Supporting information for

Determinants of Orexin Receptor Binding and Activation - a Molecular Dynamics Study

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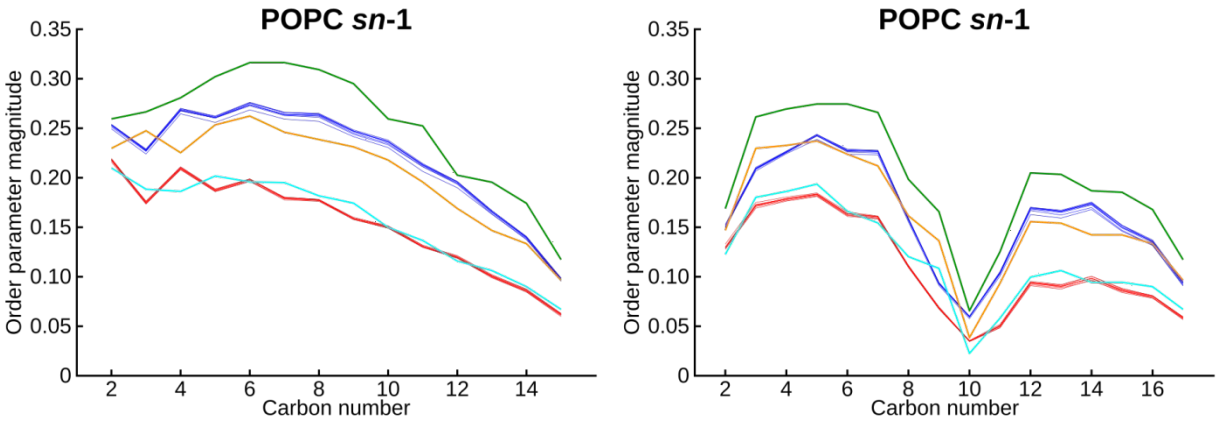


Figure S1. Order parameters for the membranes at the end of simulation. Red: simulations with the pure POPC-membrane. Blue: simulations with the 25% cholesterol membrane. Green, orange and cyan: experimental values for membranes of pure POPC, POPC-Chol (15%) and POPC-Chol (34%) respectively (Ferreira, T. M.; Coreta-Gomes, F.; Ollila, O. H. S.; Moreno, M. J.; Vaz, W. L. C.; Topgaard, D. Cholesterol and POPC Segmental Order Parameters in Lipid Membranes: Solid State ^{1}H-^{13}C NMR and MD Simulation Studies. *Phys. Chem. Chem. Phys.* **2013**, *15* (6), 1976–1989). There are eight simulations with the pure POPC-membrane and eight with the 25% cholesterol membrane. All simulations are plotted individually, but the values are that close that the curves mostly overlap.

Table S1. Average area per lipid and membrane thickness at the end of simulation.

Simulation	Ligand	Membrane	Length (µs)	POPC (Å^2)	Cholesterol (Å^2)	Thickness (Å)
1	TM5	POPC	3	61.0	–	37.3
2	TM5	POPC	1	61.4	–	36.4
3	TM5	CHOL	3	50.4	37.5	41.3
4	TM5	CHOL	1	52.0	36.6	41.1
5	TM7	POPC	3	59.8	–	37.3
6	TM7	POPC	1	60.3	–	36.9
7	TM7	CHOL	3	51.7	38.2	40.9
8	TM7	CHOL	1	51.4	39.1	41.1
9	Suvorexant	POPC	3	60.5	–	37.1
10	Suvorexant	CHOL	3	50.4	36.3	41.9
11	Nag26	POPC	3	60.4	–	36.9
12	Nag26	POPC	1	62.1	–	36.7
13	Nag26	CHOL	3	50.8	36.9	41.9
14	Nag26	CHOL	1	50.8	36.7	42.1
15	None (Apo)	POPC	3	62.1	–	36.2
16	None (Apo)	CHOL	3	51.7	38.4	40.5

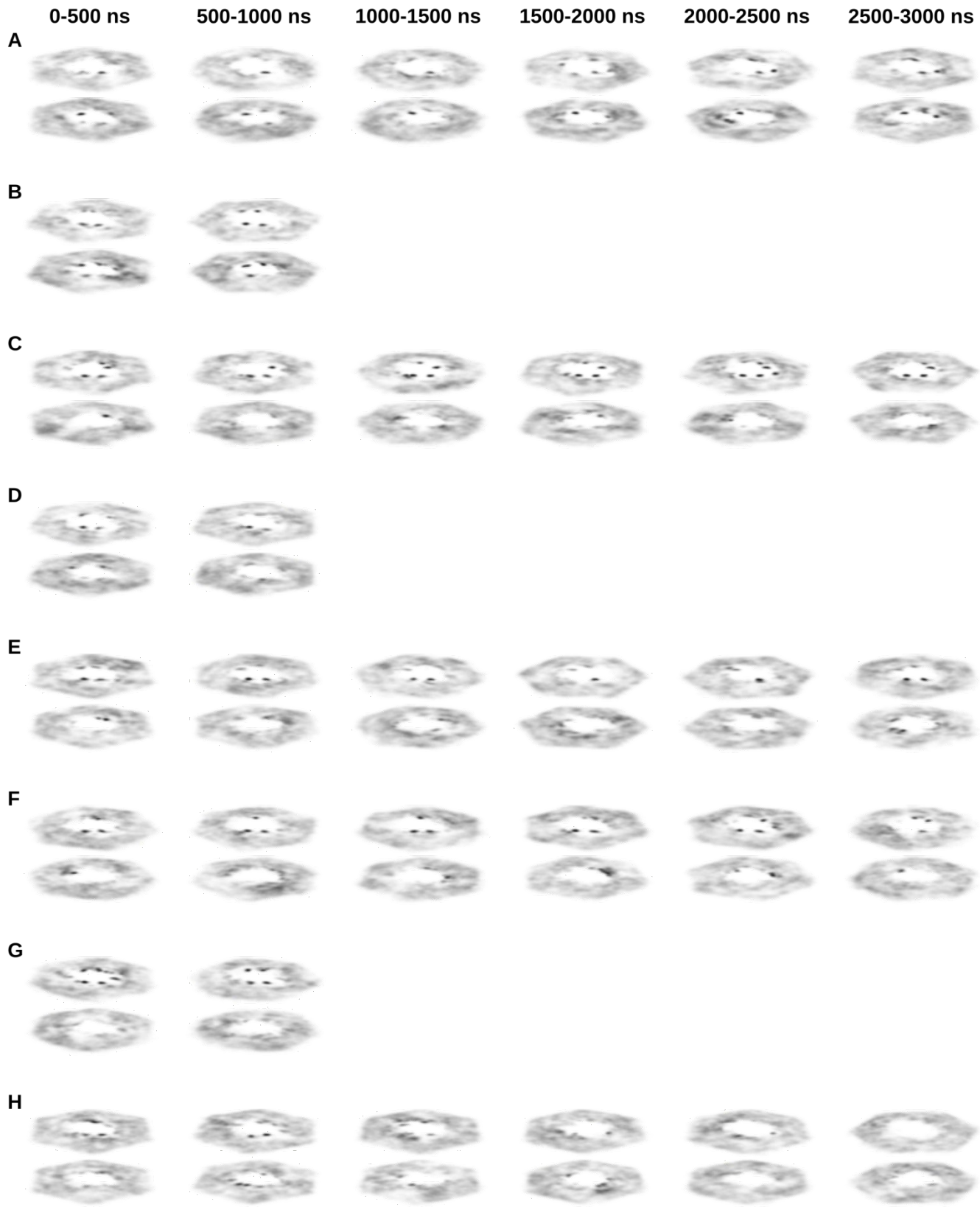


Figure S2. Evolution of cholesterol density around the receptor. **A–B)** TM5-binding mode, simulations **3–4**; **C–D)** TM7-binding mode, sim. **7–8**; **E)** Suvorexant, sim. **10**; **F–G)** Nag26, sim. **13–14**; **H)** Apo, sim. **16**. The color ranges from white (0 nm^{-3}) to black (≥ 10 nm^{-3}). The upper panels show the extracellular leaflet and the lower panels the intracellular leaflet. The viewpoint is above the extracellular membrane, with the receptor transmembrane helices TM6–7 facing the viewer, and the two most frequent locations in the extracellular leaflet flanking the TM7. Analysis produced with the Gromacs tool *densmap* with grid size of 1 Å.

