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Drug-loaded Polymeric Spherical Nucleic Acids: Enhancing Colloidal Stability and Cellular Uptake of Polymeric Nanoparticles through DNA Surface-functionalization

Resham J. Banga,[a] Sai Archana Krovi,[b,d] Suguna P Narayan,[c] Anthony J. Sprangers,[c] Guoliang Liu,[d,e] Chad A. Mirkin,[d,f] and SonBinh T. Nguyen[d,f,*]

[a]Department of Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois-60208-3113, USA; [b]Current address: UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, 301 Pharmacy Lane Beard Hall, Chapel Hill, North Carolina 27599; [c]Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois-60208-3113, USA; [d]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois-60208-3113, USA; [e]Current address: Department of Chemistry, Virginia Tech, Blacksburg, Virginia 24061, USA; [f]International Institute of Nanotechnology, Northwestern University, 2145 Sheridan Road, Evanston, Illinois-60208-3113, USA

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S1. Materials and Instrumentation

Materials. Grubbs' first-generation catalyst $(PCy_3)_2Cl_2Ru$=CHPh (**3**) was purchased from Aldrich Chemicals Co. (Milwaukee, WI, USA) and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and used without further purification, except for CD_2Cl_2 and $CDCl_3$, which were dried over calcium hydride, vacuum-transferred into air-tight solvent bulbs, and stored inside an inert-atmosphere glovebox before use. 1-[4-((Bicyclo[2.2.1]hept-5-en-2-yloxy)methyl)phenyl]-2,5,8,11,14,17-hexaoxanonadecan-19-ol (**5**)[S1] 3-((4-((1*S*,2*S*,4*S*)-bicyclo[2.2.1]hept-5-en-2-yloxy)benzyl)oxy)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopentaphenanthrene (**6**)[S2] and bicyclo[2.2.1]hept-5-en-2-yl-*N*-(3-hydroxy-2-methyl-6-([3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11hexahydrotetracen-1-yl]oxy)oxan-4-yl)carbamate (**1**)[S3] were prepared according to literature procedures.

All other reagents were purchased from Aldrich Chemicals Co. and used without further purification. Ultrapure deionized water (18.2 MΩ·cm resistivity) was obtained from a Millipore Milli-Q Biocel A10 instrument (Millipore Inc., MA, USA). Dialysis cassettes (MWCO = 3500) were purchased from Pierce Protein Research Products (IL, USA). Formvar/Carbon, 400-mesh copper TEM grids were purchased from Ted Pella, Inc. (CA, USA).

Syntheses of DNA were performed on a MerMade 48 Nucleic Acid System. DNA products were purified and analyzed on a Varian HPLC equipped with reverse-phase (RP) semi-preparative (Dynamax, 250 × 10 mm, Microsorb 300 Å/10 μm/C18) column. Absorption spectra of DNA materials were recorded on a Varian Cary UV-vis spectrophotometer (Varian, Inc., CA, USA) using quartz cuvette (path length = 10 mm, catalog # 29B-Q-10-MS, Starna cells Inc., CA, USA).

General synthesis information. All monomer and block co-polymer syntheses manipulations were performed under a dry nitrogen atmosphere using either standard Schlenk techniques or an inert-atmosphere glovebox, unless otherwise noted. HPLC-grade dichloromethane (DCM), methanol (MeOH), and dimethylformamide (DMF) were dried over neutral alumina *via* the Dow-Grubbs solvent system[S4] installed by Glass Contours (now JC Meyer Solvent Systems, Laguna Beach, CA, USA). Solvents were collected under argon, degassed under vacuum, and stored under nitrogen in Strauss flasks prior to use. All flash chromatography was carried out using a 56-mm inner diameter column containing 150-mm length of silica gel under a positive pressure of laboratory air.

Instrumentation.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 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) and peak assignments). ^{1}H and ^{13}C chemical shifts are reported in ppm downfield from tetramethylsilane (TMS).

Electrospray-ionization mass spectrometric (ESIMS) data was obtained on a Micromass Quattro II Triple Quadrupole mass spectrometer (Micromass, Inc., MA, USA). UV-vis absorption spectra for all samples were obtained on a CARY 300 Bio UV-vis spectrometer (Varian, Inc., NC, USA).

Fluorescent measurements were carried out on either a Fluorlog-3 system (HORIBA Jobin Yvon Inc., NJ, USA) or a Synergy H4 Multimode Microplate Reader. (BioTek, Winooski, VT, USA)

Polymer molecular weights relative to polystyrene standards were measured on a Waters gel-permeation chromatograph (GPC, Waters Corp., Milford, MA, USA) 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). Molecular-weight data are reported after being rounded off to the nearest 1K Dalton.

An Eppendorf centrifuge model 5804 R (Eppendorf, NY, USA) was employed for centrifugation. All PNP surface modifications were performed using a Thermomixer R platform shaker (Eppendorf, NY, USA).

Transmission electron microscopy (TEM) was performed on a Hitachi HF8100 microscope (Hitachi High Technologies America, Schaumburg, IL, USA) operating at an accelerating voltage of 200 kV. For the observation of the size and distribution of the polymer nanoparticles (PNPs) prepared in ultrapure deionized water, colloidal samples (5 μL) were deposited from aqueous dispersions of the copolymer nanoparticles onto copper EM grids (400 mesh, Formvar/carbon-coated). The grids were allowed to air-dry at atmospheric pressure and room temperature before TEM measurements.

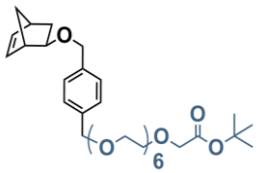
Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometric data of DNA samples were collected as negative ions using the linear mode on a Bruker AutoFlex III MALDI-ToF mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The instrument was equipped with Smartbeam™ laser technology operated at 30-40% power with a sampling speed of 10 Hz. One thousand scans were averaged for each mass spectrum. The instrument was operated using the following parameters: ion source voltage 1 = 20 kV, ion source voltage 2 = 18.5 kV, lens voltage = 8.5 kV, linear detector voltage = 0.6 kV, deflection mass = 3000 Da.

