

Supporting Information

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Materials

Reagents

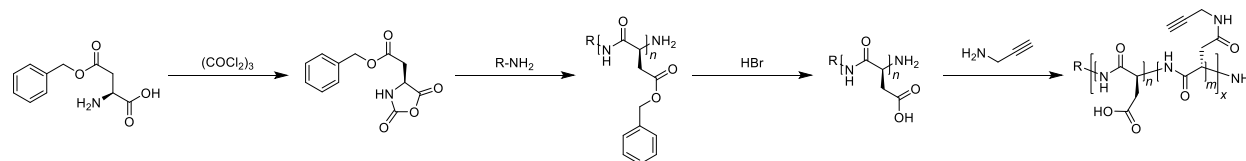
1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DSPG), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and cholesterol were purchased from Avanti. Sulfo-cyanine 3 and 7 NHS esters were purchased from Lumiprobe. Chloroform and methanol were purchased from TCI and Sigma, respectively. Whatman nuclepore polycarbonate hydrophilic membranes (400, 200, 100 and 50 nm sizes) were purchased from GE. All glassware was obtained from Chemglass. 50/15 mL Falcon tubes and 50/5/2 mL DNA loBind Eppendorf tubes were purchased from VWR. Infrared fluorescent polystyrene latex microspheres (Fluospheres), locked nucleic acid siRNAs (Silencer Select), 5 M bioreagent grade NaCl solution, and 1 M bioreagent-grade HEPES was purchased from Fisher Scientific. D02-E100-05-N and C02-E100-05-N tangential flow filtration filters were purchased from Spectrum Labs. Poly-L-arginine hydrochloride (38.5 kDa) was purchased from Alamanda Polymers. Polystyrene semi-micro cuvettes for the Malvern Zetasizer were purchased from VWR and DTS1070 folded capillary cells were purchased directly from Malvern. Black, glass bottom 364 well plates for the Wyatt DLS were purchased directly from the Peterson (1957) Nanotechnology Core Facility. Recombinant MMP9 was purchased from Enzo Life Sciences. The azido-modified biosensor peptide (sequence B(biotin)-eGvndneeGffsarK-(FAM) dGGPLGVRGKK-(N3)) was synthesized by CPC Scientific with >90% purity. mPEG-azide (5kDa) was purchased from Creative PEGWorks. Azide-functionalized iRGD was synthesized by the Biopolymers & Proteomics core facility at the Swanson Biotechnology Center at the Koch Institute for Integrative Cancer Research at MIT.

Cells

The MC26 murine colorectal cancer cell line was a kind gift from Dr. Kenneth Tanabe's lab at MGH. D8-175 murine KPC cell lines were derived from KPC (LSL-Kras^{G12D/+}; LSL Trp53^{fl/fl}; Pdx-1-Cre) transgenic mouse tumors harvested in the lab of Tyler Jacks. The OVCAR8 human ovarian cancer cell line (NCI-60) was cultured in Roswell Park Memorial Institute (RPMI) also supplemented with FBS and pen strep. Tissue culture plastic ware (T75, T25, clear and white 96 well plates), trypsin EDTA, penicillin streptomycin and RPMI 1640 media were purchased from Corning. Fetal bovine serum was purchased from Gibco. D-luciferin was purchased from Fisher Scientific.

Methods

Polymer synthesis



Scheme S1. Synthetic route of pPLD.

Synthesis of β -benzyl-L-aspartate N-carboxyanhydride (BLD NCA)

BLD NCA was prepared as described previously.^[1] Briefly, a suspension of β -benzyl-L-aspartate (25.0 g, 112 mmol), tetrahydrofuran (THF, 200 mL), and triphosgene (30.0 g, 100 mmol) was stirred and sparged with a steady stream of argon under reflux for 2 h. Hexanes (700 mL) were added and the resulting precipitate was collected by vacuum filtration, washed 4 times with hexanes, dissolved in anhydrous THF (300 mL), and recrystallized under a layer of hexanes (700

mL). The solids were collected by vacuum filtration, washed 4 times with hexanes, and dried *in vacuo* to give white crystals (25.7 g, 103 mmol, 92%).

^1H NMR (400 MHz, CDCl_3) δ 7.47 – 7.30 (m, 5H), 6.13 (s, 1H), 5.19 (s, 2H), 4.60 (dd, $J = 9.5$, 2.4 Hz, 1H), 3.09 (dd, $J = 17.7$, 3.1 Hz, 1H), 2.84 (dd, $J = 17.7$, 9.6 Hz, 1H)

$^{13}\text{C}^1\text{H}$ NMR (101 MHz, CDCl_3) δ 169.55 (s), 168.31 (s), 151.32 (s), 134.76 (s), 129.03 (s), 128.95 (s), 128.71 (s), 67.98 (s), 53.98 (s), 36.22 (s)

Synthesis of poly(β -benzyl-L-aspartate) (BPLD)

PBLD was prepared as described previously.^[1] Briefly, to a solution of BLD NCA (4.99 g, 20.1 mmol) and DMF (50 mL) was added hexylamine (26.5 μL , 0.201 mmol). The reaction was sparged with a steady stream of argon at RT for 48 h. The polymer was precipitated by addition of water and the resulting precipitate was collected by centrifugation, washed with water, and dried *in vacuo* to give a white powder (3.25 g, 15.8 mmol repeat units, 78.8%).

^1H NMR (400 MHz, DMSO): δ 8.37 – 7.98 (m, 1H), 7.51 – 7.08 (m, 5H), 5.21 – 4.92 (m, 2H), 4.74 – 4.48 (m, 1H), 2.98 – 2.54 (m, 2H).

Synthesis of poly(L-aspartate) (PLD)

PLD was prepared as described previously.^[2] Briefly, to a solution of PBLD (2.42 g, 11.8 mmol repeat units) in trifluoroacetic acid (14.5 mL) was added dropwise 33 wt% HBr in AcOH (6.0 mL). The reaction was stirred for 1 hour after which diethyl ether was added. The precipitate was collected by filtration, washed with diethyl ether, and dried *in vacuo* to yield an off-white powder (1.05 g, 9.12 mmol repeat units, 77.3%).

