Supporting Information

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Fig. S1. Nonbinary mask used for subtomogram classification. The density of the mask extends between 0 and 1. The soft transition between the inside and the outside of the mask was achieved by a Gaussian falloff. The mask is represented here as an isosurface at three different thresholds: 0.9 (A1 and A2), 0.5 (B1 and B2), and 0.1(C1 and C2) and the spherically masked average is represented as a mesh contour with docked acetylcholine receptor ribbon models. Both side views (A1–C1) and bottom view (A2–C2) are shown.



Fig. S2. Rapsyn gold immunolabeling. (*A*) Slice cross-sectioning the extracellular domain of several receptors in a tomogram of postsynaptic membranes immunogold labeled for rapsyn. (*B*) Slice with the same *xy* coordinates taken at the level of the cytoplasmic tip of the receptors. Several gold beads are visible. (*C*) The position of each gold bead relative to the nearest receptor is represented by a turquoise dot. A histogram of the position of the gold beads along the *z* axis is represented in gray; 90% of the beads were confined to a 24-nm-thick *z* slice, and 80% were confined to a 14-nm-thick *z* slice. (Scale bars: 50 nm in *A* and *B*; 10 nm in C.)



Fig. S3. Fourier shell correlation plot of the 5 class averages shown in Fig. 2. Two commonly used resolution criteria are indicated (Fourier shell correlation threshold 0.5 and 0.143).