Figure S1. α-SNAP destabilizes SNARE LD. Related to Figure 1

(A) Measured (black) and idealized (colored) extension-time trajectories of a single SNARE complex under a constant mean force of 12.5 pN in different α-SNAP concentrations. The idealized extensions were derived from three-state hidden-Markov modeling comprising the free 4HB state (state 2 in red), the α-SNAP-bound 4HB state (2α in green), and the fully folded state (1 in cyan). States 2 and 2α have identical average extensions. The extension trajectories were mean-filtered to 1 kHz, analyzed by the HMM, and plotted.

(B) Probability density distributions of the extensions shown in A. The measured distributions (symbols) were fit by a sum of three Gaussian functions derived from HMM (solid lines). The three individual Gaussian functions corresponding to the extension trajectory in the presence of 5 μM α-SNAP were plotted in dashed lines with their associated states labeled.
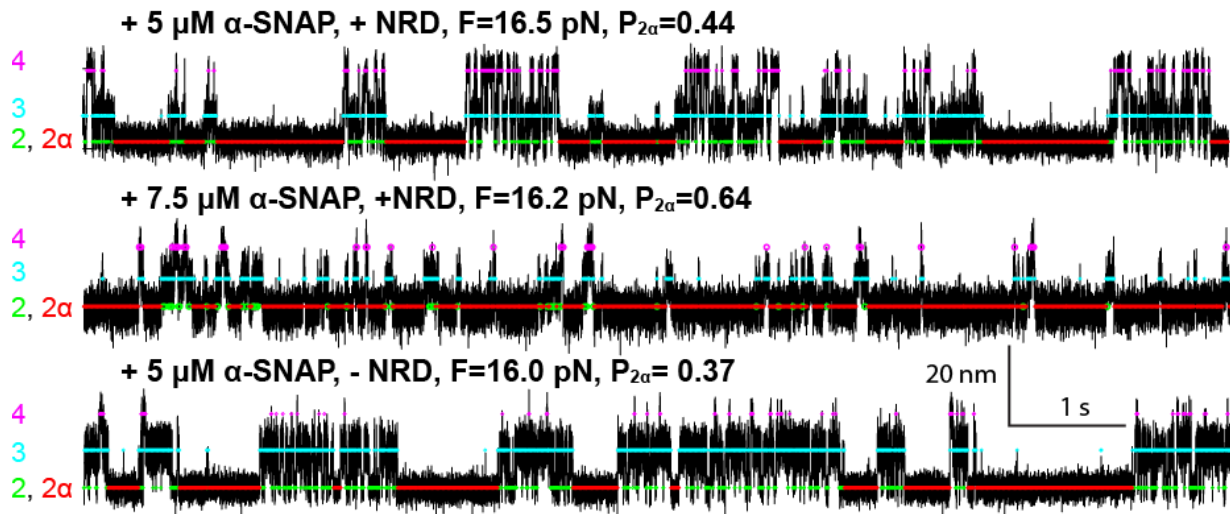
Figure S2. α-SNAP stabilizes the SNARE 4HB in an α-SNAP concentration-dependent and NRD-independent manner. Related to Figure 2

Measured (black lines) and idealized (colored circles) extension-time trajectories for SNARE complexes with (+ NRD) and without (- NRD) the N-terminal regulatory domain (NRD) in syntaxin. The average extensions of the different folding states derived from HMM are colored and numbered on the left as in Figure 1E: the free 4HB state 2 in green, the α-SNAP-bound 4HB state 2α in red, the partially-zippered state 3 in cyan, and the unzipped state 4 in magenta. The mean forces (F) and probabilities of state 2α ($P_{2\alpha}$) are indicated. Compared to the data in Figure 2A,B shown in 1 kHz, data shown here were mean-filtered to 5 kHz and plotted. Observed at a high bandwidth (>1 kHz), the partially-zippered state is further split into two sub-states with similar conformations (1). Since the lifetimes of these sub-states are much shorter than other SNARE states, we ignored the differences of these sub-states in this work to simplify our data analysis and collectively called them the partially zippered state 3.
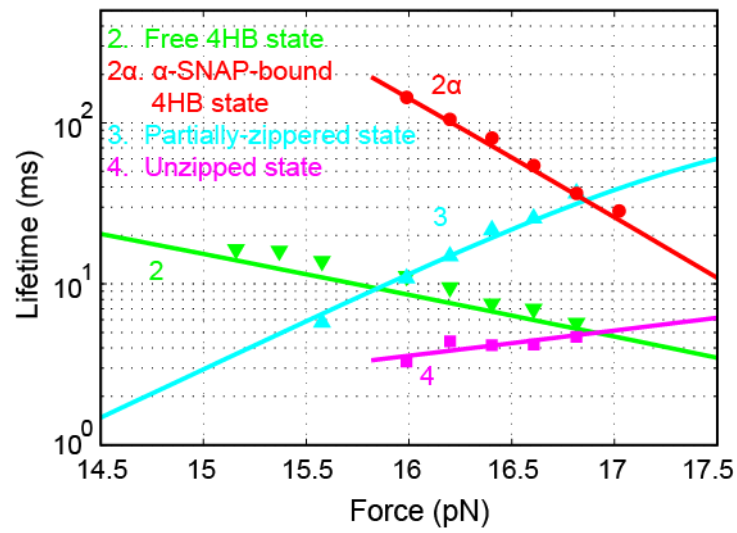
Figure S3. Force-Dependent Lifetimes of Different SNARE Folding States (symbols) and Their Best Model Fits (lines). Related to Figure 4

