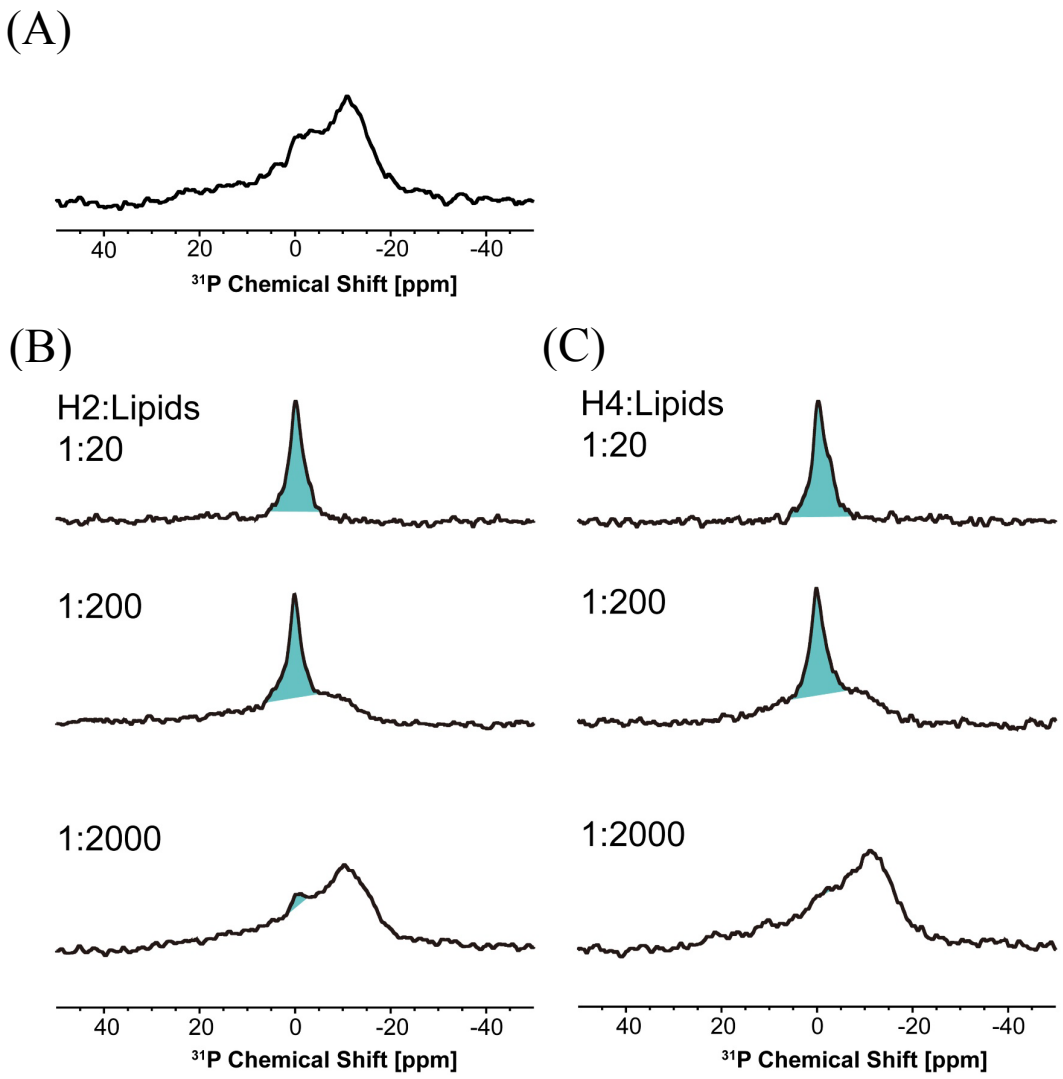


**Fig. 1.** $^{31}$P solid-state NMR spectra of *Leishmania* mimetic membrane (A) and interacting with bombinin H2 (B) and H4 peptides (C). Blue fill pattern shows isotropic NMR signal deduced form disruption of membrane. When the peptide and lipid ratio was 1:200, the isotropic components of H2 and H4 were around 40%.