Dynamic light-scattering (DLS) measurements were performed on a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) instrument equipped with a He-Ne laser (633 nm). Non-invasive backscatter method (detection at 173° scattering angle) was used. The hydrodynamic diameters (D_H) and polydispersity indices (PDI) of polymer nanoparticles were calculated by the supplied instrument software (Zetasizer DTS).

The number of particles/mL was determined using a NanoSight LM10-HS (NanoSight Ltd., Wiltshire, UK) instrument equipped with a He-Ne laser (633 nm). The size distributions of the nanoparticles, the nanoparticle concentrations, and image analysis were evaluated using the Nanoparticle Tracking Analysis (NTA) software suite.

S2. Synthesis of monomers and homopolymer 2_{15}

Synthesis of *tert*-butyl 1-(4-((-bicyclo[2.2.1]hept-5-en-2-yloxy)methyl)phenyl)-2,5,8,11,14,17,20-heptaoxadocosan-22-oate (4).



A solution of 1-[4-((bicyclo[2.2.1]hept-5-en-2-yloxy)methyl)phenyl]-2,5,8,11,14,17-hexaoxanonadecan-19-ol (**5**, 300 mg, 0.606 mmol) in dry THF (10 mL) was added *via* cannula-transfer over a period of 20 min to a suspension of NaH (26 mg, 1.09 mmol) in dry THF (10 mL) in a 100 mL Schlenk flask equipped with a magnetic stir bar. Potassium iodide (202.6 mg, 1.21 mmol) was added as a solid to the resulting mixture, followed by a solution of *tert*-butyl bromoacetate (0.134 mL, 0.909 mmol) in THF (2 mL). A water-cooled condenser was attached to the flask and the reaction mixture was allowed to reflux overnight at 80 °C. In the morning, the reaction was quenched with MeOH (10 mL); the mixture was filtered over Celite 545 to remove the NaBr byproduct, followed by rinsing with excess CH_2Cl_2 (20 mL). The combined filtrate was concentrated down and purified by flash chromatography (90:10 v/v CH_2Cl_2/MeOH) to afford monomer **4** as a bright yellow oil (369 mg, 99% yield). ^{1}H NMR ($CDCl_3$, Figure S1 (top)): δ 1.18-1.71 (m, 4H, 3- and 7-norbornenyl-H_2), 1.39 (s, 9H, C(CH_3)$_3$), 2.74 (b, 1H, 1-norbornenyl-*H*), 2.89 (b, 1H, 4-norbornenyl-*H*), 3.53-3.71 (m, 24H, OCH_2CH_2O), 4.44-4.51 (m, 7H, CH_2-C_6H_4-CH_2, OCH_2COO(CH_3)$_3$, and 2-norbornenyl-*H*), 5.85 (m, 1H, 6-norbornenyl-*H*), 6.15 (m, 1H, 5-norbornenyl-*H*), 7.22 (b, 4H, aromatic-*H*). ^{13}C NMR ($CDCl_3$, Figure S1 (bottom)): δ 27.7 (C(CH_3)$_3$), 34.4 (3-norbornenyl-*C*), 40.4 (4-norbornenyl-*C*), 45.8 (7-norbornenyl-*C*), 46.2 (1-norbornenyl-*C*), 69.3 (OCH_2COOC(CH_3)$_3$), 70.6-71.0 (m,

O*C*H_2*C*H_2O), 72.8 (CH-O-*C*H_2-Ph), 74.6 (Ph-O-*C*H_2-CH_2), 79.9 (2-norbornenyl-*C*), 82.3 (*C*(CH_3)$_3$), 127.6 (aromatic-*C*), 128.0 (aromatic-*C*), 133.1 (6-norbornenyl-*C*), 137.4 (aromatic-*C*), 138.3 (aromatic-*C*), 140.8 (5-norbornenyl-*C*), 167.8 ((OCH_2*C*OOC(CH_3)$_3$). ESIMS: *m/z* calculated for $C_{33}H_{52}O_{10}$ $(M + Na)^+$: 631.761. Found: 631.812.

