Supporting Information

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SI Text

Small-Angle Neutron Scattering (SANS) Data Analysis. The SANS data from DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) and DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) liposomes are fitted with a hollow three-shell model, i.e., as a combination of three concentric shells, where the middle layer describes the hydrophobic bilayer, and the outer and inner layers describe the outer and inner hydrophilic leaflets, respectively. The hollow three-shell model is straightforwardly derived from the Rayleigh form factor for spherical particles using the standard principles as, e.g., outlined by Pedersen (1). A weak polydispersity of the liposomes is clearly visible in the SANS data and explicitly taken into account in the model by integrating the model form factor over a Gaussian size distribution. A hydration of the hydrophilic head groups is allowed in the model. Molecular information about the chemical compositions as well as partial specific molecular volumes of the different sample constituents, respectively, DMPC, DOPC, sugar, and D_2O are used for calculating the total scattering lengths, the scattering volumes, and the resulting excess scattering length densities of the hydrophobic and hydrophilic layers of the liposomes at different sugar concentrations. The sugar concentration dependent volumes of DMPC and DOPC obtained from the densitometry measurements were used for these calculations. The macroscopic ratio of hydrophilic and hydrophobic volumes in the liposomes is fixed to the corresponding molecular ratio by assuming that the molecular volume of the PC head groups is 320 $Å^3$ (2).

This usage of molecular constraints has previously been described and tested in more detail for other amphiphilic systems (3, 4), and the approach for modeling the liposomes is similar in spirit to the approach used by Kucerka and co-workers in recent work on phospholipid bilayers (5, 6). The bottom line is that the approach reduces the number of free parameters in the model fit and ensures that only physically realistic models are fitted to the experimental data.

As a test of the robustness of the results, the data were also attempted to be fitted with a more simple approach using a hollow one-shell model and no molecular constraints. Although this yielded slightly worse model fits and slightly lower values for the liposome bilayer thickness, the overall conclusions obtained using this approach were the same, and in both cases a significant thinning of the bilayers was observed upon addition of sugar to the D_2O buffer.

The model fits are performed by means of a home-written Fortran program that calculates the scattering intensity as a function of a series of model parameters, folds the obtained scattering intensity with the resolution functions relevant for each instrumental setting, and fits the model scattering function by adjusting the model parameters using a Levenberg–Marquardt inspired steepest gradient approach (7).

The central fitting parameter from the SANS analysis is the bilayer thickness, $D_{\rm bilayer}$. As discussed in the main text, the lateral area per lipid molecule, A, may be estimated as $A = V_{\rm lip}/D_{\rm bilayer}$, where $V_{\rm lip}$ is the lipid volume from Fig. 2. Fig. S1 shows how A changes with the concentration of sugar.

Preferential Binding Parameters. The dialysis data were analyzed along the lines of the preferential interaction theory (see, e.g., refs. 8 and 9). Specifically, we calculated the preferential binding parameter Γ_3, which is defined as

$$\Gamma_3 \equiv \left(\frac{\partial m_3}{\partial m_2}\right)_{\mu_3,P,T}, \qquad \textbf{[S1]}$$

where m denotes molal concentration (mol solute/kg water) and subscript 2 and 3 identify, respectively, lipid and sugar. Γ_3 is a thermodynamic function, which provides information on the number of bound solutes. It has been extensively used in discussions of protein–solute interactions (8, 9) and is also useful for membranes (10, 11).

All samples were dissolved in 50% 1-Propanol prior to the chromatographic analysis (see main article). For the outside (lipid free) samples, the chromatographic signal was converted directly into molal concentration units by comparison with an appropriate (molal) standard curve. For the inside samples, the measured amounts of lipid and sugar were used in combination with the densitometric data to calculate the volume fraction of lipid, $\phi_{\rm lip}$, in the dialysis bag

$$\phi_{\rm lip} = \frac{V_{\rm lip} w_{\rm lip}}{V_{\rm lip} w_{\rm lip} + V_{\rm solvent}(1 - w_{\rm lip})}. \qquad \textbf{[S2]}$$

