Supplementary information: Determination of nanostructure of liposomes containing two model drugs by X-ray scattering from a synchrotron source

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Scattering model and fitting the experimental data

The X-ray scattering model employed for data analysis has been developed and described in detail by Pabst et al. (2000). Modeled phospholipid electron density profile was represented by a combination of three Gaussian profiles: two for the two head-groups and one for the tail region (Fig. 2b). The equilibrium electron density baseline corresponds to electron density of the CH_2 tail. In total nine parameters were variables in the fitting:

ρ_H - maximum density of the head group

ρ_C - minimum density of the chain group of atoms

σ_H - broadness of the head group Gaussian distribution

σ_C - broadness of the chain group Gaussian distribution

z_H - distance between the center of bilayer and the center of head group (also referred as head-to-midlayer distance)

d - repetitive distance between bilayers

N - number of bilayers in the wall

η - Caillé parameter

N_{diff} - diffusive scattering term

After the fitting performed, structural parameters could be derived from geometric relations to the bilayer profile. Main parameters of interest are:

headgroup size $\qquad d_H = \sigma(\text{FWHM})_H$

hydrocarbon chain length, $\qquad d_C = z_H - \sigma(\text{FWHM})_H / 2$

bilayer thickness, $\qquad d_{bilayer} = 2 * (z_H + \sigma(\text{FWHM})_H / 2)$

thickness of water layer, $\qquad d_{water} = d - d_{bilayer}$

where $\sigma(FWHM)_H$ is the full width at half maximum of the Gaussian representing the head-group, i.e. $2\sigma_H*2ln2$.

The lipid molecule surface area is given by:

$$A = \frac{1}{\rho_{CH2}\left(\dfrac{\rho_H}{\rho_C} - 1\right)} \left(\frac{\rho_H}{\rho_C} \frac{n_C^e}{d_C} - \frac{n_H^e}{d_H} \right) \qquad (1)$$

where n_C^e and n_H^e are the number of electrons in the chain and the head-groups, respectively. The number of electrons was calculated based on the structure of the phospholipid head group and the average molecular weight of the phospholipids, assuming that the tails consist of only CH_2-groups. The molecular weights of the phospholipid are 788 g/mol for 85G lipids and 758 g/mol for phospholipids 90G and 90NG.
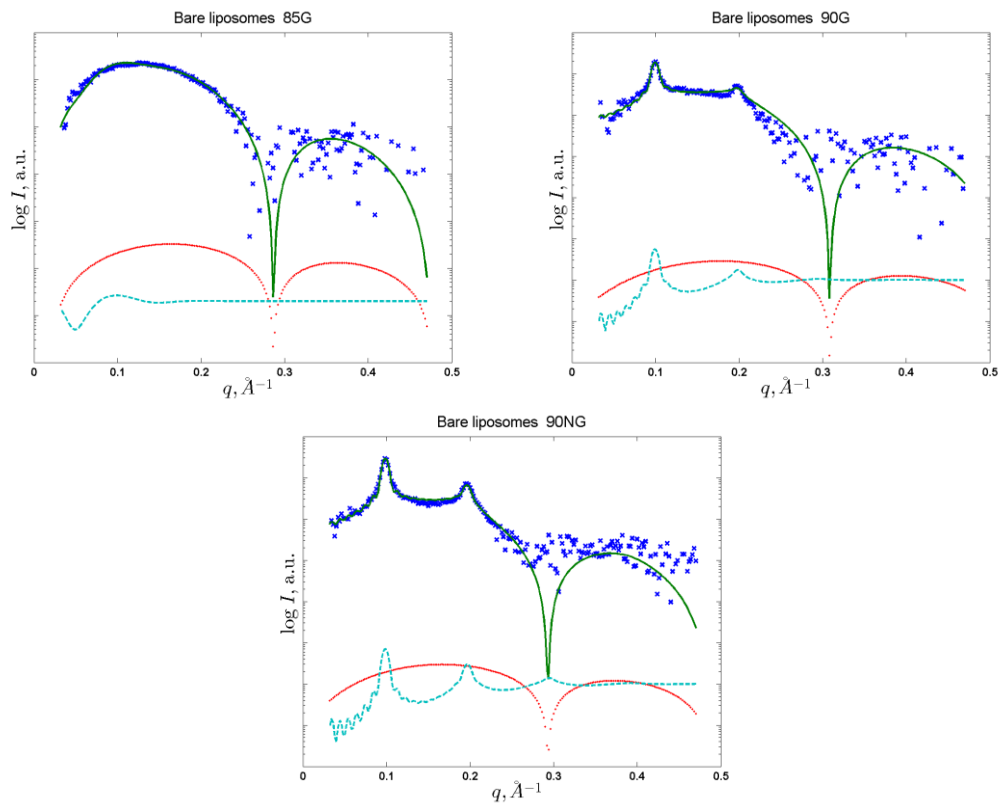
Finally, the scattering from lipid bilayer is determined by expression:

$$I(q) = \frac{1}{q^2} \left(\left| F(q) \right|^2 S(q) + N_{diff} \left| F(q) \right| \right) \qquad (2)$$
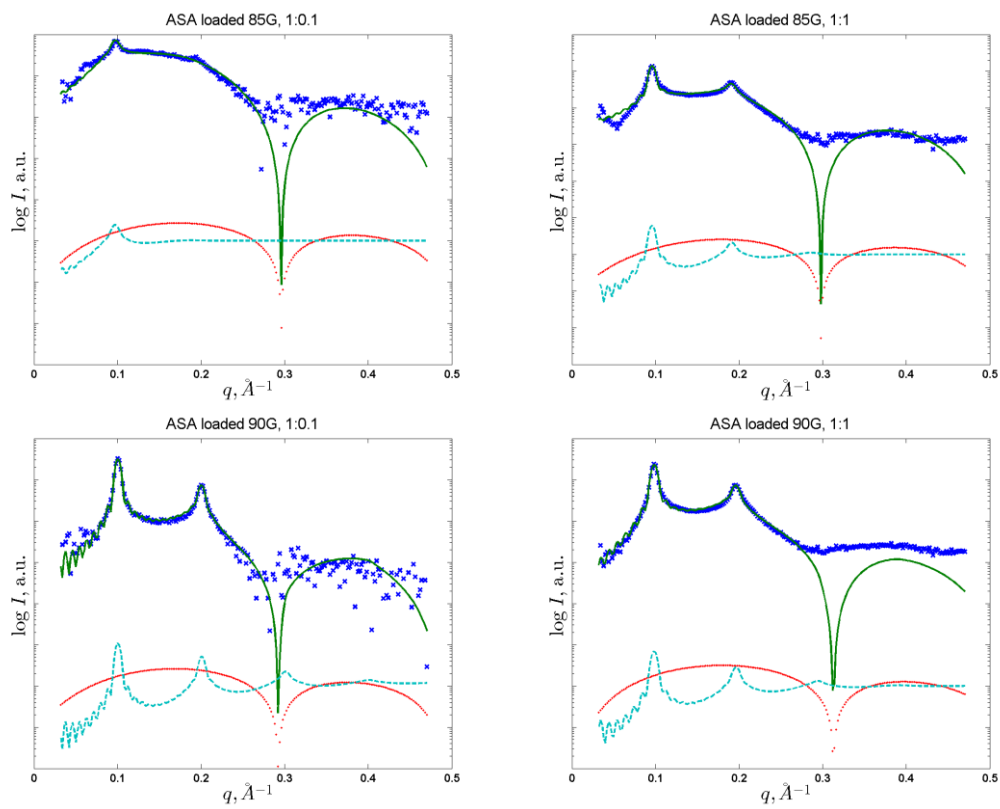
where q is the scattering vector, $F(q)$ is the bilayer scattering form factor that is calculated in the simulation based on the six parameters defined above. $S(q)$ is the wall shape factor which depends on the number of layers in the wall and on the amplitude of fluctuations of monolayers caused by mechanical stress (described by the Caillé parameter η). An additional diffusive scattering term N_{diff} is added in order to take care of internal wall defects and fluctuations.
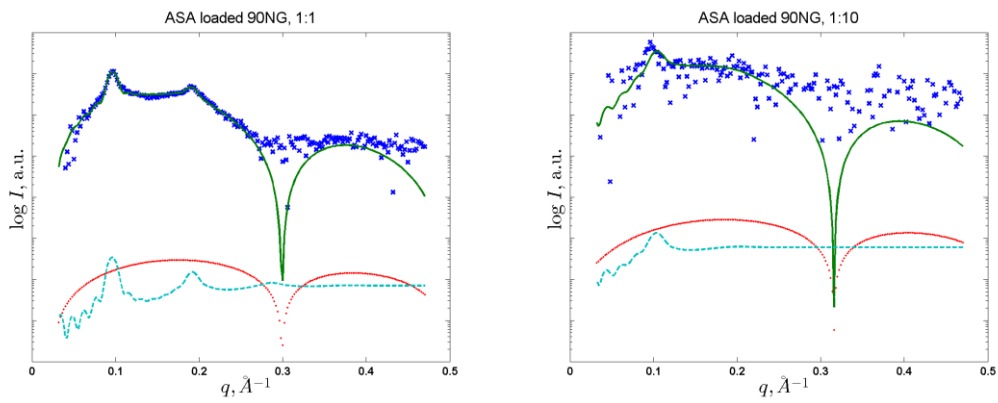
The errors in determination of fitting parameters presented in Tables S1-S3 are the highest estimated. The error bar combines both experimental and modeling uncertainties. The biggest modeling uncertainty is introduced when assumed that all liposomes have the same number of bilayers in the wall. In practice the numbers of bilayers N have some distribution. To count for it, N was varied by unity after the best fit obtained and experimental curve was fitted again with fixed value of N. The other parameters determined from such fittings and the best fitting were averaged over all the experimental curves and the error was calculated from these data. Thus, the experimental error is lower then the uncertainty which might be introduced by using current model.

