Supporting Information

Ergosterol-Induced Ordered Phase in Ternary Lipid Mixture Systems of Unsaturated and Saturated Phospholipid Membranes

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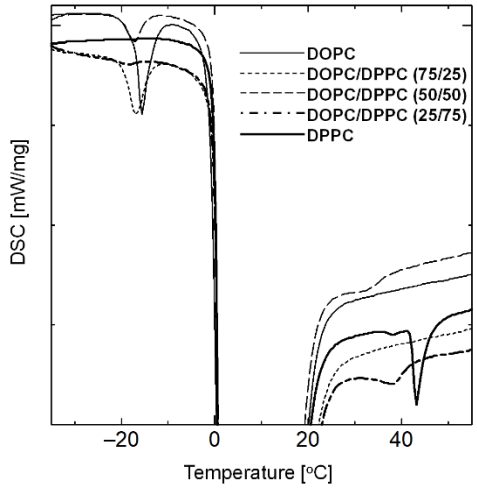
Differential scanning calorimetry analysis.

The phase transitions of DOPC/DPPC liposomes were revealed by differential scanning calorimetry (DSC) analysis (**Figure S1**). It was found that the DPPC liposome indicated a phase transition temperature (T_m) at 41 °C. The enthalpy (ΔH) for DOPC and DPPC were calculated (Table). In the case of liposome mixture ((1) DOPC liposome + DPPC liposome), the ΔH values showed a liner relationship with DPPC concentration. In contrast, the DOPC/DPPC binary mixtures (2) showed the decreased ΔH values lower than those of liposome mixtures. It is therefore suggested that DPPC molecules are not fully in solid ordered (S_o) phases.

Table S1 Phase transition enthalpy of DPPC [kcal/mol]

DOPC/DPPC	$\Delta H_{DOPC+DPPC}$ (1)	$\Delta H_{DOPC/DPPC}$ (2)	(2) - (1)
10/0	0.00	0.00	0.00
7/3	2.54	0.00	-2.54
5/5	4.92	0.49	-4.43
3/7	5.42	3.55	-1.87
0/10	7.38	7.38	0.00



	ΔH_{DOPC}	ΔH_{DPPC}	$\Delta H_{boundary}$
DOPC	-1.95 J/g	-	0
DOPC/DPPC (75/25)	-1.31 J/g (74.1%)	-	-0.45 J/g (25.9%)
DOPC/DPPC (50/50)	-0.64 J/g (40.4%)	-0.16 J/g (10.1%)	-0.79 J/g (49.5%)
DOPC/DPPC (25/75)	-0.11 J/g (7.8%)	-0.51 J/g (36.4%)	-0.78 J/g (55.8%)
DPPC	-	-1.22 J/g	0

ΔH_{DOPC} : phase transition enthalpy of DOPC
ΔH_{DPPC} : phase transition enthalpy of DPPC
$\Delta H_{boundary} = \sum(\Delta H_{DOPC} + \Delta H_{DPPC}) - (\Delta H_{DOPC} + \Delta H_{DPPC})$

Figure S1 DSC analysis of DOPC/DPPC liposomes. Based on the obtained ΔH values, the fraction of total boundary lipid in DOPC/DPPC 3/1 (75/25), 1/1 (50/50) and 1/3 (25/75) were 25.9%, 49.5%, 55.8%, respectively. The mean boundary DPPC (i.e. not in S_o state) in DOPC/DPPC 3/1, 1/1 and 1/3 was 25 mol%, 37.8 mol% and 36.0 mol%, respectively.

Estimation of phase state based on temperature-dependency of 1/P values.

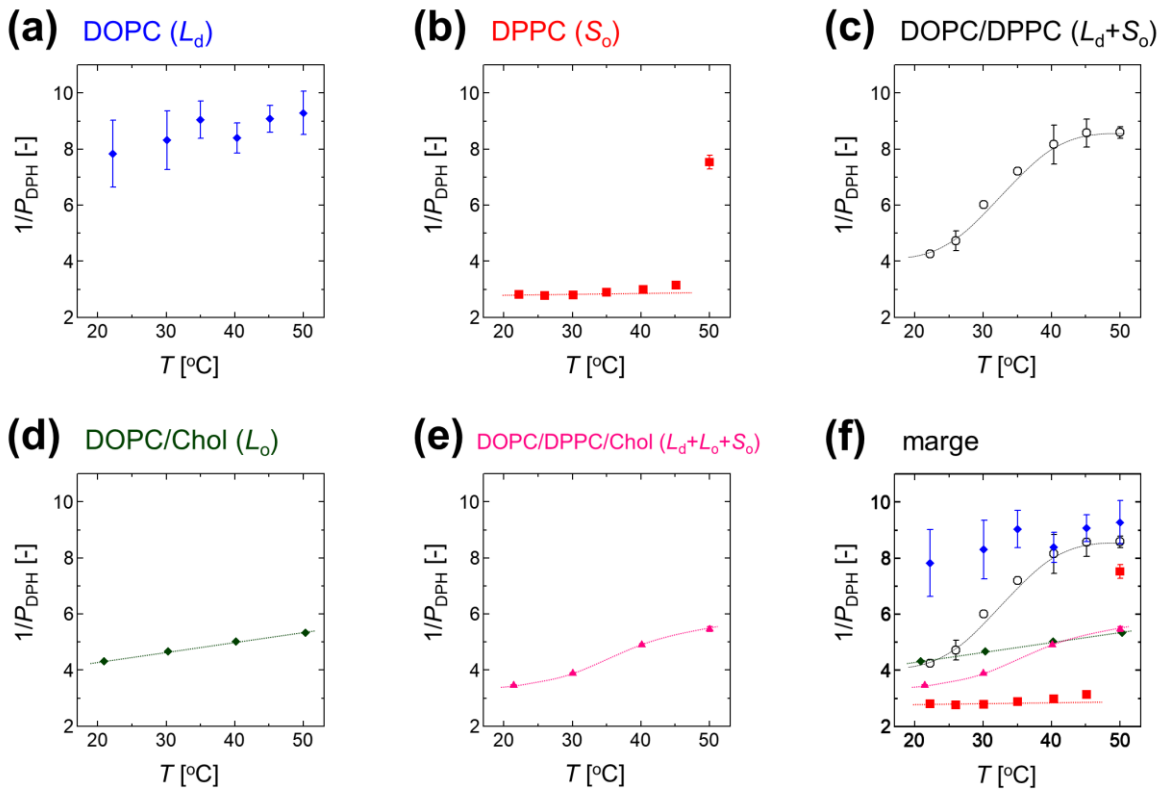


Figure S2 Temperature dependency of liposomes with different phase states. (a) DOPC, L_d phase, $1/P_{DPH}$ >6. (b) DPPC, S_o phase, $1/P_{DPH}$ ~3 (almost constant) below T_m; $1/P_{DPH}$ >6 above T_m. (c) DOPC/DPPC 1/1 (no sterol), L_d+S_o phase, T vs $1/P_{DPH}$ is sigmoidal. (d) DOPC/Chol 1/1, L_o, phase, $1/P_{DPH}$ <6, T vs $1/P$ is linear. (e) DOPC/DPPC/Chol 2/2/1, L_d+ L_o+S_o phase, $1/P_{DPH}$ <6, T vs $1/P_{DPH}$ is sigmoidal. (f) merged graph. Data are cited from Suga et al. (Suga, K. et al., *Langmuir* **2013**, *29*, 4830-4838).

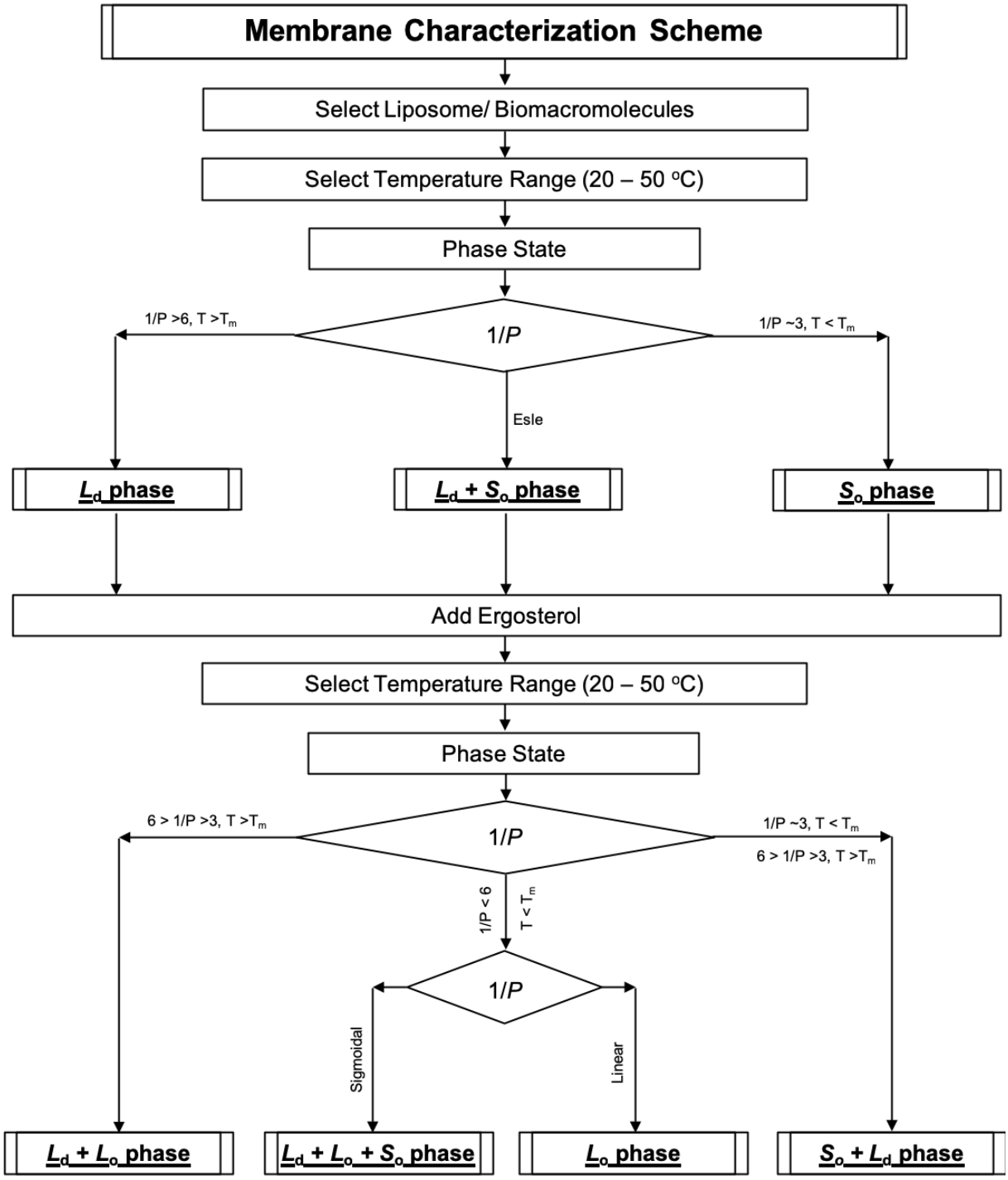


Figure S3 Scheme for constructing the phase diagram for DOPC/DPPC/Erg ternary mixtures based on membrane fluidity ($1/P_{DPH}$) and the dependence on temperatures. In the absence of Erg, the phase states of the pure or mixed phospholipid membranes was analyzed. When $T < T_m$ and $1/P_{DPH} \sim 3$, it indicated the S_o phase membrane, and when temperatures increasing over T_m, and $1/P_{DPH} > 6$, this reflected the L_d phase. If not, membrane phase was $L_d + S_o$ phase. The addition of Erg can contribute to the membrane phases. For example, the presence of Erg in S_o resulted in the $1/P_{DPH} < 6$, it indicated the mixed phase of S_o+L_o phase. Furthermore, membrane will be L_o phase, when $1/P_{DPH} < 6$, T vs $1/P_{DPH}$ is linear. While, $1/P_{DPH} < 6$, T vs $1/P_{DPH}$ is sigmoidal, the membranes in the $L_d + S_o + L_o$ phases. Otherwise, $L_d + L_o$ phase.

