Electronic Supplementary Information

for

Giant vesicle functionally expressing membrane receptors for insect pheromone

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Full experimental details

1. Reagents for GV preparation. Egg yolk lecithin, cholesterol, squalene, liquid paraffin (specific density, 0.86 − 0.89 g/mL at 20 °C), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris), sucrose, and glucose were purchased from Kanto Chemical Co. (Tokyo, Japan). Dioleoylphosphatidylethanolamine-*N*-[methoxy(polyethyleneglycol)-5000] (ammonium salt) (DOPE-PEG5000) was provided by Avanti Polar Lipids, Inc. (AL, USA). TexasRed®-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (TexasRed® DHPE) was purchased from Invitrogen (CA, USA). The cell-free protein synthesizing reagents and Transdirect™ Insect Cell were supplied from Shimadzu Co. (Kyoto, Japan). Canine pancreatic microsomal membranes were obtained from Promega Co. (WI, USA). Bombykol, (*E*,*Z*)-10,12-hexadecadien-1-ol, was provided by Prof. Shigeru Matsuyama (University of Tsukuba, Japan). These reagents were used without further purification. All lipids were stored at −20 °C under nitrogen. Water was distilled and then subjected to ion exchange with a Milli-Q water system (Millipore, Bedford, MA, USA).

2. Synthesis of mRNA from plasmids. DNA fragments encoding EGFP-BmOR1, EGFP-BmOrco, BmOR1, and BmOrco were subcloned into the EcoRV site of pTD1 (Shimadzu Co., Kyoto, Japan) using standard techniques. Each plasmid was linearized by digestion with NotI. RNAs were synthesized from the linearized plasmid using a ScriptMax Thermo T7 transcription kit (TOYOBO, Tokyo, Japan) according to manufacturer instructions. Synthesized RNAs were purified by gel filteration with MicroSpin G-25 (GE Healthcare, UK), dissolved in Tris-HCl buffered solution (50 mM, pH 8.0) and stored at −80 °C until use.

3. Preparation of GV encapsulating EGFP-tagged BmOR1. A stock solution of sucrose (4 M) was prepared using Tris-HCl buffered solution (50 mM, pH 8.0). The reagent solution (Transdirect™ Insect Cell) for cell-free protein synthesis (41 μL) was mixed on ice with the mRNA (EGFP-BmOR1) buffered solution and the sucrose stock solution to give a final volume of 50 μL and a final concentration of 0.32 g/L mRNA and 200 mM sucrose. For protein synthesis, the reagent suspension was incubated in a plastic tube at 25 ± 1 °C for 4 h. Then, the reagent suspension (50 μL) was dispersed in a 1-mL mixture of liquid paraffin/squalene (7/3, v/v) containing 7.6 g/L egg yolk lecithin, 2 mM cholesterol and 0.15 mM DOPE-PEG5000. Working on ice, a 0.3-mL aliquot of the resulting water-in-oil (W/O) emulsion was layered on 1 mL Tris-HCl

buffered solution (50 mM Tris-HCl, pH 8.0; 165 mM NaCl; and 200 mM glucose). After incubation at 0 ^{o}C for 10 min, the W/O emulsion was centrifuged at 18 800 g for 30 min at 0 °C The precipitated GVs were collected through a hole opened at the bottom of the centrifugation tube and observed under a laser scanning confocal fluorescence microscope.

4. Preparation of GVs synthesizing BmOR1 and/or BmOrco. First, 41 μL reagent solution (Transdirect^{TM} Insect Cell) for cell-free protein synthesis was mixed with 2 μL canine pancreatic microsomal membranes dispersion and brought up to a volume of 50 μL with the prepared mRNA buffered solution and sucrose stock solution at final concentrations of 0.32 g/L and 200 mM, respectively, at 0 ^{o}C (on ice). In case that two species of membrane receptors (such as EGFP-BmOR1 and BmOrco) were simultaneously synthesized inside of a GV, the final concentration of each mRNA was adjusted to 0.16 g/L. After the reagent suspension (50 μL) was dispersed in a mixture (1 mL) of liquid paraffin/squalene (7/3, v/v), containing 7.6 g/L egg yolk lecithin, 2 mM cholesterol, and 0.15 mM DOPE-PEG5000. Then, working on ice, 0.3 mL of the resulting water-in-oil (W/O) emulsion was layered on 1 mL Tris-HCl buffered solution (50 mM Tris-HCl, pH 8.0; 165 mM NaCl; 200 mM glucose). After incubating at 0 ^{o}C for 10 min, the W/O emulsion was centrifuged at 18 800 g at 0 °C for 30 min. The precipitated GVs were collected through a hole opened in the bottom of the centrifuge tube and incubated at 25 ± 1 ^{o}C for 4 h to allow protein synthesis to proceed.

5. Microscopy. Differential interference contrast/fluorescence microscopy images of GVs stained by a red-fluorescence lipid probe (TexasRed® DHPE, 5 mol%) were acquired with an Olympus IX71 microscope (Tokyo, Japan) equipped with a 4× objective lens, a CCD camera (DC-330, DAGE-MTI, IN, USA), a halogen lamp and a filter unit (λex; 520–550 nm, λem; >580 nm) for fluorescence microscopy. Laser scanning confocal fluorescence microscopy images of GVs synthesizing membrane receptors were obtained with an FV1000 microscope system (Olympus, Tokyo, Japan) equipped with a 100× oil-emersion lens (N.A. = 1.40), a 488-nm Ar^{+} laser (2.2 mW) and a detection filter (505−525 nm).

6. Western blotting of synthesized EGFP-BmOR1 and EGFP-BmOrco. EGFP-tagged BmOR1 and BmOrco were separately synthesized in plastic tubes as described above. Samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 10% to 20% gradient gel (ATTO, Tokyo, Japan). Then, the proteins were electrophoretically transferred onto a polyvinylidine difluoride membrane

(Hybond-P; GE Healthcare, UK). The membrane was blocked for 1 h with 5% blocking reagent (GE Healthcare) in PBS-T (pH 7.5, 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, and 0.1% Tween 20), incubated for 1 h with 1:1000 anti-GFP rabbit IgG fraction (Molecular Probes®; Invitrogen, MA, USA) in PBS-T, and then washed for 15 min. Following three additional washes for 5 min each, the membrane was incubated for 1 h with 1:10,000 horseradish peroxidase-conjugated anti-rabbit IgG antibody in PBS-T. The signals were visualized by incubating in ECL detection reagent (GE Healthcare) for 5 min and exposed to Hyperfilm-ECL (GE Healthcare).

7. Patch-clamp recording. The whole-cell mode patch-clamp recordings on GVs were performed at room temperature (25 ± 1 °C). Patch-electrodes (7 − 12 MΩ) were fashioned from borosilicate glass capillaries with a Flaming-Brown puller (p-97; Sutter Instrument, CA, USA). The electrode tip was polished with a custom-made microforge. The internal solution (150 mM potassium gluconate, 1 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid (EGTA), 5 mM n-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), 4 mM Na_2-ATP, 0.5 mM GTP, 2 mM NaCl, and 1 mM KCl, pH7.0) was filtered through a 0.2-μm pore diameter filter (6809-1022 Anotop 10; GE Healthcare, UK). Recording chamber (351008 easy-grip dish; BD, NJ, USA) was placed on the stage of an inverted microscope (Diaphoto 300; Nikon, Tokyo, Japan) and micromanipulation of the electrode was carried out under visual control with phase-contrast optics and a 40× objective lens. The ligand (bombykol) was dissolved in 100% dimethyl sulfoxide (DMSO) as a stock solution and diluted with bath solution (50 mM Tris-HCl, pH 8.0; 165 mM NaCl; 200 mM glucose) prior to experiments. The final concentrations of the ligand solutions used were 1 μM or 10 μM in bath solution containing 0.1% DMSO. For the application of the ligand, a perfusion valve control system (VC-6; Warner Instruments, USA) was used. Briefly, the ligand solutions were fed by gravity (approximate flow rate of 0.5-1 ml/min) into a three-way valve that was operated under remote control from an electronic pulse generator. The solutions then flowed through an application pipette into the recording chamber. Recordings were acquired with an Axopatch 1D amplifier (Molecular Devices, CA, USA) and sampled with a Digidata 1200 interface (Molecular Devices, CA, USA). These devices were controlled on a computer equipped with pCLAMP software (Molecular Devices, CA, USA). Junction potentials were nullified prior high-resistance (GΩ) seal formation. The signals were sampled at 20 kHz and filtered in low-pass mode at 2 kHz, and analyzed with Clampfit (Molecular Devices, CA, USA) and MatLab (MathWorks, MA, USA).

Fig. S1

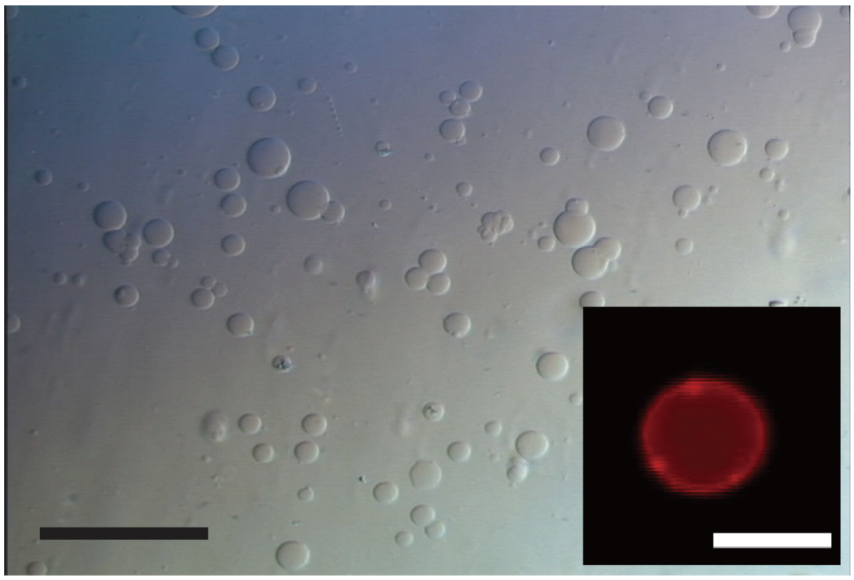

Fig. S1 Differential interference contrast image of GVs stained by a red-fluorescence lipid dye. Bar = 100 μm. Inset; fluorescence microscopy image of a GV (bar = 5 μm).

Fig. S2

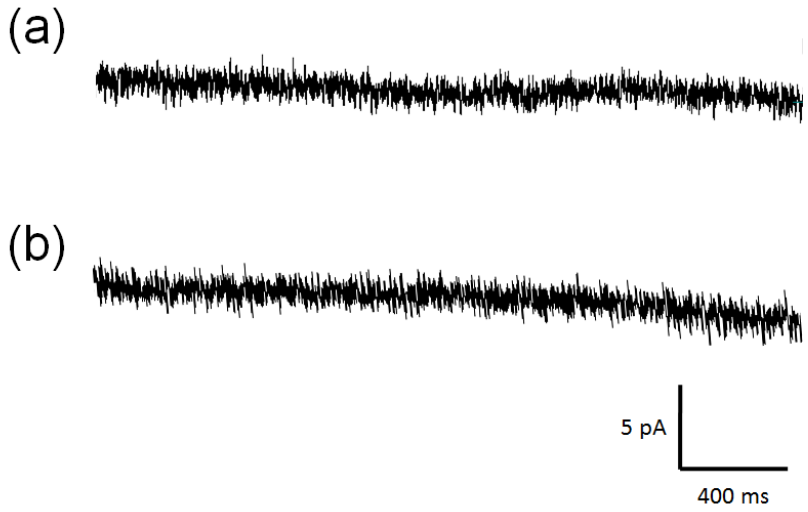

Fig. S2 Current traces of GVs containing no mRNA of BmOR1 nor BmOrco before stimulation (a) and during stimulation of 1 μM bombykol (b), recorded with a voltage clamp at −70 mV.