SUPPLEMENTARY INFORMATION

Riboswitch-Controlled Lipid Remodeling Enables Functional Membrane Asymmetry in Artificial Cells

Koki Kmaiya [1]*, Sumin Lee [1] and Kotaro Baba [1]

Graduate School of Science and Technology, Gunma University, Japan

*** Corresponding author:** Koki Kamiya

1-5-1 Tenjin-cho, Kiryu,

Gunma, Japan

Tel: +81-277-30-1342

Email: kamiya@gunma-u.ac.jp

Conjugation of alkyne to purified mCherry

The pET-28a/mCherry with a 6xHis tag at the C-terminus was transfected into E. Coli strain BL21(DE3). Cultures were grown at 37℃ in a Lysogeny broth (LB) medium containing 20 μg/mL kanamycin to OD_{600}=0.8. Protein expression was induced by adding isopropylthio-β-galactoside at a final concentration of 0.5 mM to the culture medium and shaking at 37 °C for 4 h. The culture medium was then centrifuged at 3000g for 20 min at 4℃. The collected cell pellets were stored at －80℃. The cell pellets were resuspended in a buffer solution (TE buffer (50 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.2) containing 5 mg/mL lysozyme and 500 μg/mL DNase I). After incubation for 10 min at room temperature, the TE buffer was added to the cell suspension. The cell suspension was incubated for 10 min at －80℃ and the frozen cell suspension was thawed at room temperature. The cell suspension was centrifuged at 7000g for 20 min at 4℃ and the supernatant was collected. The supernatant and Ni-NTA agarose resin equilibrated with buffer solution (10 mM Tris, 140 mM NaCl, pH 7.4) were mixed for 1 h at 4℃. After washing the resin with 20 mM Tris, 300 mM NaCl, 5 mM imidazole, pH 8.0, mCherry was eluted with 20 mM Tris, 300 mM NaCl, 200 mM imidazole, pH 8.0. The purified mCherry was concentrated using an ultrafiltration (Amicon ultracentrifugal filter (MWCO 10 kDa)), and the protein concentration was measured using a Nanodrop One (Thermo Fisher Scientific) with coefficients of molar absorbance at 280 nm. The proteins were stored at −80 °C. The mCherry solution was exchanged to 100 mM phosphate buffer (pH 7.2) containing 150 mM NaCl using the ultrafiltration. N-succinimidyl S-acetylthioacetate (SATA) and mcherry were mixed at 50:1 molar ratio and reacted for 30 min at room temperature. To remove the unreacted SATA, the reaction solution was exchanged to 100 mM phosphate buffer containing 150 mM, pH 7.2 using the ultrafiltration. The deacetylation solution (100 mM phosphate buffer (pH 7.2) containing 500 mM hydroxylamine hydrochloride, 150 mM NaCl, and 25 mM EDTA) was added to the mCherry solution to deprotect the sulfhydryl groups. After the reaction for 3 h at room temperature, the thiolated mCherry was purified to 100 mM phosphate buffer (pH 7.2) containing 150 mM NaCl using the ultrafiltration. The mixture of DBCO-maleimide and thiolated mCherry at 10:1 molar ratio was reacted for 17 h at 4°C. The unreacted DBCO-maleimide was removed from this solution using the ultrafiltration.

