



Figure 2 – figure supplement 2. NMR analysis of interactions between ^2H , ^{15}N -labeled CpxI superclamp mutant fragments and synaptobrevin-truncated SNARE complexes.

A. Expansions of ^1H - ^{15}N TROSY-HSQC spectra of D27L,E34F,R37A superclamp mutant (supcl) ^2H , ^{15}N -CpxI(26-48) in the absence (black contours) or presence (red contours) of SCA60. Because the red and black spectra are practically identical, the black spectrum was plotted at slightly lower levels to facilitate observation of the black cross-peaks behind the red ones. However, the intensities of all the cross-peaks were the same in the black and red spectra within experimental error, as illustrated by the one-dimensional traces shown above and on the right of the two-dimensional contour plots (taken at the chemical shifts indicated by the blue arrows). **B-E.** Expansions of ^1H - ^{15}N TROSY-HSQC spectra of WT ^2H , ^{15}N -CpxI(26-83) free (black contours) or bound to SCA60 (orange contours), and of D27L,E34F,R37A superclamp mutant ^2H , ^{15}N -CpxI(26-83) free (pink contours) or bound to SCA60 (blue contours). The minimal contour levels of the different spectra were adjusted to enable visualization of the weakest cross-peaks of interest; hence, cross-peak intensities are not comparable in general. However, the spectra of panel **D** were plotted at the same contour levels to allow direct comparison of cross-peak intensities for free and SCA60-bound ^2H , ^{15}N -CpxI(26-83)supcl. Selected well-resolved cross-peaks are labeled in the different panels. Note that no cross-peak from CpxI(26-83)supcl overlaps closely with the A30 and Q38 cross-peaks of WT Cpx(26-83) due to the mutations (**B,C**), and three new well-resolved cross-peaks are observed for CpxI(26-83)supcl (labeled N1-N3 in panels **B-D**). Cross-peaks N1-N3 must belong to the accessory helix where the three mutations were made and can be tentatively assigned to Q38, A30 and A37, respectively, based on their proximity to WT cross-peaks or the observed ^{15}N chemical shift (for A37). The overall changes caused by SCA60 binding are similar for WT and superclamp mutant CpxI(26-83) (**B,D,E**), including the effects on the intensities of the cross-peaks from the accessory helix.