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Supplemental Information

DPPC Bilayers in Solutions of High Sucrose Content

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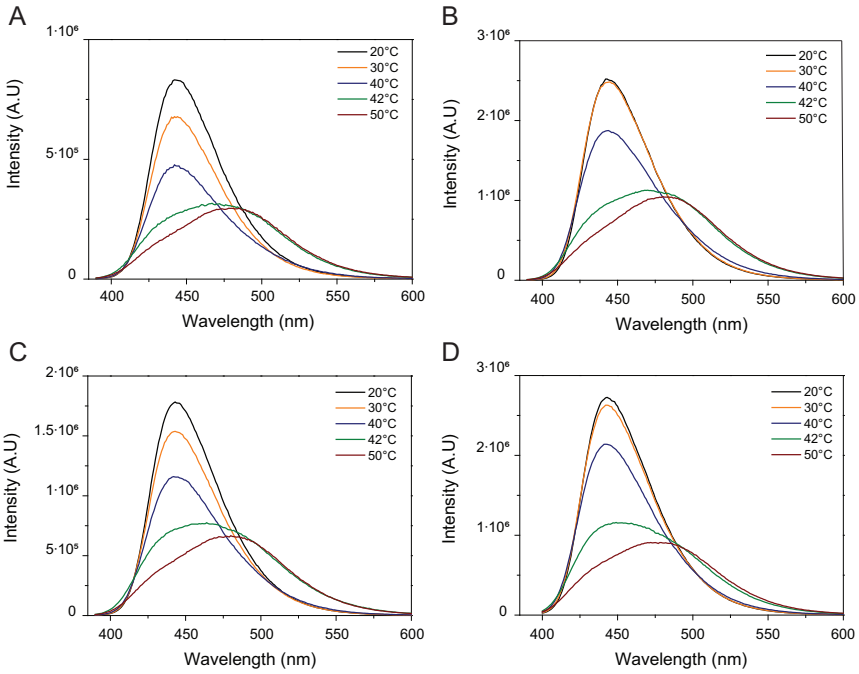

Figure S1: Comparison of emission curves of Laurdan for DPPC MLVs formed in (A) water, (B) 0.12 M sucrose, (C) 0.70 M sucrose and (D) 1.50 M sucrose at 20°C (black line), 35°C (orange line), 42°C(blue line), 50°C(green line) and 60°C (red line).

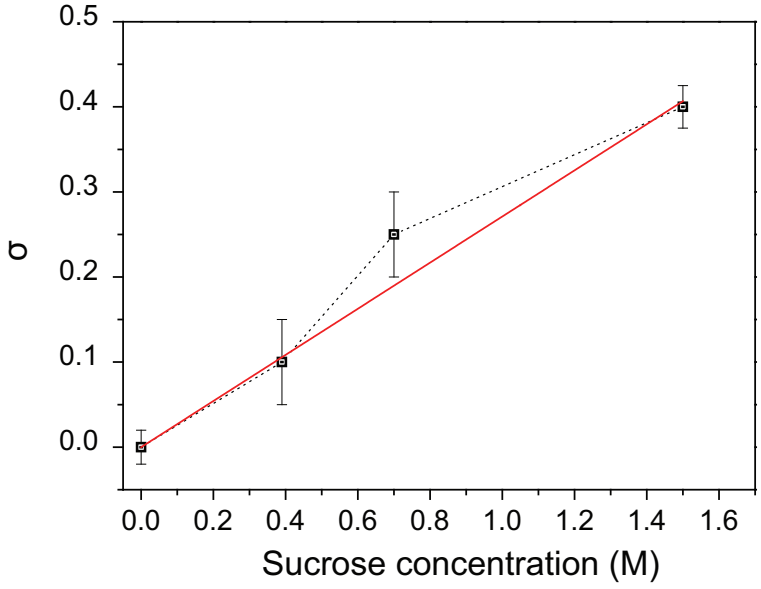

Figure S2: Dependence of sucrose coverage σ on the sucrose concentration (black squares connected by dashed lines) and linear fitting (red line). Each value of σ, which is a parameter of our model, was found by fitting the model to the experimentally obtained values of transition enthalpy.

Preparation of giant unilamellar vesicles

GUVs were prepared by electroformation following the protocol introduced by Angelova (54). Briefly, 5 μL of 2 mg/mL solution of DMPC stained with 1% mol of diI in chloroform were spread on each cathode of a custom made electroformation stage. The stage was kept under vacuum for at least one hour to ensure complete evaporation of solvent and the lipid film was subsequently hydrated using the necessary solution (water or sucrose at different concentrations) at 55 °C.

