Electronic Supplementary Information

Multimodal cellular redox nanosensors based on self-doped polyaniline nanocomposites

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Materials and methods

2.1. Materials

Polyaniline MW ~5,000, 1-pyrenebutyric acid and Tween 80 were purchased from Sigma-Aldrich Chemical Co (USA). 1-Methyl-2-Pyrrolidone (NMP) was purchased from Dae-Jung chemicals & metals (KOREA). Dulbecco's phosphate-buffered saline (DPBS, pH 7.4) was purchased from Welgene (Gyeongsan, South Korea). All other chemicals and reagents were analytical grade. Ultrapure deionized (DI) water was used for all of the synthetic processes.

2.2. Synthesis of Tween80-coated polyaniline (TPAni) nanoparticles

TPAni nanoparticles were prepared according to a previously published nano-precipitation method with some modifications. First, 5 mg of PAni was dissolved in 4 ml NMP and added to 30 ml of DI water containing 100 mg of Tween 80. The mixture was vigorously stirred at room temperature for 4 hours. After the reaction, TPAni nanoparticles were dialyzed for 48 hours (MWCO: 3,500 Da Spectra/Por® 6, SPECTRUM® LABORATORIES, INC) to remove excess impurities and filtered by centrifugation using a Centricon filter (MW cutoff: 3000 Da) for 2 h at 3000 rpm.

2.3. Synthesis of Tween80-coated polyaniline mixed 1-pyrene butyric acid (TPAba)

Detailed synthetic conditions of TPAba are shown in table S1 in Supplementary Materials. The ideal synthetic condition of TPAba are as follows: 12.5 mg of PAni and 5.39 mg of Pyba were dissolved in 4 mL of NMP. The dissolved solution was added into 30 mL of DI water solution including 100 mg of Tween 80. The mixed solution was vigorously stirred at room temperature for 4 hours. Next, the product was dialyzed using dialysis membrane (MWCO: 3,500 Da Spectra/Por® 6, SPECTRUM® LABORATORIES, INC) for 48 hours to remove excess impurities and filtered by centrifugation using a Centricon filter (MWCO: 3,000 Da) for

2 hours at 3,000 rpm.

2.4. Characterization of TPAba

After synthesis of TPAba, transmission electron microscopic (TEM) image was obtained (JEM-1011, JEOL Ltd.) and hydrodynamic diameter was also measured (ELS-Z, Otsuka Electronics). the absorbance of TPAba was analyzed UV-Vis spectrophotometer (UV-1800, Shimadzu Co), and fluorescence spectra was obtained from spectrofluorometer (FP6500, Jasco). Raman spectra were measured via Raman spectrometer (NS-100, Nanoscope Systems).

2.5. *Cell viability test of TPAba nanocomposites against breast cancer cells*

The cytotoxic effects of TPAba nanocomposites against two breast cancer cell lines (HCC-1954 and HCC-1143) were evaluated by performing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Cell Proliferation Kit I, Roche Molecular Biochemicals, Mannheim, Germany). Cells were maintained at 37℃ in a 5% CO_2 humidified atmosphere. HCC-1954 and HCC-1143 cells (5.0×10^4 cells/well) were seeded into a 96-well plate and placed at 37℃ overnight; then, cells were incubated with various concentrations of LiPani for 48 hours. The cells were washed with 100 μL of 1 mM DPBS (pH 7.4), and 100 μL of phenol red–free RPMI-1640 was added. Subsequently, the cells were treated with MTT assay solution according to the manufacturer's instructions. Cell viability was evaluated using a microplate reader (Synergy H4 Hybrid Reader, BioTek, Winooski, VT, USA) at an absorbance wavelength of 575 nm (reference wavelength of 650 nm). Cell viability was represented by normalization against cells that were not treated with LiPani, which were considered to represent 100% cell viability.

