Supporting Information

Tunable and Selective Detection of Cancer Cells using Betainized Zwitterionic Polymers with BODIPY and Graphene Oxide

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1. EXPERIMENTAL SECTION

1.1 Materials

Boron dipyrromethane (BODIPY) having benzyl chloride on the meso position was prepared according to the literature.[1] 2-(Dimethylamino) ethyl methacrylate (DMA), N-isopropyl acrylamide (NIPAAm), 2-mercaptoethanol, azobisisobutyronitrile (AIBN), 2-chloro-3',4'-dihydroxyacetophenone (CA), 1,3-propanesultone, tetrahydrofuran (THF), ethanol, trizma base (99%, Sigma), trizma HCl, (99%, Sigma), [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) all were purchased from Sigma Aldrich Korea. The NIPAAm monomer was recrystallized from hexane and dried under vacuum. LysoTracker Red DND-99 (1 mM solution in DMSO) was purchased from Molecular Probes, Invitrogen, Inc. (Eugene, OR). 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 Synthesis of poly (DMA-co-NIPAAm) (PDN)

The process of **poly (DMA-co-NIPAAm)** (**PDN**) (Mn = 36,000 determined by using gel permeation chromatography) was synthesized according to our published literature.[2-3] Briefly, DMA (30 mM) and NIPAAm (45 mM), 2-mercaptoethanol, and AIBN were dissolved in 80 ml of anhydrous tetrahydrofuran. The mixture was then stirred for 24 h at 60~70 ^{0}C. The product was purified by precipitation in hexane after rotary evaporation and dried in vacuum. The polymer was purified by dialysis (molecular weight cut-off: 1,000~1,500; Spectrum Laboratories, Inc.) for 2 days. The yield of the product was 84%.

1.3 Confocal Images

MDA-MB 231 cells, Hela cells and MDCK 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 **(CA-BODIPY)-PSMN/**rGO 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.[4-6] 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. The cell culture medium was replaced with serum-free medium prior to addition of **(CA-BODIPY)-PSMN/**rGO and Lyso Tracker Red DND-99. The cells were incubated with 0.4 ml of 50 μg/ml **(CA-BODIPY)-PSMN/**rGO and 50 nM Lyso Tracker Red in RPMI solution for 30 min at 37^{o}C. After incubation, the cell was washed three times with prewarmed PBS in a 37^{o}C water bath. Intracellular images of (**CA-BODIPY)-PSMN/**rGO and endosomes were examined by a confocal scanning microscope. Finally, the cells were examined by using an LSM510 confocal laser scanning microscope (Carl Zeiss, Germany) equipped with a 488-nm UV laser and 633-nm He Ne. L.

1.4 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 ^{o}C. 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 MDCK cells, 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 ^{o}C in a humidified 5% CO_2 containing

atmosphere. After that the media was removed and the cells were treated with different concentration of **(CA-BODIPY)-PSMN** or **(CA-BODIPY)-PSMN/**rGO 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. Absorbance was measured at the wavelength of 570 nm. The relative cell viability was measured by comparing the control well containing only the cell.

For the detection of viable cancer cells, membrane integrity was determined by DEAD cells staining using propidium iodide dye in MDA-MB 231 cancer cells. Cells were cultured and treated with **(CA-BDP)-PSMN/**rGO nanoparticles at concentration 0.5 mg/ml as described above. As control, cells were treated without given any samples. Following nanoparticles exposure, the cells were stained with propidium iodide as per the manufacturer's protocol and the membrane integrity was assessed using confocal microscopy imaging.

1.5 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 (Zetasizer Nano, Malvern-Germany). Tapping mode atomic force

microscopic (AFM) observations were performed with a digital instrument, (Nanoscope (R) IIIA), using micro fabricated cantilevers with an Amplitude Setpoint 0.7785V.

