Supporting Materials

Detailed Comparison of Deuterium Quadrupole Profiles between Sphingomyelin and Phosphatidylcholine Bilayers

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Supplementary Method

General information for synthesis:

1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine was purchased from Avanti Polar Lipids. Other chemicals and solvents were purchased from Nacalai Tesque, Aldrich, TCI, and KANTO Chemicals Inc., and used without further purification unless otherwise noted. Thin layer chromatography and column chromatography were Merck precoated silica gel 60 F-254 plates and silica gel 60 (100-200 um), respectively. Proton nuclear magnetic resonance spectra were collected on a JEOL ECA 500 (500 MHz) or a JEOL ECS 400 (400 MHz). Mass spectrometry was performed using LCQ-DECA (Tharmo quest), and high resolution mass spectra (HRMS) were recorded on a LTQ-Orbitrap XL. Voltex mixers of VOLTEX-2GENIE (scientific industries) and ultrasonic cleaner BRANSON 1510 (Yamato Inc.) were used for liposome preparation. A series of deuterated stearic acids (2-d_2-, 3-d_2-, 4-d_2-, 6-d_2-, 8-d_2-, 10-d_2-, 12-d_2-, 14-d_2-, 16-d_2-, 18-d_3-stearic acids**)** were synthesized as previously reported (1).

Synthesis of 2'-d_2-, 3'-d_2-, 4'-d_2-, 6'-d_2-, 8'-d_2-, 10'-d_2-, 12'-d_2-, 14'-d_2-, 16'-d_2-, and 18'-d_3-PSPCs

To a solution of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (52.1 mg, 0.11 mmol) and deuterated stearic acid (41.1 mg, 0.14 mmol) in dichlorometane (3.0 ml) were added 2-methyl-6-nitrobenzoic anhydride (189 mg, 0.55 mmol), *N*,*N*-dimethyl-4-aminopyridine (134 mg, 1.10 mmol). After the reaction mixture was stirred for 17 h at room temperature, the reaction was quenched with MeOH, and solvent was evaporated to give the crude products. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 3/1 to $CHCl_3$/MeOH/H_2O = 65/25/4) afforded 2'-d_2-PSPC as white solids. 3'-d_2-, 4'-d_2-, 6'-d_2-, 8'-d_2-, 10'-d_2-, 12'-d_2-, 14'-d_2-, 16'-d_2-, and 18'-d_3-PSPCs were prepared from respective deuterated stearic acid in a similar manner.

2'-d_2-PSPC: white solid (52.9 mg, 0.07 mmol, 63%). R_f 0.70 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.24 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.25

(2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.4 Hz, H3), 3.62 (2H, t, *J* = 4.8 Hz, β), 3.21 (9H, s, -N^+Me_3), 2.30 (71%*, br, H2'), 2.01 (4H, t, *J* = 6.4 Hz , H3'), 1.27 (50H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18') *not deuterated H (%); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5946**.**

3'-*d*$_2$-PSPC: white solid (38.7 mg, 0.05 mmol, 50%). R_f 0.67 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.25 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.62 (2H, t, *J* = 4.8 Hz, β), 3.21 (9H, s, -N^+Me_3), 2.30 (4H, br, H2'), 2.01 (66%*, br, H3'), 1.25 (50H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18') *not deuterated H (%); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5946**.**

4'-*d*$_2$-PSPC: white solid (44.8 mg, 0.06 mmol, 49%). R_f 0.71 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.40 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.25 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.62 (2H, t, *J* = 4.8 Hz, β), 3.20 (9H, s, -N^+Me_3), 2.30 (4H, br, H2'), 2.01 (4H, br, H3'), 1.24 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5952**.**

6'-*d*$_2$-PSPC: white solid (72.6 mg, 0.09 mmol, 90%). R_f 0.69 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.62 (2H, t, *J* = 4.8 Hz, β), 3.20 (9H, s, -N^+Me_3), 2.30 (4H, br, H2'), 2.01 (4H, br, H3'), 1.25 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5958**.**