A sinusoidal electric field (1 V peak-peak, 10 Hz) was applied for one hour while keeping the sample heated above the transition temperature. The resulting GUV suspension was kept at 20 °C (water bath) during two hours prior to use to ensure complete stabilization of the sample. Vesicles were used on the same day of preparation.

Optical Microscopy

Imaging of GUVs labelled with DiI was performed using an epifluorescence microscope Nikon Eclipse TE2000-E equipped with a x60, water immersion, NA 1.2, Nikon objective, and a Diagnostic Instrument, NI-1800, black and white camera. Prior to any observation, GUVs were submitted to an osmotic shock: the sample was diluted with $\sim$5% volume of pure water. As a consequence, GUVs underwent swelling, resulting in the removal of potential membrane tubes connected to the membrane. The samples were kept at 5 °C for at least two hours after preparation to ensure complete transition to gel (also called S_o) phase. Prior to experimental observation, GUVs were let to stabilize at 20 °C for at least one hour. 100 mL of a GUV dispersion were placed in a custom-made heating stage through which we could control the temperature of the sample with a precision of $\pm$1 °C. Sample was stabilized for at least one hour for each temperature, before acquiring images.

Giant unilamellar vesicles

Giant unilamellar vesicles of DMPC were observed in epifluorescence to probe the membrane phase behavior at the micrometric scale, at temperatures below and above T_m, known to be 25°C . Typical vesicle morphologies for each sucrose concentration at different temperatures are summarized in Fig. S3, together with statistics of phase separation.

At 20°C, DMPC vesicles appear homogenous, whatever the sucrose content. The corrugations of the membrane and steep angle defects have been previously reported for DMPC vesicles and are characteristic of a S_o gel phase (40, 41). Increasing the temperature to 25°C results in phase separation displayed by fluorescent probe partitioning. DiI has been shown to readily partition into the fluid phase (42,43), therefore the black domains observed in the micrographs are gel-phase domains in a liquid phase. In pure water, domains are still observed for both 30°C and 35°C, however they gradually decrease in size with increasing temperature. The partitioning of DiI also decreases as the contrast between domains becomes less sharp. At 40°C the membrane is in a liquid phase and displays homogenous fluorescence.

Vesicles formed in 0.20 M sucrose display a similar behavior to GUVs formed in water, however gel domains readily disappear at a lower temperature. Moreover the partitioning of DiI gradually diminishes already at 30°C, and at 35°C contrast between domains and fluid phase is very low.

For 0.39 M sucrose we observe narrower window of coexistence, since domains are only visible at 25°C, whereas at 30°C or higher a single homogeneous fluid phase is present.

It appears that for any given sucrose concentration and temperature, GUVs exhibit a similar fraction of S_o domains. This is summarized in Fig. S3B, where the temperature window of phase coexistence for

each concentration of sucrose is clearly vizualized. Though different domain typologies were observed in the GUVs, namely stripes, hexagonal and irregular, they all can be attributed to different states of tension of the individual vesicle, and be systematically associated with S_o domains (46).

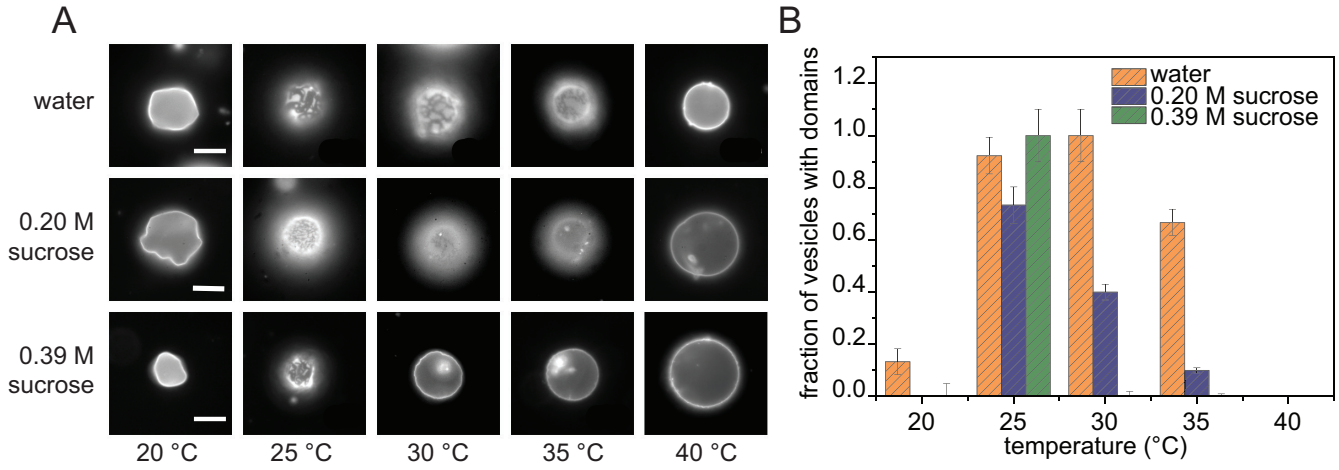

Figure S3: Summary of sucrose effects on GUVs formed with DMPC. A) Typical GUV phase behavior for DMPC in water, 0.20 M and 0.39 M sucrose in the temperature interval [20°C - 40°C]. Scale bar is 10 μm. B) Fraction of DMPC GUVs with domains for each system at the different temperatures displayed in A).

