Supplementary Information for

"Elucidating Mysteries of Phase-Segregated Membranes: Mobile-Lipid Recruitment Facilitates Pores' Passage to the Fluid Phase"

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2. The movie files **xi-0.8-phase-segregated-bilayer-top-view.mpg** and **xi-0.8-phase-segregated-bilayer-water-side-view.mpg** show the time evolution of the system (~2 μs) after a pore (ξ_0=0.8, R~1nm) was created at the centre of the L_o domain. The bilayer is depicted from above. At some point, the pore moves across the periodic y-boundary of the simulation cell. Colour code: DPPC-DIPC-CHOL = Blue-Red-Yellow. The second movie shows a lateral view of the system along the y-axis. Only water molecules were included in the rendering, to emphasize the membrane surfaces and the drifting transmembrane water channel.
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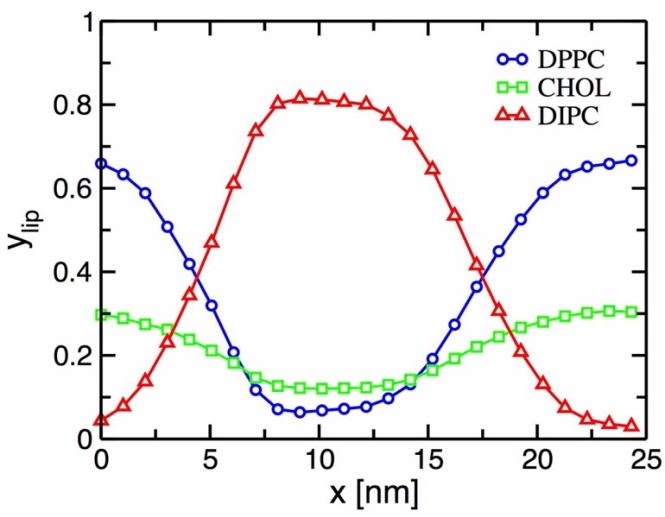

Figure S1: Membrane composition across a phase-segregated dipalmitoyl-phosphatidylcholine (DPPC), dilinoleyl-phosphatidylcholine (DIPC), and cholesterol (CHOL) membrane (DPPC:DIPC:CHOL) at T=295K. The x-direction runs perpendicular to the domain borders; $y_{lip} = N_i / (N_{DPPC} + N_{DIPC} + N_{Chol})$. The DPPC-rich L_o domain has an average composition of (0.67:0.04:0.29). The DIPC-rich L_d domain has a composition of (0.07:0.81:0.12). As noticed by Risselada et al. a temperature of 295K is optimal to observe clear phase separation within accessible simulation time scales. A higher temperature brings the system closer to the critical point making the identification of phase domains much more difficult.

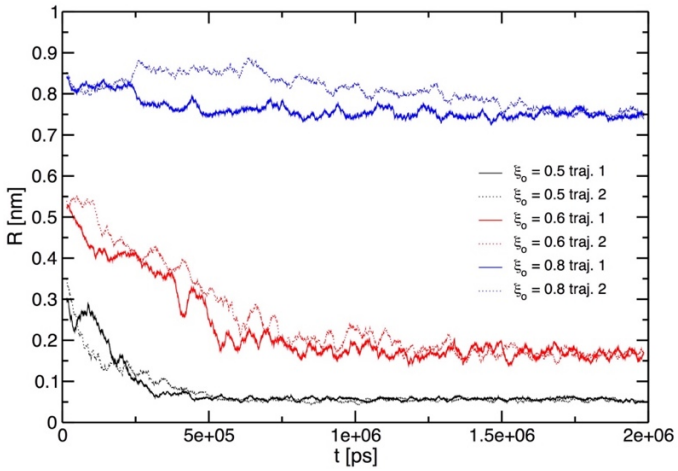

Figure S2: Pore radius, R, as a function of time for each of the 2 μs MD production runs reported in the paper. In all cases pores were created (t=0) at the centre of the L_o domain, on the final configuration of the 4 μs simulation of the phase-segregated membrane. In order to prevent spontaneous pore collapse, ξ_0 was harmonically restrained to the values annotated on the figure. Larger pores (ξ_0=0.8) do not change size when crossing the domain boundary, smaller pores do (ξ_0=0.6,0.5). For ξ_0=0.8 and 0.6 pores are toroidal and hydrophilic, whether located in L_o or L_d, and a water channel crosses the bilayer. For ξ_0=0.5, the membrane defect is hydrophobic and amounts to a local lowering of the lipids density.

