Supporting Materials

Deuterium NMR of Raft Model Membranes Reveals Domain-Specific Order Profiles and Compositional Distribution

Tomokazu Yasuda,[†‡] Hiroshi Tsuchikawa,[†] Michio Murata,[†‡] and Nobuaki Matsumori[†*]

[†]Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan; [‡]Japan Science and Technology Agency, ERATO, Lipid Active Structure Project, Toyonaka, Osaka 560-0043, Japan:

Nobuaki Matsumori's present address is Department of Chemistry, Graduate School of Sciences, Kyushu University, Higashi-ku, Fukuoka, 812-8581, Japan.

Supplementary Method

General information for synthesis:

1-oleoyl-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. Merck precoated silica gel 60 F-254 plates and silica gel 60 (100-200 um) were used for thin layer chromatography and column chromatography, respectively. Proton nuclear magnetic resonance spectra were collected on a JEOL ECS 400 (400 MHz). Mass spectrometry was performed on a LTQ-Orbitrap XL (Thermo Scientific). Voltex mixers of VOLTEX-2GENIE (Scientific Industries) and ultrasonic cleaner BRANSON 1510 (Yamato Inc.) were used for liposome preparation.

Synthesis of 11'-d_2 -DOPC

11,11-d_2-oleic acid was synthesized as previously reported (1,2). To a solution of 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (95.1 mg, 0.18 mmol) and 11,11-d_2-oleic acid (61.4 mg, 0.22 mmol) in CH_2Cl_2 (5.0 mL) were added 2-methyl-6-nitrobenzoic anhydride (310 mg, 0.90 mmol) and *N*,*N*-dimethyl-4-aminopyridine (220 mg, 1.80 mmol).

After the reaction mixture was stirred for 14 h at room temperature, the reaction was quenched with MeOH, and solvent was evaporated to give a crude product. Purification by silica gel column chromatography ($CHCl_3$/MeOH = 3/1 to $CHCl_3$/MeOH/H_2O = 65/25/4) afforded 11'-d_2-DOPC (114 mg. 145 μmol, 81%) as a white solid. R_f 0.52 (silica gel, $CHCl_3$/MeOH/H_2O = 65/25/4); ^{1}H NMR (400 MHz, CD_3OD) δ 5.38-5.27 (4H, m, H9', H10'), 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, m, H2'), 2.03 (6H, br, H8', H11), 1.60 (4H, br, H3'), 1.25 (40 H, s, -CH_2-), 0.89 (6H, t, *J* = 6.4 Hz, H18'); ESI-HRMS m/z calcd for $C_{44}H_{82}D_2NO_8PNa^+$ $(M+Na)^+$ 810.5952, found 810.5959.

Supplementary Figures

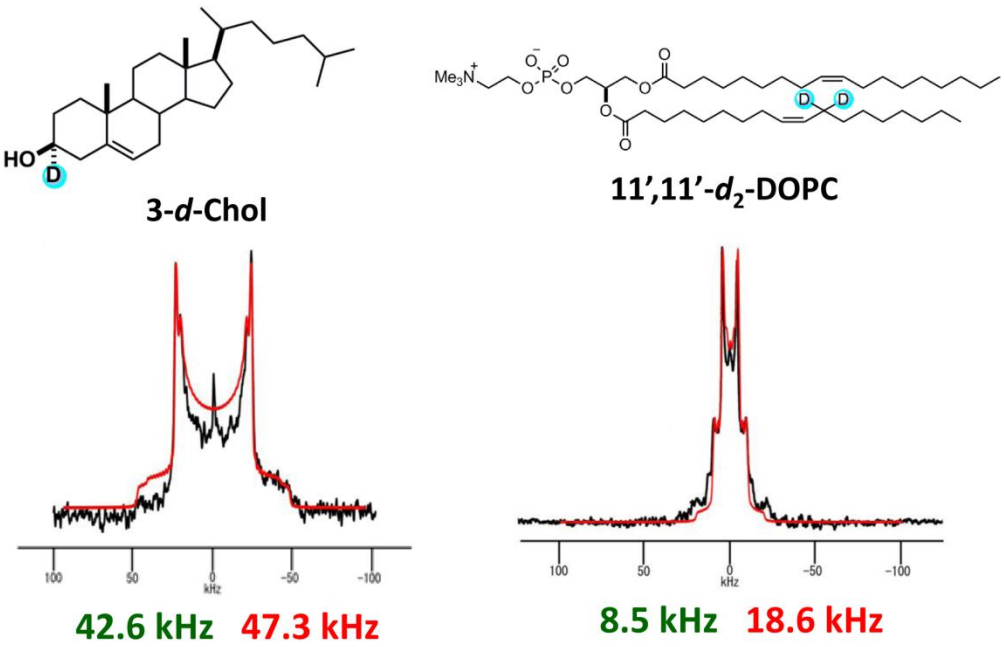

FIGURE S1 ^{2}H NMR spectra of 3-*d*-Chol and 11'-d_2-DOPC in phase-separating ternary mixture (SSM/DOPC/Chol at a ratio of 1:1:1) at 30 °C. The red traces represent the spectral simulation to evaluate the molar ratios of Chol and DOPC distributed in the L_o and L_d domains.

