# Supplementary Information

## Transformation of oil droplets into giant vesicles

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## A. Materials and methods

All commercially available reagents were purchased from Kanto Chemical Co., Inc., Sigma-Aldrich Co. or Wako Pure Chemical Industries, Ltd. and were used without further purification. $^{1}$H-NMR spectra were recorded on a JNM-ECS400 spectrometer (JEOL, Ltd., Tokyo, Japan). Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) spectra were recorded on a Voyager DE-STR spectrometer (Life Technologies, California, USA). The aggregates (droplets or vesicles) solutions were directly used without any separation step before the MALDI-TOF/MS measurement as the insolubility of the formed catalyst imine (**CI**) and vesicular membrane (**VM**). The morphological behaviour of the GVs was monitored with an IX73 differential interference contrast microscope (OLYMPUS Corp., Tokyo, Japan) equipped with 10×, 20× and 40× objective lenses. Distilled water was used to prepare aqueous solutions in all experiments.

**Turbidity measurements.** Changes in optical density were recorded using an LED-array spectrophotometer (TR-55 turbidity meter, Kasahara Chemical Instruments Corp., Kuki, Japan). After the rapid addition of the catalytic aldehyde (**CA**) or membrane precursor (**MP**) molecules to the oil-in-water emulsion, the quartz cuvette (10 mL) was gently hand-shaken three times; and the development of turbidity was monitored spectrophotometrically until a stable signal was observed.

**Differential interference contrast microscopy observation.** A vesicular dispersion was placed on a glass plate, and the plate was then immediately covered with a cover glass (thickness: approximately 0.15 mm) using a Frame-Seal$^{TM}$ incubation chamber (15 mm × 15 mm × 0.3 mm, Bio-Rad Laboratories, Inc.) as a spacer. The differential interference contrast microscopy images of the vesicles were recorded using an OLYMPUS IX73 (obj. lens 10×, 20× and 40×; N.A. = 0.55) equipped with an OLYMPUS model 12V100WHAL-L halogen lamp.

**Dynamic light scattering (DLS) measurements.** The size of the oil droplets and vesicles were measured at room temperature with an ELSZ-1000 particle analyser (OTSUKA Electronics Co., Ltd., Osaka, Japan), which is suitable for the measurement of particle sizes from 0.6 nm to 10 μm. Therefore, in this study, the data related to particle sizes from 0.6 nm to 10 μm are discussed. The particle size distribution was calculated by an inverse Laplace transformation of the raw data,

which was typically performed using the non-negative least-squares algorithm. Samples were prepared as follows: 2 mL aqueous solution of catalyst aldehyde (**CA**) was combined in a vial with octylaniline (**O**, 25 mM) in a **CA**:**O** concentration ratio of 1:5 (mol%), and the resulting mixture was gently stirred at room temperature.

## B. Formation of catalyst imine (CI) in oil droplets

An aqueous solution of **CA** was added to octylaniline (**O**) at an **O**:**CA** ratio of 5:1 (Fig. S1a). To stabilise the oil system, the dispersion was incubated for 7 h at room temperature with gentle stirring and allowed to stand for 10 min (Fig. S1b). The oil droplet was measured using a MALDI-TOF/MS spectrometer (Fig. S1c). **CI** was synthesised by hydrocondensation between **O** and **CA** in oil droplets. Fig. S1d shows the particle size distribution monitored by dynamic light scattering (DLS). As shown in Fig. S1e and Fig. S1f, NMR measurements suggested that the **CI** was generated via the consumption of the **CA** in the aqueous solution.

