nature materials

SUPPLEMENTARY INFORMATION

DOI: 10.1038/NMAT2986

Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents

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SUPPORTING MATERIALS CONTENTS

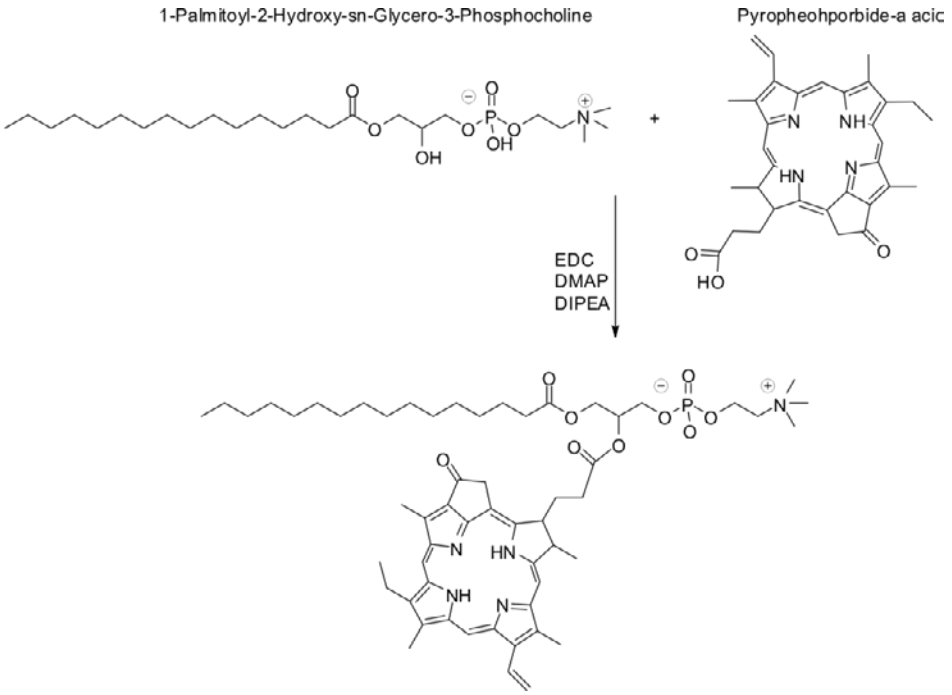
Supplementary Methods

Synthesis of pyropheophorbide-lipid

In a standard reaction, 100 nmol of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (Avanti Polar Lipids), 50 nmol pyropheophorbide (prepared from *Spirulina Pacifica* algae as described previously; Zheng *et al.*, Bioconj. Chem., 2002, *13*-392), 50 nmol of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Sigma), 25 nmol of 4-(dimethylamino) pyridine (Sigma) and 50 µL of N,N-diisopropylethylamine (Sigma) were combined in 10 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature under argon in the dark for 48 hours. The solvent was evaporated and the residue was subjected to thin layer chromatography purification (20 x 20 cm pre-coated silica gel plate with fluorescent indicator, 1.5 mm in thickness). Chloroform-methanol-glacial acetic acid-water 65:25:8:2 (volume ratio) was used as the solvent. The major band with R_f=0.4 was isolated from the plate and eluted giving a final yield of 45%. Recently, we found that improved purification could be achieved by using diol modified silica (Sorbtech) and eluting the product with 8% methanol in DCM after washing out impurities with 2% and 5% methanol in DCM. The pyropheophrobide-lipid was then dried under

DOI: 10.1038/NMAT2986

nitrogen and stored under argon at -20°C in 1 µmol aliquots. Purity (>95%) and identity (acyl-migrated regioisomer product) were confirmed with HPLC and mass spectrometry (Waters MicroMass HPLC; Phenomenex Jupiter C4 column, 0.4 mL/min flow from 25% to 95% acetonitrile over 30 minutes followed by a 10 minutes hold in 0.1 % trifluoroacetic acid, compound eluted at 32 minutes. Expected mass: 1012.3; observed mass: 1013.1).

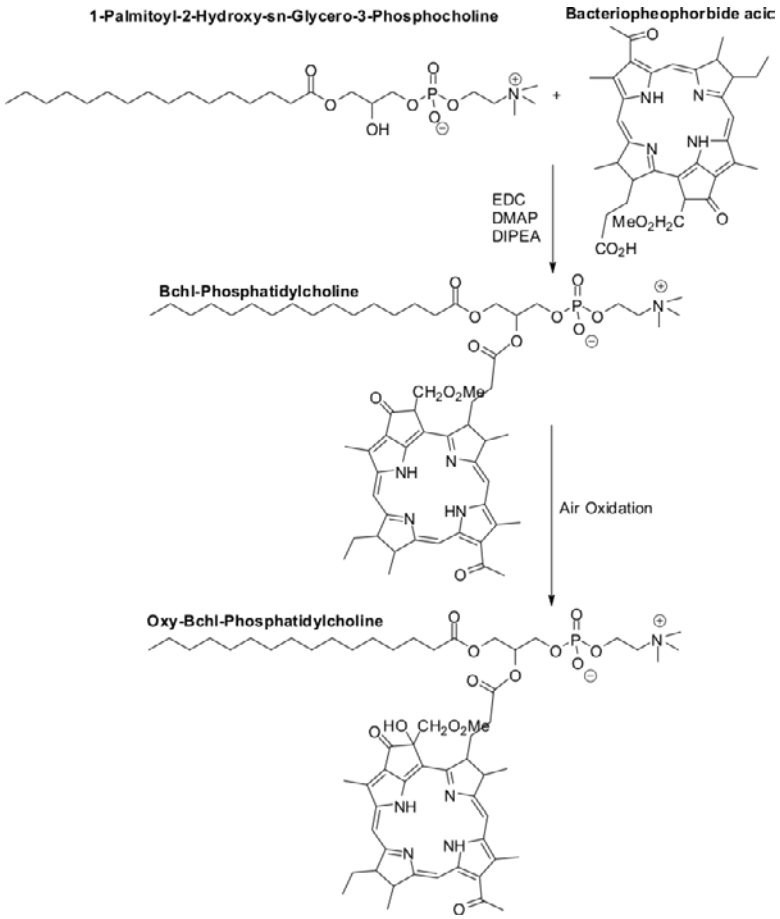


Scheme 1: Synthesis of pyropheophorbide-lipid.

