I. Materials

Catalyst (PCy₃)₂Cl₂Ru=CHPh was purchased from Aldrich Chemicals Co. (Milwaukee, WI) and used as received. Propargyl bromide was purchased from Aldrich Chemicals Co., dried over calcium hydride, vacuum-transferred into an air-tight solvent bulb, and stored under N₂ before use. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA) and used without further purification, except for CD₂Cl₂ and CDCl₃, which were dried over calcium hydride, vacuum-transferred into an air-tight solvent bulb, and stored inside an inert-atmosphere glovebox before use. N-[4-\{(bicyclo[2.2.1]hept-5-en-2-yloxy)methyl]phenyl\}methyl]-2-{1-[\{(4-chlorophenyl)carbonyl\}-5-methoxy-2-methyl-1H-indol-3-yl]acetamide (1),¹¹¹ 1-[4-\{(Bicyclo[2.2.1]hept-5-en-2-yloxy)methyl\}phenyl]-2,5,8,11,14,17-hexaoxanadecan-19-ol (3),¹¹² folate-PEG-azide,¹¹³ and gold nanoparticles functionalized with aliphatic azides were prepared according to literature procedures. Biotin-PEG-azide was purchased from Quanta BioDesign Ltd. (Powell, OH) and used as received.

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

II. General information.

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) and methylene chloride (CH₂Cl₂) were dried over neutral alumina via the Dow-Grubbs solvent system installed by Glass Contours (now SG Water USA, Nashua, NH). 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 150-mm of silica gel under a positive pressure of lab air.
1H and 13C NMR spectra were recorded on a Bruker 500 FT-NMR spectrometer (500 MHz for 1H NMR, 125 MHz for 13C NMR). 1H 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). 1H and 13C chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). Fourier-transformed infrared (FT-IR) spectroscopy was performed on a Thermo Nicolet Nexus 870 FTIR spectrometer (Thermo Nicolet, now Thermo Electron, Madison, WI), using KBr pellets for all samples. Electrospray-ionization mass spectrometry (ESIMS) data was obtained on a Micromass Quattro II Triple Quadrupole mass spectrometer (Micromass, Inc., Beverly, MA). UV-vis absorption spectra for all samples were obtained on a CARY 300 Bio UV-vis spectrometer (Varian, Inc., Cary, NC).

Polymer molecular weights relative to polystyrene standards were measured on a Waters gel-permeation chromatograph (GPC, Waters Corp., Milford, MA) 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 (TEM) was performed on a Hitachi HF8100 microscope (Hitachi High Technologies America, Schaumburg, IL) 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.

Dynamic light-scattering (DLS) measurements were performed on a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) 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 index (PDI) of polymer nanoparticles were calculated by the supplied instrument software (Zetasizer DTS).

III. Synthesis of monomers and their polymerization activity.

1-[4-({bicyclo[2.2.1]hept-5-en-2-yloxy}methyl)phenyl]-2,5,8,11,14,17,20-heptaoxatricos-22-yne (3). This synthesis is a modification of a previously reported procedure.6 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 (2, 500 mg, 1 mmol) in dry THF (10 mL) was added via cannula transfer over a period of 20 min to a suspension of NaH (44 mg, 1.82 mmol) in dry THF (10 mL) in a 50-mL Schlenk flask. Potassium iodide (334 mg, 2 mmol) was added as a solid to the resulting mixture, followed by a solution of propargyl bromide (0.135 mL, 1.52 mmol) in THF (5 mL). A water-cooled condenser was attached to the flask and the reaction mixture was allowed to reflux at 80 °C overnight. 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$_2$Cl$_2$ (20 mL). The combined filtrate was concentrated down and purified by flash chromatography (90:10 v/v CH$_2$Cl$_2$: MeOH) to afford monomer 3 as a yellow oil (330 mg, 61% yield).
NMR (CDCl₃, Figure S1 (top)): δ 1.35-1.71 (m, 4H, 3- and 7-norbornenyl-H₂), 2.39 (s, 1H, C≡CH), 2.76 (b, 1H, 1-norbornenyl-H), 2.89 (b, 1H, 4-norbornenyl-H), 3.53-3.65 (m, 24H, OCH₂CH₂O), 4.15 (s, 2H, OCH₂C≡CH), 4.44-4.50 (m, 5H, CH₂-C₆H₄-CH₂ and 2-norbornenyl-H), 5.86 (m, 1H, 6-norbornenyl-H), 6.15 (m, 1H, 5-norbornenyl-H), 7.25 (b, 4H, aromatic-H). ¹³C NMR (CDCl₃, Figure S1 (bottom)): δ 34.5 (3-norbornenyl-C), 40.4 (4-norbornenyl-C), 46.1 (7-norbornenyl-C), 46.5 (1-norbornenyl-C), 58.4 (OCH₂C≡CH), 69.1-70.1 (m, OCH₂CH₂O), 71.2 (C≡CH), 73.0 (CH-O-CH₂-Ph), 74.6 (Ph-O-CH₂-CH₂), 79.9 (2-norbornenyl-C), 80.0 (C≡CH), 127.6 (aromatic-C), 127.7 (aromatic-C), 133.2 (6-norbornenyl-C), 137.4 (aromatic-C), 138.3 (aromatic-C), 140.7 (5-norbornenyl-C). ESIMS: m/z calculated for C₃₀H₄₄O₈ (M + Na): 555.642. Found: 555.641.