8'-d_2-PSPC: white solid (70.0 mg, 0.09 mmol, 90%). R_f 0.70 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.62 (2H, t, *J* = 4.8 Hz, β), 3.21 (9H, s, -N^+Me_3), 2.30 (4H, br, H2'), 2.01 (4H, br, H3'), 1.25 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5960**.**

10'-d_2-PSPC: white solid (74.0 mg, 0.10 mmol, 95%). R_f 0.71 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.62 (2H, t, *J* = 4.8 Hz, β), 3.21 (9H, s, -N^+Me_3), 2.31 (4H, br, H2'), 2.01 (4H, br, H3'), 1.24 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5961**.**

12'-d_2-PSPC: white solid (70.9 mg, 0.09 mmol, 92%). R_f 0.70 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.61 (2H, t, *J* = 4.8 Hz, β), 3.21 (9H, s, -N^+Me_3), 2.31 (4H, br, H2'), 2.01 (4H, br, H3'), 1.25 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5960**.**

14'-d_2-PSPC: white solid (65.6 mg, 0.09 mmol, 83%). R_f 0.71 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.60 (2H, t, *J* = 4.8 Hz, β), 3.20 (9H, s, -N^+Me_3), 2.31 (4H, br, H2'), 2.01 (4H, br, H3'), 1.25 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5960**.**

16'-d_2-PSPC: white solid (65.0 mg, 0.08 mmol, 58%). R_f 0.71 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.60 (2H, t, *J* = 4.8 Hz, β), 3.20 (9H, s, -N^+Me_3), 2.31 (4H, br, H2'), 2.01 (4H, br, H3'), 1.25 (48H, s, -CH_2-), 0.88 (6H, t, *J* = 6.4 Hz,H18, H18'); ESI-HRMS m/z calcd for $C_{42}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 786.5952, found 786.5958**.**

18'-d_3-PSPC: white solid (54.7 mg, 0.07 mmol, 86%). R_f 0.71 (silica gel, $CHCl_3$/MeOH/H_2O = 65/35/8); ^{1}H NMR (400 MHz, CD_3OD) δ 5.25 (1H, br, H2), 4.41 (1H, dd, *J* = 12.4, 3.2 Hz, H1), 4.24 (2H, br, α), 4.15 (1H, dd, *J* = 12.0, 6.8 Hz, H1), 3.98 (2H, t, *J* = 6.3 Hz, H3), 3.60 (2H, t, *J* = 4.8 Hz, β), 3.20 (9H, s, -N^+Me_3), 2.31 (4H, br, H2'), 2.01 (4H, br, H3'), 1.25 (50H, s, -CH_2-), 0.88 (3H, t, *J* = 6.4 Hz,H18); ESI-HRMS m/z calcd for $C_{42}H_{81}D_3NO_8PNa^+$ $(M+Na)^+$ 787.6015, found 787.6024**.**

