Electronic Supplementary Information

Highly luminescent and photostable near-infrared fluorescent polymer dots for long-term tumor cell tracking *in vivo*

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1. Experimental section

1.1 Materials. The poly(phenylene vinylene) derivative poly[2-methoxy-5-(2-ethylhexyloxy)-1,4- phenylenevinylene] (MEH-PPV; MW: 200,000Da; polydispersity, 4.0) were purchased from ADS Dyes, Inc. (Quebec, Canada). PS-PEG-COOH (MW: 21,700 Da of the PS moiety; 1,200 Da of PEG-COOH; polydispersity, 1.25) were purchased from Polymer Source Inc. (Quebec, Canada). Silicon 2,3-naphthalocyanine bis(trihexylsilyloxide) (NIR775) and all the other chemicals were purchased from Sigma Aldrich, Inc. and used without purification.

1.2 Synthesis of NIR polymer dots

NIR775-doped NIR polymer dots were prepared according to our previously published work (*Bioconjug Chem.*, 2015, **26(5)**, 817-821). In a typical procedure, a solution of THF containing 50 μg/mL of MEH-PPV, 50 μg/mL of PS-PEG-COOH and 0.6 μg/mL of NIR775 dye was prepared. An aliquot of the mixture (5 mL) was then quickly dispersed into 10 mL of water under vigorous sonication. Extra THF was evaporated at an elevated temperature (below 90 °C) under the protection of nitrogen. The THF-free NPs solution was filtered through a 0.2-μm cellulose membrane filter.

1.3 Synthesis of Luc8-conjugated NIR polymer dots

Bioconjugation was carried out by EDC-mediated coupling reaction according to the Rao lab's work (Nat. Commun., 2012, 3:1193). In a typical conjugation reaction, 60 μL of concentrated HEPES buffer (1 M) were added to 3 mL of a solution of the NP (50 μg/mL in water) and 100 μL of a solution of the Luc8 (3 mg/mL in PBS), followed by vortex mixing. Then, 50 μl of freshly prepared aqueous EDC solution (10 mg) was added and the above mixture was magnetically stirred for 1 h at room temperature. The uncoupled free Luc8 and excess EDC were removed by four washes using a 100 K Amicon Ultra filter (Millipore Corporation) under centrifugation at 3,000 r.p.m. for 3min at 4 °C. The final complex was kept in PBS buffer at 4 °C.

1.4 *In vitro* NIR polymer dots characterization

The size and morphology of the NIR polymer dots were investigated using TEM (FEI Tecnai G2 F20 X-TWIN, 200 kV). TEM samples were prepared by dripping the NP solution onto a carbon-supported copper grid and drying at room temperature before observation. The absorption spectra were recorded on an Agilent 8453 ultraviolet-vis spectrometer. Fluorescence and bioluminescence emission spectra were collected with a FluoroMax- 3 (Jobin Yvon Inc.) and corrected for wavelength-dependent detector sensitivity as described by the company.

1.5 Cell culture, cytotoxicity and cell imaging

The HeLa cells were grown in MEM (modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C and 5% CO_2.

The *in vitro* cytotoxicity was measured using the CCK-8 assay in HeLa cell lines. Cells growing in log phase were seeded into a 96-well cell-culture plate at 1×10^4/well and

then incubated for 24 h at 37 °C under 5% CO_2. Dots (100 μL/well) at different concentrations (5, 25, 50, and 100 μg/mL) were added to the wells of the treatment group, and 100 μL/well DMEM to the negative control group, respectively. The cells were incubated for 48 h or 72 h at 37 °C under 5% CO_2. Subsequently, 10 μL of CCK-8 was added to each well of the 96 well plate and incubated for an additional 2 h at 37 °C under 5% CO_2. A Tecan microplate reader was used to measure the OD450 (A value) of each well. The following formula was used to calculate the viability of cell growth: cell viability (%) = (mean of Absorbance value of treatment group / mean of Absorbance value of control)×100.