Synthesis of Poly(propargyl-L-aspartate-co-L-aspartate) (pPLD)

PLD (1.05 g, 9.12 mmol) was added to a round bottom flask equipped with a stir bar and DMF (15 mL), water (2 mL), HOBT hydrate (ca. 20% water) (184 mg, 1.09 mmol), and propargylamine (130 μ L, 3.19 mmol) were added. EDC (216 mg, 1.09 mmol) was added portion wise with stirring. After stirring for 24 h, the DMF was removed by extraction with diethyl ether and the pH of the aqueous phase was adjusted to 7 by addition of saturated sodium bicarbonate solution. The aqueous solution was dialyzed against saturated sodium chloride followed by deionized water (MWCO 1 kDa, regenerated cellulose) and lyophilized to give a white powder (1.06 g). The degree of substitution was determined to be 12% by the ratio of protons on both sides of the side chain amide group. The number average molecular weight (M_n) of the polymer was determined by end group analysis to be 10kDa using NMR spectroscopy. The dispersity of the polymer was determined to be 1.31 by aqueous gel permeation chromatography (GPC) calibrated with narrow distributed PEG standards. Signals from the amide protons, α -proton, and propargyl proton were visible, but diminished due to deuterium exchange. ^1H NMR (400 MHz, D_2O) δ 4.73 – 4.53 (m, 1H), 3.97 (s, 0.24H), 2.86 – 2.50 (m, 2H).

Tissue culture

All media was supplemented with 1% penicillin streptomycin. All cells were allowed to adhere/grow for 24 hours before treatment with nanoparticles in a Heracell Incubator (Thermo Fisher) at 37 °C and 5% CO_2 . All cell lines were tested for mycoplasma contamination periodically, including immediately upon thawing, and over time when in culture, using the Lonza MycoAlert kit (Catalog #: LT07-318). Results were always negative for mycoplasma contamination.

Preparation of liposomes

Cholesterol and DOPE were dissolved in chloroform and DSPG was dissolved in a 65:35:8 volume ratio of chloroform, methanol and deionized water (milli-Q). Lipid mixtures composed of 66.7 mol% DSPG and 33.3 mol% cholesterol were prepared in round bottom flasks (RBFs) (10 or 50 mL depending on scale), and for DSPG-formulations methanol was added dropwise until the solution cleared. For fluorescent liposomes, the formulation was 61.7 mol% DSPG, 5 mol% DOPE and 33.3 mol% cholesterol. The lipid solution was evaporated using a BUCHI RotoVap system under heat (60 °C, water bath) until completely dry (<15 mBarr). A Branson sonicator bath was filled with reverse-osmosis water and heated until >70 °C, at which point the RBF with the lipid film was partially submerged in the bath and a volume of dH₂O was added to re-suspend the lipid film to a 1 mg lipid/mL solution. The liposome solution was sonicated for roughly 1 minute and then transferred to an Avestin LiposoFast LF-50 liposome extruder. The extruder was connected to a Cole-Parmer Polystat Heated Recirculator Bath to maintain a temperature > 65 °C. The liposome solution was extruded through successively smaller nuclepore membranes until a 50-100 nm liposome was obtained. Generally, this required 1 pass through a stack of 2-3 400, 200, 100 and 50 nm membranes. Fluorescently labeled liposomes were prepared via standard NHS-coupling of Sulfo-cyanine NHS ester to DOPE head groups according to manufacturer instructions. Liposomes were characterized for size and zeta using the techniques outlined below.

Preparation of layer-by-layer nanoparticles

Nanoparticles were layered by adding an equal volume of nanoparticle solution (not exceeding 1 mg core template/mL) to an equal volume of polyelectrolyte solution under sonication

(Branson bath sonicator, room temperature). The mixture was sonicated for roughly 5 seconds. The weight equivalents (wt. eq.) of polyelectrolyte used with respect to the liposome core were: 0.25 (first layer, PLR), 1.0 (second layer, siRNA), 0.33 (third layer, PLR), and 1.0 (fourth and final layer, pPLD). Polyelectrolyte solutions were always prepared in 50 mM HEPES and 40 mM NaCl (pH 7.4), which, upon mixing with the NP substrate (in water), were diluted 1:1 to 25 mM HEPES and 20 mM NaCl final concentration. DNA loBind tubes were used as the mixing vessels for all experiments, to prevent nonspecific adsorption of siRNA and other polymers to the plastic.

The layered particle was allowed to incubate at room temperature for 1 hour and was then purified using the tangential flow filtration method, as described previously.^[3] Briefly, crude nanoparticle solution is connected to a Spectrum Labs KrosFlo II system using masterflex, Teflon-coated tubing. D02-E100-05-N (batch volume > 5 mL) or C02-E100-05-N (batch volume < 1 mL) 100 kDa filters were used to purify the particles until 5 volume-equivalents were collected in the permeate. For cationic layers, the TFF filter was pre-treated with a solution of free polycation (same concentration used for layering) in order to minimize nonspecific adsorption of particles to the membrane walls. Samples were run at flow rates of 80 mL/min (size 16 tubing, used with D02-series filters) or 13 mL/min (size 13 tubing, used with C02-series filters). Once pure, the sample was either concentrated (by disconnecting the buffer reservoir) or recovered via reversing the direction of the peristaltic pump. For purification, the exchange buffer was always Milli-Q water. For more complete yields, 1-3 mL of water were run backwards through the tubing to recover any remaining particles. This process was repeated until the desired LbL formulation was obtained.

