

Supplementary Information

Transmembrane domain dimerization induces cholesterol rafts in curved lipid bilayers

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1) Well-tempered metadynamics convergence

To assess for well-tempered metadynamics convergence in the Ψ space while bending the lipid bilayer with subunit g helical dimer embedded, we have calculated the free energy profile as a function of the simulation time. At convergence, reconstructed profiles do not change anymore with simulation time [1-4]. Figure S1 shows that for $t \sim 1.4 \mu\text{s}$ no further significant changes are observed.

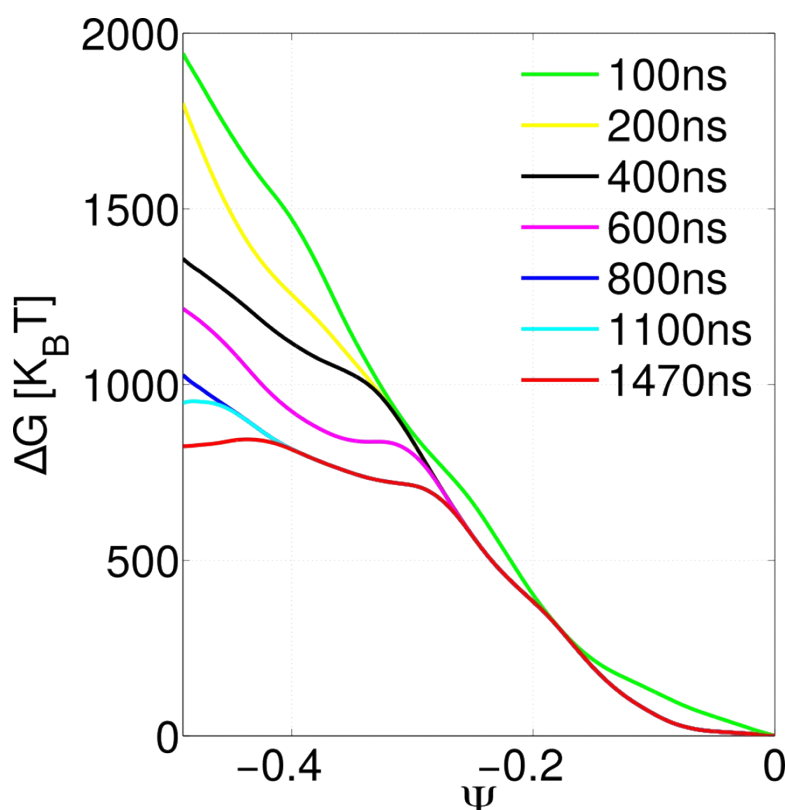


Figure S1: Free energy profile reconstruction for increasing simulation times. Convergence is reached when no further changes are observed.

To verify the convergence in a more exhaustively way, we show in figure S2 the time evolution of the collective variable that bends the bilayer (Ψ). Given that no local minima are observed in the bending process, as the time simulation increases the system freely to diffuses between curved and flat states. As expected [1,3,4], the system explores the phase space in a larger region respect to the

beginning of the simulation, and this region asymptotically tends to converge as well (see the exponential shape of the upper and lower envelopes of the function). Additionally, figure S2 shows time snapshots from metadynamics that demonstrate the diffusive behavior.

