Supplementary Information for:

Photoluminescent polymer nanoparticles for label-free cellular imaging

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1. Experimental details

Ultrasound (350 W) was introduced into the solution composed of 1.5 ml of acrylonitrile monomer, 0.5 g of sodium dodecylsulfacte and 20 ml of distilled water for 10 min. After adding 0.1 g of cerium sulphate and 0.025 g of nitroacetic acid into the solution, polymerization was performed for 10 min by aid of ultrasound irradiation. The solution was diluted by adding excess ethanol and 1M of nitric acid (to remove cerium ions) and the precipitates were dried. The products (0.1 g) were loaded into the closed vessel including sealing apparatus. The vessel was evacuated until inner pressure reaches 10^{-2} torr, and heated to 70 ^{o}C. 0.1 ml of EDA was introduced and vapor phase treatment were performed for desired time. The residual monomer and EDA vapor was removed from the reactor by additional evacuation.

The tPAN nanoparticles (5 mg) were mixed with 0.1 M of PBS buffer solution (5 ml) and F127 as a dispersant (10 mg mL^{-1}), and exposed to ultrasound for 40 min to obtain uniform dispersion. The 1 mL of Anti-ErbB2 antibody (ab2428, Abcam Inc., MA) (10 μg mL^{-1}) was added into the above solution, which had been previously incubated with EDC/NHS for 12 h (Reference: C. Wu et al. *Langmuir* 2006, **22**, 2956). The EDC/NHS conjugation method is very well-known procedure that connects carboxyl and amine group in biological experiments. EDC is a water-soluble derivative that catalyzes the formation of amide bonds between carboxylic acids and amines by activating carboxyl to form an O-urea derivative. This derivative reacts readily with nucleophiles. NHS is employed to activate carboxylic acids and amines toward amide formation, sometimes without EDC (Anal. Biochem. 218, 1994, 87). This reaction was extensively applied to couple covalently protein/enzyme molecules to self-

assemble monolayers of thiolcarboxylic acids. After 2 hours reaction at 25 ^{o}C, the residual Anti-ErbB2 is removed by centrifugal force.

TEM images were obtained with a JEOL EM-2000 EX II microscope. SEM was performed with a JEOL 6330F at an acceleration voltage of 10 kV. A Bomem MB 100 FTIR spectrometer was used to characterize the PAN and tPAN nanoparticles. UV-vis spectrum of the tPAN nanoparticles was taken with a Perkin-Elmer Lambda-20 spectrometer. The Emission spectra of PAN and tPAN nanoparticles were obtained with a JASCO FP-6500 spectrofluorometer.

Human breast cancer SK-BR-3 cell were obtained from American Type Culture Collection (Manassas, VA). SKBR-3 were cultured in RPMI-1640 medium, with 10% fetal bovine serum, 1% penicillin- streptomycin solution, 300 mg L-1 of L-glutamine, 25 mM of sodium bicarbonate, and 25 mM of 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES). Cells were incubated in a 5% CO_2 incubator at 37 ^{o}C and 100% humidity. Cells were placed in 75T flask and maintained between 1 x 10^5 and 1 x 10^6 cells mL^{-1} of medium. The medium was changed every 2-3 days or as required.

Cell viability was measured using CellTiter glow luminescent cell viability assay (Promega, Madison, WI). The assay is performed by estimating the number of viable cells in system, based on quantification of the ATP concentration. The single step procedure produces a luminescent signal, which is proportional to the quantification of ATP present in cells. This assay is depended on the alteration of beetle luciferin to oxyluciferin by a thermostable luciferase in the presence of ATP. The experiments were performed in 96-well plates. For the cell viability assay, 3000 cells per well were plated and treated with different concentrations of nanomaterials for 24 h.

