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Thermotropic Liquid Crystal Droplets Stabilized by Nanoparticles for the Optical Detection of Phospholipid Membranes: Impact of Membrane Composition on LC Ordering Transitions

Maria K. Oñate-Socarras,¹ Oscar H. Piñeres-Quiñones,¹ Lawrence M. Chen,² Sean P. Palecek,² David M. Lynn,^{2,3,*} and Claribel Acevedo-Vélez^{1,*}

¹*Department of Chemical Engineering, University of Puerto Rico-Mayagüez, Call Box 9000, Mayagüez, PR 00681-9000;* ²*Department of Chemical and Biological Engineering, 1415 Engineering Drive, University of Wisconsin-Madison, Madison, WI 53706, USA;* ³*Department of Chemistry, 1101 University Avenue, University of Wisconsin-Madison, Madison, WI 53706, USA*
Email: (D.M.L.) dlynn@engr.wisc.edu; (C.A.V.) claribel.acevedo@upr.edu

Results

Characterization of phospholipid vesicles

NMR quantification of phospholipid concentration in vesicle dispersions

Stock solutions of the phospholipids DLPC, DOPC, and DOPE were prepared at a total concentration of 5 mM in DIW, while solutions of DOPG were prepared at 8 mM. SUVs were prepared as described above. Two samples were analyzed for each phospholipid, labeled as Sample 1 or Sample 2 in **Table S1**. ³¹P[¹H] NMR data is shown in **Table S1** and **Figure S1** to **Figure S4**.

Stock solutions of mixed lipids (DLPC:DOPC, DLPC:DOPE or DLPC:DOPG) were prepared at a total concentration of 5 mM, with increasing amounts of DLPC to obtain three different lipid compositions (30:70, 40:60, or 50:50 by weight). SUVs were prepared as described above. Two samples were analyzed for each phospholipid combination, labeled as Sample 1 or Sample 2 in **Table S2**. ³¹P[¹H] NMR data is shown in **Table S2** and **Figure S5** to **Figure S13**. We note that, because DLPC and DOPC have the same type of headgroup, ³¹P[¹H] NMR cannot distinguish between these two phospholipids and, therefore, the composition of each lipid in DLPC:DOPC combinations could not be determined, as shown in **Table S2**.

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Table S1. Determination of lipid concentration in single-component vesicles from NMR data.

Lipid	Sample #	TPO Standard (mM)	NMR Measurement (ppm)	NMR Conc. (mM)	NMR Conc. Lipid (mg/mL)	Calculated Conc. (mM)	Calculated Conc. (mg/mL)	Average (mg/mL)
DLPC	1	10	0.18	188	1.119	4.045	2.515	2.655
DLPC	2	10	0.2	2.0	1.244	4.494	2.795	
DOPC	1	10	0.11	1.1	0.865	2.472	1.943	3.003
DOPC	2	10	0.23	2.3	1.808	5.169	4.063	
DOPE	1	10	0.07	0.7	0.521	1.573	1.170	0.752
DOPE	2	10	0.02	0.2	0.149	0.449	0.334	
DOPG	1	10	0.23	2.3	1.833	5.169	4.119	3.940
DOPG	2	10	0.21	2.1	1.674	4.719	3.761	

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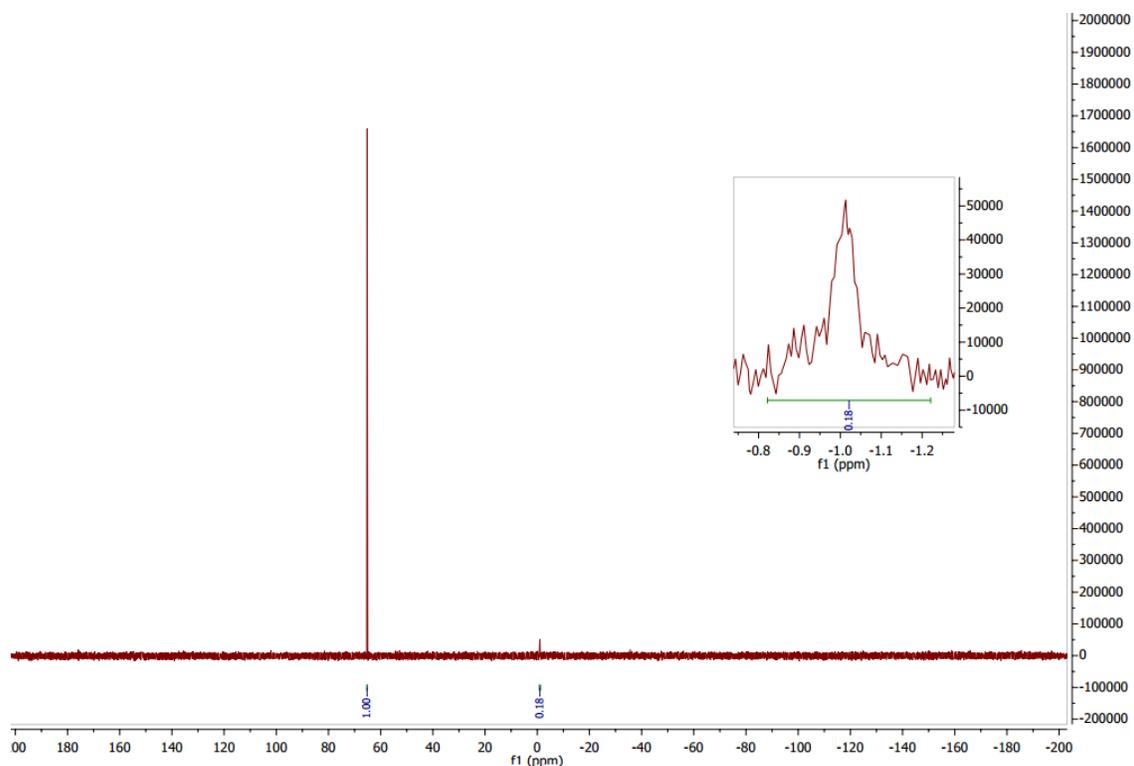


Figure S1. Representative ^{31}P [^1H] NMR spectrum of DLPC vesicles.

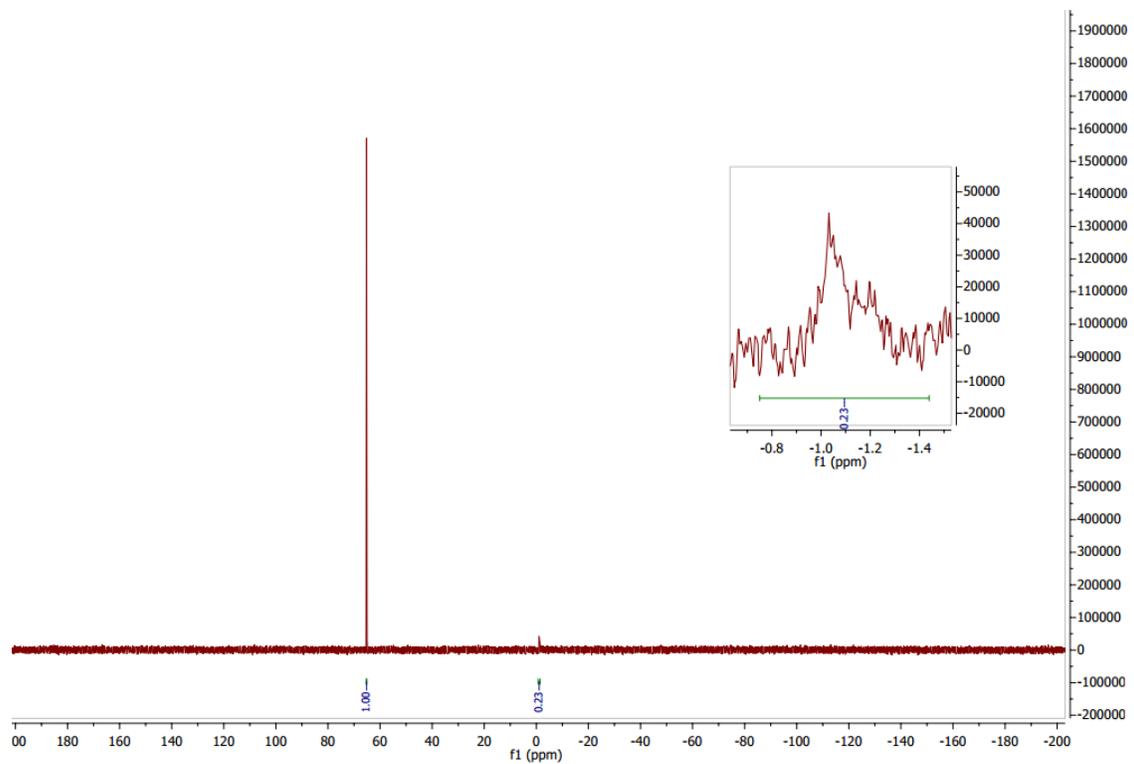


Figure S2. Representative ^{31}P [^1H] NMR spectrum of DOPC vesicles.