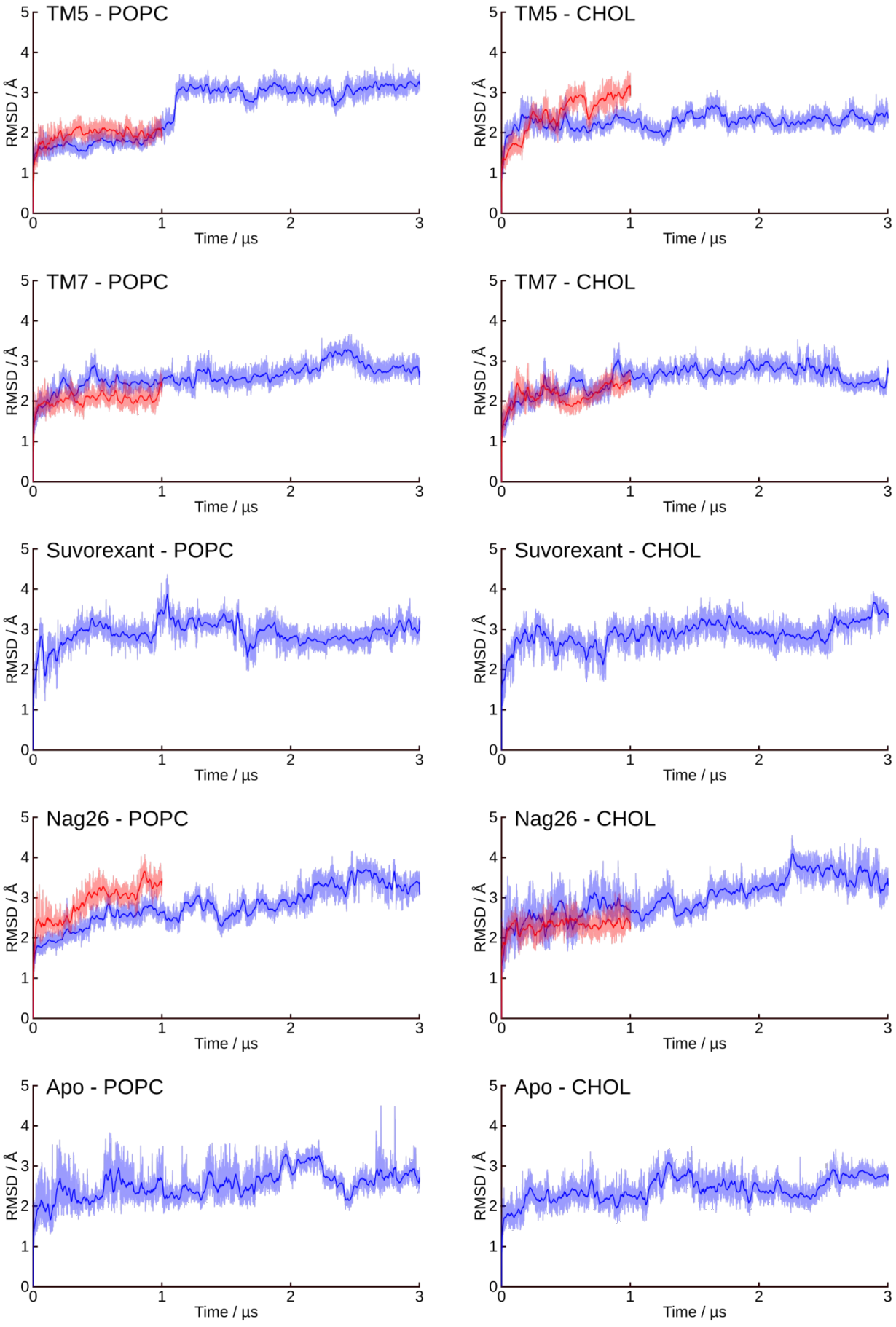


Figure S3. Receptor Cα-RMSD for all simulations. All data points shown in the transparent color, and a running average of 1000 data points in opaque color.

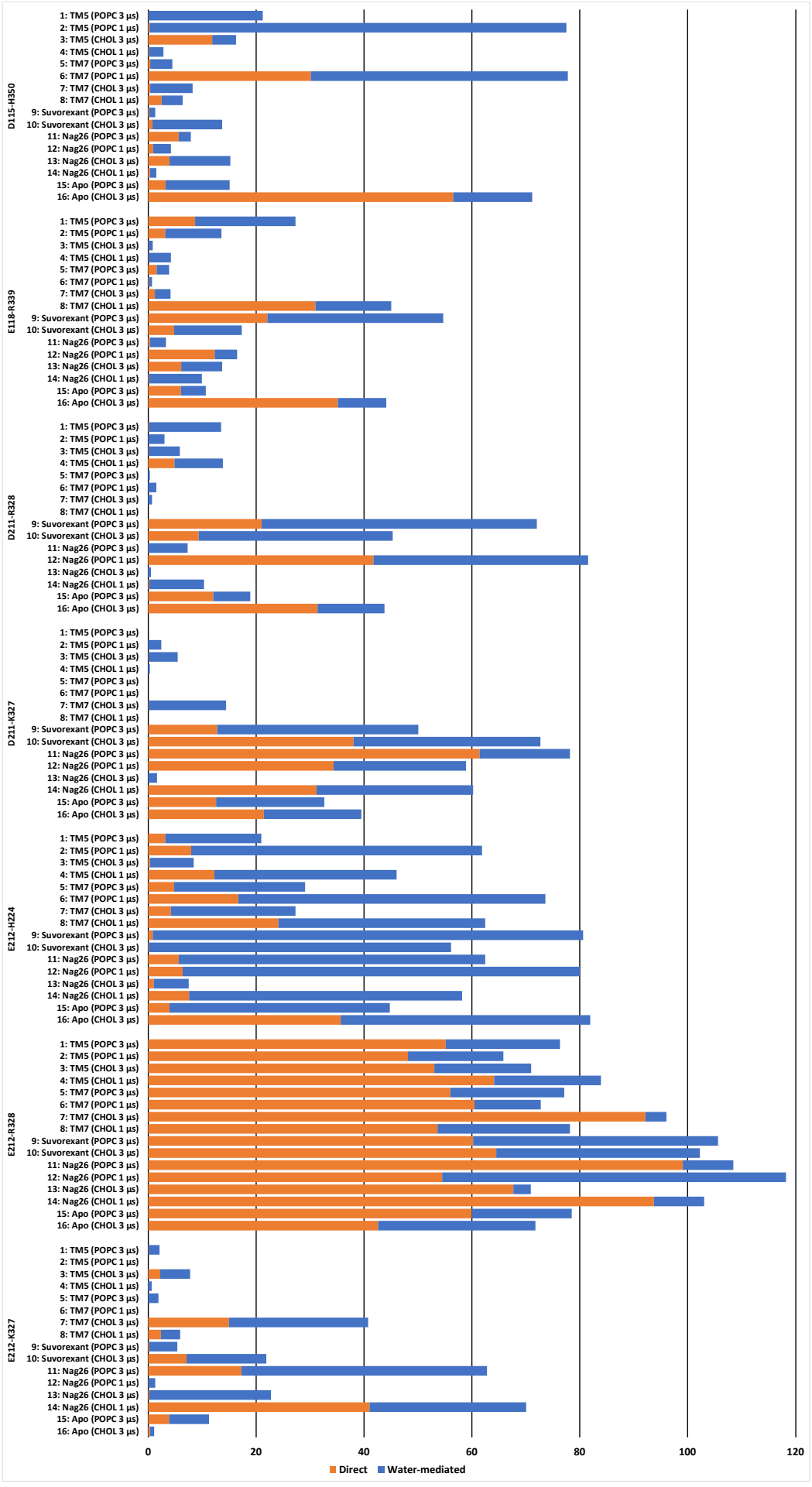


Figure S4. Frequency of intra-protein salt bridges near the ligand binding site during individual simulations. Direct salt-bridge interactions in orange, water-mediated interactions in blue.

