One-Pot Synthesis of Linear-like and Photoluminescent Polyethylenimines for Intracellular Imaging and siRNA Delivery

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Supporting Information

***Synthesis of compound b*:** All chemicals including ethylene diamine (**a**) were purchased from Sigma-Aldrich (St. Louis, USA). To synthesize compounds **b**, monomer (100 μL) was added to 5 mL of water (18 MΩ cm^{-1}) and then irradiated by X-ray at room temperature for 5 ~ 10 min. The final products were dried by lyophilizer or distillated by Kugerlrohr (BÜCHI) to remove extra starting material. Compounds **b** was 17 mg (18.9 %). Both ^{1}H NMR and ^{13}C NMR were recorded in 80 % D_2O (4.80 ppm) by a Varian MR-400 system.

***Cellular uptake*:** Lung cancer cell lines H460 were cultured in a humidified atmosphere with 5% CO_2. The cell culture medium used was RPMI 1640 (Gibco, NY, USA), supplemented with 10% fetal bovine serum (FBS; Gibco, NY, USA). For confocal microscopy, cells were plated 24 h before each experiment. After incubation with LPEIs (**b**) for 1.5 h, cells were stained with the nucleus-specific dye SYTO 59. Images were captured by an Olympus FV10i confocal spectral microscope using 60× oil immersion objective.

***Flow cytometry for cell cycle analysis*:** Lung cancer cell lines H460 with 2×10^5 cells/mL were treated with cyclin B1siRNA alone (200 nM) and compound **b**/siRNA complex ([compound **b**] = 100 ng/mL, [cyclin B1 siRNA] = 200 nM) in RPMI 1640 medium, respectively, and then incubated at 37°C, 5% $CO_{2\ (g)}$ for 1 h. Cells were

washed twice in PBS buffer solution and fixed in cold PBS solution containing 75% ethanol. After washing by PBS and then centrifuging at 1,500 rpm for 5 min, the cells were stained in propidium iodide (PI) and analyzed by FACS Calibur (BD PharMingen, NJ, USA) using WinMDI 2.9 analysis software. (The cyclin B1 siRNA sequence: sense: 5'-ACAUGAGAGCCAUCCUAAUUGTT-3, anti-sense: 5'-CAAUUAGGAUGGCUCUCAUGUTT-3', NCBI accession number of cyclin B1: NM031966)

***Cell cytotoxicity by MTT assay*:** The proliferation of human lung cancer cell lines H460 in the presence of various concentrations of our LPEIs (compound **b**) and other commercial PEIs (Aldrich), including BPEI (M_n = 1800) and the mixture of LPEI and BPEI (M_n = 423), was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT assay, Sigma, MO, USA). In a 24-well cell culture plate with 2×10^5 cells/mL of cell density per well, the final volume was 200 μL after plating cells and then allowing them to attach overnight. The cells were treated with LPEIs (**b**) and other commercial PEIs and incubated at 37°C, 5% $CO_{2\,(g)}$ for 48 h. The cells were incubated with MTT at 37°C for 1 h. After cell lysis, the intracellular formazan product was dissolved by DMSO and then quantified by a conventional ELISA reader at 540 nm.

The measurement of the quantum yield and lifetime of compound b: The compound **b** was dissolved in DI water and the quantum yield was compared by quinine (QY = 0.53) while the absorbed intensity of the solution containing either compound b or quinine was adjusted to > 0.01 and < 0.06.

The measurement of the number of secondary amines of compound b: Compound **b** (0.011 g) were dissolved in a co-solvent (20 mL, isopropyl alcohol: ethylene glycol = 1:1). After thorough mixing, the solution was either added acetic anhydride (0.2mL) to block all primary amines (1^o-amine) and secondary amine (2^o-amine) or added

salicylaldehyde (0.2 mL) to only block the primary amines, respectively. After reacting for 30 minutes, two solutions were titrated with 0.01N HCl, respectively. Figure S4A shows two titration curves from compound **b**. Their inflection points of apparent pH versus the volume of hydrochloric acid were re-plotted in Figure S4B. After being calculated, the percentage of 2^{o}-amine of the polymer was approximately 90%.