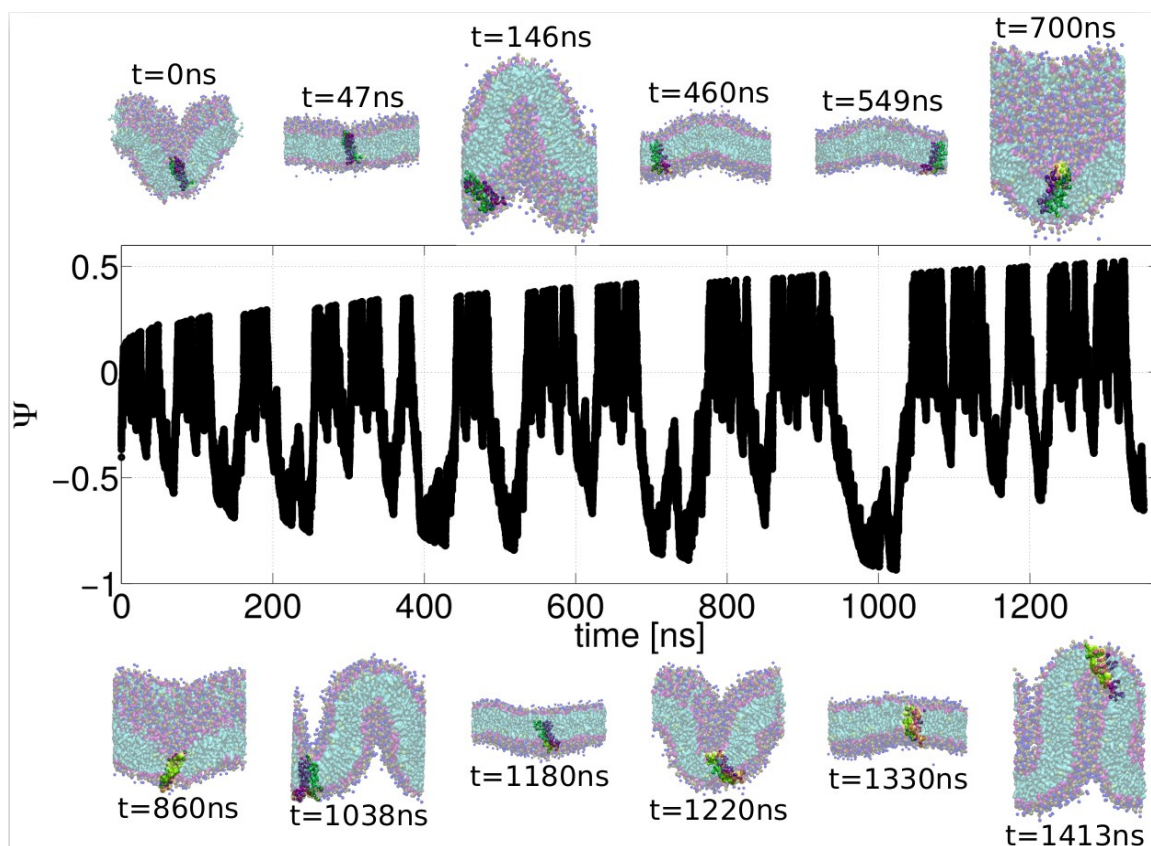


Figure S2: Time evolution of the collective variable that bends the bilayer. TM peptide dimer was included in the simulation to calculate the change in free energy to be compared with the “bilayer only” system.

2) Cholesterol clusters together in raft-like phases

We have observed that as the bilayer bends with the TM peptide dimer, cholesterol molecules tend to cluster together forming rafts. The effect is noticeable for high curvature ($\Psi \sim -0.75$) and long simulation times ($1\mu s$) as shown in figure S3, where lipid molecules (yellow) concentrate around the peptide dimer leaving the opposed high curvature region almost cholesterol depleted.

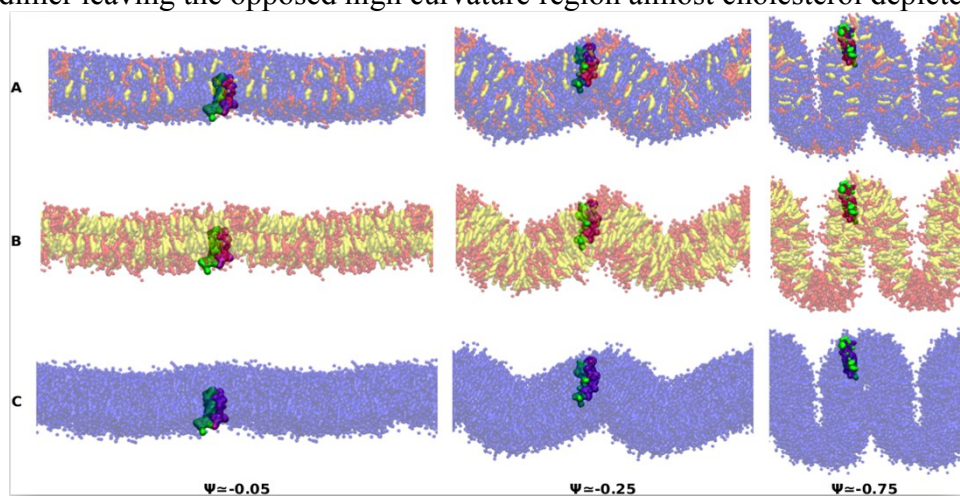
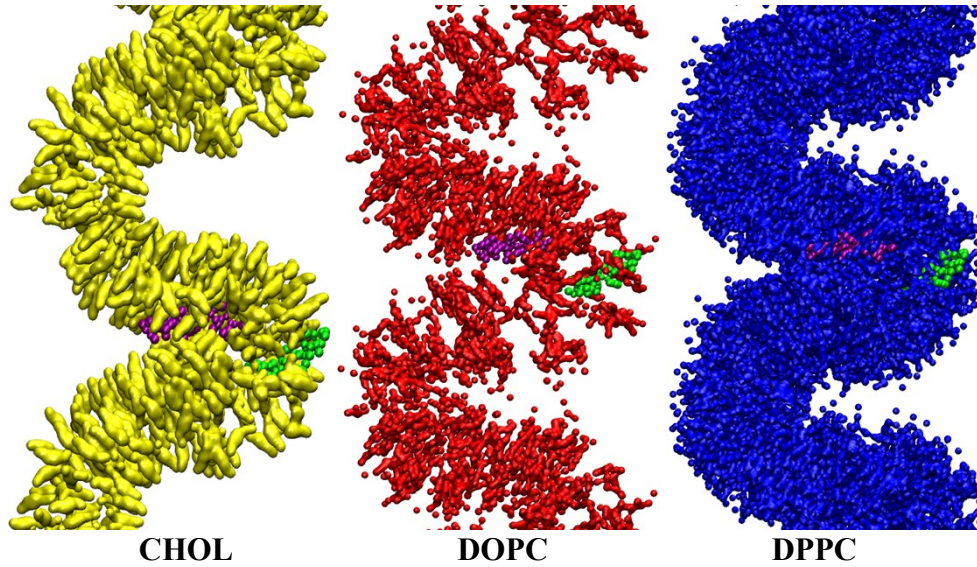


