

Electronic supplementary information

Heat-induced formation of single giant unilamellar vesicles

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Supplementary Figures

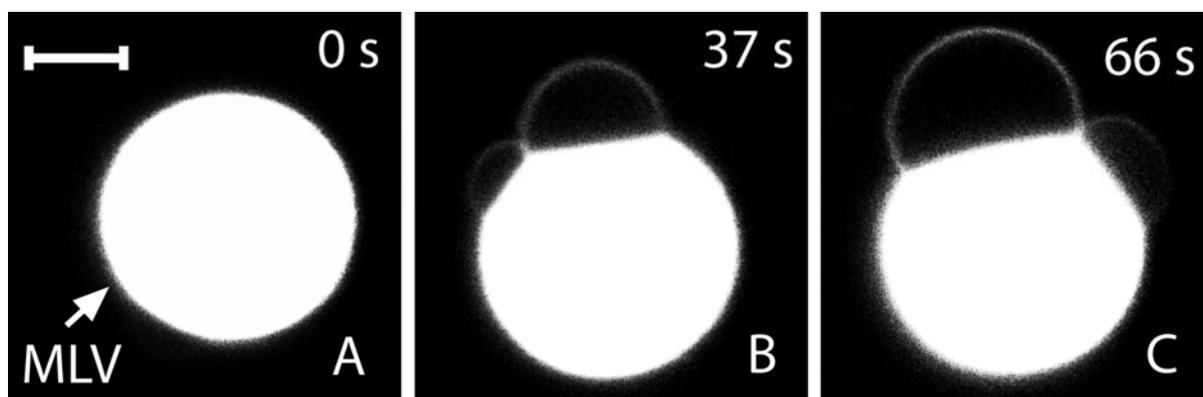


Figure S1: Heat-induced formation of GUVs from PC/PE. Confocal laser scanning micrographs of a MLV prepared from 60% PC, 39% PE and 1% Texas Red DHPE and deposited onto a borosilicate coverslip. (A) MLV before heating. The scale bar represents 5 μm . (B) MLV after 37 s of heating at 45-50°C. Formation of unilamellar vesicles starts to appear on one side. (C) After heating, the formed GUVs remain connected to the MLV. The micrographs were recorded using a 63x objective.

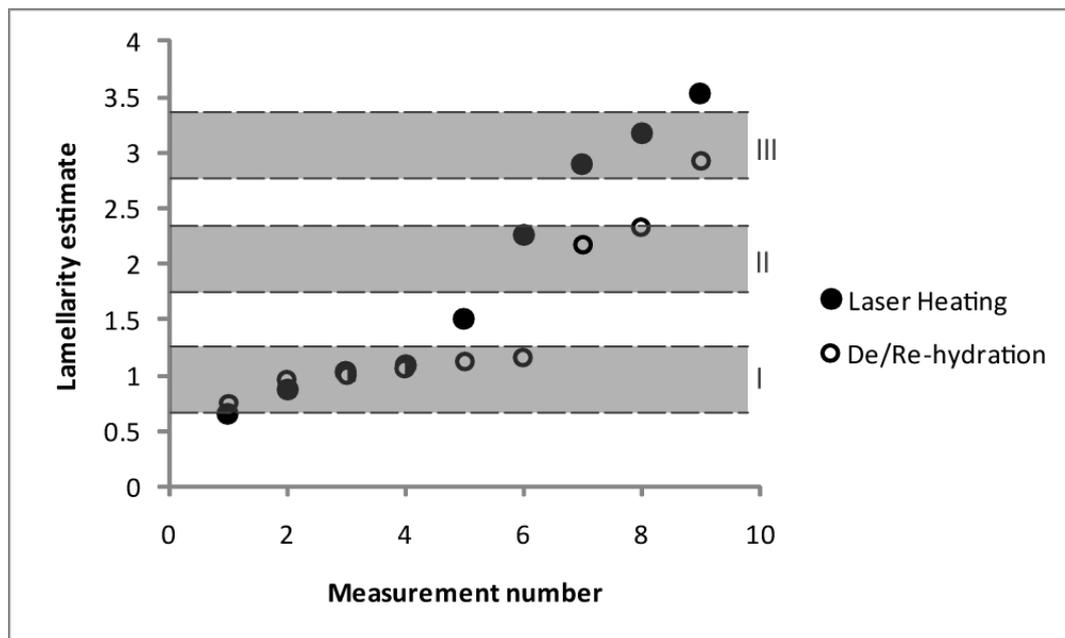


Figure S2: Distribution of lamellarity estimate of liposomes, generated using both laser heating and dehydration-rehydration techniques. The groups I-II-III are assumed uni-, bi-, tri-lamellar liposomes.

Measurement of Lamellarity

As described within the article, the lamellarity measurements were made on a total of 18 vesicles; 9 GUVs obtained by dehydration-rehydration procedure and 9 GUVs obtained by directed laser heating. Images were formed using a laser scanning confocal microscope, while maintaining the fluorescent excitation intensity and the confocal acquisition settings, for all the GUVs under observation.

Through analysis of the vesicle population created, using a similar scheme to one proposed by Akashi *et al* (1996), we were able to elucidate that the GUVs generated by directed laser heating, were observed to have the same lamellarity distribution as those GUVs occurring from the dehydration-rehydration scheme.

Assuming that the population measured was sufficiently large to capture a majority of unilamellar vesicles, ROI intensity measurements (described in the article) will indicate any occurring distribution, the lowest of which representing unilamellar. Based on this, the average intensity value, taken from the four lowest ROI intensities within each population, was used as a normalization factor, to estimate the lamellarity for each vesicle, mirroring the technique proposed by Akashi *et al* (1996).

The results of this estimation are shown in figure S2, where the grouping at each of the three lamellar levels can be distinguished. As shown by Akashi *et al* (1996), strong correlation grouping can be observed at the uni- (I), and as the lamellarity increases the variability, as does the distribution in values.

Using this data from our sample preparation, we propose that a distribution of lamellarities was generated, with the majority having a lamellarity of one, but distinct populations were observed at two and three. This distribution is apparent in both vesicles formed using the dehydration-rehydration technique, and the laser heating technique, indicating the generation technique does not significantly influence the vesicle lamellarity.