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Electronic Supplementary Information

Synthesis and Supramolecular Assembly of Clicked Anionic Dendritic Polymers into Polyion Complex Micelles

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General Methods. CH_2Cl_2, Et_3N, pyridine, and *i*-Pr_2EtN were distilled from CaH_2. DMSO was dried under 4Å molecular sieves. Column chromatography was performed with 70-230 and 230-400 mesh silica gel. Thin-layer chromatography (TLC) was done on silica aluminium-backed plates. Ultrafiltration was performed on stirred cells with Amicon® YM1 membranes. NMR chemical shifts are reported in ppm (δ units) downfield from internal tetramethylsilane ($CDCl_3$), or the HOD signal (D_2O and CD_3OD).

Dynamic Light Scattering. DLS measurements were carried out on a Malvern Nano ZS (Malvern Instruments, U.K.) operating at 633 nm with a 170º scattering angle. PIC micelles were aged for at least 24 h before measurements.

Gel Permeation Chromatography. GPC experiments were performed using a PSS Suprema Lux precolumn (10µm, 8 x 50 mm) and a PSS Suprema Lux column (10µm, 100 Å, 8 × 300 mm) with a refractive index detector. A mixture CH_3CN-H_2O (1:1) was used as eluent. Dendrimer solutions were filtered through 0.45 µm before injection. Dendrimer concentration was set at 0.25 mg/mL.

Atomic Force Microscopy. Samples for AFM imaging were prepared by depositing the PBS aqueous solution of micelles onto Si wafers. The AFM imaging was performed in air using a Multimode NanoScope V system (Veeco, Santa Barbara, CA) operated in tapping mode. Tip Characteristics: 1-10 OHm-cm Phosphorus (n) dopped Si. Cantilever T: 3.5 - 4.5 µm. Frecuency: 257-342 KHz. Tip ROC < 10 nm. Tip ROC MAx: 12.5 nm.

Sodium 3-butyn-1-sulfate (1). $ClSO_3H$ (0.47 mL, 7.13 mmol) was added dropwise to a solution of pyridine (1.27 mL, 15.68 mmol) in CCl_4 (6.5 mL) at 0 ºC. After 30 min of stirring, 3-butyn-1-ol (0.54 mL, 7.13 mmol) was added, and the reaction was left at 0 ºC for 4 h. Then, reaction was allowed to reach rt, and after 12 h of stirring, it was extracted with H_2O (3 x 15 mL). The combined aqueous phase was evaporated to half volume, and then saturated Na_2CO_3 added till no CO_2 evolution was observed. The resulting mixture was concentrated, and then triturated with hot EtOH (80 mL), and filtered. The filtrate was evaporated to give **1** as a white powder (1.22 g, 100%). ^1H NMR (250 MHz, D_2O) δ: 4.14 (t, J = 6.3 Hz, 2H), 2.63 (dt, J = 2.3 Hz, J = 6.3 Hz, 2H), 2.40 (t, J = 2.6 Hz, 1H); ^{13}C NMR (63 MHz, D_2O) δ: 80.8, 70.2, 66.5, 18.6.

Sodium 4-((prop-2-ynyl carbamoyl)methoxy)naphthalene-2,7-disulfonate (2). Et_3N (0.68 mL, 4.95 mmol) was added to a solution of bromoacetyl bromide (1.00 g, 4.95 mmol) in CH_2Cl_2 (50 mL) at -50 ºC. Then, propargyl amine (0.32 mL, 4.95 mmol) was added dropwise, and the resulting solution was allowed to reach rt and stir overnight. A white precipitate appeared, and was filtered off washing with CH_2Cl_2. The filtrate was evaporated to give a crude product that was purified by column chromatography (silica gel, 10% $MeOH/CH_2Cl_2$) to give **2-bromo-*N*-(prop-2-ynyl)acetamide** (633 mg, 73%): ^1H NMR (250 MHz, $CDCl_3$) δ: 6.68 (br s, 1H), 4.09 (dd, J = 2.5 Hz, J = 5.3 Hz, 2H), 3.90 (s, 2H), 2.29 (dt, J = 0.8 Hz, J = 2.6 Hz, 1H); ^{13}C NMR (63 MHz, $CDCl_3$) δ: 165.7, 78.6, 72.4, 30.1, 28.8. *i*-Pr_2EtN (94 μL, 0.57 mmol) and 2-bromo-*N*-(prop-2-ynyl)acetamide (100 mg, 0.57 mmol) were added to a solution of 1-naphthol-3,6-disulfonic acid disodium salt hydrate (157 mg, 0.28 mmol, 63% purity) in DMSO (0.40 mL). The resulting mixture was stirred at rt overnight. Then, it was poured into EtOAc (4 mL), and was triturated to give a solid that was collected by filtration washing with acetone. This solid crude product was dissolved in H_2O (25 mL), and was extracted with CH_2Cl_2 (3 x 25 mL). The aqueous phase was evaporated to give **2** as an off-white solid (157 mg, 70% conversion) that was directly used without further purification.