For cell imaging experiments, 2×10^5 cells per well were seeded on 18mm glass coverslips and cultured overnight before imaging with a Zeiss Axiovert 200M Microscope (excitation: 480/30 nm; dichroic beamsplitter: Q570LP; emission: D755/40M; objective: ×20; acquisition time: 1 s).

1.6 Tumor xenografts and *in vivo* fluorescence imaging

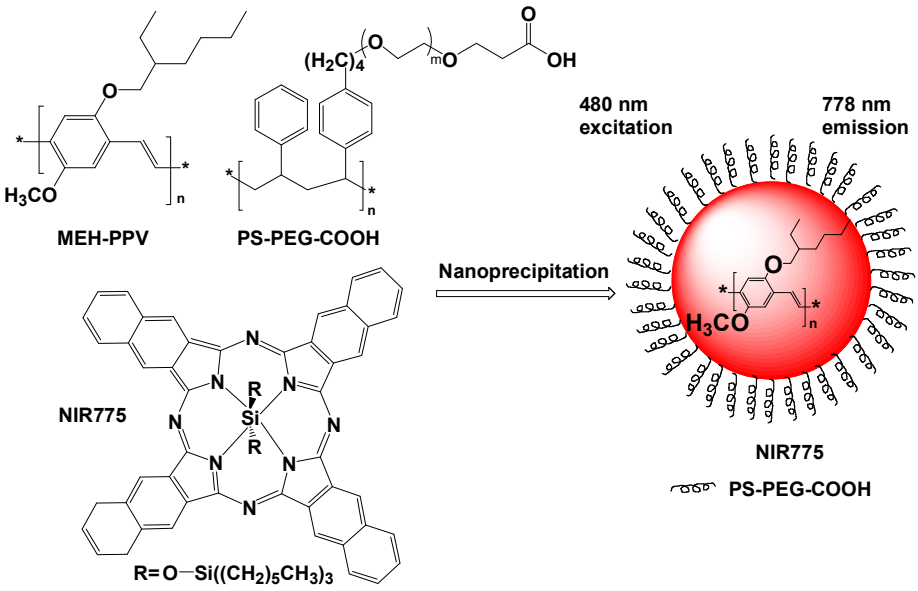
Animal procedures were reviewed and approved by the Institutional Animal Care Use Committee. Tumor cells were harvested by incubation with 0.05% trypsin-EDTA. Cells were pelleted by centrifugation and resuspended in sterile PBS. Cells (2×10^5 cells/site) were implanted subcutaneously into the five-week-old female athymic nude mice.

In vivo fluorescence imaging was performed with IVIS spectrum imaging system by using a 465 nm excitation filter and a 780 nm emission filter.

Quantify the *in vivo* fluorescent signals as total radiant efficiency ([photons/sec/cm^2/sr] / [μW/cm^2]) within a circular region of interest (ROI) using Living Image software.

1.7 Histology

Tumor tissues were collected, washed with PBS, frozen by ice and stored at −80 °C. Frozen samples were cryosectioned by microtome −20 °C into slices of 5 μm thickness, and analysed under a Zeiss Axiovert 200M Microscope.



Scheme S1 Chemical structures of MEH-PPV, PS-PEG-COOH, and NIR775, and the schematic depicts the preparation of NIR polymer dots. The NIR emission is produced based on a fluorescence resonance energy transfer (FRET) system utilizing NIR775 as an acceptor and MEH-PPV polymers as a donor.

