Supplementary information

1. Experimental section

1.1. *Materials*

4-bromophenylacetonitrile, triphenylamine, $Pd(OAc)_2$, Cs_2CO_3 and $P(t\text{-}Bu)_3$ were purchased from J&K Scientific Ltd. Triethylamine, toluene, iodine, diethyl ether, sodium, methanol, chloroform, dichloromethane and petroleum ether were purchased from Sinopharm Chemical Reagent Co Ltd. Fetal bovine serum (FBS), Roswell Park Memorial Institute-1640 (RPMI-1640) medium and Penicillin-streptomycin solution were purchased from Invitrogen (San Diego, CA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Aldrich (Milwaukee, WI). Distilled water was obtained from a Milli-Q Biocel (Millipore Corporation, Breford, USA) water purification system (18.2 MΩ·cm resistivity). KB cell line, A549 cell line and 4T1 cell line were provided by American Type Culture Collection (ATCC). UV-vis absorption spectra were obtained on a Perkin-Elmer Lambda 750 UV/vis/NIR absorbance spectroscope. The fluorescence spectra were obtained from a FluoroMax 4 (Horiba Jobin Yvon) spectrofluorimeter. Scanning electron microscopic (SEM) images were obtained on a FEI Quanta 200 FEG field emission scanning electron microscope operated at an accelerating voltage of 30 KV. Then we selected the certain area in the image and added the same scale bar as they are on the original image (scale bar=1 μm). Transmission electron microscope (TEM) images were taken by FEI Tecnai G2 F20 S-TWIN operated at an accelerating voltage of 200 kV. Dynamic light scattering

(DLS) measurements were performed on a Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.) with a 633 nm He-Ne laser.

1.2. *Synthesis of NPAFN*

Bis(4-bromophenyl) fumaronitrile was synthesized following the literature procedure. A mixture of 4-bromophenylacetonitrile (4.86 g, 24.8 mmol) and iodine (6.35 g, 25 mmol) was purged with N_2 and dry diethyl ether (100 mL) was injected via syringe. The solution was cooled to -78 ℃. Sodium methoxide (2.84 g, 52.6 mmol) and methanol (40 mL) were added slowly over a period of 30 min and stirred for 40 min. The reaction solution was then put into an ice-water bath with stirring and kept at 0 ℃ for a further 4 h. 3–6% hydrochloric acid was added dropwise and the solution was filtered to isolate the precipitate, which was rinsed with cold methanol-water solution. Filtrate was concentrated further and a second crop of product was obtained with a yield of more than 90%. A mixture of bis(4-bromophenyl) fumaronitrile (0.787 g, 2.03 mmol), triphenylamine (4.47 mmol), $CsCO_3$ (1.95 g, 5.98 mmol) and toluene (4 mL) were added inside a glove box. The reaction mixture was degassed, purged with nitrogen again and heated at 110 ℃ for 24 h. The mixture was cooled to room temperature, and water (30 mL) and dichloromethane (50 mL) were then added. An organic layer was separated and washed with brine, dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography on silica gel using 1:1 light petroleum/dichloromethane as eluant, and then the resulting solid was obtained.

1.3. *Synthesis of poly(maleic anhydride-alt-1-octadecene)-poly(ethylene glycol) (C18PMH-PEG)*

C18PMH-PEG was synthesized following the literature procedure. Briefly, 10 mg (1 eq) of Poly(maleic anhydride-alt-1-octadecene) (C18PMH, Sigma-Aldrich) and 143 mg (1 eq) of mPEG-NH_2 (5 K) (PegBio, Suzhou, China) were dissolved in 5 mL of dichloromethane with 6 μL triethylamine (TEA, Sinopharm Chemical Reagent Co.) and 11 mg of 1-Ethyl-3- (3- dimethylaminopropyl) carbodiimide (EDC, Fluka) added. After 24 h stirring, the dichloromethane solvent was blown dry by N_2. The leftover solid was dissolved in water, forming a transparent clear solution, which was dialyzed against distilled water for 2 days in a dialysis bag with molecular weight cut-off (MWCO) of 14 kDa to remove unreacted mPEG-NH_2. After lyophilization, the final product in a white solid was stored at 4 ℃ for future use.