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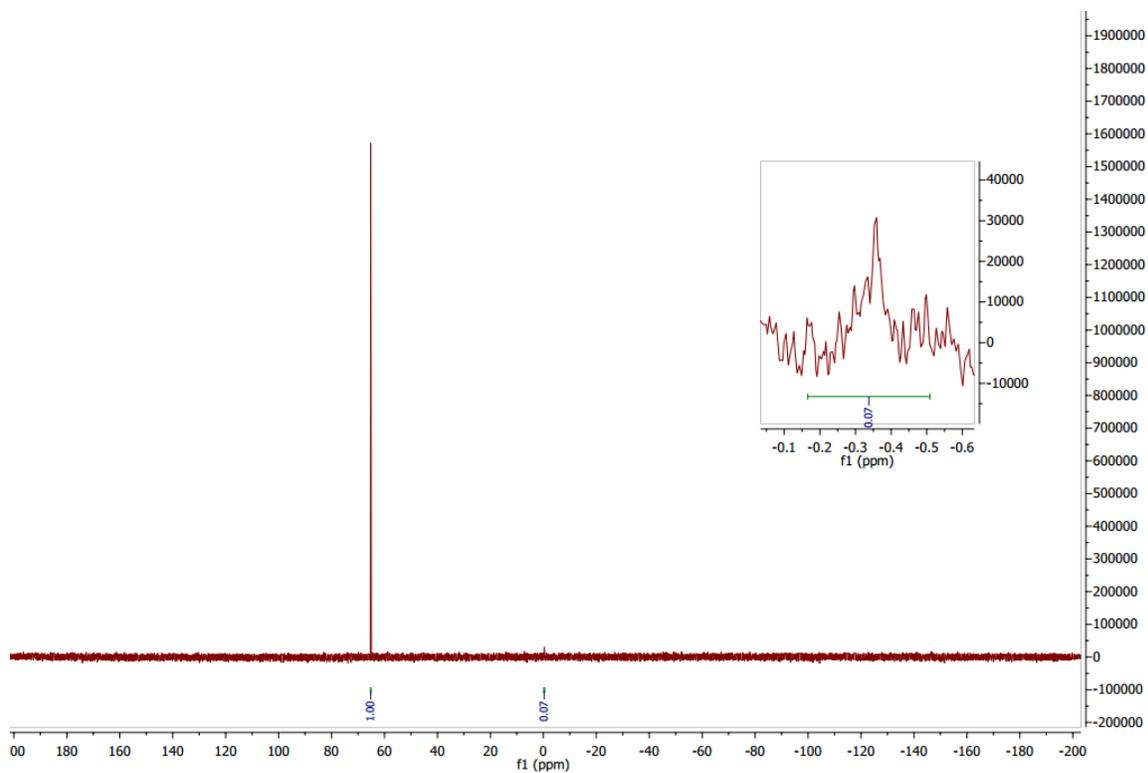


Figure S3. Representative $^{31}\text{P}[^1\text{H}]$ NMR spectrum of DOPE vesicles.

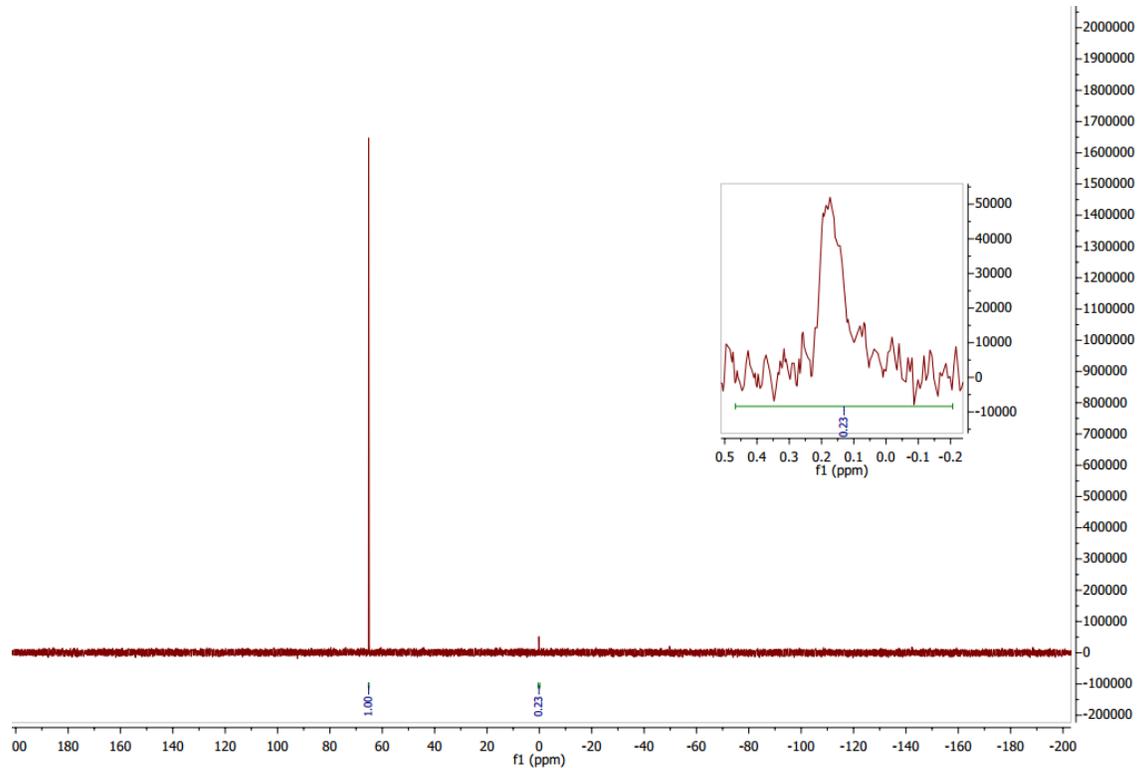


Figure S4. Representative $^{31}\text{P}[^1\text{H}]$ NMR spectrum of DOPE vesicles.

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Table S2. Determination of lipid concentration in two-component vesicles from NMR data.

Lipid Composition		Sample #	TPO Standard (mM)	Lipid 1 NMR Peak (ppm)	Lipid 1 NMR Conc. (mM)	Lipid 1 NMR Conc. (mg/mL)	Lipid 2 NMR Peak (ppm)	Lipid 2 NMR Conc. (mM)	Lipid 2 NMR Conc. (mg/mL)	Total Lipid Conc. (mM)	Total Lipid Conc. (mg/mL)	Average Conc. (mg/mL)	Composition		Average Composition	
50:50	DLPC:DOPC	1	10	0.27	2.7	1.679	0	0	0	6.067	4.271	4.429	-	-	-	-
50:50	DLPC:DOPC	2	10	0.29	2.9	1.803	0	0	0	6.517	4.588		-	-	-	-
50:50	DLPC:DOPE	1	10	0.13	1.3	0.808	0.14	1.400	1.042	6.067	4.144	4.543	48%	52%	48%	52%
50:50	DLPC:DOPE	2	10	0.15	1.5	0.933	0.16	1.600	1.190	6.966	4.942		48%	52%		
50:50	DLPC:DOPG	1	10	0.13	1.3	0.808	0.17	1.700	1.355	6.742	4.783	4.862	43%	57%	43%	57%
50:50	DLPC:DOPG	2	10	0.13	1.3	0.808	0.18	1.800	1.435	6.966	4.942		42%	58%		
40:60	DLPC:DOPC	1	10	0.27	2.7	1.679	0	0	0	6.067	4.371	4.290	-	-	-	-
40:60	DLPC:DOPC	2	10	0.26	2.6	1.617	0	0	0	5.843	4.209		-	-	-	-
40:60	DLPC:DOPE	1	10	0.11	1.1	0.684	0.13	1.300	0.967	5.393	3.749	4.062	46%	54%	43%	57%
40:60	DLPC:DOPE	2	10	0.11	1.1	0.684	0.17	1.700	1.265	6.292	4.374		39%	61%		
40:60	DLPC:DOPG	1	10	0.12	1.2	0.746	0.2	2.000	1.594	7.191	5.228	5.309	38%	63%	34%	66%
40:60	DLPC:DOPG	2	10	0.10	1.0	0.622	0.23	2.300	1.833	7.416	5.391		30%	70%		
30:70	DLPC:DOPC	1	10	0.24	2.4	1.492	0	0	0	5.393	3.974	3.477	-	-	-	-
30:70	DLPC:DOPC	2	10	0.18	1.8	1.119	0	0	0	4.045	2.980		-	-	-	-
30:70	DLPC:DOPE	1	10	0.08	0.8	0.497	0.17	1.700	1.265	5.618	3.974	4.053	32%	68%	29%	71%
30:70	DLPC:DOPE	2	10	0.07	0.7	0.435	0.19	1.900	1.414	5.843	4.133		27%	73%		
30:70	DLPC:DOPG	1	10	0.08	0.8	0.497	0.21	2.100	1.674	6.517	4.852	5.103	28%	72%	28%	72%
30:70	DLPC:DOPG	2	10	0.09	0.9	0.560	0.23	2.300	1.833	7.191	5.353		28%	72%		

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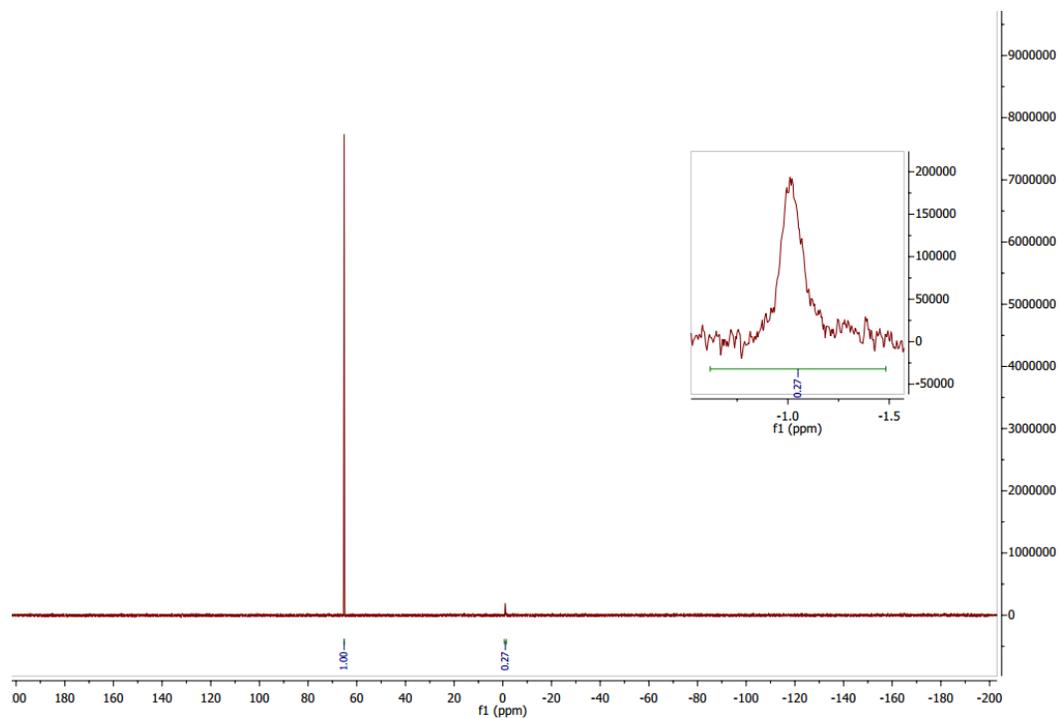


Figure S5. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPC vesicles at a 50:50 composition.