Figure S1. The ¹H (top) and ¹³C NMR (bottom) spectra for 1-[4-{(bicyclo[2.2.1]hept-5-en-2- yloxy)methyl]phenyl]-2,5,8,11,14,17,20-heptaoxatricos-22-yn (3).
1-[4-({bicyclo[2.2.1]hept-5-en-2-yloxy}methyl)phenyl]-24,24-dimethyl-2,5,8,11,14,17,20-heptaoxa-24-silapentacos-22-yne (4). Compound 3 (50 mg, 0.094 mmol) was dissolved in dry THF (15 mL) in a 50-mL Schlenk flask and cooled down to -78 °C using an acetone/dry ice bath. Lithium diisopropylamide (0.052 mL of a 2M solution in THF, 0.103 mmol) was added dropwise to this solution using a gas-tight syringe and the resulting mixture was allowed to stir for 45 min at -78 °C to fully deprotonate monomer 3. In a separate 50-mL Schlenk flask, chlorotrimethylsilane (0.024 mL, 0.188 mmol) was dissolved in dry THF (10 mL) and cannula-transferred to the lithiated reaction mixture. The resulting solution was stirred for an additional 15 min at -78 °C and allowed to warm up to room temperature overnight before being concentrated and purified by flash chromatography (90:10 v/v CH$_2$Cl$_2$: MeOH) to afford monomer 4 as a dark yellow oil (56 mg, 100% yield). 1H NMR (CDCl$_3$, Figure S2 (top)): $\delta$ 0.13 (s, 9H, CSi-(C$_3$H$_3$)$_3$), 1.35-1.71 (m, 4H, 3- and 7-norbornenyl-$H$), 2.76 (b, 1H, 1-norbornenyl-$H$), 2.89 (b, 1H, 4-norbornenyl-$H$), 3.53-3.65 (m, 24H, OCH$_2$CH$_2$O), 4.15 (s, 2H, OCH$_2$C=CSi-(CH$_3$)$_3$), 4.44-4.50 (m, 5H, CH$_2$-C$_6$H$_4$-CH$_2$ and 2-norbornenyl-$H$), 5.86 (m, 1H, 6-norbornenyl-$H$), 6.15 (m, 1H, 5-norbornenyl-$H$), 7.25 (b, 4H, aromatic-$H$). 13C NMR (CDCl$_3$, Figure S2 (bottom)): $\delta$ 0.2 (Si(CH$_3$)$_3$), 34.5 (3-norbornenyl-$C$), 40.4 (4-norbornenyl-$C$), 46.1 (7-norbornenyl-$C$), 46.5 (1-norbornenyl-$C$), 58.4 (OCH$_2$C=CSi(CH$_3$)$_3$), 69.1-70.1 (m, OCH$_2$CH$_2$O), 73.0 (CH-O-CH$_2$-Ph), 80.0 (2-norbornenyl-$C$), 91.8 (C=CSi(CH$_3$)$_3$), 101.5 (C=CSi(CH$_3$)$_3$), 127.6 (aromatic-$C$), 127.8 (aromatic-$C$), 133.2 (6-norbornenyl-$C$), 137.4 (aromatic-$C$), 138.3 (aromatic-$C$), 140.7 (5-norbornenyl-$C$). ESIMS: $m/z$ calculated for C$_{33}$H$_{52}$O$_8$Si (M + Na)$^+$: 604.736. Found: 604.723.
The $^1$H NMR (top) and $^{13}$C NMR (bottom) spectra for 1-[4-([bicyclo[2.2.1]hept-5-en-2-yloxy]methyl)phenyl]-24,24-dimethyl-2,5,8,11,14,17,20-heptaoxa-24-silapentacos-22-yn-4.

**Procedure for the polymerization of monomer 4.** In an inert-atmosphere glovebox, monomer 4 (15 mg, 0.0248 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (2 mL) in a 20-mL scintillation vial equipped with a magnetic stirring bar. A stock solution of catalyst 5 (5 mg) in CH$_2$Cl$_2$ (5 mL) was prepared, a portion of which (1.36 mL, 1.65 µmol) was added to the vial containing the solution of monomer 4 under vigorous stirring. The resulting reaction mixture was stirred for 45 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 $^1$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: $M_n = 10000$ (theoretical $M_n = 9000$), PDI = 1.25. $^1$H NMR (CDCl$_3$, Figure S3): $\delta$ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 4.15 (bs), 4.26-4.54 (bm), 5.20-5.49 (bm), 7.36 (bm).

**Figure S3.** The $^1$H NMR spectrum of the homopolymer of monomer 4.

**IV. Synthesis of block copolymers 1$_{35}$-b-4$_{15}$ and 1$_{35}$-b-3$_{15}$.**

The procedures for synthesizing 1$_{35}$-b-4$_{15}$ and 1$_{35}$-b-3$_{15}$, their associated PNPs, and the relevant analytical data are described below. Procedures and data for other copolymers and their associated PNPs are listed after page S14.

**Synthesis of block copolymer 1$_{35}$-b-4$_{15}$.** In an inert-atmosphere glovebox, monomer 1 (25 mg, 0.0439 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (2 mL) in a 20-mL scintillation vial equipped with a magnetic stirring bar. A stock solution of catalyst 5 (5 mg) in CH$_2$Cl$_2$ (5 mL) was prepared, a portion of which (1.03 mL, 1.26 µmol) was added to the vial containing the solution of monomer 1 under vigorous stirring. The resulting reaction mixture was stirred for 30 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 ($M_n = 21000$ (theoretical $M_n = 20000$), PDI = 1.13).

Immediately after aliquot removal, a solution of monomer 4 (11 mg, 0.0425 mmol) in CH$_2$Cl$_2$ (1.5 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 °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 light yellow solid. $^1$H NMR (CDCl$_3$, Figure S4): $\delta$ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57
(bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC: $M_n = 30000$ (theoretical $M_n = 29000$), PDI = 1.15. IR (KBr, Figure S7): 3309, 2861, 2173, 1680, 1593, 1517, 1477, 1356, 1321, 1224, 1034 cm$^{-1}$.

**Figure S4.** The $^1$H NMR spectrum of block copolymer $1_{35}$-$b$-$4_{15}$.

Procedure for the synthesis of the reverse block copolymer $4_{15}$-$b$-$1_{35}$. Using a similar protocol as specified above for $1_{35}$-$b$-$4_{15}$, the reverse block copolymer $4_{15}$-$b$-$1_{35}$ was synthesized starting with monomer $4$ and isolated quantitatively. $^1$H NMR (CDCl$_3$, Figure S5): δ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC for the first block: $M_n = 11000$ (theoretical $M_n = 9000$), PDI = 1.36. GPC for the block copolymer: $M_n = 32000$ (theoretical $M_n = 29000$), PDI = 1.20.

