Supporting information for:
Hydration-mediated stiffening of collective membrane dynamics by cholesterol

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S1
Molecular Dynamics Simulation Protocol

The starting configurations for our simulations were based on pre-equilibrated structures of dioleoylphosphatidylcholine (DOPC) bilayers with cholesterol (CHL) contents of 0–50 % from Jämbeck et al. All water molecules were removed and the system sizes increased by a factor of 4 compared to the pre-equilibrated bilayers, *i.e.*, the number of lipids was doubled in both lateral (x,y) dimensions, creating systems with a total of 512 lipids each (256 per leaflet). The simulation boxes were increased by 25 % in the z-direction and resolvated using the TIP4P/2005 water model, followed by energy minimization (10,000 steps of steepest descent). Care was taken not to insert water molecules in the hydrophobic core of the bilayers. The final simulation boxes contained between 21,700 and 25,797 water molecules. Figure S1 below shows a snapshot from a simulation of the pure DOPC bilayer.

![Snapshot of a simulation of the pure DOPC bilayer](image)

Figure S1: Top and side view of a snapshot from a simulation of the pure DOPC bilayer.

Table S1 lists the simulated membrane systems and their specifications.

Starting from the pre-equilibrated bilayers, we equilibrated all solvated bilayers for 25 ns (150 ns for the control simulation with 150 mN NaCl) using a 2 fs time step to integrate the equations of motion using the leap-frog integrator. Equilibrations were performed in the isothermal-isobaric ensemble (NPT) at 300 K and 1 bar using the v-rescale thermostat and Berendsen barostat, respectively, applying semi-isotropic pressure coupling. Periodic boundary conditions were applied in all three dimensions. The MD simulations were carried...
Table S1: A list of the systems studied in this work and their specifications. The area per lipid (APL) was determined as the average lateral box area at equilibrium divided by the number of lipids per leaflet $N_{\text{lipids}}$. The number of hydration water molecules $N_{\text{hydwat}}$ per lipid was determined from the number of water molecules within 5 Å of the headgroup phosphorus (DOPC) and oxygen (CHL) atoms. $N_w/N_l$ is the number of water molecules per lipid in the simulation box.

<table>
<thead>
<tr>
<th>Name</th>
<th>mol-% CHL</th>
<th>APL ($\text{Å}^2$)</th>
<th>$N_{\text{lipids}}$</th>
<th>$N_{\text{hydwat}}/\text{lipid}$</th>
<th>$N_w/N_l$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPC-ONLY</td>
<td>0</td>
<td>69.4</td>
<td>256</td>
<td>6.7</td>
<td>42</td>
</tr>
<tr>
<td>DOPC-ONLY-356</td>
<td>0</td>
<td>69.6</td>
<td>178</td>
<td>6.7</td>
<td>47</td>
</tr>
<tr>
<td>DOPC-150mM-NaCl</td>
<td>0</td>
<td>68.5</td>
<td>256</td>
<td>6.6</td>
<td>43</td>
</tr>
<tr>
<td>DOPC-HEAVY-WAT</td>
<td>0</td>
<td>69.9</td>
<td>256</td>
<td>6.8</td>
<td>42</td>
</tr>
<tr>
<td>DOPC-0.1CHL</td>
<td>10</td>
<td>63.8</td>
<td>256</td>
<td>6.1</td>
<td>45</td>
</tr>
<tr>
<td>DOPC-0.3CHL</td>
<td>30</td>
<td>52.8</td>
<td>256</td>
<td>4.9</td>
<td>46</td>
</tr>
<tr>
<td>DOPC-0.5CHL</td>
<td>50</td>
<td>44.3</td>
<td>256</td>
<td>3.7</td>
<td>50</td>
</tr>
</tbody>
</table>

out with the GROMACS 2016.3\textsuperscript{57} software package using the all-atom Slipids force field\textsuperscript{51-53} for the lipids and the TIP4P/2005 water model.\textsuperscript{54}

The SETTLE\textsuperscript{58} and LINCS\textsuperscript{59} algorithms were employed to constrain the internal degrees of freedom of water molecules and all bonds in the lipid molecules, respectively. Short-range non-bonded electrostatic and Lennard-Jones (6,12) interactions were treated with a Verlet-buffered pair list\textsuperscript{510} with potential energy functions smoothly shifted to zero at a 10 Å cut-off distance. Long-range electrostatic interactions were treated using the smooth particle mesh Ewald (PME) scheme\textsuperscript{511,512} with a grid spacing of 1.2 Å and cubic spline interpolation. Analytical dispersion corrections were applied to energy and pressure to account for truncated Lennard-Jones interactions.

We found that during the last 10 ns of the simulations the box sizes remained stable. Figure S2 shows the average box dimensions for selected systems during the last 10 ns. Error bars depict the respective standard deviations.

The lateral ordering of lipids was assessed via radial distribution functions $g(r)$ (RDF) as shown in Fig. S4. For the cholesterol containing systems the RDF was evaluated for all lipids as well as only DOPC lipids. The RDFs were determined for headgroups, tails and terminal CH$_3$ groups separately. In case of the headgroups, all phosphorus atoms (DOPC)
Figure S2: Average lengths of box vectors of the solvated lipid bilayers during the final 10 ns of the equilibration simulations. Standard deviations over 10 ns are shown as error bars.

Figure S3: Atom selections for headgroups and tails of DOPC and cholesterol lipids, respectively, used in the cross-correlation analysis.
and oxygen atoms (CHL) of the headgroups were selected.

