Supplementary Information

Poly(iohexol) Nanoparticles As Contrast Agents for In Vivo X-ray Computed Tomography Imaging

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Materials

\( \text{D,L-} \)-Lactide (LA) was purchased from TCI America (Portland, OR, USA), recrystallized three times in toluene and stored at \(-30^\circ\text{C}\) in a glovebox prior to use. (S)-2,2',2''-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayltetraacetic acid (p-SCN-Bn-DOTA) was purchased from Macrocyclics, Inc. (Dallas, TX, USA). All anhydrous solvents used in this study were purified by being passing through dry alumina columns and kept anhydrous by using molecular sieves. 5-(N-2,3-dihydroxypropylacetamido)-2,4,6-triiodo-N,N'-bis(2,3-dihydroxypropyl)isophthalamide (iohexol), hexamethylene diisocyanate and other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA) and used as received unless otherwise noted.

General

The molecular weights (MWs) of cross-linked poly(iohexol) were determined by gel permeation chromatography (GPC) (also known as size exclusion chromatography (SEC)) equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS 18-angle laser light scattering detector (Wyatt Technology, Santa Barbara, CA, USA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The wavelength of the HELEOS detector was set at 658 nm. The size exclusion columns (Phenogel columns 100 Å, 500 Å, 10^3 Å and 10^4 Å, 5 µm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) used for the analysis of polymers were serially connected on the GPC. The GPC analysis was conducted with the use of DMF containing 0.1 M LiBr as the mobile phase at a flow rate of 1 mL/min at 60 °C. The MALLS detector was calibrated using pure toluene with no need for calibration using polymer standards and was used for the determination of the absolute molecular weights (MWs). The molecular weight of polymer was determined from the \(d_0/d_c\) value.
calculated offline by means of the internal calibration system processed by the ASTRA V software (Version 5.1.7.3, Wyatt Technology). Infrared spectra were recorded on a Perkin Elmer 100 serial FT-IR spectrophotometer calibrated with polystyrene film. The NMR studies were conducted on a Varian UI500NB (500 MHz) NMR spectrometer. Low-resolution electrospray ionization mass spectrometry experiment was performed on Waters Quattro II mass spectrometer. The sizes and the particle polydispersities of the poly(iohexol) NPs were determined on a ZetaPALS dynamic light scattering (DLS) instrument (15 mW laser, incident beam = 676 nm, Brookhaven Instruments, Holtsville, NY, USA). The lyophilization of the NPs was carried out on a benchtop lyophilizer (FreeZone 2.5, Fisher Scientific, PA, USA). mPEG₅k-PLA₁₀ (PLA with degree of polymerization (DP) of 10, molecular weight (MW) of 6.3 kDa, and molecular weight distribution (MWD) of 1.07) was synthesized by following the published procedures.¹ The SEM analysis of NPs was conducted on a Hitachi-S4700 high-resolution scanning electron microscope. Human breast cancer MCF-7 cells were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were cultured in DMEM medium containing 10% Fetal Bovine Serum (FBS), 0.01 mg/ml human recombinant insulin and 100 units/mL aqueous penicillin G at 37°C in 5% CO₂ humidified air. Cells were harvested at 80% confluence with EDTA/trypsin, counted and resuspended for experiments. Female C57BL/6 or athymic nude mice were purchased from National Cancer Institute (NCI, Frederick, MD, USA). Feed and water were available ad libitum. Artificial light was provided in a 12/12 hour cycle. Micro-PET/CT imaging and fluoroscopic imaging was performed with small animal on a Siemens Inveon PET-CT system (Siemens Healthcare, USA). Ex vivo measurement of the radioactivity was conducted on a 2480 Wizard2 Automatic Gamma Counter (Perkin-Elmer).