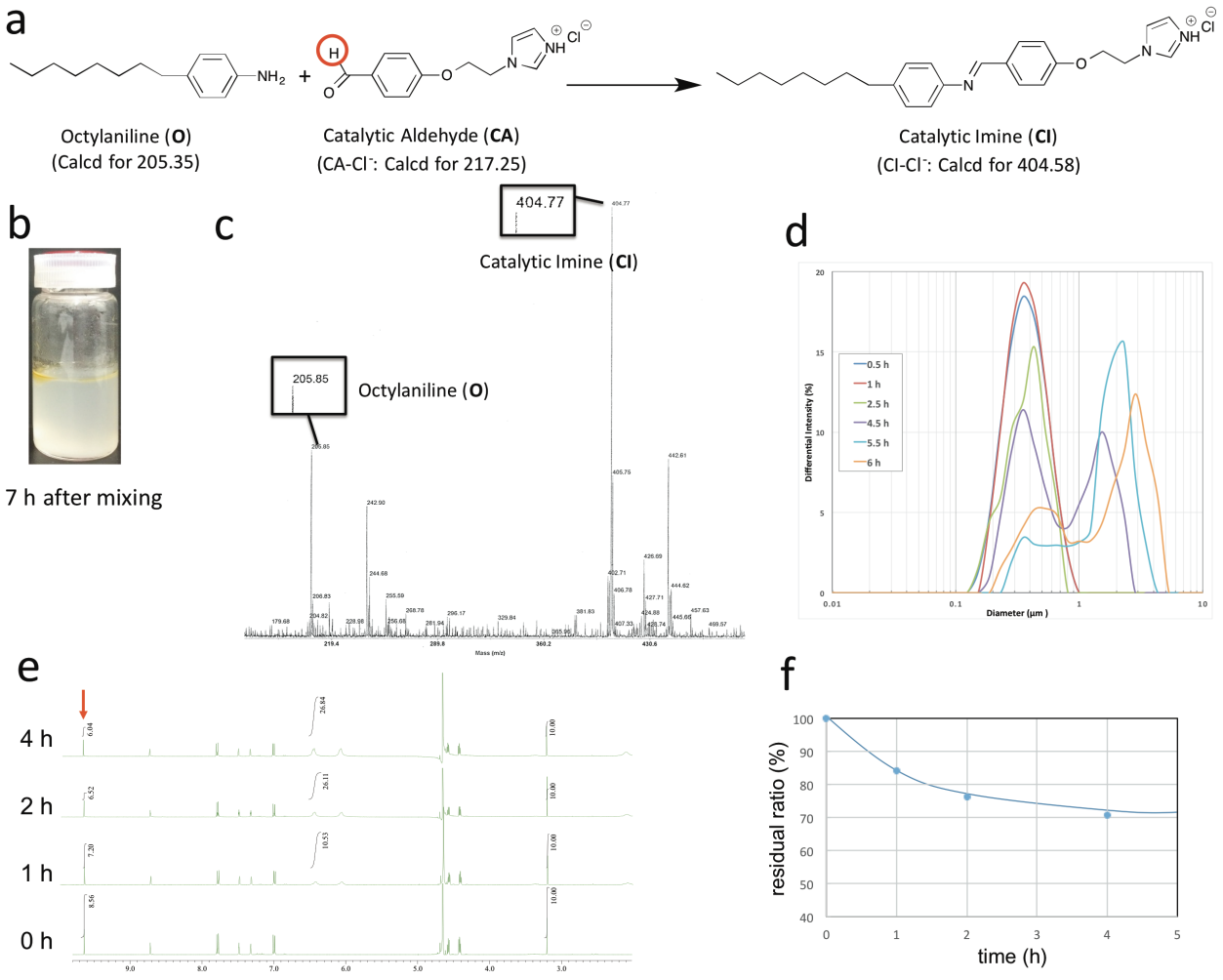


**Figure S1.** a: Scheme of the **CI** production by hydrocondensation between octylaniline (**O**) and the **CA**. b: Dispersion 7 h after **O** and **CA** were mixed and then incubated for 10 min. c: TOF-MS spectrum of an oil droplet containing **CI**. d: Changes in the distribution of DLS measurements over time (0.5, 1, 2.5, 4.5, 5.5 and 6 h after the addition of **CA**). e: $^{1}$H NMR

spectra monitoring the **CA** in the aqueous solution. The red arrow indicates the aldehyde proton peak of **CA** (red circle in Fig. S1a). f: Residual ratio of **CA** in the $D_2O$ solution versus time, as traced by $^1H$ NMR measurements.