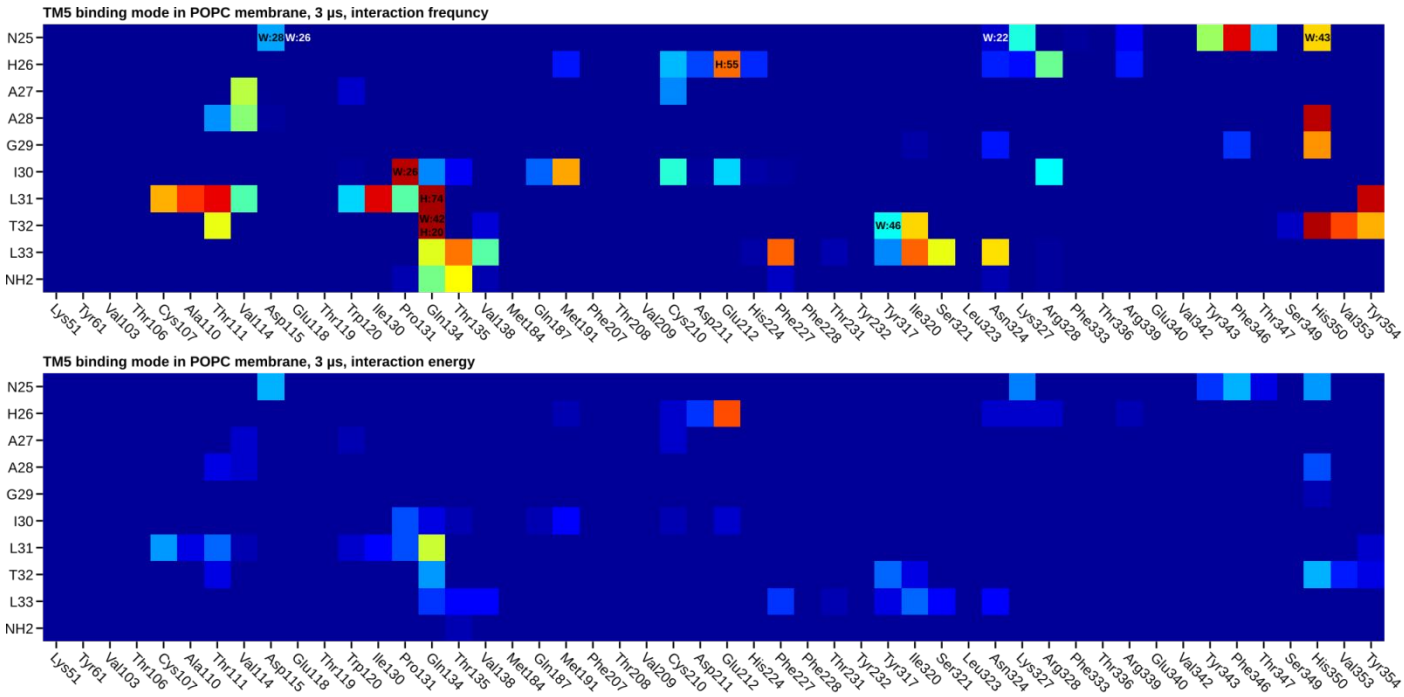


Figure S5. Interaction heatmaps for the simulation **1** (TM5-mode in POPC membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.

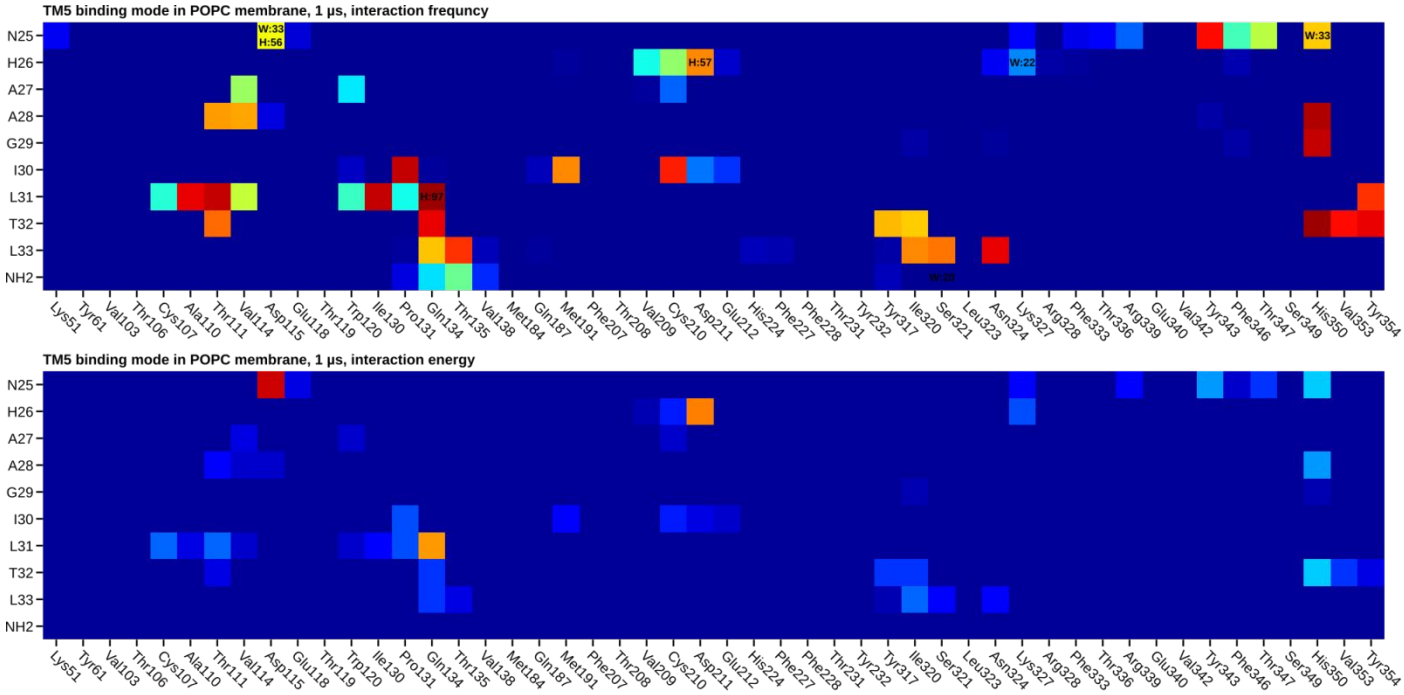
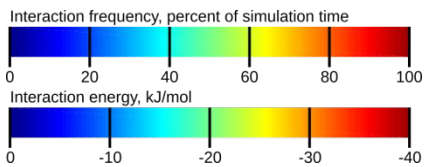


Figure S6. Interaction heatmaps for the simulation **2** (TM5-mode in POPC membrane, 1 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.