**Synthesis of poly(iohexol) cross-linked polymer**
Iohexol (500 mg, 0.6 mmol) was dissolved in DMF (20 mL) in a 50-mL round-bottom flask. Hexamethylene diisocyanate (307 mg, 1.83 mmol) was added into the flask, in the presence of dibutyltin dilaurate (10 µL, 16 µmol) as the catalyst (Figure S1). The mixture was stirred at 80 °C for 24 h. The solution was evaporated and an orange-red oily residue was obtained. The residue was dissolved and stirred in ether (50 mL) at room temperature to precipitate a pale yellow solid. The solid was isolated by centrifugation and further dried under vacuum (790 mg, 98% yield). The resulting polymer was characterized by $^1$H NMR, FT-IR and GPC. The synthesis of poly(iohexol) was monitored and confirmed by FT-IR spectroscopy (Figure S2B). At the completion of the polymerization, the isocyanate groups of hexamethylene diisocyanate (2250 cm$^{-1}$) completely disappeared and the stretching vibration band of the urethane carbonyl group (1750 cm$^{-1}$) was detected.$^2$ The $^1$H NMR spectra further confirmed the structure of the obtained polymer (Figure S3). The peak at 5.5-7.5 ppm, which is attributed to the secondary –NH proton in urethane, confirmed the urethane structure of poly(iohexol). The aliphatic CH$_2$ proton appeared at 1.0-1.5 ppm, which indicates the successful covalent conjugation of hexamethylene diisocyanate with iohexol.

**Formulation of PEGylated poly(iohexol) NPs**

A DMF solution containing the cross-linked poly(iohexol) (100 µL, 10 mg/mL) and mPEG$_{5k}$-PLA$_{10}$ (100 µL, 10 mg/mL) were added dropwise to vigorously stirred nanopure water (4 mL). The resulting PEGylated poly(iohexol) NPs were collected and washed three times with water by ultrafiltration, using a 10,000 MWCO Amicon Ultra membrane (Ultracel YM-10, Millipore Inc., Billerica, MA, USA), in order to completely remove DMF. The NPs collected from ultrafiltration were characterized by DLS and SEM.
Release study of PEGylated poly(iohexol) NPs

PEGylated poly(iohexol) NPs were prepared by nanoprecipitation as described above. The collected NPs were dispersed in human serum buffer (human serum:PBS=1:1, v/v, 5 mL), divided into 10 equal-volume portions, and added to 10 eppendorf microcentrifuge tubes (500 μL NP solution in each tube). The tubes were incubated at 37°C. At scheduled time points, two tubes were taken out, to which methanol (500 μL) was added to precipitate proteins. The precipitates were removed by centrifugation (5 min, 15000 × g). The supernatant then was injected into HPLC for analysis of the released iohexol. For the HPLC analysis, acetonitrile:water (containing 1% TFA) was used as mobile phase. The gradient for acetonitrile:water (containing 1% TFA) was kept from 25:75 for 50 min. The flow rate was 1.0 mL/min. Analytical C18 column (Luna C18, 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA, USA) was used to perform the analysis. The results were compared to the free iohexol peak identified by the same condition, and confirmed there is no release of iohexol from PEGylated poly(iohexol) NPs during 4 day’s incubation in human serum buffer at 37 °C.

Stability of PEGylated poly(iohexol) NPs

A DMF solution containing the cross-linked poly(iohexol) (100 μL, 10 mg/mL) and mPEG5k-PLA10 (100 μL, 10 mg/mL) were added dropwise to vigorously stirred nanopure water (4 mL). To evaluate the stability of NPs against dilutions upon administration, the obtained NPs were treated with a series of dilutions with human serum buffer (human serum:PBS = 1:1, v/v), and the particle size changes were measured by dynamic light scattering (DLS). For the short-term stability study, the particles were dispersed in PBS or human serum buffer (human serum:PBS = 1:1, v/v), measured by DLS at RT and followed over 60 min. For the long-term stability study, the particles were dispersed in human serum buffer, incubated at 37 °C and continuously
monitored by DLS for 4 days. The results showed that PEGylated poly(iohexol) NPs were able to keep the nanostructure against a series of dilutions and remained unchanged in 50% human serum buffer for 4 days (Figure S4A and S4B).

**Lyophilization of poly(iohexol) NPs in the presence of albumin**

A DMF solution of poly(iohexol) (10 mg/mL, 100 μL) was mixed with a DMF solution of mPEG₅₋₅LA₁₀ (10 mg/mL, 100 μL). The mixture was added dropwise to a vigorously stirred water solution (4 mL). The resulting NP solution was washed three times with water by ultrafiltration, using a 10,000 MWCO Amicon Ultra membrane (Ultracel YM-10, Millipore Inc., Billerica, MA, USA), in order to completely remove DMF. The collected NP was then analyzed by DLS. An aqueous solution of human serum albumin (HSA, 10 mg/mL, 500 μL) was added to the NP solution. The mixture was lyophilized for 16 h at −50 °C. The resulting white powder was reconstituted with nanopure water (2 mL). The solution was stirred for 5 min at room temperature. The resulting NP solution was analyzed by DLS. The results indicated that human serum albumin (HSA), an abundant protein in the blood, can be used to stabilize poly(iohexol) NPs and effectively prevent the severe aggregation of NPs during lyophilization process (Figure S4D).

**Synthesis of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-LA**

The NH₂-LA₂₅ conjugate was synthesized by following the procedures as published. In a reaction vial containing NH₂-LA₂₅ conjugates (14.4 mg, 0.004 mmol) was added an anhydrous dimethylformamide (DMF) solution (0.5 mL) of p-SCN-Bn-DOTA (5.6 mg, 0.008 mmol) and triethylamine (3.6 mg, 0.036 mmol). The reaction mixture was stirred for 4 h under nitrogen at RT. The solvent and triethylamine were removed under vacuum to give DOTA-LA₂₅, which was used directly without further purification (Figure S7).
**Synthesis of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-iohexol**

In a reaction vial containing iohexol (40.0 mg, 0.049 mmol) was added an anhydrous dimethylformamide (DMF) solution (0.5 mL) of p-SCN-Bn-DOTA (5.6 mg, 0.008 mmol). The reaction mixture was stirred overnight under nitrogen at rt. The solvent were precipitated with 50 mL ethyl ether to give DOTA-iohexol with a good yeild (>75%). ESI-MS (m/z): Calcd C_{43}H_{60}N_{8}O_{17}SI_{3} 1373.09 (M); found 1373.0 (M)^+ (Figure S8).

**Formulation of \(^{64}\)Cu labeled Poly(iohexol) NPs.**

A DMF solution containing the cross-linked poly(iohexol) (100 µL, 10 mg/mL), DOTA-LA_{25}(100 µL, 10 mg/mL) and mPEG_{5k}-PLA_{10} (100 µL, 10 mg/mL) were added dropwise to vigorously stirred nanopure water (6 mL). The resulting PEGylated DOTA-poly(iohexol) NPs were collected and washed three times with water by ultrafiltration, using a 10,000 MWCO Amicon Ultra membrane (Ultracel YM-10, Millipore Inc., Billerica, MA, USA), in order to completely remove DMF. The \(^{64}\)Cu chloride (300 µCi, obtained from Washington University at St. Louis, MO, USA) was mixed with PEGylated DOTA-poly(iohexol) NPs (3 mg) in NH$_4$OAc buffer (pH = 5.5, 0.1 M, 0.5 mL). The mixture was incubated for 1 h at 60 °C. To determine the labeling stability, the NPs were washed by ultrafiltration (10 min, 3000 × g, Ultracel membrane with 10 000 NMWL, Millipore, Billerica, MA, USA), and the radioactivity in the supernatant was measured on \(\gamma\)-counter at different time points (1 h, 4 h, and 24 h) to determine the diassociated \(^{64}\)Cu (Figure S10B). The purified \(^{64}\)Cu-labeled poly(iohexol) NPs were used for injection.

**Synthesis of \(^{64}\)Cu labeled iohexol.**
The $^{64}\text{Cu}$ chloride (300 µCi, obtained from Washington University at St. Louis, MO, USA) was mixed with DOTA-iohexol (3 mg) in NH$_4$OAc buffer (pH = 5.5, 0.1 M, 0.5 mL). The mixture was incubated for 1 h at 60 °C. To determine the labeling stability, the DOTA-iohexol was washed by ultrafiltration (10 min, 14000 × g, Ultracel membrane with 3 000 NMWL, Millipore, Billerica, MA, USA), and the radioactivity in the supernatant was measured on γ-counter at different time points (1 h, 4 h, and 24 h) to determine the diassociated $^{64}\text{Cu}$ (Figure S10B). The purified $^{64}\text{Cu}$-labeled iohexol were used for injection.

**Preparation of rabbit models**

The Animal Care and Use Committee at the University of Illinois at Chicago approved this study.