1.4. *Synthesis of NPAFN NPs*

NPAFN nanoparticles were synthesized using the previously reported method. Briefly, NPAFN was dissolved in THF to form a 1 mmol/L solution. Then dropped 150 μL as-prepared solution into 5 mL of aqueous solution by microsyringe at 0 ℃ under vigorous stirring at 1000 rpm for 5 min. The prepared solution was kept at 4 ℃.

1.5. *Preparation and functionalization of NPAFN@SiO_2 NPs*

In a typical procedure, 50 mg cethyltrimethylammonium bromide (CTAB) was

added into 30 mL of the NPAFN nanocrystals solution and then stirred vigorously for 30 min. The formation of an oil-in-water microemulsion resulted in a turbid solution. Then the mixture was heated up to 40 ℃and aged at that temperature for 10 min under stirring to evaporate the THF, resulting in a transparent NPAFN/CTAB solution. 100 μL of 2 M NaOH solution was added into the above solution and the mixture was heated up to 50 ℃under stirring. Then, 0.1 mL of tetramethylorthosilicate (TMOS) and 1 mL of ethylacetate was added to the reaction solution in sequence. After 10 min, 30 μL of APS was added and the solution was stirred for 12 h. The as-synthesized NPAFN@SiO_2 NPs were washed twice with water to remove the unreacted species and dispersed in water. Then the as-made NPAFN@SiO_2 NPs and NPAFN NPs were both dissolved in THF respectively. The concentration can be determined according to its UV-vis absorption spectrum (according to its standard Absorption/Concentration curve). After the concentrations were controlled to be same, the fluorescence intensity of NPAFN@SiO_2 NPs and NPAFN NPs in aqueous solution was compared under the same concentration.

To extract CTAB from the NPs, 10 μL HCl was added to the dispersion (pH~2.0) and stirred for 3 h at 60 ℃. For functionalization of the NPAFN@SiO_2 NPs, 30 μL of C18PMH-PEG/H_2O was added to 1 mL of NPAPF@mSiO_2 NPs solutions, and the mixture was then under ultrasonic treatment for 5 min then stored for further use. The nanoparticles size was measured by DLS at 25 ℃. The data reported represented an average of three measurements with ten scans each.

1.6. *Determination of Quantum Yields*

Fluorescence quantum yield was measured using a Perkin-Elmer Lambda 750 UV/vis/NIR Spectrometer and luminescence spectrometer. We make use of 2,7-di(4-(diphenylamino)phenyl-2,1,3-benothiadiazol-7-yl)-9,9'-spirobifluorene (*J. Am. Chem. Soc.*, 2011, 133, 5492-5499; *J. Mater. Chem. B*, 2013, 1, 3144-3151) excited at 468 nm (QY = 0.45) as standard to measure the fluorescence quantum yields of modified NPAFN NPs and NPAFN@SiO_2 NPs (remove molecule in solution by centrifugation) in solutions. Quantum yields were calculated according to the following equation:

$$\eta_s = \eta_r \left(\frac{Ar}{As}\right)\left(\frac{Is}{Ir}\right)\left(\frac{n_s^2}{n_r^2}\right)$$

η_r, η_s are fluorescence quantum yield of reference material and sample, also Ar, As are absorbance of reference and sample at excitation wavelength, Ir, Is are totally emission intensity of reference and sample at excitation wavelength, nr, ns indicate refractive of relevant solvent. Ensure the A value below 0.05, in our experiments, n_s = 1.333 (H_2O, 20 °C), n_r = 1.4 (THF, 20 °C). The QYs of NPAFN NPs and NPAFN@SiO_2 NPs were determined to be 5.8% and 8.7%.

1.7. *Cell culture*

A human nasopharyngeal epidermal carcinoma cell line (KB cell), lung cancer cells (A549 cells) and murine 4T1 breast cancer cell line (4T1 cells) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA.). The cells were cultured at 37 ℃, 5% CO_2 in RPMI-1640 medium supplemented with 10%

hear-inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin solution. The cells were pre-cultured prior to experiments until confluence was reached.