Figure S4: Radial distribution functions $g(r)$ of lipid atoms in lateral dimensions (x,y). Shown are the results for the cholesterol concentrations 0–50 mol-%. The analysis was performed for headgroups, tails, and terminal CH$_3$ groups. We computed $g(r)$ of the headgroups selecting phosphorus atoms of the DOPC lipids and for cholesterol the oxygen atom. In case of the lipid tails, all carbon atoms of the alkyl chains were selected. The data for tails are shown in gray. The RDFs of only DOPC headgroups are shown in blue and for both cholesterol and DOPC headgroups the RDFs are shown in red. The RDFs of the terminal CH$_3$ groups are shown in magenta.

The production simulations were carried out in the microcanonical ensemble (NVE). For each system, 10 configurations, spaced by 1 ns in time, were taken from which the subsequent NVE simulations were started. The NVE simulations were performed for 1 ns each with positions and velocities saved to disk every 20 fs. This high time-resolution is required to sample the collective vibrations of non-hydrogen atoms, i.e., we could resolve frequencies up to $\omega \approx 830$ cm$^{-1}$ with a resolution of $\Delta \omega \approx 0.017$ cm$^{-1}$.
Details on Analyses

The collective dynamics of the lipids were assessed via correlations of velocity fluctuations as described by Eq. 1 of the main text. A control simulation with 150 mM NaCl was performed to investigate possible ion effects on the collective headgroup dynamics (see Table S1). The lipid–lipid cross-correlation spectra (left) and dispersion curve (right) shown in Figure S5A demonstrate that the presence of 150 mM NaCl does not alter the collective motions of the lipids, in line with the negligible change in APL and \( \nu_{\text{hydrat}} \) (Table S1). Performing the cross-correlation analysis for lipid headgroups, tails and centers-of-mass. Figure S3 shows the atom selections for headgroups and tails of DOPC and cholesterol, respectively.

A control simulation with 150 mM NaCl was performed to investigate possible ion effects on the collective headgroup dynamics (see Table S1). The lipid–lipid cross-correlation spectra (left) and dispersion curve (right) shown in Figure S5A demonstrate that the presence of 150 mM NaCl does not alter the collective motions of the lipids, in line with the negligible change in APL and \( \nu_{\text{hydrat}} \) (Table S1).

Further we tested the convergence of the spectra by using only 500 ps (instead of 1 ns) for the analysis. The results are shown in Fig. S5B and demonstrate convergence of the computed spectra presented in Figures 2 and 3 of the main text.

The decay of correlation intensity was measured in terms of the correlation length \( d_{\text{corr}} \) of the collective modes, where \( d_{\text{corr}} \) is the distance at which the correlation intensity has dropped to \( 1/e \) of its value at the interchain contact distance (6 Å). Figure S7 shows the (normalized) correlation intensities as a function of lipid–lipid distance \( d \) for 0–50 mol% cholesterol for headgroups, tails, and COM. The exponential fits are shown as solid lines.

The couplings of the lipid headgroup atoms to their hydration shells were assessed by lipid–water cross-correlation spectral densities \( I(\omega, r) \propto \int v_{\text{lipid}}(t) \rho(t, r) \, dt \) using localized smooth density kernels \( \rho(t, r) \) for water oxygen atoms. We sampled localized densities of water oxygen velocities up to distances of 10 Å from the lipid headgroup surface. Analogously, we utilize the dispersion relation and obtain propagation velocities of the collective
Figure S5: Lipid–lipid cross-correlation spectra $I(\omega, d)$ of mass-weighted atomic velocities computed at different lipid atom–lipid atom distances $d$. All spectra were smoothed using Gaussian window functions of width 10 cm$^{-1}$. (A) Left: Cross-correlation spectra for the headgroups of the pure DOPC bilayer with 150 mM NaCl. Right: Dispersion relation of the negative-intensity peak trace of the spectra shown on the left. The dashed line indicates the linear fit curve (fitting up to $k = 0.6$ Å$^{-1}$). (B) Left: Cross-correlation spectra for the headgroups of the pure DOPC bilayer using only 500 ps of the simulation trajectories (instead of 1 ns). Right: Dispersion relation of the negative-intensity peak trace of the spectra shown on the left. The dashed line indicates the linear fit curve (fitting up to $k = 0.6$ Å$^{-1}$).
lipid–water motions. Figure S6 shows the dispersion curves obtained for cholesterol concentrations of 0 and 50 mol-%. In this case, we evaluated the dispersion relations for both the ordinary (0.60–0.90 Å\(^{-1}\)) and fast sound (1.00–1.60 Å\(^{-1}\)) modes.

![Dispersion curves](image)

Figure S6: Dispersion curves obtained from velocity cross-correlation spectra of lipid headgroup atoms and hydration water oxygens. Results are shown for both for the ordinary sound mode (red curve) and the fast sound mode (blue curve). The fitting ranges for ordinary and fast modes are shown as blue and red shaded areas, respectively.

![Normalized correlation intensities](image)

Figure S7: Normalized correlation intensities as a function of lipid–lipid distance for the simulated systems. The exponential fit curves are shown as colored solid lines.

Complementing our analysis of correlated motions in the lipid bilayers, the time cross-correlation functions (TCCF) were evaluated. Figure S8 shows the distance-dependent TC-CFs for the headgroups of the pure DOPC bilayer. The correlation functions were normalized with respect to the corresponding auto-correlations at zero correlation time, \(i.e.,\) in units of \(k_B T/2\). The inset shows the peak intensities over lipid atom–lipid atom distance.
Figure S8: Time cross-correlation functions of correlated velocity fluctuations of the lipid headgroup atoms in the pure DOPC bilayer. The TCCFs were normalized with respect to the corresponding auto-correlations at $t = 0$, which is proportional to the average thermal energy. The inset shows the peak intensities over lipid atom–lipid atom distance.

References


(S6) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R.