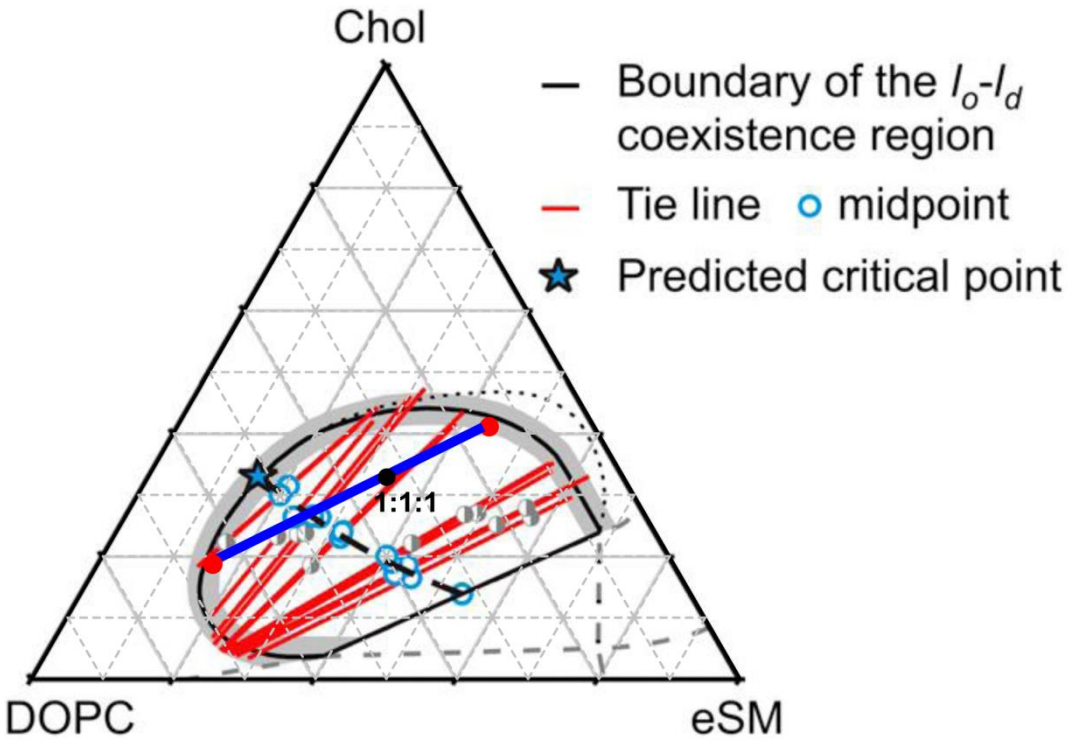

FIGURE S2 Superposition of the currently-obtained tie line (blue line) and the reported phase diagram of the DOPC/SM/Chol mixture at 23 °C. The phase diagram is from ref [3]. The solid black curve indicates the boundary of the L_o-L_d phase with ±2 mol % deviation in gray. The red points indicate the currently estimated phase boundary, and blue line is tie line containing the 1:1:1 composition.

Supplementary Tables

Table S1. Quadrupolar couplings (kHz) of deuterated SSM in membranes at 30 °C

	SSM/DOPC (1:1)	SSM/DOPC/Chol (1:1:1)
4'-d_2-SSM	29.9	34.8, 43.7
6'-d_2-SSM	30.6	38.3, 49.9
8'-d_2-SSM	29.2	38.1, 52.2
10'-d_2-SSM	29.2	36.0, 51.5
12'-d_2-SSM	25.5	29.3, 48.3
14'-d_2-SSM	16.8	24.3, 41.0
16'-d_2-SSM	11.9	16.2, 29.8

Table S2. Quadrupolar couplings (kHz) of each component in ternary membranes

	10'-d_2-SSM	11'-d_2-DOPC	3-d_1-Chol
20 °C	34.3, 53.5	8.6, 20.0	-[a]
30 °C	36.0, 51.5	8.5, 18.6	42.6, 47.0
40 °C	38.4, 49.6	9.7, 15.9	44.5
45 °C	43.3	11.4	44.4
50 °C	-[a]	-[a]	43.0

[a] Not determined.

Supporting References

[1] V. Chupin, J. A. Killian, and B. de Kruijff. 1987. ^{2}H-nuclear magnetic resonance investigations on phospholipid acyl chain order and dynamics in the gramicidin-induced hexagonal H_{II} phase. *Biophys. J.* 51:395–405.

[2] H. Chen, and E. Plettner. 2012. Site-specific ^{2}H labelled oleic acid and derived esters for use as tracers of ethyl oleate methabolism in honey bees. *Label. Compd. Radiopharm.* 55:66–70.

[3] Bezlyepkina, N., R. S. Gracià, P. Shchelokovskyy, R. Lipowsky, and R. Dimova. 2013. Phase diagram and tie-line determination for the ternary mixture DOPC/eSM/Cholesterol. *Biophys. J.* 104:1456–1464.