**Figure S5.** The $^1$H NMR spectrum of reverse block copolymer $4_{15}$-$b$-$1_{35}$. 
Procedure for the TMS deprotection of $1_{35-b-415}$ to yield $1_{35-b-315}$. In a 50-mL Schlenk flask, the TMS-protected block copolymer $1_{35-b-415}$ (20 mg) was dissolved in dry THF (10 mL). After the polymer had completely dissolved, dry MeOH (5 mL) was cannula-transferred to the solution followed by the addition of anhydrous potassium carbonate (3 mg) as a solid. The resulting reaction mixture was stirred for 1 h at room temperature. The reaction mixture was added quickly into vigorously stirred cold (-10 °C) pentanes (200 mL), and the resulting precipitate was isolated via vacuum-filtration and washed thoroughly with fresh pentanes to afford the product copolymer (90% yield) as a light yellow solid. $^1$H NMR (CDCl$_3$, Figure S6): $\delta$ 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC: $M_n = 28000$ (theoretical $M_n = 28000$), PDI = 1.10. The apparently lower PDI of $1_{35-b-315}$ compared to the parent block copolymer $1_{35-b-415}$ can be attributed to the re-isolation of the product using precipitation, which often yield polymers with slightly lower PDI. FTIR (KBr, Figure S7): 3297, 2921, 2129, 1652, 1592, 1519, 1486, 1354, 1306, 1217, 1032 cm$^{-1}$.

**Figure S6.** The $^1$H NMR spectrum of TMS-deprotected block copolymer $1_{35-b-315}$. No peaks exist at 0.18 ppm, indicating the loss of TMS groups in the copolymer composition.

**Figure S7.** FTIR spectra for copolymer $1_{35-b-415}$ (top trace) and TMS-deprotected copolymer $1_{35-b-315}$ (bottom trace). The shift of the C≡C stretch from 2173 cm$^{-1}$ to 2129 cm$^{-1}$ clearly indicates the successful removal of the TMS group.
Procedure for the click reaction with folate-PEG-azide and copolymer 1$_{35}$-b-3$_{15}$. In a 4-mL scintillation vial, a solution of CuSO$_4$·5H$_2$O in DMF (0.10 mg, 0.41 mM, 0.41 µmol) was added to a stirring solution of copolymer 1$_{35}$-b-3$_{15}$ in DMF (10 mg, 0.36 mM, 5.36 µmol of alkyne groups). A freshly prepared aliquot of sodium ascorbate in DMF (0.12 mg, 0.62 mM, 6.15 µmol) was added to the reaction mixture, followed by the addition of a solution of folate-PEG-azide in DMF (3.44 mg, 5.5 mM, 5.36 µmol). The resulting mixture was then stirred at room temperature for 12 h. The reaction mixture was added quickly into vigorously stirred cold (-10 °C) acetone (200 mL), and the resulting precipitate was isolated via vacuum-filtration to afford the product copolymer (75% yield) as a dark brown solid. Unfortunately, due to the insolubility of the “clicked” polymer in several organic solvents, $^1$H NMR and UV analyses could not be carried out. However, FTIR analysis confirmed the absence of the alkyne and azide peaks indicating that all the alkynes had reacted with folate-PEG-azide. FTIR (KBr, Figure S8): 3297, 2921, 1652, 1592, 1519, 1486, 1354, 1306, 1217, 1032 cm$^{-1}$.

Modifying copolymer 1$_{35}$-b-3$_{15}$ with folate-PEG-azide rendered it insoluble in several common organic solvents and mixtures thereof. As such, subsequent PNP formation from this folate-conjugated polymer would be a challenge. Furthermore, modifying the polymer prior to PNP formation does not ensure that all of the bioactive ligands will be present on the PNP surface. Thus, post-PNP formation modification would be a more productive strategy for making folate-functionalized PNPs.

Figure S8. FTIR spectra for alkyne copolymer 1$_{35}$-b-3$_{15}$ (top trace) and folate-“clicked” polymer (bottom trace). The absence of the C≡C stretch at 2129 cm$^{-1}$ is indicative of the successful coupling of the alkyne copolymer to folate-PEG-azide.

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V. Preparation of PNPs from block copolymer 1_{35}-b-3_{15} and subsequent PNP surface functionalization.

**General procedure for the preparation of nanoparticle dispersions.** Aqueous suspensions of the polymer nanoparticles were prepared by dialysis following a modification of the published procedure. An aliquot (2.5 mL) of a stock solution of the deprotected copolymer 1_{35}-b-3_{15} (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 (10 µL, 0.35 wt%) every 10 s using a 2-20 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. DLS analysis of the final PNP aqueous suspension revealed narrowly dispersed PNPs with an average diameter \( D_{H} \) of 128 ± 15 nm and a corresponding polydispersity index (PDI) of 0.060. TEM analysis indicated a uniform size distribution for the PNPs in the solid-state, with an average diameter of ~130 nm that is consistent with the DLS data.

**General procedure for the click reactions with folate- and biotin-PEG-azide.** In a 4-mL scintillation vial, a solution of CuSO\(_4\)·5H\(_2\)O in ultrapure deionized water (500 µL of a 10 nM solution, ~1.25 nmol) was added to an aliquot (1 mL) of the alkyne-functionalized PNP suspension (~0.1 mg of copolymer 1_{35}-b-3_{15}, ~0.052 nmol of alkyne groups). A freshly prepared aliquot of sodium ascorbate in ultrapure deionized water (500 µL of a 20 nM solution, ~1.76 nmol) was added to the reaction mixture, followed by the addition of a solution of the appropriate azide in ultrapure deionized water (50 nM, 1 mL, ~0.05 nmol). The resulting mixture was then attached to a platform shaker (Thermolyne Maxi-Mix III™ Type 65800) and allowed to agitate (200 rpm) at room temperature for 12 h. The reaction mixture was transferred to a 1.5-mL safe-lock Eppendorf tube and centrifuged for 20 min (Hermle Z 230 MA centrifuge set at 10K rpm) to a solid pellet. The supernatant, containing majority of the excess salts and unconjugated materials, was removed and the PNPs were resuspended in ultrapure deionized water (1 mL) upon mild vortexing. The suspension was then transferred to a 3-mL dialysis cassette and dialyzed against ultrapure deionized water (250 mL) with the dialate changed every 3 h, for a total time of 12 h, to further remove excess salts and azido-modified biomolecules.

