Supporting Information

Size-Dependent, Stochastic Nature of Lipid Exchange Between Nano-Vesicles and Model Membranes

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Supporting Figures

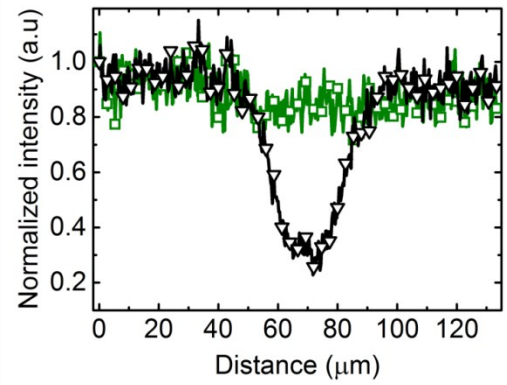

Figure S1.Fluorescence intensity profile on a cross-section through the bleach spot at t = 0 min (rectangles) and t = 2 min (triangles).

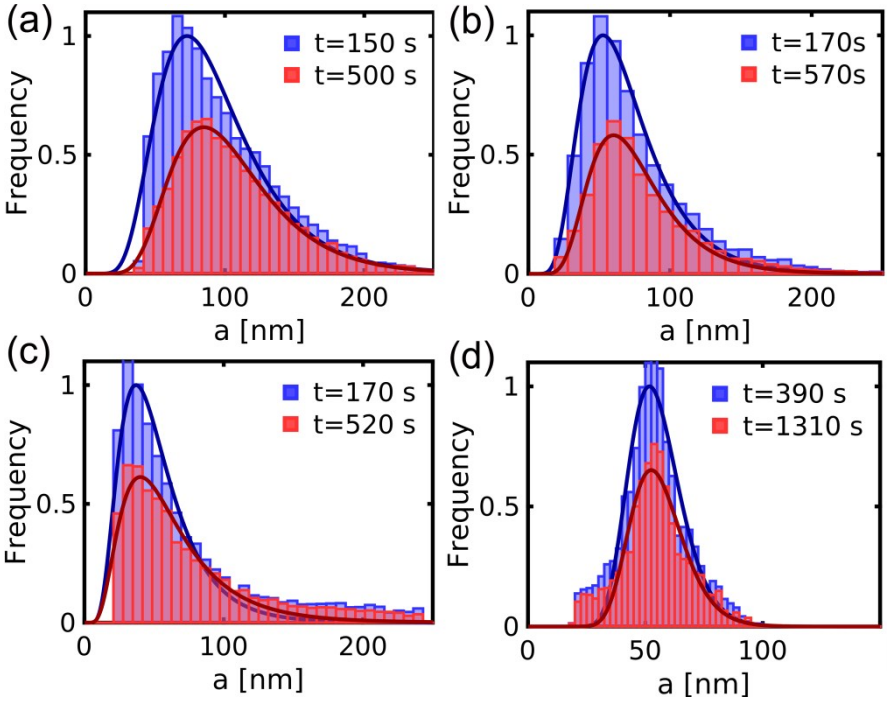

Figure S2. Size distribution at different times for the various lipid compositions. (a) 1% DOPS, 99% DOPC, (b) 5% DOPS, 95% DOPC, (c) 50% DOPS 50% DOPC, (d) 5% DOPS 95% DPPC.

