

Title	Lateral phase separation in tense membranes
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Description	

Supplementary Information for

**Lateral phase separation in tense membranes**

Tsutomu Hamada,\* Yuko Kishimoto, Takeshi Nagasaki and Masahiro Takagi

**Contents:**

1. Percentage of phase-separated vesicles as a function of the applied osmotic pressure and temperature.
2. Domain structure in DPPC/Chol binary membranes.
3. Relief of membrane tension in a single vesicle.

1. Percentage of phase-separated vesicles as a function of the applied osmotic pressure and temperature.

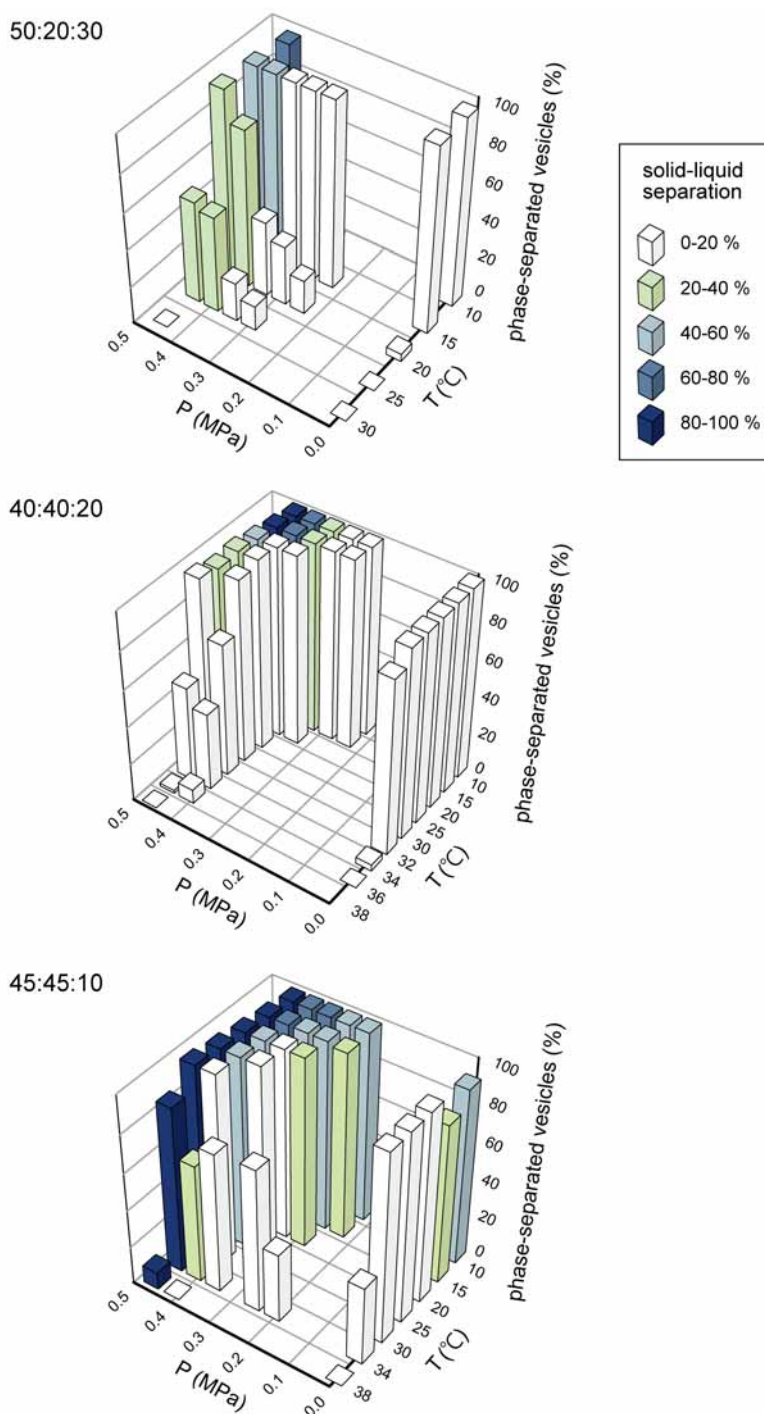


Fig. S1. Phase diagram of ternary (DOPC/DPPC/Chol=50/20/30, 40/40/20 and 45/45/10) membranes. The percentage of phase-separated vesicles is shown for the applied osmotic pressure  $P$  and temperature  $T$ . The percentage of solid-liquid separation among the phase-separated vesicles is indicated in different colors. These correspond to Figs. 5 and 9 in the article.