**Rabbit NP chemoembolization**

One male New Zealand white rabbit (weight 2.8 kg) underwent NP chemoembolization, which was performed by a single operator (R.C.G.) after the rabbit was intubated and maintained under general anesthesia. Angiography was performed with a C-arm unit (OEC Medical Systems series 9600; GE Healthcare, UK). The femoral artery was accessed through a surgical cut-down and catheterized with a 3 French vascular sheath (Cook Medical, Bloomington IN, USA). A 2 French JB1 catheter (Cook Medical) was advanced over a guide wire, and the celiac artery was selectively catheterized. The catheter was then advanced into the proper hepatic artery. Celiac and hepatic arteriography was performed via injections of iohexol (Omnipaque-300; Amersham Health, Princeton NJ, USA). After catheter position was confirmed in the proper hepatic artery, hepatic lobe chemoembolization was performed by injection of an emulsion consisting of 125 mg/kg poly(iohexol) NPs (total volume 20 mL) mixed with 10-mL ethiodized oil (Lipiodol; Geurbet, Villepente France). Under fluoroscopic visualization, the NP emulsion was injected by
hand into the catheter. Chemoembolization was continued until the entire NP emulsion was administered. Immediately after completion of chemoembolization, the rabbit was euthanized using a lethal intravenous dose of 390 mg/mL pentobarbital sodium solution (Schering-Plough, Kenilworth, NJ, USA). A computed tomography (CT) scan was obtained within 30 min of euthanasia using a BrightSpeed 16 slice scanner (GE Healthcare, UK) to delineate the anatomic distribution of injected NC mixture. The CT protocol included helical acquisition with mA = 150, kV = 120, pitch = 1.375:1, and 0.625 mm acquisition slice thickness.

**Preparation of mice models**

The study protocol was reviewed and approved by the Illinois Institutional Animal Care and Use Committee (IACUC) of the University of Illinois at Urbana–Champaign.

**MicroCT imaging**

Six female athymic nude mice bearing MCF-7 human breast cancer xenografts (6-8 weeks) were divided into two groups (n = 3), minimizing their weight differences. The two groups of mice were treated with iohexol and poly(iohexol) NPs at a dose of 50 mg/kg through intratumoral injection, respectively. MicroCT imaging was performed with a hybrid small-animal scanner (Inveon SPECT/CT; Siemens Medical Solutions USA, Inc.). Animals were placed prone on the imaging bed with legs secured in an extended position. Five minutes after injection, mice underwent high-resolution anatomic CT (360 projections, 80 kVp/500 μA penetration energy, effective pixel size of 96 μm) imaging. The microCT images were reconstructed using cone-beam algorithm with existing commercial software (Cobra Exxim) and intensity values converted to Hounsfield units (HU). The quantitative analysis was measured using Inveon Research
Workspace (Siemens Medical Solutions USA, Inc.). Briefly, complex irregular volumes of interest (VOIs) were drawn on the microCT images to determine mean counts in each VOI.

**Fluoroscopic imaging**

Female C57BL/6 mice (6-8 weeks) were divided into two groups (n = 2), minimizing their weight differences. The two groups of mice were treated with iohexol and poly(iohexol) NPs at a dose of 250 mg/kg through jugular vein injection, respectively. Fluoroscopic imaging was performed with a hybrid small-animal scanner (Inveon SPECT/CT; Siemens Medical Solutions USA, Inc.). Animals were placed prone on the imaging bed with legs secured in an extended position. High-resolution fluoroscopic imaging (80 kVp/500 μA penetration energy, effective pixel size of 96 μm) was collected at the same time with administration started. The images were collected every 10s from 0 min to 5 min post injection followed by every 10 s from 5 min to 60 min post injection. The quantitative analysis of collected images was performed using Image J. Briefly, the region of interest (ROI) was drawn on the inverted images and the mean intensity were measured and normalized by area of ROI (μm²).

**Micro-PET/CT Imaging**

Six female athymic nude mice bearing MCF-7 human breast cancer xenografts (6-8 weeks) were divided into two groups (n = 3), minimizing their weight differences. The two groups of mice were treated with iohexol and poly(iohexol) NPs at a dose of 50 mg/kg through tail vein injection, respectively. Mice were placed on the micro-CT imaging bed and kept anaesthetised with a constant isoflurane flow (∼2%). The micro-CT scan (80keV/500μA X-rays energy, 360 projections, 360 degrees, 75 μm pixel size) was used for determining the anatomical localization of different organs. Static micro-PET scans were acquired at three selected time points (1 h, 6 h, and 24 h).
and 24 h p.i.) together with micro-CT scans for anatomical co-registration. The obtained micro-PET and micro-CT images were reconstructed using ordered subset expectation maximization (OSEM) and cone-beam algorithms with existing commercial software (Inveon Acquisition Workspace and Cobra Exxim, respectively). Micro-PET images were processed using 3-D median filtering and fused with micro-CT images. To quantify the radioactivity of $^{64}$Cu in different organs, complex irregular volumes of interest (VOIs) were drawn on the micro-CT images and registered with the micro-PET images to determine mean counts in each VOI. To minimize partial volume effects, the anatomical borders of the organs were not included. The radiotracer activity from each VOI was normalized by injected dose and expressed as percent of the decay-corrected injected activity per cm$^3$ of tissue, which can be approximated as percentage %I.D./g assuming the density of tissue is $\sim$1 g/cm$^3$. The initial total injected activity was determined by dose calibrator before the injection.

**Radioactivity measurement with $\gamma$-counter**

Mice were euthanized 24 h post injection. Various organs (liver, lung, spleen, kidney, heart, small intestine, large intestine, bladder and thyroid), as well as MCF-7 subcutaneous tumors were collected, weighed and measured for radioactivity ($^{64}$Cu) with a $\gamma$-counter (Wizard2, Perkin-Elmer, USA) using appropriate energy window centered at photo peak of 511 keV. Raw counts were corrected for background, decay, and weight. Corrected counts were converted to microcurie per gram of tissue ($\mu$Ci/g) with a previously determined calibration curve by counting the $^{64}$Cu standards.$^{1b}$ The activity in each collected tissue sample was calculated as percentage of the injected dose per gram of tissue (% I.D./g). For this calculation, the tissue radioactivity was corrected for the $^{64}$Cu decay ($T(1/2)$=12.7 h) to the time of $\gamma$-well counting.
Pharmacokinetic Study of poly(iohexol) NP and iohexol.

Female athymic nude mice (6-8 weeks) were divided into two groups (n = 3), minimizing the weight differences. The two groups of mice were treated with $^{64}$Cu-labeled PEGylated poly(iohexol) NPs and $^{64}$Cu-labeled iohexol intravenously at a dose of 35 µCi, respectively. Blood was collected intraorbitally from mice at scheduled time points (5 min, 15 min, 30 min, 60 min, 2 h, 4 h, 8 h, 12 h, 24 h and 48 h post injection) for pharmacokinetic profiling. The collected blood samples were weighed and measured for radioactivity ($^{64}$Cu) with a $\gamma$-counter (Wizard2, Perkin-Elmer, USA) using appropriate energy window centered at photo peak of 511 keV. Raw counts were corrected for background, decay, and weight. Corrected counts were converted to microcurie per gram of tissue (µCi/g) with a previously determined calibration curve by counting the $^{64}$Cu standards. The activity in each collected blood sample was calculated as percentage of injected dose per gram of blood (% I.D./g). For this calculation, the blood radioactivity was corrected for the $^{64}$Cu decay to the time of $\gamma$-well counting.

$$\text{Plasma half-life} = 0.693 \times \frac{\text{time}_{interval}}{[\ln(\text{Concentration}_{\text{peak}}) - \ln(\text{Concentration}_{\text{trough}})]}$$

where time$_{interval}$ is the time between the peak and the end point, Concentration$_{peak}$ is the maximum concentration in plasma and Concentration$_{trough}$ is the concentration at the end point.

In vitro cytotoxicity evaluation

MCF-7 cells were placed in a 96-well plate for 24 h (8 000 cells per well). Cells were washed with 100 µL of prewarmed PBS. Freshly prepared PEGylated poly(iohexol) NPs with a series of concentrations (prepared in 1× PBS, 100 µL) were added to the cells. Untreated cells used as negative controls in this assay. The cells were incubated for 72 h in a 5% CO$_2$ incubator at 37 °C. The standard MTT assay protocols were followed thereafter.
**Histological evaluation**

Six female athymic nude mice (6-8 weeks) were divided into two groups (n = 3), minimizing the weight differences. In 3 mice, poly(iohexol) NPs were administered intravenously (200 μL, 30 mg/mL) via lateral tail vein to the athymic nude mice (n = 3) at a dose of 150 mg NP/kg. The other 3 mice received intravenous PBS and served as the control group. All the animals were sacrificed 24 h later, and major organs including heart, lung, liver, spleen, kidney, and intestine were collected followed by flash freezing in optimum cutting temperature (O.C.T.) compound (Sakura Finetek USA, Torrance, CA, USA). Organs were sectioned using cryostat at the thickness of 10 μm, followed by standard hematoxylin and eosin (H&E) staining. Characterization for inflammatory cell infiltrate including macrophages and neutrophils were performed on a Nanozoomer Virtual Microscopy (Hamamatsu) evaluated at 20× magnification and analyzed by an independent pathologist.
Supplementary Figures