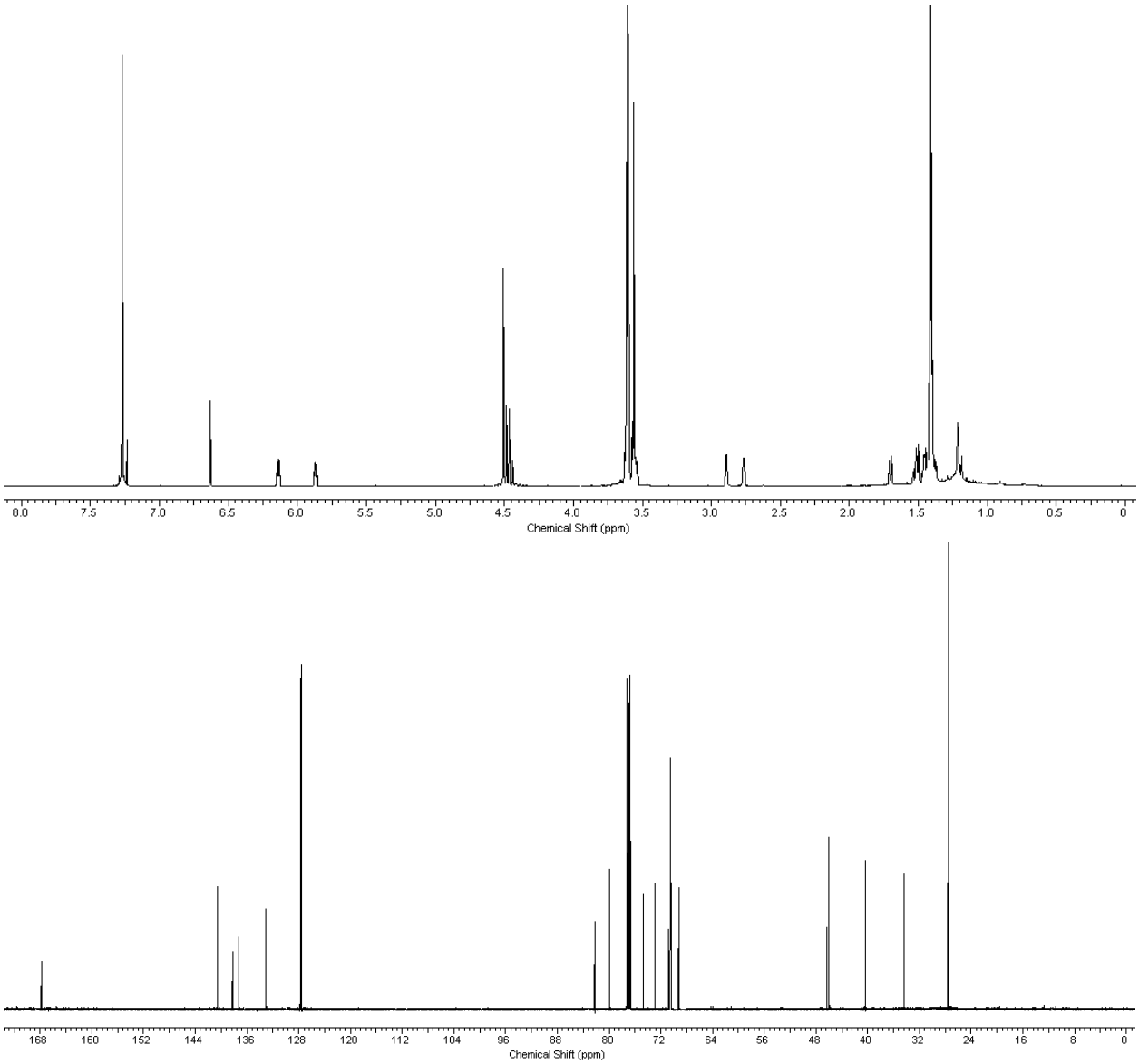
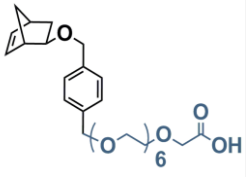


Figure S1. The ^{1}H (top) and ^{13}C NMR (bottom) spectra for *tert*-butyl 1-(4-((-bicyclo[2.2.1]hept-5-en-2-yloxy)methyl)phenyl)-2,5,8,11,14,17,20-heptaoxadocosan-22-oate (**4**).

Synthesis of 1-(4-((bicyclo[2.2.1]hept-5-en-2-yloxy)methyl)phenyl)-2,5,8,11,14,17,20-heptaoxadocosan-22-oic acid (2).



Degassed TFA (0.063 mL, 0.82 mmol) was added using a gas-tight syringe to a solution of monomer **4** (50 mg, 0.082 mmol) dissolved in dry DCM (10 mL) in a 50 mL Schlenk flask equipped with a magnetic stir bar. The resulting reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was then concentrated and purified by flash chromatography (90:10 v/v CH_2Cl_2/MeOH) to afford monomer **2** as a dark yellow oil (42 mg, 93% yield). ^{1}H NMR ($CDCl_3$, Figure S2 (top)): δ 1.18-1.71 (m, 4H, 3- and 7-norbornenyl-*H*$_2$), 2.74 (b, 1H, 1-norbornenyl-*H*), 2.89 (b, 1H, 4-norbornenyl-*H*), 3.53-3.71 (m, 24H, O*C*H_2*C*H_2O), 4.44-4.61 (m, 5H, *C*H_2-C_6H_4-*C*H_2 and 2-norbornenyl-*H*), 5.85 (m, 1H, 6-norbornenyl-*H*), 6.15 (m, 1H, 5-norbornenyl-*H*), 7.22 (b, 4H, aromatic-*H*), 8.93 (b, 1H, COO*H*). ^{13}C NMR ($CDCl_3$, Figure

S2 (bottom)): δ 34.1 (3-norbornenyl-*C*), 40.4 (4-norbornenyl-*C*), 45.9 (7-norbornenyl-*C*), 46.4 (1-norbornenyl-*C*), 68.8 (OCH_2COOCH), 70.0-70.8 (m, OCH_2CH_2O), 72.8 (CH-O-CH_2-Ph), 74.6 (Ph-O-CH_2-CH_2), 80.5 (2-norbornenyl-*C*), 128.2 (aromatic-*C*), 128.3 (aromatic-*C*), 132.9 (6-norbornenyl-*C*), 136.7 (aromatic-*C*), 137.6 (aromatic-*C*), 140.7 (5-norbornenyl-*C*), 174.3 ((OCH_2COOH). ESIMS: *m/z* calculated for $C_{29}H_{44}O_{10}$ $(M + Na)^+$: 575.652. Found: 575.675.