These measurements reveal that, for pure DMPC GUVs, a large temperature interval of phase coexistence exists, which has not been reported before in giant vesicles experiments. Although phase coexistence is expected to appear around T_m (45), the persistence of gel domains at higher temperatures may indicate that metastability is produced (46), possible because of the low rate of heating employed in the experimental setup.

The suppression by sucrose of phase coexistence for temperatures above T_m can be interpreted as a lowering of the energy involved with gel-liquid contact. The size of gel domains in liquid phase is mainly driven by the minimization of the energy involved with hydrophobic mismatch (such as a S_o/L_d coexistence) (46). Several studies have proposed that sucrose can alter the head-head distance in lipid bilayers under low hydration conditions (1, 47). Sucrose is likely to act as a reliever of hydrophobic mismatch allowing for smaller domains existence, by protecting the portion of chains exposed by the mismatch (55). It is thus possible that the presence of sugar enhances the dissolution of domains or reduces the lifetime of metastable states.

Derivation of a thermodynamic model for sucrose-lipid interaction

Cooperativity in the lipid main transition

It is assumed that the gel and fluid phases exchange their stability at a coexistence temperature T_m by means of a first order transition mechanism. Let us introduce the number of lipids N, the total Gibbs free-energies G_l, G_g, the enthalpies H_l, H_g and entropies S_l, S_g in the fluid and gel phases.

Molecular quantities are defined as $g_l = G_l/N$, $g_g = G_g/N$, $h_l = H_l/N$, $h_g = H_g/N$, $s_l = S_l/N$, $s_g = S_g/N$. Finally, we introduce the differences $\Delta g = g_l - g_g$, $\Delta h = h_l - h_g$, $\Delta s = s_l - s_g$

between the high (fluid) and low (gel) temperature phases. We denote by T the absolute temperature, and $\beta = 1/(k_B T)$ the inverse temperature factor. The probability of occurrence of a phase is proportional to $\exp(-\beta G)$. As the Gibbs free-energy scales with the number of lipids, phases cannot coexist except but in a narrow temperature interval centered around T_m.

A standard thermodynamic relation states that

$$\frac{\mathrm{d}(\beta G)}{\mathrm{d}\beta} = G + \beta \frac{\mathrm{d}}{\mathrm{d}\beta} G = G - T \frac{\mathrm{d}}{\mathrm{d}T} G = G + TS = H. \tag{S1}$$

On the other hand, at the coexistence temperature $\Delta G(T_m) = 0$. One can expand to first order in $T - T_m$ the difference in Gibbs free-energy

$$\beta \Delta G \simeq (\beta - \beta_m) \Delta H_m \simeq -\frac{T - T_m}{k_B T_m^2} \Delta H_m. \tag{S2}$$

The probability of occurrence of the gel and liquid phases reads

$$\begin{aligned} p_l(N, \beta) &= \frac{e^{-\beta G_l}}{e^{-\beta G_l} + e^{-\beta G_g}} = \frac{1}{1 + e^{\beta N \Delta g}}; \\ p_g(N, \beta) &= \frac{e^{-\beta G_g}}{e^{-\beta G_l} + e^{-\beta G_g}} = \frac{1}{1 + e^{-\beta N \Delta g}}. \end{aligned} \tag{S3}$$

The transition takes place on a temperature interval ΔT given by $\beta N \Delta g = N \Delta T \Delta h_m / k_B T_m^2 \sim 1$. It is inversely proportional to N. To provide a phenomenological description of the melting transition, one introduces a cooperativity number N_c as the effective number of lipids that share the same internal state. The system is then treated as an assembly of N/N_c "bundles" changing state independently, with $N/N_c \gg 1$. The equilibrium enthalpy at temperature T is given by:

$$H(T) = N(p_l(N_c, T) h_l + p_g(N_c, T) h_g) = N(p_l(N_c, T) \Delta h + h_g). \tag{S4}$$

where liquid-gel mismatch energy contributions are neglected and h_l and h_g are taken independent of temperature at the vicinity of the transition. Then