1.8. *In vitro cytotoxicity*

100 μL complete medium with KB/A549/4T1 cells was seeded in 96-well plates (60,000/well) and incubated for 24 h. Then, 25 μL sterilized NPAFN@SiO_2 NPs with different concentrations in phosphate buffered saline (PBS) buffer were added to each well for incubation (37 ℃, 5% CO_2). The cells were then treated with 20 μL of MTT solution (5 mg/mL in PBS) and incubated for 5 h. The medium was removed and the cells were lysed by adding 150 μL of DMSO, cell viabilities were then measured by MTT assay.

1.9. *Confocal Imaging of Cells*

KB cells (in RPMI-1640 medium) were dispersed in 24-well plates (1 mL in each well containing 1×10^5 cells per well) and grown for 24 h at 37 ℃in the humidified atmosphere with 5% CO_2. Subsequently, KB cells were cultured in NPAFN NPs and NPAFN@SiO_2 NPs at 37 ℃ for 4 h. Cells were then washed with PBS for three times to fully remove nonspecifically absorbed dye. To determine the intracellular localization, cells were stained with DAPI. Then KB cells were mounted on slides in fluoromount (Sigma, F4680) with coverslips. The cells were observed with the confocal laser microcope (Leica, TCS-SP5Ⅱ). DAPI was excited by 15% power of

diode laser ($\lambda_{excitation}$ = 405 nm); Dye were excited by 20% power of argon laser ($\lambda_{excitation}$ = 488 nm). The emissions windows were collected in the ranges of 420-480 nm, 580-700 nm for DAPI, and NPAFN@SiO_2 NPs respectively. To guarantee no cell fluorescence background was detected, we set the offset at -2%. All images were captured under the same instrumental setting and processed with image analysis software (LAS AF Lite). Then we combined the related images and added the same scale bar as they are on the original images.

1.10. *Blood circulation*

Blood circulation was measured from approximately 10 µL blood from the tail vein of BALB/c mice post injection of NPAFN@SiO_2 NPs. Each blood sample was dissolved in 1 mL of lysis buffer (1% SDS, 1% Triton X-100, 40 mM Tris Acetate). The concentration of NPAFN@SiO_2 NPs in the blood was determined by the fluorescence spectrum of each solubilized blood sample using a FluoroMax 4 fluorometer (HORIBA Jobin Yvon, France). A series of dilution of the NPAFN@SiO_2 NPs solution was measured to obtain a standard calibration curve. Blank blood sample without NPAFN@SiO_2 NPs injection was measured to determine the blood autofluorescence level, which was subtracted from the fluorescence intensities of injected samples for the concentration calculation.

1.11. *In vivo NIR fluorescence imaging*

Tumor-bearing mice were intravenously injected with 200 µL of 63 µM

NPAFN@SiO_2 NPs and imaged using a Maestro in vivo fluorescence imaging system (Cri Inc.). Green light with a peak wavelength at 523 nm was used as the excitation source. Spectral imaging from 600 nm to 750 nm (10 nm step) was carried out with various exposure times for each image frame. Autofluorescence (particularly from food residues in the stomach and intestine) was decreased by exposure time. The mouse was marked out by green line in situ corresponding with the bright-filed image.

1.12. *Biodistribution*

We used 4T1 bearing BALB/c mice as model of tumor imaging, developed by injection of 4T1 cells 6 days after inoculation of the cancer cells, the mice were just treated with NPAFN@SiO_2 NPs at a dose of 200 μL. Then we sacrificed 4T1 bearing BALB/c mice after injection and at 1, 2, 6, 12 and 24 h. Fluorescence of various organs and tissues were spectrally measured by the Maestro system. The averaged fluorescent intensity of each imaged organ (after removing the tissue autofluorescence and subtracting the background, if any, of each organ before NPAFN@SiO_2 NPs injection) was calculated for a semi-quantitative biodistribution analysis.

1.13. *Histology Analysis*

Control untreated and 200 μL of 60 μM PEGylated NPAFN@SiO_2 NPs treated mice were sacrificed 1, 7 and 14 days after treatment. Major organs from these mice were harvested, fixed in 10% neutral buffered formalin, processed routinely into paraffin, sectioned at 8 μm, stained with hematoxylin and eosin (H&E), and examined

by a digital microscope (Leica QWin). Examined tissues include liver, spleen, kidney, heart and lung.

