Supporting Information for

Lipid spontaneous curvatures estimated from temperature-dependent changes in inverse hexagonal phase lattice parameters: the effects of metal cations

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Supporting Information for

SI: 1.0 Method for estimating c_0 from the lattice expansion of inverse hexagonal phases

For an elastic deformation with principal curvatures c_1 and c_2 away from the spontaneous curvature c_0 for lipid species k, the Helfrich free energy (per unit area) is defined as:

$$E_k = \frac{\kappa}{2}\left(c_{1,k} + c_{2,k} - c_{0,k}\right)^2 + \kappa_G c_{1,k} c_{2,k} \qquad (1),$$

where κ is the mean curvature elastic modulus, and κ_G is the Gaussian curvature elastic modulus. Given that for a binary mixture of non-interacting (ideal) amphiphiles (species i and j) the total curvature elastic energy is widely assumed [1] to be a linear combination of the curvature energies of the individual components, such that

$$E_T = xE_i + (1-x)E_j \qquad (2),$$

E_i is the curvature energy of component i, and x is its mole fraction.

Assuming that for each species of the mixture c_0 is independent of temperature (i.e. for component i $c_{0,i}^{(A)} \cong c_{0,i}^{(B)} \equiv c_{0,i}$), between the temperatures A and B, the difference in the curvature energy (ΔE_T) gives the following expression:

$$\Delta E_T = \frac{x\kappa_i}{2}\left\{\left(c_{1,i}^{(A)}\right)^2 - \left(c_{1,i}^{(B)}\right)^2 + \left(c_{2,i}^{(A)}\right)^2 - \left(c_{2,i}^{(B)}\right)^2 + 2\left(c_{1,i}^{(A)}c_{2,i}^{(A)} - c_{1,i}^{(B)}c_{2,i}^{(B)}\right) - 2\left(c_{1,i}^{(A)} - c_{1,i}^{(B)} + c_{2,i}^{(A)} - c_{2,i}^{(B)}\right)c_{0,i}\right\} + \frac{(1-x)\kappa_j}{2}\left\{\left(c_{1,j}^{(A)}\right)^2 - \left(c_{1,j}^{(B)}\right)^2 + \left(c_{2,j}^{(A)}\right)^2 - \left(c_{2,j}^{(B)}\right)^2 + 2\left(c_{1,j}^{(A)}c_{2,j}^{(A)} - c_{1,j}^{(B)}c_{2,j}^{(B)}\right) - 2\left(c_{1,j}^{(A)} - c_{1,j}^{(B)} + c_{2,j}^{(A)} - c_{2,j}^{(B)}\right)c_{0,j}\right\} + x\kappa_{G,i}\left\{c_{1,i}^{(A)}c_{2,i}^{(A)} - c_{1,i}^{(B)}c_{2,i}^{(B)}\right\} + (1-x)\kappa_{G,i}\left\{c_{1,j}^{(A)}c_{2,j}^{(A)} - c_{1,j}^{(B)}c_{2,j}^{(B)}\right\} \qquad (3).$$

Since $c_2 = 0$, for hexagonal phases, and with the assumption that $\kappa_i \approx \kappa_j$, [2] equation (3) reduces to

$$\frac{2\Delta E_T}{\kappa} = x\{(c_{1,i}^{(A)})^2 - \left(c_{1,i}^{(B)}\right)^2\} + (1-x)\{\left(c_{1,j}^{(A)}\right)^2 - \left(c_{1,j}^{(B)}\right)^2\} - 2(1-x)(c_{1,j}^{(A)} - c_{1,j}^{(B)})c_{0,j} - 2x(c_{1,i}^{(A)} - c_{1,i}^{(B)})c_{0,i} \qquad (4).$$

rearranging in terms of the mixture parameters gives an equation in the linear form:

$$\frac{1}{2}\left\{\left(c_1^{(A)}\right)^2 - \left(c_1^{(B)}\right)^2\right\} = c_{0\,mix}\left\{c_1^{(A)} - c_1^{(B)}\right\} + \frac{\Delta E_T}{\kappa} \qquad (5)$$

And thus, for a given ideal binary mixture of amphiphiles in an hexagonal phase, a plot of $\frac{1}{2}\left\{\left(c_1^{(A)}\right)^2 - \left(c_1^{(B)}\right)^2\right\}$ against $\left\{c_1^{(A)} - c_1^{(B)}\right\}$

for pairs of temperatures (A and B), gives a straight line. The intercept of this line is equal to $\frac{\Delta E_T}{\kappa}$ and the slope is equal to the spontaneous curvature of the mixture, $c_{0\,mix}$.