$$\begin{aligned} \frac{\mathrm{d}H}{\mathrm{d}T} &= N \Delta h \frac{\mathrm{d}p_l}{\mathrm{d}T}, \\ &= N \Delta h \frac{-e^{\beta N_c \Delta g}}{(1 + e^{\beta N_c \Delta g})^2} \frac{\mathrm{d}\beta N_c \Delta g}{\mathrm{d}T}, \\ &= N N_c \frac{(\Delta h)^2}{k_B T^2} \frac{e^{\beta N_c \Delta g}}{(1 + e^{\beta N_c \Delta g})^2}, \\ &= n N_c \frac{(\mathcal{N}_A \Delta h)^2}{4RT^2} \frac{1}{\cosh(\beta N_c \Delta g / 2)^2}, \end{aligned} \tag{S5}$$

with $\mathcal{N}_A$ the Avogadro number, $\mathcal{N}_A \Delta h$ the molar enthalpy change at the transition, n the number of moles of lipids.

The peak maximum is at $\Delta g = 0$, $C_{p,max} = \mathrm{d}H/\mathrm{d}T|_{T=T_m} = nRN_c(\mathcal{N}_A \Delta h)^2/(4R^2T^2)$, from which N_c can be expressed in terms of molar quantities

$$N_c = \frac{C_{p,\max}}{n} \frac{4RT_m^2}{(\mathcal{N}_A \Delta h)^2} = 4 \left(\frac{C_{p,max}}{nR} \right) \left(\frac{RT_m}{\mathcal{N}_A \Delta h} \right)^2. \tag{S6}$$

Phenomenology of the lipid main transition

A simple insightful treatment of the lipid main transition was introduced by Doniach, and improved by several authors (51, 52, 55, 57, 58). It is based on a scalar order parameter showing two preferred values, one corresponding to the gel state, and the other to the fluid state. This statistical model can be implemented in practice by assigning binary variables (Ising spins) to the fixed vertices of a two dimensional lattice. Reference (52) presents in detail the historical development of the model.

In this approach, lipid molecules spontaneously adopt either a gel or a fluid conformation, depending on external thermodynamic conditions (temperature, but also isotropic pressure and membrane tension). In the Ising language, this is achieved by applying a uniform temperature (pressure, tension) dependent magnetic field that vanishes precisely at the coexistence temperature T_m, where fluid and gel are observed with equal probability. Additional nearest neighbor couplings (J parameters) are introduced to enforce cooperativity. Above a critical J_c value, the system shows phase coexistence and metastability, with thermal hysteresis upon heating and cooling cycles. Below the critical value, the system state evolves smoothly and reversibly with temperature. The latter case is therefore suitable to describe most experimental situations corresponding to a regular and reversible thermal capacity curve with a finite width, determined *e.g.* in differential scanning calorimetry (DSC) experiments.

There is some freedom left in deciding whether the binary state is assigned to a whole lipid or just a lipid chain, or which lattice is most representative (the hexagonal lattice seeming the most appropriate), with all models in the end able to describe the observed behavior (57). Several Monte-Carlo studies were shown to successfully account for various situations of interest (56).

To explain the main features of sucrose induced changes in the DPPC melting transition, we implement one such model and solve it by means of a mean-field approximation. Each binary state takes a value 0 (gel) or 1 (fluid) and describes a single lipid molecule internal state. The average internal value is therefore a real number p comprised between 0 and 1, which is readily interpreted as the probability to find a lipid molecule in the fluid conformation. For a given microscopic configuration, one introduces a configurational energy

$$\Delta\mathcal{H} = N_l(\Delta h - T\Delta s) + JN_{gl}, \tag{S7}$$

as the difference between the actual system state, and a reference state where all lipids would be in the gel state. N_l and N_{gl} are respectively the number of lipids in fluid state, and the number of lattice bonds linking lipids in a different state (state mismatch). J is the mismatch gel-fluid state penalty parameter. The quantity $\Delta\mathcal{H}$ determines the probability of the microscopic state, proportional to $\exp(-\beta\Delta\mathcal{H})$. Note that it is unusual to deal with temperature dependent "Hamiltonians" in statistical physics. The approach used here means that a coarse-graining step is performed by averaging over the inner conformations of each lipid molecule, while partitioning them into two broad classes (gel and fluid state). Eq. (S7) is something of an intermediate quantity between the true (molecular) microscopic energy and the macroscopic Gibbs free-energy.

