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Synthesis and *In Vitro* Activity of ROMP-Based Polymer Nanoparticles

DeeDee Smith,[1] Sandra H. Clark,[2] Paul A. Bertin,[1] Bernard L. Mirkin,[2,3] and SonBinh T. Nguyen[1]

[1]Department of Chemistry and The International Institute for Nanotechnology, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113, USA
[2]Department of Pediatrics, Children's Memorial Research Center, Cancer Biology and Epigenomics Program, Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614, USA.
[3]Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

General Considerations. All synthetic manipulations were performed under a dry nitrogen atmosphere using either standard Schlenk techniques or an inert-atmosphere glovebox, unless otherwise noted. HPLC-grade tetrahydrofuran (THF), *N*,*N*'-Dimethyl formamide (DMF) and methylene chloride (CH_2Cl_2) were dried over neutral alumina via the Dow-Grubbs solvent system[1] installed by Glass Contours. Solvents were collected under argon, degassed under vacuum, and stored under nitrogen in a Strauss flask prior to use. All flash chromatography was carried out using a 56-mm inner diameter column containing 200-mm of silica gel under a positive pressure of lab air.

^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 FT-NMR spectrometer (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR). ^{1}H NMR data are reported as follows: chemical shift {multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, and m = multiplet), integration, and peak assignments}. ^{1}H and ^{13}C chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). Peak assignments were made with the assistance of ACD Labs software.

High-resolution electron-impact mass spectrometry (HREIMS) and atmospheric-pressure chemical-ionization mass spectrometry (APCIMS) data were obtained on a VG70-250SE high resolution mass spectrometer. Electrospray ionization mass spectrometry (ESIMS) data was obtained on a Micromass Quattro II Triple Quadrupole mass spectrometer.

Polymer molecular weights relative to polystyrene standards were measured on a Waters gel-permeation chromatograph (GPC) equipped with Breeze software, a 717 autosampler, Shodex KF-G guard column, KF-803L and KF-806L columns in series, a Waters 2440 UV detector, and a 410 RI detector. HPLC-grade THF was used as the eluent at a flow rate of 1.0 mL/min and the instrument was calibrated using polystyrene standards (Aldrich, 15 standards, 760-1,800,000 Daltons).

Transmission Electron Microscopy. Transmission electron microscopy (TEM) was performed on a Hitachi H8100 microscope operating at an accelerating voltage of 200 kV. For the observation of the size and distribution of the copolymer nanoparticles prepared in nanopure water, colloidal samples (5 μL) were deposited from aqueous solutions of the copolymer nanoparticles onto copper EM grids (400 mesh, Formvar/carbon-coated). Water was allowed to evaporate from the grids at atmospheric pressure and room temperature. For the visualization of Chol-PNP, negative staining was performed by exposing the grids to a solution of 2 wt% uranyl acetate (5 μL) for 2 min. The grids were tapped dry with a piece of filter paper to remove the excess stain and allowed to air-dry before TEM measurements.

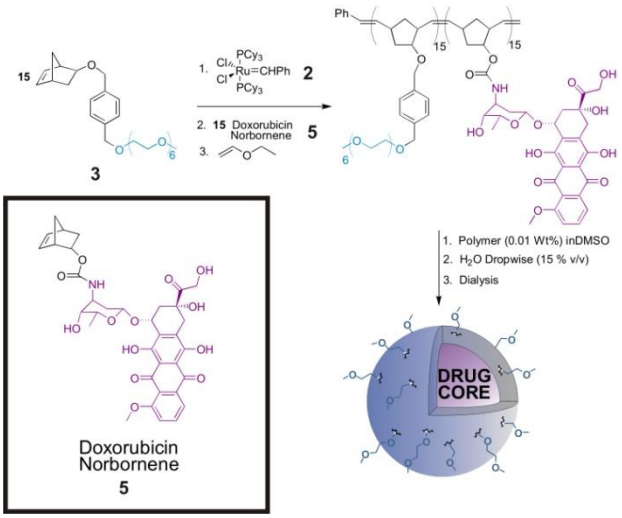
For nanoparticle samples in buffered solutions, samples were deposited onto the copper EM grids and the water was allowed to evaporate at atmospheric pressure and room temperature. Next, a drop (10 μL) of nanopure water was dropped onto the dried grids and the wetted grid was then tapped dry with a piece of filter paper. This process was repeated three times to remove excess salts.