Supporting Information for

SI: 1.1 Lattice parameter data for guest lipids in DOPE host inverse hexagonal phases

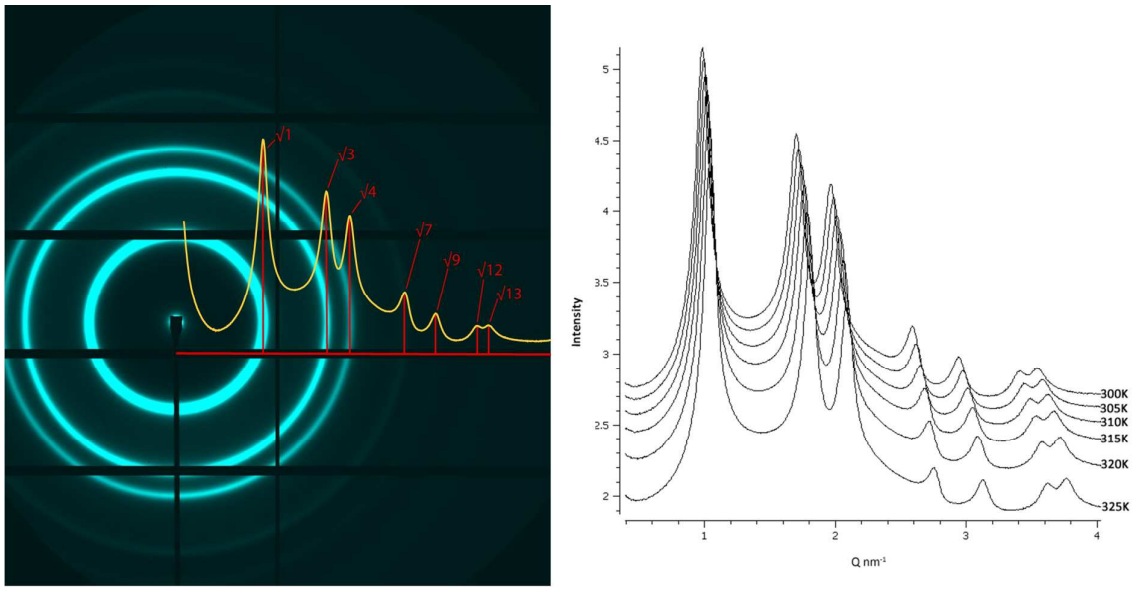

Figure S1 the temperature driven change in Q of the DOPE inverse hexagonal phase in water.

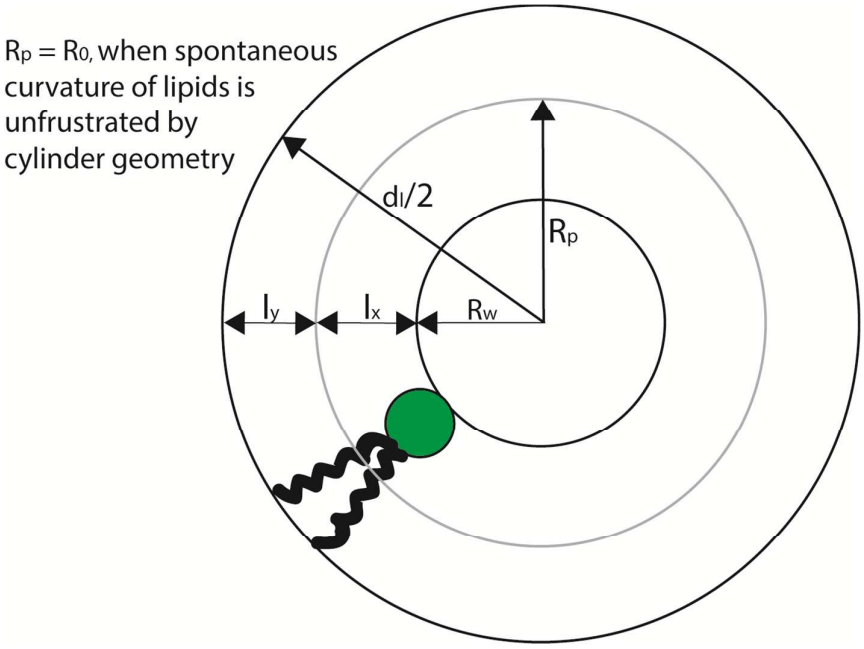

Figure S2 a summary of the relationship between the structural parameters of the inverse hexagonal cylinder and the radii of curvature of planes used to define curvature. R_p is the radius of the pivotal plane, R_w is the radius of the water cylinders and d_l is the interpore spacing (lattice parameter) of the inverse hexagonal phase. The terms l_x and l_y describe the individual portions of a lipid of length ($l = l_x + l_y$) split by the pivotal plane.

Supporting Information for

Table S1 lattice parameter data for the host (DOPE)-guest binary lipid mixtures, note in the sample ID W = water, L = low divalent cation concentration [M^{2+}] = 13 mM, M = medium divalent cation concentration [M^{2+}] = 65 mM and H = high divalent cation concentration [M^{2+}] =130 mM.

Mole fraction DOPA in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PA_0W	PA_3W	PA_6W	PA_9W
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C				
27	7.318367	7.308437	7.2692738	7.377014
32	7.247915	7.244627	7.2085587	7.280694
37	7.147983	7.144941	7.1172455	7.196467
42	7.048634	7.047201	7.0251204	7.100218
47	6.958183	6.958306	6.9413515	7.023572
52	6.907958	6.870255	6.8642198	6.94189

Mole fraction DOPA in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PA_0L	PA_3L	PA_6L	PA_9L
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C				
27	7.377309	7.383698	7.4307481	7.486057
32	7.294723	7.290803	7.3214497	7.391553
37	7.212891	7.192218	7.2282405	7.288659
42	7.119015	7.092867	7.1289138	7.184588
47	7.033387	6.998993	7.0313679	7.102539
52	6.952008	6.907237	6.9473569	7.003717