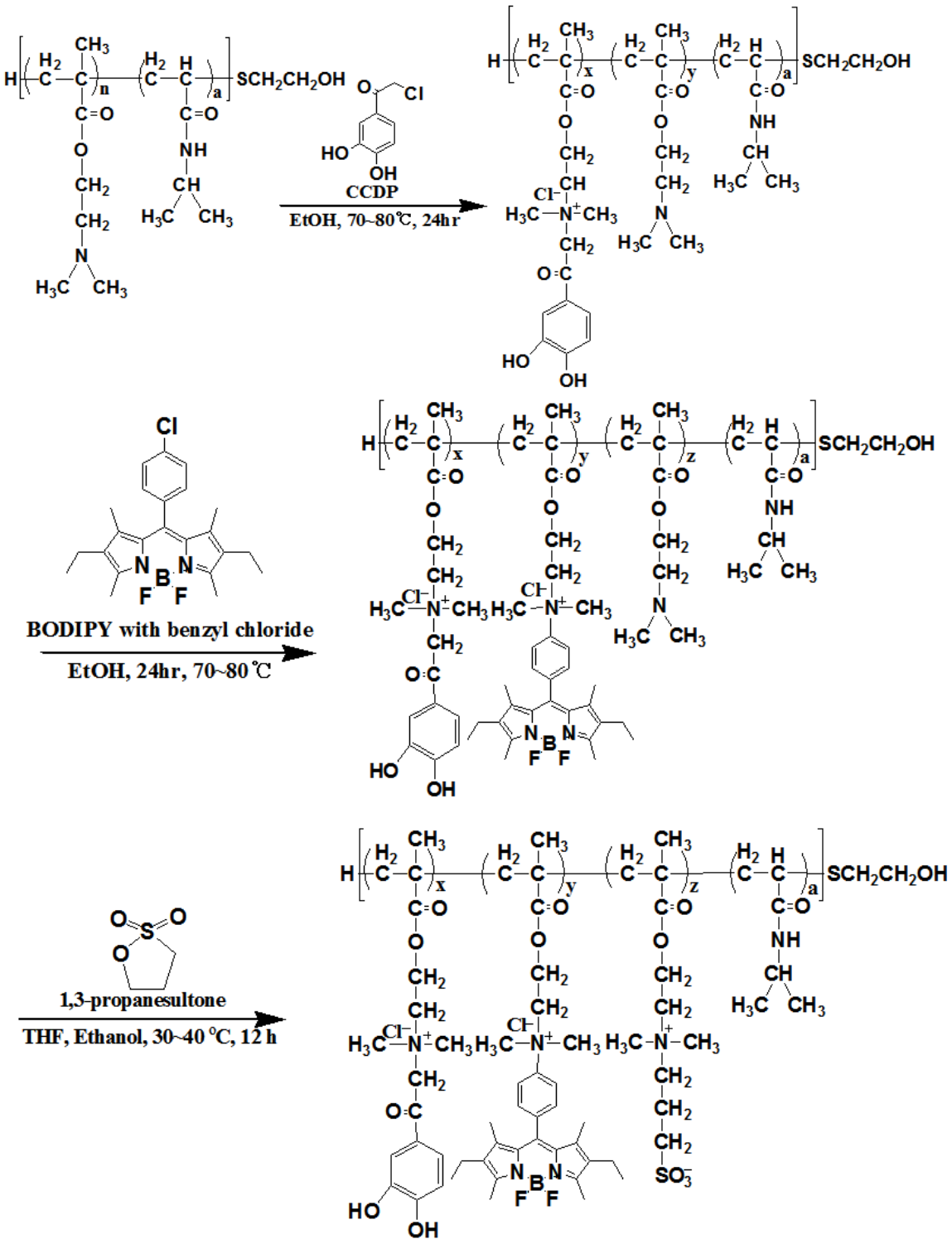

Fig. S1 Scheme illustrates the synthesis of (**CA-BODIPY**)-**PSMN**.

