Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Nonlinear concentration gradients regulated by the width of channels for observation of half maximal inhibitory concentration (IC_{50}) of transporter proteins

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Supplementary Design

We calculated the flow rates (Q) from the concentration (C) of inlets (Figure (a)).

$$Q_A + Q_C = Q'_{A1} + (Q'_{A2} + Q'_{B2}) + Q'_{B3} + (Q'_{B4} + Q'_{C4}) + Q'_{C5}$$

$$C_{1,2,3,4,5} = 10^{0,0.25,0.5,0.75,10}$$

We assumed that the flow rates of outlets were 1.

$$Q'_{A1} = (Q'_{A2} + Q'_{B2}) = Q'_{B3} = (Q'_{B4} + Q'_{C4}) = Q'_{C5} = 1$$

The flow rates of the second branches are as described follow.

$$Q_{A1} = Q'_{A1} + Q'_{A2}$$

$$Q_{B3} = Q'_{B2} + Q'_{B3} + Q'_{B4}$$

$$Q_{C5} = Q'_{C4} + Q'_{C5}.$$

$$(C_1 \times Q'_{A2} + C_3 \times Q'_{B2})/(Q'_{A2} + Q'_{B2}) = C_2$$

$$(C_3 \times Q'_{B4} + C_5 \times Q'_{C4})/(Q'_{B4} + Q'_{C4}) = C_4$$

We calculated the ratio of the flow rates in inlets (Q_A and Q_c).

$$Q_A = Q_{A1} + Q_{A3}$$

$$Q_C = Q_{C3} + Q_{C5}$$

$$(C_1 \times Q_{A3} + C_5 \times Q_{C3})/(Q_{A3} + Q_{C3}) = C_3$$

$$Q_A / Q_C = k$$

We calculated the width (W) of the horizontal channels from the flow rates (Q) at the branch point. (Figure (b) and (c)). The width of the vertical channels was W=100 μm.

$$Q_{a1}: Q_{a3} = W : W_1$$

$$Q_{c3}: Q_{c5} = W_2 : W$$

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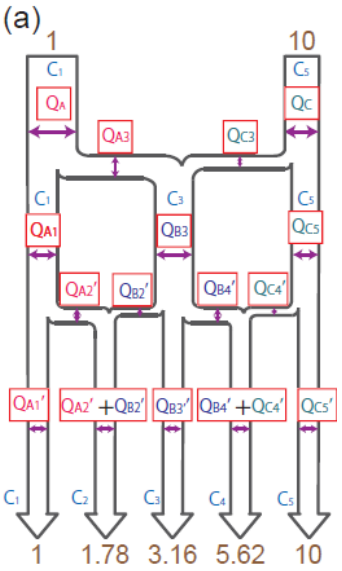