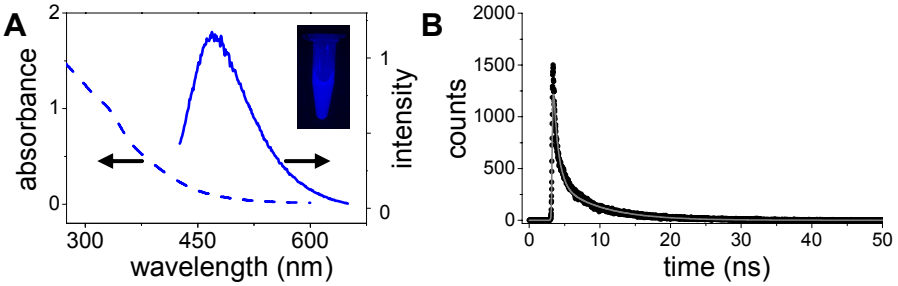

Figure S1. (A) Optical properties including UV-vis (dashed line) and emission spectra (solid line) of compound **b** indicate the maximum emission appearing at 478 nm. Inset is a white light photograph of compound **b**. (B) the photoluminescent lifetime of compound **b**, which curve (gray line) has been fitted to a biexpontential decay (black-dot line) after measuring by pulsed diode light source with 405 nm.

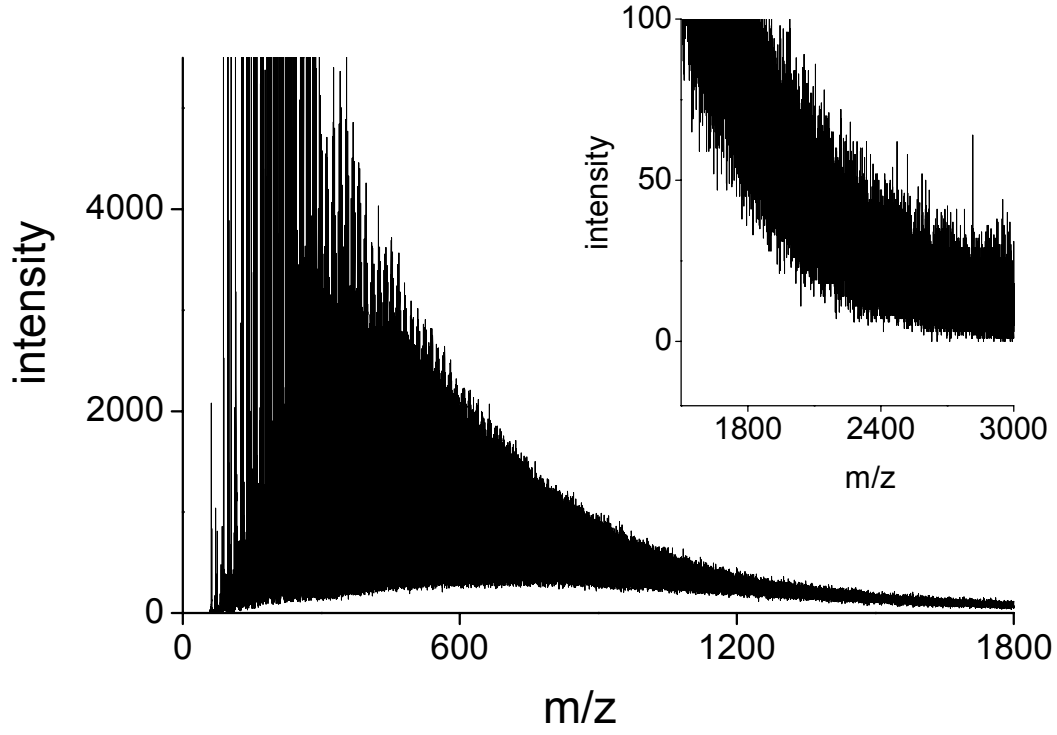

Figure S2. MALDI-TOF spectrum of compound **b**.

