Supporting Information

Materials and Methods Section

Materials. All chemicals and biological material were purchased from Sigma-Aldrich or Invitrogen unless otherwise noted.

Mice. Female BALB/c and NCr nude mice (4-6 weeks old) were purchased from Taconic and the AIN-76A purified diet was from PharmaServ/Testdiets. Mice were kept on the AIN-76A diet for at least a week before experimentation to reduce levels of body phosphorescent alfalfa. All *in vivo* experimentation was carried out under the supervision of the Division of Comparative Medicine (DCM), Massachusetts Institute of Technology, and in compliance with the Principles of Laboratory Animal Care of the National Institutes of Health. Cell lines were purchased from ATCC and were tested routinely for pathogens before use in animals via DCM.

Cell Culture. KB nasopharyngeal carcinoma cells (ATCC) and J774A.1 macrophages were used in our experiments and grown in MEM alpha media supplemented with 10% fetal bovine serum, 50 units/mL penicillin and 50 units/mL streptomycin.

Fabrication and characterization of layer-by-layer (LbL) nanoparticles. LbL nanoparticles with functional shells were prepared using a commonly used assembly technique for nanoparticle coating[1]. The negatively charged fluorescent cores used were either carboxyl functionalized gold nanoparticles (AuNPs) (~20 nm; -25 mV; see section below for synthesis) or carboxyl functionalized quantum dots (QD_{705}) (8 μM; em: 705 nm; ~20 nm; -25 mV; Invitrogen). Pol-L-lysine (15 kDa) was labeled with a near IR dye (VivoTag 800, Visen medical) following the manufacturer's instructions on ~ 5% of the primary amine side groups (based on reaction feed ratio) under aqueous conditions and at a pH of 7.4. After reaction, the labeled polymers were purified by dialysis (3.5 kDa cut-of dialysis bag) before use. For LbL assembly, nanoparticles were mixed vigorously with polyelectrolytes that were prepared at concentrations of 500 μM at pH 7.4. The mixing ratios of polyelectrolyte (PLL: poly-L-lysine (10 kDa), DXS: dextran sulfate (10 kDa), HA: hyaluronic acid (10 kDa, Lifecore Biomedical Inc.) to nanoparticle were approximately 400-500 μM : 0.05-0.1 μM. After addition of the nanoparticle to polyelectrolyte, the mixture was left stirring vigorously at pH 7-7.4 for 4 h. No salt was added to the mixture. The particles were purifed by three centrifugation (13000 rpm; 1.5 h) and re-suspension (millipore water, pH 7.4) cycles. No salt was added in the process.
All size and zeta potential measurements were made using a Zeta PALS (Brookhaven) analyzer. Tapping mode AFM characterization was performed on a Dimension 3100 instrument with Nanoscope III controller (Digital Instruments) For TEM imaging, micelle solutions were drop-cast onto carbon coated copper grids without any staining. Particle concentrations were estimated using standard

calibration graphs made from native particles. To attain equal particle concentrations, LbL nanoparticle solutions were adjusted with water based on QD_{705} fluorescence measured. For *in vivo* experiments, the concentration of QD_{705} particles used was ~ 0.5 μM given in 0.1 mL injections.

Synthesis of MUA capped gold nanoparticles. Gold nanoparticles (AuNPs) were synthesized using a published method[2]. Briefly, 100 mg of hydrogen tetra-chloroaurate(III) (HAuCl4) in water was transferred to toluene with tetraoctylammonium bromide (10 mM). The gold particles in toluene were washed three times with deionized water and transferred to a flask. Sodium borohydride (100 mg in 10 mL deionized water) was then added and the orange-colored HAuCl4 solution turned wine-red. The reaction was left stirring for 2 h. The two phases were then separated and the toluene phase was subsequently washed with 0.1 M sulfuric acid, 0.1 M sodium hydroxide, and three times with deionized water. The gold nanoparticle solution in toluene was heated to 60°C, and 5 g of 11-mercaptoundecanoic acid (MUA) in 10 mL of toluene (at 60°C) was then added to the gold nanoparticles. The gold nanoparticles settled to the bottom of the flask upon addition of MUA and the black precipitate obtained was separated and washed three times with toluene to remove any uncoordinated MUA. The precipitate was dried and redispersed in basic buffer. A wine red solution was obtained with a plasmon absorption maximum at 525 nm. The concentration of AuNPs was estimated using mass conservation considerations.

