Supplementary Material

An annular lipid belt is essential for allosteric coupling and viral inhibition of the antigen translocation complex TAP

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Computational Methods

Molecular dynamics (MD) simulations. All simulations were run with GROMACS 4.6.5, using the Amber99SB-ILDN protein force field (1,2), the Stockholm lipids force field (3,4) for POPE, POPG and POPC, and the TIP3P water model (5). Application of the SETTLE (6) (waters) and LINCS (7) (other molecules) constraint algorithms allowed for an integration timestep of 2 fs. Short-range non-bonded Coulomb and Lennard-Jones 6-12 interactions were treated with a Verlet buffered pair list (8) with potentials smoothly shifted to zero at a 10 Å cut-off. Long-range Coulomb interactions were treated with the PME method (9) with a 1.1 Å grid spacing. Analytical dispersion corrections were applied for energy and pressure to compensate for the truncation of the Lennard-Jones interactions. Periodic boundary conditions were in effect, using a cuboid cell for the lamellar bilayer and a rhombic dodecahedron for the nanodiscs. The thermodynamic ensemble was nPT. Temperature was kept constant at 300 K with a V-rescale thermostat (10) with coupling time constant 0.1 ps. For constant 1.0 bar pressure, a Berendsen barostat (11) was used with coupling time constant 0.5 ps and compressibility $4.5 \cdot 10^{-5}$ bar^{-1}. For the nanodisc simulations, isotropic pressure coupling was applied, while semi-isotropic coupling was used for the lamellar bilayer simulations.

System set-up. We simulated two different nanodisc systems, one with a POPE/POPG lipid mixture, and another with a POPC/POPG mixture. In addition, a lamellar POPC/POPG bilayer system was simulated for reference. To generate starting structures for these simulations, MSP1 scaffold coordinates were taken from the cryo-EM structure (PDB ID: 3J00) (12). For the lipids, pre-equilibrated POPE/POPG (3:1) and POPC/POPG (3:1) bilayers were generated by replacing head groups in reference POPG and POPC bilayers (respectively), taking care to keep the same number of POPE/POPC and POPG in each leaflet. After 50 ns nPT equilibration, MSP1 was inserted into these pre-equilibrated bilayers containing 222 POPE (or POPC) and 66 POPG, removing all lipid molecules either outside of or clashing with the scaffold. The structure of the coreTAP complex was taken from the homology model based on Sav1866 (13). The insertion depth and orientation of the transporter in the membrane was computed with the OPM webserver (14). Overlapping lipids were removed, leaving 96 lipid molecules (74 POPE/POPC and 22 POPG) in the initial nanodiscs. The systems were solvated after 500 steps of conjugate gradient energy-minimization and 20 ps in vacuo simulation, taking care that no water was inserted into the hydrophobic core of the membrane. Random water molecules were replaced by Na^+ and Cl^- ions to yield a concentration of 0.15 M and a neutral system charge. Total system size was about 192,000 atoms for the nanodiscs and 258,000 for the lamellar bilayers. The initial systems were once again energy-minimized (500 steps conjugate gradient) prior to simulation. Initial velocities were generated at room temperature. TAP heavy atoms were kept position-restrained (harmonic potential, force constant 1000 kJ mol^{-1} nm^{-2}) during the simulations. In the nanodisc simulations, MSP1 was also position-restrained during the first 5 ns. The reasons for restraining TAP are: i) the focus of this study is the structure of the lipid belt around TAP in the nanodisc rather than the internal structural dynamics of TAP, and ii) we wanted to exclude the possibility of large, unrealistic structural deviations that could result from the fact that only homology-based structural models are available for TAP. Finally, 50 ns trajectories were acquired for various nanodisc sizes and a model bilayer with TAP embedded, recording coordinates every 20 ps. In the

nanodisc simulations, after each 50 ns simulation, randomly-chosen lipids were removed from the system, taking care to keep an approximate 3:1 ratio for POPE/POPG and POPC/POPG. In addition, randomly-chosen ions were removed to keep a neutral system charge. Nanodisc size was thus decreased from 96 to 84, 72, 60, 50, 40, 30, and finally 22 lipids (17 POPE/POPC, 5 POPG). The systems therefore relaxed for 50 ns at each of these removal steps to allow conformational changes in the scaffold proteins to accommodate the smaller lipid belt. Taken together, total simulation times were thus 400 ns for each of the two nanodisc systems.

Analysis. Distance analysis was performed by finding, for each residue of the TAP or MSP1 dimers, the smallest distance between an atom in that residue and any atom of the other dimer. These minimal distances were then time-averaged over the entire trajectory. Phosphate densities were analyzed by slicing the system into 1 Å slabs along the membrane normal, and counting the time-averaged number of phosphate atoms within these slabs. Secondary structure was analyzed using DSSP (15); helical content was averaged over the final 25 ns of MD simulation.

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