General procedure for the anionic functionalization of [Gn]-N_3 and PEG-[Gn]-N_3: Dendrimers **[Gn]-N_3** and PEG-dendritic block copolymers **PEG-[Gn]-N_3** were dissolved in *t*-BuOH-H_2O (1:1) to give a 0.1M final concentration of terminal azides. Then, **1**, **2**, or **3** (200 mol% per terminal N_3), and freshly prepared aqueous solutions of $CuSO_4$ (5 mol% per N_3) and sodium ascorbate (25 mol% per N_3) were added. The resulting solutions were stirred at rt for 24-48 h, and then purified by ultrafiltration [Amicon® YM1, H_2O (3 x 30 mL), aqueous 1M NaCl (30 mL)], and evaporated or freeze-dried. For the synthesis of **[Gn]-3** and **PEG-[Gn]-3**, $NaHCO_3$ (110 mol% per carboxylic acid group) was added.

[G1]-1: From **[G1]-N_3** (35 mg, 0.051 mmol), **1** (53 mg, 0.307 mmol), sodium ascorbate (7.61 mg, 38.4 μmol) and $CuSO_4$ (1.92 mg, 7.7 μmol) dissolved in *t*-BuOH (0.85 mL)-H_2O (0.85 mL), and after 24 h of reaction time following the general procedure described above, **[G1]-1** (34 mg, 80%) was obtained as a white powder. IR (CsI, cm^{-1}) 2933, 1647, 1223; ^1H NMR (400 MHz, D_2O) δ: 7.90 (s, 1H), 7.80 (s, 2H), 7.14 (s, 2H), 4.56 (t, J = 4.9 Hz, 6H), 4.28-4.15 (m, 12H), 3.94-3.47 (m, 24H), 3.35 (t, J = 7.0 Hz, 2H), 3.04 (t, J = 6.3 Hz, 2H), 3.0 (t, J = 6.1 Hz, 4H), 1.63-1.60 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 172.4, 154.6, 142.7, 133.2, 127.3, 109.4, 75.1, 73.2, 72.8, 72.7, 72.6, 72.4, 72.1, 71.8, 71.7, 71.4, 70.3, 65.5, 52.9, 44.8, 28.1, 25.0, 13.6.

[G2]-1: From **[G2]-N_3** (20 mg, 8.1 μmol), **1** (25 mg, 0.145 mmol), sodium ascorbate (3.60 mg, 18.2 μmol), and $CuSO_4$ (0.91 mg, 3.6 μmol) dissolved in *t*-BuOH (0.36 mL)-H_2O (0.36 mL), and after 48 h of reaction time following the general procedure described above, **[G2]-1** (32 mg, 92%) was obtained as a white foam. IR (CsI, cm^{-1}) 2932, 1648, 1220; ^1H NMR (250 MHz, D_2O) δ: 7.90 (s, 3H), 7.80 (s, 6H), 7.14-7.04 (m, 8H), 4.61-4.48 (m, 18H), 4.31-3.47 (m, 144H), 3.27 (t, J = 7.0 Hz, 2H), 3.10-2.94 (m, 18H), 1.63-1.60 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 176.6, 154.8, 142.5, 133.3, 127.4, 109.0, 75.1, 73.2, 72.8, 72.7, 72.6, 72.4, 72.1, 71.6, 71.3, 70.1, 63.6, 53.1, 44.8, 28.4, 25.2, 13.8.

[G3]-1: From **[G3]-N$_3$** (40 mg, 5.1 μmol), **1** (47 mg, 0.275 mmol), sodium ascorbate (6.81 mg, 34.4 μmol), and $CuSO_4$ (2.03 mg, 6.9 μmol) dissolved in *t*-BuOH (0.68 mL)-H_2O (0.68 mL), and after 48 h of reaction time following the general procedure described above, **[G3]-1** (58 mg, 91%) was obtained as a white foam. IR (KBr, cm^{-1}) 2927, 1648, 1226; ^{1}H NMR (300 MHz, D_2O) δ: 8.19-7.83 (m, 27H), 7.10 (s, 26H), 4.63-4.46 (m, 54H), 4.34-3.43 (m, 468H), 3.35 (m, 2H), 3.16-2.83 (m, 54H), 1.63-1.60 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ: 169.2, 153.7, 140.7, 130.8, 125.6, 107.8, 73.6, 73.2, 71.5, 71.2, 70.5, 70.3, 69.8, 67.6, 51.8, 51.4, 40.9, 27.1, 12.0.