Click-chemistry conjugation onto LbL nanoparticles

After formulation, LbL NPs were modified with azide functionalized ligands by mixing copper II sulfate, tris-hydroxypropyltriazolylmethylamine (THPTA), and sodium ascorbate. All solutions with the exception of azide functionalized ligand were prepared in water. The azide functionalized ligand was dissolved in 0.1x PBS, though HEPES and water have also worked equally well. A matrix of conditions was tested to determine optimal conditions for NP stability by varying concentrations of LbL NPs and azide-functionalized ligands while keeping constant concentrations of other reagents. In a representative example, 0.05 mL of a 1 mg/mL LbL NP solution was mixed with 0.025 mL of azide-functionalized ligand (0.28-1.12 mM), 0.0875 mL sodium ascorbate (1 mg/mL), and 0.0875 mL CuSO₄/THPTA solution (0.016 mg/mL CuSO₄, 0.0672 mg/mL THPTA). This reaction was scaled as needed. All reactions were carried out at room temperature protected from light over 12-16 hours. Subsequently, tangential flow filtration was used to remove excess reagents and concentrate functionalized LbL NPs to desired concentration for experiments.

Characterization of nanoparticles

Nanoparticle hydrodynamic size and polydispersity were measured using dynamic light scattering (Malvern ZS90 Particle Analyzer, $\lambda = 633$ nm, material/dispersant RI 1.590/1.330). Zeta potential measurements were also acquired with the Malvern ZS90, using laser Doppler electrophoresis. Nanoparticle solutions were diluted in milli-Q water in polystyrene semi-micro cuvettes (VWR) or DTS1070 folded capillary cuvettes (Malvern) to produce samples for characterization on the Malvern SZ90. For optimization of click chemistry, the hydrodynamic size of each microbatch was assessed using high throughput dynamic light scattering (Wyatt Dyna Pro Plate Reader, $\lambda = 850$ nm), with samples diluted in milli-Q water and tested in a glass-bottom 384 well plate. siRNA

content was measured as described previously.^[4] Encapsulation efficiency (EE%) was calculated

$$\text{as: } EE\% = \frac{\text{siRNA (actual)}}{\text{siRNA (theoretical)}} \times 100.$$

In vitro MMP9 cleavage study

Biosensor functionalized LbL NPs were exposed to 12.5 nM recombinant MMP9 (Enzo Life Sciences, Inc) for 30 min at 37 °C. Afterwards, NPs were pelleted using centrifugation (30,000 RCF for 60 minutes) and the supernatant was collected. Urinary reporter was detected using an ELISA. Mouse anti-fluorescein (GeneTex) antibody was dissolved at 0.4–0.8 µg/mL overnight in 1×PBS and adsorbed to 96-well Bacti plates (Thermo). Plates were then blocked for 1 h with 1×PBS with 1% BSA (Sigma). Reporter standards were applied to blocked plates in two-fold serial dilutions in 100 µL volume for 1 h to characterize assay linearity. To detect reporters, 100 µL of 0.4 µg/mL NeutrAvidin-HRP (Pierce) was applied for 1 h. Bound HRP was exposed with 50 µL of Ultra-TMB (Pierce) for 1-5 min followed by quenching with 50 µL of 1 M HCl. Between each step, plates were washed three times with 1×PBST. Absorbance at 450 nm was measured, and then plotted against known reporter concentration. Results were normalized relative to NPs that were not exposed to MMP9.

Flow cytometry

OVCAR8 cells were seeded at 10,000 cells/well (100,000 cells/mL) into a 96 well plate. The cells were allowed to adhere for 24 hours prior to treatment with 5 µL of a 0.3 mg/mL NP solution for either 4 or 24 hours. At this point, the cells were washed 3x with warm PBS and dissociated from the well bottom using 20 µL 0.25% Trypsin-EDTA (Gibco). 150 µL warm media (RPMI w/ FBS,

P/S) were used to quench the trypsin after 3-5 min incubation at 37 °C. The samples (ex/em 715/755 nm) were analyzed using an LSR II HTS-2.

Super resolution microscopy

Chambered cover glass was coated with rat tail collagen (300 μ L of 50 μ g/mL in 0.02N acetic acid). After 5 minutes, the wells were washed with room temperature PBS and allowed to dry in a sterile environment. Wells were stored at 4 °C up to one week prior to seeding OVCAR8 cells at 8,000 cells/well. The cells were allowed to adhere for 24 hours prior to treatment with 3 μ L of a 0.5 mg/mL sulfoCy3 labeled-NP solution. The cells were incubated for 24 hours, at which time they were washed 3x with PBS and fixed in 4% methanol free formaldehyde prepared in PBS (15 min, room temperature). This was followed by three washes of ice cold Hank's Balanced Salt Solution (HBSS, ThermoFisher) at 5 min/wash. Solutions and cover glass/wells were kept covered with aluminum foil for the following steps to prevent photo bleaching of the fluorophores. 300 μ L of wheat germ agglutinin-AF647 (10 μ g/mL in HBSS) were added to each well and incubated at room temperature for 10 min. Cells were washed 3x with room temperature PBS (5 min/wash) before fixing again with 4% formaldehyde for 2 min followed by another 3x PBS wash (5 min/wash). 300 μ L of 1.25 μ g/mL Hoechst in PBS were added to each well for 2 min at room temperature before washing with 3x with room temperature PBS (5 min/wash). Finally, the wells were filled with ~5 drops of Vectashield (H-1000) and stored at 4 °C protected from light. The cells were imaged with the Delta OMX- V4 Blaze 3D structured illumination microscope (Applied Precision, now GE), equipped with 405 nm, 488 nm, 563 nm and 647 nm lasers and three sCMOS cameras. Images were acquired with a 60X, NA 1.43 oil objective, at 0.125 using 1.518 immersion oil at RT. All images were acquired under the same illumination settings and then processed with

OMX softWoRx software (Applied Precision/GE). Images were saved as tiff. Image LUTs were linearly adjusted to improve contrast on FIJI.

