Electronic Supplementary Material (ESI) for Soft Matter.
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Electronic Supplementary Information for 'On the lipid flip-flop and phase transition coupling'

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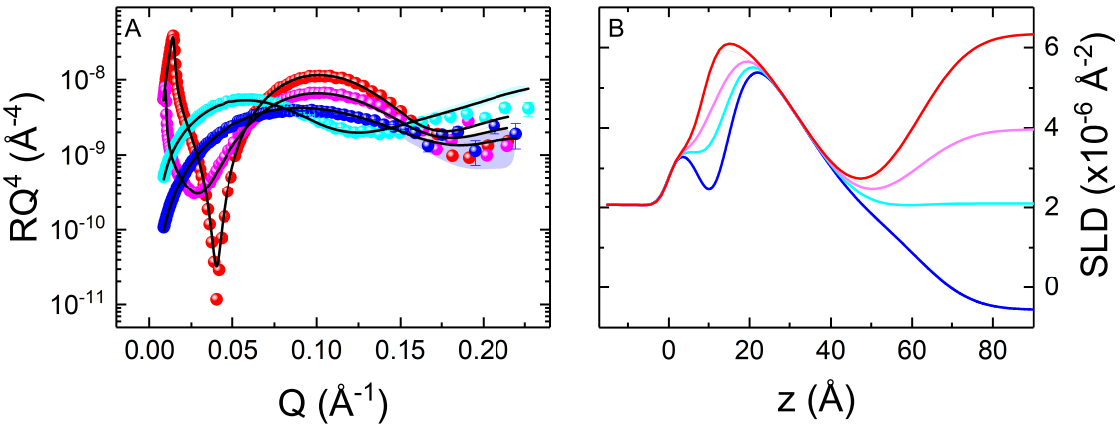

Figure **S1**: **A.** Reflectivity curves measured at 25 °C for the asymmetric sample. Contrast variation was exploited to increase the accuracy of the modeling. In particular, experimental data was collected in D_2O (red symbols), H_2O (blue symbols), a 66:34 D_2O:H_2O mixture (magenta symbols), and a 38:62 D_2O:H_2O mixture (cyan symbols). Model curves obtained by the global fit of all the experimental data are shown as black full lines, while 95% confidence intervals of the model are indicated as shaded area using the same color code as for the experimental data. **B.** Scattering length density profiles and 95% confidence intervals obtained from the modeling of the data shown in A. The same color code as in A applies. The analysis was performed with the Aurore software.[1]

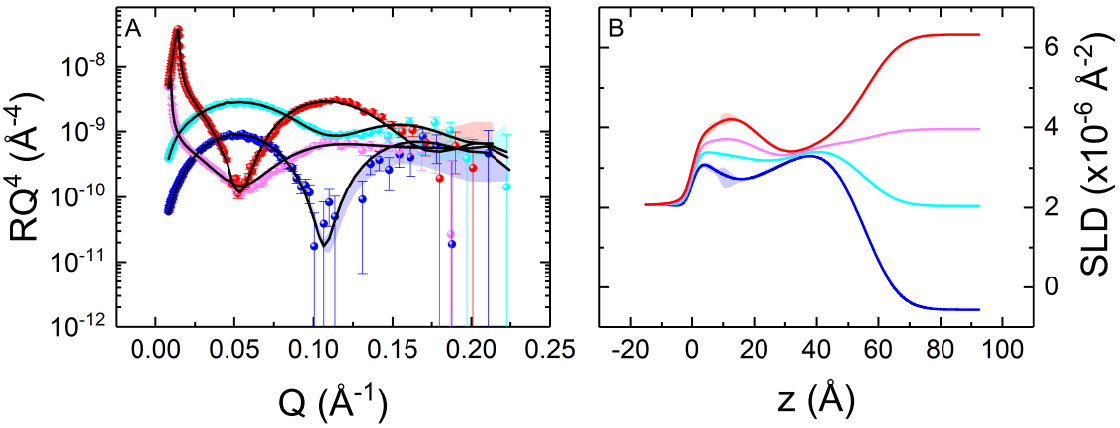

Figure **S2**: **A.** Reflectivity curves measured at 65 °C for the asymmetric sample upon loss of asymmetry (mixed state). Contrast variation was exploited to increase the accuracy of the modeling. In particular, experimental data was collected in D_2O (red symbols), H_2O (blue symbols), a 66:34 D_2O:H_2O mixture (magenta symbols), and a 38:62 D_2O:H_2O mixture (cyan symbols). Model curves obtained by the global fit of all the experimental data are shown as black full lines, while 95% confidence intervals of the model are indicated as shaded area using the same color code as for the experimental data. **B.** Scattering length density profiles and 95% confidence intervals obtained from the modeling of the data shown in A. The same color code as in A applies. The analysis was performed with the Aurore software.[1]

