Supporting material: Minimal effect of lipid charge on membrane miscibility phase behavior

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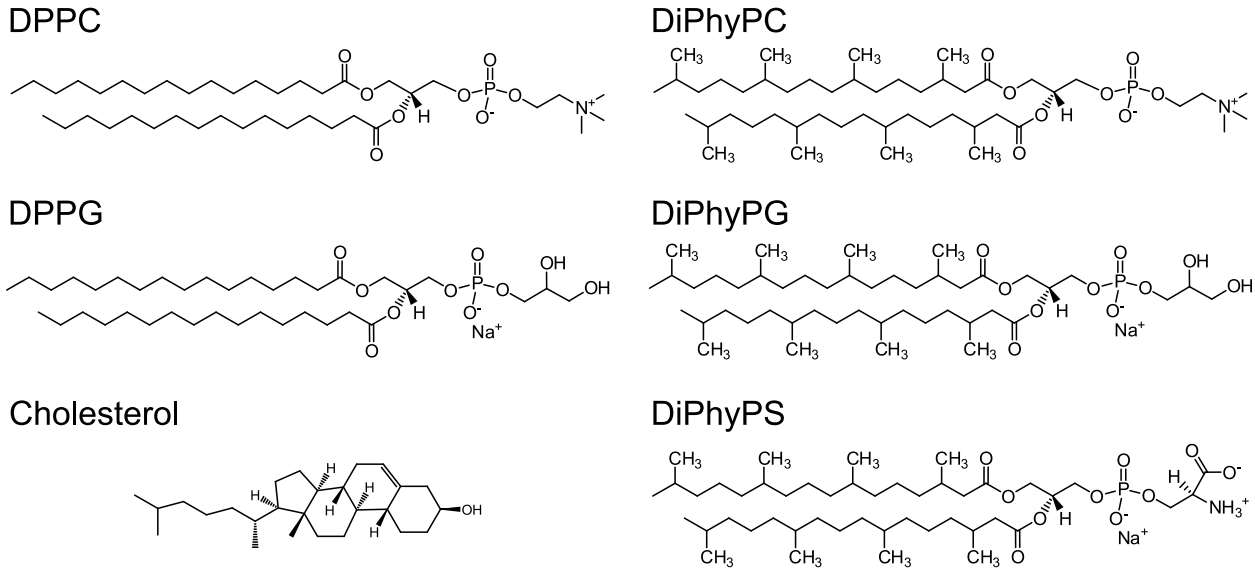

Fig. S1
Structures of lipids used in experiments. DPPG, DiPhyPG, and DiPhyPS are depicted with counterions that are diluted in the solutions used in this study.

