

## Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: Fitting of the separate C2A (magenta), C2B (grey) and SNARE (multi-color) derived from the Syt1-SNARE crystal structure (PDB code: 5CCI) onto the cryo-EM map shows that the Syt1 C2B domain concurrently binds both the SNARE proteins and the lipid membranes (yellow) on diametrically opposite surfaces under resting (1mM Mg<sup>2+</sup>-conditions).