Adhesion of soft membranes controlled by tension and interfacial polymers

Supplementary information

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FIG. 1: Schematic side view of the adhering vesicle. A. Pre-adhesion or initial state, the lower membrane resides in the shallow minimum of the interaction potential, on top of a liquid cushion. B. Soon after nucleation of the growth patch. C. Final state, the vesicle is fully adhered on the substrate.

**Vesicles and Substrates.** Giant vesicles were prepared using a 1:1 mixture of phosphocholine (14:0) and cholesterol. They were filled with sucrose at 200mM and were sedimented in Phosphate Buffer Saline (osmolarity 250 mM) on top of one of three kinds of substrates: (i) no- polymer: coated with a protein (avidin - AVI, 10 µg/ml, 30 min incubation), (ii) sparse- polymer: coated first with AVI and then an additional layer of hyaluronic acid (HA, 10 µg/ml, 30 min; MW: 700000 g/mol) or (iii) dense- polymer coated first with polylysine (PLL, 10 µg/ml, 30 min incubation) and then an additional layer of HA (10 µg/ml, 30 min).

**Microscope setup.** Observation was performed on an inverted microscope using an incoherent 120 W illuminator, (Xcite-120 illuminator, Exfo, Canada), an Antiflex (63x, NA=1.25) objective comprising a quarter wave-plate (Zeiss). Dual wavelength RICM [1] was implemented as follows: a dual video port (Zeiss) enables simultaneous imaging with two CCD cameras (PCO, Germany) using blue and green incident light which were separated by filters at λ_\text{b} = 436±10 nm λ_\text{g} = 546±12 nm.

**Geometry and notations.** See Fig. 1-SI

**Reconstruction of undulating quasi-flat membrane** We combine the effects of illumination aperture [2] and multiple reflections [3] to express, for a given incident wavelength λ, the intensity I as a function of the local membrane-substrate distance h (Eq. 1, main text). The first intensity minimum occurs at h_0 = \frac{\lambda}{4\pi n_1} \arctan \frac{\gamma \sin \delta_2}{1+\gamma \cos \delta_2}, with \delta_2 = 4\pi n_{lip} d_m / \lambda and
\[ \gamma = \frac{r_{1i}}{r_{12}} (1 - r_{12}^2) \] [3]. Here \( r_{ij} = \frac{n_i - n_j}{n_i + n_j} \) is the reflection coefficient at the interface between media i and j (0=glass, 1=outer medium, 2=membrane, 3= vesicle inner medium). Eq. 1 was checked against numerical calculation [4]. Figure 2A-SI show typical RIC micrographs of a fluctuating vesicle obtained at \( \lambda = 546 \) nm (and 436 nm as inset). INA= \( n_1 \sin \alpha_{IA} = 0.7 \) was measured independently. For each pair of frames, the measured blue intensity (\( I_b \)) vs green intensity (\( I_g \)) were compared with Eq. 1 to deduce the local membrane height h (Fig. 2B-SI). Comparison of data with theoretical curves taking into account the illumination angle (solid line) or not (INA=0, dashed line) shows a clear improvement when finite INA is used. A height map was constructed by isolating neighbouring patches of 10x10 pixels represented by a portion of the curve \( I_b vs I_g \). The membrane conformation corresponding to the patch shown in Fig. 2A-SI is represented on Fig. 2C-SI. This procedure is further used to measure the membrane undulations of weakly adhered vesicles (Fig. 2D-SI).

Reconstruction of membrane profiles with variable curvature.

If the surface whose height profile needs to be reconstructed is not approximately flat, the deviations caused by tilt or curvature of the surface have to be accounted for. The expected correction for a surface with pure tilt or constant curvature is given in [5]. However, the case of high and variable curvature, as is the case here, has not been addressed so far. We reconstruct steep membrane profiles of adhered vesicles in the vertical plane orthogonal to the adhesion rim and taking into account reflection by a curved interface with arbitrary tilt and curvature. The two dimensional approximation holds for a portion of membrane which is small compared to the radius of curvature of the adhesion rim. We assume that the profile can be described by a succession of small curved segments, each corresponding to a fringe. The reconstruction is realized fringe by fringe, starting from the adhesion zone. The location of an intensity extrema \( x_{i\text{fringe}} \) corresponds to the optical path \( \Delta_i \) (\( i=1..6 \)) which, following Eq. 1 of main text, satisfies

\[ \Delta_i = \frac{2h_0 + (i - 1)\lambda/2n}{1 - \sin^2(\alpha/2)} \] (1)