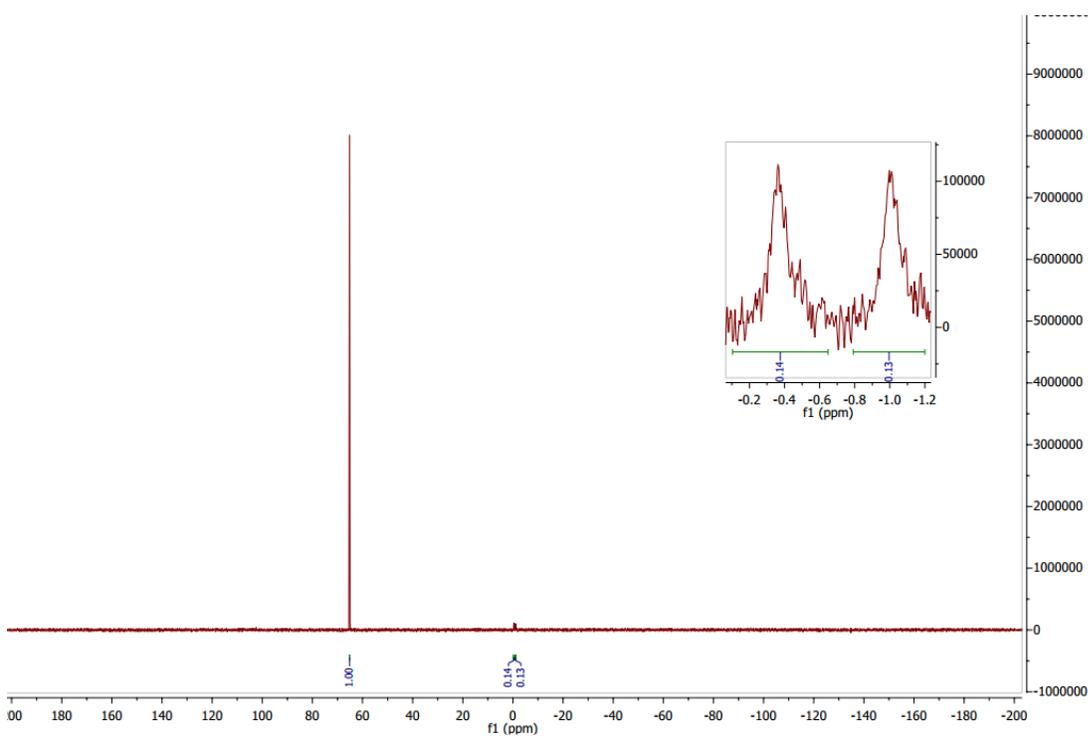


Figure S6. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPE vesicles at a 50:50 composition.

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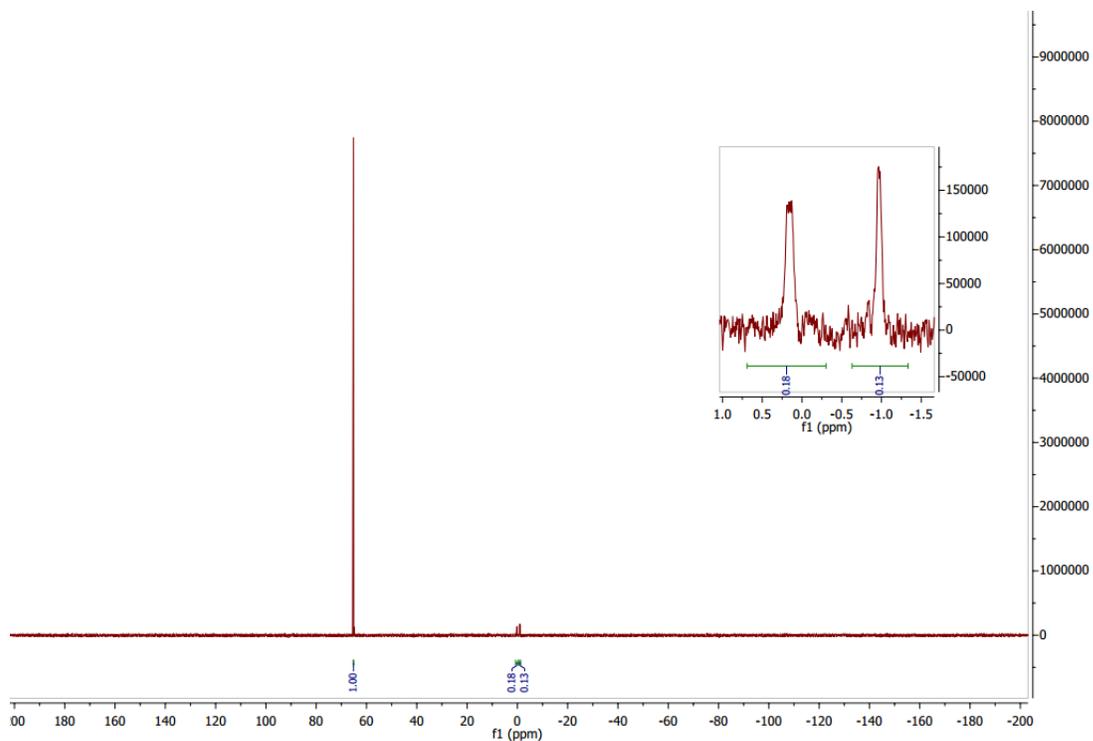


Figure S7. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPG vesicles at a 50:50 composition.

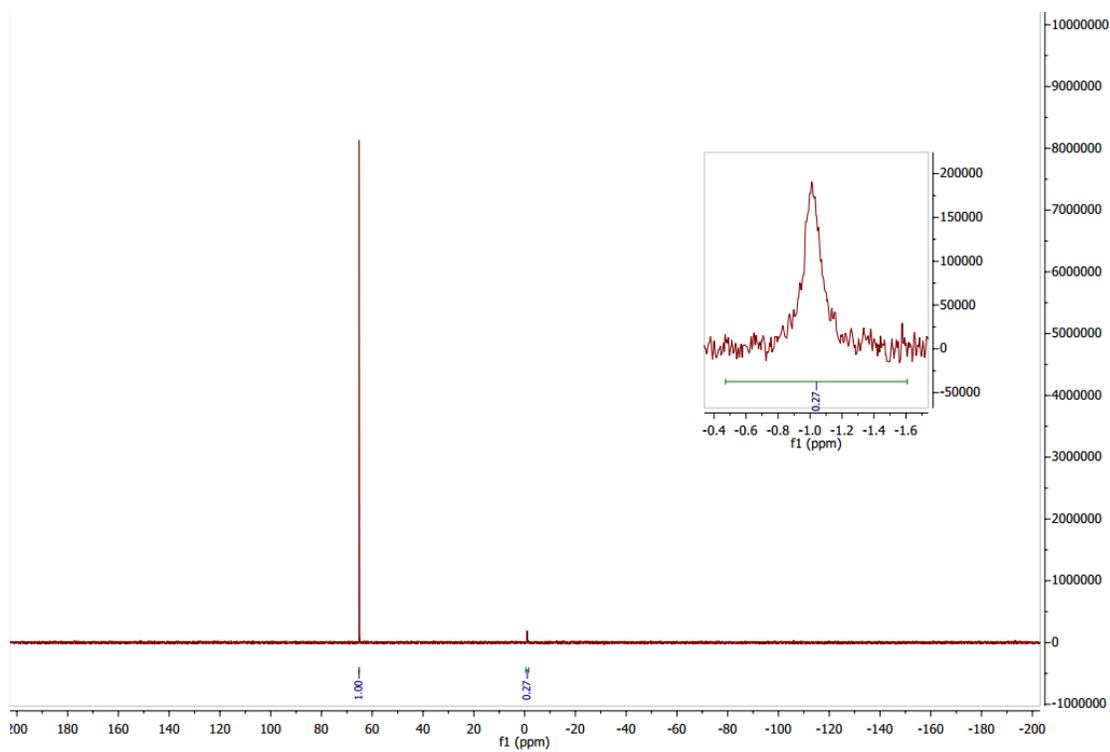


Figure S8. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPC vesicles at a 40:60 composition.

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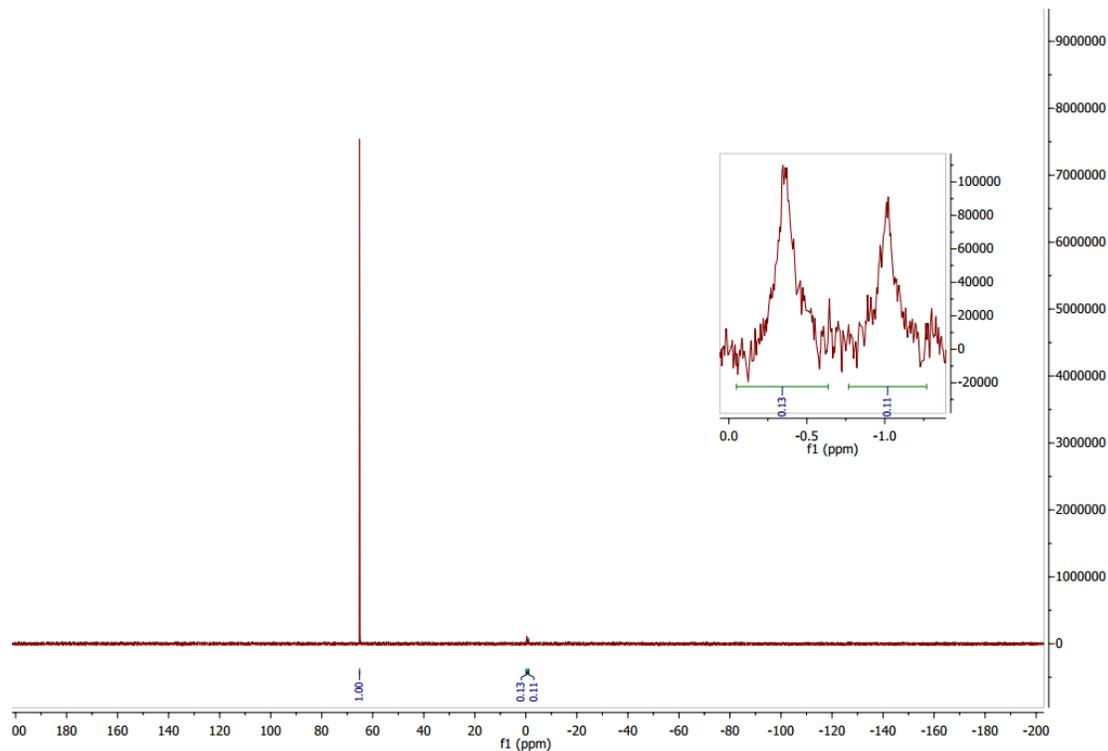


Figure S9. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPE vesicles at a 40:60 composition.