## C. MALDI-TOF-MS measurements of unprotonated imine

An aqueous solution of an unprotonated aldehyde was added to **O** at an **O**:unprotonated aldehyde molar ratio of 5:1 (Fig. S2a). To stabilise the system, the dispersion was incubated for 5 h at room temperature with gentle stirring (Fig. S2b) and then characterised by MALDI-TOF/MS (Fig. S2c). Almost no **CI** was detected, and the white aggregation consisted of **O** and the unprotonated aldehyde.

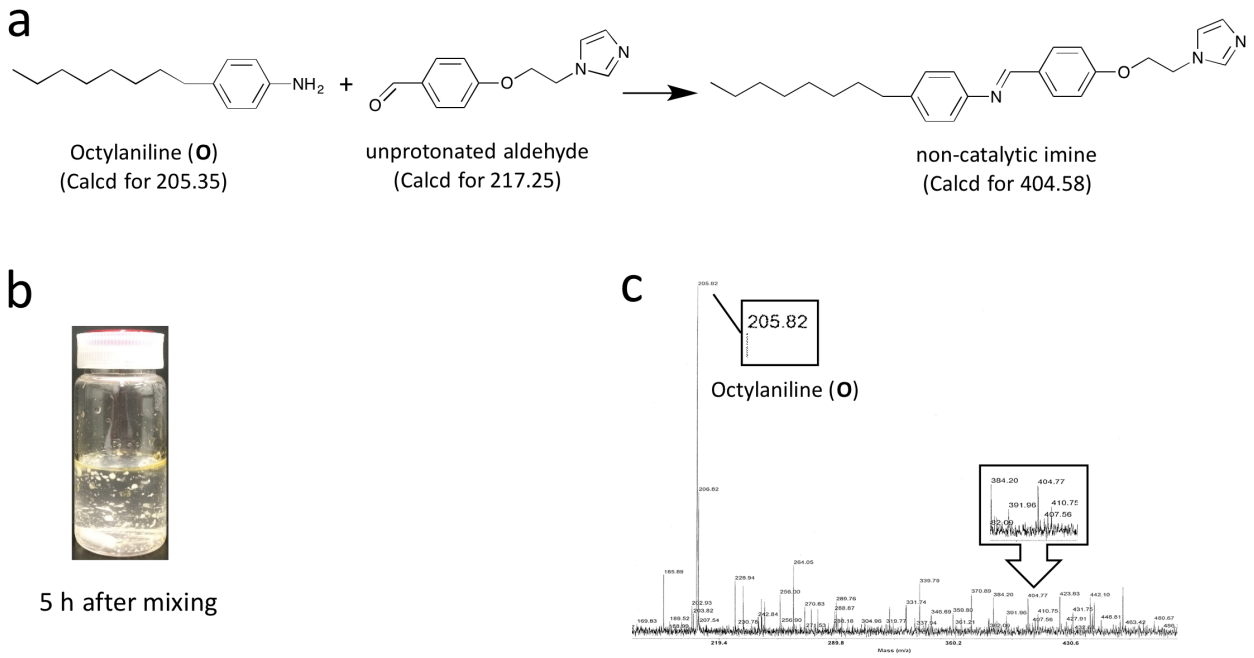


**Figure S2.** a: Scheme of the **CI** production by hydrocondensation between **O** and the unprotonated aldehyde. b: Dispersion of 5 h after mixing of **O** and the unprotonated aldehyde. c: TOF-MS spectrum of white aggregates in the dispersion.

## D. Formation of vesicular membrane (VM) molecules in giant vesicles (GVs)

An aqueous solution of the membrane precursor (**MP**) aldehyde was added to the oil droplet system at an **O**:**CA**:**MP** aldehyde molar ratio of 5:1:4 (Fig. S3a). The dispersion was incubated for 10 h to stabilise the vesicles and was then characterised by MALDI-TOF/MS (Fig. S3b). According to the TOF-MS spectra, both **VM** and **CI** molecules were detected. NMR measurements suggested that the **VM** molecule were generated via the consumption of the **MP** aldehyde in the aqueous solution (Fig. S3c).

