Electronic Supplementary Material (ESI) for Nanoscale.


# Multilamellar Nanovesicles Show Distinct Mechanical Properties Depending on their Degree of Lamellarity

# Supporting Information

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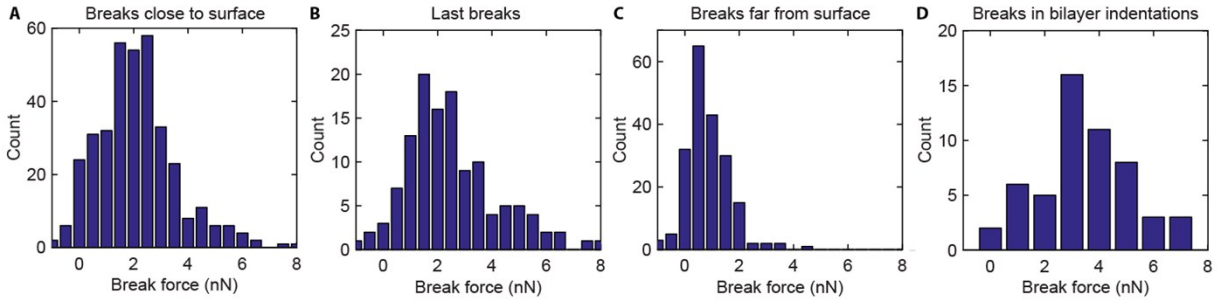


**Figure S1: Break force statistics.** Histogram of forces at which break events occur: **A)** close to the surface (2.2 $\pm$ 1.6 nN, st.d., N = 361); **B)** the last break event (2.6 $\pm$ 1.8 nN, st.d., N = 124); **C)** far from the surface (0.8 $\pm$ 0.8 nN, st.d., N = 216); and **D)** during bilayer indentations (3.5 $\pm$ 1.5 nN, st.d. N = 54). Spread in the observed break forces is generally much larger than when analyzing break distance. The force required to break through the last bilayer is typically slightly larger than the other breaks close to the glass surface. A simple explanation for this is that the force at which the AFM tip contacts the last bilayer is typically higher than for the first contacted bilayer. The force required for penetrating supported lipid bilayers is higher than the force required for the last adherent bilayer during a vesicle indentation. Potentially, this can be explained by the higher tension present in the vesicle membranes than the supported lipid bilayers, which makes penetration by the AFM tip energetically less unfavorable[1]. Finally, the breaks occurring further from the surface during vesicle indentations occur at significantly lower force than the penetration of the bilayer stack close to the surface. It is known that the penetration force required to penetrate a lipid bilayer increases with loading rate[2], and since in our experimental design the speed (in nm/s) of the AFM tip is constant, the loading rate depends on the stiffness before penetration. The low rigidity of the vesicle results in a (~5 fold) lower loading rate for the break events far from the surface, than those closer to the surface.

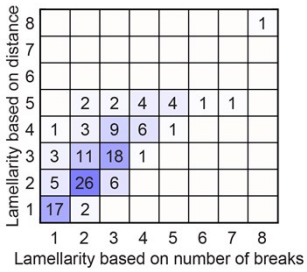


**Figure S2: Determination of the vesicle lamellarity.** In the main text we determine the lamellarity of vesicles based on the summed distance of the distance of the breaks within each curve. Alternatively, the amount of break events could be counted to determine lamellarity. Here, we compare both methods in a 2D-histogram with color (and numbers) indicating the number of vesicles in each bin. Overall, both methods yield good correspondence (~60% of vesicles are classified in the same way, and >30% differ only one lipid bilayer. We used classification based on total break distance, since we believe it represents the most unbiased approach. Some much larger breaks are present in the FDCs (see main text fig. 2D), which likely represent double (or more) bilayer penetrations. When determining the number of breaks in each curve we would have to decide what to do with each larger break event separately. (For this figure such larger breaks were counted as a single lipid bilayer; if an odd number of break events was recorded we rounded up the total.) We note that determining the lamellarity by the number of break events does not change any of the main conclusions (data not shown).

**Supplementary references:**


1 A. J. García-Sáez, S. Chiantia, J. Salgado and P. Schwille, *Biophys. J.*, 2007, **93**, 103–112.

2 A. Alessandrini, H. M. Seeger, T. Caramaschi and P. Facci, *Biophys. J.*, 2012, **103**, 38–47.