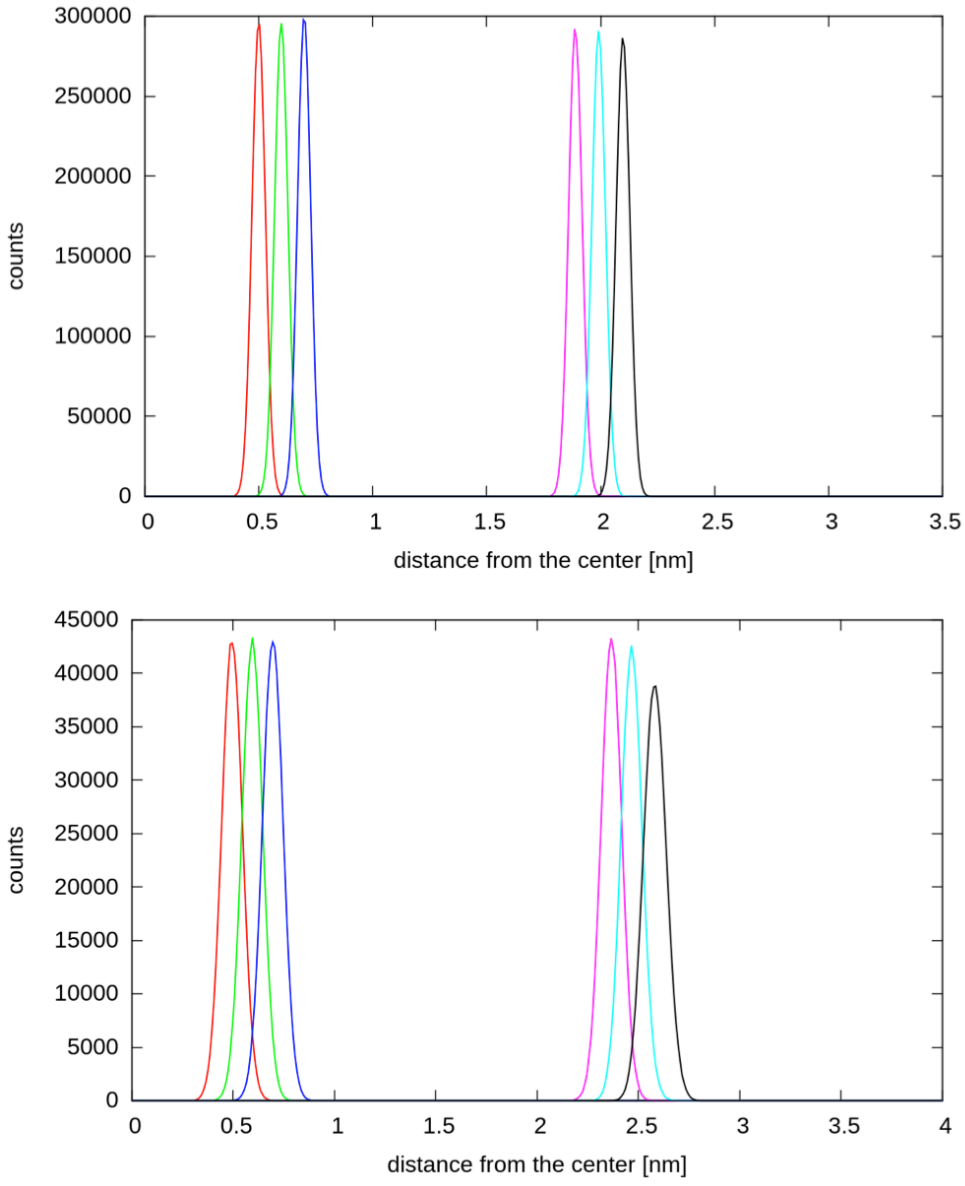
SUPPLEMENTAL MATERIAL

Umbrella Sampling histograms

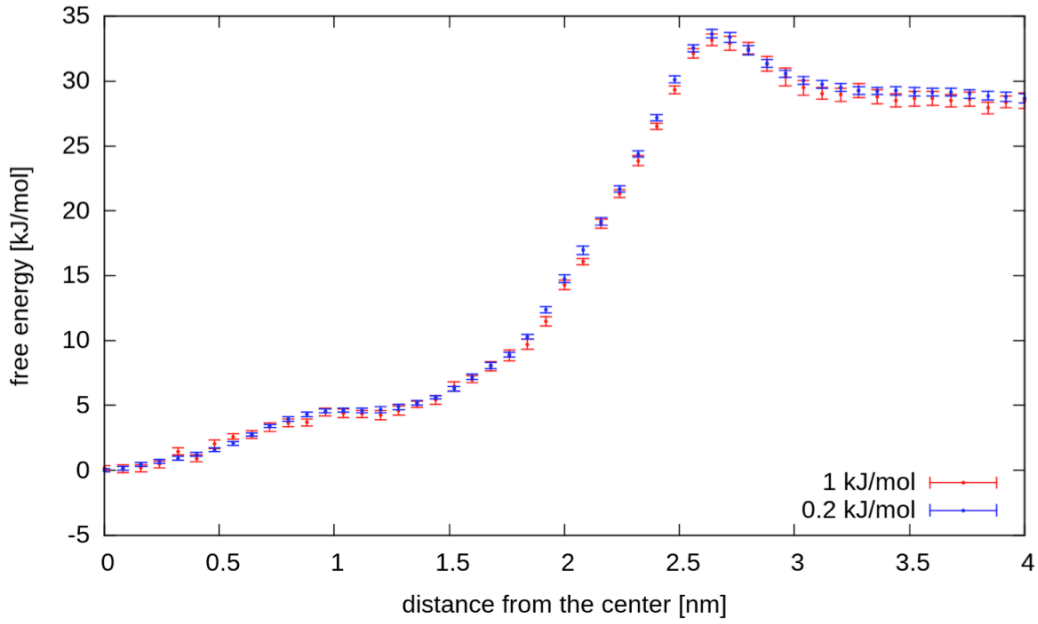
The US parameters used in this work (force constants and windows spacing) are the standard ones used in this kind of studies [1-4]. We have verified that the histograms obtained in every US calculation overlap significantly in every region of the reaction coordinate, as can be seen from Supplemental Figure 1.



