Supporting Information for Comprehensive characterization of lipid-guided G protein-coupled receptor dimerization

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## Additional information on main dimer interfaces

### NTS1 in POPC

<table>
<thead>
<tr>
<th>final dimer</th>
<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5,6/TMS,6</th>
<th>TMS5/TMS</th>
<th>TM1/TMS</th>
<th>TM1-3/TM1-3</th>
<th>TM1-4/TM4,5</th>
<th>TM1/TM1-3</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS5,6/TMS,6,</td>
<td>70</td>
<td>3.60 ± 0.11</td>
<td>92.86%</td>
<td>0.00%</td>
<td>7.14%</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TMS1/TM1</td>
<td>144</td>
<td>3.83 ± 0.32</td>
<td>0.00%</td>
<td>88.89%</td>
<td>11.11%</td>
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### NTS1 in DEPC

<table>
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<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5,6/TMS,6</th>
<th>TMS5/TMS</th>
<th>TM1/TMS</th>
<th>TM1-3/TM1-3</th>
<th>TM1-4/TM4,5</th>
<th>TM1/TM1-3</th>
<th>Other</th>
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<tr>
<td>TMS5,6/TMS,6,</td>
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<td>7.14%</td>
<td>0.00%</td>
</tr>
<tr>
<td>TMS1/TM1</td>
<td>145</td>
<td>3.92 ± 0.26</td>
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<td>0.00%</td>
<td>4.83%</td>
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<tr>
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<td>0.00%</td>
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<tr>
<td>TMS1-3/TM1-3</td>
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<td>3.72 ± 0.29</td>
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<td>24.64%</td>
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<td>TMS1-4/TM4,5</td>
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<td>TMS1/TM1-3</td>
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<td>3.91 ± 0.22</td>
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<td>1.85%</td>
<td>1.85%</td>
<td>5.58%</td>
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### NTS1 in BPL

<table>
<thead>
<tr>
<th>final dimer</th>
<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5,6/TMS,6</th>
<th>TMS5/TMS</th>
<th>TMS5/TMS (loose)</th>
<th>TM1/TM5</th>
<th>TM1-4/TM1-2</th>
<th>TM1-3/TM1-3</th>
<th>TM3,4/TM3,4</th>
<th>Other</th>
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<td>TMS5,6/TMS,6,</td>
<td>24</td>
<td>3.71 ± 0.16</td>
<td>95.83%</td>
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<td>0.00%</td>
<td>0.00%</td>
<td>4.17%</td>
<td>0.00%</td>
<td>0.00%</td>
<td></td>
</tr>
<tr>
<td>TMS5/TMS</td>
<td>49</td>
<td>3.99 ± 0.35</td>
<td>0.00%</td>
<td>79.59%</td>
<td>12.24%</td>
<td>8.16%</td>
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<tr>
<td>TMS1/TM1</td>
<td>61</td>
<td>3.85 ± 0.24</td>
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<td>6.56%</td>
<td>80.33%</td>
<td>13.11%</td>
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<tr>
<td>TMS1-4/TM1,2</td>
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<td>4.07 ± 0.36</td>
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<td>89.11%</td>
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<td>3.96%</td>
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<tr>
<td>TMS3,4/TM3,4</td>
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<td>0.00%</td>
<td>0.00%</td>
<td>3.03%</td>
<td>96.97%</td>
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### NTS1 in BPL-DHA

<table>
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<tr>
<th>final dimer</th>
<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5/TMS</th>
<th>Other</th>
</tr>
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<tbody>
<tr>
<td>TMS5/TMS</td>
<td>49</td>
<td>4.46 ± 0.17</td>
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### NTS1 BPL-DHA-mix

<table>
<thead>
<tr>
<th>final dimer</th>
<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5/TMS</th>
<th>Other</th>
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<tbody>
<tr>
<td>TMS5/TMS</td>
<td>13</td>
<td>4.28 ± 0.27</td>
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<tr>
<td>TMS1/TM1</td>
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<td>4.25 ± 0.34</td>
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<tr>
<td>TMS3,4/TM3,4</td>
<td>3</td>
<td>4.35 ± 0.08</td>
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### NTS1p in BPL