1.14. *Blood Analysis*

Nine healthy BALB/c mice were injected with 200 μL of 60 μM PEGylated NPAFN@SiO_2 NPs. The other nine mice were used as the untreated controls. Mice were sacrificed to collect the blood (0.8 mL) for blood biochemistry assay and complete blood panel test at 1, 7 and 14 days post-injection of NPs. The serum chemistry data and complete blood panel were measured in Shanghai Research Center for Biomodel Organism.

Supporting Figures

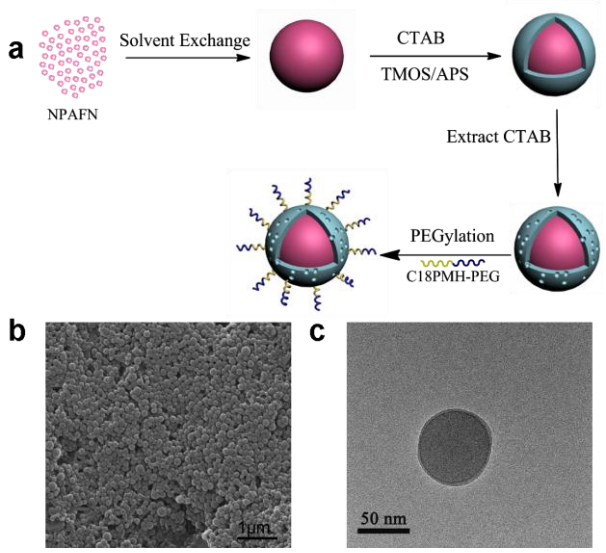

Fig. S1. (a) Schematic illustration of the synthetic procedure for NPAFN@SiO_2 NPs; (b) SEM image of NPAFN NPs; (c) TEM image of NPAFN NP before coating of silica shell.

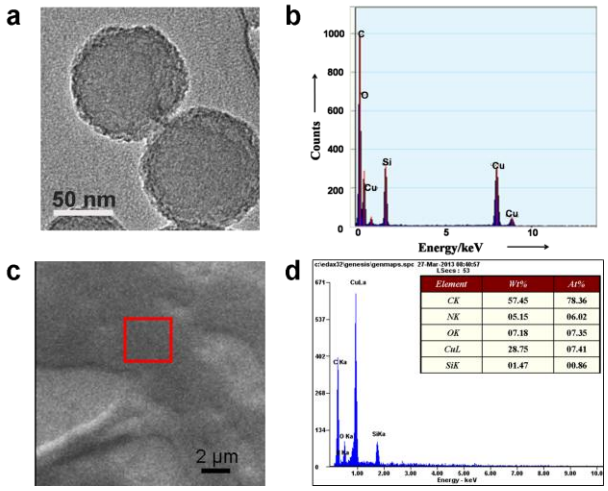

Fig. S2. (a), (b) TEM image of NPAFN@SiO_2 NPs and the corresponding EDX pattern. (c), (d) SEM and corresponding elemental ratio of NPAFN@SiO_2 NPs.

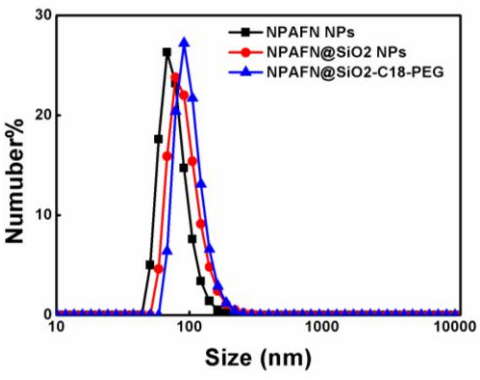

Fig. S3. Dynamic diameter of NPAFN NPs, NPAFN@SiO_2 NPs and PEGlyated NPAFN@SiO_2 NPs respectively.