Let the x and the y coordinates of the profile to be determined be \( x_i \) and \( y_i \) (corresponding to fringe \( i \)). By convention \( x_0 = 0, y_0 = 0 \) define the point O where the membrane starts to lift off the surface (Fig. 3-SI). Additionally, the initial angle between the membrane and the surface is set to be \( \phi_0 = 0 \). To reconstruct the membrane segment between fringe \( i - 1 \) and \( i \), we use the geometrical construction of Fig. 3-SI, where O is the point on the membrane giving rise to fringe \( i - 1 \) and A is the point giving rise to fringe \( i \). The tangent
FIG. 2: Conformation and undulations of a membrane patch. A. RIC micrograph of an undulating vesicle obtained at $\lambda = 546$ nm and of the $6\mu m \times 6\mu m$ patch at $\lambda = 436$ nm (inset). B. Relation between intensities at the two wavelengths and variable membrane height (boxes, in nm) obtained from Eq. (1) and $\text{INA}=0.7$ (solid line) or $\text{INA}=0$ (dashed line). Dots correspond to pixel intensities of the patch defined in A. C. Tridimensional membrane conformation of the patch after reconstruction of the height. D. Time dependent height of one $1\mu m \times 1\mu m$ subregion of the patch; bars indicate the spatial standard deviation.

to the membrane segment at $O'$ makes an angle $\varphi$ with the $x$-axis. Knowing $O'$ and $\varphi$ from the previous step, as well as the fringe distance $\delta_i = x_i^{\text{fringe}} - x_{i-1}^{\text{fringe}}$, one can determine the position of $A$, defined by the angle $\beta$ and the radius of curvature $r_c$. The light coming from $H$ is reflected into $P$ by $A$ perpendicularly to the tangent $DA$ (Fig. 3-SI). Simple geometrical considerations lead to the following relations:

$$H'A = r_c(\cos \varphi - \cos \beta)$$ (2)
FIG. 3: Reflection of the light ray HH'AP'P on the curved membrane segment O'A. Knowing O',
the initial angle $\varphi$ and the fringe position P, A is constructed by determining simultaneously
the angle $\beta$ and the radius of curvature $r_c$. The procedure is iterated for each fringe, thus building the
entire profile.

\[ O'D = H'A\left(\frac{1}{\tan \frac{\varphi + \beta}{2}} - \frac{1}{\tan \beta}\right) \]  \hspace{1cm} (3)

\[ H'A + AP' = \Delta_i - y_{O'}(1 + \frac{1}{\cos 2\beta}) \]  \hspace{1cm} (4)

where $y_{O'}$ is the y-coordinate of O'. The last relation comes from the fact that the total
optical path $\Delta_i = HA + AP$ includes the additional distance $HH' + P'P$. Using these three
relations, one has to solve for angle $\beta$ and radius of curvature $r_c$ the following couple of
nonlinear equations:

\[ H'A \frac{2\cos^2 \beta}{\cos 2\beta} = H'A + AP' = [\delta_i - O'D + y_{O'}(\tan 2\varphi - \tan 2\beta)] \sin 2\beta \]  \hspace{1cm} (5)

Incrementation for the next iteration is performed by defining: $x_{i+1} = x_i + O'H'$, $y_{i+1} = y_i + H'A$, $\varphi_{i+1} = \beta$. The system of equations 5-SI was solved numerically for each fringe and the
profiles were constructed taking typically up to 6 fringes into consideration. The algorithm
was tested on fringes obtained numerically for a spherical shape, using the formalism of
Wiegand et al. [5]. For sphere radii ranging from 14 to 20 $\mu$m and illumination numerical
TABLE I: Physical parameters used in the model of interaction potential.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{ster}}$</td>
<td>Steric repulsion length</td>
<td>0.2 nm</td>
<td>[7]</td>
</tr>
<tr>
<td>$d_{\text{lip}}$</td>
<td>Lipid head size</td>
<td>0.9 nm</td>
<td>[7]</td>
</tr>
<tr>
<td>$\Delta \rho$</td>
<td>Specific vesicle density</td>
<td>12.8 kg/m³</td>
<td>Measured</td>
</tr>
<tr>
<td>$A_H$</td>
<td>Hamacker constant</td>
<td>$2 \times 10^{-21}$ J</td>
<td>[10, 11]</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>membrane rigidity</td>
<td>$4 \times 10^{-19}$ J=100 k_BT</td>
<td>[3]</td>
</tr>
<tr>
<td>$d_m$</td>
<td>membrane thickness</td>
<td>4 nm</td>
<td>[10]</td>
</tr>
<tr>
<td>$b$</td>
<td>constant of Helfrisch repulsion</td>
<td>0.1</td>
<td>[8]</td>
</tr>
<tr>
<td>$R_f$</td>
<td>HA Flory radius</td>
<td>87 nm</td>
<td>[12]</td>
</tr>
</tbody>
</table>