***In vivo* experimentation**. BALB/c mice were used for blood circulation experiments and the NCr nudes were used for all other experiments. Mice fed on AIN-76A diet for at least a week were given single injections of the different LbL particles via the tail vein. The concentration of particles (QD_{705} and LbL nanoparticles) administered was ~ 0.5 μM given in 0.1 mL injection. Free PLL_{800} was administered at doses of 5 mg/kg. At various time points after injection, they were imaged ventrally using the IVIS system (Caliper Lifescience). Living Image software Version 3.0 (Xenogen) was used to acquire and quantitate the fluorescence. The images showing QD_{705} fluorescence was captured using Ex: 640 nm and Em: 720 nm. The images showing PLL_{800} fluorescence was captured using Ex: 710 nm and Em: 800 nm. Spectrally unmixed images were captured using a sequence of Ex: 640 nm and Em: 700 nm/720 nm/740 nm/760 nm for QD_{705}; and Ex: 745 nm and Em: 800 nm/820 nm/840 nm for PLL_{800}.
Subcutaneous tumors were induced in either the left or right hind flank of NCr nudes after injection of ~1-2 million cells (KB) in 0.1 mL media. Tumors were allowed to grow to ~100 mm^3 before experimentation. Where applicable, tissue samples were extracted for further imaging using the Licor Odyssey system. For biodistribution, tissue samples were harvested, washed, weighed, and macerated before imaging with the IVIS system for their respective fluorescence. The fluorescent data was normalized by tissue weight. Blood circulation analysis was performed by measuring the remaining QD signal from blood taken after injection with the Licor Odyssey system.

***In vitro* experimentation.** As the first examination of the stealth properties of these LbL particles, we tried opsonising them with human IgG-488. 0.2 μM LbL particles were subjected to incubation with 0.25 mg/mL of aqueous IgG-488 at pH 7.4, 37℃ for 30 min. After incubation, the particles were centrifuged down and washed with DI water three times. The amount of IgG-488 adsorbed onto the LbL particle surface was determined by the ratio of IgG-488 fluorescence to the QD_{705} fluorescence, measured using a spectrofluorometer. Subsequently, we measured their degree of uptake into J774A.1 macrophages using confocal microscopy and flow cytometry. For uptake studies, non-labeled mouse IgG was used to opsonize the particles (30 min, pH 7.4 and at 37℃) and the fluorescent QD_{705} signal was used to quantitate nanoparticle uptake. A Cy5 filter was used to detect quantum dot (em:705 nm) fluorescence on both a DeltaVision confocal microscope (Applied Precision) and FACS LSRII flow cytometer (BD Biosciences). For confocal microscopy, cells were grown until 50% confluent on chamber slides and treated with various nanoparticles. The cells were then washed repeatedly with PBS and fixed with 4% paraformaldehyde. Fluoromount-G was used to prepare the cells for confocal analysis. For FACS analysis, cells after treatment were trypsinized and washed three times with PBS to remove unbound particles before analysis. Nanoparticle aggregation was investigated by taking time dependent size measurements of different LbL nanoparticles in a solution of 20 mg/ml bovine serum albumin in PBS.

Statistical Analysis. All values shown are in mean ± SEM unless otherwise specified. Analyses were done by unpaired Student's t-test and considered significant at $P<0.05$.

1. Schneider, G.; Decher, G. *Langmuir* **2008,** 24, (5), 1778-1789.
2. Gittins, D. I.; Caruso, F. *Advanced Materials (Weinheim, Germany)* **2000,** 12, (24), 1947-1949.

