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## Supplemental Information

# The ClpS Adaptor Mediates Staged Delivery of N-End Rule Substrates to the AAA+ ClpAP Protease

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Supplemental Information Inventory

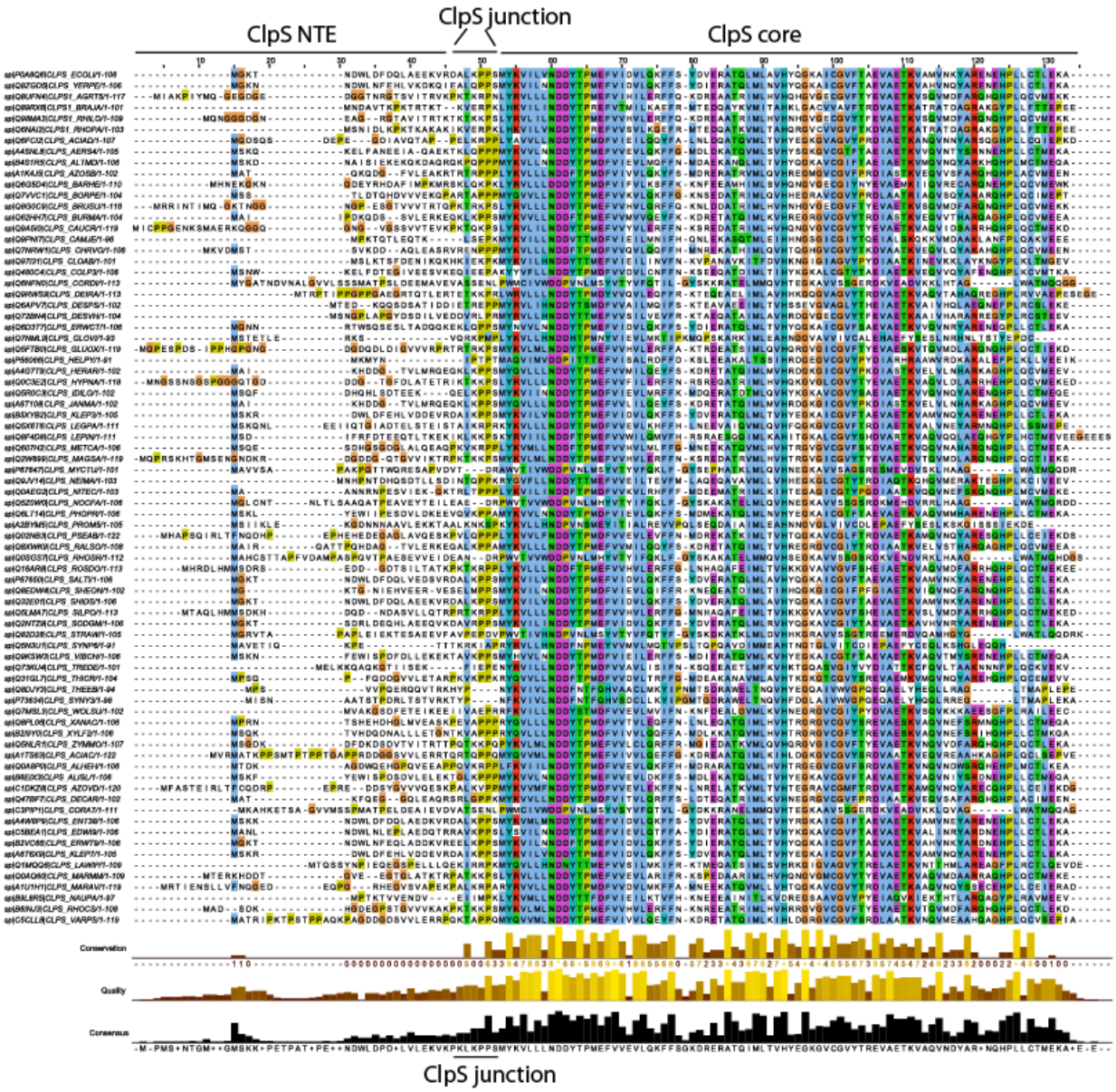


**Figure S1, related to Figure 4. Sequence alignment of several ClpS homologs.** Sequences were obtained from the Universal Protein Resource (UniProt 2010). Multiple sequence alignment was done using ClustalW. Both the sequence identity and the length of the ClpS NTE are poorly conserved. In contrast to most of the ClpS NTE, the sequence adjacent to the ClpS core (called the junction sequence: DALKPPS in *E. coli*) is moderately conserved.

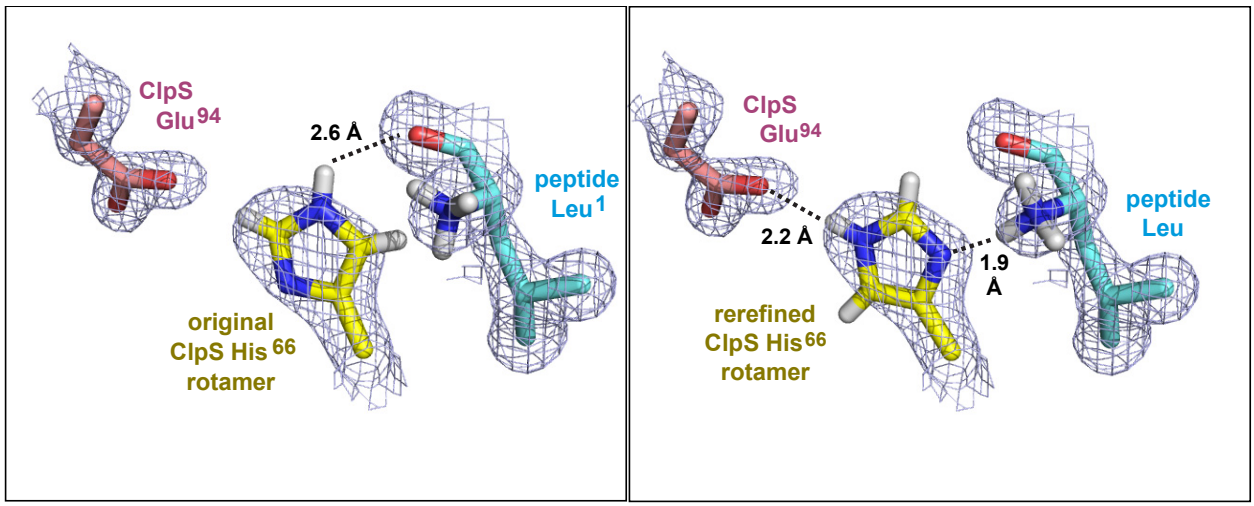


**Figure S2, related to Figure 1. Rerefinement of the 2W9R ClpS structure.** In the rerefined 2W9R structure (right panel), the His66 side chain makes good hydrogen bonds (dashed lines) with the α-NH3 group of the bound N-end peptide and with Glu94 in symmetry related subunit. In the original 2W9R structure (Schuenemann et al., 2009; left panel), His66 atoms make a poor hydrogen bond with the carbonyl oxygen of the first peptide residue, clash badly with α-NH3 protons, and positions a non-polar hydrogen very close to the Glu94 carboxylate. In both panels, the electron density (1.25σ) is from the rerefined map. For clarity, only some hydrogens are shown.

