Supplemental Material for:

Degradation of the *E. coli* antitoxin MqsA by the proteolytic complex ClpXP is regulated by zinc occupancy and oxidation

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Figure S1. ClpXP activity and stabilization of the ClpX N-terminal domain by zinc. (A) Gfp-ssrA (0.5 μM) degradation was monitored by measuring loss of fluorescence with time in reactions containing MqsR (0.5 μM), where indicated, with ClpX (0.75 μM), ClpP (1.2 μM), ATP, and an ATP-regenerating system. Fluorescence was measured using excitation and emission wavelengths of 395 and 510 nm, respectively. (B) Zinc stabilizes the conformation of the ClpX N-terminal domain. 2D [1H,15N] HSQC spectra of ClpXN in the absence (black) and presence (red) of zinc showing zinc stabilizes folding.
Figure S2. 2D $[^1H,^{15}N]$ HSQC spectra of MqsAN and MqsA1-34. (A) 2D $[^1H,^{15}N]$ HSQC of MqsA1-34 showing that MqsA1-34 is unfolded in the presence of zinc. (B) 2D $[^1H,^{15}N]$ HSQC spectrum showing folded MqsAN.
Figure S3. Titration of ClpX<sub>N</sub> with IDPs. Overlay of 2D [<sup>1</sup>H, <sup>15</sup>N] HSQC spectra of free ClpX<sub>N</sub> (blue) with Inhibitor-2<sub>10-165</sub> (I2; brown) and PNUTS<sub>376-435</sub> (pink) shown. No chemical shift perturbations are observed.
Figure S4. Characterization of WT ClpX and ClpX\textsubscript{N} variants. (A) Size exclusion chromatography (SEC) retention profiles of WT ClpX\textsubscript{N} and ClpX\textsubscript{N} variants. (B) SDS-PAGE gel of all ClpX\textsubscript{N} constructs purified in (A). (C) \textsuperscript{1}H NMR spectra of WT ClpX\textsubscript{N} and ClpX\textsubscript{N} variants. (D) SEC retention profiles of full length WT ClpX and ClpX variants. (E) ATP hydrolysis activity of ClpX wild type and N-domain variants (0.5 μM) was assayed by measuring release of phosphate with time in reactions containing ATP (5 mM) as described in Methods. Data shown is an average of at least three data sets with error indicated as S.E.M. (F) Gfp-ssrA degradation was monitored by measuring loss of fluorescence with time in reactions containing WT ClpX (gray) or ClpX N-domain variants ClpX(L12S) (yellow), ClpX(L13D) (purple), ClpX(L13A) (lavender), ClpX(H23A) (aqua), or ClpX(A30S) (red) (1.5 μM), where indicated, with ClpP (1.7 μM), ATP and an ATP-regenerating system as described in Methods. Data shown is representative of at least three data sets and error is shown as standard error.