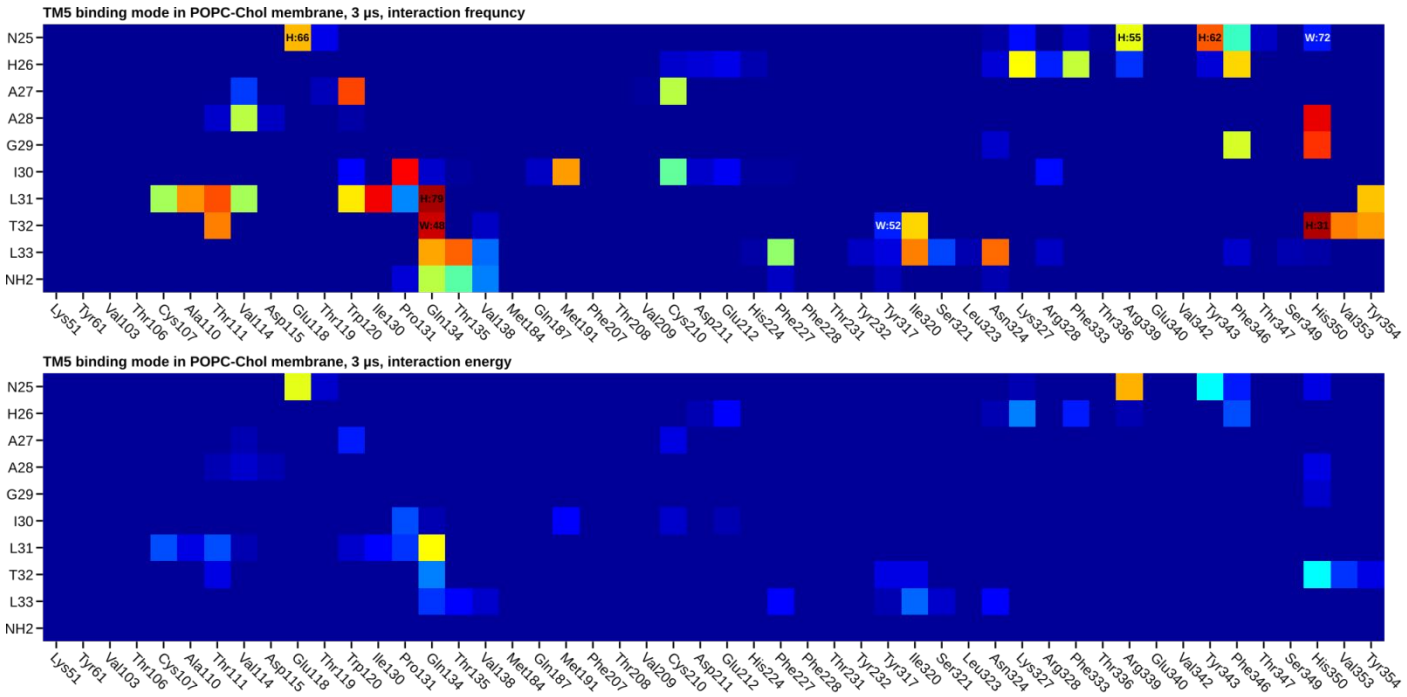


Figure S7. Interaction heatmaps for the simulation **3** (TM5-mode in POPC-cholesterol membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.

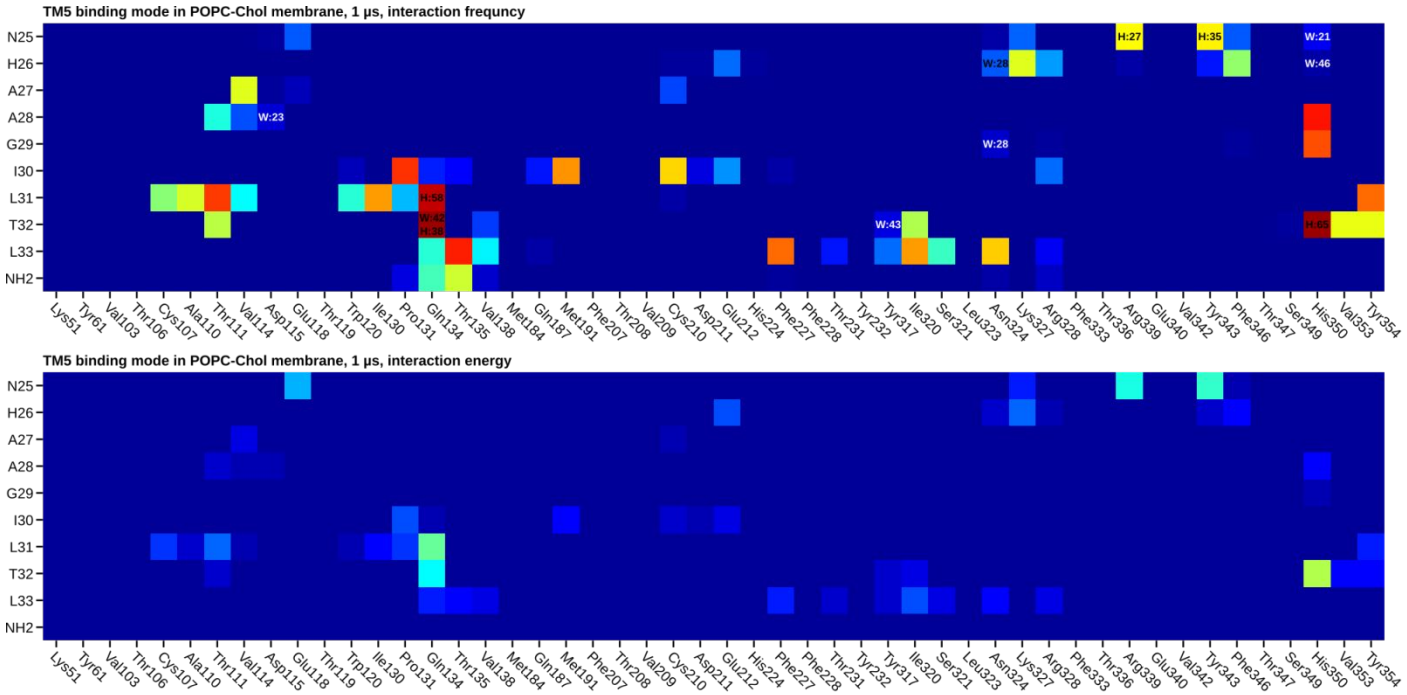
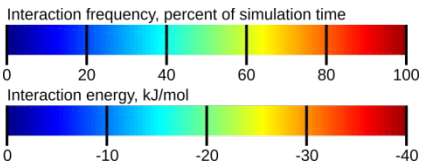


Figure S8. Interaction heatmaps for the simulation **4** (TM5-mode in POPC-cholesterol membrane, 1 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.



