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Supporting Information

Direct Observation of Bacterial Growth in Giant Unilamellar Vesicles: A Novel Tool for Bacterial Cultures

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Experimental Procedures

Materials

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[biotinyl(polyethyleneglycol)-2000] (biotin-PEG-DSPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Rhodamine-B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (rhodamine-DHPE) was purchased from Invitrogen (Carlsbad, CA, USA). Mineral oil (23334-85) was purchased from Nacalai Tesque (Kyoto, Japan). Glucose, sucrose, and sodium chloride were purchased from Wako Pure Chemicals (Tokyo, Japan). Yeast extract and bactotryptone were purchased from BD Biosciences (Franklin Lakes, NJ, USA), and deionized water was obtained from a Millipore Milli-Q system (Burlington, MA, USA).

Preculture of *E. coli*

E. coli was inoculated in 1× LB medium (1 g yeast extract, 2 g bactotryptone, and 2 g sodium chloride in 200 mL of deionized water) from a LB plate and incubated at 37°C overnight (12–14 hours). After incubation, 20 μL of culture solution was collected, transferred to fresh 1× LB medium to a total volume of 2 mL, and cultured for 2 hours.

Synthesis of Giant Unilamellar Vesicles (GUVs) Containing *E. coli*

We prepared lipid stock solutions of POPC (10 mM) and biotin-PEG-DSPE (0.1 mM) in chloroform/methanol (2:1 v/v). First, 20 μL of POPC solution and 4 μL of biotin-PEG-DSPE solution were poured into a glass tube. These two different lipid compositions were used at a POPC:biotin-PEG-DSPE molar ratio of 100:0.2. To prepare the oil phase with lipids, the organic solvent was evaporated by airflow to form a lipid film at the bottom of the glass tube, which was then placed in a desiccator and the lipid film was dried for 1 hour. Mineral oil (200 μL) was then

added to the glass tube, followed by sonication in an ASU-3D ultrasonic bath (AS ONE, Osaka, Japan) for at least 1 hour, with final concentrations of 1 mM POPC and 0.002 mM biotin-PEG-DSPE.

GUVs were prepared by adapting a previously published protocol.[1] To create an oil-water interface in a 1.5 mL-sized plastic tube, we introduced 50 μL of 1× LB medium with 200 mM glucose (external solution of GUVs) into the 1.5 mL-sized plastic tube and added 150 μL of the mineral oil containing lipids. The plastic tube was then incubated at room temperature (25°C) for 10–15 min. A glass capillary was placed in a holder and this holder set was inserted into the plastic tube. We added 2 μL of *E. coli* solution consisting of 1× LB medium with 200 mM sucrose (internal solution of GUVs) into the glass capillary. The plastic tube was then centrifuged for 10 minutes at 1600 × *g* at room temperature (25°C) in a ATT101 desktop centrifuge (Hi-Tech Co., Tokyo, Japan). The oil phase was aspirated from the plastic tube using a pipette, and the GUVs containing *E. coli* were collected from the plastic tube.

Preparation of Small Unilamellar Vesicles (SUVs)

For the preparation of the supported lipid bilayer membrane, small unilamellar vesicles (SUVs) were prepared. Using the same lipid composition for GUV preparation, 20 μL of the POPC solution and 4 μL of biotin-PEG-DSPE solution were poured into a glass tube. The organic solvent was then evaporated by airflow to form a lipid film at the bottom of the glass tube, which was then placed in a desiccator and the lipid film was dried for 1 hour. A 200-μL aliquot of 1× LB medium with 200 mM glucose (external solution of GUVs) was then added to the glass tube, which was sonicated in an ultrasonic bath for at least 1 hour, with final concentrations of 1 mM POPC and 0.002 mM biotin-PEG-DSPE. After sonication, SUVs were prepared by the extrusion method[2] using a Mini-extruder (Avanti Polar Lipids, Alabaster, AL, USA) and polycarbonate membranes with 100-nm pores (Whatman, Cambridge, UK).