Animal Studies

All animal experiments were approved by the Massachusetts Institute of Technology Committee on Animal Care (CAC) and were conducted under the oversight of the Division of Comparative Medicine (DCM). Pancreatic cancer flank models were established with a subcutaneous injection of 5×10^5 D8-175 cells to both flanks of nude mice. The liver metastatic model of colorectal cancer was established by inoculating 5×10^4 MC26 cells in the subsplenic capsule. The cells were allowed to traverse vasculature to seed the liver, and the spleen was removed to prevent ectopic tumor growth. Flank ovarian cancer models were established by subcutaneous injection of a 1:1 mixture of OVCAR8 cells and Matrigel (Corning) into both flanks of female NCR nude mice. The metastatic model of ovarian cancer was established via intraperitoneal injection of 1×10^6 OVCAR8 cells into female NCR nude mice. siRNA doses of 0.5 mg/kg were administered for knockdown studies.

Determination of urinary diagnostic function

Theranostic LbL NPs were formulated in 5% dextrose (sterilized by passing solution through 0.2 μm filter) and injected intravenously via the tail vein to dose tumor bearing or tumor free mice with 0.03 mg biosensor peptide / kg body weight. Mice were then held in specialized containers in order to collect urine. One hour after injection, urine was collected and analyzed via ELISA as described in the MMP9 cleavage study above.

Determination of in vivo gene silencing

Baseline bioluminescent signal was established for flank ovarian cancer cell models by injecting 300 μ L of 15 mg/mL D-luciferin subcutaneously. Ten minutes after D-luciferin administration, mice were imaged using an IVIS whole-animal imaging device (PerkinElmer). After the initial imaging, mice were injected systemically via the tail vein to dose 0.5 mg siRNA / kg body weight. Mice were then imaged for bioluminescent signal each day for three days following the same procedure used to establish the baseline signal. Data was analyzed using the LivingImage software to designate regions of interest over the tumors. Each tumor's luminescent signal was normalized relative to its pre-treatment baseline signal.

Biodistribution study

Metastatic ovarian cancer models were injected intraperitoneally or via the tailvein with Cy7 labeled theranostic LbL NPs formulated in 5% dextrose (sterilized by passing solution through 0.2 μ m filter) to dose 0.5 mg siRNA / kg body weight. After three days, mice were euthanized and their spleens, livers and tumors were isolated and imaged using a Li-COR Odyssey flatbed fluorescence scanner at both the 700 and 800 nm wavelengths. The tissue autofluorescence image generated by the 700 nm channel was used to identify regions of interest for quantitation using FIJI's analyze particle functionality. Fluorescence intensity in the 800 nm channel was then calculated with regions of interest and normalized by the scanned surface area.

Cryohistology

Following imaging by the Li-COR in the biodistribution study, tissues were embedded in OCT compound and flash frozen over liquid nitrogen. The cryomolds were maintained at -80 $^{\circ}$ C until

they could be sectioned by the Koch Institute's histology core facility. The slides were then imaged using a Panoramic Fluorescent Slide Scanner, using the TRITC, and Cy7 channels to detect tissue autofluorescence and nanoparticle signal, respectively.

Statistical Analysis

All statistical analysis was performed using GraphPad PRISM 7. For single comparisons, a student's t-test was used. For multiple comparison testing, one-way or two-way ANOVA was used in conjunction with the appropriate post-hoc test with $\alpha = 5\%$. Dunnett's test was used when comparing samples to a shared control group, whereas Tukey's test was used when comparing all groups. When relevant, normality tests were performed using a Shapiro-Wilk test.

