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Supporting Information

Zwitterionic fluorescence nanoparticles prepared from BODIPY conjugated polysulfobetaine for cancer cell imaging

Tamim Mosaiab,[a] Chunim Shin,[b] Pyeong Ho Choi,[a] Gyojic Shin,[c] Sangkug Lee,[c] Kyung Ho Choi,[c] Eui Sang Yoo,[d] Jihoon Lee,[e] Insik In,[e*] and Sung Young Park[a,b,*]

[a]*Department of Green Bio Engineering, Korea national University of Transportation, Chungju, 380-702, Korea Republic*

[b]*Department of Chemical & Biological Engineering, Korea National University of Transportation, Chungju, 380-702, Korea Republic*

[c]*IT Convergence Material R&D Group, Korea Institute of Industrial Technology, Hongcheon-ri, Seobuk-gu, Cheonan-si, Korea Republic*

[d]*Korea Institute of Industrial Technology, Ansan-si, 426-171, Korea republic*

[e]*Department of Polymer Science and Engineering, Korea National University of Transportation, Chungju, 380-702, Korea*

E-mail: parkchem@ut.ac.kr; Fax: +82-(0)43-841-5220; Tel: +82-(0)43-841-5225

1. Experimental Section

1.1 Materials

Boron dipyrromethane (BODIPY) having benzyl chloride on the meso position was synthesized according to the literature.[1] 2-(Dimethylamino) ethyl methacrylate (DMAEMA), N-isopropylacrylamide (NIPAAm), 2-mercaptoethanol, azobisisobutyronitrile (AIBN), 1,3-propanesultone, tetrahydrofuran (THF), ethanol, trizma base (99%, Sigma), trizma HCl, (99%, Sigma), all were purchased from Sigma Aldrich Korea. LysoTracker Blue DND-22 (1 mM solution in DMSO) was purchased from Molecular Probes, Invitrogen, Inc. (Eugene, OR). The NIPAAm monomer was recrystallized from hexane and dried under vacuum. Penicillin-streptomycin, fetal bovine serum (FBS), 0.25% (w/v) trypsin-0.03 %(w/v) EDTA solution, and RPMI-1640 medium were purchased from Gibco BRL (Carlsbad, CA, USA).

1.2 In Vitro Cytotoxicity Measurement

Human breast cancer cells MDA-MB 231 were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 U/L penicillin and 100 μg/mL streptomycin. The cells were then incubated for 3 days in a humidified 5% CO_2 containing balanced air incubator at 37 ^oC. Medium was changed for three times during incubation period. The cytotoxicity of these cells was measured using [3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide] MTT assay method. 200 μL of MDA-MB 231cells, at a density of 2 x $10^{5,}$ were placed in each well of a 96- well plate. Then the cells were incubated for 24 h at 37 ^oC in a humidified 5% CO_2 containing atmosphere. After that the media was removed and the cells were treated with different concentration of **PSMN-g-BDP** to investigate the toxicity. As blank, there were only the samples. The cells were then incubated like before for another 24 h. Then the media containing drug was removed and a total of 20 μL of a stock solution containing 15 mg of MTT in 3 mL PBS was added and incubated for another 4 h. Finally 180 μL MTT solubulizing agents were added to the cell and proper shaking for 15 min was done.

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Absorbance was measured at the wavelength of 570 nm. The relative cell viability was measured by comparing the control well containing only the cell.

1.3 Confocal Images

MDA-MB 231 cells were plated over a cover slide on a eight-well plate at a density of 2 x 10^5 cells per well and were incubated for 24 h at 37 ^{0}C in a humidified 5% CO_2 containing atmosphere. The cells were treated with **PSMN-g-BDP** at 0.01 mg/ml in fresh culture medium between pH 5.0 and 8.0. For pH control of the medium, 0.1 N HCl and 0.1 N NaOH solutions were used.[2-4] After incubation for 1 h at 37 ^{0}C, cells were washed with ice-cold PBS for several times and fixed with fresh 4% (w/v) formaldehyde solution at room temperature. Finally, the cells were examined by using an LSM510 confocal laser scanning microscope (Carl Zeiss, Germany) equipped with a 364-nm UV laser and 543-nm He Ne.

The lysosome imaging was performed with a LysoTracker Blue probe using MDA-MB 231 cancer cells. The method was adapted from the literature.[5] Cells were seeded in an eight-well plate according to the procedure abovementioned and cultured for 24 h. When cells have reached the desired confluence, remove the medium from the plates and cells were incubated with 0.01 mg/ml of **PSMN-g-BDP** at pH 5.0 and pH 7.4 in different wells for 30 min. The cells were washed thoroughly using PBS and replaced with fresh culture medium at pH 7.4. Then the cells were stained with 50 nM LysoTracker Blue and incubated the cells for 15 min at 37 ^{o}C. The dye solution was removed and the cells were gently washed twice with PBS. Finally, the cells were observed immediately at room temperature under a laser scanning confocal microscope system.

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1.4 Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 400 spectrometer operating at 400 MHz using deuterium oxide as the solvent. The copolymer was determined by gel permeation chromatography (GPC, YL9112 Isocratic pump, Younglin instrument, KOREA) with a KD-804, KD-803 column (Shodex, JAPAN) using DMF. Fluorescence measurements were recorded in 3 mL quartz cuvettes using FluoroMate FS-2 (Scienco, Korea) fluorescence spectrometer equipped with a xenon lamp excitation source. All fluorescence spectra were measured at an excitation wavelength of 526 nm. Particle size was measured by using dynamic laser light scattering (DLS) at different pH (Zetasizer Nano, Malvern-Germany). Zeta potential was obtained from zeta potential & particle size analyzer (ELS-Z) of Otsuka Electronics Corporation.

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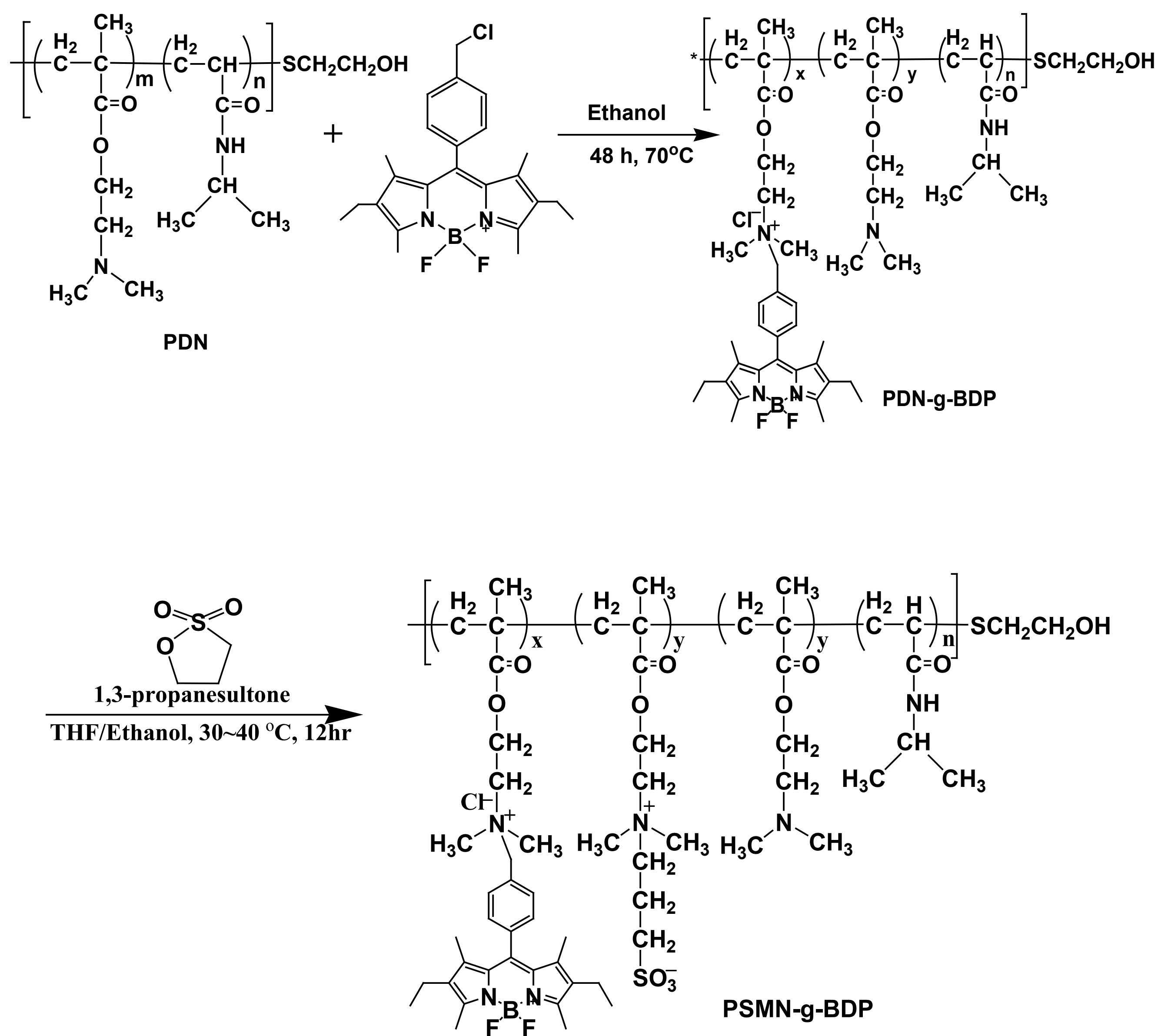

Figure S1. Scheme illustrates the synthesis of **PSMN-g-BDP**.