<table>
<thead>
<tr>
<th>final dimer</th>
<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5,6/TMS,6</th>
<th>TMS5/TMS (loose)</th>
<th>TM1/TM5</th>
<th>TM1-3/TM1-3</th>
<th>TM3,4/TM3,4</th>
<th>Other</th>
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<tbody>
<tr>
<td>TMS5,6/TMS,6,</td>
<td>31</td>
<td>3.82 ± 0.21</td>
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<td>0.00%</td>
<td>6.45%</td>
<td>0.00%</td>
<td>0.00%</td>
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<tr>
<td>TMS5/TMS</td>
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<td>3.80 ± 0.28</td>
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<tr>
<td>TMS1/TM1</td>
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<td>58.82%</td>
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<tr>
<td>TMS1/TM1-3</td>
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<td>0.00%</td>
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<td>0.00%</td>
<td>5.26%</td>
<td>5.26%</td>
<td>84.21%</td>
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### NTS1p in BPL (no palmitate)

<table>
<thead>
<tr>
<th>final dimer</th>
<th>Number of dimers</th>
<th>COM dist [nm]</th>
<th>TMS5,6/TMS,6</th>
<th>TMS5/TMS (loose)</th>
<th>TM1/TM5</th>
<th>TM3,4/TM3,4</th>
<th>Other</th>
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<td>TMS5,6/TMS,6,</td>
<td>38</td>
<td>3.66 ± 0.17</td>
<td>92.11%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>7.89%</td>
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<tr>
<td>TMS5/TMS</td>
<td>64</td>
<td>3.85 ± 0.30</td>
<td>0.00%</td>
<td>95.31%</td>
<td>4.69%</td>
<td>0.00%</td>
<td>1.56%</td>
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<tr>
<td>TMS1/TM1</td>
<td>24</td>
<td>3.92 ± 0.20</td>
<td>0.00%</td>
<td>16.67%</td>
<td>79.17%</td>
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<td>4.17%</td>
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<tr>
<td>TMS3,4/TM3,4</td>
<td>27</td>
<td>4.07 ± 0.20</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>96.30%</td>
<td>3.70%</td>
</tr>
</tbody>
</table>

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Figure S1: Comparison of initial and final NTS1 dimer interfaces. The number of dimers for each main interface at the end of simulation time is listed. The transmembrane helix bundle center-of-mass distances (COM dist) between interacting monomers for the main dimer configurations were averaged over the last 50 ns of the corresponding simulations (average ± standard deviation). Furthermore, the initial dimer configurations upon first contact (at the first frame the dimerization criterion was reached) were investigated. First dimer contacts that did not represent any of the ensemble’s main dimer interfaces are listed as Other. Typically, the initial dimer configuration was stable during the remaining simulation time.
The transmembrane helix-bundle center-of-mass distances for the main NTS1 dimer interfaces in DHA-free membranes was between ≈3.6 and 4.1 nm (Figure S1). Corresponding dimers in BPL-DHA and BPL-DHA-mix revealed increased distances (≈4.2 – 4.5 nm). As shown in Figure S2, the COM distance distribution of NTS1 dimers in DHA containing membrane environments indicates less compact dimer configurations as compared to DHA-free bilayers. While the average COM distance between monomers in the loose TM5/TM5 configuration in BPL was ≈4 nm, the COM distance between receptors in the corresponding interface in BPL-DHA was calculated to ≈4.5 nm (see Figure S1 and Figure S2B).

Figure S2: (A) Density distributions of transmembrane helix-bundle center-of-mass (COM) distances between NTS1 in dimers calculated during the last 50 ns of simulation time. (B) Comparison between loose TM5/TM5 dimer conformations in BPL and BPL-DHA.

Estimated hydrophobic mismatch

The residual hydrophobic mismatch (RHM) was estimated for all studied membrane models. As it can be seen in Figure S3, the estimated total RHM in BPL is slightly above the RHM in POPC but below the value estimated for DEPC, while the RHM in DHA containing membranes is slightly increased as compared to DEPC. Since all BPL model bilayers
contain multiple components that influence dimerization (e.g. cholesterol, anionic lipid head groups and polyunsaturated fatty acid chains), we note that the estimated RHM alone is not a sufficient descriptor to predict dimerization rates in assembly simulations in complex membrane environments.