Cartesian diagram analysis of DOPC/DPPC/Erg ternary lipid mixtures.

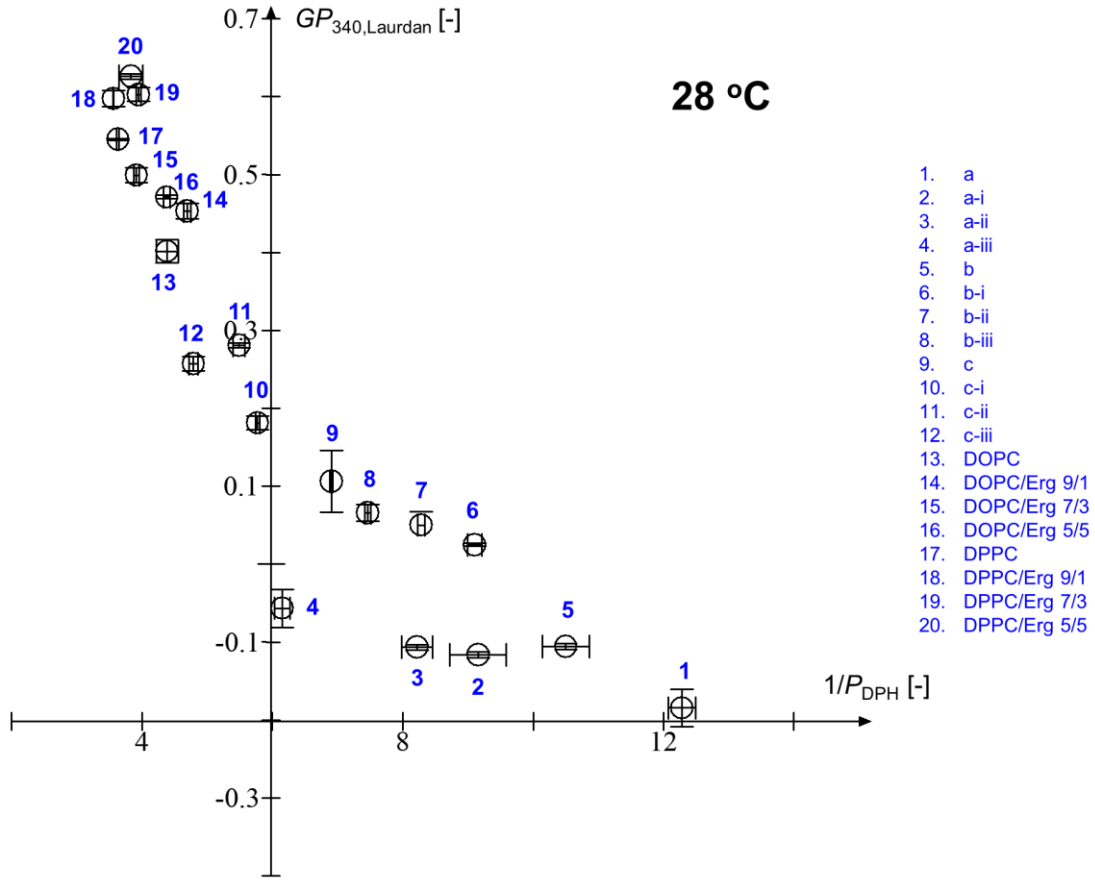


Figure S4 Cartesian diagram for DOPC/DPPC/Erg ternary mixtures at 28 °C, based on membrane fluidity ($1/P_{DPH}$) and membrane polarity ($GP_{340,Laurdan}$). When the liposome membrane becomes polar ($GP_{340,Laurdan}$ decrease), its fluidity increases ($1/P_{DPH}$ increase). The membranes showing high fluidity ($1/P_{DPH}$ >6) and in hydrophilic ($GP_{340,Laurdan}$ <0) are estimated as liquid-disordered phase (L_d). For details of Cartesian diagram analysis, see following reports (Suga, K., et al., *Langmuir* **2013**, *29*, 1899-1907; Suga, K. et al., *Langmuir* **2013**, *29*, 4830-4838; Bui, T. T. et al., *Langmuir* **2016**, *32*, 6176-6184).