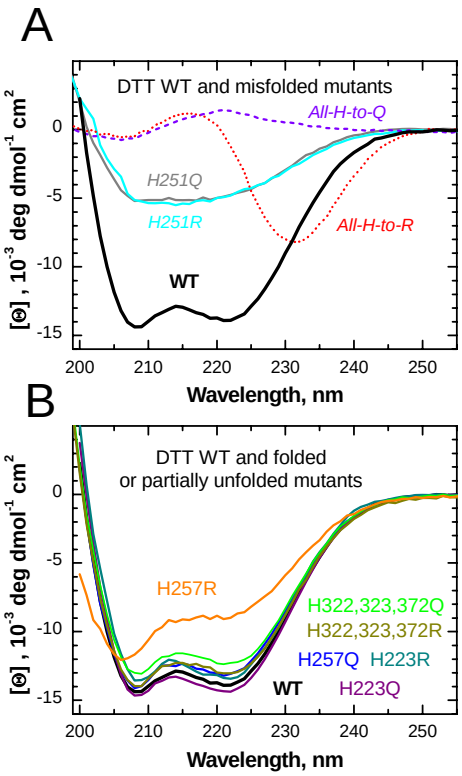

Supplement Fig. S1

Supplement Figure S1. Far-UV circular dichroism spectra of the diphtheria toxin T-domain (DTT) and its histidine mutants with removed charge (H-to-Q replacements) or introduced pH-independent charge (H-to-R replacements). CD spectra are color coded, with the names of mutants in the caption. **(A) Misfolded mutants.** Mutations of all six native histidines result in greatly distorted spectra (All-H-to-R and All-H-to-Q mutants), while either Q or R replacement of a single residue H251 produces spectra of low ellipticity without pronounced double minima characteristic of the helical structure. **(B) Folded and partially folded mutants**. Only substitutions in position 257 result in marked differences for Q- and R-replacements. In contrast, simultaneous replacements of all three C-terminal histidines (H322, H323, H372) with either R's or Q's does not lead to appreciable changes in CD appearance. **Experimental details:** WT and mutant proteins were expressed in *E. coli* and purified as described in [1]. CD measurements were collected in cuvettes of 1 mm path using Jasco-720 spectropolarimeter (Japan Spectroscopic Company, Tokyo) as described previously [2]. Samples typically contained 0.5-3 μM of T-domain in 20 mM phosphate buffer at pH 8.0.

1. Kyrychenko, A., Posokhov, Y. O., Rodnin, M. V. & Ladokhin, A. S. (2009). Kinetic intermediate reveals staggered pH-dependent transitions along the membrane insertion pathway of the diphtheria toxin T-domain. *Biochemistry* **48**, 7584-7594.
2. Rodnin, M. V., Posokhov, Y. O., Contino-Pepin, C., Brettmann, J., Kyrychenko, A., Palchevskyy, S. S., Pucci, B. & Ladokhin, A. S. (2008). Interactions of fluorinated surfactants with diphtheria toxin T-domain: testing new media for studies of membrane proteins. *Biophys J* **94**, 4348-4357.