



Figure 2 – figure supplement 1. Additional analysis of the interaction between ^2H , ^{15}N -labeled CpxI fragments and synaptobrevin-truncated SNARE complexes.

A. Chemical shift changes in the CpxI central helix of SNARE complex-bound ^2H , ^{15}N -CpxI(26-83) caused by truncation of synaptobrevin to residue 68, normalized by the changes caused by binding of ^2H , ^{15}N -CpxI(26-83) to the SC. The chemical shift changes were calculated as $\Delta\delta = [(\Delta\delta\text{HN})^2 + (0.17 \cdot \Delta\delta\text{N})^2]^{1/2}$, where $\Delta\delta\text{HN}$ and $\Delta\delta\text{N}$ are the differences in HN and N chemical shifts, respectively, between the spectra being compared. For $\Delta\delta\text{Cpx}(\text{SC}\Delta 68\text{-SC})$, we compared ^1H - ^{15}N TROSY-HSQC spectra of ^2H , ^{15}N -CpxI(26-83) bound to SC and bound to SC $\Delta 68$. For $\Delta\delta\text{Cpx}(\text{SC-free})$, we compared ^1H - ^{15}N TROSY-HSQC spectra of ^2H , ^{15}N -CpxI(26-83) free and bound to SC. **B.** Plot of $\Delta\delta\text{Cpx}(\text{SC}\Delta 68\text{-SC})$ versus $\Delta\delta\text{Cpx}(\text{SC-free})$. **C,D.** Ratio between the intensities of cross-peaks of ^1H - ^{15}N TROSY-HSQC spectra of ^2H , ^{15}N -CpxI(26-83) bound to SC $\Delta 68$ (**C**) or SC $\Delta 62$ (**D**) vs those observed for ^2H , ^{15}N -CpxI(26-83) bound to SC. To correct for small differences in protein concentrations, the cross-peaks intensities measured for each spectra were normalized with a correction factor derived by averaging the cross-peak intensities of the five C-terminal residues (residues 79-83), which were practically unaffected by the synaptobrevin C-terminal truncations. In all the plots shown in **A-D**, comparisons between chemical shifts or cross-peak intensities were made only for cross-peaks that could be identified in all the relevant spectra based on the assignments available for free and SNARE complex-bound Cpx(26-83) (Figures 2A,B) (Pabst et al., 2000; Chen et al., 2002) and the progressive movements caused by truncations in the SNARE complex (see Figure 2C).