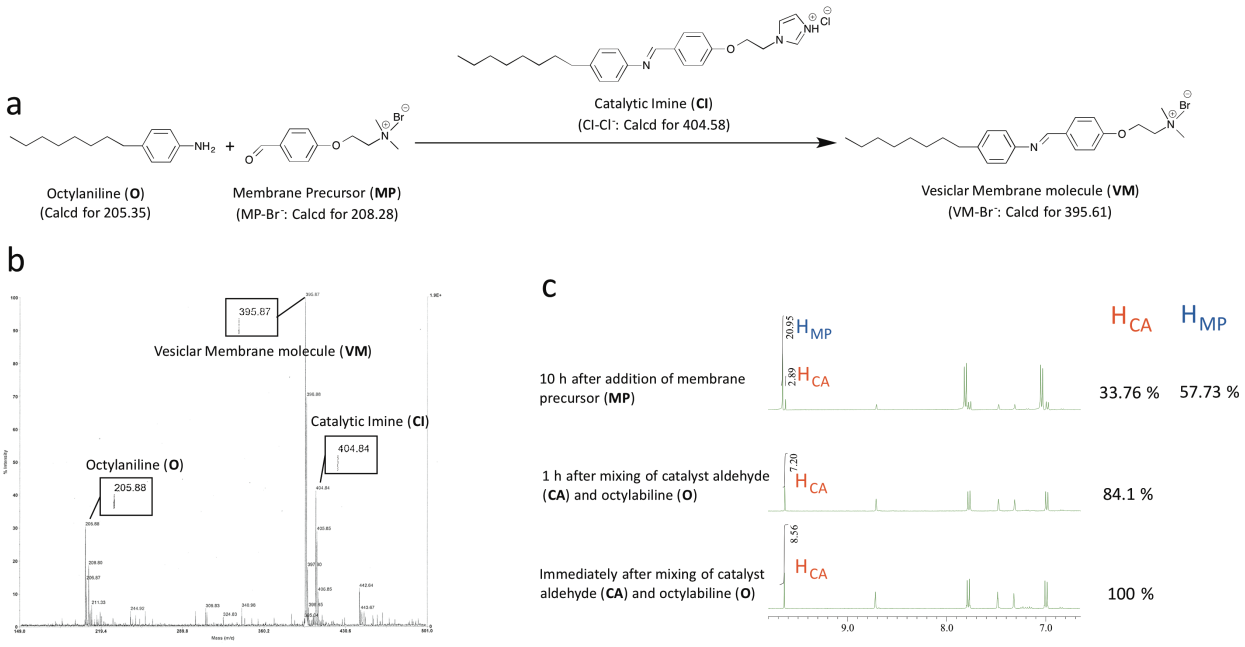


**Figure S3.** a: Scheme for the production of **VM** molecules via hydrocondensation between **O** and the **MP** aldehyde by the **CI**. b: TOF-MS spectrum of vesicles in the dispersion. c: NMR spectra of the dispersion, where $H_{CA}$ and $H_{MP}$ indicate the aldehyde proton peaks of the **CA** and **MP**, respectively.

## E. Interaction between octylaniline and membrane precursor (MP) aldehyde

**O** and **MP** at the molar ratio of 5:4 were mixed and stirred gently (Fig. S4a). The **O** and **MP** aldehydes did not actively react in the absence of a catalyst. This result is consistent with those of a previous study.[34] **O**, the **CA** and **MP** at the molar ratio of 5:1:4 were mixed simultaneously and stirred gently for 10 h, the increased turbidity was obviously observed (Fig. S4b, S4c).

