Electronic supplementary information

One-step preparation of giant lipid vesicles with high encapsulation efficiency using an electrospray technique

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Experimental section

Materials: Poly(allylamine hydrochloride) (PAH, Mw = ~56,000), and fluorescein isothiocyanate–dextran (FITC-dextran, 40k Da) were purchased from Sigma (St. Louis, MO). Green fluorescent polystyrene microspheres (0.5 μm in a diameter) were purchased as a suspension from Duke Scientific (Palo Alto, CA). D-(+)-Glucose was purchased from Nacalai Tesque Inc. (Kyoto, Japan). SYTO 9 was purchased from Invitrogen. L-α-lecithin (from soybean) that contained 95.5 wt% phosphatidylcholine and 0.3 wt% tryacylglycerol was purchased from Calbiochem (Darmstadt, Germany). All other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Electrospray: The coaxial electrospray (NF-102, MECC Co., Ogori, Japan) experimental equipment consisted of a syringe pump, a stainless steel needle, and a high-voltage generator.[7e]. Typically, an aqueous solution containing 10 wt% PAH, and ethylene glycol containing 1 wt% lecithin and 0.004 wt% L-α-phosphatidylethanolamine-*N*-(lissamine rhodamine B sulfonyl)(ammonium salt) were simultaneously sprayed from inner and outer stainless needles (cathode), respectively, into an oppositely charged citrate buffer (5 mM, pH 7) (anode) in a foil-wrapped dish to form giant lipid vesicles. During electrospraying, the citrate buffer solution in the dish was stirred continuously and gently (~ 200 rpm) using a magnetic stirrer bar. To encapsulate substrates, the substrates were added to a PAH solution (50 μg/ml calcein and 50 μg/ml FITC-dextran). A suspension of fluorescent microparticles (140 μl) was added to 0.86 ml of a PAH solution, and was subjected to electrospraying.

The feed rates for the inner and outer solution were set at 1.0 and 0.5 mL/h, respectively, and the working voltage was 15 kV. The distance from the needle to the collector was 5.0 cm. The inner/outer diameters of the inner and outer stainless needles were 130/310 μm and 1.0/2.5 mm. The fluorescently labeled giant lipid vesicles were observed using a confocal laser scanning microscope (CLSM) (FV1000-D, Olympus Co., Tokyo, Japan). Based on the microscope images, the diameters of 300 vesicles were measured using image analysis software, ImageJ (NIH).

Encapsulation of substrates: The encapsulation efficiency was determined by measuring the non-encapsulated substrate contents (calcein and FITC-dextran) in the bulk phase. The bulk phase was collected by filtering the giant-vesicle solution using a membrane filter (pore size 0.45 μm), under gentle pressure. The non-encapsulated substrates in the filtrate were quantified using a fluorescence spectrometer (LP8300, JASCO, Tokyo, Japan). Calcein and FITC-dextran were excited at 495 nm, and fluorescence emission was detected at 521 nm.

E. coli K12 (NBRC 3301) was cultured in FAB medium for 24 h. The *E. coli* suspension (1 ml) and a 10 wt% PAH aqueous solution (pH 7, adjusted by NaOHaq) were mixed, and the result was used as the inner electrospraying solution. *E. coli* cells in the giant lipid vesicles were dyed using SYTO9, and this was followed by CLSM observations at 25°C.

Results

SAXS measurement of lecithin dispersed in a phosphate buffer.

The formation of lipid bilayers was observed by small-angle X-ray scattering (SAXS) using a Nano-Viewer RA-Micro 7 (RigaKu Co., Japan). The measurements were made with a Cu Kα radiation generator (λ=0.1542 nm) operating at 40 kV and 20 mA for 15 min at room temperature. A small amount of PEG solution (1 ml) containing 1 wt% lecithin was mixed with a phosphate buffer (9 ml). The lipid bilayer formed in a buffer was subjected to SAXS measurements (Figure S1). The scattering profile (peaks repeated with the same interval at 2θ of 1.50 and 3.0°) indicates a lipid lamella structure (spacing: d was 5.9 nm).

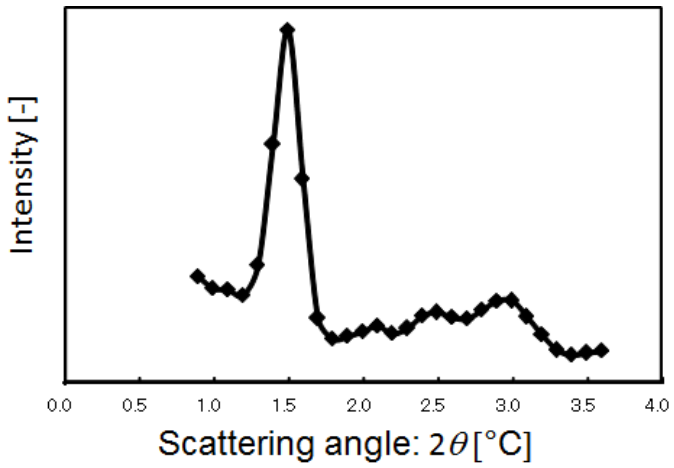

Figure S1. SAXS measurement of lecithin dispersed in a phosphate buffer.