Supplementary Figures

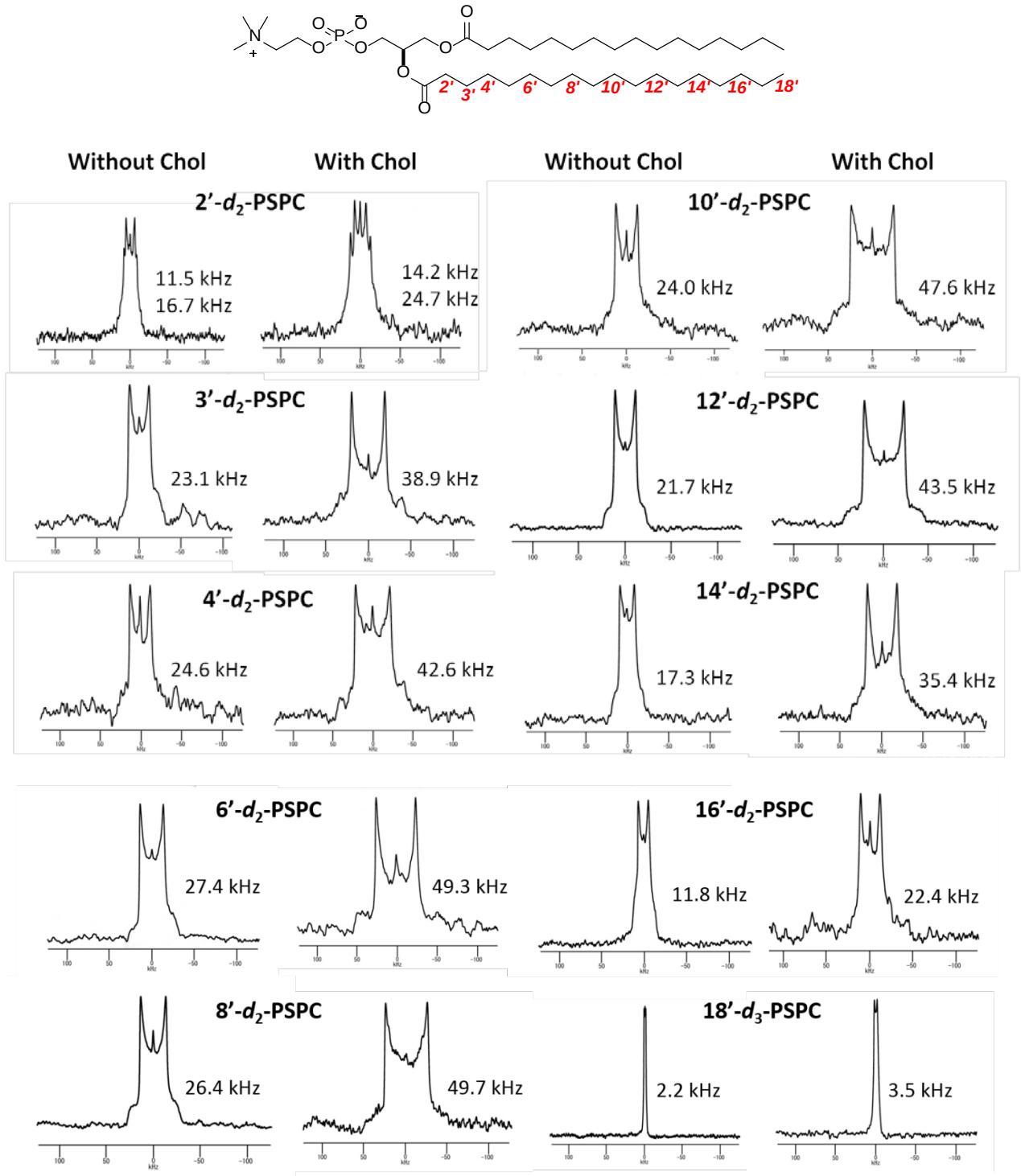

Figure S1. ^{2}H NMR spectra of deuterated PSPC bilayers in the oresence and absence of 50 mol% Chol at 50 ºC. To conserve the amount of deuterated PSPCs, we mixed commercially available unlabeled PSPC.