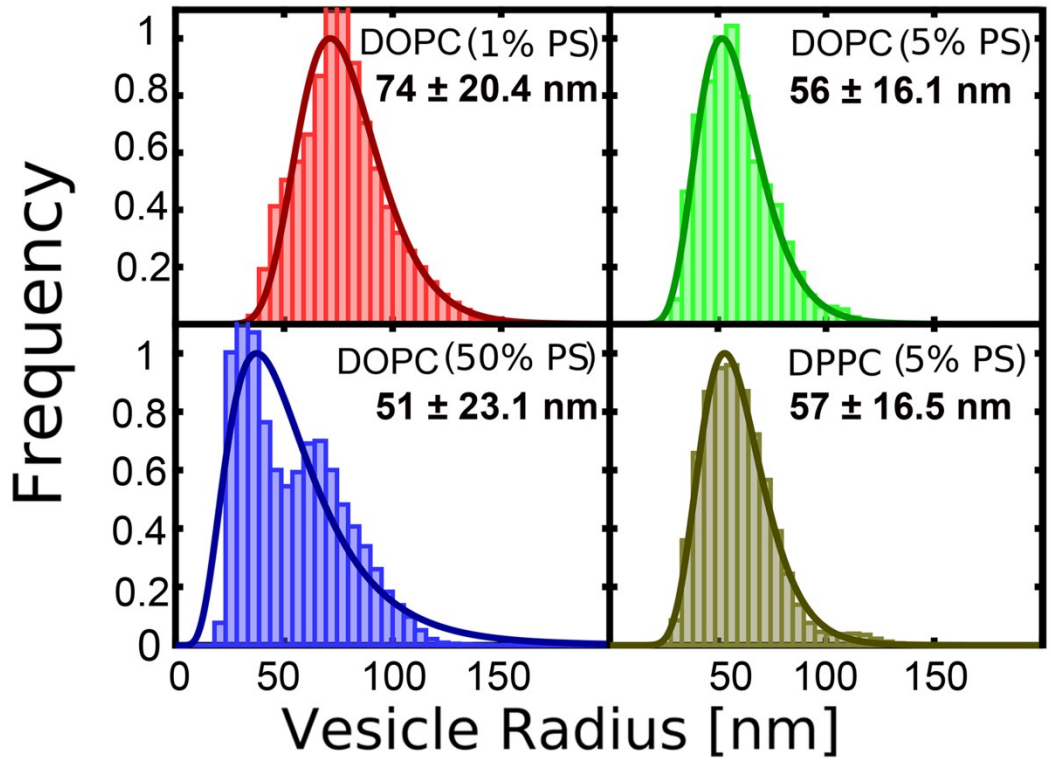

Figure S3. Size distribution for vesicles of various compositions, measured with the Nanoparticle Tracking Analysis method.

Supporting Table

Table S1. Vesicle composition, vesicle size (hydrodynamic radius a, measured by NTA), vesicle number N_0 at $t = 0$ and vesicle desorption rate k. All vesicles contain an additional 0.1 mol% rhodamine-PE. *Owing to small number density, the data for the gel-phase vesicles are accumulated from three independent image sequences.

Composition	a	N_0	k [10^{-3} s^{-1}]
1% PS, 99% DOPC	75 ± 20	1449	1.39 ± 0.05
5% PS, 95% DOPC	56 ± 16	1712	1.35 ± 0.03
50% PS, 50% DOPC	51 ± 23	1219	1.45 ± 0.03
5% PS, 95% DPPC	57 ± 17	825*	0.53 ± 0.01

Desorption Kinetics driven by Monomer Diffusion

Here we derive the time dependent SUV desorption rate, when the desorption driving charge equilibration is due to the diffusional exchange of individual (charged) lipids between the SUVs and SLB. For this purpose, let us assume that N_0 vesicles absorb on the SLB at $t = 0$ and that vesicle detachment (vesicle charge neutralization) requires the exchange of Q charged lipids, and that per unit time, each lipid has a probability k_L to be exchanged. The transfer probability rate per lipid k_L is not to be confused with the vesicle detachment rate k. Under these assumptions the probability of vesicle detachment at time t is the product of Q lipid transfer probabilities: p^Q, where each probability reads: $p = 1\text{-}\exp(-tk_L)$, giving the following time-dependent, expectation value of the normalized number of adhering vesicles:

$$\frac{N}{N_0} = 1 - \left[1 - exp\left(- k_L t\right)\right]^Q \quad . \tag{S1}$$

This relation is plotted in Figure S4a where we use $k_L = 10^{-2}$ s^{-1} and $Q = c4\pi a^2/A_L = 5\times10^2$, 2.5×10^3 and 2.5×10^4, for vesicles containing 1%, 5% and 50% negatively charged DOPS lipids respectively, $a \approx 50$ nm is the vesicle radius, $A_L \approx 0.6$ nm^2 is the membrane area per lipid molecule and c is the fraction of charged lipids, which for the three experimental cases equals $c = 0.01$, 0.05 and 0.5, respectively. Figure S4a shows that Eq. (S1) predicts a charge (Q) dependent lag-time of the order of $k_L^{-1}\log(Q)$ before N transitions from N_0 to zero during a transition time, which is of the order of k_L^{-1}. When the number of charged lipids $Q >> 1$, the lag-time is large compared to the transition time.

Next we incorporate a (log-normal) charge Q distribution among vesicles at $t = 0$, $N_0(Q)$:

$$N_0(Q) = N_0 \frac{exp\left[- \frac{(\log Q - \mu)^2}{2\sigma^2}\right]}{Q\sigma\sqrt{2\pi}} \quad . \tag{S2}$$

Here μ and σ are related to the mean Q_{mean} and the standard deviation Q_{sd} of Q as follows:

$$Q_{mean} = exp\left(\mu + \frac{\sigma^2}{2}\right), \tag{S3}$$

and:

$$Q_{sd} = Q_{mean}\sqrt{\exp\left(\sigma^2\right) - 1}, \tag{S4}$$

and the total number of vesicles is defined as the integral of $N_0(Q)$ over Q which equals N_0:

$$\int_0^\infty N_0(Q)dQ = N_0 \tag{S5}$$

.

Including the charge distribution into the desorption kinetics gives:

$$\frac{N}{N_0} = \frac{\int_0^\infty N_0(Q)\left\{1 - \left[1 - exp\left(-k_L t\right)\right]^Q\right\}dQ}{N_0} \tag{S6}$$

To apply Eq. (S6) to the experimental data requires as an input the charge distribution, which has not been measured in the present work. However to get a feel for the charge distribution, we consider the fluorophore distribution for 5 mol% fluid phase vesicles, which is determined from the measured fluorescence intensity I per vesicle. The corresponding distribution in Figure S4b has a relative standard deviation of I_{sd}/I_{mean} = 1.3. Guided by this observation we consider the effect of charge distribution on desorption kinetics in for relative deviations up to a large value of Q_{sd}/Q_{mean} = 3.45, which includes all values to be expected in reality. The resulting charge distributions [Eq. (S2)] for 5 mol% fluid phase vesicles (Q_{mean} = 2500) are plotted in Figure S4c. The corresponding desorption kinetics [Eq. (S6)] are shown in Figure S4d, which show significant lag-time, even when there is a very wide (over estimated) distribution among charge densities.

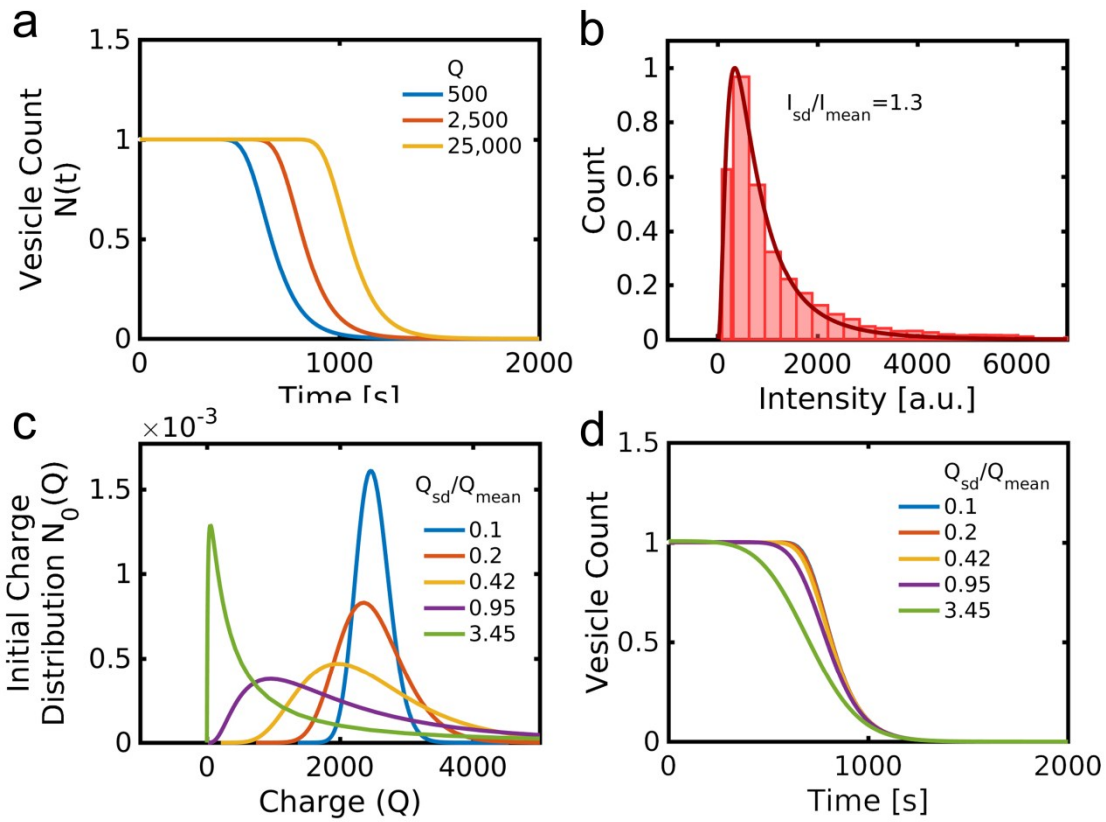

Figure S4. (a) Number of adsorbed vesicles versus time, assuming k_L = 10^{-2} s^{-1} and uniform charge density: 1% (blue), 5% (green) and 50% (red). (b) Intensity distribution, reflecting distribution of number of fluorophores in SUVs, indicative of distribution of charge composition. The distribution is log-normal with standard deviation I_{sd} relative to mean I_{mean} of I_{sd}/I_{mean} =1.3 (c) Hypothetical log-normal charge Q distribution, where Q_{mean}=2500 (5% charge density) going from Q_{sd}/Q_{mean} = 0.1 (blue peak) to 3.45 (green peak). (d) Number of adsorbed vesicles versus time, corresponding to hypothetical charge distributions in (c).

Supporting video Legend

Video S1. (Left) A representative example of a time-lapse sequence of fluorescence images of nano-vesicles adhering to the positively charged SLB surface. Vesicles diffuse in 2D on the SLB surface and detach from the SLB over time. SUVs are composed of 95 mol% zwitterionic phosphatidylcholine and 5 mol% negatively charge phosphatidylserine (PS) and stained with 1 mol% rhodamine-PE. SLB is composed of 90 mol% zwitterionic phosphatidylcholine and 10 mol% cationic DOEPC. In this movie only the SUVs are stained. *N* indicates the total number of vesicles at each frame. (Right) The number of detected vesicles as a function of time.