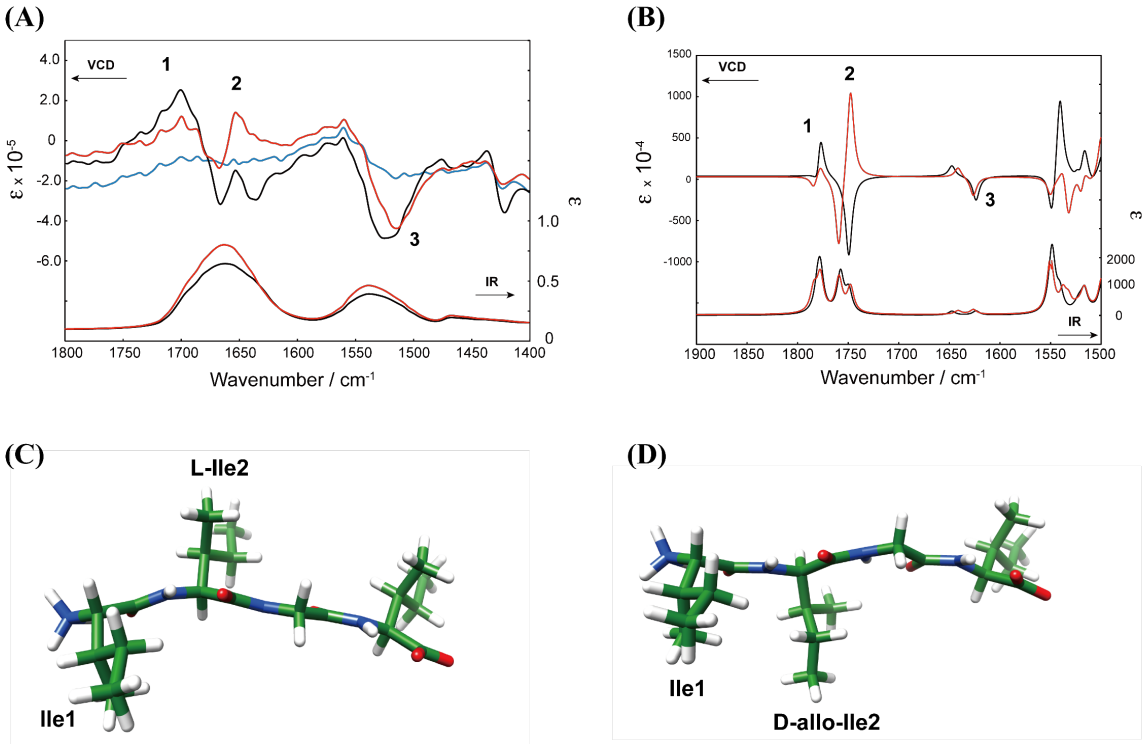


**Fig. 2.** (A) The observed VCD (upper) and IR (lower) spectra of bombinin H2 (black) and H4 (red) in KBr. Blue line is only KBr. (B) The DFT calculations of VCD (upper) and IR (lower) spectra of bombinin H2 (black) and H4 (red). The model conformation of first two side chains in N-terminal part of H2 "*trans*" (C) and H4 "*cis*" forms (D).