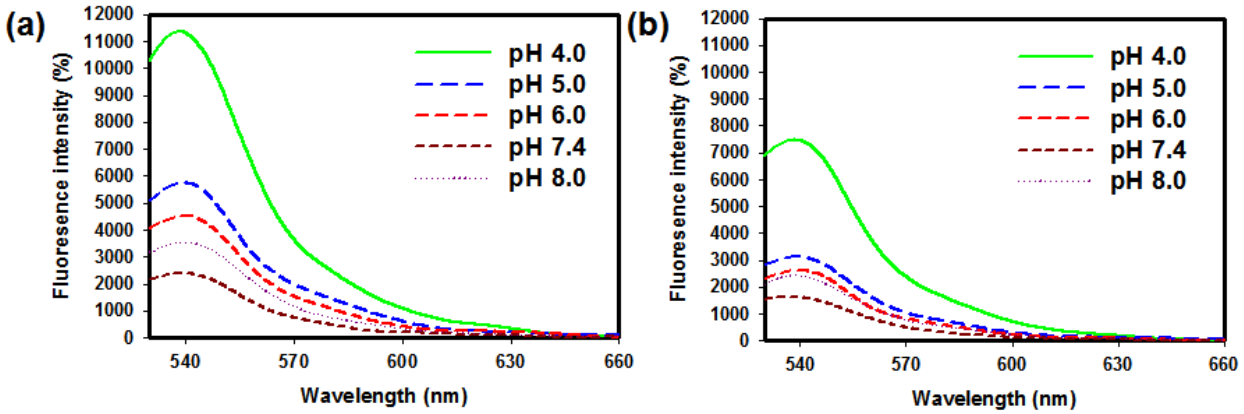

Fig. S2 Fluorescence spectra of **(CA-BODIPY)-PSMN** (0.01mg/ml) at different pH with (a) room and (b) body temperature in phosphate buffer solutions. The excitation wavelength is 526 nm.

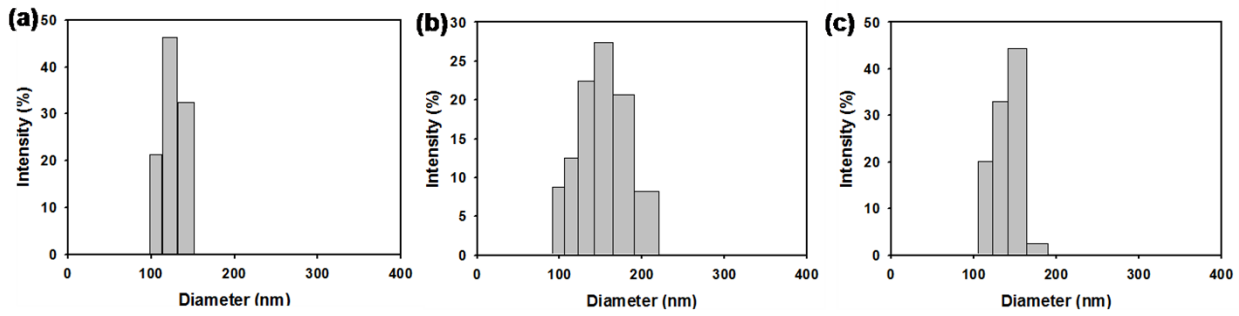

Fig. S3 Dynamic light scattering (DLS) measurements of (**CA-BODIPY**)-**PSMN**/rGO at 37 ^{o}C in different pH conditions (a) pH 5.0, (b) pH 7.4, and (c) pH 8.0 in phosphate buffer solutions respectively.

