Supporting Information

Brannigan *et al.* 10.1073/pnas.0803029105

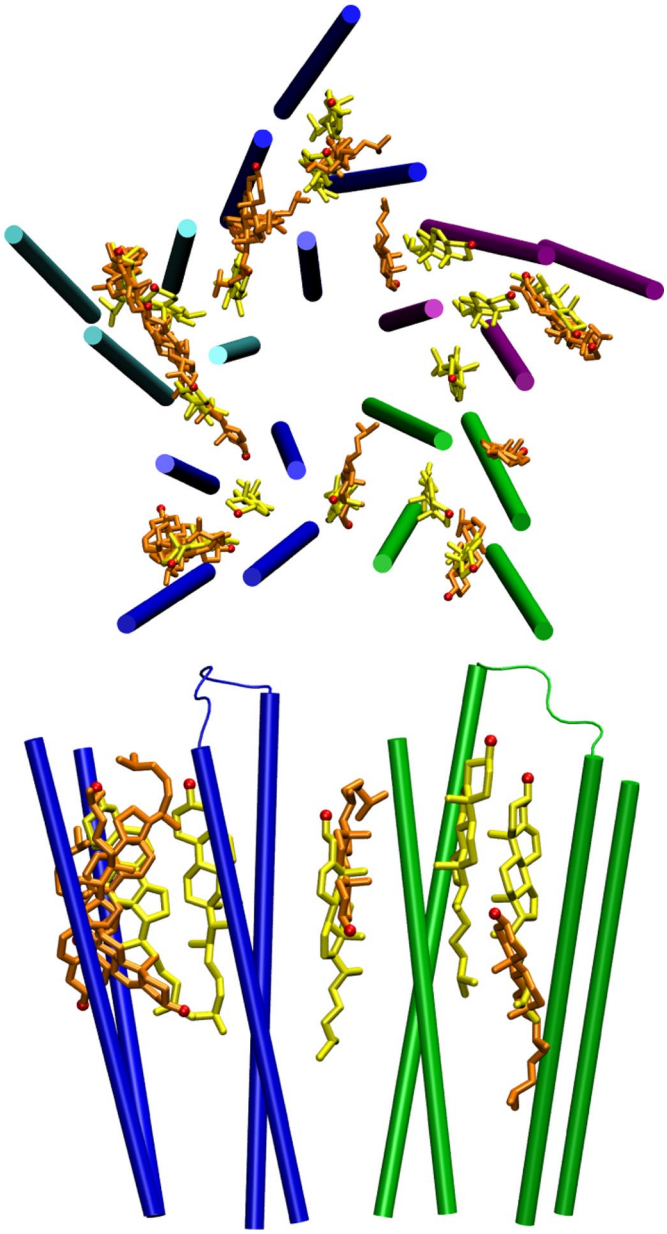

Fig. S1. Predicted binding sites of cholesterol in the acetylcholine receptor transmembrane domain. Global view along the pore axis (*Upper*) and detail of subunits δ and α_δ (*Lower*) viewed from outside the pentameric bundle, within the membrane plane. TM helices are shown as tubes colored by subunit type (α: blue, β: purple, δ: green, γ: cyan). Possible cholesterol sites predicted by Autodock are colored orange, and those resulting from manual docking are yellow, with all hydroxyl oxygen atoms shown as red spheres. Autodock-predicted sites that lie in the channel pore, the vestibule domain, or the hydrophilic interface between the TM and binding domains are not shown.