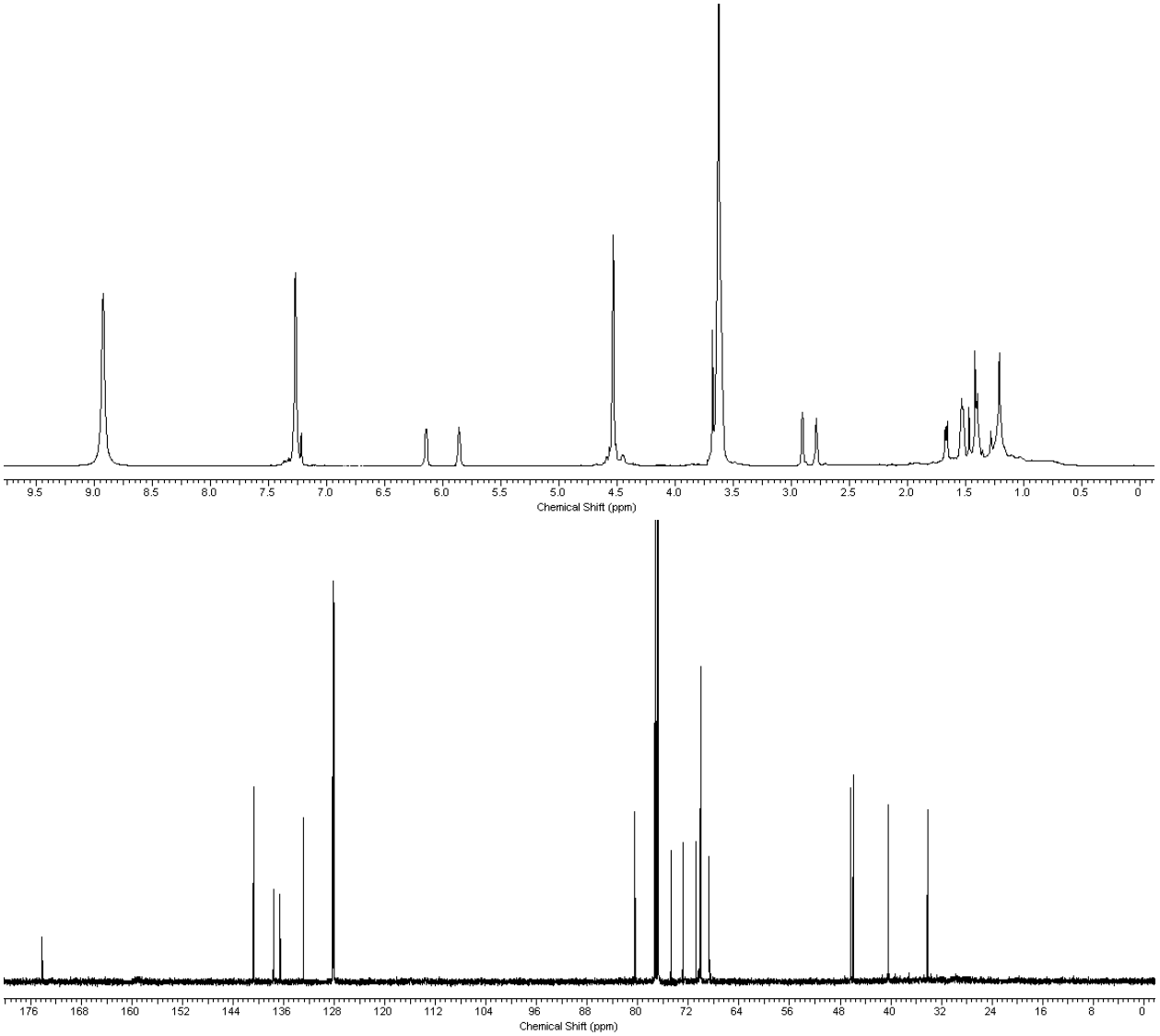
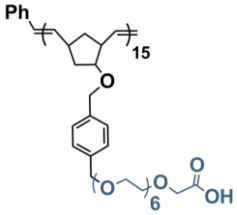


Figure S2. The ^1H (top) and ^{13}C NMR (bottom) spectra for 1-(4-((bicyclo[2.2.1]hept-5-en-2-yloxy)methyl)phenyl)-2,5,8,11,14,17,20-heptaoxadocosan-22-oic acid (**2**).

Procedure for the homopolymerization of monomer 2.



In an inert-atmosphere glovebox, monomer **2** (12 mg, 21.7 μmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) in a 20 mL scintillation vial equipped with a magnetic stir bar. A stock solution of Grubbs' first-generation catalyst (5 mg) in CH_2Cl_2 (5 mL) was prepared, a portion of which (1.19 mL, 1.45 μmol) was added to the vial containing the solution of monomer **2** under vigorous stirring. The resulting reaction mixture was stirred for 30 min at room temperature at which time an aliquot (200 μL) was removed and quenched with excess ethyl vinyl ether. A portion of this quenched aliquot was evaporated to dryness, redissolved in $CDCl_3$, and analyzed by ^1H NMR spectroscopy, which indicated complete consumption of the

monomer. The remaining portion was evaporated to dryness, dissolved in HPLC-grade THF, and subjected to GPC analysis: M_n = 9000 (theoretical M_n = 8000), PDI = 1.17. ^{1}H NMR (CD_2Cl_2, Figure S3): δ 1.02-1.42 (bm), 1.52-2.08 (bm), 2.49-2.79 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 4.35-4.62 (bm), 4.97-5.69 (bm), 7.31 (bm).