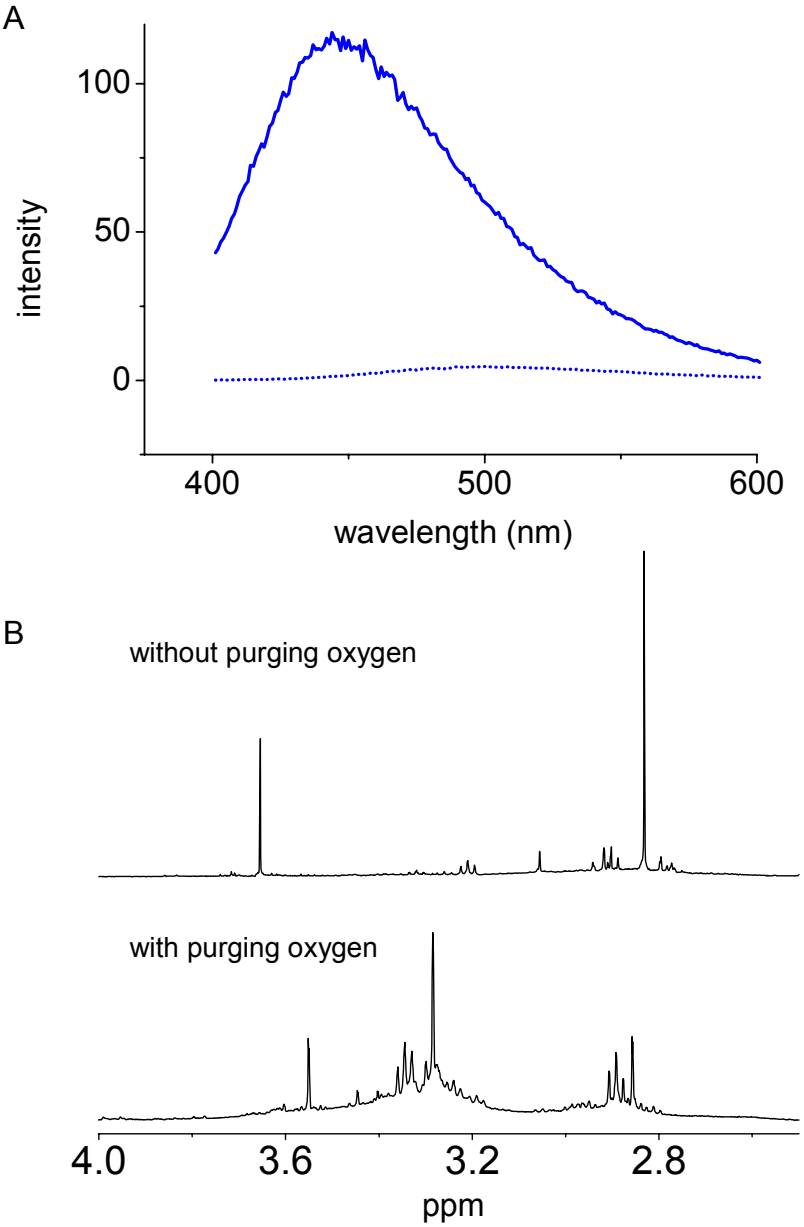

Figure S3. (A) Emission spectra of LPEIs (**b**) is in response to one typical radical scavenger ($O_{2(g)}$). Blue Solid line and dashed line for **b** are present in blowing N_2(g) and O_2(g) during synthesis, respectively. (B) ^{1}H spectra (400 MHz) show of the formation of compounds **b** without and with purging oxygen.

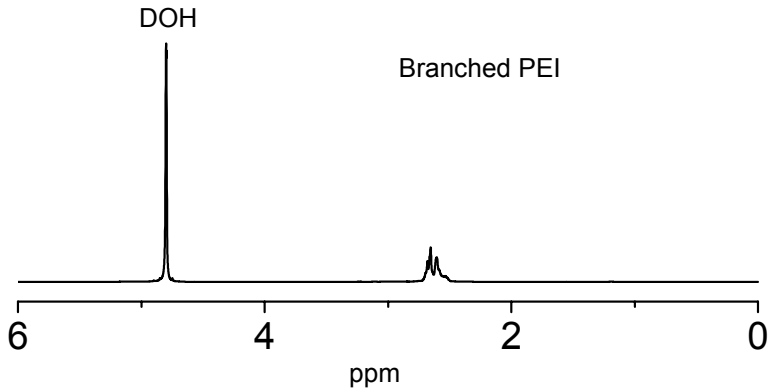

Figure S4. ^{1}H spectra of commercial BPEI.

