Modulation of raft domains in a lipid bilayer by boundary-active curcumin

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Electronic Supporting Information

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Experimental

Materials

1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and Sphingomyelin from porcine brain (SM) were purchased form Avanti Polar Lipids, Inc. (AL, USA). Texas Red® 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR-DHPE) and curcumin from Curcuma longa were purchased from Molecular Probes (OR, USA) and Sigma-Aldrich Co. (MO, USA), respectively. Dimethoxycurcumin was synthesized according to the literature.[S1] All other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used without further purification.

Preparation of giant vesicles and microscopic observation

Giant vesicles were prepared by the gentle hydration of a lipid film composed of DOPC / SM / Chol (1:1:1) containing 1 mol% of TR-DHPE with 12 mM sucrose solution at 65 °C for overnight. 40 μL of vesicular sample was subjected to the microscopic observation. A series of microscopic image was acquired using an epifluoresence microscope (Nikon TE300, Melville, NY, USA) equipped with a 40x objective lens (NA=0.6). Fluorescent images were recorded using a cooled CCD camera (SPOT RT230, Sterling Heights, MI, USA) under the irradiation of excitation light beam from a mercury lamp (Lumen Dynamics X CiteTM120, Mississauga, Canada) through an optical filter (Nikon TRITC HYQ, Melville, NY, USA). Curcumin was introduced to the aqueous phase of the vesicular sample as a form of water / ethanol (200 : 1 w/w) solution.

Preparation of planar lipid bilayer and TIRF observation

Planar lipid bilayer was formed on a clean glass substrate by the fusion of small unilamellar vesicles (SUVs). SUVs were formed by the hydration of lipid thin film composed of DOPC / SM / Chol (1:1:1) with a PBS buffer (pH=6.6, [NaCl] =100 mM) and following extrusion using a stacked polycarbonate filter with pores of 50 nm. To selectively stain l_o domains, 1 mol% of TR-DHPE was incorporated, whereas planar lipid bilayer without TR-DHPE was prepared for the direct observation of curcumin fluorescence to prevent the interference of other fluorescent dye. Suspension of liposomes was deposited onto the clean hydrophilic glass substrate and incubated for 30 minutes at 60 ^{o}C. Excess lipids were removed by the rinse with Milli-Q water.
Microscopic images of planar lipid bilayers were observed using an inverted fluorescence microscope (Olympus IX 73, Tokyo, Japan). TR-DHPE and curcumin were excited at 561 nm and 405 nm, respectively,

by a diode laser through TIRF optical system and 100x objective lens (NA = 1.49). Curcumin was introduced to the aqueous phase on the supported lipid bilayer as a form of ethanol solution. To minimize the photobleaching of the fluorescent dye, excitation laser was only irradiated during the observation. Recorded image at each observation time was analyzed using an ImageJ software.

Langmuir monolayer experiment

A series of π-A isotherms of lipid monolayers was obtained with a KSV minitrough (KSV, Finland) equipped with a teflon-coated trough with a size of 195 mm × 50 mm. Surface pressure was measured by a Wilhelmy balance upon the compression of the monolayer by two barriers. Milli-Q water was used as a subphase and lipid was deposited on the air-water interface as a form of chloroform solution. A compression rate kept constant to 10 $mm \cdot min^{-1}$. Curcumin was introduced to the subphase as an ethanol solution prior to the compression of the monolayer. Temperature of the trough was maintained at 25 ^{o}C using a circulating water bath. For the adsorption measurement, we set the surface pressure of the membrane to 35 $mN \cdot m^{-1}$ prior to the addition to curcumin.

Reference

(S1) S. V. Jovanovic, C. W. Boone, S. Steenken, M. Trinoga, R. B. Kaskey, R. B. *J. Am. Chem. Soc.* 2001, **123**, 3064.