[G3]-2: From **[G3]-N$_3$** (25 mg, 3.2 μmol), **2** (127 mg, 0.171 mmol), sodium ascorbate (47.3 mg, 0.275 mmol), and $CuSO_4$ (1.07 mg, 4.3 μmol) dissolved in *t*-BuOH (0.15 mL)-H_2O (0.15mL), and after 48 h of reaction time following the general procedure described above, **[G3]-2** (57 mg, 91%) was obtained as a white foam. ^{1}H NMR (300 MHz, D_2O) δ: 8.40-8.33 (m, 27H), 8.29 (d, J = 4.6 Hz, 27H), 8.06-8.00 (m, 27H), 7.91-7.83 (m, 54H), 7.18 (s, 26H), 6.60 (s, 27H), 4.62-4.30 (m, 54H), 4.20-3.47 (m, 468H), 3.35 (m, 2H), 1.63-1.59 (m, 2H), 0.94 (t, J = *7.4* Hz, 3H).

[G3]-3: From **[G3]-N$_3$** (37 mg, 4.7 μmol), **3** (25 mg, 0.254 mmol), $NaHCO_3$ (22 mg, 0.280 mmol), sodium ascorbate (6.30 mg, 31.8 μmol), and $CuSO_4$ (1.58 mg, 6.4 μmol) dissolved in *t*-BuOH (0.65 mL)-H_2O (0.65 mL), and after 48 h of reaction time following the general procedure described above, **[G3]-3** (51 mg, 98%) was obtained as a white foam. ^{1}H NMR (300 MHz, D_2O) δ: 7.95-7.60 (m, 27H), 7.13 (s, 26H), 4.59-4.52 (m, 54H), 4.22-4.14 (m, 78H), 3.94-3.46 (m, 380H), 3.35 (m, 2H), 2.95-2.84 (m, 54H), 2.56-2.47 (m, 54H), 1.62-1.59 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H).

PEG-[G1]-1: From **PEG-[G1]-N$_3$** (50 mg, 8.6 μmol), **1** (8.86 mg, 0.052 mmol), sodium ascorbate (1.28 mg, 6.4 mμol), and $CuSO_4$ (0.32 mg, 1.3 μmol), dissolved in *t*-BuOH (0.130 mL)-H_2O (0.130 mL), and after 48 h of reaction time according to the general procedure, **PEG-[G1]-1** (54 mg, 100 %) was obtained as a white foam. IR (KBr, cm^{-1}) 2887, 1467, 1344, 1115; ^{1}H NMR (750 MHz, D_2O)

δ: 7.92 (s, 1H), 7.90 (s, 2H), 7.19 (s, 2H), 4.64-4.50 (m, 6H), 4.35-4.12 (m, 14H), 4.03-3.44 (m, 542H), 3.41 (s, 3H), 3.29 (t, J = 4.9 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H), 3.01 (t, J = 5.8 Hz, 4H).

PEG-[G1]-2: From **PEG-[G1]-N_3** (20 mg, 3.4 μmol), **2** (25.1 mg, 20.6 μmol), sodium ascorbate (0.51 mg, 2.6 μmol), and $CuSO_4$ (0.13 mg, 0.51 μmol) dissolved in *t*-BuOH (55 μL)-H_2O (55 μL), and after 24 h of reaction time following the general procedure described above, **PEG-[G1]-2** (22 mg, 90%) was obtained as a white foam. IR (KBr, cm^{-1}) 2879, 1466, 1034; ^{1}H NMR (400 MHz, D_2O) δ: 8.40-8.33 (m, 3H), 8.29 (d, J = 4.6 Hz, 3H), 8.06-8.00 (m, 3H), 7.91-7.83 (m, 6H), 7.18 (s, 2H), 6.60 (s, 3H), 4.60-4.46 (m,6H), 4.16-4.04 (m, 8H), 4.00-3.47 (m, 548H), 3.41 (s, 3H), 3.25 (m, 2H).