Figure S3: Progressive bending of the lipid bilayer with the TM peptide dimer. Lipid types are colored as follows: CHOL (yellow), DPPC (blue) and DOPC (red). For high curvature, cholesterol clusters in raft-like phases surrounding the peptides.

The effect however vanishes if the peptides are not dimerized. Figure S4 shows the highly curved bilayer after $1\mu s$ of simulation time with TM peptides not dimerized (figure S4a) and dimerized (figure S4b). As before, the only restraint is harmonic to induce constant curvature at $\Psi=-0.75$.

a) TM peptides not dimerized:



b) TM peptides dimerized:

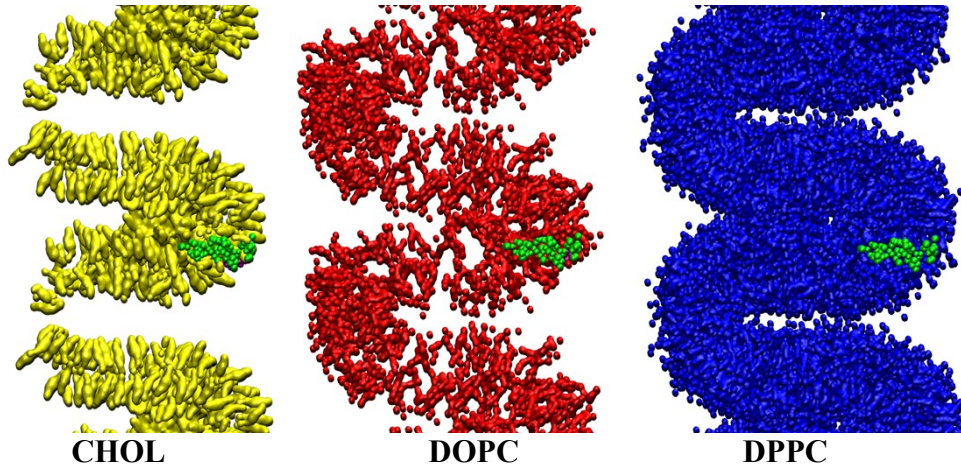


Figure S4: Lipid distributions for the high curvature regime ($\Psi=-0.75$) for both cases: a) TM peptides not dimerized and b) TM peptides dimerized. No clustering is observed when TM peptides do not dimerize.

Given that the peptides are relatively small (26 residues each) compared to the bilayer (1024 lipids), their coordination with the lipid molecules is limited the the amount of interactions between the 130 beads that compose the TM dimer and the 11064 beads of the bilayer. However, it is possible to notice that as the bilayer bends and the cholesterol clusters the peptide-cholesterol coordination number reduces its deviation (see figure S5).