Figure S3: The residual hydrophobic mismatch (with standard errors) was estimated following Mondal et al.\textsuperscript{1} The total hydrophobic mismatch was estimated as the sum of the RHM over all seven transmembrane helices.

![Estimated hydrophobic mismatch](image)

G protein-coupled dimer models

Selected NTS1 dimer configurations sampled in simulation ensembles were structurally aligned to either the $\beta_2$ adrenergic receptor ($\beta_2$AR)-G\textsubscript{s}\textsuperscript{2} or the NTS1-G\textsubscript{i1} complex\textsuperscript{3} to investigate how distinct dimer conformations could interact with G proteins (see Figure S4).

Interestingly, the larger displacements of TM6 and TM5 in the $\beta_2$AR-G\textsubscript{s} complex would likely result in steric clashes at dimer interfaces involving TM5,6, while the NTS1-G\textsubscript{i} struc-
ture appears more suitable for superimposition. In case of the POPS-stabilized symmetric TM3,4 dimer interface, both $G_s$ and $G_i$-coupled receptor structures could be aligned without steric clashes. Intriguingly, the G protein-free monomer in the TM3,4/TM3,4 dimer-$G_s$ complex appears able to interact with the $\alpha$-helical domain of the $G\alpha$-subunit.

Figure S4: Alignment of selected dimer configurations with NTS1-$G_{i1}$ (PDB: 6os9, light green) or $\beta_2$AR-$G_s$ (PDB: 3sn6, light blue) complexes. Models for G protein–receptor dimer interactions are shown for (A) TM5,6/TM5,6 interfaces sampled in POPC bilayers, (B) loose, cholesterol-stabilized TM5/TM5 dimers observed in BPL, and (C) anionic lipid-induced TM3,4/TM3,4 interfaces.
Methods

Structure preparation

There are several crystal structures available for rat NTS1 as well as a recent cryo-EM structure of human NTS1 coupled to G\textsubscript{i}, each showing all seven TM helices, however differing in resolution, thermostabilizing mutations, and the conformation of H\textsubscript{8}.\textsuperscript{3-7} Crystal structures were obtained from agonist-bound receptors and show the receptor in ’active-like’ states. In the first crystal structure of NTS1,\textsuperscript{4} H\textsubscript{8} was not resolved, while more recent structures show H\textsubscript{8} with different lengths and elevated B-factors, hinting to either a general instability of H\textsubscript{8} or increased dynamics.\textsuperscript{5,6,8} None of the currently available high-resolution structures shows a palmitoylated H\textsubscript{8}.

The protein model for NTS1 used in our simulation study was prepared based on the crystal structure 4XES\textsuperscript{6} (the structure with the currently highest resolution) taken from the Protein Data Bank (PDB).\textsuperscript{9} Here, rat NTS1 in complex with neurotensin was crystallized containing four thermostabilizing mutants A86L, E166A, G215A, V360A and the third intracellular loop was replaced with cysteine-free bacteriophage T4 lysozyme.\textsuperscript{6} Rat and human NTS1 share \approx 83\% sequence identity and the RMSD between the cryo-EM structure of human NTS1 and the 4XES rat NTS1 structure is < 1\textsubscript{Å}.

During model preparation, T4 lysozyme, the neurotensin fragment and other non-protein atoms were removed. Using MODELLER,\textsuperscript{10} thermostabilizing mutations were mutated back to the wildtype sequence and the missing ICL3 was simplified by connecting the open ends on TM5 and TM6, i.e. residues V268, P297, G298, R299, and V300 served as an artificial intracellular loop. H\textsubscript{8} of the crystal structure 4XES ends at position T383. In order to include the cysteine palmitoylation site at H\textsubscript{8}, the helix was extended by residues L384, A385 and C386 according to the NTS1 crystal structure 4BUO.\textsuperscript{5} The final NTS1 protein model contained residues A49–C386 (excluding ICL3 residues H269–E296) and was minimized using the CHARMM36m force field.\textsuperscript{11} Subsequently, the protein model was converted to the
MARTINI2.2 coarse-grained force field using *martinize.*\textsuperscript{12} The secondary and tertiary protein structure was enforced using the distance-based elastic *RubberBands* force network.\textsuperscript{13} Here, additional bonds between backbone bead pairs \((i,j)\) with a distance \((d_{ij})\)-dependent force \(F_{ij}\) were generated within a cut-off distance of 0.9 nm according to the kernel-like function:

\[
F_{ij}(d_{ij}) = f \cdot e^{-ad_{ij}^p}
\]