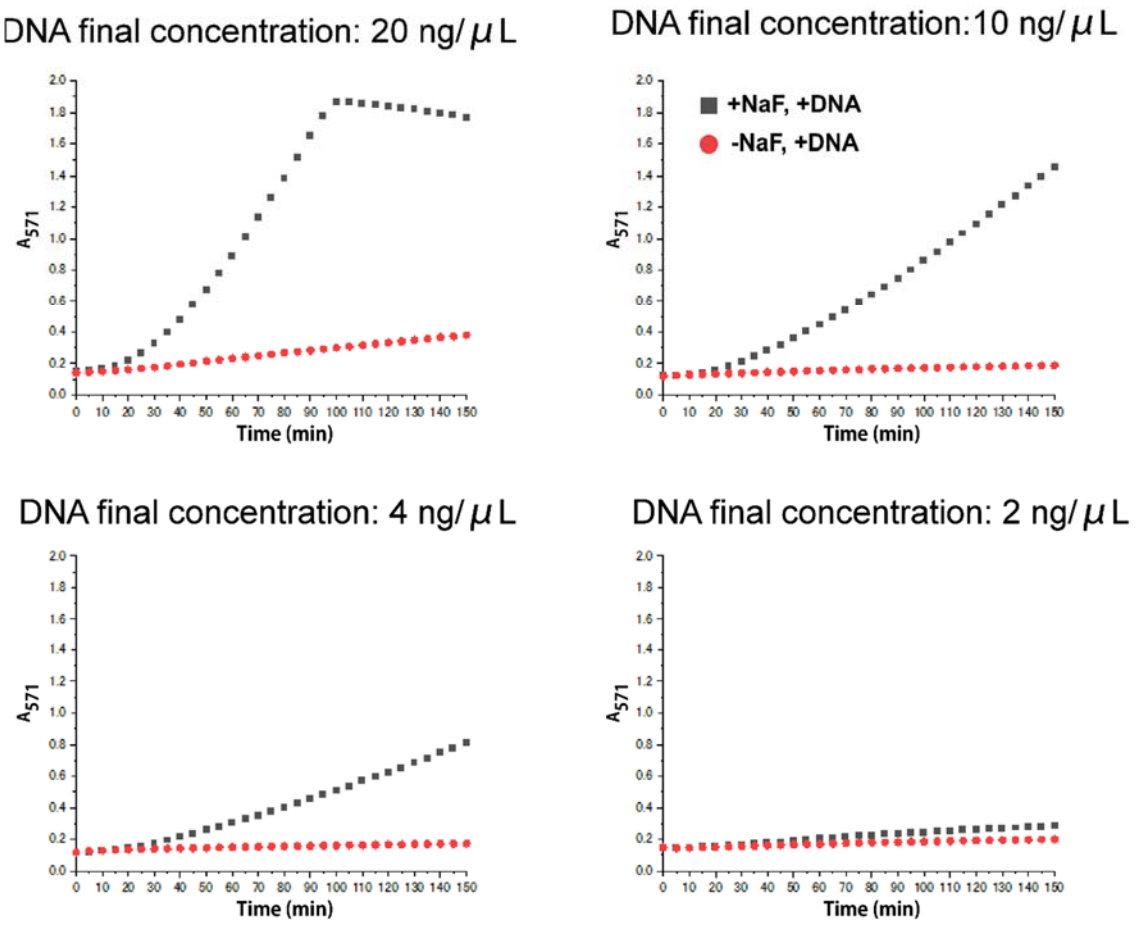

Figure S1 PLD synthesized by fluoride-responsive riboswitch of the activity assay of choline hydrolysis chaining 20 ng/μL, 10 ng/μL, 4 ng/μL, and 2 ng/μL of plasmid DNA final concentration. Three mM NaF was added to the microtube.