Supplemental Figures

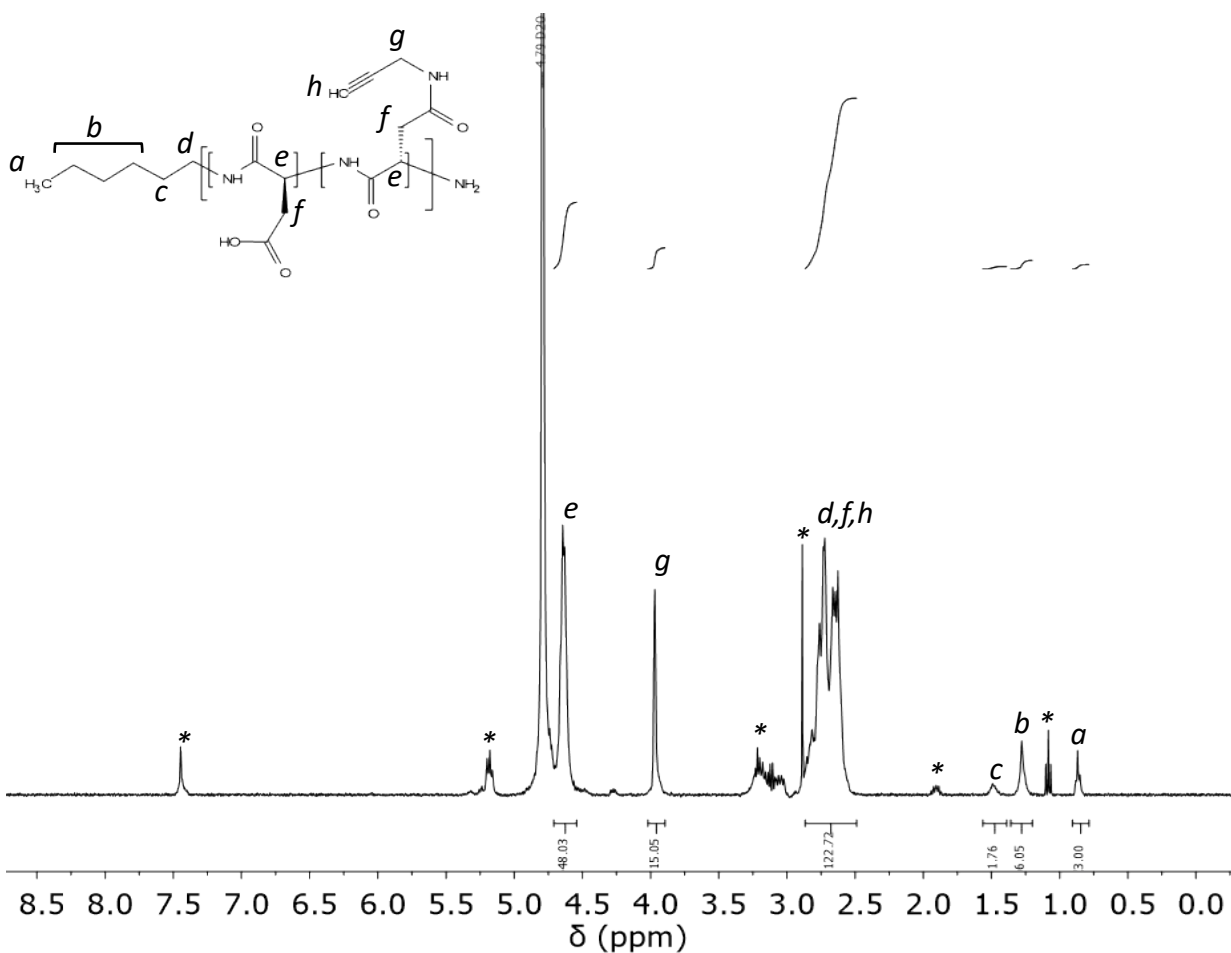


Figure S1. NMR spectrum of propargyl-modified poly-L-aspartate. Peaks from propargyl-modified poly-L-aspartate are labeled with letters. Peaks from EDC HCl and EDC urea are denoted by an asterisk. ¹H NMR (400 MHz, Deuterium Oxide) δ 4.64 (s, 46H), 3.97 (s, 15H), 2.85 – 2.50 (m, 122H), 1.49 (s, 2H), 1.28 (s, 6H), 0.86 (d, $J = 6.8$ Hz, 3H).

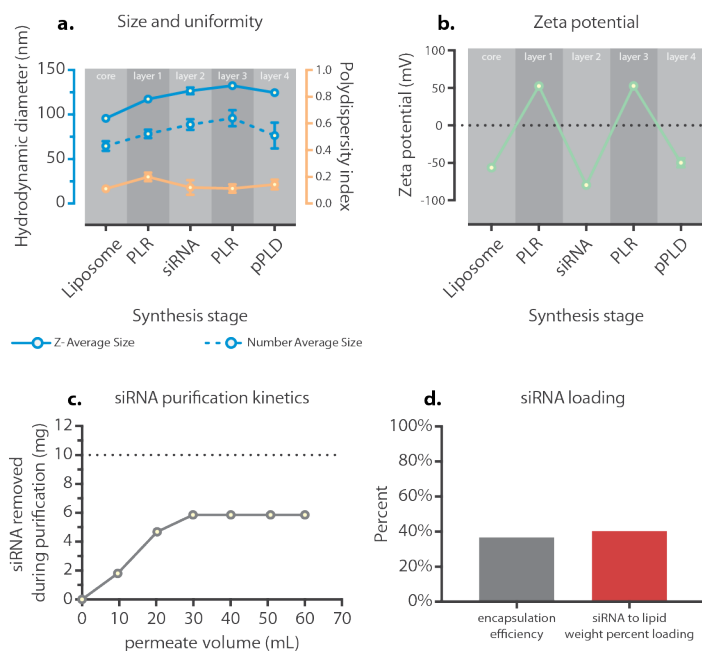


Figure S2. Click-chemistry compatible LbL nanoparticles were formed from liposomal cores, poly-L-arginine (PLR), siRNA, and propargyl-modified poly-L-aspartate (pPLD). (a) The (a) size and uniformity and (b) zeta potential of the NP was monitored during synthesis. (c) Purification kinetics of the siRNA layer were used to determine overall siRNA loading. Dashed line represents siRNA introduced into the synthesis. (d) Encapsulation efficiency of the siRNA. Error bars represent standard deviation of triplicate measurements. Where error bars are shorter than the height of the data point symbol, they are omitted.

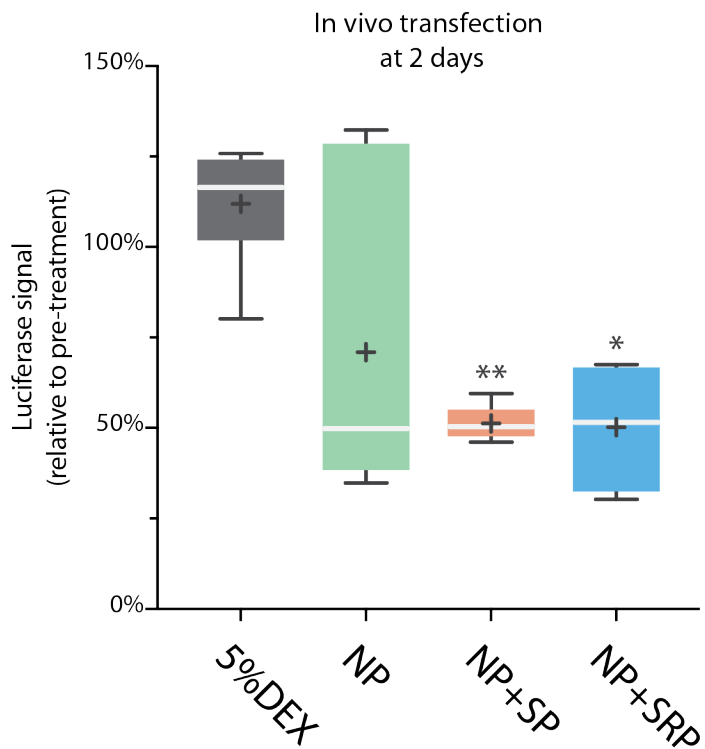


Figure S3. *In vivo* transfection of flank ovarian cancer model, two days after injection with theranostic LbL NPs. Statistical analysis was performed with a one-way ANOVA with a Tukey post-hoc test and alpha of 0.05.

