Supplementary Information

1 Vesicle Extrusion

Upon extrusion through a filter with a mean diameter of D_F, a vesicle of diameter D will be fragmented into two or more vesicles, figure 1A. A fragmentation that leads to multiple vesicles can always be divided into several sub-fragmentations each producing only two new vesicles. The filter is assumed to bias this fragmentation into vesicles with diameters similar to D_F. In order to describe this a normalized vesicle redistribution profile $f(D)$ is introduced as a tool to quantify the outcome of a successful extrusion. We model this filter by a Gaussian distribution centered at D_F and having a width given by $w = aD_F$, equation 1.

$$f(D) = \frac{1}{w\sqrt{\pi}} \exp\left(-\frac{(D - D_F)^2}{w^2}\right) \tag{1}$$

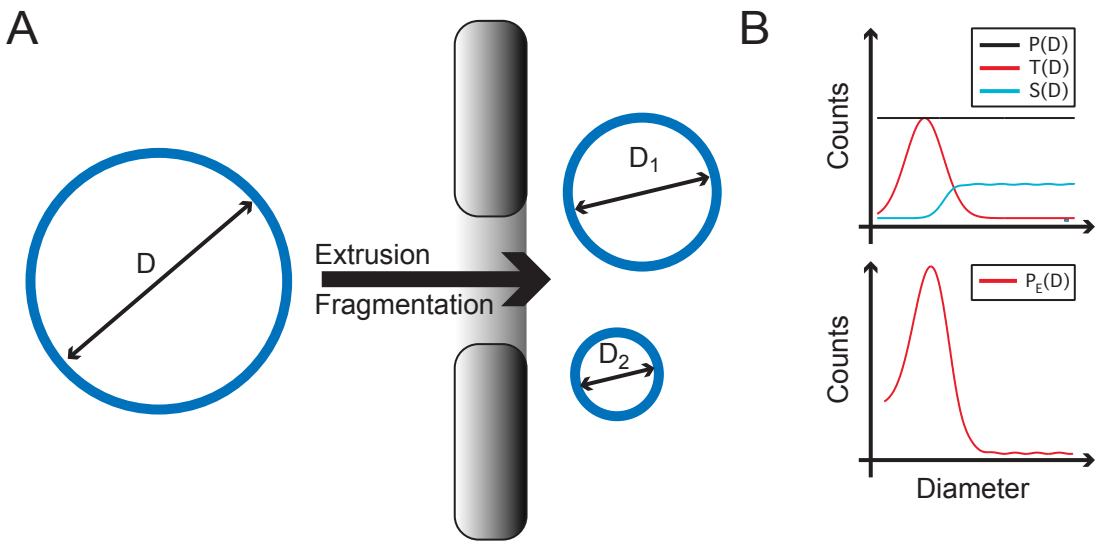

Figure 1: **Effect of extrusion on lipid vesicles: (A)** A vesicle becomes fragmented upon encountering a narrow pore, e.g. in a polycarbonate membrane. Two new and smaller vesicles are produced in order to conserve the total lipid mass. **(B)** Sketch of vesicle size distributions; initial (black, top), newly formed (red, top), fragmentation removed (turquoise, top) and extruded (red, bottom).

The constant a was estimated to 0.6 by comparing samples extruded through 100/200 nm diameter filters with theoretical calculated populations. Theoretical calculated vesicle size histograms can be obtained once an initial (polydisperse) population has been experimentally measured. Extruded populations $P_E(D)$ are generated by redistributing the initial population $P(D)$ according to the redistribution profile $f(D)$. The value $f(D)$ determines how much lipid mass is transferred from the rest of the population into the bin containing the amount of vesicles with diameter D. The transfer of lipid mass is a consequence of fragmentation-induced generation of

smaller vesicles. Let $T(D)$ be the number of new vesicles formed with diameter D, it is then given by equation 2.

$$T(D) = f(D)\frac{M_{>D_M}}{m_D} \tag{2}$$

$M_{>D_M}$ is the total lipid mass of vesicles with diameter above a certain minimum diameter D_M, while m_D is the lipid mass of one vesicle with diameter D. The minimum diameter is related to the critical diameter D_0 below which no vesicles can exist, i.e. due to membrane stress and lipid packing restraints. Thus a fragmentation cannot produce diameters below D_0. The smallest vesicles which can be fragmented into vesicles of diameter D must therefore have a minimum diameter D_M given by the expression below.