Light-Scattering Measurements. Dynamic light-scattering (DLS) measurements were performed on a Brookhaven Instruments Corp. photon correlation spectrometer (BI-200 SM goniometer) fitted with a Brookhaven Instruments BI-9000AT digital correlator and a 300-mW argon ion laser at 514 nm. The scattering angle used was 90°. A refractive index-matching bath of filtered decalin (0.2-m diameter) surrounded the scattering cell, and the temperature was fixed at 25 ˚C. Correlation data were fitted–using the method of cumulants[2]–to the logarithm of the correlation function, yielding the diffusion coefficient, D. The hydrodynamic diameters (D_H) of the nanoparticles were calculated using D and the Stokes-Einstein equation ($D = k_B T/3\pi\eta D_H$, where k_B is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity). The polydispersity factor of the nanoparticles, represented as μ_2/Γ^2, where μ_2 is the second cumulant of the decay function and Γ is the average characteristic line width, was calculated by the cumulant method. CONTIN algorithms were used in the Laplace inversion of the autocorrelation functions to confirm particle size distributions.[3] All analyses were performed with the supplied instrument software (BIC Dynamic Light Scattering Software).

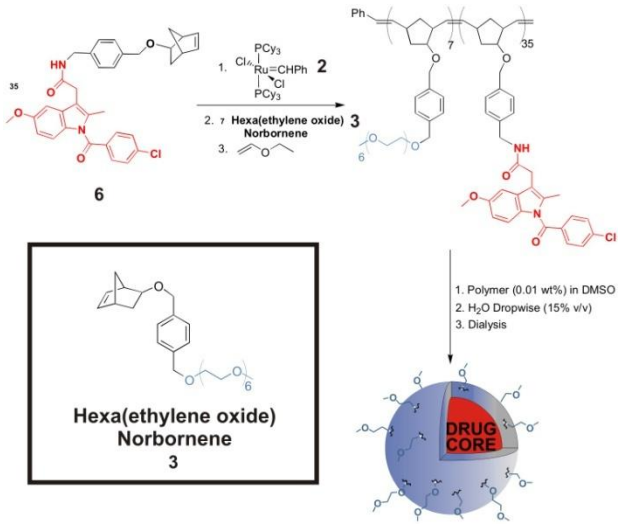
Fluorescence Microscopy. Differential interference contrast (DIC) images and red fluorescent images were taken on a Leica DM-IRB inverted microscope. A 100-W tungsten lamp equipped with neutral density filters and a color-temperature correction filter provided the transmitted light. A 100-W mercury lamp was used as the excitation source. Texas Red dichroic filters were used for fluorescence imaging (ex = 560-640, em = 630-660).

Materials. Catalyst $Cl_2(PCy_3)_2Ru{=}CHPh$ was purchased from Aldrich Chemicals and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories and used without further purification, except for $CDCl_3$, which was distilled over calcium hydride and vacuum-transferred into an air-tight solvent bulb followed by transfer to an inert-atmosphere glovebox. 5-(4-{2-*Exo*-[2-(2-{-2[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxymethyl}-benzyloxy)-bicyclo[2.2.1]hept-2-ene (PEO monomer **3**) and α-Bromo-α′-(*exo*-5-norbornene-2-ol)-*p*-xlyene (Bromoxylene monomer **7**) were prepared according to literature procedure.[4]

Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT). Doxorubicin and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO). All other reagents were purchased from Aldrich Chemicals and used without further purification unless otherwise noted. Nanopure water (18.2 MΩ•cm resistivity) was obtained from a Millipore Milli-Q Biocel A10 instrument. Spectra/Por RC (MWCO = 3500) dialysis membranes were purchased from Spectrum Laboratories. Formvar/Carbon, 400 mesh copper TEM grids were purchased from Ted Pella, Inc.



Scheme S1. Synthesis of doxorubicin-conjugated polymer nanoparticles.[5] [Hexa(ethylene glycol) monomethyl ether]-conjugated norbornene (**3**) was polymerized using Grubbs first-generation catalyst (**2**). After the polymerization was complete, carbamate-linked doxorubicin-conjugated norbornene (**5**) was added to generate the second block, and the polymerization was terminated with ethyl vinyl ether. The amphiphilic block copolymer was dissolved in DMSO, and nanoparticle formation was induced by adding water dropwise to the vigorously stirring solution. Excess DMSO was removed via dialysis against ultrapure deionized water, yielding narrowly dispersed polymer nanoparticles.



Scheme S2. Synthesis of indomethacin-conjugated polymer nanoparticles.[6] Indomethacin-conjugated norbornene (**6**) was polymerized using Grubbs first-generation catalyst (**2**). After the polymerization was complete, [hexa(ethylene glycol) monomethyl ether]-conjugated norbornene (**3**) was added to generate the second block, and the polymerization was terminated with ethyl vinyl ether. The amphiphilic block copolymer was dissolved in DMSO, and nanoparticle formation was induced by addition of water dropwise to the vigorously stirring solution. Excess DMSO was removed via dialysis against ultrapure deionized water, yielding narrowly dispersed polymer nanoparticles.

Characterization Data for Norborne-*p*-benzylether-xylenyl 3'-Amine-linked Doxorubicin Monomer (4) ^{1}H NMR (CD_3OD): δ 1.25-1.59 (bm, 8H), 1.78 (bd, 2H), 1.91 (bm, 2H), 2.02 (bd, 1H), 2.30 (d, 1H), 2.62-2.90 (bm, 4H), 3.29 (b, 1H), 3.61 (b, 1H), 3.83(b, 4H), 4.20 (b, 1H), 4.45 (bs, 1H), 4.72 (s, 2H), 5.30 (bs, 1H), 5.87 (bs, 1H), 6.16 (bs, 1H), 6.61 (bd, 1H), 6.85 (bd, 1H), 7.25 (bd, 1H), 7.52 (b, 3H), 8.08 (d, 1H). ^{13}C NMR ($CDCl_3$): δ 16.1, 29.5, 32.7, 34.2, 35.7, 45.8, 47.1, 47.3, 47.4, 47.5, 47.8, 48.0, 48.2, 48.4, 55.7, 64.5, 67.3, 68.8, 69.7, 75.5, 76.1, 101.0, 110.5, 110.8, 115.2, 118.7, 119.1, 119.6, 125.8, 132.4, 133.7, 134.1, 134.5, 135.7, 141.0, 154.5, 155.8, 156.9, 160.8, 163.9, 185.7, 186.0, 213.4. ESIMS: *m/z* 756.5 $(M+H)^+$.

Characterization Data for Cholesterol-Containing Control Monomer (1). ^{1}H NMR ($CDCl_3$): δ 0.61 (m, 3H, 18-cholesterol-CH_3) 0.81 (m, 3H, 26-cholesterol-CH_3) 0.82 (m, 3H, 27-cholesterol-CH_3) 0.87 (m, 3H, 21-cholesterol-CH_3) 0.96 (m, 3H, 19-cholesterol-CH_3) 1.03-1.97 (m, 3- and 7-norbornenyl-H_2, various cholesterol-*H*), 2.22 (m, 2H, 7-cholesterol-*H*), 2.34 (m, 2H, 4-cholesterol-*H*), 2.76 (b, 1H, 1-norbornenyl-*H*), 2.88 (b, 1H, 4-norbornenyl-*H*), 3.21 (m, 1H), 3.53 (m, 1H), 4.48 (m, 6H, 2-norbornenyl-*H*, OCH_2-*Ar*, 3-cholesterol-*H*), 5.28 (m, 1H, 6-cholesterol-*H*), 5.85 (m, 1H, 6-norbornenyl-*H*), 6.12 (m, 1H, 5-norbornenyl-*H*), 7.25 (m, 4H, aromatic-*H*). ^{13}C NMR ($CDCl_3$): δ

12.1 (18-cholesterol-*C*), 18.8 (21-cholesterol-*C*), 19.5 (19-cholesterol-*C*), 21.2 (11-cholesterol-*C*), 22.8 (27-cholesterol-*C*), 23.0 (26-cholesterol-*C*), 23.5 (23-cholesterol-*C*), 24.4 (15-cholesterol-*C*), 28.1 (25-cholesterol-*C*), 28.2 (16-cholesterol-*C*), 28.6 (13-cholesterol-*C*), 32.3 (7-cholesterol-*C*), 34.9 (20-cholesterol-*C*), 35.5 (3-norbornenyl-*C*), 36.2 (22-cholesterol-*C*), 36.8 (10-cholesterol-*C*), 37.3 (1-cholesterol-*C*), 39.5 (24-cholesterol-*C*), 39.7 (12-cholesterol-*C*), 40.0 (8-cholesterol-*C*), 40.6 (4-norborneneyl-*C*), 42.2 (4-cholesterol-*C*), 46.3 (7-norbornenyl-*C*), 46.7 (1-norbornenyl-*C*), 50.4 (9-cholesterol-*C*), 56.3 (17-cholesterol-*C*), 57.0 (14-cholesterol-*C*), 69.9 (CH_2O-norbornenyl-*C*), 71.1 (3-cholesterol-*C*), 78.6 (CH_2O-cholesterol-*C*), 80.1 (2-norbornenyl-*C*), 121.8 (6-cholesterol-*C*), 127.8 (Aryl-*C*), 127.9 (Aryl-*C*), 133.4 (6-norbornenyl-*C*), 138.3 (Aryl-*C*), 138.4 (Aryl-*C*), 140.9 (5-cholesterol-*C*), 141.1 (5-norbornenyl-*C*). ESIMS: *m/z* 598.4 $(M)^+$.

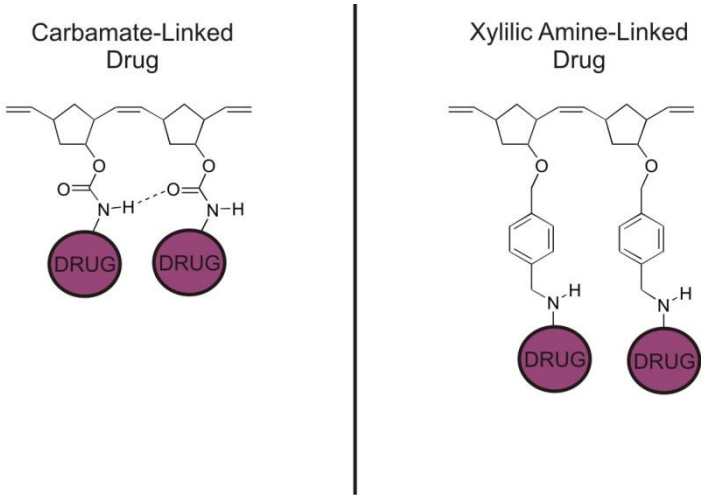


Fig. S1. (Left) Hydrogen bonding of the amine hydrogen to the carbonyl of the adjacent polymer unit. (Right) Xylilic amine hydrogens have no hydrogen bond acceptors.

Release of Doxorubicin from Amine-linked Dox-PNPs. Colloidal solutions of amine-linked Dox-PNPs were incubated for 24 h in nanopure water whose pH had been adjusted to 4 by dropwise addition of hydrochloric acid. After 24 h, the PNPs were centrifuged for 30 min at 10K rpm and the supernatant was removed. Release of doxorubicin from the PNPs into the supernatant was measured using UV-vis spectroscopy. After 24 h, there was no detectable doxorubicin in the supernatant. The supernatant was replaced and the nanoparticles were incubated in acidic media for an additional 9 days, after which there was still no detectable amount of doxorubicin in the UV-vis spectrum of the supernatant, indicating absence of release.

Storage of Dox-PNPs in DMEM Media. Colloidal solutions of the Dox-PNPs, as prepared in nanopure water, were transferred to 2-mL Eppendorf tubes and centrifuged at 10K rpm for 30 minutes. The supernatant was removed and replaced with a similar volume of DMEM and the resulting sample was stored for 2 weeks at 4 °C. To verify the integrity of the PNPs, the media was dialyzed against nanopure water and TEM was used to visualize the size and distribution. As shown in the left panel of Fig. S2, the size of the PNPs did not change with any observable deviation in the size distribution. However, samples stored for 2 months at 4 °C in DMEM did not maintain stability: after dialysis, the outline of the 'PNPs' are barely distinguishable from the background, with dense smaller particles (presumably inorganic salt precipitates from the media) mixed into the polymer (Fig. S2, right panel).

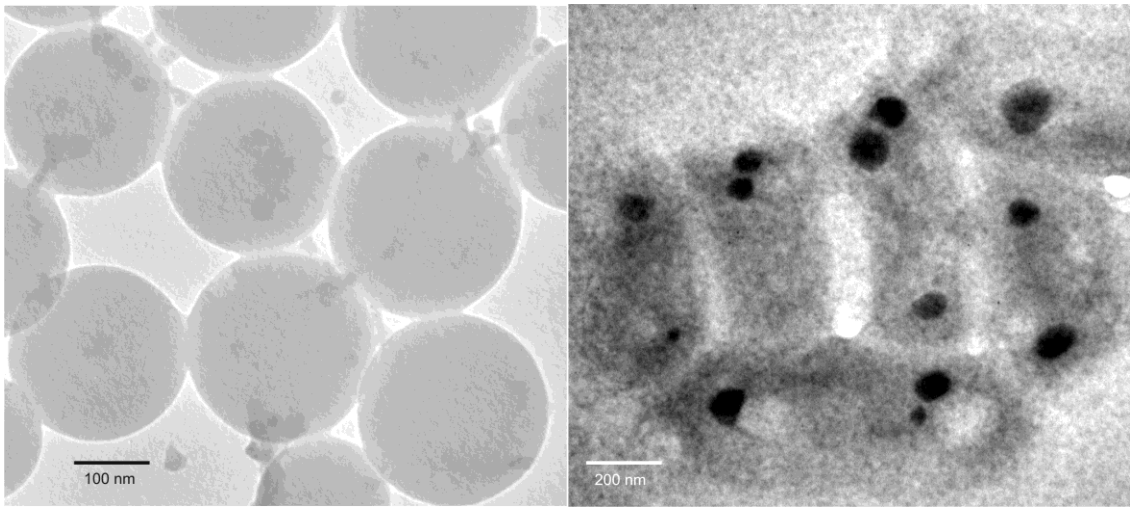


Fig. S2. TEM image of Dox-PNPs stored in DMEM after 2 weeks (left) and after 2 months (right).

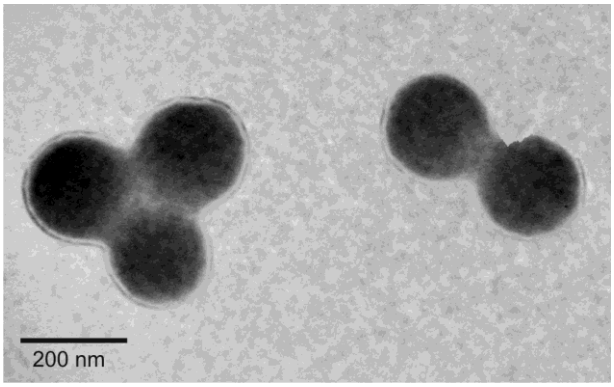


Fig. S3. TEM image of Dox-PNPs that have been resuspended in nanopure water after being stored as a lyophilized powder for several months.

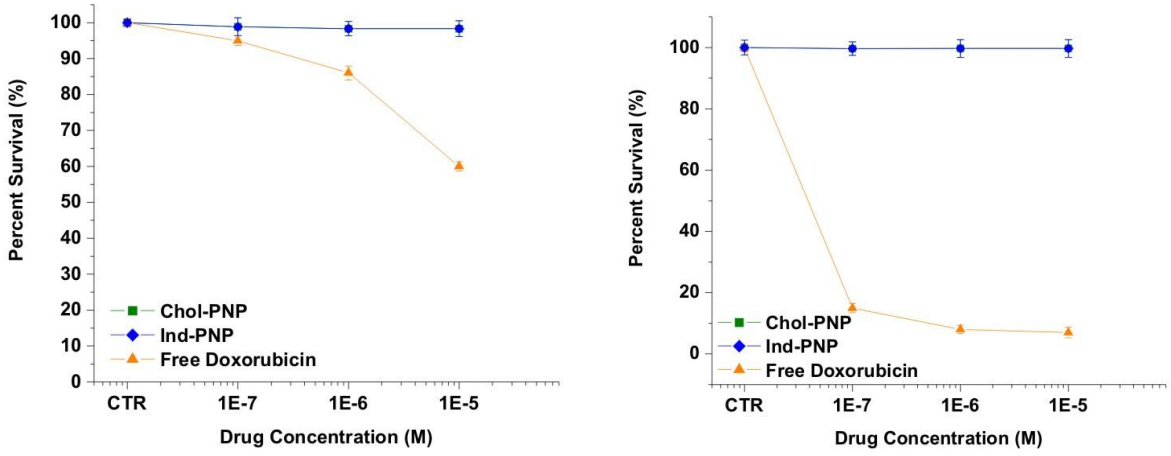


Fig. S4. Cytotoxicity profile of cholesterol-containing polymer nanoparticles (Chol-PNPs). Left: Chol-PNPs in SKrDOX6 doxorubicin-resistant neuroblastoma cell line. Right: Chol-PNPs in SKNSH wild-type neuroblastoma cell line.

Table S1. The pH of cell media during inhibition assay (the pH of DMEM was found to be 7.35).

Cell Culture	pH after 96 h
SKN-SH	7.44
SKN-SH + Dox-PNP	7.69
SKrDOX6	7.53
SKrDOX6 + Dox-PNP	7.50

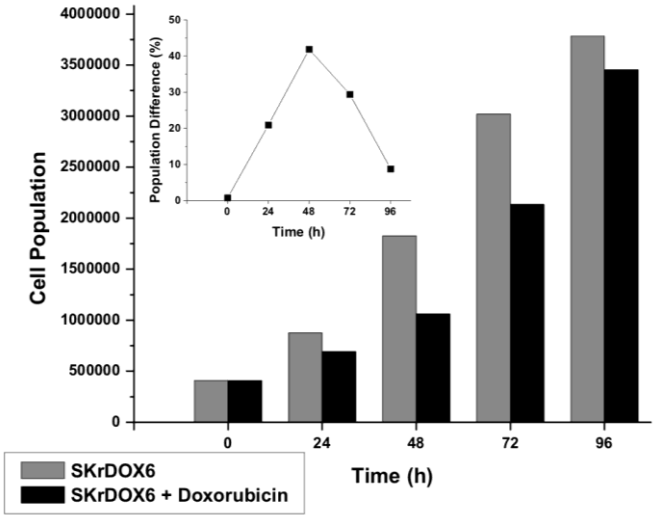


Fig. S5. Growth-inhibition profile of SKrDOX6 doxorubicin-resistant neuroblastoma cell lines with doxorubicin. Inset shows the relative population excess ($[(x_1 - x_2)/x_1]*100$), in percentage, of a culture of SKrDOX6 that has not been exposed to Doxorubicin relative to a culture that has.

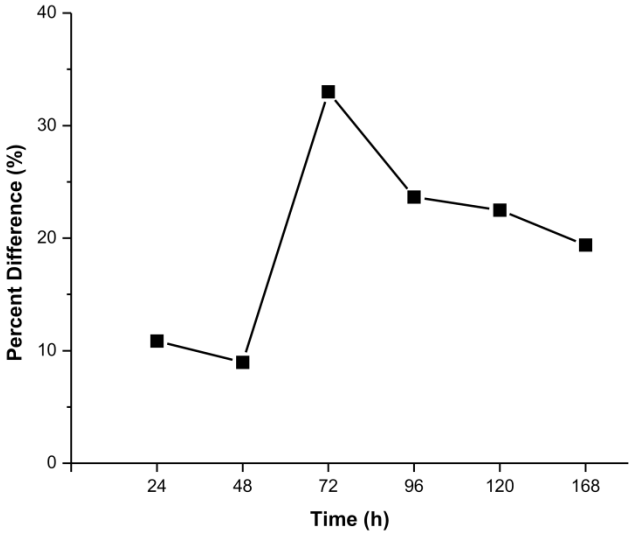


Fig. S6. The relative population excess ($[(x_1 - x_2)/x_1]*100$), in percentage, of a culture of SKrDOX6 that has not been exposed to Dox-PNPs relative to a culture that has.

Table S2. Cell inhibition data for SKNSH wild-type cells incubated with Dox-PNPs.

Time (h)	**0**	**4**	**8**	**16**	**24**	**48**	**72**	**96**	**120**	**168**
Exp. 1	226500	186000	241000	410000	376500	380000				
Exp. 2	200000				332000	287000	221500	292500		
Exp. 3	100000						207000		216000	145500

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