Supplementary Material

1 Supplementary Data

1.1 SANS data analysis

The intensity of scattered neutrons from an isotropic solution of particles is determined by the formula

\[ I(q) = A |F(q)|^2 S(q) + B, \]

where \( A \) is a scaling coefficient depending on the concentration of particles, \( B \) is the background intensity, \( |F(q)| \) is a form factor of one particle, \( S(q) \) is a structural factor that describes the interaction between particles. We have employed the Hayter-Penfold Rescaled Mean Spherical Approximation (RMSA) for the structure factor of charged spheres as available in the SASView fitting software (Hayter and Penfold, 1981; Hansen and Hayter, 1982).

The scattering form factor was calculated using the model of spherical vesicles defined as (Guinier and Fournet, 1955)

\[ F(q) = \frac{3V_1(\rho_1-\rho_2)j_1(qR_1)}{qR_1} + \frac{3V_2(\rho_2-\rho_1)j_1(qR_2)}{qR_2}, \]

where \( V_1 \) is the volume of the vesicle without the bilayer volume (i.e., solvent filled vesicle core); \( V_2 \) is the volume of the vesicle including the bilayer; \( \rho_1 \) is the neutron scattering length density (NSLD) of the solution; \( \rho_2 \) is the neutron scattering length density of the lipid bilayer; \( R_1 \) is the radius of the vesicle core, thus the inner radius of bilayer; \( R_2 \) is the outer radius of vesicle; \( j_1 \) is the spherical Bessel function of the 1st order.

Supplementary Figure S1. Vesicle geometry according to the model of spherical vesicles.
In this model, the scattering intensity is normalized to the volume of a vesicle. The NSLD of the vesicle core is the same as that of the solution outside. In addition, the bilayer-water interface is considered to be sharp since this model does not consider water molecules located in the head groups of phospholipids. This allows for avoiding possible artificial effects that are often present in more complicated multi-parameter models and makes it feasible to track subtle relative changes in the structural parameters of the lipid bilayer and vesicle upon the addition of ions. The employed simple model is justified for SANS data of protonated lipid bilayers dispersed in D2O due to the abrupt contrast between hydrogen and deuterium containing molecules (Kučerka et al., 2004).

**Supplementary Figure S2.** Neutron scattering length density distribution of POPC bilayer as used in our SANS data analysis.

The system of extruded lipid vesicles is typical of size polydispersity that we describe by Schulz distribution function (Helfrich, 1986; Hallett et al., 1991)

\[ f(R_1) = \frac{1}{R_0} (z + 1)^{x+1} \frac{1}{G(x+1)} \left( \frac{R_1}{R_0} \right)^x \exp \left( - \frac{(z+1)R_1}{R_0} \right), \]  

where

\[ z = \left( \frac{R_0}{\sigma_R} \right)^2 - 1, \]  

(4)

\( G(z) \) is the gamma function \( (z > 0) \), \( R_0 \) is the median value of this distribution. The form factor of the polydisperse vesicles is then obtained by convoluting the single-particle function with the given distribution function.

1.2 SAXS data analysis

SAXS curves were fitted following the factorization of form factor obtained for a large particle with a thin shell (Porod, 1948). Therefore, the intensity may be written as

\[ I(q) = A P_{sh}(q) |F(q)|^2 + B, \]  

(6)
where $A$ is a scaling factor, $P_{sh}(q)$ is the form-factor of the thin spherical shell with radius $R$ (vesicle), and $F(q)$ is the form-factor of the lipid bilayer. $P_{sh}(q)$ is defined as (Porod, 1948)

$$P_{sh}(q) = \int_0^\infty f(R', \sigma, R) \left( \frac{4\pi R'^2 \sin(qR')}{qR'} \right)^2 dR',$$

(7)

where $f(R', \sigma, R)$ is an asymmetric log-normal distribution describing the vesicle polydispersity as

$$f(R', \sigma, R) = \frac{1}{\sqrt{2\pi} R\sigma} \exp \left\{ -\frac{1}{2} \left( \frac{\ln R - \mu}{\sigma} \right)^2 \right\},$$

(8)

with $\mu = \ln R_m$ and $\sigma = \frac{p}{R_m}$, where $R_m$ is the median value and $p$ is the deviation from the median value. The mean size value is then $R_{mean} = \exp(\mu + \sigma^2/2)$. Log-normal distribution has proved to fit well the small-angle X-ray and neutron scattering data related to the large particles and vesicles in particular, as well as the dynamic light scattering data, while revealing the similar results when compared to other distributions (Pencer et al., 2001).

Given the symmetric lipid bilayer, $F(q)$ is calculated as the Fourier transform of the electron density profile of bilayer

$$F(q) = \int_{-d/2}^{d/2} \rho(z) \cos(qz) dz,$$

(9)

We describe $\rho(z)$ by 3 Gauss functions according to well established approach (Pabst et al., 2000)

$$\rho(z) = \rho_{head} \left[ \exp \left( -\frac{(z - \frac{z_{head}}{2})^2}{2\sigma_{head}^2} \right) + \exp \left( -\frac{(z + \frac{z_{head}}{2})^2}{2\sigma_{head}^2} \right) \right] + \rho_{tail} \left( -\frac{z^2}{2\sigma_{tail}^2} \right),$$

(10)

where $\rho_{head}$ and $\rho_{tail}$ are electron densities of lipid head groups and tails relative to the water electron density. Positions of the Gaussian peaks are at $z_i$ with the widths of $\sigma_i$. Hence, the form factor is given by

$$F(q) = 2\sqrt{2\pi} \sigma_{head} \rho_{head} \exp \left( -\frac{1}{2} (q\sigma_{head})^2 \right) + \sqrt{2\pi} \sigma_{tail} \rho_{tail} \exp \left( -\frac{1}{2} (q\sigma_{tail})^2 \right),$$

(11)
Supplementary Figure S3. Electron density distribution of POPC bilayer as used in our SAXS data analysis.

1.3 Densitometry

The specific volume of lipid $v_L$ was determined from densitometric measurements according to the formula (Pabst et al., 2007)

$$v_L = \frac{v_s - (1-w_L)v_{buffer}}{w_L},$$  

(12)

where $v_s$ is the specific volume that is inverse to the measured density of the lipid dispersion at a specific ion concentration, $v_{buffer}$ is the specific volume of the corresponding buffer that has been used for the preparation of lipid dispersions, $w_L$ is the mass fraction of lipid in the sample.

Considering the specific volume, the average molecular volume of lipids may be calculated as

$$V_L = \frac{v_L M}{N_A},$$  

(13)

where $N_A$ is the Avogadro constant, $M$ is the molar mass of POPC.

2 Supplementary References

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