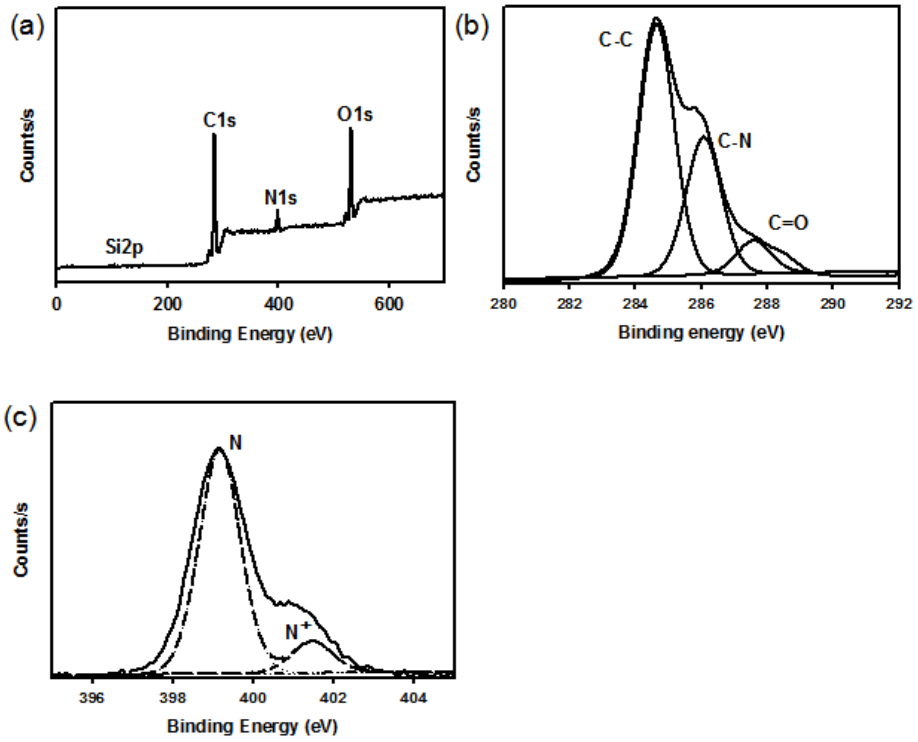

Fig. S4 XPS spectra of **(CA-BODIPY)-PSMN**/rGO (a) survey scan and (b) and (c) narrow scale scan of C1s and N1s respectively.

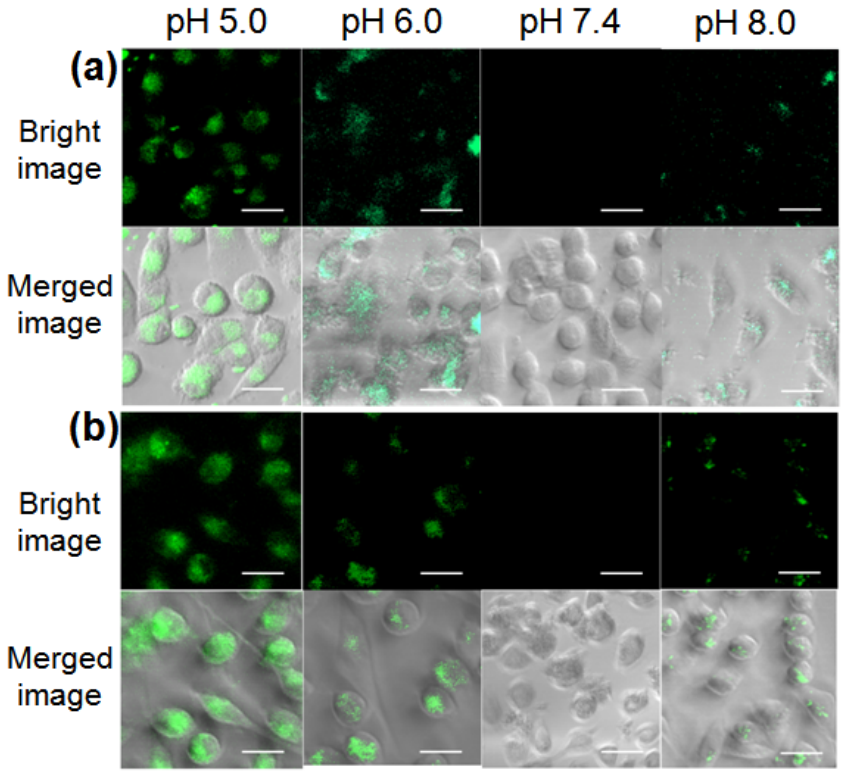

Fig. S5 Confocal microscope image of (a) Hela cancer cells (b) MDCK normal cells after incubation with **(CA-BODIPY)-PSMN**/rGO ZFNPs in fresh culture medium at different pH values. All the scale bars are 20 μm.