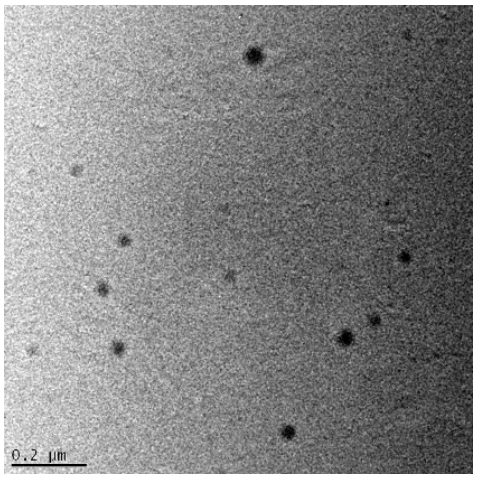


Fig. S1 The transmission electron microscopy (TEM) image of the NIR polymer dots.

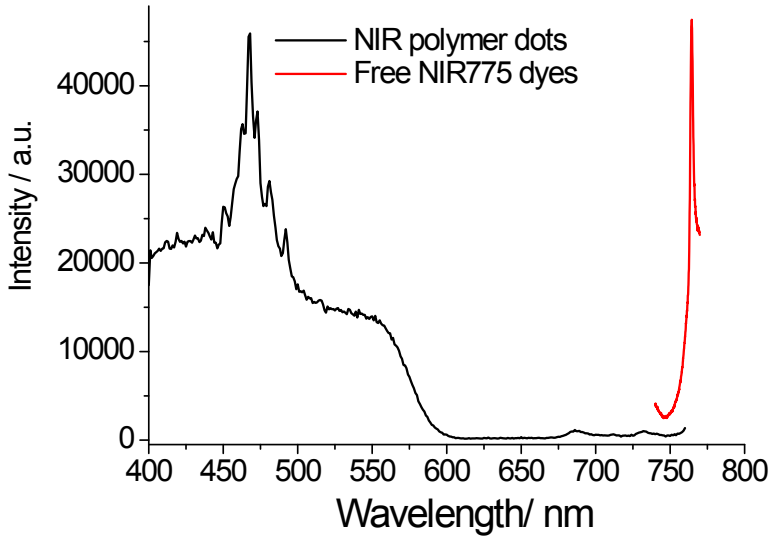


Fig. S2 Excitation spectra of the NIR polymer dots in water and free NIR775 dye in THF.

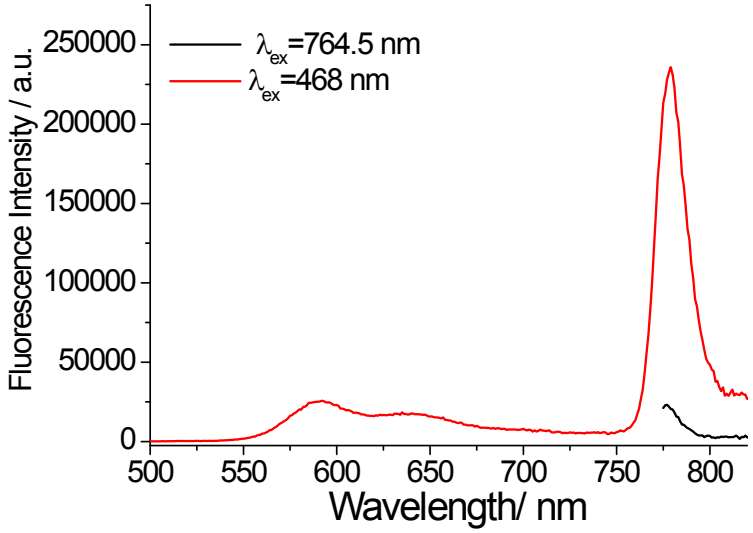


Fig. S3 Fluorescence spectra of the NIR polymer dots under 468 nm and 764.5 nm excitation, respectively.

