Electronic Supplementary Information

Dendritic Nanospace Constructed by Only Glycerol Units Enhanced Uptakes of a Fluorescent Molecule in Aqueous Solution

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Experimental Methods

Materials

Allyl chloride, 4-methylmorpholine *N*-oxide (NMO), and 50 wt % of sodium hydroxide solution were purchased from Sigma-Aldrich Co. (St. Louis. USA) and used without further purification. Tetrabutylammonium bromide (TBAB) and aqueous (4 wt%) osmium tetraoxide solution were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). 1,1,1-Tris(hydroxymethyl)propane, 4-amino-3-hydroxynaphthalene-1-sulphonic acid (AHSA), sodium hydroxide and magnesium sulfate were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were used without further purification. *t*-Butyl alchol (*t*-BuOH), acetone, ethyl acetate, toluene and methanol were reagent grade and purchased from Nacalai Tesque (Kyoto, Japan). Petroleum ether was reagent grade and purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

Synthesis and purification of PGD-G1 and G2

PGD-G1 and G2 were synthesized by two-step process based on allylation of alcohols and catalytic dihydroxylation. For preparation of PGD-G1, allylchloride (0.5 mol) was added to a solution of

1,1,1-tris(hydroxymethyl)propane (THMP) (0.1 mol alcohol equiv.), tetrabutylammonium bromide (0.01 mol) and 50 % sodium hydroxide solution (0.5 mol) in a three-necked round-bottom flask, over 22 h at 45 °C with mixing using a overhead stirrer. When the reaction reached completion, 100 mL of toluene was added to the flask. The organic phase was separated, dried with $MgSO_4$, filtered, and concentrated under vacuum. The allylated THMP was purified by silica gel column chromatography (eluent: petrolether/ethylacetate). The allylated THMP (0.1 mol equiv.) and *N*-methylmorpholine-*N*-oxide (0.11 mol) were dissolved in acetone (50 mL), distilled water (50 mL) and *t*-butanol (10 mL), and then 4 wt% OsO_4 solution in water (2 mL) was added to the mixture and stirred for 24 h at 25 °C. When the reaction was over, the volatile compounds were removed *in vacuo*. The obtained crude PGD-G1 (9.5 g) dissolved in methanol (10 mL) was applied to an acidic active alumina column (eluent: methanol/water), and the recovered crude product was then applied to silica gel chromatography (eluent: ethyl acetate/methanol), giving PGD-G1 as sticky substance with light brown color. In a similar manner, PGD-G2 was synthesized by using PGD-G1 as a starting material. The obtained PGD-G1 and G2 were identified by ^{1}H-NMR measurements using a 500 Hz FT-NMR apparatus (Bruker Advanced 500 spectrometer) and a MALDI-TOF-MS apparatus (Voyager 2000, AB SCIEX).

PGD-G1 : Yield: 84 %

^{1}H NMR (D_2O, 500 MHz): δ = 3.77 (m, 3H, HOC<u>H</u>CH_2-), 3.55-3.50 (dd, 4H, -OC<u>H_2</u>CH-), 3.45-3.40 (m, 6H, HOC<u>H_2</u>CH-), 3.39-3.34 (m. 2H, -OC<u>H_2</u>CHOH), 3.30 (q, 4H, -CC<u>H_2</u>O-), 3.22 (s, 2H, -CC<u>H_2</u>O-), 1.24 (q, 2H, -CC<u>H_2</u>CH_3), 0.73 (t, 3H, -CCH_2C<u>H_3</u>)

MALDI-TOF-MS (matrix: CHCA) m/z = 379.16 (PGD-G1 + Na^+)

PGD-G2 : Yield: 85 %

^{1}H NMR (D_2O, 500 MHz): δ = 3.76 (m, 6H, HOC<u>H</u>CH_2O-), 3.68 (m, 3H, -OC<u>H</u>CH_2O-), 3.64-3.53 (m, 12H, HOCHC<u>H_2</u>O-), 3.53-3.48 (m, 12H, HOC<u>H_2</u>CH-), 3.48-3.38 (m, 12H, -OC<u>H_2</u>CHO-), 3.29 (q, 4H, -CC<u>H_2</u>O-), 3.22 (s, 2H, -CC<u>H_2</u>O-), 1.25 (q, 2H, -CC<u>H_2</u>CH_3), 0.74 (t, 3H, -CCH_2C<u>H_3</u>)

MALDI-TOF-MS (matrix: CHCA) m/z = 823.12 (PGD-G2 + Na^+)

Fluorescent measurements of AHSA in the presence of various concentration of PGDs

AHSA (3 mg, 13 μmol) was dissolved in 100 mL of 10 mM acetate buffer (pH 5.0) as a stock solution. PGD-G2 (2, 4, 9, 18, 35, 70 mmol) was dissolved in 1 mL of 10 mM acetate buffer (pH 5.0). The stock solution of AHSA (30 μL) was added to each PGD-G2 solution (final concentration; AHSA: AHSA: 0.4 μM, PGD-G2: 2, 4, 8, 16, 31, 64 □M). The mixture of AHSA and PGD-G2 was incubated for 30 min at 4˚C in a quartz cell, and emission spectra of the solution were measured (excitation wavelength: 342 nm) by a spectrofluorometer (F-2500, HITACHI, Ltd., Japan) at 4˚C under nitrogen atmosphere. The same experiments were performed using PGD-G1 instead of PGD-G2. Glycerol was used as a control sample of PGDs.

^{1}H-NMR titration of AHSA toward PGDs

PGD-G2 or -G1 was dissolved in 10 mM acetate buffer prepared by D_2O, p*D* of which was adjusted by NaOD and acetic acid-d_4 (concentration: 7.37 mM). AHSA dissolved in the buffer was added to the PGD-dissolved solution to be 4.93, 9.87, 19.7 and 49.3 mM (final concentration of AHSA: 49.3 mM). ^{1}H-NMR spectra of each solution were measured using a 500 Hz FT-NMR apparatus (Bruker Advanced 500).

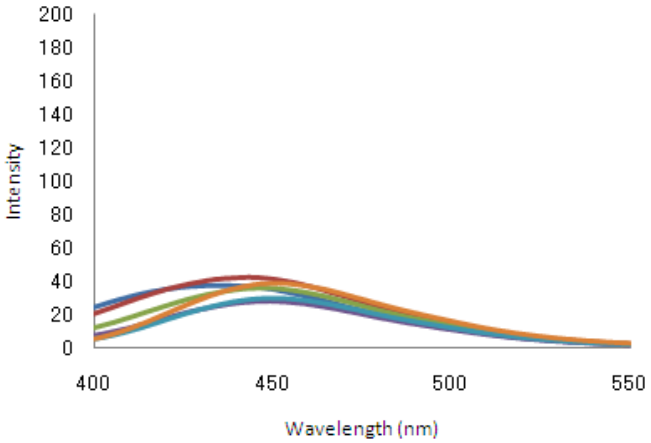

Fig. S1 Fluorescent spectral change for a solution of AHSA (0.4 μM) on the addition of PGD-G1 in 10 mM acetate buffer (pH 5.0) at 4 °C: PGD-G1 concentration = 4, 9, 18, 35, 70, 140 μM (from bottom to top).

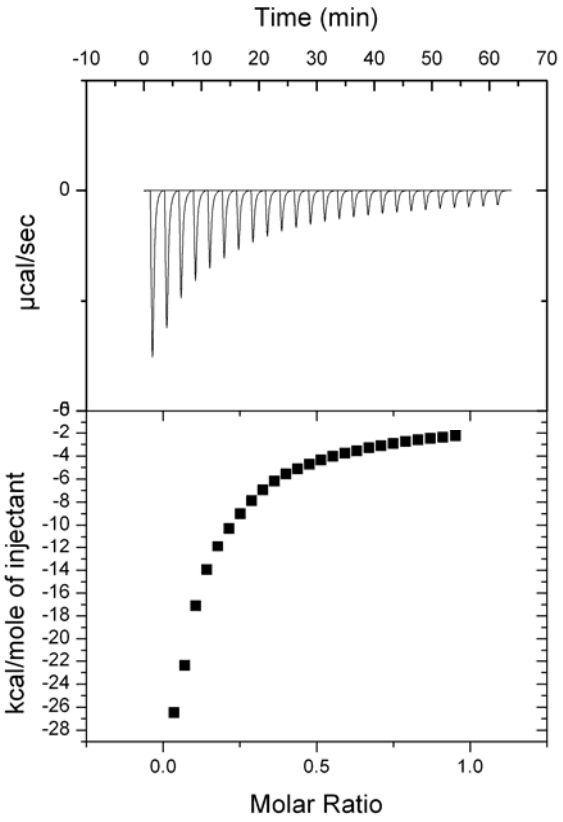

Fig. S2 ITC titration curves for PGD-G1 and AHSA in water. ITC measurements were performed to establish solution binding constants. In this experiment, a solution of PGD-G1 (0.1 mM) was titrated with a solution of AHSA (total volume: 0.5 mM).