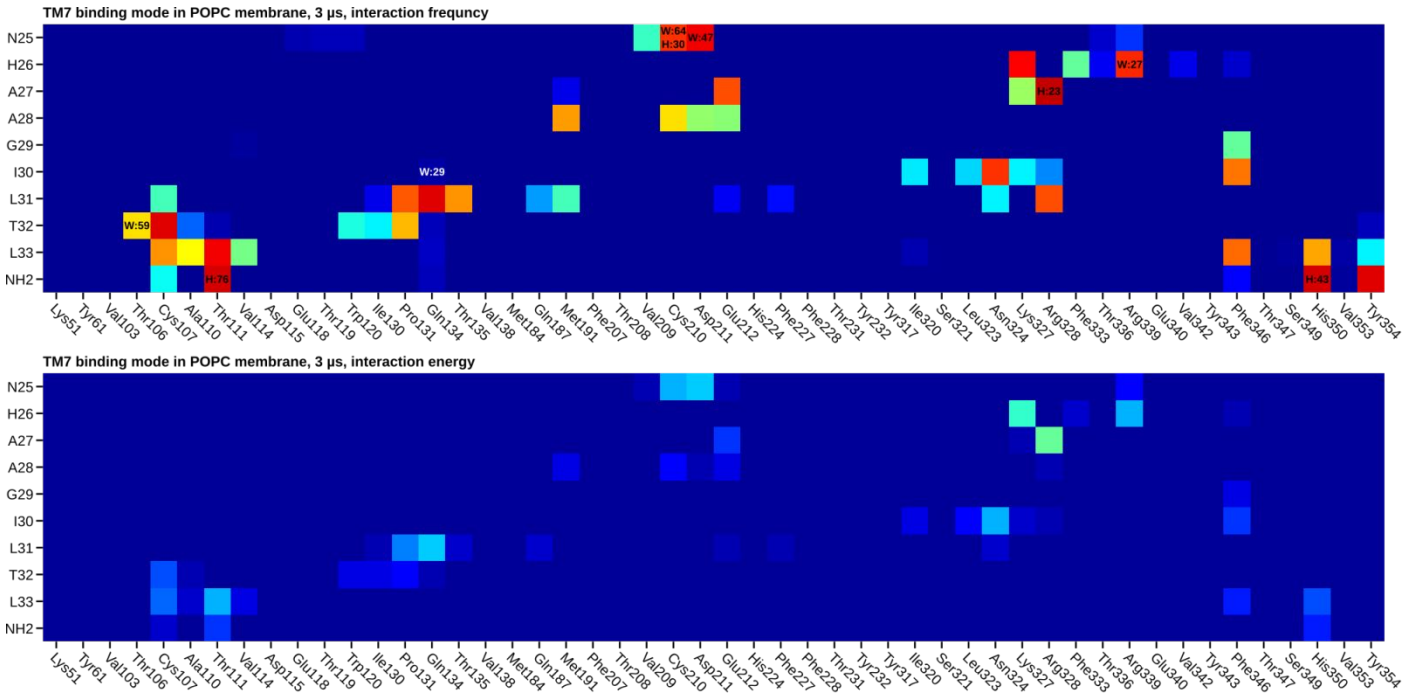


Figure S9. Interaction heatmaps for the simulation **5** (TM7-mode in POPC membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.

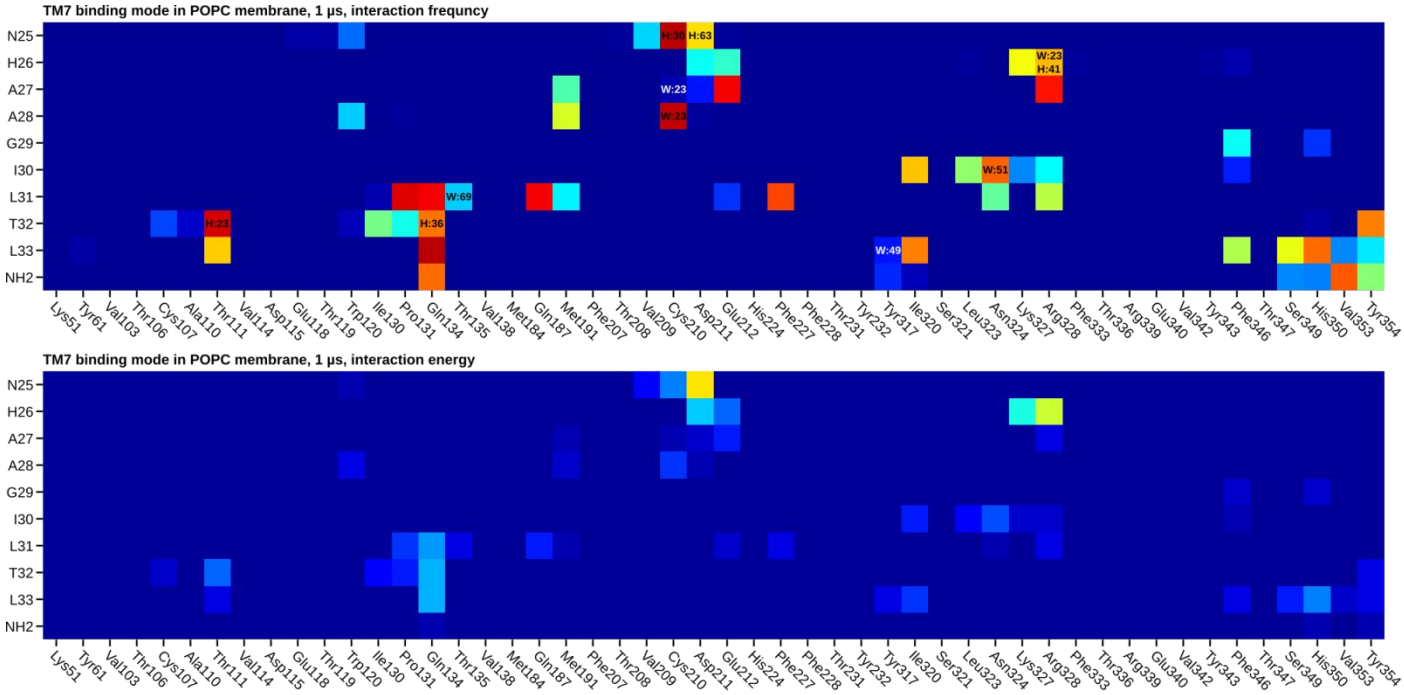
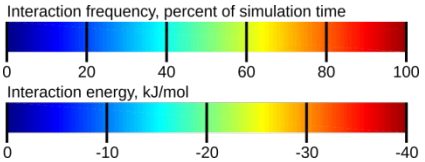


Figure S10. Interaction heatmaps for the simulation **6** (TM7-mode in POPC membrane, 1 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.



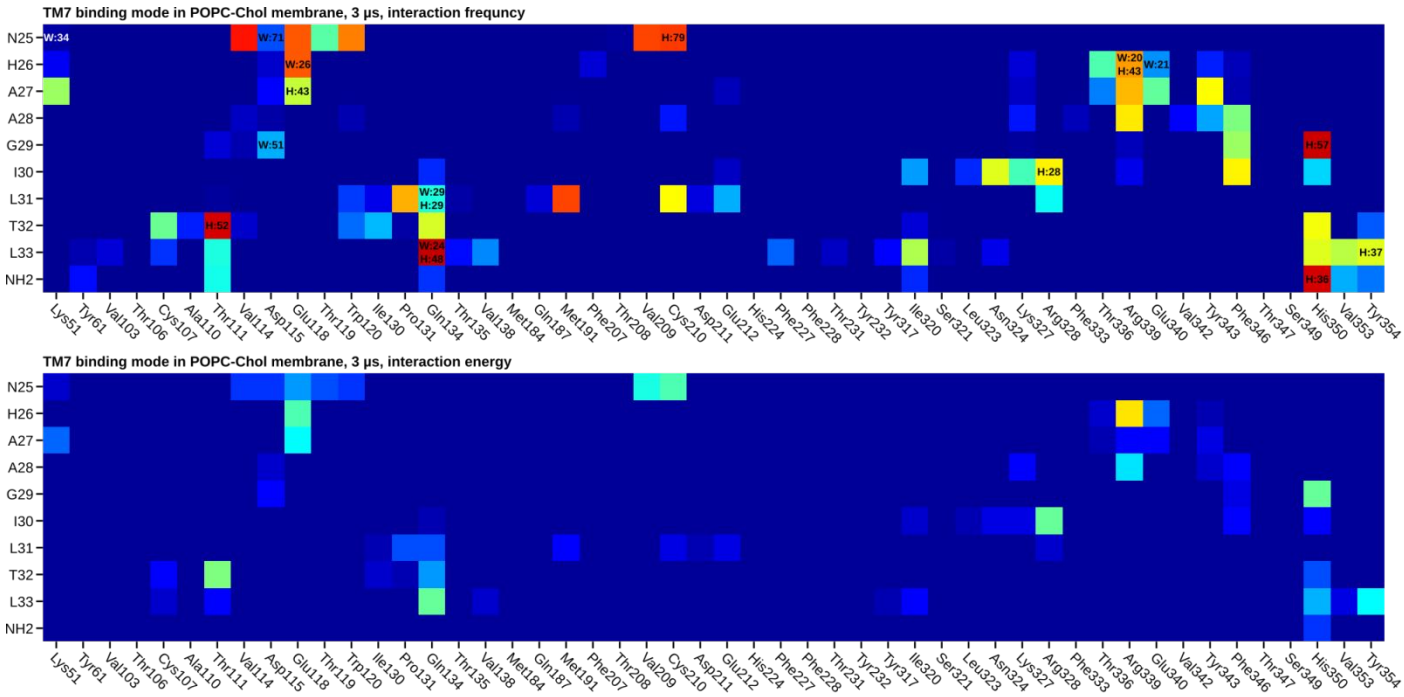


Figure S11. Interaction heatmaps for the simulation **7** (TM7-mode in POPC-cholesterol membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.