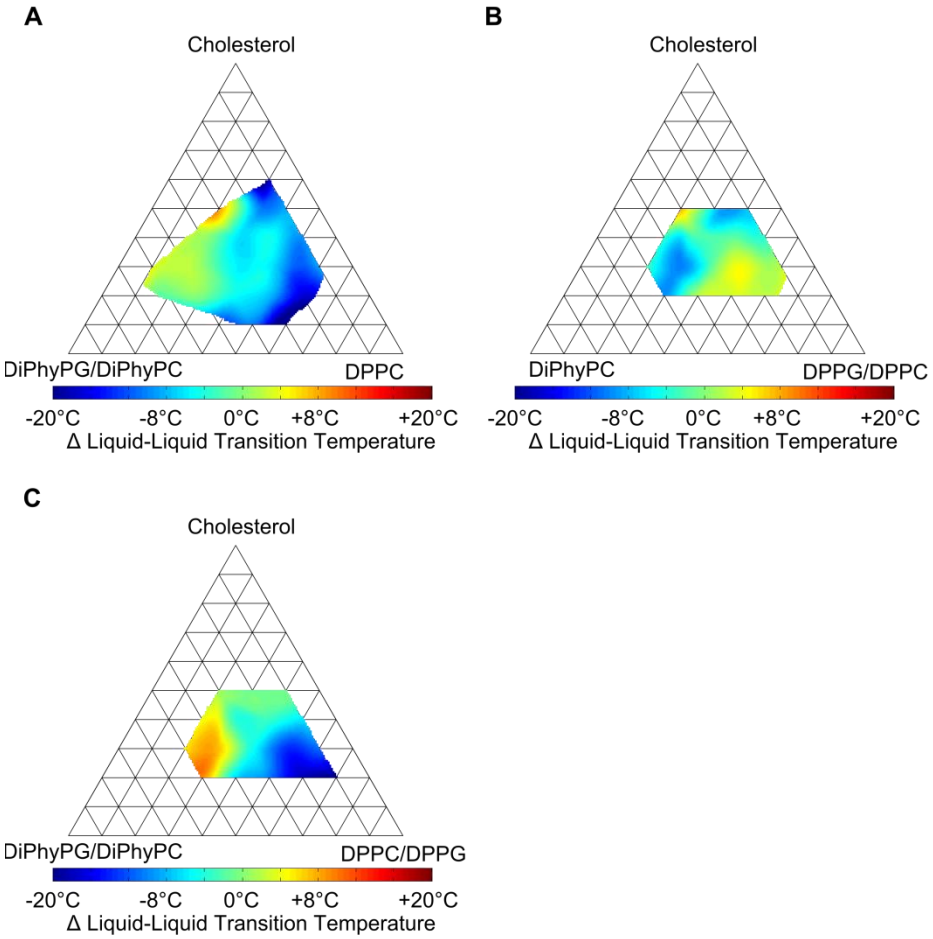

Fig. S2

Difference in miscibility transition temperatures (T_{mix}) for different systems of DiPhyX:DPX:Chol, where "X" denotes either a PC-lipid or a PG-lipid. Given that uncertainties in T_{mix} at each composition are ±3 ˚C, uncertainties in T_{mix} in the figures above are ±4 ˚C at each point. A) DiPhyPG:DPPC:Chol minus DiPhyPC:DPPC:Chol. B) DiPhyPC:DPPG:Chol minus DiPhyPC:DPPC:Chol. For both A and B, positive values correspond to higher transition temperatures in the charged system. C) DiPhyPG:DPPC:Chol minus DiPhyPC:DPPG:Chol. For A and B, the compositions with the highest values of T_{mix} shift towards the vertex of the charged lipid. For C, the two trends in A and B are additive, and the compositions with the highest values of T_{mix} shift strongly towards the vertex of the low temperature lipid.

