Supporting Information

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SI Text

Materials and Methods. ***Chemicals and instrumentation.*** Commercially available chemical reagents, solvents, and silica gel (60Å, 230–400 mesh) were used without further purification. For samples in solution, absorption spectra were recorded with a Hewlett-Packard HP8452A diode-array spectrophotometer and corrected emission spectra were obtained with an ISS PC1 steady state fluorometer. Matrix assisted laser desorption ionization-time of flight mass spectra (MALDI-TOF MS) were recorded using a PE Voyager DE-Pro MALDI-TOF mass spectrometer using α-cyano-4-hydroxycinnamic acid as the matrix. Fluorescence in cell cultures was imaged with a Zeiss LSM-510 and LSM-710 confocal fluorescence microscopes. Emission spectra of live cells were recorded with a Molecular Devices Gemini EM Microplate Spectrophotometer. Whole body fluorescence images and x-rays were acquired with a Kodak IS4000MM multimodal small animal imager.

Porphyrazine synthesis. (5R, 6R)2,3-dicyano-5,6-dimethoxy-5,6-dimethyl-1,4-diox-2-ene 1 (S1) and 4,7-bis(isopropyloxy)-1,3-diiminoisoindoline 2 (S2, S3) were prepared as described. $Mg(OPr)_2$ was prepared by adding magnesium turnings (0.04 g, 1.6×10^{-3} mol) and I_2 (0.01 g, 4×10^{-5} mol) to *n*-propanol (40 mL) and refluxing for 24 h. The solution was cooled to room temperature, 2 (0.466 g, 1.79 mmol, 4 eq) and 1 (0.100 g, 0.446 mmol, 1 eq) were added, and the reaction mixture was refluxed for 14 h. Solvent was removed en vacuo, the solid was dissolved in CH_2Cl_2 (100 mL), and filtered through celite to remove magnesium salts. The volume was reduced to 50 mL, 50 mL glacial acetic acid was added, and the reaction stirred for 24 h. Ice (25 g) was added, the solution was neutralized with 1N $NaOH_{(aq)}$, washed with H_2O (3×25 mL), dried over $MgSO_4$, and filtered. Pz separation was achieved by column chromatography on silica gel (CH_2Cl_2:EtOAc, 9:1 v/v).

Fluorescence quantum yield determination. Corrected emission spectra for quantum yield determination were recorded with a 4 nm bandwidth and photomultiplier tubes were cooled to 4 °C. Solutions were prepared to absorb less than 1 AU so as to prevent re-absorption and self-quenching. Quantum yields for the S_1 emission were determined with the relative method using cresyl violet ($\Phi_f = 0.54$, methanol) (S4) as the standard and Eq. **1** where x and r denote the sample and standard, respectively, A is the absorption at the excitation wavelength, F is the integrated fluorescence intensity, and n is the refractive index of the solvent. Cross-calibration between standards yielded less than 10% error for this method and instrumentation.

$$\Phi_x = \Phi_r(A_r/A_x)(F_x/F_r)(n_x^2/n_r^2) \qquad \textbf{[S1]}$$

Cell culture. Human mammary epithelial cells and A549 lung carcinoma cells were obtained from American Type Culture Collection (Rockville, MD). MDA-MB-231 and BT-549 cells were cultured in Alpha-medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan UT) non-essential amino acids, 1 mM sodium pyruvate and 10 mM HEPES pH 7, 2 mM L-glutamine, 50 μg/mL gentamicin and 1 μg/mL insulin (Sigma-Aldrich). MCF7 and BT-20 cells were cultured in minimal essential medium supplemented with Earl's salts and 10% FBS. T47D and A549 cells were cultured in RPMI 1640 supplemented with 10% FBS. Hs578T cells were cultured in Dulbecco's minimal essential medium supplemented with 10% FBS. Cells were grown as a monolayer in a humidified chamber of 5% CO_2 at 37 °C. Unless otherwise noted, cells were incubated with pz in this chamber and washed with PBS at room temperature.

Uptake of 247 in mammary tumor cells. MDA-MD-231, BT-549, MCF7, T47D, BT-20, and Hs578T cells were plated on sterile glass coverslips at a density of 3×10^5 cells per well in 6-well dishes containing complete media. For uptake studies, cells were incubated with 50 μM 247 for 1 h. Dosing media was removed and cells were washed once with media before incubation in fresh media for an additional 24 h before imaging. For serum-free uptake studies, cells were pre-incubated in serum-free media for 18 h before 1 h dosing with 247. For all live-cell imaging experiments cells were washed twice with 1 X PBS and the slide prepared for imaging using imaging buffer (phenol red-free media, 2 mg/mL D-glucose, 2 mg/mL BSA, 15 mM NaCl, 20 mM HEPES, 1 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$) to maintain the pH of the media (S5). Coverslips were sealed to the slides and images acquired.

Dual staining of tumor cells with 247 and organelle-specific dyes. MDA-MB-231 cells were plated as previously described. A549 cells were plated on glass coverslips and allowed to grow to ~60% confluency. Prior to incubation with organelle-specific dyes, MitoTracker, CellTracker, ER-Tracker and LysoTracker (Invitrogen) cells were incubated with 50 μM 247 for 1 h, 24 h prior to imaging as described. Prior to incubation with DAPI, A549 cells were incubated with 25 μM 247 for 24 h. Cells were then incubated with either 3 μM ER-Tracker for 30 min, 5 μM LysoTracker for 2 h, 500 nM MitoTracker for 30 min, 500 nM CellTracker for 30 min, or 300 nm DAPI for 5 min at 37 °C.

Coincubation of 247 with serum proteins LDL and BSA. For LDL colocalization with 247, MDA-MB-231 cells were coincubated with 50 μg/mL LDL-BODIPY (Invitrogen) and 50 μM 247 in complete media for 5 h prior to imaging. Cells were washed twice with 1 X PBS and prepared for imaging. For the identification of the serum component that specifies tumor localization MDA-MB-231 and BT-549 cells were incubated in serum-free media for 18 h prior to the addition of 247. Cells were then incubated with 3 μM 247 in the presence of 100 μg of BSA or LDL for 5 h. Cells were rinsed twice with PBS and live cell images acquired as described above.

Inhibition of LDL-mediated uptake of 247. The inhibition of LDL-mediated uptake of 247 into cells in complete media was performed by plating MDA-MB-231 cells on glass coverslips and incubating cells with 0 or 25 mg/mL heparin (Sigma) for 5 h in complete media. Inhibition of LDL-mediated uptake of 247 in serum-free media was performed by plating MDA-MB-231, MCF7 or HS578T cells on glass coverslips in complete media. After 24 h cells were washed and incubated in serum-free media for 18 h prior to the addition of 3 μM 247 in the presence of 0 or 100 μg of LDL or BSA in serum-free media with 5 mg/mL heparin for 5 h as indicated in the figure legends. Cells were rinsed once with serum-free media and incubated for an additional 24 h in

serum-free media supplemented with 5 mg/mL heparin before images were acquired.

LDL-BODIPY/247 FRET. FRET was achieved for the LDL-BODIPY/247 system with the LDL-BODIPY as the donor and 247 as the acceptor. LDL-BODIPY (50 μg/mL in H_2O) fluorescence was observed with 490 nm excitation. 247 was then added (50 μM) from a 5 mM DMSO stock solution. The LDL-BODIPY/247 solution was stirred for 15 min to ensure complete binding of 247 to LDL and the emission spectrum was collected. Efficiency was determined to be 87% by a comparison of the quantum yields of fluorescence of the LDL-BODIPY in the absence and presence of 247.

In vitro fluorescence spectroscopy. Fluorescence spectra of MDA-MB-231 cells were collected with 650 nm excitation with emission detected from 690–750 nm. Cells were seeded in a 96-well Special Optics Low Fluorescence Assay Plates (Corning, NY, US). Cells were stained with 25 μM 247 for 24 h before collecting emission spectra. Vehicle control cells were treated with an equivalent amount of DMSO in biological media. Pz 247 in biological media without cells was also loaded into the 96-well plate as another negative control. Fluorescence intensities were recorded at 1 nm increments.

Ex vivo tumor imaging. Following in vivo imaging, the animals were killed and tumors removed. Tumors were cut into 2 mm^3 pieces and processed for histological analysis using a PELCO (Redding, CA) Biowave tissue processor according to the manufacturer's protocols. The resulting paraffin embedded tumor blocks were cut into 4 μm thick sections, mounted on microscope slides and stained for nuclei with DAPI. Fluorescence was recorded using 543 nm excitation and LP 560 nm emission filters for RFP, 633 nm excitation and 700–1,000 nm BP emission filters for 247, and 745 nm two-photon excitation and 435–485 nm emission filters for DAPI.

In a separate experiment, after killing the animals and removing the tumors, the internal organs were removed and imaged using the same filter sets as for the whole animals [Fig. S5(*e*)].

1. Bellec N, et al. (2000) Porphyrazinediols: Synthesis, Characterization, and Complexation to Group IVB Metallocenes. *J Org Chem* 65(6):1774–1779.
2. Lee S, White AJP, Williams DJ, Barrett AGM, Hoffman BM (2001) Synthesis of Near-IR Absorbing/Emitting Porphyrazine Derivatives with Tunable Solubility. *J Org Chem* 66(2):461–465.
3. Forsyth TP, et al. (1998) A Facile and Regioselective Synthesis of *trans*-Heterofunctionalized Porphyrazine Derivatives. *J Org Chem* 63(2):331–336.
4. Magde D, Brannon JH, Cremers TL, and Olmsted J (1979) Absolute luminescence yield of cresyl violet. A standard for the red. *J Phys Chem* 83(6): 696–699.
5. Brown PS WE, Aroeti B, Chapin SJ, Moston KE, Dunn KW (2000) Definition of distinct compartments in polarized Madin-Darby canine kidney (MDCK) cells for membrane-volume sorting, polarized sorting and apical recycling. *Traffic* 1(2):124–140.

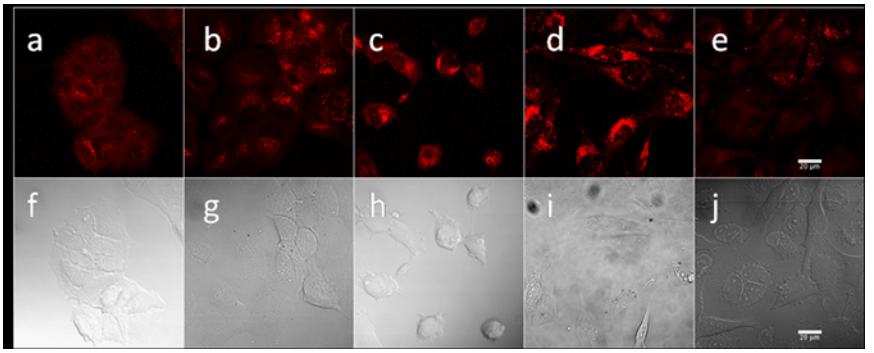

Fig. S1. Uptake of 247 in cancer cell lines. Cells were treated with 50 μM 247 for 1 h. Fluorescence and transmitted light images were acquired 24 h after removal of 247. (a, f) MCF7, (b, g) T47D, (c, h) Hs578T, (d, i) BT-549, and (e, j) HeLa cells.

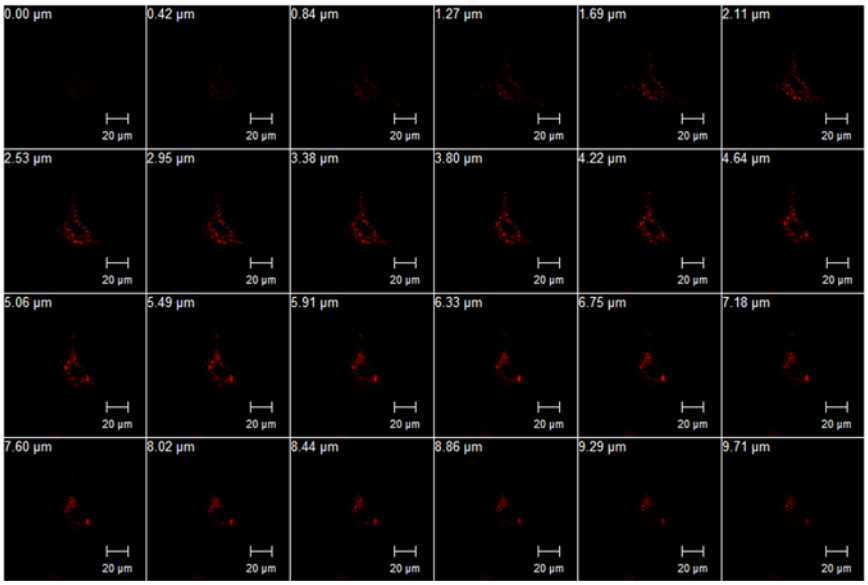

Fig. S2. Internalization of 247 (25 μM, 18 h stain) within MDA-MB-231 cells as shown by confocal z-stack images captured at 0.42 μm increments.

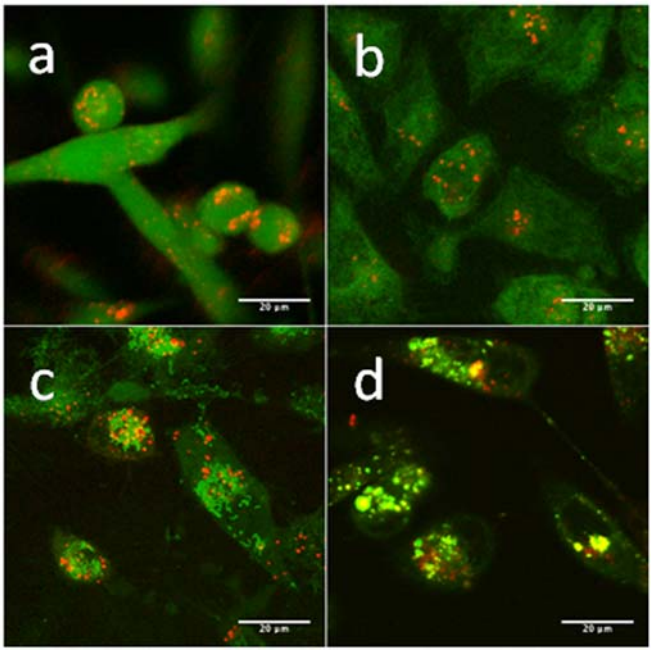

Fig. S3. Subcellular localization of 247. MDA-MB-231 cells incubated with 50 μM 247 for 1 h. Cells were also incubated with (a) Celltracker green, (b) Mitotracker green, (c) ER tracker green, and (d) Lysotracker green prior to imaging.

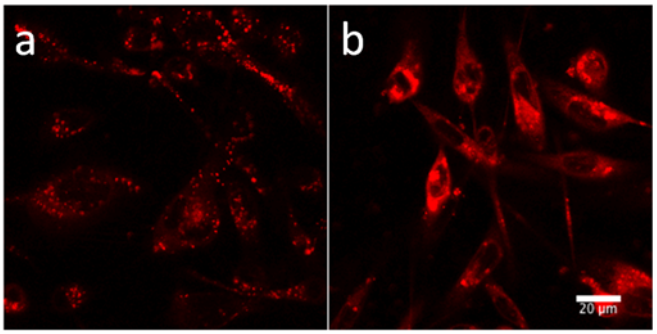

Fig. S4. Inhibition of 247 uptake by heparin in complete media. MDA-MB-231 cells incubated with 50 μM 247 in (a) complete media or (b) complete media with 25 mg/mL heparin for 1 h and visualized 24 h after removal of 247.

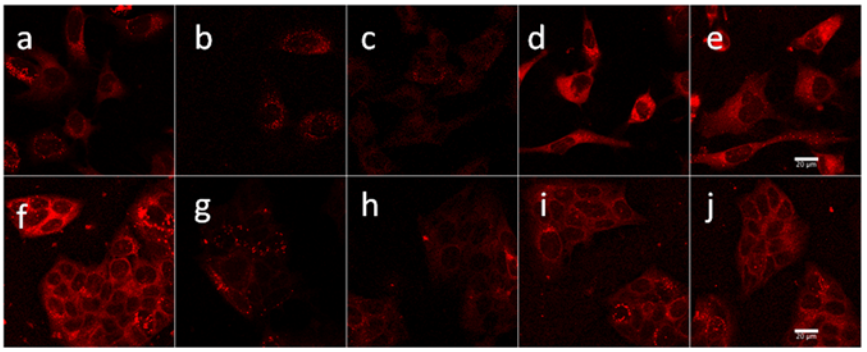

Fig. S5. Inhibition of LDL-mediated uptake of 247 by heparin. (a- e) Hs578T and (f- j) MCF7 cells incubated with 3 μM 247 in serum-deficient media (a, f) alone, (b, g) 100 μg/mL LDL, (c, h) 100 μg/mL LDL with 5 mg/mL heparin, (d, i) 100 μg/mL BSA or (e, j) 100 μg/mL BSA with 5 mg/mL heparin.

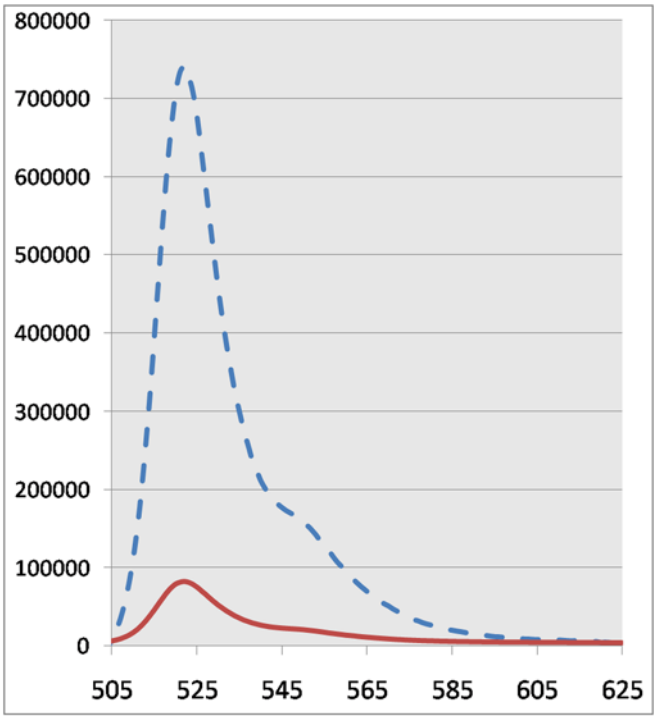

Fig. S6. FRET with LDL-BODIPY as the donor and 247 as the acceptor. LDL-BODIPY emission (dashed) and LDL-BODIPY emission in the presence of 247 (solid). Energy transfer was found to be 87% efficient.

Table S1. Effect of the local polar environment on the S_1 emission of 247

Polar environment	Δf	λ_{em}	ϕ_f
Hexane	0.00	703	0.090
Cyclohexane	0.00	704	0.087
Ethyl acetate	0.20	713	0.059
THF	0.21	718	0.024
Acetone	0.28	722	0.010
Methanol	0.31	733	0.006
PC liposomes	n.a.	710	n.a.
MDA-MB-231 cells*	n.a.	705	n.a.

The emission maximum (λ_{em}) shifts to longer wavelengths and the quantum yield of fluorescence (ϕ_f) decreases with increasing solvent polarizability $(\Delta f) \cdot \Delta f = (\epsilon - 1)/(2\epsilon + 1) - (n^2 - 1)/(2n^2 + 1)$ where ϵ and n are the dielectric constant and refractive index, respectively, for the solvent.