Methods

Simulation protocol

The system was described using the MARTINI coarse grain (CG) model \(^1\). Here, on average, four heavy atoms or four water molecules are represented by one CG bead. Covalent bonds of lipids are modeled by springs, and the stiffness of the lipid tails is provided by angle potentials. The polarity of the groups is modeled by effective Lennard-Jones (LJ) parameters. LJ interactions are truncated at 1.2 nm and shifted between 0.9 to 1.2 nm. The zwitterionic character of the lipid molecules is modeled by charges on the choline and phosphate groups. These charged groups interact via a Coulomb potential with a relative dielectric constant \(\epsilon = 15\) and shifted between 0
and 1.2 nm to mimic the effect of distance depending screening. A 1.2 nm cut-off was used for the neighbor list updated every 10 time steps. The time step used was 160 fs. Here it should be noted that, due to the smoothness of the potential, the effective timescale based on the diffusion of lipid molecules is a factor of four larger than the nominal timescale; all time intervals given in this paper are based on the effective timescale. A temperature of 300 K (or 380 K) was maintained by coupling the lipids and the water separately to a heat bath using a Berendsen thermostat \(^2\) with a relaxation time of 0.4 ps. The dimensions of the box normal and lateral to the bilayer were scaled independently to maintain a pressure of 1 bar in each direction corresponding to zero tension using a Berendsen barostat \(^2\) with a relaxation time of 0.8 ps. All simulations were performed with the GROMACS software package, version 3.3 \(^3\).

**Determination of 2D PMF**

Here the derivation of Eq. 2 for computing the 2D PMF shall be provided. Given the reaction coordinates \(x\) and \(y\), the 1D PMF along \(x\) shall be denoted as \(G_1(x)\) and the 2D PMF as \(G_2(x, y)\). The normalized histogram for \(y\) for given \(x\) is denoted as \(H_x(y)\). Note that only \(x\) but not \(y\) is imposed in the simulations. That is, \(H_x(y)\) is obtained from the spontaneous fluctuations in \(y\) for given \(x\). The PMF along \(y\) for given \(x\), \(G_x(y)\), obeys

\[
G_x(y) = -k_B T H_x(y) + c_x \equiv G_2(x, y)
\]

with \(c_x\) depending on \(x\). As

\[
\int dy \exp(-G_2(x, y)/k_B T) = \exp(-G_1(x)/k_B T)
\]

and \(\int dy H_x(y) = 1\) by definition, we obtain \(c_x = \Delta G_1(x)\), as had to be proved.
**Results: Minimal distance and center-of-mass distances**

Here the relation between the minimal distance between the hydrophobic cores of the bilayers, \( u \), used as the second reaction coordinate, and the respective center-of-mass distance, \( d_b \), as well as the center-of-mass distance between the distal leaflets, \( d_l \), shall be discussed. Figure 1 compares time evolutions for the three observables during a stalk formation event. Stalk formation is indicated by a sharp transition in \( u \) from \( u = 1.5 \pm 0.1 \) nm to \( u = 0.45 \pm 0.01 \) nm. The latter indicates van der Waals contacts between the hydrophobic cores of the bilayers. During the transition, \( d_l \) shows a smaller and gradual decrease over a time period of 50 ns (transition time) after which a plateau region is reached. The distance \( d_b \) behaves similarly during the transition time but decreases further, reflecting the mixing of lipids between the proximal leaflets via diffusion through the stalk.

**Additional information**

The full author list of Ref. 37 in the main paper is given in 4.

**References**


H.; Schenck, S.; Brüegger, B.; Ringler, P.; Müller, S. A.; Rammner, B.; Gräter, F.; Hub,  
J. S.; de Groot, B. L.; Mieskes, G.; Moriyama, Y.; Klingauf, J.; Grubmüller, H.; Heuser, J.;  
Figure 1: Time evolution of the center-of-mass distance for the hydrophobic cores (green), and distal leaflets (cyan) of the bilayers as well as the minimal distance (red) are plotted. The stalk was formed in the window with the lipid tail restrained close to its equilibrium position, $z_T = 2\,\text{nm}$. Fourier components with frequencies above $0.1\,\text{ms}^{-1}$ are filtered out using a Fast Fourier Transformation filter.