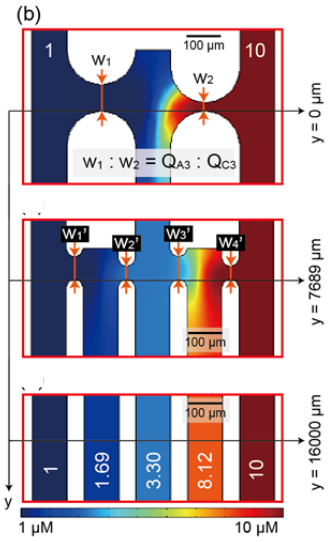

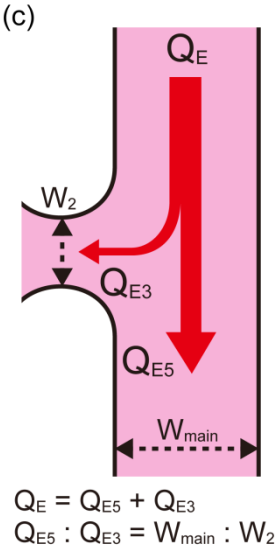

Supplementary Figures

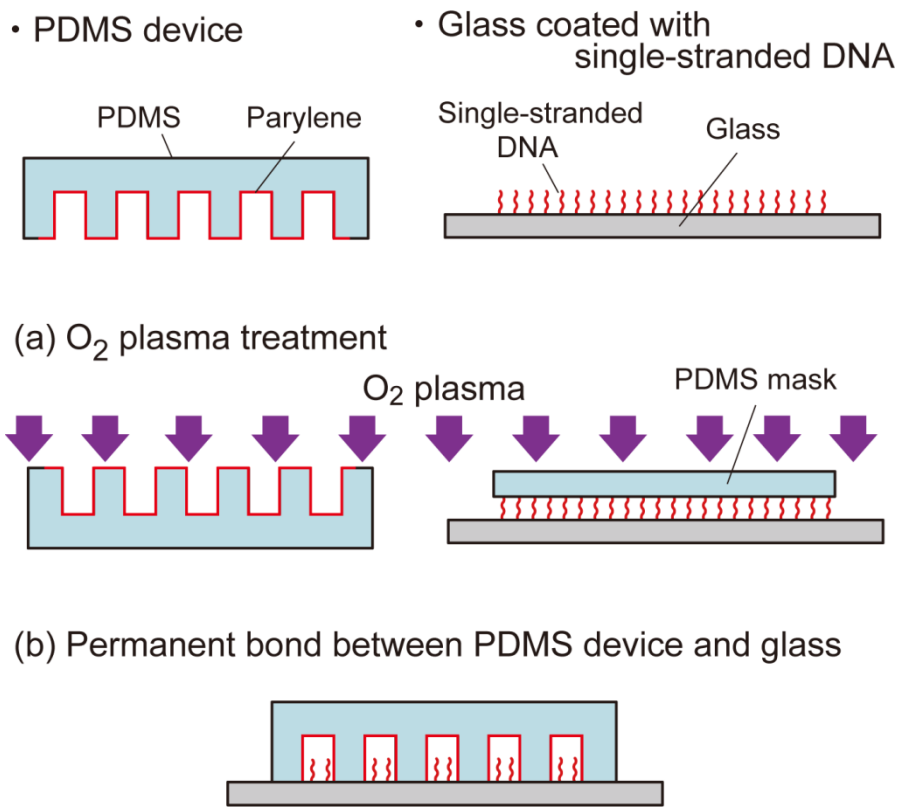

Figure S1 Schematic illustration of the process of (a) the O_2 plasma treatment and (b) the permanent bond.

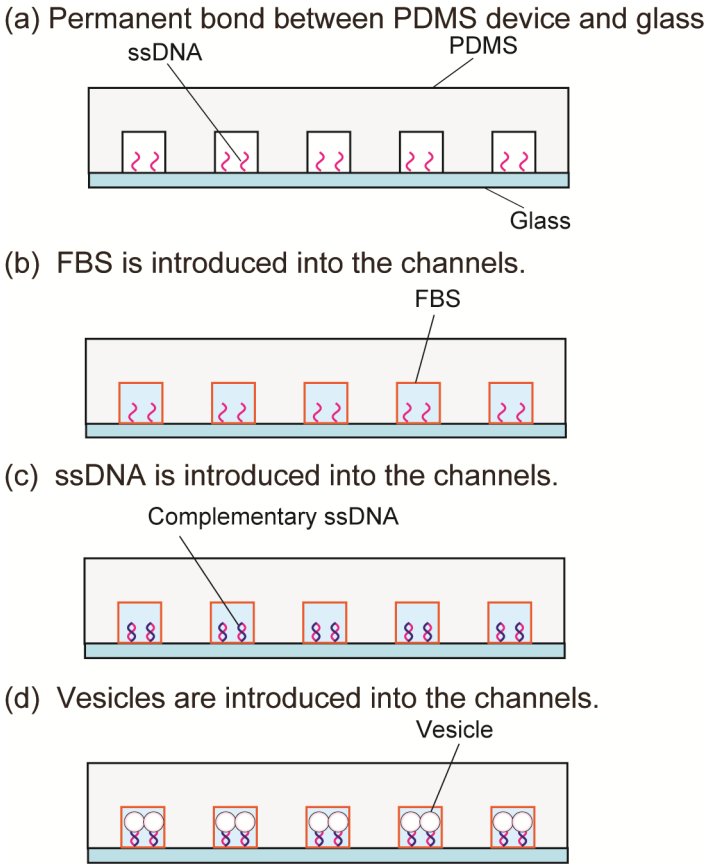

Figure S2 Schematic illustration of the vesicle immobilization process. (a) Permanent bond between the PDMS device and the glass. (b) Ten percent (v/v) fetal bovine serum (FBS) in phosphate buffered saline (PBS) buffer was introduced for 40 min into the channels. (c) A cholesterol-modified complimentary ssDNA was introduced for 45 min. (d) Vesicles were injected into the channels.

