Supporting Information

Hydrodynamic Propulsion of Liposomes Electrostatically Attracted to a Lipid Membrane Reveals Size-Dependent Conformational Changes

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Relation Between Liposome Radius, Drift Velocity and Diffusivity

The shear-induced velocity of the liposome is governed by two forces; i.e. the viscous friction with the solvent $F_S$ and the viscous friction with the underlying membrane $F_M$, respectively. Without loss of generality these forces are expressed as products of a friction coefficient and a velocity difference, i.e. $F_S = \mu_S(\gamma a - U)$ and $F_M = \mu_M U$, where $\mu_S$ and $\mu_M$ are the friction coefficients between the liposome and the solvent and the friction coefficient between the liposome and the membrane, respectively, $a$ is the particle radius, $\gamma$ is the shear rate and $U$ is the liposome velocity. Under steady conditions, the two forces balance.

$$\mu_S(\gamma a - U) = \mu_M U. \quad (S1)$$

In Eq. (S1) it is assumed that the underlying membrane is non-moving. In reality however the shear stress on the supported bilayer results in bilayer motion, with its upper leaflet moving with a velocity $U_L$ over the stationary lower leaflet. This motion is in the literature referred to as tank-treading. Here we show that $U_L$ is negligible compared to the liposome velocity $U$, by estimating $U_L$ from equating the inter-leaflet shear stress $bU_L$ to the viscous shear stress $\eta\gamma$. Here $\eta = 10^{-3}$ kg·m$^{-1}$ s$^{-1}$ is the bulk solvent viscosity, $\gamma \approx 2\times10^2$ s$^{-1}$ is the shear rate and $b \approx 2\times10^7$ kg·m$^{-2}$ s$^{-1}$ is the inter-leaflet friction coefficient. This shows that $U_L = \eta\gamma/b \approx 10^{-8}$ m·s$^{-1}$ is small compared to the liposome velocity $U \approx 10^{-6}$ m·s$^{-1}$ (see Figure 1f in the main text). Consequently $U_L$ can be ignored in the present analysis. For smaller objects, however, such as proteins: $U_L/U \approx 1$, and $U_L$ must be taken into account. It is further noted that in our experiment the fluid velocity at one particle radius away from the SLB surface $\gamma a \approx 10^{-5}$ m·s$^{-1}$ (using $a \approx 5\times10^{-8}$ m and $\gamma \approx 2\times10^2$ s$^{-1}$) is an order of magnitude larger than the particle velocity itself $U \approx 10^{-6}$ m·s$^{-1}$. This condition allows ignoring $U$ on the left hand side of Eq. (S1) and it further implies that the friction between the liposomes and the SLB [right hand side of Eq. (S1)] is an order of
magnitude larger than the friction between the liposomes and the solvent [left hand side of Eq. (S1)], i.e. $\mu_S \ll \mu_M$. Therefore the diffusivity of the liposomes is governed by $\mu_M$ according to the fluctuation-dissipation theorem: $D = k_B T/\mu_M$. This simplification is further supported by the measured value for $D = 3 \times 10^{-13}$ m$^2 \cdot $s$^{-1}$ (see Figure 1e in the main text), which shows that the friction between the liposomes and the SLB: $\mu_M = k_B T/D \approx 1 \times 10^{-8}$ kg$\cdot$s$^{-1}$ is an order of magnitude larger than the friction between the liposomes and the solvent $\mu_S = C_F \times 6 \pi a \eta \approx 2 \times 10^{-9}$ kg m, where the solvent friction factor $C_F = 1.7$ for a shear flow past a stagnant sphere attached to a wall. Combining Eq. (S1) with $D = k_B T/\mu_M$ and $\mu_S = C_F \times 6 \pi a \eta$ results in:

$$C_F = \frac{k_B T U}{6 \pi a^2 \eta D}.$$  \hfill (S2)

It is re-emphasized that in the derivation towards Eq. (S2) it has been assumed that the liposomes are sufficiently large, such that they move much faster than the SLB, while at the same time, they are assumed sufficiently small, such that they move much slower than the local fluid, i.e. the friction between the liposomes and the SLB is much larger than the friction between the liposomes and the solvent. Based on the above mentioned parameter values, these conditions correspond to $a >> 1$ nm and $a << 10^3$ nm, respectively, which are clearly satisfied in the present work.

**2D-FN accuracy**

Here we discuss the accuracy of the two dimensional flow nanometry (2D-FN) method by studying the statistics of the measured diffusivity and velocity due to the stochastic nature of the liposome motion. For this purpose we consider a trajectory with a time span $t$. During this time the corresponding liposome displaces $Ut$ due to drift and it displaces $\Delta x \sim (Dt)^{1/2}$ due to diffusion, which may be “misinterpreted” by the 2D-FN algorithm as drift. This misinterpretation
results in a deviation of the apparent diffusivity and velocity of $D \sim \Delta x^2/t \sim D$, and $U = \Delta x/t \sim (D/t)^{1/2}$. As an effect, the relative spread in the diffusivity is of order one: $\Delta D/D \sim 1$ and the relative spread in the velocity: $\Delta U/U \sim (D/U^2 t)^{1/2}$ decreases with increasing drift velocity $U$. From these considerations it is clear that for an optimal determination of the drift velocity, one should optimize the parameter $U(t/D)^{1/2}$, by maximizing the total trajectory time $t$ and the applied flow rate. For this reason we refer to this parameter as the trajectory quality $Q$:

$$Q = \sqrt{\frac{U^2 t}{D}}.$$  \hspace{1cm} (S3)