PEG-[G1]-3: From **PEG-[G1]-N_3** (20 mg, 3.4 μmol), **3** (2.68 mg, 20.1 μmol), $NaHCO_3$ (1.90 mg, 20.6 μmol), sodium ascorbate (0.51 mg, 2.6 μmol), and $CuSO_4$ (0.13 mg, 0.51 μmol) dissolved in *t*-BuOH (55 μL)-H_2O (55 μL), and after 48 h of reaction time following the general procedure described above, **PEG-[G1]-3** (19 mg, 91%) was obtained as a white foam. ^{1}H RMN (400 MHz, D_2O) δ: 7.79 (s, 1H), 7.77 (s, 2H), 7.18 (s, 2H), 4.59-4.51 (m, 6H), 4.28-4.15 (m, 8H), 3.99-3.51 (m, 542 H), 3.41 (s, 3H), 3.28 (t, J = 4.89 Hz, 2H), 2.94-2.84 (m, 6H), 2.57-2.45 (m, 6H).

PEG-[G2]-1: From **PEG-[G2]-N_3** (25 mg, 3.3 μmol), **1** (10.17 mg, 0.059 mmol), sodium ascorbate (1.46 mg, 7.4 mμol), and $CuSO_4$ (0.37 mg, 1.5 μmol), dissolved in *t*-BuOH (0.150 mL)-H_2O (0.150 mL), and after 48 h of reaction time according to the general procedure, **PEG-[G2]-1** (28 mg, 92%) was obtained as a white foam. IR (KBr, cm^{-1}) 2887, 1467, 1344, 1115; ^{1}H NMR (400 MHz, D_2O) δ: 7.87 (br s, 9H), 7.09 (s, 8H), 4.60-4.51 (m, 18H), 4.31-4.00 (m, 44H), 4.00-3.50 (m, 620H), 3.41 (s, 3H), 3.26 (t, J = 4.9 Hz, 2H), 3.04-3.01 (m,18H).

PEG-[G3]-1: From **PEG-[G3]-N_3** (20 mg, 1.5 μmol), **1** (14 mg, 0.083 mmol), sodium ascorbate (1.03 mg, 5.2 μmol), and $CuSO_4$ (0.26 mg, 1.0 μmol), dissolved in *t*-BuOH (0.21 mL)-H_2O (0.21 mL), and after 48 h of reaction time according to the general procedure, **PEG-[G3]-1** (27 mg, 100%)

was obtained as a white foam. IR (KBr, cm^{-1}) 2877, 1458, 13343, 1107; ^1H NMR (400 MHz, D_2O) δ: 7.85 (br s, 27H), 7.10 (s, 26H), 4.61-4.47 (m, 54H), 4.30-4.00 (m, 134H), 4.00-3.49 (m, ~966H), 3.42-3.35 (m, 5H), 3.09-2.97 (m, 54H).

PEG-[G3]-2: From **PEG-[G3]-N_3** (12 mg, 0.9 μmol), **2** (61 mg, 0.050 mmol), sodium ascorbate (1.23 mg, 6.2 μmol), and $CuSO_4$ (0.31 mg, 1.2 μmol), dissolved in *t*-BuOH (0.125 mL)-H_2O (0.125 mL), and after 48 h of reaction time according to the general procedure, **PEG-[G3]-2** (23 mg, 100%) was obtained as a white foam. ^1H NMR (400 MHz, D_2O) δ: 8.45-8.33 (m, 27H), 8.29 (m, 27H), 8.06-8.00 (m, 27H), 7.91-7.83 (m 54H), 7.18 (s, 26H), 6.60 (s, 27H), 4.63-4.34 (m, 110H), 4.01-3.48 (m, ~1023H), 3.41 (m, 5H).

PEG-[G3]-3: From **PEG-[G3]-N_3** (34 mg, 2.6 μmol), **3** (14 mg, 0.141 mmol), $NaHCO_3$ (13 mg, 0.155 mmol), sodium ascorbate (3.49 mg, 17.6 μmol), and $CuSO_4$ (0.88 mg, 3.5 μmol), dissolved in *t*-BuOH (0.35 mL)-H_2O (0.35 mL), and after 48 h of reaction time according to the general procedure, **PEG-[G3]-3** (41 mg, 98%) was obtained as a white foam. ^1H NMR (400 MHz, D_2O) δ: 7.87-7.71 (m, 27H), 7.10 (s, 26H), 4.59-4.43 (m, 54H), 4.30-4.12 (m, 80H), 4.00-3.47 (m, ~966H), 3.41 (m, 5H), 2.95-2.83 (m, 54H), 2.63-2.40 (m, 54H).

Preparation of PIC micelles. PEG-[G3]-1 and **PLL** (M_n 12400, M_w 16100, DP = 77, by LALLS) were separately dissolved in either H_2O or 10 mM PBS pH 7.4, and filtered through 0.45 μm nylon filters. PIC micelles were prepared by mixing these solutions at an equal ratio of L-lysine repetition units and sulfate residues (total concentration: 0.98 mg/mL). Solutions were aged for at least 24 h, and filtered (0.45 μm) before any measurement being performed.