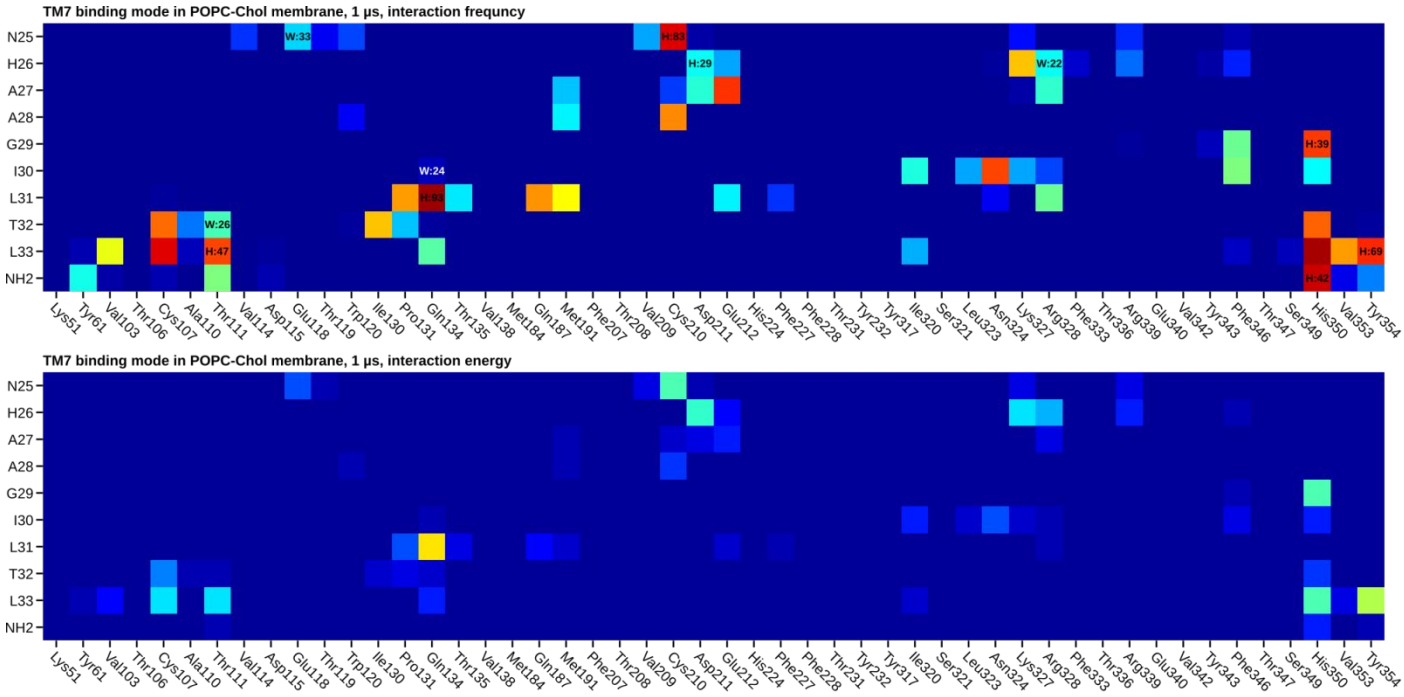
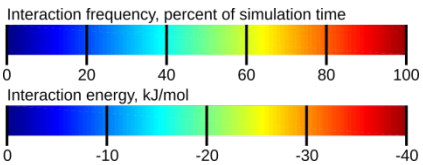


Figure S12. Interaction heatmaps for the simulation **8** (TM7-mode in POPC-cholesterol membrane, 1 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant residues. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales.



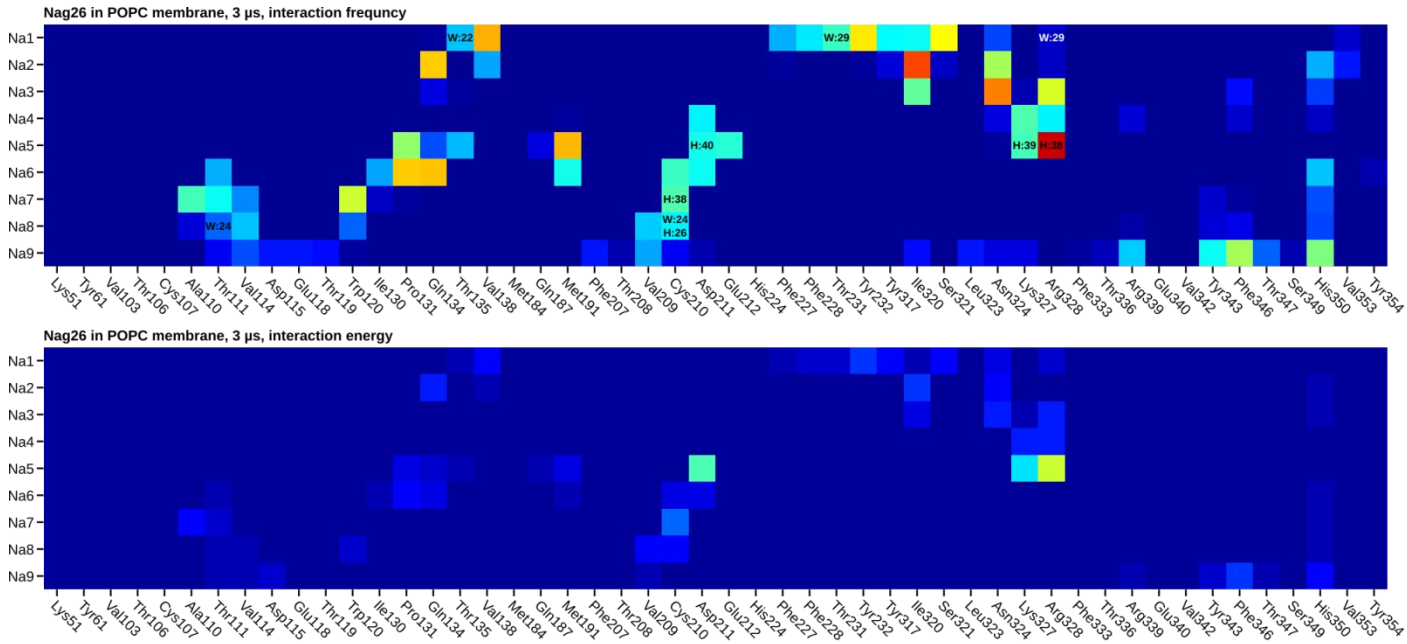


Figure S13. Interaction heatmaps for the simulation **11** (Nag26 agonist in POPC membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant receptor residue and ligand group. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales and ligand groups.