Supplemental Figures

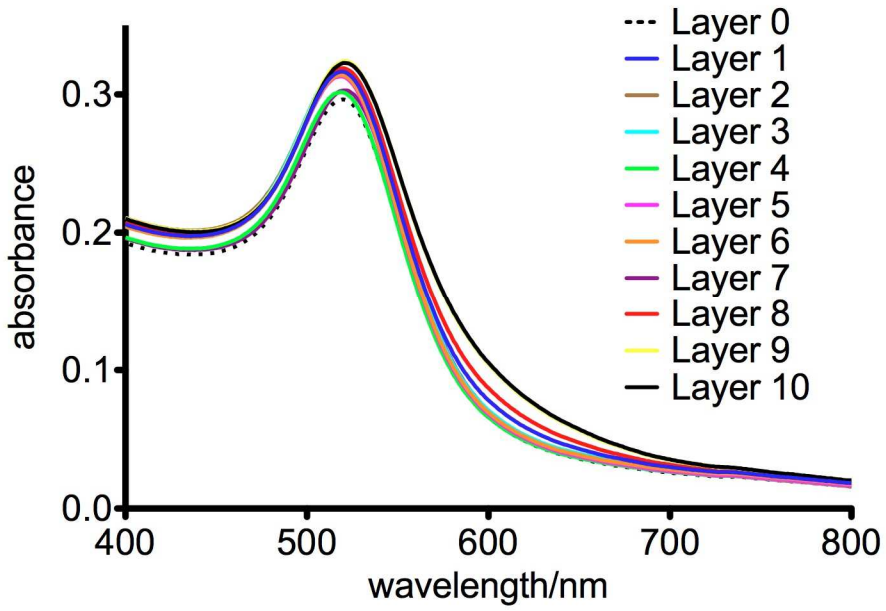

Fig. S1. UV/vis spectra of the layer-by-layer (LbL) gold nanoparticles (AuNPs) after the deposition of each layer of polyelectrolyte. (poly-L-lysine or dextran sulfate).

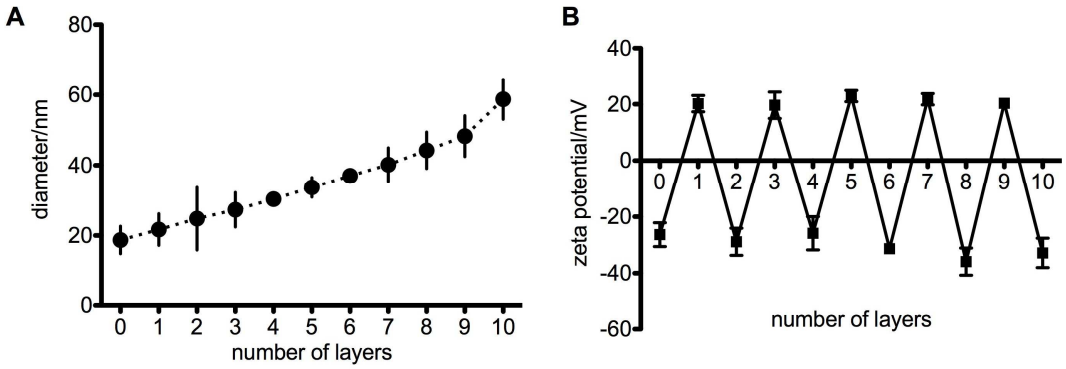

Fig. S2. A) The growth curve of PLL/DXS (poly-L-lysine/dextran sulfate) nanofilms deposited carboxyl functionalized quantum dots (QD_{705}). Each layer is ~ 2 nm thick. B) The zeta potential of LbL particle after deposition of each PLL or DXS layer show complete reversal of charge.