Construction of the GUV Observation System (Bacterial Culture System)

GUVs were observed using a handmade chamber system. A chamber with a double-faced seal (10 × 10 × 1 mm) was drilled to create a 5-mm hole and pasted on a cover glass (40 × 30 mm, thickness 0.25–0.35 mm). For the preparation of the supported lipid bilayer membrane at the hole of the spacer, 20 μL of SUV-containing solution was added to the hole and incubated at room temperature (25°C) for 30 minutes. The hole was washed twice with 1× LB medium containing 200 mM glucose, and then 10 μL of neutravidin (1 mg/mL; Thermo Fisher Scientific, Waltham, MA, USA) was introduced into the hole and incubated at room temperature (25°C) for 15 minutes, followed by two washes with 1× LB medium containing 200 mM glucose. The solution containing GUVs was then added into the hole of the chamber and the chamber was sealed with a cover glass (18 × 18 mm, thickness 0.12–0.17 mm).

Fluorescence Imaging of GUVs Containing *E. coli*

Confocal laser-scanning imaging of GUVs containing *E. coli* (Figure 2a and d) was performed using an IX-73 inverted microscope (Olympus, Tokyo, Japan) equipped with a CSU10 spinning disk confocal scanning unit (Yokogawa, Tokyo, Japan), and LUCPLFLN 40×/0.6 NA and 60×/0.7 NA objective lenses (Olympus). Images were captured using a Zyla 4.2 plus sCMOS camera (Andor, Belfast, UK), and images shown in Figure 2b and c were captured with an inverted microscope (Eclipse Ti-E, Nikon, Tokyo, Japan) equipped with an A1R laser-scanning confocal microscopy system (Nikon), a GaAsP detector (Nikon), and an CFI Apo 60×/1.4 NA oil λs oil-immersion objective lens (Nikon). Sectioned images were deconvoluted by the imaging software NIS-Elements version 4.51 (Nikon). All confocal images of Z-projection were processed with ImageJ. For the imaging of GUVs containing *E. coli*, rhodamine-DHPE was used to stain the GUV membrane, and rhodamine-DHPE was mixed with POPC and biotin-PEG-DSPE in preparation

of the oil phase at a final molar ratio of 0.001 against POPC. *E. coli* cells were stained using SYTO9 as follows: 50 µL of *E. coli* solution (1× LB medium with 200 mM sucrose) with 5 µM SYTO9 was incubated at room temperature (25°C) for 20 minutes before encapsulation. After incubation, encapsulation into GUVs was carried out as described above.

To detect cell viability, *E. coli*-containing solution was mixed with 5 µM of SYTO9 and propidium iodide (PI) (Dojindo, Kumamoto, Japan) and incubated at room temperature (25°C) for 20 minutes. Dead *E. coli* cells were prepared by treatment with 100% methanol for 1 minute[3]. We checked the cell viability of *E. coli* before and after encapsulation in GUVs.

Bacterial Culture Inside GUVs Against Antibiotic Compounds

To evaluate the protection of *E. coli* within GUVs against external antibiotics, 1 µL of ampicillin (1 µg/mL, Wako Pure Chemicals, Osaka, Japan) was added to *E. coli*-containing GUVs in the hole of chamber, and the chamber was sealed with a cover glass. The *E. coli*-containing GUVs were incubated at 37°C on a microscopic heating stage system by using a TP-110R-100 thermoplate (TOKAI HIT, Shizuoka, Japan). Bacterial growth was captured with a Zyla 4.2 plus sCMOS camera (Andor, Belfast, UK).

Image Processing and Quantification of *E. coli* Growth

To quantify *E. coli* growth, the original phase-contrast microscopic images were used and analyzed with ImageJ (Figure S8a). We determined the edges of *E. coli* cells inside a GUV (Figure S8b). The image was binarized and the region excluding *E. coli* (A_{ex}) was measured (Figure S8c). The area occupied by *E. coli* ($A_{E.\ coli}$) inside a GUV was calculated as $A_{E.coli}$ (%) = ($A_{GUV} - A_{ex}$) / A_{GUV} × 100.

References

1. M. Morita, H. Onoe, M. Yanagisawa, H. Ito, M. Ichikawa, K. Fujiwara, H. Saito, M. Takinoue, *ChemBioChem* **2015**, *16*, 2029–2035.
2. D. J. Lestage, M.W. Urban, *Langmuir* **2005**, *21*, 4266–4267.
3. D. K. Kang, X. Gong, S. Cho, J. Y. Kim, J. B. Edel, S. I. Chang, J. Choo, A. J. deMello, *Anal. Chem.* **2015**, *87*, 10770–10778.

Supporting Figures

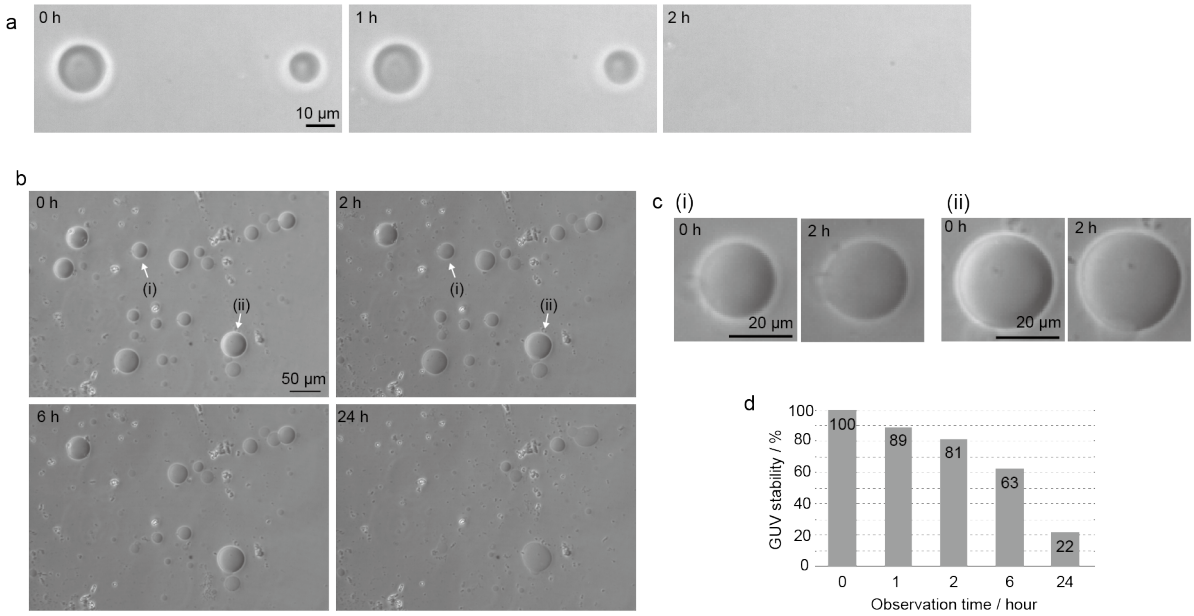


Figure S1

a) Rupture of GUVs on an uncoated glass substrate after a 2-hour incubation at 37°C. b) Time-lapse images of the stability of GUVs immobilized on a glass substrate coated with BSA. c) High-magnification images showing deformation of GUVs by binding to BSA, which are indicated as (i) and (ii) with white arrows in image b. d) The percentage of intact GUVs remaining at each incubation time.

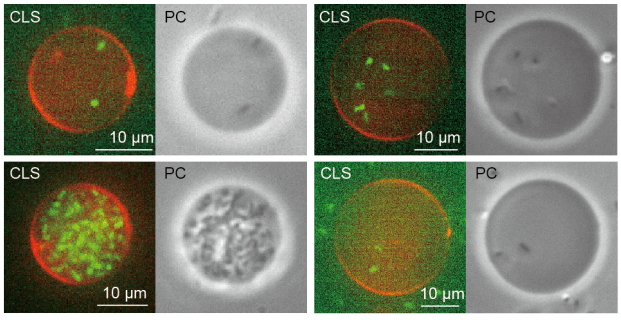


Figure S2

Confocal laser-scanning (CLS) and phase-contrast (PC) microscopic images in the equatorial plane of a GUV containing SYTO9-stained *E. coli*.

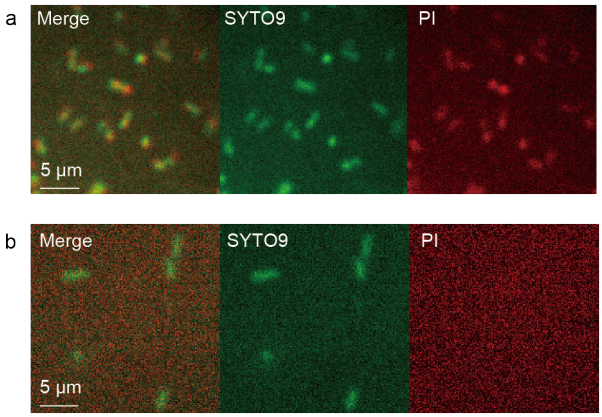


Figure S3

Fluorescence microscopic image of *E. coli*. a) Dead *E. coli* cells stained with SYTO9 and PI. b) Live *E. coli* cells stained with SYTO9 and PI in 1× LB medium. There are no *E. coli* cells positive for PI staining because all *E. coli* cells are living.

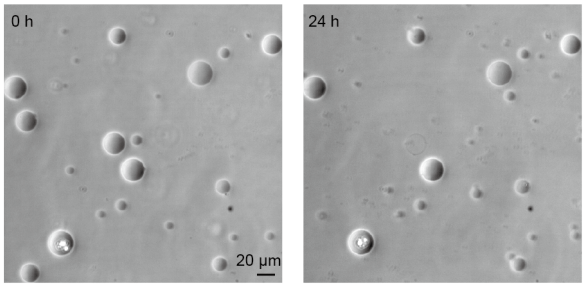


Figure S4

Microscopic image of GUVs after external addition of ampicillin (1 µg/mL). The image on the left shows GUVs before the addition of ampicillin, while the image on the right shows the same field 24 hours after the addition of ampicillin.

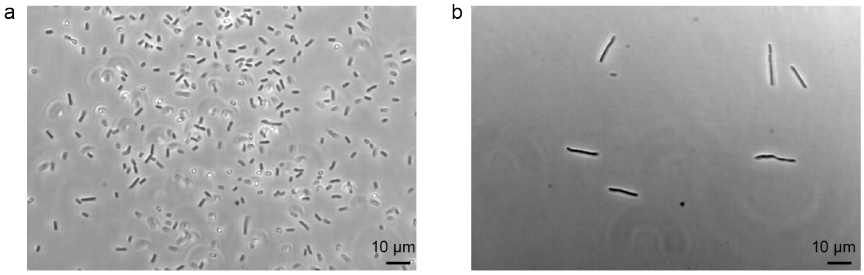


Figure S5

Microscopic image of cell shapes of *E. coli* after 1 hour a) in the absence of ampicillin or b) in the presence of ampicillin (1 µg/mL).

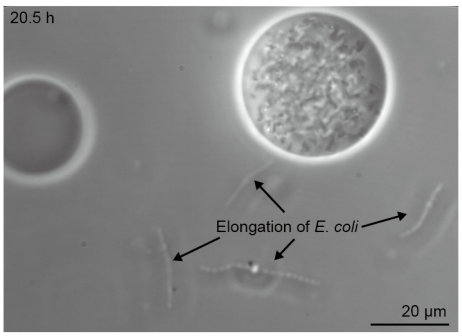


Figure S6

Microscopic image showing elongation of *E. coli* outside GUVs as a result of ampicillin exposure (1 µg/mL) for 20.5 hours.

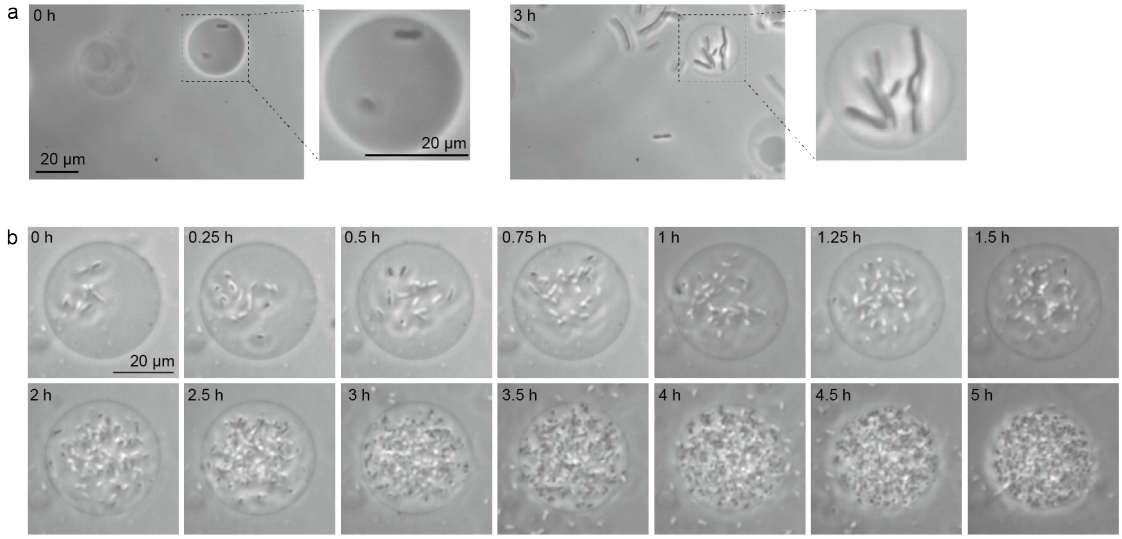


Figure S7

The growth state of *E. coli* inside a defective GUV. a) Elongation of *E. coli* inside a defective GUV following exposure to ampicillin (1 μg/mL). b) Time-course series of images showing the growth of *E. coli* inside a defective GUV without ampicillin.

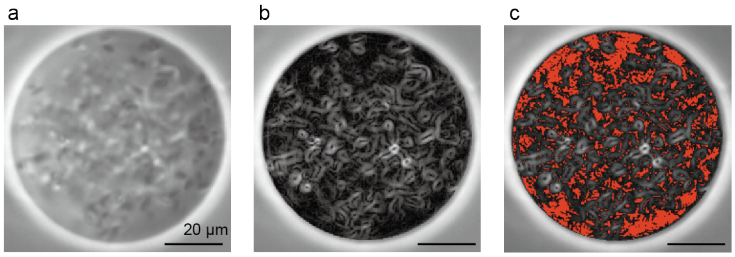
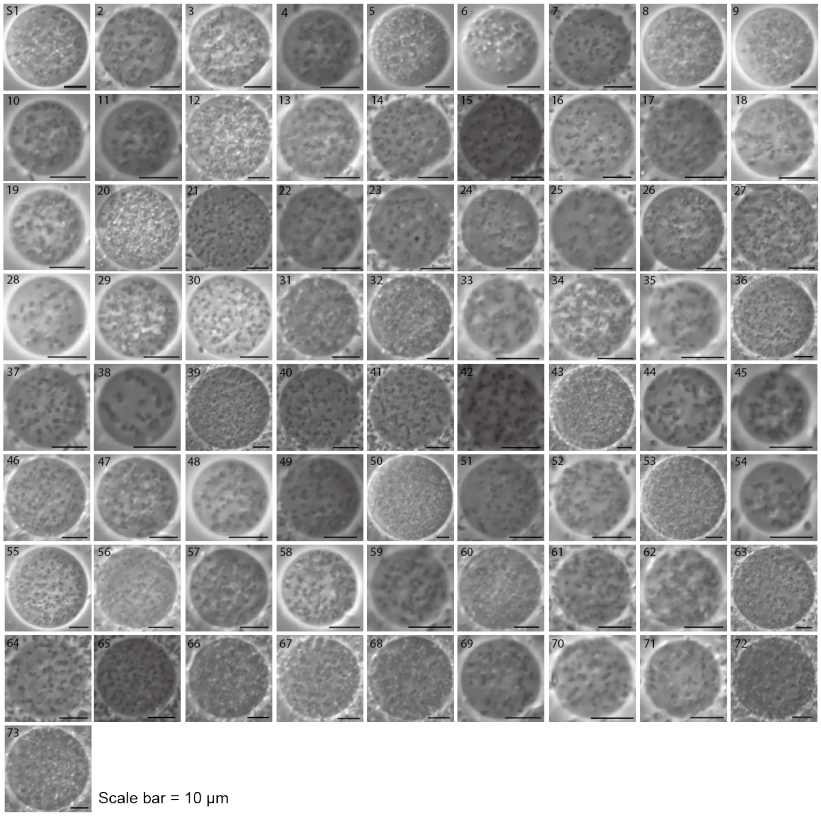


Figure S8

Image processing before analysis of the area occupied by *E. coli* inside a GUV. a) Original image; b) determination of the edges of *E. coli* cells; and c) binarized image from b.



	Size (µm)	Area (%)			
S1	36	77.8	S37	22.6	43.2
S2	26.8	59.1	S38	17.9	35.6
S3	28.8	79.6	S39	49.1	71.7
S4	14.4	53	S40	30.9	55.6
S5	32.1	78.5	S41	34.3	63.9
S6	22.3	58.3	S42	20.1	47.2
S7	27.2	44.8	S43	55.3	83.3
S8	31.1	77.4	S44	21.9	42.6
S9	30.9	76.4	S45	16.5	62.5
S10	20.5	59.1	S46	30.7	70.4
S11	19.1	52.7	S47	28	66.5
S12	38.6	85	S48	19.3	54.7
S13	22.3	55	S49	23.2	43.3
S14	25	55.4	S50	66.2	72.7
S15	26	48	S51	23.6	40.7
S16	28.8	43.6	S52	19.1	41.5
S17	20.1	50.1	S53	61.3	79.8
S18	21.7	54.9	S54	16.1	40.9
S19	21.3	62.3	S55	40	66.3
S20	45.9	81.7	S56	30.9	66.2
S21	37.6	39	S57	26.8	61
S22	20.5	55.8	S58	24.2	71.7
S23	26.2	46.8	S59	17.7	53.4
S24	26.8	42.5	S60	31.9	73.4
S25	20.9	37.9	S61	22.8	65.3
S26	32.1	60.1	S62	19.5	70.7
S27	30.5	65.9	S63	48.8	71
S28	19.9	43.5	S64	26.8	56.2
S29	22.6	80.8	S65	28.8	59.5
S30	28.6	66.9	S66	36.2	65.2
S31	27	72.9	S67	35.1	67.1
S32	35.3	74	S68	38.2	66.7
S33	16.9	46.5	S69	21.9	56.6
S34	22.3	76.1	S70	18.3	47.9
S35	16.7	35.4	S71	22.3	49.9
S36	43.3	67.2	S72	38.8	70.4
			S73	45.5	73.3

Figure S9

The occupied area of *E. coli* within 73 different GUVs was analyzed. The image of S1 is identical to the image shown in Figure 4A.

Supporting Movie Captions

Supporting Movie 1. 3D reconstruction of a GUV containing SYTO9-stained *E. coli*.

Supporting Movie 2. *E. coli* moving inside a GUV.

Supporting Movie 3. *E. coli* division inside a GUV.