Cells were seeded at a density of 2000 cells per well, in 8–well Lab–Tek™ II Chambered Coverglass (Nunc, Thermo Fisher Scientific, NY) and treated with PAN nanomaterials (10 μg mL^{-1}). After incubation with PAN nanomaterials for 24 h, the plates were observed under a DeltaVision® RT imaging system (Applied Precision, Issaquah, WA) with temperature (37^{o}C) and CO_2 (5%) control and photographed using a Cascade II EMCCD camera.

Polyethylene glycol-N-hydroxysuccinimide (PEG-NHS; Nanocs Inc., NY) was used for surface functionalization of the tPAN nanoparticles that could reduce the non-specific uptake on the cells due to the electrostatic attraction between positive charge of tPAN surface and negative charge of cell lipid layer. NHS is highly reactive compounds suitable for the modification of amino groups. NHS is the most common type of activated esters. The reaction of PEG-NHS with amines is conducted as manufacturer's instructions. Briefly, tPAN nanoparticles (10 mg) were mixed with 0.1 M of PBS (10 mL). Then, 4 mM of PEG-NHS in DMSO was added into the tPAN solution. After 3 h reaction at 25 ^{o}C, the product was washed thoroughly by 0.1 M PBS.

2. Dispersity of PAN and tPAN nanoparticles.

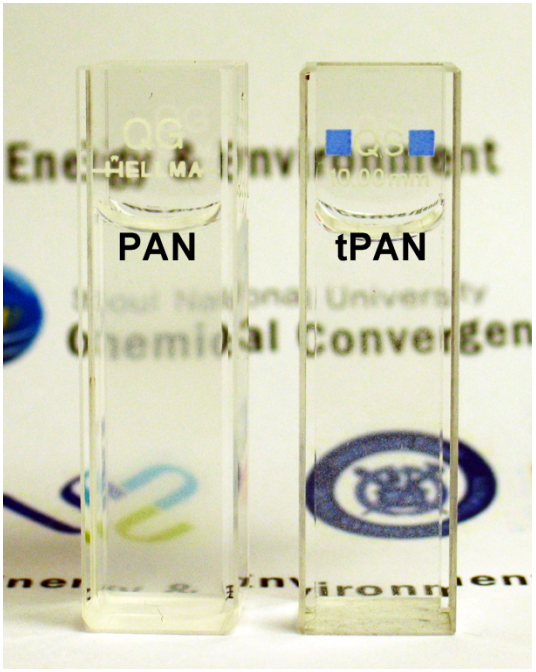

Figure S1. Photograph of aqueous media containing PAN and tPAN nanoparticles (0.1 mg mL-1)

3. FTIR peak assignments

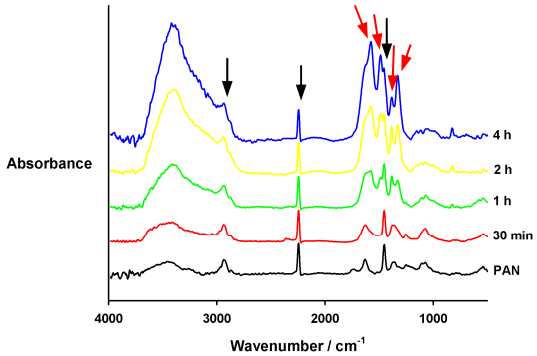

Figure S2. FTIR spectra of PAN and tPAN nanoparticles as a function of reaction time. The time increasing (30 min, 1, 2, and 4 h) represents the EDA treatment time on the PAN nanoparticles.

Table S1. FTIR assignment of PAN and tPAN nanoparticles

Materials	Wavenumber[a]	Assignments
PAN nanoparticles	2872	C−H stretching
	2241	C≡N stretching
	1446	C−H deformation
EDA Treated PAN nanoparticles	1568	N−H deformation of amine group
	1479, 1375	C−H stretching conjugated with EDA
	1319	C−N stretching
	815	N −H rocking