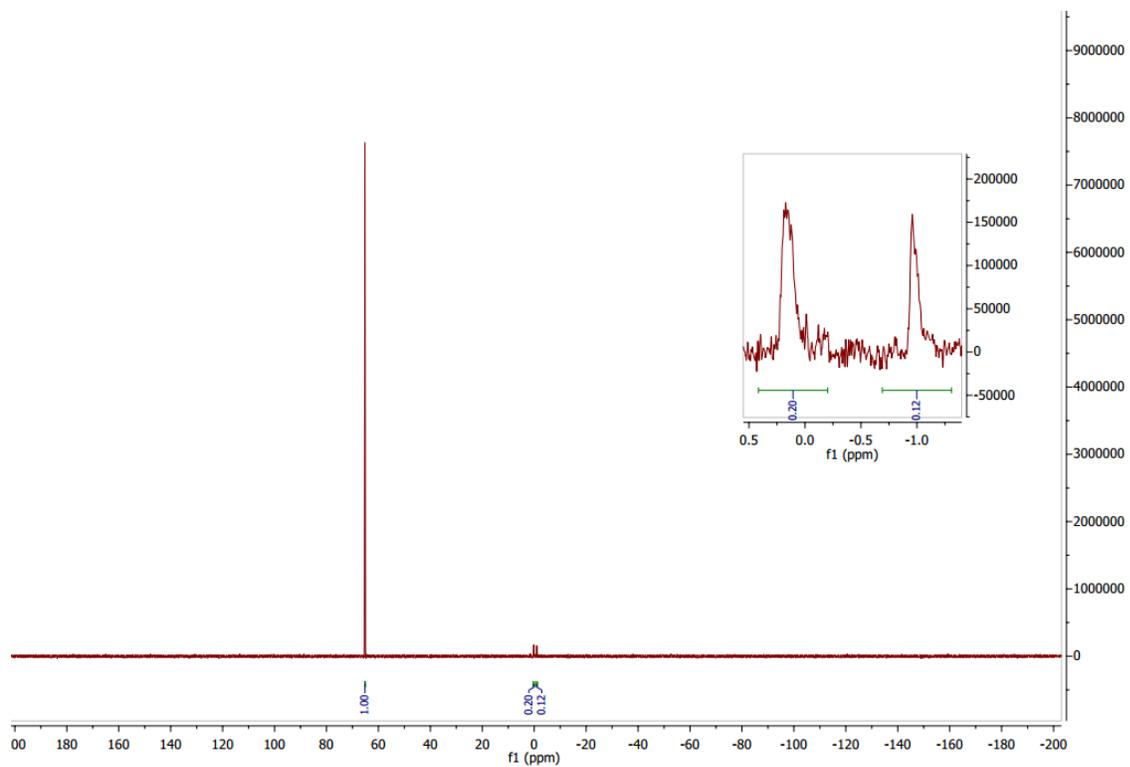


Figure S10. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPG vesicles at a 40:60 composition.

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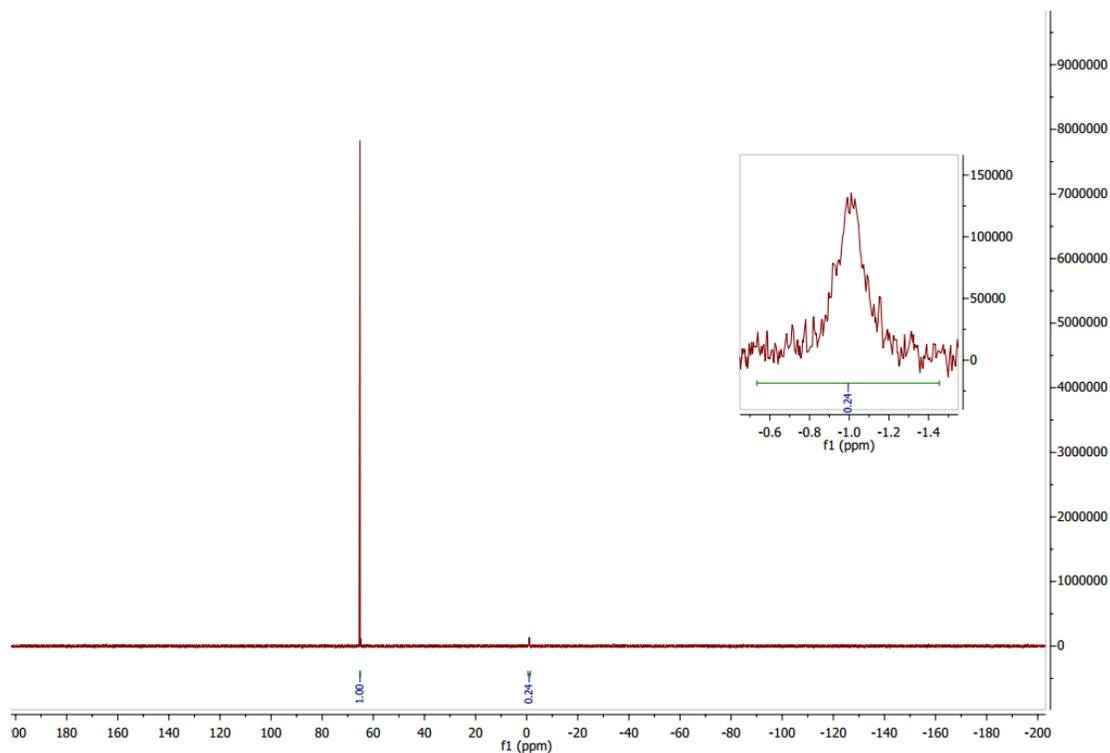


Figure S11. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPC vesicles at a 30:70 composition.

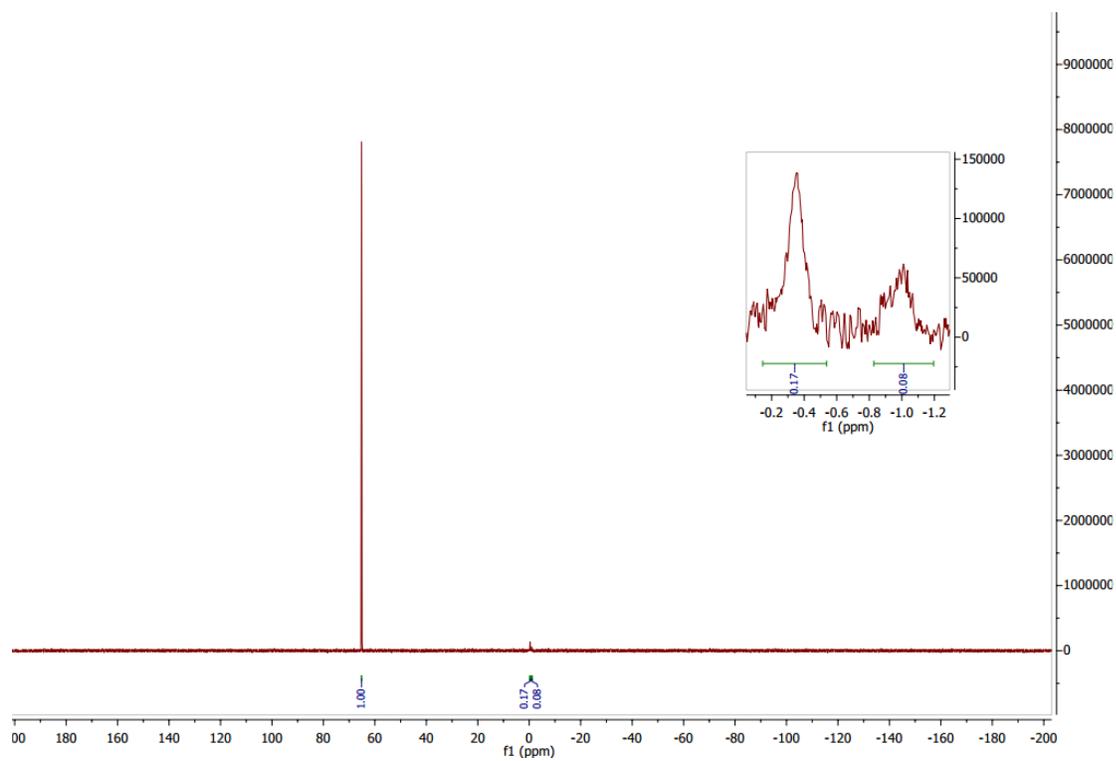


Figure S12. Representative ^{31}P [^1H] NMR spectrum of DLPC:DOPE vesicles at a 30:70 composition.