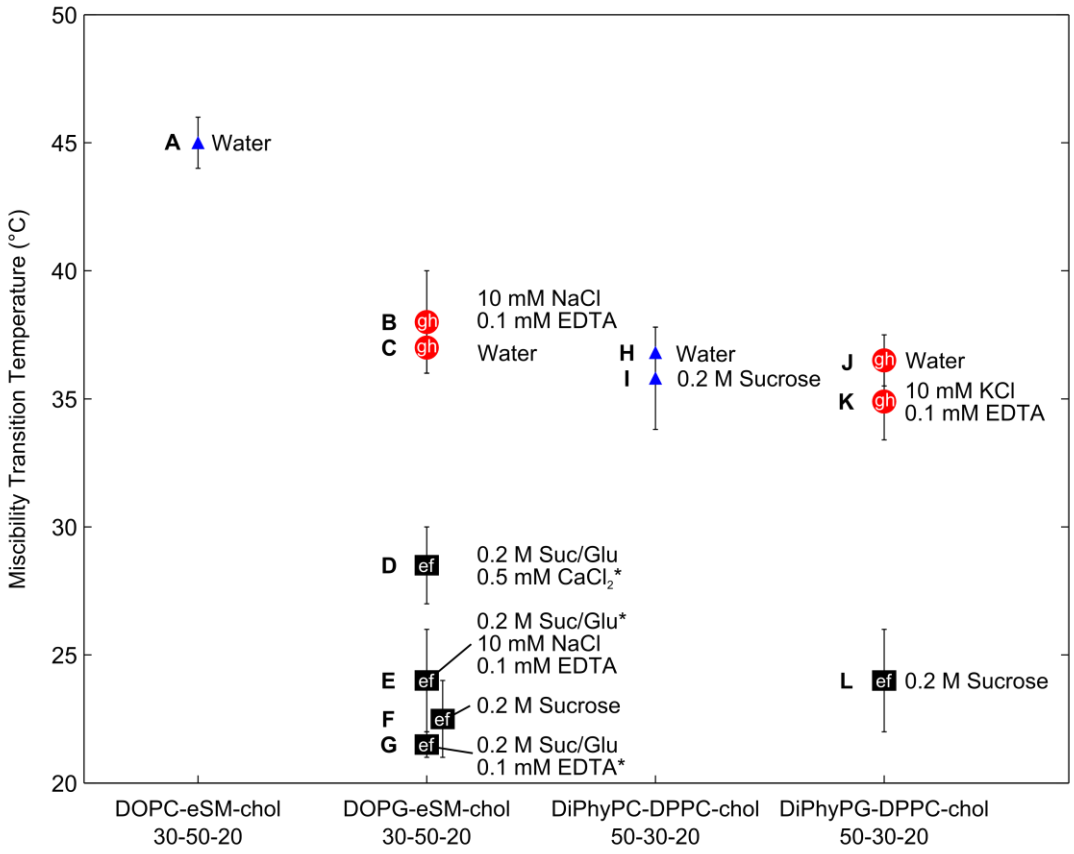

Fig. S3

This figure shows transition temperatures of vesicles prepared from four different lipid compositions by different methods and in different solutions. "Suc/Glu" denotes 0.2M sucrose inside of vesicles and 0.2M glucose outside. ef denotes vesicles prepared by electroformation that contain PG lipids as the low melting temperature species and that are in solutions with 0.2M sugar. gh denotes vesicles prepared by gentle hydration that contain PG lipids as the low melting temperature species. ▲ denotes vesicles formed by electroformation that contain PC as the low melting temperature lipid (that is, they contain no anionic lipids). The symbol * denotes data from C.C. Vequi-Suplicy et al. (1).

The major finding of this figure is that the difference in miscibility transition temperature between charged vesicles produced by electroformation and those produced by gentle hydration is larger than the effect of adding: monovalent salt, divalent salt, or replacing PG with PC. Specifically, the method of vesicle preparation of charged vesicles (electroformation in sucrose vs. gentle hydration) has a very large effect on miscibility transition temperatures, on the order of 10 ˚C (compare point C to F, B and E, or point J to L). This effect of preparation method is larger than of adding monovalent salt (compare point B to point C, E to G, or J to K), and even than of adding divalent cations (compare point D to point G). Similarly, the effect of preparation method is larger than of replacing PC-lipids with PG-lipids (compare point H to point J, or A to C).

The figure above shows that we reproduce the general results of Vequi-Suplicy et al. because our data point F is within experimental uncertainty of point G from Vequi-Suplicy et al. Taken together, the data in the figure imply that the stunning, >20 ˚C drop in miscibility transition temperatures reported by Vequi-Suplicy et al. (from point A to point G) is valid, and is mostly attributable to the method of preparing charged vesicles by electroformation in sucrose vs. by gentle hydration in water (compare points C and G). Although a mechanistic explanation is beyond the scope of this study, a conclusion that is consistent with the results above is that sucrose interacts much more significantly with PG headgroups than with PC headgroups (compare points H and I).

Phospholipid Ratio	
Ion Polarity	Negative
Ion Source Type	ESI
Capillary	2.60 kV
Cone	25.00 V
Extractor	3.00 V
RF Lens	0.2 V
Source Temperature	120 °C
Desolvation Temperature	400 °C
Cone Gas Flow	25 L/Hr
Desolvation Gas Flow	900 L/Hr
LM Resolution	14.0
HM Resolution	14.0
Ion Energy 1	1.0
Entrance	-2
Collision	2
Exit	1
LM 2 Resolution	15.0
HM 2 Resolution	15.0
Ion Energy 2	2.5
Multiplier	650
Syringe Pump Flow	20 µL/min

Cholesterol Ratio:	
Ion Polarity	Positive
Ion Source Type	ESI
Trap Drive	45.0
Octopole RF Amplitude	100.0 V
Lens 2	-60.0 V
Capillary Exit	105.0 V
Lens 1	-5.0 V
Dry Temp	250 °C
Nebulizer	9.00 psi
Dry Gas	5.00 L/min
HV Capillary	4000 V
HV End Plate Offset	-500 V
Fragmentation Width	10.00 m/z
Fragmentation Time	40000 µs
Fragmentation Delay	5000 µs

Table S1
Instrument parameters for mass spectrometry. Parameters are specific to the instrument used.

Supporting References

1. Vequi-Suplicy, C. C., K. A. Riske, R. L. Knorr, and R. Dimova. 2010. Vesicles with charged domains. Biochim. Biophys. Acta, Biomembr. 1798:1338-1347.