Supplementary Material (ESI) for Soft Matter
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Membrane fusion of pH-sensitive liposomes - A quantitative study using giant unilamellar vesicles

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Electronic Supplementary Information (ESI)

Derivation of Equation 1 in the main article. Assuming that the fusogenic liposomes are unilamellar, then the number of fluorophore lipids in each fusogenic LUV, n_{LUV}, is given by

$$n_{LUV} = x_{LUV}\frac{A_{LUV}}{\bar{a}_{LUV}} \tag{S.1}$$

where x_{LUV} is the rhodamine concentration in the LUV membrane (mole fraction), A_{LUV} is the total surface area of the LUV membrane and $\bar{a}_{LUV}$ is the weighed average of the areas of lipids in the LUV, *i.e.* DOPE, OA and Rho-DOPE.

The number of fluorophore lipids in the GUV membrane after fusion, n_{GUV}, is given by

$$n_{GUV} = x_{GUV}\frac{A_{GUV}}{\bar{a}_{GUV}} \tag{S.2}$$

where x_{GUV} is the rhodamine concentration in the GUV membrane (mole fraction), A_{GUV} is the total surface area of the GUV membrane and $\bar{a}_{GUV}$ is the weighed average of the areas of POPC, Chol, DOTAP and Rho-DOPE.

As the number of fluorophore lipids is conserved during fusion, the number of fusion liposomes, n_{LM}, that have fused with a GUV is given by

$$\begin{aligned} n_{LM} &= \frac{n_{GUV}}{n_{LUV}} \\ &= \frac{x_{GUV}}{x_{LUV}}\frac{A_{GUV}}{A_{LUV}}\frac{\bar{a}_{LUV}}{\bar{a}_{GUV}} \\ &= \frac{x_{GUV}}{x_{LUV}}\left(\frac{r_{GUV}}{r_{LUV}}\right)^2\frac{\bar{a}_{LUV}}{\bar{a}_{GUV}} \end{aligned} \tag{S.3}$$

where r_{GUV} is the radius of the GUV after fusion, r_{LUV} is the average radius of the fusogenic LUVs and the subscript in n_{LM} signifies that this value was found using the lipid mixing assay.

As LUVs and GUVs contain equal amounts of non-phospholipids (OA and Chol), which occupy approximately the same area[1,2], and assuming that the fluorophore lipids occupy the same area in LUVs and GUVs, then $\frac{\bar{a}_{LUV}}{\bar{a}_{GUV}}$ is approximately 1, giving

$$n_{LM} \approx \frac{x_{GUV}}{x_{LUV}}\left(\frac{r_{GUV}}{r_{LUV}}\right)^2 \tag{S.4}$$

Eq. S.4 equals eq. 1 in the main article.

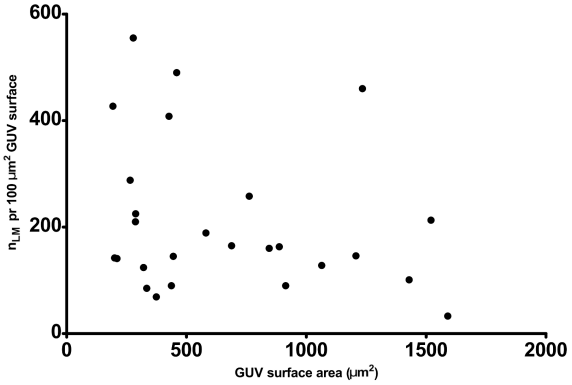

Fig. S 1 Number of fusion liposomes, n_{LM}, to fuse with each GUV per GUV surface area, shown for different sizes of GUVs with 10% positively charged lipids. There is no clear correlation between fusogenic abilities and size of the GUV.

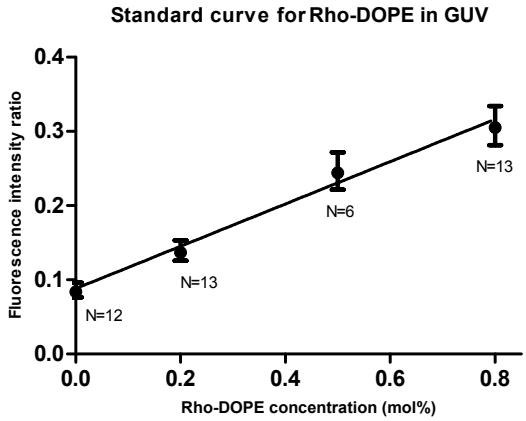

Fig. S 2 Standard curve of the relationship between Rho-DOPE content in a GUV membrane and the FRET ratio (Rho-signal divided by NBD-signal when only NBD is excited). This standard curve is used to calculate the Rho-DOPE concentration in a GUV membrane after fusion with fusogenic liposomes labeled with rhodamine. Error bars are SDOM.

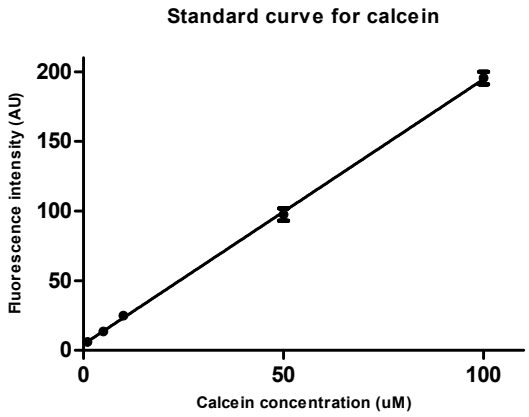

Fig. S 3 Standard curve of calcein dissolved in buffer, where the fluorescence intensity is measured on confocal images. This standard curve is used to convert calcein fluorescence intensity to a concentration. At higher concentrations, calcein becomes self-quenching, but in the range shown the intensity increases linearly with concentration. The fluorescence intensity is dependant on fluctuations in laser power, so the standard curve was measured the same day as experiments were performed. All points were measured in triplicates, and error bars are SDOM.

Table 1 Average calcein concentration for the pure LUV solution after addition of triton, and for the same solution diluted 5 times in glycine buffer. The calcein concentrations shown are found using a standard curve, similar to the one shown in fig.3. These concentrations are used to find the encapsulated concentration of calcein inside the fusogenic LUVs. Values are averages of 3 measurements, and are shown ± SDOM

Name	Intensity (AU)	Calcein concentration (μM)
LUV solution	70.5 ± 0.9	123.9 ± 1.6
LUVs diluted 5x	16.6 ± 0.4	24.1± 0.7

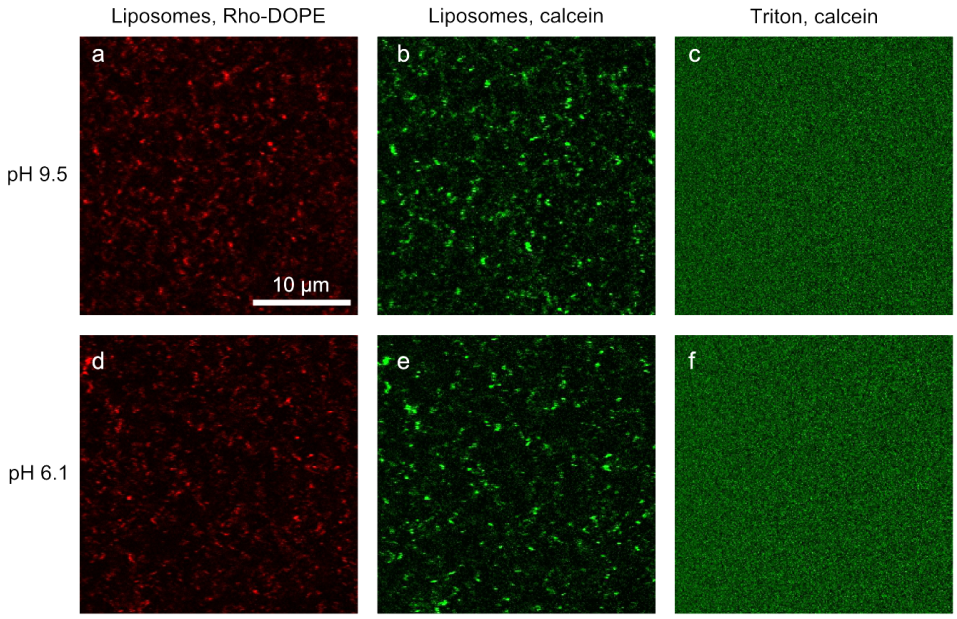

Fig. S 4 Confocal images of fusogenic LUVs labeled with Rho-DOPE (red), and with encapsulated calcein (green). The fusogenic liposomes are shown at pH 9.5 (a-c) or pH 6.1 (d-f), before (a,b,d,e) and after disruption with Triton-X100 (c and f). Calcein is encapsulated at a self-quenching concentration in the LUVs, so the signal increases upon disruption of the liposomes and release of calcein. The increase is approximately the same at the two pH-values (a factor 2.9 at pH 9.5 and a factor 2.7 at pH 6.1, measured using Leica software), which shows that almost all calcein is retained in the LUVs, even at pH 6.1. All images have the same size.

References

1 G. H. Peters, F. Y. Hansen, M. S. Møller and P. Westh, *J. Phys. Chem. B*, 2009, **113**, 92–102.

2 D. Ghosh and J. Tinoco, *Biochim. Biophys. Acta*, 1972, **266**, 41–49.