Figure S1: Box size dependence of tension fluctuations



Figure S1: In the NVT ensemble, the bilayer tension fluctuates around its average value. The standard deviation of the tension values plotted as a function of the linear size L_{\parallel} of the simulation box. The magnitude of the fluctuations decreases strongly with increasing system size up to $L_{\parallel} \simeq 30r_0$, and then seems to approach a constant value.

Figure S2: Self-assembly of vesicle



Figure S2: Simulation snapshots from the self-assembly of a bilayer vesicle from a random initial configuration of ~ 1500 lipids with $H_3(C_4)_2$ architecture and interaction parameters as given in Table 1b in a box with sidelength $32 r_0$. The corresponding number of simulation timesteps Δt is shown under each snapshot. As further simulations are started from pre-assembled states it is important to ensure that the model lipids form stable bilayers.

Figure S3: Bead density profile across bilayer membrane



Figure S3: The bead density profile ρr_0^3 of a tensionless bilayer as a function of the Cartesian coordinate z. The bilayer midplane is located at z = 0. The water density (solid line) drops to zero in the bilayer center and the head beads (red/dashed peaks) accumulate at the interface between hydrophobic tails (green/dotted) and the water.

Figure S4: Lateral stress profiles of tensionless bilayer



Figure S4: Stress profiles s(z) of an approximately tensionless bilayer as a function of the Cartesian coordinate z. The bilayer midplane is located at z = 0. (a) The total stress profile and (b) The individual contributions to the total stress. Solid line/red: head-water interactions, dash-dotted/green: tail-water interactions, dotted/black: bond forces and dashed/grey: bond-pair forces. In (a), the positive peaks to the sides arise from head and tail bead interactions with the solvent, while the bond potentials dominate the negative contributions in the bilayer centre.

Figure S5: Parameter dependence of the membrane's stretching behavior



Figure S5: (a) The effect of individual parameter changes on the stretching behavior. Each plot shows the bilayer tension $\bar{\Sigma}$ versus area per molecule \bar{A} under variation of the a_{ij} parameter specified in the inset. The values of all other parameters are the same as in the old parameter set, which is shown (b). The graph corresponding to this parameter set are drawn in red in each plot. The last frame shows the effects of variation of the $a_{\rm HC}$ parameter in the new parameter set given in (c). Changing this parameter barely affects the stretching behavior. Note that these graphs are recorded for short lipids with 3 beads per chain, for which the bilayer structure is not stable at low values of \bar{A} . This is the reason, why there is no state with zero tension in many cases.

Data S1 - DPD Simulation Method

Dissipative particle dynamics (DPD) is a coarse grained, particle-based simulation technique. This appendix summarizes the main features of the simulation method, followed by a more detailed description of the simulation setup.

The system in our DPD model is build up from N spherical simulation particles or 'beads' labeled i = 0, ..., N - 1. These represent small fluid volumes and thus contain several atoms and interact via soft potentials. A bead's position \mathbf{r}_i and velocity \mathbf{v}_i are defined for its center of mass, and its size is determined by the cutoff radius of the interactions. Two beads *i* and *j* experience three types of forces: (i) a conservative force \mathbf{F}_{ij}^C , which represents a coarsegrained description of the intermolecular interaction between the atoms or molecules contained in the beads; (ii) a random force \mathbf{F}_{ij}^R , which represents thermal noise; and (iii) a dissipative force \mathbf{F}_{ij}^D , which must be added in order to ensure the fluctuation-dissipation relation in equilibrium (?). The force on a pair of beads *i* and *j*, with separation $r_{ij} \equiv |\mathbf{r}_j - \mathbf{r}_i|$ resulting from the conservative interaction is given by

$$\mathbf{F}_{ij}^C = a_{ij}(1 - r_{ij}/r_0)\hat{\mathbf{r}}_{ij} \quad \text{for} \quad r_{ij} \le r_0.$$
(1)

It is characterized by two parameters: (i) the force amplitude $a_{ij} > 0$, which depends on the species of the two beads, and (ii) the range r_0 , which is taken to be independent of the bead pair. All equilibrium properties of the system are determined by the properties of F^C .

