Molecular View of the Role of Fusion Peptides in Promoting Positive Membrane Curvature

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Supporting information for: Molecular view of the role of fusion peptides in promoting positive membrane curvature

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This supplementary material contains details about the parametrization of the influenza HA fusion peptide and its G1V and W14A mutant, as well as a more detailed version of the phase diagram presented in the main article. In addition, we present of the peptides’ effect on stalk formation in lamellar systems at low hydration.

Models for the fusion peptides

The models for the fusion peptides were based on NMR structures obtained in micelles at fusogenic pH. Particle types and bonded interactions were set according to the standard MARTINI protocol. Note that the secondary structure is pre-assigned in the model, based on the DSSP code. Details and further alterations for the individual peptides are given below. The complete parameter files are available from the MARTINI website (http://cgmartini.nl).

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**Wildtype at pH 5.0:** The model for the influenza HA fusion peptide at fusiogenic pH (pH 5.0) was optimized to match the fifth structure contained in the PDB entry 1IBN. The parameters were set corresponding to the sequence GLFGAIAGFIENGWEGMIDG and a secondary structure alpha-helical from residue 2-10 and 15-18 with a turn for residues 11-14 (corresponding to the DSSP code ∼HHHHHHHHHTTTTHHH∼). To reproduce the fixed angle between the two helices, the values for the bond, angle and dihedral potentials of the backbone beads of residue 8 to 16 were altered: the force constants for the bonds were raised to 1250 kJ mol$^{-1}$, the force constants for the angles were raised to 700 kJ mol$^{-1}$ and the minimum of the angle potential set to the corresponding angles found for the alpha carbons of the experimental structure. Additional dihedral angle potentials were introduced with a force constant of 400 kJ mol$^{-1}$, and a multiplicity of 1. Here also the minimum was set to the corresponding angle of the alpha carbons in the experimental structure.

**G1V mutant:** The secondary structure for the G1V mutant has been experimentally determined as alpha-helical over the whole length. Since standard parameters for alpha-helical regions are included in the MARTINI forcefield, there was no need for further optimization with respect to the experimental structure. The topology was obtained based on the sequence VLFGAIAGFIENGWEGMIDG and a secondary structure fully alpha-helical except for the two terminal residues at both ends of the peptide. No additional potentials were introduced.

**W14A mutant:** For the W14A mutant, the secondary structure contains several motifs not supported in the MARTINI model. To represent these structures using the current MARTINI model, a provisional topology was obtained using the correct sequence (GLFGAIAGFIENGGAEGMIDG) but the secondary structure of the wildtype. This topology was then adapted to match the tenth structure contained in the PDB entry for the mutant peptide (2DCI) by setting the minimum values for the angle and dihedral potentials of the backbone to match the values found for the alpha-carbons in the experimental structure. The force constants for bond, angle and dihedral potentials connecting the backbone beads were set to 1250, 700 and 400 kJ mol$^{-1}$, respectively, except for

S2
the termini, for which the originally assigned values were kept.

**Wildtype at pH 7.4:** For the model of the wildtype peptide at non-fusogenic pH (pH 7.4) we used the first structure in the PDB entry 1IBO. A topology was obtained according to the DSSP code ~HHHHHHHHTTTTSS~~~. To constrain the structure in the non-helical regions, the minimum values for the angle and dihedral potentials of the backbone were set to match the values found for the alpha-carbons in the experimental structure. The force constants for bond, angle and dihedral potentials connecting the backbone beads were set to 1250, 700 and 400 kJ mol$^{-1}$, respectively, except for the termini, for which the originally assigned values were kept.

**Phase Diagram**

The simulations of spontaneous aggregation presented in Fig. 3 in the main article were performed at different temperatures in the range of 270–315 K. Figure 1 shows the results as a two-dimensional bar-graph, indicating the temperature as well as the exact hydration level at which the simulations were performed.

**Stalk formation**

To assess the ability of the influenza HA fusion peptide to induce stalks in lamellar systems, we performed three sets of simulations of two closely apposed DOPE (dioleoylphosphatidylethanolamine) bilayers: one without peptides, one with peptides in all monolayers, and one with peptides only in the monolayers facing one of the two water layers (cf. Figure 2).