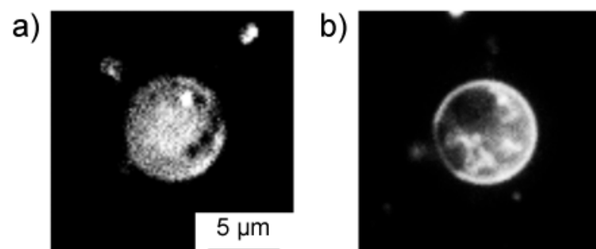

Fig. S1 Fluorescence microscopic observation of domains in a giant vesicle consisting of DOPC / SM / Chol. Images were acquired at (a) 0 and (b) 120 minutes after the addition of water / ethanol (200:1 w/w) solution without curcumin. Dark and bright regions are corresponding to the liquid-ordered (l_o) raft domains and liquid-disordered (l_d) PC-rich membrane, respectively.

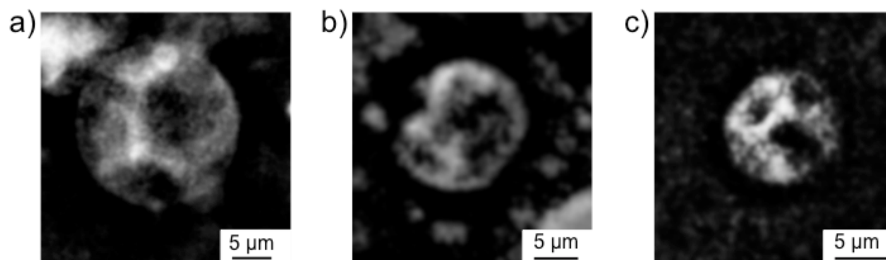

Fig. S2 Effect of curcumin amount on the domain fusion. Images were acquired at 120 minutes after the addition of (a) 0.2, (b) 0.1 and (c) 0.05 mole % of curcumim. Dark and bright regions are corresponding to the liquid-ordered (l_o) raft domains and liquid-disordered (l_d) PC-rich membrane, respectively.

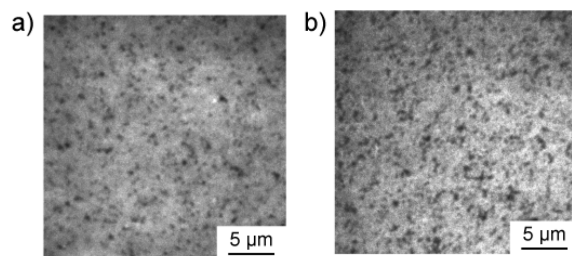

Fig. S3 TIRF images of a planar lipid bilayer consisting of DOPC/SM. Images were acquired at (a) just after the sample preparation and (b) 120 minutes later. Dark and bright regions correspond to s_o and l_d phases, respectively.

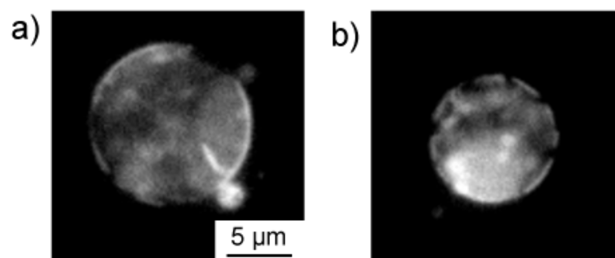

Fig. S4 Fluoresence microscopic observation of domains in a giant vesicle consisting of DOPC / SM / Chol. Images were acquired at (a) 0 and (b) 120 minutes after the addition of curcumin. Dark and bright regions are corresponding to the liquid-ordered (l_o) raft domains and liquid-disordered (l_d) PC-rich membrane, respectively. [curcumin] / [lipid] = 8×10^{-6}.

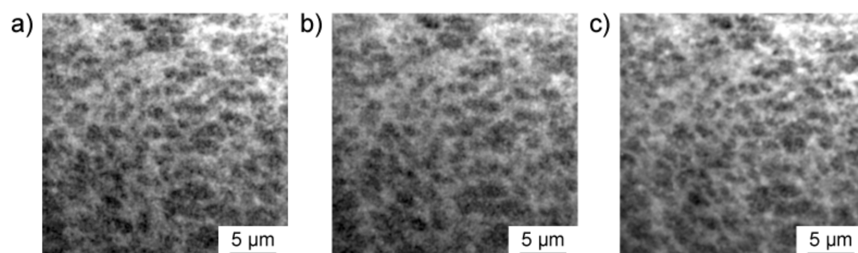

Fig. S5 TIRF images of a planar lipid bilayer consisting of DOPC/SM. Images were acquired at (a) 0, (b) 10 and (c) 60 minutes after the addition of curcumin. Dark and bright regions correspond to s_o and l_d phases, respectively. [curcumin] / [lipid] = 8×10^{-6}.

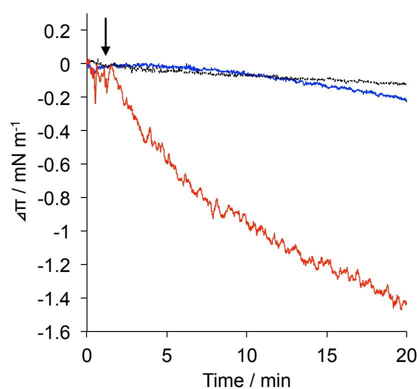

Fig. S6 Time-course of the surface pressure difference (Δπ) of DOPC / SM / Chol (Red), SM / Chol (black) and DOPC (blue) monolayers upon the addition of curcumim. The arrow indicates addition of curcumin to the subphase. [curcumin] / [lipid] = 8×10^{-6}, at 25 °C.

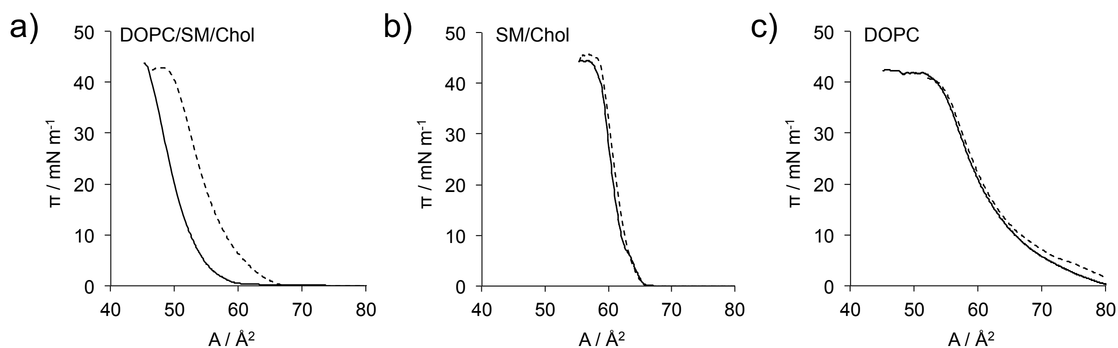

Fig. S7 Effect of curcumin on the surface pressure (π) – molecular area (A) isotherm of (a) DOPC / SM / Chol, (b) SM / Chol and (c) DOPC monolayers. Dashed and solid lines correspond to the absence and the presence of curcumin, respectively. [curcumin] / [lipid] = 8×10^{-6}, at 25 °C.

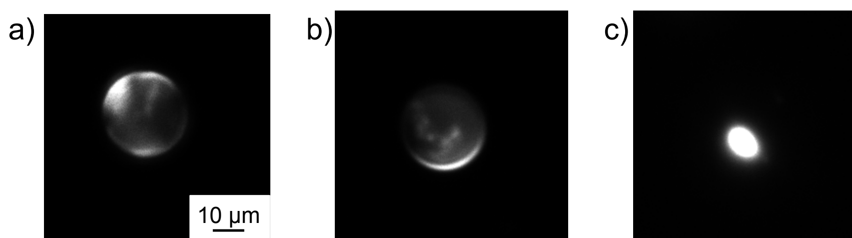

Fig. S8 Fluorescence microscopic observation of a giant vesicle consisting of DOPC / SM / Chol. Images were acquired at (a) 0, (b) 180 and (c) 405 seconds after the addition of dimethoxycurcumin. Dark and bright regions are corresponding to the liquid-ordered (l_o) raft domains and liquid-disordered (l_d) PC-rich membrane, respectively. [dimethoxycurcumin] / [lipid] = 3×10^{-3} at 25 °C.