[a] unit: cm^{-1}

4. Emission spectra of tPAN nanoparticles and DAPI

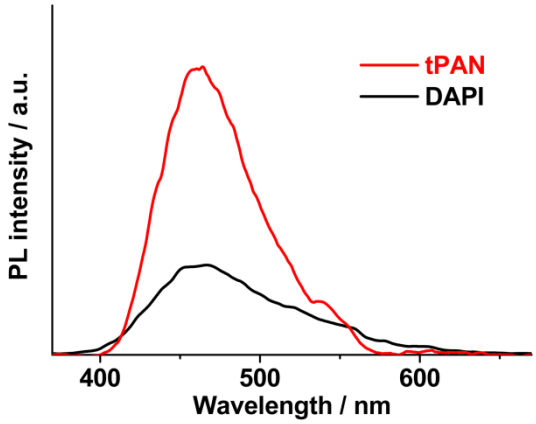

Fig. S3. Emission spectra of tPAN nanoparticles in aqueous solution and DAPI solution.

The PL quantum yields of tPAN nanoparticles were determined by a comparison method of the fluorescence emission with the standard reference sample of DAPI (D1306, Molecular Probes Inc., OR) in solutions. Basically, the fluorescence emission of a sample can be calculated with following formula with related parameters (Appl. Opt. 34, 1995, 1989).

$$F = K\Phi c\sigma l I$$

where Φ is the fluorescence quantum yield of the sample, c is the concentration of the sample, σ is the one-photon absorption cross section, l is the length of the path in which photons are absorbed, I is the flux of incident photons (photons/cm^2 s), F is the integrated fluorescence signal in the emission region, and K is a parameter constant of the instrument. By measuring the emission spectra as represented in Fig. S3, the F_P (tPAN) and F_D (DAPI), corresponding to integration of the emission regions in Fig. S3, respectively, were obtained. The fluorescence signals were measured under 287 nm with the same experimental conditions in the same system, so that the K, l, and I are the same for tPAN and DAPI samples. Using the above formula for tPAN and DAPI, respectively, the PL quantum yield Φ_P of tPAN nanoparticles can then be calculated by comparing with the known Φ_D of DAPI as follows:

$$\Phi_P = \frac{F_P}{c\sigma} \times \frac{c_D \sigma_D}{F_D} \times \Phi_D$$

Since the cσ represents the absorption of the sample, the term of $c_D\sigma_D/c\sigma$ can be replaced by A_D/A_P. The A is the absorption coefficient of the sample at the excitation wavelength. Then the formula turns to the simple form.

$$\Phi_P = \frac{F_P}{A_P} \times \frac{A_D}{F_D} \times \Phi_D$$

When the values of A_D and A_P were measured, the Φ_P of tPAN can be obtained. The value of F was calculated based on emission spectra in Fig. S3. We measured tPAN and DAPI, at the excitation wavelength of 287 nm in a spectrometer (JASCO FP-6500) and then determined the Φ_P according to the formula.