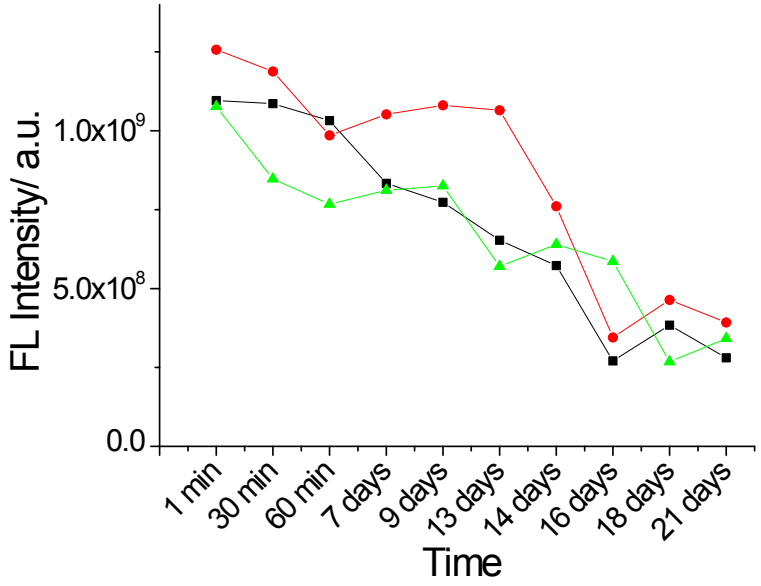


Fig S4. Region of interest (ROI) analysis of the fluorescence intensity of the NIR polymer dots (~20 μg) incubated HeLa cells in the mice over time (n=3).

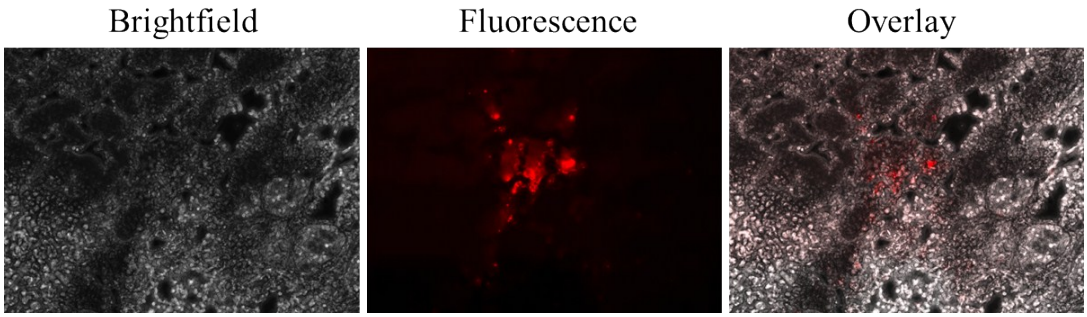


Fig. S5 *Ex vivo* fluorescence imaging of the tumor tissue excreted from the mouse after 20 days injection with the NIR polymer dots (~5μg) incubated HeLa cells. (Excitation filter: 465±15 nm; emission filter: 780±10 nm).