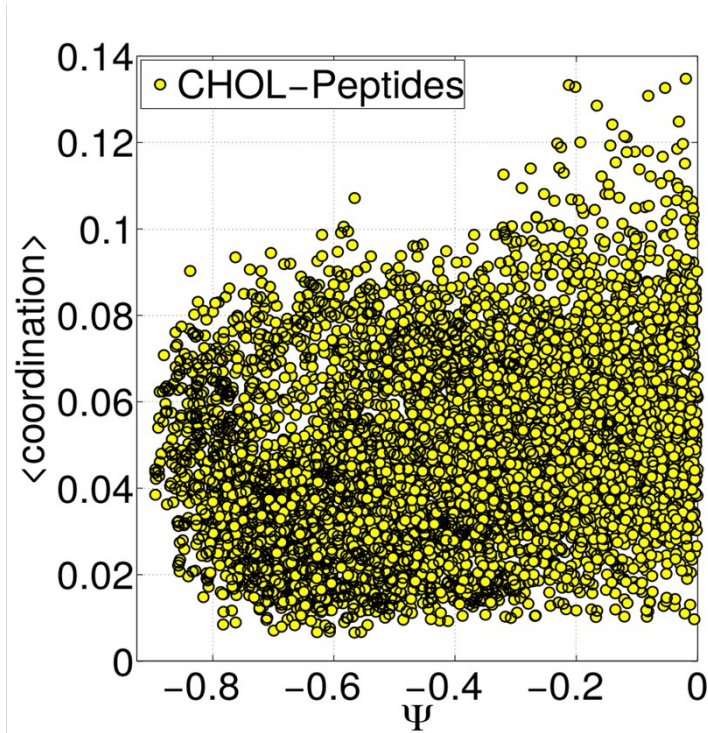


Figure S5: Coordination numbers for TM peptide dimer and cholesterol molecules as the bilayer bends.

As previously noticed, if the peptides are not present while the bilayer bends no cholesterol raft forms. Then, self-coordination numbers for DPPC, DOPC and CHOL should exhibit a rather different behavior. Figure S6 is analogous to figure 3 in the manuscript but with no peptides included in the simulations. It is observed that for the three species present in the membrane, there is no significant change in the coordinations as a function of the bilayer curvature.

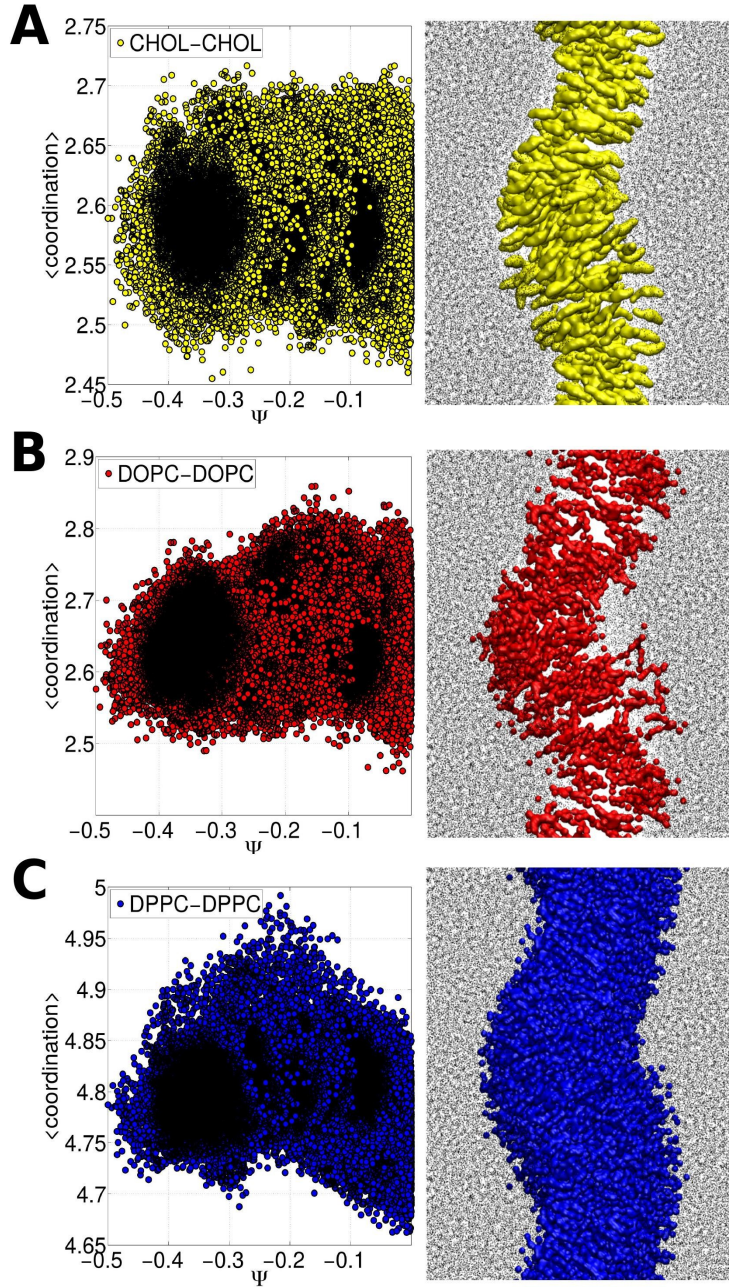


Figure S6: Lipid distribution and membrane bending. Self-coordination numbers were calculated for each specie (DPPC, DOPC and CHOL) in the ternary membrane in presence of the TM peptide dimer as a function of Ψ . Panel A: CHOL, panel B: DOPC and panel C: DPPC. In all three cases water molecules are shown as black dots.