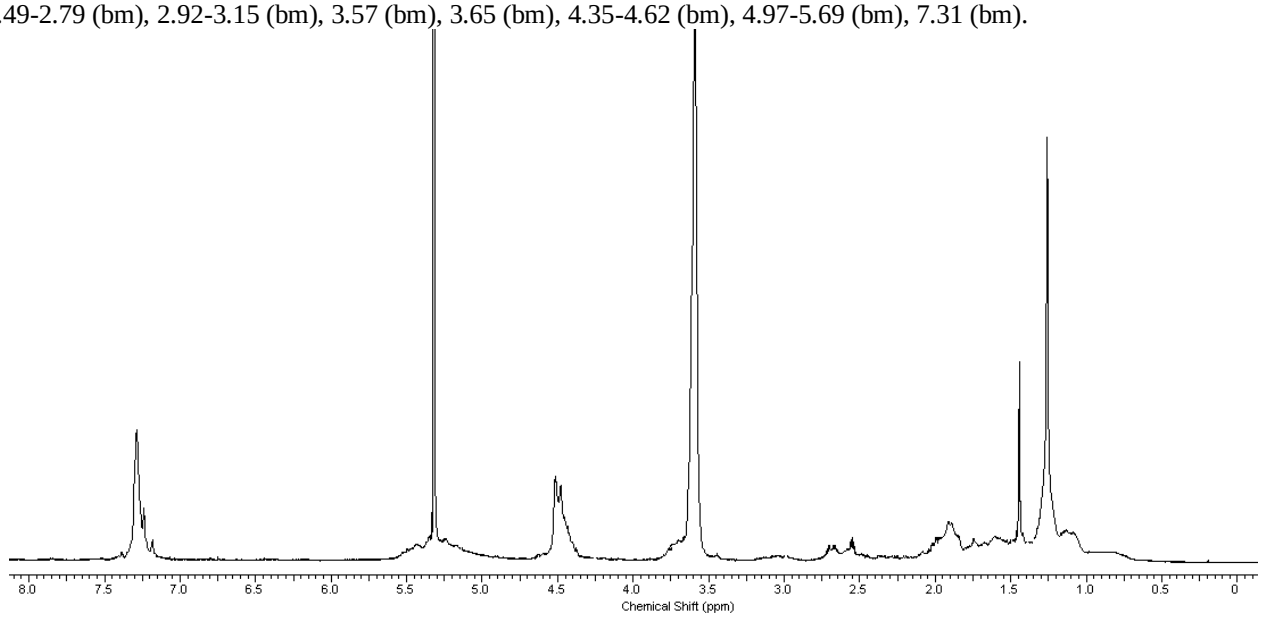
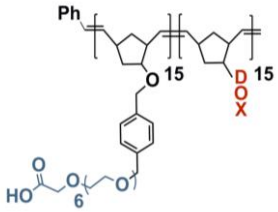


Figure S3. The ^{1}H NMR spectrum of the homopolymer $\mathbf{2}_{15}$.

S3. Synthesis of block copolymers $\mathbf{2}_{15}$-*b*-$\mathbf{1}_{15}$ and $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$

Synthesis of block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{1}_{15}$.



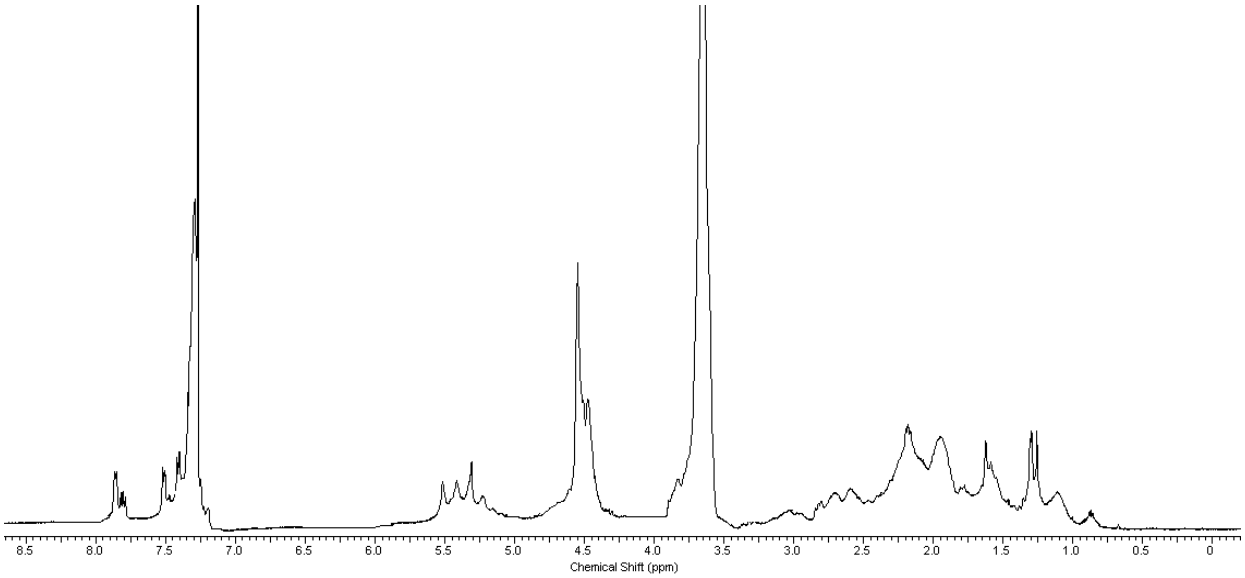
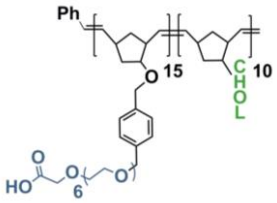


Figure S4. The ^{1}H NMR spectrum of block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{1}_{15}$.

Synthesis of block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$.



In an inert-atmosphere glovebox, monomer **2** (15 mg, 27.1 μmol) was dissolved in an anhydrous mixture of $CHCl_3$ (1 mL) in a 20 mL scintillation vial equipped with a magnetic stir bar. A stock solution of Grubbs' first-generation catalyst (5 mg) in $CHCl_3$ (5 mL) was prepared, a portion of which (1.48 mL, 1.81 μmol) was added to the vial containing the solution of monomer **2** under vigorous stirring. The resulting reaction mixture was stirred for 45 min at room temperature at which time an aliquot (100 μL) was removed and quenched with excess ethyl vinyl ether. A portion of this quenched aliquot was evaporated to dryness, redissolved in $CDCl_3$, and analyzed by ^{1}H NMR spectroscopy, which indicated complete consumption of the monomer. The remaining portion was evaporated to dryness, dissolved in HPLC-grade THF, and subjected to GPC analysis (M_n = 9000 (theoretical M_n = 8000), PDI = 1.18).

Immediately after aliquot removal, a solution of monomer **6** (10.8 mg, 18.1 μmol) in $CHCl_3$ (1 mL) was added to the reaction vial and the resulting polymerization mixture was stirred for an additional 45 min before being terminated with the addition of ethyl vinyl ether (1 mL). The reaction mixture was added quickly into vigorously stirred cold (-10 ^{o}C) pentanes (200 mL), and the resulting precipitate was isolated *via* vacuum-filtration and washed thoroughly with fresh pentanes to afford the product copolymer quantitatively as a dark yellow solid. ^{1}H NMR ($CDCl_3$, Figure S5): δ 0.79 (bs), 0.97-1.32 (bm), 1.35-1.57 (bm), 1.69-2.03 (bm), 2.44-2.69 (bm), 3.18 (bs), 3.34-3.47 (bm), 3.49-3.74 (bm), 4.35-4.51 (bm), 4.58-4.69 (bm), 5.02-5.52 (bm), 7.20 (bs). GPC: M_n = 15000 (theoretical M_n = 14000), PDI = 1.12.