Mole fraction DOPA in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PA_0M	PA_3M	PA_6M	PA_9M
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C				
27	7.415363	7.405248	7.2079186	7.030101
32	7.327964	7.300216	7.1124913	6.938372
37	7.230668	7.215054	7.0366683	6.852102
42	7.140361	7.110672	6.9531241	6.743155
47	7.049752	7.024761	6.858089	6.662996
52	6.958172	6.925956	6.7864589	6.56156

Supporting Information for

Mole fraction DOPA in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PA_0H	PA_3H	PA_6H	PA_9H
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.464370932	7.418741	7.300553	7.206117
32	7.37591908	7.326673	7.2221116	7.130053
37	7.294245852	7.24318	7.1240395	7.04467
42	7.195232124	7.15134	7.0478415	6.967482
47	7.104148247	7.074437	6.9628076	6.879665
52	7.029605556	6.992024	6.8683479	6.803837

Mole fraction DOPS in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PS_0W	PS_3W	PS_6W	PS_9W
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.318367	7.320729	7.3655772	7.479648
32	7.247915	7.233812	7.2843121	7.394892
37	7.147983	7.140834	7.1908786	7.311123
42	7.048634	7.053119	7.093087	7.206422
47	6.958183	6.959945	7.0099663	7.130331
52	6.907958	6.882221	6.9138694	7.052747

Mole fraction DOPS in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PS_0L	PS_3L	PS_6L	PS_9L
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.377309	7.342283	7.507822	7.70083
32	7.294723	7.279478	7.4262625	7.607004
37	7.212891	7.173369	7.343589	7.511524
42	7.119015	7.082848	7.2449421	7.435543
47	7.033387	7.007373	7.1662438	7.359277
52	6.952008	6.930374	7.0806643	7.27198

Supporting Information for

Mole fraction DOPS in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PS_0M	PS_3M	PS_6M	PS_9M
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.415363	7.374131	7.3591407	7.19073
32	7.327964	7.286111	7.2853921	7.139247
37	7.230668	7.216116	7.1833717	7.074839
42	7.140361	7.108306	7.0885684	6.991963
47	7.049752	7.032981	7.0020086	6.904628
52	6.958172	6.940901	6.9194905	6.834326

Mole fraction DOPS in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	PS_0H	PS_3H	PS_6H	PS_9H
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.464370932	7.419646	7.3885718	7.357927
32	7.37591908	7.356647	7.3187617	7.292476
37	7.294245852	7.290321	7.2351828	7.200502
42	7.195232124	7.159409	7.1352302	7.115207
47	7.104148247	7.092331	7.0399328	7.036205
52	7.029605556	7.009046	6.968704	6.963962

Mole fraction MO in DOPE	0	0.01	0.03	0.06
Sample ID/ Buffer	MO_0W	MO_1W	MO_3W	MO_9W
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.318367	7.319049	7.2590416	7.21647
32	7.247915	7.212133	7.1728754	7.128867
37	7.147983	7.122182	7.0823894	7.03826
42	7.048634	7.040904	6.996525	6.957805
47	6.958183	6.96171	6.9119886	6.876689
52	6.907958	6.868562	6.8206048	6.786829

Mole fraction	0	0.03	0.06	0.09

Supporting Information for

DiPHYPE in DOPE Sample ID/ Buffer	DiPHY_0W	DiPHY _1W	DiPHY _3W	DiPHY _9W
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.318367	7.188062	7.1037345	7.010862
32	7.247915	7.126846	7.0268301	6.937093
37	7.147983	7.065472	6.9522802	6.864376
42	7.048634	6.973788	6.861591	6.786048
47	6.958183	6.896397	6.7783605	6.709736
52	6.907958	6.815249	6.6990233	6.621081

Mole fraction DD in DOPE Sample ID/ Buffer	0	0.05	0.20	0.30
	DD_0W	DD _5W	DD _20W	DD _30W
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.318367	7.160205	6.7213444	6.137732
32	7.247915	7.066511	6.6535377	6.086439
37	7.147983	6.973425	6.583103	6.012014
42	7.048634	6.873107	6.5174699	5.965222
47	6.958183	6.82305	6.4530165	5.89421
52	6.907958	6.736387	6.3778787	5.850239

Mole fraction HD in DOPE Sample ID/ Buffer	0	0.05	0.20	0.30
	HD_0W	HD _5W	HD _20W	HD _30W
TEMP/ °C	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
27	7.318367	7.225091	7.0383737	6.535801
32	7.247915	7.129953	6.9705652	6.46393
37	7.147983	7.033697	6.8798967	6.39424
42	7.048634	6.947037	6.7852082	6.326984
47	6.958183	6.870628	6.6968197	6.273952
52	6.907958	6.790145	6.6131831	6.207613

Supporting Information for

Mole fraction tRA in DOPE	0	0.06	0.09
Sample ID/ Buffer	tRA _0W	tRA _6W	tRA _9W
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C			
27	7.318367	7.063724	7.023688
32	7.247915	6.997493	6.930266
37	7.147983	6.939133	6.861241
42	7.048634	6.835805	6.765519
47	6.958183	6.766886	6.69839
52	6.907958	6.686818	6.626895

Mole fraction tR in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	tR _0W	tR _3W	tR _6W	tR _9W
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C				
27	7.318367	7.0717212	6.503043	6.405972
32	7.247915	7.0104137	6.479496	6.377027
37	7.147983	6.9357985	6.446094	6.326136
42	7.048634	6.8704673	6.431951	6.288138
47	6.958183	6.7990597	6.39634	6.246173
52	6.907958	6.7231687	6.394455	6.217585