a)

Bare liposomes 85G

Bare liposomes 90G

Bare liposomes 90NG

$\log I$, a.u.

$q, \mathring{A}^{-1}$

b)

ASA loaded 85G, 1:0.1

ASA loaded 85G, 1:1

ASA loaded 90G, 1:0.1

ASA loaded 90G, 1:1

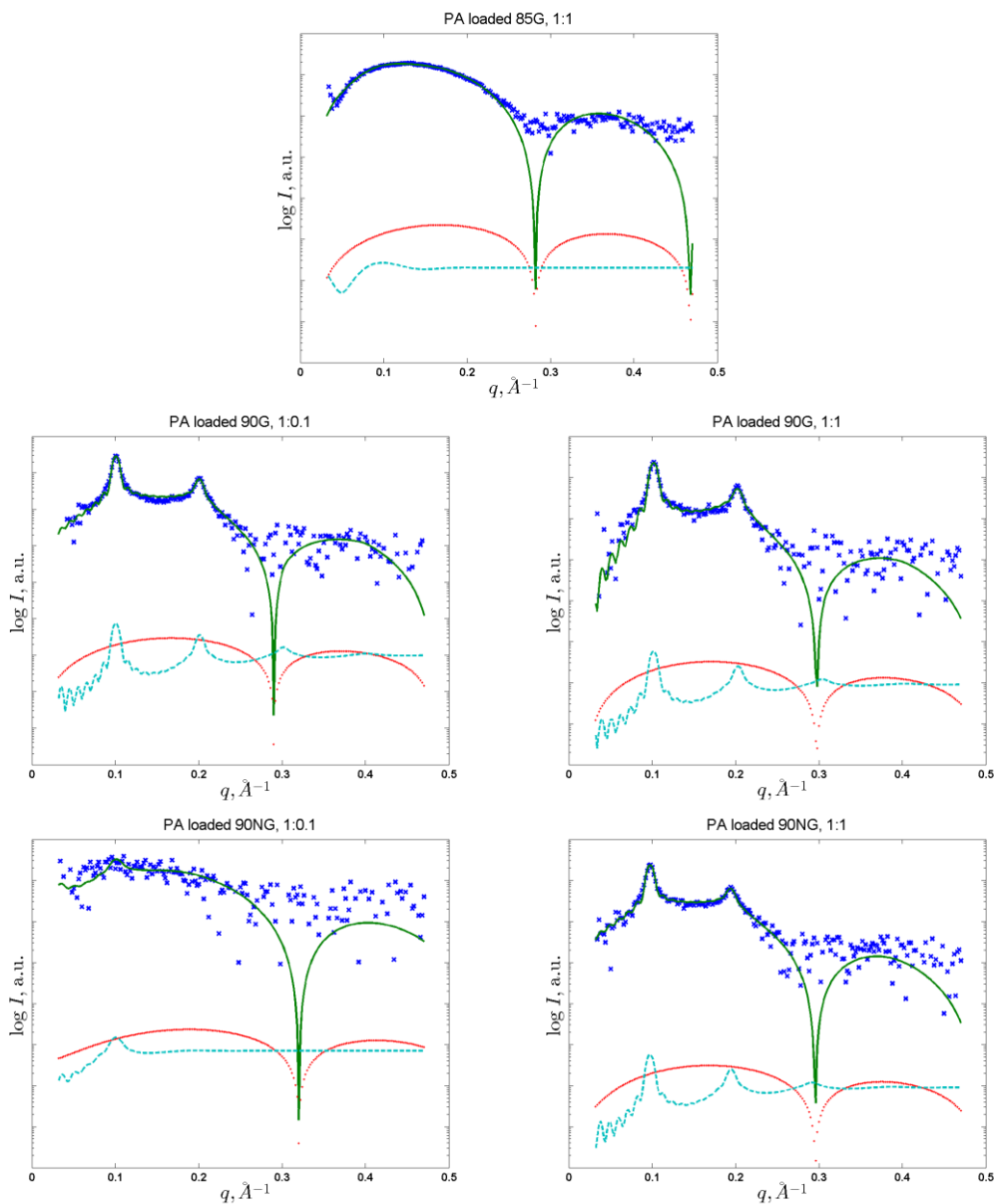$\log I$, a.u.