$\beta\Delta\mathcal{H}$ can be expanded around the coexistence temperature:

$$\beta\Delta\mathcal{H} = -\frac{\mathcal{N}_A\Delta h}{RT_m^2}(T - T_m)N_l + \frac{\mathcal{N}_A J}{RT_m}N_{gl}. \tag{S8}$$

The Gibbs free-energy associated to the configurational energy above reads in the large N limit:

$$\beta\Delta G = \beta\langle\Delta\mathcal{H}\rangle - \mathcal{S}/k_B, \tag{S9}$$

where appears the entropy $\mathcal{S}$ associated to the many internal gel-fluid microscopic configurations of the system. The above expression can be expressed at mean-field level by introducing the probability p of

finding each lipid in the fluid state, and the average coordination z of a site in the lattice (average number of neighboring lipid molecules, 6 for an hexagonal lattice). It reads

$$\beta\Delta G = -\frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T_m)Np + \frac{\mathcal{N}_A J}{RT_m}zNp(1-p) + N[p\ln(p)+(1-p)\ln(1-p)]. \tag{S10}$$

The $\mathcal{S}/k_B = -N[p\ln(p)+(1-p)\ln(1-p)]$ expression is characteristic of the statistical entropy of N independent binary variables. The mean-field self-consistent equations result from minimizing $\beta\Delta G$ with respect to p, in order to find the best compromise between the number of configurations $\exp(\mathcal{S}/k_B)$ and the energy penalty $\langle\Delta\mathcal{H}\rangle$. One obtains

$$\ln(p) - \ln(1-p) + \frac{\mathcal{N}_A J}{RT_m}z(1-2p) - \frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T_m) = 0, \tag{S11}$$

or equivalently

$$\frac{p}{1-p} = \exp\left(\frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T_m) + \frac{\mathcal{N}_A J}{RT_m}z(2p-1)\right). \tag{S12}$$

Self-consistent equations are trivially satisfied by

$$p(T) = \frac{\exp\left(\frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T_m)\right)}{1+\exp\left(\frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T_m)\right)} \tag{S13}$$

at vanishing coupling $J=0$, and must be numerically solved in the general case.

There is freedom in deciding if the interaction term $-Jzp(1-p)$ is of enthalpic or entropic origin. Assuming that J is enthalpic and does not depend on temperature T, one derives the mean-field enthalpy difference

$$\Delta H(T) = Np(T)\Delta h + JzNp(T)(1-p(T)) \tag{S14}$$

that can be compared with the experimental DSC thermograms once the solution of eq. (S12) is obtained. Moreover, one observes that, irrespective of the choice done regarding the interaction term, the difference $\Delta H(p=1) - \Delta H(p=0)$ reaches the expected limit value $N\Delta h$, corresponding to the total latent heat upon melting completely the system from the gel to the fluid state.

Introducing the dimensionless coupling $\tilde{J} = \frac{\mathcal{N}_A J}{RT_m}z$, one finds that the critical value separating reversible and temperature hysteresis is $\tilde{J}_c = 2$ in the mean-field approximation. A quite sharp specific heat peak can therefore be obtained with $\tilde{J}_c \simeq 1.94$.

In the practical situation of a DPPC bilayer, when assigning binary variables to lipid molecules (not chains), treating lipids as basic degrees of freedom coupled with $\tilde{J}_c = 1.94$, and taking $\mathcal{N}_A\Delta h$ equal to the experimental value $38\ \mathrm{kJ\cdot mol^{-1}}$ (9.1 kcal $\cdot\mathrm{mol}^{-1}$) leads to a non negligible amount of the minor component into the major component around the location of the phase transition. This means that the area under the peaked curve $\mathrm{d}\Delta H/\mathrm{d}T$ on a 10°C temperature interval centered around $T_m = 273.15 + 41.8 = 314.95$K gives a value 28 kJ·mol^{-1}, smaller than the experimental one. This is inherent to the "Ising" like treatment of the internal degrees of freedom, and is also true for Monte-Carlo "exact" sampling of the configurations. To get around this shortcoming, one can decide on a phenomenological ground to assign a larger value to the constant $\mathcal{N}_A\Delta h$. We found that at mean field level, with $\tilde{J}_c = 1.94$, the correct $\Delta H(T_m+5) - \Delta H(T_m-5) = 38\ \mathrm{kJ\cdot mol^{-1}}$ value is recovered for $\mathcal{N}_A\Delta h = 56\ \mathrm{kJ\cdot mol^{-1}}$ (13.4 kcal·mol^{-1}). There is then 16% of fluid lipid at $T_m - 5 = 36.8$°C and 84% at $T_m + 5 = 46.8$°C.

Influence of the sucrose on the gel transition

Increasing concentrations of sucrose in solution lead to a noticeable drop in latent heat (area under the specific heat curve) with only a tiny increase in the appearent melting temperature (of the order of 1 K).

In first order phase transitions, the coexistence temperature T_m coincides with the ratio $\Delta h/\Delta s$. If the sucrose was only acting on changing the enthalpy jump $\Delta h'$, then keeping the melting temperature constant by 1 part in 300 would require a quasi-perfect matching of the entropy variation $\Delta s'$, with $T_m = \Delta h/\Delta s \simeq \Delta h'/\Delta s'$. On the other hand, it is well known that lipid melting temperature is extremely sensitive to molecular details. Perdeuteration of the DPPC alkyl chains, for instance, lowers the transition temperature by 4°C. Shifting one C16 fatty acid chain link with glycerol from *sn*-2 to *sn*-3 position has the same consequence. Going from *cis* to *trans* double bond insaturations raise the melting transition of DOPC by 60 K.

If one thinks of the action of sucrose as simply dehydrating the lipid headgroups, then a strong elevation of the melting transition temperature would be expected. Yet, the observed change goes in this direction, but in much weaker proportions. In addition, hydrophilic sucrose molecules are not really expected to interact with the bulk of hydrophobic alkyl chains region, which is where the largest part of the contribution to the enthalpy change Δh is expected to arise from.

Drops in latent heat at the transition can be alternatively explained by the presence of domains. If one assumes that in the presence of sucrose, lipids get separated into sucrose-depleted and sucrose enriched domains, and that only sucrose depleted domains melt as usual, with other domains remaining in the gel phase, then the result would also be a neat decrease in experimental latent heat. However, here is no clear reason for such domains to form, and this mechanism lacks experimental support.

We propose here an alternative mechanism where sucrose adsorbs indistinctly in the gel and fluid phases. Lipids that are in close contact with sucrose molecules are assumed to melt at a slightly higher temperature T'_m, and more importantly, to behave in a less cooperative way than in pure lipid water solutions. This could be justified for instance by saying that gel-fluid mismatch configurations are eased by surrounding sucrose molecules.

Adapting the previous model, the configurational energy becomes

$$\Delta\mathcal{H} = (\Delta h - T\Delta s)N_l + (\Delta h' - T\Delta s')N'_l + JN_{gl} + J'N'_{gl} + J''N''_{gl}, \tag{S15}$$

with N_l the number of free lipids in fluid state, N'_l the number of lipid in fluid state in contact with sucrose, N_{gl} the number of unlike gel-fluid free lipid pairs, N'_{gl} the number of unlike gel-fluid lipid pairs, both in contact with sucrose and N''_{gl} the number of unlike gel-fluid pairs with one lipid free and one lipid in contact with sucrose, J, J', J'' being the corresponding mismatch penalties.

We assume now that the probability for a lipid to be in contact with sucrose is σ, that p is the average probability of finding free lipids in fluid state, and p' the average probability of finding lipids in contact with sucrose in fluid state. The average configurational energy can be expressed in the mean-field limit.

$$\begin{aligned}\langle\beta\Delta\mathcal{H}\rangle &= -\frac{\mathcal{N}_A\Delta h}{RT_m^2}(T - T_m)(1-\sigma)Np - \frac{\mathcal{N}_A\Delta h}{RT_m^2}(T - T'_m)\sigma Np' \\ &\quad + N\tilde{J}(1-\sigma)^2p(1-p) + N\tilde{J}'\sigma^2p'(1-p') \\ &\quad + N\tilde{J}''\sigma(1-\sigma)[p(1-p') + p'(1-p)]\end{aligned} \tag{S16}$$

where for simplicity we assume $\Delta h \simeq \Delta h'$, $T_m \simeq T'_m$ at the first order of the temperature expansion. The mean field configurational entropy then becomes:

$$\begin{aligned} -\mathcal{S}/k_B &= N\Big[\sigma p' \ln(\sigma p') + \sigma(1-p')\ln[\sigma(1-p')] \\ &\quad +(1-\sigma)p\ln[(1-\sigma)p] + (1-\sigma)(1-p)\ln[(1-\sigma)(1-p)]\Big], \\ &= N\Big[\sigma\ln(\sigma) + (1-\sigma)\ln(1-\sigma) + \sigma[p'\ln(p') + (1-p')\ln(1-p')] \\ &\quad +(1-\sigma)[p\ln(p) + (1-p)\ln(1-p)]\Big]. \end{aligned} \tag{S17}$$

The Gibbs free-energy $\beta\Delta G(p,p',\sigma,T) = \langle\beta\Delta\mathcal{H}\rangle - \mathcal{S}/k_B$ must now be minimized with respect to p and p'. We do not perform a minimization over σ because we assume σ imposed by the sucrose molarity ([Sucrose]) of the hydrating solution.

The self-consistent equations become

$$\begin{aligned} \ln\left(\frac{p}{1-p}\right) - \frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T_m) + \tilde{J}(1-\sigma)(1-2p) + \tilde{J}''\sigma(1-2p') &= 0; \\ \ln\left(\frac{p'}{1-p'}\right) - \frac{\mathcal{N}_A\Delta h}{RT_m^2}(T-T'_m) + \tilde{J}'\sigma(1-2p') + \tilde{J}''(1-\sigma)(1-2p) &= 0. \end{aligned} \tag{S18}$$

With the numerical solution for $p(T), p'(T)$ determined, the temperature dependent enthalpy is readily obtained from eq. (S16).

In practice, equations (S18) are solved for each temperature T using the Newton-Raphson iteration scheme, starting initially from the exact solution at $\tilde{J} = \tilde{J}' = \tilde{J}'' = 0$, and iteratively converged for increasing values of the coupling constant. Below critical coupling $\tilde{J}_c = 2$, the method is fast and accurate.

An interesting behavior is obtained for the following choice of parameters:

- $T_m = 273.15 + 41.8$ K, $T'_m = 273.15 + 41.8 + 5.0$ K,
- $\tilde{J} = 1.94$; $\tilde{J}' = \tilde{J}'' = 0.97$,
- $\mathcal{N}_A\Delta h = \mathcal{N}_A\Delta h' = 56$ kJ $\cdot$ mol^{-1}.

The three graphs below explains how the decreased cooperativity mechanism works:

0.1 Connection with previous work and correspondence with the usual Ising model

Ising variables are usually binary variables s taking the values ± 1. The order parameter $m = \langle s\rangle$ is a real number comprised between -1 and 1. The correspondence between p and m is

$$m = 2p - 1 \Leftrightarrow p = (1+m)/2. \tag{S19}$$

At T_m, eq. (S12) can be rewritten

$$\frac{1+m}{1-m} = \exp(\tilde{J}m), \tag{S20}$$

which can be inverted as

$$m = \frac{e^{\tilde{J}m} - 1}{e^{\tilde{J}m} + 1} = \tanh(\tilde{J}m/2). \tag{S21}$$

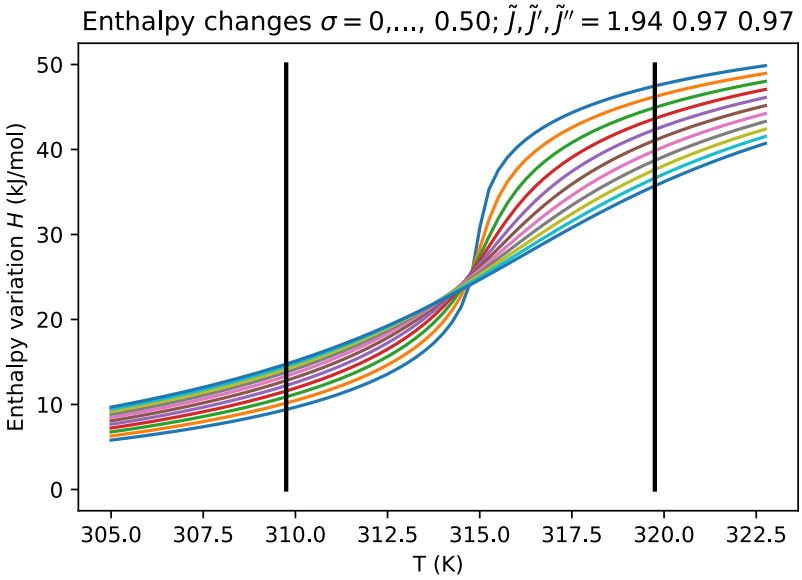

Figure S4: Enthalpy variation with temperature as σ increases from 0 to 0.5. We integrate the area under the specific heat curve from $T_m - 5$ K to $T_m + 5$ K.