Mole fraction cR in DOPE	0	0.03	0.06	0.09
Sample ID/ Buffer	cR _0W	cR _3W	cR _6W	cR _9W
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C				
27	7.318367	6.9751271	6.584616	6.237239
32	7.247915	6.9168019	6.551888	6.203299
37	7.147983	6.856882	6.519052	6.19719
42	7.048634	6.8093628	6.494948	6.161065
47	6.958183	6.7170249	6.452588	6.144797
52	6.907958	6.6514535	6.432315	6.129206

Mole fraction	0	0.005	0.01	0.03	0.05

Supporting Information for

Chol in DOPE

Sample ID/ Buffer	Chol _0W	Chol _05W	Chol _1W	Chol _3W	Chol_5W
	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm	Lattice parameter / nm
TEMP/ °C					
27	7.318367	7.323842	7.3236703	7.322342	7.265006
32	7.247915	7.254932	7.2344307	7.22784	7.201074
37	7.147983	7.150451	7.1586196	7.130851	7.1085
42	7.048634	7.071813	7.0683478	7.040613	7.020326
47	6.958183	6.957302	6.9821443	6.971696	6.927977
52	6.907958	6.883017	6.8925935	6.864535	6.842135

Supporting Information for

SI 1.2 Validation of the method of estimating c_0 from the temperature dependence of the lattice parameter of DOPE: anionic lipid mixtures

To confirm that the changes, we report, in the spontaneous curvature of DOPE: DOPA and DOPE: DOPS mixtures were not artefacts of the method of estimating c_0 we carried out an independent test. For this test we calculated the value of c_{0mix} from previously published data [3] that showed the variance of the lattice parameters of inverse hexagonal phases of DOPE: anionic lipid mixtures with temperature, in the presence absence of buffer/ divalent cations. Critically, in the published study where we have taken data from, the authors calculate the spontaneous curvature of the lipid mixtures using a traditional method where they reconstruct the electron density profile of hexagonal unit cells. These data are shown in Figures S3 to S5 and clearly show that within experimental error our method reproduces the c_0 values obtained by this traditional method over the composition range, i.e. up to 0.09 mol %, we have studied.

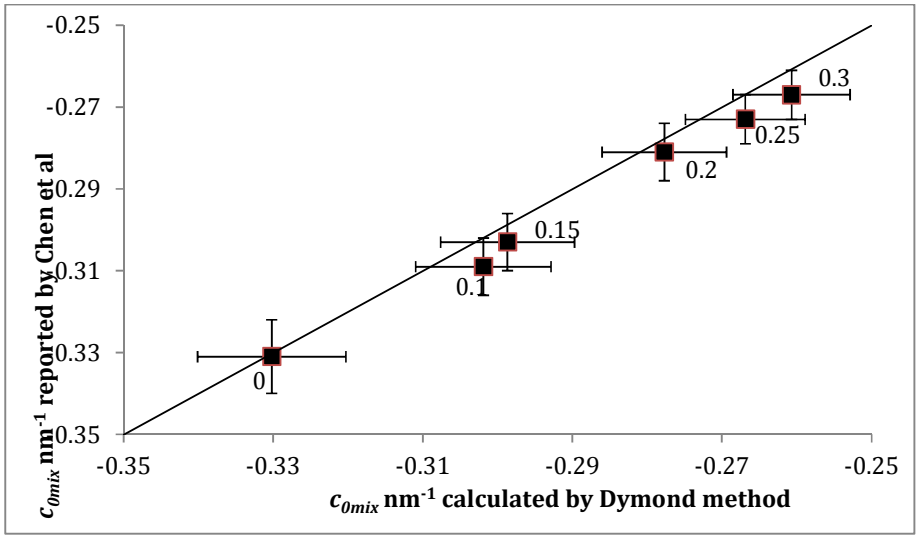

Figure S3 comparing c_0 values of DOPE:DOPA lipid mixtures in water obtained by Chen et al.[3] at 20°C to c_0 values calculated using our method (Dymond et al. method) but using the lattice parameter data published by Chen et al.[3]. The solid diagonal line shows where the two methods give identical results and data labels show the mole fraction of DOPA.