5. In-situ images

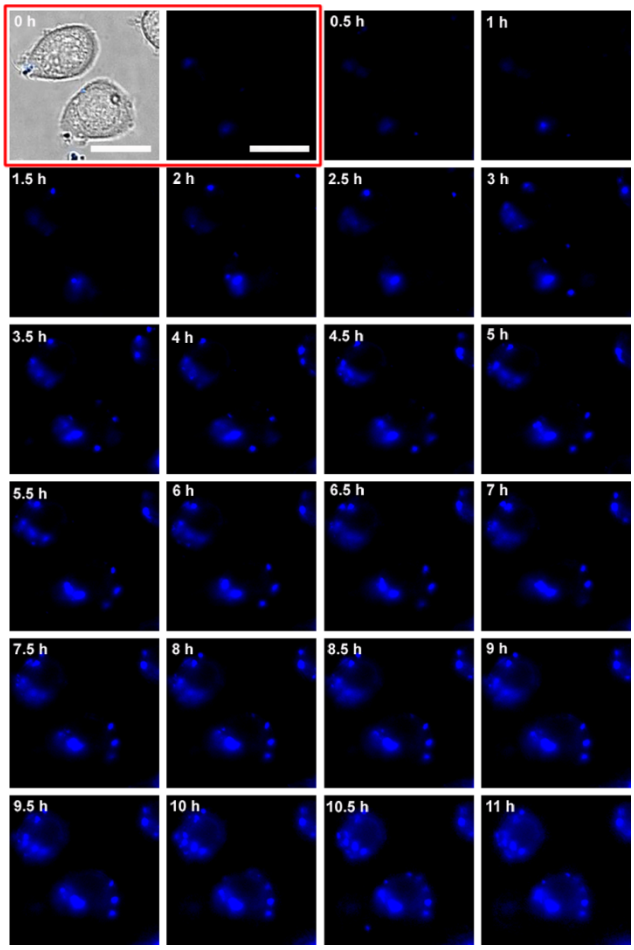

Figure S4. Time lapse live cell fluorescent images of antibody conjugated tPAN nanoparticles in SK-BR-3 cells over 11 h. The blue represents nanoparticles in cells. The scale of each square is 50 μm. The images are taken at 30 min interval for 11 h.

When the incubation time was 1 h, weak fluorescence was detected on the cells. However, the cell shapes were obvious over 8 h due to completed uptake of the antibody conjugated tPAN nanoparticles. Furthermore, the PL intensity at 11 h was not altered compared to initial PL intensity.

