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Supporting Material:
Bending and puncturing the influenza envelope

With the following figures we show:
i) How much the SUVs are deformed during the measurements.
ii) At the relatively high deformations of 20 % used in our experiments, the deformation was fully reversible and elastic. Non-reversibility was mainly observed at deformations higher than 50 %.
iii) When the same data was analyzed at lower deformations, the calculated elastic properties agreed within 15 %
iv) The effects of the variable boundary conditions (the flat surface and the AFM tip) are visualized, based on the results obtained with our finite element model.

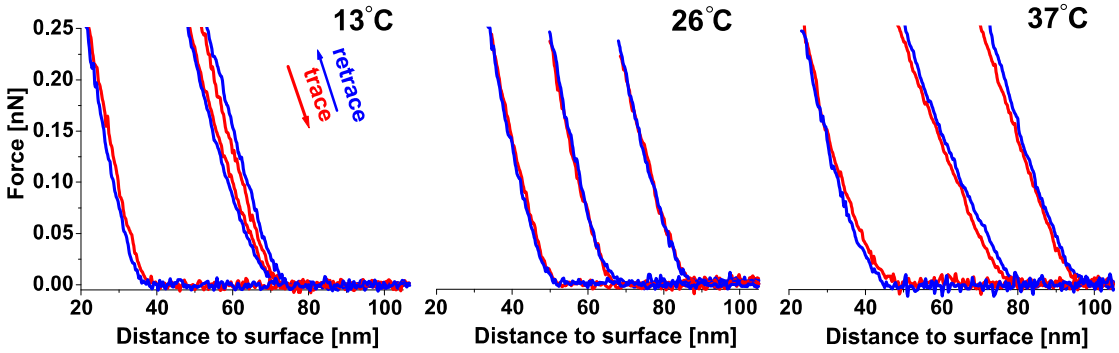

Figure S1: We compared the indentation curves obtained for Influenza SUV at 13, 26 and 37 °C after pushing (trace) and retraction (retrace) of the cantilever. Shown are 9 example curves of trace and retrace from single particles between 40 and 100 nm that present no discontinuities: The deformation was fully reversible without hysteresis in the 0-0.25 nN range, thus elastic, as expected for a fluid phase bilayer. Each curve is the average of up to 4 curves that were obtained on top of a single particle (see also Materials and methods).

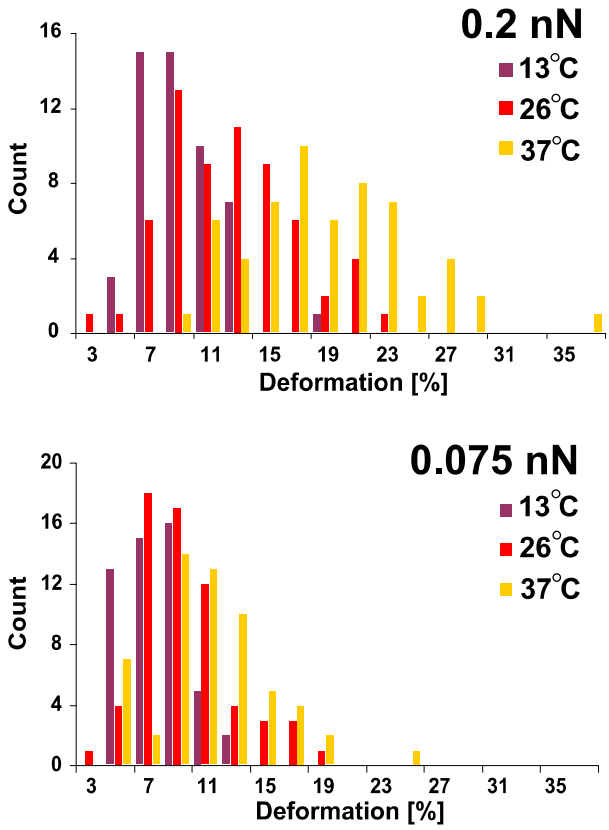

Figure S2: Deformation of influenza SUV (as % of the vesicle height) at 0.2 nN (top) and 0.075 nN (bottom). The 0.2 nN limit was used for the 'high force' analysis of the data as described in the report, the lower limit of 0.075 nN was used for the alternative 'low force' analysis, that is presented in Fig. S3.

Indentation/SUV	indentation at 0.075 nN avg ± s.e	indentation at 0.20 nN avg ± s.e
Influenza 13°C	5.9 ± 0.2 nm *(7.8 ± 0.3 %)*	10.3 ± 0.4 nm *(13.4 ± 0.4 %)*
Influenza 26°C	7.9 ± 0.3 nm *(9.5 ± 0.4 %)*	13.9 ± 0.4 nm *(16.4 ± 0.5 %)*
Influenza 37°C	9.8 ± 0.6 nm *(11.3 ± 0.5 %)*	16.3 ± 0.8 nm *(19 ± 0.7 %)*

Table S1:
The indentation of influenza particles at the maximum forces used for the low- and high force analysis. At 0.2 nN force the average deformations were up to 19 % of the liposome diameter. At 0.075 nN the average deformations were up to 11 % of the liposome diameter and the average indentation depths were 6-10 nm. At the same force the

indentation depth was 5.9 nm on average and always less than 9.7 nm for particles < 50 nm.