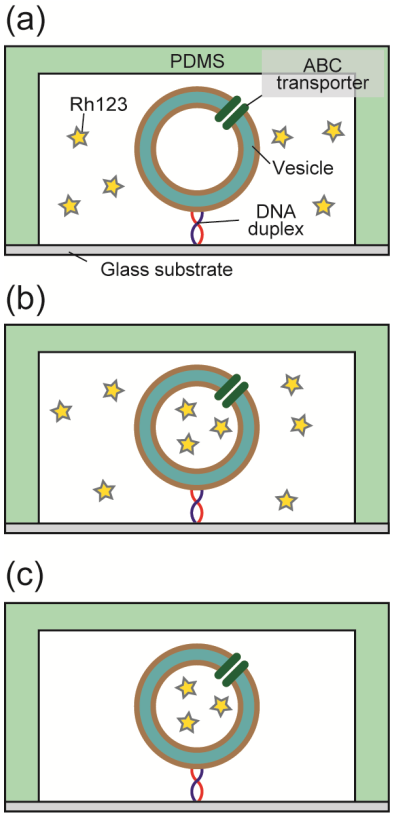

Figure S3 Schematic illustration of the experimental process for Rh123 incubation to the ABC-transporter vesicles. (a) Rh123 is introduced into the channels. (b) Rh123 is transported to the vesicles by the ABC-transporters. (c) The channels are washed using buffer solution.