Note: A ratio of 15:10 for monomers **2**:**6** was chosen to restrict the molecular weight of the polymers to be <45 kDa to ensure renal clearance.[S5] In addition, the PNPs derived from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$ had similar diameters to the PNPs formed from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{1}_{15}$ (*vide infra*), allowing us to compare both sets of PNPs.

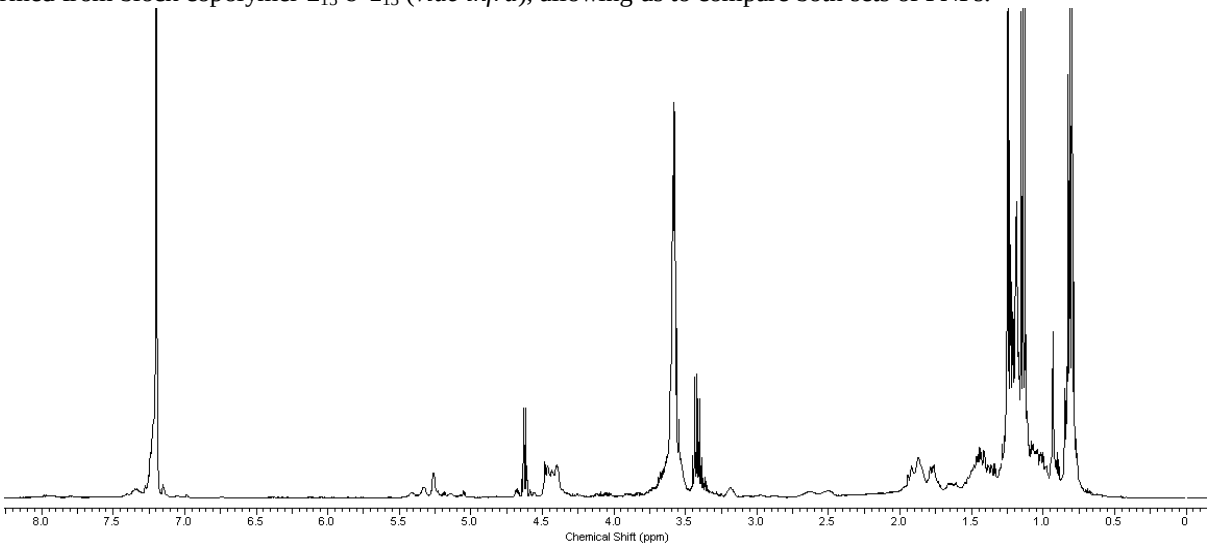
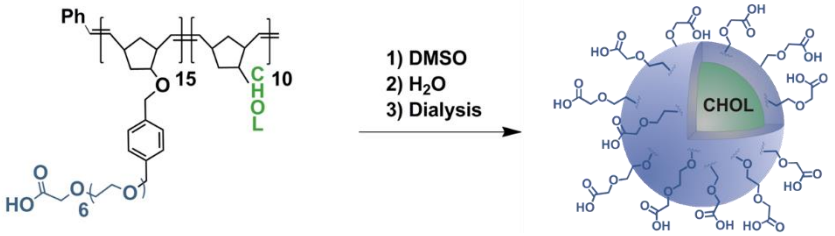


Figure S5. The ^{1}H NMR spectrum of block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$.

S4. Preparation of cholesterol-loaded PNPs in the 60-75 nm size range from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$



An aqueous suspensions of the polymer nanoparticles were prepared by dialysis following a modification of the published procedure.[S6] An aliquot (2.5 mL) of a stock solution of block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$ (0.01 wt %) in DMSO was transferred to a 4 mL scintillation vial and set to stir vigorously. Ultrapure deionized water was added to this stirring

copolymer solution at a rate of 1 drop (50 µL, 1.75 wt %) every 1 s using a 20-100 µL micro-pipette until the mixture contained 18 wt % water. The resulting cloudy mixture was placed in a 3 mL dialysis cassette and dialyzed against ultrapure deionized water (500 mL), with the dialate changed every 2 h. Complete absence of DMSO in the dialate after 48 h was verified by UV-vis spectroscopy as indicated by the disappearance of the UV cut-off for DMSO at 268 nm.

Characterization of PNPs derived from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$. DLS analysis of the final PNP aqueous suspension derived from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$ revealed narrowly dispersed PNPs with an average diameter (D_H) of 72 ± 7 nm and a corresponding polydispersity index (PDI) of 0.072 (Figure S6a). TEM analysis indicated a uniform size distribution for the PNPs in the solid-state, with an average diameter of ~65 nm (Figure S6b and S6c) that is consistent with the DLS data. The zeta potential of these PNPs was determined to be -6.83 ± 0.5 mV.

Note: Although block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{15}$ was synthesized and the corresponding PNPs were successfully prepared, it did not afford the desired PNP size (*i.e.*, ~70 nm) for systematic comparison with the PNPs derived from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{1}_{15}$.

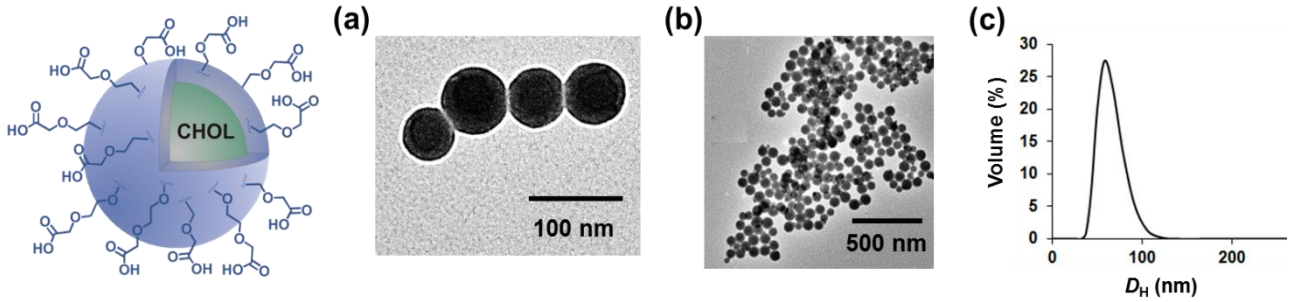


Figure S6. Left: A schematic representation of the PNPs derived from block copolymer $\mathbf{2}_{15}$-*b*-$\mathbf{6}_{10}$. Right panels: (a) a representative TEM image of these PNPs in ultrapure deionized water; (b) a low-magnification TEM image of this material; and (c) the corresponding D_H distribution of these PNPs as measured by DLS.

S5. Cell-viability study for cholesterol-loaded PSNAs

The cholesterol-loaded PSNAs were prepared from the cholesterol-loaded PNPs and characterized using the same protocols described in the main manuscript. Cell-viability data were also obtained using similar conditions described in the main manuscript for the drug-loaded PSNAs.

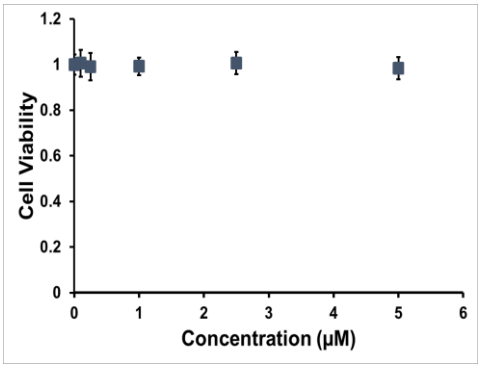


Figure S7. Cell viability data for cholesterol-loaded PSNAs.

Table S1. Sequences of the DNA single strands used in this study.

DNA sequences	
T_{20}	T_{20}-(Spacer 18)$_2$-NH_2-3'
Cy5-T_{20}	Cy5-T_{20}-(Spacer 18)$_2$-NH_2-3'
Melt A	AAC GAC TCA TAT TAA CAA- T_5-(Spacer 18)$_2$-NH_2-3
Melt B	TTG TTA ATA TGA GTC GTT- T_5-(Spacer 18)$_2$-NH_2-3

S6. Stability of Dox-PSNAs in serum

A serum-stability analysis for Dox-PSNAs was carried out by incubating the Dox-PSNAs in a 1× PBS solution comprising 10 vol% human serum (final doxorubicin concentration = 2.5 µM, final volume = 1 mL) at 37 °C. The release of the drug,

as represented by the intensity of the solution fluorescence at 590 nm, was monitored continuously at 5 min intervals with sample excitation at 470 nm. As a negative control experiment, Dox-PSNAs were also incubated in 1× HBS at 37 °C and monitored simultaneously under the same condition. As a positive control, Dox-PSNAs were also incubated in 0.1 M HCl solution and monitored simultaneously under the same condition.

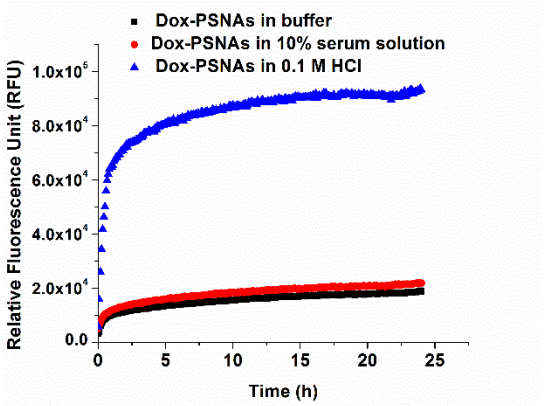


Figure S8. Doxorubicin-based fluorescent profiles for Dox-PSNAs being incubated in PBS buffer, 10 vol % human serum, and 0.1 M HCl (aq). Time 0 is the reading before the addition of serum solution and 0.1 M HCl into the respective samples.

S7. pH-dependent drug release profiles of Dox-PSNAs

The pH dependent release of doxorubicin from dox-PSNAs was evaluated by incubating dox-PSNAs in 1× PBS solutions at different pH (7.4, 6, 5, 4, 3; final doxorubicin concentration in solution 2 μM, final volume 100 μL) at 37 °C. The pH dependent release of doxorubicin from the PSNAs was monitored continuously for a period of 12 h by measuring the solution fluorescence at 590 nm with sample excitation at 470 nm on a Synergy H4 Multimode Microplate Reader.

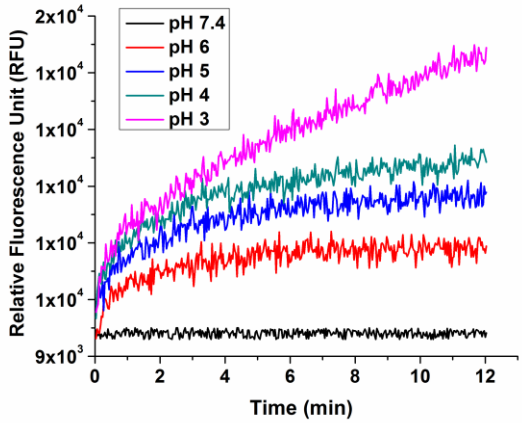


Figure S9. pH release profile of doxorubicin-loaded PSNAs.

S8. Stability of Dox-PSNAs in cell culture media.