The lifetimes were calculated based on the transition rates shown in Figure 4C (see also 'Experimental Procedures').
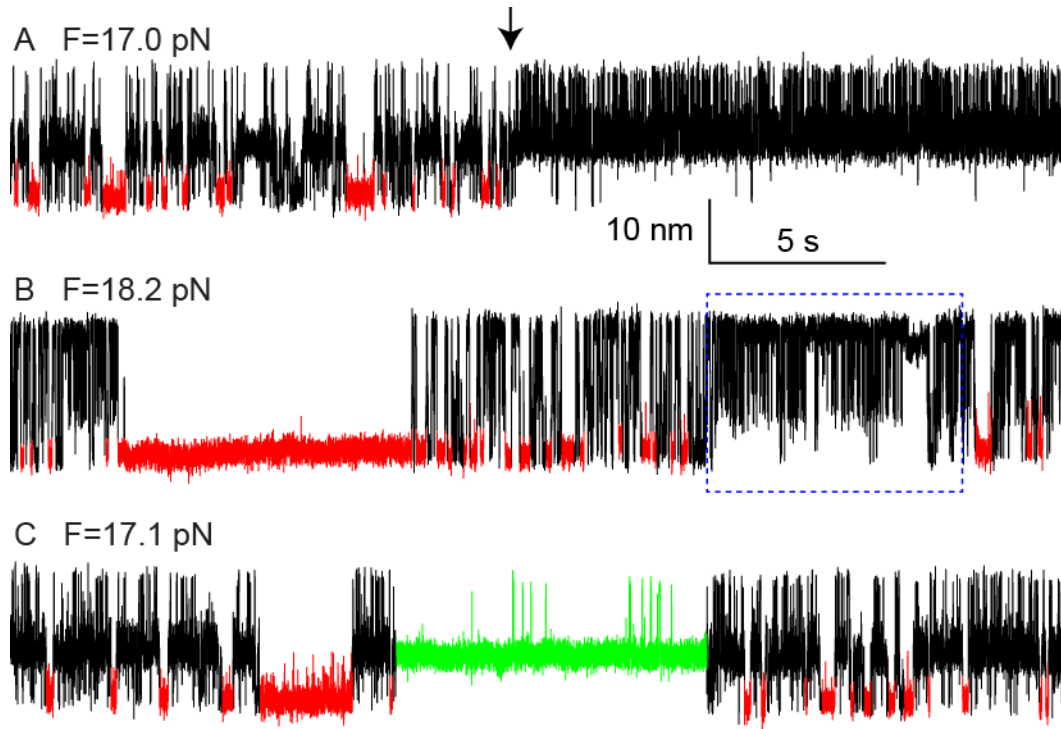
Figure S4. Extension-time trajectories showing kinetic heterogeneity of α-SNAP-SNARE binding. Related to Figure 5

The α-SNAP-bound 4HB and the partially zippered states are highlighted in red and green, respectively. Sudden kinetic switching is indicated by a black arrow in A and a dashed rectangle in B.

References

1. Ma L, Rebane AA, Yang G, Xi Z, Kang Y, Gao Y, Zhang YL (2016) Munc18-1-regulated stage-wise SNARE assembly underlying synaptic exocytosis. *eLIFE* 4:e09580.