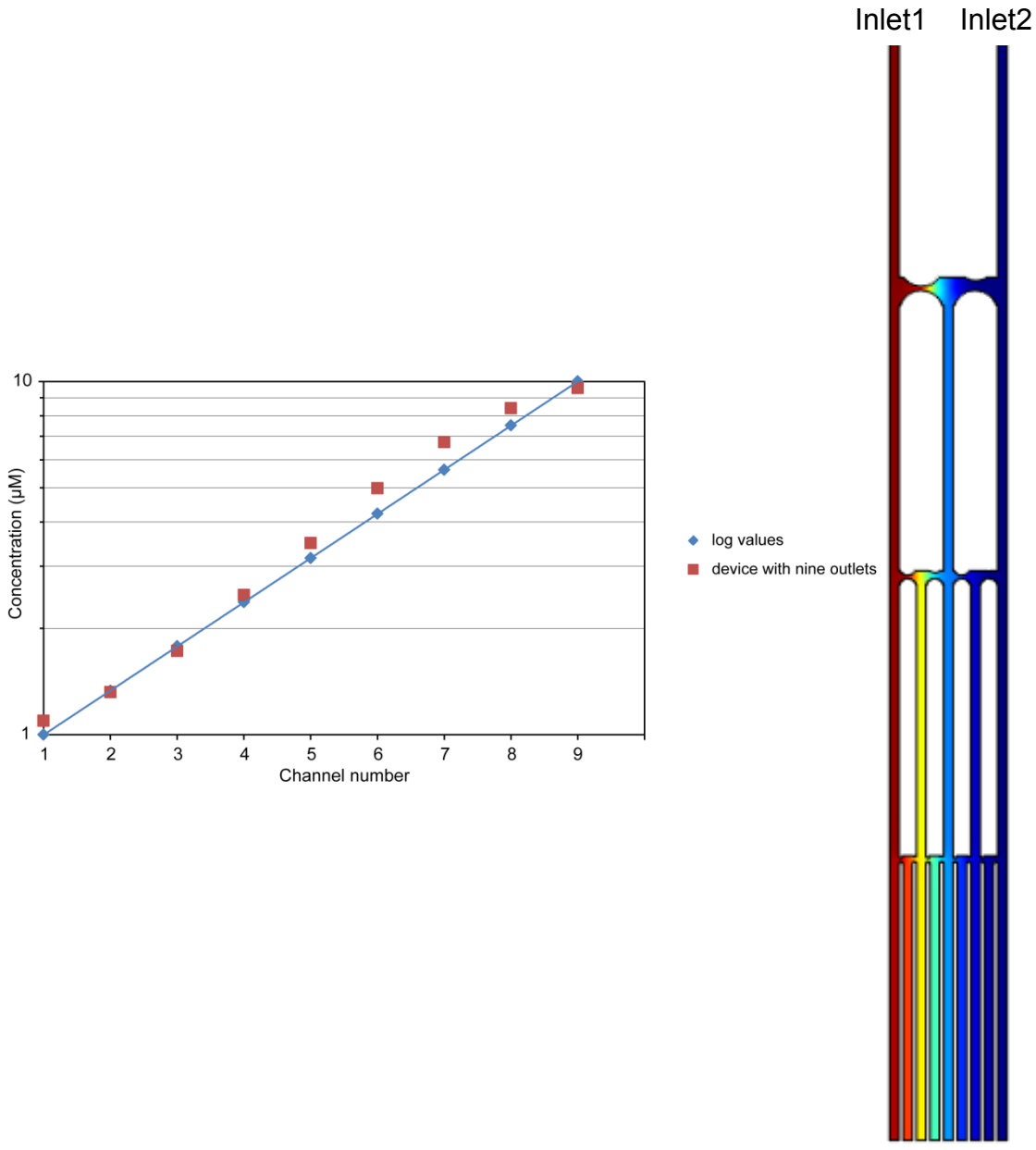

Figure S4 COMSOL simulation result of a logarithmic concentration gradient generator with nine outlets. The flow rates of inlet 1 and inlet 2 were 0.5 and 0.75 μL/h, respectively. The concentrations of inlet1 and 2 were 10 and 1.0μM**.** The generated concentrations for each channel were 9.59, 8.40, 6.74, 4.99, 3.49, 2.49, 1.73, 1.32, and 1.1 μM

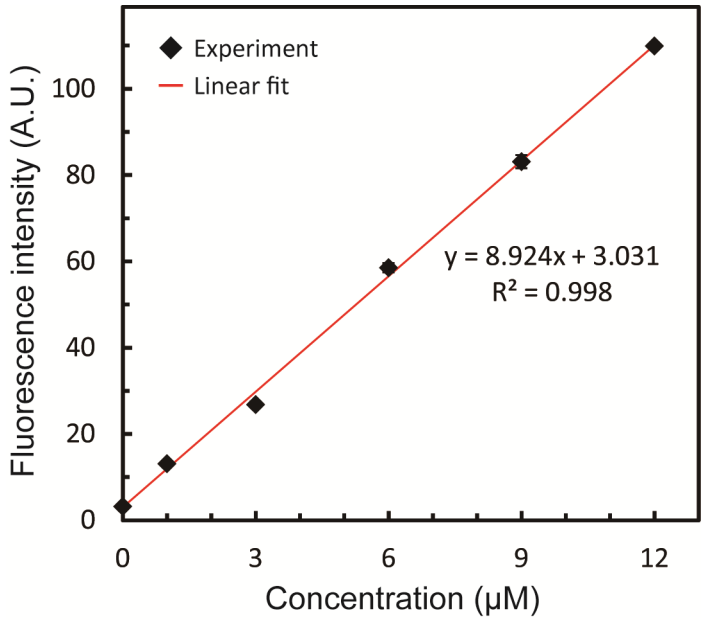

Figure S5 Calibration curve of the fluorescence intensity for different Rh123 concentrations. The concentrations generated by the logarithmic and linear devices were calculated using the calibration curve.

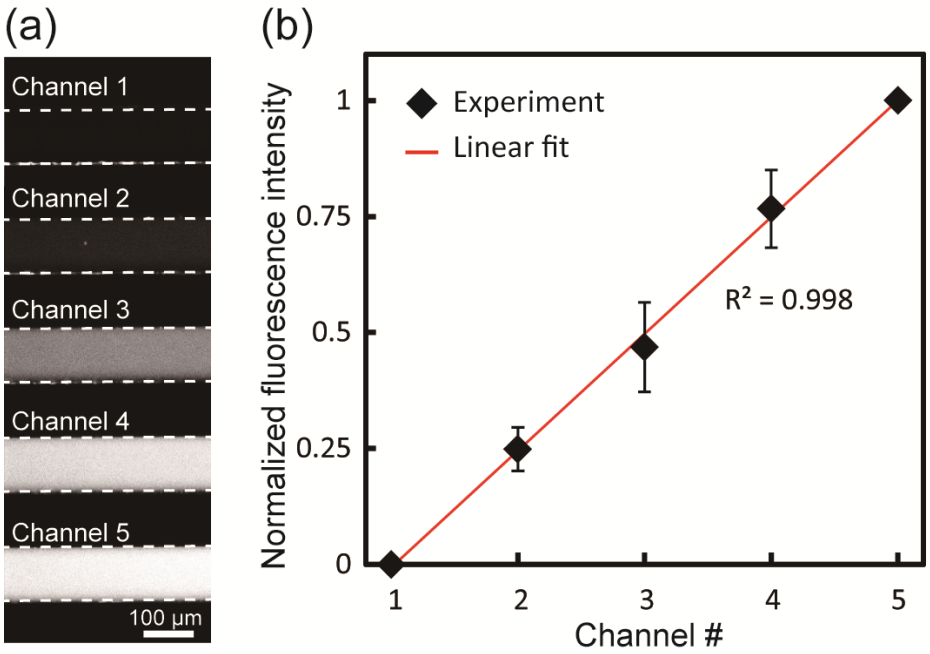

Figure S6 Linear concentration gradient generator capable of generating five concentration gradients was used in the experiments. (a) Fluorescence image of Rh123 downstream of the linear gradient device. (b) Graph of normalized fluorescence intensities for the linear concentration gradient device. The error bars indicate the standard deviations for the different channels.

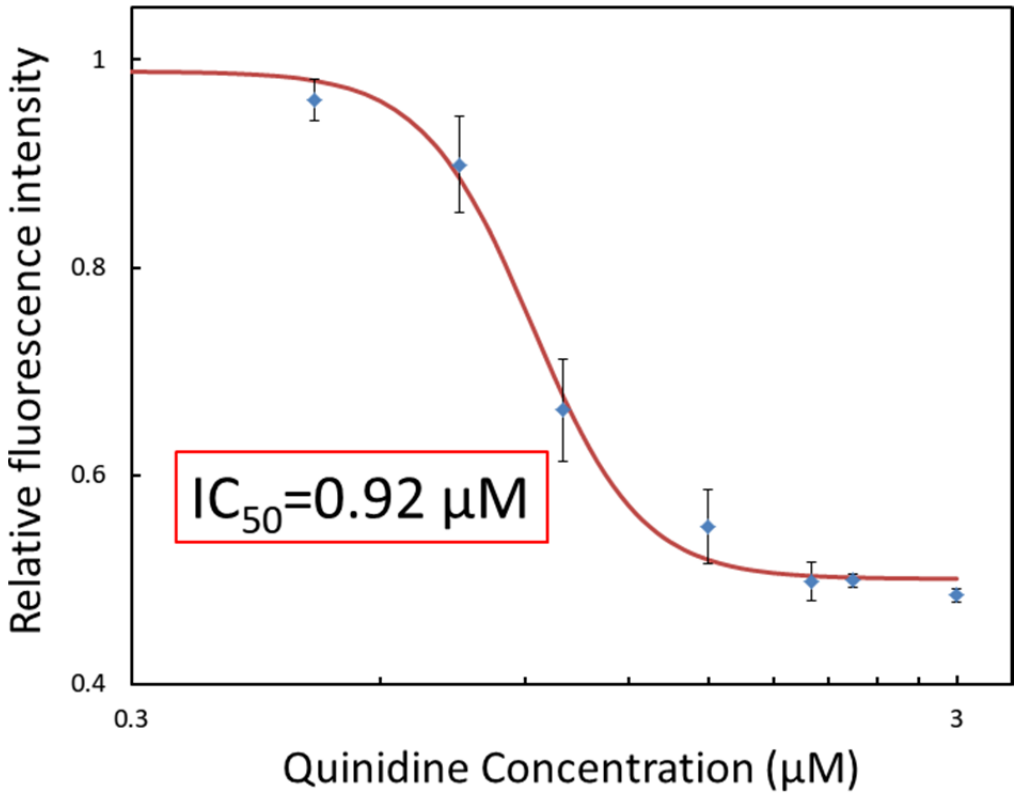

Figure S7 Linear concentration gradient generator was used in the experiments. Graph of normalized fluorescence intensities for the linear concentration gradient device. The error bars indicate the standard deviations for the different channels (N=3, n>10).