6. Z-sectional Images

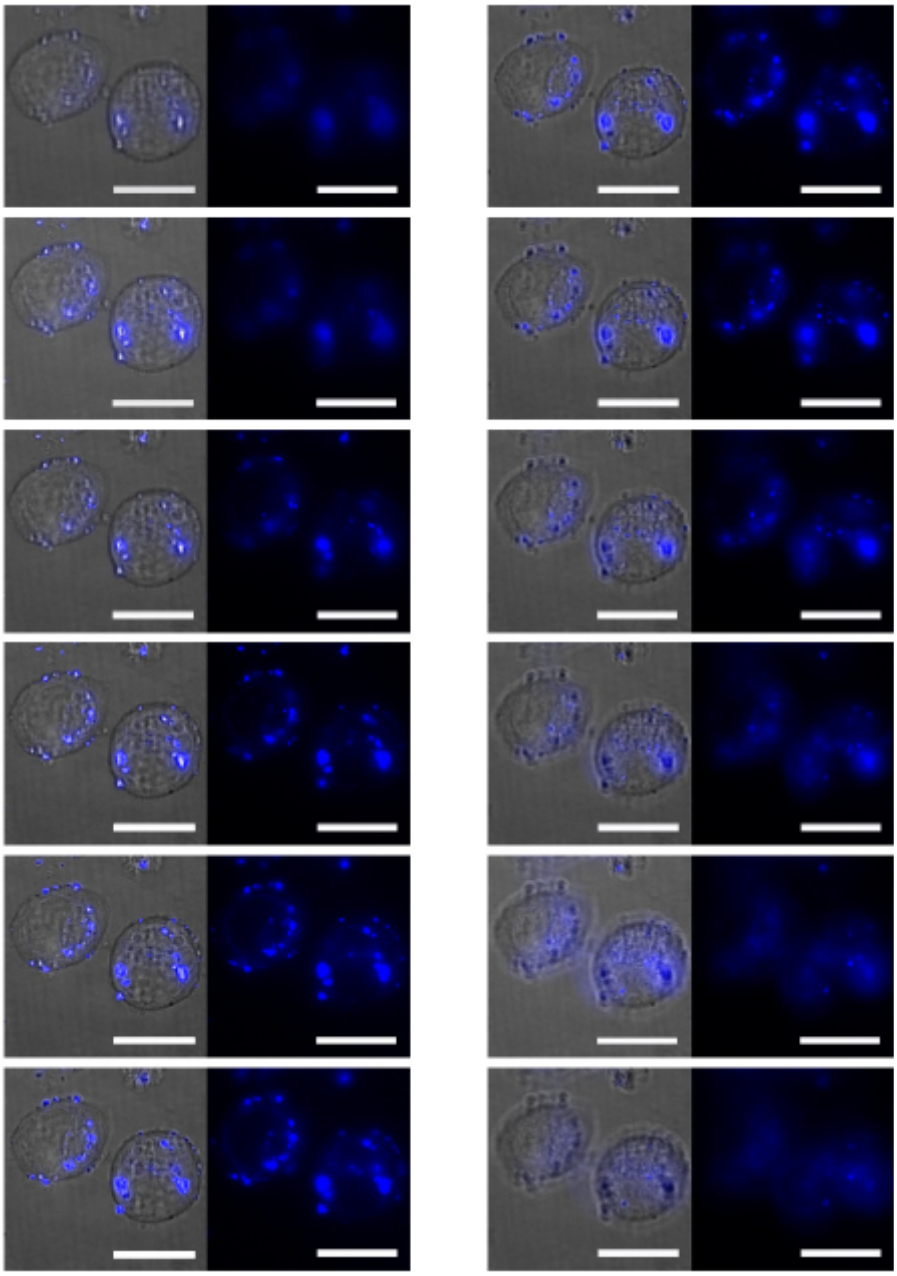

Figure S5. Differential interface contrast (DIC) and fluorescent z-sectioning images of SK-BR-3 cell treated with antibody conjugated tPAN nanoparticles for 24 h. The sections are ordered from apex of the cell to the near substrate plane. Z-scan step width is 0.1μm. (Scale bars: 20 μm)

7. PEG-treated tPAN nanoparticles

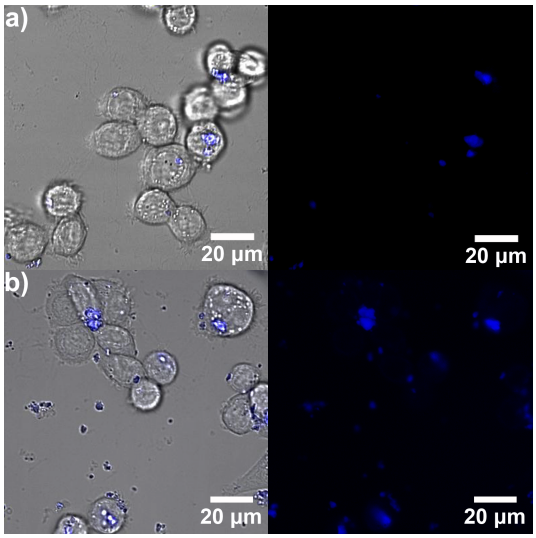

Fig. S6. Live cell microscopic images of SK-BR-3 cells incubated with PEG-treated tPAN nanoparticles of a) 5 μg mL^{-1}, b) 25 μg mL^{-1} (left slot: DIC images, right slot: fluorescent images).

In order to improve non-specific endocytosis of the tPAN, polyethylene glycol (PEG) treatment was performed on the tPAN. PEG-treated tPAN (termed PEG-tPAN) that was used for reducing its non-specificity. Fig. S6 represented live cell microscopic images SK-BR-3 cells incubated with PEG-tPAN for 24 h. In Fig. S6a, the majority of the PEG-tPAN located in the surface of cells, not in the cells. Furthermore, the emission intensity and population were relatively smaller than those of tPAN (Fig. 4). Some of the PEG-tPAN remained outside of the cells. It was obvious that PEGylation on tPAN inhibited endocytosis of the nanoparticles into the cells due to minimizing the electrostatic interaction between the particle and cell surface. Moreover, more particles located outside of the cells when the dose of the PEG-tPAN increased to 25 μg mL^{-1} (Fig. S6b). Judging from these results, the non-specific uptake of positive-charged nanoparticles (tPAN) could be reduce through the surface functionalization of the PEGylation. Labeling for active targeting after the surface functionalization can be performed by further research.