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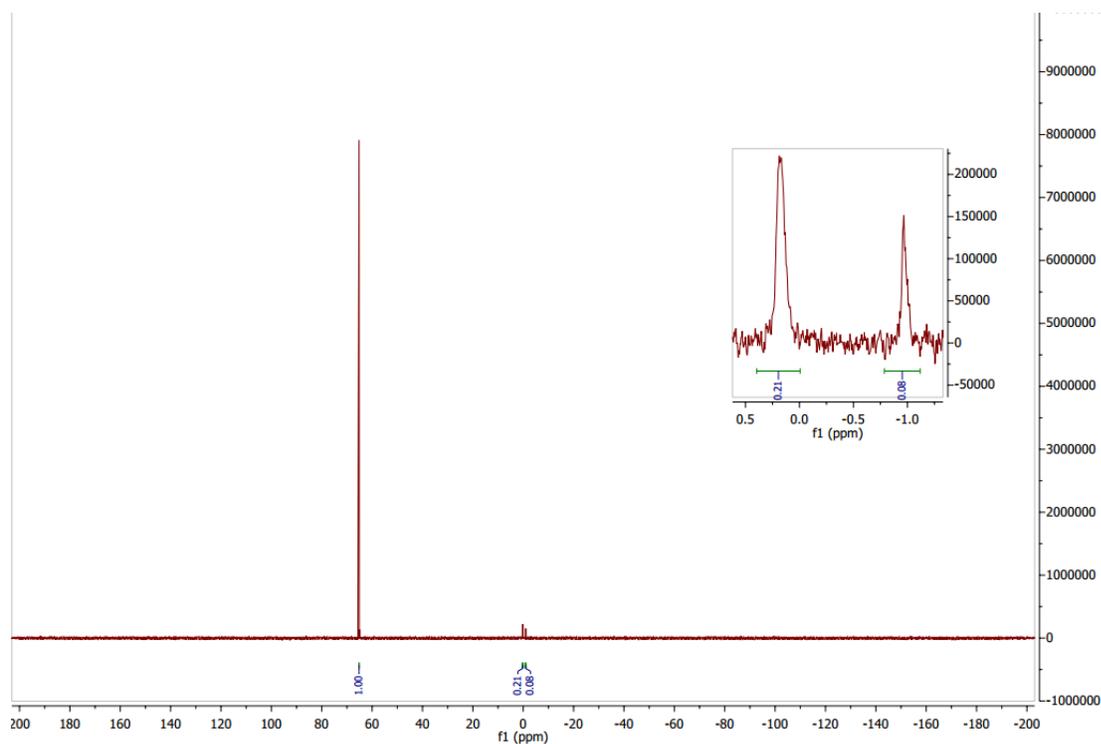


Figure S13. Representative $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of DLPC:DOPG vesicles at a 30:70 composition.

Characterization of vesicle size using DLS measurements

Table S3. Hydrodynamic diameter (nm) of single-component phospholipid vesicles. The representative values reported in the table correspond to three measurements using the same sample. The experiment was conducted using triplicate samples.

Phospholipid	Effective Diameter (nm)	Polydispersity	Mean (nm)	Standard Deviation
DLPC	120.5	0.12	119.9	0.6
	119.3	0.13		
	119.9	0.12		
DOPC	158.2	0.15	157.4	1.2
	158.1	0.16		
	156.1	0.15		
DOPE	145.2	0.15	144.6	1.3
	143.1	0.20		
	145.6	0.17		
DOPG	101.6	0.28	101.1	1.6
	102.4	0.26		
	99.3	0.82		

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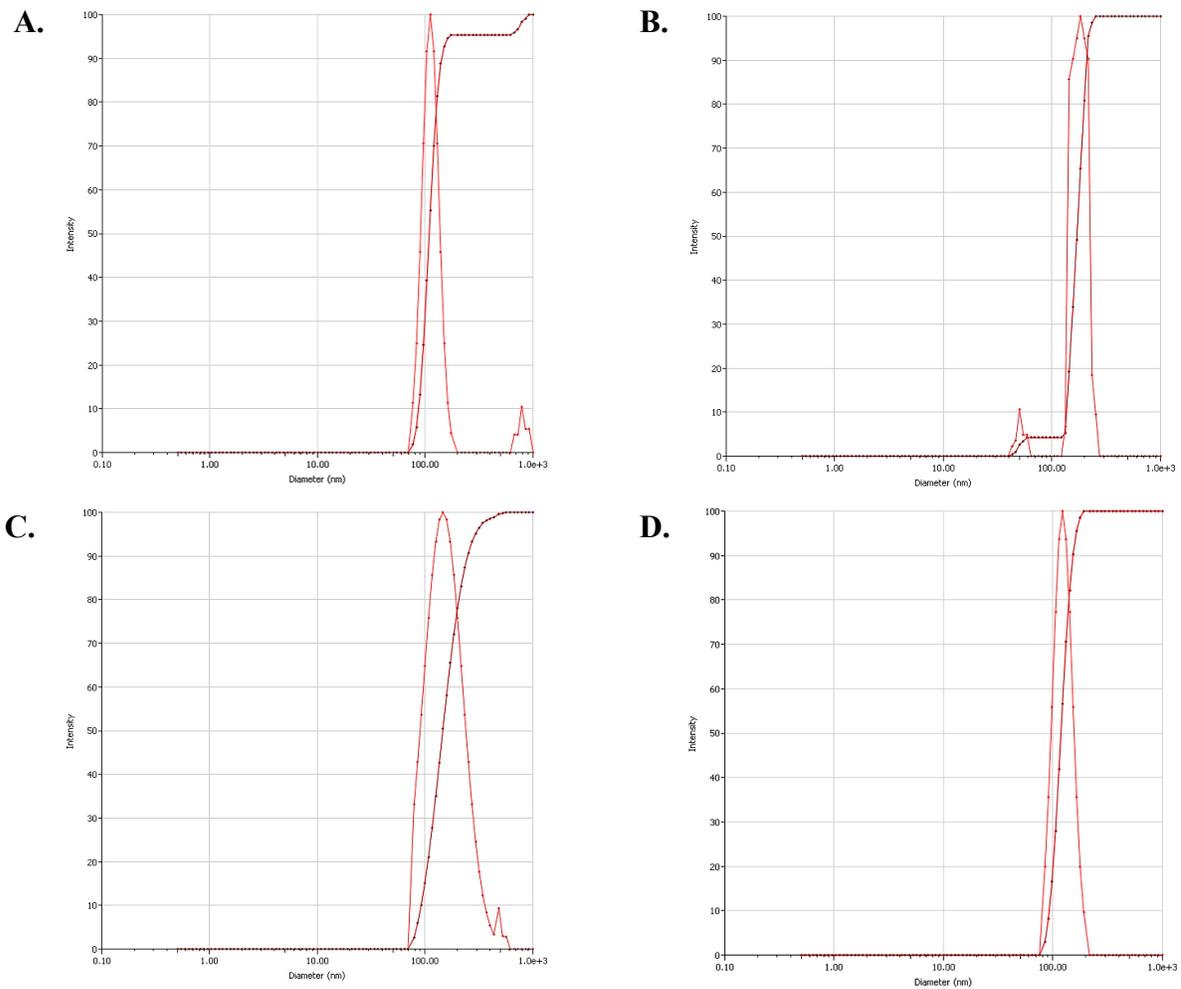


Figure S14. Representative size distribution curves obtained using DLS measurements for single-component vesicles of (A) DLPC, (B) DOPC, (C) DOPE, and (D) DOPG phospholipids.

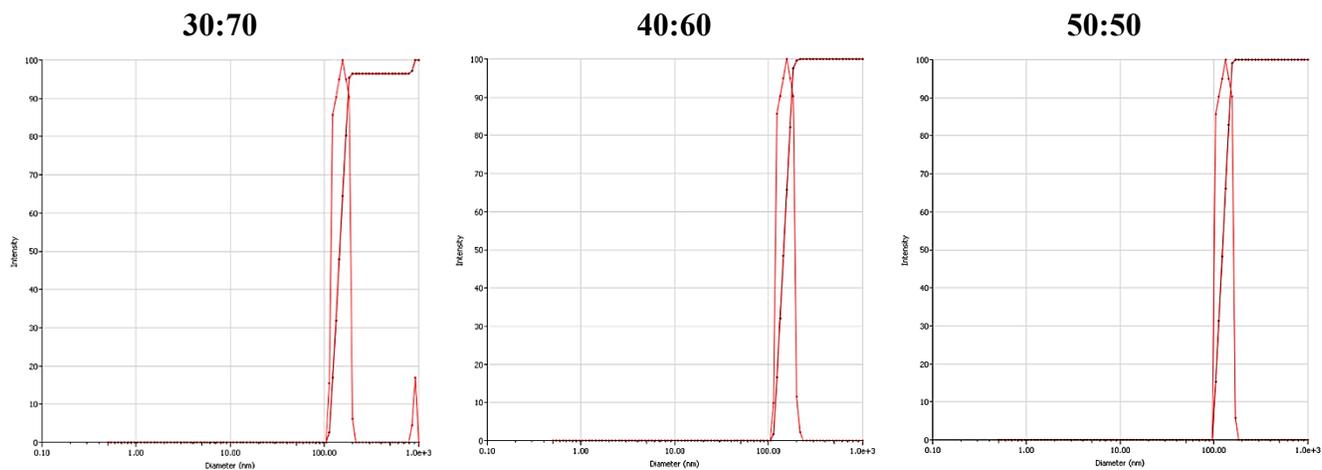
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Table S4. Hydrodynamic diameter (nm) of two-component phospholipid vesicles. The representative values reported in the table correspond to three measurements using the same sample. The experiment was conducted using triplicate samples.