An aliquot of Dox-PSNAs (50 μL) was added to an Eppendorf tube containing OptiMEM media (See cell viability section in main manuscript, final volume 500 μL). The size of the Dox-PSNA was analyzed after 24 h incubation period at 37 C using DLS. The Dox-PSNA do not know any signs of aggregation.

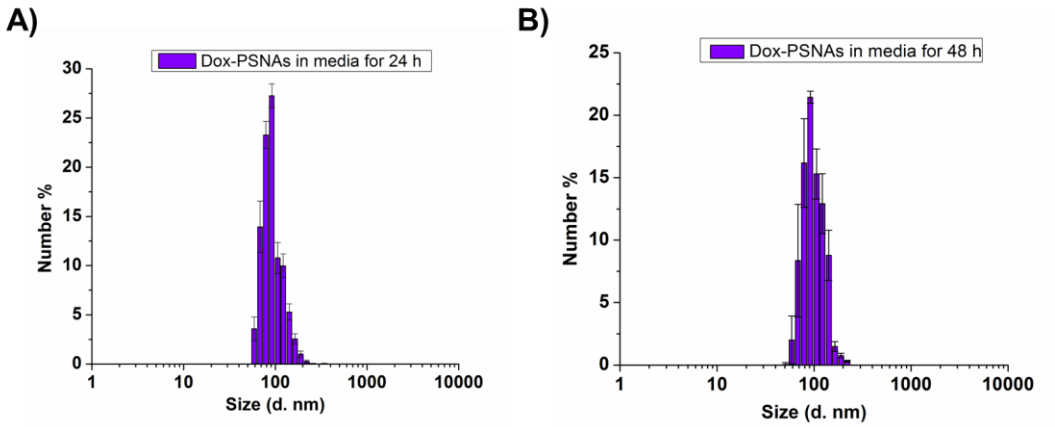


Figure S10. DLS profiles of doxorubicin-loaded PSNAs confirming their stability after 24 and 48 h incubation in cell culture media

S9. Quantification of cellular uptake of Dox-PSNAs and free doxorubicin.

SKOV3 cells (~ 10,000 cell/well) were plated on a 96 well plate in corresponding cell media (see cell culture studies section in the materials and method section of the manuscript). The following day, free doxorubicin and Dox-PSNAs at different doxorubicin concentrations (0.1, 0.25, 5, 1, 1.5, 2 μM) were added and the cells were incubated for 24 h. At the end of incubation period, the cells were washed 3 times with 1× PBS containing 0.01 vol % of Tween-20 and then trypsinized to form a suspension. The resulting cell suspension was subjected to flow cytometry using the doxorubicin intensity channel on a Guava easyCyte 8HT instrument (Millipore, Billerica, MA, USA). The fluorescence was normalized using untreated cells as a negative control. Error values were calculated using the standard error of the mean of median signal from different wells representing one type of sample.

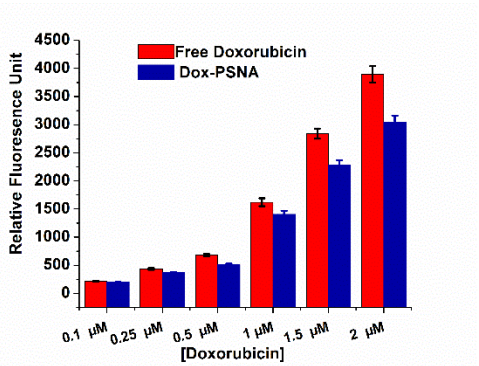


Figure S11. Flow cytometry analysis of SKOV-3 cells that have been incubated with free doxorubicin (red bars) and dox-PSNAs after 24 h (blue bars), showing a higher fluorescent intensity for the cells that have been incubated with free doxorubicin. This can be attributed to a couple of reasons: i) free doxorubicin can be transport faster across the cell membrane and ii) the doxorubicin inside the Dox-PSNAs is partially self-quenched due to higher packing density.

S10. Melt analysis of the [Dox-PSNAs + non-complementary Au-core SNAs] aggregate

A control melt analysis was carried out on a two nanoparticle-component system using the same conditions described in the materials and method section. Dox-PSNAs functionalized with 5'-T_{20}-NH_2-3' strands were prepared as described above. 13 nm Au-core SNAs functionalized with the complementary 5'-T_{20}-SH-3' strands (Table S1) were prepared following a protocol described the material and method section. The two particles were mixed the in a 1:1 ratio (total DNA concentration = 1.5 μM, total volume = 1 mL). The solution were then subjected to a gradually temperature ramp from 20 to 65 ℃ (rate of 0.25 ℃/min) while the absorbance for the solution was continuously monitored at 260 nm.

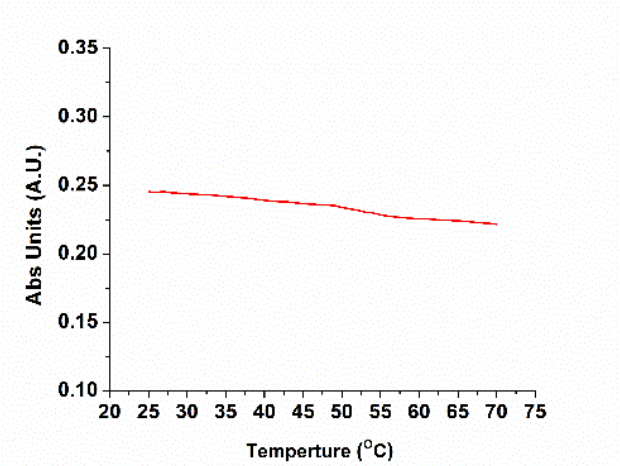


Figure S12. The temperature-dependent absorption (monitored at λ = 260 nm) profile of a mixture comprising Dox-PSNAs and non-complementary Au-core SNAs. The flatness nature of this profile suggests that aggregates did not form. This is in stark contrast to the profile shown in Figure 3D of the manuscript where a cooperative melting profile is observed for the [Dox-PSNAs + complementary Au-core SNAs] aggregate, attributable to the presence of cooperative behavior.

S11. References

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