Control experiments were carried out using the same starting materials and reaction conditions as specified above but in the absence of CuSO\(_4\)·5H\(_2\)O and sodium ascorbate.
Procedure for evaluating the potential for Glaser-type homocoupling of terminal alkynes to occur in copolymer 1_{35}-b-3_{15}. To a stirring solution of copolymer 1_{35}-b-3_{15} in DMF (10 mg, 0.36 mM, 5.36 µmol of alkyne groups) in a 10-mL round-bottomed flask were sequentially added CuI (1.02 mg, 5.36 µmol), Na₂CO₃ (0.9 mg, 10.72 µmol), and iodine (1.36 mg, 5.36 µmol) as solids following a previously reported procedure. A water-cooled condenser was attached to the flask and the reaction mixture was allowed to stir at 80 °C in air overnight. In the morning, the mixture was cooled to room temperature and subsequently filtered over Celite 545 to remove the salt byproducts. The filtrate was then added quickly into vigorously stirred cold (-10 °C) acetone (200 mL), and the resulting precipitate was isolated via vacuum-filtration to afford a dark yellow solid (75% yield). IR (KBr, Figure S9): 3297, 2921, 2129, 1652, 1592, 1519, 1486, 1354, 1306, 1217, 1032 cm⁻¹.

A potential concern in carrying out click chemistry on alkyne-functionalized PNPs is that Cu-catalyzed alkyne homocoupling (i.e., Glaser-type coupling) could occur, either between the surface alkynes on a single particle or between different alkyne-functionalized PNPs. Such homocouplings often require high concentrations of reactants, the presence of excessive oxidants, an inorganic base, and a high reaction temperature. Since such conditions were not employed in our click protocol, oxidative coupling in our alkyne-functionalized PNPs should be negligible. Furthermore, severe aggregation of PNPs would be observed if Glaser-type couplings were to occur between the particles. Given that DLS measurements of the folate- and biotin-modified PNPs do not reveal broad size distributions and PDIs (see below), we are confident that oxidative couplings between PNPs in our dilute reactions are insignificant.

To evaluate the possibility that oxidative coupling may occur between the alkyne groups on the particle surface, we subjected the alkyne-functionalized copolymer 1_{35}-b-3_{15} to the Glaser-type homocoupling reaction conditions as outlined above. After ~12 h, there was no observable change in the polymer starting material and we did not observe any IR stretch in the range of 2200-2300 cm⁻¹ (attributed to dialkyne, see Section IV, Figure S9). Since oxidative homocoupling does not occur under these “harsher-than-click” conditions in our polymer, where the alkyne groups are relatively free to couple, we are confident that it did not occur in our click experiments on the surface of the alkyne-functionalized PNPs, where the alkynes are more constrained. In addition, the click coupling of this alkyne-functionalized copolymer with folate did not display the characteristic dialkyne IR stretch (Section IV, Figure S8), further supporting the unlikelihood of alkyne homocoupling under our click reaction conditions.

Figure S9. FTIR spectra for alkyne copolymer 1_{35}-b-3_{15} (top trace) and the same polymer subjected to Glaser-type reaction conditions (bottom trace). Retention of the C≡C stretch at 2129 cm⁻¹ and lack of any stretches in the range of 2200-2300 cm⁻¹ (attributed to C≡C—C≡C) are indicative of the unsuccessful alkyne homocoupling.
Characterization of folate-modified PNPs. Folate-modified PNPs retained the narrow size distribution of the parent PNPs (PDI = 0.060 with $D_H = 137 \pm 10$ nm), as indicated by DLS and TEM (Figure S10). To assess the degree of modification, a calibration curve (Figure S11) of folic acid absorbance ($\varepsilon = 27022$ cm$^{-1}$ M$^{-1}$ at $\lambda_{\text{max}} = 278$ nm) in water was constructed with several folic acid concentrations (0, 0.005, 0.01, 0.025, 0.05 mmol/L). An aliquot (1 mL) of the purified folate-modified PNP suspension was pipetted into a 1-mL cuvette and its absorbance was recorded. The folate-conjugated PNPs contained ~67 folate groups as determined by UV-vis spectroscopy. As expected, PNPs obtained from the control experiment (in the absence of CuSO$_4\cdot$5H$_2$O and sodium ascorbate) showed no significant absorbance in the folate region.

To quantify the azide-alkyne coupling in a more accurate manner, the absorbance of an aliquot (500 µL) of folate-conjugated PNPs, that were lyophilized and re-dissolved in DMSO, was measured. Based on the calibration curve, it was determined that ~71 folate groups were coupled to the surface of PNPs. Because the number of folate groups obtained by this method closely matched the number previously obtained for intact PNP, we decided to use the initially determined value directly without the need to apply a correction factor (cf. the biotin example below).

**Figure S10.** Left: A schematic representation of the folate-conjugated PNPs after click ligation and subsequent purification. Right: (a) A representative TEM image of the resulting PNPs. (b) $D_H$ distribution of the PNPs as measured by DLS.

**Figure S11.** The UV-vis calibration curve for folic acid ($\varepsilon = 27022$ cm$^{-1}$ M$^{-1}$ at $\lambda_{\text{max}} = 278$ nm) in water, constructed with several folic acid concentrations (0, 0.005, 0.01, 0.025, 0.05 mmol/L).

