Electronic Supplementary Information

# DNA-Induced Fusion between Lipid Domains of Peptide-Lipid Hybrid Vesicles

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**CONTENTS**

## SI-1. Materials and Methods

### Materials

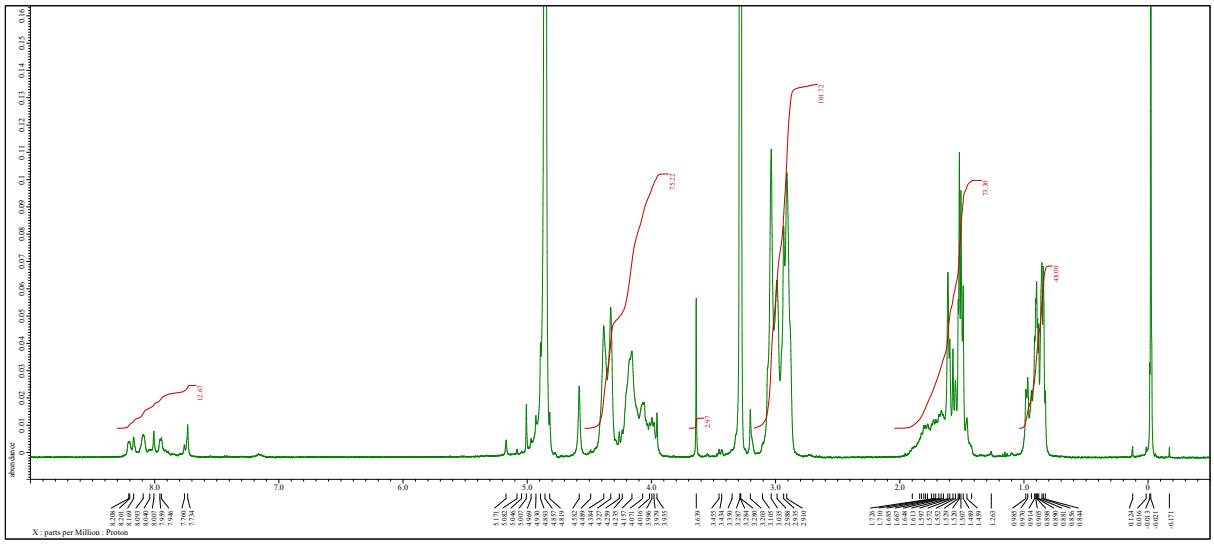
All amino acids and condensation reagents were purchased from Watanabe Chemical Co., Ltd., Japan. DNA-cholesterol conjugates were purchased from Tsukuba Oligo Service Co., Ltd., Japan. The sequences of the DNA-A-chol and DNA-A′-chol conjugates are: cholesterol-TEG-5′-TGT GGA AGA AGT TGG TGA-3′ and 5′-TCA CCA ACT TCT TCC ACA-3′-TEG-cholesterol, respectively, where TEG = triethylene glycol.

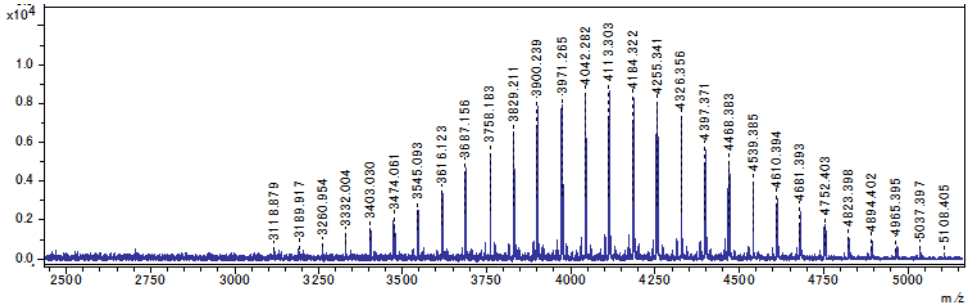
### Synthesis of amphiphilic polypeptide, S35L16

Amphiphilic polypeptide poly(Sar)$_{35}$-*b*-(L-Leu-Aib)$_8$ (S35L16) (Fig. 1) was synthesized as reported previously.[1-3] The synthesized compound was characterized by $^1$H NMR spectroscopy and MALDI-TOF mass spectrometry.