Figure S1. Synthetic route of cross-linked poly(iohexol).
Figure S2. (A) GPC analysis of cross-linked poly(iohexol) at different molar ratios of iohexol (I)/Hexamethylene diisocyanate (H). (B) FT-IR spectra of hexamethylene diisocyanate (a), iohexol (b), and poly(iohexol) (c) at the I/H ratio of 1/3 (P3).
Figure S3. $^1$H NMR spectrum of cross-linked poly(iohexol) in $d_6$-DMSO.
Figure S4. Formulation and characterization of PEGylated poly(iohexol) NPs. (A) The size changes of poly(iohexol) NP with/without dilutions in human serum (HS) buffer (HS:PBS =1:1, v/v), as determined by dynamic light scattering (DLS). (B) Long-term stability of poly(iohexol) NP in HS buffer at 37 °C. (C) DLS analysis on the stability of NPs in Dulbecco’s Modified Eagle Medium (DMEM); particle size and size distribution at day 0 and day 3. (D) DLS analysis of NP reconstitution; monodispersed NPs mixed with human serum albumin (HSA) in water before lyophilization, and the reconstituted NPs after lyophilization in the presence of HSA (HSA: NC =10 : 1, wt/wt).
Figure S5. (A) HU measurements of poly(iohexol) NP ex vivo. The plot showed linear correlation between concentration of the poly(iohexol) NPs and CT calculated solution attenuation measured in Hounsfield units (HU). (B) Axial CT images after transarterial chemoembolization using poly(iohexol) NPs in a New Zealand white rabbit. Dose administered was approximately 125 mg/kg. After injection of 30 mL of nanoparticle emulsion (consisting of 20 mL NP solution mixed with 10 mL poppy seed oil), cross-sectional imaging reveals accumulation of nanoparticles within liver parenchyma, evidenced by focal areas of high tissue attenuation (arrowheads). Arrow indicated high attenuation of vascular structures.
Figure S6. C57BL/6 mice followed jugular vein injection of 200 μL of conventional iodinated contrast agent (iohexol) solution and poly(iohexol) NP solution at 250 mg iohexol/ kg. Mean density of different organs (heart, liver and kidney) normalized by area of organs (μm²) at selected time points (0 min and 60 min) after injection of iohexol or poly(iohexol) NP, quantified based on inverted images by Image J.
Figure S7. Synthesis of $^{64}$Cu labeled poly(iohexol) NP and $^{64}$Cu labeled iohexol.
Figure S8. ESI-MS spectra of DOTA-iohexol.
Figure S9. *In vivo* biodistribution of poly(iohexol) NP and iohexol. (A) *In vivo* whole-body dynamic PET/CT imaging of mice treated with $^{64}$Cu labeled poly(iohexol) NP (upper panel) and iohexol (bottom panel). Images were taken at 1 h, 4 h and 24 h after tail vein injection of poly(iohexol) NP and iohexol, respectively. (B) The accumulation of $^{64}$Cu labeled poly(iohexol) NP (upper panel) and iohexol (bottom panel) in various organs at 1 h, 4 h and 24 h after tail vein injection of poly(iohexol) NP and iohexol, respectively. Data was obtained from direct analysis of PET/CT imaging. (C) The kinetics of tissue distribution of $^{64}$Cu labeled poly(iohexol) NP and iohexol in heart, liver, lung, spleen, kidney, bladder and tumors at 1 h, 4 h and 24 h after tail vein injection. (D) Methodology of assessing *in vivo* biodistribution of poly(iohexol) NP and iohexol.
by PET/CT imaging though quantifying radioactivity of various organs (heart, lung, liver, kidney, spleen and bladder) in the axial PET/CT imaging sections.

**Figure S10.** (A) *In vivo* biodistribution of $^{64}$Cu labeled poly(iohexol) NP (red bar) and iohexol (blue bar) in MCF-7 xenografts bearing athymic nude mice, assessed by $\gamma$-counter. All the data was presented as percentage of injected dose per gram of tissues (% I.D. /g). (B) Stability of $^{64}$Cu labeling with poly(iohexol) NP and iohexol in serum.
Figure S11. *In vitro* toxicity of poly(iohexol) NP against MCF-7 cells (37 °C, 72 h), evaluated by MTT assay (37 °C, 72 h).
Figure S12. Histopathology of mouse tissues following intravenous injection of poly(iohexol) NPs. Selected organs were taken from control mice receiving PBS and mice receiving 300 mg/kg of NPs 24 h post injection, and representative sections were stained by hematoxylin and eosin. No acute inflammation was found in animals receiving poly(iohexol) NPs.
Reference:

