

Supporting Information

**pH-Sensitive Brush Polymer-Drug Conjugates by Ring-Opening
Metathesis Copolymerization**

Jiong Zou,[†] Goran Jafr,[‡] Efrosyni Themistou,[†] Yoonsing Yap,[†] Zachary A. P. Wintrob,[‡]
Paschalis Alexandridis,[†] Alice C. Ceacareanu,[‡] and Chong Cheng^{*,†}

[†]*Department of Chemical and Biological Engineering, and* [‡]*Department of Pharmacy
Practice, University at Buffalo, The State University of New York, Buffalo, NY 14260*

*Author to whom correspondence should be addressed:

Tel: (716)645-1193; Fax: (716)645-3822; Email: ccheng8@buffalo.edu (C. Cheng)

Experimental Section

Materials. 5-NB-2-carboxylic acid (*endo/exo* mixture; 98%), iodine (99.8%), potassium iodide (99%), sodium hydroxide (97%), sodium bicarbonate (99+%), sodium thiosulfate (99%), *N,N'*-dicyclohexyl-carbodiimide (DCC; 99%), 4-dimethylaminopyridine (DMAP; 99+%), 1,6-hexanediol (99%), pyridinium chlorochromate (PCC; 98%), 3-bromopyridine (99%), ethylene oxide (99.5%), potassium (99.5%), the 1st generation Grubbs catalyst (**7a**) and the 2nd generation Grubbs catalyst were purchased from Sigma-Aldrich. L-Malic acid (99%), boron trifluoride diethyl etherate (98%), 1,1-diphenylethylene (99%) and ethyl vinyl ether (99%) were purchased from Acros. Diethyl ether (HPLC), tetrahydrofuran (THF; HPLC), dichloromethane (DCM; HPLC), ethyl acetate (HPLC), and hexane (HPLC) were purchased from Fisher Chemical. Paclitaxel (PTXL; 99+%) was purchased from AvaChem. 7-Diethylaminocoumarin-3-carbonyl azide (**10**) was purchased from molecular probe. Toluene (99.5+%) was purchased from VWR. Ethylene oxide, DCM, hexane, and toluene were dried by distillation over CaH₂; THF was dried by refluxing overnight over CaH₂ and then distillation from sodium naphthalene. All chemicals were used without further purification unless stated otherwise.

exo-5-NB-2-carboxylic acid (**3**), *exo*-5-NB-2-methanol, and the 3rd generation Grubbs catalyst (**7b**) were obtained respectively following literature methods.¹⁻³ 1,4-Dipotassio-1,1,4,4-tetraphenylbutane (~0.25 M) was prepared by the reaction of

1,1-diphenylethylene (2.04 g, 11.3 mmol) with an excess of potassium (0.98 g, 25.1 mmol) in 20 mL of dry THF at room temperature overnight under nitrogen atmosphere.⁴

Measurements. ¹H NMR spectra were recorded at 500 MHz on solutions in CDCl₃ on a Varian INOVA-500 spectrometer maintained at 25 °C, with tetramethylsilane (TMS) as an internal reference standard. ¹³C NMR spectra were recorded at 125.7 MHz on solutions in CDCl₃ on a Varian INOVA-500 spectrometer maintained at 25 °C, with the solvent carbon signal as standard.

Absolute molecular weight and molecular weight distribution were determined by gel permeation chromatography (GPC). GPC was conducted using Viscotek GPC system equipped with a VE-3580 refractive index (RI) detector, a 270 dual detector system having a viscometer detector and a dual-angle (7° and 90°) laser light scattering detector, a VE 1122 pump, and two mixed-bed organic columns (PAS-103M and PAS-105M). *N,N'*-dimethylformamide (HPLC grade) with 0.1 M LiBr was used as solvent for polymers and eluent for GPC with a flow rate of 0.35 mL/min at 55°C. Polymer solutions were prepared at a known concentration (ca. 3 mg/mL) and an injection volume of 100 μL was used. The system was calibrated with linear polystyrene standards with narrow polydispersities (PDI < 1.1; Polymer Laboratories, Varian Inc.).