Supporting Information for

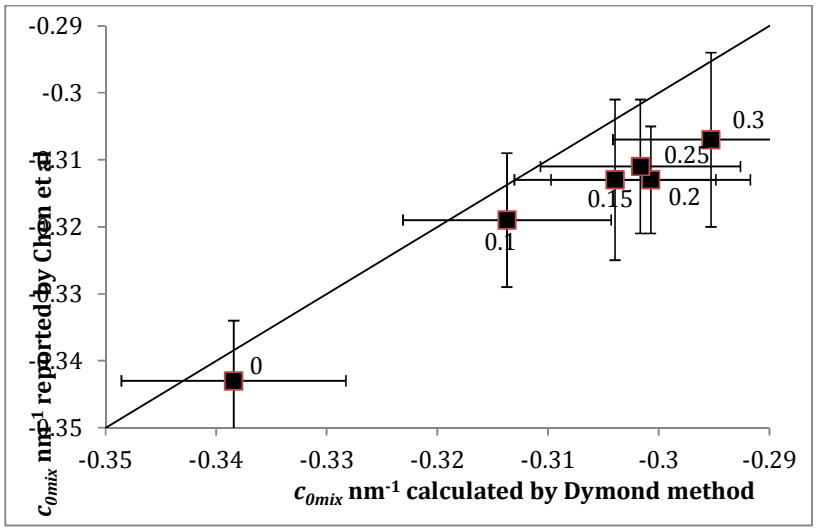

Figure S4 comparing c_0 values of DOPE:DOPA lipid mixtures in 10 mM HEPES, 50 mM Ca^{2+} obtained by Chen et al.[3] at 20°C to c_0 values calculated using our method (Dymond et al. method) but using the lattice parameter data published by Chen et al. [3]. The solid diagonal line shows where the two methods give identical results and data labels show the mole fraction of DOPA.

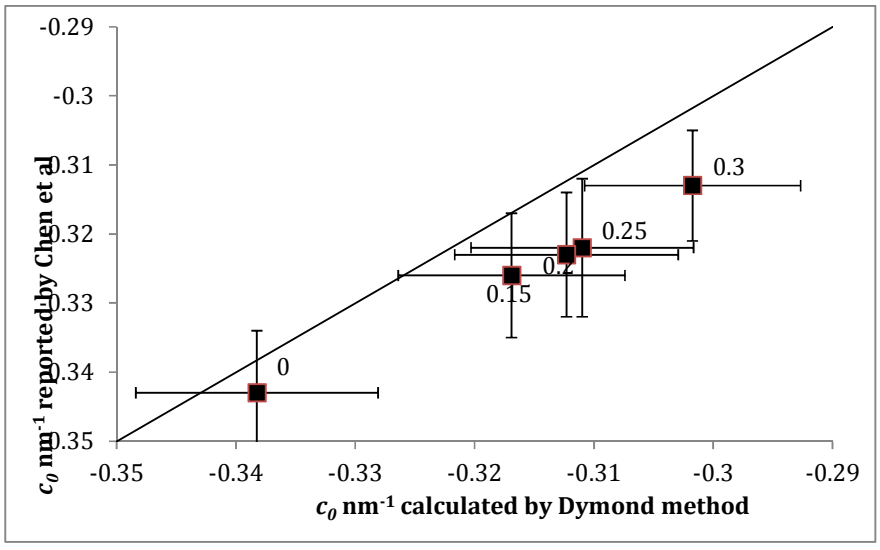

Figure S5 comparing c_0 values of DOPE:DOPA lipid mixtures in 10 mM HEPES, 70 mM Ca^{2+} obtained by Chen et al.[3] at 20°C to c_0 values calculated using our method (Dymond et al. method) but using the lattice parameter data of Chen et al. [3]. The solid diagonal line shows where the two methods give identical results and data labels show the mole fraction of DOPA.

Supporting Information for

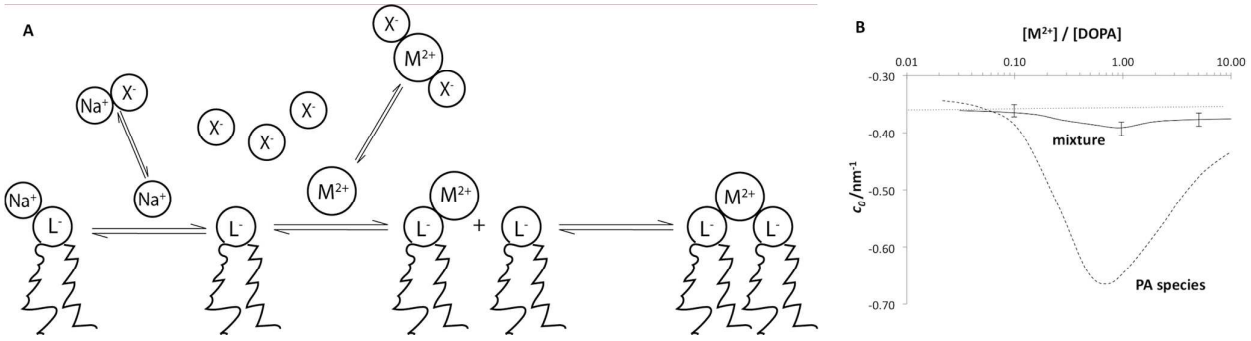

Figure S6A. Schematic representation of the different lipid species that comprise c_{0j} for a generic anionic lipid (L^-) interacting with divalent (M^{2+}) and monovalent (Na^+) cation salts, where X^- is a generic salt counterion. These interactions were taken as the basis of the equilibrium model. S6B the total spontaneous curvature (c_{0j}) of DOPA (dashed line) as a function of divalent cation concentration, data are determined from the equilibrium association model (Fig S6A) for 9 mol% DOPA such that c_{0j} of DOPA is sum of multiple lipid species (DOPA-Na, $DOPA^-$, $DOPA\text{-}M^+$, $(DOPA)_2M$). The solid curve is the fit to the experimental data for the DOPE: DOPA mixtures i.e. c_{0mix} versus divalent cation concentration. The horizontal dotted line denotes the value of c_0 for DOPE.