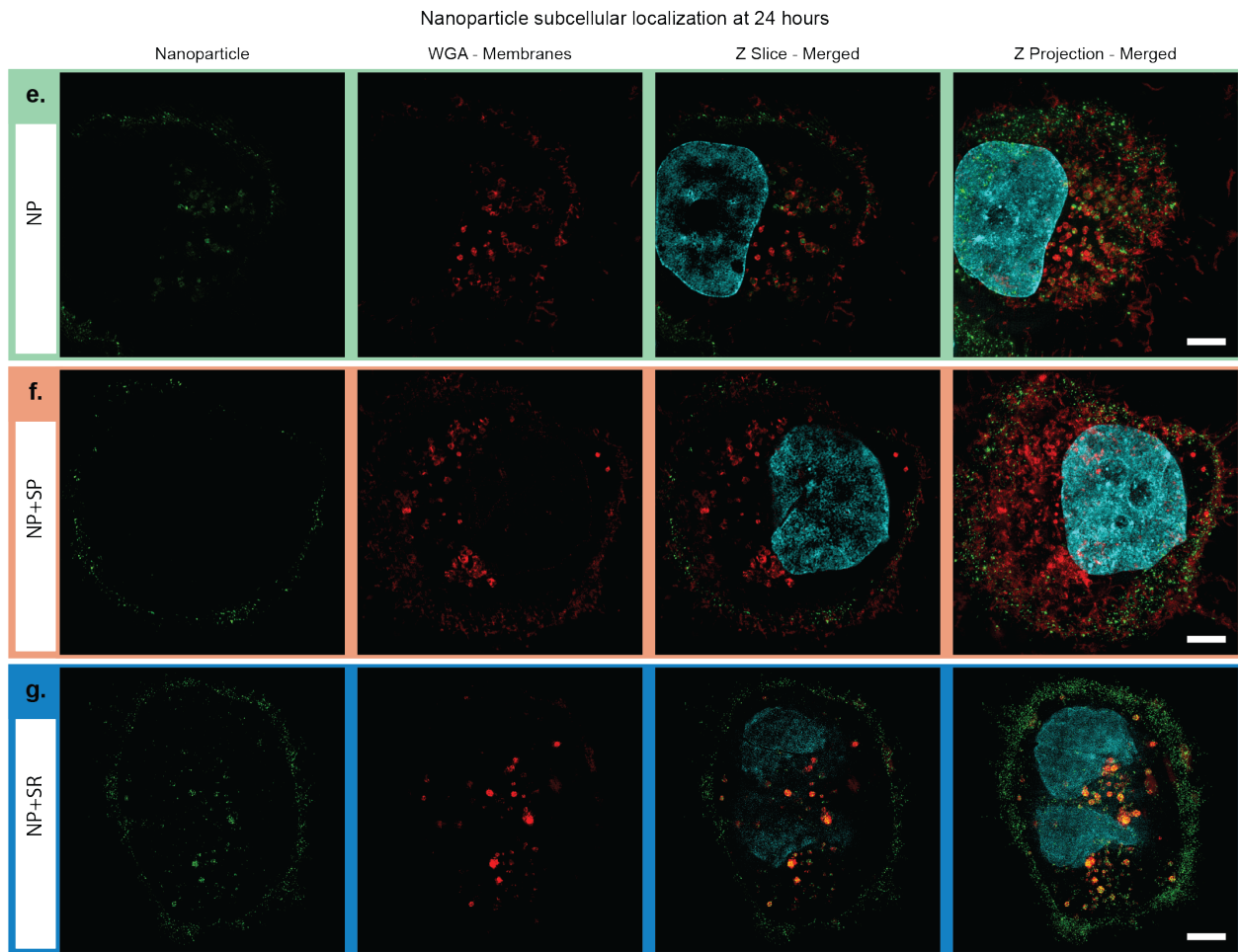


Figure S4. Single channel and merged OMX SIM images of OVCAR8 cells incubated with sulfoCy-3 labeled LbL NPs for 24 hours. Wheat germ agglutinin, shown in red, was used to stain membranes. NPs are shown in green and nuclei in cyan. Scale bar = 10 μm .