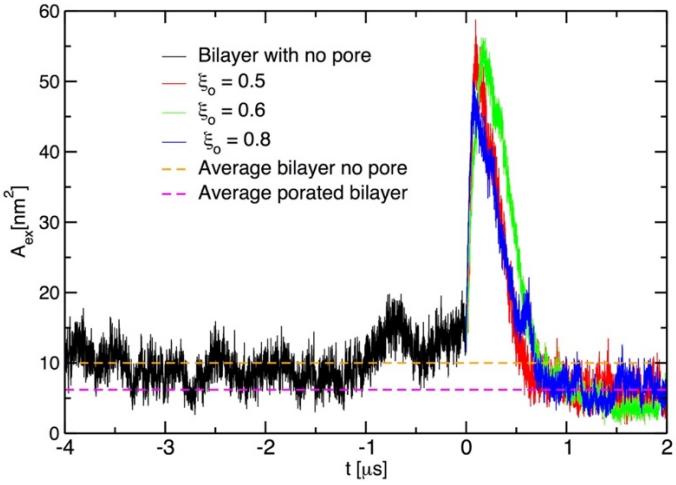

Figure S3: Time evolution of membrane excess area. The excess area is the difference between the actual surface area of the undulated membrane and the simulation box's X-Y area. The area of the undulated membrane was computed by fitting a geometric surface to the phosphate groups of DPPC and DIPC, as described in ref. 29, and implemented in g_lomepro.[2] This utility code also allowed us to correlate pore position with local membrane curvature. The black line (spanning negative times) corresponds to A_{ex} evaluated on the non-porated phase-segregated bilayer. Pores with ξ_0=0.8(blue), ξ_0=0.6(green), and ξ_0=0.5(red), created at t=0, immediately induce membrane bending resulting in larger values of A_{ex}. The deformation relaxes as stress is released while the pores drift and reach the centre of the L_d phase.

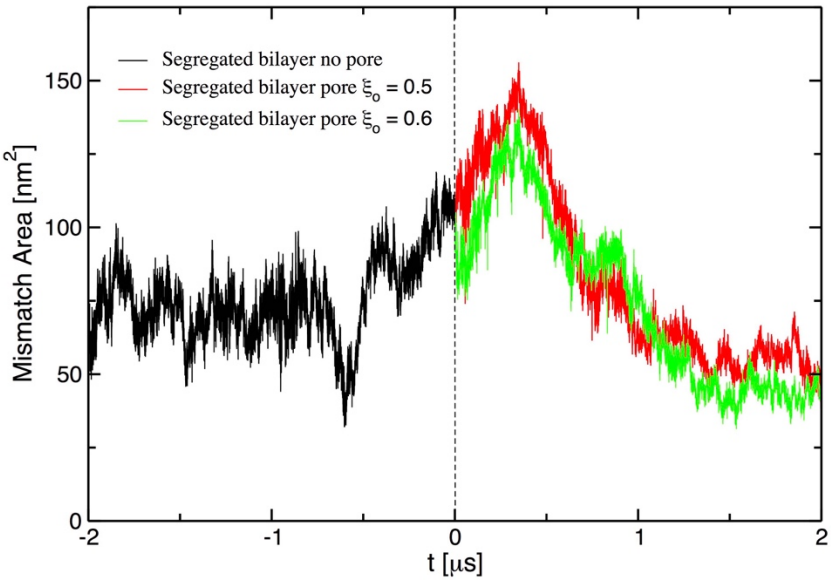

Figure S4: The mismatch area quantifies the overall extent to which the domains' upper and lower leaflets are out of kilter. The positive value shown by the non-porated

[2] http://www3.mpibpc.mpg.de/groups/de_groot/g_lomepro.html

membrane (black line) is due to a small surface tension that controls the degree of registration between the two domain leaflets (see ref. 3). Pores are created in L_o at t=0, inducing membrane curvature and pushing the upper and lower leaflets out of kilter. The mismatch area goes back to normal once the pores reach L_d. Only one trajectory for ξ_0=0.6(green) and ξ_0=0.5(red) is shown, the others depict similar trends.

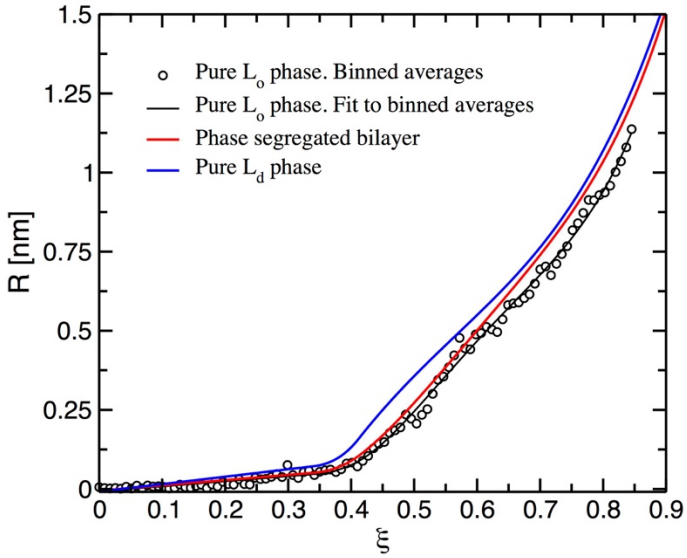

Figure S5: R(ξ) functions computed by Monte Carlo (see paper) from the various simulated systems, as annotated on Figure.

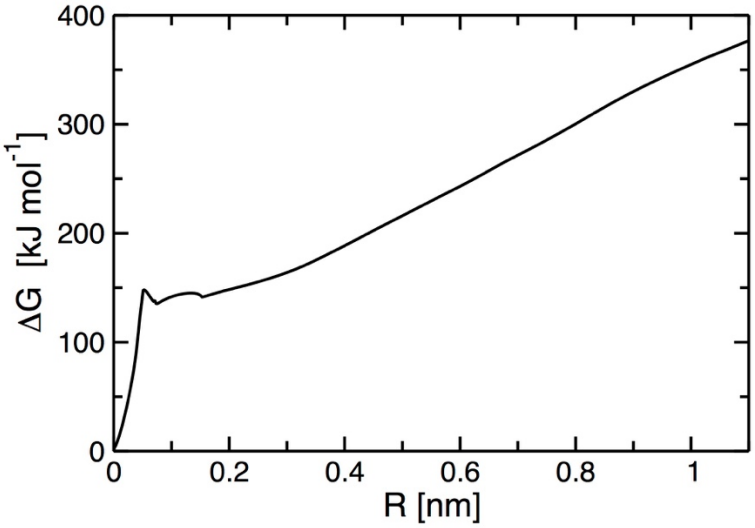

Figure S6: Difference in free energy cost for creating a pore of radius R in the bulk of L_o or L_d, i.e $\Delta G(R)=\Delta G_{Ld}(R)- \Delta G_{Lo}(R)$. $-\Delta G(R)$ is the driving force that pushes the pore from L_o to L_d.

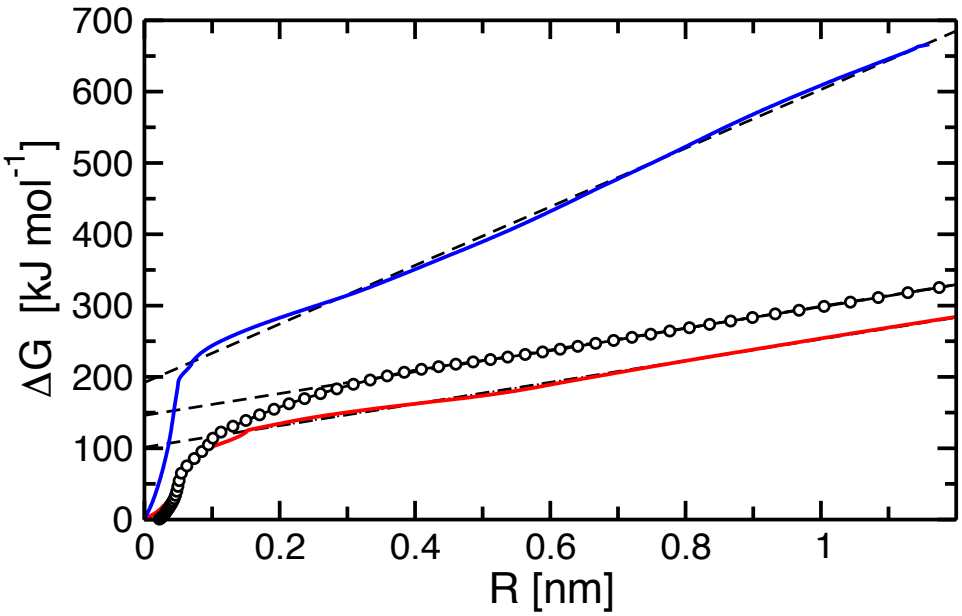

Figure S7: Free-energy vs. pore radius, as shown in the bottom panel of Figure 4. The dashed lines are least-square fittings to the Classical Nucleation Theory expression for the pore energy (equation 1 in the manuscript, with $\gamma_s = 0$) in the R$\in$ (0.3-1.3)nm interval. In each case, the ordinate of the origin provides ΔG_n , the slope allows to compute γ_L.