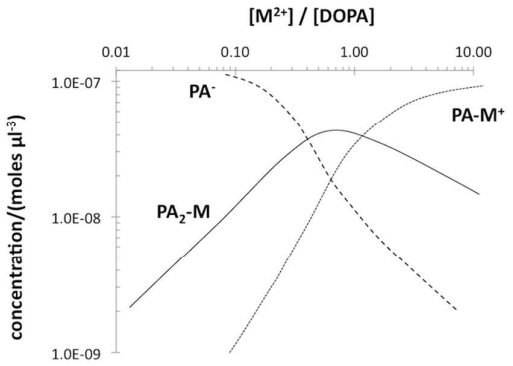

Figure S7. Contribution of individual DOPA lipid species ($DOPA^-$, $DOPA\text{-}M^+$, $(DOPA)_2M$) to the spontaneous curvature c_{0j}, as a function of divalent cation concentration. Data are from the anionic species equilibria model for a lipid concentration of 4.0×10^{-8} moles μL^{-1} (3 mol% DOPA).

SI 1.3 Compositional dependence of c_{0mix} values in DOPE host templates

To confirm that the trends that we observe in our c_{0mix} values as a function of the increasing both anionic lipid and divalent cation concentration, shown in Figure 3A and B, are not artefactual we also independently calculated the values of c_{0mix} for these samples. This was achieved using the frequently used relationships [3–5] shown in Equations 6 and 7,

$$\emptyset_w = \frac{2\pi R_w^2}{\sqrt{3}{d_l}^2} \quad (6)$$

$$R_p = d_l\sqrt{\frac{\sqrt{3}}{2\pi}\left(1 - \frac{V_p}{V_l}\emptyset_l\right)} \quad (7)$$

Where $\emptyset_w$ is the total water volume fraction, and $\emptyset_l$ is the total lipid volume fraction, d_l is the lattice parameter, R_w is the radius of the water cylinders, V_p is the lipid volume across the distance l_y and V_l is the total lipid volume. Critically since the ratio of V_p/V_l does not change with up to 30 mol % anionic lipid [6] and its temperature dependence is slight but well detailed [4] we can calculate R_p, if we know $\emptyset_l$ and/ or $\emptyset_w$. We set up our studies above limiting hydration and since there are extensive experimental details in the literature of the limiting hydration value of $\emptyset_w$ in DOPE: DOPA and DOPE: DOPS lipid mixtures then using these literature values we can calculate R_p for our data. We have carried out this calculation using the lattice parameter data we obtained experimentally (Table S1) and compare the answers to those reported in the main document text.

Initially we calculate $\emptyset_w$ to assess the extent to which it changes over the range of divalent cation and anionic lipid concentrations studied. We do this for DOPE: DOPS and DOPE: DOPA in water using the published data for the limiting hydration of these lipid mixtures in water. These published data show that in water $\emptyset_w$ ranges from 0.27 in pure DOPE to 0.3 in DOPE: DOPA (88:12) [7] and 0.31 in DOPE: DOPA (92:08) [5], these data show that $\emptyset_w$ has a linear dependence compositional dependence with increasing mole fraction of DOPA or DOPS. It is also possible to calculate the values of $\emptyset_w$, using Equation 6, from the work of Chen et al. [3], who report both d_l and R_w for DOPE: DOPA compositions at divalent cation concentrations of up to 100 mM. This calculation shows that $\emptyset_w$has a non-linear relationship with increasing divalent cation concentration and DOPA composition, however the variance is slight ranging from 0.24 to 0.27 with an average and standard deviation of 0.26 ± 0.01. Since $\emptyset_l = 1 - \emptyset_w$ we calculated the individual values of $\emptyset_l$ and used these values in Equation 7, at the divalent cation concentrations that were closest to those used in our studies. Under these conditions the R_p that emerges from Equation 7 is c_{0mix} which we then use to generate the same plot as is shown in Figure 3B. This is shown

in Figure S8 where we see the same dependence of c_{0mix} on [M^{2+}]/[DOPA], shown in Figure S6 and similar c_{0mix} values.

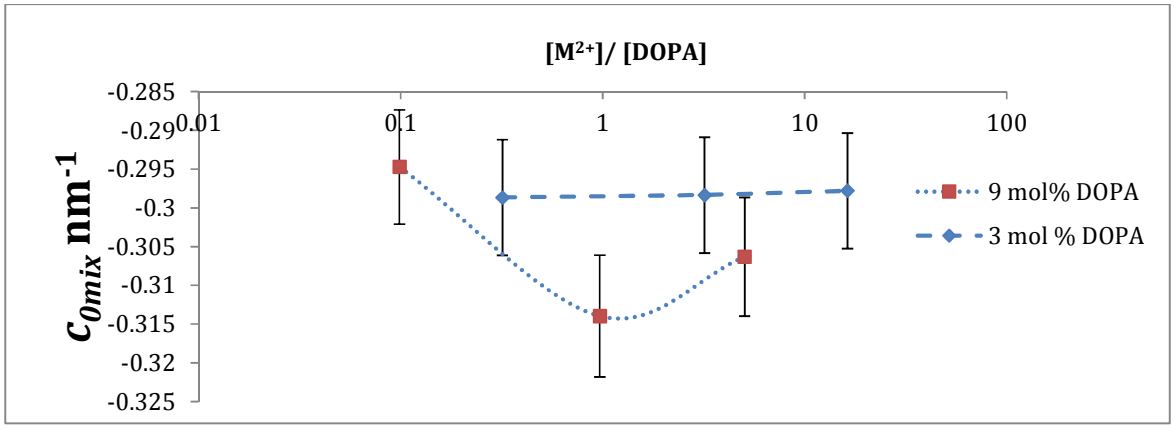

Figure S8 the change in c_{0mix} with DOPE: DOPA composition and total divalent cation concentration. Data were determined using a 'traditional' method to obtain c_{0mix} as detailed in the SI text and show identical trends to those data in Figure 3A, where c_{0mix} was determined using our method.