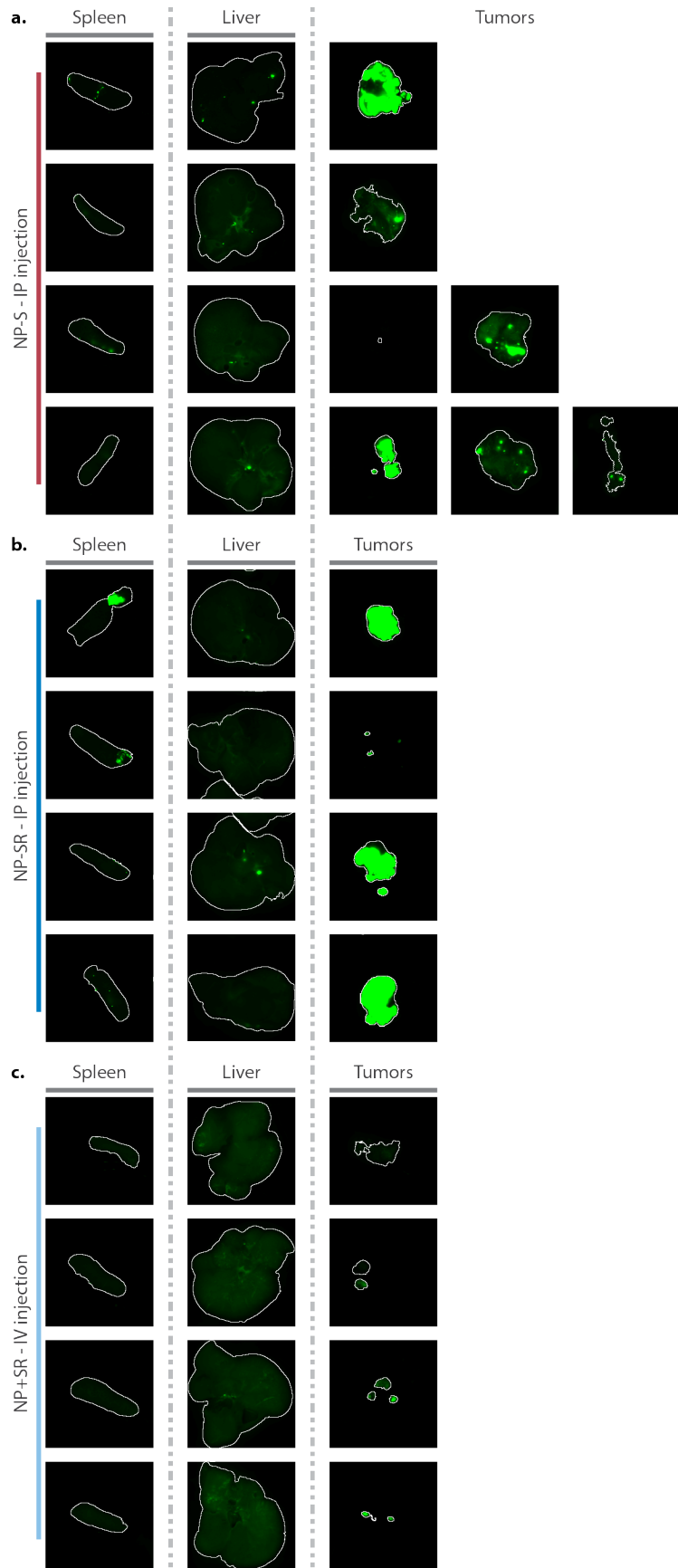


Figure S5. Spleens, livers, and tumors from mice treated with Cy7-labeled theranostic LbL NP. White outlines denote the region of interest as defined by tissue autofluorescence in the 700 nm channel. Green signal corresponds to fluorescence from LbL liposomes in the 800 nm channel.

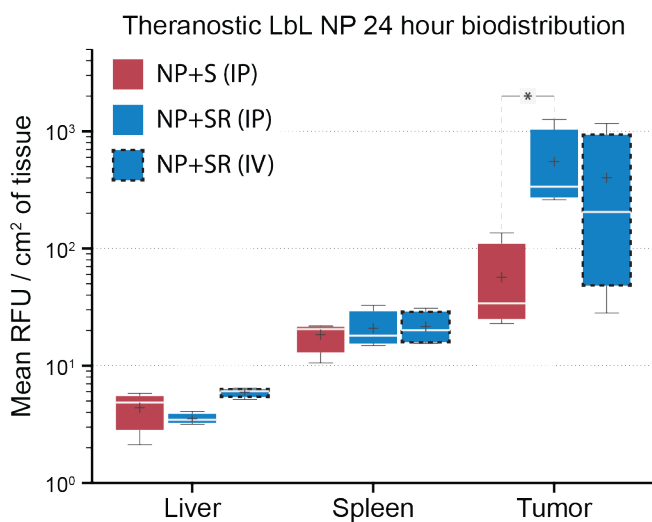


Figure S6. Quantification of NP accumulation in liver, spleen, and tumors 3 days post-administration using results from a fluorescent flatbed scanner (Li-COR Odyssey).