4) Self-coordination numbers

To measure the formation of cholesterol rafts we have used a self coordination function as implemented in PLUMED under the instruction COORDINATIONNUMBER, with a rational switching function, which calculates self interactions as:

$$s_{ij}(r) = \frac{1 - \left(\frac{r_{ij} - d_0}{r_0} \right)^6}{1 - \left(\frac{r_{ij} - d_0}{r_0} \right)^{12}} \quad (1)$$

where, r_{ij} are the 3D distances between MARTINI beads of cholesterol, r_0 is the cut-off distance set to 0.3 nm and parameter $d_0 = 0.215 \text{ nm}$ corresponds to the mean vdW radii of the cholesterol beads

($d_0=0.235\text{nm}$ for DPPC and DOPC). Then, s_{ij} is ~ 1 if the contact between beads i and j is formed and is ~ 0 otherwise (if any two beads i and j are separated by a large distance $r_{ij} \gg r_0$ then the denominator to the 12^{th} power makes the total ratio to be ~ 0 , on the other hand, if $r_{ij} \sim r_0$ then both numerator and denominator are comparable making the total ratio to be ~ 1). Figure S7 plots equation 1 as a function of the bead-bead distance (r_{ij}) with the specified r_0 and d_0 parameters.

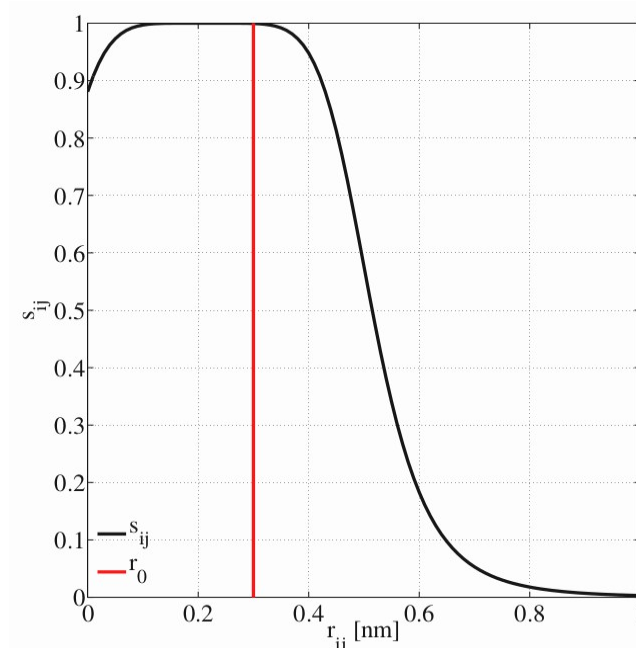


Figure S7: Plot of the coordination number function in equation 1 for CHOL parameters. Red line highlights cut off distance.

For the systems studied here coordination has been calculated for each lipid species, namely DPPC, DOPC or CHOL, using the described parameters. Then, for a given lipid type the self-coordination number is the count of how many beads belonging to the same lipid type are within the range determined by the cut-off distance (d_0). The sum of all counted interactions at each molecular dynamics snapshot gives an idea of the proximity of the the lipid molecules of each type included in the system as a function of some parameter of the simulation (in our case we have used Ψ , the collective variable that bends the bilayer). Reported coordination numbers correspond to mean values in all cases.

References:

- [1] **Metadynamics.** Barducci A., Bonomi M, and Parrinello M. *WIREs Comput Mol Sci*, 2011, 1:826-843.
- [2] **Well-Tempered Metadynamics: A Smoothly Converging and Tunable Free-Energy Method.** Barducci A, Bussi G, and Parrinello M. *Phys. Rev. Lett.*, 2008, 100, 020603.
- [3] **Metadynamics: a method to simulate rare events and reconstruct the free energy in biophysics, chemistry and material science.** Laio A and Gervasio FL. *Rep. Prog. Phys.*, 2008, 71 126601.
- [4] **Assessing the Accuracy of Metadynamics.** Laio A, Rodriguez-Forte A, Gervasio FL, Ceccarelli M, and, and Parrinello M. *The Journal of Physical Chemistry B*, 2005, 109 (14), 6714-6721.