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Supporting Information

Antibody-Mediated Functional Control of Dansylated Polytheonamide Mimic

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Fluorescence Measurements

Preparation of LUVs

Large unilamellar vesicles (LUVs) for fluorescence measurements were prepared according to the thin-film hydration method,[S1] followed by extrusion through Nuclepore polycarbonate filters mounted in the mini-extruder aparatus.[S2] A solution of egg yolk phosphatidylcholine (EYPC, 22.0 mg, 28.6 μmol) and cholesterol (5.3 mg, 13.7 μmol) in $CHCl_3$ (1.1 mL) was evaporated, and dried under vacuum to form a lipid thin film. The thin film was hydrated and suspended in 1.6 mL of pH 6.5 HEPES buffer solution (15 mM HEPES, 200 mM NaCl) by voltexing and sonication. After five-times freeze-thaw cycles, the lipid suspension was extruded 39 times through a polycarbonate filter with 0.1 μm of pore size in the diameter. The LUVs was purified through size exclusion chromatography using a disposable PD-10 column with pH 6.5 HEPES buffer solution (15 mM HEPES, 200 mM NaCl) as an eluent. Lipid concentration of the resulting LUV suspension was deduced from the phosphatidylcholine concentration determined by using Phospholipid C-Test Wako (Wako Pure Chemical Industries, Osaka, Japan).[S3] The lipid concentration of LUV suspension was adjusted to 480 μM with pH 6.5 HEPES buffer solution, then adjusted to 16 μM with the pH 7.5 HEPES buffer solution (15 mM HEPES, 200 mM NaCl) to generate pH-gradient. A suspension of the LUVs was immediately used for the fluorescence measurements.

Fluorescence Measurements

A solution of anti-dansyl IgG (polyclonal anti-dansyl antibody, A-6398, 1 mg/mL in pH7.2 PBS 5 mM NaN_3, Life Technologies Co., CA, USA) was diluted in pH 7.2 buffer (PBS, 5 mM NaN_3) or a suspension of LUVs. The concentrations of IgG were adjusted to 31.3, 62.5, 125, and 250 nM in the pH 7.2 buffer or 125 nM in the suspension of LUVs, respectively. 10 μM peptide solution in 20% aqueous DMSO (1 μL) was diluted in the buffer containing IgG, a suspension of LUVs containing IgG, IgG-free buffer or an IgG-free suspension of LUVs. After 3-min incubation, emission spectra (excitation at 330 nm) of the solutions were measured on a JASCO FP-6500 spectrofluorometer equipped with EHC-573 Peltier thermostatted cell holder (JASCO Co., Tokyo, Japan) at 25 °C. The background fluorescences were canceled by subtracting corresponding control spectra. A FMM-100 micro quartz cell with a FMM-110 micro cell holder (JASCO Co., Tokyo, Japan) was used for all measurements.

Liposome Assay

Preparation of Pyranine-Encapsulated LUVs [S4]

Trisodium 8-hydroxypyrene-1,3,6-trisulfonate (pyranine)-encapsulated LUVs were prepared according to the thin-film hydration method, followed by extrusion through Nuclepore polycarbonate filters mounted in the mini-extruder aparatus. A solution of egg yolk phosphatidylcholine (EYPC,

17.0 mg, 22.1 μmol) and cholesterol (4.1 mg, 10.6 μmol) in $CHCl_3$ (0.85 mL) was evaporated, and dried under vacuum to form a lipid thin film. The thin film was hydrated and suspended in 1.28 mL of pyranine-containing pH 6.5 HEPES buffer solution (15 mM HEPES, 200 mM NaCl, 1 mM pyranine) by voltexing and sonication. After five-times freeze-thaw cycles, the lipid suspension was extruded 39 times through a polycarbonate filter with 0.1 μm of pore size in the diameter. The external pyranine-containing buffer was replaced by pyranine-free pH 6.5 HEPES buffer solution (15 mM HEPES, 200 mM NaCl) through size exclusion chromatography using a disposable PD-10 column. Lipid concentration of the resulting pyranine-encapsulated LUV suspension was deduced from the phosphatidylcholine concentration determined by using Phospholipid C-Test Wako. The lipid concentration of LUV suspension was adjusted to 480 μM with pyranine-free pH 6.5 HEPES buffer solution, then adjusted to 16 μM with the pyranine-free pH 7.5 HEPES buffer solution (15 mM HEPES, 200 mM NaCl) to generate pH-gradient. A suspension of the LUVs was immediately used for the H^+/Na^+ exchange assay.