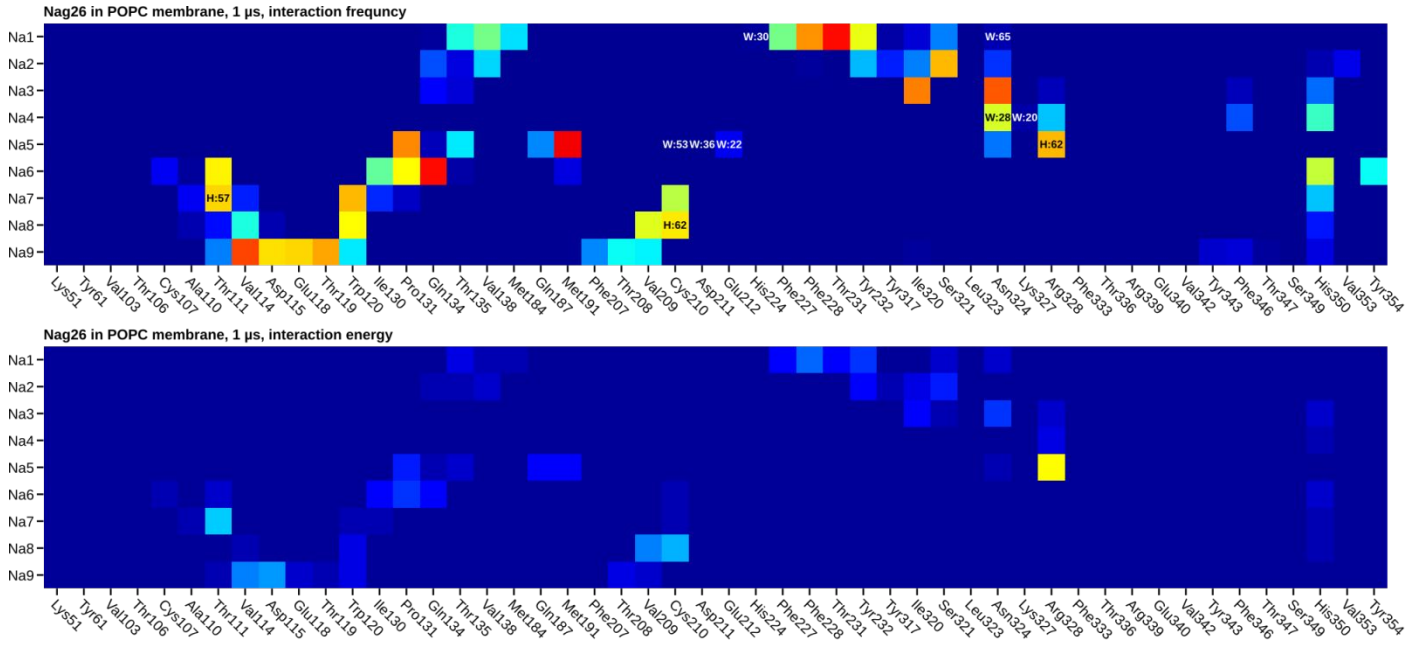
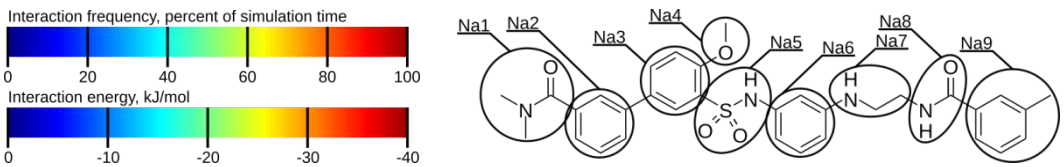


Figure S14. Interaction heatmaps for the simulation **12** (Nag26 agonist in POPC membrane, 1 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant receptor residue and ligand group. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales and ligand groups.



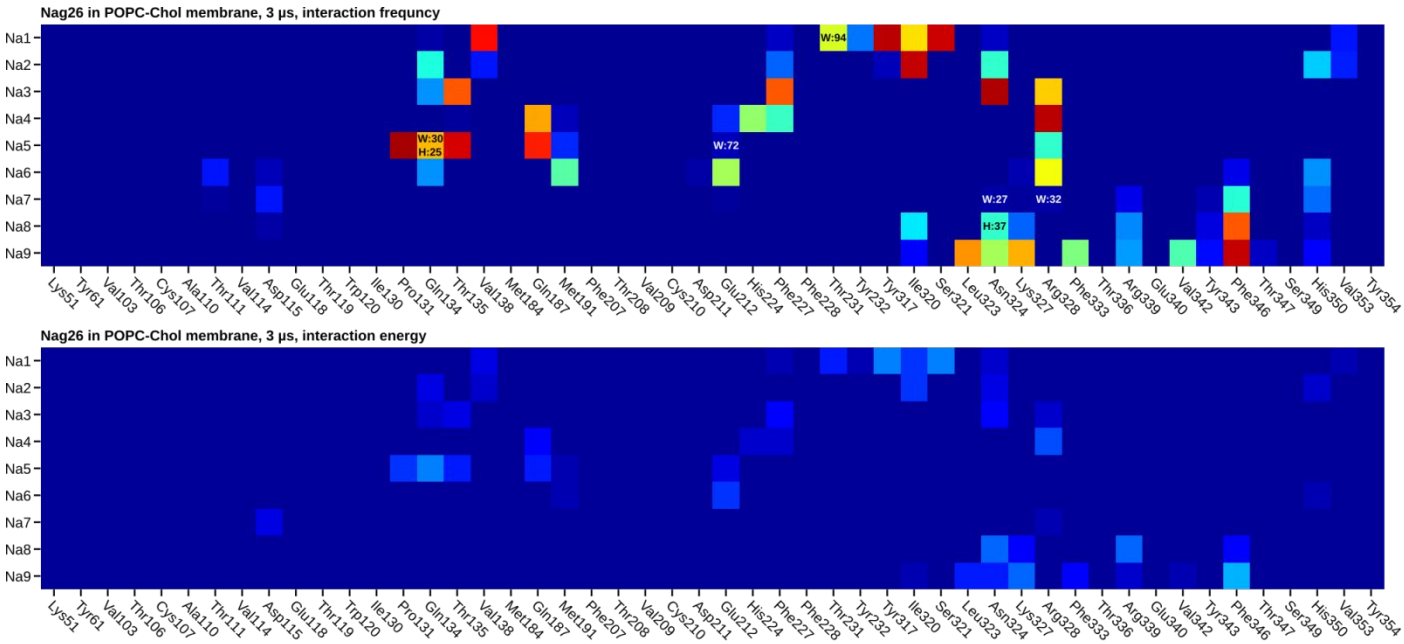


Figure S15. Interaction heatmaps for the simulation **13** (Nag26 agonist in POPC-cholesterol membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant receptor residue and ligand group. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales and ligand groups.