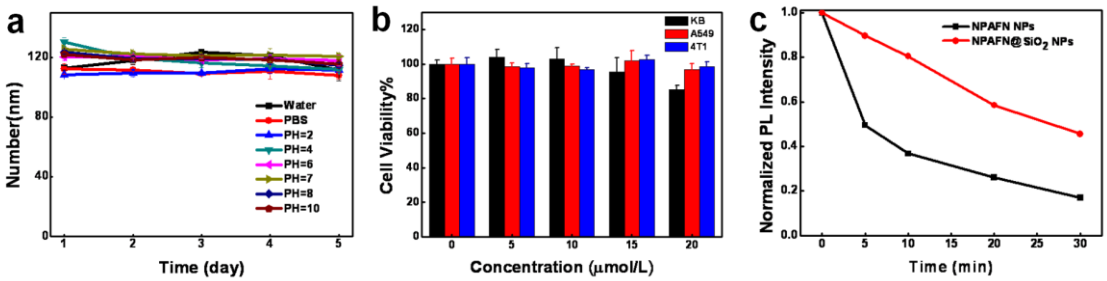

Fig. S4. (a) Size stability of modified NPs in different conditions; (b) Cell toxicity of modified NPs with KB/A549/4T1 cells; (c) Photostability of NPAFN NPs and NPAFN@SiO_2 NPs at the same concentrations. Samples are continuously irradiated by a 300 W xenon lamp.

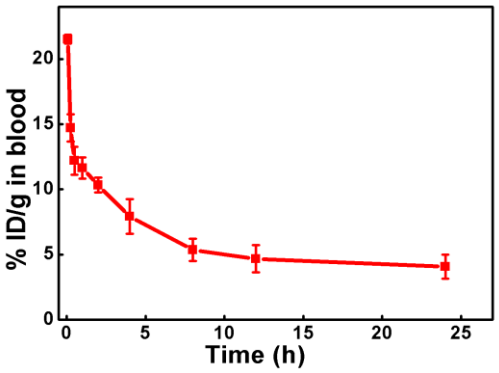

Fig. S5. The blood circulation curve of NPAFN@SiO_2 NPs determined by measuring NPAFN fluorescence in the blood at different time points post injection. The unit was a percentage of injected dose per gram tissue (% ID/g).

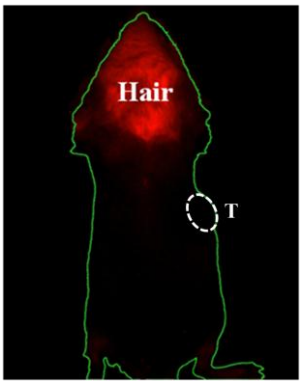

Fig. S6. In vivo imaging of a 4T1-tumor-bearing BALB/c mouse before NPAFN@SiO_2 NPs injection.

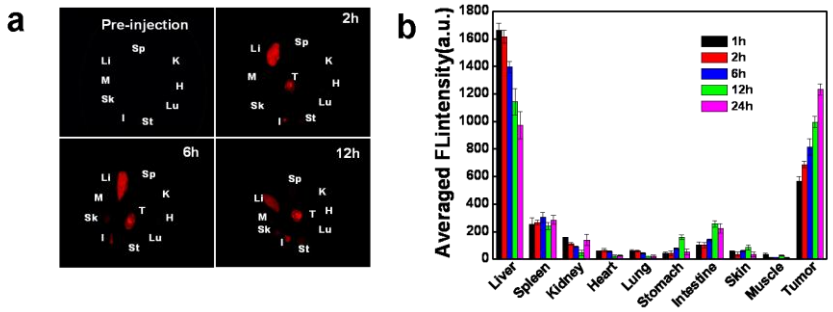

Fig. S7. (a) Spectrally resolved ex vivo fluorescence images of organs before injection and 2, 6, and 12 h after injection of NPAFN@SiO_2 NPs. Sk: skin, M: muscle, I: intestine, H: heart, Lu: lung, Li: liver, K: kidney, Sp: spleen, St: stomach, and T: tumor; (b) Semi-quantitative biodistribution of NPAFN@SiO_2 NPs in mice determined by the averaged fluorescence intensity of each organ (after subtraction of the fluorescence intensity of each organ before injection). Error bars were based on three mice per group.