H^+/Na^+ Exchange Assay with Anti-dansyl IgG

A suspension of pyranine-encapsulated pH-gradient LUVs containing a solution of anti-dansyl IgG was placed in a micro quartz cuvette (790 μL). Concentration of IgG was adjusted to 62.5, 125 or 250 nM in each entry. The suspension was stirred gently with a magnetic stir bar in a JASCO FP-6500 spectrofluorometer. The fluorescence emission was measured at 520 nm with the excitation at 460 nm. After equilibration at 25°C for 10 min, the fluorescence emission was started to be recorded. After 60 s, 10 μM peptide solution in 20% aqueous DMSO (10 μL) was added via an injection port to the stirring LUV suspension. The fluorescence emission was recorded for 10 min at a sampling rate of 1 s, and then the remaining LUVs were completely lysed by addition of 2% Triton X-100 aqueous solution (5 μL). The background drift from DMSO addition was canceled by subtracting the control trace obtained from 20% aqueous DMSO without peptides. The data were smoothed by moving average. Ion-transport activity of tested peptides (I) were normalized against 100% lysis by Triton X-100 (I_∞) as following: $I = 100 \times (I_0 - I_t)/(I_0 - I_\infty)$, where I_t is the fluorescence intensity at time t, and I_0 is the fluorescence intensity before the addition of peptides. Final values of H^+/Na^+ exchange were deduced as the average values of 250–300 s to be 45% (**2**), 42% (**2** + 62.5 nM IgG), 29% (**2** + 125 nM IgG), 17% (**2** + 250 nM IgG), 48% (**1**), and 51% (**1** + 250 nM IgG), respectively.

Cytotoxicity Assay

Cell Culture

P388 mouse leukemia cells were obtained from Institute of Development Aging and Cancer (Tohoku University). Cells were maintained in RPMI1640 growth medium [RPMI1640 with phenol red (Wako Pure Chemical Industries, Osaka, Japan), 10% fetal bovine serum, penicillin G

(100 units/mL), streptomycin (100 μg/mL)] under atmosphere of 5% CO_2 at 37°C.

Cytotoxicity Neutralization Assay

Compounds **1** or **2** were diluted in RPMI1640 growth medium [RPMI1640 with phenol red (Wako Pure Chemical Industries, Osaka, Japan), 10% fetal bovine serum, penicillin G (100 units/mL), streptomycin (100 μg/mL)] with 2% DMSO to 0.4 and 1.2 nM for **1** or 40 and 120 nM for **2**. A solution of anti-dansyl IgG (A-6398, 1 mg/mL in pH7.2 PBS 5 mM NaN_3) were diluted in pH 7.2 buffer (PBS, 5 mM NaN_3) to various concentrations (3-fold serial dilution, 6.7–0.25 μM). The pH 7.2 buffer (PBS, 5 mM NaN_3) was also used as a vehicle control. P388 cells were harvested at 4 °C by centrifugation at 1,000 rpm for 3 min using a MRX-150 centrifugator equipped with a TMA-3 rotor (Tomy, Tokyo, Japan). The collected cells were resuspended into the growth medium at 2 x 10^4 cells/mL. Aliquots of the medium containing the compounds **1** or **2** (2% DMSO, 100 μL) were mixed with the serial dilution of IgG (5.38 μL) or the pH 7.2 buffer (PBS, 5 mM NaN_3, 5.38 μL) in 96-well plates. The medium containing P388 cells (100 μL) were added to the 96-well plates. The final concentrations of the compounds **1** or **2** were 0.2 and 0.6 nM for **1** or 20 and 60 nM for **2**, respectively. The final range of the concentration of IgG was 6.7–180 nM. The final concentration of P388 cells and DMSO were 1 x 10^4 cells/mL and 1%, respectively. After incubation for 92 h under atmosphere of 5% CO_2 at 37°C, the numbers of viable cells were determined by 3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-*bis*(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT) assay. Phenazine methosulfate (PMS)/XTT solution in the growth medium (PMS 1.53 mg/mL in Milli-Q/XTT 50 mg/mL in Milli-Q/growth medium = 1:2:47 v/v, 50 μL)[S5] were added to the each well and further incubated for 4 h under atmosphere of 5% CO_2 at 37°C. The absorbance of each well at 490 nm was measured using a Bio-Rad Benchmark microplate reader (Bio-Rad, CA, USA). The cell viabilities were evaluated by means of three replicates and normalized as the % of control (the cells in the control wells were cultured under toxin-free and pH 7.2 buffer-free conditions). Toxicities of the pH 7.2 buffer and IgG were negligible (Figure S1).

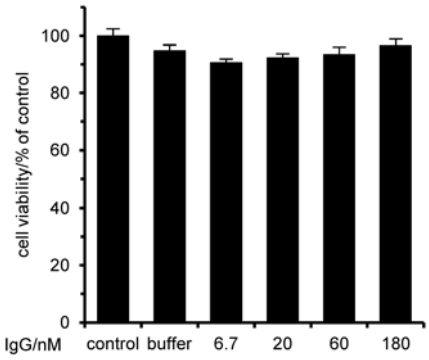

Figure S1. Evaluation of toxicities of the pH 7.2 buffer (PBS, 5 mM NaN_3) and anti-dansyl IgG. Cell viability was determined by XTT method.

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