Conjugated polymer nanoparticles for biomedical in vivo imaging

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Experimental Details

Synthesis of cvPDs. All cyanovinylene-backboned polymer dots (cvPDs) were prepared by *in situ* Knoevenagel polymerization of equimolar dialdehyde and diacetonitrile monomers (10.82 mg in total) in aqueous micelles of Tween 80 (0.3-0.6 g in 5 mL water), as described for NIR-cvPDs in the following. 2,5-Bis(octyloxy)terephthalaldehyde (25.8 mg, 60 mmol, Aldrich Chemical) and *p*-xylylene dicyanide (10.3 mg, 60 mmol, TCI) were dissolved in Tween 80 (1 g, Sigma Chemical) with gentle heating. The cooled mixture (0.3 g) was mixed with Milli-Q water (5 mL) under sonication into a transparent micellar dispersion. Polymerization was initiated by adding 0.2 mL of tetrabutylammonium hydroxide (1.0 M in methanol, Aldrich Chemical) and conducted overnight under magnetic stirring at room temperature. Unreacted monomers, catalyst and excess surfactant were removed by dialyzing the reaction mixture against Milli-Q water in a cellulose ester membrane (cutoff=300 kDa) for 48 h, to afford a red-colored clear dispersion of fluorescent polymer nanoparticles that can be used as is for the *in vivo* applications. For characterization purpose, the obtained aqueous dispersion was freeze-dried into a mixture of agglomerates of polymer nanoparticles and excess Tween 80 residue (1:9.9 by weight) that is no more dispersible in water and hardly soluble in most organic solvents. From the dried polymer powder amount after ethanol washing of Tween 80, the polymerization yield was estimated to be 89%. In FT-IR spectroscopic analysis of the dried polymer powder and two monomers (KBr, Spectrum GX, PerkinElmer), two C-H stretching vibration bands of the monomeric aldehyde at 2762 and 2881 cm^{-1} were disappeared and the nitrile stretching vibration band was shifted from 2251 cm^{-1}

(aliphatic nitrile, monomeric) to 2211 cm^{-1} (conjugated nitrile, polymeric), confirming semiconducting polymer formation and complete removal of monomers.

Characterization of cvPDs. TEM images were obtained with a CM30 electron microscope (FEI/Philips) operated at 200 kV. Absorption (transmittance) and photoluminescence spectra of cvPDs dispersed in water or 100% serum were acquired using a UV-visible spectrometer (Agilent 8453) and a fluorescence spectrophotometer (Hitachi F-7000, wavelength calibrated for excitation and emission), respectively. The fluorescence quantum yields were determined using a methanol solution of rhodamine B as a reference. Unfiltered true-color photographs of water-dispersed cvPDs or cvPD-injected mice were taken with a general-purpose digital camera under room light or a hand-held UV lamp at 365 nm (Spectroline, ENF-240C/FE, Spectronics, 1-2 mW/cm^2).

Animal experiments. The animal study has been approved by the animal care and use committee of Korea Institute of Science and Technology and all handling of mice was performed in accordance with the institutional regulations.

SLN mapping by *in vivo* imaging. For animal experiments, BALB/c nude mice (male, 5 weeks of age; Institute of Medical Science, Tokyo) were initially anaesthetized with intraperitoneal injection of 0.5% pentobarbital sodium (0.10 mL/10 g). NIR-cvPDs in an as-dialyzed aqueous dispersion (10 μL, 0.17-1.7 mg/mL) were intradermally injected into the forepaw pad. NIR fluorescence snapshots were imaged at predetermined time points after injection, using a 12-bit CCD camera (Kodak Image Station 4000MM) equipped with a 150-W quartz halogen illuminator (Fiber-Lite, PL900, Dolan-Jenner) and a filter set of x535 for excitation and e700WA for emission. The real-time fluorescence video of SLN mapping was recorded using a 580-nm filtered CCD camera under the illumination at 460-490 nm, implemented in a high-sensitivity imaging system (Olympus OV100).