1H NMR (400 MHz, $CD_3OD$) δ (ppm) 8.2–7.7 [m, 14H, amide], 7.2 [s, 1H, urethane], 4.5–3.9 [br, 78H, LeuC$^{\alpha}H$, (SarC$H_2$)$_{35}$], 3.64 [s, 3H, OC$H_3$], 3.2–2.8 [m, 105H, (SarC$H_3$)$_{35}$], 2.0–1.4 [m, 72H, LeuC$H_2$, LeuC$^{\gamma}H$, Aib(C$H_3$)$_2$], 1.0–0.8 [m, 48H, Leu(C$H_3$)$_2$].

MALDI-TOF MS calculated $C_{188}H_{325}N_{51}O_{54}Na^+$ $[M+Na]^+$ *m/z* 4184.41, found: 4184.32.






**Preparation of molecular assemblies**

Stock solutions were prepared by dissolving polypeptides (20 mg) and lipids (20 mg) in ethanol (400 μL). Peptide (4.8 μL) and lipid (0.8 μL) stock solutions were mixed and injected with rapid one-shot into saline (Otsuka Normal Saline, Otsuka Pharmaceutical Factory, Inc., Tokyo, Japan) (1 mL) which has about 0.9% NaCl concentration while stirring. After stirring at room temperature for 30 min, the dispersion was heated at 90 °C for 1 h. DNA-labeled PLHVs (PLHV-A and PLHV-A′) were prepared by rapidly adding DNA-A-chol or DNA-A′-chol solutions into the PLHV dispersions during the stirring process, before heat treatment. The final

concentrations of lipid and DNA were 59 μM and 0.1 μM, respectively (lipid/DNA ratio of 590:1).

The DNA concentration referred the Vogel group's paper.[4] In their paper, the authors chose 46 μM lipid (DOPC+DOPE+cholesterol) and 0.105 μM DNA. We used the same concentration (0.105 μM) of DNA to label our peptide/lipid hybrid assembly. Because the assembly composed of peptide (0.24 mg, 57 nmol) and lipid (0.04 mg, 59 nmol), calculated lipid/DNA ratio was 590:1. We used the DNA-labelled sample for fusion experiment because the ratio was not so far from Vogel group's value.

**Transmission electron microscopy (TEM)**

TEM images were taken using a JEOL JEM-1230 instrument at an accelerating voltage of 80 kV. A drop of dispersion was mounted on a carbon-coated Cu grid (Okenshoji Co., Ltd., Japan). Negative staining was performed with 2% samarium acetate, followed by removal of excess fluid with a filter paper.

**Laurdan Test**

The membrane fluidities of the peptide-lipid hybrid vesicles, S35L16 assemblies, and dimyristoyl phosphatidylcholine (DMPC) liposomes were measured using *N*,*N*-dimethyl-6-dodecanoyl-2-naphthylamine (Laurdan), which is a fluorescent dye that is sensitive to the polarity of its surrounding environment. A general polarization value, $GP_{340}$, was calculated

using the equation $GP_{340} = (I_{440} - I_{490})/(I_{440} + I_{490})$, where $I_{440}$ and $I_{490}$ are the Laurdan emission intensities at 440 and 490 nm (at 340 nm excitation), respectively.

**Dynamic light scattering (DLS)**

The hydrodynamic diameters of the molecular assemblies in saline were analyzed using a ELSZ-2PL instrument (Photal Otsuka Electronics, Japan) with a He-Ne laser. All measurements were performed at 25 °C. The histogram of hydrodynamic diameter was weighted by the intensity.

**Fluorescence spectroscopy**

The fluorescence spectra of the dispersions were measured using a JASCO FP-6500 spectrofluorometer at 25 °C with a transmission cell.