The dissipative force is linearly related to the relative velocity ${\bf v}_{ij}={\bf v}_j-{\bf v}_i$ and takes the form

$$\mathbf{F}_{ij}^{D} = -\gamma_{ij} (1 - r_{ij}/r_0)^2 (\hat{\mathbf{r}}_{ij} \cdot \mathbf{v}_{ij}) \hat{\mathbf{r}}_{ij}$$
(2)

with friction coefficient γ_{ij} . Finally, the random force \mathbf{F}_{ij}^R is given by

$$F_{ij}^{R} = \sqrt{2\gamma_{ij}k_{\rm B}T}(1 - r_{ij}/r_0)\zeta_{ij}\hat{\mathbf{r}}_{ij}$$
(3)

with thermal energy $k_{\rm B}T$ and random variable $\zeta_{\rm ij}$. These random variables are distributed uniformly with $\langle \zeta_{\rm ij}(t) \rangle = 0$ and $\langle \zeta_{\rm ij}(t) \zeta_{\rm i'j'}(t') \rangle = (\delta_{\rm ii'} \delta_{\rm jj'} + \delta_{\rm ij'} \delta_{\rm ji'}) \delta(t-t')$ and symmetrically, so that $\zeta_{\rm ij}(t) = \zeta_{\rm ji}(t)$.

All three force contributions act along the vector connecting the beads' centres, $\hat{\mathbf{r}}_{ij}$, and vanish outside the cutoff radius r_0 . They obey the symmetry

 $\mathbf{F}_{ij} = -\mathbf{F}_{ji}$, *i.e.* their effect on the two beads *i* and *j* is equal and opposite, ensuring local momentum conservation in the system and producing the correct hydrodynamic behavior. The total force acting on a bead is given by the sum over the contributions from all beads within the range r_0 and the system is updated according to the original integration scheme as introduced by Hoogebrugge and Koelman (?) and developed by Groot and Warren (?).

Larger molecules are build up from several beads connected with harmonic potentials of the form $U_2 = (1/2)k_2(r_{i,i+1}-l_0)^2$, which are parametrized by the spring constant k_2 and the unstretched length l_0 . In addition, molecules such as the hydrocarbon chains can be stiffened by means of a three-body potential $U_3 = k_3 [1 - \cos(\psi - \psi_0)]$ between three consecutive beads along the chain, where k_3 is the bending constant and ψ and ψ_0 are the tilt angle defined by the scalar product of the two bonds and the preferred value of that angle, respectively.

Data S2 - Stress Tensor calculation

To obtain the membrane tension in a simulation the components of a microscopic stress tensor $\sigma^{\alpha\beta}$ at position $\mathbf{R} \equiv (X, Y, Z)$ are calculated, and averaged over time. The interaction potential is devided into m-body interaction clusters with m-body interaction Potential $U^{(m)}$. The contribution to the microscopic stress tensor from m-body interactions, $[\sigma^{\alpha\beta}]^{(m)}(Z)$, is given by (?)

$$\begin{bmatrix} \sigma^{\alpha\beta} \end{bmatrix}^{(m)} (Z) = -\frac{1}{m} \frac{1}{L_{\parallel}^2} \sum_{\langle j \rangle} \sum_{\langle k,l \rangle} (\nabla^{\alpha}_{j_k} U^{(m)} - \nabla^{\alpha}_{j_l} U^{(m)})$$

$$\times \int_{\mathscr{C}_{j_k j_l}} d\ell^{\beta}_{kl} \, \delta(Z - \ell^z_{kl})$$

$$(4)$$

Here L_{\parallel} is the lateral size of the membrane, $\langle j \rangle$ denotes summation over all m-clusters and $\langle k, l \rangle$ the sum over all possible pairs of particles within a given m-cluster and the integral is over a straight-line contour $\ell_{\rm kl}$ connecting particles l and k.

In the method introduced by Goetz and Lipowsky, to obtain the stress profile in simulations, the simulation box is subdivided into thin slices and the components are calculated for each one by multiplying the above expression with two Heaviside step functions.

Instead of this subdivision, the components of the stress tensor can be directly integrated over the Z coordinate of the simulation box. The contour ℓ_{kl} is parametrized by λ as $\ell_{kl} = \mathbf{r}_{j_k} + \lambda(\mathbf{r}_{j_l} - \mathbf{r}_{j_k}) \equiv \mathbf{r}_{j_k} + \lambda \mathbf{r}_{j_k j_l}$ with $0 \le \lambda \le 1$, so that it starts from particle j_k with position \mathbf{r}_{j_k} for $\lambda=0$ and ends at j_l with position \mathbf{r}_{j_l} for $\lambda=1$. Changing variables from ℓ^{β} to λ in equation 4 produces an additional factor $r_{j_k j_l}^{\beta}$ in the integral now given by

$$\begin{bmatrix} \sigma^{\alpha\beta} \end{bmatrix}^{(m)} (Z) = -\frac{1}{m} \frac{1}{L_{\parallel}^2} \sum_{\langle j \rangle} \sum_{\langle k,l \rangle} (\nabla^{\alpha}_{j_k} U^{(m)} - \nabla^{\alpha}_{j_l} U^{(m)})$$

$$\times \int_0^1 d\nu \, r^{\beta}_{j_k j_l} \, \delta(Z - r^z_{jk} - \lambda r^z_{j_k j_l})$$

$$(5)$$

Integration over the Z coordinate yields the z-integrated components of the stress tensor for the m-body interactions, $I_m^{\alpha\beta}$:

$$I_{m}^{\alpha\beta} \equiv \int_{0}^{L_{\perp}} dZ \, \left[\bar{\sigma}^{\alpha\beta}\right]^{(m)}(Z) \qquad (6)$$
$$= -\frac{1}{L_{\parallel}^{2}} \frac{1}{m} \sum_{\langle j \rangle} \sum_{\langle k,l \rangle} [\nabla_{j_{k}}^{\alpha} U^{(m)} - \nabla_{j_{l}}^{\alpha} U^{(m)}] (r_{j_{l}}^{\beta} - r_{j_{k}}^{\beta})$$

Data S3 - Simulated Systems

The Simulations described here use different starting structures. Overall, four types of simulations were performed. All of them are done in the NVT ensemble with a density $\rho = 3$ beads/unit volume.

(i) a random mixture of lipids and solvent to test whether the lipids self-assemble to a bilayer structure. This initial state starts from a random distribution of the beads in a $32 r_0^3$ simulation box, subject only to the constraint that adjacent beads of an amphiphile cannot be separated by more than a bead diameter to avoid artificially large forces.

(ii) Simulations characterizing the membrane properties and their parameter dependence start from a pre-assembled planar bilayer. The bilayer patch is assembled to have a specific area per lipid A. As the box cross section is fixed, A determines the number of lipids in the system. Most of these simulations run in a cubic box with sides of $32 r_0$, but test in a larger box with area $(72 r_0)^2$ as used in the fusion simulations show that the results hold also for these bilayers.

(iii) For fusion simulations a vesicle is assembled in close proximity to a planar bilayer patch. Similar to the planar bilayer, the vesicles are assembled with a prescribed area per molecule. The lipids are distributed on two spherical shells defined by the outer radius of the vesicle and the bilayer thickness, which yields the radius of the inner surface. To obtain the target area per molecule $(A)_{\rm ve}$ on both surfaces, $N_{\rm ex} = \frac{4\pi R_{\rm ex}^2}{(A)_{\rm ve}}$ and $N_{\rm in} = \frac{4\pi R_{\rm in}^2}{(A)_{\rm ve}}$ lipids are distributed on the outer and inner monolayer respectively. These are arranged as equally spaced as possible on the spheres, with their head groups placed on the spherical shells defining the surfaces and their tails pointing radially in and out, respectively, towards the bilayer midplane. In these simulations, fusion is induced by applying a lateral tension to the planar bilayer. This is achieved by increasing the A which is directly related to the bilayer tension. Most fusion simulations have been done for two different vesicle diameters, $20r_0$ and $40r_0$, corresponding to approximately 14 and 28 nm, respectively. The simulation box for these vesicle sizes had a box area of $(72 r_0)^2$, *i.e.* ~ $(50 \text{ nm})^2$, and heights of $52 r_0$ and $72 r_0$ for the $20 r_0$ and the $40 r_0$ vesicle, respectively. Additional simulations of a vesicle with diameter $20 r_0$ in a smaller box of side length $36 r_0$ were performed to further explore the dependence of the results on the vesicle size.

(iv) Finally, we use two adhering bilayers are assembled in contact with each other to measure the work required to flipflop lipid tails between the bilayers in the force spectroscopy simulations. To measure the energy barrier for this process, a single lipid from the lower bilayer is selected and a slowly moving, external harmonic potential is applied to one of its tail beads. The potential starts out centered on the instantaneous z-coordinate of the selected tail bead, which is chosen as the origin of the bead's position coordinate, $z_{\text{bead}} = 0$. The potential moves slowly upwards at constant speed $0.0004r_0/\Delta t$ or 0.009 nm/ns, until the tail has flipped into the other bilayer and the lipid has assumed an extended or splayed conformation. During this process, the harmonic force and the beads position $z_{\text{bead}} = 0$ are recorded. Once the bead has overcome the energy barrier, the harmonic force becomes negative. To obtain the work expended to flip the lipid, the force is integrated over z_{bead} up to the point where it becomes negative.

Data S4 - Geometry of Tense Membranes

Fluid bilayer membranes typically can have a large variety of different shapes, which are determined by the bending elasticity and spontaneous curvature under the constraints of constant membrane area and constant volume adapted to the osmotic conditions. This makes vesicle equilibrium configurations, in general, fundamentally different from those of liquid interfaces, as they are not governed by interfacial tension.

In tense membranes, on the other hand, the effects of membrane tension become relevant. The shape of an adhering vesicle with volume $V_{\rm ve}$ and surface area $A_{\rm me}$ with corresponding membrane tension $\Sigma_{\rm me}$, is determined by the free energy functional

$$\tilde{\mathcal{F}}_{\rm ve} = V_{\rm ve}\Delta P + A_{\rm me}\Sigma_{\rm me} + \tilde{\mathcal{F}}_{\rm be} + \tilde{\mathcal{F}}_{\rm ad}.$$
(7)

The last two terms represent the bending energy $\tilde{\mathcal{F}}_{be}$ and the adhesion energy $\tilde{\mathcal{F}}_{ad}$ (?).

Minimization gives a transversality condition for the contact principle curvature $C_{\rm co} = (2|W|/\kappa)^{1/2}$ (?). If the radius of this contact curvature, $R_{\rm co} = (\kappa/2|W|)^{1/2}$, is small compared to the linear size, i.e. the radius, of the spherical vesicle, $(\kappa/2|W|) \ll R_{\rm ve}$, the bending energy contribution becomes negligible and membrane shapes are dominated by the tension. This is the case either for large adhesion strength |W| compared to the bending energy κ , or for large vesicles. In that case, vesicles have shapes approaching spheres or spherical segments, comparable to those of liquid interfaces of droplets. An effective contact angle $\theta_{\rm eff}$ can be defined and the membranes obey effective Young and Laplace equations (?).

However there are important differences to the shape of a droplet. The droplet's volume is constant and its surface area can change. The membrane area of a vesicle on the other hand, is fixed. Furthermore, the contact angle of a droplet is fixed by the surface tensions, which depends only on material properties, whereas for a vesicle the membrane tension depends on the geometry, so that the angle can change.

Since the simulated membranes are more stretchable and their area can be increased by about 20 % before they rupture the membrane shapes are tension dominated despite their small size. In the simulation snapshots, the DPD membranes assume shapes, which can be approximated as spherical caps. In our fusion setup, the geometry consist of a planar membrane in contact with an originally spherical vesicle. Some simulations result in adhesion or hemifusion between the vesicle and planar bilayer patch in mechanical equilibrium. The geometry of both states consist of three membrane segments, two spherical cap regions for the vesicle and the contact or hemifused area, and a segment of planar membrane (Fig. S6).

In the case of mechanically stable adhesion, the forces on the contact line arising from the adhesion energy |W| and the membrane tensions must be balanced. Assuming the membrane tensions $\Sigma_{\rm pl}$ and $\Sigma_{\rm ve}$ are uniform throughout the bilayer patch and the vesicle membrane, this force balance is given by the Neumann equations

$$(\Sigma_{\rm pl} + \Sigma_{\rm ve} - |W|)\cos(\theta_1) + \Sigma_{\rm ve}\cos(\theta_2) - \Sigma_{\rm pl} = 0$$

$$(\Sigma_{\rm pl} + \Sigma_{\rm ve} - |W|)\sin(\theta_1) - \Sigma_{\rm ve}\sin(\theta_2) = 0$$
(8)

A further constraint arises because the time scales of the simulation the membranes are water impermeable, so that the interior volume of the vesicles is constant. These conditions define the equilibrium shape of the membranes, if the material properties $\Sigma_{\rm pl}$, $\Sigma_{\rm ve}$ and |W| are known. However, at least |W| is unknown initially.

In the simulations, the initial molecular areas, \overline{A} and $(\overline{A})_{ve}$, are prescribed. The number of molecules in the bilayer patch and vesicle, N_{pl} and N_{ve} , are constant and thus, from the geometry of the system, the molecular areas in the final state are known. As the relation between membrane tension and molecular area is explicitly measured for planar bilayers, Σ_{pl} can be deduced and the force balance equations can be solved for |W| and Σ_{ve} in terms of Σ_{pl} .

Therefore, to analyse the simulation results, circular arcs are fitted to the simulation snapshots, as in Figure S6. These fits give values for both |W| and the tension-free area per molecule in the vesicle, \bar{A}_{ve} , which might differ from that of a planar bilayer, because of the relatively strong curvature.

