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Supplementary Information

An amphiphilic graft copolymer-based nanoparticle platform for reduction-responsive anticancer and antimalarial drug delivery

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EXPERIMENTAL SECTION

Materials. Methyl trifluoromethanesulfonate (≥ 98%, Sigma), 2-methyl-2-oxazoline (≥ 98%, Sigma), ε-caprolactone (≥ 99%, Sigma), potassium thioacetate (98%, Sigma), triphenylphosphine (99%, Sigma), calcium hydride (95%, Sigma), Tin(II) 2-ethylhexanoate (~95%, Sigma), Pd/C (10 wt. %, Sigma), 2-pyridylthio cysteamine hydrochloride (≥ 97%, Activate Scientific), triethylamine (≥ 99.5%, sigma), *N*,*N*'-dicyclohexylcarbodiimide (99%, sigma), 4-dimethylaminopyridine (≥ 99%, sigma), Dox.HCl (98%, Beijing Zhongshuo Pharmaceutical Technology Development Co., Ltd.), acetic acid (≥ 99.8%, Fluka), anhydrous MeOH (≥99.5%, Sigma), anhydrous EtOH (≥99.5%, Sigma), diethyl ether (≥99.9%, Sigma), ethyl acetate (≥99.8%, Sigma), dichloromethane (≥99.9%, Sigma), acetonitrile (≥99.9%, Sigma) and toluene (≥99.9%, Sigma), PBS buffer (pH=7.2, life technologies). ε-caprolactone, 2-methyl-2-oxazoline, dichloromethane, acetonitrile, and toluene were distilled from CaH_2 before use. Other chemicals were used directly without any purification.

Synthesis of poly(2-methyl-2-oxazoline) thioacetate (PMOXA-SAc). Methyl trifluoromethanesulfonate (120 mg, 0.73 mmol) and 2-methyl-2-oxazoline (5 g, 58.8 mmol) in distilled acetonitrile (30 mL) were polymerized at 80 °C for 18 h under argon atmosphere. The polymerization was terminated by addition of potassium thioacetate (0.6 g, 3.74 mmol) at RT. The solution was further continuously stirred for another 24 h under argon. Potassium thioacetate was filtered away and the product was purified by precipitation from cold diethyl ether. The obtained precipitate was dissolved in ddH_2O and was dialyzed against ddH_2O for 48 h to yield 4.6 g colorless PMOXA-SAc with a yield of 92%. ^{1}H NMR (d_6-DMSO): δ = 1.98 ppm (C***H***$_3$-(C=O)), 2.34-2.37 ppm (S(O=C)-C***H***$_3$), 2.94 ppm (-C***H***$_2$-S-), 2.99 ppm (C***H***$_3$-N-), 3.35-3.47 ppm (-C***H***$_2$-C***H***$_2$-N).

Synthesis of thiolated poly(2-methyl-2-oxazoline) (PMOXA-SH). PMOXA-SAc (200 mg, 0.03 mmol) was dissolved in anhydrous MeOH (10 mL) and triphenylphosphine (56 mg, 0.22 mmol) was added. Finally, the solution was stirred for another 48 h at RT under argon atmosphere. Afterwards, the reaction mixture was precipitated from cold diethyl ether twice, and 170 mg light pink color PMOXA-SH was obtained with a yield of 85%. ^{1}H NMR (d_6-DMSO): δ

= 1.98 ppm (C***H***$_3$-(C=O)), 2.94 ppm (-C***H***$_2$-S-), 2.99 ppm (C***H***$_3$-N-), 3.34-3.74 ppm (-C***H***$_2$-C***H***$_2$-N).

Synthesis of the poly(ε-caprolactone)-*co*-poly(α-benzyl carboxylate-ε-caprolactone) (PCL-*co*-PBCL). Anhydrous EtOH (20.5 μL) was mixed with freshly distilled toluene (25 mL), followed by addition of ε-caprolactone (4.12 g, 36.1 mmol) and α-benzyl carboxylate-ε-caprolactone (0.85 g, 3.4 mmol) which was synthesized according to the published report.[1] Subsequently, Zn(II) 2-ethylhexanoate (4 μL) was added to the above reaction mixture. Finally, the reaction was carried out at 110 °C under argon atmosphere for 24 h. After the polymerization, the polymer was precipitated twice from diethyl ether to obtain 4.1 g colorless poly(ε-caprolactone)-*co*-poly(α-benzyl carboxylate-ε-caprolactone) (PCL-*co*-PBCL) with a yield of 82%. The block ratio of PCL-*co*-PBCL was calculated by comparing integrals of signals at 5.23 ppm (methylene protons on benzyl in α-benzyl carboxylate-ε-caprolactone units), 3.33 ppm (methylene protons on ε-caprolactone units) and 1.19 ppm (protons on the end methyl group). ^{1}H NMR ($CDCl_3$): δ = 1.19 ppm (C***H***$_3$-CH_2-O-), 1.31 ppm ((C=O)-CH_2-CH_2-C***H***$_2$-CH_2-CH_2-O-), 1.56 ppm ((C=O)-CH_2-C***H***$_2$-CH_2-C*H*$_2$-CH_2-O-)1.87 ppm (C_6H_6-CH_2-(COO)-CH-C***H***$_2$-), 2.22 ppm ((C=O)-C***H***$_2$-CH_2-CH_2-CH_2-CH_2-O-), 3.33 ppm (C_6H_6-CH_2-(COO)-C***H***-CH_2-), 3.97 ppm ((C=O)-CH_2-CH_2-CH_2-CH_2-C***H***$_2$-O-), 5.23 ppm (C_6H_6-C***H***$_2$-), 7.26 ppm (C_6***H***$_6$-CH_2-).

Synthesis of the poly(ε-caprolactone)-*co*-poly(α- carboxylic acid-ε-caprolactone) (PCL-*co*-PCCL). PCL-*b*-PBCL (2 g, 0.11 mmol) was first dissolved in argon bubbled ethyl acetate (20 mL), then Pd/C (600 mg) was added. Finally, the above solution was stirred for 42 h at RT under hydrogen atmosphere. After the reaction, the sample was centrifuged to remove the catalyst Pd/C and precipitated from cold diethyl ether twice to purify the polymer. The 1.70 g colorless PCL-*b*-PCCL was obtained with a yield of 85%. ^{1}H NMR ($CDCl_3$): δ = 1.27 ppm (C***H***$_3$-CH_2-O-), 1.41 ppm ((C=O)-CH_2-CH_2-C***H***$_2$-CH_2-CH_2-O-), 1.67 ppm ((C=O)-CH_2-C***H***$_2$-CH_2-C***H***$_2$-CH_2-O-), 1.95 ppm (HOOC-CH-C***H***$_2$-), 2.34 ppm ((C=O)-C***H***$_2$-CH_2-CH_2-CH_2-CH_2-O-), 3.37 ppm (HOOC-C***H***-CH_2-), 4.07 ppm ((C=O)-CH_2-CH_2-CH_2-CH_2-C***H***$_2$-O-).

Synthesis of the poly(ε-caprolactone)-*co*-poly(α-pyridyldisulfide-ε-caprolactone) (PCL-*co*-PPCL). PCL-*co*-PCCL (500 mg, 0.028 mmol), 2-pyridylthiol cysteamine hydrochloride (60

mg, 0.27 mmol) and triethylamine (104 μL) were first dissolved in freshly distilled dichloromethane (20 mL), then *N,N*'-dicyclohexylcarbodiimide (100 mg, 0.48 mmol) and 4-dimethylaminopyridine (10 mg, 0.082 mmol) were added into the reaction medium. The reaction was carried out at RT under argon atmosphere for 60 h. The mixture was precipitated from cold diethyl ether twice, obtaining 300 mg colorless PCL-*co*-PPCL with a yield of 60%. ^{1}H NMR ($CDCl_3$): δ = 1.26 ppm (C***H***$_3$-CH_2-O-), 1.34 ppm ((C=O)-CH_2-CH_2-C***H***$_2$-CH_2-CH_2-O-), 1.65 ppm ((C=O)-CH_2-C***H***$_2$-CH_2-C*H*$_2$-CH_2-O-), 1.98 ppm (-NH-(C=O)-CH-C***H***$_2$-), 2.30 ppm ((C=O)-C***H***$_2$-CH_2-CH_2-CH_2-CH_2-O-), 2.93 ppm (-SS-C***H***$_2$-CH_2-), 3.54 ppm (-NH-(C=O)-C***H***-CH_2-), 3.65 ppm (-SS-CH_2-C***H***$_2$-), 4.05 ppm ((C=O)-CH_2-CH_2-CH_2-CH_2-C***H***$_2$-O-), 7.0-8.6 ppm (-SS-C_6***H***$_4$N).

Synthesis of poly(2-methyl-2-oxazoline)-*graft*(SS)-poly(ε-caprolactone) (PMOXA-*g*(SS)-PCL). PCL-*co*-PPCL (120 mg, 0.0067 mmol) and PMOXA-SH (290 mg, 0.038 mmol) were dissolved in dimethylformamide (10 mL), and acetic acid (200 μL) was continuously added. The solution was stirred at RT under argon atmosphere for 72 h. The copolymer was purified by precipitation from cold MeOH and finally 100 mg of a white solid product was obtained with a yield of 38%. ^{1}H NMR ($CDCl_3$): δ = 1.26 ppm (C***H***$_3$-CH_2-O-), 1.38 ppm ((C=O)-CH_2-CH_2-C***H***$_2$-CH_2-CH_2-O-), 1.64 ppm ((C=O)-CH_2-C***H***$_2$-CH_2-C***H***$_2$-CH_2-O-), 2.11 ppm (-(C=O)-C***H***$_3$), 2.30 ppm ((C=O)-C***H***$_2$-CH_2-CH_2-CH_2-CH_2-O-), 2.84 ppm (-C***H***$_2$-SS-C***H***$_2$-), 3.05 ppm (C***H***$_3$-N(C=O)-), 3.46 ppm (-N-C***H***$_2$-C***H***$_2$-N-), 4.06 ppm ((C=O)-CH_2-CH_2-CH_2-CH_2-C***H***$_2$-O-).

Synthesis of PMOXA-OH. Methyl trifluoromethanesulfonate (109 μL, 0.99 mmol) was dissolved in anhydrous acetonitrile (100 ml), then 2-methyl-2-oxazoline (10 mL) was added into the above solution. After changing argon three times, the polymerization was initiated and further carried out at 80 °C for 24 h under argon. After polymerization, the solution was cooled down to RT and a KOH/MeOH solution (0.5 M) (10 mL) was added to quench the reaction. 9 g of a colorless solid polymer were obtained by precipitation from diethyl ether with a yield of 90%.

Synthesis of PMOXA-*b*-PCL. Lyophilized PMOXA-OH (3.18 g) was first mixed with freshly distilled ε-caprolactone (4.53 mL, 41.2 mmol), then, stannous octoate (30 μL, 0.09mmol) was added to the polymerization medium. After changing argon three times, the bulk polymerization

was carried out at 110 °C for 0.5 h under argon. 6 g of a colorless solid were obtained by precipitation from diethyl ether twice with a yield of 78%. See Scheme S1 and Fig. S5.

Characterization Methods. Fourier transform infrared spectroscopy (FTIR) (PerkinElmer Spectrum 100 FTIR Spectrometer) was used to characterize the presence of specific chemical groups. Polymer samples were measured with 256 scans and 2 cm^{-1} resolution. The spectra were measured from 400 to 4000 cm^{-1}.

^1H NMR was recorded on a Bruker DPX-400 MHz spectrometer in d_6-DMSO and $CDCl_3$ with tetramethysilane as a standard. The ^1H NMR analysis was performed by using MestReNova software.

The molecular weight and polydispersity index (PDI) of PCL-*co*-PBCL and PMOXA-*g*(SS)-PCL were determined by using a Viscotek GPC max system equipped with four Agilent PL gel columns (10 μm guard; mixed C; 10 μm, 100 Å; 5 μm, 103 Å). THF was used as eluent at a flow rate of 1 mL min^{-1} at 40 °C. Signals were recorded with a refractive-index detector and calibrated against polystyrene standards (Agilent).

Transmission electron microscopy (TEM) was used to analyze the supramolecular assemblies of block copolymers. Sample solutions were deposited on a carbon-coated copper grid and negatively stained using 2% uranyl acetate solution. The samples were examined on a Philips Morgagni 268D TEM or a Philips CM100, both operated at 80 kV.

The particle size was characterized by dynamic light scattering (DLS) at a fixed angle of $\theta = 173^{o}$ using a Zetasizer Nano (Malvern, Worcestershire, UK) with a laser beam wavelength of 633 nm.

Fluorescence emission spectroscopy was performed on a Perkin Elmer, LS55.

UV-Vis absorbance was measured using a Spectramax M5e (Molecular Device) 96-well plate reader.

Fluorescence correlation spectroscopy (FCS) and confocal laser scanning microscopy (CLSM) were performed on a commercial Zeiss LSM 510 META/Confcor2. For FCS, a HeNe laser (633nm), a 40x water-immersion objective (Zeiss C/Apochromat, NA 1.2) and the appropriate filter sets were used. For CLSM, a laser diode (405 nm), argon laser (488 nm), helium/neon laser (633nm) and a 40× water-immersion objective (Zeiss C/Apochromat, NA 1.2) were used. The

cells were imaged in multitrack mode, whereas the channel for Doxorubicin was fixed to the same settings over all images. The images were processed using LSM Image Browser (Zeiss).

Fluorescence micrographs of iRBCs and RBCs were taken on a Leica DM 5000B fluorescence microscope. Images were cropped using GIMP software; no other adjustments of original images were performed.

Nanoparticle Formation and Critical Micelles Concentration (CMC). Nanoparticles of PMOXA-*g*(SS)-PCL were prepared by first dissolving 5 mg of copolymer in 300 μL DMF, then 1 mL of filtered PBS buffer was added drop wise under continuous stirring. The solution was subsequently dialyzed against PBS buffer for 24 h with changing PBS buffer solution three times.

The critical micelle concentration (CMC) values of three PMOXA-*g*(SS)-PCL samples were determined by using pyrene as the fluorescent probe. The concentration of graft copolymers was varied from $1*10^{-5}$ mg/mL to 0.5 mg/mL, while preserving the same concentration of pyrene at 0.8 μM. After mixing the graft copolymer solution with pyrene, the solution was kept in the dark at 37 °C for 12 h before measuring. Fluorescence spectra were recorded using fluorescence spectroscopy at an excitation wavelength of 330 nm. Fluorescence emission at 372 and 383 nm were monitored. The CMC was estimated as the cross-point when extrapolating the intensity ratio I_{383} / I_{372} at low and high concentration regions.

Loading of Nanoparticles Formed by PMOXA-*g*(SS)-PCL and PMOXA-*b*-PCL. DOX-loaded PMOXA-*g*(SS)-PCL nanoparticles were obtained by drop wise addition of 1 mL PBS into the mixture 0.2 mL PMOXA-*g*(SS)-PCL or PMOXA-*b*-PCL DMF solution (5 mg/mL) and 10 μl, 30 μl and 50 μl DOX in DMSO (5 mg/mL) under stirring at RT, followed by dialysis against PBS with MWCO 3500 dialysis tubes at RT in the dark and changing PBS buffer solution three times during 24 h. The amount of DOX was determined using fluorescence measurement with excitation at 480 nm and emission at 560 nm. For determination of the amount of loaded drug, the DOX loaded nanoparticle samples were first lyophilized, then dissolved in 1 mL DMSO/DMF mixture (V_{DMSO} /V_{DMF} = 2 : 1) and analyzed using fluorescence spectroscopy. The DOX standard calibration curve in DMSO/DMF solvent mixture (V_{DMSO} /V_{DMF}

= 2 : 1) was also obtained by fluorescence spectroscopy (Fig. S9). Drug loading efficiency (DLE) and drug loading content (DLC) were calculated according to the following formulas:

DLE (%) = (weight of loaded drug / weight in feed) * 100%

DLC (wt%) = (weight of loaded drug / weight of polymer+drug) * 100%

SHMT inhibitor (±)-**1**-, Bodipy630-, and NileRed were loaded into PMOXA-*g*(SS)-PCL and PMOXA-*b*-PCL by the solvent switch technique. First, PMOXA-*g*(SS)-PCL was dissolved in DMF (150 μL), and PMOXA-*b*-PCL in THF (150 μL). Subsequently, the drug or dye was dissolved in DMSO (SHMT inhibitor (±)-**1**, 10 mM, Bodipy630, 7.9 mM) or THF (NileRed, 8 mM) and added to the dissolved polymers in the desired ratio. In case of PMOXA-*g*(SS)-PCL, 0.5 mL PBS was then added dropwise under stirring. For PMOXA-*b*-PCL, the polymer-drug/dye mixture was dropwise added to 0.5 mL stirred PBS. The mixtures were transferred into dialysis tubes (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA, MWCO 300 kDa) and extensively dialyzed against 0.15 M NaCl for at least three days, while changing the solution at least eight times, and in the last step of purification dialysis was performed against PBS (Sigma). After purification, samples were sterile filtered using sterile 0.45 μm syringe filters. Loading content in SHMT inhibitor (±)-**1**-, Bodipy630-, and NileRed-loaded nanoparticles was determined using UV-Vis absorption measurements at corresponding absorbance maxima and comparing to drug/dye calibration curves in PBS (Fig. S11).

Reduction-Triggered Destabilization of PMOXA-*g*(SS)-PCL Nanoparticles. The size of the nanoparticles in response to 10 mM DTT in PBS was traced by DLS measurements. Briefly, 1 mL of a PMOXA-*g*(SS)-PCL nanoparticle solution (1 mg/mL) in PBS was transferred into a DLS measurement cell, to which concentrated DTT PBS buffer solution was added to adjust the final concentration of DTT to 10 mM. At different time intervals, the size of the nanoparticles was measured by DLS.

Reduction-Triggered Release and Medium Stability of Dye-loaded Nanoparticles Formed from PMOXA-*g*(SS)-PCL and PMOXA-*b*-PCL. Bodipy630-loaded PMOXA-*g*(SS)-PCL and PMOXA-*b*-PCL nanoparticles were prepared as described above using theoretical final dye

concentrations in the nanoparticle stock solutions of 1 μM for FCS release studies, 25 μM and 140 μM for FCS studies in cell media. For release measurements, aliquots of 20 μL nanoparticle solution (containing 1 μM Bodipy630) were added to 200 μl pre-warmed (37 °C) PBS, 11 mM DTT in PBS, or 11 mM glutathione (GSH) in 0.1 M phosphate buffer (+ 50 mM NaCl, pH 7) and subsequently incubated under shaking (500 RPM) at 37 °C in an Eppendorf Thermomixer Comfort. 5 μL of these mixtures were transferred for subsequent FCS measurements at each time point. 20 x 5 s FCS curves were recorded for each sample at each time point. Resulting FCS curves were fitted with a two-component system, whereas one diffusion parameter was fixed to free dye diffusion (typically about 50 – 60 μs). FCS curves that could not be fitted correctly by the program were excluded from the average (less than 1% of all curves). The following formula for autocorrelation curve fitting was used for one-component fits (for *e.g.* calibration measurements with free dye):

$$G(\tau)_{1\text{comp-fit}} = 1 + \frac{1}{N}\left[\frac{1}{1+\frac{\tau}{\tau_D}}\frac{1}{\sqrt{1+R^2\frac{\tau}{\tau_D}}}\right]$$

Two-component fits were used for nanoparticle stability and release measurements, with one diffusion component being fixed to free dye diffusion (typically about 50 – 60 μs) for release and stability measurements in PBS, or protein-dye complex diffusion (typically about 300 μs) for stability measurements in cell media, respectively:

$$G(\tau)_{2\text{comp-fit}} = 1 + \frac{1}{N}\left[\frac{f_1}{1+\frac{\tau}{\tau_{D1}}}\frac{1}{\sqrt{1+R^2\frac{\tau}{\tau_{D1}}}}\right] + \frac{1}{N}\left[\frac{f_2}{1+\frac{\tau}{\tau_{D2}}}\frac{1}{\sqrt{1+R^2\frac{\tau}{\tau_{D2}}}}\right]$$

with diffusion times (τ_{D1} and τ_{D2}), number of particles N, the fraction f of molecules with the corresponding diffusion time, and R, the structural parameter, which was always fixed to 5. The relation between τ_D and the x-y dimension of the confocal volume (ω_{xy}) was used for calibration and to calculate diffusion coefficients (D):

$$\tau_D = \frac{{\omega_{xy}}^2}{4D}$$

D and Einstein-Stokes equation were needed to calculate hydrodynamic radii (R_H) and diameters (D_H) using the Boltzmann's constant (k_B), the absolute temperature (T), and the viscosity of the surrounding medium (η):

$$D = \frac{k_B T}{6\pi\eta R_H}$$

Number of dye molecules per nanoparticle was calculated based on the molecular brightness (counts per molecule, CPM in kHz) when only one component was found (medium stability measurements):

$$\frac{\#\text{dye}}{\text{nanoparticle}} = \frac{\text{CPM}_{\text{nanoparticle}}}{\text{CPM}_{\text{dye}}}$$

Percentage of free dye is presented as average ± SEM from three independent measurements using two independent samples for each copolymer. Hydrodynamic diameters (D_H) were calculated using Einstein-Stokes equation; diffusion constants obtained for the nanoparticles and free Atto655 in PBS as a calibration for the confocal volume.

For FCS measurements in cell medium, the following four representative dye-loaded nanoparticle stock solutions were used: PMOXA-*g*(SS)-PCL 2 (2.5 μM Bodipy630), PMOXA-*g*(SS)-PCL 3 (56 μM Bodipy630) and PMOXA-*b*-PCL (2.5 μM, and 133 μM Bodipy630), with the following dilutions in the corresponding pre-warmed (37 °C) cell culture medium: 1 to 10 (2.5 μM stocks), and 1 to 50 (56 μM, 133 μM stocks). Samples were kept under constant shaking (500 RPM) at 37 °C in an Eppendorf Thermomixer Comfort for four days. At each time point, 5 μl of these mixtures were transferred for subsequent FCS measurements of 30 x 5 s. Each curve was fitted using a two-component model with one component being fixed to protein-dye complex (typically about 300 μs) obtained from measurements of a Bodipy630 solution in cell culture media. Few curves with bright and slow diffusing aggregates were excluded from the average (< 1% of all curves), due to their disproportional effect on the average.[2] Average values for 30 curves at each time point and corresponding standard deviation (SD) are presented. R Statistics and Excel were used for preparing graphs.

Cancer Cell Culture. HeLa cells were grown in 75 cm^2 cell culture flasks (BD Bioscience) at 37 °C under a 5% CO_2 atmosphere in the cell culture medium (10 % FBS, normal cell grow conditions). When cells reached a confluency of about 80%, they were split in a 1 : 10 ratio. To prepare the cell culture medium 50 ml fetal bovine serum (FBS, BioConcept AG), 10 ml 10'000 U/ml penicillin and 10'000 µg/ml streptomycin (Gibco) were filled up to 500 ml with fresh Dulbecco's modified eagle's medium (DMEM) (Sigma). The medium was sterile filtered through a 0.2 µm vacuum filter (Millipore). The cells were subcultured by trypsinization.

Preparation of Cancer Cells for Live Cell Imaging. For live images 50'000 HeLa cells were seeded into each 8-well microscope chamber slides (Nunc) for 24 h in a normal cell culture conditions. Before adding the samples to the cells, old medium was removed; new medium was added to such an amount that in the end the total volume with sample resulted to be 100 µl. After 1 h, 2 h, 4 h or 8 h incubation under normal cell growing conditions cells were washed with PBS and 400 µl DMEM was added. To stain nucleus and cell membrane, cells were incubated with 0.2 µg/ml Hoechst 33342 (Invitrogen) for 25 min and 2.5 µg/ml CellMask Deep Red (Invitrogen) for 5 min. Subsequently, cells were washed with PBS and wells were filled up with 400 µl PBS.

MTS assay. Into each vial of a 96-well plate (Falcon) 10'000 cells were seeded, filled up to 100 µl with cell culture medium and incubated for 24 h under normal cell grow conditions. Prior to addition of the sample, the old medium was removed, DOX-loaded PMOXA-*g*(SS)-PCL nanoparticles, DOX-loaded PMOXA-*b*-PCL nanoparticles, free DOX or PBS were added to a volume of 20 µl and filled up to 100 µl with cell growing medium. The samples had a final DOX concentration of 0.9 μg/ml, 2.7 μg/ml, 4.5 μg/ml and 9 μg/ml, or blank PMOXA-*g*(SS)-PCL nanoparticles. As a control, 20 µl PBS was added and filled up to 100 µl with cell growing medium and the blank measurement was prepared the same but without cells. The cells were then incubated for 48 h at normal cell growing conditions. Subsequently, 10 µl of MTS (Promega) was added to each well and incubated for another 4 h. The absorbance at 490 nm was measured with a plate reader Spectramax M5e (Molecular Device). To calculate the cell viability the average value from the blank was subtracted from the measured sample values and normalized to the average value of cells only. Data are presented as average ± SD (n = 8).

Malaria Parasite Culturing and Growth Inhibition Assays. Malaria parasites (*Plasmodium falciparum* 3D7 and NF54 strain) were maintained in culture using human erythrocytes as host cells and malaria culture medium (MCM) consisting of RPMI 1640 medium completed with 0.5 % Albumax, 25 mM Hepes, 25 mM $NaHCO_3$ buffer (pH = 7.3), 0.36 mM hypoxanthine, and 100 μg/mL neomycin as described elsewhere.[3,4]

Four-day suspension inhibition assay and three-day static inhibition assay were performed using established assays.[5-7] 10 mM SHMT inhibitor (±)-**1** stock in DMSO and SHMT inhibitor (±)-**1**-loaded nanoparticle samples in PBS were diluted in PBS immediately before use in the assays. Briefly, four-day suspension assays were conducted in 24-well plates under continuous agitation at 37 °C. Hematocrit was 5 % and starting parasitemia (% of iRBCs, 3D7 strain) was about 0.1 %. After 96 hours, control wells (PBS added) reached about 2 - 4 % iRBCs as measured by flow cytometry (FACSCalibur, BD Biosciences). For each well, 100'000 cells were counted and parasitemia was compared to PBS control wells to obtain mean growth ± standard error (SEM). Each sample was tested in at least three independent assays in duplicates. Statistical comparison was performed using Student's *t*-test (two-tailed, type 2). Graphs were prepared using QtiPlot (http://soft.proindependent.com/qtiplot.html). To obtain IC_{50} values, experimental growth inhibition curves were fitted using logistic fits in QtiPlot with fixed maximum value A1 = 100.

Three-day static assays were performed in 96-well plates using 1.25 % hematocrit and 0.3 % parasitemia (NF54 strain) at the beginning. After 48 h, 0.25 μCi [^{3}H]hypoxanthine was added to each well, and the assay was continued for an additional 24 h. After a total of three days incubation, parasites were harvested onto glass-fiber filters, and radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich). The results were recorded and expressed as a percentage of the untreated controls. Fifty percent inhibitory concentrations (IC_{50}) were estimated by linear interpolation.[8]

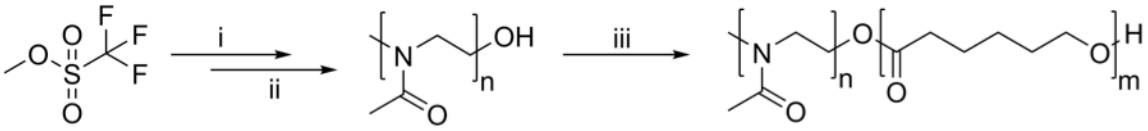
One-day suspension inhibition assay was performed and evaluated similarly to the four-day suspension assay but using 10 % hematocrit and 1 % starting parasitemia with late stage parasites (3D7 strain). IC_{50} values had to be baseline-corrected in this case, because dead parasites remained within the culture for this short one-day incubation periods and therefore still appeared in the flow cytometry measurements. Thin blood smears revealed only dead late stages and no ring stage parasites at high drug concentration after one-day incubation, which confirms

that the slightly increased baseline was due to remaining dead parasites (iRBCs) and not due to incomplete drug action.

Preparation of Malaria Parasite Cultures for Imaging. 0.36 ml malaria parasite culture (4% parasitemia, late stages, 5% hematocrit) in MCM was transferred to Eppendorf tubes and 40 μl of 25 μM NileRed solution in PBS (freshly prepared from a 10 mM stock in THF and immediately added), 25 μM NileRed in PMOXA-*g*(SS)-PCL 3 nanoparticles, and 25 μM NileRed in PMOXA-*b*-PCL nanoparticles. The gas in the tubes was exchanged by the culturing gas mixture and then incubated under shaking conditions at 37 °C for 1.5 h. Then, 1 μl of 1 mg/ml Hoechst DNA-stain was added and incubation was continued under static condition for 0.5 h. After centrifugation, 5 μl of the pellet was mounted on a microscopy slide and images were taken using a 60x oil immersion objective. First, the appropriate filters and exposure times were adjusted for the free dye sample. Then, parasites were searched in DIC mode and automatic overlays were taken for each sample using the exact same setting in order to have comparable brightness data.

SUPPORTING SCHEMES, FIG.S, TABLES

Scheme S1. The synthetic route for PMOXA-*b*-PCL.[a]



[a] Reagents and conditions: (i) methyl trifluoromethanesulfonate, 2-methyl-2-oxazoline, acetonitrile, 80 °C for 24 h under argon; (ii) KOH MeOH solution (0.5 M), RT for 24 h; (iii) ε-caprolactone, Zn(II) 2-ethylhexanoate, 110 °C for 0.5 h under argon.

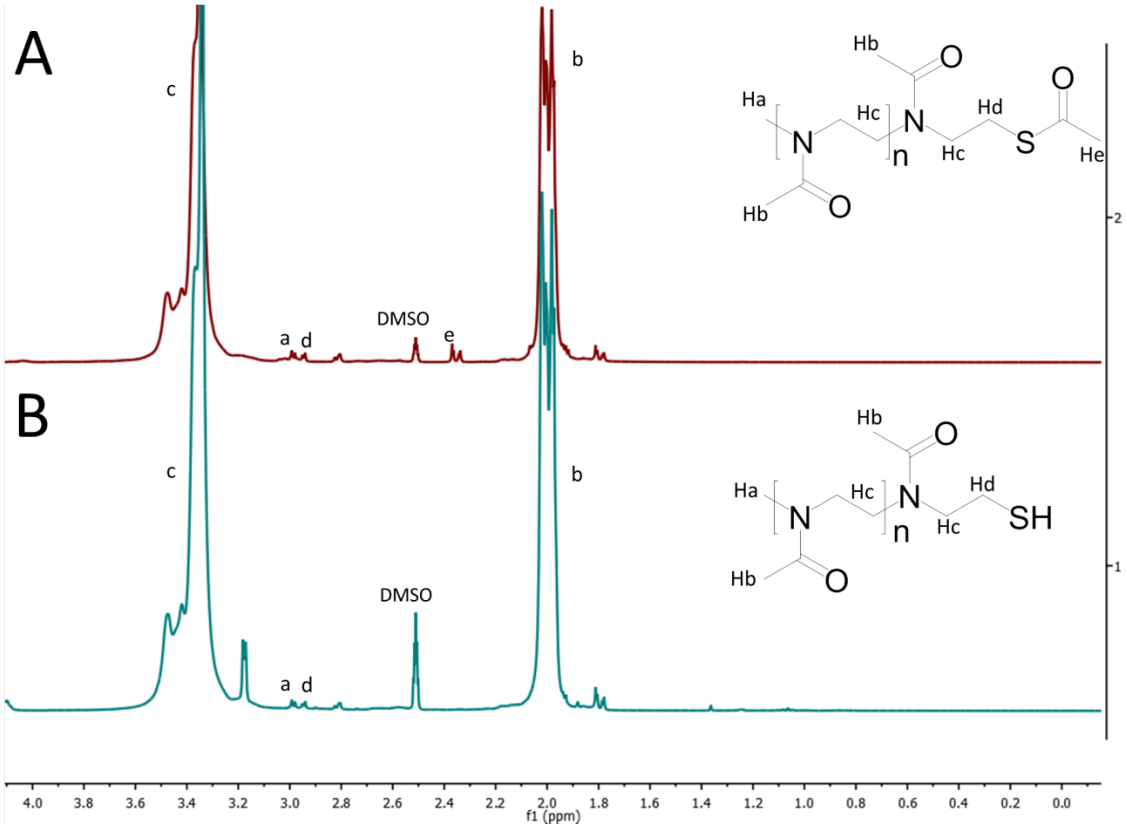


Fig. S1 ^{1}H NMR spectrum of PMOXA-SAc (A) and PMOXA-SH (B) in d_6-DMSO.

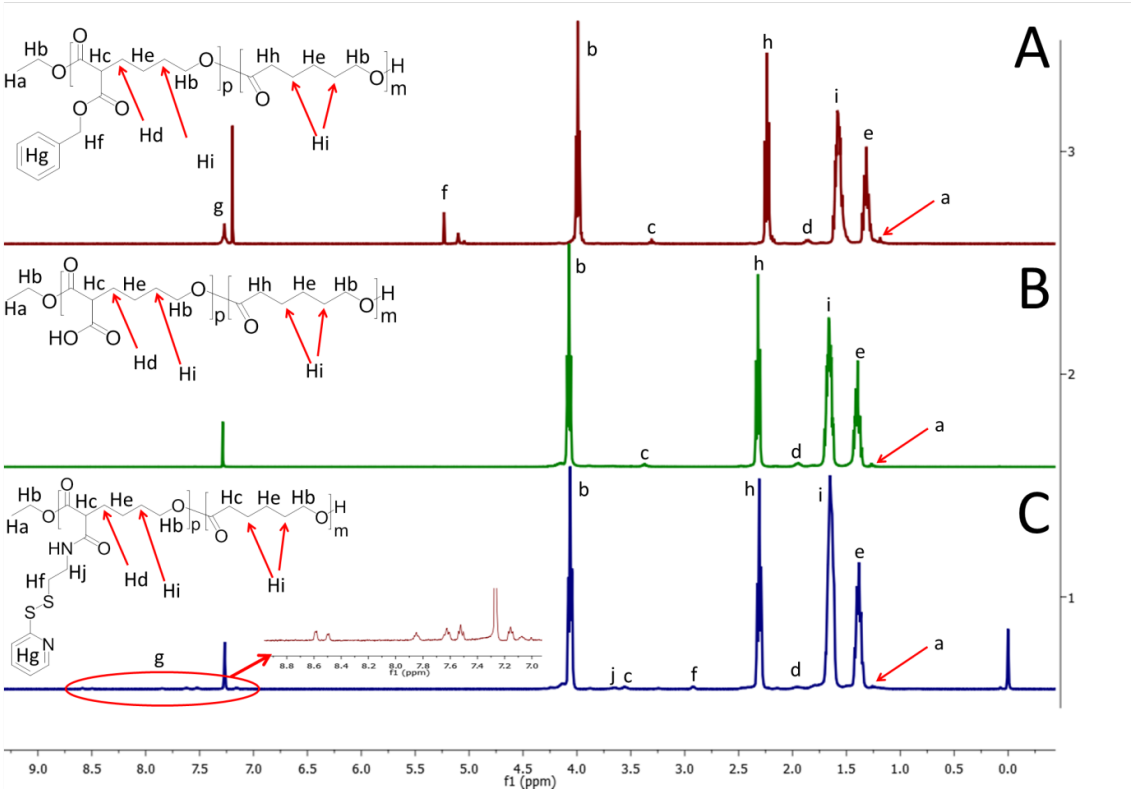


Fig. S2 ^{1}H NMR spectrums of PCL-*co*-PBCL (A), PCL-*co*-PCCL (B) and PCL-*co*-PPCL (C) in $CDCl_3$.

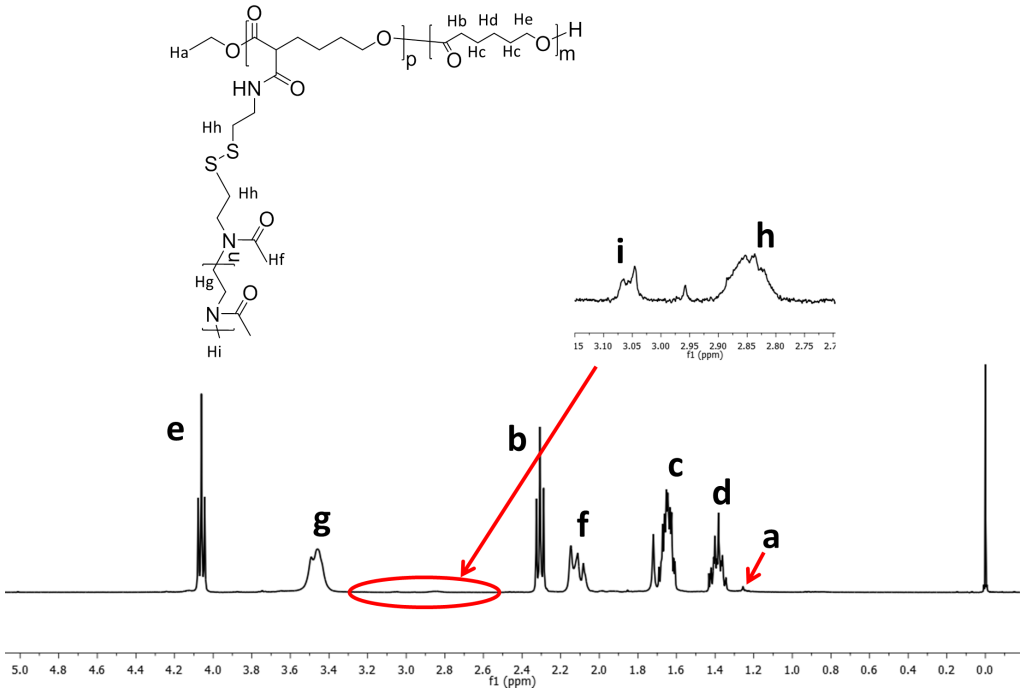


Fig. S3 The ^{1}H NMR spectrum of PMOXA-*g*(SS)-PCL copolymer in $CDCl_3$.

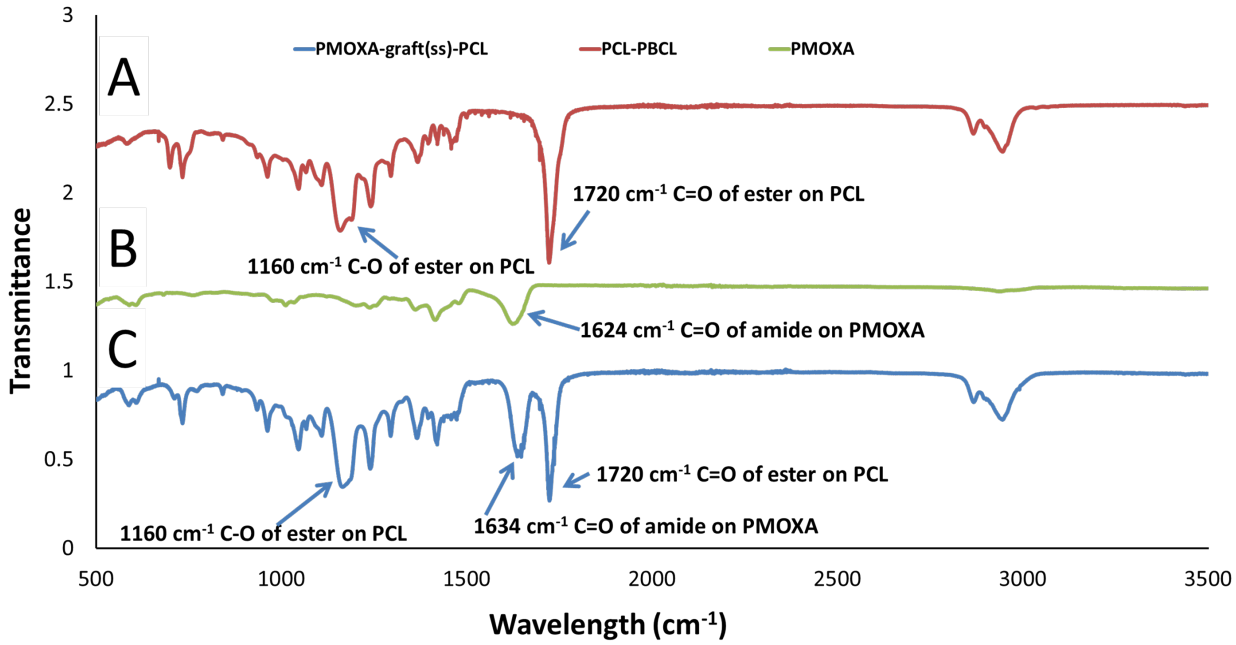


Fig. S4 The FTIR spectra of PCL-*co*-PBCL (A), PMOXA-SH (B) and PMOXA-*g*(SS)-PCL (C).

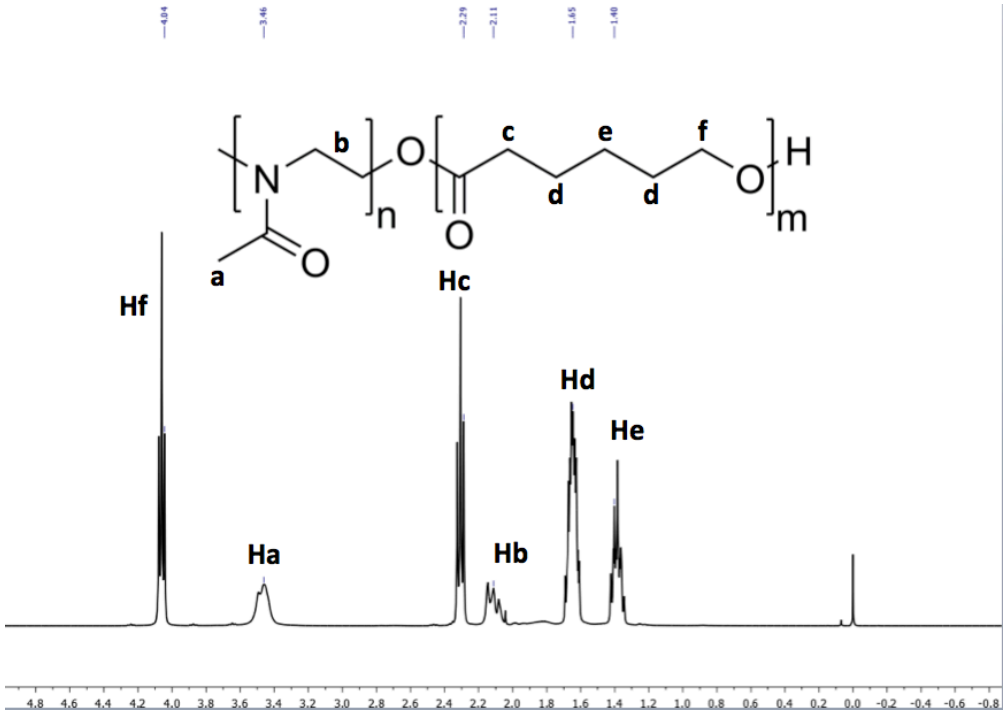


Fig. S5 The ^{1}H NMR spectrum of PMOXA-*b*-PCL in $CDCl_3$.

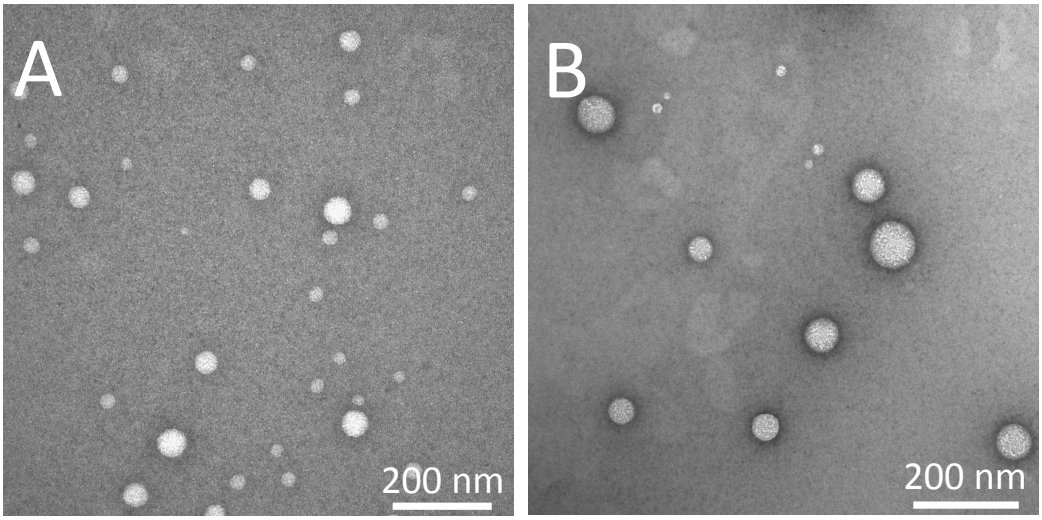


Fig. S6 TEM images of nanoparticles self-assembled from (A) PMOXA-*g*(SS)-PCL 2, and (B) PMOXA-*g*(SS)-PCL 3.

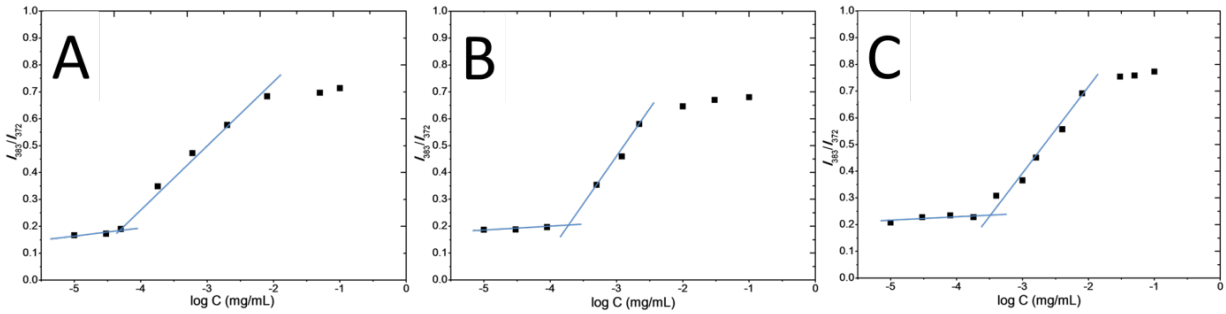


Fig. S7 The fluorescence intensity ratio I_{383}/I_{372} of pyrene as a function of PMOXA-*g*(SS)-PCL copolymers concentration. Blue lines are linear fits and the crossing-points are the values of CMC. (A) PMOXA-*g*(SS)-PCL 2; (B) PMOXA-*g*(SS)-PCL 1; and (C) PMOXA-*g*(SS)-PCL 3.

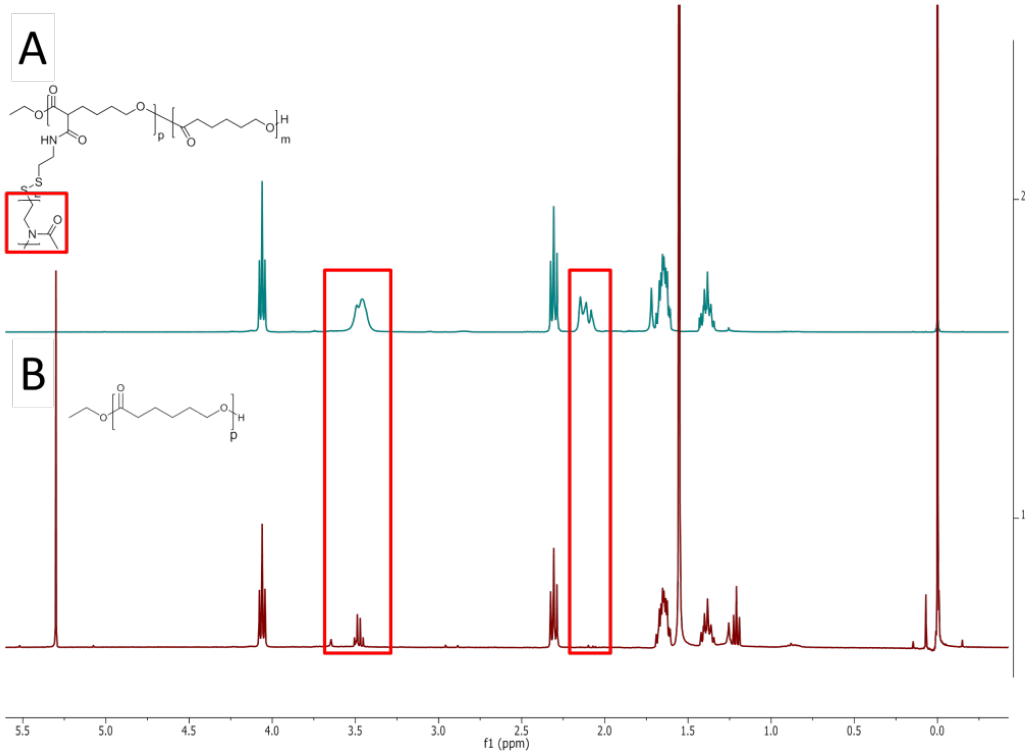


Fig. S8 The ^{1}H NMR spectrum of (A) PMOXA-*g*(SS)-PCL 1 and (B) of the white precipitate obtained in the nanoparticles solution after treatment with 10 mM DTT.

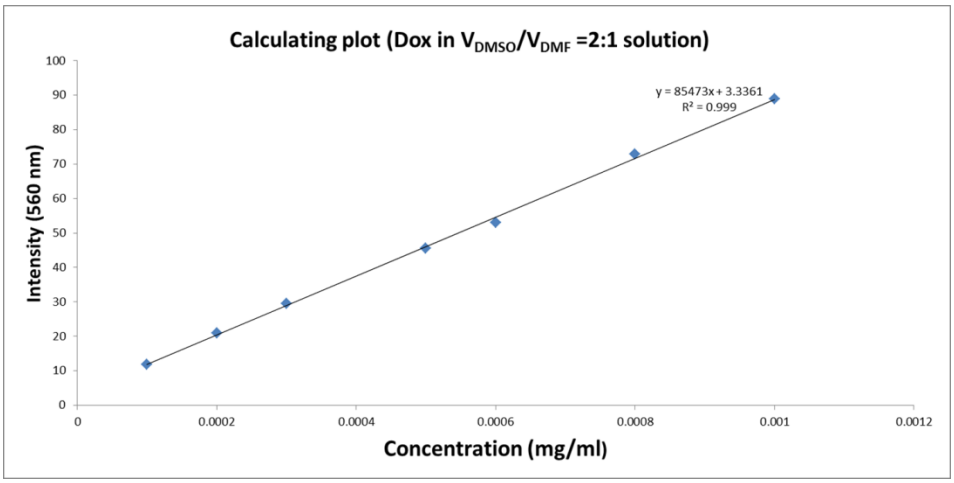


Fig. S9 The DOX concentration calibration curve in the mixture solution of DMSO and DMF (V_{DMSO}/V_{DMF}=2:1).

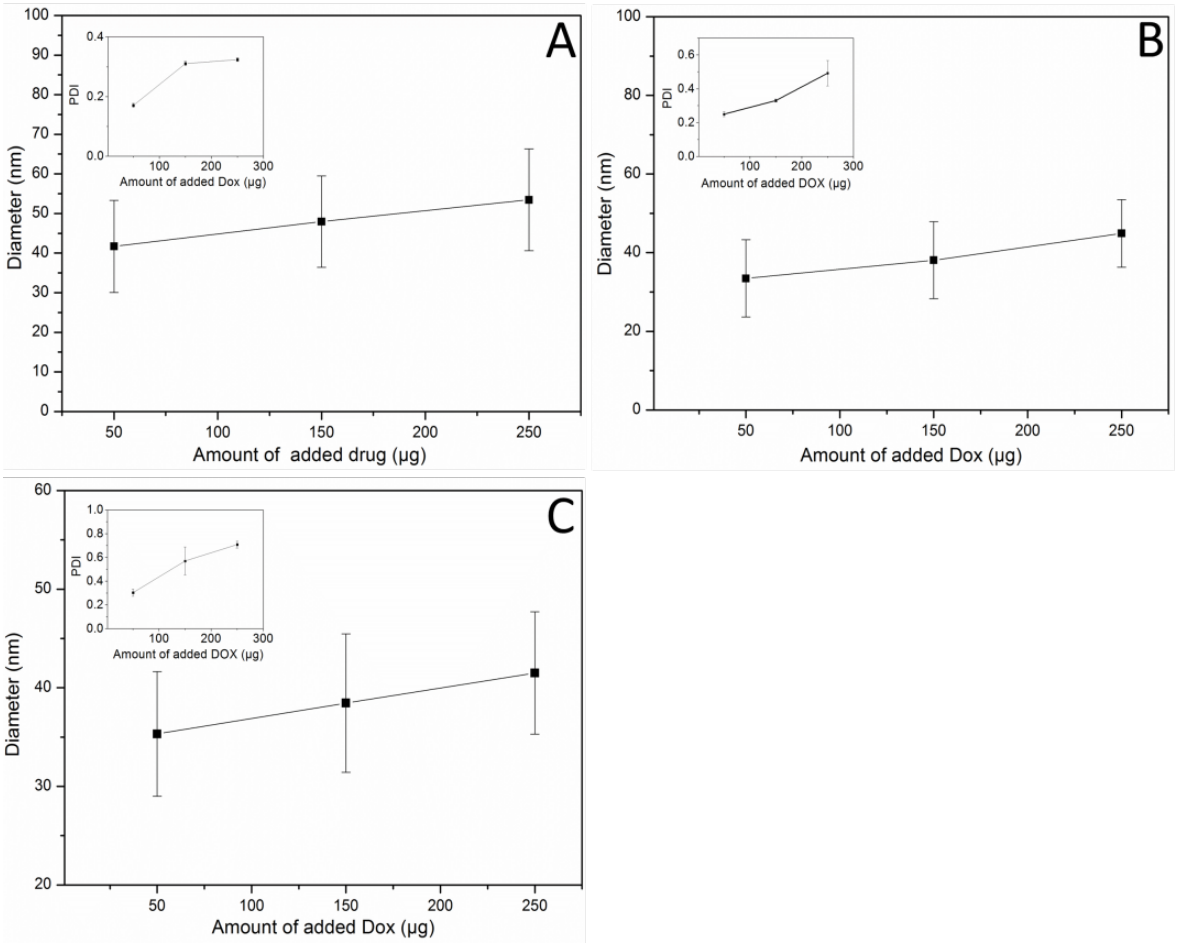


Fig. S10 The influence of different feed amounts of DOX on nanoparticle diameters and PDI (inset) measured by DLS measurements. (A): PMOXA-*g*(SS)-PCL 1; (B): PMOXA-*g*(SS)-PCL 2; (C): PMOXA-*g*(SS)-PCL 3.

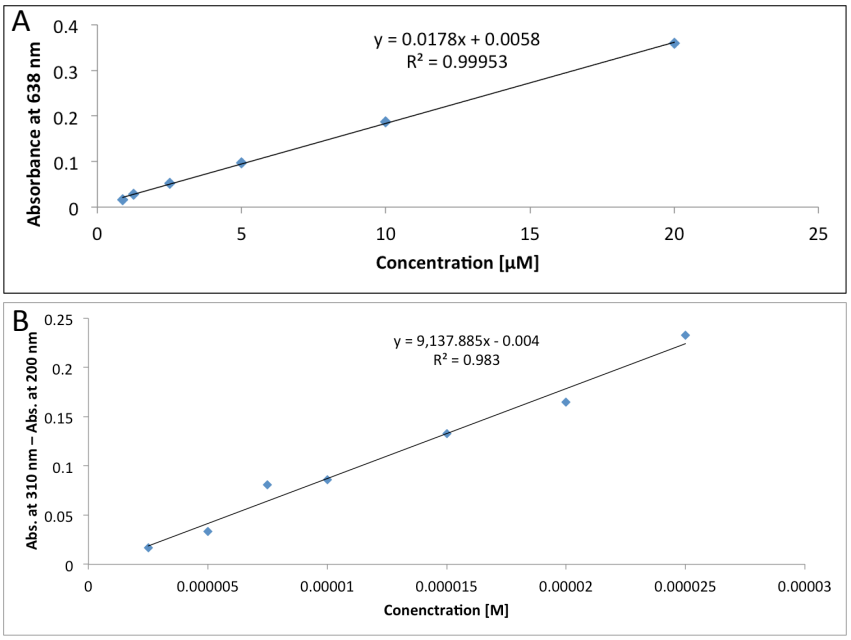


Fig. S11 Calibration curve for Bodipy630 in PBS and SHMT inhibitor (±)-**1** in PBS used to calculate amount of encapsulated dye/drug in nanoparticle samples. Due to drug aggregation, the values for SHMT inhibitor (±)-**1** absorbance at peak maximum (310 nm) had to be corrected for light scattering by subtracting the value at 200 nm; nanoparticle calculations were performed similarly.

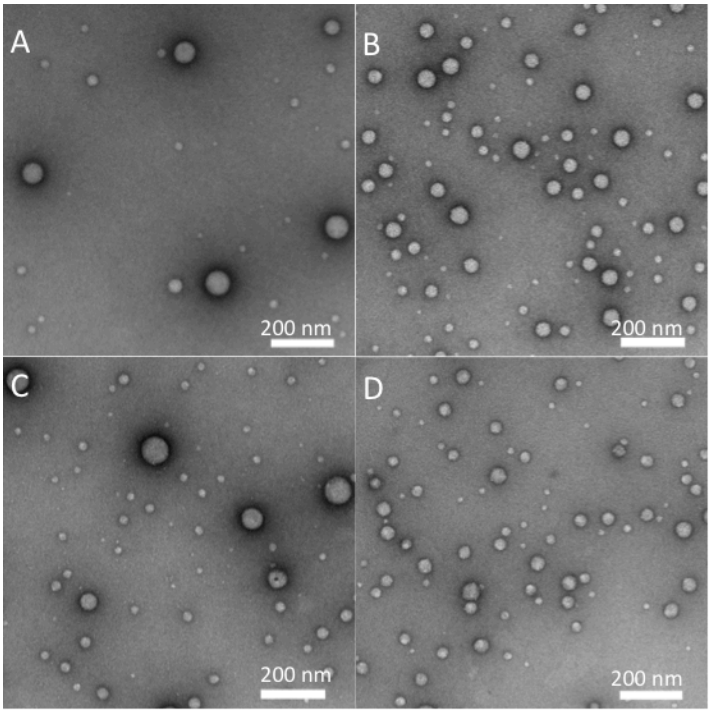


Fig. S12 TEM images of Bodipy630-loaded nanoparticles (1 μM Bodipy630) – used for triggered release studies – self-assembled from (A) PMOXA-*b*-PCL, (B) PMOXA-*g*(SS)-PCL 1, (C) PMOXA-*g*(SS)-PCL 2, and (D) PMOXA-*g*(SS)-PCL 3.

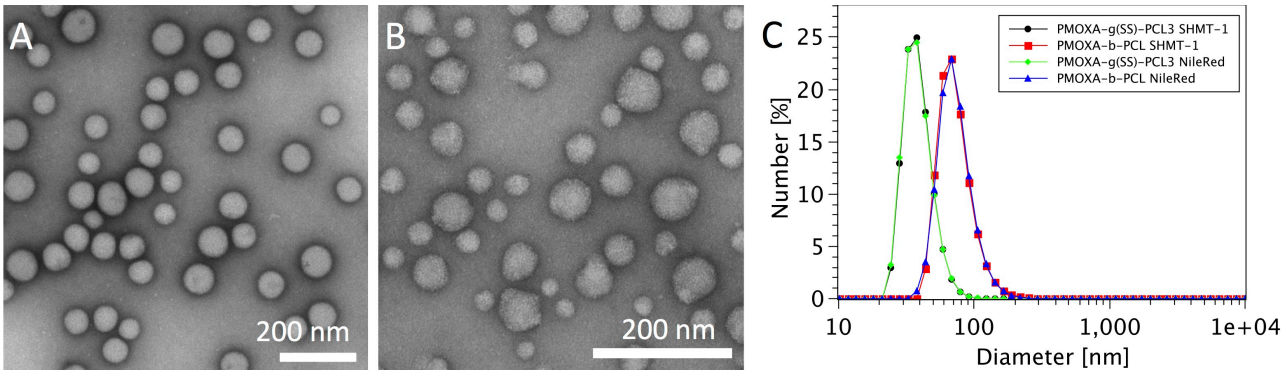


Fig. S13 Morphology of drug-loaded (SHMT inhibitor (±)-**1**) nanoparticles self-assembled from PMOXA-*b*-PCL (A), and PMOXA-*g*(SS)-PCL 3 recorded by transmission electron microscopy (TEM). (C) Hydrodynamic diameter and number distribution of dye- (NileRed) and drug-loaded (SHMT inhibitor (±)-**1**) nanoparticles in PBS measured by DLS.

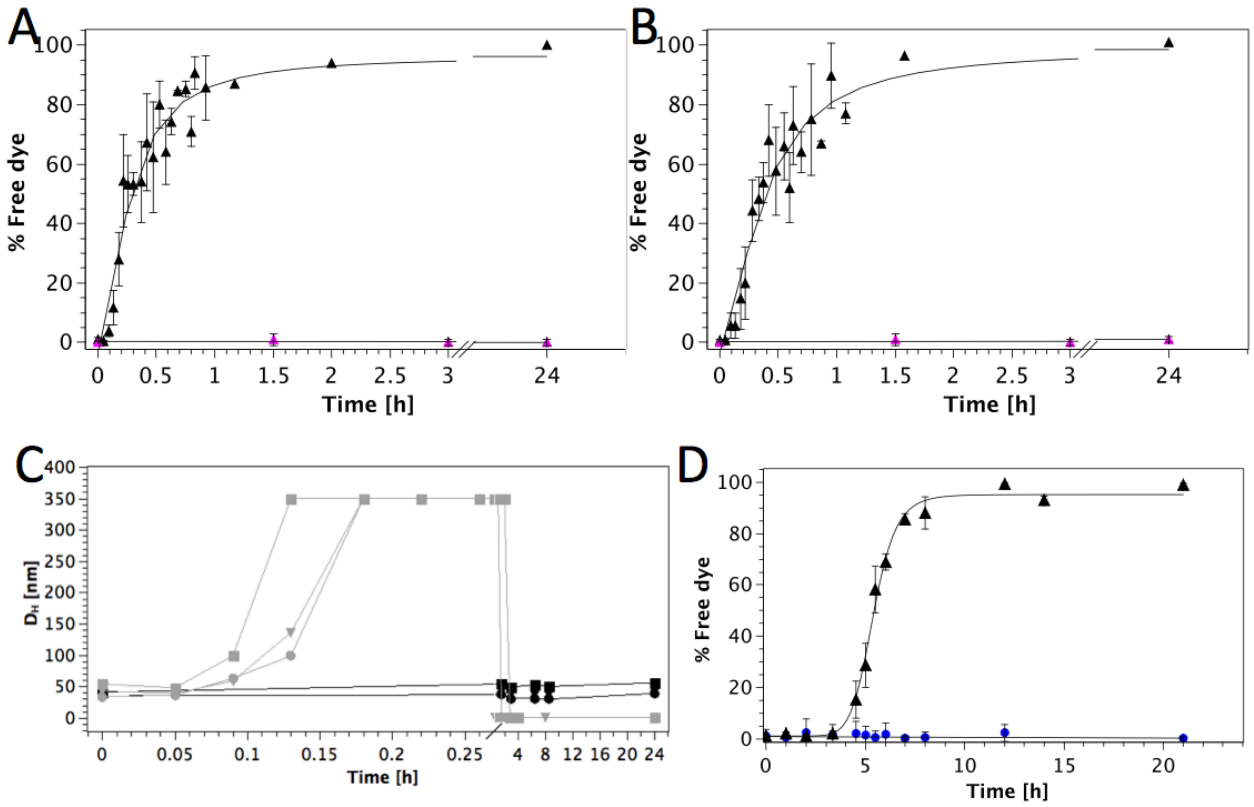


Fig. S14 (A) Time series of the percentage of free dye (Bodipy630) fraction from FCS curves of nanoparticles with the following conditions: PMOXA-*g*(SS)-PCL 2 in PBS without DTT at 37 °C (magenta triangles), PMOXA-*g*(SS)-PCL 2 in PBS containing 10 mM DTT at 37 °C (black triangles). (B) Same as (A) but for PMOXA-*g*(SS)-PCL 3. Values are mean of three independent measurements ± SEM for each time point. (C) Development of hydrodynamic diameters calculated from FCS diffusion times: PMOXA-*g*(SS)-PCL 1 (down triangles), PMOXA-*g*(SS)-PCL 2 (squares), PMOXA-*g*(SS)-PCL 3 (circles) in PBS containing 10 mM DTT at 37°C under shaking condition (grey symbols and curves), and in PBS without DTT at 37°C under shaking

condition (black symbols and curves). Diameters of aggregates above 350 nm are shown at 350 nm, because accurate size information is only possible for particles/aggregates smaller than the confocal volume. (D) Time series of the percentage of free dye (Bodipy630) fraction from FCS curves of nanoparticles with the following conditions: PMOXA-*g*(SS)-PCL 3 (black triangles) and PMOXA-*b*-PCL (blue circles) in 100 mM phosphate buffer (+50 mM NaCl) containing 10 mM glutathione (GSH) at 37 °C.

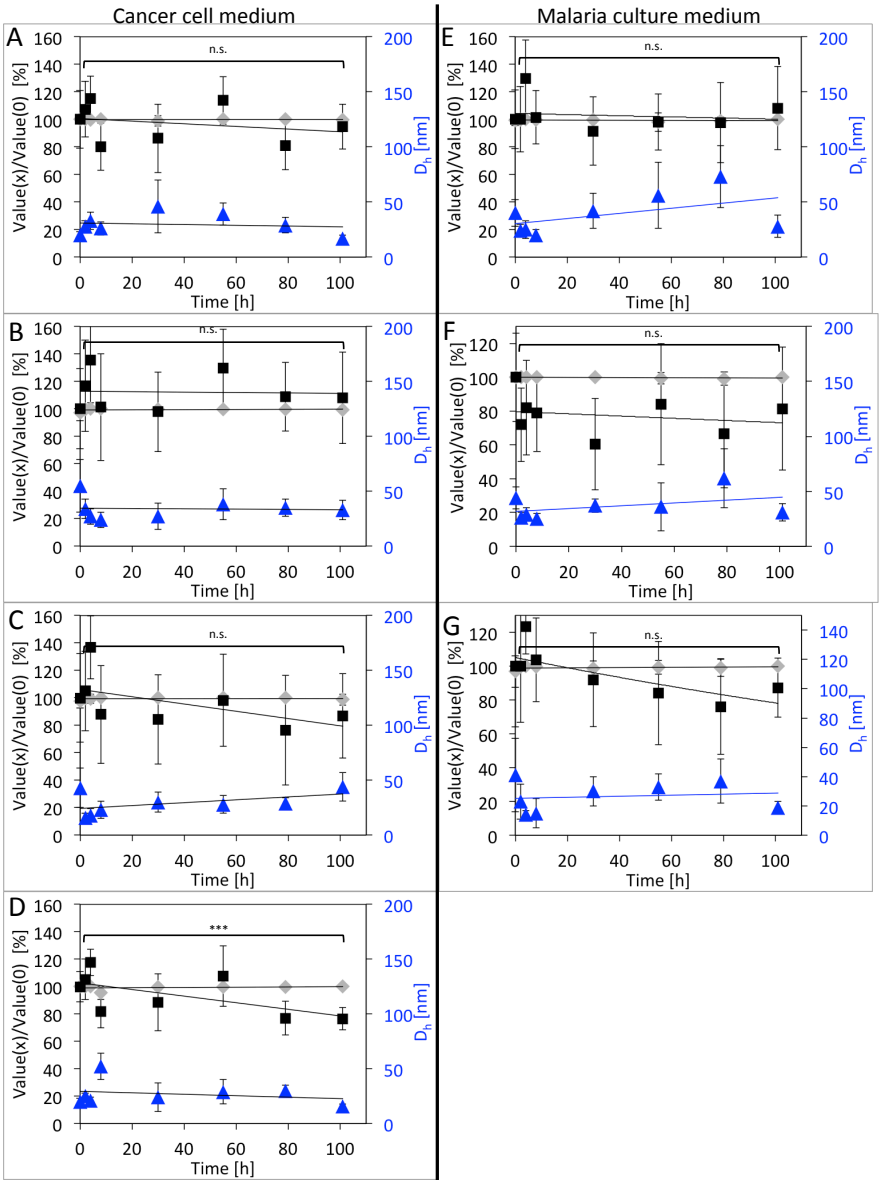


Fig. S15 Stability of dye-loaded (Bodipy630) nanoparticles in cancer cell medium (left) and malaria culture medium (right) at 37 °C under shaking condition for 4 days: nanoparticle fraction from two-component autocorrelation curve fitting compared to time point 0 (grey diamonds), change in number of dye molecules per nanoparticles calculated from molecular brightness (CPM) data compared to time point 0 (black squares), and hydrodynamic nanoparticle diameter calculated from obtained diffusion times (blue triangles). (A,E) PMOXA-*b*-PCL nanoparticles (stock containing 2.5 μM Bodipy630). (B,F) PMOXA-*b*-PCL nanoparticles (133 μM

Bodipy630). (C,G) PMOXA-*g*(SS)-PCL 2 nanoparticles (2.5 μM Bodipy630). (D) PMOXA-*g*(SS)-PCL 3 nanoparticles (56 μM Bodipy630). Values are mean of 30 curves ± SD at each time point. Statistics were analyzed using two-tailed Student's *t*-test.

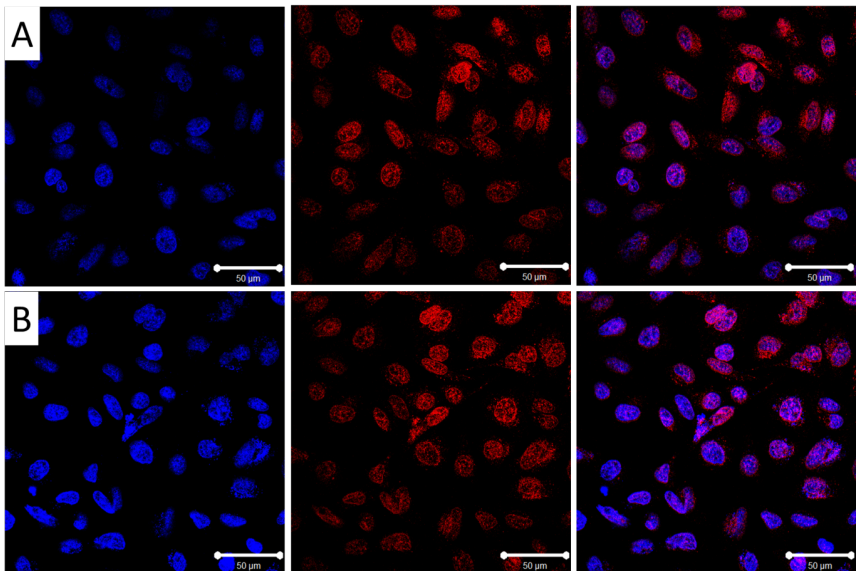


Fig. S16 CLSM images of intracellular DOX release from the reduction-responsive DOX-loaded PMOXA-*g*(SS)-PCL 2 nanoparticles using HeLa cells after 2 h incubation. For each panel, images from left to right show cell nuclei stained by Hoechst 33342 (blue), DOX fluorescence in cells (red) and overlays of the two images. A: DOX-loaded PMOXA-*g*(SS)-PCL 2 nanoparticles (0.25 μg/mL DOX); (B) free DOX as control (0.25 μg/mL DOX). (scale bars are 50 μm).

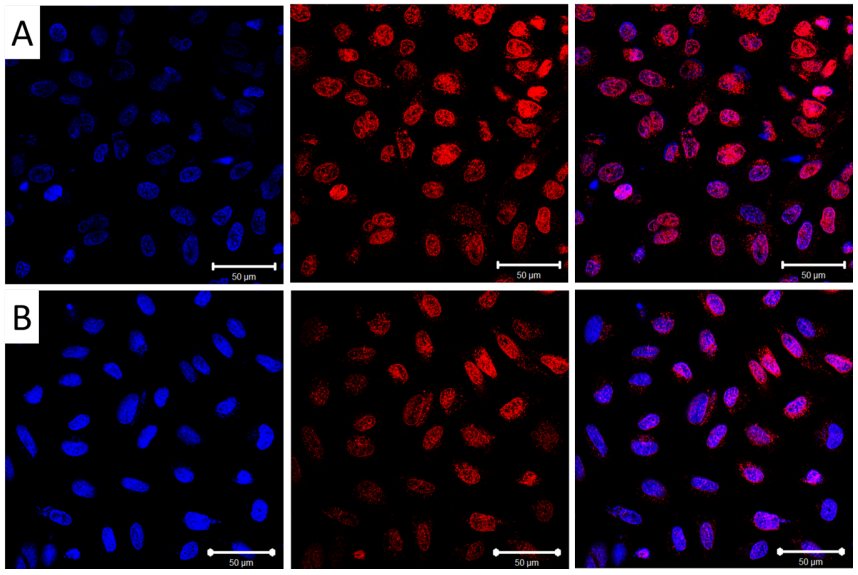


Fig. S17 CLSM images of intracellular DOX release from the reduction-responsive DOX-loaded PMOXA-*g*(SS)-PCL 2 nanoparticles using HeLa cells after 4 h incubation. For each panel,

images from left to right show cell nuclei stained by Hoechst 33342 (blue), DOX fluorescence in cells (red) and overlays of the two images. A: DOX-loaded PMOXA-*g*(SS)-PCL 2 nanoparticles (0.25 μg/mL DOX); (B) free DOX as control (0.25 μg/mL DOX). (scale bars are 50 μm)

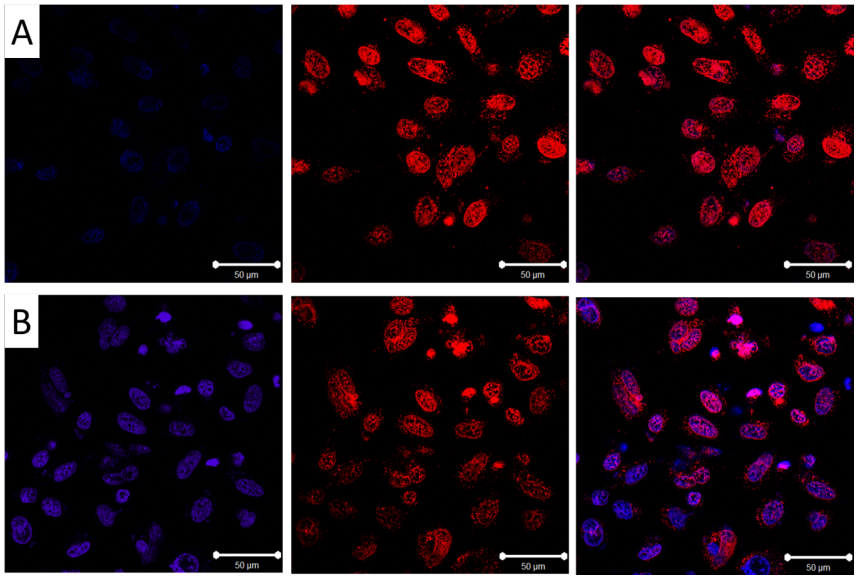


Fig. S18 CLSM images of intracellular DOX release from the reduction-responsive DOX-loaded PMOXA-*g*(SS)-PCL 2 nanoparticles using HeLa cells after 8 h incubation. For each panel, images from left to right show cell nuclei stained by Hoechst 33342 (blue), DOX fluorescence in cells (red) and overlays of the two images. A: DOX-loaded PMOXA-*g*(SS)-PCL 2 nanoparticles (0.25 μg/mL DOX); (B) free DOX as control (0.25 μg/mL DOX). (scale bars are 50 μm)

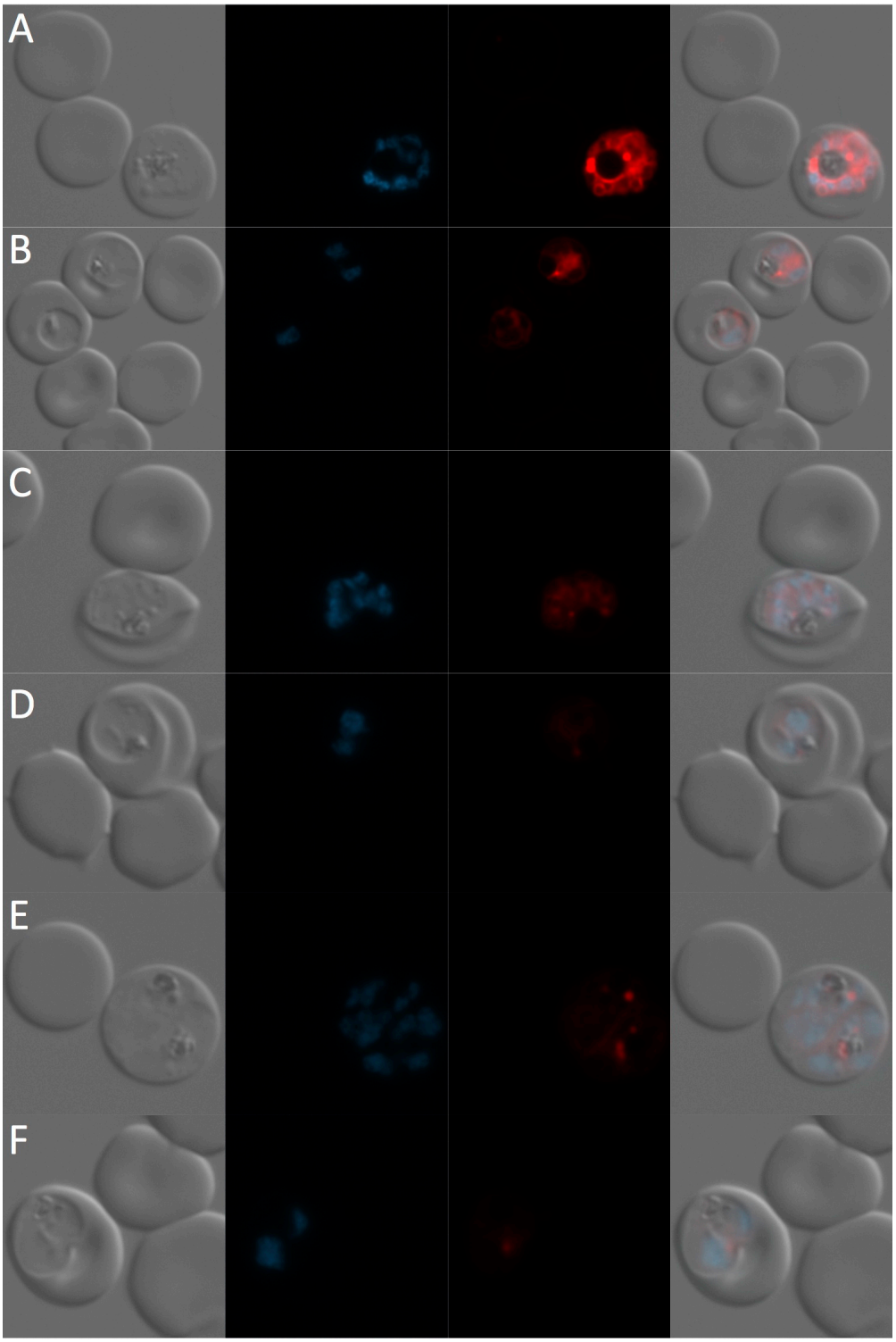


Fig. S19 Fluorescence imaging demonstrating delivery of hydrophobic model molecule, NileRed, to iRBCs using no carrier (A, B); PMOXA-*g*(SS)-PCL 3 nanoparticles (C, D); PMOXA-*b*-PCL nanoparticles (E, F): DIC (left), Hoechst DNA-stain (middle left), NileRed signal (middle right), merge (right). Same dye concentrations (final 2.5 μM) and microscopy settings were used for all images.

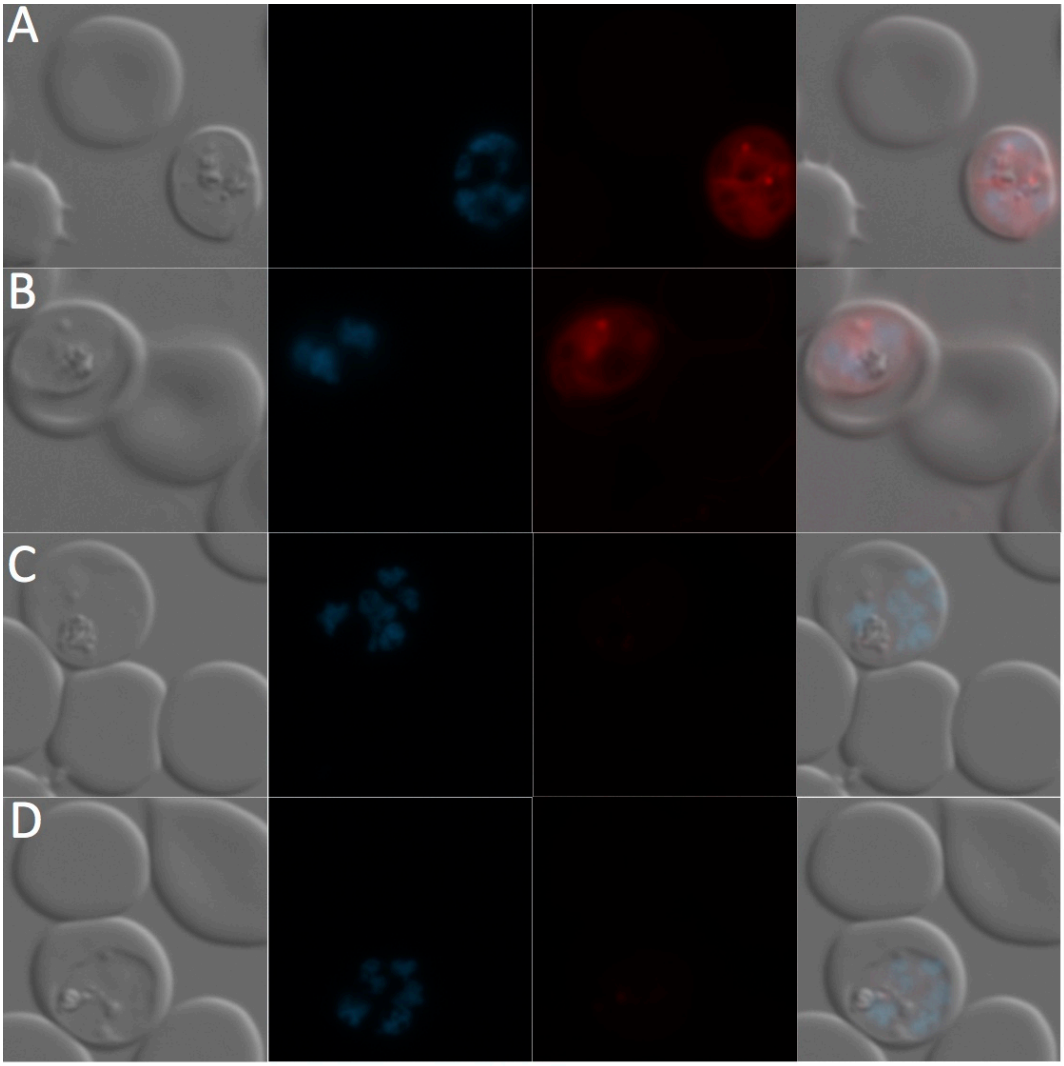


Fig. S20 Fluorescence imaging demonstrating uptake of hydrophobic dye by iRBCs using freshly prepared NileRed solution in PBS (A, B), and 1 day incubated NileRed solution in PBS: DIC (left), Hoechst DNA-stain (middle left), NileRed signal (middle right), merge (right). Same dye concentrations (final 2.5 μM) and microscopy settings were used for all images.

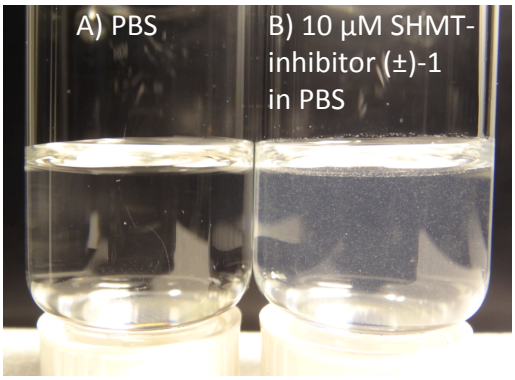


Fig. S21 Digital image of a (A) PBS solution and a (B) 10 μM SHMT inhibitor (±)-**1** in PBS after 30 minutes at RT, prepared from a 10 mM stock in DMSO, demonstrating precipitation of the hydrophobic drug in aqueous solution.

Table S1 DOX Loading Content (DLC), Loading Efficiency (DLE), Size and PDI of DOX-loaded PMOXA-*graft*(SS)-PCL Nanoparticles.

Polymer	DLC (wt %)		DLE (%)	DLS	
	in feed	determined		Size (nm)	PDI
PMOXA-*graft*(SS)-PCL 1	4.8	1.9±0.1	38.2±0.6	42±12	0.17
	13	5.8±0.2	40.8±1.6	48±12	0.31
	20	9.0±0.2	39.4±1.1	53±13	0.33
PMOXA-*graft*(SS)-PCL 2	4.8	1.7±0.1	35.7±1.3	33±10	0.26
	13	5.9±0.1	41.5±0.7	38±10	0.34
	20	9.9±0.3	41.5±1.0	45±9	0.53
PMOXA-*graft*(SS)-PCL 3	4.8	1.8±0.1	36.9±1.6	35±6	0.33
	13	5.9±0.1	41.1±0.9	33±7	0.43
	20	8.6±0.5	36.0±2.2	42±6	0.74

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