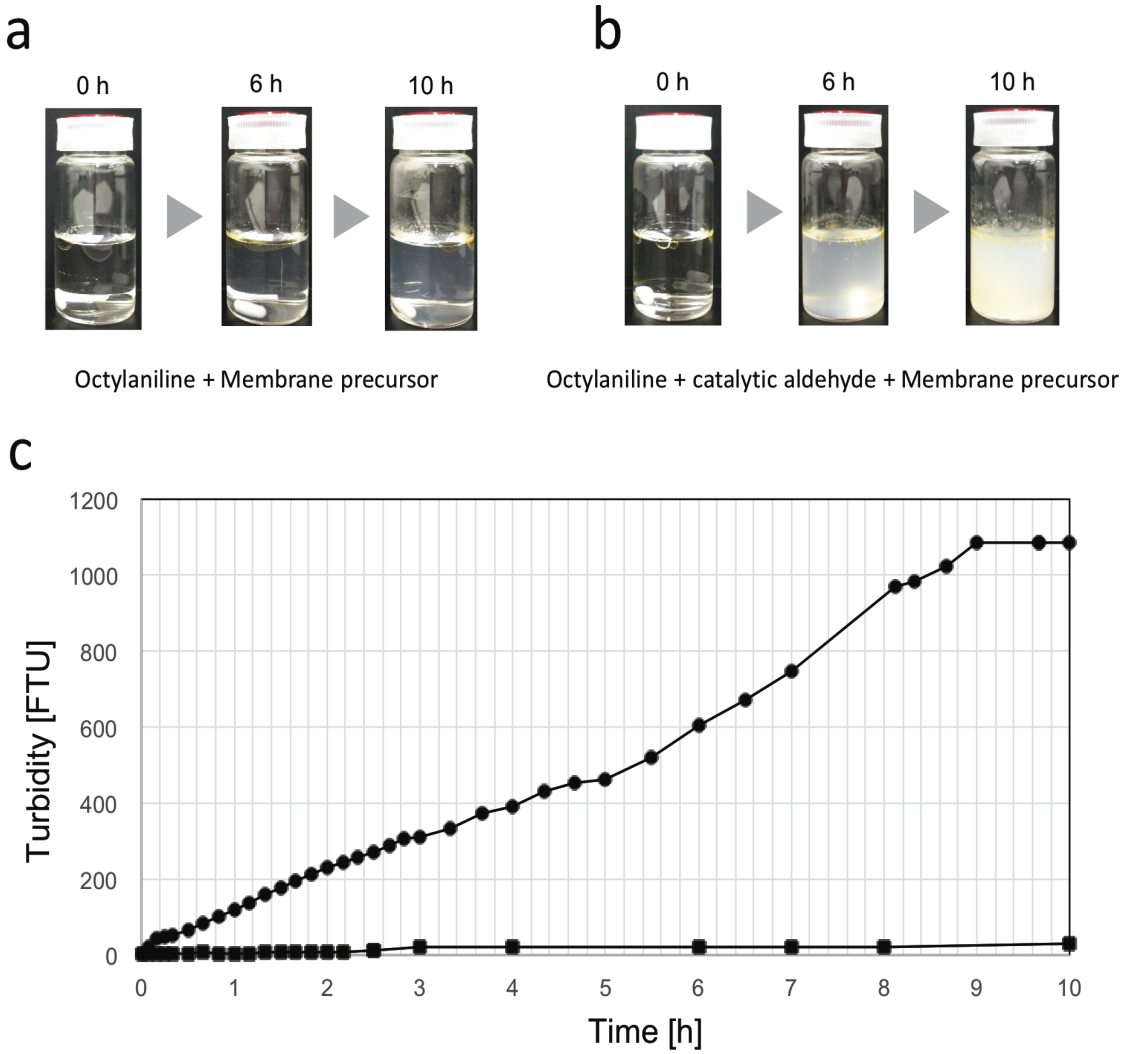


**Figure S4.** a: Observation of turbidity in the vial containing a mixture of **O** and **MP** aldehydes. The yellow oil layer of **O** was observed even after 10 h. b: Observation of turbidity in the vial containing a mixture of **O** and two different aldehydes. c: Turbidity measurements of the dispersion. Squares: Changes in turbidity when the **MP** and **O** were mixed together. Dispersion of a mixture between **O** and **MP** at a concentration ratio of 1:1 (mol%). Circles: Results of turbidity measurements of GVs after mixing **O** and two different aldehydes simultaneously.