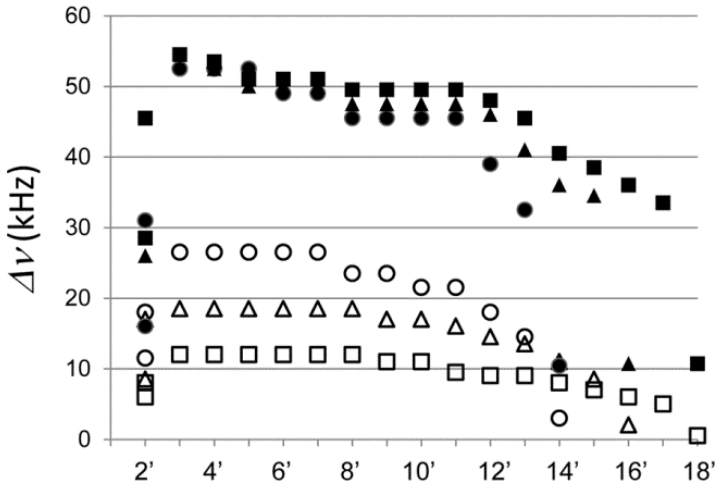

Figure S2. Reported quadrupole splitting profiles of *sn*-2 chain of DMPC, DPPC, and DSPC determined from perdeuterated acyl chains. The data were obtained from DMPC-d_{54} (○, ●), DPPC-d_{62} (△, ▲), and DSPC-d_{70} (□, ■) in the presence (●, ▲, ■) and absence (○, Δ, □) of 50 mol% Chol, at 35 °C for DMPC-d_{54}, at 52 °C for DPPC-d_{62}, and at 63 °C for DSPC-d_{70}. Data from Supporting Ref 2.

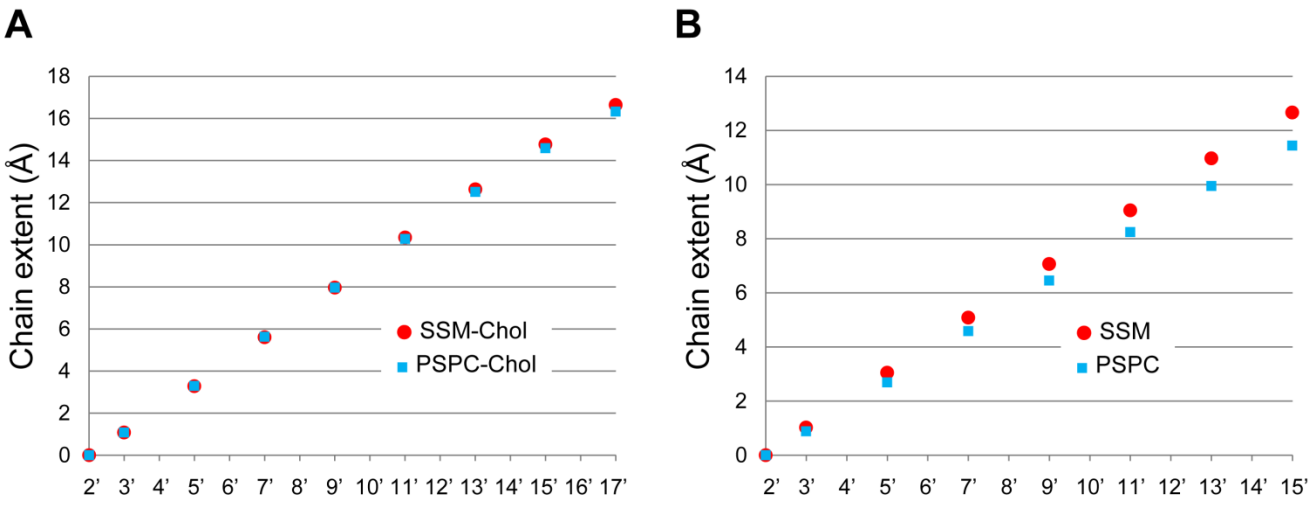

Figure S3. Segmental projections onto the bilayer normal for Chol-containing (A) and pure (B) bilayers at 50 ºC. The calculation was made from quadrupole splitting values on the basis of the first-order mean-torque model (3). The abscissa axes start from the C2' carbon, and end at the C17' carbon for cholesterol-containing membranes (A) and at the C15' carbon for pure PSPC and SSM membranes (B). The reason why the abscissa axes don't terminate at the C18' carbon is that we don't have the order parameter of C17' that is necessary to obtain the full chain projection including C18'. In addition, since the absolute values of order parameters of the C16' for pure SSM and PSPC membranes are below 0.125 that is the criteria for the application of the first-order mean-torque model, the abscissa ends at the C15' carbon in panel B.