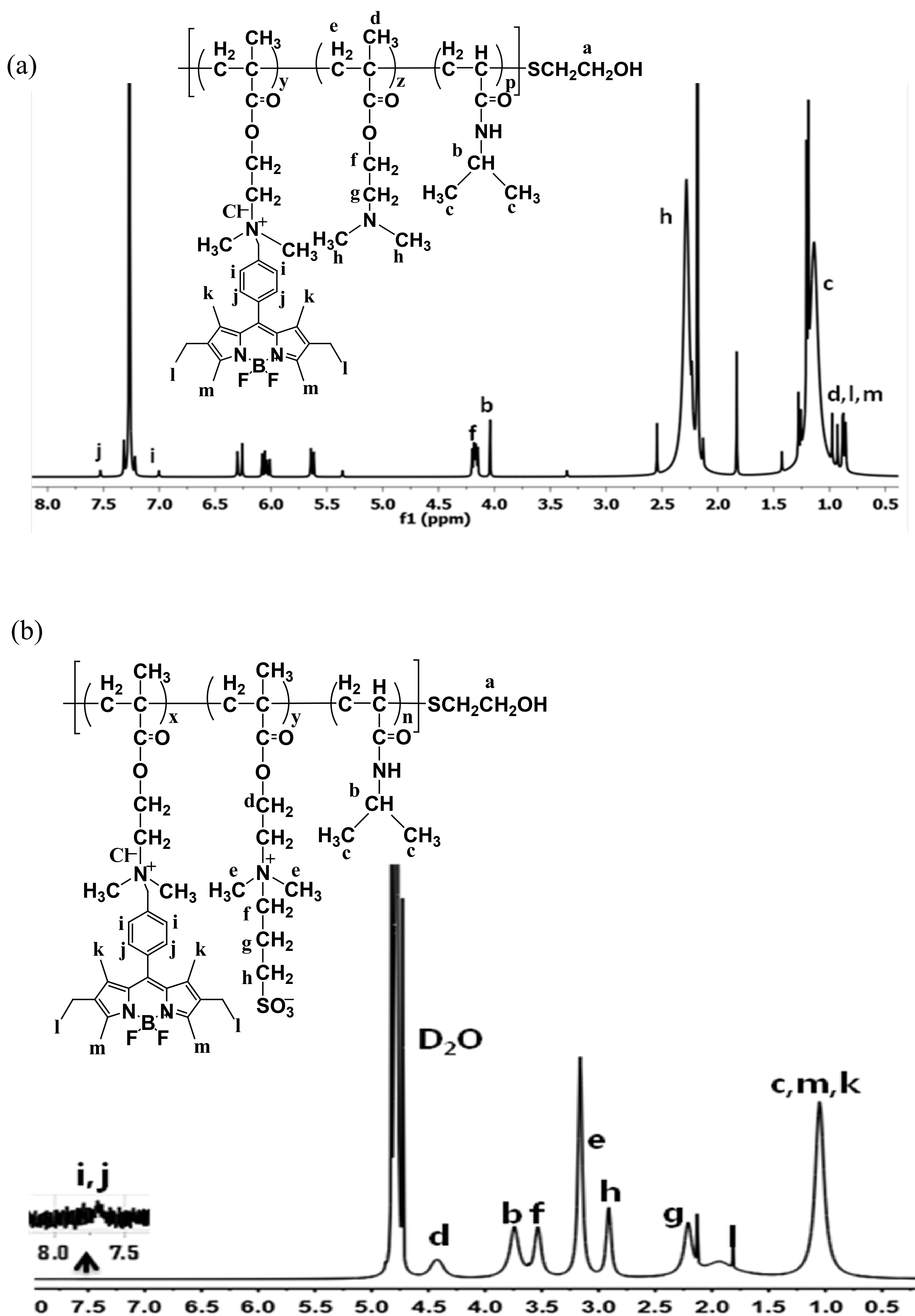

Figure S2. ^{1}H-NMR spectrum of (a) **PDN-g-BDP** in $CDCl_3$ and (b) **PSMN-g-BDP** in D_2O.