$$D_M = \sqrt{D^2 + D_0^2} \tag{3}$$

It is now possible to reformulate equation 2 as a sum over all the fragmentation-available vesicle sizes present in the initial population:

$$T(D) = \frac{f(D)}{m_D} \sum_{D_M \,\leq\, i} m_i P(i) = \frac{f(D)}{D^2} \sum_{\sqrt{D^2+D_0^2} \,\leq\, i} i^2 P(i) \tag{4}$$

Now having established the number of vesicles produced by fragmentation it is necessary also to calculate how many vesicles are removed from the initial population in order to form the new. The number of vesicles with diameter D removed by fragmentation, $S(D)$, is given by collecting the total fraction of lipid mass transferred from D into smaller vesicles:

$$S(D) = P(D) \sum_{D_0 \,\leq\, i \,<\sqrt{D^2-D_0^2}} f(i) \tag{5}$$

The vesicle redistribution profile along with the initial population thus exclusively defines two new vesicle distributions; the population of new vesicles formed $T(D)$ and initial vesicles destroyed by fragmentation $S(D)$. The extruded population profile is thus constructed by adding and subtracting $T(D)$ and $S(D)$ respectively from $P(D)$, equation 6, as sketched in figure 1B:

$$P_E(D) = P(D) + T(D) - S(D) \tag{6}$$

2 Intensity Calibration of TetraSpeck Microspheres

The degree of staining of colloidal beads with diameters of 100 nm, 210 nm, 500 nm and 1000 nm were investigated by spectroscopic bulk measurements. Fluorescence spectra were obtained from each bead population and is shown in figure 3.
The bulk samples were excited at 488 nm and fluorescence emission was collected from 510 - 550 nm, thus matching the excitation/emission settings from the confocal microscope setup. The fluorescence spectra were integrated in order to obtain the total intensity in the given wavelength interval. The fluorescence density σ_F (intensity/volume) was calculated from the integrated intensity I_T, the bead bulk concentration C and the colloidal volume V according to equation 7.

$$\sigma_F = \frac{I_T}{C\ V} = \frac{6I_T}{\pi C D^3} \tag{7}$$

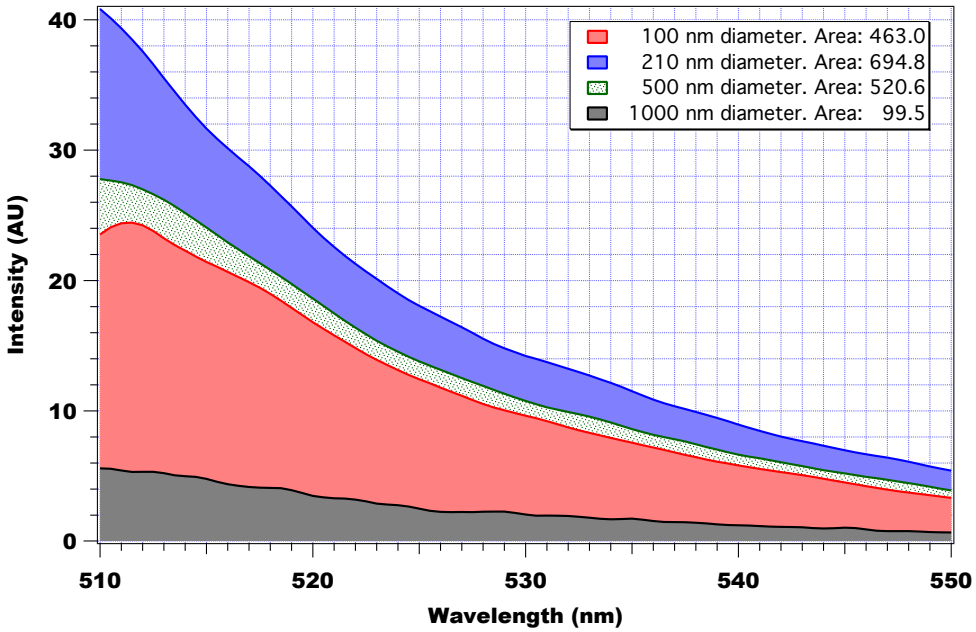

Figure 2: **Fluorescence spectroscopy of colloidal particles**: Fluorescence emission obtained by excitation at 488 nm and shown from 510-550 nm of 100 nm diameter beads (red), 210 nm diameter (blue), 500 nm diameter (green) and 1000 nm diameter (black). The total integrated intensity is shown in the legend for each bead population.