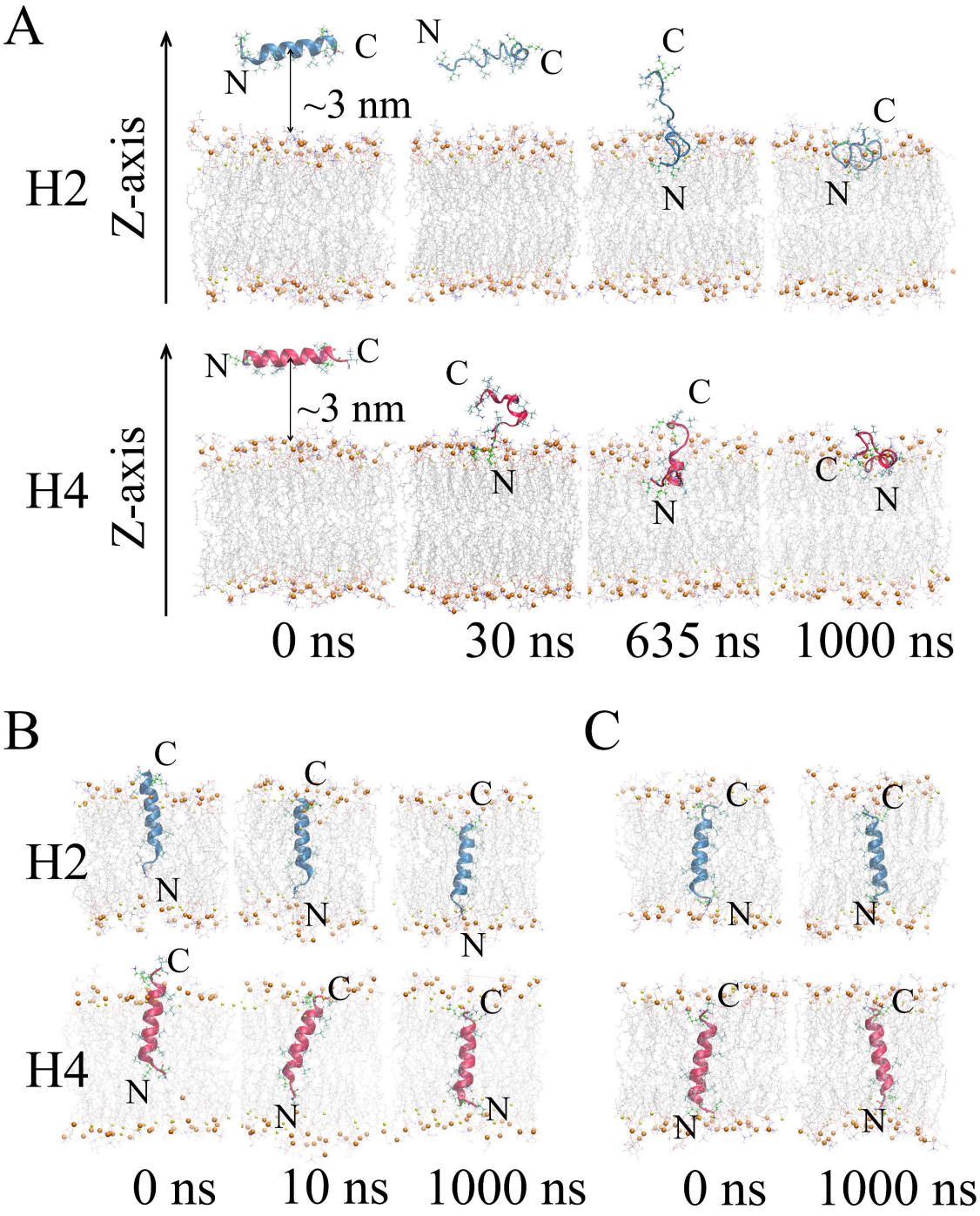


**Fig. 3.** The snapshots of bombinin H2 and H4 in A, B and C types of simulations. Bombinin H2 and H4 are initially placed on different positions as, Type A: the peptides are placed in water region above the membrane surface, Type B: The peptides are placed as pseudo transmembrane. Amino acid residues of 18 and 19 lysine are placed at the interface between the membrane surface and water, and Type C: The peptides are placed as transmembrane. Water molecules and ions are not shown in this figure for the clarity.