The general force \(f\) was set to 500 kJ/mol nm\(^2\) and parameters \(a\) and \(p\) defining the exponential decay profile of \(F_{ij}\) were chosen as 3 and 6, respectively.\textsuperscript{14} *RubberBands* are similar to ElNeDyn\textsuperscript{15} network and are implemented in *martinize*.

Parameters for palmitoylated cysteine can be found in the currently available CHARMM36 force field,\textsuperscript{11,16–18} corresponding MARTINI parameters were reported by Atsmon-Raz *et al.*\textsuperscript{19}

**Simulation setup**

**Coarse-grained simulations.** Dimerization simulation ensembles (see Table 1 of manuscript) were generated using the DAFT protocol,\textsuperscript{13} i.e. in every simulation two initially separated, randomly rotated (along z-axis) coarse-grained (CG) proteins were placed in a rhombic box at a defined starting distance (\(\approx 3.6\) nm minimum distance). Next, CG lipid bilayers\textsuperscript{20} and water molecules were added using *insane.*\textsuperscript{21} Resulting simulation systems contained roughly 400–500 lipid molecules and 7,100–7,700 water beads. In case of BPL membrane models containing anionic lipids, CG sodium ions were added to neutralize the system net charge. Parameters for SDPE/PS lipids were built following to the classic MARTINI scheme,\textsuperscript{20} bond and angle parameters were refined in comparison to atomistic simulations (see next Section). Resulting simulation systems underwent the *martinate* protocol,\textsuperscript{22} i.e. a steepest-descent minimization for 1,500 steps prior to a short 100 ps *NPT* equilibration (constant particle number \(N\), constant pressure \(P\) and temperature \(T\)). Final production MD simulations were performed in *NPT* ensembles with an integration time step of 20 fs at 310 K using
the v-rescale thermostat\textsuperscript{23} with a coupling time constant of 1 ps, and at 1 bar applying the Berendsen barostat\textsuperscript{24} with a coupling time constant of 3 ps in a semi-isotropical manner. The 12-6 Lennard-Jones potential was shifted to zero between 0.9 and 1.2 nm, Coulomb interactions were shifted to zero between 0 and 1.2 nm, and a relative dielectric constant of 15 was used as suggested for the MARTINI force field. Cholesterol-binding to NTS1 and NTS1p in BPL was investigated using ten 2 $\mu$s simulations containing one protein only, following the same set-up as described for dimerization simulations. CG simulations were performed and analyzed using GROMACS 4.6.\textsuperscript{25}

**Atomistic simulations.** Atomistic simulations of palmitolyated and non-lipidated NTS1 were first set up as CG systems. A CG monomer was embedded in a BPL-DHA bilayer using *insane* and the system was minimized for 500 steps prior to a 5 ns MD simulation with position restraints on the protein structure using the same simulation parameters as described before. Subsequently, the system was converted back to atomistic resolution for simulation with CHARMM36m\textsuperscript{11} using *backward* and the *initram* relaxation protocol.\textsuperscript{26} Ions were added to reach a salt concentration of 150 mM and a system net charge of zero. The backmapped systems contained roughly 600 lipids, 50,000 water molecules and 400 ions. Next, the prepared NTS1 model was fitted onto the backmapped structure and the system was first minimized for 1,000 steps with a fixed protein structure before undergoing another 200 steps of general minimization. Prior to the production run, the system was simulated for 2 ns in an *NPT* ensemble with position restraints applied on the protein backbone. Atomistic MD simulations were carried out with a 2 fs time step at 310 K using the v-rescale thermostat with a coupling time constant of 0.5 ps and at 1 bar applying the Parrinello-Rahman barostat\textsuperscript{27} with a coupling time constant of 5 ps in a semi-isotropical manner. The Lennard-Jones potential was switched to zero between 0.8 and 1.2 nm and electrostatic interactions between particles separated by more than 1.2 nm were computed using the particle mesh-Ewald summation.\textsuperscript{28} Production runs were computed for 1 $\mu$s using GROMACS 5.1.\textsuperscript{29} In case of the atomistic simulation of NTS1 in BPL with 4% SDPE/PS, the CG system was
converted to atomistic resolution after 5 µs of CG simulation and subsequently simulated for 500 ns using the CHARMM36m\textsuperscript{11} force field and GROMACS 2018 with the simulation parameters as described above. Lipid parameters for the CHARMM36 force field can be found in the CHARMM-GUI web-server.\textsuperscript{30–32}