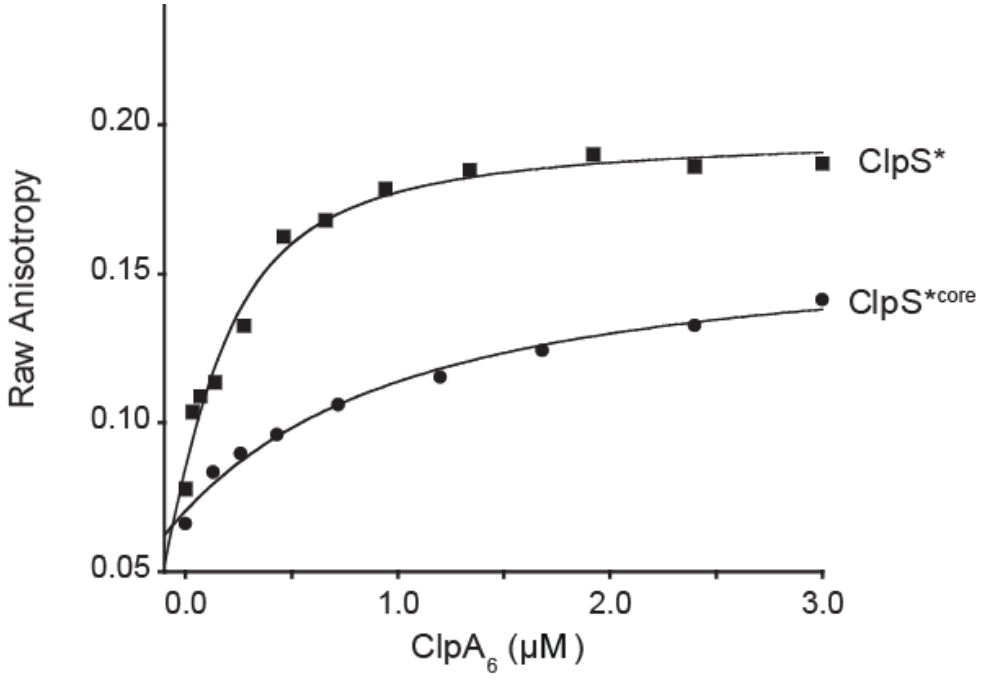


**Figure S3, related to Figure 3. "ClpS binds $ClpA_6$ more weakly when it lacks its N-terminal extension (NTE)".** A fluorescent variant of the ClpS adaptor without its N-terminal extension ($ClpS^{*core}$) binds at least 10-fold weaker to $ClpA_6$ than full length ClpS (ClpS*).

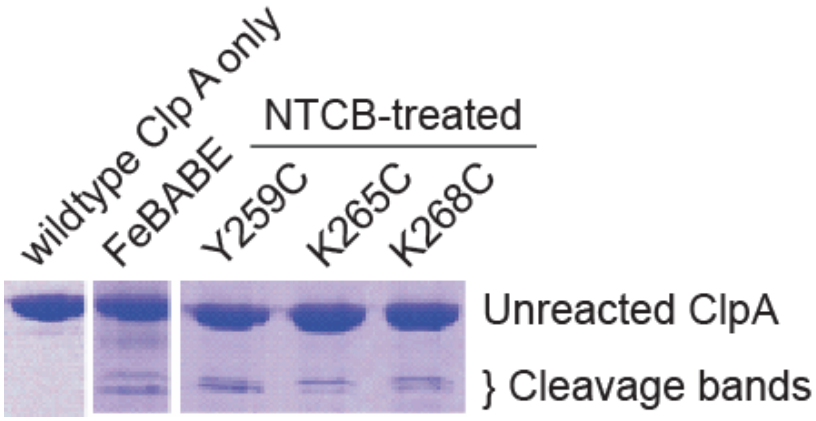


**Figure S4, related to Figure 5. "Size estimation of ClpSQ12C/FeBABE/ClpA-cleavage reaction products."** The larger cleavage bands are shown next to ClpA fragments generated from NTCB digestions of ClpA cysteines in the pore. On the left-hand side is unmodified wild-type ClpA. The FeBABE experiments mapped residues 259-268 of ClpA as the residues physically contacting the ClpS NTE. These residues are located in the D1 AAA+ ring of ClpA, near the axial translocation pore.