$q, \mathring{A}^{-1}$

c)

Figure S1 Examples of fitted model to the experimental scattering data: a) bare liposomes, b) acetylsalicylic acid loaded liposomes, c) paracetamol loaded liposomes. Blue crosses (×) represent experimental data, green solid line (—) is fitted model, red dots (⋯) indicate scattering form factor and cyan dash line (– –) indicates scattering shape factor.

Table S1 Fitted parameters for different types of bare liposomes in water

Phospholipid type	85G	90G	90NG
Number of analyzed curves	7	5	7
σ_H, Å	3.3±0.4	3.0±0.3	3.2±0.3
σ_C, Å	9.9±0.3	10.3±0.5	9.8±0.3
ρ_H, $e/\text{Å}^3$	0.11±0.01	0.11±0.01	0.10±0.01
ρ_C, $e/\text{Å}^3$	-0.08±0.01	-0.08±0.01	-0.08±0.01
z_H, Å	16.4±0.8	16.6±0.6	15.9±0.5
d, Å	60.1±0.2	63.4±0.3	63.8±0.6
N	2±1	9.6±2	9.8±2
Caillé parameter η	0.45±0.24	0.47±0.29	0.13±0.05
N_{diff}	4.0±0.8	6.2±1.3	3.1±0.7

Table S2 Fitted parameters for acetylsalicylic acid-loaded liposomes

Phospholipid type	85G	85G	90G	90G	90NG	90NG
Ratio of lipid to drug	1:0.1	1:1	1:0.1	1:1	1:1	1:10
Number of analyzed curves	2	3	2	3	5	2
σ_H, Å	2.4±0.5	2.7±0.1	3.1±0.1	3.4±0.3	2.9±0.1	3.1±0.2
σ_C, Å	10.6±0.2	11.0±0.4	9.8±0.1	9.7±0.2	9.8±0.1	9.6±0.1
ρ_H, $e/\text{Å}^3$	0.10±0.01	0.13±0.01	0.10±0.01	0.12±0.01	0.12±0.01	0.12±0.01
ρ_C, $e/\text{Å}^3$	-0.05±0.01	-0.06±0.01	-0.07±0.01	-0.09±0.01	-0.07±0.01	-0.07±0.01
z_H, Å	16.9±0.1	15.8±0.2	16.2±0.3	15.1±0.1	15.9±0.1	15.4±0.3
d, Å	64.0±0.2	65.6±0.1	62.6±0.1	63.9±0.1	65.3±0.1	64.8±0.1
N	10±1	10±1	12±1	10±1	7±1	5.5±1
Caillé parameter η	0.52±0.32	0.20±0.03	0.10±0.01	0.15±0.02	0.18±0.01	0.32±0.02
N_{diff}	12.3±8.6	4.6±0.3	≤0.1	0.98±0.32	5.7±1.4	≤0.01

Table S3 Fitted parameters for paracetamol-loaded liposomes

Phospholipid type	85G	90G	90G	90NG	90NG
Ratio of lipid to drug	1:1	1:0.1	1:1	1:0.1	1:1
Number of analyzed curves	3	2	3	1	2
σ_H, Å	2.8±0.3	3.4±0.2	3.6±0.2	3.1±0.2	3.5±0.1
σ_C, Å	10.0±0.1	9.7±0.1	9.7±0.1	10.0±0.1	9.6±0.1
ρ_H, $e/\text{Å}^3$	0.11±0.01	0.11±0.01	0.12±0.01	0.11±0.01	0.11±0.01
ρ_C, $e/\text{Å}^3$	-0.06±0.02	-0.08±0.01	-0.08±0.01	-0.07±0.01	-0.08±0.02
z_H, Å	16.7±0.2	16.3±0.2	16.2±0.3	15.6±1.2	16.2±0.2
d, Å	60.2±0.3	62.4±0.1	61.9±0.1	62.6±4.5	64.5±0.1
N	2±1	10±1	9±1	7±1	9±1
Caillé parameter η	0.47±0.20	0.11±0.03	0.11±0.03	0.70±0.23	0.14±0.02
N_{diff}	9.9±3.8	3.2±0.5	1.5±0.3	3.8±0.7	3.9±0.45