SUPPLEMENTAL DATA

CHARGE REQUIREMENTS FOR PROTON GRADIENT-DRIVEN TRANSLOCATION OF ANTHRAX TOXIN

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Running head: Proton-gradient driven translocation

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Experimental Procedures

Equilibrium chemical denaturation titrations. Guanidinium chloride titrations carried out on LF_N mutants in 10 mM sodium phosphate, 1 M glucose, pH 7.5, 20 °C as described (1,2). Each titration point is monitored by circular dichroism (CD) spectroscopy at 222 (±2) nm using a Jasco J-810 spectropolarimeter (Easton, MD). The CD-probed curves fit to a four-state thermodynamic model ($N \leftrightarrow I \leftrightarrow J \leftrightarrow U$), where native ($N$), two intermediates ($I$ and J), and an unfolded (U) state are populated (2). We use the thermodynamic difference between the N and I states (ΔG_{NI}) to assess the stability of the protein.

REFERENCES

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2. Krantz, B. A., Trivedi, A. D., Cunningham, K., Christensen, K. A., and Collier, R. J. (2004) *J. Mol. Biol.* **344**, 739-756

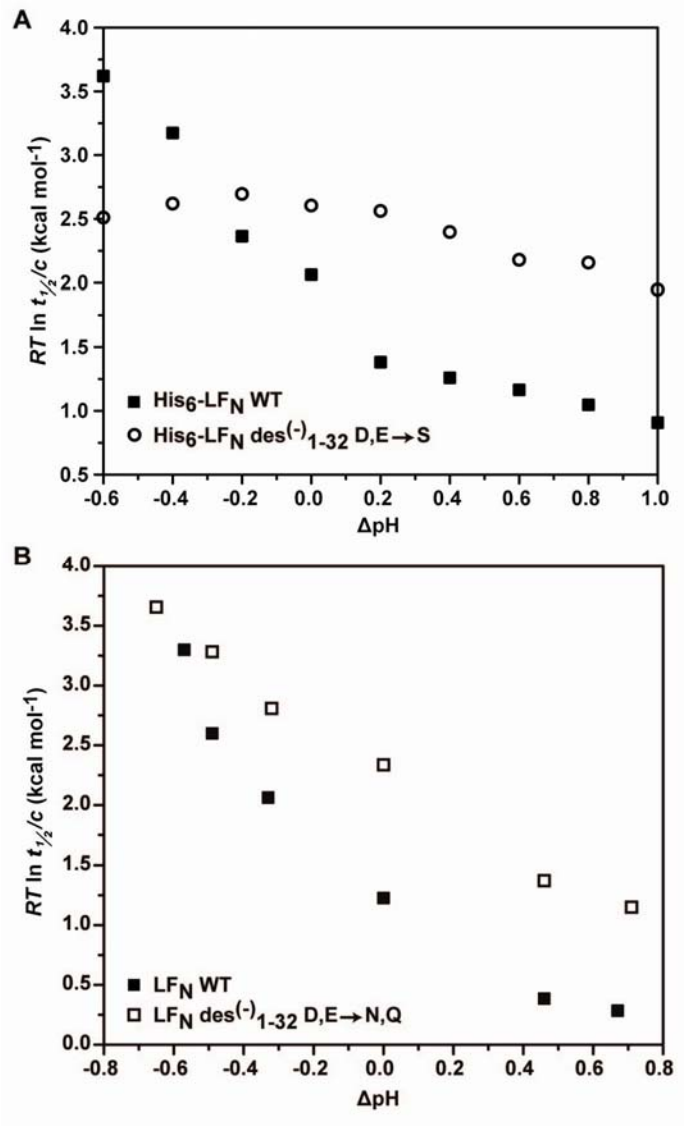

Fig. S1. LF_N $des^{(-)}_{1-32}$ has reduced ΔpH dependence relative to LF_N WT regardless of replacement residue or His_6 tag. The LF_N $des^{(-)}_{1-32}$ construct changes the aspartates and glutamates in LF_N's presequence to (A) serines or (B) their respective nonpolar analogs asparagines and glutamines. Additionally, in (B) the His_6 tags have been removed from the proteins. $\Delta G‡$ versus ΔpH profiles are shown for (A) His_6-LF_N WT (■) and His_6-LF_N $des^{(-)}_{1-32}{}^{D,E\rightarrow S}$ (○) or (B) LF_N WT (■) and LF_N $des^{(-)}_{1-32}{}^{D,E\rightarrow N,Q}$. In both cases, conditions are $\Delta\Psi$ = 60 mV and pH_{cis} = 5.6.