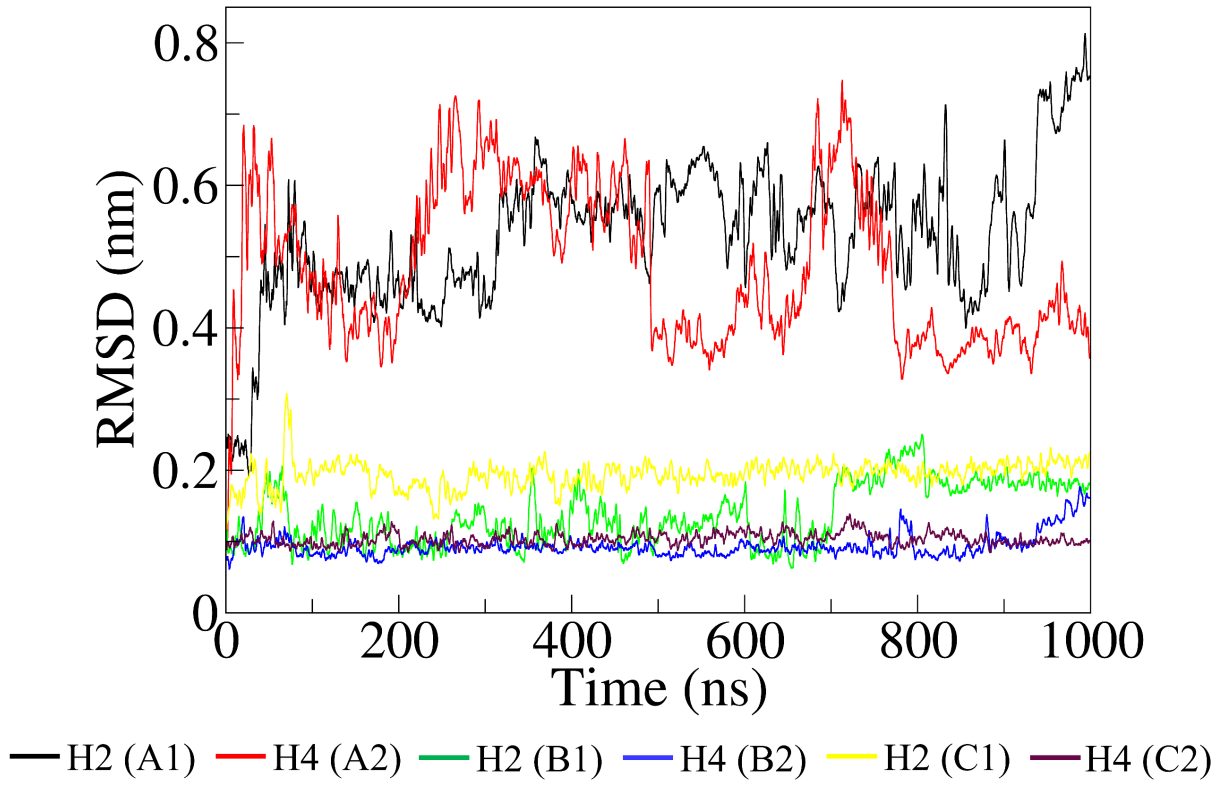


**Fig. 4.** Root-mean-square deviation (RMSD) of the backbone atoms of H2 and H4 peptides calculated from their initial conformations as a function of time. The black, green and yellow lines indicate H2 in A1, B1, and C1 simulations; the red, blue and brown lines indicate H4 in A2, B2, and C2 simulations, respectively. For the clarity, data were averaged in every 1000 points.