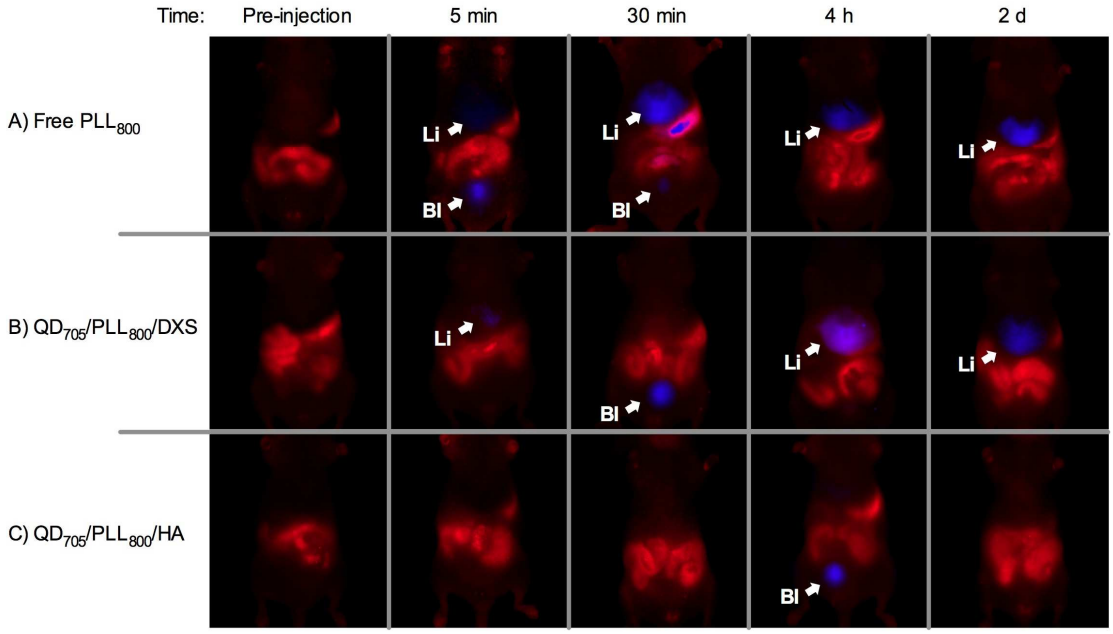

Fig. S3. Spectrally unmixed images of mice representative of the *in vivo* events that occur to near-IR labeled poly-L-lysine (PLL, 15 kDa) after injection. PLL_{800} is injected in free form in (A) and as part of a single bilayer LbL nanoparticle in (A) and (C). Images show PLL_{800} fluorescence in blue and tissue auto fluorescence in red. A PLL_{800} signal detected in the bladder (Bl) indicates destabilization of LbL nanoparticles. Li = liver; Bl = bladder. Note: these mice were not maintained on the alfalfa free AIN-76A diet.

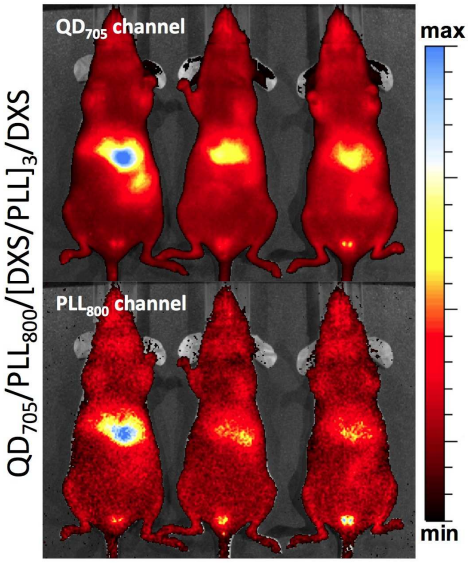

Fig. S4. Co-localization of both nanoparticle film (PLL_{800}) and core (QD_{705}) in the livers of a set of 3 mice after injection with $QD_{705}/PLL_{800}/[DXS/PLL]_3/DXS$ shows stability and liver uptake of entire LbL nanoparticle.

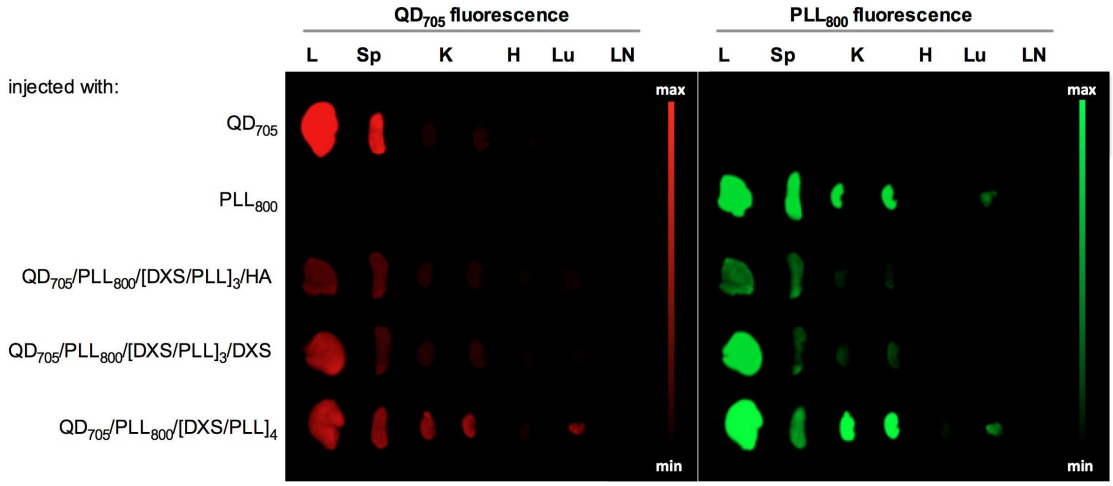

Fig. S5. Tissue harvested from mice at 4 h after different agents were administered. QD_{705} and PLL_{800} fluorescence were detected from the same set of tissue. This image gives a visual representation of the biodistribution of each system, tracked on both fluorescent channels. Li=Liver, Sp=spleen, Ki=kidneys, H=heart, Lu=lungs and LN=lymph node.

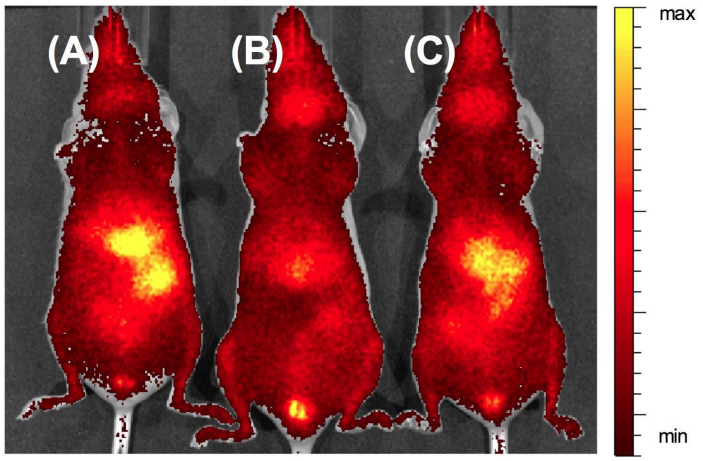

Fig. S6. Competition of liver uptake of QD_{705}/PLL/$[DXS/PLL]_3$/DXS (A) with free DXS and HA (10 mg/kg each). B= (A) + DXS, C= (A) + HA. A representative mouse from each treatment is imaged on a single viewing field.

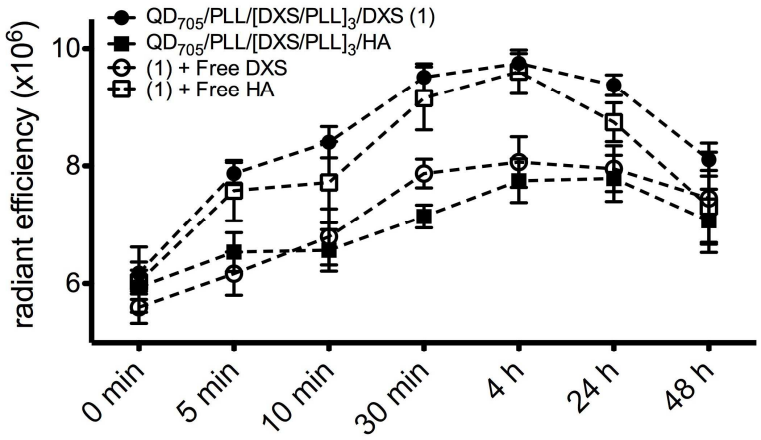

Fig. S7. The time dependant accumulation of HA and DXS terminated particles in the liver, with co-injections of free DXS and HA (10 mg/kg). Data is given in mean±SEM, n = 3.

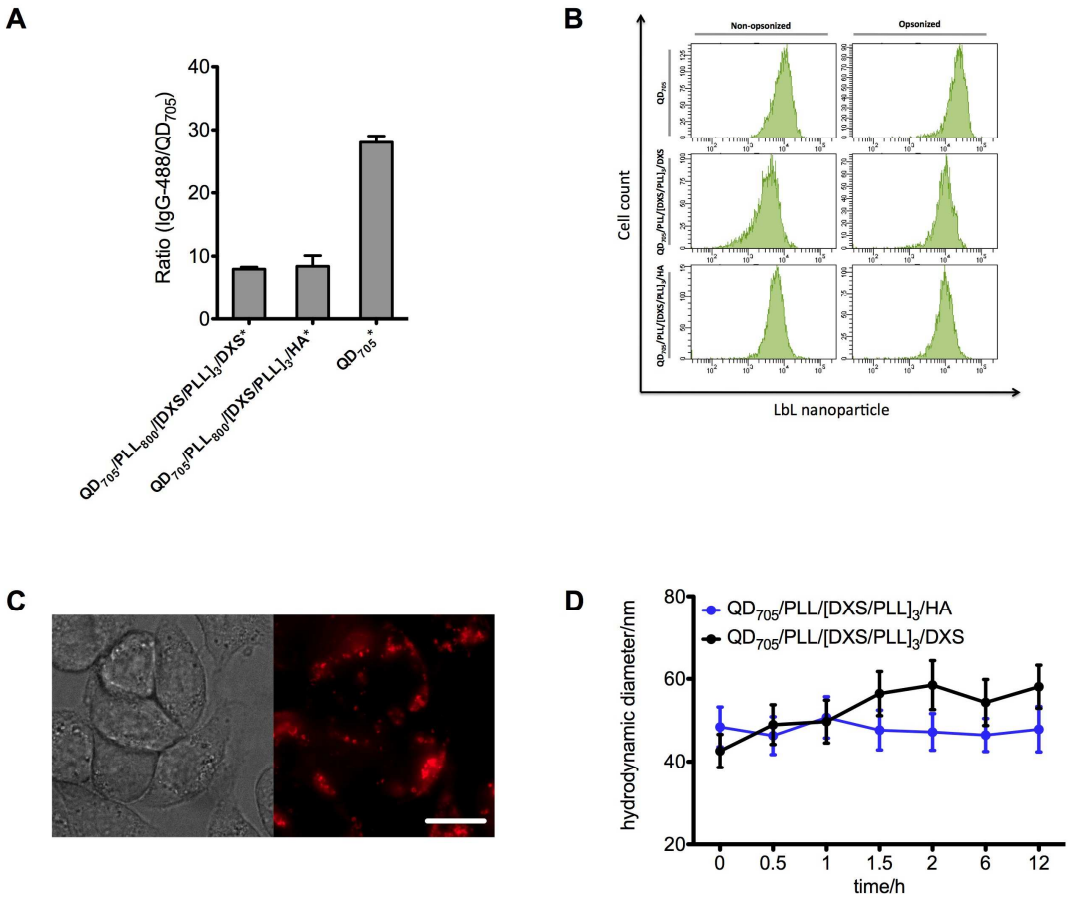

Fig. S8. Examination of RES involvement in liver uptake of DXS and HA terminated LbL nanoparticles. A) The degree of opsonization with IgG-AF488 measured by taking the ratio of AF488/QD_{705}. Free QD_{705} bound to more IgG without an antifouling terminal layer while there were no differences in the degree of opsonization of HA and DXS terminated films. B) Raw histograms showing the cell associated fluorescence after treatment of each of each condition in Fig 4C. C) Representative confocal image showing the uptake of LbL nanoparticles after opsonization into mouse macrophage J774A.1 cells. Scale bar = 10 μm. D) Time dependent increase of the hydrodynamic diameter of LbL nanoparticles in a solution of 20 mg/ml bovine serum albumin in PBS.