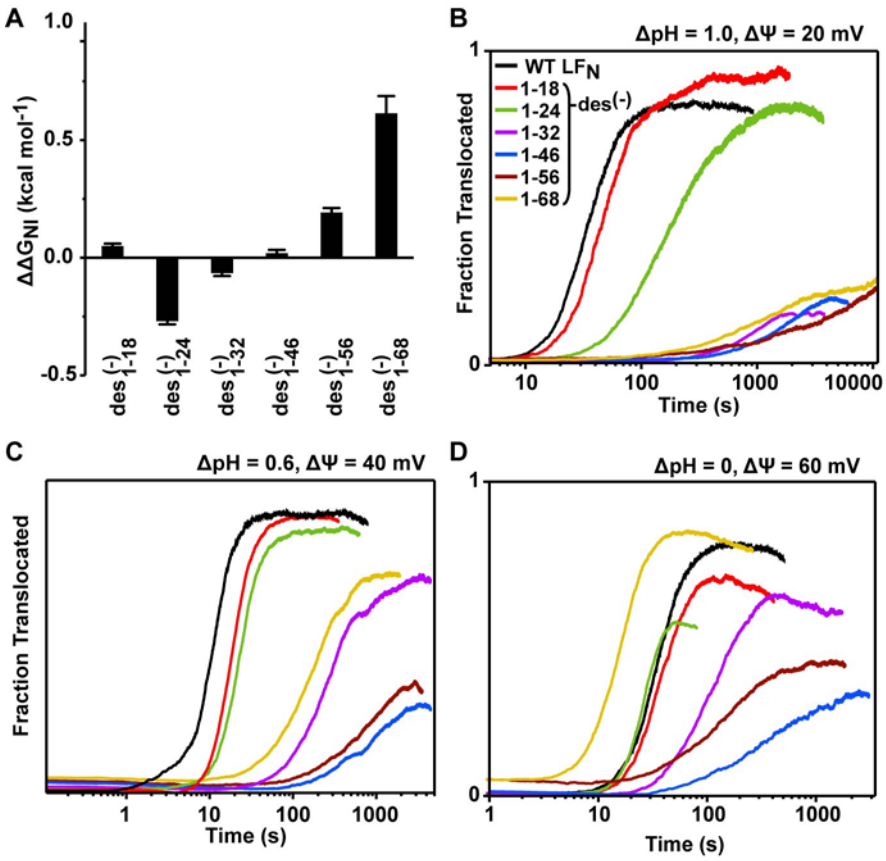

Fig. S2. Acidic residues within LF_N's folded domain are also critical to ΔpH-driven translocation. (A) The change in protein stability ($\Delta\Delta G_{NI}$) for the His_6-LF_N $des^{(-)}$ series relative to His_6-LF_N WT estimated from guanidinium chloride denaturant melts probed by circular dichroism. The thermodynamic quantity, $\Delta\Delta G_{NI}$, compares difference in the *N* and *I* state free energies of the mutant (MUT) to WT as follows: $\Delta\Delta G_{NI} = \Delta G_{NI}(MUT) - \Delta G_{NI}(WT)$. Error bars are the mean ±s.d ($n = 2$). (B-D) Translocation records for His_6-LF_N WT (black), His_6-LF_N $des^{(-)}_{1-18}$ (red), His_6-LF_N $des^{(-)}_{1-24}$ (green), His_6-LF_N $des^{(-)}_{1-32}$ (purple), His_6-LF_N $des^{(-)}_{1-46}$ (blue), His_6-LF_N $des^{(-)}_{1-56}$ (brown), and His_6-LF_N $des^{(-)}_{1-68}$ (gold) under different ΔpH and ΔΨ driving force conditions: (B) ΔpH = 1.0, $pH_{cis} = 5.6$, ΔΨ = 20 mV; (C) ΔpH = 0.6, $pH_{cis} = 5.6$, ΔΨ = 40 mV; and (D) ΔpH = 0, $pH_{cis} = 5.6$, and ΔΨ = 60 mV, as summarized in Fig. 4B.

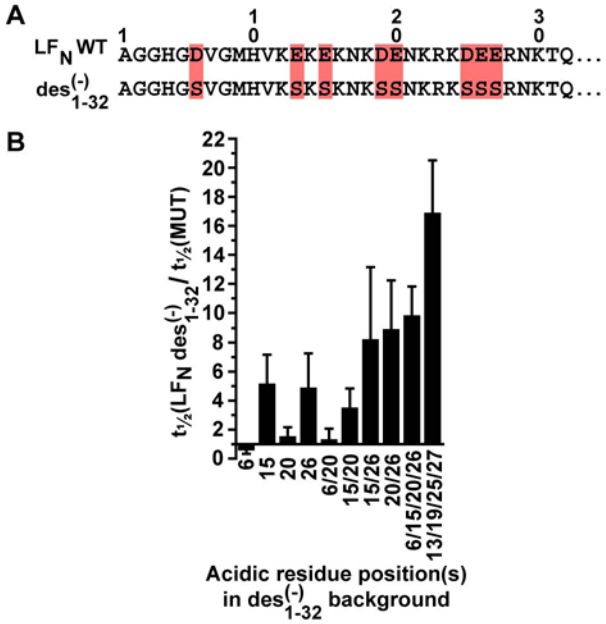

Fig S3. Acidic-residue positions in LF_N's presequence are most critical to ΔpH-driven translocation. Acidic residues were reintroduced in their wild-type positions into the His_6-LF_N $des^{(-)}_{1-32}$ background. (A) The sequences of the first 32 residues of LF_N WT and LF_N $des^{(-)}_{1-32}$ are shown, where acidic residues in the WT sequence are shaded red. (B) The relative translocation $t_{1/2}$ times for ΔpH-driven translocation are given as the ratio $t_{1/2}(des^{(-)}_{1-32}) / t_{1/2}(MUT)$, where the mutant (MUT) is the construct with the reintroduced acidic residue(s). The numbers on the *x*-axis indicate the position(s) in which acidic residues are reintroduced into the His_6-LF_N $des^{(-)}_{1-32}$ backgrounds. Multisite acidic-residue reintroductions are indicated with slashes separating the residue number. ΔpH-driven translocation conditions were ΔpH = 0.6, pH_{cis} = 5.6, and $\Delta\psi$ = 35 mV. Error bars are the mean ±s.d. (n = 2). For reference, the relative translocation time for LF_N WT compared to LF_N $des^{(-)}_{1-32}$, $t_{1/2}(des^{(-)}_{1-32}) / t_{1/2}(WT)$, is 14.8 (±3.0).