**Förster resonance energy transfer (FRET) analysis**

FRET analysis was carried out using a fluorometer (Microplate Flash Reader, PerkinElmer, 2013, USA). For the NBD-chol/Rhod-PE (donor/acceptor) pair, the excitation wavelength was set at 460 nm and emission spectra were collected from 480 to 650 nm. FRET was measured in vesicles containing known concentrations of donor and acceptor probes.

PLHVs were labeled with 1 mol% of NBD-chol and 1 mol% of Rhod-PE. Briefly, for hybrid vesicles composed of polypeptides and lipid, 0.05 mg/μL ethanol stock solutions of S35L16,

DMPC, NBD-chol, and Rhod-PE were mixed in the desired ratio (S35L16, DMPC, NBD-chol, Rhod-PE = 73, 73, 0.7, 0.7 nmol) and then injected into saline (1 mL) in one shot. The mixture was then stirred and heated in the same manner as described above (for the preparation of molecular assemblies). Similarly, DMPC liposomes containing NBD-chol and Rhod-PE were prepared as a control sample (DMPC, NBD-chol, Rhod-PE = 73, 0.7, 0.7 nmol). In addition, respective donor-labeled (NBD-chol only) vesicles were prepared to calculate the FRET efficiency ($E$) in the absence and presence of the acceptor according to: $E$ (%) = ($I_D - I_{DA}$) / ($I_D$) × 100, where $I_D$ and $I_{DA}$ are the donor intensities of samples with only donor-labeled vesicles and samples with both donor- and acceptor-labeled vesicles, respectively.

**Fusion test**

Fusogenicity was evaluated by FRET analysis. For this analysis, PLHV-A vesicles were labeled with 1 mol% of NBD-PE and 1 mol% of Rhod-PE. 0.05 mg/μL ethanol stock solutions of S35L16, DMPC, NBD-PE, and Rhod-PE were mixed in the desired ratio (S35L16, DMPC, NBD-PE, Rhod-PE = 73, 73, 0.7, 0.7 nmol) and then injected into saline (1 mL) at one shot. The mixture was then stirred and heated. Dispersions of labeled PLHV-A and PLHV-A′ (or pure PLHV, as a negative control) were mixed in a volume ratio of 1:3 and stirred. The mixture was heated at 90 °C for 15 min and then cooled to room temperature, and this procedure was repeated 3 times. Before and after this procedure, fluorescence spectra were recorded from 480 and 650 nm at an excitation wavelength at 460 nm. Finally, a Triton X-100 solution was added

to the dispersion to induce the micellization of the PLHVs to estimate the 100% emission intensity of NBD-PE.

**SI-2. Size histogram of PLHVs before and after fusion (Fig. S1)**

The histogram of PLHV assembly diameter in TEM images was made. PLHV, PLHV-A and PLHV-A’ did not show any difference. Also, after fusion treatment, the combination of PLHV-A and PLHV-A’ slightly showed the peak shift from 40 nm to 50 nm.