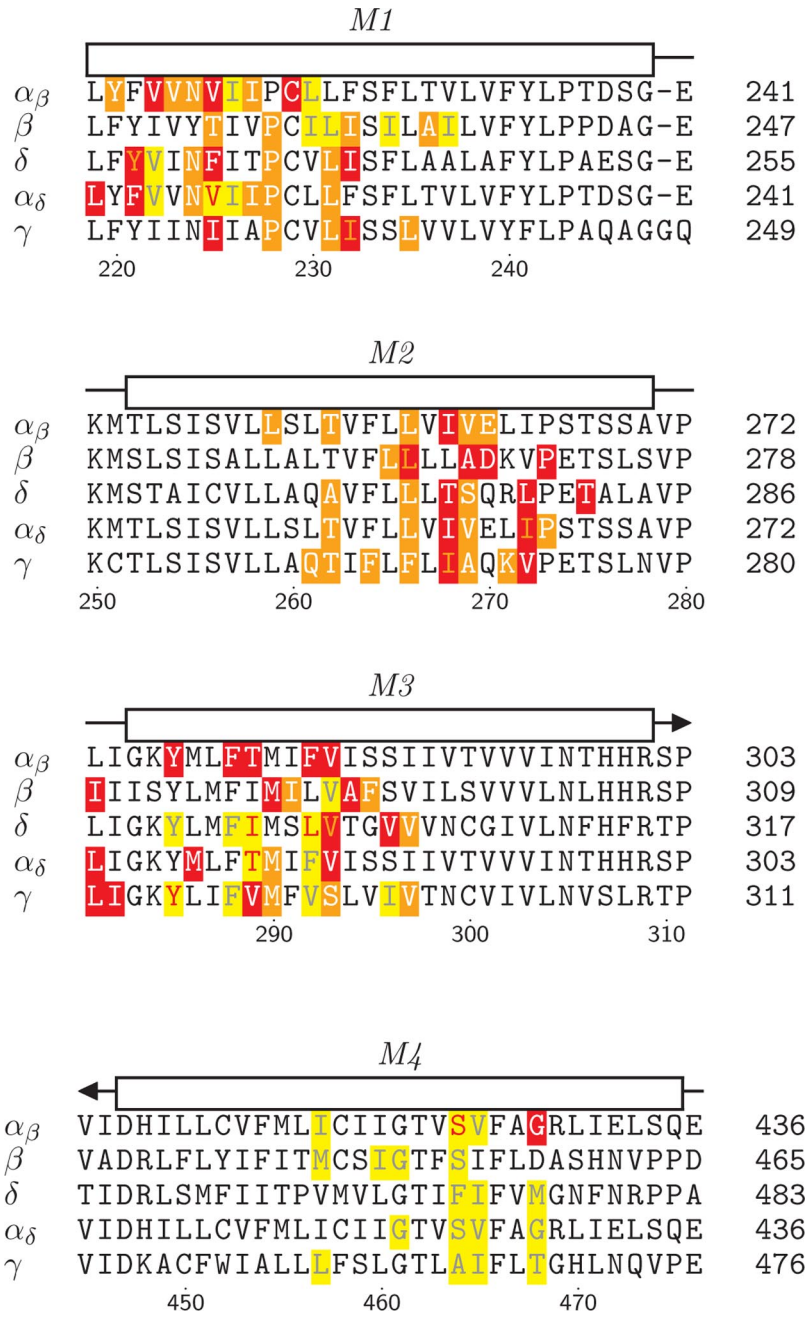

Fig. S2. Residues in TM helices contacting cholesterol in A sites (yellow shading, gray text), B sites (orange shading, white text), C sites (red shading, white text), both A and C sites (yellow shading, red text), and both B and C sites (red shading, orange text). Contact residues were defined as those for which the distance of closest approach between the residue and a given cholesterol molecule was less than 2.5 Å for at least 55% of the trajectory (not including the first 10 ns of restrained dynamics). The contact residues did not change significantly when the trajectory was broken into 5-ns blocks, but did exhibit substantial oscillations over much shorter time scales. Over 100 residues between the M3 and M4 helices are not shown.

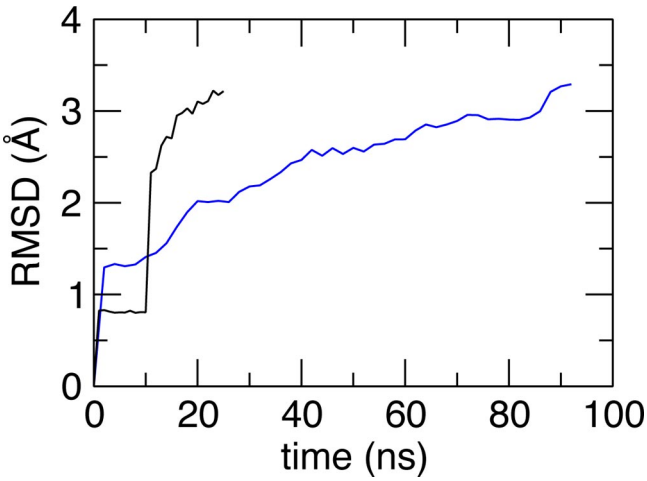

Fig. S3. RMSD of Cα atoms in the TM domain of two control simulations differing only by their equilibration protocol and random seed. The fast-equilibration protocol (black) applied restraints to all Cα atoms for 10 ns, and the slow-equilibration protocol (blue) slowly released restraints over Cα atoms in the noninterfacial loops, then the M1 and M3 helices and the β-sheets of the agonist-binding domain, then M2 helices, then the interfacial loops of the agonist-binding and TM domains, and finally, the M4 helices. In the fast-equilibration protocol, restraints were lifted from 1 kcal/mol/Å^2 instantaneously, whereas in the slow-equilibration protocol releasing of restraints occurred at a rate of 0.1 kcal/mol/Å^2/ns. Both trajectories contain ≈15 ns of unrestrained (except for the vestibule domain) dynamics at the end of the simulations.