Characterization of biotin-modified PNPs. Biotin-modified PNPs retained the narrow size distribution of the parent PNPs (PDI = 0.032 with $D_H = 138 \pm 10$ nm), as indicated by DLS and TEM (Figure S12). The number of biotin groups present on the PNP surface was analyzed using a Pierce biotin quantitation kit following the protocol...
A pre-weighed measure of HABA₄-avidin complex (HABA = \([2\text{-}4'\text{-hydroxyazobenzene}\) benzoic acid]) in the biotin quantitation kit was dissolved in ultrapure deionized water (900 µL). This entire HABA₄-avidin solution was pipetted into a 1-mL cuvette, and the background absorbance was measured at 500 nm (λ\(_{\text{max}}\) for HABA₄-avidin, \(\varepsilon = 35000\text{ cm}^{-1}\text{ M}^{-1}\)) using a UV-vis spectrometer. An aliquot (100 µL) of the biotinylated-PNPs was then added to this solution and the resulting mixture was mixed by repeated inversion of the cuvette for ~10 s before the absorbance at 500 nm was re-recorded (Figure S13). Upon introduction of the biotinylated-PNPs, HABA is displaced from the HABA₄-avidin complex, resulting in a decrease in the absorbance that can be correlated to the amount of biotin in the sample. Using the online calculator (http://www.piercenet.com/haba/habacalccuv.cfm), it was determined that there were ~40 biotin molecules/PNP. Analysis of the control experiment (in the absence of CuSO₄·5H₂O and sodium ascorbate) resulted in an average of 2 biotin molecules/PNP which could be attributed to incomplete dialysis.

When the aforementioned biotin/PNP number was compared to the higher level of functionalization obtained with folate-PEG-azide (see above), it appears that biotin-PEG-azide may have coupled to alkyne-PNPs with low efficiency. As mentioned in the manuscript, this discrepancy can be attributed to the fact that some biotin groups might be inaccessible to avidin binding, resulting in imprecise biotin quantification on the PNP surface. To address this issue, we conducted an experiment similar to the PNP-dissolution experiment outlined above for the folate-conjugated PNP. A pre-weighed measure of HABA₄-avidin complex in the biotin quantitation kit was dissolved in ultrapure deionized water (900 µL). This entire HABA₄-avidin solution was pipetted into a 1-mL cuvette, and the background absorbance was measured at 500 nm using a UV-vis spectrometer. An aliquot (100 µL) of the as-prepared biotin-conjugated PNPs was then lyophilized, re-dissolved in DMSO (100 µL), and added to this HABA₄-avidin solution. The resulting mixture was mixed thoroughly using a pipette before the absorbance at 500 nm was re-recorded (Figure S13). Compared to the absorbance recorded in the biotin quantitation of aqueous biotinylated PNPs (see above), a greater decrease in absorbance was observed for the dissolved PNP, clearly indicating that some biotin groups on the intact biotinylated PNPs were sterically hindered and unable to bind avidin. Using the online calculator, it was determined that there actually were ~65 biotin molecules/dissolved PNP. Thus, a correction factor of 1.63 will need to be applied to the apparent biotin conjugation level for intact biotinylated PNPs to obtain the actual number of biotin-modified alkyne groups per PNP.

Figure S12. Left: A schematic representation of the biotin-conjugated PNPs after click ligation and subsequent purification. Right: (a) A representative TEM image of the resulting PNPs. (b) \(D_\text{h}\) distribution of the PNPs as measured by DLS.
Figure S13. UV-vis spectra of the HABA-avidin complex, intact biotin-modified PNPs (after purification), control PNPs (exposed to biotin-PEG-azide in the absence of copper catalyst), and dissolved biotin-modified PNPs (after being dissolved in organic media that disrupted the core-shell structure). The reduced absorbance for the dissolved PNPs clearly indicates that some surface-bound biotin moieties were sterically hindered and unable to bind avidin.

Procedure for the click reactions with azide-functionalized AuNPs. In a 4-mL scintillation vial, a solution of CuSO₄·5H₂O in ultrapure deionized water (500 µL of a 10 nM solution, 1.25 nmol) was added to an aliquot (1 mL) of the alkyne-functionalized PNP suspension (0.1 mg of copolymer 1₃₅-b-3₅₁₅, ~0.052 nmol of alkyne groups). A freshly prepared aliquot of sodium ascorbate in ultrapure deionized water (500 µL of a 20 nM solution, 1.76 nmol) was added to the reaction mixture, followed by the addition of a solution of the 13-nm N₃-modified AuNPs in ultrapure deionized water (100 µL). The resulting suspension was then attached to a platform shaker (Thermolyne Maxi-Mix III™ Type 65800) and allowed to agitate (200 rpm) at room temperature for 12 h. The reaction mixture was transferred to a 1.5-mL safe-lock Eppendorf tube and centrifuged for 20 min (Hermle Z 230 MA centrifuge set at 13.2K rpm) to a solid pellet. The supernatant was removed and the AuNP-conjugated PNPs were resuspended in ultrapure deionized water (1 mL). This process was repeated two more times to remove any unconjugated (i.e., excess, unreacted) AuNPs.⁷⁹

Control experiments were carried out using the same starting materials and reaction conditions as specified above but in the absence of CuSO₄·5H₂O and sodium ascorbate.

Characterization for the AuNPs-N₃-modified PNPs. TEM analysis of an aliquot (5 µL) of the AuNP-PNP dispersion showed the gold nanoparticles bound to the surface of the PNPs (Figure 2a in main text). The corresponding control experiment confirmed that minimal non-specific interactions exist between AuNPs and PNPs. (Figure 2b in main text).

Kinetic evaluation of click reaction as a function of time. Following the procedure described above for the click reaction between alkyne-functionalized PNPs and either biotin- or folate-PEG-azide, the stoichiometric ratio of the azide was varied in three different reactions (0.025, 0.05, and 0.1 nmol of the azide, corresponding to 0.5, 1, and 2
equiv, respectively, was added). The reaction was monitored at 0, 6, 12, 18, 24 and 48 h using the quantification protocols described above. Treating the alkyne PNPs with 0.5, 1, and 2 equiv of folate-PEG-azide resulted in a maximum of 25, 69, and 72 folate groups, respectively. The reaction profile approached a plateau at 12 h for 0.5 equiv and 6 h for 1 and 2 equiv (Figure 1b in main text and S14 herein). Reaction of alkyne PNPs with 0.5, 1, and 2 equiv biotin-PEG-azide resulted in a maximum of 14, 40, and 42 apparent biotin groups, respectively (Figure 1b in main text and S14 herein). In all 3 cases, saturation was achieved at the 24 h time point.

Figure S14. Reaction profiles of click ligation between alkyne PNPs and (a) 0.5 equiv azide; (b) 1 equiv azide; (c) 2 equiv azide; (d) 1 equiv azide with direct dialysis.