Supplementary Tables

Table S1. Quadrupolar couplings (kHz) of 2'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	-
30 °C	-	13.0, 24.7
40 °C	-	14.0, 25.4
50 °C	11.5, 16.7	14.2, 24.7
55 °C	11.3, 15.7	14.4, 24.1

Table S2. Quadrupolar couplings (kHz) of 3'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	44.5
30 °C	-	43.1
40 °C	-	41.4
50 °C	23.1	38.9
55 °C	22.5	37.9

Table S3. Quadrupolar couplings (kHz) of 4'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	49.2
30 °C	-	46.8
40 °C	-	44.3
50 °C	24.6	42.6
55 °C	23.4	

Table S4. Quadrupolar couplings (kHz) of 6'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	55.7
30 °C	-	54.1
40 °C	-	50.5
50 °C	27.4	49.3
55 °C	25.6	48.0

Table S5. Quadrupolar couplings (kHz) of 8'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	55.6
30 °C	-	53.8
40 °C	-	52.1
50 °C	26.4	49.7
55 °C	24.7	

Table S6. Quadrupolar couplings (kHz) of 10'-d_2-PSPC membranes

	PSPC	PSPC/Chol (50 mol%)	PSPC/Chol (33 mol%)	PSPC/Chol (20 mol%)
20 °C	-	52.1	57.2	-
30 °C	-	53.2	54.8	-
40 °C	-	51.2	52.4	54.0
45 °C	-	49.8		54.0
50 °C	24.0	47.6	48.3	40.8
55 °C	22.2		44.8	36.4

Table S7. Quadrupolar couplings (kHz) of 12'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	50.9
30 °C	-	49.3
40 °C	-	47.0
50 °C	21.7	43.5
55 °C	19.4	

Table S8. Quadrupolar couplings (kHz) of 14'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	44.2
30 °C	-	41.0
40 °C	-	38.1
50 °C	17.3	35.4
55 °C	15.3	34.4

Table S9. Quadrupolar couplings (kHz) of 16'-d_2-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	30.8
30 °C	-	29.1
40 °C	-	25.4
50 °C	11.8	22.4
55 °C	10.0	21.6

Table S10. Quadrupolar couplings (kHz) of 18'-d_3-PSPC membranes

	PSPC	PSPC/Chol
20 °C	-	5.6
30 °C	-	4.8
40 °C	-	4.3
50 °C	2.2	3.5
55 °C	1.9	2.9

Table S11. Quadrupolar couplings (kHz) of 10'-d_2-SSM membranes

	SSM/Chol (50 mol%)	SSM/Chol (33 mol%)	SSM/Chol (20 mol%)
20 °C	54.8	55.5	-
30 °C	54.3	54.2	53.3
40 °C	52.9	52.6	49.2
45 °C	51.9	51.2	47.0
50 °C	50.8	49.8	43.1

Supporting References

1. Matsumori, N., T. Yasuda, H. Okazaki, T. Suzuki, T. Yamaguchi, H. Tsuchikawa, M. Doi, T. Oishi, and M. Murata. 2012. Comprehensive molecular motion capture for sphingomyelin by site-specific deuterium labeling. *Biochemistry.* 51:8363–8370.
2. Sankaram, M. B., and T. E. Thompson. 1990. Modulation of phospholipid acyl chain order by cholesterol. A solid-state ^{2}H nuclear magnetic resonance study. *Biochemistry.* 29:10676–10684.
3. Bartels, T., R. S. Lankalapalli, R. Bittman, K. Beyer, and M. F. Brown. 2008. Raftlike mixtures of sphingomyelin and cholesterol investigated by solid-state ^{2}H NMR spectroscopy. *J Am Chem Soc.* 130:14521–14532.