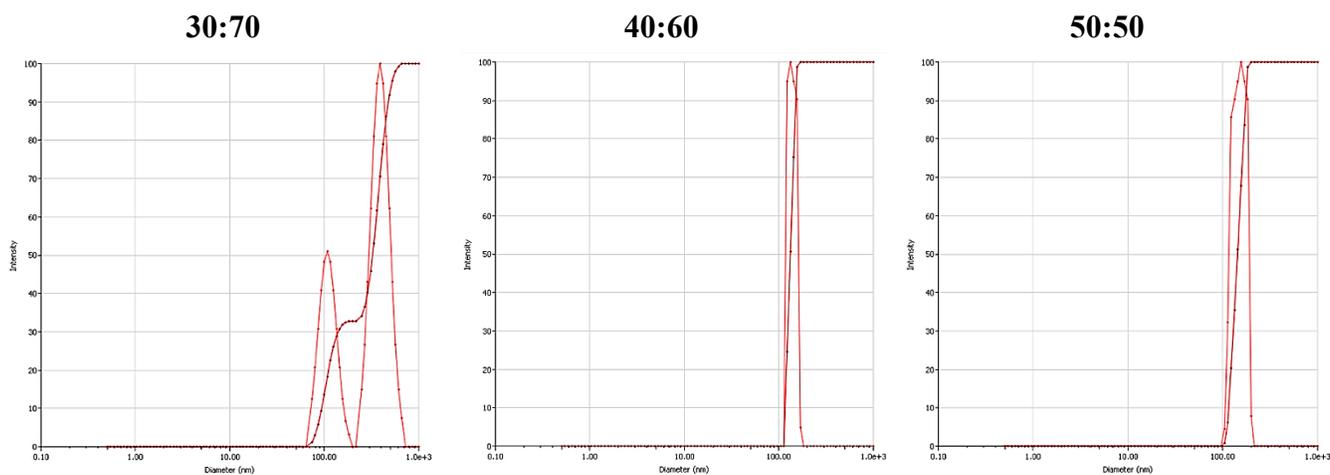
Phospholipid Composition		Effective Diameter (nm)	Polydispersity	Mean (nm)	Standard Deviation
DLPC:DOPC	30:70	159.1	0.18	159.5	0.91
		160.6	0.12		
		158.9	0.14		
	40:60	146.2	0.12	145.2	1.23
		145.5	0.13		
		143.8	0.13		
	50:50	132.7	0.13	132.3	0.48
		131.7	0.12		
		132.4	0.11		
DLPC:DOPE	30:70	228.1	0.28	230.0	2.19
		232.4	0.26		
		229.5	0.27		
	40:60	133.5	0.09	133.7	1.08
		134.9	0.09		
		132.7	0.07		
	50:50	143.5	0.14	143.6	0.13
		143.6	0.13		
		143.8	0.09		
DLPC:DOPG	30:70	161.6	0.24	161.8	0.26
		162.8	0.26		
		160.9	0.28		
	40:60	167.2	0.28	165.6	0.278
		163.9	0.28		
		165.8	0.28		
	50:50	158.5	0.24	158.0	0.23
		158.7	0.21		
		156.7	0.25		

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A. DLPC:DOPC



B. DLPC:DOPE



C. DLPC:DOPG

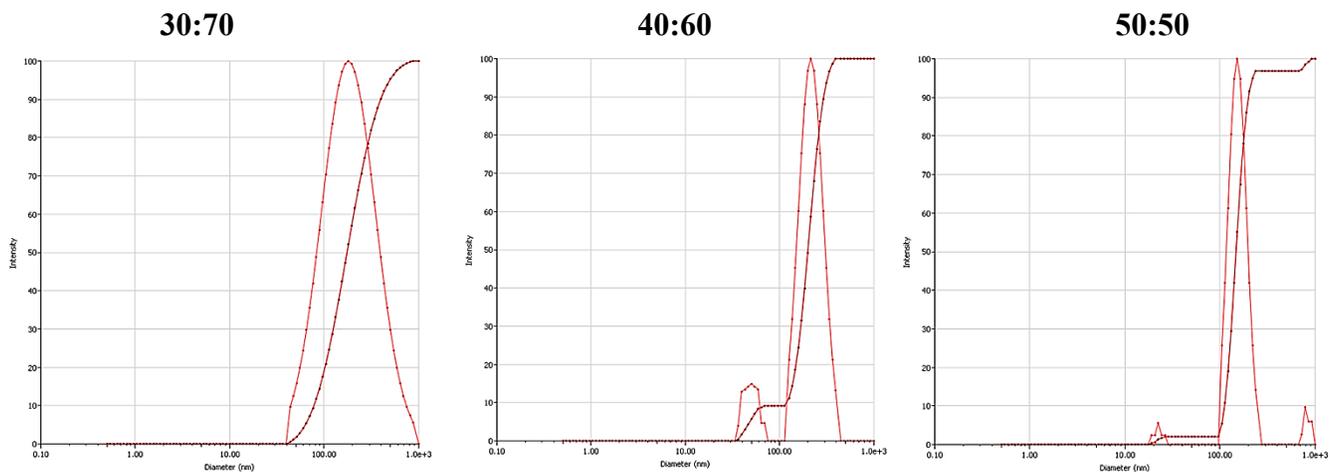


Figure S15. Representative size distribution curves obtained using DLS measurements for two-component vesicles of (A) DLPC:DOPC, (B) DLPC:DOPE, and (C) DLPC:DOPG.

Optical characterization of the LC ordering transitions induced by phospholipid vesicles

We define a transition from a “bipolar” to a “non-bipolar” state as the concentration of lipid at which at least 50% of the droplets analyzed in a sample are no longer observed to be in their initial bipolar configuration (**Figure S16**; see also additional discussion in the main text). The designation “B” and “NB” represent bipolar and non-bipolar configurations, respectively. “NB” represents the condition for which at least 50% of the analyzed LC droplet population observed in a sample exhibited a mixture of bipolar, pre-radial, escaped radial, and radial configurations. The results presented here correspond to samples analyzed on the same day of LC emulsion preparation, for at least 100 droplets.

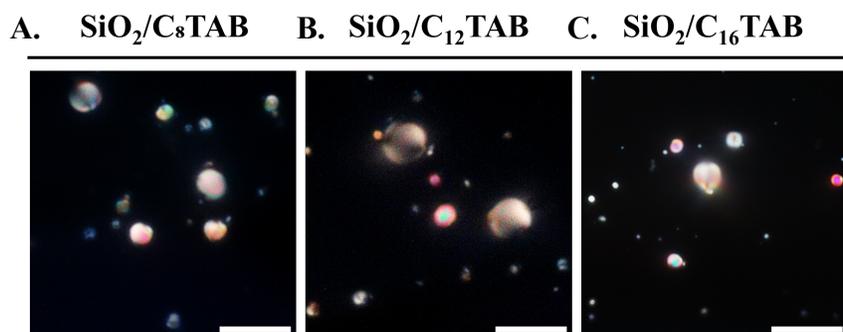


Figure S16. Representative polarized light micrographs showing the “bipolar” configuration adopted by LC droplets stabilized using SiO₂/C_nTAB complexes with **(A)** C₈TAB, **(B)** C₁₂TAB, and **(C)** C₁₆TAB when dispersed in water. Scale bars on the images represent a length of 20 μm.

Table S5. Configurations adopted by SiO₂/C_nTAB-stabilized LC droplets when exposed to DLPC vesicles at increasing total lipid concentration. The designation “B” and “NB” represent bipolar and non-bipolar configurations, respectively.

DLPC											
	SiO₂		SiO₂/C₈TAB			SiO₂/C₁₂TAB			SiO₂/C₁₆TAB		
Lipid Concentration (μg/mL)	12.4	31.1	15.5	31.1	62.2	6.21	24.5	31.1	3.1	18.7	31.1
LC Configuration	B	NB	B	NB	NB	B	NB	NB	B	NB	NB

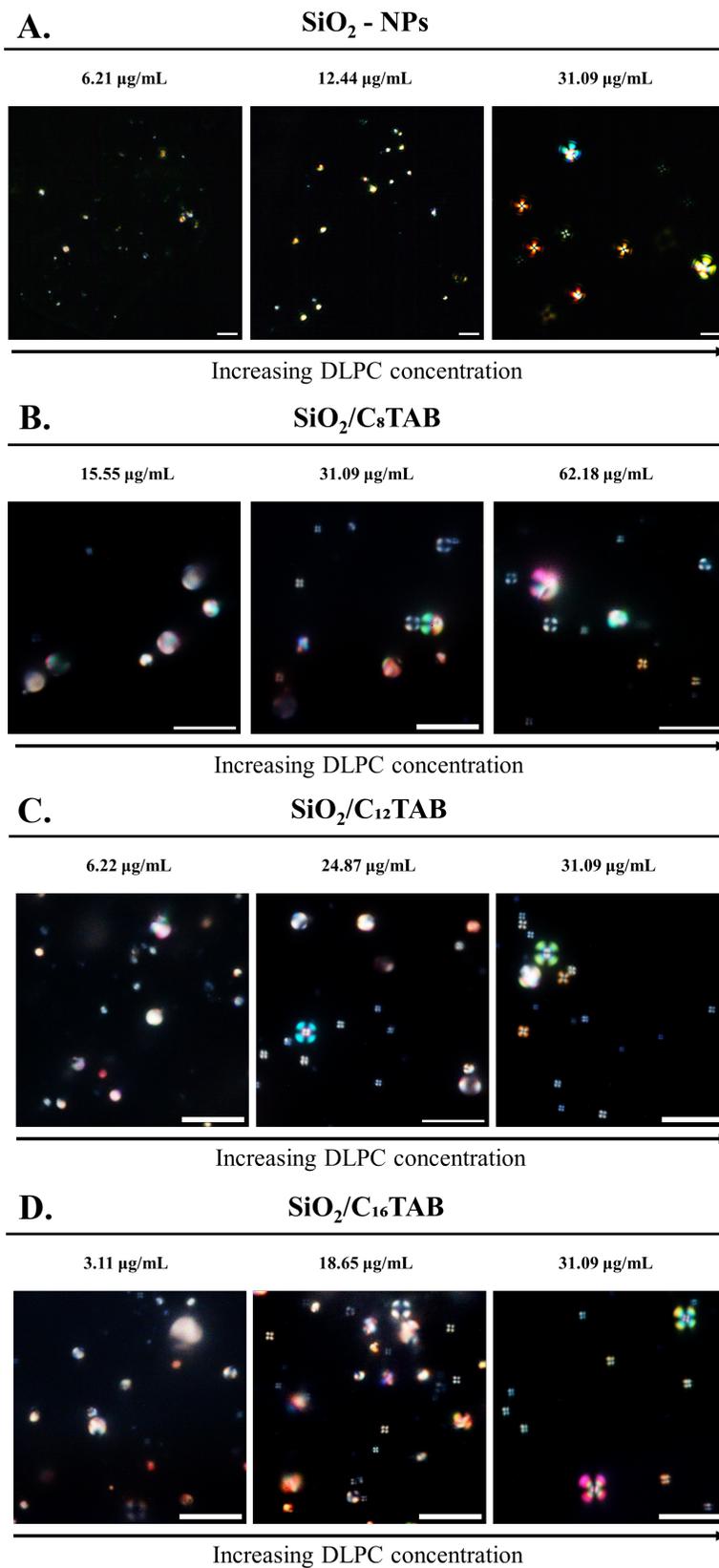


Figure S17. Representative optical micrographs showing the “bipolar” to “non-bipolar” LC ordering transitions in LC droplets stabilized using (A) SiO_2 - NPs and $\text{SiO}_2/\text{C}_n\text{TAB}$ complexes with (B) C_8TAB , (C) C_{12}TAB , and (D) C_{16}TAB upon exposure to DLPC vesicles. The scale bars correspond to 20 μm .