Supplemental Figure 1. US histograms for PE dimer in the atomistic (top) and CG case (down). For clarity, only a few histograms are shown: the ones corresponding to three consecutive windows inside the hydrophobic core of the membrane and to three consecutive windows in the lipid heads region.

Metadynamics parameters

In MT calculations, the width of the gaussians was chosen small enough to be able to distinguish the finest detail of the FES, i.e. the small barrier in the lipid heads region. Once the width has been fixed, the height of the gaussians came from a coarse preliminary optimization procedure, in which we tried a few values, decreasing it from 2 kJ/mol to 0.2 kJ/mol.A comparison between the results obtained with the value used in the work (0.2 kJ/mol) and the ones obtained with higher gaussians (1 kJ/mol) in the CG case is shown in Supplemental Figure 2.



Supplemental Figure 2. Comparison between free energy profiles obtained with two different heights of the gaussians, in the case of the PE CG dimer. The procedure for the calculation of the error bars is the one described in the article, and the total simulation time is the same in both cases (8 μs).

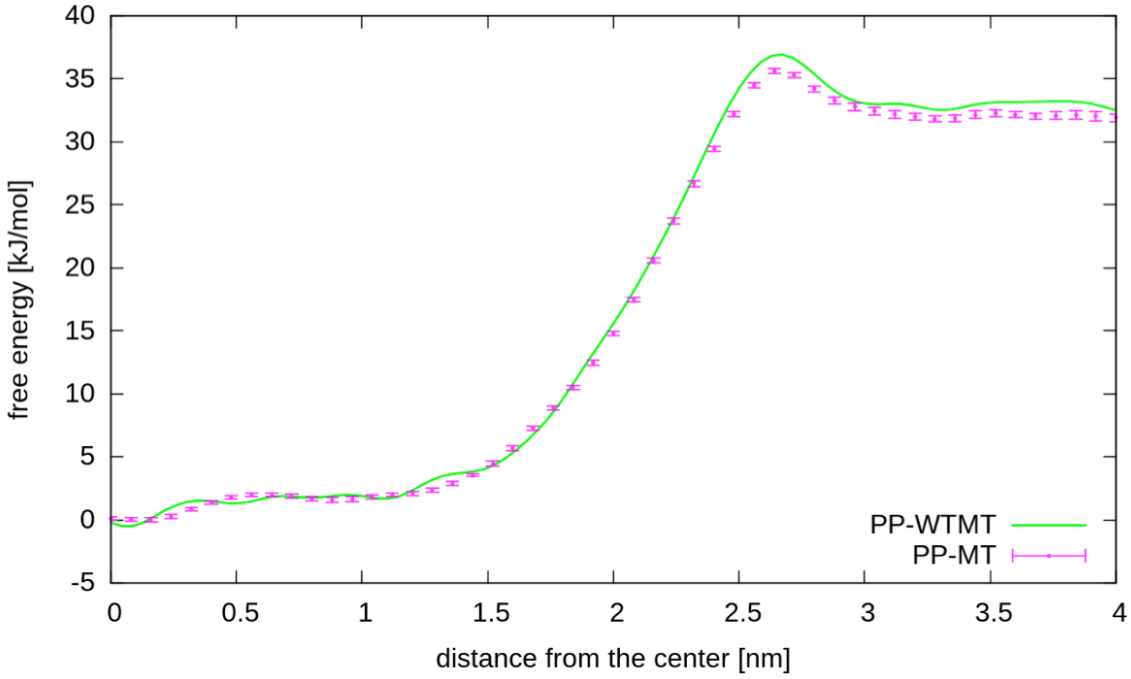
Well Tempered vs Standard Metadynamics

We have considered also the option to use Well Tempered Metadynamics (WT-MT) [5] instead of standard Metadynamics. The WT-MT algorithm requires the setting (and thus the optimization) of an additional parameter: the bias-factor, which controls the rate of the decrease of the Gaussian heights during the MT run.

We obtained our best WT-MT free energy profile using a bias factor of 14 (the range we tested is 10-20). In Supplemental Figure 3, we compare it with the result obtained with standard MT. It can be noticed that, within the same simulation time, the WT-MT profile still presents spurious local oscillations that have an amplitude bigger than the MT error bars. If the simulation time is increased, these oscillations will disappear, leading sooner or later to a smooth profile as the one produced with standard MT, but the efficiency will

be lower. Furthermore, there would still be need of a practical criterion to derive the error bars.

An additional problem would come out in the atomistic case, where the kinetics is slower and the head group region is more difficult to cross. This leads to shifts in the free energy difference between the two regions, which are damped by the averaging method we use in standard MT. In WT-MT, where, as the Reviewer points out, the averaging of the profiles is not appropriate, these shifts would take a long time to disappear.



Supplemental figure 3. Comparison, in the case of the PP CG dimer, between the profile obtained with standard MT and the procedure described in the article (pink error bars) and the profile obtained with Well Tempered Metadynamics (green line) at the end of the same simulation time (8 μs).

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