Synthesis of bacteriochlorophyll-lipid

In a standard reaction, 100 nmol of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine, 50 nmol of bacteriochlorophyll acid (prepared as described previously; Kozyrev *et al*., J. Org. Chem., 2006, *71*-1949) 50 nmol of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, 25 nmol 4-(dimethylamino) pyridine and 50 µL of N,N-diisopropylethylamine were combined in to 10 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature under argon in dark for 48 hours. The solvent was

evaporated and the residue was subjected to thin layer chromatography plate purification (20 x 20 cm pre-coated silica gel TLC plate with fluorescence indicator, 1.5 mm in thickness). Chloroform-methanol-glacial acetic acid-water 65:25:8:2 (volume ratio) was used as the developing system. The final product was obtained in 38% yield with R_f=0.4. The final product spontaneously oxidized to yield an oxidized derivative of bacteriochlorophyll-lipid, which was verified by mass spectrometry and the expected structure is shown in Scheme 2. After purification, the lipid was aliquoted, dried and stored under argon at -20° C. The purity (>95%) and identity (acyl-migrated regioisomer product) were confirmed by analytical HPLC and mass spectrometry. (Same protocol as pyropheophorbide-lipid. Compound eluted at 32 minutes. Expected mass: 1104.3; observed mass: 1104.8).



Scheme 2: Synthesis of bacteriochlorophyll-lipid.

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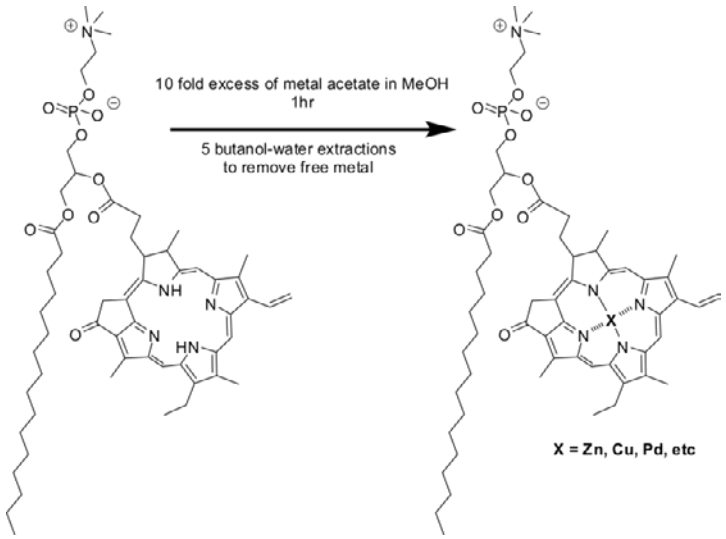
Generation of metallic pyropheophorbide-lipid

To generate porphyrin-lipid conjugates with a chelated metal, 10 fold excess free zinc acetate (Bioshop Canada) was incubated with pyro-lipid in methanol for 1 hour at room temperature under argon. The same protocol was repeated with copper acetate and palladium acetate. Free metal was removed with 5 butanol/water extractions. The metal porphyrin-lipid was then aliquoted, dried and stored under argon at -20° C. The stable metal incorporation, purity (>95%) and identity of the porphyrin lipids were confirmed by HPLC and mass spectrometry (elution time 31 min; expected mass: 1075.6; observed mass: 1075.0).



Scheme 3: Generation of metallic pyro-lipid. This approach was possible for several metals, including zinc, copper and palladium.

Formation of porphysomes

Porphyrin-lipid films were prepared in 12 mm x 75 mm borosilicate test tubes (Fisher Scientific) by combining 95 molar % porphyrin-lipid with 5 molar % distearoyl-*sn*-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol) (PEG-2000-PE, Avanti Polar Lipids) in chloroform. For folate conjugated porphysomes, 4 molar % PEG-2000-PE was supplemented with 1 molar % 1,2-distearoyl-*sn*-glycero-3-

phosphoethanolamine-N-folate(polyethylene glycol) (Folate-PEG-PE, Avanti Polar Lipids) in chloroform. Films were dried under a stream of nitrogen gas and further dried under vacuum for 1 hour. The lipid film was stored at -20° C under argon until hydration with phosphate PBS (150 mM NaCl, 10 mM phosphate, pH 7.4) and was then subjected to five freeze-thaw cycles, by freezing the test tube in liquid nitrogen and thawing it in water heated to 65° C. The porphysome suspension was extruded 15 times using a Mini-Extruder (Avanti Polar Lipids) through a 100 nm pore size polycarbonate membrane (Avanti Polar Lipids) at 65° C. Porphysomes were usually formed at 1 or 0.5 mg/mL combined porphyrin-lipid and PEG-lipid concentration. Final porphysome concentration was assessed after their extrusion by measuring the absorption of a dilute sample in methanol (Bioshop Canada) and using extinction coefficients of 97,000 $M^{-1}cm^{-1}$ at 410 nm for pyropheophorbide-lipid and 37,000 $M^{-1}cm^{-1}$ at 750 nm for bacteriochlorophyll-lipid and assuming 83,000 porphyrin-lipids per 100 nm porphysome containing 95% porphyrin-lipid[24]. Usually 1-2 µL of porphysomes were diluted in 1 mL of methanol for the measurement. Porphysomes were stored at 4° C under argon until use. For the large scale porphysomes used for *in vivo* toxicity assessment, porphyrin-lipid was combined with PEG-lipid in a 50 mL round bottom flask and the organic solvent was evaporated under reduced pressure. The flask was then hydrated with approximately 10 mL of PBS (for ~75 mg lipid) and the solution was subjected to 5 freeze-thaw cycles. Porphysomes were then formed by sonicating the flask at 55° C for 1 hour. Porphysomes were then filtered through a 0.2 µm filter (Acrodisc filter, Pall) and concentrated with a centrifugal conical tube concentrator with 100 kDa membrane pore size (Millipore). Final size (125 nm) was assessed by dynamic light scattering and concentration was determined by absorption. To form small 30 nm porphysomes, a pure porphyrin-lipid film was generated with 0.1 mg porphyrin-lipid and dried under nitrogen and vacuum. The film was rehydrated with 200 µL of water and was sonicated for 10 minutes at 55° C.

Characterization of size and shape of porphysomes

DOI: 10.1038/NMAT2986

Liposome and porphysome size was measured using a Malvern Nanosizer ZS90 (Malvern Instruments). Liposome and porphysome solutions were diluted to 6 μg/mL in PBS and three measurements were performed with 15 runs each and the results averaged. Electron microscopy specimens were prepared by incubating 0.05 mg/mL pyropheophorbide porphysomes (5% PEG-lipid, 95% pyro-lipid) on glow discharged carbon coated grids for 2 minutes, rinsing three times with milli-Q water and staining with 2% uranyl acetate. Samples were then visualized with a Tecnai F20 electron microscope (FEI Company) operating at 200 kV and images were recorded with a Tietz F114 CCD (TVIPS).