Having obtained the fluorescence density from each population it is possible to compare and correct for differences in staining. All populations were normalized to the 210 nm sample by applying a correction factor k_F, table 1. This factor was multiplied on the integrated intensities obtained from the confocal fluorescence micrographs prior to applying the size conversion. This ensured that the corrected integrated intensities were directly comparable with each other and hence the obtained bead diameters.

Catalog no.	**C (ml^{-1})**	**Bead diameter (nm)**	I_T **(AU)**	σ_F **(AU/nm^3)**	k_F
T7279	$1.8 \cdot 10^{13}$	100 ± 6	463.0	$1.228 \cdot 10^{-15}$	1.77
T7280	$2.0 \cdot 10^{12}$	210 ± 21	694.8	$2.171 \cdot 10^{-15}$	1.00
T7281	$1.5 \cdot 10^{11}$	500 ± 20	520.6	$1.473 \cdot 10^{-15}$	1.47
T7282	$1.8 \cdot 10^{10}$	1000 ± 32	99.5	$1.320 \cdot 10^{-15}$	1.64

Table 1: **Table of spectroscopic data**: From left to right (i) Molecular Probes catalog number, (ii) number of beads per ml solution, (iii) nominal bead diameter, (iv) integrated spectral intensity, (v) brightness per bead volume and (vi) intensity correction factor.

3 Vesicle Structure

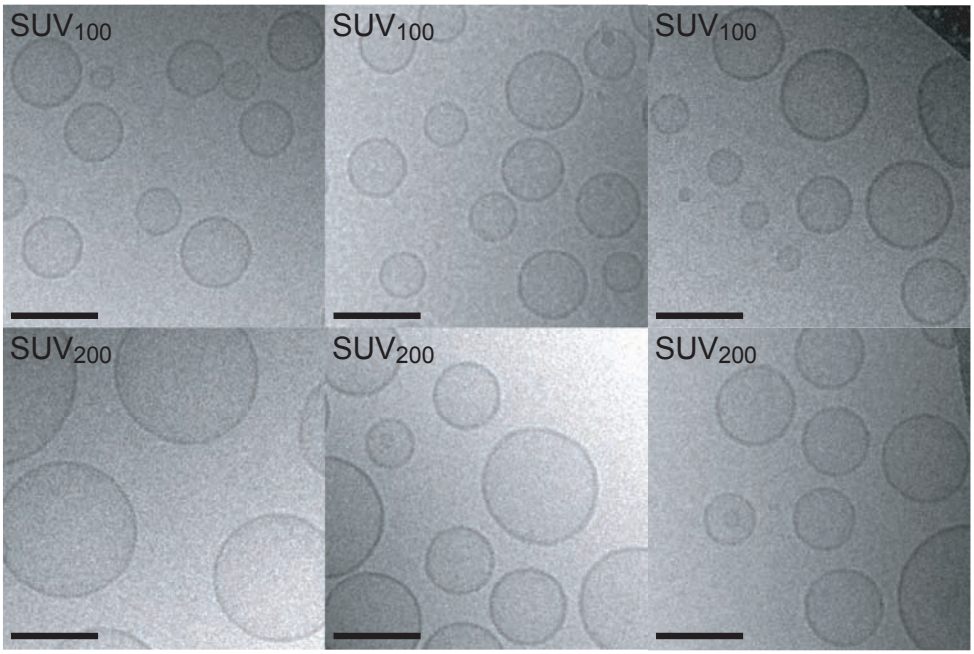

Figure 3: **Cryogenic Transmission Electron Micrographs of Extruded Vesicles**: (Top row) Representative micrographs of vesicles extruded through a polycarbonate membrane with filter pore diameters of 100 nm and (Bottom row) 200 nm. The shape of the vesicles are observed to be approximately spherical. All scale bars are 100 nm.