2.6. Dark field microscopy imaging of breast cancer cell lines

HCC-1954 and HCC-1143 cells (1.0×10^6 cells/well) were seeded into 6-well plates with cover-glass bottoms and incubated for 4 hours at 37℃. TPAba nanocomposites were added to RPMI and incubated with the cells for 48 hours at 37℃. TPAba nanocomposites in the cells was observed by recording light scattering images using an inverted microscope (Olympus BX51, Japan) with a highly numerical dark field condenser (U-DCW, Olympus), which delivers a very narrow beam of white light from a tungsten lamp to the cell surface. Immersion oil (nd 1.516, Olympus) was used to narrow the gap between the condenser and the glass slide and to balance the refractive index.

2.7. Spectra measurements of cancer cells treated with TPAba nanocomposites

HCC-1954 and HCC-1143 cells (1.0×10^6 cells/well) were seeded in 6-well plates and incubated for 24 hours at 37℃. TPAba nanocomposites were added to phenol red–free medium, and then incubated with the plated cells for 48 hours at 37℃ with 5% CO_2 atmosphere. The supernatant was transferred to new centrifuge tubes and centrifuged at 15,000 rpm for 30 minutes to remove any remaining cell debris. Then, the sample absorbance and fluorescence were measured using a microplate reader (Synergy H4 Hybrid reader, BioTek), and analyzed with Gene5 software (v 3.08, BioTek), and Raman spectra of cells with TPAba nanocomposites were measured via Raman spectrometer (NS-100, Nanoscope Systems).