Characterization of porphysome self-quenching

Porphysomes and liposomes were formed by first creating separate stock solutions of porphyrin-lipid, egg yolk phosphatidylcholine (EYPC) and cholesterol in chloroform. Free pyrophephorbide was dissolved in methanol. These constituents were combined at the indicated molar ratios (with a constant EYPC:CHOL ratio, and increasing amounts of pyro-lipid or free pyrophephorbide) in separate test tubes. The organic solvent was then evaporated under a nitrogen stream and trace organic solvent was removed by drying the films under vacuum. The separate lipid films containing all the indicated components were then hydrated with PBS, freeze-thawed and extruded as described above. Emission spectra were recorded with a Fluoromax fluorometer (Horiba Jobin Yvon) using 2 nm slit widths. Porphysome solutions were diluted to 0.02 μg/mL in PBS and those containing free pyropheophorbide or pyropheophorbide-lipid were excited at 420 nm and emission was measured and integrated from 600 nm to 750 nm. Background subtraction of an equal concentration of 100 nm egg phosphatidyl choline:cholesterol (3:2) liposomes was performed. NBD liposomes were formed in the same manner as porphysomes, but by replacing the porphyrin-lipid with 1-palmitoyl-2-{12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]lauroyl}-sn-glycero-3-phosphocholine (Avanti Polar Lipids). NBD liposomes were excited at 470 nm and emission was measured and integrated from 500 nm to 600 nm. The fluorescence self-quenching F_{DET}/F_0 of each sample was determined by ratio of the integrated fluorescence emission in the presence or absence of 0.5% Triton X-100 (Bioshop) over four measurements from separate preparations.

Resonance light scattering of porphysomes

Pyropheophorbide porphysomes and gold nanorods (40 nm length by 15 nm width, estimated ε_{680} = 3.5×10^{9} $M^{-1}cm^{-1}$, based on Orendorff and Murphy, 2006, J. Phys Chem. B., *110*-3990) kindly provided by the Kumacheva lab, University of Toronto) were adjusted to the same absorbance at 680 nm of 0.067 in PBS. Excitation and emission were then set to the same wavelength using 1 nm slit widths and scanned from 400 nm to 700 nm. After blank subtraction, the resonance scatter of the two samples was divided. Similar results were obtained with commercial 650 nm wavelength nanorods (Nanopartz).

Differential scanning calorimetry

Differential scanning calorimetry was performed on 5 mg/mL samples of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), hydrogenated soy phosphatidyl choline (HSPC) and pyropheophorbide porphysomes using a 6100 Nano Differential Scanning Calorimeter (Calorimetry Sciences Corporation). Samples were prepared by forming 5 mg lipid films, rehydrating in 1 mL of PBS and sonicating at 60°C for 15 minutes. Samples were degassed in a vacuum for 30 minutes prior to measurement and scanned at a rate of 1°C/min. PBS was used as the reference and one heating and cooling cycle as the baseline. For each sample, three cooling and heating scans from 5°C to 95°C were performed and the results averaged to determine the phase transition temperature of the lipid.

Photothermal properties of porphysomes

5 μL drops were placed on a piece of parafilm. All solutions were measured in PBS, with liposomes and porphysomes normalized to 0.5 mg/mL concentration. Porphysomes and gold nanorods were also normalized to an optical density at 680 nm of 0.8. Samples were irradiated with a 673 nm diode laser with 150 mW output and the temperature equilibrated within 60 seconds. Surface temperature was then imaged using a temperature calibrated infrared camera (Mikroshot).

Characterization of photoacoustic properties of porphysomes

DOI: 10.1038/NMAT2986

Photoacoustic measurements were carried out using a Ti:Saphire tunable laser setup with a ultrasound transducer as previously described (see Cho *et al.*, J. Phys. Chem., 2009, *113*-9023). The light fluence was less than 7 mJ/cm^2 for photoacoustic measurement, within the ANSI limit. The axial and transverse resolutions of the system were 150 μm and 590 μm, respectively. By measuring the arrival times of generated photoacoustic signals, one-dimensional depth-resolved images (called A-lines) were acquired. Additional raster scanning along two transverse directions provided the three-dimensional images. The acquired volumetric data was processed in a form: a maximum amplitude projection - a projection of the maximum photoacoustic signal along each A-line onto the corresponding plane. Measurements were carried out at 760 nm using bacteriochlorophyll porphysomes in PBS solution. For structural dependent studies, the photoacoustic signal of porphysomes was compared to porphysomes that had been lysed with 0.5% Triton X-100.

Animal experiments were performed in compliance with Washington University guidelines. *In vivo* lymphatic mapping with porphysomes was performed using Sprague-Dawley rats (~200 g) and a 100μL injection of 9 nM bacteriochlorophyll porphysomes on the left forepaw. The region of interest was shaved prior to injection and photoacoustic measurements. After 2.5 hours, animals were sacrificed and first draining lymph node photoacoustic signal was confirmed *ex-vivo* (data not shown). Data shown is representative of 3 experiments.

Fluorescence activation of porphysomes with KB cells

KB cells were cultured in folate negative RPMI 1640 media (Invitrogen) with 10% FBS and seeded in an 8 well glass chamber (Lab-tek Chamber Coverglass, Nunc) with 30,000 cells in 200 μL media per well two days prior to imaging. Cells were incubated with pyropheophobide porphysomes (30 pM porphysome concentration) for 3 hours at 37° C in the media without serum and imaged with confocal microscopy (Olympus FluoView 1000) using 633 nm laser excitation. The porphysome containing media was not removed prior to imaging and 0.5 μL of 5 mg/mL Hoechst 33258 stain (Sigma) was added to visualize

cell nuclei using 405 nm laser excitation. Data shown is representative of over 10 experiments, and specific folate mediated uptake was also confirmed by flow cytometry (data not shown). For colocalization studies, cells were also incubated with Alexa 488 transferrin (Invitrogen) or lysotracker (Invitrogen), as well as Hoechst 33258 prior to live-cell confocal microscopy. Cell viability was assessed by incubating porphysomes overnight with KB cells in media lacking serum. 20 μL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Invitrogen, 5 mg/mL) was then added to each well and the plate was incubated with cells 1 for hour. Media was replaced with 150 μL of 70% isoproponal in 0.1 M HCl, shaken for 20 minutes and absorbance was measured at 570 nm to determine viability relative to an untreated control. Animal experiments were performed in compliance with University Health Network guidelines. $3x10^6$ KB cells were inoculated subcutaneously in nude mice and the xenograft grew for 2-3 weeks. Mice (weighing approximately 30 g) were then injected via tail vein with bacteriochlorophyll porphysomes (7.5 pmol). Imaging was performed using a Maestro imaging system (CRI) using a 710 to 760 nm bandpass excitation filter and an 800 nm longpass emission filter with 2 second exposure time. Data shown is representative of 3 experiments.