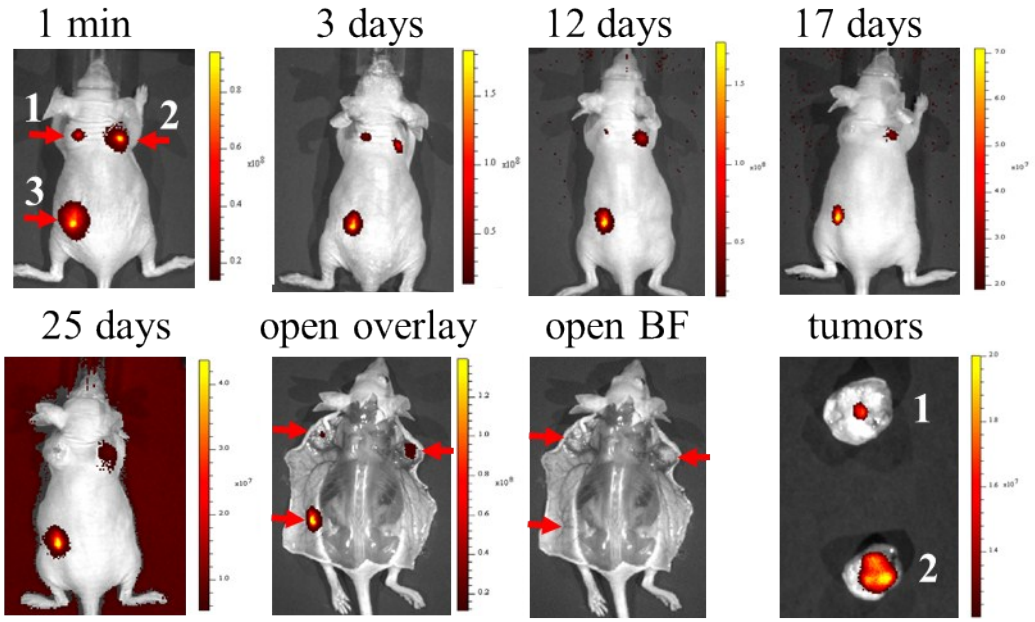


Fig. S6 Time-dependent *in vivo* fluorescence imaging of the mouse injected with different concentration of the NIR polymer dots (upper left 1: ~5 μg, upper right 2: ~10 μg, down left 3: ~20 μg) incubated HeLa cells and *ex vivo* fluorescence imaging of the tumors excreted from the mouse after 25 days injection.

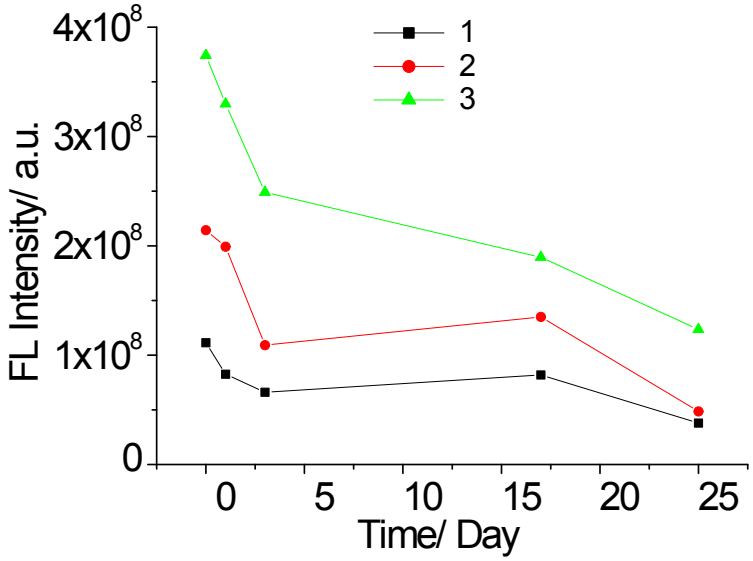


Fig. S7 Region of interest (ROI) analysis of the fluorescence intensity of the NIR polymer dots (upper left 1: ~5 μg, upper right 2: ~10 μg, down left 3: ~20 μg) incubated HeLa cells in the mouse over time.

Counts	~3 μg (Ⅰ)	~0.3 μg (Ⅱ)	~0.6 μg (Ⅲ)	~0.06 μg (Ⅳ)	Background
FL intensity	668900	283500	532000	186600	180600
Ratio (signal/bk)	3.7	1.5	2.9	1.0	
	~3 μg	~0.3 μg	~0.6 μg	~0.06 μg	Background
BL open intensity	450000	14970	26100	8066	1288
Ratio (signal/bk)	349.3	11.6	20.2	6.2	
BL filter intensity	20220	6398	9314	3058	1643
Ratio (signal/bk)	12.3	3.8	5.6	1.8	

Table S1. Signal to background (bk) ratio analysis of the bioluminescence (BL) and fluorescence (FL) intensity of the Luc8-conjugated NIR polymer dots.