Supplementary information:

The intrinsically disordered N-terminal extension of the ClpS adaptor reprograms its partner AAA+ ClpAP protease

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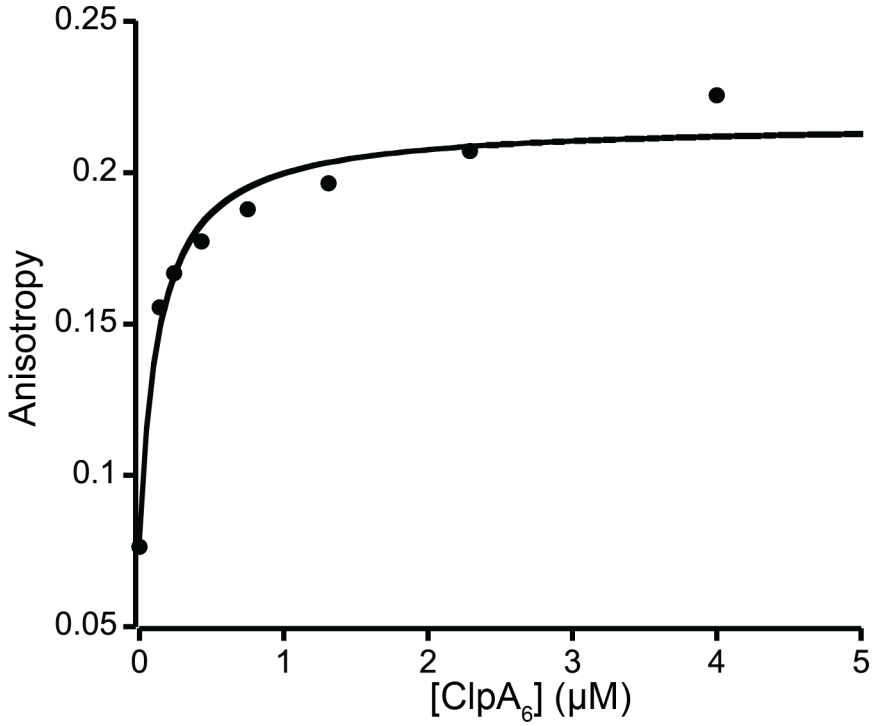
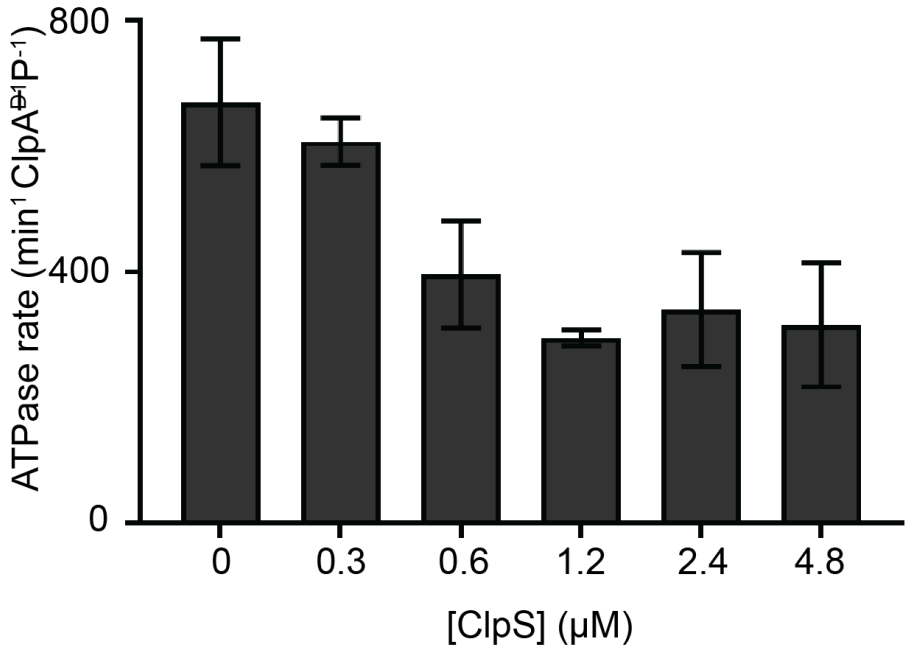
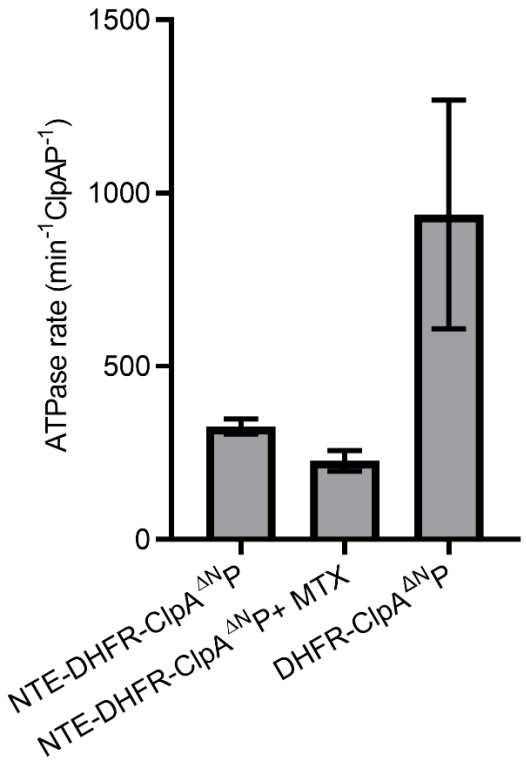
Running head: *ClpS reprogramming of ClpAP*