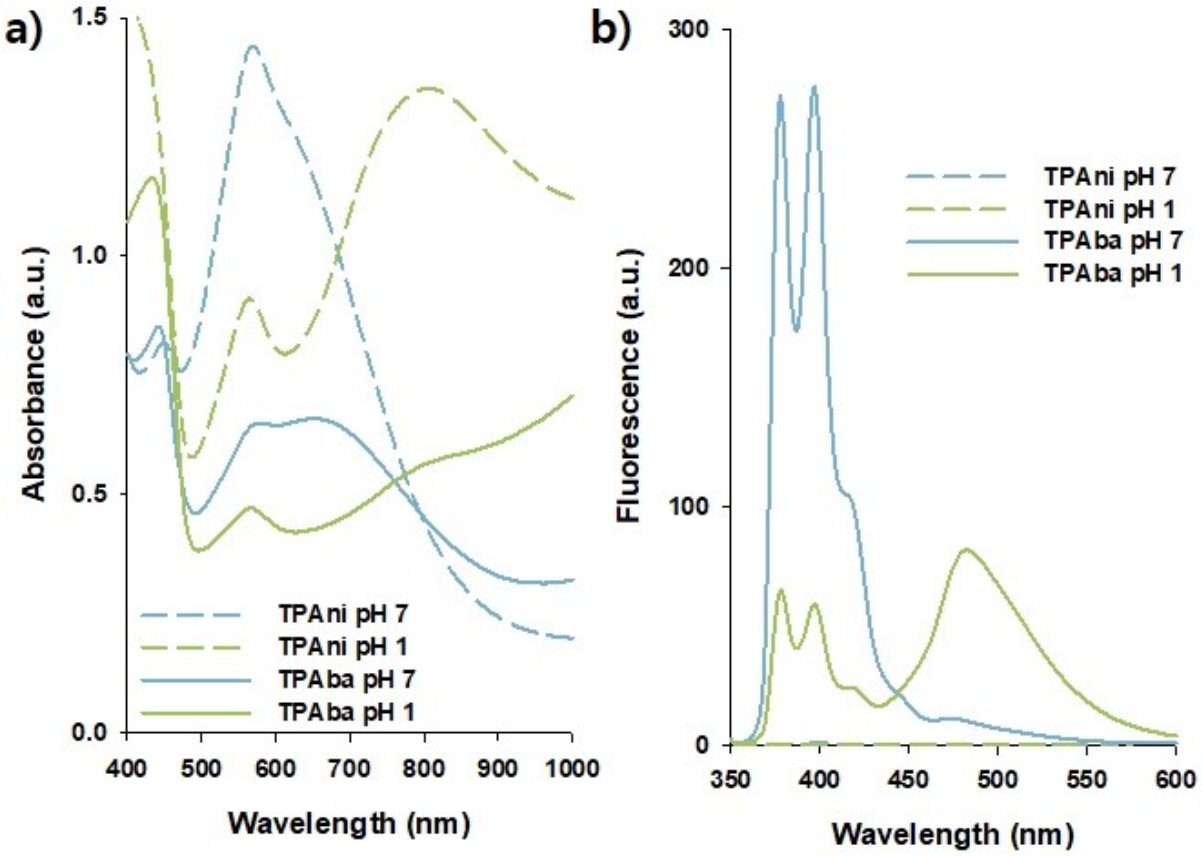

Figure. S1. a) Absorbance and b) fluorescence spectra of TPAni nanoparticles and TPAba nanocomposites in the pH 7 and pH 1.

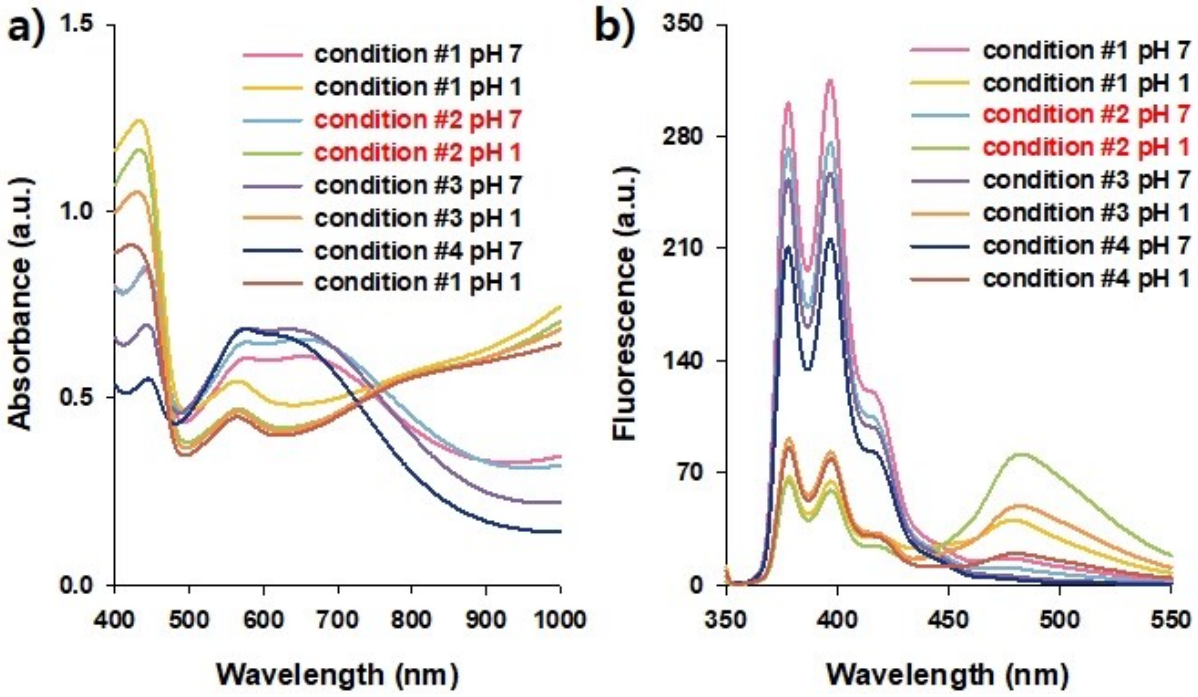

Figure. S2. a) absorbance and fluorescence spectra of TPAba nanocoposites at indicated synthetic conditions. The synthetic conditions are described at Table S1. The optimal condition for synthesis of TPAba nanocomposites is synthetic condition #2.

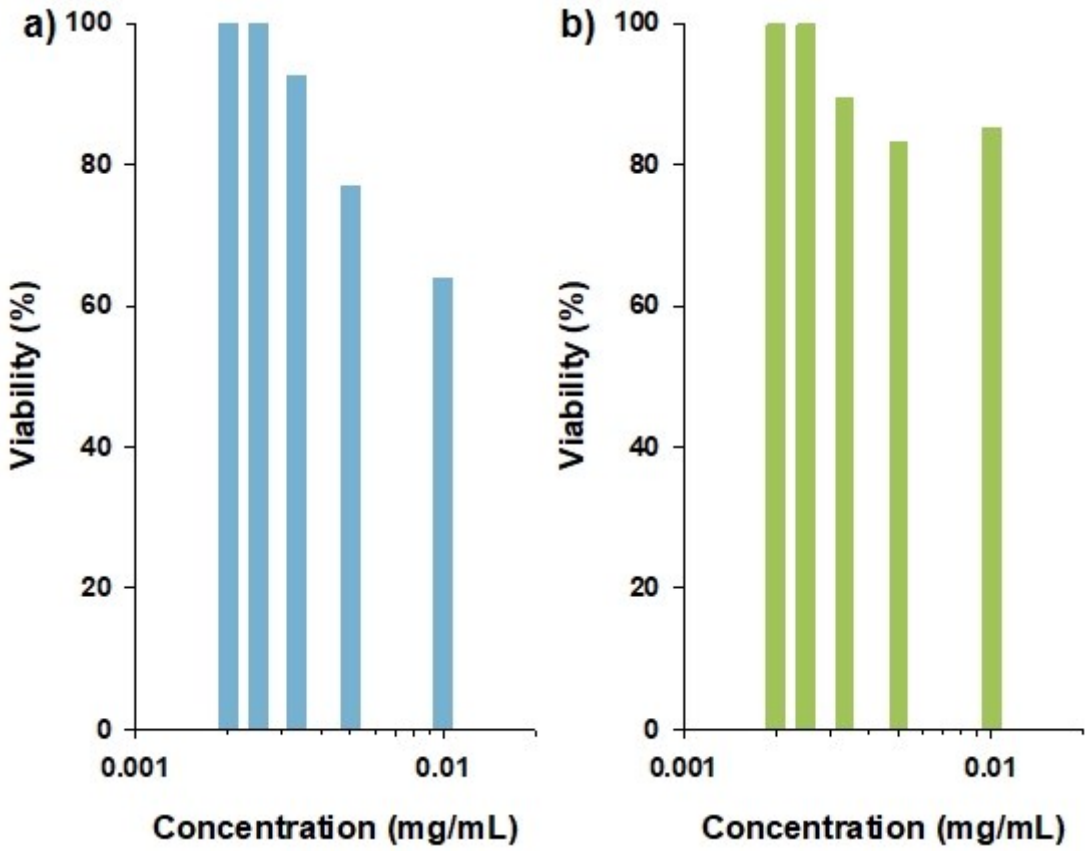

Figure. S3. Cell viability test of a) HCC-1143 and b) HCC-1954 cancer cell lines for TPAba nanocomposites.

Synthetic conditions	Pyrenebutyric acid (mg)	Polyaniline (mg)	TWEEN 80 (mg)	NMP (mL)
1	10.80	12.50	100.00	4.00
2	5.39	12.50	100.00	4.00
3	2.70	12.50	100.00	4.00
4	1.35	12.50	100.00	4.00

Table S1. Synthetic conditions for the fabrication of TPAba nanocomposites.

Raman shift (cm^{-1})	Description
525	Skeletal stretching (pyrene)
749	Quinoid ring deformation (EB)
859	Quinoid ring deformation (EB)
1166	C-H bending in quinoid ring (EB)
1228	C=C stretching/ C-H in-plane bending (pyrene)
1343	**Radical cation by protonation (ES)**
1418	C=C stretching/ring stretching (pyrene)
1457	**C=N stretching in quinoid ring (EB)**
1508	N-H bending (ES)
1597	C=C stretching in quinoid ring (EB)

Table S2. Description of the observed Raman peaks.