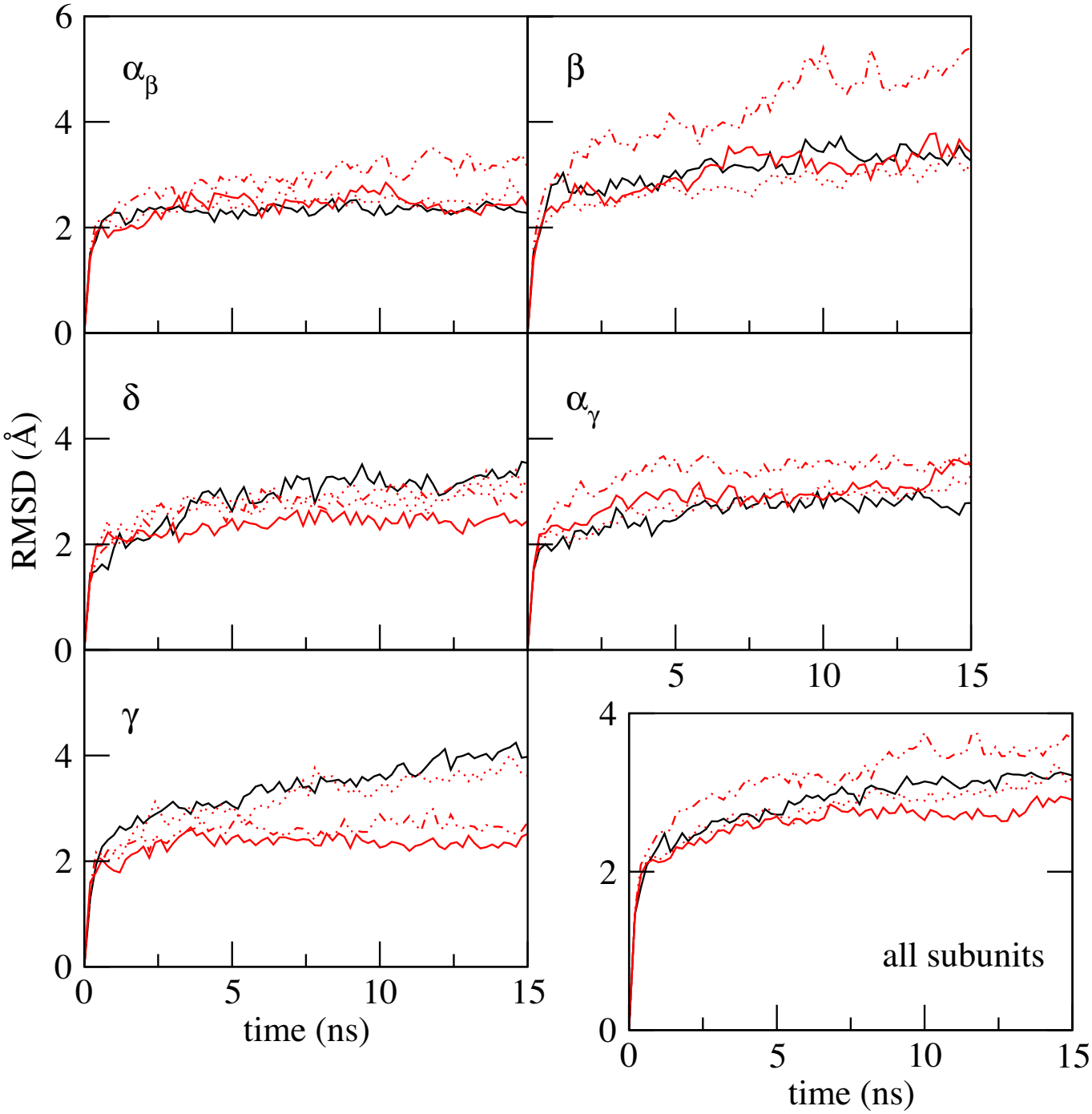

Fig. S4. RMSD of Cα in the TM domain atoms over time, calculated when protein is aligned to minimize deviations between TM domain helices and the starting structure, for systems with no cholesterol (black), five cholesterol molecules in A sites (red dot), 10 cholesterol molecules in A and B sites (red dash-dot), and 15 cholesterol molecules in A, B, and C sites (red solid). Division into subunits represents contribution of each subunit to overall RMSD. Ten nanoseconds of simulation in which the Cα atoms were harmonically restrained to the starting structure are not shown.

