



**Figure 1 – figure supplement 2. High B-factors in the accessory helix in the crystal structure of the CpxI(26-83) superclamp mutant bound to SCA60.** A. Electron density images of the crystal structure of CpxI(26-83) superclamp mutant bound to SCA60 (PDB accession code 3RK3; Kummel et al. 2011). Superimposed on the coordinates from 3RK3 is the map with coefficients  $2|mF(\text{obs}) - DF(\text{calc})|$ , contoured at the r.m.s.d. level of the map. The phases and map were calculated from the deposited 3RK3 structure factors in the program PHENIX (Afonine et al. 2010). Note that little electron density is observed for the side chains of the accessory helix, even for the hydrophobic residues in the interface with the SNAREs (the native L41 and the mutant L27, F34 and A37; upper panel). In contrast, clear electron density is observed for the side chains of the central helix that contact the SNARE complex (lower panel; some of these residues are labeled). B. Plot of average atomic displacement parameters (ADP or "B-factors") over the residues of the CpxI(26-83) superclamp molecule in 3RK3. The plot is color coded from blue as the minimum to red as the maximum value of the average B-factors. Note that the B-factors are much higher for the accessory helix than for the residues of the central CpxI helix that contact the truncated SNARE complex (residues 59 to 72). Reference: Afonine, P.V., Mustyakimov, M., Grosse-Kunstleve, R.W., Moriarty, N.W., Langan, P., and Adams, P.D. (2010). Joint X-ray and neutron refinement with phenix.refine. *Acta Crystallogr. D. Biol. Crystallogr.* 66, 1153-1163.