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Table S6. Configurations adopted by SiO₂/C₁₆TAB-stabilized LC droplets when exposed to DOPC, DOPE, or DOPG vesicles at increasing total lipid concentration. The designation “B” and “NB” represent bipolar and non-bipolar configurations, respectively.

Phospholipid	DOPC			DOPE			DOPG		
Concentration (µg/mL)	628.9	786.1	2,358.5	744.03	1,488.1	2,232.1	797.03	1,992.6	6,376.2
LC Configuration	B	NB	NB	B	B	B	B	B	B

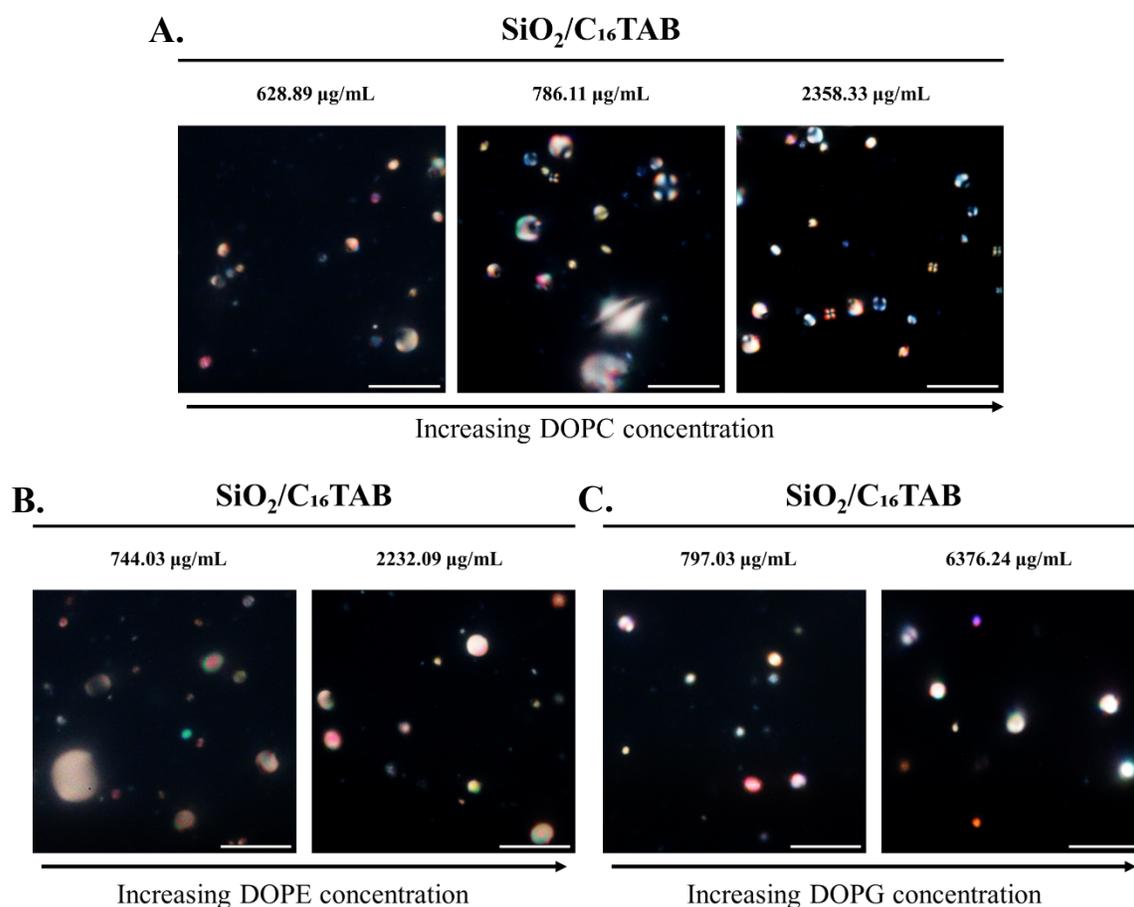


Figure S18. Representative optical micrographs showing the configurations adopted by LC droplets stabilized using SiO₂/C₁₆TAB complexes upon exposure (A) DOPC, (B) DOPE, and (C) DOPG vesicles. The scale bar corresponds to 20 µm.

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Table S7. Configurations adopted by SiO₂/C₁₆TAB-stabilized LC droplets when exposed to DLPC:DOPC vesicles at different lipid compositions and increasing total lipid concentrations. The designation “B” and “NB” represent bipolar and non-bipolar configurations, respectively.

DLPC:DOPC						
Lipid Composition	30:70		40:60		50:50	
Total Lipid Concentration (μg/mL)	200	300	100	200	150	200
LC Configuration	B	NB	B	NB	B	NB

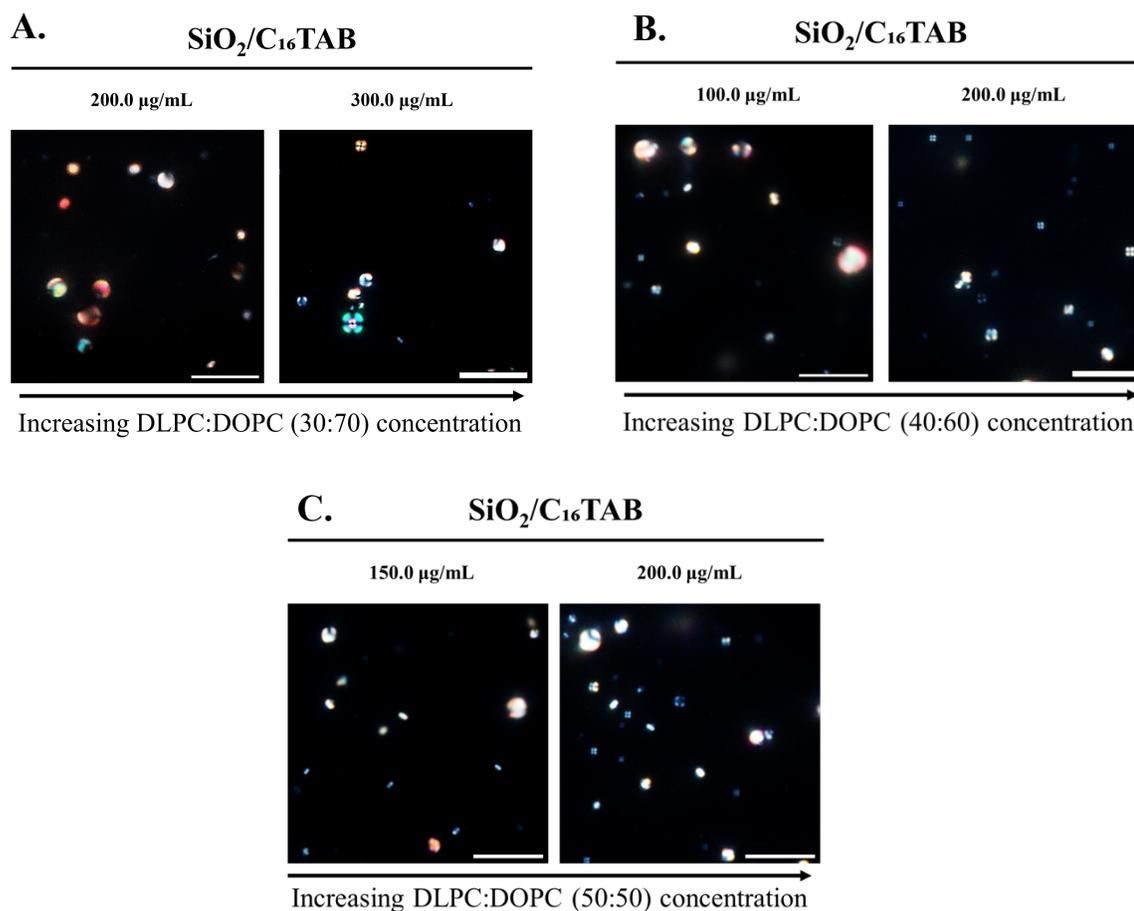


Figure S19. Representative optical micrographs showing the configurations adopted by LC droplets stabilized using SiO₂/C₁₆TAB complexes upon exposure to DLPC:DOPC vesicles at (A) 30:70, (B) 40:60, and (C) 50:50 compositions. The scale bar corresponds to 20 μm.

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Table S8. Configurations adopted by SiO₂/C₁₆TAB-stabilized LC droplets when exposed to DLPC:DOPE vesicles at different lipid compositions and increasing total lipid concentrations. The designation “B” and “NB” represent bipolar and non-bipolar configurations, respectively.