While our purification of the “click”-conjugation experiments involves centrifugation to remove majority of the catalysts and non-conjugated materials, we were curious if significant additional click reaction would occur if the work up only consisted of dialysis. For cases where the PNP may not be stable enough to survive the centrifugation, dialysis would be the only viable method for isolating the conjugated materials away from all the remaining reagents and catalysts. In such cases, the dialysis time (~1 week) would be much longer than the centrifugation time (20 min) and it is possible that additional click reaction would occur during this longer time period. Hence, we utilized the aforementioned kinetic evaluation procedure to determine the extent of click reaction occurring during long dialysis times. A series of kinetic experiments similar to that described above were then carried out but without the centrifugation step in the purification process. Instead, the reaction mixtures at the appropriate time points were placed directly into dialysis cassettes and dialyzed against ultrapure deionized water, with dialate changed every 2 h during the first 10 h and every 8 h subsequently, for the total time of 1 week. This slight change in protocol yielded similar results to the ones obtained with centrifugation (Figure S14d; data shown only for 1 equiv azide), assuring that little additional click reactions have occurred during dialysis. Presumably, the reaction
mixture becomes highly dilute during dialysis, resulting in a rapid decrease in reactivity.

VI. Synthesis of other copolymers and their associated PNPs.

**Procedure for the synthesis of the block copolymer 120\text{-}b\text{-}315.** Using a similar protocol as that specified in section IV for 135\text{-}b\text{-}415, block copolymer 120\text{-}b\text{-}415 was synthesized and isolated quantitatively. $^1$H NMR (CDCl$_3$, Figure S15 top): $\delta$ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC for the first block: $M_n = 10000$ (theoretical $M_n = 9000$), PDI = 1.25. GPC for the block copolymer: $M_n = 21000$ (theoretical $M_n = 20000$), PDI = 1.06.

Block copolymer 120\text{-}b\text{-}315 was then synthesized following a similar procedure as that outlined in section IV (Procedure for the TMS deprotection of 135\text{-}b\text{-}415 to yield 135\text{-}b\text{-}315). $^1$H NMR (CDCl$_3$, Figure S15 bottom): $\delta$ 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC: $M_n = 22000$ (theoretical $M_n = 19000$), PDI = 1.29.

**Figure S15.** $^1$H NMR spectra of the TMS-protected copolymer 120\text{-}b\text{-}415 (top) and deprotected copolymer 120\text{-}b\text{-}315 (bottom).
Procedure for the synthesis of the block copolymer 1_{50}-b-3_{15}. Using a similar protocol as that specified in section IV for 1_{35}-b-4_{15}, block copolymer 1_{50}-b-4_{15} was synthesized and isolated quantitatively. $^1$H NMR (CDCl$_3$, Figure S16 top): $\delta$ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC for the first block: $M_n = 10000$ (theoretical $M_n = 9000$), PDI = 1.28. GPC for the block copolymer: $M_n = 39000$ (theoretical $M_n = 38000$), PDI = 1.05.

Block copolymer 1_{50}-b-3_{15} was then synthesized following a similar procedure as that outlined in section IV (Procedure for the TMS deprotection of 1_{35}-b-4_{15} to yield 1_{35}-b-3_{15}). $^1$H NMR (CDCl$_3$, Figure S16 bottom): $\delta$ 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC: $M_n = 38000$ (theoretical $M_n = 37000$), PDI = 1.11.

Figure S16. $^1$H NMR spectra of the TMS-protected copolymer 1_{50}-b-4_{15} (top) and deprotected copolymer 1_{50}-b-3_{15} (bottom).
Procedure for the synthesis of the block copolymer 1_{35}-b-3_{15}. Using a similar protocol as that specified in section IV for 1_{35}-b-4_{15}, block copolymer 1_{35}-b-4_{15} was synthesized and isolated quantitatively. $^1$H NMR (CDCl$_3$, Figure S17 top): $\delta$ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC for the first block: $M_n = 3000$ (theoretical $M_n = 3000$), PDI = 1.20. GPC for the block copolymer: $M_n = 25000$ (theoretical $M_n = 23000$), PDI = 1.13.

Block copolymer 1_{35}-b-3_{15} was then synthesized following a similar procedure as that outlined in section IV (Procedure for the TMS deprotection of 1_{35}-b-4_{15} to yield 1_{35}-b-3_{15}). $^1$H NMR (CDCl$_3$, Figure S17 bottom): $\delta$ 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC: $M_n = 25000$ (theoretical $M_n = 23000$), PDI = 1.23.

Figure S17. $^1$H NMR spectra of the TMS-protected copolymer 1_{35}-b-4_{15} (top) and deprotected copolymer 1_{35}-b-
Procedure for the synthesis of the block copolymer 135-b-325. Using a similar protocol as that specified in section IV for 135-b-415, block copolymer 135-b-425 was synthesized and isolated quantitatively. $^1$H NMR (CDCl$_3$, Figure S18 top): $\delta$ 0.18 (bs), 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC for the first block: $M_n = 16000$ (theoretical $M_n = 15000$), PDI = 1.49. GPC for the block copolymer: $M_n = 36000$ (theoretical $M_n = 35000$), PDI = 1.13.

Block copolymer 135-b-325 was synthesized following a similar procedure as that outlined in section IV (Procedure for the TMS deprotection of 135-b-425 to yield 135-b-325). $^1$H NMR (CDCl$_3$, Figure S18 bottom): $\delta$ 1.10-1.44 (bm), 1.57-1.93 (bm), 2.27 (bs), 2.57-2.68 (bm), 2.92-3.15 (bm), 3.57 (bm), 3.65 (bm), 3.74 (bs), 4.15 (bm), 4.26-4.54 (bm), 5.20-5.49 (bm), 6.64-7.61 (bm), 7.79 (bd). GPC: $M_n = 37000$ (theoretical $M_n = 33000$), PDI = 1.19.
Procedure for the preparation of nanoparticle dispersions. Aqueous suspensions of polymer nanoparticles were prepared from several block copolymers following the protocol as that specified in section V (General procedure for the preparation of nanoparticle dispersions). The internal contrast in the TEM images shown below are most likely due to focus differences during imaging. However, it may also be due to the relative difference in electron densities from the different block ratios in the copolymers. When the proportion of the hydrophobic block segment is higher, the PNPs appear to be darker due to the electron-rich indomethacin-containing hydrophobic segments. On the other hand, as the proportion of the less-electron-rich PEG-containing hydrophilic block increases, the PNPs appear lighter in contrast.