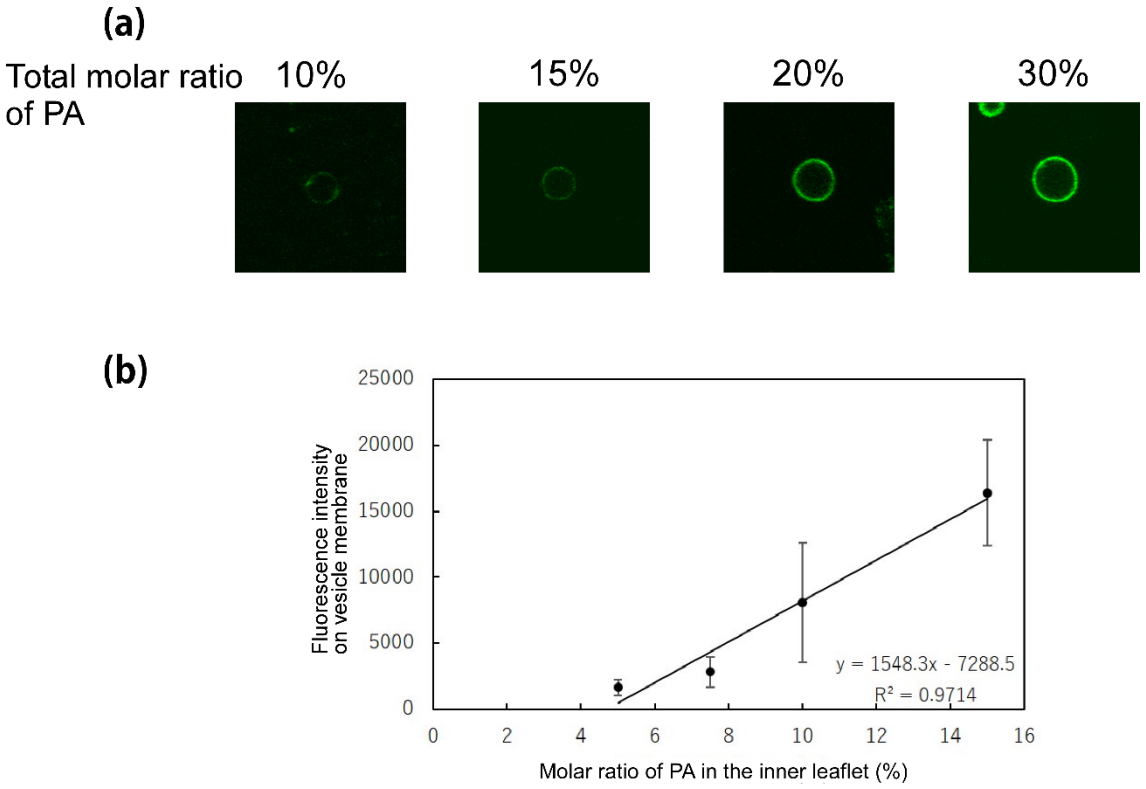

Figure S2 PLD synthesized by fluoride-responsive riboswitch of the activity assay of choline hydrolysis chaining 20 ng/μL, 10 ng/μL, 4 ng/μL, and 2 ng/μL of plasmid DNA final concentration. Three mM NaF was added to the microtube.