The results for |W| can then be used with the equations 8, and the volume constraint to predict the deformation of an adhering vesicle and the size of the adhesion area for different tensions.

The estimates represents a lower limit of |W| and the tension-free A_{ve} , as other contributions, such as bending energies, also contribute to reduce the deformation.



Figure S6: The membrane configurations of mechanically stable adhesion (left) and hemifusion (right), between the vesicle and planar bilayer patch. The geometry of both states consist of three membrane segments, two spherical cap regions for the vesicle and the contact or hemifused area, and a segment of planar membrane. The figure illustrates the spherical fits to the vesicle membrane (blue) and originally planar membrane (red), and the effective contact angles θ_1 and θ_2 that these make with the bilayer plane. In practice, for stable adhesion only the planar membrane shape is used, to obtain the adhesion strength |W| and the tension free \bar{A}_{ve} . These values are then used together with the constraint of constant vesicle volume to predict the vesicle shape for given initial molecular areas of the planar bilayer and vesicle membranes. To describe the partially hemifused configuration (B), in addition the extension of the hemifused diaphragm $a_{\rm HF}$, (green) has to be measured. From the fully hemifused conformation (A), one can estimate line energy Λ of the membrane junction.

For a hemifused vesicle, on the other hand, the number of molecules in the aggregates is not fixed, as the bilayers merge in the hemifused patch. Only the total number of lipids in the system, N_{total} and the vesicle volume remain constant. Since the individual values of \bar{A} are not accessible it can be instructive to calculate the net tension $\Sigma_{\text{net}} \equiv K_A / A_0 (A_{\text{total}} / N_{\text{total}} - \bar{A}_0)$ from the total area A_{total} of all monolayers to see if the bilayers in the system are tension-free.

There are two possible geometries, illustrated in Figure S6, that a vesicle hemifused to a planar bilayer patch can assume: the hemifused region can span the entire contact area or the hemifused diaphragm has formed within a region where the two membranes adhere. Since in the first case there is no area of adhesion the adhesion energy |W| is no longer involved. In the second case, the adhesive strength and the tension in the hemifused patch have to be balanced.

On long time scales, cross monolayer exchange of lipids across small pores enables equilibration of the monolayers. Therefore, in an equilibrated system the tensions in all membrane segments should have the same magnitude Σ , so that the force balance at the three-membrane contact line reduces to

$$\Sigma \left(\cos(\theta_1) + \cos(\theta_2) - 1 \right) = 0 \tag{9}$$

As a consequence, either $\Sigma = 0$ due to the area gained from hemifusion or $\theta_1 = \theta_2 = \theta_{\rm HF} = 60^\circ$. In the latter case, different values of θ would suggest the influence of a line tension Λ of the three-membrane line and allow to infer its value by setting the above force balance (10) equal to $\frac{2}{a}\Lambda$:

$$\Sigma \left(\cos(\theta_1) + \cos(\theta_2) - 1 \right) = \frac{2}{a} \Lambda$$
(10)

Figure S7: Work distributions for enforced interbilayer flips of lipid molecules



Figure S7: Histograms showing the work distribution used for the Jarzinsky relation for the molecular areas $\bar{A} = 1.25$, $\bar{A} = 1.3$, $\bar{A} = 1.35$, $\bar{A} = 1.4$, $\bar{A} = 1.45$ and $\bar{A} = 1.5$. From the shape of these distributions, their exact nature is difficult to determine. However, the very slow pulling rates leads to the expectation that they are gaussian distributions, and the fact that cumulants of orders greater than two are negligibly small seem to confirm this expectation.

Figure S8: Vesicle size dependence of average fusion time



Figure S8: The average fusion times $\langle t_{\rm fu} \rangle_{\rm a}$ as functions of the area per molecule \bar{A} for three different systems: (i) a vesicle with a diameter of 28 nm in a box with side length $L_{\parallel} = 50$ nm (diamonds), (ii) a vesicle with a diameter of 14 nm in a box with side length $L_{\parallel} = 50$ nm (crosses) and (iii) a vesicle with a diameter of 14 nm in a box with side length $L_{\parallel} = 25$ nm (open diamonds). Note that the radius of the vesicle, $R_{\rm ve} = 14$ nm is the same in systems (ii) and (iii) , whereas the ratio $R_{\rm ve}/L_{\parallel}$ is the same for (i) and (iii). The data shows that the fusion times of the two systems with the same ratio $R_{\rm ve}/L_{\parallel}$ between the vesicle radius and the linear size of the bilayer are much more similar than for those which with the same vesicle radius.