Supplementary Figure 1: ClpS$^{\Delta N17*fl}$ binding to ClpA. Fluorescence anisotropy of ClpS$^{\Delta N17*fl}$ (0.2 μM) in the presence of λ-ssrA (30 μM), 2 mM ATPγS, as assayed at increasing concentrations of ClpA. ClpS$^{\Delta N17*fl}$ is a ClpS variant that contains a single cysteine and lacks the N-terminal 17 residues. The line is fit to a quadratic equation for binding with 50% binding (K_D) at 210 ± 120 nM. The K_D is an average ± SD (n=3).



Supplementary Figure 2: Suppression of ClpA$^{\Delta 1}$P ATPase rate by ClpS. ATP hydrolysis rates by ClpA$^{\Delta 1}$P (0.4 μM ClpA$^{\Delta 1}{}_6$, 0.8 μM ClpP$_{14}$) were determined in the presence of 30 μM λ-ssrA at increasing ClpS concentrations. Values are averages ± 1 SD (n = 3).



Supplementary Figure 3: NTE suppresses the ATPase rate of ClpA. ATP hydrolysis rates of NTE-DHFR-ClpA$^{\Delta N}$ and DHFR- ClpA$^{\Delta N}$ chimeras (0.4 μM ClpA$^{\Delta N}$ chimera, 0.8 μM ClpP$_{14}$) were determined. NTE-DHFR-ClpA$^{\Delta N}$ has a lower ATPase rate compared to DHFR-ClpA$^{\Delta N}$, supporting the model whereby NTE interactions with the translocation machinery suppress the rate of ATP hydrolysis by ClpA. Values are averages ± 1 SD (n = 3).

Highlights:

- ClpS adaptor enhances and inhibits degradation by ClpAP, tuning substrate choice.
- ClpS impedes degradation of ssrA-substrates, however the mechanism has been unclear.
- In one mechanism, ClpS acts non-competitively to decrease ssrA-tag affinity to ClpA.
- ClpS also reduces the ClpA ATPase, thereby slowing protein unfolding/translocation.
- To inhibit, ClpS's intrinsically disordered "domain" is necessary and sufficient.
- ClpS is multi-faceted, controlling both substrate binding and enzyme activity.