Aperture 0.5 to 0.7, the shape retrieved from 6 fringes differed by less than 4% from the input sphere. For experimental profiles of vesicles, the reconstruction is very sensitive to the location of the lift-off point of the membrane (point O). Practically, it was determined in order to fit as well as possible a membrane profile described by the balance of adhesion and tension, and therefore following the equation: 

$$y = x \sin \theta - \Lambda \sin \theta [1 - \exp(-x/\Lambda)] .$$

These profiles were used to extract the values of $\theta$ and $\Lambda$ (see Fig. 1-SI) to finally calculate the tension $\tau$ and the adhesion energy $W$. We verified that an estimation of the adhesion energy from the contact radius of curvature [6] gives very similar results.

**Interaction potential.** The interaction potential $V(h)$ with the underlying substrate is constructed as sum of five terms all of which are a function of $h$. The terms are (see table for definitions and numerical values used for constants):

(i) the short range steric repulsion $V_S = \frac{2kT}{d_{\text{lip}}} \exp(-h/L_{\text{ster}})$ [7]

(ii) the vesicle weight under gravity $g$ $V_g = 2R_V \Delta \rho gh$, with the vesicle radius $R_V$ (5-25 µm) [10]

(iii) the Van der Waals attraction $V_{\text{vdW}} = - \frac{A_H}{12 \pi h^2} [1 - \frac{1}{(h+d_m)^2}]$ [10]

(iv) the repulsion due to membrane undulations accounting for the two limits of rigidity or tension dominated regimes: $V_U = \frac{6(k BT)^2}{\kappa h^2} \frac{\epsilon^2}{\sinh(\epsilon)^2}$ with $\epsilon = \sqrt{\sigma / bkTh / 2}$ [8]

(v) the steric repulsion by the polymer, assuming that HA is grafted to the surface: for $h > R_f$, $V_{P, \text{high}} = -k_B T c_{\text{pol}} \ln \left[1 - 2 \exp \left(-\frac{3}{2} \frac{\hbar^2}{2 R_f^2} \right)\right]$ and for $h < R_f$, $V_{P, \text{low}} = k_B T c_{\text{pol}} \left[0.5 \ln \left(\frac{3}{8 \pi} \frac{\hbar^2}{R_f} \right) + \frac{\pi^2 R_f^2}{3 h^2}\right]$, with $R_f = 87nm$ the Flory radius of HA and $c_{\text{pol}}$ the surface density [9].
Undulations. In the state of weak adhesion, the membrane exhibits strong membrane undulations (see Fig. 2-SI). In the approximation of an harmonic potential, the mean square amplitude of fluctuations reads \( \xi_\perp = l_\sigma \Omega(\sigma/\sigma^*) \) [13] with \( \sigma^* = \sqrt{4Kv_2} \), \( l_\sigma = \sqrt{\frac{k_B T}{2\pi\sigma}} \).  

\( v_2 = \frac{\sigma^2 V}{m^2} \) is the second derivative of the potential at the weak adhesion minimum and \( \Omega(y) = \arctan(\sqrt{y^{-2} - 1})/\sqrt{y^{-2} - 1} \) for \( y < 1 \) and \( \Omega(y) = \arctanh(\sqrt{1 - y^{-2}})/\sqrt{1 - y^{-2}} \) for \( y > 1 \). We verified that \( \xi_\perp \) is mainly determined by the tension \( \sigma \), because \( v_2 \) is largely independent of the values of the parameters. Typically, \( \xi_\perp \simeq 15 \text{ nm} \) before spreading correspond to a the initial tension of the vesicle \( \sigma \sim 10^{-5} \text{ N/m} \).

Deviation from spherical cap. For an adhered vesicle having the shape of a spherical cap of radius \( R_V \) and adhesion radius \( R_a \), the contact angle is \( \theta_{sc} = \arcsin(R_a/R_v) \). The contact angle \( \theta_m \) of a vesicle can also be directly measured by fitting a asymptote to the membrane profile reconstructed bit by bit as explained above. To assess the validity of spherical cap shape approximation, we measured the ratio \( \theta_{sc}/\theta_m \) as a function of the adhesion energy (Fig. 4-SI). It is clear that the lower the adhesion energy, the larger is the discrepancy between the real shape and the spherical cap. This could be at the origin of an error in estimation of the contact angle in weak adhesion, leading to a discrepancy with Eq. 3 of main text.