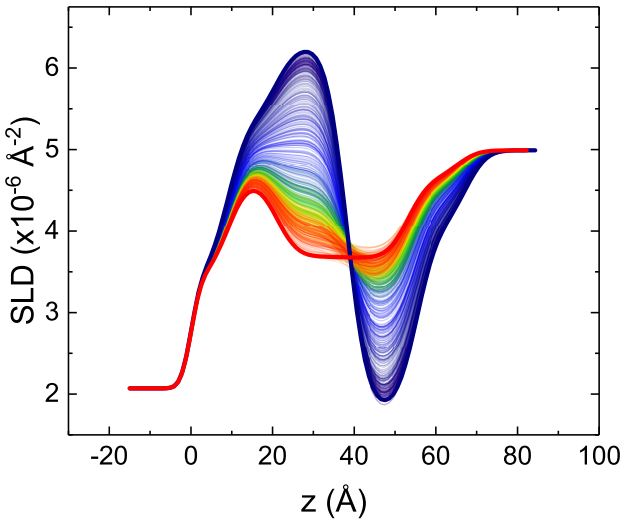

Figure **S3**: Scattering length density profiles obtained from the analysis of TTR-NR data measured during the slow temperature scan. The profile related to the lower temperature (asymmetric composition) is shown in blue and the profile of the final mixed SLB is plotted in red. Intermediate colors correspond to intermediate temperatures as described in the main text.

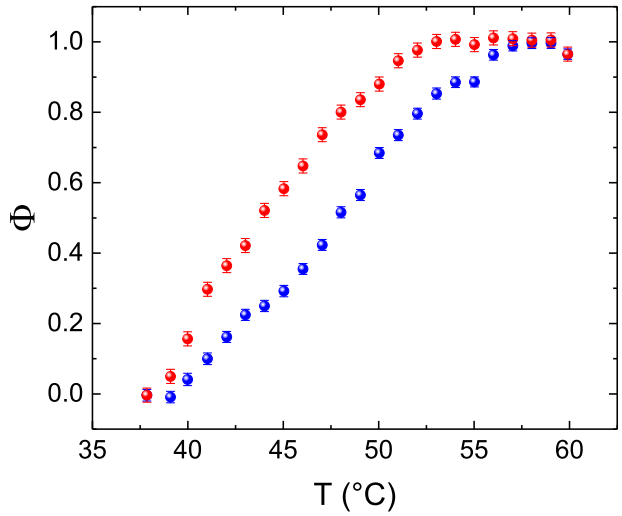

Figure **S4**: Temperature dependence of the amount of fluid lipids in a DPPC bilayer during the main $L_\beta - L_\alpha$ phase transition. These data were not obtained from an asymmetric samples, but from a symmetric one. Reproduced with permission from reference.[2]

