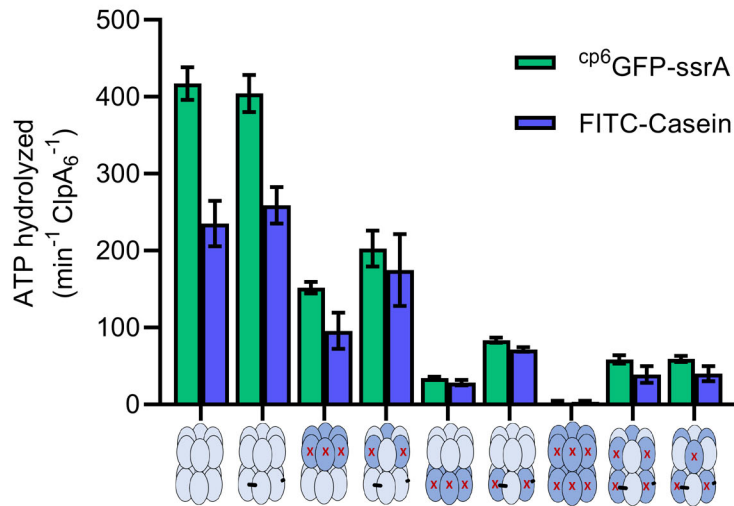
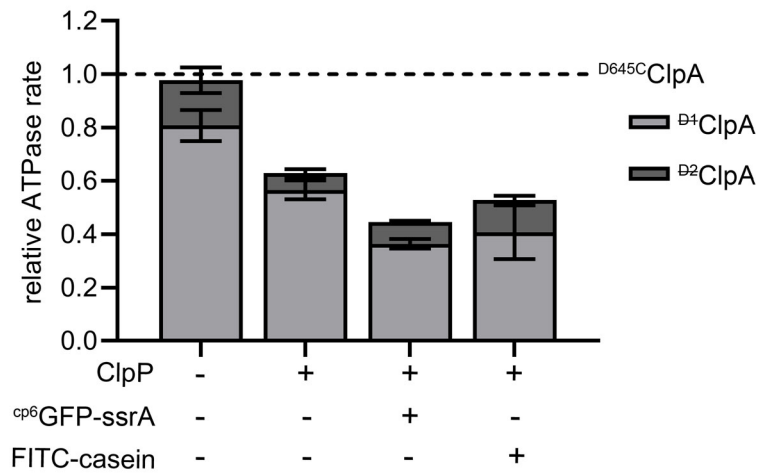


## SI Appendix



**S1. ATP hydrolysis by the ClpA/ClpP variants with substrates.** Rates of hydrolysis of ATP (5 mM) by ClpAP variants in the presence of <sup>cp6</sup>GFP-ssrA (20  $\mu$ M; green) or FITC-casein (50  $\mu$ M; blue). Experiments were performed using 0.25  $\mu$ M of each ClpA variant and 0.75  $\mu$ M ClpP. Values are averages ( $n = 6$ )  $\pm$  1 SD.



**S2. Relative contributions of the D1 and D2 rings to ATP hydrolysis under different conditions.** The relative ATPase rates of  $D^1$ ClpA and  $D^2$ -ClpA roughly add up to the ATP-hydrolysis rate of the parental enzyme ( $D^{645C}$ ClpA) in the absence of ClpP and substrates. However, the summed ATPase rate is lower than predicted (44-62%) of this rate when ClpP or ClpP and protein substrates were present. Values are averages ( $n \geq 4$ )  $\pm$  1 SD.

ClpA variant	Active D1 modules	Active D2 modules	$\Sigma$			
			-ClpP	+ClpP	+ClpP + <sup>cp6</sup> GFP- ssrA	+ClpP +FITC- casein
D645C ClpA, ClpA <sup>x</sup>	6	6	6	6	6	6
D <sup>1</sup> ClpA	0	6	5.0	5.4	4.9	4.6
D <sup>2</sup> ClpA	6	0	1	0.6	1.1	1.4
altD1 ClpA <sup>x</sup>	3	6	5.5	5.7	5.4	5.3
altD2 ClpA <sup>x</sup>	6	3	3.5	3.3	3.6	3.7
D <sup>1</sup> /D <sup>2</sup> ClpA	0	0	0	0	0	0
altD1/altD2/cis ClpA <sup>x</sup> , altD1/altD2/trans ClpA <sup>x</sup>	3	3	3	3	3	3

**Table S1.** Table listing calculated values of  $\Sigma$  for ClpA variants.  $\Sigma$  was calculated as the sum of number of active D1 sites (column 1) multiplied by the fractional contribution determined for each condition in Fig. 6B (0.17, 0.10, 0.18, 0.23 for -ClpP, +ClpP, +ClpP and <sup>cp6</sup>GFP-ssrA, and +ClpP and FITC-casein, respectively) plus the number of active D2 sites (column 2) multiplied by the fractional contribution determined in Fig. 6B (0.83, 0.90, 0.82, 0.77 for -ClpP, +ClpP, +ClpP and <sup>cp6</sup>GFP-ssrA, and +ClpP and FITC-casein, respectively). These  $\Sigma$  values were used as the x-axis in Fig. 6C-F.