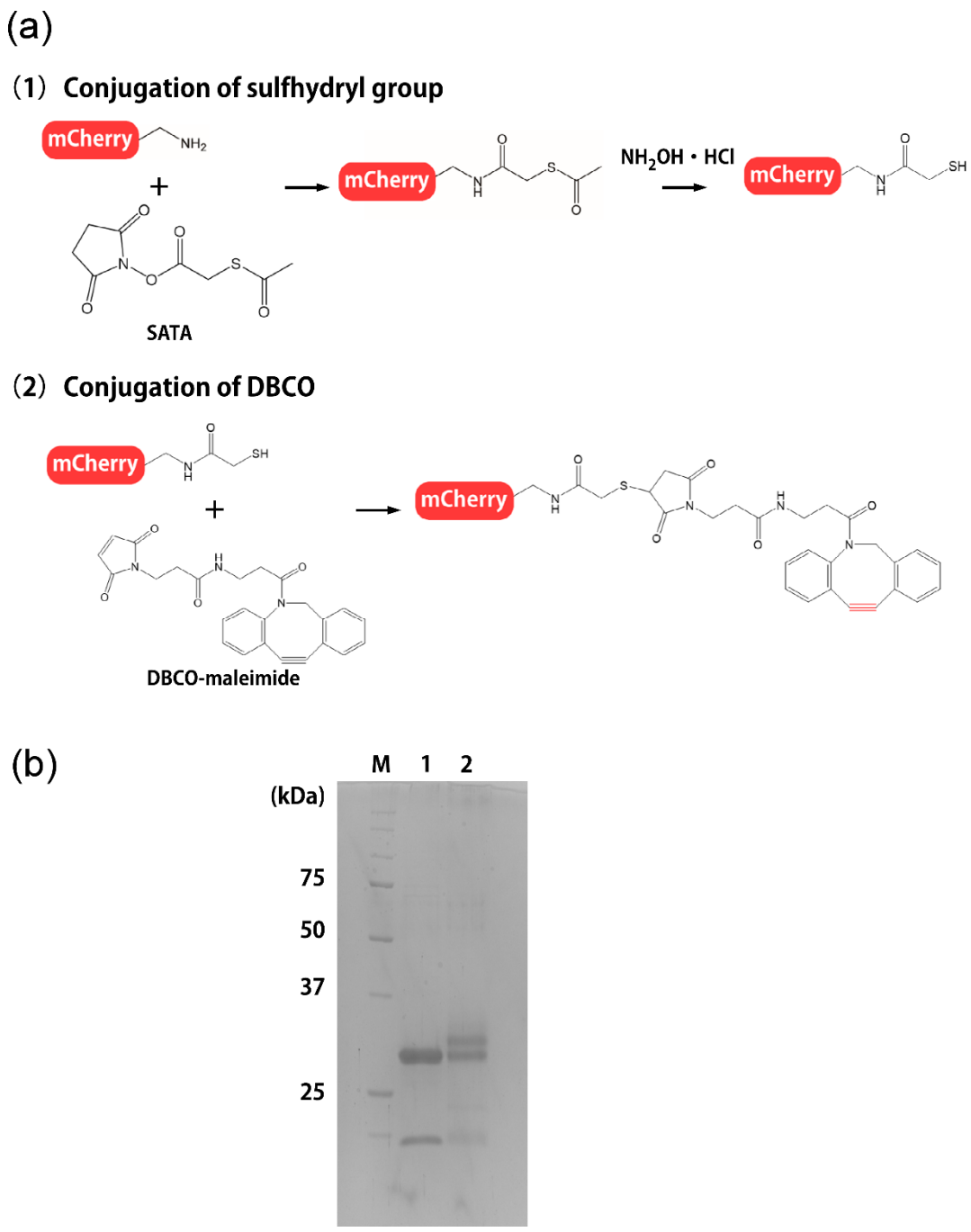

Figure S3 (a) scheme of DBCO-conjugated mCherry. (b) SDS-PAGE analysis for confirming the conjugation of DBCO to mCherry. M: Marker, Lane 1: mCherry, Lane 2: DBCO-conjugated mCherry.

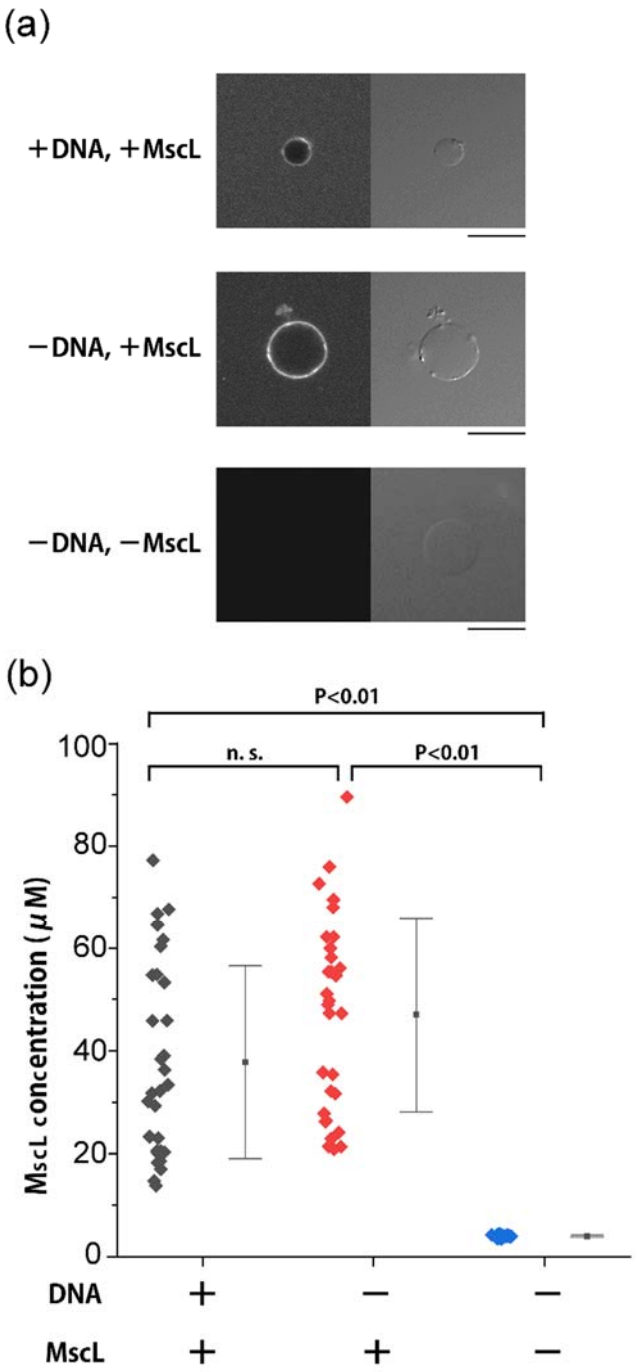

Figure S4 (a) Confocal laser scanning microscopy fluorescence images of the lipid vesicles with/without MscL. Scale bar, 10 um. (b) Concentration of MscL on the lipid vesicles with DNA and MscL (n=30 vesicles, N= 3 experiments), with MscL (No DNA) (n=30 vesicles, N= 3 experiments), and without DNA and MscL (n=30 vesicles, N= 3 experiments).