In Eq. **S2** $w_{\rm lip}$ is the weight fraction of the lipid, which is also known from the HPLC trials. $V_{\rm lip}$ is the partial specific volume of lipid given in Fig. 2. $V_{\rm solvent}$ is the specific volume of the aqueous phase (binary sugar–water mixture), which was also measured in this work. This function may be expressed by a polynomial fit to the experimental data; $V_{\rm solvent}(w_{\rm sugar}) = a w_{\rm sugar}{}^2 + b w_{\rm sugar} + c$. The parameters a, b, and c for the different sugars and the range of validity of the fit are given in Table S1

The volume fraction of the aqueous solution inside the dialysis bag (i.e., the nonlipid volume) is $\phi_{\rm solvent} = 1 - \phi_{\rm lip}$, and it follows that the chromatographic signal must be divided by $1 - \phi_{\rm lip}$ to get the same unit (molal) as the lipid free sample taken outside the dialysis bag.

Using the corrected value, we calculated the difference in sugar concentration across the dialysis bag, $\Delta m_3 = {}^{\rm in}m_3 - {}^{\rm out}m_3$. Initial trials showed that at a constant concentration of sugar, Δm_3 scaled proportionally with the lipid concentration, m_2, in the investigated range [20–60 mmol lipid/(kg water)]. It follows that Γ_3 is independent on the lipid concentration and the differential in Eq. **S1** may be approximated $\Gamma_3 \approx \Delta m_3/m_2$. The preferential binding parameter Γ_3 was calculated according to this approximation and plotted as a function of the free sugar concentration, in Fig. 3.

The preferential binding parameters measured here can be converted into a partitioning coefficient, P, for the distribution of sugar between the hydration layer near the membrane interface and the aqueous bulk as described elsewhere (11–13). In Table S2, P values are calculated for selected sugar concentrations and compared to literature data.

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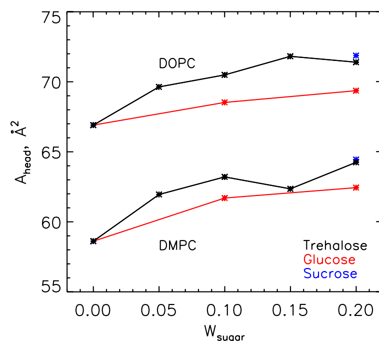

Fig. S1. Lateral area per lipid molecule for DMPC and DOPC plotted as a function of the weight fraction of sugar. The areas were calculated as the ratio of membrane volume (Fig. 2) and thickness (Fig. 1).

Table S1. Parameters for the determination of the specific volume of binary aqueous sugar solutions

Sugar:	Trehalose	Glucose	Sucrose
Maximal valid w_{sugar}	0.35	0.42	0.41
a	0.021245	0.025257	0.015631
b	−0.39484	−0.38225	−0.3852
c	1.00798	1.00798	1.00798

The experimental data at 40 °C are fitted to the polynomial $V_{solvent}(w_{sugar}) = aw_{sugar}^2 + bw_{sugar} + c$, where w_{sugar} is the weight fraction of the sugar. The expressions are valid from $w_{sugar} = 0$ to the value listed in the second row.

Table S2. The partitioning between membrane interface and bulk for small sugars

Sugar	Bulk sugar concentration mol $(kg\,H_2O)^{-1}$	Lipid	P	Method	Reference
Glucose	1.6*	DMPC	0.61	SANS	(1)
Glucose	1.1*	DOPE	0.42	SANS/SAXS	(2)
Glucose	1.2*	DPPC	0.38	SANS	(3)
Glucose	1–2	DMPC	0.5	Vapor pressure	(4)
Sucrose	1–1.5	DMPC	0.2	Vapor pressure	(4)
Trehalose	1–2.5	DMPC	0.2	Vapor pressure	(4)
Glucose	0.15/1.0	DMPC	1.9/0.7	Dialysis equilibrium	Current work
Trehalose	0.15/0.75	DMPC	2.5/0.6	Dialysis equilibrium	Current work
Sucrose	0.15/1	DMPC	2.3/0.8	Dialysis equilibrium	Current work

The partitioning coefficient, $P = [\text{sugar}]_{local}/[\text{sugar}]_{bulk}$ specifies the ratio of sugar concentrations in these two domains, and it may be translated into a free energy of transfer as $\Delta G^{\circ} = -RT \ln P$. For the current data, P was calculated as described in ref. 4.

*Estimated from volume fractions given in the reference and the density data in Table S1.

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