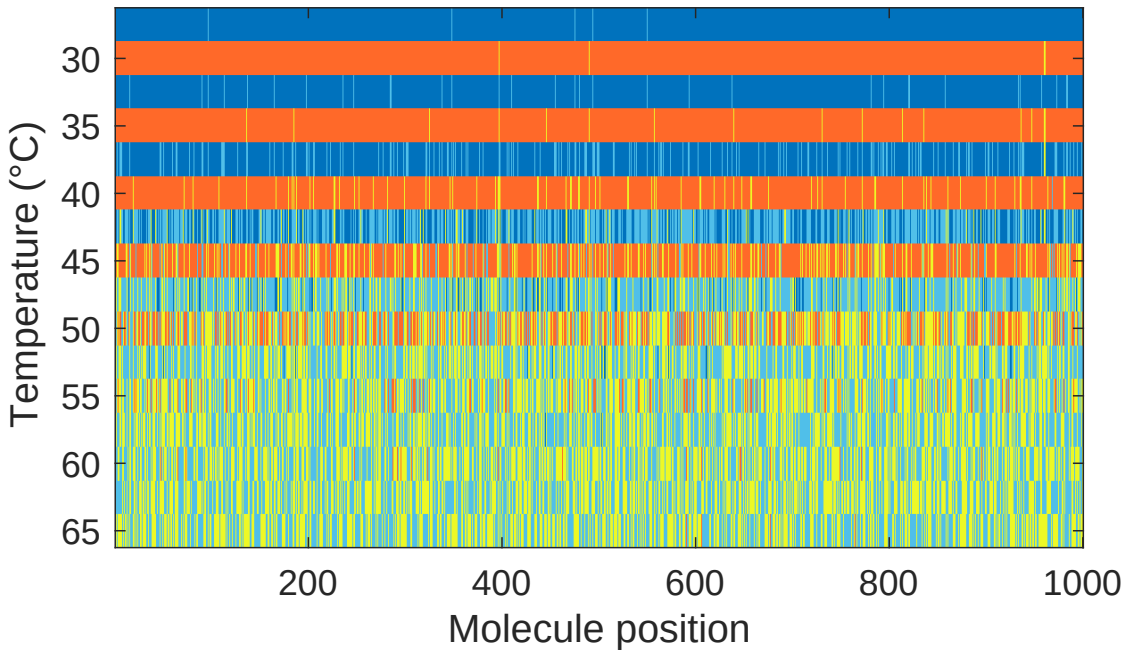

Figure **S5**: Evolution of the state of lipid molecules in an asymmetric bilayer determined by Monte Carlo simulation. At the lower temperature, the two leaflets were populated either by deuterated (blue) or hydrogenated (orange) molecules in the gel state, without any initial degree of mixing. As temperature increased, phase transition of individual molecules was possible. This phase transition was described in terms of the Φ_{prox} and Φ_{dist} parameters as described in the main text. Once in fluid phase (yellow for the hydrogenated molecules, cyan for the deuterated ones), molecules were allowed to undergo flip-flop events, i.e. to translocate from one leaflet to the other one. MC simulations were performed using a temperature ramp going from 25 °C to 65 °C with constant temperature steps (1 °C), during which the system was sampled for different times. Only if the sampling time was not long enough to allow for a large number of LFF events, the system did not mix. In all other cases, the temperature dependence of the time-averaged SLD overlapped (see Figure **S6**). Spatial constraints were tested but did not have any clear impact on the overall LFF behaviour.

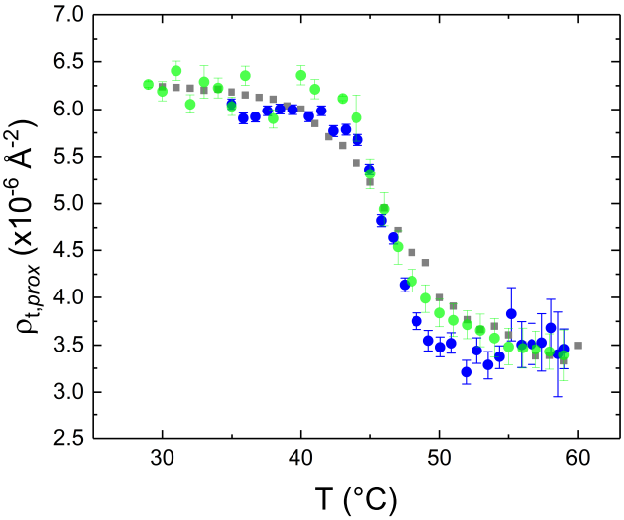

Figure **S6**: Temperature dependence of the SLD of the hydrophobic region of the proximal leaflet obtained from the analysis of TTR-NR data for the fast (blue circles) and slow (green dots) temperature scans. The temperature evolution of the parameter obtained from a Monte Carlo simulation is plotted with gray squares. Since the slow scan included constant temperature steps, the average value is reported. To account for initial compositional differences, the system for the simulation was prepared with the original level of mixing as the one reported in the main text.

References

(1) Gerelli, Y. Aurore : new software for neutron reflectivity data analysis. *Journal of Applied Crystallography* **2016**, *49*, 330–339.

(2) Gerelli, Y. Phase Transitions in a Single Supported Phospholipid Bilayer: Real-Time Determination by Neutron Reflectometry. *Physical Review Letters* **2019**, *122*, 248101.