**Refinement of coarse-grained DHA parameters**

Parameters of docosahexaenoic acid (DHA) for SDPE and SDPS lipids were generated according to the classic mapping scheme of the MARTINI lipid force field.\textsuperscript{20} An atomistic simulation of a BPL-DHA bilayer (containing roughly 500 lipids) was computed for 200 ns using the CHARMM36 force field.\textsuperscript{11,16–18} Subsequently, distances and angles between heavy atom groups reflecting the CG mapping scheme were calculated and compared to corresponding CG parameter models used for 200 ns CG simulations. Final SDPE MARTINI parameters as well as distance and angle distributions between mapped atom groups or CG beads are presented in Figure S5.

**Analysis**

**Dimerization criterium.** In order to distinguish between receptor monomers and dimers in simulation ensembles a simple protein-protein interaction energy (estimated as the sum of Lennard-Jones and Coulomb interactions between TM segments) cutoff of -50 kJ/mol was used. As reported previously, this dimerization criterium was used to identify direct protein-protein contacts in CG simulations.\textsuperscript{14}

**Diffusion coefficients.** Diffusion coefficients of NTS1 in different simulation ensembles were estimated for receptors during the first 200 ns of those simulations that did not show dimer interactions until 250 ns. The lateral self-diffusion $D_s$ of a receptor was computed from the slope of the mean-square displacement (MSD) averaged over all receptor beads,
Figure S5: (A) Distance and angle distributions between example atomistic heavy atom mapping groups and corresponding CG beads of DHA. Mean distances $d$ and angles $\gamma$ are given for atomistic and CG parameters. (B) Parameter file for SDPE used in CG simulations.

\[
D_s = \lim_{t \to \infty} \frac{\langle (\Delta r(t))^2 \rangle}{4t} \tag{2}
\]

Accordingly, the distance $\Delta r(t)$ a bead moved in time $t$ was monitored, and the slopes of MSD curves were fitted on the time window between 5-20 ns. The MSD analysis was corrected for the center of mass motion of the protein-membrane system.

**Orientational analysis.** The dimeric configurational space was determined by computing relative binding angles ($\beta$, $\chi$) between receptors in dimer complexes.$^{13,33}$ Briefly, an internal
A coordinate system with origin at the receptor center of mass was constructed according to the principal components of the protein (see Figure 1B, main manuscript). Superimposing two receptor coordinate systems yielded the relative angles between both proteins. In a system with two receptors (monomer A and monomer B), monomer A serves as the reference structure. The angle $\beta$ describes the rotation of monomer B around the z-axis of the reference structure A, i.e. provides the TM segment of monomer A involved at the dimer interface.

The dimer interaction site of monomer B was described by the angle $\chi$, calculated by $\chi = (180^\circ + \beta - \phi) \mod 360$, where $\phi$ is the rotation of monomer B around its z-axis. Relative binding angles were calculated between receptors in dimeric complex for the last 50ns of simulation time.

Subsequently, dimer $(\beta, \chi)$-coordinates of simulation ensembles were used to compute 2-dimensional kernel-densities as represented in Figure 1C (main manuscript). These angle height-fields rationalize the dimeric configurational space between receptors in simulation ensembles. Dimer structures with $(\beta, \chi)$-configurations closest to the height-field maximum and strong interaction energies were selected as representative models and converted back to atomistic resolution (for more information see previous studies Pluhakova et al. and Gahbauer et al.).

