Supporting Material

Membrane Interface Composition Drives the Structure and the Tilt of the Single Transmembrane Helix Protein PMP1: MD Studies

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Materials and Methods

The CHARMM program (1) was used for molecular dynamics simulation with the PAR22 for proteins and PAR27 for lipids all-atom force fields ((2) and (3) respectively), including parameters for POPC and POPS phospholipids and the TIP3P water potential (4). All calculations were performed on 16 Pentium 4 processors and on the large scale facilities of the "Centre de Calcul et de Recherche Technologique" (CCRT) of the French Nuclear Agency (CEA).

The *construction* of the initial hydrated phospholipid bilayer comprising 242 POPC (121 POPC on each layer) was carried out using a protocol developed by Woolf and Roux (5,6). We carried out several changes, mainly by developing a library of 50 pre-equilibrated and prehydrated POPC phospholipids and by introducing the peptide only once the bilayer was equilibrated. The fixed dimensions (X, Y, Z) of the primary cell are 88 Å, 88 Å, 74 Å. The X and Y dimensions of the simulation box corresponded to 64 $Å^2$ for POPC lipid cross-section at 310K (7-9) and the Z dimension, to five water layers on each membrane side, enough to hydrate the membrane (i.e. 34.7 water molecules per POPC, (8)) and to shield molecular interactions between membranes above each other. The system consisted of 57673 atoms (242 lipids and 8415 water molecules).

Equilibration was carried out at constant volume, with Langevin dynamics, using a friction coefficient of 3.0 ps^{-1} on all atoms except hydrogens and a bath temperature of 310 K. Electrostatic and van der Waals interactions were truncated at a cutoff distance of 12 Å with a smooth switching function on electrostatic forces and with a shifting function on van der Waals potential over a 4 Å interval (this truncation scheme has been shown to be efficient and accurate in our previous study (10)). A cutoff distance of 14 Å was used to calculate the nonbonded lists and image lists. The lengths of all bonds involving hydrogen atoms were constrained using the SHAKE algorithm (11), which allows a 2 fs time step to be used for the numerical integration of the equation of motion. Periodic boundary conditions were applied in the three dimensions to model an infinite multilayer system. Planar constraints on lipid glycerol C2 carbon were applied at z = +/- 18 Å and the water molecules were prevented from penetrating the membrane hydrophobic core. These constraints were gradually decreased to zero during equilibration.

Initial structure: After 1 ns of equilibration, *the membrane protein (PMP1) was introduced into the pure POPC bilayer*. The PMP1 starting structure was derived from ^{1}H-NMR data obtained in micelles. The structure comprises a single transmembrane helix (from the N-terminus up to R33) and a 5-residue C-terminal segment, which folds back toward the membrane interior (12). We conserved the C16S mutation for simulation. Two POPC (one on each side of the bilayer) were removed to fit the PMP1 transmembrane helix, and the C-terminal segment was located in the bottom leaflet side. Using the PMP1 inserted into the pure POPC bilayer construction, *a mixed POPC-POPS bilayer* was generated by replacing the eight POPC headgroups surrounding the PMP1 C-terminal segment by eight POPS headgroups, without changing the rest of the concerned phospholipids. Thus, both the starting structure and positioning of PMP1 were exactly the same in both membranes. The only difference being the membrane interface composition. Polar headgroups for the eight POPCs in direct contact with the PMP1 C-terminal segment were replaced. This was carried out based on previous ^{2}H-NMR experiments that show that the PMP1 fragment specifically segregates eight POPS when inserted in mixed POPC/POPS bilayers (13). Statistical

replacement of the polar heads would have required a simulation time of several microseconds to reach a distribution equilibrium because of the slow diffusion of lipids. Ions were finally added to neutralize the systems (5 Cl^- in the pure POPC and 3 Na^+ in the mixed POPC-POPS bilayer). The systems consist of 57767 atoms in the case of PMP1 in the pure POPC bilayer and of 57706 atoms in the case of PMP1 in the mixed POPC-POPS bilayer. Minimization and equilibration were performed for a further 700 ps with constraints on the peptide and on the lipid glycerol C2 carbon, to fix them. These constraints were gradually decreased to zero during equilibration.

Finally, *production* at 310 K with a constant volume and the Leap Verlet algorithm was performed over the course of 50 ns for both systems. We ensured that the temperature and potential energy remained constant during the simulation time. Several variables were also derived from the simulation and were compared to experimental data, to validate the protocol used (data not shown). The average membrane thickness, measured from the average distance between the two planes formed by the phosphate groups, during simulation was 40 Å, in general agreement with previous experimental results (8). The distribution profiles along the z axis of various atoms (water oxygen atoms, lipid nitrogen atoms, lipid phosphate atoms, carbons of lipid glycerol groups, carbons of aliphatic chain: CH_2, CH and CH_3 groups) show good agreement with previous neutron diffraction experimental results (14). Less than 2% of water molecules are inserted into the hydrophobic core of the membrane, without creating any gaps. We checked that the aliphatic chain dihedral angle distribution, C-C angle values and also aliphatic chain order parameter were in good agreement with experimental data (15).

Number of contacts: we computed the number of contacts to investigate the structural motifs and specific interactions within the protein and with lipids. We calculated it throughout the simulation time, by counting each time the groups of atoms of interest were closer than a cut off distance of 3.5 Å (16). This calculation was performed on 5000 frames for 50 ns of simulation, i.e. every 10 ps. We defined different groups of atoms for each residue: the backbone CO and NH groups; for side chains, we focused only on donor and acceptor groups (NH and both NH_2 for Arg, NH_2 and separately CO for Gln, NH_3^+ for Lys, NH for Trp, OH for Tyr); for lipid molecules, we defined a carbonyl group for both CO of the aliphatic chains, a phosphate group (PO_4^-) and a $N(CH_3)_3^+$ group for the POPC headgroup, and NH_3^+ and COO^- groups for the POPS headgroup. The number of contacts was finally normalized to facilitate comparison and interpretation. We decided to count contacts and not hydrogen bonds, in order to be less restrictive. Indeed, the contact number provides a supplementary information on the chemical environment of the group of atoms of interest. Moreover, we checked that when the number of contacts between a donor and an acceptor group is maximal i.e. equal to 1, it effectively corresponds to a hydrogen bond.

Residence time: the residence time of the lipid sn-2 aliphatic chain in the groove formed on one side of the helix by a series of short side chain residues was calculated as the time during which the distance between the center of mass of the aliphatic chain carbons and the center of mass of the short side chain residue Cα is less than 8 Å. We verify that above this distance, the lipid chain is no more lying in the groove.

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