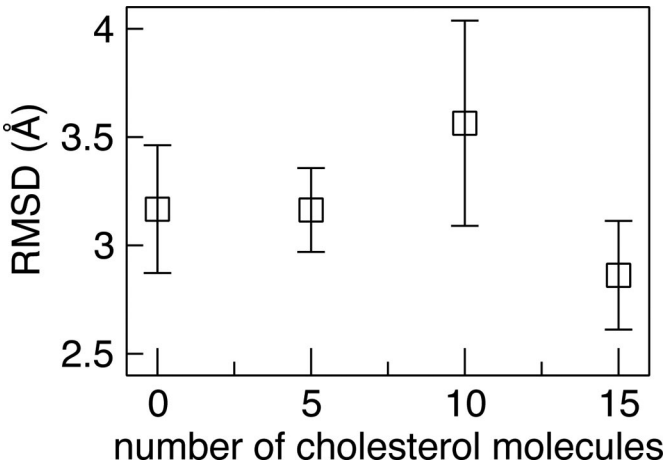

Fig. S5. RMSD of TM Cα atoms at the end of 25 ns of simulation (15 ns unrestrained), for different cholesterol treatments, aligned as in Fig. S4. Error bars reflect the distribution of the final frame across subunits rather than RMSD time evolution. Full occupancy by 15 cholesterol molecules is most effective at reducing RMSD, but taken alone, the effect is not statistically significant. Other measures yield more insight into the direct effect of bound cholesterol on nAChR dynamics and stability, indicating that cholesterol reduces drift in areas of the protein deemed fundamental for function (such as interfaces) but increases fluctuations in some other regions.

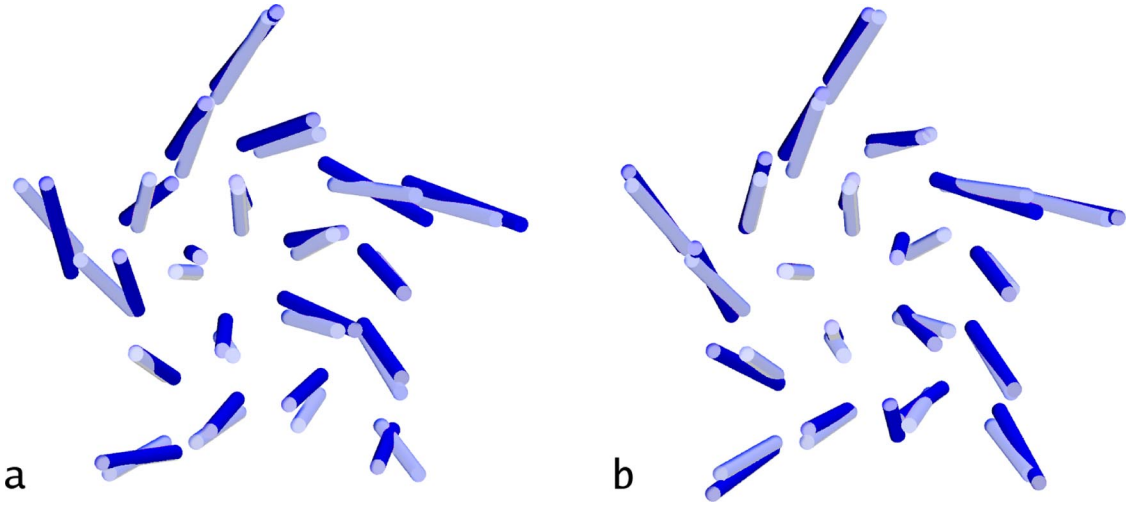

Fig. S6. Comparison between starting structure and structure after 15 ns of unrestrained dynamics (25 ns total) for TM helices in a system without cholesterol (*A*) and a system with cholesterol bound in A, B, and C sites (*B*). For each helix, the starting position (light blue) and ending position (blue) are shown; helices interrupted by a kink appear as two cylinders. The system without cholesterol displays a significantly larger deviation from the starting structure, as indicated by differences in both position and orientation of corresponding helices.

Other Supporting Information Files

Dataset S1 (PDB)