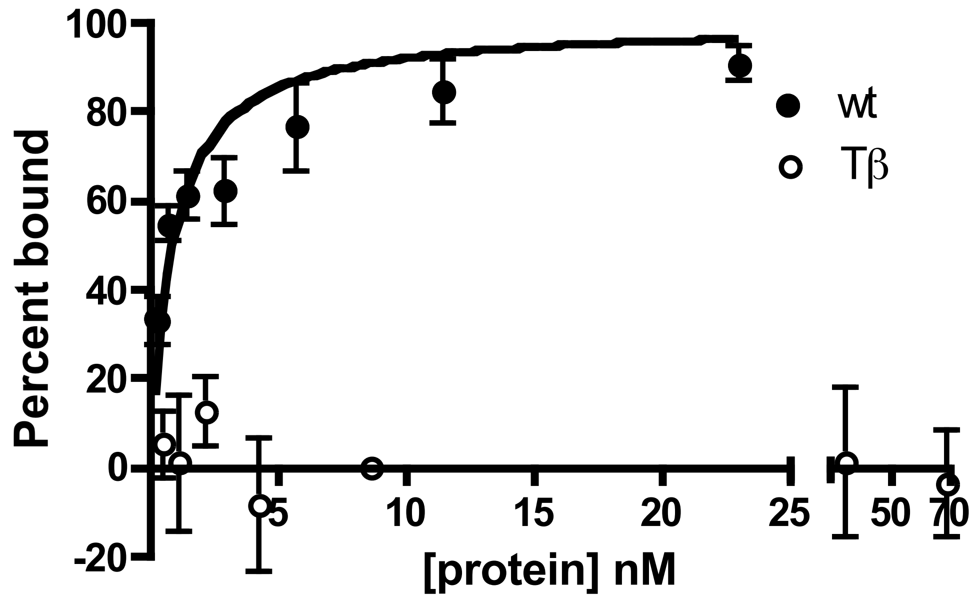
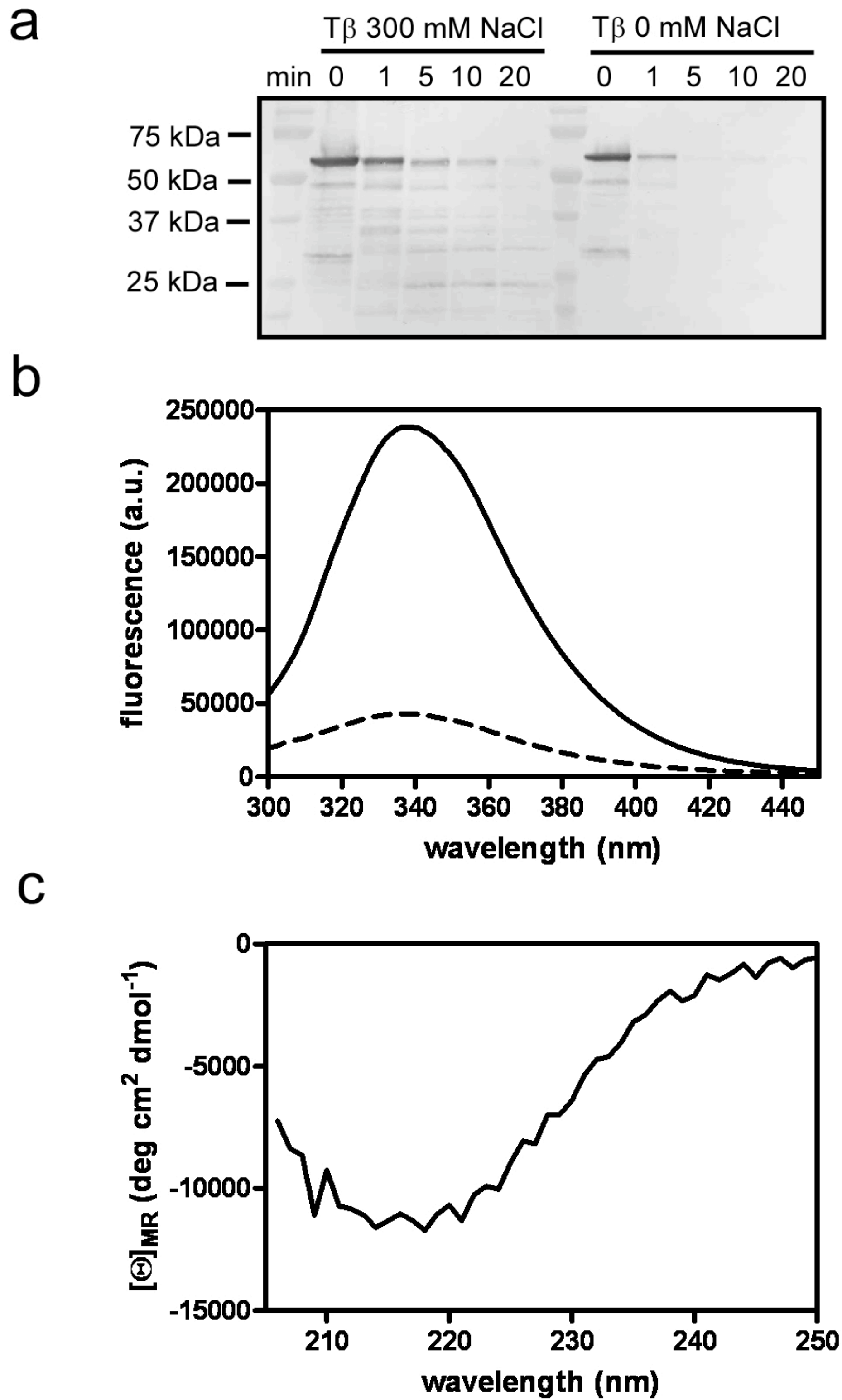
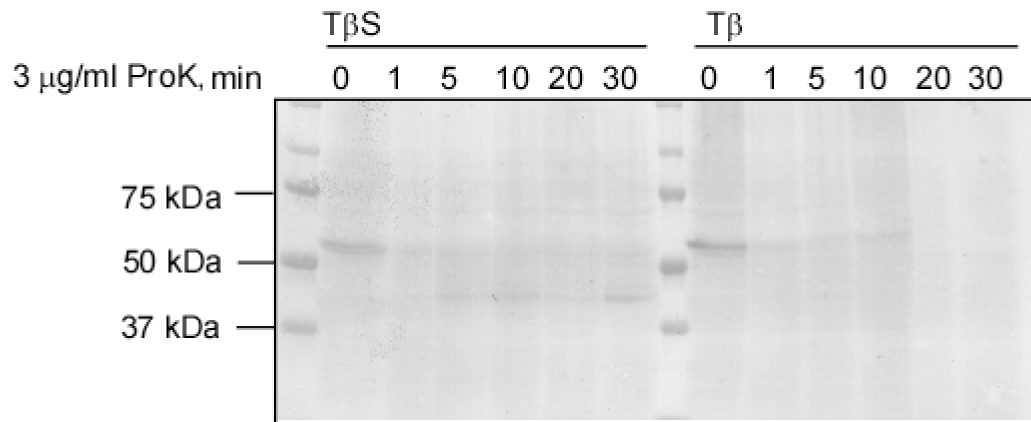