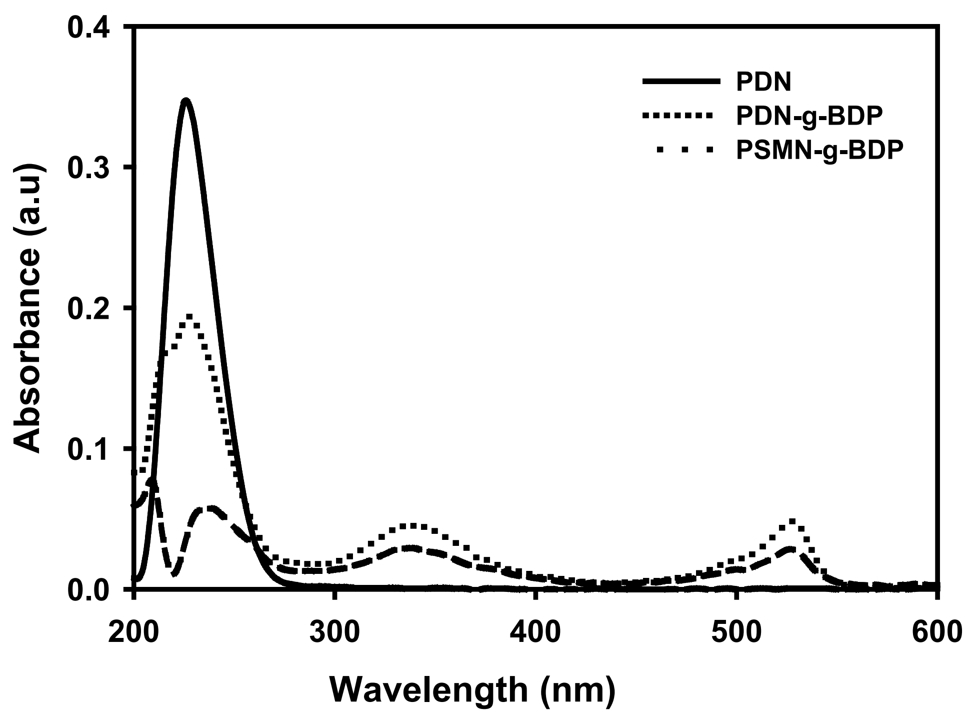

Figure S3. UV-vis spectra of the prepared zwitterionic **PSMN-g-BDP** at 37 ^{o}C in phosphate buffer solutions at pH 7.4 with concentration 0.01 mg/ml.

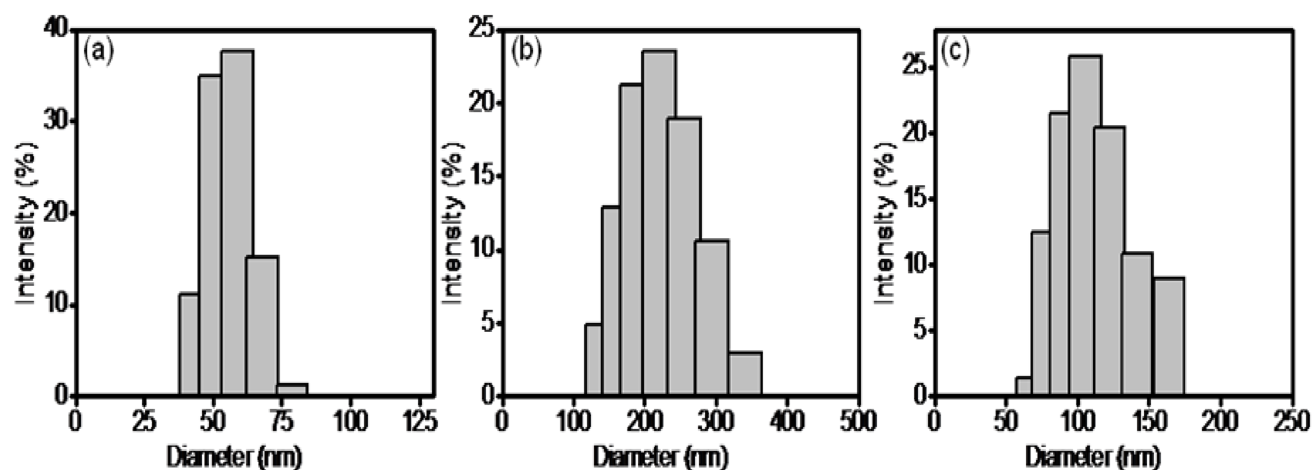

Figure S4. Dynamic light scattering (DLS) measurements of **PSMN-g-BDP** at 37 ^{o}C in different pH conditions (a) pH 5.0, (b) pH 7.4, and (c) pH 8.0 in phosphate buffer solutions respectively.

References and Notes

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