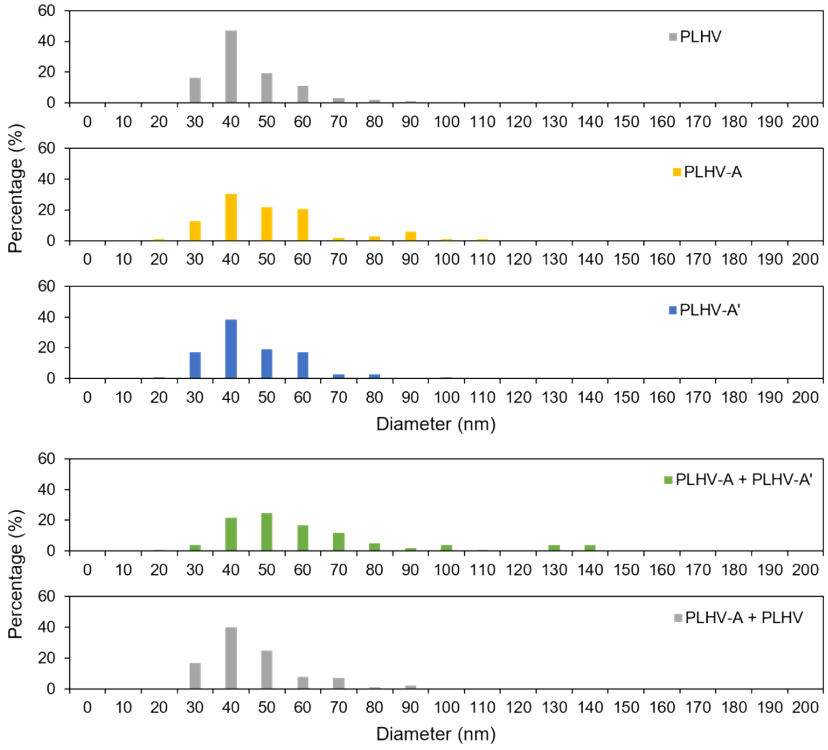



Fig. S1. Size histogram of PLHV assemblies (PLHV: n = 98, PLHV-A: n = 101, PLHV-A’: n = 104, PLHV-A+PLHV-A’: n = 101, PLHV-A+PLHV: n = 101) in TEM images.

**SI-3. TEM images of assemblies after fusion treatment (Fig. S2 and S3)**

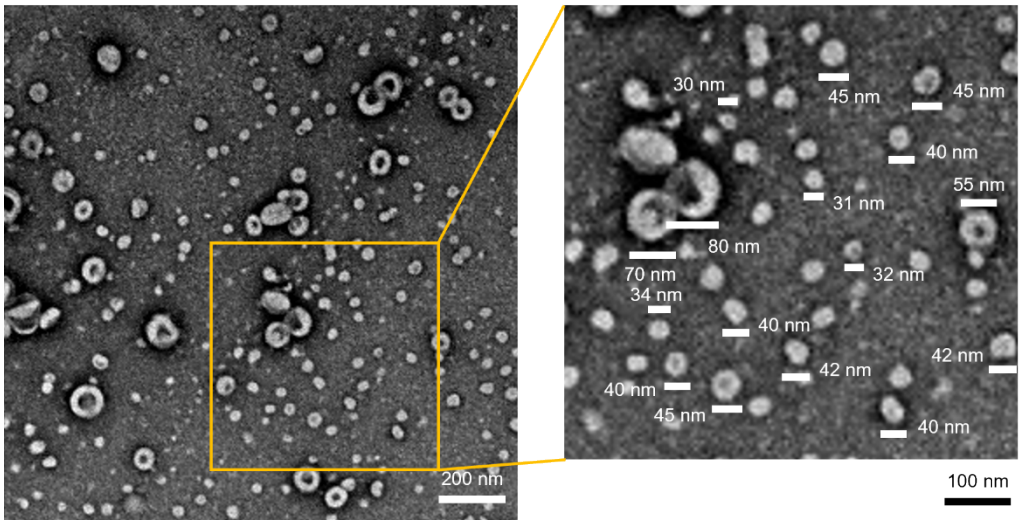



Fig. S2 The magnified TEM images of PLHV-A + PLHV-A’ assembly after fusion treatment.

This is magnified images of Fig.4D.

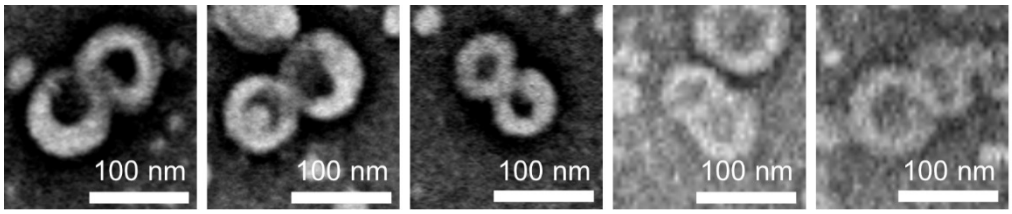



Fig. S3. TEM images of connected vesicles observed in the PLHV-A + PLHV-A′ assembly

mixture after fusion treatment.

## Reference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