DLPC:DOPE									
Lipid Composition	30:70			40:60			50:50		
Total Lipid Concentration (μg/mL)	120	150	200	50	100	200	50	100	120
LC Configuration	NB	NB	NB	B	NB	NB	B	NB	NB

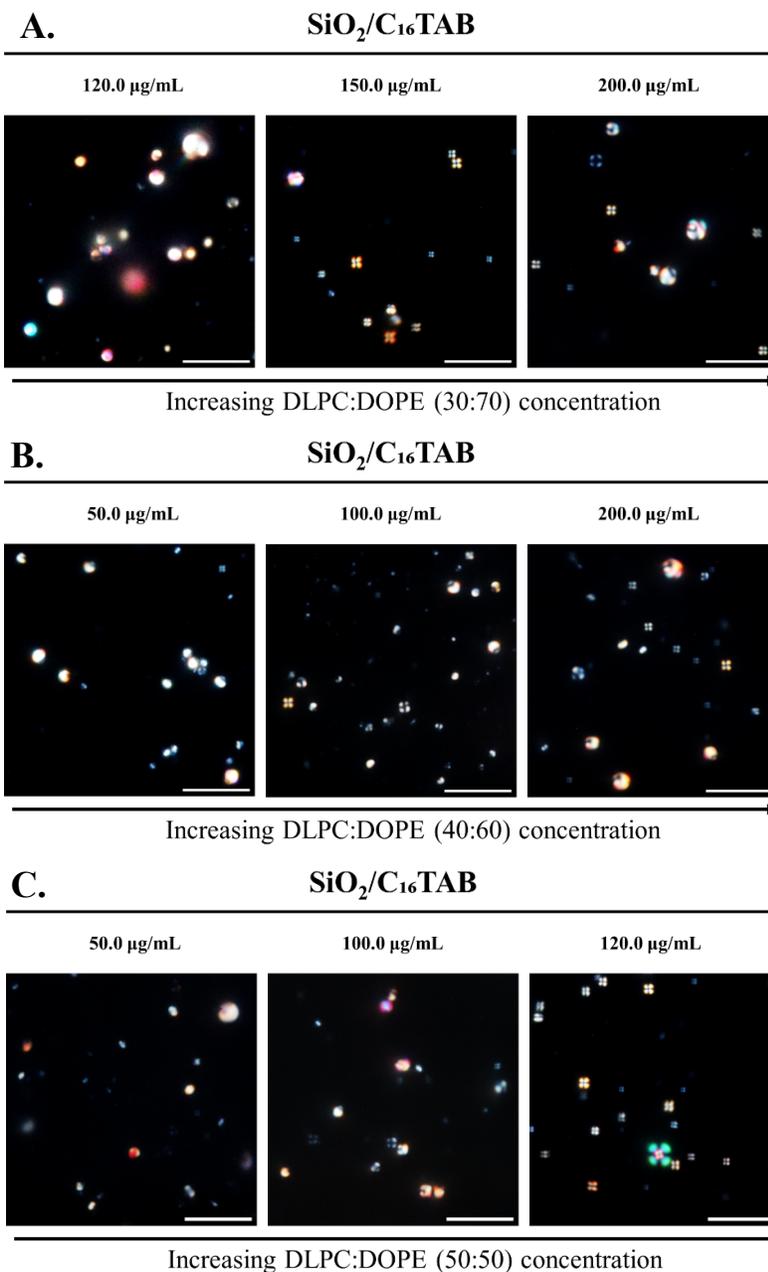


Figure S20. Representative optical micrographs showing the configurations adopted by LC droplets stabilized using SiO₂/C₁₆TAB complexes upon exposure to DLPC:DOPE vesicles at (A) 30:70, (B) 40:60, and (C) 50:50 compositions. The scale bar corresponds to 20 μm.

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Table S9. Configurations adopted by SiO₂/C₁₆TAB-stabilized LC droplets when exposed to DLPC:DOPG vesicles at different lipid compositions and increasing total lipid concentrations. The designation “B” and “NB” represent bipolar and non-bipolar configurations, respectively.

DLPC:DOPG									
Lipid Composition	30:70			40:60			50:50		
Total Lipid Concentration (μg/mL)	120	200	300	50	100	200	100	120	200
LC Configuration	B	NB	NB	B	NB	NB	B	NB	NB

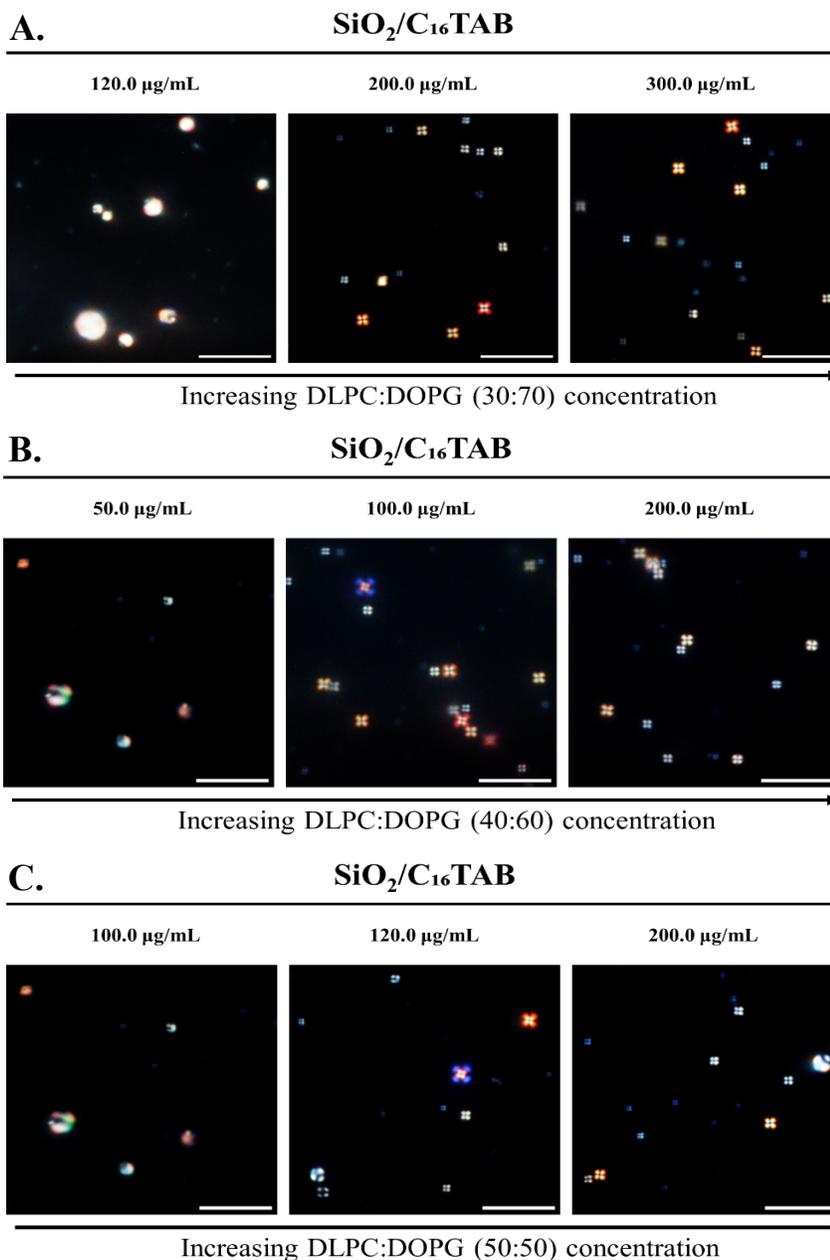


Figure S21. Representative optical micrographs showing the configurations adopted by LC droplets stabilized using SiO₂/C₁₆TAB complexes upon exposure to DLPC:DOPE vesicles at (A) 30:70, (B) 40:60, and (C) 50:50 compositions. The scale bar corresponds to 20 μm

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Table S10. Percentage of LC droplets with an observable “bipolar” to “non-bipolar” optical transition in two-component vesicle systems at different compositions, reported at the lowest total lipid concentration that triggered this transition ($\mu\text{g/mL}$, shown in the parentheses). These results represent observations of at least 100 droplets.

Lipid Composition	30:70	40:60	50:50
DLPC:DOPC	61% (300)	65% (200)	65% (200)
DLPC:DOPE	68% (150)	65% (100)	73% (100)
DLPC:DOPG	73% (200)	98% (100)	65% (120)

Table S11. Configurations adopted by $\text{SiO}_2/\text{C}_{16}\text{TAB}$ -stabilized LC droplets when exposed to two-component vesicle systems that do not include DLPC. The designation “B” represents the bipolar configuration.

Vesicle System	DOPE:DOPC	DOPG:DOPC	DOPG:DOPE
Lipid Composition	40:60	40:60	40:60
Total Lipid Concentration ($\mu\text{g/mL}$)	3,845.4	1,517.7	1,274.6
LC Configuration	B	B	B

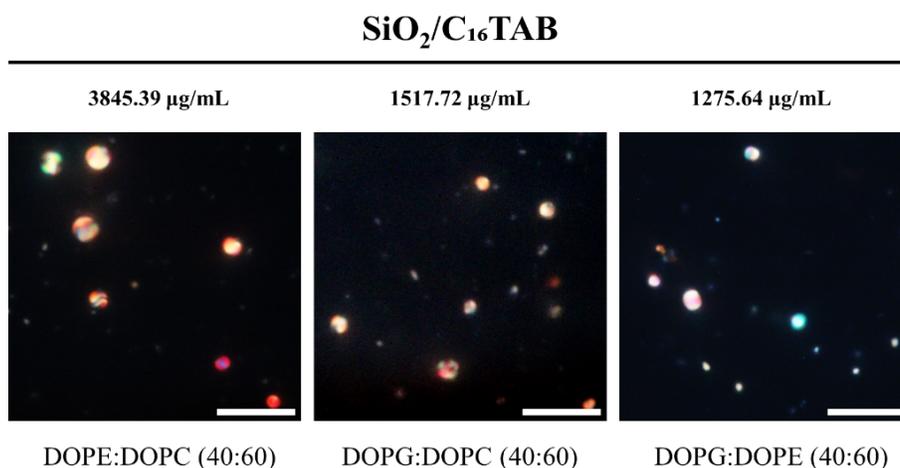


Figure S22. Representative optical micrographs showing the configurations adopted by LC droplets stabilized using $\text{SiO}_2/\text{C}_{16}\text{TAB}$ complexes upon exposure to DOPE:DOPC (left panel), DOPG:DOPC (center panel), and DOPG:DOPE (right panel) at a 40:60 lipid composition. The droplets remained in a bipolar configuration at the concentrations tested. The scale bar corresponds to 20 μm .