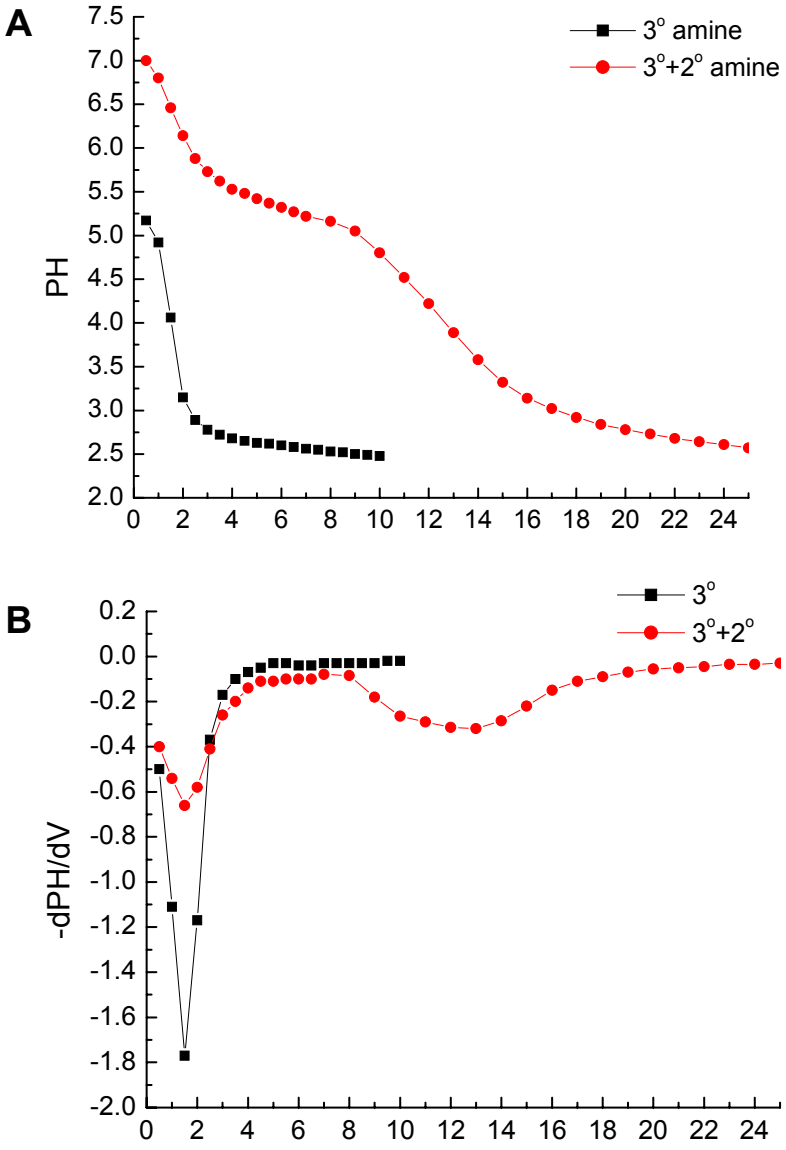

Figure S5. (A) The titration curves and (B) difference curves of (A) compound **b** were measured by according to the previous report.[1]

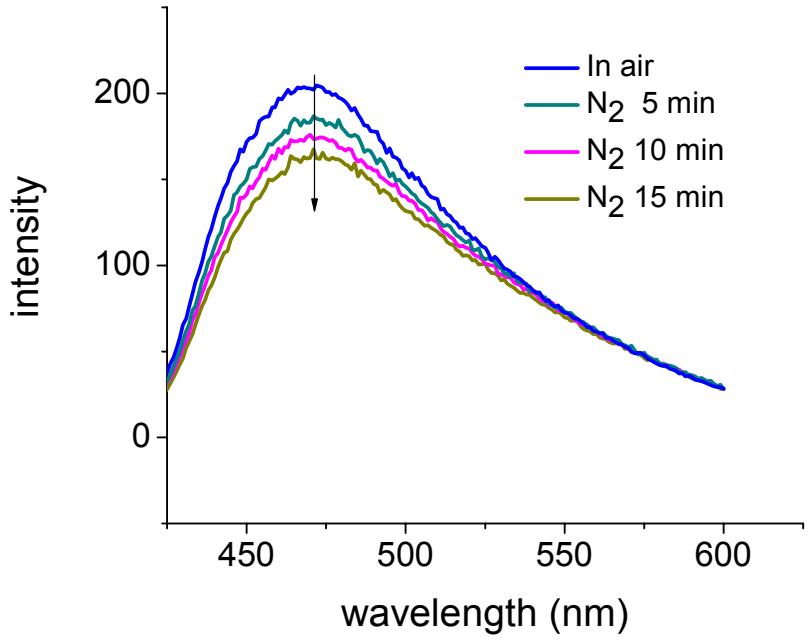

Figure S6. Emission spectra of compound **b** in water were carried out in air and $N_{2(g)}$-bubbling.

Reference in supporting information:

1. S. Siggia and J. G. Hanna, In Quantitative Organic Analysis via Functional Groups, 4th ed.; Krieger: Malabar, FL, **1988**; pp 569–572.