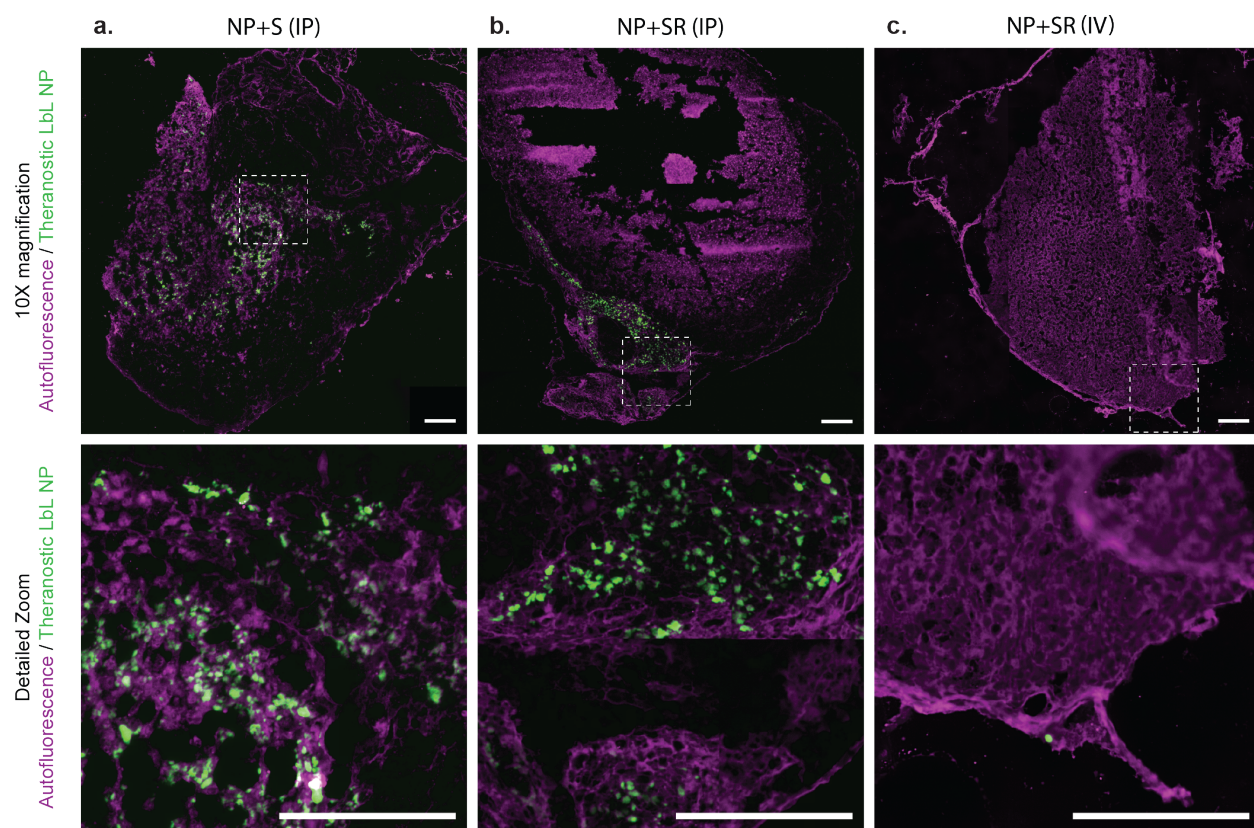


Figure S7. Low and high magnification images. Cryohistology was performed on tumor tissue and imaged using a fluorescent slide scanner to detect tissue autofluorescence (pseudo-colored magenta) and NP signal (pseudo-colored green). These representative images of LbL NP distribution through tumors when administered (a-b) IP and (c) intravenously. Scale bar = 100 μm .

Supplemental Tables

Table S1. Summary of reaction conditions for NP click functionalization. Azide-functionalized ligands were prepared in 0.1X PBS and NPs in water. The ligand solution was 10% of the final well volume, resulting in the final concentration being 0.01X PBS. Conditions resulting in particles with stable size (diameter < 300 nm) are shown in blue. Conditions resulting in particle destabilization are shown in red. Particles > 1000 nm in diameter are represented as such. If the instrument was unable to report a reliable size due to particle aggregation, this was tabulated as N/A in the table below.

[NP] (mg/mL)	Azide-functionalized peptide (mM)									
	-	[iRGD]			[Biosensor]			[1:1 combination]		
	-	0.28	0.56	1.12	0.28	0.56	1.12	0.28	0.56	1.12
1	125	152	133	223	172	N/A	118	145	162	274
0.5	125	210	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.25	127	N/A	N/A	720	499	374	393	N/A	N/A	759
0.125	125	141	208	277	N/A	937	N/A	N/A	N/A	812

Table S2. Summary of reaction conditions for NP click functionalization. Conditions resulting in particles with an acceptable PDI (< 0.3) are shown in blue. Conditions resulting in particle destabilization are shown in red. Particles that were reported as ‘multimodal’ by the instrument are reported as N/A in the table below.

[NP] (mg/mL)	Azide-functionalized peptide (mM)									
	-	[iRGD]			[Biosensor]			[1:1 combination]		
	-	0.28	0.56	1.12	0.28	0.56	1.12	0.28	0.56	1.12
1	0.24	0.24	0.24	0.36	0.10	N/A	0.24	0.24	0.24	N/A
0.5	0.24	0.24	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.25	0.26	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.125	0.24	0.20	0.24	0.28	N/A	N/A	N/A	0.46	N/A	N/A

Table S3. Family-wise error adjusted P values for NP uptake at 4 hours from Figure 3c.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CML vs. NP+SR	-1128	-1595 to -660	Yes	****	<0.0001
CML vs. NP+SRP	-995.7	-1463 to -528	Yes	****	<0.0001
CML vs. NP+SP	-864.3	-1332 to -396.7	Yes	****	<0.0001
CML vs. NP+S	-988	-1456 to -520.3	Yes	****	<0.0001
CML vs. NP	-533.2	-1001 to -65.49	Yes	*	0.0182
NP+SR vs. NP+SRP	132	-335.7 to 599.7	No	ns	0.9534
NP+SR vs. NP+SP	263.3	-204.3 to 731	No	ns	0.5345
NP+SR vs. NP+S	139.7	-328 to 607.3	No	ns	0.9413
NP+SR vs. NP	594.5	126.8 to 1062	Yes	**	0.0066
NP+SRP vs. NP+SP	131.3	-336.3 to 599	No	ns	0.9544
NP+SRP vs. NP+S	7.667	-460 to 475.3	No	ns	>0.9999
NP+SRP vs. NP	462.5	-5.179 to 930.2	No	ns	0.054
NP+SP vs. NP+S	-123.7	-591.3 to 344	No	ns	0.9645
NP+SP vs. NP	331.2	-136.5 to 798.8	No	ns	0.2885
NP+S vs. NP	454.8	-12.85 to 922.5	No	ns	0.0603

Table S4. Family-wise error adjusted P values for NP uptake at 4 hours from Figure 3d.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CML vs. NP+SR	-14606	-19056 to -10156	Yes	****	<0.0001
CML vs. NP+SRP	-11719	-16169 to -7270	Yes	****	<0.0001
CML vs. NP+SP	-5403	-9853 to -953	Yes	*	0.0104
CML vs. NP+S	-11285	-15735 to -6836	Yes	****	<0.0001
CML vs. NP	-4401	-8850 to 49.31	No	ns	0.054
NP+SR vs. NP+SRP	2887	-1563 to 7336	No	ns	0.3805
NP+SR vs. NP+SP	9203	4753 to 13653	Yes	****	<0.0001
NP+SR vs. NP+S	3321	-1129 to 7770	No	ns	0.2376
NP+SR vs. NP	10206	5756 to 14655	Yes	****	<0.0001
NP+SRP vs. NP+SP	6317	1867 to 10766	Yes	**	0.002
NP+SRP vs. NP+S	434	-4016 to 4884	No	ns	0.9997
NP+SRP vs. NP	7319	2869 to 11769	Yes	***	0.0003
NP+SP vs. NP+S	-5883	-10332 to -1433	Yes	**	0.0044
NP+SP vs. NP	1002	-3447 to 5452	No	ns	0.9823
NP+S vs. NP	6885	2435 to 11335	Yes	***	0.0007

References

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