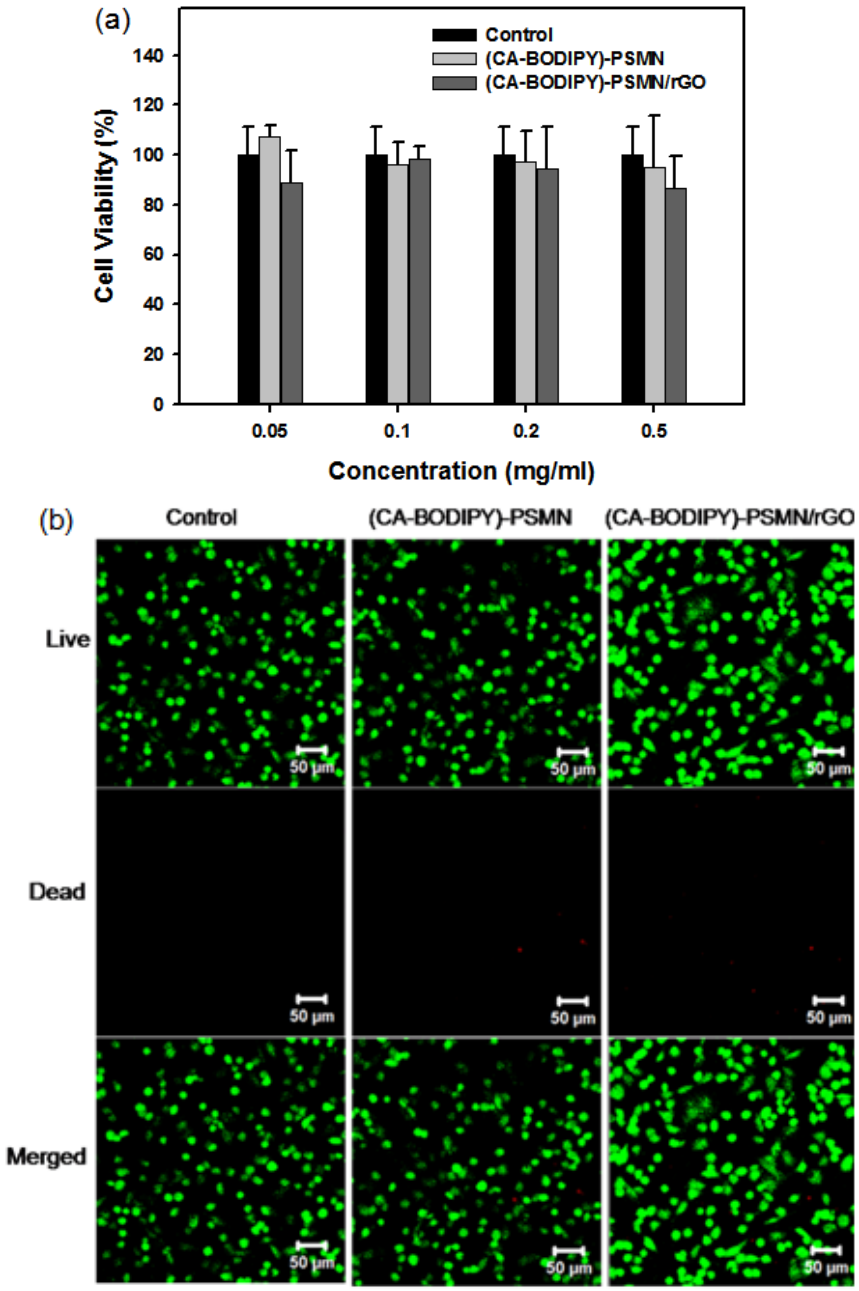

Fig. S6 (a) *In vitro* cytotoxicity of **(CA-BODIPY)-PSMN** and (**CA-BODIPY**)-**PSMN**/rGO determined by MTT assays in MDA-MB 231 cells. The cells were incubated with sample at different concentrations for 24 h. Data are presented as well as the average ± standard deviation (n = 6). (b) Fluorescence microscopy images of MDA-MB 231 cells after treatment with (**CA-BODIPY**)-**PSMN** and (**CA-BODIPY**)-**PSMN**/rGO for 24 h at 37 ^{o}C and staining with calcein AM for live cells (green) and propidium iodide for dead cells (red). All the scale bars are 50 um.

References

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