For 120-b-315 (m = 20, n = 15). DLS analysis of the final PNP aqueous suspension revealed narrowly dispersed PNPs with an average $D_H$ of $117 \pm 9$ nm and a corresponding PDI of 0.027. TEM analysis indicated a uniform size distribution for the PNPs in the solid-state, with an average diameter of ~115 nm that is consistent with the DLS data (Figure S19).

For 150-b-315 (m = 50, n = 15). DLS analysis of the final PNP aqueous suspension revealed narrowly dispersed PNPs with an average $D_H$ of $214 \pm 20$ nm and a corresponding PDI of 0.033. TEM analysis indicated a uniform size distribution for the PNPs in the solid-state, with an average diameter of ~210 nm that is consistent with the DLS data (Figure S19).

For 135-b-35 (m = 35, n = 5). DLS analysis of the final PNP aqueous suspension revealed narrowly dispersed PNPs with an average $D_H$ of $149 \pm 14$ nm and a corresponding PDI of 0.019. TEM analysis indicated a uniform size distribution for the PNPs in the solid-state, with an average diameter of ~150 nm that is consistent with the DLS data (Figure S19).

For 135-b-325 (m = 35, n = 25). DLS analysis of the final PNP aqueous suspension revealed narrowly dispersed PNPs with an average $D_H$ of $111 \pm 11$ nm and a corresponding PDI of 0.063. TEM analysis indicated a uniform size distribution for the PNPs in the solid-state, with an average diameter of ~100 nm that is consistent with the DLS data (Figure S19).
In PNP fabrication, when the length of either hydrophilic or hydrophobic block of the copolymer starting materials is varied, a change in the size of the resulting PNPs is expected. In this manuscript, two sets of copolymers were explored—one with varied hydrophobic block length, keeping the hydrophilic block constant, and the second with the opposite block size variation. When the hydrophilic, PEG-alkyne block length is constant \((n = 15)\) and the hydrophobic indomethacin block length is increased \((m = 20, 35, 50)\), the size of the fabricated PNPs is expected to increase due to increase in the proportion of hydrophobic core material per polymer chain. Such a trend is indeed observed based on both DLS and TEM data (Figure S19), consistent with previous observations in our group that copolymers with higher hydrophobic:hydrophilic block ratios afforded PNPs with larger diameters.\(^{S1}\)

However, when the hydrophilic block length is lengthened \((n = 5, 15, 25)\), a decrease in the size of the PNPs, albeit much smaller than the change seen in increasing the hydrophobic block, is anticipated because the lengthening hydrophilic blocks now pack better together in the shell, limiting the size of the hydrophobic core. This decrease in the size of PNPs was apparent in our DLS and TEM data (Figure S19). Similar observations have been reported by Zhang and coworkers.\(^{S10}\) We note that Bertin and coworkers also reported that variation of the hydrophilic block length, while keeping the hydrophobic block length constant, did not result in a significant change in the mean diameters of the fabricated PNPs.\(^{S1}\) Due to a lack of monodisperse PNP samples in this latter work, a clear trend was not apparent.

**Figure S19.** Representative TEM images (left) and \(D_H\) distribution as measured by DLS (right) of the PNPs derived from copolymers \(1_m-b-3_n\), indicating well-defined, narrowly dispersed nanoparticles.

**Procedure for the click reactions with folate- and biotin-PEG-azide on PNPs.** “Click” reactions of PNPs derived from different copolymers with folate- and biotin-PEG-azide were performed in a manner similar to the one...
outlined in section V (General procedure for the click reactions with folate- and biotin-PEG-azide).

PNPs made from 120-b-315 (m = 20, n = 15). Folate-modified PNPs (PDI = 0.052 with \(D_H = 120 \pm 11\) nm) retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). The folate-conjugated PNPs contained ~60 folate groups as determined by UV-vis spectroscopy.

Biotin-modified PNPs (PDI = 0.026 with \(D_H = 124 \pm 12\) nm) also retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). Based on the HABA-avidin assay, it was determined that there were ~36 biotin molecules/PNP. Applying the correction factor to the apparent biotin conjugation level yielded an actual number of ~59 biotin groups/PNP.

PNPs made from 150-b-315 (m = 50, n = 15). Folate-modified PNPs (PDI = 0.045 with \(D_H = 205 \pm 22\) nm) retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). The folate-conjugated PNPs contained ~59 folate groups as determined by UV-vis spectroscopy.

Biotin-modified PNPs (PDI = 0.016 with \(D_H = 204 \pm 15\) nm) also retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). Based on the HABA-avidin assay, it was determined that there were ~38 biotin molecules/PNP. Applying the correction factor to the apparent biotin conjugation level yielded an actual number of ~62 biotin groups/PNP.

PNPs made from 135-b-35 (m = 35, n = 5). Folate-modified PNPs (PDI = 0.032 with \(D_H = 144 \pm 16\) nm) retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). The folate-conjugated PNPs contained ~25 folate groups as determined by UV-vis spectroscopy.

Biotin-modified PNPs (PDI = 0.003 with \(D_H = 145 \pm 11\) nm), also retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). Based on the HABA-avidin assay, it was determined that there were ~17 biotin molecules/PNP. Applying the correction factor to the apparent biotin conjugation level yielded an actual number of ~28 biotin groups/PNP.

PNPs made from 135-b-325 (m = 35, n = 25). Folate-modified PNPs (PDI = 0.037 with \(D_H = 108 \pm 11\) nm) retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). The folate-conjugated PNPs contained ~137 folate groups as determined by UV-vis spectroscopy.