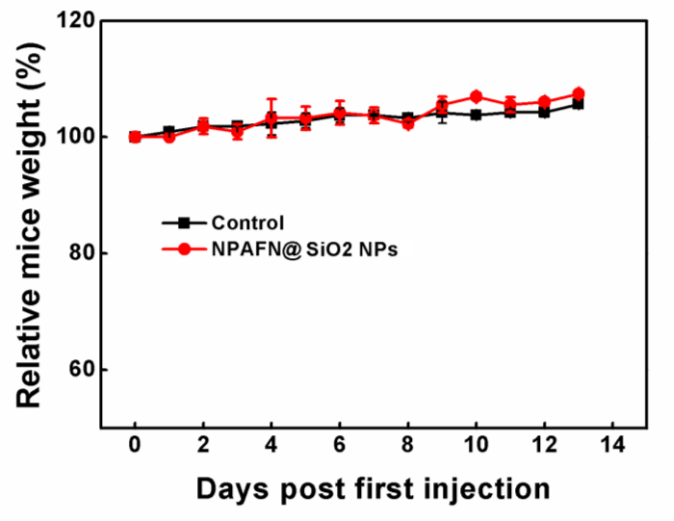

Fig. S8. Change in body weight obtained from mice injected with the NPAFN@SiO_2 NPs and physiologic saline (control groups), respectively.

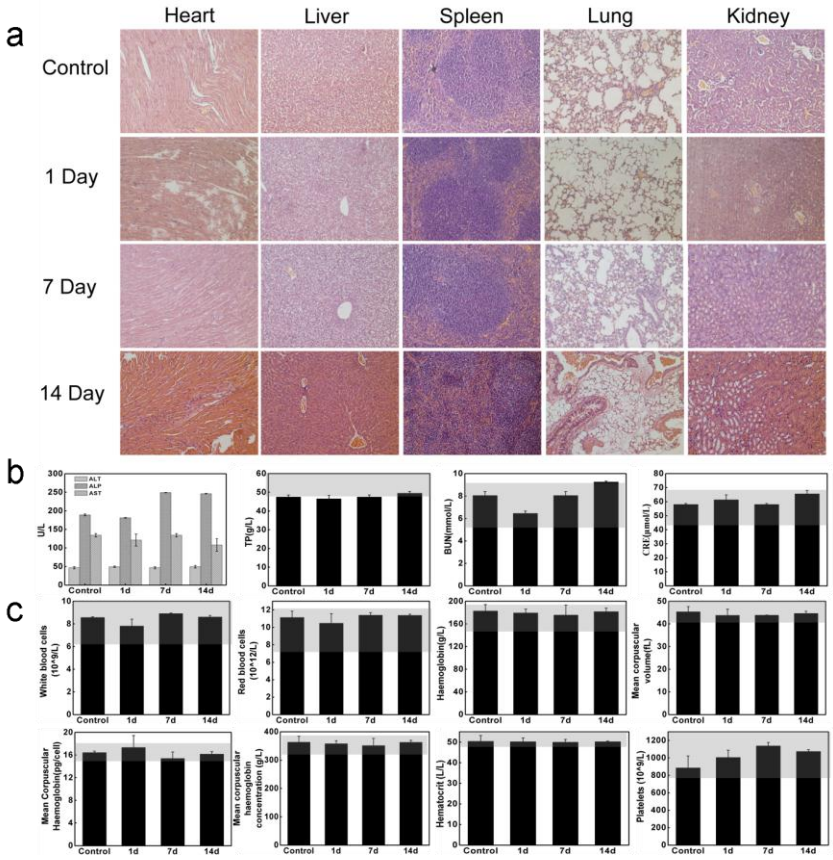

Fig. S9. (a) Representative organ histology for control and treated animals. Heart, liver, spleen, lung, kidney are shown. (b) Serum biochemistry and (c) hematology results from animals treated with NPAFN@SiO_2 NPs and physiologic saline for 1, 7, 14 days. Related serum biochemistry indicators include ALT, AST, TP, ALP, BUN and CRE. Related hematology indicators include WBC, RBC, HGB, MCV, MCH, HCT and PLT.