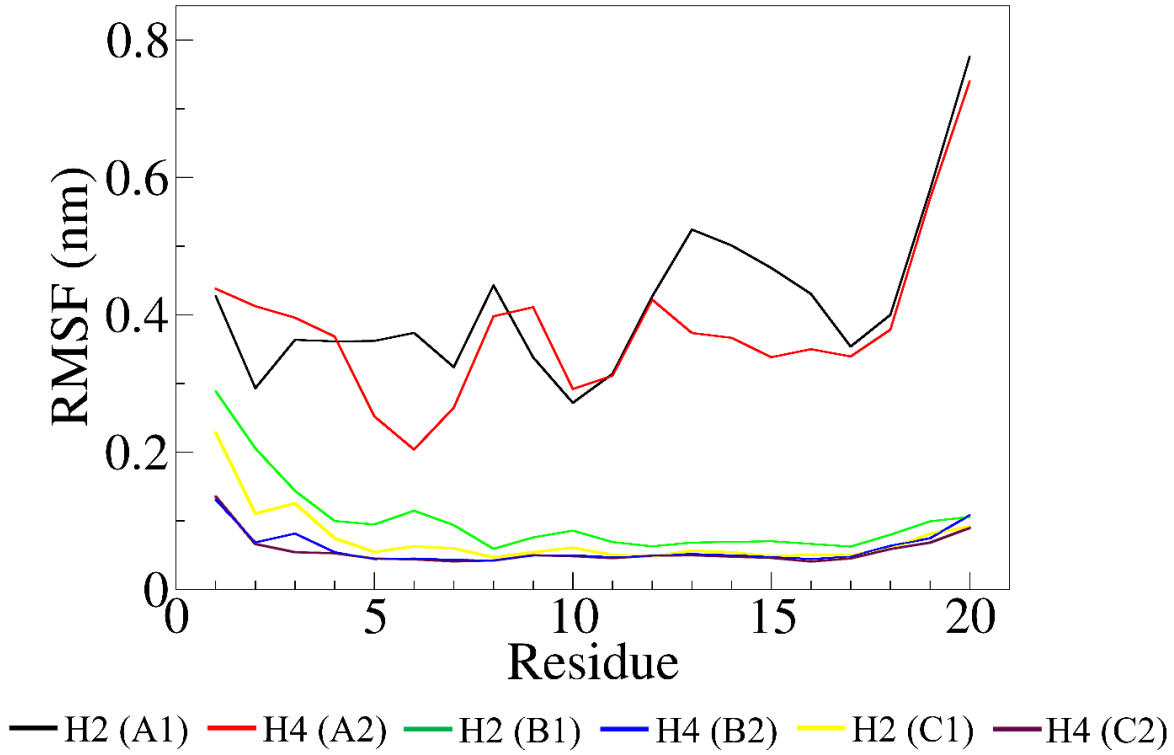


**Fig. 5.** Root-mean-square fluctuation (RMSF) of the backbone atoms for each residue of H2 and H4 peptides averaged over the whole simulation times. The black, green and yellow lines indicate H2 in A1, B1, and C1 simulations; the red, blue and brown lines indicate H4 in A2, B2, and C2 simulations, respectively.

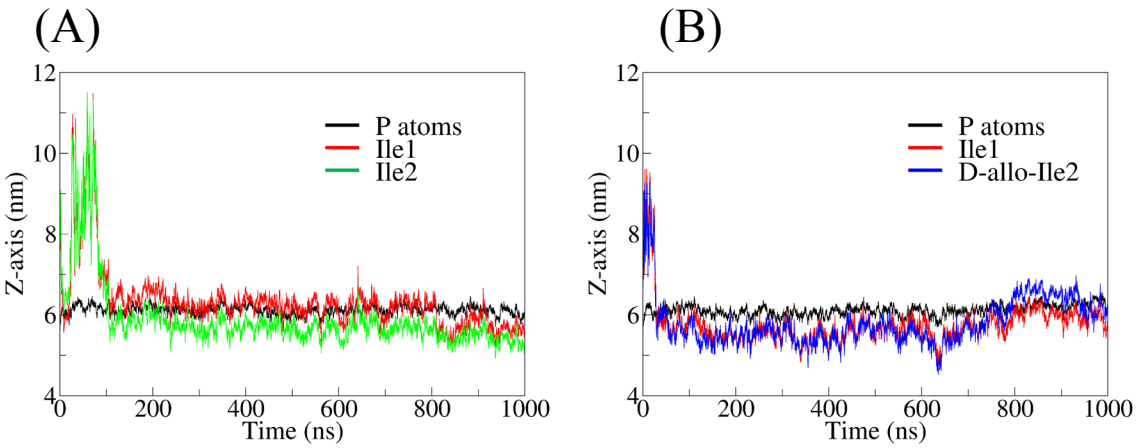


**Fig. 6.** Time courses of the location of Ile1 (red), Ile2 (green), D-allo-Ile2 (blue) residues along to the z-axis from the membrane surface during the A1 (A) and A2 (B) simulations. The locations of the residues of the peptides were calculated using the average of Z coordinates of the side chain atoms of Cβ, Cγ1, and Cγ2. The location of the membrane surface was estimated by the average Z coordinates of phosphorus atoms in the upper leaf of the membrane (Black lines). For the clarity of the graphs, data were averaged every 1000 points.

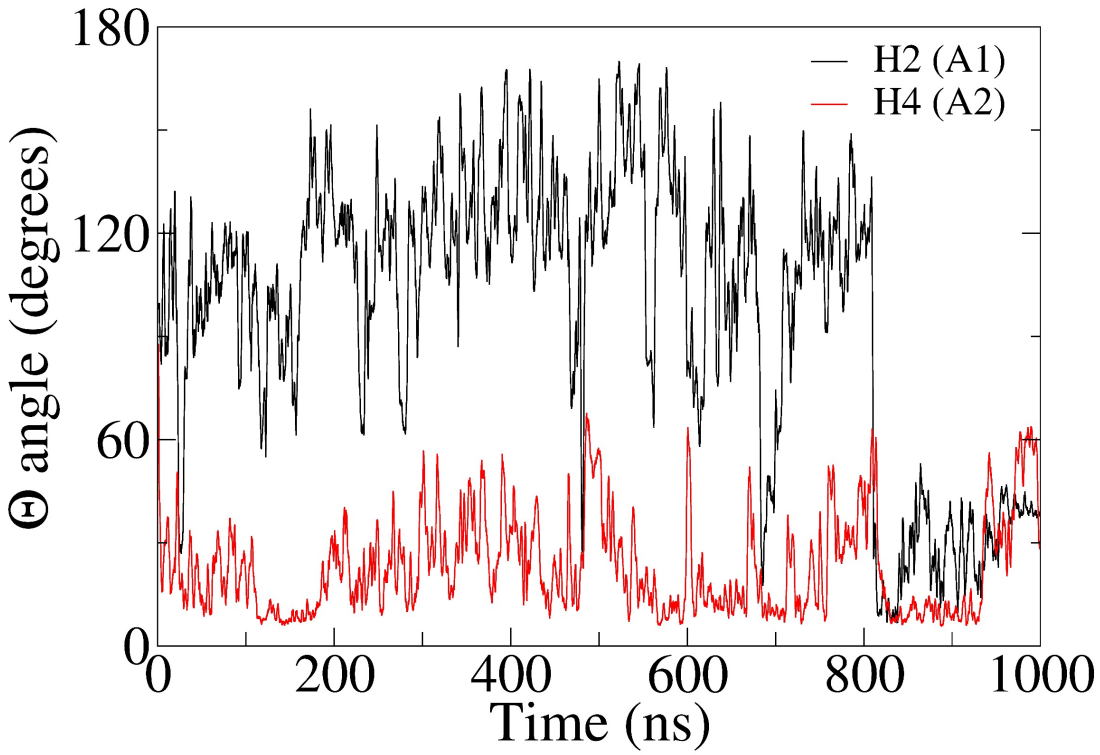


**Fig. 7.** Pseudo-dihedral angles between Ile1 and Ile2 of H2 and Ile1 and D-allo-Ile2 of H4 were shown according to the time during the A1 (black line) and A2 (red line) simulations. For the clarity of the graph, data were averaged every 1000 points.

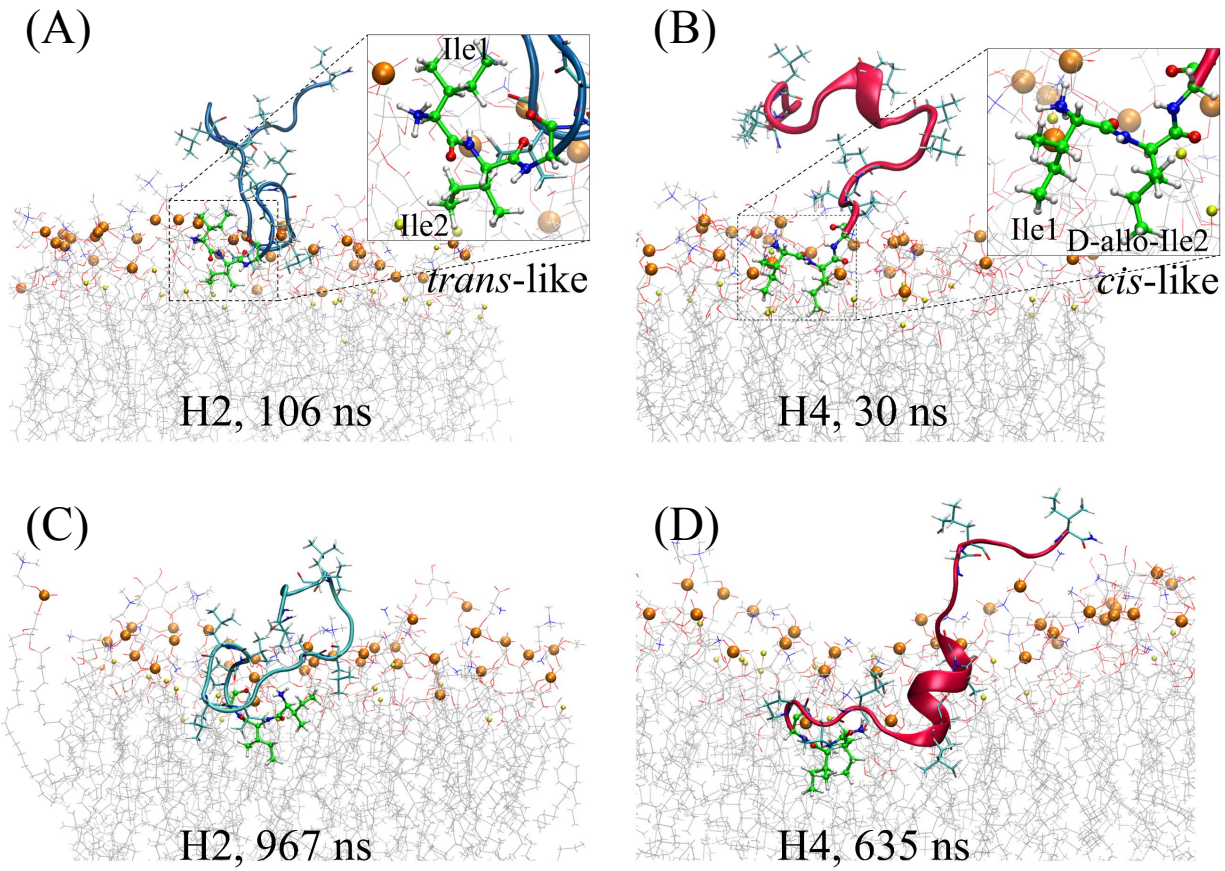


**Fig. 8.** The snapshots of the H2 and H4 peptides, which were inserted to the membrane for the first time, were shown in (A) and (B), respectively. The conformations of the residues 1 and 2 were represented using ball and stick model in the insets. The snapshots at the deepest penetrations of H2 and H4 were also shown in (C) and (D), respectively. Phosphorus atoms of the phospholipids and oxygen atoms of ergosterols were shown by orange and yellow balls. Hydrophobic side chains of the peptides were drawn as licorice model with VMD software.