Biotin-modified PNPs (PDI = 0.042 with \(D_H = 108 \pm 12\) nm) also retained the narrow size distribution of the parent PNPs, as indicated by DLS and TEM (Figure S20). Based on the HABA-avidin assay, it was determined that there were ~76 biotin molecules/PNP. Applying the correction factor to the apparent biotin conjugation level yielded an actual number of ~124 biotin groups/PNP.
Figure S20. Representative TEM images (top) and $D_{44}$ distribution (as measured by DLS, bottom) for biotin- and folate-conjugated PNPs derived from copolymers $1_m$-b-$3_n$. Both sets of data indicate retention of the original well-defined, narrowly dispersed spherical morphology.

Evaluation of surface conjugation efficiencies for PNPs derived from copolymers with the same alkyne-functionalized block length yielded similar results (Figure S21). Such a trend is anticipated since this set of PNP dispersions has an equivalent density of surface functional groups.

Figure S21. Similar conjugation profiles achieved for PNPs derived from copolymers with a constant hydrophilic block length.

VII. Miscellaneous experiments.
Attempted polymerization of monomer 3. In an inert-atmosphere glovebox, monomer 3 (10 mg, 0.0188 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (2 mL) in a 20-mL scintillation vial equipped with a magnetic stirring bar. A stock solution of catalyst 5 (5 mg) in CH$_2$Cl$_2$ (5 mL) was prepared, a portion of which (1.033 mL, 1.26 µmol) was added to the vial containing the solution of monomer 3 under vigorous stirring. The resulting reaction mixture was stirred for 1 h 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 incomplete consumption of the monomer (Figure S22). The remaining portion was evaporated to dryness, dissolved in HPLC-grade THF, and subjected to GPC analysis: $M_n = 4000$ (theoretical $M_n = 8000$), PDI = 1.39.

Figure S22. The $^1$H NMR spectrum of the attempted polymerization of monomer 3 by catalyst 5 clearly shows left-over norbornene monomer peaks (red box), indicating incomplete polymerization.

Attempted polymerization of monomer 1 in the presence of phenylacetylene. In an inert-atmosphere glovebox, monomer 1 (10 mg, 0.0176 mmol) was dissolved in CD$_2$Cl$_2$ (2 mL) in a 20-mL scintillation vial equipped with a magnetic stirring bar. A solution of phenylacetylene (100 µL, 0.9106 mmol) in CD$_2$Cl$_2$ (1 mL) was added to the vial containing the solution of monomer 1. A stock solution of catalyst 5 (5 mg) in CD$_2$Cl$_2$ (5 mL) was prepared, a portion of which (0.413 mL, 0.502 µmol) was added to the vial containing the mixture of monomer 1 and phenylacetylene under vigorous stirring. The resulting reaction mixture was stirred for 1 h at room temperature at which time an aliquot (500 µL) was removed and quenched with excess ethyl vinyl ether. This quenched aliquot was evaporated to dryness, redissolved in CDCl$_3$, and analyzed by $^1$H NMR spectroscopy, which indicated incomplete consumption of the monomer (Figure S23). This suggests that presence of free acetylene groups inhibits polymerization of norbornene-based monomers.
Figure S23. The $^1$H NMR spectrum of the attempted polymerization of monomer 1 by catalyst 5 in the presence of phenylacetylene clearly shows left-over norbornene monomer peaks (red box), indicating that polymerization was inhibited.

Formation of PNPs derived from TMS-protected copolymer 1$_{35-b-415}$. Aqueous suspensions of TMS-protected PNPs containing 0.01 wt% of 1$_{35-b-415}$ copolymer were prepared following a similar procedure as described in section V (General procedure for the preparation of nanoparticle dispersions). A representative TEM image (Figure S24a) indicated a spherical morphology in good agreement with DLS data ($D_{h} = 186 \pm 22$ nm, PDI = 0.013) (Figure S24b).

Figure S24. Left: A schematic representation of the TMS-protected PNPs derived from the parent copolymer 1$_{35-b-415}$. Right: (a) A representative TEM image of the resulting PNPs, indicating a well-defined, narrowly dispersed spherical morphology. (b) $D_{h}$ distribution of the PNPs as measured by DLS.

Attempted one-pot in situ TMS-deprotection/CuAAC on PNPs derived from parent copolymer 1$_{35-b-415}$. In a
4-mL scintillation vial, an aliquot of AgBF₄ in ultrapure deionized water (1 µL of a stock solution of 1.2 mg in 200 mL water, 30.3 nmol) was added to an aliquot of the TMS-protected PNP suspension (~0.1 mg of copolymer 1₃₅-b-4₁₅, ~0.052 nmol of TMS-protected alkyne groups). This resulting mixture was then attached to a platform shaker (Thermolyne Maxi-Mix III™ Type 65800) and allowed to agitate (200 rpm) at room temperature for ~10 min. A solution of CuSO₄·5H₂O in ultrapure deionized water (500 µL of a 10 nM solution, ~1.25 nmol) was added to the reaction mixture, followed by the addition of a freshly prepared aliquot of sodium ascorbate in ultrapure deionized water (500 µL of a 20 nM solution, ~1.76 nmol) was added to the reaction mixture and allowed to agitate at room temperature for 12 h. The suspension was then transferred to a 3-mL dialysis cassette and dialyzed against ultrapure deionized water (250 mL) with the dialate changed every 3 h to remove excess salts and azido-modified biomolecules. Unfortunately, attempts at performing a one-pot in situ TMS-deprotection/CuAAC resulted in loss of the original well-defined morphology of the parent PNPs as observed by TEM and DLS (Figure S25b: folate-treated PNPs $D_\text{H} = 328$ nm, PDI = 0.909; Figure S25d: biotin-treated PNPs $D_\text{H} = 1476$ nm, PDI = 0.937).

Figure S25. Left: Proposed schematic illustration of PNPs obtained after a one-pot in situ TMS-deprotection/CuAAC on 186-nm PNPs derived from copolymer 1₃₅-b-4₁₅. Right top panels: (a) TEM and (b) DLS data of the PNPs after folate treatment. Right bottom panels: (c) TEM and (d) DLS data of the PNPs after biotin treatment, clearly indicating loss of the original well-defined, narrowly dispersed spherical morphology in both cases (as compared to Figure S24).

VIII. References:


