Effect of the HIV-1 fusion peptide on the mechanical properties and leaflet coupling of lipid bilayers

Supplementary material

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1. Analysis of micropipette aspiration experiment data

The software for analysis of micropipette aspiration images is written in Python using the package wxPython for graphical user interface, PIL for image loading, matplotlib for outputs plotting and SciPy for numerical analysis. Since the studied system, vesicle aspirated in a cylindrical pipette, is axis-symmetric, the exact geometry is defined by a few parameters only and there is no need of finding the exact contour of the vesicle. The position of the pipette walls defining the internal diameter of the capillary is obtained from an intensity line profile perpendicular to the pipette axis; see blue line in Figure S1. The axis of the vesicle-pipette system is calculated and the brightness line profile along this axis is extracted; see yellow line in Figure S1. The latter provides the following characteristic features defining the system geometry: the position of the pipette tip, the position of the aspirated part of the vesicle and the position of the outside part of the vesicle. To exclude errors caused by possible flickering of the membrane several (usually 5) images were recorded for each pressure for further averaging during analysis. The waiting time between a pressure change and image acquisition is about 1 min, which for the soft membranes explored in this work is sufficient to establish equilibrium.

1.1. Locating the inner walls of the pipette

To find the pipette inner walls, the user supplies approximate range of pipette wall positions, after which several brightness line profiles across the pipette are extracted; see blue line in Figure S1. The minimal value of the brightness inside user-supplied ranges is taken as a position of the wall.
**Figure S1.** Example of a vesicle aspirated in a micropipette viewed with phase contrast microscopy. The blue line is an intensity line profile across the pipette, i.e., perpendicular to the pipette axis. The inner pipette walls correspond to specific local minima in the profile, which are used to determine the pipette inner diameter. The yellow line is an intensity line profile along the pipette axis. The features of interest described in the text are clearly distinguishable.

**1.2. Defining the pipette axis and radius**

The brightness profiles along two cross-sections of the pipette produce 4 points, 2 for each pipette wall. The pipette inner diameter is calculated as an average distance from each of the points to the line defined by points on the opposite pipette wall. The axis of the pipette is found as a bisector between the lines defining the pipette walls.

**1.3. Extracting characteristic features of the vesicle geometry**

The brightness profile along the pipette axis is extracted; see yellow line in Figure S1. The position of the pipette tip is defined as the brightest point in the user-defined vicinity of the pipette mouth. To find the positions of the vesicle tips inside and outside the pipette, the intensity line profile is smoothed using Savitzky-Golay filter [1, 2] and the absolute value of the gradient of the profile is calculated. The vesicle positions are then defined as the points with maximal absolute gradient left and right of the user-defined pipette mouth vicinity.

**1.4. Calculating the vesicle geometry**

The extracted positions of the pipette tip $x_p$, the tip of the aspirated part of the vesicle $x_a$ and the tip of the outside part of the vesicle $x_v$ yield the aspirated length $L_a = |x_a - x_p|$ and the outside vesicle projection length $L_v = |x_v - x_p|$. The radius of the pipette $R_p$
is calculated during the location of the the positions of the pipette walls. The radius of the outside part of the vesicle is

\[ R_v = \frac{L_v^2 + R_p^2}{2L_v} \]  

(S1)

The vesicle surface tension \( \tau \) is calculated after [3] as

\[ \tau = \frac{PR_p}{2 \left(1 - \frac{R_p}{R_v}\right)}, \]  

(S2)

where \( P \) is the pressure applied to the pipette. The relative change in the vesicle surface area, \( \alpha \), is calculated taking into account the correction for initial aspiration of the vesicle into the pipette [4]:

\[ \alpha = \left[ \frac{1}{2} \left( \frac{R_p}{R_{v0}} \right)^2 \frac{L_a}{R_p} + \left[ 1 - \frac{3}{4} \left( \frac{R_p}{R_{v0}} \right)^3 \frac{L_a}{R_p} \right] \right], \]  

(S3)

where \( \gamma = 1 - \frac{2R_pL_{a0} + R_p^2}{4R_{v0}^2} \), with \( L_{a0} \) being the initial aspirated length and \( R_{v0} \) the initial radius of the outside part of the vesicle.

1.5. Obtaining the bending rigidity \( \kappa \) and the stretching elasticity modulus \( K \)

The obtained dependence of the area dilation \( \alpha \) as a function of the membrane tension \( \tau \) is fitted piecewise with the corresponding model - logarithmic for low tensions (\( \tau < 0.5 \) mN/m) and linear for high tensions (\( \tau > 0.5 \) mN/m). Examples for data from these two tension regimes are given in Figure S2 and Figure S3. The dependence of the area change in low tension regime is described by the expression [5, 6]

\[ \alpha = \frac{k_B T}{8\pi \kappa} \ln \left( \frac{\tau}{\tau_0} \right) \]  

(S4)

where \( \tau_0 \) is the initial tension. In the high tension regime, the fitting follows [4]

\[ \alpha = \frac{\tau}{K} + \alpha_0, \]  

(S5)

where \( \alpha_0 \) is the initial dilation. Since both \( \alpha \) and \( \tau \) have their respective errors, fitting of the experimental data is performed with orthogonal distance regression (full least square fitting) using ODR package of SciPy [7].

Measured values for stretching elasticity are shown in Figure S4. The big spread and no observable dependence from mole fraction of FP23 allow for no conclusion for the effect of the peptide on stretching elasticity.

2. Fluctuation analysis

For a detailed description of the fluctuation analysis see [8, 9]. Briefly, the contour of a vesicle is located on each collected image and decomposed into undulations of various modes around a spherical shape with coefficients of the decomposition being dependent on the value of the bending rigidity \( \kappa \). Then, the values for \( \kappa \) for each of
**Figure S2.** Relative dilation vs tension in the low tension regime for two different vesicles, one made of pure DOPC and one of DOPC doped with 2%FP23. Note the logarithmic scale for the tension. The lines correspond to ODR fits, with slope defining the bending rigidity $\kappa$, see Equation (S4).

**Figure S3.** Dilation vs tension in the high tension regime for two different vesicles, one made of pure DOPC and one of DOPC doped with 1%FP23. The lines correspond to ODR fits, with slope defining the stretching elasticity $K$, see Equation (S5).
Figure S4. Micropipette aspiration data for the stretching elasticity of DOPC bilayer as a function of FP23 content. The error bars correspond to standard errors.

Figure S5. Mode number dependence of the bending rigidity $\kappa$ for a vesicle made of DOPC doped with 1%FP23 with a radius of $\approx 20\mu$m. The error bars are given only for the bending-governed regime used to estimate $\kappa$, $7 \leq q \leq 15$ in this particular case. The horizontal line indicates the corresponding average of $10.2 \ k_B T$.

The mode numbers is obtained from a non-linear fit. The obtained dependence of $\kappa$ vs the mode number (see Figure S5) exhibits a specific region of mode numbers where the undulations are primarily governed by bending rigidity. The values for $\kappa$ in this range only are averaged out to produce the final output.
3. Data processing

3.1. Filtering off pure lipid vesicles

As indicated in the main text, the distinguishable experimental data clustering (see Figure 3 in the main paper) suggests the presence of pure lipid vesicles in samples prepared from mixtures of the lipid and FP23. In order to get rid of this contribution we performed the following filtering of the data. First, the average of the considered parameter $x$ (in our case, the bending rigidity measured with one of the methods) is calculated for vesicles prepared from pure lipid, yielding a value for the average $\bar{x}$ and the corresponding standard error $\sigma$. Then, in the analysis of vesicles containing the peptide only values smaller than those measured for pure lipid vesicles, i.e. $x_i < \bar{x} - \sigma$, are considered for averaging (compare Figure 2 with the unfiltered data from the main paper and Figure S6 with data filtered as described above).

References

Supplementary material for “Effect of the HIV-1 fusion peptide...”