***Ex vivo* imaging and histologic analysis.** After *in vivo* imaging studies, *ex vivo* NIR fluorescence images of resected organs, lymph nodes and the rest of the body were taken by a Kodak imaging system with the same acquisition setup as used for *in vivo* imaging. For optical and fluorescent histologic inspection, hematoxylin-eosin (H&E)-stained sections (20 μm, CM1900 microtome, Leica) of the OCT-embedded lymph nodes were photographed on microscopes (Olympus BX51 and Ziess Axioskop2 FS Plus). Histologic TEM imaging was performed at Korea Basic Science Institute, where Epon812-embedded lymph nodes were sectioned at a thickness of 70 nm by using an ultra-microtome (Ultracut UCT, Leica) and electron micrographs were taken on a Bio-TEM (Tecnai G2 spirit, FEI).

Supplementary Figures

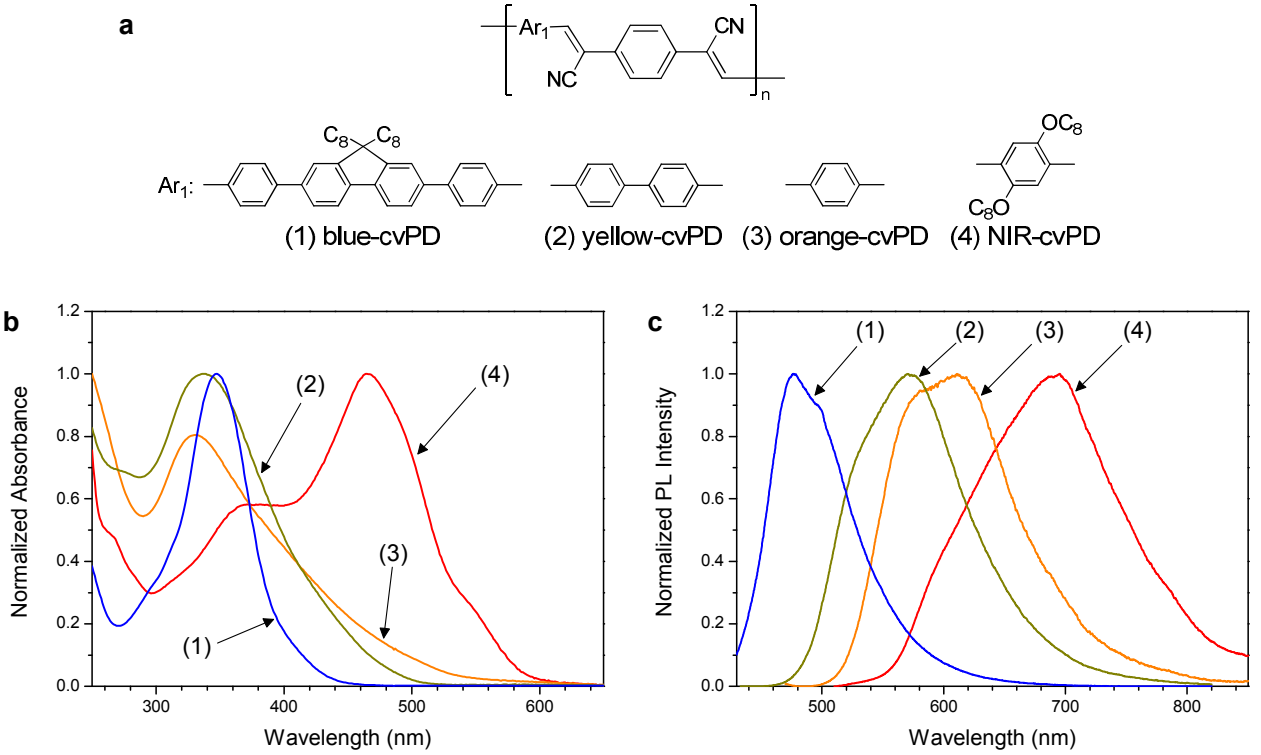


Fig. S1 Chemically tuned fluorescence color of cvPDs by varying the chemical structure of monomers. (a) Chemical structures of cvPDs, synthesized in this study. In the structures of aromatic dialdehyde and diacetonitrile monomers in Figure S1, Ar_2 is phenylene and Ar_1 is varied as shown above, to tune the fluorescence color throughout the visible-to-NIR optical range. (b, c) Normalized absorption and photoluminescence spectra of water-dispersed cvPDs. The fluorescence quantum yields were measured to be 0.56 (blue-cvPD), 0.61 (yellow-cvPD), 0.25 (orange-cvPD), and 0.21 (NIR-cvPD), respectively.