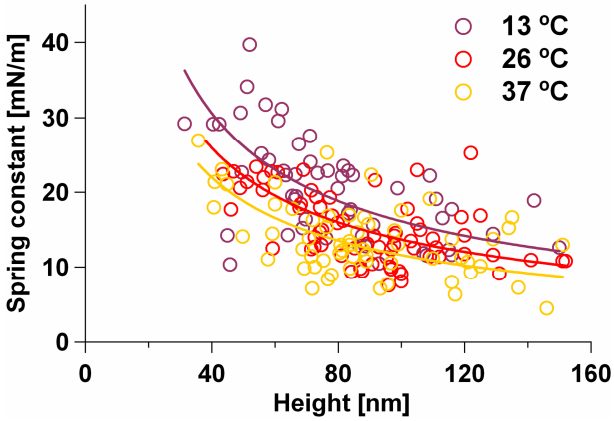

Figure S3: Another way to verify the elasticity of SUV is to compare the response of liposomes in two different force ranges. Therefore we determined the stiffness of the same influenza SUV data set by fitting the force curves between 0.025-0.075 nN with a linear function, and compared the response at this low force to that obtained in the 0.1-0.2 nN range. Although the spread in the data is larger than when analyzed with the high force fit (0.1-0.2 nN, Fig. 4 in the main report), the difference between the three temperatures is still clear. The solid line shows the fit to the data. The empirical fit function was obtained in a similar way as eq. 5 in the report. The FEM deformation curves were fitted between 25 and 75 pN, which followed within 8 %: $k \approx 27e4 * E^{0.63} * t^2 / d^{0.70}$

	0.025-0.075 nN fit	0.1-0.2 nN fit
13 ° C	59 MPa	68 MPa
26 ° C	45 MPa	45 MPa
37 ° C	35 MPa	37 MPa

Table S2:
To test whether the response is also quantitatively identical at low and high force analysis, we calculated the Young's moduli obtained from the fits at low force (from Fig. S3) and compared these values with those presented in the main report. The differences between the three temperatures remain clear, the absolute values agree within 15 %.

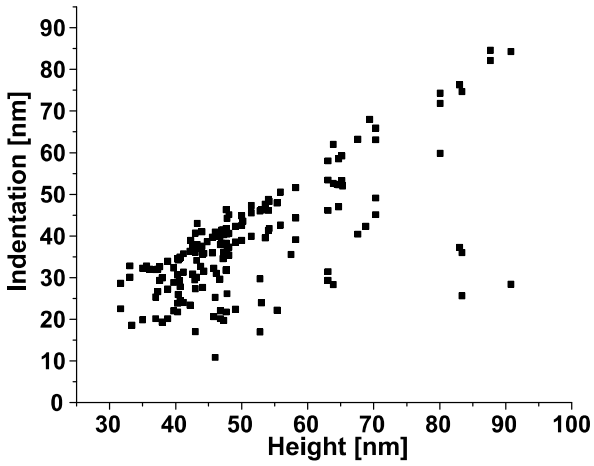

Figure S4: The indentation at which the particles are punctured is plotted vs the particle height (Influenza SUV). A puncture event is an indicator for the transition from elastic to non-elastic deformation. Most punctures occurred at a very high indentation and low distance to the surface. Only 4 % of the vesicles showed puncture events between 10 and 20 nm indentations (marked in red): Also for these 4 % the punctures always occurred at forces superior to 0.2 nN. This further supports our conclusion that the deformation of influenza SUV is reversible when the applied force does not exceed 0.2 nN.

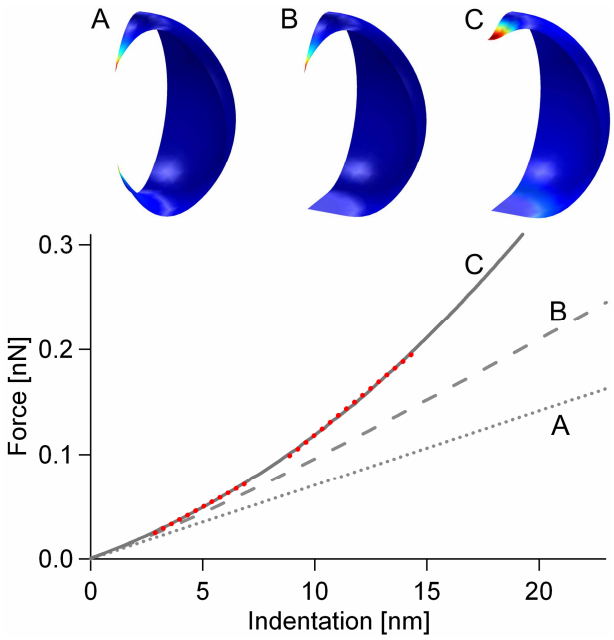

Figure S5: The modeled indentation of the liposome depends on the applied boundary conditions.
A) A thin shell model (100 nm diameter (*d*), 40 MPa (*E*), 3.1 nm shell thickness (*t*)) is symmetrically indented by two point forces. The force vs indentation curve is linear and the slope follows: $E^{*}t^{2}/d$.
B) The model is supported by a planar surface and indented by a point force. During indentation the liposome gets flattened onto the planar surface. This increasing contact area is visible in the curve as an increase in slope during the indentation.
C) The model is supported by a planar surface and indented by a hyperbolic tip. Because of the increasing contact area during indentation of both the surface and the tip, the slope increases even more with the indentation. For the analysis of our data we used this model as it most realistically describes the experimental geometry. The red dotted lines between 0.025-0.075 and between 0.10-0.20 nN, indicate the linear fits we used to define the stiffness of the liposomes.