Biodegradation of porphysomes

Pyropheophorbide porphysomes (with pyropheophorbide-lipid concentration of 400 μM) were incubated with 200 U lipase (from *Rhizomucor miehei*, Sigma) for 24 hours at 37° C in PBS containing 0.5% Triton X-100 and 10 mM $CaCl_2$. The solution was then subjected to HPLC-MS analysis as described for porphyrin-lipid purification and absorption was analyzed at 400 nm. Following previously described methods[33], 100 μM pyropheophorbide was then incubated in 0.25% Triton X-100 with 25 units of horseradish peroxidase (type II, Sigma), 250 μM of hydrogen peroxide and 500 μM 2,4-dichlorophenol (Sigma), and absorption loss at 700 nm was monitored. After 1 hour, another 250 μM hydrogen peroxide was added and the reaction was monitored for another hour.

Toxicity, biodistribution and blood clearance of porphysomes

DOI: 10.1038/NMAT2986

Animal experiments were performed in accordance with University Health Network guidelines. 6 week male BALB/c mice were obtained from Charles River. Blood was sampled from the saphenous vein approximately 6 hours before porphysome or saline injection. Blood was subjected to the Mammalian Liver Profile tests (Abaxis), and MASCOT hematology profiling (Drew Scientific) according to manufacturer protocol. The total bilirubin value for the Liver Profile test was excluded since several readings gave errors. Mice were injected via tail vein with porphysomes (1000 mg/kg) or an equal volume of PBS. Over a two week period, mice were observed for behavioral changes and weight was monitored. Mice were then sacrificed, after cardiac puncture to obtain blood for analysis. Mice carcasses were placed in a 10% formalin solution and sent to Ontario Veterinary College (Guelph, Ontario) for histopathology analysis. Tissues examined included: trachea, esophagus, thyroid gland, thymus, heart, lungs, liver, kidneys, spleen, small intestine, cecum, colon, urinary bladder, prostate, seminal vesicles, testes, epididymus, skin, femur, bone marrow, skeletal muscle, head, eyes, ears, and brain.

For biodistribution, female nude mice (~23 g) bearing KB tumors were injected with porphysomes (with 5% PEG-lipid; with or without 30 molar % cholesterol) containing 100 nmol pyro-lipid (n=5 in each group). Mice were sacrificed 24 hours post-injection and organs were collected. 30 mg of tissue were weighed and homogenized in 1 mL PBS on ice for 2 minutes. Triton X-100 was added to a final concentration of 1 %, and the mixtures were vortexed for 2 minutes and then centrifuged at 13,200 rpm for 15 minutes (5415D Microcentrifuge, Eppendorf). Fluorescence of the supernatant was then measured (excitation, 410 nm; emission, 675 nm; slit width, 5nm), and the % of injected dose per gram of tissue was calculated based on a standard curve to calibrate pyro-lipid concentration.

For blood clearance, female nude mice (~20 g) were injected with regular porphysomes (95% pyro-lipid, 5% PEG-lipid) or cholesterol porphysomes (65% pyro-lipid, 5% PEG-lipid, 30% cholesterol) via tail vein based on an injection dose of 100 nmol pyro-lipid (n=4 for each group). Blood was sampled from the saphenous vein using a 25 gauge needle to puncture the vein and heparinized capillirary tubes (Fisher) to collect the blood up to 72 hours post-injection, and centrifuged at 3000 rpm (5415D Microcentrifuge,

Eppendorf) for 10 minutes to isolate plasma. The porphysome concentrations were measured based on the fluorescence (excitation, 410 nm; emission, 675 nm; slit width, 5nm). The logarithm values of plasma concentrations were plotted as a function of time, showing that it is a one compartment model. GraphPad Prism was used for data analysis for the best-fit line and half-life.

Fluorophore and drug loading of porphysomes

To encapsulate 5(6)carboxyfluorescein (Anaspec), a 1 mg porphysome film with or without 30 molar % cholesterol was hydrated with 250 mM carboxyfluorescein, 10 mM Tris pH 8 (pH was adjusted with sodium hydroxide). After freeze-thaw and extrusion, free carboxyfluorescein was removed by gel filtration using a PD-10 column (GE Healthcare) equilibrated with PBS. 300 µL fractions were collected and a 20 µL aliquot of each fraction was added to a 300 µL solution of 0.5% Triton X-100 and 10 mM Tris pH 8. Fluorescence of the fractions was then analyzed with a SpectraMax fluorometer (Molecular Devices) by measuring the porphyrin fluorescence with 415 nm excitation and 685 nm emission, and measuring the carboxyfluorescein fluorescence with 485 nm excitation and 525 nm emission. Relative carboxyfluorescein incorporation was determined by first summing the total carboxyfluorescein fluorescence in the excluded, porphysome-containing fractions. Fluorescence measurements of the different types of porphysomes were performed at the same time. Carboxyfluorescein incorporation was then determined (relative to the non-cholesterol porphysomes) by dividing the carboxyfluorescein fluorescence of the cholesterol-containing porphysomes by the non-cholesterol containing porphysomes. To incorporate doxorubicin, a 0.45 mg/mL (0.78 mM) solution of doxorubicin hydrochloride (Sigma Aldrich) with 0.078 mM NaOH was loaded into porphysomes with or without 50 molar % cholesterol. A 1 mg film was hydrated with 1 ml 155mM ammonium sulfate pH 5.5 and subject to freeze-thaw cycles and extrusion. Free ammonium sulfate was removed by gel filtration using a PD-10 column (GE Healthcare) equilibrated with PBS and the porphysome containing fractions were collected in 2 mL. A 500 µL aliquot was incubated with doxorubicin (25% of the pyro-lipid concentration) for 2 hours at 37° C. Following incubation, free doxorubicin was removed by gel filtration using a PD-10 column

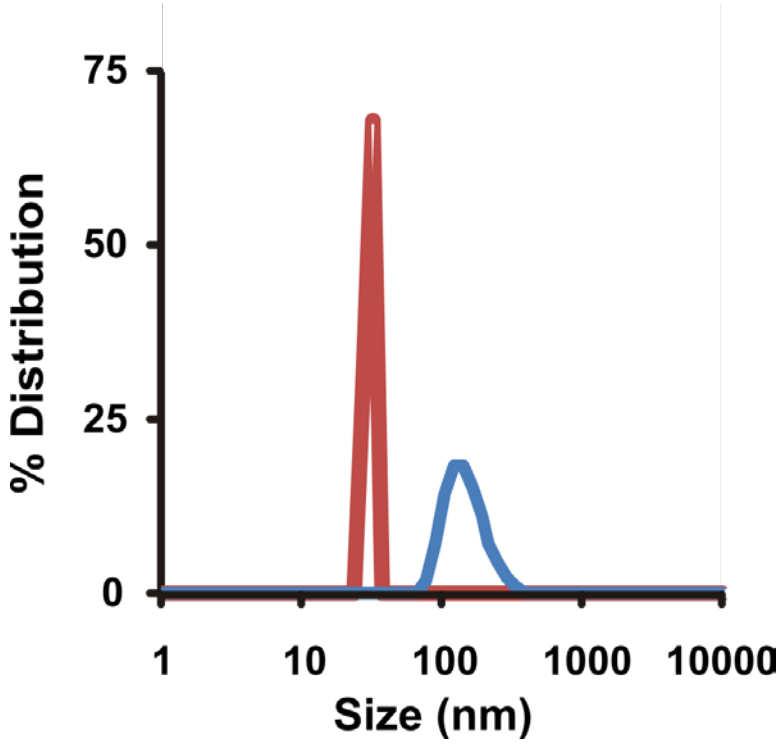
DOI: 10.1038/NMAT2986

equilibrated with PBS. 95 300µl fractions were collected and for each fraction, a 20µL aliquot was added to 280 µL 0.5% Triton X-100. Porphyrin and doxorubicin fluorescence in each fraction were then measured with a SpectraMax fluorometer (Molecular Devices) using wavelengths of 420 nm excitation and 680 nm emission for the porphyrin and 485 nm excitation and 595 nm emission for doxorubicin. Doxorubucin incorporation was determined by dividing the sum of the doxorubicin fluorescence in the excluded, porphysome-containing fractions by the sum of doxorubicin fluorescence from all collected fractions.

Photothermal therapy using porphysomes

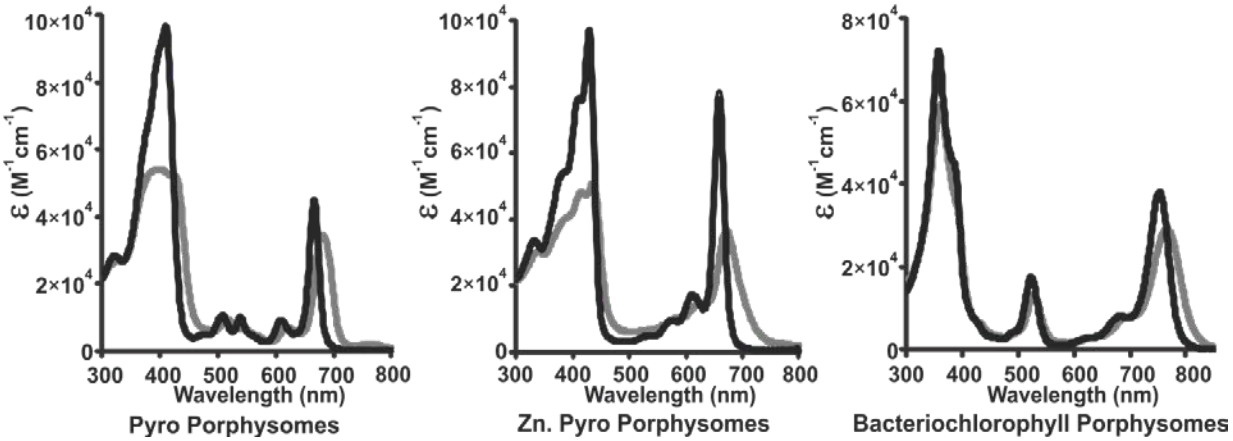
KB tumors were generated in female nude mice by injecting 2×10^6 cells into the right flank of female nude mice (~23 g). When tumor volumes reached 4-5 mm, 42 mg/kg of porphysomes containing 30 molar % cholesterol were injected via tail vein. 24 hours later, mice were anesthetized with 2% (v/v) isofluorane and tumors were irradiated with a 658 nm laser (Orion, Laserglow Technologies). Laser output at 660 nm was measured as 750 mW and the spot size was 5 mm by 8 mm. Tumor temperatures were recorded with an infrared camera (Mikroshot). For one week following treatment, all mice received enrofloxacin (0.25 mg/mL) in their drinking water. Tumor volume was measured daily and mice were sacrificed once tumor size reached 10 mm.

SUPPLEMENTARY FIGURES

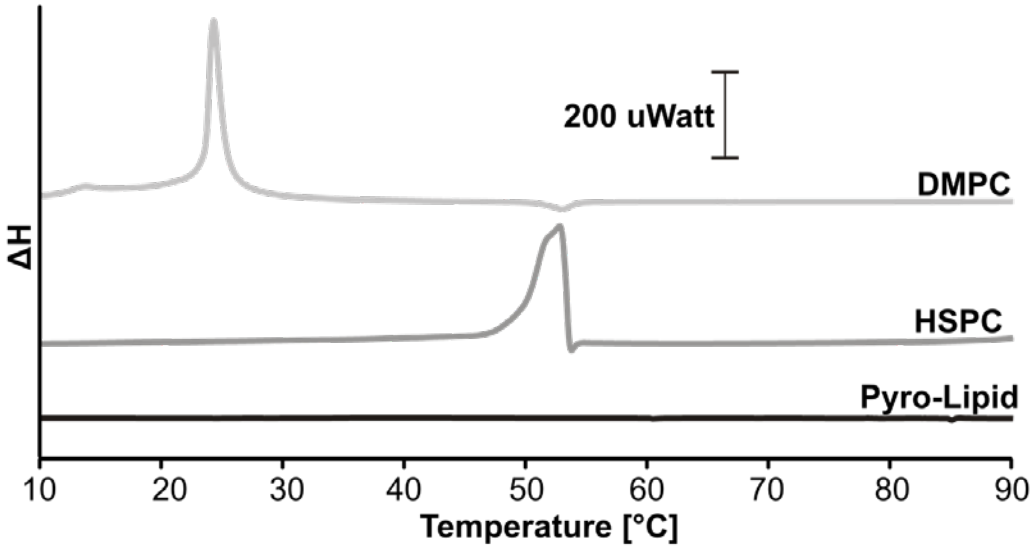


Supplementary Figure S1 Generation of 30 nm porphysomes. Dynamic light scattering measurements show that pyropheophorbide-lipid that was rehydrated and sonicated in water (red) generated small, 30 nm porphysomes. Porphysomes that were created through extrusion through a 100 nm polycarbonate membrane were larger in size (blue).

DOI: 10.1038/NMAT2986

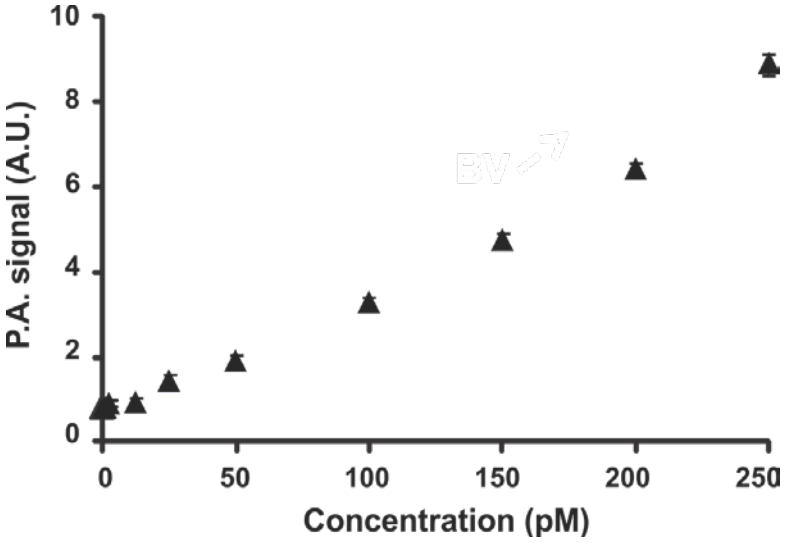


Supplementary Figure S2 Optical extinction of porphyrin-lipid subunits in organic and aqueous solvent. The absorbance is shown for the indicated porphyrin-lipid in methanol (black). For reference, the absorbance of porphysomes (composed of the porphyrin-lipid incorporated into 100 nm porphysomes measured in PBS) is also shown in gray.

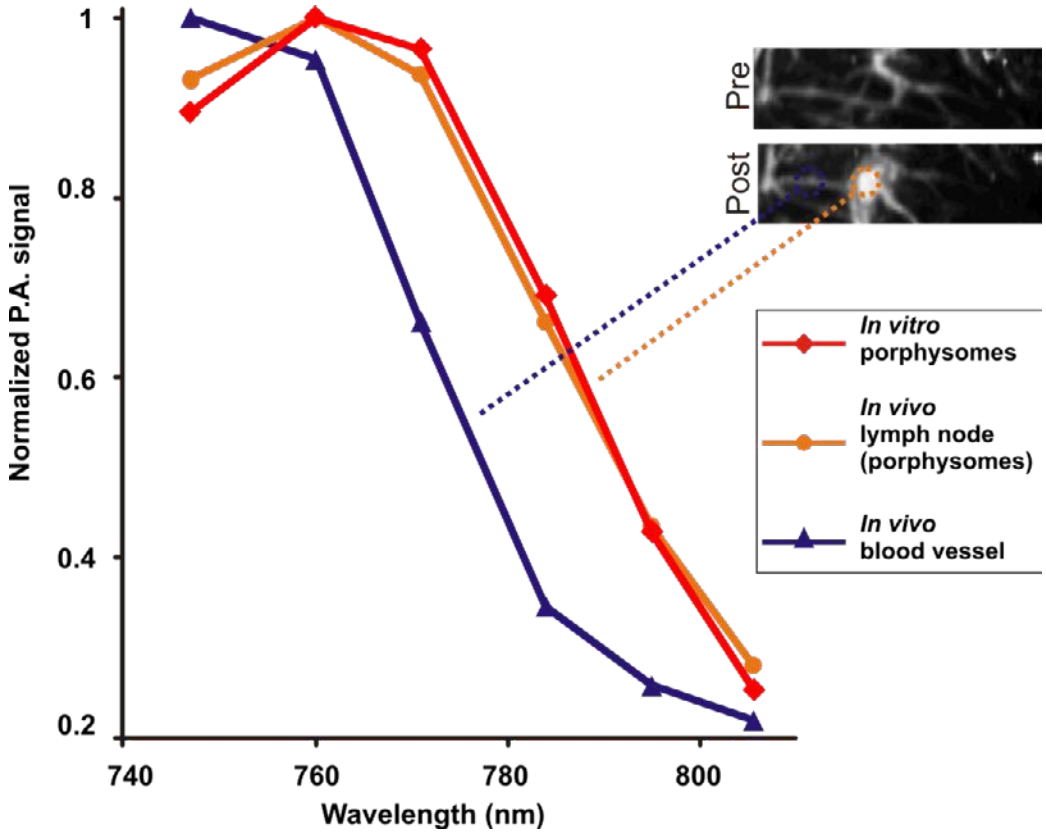


Supplementary Figure S3 Porphyrin-lipid lacks a conventional transition temperature. Differential scanning calorimetry revealed that while hydrogenated soy phosphatidyl choline and dimiristyol phosphatidyl choline have clear transition temperatures, pyropheophorbide-lipid does not. The calorimetry was performed in PBS with a lipid concentration of 5 mg/mL.

DOI: 10.1038/NMAT2986



Supplementary Figure S4 Photoacoustic detection of porphysomes. Photoacoustic transduction of bacteriochlorophyll porphysomes measured in PBS as a function of porphysome concentration (mean +/- SEM from 20 measurements).



Supplementary Figure S5 Distinct spectral responses of blood and porphysomes *in vivo.* Normalized photoacoustic response for the indicated portion of the image shown in the inset. The *in vivo* porphysomes that accumulated in the lymph node have the same spectral response as porphysomes in solution placed in tubing and measured *in vitro.*

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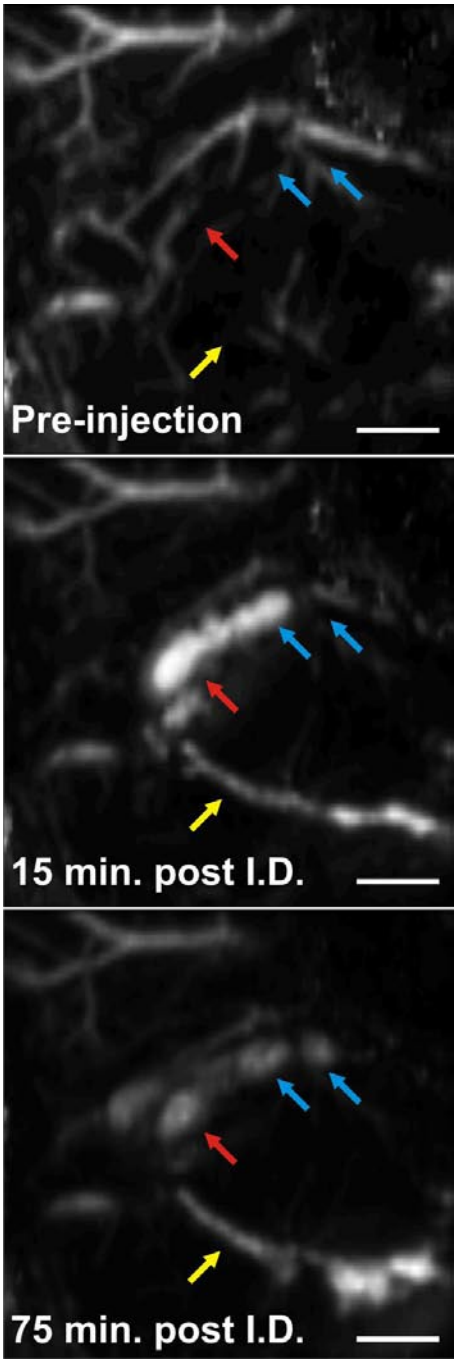
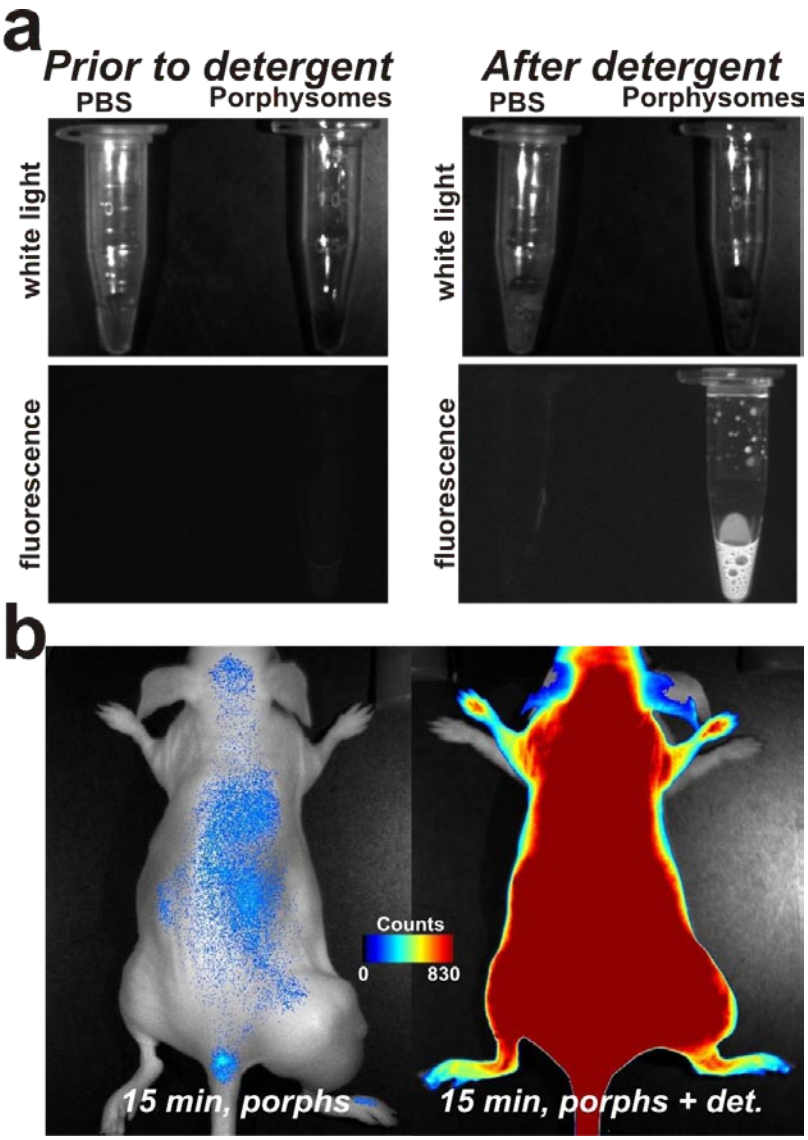


Supplementary Figure S6 Photoacoustic mapping of multiple lymph nodes using porphysomes. Secondary and tertiary lymph nodes became detectable in a rat with intradermal injection of 2.3 pmol of porphysomes. Yellow arrow indicates inflowing lymph vessel. Red and blue arrows indicate the first draining and subsequent lymph nodes, respectively. 5 mm scale bar is indicated.



Supplementary Figure S7 Low fluorescence background of porphysomes. **a,** Porphysome and saline solutions prior to and after detergent addition (10% Cremophore EL). **b,** Fluorescence imaging demonstrating the fluorescence signal that appeared in **Fig 3c-ii** after injection of porphysomes (left) or injection of the same concentration of porphysomes that had been disrupted with Cremophore EL prior to injection (right). Note the mouse shown on the right moved slightly between the white light and fluorescence imaging.

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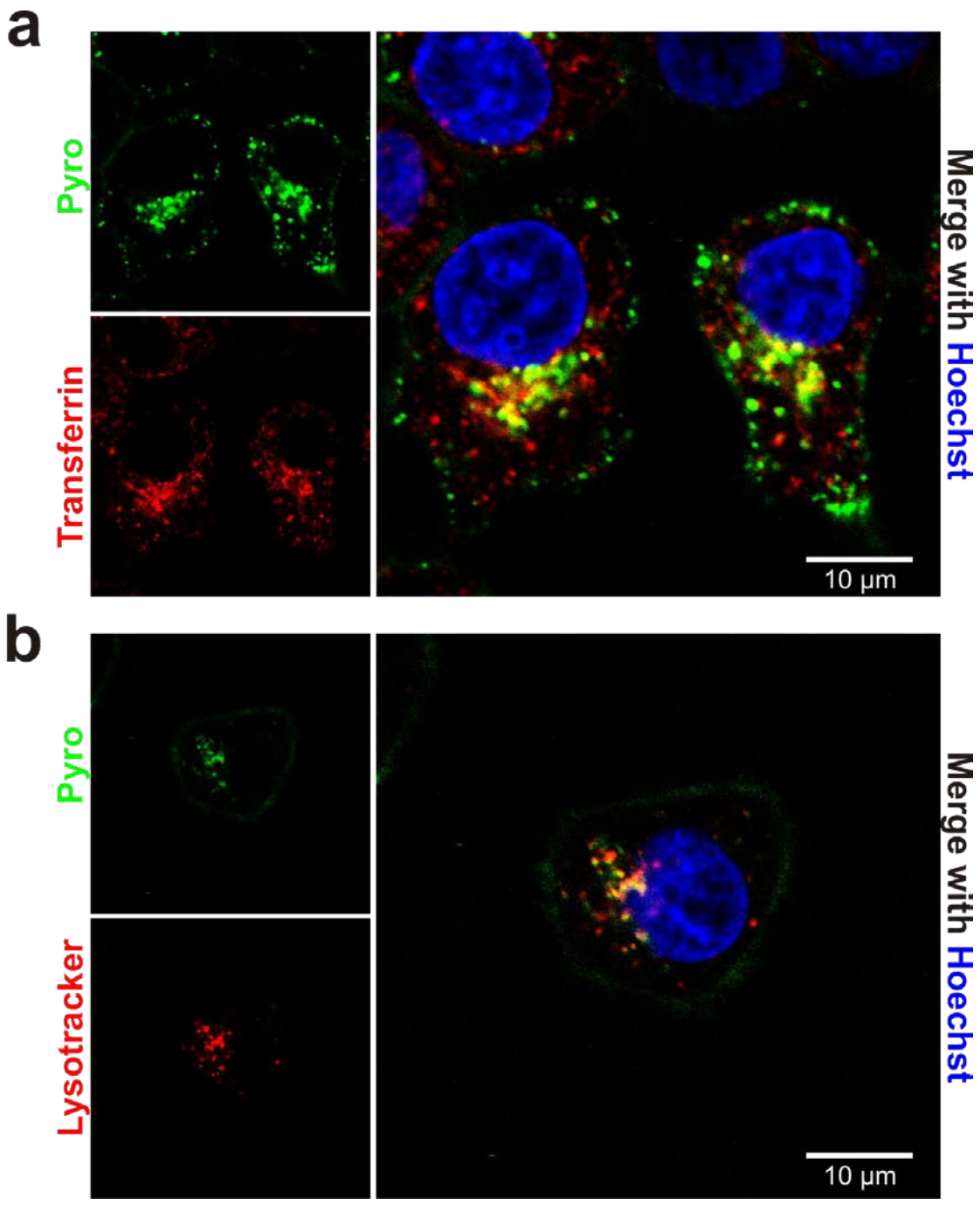
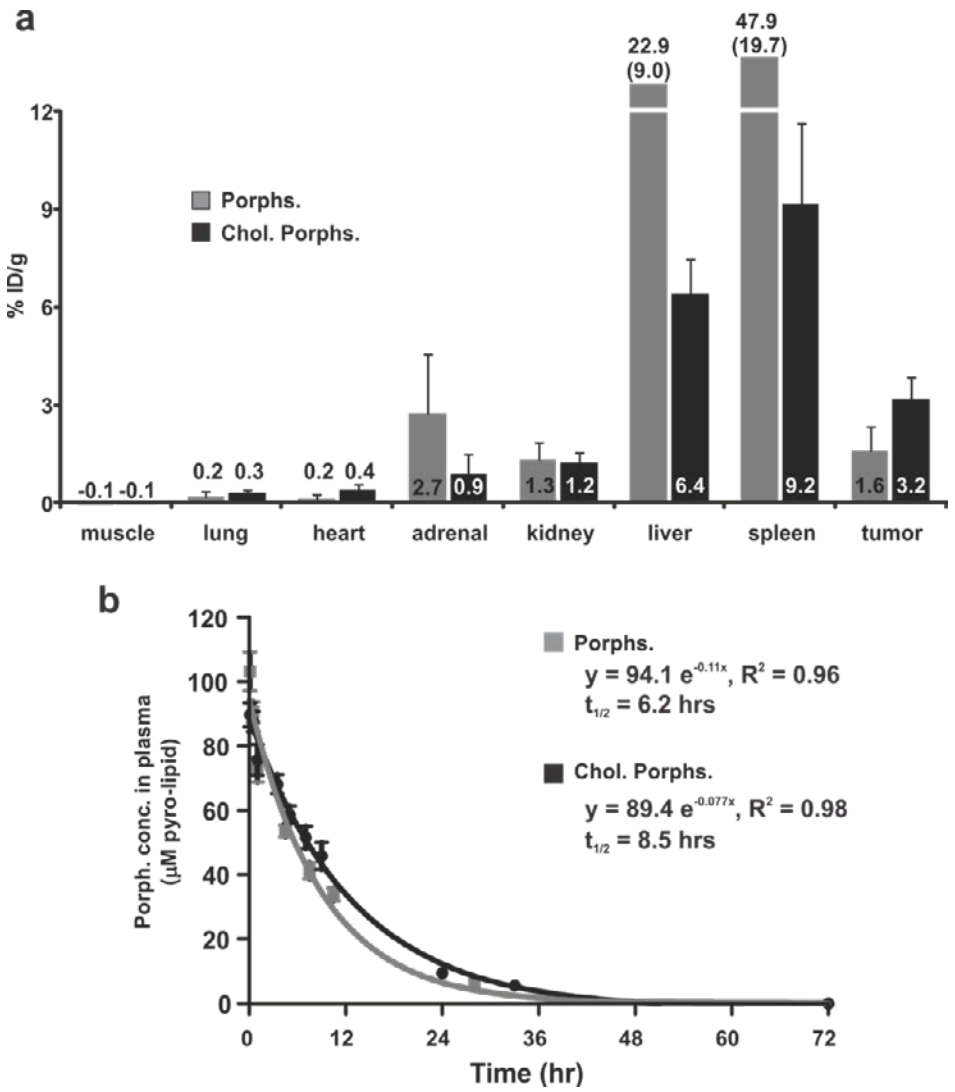


Supplementary Figure S8 Colocalization of porphysomes in early endosomes and lysosomes. **a,** KB cells were co-incubated with porphysomes containing 1 molar % folate-PEG-lipid, and Alexa 488 transferrin for 3 hours prior to live cell confocal microscopy. Channels are colored as indicated. **b,** KB cells were incubated with porphysomes containing 1 molar % folate-PEG-lipid for 3 hours, then with lysotracker for 30 minutes prior to confocal imaging. Channels are colored as indicated.



Supplementary Figure S9 Biodistribution and blood clearance of porphysomes. **a,** Biodistribution of porphysomes (95% pyro-lipid, 5% PEG-lipid) or cholesterol porphysomes (30% cholesterol, 5% PEG-lipid, 65% pyro-lipid) 24 hours following I.V. injection of porphysomes containing a total of 100 nmol pyro-lipid. Mean values are indicated on the graph and standard deviations are shown for the liver and spleen samples of regular porphysomes in brackets (mean +/- SD from 5 mice in per group). **b,** Blood clearance of porphysomes. Mice were injected intravenously with porphysomes with a total of 100 nmol pyro-lipid. 30 μL blood was collected from the saphenous vein at the indicated time points and pyro-lipid concentration was assessed by fluorescence measurements (mean +/- SD from 4 mice per group)