Dialdehyde monomers (4,4'-biphenyldicarboxaldehyde, terephthalaldehyde and 2,5-bis(octyloxy)terephthalaldehyde), diacetonitrile monomer (*p*-xylylene dicyanide) and other reagents were purchased from Aldrich Chemical or TCI, and used as received. 4,4'-(9,9-Dioctyl-fluorene-2,7-diyl)dibenzaldehyde was prepared by Suzuki coupling between 9,9-dioctyl-2,7-dibromofluorene and 4-formylphenylboronic acid in toluene, following the standard scheme with a palladium catalyst ($Pd(PPh_3)_4$) and aq. Na_2CO_3. ^{1}H NMR ($CDCl_3$): δ 10.09 (s, 2H), 8.00 (d, 4H, *J*=8.1 Hz), 7.85 (d, 4H, *J*=8.1 Hz), 7.84 (d, 2H, *J*=8.1 Hz), 7.66 (dd, 2H, *J*=8.1, 1.7 Hz), 7.62 (m, 2H), 2.10-2.04 (m, 4H), 1.23-1.02 (m, 20H), 0.78 (t, 6H, *J*=6.8 Hz), 0.83-0.63 (m, 4H).

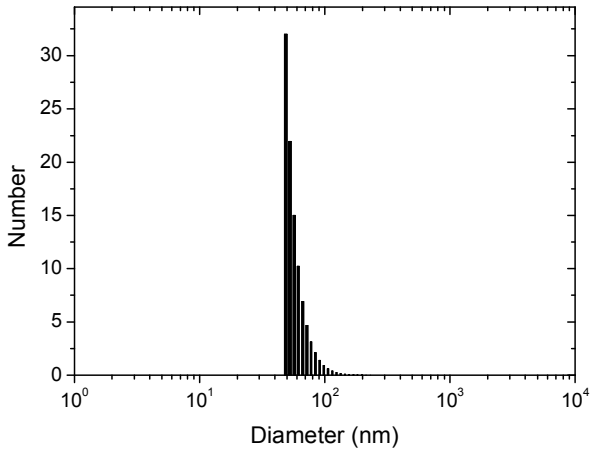


Fig. S2 Hydrodynamic size of NIR-cvPDs, determined by dynamic light scattering (Otsuka ELS-Z2 with a detection angle of 165°), which presented a number-weighted size distribution of 60.3±14.2 (mean ± s.d.).

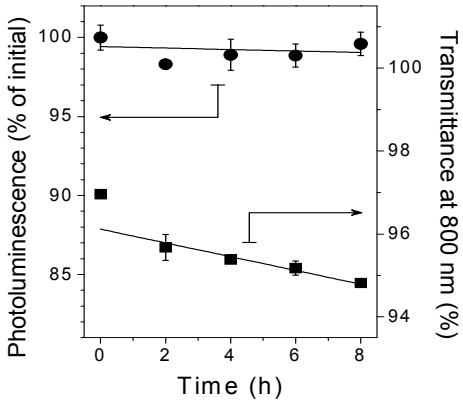
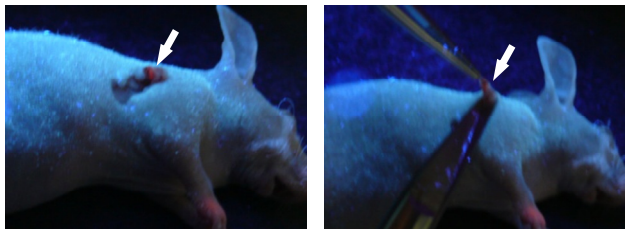


Fig. S3 Chemical and colloidal stability of NIR-cvPDs in 100% FBS at 37 °C. Transmittance at absorption-free wavelength (800 nm) represents a measure of light loss by scattering.

Axillary Lymph Node Resection



Lateral Thoracic Lymph Node Resection

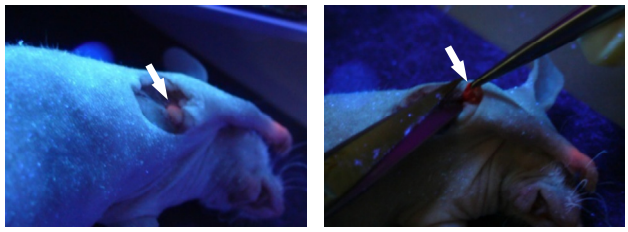


Fig. S4 True-color photographs showing fluorescence-guided surgery of a cvPD-injected mouse (10 μL of 0.17 mg/mL, intradermally into the right paw), taken with a general-purpose digital camera under UV excitation. At 60 min post-injection, SLNs of the anaesthetized mouse were precisely located and resected in a minimally invasive way, through intraoperative guidance by fluorescence under the illumination with a 365-nm handheld UV lamp (1-2 mW/cm^2). SLNs are indicated by arrows.

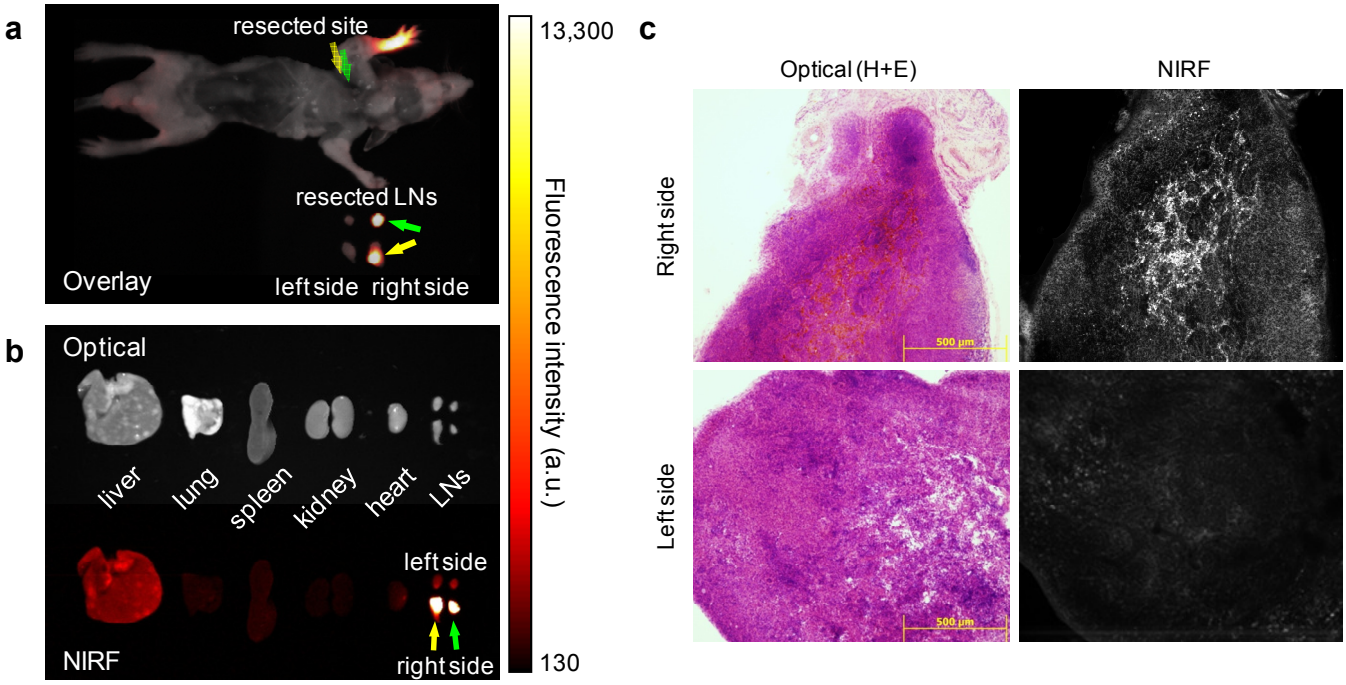


Fig. S5 (a-b) Optical, NIR fluorescence (NIRF) and overlay images showing the *ex vivo* biodistribution of NIR-cvPDs in a mouse. Lymph nodes (LNs) and organs in (b) were collected from the mouse body shown in (a), a week after intradermal injection into the right forepaw. The resected SLNs and the corresponding resection sites in the body are indicated by bright and shaded arrows for axillary (yellow) and lateral thoracic (green) nodes, respectively. Complete excision of