### Relation between Diffusivity and Inter-Membrane Spacing

Here we analyze the relation between the inter-membrane spacing $\delta$ and the diffusivity $D$ of liposomes, that are electrostatically adhering to a supported lipid bilayer (SLB) surface. In particular we will estimate the increase in inter-membrane separation, that is required to increase the diffusivity of a 75 nm radius liposome by 50% (from 0.2 to 0.3 $\mu$m²·s⁻¹), as was observed in Figure 4b in the main text, which is attributed to a shear induced lift force. To this end, we use the Einstein relation: $D = k_B T/\mu_M$, with $k_B T$ the Boltzmann energy, to translate the diffusivity, measured before lift (0.2 $\mu$m²·s⁻¹), into a friction coefficient: $\mu_M = k_B T/D = 2 \times 10^{-8}$ kg·s⁻¹, and we model the friction between the liposomes and the SLB as that of a disk-shaped membrane inclusion: $\mu_M = 2\pi a_C^2 b^4$.

$$D = \frac{k_B T}{2\pi a_C^2 b^4}. \hspace{1cm} (S4)$$

Here $a_C$ is the radius of the membrane inclusion, which is assumed to be electrostatically coupled to the liposome. For the applicability of the limiting expression [Eq. (S4)] $a_C$ must be larger than the characteristic length: $a^* = (\eta_M/b)^{1/2} = 3$ nm, where: $\eta_M = \frac{1}{2} \times 4 \times 10^{-10}$ kg·s⁻¹, is the
monolayer (half bilayer) viscosity, and: \( b = 2 \times 10^7 \) kg s\(^{-1}\)m\(^{-2}\), is the inter-leaflet friction coefficient.\(^1\) Eq. (S4) predicts an inclusion radius of \( a_c = 13 \) nm, which is larger than \( a^* \), such that the applicability of Eq. (S4) is guaranteed.

The next step is to derive an expression between \( a_c \) and the inter-membrane distance \( \delta \) (between the liposome and the SLB). To this end we model the contact area as a circular region of the SLB membrane, that is within one Debye length: \( \lambda = (ek_BT/ne^2)^{1/2} \) of the outer surface of the spherical liposome membrane (radius \( a \)). Here \( e \) is the unit charge, \( \varepsilon \) is the electric permittivity of water, and \( n = 2 \times 10^3NA_c \) is the number of counter-ions per unit volume, where \( c \) is the NaCl molarity and \( N_A \) is Avogadro’s constant. Approximating the liposome as a sphere, that hovers a distance \( \delta \) (inter-membrane spacing) above the SLB surface, and assuming that \( \lambda/a \ll 1 \), we approximate the liposome surface with:

\[
y = \delta + \frac{r^2}{2a}, \quad (S5)
\]

where \( y \) is the height above the supported membrane and \( r \) is the horizontal distance to the center of the liposome. Inserting \( y = \lambda \) (beyond this length electrostatic forces are screened) into Eq. (S5) and solving for \( r \) (which is the contact radius \( a_c \)) gives:

\[
a_c = \sqrt{2a(\lambda - \delta)}. \quad (S6)
\]

Using \( a = 75 \) nm (see Figure 3b in the main text), \( \lambda = 0.8 \) nm (150 mM ionic strength) and \( \delta = 0 \) nm (point contact, prior to shear-induced separation), Eq. (S6) predicts: \( a_c = 8 \) nm, which agrees reasonably with the estimate \( a_c = 13 \) nm, which was obtained by applying Eq. (S4) to the measured diffusivity. This agreement supports the validity of Eq. (S6).

Finally Eqs. (S4) and (S6) are combined into the following expression for the change in the diffusivity \( \Delta D \) due to a change in the inter-membrane spacing \( \Delta \delta \):
\[ \frac{\Delta D}{D} \approx \frac{\Delta \delta}{\lambda}. \]  

For \( \lambda = 0.8 \) nm, this relation predicts that a 0.4 nm change in the inter-membrane spacing produces a 50% increase in the diffusivity.

**Supporting Video Legend**

**Video S1.** (Left) A representative example of a time-lapse sequence of fluorescence images of nano-liposomes adhering to a positively charged supported lipid bilayer (SLB) showing that the liposomes diffuse in 2D on the SLB. Liposomes are composed of 95 mol% zwitterionic phosphatidylcholine (DOPC) and 5 mol% negatively charged phosphatidylserine (DOPS) and stained with 1 mol% rhodamine-PE. The SLB is composed of 90 mol% zwitterionic DOPC and 10 mol% cationic 1,2-distearoyl-sn-glycero-3-ethylphosphocholine (chloride salt) (DOEPC). In this movie only the liposomes are stained. (Right) The corresponding processed video used for analysis. Liposomes are identified as clusters of more than three and less than 100 pixels, whose fluorescence intensities exceed two times the intensity noise level.

**Video S2.** (Left) A representative time-lapse sequence of fluorescence images showing the hydrodynamic propulsion of liposomes electrostatically attracted to a lipid membrane (flow rate: \( \Phi = 23 \) \( \mu \)L/s). (Right) The corresponding processed video used for analysis.

**Supporting References**