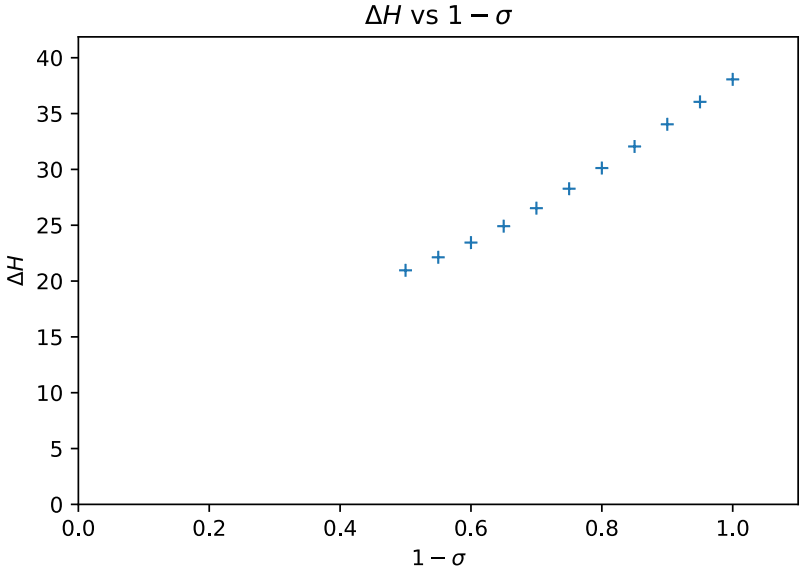

Figure S5: Resulting enthalpy change vs $1 - \sigma$ (pure water at the right of the graph). We note that for the selected values, the ΔH curves seem initially to decrease linearly with $1 - \sigma$.

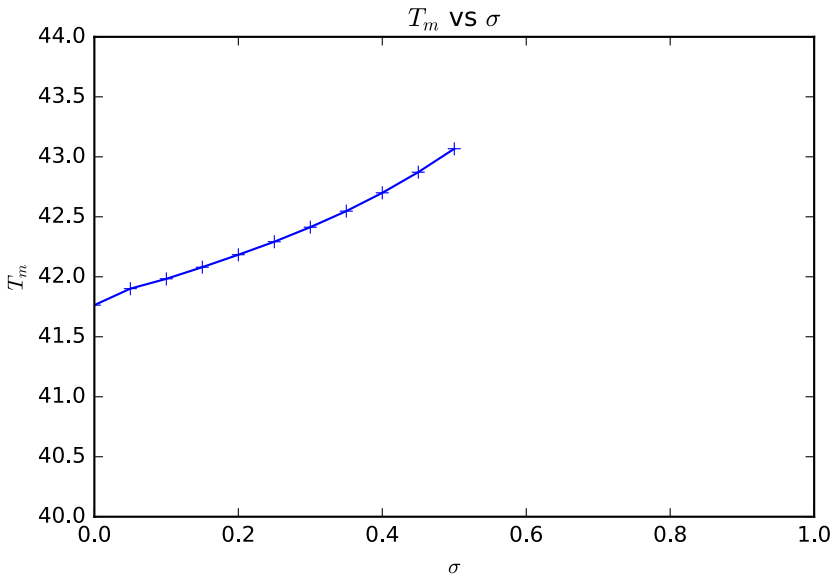

Figure S6: Variation of the apparent melting temperature (inflection point of $\Delta H(T)$)

A comparison with the usual self-consistent Ising equation $m = \tanh(\beta J_{\text{Ising}} z m)$ shows that $J = 2J_{\text{Ising}}$. One could also have deduced it from the mismatch energy associated with two antiparallel spins around a bond ($2J_{\text{Ising}}$) which equals our mismatch energy J. Eq. (S21) leads to the mean-field value $\beta J_{\text{Ising}} z = \tilde{J}/2 = 1$.

By comparison, the exact value of the critical point on a 2d hexagonal lattice Ising model is $\beta J_{\text{Ising}} = 0.2746\ldots$ (see (59), page 671). In our notations, this corresponds to $\tilde{J} = 0.5432$. Back to the original parameter J, one finds (with $z = 6$, and 1 cal=4.18 J):

- mean-field: $\mathcal{N}_A J = \frac{2}{z} RT_m = 860 \text{ J} \cdot \text{mol}^{-1} = 205 \text{ cal} \cdot \text{mol}^{-1}$,
- exact: $\mathcal{N}_A J = 2 \times 0.2746 \times RT_m = 1414 \text{ J} \cdot \text{mol}^{-1} = 338 \text{ cal} \cdot \text{mol}^{-1}$.

The mean-field approximation underestimates the magnitude of the coupling constant that is needed to correlate the spins to a given degree.

We can compare now the values used in this study to those of Jerala et al. (55). In Jerela et al., the gel-fluid mismatch penalty is noted $\omega = J$. The proposed value for fitting the DSC curve of DPPC systems is $\omega = 282$ cal, when internal degrees of freedom are associated to whole lipids. This corresponds to a ratio $J/J_c = 282/338 = 0.8343$.

Transposed to the mean-field critical value $205 \text{ cal} \cdot \text{mol}^{-1}$, this would corresponds to a $J \simeq \omega \simeq 0.8343 \times 205 = 171 \text{ cal} \cdot \text{mol}^{-1}$ (715 J ·mol^{-1}). By comparison, we use in the above approach $J = 199 \text{ cal} \cdot \text{mol}^{-1}$ (830 J·mol^{-1}) and $J' = J'' = 100 \text{ cal} \cdot \text{mol}^{-1}$ (415 J·mol^{-1}). We therefore globally operate closer to the critical point.