In this context it is interesting to ask what drives this change in c_{0mix} i.e. changes in $\emptyset_l$ or the lattice parameter d_l. This question is easily answered by setting $\emptyset_l$ constant or by randomly varying $\emptyset_l$ over the range observed. In both instances the same trend of c_{0mix} varying with [M^{2+}]/ [DOPA] is seen, which demonstrates that variance in d_l drives the change we report in c_{0mix}. In in other words changes in d_l that we observe originate from the change in spontaneous curvature of mixtures and not as artefacts due to our assumption that l_y does not change significantly with temperature or composition.

Supporting Information for

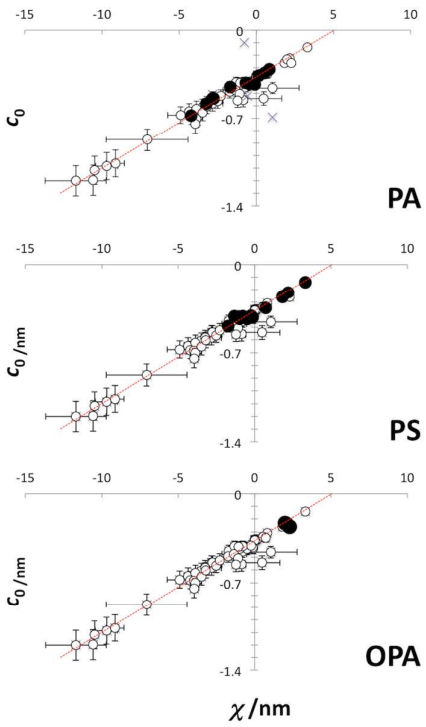

Figure S9. Range of spontaneous curvatures determined from XRD data for the anionic lipids DOPA, DOPS and lysoOPA. Empty, light shaded and dark shaded circles correspond to data for lipid concentrations of 4.0×10^{-8} moles μL^{-1}, 1.3×10^{-7} moles μL^{-1} and 1.3×10^{-6} moles μL^{-1}.

Supporting Information for

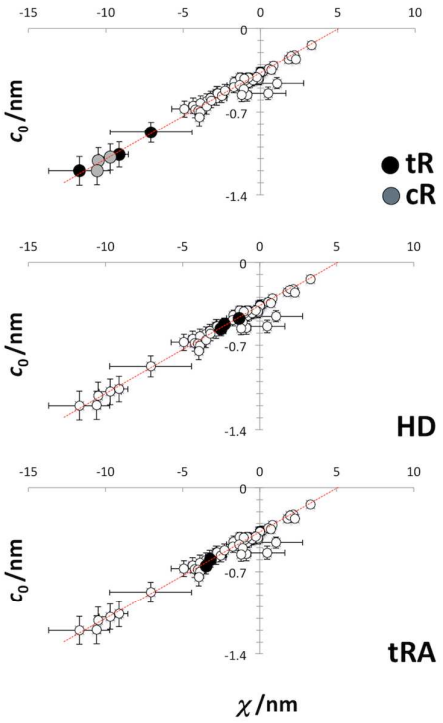

Figure S10. Range of spontaneous curvatures determined from XRD data for the lipids cR, tR, HD and tRA.

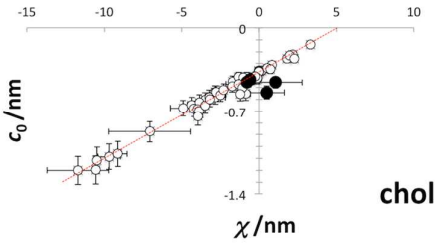

Figure S11 Range of spontaneous curvatures determined from XRD data for the lipid chol.

Supporting Information for

Table S2, spontaneous curvature data for guest lipids in an inverse hexagonal DOPE host phase; the error in the c_{0j} or R_{0j} values is estimated to be ± 7.5%. Where $Mf_{(j)}$, is the mole percent fraction of the guest lipid, j, in the host DOPE inverse hexagonal phase, $c_{0\,mix}$ is the gradient determined from Eq. 1, R_0 is the radius of spontaneous curvature of the guest lipid, j, calculated using our method, c_0 is the spontaneous curvature of the guest lipid, j, and χ_j is the curvature power of the guest lipid j, determined from Eq. 7.