## 2. Domain structure in DPPC/Chol binary membranes.

We investigated the response of DPPC/Chol (8/2, 7/3 and 6/4) binary membranes under membrane tension. Without tension, vesicles do not show phase separation at 20 °C (Fig. S2a), as reported elsewhere (1). It was found that DPPC/Chol=7/3 vesicles produced domains when under tension by an osmotic pressure of  $\Delta c=180$  mM (Fig. S2b). The membrane surface was covered by several small domains with bright fluorescence, which indicates that fluid domains were separated from the ordered matrix region. The behavior of microscopic phase separation with typically smaller sized domains is similar to that of monolayer domains in the  $\beta$  region observed in the same DPPC/Chol mixture (1). Notably, there were no domains in DPPC/Chol=8/2 and 6/4 membranes under tension in our experiment.

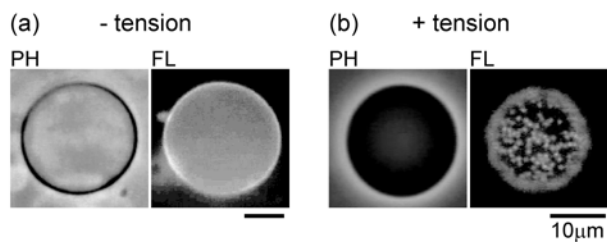


Fig. S2. Tension-induced phase organization in DPPC/Chol=7/3 vesicles. Typical phase-contrast (PH) and fluorescent (FL) images of vesicles without tension ( $\Delta c=0$  mM) (a) and with tension ( $\Delta c=180$  mM) (b). In the case of tense vesicles, the PH image shows high contrast because of the difference in the glucose concentration across the bilayer.

### 3. Relief of membrane tension in a single vesicle.

We demonstrated the real-time monitoring of phase transition of a single vesicle with a change in tension. We prepared a vesicle containing a synthesized photoresponsive amphiphile, KAON, the molecular conformation of which can be switched by light irradiation (2,3). We reported that cis- or trans-isomerization of KAON increase or decrease the equilibrium surface area per molecule of the vesicle without a change in the vesicular volume, respectively (2,3). Here, we used this system for the controllable relief of membrane tension. We prepared vesicles with 80 mol% lipids (DOPC/DPPC/Chol=40/40/20) and 20 mol% KAON, and situated them under tension by the osmotic pressure of  $\Delta c=180$  mM, where the KAON isomer is in a trans form. The DOPC/DPPC/Chol/KAON vesicles showed essentially the same phase behavior upon osmotic stress, i.e., tension-induced phase separation, as the DOPC/DPPC/Chol vesicles. Then, we irradiated UV light on the phase-separated tense vesicle to achieve cis-isomerization. It was found that the domains disappeared and the vesicle transformed into homogeneous phase (Fig. S3A). The bulky structure of cis-isomer increased the equilibrium surface area of the vesicle, and relieved membrane stretching, i.e., tension decreased. The vesicle again phase separate by increase in tension under trans-isomerization (Fig. S3B). As a control, we confirmed that the photoisomerization did not change phase organization in the vesicles without tension ( $\Delta c=0$  mM).

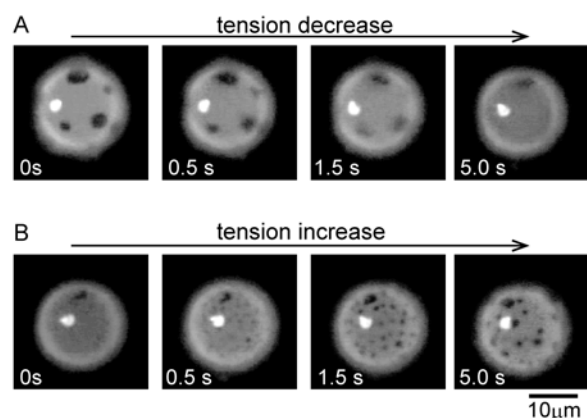


Fig. S3. Microscopic image sequence of membrane phase behavior with a change in tension using a synthesized photoresponsive amphiphile, where  $T = 30$  °C and  $\Delta c = 180$  mM. (A) Cis-isomerization by UV light increases the equilibrium surface area of the vesicle, leading to the relief of tension. (B) The decreased tension goes back to increase under trans-isomerization. UV light was irradiated through a band-pass filter (Olympus, 330–385 nm) with the use of an extra-high-pressure mercury lamp (100 W).

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