SLNs by fluorescence-guided surgery is confirmed by the whole body NIRF image in (a) that shows no remnant signal at the surgical site. (c) Optical and NIRF micrographs of hematoxylin (H) and eosin (E) stained sections of the resected lymph nodes in (a). Samples resected from the injected (right) and non-injected (left) sides of the body correspond to the polymer dot-entrapped and the dot-free control lymph nodes, respectively. Sinuses of the injected side LN appear to be filled with orange color and NIRF of NIR-cvPDs.

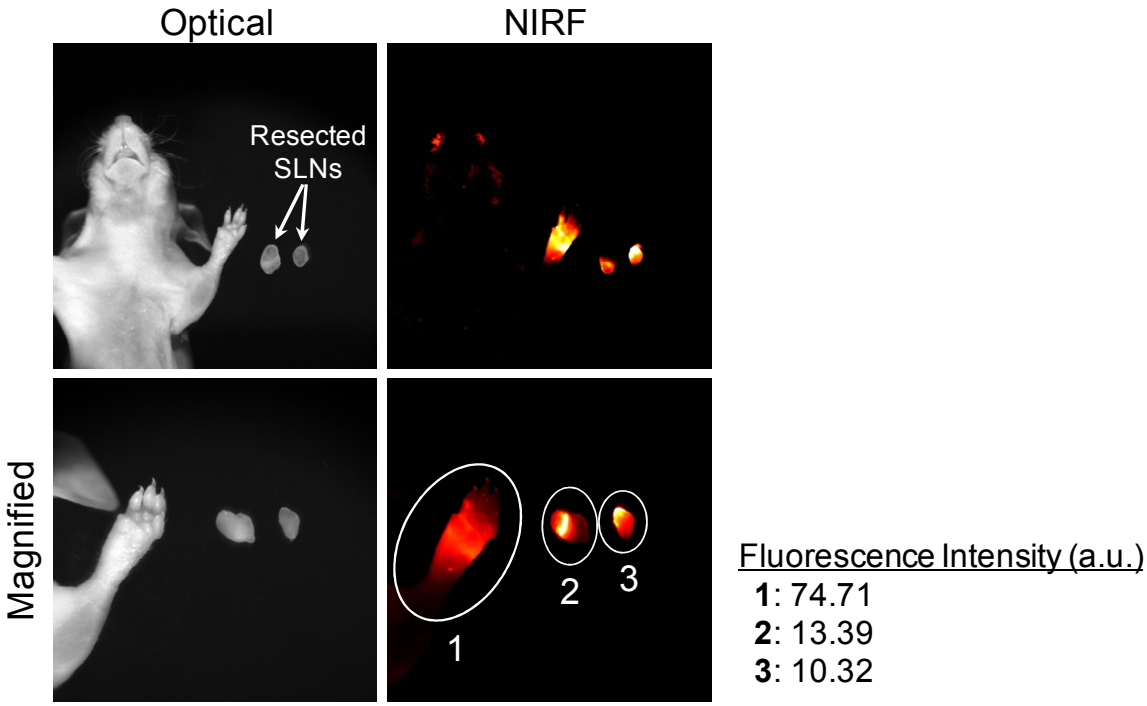


Fig. S6 Optical and NIR fluorescence (NIRF) images of an anaesthetized mouse injected with NIR-cvPD (10 μL of 0.17 mg/mL, intradermally into the right paw) and NIR-cvPD-entrapped SLNs resected from the mouse at 60 min post-injection. Circles in the magnified NIRF image indicate regions of interest (ROIs) for integration of fluorescence intensity, the values of which are shown. From the intensity ratio between the dot-injected forepaw (**1**) and the two nodes (**2**, **3**), the SLN uptake efficiency was evaluated to be 24% of the injected dose.