lipid j	Mf_j (in DOPE)	$c_{0\,mix}$ / nm^{-1}	R_{0j} / nm	R_{0j} error (±)	c_{0j} / nm^{-1}	c_{0j} error (±)	χ_j / nm^{-1}	mean c_{0j}	sd
chol	0.05	-0.37	-1.84	0.14	-0.54	0.04	0.49	-0.47	0.05
	1	-0.37	-2.18	0.16	-0.46	0.03	1.07		
	3	-0.37	-2.29	0.17	-0.44	0.03	-0.57		
	5	-0.37	-2.20	0.17	-0.45	0.03	0.79		
MO	1	-0.37	-1.79	0.13	-0.56	0.04	-0.86	-0.54	0.03
	3	-0.37	-1.80	0.14	-0.55	0.04	-1.09		
	6	-0.38	-1.98	0.15	-0.51	0.04	-1.22		
diPhyPE	3	-0.38	-1.48	0.11	-0.67	0.05	-4.91	-0.65	0.02
	6	-0.38	-1.55	0.12	-0.65	0.05	-4.35		
	9	-0.39	-1.60	0.12	-0.63	0.05	-3.87		
OA	2	-0.44	-1.46	0.11	-0.69	0.05	-4.00	-0.71	0.03
	3	-0.47	-1.43	0.11	-0.70	0.05	-3.88		
	4	-0.52	-1.34	0.10	-0.75	0.06	-3.98		
cR	3	-0.39	-0.92	0.07	-1.08	0.08	-9.70	-1.13	0.06
	6	-0.41	-0.89	0.07	-1.12	0.08	-10.48		
	9	-0.44	-0.84	0.06	-1.20	0.09	-10.56		
DD	5	-0.38	-1.65	0.12	-0.61	0.05	-3.49	-0.63	0.05
	20	-0.41	-1.71	0.13	-0.58	0.04	-2.82		
	30	-0.46	-1.46	0.11	-0.69	0.05	-3.79		
HD	5	-0.38	-1.91	0.14	-0.52	0.04	-2.29	-0.52	0.04
	20	-0.39	-2.11	0.16	-0.47	0.04	-1.34		
	30	-0.43	-1.77	0.13	-0.56	0.04	-2.51		
tR	3	-0.38	-1.14	0.09	-0.87	0.07	-7.07	-1.05	0.16
	6	-0.42	-0.83	0.06	-1.20	0.09	-11.70		
	9	-0.43	-0.94	0.07	-1.06	0.08	-9.13		
tRA	6	-0.39	-1.53	0.11	-0.65	0.05	-3.19	-0.63	0.04
	9	-0.39	-1.66	0.12	-0.60	0.05	-1.30		

Supporting Information for

Table S3 spontaneous curvature data for guest phospholipids in inverse hexagonal DOPE host phases, we estimate a ± 7.5% error in the c_{0j} or R_{0j} values using this methodology. Where $Mf_{(j)}$, is the mole percent fraction of the guest lipid, j, in the host DOPE inverse hexagonal phase, $c_{0\,mix}$ is the gradient determined from Eq. 1, R_0 is the radius of spontaneous curvature of the guest lipid, j, calculated using our method, c_0 is the spontaneous curvature of the guest lipid, j, and χ_j is the curvature power of the guest lipid j, determined from Eq. 7.

lipid j	$[M^{2+}]$ / mM	Mf_j (in DOPE)	$c_{0\,mix}$ / nm^{-1}	R_{0j} / nm	R_{0j} error (±)	c_{0j} / nm^{-1}	c_{0j} error (±)	χ_j / nm^{-1}
DOPE	0	0	-0.37	-2.71	0.20	-0.37	0.03	0.00
	13	0	-0.37	-2.73	0.20	-0.37	0.03	0.00
	65	0	-0.36	-2.74	0.21	-0.36	0.03	0.00
	130	0	-0.36	-2.77	0.21	-0.36	0.03	0.00
DOPS	0	3	-0.37	-2.45	0.18	-0.41	0.03	-0.24
		6	-0.37	-2.88	0.22	-0.35	0.03	0.72
		9	-0.36	-3.85	0.29	-0.26	0.02	1.82
DOPS	13	3	-0.37	-2.43	0.18	-0.41	0.03	-1.32
		6	-0.36	-4.45	0.33	-0.22	0.02	2.18
		9	-0.35	-7.21	0.54	-0.14	0.01	3.31
DOPS	65	3	-0.37	-2.32	0.17	-0.43	0.03	-0.49
		6	-0.37	-2.42	0.18	-0.41	0.03	-0.79
		9	-0.38	-2.04	0.15	-0.49	0.04	-1.73
DOPS	130	3	-0.36	-2.38	0.18	-0.42	0.03	-0.13
		6	-0.37	-2.31	0.17	-0.43	0.03	-0.98
		9	-0.37	-2.39	0.18	-0.42	0.03	-1.04
DOPA	0	3	-0.37	-2.33	0.18	-0.43	0.03	-0.10
		6	-0.37	-2.37	0.18	-0.42	0.03	-0.51
		9	-0.37	-2.95	0.22	-0.34	0.03	0.54
DOPA	13	3	-0.37	-2.39	0.18	-0.42	0.03	-0.69
		6	-0.37	-2.79	0.21	-0.36	0.03	0.16
		9	-0.36	-3.22	0.24	-0.31	0.02	0.84
DOPA	65	3	-0.37	-2.36	0.18	-0.42	0.03	-0.52
		6	-0.38	-1.72	0.13	-0.58	0.04	-3.23
		9	-0.39	-1.47	0.11	-0.68	0.05	-4.21
DOPA	130	3	-0.36	-2.22	0.17	-0.45	0.03	-1.70
		6	-0.37	-1.85	0.14	-0.54	0.04	-2.84
		9	-0.38	-1.85	0.14	-0.54	0.04	-2.77