Hydrodynamic diameters (D_h) and size distributions of BPDCs in aqueous solutions were determined by dynamic light scattering (DLS). DLS measurements were performed on a Brookhaven Goniometer (BI-200SM Ver.2.0; Brookhaven Instruments,

Holtsville, NY) maintained at 25 °C at 514 nm laser light irradiation. The photon count rate was measured at a fixed angle perpendicular to the incident light (90°). The correlation function was fitted with NNLS routine to provide size distributions. Dilute solutions of brush polymer-drug conjugates (BPDCs; 1.0 mg/mL in water) were passed through 450 nm low protein binding hydrophilic LCR (PTFE) membrane filter (Millex-LCR, Millipore) for dust free process prior to the DLS measurements.

Transmission electron microscopy (TEM) images were obtained by using a JEOL 2010 microscope. TEM samples were prepared by dip coating 300 mesh carbon-coated copper grids with dilute solutions of BPDCs (1.0 mg/mL). Fresh prepared 0.2 % solution of ruthenium tetroxide prepared by reaction between sodium periodate and hydrated ruthenium dioxide in water was used as the staining agent for TEM samples.⁵ The dried samples were treated with the volatile vapors of ruthenium tetroxide for 5 h prior to TEM measurements.

The profiles of drug release from BPDC was acquired by combined TLC separation and HPLC method.⁶ The BPDC with 0.04 mg/ml concentration was prepared in distilled water (pH = 7.0) and acid condition (pH = 5.5, 20 mM; acetate buffer). Based on the low water-solubility of PTXL, the solution concentration was selected to avoid precipitation of PTXL released from BPDC. At different time intervals, every time 5 mL of BPDC solution was taken out, and then extracted by chloroform (3 × 5 ml). The extracted free PTXL-based moieties in chloroform were separated by TLC and

concentrated for HPLC measurements. TLC separation was operated on a PE silica gel plate (PE SIL G/UV; Whatman), eluted with 10% MeOH in DCM. The silica gel on the TLC plate within the R_f range corresponding to PTXL-based moieties ($0.5 < R_f < 0.8$) was scratched off, and washed by DCM several times. After solvent evaporation, the dried PTXL-based moieties were dissolved in 0.1 mL acetonitrile for HPLC measurement. HPLC analysis was performed on Agilent 1100 series HPLC system with G1322A online degasser, G1312A binary pump, and G1313A autosampler. Chromatographic separations were achieved using a reversed phase C18-column (ZORBAX SB-C18, 250×4.6 mm, 5μ) at 25°C . The mobile phase consisted of deionized water and HPLC grade acetonitrile, with linear gradients of acetonitrile/water (1:9~4:6 v/v 0~10 min, 4:6~6:4 v/v 10~15 min, 6:4~4:6 v/v 15~22 min, 4:6~1:9 22~24 min) at a flow rate of 1.0 ml/min. The UV wavelength for the detection of PTXL moieties was set at 227 nm.⁶ It should be noted that TLC process to obtain the released PTXL moieties was required because polymer scaffolds potentially may clog the HPLC column.

6-Hydroxyhexyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (4). In a 100 mL flask with a stirring bar, 1,6-hexanediol (2.4 g, 20 mmol), DCC (0.922 g, 4.47 mmol) and DMAP (49.2 mg, 0.41 mmol) were added and dissolved in 30 mL CHCl_3 . Then **3** (563 mg, 4.07 mmol) dissolved in 10 mL CHCl_3 was added dropwise into the reaction mixture under reflux within 30 min. After stirring under reflux for 48 h, the reaction mixture was allowed to cool to room temperature. The white precipitate was removed by

filtration and the filtrate was concentrated in vacuo and purified by column chromatography (silica gel, eluting with hexane, ethyl acetate in gradient; **4** had a R_f value of 0.3 in 1:1 (v/v) of hexane-ethyl acetate) to obtain **4** as white solid. Yield: 0.536 g (55%). ¹H NMR (500 MHz, CDCl₃, ppm): δ 1.34-1.97 (m, 12H, 6 × CH₂), 2.21 (d of d, 1H, *J* = 4.5 Hz and *J* = 10.2 Hz, CH), 2.92 (s, 1H, CH), 3.03 (s, 1H, CH), 3.65 (m, 2H, CH₂OH), 4.08 (t, 2H, *J* = 6.5 Hz, CH₂OCO), 6.10-6.15 (m, 2H, CH=CH). ¹³C NMR (125.7 MHz, CDCl₃, ppm) δ 25.4, 25.7, 28.7, 30.3, 32.6, 41.5, 43.2, 46.3, 46.6, 62.8, 64.4, 135.7, 138.0, 176.4.

6-Oxohexyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (5). In a 100 mL flask with a stirring bar, **4** (495 mg, 1.83 mmol) was dissolved in 35 mL DCM. Then PCC (592 mg, 2.75 mmol) was added directly to the mixture. After stirring under r. t. for 1 h, the reaction mixture was filtered and the filtrate was concentrated in vacuo. The mixture was purified by column chromatography (silica gel, eluting with hexane, ethyl acetate in gradient; **5** had a R_f value of 0.5 in 1:1 (v/v) of hexane-ethyl acetate) and gave **5** as colorless liquid. Yield: 395 mg (80 %). ¹H NMR (500 MHz, CDCl₃, ppm): δ 1.36-1.97 (m, 10H, 5 × CH₂), 2.21 (dd, 1H, *J* = 4.5 Hz and *J* = 10.2 Hz, CH), 2.45 (t, 2H, *J* = 7.0, CH₂CHO), 2.92 (s, 1H, CH), 3.03 (s, 1H, CH), 4.09 (t, 2H, *J* = 6.5 Hz, CH₂OCO), 6.10-6.15 (m, 2H, CH=CH), 9.77 (s, 1H, CH₂CHO). ¹³C NMR (125.7 MHz, CDCl₃, ppm) δ 21.6, 25.5, 28.4, 30.3, 41.5, 43.2, 46.3, 46.6, 43.6, 64.3, 135.6, 137.9, 174.7, 202.9.

2-((4R)-2-(5-(Bicyclo[2.2.1]hept-2-enecarbonyloxy) pentyl)-5-oxo-1,3-

dioxolan-4-yl)acetic acid (6). In a 10 mL flask with a stirring bar, **5** (120 mg, 0.508 mmol) was added into a 5 mL Et₂O solution of L-malic acid (75 mg, 0.56 mmol), and after stirring the mixture at r. t. for 10 min, BF₃OEt₂ (355 mg, 2.5 mmol) was added. The reaction was conducted in r. t. for 60 h under N₂ atmosphere. The reaction mixture was purified by column chromatography (silica gel, eluting with hexane, ethyl acetate in gradient; **6** had a R_f value of 0.2 in 1:1 (v/v) of hexane-ethyl acetate) and gave **6** as colorless liquid. Yield: 108 mg (61 %). ¹H NMR (500 MHz, CDCl₃, ppm): δ 1.35-1.93 (m, 12H, 6 × CH₂), 2.21 (d of d, 1H, *J* = 4.5 Hz and *J* = 10.2 Hz, CH), 2.84-3.05 (m, 4H, 2 × CH and CH₂COOH), 4.08 (t, 2H, *J* = 6.5 Hz, CH₂OCO), 4.61 (t, 1H *cis*, *J* = 4.8 Hz, CHCH₂COOH), 4.65 (t, 1H *trans*, *J* = 4.8 Hz, CHCH₂COOH), 5.57 (t, 1H *cis*, *J* = 4.8 Hz, OCH(CH₂)O), 5.73 (t, 1H *trans*, *J* = 4.8 Hz, OCH(CH₂)O), 6.10-6.15 (m, 2H, CH=CH). ¹³C NMR (125.7 MHz, CDCl₃, ppm) δ 22.3, 25.5, 28.4, 30.3, 33.5, 34.7, 35.3, 41.5, 43.2, 46.3, 46.6, 64.3, 70.1, 71.2, 104.7, 105.9, 135.6, 137.9, 172.1, 173.9, 176.5.

NB-PTXL conjugated monomer (1). In a 25 mL flask with a stirring bar, **6** (108 mg, 0.306 mmol), paclitaxel (274 mg, 0.321 mmol), DCC (69.4 mg, 0.337 mmol) and DMAP (8 mg, 0.061 mmol) were added separately and dissolved in 10 mL DCM. The reaction mixture was stirred at r. t. for 48 h, filtrated, and concentrated and then separated by flash chromatography (on silica gel; eluting with hexane, ethyl acetate in gradient; **1** had a R_f value of 0.3 in 1:1 (v/v) of hexane-ethyl acetate), giving 310 mg of **1** as a white solid. Yield: 90 %. ¹H NMR (500 MHz, CDCl₃, ppm): δ 1.13 (s, 3H, CH₃), 1.25 (s, 3H,

CH_3), 1.26-2.00 (m, 19H, CH , $2 \times CH_3$, and $6 \times CH_2$), 2.12-2.42 (m, 6H, CH , CH_2 , $OCOCH_3$), 2.42-2.59 (m, 5H, OH , CH , and $OCOCH_3$), 2.90-3.01 (m, 4H, $2 \times CH$, and CH_2), 3.47 (s, 1H, OH), 3.65 (s, 1H, OH), 3.82 (d, 1H, $J = 7.1$ Hz, CH), 4.03 (t, 2H, $J = 6.5$ Hz, CH_2OCO), 4.18-4.36 (m, 2H, CH_2O), 4.44 (m, 1H, $CHOH$), 4.54 (t, 1H *cis*, $J = 4.8$ Hz, CH), 4.56 (t, 1H *trans*, $J = 4.8$ Hz, CH), 4.97 (d, 1H, $J = 8.0$, $CHOCH_2$), 5.46 (m, 1H, $OCHO$), 5.51 (s, 1H, $CHOH$), 5.68 (d, 1H, $J = 7.1$ Hz, $CHOCOPh$), 6.00 (dd, 1H, $J = 9.2$ Hz, $J = 3.0$ Hz, $PhCH$), 6.09-6.12 (m, 2H, $CH=CH$), 6.23-6.34 (m, 2H, $2 \times CHOCO$), 7.01 (1H, d, $J = 9.2$ Hz, NH), 7.45 (m, 10H, $Ar-H$), 7.61 (t, 1H, $J = 7.2$ Hz, $Ar-H$), 7.80 (2H, d, $J = 7.4$ Hz, $Ar-H$), 8.15 (2H, d, $J = 7.2$ Hz, $Ar-H$). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm) δ 9.6, 14.8, 20.8, 22.5, 24.9, 25.5, 26.7, 28.3, 30.3, 33.1, 33.9, 35.3, 35.6, 41.6, 43.2, 45.6, 46.3, 46.6, 52.8, 58.5, 64.1, 71.3, 71.8, 71.9, 72.1, 74.8, 75.1, 75.5, 76.4, 79.1, 81.0, 84.4, 105.0, 126.5, 127.3, 128.5, 128.6, 128.8, 129.1, 129.2, 130.2, 132.0, 132.8, 133.4, 133.7, 135.6, 136.8, 138.0, 138.1, 142.7, 167.0, 167.4, 167.7, 169.9, 171.3, 171.9, 176.4, 203.8.

PEG-based NB-functionalized macromonomer (2). The preparation of **2** was based on a literature method with modification.³ In a 100 mL flask, a THF solution of *exo*-5-NB-2-methanol (0.55 g, 4.4 mmol; 20 mL) was titrated with a fresh prepared THF solution of 1,4-dipotassio-1,1,4,4-tetraphenylbutane (~0.25 M) under nitrogen atmosphere, using the characteristic red color of the latter as indicator. After *exo*-5-NB-2-methanol was quantitatively deprotonated, the solution was cooled with dry

ice-acetone bath, and 9.0 g of ethylene oxide (205 mmol) was added. After cooling with dry ice-acetone bath for 5 h, the solution was allowed to rise to room temperature overnight. After keeping at room temperature for over 40 h, the reaction medium was deactivated by addition of acidified methanol. The reaction mixture was concentrated by solvent evaporation, and then precipitated in diethyl ether to give 7.6 g of **2** as a white solid. Yield: 80 %. $DP_{\text{PEG}}^{\text{NMR}} = 50$ (determined based on the resonance intensities of ethylene oxide protons at 3.32-3.90 ppm relative to NB alkene protons at 6.04-6.15 ppm), $M_n^{\text{NMR}} = 2.4$ kDa, $PDI^{\text{GPC}} = 1.1$ (based on signal from the RI detector of GPC). ^1H NMR (500 MHz, CDCl_3 , ppm) : δ 1.00-1.35 (m, 4H, $2 \times \text{CH}_2$), 1.70 (m, 1H, CH), 2.74-2.88 (m, $2 \times \text{CH}_2$ and OH), 3.32-3.90 (m, 202H, CH_2O and $50 \times \text{CH}_2\text{CH}_2\text{O}$), 6.04-6.15 (m, 2H, $\text{CH}=\text{CH}$). ^{13}C NMR (125.7 MHz, CDCl_3 , ppm) δ 29.6, 38.7, 41.5, 43.6, 44.9, 61.5, 70.2, 70.3, 70.5, 72.5, 75.9, 124.0, 136.5.

General procedure for the synthesis of BPDCs (8). To a 10 mL Schlenk flask with a magnetic stir bar were added monomer **1**, macromonomer **2**, and DCM (2.0-4.0 mL) as the solvent under nitrogen atmosphere. After **1** and **2** were completely dissolved, a DCM solution of Grubbs catalyst (either **7a** or **7b**; <1.2 mL) was added to start polymerization at room temperature. When the polymerization time was reached, the polymerization solution was quenched by the addition of ethyl vinyl ether (1.0-2.0 mL). An aliquot of the resulting polymerization solution was analyzed by ^1H NMR and GPC analysis. Full conversions of **1** and **2** were confirmed by the absence of the resonances of

exo-NB alkene protons at 6.1 ppm on the ^1H NMR spectrum and the absence of GPC peaks corresponding to **1** (peak at 52.6 min) and **2** (peak at 57.4 min) on the GPC curve. The polymerization solution was precipitation in diethyl ether and gave BPDC **8** as a white solid. ^1H NMR (500 MHz, CDCl_3 , ppm): 1.00-3.20 (br m, all *CH*, *CH*₂, *OCOCH*₃ from **1** and **2** units except those identified otherwise), 3.20-4.09 (br m, all *CH*₂*O* and *OCH*₂*CH*₂*O* from **2** units, *CH* and $2 \times$ *OH* from **1** units), 4.09-4.64 (br m, *CH*, *CH*₂, *CHOH*, and *CH*₂*OCO* from **1** units), 4.97 (br, *CHOCH*₂ from **1** units), 5.04-5.58 (br m, all *CH=CH* from PNB, *OCHO* and *CHOH* from **1** units), 5.68 (br, *CHOCOPh* from **1** units), 6.00 (br, *PhCH* from **1** units), 6.20-6.35 (br m, $2 \times$ *CHOCO* from **1**), 7.11 (br, *NH* from **1** units), 7.30-8.20 (br m, all *Ar-H* from **1** units). ^{13}C NMR (125.7 MHz, CDCl_3 , ppm) δ 9.8, 14.8, 21.0, 21.4, 22.9, 34.1, 35.8, 43.2, 61.7, 70.3, 70.5, 72.6, 105.1, 123.9, 126.9, 127.3, 128.6, 129.1, 130.0, 167.0.

BPDC 8a. Sample **8a** was prepared from the polymerization mixture of monomer **1** (200 mg, 0.168 mmol), macromonomer **2** (402 mg, 0.168 mmol), and 1st generation Grubbs' catalyst **7a** (5.54 mg, 0.00673 mmol) in DCM. The polymerization was quenched at 3 h, and the isolation yield was 90%. $M_n^{\text{GPC}} = 104$ kDa, $\text{PDI}^{\text{GPC}} = 1.11$.

BPDC 8b. Sample **8b** was prepared from the polymerization mixture of monomer **1** (50 mg, 0.042 mmol), macromonomer **2** (100 mg, 0.042 mmol), and 2nd generation Grubbs' catalyst **7b** (1.48 mg, 0.00168 mmol) in DCM. The polymerization was quenched at 15 min, and the isolation yield was 85%. $M_n^{\text{GPC}} = 127$ kDa, $\text{PDI}^{\text{GPC}} = 1.04$.

BPDC 8c. Sample **8c** was prepared from the polymerization mixture of monomer **1** (100 mg, 0.084 mmol), macromonomer **2** (201 mg, 0.084 mmol), and 1st generation Grubbs' catalyst **7a** (0.69 mg, 0.00084 mmol) in DCM. The polymerization was quenched at 19 h, and the isolation yield was 90%. $M_n^{\text{GPC}} = 571$ kDa, $\text{PDI}^{\text{GPC}} = 1.34$.

BPDC 8d. Sample **8d** was prepared from the polymerization mixture of monomer **1** (50 mg, 0.042 mmol), macromonomer **2** (100 mg, 0.042 mmol), and 1st generation Grubbs' catalyst **7a** (0.37 mg, 0.00042 mmol) in DCM. The polymerization was quenched at 1.5 h, and the isolation yield was 87%. $M_n^{\text{GPC}} = 390$ kDa, $\text{PDI}^{\text{GPC}} = 1.09$.

Supporting Figures

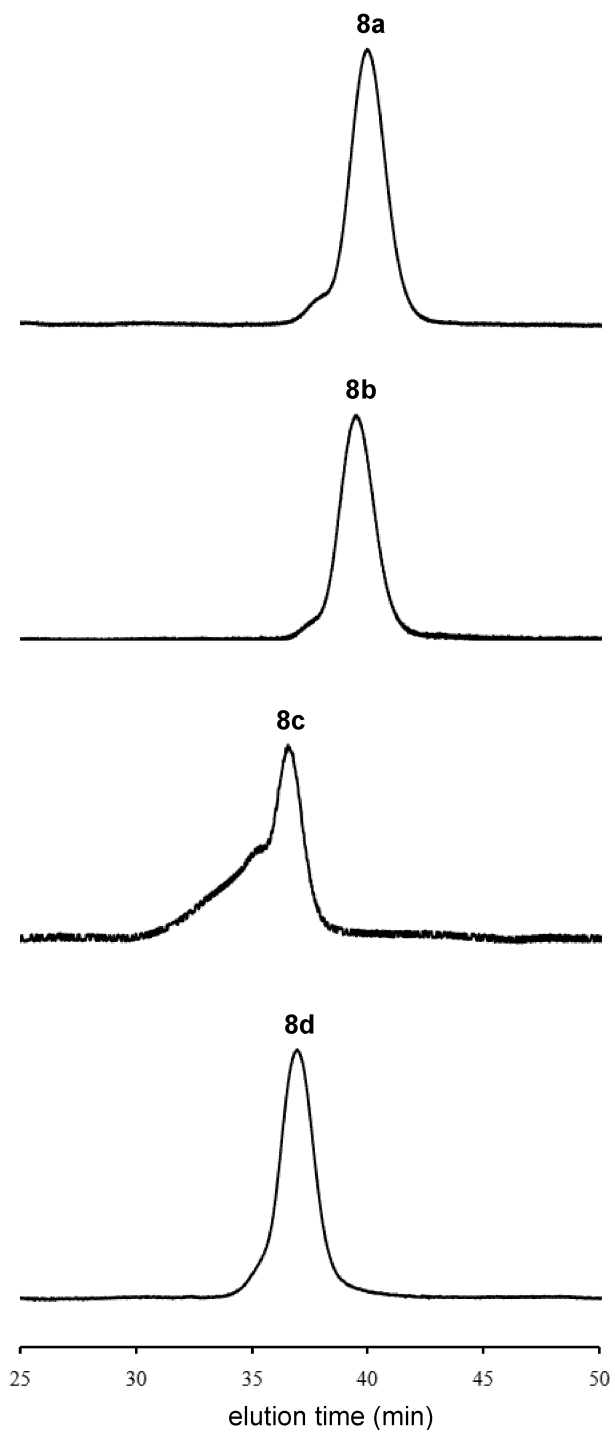


Figure S1. GPC curves of BPDCs **8a-8d**.

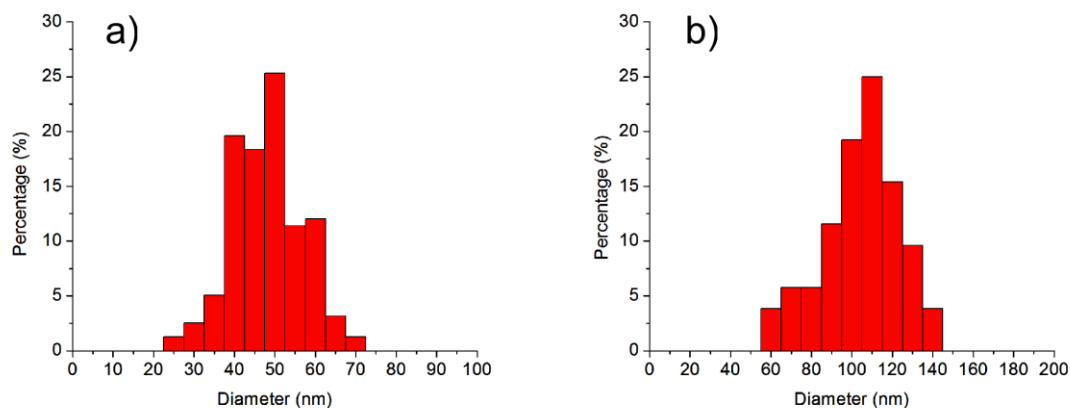


Figure S2. Histograms of fractions of particles vs particle surface diameters (nm) of for a) **8a** and b) **8d** on TEM grids (samples were prepared by dip coating from aqueous BPDC solutions).

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