**Bilayer thickness.** The membrane thickness was estimated by substracting the z-positions of CG lipid PO4 beads of opposing leaflets. Protein-free membrane systems were set up using insanely, contained roughly 400-500 lipid molecules and were simulated for 200ns. The bilayer thickness in vicinity of proteins in simulation ensembles was computed during 150-200ns for lipids within 1 nm around receptor monomers that did not show dimerization until 250 ns. 

**Residual hydrophobic mismatch.** The residual hydrophobic mismatch was estimated following the approach presented in Mondal et al.. The surface area of residues participating in unfavorable hydrophobic-polar interactions was penalized by $0.028 \text{ kcal/(mol} \cdot \text{Å}^2)$. For hydrophobic residues, the surface exposed *outside* the hydrophobic bilayer core (between C2, D2, or R1 beads of opposing leaflets, for POPC/PE/PS, SDPE/PS (DHA), or cholesterol,
respectively) was calculated as the solvent accessible surface area (SASA). Accordingly, the surface exposed to the bilayer hydrophobic core was taken to penalize unfavorable interactions of hydrophilic residues. The SASA was calculated using a probe radius of 5.2 Å, between 200 ns and 500 ns for each monomers in 10 ensemble simulations that did not show receptor dimers until 1 µs of simulation time. To describe the hydrophobic mismatch of known interfacial residues, i.e. Trp, Arg and Lys, the molecules were divided into polar and unpolar segments (beads) which were individually penalized as described above. Finally, the TM hydrophobic mismatch was calculated by adding up the RHM of all residues belonging to each helix.

**Protein-lipid interactions.** Binding of cholesterol was calculated from ten 2 µs CG simulations of a single NTS1 receptor embedded in BPL bilayers. A residue was considered as occupied by cholesterol if at least one of the 8 cholesterol beads was located within 0.62 nm. The first 200 ns were discarded to exclude random protein-cholesterol contacts during bilayer construction with insane. The occupancy (averaged over all 10 simulations) of each residue was determined as the fraction of simulation time the residue was in contact with cholesterol. The spatial distribution of cholesterol around NTS1 (shown in Figure 3A, main manuscript) were calculated for the five nearest cholesterol molecules.

Binding of different lipid types to NTS1 was estimated by calculating the occupancy of extra- and intracellular ends of TM helices (or H8) by head group beads of different lipids (i.e. PO4 with NC3, NH3, or CNO for PC, PE, or PS lipids, respectively). On each helix end, 8 residues were selected corresponding to two helical turns. A helix end was considered occupied by a specific lipid type if one head group bead was located within 0.65 nm (reflecting the size of the first solvation shell of lipid molecule beads around the protein computed from the radial distribution function). Relative lipid occupancies were computed for a time interval from 200 ns to 800 ns from dimerization ensemble simulations in BPL that did not show dimerization until 1 µs and finally scaled by the concentration of lipid types in the model bilayer.
Concentrations of saturated and polyunsaturated lipids around NTS1 were estimated by monitoring the relative amounts of corresponding lipid head group PO4 beads 1 nm around NTS1 in BPL-DHA-mix bilayers (see Figure 4 B, main manuscript). Calculations were carried out for 100 ensemble simulations during the first 1 µs that did not show dimerization until 2 µs. Accumulation of different lipid types around NTS1 was additionally analyzed in CG and atomistic simulations of NTS1 embedded in BPL with 4% SDPE/PS (see Figure 4 C and D, main manuscript). The enrichment factor was calculated by comparing the concentrations of lipid type head group phosphates (PO4 in CG, P in atomistic systems) 1 nm around NTS1 to the lipid bulk concentration. The accommodation of lipid tails to the surface of NTS1 was investigated by averaging the minimum distances of carbon atoms belonging to individual fatty acid hydrocarbon chains of SDPE/PS lipids bound to NTS1.
References


cholesterol-conditioned dimerization of the G protein coupled chemokine receptor type