Supplementary Figure S1: *Representative tailspike competition ELISA data*. Measurements of binding of mAb 155 to native wild type tailspike trimer and Tβ. mAb 155 binds to tailspike with an apparent K_d of 0.845 nM, but has no apparent binding affinity for Tβ.



Supplementary Figure S2: *Tβ refolded under low salt conditions has native-like spectral signatures, but no protease-resistant fragment.* **(a)** Western blot of proteinase K

digestion of Tβ refolded in refolding buffer (300 mM NaCl, 50 mM phosphate, 2 mM EDTA, 3 μM 2-mercaptoethanol, pH 8.0)), or 50 mM phosphate, pH 7.0. Proteinase K was added at a final concentration of 1 μg/ml and digestions were performed for the indicated times at 4ºC. **(b)** Fluorescence emission spectra for Tβ refolded under low-salt conditions (dashed line), and native tailspike (solid line). **(c)** Far-UV circular dichroism spectrum of Tβ refolded under low-salt conditions.



Supplementary Figure S3: *Proteolysis of Tβ refolded in an* E. coli *cell lysate supernatant.* Similar concentrations of Tβ refolded in the presence of a cleared lysate and TβS were subjected to proteinase K digestion for the indicated times at a final proteinase K concentration of 3 μg/ml. Cleared lysate was added to the TβS sample mixture prior to digestion in order to normalize digestion conditions.

Supplementary Methods

Refolding of Tβ in Lysate

Concentrated Tβ in 6M GdnHCl was refolded by rapid 1:50 dilution and rapid mixing with a micropipette into an *E. coli* cell lysate supernatant. The refolding solution was incubated at 0ºC for at least 48 hours prior to use.

Protease Digestions of Tβ Under Alternative Conditions

Purified Tβ refolded under either physiological or low salt conditions was digested with 1 μM proteinase K for the indicated times, following the protocol described (Methods). For the digestion of TβS and Tβ refolded in lysate, a concentration of 3 μg/ml proteinase K was used, and *E. coli* lysate supernatant was added to the purified ribosome sample. Digestion products were detected as described (Fig. 3a).

Fluorescence Measurements

All fluorescence measurements were made using a PTI QM-6 fluorimeter (Birmingham, NJ). Each protein concentration was 10 μg/ml. Samples were measured at 10ºC in 50 mM phosphate buffer, with an excitation wavelength of 280 nm, and integration time of 1 sec. Spectra shown are an average of three scans.

Circular Dichroism Spectroscopy

All CD spectra were collected using an Aviv 62DS CD Spectrometer (Lakewood, NJ). All measurements were made at 10ºC, in 50 mM phosphate buffer, with a protein

concentration of 0.4 mg/ml. A step size of 1 nm and an averaging time of 5 sec were used.