DOPE was chosen as a lipid with a high spontaneous negative mean curvature, giving the system a strong “incentive” to leave the lamellar phase and potentially reducing the energy of stalks in accordance with their overall negative mean curvature.
Figure 1: Overview of the phases adopted after 12 µs in dependence on temperature and level of hydration (W/L) for pure DOPE, a 1:1 mixture of DOPE and DOPC, and pure DOPC. Results are shown for simulations containing no peptides, the influenza HA fusion peptide (WT), and the non-fusogenic G1V and W14A mutants. Green indicates an inverted hexagonal phase, red a stalk phase, blue a lamellar phase, cyan a single bicontinuous cubic diamond phase, and magenta a double bicontinuous cubic diamond phase. Note that only colored boxes present performed simulations; white boxes merely serve as placeholders to guide the eye.
Figure 2: The three principal setups for our simulations of stalk formation. All setups contain two parallel bilayers consisting of 98 lipids each, separated by two water layers with equal numbers of water beads. The difference is the distribution of fusion peptides: we performed simulations without peptides (NP), with one peptide in each monolayer (SP), and with one peptide only in the monolayers facing one of the two water layers (AP). For all setups, multiple simulations were performed using different hydration levels and initial velocities.

**Simulation setup**

The simulations were started from a configuration with two bilayers of 98 DOPE lipids each with an equal amount of lipids per monolayer, separated by two water slabs consisting of an identical number of beads (including sodium ions to counter the charges in the simulations containing peptides). As illustrated in Figure 2, three sets of simulations were run containing either no peptides, one peptide in every monolayer or one peptide only in the monolayers adjacent to one of the two water layers. For every set of conditions, a total of four simulations starting with different initial velocities were run. Two different hydration levels were used; one at which the peptide-free reference simulations readily formed stalks within the simulation time (2.24 water molecules per lipid), and a slightly higher hydration level (2.65 water molecules per lipids) at which the peptide-free system only formed stalks in one of the four simulations performed.

The simulations were carried out at a reference temperature of 323 K with a coupling time constant of 0.5 ps, and with semi-isotropic pressure coupling with a compressibility of $1 \times 10^{-5}$ bar$^{-1}$, a coupling time constant of 1.2 ps and a reference pressure of 1.0 bar for all directions.
using the Berendsen scheme.\textsuperscript{6} The simulations were run for 25,000,000 steps, corresponding to an effective time of 4 $\mu$s.

To avoid premature formation of stalks due to insufficient equilibration in systems of low hydration, the systems were prepared starting at the higher hydration levels, gradually removing a small number of water beads and equilibrating the system to obtain the systems at low hydration.

**Results**

In our simulations, the presence of fusion peptides in both monolayers of the lamella did not act to facilitate stalk formation. In fact, the peptides were rather unexpectedly found to prevent stalks at a hydration level of 2.24 water molecules per lipid, at which stalks spontaneously formed in the absence of peptides. Systems with an asymmetric distribution in which the peptides were only present in the monolayers around one of the two water layers of the systems (\textit{cis} monolayers) formed stalks at an efficiency comparable to the peptide-free reference. However, the initial stalk to form in these simulations always occurred between the \textit{trans} monolayers not containing the peptides. Stalks between the \textit{cis} monolayers appeared afterwards at the lower of the two hydration levels, and not at the higher.

To complement our results, we used trajectories of conditions at which no stalks had formed to determine the peptides’ effects on the properties of isolated bilayers as shown in Table 1. For these, we also included similar simulations with bilayers consisting of DOPC (dioleoylphosphatidylcholine). Bilayers of both DOPE and DOPC display an increase of surface area accompanied by a reduced thickness and an increased inter-bilayer distance in the presence of the fusion peptides.

**References**


Table 1: Properties of lipid bilayers consisting of 98 lipids of DOPE or DOPC at 323 K at a hydration level of 2.65 water molecules per lipid in the presence of one peptide in both monolayers (SP) and in the absence of peptides (NP). Shown are the box volume $V_{\text{box}}$, the area of the bilayers $A_{XY}$, the height of the box in the dimension perpendicular to the bilayers $Z_{\text{box}}$, the bilayer thickness $D$ (defined as the average distance between the peaks representing phosphate in the density plot) and the distance between the bilayers $d_{\text{im}}$ (calculated as $Z_{\text{box}}/2 - D$). In addition, the difference between the values in the presence and absence of the peptides is indicated ($\Delta$). The errors given are error estimates obtained via block-averaging.\(^7\)

<table>
<thead>
<tr>
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<th>$V_{\text{box}}$ (nm(^3))</th>
<th>$A_{XY}$ (nm(^2))</th>
<th>$Z_{\text{box}}$ (nm)</th>
<th>$D$ (nm)</th>
<th>$d_{\text{im}}$ (nm)</th>
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<td>-0.021</td>
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<tr>
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