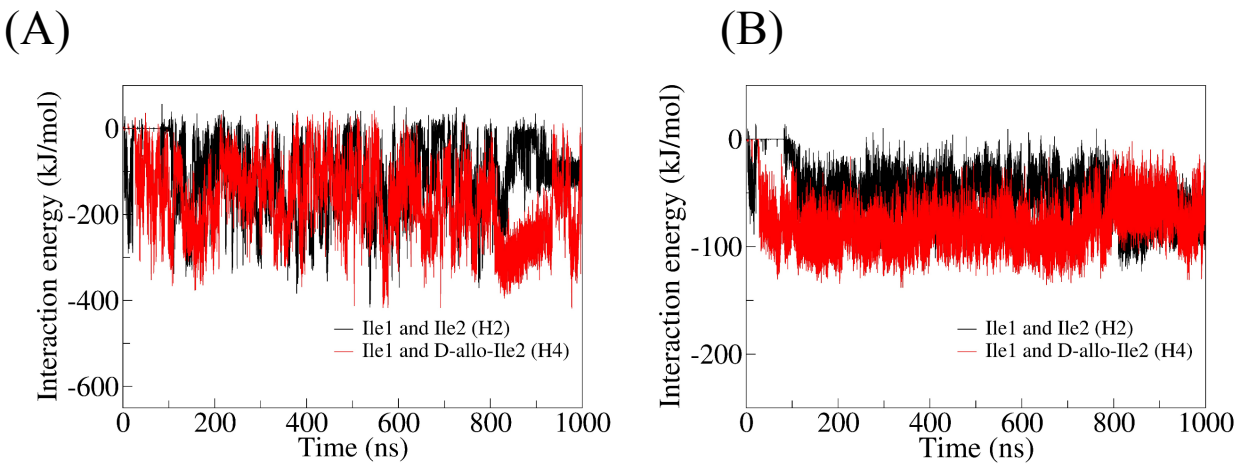


**Fig. 9.** Electrostatic (A) and van der Waals (B) interactions between first two side chains and the membrane molecules.

**Table 1.** The ID of the simulation systems, simulation lengths, peptide spices, and the number of molecules in the systems.

| ID | Peptide | System | Lipids | | | | | $Na^+$ ions | $Cl^-$ ions | $H_2O$ molecules |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | DOPE | DOPC | DOPI | DOPS | ERG | | | |
| A1 | H2 | A | 24 | 24 | 12 | 6 | 36 | 34 | 19 | 5954 |
| B1 | H2 | B | 16 | 16 | 8 | 4 | 26 | 11 | 2 | 2963 |
| C1 | H2 | C | 16 | 16 | 8 | 4 | 24 | 9 | 0 | 1888 |
| A2 | H4 | A | 24 | 24 | 12 | 6 | 36 | 34 | 19 | 5943 |
| B2 | H4 | B | 16 | 16 | 8 | 4 | 26 | 11 | 2 | 3147 |
| C2 | H4 | C | 16 | 16 | 8 | 4 | 24 | 9 | 0 | 1982 |

**Table 2.** The *cis*-like conformation percentages of residue 1 and 2 in the three different time regions and total simulation time.

| ID | Peptide | 0-200 ns, % | 200-800 ns, % | 800-1000 ns, % | Total, % |
|---|---|---|---|---|---|
| A1 | H2 | 36.13 | 20.00 | 94.94 | 38.52 |
| A2 | H4 | 99.63 | 99.94 | 99.97 | 99.88 |

**Table 3.** Interaction energies of the electrostatic and van der Waals terms between the first two residues of the peptides and the membrane molecules over the last 500 ns of A1 and A2 simulations. The more detail tables which include the standard deviation are shown in Table S2.

| Interaction energy* | Peptide/Amino acids | DOPE | DOPC | DOPI | DOPS | ERG | All membrane molecules |
|---|---|---|---|---|---|---|---|
| Electrostatic | Ile1-Ile2 (H2) | -1.2 | -50.1 | -47.6 | -7.9 | -0.5 | -107.3 |
| | Ile1-D-allo-Ile2 (H4) | -11.8 | -64.4 | -58.5 | -50.5 | -10.2 | -195.4 |
| van der Waals | Ile1-Ile2 (H2) | -1.1 | -31.5 | -15.6 | -0.1 | -12.6 | -60.9 |
| | Ile1-D-allo-Ile2 (H4) | -3.8 | -32.3 | -23.2 | -11.3 | -9.3 | -80.0 |

* All interaction energy values are expressed as [kJ/mol].