Dispersion of a mixture between **CA**, **O** and **MP** at a concentration ratio of 1:5:4 (mol%).

## F. Formation of catalyst in giant vesicles by mixing three molecules

To investigate the composition of a vesicle via simultaneously mixing octylaniline and two aldehydes, **O**, **CA** and **MP** were mixed in a vial at room temperature at an **O**:**CA**:**MP** molar ratio of 5:1:4 (Fig. S5a). According to the NMR monitoring of reduction of the aldehydes over the course of time, **CI** and **VM** were generated via consumption of **CA** and **MP** in the $D_2O$ solution (Fig. S5c). To stabilise the vesicle, we incubated the dispersion for 10 h and then characterised it by MALDI-TOF/MS (Fig. S5b). According to TOF-MS spectra, both **VM** and **CI** molecules were detected.

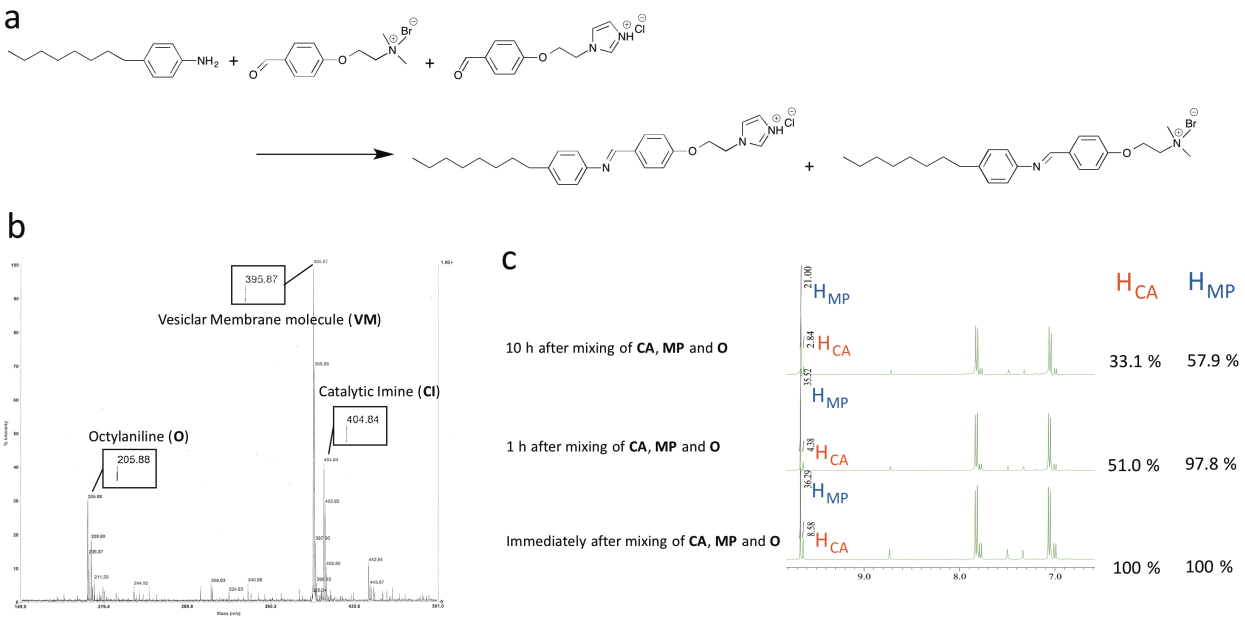


**Figure S5.** a: Scheme showing the production of **CI** and **VM** molecules. b: TOF-MS spectrum of vesicles in the dispersion. c: NMR spectra of the dispersion. $H_{CA}$ and $H_{MP}$ indicate the aldehyde proton peaks of the **CA** and **MP**, respectively.