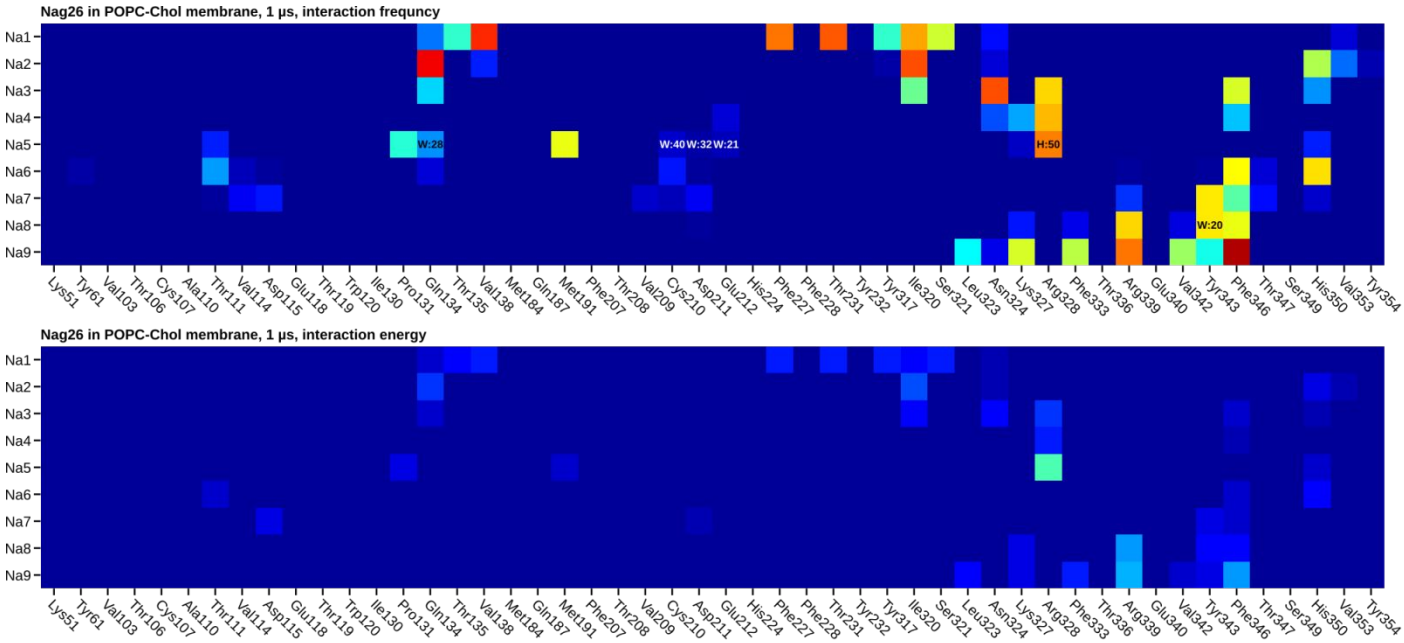
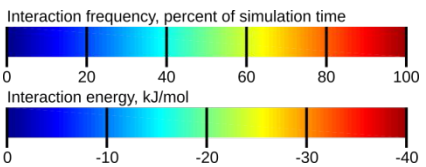
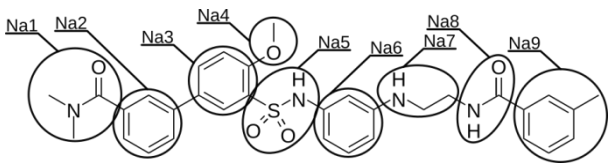


Figure S16. Interaction heatmaps for the simulation **14** (Nag26 agonist in POPC-cholesterol membrane, 1 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant receptor residue and ligand group. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales and ligand groups.





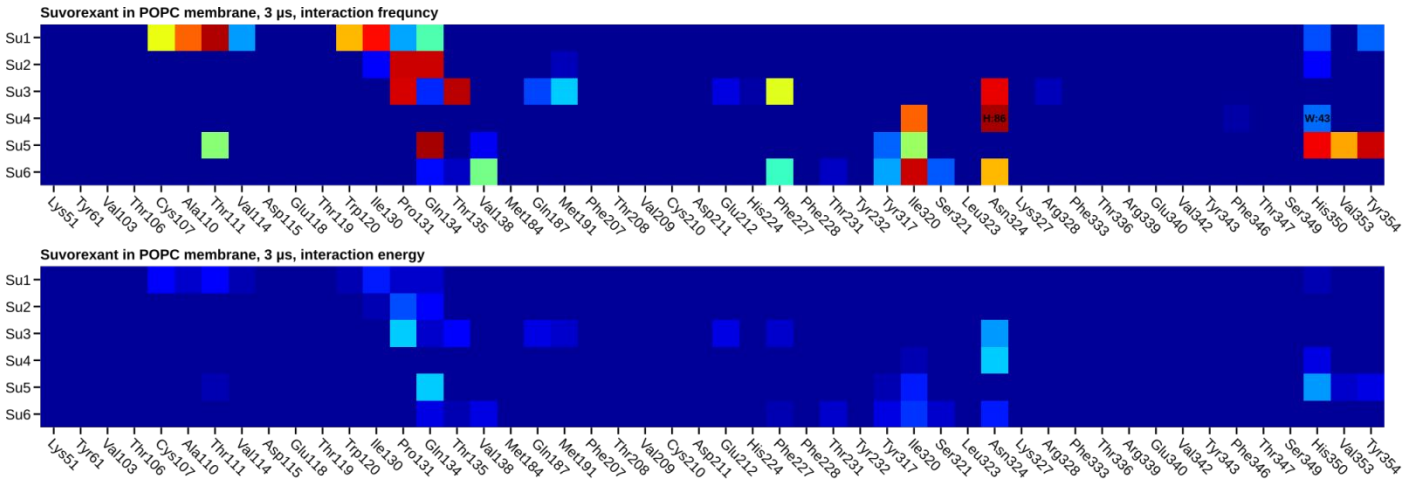


Figure S17. Interaction heatmaps for the simulation **9** (the antagonist suvorexant in POPC membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant receptor residue and ligand group. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales and ligand groups.

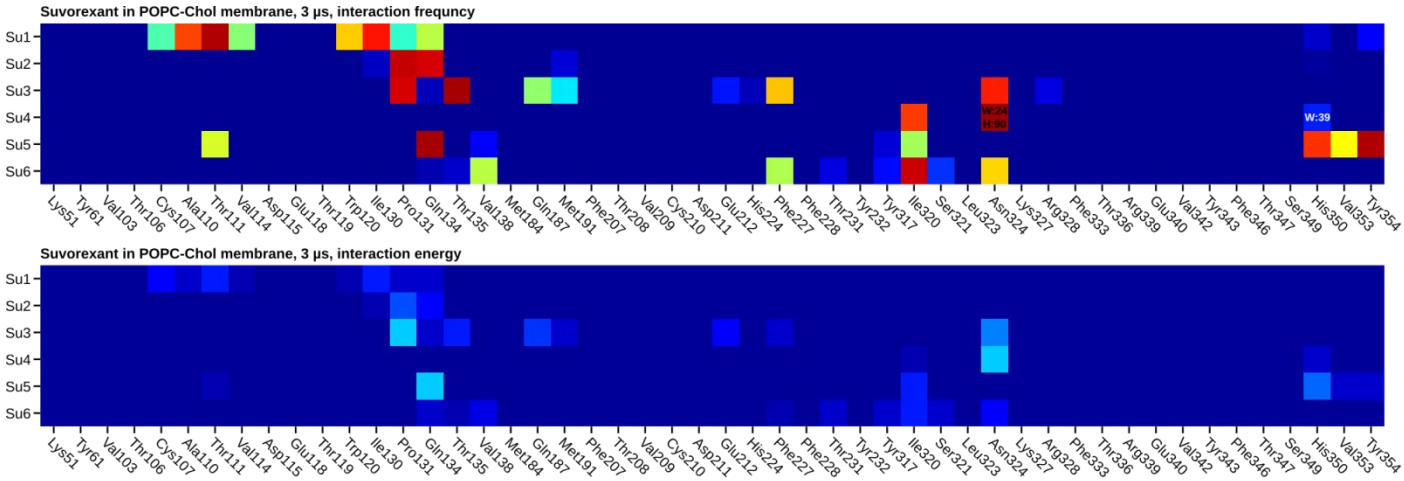


Figure S18. Interaction heatmaps for the simulation **10** (the antagonist suvorexant in POPC-cholesterol membrane, 3 µs). The upper map shows the frequency of any pairwise heavy-atom distance of <4Å between the relevant receptor residue and ligand group. H and W denote the frequencies of direct and water-mediated hydrogen bonds, respectively (shown only if above 20%). The lower map shows the average pairwise interaction energy. See bottom of page for color scales and ligand groups.